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Synthesis of novel types of acyclic nucleoside phosphonates and preparation of prodrugs and drug delivery systems

Syntéza nového typu acyklických nukleosid fosfonátů a příprava proléčiv a systémů doručení léčiva.

Doctoral thesis

Supervisor: Ing. Zlatko Janeba, CSc.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze dne 10. 6. 2021 .....

Podpis

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Abstract

First part of this thesis was focused on the previously overlooked field of C1'-branched acyclic nucleoside phosphonates (ANPs). Five diverse synthetic approaches were developed/optimized affording key 6-chloropurine intermediates bearing N^9-phosphonomethoxyethyl (PME) branched at C1' position in 2–4 steps. It was demonstrated that these intermediates can be further vastly diversified into ANPs bearing both natural and unnatural nucleobases. Single enantiomers as well as racemates of final C1'-branched ANPs (overall 48 final compounds) were prepared and selected compounds were evaluated with respect to their biological properties. The aforementioned ANPs showed no antiviral potency against studied viruses and only weak to moderate cytostatic activity. Adenine C1'-branched ANPs proved to be the most potent currently known inhibitors of *Trypanosoma brucei* adenine phosphoribosyl transferase (*Tbr*APRT), an enzyme involved in purine salvage pathway (PSP) of *T. brucei*. Further biological evaluation of prepared compounds is in progress.

Second part of this thesis was focused on development of novel prodrug moieties with higher selectivity index (*i.e.* toxicity/potency ratio – SI) based on so-called ProTide prodrugs where phenol (present in ProTides) was replaced by tyrosine derivatives. Tenofovir was selected as suitable model compound and the efficiency of developed prodrugs was evaluated against human immunodeficiency virus (HIV) and hepatitis B virus (HBV), and compared to ProTide prodrug of tenfovir (tenofovir alafenamide fumarate – TAF). Three series were developed affording overall 27 prodrugs. The prodrugs were resolved using chiral HPLC column to fast-eluting epimer (FEE) and slow-eluting epimer (SEE) as a new chiral center on the phosphorous atom was formed during the synthesis. The synthesis was optimized to obtain prodrugs in one-pot manner with up to 86% yield. The developed prodrugs reached up to single-digit picomolar potency against HIV and nearly 300-fold higher SI compared to TAF. Regarding HBV, the most efficient prodrug reached over 25-fold higher SI compared to TAF. The developed prodrugs showed slightly higher chemical stability, significantly higher plasma stability, but considerably lower microsomal stability than TAF. The metabolic studies demonstrated markedly higher cellular uptake of the novel prodrugs and substantially higher levels of released tenofovir inside the cells compared to TAF. Sharp differences between the metabolism of FEEs and SEEs were further revealed. These promising results provide a strong foundation for further development of these novel prodrugs.

Abstrakt

První část této práce se zabývá dříve přehlíženými C1'-větvenými acyklickými nukleosid fosfonáty (ANPs). Bylo vyvinuto/optimalizováno pět různých syntetických přístupů vedoucích ve 2–4 krocích ke klíčovému 6-chlorpurinovému intermediátu nesoucímu N^9-fosfonomethoxyethyl (PME) větvený v poloze C1'. Bylo prokázáno, že tyto intermediáty mohou být dále rozsáhle diversifikovány na ANPs nesoucí jak přírodní tak modifikované nukleobáze. Byly připraveny jednotlivé enantiomery i racemáty finálních C1'-větvených ANPs (celkem 48 finálních látek) a vybrané látky byly testovány pro jejich biologické vlastnosti. Zmíněné ANPs neprokázaly žádnou aktivitu vůči studovaným virům a pouze slabou nebo mírnou cytostatickou aktivitu. Adeninové C1'-větvené ANPs se prokázaly být nejaktivnějšími dosud známými inhibitory adenin fosforibosyl transferásy *Trypanosomy brucei* (*Tbr*APRT), enzymu záchytného mechanismu purinů. Další hodnocení biologických vlastností stále probíhá.

Druhá část této práce se zabývá vývojem nových proléčiv s vyšším indexem selektivity (tzn. poměr toxicity a aktivity – SI), která jsou založená na tzv. ProTide proléčivech, a která obsahují deriváty tyrosinu namísto fenolu (přítomného v ProTidech). Tenofovir byl vybrán jako vhodná modelová látka, proto byla efektivita vyvinutých proléčiv hodnocena proti lidskému imunodeficientnímu viru (HIV) a viru hepatitidy B (HBV) a byla porovnána s ProTide proléčivem tenofoviru – tenofovirem alafenamide fumarátem (TAF). Byly vyvinuty tři série a celkově 27 proléčiv. Proléčiva byla rozdělena na chirální HPLC koloně na rychle eluovaný epimer (FEE) a pomalu eluovaný epimer (SEE), protože v průběhu syntézy bylo vytvořeno nové chirální centrum na atomu fosforu. Syntéza byla optimalizována, aby byly požadovaná proléčiva získána "one-pot" s výtěžkem až 86 %. Vyvinutá proléčiva dosáhla aktivity proti HIV v řádu jednotek picomolů a téměř 300-krát vyšší SI než TAF. Proti HBV dosáhlo nejnadějnější proléčivo 25-krát vyšší SI než TAF. Vyvinutá proléčiva jsou chemicky mírně stabilnější, výrazně stabilnější v plasmě, ale značně labilnější v mikrosomech než TAF. Metabolické studie odhalily znatelně vyšší prostup do buňek nových proléčiv a podstatně vyšší hladiny uvolněného tenofoviru v buňkách než TAF. Dále byly odhaleny výrazné rozdíly mezi metabolismem FEE a SEE. Tyto nadějné výsledky jsou silným základem pro další vývoj těchto nových proléčiv.

List of abbreviations

AA	aldehyde approach
AIDS	acquired immunodeficiency syndrome
ANP	acyclic nucleoside phosphonate
ANPpp	acyclic nucleoside phosphono diphosphate
APRT	adenine phosphoribosyl transferase
ara-CMP	cytarabine monophosphate
ATP	adenosine triphosphate
AUC	area under the curve
B16	B16 melanoma cell line
BOC	*tert*-butyloxycarbonyl
CA	condensation approach
cAMP	3′,5′-cyclic adenosine monophosphate
CC_{50}	50% cytotoxic concentration
CCRF-CEM	T-lymphoblastoid cell line
CD	circular dichroism
CHIKV	chikungunya virus
COSY	correlation spectroscopy
cPrPMEDAP	9-((2-phosphonomethoxy)ethyl)-N^6-cyclopropyl-2,6,diaminopurine
CycloAmb	cycloaminobenzyl
CycloSal	cyclosaligenyl
CYP	cytochrome P_{450}
CYP3A4	cytochrome P_{450} isoenzyme 3A4
DABCO	1,4-diazabicyclo[2.2.2]octane
dAMP	deoxyadenosine monophosphate
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMAM	dimethylaminomethylene
DMF	*N*,*N*-dimethylformamide
DMF DMA	*N*,*N*-dimethylformamide dimethyl acetal
DNA	deoxyribonucleic acid
DTE	*S*-((2-hydroxyethyl)sulfidyl)-2-thioethyl
EC_{50}	50% effective concentration
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EV71	enterovirus 71
FDA	U.S. Food and Drug Administration

FEE	fast-eluting epimer
GA	glycerol approach
GC-MS	gas chromatography coupled with mass spectrometer
GI	gastrointestinal
GTP	guanosine triphosphate
HA	hemiaminal approach
HAT	human African trypanosomiasis
HATU	(2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HCV	hepatitis C virus
HDP	hexadecyloxypropyl
HeLa S3	cervical cancer cell line
HepG2	liver cancer cell line
HG(X)PRT	hypoxanthine-guanine-(xanthine) phosphoribosyl transferase
HIV	human immunodeficiency virus
HL60	human leukemia cell line
HMBC	heteronuclear multiple quantum coherence
HPLC	high-performance liquid chromatography
(*S*)-HPMPC	((*S*)-1-((3-hydroxy-2-phosphonomethoxy)propyl)cytosine
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
K_i	inhibitory constant
L1210	leukemia cell line
LPLs	lysophospholipids
LTQ	linear trap quadrupole
MNV	murine norovirus
MRP	multidrug resistance protein
MW	microwave
NB	nucleobase
NECT	nifurtimox eflornithine combination therapy
NMR	nuclear magnetic resonance
OAT	organic anion transporter
PDA	photodiode-array
PLs	phospholipids
PME	(phosphonomethoxy)ethyl
PMEA	9-((phosphonomethoxy)ethyl)adenine
PMEG	9-((phosphonomethoxy)ethyl)guanine
PMEGpp	9-((phosphonomethoxy)ethyl)guanine diphosphate
(*R*)-PMPA	((*R*)-9-((2-phosphonomethoxy)propyl)adenine

PO	oral administration
POC	isopropyloxymethyl carbonate
POM	pivaloyloxymethyl
ProTide	commonly used abbreviation for aryloxy phosphonamidate prodrugs (PROdrug of nucleoTIDE)
PRT	phosphoribosyl transferase
PSP	purine salvage pathway
RNA	ribonucleic acid
RSV	respiratory syncytial virus
SA	substitution approach
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SATE	*S*-acyl-2-thioethyl
SEE	slow-eluting epimer
SI	selectivity index, *i.e.* toxicity/potency ratio
SMR	starting material recovery
SQD	single quadrupole detection
TAF	tenofovir alafenamide fumarate
*Tbr*APRT	*Trypanosoma brucei* adenine phosphoribosyl transferase
TDF	tenofovir disoproxil fumarate
TFA	trifluoroacetic acid
TFV	tenofovir
TFVpp	tenofovir diphosphate
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	trimethylsilyl
TMSBr	trimethylsilyl bromide
TMSOTf	trimethylsilyl triflate
UHPSFC-MS	ultrahigh-performance supercritical fluid chromatography with mass spectrometer
UPC2	ultra-performance convergence chromatograph
UPLC	ultra-performance liquid chromatography
WHO	World Health Organization
YFV	yellow fever virus

CONTENTS

Introduction

Nucleotides (**Figure 1**) are absolutely essential for all living organisms. They play the central role in energy metabolism as fundamental molecules for energy transfer (ATP, GTP). They are components of cofactors necessary for the activity of many enzymes. Finally, they are crucial for information transfer either as messenger molecule (cyclic adenosine monophosphate – cAMP) or as the building blocks of DNA and RNA.

A number of different nucleoside analogues is currently used in clinic as therapeutics against viral infections or cancer.[1] Inside the cell, most of the nucleoside analogues need to undergo phosphorylation to the corresponding triphosphates in order to exert an activity.[2,3] Modified nucleosides are inherently different from natural nucleosides, hence, the kinases ensuring the first, most selective phosphorylation often generate the modified monophosphates less efficiently.[2,4,5] This has been identified as the rate-limiting step in the bioactivation of these molecules.[6] To bypass this drawback, analogues of nucleoside monophosphates (nucleotides, **Figure 1**) have been synthesized. They are, however, unstable *in vivo* due to the chemically and enzymatically labile P–O bond.[7] To address this issue, nucleoside phosphonates equipped with more stable P–C bond were developed and the development eventually led up to clinically used drugs.[8]

Acyclic nucleoside phosphonates

Acyclic nucleoside phosphonates (ANPs) are chemically and metabolically stable analogues of nucleotides. ANPs were first developed in the group of prof. Antonín Holý more than 30 years ago.[8] In contrast to the canonical nucleotides, the molecules of ANPs possess the phosphonate moiety instead of the phosphate moiety and an aliphatic chain containing an oxygen atom instead of the cyclic sugar (**Figure 1**).[9–11]

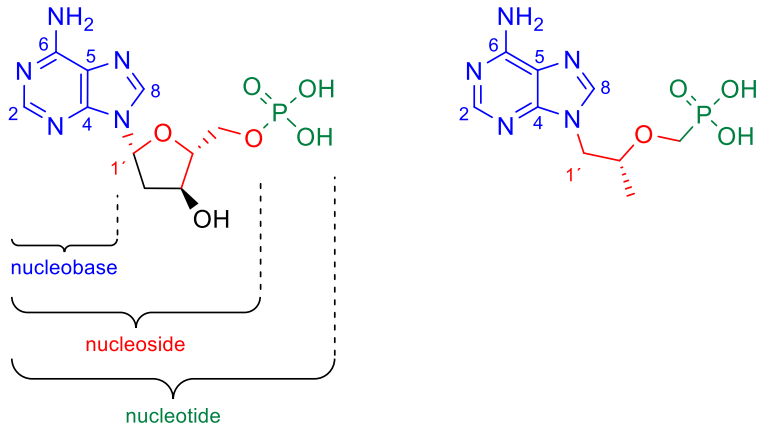


Figure 1. Deoxyadenosine monophosphate – dAMP (left) and analogous ANP tenofovir (right).

The major advantages of ANPs include the presence of the C-P linkage which is enzymatically and chemically more stable compared to the O-P linkage in natural nucleotides, and the presence of the phosphonate moiety which bypasses the first, most critical, and rate-limiting phosphorylation.[7] Another advantage is the high flexibility of the acyclic scaffold of the molecule which, on one hand increases entropic penalty during the enzyme-binding process, but on the other hand reduces the risk of resistance development.

Biological activity of ANPs

ANPs are mostly known as a class of antiviral and anticancer agents although the antiprotozoal activity should not be forgotten.[3,12] Under physiological conditions, phosphonate is deprotonated and the polar ANP molecule is unable to effectively penetrate lipophilic cell membranes.[13] Therefore, a suitable prodrug strategy[14–16] has to be utilized to transport ANPs into cells, where the prodrug moiety is cleaved off and the parent ANP is further phosphorylated to active phosphono diphosphate (ANPpp).[3,10,11]

Antiviral activity

Following an antiviral activity of ANPs ultimately led to the development of three compounds which are currently commercially available as antiviral drugs (some of them in the form of prodrugs) – adefovir, cidofovir, and tenofovir (**Figure 2**). Adefovir ((9-((2-phosphonomethoxy)ethyl)adenine – PMEA) was first reported in 1987 by Holý and Rosenberg[17], and is used for the treatment of chronic hepatitis B infections.[18] Cidofovir ((*S*)-1-((3-hydroxy-2-phosphonomethoxy)propyl)cytosine – (*S*)-HPMPC) was first described by De Clercq et al.[19] in 1987 and is currently approved for the treatment of human cytomegalovirus (HCMV) retinitis in AIDS patients but is also effective against adeno-, herpes-, papilloma-, and poxvirus infections.[20] Finally, tenofovir ((*R*)-9-((2-phosphonomethoxy)propyl)adenine – (*R*)-PMPA) which was first mentioned by Balzarini et al.[21] in 1993 is effective against retroviruses and is used for the treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections.[22]

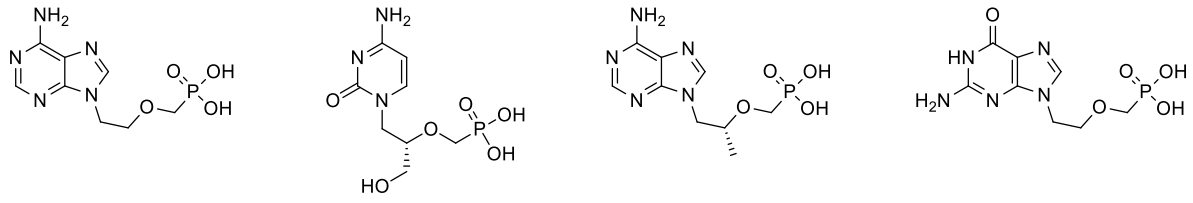


Figure 2. Structures of adefovir ((9-((2-phosphonomethoxy)ethyl)adenine – PMEA), cidofovir ((*S*)-1-((3-hydroxy-2-phosphonomethoxy)propyl)cytosine – (*S*)-HPMPC), tenofovir ((*R*)-9-((2-phosphonomethoxy)propyl)adenine – (*R*)-PMPA), and PMEG ((9-((2-phosphonomethoxy)ethyl)guanine).

The reason for the significant antiviral activity is very selective affinity of the diphosphorylated metabolite (ANPpp) to the viral DNA polymerase compared to the human α, β, and γ polymerases.[13] The diphosphorylated metabolite acts as a competitive inhibitor of the viral DNA and/or RNA polymerase (or reverse transcriptase in the case of retroviruses) but also as a substrate for the viral DNA polymerase. It can be therefore incorporated into the growing DNA strand thus terminating the chain elongation.[9,13]

Antitumor activity

Cidofovir, adefovir, and the guanine counterpart of adefovir – PMEG (9-((2-phosphonylmethoxy)ethyl)guanine) also exhibit remarkable antitumor activity (**Figure 2**).[23,24] The antiproliferative activity of PMEG was confirmed *in vivo* in murine tumor model against both P388 leukemia and B16 melanoma.[25] PMEG was found to act via the diphosphorylated metabolite (PMEGpp) which exhibits high affinity towards human DNA polymerases α and ε involved in DNA replication. It acts either as an inhibitor of these polymerases or as a chain terminator upon incorporation in the growing DNA strand which arrests the S phase during the cell replication and leads to subsequent apoptosis.[26,27] However, PMEG showed very narrow therapeutic window and *in vivo* experiments revealed high prevalence of nephrotoxicity and gastrointestinal toxicity, and the last two decades have been dedicated to efforts for development of suitable prodrug, none of which so far reached past phase II of clinical trials.[24]

Antiprotozoal activity

Recently, ANPs were found to inhibit purine phosphoribosyl transferases (PRTs) – the crucial enzymes in the purine salvage pathway (PSP).[28–34] As some pathogens (e.g. *Plasmodium* or *Trypanosoma*) are unable to synthesize purines *de novo*, the inhibition of enzymes involved in PSP of these pathogens is considered to be an interesting medicinal target. Despite the complexity and redundancy of enzymes involved in PSP, it has been shown that inhibition of hypoxanthine-guanine-(xanthine) phosphoribosyl transferases (HG(X)PRTs) using ANPs leads to growth inhibition of *Plasmodium falciparum*[28–32] and *Trypanosoma brucei*[33,34].

Trypanosoma brucei

According to WHO, *Trypanosoma brucei* (**Figure 3**) accounts for 98% reported cases of human African trypanosomiasis (HAT) also known as sleeping sickness.[35] There had been several major epidemics in the past century and this protozoan parasitic disease still puts 65 millions of people at risk. However, due to intensive efforts the number of cases fortunately steadily decreases.[36]

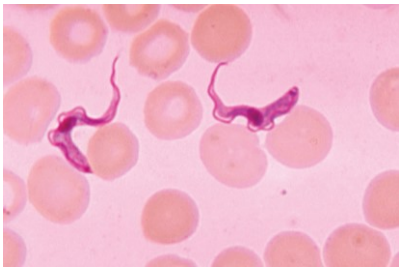


Figure 3. *Trypanosoma* in the blood of a patient suffering HAT (image by Kennedy[37]).

HAT is transmitted by tsetse fly through saliva during the feeding and has two forms in humans, each caused by different subspecies – *T. brucei gambiense* is responsible for the more common form while *T. brucei rhodesiense* for the less common form.[38] There are two stages recognized during HAT. The early stage can last months or even years, however, in case of *T. brucei rhodesiense*, the early stage is more rapid leading to the late stage in the time frame of weeks. The late stage is defined by the penetration of the trypanosomas into the central nervous system which is associated with increased neurological disorders including sleeping disorders (observed in approx. 75% of patients) giving the disease its name.[39] Without the treatment, the disease leads inevitably to coma and death.

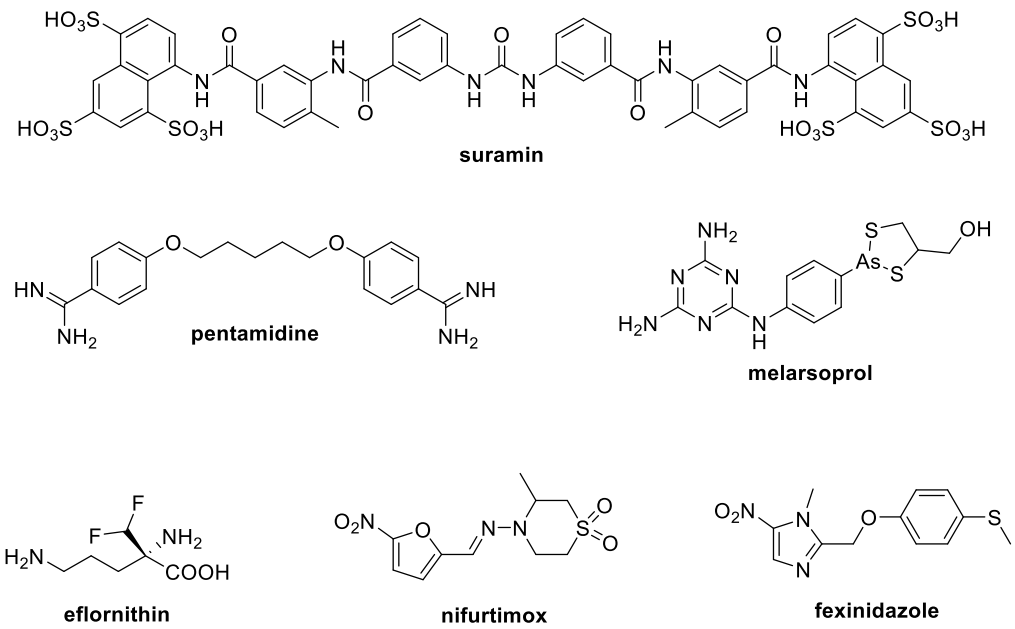


Figure 4. Currently used agents for treatment of HAT.

Current treatment for early stage relies mainly on pentamidine or suramin while late stage is treated with melarsoprol or nifurtimox/eflornithin combination (**Figure 4**).[40] Pentamidine and suramin represent the first line treatment for *gambiense* and *rhodesiense* HAT, respectively. Both pentamidine and suramin are associated among others with

nephrotoxicity and gastrointestinal symptoms.[40] Melarsoprol is a very toxic arsenic drug causing reactive encephalopathy in 10% of patients half of which eventually die.[37] Sadly, melarsoprol is still the only treatment for the late stage *rhodesiense* HAT. Since 1981, eflornithin replaced melarsoprol in the treatment of late stage *gambiense* HAT. While being less toxic than melarsoprol, eflornithin still exhibits serious adverse effects including hypertension, macular rash, peripheral neuropathy, tremor, and gastrointestinal problems.[41] Moreover, due to low plasma stability ($t_{1/2}$ = 3 h), eflornithin needs to be administered very frequently. Enhancement of the therapy was achieved by nifurtimox eflornithine combination therapy (NECT) as nifurtimox is administered PO and eflornithin is administered less frequently. Nevertheless, NECT exerts higher incidence of tremors, anorexia, and nausea compared to eflornithin therapy.[40] Recently, a new promising oral drug fexinidazole (**Figure 4**) was approved for treatment of early stage and non-severe late stage *gambiense* HAT. Although less effective than NECT, fexinidazole exhibit less severe effects like vomiting, headache, and tremors.[42] Due to the aforementioned drawbacks of current treatment the need for new therapeutics against *T. brucei* persists.

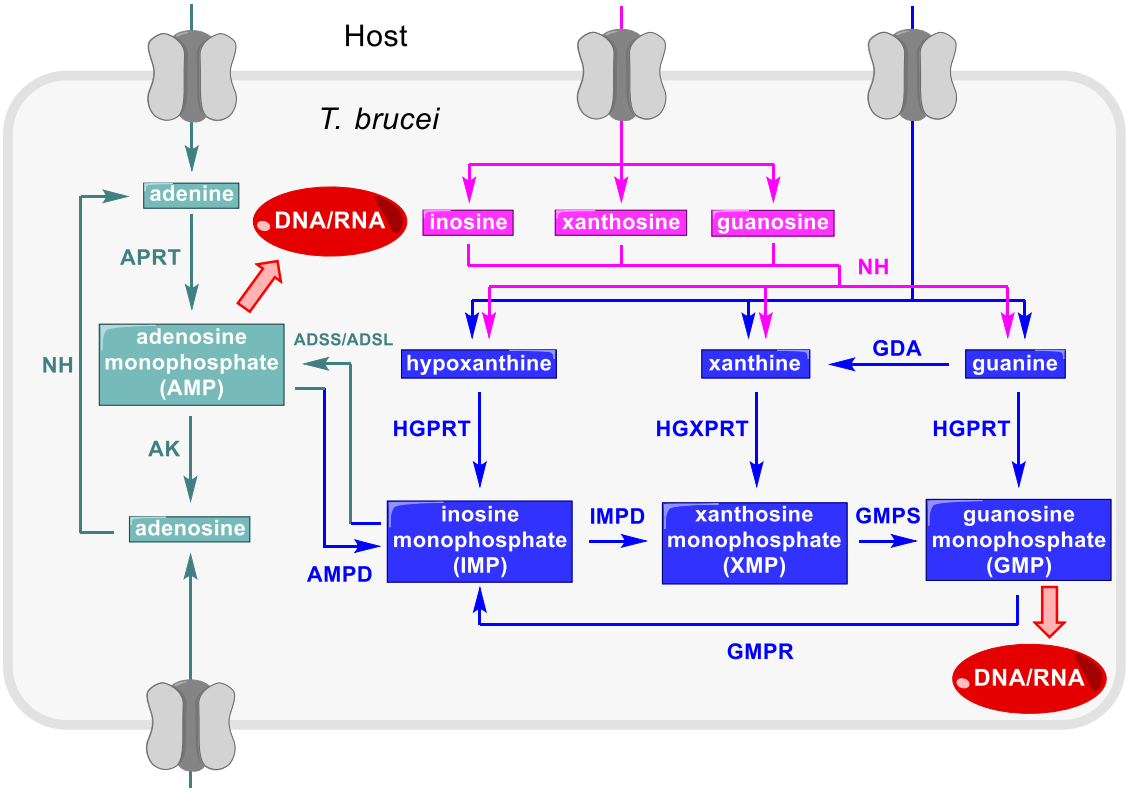


Figure 5. Purine salvage pathway of *Trypanosoma brucei*. AK – adenosine kinase, ADSL – adenylosuccinate lyase, ADSS – adenylosuccinate synthase, AMPD – AMP deaminase, APRT – adenine phosphoribosyl transferase, GDA – guanine deaminase, GMPR – GMP reductase, GMPS – GMP synthase, HG(X)PRT – hypoxanthine–guanine–(xanthine) phosphoribosyl transferase, IMPD – inosine-5′-monophopshate dehydrogenase, NH – nucleoside hydrolase.[33]

As extracellular parasite, *T. brucei* remains in body fluids where it multiplies by binary fission and the essential nucleotides for the reproduction are accessible for *T. brucei* solely via PSP (**Figure 5**).[37] As mammalian cells are able to obtain purines *de novo*, inhibition of PSP enzymes is thought to be a promising target. Phosphoribosyltransferases (PRTs) catalyze the formation of purine nucleoside monophosphate from purine nucleobase and 5-phospho-D-ribosyl-1-pyrophosphate (**Figure 6**) and as crucial enzymes in PSP, PRTs were recently investigated as potential targets.[33,34,43] Despite the redundancy and complexity of *T. brucei* PSP, hypoxanthine and guanine ANPs were able to inhibit hypoxanthine-guanine-(xanthine) phosphoribosyl transferases (HG(X)PRTs) *in vitro* thus inhibiting the growth of *T. brucei*.[33]

Since the versatility of PSP might eventually lead to evolution of resistance, the design of selective adenine phosphoribosyl transferase (APRT) inhibitors nicely complements HG(X)PRT inhibitors and might lead to a complex restriction of parasite growth. Very recently, Doleželová et al. showed that even though APRT1/2 are not essential for *T. brucei*, adenine ANPs were able to inhibit *in vitro* the growth of *T. brucei*.[43]

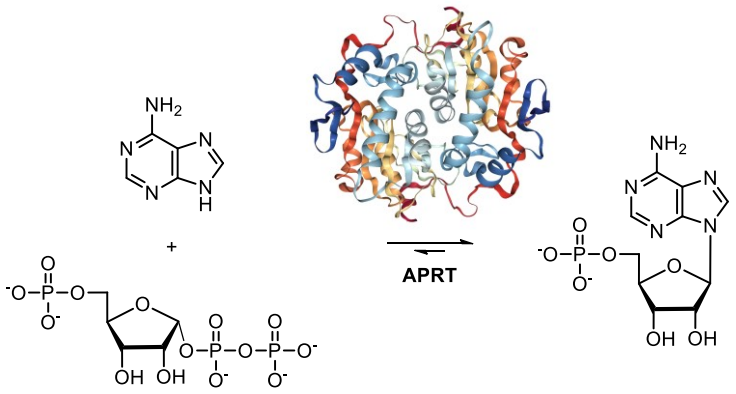
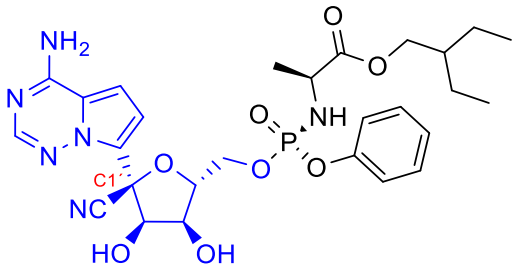


Figure 6. General transformation catalyzed by purine PRTs shown on the example of *Tbr*APRT[44].

C1'-Modified nucleotides

Extensive structure-activity relationship studies on ANPs were performed over the years in which both nucleobase and/or aliphatic linker were modified.[12,45] Interestingly, the field of C1′-modified ANPs has been far less explored compared to the other structural modifications. Currently only a handful of C1′-branched ANPs is known and all of them are only adenine derivatives.[46–49] This is surprising since C1′-branched ANPs, lacking the labile glycosidic bond, should be more chemically and enzymatically stable compared to analogous C1′-substituted (deoxy)ribose containing nucleos(t)ide analogues.[50] Recent discoveries exposed the potential of such compounds as Warren et al.[51] described the potent antiviral C1′-branched C-nucleotide prodrug GS-5734 (currently know under the name of remdesivir) (**Figure 7**) where the cyano substituent at the position C1′ is thought to increase the potency and selectivity towards the viral RNA polymerase.



GS-5734
remdesivir

Figure 7. Structure of remdesivir (parent nucleoside depicted in blue).

Remdesivir is a prodrug of the parent nucleoside (**Figure 7**, blue), which was introduced by Cho et al.[52] in 2012 as a part of a research of antiviral efficacy of C1′-modified nucleosides. The parent nucleoside was shown to be effective against hepatitis C virus (HCV) and other RNA viruses, and remdesivir showed remarkable efficiency (nanomolar EC_{50}) against Ebola virus, HCV, and respiratory syncytial virus (RSV) *in vitro* using human cell lines.[51,53] Moreover, this compound was also effective against Ebola virus *in vivo* in rhesus monkeys.[51] Since remdesivir proved to be an effective inhibitor of RNA-dependent RNA-polymerases of various viral families (including Coronaviridae), it has been most recently studied as a potential therapy against SARS-CoV-2 (causing the ongoing COVID-19 pandemic).[54–57]

Hence, the exploration of chemistry and biological properties of C1′-branched ANPs represents an interesting opportunity for a medicinal chemistry research.

Prodrugs

Both nucleoside phosphates and nucleoside phosphonates are deprotonated at physiological pH, thus, forming a dianionic species which significantly complicates their transport through biomembranes. Prodrug technologies have been developed to mask the charged nature of nucleotide analogues and to consequently circumvent the bioavailability obstacle. Prodrug moieties enable an efficient transport into the target cells where the prodrug moiety is cleaved off, thus releasing the parent active compound (**Figure 8**). A successful prodrug has to be stable in gastrointestinal (GI) tract and be efficiently absorbed in the systemic circulation where it must survive long enough to reach the target cells. Hence, both chemical and enzymatic stability needs to be thoroughly evaluated early on.[58]

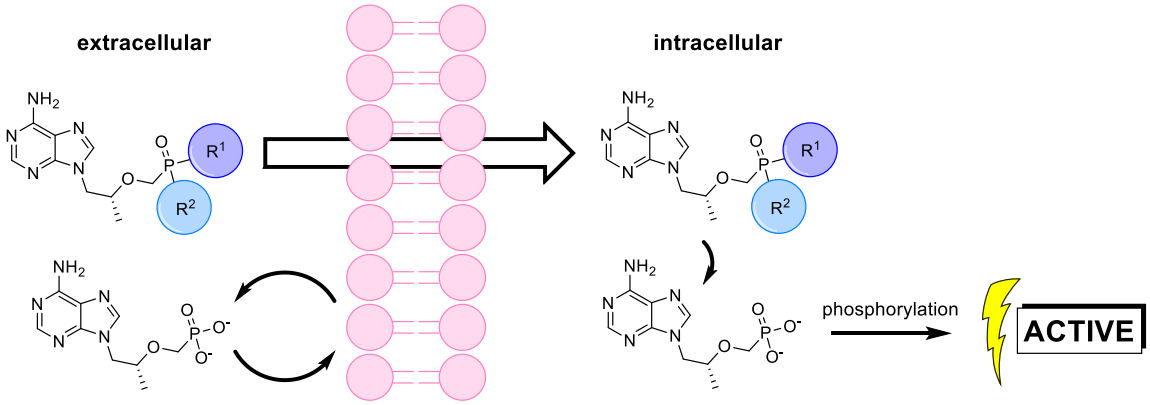


Figure 8. General principle of prodrug approach shown on the example of tenofovir.

Numerous diverse prodrug approaches have been developed over the years (**Figure 9**) and the concept of prodrugs proved to be extremely beneficial. The most successful prodrugs are currently components of several approved drugs.

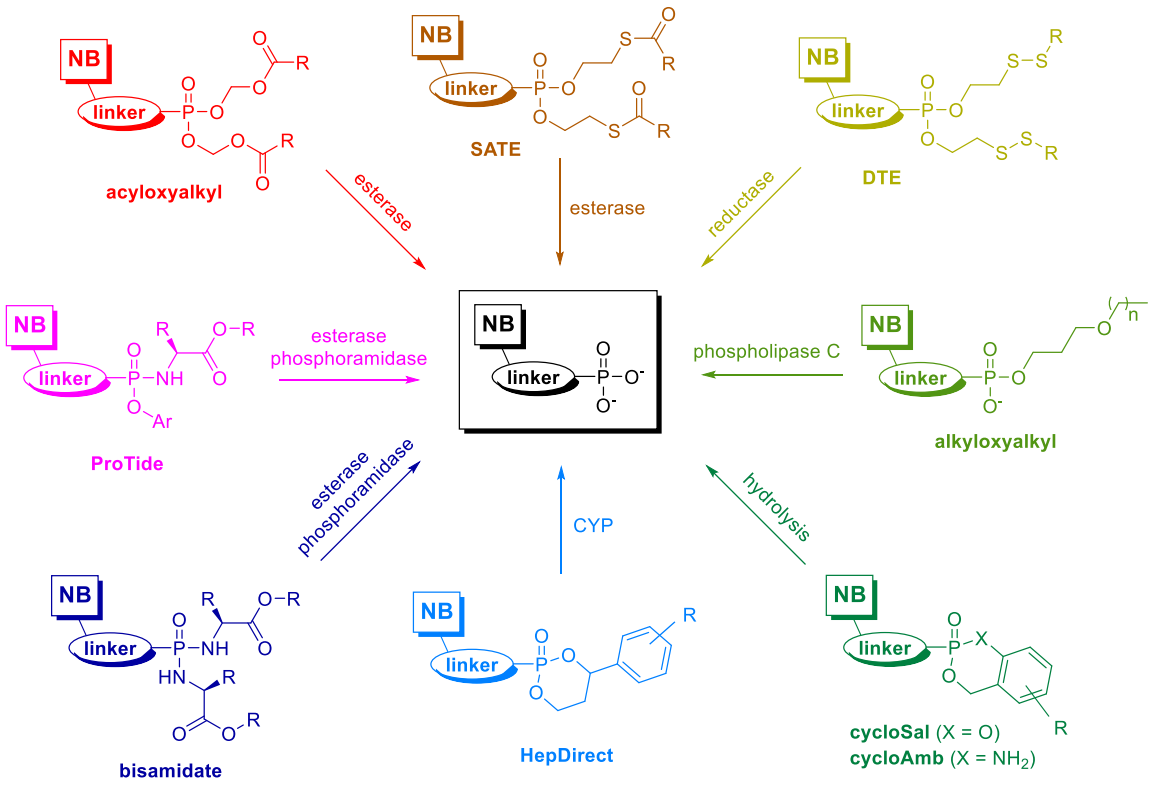


Figure 9. The overview of developed prodrug approaches (NB – nucleobase).

Alkyl prodrugs

The efforts to develop efficient prodrugs started with adopting simple alkyl groups.[59] Carboxylic esters are *in vivo* rapidly cleaved by carboxyesterases, however, alkyl esters

of phosph(on)ates are cleaved far less readily. This prevents their use as prodrugs since the high metabolic stability blocks the release of the parent active molecule. Especially, mono-alkyl esters proved to be unsuitable due one persisting charge combined with slow cleavage of the ester. Rosowsky et al.[59] prepared mono-alkyl prodrugs of ara-CMP and observed substantial drop of cytostatic activity compared to parent ara-CMP both *in vivo* and *in vitro* (in L1210 leukemia and B16 melanoma cells). Starrett et al.[60] prepared mono-alkyl and bis-alkyl prodrugs of PMEA and observed in accordance with previous results poor bioavailability for mono-alkyl prodrugs. Despite good absorption, the bis-alkyl prodrugs also exhibited low efficacy thus confirming the high enzymatic stability of bis-alkyl phosphonate esters which prevents the liberation of the parent active PMEA.[60]

Acyloxyalkyl prodrugs

The hydrolytic resistance of alkyl ester prodrugs directed further efforts to acyloxyalkyl esters bearing most often bis(pivaloyloxymethyl) (POM) or bis(isopropyloxymethyl carbonate (POC) moiety (**Figure 10**). The first step in metabolism of both POM and POC prodrugs is cleavage by an esterase. Metabolites undergo spontaneous degradation thus producing phosph(on)ate mono-ester, formaldehyde, and in case of POC also carbon dioxide. The second cycle yields the parent phosph(on)ate (**Figure 10**).

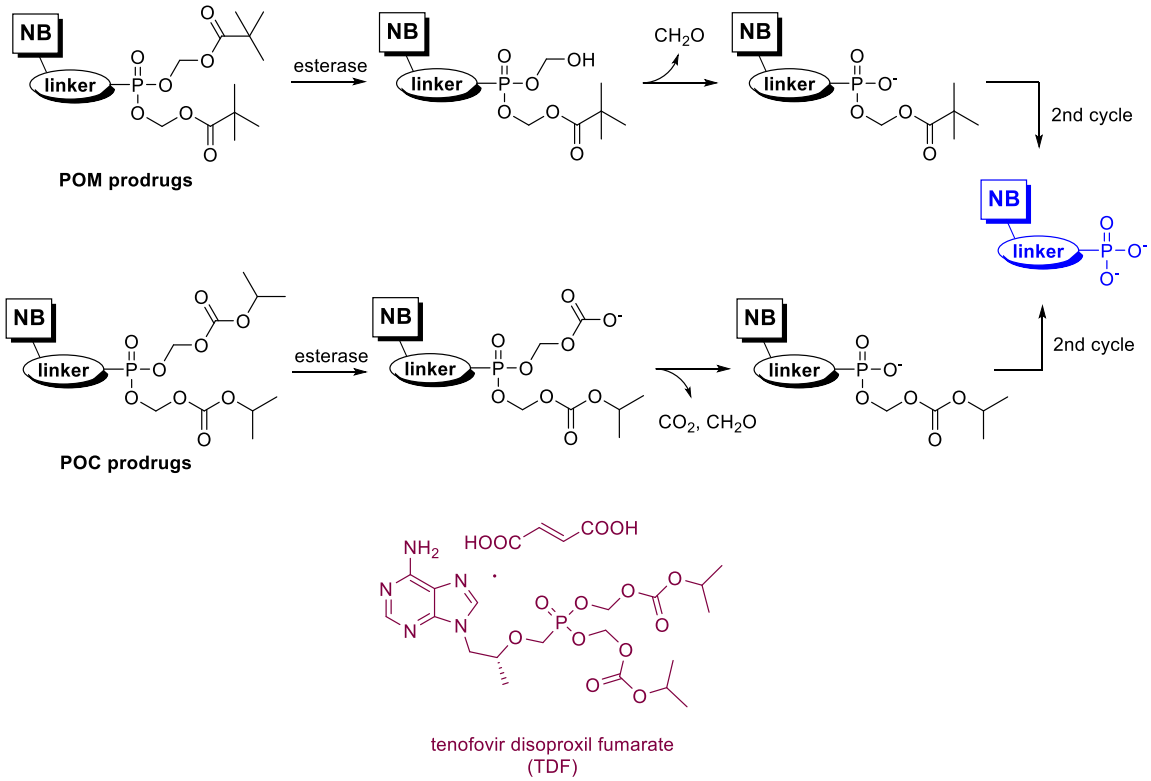


Figure 10. General structure of acyloxyalkyl (POM and POC) prodrugs, their metabolism and the structure of tenofovir disoproxil fumarate (TDF).

Starrett et al.[60] also prepared the bis(acyloxy)alkyl prodrugs of PMEA and observed significant increase of bioavailability both *in vitro* and *in vivo* which was attributed to the increased cellular uptake of the prodrug. Bis(POM) prodrug of PMEA (adefovir dipivoxil) was initially evaluated against HIV, however, the development was stopped due to high nephrotoxicity observed.[15,61] After further investigation, FDA approved in 2002 adefovir dipivoxil for treatment of HBV infections.[62] Another promising antiviral compound - tenofovir[21] - was further developed to achieve higher bioavailability. Bis(POC) prodrug of tenofovir (tenofovir disoproxil fumarate – TDF) exhibited more than 100-fold higher activity against HIV compared to parent tenofovir due to significantly increased (over 100-fold) cellular uptake in target lymphocytes.[63] After oral administration in mice, TDF showed 20% bioavailability and relatively low toxicity.[64] The promising results were confirmed in humans.[64] Eventually, TDF (Viread) was approved for the treatment of HIV/AIDS (in 2001) and chronic hepatitis B (in 2008).[65,66]

S-Acyl-2-thioethyl (SATE) and *S*-((2-hydroxyethyl)sulfidyl)-2-thioethyl (DTE) prodrugs

Both SATE and DTE prodrugs are connected by thioethyl linker to an enzymatically labile moiety. SATE prodrugs utilize esterase-labile thioacetate while DTE prodrugs utilize reductase-labile disulfide moiety. Upon enzymatic cleavage, the unstable metabolite decomposes to ethylene sulfide and mono(SATE)/mono(DTE) phosph(on)ate (**Figure 11**).[67] The second cycle releases the parent phosph(on)ate. The usage of SATE and DTE prodrugs has been limited to *in vitro* experiments due to toxicity concerns regarding ethylene sulfide which is formed during the metabolism.[14]

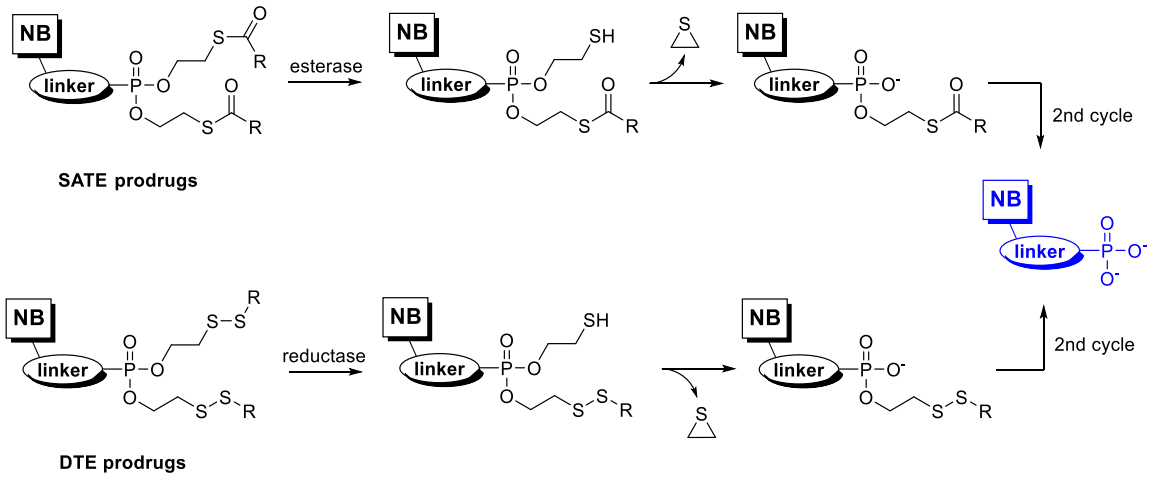


Figure 11. General structure of SATE and DTE prodrugs and their metabolism.

Alkoxyalkyl prodrugs (Hostetler)

Lysophospholipids (LPLs) are bearing only one fatty acyl (**Figure 12**) as opposed to phospholipids (PLs) which are bearing two fatty acyls. The lack of one fatty acyl causes a conic shape of LPLs which increases the mobility through phospholipid bilayer membrane.[68] Inspired by the fast movement of LPLs through membranes, Hostetler et al. developed alkoxyalkyl prodrugs composed of one hexadecyloxypropyl or octadecyloxypropyl moiety on phosphonate thus mimicking the LPL (**Figure 12**).[69] The metabolism of these prodrugs is ensured by phospholipase C which releases the parent phosphonate.

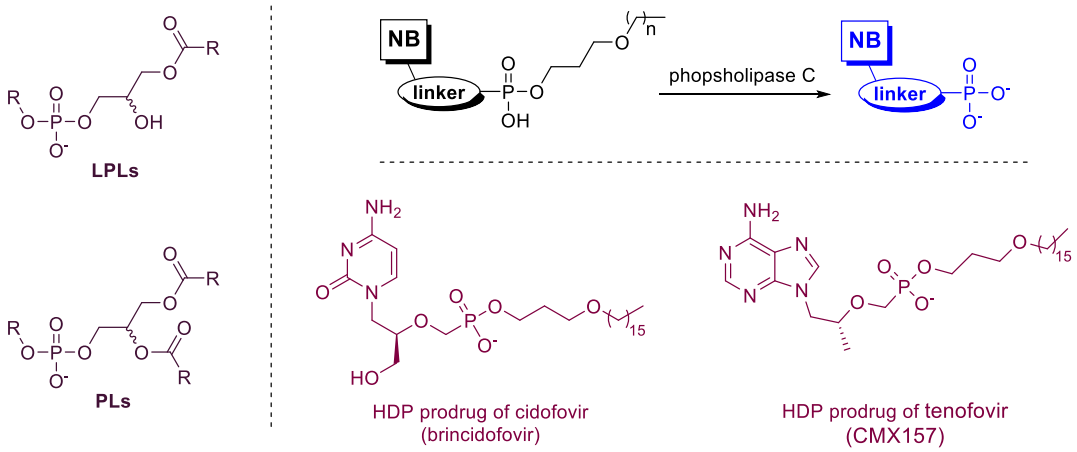


Figure 12. General structure of lysophospholipids (LPLs) and phospholipids (PLs) (left), general structure of alkyloxyalkyl produgs and their metabolism (top, right), and the structure of brincidofovir, and structure of CMX157 (bottom, right).

The optimal length of the chain was studied by Wan et al.[70] who compared 12–24 atoms long chains. It was observed that the optimal length for efficient cellular uptake translated to high antiviral activity is 20 atoms. Hence, 16 atoms from hexadecyl moiety plus 4 atoms from oxypropyl moiety showed the best results. Both longer and shorter chains than 20 atoms exhibited sharp drop of antiviral potency.[70] This approach was developed for phosphonates and proved to be very effective. In case of cidofovir, the hexadecyloxypropyl (HDP) prodrug (**Figure 12**) substantially increased antiviral activity against poxviruses, herpes viruses, adenoviruses, and polyoma virus *in vitro*.[69] The HDP prodrug of cidofovir has been developed by Chimerix, Inc. under generic name brincidofovir and is currently evaluated by FDA for treatment of smallpox.[71] This approach was also applied to tenofovir. HDP prodrug of tenofovir (also known as CMX157) (**Figure 12**) showed increased activity against HIV and HBV *in vitro* while exhibiting no toxicity and good bioavailability *in vivo*.[72] CMX157 was further pursued for the treatment of HBV infection and is currently in phase II clinical studies.[73]

Cyclosaligenyl (CycloSal) and cycloaminobenzyl (CycloAmb) prodrugs

Unlike previously mentioned prodrugs, CycloSal and CycloAmb prodrugs do not depend on enzymatic cleavage and rely on chemical hydrolysis and subsequent spontaneous decay to free parent phosph(on)ate and benzyl alcohol derivatives (salicylic alcohol derivatives in case of cycloSal prodrugs or 2-aminobenzyl alcohol derivatives in case of cycloAmb prodrugs) (**Figure 13**).[74] These prodrugs were developed by Meier et al. who applied this approach among others to PMEA.[75] The CycloSal prodrugs of PMEA proved to be hydrolytically highly unstable. The stability was increased using cycloAmb prodrugs, however, despite significantly lower cytotoxicity, cycloAmb PMEA showed 2–3-fold lower activity against HIV compared to PMEA *in vitro*.[75] Still, the selectivity index (SI - toxicity/potency ratio) of the most promising prodrug achieved 9.7 while SI of previously described bis(POM) PMEA reached in the same assay 4.1.[75]

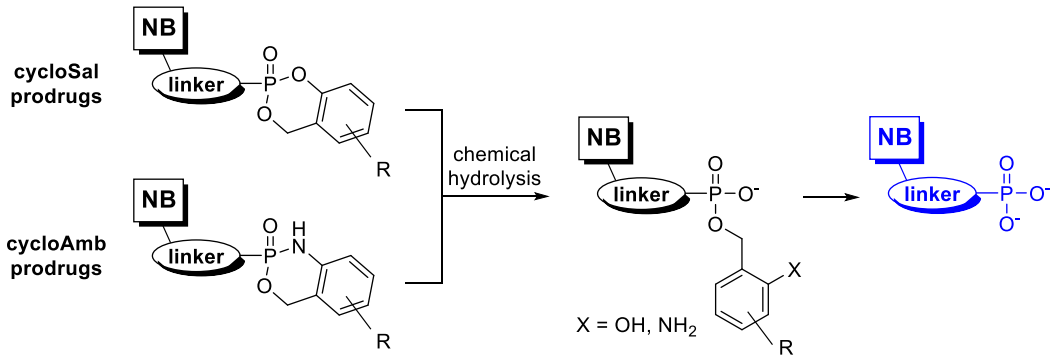


Figure 13. General structure of cycloSal and cycloAmb prodrugs and their metabolism.

HepDirect Prodrugs

The cyclic 1-aryl-1,3-propyl ester of phopsh(on)ates are prodrugs developed to target liver. The cleavage of the prodrug moiety is ensured via cytochrome P_{450} (CYP) mediated hydroxylation (**Figure 14**). The unstable oxidized metabolite undergoes opening and subsequent β-elimination to release the parent compound.[76]

It was observed that activation of the prodrug is highly stereospecific. Hence, the activation proceeded only in case of *cis* configuration between aryl and nucleoside moiety (i. e. R_PS or S_PR diastereoisomers) as shown in the molecule of pradefovir (**Figure 14**).[76] The liver selectivity is related to predominant cleavage of these prodrugs by liver specific cytochrome isoenzyme CYP3A4.[76] Moreover, the liver selectivity is highly desirable for treatment of HBV infections. The HepDirect prodrug of PMEA (known as pradefovir) is evaluated for the treatment of HBV infections and proved to be effective and safe in phase I clinical trial.[77]

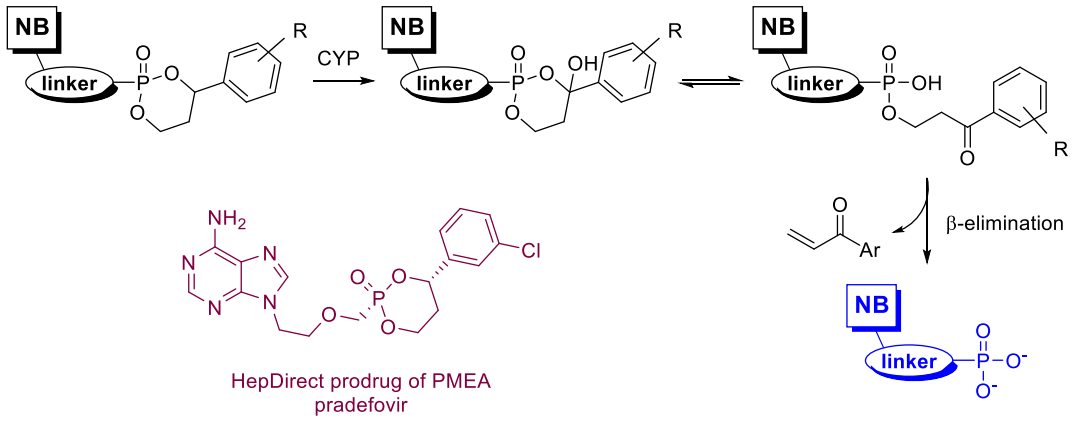


Figure 14. General structure of HepDirect produgs, their metabolism, and the structure of pradefovir.

Phosphoramidate and phosphonamidate prodrugs

Bisamidate prodrugs

Bisamidate prodrugs are bearing two amino acids connected via P-N bonds (**Figure 15**). The advantage of this approach is release of solely non-toxic (natural) amino acids upon the metabolism and employment of two identical amino acids prevents formation of chirality on the phosphorous atom. The metabolism is thought to be analogous to that of ProTide prodrugs (**Figure 18**), which are thoroughly discussed below.

The bisamidate approach was applied in compound GS-9191 (**Figure 15**) which was investigated in regard to its utilization as a topical treatment of human papilloma virus (HPV)-associated lesions.[78] Interestingly, GS-9191 is a prodrug within a prodrug. Cleavage of the two isobutyl-(*S*)-phenylalanine units releases cPrPMEDAP (**Figure 15**) which has been recognized as a prodrug of aforementioned PMEG due to enzymatic deamination.[79] The bisamidate approach has been successfully employed also in our research group.[30,31,80,81]

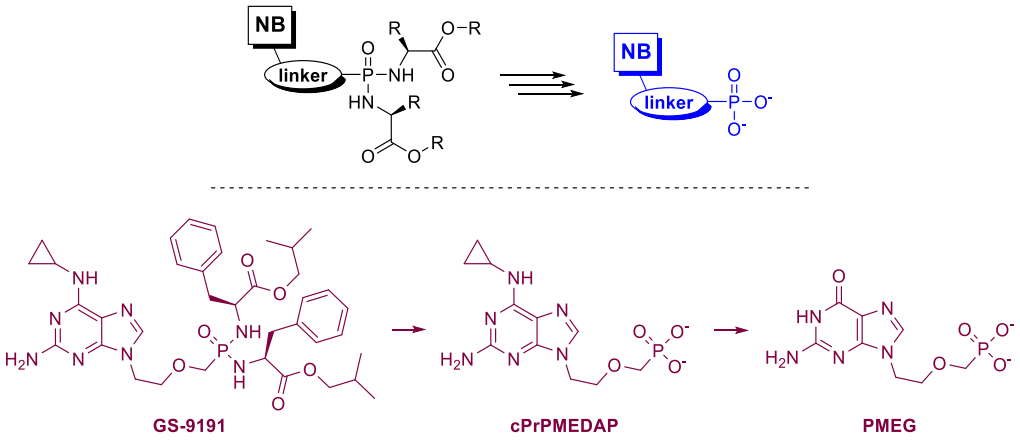


Figure 15. General structure of bisamidate prodrugs and the metabolism of GS-9191.

ProTide prodrugs

Replacing one of the amino acids in bisamidate prodrugs by an aryloxy moiety yields so-called ProTide prodrugs which are currently considered to be the leading approach in the field of prodrugs of modified nucleotides. ProTide approach was originally invented by prof. McGuigan in the early 1990s[82] and is currently applied in various clinical candidates and three approved drugs – sofosbuvir, remdesivir (approved for an emergency use in patients suffering SARS-CoV-2 infection), and the aforementioned tenofovir alafenamide fumarate (TAF) (**Figure 16**).[1,4] To be absolutely accurate, it is necessary to note that TAF is produced in the form of hemifumarate (*i.e.* 1 mol of fumarate for 2 mol of tenofovir alafenamide).

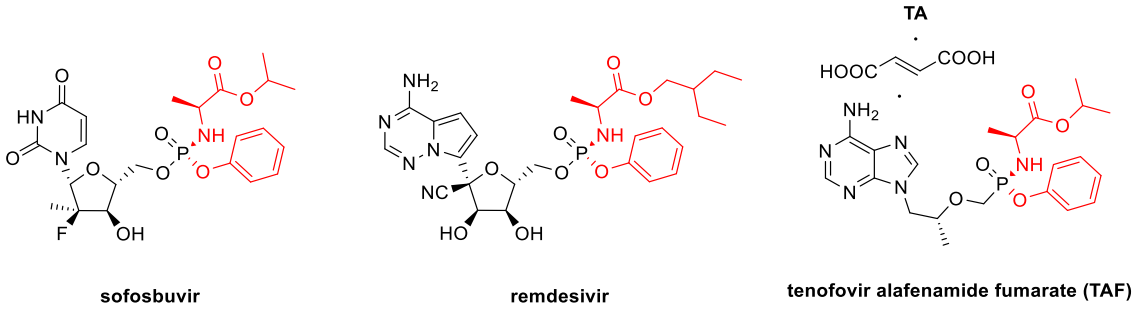


Figure 16. Structure of sofosbuvir, remdesivir and tenofovir alafenamide fumarate (TAF) with the ProTide moiety depicted in red. TA – tenofovir alafenamide

TAF is the successor of the first tenofovir prodrug (bis(POC)) – tenofovir disoproxil fumarate (TDF) (**Figure 10**). The treatment with TDF required high doses in order to exhibit desired activity. This was caused by the low plasma stability, therefore, low amount of drug reaching the target cells (**Figure 17**).[83] Nevertheless, the low bioavailability was not the major problem of TDF as the lability of TDF led to high levels of circulating tenofovir (TFV) in plasma, which is associated with nephrotoxicity and bone toxicity.[84,85]

Renal elimination of TFV is mediated by organic anion transporters (OAT) 1 and 3 securing the uptake while the efflux into urine is insufficiently mediated by multidrug resistance protein type 4 (MRP4).[86] The imbalance between rapid uptake of TFV from plasma and slow efflux into urine leads to TFV accumulation in proximal-tubule cells and thus to nephrotoxicity. The mechanism of bone toxicity (reduction of mineral density) is not fully clarified yet, however, no direct effect of TFV on osteoblasts was observed *in vitro*.[87]

The drawbacks of TDF were substantially reduced with the ProTide of tenofovir – TAF requires 30-fold lower dose compared to TDF to achieve the same efficacy as TDF thus reducing the risk of toxicity by the factor of 30 as well.[88] TAF proved to be more stable while reaching higher levels of pharmacologically relevant TFV diphosphate (TFVpp) due to highly efficient metabolization in target cells (**Figure 17**).[89] Released metabolites

have ionic nature and are therefore trapped inside the cell which results in rapid accumulation in target cells. The crucial levels of TFV in plasma during treatment with TAF decreased to 10% compared to patients treated with TDF.[90] In 2016, TAF (Vemlidy) has been approved for the treatment of HIV/AIDS as safer and more potent alternative to TDF.

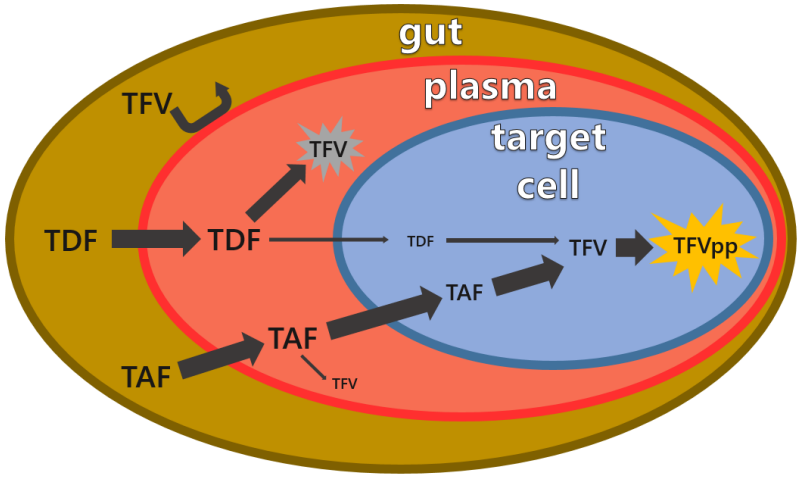


Figure 17. Transport of tenofovir (TFV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) through membranes to the target cell and conversion of prodrugs to biologically active TFV diphosphate (TFVpp).

