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SYNTHESIS, CONFORMATIONAL STUDY AND BIOLOGICAL EVALUATION OF PENTOPYRANOSIDE NUCLEOSIDE PHOSPHONATES

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Promotor:
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Summary

Synthetic analogues of nucleosides, nucleotides and oligonucleotides have found application in the life-saving treatment of cancers and viral infections. High-affinity binding oligonucleotides have potential in the development of antisense, siRNA, aptamer and xeno nucleic acids (XNAs) research. These synthetic mimics usually carry modifications of either one or more of the essential components of the nucleic acid, such as nucleobase, sugar and phosphate. The vast amount of synthetic data and their biochemical properties are available to the research community for the base and sugar modified synthetic nucleosides. Sugar modification along with modification in internucleotide phosphate bond is relatively still unexplored.

Hexitol nucleosides are the only six-membered sugar analogues which showed promising antiviral activities to date. In addition, with regard to the corresponding oligomers, hexitol nucleic acids (HNA) demonstrated the ability to fold into helical structures as well as form stable hybrids with natural counterparts, by reproducing the A-type form of dsRNA. HNA has also been proven to act as xenobiotic genetic material (XNA) capable of evolution *in vitro* while directing DNA synthesis in *E. coli*.

In this PhD thesis, we describe the synthesis of the phosphonate mimetics of hexitol nucleoside monophosphate. Preliminary antiviral activities and incorporation tests are also investigated.

In **Chapter 2**, design and synthesis of the sugar scaffolds for the synthesis of thymine pentopyranoside nucleoside phosphonate are described. Different strategies were explored and discussed. Notably, the stereochemical outcome of the Vorbrüggen glycosylation step utilised en route to the thymine analogue clearly suggests the absence of anchimeric assistance, as opposed to what is usually observed during nucleoside synthesis using protected furanose precursors.

In the second part of this thesis (**Chapter 3**), synthesis of D-*threo*-pentopyranoside phosphonate containing adenine as the nucleobase is explored. Strategies used in thymine nucleoside phosphonate were not successful for the introduction of the purine nucleobase. Moreover, the Vorbrüggen glycosylation on adenine nucleoside failed to give the desired phosphonate glycoside.

The synthesis of the adenine analogue relied on a linear approach for building the purine base on a phosphonometylated 4-amino pentopyranoside synthon. This method allowed for an improved stereoselectivity and could potentially be extended to the stepwise construction of other purine as well as pyrimidine bases.

In the third part of this thesis (**Chapter 4**), two more D-*threo*-pentopyranoside phosphonates bearing uracil and hypoxanthine analogues are presented. Their synthesis followed the analogous strategies developed for the thymine and adenine. Detailed solution conformational analyses of all the four final nucleoside phosphonate products were carried out using NMR spectroscopy. According to NMR studies, the solution conformation of such pentopyranoside nucleoside phosphonates prefers an equatorial orientation of the nucleobase that circumvents unfavourable 1,3-diaxial interactions, in contrast to HNA nucleosides. Antiviral tests for adenine and thymine nucleoside phosphonates revealed that they are devoid of activity. Preliminary primer incorporation reactions using a mesophilic and thermophilic polymerase revealed that these phosphonate mimics are potentially useful monomers for medical and biotechnological applications.

Samenvatting

Synthetische analogen van nucleosiden, nucleotiden en oligonucleotiden worden reeds gebruikt in levensreddende behandelingen van kanker en virale infecties. Oligonucleotiden die een hoge bindingsaffiniteit bezitten voor hun doelwit, zouden kunnen gebruikt worden in de antisense, siRNA en aptameer onderzoek. Deze synthetische varianten hebben modificaties in één of meerdere componenten van het oorspronkelijke nucleïnezuur, zoals de nucleobase-, de suiker- of de fosfaatgroep. Er zijn zeer veel voorbeelden van base- en suikergemodificeerde synthetische nucleosiden en hun synthese en werkingsmechanisme zijn goed gekend. Suikermodificatie in combinatie met modificatie van de internucleotide fosfaatgroep is tot op heden relatief weinig bestudeerd.

Hexitolnucleosiden zijn interessante voorbeelden van suikergemodificeerde nucleosiden met antivirale activiteit. Wanneer men ze gebruikt voor oligonucleotidesynthese worden polymeren gevormd, "*Hexitol Nucleic Acids"* (HNA), die zich vouwen in helicale structuren die stabiele hybridestructuren vormen met natuurlijke nucleïnezuren. Dit HNA werd getest als xenobiotisch erfelijk materiaal voor *in vitro* evolutie en als templaat voor DNA synthese *in vivo*. Het heeft echter niet de nodige eigenschappen om als echt orthogonaal informatiesysteem te kunnen functioneren.

De synthese van de fosfonaatanalogen van hexitolnucleosidemonofosfaat wordt in deze doctoraatsthesis besproken. Preliminaire evaluatie van antivirale activiteiten en DNA-incorporatie-eigenschappen werden ook onderzocht.

In **Hoofdstuk 2** wordt het ontwerp en de synthese van de suikergedeeltes voor de algemene synthese van thyminepentopyranosidenucleosidefosfonaat onderzocht en besproken. Verschillende strategieën werden onderzocht en besproken. Vooral het stereochemisch resultaat van de Vorbrüggen-glycosylatiereactie, die gebruikt werd tijdens de synthese van het thymine-analoog, suggereert de afwezigheid van anchimere hulp van de beschermde suiker bij die reactie, dit in tegenstelling tot wat er normaal geobserveerd wordt indien men gebruik maakt van beschermde furanoseprecursoren tijdens de synthese van nucleosiden.

In het tweede deel van deze thesis (**Hoofdstuk 3**) wordt de synthese van D-*threo*-pentopyranosidefosfonaat met adenine als nucleobase onderzocht. De strategieën uitgewerkt voor de synthese van het thyminenucleosidefosfonaat werkte niet voor het invoeren van de purinenucleobase. Daarenboven gaf de Vorbrüggen-glycosylatie op het adeninenucleoside, het onjuiste fosfonaatglycoside. Voor de synthese van het adenine-analoog werd daarom een lineaire werkwijze om de purinebase op te bouwen op een fosfonogemethyleerd 4-amino-pentopyranosidesynthon gebruikt. Deze methode geeft een betere stereoselectiviteit en kan eventueel verder gebruikt worden in de stapsgewijze opbouw van andere purinebasen alsook pyrimidinebasen.

In het derde en laatste deel van deze thesis (**Hoofdstuk 4**) wordt de synthese van twee additionele D-*threo*-pentopyranosidefosfonaten, die respectievelijk uracil en hypoxanthine als nucleobase dragen, beschreven. Hun synthese volgde gelijkaardige strategieën die ontwikkeld werden tijdens de synthese van de thymine- en adeninenucleosidefosfonaten. NMR spectroscopie werd gebruikt om de conformatie te bepalen van de vier verkregen nucleosidefosfonaten. Volgens NMR-studies wordt de voorkeur gegeven aan een equatoriale positie van de nucleobase zodat ongewenste 1,3-diaxiale interacties vermeden worden. Antivirale testen voor de adenine- en thyminenucleosidefosfonaten tonen een afwezigheid van activiteit aan. Preliminaire DNA-incorporatiereacties, gebruik makende van een mesofiel en thermofiel polymerase, tonen aan dat de difosfaten van deze fosfonaatnucleosiden als substraat kunnen fungeren, hetgeen hoopgevend is voor hun potentieel gebruik in synthetische biologie.

Abbreviation

2D	*Two dimensional*
A	*Adenine*
Ac	*Acetyl*
AcCN	*Acetonitrile*
AIBN	*Azobisisobutyronitrile*
ANA	*Arabino nucleic acid*
AZT	*3′-Azido-3′-deoxythymidine*
Bn	*Benzyl*
Bu_3SnH	*Tributyltin hydride*
Bz	*Benzoyl*
C	*Cytosine*
CDI	*1,1′-carbonyldiimidazole*
CeNA	*Cyclohexene nucleic acid*
CNA	*Cyclohexanyl nucleic acid*
COSY	*Correlation spectroscopy*
CST	*Compartmentalised self-tagging*
DBU	*1,8-diazabicyclo[5.4.0]undec-7-ene*
DCM	*Dichloromethane*
DEAD	*Diethyl azodicarboxylate*
DF	*Defibrotide*
DIAD	*Diisopropyl azodicarboxylate*
DIBAL	*Diisobutylaluminium*
DIPEA	*N,N-diisopropylethylamine*
DMAP	*4-dimethylaminopyridine*
DMF	*N,N-dimethylformamide*
DMPU	*N,N′-Dimethylpropyleneurea*
DMSO	*Dimethyl sulfoxide*
DNA	*Deoxyribonucleic acid*
EBV	*Epstein–Barr virus*
ESI	*Electrospray ionization*
Et	*Ethyl*
FANA	*2′-fluoro-arabino-nucleic acid*
FDA	*Food and Drug Administration*
G	*Guanine*
GNA	*Glycerol nucleic acid*
HBV	*Hepatitis B virus*
HCMG	*(+/-)-9-[[(Z)-2-(hydroxymethyl)cyclopropyl]methyl]guanine*
HCMV	*Human cytomegalovirus*
HCV	*Hepatitis C virus*
HEL	*Human embryonic lung cells*

HIV	*Human immunodeficiency virus*
HMBC	*Heteronuclear multiple bond correlation*
HNA	*Hextitol nucleic acid*
HPV	*Human papillomavirus*
HPMPC	*(S)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine*
HRMS	*High resolution mass spectrometry*
HSQC	*Heteronuclear single quantum coherence*
HSV	*Herpes simplex virus*
Hx	*Hypoxanthine*
IUdR/Idoxuridine	*5-Iodo-2'-deoxyuridine*
LBV	*Lobucavir*
LNA	*Locked nucleic acid*
MCC	*Minimum cytotoxic concentration*
Me	*Methyl*
MEM	*Methoxyethoxyethyl*
MMTrCl	*4-methoxytriphenylmethyl chloride*
MOE	*Methoxyethyl*
MsCl	*Methanesulfonyl chloride*
NBS	*N-Bromosuccinimide*
NDP	*Nucleoside diphosphate*
NMP	*Nucleoside monophosphate*
NMR	*Nuclear magnetic resonance*
NTP	*Nucleoside triphosphate*
ONA	*Oxepane nucleic acids*
OXT	*Oxetanocin*
PFU	*Plaque forming units*
PMCG	*9-[1-(Phosphonomethoxycyclopropyl)methyl]guanine*
ppm	*Parts per million*
PTFAI	*N-phenyltrifluoroacetimidates*
RNA	*Ribonucleic acid*
RP-HPLC	*Reversed-phase high-performance liquid chromatography*
RT	*Reverse transcriptase*
rt	*Room temperature*
T	*Thymine*
TAF	*Tenofovir alafenamide fumarate*
TBAF	*Tetra-n-butylammonium fluoride*
TBDMSCl/TBSCl	*Tert-Butylchlorodimethylsilane*
TBDPSCl	*Tert-Butylchlorodiphenylsilane*
TCAI	*Trichloroacetimidates*
TCDI	*1,1'-thiocarbonyldiimidazole*
TDF	*Tenofovir disoproxil fumarate*
TEA	*Triethylamine*
TEAB	*Triethylammonium bicarbonate*

TEMPO	*(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl*
TFA	*Trifluoroacetic acid*
Tgo	*Thermococcus gorgonarius*
THF	*Tetrahydrofuran*
TK	*Thymidine kinase*
TLC	*Thin layer chromatography*
T_m	*Melting temperature*
TMSBr	*Bromotrimethylsilane*
TMSCl	*Chlorotrimethylsilane*
TMSI	*Iodotrimethylsilane*
TMSOTf	*Trimethylsilyl trifluoromethanesulfonate*
TNA	*Threose nucleic acid*
Trifluridine	*5-trifluoromethyl-2'-deoxyuridine*
TsCl	*4-Toluenesulfonyl chloride*
U	*Uracil*
VZV	*Varicella zoster virus*
XNA	*Xenobiotic nucleic acid*

Contents

Chapter 1. Application and approaches in nucleosides, nucleotides and oligonucleotides research

1.1 General introduction

Virus infections and human cancer are still an important cause of morbidity and mortality all around the world. Since the first approval of Idoxuridine (5-Iodo-2′-deoxyuridine, IUdR) as an anti-herpesvirus drug in 1963, a vast number of nucleosides and nucleotides have found applications in the fields of viral infections and cancer research [1]. In addition, the development of modified nucleosides found their use in research areas like diagnostic probes, antibiotics, and radiopharmaceuticals.[2] Over the years thousands of modified nucleosides/nucleotides have been synthesised, though only less than 30 have been approved for therapeutic use. Within these approved nucleoside analogues, only nine infections are targeted even though more than 200 viruses are found in human.[1] Thus, the design and synthesis of nucleosides/nucleotides with a novel structure is still an important research area in medicinal chemistry.

Steady progress is also made in the oligonucleotides field, which has found their application as constructs of custom antisense, siRNA and aptamers research.[3] These oligonucleotides carry modified nucleobase, sugar or phosphate groups to screen their usefulness in therapy. Searching for new structural diversity of oligonucleotides is a challenging task. Besides potential applications in therapeutics and diagnostics, the study of new nucleic acid analogues may contribute to the understanding of the chemical etiology of nucleic acid structures, or to the better understanding the natural genetic system and the exploration of artificial living system bearing xenobiotic nucleic acid. These quests can be approached by designing new orthogonal oligonucleotides capable of carrying and passing genetic information.

The main objective of the work presented in this project is the synthesis of novel nucleotide analogues which may be used as antivirals and/or as a constituent of oligomers. More specifically, this project will focus on pyranosyl nucleoside phosphonates.

1.2 Nucleoside and nucleoside phosphonate analogues as antiviral agents.

The development of chemotherapy towards viruses was very slow at the beginning. Several reasons account for this: viruses utilise host cell cellular enzyme/machinery for protein synthesis and their replication, which makes the search for selective substrates (less toxicity) problematic; the success of some vaccines that work via immunization seemed more promising during the early year.[4] It was

only with the identification of HIV as the causative agents of AIDS that the chemotherapy for viruses came to a dramatically changing era. Since the approval of idoxuridine by the FDA in 1963, thousands of compounds were reported with antiviral activities. However, only 90 drugs were formally approved to treat human-related virus infection.[1]

Despite achievements made in the field of antiviral agents, the development of novel and successful antiviral treatment remains urgent and challenging. At first, the fast mutating nature of the virus genome (HIV and influenza infection etc.) can lead to drug resistance and decrease in the effectiveness of the treatment.[5-6] Second, the adverse effects are still big hurdles to overcome, especially for the viruses that can integrate their genomes into the host cell genome and those viruses using the host cell polymerases for their replication.[7-8] Third, there are still more than 200 viruses lacking efficient treatments (antivirals or vaccines), and new infections are on the rise. (Ebola, Zika etc.).[9]

This part will give an overview of approved nucleoside and nucleoside phosphonate drugs (several representative agents under clinical trials will also be included). Their structural modification strategies, the general mode of action, targets for antiviral therapy and representative drugs is discussed.

1.2.1 General modification strategy of natural nucleosides/nucleotides

Currently, there are about 25 nucleoside related drugs that are approved as antivirals (**Table 1-1** and **Table 1-2**), which are either modified nucleosides or nucleoside phosphonates. These modified agents have been the most successful antivirals targeting HIV, HBV, HCV, HPV, HSV, HCMV.[1, 10]

Successful modification strategies have been applied to mimic all the three moieties of nucleotides (**Figure 1-1**). Among these modifications, the ribose part has been changed the most, which include ring size change, ring oxygen replacement, ring opening, inversion of configuration, azotation, halogenation *etc*. While the nucleobase modification often encompasses halogenation, *N*-conjugation and azotation, modification of phosphate moiety utilise strategies like phosphonate mimics, phosphorothioates, phosphoramidate, phosphonobisamidate and P-boronated mimics.[2, 11 12]

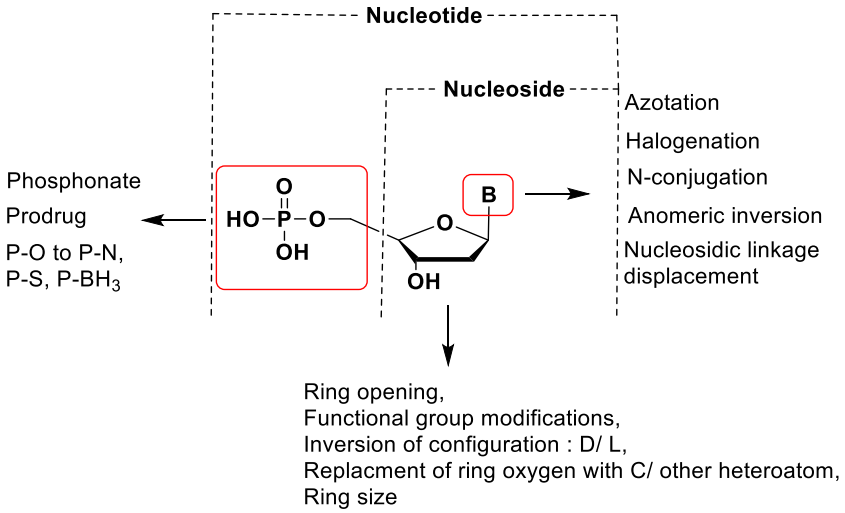


Figure 1-1. Modification strategies of nucleosides/nucleotides

1.2.2 Mode of action

The bioconversion of a nucleoside to a nucleotide is controlled by cellular or viral kinases. These kinases can convert the nucleoside to monophosphate (NMP), diphosphate (NDP), and triphosphate (NTP) step by step. In most cases, the nucleoside analogue is converted to its triphosphate form. The nucleoside triphosphate (NTP) acts as either a competitive inhibitor of viral polymerase or a chain terminator of viral DNA/RNA elongation/synthesis, exhibiting antiviral activity. The main challenge lies in the similarity of these nucleoside analogues with their natural counterparts, which has the possibilities for interference with normal cellular transcription and translation. This interference will eventually lead to host cell genome toxicity, which may explain the adverse effects of antiviral nucleoside analogues. Therefore, the discovery of orthogonal nucleoside analogues is of importance to minimise the host cell genomic toxicity, to achieve a broader therapeutic window.[13-14]

In principle, a broader therapeutic window can be achieved by two differentiating steps, the kinase-mediated phosphorylation and the polymerases mediated polymerisation, depending on the structural differentiation between virus enzymes and natural human ones.[15] However, most of the viruses (except for HSV, which uses its own kinase), use human cellular kinases for phosphorylation, thus selectively can only be achieved at the polymerisation step. Retroviruses (RNA viruses) and some DNA viruses (HBV, HCMV, HSV, and VZV) are capable of inserting their genomic copy into the DNA of host cell, thus changing the genome of the host cell. These invaded cells are now capable of transcription for the synthesis of polymerases required for the viral replication. In the case of these viral infections, selective substrates may be obtained by the structural modification of nucleoside analogues. For other DNA viruses, ‘HPV’ for example, uses cellular polymerases for replication, here the selectively is not possible.

During the activation of nucleoside analogues to NTP, the first phosphorylation step to monophosphate is the rate-limiting step, which may hinder the activation of these nucleoside analogues. While the phosphodiester bond in pre-prepared nucleoside phosphate could be easily dephosphorylated during membrane transportation and inside the cytoplasm. Two most successful strategies have been used to overcome this limitation, the prodrug-approach[16] and the phosphonate approach[17]. The prodrug approach uses functional group, normally protected amino acids, to mask the phosphate towards membrane transport. While phosphonate approach uses a P-C-O bond to replace the P-O-C bond in natural phosphate. The phosphonate group is a bioisostere of the phosphate group with similar chemical and biological properties, which can be further phosphorylated *in vivo*. The advantage of the phosphonate group lies in its improved metabolic stability as compared to its phosphate counterpart. Likewise, these nucleoside phosphonates need to be phosphorylated to their diphosphates (mimetics of triphosphates) to act as the substrates of viral polymerases.

1.2.3 Targets for antiviral chemotherapy[18-20]

One of the most important concepts in the design of bioactive agents is the rational drug design. For structure-based rational drug design, the structure of targeted enzyme or protein needs to be identified, and then a suitable structure which fits the active site of this target will be designed, synthesised and tested. Unlike the traditional trial-and-error based approach, it is logical that the structure based rationale will improve the possibilities of finding the hit compound.

The fast evolution in the field of biochemistry has greatly enriched our understanding of the viral replication cycle and viral-host interaction. These proceedings not only helped researchers to better understand how existing antiviral agents exhibit their effect, but also reveal possible proteins/enzymes which could be used as potential targets for future antivirus drug design.

The cumulative virus-specific events involved in replication cycle can be considered as the potential targets; these events include: adsorption, fusion and uncoating occurring near the membrane of the host cell; release, transport, replication, transcription, post-transcriptional modification, translation inside the host cell; and the final assembly and release of the mature virions from the host cell. In addition, some host enzymes and proteins may also be involved and thus could be targeted. Among all these potential targets, viral specific enzymes are the most important ones, and some representatives include, human herpes viruses, protease and HIV reverse transcriptase.

It is evident that most of the approved antiviral nucleoside/nucleotide drugs were not developed through target-based rational design. Instead, these compounds were discovered by chance and were first proved active while afterwards their mode of action was elucidated. Efforts are still needed to understand and design novel target-based agents.

1.2.4 Approved nucleoside and nucleoside phosphonate analogues

Till now, approved nucleoside or nucleotide analogues either fall into acyclic or 5-membered ring categories. For nucleoside analogues, both sugar and/nucleobase modifications have representative drugs, while nucleotide analogues (including phosphate modification) are all acyclic nucleoside phosphonates. In this section, an overview of these approved agents from a structural rational is presented.

1.2.4.1 Acyclic nucleoside and nucleoside phosphonate analogues

In acyclic nucleoside and nucleoside phosphonate analogues, an acyclic chain replaces the ribose sugar ring. These analogous have a higher order of structural flexibility. This is an important class of drugs with low mammalian toxicity and good drug tolerance. Concurrently, acyclic nucleoside and nucleoside phosphonates have found application towards several viral targets, HIV, HSV, HBV, HCV, VZV and HCMV. Representative acyclic nucleoside and nucleoside phosphonate are divided into five types by Hai-M and co-workers, depending on where the sugar ring is cut.[21] The modification of acyclic analogues normally focuses on the following strategies (**Figure 1-2**): acyclic chain oxygen replacement with S, N or CH_2; add or replace substitution on the acyclic chain; and the modification of nucleobases. Noteworthy, the prodrug approach is often used to improve the membrane penetration and bioavailability, which reduces the effective dosage, thereby reducing their cytotoxicity.[22] Representative antiviral acyclic nucleosides and nucleoside phosphonates are listed in **Table 1-1**.

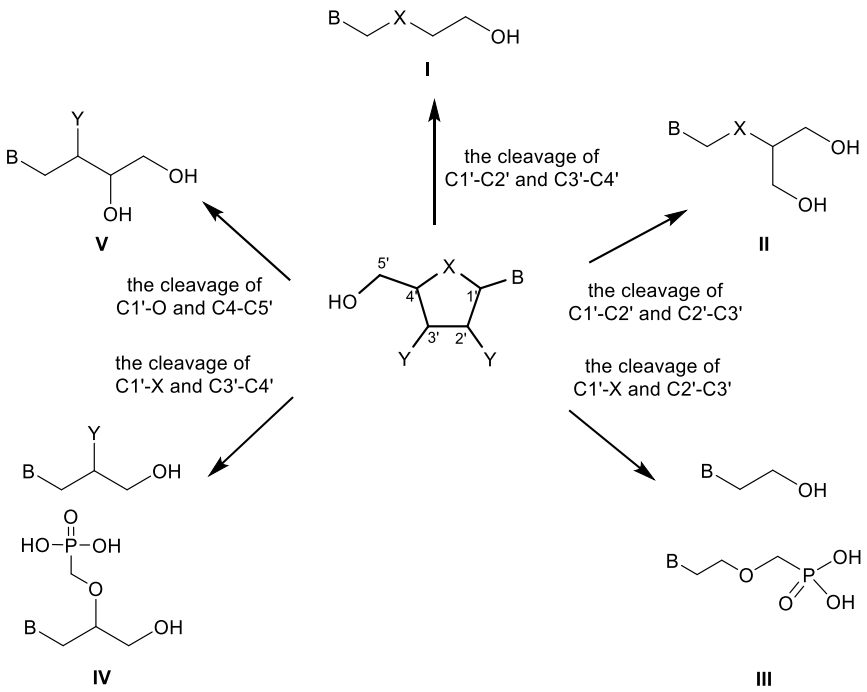
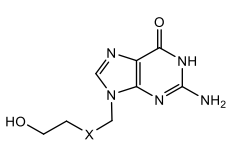
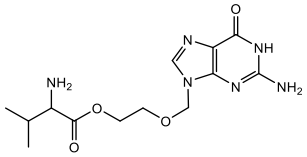
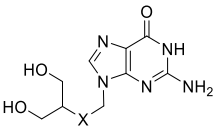
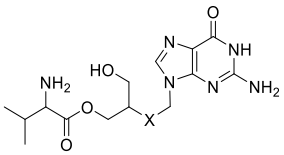
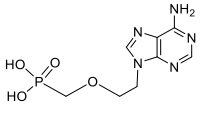
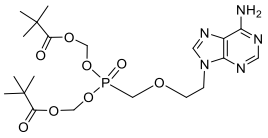
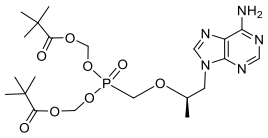
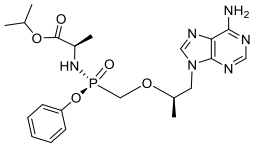
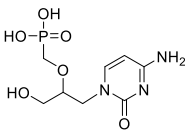
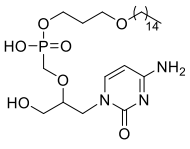
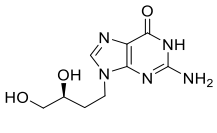
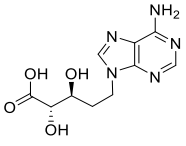


Figure 1-2. Acyclic nucleoside and nucleoside phosphonates

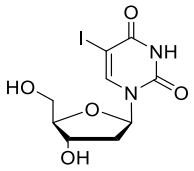
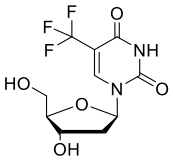
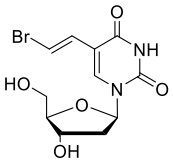
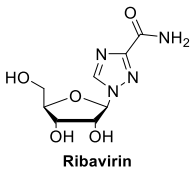
Table 1-1. Representative antivirus acyclic nucleosides and nucleoside phosphonates

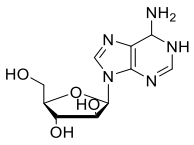
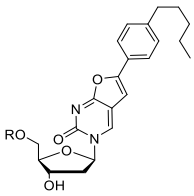
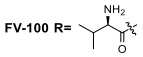
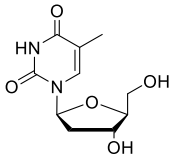
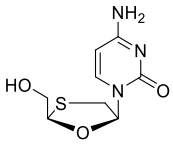
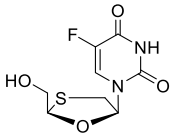
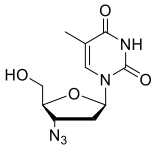
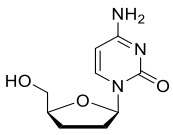
Category	Structure	Virus
Type I	 X=O **Acyclovir** X=CH_2 **HBG**  **Valaciclovir**	HSV
Type II	 X=O **Ganciclovir** X=C **Penciclovir**  X=O **Valganciclovir** X=C **Famciclovir**	GCV & VGCV for HCMV PCV & FCV for HSV
Type III	 **Adefovir**  **Adefovir dipivoxil**  **Tenofovir dipivoxil (TDF)**  **Tenofovir alafenamide (TAF)**	Adefovir dipivoxil for HBV TDF & TAF for HIV & HBV
Type IV	 **Cidofovir**  **Brincidofovir**	HCMV in AIDS
Type V	 Buciclovir  D-eritadenine	Not approved

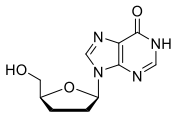
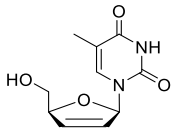
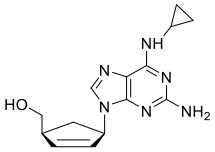
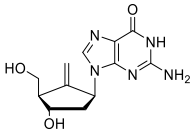
1.2.4.2 5-membered nucleoside and nucleoside phosphonate analogues[1, 10]

Most of the approved nucleos(t)ide analogues fall into the 5-membered category, which is also the one, nature selected in DNA and RNA. These approved 5-membered drugs are all nucleoside analogues, which can be arbitrarily divided into four categories depending on their structure: Type I; ribose or 2′-deoxyribose related antiviral agents, which focuses on the nucleobases modification substituted on ribose or deoxyribose. Type II; L-nucleoside analogues, which successfully applies the L-enantiomers as antivirals, the replacement of the sugar CH_2 with heteroatom is the other highlight in this category. Type III; 2′, 3′-dideoxyribose modified nucleosides related antiviral agents, focused on the modification of furanose, either removing the hydroxyl at 2′ and/or 3′ position or replacing it with other bioisosteres. Type IV; 5-membered cyclitol related nucleoside drugs, which are metabolically more stable carbocycles. (**Table 1-2**)

Table 1-2. Representative 5-membered antiviral agents

Category	Structure	Target virus
Type I Nucleobase modification	 **Idoxuridine**	HSV, VZV
	 **Trifluridine**	HSV
	 **Brivudine**	HSV, VZV
	 **Ribavirin**	HCV infections, RSV and viral hemorrhagic fevers

	 Vidarabine	HSV, VZV
	 CF-173 R=H  FV-100 R=	VZV*
Type II L-nucleosides	 Telbivudine	HBV
	 Lamivudine	HIV, HBV
	 Emtricitabine	HIV
Type III 2′, 3′-dideoxyribose	 Zidovudine	HIV
	 Zalcitabine	HIV

	 Didanosine	HIV
	 Stavudine	HIV
Type IV 5-membered cyclitol	 Abacavir	HIV
	 Entecavir	HBV

***** Phase 3 clinical trial of the FV-100 versus Valacyclovir for the Prevention of Post-Herpetic Neuralgia was terminated in December 2017 since reassessment of market opportunity. (ClinicalTrials.gov registration number NCT02412917).

1.2.5 3-membered carbocyclic nucleoside and nucleoside phosphonate analogues

Currently, there is no approved drug in this class; some of these structures (**Figure 1-3** and **Table 1-3**) are very interesting and exhibit various activities against different viral infections.

Based on the association between pseudo-sugar (the 3-membered ring) and the nucleobase, cyclopropane nucleosides are classified into three groups (**Figure 1-3**), directly linked (Type A), separated by methylene spacer (Type B), and separated by the unsaturated linker (Type C).[23] The Type A nucleosides are devoid of any antiviral activity, however, Type B and Type C have shown interesting biological activity against various viral infections (e.g., HSV, VZV, HCMV, EBV).[24-26]

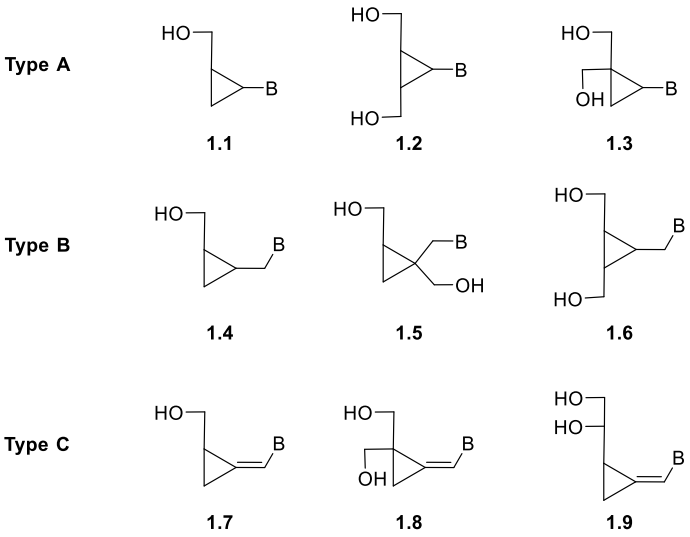
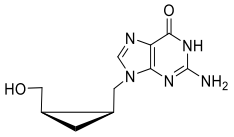
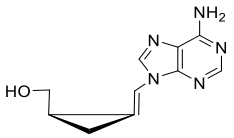
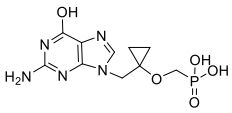
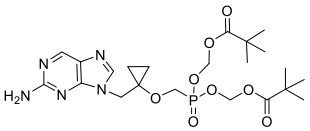
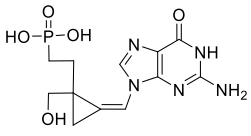
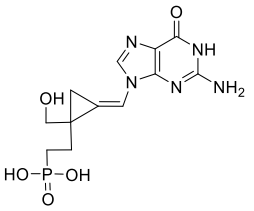
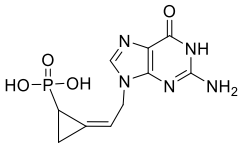
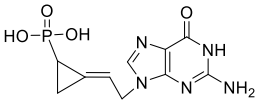


Figure 1-3. 3-membered nucleoside analogues

The 3-membered nucleoside phosphonate can also be classified into two groups based on similar rules (to avoid confusion, we did not group them with nucleosides), Type I is also characterized by the methylene linkage, while Type II has a double bond linker (**Table 1-3**). Among the Type I nucleoside phosphonates 9-[1-(Phosphonomethoxycyclopropyl)methyl]-guanine (PMCG) can be considered as the cyclopropyl analogues of adefovir. Prodrug strategy was also successfully applied to Type I phosphonate.[27] Type II phosphonates contain an exocyclic double bond as a linkage between nucleobase and cyclopropane. Like their nucleoside counterpart, this type of nucleoside phosphonate has shown broad-spectrum antiviral activities.[28-29] Noteworthy, a significant antiviral activity difference was noticed between the Z-isomer and E-isomer of nucleosides and nucleoside phosphonates (**Table 1-3**).

Table 1-3. Representative 3-membered antiviral agents

Category	Structure	Target virus
Nucleoside Type B	 HCMG	herpes virus
Nucleoside Type C	 Synadenol B = Adenine Synguanol B = Guanine	HCMV and EBV Synguanol moderate active towards HIV Z-isomers broad-spectrum activity while E-isomer is devoid of activities

Nucleoside phosphonate Type I	 PMCG	 Besifovir	HBV
Nucleoside phosphonate Type II	 1.10	 1.11	Z-isomer **1.10** is active against HCMV, VZV and EBV, while E-isomer **1.11** is devoid of activity.
	 1.12	 1.13	E-type **1.13** is more active than Z-type **1.12** against EBV

1.2.6 4-membered cyclic nucleoside and nucleoside phosphonate analogues

Unlike the strained, and reactive 3-membered ring, epoxide nucleoside, it is possible to construct, oxygen-containing the 4-membered cyclic nucleoside. The best example of this class is oxetanocin A which is a natural oxetane nucleoside isolated from the culture filtrate of *Bacillus megaterium*.[30]

The modification of oxetanocin-A **(Figure 1-4)**, focuses on the exploration of different nucleobases. Oxetanocin A and its base modified counterparts have shown significant anti-HIV activity. Also, OXT-G exhibit potent activity against HCMV, whereas OXT-T is an inhibitor of HSV and VZV.[31]

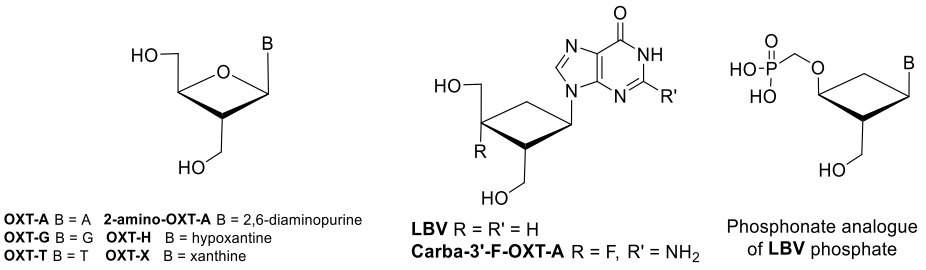


Figure 1-4. OXT-A and related nucleosides

The second site of variation is the ring oxygen atom when replaced by a carbon gives cyclobutane analogues with improved stability toward hydrolysis. Compared to their oxo-counterparts, some of these carbocyclic oxetanocins exhibit improved bioavailability as well as better antiviral activity. Among them, cyclobut-G (lobucavir, LBV) **(Figure 1-4)** showed broad-spectrum antiviral activity against various viruses. It is comparable to acyclovir as anti-HSV-1 and HSV-2 agent, and more potent against VZV and EBV.[32-34] LBV has undergone clinical trials as an anti-HBV agent, which

were unfortunately suspended due to oncogenicity in rodents.[35]

The phosphonate analogues of LBV monophosphate **(Figure 1-4)** were also synthesised. However, these phosphonates were far less potent than their nucleoside counterpart LBV.[36]

1.2.7 6-membered nucleoside analogues

Although once considered as exotic structures, pyranose nucleoside analogues are also found in nature like more common pentofuranose nucleosides. Of these natural pyranose nucleosides, blasticidin was known as early as in 1966, to the most recent amipurimycin in 1997. They are nucleoside antibiotics exhibiting a variety of biological activities. Noteworthy, the first chemically synthesised nucleosides in this class are gluco-pyranosyl adenine nucleosides.[37]

The systematic exploration of 6-membered nucleoside analogues was started by Eschenmoser in the 1980s; this work was mainly focused on the chemical etiology.[38] At the same time, active investigation of pyranosyl nucleosides/nucleotides as antivirals was carried out by Herdewijn's group. The earlier exploratory years of pyranosyl nucleosides as antivirals, however, were not fruitful. In the early 1990s, Herdewijn and co-workers synthesised several unsaturated (3-eno **1.14** and 2-eno **1.15**) and 1´-*O*-4-heteroatom-pyranosyl nucleoside analogues **1.16 (Figure 1-5)**, to study SAR (structure-activity relationship) against HIV. However, none of these six-membered nucleosides were active.[39-41] Given the alienation of pyranose ring, these compounds could probably be a poor substrate for intracellular phosphorylating kinases, which might be rationalised for the lack of their antiviral activity.[42]

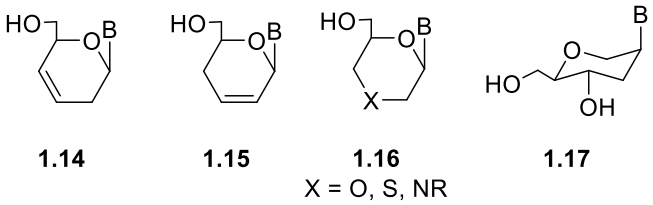


Figure 1-5. Pyranosyl nucleoside analogues

The relationship between the geometry (conformation) of the pyranose ring and antiviral activity was then re-considered and explored. By shifting the nucleobase from anomeric 1′ position to 2′ position, which will then be devoid of the anomeric effect, influencing the orientation of nucleobase in hexitol nucleoside. As a consequence, unlike other pyranosyl nucleosides, these hexitol nucleosides (2′-nucleobase) adopt a conformation with an axial nucleobase. Besides, this modification leads to chemical and enzymatic stability as compared to the originally anomerically placed nucleobase in pyranosyl nucleosides. This novel series of 1,5-anhydro-2,3-dideoxy-β-D-arabinohexitol nucleosides has shown selective antiherpes activity (HSV-1 and HSV-2). Whereas cytosine and guanine analogues are broad range antivirals. In hexitol nucleosides, when the 2′-

nucleobase is adenine, cytosine or guanine, equal activity against thymine kinase$^+$ and thymine kinase$^-$ is found. Thus, virus-encoded thymine kinase is not a pre-requisite for phosphorylation.[42]

Inspired by these results, their carbocyclic analogues like cyclohexanyl and cyclohexenyl nucleosides were also synthesised and explored. None of the cyclohexanyl congeners showed activity against the tested viruses, which may be explained by the conformation differences between these carbocyclic analogues and their hexitol mimics.[43] However, the insertion of a double bond led to more attractive cyclohexenyl congeners. By combining the improved stability induced by the absence of anomeric centre and the conformational variability derived from the double bond, this pseudosugar nucleoside can be regarded as a (bio)isostere of the natural ribo-furanose ring adopting similar S- and N-type conformations (**Figure 1-6**). Both D- and L- cylcohexenyl guanine enantiomers were active against a whole range of herpes viruses with comparable potency and virus spectrum. More interestingly, conformation and modelling study showed that these two enantiomeric nucleosides interact with the same amino acid residues in the active site of HSV-1 thymidine kinase.[44]

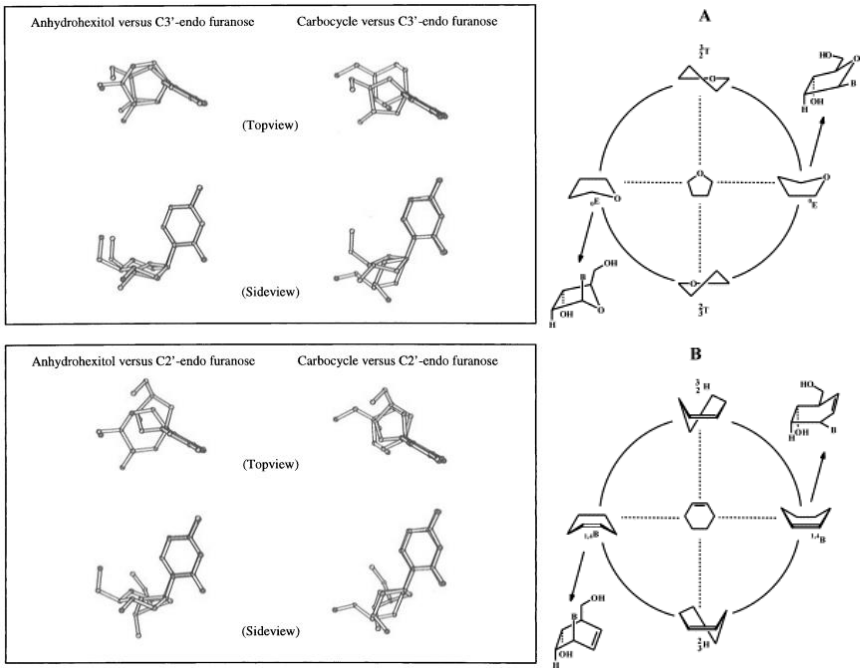


Figure 1-6. Left: Comparison between the three-dimensional conformations of the six-membered sugar ring nucleosides (anhydrohexitol and carbocyclic) versus a normal nucleoside with its sugar moiety modelled in the two most common puckering conformations (C2′-endo and C3′-endo). **Right:** Pseudo-rotational cycle of cyclohexene **B** vs furanose **A**.(Image adapted from reference[44])

Hexitol and cyclohexenyl related nucleoside phosphonates (**Figure 1-7**) were also explored. In the 1990s, in parallel with the research on hexitol and cyclohexenyl nucleosides, their 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydropentopyranosyl nucleosides bearing a 1,4-cis phosphonomethyl moiety and nucleobase were synthesised and tested against different herpesviruses and/or HIV.

However, none of these nucleoside phosphonates was active.[44-46] A good viable synthetic route for these analogues is not yet well documented or investigated.

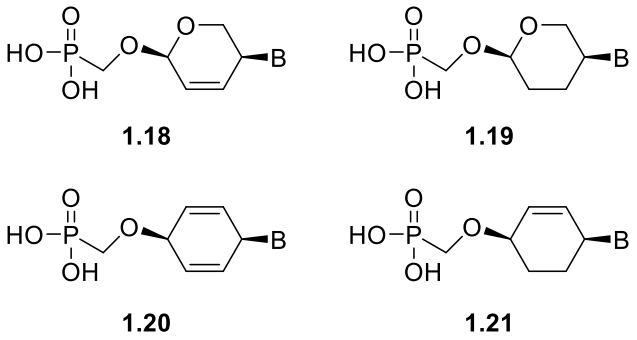


Figure 1-7. Hexitol nucleoside phosphonates and cyclohexenyl nucleoside phosphonates.

1.2.8 Oxepane (7-membered) nucleoside analogues[47]

Like, six-membered pyranose/hexitol scaffolds, 7-membered oxepane ring analogues are also found in nature and has shown synthetic and biological interest. Chemical synthesis of seven-member oxepane by ring expansion is challenging chemistry. The synthesis of 7-membered oxepane nucleoside mimics was developed by Masad J. Damha's group in 2007. Following their work, several oxepane nucleoside analogues have been synthesised and reported as potential antivirals and glycosidase inhibitors (**Figure 1-8**). Meanwhile, their work also led to the development of a new series of nucleic acids, oxepane nucleic acids (ONAs). With more flexible sugar mimic relative of hexitol nucleic acid, ONA is able to trigger ribonuclease H (RNase H) mediated degradation of a complementary RNA strand, which could be of great value in the antisense field. The exploration of related 7-membered nucleosides and oligonucleotides are still ongoing.

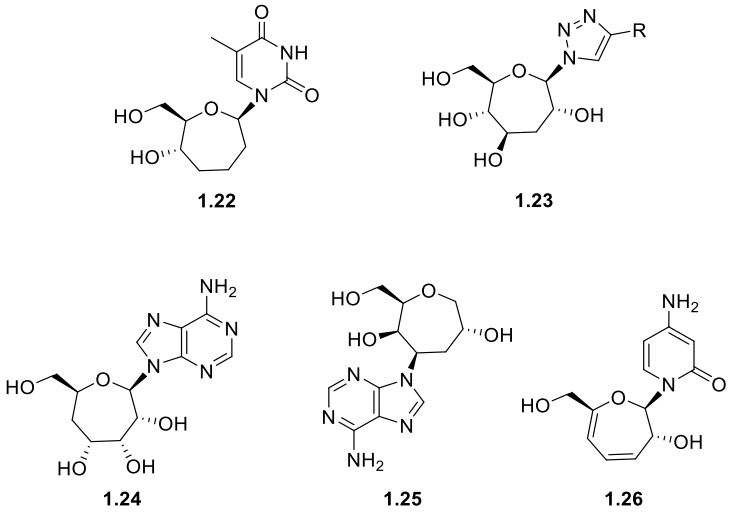


Figure 1-8. Oxepane nucleosides.

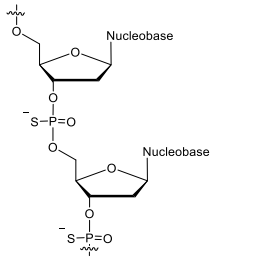
1.3 Synthetic oligonucleotides

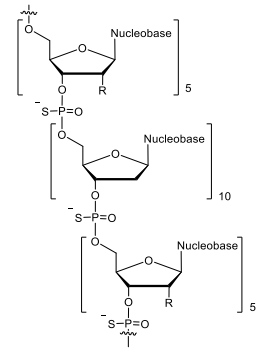
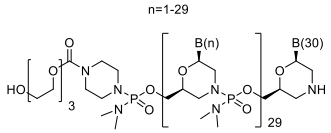
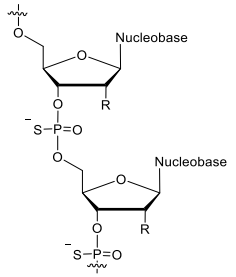
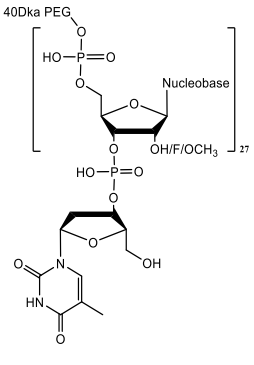
In the late 1970s, thanks to the extensive knowledge gained in the structural and functional characterisation of natural nucleic acids (especially RNAs), the search for therapeutically useful oligonucleotides was initiated by Stephenson and Zamecnik in the field of antisense agents. Early explorations in antisense oligonucleotides proved that natural DNA or RNA could not be used directly as therapeutics for several reasons, such as the stability of phosphodiester bonds against endogenous nucleases, limited cellular or tissue uptake.[48] Attempts to overcome these hindrances lead to the synthesis of numerous synthetically modified oligonucleotide analogues.[48]. After more than 30 years in the field of antisense oligonucleotides and aptamers, as well as more than 15 years in the field of siRNAs, therapeutic oligonucleotides have found their applications: in controlling/interfering with various cellular functions; antigene/antisense oligonucleotides and siRNA that bind to RNA or DNA; aptamers that bind to functional proteins.[49]

1.3.1 Approved oligonucleotides[49]

The exploration of synthetic oligonucleotide analogues not only enriched our understanding of some of the key biochemical processes, but also led to the approval of several promising oligonucleotides, either as antisense agents or aptamers. (**Table 1-4**)

Table 1-4. Modified Oligonucleotides having a therapeutic application

Category	Oligonucleotide	Modification	Target
antisense		Phosphorothioates	HCMV in immune-compromised patients

	 20 mer : 5'-G* mC* mC* mU* mC* dA dG dT dmC dT dG dmC dT dT dC dG* mC* A* mC* mC*-3' * R= m = 5-methyl d = 2'-deoxy **Mipomersen**	Phosphorothioate; 2′-MOE ribose gapmer	homozygous familial hyper-cholesterolemia
	 **Eteplirsen**	Phosphomorpholidate	Duchenne muscular dystrophy
	 18 mer : 5'-mU mC A mC mU mU - mU mC A mU A A mU G mC mU G G-3' R = m = 5-methyl **Nusinersen**	Phosphorothioate; 2′-O-MOE ribose and 5-Me cytidines	infants with spinal muscular atrophy
aptamer	 **Pegaptanib**	2′-O-Me or 2′-F ribose	age-related macular degeneration

Here it is worth to mention, FDA has approved a natural oligonucleotide purified from controlled depolymerisation of intestinal mucosa of the pig, named Defibrotide (DF) in the year 2016. DF was

indicated for the veno-occlusive disease of the liver occurring after bone marrow transplantation. DF's mechanism of action is still not clear. It is proposed that the active oligomers in DF are those that are double-stranded (10%), by their ability to form intrastrand stem-loop structures (hairpin), or inter-strand concatamers.[49] These well-organised structures could provide resistance to nuclease degradation, and stabilising the individual strands for long enough for them to reach the liver.

1.3.2 Xenobiotic nucleic acids (XNAs)

Xenobiotic nucleic acids (XNAs) are an emerging field of synthetic oligonucleotides, aiming to achieve orthogonal synthetic information storage system, as an alternative for natural DNAs and RNAs. Xenobiology is a new term used to describe artificial biological systems which are different from the canonical "central dogma" system (DNA-RNA-20 amino acids). For example, xenobiology attempts to search for xenobiotic nucleic acids (XNAs) as unnatural information carriers. It also explores the expansion of natural three-letter codon for amino acids and/or the incorporation of non-proteinogenic (non-coded) amino acids. These XNAs are artificial alternatives of natural DNA or RNA that might differ in the three counterparts (sugar, nucleobases and/or phosphate) while still being able to store and pass on the information.[50]

The previous exploration of XNAs mostly focussed on the modification of the sugar backbone and led to the discovery of ANA, FANA, TNA, HNA, CeNA, and LNA **(Figure 1-9)**.[51] Holliger and collaborators demonstrated that the genetic information not only can be stored but can also be retrieved from these sugar modified synthetic oligonucleotides with engineered polymerases. HNA aptamers against HIV trans-activating response RNA and hen egg lysozyme were synthesised and selected with high specificity and affinity.[51]

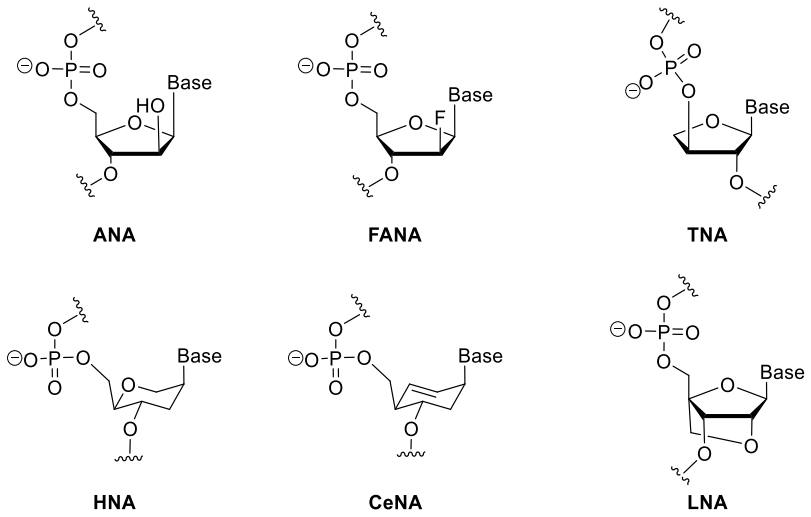


Figure 1-9. Sugar modified XNAs

More recently, Holliger's group has developed XNAzymes capable of ligase and in *trans* RNA

endonuclease activity (an essential functional counterpart of natural DNAzymes/RNAzymes).[52] *In vivo* studies demonstrated that constructed plasmids carrying XNA nucleotides thyA active codons were capable of serving as a template for *E. coli* replication.[53] These recent discoveries not only demonstrate that central dogma could be expanded and even redesigned but also raise the possibility that life could have begun with something other than RNA or DNA.

1.3.3 Six-membered nucleic acids

The rationale behind the investigation of six-membered hexitol/pyranosyl oligonucleotides is based on the considerations of their favourable physicochemical properties over five-membered furanosyl oligomers. Apart from the environmental influence, the formation of the duplex by two single-stranded oligomers is governed by two factors, enthalpy and entropy. Assuming that inter-strand cross-link forces in a pyranosyl oligonucleotide and its furanosyl counterpart are the same stacking force of nucleobases and hydrogen bonding, the enthalpy change can be assumed as comparable. In the case of pre-organized six-membered oligonucleotides with a more rigid ring, the loss in entropy during the formation of the duplex is less. Thus they might have a free-energy advantage.[38]

A lot of works have been done based on this assumption, which can be broadly divided into two categories: the first one was primarily initiated by Eschenmoser's group, which aimed to explore the chemical etiology of natural nucleic acids, e.g. homo-DNA, pyranosyl RNA (pRNA) and related oligonucleotides **(Figure 1-10)**.[38] The second one was carried out by Herdewijn's group to investigate the possible application of oligomers containing six-membered sugar mimetics, not only in antisense field but also as synthetic functional alternatives of natural DNA/RNA in XNA field (synthetic biology), this work led to the exploration of hexitol nucleic acids and cyclohexenyl nucleic acids.[54-55]

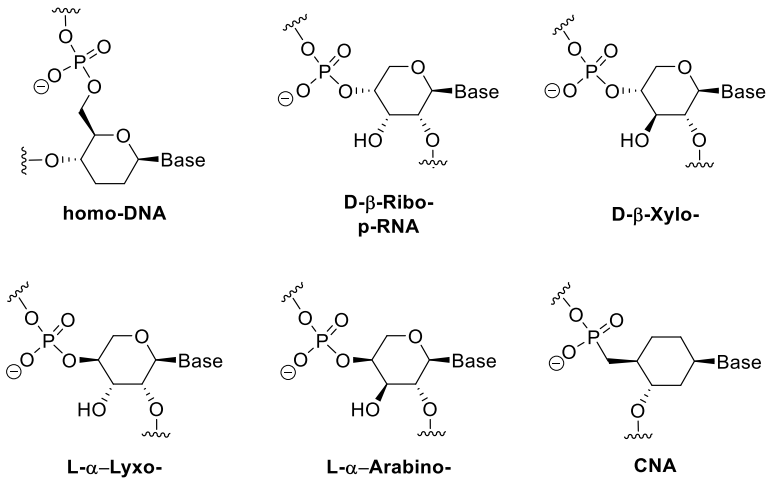


Figure 1-10. Six-membered nucleic acids

1.3.3.1 homo-DNA, pRNA and related nucleic acids[38]

In the late 1980s, to answer the chemical etiology of natural DNA and RNA, Eschenmoser and co-workers initiated the synthesis and examination of hexo-pyranosyl nucleic acids. One of the most important parts of Eschenmoser's work was that he proposed the strategies, principles and stages of the research within the chemical etiology field. First, he visualised that during the early days of nucleic acid evolution, the abundance of alternative aldo-sugars beyond (deoxy) ribose in nature means it is more likely that different nucleic acids with alternative sugars could have co-existed. And also, given the special structure needed to form hydrogen bonding between nucleobases and the unique negative electronic properties of phosphate linkage, these two parts are less changeable. Thus, the base-pairing properties of different sugar-modified oligonucleotides systems could be synthesised and tested. During this step, those alternatives without pairing ability can be dropped. Secondly, the self-replication ability of those selected needs to be evaluated since it is pre-requisite for acting as genetic material. Third, the capability to express phenotype should be judged. All these capabilities together determine whether the candidate nucleic acids under investigation are selectable in competition with natural DNA.

With the quest of chemical etiology, the search for oligonucleotides bearing alternative natural sugar backbone and answering, "why pentose, not hexose?", their pioneer work led to the discovery of homo-DNA and several related synthetic oligonucleotides, which are among the first series of six-membered nucleic acid mimics.