The proposed mechanism of ProTide metabolism leading to the release of parent molecule starts with the cleavage of the amino acid ester (**Figure 18**). Cathepsin A, a ubiquitous carboxypeptidase highly expressed in lymphoid cells, has been recognized as the major esterase involved in this step.[91] Generated carboxylate undergoes intramolecular cyclisation, thus, displacing the aryl moiety and forming a cyclic intermediate which opens upon hydrolysis and a phosphoramidase-type enzyme releases the parent compound. While this is generally accepted mechanism, there had been no experimental evidence of the cyclic intermediates in the ProTide metabolism until recently Procházková et al.[92] were able to confirm such species using NMR spectroscopy and mass spectrometry coupled with infrared spectroscopy, therefore supporting the rightness of the postulated mechanism.

During the ProTide metabolism, an alcohol, an amino acid, and phenol are released. The influence of the amino acid ester was studied by McGuigan et al.[93] This work indicated that more lipophilic ester may increase the activity via higher cellular uptake, however, it also demonstrated that not only lipophilicity determines the resulting activity. The substrate activity of the compounds towards esterases also plays an important role. In particular, *t*-butyl ester was hydrolyzed significantly slower compared to methyl, ethyl, or isopropyl derivatives. As another example, phenyl ester was cleaved faster compared to more lipophilic ethyl- and propylphenyl analogues.[93]

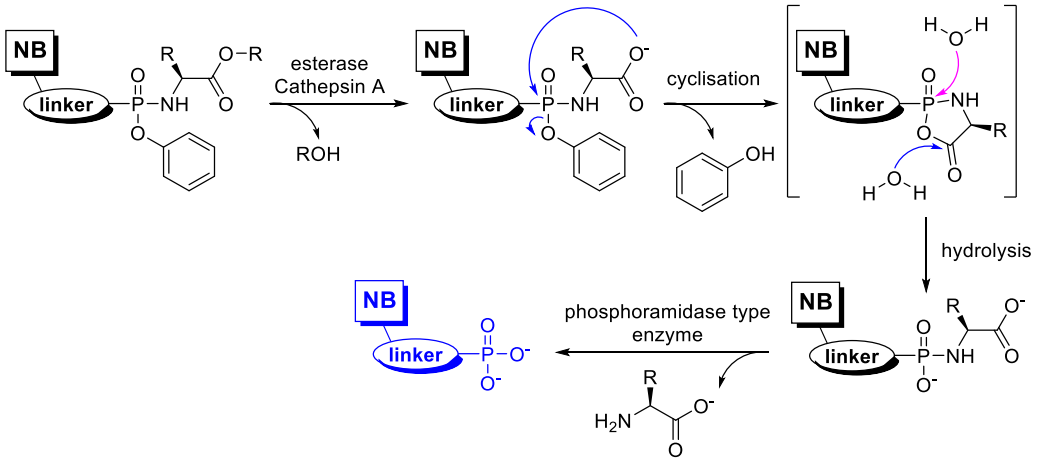


Figure 18. The mechanism of ProTide metabolism.

The role of the amino acid was also studied. It has been shown that cathepsin A is not able to hydrolyze bulkier amino acids.[91] Studies suggest alanine as the most eligible option.[91,94]

Regarding the aryl ring, the substitution was studied by Siddiqui et al.[95] While mainly electron-withdrawing groups did increase the activity, the toxicity was higher compared to the original ProTide with phenol.[95] This is possibly the reason why mostly phenol is utilized in the clinical candidates and approved drugs. However, the release of phenol during the metabolism of ProTides has been recognized as problematic due to its apparent toxicity.[96,97]

The replacement of phenol for less toxic moiety might result in even safer prodrug moiety. Part of this thesis focuses on the development of modified ProTides with tyrosine derivatives instead of phenol. Strikingly, only two tyrosine derivatives of ProTides are known in the literature as a part of an effort to increase aqueous solubility by Siddiqui et al.[96] Substitution of phenol for tyrosine methyl ester indeed increased aqueous solubility 100-fold, however, decreased the lipophilicity, and therefore, the potency was reduced dramatically.[96] Nevertheless, the same compound with *t*-butyl carbamate (BOC) on the N-terminus showed the lowest toxicity in the study and partially recovered the activity, which was approx. 10-fold lower compared to the original compound bearing phenol. No subsequent study of the phenol replacement with tyrosine derivatives has been done.

Aims of the study

The first general aim of this thesis was to explore previously neglected field of C1′-branched acyclic nucleoside phosphonates (ANPs) regarding both synthetic and biological aspects. Biological evaluation of ANPs is very often dependent on a prodrug application, therefore, the second general aim of this study was to develop novel prodrug moiety (preferably with enhanced properties) using tenofovir as suitable and available model compound representing ANPs.

The specific aims of this thesis were:

1 to develop suitable synthetic approaches towards C1′-branched ANPs

2 to evaluate biological activity of prepared C1′-branched ANPs

3 to design and synthesize novel prodrugs with tenofovir as model active compound

4 to develop suitable method for separation of epimers formed during the synthesis of prodrugs

5 to evaluate the efficacy of developed prodrugs against HIV and HBV, and to compare them to clinically used analog tenofovir alafenamide fumarate (TAF)

6 to evaluate both chemical and enzymatic stability of promising prodrugs

Results and discussion

As a multidisciplinary field, medicinal chemistry requires collaboration of experts from various areas. I would like to acknowledge the work of my colleagues from biochemical areas and their contribution to this study.

Team of Prof. Johan Neyts (The Neyts-lab of Virology, Antiviral Drug & Vaccine Research, Rega Institute, KU Leuven, Belgium) performed antiviral evaluation of C1′-branched ANPs.

Gilead Sciences, Inc. (Foster City, CA, USA) evaluated antiviral properties of C1′-branched ANPs.

Team of Dr. Alena Zíková (Molecular Parasitology, Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic) performed biological evaluation of C1′-branched ANPs against *Tbr*APRT.

Team of Dr. Jan Weber (Virology, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic), namely Michala Zgarbová and Jan Hodek carried out the assays evaluating the cytotoxicity and potency against HIV and HBV.

Team of Dr. Helena Mertlíková-Kaiserová (Biochemical Pharmacology, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic) carried out the assays evaluating cytotoxicity, enzymatic stability, and metabolic studies.

C1′-Branched ANPs

The idea of synthesis and subsequent biological evaluation of C1′-branched ANPs bearing 9-phosphonomethoxyethyl (PME) moiety was inspired by ANP tenofovir[21] and by C1′-modified cyclic nucleotide GS-5734[52,98,99] (currently known as remdesivir) (**Figure 19**) whose biological properties were described in the Introduction. C1′-Branched ANPs lack the labile phosphate group and are therefore chemically and enzymatically more stable compared to canonical nucleotides. Moreover, given the structural proximity of C1′-branched ANPs to tenofovir and remdesivir, these compounds hold a potential to exert promising biological activities.

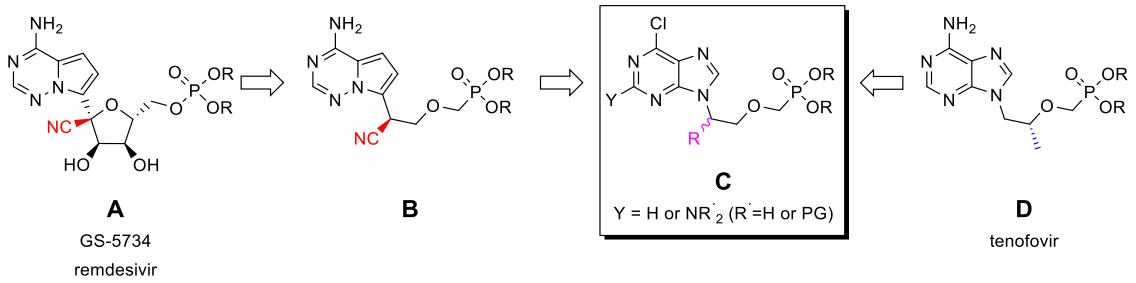


Figure 19. Structure of remdesivir (**A**), an analogous C1′-branched ANP (**B**), the general structure of target intermediates for synthesis of C1′-branched ANPs (**C**), and the structure of tenofovir (**D**).

Synthesis

6-Chloropurine derivatives (structure **C**, **Figure 19**) were selected as target compounds for the synthesis since these intermediates can be further vastly diversified. Ammonolysis secures adenine derivatives, hydrolysis secures hypoxanthine or guanine derivatives. But the chlorine atom can be displaced by various other *O*-nucleophiles and *N*-nucleophiles. Moreover, these intermediates can undergo cross-coupling reactions. All these transformations hence potentially lead to endless number of derivatives.

Since the field of C1′-branched ANPs was neglected, it was necessary to establish eligible synthetic approaches securing the target compounds **C**. Up to date, only a handful of C1′-branched purine ANPs and only adenine derivatives have been reported. Starting from chiral aminoalcohol, Jeffery et al.[48] prepared (*R*)-C1′-methyl derivative of adefovir (structure **I**, **Figure 20**) in 5 steps with 30% overall yield. The same compound was synthesized later as a demonstration of synthetic utility of reported asymmetric hydrogenation of α-purine acrylates (5 steps, 32% overall).[47] Wu et al.[46] prepared laboriously C1′-ethyl derivatives of adefovir (compounds **II**, **Figure 20**) as acyloxyalkyl prodrugs in 8–16 steps with overall yield of 0.07–1.07%. Finally, Kundarapu et al.[100] prepared C1′-ethynyl derivative via nucleophilic addition to an aldehyde and subsequent Mitsunobu reaction (structure **III**, **Figure 20**) in 8 steps (yield not reported).

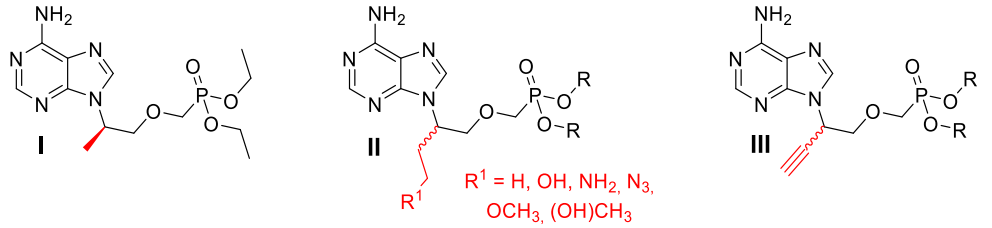
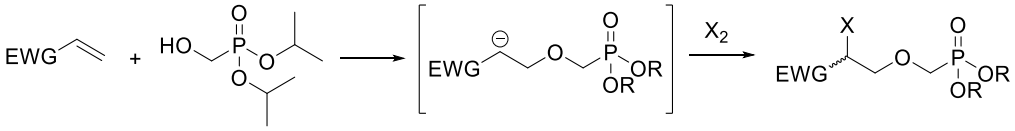


Figure 20. Structures of previously reported C1′-branched ANPs **I**, **II**, and **III**.

Tandem reaction

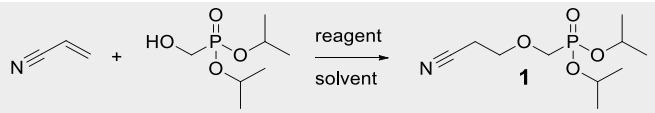
In the very beginning, I focused on the possibility of a tandem reaction which would elegantly secured valuable branched halides for the nucleophilic displacement by 6-chloropurine (**Scheme 1**). Specifically, (hydroxymethyl)phosphonate would serve as a nucleophile in an oxa-Michael addition thus generating a stabilized carbanion which would attack a halogen molecule.



Scheme 1. General scheme of the studied tandem reaction.

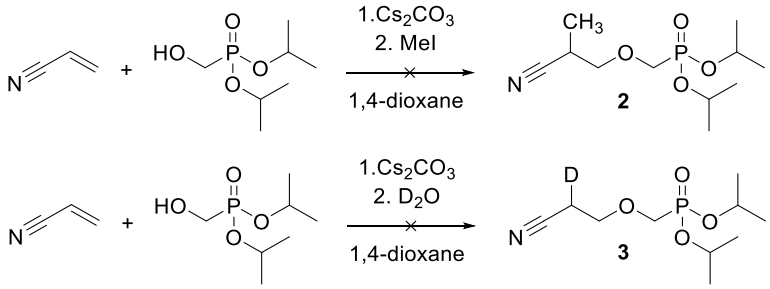
This approach was studied on the reaction of diisopropyl (hydroxymethyl)phosphonate with acrylonitrile. First, the conditions for the oxa-Michael addition were optimized (conversions estimated based on GC-MS). The reaction did not proceed without catalysis (**Table 1**, Entry 1). Activation of the electrophile using both Brønsted acid (Tf_2NH)[101] or Lewis acid ($BF_3 \cdot Et_2O$)[102] resulted again in 0% conversion. An activation of the electrophile using Bu_3P[103] improved the conversion to 20%. Since (hydroxymethyl)phosphonate is truly a poor nucleophile, the strategy of activating the donor was more promising. Indeed, an addition of suitable base (Cs_2CO_3 or KOH) afforded up to 100% conversions in less than 1 hour.[104] 1,4-Dioxane proved to be a suitable solvent as analogous reactions in *t*-BuOH led to lower conversions. Hence, Entry 6 was selected as the most efficient and convenient method.

Table 1. Optimization of the oxa-Michael addition of diisopropyl (hydroxymethyl)phosphonate to acrylonitrile.



Entry	Reagent	Amount	Solvent	Conversion after 1h	Conversion after 18h
1	none	N/A	1,4-dioxane	0%	0%
2	Tf_2NH	20 mol%	1,4-dioxane	0%	0%
3	$BF_3 \cdot Et_2O$	20 mol%	1,4-dioxane	0%	0%
4	Bu_3P	20 mol%	1,4-dioxane	9%	20%
5	K_2CO_3	20 mol%	1,4-dioxane	55%	55%
6	Cs_2CO_3	20 mol%	1,4-dioxane	100%	100%
7	KOH	20 mol%	1,4-dioxane	100%	100%
8	Cs_2CO_3	1 eq.	1,4-dioxane	100%	100%
9	KOH	1 eq.	1,4-dioxane	100%	100%
10	Cs_2CO_3	1 eq.	*t*-BuOH	100%	67%
11	KOH	1 eq.	*t*-BuOH	33%	0%

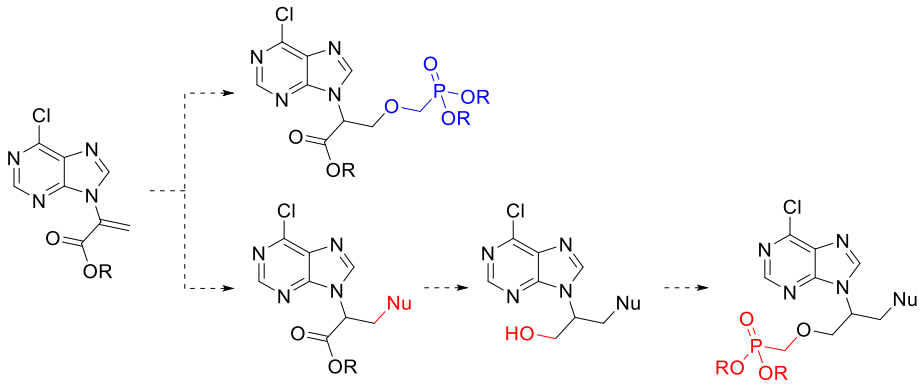
With the optimized reactions, I proceeded to the tandem reaction. In order to verify whether this is a viable approach, I performed the tandem reaction with MeI as a strong electrophile (**Scheme 2**). Unfortunately, the desired adduct **2** was not observed. Quenching the reaction with D_2O also did not result in deuteration of the product (**3**) (**Scheme 2**). Based on these results, the tandem reaction was dismissed.



Scheme 2. The unsuccessful attempts for tandem reaction.

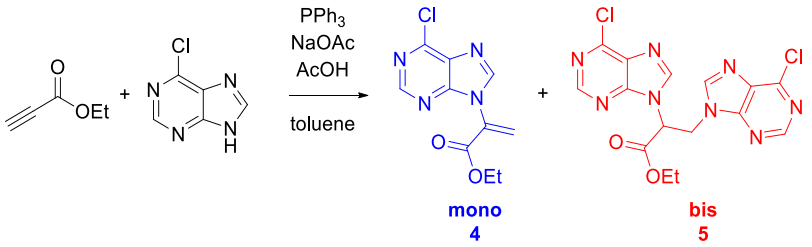
Purine-acrylate approach

(*R*)-C1′-Methyl derivative of adefovir (structure **I**, **Figure 20**) was prepared via asymmetric hydrogenation of *α*-purine acrylates obtained via PPh_3-catalyzed reaction of purines with esters of propiolate.[47,105] I considered the *α*-purine acrylate as a suitable intermediate for synthesis of various C1′-branched ANPs. First, *α*-purine acrylate could react in an oxa-Michael reaction with (hydroxymethyl)phosphonate and the ester function could be subsequently further derivatized (**Scheme 3**, blue). Or second, *α*-purine acrylate would react with various nucleophiles and the ester function would be subsequently reduced to an alcohol which would be used for the connection of the phosphonate moiety (**Scheme 3**, red).



Scheme 3. Possible utilization of *α*-purine acrylate in the synthesis of C1′-branched ANPs.

Sun et al.[47] reported a procedure where the reaction mixture was heated at 110 °C for 24 h. When I attempted to reproduce this procedure, however, I observed a formation of substantial amount of undesirable bis-adduct **5** (**Scheme 4**, red). Further experiments confirmed that at elevated temperatures, the desired mono-adduct **4** is reactive enough to undergo second addition, thus forming the aforementioned bis-adduct **5** (**Table 2**). Even without heating, the bis-adduct **5** was formed in relatively high amount. The decrease of temperature (to 15 °C) and reaction time (to 10 min) finally afforded predominantly the desired product **4** (**Table 2**).



Scheme 4. Synthesis of ethyl *α*-purine acrylate.

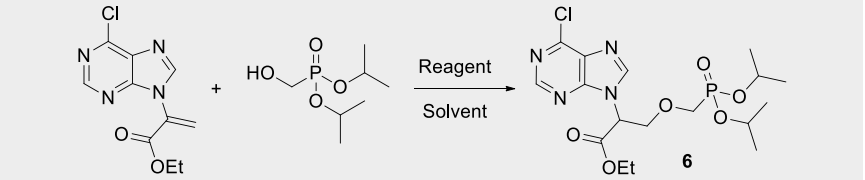
Table 2. Optimization of ethyl α-purine acrylate synthesis.

Entry	Temperature	Reaction time	Ratio [%] mono:bis
1	110 °C	17 h	50:50
2	110 °C	1 h	72:28
3	110 °C	3 h	67:33
4	25 °C	15 min	97:3
5	25 °C	1 h	83:17
6	25 °C	3 h	83:17
7	15 °C	10 min	99:1

However, eliminating the formation of bis-adduct **5** during the reaction was not the end of surprises as several other problems came into light during the purification. For instance, when the starting 6-chloropurine was present in the mixture during adsorption on silica gel, the acidity of silica gel catalyzed further reaction and the formation of bis-adduct **5** was observed. This was easily prevented by extraction of 6-chloropurine with water. Adsorption of the crude mixture on silica gel in MeOH containing 1% of Et_3N resulted in complete conversion of the product to a methoxy-adduct. This was also easily prevented and final work-up of the reaction consisted of extraction of 6-chloropurine with water, followed by adsorption of the crude mixture on silica gel in EtOAc. This way, no bis-adduct **5** was observed before separation. However, after the silica gel chromatography (using cyclohexane/EtOAc as a mobile phase), the bis-adduct **5** was observed in substantial amount which resulted in dramatic decrease of desired mono-adduct **4** yield to 7%. These results illustrate high reactivity of α-purine acrylate.

In contrast with previous observations, the subsequent oxa-Michael addition with (hydroxymethyl)phosphonate with compound **4** did not proceed nearly as well as with acrylonitrile. The previous optimization of oxa-Michael addition (**Table 2**) showed Cs_2CO_3 or KOH to be the most suitable bases giving full conversion. In this case, however, the highest observed conversion of compound **6** reached unsatisfactory 20% (**Table 3**). Stronger bases such as NaH or Na led to decomposition to 6-chloropurine and a high number of side products. It became apparent that α-purine acrylates are weaker electrophiles than acrylonitrile and combined with the very low nucleophilicity of (hydroxymethyl)phosphonate, this translates into the poor conversion. Based on these results combined with the low yield of compound **4**, I decided to skip the exploration of the more laborious derivatizations (**Scheme 3**, red) and I turned my attention to different direction.

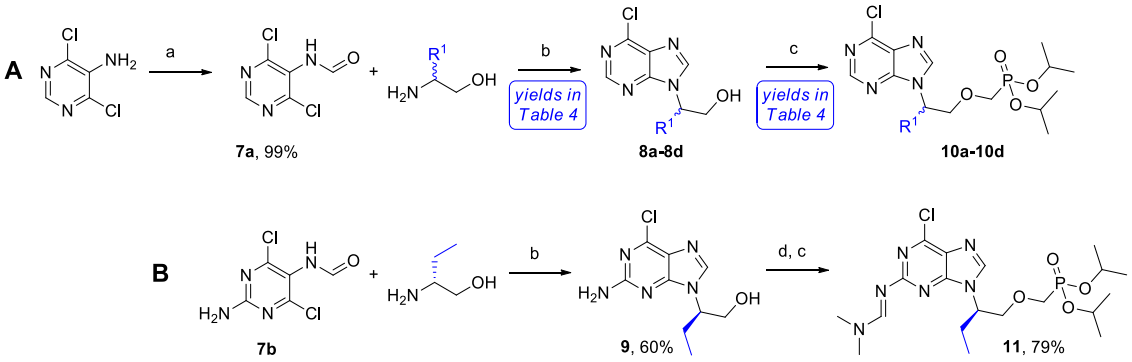
Table 3. Conditions for the oxa-Michael addition of diisopropyl (hydroxymethyl)phosphonate to ethyl α-purine acrylate.



Entry	Reagent	Amount	Solvent	Conversion
1	KOH	20 mol%	1,4-dioxane	0%
2	Cs_2CO_3	20 mol%	1,4-dioxane	5%
3	Cs_2CO_3	1 eq.	1,4-dioxane	20%
4	NaH	1 eq.	DMF	0%
5	Na	20 mol%	THF	0%

Condensation approach

I further considered the possibility of building up the purine base. Jeffery et al. reported a procedure utilizing (*R*)-alaninol to obtain (*R*)-C1′-methyl derivative of adefovir (structure **I**, **Figure 20**).[48] I modified the synthesis using the one-pot procedure published by Dejmek et al.,[106] where the nucleophilic displacement of the chlorine atom and imidazole ring closure were carried out in 1 step (**Scheme 5**). The starting compound **7a** was quantitatively prepared from 5-amino-4,6-dichloropyrimidine and subsequently employed in the condensation with suitable enantiomerically pure amino alcohol. The condensation reaction was carried out in a microwave reactor under relatively energetic conditions and secured compounds **8a-8d** in moderate yields (typically around 60%) (**Scheme 5**, **A**, **Table 4**).



Scheme 5. Synthesis of target 6-chloropurine (**A**) and protected 2-amino-6-chloropurine (**B**) derivatives via the condensation approach. Reagents and conditions: a) HCOOH, Ac_2O, 25 °C, 16 h; b) DIPEA, 1,4-dioxane, 160 °C, 2 h, MW; c) $TfOCH_2P(O)(OiPr)_2$, *n*-BuLi, THF, −78 °C, 2 h; d) DMF DMA, 80 °C, 15 min.

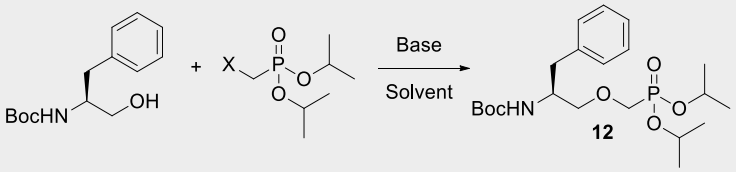
Table 4. Compounds prepared via the condensation approach (**Scheme 5**).

R^1	Compound	Yield	Compound	Yield
Et[a]	(*R*)-**8a**	56%	(*R*)-**10a**	73%
*i*Pr	(*S*)-**8b**	91%	(*S*)-**10b**	91%
*i*Pr	(*R*)-**8b**	63%	(*R*)-**10b**	80%
*c*Pr	(*R*)-**8c**	66%	(*R*)-**10c**	69%
BnOMe	(*S*)-**8d**	53%	(*R*)-**10d**	36%

[a]Et, ethyl; *i*Pr, isopropyl; *c*Pr, cyclopropyl; BnOMe, benzyloxymethyl.

The connection of phosphonate moiety was studied on a model reaction with Boc-(*S*)-phenylalaninol. This simple nucleophilic displacement proved to be quite challenging since the conversion of compound **12** reached a maximum of 5% under various conditions (**Table 5**). Fortunately, deprotonation using *n*-BuLi and utilization of diisopropyl triflyloxymethanephosphonate afforded the desired 6-chloropurine derivatives **10a**-**10d** in satisfactory yield (typically around 75%) (**Scheme 5**, **A**, **Table 4**), hence substantially improving the phosphonate moiety introduction reported previously by Jeffery et al. (41%) and by Wu et al. (52% average).[46,48]

Table 5. Optimization of the phosphonate moiety connection via nucleophilic displacement.



Entry	X	Base	Solvent	Temperature	Conversion
1	OTs (1.2 eq.)	NaH (1.5 eq.)	DMF	25°C	5%
2	Br (1.2 eq.)	NaH (1.5 eq.)	DMF	25°C	5%
3	OTs (2 eq.)	DIPEA (2.5 eq.)	MeCN	75°C	0%
4	Br (2 eq.)	DIPEA (2.5 eq.)	MeCN	75°C	0%
5	OTs (10 eq.)	Ag_2O (5 eq.)	DCM	40°C	0%
6	Br (10 eq.)	Ag_2O (5 eq.)	DCM	40°C	0%
7	OTs (1.2 eq.)	Cs_2CO_3 (1 eq.)	1,4-dioxane	75°C	0%
8	Br (1.2 eq.)	Cs_2CO_3 (1 eq.)	1,4-dioxane	75°C	0%
9	OH (1 eq.)	PPh_3, DIAD (1 eq.)	THF	70°C	0%
10	OTf (1.2 eq.)	NaH (1.5 eq.)	DMF	25°C	0%

This synthetic sequence was also validated for 2-aminopurine derivatives. Starting from commercially available compound **7b**, the condensation reaction with 2-amino-6-chloropurine afforded compound **9** in a similar yield as compounds **8a**-**8d**. The 2-amino group was protected using dimethylaminomethylene (DMAM) group prior to the nucleophilic displacement which secured compound **11**.

This approach is convenient for the synthesis of enantiomerically pure C1′-branched ANPs since starting 2-aminoalcohols are often available as single enantiomers or can be conveniently obtained from corresponding amino acids. For instance, (*S*)-2-amino-3-(benzyloxy)propan-1-ol (*O*-benzyl-D-serinol) used in the synthesis of (*S*)-**8d** was obtained in a 88% yield via the reduction of (*R*)-2-amino-3-(benzyloxy)propionic acid (*O*-benzyl-D-serine) with *in situ* prepared BH_3.

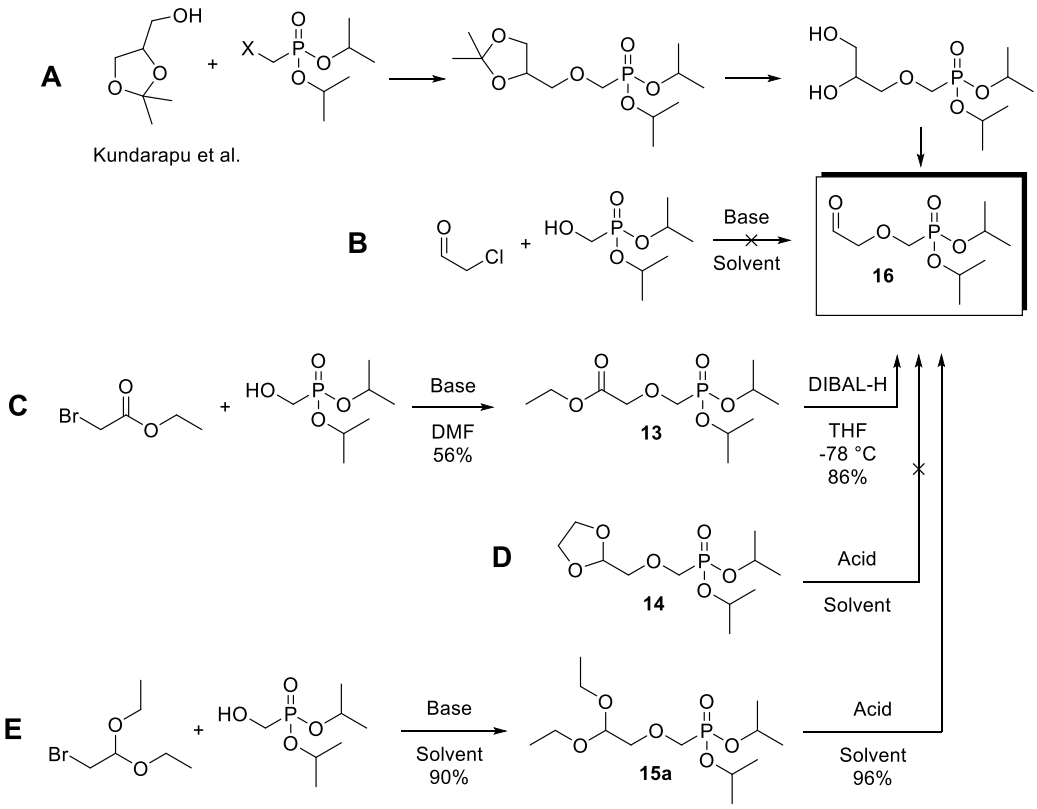
Aldehyde approach

Aldehyde of PME (**16**) was one of the main aims in the development of synthetic approaches since an addition of various nucleophiles affords secondary alcohols suitable for subsequent Mitsunobu reaction. The aldehyde was previously synthesized in 3 steps (yield not reported).[100] Kundarapu et al. alkylated and subsequently hydrolyzed solketal. The obtained diol was oxidatively cleaved using $NaIO_4$ (**Scheme 6**, **A**). The resulting alcohol was obtained in 48% yield after addition of TMS-protected acetylene.

I was aiming to increase the efficiency and therefore performed a screening of possible synthetic routes. The simplest way was to connect diisopropyl (hydroxymethyl)phosphonate to chloroacetaldehyde which, however, gave no conversion under the studied reaction conditions (**Scheme 6**, **Table 6**, **B**).

Other possibility I considered was to employ ethyl bromoacetate to prepare and subsequently reduce compound **13** to the desired aldehyde **16**. The alkylation had to be slightly optimized to achieve satisfactory conversion 80% which resulted in 56% yield (**Scheme 6**, **Table 6**, **C**). The reduction using DIBAL-H successfully secured to desired aldehyde in 86% yield (48% overall).

I also considered a utilization of suitable acetal which would upon acidic hydrolysis yield the desired aldehyde **16**. My colleague Jan Frydrych prepared cyclic acetal **14** which I attempted to hydrolyze using various acids (**Scheme 6**, **Table 6**, **D**). But the cyclic acetal proved to be very stable. I therefore prepared acyclic acetal **15a** starting from bromoacetaldehyde diethyl acetal. The alkylation was optimized (**Scheme 6**, **Table 6**, **E1**) to obtain almost quantitative conversion and 90% isolated yield. Obtained acyclic acetal **15a** proved to be considerably more labile compared to its cyclic counterpart as hydrolysis with H_2SO_4 afforded the desired aldehyde **16** in a quantitative conversion and 96% yield (**Scheme 6**, **Table 6**, **E2**).



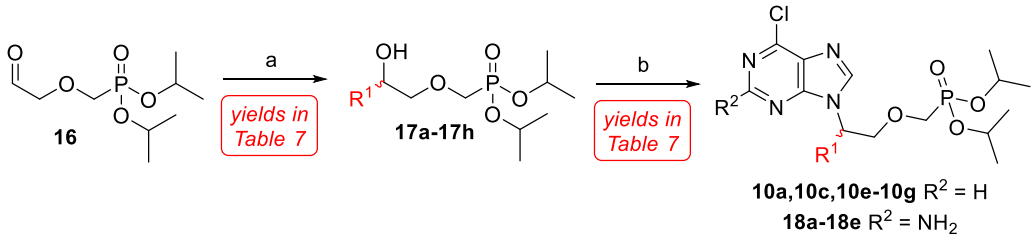
Scheme 6. Screening of suitable synthetic routes towards PME aldehyde **16**.

Table 6. Conditions applied in the screening of suitable synthetic route towards PME aldehyde **16** (**Scheme 6**).

	Entry	1	2	3	4
B	Base	KOH	Cs_2CO_3	Cs_2CO_3	
	Solvent	DMF	1,4-dioxane	neat	
C	Base	NaH (3 eq.)	NaH (1 eq.)	NaH (1 eq.)	
	Temperature	25 °C	70 °C	25 °C	
	Conversion	0%	35%	80%	
D	Acid	DOWEX	HCl	H_2SO_4	$In(OTf)_3$
	Solvent	H_2O/MeOH	THF	H_2O/THF	acetone
	Temperature	25 °C → 50 °C	25 °C → 50 °C	25 °C → 50 °C	25 °C → 50 °C
E_1	Base	NaH (5 eq.)	NaH (3 eq.)	NaH (1 eq.)	Cs_2CO_3
	Solvent	DMF	DMF	DMF	1,4-dioxane
	Conversion	16%	9%	95%	0%
E_2	Acid	DOWEX	H_2SO_4		
	Solvent	H_2O/MeOH	H_2O/THF		
	Conversion	0%	100%		

The synthetic route via hydrolysis of acyclic acetal employed cheap and readily accessible reagents and proved to be both most convenient and most efficient as it afforded aldehyde **16** in an overall 86% yield. Moreover, this synthesis was suitable for multigram scale which was highly desirable and aldehyde **16** was prepared in over 60 g scale. The aforementioned protocol substantially enhances the previously reported procedure.[100]

Aldehyde **16** was the key intermediate for synthesis of various derivatives. An addition of diverse nucleophiles (e.g. Grignard reagents, cyanide, trifluoromethyltrimethysilane) to the aldehyde group of **16** offered secondary alcohols **17** in excellent yields (80–96%, except for (*RS*)-**17g**) (**Scheme 7**, **Table 7**). This approach substantially enhances the procedure reported by Kundarapu et al.[100] who prepared TMS-protected (*RS*)-**17d** in 4 steps with 48% overall yield. This approach yields compound (*RS*)-**17d** in 3 steps with overall yield of 71%, and without the need for protection of the 2-aminopurine group.



Scheme 7. Reagents and conditions: a) various nucleophiles; b) 6-chloropurine or 2-amino-6-chloropurine, DIAD, PPh_3, THF, 70 °C, 1 h.

Table 7. Compounds prepared via aldehyde approach (**Scheme 7**).

R^1	Compound	Yield	Compound (R^2= H)	Yield	Compound (R^2= NH_2)	Yield
Me	(*RS*)-**17a**	80%	(*RS*)-**10e**	53%	(*RS*)-**18a**	74%
Et	(*RS*)-**17b**	80%	(*RS*)-**10a**	61%	(*RS*)-**18b**	65%
Vinyl	(*RS*)-**17c**	92%	(*RS*)-**10f**	80%	(*RS*)-**18c**	40%
Ethynyl	(*RS*)-**17d**	83%	(*RS*)-**10g**	91%	(*RS*)-**18d**	43%
*c*Pr	(*RS*)-**17e**	91%	(*RS*)-**10c**	30%	(*RS*)-**18e**	25%
CF_3-	(*RS*)-**17f**	96%	n.r[a]	--	n.r.	--
NO_2CH_2-	(*RS*)-**17g**	54%	n.r.	--	n.r.	--
CN-	(*RS*)-**17h**	89%	n.r.	--	n.r.	--
$BzOCH_2$-[b]	(*RS*)-**17i**[b]	--	(*RS*)-**10h**	68%	--	--

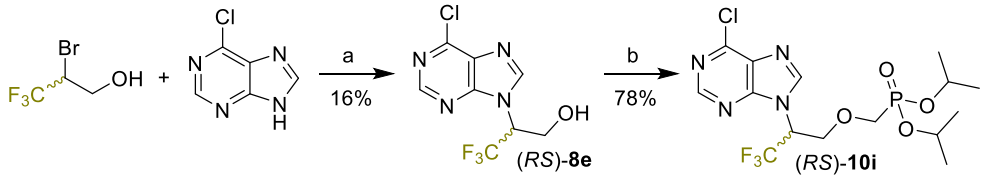
[a] n.r., no reaction observed. [b] not prepared via aldehyde approach, prepared by my colleague Dr. Špaček

Alcohols **17** (including (*RS*)-**17i** which was prepared by my colleague Dr. Špaček) reacted in Mitsunobu reaction with the corresponding purines to give products **10** and **18** in 25–91% yields (**Scheme 7**, **Table 7**). However, the Mitsunobu reaction did not proceed with secondary alcohols bearing electron-withdrawing groups (*e.g.* CF_3, CH_2NO_2 or CN). Since the alcohol did not undergo elimination under the reaction conditions, the reason

for the reaction failure was suggested to be the lack of electron density on the oxygen atom. This apparently reduced the ability of the oxygen atom to attack the phosphonium intermediate thus preventing it to become a good leaving group. Nevertheless, in other cases, this approach proved to be very efficient.

Substitution approach

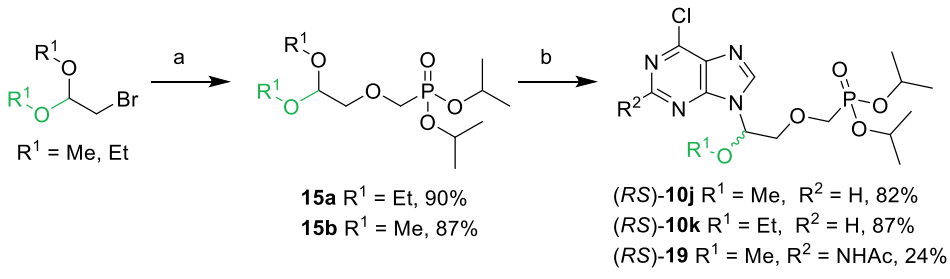
Since compound (*RS*)-**17f** did not react in Mitsunobu reaction, the trifluoromethyl derivative ((*RS*)-**10i**) was obtained via nucleophilic displacement of secondary bromide with 6-chloropurine. As expected, this was an inefficient reaction affording compound (*RS*)-**8e** only in a 16% yield (**Scheme 8**). Nevertheless, after alkylation with triflyloxymethanephosphonate, the desired trifluoromethyl derivative (*RS*)-**10i** was obtained in a 12% yield (over 2 steps).



Scheme 8. Reagents and conditions: a) K_2CO_3, DMF, 60 °C, 24 h, b) $TfOCH_2P(O)(OiPr)_2$, *n*-BuLi, THF, −78 °C, 2 h.

Hemiaminal approach

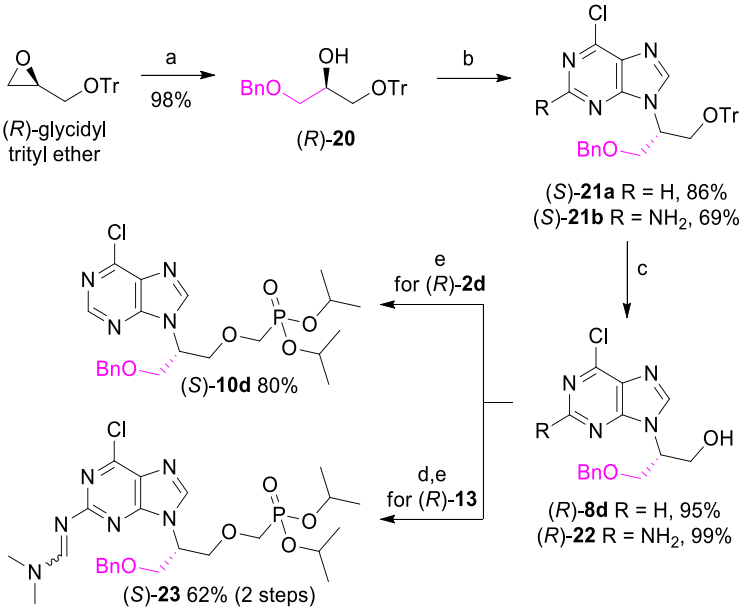
Using a reaction developed by Frydrych et al.[107], the hemiaminal derivatives could be efficiently obtained using previously developed acyclic acetals. First, the phosphonate acetals **6** were prepared by the aforementioned alkylation of diisopropyl hydroxymethanephosphonate with bromoacetaldehyde dialkylacetal. Treatment of **15** with the corresponding purines, Ac_2O and TMSOTf afforded compounds **10** in high yields (over 80%), while 2-aminopurine analogue **19** in a 24% yield only (**Scheme 9**). The *N*-acetyl group of **19** can be cleaved under basic conditions or can be kept for the subsequent synthesis.



Scheme 9. Reagents and conditions: a) NaH, $OHCH_2P(O)(OiPr)_2$ DMF, 25 °C, 19 h; b) 6-chloropurine or 2-amino-6-chloropurine, TMSOTf, Ac_2O, MeCN, 25 °C, 15 min.

Glycerol approach

The last developed approach towards C1′-branched ANPs starts with an opening of optically pure (*R*)-glycidyl trityl ether with sodium benzyloxide to give protected glycerol (*R*)-**20**,[108] which was then attached to the corresponding purine using Mitsunobu reaction (**Scheme 10**). The trityl group was removed under acidic conditions and the methylphosphonate moiety was introduced by the optimized alkylation to afford compounds (*S*)-**10d** and (*S*)-**23** in high yields (**Scheme 10**). In case of 2-amino-6-chloropurine, the protection of the 2-amino group with DMAM was necessary prior to the last step. After displacement of the chlorine atom, the benzyl group can be easily removed to yield the corresponding hydroxymethyl derivative (data not shown). Moreover, the first step (the epoxide opening) can be presumably performed with other nucleophiles leading to different substituents at the C1′ position.



Scheme 10. Reagents and conditions: a) NaH, BnOH, DMF, 100 °C, 3 h; b) 6-chloropurine or 2-amino-6-chloropurine, PPh_3, DIAD, THF, 70 °C, 1 h; c) TFA, DCM, 25 °C, 30 min; d) DMF DMA, 80 °C, 15 min; e) $TfOCH_2P(O)(OiPr)_2$, *n*-BuLi, THF, −78 °C, 2 h.

Comparison of synthetic approaches

Obviously, when a pure enantiomer is required, the condensation approach (CA) and glycerol approach (GA) are preferable. Nevertheless, the aldehyde approach (AA) can be still employed to afford single enantiomers, but chiral separations have to be carried out at the end of the synthesis. This can be beneficial when both (*S*) and (*R*) enantiomers are needed in order to identify the more active one. It is noteworthy that enantiomers of ANPs in the form of diisopropyl esters can be easily separated with a chiral column using normal phase, however, separation of enantiomers of free phosphonic acids using reversed phase is very difficult and laborious.

CA (for (*R*)-**10a** and (*R*)-**10c**, **Scheme 5**) consists of 3 steps, while AA (for (*RS*)-**10a** and (*RS*)-**10c**, **Scheme 7**) of 4 steps. Nevertheless, aldehyde **16** (AA) could be efficiently prepared in a large scale and the reaction of secondary alcohols with purines did not require DMAM protection of the 2-aminopurine group. Both approaches gave similar overall yields of target 6-chloropurines with C1′-ethyl group (~ 40–45%), but in case of C1′-cyclopropyl analogues, CA gave 2-fold higher yield of target intermediate **C** (**Figure 19**) (~ 46% vs 23%) compared to AA, due to the lower yield of the Mitsunobu reaction.

The (*R*) and (*S*) enantiomers of the C1′-benzyloxymethyl analogues (**10d**) were synthesized via CA and GA, respectively. The number of synthetic steps towards target 6-chloropurine intermediate is lower in CA compared to GA (3 steps vs 4 steps). However, when comparing the yields, GA exhibits considerably higher yield (~ 64%) compared to CA (~ 19%), thus representing more efficient route despite higher number of synthetic steps.

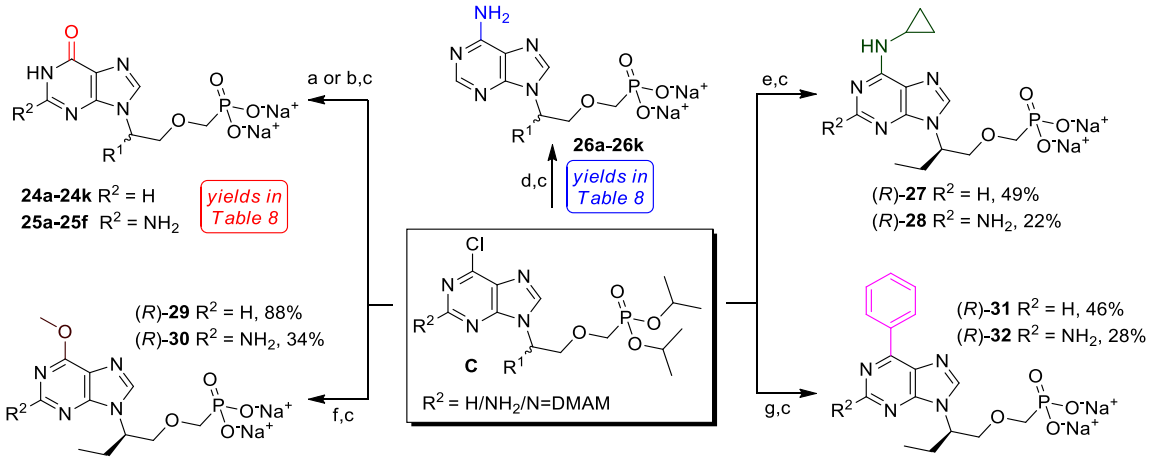
The hemiaminal approach (similarly to AA) inherently leads to racemic mixtures but is essential for the synthesis of the C1′-alkoxy derivatives and is very efficient especially in the case of 6-chloropurine analogues (70–80%, 2 steps).

The substitution approach can find use in some special cases, e.g. in the synthesis of the C1′-trifluoromethyl derivatives. Thus, compound (*RS*)-**10i**, which could not be prepared by AA, was obtained via nucleophilic substitution (**Scheme 8**).

Further modifications

Once the compounds of general structure **C** (**Figure 19**) are obtained via any of the aforementioned approaches, they can be easily modified by a replacement of the chlorine atom at position C6. Finally, deprotection of phosphonate esters using TMSBr is usually the last step of the synthesis.[109] Standard solvent used for this reaction is MeCN, nevertheless, acid-labile derivatives were deprotected in pyridine in order to avoid acidic environment. After purification, all final products passed through DOWEX 50 (Na^+ cycle) to obtain ANPs as unified sodium salts.

Hence, acidic hydrolysis of the intermediates **C** **(Figure 19)** was employed to prepare ANPs bearing hypoxanthine (compounds **24**, **Scheme 11**, **Table 8**) and guanine (compounds **25**, **Scheme 11**, **Table 8**), however, basic hydrolysis had to be used in the case of C1′-alkoxy analogues containing the acid-labile hemiaminal moiety. Adenine-based ANPs (compounds **26**, **Table 8**), were obtained by microwave-assisted ammonolysis. Other nucleophiles, cyclopropylamine (*c*$PrNH_2$) and potassium methoxide (MeOK), were used to replace the 6-chloro substituent in order to obtain compounds (*R*)-**27**, (*R*)-**28** and (*R*)-**29**, (*R*)-**30**, respectively (**Scheme 11**). Intermediates **C** were also used for the introduction of the phenyl moiety using Suzuki coupling in order to prepare compounds (*R*)-**31** and (*R*)-**32** (**Scheme 11**). Evidently, intermediates **C** can be used for the synthesis of a library of C1′-branched ANPs bearing either natural or modified nucleobases.



Scheme 11. Reagents and conditions: a) TFA/H_2O, 25 °C, 24 h; b) DABCO, K_2CO_3, 1,4-dioxane/H_2O, 90 °C, 2 h; c) TMSBr, DOWEX 50 (Na^+ cycle), MeCN or pyridine, 25 °C, 24 h; d) EtOH NH_3/aq. NH_3 (1:1), 120 °C, MW, 30 min; e) *c*PrNH$_2$, MeCN, 80 °C, 16 h; f) MeO^-K^+, MeOH, 25 °C, 2-16 h; g) $PhB(OH)_2$, Cs_2CO_3, $Pd(PPh_3)_4$, 1,4-dioxane/H_2O, 110 °C, 1 h.

Table 8. Yields of ANPs bearing hypoxanthine (**24**), guanine (**25**), and adenine (**26**) (**Scheme 11**).

Compound	R^1	R^2	Yield (2 steps)	Compound	R^1	Yield (2 steps)
(*RS*)-**24a**	Me	H	62%	(*RS*)-**26a**	Me	61%
(*RS*)-**24b**	Et	H	38%	(*RS*)-**26b**	Et	25%
(*R*)-**24b**	Et	H	69%	(*R*)-**26b**	Et	66%
(*S*)-**24c**	*i*Pr	H	36%	(*S*)-**26c**	*i*Pr	44%
(*R*)-**24c**	*i*Pr	H	42%	(*R*)-**26c**	*i*Pr	47%
(*RS*)-**24d**	Vinyl	H	30%	(*RS*)-**26d**	Vinyl	29%
(*RS*)-**24e**	Ethynyl	H	56%	(*RS*)-**26e**	Ethynyl	27%[a]
(*RS*)-**24f**	*c*Pr	H	87%	(*RS*)-**26f**	*c*Pr	53%
(*R*)-**24f**	*c*Pr	H	88%	(*R*)-**26f**	*c*Pr	69%
(*RS*)-**24g**	CF_3-	H	41%	(*RS*)-**26g**	CF_3-	42%
(*S*)-**24h**	BnOMe	H	30%	(*S*)-**26h**	BnOMe	70%
(*R*)-**24h**	BnOMe	H	28%	(*R*)-**26h**	BnOMe	56%
(*R*)-**24i**	$HOCH_2$-	H	33%	(*RS*)-**26i**	$HOCH_2$-	37%
(*RS*)-**24j**	Methoxy	H	33%	(*R*)-**26i**	$HOCH_2$-	27%[a]
(*RS*)-**24k**	Ethoxy	H	20%	(*RS*)-**26j**	Methoxy	50%
(*RS*)-**25a**	Me	NH_2	61%	(*S*)-**26j**	Methoxy	63%
(*RS*)-**25b**	Et	NH_2	57%	(*R*)-**26j**	Methoxy	63%
(*R*)-**25b**	Et	NH_2	29%	(*RS*)-**26k**	Ethoxy	23%
(*RS*)-**25c**	Vinyl	NH_2	26%	(*S*)-**26k**	Ethoxy	65%
(*RS*)-**25d**	Ethynyl	NH_2	27%	(*R*)-**26k**	Ethoxy	63%
(*RS*)-**25e**	*c*Pr	NH_2	50%			
(*RS*)-**25f**	MeO-	NH_2	11%			

[a] over three steps

Final ANPs were obtained in a wide range of yields (11–88% in 2 steps: modification at C6 position and ester groups removal). In general, ANPs lacking 2-aminopurine group were obtained in higher yields (20–88%, 2 steps) compared to their counterparts bearing the 2-aminopurine group (11–61%, 2 steps). This may, partially, be explained by the necessity to protect and deprotect the 2-aminopurine group in CA and GA. However, even AA, which did not require the protection/deprotection strategy, gave lower yields of the target 2-aminopurine analogues.

Biological evaluation

Antiviral activity

Out of the prepared C1′-branched ANPs, 26–31 compounds bearing adenine, hypoxanthine, or guanine nucleobase were selected and tested against human immunodeficiency virus (HIV), hepatitis C virus (HCV), enterovirus 71 (EV71), chikungunya virus (CHIKV), murine norovirus (MNV), and yellow fever virus (YFV).

Table 9. Antiviral activity of tested C1′-branched ANPs (except for **26a**).

Virus	EC_{50} [μM]	CC_{50} [μM]
HIV	>50	>50
HCV	>44	>44
EV71	>100	>20
CHIKV	>100	>100
MNV	>300	>300
YFV	>100	>100

The tested compounds showed no cytotoxicity but also no activity against studied viruses (**Table 9**). The only exception was compound **26a** exhibiting weak activity against HIV (EC_{50} = 17 μM) and HCV (EC_{50} = 37–41 μM). Although compound **26a** is an isomer of tenofovir (C1′-methyl instead of C2′-methyl) it exhibited more than 3-fold drop of potency against HIV.[84]

These finding are unfortunately in an agreement with the available antiviral data reported for the handful of C1′-branched ANPs which showed poor activity against HIV, HCV, and RSV for compound **II** (**Figure 20**) and low anti-HCV activity for compounds **III** (**Figure 20**).[46,100]

Cytostatic activity

PMEG (9-((2-phosphonylmethoxy)ethyl)guanine), which is structurally closely related to the prepared guanine C1′-branched ANPs (**Figure 21**), exerts significant antiproliferative and cytostatic potency against solid tumor and leukemia cell lines.[24] The guanine derivatives **25a**-**25e** were therefore evaluated regarding their cytostatic potency against cancer cell lines (HepG2, HL60, HeLa S3, and CCRF-CEM) and compared to PMEG (**Figure 21**).

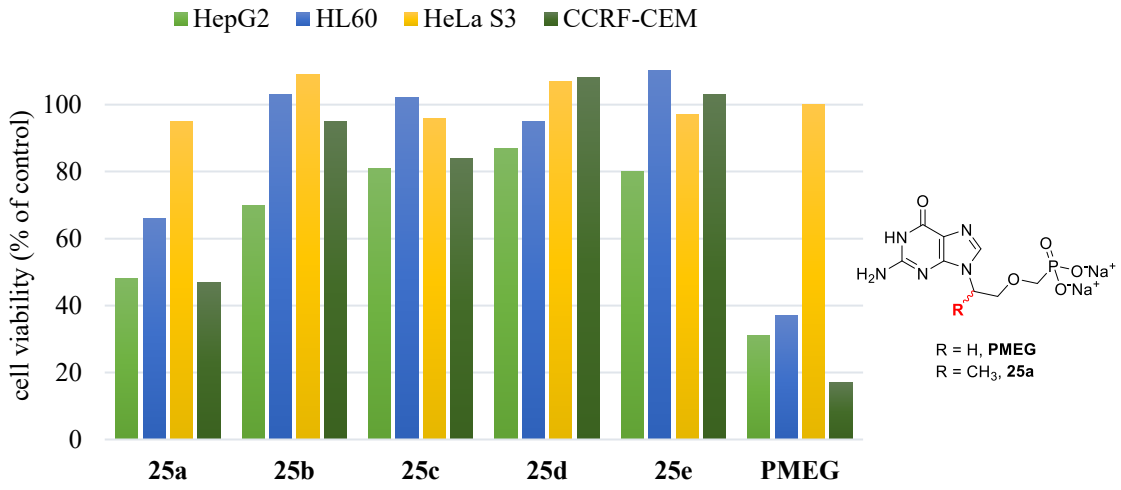


Figure 21. Cytostatic activity of guanine C1′-branched ANPs **25a**-**25e** and structure of PMEG/the most potent compound **25a**.

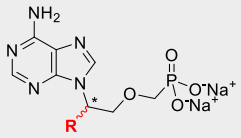
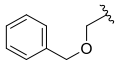
The most potent guanine C1′-branched ANP **25a** was still considerably less active compared to PMEG. The observed cytostatic potency of prepared guanine C1′-branched ANPs was only moderate to weak (**Figure 21**).

Antiprotozoal activity

Prepared adenine C1′-branched ANPs **26** were tested as potential inhibitors of *Trypanosoma brucei* adenine phosphoribosyl transferase 1 (*Tbr*APRT1). The active recombinant *Tbr*APRT1 was obtained via overexpression in *E. coli*.

It was found that most of compounds **26** are inhibitors of *Tbr*APRT1 with K_i values < 20 μM. The worst potency was exerted by derivatives bearing methyl (**26a**), ethynyl (**26e**) and trifluoromethyl (**26g**) at the position C1′ (**Table 10**).

Table 10. The inhibitory activity of prepared adenine C1′-branched ANPs **26** against *Tbr*APRT1.

 R=	Compound	*Tbr*APRT1 K_i [μM]
	(*RS*)-**26a**	> 30
	(*RS*)-**26b**	11.8 ± 1.4
	(*R*)-**26b**	**3.18 ± 0.40**
	(*S*)-**26c**	17.5 ± 3.1
	(*R*)-**26c**	**0.57 ± 0.07**
	(*RS*)-**26d**	16.3 ± 0.9
	(*RS*)-**26e**	26.7 ± 1.8
	(*RS*)-**26f**	5.84 ± 0.62
	(*R*)-**26f**	**2.95 ± 0.28**
	(*RS*)-**26g**	> 30
	(*S*)-**26h**	> 30
	(*R*)-**26h**	**0.39 ± 0.01**
	(*RS*)-**26i**	19.7 ± 2.4
	(*R*)-**26i**	15.4 ± 1.9
	(*RS*)-**26j**	13.1 ± 0.5
	(*S*)-**26j**	> 30
	(*R*)-**26j**	9.88 ± 1.08
	(*RS*)-**26k**	3.95 ± 0.59
	(*S*)-**26k**	> 30
	(*R*)-**26k**	**2.39 ± 0.43**

Based on the data, it was clearly evident that the configuration on C1′ chiral center strongly influences the activity. The (*R*) enantiomers proved to be superior inhibitors compared to (*S*) enantiomers or racemates. This effect was most pronounced in the most potent compound **26h** where the (*R*) enantiomer reached submicromolar activity (K_i = 0.39 μM) while the (*S*) enantiomer did not exert activity even at 30 μM concentration (**Table 10**). The second submicromolar inhibitor was compound (*R*)-**26c** (K_i = 0.57 μM) bearing C1′-isopropyl substituent. Other promising compounds with a single-digit micromolar activity were compounds bearing C1′-ethyl ((*R*)-**26b**) and C1′-cyclopropyl ((*R*)-**26f**) (**Table 10**).

To further verify the observed selectivity towards (*R*) enantiomers, I performed resolution of additional two racemates (diisopropyl phosphonate esters of **26j** and **26k**), on a chiral HPLC column and then transformed the pure enantiomers to the free phosphonic acids. Again, in all cases the (*R*) enantiomers were more potent inhibitors than (*S*) enantiomers (**Table 10**).

The reason for the observed selectivity towards (*R*) enantiomers is currently being investigated using *in silico* docking studies and the crystallization of *Tbr*APRT with the presented inhibitors is also being pursued. However, the observed selectivity can be presumably accounted for steric factors rather than electronic as the C1′-isopropyl derivative (**26c**) cannot form significant hydrogen bonding.

Modified ProTide prodrugs

The topic of ANPs is closely related to prodrugs. Both nucleoside phosphates and nucleoside phosphonates are deprotonated at physiological pH and their ionic character significantly reduces their ability to penetrate through biomembranes. In order to address this issue many prodrug moieties have been developed (as described in the Introduction). Prodrug moiety masks the ionic character of the parent molecule and substantially enhances its delivery to the target cell. Once inside the cell, the prodrug moiety is cleaved off thus releasing the parent active compound. Currently, the so-called ProTides are considered to be the leading approach in the field of prodrugs of modified nucleotides.

I used tenofovir as a suitable and available model compound for the purposes of this study. In a cell-based assay, tenofovir exhibits single-digit μM potency against human immunodeficiency virus (HIV) while the first generation prodrug – tenofovir disoproxil fumarate (TDF) exhibits 100-fold higher potency, and the second generation prodrug (ProTide) – tenofovir alafenamide fumarate (TAF) exhibits 1000-fold higher potency (**Figure 22**).[21,84]

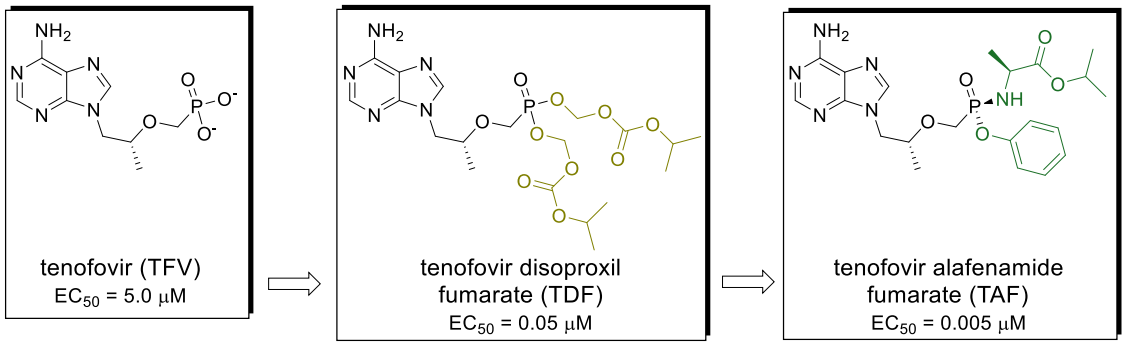


Figure 22. The influence of the prodrug approach implementation on the potency of tenofovir against HIV-1 *in vitro*.[84]

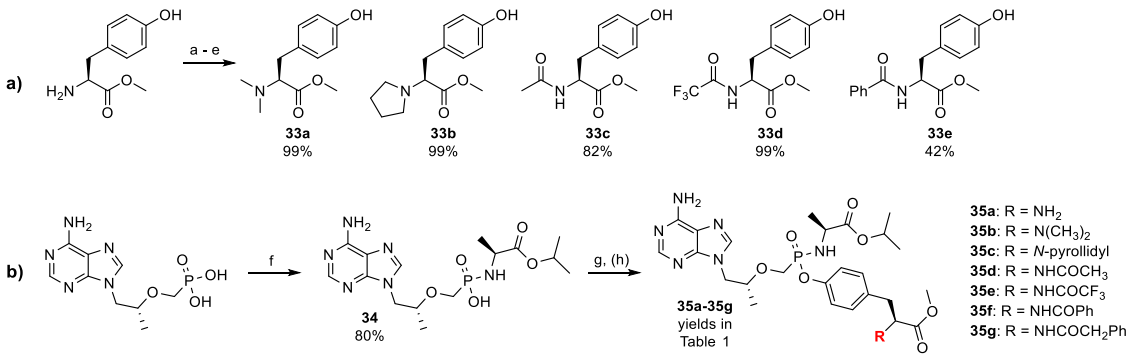
The ProTide of tenofovir (TAF) contains (*S*)-alanine isopropyl ester and phenol as the masking moieties. Both masking moieties are cleaved off during the metabolism and since phenol exhibits relatively high toxicity, I decided to replace phenol for (*S*)-tyrosine derivatives and I aimed for higher selectivity index (SI - toxicity/potency ratio) compared to TAF.

First series

In order to reduce the complexity and possible side reactions, I prepared the final compounds sequentially (**Scheme 12b**). However, it is certainly desirable to synthesize prodrugs in a one-pot manner and later in the study, I did optimize the one-pot reaction affording up to 86% yield.

The first series of final compounds consisted of (*S*)-tyrosine methyl esters modified on the N-terminus (**Scheme 12a**). First, compounds **33a**-**33e** were prepared from (*S*)-tyrosine

methyl esters using standard procedures. Compound **33a** was obtained via double reductive amination with formaldehyde. Compound **33b** was obtained via double nucleophilic displacement of 1,4-dibromobutane. Compound **33c** and **33d** were obtained under basic conditions using acetic and trifluoroacetic anhydride, respectively. Compound **33e** was obtained via amide coupling with HATU. Compound **33f** was available commercially.



Scheme 12. Synthesis of prodrugs in the first series. Reagents and conditions: a) for **33a**: CH_2O, Na_2SO_4, AcOH, $NaBH_3CN$, MeOH, 25 °C, 1 h; b) for **33b**: 1,4-dibromobutane, $NaHCO_3$, toluene, 115 °C, 3 h; c) for **33c**: Ac_2O, Na_2CO_3, acetone/H_2O, 25 °C, 30 min; d) for **33d**: $(CF_3CO)_2O$, pyridine, DCM, 25 °C, 5 min; e) for **33e**: PhCOOH, HATU, DIPEA, DMF, 25 °C, 1 h; f) EDC, Et_3N, H_2O, 40 °C, 16 h; g) compound **1**, Et_3N, PPh_3, 2,2′-dipyridyl disulfide, pyridine, 65 °C, 16 h; h) to obtain **35a**: **35g**, H_2, Pd/C, phosphate buffer (pH 7.4), 25 °C, 1 h.

Compound **34** was prepared starting from tenofovir according to previously reported procedure with EDC.[110] The key connection of the modified tyrosines (**33a-33f**) and compound **34** was performed using PPh_3 and 2,2′-dipyridyl disulfide (Aldrithiol™-2) (**Scheme 12b**) to afford prodrugs **35a-35g**. This key connection is discussed in detail below during the one-pot reaction optimization.

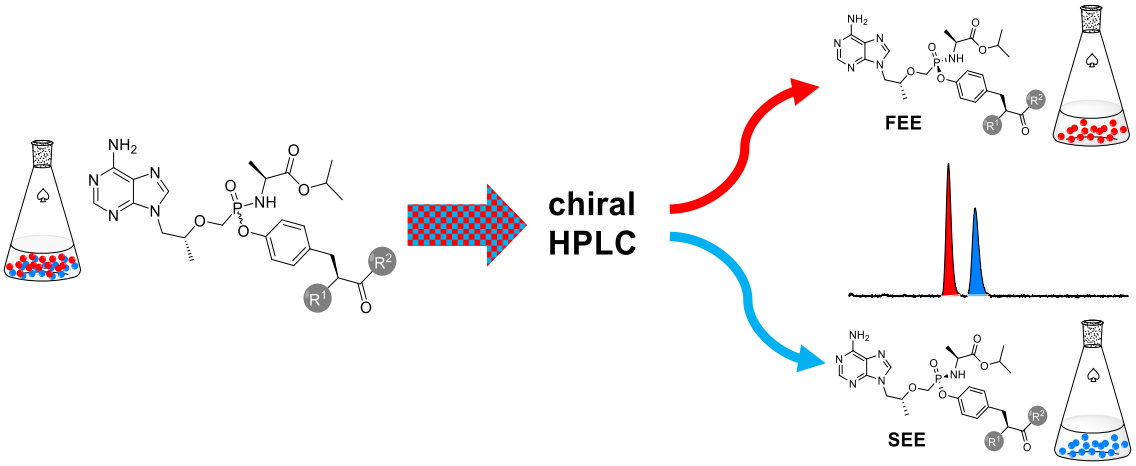
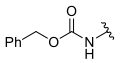


Figure 23. Illustration of chiral separation of fast-eluting epimer (FEE) and the slow-eluting epimer (SEE).

During the synthesis, a new chiral center was formed on the phosphorous atom. In order to resolve the mixture of two epimers, a chiral HPLC was employed thus separating the fast-eluting epimer (FEE) and the slow-eluting epimer (SEE) (**Figure 23**). The separation was optimized for resolution of up to 250 mg. The most eligible column proved to be YMC Chiral-Art Amylose SA with *n*-heptane/EtOH/MeCN as a mobile phase.

Table 11. The first series of final prodrugs, their activity against HIV-1 and cytotoxicity.

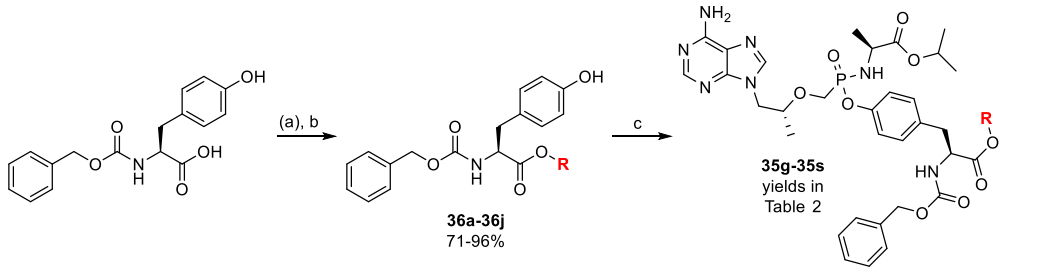
compound	R	yield [%]	EC_{50} [µM]		CC_{50} [µM]	
			FEE	SEE	FEE	SEE
TAF	--	--	0.0064[a]		35[a]	
35a	NH_2	23[b]	0.65	0.35	>50	>50
35b		21	2.5	0.048	>50	38
35c		54	0.011	0.016	24	27
35d		30	2.2	0.72	>50	>50
35e		5	0.54	0.15	>50	>50
35f		59	0.072	0.084	>50	>50
35g		49	0.030	0.016	8.5	16

[a] Data for S_p-epimer; [b] over 2 steps

The activities against HIV-1 indicated the SEE to be usually the more potent isomer (**Table 11**). This suggests that SEE might have S_p configuration since Chapman et al. reported the S_p isomer of TAF to be approx. 10-fold more potent compared to R_p isomer.[113] The circular dichroism (CD) spectra also suggest the SEE to have S_p configuration as the spectra align in some regions with the spectra of TAF while FEE spectra do no align in any region. However, there is no definitive proof of the absolute configuration yet. Crystallization of these scaffolds (either as a free base or as hydrochloride/fumarate salts) proved to be very challenging. Various techniques (including slow evaporation, solvent diffusion, vapor diffusion, and slow cooling) have been employed to date resulting in nearly 200 failed experiments. Most often, the compounds either precipitated or oiled-out from the solution after reaching the critical concentration. Small non-crystalline grains or very small needle-like microcrystals unsuitable for X-ray diffraction were obtained in the best case.

Second series

The biological data from the first series identified prodrugs **35c** and **35g** as the most promising (**Table 11**). Due to concerns regarding the potential metabolic instability of pyrrolidine[114] and generally good stability of carbamates[115,116], compound **35g** was selected as a template for the second series which explored modifications on the C-terminus. Hence, the second series compounds were bearing carboxybenzyl moiety on the N-terminus of tyrosine while the C-terminus was modified. One tyrosine ester (**36a**) was prepared using $SOCl_2$, nine tyrosine esters (**36b-36j**) were prepared using Mitsunobu reaction[117] and two tyrosine esters were available commercially. All *N*-((benzyloxy)carbonyl)-(*S*)-tyrosine esters reacted with compound **34** to afford 12 final prodrugs (**35h-35s**) (**Scheme 13**, **Table 12**).



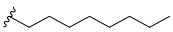
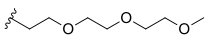
36a: R = isopropyl
36b: R = isoamyl
36c: R = cyclopentyl
36d: R = *n*-hexyl
36e: R = cyclohexyl
36f: R = 2-ethylbutyl
36g: R = *n*-octyl
36h: R = 2-(2-(2-methoxyethoxy)ethoxy)ethyl
36i: R = benzyl
36j: R = thiophen-2-ylmethyl

35h: R = ethyl
35i: R = isopropyl
35j: R = *tert*-butyl
35k: R = isoamyl
35l: R = cyclopentyl
35m: R = *n*-hexyl
35n: R = cyclohexyl
35o: R = 2-ethylbutyl
35p: R = *n*-octyl
35q: R = 2-(2-(2-methoxyethoxy)ethoxy)ethyl
35r: R = benzyl
35s: R = thiophen-2-ylmethyl

Scheme 13. Synthesis of final compounds in the second series. Reagents and conditions: a) for R=*i*Pr, $SOCl_2$, *i*PrOH, 25 °C, 72 h, 79%; b) ROH, PPh_3, DIAD, THF, 0–25 °C, 30 min, 71% – 96%; c) compound **34**, Et_3N or DIPEA, PPh_3, 2,2′-dipyridyl disulfide, pyridine, 65 °C, 16 h.

The second series again confirmed the more efficient metabolization and consequently higher activities of SEEs (**Table 12**). Nevertheless, no correlation between potency and lipophilicity was observed. For instance, the 4-carbon long *t*-butyl ester (**35j**), 6-carbon long cyclohexyl ester (**35n**) and 8-carbon long *n*-octyl ester (**35p**) exhibited one order of magnitude lower potency compared to TAF. In contrary, 2-carbon long ethyl ester (**35h**), 3-carbon long isopropyl ester (**35i**) 5-carbon long isoamyl (**35k**) and cyclopentyl (**35l**) esters, and 6-carbon long *n*-hexyl ester (**35m**) exhibited higher potency compared to TAF reaching up to two orders of magnitude higher potency.
The biological data further revealed three prodrugs exerting considerably higher SI compared to TAF, one of which (**35l**-SEE) exhibited 26-fold higher SI (**Figure 24**). Prodrug **35l**-SEE therefore became the lead and was further thoroughly evaluated.

Table 12. The second series of final prodrugs, their activity against HIV-1 and cytotoxicity.

compound	R	yield [%]	EC_{50} [µM]		CC_{50} [µM]	
			FEE	SEE	FEE	SEE
TAF	--	--	0.0064[a]		35[a]	
35h		32	0.02	0.0033	13	11
35i		24	0.027	**0.0006**	13	10
35j		23	0.013[b]		6.6[b]	
35k		19	0.011	**0.00056**	24	8.0
35l		37	0.016	**0.00007**	17	10
35m		37	0.0018[b]		8.4[b]	
35n		53	0.019	0.016	17	13
35o		56	0.021	0.0084	14	9.1
35p		43	0.036	0.050	38	38
35q		32	0.473[b]		>50[b]	
35r		40	0.009[b]		10[b]	
35s		10	0.0034[b]		8.2[b]	

[a] Data for S_p-epimer; [b] Data for the mixture of epimers

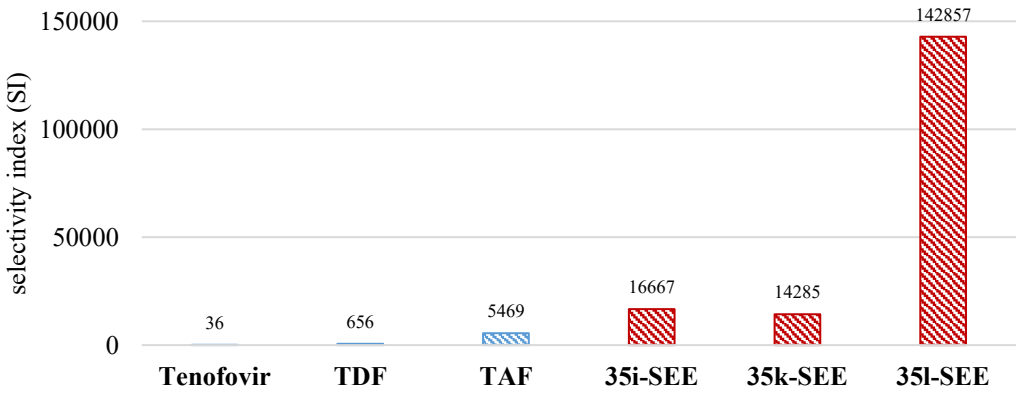


Figure 24. Selectivity indexes (SIs) of the three most efficient prodrugs from the second series compared to tenofovir and its prodrugs (TDF and TAF).

Evaluation of 35l-SEE

To assess the importance of the chiral center present in tyrosine moiety, the opposite enantiomer (*R*) was prepared (**37**, see Experimental part) and introduced to compound **34** (**Scheme 12**), thus forming prodrug **35t** (**Figure 25**). The synthesis of prodrug **35t** was identical to synthesis of prodrug **35l**. The biological data suggest that the chiral center in tyrosine is indeed important for the efficacy of the prodrug since **35t**-SEE exerted one order of magnitude lower potency. Interestingly, the potency of **35t**-SEE proved to be twice lower compared to **35t**-FEE which is in contrast with previous results of compounds bearing (*S*) configuration in tyrosine (**Figure 25**).

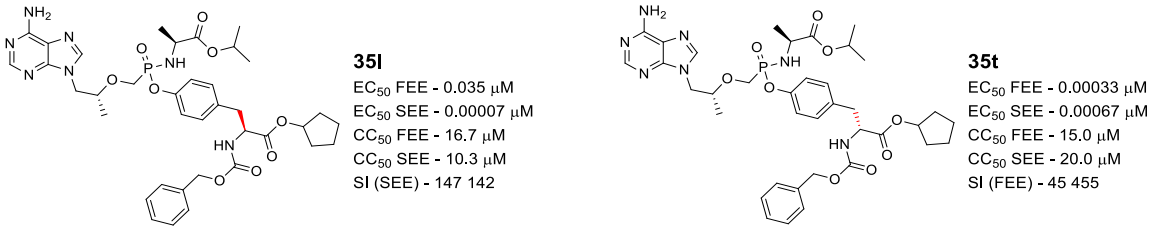
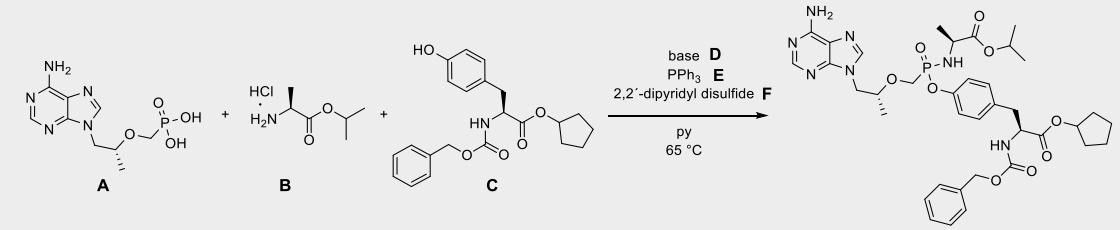


Figure 25. The effect of the chiral center in tyrosine on the activity and cytotoxicity.

Table 13. Optimized one-pot reaction securing the novel prodrugs.



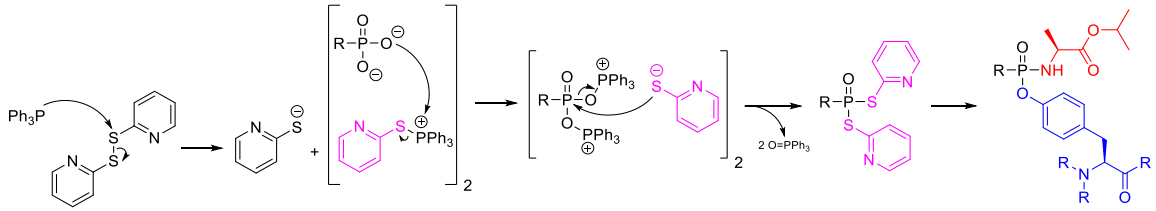
Entry	A	B	C	D	E	F	Yield
1	1 eq.	1.5 eq.	1.8 eq.	10 eq. TEA	8 eq.	8 eq.	**58%**
2	1 eq.	1.5 eq.	2 eq.	10 eq. TEA	8 eq.	8 eq.	**56%**
3	1 eq.	1.2 eq.	1.8 eq.	10 eq. DIPEA	7 eq.	7 eq.	**77%**
4	1 eq.	1 eq.	1.8 eq.	10 eq. DIPEA	7 eq.	7 eq.	**78%**
5	1 eq.	1 eq.	1.8 eq.	10 eq. DIPEA	4 eq.	4 eq.	**86%**
6	1 eq.	1 eq.	1.8 eq.	10 eq. DIPEA	2 eq.	2 eq.	**1%**

Prodrug **35l** was further used for an optimization of the one-pot reaction securing the prodrugs. The experience from previous reactions reduced the optimization effort to a minimum. One of the most important factors was the ratio between the *N*-nucleophile ((*S*)-alanine isopropyl ester) and *O*-nucleophile ((*S*)-tyrosine derivatives). Once the amount of *N*-nucleophile reached higher than 1 eq., the undesirable bisamidate side product was formed. To avoid this side reaction, the level of *N*-nucleophile was reduced

to 1 eq. while the level of *O*-nucleophile was set to 1.8 eq. It was further found, that 4 eq. of both PPh_3 and 2,2′-dipyridyl disulfide are optimal for efficient catalysis while 2 eq. are not sufficient and yield only traces of the product (**Table 13**).

The best results were obtained when tenofovir was mixed with 1 eq. of (*S*)-alanine isopropyl ester hydrochloride, 1.8 eq. of (*S*)-tyrosine derivative, and 10 eq. of a base. This mixture was heated to 65 °C and stirred. Subsequently, 4 eq. of PPh_3 and 4 eq. of 2,2′-dipyridyl disulfide were mixed in a separate flask and then added to the reaction mixture as a solution. This procedure secured the prodrugs in one-pot in a very good yield (86%).

The proposed mechanism of this key reaction is based on the knowledge of PPh_3 reaction with 2,2′-dipyridyl disulfide[111] and subsequent formation of phosphonate bis(pyridine-2-yl)thioesters[112] which firstly undergo reaction with the more nucleophilic (*S*)-alanine isopropyl ester followed by the reaction with the less nucleophilic (*S*)-tyrosine derivatives (**Scheme 14**).



Scheme 14. The proposed mechanism of the key reaction yielding the prodrugs.

Prior to the evaluation of chemical and metabolic stability, the water-solubility of prodrug **35l**-SEE was increased via formation of corresponding fumarate and hydrochloride salts which were obtained via heating of prodrug **35l**-SEE in THF with fumaric and hydrochloric acid, respectively. The formation of corresponding salts was confirmed by ^{1}H NMR. Stronger ionic pair in **35l**-SEE-HCl caused shift of purine hydrogens from 8.14 ppm (for *H2*) and 8.09 ppm (for *H8*) to 8.47 ppm and 8.43 ppm, respectively (**Figure 26**). Weaker ionic pair in **35l**-SEE-fum shifted purine hydrogens only insignificantly (0.004-0.005 ppm downfield). Nevertheless, the signal of fumarate (6.63 ppm) in the molecule suggests successful formation of the salt.

The water-solubility of studied compounds was determined by a method exploiting the compounds absorption at 254 nm using HPLC (for details, see Experimental part). The area under the curve (AUC) was correlated to the corresponding calibration curve in order to calculate the concentration of dissolved compound.

Original **3l**-SEE reached the maximum of 53.50 μM concentration in water while TAF reached >1000 μM. Hence, the salts were prepared to increase the solubility. The fumarate (**35l**-SEE-fum) and hydrochloride (**35l**-SEE-HCl) salts of **35l**-SEE increased water-solubility 2-fold (103.71 μM) and 4-fold (209.79 μM), respectively (**Figure 27**).

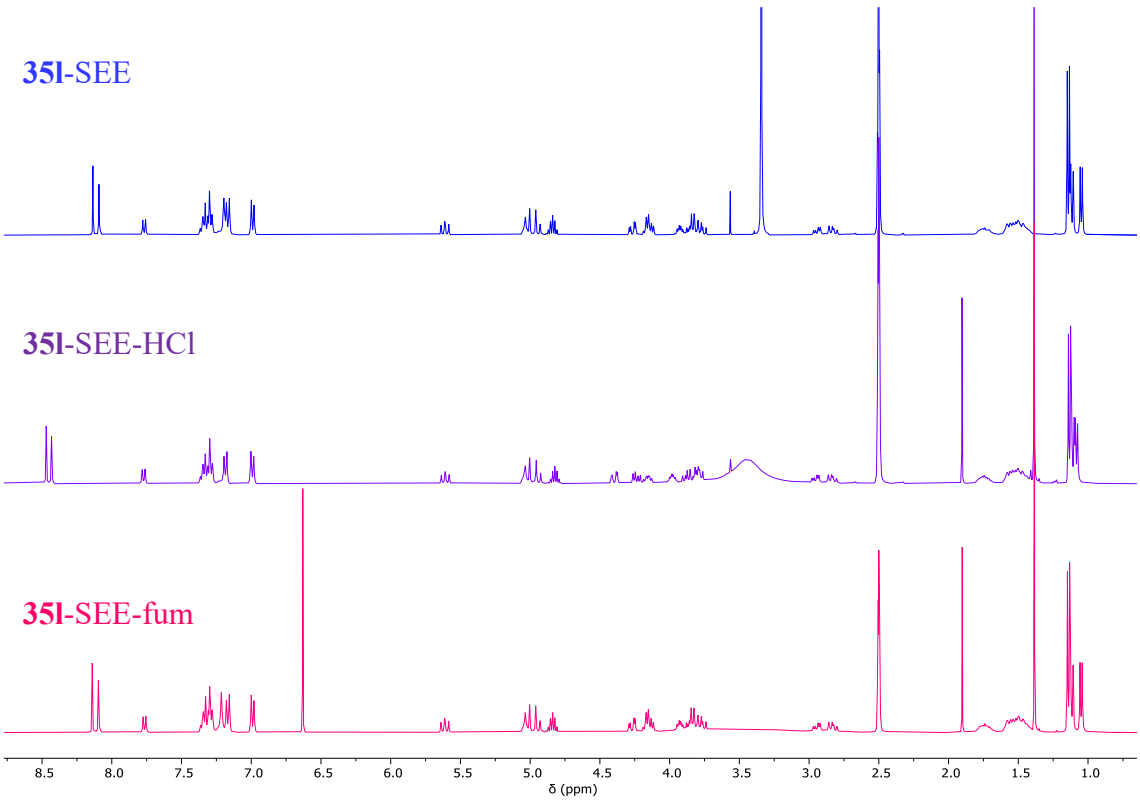


Figure 26. ^{1}H NMR spectra of **35l**-SEE compared to **35l**-SEE-HCl and **35l**-SEE-fum (spectra of the salts contain signals of residual acetone (1.90 ppm) and cyclohexane (1.39 ppm)).

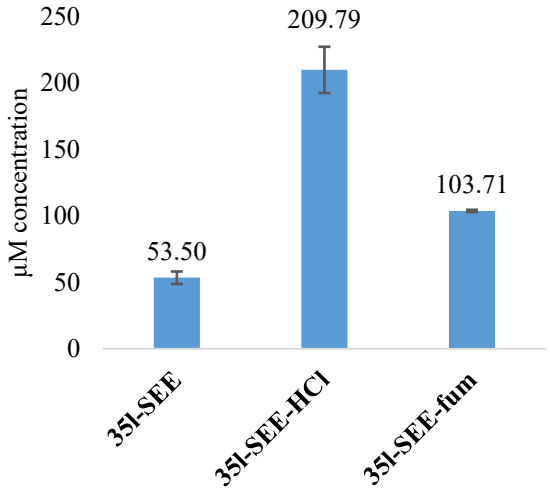


Figure 27. Water-solubility of prodrug **35l**-SEE and its fumarate and hydrochloride salts. Bars are means ± SEM obtained by averaging results of three experiments for each prodrug.

The chemical stability was evaluated at five different pH values (1.5 – 10.0) covering all the possible physiological pH values. As expected, the stability was temperature-dependent (for details regarding the chemical stability assay, see Experimental part). The most labile bonds in the molecule are the phosphorous-heteroatom bonds. Specifically, in an acidic environment, the P-N bond is cleaved thus disconnecting alanine from the molecule while in basic environment, the P-O bond is cleaved thus disconnecting tyrosine from the molecule (**Figure 28**).

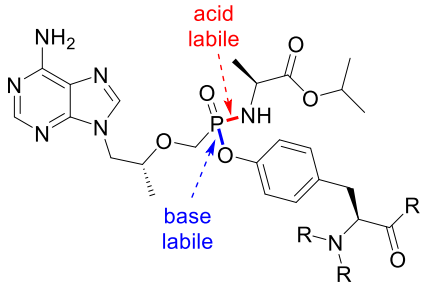


Figure 28. Identification of the most labile bonds in prodrugs.

Overall, prodrug **35l**-SEE proved to be slightly more stable compared to TAF. Both TAF and **35l**-SEE exhibited very similar stability profiles showing lower stability in basic environment. The lowest stability was observed at pH 10.0 followed by pH 1.5. In contrary, both types of prodrugs showed the highest stability at pH 4.5 with no degradation even at 37 °C (**Figure 29**).

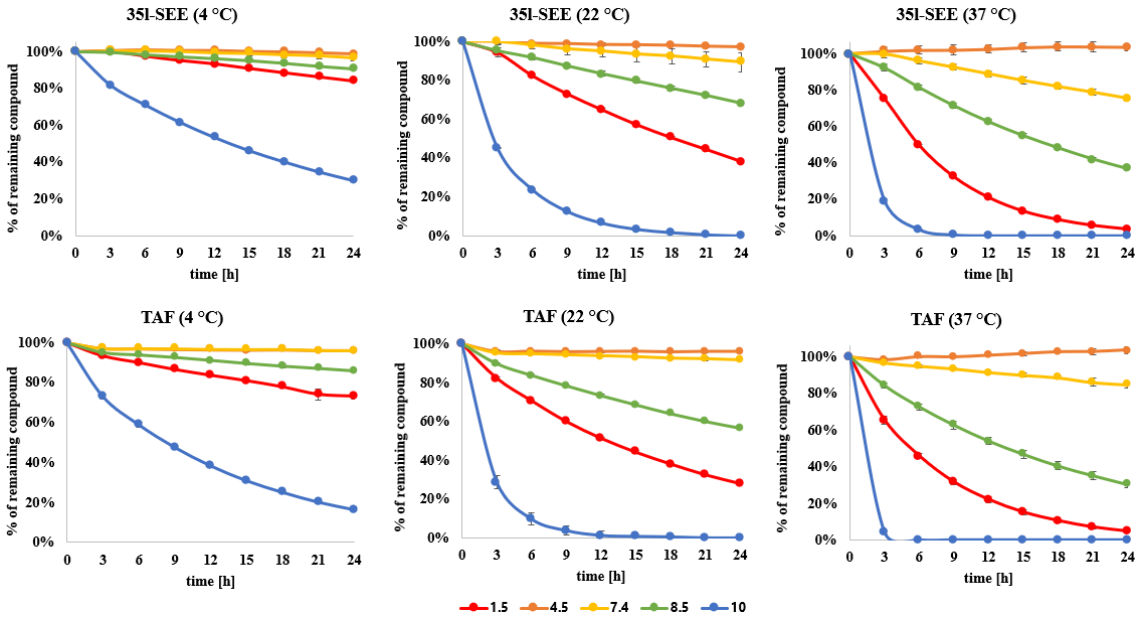


Figure 29. The pH stability of prodrug **35l**-SEE compared to TAF.

Plasma stability and microsomal stability were further determined to evaluate enzymatic stability of the novel prodrugs. FEE of prodrug **35l** is apparently more prone to degradation in plasma ($t_{1/2}$ = 277 min) compared to SEE of prodrug **35l** ($t_{1/2}$ = 578 min). Most importantly, **35l**-SEE proved to be significantly more stable in plasma compared to TAF ($t_{1/2}$ = 207 min) (**Figure 30**, left). This is crucial since degradation in plasma leads to high levels of tenofovir in systemic circulation which is associated with nephrotoxicity and bone toxicity.[83–85]

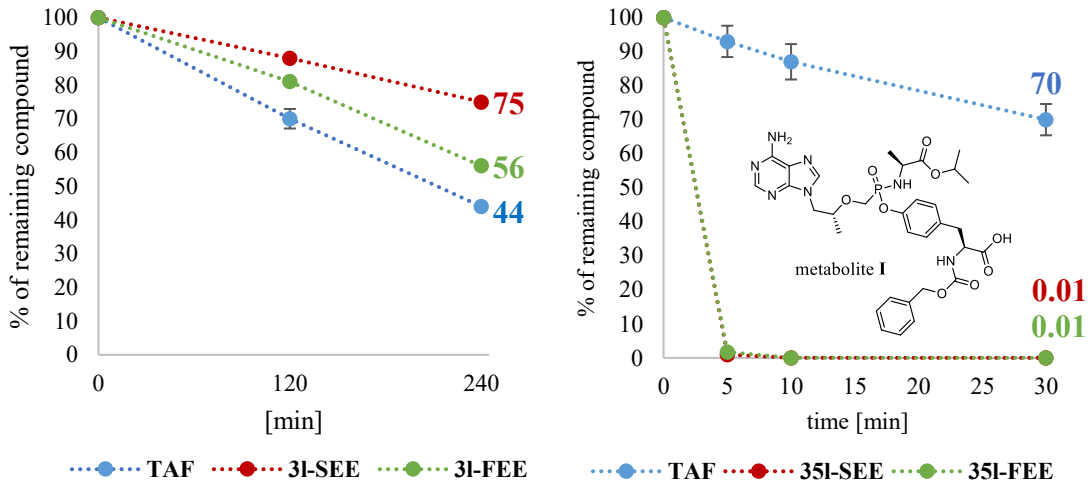


Figure 30. The plasma stability (left) and microsomal stability (right) of prodrug **35l**-SEE compared to TAF.

An obstacle appeared in the form of microsomal stability as both epimers of prodrug **35l** degraded almost instantly in human hepatic microsomes suggesting high first-pass effect. While investigating the arising metabolites, I identified metabolite **I** lacking the cyclopentyl ester as the major metabolite formed (**Figure 30**, right).

Third series

To address the low microsomal stability, I designed and prepared the third series composed of three prodrugs where the ester function was replaced by a more stable isosteric moiety. Instead of the ester group, prodrug **35u** was bearing an isosteric amide while prodrugs **35v** and **35w** were bearing an isosteric ketone (**Figure 31**). I also prepared two other derivatives having the isosteric amido (**35x**) or amino (**35y**) moiety instead of the carbamate group on the N-terminus (**Figure 31**).

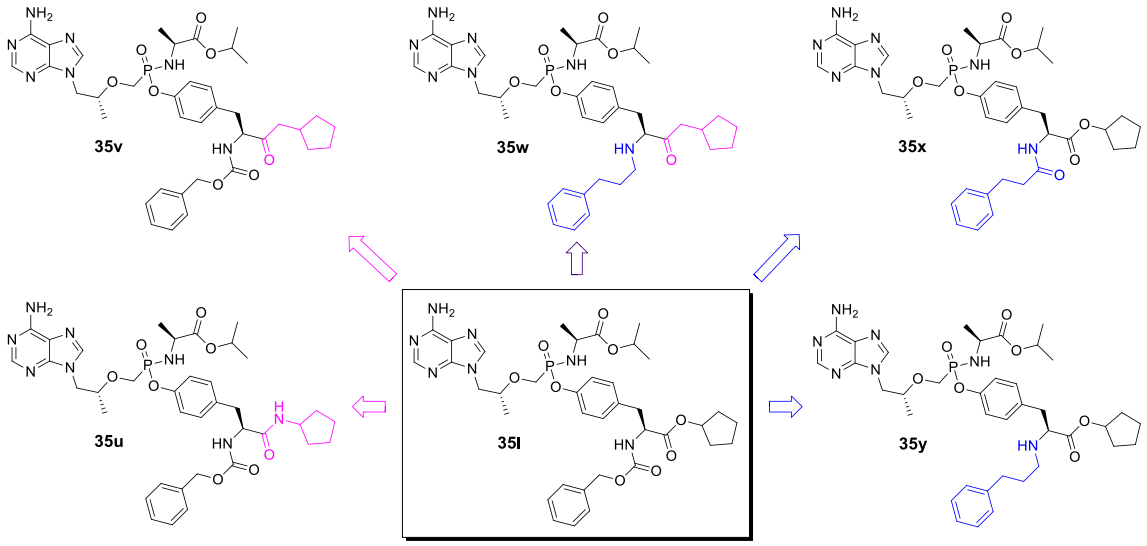
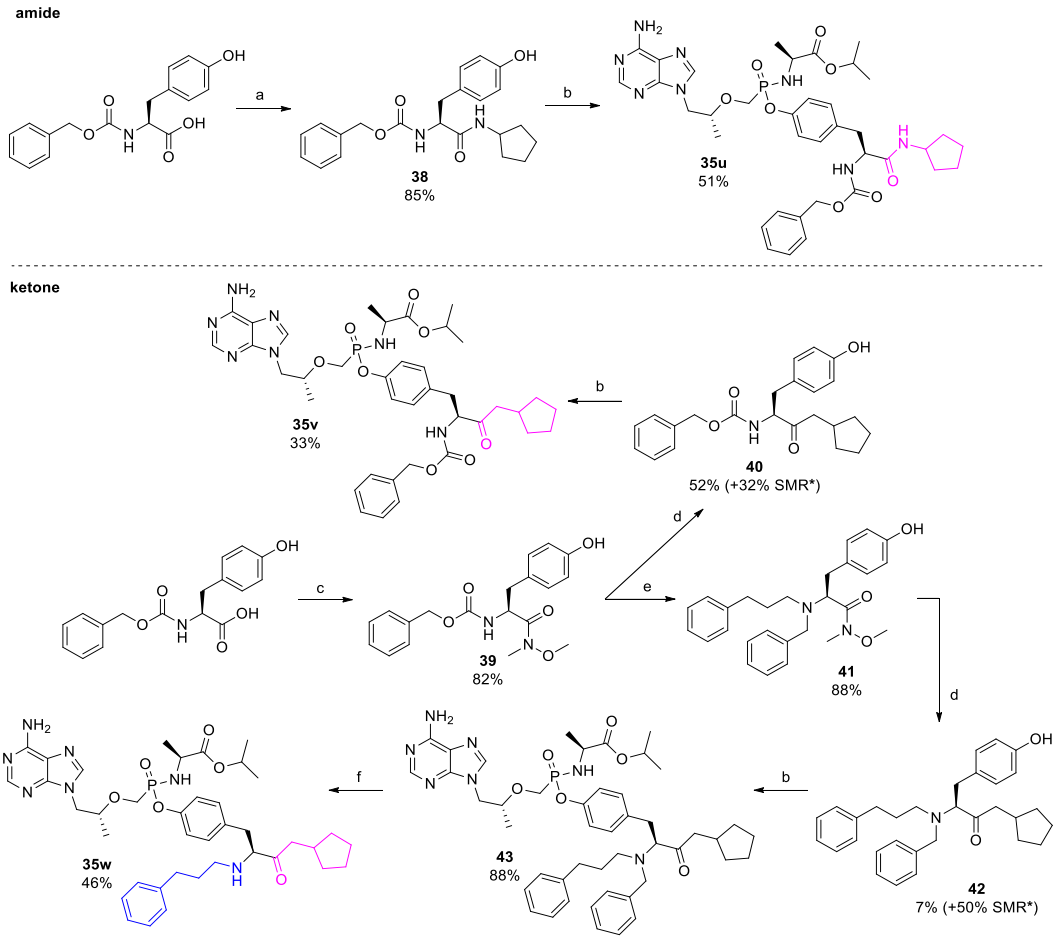


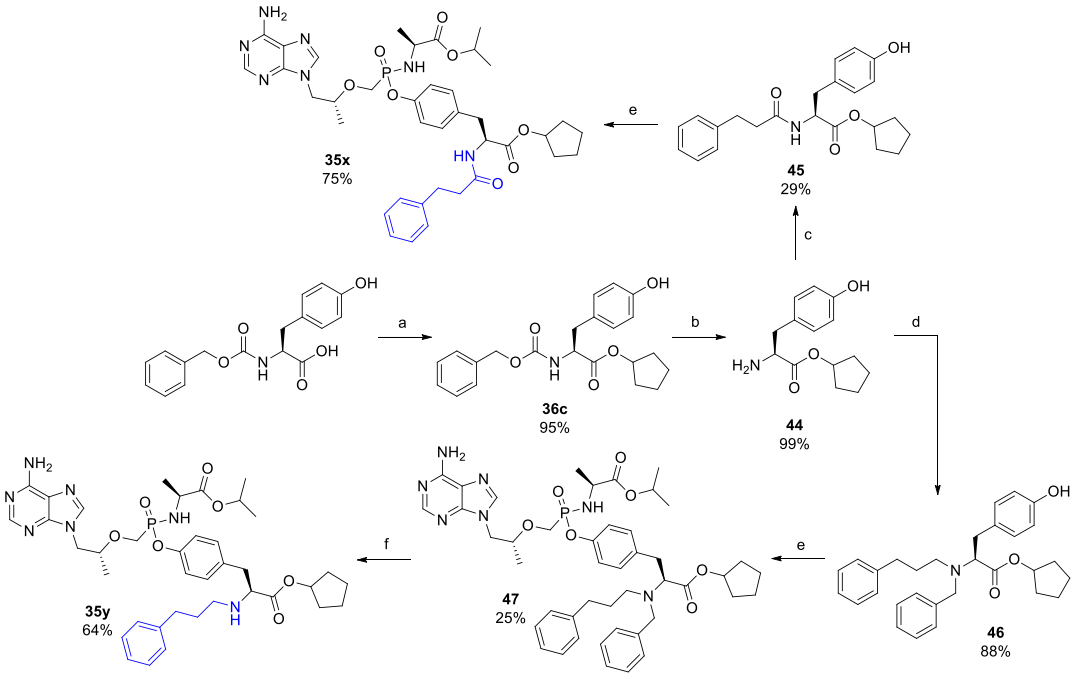
Figure 31. Structures of target prodrugs for the third series based on prodrug **35l**.

To obtain prodrug **35u**, *N*-((benzyloxy)carbonyl)-(*S*)-tyrosine was coupled with cyclopentyl amine using HATU and the following reaction with compound **34**, PPh_3 and 2,2′-dipyridyl disulfide yielded desired **35u** (**Scheme 16**). The synthesis of compounds bearing ketone also started from *N*-((benzyloxy)carbonyl-(*S*)-tyrosine which was converted to a so-called Weinreb amide **39**. Compound **39** reacted with freshly prepared cyclopentylmethylmagnesium bromide to afford **40**, which reacted with compound **34** to obtain final prodrug **35v** (**Scheme 16**). To afford compound **35w**, **39** underwent catalytic hydrogenation followed by double asymmetric reductive amination thus yielding compound **41**, which was treated with freshly prepared cyclopentylmethylmagnesium bromide thus obtaining compound **42**. Compound **42** reacted with compound **34** to obtain **43**. Final deprotection of compound **43** using catalytic hydrogenation had to be carried out in phosphate buffer (pH 7.4) to prevent degradation of final prodrug **35w** (**Scheme 16**).



Scheme 16. Synthesis of prodrugs bearing isosteric amide or ketone instead of ester on the C-terminus of tyrosine. Reagents and conditions: a) cyclopentyl amine, HATU, DIPEA, DMF, 25 °C, 30 min; b) compound **34**, Et_3N or DIPEA, PPh_3, 2,2′-dipyridyl disulfide, pyridine, 65 °C, 16 h; c) $CH_3ONHCH_3 \cdot HCl$, HATU, DIPEA, DMF, 25 °C, 30 min; d) cyclopentylmethylmagnesium bromide, THF, 0 – 50 °C, 16 h; e) H_2, Pd/C, MeOH, 25 °C, 1 h, then PhCHO, 3-phenylpropanal, Na_2SO_4, AcOH, $NaBH_4$, MeOH, 25 °C, 2 h; f) H_2, Pd/C, phosphate buffer pH 7.4, 25 °C, 1 h. *SMR – starting material recovery

Synthesis of prodrug **35x** started with the cleavage of (benzyloxy)carbonyl from compound **36c** thus yielding **44**. Compound **44** was coupled with 3-phenylpropionic acid using HATU to obtain **45** which was attached to compound **34** to afford desired prodrug **35x** (**Scheme 17**). Compound **44** underwent double asymmetric reductive amination thus yielding compound **46**, which was connected to compound **34** to afford compound **47**. The desired prodrug **35y** was obtained after catalytic hydrogenation of **47** in phosphate buffer (pH 7.4) (**Scheme 17**).



Scheme 17. Synthesis of prodrugs bearing isosteric amide or amine instead of carbamate on the N-terminus of tyrosine. Reagents and conditions: a) cyclopentanol, PPh_3, DIAD, THF, 0 – 25 °C, 30 min; b) H_2, Pd/C, MeOH, 25 °C, 1 h; c) 3-phenylpropionic acid, HATU, DIPEA, DMF, 25 °C, 30 min; d) PhCHO, 3-phenylpropanal, Na_2SO_4, AcOH, $NaBH_4$, MeOH, 25 °C, 2 h; e) compound **34**, Et_3N, PPh_3, 2,2′-dipyridyl disulfide, pyridine, 65 °C, 16 h; f) H_2, Pd/C, phosphate buffer pH 7.4, 25 °C, 1 h.

The third series revealed prodrugs with an exceptional potency (**Table 14**). Especially interesting were SEEs of prodrugs **35v**, **35w**, and **35x**. Prodrug **35v**-SEE exhibited the highest SI in the whole study reaching nearly 300-fold higher SI compared to TAF. Prodrug **35w**-SEE showed considerably lower toxicity while exerting 150-fold higher SI compared to TAF and similarly high SI was observed in the case of prodrug **35x**-SEE. Interestingly, the substitution of ester for amide led to significant drop of efficacy (**35u**).

Table 14. Activity against HIV-1, cytotoxicity, and selectivity indexes of prodrugs from the third series.

compound	EC_{50} [μM]		CC_{50} [μM]		SI (for SEE)
	FEE	SEE	FEE	SEE	
35u	0.01	0.013	17	28	2 154
35v	0.00015	0.000006	7.6	9.2	**1 533 333**
35w	0.0096	0.00006	7.3	48	**800 000**
35x	0.00036	0.00001	11	8.8	**880 000**
35y	0.00068	0.00028	7.6	6.0	21 429

Prodrug with the highest SIs (**35v**, **35w**, and **35x**) were selected for subsequent enzymatic stability evaluation. In agreement with previous data, the FEEs were more prone to degradation in plasma compared to SEEs. Most importantly, plasma stability of SEEs of the three selected prodrugs remained substantially higher compared to TAF (**Figure 32**, left). The microsomal stability confirmed to be very low in case the ester remained in the molecule (**35x**). After substitution of the ester for an isosteric ketone, the microsomal stability did increase, however, still remained considerably lower compared to TAF (**Figure 32**, right). The investigation of **35w** revealed oxidized metabolites and compound **34** as major metabolites.

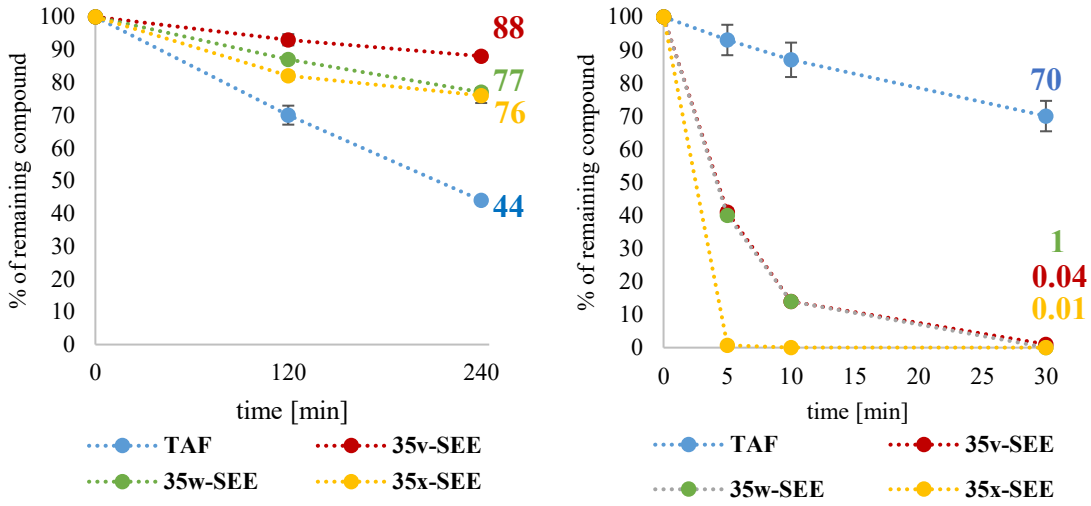


Figure 32. The plasma stability (left) and microsomal stability (right) of compounds **35v**-SEE, **35w**-SEE, and **35x**-SEE compared to TAF.

The reason for the exceptionally high activities was further investigated. The contribution of tyrosine derivatives (released during the metabolism) to the anti-HIV effect was excluded as all of the tyrosine moieties from compounds **35l**, **35v**, **35w**, **35x**, and **35y** showed no activity against HIV-1 (EC_{50} > 40 μM). Different rate of prodrug cleavage by cathepsin A was also found to be an unlikely reason for the marked potency differences between TAF and the novel prodrugs since prodrugs **35l**, **35v**, **35w**, and **35x** exhibited comparable substrate activity towards cathepsin A as TAF (**Table 15**).

Table 15. Substrate activity of prodrugs **35l**, **35v**, **35w**, **35x** and TAF towards cathepsin A.

Prodrug	cathepsin A spec. activity (pmol · min^{-1} · μg^{-1})	
	FEE	SEE
TAF	25,8 ± 1,7 (S_P)	
35l	35,9 ± 2,6	33,6 ± 9,1
35v	30,3 ± 3,8	39,7 ± 2,0
35w	10,2 ± 2,4	16,3 ± 5,9
35x	29,5 ± 0,1	26,0 ± 0,6

The metabolic studies in intact lymphoid cells (CCRF-CEM) suggested that the superior antiviral efficacy were achieved due to highly efficient cellular uptake and rapid metabolization to free tenofovir (TFV) inside the cells (**Figure 33**). In particular, the prodrugs lacking the ester moiety (**35v** and **35w**) exhibited substantially higher levels of both intact prodrug and released tenofovir (TFV) inside the cells compared to TAF (**Figure 33**). While the SEEs of prodrugs bearing the ester group (**35l** and **35x**) also showed considerably higher concentration of TFV than TAF, they were less effective than prodrugs **35v** and **35w**. This can be explained by their lower metabolic stability which is also reflected in lower levels of intact prodrugs in the cells (**Figure 33**). Along with high cellular uptake of both FEEs and SEEs, the metabolic study also exposed sharp differences in the cleavage of the two epimers. Clearly, the SEEs are metabolized to the parent TFV significantly faster than FEEs. This is in accordance with the higher observed activities of SEEs against HIV-1.

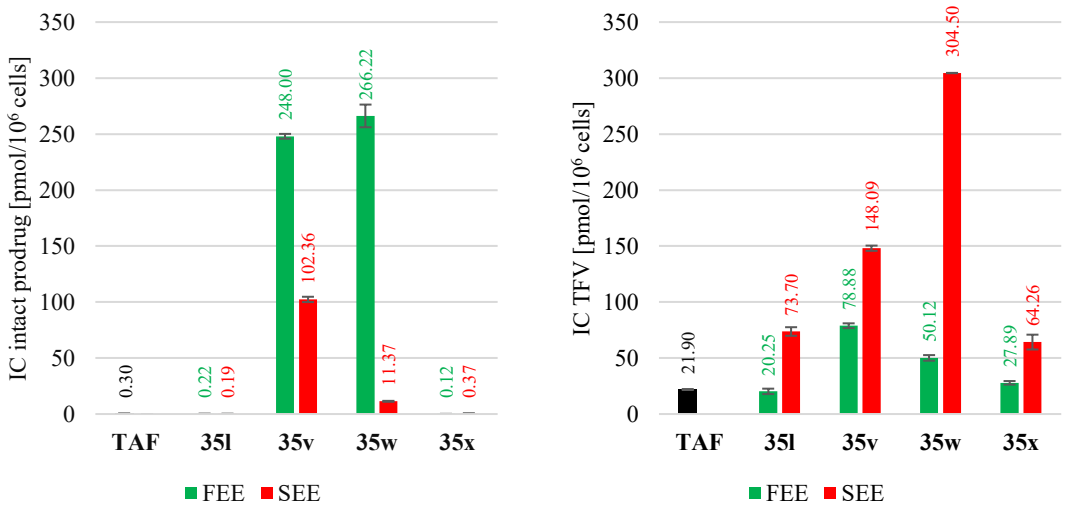


Figure 33. Intracellular content (IC) (pmol/10^6 cells) of intact prodrugs (left) and released tenofovir (TFV) (right) in CCRF-CEM lymphoblast cells after 45 min incubation. Bars are means ± SEM obtained by averaging results of two experiments for each prodrug.

The marked differences in metabolic fate of the two epimers were further studied with prodrug **35w** (**Figure 34**, **Table 16**). In agreement with previous data, SEE released 6-fold higher amount of TFV than FEE. Moreover, the key metabolite **M2** which is present in the original ProTide metabolism[118] was 11-fold more abundant in SEE than in FEE thus suggesting standard ProTide metabolic pathway for SEE. However, the 5-fold higher levels of metabolite **M1** in FEE suggested an additional metabolic pathway for FEE since **M1** is absent in ProTide metabolism. Moreover, FEE metabolism resulted in a considerably higher amount of observed metabolites (**Figure 34**, **Table 16**). This can be explained by the evidently slower cleavage of FEE to the free TFV, resulting in an intracellular accumulation of the intact prodrug which can be further transformed by various enzymes thus leading to high number of metabolites as opposed to SEE which is rapidly cleaved to the parent TFV thus leaving only a low amount of intact prodrug available for sideways metabolic transformations.

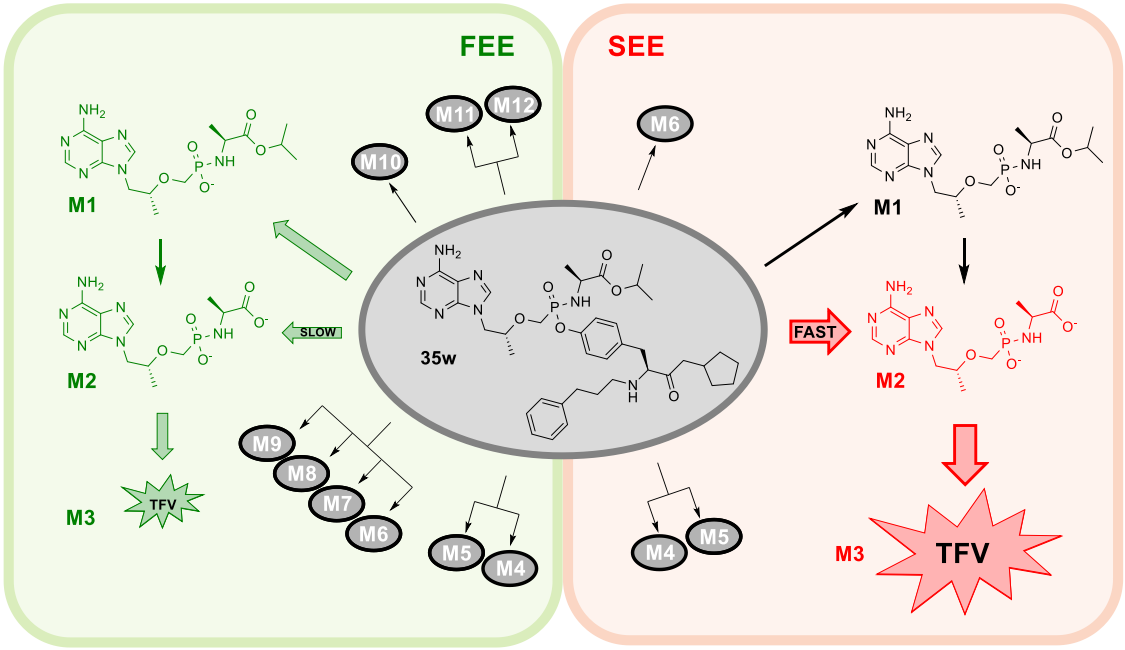


Figure 34. Intracellular metabolization of compound **35w** demonstrating the sharp differences in the metabolism of FEE and SEE.

Table 16. The observed metabolites of FEE and SEE of prodrug **35w**.

Metabolite	Most possible transformation	Change	Exact mass	Most possible formula	FEE/SEE abundance
M1	tyrosine cleavage	– $C_{24}H_{29}NO$	400.162	$C_{15}H_{25}N_6O_5P$	5-fold ↑ in FEE
M2 (**34**)	*i*Pr ester cleavage → cyclisation → hydrolysis	– $C_{27}H_{35}NO$	358.116	$C_{12}H_{19}N_6O_5P$	11-fold ↑ in SEE
M3 (TFV)	alanine cleavage	– $C_{30}H_{40}N_2O_2$	287.078	$C_9H_{14}N_5O_4P$	6-fold ↑ in SEE
M4	reduction	+ H_2	749.403	$C_{39}H_{56}N_7O_6P$	13.5-fold ↑ in FEE
M5	reduction	+ H_2	749.403	$C_{39}H_{56}N_7O_6P$	1.5-fold ↑ in FEE
M6	desaturation, oxidation	– H_2 + O	761.367	$C_{39}H_{52}N_7O_7P$	7-fold ↑ in FEE
M7	desaturation, oxidation	– H_2 + O	761.367	$C_{39}H_{52}N_7O_7P$	only in FEE
M8	desaturation, oxidation	– H_2 + O	761.367	$C_{39}H_{52}N_7O_7P$	only in FEE
M9	desaturation, oxidation	– H_2 + O	761.367	$C_{39}H_{52}N_7O_7P$	only in FEE
M10	oxidative deamination	– NH + O	748.371	$C_{39}H_{53}N_6O_7P$	only in FEE
M11	oxidation	+ O	763.382	$C_{39}H_{54}N_7O_7P$	only in FEE
M12	oxidation	+ O	763.382	$C_{39}H_{54}N_7O_7P$	only in FEE

The presented prodrugs were also very efficient in hepatocytes against HBV. Thirteen prodrugs exhibited higher efficacy than TAF (**Table 17**) and in most cases, the prodrugs did not exert any toxicity. The most promising prodrug in the hepatocyte assay proved to be **35k** exerting more than 25-fold higher SI compared to TAF.

Table 17. Activity against HBV and cytotoxicity of prodrugs exhibiting higher EC_{50} compared to TAF.

compound	EC_{50} [µM]		CC_{50} [µM]	
	FEE	SEE	FEE	SEE
TAF	0.0053[a]		>50[a]	
35d	0.0072	0.0032	>50	>50
35e	0.0016	0.00027	>50	>50
35g	0.0006[b]		15[b]	
35h	0.0036[b]		>50[b]	
35i	0.0042[b]		>50[b]	
35k	**0.0002**[b]		>50[b]	
35l	0.0007[b]		>50[b]	
35m	0.0014[b]		>50[b]	
35v	0.0034	0.0028	>50	>50
35x	0.0014	0.0044	>50	>50
35y	0.004	0.019	>50	29
43	0.0013[b]		46[b]	
47	0.0012[b]		>50[b]	

[a] Data for S_p-epimer; [b] Data for the mixture of epimers

These promising results proved the presented concept to be viable and they lay down a strong foundation for further development of next generation tyrosine prodrugs aiming for higher microsomal stability while retaining the extraordinary SIs.

Conclusions

This study explored the previously overlooked field of C1′-branched acyclic nucleoside phosphonates (ANPs). Five diverse synthetic approaches were developed/optimized affording the key 6-chloropurine intermediates containing N^9-phosphonomethoxyethyl (PME) moiety in 2–4 steps. As reviewed in the chapter Results and Discussion, each approach offers different options regarding stereospecifity, possibility for derivatization during the synthesis, or yield. It was further demonstrated that these intermediates can be further vastly diversified to various final compounds bearing free phosphonate. Hydrolysis secured hypoxanthine and guanine derivatives. Acidic hydrolysis was employed in most cases, however, derivatives bearing acid-labile hemiaminal moiety (C1′-alkoxy) had to be hydrolysed under basic conditions. Ammonolysis afforded adenine derivatives. Other *O*- and *N*-nucleophiles or coupling reactions can be used to prepare ANPs with unnatural nucleobases. Overall, 48 final C1′-branched ANPs in the form of sodium salts was synthesized.

Selected adenine, hypoxanthine, and guanine C1′-branched ANPs were tested against various viruses. With agreement with the handful of cases reported, we observed no significant antiviral activity against human immunodeficiency virus (HIV), hepatitis C virus (HCV), enterovirus 71 (EV71), chikungunya virus (CHIKV), murine norovirus (MNV), and yellow fever virus (YFV).

Selected guanine C1′-branched ANPs were tested for their cytostatic potency and compared to PMEG. All tested compounds were less potent compared to PMEG and exhibited only moderate or weak cytostatic potency in HepG2, HL60, HeLa S3, and CCRF-CEM cell lines.

Adenine C1′-branched ANPs proved to be potent inhibitors of adenine phosphoribosyl transferases of *Trypanosoma brucei* (*Tbr*APRT). The enzymatic assays with active recombinant protein revealed compounds exerting up to submicromolar inhibitory constant (K_i). The data further exposed a very strong influence of the C1′ chiral center configuration on the potency. The (*R*) enantiomers were substantially more potent compared to (*S*) enantiomers or racemates. To validate the observed selectivity, additional racemates were resolved using chiral HPLC column. As in previous cases, the (*R*) enantiomers proved to be significantly more potent. The adenine (*R*)-C1′-branched ANPs are up to date the most potent known inhibitors of *Tbr*APRT.

The second part of this study was focused on prodrugs which are closely related to ANPs. Based on ProTide prodrugs, modified prodrug moieties were developed bearing (*S*)-tyrosine derivatives instead of phenol. The design was initially based on the goal to reduce the toxicity since the original ProTides release phenol during the metabolism. However, the major aim was to increase the selectivity index (SI - toxicity/potency ratio). Tenofovir was selected as suitable model compound and the efficiency of the novel prodrugs was therefore evaluated against HIV-1 and compared to the ProTide prodrug of tenofovir (tenofovir alafenamide fumarate – TAF). The synthesis was performed sequentially in order to reduce the complexity and possible side reactions. However, it is highly desirable

to synthesize prodrugs in a one-pot manner. Hence, the one-pot reaction affording the prodrugs was eventually optimized up to 86% yield which was considerably higher than the yield of sequential synthesis.

During the synthesis, a new chiral center was formed on the phosphorous atom. The two foremd epimers were resolved using chiral HPLC column, thus obtaining the fast-eluting epimer (FEE) and the slow-eluting epimer (SEE). Numerous attempts to crystallize the obtained prodrugs either in the form of a free base or in the form of salts failed, therefore, the absolute configuration has not been assigned yet.