As the first backbone-modified pyranosyl nucleic acids, β-homo-DNA showed a much stronger Watson-crick base pairing property than DNA, while reversed Hoogsteen base-pairing mode of adenine and guanine self-pairing was also a pronounced possibility. Since the sugar part in homo-DNA is not found in nature, using homo-DNA as a model system, other members of (4′-6′) hexopyranosyl oligonucleotides with more hydroxyl groups on the sugar ring were also studied. However, maybe because of the intra-strand steric hindrance originated from the extra hydroxyl and the neighbouring nucleobase, either none or much weaker base-pairing ability was observed.

Encouraged by the interesting properties of homo-DNA and to find the answer to the question "why ribose, and why ribofuranose and not ribopyranose?" Eshchenmoser and co-workers synthesised and tested the pairing properties of a series of (2′-4′) oligonucleotide systems containing different pentopyranosyl units, D-β-ribo-, D-β-xylo-, L-α-lyxo- and L-α-arabino- **(Figure 1-10)**. The conformational analysis revealed all members in these pentopyranosyl adopt a conformation with an equatorial nucleobase. These pentopyranosyl oligomers are stronger Watson-crick base-pairing systems than RNA and did not cross-pair with RNA. All the members in this family were able to cross-pair with each other, which demonstrated their remarkable capacity of adopting a common conformation for pairing. The most promising system in this family is the pyranosyl analogues

(pRNA) of natural RNA with more selective pairing modes, without the Hoogsteen or reverse-Hoogsteen mode.

1.3.3.2 Hexitol nucleic acids

Learnt from Eschenmoser's pioneer work on homo-DNA, Herdewijn's group realised that Watson-Crick base-pairing rules are although governed by nucleobase sequences, this pairing ability has a significant influence from sugar backbone.[55]. Unlike, Eschenmoser's work which was focused only on pentopyranose sugar mimics (anomeric nucleobase), Herdewijn's group has synthesised and compared hexitol nucleic acids to search for a system capable of high cross-communication from a functional point of view.

To start, the first question is which hexitol nucleotide analogues can form a helical duplex with DNA/RNA in a manner similar to natural DNA/RNA duplex. Based on the X-ray structures of 13 available pyrimidine nucleosides, sugar conformation or ring puckering were studied to generate the information on their conformational preference. The molecular-modelling studies were used to select the most favourable hexitol oligonucleotides that can hybridise with natural nucleic acids. From this study, two structure and several other related structural analogues were selected and synthesised. Later, two fully modified oligomers were also synthesised and evaluated to hybridise with complementary DNAs. This work revealed that modified hexitol oligonucleotides could form a duplex with natural oligomers.[54]

In parallel with the molecular modelling, several structural representatives of hexitol oligomers were also synthesised and annealed to natural DNA. The thermal stability of these duplexes was investigated.[55-56] Later, it was shown that some of the hexitol nucleotides are recognised by polymerases, widening the application scope of these pyranosyl oligomers.[57]

Among the various six-membered backbones, hexitol nucleosides and nucleic acids are the most promising candidates. Hexitol nucleosides are DNA analogues formed by the insertion of an extra methylene (-CH_2-) group between ring oxygen and 1′-carbon of deoxyribose. Thus there is no anomeric carbon in these nucleoside mimics. These 1, 5-anhydrohexitol nucleosides adopt a slightly distorted chair conformation with axial nucleobase and equatorial -CH_2OH, which is similar to the N-type conformation of furanose nucleosides.[42]

Duplexes formed by HNA and DNA/RNA are very stable. The thermal stability study of HNA containing duplex is observed in the order of HNA:HNA > HNA:RNA > HNA:DNA. Better mismatch discrimination and improved phosphodiesterase stability is observed in HNAs.[58] The solution-structure of HNA-RNA duplex was also investigated, which shows A-form ds-RNA like antiparallel conformation following standard Watson-crick rules and N-type sugar pucker of the ribose.[59] The N-type sugar puckering may explain why HNA can form a more stable duplex with

RNA than DNA. HNAs are relatively more stable to the endonuclease activity of RNase H, due to rigid chair conformation of pyranose imparting relatively weak interaction with the RNase H.[60-61]

To explore the function of HNA oligomers, Holliger and coworkers successfully developed a method named compartmentalised self-tagging (CST) strategy. *Thermococcus gorgonarius* (Tgo) polymerase was randomly mutated to construct a library, which was then used to select variants capable of synthesising HNA oligomers from DNA templates. Up to 72 consecutive hexitol nucleoside triphosphates could be incorporated based on a DNA template using engineered Po16G12 DNA polymerase. To retrieve information from HNA polymers back into DNA, reverse transcriptase RT521 (TgoT mutant), was selected as a proficient HNA RT. Moreover, the two selected enzymes were successfully used for the *in vitro* evolution of HNA aptamers against hen egg lysozyme (HEL, protein target for RNA aptamers) and HIV trans-activating response RNA (TAR, the target for DNA aptamers). Both aptamers showed high affinity and specificity toward binding with their targets, which proves that HNA can fold into defined structures with very specific ligand binding site.[51]

Moreover, *in vivo* study demonstrated that up to six contiguous HNA nucleotides (2 codons) could serve as a template for *E. coli* replication enzyme. This established a binary genetic cassette based on HNA, which can convey hereditary information to DNA and used for selecting XNA-Templated DNA synthesis *in vivo*.[53]

More recently, after demonstrating XNAs are able to bind enzymes and fold into defined structures, the researchers explored XNAs's ability as catalytic XNAzymes. They sequentially developed XNAzymes that can cleave a phosphodiester bond, *i.e.* XNA endonuclease. In their final demonstration, they successfully developed an XNAzyme with RNA-endonuclease activity and XNAzyme with ligase activity, which empowered XNAs as full synthetic catalytic system.[52]

1.3.3.3 Cyclohexenyl nucleic acids

The other promising oligonucleotide candidate also developed by Herdewijn's group are the cyclohexenyl nucleic acids (CeNA). This part of work on six-membered carbocyclic oligonucleotides was started with the investigation of cyclohexanyl nucleic acids (CNA) **(Figure 1-10)**, which is the carba-analogue of HNA. dsCNA forms more stable duplexes than the corresponding DNA duplex, whereas, CNA hybridises more strongly with RNA than DNA. However, the affinity of CNA to RNA is weaker compared to that of HNA and ANA.[62-63] Most probably due to higher 1,3-diaxial interaction originating from the replacement of oxygen with CH_2, CNA tends to avoid this, it has a propensity to align the nucleobases in equatorial conformation. As a consequence, this flexibility is not favoured during duplex formation with DNA/RNA.[64]

By introducing a double bond in the cyclohexane ring, the conformational properties can be altered. The cyclohexene ring in cycleohexenyl nucleic acids (CeNA) can be considered as a bioisostere of the furanose ring in canonical DNA/RNA with similar conformational behaviour.[44, 65]

Being carbocyclic nucleosides, they have stability against enzymatic degradation. CeNA has conformational mobility which is much similar to that in DNA/RNA (CeNA:RNA hybridise in A-form conformation while in CeNA:DNA it is B-form). Unlike HNA, CeNA does not seem to induce a conformational change of a double strand DNA when it is incorporated into a DNA chain. Being able to accommodate well in a variety of duplexes, CeNA is also characterized by its universal base-pairing behaviour. Not only with DNA and RNA, but it can also hybrid with α- and β-homo DNA, HNA and CNA.[66-67]

The kinetic parameters of cyclohexenyl adenine triphosphate incorporation study show that it is recognised by various polymerases, indicating CeNA might be a suitable candidate as an alternative nucleic acid system for the development of XNAs (as orthogonal nucleic acids). [68] The insertion of CeNA in modified siRNA is also promising, desired biological responses are enhanced in most cases. In addition, the capability of CeNA to induce RNase H activity is also striking, making it the second (after arabino-pyranosyl nucleic acids) and also the most promising six-membered candidate in antisense therapeutic research.[66,69]

Moreover, like HNA, CeNA is also able to bind ligands and fold into the defined structure (capable of heredity and evolution),[51] as well as act as XNAzyme[52] with endonuclease and ligase activity. Further, CeNAs are also applicable for binary genetic cassettes test *in vivo* mentioned above in HNAs.[53]

1.4 Nucleoside phosphonate analogues: synthetic approach

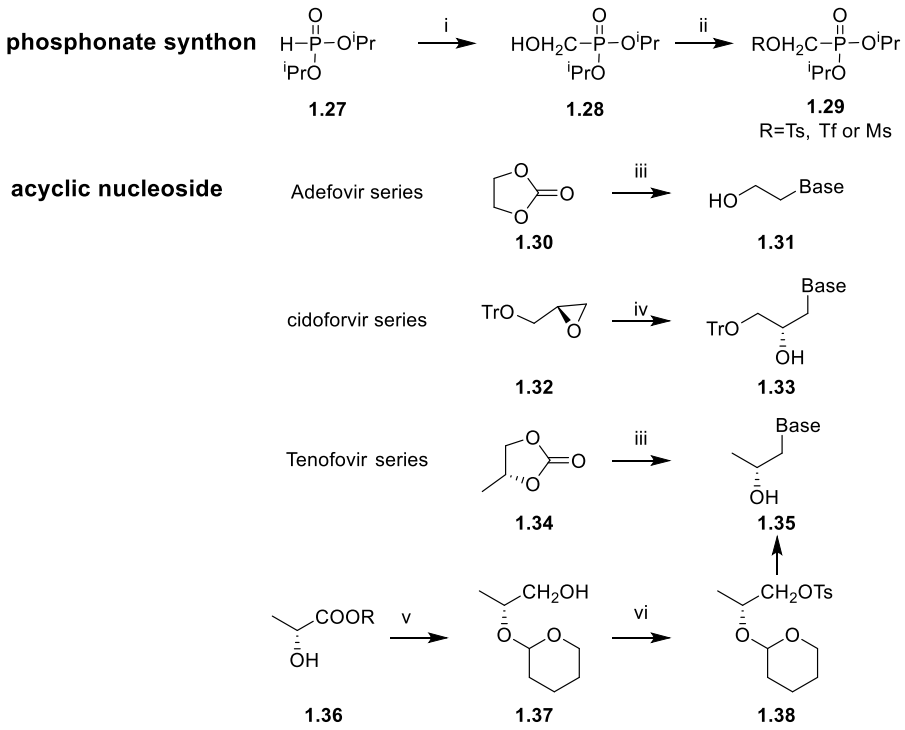
Classically, structural modifications, employed in modified nucleoside phosphonates can be divided into three moieties: pseudo-sugar, nucleobase and the phosphonates. There are mainly two synthetic strategies based on the sequence in which a convergent synthetic approach interlinks these three parts. In the first method, the initial *N*-glycosylation reaction of nucleobase on the glycone moiety will form the nucleoside analogue, followed by phosphonomethylation using functional methylphosphonic acid diester derivative (phosphonate synthon) to introduce the phosphonate moiety. Or *vice versa*, phosphonate moiety will be first incorporated on the pseudo-sugar to form a synthon, which is then subjected to *N*-alkylation with (protected) heterocyclic bases or N-glycosylation with nucleobase. This section briefly gives an overview of synthetic strategies for therapeutically important nucleoside phosphonates.

1.4.1 Acyclic nucleoside phosphonates[70]

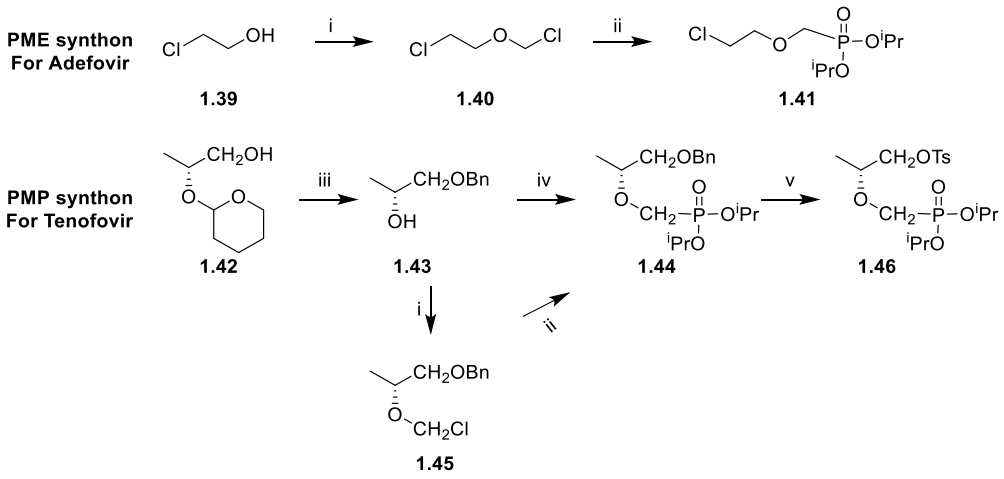
Holý A, De Clercq E. and co-workers have done pioneering work in the exploration of acyclic nucleoside phosphonates.

In the first method, suitably protected nucleobase is *N*-alkylated to form the corresponding hydroxyalkyl nucleoside (**1.31, 1.33, and 1.35**). The phoshonomethylation of the free hydroxyl moiety on the acyclic nucleoside is then carried out with activated electrophilic methylphosphonic acid diester (**1.29**) bearing a suitable leaving group (bromide, tosylate, mesylate, triflate) in the presence of a strong base.

In the second route, phoshonomethylated alkyl synthons (**1.41**, **1.44**) are prepared. Likewise, nucleobase is introduced by the nucleophilic substitution by the nucleobase nitrogen on to a leaving group on the side chain. Examples are shown in **Scheme 1-1** and **Scheme 1-2**.



Scheme 1-1. Synthesis of acyclic nucleoside then introduction on phosphonomethyl moiety. *Reagents and conditions*: (i) $(CH_2O)n$, Et_3N; (ii) sulfonyl chloride or anhydride, Et_3N or pyridine; (iii) NaOH, DMF, nucleobase; (iv) Nucleobase, Cs_2CO_3; (v) (a) 3,4-Dihydropyrane, HCl, DMF; (b) Red-Al; (vi) TsCl, pyridine

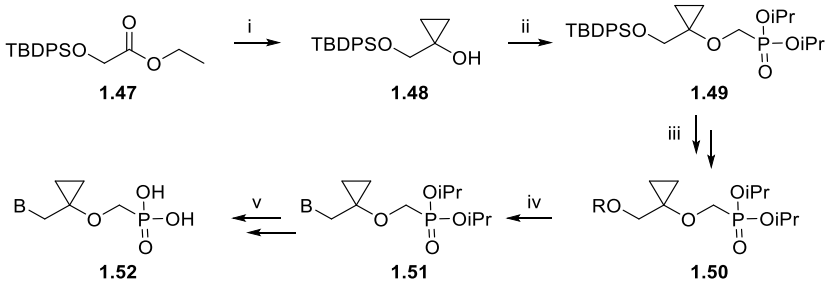


Scheme 1-2. Phosphonomethyl ether Synthon (PME). *Reagents and conditions*: (i) 1,3,5-trioxane, HCl; (ii) Triisopropyl phosphite; (iii) (a) BnBr, NaH; (b) 0.25M sulfuric acid; (iv) NaH, phosphonate synthon; (v) (a) H_2, Pd/C; (b) TsCl, pyridine.

1.4.2 Cyclopropyl or Cyclobutyl (3- and 4- membered carbocyclic) nucleoside phosphonates

3- and 4-membered cyclic nucleoside phosphonates have attracted attention recently. The rationale for 3-membered cyclic nucleoside phosphonates lies in the promising bio-activities of adefovir and acyclovir. Since all the five bonds of acyclic adefovir and acyclovir are rotatable, and introducing a ring will decrease the molecular flexibility, will improve the entropic factor and might improve the biologically active.[27] There are two types of 3-membered cyclic nucleoside phosphonates.

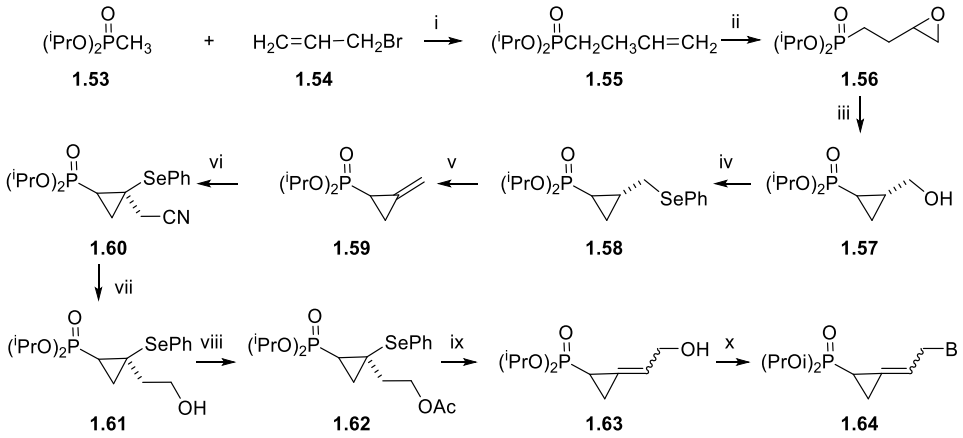
For example, the synthesis of 3-membered cyclopropyl nucleoside phosphonates is reported by Choi and co-workers in 2004.[27] The synthesis started with the construction of cyclopropanol (**1.48**) by Kulinkovich reaction utilizing transmetallation of Grignard reagent bearing beta hydrogen atom, followed by sequential incorporation of the phosphonate (**1.49**) and nucleobase moieties by nucleophilic substitution. **(Scheme 1-3)**



Scheme 1-3. Cyclopropyl nucleosides. *Reagents and conditions*: (i) CH_3CH_2MgBr, $Ti(O^iPr)_4$ (0.25 equiv), THF, 0 °C to 25 °C, 10 h; (ii) $BrCH_2P(O)(O^iPr)_2$, LiOt-Bu, LiI(cat.), DMF, THF, 60 °C, 4 h; (iii) (a) NH_4F, MeOH, reflux, 10 h; (b) MsCl, TEA, MDC, 0 °C to 25 °C; (iv) 6-chloroguanine, NaH, DMF, 80 °C,4 h; (v)

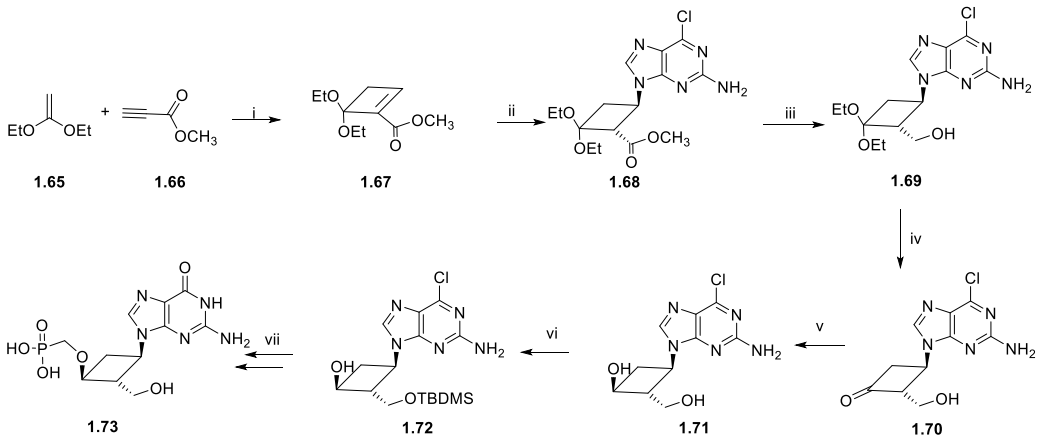
For 6-deoxyguanine analogue (a) H_2, 5% Pd on C, THF, 1 atm, 18 h; (b) TMSBr, MDC, reflux, 18 h; and for guanine analogue (a) TMSBr, MDC, reflux, 18 h; (b) 2 N HCl, reflux, 6 h;

The synthesis of cyclopropyl nucleoside phosphonates with exocyclic double bond is reported by Zhao and co-workers in 2005.[29] The cyclopropane ring was obtained by intramolecular epoxide ring opening by carbanion generated by *n*-butyl lithium (**1.57**). Key steps include β-elimination of selenoxide (**1.59**), phosphonate group directed addition, chain elongation using nitrile, H_2O_2 oxidation and selenoxide elimination (**1.63**). The nucleobases were incorporated on the bromo derivatives smoothly by simple nucleophilic attack. **(Scheme 1-4)**



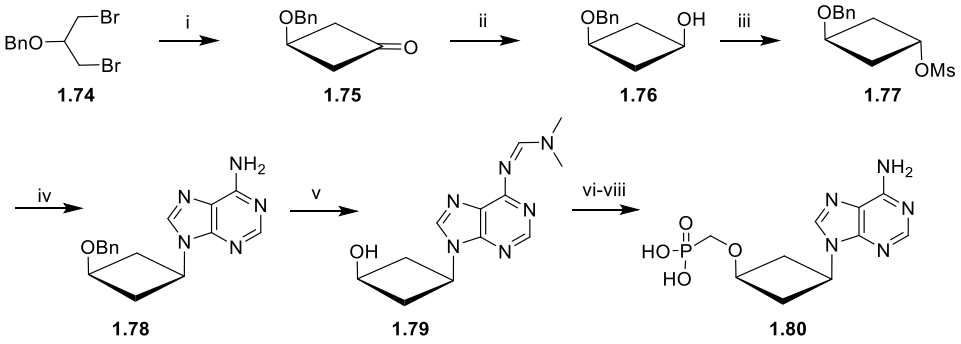
Scheme 1-4. *Reagents and conditions*: (i) (a) nBuLi, THF, -78 °C; (ii) mCPBA, CH_2Cl_2; (iii) Ph_3P, Br_2, CH_2Cl_2; (iv) (a) $(PhSe)_2$, EtOH; (b)NaOH, $NaBH_4$; (v) (a) H_2O_2, THF (b) $(^iPr)_2NEt$, toluene; (vi) (a) $(PhSe)_2$, NBS, CH_2Cl_2; (b)Me_3SiCN, Bu_4NF, MeCN; (vii) (a)HCl(g), MeOH; (b) $LiBH_4$, THF; (viii) (a)Ac_2O, pyridine; (ix) (a) H_2O_2, THF; (b) K_2CO_3, MeOH/H_2O; (x) (a) Ph_3P, CBr_4, CH_2Cl_2; (b) B-H, K_2CO_3, DMF; (c) 6M HCl.

The synthesis of 4-membered carbocyclic nucleoside phosphonates was started by Daniel and co-workers in the year 1992.[36] The key steps include [2+2] cycloaddition to form the cyclobutene ring (**1.67**) and a Michael-type addition of the nucleobase for the introduction of a nucleobase (**1.68**) on the ring. **(Scheme 1-5)**



Scheme 1-5. *Reagents and conditions*: (i) CH_2Cl_2, reflux; (ii) 2-amino-6-chloropurine, DBU; (iii) LAH; (iv) 1N HC1; (v) $NaBH_4$; (v) t-butyldimethylsilyl chloride, imidazole; (vii) (a) sodium hydride, diethylphosphonomethyl triflate; (b) TMSBr, then 1N HC1;

Later in 2014, Liotta and co-workers developed a method for the synthesis cyclobutyl ring (**1.76**) using benzyl protected 1,3-dibromide and methyl (methylsulfinyl)methyl sulfide as the starting materials **(Scheme 1-6)**. The nucleobase was introduced by nucleophilic displacement of a leaving group on the cyclobutyl ring by the nucleobase nitrogen (**1.78**). Likewise, after debenzylation, the phosphonomethyl moiety (**1.80**) is introduced. [71]



Scheme 1-6. *Reagents and conditions*: (i) (a) *n*-butyl lithium, THF, methyl methylsulfinylmethyl sulfide, -78 °C; (b) 35% perchloric acid, diethyl ether; (ii) 1M lithium tri-sec-butyl borohydride, THF; (iii) (a) PPh_3, 4-nitrobenzoic acid, DIAD, THF; (b) 1,4-dioxane, 0.4M NaOH; (iv) MsCl, Et_3N, CH_2Cl_2; (v) adenine, 18-crown-6, K_2CO_3; (vi) BCl_3, CH_2Cl_2; (vii) 1,1-diethoxy-*N*,*N*-dimethylmethanamine, DMF, rt; (viii) (a) NaH, diethyl phosphonomethyl tosylate, DMF; (b) 7N ammonia in methanol; (ix) TMSBr, AcCN.

1.4.3 Pentofuranose/tetrofuranose (5-membered) nucleoside phosphonates

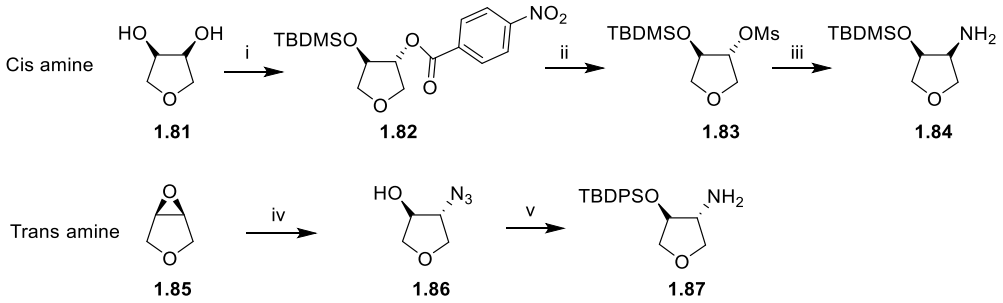
5-membered nucleoside phosphonates can be arbitrarily divided into six types depending on the relative position of the nucleobase and phosphonate moieties: 3′,4′-disubstituted tetrahydrofuran **type I**, 2′-phosphonate-3′-nucleobase tetrahydrofuran **type II**, 2′-nucleobase-5′-phosphonate

tetrahydrofuran **type III**, 2′-nucleobase-4′-phosphonate tetrahydrofuran (threose) **type IV**, 2′-phosphonate-4′-nucleobase tetrahydrofuran (iso-threose) **type V**, adefovir derived **type VI**.

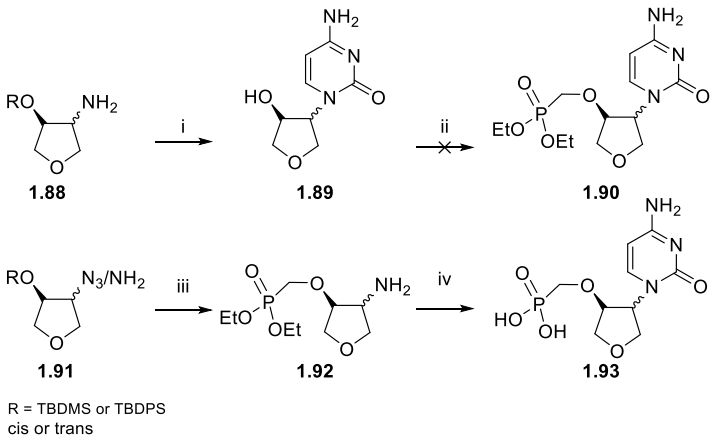
1.4.3.1 3,4-disubstituted pentofuranose derived nucleoside phosphonates[72]

The philosophy of this type of nucleosides lies in the potent antiviral activities of acyclic (S)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC). In an attempt to explore further nucleotide analogues that maintain the stable phosphonate bond, and reduce the entropy of the acyclic system, Wang and co-workers in 1997, have synthesised cyclic analogues of HPMPC by tethering 1′ and 2′ carbons in HPMPC with a - CH_2OCH_2- moiety. The ring opening of 3,4-epoxide (**1.85**) or Mitsunobu coupling of nucleobases and alcohol in the case cis-3,4-diol (**1.81**) were not successful. Alternatively, nucleophilic displacement by azide, a useful precursor for the amino group (**1.87**) and building a nucleobase on this nitrogen (**1.89, 1.93**) worked well. **(Scheme 1-7 and Scheme 1-8)**

However, after the construction of the nucleobase, unexpectedly, efforts to link the phosphonate moiety were not successful. Thus the researchers inversed the reaction sequences as shown in **Scheme 1-8.**



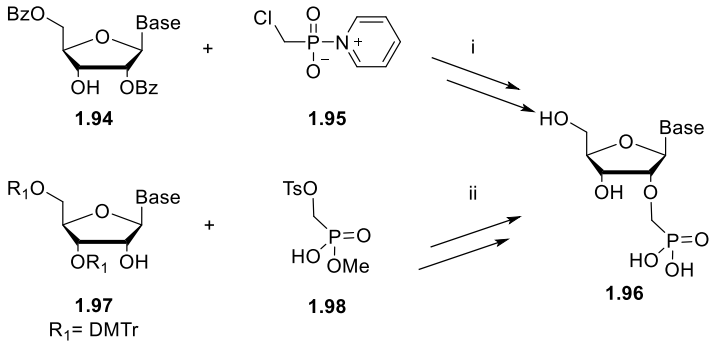
Scheme 1-7. *Reagents and conditions*: Cis amine (i) (a) TBDMSCl, Et_3N, CH_2Cl_2; (b) PPh_3, 4-nitrobenzoic acid, DEAD, benzene; (ii) (a) K_2CO_3, MeOH; (b) MsCl, lutidine, Hünig's base, CH_2Cl_2; (iii) (a) LiN_3, *n*-Bu_4NI, DMF, 120 °C; (b) Pd/C, H_2. Trans amine (iv) NaN_3, H_2O, NH_4Cl, $CH_3OCH_2CH_2OH$; (v) (a) TBDPSCl, imidazole; (b) Pd/C, H_2.