The first series of prodrugs was modified on the N-terminus of tyrosine while the second series was modified on the C-terminus of tyrosine. The anti-HIV data showed the SEEs to be the more efficient epimers. In the second series, a promising prodrug **35l-**SEE was discovered with a double-digit picomolar EC_{50} and 26-fold higher SI compared to TAF. Prodrug **35l-**SEE thus became a lead and was thoroughly evaluated. It was observed that the chiral center in tyrosine plays an important role as the analogous prodrug to **35l** bearing (*R*)-tyrosine (**35t**) exhibited one order of magnitude lower efficacy. The water-solubility of **35l-**SEE was increased 2-fold and 4-fold via formation of fumarate and hydrochloride salts, respectively. Compound **35l-**SEE was further evaluated with respect to chemical and enzymatic stability. While exhibiting similar stability profiles as TAF, **35l-**SEE proved to be slightly more stable at the five observed pHs. The plasma stability of **35l-**SEE was substantially higher compared to TAF. Nevertheless, a drawback was spotted during microsomal stability studies as compound **35l-**SEE degraded almost immediately in human microsomes. The major metabolite lacking the cyclopentyl ester was identified. The ester function was replaced by an isosteric amide or ketone moiety to address this issue. While the substitution of the ester for an amide (**35u**) led to dramatic drop of efficacy, the substitution of the ester for a ketone (**35v**) resulted in a single-digit picomolar EC_{50} and nearly 300-fold increase of SI compared to TAF.

Selected prodrugs from the last series again proved to be significantly more stable in plasma compared to TAF. The critical microsomal stability was increased, however, remained considerably lower compared to TAF.

The reason for the outstanding antiviral potency of studied prodrugs was investigated. The tyrosine parts of the prodrugs, which are released during the metabolism exhibited no activity against HIV-1 and therefore could not be the reason for the high potency. Faster metabolization by cathepsin A was also excluded as a reason since the novel prodrugs proved to be comparable substrates for cathepsin A as TAF. The reason for the exceptional potency appears to be very high cellular uptake of these prodrugs and their rapid metabolization resulting in high levels of tenofovir in the target cells. The metabolic studies further revealed substantial differences in metabolism of FEE and SEE.

The presented prodrugs were also studied against HBV in hepatocytes. Thirteen prodrugs demonstrated higher potency compared to TAF while showing no cytotoxicity. The most potent prodrug in the study against HBV proved to be **35k** exhibiting over 25-fold higher SI compared to TAF.

List of author´s publications related to this thesis

(1) **Kalčic, F.**; Dračínský, M.; Janeba, Z. Diverse Synthetic Approaches towards C1′-Branched Acyclic Nucleoside Phosphonates. *Org. Biomol. Chem.* **2021**.

(2) **Kalčic, F.**; Frydrych, J.; Doleželová, E.; Slapničková, M.; Poštová Slavětínská, L.; Pachl, P.; Dračínský, M.; Hocková, D.; Zíková, A.; Janeba, Z. C1'-Branched Acyclic Nucleoside Phosphonates Mimicking Adenosine Monophosphate: Inhibitors of *Trypanosoma brucei* Adenine Phosphoribosyltransferase, **2021**, *(close to submission)*

(3) **Kalčic, F.**; Zgarbová, M.; Hodek, J.; Chalupský K.; Dračínský, M.; Dvořáková A.; Strmeň, T.; Baszczyňski, O.; Weber, J.; Mertlíková-Kaiserová, H.; Janeba, Z. Development of Modified ProTide Prodrugs of Tenofovir with Enhanced Properties. **2021**, *(close to submission)*

Experimental part

General information

All solvents and reagents were purchased from commercial suppliers and were not further purified. Reactions were monitored using thin-layer chromatography (TLC) on silica gel 60 F254 plates (Merck KGaA, Germany) and/or ultra-high performance liquid chromatography with mass spectrometer (UPLC-MS Acquity Waters, USA, H-Class Core System with Waters Acquity UPLC BEH C18 1.7 μm, 2.1 × 100 mm column, Waters Acquity UPLC PDA detector, and mass spectrometer Waters SQD2, linear gradient elution with 0–100 % MeCN in water with 0.1% HCOOH). The column and flash chromatography (ISCO Teledyne, USA) were performed on 60A silica gel (Acros Organics, Belgium). Solvents were evaporated using rotary evaporator at 40 – 70 °C/2 mbar.

Microwave (MW) heating was performed using microwave reactor Discover (CEM, USA) with the Explorer module. The reactor frequency was 2.45 GHz and radiation power up to 300 W. Reactions were stirred in the reactor. Temperature and pressure were monitored by an infrared temperature sensor (outside the reaction mixture) and CEM Explorer pressure sensor, respectively.

NMR spectra were measured using Bruker Avance III HD 400 MHz equipped with Prodigy cryoprobe operating at 9.39 T. ^{1}H, ^{13}C, ^{19}F, and ^{31}P spectra were acquired at 400, 100, 377, and 162 MHz, respectively. Two-dimensional spectra ^{1}H^{1}H COSY, ^{1}H^{13}C HSQC, ^{1}H^{13}C HMBC were acquired for assignment purposes. Chemical shifts (δ) are listed in ppm, coupling constants (J) in Hz. NMR multiplicities are abbreviated as follows – singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of doublets of doublets (ddd), doublet of doublets of doublets of doublets (dddd), doublet of triplets (dt), doublet of quartets (dq), doublet of pentets (dp), triplet of doublets (td), and multiplet (m). All spectra were referenced to residual signal of DMSO (2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C), $CDCl_3$ (7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C), or 1,4-dioxane (3.75 ppm for ^{1}H and 67.19 ppm for ^{13}C, for samples measured in D_2O).

High-resolution mass spectra were obtained using LTQ Orbitrap XL (Thermo Fisher Scientific, USA) for ESI ionization.

Optical purity was determined using Waters UPC2 UHPSFC/MS system with PDA detector (YMC Alcyon columns, 150 x 3 mm, 3 um, linear gradient elution 2–40% *i*PrOH in CO_2). The optical rotation was determined using Autopol IV polarimeter (Rudolph Research Analytical, USA). The value of optical rotation for compounds (*R*)-**24b**, (*R*)-**25b**, and (*R*)-**27**–(*R*)-**32** had to be extrapolated to 66 – 70% *ee*, as it was retrospectively revealed that the starting compound from the commercial supplier, (*R*)-2-aminobutan-1-ol, contained 15 – 17% of (*S*)-enantiomer and the free phosphonates did not separate on the chiral column in reversed phase. On the contrary, the precursors of these compounds (*i.e.* compounds (*R*)-**8a**, (*R*)-**9**, (*R*)-**10a**, and (*R*)-**11**) did separate well on the chiral column

in normal phase and were therefore separated to determine *ee* and to obtain accurate optical rotation data. The CD spectra were measured using Jasco 1500 spectropolarimeter. The CD spectra were measured in spectral range 190–300 nm in the quartz cell with 0.2 mm path length, a scanning speed of 10 nm/min, a response time of 8 seconds, a scanning step 0.5 nm with the sample of the concentration 4.4*10-^{4}M in MeOH. After baseline correction, the final spectra were expressed as differencial extinction (*De*) (l.cm^{-1}.mol^{-1}).

Purity of target compounds was ≥ 95%. Purity was determined by the combination of UPLC−PDAMS, NMR, and HRMS. Yields were determined based on the amount of isolated compound.

Standard procedures

Standard procedure A

Freshly prepared compound **7a** or commercially available **7b** (1.2 mmol) were mixed with suitable amino alcohol (1.0 mmol) and DIPEA (2.0 mmol or 3.0 mmol when starting from a hydrochloride) in the appropriate solvent. The mixture was heated in a MW reactor for 1 h and then separated using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure B

The starting purine (1.0 mmol) was dissolved in anhydrous THF and the solution was cooled to −78 °C. *n*-BuLi (1.1 mmol) was added dropwise, the mixture was stirred at −78 °C for 10 min, and finally diisopropyl triflyloxymethanephosphonate (1.5 mmol) was added. The mixture was stirred at −78 °C and after the consumption of the starting purine, the reaction was quenched with sat. NH_4Cl solution, concentrated, and extracted with EtOAc (3 × 50 mL). Organic fractions were combined, washed with brine (1 × 50 mL), dried over $MgSO_4$, and concentrated. The residue was purified using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure C

6-Chloropurine or 2-amino-6-chloropurine (1.0 mmol) was mixed with Ac_2O (1.0 mmol) and compound **15a** or **15b** (1.0 mmol) in anhydrous MeCN (5 mL). TMSOTf (1.5 mmol) was added and the mixture was stirred at 25 °C for 15 min. The reaction was quenched with H_2O and extracted with DCM (3 × 50 mL). Organic fractions were combined, washed with brine (1 × 50 mL), dried over $MgSO_4$, and concentrated. The residue was purified using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure D

To a solution of compound **16** (1.0 mmol) in anhydrous THF (2 mL) was dropwise added at −78 °C Grignard reagent (2.0 mmol). The mixture was stirred at −78 °C for 30 min, allowed to warm up to 25 °C over the period of 20 min and then quenched with sat. NH_4Cl solution. The mixture was concentrated, extracted with DCM (3 × 50 mL). Organic fractions were combined, washed with brine (1 × 50 mL), dried over $MgSO_4$, and concentrated to yield the desired compound.

Standard procedure E

A mixture of 6-chloropurine or 2-amino-6-chloropurine (1.0 mmol), PPh_3 (1.05 mmol), and corresponding alcohol **17** (1.05 mmol) in THF (7 mL) was cooled to 0 °C. DIAD (1.05 mmol) was added and the mixture was allowed to warm up to 25 °C and stirred for 15 min. The mixture was concentrated and purified using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure F

Starting compound was stirred in a TFA:H_2O (3:1 ratio) mixture at 25 °C for 24 h, concentrated, and co-distilled twice with H_2O. The residue was purified using silica gel flash chromatography (linear gradient elution 0–20% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure G

To a solution of starting phosphonate diester (0.5 mmol) in 5 mL of anhydrous MeCN (or pyridine in case of acid labile compounds) was added TMSBr (500 μL). The mixture was stirred at 25 °C until full conversion (16–60 h), concentrated, and co-distilled 2 × with toluene to ensure complete removal of residual TMSBr. The residue was added to a MeOH:H_2O (1:1 ratio) mixture and stirred at 25 °C for 15 min. Solvents were evaporated and the residue was dissolved in 2M TEAB (5 mL). After evaporation, the residue was separated using C_{18}-reversed phase flash chromatography (linear gradient elution 0–50% MeOH in water). Purified product was taken through DOWEX 50 (Na^+ cycle) to secure unified sodium salt.

Standard procedure H

Starting compound (1.0 eq.), DABCO (0.8 eq.), and K_2CO_3 (1.0 eq.) were stirred in a 1,4-dioxane:H_2O (5:1 ratio) mixture at 90 °C for 2 h, concentrated, and co-distilled with EtOH (2 ×). The residue was purified using silica gel flash chromatography (linear gradient elution 0–20% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure I

A 1,4-dioxane:water (4:1) mixture was bubbled with argon for 2 min. Starting compound (1.0 eq.), corresponding boronic acid (1.5 eq.), Cs_2CO_3 (2.5 eq.) and $Pd(Ph_3P)_4$ (5 mol%)

were added and the reaction mixture was stirred at 110 °C for 30 min. Solvents were evaporated, the residue was co-distilled with EtOH (2 ×) and purified using silica gel flash chromatography (linear gradient elution 0–50% (EtOAc:MeOH 9:1) in hexane) to afford the desired product.

Standard procedure J

Starting compound and cyclopropyl amine (10 eq.) were stirred in anhydrous MeCN at 70 °C for 24 h. The mixture was concentrated and the residue purified using silica gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford the desired product.

Standard procedure K

Starting compound **10** or **18** was dissolved in a mixture of ethanolic and aqueous ammonia (1:1 ratio), and stirred in a MW reactor at 120°C for 30 min. The mixture was concentrated and separated using silica gel chromatography (linear gradient elution 0–20 % MeOH in $CHCl_3$) to obtain the desired product.

Standard procedure L

A starting compound (1.00 mmol), an alcohol (1.05 mmol), and PPh_3 (1.05 mmol) were mixed in THF (5 mL) under argon atmosphere. The mixture was cooled to 0 °C and DIAD (1.05 mmol) was added dropwise. The mixture was allowed to warm up to 25 °C and stirred for 30 min. The mixture was concentrated and separated silica using gel flash chromatography (linear gradient elution 0–50% EtOAc/MeOH (9:1 ratio) in hexane) to afford the desired compound.

Standard procedure M

An acid (1.00 mmol), an amine (1.20 mmol), and HATU (1.50 mmol) were dissolved in DMF (5 mL) under argon atmosphere. Et_3N or DIPEA (5.00 mmol) was added, the mixture was stirred at 25 °C for 30 min, concentrated, diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ (3 × 25 mL) and brine (25 mL). The organic fraction was dried over $MgSO_4$, filtered, concentrated and purified using silica gel flash chromatography (linear gradient elution 0–50% EtOAc/MeOH (9:1 ratio) in hexane) to afford the desired compound.

Standard procedure N

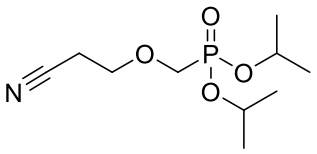
Compound **34** (1.00 mmol) and an L-tyrosine derivative (1.50–2.00 mmol) were mixed in pyridine (5 mL) under argon atmosphere. Base (8.00–10.00 mmol) was added and the mixture was stirred at 65 °C for 10 min. A solution of PPh_3 (6.00–8.00 mmol) and Aldrithiol™ (6.00–8.00 mmol) in pyridine (5 mL) was added and the mixture was stirred at 65 °C for 16 h. After evaporating the volatiles, the residue was co-distilled with toluene twice and purified using silica gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford the desired compound.

Standard procedure O

Tenofovir (1.00 mmol), L-alanine isopropyl ester hydrochloride (1.00 mmol), and an L-tyrosine derivative (1.80 mmol) were mixed in pyridine (6 mL) under argon atmosphere. DIPEA (10.00 mmol) was added and the mixture was stirred at 65 °C for 10 min. A solution of PPh_3 (4.00 mmol) and Aldrithiol^{TM} (4.00 mmol) in pyridine (6 mL) was added and the mixture was stirred at 65 °C for 16 h. After evaporating the volatiles, the residue was co-distilled with toluene twice and purified using silica gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford the desired compound.

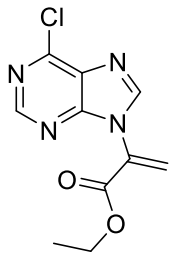
Spectral data and characterization of prepared compounds

Diisopropyl ((2-cyanoethoxy)methyl)phosphonate (**1**)



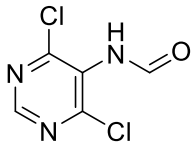
(Hydroxymethyl)phosphonate (25 mg, 0.13 mmol) and acrylonitrile (85 µL, 1.30 mmol) were added to a suspension Cs_2CO_3 (10 mg, 0.03 mmol) in 1,4-dioxane (2 mL). The mixture was stirred at 25 °C for 18h, concentrated, the residue re-suspended in EtOAc (10 mL) and washed with H_2O (3 × 10 mL) to obtain compound **1** (29 mg, 91%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 4.68–4.55 (m, 2H), 3.80 (d, J = 8.4 Hz, 2H), 3.70 (t, J = 6.0 Hz, 2H), 2.78 (t, J = 5.9 Hz, 2H), 1.26 (d, J = 1.5 Hz, 6H), 1.25 (d, J = 1.5 Hz, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 119.02, 70.27 (d, J = 6.6 Hz), 67.11 (d, J = 11.7 Hz), 64.59 (d, J = 164.3 Hz), 23.85 (d, J = 3.7 Hz), 23.74 (d, J = 5.1 Hz), 17.85. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.57. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{10}H_{20}O_4NNaP$ 272.10222, found 272.10226.

Ethyl 2-(6-chloro-9*H*-purin-9-yl)acrylate (**4**)



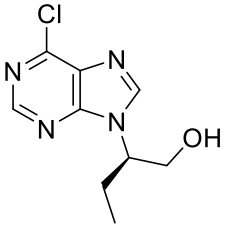
6-Chloropurine (2.63 g, 16.99 mmol), PPh_3 (892 mg, 3.40 mmol), NaOAc (279 mg, 3.40 mmol) were mixed with toluene (35 mL) and cooled to 15 °C. AcOH (194 µL, 3.40 mmol) and ethyl propiolate (2.58 mL, 25.49 mmol) were added, the mixture was stirred at 15 °C for 10 min, and washed with H_2O (3 × 50 mL). The organic fraction was dried over $MgSO_4$, concentrated and the residue was purified using silica gel flash chromatography (linear gradient elution 0–100% EtOAc in hexane) to afford the desired compound **4** (297 mg, 7%) as an orangish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.82 (s, 1H), 6.76 (d, J = 1.3 Hz, 1H), 6.52 (d, J = 1.3 Hz, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 162.04, 152.78, 152.48, 149.98, 147.61, 131.11, 127.46, 62.68, 14.34. **MS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{10}H_{10}O_2N_4Cl$ 253.05, found 253.07.

4,6-Dichloro-5-formamidopyrimidine (**7a**)



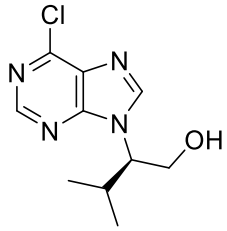
Compound **7a** was prepared from 4,6-dichloro-5-aminopyrimidine according to previously published procedure.[119] **^{1}H NMR** (401 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.86 (s, 1H), 8.36 (s, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.94, 159.24, 155.98, 128.07. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_5H_2ON_3Cl_2$ 189.95804, found 189.95797.

(*R*)-2-(6-Chloro-9*H*-purin-9-yl)butan-1-ol ((*R*)-**8a**)



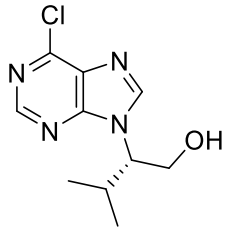
Following standard procedure A, compound **7a** (1.70 g, 8.85 mmol), (*R*)-2-aminobutan-1-ol (0.70 mL, 7.38 mmol), and DIPEA (2.57 mL, 14.76 mmol) were stirred in 1,4-dioxane (15 mL) at 160 °C for 1 h to obtain (*R*)-**8a** (0.93 g, 56%) as a orangish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.75 (s, 1H), 5.02 (t, J = 5.6 Hz, 1H), 4.55 (dddd, J = 9.7, 7.6, 5.6, 4.3 Hz, 1H), 3.90 (ddd, J = 11.5, 7.7, 5.6 Hz, 1H), 3.75 (ddd, J = 11.5, 5.6, 4.3 Hz, 1H), 2.09–1.87 (m, 2H), 0.75 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.30, 151.22, 148.89, 146.92, 131.05, 62.04, 60.27, 22.99, 10.37. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_9H_{12}ON_4Cl$ 227.06942, found 227.06942. **$[\alpha]^{25}_D$** = +24.1 (c 0.299 g/100 mL, $CHCl_3$/MeOH 1/1).

(*R*)-2-(6-Chloro-9*H*-purin-9-yl)-3-methylbutan-1-ol ((*R*)-**8b**)



Following standard procedure A, compound **7a** (1.16 g, 6.04 mmol), (*R*)-valinol (0.52 g, 5.03 mmol), and DIPEA (1.75 mL, 10.06 mmol) were stirred in 1,4-dioxane (12 mL) at 160 °C for 1 h to obtain (*R*)-**8b** (0.76 g, 63%) as a yellowish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) and **^{13}C NMR** (101 MHz, DMSO-d_6) spectra were identical to those of (*S*)-**8b**. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{10}H_{14}ON_4Cl$ 241.08507, found 241.08503. **$[\alpha]^{25}_D$** = +31.2 (c 0.173 g/100 mL, $CHCl_3$/MeOH 1/1).

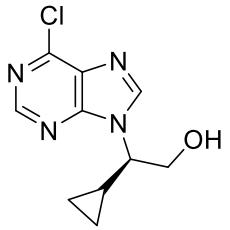
(*S*)-2-(6-Chloro-9*H*-purin-9-yl)-3-methylbutan-1-ol ((*S*)-**8b**)



Following standard procedure A, compound **7a** (1.16 g, 6.04 mmol), (*S*)-valinol (0.52 g, 5.03 mmol), and DIPEA (1.75 mL, 10.06 mmol) were stirred in 1,4-dioxane (12 mL) at 160 °C for 1 h to obtain (*S*)-**8b** (1.10 g, 91%) as a yellowish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.75 (s, 1H), 4.36 (ddd, J = 9.2, 8.1, 3.8 Hz, 1H), 4.02 (dd, J = 11.6, 8.2 Hz, 1H), 3.83 (dd, J = 11.6, 3.8 Hz, 1H), 2.39 (d of septets, J = 9.1, 6.7 Hz, 1H), 1.02 (d, J = 6.7 Hz, 3H), 0.66 (d, J = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.49, 151.26, 148.91, 147.10, 130.83, 64.26, 60.29, 28.74,

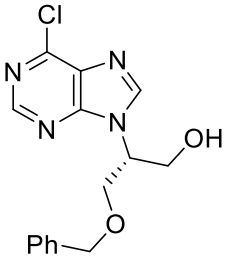
19.64, 19.22. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{10}H_{14}ON_4Cl$ 241.08507, found 241.08462. **[α]$^{25}_D$** = −26.5 (c 0.268 g/100 mL, $CHCl_3$/MeOH 1/1).

(*R*)-2-(6-Chloro-9*H*-purin-9-yl)-2-cyclopropylethan-1-ol ((*R*)-**8c**)



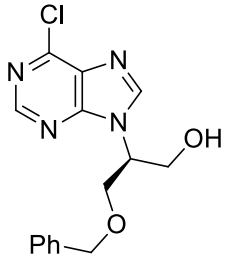
Following standard procedure A, compound **7a** (1.59 g, 8.28 mmol), (*R*)-2-amino-2-cyclopropylethan-1-ol hydrochloride (0.95 mg, 6.90 mmol), and DIPEA (3.61 mL, 20.70 mmol) were stirred in 1,4-dioxane (15 mL) at 160 °C for 1 h to obtain (*R*)-**8c** (1.08 g, 66%) as a yellowish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.76 (s, 1H), 4.09–4.04 (m, 1H), 3.93–3.83 (m, 2H), 1.64–1.55 (m, 1H), 0.69 (tdd, J = 8.6, 6.0, 4.4 Hz, 1H), 0.55–0.48 (m, 1H), 0.47–0.41 (m, 1H), 0.25 (ddt, J = 9.3, 6.0, 4.5 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.06, 151.20, 148.89, 146.94, 131.06, 63.81, 61.96, 11.91, 4.70, 3.11. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{10}H_{12}ON_4Cl$ 239.06942, found 239.06944. **[α]$^{25}_D$** = +36.2 (c 0.398 g/100 mL, $CHCl_3$/MeOH 1/1).

(*R*)-3-(Benzyloxy)-2-(6-chloro-9*H*-purin-9-yl)propan-1-ol ((*R*)-**8d**)



Compound (*S*)-**21a** (0.50 g, 0.89 mmol) was stirred at 0 °C in DCM/TFA (5 mL, 4:1 ratio) for 30 min. The mixture was diluted with 50 mL of DCM and TFA was washed away with H_2O (3 × 50 mL). DCM was washed with brine and dried over $MgSO_4$. The residue was purified using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford (*R*)-**8d** (269 mg, 95%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) and **^{13}C NMR** (101 MHz, DMSO-d_6) spectra were identical to those of (*S*)-**8d**. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{15}H_{15}O_2N_4ClNa$ 341.07757, found 341.07735. **[α]$^{25}_D$** = +3.5 (c 0.255 g/100 mL, $CHCl_3$/MeOH 1/1).

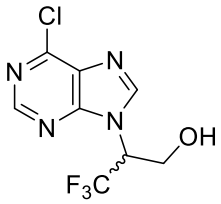
(*S*)-3-(Benzyloxy)-2-(6-chloro-9*H*-purin-9-yl)propan-1-ol ((*S*)-**8d**)



(*R*)-2-Amino-3-benzyloxypropionic acid (1.50 g, 7.68 mmol) was mixed with $NaBH_4$ (0.72 g, 19.20 mmol) in anhydrous THF (10 mL). The mixture was cooled to 0 °C and I_2 (1.95 g, 7.68 mmol) was added. The mixture was stirred at 0 °C for 10 min and then at 55 °C for 19 h. The reaction was quenched with H_2O, concentrated and extracted with EtOAc (3 × 50 mL). Organic fractions were combined, washed with brine (1 × 50 mL), dried over $MgSO_4$, and concentrated to afford (*R*)-2-amino-3-(benzyloxy)propan-1-ol (1.22 g, 88%) as a colorless oil which was directly used in the following reaction. Following standard procedure A, compound **7a**

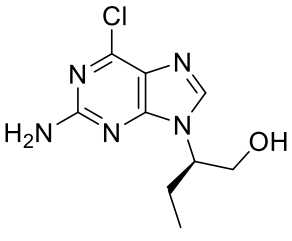
(1.46 g, 7.62 mmol), (*R*)-2-amino-3-(benzyloxy)propan-1-ol (1.15 mg, 6.35 mmol), and DIPEA (2.21 mL, 12.70 mmol) were stirred in 1,4-dioxane (15 mL) at 160 °C for 1 h to obtain (*S*)-**8d** (1.08 g, 53%) as a yellowish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.74 (s, 1H), 7.28–7.21 (m, 3H), 7.15–7.09 (m, 2H), 5.13 (t, J = 5.5 Hz, 1H), 4.91 (ddt, J = 8.1, 7.1, 4.7 Hz, 1H), 4.52–4.39 (m, 2H), 4.04 (dd, J = 10.4, 8.2 Hz, 1H), 3.96 (ddd, J = 11.5, 7.1, 5.4 Hz, 1H), 3.91–3.82 (m, 2H). **^{13}C NMR** 101 MHz, DMSO-d_6) δ 152.23, 151.25, 148.90, 146.97, 137.79, 130.92, 128.16, 127.49, 127.35, 71.98, 67.57, 59.62, 57.89. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{16}O_2N_4Cl$ 319.09563, found 319.09567. **$[\alpha]^{25}_D$** = +26.9 (c 0.104 g/100 mL, $CHCl_3$/MeOH 1/1).

2-(6-Chloro-9*H*-purin-9-yl)-3,3,3-trifluoropropan-1-ol ((*RS*)-**8e**)



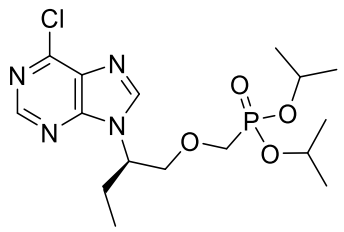
To a solution of 6-chloropurine (0.58 g, 3.75 mmol) in anhydrous DMF (10 mL) was added K_2CO_3 (0.78 g, 5.63 mmol). The mixture was stirred at 25 °C for 10 min and 2-bromo-3,3,3-trifluoropropan-1-ol (0.39 mL, 3.75 mmol) was added. The mixture was stirred at 60 °C for 24 h, filtered through Celite, and concentrated. The residue was dissolved in EtOAc (50 mL), washed with H_2O (3 × 50 mL), with brine (1 × 50 mL), dried over $MgSO_4$, and concentrated. The residue was purified using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford (*RS*)-**8e** (165 mg, 16%) as colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.71 (s, 1H), 6.84–6.76 (m, 1H), 4.64–4.54 (m, 2H), 4.52–4.42 (m, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.11, 151.69, 149.04, 148.07, 131.11, 124.67 (q, J = 283.2 Hz), 66.71 (q, J = 30.0 Hz), 43.64. **^{19}F NMR** (377 MHz, DMSO-d_6) δ −76.97 (d, J = 7.1 Hz). **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_8H_5ON_4ClF_3$ 265.01095, found 265.01097.

(*R*)-2-(2-Amino-6-chloro-9*H*-purin-9-yl)butan-1-ol ((*R*)-**9**)



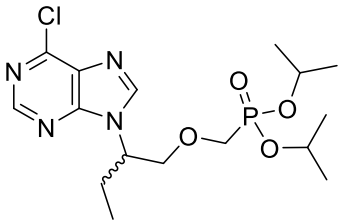
Following standard procedure A, compound **7b** (commercially available, 5.00 g, 24.15 mmol), (*R*)-2-aminobutan-1-ol (1.90 mg, 20.13 mmol), and DIPEA (7.01 mL, 40.26 mmol) were stirred in H_2O:EtOH (1:1 ratio) (50 mL) at 120 °C for 1 h to obtain (*R*)-**9** (2.91 g, 60%) as a yellowish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.16 (s, 1H), 6.84 (s, 2H), 5.01 (t, J = 5.4 Hz, 1H), 4.34–4.22 (m, 1H), 3.81 (ddd, J = 11.2, 7.2, 5.2 Hz, 1H), 3.67 (dt, J = 11.2, 4.8 Hz, 1H), 1.97–1.82 (m, 2H), 0.75 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.54, 154.46, 149.21, 142.55, 123.57, 61.99, 58.95, 23.01, 10.43. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_9H_{13}ON_5Cl$ 242.08031, found 242.08034. **$[\alpha]^{25}_D$** = −5.6 (c 0.266 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl (*R*)-((2-(6-chloro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**10a**)



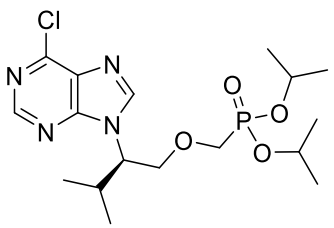
Following standard procedure B, compound (*R*)-**8a** (0.90 g, 3.97 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 1.75 mL, 4.37 mmol) and diisopropyl triflyloxymethanephosphonate (1.95 g, 5.96 mmol) in anhydrous THF (10 mL) to afford (*R*)-**10a** (1.17 g, 73%) as a light orange viscose oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.77 (s, 2H), 4.82–4.75 (m, 1H), 4.45–4.30 (m, 2H), 4.11 (dd, J = 10.4, 8.6 Hz, 1H), 3.89 (dd, J = 10.4, 4.0 Hz, 1H), 3.77 (dd, J = 13.9, 8.3 Hz, 1H), 3.68 (dd, J = 13.9, 8.3 Hz, 1H), 2.11–1.87 (m, 2H), 1.13–1.07 (m, 6H), 1.05–0.98 (m, 6H), 0.77 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.14, 151.36, 149.05, 146.63, 130.97, 72.66 (d, J = 11.4 Hz), 70.15–69.95 (m), 64.65 (d, J = 164.0 Hz), 57.13, 23.62 (d, J = 3.7 Hz), 23.50–23.38 (m), 23.11, 10.20. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.22. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{16}H_{27}O_4N_4ClP$ 405.14530, found 405.14554. **$[\alpha]^{25}_D$** = +18.3 (c 0.142 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl ((2-(6-chloro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*RS*)-**10a**)



Following standard procedure E, compound (*RS*)-**17b** (390 mg, 1.45 mmol) reacted with 6-chloropurine (213 mg, 1.38 mmol), PPh_3 (380 mg, 1.45 mmol), and DIAD (284 µL, 1.45 mmol) in anhydrous THF (10 mL) to afford (*RS*)-**10a** (342 mg, 61%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.77 (s, 2H), 4.82–4.75 (m, 1H), 4.44–4.31 (m, 2H), 4.12 (dd, J = 10.4, 8.6 Hz, 1H), 3.89 (dd, J = 10.3, 4.0 Hz, 1H), 3.77 (dd, J = 14.0, 8.3 Hz, 1H), 3.68 (dd, J = 13.9, 8.2 Hz, 1H), 2.11–1.87 (m, 2H), 1.13–1.08 (m, 6H), 1.07–0.98 (m, 6H), 0.76 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.14, 151.35, 149.05, 146.62, 130.97, 72.66 (d, J = 11.3 Hz), 70.17–69.93 (m), 64.65 (d, J = 163.7 Hz), 57.13, 23.62 (d, J = 3.9 Hz), 23.51–23.34 (m), 23.11, 10.19. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.22. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{25}O_4N_4ClNaP$ 427.12724, found 427.12702.

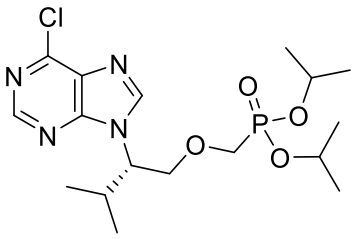
Diisopropyl (*R*)-((2-(6-chloro-9*H*-purin-9-yl)-3-methylbutoxy)methyl)phosphonate ((*R*)-**10b**)



Following standard procedure B, compound (*R*)-**8b** (0.73 g, 3.03 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 1.33 mL, 3.33 mmol) and diisopropyl triflyloxymethanephosphonate (1.49 g, 4.55 mmol) in anhydrous THF (10 mL) to afford (*R*)-**10b** (1.02 g, 80%) as a light orange viscose oil. **^{1}H NMR** (401

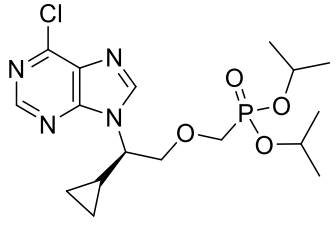
MHz, DMSO-d_6) and **^{13}C NMR** (101 MHz, DMSO-d_6) were identical to those of (*R*)-**10b**. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.23. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{17}H_{28}O_4N_4ClNaP$ 441.14289, found 441.14345. **$[\alpha]^{25}_D$** = +13.1 (c 0.245 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl (*S*)-((2-(6-chloro-9*H*-purin-9-yl)-3-methylbutoxy)methyl)phosphonate ((*S*)-**10b**)



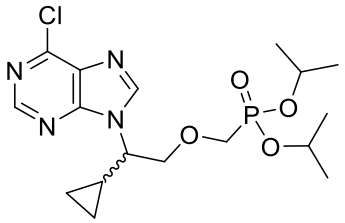
Following standard procedure B, compound (*S*)-**8b** (1.00 g, 4.15 mmol) reacted with *n*-BuLi (1.6 M in hexanes, 2.86 mL, 4.57 mmol) and diisopropyl triflyloxymethanephosphonate (2.04 g, 6.23 mmol) in anhydrous THF (12 mL) to afford (*S*)-**10b** (1.59 g, 91%) as a light-yellow viscose oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.76 (s, 1H), 4.64–4.55 (m, 1H), 4.42–4.32 (m, 2H), 4.23 (dd, *J* = 10.4, 8.8 Hz, 1H), 3.95 (dd, *J* = 10.5, 3.7 Hz, 1H), 3.80–3.63 (m, 2H), 2.44–2.32 (m, 1H), 1.13–1.06 (m, 6H), 1.06–0.99 (m, 9H), 0.68 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.32, 151.40, 149.08, 146.73, 130.75, 71.19 (d, *J* = 11.8 Hz), 70.18–69.85 (m), 64.65 (d, *J* = 164.1 Hz), 61.12, 29.17, 23.67–23.53 (m), 23.51–23.35 (m), 19.42, 19.15. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.23. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{17}H_{28}O_4N_4ClNaP$ 441.14289, found 441.14325. **$[\alpha]^{25}_D$** = −11.0 (c 0.371 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl (*R*)-((2-(6-chloro-9*H*-purin-9-yl)-2-cyclopropylethoxy)methyl)phosphonate ((*R*)-**10c**)



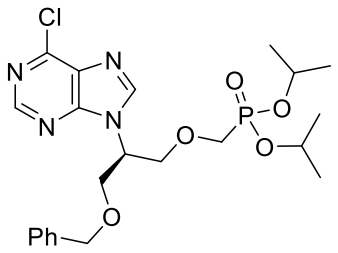
Following standard procedure B, compound (*R*)-**8c** (0.95 g, 3.98 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 1.75 mL, 4.38 mmol) and diisopropyl triflyloxymethanephosphonate (1.96 g, 5.97 mmol) in anhydrous THF (10 mL) to afford (*R*)-**10c** (1.15 g, 69%) as a light orange viscose oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.77 (s, 1H), 4.43–4.32 (m, 2H), 4.32–4.24 (m, 1H), 4.13 (ddd, *J* = 10.1, 8.6, 3.7 Hz, 1H), 3.99 (dd, *J* = 10.2, 3.7 Hz, 1H), 3.77 (dd, *J* = 13.9, 8.3 Hz, 1H), 3.69 (dd, *J* = 13.9, 8.2 Hz, 1H), 1.63–1.54 (m, 1H), 1.13–1.06 (m, 6H), 1.06–0.97 (m, 6H), 0.75–0.63 (m, 1H), 0.63–0.55 (m, 1H), 0.48–0.41 (m, 1H), 0.32–0.26 (m, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 151.88, 151.36, 149.07, 146.60, 130.94, 72.48 (d, *J* = 11.6 Hz), 70.13–69.96 (m), 64.66 (d, *J* = 164.1 Hz), 60.51, 23.61 (d, *J* = 3.7 Hz), 23.48–23.33 (m), 11.97, 4.48, 3.26. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.22. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{17}H_{27}O_4N_4ClP$ 417.14530, found 417.14535. **$[\alpha]^{25}_D$** = +12.7 (c 0.456 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl ((2-(6-chloro-9*H*-purin-9-yl)-2-cyclopropylethoxy)methyl)phosphonate ((*RS*)-**10c**)



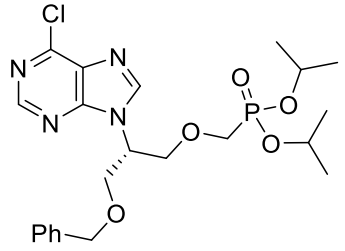
Following standard procedure E, compound (*RS*)-**17e** (1.20 g, 4.28 mmol) reacted with 6-chloropurine (0.63 g, 4.08 mmol), PPh_3 (1.12 g, 4.28 mmol), and DIAD (0.84 mL, 4.28 mmol) in anhydrous THF (20 mL) to afford (*RS*)-**10c** (0.51 g, 30%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6), **^{13}C NMR** (101 MHz, DMSO-d_6) and **^{31}P NMR** (162 MHz, DMSO-d_6) were identical with those of (*R*)-**10c**. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{17}H_{27}O_4N_4ClP$ 417.14530, found 417.14500.

Diisopropyl (*R*)-((3-(benzyloxy)-2-(6-chloro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*R*)-**10d**)



Following standard procedure B, compound (*S*)-**8d** (0.95 g, 2.98 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 1.31 mL, 3.28 mmol) and diisopropyl triflyloxymethanephosphonate (1.47 g, 4.47 mmol) in anhydrous THF (10 mL) to afford (*R*)-**10d** (0.53 g, 36%) as a light yellow-orange viscose oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.75 (s, 1H), 7.29–7.22 (m, 3H), 7.15–7.10 (m, 2H), 5.13 (tt, J = 8.5, 4.4 Hz, 1H), 4.52–4.33 (m, 4H), 4.20 (dd, J = 10.4, 8.3 Hz, 1H), 4.05–3.95 (m, 2H), 3.86 (dd, J = 10.4, 4.6 Hz, 1H), 3.79 (dd, J = 14.0, 8.3 Hz, 1H), 3.72 (dd, J = 14.0, 8.2, 1H), 1.13–1.09 (m, 6H), 1.05–1.01 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.11, 151.40, 149.05, 146.73, 137.67, 130.84, 128.19, 127.57, 127.40, 72.05, 70.24–70.05 (m), 69.86 (d, J = 7.2 Hz), 67.52, 64.75 (d, J = 163.3 Hz), 55.08, 23.66 (d, J = 3.6 Hz), 23.56–23.39 (m). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.26. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{22}H_{31}O_5N_4ClP$ 497.17151, found 497.17142. **$[\alpha]^{25}_D$** = −5.1 (c 0.295 g/100 mL, $CHCl_3$/MeOH 1/1).

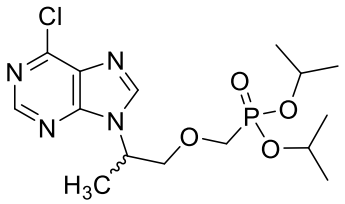
Diisopropyl (*S*)-((3-(benzyloxy)-2-(6-chloro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*S*)-**10d**)



Following standard procedure B, compound (*R*)-**8d** (95 mg, 0.30 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 131 µL, 0.33 mmol) and diisopropyl triflyloxymethanephosphonate (148 mg, 0.45 mmol) in anhydrous THF (2 mL) to afford (*S*)-**10d** (119 mg, 80%) as a as a colorless viscose oil. **^{1}H NMR** (400 MHz, DMSO-d_6), **^{13}C NMR** (101 MHz, DMSO-d_6) and **^{31}P NMR** (162 MHz, DMSO-d_6) spectra were identical to those of (*R*)-**10d**.

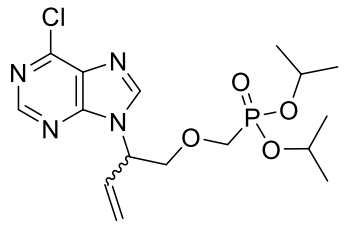
HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{30}O_5N_4ClNaP$ 519.15346, found 519.15254. **$[\alpha]^{25}_D$** = +3.0 (c 0.919 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl ((2-(6-chloro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*RS*)-**10e**)



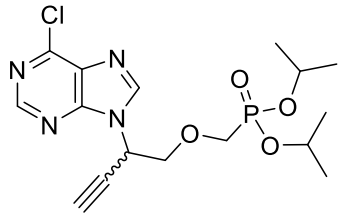
Following standard procedure E, compound (*RS*)-**17a** (600 mg, 2.36 mmol) reacted with 6-chloropurine (348 mg, 2.25 mmol), PPh_3 (619 mg, 2.36 mmol), and DIAD (463 µL, 2.36 mmol) in anhydrous THF (12 mL) to afford (*RS*)-**10e** (463 mg, 53%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.78 (s, 1H), 5.03–4.95 (m, 1H), 4.46–4.30 (m, 2H), 4.06 (dd, J = 10.3, 8.4 Hz, 1H), 3.87 (dd, J = 10.3, 4.1 Hz, 1H), 3.78 (dd, J = 13.9, 8.3 Hz, 1H), 3.69 (dd, J = 13.9, 8.3 Hz, 1H), 1.56 (d, J = 7.1 Hz, 3H), 1.13–1.08 (m, 6H), 1.06–1.00 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 151.82, 151.27, 148.98, 146.37, 131.03, 73.75 (d, J = 11.3 Hz), 70.30–69.81 (m), 64.66 (d, J = 164.1 Hz), 51.08, 23.63 (d, J = 3.6 Hz), 23.51–23.36 (m), 16.25. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.23. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{15}H_{24}O_4N_4ClNaP$ 413.11159, found 413.11210.

Diisopropyl (((2-(6-chloro-9*H*-purin-9-yl)but-3-en-1-yl)oxy)methyl)phosphonate ((*RS*)-**10f**)



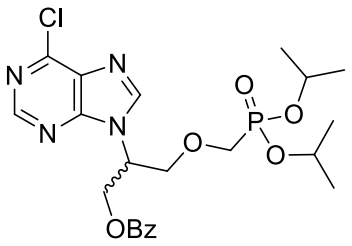
Following standard procedure E, compound (*RS*)-**17c** (600 mg, 2.25 mmol) reacted with 6-chloropurine (331 mg, 2.14 mmol), PPh_3 (590 mg, 2.25 mmol), and DIAD (442 µL, 2.25 mmol) in anhydrous THF (12 mL) to afford (*RS*)-**10f** (688 mg, 80%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.79 (s, 1H) 6.22 (ddd, J = 17.0, 10.6, 6.2 Hz, 1H), 5.57–5.51 (m, 1H), 5.33 (dt, J = 10.6, 1.1 Hz, 1H), 5.21 (ddd, J = 17.2, 1.5, 0.9 Hz, 1H), 4.46–4.34 (m, 2H), 4.25 (dd, J = 10.5, 8.9 Hz, 1H), 3.99 (dd, J = 10.5, 4.4 Hz, 1H), 3.82 (dd, J = 13.9, 8.4 Hz), 3.74 (d, J = 13.9, 8.3 Hz), 1.12 (d, J = 6.2 Hz, 3H), 1.11 (d, J = 6.2 Hz), 1.04 (d, J = 6.2 Hz, 3H), 1.03 (d, J = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 151.80, 151.51, 149.17, 146.50, 132.59, 130.87, 119.34, 71.97 (d, J = 12.1 Hz), 70.22–70.01 (m), 64.64 (d, J = 164.2 Hz), 56.96, 23.63 (d, J = 3.8 Hz), 23.51–23.36 (m). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.17. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{24}O_4N_4ClNaP$ 425.11159, found 425.11218.

Diisopropyl (((2-(6-chloro-9*H*-purin-9-yl)but-3-yn-1-yl)oxy)methyl)phosphonate ((*RS*)-**10g**)



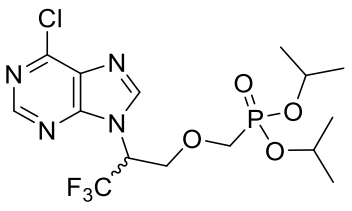
Following standard procedure E, compound (*RS*)-**17d** (600 mg, 2.27 mmol) reacted with 6-chloropurine (334 mg, 2.16 mmol), PPh_3 (595 mg, 2.27 mmol), and DIAD (446 µL, 2.27 mmol) in anhydrous THF (12 mL) to afford (*RS*)-**10g** (791 mg, 91%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.80 (s, 1H), 5.92 (ddd, J = 8.2, 4.3, 2.5 Hz, 1H), 4.46–4.35 (m, 2H), 4.27 (dd, J = 10.5, 8.1 Hz, 1H), 4.05 (dd, J = 10.5, 4.4 Hz, 1H), 3.89–3.72 (m, 3H), 1.14–1.08 (m, 6H), 1.07–1.01 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 151.79, 151.25, 149.37, 146.17, 130.90, 78.22, 77.16, 72.03 (d, J = 11.7 Hz), 70.17 (d, J = 6.5 Hz), 64.65 (d, J = 163.9 Hz), 46.16, 23.64 (d, J = 3.7 Hz), 23.53–23.38 (m). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 20.96. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{22}O_4N_4ClNaP$ 423.09594, found 423.09655.

2-(6-Chloro-9*H*-purin-9-yl)-3-((diisopropoxyphosphoryl)methoxy)propyl benzoate ((*RS*)-**10h**)



Following standard procedure E, compound (*RS*)-**17i** (547 mg, 1.58 mmol) reacted with 6-chloropurine (232 mg, 1.50 mmol), PPh_3 (414 mg, 1.58 mmol), and DIAD (308 µL, 1.58 mmol) in anhydrous THF (10 mL) to afford (*RS*)-**10g** (521 mg, 68%), as a yellowish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.79 (s, 1H), 7.84–7.78 (m, 2H), 7.66–7.44 (m, 3H), 5.40–5.31 (m, 1H), 4.86–4.71 (m, 2H), 4.50–4.40 (m, 2H), 4.37 (dd, J = 10.5, 8.2 Hz, 1H), 4.15 (dd, J = 10.5, 4.5 Hz, 1H), 3.87–3.77 (m, 2H), 1.15–1.04 (m, 12H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.14, 152.22, 151.55, 149.13, 146.61, 133.62, 130.84, 129.17, 128.88, 128.75, 70.28–70.08 (m), 69.84 (d, J = 11.8 Hz), 64.83 (d, J = 164.3 Hz), 62.90, 54.31, 23.74–23.41 (m). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.22. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{22}H_{29}O_6N_4ClP$ 511.15078, found 511.15003.

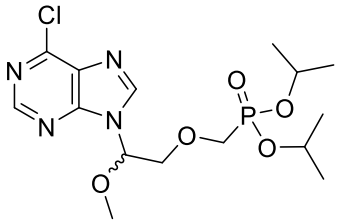
Diisopropyl ((2-(6-chloro-9*H*-purin-9-yl)-3,3,3-trifluoropropoxy)methyl)phosphonate ((*RS*)-**10i**)



Following standard procedure B, compound (*RS*)-**8e** (145 mg, 0.54 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 237 µL, 0.59 mmol) and diisopropyl triflyloxymethanephosphonate (266 mg, 0.81 mmol) in anhydrous THF (10 mL) to afford (*RS*)-**10i** (182 mg, 78%)

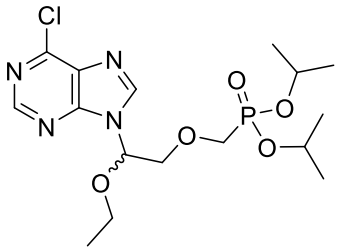
as a orangish solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.66 (s, 1H), 4.81–4.70 (m, 2H), 4.70–4.61 (m, 1H), 4.58–4.43 (m, 2H), 4.07–3.93 (m, 2H), 1.20 (d, J = 6.1 Hz, 3H), 1.19–1.15 (m, 6H), 1.10 (d, J = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.62, 152.23, 149.55, 148.29, 131.04, 124.48 (q, J = 285.0 Hz), 76.71–76.10 (m), 71.13–70.85 (m), 66.32 (d, J = 165.7 Hz), 41.70, 24.24–23.81 (m). **^{19}F NMR** (377 MHz, DMSO-d_6) δ −74.65 (d, J = 6.9 Hz). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 19.64. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{22}O_4N_4ClF_3P$ 445.10138, found 445.10126.

Diisopropyl ((2-(6-chloro-9*H*-purin-9-yl)-2-methoxyethoxy)methyl)phosphonate ((*RS*)-**10j**)



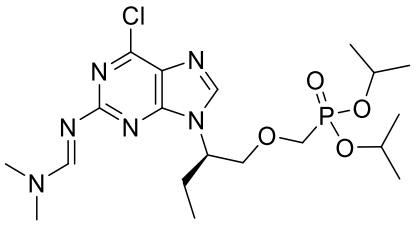
Following standard procedure C, compound **15b** (299 mg, 1.05 mmol) reacted with 6-chloropurine (162 mg, 1.05 mmol), Ac_2O (99 µL, 1.05 mmol), and TMSOTf (286 µL, 1.58 mmol) to afford (*RS*)-**10j** (351 mg, 82%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.82 (s, 1H), 5.95 (dd, J = 7.0, 4.8 Hz, 1H), 4.47–4.36 (m, 2H), 4.21 (dd, J = 10.6, 7.1 Hz, 1H), 4.08 (dd, J = 10.6, 4.8 Hz, 1H), 3.88–3.72 (m, 2H), 3.27 (s, 3H), 1.15–1.09 (m, 6H), 1.09–1.01 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.22, 151.89, 149.30, 146.01, 131.06, 84.14, 71.20 (d, J = 12.1 Hz), 70.17 (d, J = 6.5 Hz), 70.15 (d, J = 6.5 Hz), 65.08 (d, J = 164.3 Hz), 56.48, 23.64 (d, J = 3.7 Hz), 23.44 (d, J = 4.4 Hz). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.04. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{15}H_{24}O_5N_4ClNaP$ 429.10651, found 429.10693.

Diisopropyl ((2-(6-chloro-9*H*-purin-9-yl)-2-ethoxyethoxy)methyl)phosphonate ((*RS*)-**10k**)



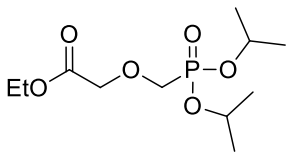
Following standard procedure C, compound **15a** (1.01 g, 3.23 mmol) reacted with 6-chloropurine (0.50 g, 3.23 mmol), Ac_2O (305 µL, 3.23 mmol), and TMSOTf (0.88 mL, 4.85 mmol) to afford (*RS*)-**10k** (1.18 g, 87%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.82 (s, 1H), 6.05 (dd, J = 7.0, 4.9 Hz, 1H), 4.50–4.34 (m, 2H), 4.19 (dd, J = 10.6, 7.1 Hz, 1H), 4.07 (dd, J = 10.6, 4.9 Hz, 1H), 3.88–3.73 (m, 2H), 3.58 (dq, J = 9.6, 7.0 Hz, 1H), 3.41 (dq, J = 9.6, 7.0 Hz, 1H), 1.12 (d, J = 6.2 Hz, 6H), 1.10–1.02 (m, 9H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 152.12, 151.87, 149.26, 145.93, 131.00, 82.48, 71.42 (d, J = 11.9 Hz), 70.15 (d, J = 6.7 Hz), 65.06 (d, J = 164.3 Hz), 64.56, 23.63 (d, J = 3.8 Hz), 23.44 (d, J = 4.6 Hz), 14.62. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.05. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{26}O_5N_4ClNaP$ 443.12216, found 443.12164.

Diisopropyl (*R*)-((2-(6-chloro-2-(((dimethylamino)methylene)amino)-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**11**)



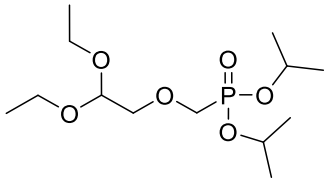
Compound (*R*)-**9** (2.80 g, 11.59 mmol) was stirred with DMF DMA (4.63 mL, 34.77 mmol) in DMF (15 mL) at 80 °C for 1 h. The mixture was concentrated and co-distilled 3 × with toluene to yield (*R*)-*N′*-(6-chloro-9-(1-hydroxybutan-2-yl)-9*H*-purin-2-yl)-*N*,*N*-dimethylformimidamide (3.32 g, 97%), which was used directly in the following reaction. Following standard procedure B, (*R*)-*N′*-(6-chloro-9-(1-hydroxybutan-2-yl)-9*H*-purin-2-yl)-*N*,*N*-dimethylformimidamide (0.95 g, 2.98 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 1.31 mL, 3.28 mmol) and diisopropyl triflyloxymethanephosphonate (1.47 g, 4.47 mmol) in anhydrous THF (10 mL) to afford (*R*)-**11** (3.91 g, 81%) as a light orange viscose oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.38 (s, 1H), 4.70–4.60 (m, 1H), 4.41 (d of septets, J = 7.7, 6.1 Hz, 2H), 4.08 (dd, J = 10.3, 8.3 Hz, 1H), 3.89–3.83 (m, 1H), 3.83–3.65 (m, 2H), 3.16 (s, 3H), 3.04 (s, 3H), 2.04–1.82 (m, 2H), 1.15–1.09 (m, 6H), 1.08–1.02 (m, 6H), 0.77 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 161.71, 158.46, 153.91, 148.75, 144.05, 126.28, 72.82 (d, J = 11.7 Hz), 70.23–69.99 (m), 64.69 (d, J = 163.7 Hz), 56.15, 40.48, 34.66, 23.66 (d, J = 4.1 Hz), 23.52–23.39 (m), 23.14, 10.25. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.27. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{19}H_{33}O_4N_6ClP$ 475.19839, found 475.19871. **$[\alpha]^{25}_D$** = −10.3 (c 0.068 g/100 mL, $CHCl_3$/MeOH 1/1).

Ethyl 2-((diisopropoxyphosphoryl)methoxy)acetate (**13**)



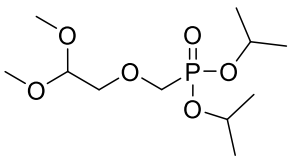
NaH (60% in mineral oil, 20 mg, 0.50 mmol) was mixed with DMF (2 mL) under argon atmosphere and cooled to 0 °C. Diisopropyl (hydroxymethyl)phosphonate (98 mg, 0.50 mmol) was added and the mixture was stirred at 0 °C for 10 min. Ethyl bromoacetate (66 µL, 0.60 mmol) was added, the mixture was allowed to warm up to 25 °C, stirred for 18 h and concentrated. The residue was mixed with DCM (20 mL), washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), the organic fraction was dried over $MgSO_4$ and concentrated to afford compound 13 (79 mg, 56%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 4.67–4.55 (m, 2H), 4.21 (d, J = 1.0 Hz, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.84 (d, J = 8.6 Hz, 2H), 1.27–1.23 (m, 12H), 1.20 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 169.45, 70.30 (d, J = 6.6 Hz), 68.82 (d, J = 13.2 Hz), 64.78 (d, J = 164.3 Hz), 60.28, 23.90–23.63 (m), 14.06. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.36. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{11}H_{24}O_6P$ 283.13050, found 283.13053.

Diisopropyl ((2,2-diethoxyethoxy)methyl)phosphonate (**15a**)



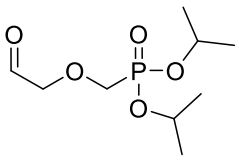
A suspension of NaH (11.72 g, 293.10 mmol, 60% in mineral oil) in anhydrous DMF (20 mL) was cooled to 0 °C. Diisopropyl (hydroxymethyl)phosphonate (57.50 g, 293.10 mmol) was added dropwise over the period of 20 min. The mixture was stirred at 0 °C for 20 min followed by addition of acetaldehyde dimethyl acetal (52.91 mL, 351.72 mmol). The reaction mixture was allowed to warm up to 25 °C and further stirred for 19 h. After quenching with H_2O, the mixture was concentrated and extracted with DCM (3 × 600 mL). Organic fractions were combined, washed with brine (1 × 500 mL), dried over $MgSO_4$, and concentrated to afford **15a** (82.36 g, 90%) as a yellowish oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 4.75 (d of septets, J = 7.7, 6.2 Hz, 2H), 4.62 (t, J = 5.2 Hz, 1H), 3.82 (d, J = 8.1 Hz, 2H), 3.69 (dq, J = 9.4, 7.1 Hz, 2H), 3.61 (dd, J = 5.2, 0.5 Hz, 2H), 3.55 (dq, J = 9.3, 7.0 Hz, 2H), 1.35–1.30 (m, 12H), 1.20 (t, J = 7.0 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 101.25, 73.63 (d, J = 11.0 Hz), 71.16 (d, J = 6.5 Hz), 66.43 (d, J = 166.9 Hz), 62.62, 24.07–24.30 (m), 15.46. **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.95. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{13}H_{29}O_6NaP$ 335.15940, found 335.15935.

Diisopropyl ((2,2-dimethoxyethoxy)methyl)phosphonate (**15b**)



A suspension of NaH (0.41 g, 10.19 mmol, 60% in mineral oil) in anhydrous DMF (20 mL) was cooled to 0 °C. Diisopropyl (hydroxymethyl)phosphonate (2.00 g, 10.19 mmol) was added dropwise over the period of 5 min. The mixture was stirred at 0 °C for 10 min followed by addition of acetaldehyde dimethyl acetal (1.44 mL, 12.23 mmol). The reaction mixture was allowed to warm up to 25 °C and further stirred for 19 h. After quenching with H_2O, the mixture was concentrated and extracted with DCM (3 × 70 mL). Organic fractions were combined, washed with brine (1 × 70 mL), dried over $MgSO_4$, and concentrated to afford **15b** (2.51 g, 87%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 4.81–4.69 (m, 2H), 4.51 (t, J = 5.2 Hz, 1H), 3.86–3.77 (m, 2H), 3.62 (dd, J = 5.2, 0.6 Hz, 2H), 3.38 (s, 6H), 1.38–1.30 (m, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 102.85, 72.62 (d, J = 10.0 Hz), 71.22 (d, J = 6.6 Hz), 66.39 (d, J = 167.2 Hz), 54.16, 24.34–23.96 (m). **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.86. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{11}H_{25}O_6NaP$ 307.12810, found 307.12832.

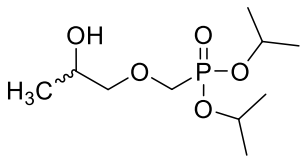
Diisopropyl ((2-oxoethoxy)methyl)phosphonate (**16**)



To a solution of compound **15a** (83.00 g, 265.74 mmol) in H_2O:1,4-dioxane (1:1 ratio) was slowly added H_2SO_4 (14.88 mL, 279.03 mmol). The mixture was stirred at 80 °C for 30 min, poured

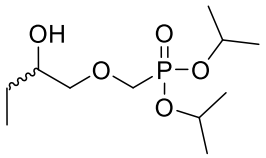
into a sat. $NaHCO_3$ solution, and extracted with DCM (3 × 500 mL). Organic fractions were combined, washed with brine (1 × 500 mL), dried over $MgSO_4$, and distilled in vacuo to afford **16** (60.78 g, 96%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 9.72 (s, 1H), 4.86–4.70 (m, 2H), 4.27 (d, J = 0.9 Hz, 2H), 3.86 (d, J = 8.1 Hz, 2H), 1.35 (d, J = 6.3 Hz, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 199.66, 77.80 (d, J = 8.9 Hz), 71.58 (d, J = 6.6 Hz), 66.52 (d, J = 167.4 Hz), 24.23 (d, J = 3.7 Hz), 24.19 (d, J = 4.5 Hz). **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.79. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_9H_{20}O_5P$ 239.10429, found 239.10431.

Diisopropyl ((2-hydroxypropoxy)methyl)phosphonate ((*RS*)-**17a**)



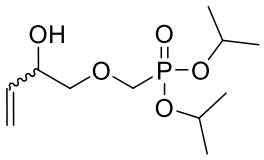
Following standard procedure D, compound **16** (1.15 g, 4.83 mmol) reacted with 3.0 M methylmagnesium bromide (3.0 M in Et_2O, 3.22 mL, 9.66 mmol) in anhydrous THF (10 mL) to yield (*RS*)-**17a** (0.98 g, 80%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 4.82–4.70 (m, 2H), 3.99 (dqd, J = 8.1, 6.4, 2.9 Hz, 1H), 3.79 (d, J = 8.1 Hz, 2H), 3.61 (ddd, J = 9.9, 2.8, 0.6 Hz, 1H), 3.42–3.33 (m, 1H), 1.38–1.29 (m, 12H), 1.14 (d, J = 6.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 79.39 (d, J = 9.5 Hz), 71.38 (d, J = 6.6 Hz), 71.34 (d, J = 6.7 Hz), 66.39, 66.37 (d, J = 167.9 Hz), 24.23 (d, J = 3.7 Hz), 24.16 (d, J = 4.9 Hz), 18.50. **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.32. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{10}H_{23}O_5PNa$ 277.11753, found 277.11763.