Scheme 1-8. *Reagents and conditions*: (i) (a) isocyanate, toluene; (b) 2N H_2SO_4, dioxane, reflux; (c) Ac_2O, DMAP, pyridine; (d) Lawesson's reagent; (e) NH_3, EtOH, 120 °C; (ii) diethylphosphonomethyltriflate, NaH, DMF; (iii) (a) TBAF, THF; (b) diethylphosphonomethyltriflate, NaH, DMF; (c) Pd/C, H_2; (iv) (a) isocyanate, toluene; (b) 2N H_2SO_4, dioxane, reflux; (c) *p*-methoxyphenyl dichlorophosphate, 1,2,4-triazole, pyridine; (d) NH_3, H_2O/dioxane; (v) TMSBr, AcCN.

1.4.3.2 2′-*O*-methylphosphonate ribonucleoside

Rosenberg and co-workers in the early 1980s reported the synthesis of 2′-*O*-methylphosphonate ribonucleoside. These are phosphonate bioisostere at 2′-position instead of 5′- or 3′-position found in natural nucleotide or oligonucleotide. The 3′-hydroxyl free moiety in suitably protected ribonucleoside is treated with chloromethanephosphoryl chloride in the presence of pyridine (**1.95**) and water (hydrolysed chloromethanephosphonyl chloride), which goes via a cyclic monoester intermediate under the mild aqueous basic condition to afford 2′-*O*-methylphosphonate ribonucleoside (**1.96**).[73-74] Later, tosyloxymethanephosphonate was also successfully used as a phosphonylation reagent on protected nucleoside.[75] **(Scheme 1-9)**

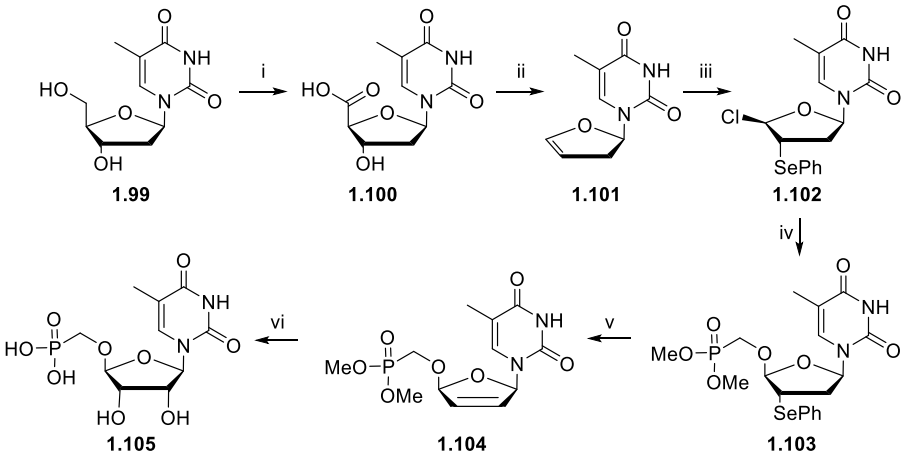


Scheme 1-9. *Reagents and conditions*: (i) (a) pyridine, H_2O; (b) LiOH in 40% aqueous dioxane, 40 °C; (ii) (a) NaH, DMF, rt; (b) 80% AcOH, rt; (c) TMSBr, 2,6-lutidine, AcCN.

1.4.3.3 4′-*O*-phosphonate tetrahydrofuran nucleoside

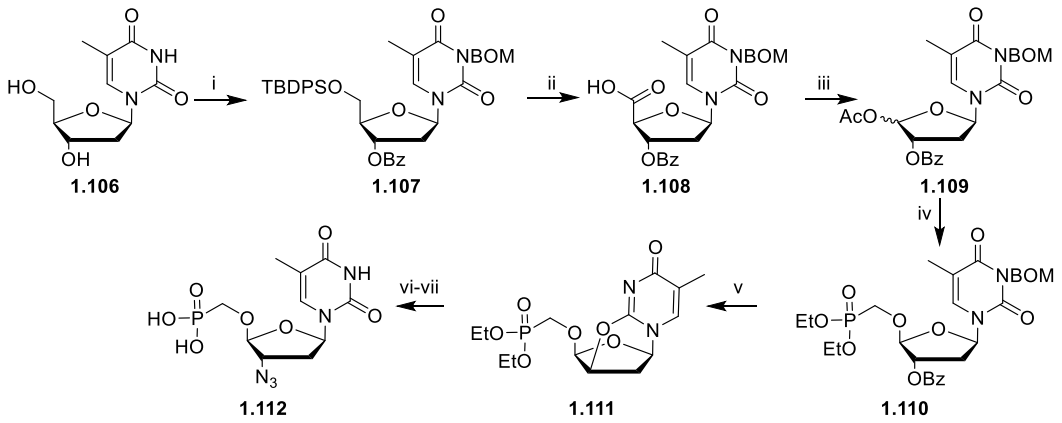
This type of nucleoside phosphonate can be seen as the 4′-phosphonate bioisosteres of natural ribonucleotide (5′-*O*-phosphate). However, this type of phosphonate was not easy to obtain due to inherent two anomeric centre resulting in chemical instabilities like epimerization of the anomeric centre or a ring-opening issue.

As shown in **Scheme 1-10**, the key glycal (**1.101**) was obtained by oxidation and decarboxylation of natural deoxy-nucleoside. From the glycal, chlorophenyl selenyl adduct (**1.102**) was obtained which serves as glycosyl halide. Silver mediated etherification of the glycosyl donor successfully gives 4′-*O*-phosphonomethyl tetrahydrofuran nucleoside. The oxidation of 3′-phenylselenyl resulted in 2′,3′ double bond formation, which can be either reduced or dihydroxylated using catalytic osmium tetraoxide.[76-77]



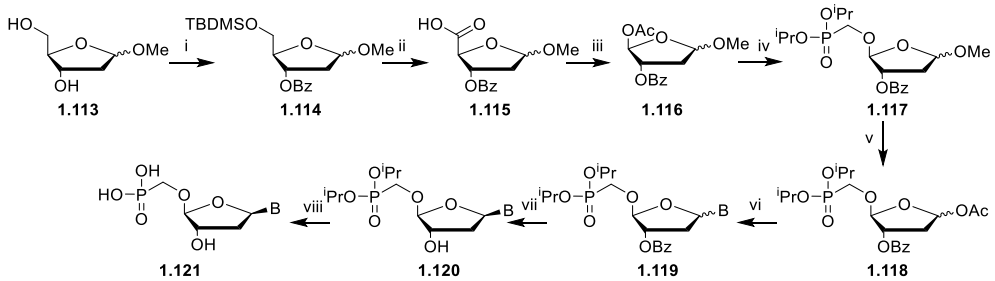
Scheme 1-10. *Reagents and conditions*: (i) reduced platinum oxide, oxygen, pH 8.8 solution of sodium hydrogen carbonate and sodium carbonate decahydrate in water ; (ii) dimethylformamide dineopentyl acetal, DMF, 80 °C; (iii) PhSeCl, DCM, -70 °C; (iv) silver perchlorate, protected phosphonomethanol, AcCN, -70 °C; (v) $NaIO_4$, MeOH; (vi) (a) H_2, Pd/C then TMSBr, AcCN or (b) OsO_4, 4-methylmorpholine *N*-oxide then TMSBr.

Later in 2007, Mackman and co-workers also explore the synthesis of these analogues using deoxy-nucleoside **1.106**, as depicted in **Scheme 1-11**, which under anchimeric assistance afforded **1.110**. However, prolonged reaction duration resulted in an anomerization at the C1 centre, which makes the yield of this phosphonomethnolation step low (14%, from **1.109** to **1.110**).[78]



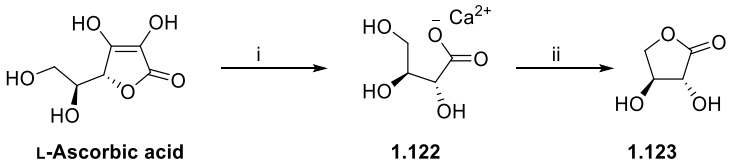
Scheme 1-11. *Reagents and conditions*: (i) (a) TBDPSCl, DMAP, pyridine; (b) BOMCl, DBU; (c) BzCl, DMAP, pyridine; (ii) (a) TBAF; (b) BAIB, TEMPO, H_2O, AcCN; (iii) $Pb(OAc)_4$, pyridine; (iv) TMSOTf, protected phosphonomethanol; (v) (a) $Pd(OH)_2$, H_2; (b) NH_4OH; (vi) DAST; (vii) (a) NaN_3, PhCOOH, 120°C; (b) TMSBr, 2,6-lutidine, AcCN.

Herdewijn's group has also done work related to this type of structure. They synthesised this type of nucleoside phosphonate in a reverse way based on protected sugar scaffold, first using *Vorbrüggen* condition to introduce the phosphonomethanol linkage to get **1.117**, and then the nucleobase was incorporated under Lewis acid condition as a mixture of α- and β- isomer (**1.119**). **(Scheme 1-12)**. [79]



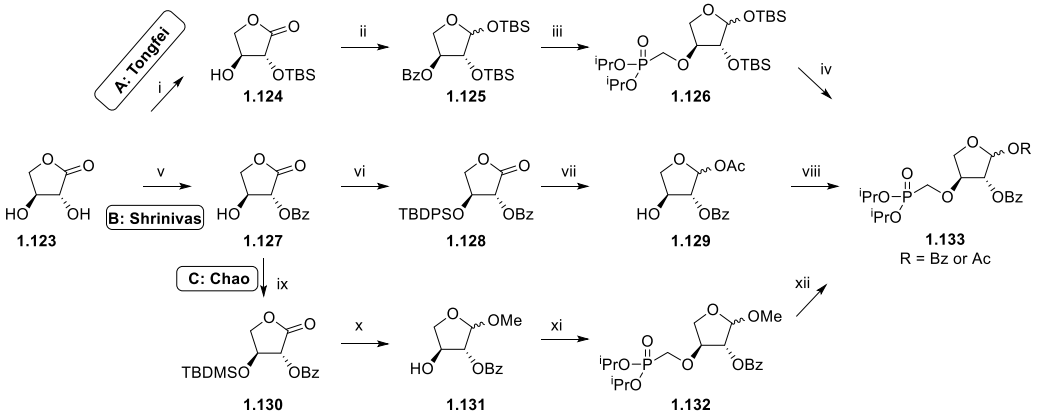
Scheme 1-12. *Reagents and conditions*: (i) (a) TBDMSCl, pyridine, 0 °C; (b) benzoyl chloride, DMAP; (ii) (a) TBAF, AcOH, THF; (b) $PhI(OAc)_2$, TEMPO, CH_3CN/H_2O (1/1, v/v), rt; (iii) $Pb(OAc)_4$, pyridine, THF, rt; (iv) TMSOTf, hydroxymethyl phosphonic acid diisopropyl ester, DCM; (v) Ac_2O, AcOH, H_2SO4, DCM , 0°C; (vi) *N*,*O*-bis(trimethylsilyl)acetamide, base, TMSOTf; (vii) 7N NH_3 in MeOH, rt, overnight; (viii) HMDS, TMSI, CH_3CN/CH_2Cl_2(1/1, v/v), 0 °C, 1 hr.

1.4.3.4 1′-nucleobase-3′-phosphonate tetrahydrofuran (threose) nucleoside phosphonates



Scheme 1-13. *Reagents and conditions*: (i) 30% aq. H_2O_2, $CaCO_3$, H_2O; (ii) (a) Dowex 50WX-50, H_2O; (b) 0.01M TsOH, AcCN, reflux

Eschenmoser's group[80] has developed the most frequently used threonolactone scaffold **1.123 (Scheme 1-13)** for the synthesis of threose nucleic acids (TNA). Starting from this lactone, nucleoside phosphonate can be obtained by first incorporation of the phosphonate followed by the nucleobase, or *vice versa*. The synthetic work, applying both strategies, was mainly conducted in Herdewijn's group.



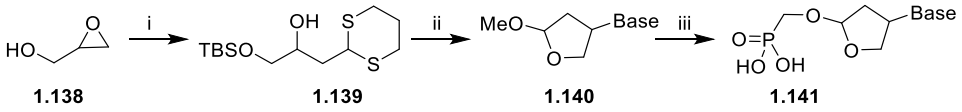
Scheme 1-14. *Reagents and conditions*: (i) TBSCl, imidazole, AcCN; (ii) (a) BzCl, pyridine; (b) DIBAL-H, THF, -78 °C; (c) TBSCl, imidazole, AcCN; (iii) (a) sat. NH_3 in MeOH; (b) $(iPrO)_2POCH_2OTf$, NaH, THF; (iv) (a) TFA/H_2O; (b) BzCl, pyridine; (v) BzCl, imidazole, AcCN; (vi) TBDPSCl, cat. DMAP, imidazole, AcCN; (vii) (a) DIBAL-H, THF, -70 °C; (b) Ac_2O, Et_3N, DCM; (viii) (a) $Et_3N{\cdot}3HF$, THF; (b) phosphonomethyl sulfonate, NaH; (ix) TBDMSCl, cat. DMAP, imidazole, AcCN; (x) (a) DIBAL-H, THF, -78 °C; (b) Acetic chloride, MeOH, rt; (xi) phosphonomethyl sulfonate, NaH, THF; (xii) Acetic anhydride, H_2SO_4, CH_2Cl_2, rt

The first strategy was reported by Tongfei *et al.* **(Scheme 1-14A)**.[81] This overall strategy involved several protection-deprotection-reprotection sequences in order to have 1,2-diacyl scaffold for *Vorbrüggen glycosylation.* 2-OH group of **1.123** was selectively protected by bulky silyl group, followed by 3-O-benzoylation. Reduced anomeric hydroxyl was then protected, which can give free 3-OH after debenzoylation. The phosphonomethanol was introduced by nucleophilic substitution via its sulfonate form. Optimisations were performed by Shrinivas *et al.* **(Scheme 1-14B)**[82] and Chao *et al.* **(Scheme 1-14C)** in 2016.[83] In their work, **1.128** and **1.131** key intermediates

were readily obtained from threonolatone. These intermediates are easily functionalised for the synthesis of nucleosides and phosphoramidite precursors for the synthesis of TNA**.**

1.4.3.5 1′-phosphonate-4′-nucleobase tetrahydrofuran (*iso*-Threose) nucleoside phosphonates[84]

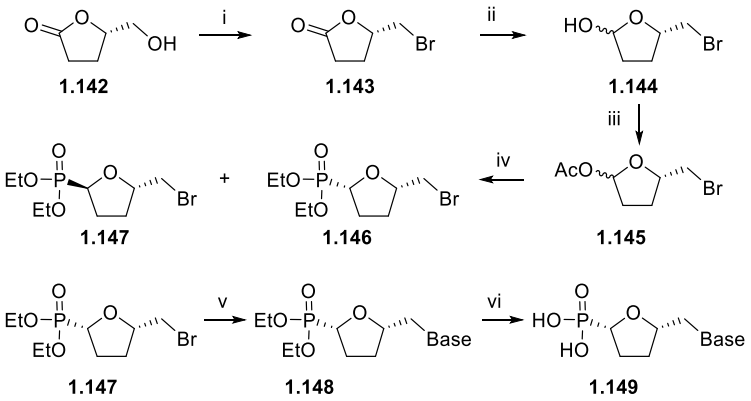
These are the *iso*-type analogues of threose nucleoside phosphonates, characterised by 1′-phosphonomethyl group and 3′-nucleobase (the position of nucleobase and phosphonate is interchanged as compared to that in threose nucleosides). Iso-threose nucleoside phosphonates are synthesised from silyl protected glycidol, followed by epoxide ring opening by 1,3-dithiane umpolung to obtain **1.139**. Mitsunobu coupling followed by silyl deprotection and *in situ* 1,3 dithiane cyclisation, methyl furanoside **1.140** was obtained. As usual, Lewis acid catalysed phosphonomethylation affords *iso*-threose nucleoside phosphonates. **(Scheme 1-15)**



Scheme 1-15. (i) (a) TBDMSCl, DMAP, Et_3N, CH_2Cl_2; (b) *n*-BuLi, 1,3-dithiane, DMPU, THF; (ii) (a) Base, DIAD, dioxane; (b) HCl, EtOH; (c) $PhI(OTFA)_2$, MeOH; (iii) (a) TMSOTf, protected phosphonomethanol; ((b) TMSBr, AcCN.

1.4.3.6 Adefovir derivative derived nucleoside phosphonates[85]

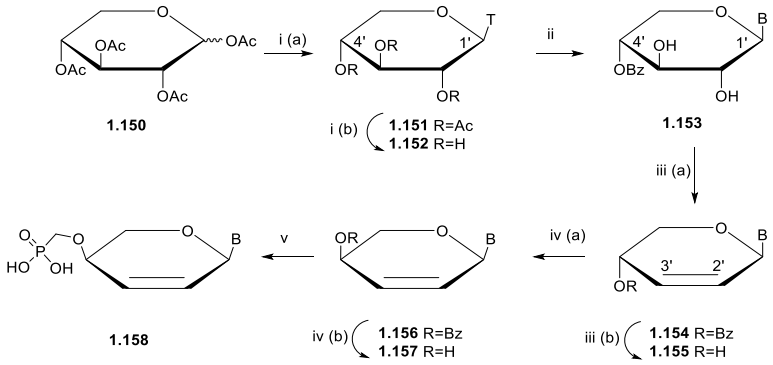
The motivation behind the design of this structure (cyclic analogue of Type III acyclic nucleoside, **Figure 1-2**) lies in improving the anti-HCMV activity and reduce cytotoxicity. This work was primarily done by Ngusen-Ba's group. From commercially available (-)-(5S)-5-hydroxymethyl-tetrahydrofuran-2-one, corresponding bromide **1.143** was obtained, followed by reduction and the formation of easily leaving acetate **1.145**. Lewis catalysed Arbuzov reaction provides the corresponding phosphonate as 1:1 diastereomeric mixture (**1.147** and **1.146**). The nucleobase was then smoothly introduced by halide displacement by 2-amino-6-chloropurine in the presence of cesium carbonate. **(Scheme 1-16)**



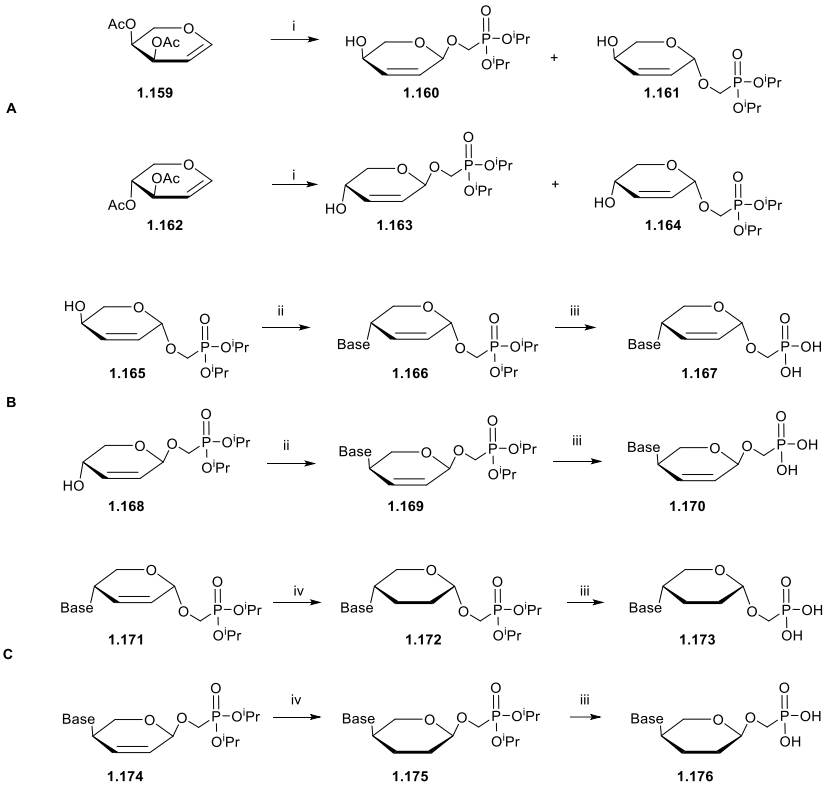
Scheme 1-16. *Reagents and conditions*: (i) CBr_4, Ph_3P, AcCN; (ii) DIBAL-H, toluene, -78 °C; (iii) Ac_2O, pyridine, DMAP; (iv) $TiCl_4$, $P(OEt)_3$, DCM, -30 °C; (v) Cs_2CO_3, nucleobase, DMF, 95 °C; (vi) TMSBr, DCM.

1.4.4 Pyranose-modified nucleoside phosphonates

The literature reporting the synthesis of pyranose-modified nucleoside phosphonates is limited. The first type of pyranosyl nucleoside phosphonate was synthesised in 1994 bearing nucleobase at the anomeric centre and phosphonate at 4′ position **(Scheme 1-17)**. The strategy involves TMSOTf catalysed reaction of peracetylated D-xylose and silylated nucleobase to afford *β*-D-pyranosyl nucleoside **1.151**. The nucleoside was then deacetylated and selectively protected at 4′ OH by treatment with Bu_2SnO followed by BzCl (**1.153)**. Using chlorodiphenylphosphine/iodine/imidazole and Zn vicinal diol was converted into corresponding olefin **1.154**. The 4′-OH configuration was inverted under mitsunobu conditions (**1.157**) and later the phoshonomethyl moiety was introduced (**1.158**).[45]

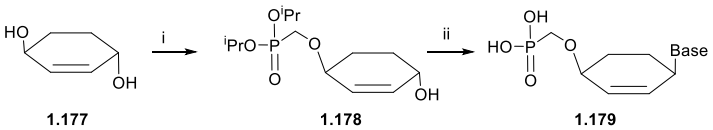


Scheme 1-17. *Reagents and conditions*: (i) (a) silylated nucleobase, TMSOTf; (b) NaOMe/MeOH; (ii) Bu_2SnO then BzCl; (iii) (a) chlorodiphenylphosphine/iodine/imidazole, toluene/AcCN, Zn, pyridine; (b) ammonia in methanol; (iv) (a) Benzoic acid, mitsunobu condition; (b) ammonia in methanol; (v) diisopropyl[(p-tolylsulfonyl)oxy] methanephosphonate, NaH; (vi) TMSBr, DCM.



Scheme 1-18. *Reagents and conditions*: (i) (a) $(^{i}PrO)_2POCH_2OH$, TMSOTf, AcCN; (b) ammonia in methanol; (ii) Nucleobase, Ph_3P, DEAD, dioxane; (iii) TMSBr, DCM; (iv) H_2, 10% Pd/C, MeOH.

In the second type of pyranosyl nucleoside phosphonate anomeric oxygen carries a phosphonate moiety and the nucleobase is at the 4′-position. Herdewijn and co-workers have reported the synthesis of phosphonate derivatives of tetrahydro- (**1.173** and **1.176**) and 2,5-cis-dihydro-2*H*-pyranyl nucleoside phosphonates (**1.167** and **1.170**) by Ferrier Rearrangement (**Scheme 1-18)**. Starting from glycal 3,4-di-*O*-acetyl-D-xylal, two isomeric glycosides were obtained in the ratio of 1:3, using diisopropyl ester of hydroxymethyphosphonic acid as an acceptor in the presence of Lewis acid. The nucleobase was then introduced under Mitsunobu condition. Palladium-catalysed hydrogenation of the dihydro-2*H*-pyranyl nucleosides afforded the corresponding tetrahydro-counterparts. Similarly, starting from 3,4-di-*O*-acetyl-L-arabinal, other isomers were obtained.[46] Further, their carbocyclic analogue (**Scheme 1-19**), cyclohexenyl nucleoside phosphonate were also synthesised starting from 1,4-trans-cyclohexenediol. Sequential key synthetic steps are monotritylation of **1.177**, nucleophilic phosphonomethylation and Mitsunobu condition mediated nucleobase introduction.[44]



Scheme 1-19. *Reagents and conditions*: (i) (a) TrCl, Et_3N, DMAP, CH_2Cl_2;; (b) $(^{i}PrO)_2POCH_2OTs$,NaH,

DMF; (c) 80% HOAc; (ii) (a)Nucleobase, Ph_3P, DEAD, dioxane; (b) TMSBr, DCM;

1.5 Objectives of the research

Although HNA oligonucleotides are resistant against cellular nucleases, their phosphorylated nucleoside precursors might be dephosphorylated by phosphatases in a cellular context. On the other hand, hexitol nucleosides are phosphorylated only in a herpes virus-infected cell by the viral thymidine kinase, and not in non-infected cells. These factors will thus limit their application in the field of antivirals and XNAs.

A major category of the approved nucleotide analogues are nucleoside phosphonates. The phosphonate moiety is a bioisostere of phosphate with similar polar and electronic properties, which allows it to function as an antiviral agent and as a part of artificial information system just like its phosphate counterpart. The improved physiological stability of phosphonate mimics will further lead to prolonged intracellular half-lives.

Based on the promising antiviral activity of hexitol nucleoside and properties of HNAs in the xenobiotic information system, we became interested in the synthesis and conformational studies of the 1′-phosphonate pentopyranose analogues of hexitol nucleoside phosphate **(Figure 1-11)**.

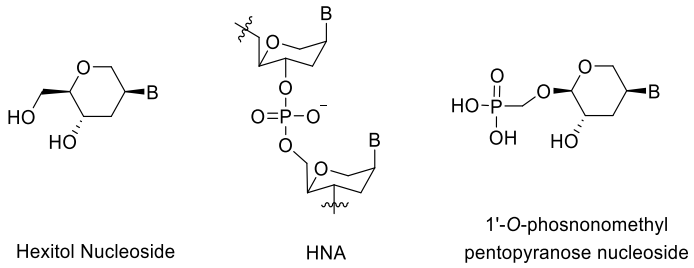


Figure 1-11. Structures of a hexitol nucleoside, HNA, and 6-membered nucleoside phosphonate

Further, in hexitol nucleosides nucleobase (at 2′) is axially oriented. This may or may not be the case in the proposed 1′-phosphonomethyl pentopyranose nucleoside **(Figure 1-12)**. Here probably the preferred conformation will be governed by avoiding the 1,3-diaxial steric interaction among bulky substituents and the steric interaction between the lone pairs of ring oxygen and anomeric oxygen and direction of their dipole moment.

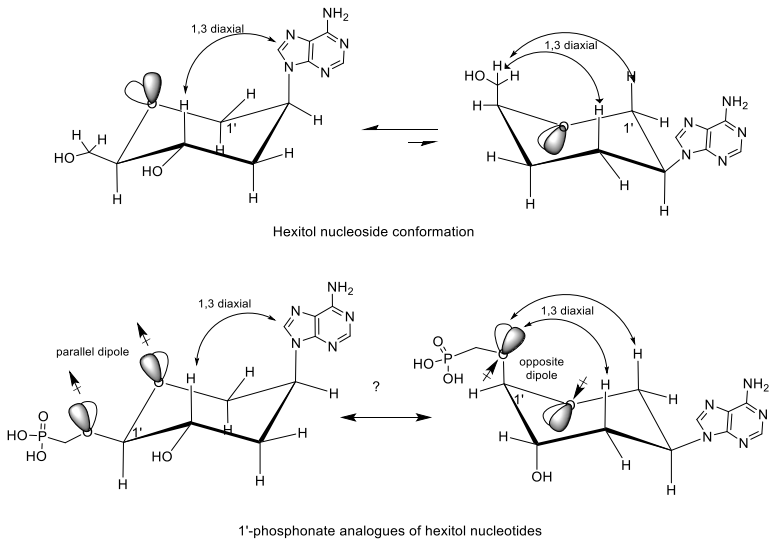


Figure 1-12. The chair conformation of hexitol nucleosides and 1′-phosphonate analogues of hexitol nucleotides

This doctoral thesis describes the exploration of a suitable synthetic route for the proposed 1′-*O*-phosphonomethyl pentopyranose nucleoside in **Chapter 2** and **Chapter 3**. **Chapter 2** describes the explored chemical methodology toward nucleoside phosphonate bearing thymine. **Chapter 3** describes the results obtained during the synthesis of analogue with adenine nucleobase. In **Chapter 4**, the expansion of the synthetic knowledge gained for the synthesis of analogues with other nucleobases, results of the anti-herpesvirus assay, and study as substrate of polymerases is presented.

1.6 References

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Chapter 2. Synthesis of Phosphonomethyl 3,4-dideoxy-4-(thymid-1-yl)-*α*-D-*threo*-pentopyranoside

Abstract: The synthesis of a pentopyranoside nucleoside bearing thymine with a phosphonate functionality at the 1′-anomeric oxygen has been performed. The key functionalised L-*glycero*-pentopyranose carbohydrate synthon was prepared from L-arabinose and further elaborated into the final six-membered ring nucleoside phosphonate via nucleobase incorporation and phosphonomethylation reactions. The stereochemical outcome of the Vorbrüggen type phosphonylation step utilised en route to the thymine analogue clearly suggests the absence of anchimeric assistance, as opposed to what is usually observed during nucleoside synthesis using protected furanose precursors.

2.1 Introduction

Several methods are available in the literature for the preparation of five-membered cyclic as well as acyclic nucleoside phosphonates (**Chapter 1, Section 1.5**), however, in the case of six-membered ring analogues, the synthesis is complicated by the greater conformational diversity arising from the pyranosyl moiety. A general method to generate these proposed 1′-phosphonomethylated pentopyranosides bearing 4′-nucleobase derivatives is not yet reported. The knowledge of the properties of these monomers/oligomers will add valuable data in the field of antivirals and oligonucleotides.

Based on the existing knowledge on synthetic strategies for acyclic, 3-, 4- and 5-membered sugar nucleoside phosphonates, the synthesis of pyranosyl nucleoside phosphonates could be planned by sequential insertion of nucleobase and later phosphonomethylation reaction (S_N2 reaction or by glycosylation reaction) or vice versa on key sugar synthon. Although the synthetic protocols could be designed based on existing analogous chemical synthetic pathways, the success or the feasibility of protocols is always governed by the actual chemical substrate undergoing the chemical transformation, which is always unpredictable imposing untoward synthetic challenges. The proposed pentopyranosyl phosphonosides carries three chiral centres, which could be easily derived starting from the chiral pool of commercially available carbohydrate or reduced sugar. The sugar is chemically modified to obtain the key sugar scaffolds and later easily elaborated into the designed nucleoside.

2.1.1 Incorporation of the nucleobase

As in the proposed nucleosides, the nucleobase is positioned at carbon 4 of the pentopyranose (**Figure 1-11**), similar protocols as those employed in the case of hexitol nucleic acid monomers, and acyclic/carbocyclic nucleotides are applicable. These include Mitsunobu coupling[1] of 4-positioned hydroxyl moiety, nucleophilic substitution[2-3] based on sulfonate leaving group (tosylate, mesylate or triflate), ring opening of epoxide[4] and construction of nucleobase from 4-positioned amino moiety[5-6] (**Figure 2-1**).

In the former literature references[7-8] for the synthesis of hexitol nucleoside bearing pyrimidine nucleobase, Mitsunobu coupling gave a better yield than a nucleophilic attack on sulfonate. Further, the regioselectivity between O^2- vs N^1-alkylation of the pyrimidine nucleobase also needs to be taken into consideration during the design of the synthetic protocol .[7-8]

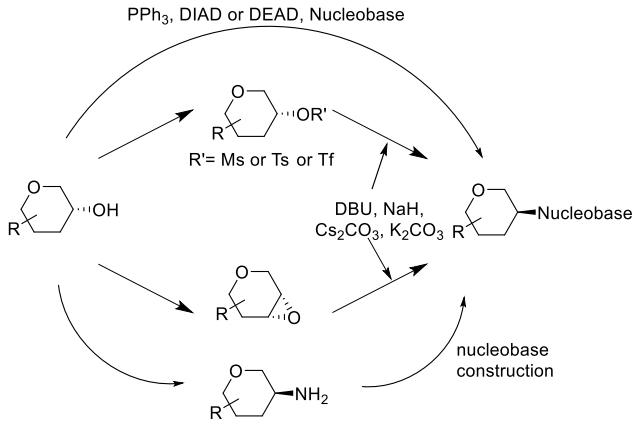


Figure 2-1. Possible strategies for the introduction of the nucleobase at a non-anomeric secondary carbon.

2.1.2 Phosphonomethanol glycosylation

The phosphonomethanol group in the proposed nucleoside phosphonates is located at the anomeric centre, and it can be introduced by selecting suitable glycosylation condition. In classical glycosylation reaction, anomeric oxygen is exchanged by aglycone (e.g. nucleobase, phosphonomethanol or another substituent) by replacement of activator in glycosyl donor. The methods[9] for the introduction of a substituent at the anomeric carbon can be arbitrarily divided into four groups, as summarised in **Figure 2-2**. The first method starts with pyranosyl halides and takes advantage of halophilicity of silver or mercury salts as a promotor for pyranosyl halide activator for the formation of the glycosidic bond. Alternatively in-situ sodium salt of aglycone can also serve as a nucleophile for the displacement of the anomeric halide. The second scaffold that can be used are glycals (**Scheme 1-19**, **Chapter 1**). Glycals are relatively more stable than halides. However, the reaction yield is usually low, and the stereocontrol of the reaction is poor.[10] In 1994, Castillon S. *et al.* developed selenium mediated synthesis of pyranose nucleoside form glycal via a selenoium

intermediate[11-12]. The third method is Vorbrüggen type reactions (silyl-Hilbert-Johnson glycosylation).[13] This procedure allows the use of the more stable 1-*O*-activated sugar as a glycosylation donor, for example acetyl, benzoyl, trichloroacetimidates (TCAI)[14] or N-phenyltrifluoroacetimidates (PTFAI)[15] as the leaving group. With suitable Lewis acids, under mild conditions, these donors will react with the nucleophilic acceptor. Besides these thioglycosides may also be considered as the glycosylation donor. Their use is well documented in the field of oligosaccharide synthesis. However, nowadays their use is avoided due to unpleasant smell and side reactions.[16] The advantage of thioglycosides is their stability under a wide range of reaction conditions, especially when multiple steps of protection and deprotection are required in the synthetic design. The most commonly used condition for the glycosylation of thioglycosides is *N*-iodosuccinimide /AgOTf.

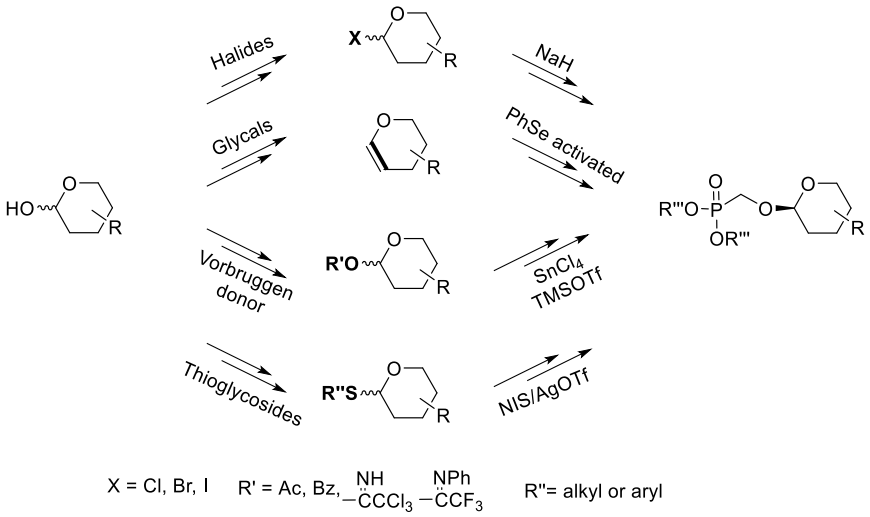


Figure 2-2. Possible strategies for the phosphonomethanol glycosylation

In the field of nucleoside chemistry, there is a vast amount of literature referring to phosphonylation of nucleosides on pseudo-sugar (acyclic, 4-membered or 5-membered), however there is only one report describing phosphonomethylation of a six-membered nucleoside via a Michael addition[10] on glycal, which is low yielding, and later stereocontrolled functionalization of the double bond is not feasible. Former literature on 6-membered sugar scaffold is well documented for the synthesis of pyranose nucleosides (**Section 1.2.7** in **Chapter** 1, without phosphate counterpart)[9] and the synthesis of oligosaccharides[11, 17]. However, the synthesis of the proposed 1-*O*-phosphonomethyl

pentopyranose will require the design and exploration of different sugar scaffolds and glycosylation strategies.

Conformationally, pyranosyl nucleosides are more rigid than furanose nucleosides.[18] The influence of the nucleobase ring on the conformation of the pyranose ring is not predictable.[18] The anchimeric assistance is a useful stereoselective strategy for the glycosylation of furanose sugars, which might also be suitable for pyranose sugars.

To conclude, the challenges for the synthesis of this type of nucleoside phosphonates include: search for a suitable commercially available sugar and modify it to the versatile scaffold; finding suitable chemical conditions for the introduction of nucleobase(s) on the non-anomeric carbon; and to screen suitable glycosylation donor and Lewis acid for the replacement of anomeric oxygen by phosphonomethanol.

In this context, we set out to investigate plausible synthetic pathways that would enable the construction of a suitably functionalized pentopyranoside scaffold as a key intermediate for further nucleobase incorporation and glycosylation reactions.

2.2 Results and discussion

The key disconnections in our retrosynthetic analysis of target D-*threo*-pentopyranoside nucleoside phosphonates **I** are shown in **Figure 2-3**. For both proposed routes A and B, it was envisaged that the synthesis of a suitably functionalized L-*glycero*-pentopyranose (**III)** or L-*erythro*-pentopyranose (**V**) synthon could begin from a five-carbon L-sugar. Thus, L-arabinose **2.1** was chosen as a common starting material.

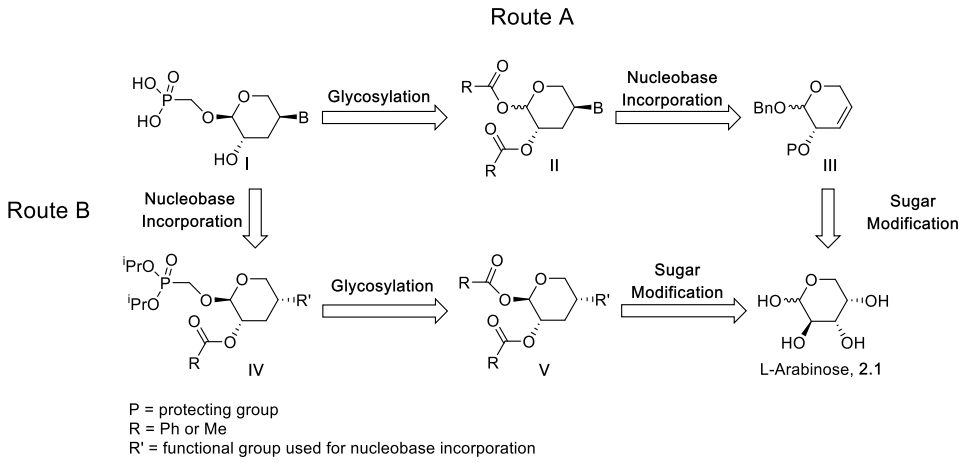
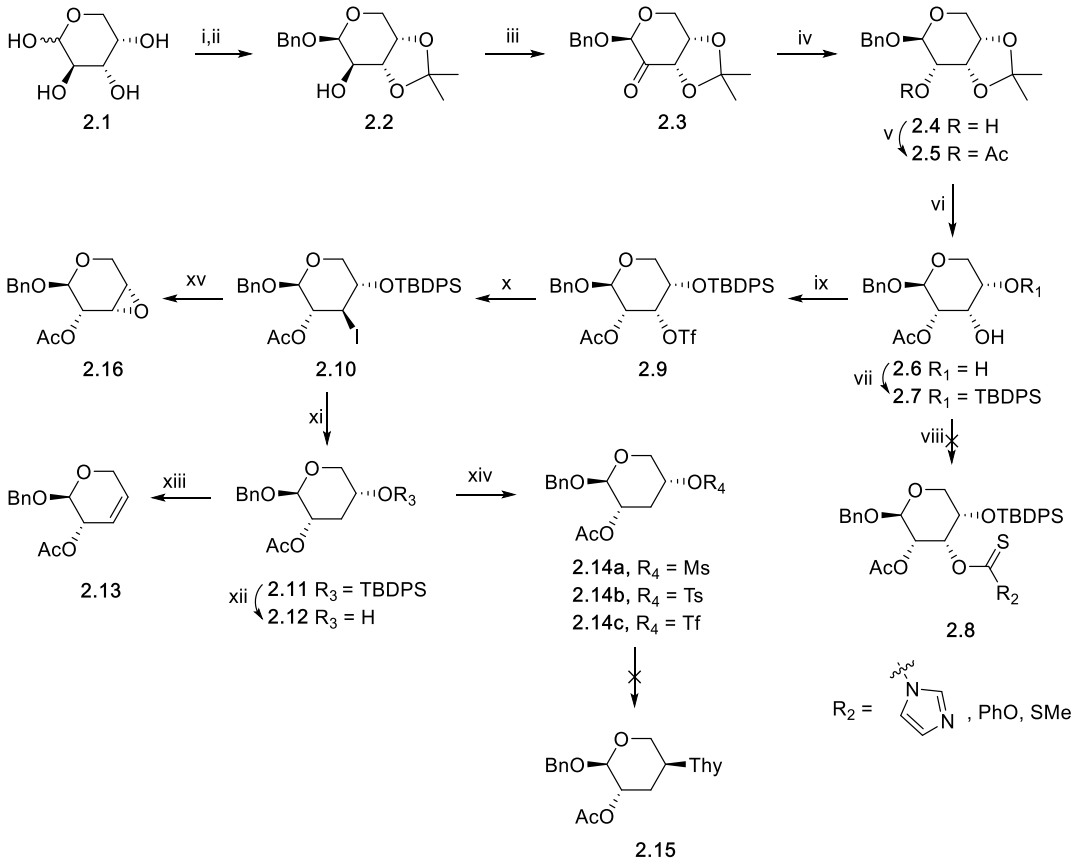


Figure 2-3. Retrosynthetic analysis of planned D-*threo*-pentopyranoside nucleoside phosphonates **I**.

Since the glycosidic linkage between the anomeric OH and the phosphonate functionality might be susceptible to epimerization during other synthetic manipulations, that need to be performed along the overall synthetic path, route A in **Figure 2-3**, which entails the introduction of this group towards the end of the synthetic sequence was initially pursued. Thus, benzyl isopropylidene arabinoside **2.2** was readily synthesised from L-arabinose **2.1** in two steps and used without the need of chromatographic purification (**Scheme 2-1**).[19] Oxidation of **2.2** with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) followed by direct reduction of the resultant 2-lactone **2.3** furnished 1,2-*trans* configured sugar **2.4**. Given that the glycosylation step for the introduction of the phosphonomethyl moiety usually requires the anchimeric assistance from a neighbouring 2-*O*-acyl group, the 2-hydroxyl of compound **2.4** was protected as an acetate, affording **2.5** in excellent yield. Initial attempts to hydrolyse the isopropylidene acetal of **2.5** in the presence of *p*-toluenesulfonic acid resulted in the concurrent cleavage of the 2-*O*-acetyl group. The use of 80% aq. acetic acid was found to allow for selective cleavage of the acetal functionality to afford diol **2.6**. The next step in the synthesis entailed the regioselective protection of the equatorially oriented 4-hydroxy group. However, only an inseparable mixture of products was obtained in the presence of TBDMSCl due to concurrent silylation at different positions of the sugar ring. To minimise 3-*O*-silylation, a bulkier reagent such as TBDPSCl was employed, which successfully led to the highly predominant formation of the desired compound. Subsequently, 3-hydroxyglycoside **2.7** was subjected to Barton-McCombie deoxygenation. However, no desired product (**2.8**) was formed

upon reaction with a variety of sulfur reagents such as CS_2 MeI, 1,1′-dithiocarbonydiimidazole (TCDI), and *O*-phenyl chlorothionoformate. This result may be attributed to the axial orientation as well as the increased steric hindrance of the bulky 4-OTBDPS moiety, which might prevent the formation of the corresponding *O*-thiocarbonyl intermediate **2.8**. Alternatively, compound **2.7** was converted to 3-*O*-triflate derivative **2.9**, which underwent successive S_N2 iodination and radical reduction to furnish fully protected sugar synthon **2.11**. Later, desilylation at the 4-position afforded coupling partner **2.12,** required for the construction of 1′-*O*-phosphonomethyl-D-*threo*-pentapyranosides carrying a nucleobase at the 4′-position.



Scheme 2-1. Initial routes for the preparation of functionalized L-*glycero*-pentopyranoside scaffolds **2.12**, **2.14a-c**, and **2.16**. *Reagents, conditions and yields:* (i) BnOH, AcCl, 75%; (ii) DMP, CSA, DMF, 96%; (iii) TEMPO, NaClO, KBr, DCM, H_2O, 90%; (iv) $NaBH_4$, CH_3OH, 90%; (v) Ac_2O, Et_3N, DMAP, DCM, 93%; (vi) 80% aq. AcOH, 83%; (vii) TBDPSCl, imidazole, DCM, 71%; (viii) TCDI or PhOC(S)Cl, base, with or without $(Bu_3Sn)_2O$ as an activator; or CS_2 , MeI, NaH; (ix) Tf_2O, pyridine, DCM, 95%; (x) NaI, DMF, 96%; (xi) Bu_3SnH, AIBN, toluene, 94%; (xii) TBAF, THF, 87%; (xiii) thymine or N^3-

benzoylthymine, PPH_3, DIAD or DEAD, THF or dioxane, rt; (xiv) MsCl or TsCl, Et_3N/pyridine, DCM; (xv) TBAF, THF, 95%.

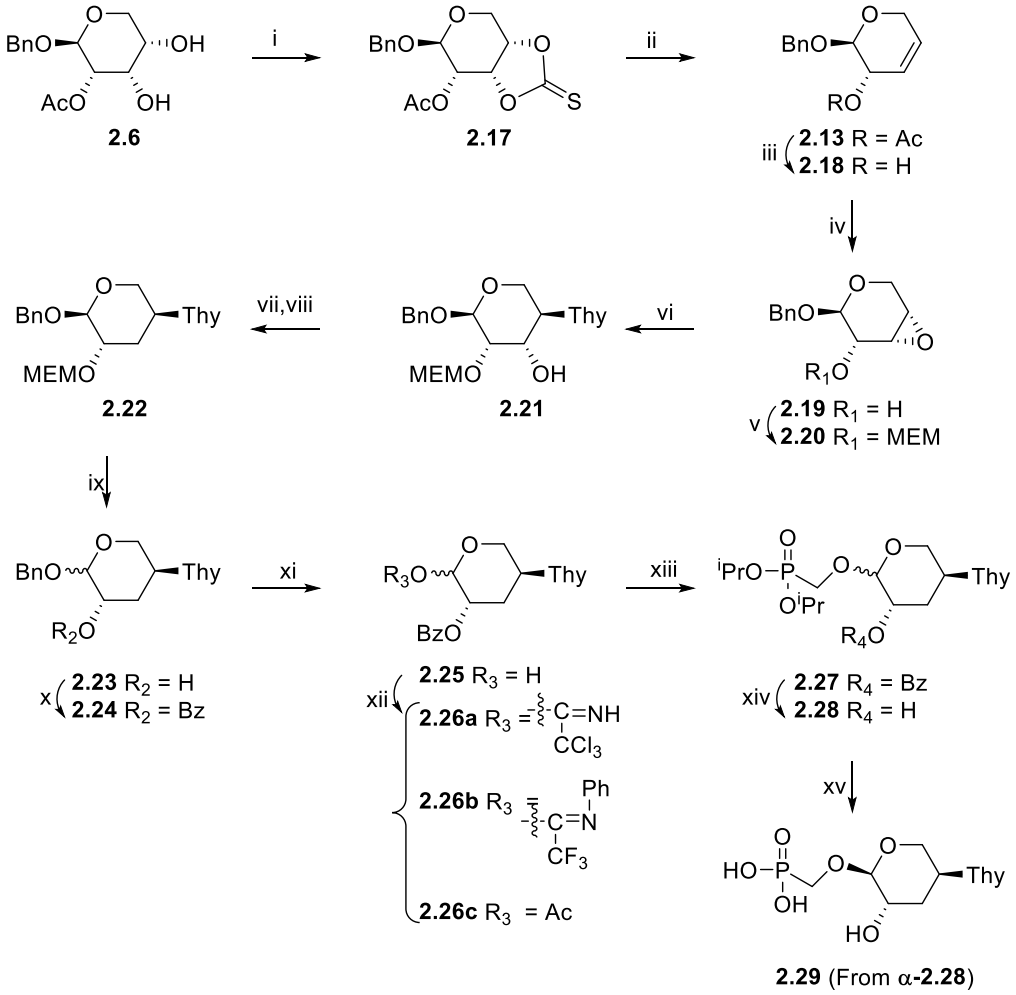
All attempts to introduce thymine or N^3-benzoyl protected thymine as nucleobase by reacting **2.12** under Mitsunobu conditions[8] resulted in the formation of elimination product **2.13** rather than the desired nucleoside, most likely due to the axial orientation of the 4-hydroxyl group (for detailed conditions, see Experimental Section). Further efforts to achieve the nucleophilic substitution of 4-OTs and 4-OMs substituted analogues **2.14a, b** by thymine in the presence of different bases such as DBU, NaH, Cs_2CO_3, and K_2CO_3 alone or in combination with a variety of crown ethers were also unsuccessful. Moreover, the conversion of the 4-OH of **2.12** into a better leaving group using triflic anhydride did not provide the expected activated product, but rather led to a 3,4-elimination reaction (**2.13**) and nucleophilic substitution with pyridine at the 4-position. Therefore, another option for the introduction of the nucleobase was investigated focusing on the preparation of a 3,4-epoxy sugar, which could in principle undergo a ring-opening reaction upon the nucleophilic attack of heterocyclic nitrogen. Initially, the envisaged epoxide **2.16** was directly obtained in one step from 3-iodo derivative **2.10** upon removal of the 4-*O*-silyl protecting group (**Scheme 2-1**). It was found that the basic conditions, due to the use of the deprotecting reagent TBAF, favoured efficient epoxide formation once the 4-hydroxyl group was unmasked. However, when epoxide **2.16** was reacted with thymine under basic (DBU) and high temperature (110 °C) conditions, conversion to the desired nucleoside occurred concomitantly with the migration and hydrolysis of the 2′-acetyl group leading to a complex mixture of products.

This problem was addressed by adopting an alternative synthetic strategy that would allow generating a differently substituted key sugar intermediate with higher stability under base-sugar coupling conditions (**Scheme 2-2**). In particular, a Corey-Winter olefination was employed to install the alkene at the 3,4-position to obtain the L-*glycero*-pentopyranose ring. Treatment of **2.6** with TCDI afforded cyclic thionocarbonate **2.17**, which subsequently underwent a *syn*-elimination in the presence of triethyl phosphite to furnish 3,4-unsaturated pentose **2.13** in good yield (84% over two steps). It should be noted that in this case, the standard use of trimethyl phosphite was ineffective to promote the desulfurisation step. Epoxidation of **2.13** using either *m*-CPBA or H_2O_2

did not proceed stereoselectively while resulting in a mixture of diastereomeric epoxides. Hydrolysis of the 2-*O*-acetyl group of **2.13** prior to epoxidation was, therefore, necessary, in order for the corresponding free hydroxyl moiety to establish a hydrogen bonding interaction with the oxidising agent that allowed for the reaction to selectively take place from the same side of the 2-hydroxy group. Thus, epoxide **2.19** was obtained in good yield and successively subjected to MEM protection, epoxide ring opening with thymine, and Barton deoxygenation to yield 4-thymidylated sugar **2.22**. The MEM protecting group was then removed under acidic conditions affording a 15:1 mixture of α- and β-anomers **2.23a** and **2.23b**, which were characterised after column chromatography separation, and further benzoylated at the 2′ position to furnish **2.24a** and **2.24b** in an overall 90% yield. While the Pd/C-catalysed hydrogenolysis of the anomeric OBn functionality proceeded sluggishly in either methanol or acetic acid (3 days), a significant enhancement of the reaction rate was observed when the debenzylation was conducted in THF as solvent (8 h). Later, we found that treatment of a mixture of **2.24a** and **2.24b** under transfer hydrogenation conditions (cyclohexene in ethanol) produced the desired glycone **2.25** in 90% yield.

For the final glycosylation step, glycone **2.25** was first activated at the anomeric position either as a *N*-phenyltrifluoroacetimidate (PTFAI, **2.26a**), trichloroacetimidate (TCAI, **2.26b**), or acetate (**2.26c**), while diisopropyl phosphonomethanol was used as glycosyl acceptor. A variety of standard conditions for the formation of the O-C-P bond were screened and are summarised in **Table 2-1**. Glycosylated product **2.27** could not be isolated as a pure compound by silica gel column chromatography at this stage due to diisopropylphosphonomethanol contamination, thus it was used as such in the following debenzoylation step, and product characterisation was carried out at the level of compound **2.28**. Surprisingly, glycosyl donors **2.26a** and **2.26b** yielded either starting material **2.25** (entries 1 and 3, **Table 2-1**) or a product with undesired stereochemistry at the anomeric centre (**β-2.28**, entries 2 and 4, **Table 2-1**). The formation of **β-2.28** suggests the non-participation of the 2′-*O*-acyl moiety, with the aglycone acceptor approaching the oxocarbenium intermediate from the β-face to form the 1,4-*anti* conformer. In contrast, 1′-acetylated nucleoside donor **2.26c** furnished the desired **α-2.28** isomer as major compound (**α-2.28**:**β-2.28** = 2:1) in the presence of a strong Lewis acid (TMSOTf) at room temperature. It can be postulated that at low

temperature (-78 ºC) the presence of aglycone moieties with an increased leaving group ability as those in **2.26a** and **2.26b** support the formation of product **β-2.28** as a result of the absence of neighbouring group assistance, whereas in the case of **2.26c** bearing a poorer leaving group the reaction favours isomer **α-2.28**. Final removal of the diisopropyl ester functionalities under standard conditions afforded nucleoside phosphonate **2.29** in 40% yield (0.73% overall yield).

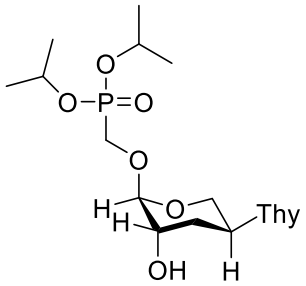


Scheme 2-2. Synthesis of thymine *α*-D-*threo*-pentopyranoside nucleoside phosphonate **2.29**. *Reagents, conditions and yields*: (i) TCDI, DCM, 83%; (ii) triethylphosphite, 140 °C, 85%; (iii) MeONa, methanol, 91%; (iv) *m*-CPBA, DCM, 89%; (v) 2-Methoxyethoxymethyl chloride (MEMCl), DCM, 91%; (vi) thymine, DBU, DMF, 85%; (vii) TCDI, DMAP, DCM; (viii) Bu_3SnH, AIBN, toluene, 83% over two steps; (ix) 1M HCl in dioxane, 75% for ***α*-2.23** and 5% for ***β*-2.23**; (x) BzCl, pyridine, 0 °C, 92%; (xi) Pd/C, cyclohexene, ethanol, 88%; (xii) Ac_2O, pyridine, 85%; (xiii) TMSOTf, hydroxymethyl phosphonic acid

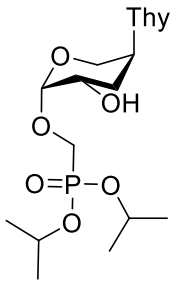
diisopropyl ester, CH_2Cl_2; (xiv) 7 M NH_3 in methanol, 20% over two steps for ***α*-28**; (xv) TMSBr, 2,6-lutidine, acetonitrile, 40%.

Table 2-1. Optimisation of the Glycosylation Step.

entry	glycosyl donor	Lewis acid and T (°C)	product (yield%)
1	**2.26a**[b]	TMSOTf, -78 °C	**2.25**[c]
2		$BF_3.Et_2O$, -78 °C	**β-2.28** (30%)[a]
3	**2.26b**[b]	TMSOTf, -78 °C	**2.25**[c]
4		$BF_3.Et_2O$, -78 °C	**β-2.28** (40%)[a]
5	**2.26c**	TMSOTf, rt	**α-2.28**/**β-2.28** (20/10%)[a]
6		$BF_3.Et_2O$, rt	**β-2.28** (traces)[a]



α-2.28



β-2.28

[a]Yields refer to the glycosylated product isolated after the following debenzoylation step. [b]Experimental procedures for the phosphonylation of **2.26a** and **2.26b** are detailed in the Experimental Information. [c]Yield was not determined since it was obtained as crude and not able to purified.