Diisopropyl ((2-hydroxybutoxy)methyl)phosphonate ((*RS*)-**17b**)



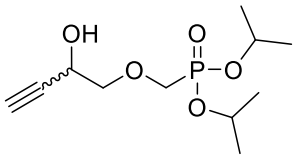
Following standard procedure D, compound **16** (476 mg, 2.00 mmol) reacted with ethylmagnesium bromide (3.0 M in Et_2O, 1.33 mL, 4.00 mmol) in anhydrous THF (4 mL) to yield (*RS*)-**17b** (428 mg, 80%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 4.83–4.67 (m, 2H), 3.78 (d, J = 8.1 Hz, 2H), 3.76–3.68 (m, 1H), 3.64 (dd, J = 9.9, 2.8 Hz, 1H), 3.46–3.39 (m, 1H), 1.51–1.42 (m, 2H), 1.36–1.31 (m, 12H), 0.95 (t, J = 7.5 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 77.94 (d, J = 9.8 Hz), 71.68, 71.50–71.32 (m), 66.36 (d, J = 168.0 Hz), 25.96, 24.22 (d, J = 4.4 Hz), 24.14 (d, J = 4.3 Hz), 10.04. **^{31}P NMR** (162 MHz, $CDCl_3$) δ 22.38. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{11}H_{25}O_5PNa$ 291.13318, found 291.13307.

Diisopropyl (((2-hydroxybut-3-en-1-yl)oxy)methyl)phosphonate ((*RS*)-**17c**)



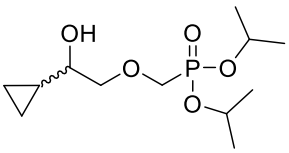
Following standard procedure D, compound **16** (1.15 g, 4.83 mmol) reacted with vinylmagnesium bromide (0.7 M in THF, 13.80 mL, 9.66 mmol) in anhydrous THF (10 mL) to yield (*RS*)-**17c** (1.19 g, 92%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 5.81 (ddd, J = 17.3, 10.6, 5.4 Hz, 1H), 5.37 (dt, J = 17.2, 1.6 Hz, 1H), 5.20 (dt, J = 10.6, 1.5 Hz, 1H), 4.82–4.69 (m, 2H), 4.37–4.32 (m, 1H), 3.81 (d, J = 8.0 Hz, 2H), 3.70 (ddd, J = 10.2, 3.1, 0.7 Hz, 1H), 3.48 (dd, J = 10.2, 7.9 Hz, 1H), 1.38–1.30 (m, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.26, 116.70, 77.71 (d, J = 8.9 Hz), 71.58–71.26 (m), 67.31, 65.64, 24.24 (dd, J = 4.0 Hz), 24.17 (d, J = 4.5 Hz). **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.28. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{11}H_{23}O_5PNa$ 289.11753, found 289.11754.

Diisopropyl (((2-hydroxybut-3-yn-1-yl)oxy)methyl)phosphonate ((*RS*)-**17d**)



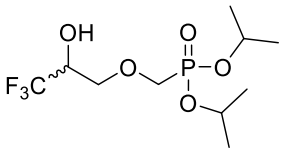
Following standard procedure D, compound **16** (1.15 g, 4.83 mmol) reacted with ethynylmagnesium bromide (0.5 M in THF, 19.34 mL, 9.66 mmol) in anhydrous THF (10 mL) to yield (*RS*)-**17d** (1.05 g, 83%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 4.8–4.71 (m, 2H), 4.58–4.55 (m, 1H), 3.93–3.78 (m, 3H), 3.70 (ddd, J = 10.2, 7.3, 1.9 Hz, 1H), 2.43 (d, J = 2.3 Hz, 1H), 1.39–1.24 (m, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 77.38, 73.86 (d, J = 13.5 Hz), 71.82–71.53 (m), 66.85 (d, J = 168.0 Hz), 61.76 (d, J = 2.9 Hz), 24.18 (d, J = 4.9 Hz). **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.34. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{11}H_{21}O_5PNa$ 287.10188, found 287.10208.

Diisopropyl ((2-cyclopropyl-2-hydroxyethoxy)methyl)phosphonate ((*RS*)-**17e**)



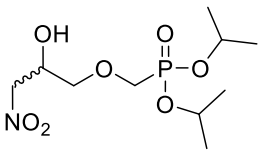
Following standard procedure D, compound **16** (2.00 g, 8.40 mmol) reacted with cyclopropylmagnesium bromide (1.0 M in 2-MeTHF, 16.80 mL, 16.80 mmol) in anhydrous THF (15 mL) to yield (*RS*)-**17e** (2.15 g, 91%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.82–4.67 (m, 2H), 3.88–3.73 (m, 3H), 3.56 (dd, J = 10.0, 7.9 Hz, 1H), 3.12 (td, J = 8.1, 2.8 Hz, 1H), 1.38–1.29 (m, 12H), 0.84 (qt, J = 8.2, 4.9 Hz, 1H), 0.59–0.49 (m, 1H), 0.49–0.42 (m, 1H), 0.38 (dtd, J = 9.1, 5.2, 3.9 Hz, 1H), 0.22 (dtd, J = 9.1, 5.3, 4.1 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 77.94 (d, J = 9.5 Hz), 74.83, 71.37 (d, J = 6.6 Hz), 71.34 (d, J = 6.6 Hz), 66.41 (d, J = 167.9 Hz), 24.22 (d, J = 3.8 Hz), 24.15 (d, J = 4.4 Hz), 13.33, 2.63, 1.85. **^{31}P NMR** (162 MHz, $CDCl_3$) δ 22.08. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{12}H_{25}O_5PNa$ 303.13318, found 303.13345.

Diisopropyl ((3,3,3-trifluoro-2-hydroxypropoxy)methyl)phosphonate ((*RS*)-**17f**)



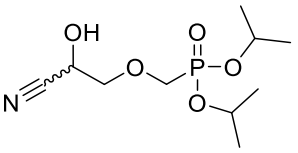
$TMSCF_3$ (522 µL, 3.53 mmol) and TBAF (1M in THF, 291 µL, 0.29 mmol) were added to a solution of compound **16** (700 mg, 2.94 mmol) in anhydrous THF (10 mL). The mixture was stirred at 25 °C for 1 h, quenched with H_2O, and extracted with EtOAc (3 × 70 mL). Combined organic fractions were washed with brine (1 × 70 mL), dried over $MgSO_4$, and concentrated to afford (*RS*)-**17f** (867 mg, 96%) as a brownish oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.82–4.65 (m, 2H), 4.20–4.09 (m, 1H), 3.92–3.72 (m, 4H), 1.36–1.28 (m, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 124.40 (q, J = 282.3 Hz), 72.25–72.04 (m), 72.00–71.83 (m), 69.80 (q, J = 30.6 Hz), 66.95 (d, J = 168.1 Hz), 24.34–23.89 (m). **^{19}F NMR** (376 MHz, $CDCl_3$) δ −77.47. **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.92. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{10}H_{20}O_5F_3PNa$ 331.08927, found 331.08930.

Diisopropyl ((2-hydroxy-3-nitropropoxy)methyl)phosphonate ((*RS*)-**17g**)



Nitromethane (68 µL, 1.26 mmol) was added to a suspension of NaH (50 mg, 1.26 mmol, 60% in mineral oil) in anhydrous THF (1 mL). The mixture was stirred for 10 min and the solution of compound **16** (100 mg, 0.42 mmol) in anhydrous THF (1 mL) was added. The mixture was stirred at 25 °C for 1 h, quenched with H_2O, and extracted with EtOAc (3 × 30 mL). Combined organic fractions were washed with brine (1 × 30 mL), dried over $MgSO_4$, and concentrated to afford (*RS*)-**17g** (68 mg, 54%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.80–4.68 (m, 2H), 4.54–4.45 (m, 3H), 3.86–3.67 (m, 4H), 1.33 (d, J = 6.3, 12H). **^{13}C NMR** (101 MHz, CDCl3) δ 77.98, 74.52 (d, J = 8.9 Hz), 71.78 (d, J = 6.6 Hz), 71.74 (d, J = 6.7 Hz), 67.94, 66.75 (d, J = 168.0 Hz), 24.32–24.01 (m). **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.82. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{10}H_{22}O_7NPNa$ 322.10261, found 322.10283.

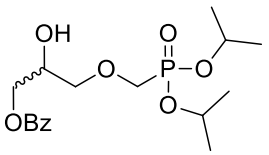
Diisopropyl ((2-cyano-2-hydroxyethoxy)methyl)phosphonate ((*RS*)-**17h**)



KCN (684 mg, 10.50 mmol) was added to a solution of compound **16** (50 mg, 0.21 mmol) in H_2O/1,4-dioxane (1:1 ratio) (5 mL). The mixture was stirred at 25 °C for 1 h and extracted with EtOAc (3 × 30 mL). Combined organic fractions were washed with brine (1 × 30 mL), dried over $MgSO_4$, and concentrated to afford (*RS*)-**17h** (50 mg, 89%) as a colorless oil. **^{1}H NMR** (401 MHz, $CDCl_3$) δ 4.89–4.71 (m, 2H), 4.59 (dd, J = 4.8, 3.1 Hz, 1H), 4.04–3.92 (m, 2H), 3.87–3.78 (m, 2H), 1.37–1.32 (m, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 118.05, 75.82 (d, J = 6.7 Hz), 72.65 (d, J = 7.1 Hz), 72.15 (d, J = 7.1 Hz), 67.73 (d, J = 168.1 Hz), 61.69, 24.29–24.05 (m). **^{31}P NMR** (162 MHz,

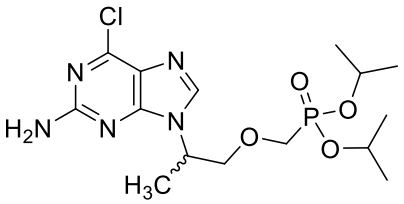
$CDCl_3$) δ 22.54. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{10}H_{20}O_5NPNa$ 288.09713, found 288.09721.

3-((Diisopropoxyphosphoryl)methoxy)-2-hydroxypropyl benzoate ((*RS*)-**17i**)



NaH (60% in mineral oil, 2.00 g, 138.89 mmol) was mixed with THF (200 mL) under argon atmosphere and cooled to 0 °C. Diisopropyl (hydroxymethyl)phosphonate (10.00 g, 50.97 mmol) was added and the mixture was stirred at 0 °C for 10 min. Epibromhydrin (4.30 mL, 50.97 mmol) was added, the mixture was allowed to warm up to 25 °C, stirred for 18 h and concentrated. The residue was mixed with DCM (20 mL), washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), the organic fraction was dried over $MgSO_4$ and distilled under vacuum to afford diisopropyl ((oxiran-2-ylmethoxy)methyl)phosphonate (6.02 g, 23.78 mmol) which was dissolved in anhydrous MeCN (250 mL) and to the solution, PhCOOH (3.48 g, 28.54 mmol) and TBAB (229 mg, 0.71 mmol) was added and the mixture was stirred in MW-reactor at 180°C for 6 h. The mixture was concentrated, re-suspended in 5% K_2CO_3 solution and extracted with $CHCl_3$ (3 × 300 mL). The combined organic fractions were washed with brine (1 × 300 mL), dried over $MgSO_4$ and purified using silica gel flash chromatography (linear gradient elution 0–20% MeOH in $CHCl_3$) to afford (*RS*)-**17i** as a colorless oil (5.97 g, 31% over 2 steps). *Note: prepared by my colleague Dr. Špaček.* **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.07–8.02 (m, 2H), 7.59–7.53 (m, 1H), 7.47–7.39 (m, 2H), 4.81–4.68 (m, 2H), 4.43–4.34 (m, 2H), 4.21–4.11 (m, 1H), 3.84–3.69 (m, 4H), 1.33 (d, J = 6.1 Hz, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.68, 133.26, 129.99, 129.83, 128.52, 74.99 (d, J = 8.8 Hz), 71.60–71.46 (m), 68.99, 66.72 (d, J = 168.0 Hz), 65.73, 24.26–24.08 (m). **^{31}P NMR** (162 MHz, $CDCl_3$) δ 21.99. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{17}H_{28}O_7P$ 375.15672, found 375.15741.

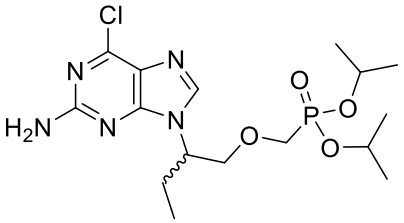
Diisopropyl ((2-(2-amino-6-chloro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*RS*)-**18a**)



Following standard procedure E, compound (*RS*)-**17a** (300 mg, 1.18 mmol) reacted with 2-amino-6-chloropurine (190 mg, 1.12 mmol), PPh_3 (310 mg, 1.18 mmol), and DIAD (232 µL, 1.18 mmol) in anhydrous THF (6 mL) to afford (*RS*)-**18a** (336 mg, 74%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.19 (s, 1H), 6.86 (s, 2H), 4.75–4.64 (m, 1H), 4.50–4.38 (m, 2H), 3.97 (dd, J = 10.2, 8.0 Hz, 1H), 3.84–3.66 (m, 3H), 1.46 (d, J = 7.0 Hz, 3H), 1.18–1.12 (m, 6H), 1.12–1.04 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.56, 153.94, 149.27, 141.79, 123.49, 73.82 (d, J = 11.5 Hz), 70.98–69.41 (m), 64.76 (d, J = 163.7 Hz), 49.83, 23.70 (d, J = 3.7 Hz), 23.52 (d, J = 4.4 Hz), 16.41. **^{31}P**

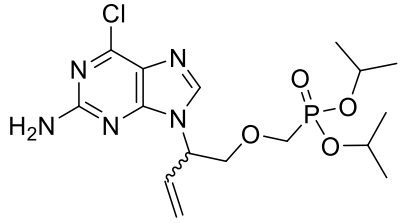
NMR (162 MHz, DMSO-d_6) δ 21.24. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{15}H_{25}O_4N_5ClNaP$ 428.12249, found 428.12297.

Diisopropyl ((2-(2-amino-6-chloro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*RS*)-**18b**)



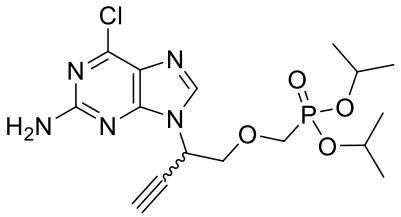
Following standard procedure E, compound (*RS*)-**17b** (400 mg, 1.49 mmol) reacted with 2-amino-6-chloropurine (241 mg, 1.42 mmol), PPh_3 (391 mg, 1.49 mmol), and DIAD (292 µL, 1.49 mmol) in anhydrous THF (10 mL) to afford (*RS*)-**18b** (385 mg, 65%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.18 (s, 1H), 6.86 (s, 2H), 4.52–4.35 (m, 3H), 4.02 (dd, J = 10.3, 8.2 Hz, 1H), 3.84–3.64 (m, 3H), 1.99–1.79 (m, 2H), 1.18–1.11 (m, 6H), 1.11–1.04 (m, 6H), 0.76 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.59, 154.33, 149.28, 142.12, 123.42, 72.78 (d, J = 11.6 Hz), 70.34–70.05 (m), 64.73 (d, J = 163.7 Hz), 55.89, 23.69 (d, J = 3.7 Hz), 23.49 (d, J = 4.6 Hz), 23.11, 10.24. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.21. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{27}O_4N_5ClNaP$ 442.13814, found 442.13807.

Diisopropyl (((2-(2-amino-6-chloro-9*H*-purin-9-yl)but-3-en-1-yl)oxy)methyl)phosphonate ((*RS*)-**18c**)



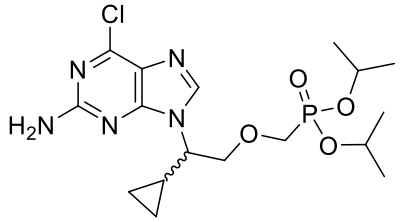
Following standard procedure E, compound (*RS*)-**17c** (300 mg, 1.13 mmol) reacted with 2-amino-6-chloropurine (183 mg, 1.08 mmol), PPh_3 (296 mg, 1.13 mmol), and DIAD (221 µL, 1.13 mmol) in anhydrous THF (6 mL) to afford (*RS*)-**18c** (182 mg, 40%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.20 (s, 1H), 6.90 (s, 2H), 6.14 (ddd, J = 17.3, 10.5, 5.9 Hz, 1H), 5.28 (dt, J = 10.5, 1.2 Hz, 1H), 5.22 (dddt, J = 8.7, 5.8, 4.2, 1.5 Hz, 1H), 5.09 (dt, J = 17.2, 1.2 Hz, 1H), 4.51–4.39 (m, 2H), 4.16 (dd, J = 10.4, 8.7 Hz, 1H), 3.92 (dd, J = 10.4, 4.3 Hz, 1H), 3.81 (dd, J = 13.9, 8.4 Hz, 1H), 3.74 (dd, J = 13.9, 8.4 Hz, 1H), 1.17–1.13 (m, 6H), 1.11–1.06 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.68, 153.99, 149.41, 141.97, 133.18, 123.25, 118.54, 72.12 (d, J = 11.3 Hz), 70.24 (d, J = 6.1 Hz), 64.70 (d, J = 164.3 Hz), 55.90, 23.69 (d, J = 3.8 Hz), 23.50 (d, J = 4.7 Hz). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.15. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{25}O_4N_5ClNaP$ 440.12249, found 440.12295.

Diisopropyl (((2-(2-amino-6-chloro-9*H*-purin-9-yl)but-3-yn-1-yl)oxy)methyl)phosphonate ((*RS*)-**18d**)



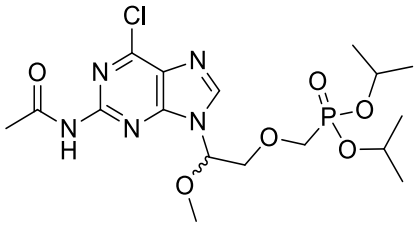
Following standard procedure E, compound (*RS*)-**17d** (300 mg, 1.14 mmol) reacted with 2-amino-6-chloropurine (185 mg, 1.09 mmol), PPh_3 (299 mg, 1.14 mmol), and DIAD (224 µL, 1.14 mmol) in anhydrous THF (6 mL) to afford (*RS*)-**18d** (139 mg, 43%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.00 (s, 2H), 5.54 (ddd, J = 7.9, 4.4, 2.5 Hz, 1H), 4.52–4.38 (m, 2H), 4.21 (dd, J = 10.4, 8.0 Hz, 1H), 3.98 (dd, J = 10.4, 4.4 Hz, 1H), 3.88–3.74 (m, 2H), 3.70 (d, J = 2.5 Hz, 1H), 1.17–1.13 (m, 6H), 1.11–1.06 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.79, 153.43, 149.61, 141.51, 123.15, 77.78, 77.54, 71.98 (d, J = 11.9 Hz), 70.27 (d, J = 6.6 Hz), 70.24 (d, J = 6.4 Hz, 1H), 64.72 (d, J = 164.0 Hz), 45.11, 23.69 (d, J = 3.8 Hz), 23.58–23.43 (m). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 20.91. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{23}O_4N_5ClNaP$ 438.10684, found 438.10743.

Diisopropyl ((2-(2-amino-6-chloro-9*H*-purin-9-yl)-2-cyclopropylethoxy)methyl)phosphonate ((*RS*)-**18e**)



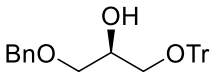
Following standard procedure E, compound (*RS*)-**17e** (600 mg, 2.14 mmol) reacted with 2-amino-6-chloropurine (346 mg, 2.04 mmol), PPh_3 (561 mg, 2.14 mmol), and DIAD (420 µL, 2.14 mmol) in anhydrous THF (10 mL) to afford (*RS*)-**18e** (221 mg, 25%), as a yellow oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.24 (s, 1H), 6.85 (s, 2H), 4.48–4.36 (m, 2H), 4.20 (dd, J = 10.3, 8.6 Hz, 1H), 3.91 (dd, J = 10.2, 3.8 Hz, 1H), 3.84–3.78 (m, 1H), 3.78–3.65 (m, 2H), 1.48 (dddd, J = 12.8, 9.8, 7.9, 4.9 Hz, 1H), 1.16–1.11 (m, 6H), 1.09–1.03 (m, 6H), 0.71–0.59 (m, 1H), 0.52–0.39 (m, 2H), 0.32–0.21 (m, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.59, 154.09, 149.29, 142.18, 123.41, 72.59 (d, J = 11.7 Hz), 70.36–69.81 (m), 64.73 (d, J = 163.7 Hz), 59.35, 23.68 (d, J = 3.8 Hz), 23.48 (d, J = 4.4 Hz), 12.01, 4.31, 3.17. **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.18. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{17}H_{27}O_4N_5ClNaP$ 454.13814, found 454.13776.

Diisopropyl ((2-(2-amino-6-chloro-9*H*-purin-9-yl)-2-methoxyethoxy)methyl)phosphonate ((*RS*)-**19**)



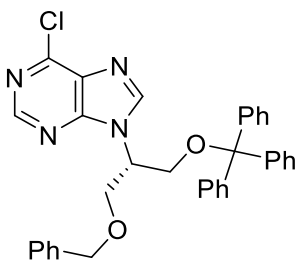
Following standard procedure C, compound **15b** (284 mg, 1.00 mmol) reacted with 2-amino-6-chloropurine (170 mg, 1.00 mmol), Ac_2O (94 µL, 1.00 mmol), and TMSOTf (271 µL, 1.50 mmol) to afford (*RS*)-**19** (101 mg, 24%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.65 (s, 1H), 5.79 (dd, J = 7.2, 4.9 Hz, 1H), 4.50–4.37 (m, 2H), 4.23 (dd, J = 10.6, 7.2 Hz, 1H), 4.08 (td, J = 10.6, 4.9 Hz, 1H), 3.87–3.74 (m, 2H), 3.27 (s, 3H), 2.21 (s, 3H), 1.17–1.10 (m, 6H), 1.09–1.03 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 169.29, 153.49, 152.67, 149.69, 145.41, 127.79, 84.43, 71.47 (d, J = 12.4 Hz), 70.65 (d, J = 6.7 Hz), 70.61 (d, J = 6.7 Hz), 65.57 (d, J = 164.3 Hz), 56.92, 25.09, 24.12 (d, J = 3.7 Hz), 23.92 (d, J = 4.6 Hz). **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{17}H_{27}O_6N_5ClNaP$ 486.12797, found 486.12822.

(*R*)-1-(Benzyloxy)-3-(trityloxy)propan-2-ol (**20**)



To a suspension of NaH (3.79 g, 94.82 mmol) in anhydrous DMF (85 mL) was at 25 °C added BnOH (9.81 mL, 94.82 mmol) over the period of 15 min. The mixture was stirred for further 10 min at 25 °C. A solution of (*R*)-glycidyl trityl ether (25.00 g, 79.02 mmol) in anhydrous DMF (40 mL) was added over the period of 15 min and the mixture was stirred at 100 °C for 3 h. The reaction was quenched with H_2O and extracted with EtOAc (3 × 300 mL). Organic fractions were combined, washed with brine (1 × 200 mL), dried over $MgSO_4$, and concentrated. The residual BnOH was removed using in vacuo distillation to afford **20** (32.93 g, 98%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 7.43–7.17 (m, 20H), 4.96 (d, J = 5.5 Hz, 1H), 4.45 (s, 2H), 3.87–3.77 (m, 1H), 3.53–3.40 (m, 2H), 3.01–2.93 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 143.93, 138.50, 128.45–126.68 (m), 85.71, 72.15, 71.71, 68.67, 65.12. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{29}H_{28}O_3Na$ 447.19307, found 447.19295. **$[\alpha]^{25}_D$** = +1.7 (c 0.356 g/100 mL, $CHCl_3$/MeOH 1/1).

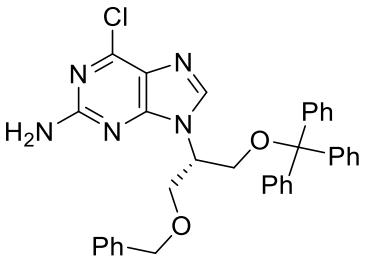
(*S*)-9-(1-(Benzyloxy)-3-(trityloxy)propan-2-yl)-6-chloro-9*H*-purine ((*S*)-**21a**)



Following standard procedure E, compound **20** (550 mg, 1.30 mmol) reacted with 6-chloropurine (192 mg, 1.24 mmol), PPh_3 (340 g, 1.30 mmol), and DIAD (255 µL, 1.30 mmol) in anhydrous THF (8 mL) to obtain (*S*)-**21a** (600 mg, 86%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.64 (s, 1H), 7.26–7.05 (m, 20H), 5.16–5.10 (m, 1H), 4.47 (d, J

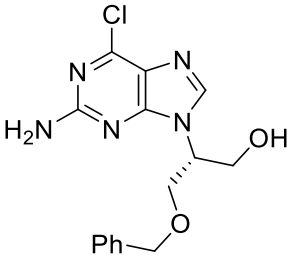
= 3.4 Hz, 2H), 4.19 (dd, *J* = 10.4, 8.2 Hz, 1H), 3.97 (dd, *J* = 10.3, 5.2 Hz, 1H), 3.56 (dd, *J* = 9.9, 7.3 Hz, 1H), 3.46 (dd, *J* = 9.9, 3.9 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 151.92, 151.20, 149.02, 147.03, 143.02, 137.69, 130.95, 128.56–126.57 (m), 86.12, 71.96, 66.83, 61.59, 56.10. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{34}H_{29}O_2N_4ClNa$ 583.18713, found 583.18640. **$[\alpha]^{25}_D$** = +8.9 (c 0.293 g/100 mL, $CHCl_3$/MeOH 1/1).

(*S*)-9-(1-(Benzyloxy)-3-(trityloxy)propan-2-yl)-6-chloro-9*H*-purin-2-amine ((*S*)-**21b**)



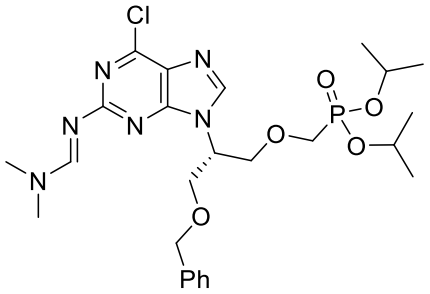
Following standard procedure E, compound **20** (550 mg, 1.30 mmol) reacted with 2-amino-6-chloropurine (210 mg, 1.24 mmol), PPh_3 (340 g, 1.30 mmol), and DIAD (255 µL, 1.30 mmol) in anhydrous THF (8 mL) to obtain (*S*)-**21b** (495 mg, 69%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.34–7.09 (m, 20H), 6.87 (s, 2H), 4.87–4.81 (m, 1H), 4.48 (s, 2H), 4.10 (dd, *J* = 10.2, 7.7 Hz, 1H), 3.90 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.45 (dd, *J* = 9.7, 7.0 Hz, 1H), 3.32–3.25 (m, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.65, 154.36, 149.34, 143.15, 142.39, 137.81, 128.69–126.67 (m), 123.33, 86.08, 72.02, 67.22, 62.07, 54.71. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{34}H_{30}O_2N_5ClNa$ 598.19802, found 598.19763. **$[\alpha]^{25}_D$** = −10.1 (c 0.345 g/100 mL, $CHCl_3$/MeOH 1/1).

(*R*)-2-(2-Amino-6-chloro-9*H*-purin-9-yl)-3-(benzyloxy)propan-1-ol ((*R*)-**22**)



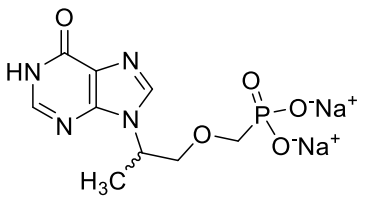
Compound (*S*)-**21b** (180 mg, 0.31 mmol) was stirred at 0 °C in DCM/TFA (2.5 mL, 4:1 ratio) for 30 min. The mixture was diluted with 25 mL of DCM and TFA was washed away with H_2O (3 × 25 mL). DCM was washed with brine and dried over $MgSO_4$. The residue was purified using silica gel flash chromatography (linear gradient elution 0–10% MeOH in $CHCl_3$) to afford (*R*)-**22** (102 mg, 99%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.33–7.20 (m, 3H), 7.20–7.15 (m, 2H), 6.86 (s, 2H), 5.12 (t, *J* = 5.4 Hz, 1H), 4.63 (ddt, *J* = 7.8, 6.7, 4.9 Hz, 1H), 4.49 (d, *J* = 12.2 Hz, 1H), 4.44 (d, *J* = 12.1 Hz, 1H), 3.96–3.74 (m, 4H), 3.13–2.91 (m, 4H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.56, 154.38, 149.22, 142.49, 137.93, 128.23, 127.51, 127.38, 123.37, 72.04, 67.87, 59.68, 56.59. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{15}H_{16}O_2N_5ClNa$ 356.08847, found 356.08820. **$[\alpha]^{25}_D$** = +15.8 (c 0.277 g/100 mL, $CHCl_3$/MeOH 1/1).

Diisopropyl (*S*)-((3-(benzyloxy)-2-(6-chloro-2-(((dimethylamino)methylene)amino)-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*S*)-**23**)



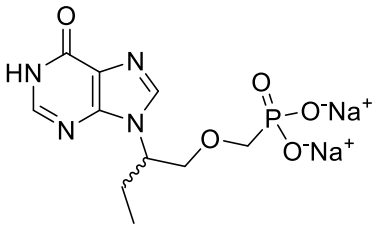
Compound (*R*)-**22** (50 mg, 0.15 mmol) was stirred with DMF DMA (60 µL, 0.45 mmol) in DMF (1 mL) at 80 °C for 15 min. The mixture was concentrated and co-distilled 3 × with toluene to yield (*R*)-*N*′-(9-(1-(benzyloxy)-3-hydroxypropan-2-yl)-6-chloro-9*H*-purin-2-yl)-*N*,*N*-dimethylformimidamide (58 mg, 99%) as a white solid which was used directly in the following reaction. Following standard procedure B, (*R*)-*N*′-(9-(1-(benzyloxy)-3-hydroxypropan-2-yl)-6-chloro-9*H*-purin-2-yl)-*N*,*N*-dimethyl formimidamide (58 mg, 0.15 mmol) reacted with *n*-BuLi (2.5 M in hexanes, 69 µL, 0.17 mmol) and diisopropyl triflyloxymethanephosphonate (75 mg, 0.23 mmol) in anhydrous THF (1 mL) to afford (*S*)-**23** (53 mg, 62%) as a colorless viscose oil. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.38 (s, 1H), 7.31–7.22 (m, 3H), 7.21–7.13 (m, 2H), 5.00 (tt, J = 7.8, 4.6 Hz, 1H), 4.53–4.39 (m, 4H), 4.16 (dd, J = 10.4, 8.1 Hz, 1H), 4.01–3.91 (m, 2H), 3.90–3.70 (m, 3H), 3.15 (s, 3H), 3.04 (s, 3H), 1.15–1.12 (m, 6H), 1.06 (d, J = 6.1 Hz, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 161.73, 158.45, 153.81, 148.75, 144.09, 137.76, 128.18, 127.52, 127.38, 126.11, 72.05, 70.48 (d, J = 11.6 Hz), 70.24–69.91 (m), 67.28, 64.77 (d, J = 163.5 Hz), 54.13, 40.45, 34.62, 23.65 (d, J = 3.7 Hz), 23.47 (d, J = 4.4 Hz). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 21.04. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{25}H_{36}O_5N_6ClNaP$ 589.20655, found 589.20581. **$[\alpha]^{25}_D$** = +4.9 (c 0.061 g/100 mL, $CHCl_3$/MeOH 1/1).

Sodium ((2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*RS*)-**24a**)



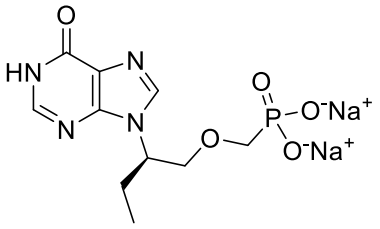
Following standard procedure F, compound (*RS*)-**10e** (180 mg, 0.46 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (145 mg, 0.39 mmol) was stirred in anhydrous MeCN (3.9 mL) and TMSBr (390 µL) at 25 °C for 16 h to yield (*RS*)-**24a** (108 mg, 71% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.31 (s, 1H), 8.17 (s, 1H), 4.97–4.87 (m, 1H), 4.02–3.93 (m, 2H), 3.50–3.44 (m, 2H), 1.58 (d, J = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 159.23, 149.34, 145.97, 141.44, 123.90, 75.07 (d, J = 10.3 Hz), 67.96 (d, J = 155.5 Hz), 51.93, 16.84. **^{31}P NMR** (162 MHz, D_2O) δ 18.04. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{12}O_5N_4P$ 287.05508, found 287.05535.

Sodium ((2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*RS*)-**24b**)



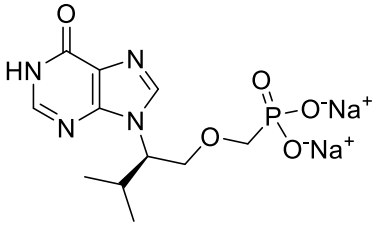
Following standard procedure F, compound (*RS*)-**10a** (121 mg, 0.30 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (65 mg, 0.17 mmol) was stirred in anhydrous MeCN (1.7 mL) and TMSBr (170 µL) at 25 °C for 16 h to yield (*RS*)-**24b** (40 mg, 38% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.31 (s, 1H), 8.17 (s, 1H), 4.75–4.66 (m, 1H), 4.05 (dd, J = 11.0, 7.8 Hz, 1H), 3.99 (dd, J = 11.0, 4.0 Hz, 1H), 3.49 (d, J = 8.3 Hz, 2H), 2.05–1.92 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 159.35, 149.84, 146.06, 141.78, 123.88, 74.14, 67.72 (d, J = 156.6 Hz), 57.99, 24.49, 10.11. **^{31}P NMR** (162 MHz, D_2O) δ 18.15. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{14}O_5N_4P$ 301.07073, found 301.06995.

Sodium (*R*)-((2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**24b**)



Following standard procedure F, compound (*R*)-**10a** (174 mg, 0.43 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivatives (125 mg, 0.32 mmol) was stirred in anhydrous MeCN (3.2 mL) and TMSBr (320 µL) at 25 °C for 16 h to yield (*R*)-**24b** (103 mg, 69% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.31 (s, 1H), 8.17 (s, 1H), 4.70 (dddd, J = 9.0, 7.7, 6.0, 4.3 Hz, 1H), 4.09–3.95 (m, 2H), 3.48 (d, J = 8.3 Hz, 2H), 2.07–1.90 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 159.47, 149.85, 146.18, 141.85, 123.88, 73.89 (d, J = 9.5 Hz), 69.36 (d, J = 151.2 Hz), 58.15, 24.71, 10.10. **^{31}P NMR** (162 MHz, D_2O) δ 16.23. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{14}O_5N_4P$ 301.07073, found 301.07027. **$[\alpha]^{25}_D$** = +3.8 (c 0.280 g/100 mL, H_2O/MeOH 1/1).

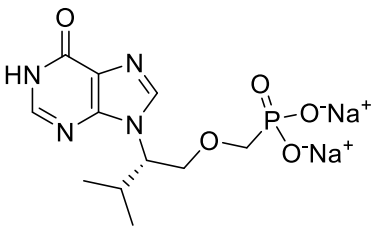
Sodium (*R*)-((3-methyl-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**24c**)



Following standard procedure F, compound (*R*)-**10b** (235 mg, 0.56 mmol) was converted to 6-oxo derivative. Following standard procedure G, 6-oxo derivatives (140 mg, 0.35 mmol) was stirred in anhydrous MeCN (3.5 mL) and TMSBr (350 µL) at 25 °C for 16 h to yield (*R*)-**24c** (84 mg, 42% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.33 (s, 1H), 8.17 (s, 1H), 4.49 (td, J = 8.6, 3.5 Hz, 1H), 4.16

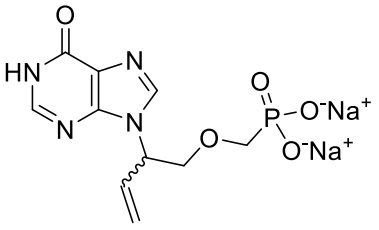
(dd, *J* = 11.1, 8.2 Hz, 1H), 4.04 (dd, *J* = 11.1, 3.5 Hz, 1H), 3.56–3.47 (m, 2H), 2.34 (d of septets, *J* = 8.9, 6.7 Hz, 1H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.72 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 159.39, 149.97, 146.11, 142.10, 123.64, 72.33 (d, *J* = 10.2 Hz), 68.68 (d, *J* = 153.2 Hz), 62.12, 30.48, 19.51, 18.97. **^{31}P NMR** (162 MHz, D_2O) *δ* 16.95. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{16}O_5N_4P$ 315.08638, found 315.08615. **$[\alpha]^{25}_D$** = +4.7 (c 0.232 g/100 mL, H_2O/MeOH 1/1).

Sodium (*S*)-((3-methyl-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*S*)-**24c**)



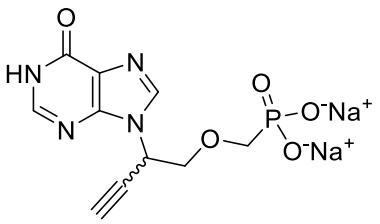
Following standard procedure F, compound (*S*)-**10b** (209 mg, 0.50 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (135 mg, 0.30 mmol) was stirred in anhydrous MeCN (3.0 mL) and TMSBr (300 µL) at 25 °C for 16 h to yield (*S*)-**24c** (65 mg, 36% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) *δ* 8.26 (s, 1H), 8.09 (s, 1H), 4.41 (td, *J* = 8.6, 3.5 Hz, 1H), 4.07 (dd, *J* = 11.1, 8.2 Hz, 1H), 3.97 (dd, *J* = 11.1, 3.6 Hz, 1H), 3.40 (d, *J* = 8.3 Hz, 2H), 2.26 (d of septets, *J* = 9.1, 6.8 Hz, 1H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.65 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 159.45, 150.01, 146.13, 142.12, 123.66, 72.32 (d, *J* = 10.1 Hz), 69.02 (d, *J* = 152.4 Hz), 62.15, 30.51, 19.55, 18.95. **^{31}P NMR** (162 MHz, D_2O) *δ* 16.56. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{16}O_5N_4P$ 315.08638, found 315.08628. **$[\alpha]^{25}_D$** = −3.7 (c 0.240 g/100 mL, H_2O/MeOH 1/1).

Sodium (((2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)but-3-en-1-yl)oxy)methyl)phosphonate ((*RS*)-**24d**)



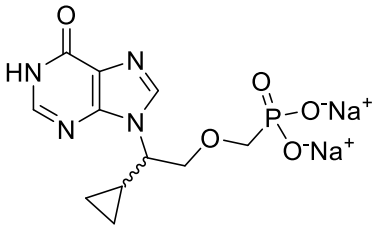
Following standard procedure F, compound (*RS*)-**10f** (209 mg, 0.52 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (70 mg, 0.18 mmol) was stirred in anhydrous MeCN (1.8 mL) and TMSBr (180 µL) at 25 °C for 16 h to yield (*RS*)-**24d** (53 mg, 30% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) *δ* 8.32 (s, 1H), 8.16 (s, 1H), 6.18 (ddd, *J* = 17.3, 10.6, 5.5 Hz, 1H), 5.45–5.40 (m, 1H), 5.39–5.33 (m, 1H), 5.13–5.05 (m, 1H), 4.18 (dd, *J* = 10.9, 7.8 Hz, 1H), 4.12 (dd, *J* = 10.9, 4.5 Hz, 1H), 3.59 (d, *J* = 8.3 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) *δ* 159.32, 149.42, 146.22, 142.02, 133.11, 123.94, 119.41, 73.09 (d, *J* = 10.3 Hz), 68.73 (d, *J* = 153.3 Hz), 57.81. **^{31}P NMR** (162 MHz, D_2O) *δ* 17.41. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{12}O_5N_4P$ 299.05508, found 299.05441.

Sodium (((2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)but-3-yn-1-yl)oxy)methyl)phosphonate ((*RS*)-**24e**)



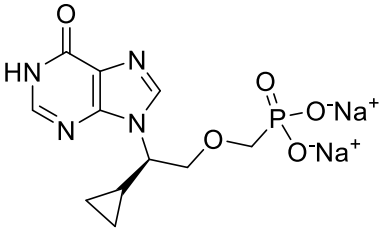
Following standard procedure F, compound (*RS*)-**10g** (184 mg, 0.46 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (120 mg, 0.31 mmol) was stirred in anhydrous MeCN (3.1 mL) and TMSBr (310 µL) at 25 °C for 16 h to yield (*RS*)-**24e** (88 mg, 56% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.38 (s, 1H), 8.18 (s, 1H), 5.70 (ddd, J = 6.9, 4.7, 1.9 Hz, 1H), 4.20–4.06 (m, 2H), 3.64–3.53 (m, 2H), 3.07 (d, J = 2.5 Hz, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 159.25, 148.94, 146.43, 141.88, 124.12, 77.43, 77.20, 77.16, 73.85 (d, J = 10.4 Hz), 68.96 (d, J = 154.1 Hz), 47.52. **^{31}P NMR** (162 MHz, D_2O) δ 17.35. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{10}O_5N_4P$ 297.03943, found 297.03932.

Sodium ((2-cyclopropyl-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)ethoxy)methyl)phosphonate ((*RS*)-**24f**)



Following standard procedure F, compound (*RS*)-**10c** (100 mg, 0.24 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (90 mg, 0.23 mmol) was stirred in anhydrous MeCN (2.3 mL) and TMSBr (230 µL) at 25 °C for 16 h to yield (*RS*)-**24f** (75 mg, 87% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.39 (s, 1H), 8.15 (s, 1H), 4.20–4.08 (m, 2H), 4.05–3.98 (m, 1H), 3.46 (d, J = 8.3 Hz, 2H), 1.58–1.48 (m, 1H), 0.82–0.73 (m, 1H), 0.60–0.47 (m, 2H), 0.32–0.24 (m, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 159.67, 149.59, 146.27, 141.97, 123.84, 73.93 (d, J = 9.5 Hz), 69.75 (d, J = 150.4 Hz), 61.62, 12.76, 5.15, 3.26. **^{31}P NMR** (162 MHz, D_2O) δ 17.82. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{14}O_5N_4P$ 313.07073, found 313.07028.

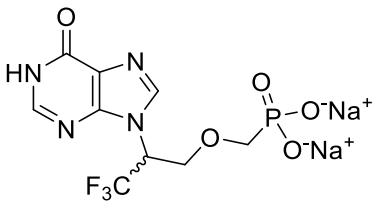
Sodium (*R*)-((2-cyclopropyl-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)ethoxy)methyl)phosphonate ((*R*)-**24f**)



Following standard procedure F, compound (*R*)-**10c** (100 mg, 0.24 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (88 mg, 0.22 mmol) was stirred in anhydrous MeCN (2.3 mL) and TMSBr (230 µL) at 25 °C for 16 h to yield (*R*)-**24f** (76 mg, 88% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.38 (s, 1H), 8.15 (s, 1H), 4.18 (dd, J = 11.1, 7.7 Hz, 1H),

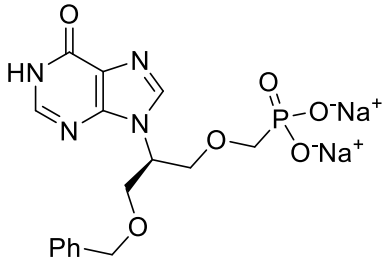
4.14–4.02 (m, 1H), 4.02–3.96 (m, 1H), 3.62–3.48 (m, 2H), 1.58–1.49 (m, 1H), 0.78 (tdd, J = 9.4, 4.5, 3.1 Hz, 1H), 0.57–0.47 (m, 2H), 0.29 (tdd, J = 9.3, 6.8, 4.1 Hz, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 159.33, 149.51, 146.03, 141.94, 123.83, 74.06 (d, J = 10.2 Hz), 68.59 (d, J = 154.1 Hz), 61.55, 12.58, 5.13, 3.32. **^{31}P NMR** (162 MHz, D_2O) δ 17.28. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{14}O_5N_4P$ 313.07073, found 313.07041. **$[\alpha]^{25}_D$** = +7.3 (c 0.246 g/100 mL, H_2O/MeOH 1/1).

Sodium ((3,3,3-trifluoro-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*RS*)-**24g**)



Following standard procedure F, compound (*RS*)-**10i** (67 mg, 0.15 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (48 mg, 0.11 mmol) was stirred in anhydrous MeCN (1.1 mL) and TMSBr (110 µL) at 25 °C for 16 h to yield (*RS*)-**24g** (24 mg, 41% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.35 (s, 1H), 8.19 (s, 1H), 4.71 (dd, J = 15.2, 4.3 Hz, 1H), 4.61 (dd, J = 15.1, 6.0 Hz, 1H), 4.52–4.40 (m, 1H), 3.81 (dd, J = 12.3, 9.3 Hz, 1H), 3.58 (dd, J = 12.4, 9.1 Hz, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 159.42, 149.64, 146.52, 143.92, 124.87 (q, J = 250.1 Hz), 123.29, 78.01–76.54 (m), 71.15 (d, J = 150.7 Hz), 42.76. **^{31}P NMR** (162 MHz, D_2O) δ 14.46. **^{19}F NMR** (377 MHz, D_2O) δ −75.39 (d, J = 6.3 Hz). **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_9O_5N_4F_3P$ 341.02681, found 341.02600.

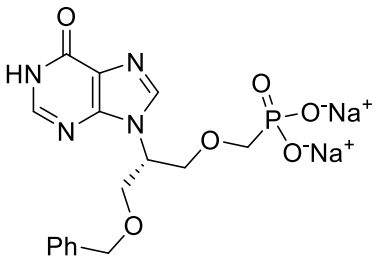
Sodium (*R*)-((3-(benzyloxy)-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*R*)-**24h**)



Following standard procedure F, compound (*R*)-**10d** (89 mg, 0.18 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (80 mg, 0.17 mmol) was stirred in anhydrous MeCN (1.7 mL) and TMSBr (170 µL) at 25 °C for 60 h to yield (*R*)-**24h** (22 mg, 28% over 2 steps) as a white solid (and (*R*)-**24i** (21 mg, 33% over 2 steps) as a white solid). [Note: Shorter reaction time preferentially leads to the cleavage of phosphonate esters while longer reaction time leads also to the product with cleaved benzyl group.] **^{1}H NMR** (401 MHz, D_2O) δ 8.19 (s, 1H), 7.96 (s, 1H), 7.25–7.12 (m, 3H), 7.03–6.96 (m, 2H), 4.89 (tt, J = 7.5, 4.6 Hz, 1H), 4.49 (d, J = 12.2 Hz, 1H), 4.38 (d, J = 12.2 Hz, 1H), 4.13–3.96 (m, 4H), 3.52 (d, J = 8.5 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 159.16, 149.28, 145.69, 142.30, 137.17, 128.94, 128.84, 128.68, 123.98, 73.24, 70.75 (d, J = 10.6 Hz), 69.05 (d, J = 153.0 Hz), 67.85, 56.63. **^{31}P NMR** (162 MHz, D_2O) δ 16.57. **HRMS** (ESI) *m/z*

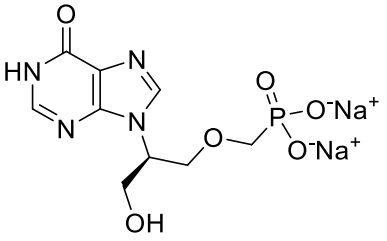
[M−H]$^-$ calcd for $C_{16}H_{18}O_6N_4P$ 393.09694, found 393.09641. **[α]$^{25}_D$** = +15.3 (c 0.183 g/100 mL, H_2O/MeOH 1/1).

Sodium (*S*)-((3-(benzyloxy)-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*S*)-**24h**)



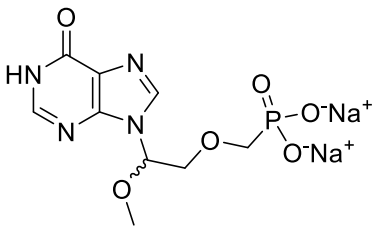
Following standard procedure F, compound (*S*)-**10d** (119 mg, 0.24 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (100 mg, 0.21 mmol) was stirred in anhydrous MeCN (2.1 mL) and TMSBr (210 µL) at 25 °C for 16 h to yield (*S*)-**24h** (32 mg, 30% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.19 (s, 1H), 7.96 (s, 1H), 7.28–7.15 (m, 3H), 7.00–6.97 (m, 2H), 4.89 (tt, *J* = 7.6, 4.6 Hz, 1H), 4.50 (d, *J* = 12.3 Hz, 1H), 4.38 (d, *J* = 12.2 Hz, 1H), 4.11–3.96 (m, 4H), 3.53 (d, *J* = 8.4 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 149.29, 145.69, 142.30, 137.18, 128.94, 128.84, 128.69, 123.98, 73.25, 70.76 (d, *J* = 10.5 Hz), 68.96 (d, *J* = 152.8 Hz), 67.85, 56.63. **^{31}P NMR** (162 MHz, D_2O) δ 16.68. **HRMS** (ESI) *m/z* [M−H]$^-$ calcd for $C_{16}H_{18}O_6N_4P$ 393.09694, found 393.09663. **[α]$^{25}_D$** = −5.4 (c 0.239 g/100 mL, H_2O/MeOH 1/1).

Sodium (*R*)-((3-hydroxy-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*R*)-**24i**)



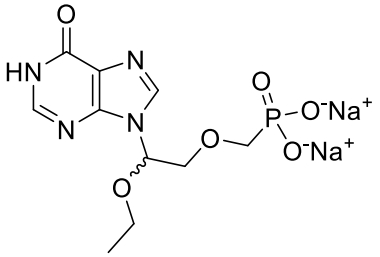
Following standard procedure F, compound (*R*)-**10d** (89 mg, 0.18 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (80 mg, 0.17 mmol) was stirred in anhydrous MeCN (1.7 mL) and TMSBr (170 µL) at 25 °C for 60 h to yield (*R*)-**24i** (21 mg, 33% over 2 steps) as a white solid (and (*R*)-**24h** (22 mg, 28% over 2 steps) as a white solid). [Note: Shorter reaction time preferentially leads to the cleavage of phosphonate esters while longer reaction time leads also to the derivative with cleaved benzyl group.] **^{1}H NMR** (401 MHz, D_2O) δ 8.36 (s, 1H), 8.18 (s, 1H), 4.90 (tt, *J* = 6.7, 4.8 Hz, 1H), 4.15–3.99 (m, 4H), 3.56 (d, *J* = 8.4 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 159.35, 149.77, 146.21, 142.03, 123.78, 71.00 (d, *J* = 10.4 Hz), 69.06 (d, *J* = 152.9 Hz), 61.09, 57.44. **^{31}P NMR** (162 MHz, D_2O) δ 16.71. **HRMS** (ESI) *m/z* [M−H]$^-$ calcd for $C_9H_{12}O_6N_4P$ 303.04999, found 303.04962. **[α]$^{25}_D$** = +3.7 (c 0.214 g/100 mL, H_2O/MeOH 1/1).

Sodium ((2-methoxy-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)ethoxy)methyl)phosphonate ((*RS*)-**24j**)



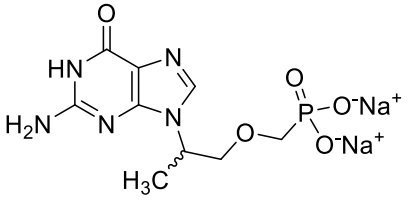
Following standard procedure H, compound (*RS*)-**10j** (281 mg, 0.69 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (150 mg, 0.39 mmol) was stirred in anhydrous pyridine (3.9 mL) and TMSBr (390 µL) at 25 °C for 16 h to yield (*RS*)-**24j** (78 mg, 33% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.25 (s, 1H), 8.12 (s, 1H), 5.80 (t, J = 5.2 Hz, 1H), 4.04 (dd, J = 11.0, 5.4 Hz, 1H), 3.99 (dd, J = 11.0, 5.1 Hz, 1H), 3.62–3.53 (m, 2H), 3.29 (s, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 159.33, 149.55, 146.63, 141.34, 124.30, 85.38, 72.84 (d, J = 10.9 Hz), 68.52 (d, J = 155.0 Hz), 57.13. **^{31}P NMR** (162 MHz, D_2O) δ 17.42. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{12}O_6N_4P$ 303.04999, found 303.05003.

Sodium ((2-ethoxy-2-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)ethoxy)methyl)phosphonate ((*RS*)-**24k**)



Following standard procedure H, compound (*RS*)-**10k** (555 mg, 1.32 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (250 mg, 0.62 mmol) was stirred in anhydrous pyridine (6.2 mL) and TMSBr (620 µL) at 25 °C for 16 h to yield (*RS*)-**24k** (94 mg, 20% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.35 (s, 1H), 8.19 (s, 1H), 5.98 (t, J = 5.1 Hz, 1H), 4.14–4.02 (m, 2H), 3.72–3.66 (m, 1H), 3.66–3.61 (m, 2H), 3.52 (dq, J = 9.5, 7.1 Hz, 1H), 1.15 (t, J = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 159.30, 149.46, 146.59, 141.33, 124.15, 83.76, 73.04 (d, J = 11.1 Hz), 68.63 (d, J = 154.8 Hz), 66.25, 14.46. **^{31}P NMR** (162 MHz, D_2O) δ 17.31. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{14}O_6N_4P$ 317.06564, found 317.06561.

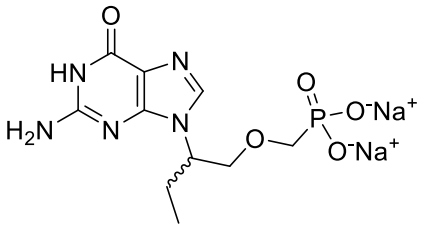
Sodium ((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)propoxy)methyl)phosphonate ((*RS*)-**25a**)



Following standard procedure F, compound (*RS*)-**18a** (219 mg, 0.54 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (160 mg, 0.41 mmol) was stirred in anhydrous MeCN (4.1 mL) and TMSBr (410 µL) at 25 °C for 16 h to yield (*RS*)-**25a** (115 mg, 61% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 7.97 (d, J = 0.5 Hz, 1H), 4.74–4.64 (m, 1H), 3.94 (dd, J = 10.8, 7.5 Hz,

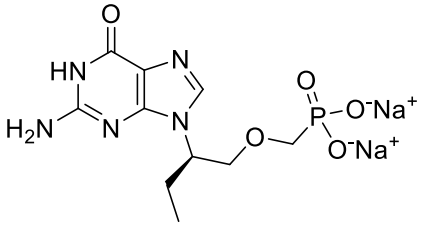
1H), 3.89 (dd, *J* = 10.8, 4.6 Hz, 1H) 3.63–3.52 (m, 1H), 1.50 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 159.60, 154.20, 152.02, 138.99, 116.46, 75.06 (d, *J* = 10.0 Hz), 68.55 (d, *J* = 153.9 Hz), 50.87, 17.01. **^{31}P NMR** (162 MHz, D_2O) *δ* 17.32. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{13}O_5N_5P$ 302.06598, found 302.06579.

Sodium ((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*RS*)-**25b**)



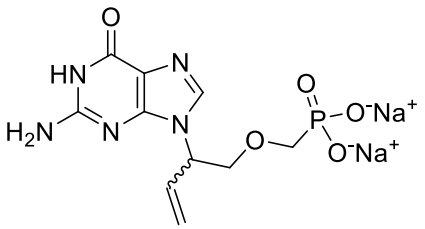
Following standard procedure F, compound (*RS*)-**18b** (327 mg, 0.78 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (269 mg, 0.67 mmol) was stirred in anhydrous MeCN (6.7 mL) and TMSBr (670 µL) at 25 °C for 16 h to yield (*RS*)-**25b** (161 mg, 57% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) *δ* 7.97 (s, 1H), 4.50 (dddd, *J* = 8.9, 7.6, 6.0, 4.1 Hz, 1H), 4.04–3.87 (m, 2H), 3.67–3.52 (m, 2H), 2.00–1.81 (m, 2H), 0.80 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 159.65, 154.24, 152.60, 139.38, 116.42, 74.23 (d, *J* = 10.3 Hz), 67.93 (d, *J* = 156.1 Hz), 56.88, 24.44, 10.08. **^{31}P NMR** (162 MHz, D_2O) *δ* 18.04. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{15}O_5N_5P$ 316.08163, found 316.08138.

Sodium (*R*)-((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**25b**)



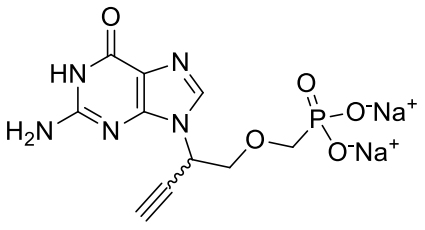
Following standard procedure F, compound (*R*)-**11** (252 mg, 0.53 mmol) was converted to 6-oxo-2-amino derivative. Following standard procedure G, the 6-oxo-2-amino derivative (160 mg, 0.40 mmol) was stirred in anhydrous MeCN (4.0 mL) and TMSBr (400 µL) at 25 °C for 16 h to yield (*R*)-**25b** (55 mg, 29% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) *δ* 7.98 (s, 1H), 4.56–4.44 (m, 1H), 4.03–3.89 (m, 2H), 3.49 (d, *J* = 8.3 Hz, 2H), 2.00–1.79 (m, 2H), 0.78 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 159.76, 154.34, 152.57, 139.39, 116.45, 73.99 (d, *J* = 9.5 Hz), 69.59 (d, *J* = 150.1 Hz), 57.04, 24.68, 10.03. **^{31}P NMR** (162 MHz, D_2O) *δ* 16.04. **HRMS** (ESI) *m/z* $[M-H]^-$calcd for $C_{10}H_{15}O_5N_5P$ 316.08163, found 316.08124. **$[\alpha]^{25}_D$** = +23.4 (c 0.513 g/100 mL, H_2O/MeOH 1/1).

Sodium (((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)but-3-en-1-yl)oxy)methyl)phosphonate ((*RS*)-**25c**)



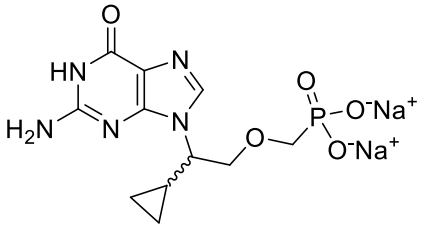
Following standard procedure F, compound (*RS*)-**18c** (167 mg, 0.40 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (52 mg, 0.13 mmol) was stirred in anhydrous MeCN (1.3 mL) and TMSBr (130 µL) at 25 °C for 16 h to yield (*RS*)-**25c** (37 mg, 26% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 7.99 (s, 1H), 6.11 (ddd, J = 17.2, 10.6, 5.2 Hz, 1H), 5.33 (dd, J = 10.7, 1.7 Hz, 1H), 5.21 (dtt, J = 8.1, 4.8, 1.8 Hz, 1H), 5.03 (dd, J = 17.3, 1.7 Hz, 1H), 4.12 (dd, J = 10.9, 7.8 Hz, 1H), 4.06 (dd, J = 10.9, 4.6 Hz, 1H), 3.68–3.59 (m, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 159.58, 154.31, 152.14, 139.52, 133.42, 118.97, 116.39, 73.13 (d, J = 10.2 Hz), 68.37 (d, J = 154.6 Hz), 56.77. **^{31}P NMR** (162 MHz, D_2O) δ 17.36. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{13}O_5N_5P$ 314.06598, found 314.06576.

Sodium (((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)but-3-yn-1-yl)oxy)methyl)phosphonate ((*RS*)-**25d**)



Following standard procedure F, compound (*RS*)-**18d** (166 mg, 0.40 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (55 mg, 0.14 mmol) was stirred in anhydrous MeCN (1.4 mL) and TMSBr (140 µL) at 25 °C for 16 h to yield (*RS*)-**25d** (38 mg, 27% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.06 (s, 1H), 5.48 (ddd, J = 7.0, 4.8, 2.5 Hz, 1H), 4.16–4.03 (m, 2H), 3.73–3.56 (m, 2H), 3.02 (d, J = 2.5 Hz, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 159.53, 154.41, 151.63, 139.33, 116.44, 78.01, 76.71, 73.73 (d, J = 10.3 Hz), 69.15 (d, J = 152.6 Hz), 46.53. **^{31}P NMR** (162 MHz, D_2O) δ 16.59. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{11}O_5N_5P$ 312.05033, found 312.05008.

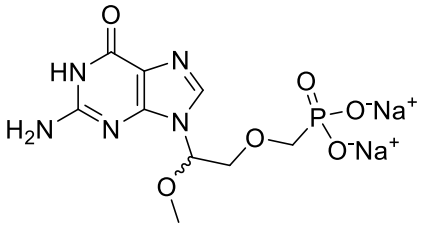
Sodium ((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2-cyclopropylethoxy)methyl)-phosphonate ((*RS*)-**25e**)



Following standard procedure F, compound (*RS*)-**18e** (181 mg, 0.42 mmol) was converted to 6-oxo derivative. Following standard procedure G, the 6-oxo derivative (125 mg, 0.30 mmol) was stirred in anhydrous MeCN (3.0 mL) and TMSBr (300 µL) at 25 °C for 16 h to yield (*RS*)-**25e** (78 mg, 50% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.06 (s, 1H), 4.15–3.99 (m, 2H), 3.82

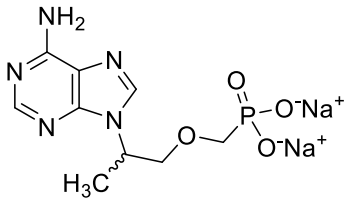
(ddd, J = 9.9, 7.6, 4.0 Hz, 1H), 3.68–3.50 (m, 2H), 1.46 (dtt, J = 10.0, 7.9, 5.0 Hz, 1H), 0.85–0.70 (m, 1H), 0.54–0.47 (m, 2H), 0.33–0.22 (m, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 152.10, 139.67, 116.27, 74.21 (d, J = 10.1 Hz), 68.28 (d, J = 155.2 Hz), 60.43, 12.47, 5.05, 3.16. **^{31}P NMR** (162 MHz, D_2O) δ 17.73. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{15}O_5N_5P$ 328.08163, found 328.08163.

Sodium ((2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2-methoxyethoxy)methyl)phosphonate ((*RS*)-**25f**)



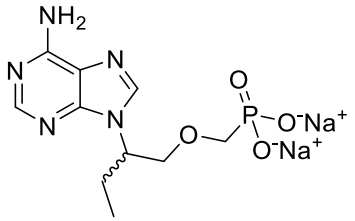
Following standard procedure H, compound (*RS*)-**19** (76 mg, 0.18 mmol) was converted to 6-oxo derivative. Following standard procedure G, 6-oxo derivative (30 mg, 0.07 mmol) was stirred in anhydrous pyridine (0.7 mL) and TMSBr (70 µL) at 25 °C for 16 h to yield (*RS*)-**25f** (7 mg, 11% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 7.99 (s, 1H), 5.68 (t, J = 5.3 Hz, 1H), 4.15–3.95 (m, 2H), 3.70–3.60 (m, 2H), 3.34 (s, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 159.70, 152.41, 138.91, 116.70, 84.54, 72.79 (d, J = 10.7 Hz), 68.56 (d, J = 155.2 Hz), 56.86. **^{31}P NMR** (162 MHz, D_2O) δ 17.43. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{13}O_6N_5P$ 318.06089, found 318.06067.

Sodium ((2-(adenin-9-yl)propoxy)methyl)phosphonate ((*RS*)-**26a**)[48]



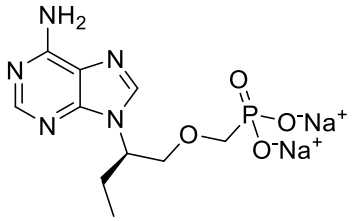
Following standard procedure K, compound (*RS*)-**10e** (156 mg, 0.40 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (115 mg, 0.31 mmol) was stirred in anhydrous MeCN (3.1 mL) and TMSBr (310 µL) at 25 °C for 16 h to yield (*RS*)-**26a** (81 mg, 61% over 2 steps) as an off-white solid. **^{1}H NMR** (500 MHz, D_2O) δ 8.28 (s, 1H), 8.15 (s, 1H), 4.87 (pd, J = 7.1, 4.2 Hz, 1H), 4.02 (dd, J = 10.9, 7.5 Hz, 1H), 3.93 (dd, J = 10.9, 4.2 Hz, 1H), 3.61–3.54 (m, 2H), 1.57 (d, J = 7.0 Hz, 3H). **^{13}C NMR** (126 MHz, D_2O) δ 155.85, 152.52, 149.27, 141.74, 119.01, 74.83 (d, J = 10.5 Hz), 68.21 (d, J = 155.3 Hz), 51.64, 16.87. **^{31}P NMR** (202 MHz, D_2O) δ 15.74. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{13}O_4N_5P$ 286.07106, found 286.07097.

Sodium ((2-(adenin-9-yl)butoxy)methyl)phosphonate ((*RS*)-**26b**)



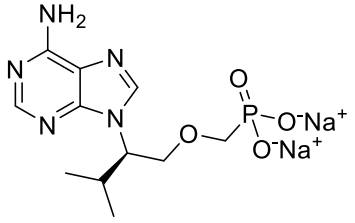
Following standard procedure K, compound (*RS*)-**10a** (134 mg, 0.33 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (40 mg, 0.10 mmol) was stirred in anhydrous MeCN (1.0 mL) and TMSBr (100 µL) at 25°C for 16 h to yield (*RS*)-**26b** (29 mg, 25 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.31 (s, 1H), 8.21 (s, 1H), 4.72–4.64 (m, 1H), 4.09 (dd, J = 11.0, 7.8 Hz, 1H), 3.96 (dd, J = 11.0, 3.9 Hz, 1H), 3.66–3.52 (m, 2H), 2.05–1.93 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 155.54, 151.99, 149.78, 142.40, 119.01, 73.95 (d, J = 10.8 Hz), 67.77 (d, J = 156.3 Hz), 57.76, 24.44, 10.11. **^{31}P NMR** (162 MHz, D_2O) δ 18.13. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{15}O_4N_5P$ 300.08671, found 300.08696.

Sodium (*R*)-((2-(adenin-9-yl)butoxy)methyl)phosphonate ((*R*)-**26b**)



Following standard procedure K, compound (*R*)-**10a** (328 mg, 0.81 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (250 mg, 0.65 mmol) was stirred in anhydrous MeCN (6.5 mL) and TMSBr (650 µL) at 25°C for 16 h to yield (*R*)-**26b** (185 mg, 66 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.31 (s, 1H), 8.19 (s, 1H), 4.72–4.61 (m, 1H), 4.07 (dd, J = 11.0, 7.8 Hz, 1H), 3.97 (dd, J = 11.0, 4.1 Hz, 1H), 3.52 (d, J = 8.3 Hz, 2H), 2.06–1.90 (m, 2H), 0.78 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 156.10, 152.79, 149.83, 142.24, 119.06, 73.85 (d, J = 10.0 Hz), 68.88 (d, J = 153.1 Hz), 57.78, 24.58, 10.14. **^{31}P NMR** (162 MHz, D_2O) δ 16.86. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{15}O_4N_5P$ 300.08671, found 300.08661. $[\alpha]^{25}_D$ = + 6.5 (c 0.294 g/100 mL, H_2O/MeOH 1/1).

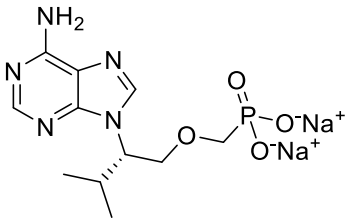
Sodium (*R*)-((2-(Adenin-9-yl)-3-methylbutoxy)methyl)phosphonate ((*R*)-**26c**)



Following standard procedure K, compound (*R*)-**10b** (209 mg, 0.50 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (120 mg, 0.30 mmol) was stirred in anhydrous MeCN (3.0 mL) and TMSBr (300 µL) at 25°C for 16 h to yield (*R*)-**26c** (85 mg, 47 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.36 (s, 1H), 8.20 (s, 1H), 4.54–4.39 (m, 1H), 4.17 (dd, J = 11.1, 8.1 Hz, 1H), 4.03 (dd, J = 11.0, 3.4 Hz, 1H), 3.50 (d, J = 7.7 Hz, 2H), 2.41–

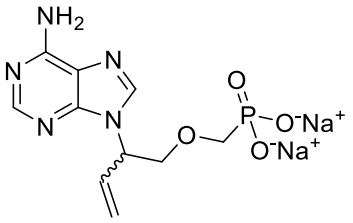
2.27 (m, 1H), 1.04 (d, J = 6.7 Hz, 3H), 0.70 (d, J = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 156.19, 152.86, 149.57, 142.56, 72.29 (d, J = 9.5 Hz), 68.94 (d, J = 153.1 Hz), 61.82, 30.42, 19.56, 19.01. **^{31}P NMR** (162 MHz, D_2O) δ 16.78. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{17}O_4N_5P$ 314.10236, found 314.10231. **$[\alpha]^{25}_D$** = + 8.4 (c 0.380 g/100 mL, H_2O/MeOH 1/1).

Sodium (*S*)-((2-(Adenin-9-yl)-3-methylbutoxy)methyl)phosphonate ((*S*)-**26c**)



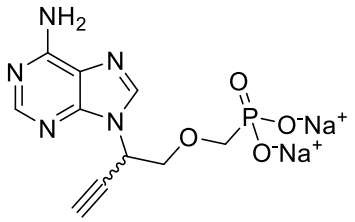
Following standard procedure K, compound (*S*)-**10b** (209 mg, 0.50 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (120 mg, 0.30 mmol) was stirred in anhydrous MeCN (3.0 mL) and TMSBr (300 µL) at 25°C for 16 h to yield (*S*)-**26c** (80 mg, 44 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.36 (s, 1H), 8.21 (s, 1H), 4.50–4.41 (m, 1H), 4.16 (dd, J = 11.1, 8.2 Hz, 1H), 4.03 (dd, J = 11.1, 3.5 Hz, 1H), 3.47 (d, J = 8.3 Hz, 2H), 2.41–2.28 (m, 1H), 1.05 (d, J = 6.7 Hz, 3H), 0.71 (d, J = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 156.25, 152.92, 150.12, 142.57, 118.30, 72.28 (d, J = 9.7 Hz), 69.16 (d, J = 151.9 Hz), 61.85, 30.43, 19.59, 19.00. **^{31}P NMR** (162 MHz, D_2O) δ 16.47. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{17}O_4N_5P$ 314.10236, found 314.10223. **$[\alpha]^{25}_D$** = – 3.5 (c 0.317 g/100 mL, H_2O/MeOH 1/1).

Sodium (((2-(Adenin-9-yl)but-3-en-1-yl)oxy)methyl)phosphonate ((*RS*)-**26d**)



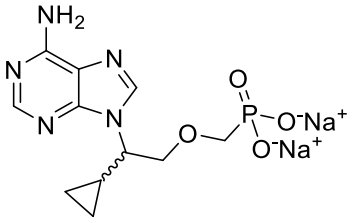
Following standard procedure K, compound (*RS*)-**10f** (205 mg, 0.51 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (70 mg, 0.18 mmol) was stirred in anhydrous MeCN (1.8 mL) and TMSBr (180 µL) at 25°C for 16 h to yield (*RS*)-**26d** (50 mg, 29 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.30 (s, 1H), 8.14 (s, 1H), 6.15 (ddd, J = 17.3, 10.5, 5.5 Hz, 1H), 5.40–5.36 (m, 1H), 5.34 (dd, J = 10.5, 1.7 Hz, 1H), 5.06 (dd, J = 17.3, 1.7 Hz, 1H), 4.20 (dd, J = 10.9, 7.9 Hz, 1H), 4.10 (dd, J = 11.0, 4.3 Hz, 1H), 3.71–3.60 (m, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 155.75, 152.52, 149.31, 142.22, 133.00, 119.34, 118.88, 73.04 (d, J = 11.0 Hz), 67.93 (d, J = 155.5 Hz), 57.51. **^{31}P NMR** (162 MHz, D_2O) δ 17.84. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{13}O_4N_5P$ 298.07106, found 298.07114.

Sodium (((2-(adenin-9-yl)but-3-yn-1-yl)oxy)methyl)phosphonate ((*RS*)-**26e**)



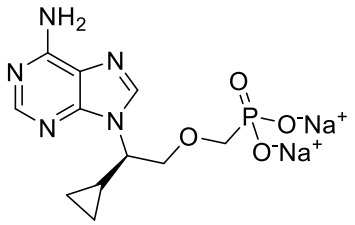
Compound (*RS*)-**10g** (236 mg, 0.59 mmol) and *p*-methoxybenzylamine were stirred in MeOH at 50°C for 2 h. The mixture was concentrated, then dissolved in TFA and stirred at 50°C for 48 h. The mixture was concentrated, co-distilled with H_2O twice and with EtOH once. The residue was separated using silica gel chromatography (linear gradient elution 0–20 % MeOH in $CHCl_3$) to obtain 6-amino derivative. Following standard procedure G, the 6-amino derivative (130 mg, 0.34 mmol) was stirred in anhydrous MeCN (3.4 mL) and TMSBr (340 µL) at 25°C for 16 h to yield (*RS*)-**26e** (55 mg, 27 % over 3 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.36 (s, 1H), 8.19 (d, J = 1.0 Hz, 1H), 5.65 (ddd, J = 7.0, 4.5, 2.5 Hz, 1H), 4.17 (dd, J = 10.9, 7.0 Hz, 1H), 4.09 (dd, J = 10.9, 4.5 Hz, 1H), 3.65–3.60 (m, 2H), 3.06 (d, J = 2.5 Hz, 1H) . **^{13}C NMR** (101 MHz, D_2O) δ 156.06, 152.92, 148.86, 142.14, 119.10, 77.45, 77.10, 73.67 (d, J = 10.5 Hz), 68.30 (d, J = 156.0 Hz), 47.26. **^{31}P NMR** (162 MHz, D_2O) δ 17.50. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{10}H_{11}O_4N_5P$ 296.05541, found 296.05552.

Sodium ((2-(adenin-9-yl)-2-cyclopropylethoxy)methyl)phosphonate ((*RS*)-**26f**)



Following standard procedure K, compound (*RS*)-**10c** (100 mg, 0.24 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (68 mg, 0.17 mmol) was stirred in anhydrous MeCN (1.7 mL) and TMSBr (170 µL) at 25°C for 16 h to yield (*RS*)-**26f** (46 mg, 53 % over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.39 (s, 1H), 8.20 (s, 1H), 4.20 (dd, J = 10.6, 8.0 Hz, 1H), 4.08 (dd, J = 10.6, 3.6 Hz, 1H), 4.01–3.94 (m, 1H), 3.56 (d, J = 6.8 Hz, 2H), 1.61–1.48 (m, 1H), 0.82–0.72 (m, 1H), 0.60–0.48 (m, 2H), 0.37–0.22 (m, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 156.01, 152.87, 149.49, 142.50, 119.31, 73.96 (d, J = 11.1 Hz), 61.22, 12.49, 5.05, 3.29. **^{31}P NMR** (162 MHz, D_2O) δ 15.89. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{15}O_4N_5P$ 312.08671, found 312.08661.

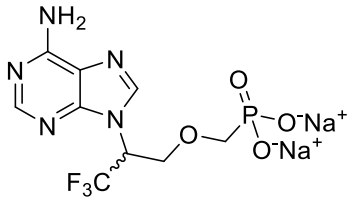
Sodium (*R*)-((2-(adenin-9-yl)-2-cyclopropylethoxy)methyl)phosphonate ((*R*)-**26f**)



Following standard procedure K, compound (*R*)-**10c** (258 mg, 0.62 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (200 mg, 0.50 mmol) was stirred in anhydrous MeCN (5.0 mL) and TMSBr (500 µL) at 25°C for 16 h to yield (*R*)-**26f** (153 mg, 69 % over 2 steps) as an off-white

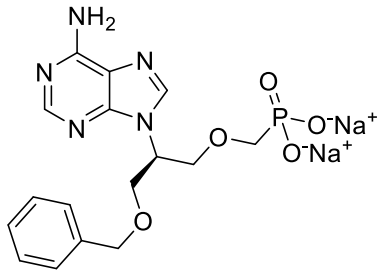
solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.29 (s, 1H), 8.08 (s, 1H), 4.10 (dd, J = 11.0, 7.7 Hz, 1H), 3.99 (dd, J = 11.0, 3.8 Hz, 1H), 3.94–3.84 (m, 1H), 3.44 (d, J = 8.4 Hz, 2H), 1.55–1.38 (m, 1H), 0.74–0.62 (m, 1H), 0.50–0.35 (m, 2H), 0.24–0.12 (m, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 156.05, 152.73, 149.54, 142.34, 119.01, 73.93 (d, J = 10.3 Hz), 68.74 (d, J = 154.0 Hz), 61.21, 12.58, 5.02, 3.29. **^{31}P NMR** (162 MHz, D_2O) δ 17.17. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{15}O_4N_5P$ 312.08671, found 312.08679. **$[\alpha]^{25}_D$** = + 13.0 (c 0.261 g/100 mL, H_2O/MeOH 1/1).

Sodium ((2-(adenin-9-yl)-3,3,3-trifluoropropoxy)methyl)phosphonate ((*RS*)-**26g**)



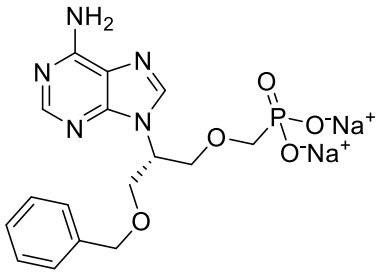
Following standard procedure K, compound (*RS*)-**10i** (70 mg, 0.16 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (32 mg, 0.08 mmol) was stirred in anhydrous MeCN (0.8 mL) and TMSBr (80 µL) at 25°C for 16 h to yield (*RS*)-**26g** (26 mg, 42 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.35 (s, 1H), 8.21 (s, 1H), 4.67 (dd, J = 15.1, 4.1 Hz, 1H), 4.57 (dd, J = 15.1, 6.2 Hz, 1H), 4.48–4.36 (m, 1H), 3.81 (dd, J = 12.2, 9.4 Hz, 1H), 3.55 (dd, J = 12.2, 9.3 Hz, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 156.16, 153.10, 149.59, 144.16, 124.71 (d, J = 284.1 Hz), 118.67, 77.46–76.93 (m), 71.03 (d, J = 151.2 Hz), 42.46. **^{19}F NMR** (377 MHz, D_2O) δ -75.41 (d, J = 6.0 Hz). **^{31}P NMR** (162 MHz, D_2O) δ 14.65. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{10}O_4N_5F_3P$ 340.04280, found 340.04263.

Sodium (*R*)-((2-(adenin-9-yl)-3-(benzyloxy)propoxy)methyl)phosphonate ((*R*)-**26h**)



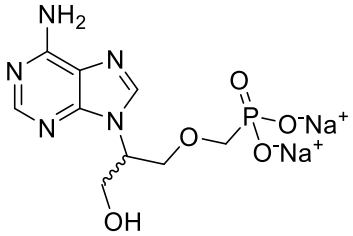
Following standard procedure K, compound (*R*)-**10d** (65 mg, 0.13 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (50 mg, 0.10 mmol) was stirred in anhydrous MeCN (1.0 mL) and TMSBr (100 µL) at 25°C for 16 h to yield (*R*)-**26h** (32 mg, 56 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.21 (s, 1H), 8.00 (s, 1H), 7.18–7.07 (m, 3H), 6.95–6.90 (m, 2H), 4.91–4.83 (m, 1H), 4.42 (dd, J = 41.4, 12.3 Hz, 2H), 4.12–3.97 (m, 4H), 3.49–3.43 (m, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 156.00, 152.47, 149.29, 142.66, 137.00, 128.89–128.49 (m), 119.18, 73.16, 70.56 (d, J = 2.6 Hz), 69.86 (d, J = 157.7 Hz), 67.63, 56.35. **^{31}P NMR** (162 MHz, D_2O) δ 15.73. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{16}H_{18}O_5N_5NaP$ 414.09487, found 414.09406. **$[\alpha]^{25}_D$** = + 10.2 (c 0.254 g/100 mL, H_2O/MeOH 1/1).

Sodium (*S*)-((2-(adenin-9-yl)-3-(benzyloxy)propoxy)methyl)phosphonate ((*S*)-**26h**)



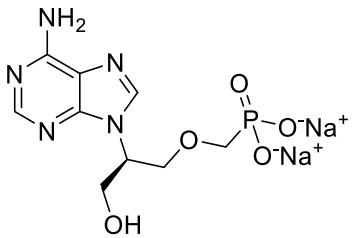
Following standard procedure K, compound (*S*)-**10d** (184 mg, 0.37 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (160 mg, 0.34 mmol) was stirred in anhydrous MeCN (3.4 mL) and TMSBr (340 µL) at 25°C for 16 h to yield (*S*)-**26h** (114 mg, 70 % over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.21 (s, 1H), 8.02 (s, 1H), 7.22–7.10 (m, 3H), 6.98–6.92 (m, 2H), 4.92–4.84 (m, 1H), 4.43 (dd, J = 40.7, 12.2 Hz, 2H), 4.12–3.98 (m, 4H), 3.50 (dd, J = 8.5, 1.1 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 156.02, 152.51, 149.34, 142.59, 137.08, 128.86–128.61 (m), 119.15, 73.19, 70.71 (d, J = 10.6 Hz), 69.25 (d, J = 152.4 Hz), 67.72, 56.24. **^{31}P NMR** (162 MHz, D_2O) δ 16.39. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{20}O_5N_5NaP$ 416.10943, found 416.10931. **$[\alpha]^{25}_D$** = – 8.8 (c 0.295 g/100 mL, H_2O/MeOH 1/1).

Sodium ((2-(adenin-9-yl)-3-hydroxypropoxy)methyl)phosphonate ((*RS*)-**26i**)[120]



Following standard procedure K, compound (*RS*)-**10h** (143 mg, 0.28 mmol) was converted to the 6-amino derivative. Following standard procedure G, the 6-amino derivative (55 mg, 0.14 mmol) was stirred in anhydrous MeCN (1.4 mL) and TMSBr (140 µL) at 25°C for 16 h to yield (*RS*)-**26i** (36 mg, 37 % over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.32 (s, 1H), 8.14 (s, 1H), 4.91–4.82 (m, 1H), 4.09–3.90 (m, 4H), 3.65 (d, J = 8.7 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 155.70, 152.52, 149.58, 142.23, 118.75, 71.10 (d, J = 11.7 Hz), 68.12 (d, J = 155.5 Hz), 60.95, 57.18. **^{31}P NMR** (162 MHz, D_2O) δ 15.85. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_9H_{13}O_5N_5P$ 302.06598, found 302.06592.

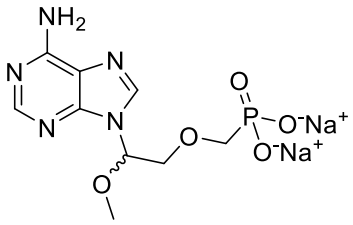
Sodium (*R*)-((adenin-9-yl)-3-hydroxypropoxy)methyl)phosphonate ((*R*)-**26i**)



Following standard procedure K, compound (*R*)-**10d** (243 mg, 0.49 mmol) was converted to the 6-amino derivative. This compound (186 mg, 0.39 mmol) was dissolved in AcOH and catalytic amount of Pd/C was added under the stream of argon. The mixture was stirred under H_2 atmosphere at 25°C for 1 h. The mixture was filtered, concentrated, co-distilled twice with H_2O, to afford deprotected hydroxy derivative. Following standard procedure G, the 6-amino derivative (150 mg, 0.39 mmol) was stirred in anhydrous MeCN (3.9 mL) and TMSBr (390 µL) at 25°C for 16 h to yield (*R*)-**26i**

(46 mg, 27 % over 3 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.39 (s, 1H), 8.21 (s, 1H), 4.90–4.82 (m, 1H), 4.16–3.98 (m, 4H), 3.51 (d, J = 8.3 Hz, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 156.16, 152.95, 149.76, 142.45, 118.95, 70.86 (d, J = 9.8 Hz), 69.72 (d, J = 150.6 Hz), 60.98, 57.08. **^{31}P NMR** (162 MHz, D_2O) δ 16.03. **HRMS** (ESI) *m/z* [M-H]$^-$ calcd for $C_9H_{13}O_5N_5P$ 302.06598, found 302.06591. **$[\alpha]^{25}_D$** = + 7.6 (c 0.261 g/100 mL, H_2O/MeOH 1/1).

Sodium ((2-(adenin-9-yl)-2-methoxyethoxy)methyl)phosphonate ((*RS*)-**26j**)



Following standard procedure K, compound (*RS*)-**10j** (480 mg, 1.18 mmol) was converted to the 6-amino derivative which was separated to (*R*) and (*S*) enantiomers using chiral HPLC (isocratic elution, 78:22 *n*-heptane/*i*PrOH containing 0.3% of DEA, YMC CHIRAL ART Cellulose SC (5 μm, 20 × 250 mm) column). Following standard procedure G, the racemic 6-amino derivative (320 mg, 0.83 mmol) was stirred in anhydrous pyridine (8.3 mL) and TMSBr (830 μL) at 25°C for 16 h to yield (*RS*)-**26j** (207 mg, 50 % over 2 steps) as an off-white solid. The (*R*) and (*S*) enantiomers were prepared in the same manner in 63% and 63% yield, respectively.

Spectral data and characterization of (*RS*)-**26j**:

^{1}H NMR (401 MHz, D_2O) δ 8.34 (s, 1H), 8.11 (s, 1H), 5.84 (t, J = 5.1 Hz, 1H), 4.20–4.11 (m, 2H), 3.82 (d, J = 8.7 Hz, 2H), 3.33 (s, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 155.26, 152.36, 149.25, 141.49, 118.62, 84.80, 72.68 (d, J = 11.7 Hz), 68.42 (d, J = 156.3 Hz), 57.17. **^{31}P NMR** (162 MHz, D_2O) δ 15.79. **HRMS** (ESI) *m/z* [M-H]$^-$ calcd for $C_9H_{13}O_5N_5P$ 302.06598, found 302.06584.

Spectral data and characterization of (*R*)-**26j**:

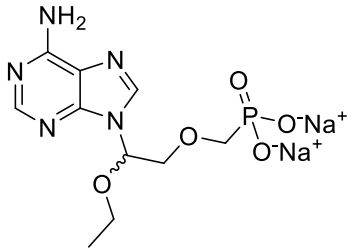
^{1}H NMR (401 MHz, D_2O) δ 8.39 (s, 1H), 8.23 (s, 1H), 5.86 (dd, J = 5.6, 4.6 Hz, 1H), 4.11 (dd, J = 10.9, 5.6 Hz, 1H), 4.04 (dd, J = 10.9, 4.7 Hz, 1H), 3.54 (d, J = 8.6 Hz, 2H), 3.34 (s, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 156.32, 153.38, 149.72, 141.71, 119.13, 84.85, 72.77 (d, J = 11.0 Hz), 70.22 (d, J = 149.7 Hz), 56.95. **^{31}P NMR** (162 MHz, D_2O) δ 15.52. **HRMS** (ESI) *m/z* [M-H]$^-$ calcd for $C_9H_{13}O_5N_5P$ 302.06598, found 302.06565. **$[\alpha]^{25}_D$** = + 14.3 (c 0.300 g/100 mL, H_2O/MeOH 1/1).

Spectral data and characterization of (*S*)-**26j**:

^{1}H NMR (401 MHz, D_2O) δ 8.39 (s, 1H), 8.23 (s, 1H), 5.86 (dd, J = 5.6, 4.6 Hz, 1H), 4.11 (dd, J = 10.9, 5.6 Hz, 1H), 4.03 (dd, J = 10.9, 4.6 Hz, 1H), 3.54 (d, J = 8.6 Hz, 2H), 3.34 (s, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 156.31, 153.37, 149.71, 141.71, 119.11, 84.85, 72.77 (d, J = 11.0 Hz), 70.25 (d, J = 149.7 Hz), 56.95. **^{31}P NMR** (162 MHz, D_2O) δ 15.50.

HRMS (ESI) *m/z* [M-H]$^-$ calcd for $C_9H_{13}O_5N_5P$ 302.06598, found 302.06550. **[α]$^{25}_D$** = – 7.5 (c 0.279 g/100 mL, H_2O/MeOH 1/1).

Sodium ((2-(adenin-9-yl)-2-ethoxyethoxy)methyl)phosphonate ((*RS*)-**26k**)



Following standard procedure K, compound (*RS*)-**10k** (105 mg, 0.25 mmol) was converted to the 6-amino derivative which was separated to (*R*) and (*S*) enantiomers using chiral HPLC (isocratic elution, 78:22 *n*-heptane/*i*PrOH containing 0.3% of DEA, YMC CHIRAL ART Cellulose SC (5 µm, 20 × 250 mm) column). Following standard procedure G, the racemic 6-amino derivative (70 mg, 0.17 mmol) was stirred in anhydrous pyridine (1.7 mL) and TMSBr (170 µL) at 25°C for 16 h to yield (*RS*)-**26k** (21 mg, 23 % over 2 steps) as an off-white solid. The (*R*) and (*S*) enantiomers were prepared in the same manner in 63% and 65% yield, respectively.

Spectral data and characterization of (*RS*)-**26k**:

^{1}H NMR (401 MHz, DMSO) *δ* 8.33 (s, 1H), 8.11 (s, 1H), 5.80 (t, *J* = 5.0 Hz, 1H), 3.98–3.85 (m, 2H), 3.55–3.20 (m, 4H), 0.99 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO) *δ* 156.56, 153.82, 150.35, 141.78, 119.08, 83.34, 73.12–72.71 (m), 70.31 (d, *J* = 151.0 Hz), 65.90, 15.50. **^{31}P NMR** (162 MHz, DMSO) *δ* 14.98. **HRMS** (ESI) *m/z* [M-H]$^-$ calcd for $C_{10}H_{15}O_5N_5P$ 316.08163, found 316.08194.

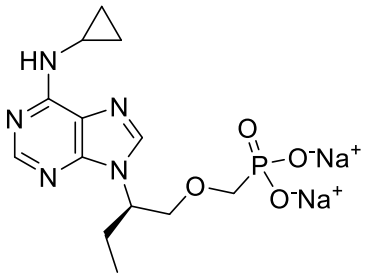
Spectral data and characterization of (*R*)-**26k**:

^{1}H NMR (401 MHz, D_2O) *δ* 8.40 (s, 1H), 8.22 (s, 1H), 5.95 (t, *J* = 5.0 Hz, 1H), 4.13–4.01 (m, 2H), 3.72–3.62 (m, 1H), 3.58 (d, *J* = 8.6 Hz, 2H), 3.53–3.41 (m, 1H), 1.14 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 156.26, 153.32, 149.63, 141.65, 119.01, 83.22, 73.04 (d, *J* = 11.0 Hz), 69.58 (d, *J* = 151.9 Hz), 66.07, 14.48. **^{31}P NMR** (162 MHz, DMSO) *δ* 16.28. **HRMS** (ESI) *m/z* [M-H]$^-$ calcd for $C_{10}H_{15}O_5N_5P$ 316.08163, found 316.08136. **[α]$^{25}_D$** = + 14.8 (c 0.332 g/100 mL, H_2O/MeOH 1/1).

Spectral data and characterization of (*S*)-**26k**:

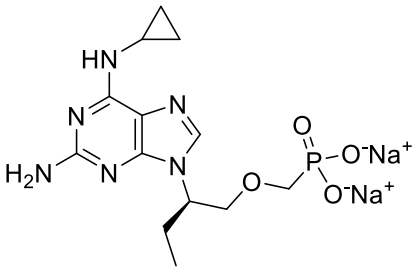
^{1}H NMR (401 MHz, D_2O) *δ* 8.38 (s, 1H), 8.21 (s, 1H), 5.95 (t, *J* = 5.0 Hz, 1H), 4.13–4.02 (m, 2H), 3.72–3.58 (m, 3H), 3.53–3.41 (m, 1H), 1.14 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) *δ* 156.21, 153.27, 149.58, 141.60, 119.00, 83.22, 73.03 (d, *J* = 11.0 Hz), 69.20 (d, *J* = 153.3 Hz), 66.08, 14.48. **^{31}P NMR** (162 MHz, DMSO) *δ* 16.73. **HRMS** (ESI) *m/z* [M-H]$^-$ calcd for $C_{10}H_{15}O_5N_5P$ 316.08163, found 316.08133. **[α]$^{25}_D$** = – 14.8 (c 0.384 g/100 mL, H_2O/MeOH 1/1).

Sodium (*R*)-((2-(6-(cyclopropylamino)-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**27**)



Following standard procedure J, compound (*R*)-**10a** (36 mg, 0.09 mmol) reacted with cyclopropylamine (62 µL, 0.90 mmol) to afford 6-cyclopropylamine derivative. Following standard procedure G, the 6-cyclopropylamine derivative (35 mg, 0.08 mmol) was stirred in anhydrous MeCN (0.8 mL) and TMSBr (80 µL) at 25 °C for 16 h to yield (*R*)-**27** (17 mg, 49% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.28 (s, 1H), 8.25 (s, 1H), 4.72–4.61 (m, 1H), 4.09–3.92 (m, 2H), 3.49–3.40 (m, 2H), 2.86 (s, 1H), 2.09–1.90 (m, 2H), 0.99–0.85 (m, 2H), 0.76 (t, J = 7.4 Hz, 3H), 0.70–0.61 (m, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 156.29, 152.80, 141.84, 119.48, 73.84 (d, J = 9.5 Hz), 69.67 (d, J = 150.4 Hz), 57.82, 24.66, 23.80, 10.15, 7.21. **^{31}P NMR** (162 MHz, D_2O) δ 15.92. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{13}H_{19}O_4N_5P$ 340.11801, found 340.11768. **$[\alpha]^{25}_D$** = +5.6 (c 0.324 g/100 mL, H_2O/MeOH 1/1).

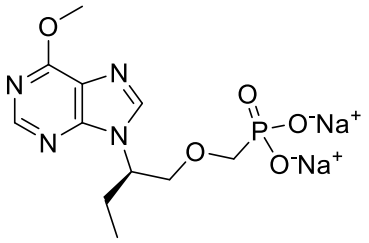
Sodium (*R*)-((2-(2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**28**)



Following standard procedure J, compound (*R*)-**5** (200 mg, 0.42 mmol) reacted with cyclopropylamine (291 µL, 4.20 mmol) to afford 6-cyclopropylamine derivative. To cleave DMAM protecting group, the residue was prior to the separation dissolved in EtOH (2.5 mL) containing 0.25 mL conc. HCl and stirred at 70 °C for 1h. The mixture was neutralized with KOH, concentrated, and separated to afford 2-amino-6-cyclopropylamine derivative. [Note: The yield was considerably lowered due to the formation of 6-dimethylaminopurine derivative (presumably originating from the unstable DMAM protecting group) which was formed approx. in the same amount as the desired 6-cyclopropylamine derivative. Therefore, it might be eligible in this case to remove DMAM group first and then introduce the cyclopropylamine group (or any other amine).] Following standard procedure G, compound 2-amino-6-cyclopropylamine derivative (50 mg, 0.11 mmol) was stirred in anhydrous MeCN (1.1 mL) and TMSBr (110 µL) at 25 °C for 16 h to yield (*R*)-**28** (37 mg, 22% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 7.98 (s, 1H), 4.50 (ddd, J = 12.1, 8.6, 5.1 Hz, 1H), 4.01–3.87 (m, 2H), 3.51–3.42 (m, 2H), 2.84 (bs, 1H), 2.01–1.83 (m, 2H), 0.89–0.82 (m, 2H), 0.77 (t, J = 7.5 Hz, 3H), 0.69–0.61 (m, 2H). **^{13}C NMR** (101 MHz, D_2O) δ 160.71, 156.99, 151.53, 139.01, 113.79, 74.19 (d, J = 9.6 Hz), 69.52 (d, J = 150.9 Hz), 56.78, 24.69, 23.79, 10.11, 7.34. **^{31}P NMR** (162 MHz, D_2O) δ 16.27.

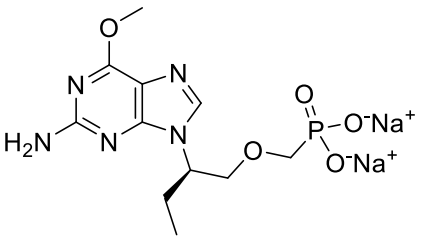
HRMS (ESI) *m/z* $[M-H]^-$ calcd for $C_{13}H_{20}O_4N_6P$ 355.12891, found 355.12827. **$[\alpha]^{25}_D$** = +27.9 (c 0.338 g/100 mL, H_2O/MeOH 1/1).

Sodium (*R*)-((2-(6-methoxy-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**29**)



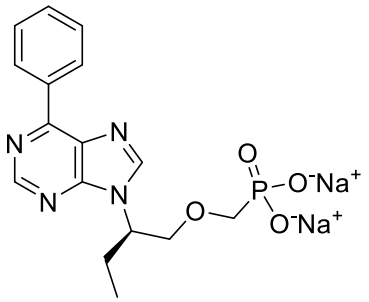
Compound (*R*)-**10a** (9 mg, 0.02 mmol) reacted with MeOK (4 mg, 0.06 mmol) in MeOH (0.5 mL) at 25 °C for 2 h. The mixture was concentrated and the residue purified using silica gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford the 6-methoxy derivative. Following standard procedure G, the 6-methoxy derivative (9 mg, 0.02 mmol) was stirred in anhydrous pyridine (0.2 mL) and TMSBr (20 µL) at 25 °C for 16 h to yield (*R*)-**29** (7 mg, 88% over 2 steps) as a white solid. [Note: The synthetic sequence can be reversed, i.e. the phosphonate isopropyl esters can be first cleaved with TMSBr leading to 6-bromopurine derivative in only 3 h and then the methoxy group can be introduced to the position C6, thus forming compound (*R*)-**29**. **^{1}H NMR** (401 MHz, D_2O) δ 8.49 (s, 1H), 8.48 (s, 1H), 4.76–4.70 (m, 1H), 4.19 (s, 3H), 4.12–3.97 (m, 2H), 3.48–3.39 (m, 2H), 2.08–1.94 (m, 2H), 0.78 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 161.64, 152.27, 152.17, 144.20, 121.25, 73.70 (d, J = 9.3 Hz), 69.78 (d, J = 150.0 Hz), 58.29, 55.51, 24.62, 10.15. **^{31}P NMR** (162 MHz, D_2O) δ 15.74. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{16}O_5N_4P$ 315.08638, found 315.08601. **$[\alpha]^{25}_D$** = +8.9 (c 0.268 g/100 mL, H_2O/MeOH 1/1).

Sodium (*R*)-((2-(2-amino-6-methoxy-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**30**)



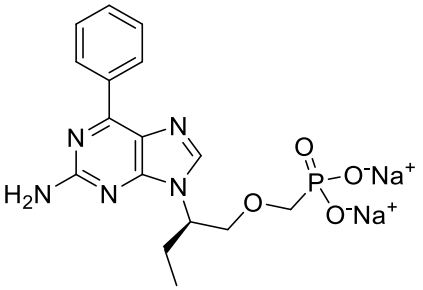
Compound (*R*)-**5** (190 mg, 0.40 mmol) and TMSBr (400 µL) were stirred at 25 °C for 3 h. The mixture was concentrated, co-distilled 2 × with toluene, then re-dissolved in MeOH, and MeOK (84 mg, 1.20 mmol) added. The mixture was stirred at 25 °C for 19 h. Solvents were evaporated and the residue was dissolved in 2M TEAB (2 mL). After evaporation, the residue was separated using C_{18}-reversed phase flash chromatography (linear gradient elution 0–50% MeOH in water). Purified product was taken through Na^+ DOWEX to yield (*R*)-**30** (51 mg, 34% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.09 (s, 1H), 4.61–4.50 (m, 1H), 4.08 (s, 3H), 4.04–3.89 (m, 2H), 3.51 (d, J = 8.3 Hz, 2H), 2.02–1.86 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 162.24, 160.52, 154.28, 140.73, 114.71, 74.07 (d, J = 9.7 Hz), 68.99 (d, J = 152.5 Hz), 57.10, 55.00, 24.53, 10.14. **^{31}P NMR** (162 MHz, D_2O) δ 16.83. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{11}H_{17}O_5N_5P$ 330.09728, found 330.09683. **$[\alpha]^{25}_D$** = +35.6 (c 0.296 g/100 mL, H_2O/MeOH 1/1).

Sodium (*R*)-((2-(6-phenyl-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**31**)



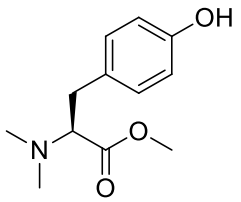
Following standard procedure I, compound (*R*)-**10a** (40 mg, 0.10 mmol) reacted with $PhB(OH)_2$ (18 mg, 0.15 mmol), Cs_2CO_3 (81 mg, 0.25 mmol), and $Pd(PPh_3)_4$ (6 mg, 0.005 mmol) to afford 6-phenyl derivative. Following standard procedure G, the 6-phenyl derivative (45 mg, 0.10 mmol) was stirred in anhydrous MeCN (1.0 mL) and TMSBr (100 μL) at 25 °C for 16 h to yield (*R*)-**31** (19 mg, 46% over 2 steps) as a white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.80 (s, 1H), 8.67 (s, 1H), 8.18–8.08 (m, 2H), 7.64–7.52 (m, 3H), 4.88–4.81 (m, 1H), 4.12 (dd, J = 11.0, 7.3 Hz, 1H), 4.01 (dd, J = 10.9, 4.0 Hz, 1H), 3.55 (d, J = 8.4 Hz, 2H), 2.13–1.98 (m, 2H), 0.84 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 156.02, 152.55, 152.00, 146.98, 134.70, 131.93, 130.98, 130.07, 129.42, 73.58 (d, J = 10.2 Hz), 68.87 (d, J = 153.1 Hz), 57.96, 24.48, 10.31. **^{31}P NMR** (162 MHz, D_2O) δ 16.93. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{16}H_{18}O_4N_4P$ 361.10711, found 361.10672. **$[\alpha]^{25}_D$** = +0.6 (c 0.484 g/100 mL, H_2O/MeOH 1/1).

Sodium (*R*)-((2-(2-amino-6-phenyl-9*H*-purin-9-yl)butoxy)methyl)phosphonate ((*R*)-**32**)



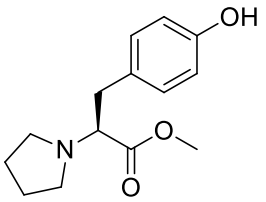
Following standard procedure I, compound (*R*)-**5** (400 mg, 0.84 mmol) reacted with $PhB(OH)_2$ (154 mg, 1.26 mmol), Cs_2CO_3 (684 mg, 2.10 mmol), and $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) to afford 6-phenyl derivative. To cleave DMAM protecting group, the residue was prior to the separation dissolved in EtOH (5 mL) containing 0.5 mL conc. HCl and stirred at 70 °C for 1h. The mixture was neutralized with KOH, concentrated, and separated to afford 2-amino-6-phenyl derivative. Following standard procedure G, 2-amino-6-phenyl derivative (250 mg, 0.54 mmol) was stirred in anhydrous MeCN (5.4 mL) and TMSBr (540 μL) at 25 °C for 16 h to yield (*R*)-**32** (100 mg, 28% over 2 steps) as an off-white solid. **^{1}H NMR** (401 MHz, D_2O) δ 8.30 (s, 1H), 8.14–8.03 (m, 2H), 7.62–7.48 (m, 3H), 4.68–4.57 (m, 1H), 4.11–3.88 (m, 2H), 3.55 (d, J = 8.4 Hz, 2H), 2.09–1.87 (m, 2H), 0.83 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 160.31, 157.46, 154.69, 143.88, 134.97, 131.70, 129.86, 129.25, 125.02, 73.85 (d, J = 9.7 Hz), 68.87 (d, J = 153.2 Hz), 56.93, 24.42, 10.24. **^{31}P NMR** (162 MHz, D_2O) δ 17.03. **HRMS** (ESI) *m/z* $[M-H]^-$ calcd for $C_{16}H_{19}O_4N_5P$ 376.11801, found 376.11746. **$[\alpha]^{25}_D$** = +27.4 (c 0.334 g/100 mL, H_2O/MeOH 1/1).

N,N-Dimethyl-L-tyrosine methyl ester (**33a**)



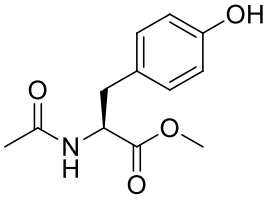
L-Tyrosine methyl ester (500 mg, 2.56 mmol), CH_2O (37% in H_2O, 1.27 mL, 10.24 mmol), and Na_2SO_4 (3.64 g, 25.60 mmol) were mixed in MeOH (15 mL) under argon atmosphere. AcOH (366 µL, 6.40 mmol) and $NaBH_3CN$ (643 mg, 10.24 mmol) were added and the mixture was stirred at 25 °C for 1 h, filtered, concentrated, and separated using silica using gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford **33a** (568 mg, 99%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.17 (bs, 1H), 7.00–6.90 (m, 2H), 6.69–6.57 (m, 2H), 3.53 (s, 3H), 3.33 (dd, J = 9.0, 6.4 Hz, 1H), 2.81 (dd, J = 13.5, 9.0 Hz, 1H), 2.71 (dd, J = 13.5, 6.4 Hz, 1H), 2.24 (s, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.28, 155.71, 129.87, 128.14, 115.02, 68.76, 50.65, 41.33, 34.28. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{12}H_{18}O_3N$ 224.12812, found 224.12796. **$[\alpha]^{25}_D$** = +43.3 (c 0.549 g/100 mL, $CHCl_3$/MeOH 1/1).

(*S*)-Methyl 3-(4-hydroxyphenyl)-2-(pyrrolidin-1-yl)propanoate (**33b**)



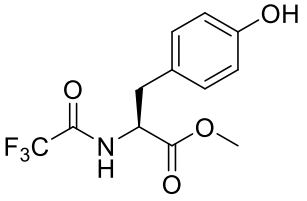
L-Tyrosine methyl ester (220 mg, 1.13 mmol) and $NaHCO_3$ (190 mg, 2.26 mmol) were mixed in toluene (4 mL). 1,4-Dibromobutane (148 µL, 1.24 mmol) was added, the mixture was stirred at 110 °C for 3 h, filtered, concentrated, and separated using silica using gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford **33b** (278 mg, 99%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.01–6.90 (m, 2H), 6.68–6.58 (m, 2H), 3.49 (s, 3H), 3.40–3.36 (m, 1H), 2.89–2.76 (m, 2H), 2.68–2.59 (m, 2H), 2.59–2.52 (m, 2H), 1.71–1.61 (m, 4H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.90, 155.76, 129.83, 127.96, 115.02, 67.09, 50.80, 49.42, 35.94, 23.07. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{14}H_{20}O_3N$ 250.14377, found 250.14366. **$[\alpha]^{25}_D$** = +36.5 (c 0.351 g/100 mL, $CHCl_3$/MeOH 1/1).

N-Acetyl-L-tyrosine methyl ester (**33c**)



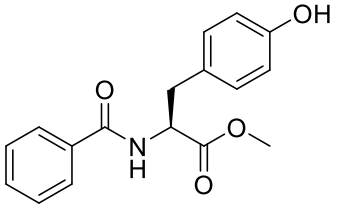
Na_2CO_3 (212 mg, 2.00 mmol) and L-tyrosine methyl ester (380 mg, 2.00 mmol) were mixed with acetone/H_2O (3:1 ratio, 8 mL). Ac_2O (208 µL, 2.20 mmol) was added and the mixture was stirred at 25 °C for 1 h. The mixture was concentrated and separated using using silica gel flash chromatography (linear gradient elution 0–15 % MeOH in $CHCl_3$) to afford **33c** (391 mg, 82%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.22 (s, 1H), 8.26 (d, J = 7.7 Hz, 1H), 7.01–6.96 (m, 2H), 6.69–6.61 (m, 2H), 4.35 (ddd, J = 9.0, 7.7, 5.7 Hz, 1H), 3.57 (s, 3H), 2.87 (dd, J = 13.8, 5.7 Hz, 1H), 2.74 (dd, J = 13.8, 9.0 Hz, 1H), 1.79 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.36, 169.31, 155.98, 129.94, 127.22, 115.04, 53.99, 51.73, 36.05, 22.25. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{12}H_{15}O_4NNa$ 260.08933, found 260.08920. **$[\alpha]^{25}_D$** = +48.9 (c 0.356 g/100 mL, $CHCl_3$/MeOH 1/1).

N-(2,2,2-Trifluoroacetyl)-L-tyrosine methyl ester (**33d**)



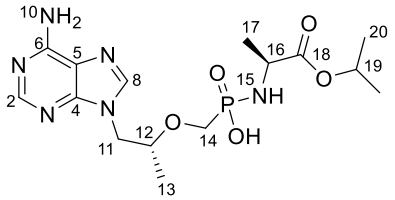
L-tyrosine methyl ester (380 mg, 2.00 mmol) was mixed with DCM (8 mL). Pyridine (161 µL, 2.10 mmol) and $(CF_3CO)_2O$ (292 µL, 2.10 mmol) were added, the mixture was stirred for 5 min, concentrated, and separated using using silica gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford **33d** (578 mg, 99%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.85 (d, J = 8.1 Hz, 1H), 9.25 (s, 1H), 7.06–6.98 (m, 2H), 6.69–6.61 (m, 2H), 4.50 (ddd, J = 10.4, 8.1, 5.0 Hz, 1H), 3.66 (s, 3H), 3.06 (dd, J = 13.9, 5.0 Hz, 1H), 2.89 (dd, J = 14.0, 10.4 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.56, 156.46, 156.07, 129.98, 126.75, 115.69 (q, J = 288.0 Hz), 115.09, 54.31, 52.35, 34.85. **^{19}F NMR** (377 MHz, DMSO-d_6) δ – 74.25. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C12H12O4NF_3Na$ 314.06106, found 314.06092. **$[\alpha]^{25}_D$** = +24.9 (c 0.193 g/100 mL, $CHCl_3$/MeOH 1/1).

N-Benzoyl-L-tyrosine methyl ester (**33e**)



Following standard procedure M, L-tyrosine methyl ester (500 mg, 2.56 mmol), benzoic acid (375 mg, 3.07 mmol), HATU (4.46 g, 3.84 mmol), and DIPEA (2.23 mL, 12.80 mmol) were stirred in DMF (10 mL) to afford **33e** (319 mg, 42%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.22 (bs, 1H), 8.78 (d, J = 7.8 Hz, 1H), 7.86–7.77 (m, 2H), 7.58–7.40 (m, 3H), 7.13–7.05 (m, 2H), 6.69–6.61 (m, 2H), 4.58 (ddd, J = 9.9, 7.8, 5.4 Hz, 1H), 3.63 (s, 3H), 3.09–2.93 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.39, 166.50, 155.96, 133.74, 131.52, 130.04, 128.32, 127.72, 127.43, 115.08, 54.73, 51.92, 35.55. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{17}H_{18}O_4N$ 300.12303, found 300.12298. **$[\alpha]^{25}_D$** = +6.0 (c 0.252 g/100 mL, $CHCl_3$/MeOH 1/1).

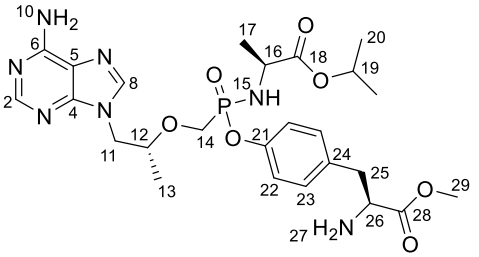
P-((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)-*N*-((*S*)-1-isopropoxy-1-oxopropan-2-yl)phosphonamidic acid (**34**)



Tenfovir (500 mg, 1.74 mmol), L-alanine isopropyl ester hydrochloride (1.46 g, 8.71 mmol), and DIPEA (1.52 mL, 8.71 mmol) were mixed in H_2O (20 mL). EDC (1.35 g, 8.71 mmol) was added, the mixture was stirred at 40 °C for 24 h, concentrated, and separated using C_{18}-reversed phase flash chromatography (linear gradient elution 0–100 % MeOH in water) to afford **34** (558 mg, 80%) as a white solid. **^{1}H NMR** (500 MHz, D_2O) δ 8.22 (s, 2H, *H2*, *H8*), 4.69 (hept, J = 6.3 Hz, 1H, *H19*), 4.36 (dd, J = 14.8, 3.0 Hz, 1H, *H11a*), 4.20 (dd, J = 14.8, 7.9 Hz, 1H, *H11b*), 3.95–3.88 (m, 1H, *H12*), 3.62–3.57 (m, 2H, *H14a*, *H16*), 3.31 (dd, J = 12.6, 9.7 Hz, 1H, *H14b*), 1.21–1.05 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (126 MHz, D_2O) δ 177.13 (d, J = 3.3 Hz, *C18*), 156.12 (*C6*), 152.91 (*C2*), 149.85

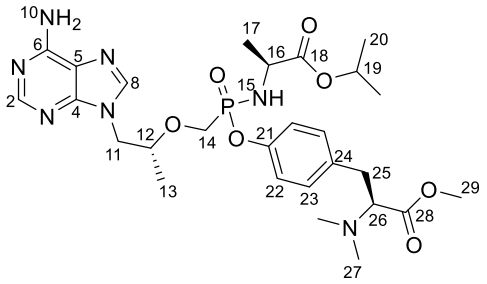
(*C4*), 144.11 (*C8*), 118.90 (*C5*), 76.34 (d, *J* = 12.4 Hz, *C12*), 70.41 (*C19*), 67.19–66.01 (m, *C14*), 50.66 (*C16*), 49.02 (*C11*), 21.30 and 21.28 (*C20*), 21.01 (d, *J* = 5.4 Hz, *C17*), 16.37 (*C13*). **^{31}P NMR** (162 MHz, D_2O) *δ* 19.69. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{26}O_5N_6P$ 401.16968, found 401.17043.

Methyl (2*S*)-2-amino-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)propanoate (**35a**)



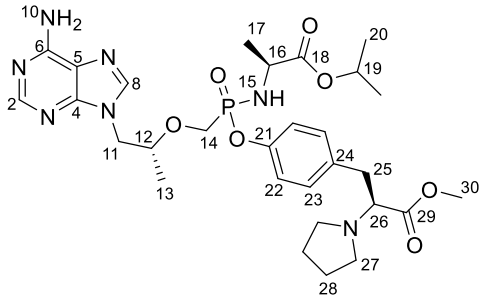
Compound **35g** (40 mg, 0.06 mmol) was mixed with Pd/C in phosphate buffer (0.01 M, pH 7.4)/MeOH (4:1 ratio) under argon atmosphere. The flask was evacuated and flushed with H_2 (3 ×) and the mixture was stirred under H_2 atmosphere at 25 °C for 1 h. After filtration, the volatiles were evaporated and the resiude was purified using C_{18}-reversed phase flash chromatography (linear gradient elution 0–100 % MeOH in water) to afford **35a** (15 mg, 47%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) *δ* 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.20 and 7.19 (bs, 2H, *H10*), 7.14–7.07 (m, 2H, *H23*), 7.03–6.92 (m, 2H, *H22*), 5.60 and 5.47 (dd, *J* = 11.9, 10.4 and 12.4, 10.0 Hz, 1H, *H15*), 4.89–4.78 (m, 1H, *H19*), 4.31–4.08 (m, 2H, *H11*), 4.01–3.90 (m, 1H, *H12*), 3.89–3.72 (m, 3H, *H14*, *H16*), 3.57 (s, 3H, *H29*), 3.54–3.51 (m, 1H, *H26*), 2.84–2.79 and 2.76–2.69 (m, 1H, *H25*), 1.17–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) *δ* 175.12 (*C28*), 173.04 and 172.90 (d, *J* = 4.0 and 3.7 Hz, *C18*), 155.98 and 155.96 (*C6*), 152.42 and 152.41 (*C2*), 149.84 and 149.79 (*C4*), 148.80–148.58 (m, *C21*), 141.46 and 141.41 (*C8*), 133.89 and 133.83 (*C24*), 130.21 (*C23*), 120.37 and 120.16 (d, *J* = 4.4 and 4.5 Hz, *C22*), 118.38 and 118.33 (*C5*), 75.80–75.31 (m, *C12*), 67.92 (*C19*), 64.15 and 64.10 (d, *J* = 154.6 and 155.0 Hz, *C14*), 55.62 (*C26*), 51.41 (*C29*), 49.07 and 49.01 (*C16*), 46.82 and 46.72 (*C11*), 21.46–21.39 (m, *C20*), 20.29 and 19.92 (d, *J* = 5.2 and 5.5 Hz, *C17*), 16.78 and 16.64 (*C13*). **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) *δ* 22.82 and 22.01. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{25}H_{36}O_7N_7NaP$ 600.23060, found 600.23035.

Methyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(dimethylamino)propanoate (**35b**)



Following standard procedure N, compound **34** (300 mg, 0.75 mmol), **33a** (301 mg, 1.35 mmol), DIPEA (1.05 mL, 6.00 mmol), PPh_3 (787 mg, 3.00 mmol), and Aldrithiol™ (661 mg, 3.00 mmol) were stirred in pyridine (7 mL) to afford **35b** (94 mg, 21%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.14 (s, 1H, *H2*), 8.11 and 8.11 (s, 1H, *H8*), 7.22 and 7.21 (bs, 1H, *H10*), 7.18–7.09 (m, 2H, *H23*), 7.03–6.93 (m, 2H, *H22*), 5.62 and 5.49 (dd, *J* = 12.0, 10.4 and 12.3, 9.9 Hz, 1H, *H15*), 4.90–4.78 (m, 1H, *H19*), 4.32–4.10 (m, 2H, *H11*), 4.01–3.71 (m, 4H, *H12*, *H14*, *H16*), 3.54 (s, 3H, *H29*), 3.44–3.38 (m, 1H, *H26*), 2.93–2.77 (m, 2H, *H25*), 2.25 (s, 6H, *H27*), 1.18–1.00 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.05 and 172.93 (d, *J* = 4.0 and 4.0 Hz, *C18*), 171.13 (*C28*), 156.01 and 155.99 (*C6*), 152.45 (*C2*), 149.87 and 149.83 (*C4*), 148.70–148.49 (m, *C21*), 141.49 and 141.44 (*C8*), 134.38 and 134.30 (*C24*), 130.00 (*C23*), 120.44 and 120.24 (d, *J* = 4.3 and 4.4 Hz, *C22*), 118.42 and 118.36 (*C5*), 75.75–75.41 (m, *C12*), 68.23 (*C26*), 67.94 (*C19*), 64.21 and 64.14 (d, *J* = 155.1 and 154.8 Hz), 50.75 (*C29*), 49.11 and 49.03 (*C16*), 46.84 and 46.74 (*C11*), 41.27 (*C27*), 34.21 (*C25*), 21.48–21.39 (m, *C20*), 20.28 and 19.91 (d, *J* = 5.1 and 5.5 Hz, *C17*), 16.76 and 16.65 (*C13*). **^{31}P NMR** (DMSO-d_6, mixture of epimers) δ 25.53 and 24.73. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{41}O_7N_7P$ 606.27996, found 606.27982.

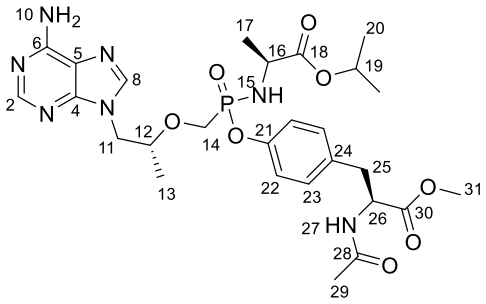
Methyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(pyrrolidin-1-yl)propanoate (**35c**)



Following standard procedure N, compound **34** (188 mg, 0.47 mmol), **33b** (209 mg, 0.84 mmol), DIPEA (655 µL, 3.76 mmol), PPh_3 (493 mg, 1.88 mmol), and Aldrithiol™ (414 mg, 1.88 mmol) were stirred in pyridine (5 mL) to afford **35c** (160 mg, 54%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.22 and 7.20 (bs, 2H, *H10*), 7.17–7.06 (m, 2H, *H23*), 7.03–6.92 (m, 2H, *H22*), 5.62 and 5.48 (dd, *J* = 12.0, 10.4 and 12.4, 10.0 Hz, 1H, *H15*), 4.89–4.78 (m, 1H, *C19*), 4.31–4.09 (m, 2H, *H11*), 3.99–3.72 (m, 4H, *H12*, *H14*, *H16*), 3.50 (s, 3H, *H30*), 3.47–3.44 (m, 1H, *H26*), 2.93–2.87 (m, 2H, *H25*), 2.66–2.55 (m, 4H, *H27*), 1.68–1.62 (m, 4H, *H28*), 1.18–1.02 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of

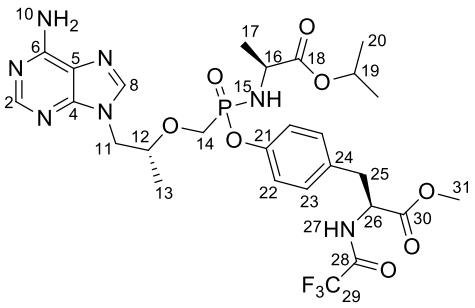
epimers) δ 173.03 and 172.90 (d, J = 4.1 and 3.9 Hz, *C18*), 171.69 (*C28*), 155.99 and 155.97 (*C6*), 152.43 (*C2*), 149.85 and 149.81 (*C4*), 148.84–148.54 (m, *C21*), 141.46 and 141.41 (*C8*), 134.14 and 134.05 (*C24*), 129.94 (*C23*), 120.44 and 120.20 (d, J = 4.3 and 4.5 Hz, *C22*), 118.40 and 118.35 (*C5*), 75.75–75.31 (m, *C12*), 67.91 (*C19*), 66.34 (*C26*), 64.18 and 64.12 (d, J = 154.4 and 155.1 Hz, *C14*), 50.86 (*C30*), 49.28 (*C27*), 49.09 and 49.00 (*C16*), 46.82 and 46.71 (*C11*), 35.83 (*C25*), 23.11 (*C28*), 21.48–21.36 (m, *C20*), 20.24 and 19.89 (d, J = 5.2 and 5.5 Hz, *C17*), 16.76 and 16.62 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.52 and 24.67. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{29}H_{43}O_7N_7P$ 632.29561, found 632.29538.