2.3 Conclusion

In summary, D-*threo*-pentopyranoside nucleoside analogue bearing a phosphonate functionality rather than a nucleobase (i.e., in natural nucleosides) at the anomeric centre has been synthesised

starting from L-arabinose. Different synthetic strategies were explored. Specifically, stereocontrolled routes to suitably functionalized key carbohydrate scaffolds, an epoxide, have been established and optimised. Such intermediates were further assembled and elaborated into the final six-membered ring nucleosides using the initial insertion of the nucleobase on the glycone moiety followed by phosphonomethylation. Notably, when thymine containing pentopyranose glycosyl donor was subjected to Vorbrüggen glycosylation conditions, the reaction proceeded without neighbouring group participation, in contrast to the usual behaviour of protected furanose nucleoside precursors. Details about the NMR data will be discussed in **Chapter 4.**

2.4 Experimental section

General Information. All reagents and solvents were purchased from commercial sources and used as obtained. Moisture sensitive reactions were performed using oven-dried glassware under a nitrogen or argon atmosphere. NMR spectra were recorded on a Bruker Advance 300 MHz (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz; ^{31}P NMR, 121 MHz), 500 MHz (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz; ^{31}P NMR, 202 MHz), or 600 MHz (^{1}H NMR, 600 MHz; ^{13}C NMR, 150 MHz) spectrometer with tetramethylsilane as internal standard or referenced to the residual solvent signal, and 85% H_3PO_4 for ^{31}P NMR. All intermediates and final compounds were characterised by using 2D NMR (^{1}H-COSY, HSQC, NOESY, and HMBC) spectroscopic techniques. For NMR assignment of sugar protons and carbons, prime numbering is used. High-resolution mass spectra [HRMS (ESI)] were obtained on a quadruple orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min, and spectra were obtained in positive (or in negative) ionisation mode with a resolution of 15000 (fwhm) using leucine enkephalin as lock mass. Precoated aluminium sheets (254 nm) were used for TLC. Products were purified by column chromatography on silica gel (60 Å, 0.035−0.070 mm, Acros Organics). Preparative RP-HPLC purifications were carried out on a Phenomenex Gemini 110A column (C18, 10 μm, 21.2 mm × 250 mm) using CH_3CN/0.05 M TEAB buffer or H_2O/ CH_3CN as eluent gradient.

Benzyl 2-*O*-acetyl-3,4-*O*-isopropylidene-*β*-L-ribopyranoside (2.5). Benzyl 3,4-*O*-isopropylidene-*β*-L-ribopyranoside **2.4** was prepared in 4 steps from L-arabinose **2.1** following a

literature procedure that did not require column chromatography purification.[19] Next, to a stirred solution of **2.4** (34.7 g, 123.8 mmol), DMAP (0.76 g, 6 mmol) and triethylamine (69.0 mL, 495.2 mmol) in DCM (500 mL), acetic anhydride (23.4 mL, 247.6 mmol) was added dropwise at 0 ºC. The reaction mixture was slowly warmed to room temperature and stirred for 2 h. After completion of the reaction, the mixture was cooled to 0 ºC and quenched with saturated aq. $NaHCO_3$. The organic layer was washed with brine (200 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (10:1, hexane/EtOAc, R_f = 0.3) to afford **2.5** (37.3 g, 93% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5H, Ph), 5.00 (dd, *J* = 6.1, 3.3 Hz, 1H, H-2), 4.93 (d, *J* = 6.2 Hz, 1H, H-1), 4.80 (d, *J* = 12.3 Hz, 1H, C*H*$_2$Ph), 4.80 (d, *J* = 12.3 Hz, 1H, C*H*$_2$Ph), 4.57 (dd, *J* = 7.0, 3.2 Hz, 1H, H-3), 4.56 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.32 (dt, *J* = 7.0, 1.9 Hz, 1H, H-4), 3.87 (dd, *J* = 13.1, 2.5 Hz, 1H, H-5), 3.71 (dd, *J* = 13.1, 1.6 Hz, 1H, H-5′), 2.13 (s, 3H, C*H*$_3$CO), 1.53 (s, 3H, [C(C*H*$_3$)$_2$]), 1.33 (s, 3H, [C(C*H*$_3$)$_2$]); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (CH_3*C*O), 137.6, 128.5, 127.8, 127.7 (Ph), 110.4 [*C*(CH$_3$)$_2$], 96.9 (C-1), 73.6 (C-2), 71.8 (C*H*$_2$Ph), 70.3 (C-3), 69.5 (C-4), 62.5 (C-5), 26.5 [C(*C*H$_3$)$_2$], 25.3 [C(*C*H$_3$)$_2$], 21.2 (*C*H$_3$CO); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{17}H_{22}O_6Na$ 345.1309; Found 345.1308.

Benzyl 2-*O*-acetyl-*β*-L-ribopyranoside (2.6). A solution of **2.5** (20.0 g, 62.04 mmol) in 80% aq. acetic acid (400 mL) was stirred at 60 ºC for 2 h. After removal of all the volatiles under reduced pressure, the residue was partitioned between water (200 mL) and EtOAc (300 mL). The water layer was extracted with EtOAc (1 × 200 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (100:5 DCM/methanol, R_f = 0.25) to afford **2.6** (14.6 g, 83%) as a white solid. ^{1}H NMR (500 MHz, DMSO) δ 7.41–7.18 (m, 5H, Ph), 5.10 (d, *J* = 4.7 Hz, 1H, OH-3), 4.76 (d, *J* = 7.0 Hz, 1H, H-1), 4.73 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J* = 7.0 Hz, 1H, OH-4), 4.55 (dd, *J* = 6.8, 2.9 Hz, 1H, H-2), 4.54 (d, *J* = 12.3 Hz, 1H, C*H*$_2$Ph), 3.96 (dt, *J* = 4.3, 2.6 Hz, 1H, H-3), 3.69–3.55 (m, 3H, H-4, H-5 and H-5′), 2.11 (s, 3H, C*H*$_3$CO); ^{13}C NMR (125 MHz, DMSO) δ 169.6 (CH_3*C*O), 137.8, 128.3, 127.5, 127.4 (Ph), 97.1 (C-1), 72.0 (C-2), 69.5 (*C*H$_2$Ph), 67.8 (C-3), 66.7 (C-4), 63.5 (C-5), 20.9 (*C*H$_3$CO); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{14}H_{18}O_6Na$

305.0996; Found 305.0998.

Benzyl 2-*O*-acetyl-4-*O*-*tert*-butyldiphenylsilyl-*β*-L-ribopyranoside (2.7). To a stirred solution of **2.6** (10.0 g, 35.41 mmol) and imidazole (6.03 g, 88.5 mmol) in dry acetonitrile (300 mL) at 0 °C, a solution of *tert*-butyldiphenylchlorosilane (10.1 mL, 38.9 mmol) in anhydrous acetonitrile (50 mL) was added dropwise. The reaction mixture was stirred at room temperature for 8 h. After removal of all the volatiles under reduced pressure, the resulting residue was partitioned between water (100 mL) and EtOAc (200 mL). The water layer was extracted with EtOAc (1 × 200 mL). The combined organic layer was washed with brine (150 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (10:1 hexane/EtOAc, R_f = 0.3) to afford **2.7** (13.0 g, 71% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75–7.62 (m, 4H, Ph), 7.47–7.36 (m, 6H, Ph), 7.32–7.22 (m, 5H, Ph), 4.92 (d, *J* = 5.2 Hz, 1H, H-1), 4.82 (dd, *J* = 5.1, 3.6 Hz, 1H, H-2), 4.71 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.50 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.10 (t, *J* = 3.3 Hz, 1H, H-3), 3.92 (dt, *J* = 5.9, 3.9 Hz, 1H, H-4), 3.63–3.49 (m, 2H, H-5 and H-5′), 2.12 (s, 3H, C*H*$_3$CO), 1.09 (s, 9H, [C(C*H*$_3$)$_3$]); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4 (CH_3*C*O), 137.3, 135.8, 135.75, 133.2, 132.5, 130.3, 130.2, 128.5, 128.1, 128.0, 127.8 , 127.6 (Ph), 97.3 (C-1), 71.3 (C-2), 70.1 (*C*H$_2$Ph), 69.1 (C-4), 67.6 (C-3), 63.1 (C-5), 27.0 [C(*C*H$_3$)$_3$], 21.2 (*C*H$_3$CO), 19.4 [*C*(CH$_3$)$_3$]; HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{30}H_{36}O_6SiNa$ 543.2174; Found 543.2167.

Experimental procedure for the synthesis of thiocarbonyl compound 2.8. When using TCDI and PhOC(S)Cl as reagents: To a solution of **7** (1 eq) and base (2.0 eq triethylamine, pyridine, or DIPEA) in anhydrous DCM or chloroform TCDI or PhOC(S)Cl (2 eq) was slowly added at 0 ºC. The reaction was stirred either at room temperature, 40 ºC (for DCM), or 65 ºC (for chloroform) under a nitrogen atmosphere. No product was formed, as confirmed by TLC or NMR analysis of the crude reaction mixture.

When using $(Bu_3Sn)_2O$ as an activator, this reagent was added before the addition of TCDI or PhOC(S)Cl , and the mixture was stirred either at room temperature or under reflux. However, TLC did not show the formation of a tin intermediate.

When using CS_2 + MeI as reagents: To a stirred solution of **2.7** (1 eq) and CS_2 (5 eq) in anhydrous

DCM at -20 ºC, NaH (1.0 eq) was slowly added. The reaction was stirred at room temperature and monitored by TLC. Acetyl migration and hydrolysis were observed in this case. Thus this route was discontinued.

Benzyl 2-*O*-acetyl-4-*O*-*tert*-butyldiphenylsilyl-3-*O*-trifluoromethanesulfonyl-*β*-L-ribopyranoside (2.9). To a stirred solution of **2.7** (10.0 g, 19.22 mmol) and pyridine (4.8 mL, 57.7 mmol) in dry DCM (300 mL) at 0 °C, triflic anhydride (6.5 mL, 38.44 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was cooled to 0 ºC and quenched with saturated aq. $NaHCO_3$. The reaction mixture was further washed with saturated aq. $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (silica gel was presoaked with 0.5% of triethylamine in hexane; chromatography was performed using 15:1 hexane/EtOAc, R_f = 0.2) to afford **2.9** (11.9 g, 95% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79–7.58 (m, 4H, Ph), 7.54–7.15 (m, 11H, Ph), 5.31 (dd, *J* = 2.4, 2.2 Hz, 1H, H-2), 4.82 (dd, *J* = 7.3, 2.4 Hz, 1H, H-3), 4.74 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.72 (d, *J* = 7.2 Hz, 1H, H-1),4.50 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 3.94 (ddd, *J* = 9.4, 5.8, 2.3 Hz, 1H, H-4), 3.56 (dd, *J* = 11.6, 9.5 Hz, 1H, H-5), 3.39 (dd, *J* = 11.6, 4.8 Hz, 1H, H-5′), 2.08 (s, 3H, C*H*$_3$CO), 1.08 (s, 9H, [C(C*H*$_3$)$_3$]); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6 (CH_3*C*O), 135.9, 135.85, 133.0, 130.6, 130.5, 128.6, 128.3, 128.1, 127.7 (Ph), 120.8 (*C*F$_3$), 97.2 (C-1), 86.0 (C-3), 70.9 (C-2), 68.7 (*C*H$_2$Ph), 67.3 (C-4), 63.7 (C-5), 26.8 [C(*C*H$_3$)$_3$], 20.7 (*C*H$_3$CO), 19.2 [*C*(CH$_3$)$_3$]; HRMS (ESI-TOF) m/z: $[M+NH_4]^+$ calcd for $C_{31}H_{39}NF_3O_8SSi$ 670.2118; Found 670.2119.

Benzyl 2-*O*-acetyl-3-deoxy-3-iodo-4-*O*-*tert*-butyldiphenylsilyl-*β*-L-xylopyranoside (2.10). A suspension of **2.9** (4.00 g, 6.12 mmol) and NaI (2.76 g, 18.38 mmol) in THF (70 mL) was stirred at room temperature for 12 h. After removal of all the volatiles under reduced pressure, the resulting residue was separated between EtOAc (100 mL) and saturated aq. $NaHCO_3$ (50 mL). The water layer was extracted with EtOAc (50 mL) once. The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (15:1 hexane/EtOAc) to afford **2.10** (3.71 g, 96% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94–7.62 (m, 4H, Ph), 7.56–7.10 (m, 11H, Ph), 5.06 (dd, *J* =

10.5, 7.2 Hz, 1H, H-2), 4.76 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.49 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.29 (d, *J* = 7.2 Hz, 1H, H-1), 4.14–3.87 (m, 2H, H-3 and H-4), 3.64 (dd, *J* = 11.6, 4.4 Hz, 1H, H-5), 3.28–3.03 (m, 1H, , H-5′), 2.07 (s, 3H, C*H*$_3$CO), 1.09 (s, 9H, [C(C*H*$_3$)$_3$]); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0 (CH_3*C*O), 136.3, 135.9, 130.3, 130.1, 128.5, 128.0, 127.9, 127.8, 127.7 (Ph), 100.4 (C-1), 73.7 (C-2), 73.1 (C-4), 70.4 (*C*H_2Ph), 68.2 (C-5), 33.5 (C-3), 27.2 [C(*C*H$_3$)$_3$], 21.0 (*C*H_3CO), 19.7 [*C*(CH$_3$)$_3$]; HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{30}H_{35}IO_5SiNa$ 653.1193; Found 653.1196.

Benzyl 2-*O*-acetyl-3-deoxy-4-*O*-*tert*-butyldiphenylsilyl-*β*-L-*erythro*-pentopyranoside (2.11). To a stirred solution of **2.10** (2.00 g, 3.17 mmol) and AIBN (0.26 g, 1.59 mmol) in toluene (100 mL), tributyltin hydride (1.7 mL, 6.34 mmol) was added dropwise. The reaction mixture was heated at 110 °C for 1 h. After removal of all the volatiles under reduced pressure, the resulting crude residue was purified by column chromatography on silica gel (15:1 hexane/EtOAc, R_f = 0.2) to afford **2.11** (1.5 g, 94% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70–7.60 (m, 4H, Ph), 7.45–7.34 (m, 6H, Ph), 7.33–7.25 (m, 5H, Ph), 4.75 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.72–4.65 (m, 1H, H-2), 4.56 (d, *J* = 5.3 Hz, 1H, H-1), 4.52 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 3.88–3.78 (m, 1H, H-4), 3.74 (ddd, *J* = 11.4, 3.7, 1.2 Hz, 1H, H-5a), 3.32 (dd, *J* = 11.4, 6.3 Hz, 1H, H-5e), 2.21 (dtd, *J* = 13.2, 4.6,1.2 Hz, 1H, H-3a), 2.04 (s, 3H, C*H*$_3$CO), 1.77 (dt, *J* = 13.2, 7.6 Hz, 1H, H-3e), 1.06 (s, 9H, [C(C*H*$_3$)$_3$]); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2 (CH_3*C*O), 137.6, 135.8, 133.9, 133.6, 123.0, 128.5, 127.9, 127.8 (Ph), 99.2 (C-1), 69.7 (*C*H_2Ph), 68.6 (C-2), 67.4 (C-5), 65.5 (C-4), 34.3 (C-3), 27.0 [C(*C*H$_3$)$_3$], 21.3 (*C*H_3CO), 19.3 [*C*(CH$_3$)$_3$]; HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{30}H_{36}O_5SiNa$ 527.2224; Found 527.2216.

Benzyl 2-*O*-acetyl-3-deoxy-*β*-L-*erythro*-pentopyranoside (2.12). To a stirred solution of **2.11** (1.00 g, 1.98 mmol) in THF (40 mL) at 0 °C, TBAF (1 M in THF, 3.96 mL, 3.96 mmol) was added dropwise. The reaction mixture was slowly warmed to room temperature and stirred for 5 h. After completion of the reaction, all volatiles were removed *in vacuo*. The resulting residue was separated between EtOAc (40 mL) and saturated aq. $NaHCO_3$ (20 mL). The water layer was extracted with EtOAc (30 mL) once. The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 5:1, v/v; 1:1, v/v) affording **2.12** (0.46 g, 87%) as a colorless oil. ^{1}H

NMR (600 MHz, $CDCl_3$) δ 7.41–7.30 (m, 5H, Ph), 4.90 (dt, *J* = 3.2, 2.3 Hz, 1H, H-2), 4.78 (bs, 1H, H-1e), 4.77 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 4.56 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 4.01 (dd, *J* = 12.0, 1.8 Hz, 1H, H-5a), 3.75 (dq, *J* = 2.7, 2.4 Hz, 1H, H-4e), 3.67 (dt, *J* = 11.9, 2.3 Hz, 1H, H-5e), 2.22 (td, *J* = 15.0, 3.7 Hz, 1H, H-3a), 2.1 (s, 3H, C*H*$_3$CO), 1.96 (ddtd, *J* = 15.0, 3.2, 2.4, 0.93 Hz, 1H, H-3e); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.5 (CH_3*C*O), 137.0, 128.5, 127.9, 127.8 (Ph), 95.8 (C-1), 69.3 (*C*H_2Ph), 68.5 (C-2), 64.5 (C-5), 64.2 (C-4), 29.3 (C-3), 21.2 (*C*H_3CO); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{14}H_{19}O_5Na$ 289.1046; Found 289.1045.

Benzyl 2-O-acetyl-*β*-l-*glycero*-pent-3-enopyranoside (2.13). This compound was first obtained as a side product during the Mitsunobu reaction (see **Scheme 2-1**), and it was later synthesised by using the triethyl phosphite method.

Mitsunobu conditions: To a stirred suspension of **2.12** (1 eq), thymine or *N*^{3}-Bz-thymine (2 eq), and triphenylphosphine (2.2 eq) in anhydrous dioxane or THF, a solution of DEAD or DIAD in 2 mL of THF or dioxane was slowly added. The mixture was stirred until the disappearance of starting material **2.12**. After removal of all the volatiles under reduced pressure, the resulting residue was separated between EtOAc and saturated aq. $NaHCO_3$. The water layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using 15:1(v/v) hexane/ EtOAc. The reaction was conducted at different temperatures (-78, -20, 0 °C, and rt), however only the elimination product **2.13** was formed instead of the desired nucleoside.

Triethyl phosphite conditions: A stirring solution of **2.17** (8.00 g, 24.7 mmol) in triethylphosphite (250 mL) was maintained at 140 ºC for 14 h and the reaction was monitored by TLC for completion. After removal of all the volatiles under reduced pressure, the crude residue was subjected to column chromatography on silica gel (15:1 hexane/EtOAc) to give **2.13** (5.20 g, 85% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.27 (m, 5H, Ph), 6.08 (td, J = 10.3, 2.4 Hz, 1H, H-3), 5.95–5.71 (m, 1H, H-4), 5.11–4.94 (m, 1H, H-2), 4.90 (bs, 1H, H-1), 4.81 (d, J = 12.0 Hz, 1H, CH2Ph), 4.62 (d, J = 12.0 Hz, 1H, CH2Ph), 4.37–4.09 (m, 2H, H-5 and H-5′), 2.07 (s, 3H, CH3CO); 13C NMR (75 MHz, CDCl3) δ 170.4 (CH3CO), 137.4, 131.3, 128.6, 128.0 (Ph), 127.9 (C-3), 120.4

(C-4), 96.8 (C-1), 69.9 (C-2), 66.1 (CH2Ph), 59.7 (C-5), 21.2 (CH3CO); HRMS (ESI-TOF) m/z: [M+H]+ calcd for C14H17O4 249.1121; Found 249.1123.

General procedure for the synthesis of benzyl 2-*O*-acetyl-3-deoxy-4-sulfonate-*β*-L-*erythro*-pentopyranosides 2.14a-b. To a stirred solution of **2.12** (0.2 g, 0.75 mmol) and pyridine (0.18 mL, 2.25 mmol) in DCM (10 mL) at 0 °C, a solution of a sulfonyl chloride in DCM (2 mL) was added dropwise. The progress of the reaction was monitored by TLC, and 0.5 mL of saturated aq. $NaHCO_3$ was added to quench the reaction at 0 °C. The reaction mixture was washed with saturated aq. $NaHCO_3$ (5 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude mesylate or tosylate was purified by column chromatography on silica gel to afford the desired sulfonate, after prewashing the column with 0.1% of triethylamine in hexane.

*Mesylate (**2.14a**).* Following the general procedure in the presence of mesyl chloride (0.12 mL, 1.5 mmol), a crude product was obtained, which was purified by column chromatography using 5:1 hexane/EtOAc as eluent to afford **2.14a** (0.23 g, 87% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.27 (m, 5H, Ph), 4.84–4.70 (m, H-1, H-2, H-4 and C*H*$_2$Ph), 4.54 (d, *J* = 12.1 Hz, 1H, C*H*$_2$Ph), 4.03 (dd, *J* = 13.0, 1.8 Hz, 1H, H-5), 3.82 (d, *J* = 13.0 Hz, 1H, H-5′), 3.01 (s, 3H, C*H*$_3$ SO_2), 2.29–2.21 (bs, 2H, H-3 and H-3′), 2.07 (s, 3H, C*H*$_3$CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.9 (CH_3*C*O), 136.7, 128.3, 127.8, 127.7 (Ph), 95.9 (C-1), 72.5 (C-2), 69.2 (*C*H_2Ph), 66.2 (C-4), 61.5 (C-5), 38.4 (*C*H_3 Ms), 27.8 (C-3), 20.8 (*C*H_3CO); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{15}H_{20}O_7S_1Na$ 367.0822; Found 367.0816.

*Tosylate (**2.14b**).* Following the general procedure in the presence of tosyl chloride (0.29 g, 1.5 mmol), a crude product was obtained, which was purified by column chromatography using 10:1 hexane/EtOAc to afford **2.14b** (0.267 g, 85% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.1 Hz, 2H, Ph), 7.45–7.18 (m, 7H, Ph), 4.84–4.66 (m, 3H, H-1, H-2 and C*H*$_2$Ph), 4.61– 4.56 (m, 2H, H-4 and C*H*$_2$Ph), 3.95 (d, *J* = 12.8 Hz, 1H, H-5), 3.68 (d, *J* = 12.8 Hz, 1H, H-5′), 2.45 (s, 3H, C*H*$_3$ Ts), 2.24–2.06 (m, 2H, H-3 and H-3′), 2.04 (s, 3H, C*H*$_3$CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (CH_3*C*O), 145.0, 137.0, 134.2, 130.0, 128.6, 128.1, 128.0, 127.8 (Ph), 96.3 (C-1), 73.0 (C-2), 69.6 (*C*H_2Ph), 66.6 (C-4), 61.9 (C-5), 28.0 (C-3), 21.7 (*C*H_3 Ts), 21.2 (*C*H_3CO);

HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{21}H_{24}O_7S_1Na$ 443.1135; Found 443.1135.

Benzyl 2-*O*-acetyl-3,4-anhydro-*β*-L-ribopyranoside (2.16). Following a similar procedure as that used for the synthesis of **2.12**, epoxide **2.16** was obtained starting from **2.10** (0.57 g, 0.32 mmol), TBAF (0.63 mL, 0.63 mmol, 1 M in THF) in THF (10 mL). The crude residue was purified by column chromatography using hexane/EtOAc (8:1, v/v; 3:1 v/v) to give **2.16** as a white solid (0.2 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.24 (m, 5H, Ph), 4.95 (dd, *J* = 4.7, 3.2 Hz, 1H, H-2), 4.74 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.65 (d, *J* = 3.0 Hz, 1H, H-1), 4.52 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.08 (dd, *J* = 13.3, 1.6 Hz, 1H, H-5), 3.98 (d, *J* = 13.3 Hz, 1H, H-5′), 3.59 (appt, *J* = 4.0 Hz, 1H, H-3), 3.34 (dd, *J* = 3.9, 1.2 Hz, 1H, H-4), 2.14 (s, 3H, C*H*$_3$CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (CH$_3$*C*O), 137.0, 128.6, 128.1, 127.9 (Ph), 95.8 (C-1), 69.9 (C-2), 68.3 (*C*H$_2$Ph), 58.7 (C-5), 51.2 (C-3), 49.8 (C-4), 20.9 (*C*H$_3$CO); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{14}H_{16}O_5Na$ 287.0890; Found 287.0894.

Benzyl 2-*O*-acetyl-3,4-*O*-thiocarbonyl-*β*-L-ribopyranoside (2.17). A solution of **2.6** (10.0 g, 35.42 mmol) and TCDI (12.6 g, 70.9 mmol) in anhydrous DCM (500 mL) was stirred at room temperature for 10 h. The reaction mixture was washed with saturated aq. $NaHCO_3$ (200 mL). The organic layer was separated, and the aqueous layer was extracted with DCM. The combined organic layer was washed with brine and dried over Na_2SO_4. After removal of all the volatiles under reduced pressure, the crude residue was purified by column chromatography (3:1 DCM/hexane) to afford **2.17** (9.60 g, 83% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.30 (m, 5H, Ph), 5.22 (dd, *J* = 7.7, 4.0 Hz, 1H, H-4), 5.11 (appt, *J* = 4.2 Hz, 1H, H-2), 5.00 (d, *J* = 4.6 Hz, 1H, H-1), 4.98 (dd, *J* = 7.8, 1.2 Hz, 1H, H-3), 4.78 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.60 (d, *J* = 11.9 Hz,, 1H, C*H*$_2$Ph), 4.10 (dd, *J* = 14.1, 0.7 Hz, 1H, H-5), 4.01 (dd, *J* = 14.1, 2.0 Hz, 1H, H-5′), 2.15 (s, 3H, C*H*$_3$CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.2 (*C*S), 169.9 (CH$_3$*C*O), 136.5, 128.7, 128.4, 128.1 (Ph), 96.1 (C-1), 78.4 (C-4), 75.6 (C-3), 70.1 (*C*H$_2$Ph), 66.7 (C-2), 58.6 (C-5), 20.8 (*C*H$_3$CO); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{15}H_{17}O_6S_1$ 325.0740; Found 325.0740.

Benzyl *β*-L-*glycero*-pent-3-enopyranoside (2.18). To a stirred solution of **2.13** (3.75 g, 15.1 mmol) in methanol (150 mL) at 0 °C, a 30% NaOMe solution in MeOH (5.59 mL, 30.2 mmol) was added

dropwise. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, all the volatiles were removed *in vacuo*. The remaining residue was partitioned between EtOAc (100 mL) and saturated aq. $NaHCO_3$ (100 mL). The water layer was extracted with EtOAc (1 × 50 mL). The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (4:1 hexane/EtOAc) to give **2.18** (2.86 g, 91% yield) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.24 (m, 5H, Ph), 6.08–5.78 (m, 2H, H-3 and H-4), 4.80 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.77 (d, *J* = 2.6 Hz, 1H, H-1), 4.58 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.27–4.03 (m, 2H, H-5 and H-5′), 3.89 (bs, 1H, H-2), 2.61 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.4, 128.7, 128.5, 128.0 (Ph), 127.9 (C-3), 124.6 (C-4), 99.9 (C-1), 70.0 (*C*H_2Ph), 64.8 (C-2), 60.8 (C-5); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{12}H_{15}O_3$ 229.08353; Found 229.0842.

Benzyl 3,4-anhydro-*β*-L-ribopyranoside (2.19). A solution of *m*-chloroperbenzoic acid (*m*CPBA, 77%, 4.12 g, 9.46 mmol) in DCM (5 mL) was added dropwise to a stirring solution of **2.18** (2.60 g, 12.6 mmol) in dry DCM (120 mL) at 0 °C. After the addition was completed, the reaction mixture was stirred at 8-9 °C for 24 h. It was then cooled to 0 °C and 20% aq. $Na_2S_2O_3$ (25 mL) was added, and the stirring was continued for 2 h. The organic layer was separated and successively washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3:1 hexane/EtOAc) to afford **2.19** (2.50 g, 89% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.22 (m, 5H, Ph), 4.72 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.58 (d, *J* = 2.3 Hz, 1H, H-1), 4.49 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.01 (dd, *J* = 13.4, 1.5 Hz, 1H, H-5), 3.93 (d, *J* = 13.4 Hz, 1H, H-5′), 3.82 (dd, *J* = 4.5, 2.4 Hz, 1H, H-2), 3.50 (appt, *J* = 4.5 Hz, 1H, H-3), 3.33 (ddd, *J* = 4.2, 1.5, 0.6 Hz, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.0, 128.6, 128.1, 128.0 (Ph), 97.9 (C-1), 69.7 (*C*H_2Ph), 64.7 (C-2), 58.0 (C-5), 51.8 (C-3), 51.4 (C-4); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{12}H_{14}O_4Na$ 245.0785; Found 245.0791.