Methyl (2*S*)-2-acetamido-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)propanoate (**35d**)



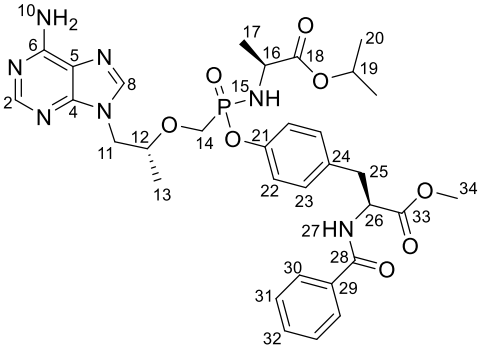
Following standard procedure N, compound **34** (120 mg, 0.30 mmol), **33c** (85 mg, 0.36 mmol), Et_3N (335 µL, 2.40 mmol), PPh_3 (315 mg, 1.20 mmol), and Aldrithiol^{TM} (264 mg, 1.20 mmol) were stirred in pyridine (5 mL) to afford **35d** (55 mg, 30%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) δ 8.35 and 8.33 (bs, 1H, *H27*), 8.13 and 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.26–7.12 (m, 4H, *H10*, *H23*), 7.04–6.95 (m, 2H, *H22*), 5.68–5.60 and 5.55–5.46 (m, 1H, *H15*), 4.90–4.78 (m, 1H, *H19*), 4.45–4.37 (m, 1H, *H26*), 4.31–4.10 (m, 2H, *H11*), 4.00–3.89 (m, 1H, *H12*), 3.87–3.71 (m, 3H, *H14*, *H16*), 3.58 and 3.56 (s, 3H, *H31*), 3.00–2.79 (m, 2H, *H25*), 1.78 and 1.78 (s, 3H, *H29*), 1.19–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) δ 173.07 and 172.93 (d, J = 4.1 and 4.0 Hz, *C18*), 172.23 (*C30*), 169.36 (*C28*), 156.00 and 155.98 (*C6*), 152.46 and 152.44 (*C2*), 149.85 and 149.81 (*C4*), 149.09–148.72 (m, *C21*), 141.47 and 141.43 (*C8*), 133.35 and 133.25 (*C24*), 130.09 (*C23*), 120.53 and 120.25 (d, J = 4.1 and 4.6 Hz, *C22*), 118.38 and 118.34 (*C5*), 75.82–75.31 (m, *C12*), 67.94 (*C19*), 64.21 and 64.13 (d, J = 154.8 and 155.2 Hz, *C14*), 53.64 (*C26*), 51.85 (*C31*), 49.09 and 49.00 (*C16*), 46.79 and 46.68 (*C11*), 36.00 (*C25*), 22.25 (*C29*), 21.48–21.41 (m, *C20*), 20.28 and 19.92 (d, J = 5.2 and 5.4 Hz, *C17*), 16.82 and 16.67 (*C13*). **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) δ 22.90 and 22.03. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{27}H_{38}O_8N_7NaP$ 642.24117, found 642.24054.

Methyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(2,2,2-trifluoroacetamido)propanoate (**35e**)



Following standard procedure N, compound **34** (150 mg, 0.37 mmol), **33d** (216 mg, 0.74 mmol), Et_3N (413 µL, 2.96 mmol), PPh_3 (388 mg, 1.48 mmol), and Aldrithiol™ (326 mg, 1.48 mmol) were stirred in pyridine (6 mL) to afford **35e** (13 mg, 5%) as an off-white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) δ 9.90 (bs, 1H, *H27*), 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.24–7.16 (m, 4H, *H10*, *H23*), 7.05–6.99 (m, 2H, *H22*), 5.68–5.60 (m) and 5.48 (dd, J = 12.4, 10.0 Hz, 1H, *H15*), 4.88–4.78 (m, 1H, *H19*), 4.63–4.55 (m, 1H, *H26*), 4.30–4.11 (m, 2H, *H11*), 3.99–3.89 (m, 1H, *H12*), 3.88–3.72 (m, 3H, *H14*, *H16*), 3.67 and 3.67 (s, 3H, *H31*), 3.19–3.13 and 3.01–2.95 (m, 1H, *H25*), 1.18–1.02 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) δ 173.02 and 172.86 (d, J = 4.3 and 4.0 Hz, *C18*), 170.38 (*C30*), 156.32 and 156.30 (q, J = 36.6 and 36.2 Hz, *C28*), 155.97 and 155.96 (*C6*), 152.43 and 152.41 (*C2*), 149.84 and 149.80 (*C4*), 149.20–148.77 (m, *C21*), 141.44 and 141.40 (*C8*), 132.88 and 132.73 (*C24*), 130.09 (*C23*), 120.61 and 120.23 (d, J = 4.2 and 4.6 Hz, *C22*), 118.37, 118.33 (*C5*), 115.65 (q, J = 288.0 Hz, *C29*), 75.78–75.39 (m, *C12*), 67.89 (*C19*), 64.21 and 64.11 (d, J = 155.0 and 154.9 Hz, *C14*), 53.91 (*C26*), 52.42 (*C31*), 49.06 and 48.97 (*C16*), 46.75 and 46.67 (*C11*), 34.80 (*C25*), 21.46–21.32 (m, *C20*), 20.18 and 19.83 (d, J = 5.1 and 5.2 Hz, *C16*), 16.77 and 16.63 (*C13*). **^{19}F NMR** (470 MHz, DMSO-d_6, mixture of epimers) δ -74.26 and -74.28. **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) δ 22.93 and 22.02. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{27}H_{35}O_8N_7F_3NaP$ 696.21290, found 696.21216.

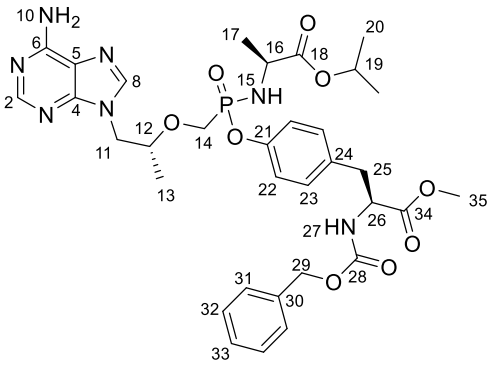
Methyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-benzamidopropanoate (**35f**)



Following standard procedure N, compound **34** (188 mg, 0.47 mmol), **33e** (251 mg, 0.84 mmol), DIPEA (655 µL, 3.76 mmol), PPh_3 (493 mg, 1.88 mmol), and Aldrithiol™ (414 mg, 1.88 mmol) were stirred in pyridine (5 mL) to afford **35f** (189 mg, 59%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.83 and 8.83 (d, J = 7.8 and 7.8 Hz, 1H, *H27*), 8.14 and 8.13 (s, 1H, *H2*), 8.09 and 8.08 (s, 1H, *H8*), 7.83–7.76 (m, 2H, *H30*), 7.56–7.48 (m, 1H, *H32*), 7.50–7.40 (m, 2H, *H31*), 7.30–7.16 (m, 4H, *H10*, *H23*), 7.06–6.97 (m, 2H, *H22*), 5.60 and 5.45 (dd, J = 12.0, 10.3 and

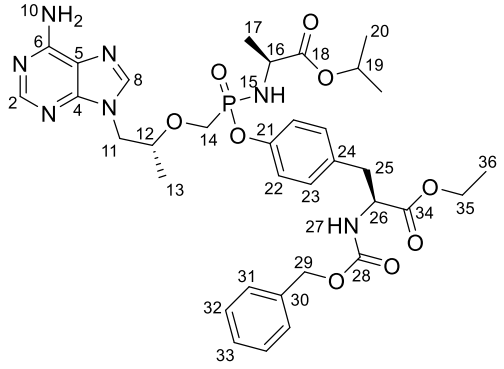
12.5, 10.0 Hz, 1H, *H15*), 4.85–4.75 (m, 1H, *H19*), 4.68–4.61 (m, 1H, *H26*), 4.29–4.08 (m, 2H, *H11*), 3.96–3.69 (m, 4H, *H12*, *H14*, *H16*), 3.64 and 3.64 (s, 3H, *H34*), 3.20–3.03 (m, 2H, *H25*), 1.14–0.98 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.01 and 172.87 (d, J = 4.2 and 3.9 Hz), 172.19 (*C33*), 166.50 and 166.46 (*C28*), 156.00 and 155.98 (*C6*), 152.47 and 152.43 (*C2*), 149.84 and 149.82 (*C4*), 149.06–148.61 (m, *C21*), 141.46 and 141.42 (*C8*), 133.83–133.60 (m, *C24*, *C29*), 131.50 (*C32*), 130.12 (*C23*), 128.27 (*C31*), 127.40 and 127.41 (*C30*), 120.55 and 120.22 (d, J = 4.3 and 4.5 Hz), 118.39 and 118.35 (*C5*), 75.78–75.33 (m, *C12*), 67.89 (*C19*), 64.19 and 64.13 (d, J = 154.8 and 155.2 Hz, *C14*), 54.29 (*C26*), 51.98 (*C34*), 49.07 and 48.97 (*C16*), 46.77 and 46.67 (*C11*), 35.50 (*C25*), 21.41–21.38 (m, *C20*), 20.21 and 19.87 (d, J = 5.1 and 5.3 Hz, *C17*), 16.78 and 16.62 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.55 and 24.67. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{32}H_{41}O_8N_7P$ 682.27487, found 682.27467.

Methyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35g**)



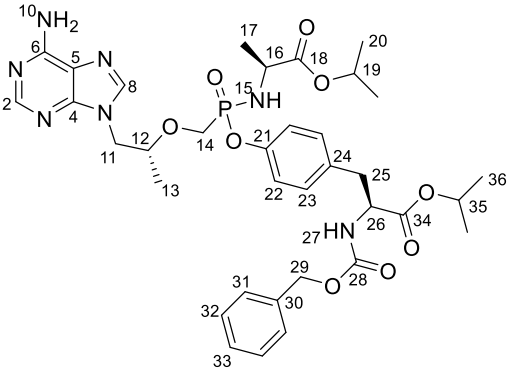
Following standard procedure N, compound **34** (140 mg, 0.35 mmol), *N*-((benzyloxy)carbonyl)-L-tyrosine methyl ester (231 mg, 0.70 mmol), Et_3N (390 µL, 2.80 mmol), PPh_3 (367 mg, 1.40 mmol), and Aldrithiol^{TM} (308 mg, 1.40 mmol) were stirred in pyridine (6 mL) to afford **35g** (121 mg, 49%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.17 and 8.17 (s, 1H, *H2*), 8.14 and 8.13 (s, 1H, *H8*), 7.82 and 7.82 (d, J = 8.2 and 8.2 Hz, 1H, *H27*), 7.44–7.24 (m, 7H, *H10*, *H31*, *H32*, *H34*), 7.23–7.14 (m, 2H, *H23*), 7.06–6.97 (m, 2H, *H22*), 5.62 and 5.48 (dd, J = 12.0, 10.3 and 12.4, 9.9 Hz, 1H, *H15*), 5.04–4.91 (m, 2H, *H29*), 4.87–4.77 (m, 1H, *H19*), 4.33–4.11 (m, 3H, *H11*, *H26*), 4.00–3.73 (m, 4H, *H12*, *H14*, *H16*), 3.62 (s, 3H, *H35*), 3.05–2.95 and 2.88–2.78 (m, 2H, *H25*), 1.16–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.02 and 172.89 (d, J = 4.2 and 3.9 Hz, *C18*), 172.30 (*C34*), 155.96 (*C28*), 155.40 and 155.36 (*C6*), 151.69 and 151.67 (*C2*), 149.74 and 149.70 (*C4*), 149.01–148.70 (m, *C21*), 141.77 and 141.73 (*C8*), 136.85 (*C30*), 133.48 and 133.37 (*C24*), 130.12 (*C23*), 128.33 (*C32*), 127.81 (*C33*), 127.63 and 127.60 (*C31*), 120.47 and 120.24 (d, J = 4.3 and 4.5 Hz, *C22*), 118.32 and 118.28 (*C5*), 75.78–75.29 (m, *C12*), 67.92 (*C19*), 65.47 and 65.45 (*C29*), 64.22 and 64.13 (d, J = 154.8 and 154.8 Hz, *C14*), 55.56 (*C26*), 51.93 (*C35*), 49.05 and 49.00 (*C16*), 46.82 and 46.77 (*C11*), 35.72 (*C25*), 21.42 and 21.38 (*C20*), 20.26 and 19.90 (d, J = 5.0 and 5.6 Hz, *C17*), 16.77 and 16.65 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.55 and 24.74. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{33}H_{42}O_9N_7NaP$ 734.26738, found 734.26707.

Ethyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35h**)



Following standard procedure N, compound **34** (200 mg, 0.50 mmol), *N*-((benzyloxy)carbonyl)-L-tyrosine ethyl ester (343 mg, 1.00 mmol), Et_3N (558 µL, 4.00 mmol), PPh_3 (787 mg, 3.00 mmol), and Aldrithiol™ (661 mg, 3.00 mmol) were stirred in pyridine (8 mL) to afford **35h** (117 mg, 32%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.82–7.78 (m, 1H, *H27*), 7.38–7.26 (m, 5H, *H31*, *H32*, *H33*), 7.25–7.13 (m, 4H, *H10*, *H23*), 7.06–6.97 (m, 2H, *H22*), 5.62 and 5.49 (dd, *J* = 12.0, 10.3 and 12.4, 10.0 Hz, 1H, *H15*), 5.05–4.92 (m, 2H, *H29*), 4.88–4.78 (m, 1H, *H19*), 4.31–4.11 (m, 3H, *H11*, *H26*), 4.06 (q, *J* = 7.0 Hz, 2H, *H35*), 3.99–3.74 (m, 4H, *H12*, *H14*, *H16*), 3.03–2.94 and 2.89–2.80 (m, 2H, *H25*), 1.16–1.01 (m, 15H, *H13*, *H17*, *H20*, *H36*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.05 and 172.90 (d, *J* = 4.0 and 4.0 Hz, *C19*), 171.82 (*C34*), 155.97–155.93 (m, *C6*, *C28*), 152.39 (*C2*), 149.84 and 149.82 (*C4*), 149.08–148.68 (m, *C21*), 141.47 and 141.43 (*C8*), 136.90 (*C30*), 133.48 and 133.37 (*C24*), 130.15 (*C23*), 128.33 (*C32*), 127.81 (*C33*), 127.63 and 127.61 (*C31*), 120.47 and 120.25 (d, *J* = 4.3 and 4.5 Hz), 118.39 and 118.34 (*C5*), 75.78–75.36 (m, *C12*), 67.92 (*C19*), 65.44 (*C29*), 64.22 and 64.14 (d, *J* = 155.5 and 156.3Hz), 60.59 (*C35*), 55.64 (*C26*), 49.07 and 49.01 (*C16*), 46.77 and 46.71 (*C11*), 35.78 (*C25*), 21.42 and 21.40 (*C20*), 20.27 and 19.89 (d, *J* = 5.1 and 5.1 Hz, *C16*), 16.79 and 16.68 (*C13*), 14.01 (*C36*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.55 and 24.74. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{34}H_{45}O_9N_7P$ 726.30109, found 726.30136.

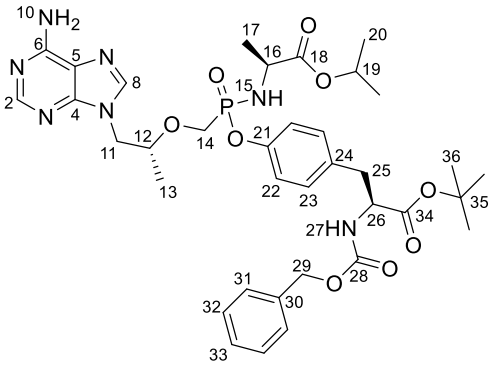
Isopropyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35i**)



Following standard procedure N, compound **34** (210 mg, 0.52 mmol), **36a** (373 mg, 1.04 mmol), Et_3N (580 µL, 4.16 mmol), PPh_3 (546 mg, 2.08 mmol), and Aldrithiol™ (458 mg, 2.08 mmol) were stirred in pyridine (8 mL) to afford **35i** (92 mg, 24%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) δ 8.13 and 8.13 (s, 1H, *H2*), 8.11 and 8.09 (s, 1H, *H8*), 7.80 and 7.78 (bs, 1H, *H27*), 7.38–7.25 (m, 5H, *H31*, *H32*, *H33*), 7.24–7.15 (m, 4H, *H10*, *H23*), 7.05–6.95 (m,

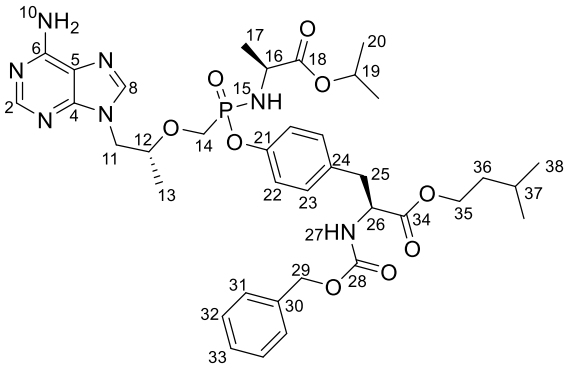
2H, *H22*), 5.67–5.59 and 5.53–5.46 (m, 1H, *H15*), 5.06–4.73 (m, 4H, *H19*, *H29*, *H35*), 4.31–4.07 (m, 3H, *H11*, *H26*), 3.98–3.71 (m, 4H, *H12*, *H14*, *H16*), 3.00–2.90 and 2.87–2.78 (m, 2H, *H25*), 1.18–0.94 (m, 18H, *H13*, *H17*, *H20*, *H36*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) δ 173.13 and 172.98 (d, J = 4.0 and 3.9 Hz, *C18*), 171.41 (*C34*), 156.01 (*C6*, *C28*) 152.49 (*C2*), 149.87 and 149.84 (*C4*), 149.06–148.72 (m, *C21*), 141.53 and 141.49 (*C8*), 136.98 (*C30*), 133.51 and 133.40 (*C24*), 130.24 (*C23*), 128.40 (*C32*), 127.88 (*C33*), 127.70 and 127.68 (*C31*), 120.54 and 120.30 (d, J = 4.1 and 4.4 Hz, *C22*), 118.40 and 118.36 (*C5*), 75.81–75.43 (m, *C12*), 68.15 and 68.00 and 67.99 (*C19*, *C35*), 65.46 and 65.44 (*C29*), 64.23 and 64.16 (d, J = 154.9 and 154.8 Hz, *C14*), 55.80 and 55.78 (*C26*), 49.11 and 49.07 (*C16*), 46.80 and 46.73 (*C11*), 35.82 (*C25*), 21.58–21.43 (m, *C20*, *C36*), 20.34 and 19.94 (d, J = 5.1 and 5.5 Hz, *C17*), 16.84 and 16.72 (*C13*). **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) δ 22.92 and 22.09. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{35}H_{47}O_9N_7P$ 740.31674, found 740.31697.

Tert-butyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35j**)



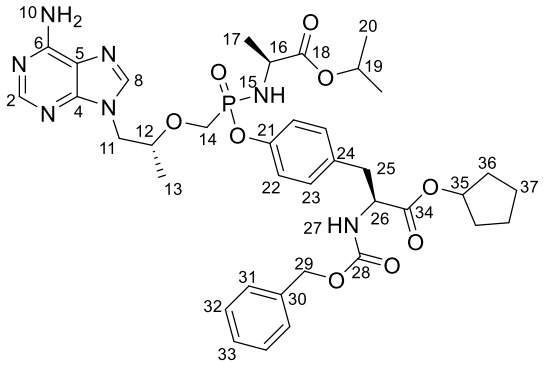
Following standard procedure N, compound **34** (180 mg, 0.45 mmol), *N*-((benzyloxy)carbonyl)-L-tyrosine *t*-butyl ester monohydrate (351 mg, 0.90 mmol), Et_3N (501 µL, 3.60 mmol), PPh_3 (708 mg, 2.70 mmol), and Aldrithiol™ (595 mg, 2.70 mmol) were stirred in pyridine (8 mL) to afford **35j** (154 mg, 45%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.69 (bd, J = 8.0 Hz, 1H, *H27*), 7.38–7.15 (m, 9H, *H10*, *H23*, *H31*, *H32*, *H33*), 7.07–6.96 (m, 2H, *H23*), 5.61 and 5.48 (dd, J = 12.0, 10.3 and 12.3, 9.9 Hz, 1H, *H15*), 5.06–4.91 (m, 2H, *H29*), 4.89–4.78 (m, 1H, *H19*), 4.32–4.14 (m, 2H, *H11*), 4.13–4.06 (m, 1H, *H26*), 3.99–3.74 (m, 4H, *H12*, *H14*, *H16*), 3.00–2.89 and 2.87–2.76 (m, 2H, *H25*), 1.33 (s, 9H, *H36*), 1.18–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.04 and 172.88 (d, J = 4.1 and 4.0 Hz, *C18*), 170.94 (*C34*), 155.99–155.90 (m, *C6*, *C28*), 152.42 (*C2*), 149.83 and 149.80 (*C4*), 148.94–148.70 (m, *C21*), 141.44 and 141.40 (*C8*), 136.95 (*C30*), 133.59 and 133.49 (*C24*), 130.16 (*C23*), 128.31 (*C32*), 127.79 (*C33*), 127.66 and 127.64 (*C31*), 120.42 and 120.18 (d, J = 4.2 and 4.1 Hz, *C22*), 118.37 and 118.34 (*C5*), 80.67 (*C35*), 75.78–75.35 (m, *C12*), 67.90 (*C19*), 65.36 (*C29*), 64.19 and 64.15 (d, J = 155.5 and 155.2 Hz, *C14*), 56.14 and 56.12 (*C26*), 49.05 and 49.01 (*C16*), 46.75 and 46.69 (*C11*), 35.92 (*C25*), 27.55 (*C36*), 21.41 and 21.38 (*C20*), 20.28 and 19.88 (d, J = 5.4 and 5.4 Hz), 16.79 and 16.66 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.53 and 24.74. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{36}H_{49}O_9N_7P$ 754.33239, found 754.33278.

Isopentyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35k**)



Following standard procedure N, compound **34** (190 mg, 0.47 mmol), **36b** (362 mg, 0.94 mmol), Et_3N (523 µL, 3.76 mmol), PPh_3 (493 mg, 1.88 mmol), and Aldrithiol^{TM} (414 mg, 1.88 mmol) were stirred in pyridine (8 mL) to afford **35k** (68 mg, 19%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) δ 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H. *H8*), 7.83 and 7.81 (bs, 1H, *H27*), 7.37–7.26 (m, 5H, *H31*, *H32*, *H33*), 7.25–7.14 (m, 4H, *H10*, *H23*), 7.05–6.97 (m, 2H, *H22*), 5.68–5.60 and 5.56–5.47 (m, 1H, *H15*), 5.03–4.91 (m, 2H, *H29*), 4.86–4.77 (m, 1H, *H19*), 4.32–4.09 (m, 3H, *H11*, *H26*), 4.05 (t, *J* = 6.7 Hz, 2H, *H35*), 3.99–3.73 (m, 4H, *H12*, *H14*, *H16*), 3.02–2.94 and 2.87–2.79 (m, 2H, *H25*), 1.63–1.53 (m, 1H, *H37*), 1.44–1.37 (m, 2H, *H36*), 1.19–0.77 (m, 18H, *H13*, *H17*, *H20*, *H38*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) δ 173.11 and 172.96 (d, *J* = 4.1 and 3.9 Hz, *C18*), 171.91 (*C34*), 156.04–155.99 (m, *C6*, *C28*), 152.48 (*C2*), 149.87 and 149.84 (*C4*), 148.97 and 148.84 (d, *J* = 8.9 and 8.9 Hz, *C21*), 141.51 and 141.47 (*C8*), 136.91 (*C30*), 133.56 and 133.45 (*C24*), 130.18 (*C23*), 128.39 (*C32*), 127.89 (*C33*), 127.71 and 127.68 (*C31*), 120.55 and 120.31 (d, *J* = 4.1 and 4.3 Hz, *C22*), 118.40 and 118.36 (*C5*), 75.76–75.45 (m, *C12*), 67.98 and 67.96 (*C19*), 65.49 and 65.47 (*C29*), 64.25 and 64.16 (d, *J* = 154.9 and 155.3 Hz, *C14*), 63.07 (*C35*), 55.74 (*C26*), 49.10 and 49.04 (*C16*), 46.77 and 46.71 (*C11*), 36.78 (*C36*), 35.74 (*C25*), 24.41 (*C37*), 22.34 and 22.28 (*C38*), 21.47 and 21.44 (*C20*), 20.32 and 19.95 (d, *J* = 5.0 and 5.5 Hz, *C17*), 16.84 and 16.72 (*C13*). **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) δ 22.90 and 22.07. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{37}H_{51}O_9N_7P$ 768.34804, found 768.34815.

Cyclopentyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35l**)



Following standard procedure N, compound **34** (210 mg, 0.52 mmol), **36c** (399 mg, 1.04 mmol), Et_3N (580 µL, 4.16 mmol), PPh_3 (546 mg, 2.08 mmol), and Aldrithiol™ (458 mg, 2.08 mmol) were stirred in pyridine (8 mL) to afford **35l** (147 mg, 37%) as a white solid. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{37}H_{49}O_9N_7P$ 766.33239, found 766.33223.

NMR data and characterization of **35l-FEE**:

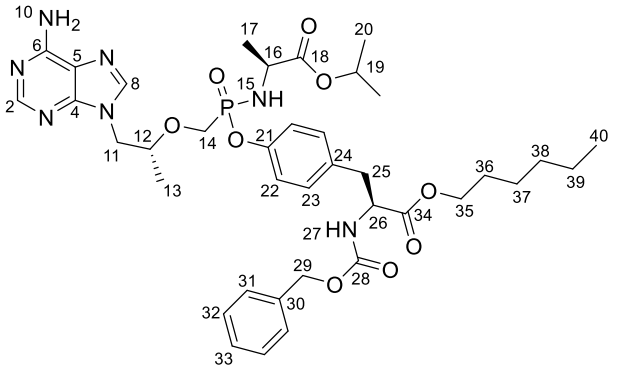
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H, *H2*), 8.10 (s, 1H, *H8*), 7.76 (d, *J* = 7.9 Hz, 1H, *H27*), 7.38–7.26 (m, 5H, *H31*, *H32*, *H33*), 7.21–7.17 (m, 4H, *H10*, *H23*), 7.05–7.00 (m, 2H, *H22*), 5.48 (dd, *J* = 12.3, 10.0 Hz, 1H, *H15*), 5.07–4.92 (m, 3H, *H29*, *H35*), 4.82 (hept, *J* = 6.2 Hz, 1H, *H19*), 4.28 (dd, *J* = 14.3, 3.9 Hz, 1H, *H11a*), 4.22–4.12 (m, 2H, *H11b*, *H26*), 4.00–3.92 (m, 1H, *H12*), 3.90–3.77 (m, 3H, *H14*, *H16*), 2.95 (dd, *J* = 13.8, 5.5 Hz, 1H, *H25a*), 2.83 (dd, *J* = 13.9, 9.6 Hz, 1H, *H25b*), 1.81–1.39 (m, 8H, *H36*, *H37*), 1.15–1.00 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 173.05 (d, *J* = 4.4 Hz, *C18*), 171.50 (*C34*), 155.98–155.94 (*C28*, *C6*), 152.42 (*C2*), 149.84 (*C4*), 148.79 (d, *J* = 9.5 Hz, *C21*), 141.45 (*C8*), 136.92 (*C30*), 133.48 (*C24*), 130.14 (*C23*), 128.33 (*C32*), 127.81 (*C33*), 127.63 (*C31*), 120.47 (d, *J* = 4.4 Hz, *C22*), 118.34 (*C5*), 77.21 (*C35*), 75.63 (d, *J* = 12.5 Hz, *C12*), 67.92 (*C19*), 65.41 (*C29*), 64.14 (d, *J* = 154.8 Hz, *C14*), 55.67 (*C26*), 49.01 (*C16*), 46.70 (*C11*), 35.73 (*C25*), 32.08 (*C36*), 32.05 (*C36*), 23.24 (*C37*), 23.21 (*C37*), 21.47 (*C20*), 21.45 (*C20*), 19.89 (d, *J* = 5.9 Hz, *C17*), 16.79 (*C13*). **^{31}P NMR** (162 MHz, DMSO) δ 25.55.

NMR data and characterization of **35l-SEE**:

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H, *H2*), 8.09 (s, 1H, *H8*), 7.77 (d, *J* = 7.9 Hz, 1H, *H27*), 7.39–7.26 (m, 5H, *H31*, *H32*, *H33*), 7.22–7.15 (m, 4H, *H10*, *H23*), 7.02–6.96 (m, 2H, *H22*), 5.61 (dd, *J* = 12.0, 10.4 Hz, 1H, *H15*), 5.07–4.92 (m, 3H, *H29*, *H35*), 4.84 (hept, *J* = 6.2 Hz, 1H, *H19*), 4.27 (dd, *J* = 14.4, 3.9 Hz, 1H, *H11a*), 4.20–4.09 (m, 2H, *H11b*, *H26*), 3.97–3.90 (m, 1H, *H12*), 3.89–3.73 (m, 3H, *H14*, *H16*), 2.94 (dd, *J* = 13.8, 5.7 Hz, 1H, *H25a*), 2.83 (dd, *J* = 13.9, 9.5 Hz, 1H, *H25b*), 1.82–1.38 (m, 8H, *H36*, *H37*), 1.17–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 172.90 (d, *J* = 3.7 Hz, *C18*), 171.51 (*C34*), 156.00–155.95 (m, *C28*, *C6*), 152.44 (*C2*), 149.82 (*C4*), 148.93 (d, *J* = 8.8 Hz, *C21*), 141.42 (*C8*), 136.91 (*C30*), 133.38 (*C24*), 130.13 (*C23*), 128.33 (*C32*), 127.82 (*C33*), 127.65 (*C31*), 120. 23 (d, *J* = 4.4 Hz, *C22*), 118.38 (*C5*), 77.21 (*C35*), 75.52 (d, *J* = 12.5 Hz, *C12*), 67.91 (*C19*), 65.42 (*C29*), 64.20 (d, *J* = 154.1 Hz, *C14*), 55.65 (*C26*), 49.06 (*C16*), 46.76 (*C11*), 35.73 (*C25*), 32.08 (*C36*), 32.05 (*C36*),

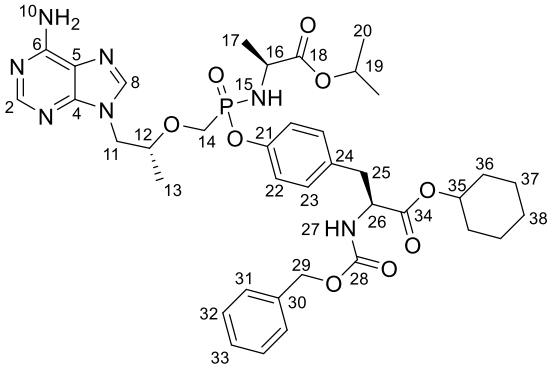
23.24 (*C37*), 23.21 (*C37*), 21.43 (*C20*), 21.39 (*C20*), 20.28 (d, *J* = 5.1 Hz, *C17*), 16.68 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 24.75.

n-Hexyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35m**)



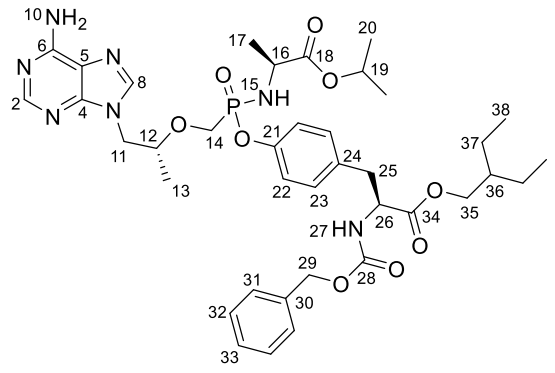
Following standard procedure N, compound **34** (200 mg, 0.50 mmol), **36d** (399 mg, 1.00 mmol), Et_3N (558 µL, 4.00 mmol), PPh_3 (525 mg, 2.00 mmol), and Aldrithiol^{TM} (441 mg, 2.00 mmol) were stirred in pyridine (8 mL) to afford **35m** (144 mg, 37%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.11 and 8.09 (s, 1H, *H8*), 7.80 and 7.80 (d, *J* = 8.0 and 8.1 Hz, 1H, *H27*), 7.38–7.25 (m, 5H, *H31*, *H32*, *H33*), 7.24–7.13 (m, 4H, *H10*, *H23*), 7.06–6.97 (m, 2H, *H22*), 5.61 and 5.48 (dd, *J* = 12.0, 10.4 and 12.4, 9.9 Hz, 1H, *H15*), 5.06–4.90 (m, 2H, *H29*), 4.88–4.77 (m, 1H, *H19*), 4.32–4.11 (m, 3H, *H11*, *H26*), 4.06–3.73 (m, 6H, *H12*, *H14*, *H16*, *H35*), 3.04–2.94 and 2.89–2.79 (m, 2H, *H25*), 1.55–1.45 (m, 2H, *H36*), 1.31–1.20 (m, 6H, *H37*, *H38*, *H39*), 1.15–1.01 (m, 12H, *H13*, *H17*, *H20*), 0.88–0.79 (m, 3H, *H40*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.04 and 172.90 (d, *J* = 4.4 and 3.7 Hz, *C18*), 171.85 (*C34*), 156.04–155.83 (m, *C28*, *C6*), 152.41 (*C2*), 149.84 and 149.82 (*C4*), 149.10–148.73 (m, C21), 141.47 and 141.43 (*C8*), 136.88 (*C30*), 133.52 and 133.40 (*C24*), 130.12 (*C23*), 128.33 (*C32*), 127.81 (*C33*), 127.63 and 127.60 (*C31*), 120.50 and 120.26 (d, *J* = 4.3 and 4.5 Hz, *C22*), 118.39 and 118.34 (*C5*), 75.87–75.40 (m, *C12*), 67.93 and 67.92 (*C19*), 65.45 and 65.43 (*C29*), 64.55 (*C35*), 64.24 and 64.15 (d, *J* = 154.1 and 155.5 Hz, *C14*), 55.69 (*C26*), 49.07 and 49.01 (*C16*), 46.76 and 46.70 (*C11*), 35.76 (*C25*), 30.83 (*C38*), 28.01 (*C36*), 24.90 (*C37*), 21.99 (*C39*), 21.42 and 21.39 (*C20*), 20.28 and 19.91 (d, *J* = 5.1 and 5.4 Hz, *C17*), 16.79 and 16.68 (*C13*), 13.88 (*C40*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.55, 24.73. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{38}H_{52}O_9N_7NaP$ 804.34563, found 804.34537.

Cyclohexyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35n**)



Following standard procedure N, compound **34** (110 mg, 0.27 mmol), **36e** (163 mg, 0.41 mmol), Et_3N (376 µL, 2.16 mmol), PPh_3 (283 mg, 1.08 mmol), and Aldrithiol™ (238 mg, 1.08 mmol) were stirred in pyridine (5 mL) to afford **35n** (112 mg, 53%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.78 and 7.76 (bs, 1H, *H27*), 7.39–7.25 (m, 5H, *H31*, *H32*, *H33*), 7.26–7.14 (m, 4H, *H10*, *H23*), 7.07–6.96 (m, 2H, *H22*), 5.62 and 5.48 (dd, *J* = 12.0, 10.4 and 12.4, 9.9 Hz, 1H, *H15*), 5.06–4.90 (m, 2H, *H29*), 4.89–4.75 (m, 1H, *H19*), 4.71–4.60 (m, 1H, *H35*), 4.33–4.10 (m, 3H, *H11*, *H26*), 4.00–3.72 (m, 4H, *H12*, *H14*, *H16*), 3.03–2.91 and 2.89–2.78 (m, 2H, *H25*), 1.77–1.17 (m, 10H, *H36*, *H37*, *H38*), 1.17–1.00 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.05 and 172.90 (d, *J* = 4.1 and 4.0 Hz, *C18*), 171.18 (*C34*), 156.06–155.88 (m, *C28*, *C6*), 152.44 (*C2*), 149.84 and 149.82 (*C4*), 149.07–148.66 (m, *C21*), 141.46 and 141.43 (*C8*), 136.94 (*C30*), 133.51 and 133.41 (*C24*), 130.15 (*C23*), 128.32 (*C32*), 127.81 (*C33*), 127.66 and 127.64 (*C31*), 120.49 and 120.25 (d, *J* = 4.3 and 4.4 Hz, *C22*), 118.39 and 118.35 (*C5*), 75.80–75.35 (m, *C12*), 72.49 (*C35*), 67.93 (*C19*), 65.41 (*C29*), 64.24 and 64.15 (d, *J* = 155.0 and 155.5 Hz, *C14*), 55.80 and 55.78 (*C26*), 49.07 and 49.02 (*C16*), 46.77 and 46.72 (*C11*), 35.79 (*C25*), 30.91 and 30.76 (*C36*), 24.84 (*C38*), 22.90 (*C37*), 21.43 and 21.40 (*C20*), 20.29 and 19.90 (d, *J* = 5.2 and 5.6 Hz, *C17*), 16.79 and 16.67 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.56 and 24.75. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{38}H_{51}O_9N_7P$ 780.34804, found 780.34815.

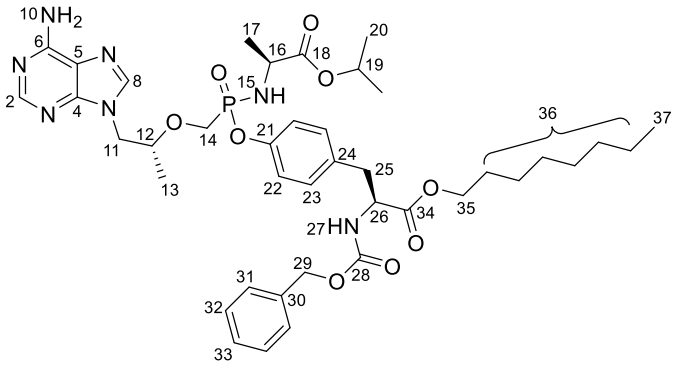
2-Ethylbutyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35o**)



Following standard procedure N, compound **34** (110 mg, 0.27 mmol), **36f** (164 mg, 0.41 mmol), Et_3N (376 µL, 2.16 mmol), PPh_3 (283 mg, 1.08 mmol), and Aldrithiol™ (238 mg, 1.08 mmol) were stirred in pyridine (5 mL) to afford **35o** (119 mg, 56%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09

(s, 1H, *H8*), 7.80 and 7.80 (d, *J* = 8.2 and 8.2 Hz, 1H, *H27*), 7.39–7.24 (m, 5H, *H31*, *H32*, *H33*), 7.25–7.14 (m, 4H, *H10*, *H23*), 7.07–6.97 (m, 2H, *H22*), 5.61 and 5.48 (dd, *J* = 12.0, 10.4 and 12.4, 10.0 Hz, 1H, *H15*), 5.05–4.90 (m, 2H, *H29*), 4.89–4.75 (m, 1H, *H19*), 4.32–4.10 (m, 3H, *H11*, *H26*), 4.00–3.73 (m, 6H, *H12*, *H14*, *H16*, *H35*), 3.04–2.94 and 2.90–2.78 (m, 2H, *H25*), 1.49–1.37 (m, 1H, *H36*), 1.31–1.20 (m, 4H, *H37*), 1.17–1.01 (m, 12H, *H13*, *H17*, *H20*), 0.88–0.74 (m, 6H, *H38*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.04 and 172.89 (d, *J* = 4.2 and 3.9 Hz, *C18*), 171.87 (*C34*), 156.00–155.94 (m, *C28*, *C6*), 152.43 (*C2*), 149.85 and 149.81 (*C4*), 149.07–148.70 (m, *C21*), 141.45 and 141.41 (*C8*), 136.84 (*C30*), 133.54 and 133.43 (*C24*), 130.09 (*C23*), 128.33 (*C32*), 127.82 (*C33*), 127.67 and 127.63 (*C31*), 120.50 and 120.26 (d, *J* = 4.3 and 4.4 Hz, *C22*), 118.38 and 118.34 (*C5*), 75.82–75.29 (m, *C12*), 67.92 and 67.91 (*C19*), 66.16 (*C35*), 65.47 and 65.45 (*C29*), 64.23 and 64.14 (d, *J* = 154.8 and 154.8 Hz, *C14*), 55.72 (*C26*), 49.06 and 49.01 (*C16*), 46.74 and 46.69 (*C11*), 39.69 (*C36*), 35.70 (*C25*) 22.63 and 22.59 (*C37*), 21.42 and 21.38 (*C20*), 20.27 and 19.90 (d, *J* = 5.5 and 5.4 Hz, *C17*), 16.78 and 16.68 (*C13*), 10.85 and 10.81 (*C38*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.55 and 24.75. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{38}H_{53}O_9N_7P$ 782.36369, found 782.36380.

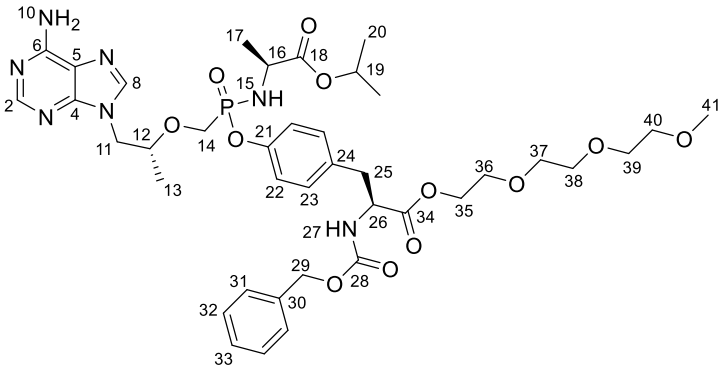
n-Octyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35p**)



Following standard procedure N, compound **34** (190 mg, 0.47 mmol), **36g** (402 mg, 0.94 mmol), Et_3N (523 µL, 3.76 mmol), PPh_3 (493 mg, 1.88 mmol), and Aldrithiol™ (414 mg, 1.88 mmol) were stirred in pyridine (8 mL) to afford **35p** (165 mg, 43%) as a white solid. **^{1}H NMR** (400 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.79 and 7.78 (d, *J* = 8.1 and 8.3 Hz, 1H, *H27*), 7.36–7.15 (m, 9H, *H10*, *H23*, *H31*, *H32*, *H33*), 7.05–6.98 (m, 2H, *H22*), 5.60 and 5.47 (dd, *J* = 12.1, 10.4 and 12.3, 9.9 Hz, 1H, *H15*), 5.04–4.91 (m, 2H, *H29*), 4.88–4.78 (m, 1H, *H19*), 4.32–4.11 (m, 3H, *H11*, *H26*), 4.05–3.74 (m, 6H, *H12*, *H14*, *H16*, *H35*), 3.02–2.95 and 2.88–2.80 (m, 2H, *H25*), 1.54–1.17 (m, 12H, *H36*), 1.16–1.02 (m, 12H, *H13*, *H17*, *H20*), 0.87–0.81 (m, 3H, *H37*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.01–172.83 (m, *C18*), 171.80 (*C34*), 155.93 (*C6*, *C28*), 152.37 (*C2*), 149.82 and 149.79 (*C4*), 148.98–148.75 (m, *C21*), 141.42 and 141.38 (*C8*), 136.85 (*C30*), 133.48 and 133.37 (*C24*), 130.08 (*C23*), 128.29 (*C32*), 127.77 (*C33*), 127.57 and 127.55 (*C31*), 120.45 and 120.22 (d, *J* = 4.2 and 3.7 Hz, *C22*), 118.37 and 118.32 (*C5*), 75.67–75.43 (m, *C12*), 67.88 and 67.86 (*C19*), 65.39 (*C29*), 64.51 (*C35*), 64.98–63.37 (m, *C14*), 55.64 (*C26*), 49.04 and 48.98

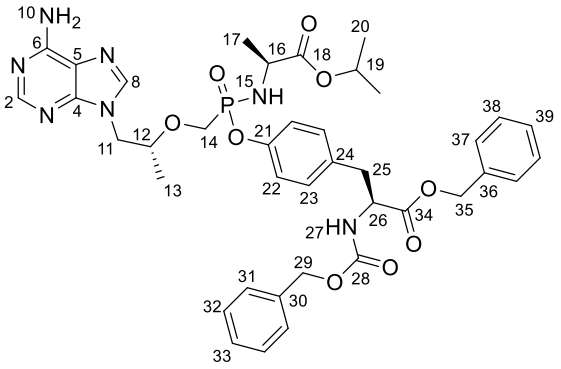
(*C16*), 46.73 and 46.68 (*C11*), 35.73 (*C25*), 31.18 and 28.55 and 28.02 and 25.20 and 22.05 (*C36*), 21.39 and 21.36 (*C20*), 20.28–19.84 (m, *C16*), 16.76 and 16.65 (*C13*), 13.92 (*C37*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.29 and 24.48. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{40}H_{57}O_9N_7P$ 810.39499, found 810.39485.

2-(2-(2-Methoxyethoxy)ethoxy)ethyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35q**)



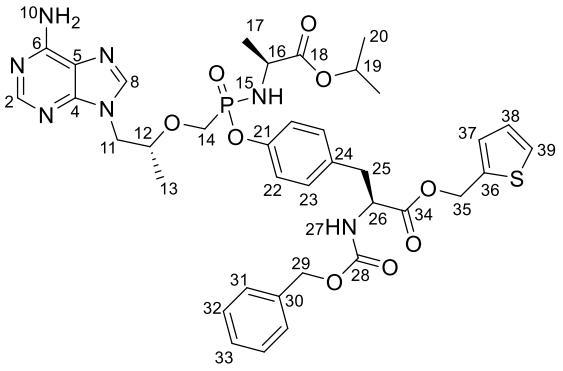
Following standard procedure N, compound **34** (210 mg, 0.52 mmol), **36h** (480 mg, 1.04 mmol), Et_3N (580 µL, 4.16 mmol), PPh_3 (546 mg, 2.08 mmol), and Aldrithiol^{TM} (458 mg, 2.08 mmol) were stirred in pyridine (8 mL) to afford **35q** (141 mg, 32%) as an off-white solid. **^{1}H NMR** (400 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.80 and 7.79 (d, J = 8.1 and 8.1 Hz, 1H, *H27*), 7.39–7.25 (m, 5H, *H31*, *H32*, *H33*), 7.25–7.14 (m, 4H, *H10*, *H23*), 7.09–6.94 (m, 2H, *H22*), 5.60 and 5.47 (dd, J = 12.0, 10.3 and 12.4 and 10.0 Hz, 1H, *H15*), 5.04–4.91 (m, 2H, *H29*), 4.89–4.75 (m, 1H, *H19*), 4.33–4.08 (m, 5H, *H11*, *H26*, *H35*), 4.01–3.72 (m, 4H, *H12*, *H14*, *H16*), 3.63–3.36 (m, 10H, *H36*, *H37*, *H38*, *H39*, *H40*), 3.05–2.96 and 2.90–2.79 (m, 2H, *H25*), 1.17–1.00 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.04 and 172.90 (d, J = 4.0 and 3.9 Hz, *C18*), 171.80 (*C34*), 156.08–155.88 (m, *C6*, *C28*), 152.44 (*C2*), 149.86 and 149.82 (*C4*), 149.10–148.70 (m, *C21*), 141.47 and 141.43 (*C8*), 136.86 (*C30*), 133.48 and 133.37 (*C24*), 130.14 (*C23*), 128.34 (*C32*), 127.81 (*C33*), 127.63 and 127.61 (*C31*), 120.48 and 120.26 (d, J = 4.3 and 4.5 Hz, *C22*), 118.39 and 118.35 (*C5*), 75.79–75.38 (m, *C12*), 71.27 (*C40*), 69.89–69.57 (m, *C37*, *C38*, *C39*), 68.15 (*C36*), 67.94 and 67. 92 (*C19*), 65.48 and 65.46 (*C29*), 64.25 and 64.16 (d, J = 156.3 and 155.5 Hz, *C14*), 64.00 (*C35*), 58.05 (*C41*), 55.60 (*C26*), 49.07 and 49.03 (*C16*), 46.75 and 46.72 (*C11*), 35.73 (*C25*), 21.42 and 21.39 (*C20*), 20.27 and 19.90 (d, J = 5.1 and 5.3 Hz, *C17*), 16.79 and 16.67 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.33 and 24.53. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{39}H_{54}O_{12}N_7NaP$ 866.34603, found 866.34574.

Benzyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35r**)



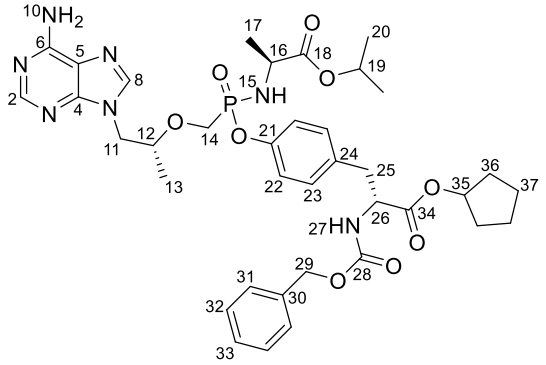
Following standard procedure N, compound **34** (195 mg, 0.49 mmol), **36i** (397 mg, 0.98 mmol), Et_3N (547 µL, 3.92 mmol), PPh_3 (514 mg, 1.96 mmol), and Aldrithiol™ (432 mg, 1.96 mmol) were stirred in pyridine (8 mL) to afford **35r** (153 mg, 40%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.14 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.86 and 7.86 (d, J = 8.1 and 8.1 Hz, 1H, *H27*), 7.40–7.25 (m, 10H, *H31*, *H32*, *H33*, *H37*, *H38*, *H39*), 7.24–7.14 (m, 4H, *H10, H23*), 7.05–6.97 (m, 2H, *H22*), 5.62 and 5.49 (dd, J = 12.0, 10.3 and 12.4, 10.0 Hz, 1H, *H15*), 5.18–5.06 (m, 2H, *H35*), 5.05–4.90 (m, 2H, *H29*), 4.88–4.77 (m, 1H, *H19*), 4.34–4.11 (m, 3H, *H11*, *H26*), 4.01–3.73 (m, 4H, *H12*, *H14*, *H16*), 3.08–2.99 and 2.92–2.80 (m, 2H, *H25*), 1.17–0.99 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.05 and 172.90 (d, J = 3.7 and 3.7 Hz, *C18*), 171.71 (*C34*), 156.08–155.95 (m, *C6*, *C28*), 152.44 (*C2*), 149.85 and 149.82 (*C4*), 149.08–148.75 (m, *C21*), 141.46 and 141.43 (*C8*), 136.84 (*C30*), 135.83 (*C36*), 133.42 and 133.31 (*C24*), 130.17 (*C23*), 128.44 (*C38*), 128.35 (*C32*), 128.05 (*C39*), 127.83 (*C33*), 127.80 and 127.78 (*C37*), 127.67 and 127.63 (*C31*), 120.50 and 120.28 (d, J = 4.3 and 4.4 Hz, *C22*), 118.39 and 118.35 (*C5*), 75.74–75.41 (m, *C12*), 67.92 and 67.93 (*C19*), 66.08 (*C35*), 65.49 and 65.51 (*C29*), 64.25 and 64.16 (d, J = 154.4 and 154.8 Hz, *C14*), 55.71 (*C26*), 49.07 and 49.02 (*C16*), 46.76 and 46.71 (*C11*), 35.67 (*C25*), 21.43 and 21.39 (*C20*), 20.28 and 19.91 (d, J = 5.1 and 5.1 Hz, *C17*), 16.80 and 16.68 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.56 and 24.76. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{39}H_{46}O_9N_7NaP$ 810.29868, found 810.29850.

Thiophen-2-ylmethyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35s**)



Following standard procedure N, compound **34** (210 mg, 0.52 mmol), **36j** (428 mg, 1.04 mmol), Et_3N (580 µL, 4.16 mmol), PPh_3 (546 mg, 2.08 mmol), and Aldrithiol^{TM} (458 mg, 2.08 mmol) were stirred in pyridine (8 mL) to afford **35s** (41 mg, 10%) as a white solid. **^{1}H NMR** (400 MHz, DMSO-d_6, mixture of epimers) δ 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.82 and 7.82 (d, J = 8.1 and 8.0 Hz, 1H, *H27*), 7.56 (dd, J = 5.1, 1.3 Hz, 1H, *H39*), 7.37–7.24 (m, 5H, *H31*, *H32*, *H33*), 7.20–7.13 (m, 5H, *H10*, *H23*, *H37*), 7.04–6.95 (m, 3H, *H22*, *H38*), 5.60 and 5.47 (dd, J = 12.0, 10.3 and 12.4, 10.0 Hz, 1H, *H15*), 5.29 (s, 2H, *H35*), 5.03–4.90 (m, 1H, *H29*), 4.87–4.77 (m, 1H, *H19*), 4.31–4.11 (m, 3H, *H11*, *H26*), 3.99–3.74 (m, 4H, *H12*, *H14*, *H16*), 3.03–2.95 and 2.87–2.78 (m, 2H, *H25*), 1.15–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.03–172.84 (m, *C18*), 171.52 (*C34*), 155.95 (*C6*, *C28*), 152.42 (*C2*), 149.84 and 149.81 (*C4*), 141.45 and 141.41 (*C8*), 137.56 (*C36*), 136.82 (*C30*), 133.32 and 133.20 (*C24*), 130.12 (*C23*), 128.58 (*C37*), 128.33 (*C32*), 127.80 (*C33*), 127.66–127.55 (m, *C31*, *C39*), 126.87 (*C38*), 120.47 and 120.25 (d, J = 3.8 and 3.6 Hz, *C22*), 118.37 and 118.34 (*C5*), 75.77–75.36 (m, *C12*), 67.91 (*C19*), 65.48 (*C29*), 65.00–63.37 (m, *C14*), 60.71 (*C35*), 55.58 (*C26*), 49.05 and 49.01 (*C16*), 46.73 (*C11*), 35.60 (*C25*), 21.41 and 21.38 (*C20*), 20.26 and 19.89 (d, J = 5.1 and 5.4 Hz, *C17*), 16.79 and 16.67 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.31 and 24.52. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{37}H_{45}O_9N_7PS$ 794.27316, found 794.27339.

Cyclopentyl (2*R*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(((benzyloxy)carbonyl)amino)propanoate (**35t**)



Following standard procedure O, tenofovir (112 mg, 0.39 mmol), L-alanine isopropyl ester hydrochloride (65 mg, 0.39 mmol), **37** (270 mg, 0.70 mmol), DIPEA (679 µL, 3.90 mmol), PPh_3 (409 mg, 1.56 mmol), and Aldrithiol^{TM} (344 mg, 1.56 mmol) were stirred in pyridine (8 mL) to afford **35t** (244 mg, 82%) as a white solid. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{37}H_{49}O_9N_7P$ 766.33239, found 766.33272.

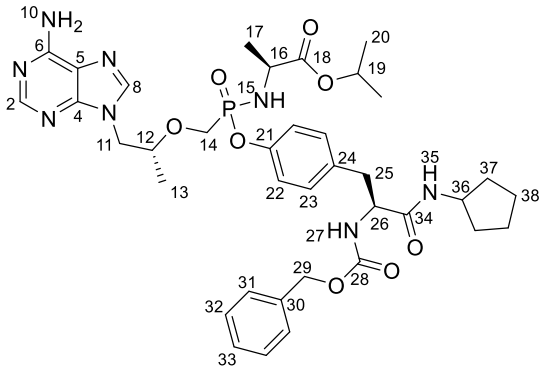
NMR data and characterization of **35t-FEE**:

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.14 (s, 1H, *H2*), 8.12 (s, 1H, *H8*), 7.76 (bd, J = 8.0 Hz, 1H, *H27*), 7.38–7.27 (m, 5H, *H31*, *H32*, *H33*), 7.25–7.18 (m, 3H, *H10*, *H23*), 7.05–6.99 (m, 2H, *H22*), 5.48 (dd, J = 12.4, 10.0 Hz, 1H, *H15*), 5.06–4.90 (m, 3H, *H35*, *H29*), 4.82 (hept, J = 6.3 Hz, 1H, *H19*), 4.28 (dd, J = 14.4, 4.0 Hz, 1H, *H11a*), 4.22–4.12 (m, 2H, *H11b*, *H26*), 4.00–3.93 (m, 1H, *H12*), 3.90–3.77 (m, 3H, *H14*, *H16*), 2.95 (dd, J = 14.1, 5.5 Hz, 1H, *H25a*), 2.83 (dd, J = 13.8, 9.7 Hz, 1H, *H25b*), 1.84–1.39 (m, 8H, *H36*, *H37*), 1.15–1.02 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.04 (d, J = 4.0 Hz, *C18*), 171.50 (*C34*), 155.96 (*C6*), 155.84 (*C28*), 152.29 (*C2*), 149.82 (*C4*), 148.80 (d, J = 8.6 Hz, *C21*), 141.51 (*C8*), 136.90 (*C30*), 133.48 (*C24*), 130.13 (*C23*), 128.32 (*C32*), 127.81 (*C33*), 127.64 (*C31*), 120.45 (d, J = 4.3 Hz, *C22*), 118.32 (*C5*), 77.21 (*C35*), 75.62 (d, J = 12.5 Hz, *C12*), 67.91 (*C19*), 65.40 (*C29*), 64.14 (d, J = 155.1 Hz, *C14*), 55.66 (*C26*), 49.01 (*C16*), 46.72 (*C11*), 35.71 (*C25*), 32.08 (*C36*), 32.05 (*C36*), 23.24 (*C37*), 23.20 (*C37*), 21.42 (*C20*), 21.39 (*C20*), 19.88 (d, J = 5.9 Hz, *C16*), 16.79 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 25.55.

NMR data and characterization of **35t-SEE**:

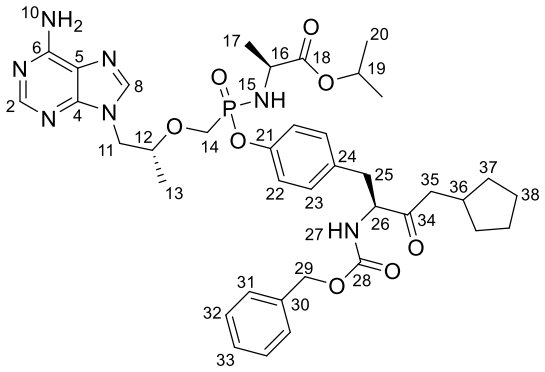
^{1}H NMR (401 MHz, DMSO-d_6) δ 8.14 (s, 1H, *H2*), 8.10 (s, 1H, *H8*), 7.77 (bd, J = 8.0 Hz, 1H, *H27*), 7.37–7.26 (m, 5H, *H31*, *H32*, *H33*), 7.21 (bs, 2H, *H10*), 7.20–7.14 (m, 2H, *H23*), 7.02–6.97 (m, 2H, *H22*), 5.61 (dd, J = 12.0, 10.4 Hz, 1H, *H15*), 5.07–4.92 (m, 3H, *H35*, *H29*), 4.84 (hept, J = 6.3 Hz, 1H, *H19*), 4.27 (dd, J = 14.4, 3.8 Hz, 1H, *H11a*), 4.18–4.11 (m, 2H, *H11b*, *H26*), 3.96–3.74 (m, 4H, *H12*, *H14*, *H16*), 2.95 (dd, J = 13.9, 5.6 Hz, 1H, *H25a*), 2.83 (dd, J = 13.9, 9.7 Hz, 1H, *H25b*), 1.81–1.40 (m, 8H, *H36*, *H37*), 1.15–1.04 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.98 (d, J = 4.0 Hz, *C18*), 171.50 (*C34*), 155.96 (*C6*), 155.94 (*C28*), 152.41 (*C2*), 149.82 (*C4*), 148.95 (d, J = 8.7 Hz, *C21*), 141.51 (*C8*), 136.91 (*C30*), 133.39 (*C24*), 130.14 (*C23*), 128.33 (*C32*), 127.81 (*C33*), 127.63 (*C31*), 120.22 (d, J = 4.4 Hz, *C22*), 118.36 (*C5*), 77.21 (*C35*), 75.50 (d, J = 12.4 Hz, *C12*), 67.90 (*C19*), 65.40 (*C29*), 64.20 (d, J = 154.7 Hz, *C14*), 55.67 (*C26*), 49.05 (*C16*), 46.76 (*C11*), 35.73 (*C25*), 32.09 (*C36*), 32.05 (*C36*), 23.25 (*C37*), 23.21 (*C37*), 21.42 (*C20*), 21.40 (*C20*), 20.27 (d, J = 5.1 Hz, *C16*), 16.67 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6) δ 24.74.

Isopropyl (((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(4-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(cyclopentylamino)-3-oxopropyl)phenoxy)phosphoryl)-L-alaninate (**35u**)



Following standard procedure N, compound **34** (110 mg, 0.27 mmol), **38** (157 mg, 0.41 mmol), DIPEA (376 µL, 2.16 mmol), PPh_3 (283 mg, 1.08 mmol), and Aldrithiol™ (238 mg, 1.08 mmol) were stirred in pyridine (5 mL) to afford **35u** (106 mg, 51%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.88 and 7.89 (d, J = 7.3 and 7.3 Hz, 1H, *H27*), 7.46–7.14 (m, 10H, *H10*, *H23*, *H31*, *H32*, *H33*, *H35*), 7.08–6.94 (m, 2H, *H22*), 5.60 and 5.47 (dd, J = 12.0, 10.3 and 12.4, 9.9 Hz, 1H, *H15*), 5.04–4.88 (m, 2H, *H29*), 4.88–4.77 (m, 1H, *H19*), 4.35–4.08 (m, 3H, *H11*, *H26*), 4.03–3.73 (m, 5H, *H12*, *H14*, *H16*, *H36*), 2.91–2.78 and 2.78–2.63 (m, 2H, *H25*), 1.85–1.21 (m, 8H, *H37*, *H38*), 1.18–1.00 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.02 and 172.89 (d, J = 4.0 and 3.9 Hz, *C18*), 170.60 (*C34*), 155.95 (*C6*), 155.73 (*C28*), 152.41 (*C2*), 149.84 and 149.80 (*C4*), 148.80–148.57 (m, *C21*), 141.43 and 141.39 (*C8*), 137.03 (*C30*), 134.10 and 133.98 (*C24*), 130.21 (*C23*), 128.29 (*C32*), 127.69 (*C33*), 127.52 and 127.49 (*C31*), 120.23 and 120.01 (d, J = 4.3 and 4.3 Hz), 118.36 and 118.33 (*C5*), 75.69–75.45 (m, *C12*), 67.89 (*C19*), 56.04 and 56.01 (*C26*), 50.25 (*C36*), 49.04 and 49.01 (*C16*), 46.69 (*C11*), 37.15 (*C25*), 32.30 and 32.06 (*C37*), 23.44 and 23.40 (*C38*), 21.42 and 21.38 (*C20*), 20.27 and 19.88 (d, J = 5.1 and 5.3 Hz, *C17*), 16.79 and 16.69 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.46 and 24.67. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{37}H_{50}O_8N_8P$ 765.34837, found 765.34848.

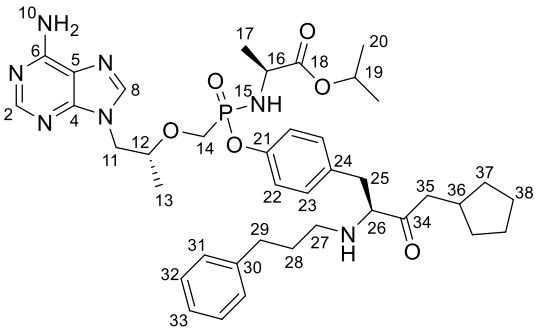
Isopropyl (((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(4-((*S*)-2-(((benzyloxy)carbonyl)amino)-4-cyclopentyl-3-oxobutyl)phenoxy)phosphoryl)-L-alaninate (**35v**)



Following standard procedure N, compound **34** (200 mg, 0.50 mmol), **40** (381 mg, 1.00 mmol), Et_3N (558 µL, 4.00 mmol), PPh_3 (525 mg, 2.00 mmol), and Aldrithiol™ (441 mg, 2.00 mmol) were stirred in pyridine (8 mL) to afford **35v** (126 mg, 33%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.11 and 8.09 (s, 1H, *H8*), 7.72 and 7.72 (d, J = 8.2 and 8.2 Hz, 1H, *H27*), 7.38–7.24 (m, 5H, *H31*, *H32*,

H33), 7.24–7.14 (m, 4H, H10, H23), 7.05–6.95 (m, 2H, *H22*), 5.62 and 5.48 (dd, *J* = 12.0, 10.3 and 12.5, 9.9 Hz, 1H, *H15*), 5.04–4.90 (m, 2H, *H29*), 4.89–4.77 (m, 1H, *H19*), 4.32–4.10 (m, 3H, *H11*, *H26*), 4.01–3.72 (m, 4H, *H12*, *H14*, *H16*), 3.03–2.92 and 2.70–2.59 (m, 2H, *H25*), 2.59–2.37 (m, 2H, *H35*), 2.17–2.05 (m, 1H, *H36*), 1.76–1.37 (m, 6H, *H37*, *H38*), 1.19–0.94 (m, 14H, *H13*, *H17*, *H20*, *H38*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 209.12 (*C34*), 173.05 and 172.86 (d, *J* = 4.0 and 3.9 Hz, *C19*), 156.06–155.88 (m, *C6*, *C28*), 152.41 (*C2*), 149.84 and 149.80 (*C4*), 148.92–148.55 (m, *C21*), 141.43 and 141.39 (*C8*), 136.99 (*C30*), 134.05 and 133.93 (*C24*), 130.14 (C*23*), 128.31 (*C32*), 127.78 (*C33*), 127.57 and 127.54 (*C31*), 120.43 and 120.17 (d, *J* = 4.3 and 4.0 Hz, *C22*), 118.37 and 118.33 (*C5*), 75.84–75.28 (m, *C12*), 67.89 (*C19*), 65.37 (*C29*), 64.21 and 64.13 (d, *J* = 154.9 and 155.7 Hz, *C14*), 61.30 (*C26*), 49.05 and 48.99 (*C16*), 46.73 and 46.68 (*C11*), 44.79 (*C35*), 34.63 (*C36*), 34.39 (*C25*), 32.02 and 32.00 (*C37*), 24.46 (*C38*), 21.41 and 21.38 (*C20*), 20.25 and 19.89 (d, *J* = 4.5 and 5.4 Hz, *C17*), 16.78 and 16.67 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.52, 24.70. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{38}H_{50}O_8N_7NaP$ 786.33507, found 786.33508.

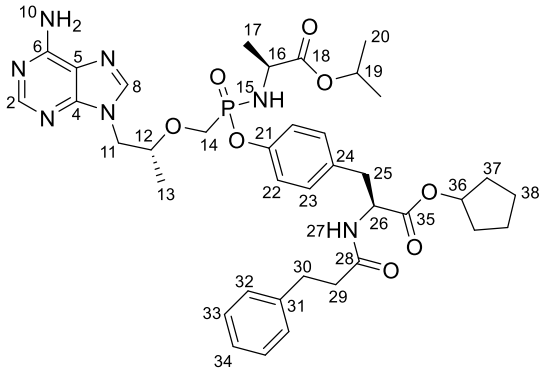
Isopropyl (((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(4-((*S*)-4-cyclopentyl-3-oxo-2-((3-phenylpropyl)amino)butyl)phenoxy)phosphoryl)-L-alaninate (**35w**)



Compound **43** (125 mg, 0.15 mmol) was mixed with Pd/C in phosphate buffer (0.01 M, pH 7.4)/MeOH (4:1 ratio) under argon atmosphere. The flask was evacuated and flushed with H_2 (3 ×) and the mixture was stirred under H_2 atmosphere at 25 °C for 1 h. After filtration, the volatiles were evaporated and the resiude was purified using C_{18}-reversed phase flash chromatography (linear gradient elution 0–100 % MeOH in water) to afford **35w** (52 mg, 46%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.28–7.08 (m, 9H, *H10*, *H23*, *H31*, *H32*, *H33*), 7.04–6.94 (m, 2H, *H22*), 5.61 and 5.47 (dd, *J* = 11.9, 10.4 and 12.3, 9.9 Hz, 1H, *H15*), 4.90–4.76 (m, 1H, *H19*), 4.32–4.09 (m, 2H, *H11*), 4.01–3.67 (m, 4H, *H12*, *H14*, *H16*), 3.40–3.29 (m, 1H, *H26*), 2.82–2.71 and 2.71–2.61 (m, 2H, *H25*), 2.56–2.24 (m, 6H, *H27*, *H29*, *H35*), 2.11–2.00 (m, 1H, *H36*), 1.71–1.38 (m, 8H, *H28*, *H37*, *H38*), 1.18–1.01 (m, 12H, *H13*, *H17*, *H20*), 1.01–0.89 (m, 2H, *H37*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 212.80 (*C34*), 173.02 and 172.87 (d, *J* = 4.1 and 4.0 Hz, *C18*), 155.98 and 155.95 (*C6*), 152.42 (*C2*), 149.84 and 149.80 (*C4*), 148.76–148.38 (m, *C21*), 142.00 (*C30*), 141.42 and 141.38 (*C8*), 134.51 and 134.40 (*C24*), 130.14 (*C23*), 128.24 and 128.16 (*C31*, *C32*), 125.57 (*C33*), 120.36 and 120.07 (d, *J* = 4.2 and 4.4 Hz, *C22*), 118.38 and 118.33 (*C5*), 75.92–75.29 (m, *C12*), 68.17 (*C26*), 67.89 (*C19*), 64.12 (d, *J* = 155.1 Hz, *C14*), 49.06 and 48.99 (*C16*), 46.78 and 46.68 (*C11*), 46.48 (*C27*), 45.25 and 45.20 (*C35*), 36.70 (*C25*), 34.57 (*C36*), 32.65 (*C29*), 32.06 and 31.99 (*C37*),

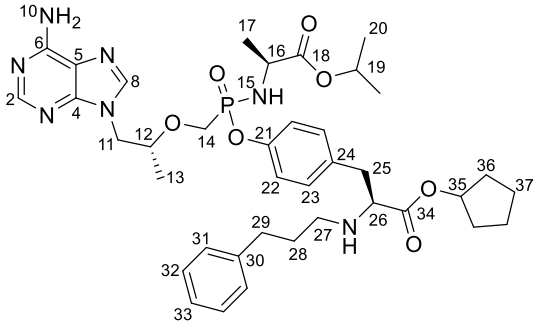
31.47 (*C28*), 24.45 (*C38*), 21.42 and 21.38 (*C20*), 20.31–19.87 (m, *C17*), 16.77 and 16.62 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.49 and 24.64. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{39}H_{55}O_6N_7P$ 748.39460, found 748.39434.

Cyclopentyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(3-phenylpropanamido)propanoate (**35x**)



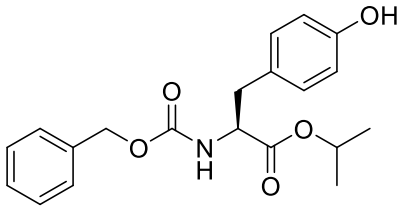
Following standard procedure N, compound **34** (100 mg, 0.25 mmol), **45** (191 mg, 0.50 mmol), Et_3N (278 µL, 2.00 mmol), PPh_3 (262 mg, 1.00 mmol), and Aldrithiol^{TM} (220 mg, 1.00 mmol) were stirred in pyridine (7 mL) to afford **35x** (143 mg, 75%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.29 and 8.29 (bd, *J* = 7.6 and 7.6 Hz, 1H, *H27*), 8.14 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.29–7.08 (m, 9H, *H10*, *H23*, *H32*, *H33*, *H34*), 7.04–6.95 (m, 2H, *H22*), 5.61 and 5.47 (dd, *J* = 12.0, 10.4 and 12.4, 9.9 Hz, 1H, *H15*), 5.05–4.98 (m, 1H, *H36*), 4.89–4.77 (m, 1H, *H19*), 4.42–4.33 (m, 1H, *H26*), 4.31–4.10 (m, 2H, *H11*), 4.00–3.72 (m, 4H, *H12*, *H14*, *H16*), 2.97–2.79 (m, 2H, *H25*), 2.77–2.71 (m, 2H, *H30*), 2.39–2.35 (m, 2H, *H29*), 1.85–1.37 (m, 8H, *H37*, *H38*), 1.18–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.04 and 172.88 (d, *J* = 4.1 and 4.1 Hz, *C18*), 171.47 and 171.46 (*C28*), 171.29 and 171.28 (*C35*) 155.97 and 155.95 (*C6*), 152.41 (*C2*), 149.83 and 149.80 (*C4*), 148.98–148.68 (m, *C21*), 141.42 and 141.38 (*C8*), 141.17 (*C31*), 133.30 and 133.18 (*C24*), 130.07 (*C23*), 128.24 (*C33*), 128.13 (*C32*), 125.87 (*C34*), 120.48 and 120.18 (d, *J* = 4.2 and 4.5 Hz), 118.38 and 118.33 (*C5*), 77.09 (*C36*), 75.79–75.40 (m, *C12*), 67.89 (*C19*), 65.05–63.34 (m, *C14*), 53.62 (*C26*), 49.04 and 49.00 (*C16*), 46.75 and 46.69 (*C11*), 36.54 (*C29*), 36.03 (*C25*), 32.03 (*C37*), 30.91 (*C30*), 23.25 and 23.22 (*C38*), 21.42 and 21.39 (*C20*), 20.29 and 19.90 (d, *J* = 5.0 and 5.6 Hz, *C16*), 16.81 and 16.65 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.55 and 24.73. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{38}H_{51}O_8N_7P$ 764.35312, found 764.35345.

Cyclopentyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-((3-phenylpropyl)amino)propanoate (**35y**)



Compound **47** (65 mg, 0.08 mmol) was mixed with Pd/C in phosphate buffer (0.01 M, pH 7.4)/MeOH (4:1 ratio) under argon atmosphere. The flask was evacuated and flushed with H_2 (3 ×) and the mixture was stirred under H_2 atmosphere at 25 °C for 1 h. After filtration, the volatiles were evaporated and the resiude was purified using C_{18}-reversed phase flash chromatography (linear gradient elution 0–100 % MeOH in water) to afford **35y** (37 mg, 64%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.29–7.06 (m, 9H, *H10*, *H23*, *H31*, *H32*, *H33*), 7.03–6.92 (m, 2H, *H22*), 5.60 and 5.46 (dd, *J* = 11.9, 10.3 and 12.3, 9.9 Hz, 1H, *H15*), 5.03–4.93 (m, 1H, *H35*), 4.91–4.75 (m, 1H, *H35*), 4.32–4.09 (m, 2H, *H11*), 4.00–3.70 (m, 4H, *H12*, *H14*, *H16*), 3.31–3.24 (m, 1H, *H26*), 2.86–2.68 (m, 2H, *H25*), 2.58–2.31 (m, 4H, *H27*, *H29*), 1.79–1.27 (m, 10H, *H28*, *H36*, *H37*), 1.17–0.99 (m, 12, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.84 (*C34*), 173.04 and 172.87 (d, *J* = 4.0 and 4.0 Hz, *C18*), 155.97 and 155.95 (*C6*), 152.41 (*C2*), 149.83 and 149.80 (*C4*), 148.87–148.45 (m, *C21*), 142.03 (*C30*), 141.42 and 141.38 (*C8*), 133.96 and 133.87 (*C24*), 130.13 (*C23*), 128.26 and 128.19 (*C31*, *C32*), 125.59 (*C33*), 120.31 and 120.04 (d, *J* = 4.3 and 4.4 Hz, *C22*), 118.38 and 118.33 (*C5*), 76.45 (*C35*), 75.78–75.33 (m, *C12*), 67.88 (*C19*), 64.10 (d, *J* = 153.3 Hz, *C14*), 62.54 (*C26*), 49.04 and 49.00 (*C16*), 46.76 and 46.68 (*C11*), 46.47 (*C27*), 38.07 (*C25*), 32.73 (*C29*), 32.14 and 32.06 (*C36*), 31.34 (*C28*), 23.19 and 23.14 (*C37*), 21.41 and 21.38 (*C20*), 20.38–19.73 (m, *C17*), 16.78 and 16.64 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.47 and 24.67. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{38}H_{53}O_7N_7P$ 750.37386, found 750.37366.

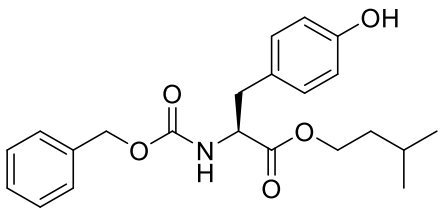
N-((benzyloxy)carbonyl)-L-tyrosine isopropyl ester (**36a**)



N-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol) was dissolved in *i*PrOH (5 mL), $SOCl_2$ (231 µL, 3.18 mmol) was added, and the solution was stirred at 25 °C for 72 h. The mixture was concentrated and the residue was purified using silica gel flash chromatography (linear gradient elution 0–15% MeOH in $CHCl_3$) to afford **36a** (449 mg, 79%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.29 (s, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.41–7.19 (m, 5H), 7.08–6.99 (m, 2H), 6.72–6.64 (m, 2H), 5.08–4.93 (m, 2H), 4.93–4.81 (m, 1H), 4.14 (ddd, J = 9.5, 7.9, 5.7 Hz, 1H), 2.89 (dd, J = 13.8, 5.7 Hz, 1H), 2.78 (dd, J = 13.8, 9.5 Hz, 1H), 1.16 (d, J = 6.2 Hz, 3H), 1.08

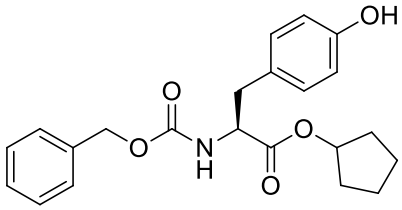
(d, J = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.64, 156.10, 156.07, 137.10, 130.18, 128.42, 127.87, 127.65, 127.44, 115.12, 68.06, 65.44, 56.20, 35.99, 21.62, 21.43. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{20}H_{23}O_5NNa$ 380.14684, found 380.14694. **$[\alpha]^{25}_D$** = +7.7 (c 0.404 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine isoamyl ester (**36b**)



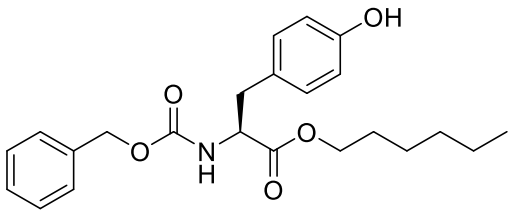
Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), isoamyl alcohol (181 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **36b** (509 mg, 83%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.22 (s, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.38–7.24 (m, 5H), 7.06–6.95 (m, 2H), 6.70–6.61 (m, 2H), 5.04–4.93 (m, 2H), 4.14 (ddd, J = 9.7, 7.9, 5.7 Hz, 1H), 4.09–3.98 (m, 2H), 2.88 (dd, J = 13.8, 5.7 Hz, 1H), 2.75 (dd, J = 13.8, 9.7 Hz, 1H), 1.63–1.47 (m, 1H), 1.42–1.34 (m, 2H), 0.85 (d, J = 3.9 Hz, 3H), 0.84 (d, J = 3.9 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.50, 156.47, 156.39, 137.41, 130.47, 128.78, 128.25, 128.03, 127.79, 115.46, 65.81, 63.33, 56.51, 37.21, 36.30, 24.80, 22.76, 22.68. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{27}O_5NNa$ 408.17814, found 408.17820. **$[\alpha]^{25}_D$** = +8.1 (c 0.099 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine cyclopentyl ester (**36c**)



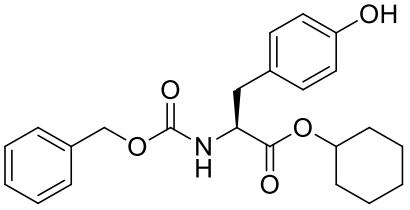
Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (1.58 g, 5.00 mmol), cyclopentanol (476 µL, 5.25 mmol), PPh_3 (1.38 g, 5.25 mmol), and DIAD (1.03 mL, 5.25 mmol) were stirred in THF (40 mL) to afford **36c** (1.82 g, 95%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.42–7.16 (m, 5H), 7.06–6.94 (m, 2H), 6.72–6.58 (m, 2H), 5.07–4.87 (m, 3H), 4.10 (ddd, J = 9.4, 7.9, 6.0 Hz, 1H), 2.85 (dd, J = 13.8, 6.0 Hz, 1H), 2.75 (dd, J = 13.8, 9.4 Hz, 1H), 1.83–1.65 (m, 2H), 1.63–1.37 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.70, 155.98, 155.93, 136.97, 130.02, 128.32, 127.77, 127.57, 127.28, 114.96, 77.05, 65.32, 55.99, 35.82, 32.07, 32.03, 23.24, 23.18. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{35}O_5NNa$ 406.16249, found 406.16317. **$[\alpha]^{25}_D$** = +5.9 (c 0.136 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine *n*-hexyl ester (**36d**)



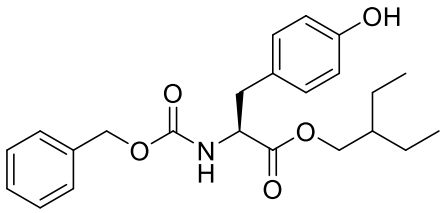
Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), *n*-hexanol (210 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **36d** (533 mg, 84%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.24 (bs, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.38–7.24 (m, 5H), 7.05–6.97 (m, 2H), 6.69–6.61 (m, 2H), 4.98 (d, J = 2.7 Hz, 2H), 4.15 (ddd, J = 9.6, 8.1, 5.6 Hz, 1H), 4.06–3.95 (m, 2H), 2.89 (dd, J = 13.8, 5.6 Hz, 1H), 2.76 (dd, J = 13.9, 9.6 Hz, 1H), 1.54–1.43 (m, J = 7.2, 6.7 Hz, 2H), 1.31–1.14 (m, 6H), 0.85 (t, J = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.08, 156.05, 155.95, 136.96, 130.02, 128.33, 127.78, 127.56, 127.32, 115.02, 65.35, 64.44, 56.05, 35.88, 30.85, 28.01, 24.92, 21.99, 13.90. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}O_5N$ 400.21185, found 400.21199. **$[\alpha]^{25}_D$** = +5.8 (c 0.449 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine cyclohexyl ester (**36e**)



Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (536 mg, 1.70 mmol), cyclohexanol (186 µL, 1.79 mmol), PPh_3 (469 mg, 1.79 mmol), and DIAD (351 µL, 1.79 mmol) were stirred in THF (8 mL) to afford **36e** (572 mg, 85%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.24 (bs, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.45–7.16 (m, 5H), 7.06–6.98 (m, 2H), 6.69–6.61 (m, 2H), 5.06–4.93 (m, 2H), 4.73–4.59 (m, 1H), 4.12 (ddd, J = 9.6, 7.9, 5.7 Hz, 1H), 2.88 (dd, J = 13.9, 5.7 Hz, 1H), 2.76 (dd, J = 13.9, 9.6 Hz, 1H), 1.77–1.49 (m, 4H), 1.49–1.12 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.37, 156.00, 155.96, 137.00, 130.04, 128.32, 127.76, 127.57, 127.36, 115.00, 72.33, 65.31, 56.15, 35.87, 30.92, 30.74, 24.85, 22.90. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}O_5N$ 398.19620, found 398.19620. **$[\alpha]^{25}_D$** = +6.8 (c 0.310 g/100 mL, $CHCl_3$/MeOH 1/1).

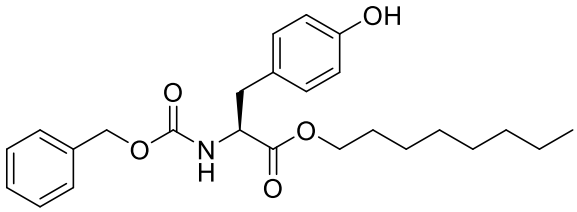
N-((benzyloxy)carbonyl)-L-tyrosine 2-ethylbutyl ester (**36f**)



Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (536 mg, 1.70 mmol), 2-ethylbutanol (220 µL, 1.79 mmol), PPh_3 (469 mg, 1.79 mmol), and DIAD (351 µL, 1.79 mmol) were stirred in THF (8 mL) to afford **36f** (654 mg, 96%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.39–7.23 (m, 5H), 7.06–6.98 (m, 2H), 6.70–6.62 (m, 2H), 5.04–4.92 (m, 2H), 4.16 (ddd, J = 9.7, 8.1, 5.6 Hz, 1H), 3.98–

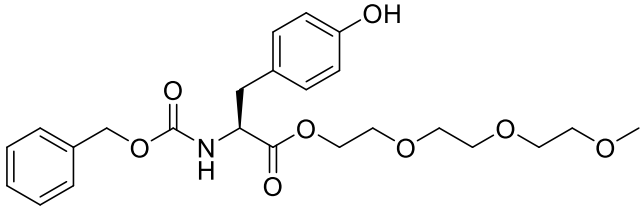
3.88 (m, 2H), 2.89 (dd, J = 13.8, 5.6 Hz, 1H), 2.76 (dd, J = 13.8, 9.7 Hz, 1H), 1.48–1.34 (m, 1H), 1.30–1.14 (m, 4H), 0.82 (td, J = 7.5, 2.2 Hz, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.10, 156.02, 155.96, 136.94, 129.99, 128.33, 127.80, 127.61, 127.37, 115.03, 66.06, 65.38, 56.11, 39.70, 35.84, 22.64, 22.60, 10.87, 10.82. **HRMS** (ESI) m/z $[M+H]^+$ calcd for $C_{23}H_{30}O_5N$ 400.21185, found 400.21194. **$[\alpha]^{25}_D$** = +6.0 (c 0.466 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine *n*-octyl ester (**36g**)



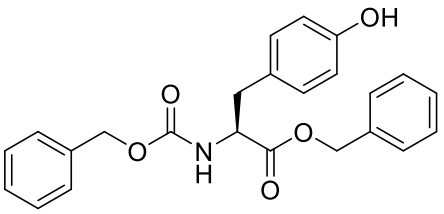
Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), *n*-octanol (261 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **36g** (565 mg, 83%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.25 (bs, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.39–7.26 (m, 5H), 7.06–6.99 (m, 2H), 6.70–6.63 (m, 2H), 4.99 (d, J = 2.8 Hz, 2H), 4.16 (ddd, J = 9.8, 8.0, 5.6 Hz, 1H), 4.06–3.97 (m, 2H), 2.90 (dd, J = 13.8, 5.5 Hz, 1H), 2.77 (dd, J = 13.9, 9.7 Hz, 1H), 1.58–1.43 (m, 2H), 1.24 (s, 10H), 0.86 (t, J = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.07, 156.04, 155.96, 136.96, 130.01, 128.33, 127.79, 127.55, 127.34, 115.02, 65.36, 64.45, 56.06, 35.87, 31.24, 28.61, 28.05, 25.26, 22.11, 13.98. **HRMS** (ESI) m/z $[M+H]^+$ calcd for $C_{25}H_{34}O_5N$ 428.24315, found 428.24323. **$[\alpha]^{25}_D$** = +10.6 (c 0.377 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine 2-(2-(2-methoxyethoxy)ethoxy)ethyl ester (**36h**)



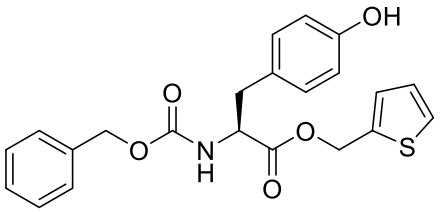
Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), 2-(2-(2-methoxyethoxy)ethoxy)ethanol (261 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **36h** (588 mg, 80%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.38–7.25 (m, 5H), 7.08–7.00 (m, 2H), 6.69–6.63 (m, 2H), 4.99 (s, 2H), 4.23–4.05 (m, 3H), 3.62–3.45 (m, 8H), 3.43–3.38 (m, 2H), 3.22 (s, 3H), 2.92 (dd, J = 13.9, 5.2 Hz, 1H), 2.76 (dd, J = 13.9, 9.8 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.00, 156.01, 155.96, 136.94, 130.05, 128.33, 127.77, 127.56, 127.35, 115.02, 71.27, 69.84, 69.74, 69.61, 68.16, 65.37, 63.89, 58.05, 55.96, 35.80. **HRMS** (ESI) m/z $[M+H]^+$ calcd for $C_{24}H_{32}O_8N$ 462.21224, found 462.21241. **$[\alpha]^{25}_D$** = +6.7 (c 0.624 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine *n*-octyl ester (**36i**)



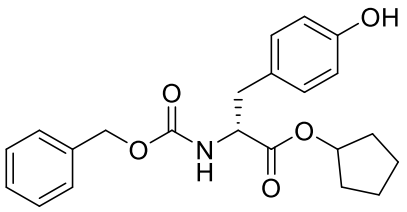
Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), benzyl aclohol (174 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **36i** (491 mg, 76%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 7.80 (d, J = 8.1 Hz, 1H), 7.39–7.24 (m, 10H), 7.05–6.96 (m, 2H), 6.68–6.60 (m, 2H), 5.09 (s, 2H), 5.01–4.94 (m, 2H), 4.28–4.17 (m, 1H), 2.92 (dd, J = 13.8, 5.5 Hz, 1H), 2.77 (dd, J = 13.8, 9.7 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.91, 156.31, 155.98, 136.91, 135.84, 130.03, 128.39, 128.33, 128.01, 127.75, 127.58, 115.10, 65.94, 65.39, 56.08, 35.78. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{24}O_5N$ 406.16490, found 406.16495. **$[\alpha]^{25}_D$** = –2.6 (c 0.377 g/100 mL, $CHCl_3$/MeOH 1/1).

N-((benzyloxy)carbonyl)-L-tyrosine thiophen-2-ylmethyl ester (**36j**)



Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), 2-thienylmethanol (159 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **36j** (464 mg, 71%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.56 (dd, J = 5.1, 1.3 Hz, 1H), 7.38–7.23 (m, 5H), 7.14 (dd, J = 3.5, 1.3 Hz, 1H), 7.02 (dd, J = 5.1, 3.5 Hz, 1H), 7.00–6.93 (m, 2H), 6.67–6.59 (m, 2H), 5.28 (s, 2H), 4.98 (d, J = 2.6 Hz, 2H), 4.18 (ddd, J = 10.0, 8.2, 5.1 Hz, 1H), 2.89 (dd, J = 13.9, 5.1 Hz, 1H), 2.74 (dd, J = 13.9, 10.0 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.19, 156.46, 156.42, 138.05, 137.37, 130.49, 129.04, 128.80, 128.23, 128.01, 127.66, 127.31, 115.49, 65.84, 61.09, 56.42, 36.14. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}O_5NS$ 412.12132, found 412.12140. **$[\alpha]^{25}_D$** = –5.5 (c 0.237 g/100 mL, $CHCl_3$/MeOH 1/1).

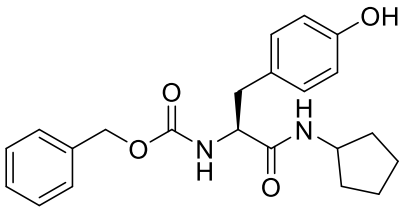
N-((benzyloxy)carbonyl)-D-tyrosine cyclopentyl ester (**37**)



Following standard procedure L, *N*-((benzyloxy)carbonyl)-L-tyrosine (500 mg, 1.59 mmol), cyclopentanol (152 µL, 1.67 mmol), PPh_3 (438 mg, 1.67 mmol), and DIAD (328 µL, 1.67 mmol) were stirred in THF (8 mL) to afford **37** (283 mg, 46%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.42–7.15 (m, 5H), 7.05–6.97 (m, 2H), 6.70–6.62 (m, 2H), 5.06–5.01 (m, 1H), 4.99 (d, J = 5.6 Hz, 2H), 4.10 (ddd, J = 9.4, 7.9, 5.9 Hz, 1H), 2.85 (dd, J = 13.9, 5.9 Hz, 1H), 2.75 (dd, J = 13.9, 9.4 Hz, 1H), 1.83–1.64 (m, 2H), 1.64–

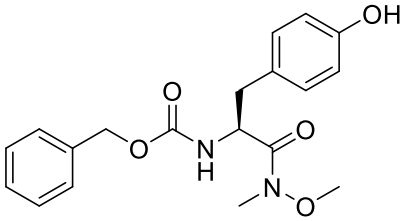
1.36 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.70, 155.98, 155.92, 136.97, 130.02, 128.31, 127.77, 127.58, 127.28, 114.96, 77.05, 65.32, 55.99, 32.07, 32.03, 23.25, 23.18. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{35}O_5NNa$ 406.16249, found 406.16322. **$[\alpha]^{25}_D$** = –9.8 (c 0.153 g/100 mL, $CHCl_3$/MeOH 1/1).

Benzyl (*S*)-(1-(cyclopentylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (**38**)



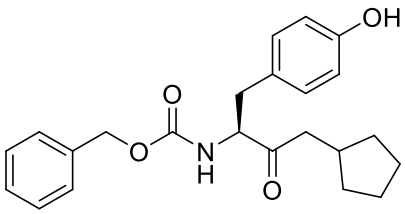
Following standard procedure M, *N*-((benzyloxy)carbonyl)-L-tyrosine (536 mg, 1.70 mmol), cyclopentyl amine (202 µL, 2.04 mmol), HATU (970 mg, 2.55 mmol), and DIPEA (1.48 mL, 8.50 mmol) were stirred in DMF (10 mL) to afford **38** (550 mg, 85%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.17 (s, 1H), 7.82 (d, J = 7.3 Hz, 1H), 7.39–7.23 (m, 6H), 7.06–6.99 (m, 2H), 6.69–6.60 (m, 2H), 4.96 (d, J = 1.5 Hz, 2H), 4.17–4.07 (m, 1H), 4.02–3.89 (m, 1H), 2.77 (dd, J = 13.7, 5.1 Hz, 1H), 2.63 (dd, J = 13.7, 9.7 Hz, 1H), 1.83–1.68 (m, 2H), 1.65–1.54 (m, 2H), 1.53–1.43 (m, 2H), 1.40–1.32 (m, 1H), 1.32–1.22 (m, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.26, 156.20, 156.17, 137.61, 130.61, 128.75, 128.46, 128.11, 127.87, 115.24, 65.56, 56.80, 50.68, 37.67, 32.77, 32.50, 23.92, 23.86. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{26}O_4N_2Na$ 405.17848, found 405.17852. **$[\alpha]^{25}_D$** = +5.7 (c 0.632 g/100 mL, $CHCl_3$/MeOH 1/1).

Benzyl (*S*)-(3-(4-hydroxyphenyl)-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (**39**)



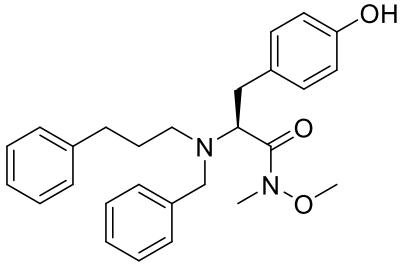
Following standard procedure M, *N*-((benzyloxy)carbonyl)-L-tyrosine (5.00 g, 15.86 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (1.86 g, 19.03 mmol), HATU (9.05 g, 23.79 mmol), and DIPEA (13.81 mL, 79.30 mmol) were stirred in DMF (100 mL) to afford **39** (4.67 g, 82%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.21 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.39–7.22 (m, 5H), 7.07–6.99 (m, 2H), 6.70–6.62 (m, 2H), 4.95 (s, 2H), 4.63–4.49 (m, 1H), 3.71 (s, 3H), 3.10 (s, 3H), 2.77 (dd, J = 13.7, 4.4 Hz, 1H), 2.62 (dd, J = 13.8, 9.8 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 155.96, 155.89, 137.01, 130.04, 128.31, 127.72, 127.55, 114.97, 65.24, 53.23, 35.75. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}O_5N_2$ 360.16797, found 360.16781. **$[\alpha]^{25}_D$** = +10.1 (c 0.357 g/100 mL, $CHCl_3$/MeOH 1/1).

Benzyl (*S*)-(4-cyclopentyl-1-(4-hydroxyphenyl)-3-oxobutan-2-yl)carbamate (**40**)



I_2 (249 mg, 0.98 mmol) and Mg (356 mg, 14.63 mmol) were mixed in THF (9.75 mL) under argon atmosphere. (Bromomethyl)cyclopentane (1.25 mL, 9.75 mmol) was added and the mixture was stirred at 25 °C. Approximately after 1 min, the mixture turned clear in color and the exothermic reaction was triggered. The reaction mixture was stirred at 25 °C for 30 min and at 50°C for 1 h. The freshly prepared cyclopentylmethylmagnesium bromide (1 M in THF, 9.75 mL, 9.75 mmol) was added dropwise at 0 °C to a solution of compound **39** (700 mg, 1.95 mmol) in THF (10 mL). The mixture was allowed to warm up to 25 °C, stirred for 16 h, quenched slowly with 1 M aqueous HCl, and concentrated. The resiude was diluted in $CHCl_3$, washed with H_2O (3 × 50 mL), washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified using silica using gel flash chromatography (linear gradient elution 0–50% EtOAc/MeOH (9:1 ratio) in hexane) to afford **40** (386 mg, 52% + 241 mg, 32% of the starting **39**) as an off-white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.20 (s, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.38–7.22 (m, 5H), 7.07–6.95 (m, 2H), 6.69–6.60 (m, 2H), 5.04–4.88 (m, 2H), 4.13 (ddd, J = 10.0, 8.1, 5.0 Hz, 1H), 2.87 (dd, J = 14.0, 5.0 Hz, 1H), 2.62–2.52 (m, 1H), 2.40 (dd, J = 17.1, 7.1 Hz, 1H), 2.18–2.02 (m, 1H), 1.76–1.63 (m, 2H), 1.57–1.37 (m, 4H), 1.07–0.92 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 209.91, 156.44, 156.30, 137.55, 130.52, 128.77, 128.50, 128.20, 127.94, 126.88, 115.44, 65.75, 62.10, 45.43, 35.05, 34.98, 32.50, 32.46, 24.93. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{23}H_{27}O_4NNa$ 404.18323, found 404.18388. **$[\alpha]^{25}_D$** = +9.3 (c 0.107 g/100 mL, $CHCl_3$/MeOH 1/1).

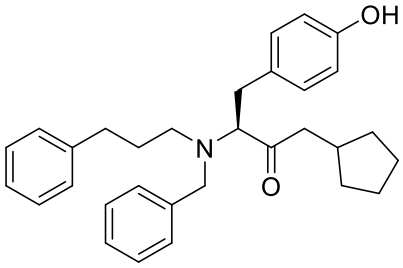
(*S*)-2-(benzyl(3-phenylpropyl)amino)-3-(4-hydroxyphenyl)-*N*-methoxy-*N*-methylpropanamide (**41**)



Compound **39** (4.00 g, 11.16 mmol) was dissolved in MeOH (25 mL) and Pd/C was added under argon atmosphere. The flask was evacuated and flushed with H_2 (3 ×) and the mixture was stirred under H_2 atmosphere at 25 °C for 1 h. The mixture was filtrated, concentrated and mixed with PhCHO (1.25 mL, 12.27 mmol), and Na_2SO_4 (7.92 g, 55.75 mmol) in MeOH (50 mL) under argon atmosphere. AcOH (765 µL, 13.38 mmol) and $NaBH_3CN$ (1.40 g, 22.30 mmol) were added and the mixture was stirred at 25 °C for 30 min. 3-Phenylpropanal (2.94 mL, 22.30 mmol), AcOH (765 µL, 13.38 mmol), and $NaBH_3CN$ (1.40 g, 22.30 mmol) were added. The mixture was stirred at 25 °C for 1 h, filtered, concentrated, and separated using silica using gel flash chromatography (linear gradient elution 0–40% EtOAc/MeOH (9:1 ratio) in hexane) to afford **41** (4.22 g, 88%) as a colorless oil.

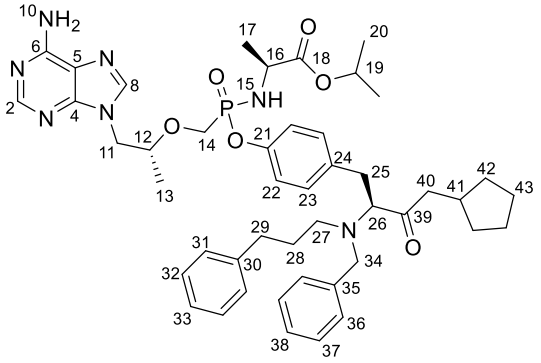
^{1}H NMR (401 MHz, DMSO-d_6) δ 9.16 (s, 1H), 7.31–7.17 (m, 7H), 7.16–7.10 (m, 1H), 7.08–7.02 (m, 2H), 6.96–6.88 (m, 2H), 6.69–6.61 (m, 2H), 4.00 (s, 1H), 3.83 (d, J = 14.4 Hz, 1H), 3.68 (d, J = 14.4 Hz, 1H), 3.29–3.13 (m, 3H), 3.01 (s, 3H), 2.91 (dd, J = 13.2, 9.2 Hz, 1H), 2.77 (dd, J = 13.2, 5.4 Hz, 1H), 2.68–2.53 (m, 2H), 2.46–2.36 (m, 2H), 1.68–1.55 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 155.57, 142.27, 140.49, 130.07, 128.78, 128.45, 128.17, 127.95, 126.59, 125.52, 114.98, 60.89, 59.50, 54.41, 50.14, 32.82, 32.58, 31.45, 29.90. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{33}O_3N_2$ 433.24857, found 433.24872. **$[\alpha]^{25}_D$** = +9.8 (c 0.589 g/100 mL, $CHCl_3$/MeOH 1/1).

(*S*)-3-(benzyl(3-phenylpropyl)amino)-1-cyclopentyl-4-(4-hydroxyphenyl)butan-2-one (**42**)



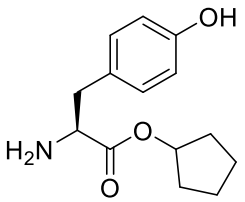
I_2 (586 mg, 2.31 mmol) and Mg (842 mg, 34.65 mmol) were mixed in THF (23.1 mL) under argon atmosphere. (Bromomethyl)cyclopentane (2.96 mL, 23.10 mmol) was added and the mixture was stirred at 25 °C. Approximately after 1 min, the mixture turned clear in color and the exothermic reaction was triggered. The reaction mixture was stirred at 25 °C for 30 min and at 50°C for 1 h. The freshly prepared cyclopentylmethylmagnesium bromide (1 M in THF, 23.1 mL, 23.10 mmol) was added dropwise at 0 °C to a solution of compound **41** (2.00 g, 4.62 mmol) in THF (25 mL). The mixture was allowed to warm up to 25 °C, stirred for 16 h, quenched slowly with 1 M aqueous HCl, and concentrated. The resiude was diluted in $CHCl_3$, washed with H_2O (3 × 150 mL), washed with brine (150 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified using silica using gel flash chromatography (linear gradient elution 0–50% EtOAc/MeOH (9:1 ratio) in hexane) to afford **42** (153 mg, 7% + 1.02 g, 50% of the starting **41**) as an off-white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.11 (s, 1H), 7.35–7.20 (m, 7H), 7.17–7.07 (m, 3H), 6.91–6.85 (m, 2H), 6.62–6.57 (m, 2H), 3.76 (d, J = 13.8 Hz, 1H), 3.58 (d, J = 13.8 Hz, 1H), 3.45 (dd, J = 9.3, 4.1 Hz, 1H), 2.85 (dd, J = 13.3, 9.3 Hz, 1H), 2.66 (dd, J = 13.4, 4.1 Hz, 1H), 2.60–2.26 (m, 6H), 1.89–1.77 (m, 1H), 1.75–1.62 (m, 2H), 1.52–1.28 (m, 6H), 0.91–0.75 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 209.25, 155.33, 142.04, 139.78, 130.05, 129.42, 128.72, 128.23, 128.18, 126.95, 125.63, 114.95, 68.46, 54.69, 49.51, 46.68, 35.08, 32.86, 31.88, 31.72, 29.92, 27.98, 24.31. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{31}H_{38}O_2N$ 456.28971, found 456.28952. **$[\alpha]^{25}_D$** = –46.5 (c 0.187 g/100 mL, $CHCl_3$/MeOH 1/1).

Isopropyl (((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(4-((*S*)-2-(benzyl(3-phenylpropyl)amino)-4-cyclopentyl-3-oxobutyl)phenoxy)phosphoryl)-L-alaninate (**43**)



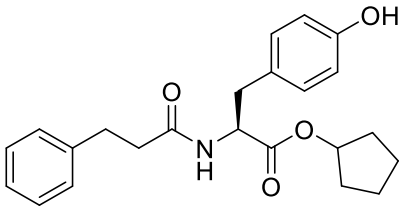
Following standard procedure N, compound **34** (96 mg, 0.24 mmol), **42** (109 mg, 0.24 mmol), DIPEA (334 µL, 1.92 mmol), PPh_3 (252 mg, 0.96 mmol), and Aldrithiol^{TM} (211 mg, 0.96 mmol) were stirred in pyridine (5 mL) to afford **43** (177 mg, 88%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.37–6.90 (m, 16H, *H10*, *H22*, *H23*, *H31*, *H32*, *H33*, *H36*, *H37*, *H38*), 5.59 and 5.46 (dd, *J* = 12.0, 10.4 and 12.2, 10.0 Hz, 1H, *H15*), 4.91–4.73 (m, 1H, *H19*), 4.33–4.09 (m, 2H, *H11*), 3.99–3.55 (m, 6H, *H12*, *H14*, *H16*, *H34*), 3.51–3.45 (m, 1H, *H26*), 2.97–2.87 and 2.79–2.71 (m, 2H, *H25*), 2.63–2.28 (m, 6H, *H27*, *H29*, *H40*), 1.88–1.78 (m, 1H, *H41*), 1.76–1.27 (m, 8H, *H28*, *H42*, *H43*), 1.15–0.99 (m, 12H, *H13*, *H17*, *H20*), 0.92–0.75 (m, 1H, *H42*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 209.12 and 209.07 (*C39*), 173.00 and 172.85 (d, *J* = 4.2 and 3.9 Hz, *C18*), 155.98 and 155.96 (*C6*), 152.41 (*C2*), 149.83 and 149.79 (*C4*), 148.40–148.05 (m, *C21*), 141.98 (*C30*), 141.42 and 141.38 (*C8*), 139.62 (*C35*), 135.73 and 135.59 (*C24*), 130.15 (*C23*), 128.74 (*C36*), 128.23 and 128.21 and 128.17 (*C31*, *C32*, *C37*), 126.97 (*C38*), 125.63 (*C33*), 120.43 and 120.12 (d, *J* = 4.1 and 4.3 Hz, *C22*), 118.38 and 118.33 (*C5*), 75.74–75.38 (m, *C12*), 68.45 and 68.44 (*C26*), 67.85 (*C19*), 64.14 and 64.07 (d, *J* = 154.9 and 155.4 Hz, *C14*), 54.72 (*C34*), 49.51 (*C27*), 49.04 and 48.98 (*C16*), 46.74 and 46.66 (*C11*), 46.49 (*C40*), 35.03 and 35.01 (*C41*), 32.86 (*C29*), 31.88 and 31.73 (*C42*), 29.89 (*C28*), 28.05 (*C25*), 24.32 and 24.29 (*C43*), 21.39 and 21.37 (*C20*), 20.26 and 19.88 (d, *J* = 5.5 and 5.7 Hz, *C17*), 16.77 and 16.64 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.45 and 24.64. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{46}H_{61}O_6N_7P$ 838.44155, found 838.44165.

L-tyrosine cyclopentyl ester (**44**)



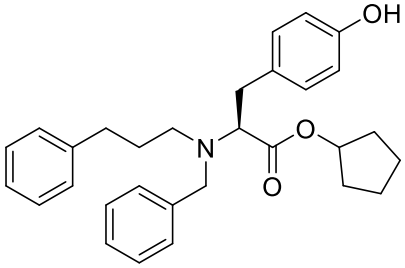
Compound **36c** (1.95 g, 5.08 mmol) was dissolved in MeOH (10 mL) and Pd/C was added under argon atmosphere. The flask was evacuated and flushed with H_2 (3 ×) and the mixture was stirred under H_2 atmosphere at 25 °C for 1 h. After filtration, compound **44** (1.26 g, 99%) was obtained as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.18 (s, 1H), 6.96–6.92 (m, 2H), 6.67–6.62 (m, 2H), 5.01–4.95 (m, 1H), 3.42 (t, *J* = 6.8 Hz, 1H), 2.67 (d, *J* = 6.7 Hz, 2H), 1.80–1.69 (m, 2H), 1.60–1.37 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.82, 155.83, 130.09, 127.79, 114.86, 76.37, 55.93, 32.09, 32.05, 23.27, 23.20. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{14}H_{20}O_3N$ 250.14377, found 250.14396. **$[\alpha]^{25}_D$** = +3.0 (c 0.549 g/100 mL, $CHCl_3$/MeOH 1/1).

N-(3-phenylpropanoyl)-L-tyrosine cyclopentyl ester (**45**)



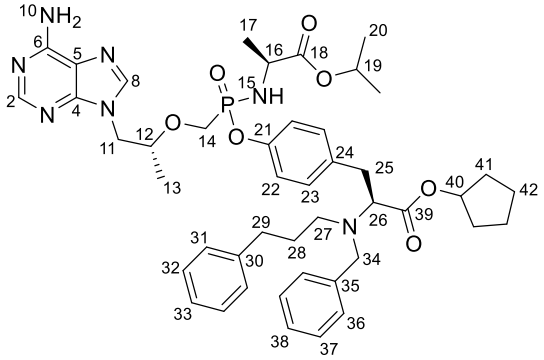
Following standard procedure M, compound **44** (450 mg, 1.80 mmol), 3-phenyl propionic acid (270 mg, 1.80 mmol), HATU (1.03 g, 2.70 mmol), and DIPEA (1.57 mL, 9.00 mmol) were stirred in DMF (10 mL) to afford **45** (197 mg, 29%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.23 (d, J = 7.6 Hz, 1H), 7.28–7.21 (m, 2H), 7.21–7.13 (m, 3H), 7.00–6.92 (m, 2H), 6.68–6.60 (m, 2H), 5.04–4.95 (m, 1H), 4.36–4.26 (m, 1H), 2.82 (dd, J = 13.8, 6.5 Hz, 1H), 2.78–2.70 (m, 3H), 2.42–2.34 (m, 2H), 1.82–1.65 (m, 1H), 1.64–1.34 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.50, 171.43, 155.98, 141.21, 129.99, 128.25, 128.15, 127.11, 125.85, 114.96, 76.95, 53.97, 36.53, 36.15, 32.04, 30.91, 23.27, 23.20. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{23}H_{27}O_4NNa$ 404.18323, found 404.18392. **$[\alpha]^{25}_D$** = +12.2 (c 0.147 g/100 mL, $CHCl_3$/MeOH 1/1).

N-benzyl-*N*-(3-phenylpropyl)-L-tyrosine cyclopentyl ester (**46**)



Compound **44** (500 mg, 2.01 mmol), PhCHO (225 µL, 2.21 mmol), and Na_2SO_4 (1.43 g, 10.05 mmol) were mixed in MeOH (10 mL) under argon atmosphere. AcOH (138 µL, 2.41 mmol) and $NaBH_3CN$ (253 mg, 4.02 mmol) were added and the mixture was stirred at 25 °C for 30 min. 3-Phenylpropanal (529 µL, 4.02 mmol), AcOH (138 µL, 2.41 mmol), and $NaBH_3CN$ (253 mg, 4.02 mmol) were added. The mixture was stirred at 25 °C for 1 h, filtered, concentrated, and separated using silica using gel flash chromatography (linear gradient elution 0–40% EtOAc/MeOH (9:1 ratio) in hexane) to afford **46** (805 mg, 88%) as a colorless oil. **^{1}H NMR** (401 MHz, DMSO-d_6) δ 9.17 (s, 1H), 7.30–7.10 (m, 8H), 7.10–7.02 (m, 2H), 6.93–6.85 (m, 2H), 6.68–6.59 (m, 2H), 5.09–5.01 (m, 1H), 3.91 (d, J = 14.3 Hz, 1H), 3.51 (d, J = 14.3 Hz, 1H), 3.44 (dd, J = 8.4, 6.8 Hz, 1H), 2.87 (dd, J = 13.7, 8.4 Hz, 1H), 2.73 (dd, J = 13.7, 6.8 Hz, 1H), 2.70–2.60 (m, 1H), 2.49–2.36 (m, 3H), 1.85–1.66 (m, 2H), 1.66–1.40 (m, 8H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.36, 155.67, 142.12, 139.93, 130.00, 128.29, 128.27, 128.17, 128.04, 114.86, 76.42, 64.06, 54.67, 49.83, 34.24, 32.72, 32.43, 32.21, 29.64, 23.18, 23.15. **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd for $C_{30}H_{36}O_3N$ 458.26897, found 458.26966. **$[\alpha]^{25}_D$** = –55.6 (c 0.250 g/100 mL, $CHCl_3$/MeOH 1/1).

Cyclopentyl (2*S*)-3-(4-((((((*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)(((*S*)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(benzyl(3-phenylpropyl)amino)propanoate (**47**)



Following standard procedure N, compound **34** (200 mg, 0.50 mmol), **46** (458 mg, 1.00 mmol), Et_3N (558 µL, 4.00 mmol), PPh_3 (525 mg, 2.00 mmol), and Aldrithiol^{TM} (441 mg, 2.00 mmol) were stirred in pyridine (8 mL) to afford **47** (107 mg, 25%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.29–6.94 (m, 16H, *H10*, *H22*, *H23*, *H31*, *H32*, *H33*, *H36*, *H37*, *H38*), 5.61 and 5.47 (dd, J = 12.0, 10.4 and 12.4, 9.9 Hz, 1H, *H15*), 5.10–5.05 (m, 1H, *H40*), 4.88–4.77 (m, 1H, *H19*), 4.31–4.11 (m, 2H, *H11*), 4.02–3.73 (m, 5H, *H12*, *H14*, *H16*, *H34a*), 3.55–3.46 (m, 2H, *H26*, *H34b*), 2.98–2.78 (m, 2H, *H25*), 2.70–2.35 (m, 4H, *H27*, *H29*), 1.85–1.46 (m, 10H, *H28*, *H41*, *H42*), 1.15–1.01 (m, 12H, *H13*, *H17*, *H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.03 and 172.86 (d, J = 4.0 and 4.0 Hz, *C18*), 171.20 (*C39*), 155.96 and 155.94 (*C6*) 152.39 (*C2*), 149.84 and 149.80 (*C4*), 148.67–148.36 (m, *C21*), 142.05 (*C30*), 141.43 and 141.38 (*C8*), 139.75 and 139.73 (*C35*), 134.54 and 134.40 (*C24*), 130.13 (*C23*), 128.28 (*C36*), 128.16 and 128.07 (*C31*, *C32*, *C37*), 126.72 (*C38*), 125.55 (*C33*), 120.35 and 120.02 (d, J = 4.2 and 4.4 Hz, *C22*), 118.37 and 118.33 (*C5*), 76.58 (*C40*), 75.79–75.34 (m, *C12*), 67.87 (*C19*), 64.92–63.34 (m, *C14*), 63.65 and 63.58 (*C26*), 54.68 (*C34*), 49.78 and 49.71 (*C27*), 49.05 and 49.01 (*C16*), 46.71 and 46.66 (*C11*), 34.11 (*C25*), 32.72 (*C29*), 32.48 and 32.19 (*C41*), 29.61 and 29.59 (*C28*), 23.20 and 23.14 (*C42*), 21.40 and 21.38 (*C20*), 20.28 and 19.89 (d, J = 5.0 and 5.3 Hz, *C16*), 16.80 and 16.66 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.49, 24.65. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{45}H_{59}O_7N_7P$ 840.42081, found 840.42106.

Solubility measurements

The stock solution of studied compound (0.5 mM in DMSO) was prepared, out of which 50 µL was pipetted in a vial containing 950 µL of MilliQ water affording 25 µM concetration. Using HPLC (Agilent 1260/1290 Infinity II, Reprosil 5 µm, 3 × 200 mm, C_{18} with guard, 40 °C, flow rate 1 mL/min, modified gradiend 5-100% of MeCN in H_2O), this vial was used to measure an 8-point calibration curve (based on the UV absorption at 254 nm) ranging from 1.35 µM to 150 µM via different injection volumes.

Studied compounds were mixed with MilliQ water to hit hypothetical 1 mM concentration to secure oversaturation of the mixture. Compounds were incubated at 25°C for 24h in order to reach equilibrium. The remaining solid was removed using centrifugation (30 000 rcf for 15 min). The supernatants were analyzed and the concentration of the solute was determined. The AUCs were correlated to corresponding calibration curves to calculate the concentration in the samples.

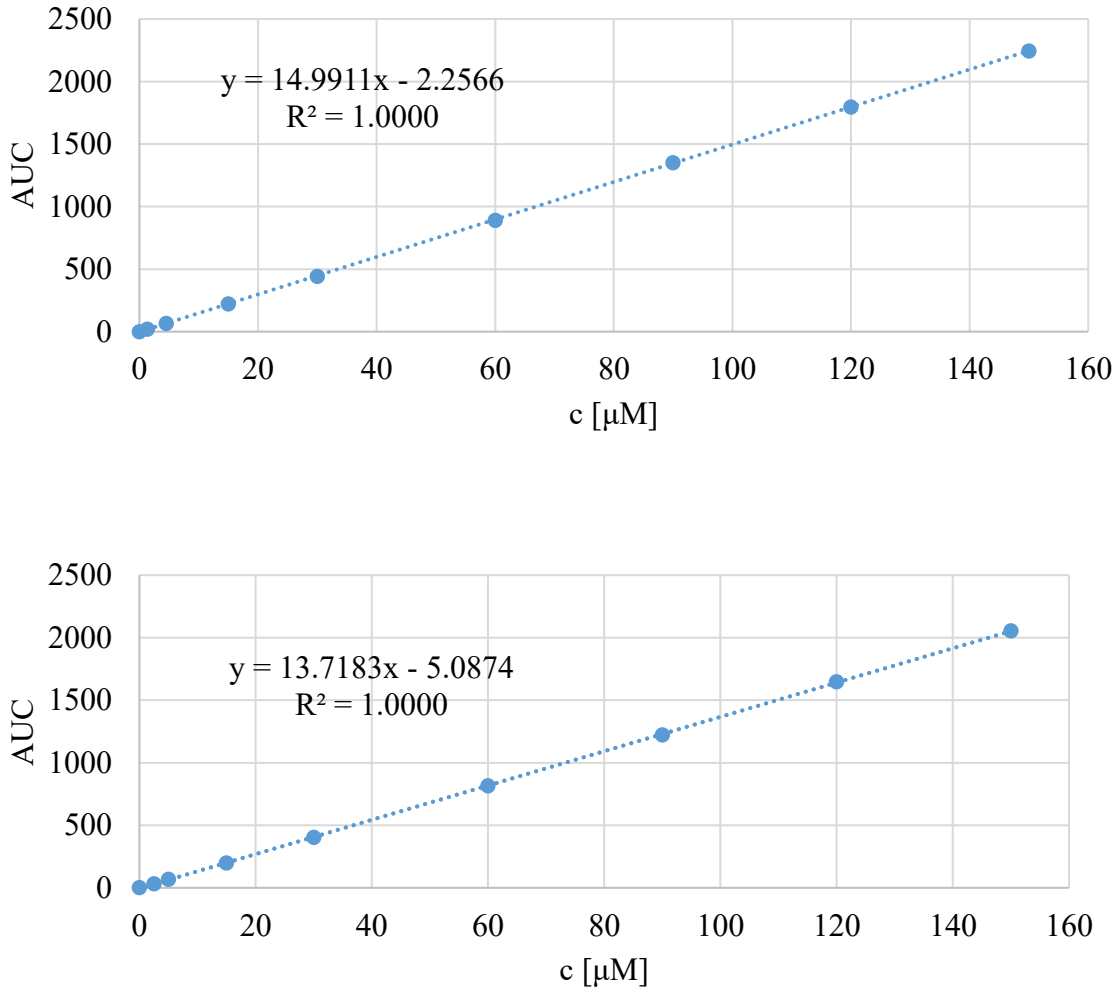


Figure 35. Calibration curves for TAF (top) and **35l**-SEE (bottom).

pH stability assay

Using Jenway 3505 pH meter, the stock solutions of 0.01 M buffers were prepared (NaCl-HCl buffer pH – 1.5, citrate buffer – pH 4.5, phosphate buffer – pH 7.4, Tris-HCl buffer – pH 8.5, and NaOH-glycine buffer – pH 10.0). The stock solution of studied compound (0.5 mM in DMSO) was prepared, out of which 50 μL was pipetted in a vial containing 950 μL of MilliQ water affording 25 μM concetration. Using HPLC (Agilent 1260/1290 Infinity II, Reprosil 5 μm, 3 × 200 mm, C_{18} with guard, 40 °C, flow rate 1 mL/min, modified gradiend 5-100% of MeCN in H_2O), this vial was used to measure an 8-point calibration curve (based on the UV absorption at 254 nm) ranging from 1.35 μM to 150 μM via different injection volumes.

Out of the stock solution of studied compound (0.5 mM in DMSO), 50 μL was pipetted in a vial containing 950 μL of appropriate buffer affording 25 μM concetration. Each sample was incubated at given temperature (4 °C, 22 °C, or 37 °C) for 24 h while analyzing the sample every 3 h. Each experiment was conducted in triplicate, at three temperatures, at five different pH thus resulting in 45 experiments for each compound.

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