Benzyl 3,4-anhydro-2-*O*-methoxyethoxymethyl-*β*-L-ribopyranoside (2.20). To a stirred solution of **2.19** (2.10 g, 9.45 mmol) and DIPEA (4.90 mL, 28.4 mmol) in dry DCM (50 mL) at 0 °C was added MEMCl (2.16 mL, 18.9 mmol). After the addition was completed, the reaction mixture was stirred at room temperature for 1 h, and later at 40 ºC for 8 h. The reaction mixture was washed

with saturated aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The remaining residue was purified by column chromatography on silica gel (15:1 hexane/EtOAc) to afford **2.20** (2.68 g, 91% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.20 (m, 5H, Ph), 4.89 (s, 2H, OC*H*$_2$O), 4.75 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.61 (d, *J* = 4.0 Hz, 1H, H-1), 4.50 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.07 (dd, *J* = 13.4, 2.3 Hz, 1H, H-5), 3.92 (d, *J* = 13.4 Hz, 1H, H-5′), 3.92–3.79 (m, 2H, H-2 and OC*H*$_2$C*H*$_2$O), 3.76–3.63 (m, 1H, OC*H*$_2$C*H*$_2$O), 3.56–3.47 (m, 3H, H-3 and OC*H*$_2$C*H*$_2$O), 3.39–3.31 (m, 1H, H-4), 3.35 (s, 3H, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.1, 128.3, 127.7, 127.7 (Ph), 97.6 (O*C*H$_2$O), 95.1 (C-1), 71.7 (C-2), 71.6 (OCH$_2$*C*H$_2$O), 69.9 (*C*H$_2$Ph), 67.1 (O*C*H$_2$CH$_2$O), 59.9 (C-5), 58.8 (O*C*H$_3$), 51.9 (C-3), 51.7 (C-4); $[M+Na]^+$ calcd for $C_{16}H_{22}O_6Na$ 333.1309; Found 333.1317.

Benzyl 4-deoxy-2-*O*-(2-methoxyethoxymethyl)-4-(thymid-1-yl)-*α*-D-lyxopyranoside (2.21). To a stirred suspension of **2.20** (1.90 g, 6.12 mmol) and thymine (2.32 g, 18.4 mmol) in dry DMF (50 mL) at 0 °C was slowly added DBU (3 mL, 18.87 mmol). The suspension was stirred at room temperature for 30 min until all the solid dissolved and then heated at 80 °C for 8 h. After removal of all the volatiles under reduced pressure, the remaining syrup was dissolved in EtOAc and washed with saturated aq. $NaHCO_3$. The organic phase was collected, dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by silica gel column chromatography (100:2 DCM/Methanol) to afford **2.21** (2.27 g, 85% yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.74 (s, 1H, NH), 7.35 (dd, *J* = 20.3, 6.9 Hz, 5H, Ph), 7.03 (s, 1H, H-6), 5.02 (d, *J* = 1.5 Hz, 1H, H-1′), 4.87 (d, *J* = 7.2 Hz, 1H, OC*H*$_2$O), 4.80 (d, *J* = 7.2 Hz, 1H, OC*H*$_2$O), 4.76 (d, *J* = 11.7 Hz, 1H, C*H*$_2$Ph), 4.54 (d, *J* = 11.7 Hz, 1H, C*H*$_2$Ph), 4.28 (appt, *J* = 7.9 Hz, 1H, H-3′), 3.98 (appt, *J* = 2.4 Hz, 1H, H-2′), 3.89–3.66 (m, 4H, H-5′, H-5′ and OCH$_2$C*H*$_2$O), 3.51 (s, 2H, OC*H*$_2$CH$_2$O), 3.34 (s, 3H, OC*H*$_3$), 1.88 (s, 3H, T C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.9 (C-4), 151.8 (C-2), 137.1 (C-6), 137.0, 128.6, 128.1, 127.9 (Ph), 111.0 (C-5), 98.3 (C-1′), 96.7 (O*C*H$_2$O), 78.9 (C-2′), 71.5 (OCH$_2$*C*H$_2$O), 69.4 (*C*H$_2$Ph), 67.8 (O*C*H$_2$CH$_2$O), 65.9 (C-3′), 60.2 (C-5′), 58.9 (O*C*H$_3$), 54.7 (C-4′), 12.5 (T *C*H$_3$); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{21}H_{28}N_2O_8Na$ 459.1738; Found 459.1731.

Benzyl 3,4-dideoxy-2-*O*-(2-methoxyethoxymethyl)-4-(thymid-1-yl)-*α*-D-*threo*-pentopyranoside (2.22). To a solution of **2.21** (1.40 g, 2.6 mmol) and DMAP (100 mg, 0.8 mmol)

in anhydrous DCM (30 mL) was added TCDI (940 mg, 5.20 mmol) at room temperature. The reaction mixture was stirred at 40 °C overnight. It was then washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was dissolved in toluene (50 mL), and AIBN (170 mg, 1.10 mmol) was added followed by tributyltin hydride (1.70 mL, 4.20 mmol). The reaction mixture was refluxed for 1 h. After removal of all the volatiles under reduced pressure, the crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 100:0, v/v; 100:2, v/v; 30:1, v/v) to afford **2.22** (1.10 g, 83% yield over two steps) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.95 (s, 1H, NH), 7.47–7.19 (m, 5H, Ph), 7.06 (s, 1H, H-6), 5.10–4.89 (m, 1H, H-4′), 4.80 (m, 4H, OC*H*$_2$O, C*H*$_2$Ph and H-1′), 4.59 (d, *J* = 11.1 Hz, 1H, C*H*$_2$Ph), 3.93 (bs, 1H, H-2′), 3.85–3.64 (m, 4H, H-5′, H-5′, OCH_2CH_2O), 3.51 (dd, *J* = 5.4, 3.6 Hz, 2H, OCH_2CH_2O), 3.34 (s, 3H, OC*H*$_3$), 2.30 (td, *J* = 12.7, 2.8 Hz, 1H, H-3′), 2.03 (dt, *J* = 12.6, 3.1 Hz, 1H, H-3″), 1.90 (s, 3H, T C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3 (C-4), 151.4 (C-2), 137.5 (C-6), 137.0, 128.8, 128.20, 128.25 (Ph), 111.2 (C-5), 96.7 (OCH_2O), 95.2 (C-1′), 73.0 (C-2′), 71.9 (OCH_2CH_2O), 69.5 (CH_2Ph), 67.5 (OCH_2CH_2O), 61.3 (C-5′), 59.2 (OCH_3), 47.6 (C-4′), 29.2 (C-3′), 12.8 (T CH_3); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{21}H_{29}N_2O_7$ 421.1969; Found 421.1964.

Benzyl 3,4-dideoxy-4-(thymid-1-yl)-*α*/*β*-D-*threo*-pentopyranoside 2.23. Hydrochloric acid in 1,4-dioxane (4 M, 8 mL) was added dropwise to a solution of **2.22** (1.20 g, 2.86 mmol) in 1,4-dioxane (24 mL), and the reaction mixture was stirred at room temperature for 8 h. After removal of all the volatiles under reduced pressure, the remaining syrup was co-evaporated with triethylamine (5 mL) once and then subjected to column chromatography on silica gel to give *α*/*β*-**2.23**. Data for the *α*-**2.23**: (0.72 g, 75% yield**)**; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.96 (s, 1H, NH), 7.46–7.29 (m, 5H, Ph), 7.12 (d, *J* = 1.1 Hz, 1H, H-6), 5.13–4.97 (m, 1H, H-4′), 4.80 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.71 (d, *J* = 2.4 Hz, 1H, H-1′), 4.57 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 3.96 (bs, 1H, H-2′), 3.82 (m, 2H, H-5′ and H-5″), 3.67 (d, *J* = 6.1 Hz, 1H, OH), 2.27 (appt d, *J* = 13.0, 3.3 Hz, 1H, H-3′), 2.01 (d appt, *J* = 13.3, 4.3 Hz, 1H, H-3′), 1.88 (d, *J* = 1.1 Hz, 3H, T C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.8 (C-4), 151.3 (C-2), 137.0 (C-6), 136.6, 128.5, 128.0, 128.0 (Ph), 111.0 (C-5), 98.3 (C-1′), 69.5 (CH_2Ph), 67.0 (C-2′), 61.4 (C-5′), 46.8 (C-4′), 30.8 (C-3′), 12.5 (T CH_3); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{21}N_2O_5$ 333.1445; Found 333.1443.

Data for the *β*-**2.23**: (48 mg, 5% yield**)**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.52 (s, 1H, NH), 7.61 (d, *J* = 1.2 Hz, 1H, H-6), 7.42–7.29 (m, 5H, Ph), 4.92 (d, *J* = 2.9 Hz, 1H, H-1′), 4.84 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.76–4.52 (m, 2H, C*H*$_2$Ph, H-4′), 4.13 (dd, *J* = 13.0, 3.9 Hz, 1H, H-5′), 3.94–3.73 (m, 2H, H-5′, H-2′), 2.48 (d, *J* = 8.3 Hz, 1H, OH), 2.21– 2.17 (dT, *J* = 12.9, 3.8 Hz, 1H, H-3′), 2.15– 2.09 (m, 1H, H-3′′), 1.95–1.86 (d, *J* = 1.2 Hz, 3H, T C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.0 (C-4), 150.9 (C-2), 138.2, 136.4 (C-6), 128.5, 128.1, 128.0 (Ph), 110.6 (C-5), 97.3 (C-1′), 69.9 (*C*H_2Ph), 64.4 (C-2′), 61.1 (C-5′), 51.2 (C-4′), 31.1 (C-3′), 12.5 (T *C*H_3).

Benzyl 2-*O*-benzoyl-3,4-dideoxy-4-(thymid-1-yl)-*α*/*β*-D-*threo*-pentopyranoside (2.24). To a solution of *α*/*β*-**2.23** (400 mg, 1.20 mmol) in pyridine (15 mL) at 0 °C, benzoyl chloride (0.24 mL, 1.81 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h. After removal of all the volatiles under reduced pressure, the remaining residue was partitioned between DCM (30 mL) and saturated aq. $NaHCO_3$ (20 mL). The aqueous layer was extracted again with DCM (20 mL). The combined organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3:1 hexane/EtOAc) to afford *α*/*β*-**2.24** (483 mg, 92% yield) as a colourless oil.

Data for *α*-**2.24**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.17 (s, 1H, NH), 8.09 (m, 2H, Ph), 7.64–7.30 (m, 8H, Ph), 7.04 (d, *J* = 1.2 Hz, 1H, H-6), 5.31 (td, *J* = 3.5, 3.2 Hz, 1H, H-2′), 5.09 (tt, *J* = 11.7, 5.0 1H, H-4′), 4.92 (d, *J* = 1.6 Hz, 1H, H-1′), 4.82 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.62 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 3.87 (appt, *J* = 10.8 Hz, 1H, H-5′), 3.81 (ddd, *J* = 10.7, 5.0, 1.9 Hz, 1H, H-5′), 2.49 (td, *J* = 13.2, 3.2 Hz, 1H, H-3′), 2.26–2.04 (dt, *J* = 13.8, 3.6 Hz, 1H, H-3′′), 1.92 (d, *J* = 1.2 Hz, 3H, T C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.5 (Ph*C*O), 163.5 (C-4), 150.8 (C-2), 136.8, 136.1 (C-6), 133.4, 129.8, 129.4(Ar-C), 128.6, 128.5, 128.3, 128.1, 127.9 (Ph), 111.3 (C-5), 95.0 (C-1′), 69.5 (*C*H_2Ph), 69.1 (C-2′), 60.7 (C-5′), 47.0 (C-4′), 28.2 (C-3′), 12.5 (T *C*H_3); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{24}H_{25}N_2O_6$ 437.1707; Found 437.1700.

Data for *β*-**2.24**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.36 (s, 1H, NH), 8.18–7.72 (m, 2H, Ph), 7.46 (m, 3H, Ph), 7.19 (m, 5H, Ph), 5.29 (dd, *J* = 6.9, 9.9 Hz, 1H, H-2′), 5.01 (td, *J* = 5.9, 10.9 Hz, 1H, H-4′), 4.85 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.63 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.58 (d, *J* = 7.4 Hz, 1H,

H-1′), 4.11 (dd, *J* = 3.7, 11.1 Hz, 1H, H-5′), 3.60 (appt, *J* = 11.0 Hz, 1H, H-5″), 2.18–1.74 (m, 5H, , H-3′, , H-3″ and T C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4 (Ph*C*O), 163.6 (C-4), 151.4 (C-2), 136.8 , 136.0 (C-6), 133.5, 130.0, 129.0, 128.5, 128.4, 127.9, 127.9 (Ph), 111.7 (C-5), 100.6 (C-1′), 71.1 (C-2′), 70.3 (*C*H$_2$Ph), 62.6 (C-5′), 54.0 (C-4′), 30.8 (C-3′), 12.7 (T *C*H$_3$).

2-*O*-Benzoyl-3,4-dideoxy-4-(thymid-1-yl)-*α*-D-*threo*-pentopyranose (2.25). To a solution of *α*/*β*-**2.24** (300 mg, 0.69 mmol) in ethanol (10 mL), Pd/C (366 mg, 0.34 mmol) and cyclohexene (1.39 mL, 13.8 mmol) were added. The reaction mixture was stirred at 80 ºC for 6 h. It was then cooled and filtered through a pad of Celite and concentrated under reduced pressure to give **2.25** (209 mg, 88%) as a white solid. ^{1}H NMR (600 MHz, DMSO) δ 11.3 (s, 1H, NH), 8.01 (m, 2H, Ph), 7.72–7.68 (m, 1H, Ph), 7.65 (d, *J* = 1.2 Hz, 1H, H-6), 7.59–7.56 (m, 2H, Ph), 7.00 (d, *J* = 4.7 Hz, 1H, OH), 5.04 (td, *J* = 4.7, 3.6 Hz, 1H, H-2′), 5.01 (dd, *J* = 4.7, 1.4 Hz, 1H, H-1′), 4.79 (tt, *J* = 10.7, 4.3 Hz, 1H, H-4′), 4.03 (appt, *J* = 10.8 Hz, 1H, H-5′), 3.59 (ddd, *J* = 10.5, 4.4, 1.7 Hz, 1H, H-5″), 2.59 (td, *J* = 13.4, 3.1 Hz, 1H, H-3′), 2.01 (dt, *J* = 13.4, 4.5 Hz, 1H, H-3′), 1.79 (d, *J* = 0.9 Hz, 3H, T C*H*$_3$); ^{13}C NMR (151 MHz, DMSO) δ 165.0 (Ph*C*O), 163.8 (C-4), 151.0 (C-2), 138.0, 133.7 (C-6), 129.7, 129.4, 129.3, 128.9, 128.8 (Ph)**,** 109.2 (C-5), 89.7 (C-1′), 70.9 (C-2′), 59.8 (C-5′), 47.3 (C-4′), 27.3 (C-3′), 12.1 (T *C*H$_3$); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{19}N_2O_6$ 347.1238; Found 347.1241.

General procedure for the synthesis of trichloroacetimidate and *N*-phenyltrifluoroacetimidate glycosylation donors 2.26a and 2.26b. To a stirred solution of **2.25** (100 mg, 0.29 mmol) and K_2CO_3 (44 mg, 0.32 mmol) in acetone (3 mL) at 0 ºC, *N*-aryltrifluoroacetimidoyl chloride (120 mg, 0.58 mmol) or trichloroacetonitrile (0.06 mL, 0.58 mmol) was added dropwise. The reaction was monitored by TLC for completion. The reaction mixture was then filtered through a pad of Celite and concentrated under reduced pressure to afford the corresponding crude donor.

Data for **2.26a**: ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 9.27 (s, 1H, NH), 8.00–7.97 (m, 2H, Ph), 7.59–7.50 (m, 1H, Ph), 7.42 (m, 2H, Ph), 7.31 (s, 1H, H-6), 6.21 (bs, 1H, H-1′), 5.31 (bs, 1H, H-2′), 4.89 (m, 1H, H-4′), 4.14 (t, *J* = 10.8 Hz, 1H, H-5′), 3.88 (m, 1H, H-5″), 2.76 (t, *J* = 13.3 Hz, 1H, H-3′),

2.29 (d, *J* = 13.6 Hz, 1H, H-3′), 1.68 (s, 3H, T C*H*$_3$), ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 165.8 (C-4), 151.8 (C-2), 138.6, 134.4 (C-6), 130.5, 129.5 (Ph), 110.9 (C-5), 93.9 (C-1′), 69.4 (C-2′), 63.1 (C-5′), 49.2 (C-4′), 28.5 (C-3′), 12.4 (T *C*H$_3$).

Data for **2.26b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18–8.00 (m, 2H, Ph), 7.61 (m, 1H, Ph), 7.48 (m, Ph), 7.32 (m, 2H, Ph), 7.13 (m, 1H, Ph), 6.96 (bs, 1H, H-6), 6.88 (d, *J* = 7.7 Hz, 2H, Ph), 6.27 (bs, 1H, H-1′), 5.46 (bs, 1H, H-2′), 5.12 (m, 1H, H-4′), 3.95 (bs, 2H, H-5′, H-5′), 2.54 (m, 1H, H-3′), 2.31 (m, 1H, H-3′), 1.96 (d, *J* = 1.0 Hz, 3H, T C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 163.1 (C-4), 150.7(C-2), 143.2, 135.9, 133.9 (C-3), 130.1, 129.2, 129.0, 128.8, 124.9 (Ph), 119.7 (*C*F$_3$), 111.9 (C-5), 92.4 (C-1′), 67.9 (C-2′), 62.4 (C-5′), 47.0 (C-4′), 28.3 (C-3′), 12.7 (T *C*H$_3$); $[M+Na]^+$ calcd for $C_{25}H_{22}F_3N_3O_6Na$ 540.1353; Found 540.1362.

Experimental procedure for the phosphonylation of 2.26a and 2.26b. To a stirred solution of phosphonylation donor **2.26a** or **2.6b** (1 eq) and diisopropylphosphonomethanol (2 eq) in dry DCM was added 4 Å molecular sieves, and the mixture was stirred for 30 min at room temperature. The reaction mixture was cooled to -78 ºC and Lewis acid (TMSOTf or $BF_3.Et_2O$, 1.1 eq) was added. The mixture was kept stirring at -78 ºC and monitored by TLC for completion (very fast, normally within 10 min). The reaction was quenched by addition of saturated aq. $NaHCO_3$ at 0 ºC. It was then diluted with DCM and saturated aq. $NaHCO_3$. The aqueous layer was extracted twice with DCM. The combined organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford **2.25** or ***β*-2.27**, respectively, as shown in **Table 2-1**, along with residual diisopropylphosphonomethanol.

Acetyl 2-*O*-benzoyl-3,4-dideoxy-4-(thymid-1-yl)-*α*-D-*threo*-pentopyranoside (2.26c). To a solution of **2.25** (150 mg, 0.43 mmol) in pyridine (5 mL) at 0 °C, acetic anhydride (0.082 mL, 0.87 mmol) was added dropwise. The reaction mixture was slowly warmed to room temperature and left stirring for 2 h. After removal of all the volatiles under reduced pressure, the remaining residue was taken up with DCM (10 mL), and 10 mL of saturated aq. $NaHCO_3$ was added. The aqueous layer was then extracted with DCM (2 × 10 mL). The combined organic layer was washed with saturated

aq. $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2:1 hexane/EtOAc) to give **2.26c** (142.8 mg, 85%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.26 (s, 1H, NH), 8.17–8.01 (m, 2H, Ph), 7.63–7.54 (m, 1H, Ph), 7.53–7.40 (m, 2H, Ph), 7.08 (d, *J* = 1.2 Hz, 1H, H-6), 6.10 (d, *J* = 1.9 Hz, 1H, H-1′), 5.28 (td, *J* = 3.4, 1.9 Hz, 1H, H-2′), 4.93 (tt, *J* = 10.4, 4.3 Hz, 1H, H-4′), 4.02 (appt, *J* = 10.8 Hz, 1H, H-5′), 3.92 (ddd, *J* = 11.0, 4.7, 1.8 Hz, 1H, H-5″), 2.61 (ddd, *J* = 13.6, 12.3, 3.3 Hz, 1H, H-3′), 2.27 (dt, *J* = 13.6, 3.7 Hz, 1H, H-3″), 2.21 (s, 3H, C*H*$_3$CO), 1.95 (d, *J* = 1.1 Hz, 3H, T C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.8 (CH_3*C*O), 165.4 (Ph*C*O), 163.7 (C-4), 150.9 (C-2), 136.9 (C-6), 133.8, 130.0, 129.2, 128.7 (Ph), 111.6 (C-5), 89.6 (C-1′), 68.3 (C-2′), 62.5 (C-5′), 48.7 (C-4′), 28.5 (C-3′), 21.1 (*C*H_3CO)), 12.7 (T *C*H_3); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{19}H_{20}N_2O_7Na$ 411.1163; Found 411.1157.

Diisopopylphosphonomethyl 2-*O*-benzoyl-3,4-dideoxy-4-(thymid-1-yl)-*α*-D-*threo*-pentopyranoside (2.27). To a stirred solution of **2.26c** (120 mg, 0.31 mmol) and diisopropylphosphonomethanol (121 mg, 0.62 mmol) in dry DCM (3 mL) was added 4 Å molecular sieves, and the mixture was stirred for 30 min at room temperature. The reaction mixture was cooled to 0 ºC, and TMSOTf (0.073 mL, 0.40 mmol) was added. The mixture was warmed to room temperature and stirred for 10 h. The reaction was quenched by addition of saturated aq. $NaHCO_3$ at 0 ºC. It was then diluted with DCM (10 mL) and saturated aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with DCM (2 × 10 mL). The combined organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford **2.27** as a mixture of α and *β* isomers, along with residual diisopropylphosphonomethanol. This material was used in the next step without further purification. HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{24}H_{33}N_2O_9P_1Na$ 547.1816; Found 547.1801.

Diisopopylphosphonomethyl 3,4-dideoxy-4-(thymid-1-yl)-*α*/*β*-D-*threo*-pentopyranoside (2.28). A solution of crude **2.27** in 7N NH_3 in MeOH (5 mL) was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (20:1 CH_2Cl_2/MeOH) to give *α*/*β*-**2.28** as a white foam.

Data for α-**2.28**: 25 mg, 20% over two steps; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.69 (s, 1H, NH), 7.24 (s, 1H, H-6), 5.01 (tt, *J* = 10.3, 4.0 Hz, 1H, H-4′), 4.84–4.73 (m, 2H, [C*H*(CH_3)$_2$]), 4.68 (d, *J* = 2.6 Hz, 1H, H-1′), 4.05–3.93 (m, 2H, PC*H*$_2$ and H-2′), 3.86 (td, *J* = 4.2, 10.8 Hz, 1H, H-5′), 3.80–3.70 (m, 2H, H-5″ and PC*H*$_2$), 2.27 (ddd, *J* = 12.8, 1.2 Hz, 1H, H-3′), 2.05–1.97 (dt, *J* = 13.2, 4.3 Hz,1H, H-3″), 1.91 (d, *J* = 1.1 Hz, 3H, T C*H*$_3$), 1.41–1.29 (m, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.9 (C-4), 151.3 (C-2), 137.0 (C-6), 111.2 (C-5), 100.4 (d, $^{3}J_{P,C}$ = 11.1 Hz, C-1′), 71.6 [*C*H(CH_3)$_2$], 66.5 (C-2′), 61.9 (C-5′), 61.8 (d, $^{1}J_{P,C}$ = 171.8Hz, P*C*H$_2$), 47.0 (C-4′), 31.0 (C-3′), 24.2 [CH(*C*H$_3$)$_2$], 12.6 (T *C*H$_3$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 20.0; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{30}N_2O_8P_1$ 421.1734; Found 421.1735.

Data for *β*-**2.28**: 13 mg, 10% over two steps; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (s, 1H, NH), 7.64 (d, *J* = 1.2 Hz, 1H, H-6), 4.94 (d, *J* = 2.6 Hz, 1H, H-1′), 4.79 (tdd, *J* = 12.4, 6.2, 1.3 Hz, 2H, [C*H*(CH_3)$_2$]), 4.62 (tt, *J* = 4.4, 3.7 Hz, 1H, H-4′), 4.17 (dd, *J* = 13.2, 3.9 Hz, 1H, H-5′), 4.03 (dd, *J* = 13.8, 9.4 Hz, 1H, PC*H*$_2$), 3.90–3.67 (m, 3H, H-5″, PC*H*$_2$ and H-2′), 2.19–2.16 (dt, *J* = 13.8, 4.9 Hz, 1H, H-3′), 2.19–2.06 (ddd, *J* = 13.8, 9.8, 3.9 Hz, 1H, H-3″), 1.93 (d, *J* = 1.1 Hz, 3H, T C*H*$_3$), 1.34 (m, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.9 (C-4), 151.0 (C-2), 138.2 (C-6), 110.8 (C-5), 99.8 (d, $^{3}J_{P,C}$ = 9.9 Hz, C-1′), 71.8, 71.7 (d, $^{2}J_{P,C}$ = 6.6 Hz, [*C*H(CH_3)$_2$]), 64.3 (C-2′), 62.6 (d, $^{1}J_{P,C}$ = 171.0Hz, P*C*H$_2$), 61.2 (C-5′), 51.2 (C-4′), 30.9 (C-3′), 24.2 [CH(*C*H$_3$)$_2$], 12.8 (T *C*H$_3$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 20.0.

Phosphonomethyl 3,4-dideoxy-4-(thymid-1-yl)-*α*-D-*threo*-pentopyranoside (2.29). To a solution of α-**2.28** (20 mg, 0.048 mmol) and 2,6-lutidine (0.04 mL, 0.38 mmol) in dry CH_3CN (2 mL) was added bromotrimethylsilane (0.05 mL, 0.38 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight and quenched with 1.0 M aq. TEAB solution (1 mL). After removal of all the volatiles under reduced pressure, the remaining residue was partitioned between water and EtOAc/ether (1:1) and the water layer was lyophilised. The crude residue was first purified by silica gel column chromatography (10:1:0 to 10:5:1, CH_2Cl_2/MeOH/1.0 M aq. TEAB) and then by preparative reverse phase HPLC with a gradient of CH_3CN in 0.05 M TEAB ranging from 2 to 30% to give **2.29** (**2.29**·0.67 Et_3N salt, 8.3 mg, 40%) as a white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.66 (s, 1H, H-6), 4.79 (tt, *J* = 10.8, 4.8 Hz, 1H, H-4′), 4.68 (d, *J* = 2.2 Hz, 1H, H-1′), 4.04

(td, *J* = 3.0, 3.6 Hz, 1H, H-2′), 3.91 (appt t, *J* = 10.7 Hz, 1H, H-5′), 3.87 (dd, *J* = 13.1, 9.3 Hz, 1H, PC*H*$_2$), 3.73 (ddd, *J* = 11.2, 4.7, 1.4 Hz, 1H, H-5″), 3.63 (dd, *J* = 13.2, 9.3 Hz,1H, PC*H*$_2$), 3.18 (q, *J* = 7.4 Hz, 4H, C*H*$_2$CH$_3$), 2.38 (ddd, *J* = 13.8, 12.1, 3.0 Hz, 1H, H-3′), 1.93 (dtd, *J* = 13.1, 3.9, 1.2 Hz, 1H, H-3′), 1.88 (s, 3H, T C*H*$_3$), 1.26 (t, *J* = 7.3 Hz, 6H, CH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D$_2$O) δ 166.1 (C-4), 151.9 (C-2), 139.1 (C-6), 110.7 (C-5), 99.3 (d, $^3J_{P,C}$ = 12.0 Hz, C-1′), 99.3 (C-1′), 65.7 (C-2′), 62.9 (d, $^1J_{P,C}$ = 156.6 Hz, P*C*H$_2$), 60.4 (C-5′), 46.7 (C-4′), 29.2 (C-3′), 11.0 (T *C*H$_3$); ^{31}P NMR (121 MHz, D$_2$O) δ 15.7; HRMS (ESI-): [M-H]$^-$ calcd for $C_{11}H_{16}N_2O_8P_1$ 335.0650; Found 335.0654.

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Chapter 3. Synthesis of Phosphonomethyl 4-(adenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside

Abstract: The synthesis of a pentopyranoside adenine nucleoside with a phosphonate functionality at the 1′-anomeric oxygen has been performed. Different synthetic strategies were explored. Key functionalized L-*erythro*-pentopyranose carbohydrate synthon was prepared and further elaborated into the final six-membered ring nucleoside phosphonate via phosphonomethylation and adenine nucleobase construction from an amino moiety. Notably, attempts for direct anomeric phosphonylation of adenine nucleoside were not successful, which indicates the important role played by steric effect and conformation of the nucleoside, on the outcome of the glycosylation (phosphonylation) reaction with the pyranosyl adenine nucleoside.

3.1 introduction

The protocols for the nucleobase incorporation on non-anomeric carbon atoms and the glycosylation by phosphonomethanol as acceptor, presented in **Chapter 2** are also applicable for the synthesis of the pentopyranosyl adenine nucleoside phosphonate. However, in the case of purine nucleobases, an additional obstacle for the synthesis of *N*-9 purine nucleosides is the potential side reaction at the 7-nitrogen of adenine occurs to deliver undesired *N*-7 minor isomer.[1]

The introduction of adenine can be done following one of the three methods as depicted in **Figure 3-1** (Route A, B and C). Route A follows similar strategies as described in **Chapter 2 (Figure 2-3)**, where adenine is a nucleophile, and the activated carbohydrate scaffold is an electrophile (sulfonate or halide displacement or Mitsunobu condensation or epoxide opening**).** Route B employs an amino sugar as a nucleophile and derivatised pyrimidine as an electrophile, which is a common approach in the synthesis carbocyclic nucleoside.[2] Route C is seldomly used since the conversion from imidazole to purine requires high-temperature reaction conditions.[3-4]

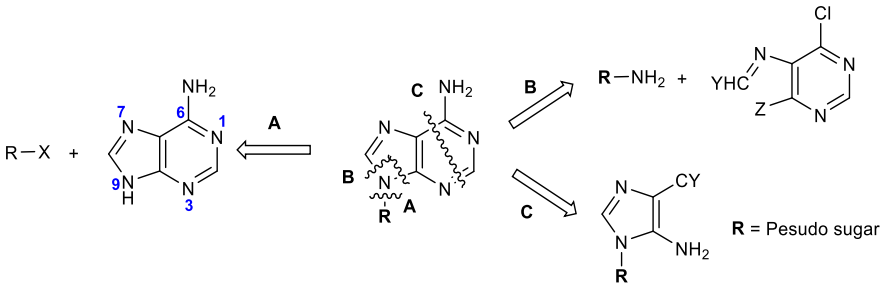
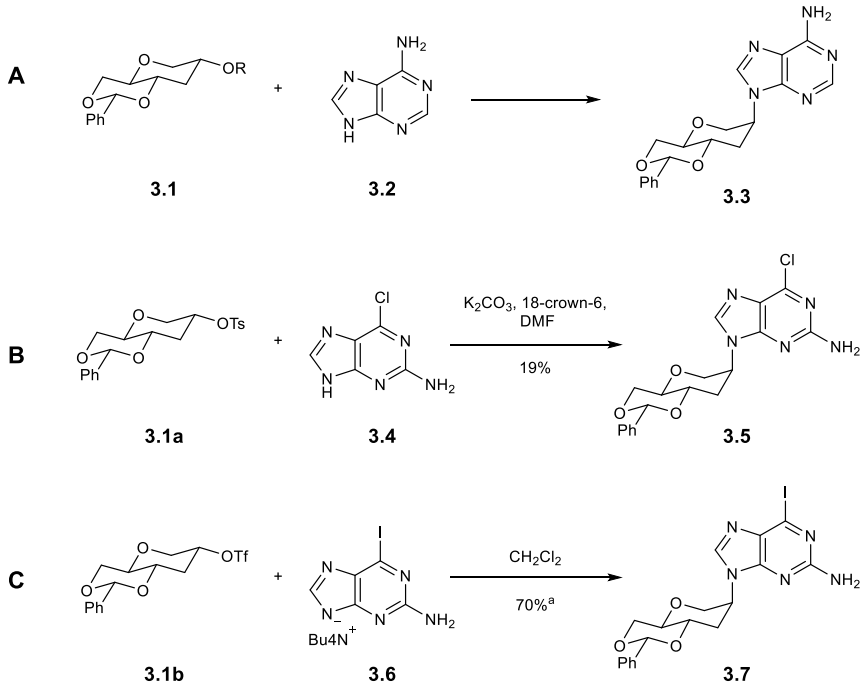


Figure 3-1: Possible route for the synthesis of adenine nucleoside

Alkylation and glycosylation of purines are usually not regioselective and gives mixtures of *N*-9 and *N*-7 products. Alkylation of the adenine anion with alkyl mesylates in DMF at 80 °C in the presence of 18-crown-6 affords both *N*-9 and *N*-7 isomers in 61% and 9% of yield respectively. Glycosylation of persilylated 6-*N*-benzoyladenine with a dioxabicylco[3.2.1]octane at ambient temperature gives both *N*-9 and *N*-7 products, and it was suggested that the *N*-7 isomer is the kinetic product. This undesired isomer was converted into the *N*-9 isomer at elevated temperature in the presence of TMSOTf[5-6]. However, in other cases increasing the temperature of the reaction did not

affect the ratio of *N*-9 vs *N*-7 isomers.[7] The mechanisms and the thermodynamic nature of the isomerisation reaction are only partially studied. The reaction solvents (polar vs non-polar), the nature of glycosyl donor, the size of the substituents at position 6 of the purine ring[8] and catalyst (acid or base) contributes to the regioselectivity of the glycosylation.[6, 9-10]

Table 3-1 summarises the reports from the literature references for the alkylation of adenine during the synthesis of hexitol nucleoside; different sugar sulfonates (triflate or tosylate), bases (LiH, NaH, or K_2CO_3) and ligands (with or without 18-crown-6 or 12-crown-4) were screened. This optimisation study led to the reaction circumstances in which the formation of the *N*-7 isomer is significantly reduced or completely avoided by appropriate choice of alkylating agent and base. **(Scheme 3-1** and **Table 3-1**).[11-12]



Scheme 3-1. Alkylation of hexitol 2-amino-6-halo purine nucleoside. A: adenine as nucleobase, detailed conditions see Table 3-1; B: 2-amino-6-Cl-purine alkylation; C: optimized 2-amino-6-I-purine alkylation. [a]no N-7 isomer was noticed.

Table 3-1. Conditions for the alkylation of adenine on the hexitol scaffold

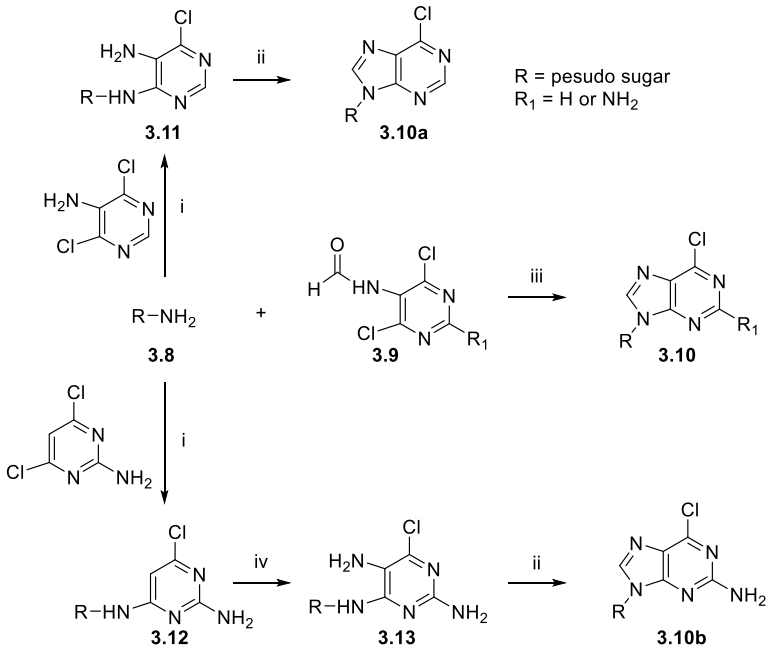
R	Base	Ligand	Yield (%)
Ts	NaH	-	56
Ts	LiH	12-crown-4	82[a]
Ts	LiH	18-crown-6	61
Tf	Tetrabutylammonium hyrdoxide[b]	-	66[c]

[a]No N-7 isomer was noticed

[b]Tetrabutylamonium salt was presynthesized before the reaction

[c]Scale up of triflate reaction was problematic because of the instability

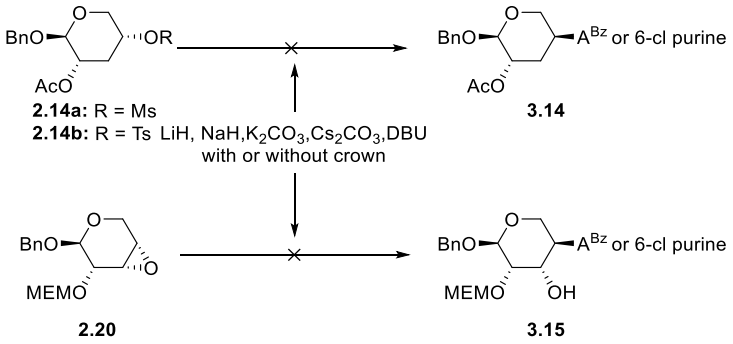
Introduction of the bulky group/protecting group at the C-6 position was also utilised to achieve a better selectivity for the formation *N*-9 isomer.[13-14] Another possible strategy is to construct the desired purine ring on the sugar-scaffold carrying an amino group (**Figure 3-1 route B**).[15-16] Nowadays, the commercially available 5-amino-4,6-dichloro pyrimidine is the most frequently used synthon for the construction of adenine core structure.[17] (**Scheme 3-2**)



Scheme 3-2. Construction of purine nucleobase. *Reagents, conditions and yields*: (i) BuOH, Et_3N, reflux; (ii) (a) $AcOCH(OEt)_2$; (b) 0.5N HCl, MeOH; (iii) BuOH, DIPEA, reflux; (iv) (a) $4\text{-}ClC_6H_4N_2Cl$; (b) Zn, AcOH.

3.2 Results and discussion

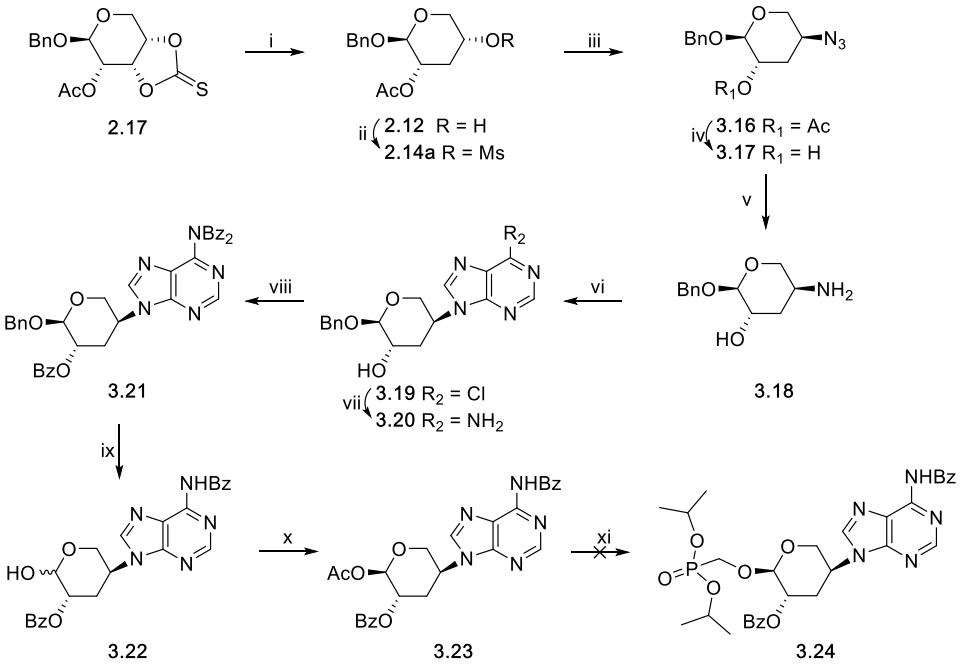
As discussed above about the strategies for the introduction of the purine base on an hexitol scaffold[11-12], and in analogy to the work described for the synthesis of thymine D-*threo*-pentopyranoside phosphonate **2.29** in **Chapter 2**, we initially investigated the nucleophilic substitution of 4-sulfonate activated sugars **2.14a-b** as well as ring opening reactions of epoxide **2.20** with 6-chloropurine and N^6-benzoyl protected adenine (**Scheme 3-3**). However, all attempts to directly introduce the purine base at the 4-position using various additives failed.



Scheme 3-3. Attempted reactions for the introduction of purine nucleobases.

Therefore, we opted for the construction of the purine ring via a linear approach. As shown in **Scheme 3-4**, radical deoxygenation of previously synthesised thionocarbonate derivative **2.17** afforded 3-deoxy compound **2.12** in 60% yield along with small amounts of the undesired 4-deoxy product (10%), under high dilution reaction conditions (0.02 mol/L). It should be noted that when the reaction was conducted at a concentration of 0.10 mol/L, the reduction of the C=S bond to a methylene moiety ($-CH_2$) took place instead of the desired deoxygenation reaction at the 3-position. Mesylation and subsequent azidation of the 4-hydroxyl group occurred smoothly to afford 4-azido compound **3.16** in good yield. After deacetylation at the 2-position, compound **3.17** was reacted under Staudinger conditions to give 4-amino-D-*threo*-pentopyranoside **3.18**. Next, a literature procedure was employed for the construction of the purine ring starting from precursor **3.18** and 4,6-dichloro-5-formamidopyrimidine. [17] The initially formed pyrimidine nucleoside intermediate

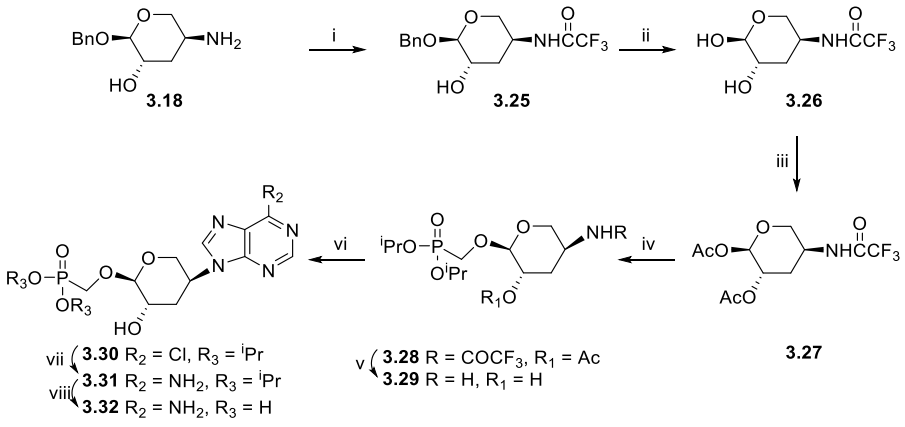
underwent *in situ* cyclization under basic conditions to smoothly provide 6-chloropurine pyranoside **3.19**. Subsequent amination at the 6-position of purine in **3.19** yielded adenine analogue **3.20** in excellent yield (95%), whose further reaction with an excess of benzoyl chloride furnished fully protected derivative **3.21**. Following attempts to achieve the removal of the 1′-benzyl protecting group along with mono-debenzoylation at the primary amino group of **3.21** under different hydrogenation conditions in the presence of a range of Pd catalysts (10% Pd/C, 20% $Pd(OH)_2/C$, $Pd(OAc)_2$, and Pd-black) did not deliver chemoselectively glycone **3.22**, due to the contamination of the desired compound with other fully and partially *N*-debenzoylated by-products. However, the use of a Lewis-acid such as boron trichloride enabled benzyl removal with the exclusive formation of the desired anomeric hydroxyl glycone **3.22** in good yield. After acetylation of the anomeric 1′-OH, the resulting glycosyl donor **3.23** was reacted with diisopropyl phosphonomethanol under conditions similar to those developed earlier for the phosphomethylation of thymine analogue **2.29** (**Table 2-1**). To our dismay, none of these conditions provided the desired target phosphonate glycoside **3.24** and similar attempts to prepare a 6-chloropurine containing nucleoside starting from **3.19** through a similar sequence of steps also met with failure.



Scheme 3-4. The building of the purine ring on 4-amino-D-*threo*-pentopyranoside Sugar **3.18**. *Reagents, conditions and yields*: (i) Bu_3SnH, AIBN, toluene; (ii) MsCl, TEA, DMAP, DCM, 54% over two

steps; (iii) NaN_3, DMF, 91%; (iv) MeONa, MeOH, 95%; (v) triphenyl phosphine, THF then water, 92%; (vi) 4,6-dichloro-5-formamidopyrimidine, DIPEA, BuOH, 75%; (vii) NH_3 in ethanol, 95%; (viii) BzCl, pyridine, 88%; (ix) 1 M BCl_3, DCM, -78 to 0 °C, 80%; (x) Ac_2O, pyridine, 78%; (xi) TMSOTf, hydroxymethyl phosphonic acid diisopropyl ester, CH_2Cl_2.

In view of these results, we, therefore, decided to turn to route B in our retrosynthetic plan (**Figure 2-3**). As illustrated in **Scheme 3-5**, the amino moiety of sugar scaffold **3.18** was first protected with a trifluoroacetyl group to yield **3.25**, whose benzyl group was later cleaved by Pd/C catalytic hydrogenation. Diol **3.26** was then transformed into 1,2-diacetyl glycosyl donor **3.27**, which underwent smooth glycosylation with diisopropyl phosphonomethanol at the 1-position affording **3.28**.[18] Subsequent trifluoroacetyl deprotection under basic conditions provided key amino sugar intermediate **3.29**, which served as a synthon for the stepwise construction of the purine nucleobase. The final adenine nucleoside phosphonate **3.32** was successfully obtained in 40% yield (1.4%, overall yield) following the cleavage of the diisopropyl esters with TMSBr in the presence of lutidine. It is worth mentioning that 1-*O*-diisopopylphosphonomethyl-3,4-dideoxy-4-amino-*α*-D-*threo*-pentopyranoside **3.29** might constitute a versatile intermediate for the construction of a variety of purine or pyrimidine nucleobases. [17]



Scheme 3-5. Synthesis of adenine *α*-D-*threo*-pentopyranoside nucleoside phosphonate **3.32.** *Reagents, conditions and yields*: (i) ethyl trifluoroacetate, TEA, methanol, 93%; (ii) Pd/C, H_2, methanol, 90%; (iii) Ac_2O, pyridine, 80%; (iv) hydroxymethyl phosphonic acid diisopropyl ester, TMSOTf, DCM, rt; (v) 7 M NH_3 in methanol, 45% over two steps; (vi) 4,6-dichloro-5-formamidopyrimidine, DIPEA, BuOH, 80%; (vii) NH_3 in ethanol, 90%; (viii) TMSBr, acetonitrile, 40%.

3.3 Conclusion

In summary, D-*threo*-pentopyranoside nucleoside analogue bearing a phosphonate functionality rather than a nucleobase (i.e., in natural nucleosides) at the anomeric centre have been synthetized starting from L-arabinose. Different chemical pathways were explored. Specifically, stereocontrolled routes to suitably functionalized key carbohydrate scaffolds have been established and optimised. The synthesis of the adenine analogue relied on a linear building of nucleobase on a phosphonometylated 4-amino pentopyranoside synthon. This method paves the way for an improved stereoselectivity and can potentially be extended to the stepwise construction of other purine as well as pyrimidine bases.

3.4 Experimental section

General Information. All reagents and solvents were purchased from commercial sources and used as obtained. Moisture sensitive reactions were performed using oven-dried glassware under a nitrogen or argon atmosphere. NMR spectra were recorded on a Bruker Advance 300 MHz (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz; ^{31}P NMR, 121 MHz), 500 MHz (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz; ^{31}P NMR, 202 MHz), or 600 MHz (^{1}H NMR, 600 MHz; ^{13}C NMR, 150 MHz) spectrometer with tetramethylsilane as internal standard or referenced to the residual solvent signal, and 85% H_3PO_4 for ^{31}P NMR. All intermediates and final compounds were characterised by using 2D NMR (^{1}H-COSY, HSQC, NOESY, and HMBC) spectroscopic techniques. For NMR assignment of sugar protons and carbons, prime numbering is used. High-resolution mass spectra [HRMS (ESI)] were obtained on a quadruple orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min, and spectra were obtained in positive (or in negative) ionization mode with a resolution of 15000 (fwhm) using leucine enkephalin as lock mass. Precoated aluminium sheets (254 nm) were used for TLC. Products were purified by column chromatography on silica gel (60 Å, 0.035−0.070 mm, Acros Organics). Preparative RP-HPLC purifications were carried out on a Phenomenex Gemini 110A column (C18, 10 μm, 21.2 mm × 250 mm) using CH_3CN/0.05 M TEAB buffer or H_2O/ CH_3CN as eluent gradient.

Benzyl 2-*O*-acetyl-3-deoxy-*β*-L-*erythro*-pentopyranoside (2.12). A solution of **2.17** (2.00 g, 6.20 mmol) and AIBN (306 mg, 1.86 mmol) in toluene (310 mL) was heated at 100 °C until complete dissolution of the reagents. To this solution, Bu_3SnH (3.34 mL, 12.4 mmol) was added, and the mixture was stirred for 30 min at 100 °C under an argon atmosphere. After removal of all the volatiles under reduced pressure, the remaining residue was directly subjected to silica gel column chromatography to afford a mixture of 3-deoxy (60%) and 4-deoxy (10%) products, which were separated after the next mesylation step.

Benzyl 2-*O*-acetyl-4-azido-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.16). A suspension of **2.14a** (5.60 g, 16.3 mmol) and sodium azide (2.11 g, 32.52 mmol) in DMF (150 mL) was stirred at 90 °C for 8 h. After removal of all the volatiles under reduced pressure, the resulting residue was taken up with DCM (150 mL), and 100 mL of saturated aq. $NaHCO_3$ was added. The aqueous layer was extracted with DCM (2 × 75 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (15:1 hexane/EtOAc) to give **3.16** (4.30 g, 91%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44–7.21 (m, 5H, Ph), 4.94 (dt, *J* = 2.9, 3.3 Hz, 1H, H-2), 4.72 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.69 (d, *J* = 1.6 Hz, 1H, H-1), 4.49 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 3.77 (tt, *J* = 11.1, 5.5 Hz, 1H, H-4), 3.70 (ddd, *J* = 10.7, 4.9, 1.9 Hz, 1H, H-5), 3.60 (appt, *J* = 10.8 Hz, 1H, H-5′), 2.21–1.90 (m, 5H, H-3, H-3′, C*H*$_3$CO); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.7 (CH$_3$*C*O), 136.9, 128.4, 127.8, 127.7 (Ph), 94.8 (C-1), 69.0 (*C*H$_2$Ph), 68.6 (C-2), 61.4 (C-5), 52.1 (C-4), 29.1 (C-3), 20.9 (*C*H$_3$CO); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{14}H_{17}N_3O_4Na$ 314.1111; Found 314.1113.

Note: Concentrating azide-containing reaction mixtures and products through rotary evaporation have caused documented explosions. NaN_3 is known to decompose violently causing explosions at 275 °C

Benzyl 4-azido-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.17). A methanolic solution of NaOMe (5.4 M, 5.09 mL, 27.5 mmol) was added dropwise to a solution of **3.16** (4.00 g, 13.73 mmol) in methanol (150 mL). The reaction was stirred at room temperature for 3 h. After removal of all the volatiles under reduced pressure, the resulting residue was taken up with DCM (100 mL), and 50 mL of saturated aq. $NaHCO_3$ was added. The aqueous layer was extracted again with DCM (50

mL). The combined organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10:1 hexane/EtOAc) give **3.17** (3.25 g, 95%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.25 (m, 5H, Ph), 4.79 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.50 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.47 (d, *J* = 4.2 Hz, 1H, H-1), 3.80 (td, *J* = 5.3, 2.0 Hz, 1H, H-2), 3.76 (m, 1H, H-4), 3.74–3.69 (dd, *J* = 7.3, 11.2 Hz, 1H, H-5), 3.63 (ddd, *J* = 11.0, 3.8, 1.2 Hz, 1H, H-5′), 2.68 (d, *J* = 3.0 Hz, 1H, OH), 2.06–1.97 (dddd, *J* = 13.5, 8.9, 3.7, 1.0 Hz,, 1H, H-3), 1.90 (dddd, *J* = 13.5, 6.3, 4.2, 1.1 Hz, 1H, H-3′); ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.0, 128.4, 128.3, 127.9 (Ph), 99.6 (C-1), 69.5 (*C*H_2Ph), 66.5 (C-2), 63.4 (C-5), 53.4 (C-4), 32.1 (C-3); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{12}H_{15}N_3O_3Na$ 272.1006; Found 272.1003.

Benzyl 4-amino-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.18). To a solution of **3.17** (2.00 g, 8.02 mmol) in dry THF (80 mL) was added Ph_3P (0.46 g, 1.75 mmol). The mixture was stirred at room temperature for 1 h. Water (2 mL) was added and the mixture was stirred at 50 ºC for 6 h. It was then evaporated, and the resulting residue was purified by silica gel column chromatography (100/15 DCM/MeOH) to give **3.18** (1.65 g, 92%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46–7.28 (m, 5H, Ph), 4.80 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.52 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.50 (d, *J* = 3.3 Hz, 1H, H-1), 3.80 (dt, *J* = 5.9, 3.5 Hz, 1H, H-2), 3.60 (ddd, *J* = 11.0, 4.1, 1.3 Hz, 1H, H-5), 3.50 (dd, *J* = 11.0, 8.0 Hz, 1H, H-5), 3.15 (tt, *J* = 12.7, 4.2 Hz, 1H, H-4), 2.05–1.53 (m, 5H, H-3, H-3, OH, NH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.3, 128.4, 127.9, 127.8 (Ph), 99.7 (C-1), 69.4 (*C*H_2Ph), 67.6 (C-5), 66.9 (C-2), 43.6 (C-4), 36.4 (C-3); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{12}H_{18}N_1O_3$ 224.1281; Found 224.1283.

Benzyl 4-(6-chloropurin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.19). 4,6-Dichloro-5-formamidopyrimidine (860 mg, 4.48 mmol) and DIPEA (1.17 mL, 6.72 mmol) were added to a solution of pentopyranoside **3.18** (500 mg, 2.24 mmol) in *n*-butanol (25 mL). The reaction mixture was stirred for 2 h at 100 °C, the temperature was then raised to 140 °C and maintained at this temperature for 10 h. After removal of all the volatiles under reduced pressure, the remaining residue was purified by silica gel column chromatography (gradient CH_2Cl_2/MeOH, 100:0, v/v; 99:1, v/v; 20:1, v/v) to give **3.19** (605 mg, 75%) as a colorless oil. ^{1}H NMR (600 MHz, DMSO) δ

8.25 (s, 1H, H-8), 8.16 (s, 1H, H-2), 7.45–7.25 (m, 5H, Ph), 5.34 (d, *J* = 3.9 Hz, 1H, OH), 4.92 (tt, *J* = 11.1, 4.2 Hz, 1H, H-4′), 4.76 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.66 (d, *J* = 1.8 Hz, 1H, H-1′), 4.54 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.10 (appt, *J* = 10.6 Hz, 1H, H-5′), 3.82 (dt, *J* = 6.0, 3.3 Hz, 1H, H-2′), 3.76 (ddd, *J* = 10.6, 4.4, 1.6 Hz, 1H, H-5′), 2.71 (td, *J* = 12.6, 3.0 Hz, 1H, H-3′), 1.99 (dt, *J* = 12.8, 3.8 Hz, 1H, H-3′); ^{13}C NMR (150 MHz, DMSO) δ 156.2 (C-6), 152.4 (C-2), 149.6 (C-4), 139.6 (C-8), 137.9, 128.4, 127.9, 127.7 (Ph), 119.1 (C-5), 98.1 (C-1′), 68.2 (*C*H$_2$Ph), 65.7 (C-2′), 61.7 (C-5′), 46.7 (C-4′), 31.6 (C-3′); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{18}Cl_1N_4O_3$ 361.1061; Found 361.1056.

Benzyl 4-(adenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.20). A solution of **3.19** (300 mg, 0.83 mmol) in saturated ammonia in ethanol (10 mL) was stirred in a sealed flask at 60 ºC for 8 h. After removal of all the volatiles under reduced pressure, the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 15:1) to give **3.20** (269 mg, 95%) as a colorless oil. ^{1}H NMR (500 MHz, DMSO) δ 8.82 (s, 1H, H-8), 8.79 (s, 1H, H-2), 7.46–7.29 (m, 5H, Ph), 5.38 (d, *J* = 4.0 Hz, 1H, OH), 5.07 (tt, *J* = 11.5, 4.3 Hz, 1H, H-4′), 4.76 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J* = 1.8 Hz, 1H, H-1′), 4.55 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.16 (appt, *J* = 10.6 Hz, 1H, H-5′), 3.99–3.74 (m, 2H, H-5′ and H-2′), 2.76 (td, *J* = 12.5, 3.0 Hz, 1H, H-3′), 2.06 (ddd, *J* = 12.7, 5.2, 2.8 Hz, 1H, H-3″); ^{13}C NMR (126 MHz, DMSO) δ 152.0 (C-4), 151.4 (C-2), 149.2 (C-6), 146.4 (C-8), 137.8, 131.2 (C-5), 128.4, 127.8, 127.6 (Ph), 98.0 (C-1′), 68.3 (*C*H$_2$Ph), 65.6 (C-2′), 61.2 (C-5′), 47.9 (C-4′), 31.4 (C-3′); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{20}N_5O_3$ 342.1561; Found 342.1566.

Benzyl 2-*O*-benzoyl-4-(*N*^{6},*N*^{6}-dibenzoyladenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.21). To a solution of **3.20** (200 mg, 0.59 mmol) in dry pyridine (6 mL) at 0 ºC was added benzoyl chloride (0.46 mL, 3.52 mmol), and the reaction mixture was warmed to room temperature and stirred for 3 h. After removal of all the volatiles under reduced pressure, the resulting residue was partitioned between saturated aq. $NaHCO_3$ (10 mL) and DCM (20 mL). The water layer was further extracted with DCM (1 × 10 mL). The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (gradient hexane/EtOAc, 10:1, v/v; 3:1) to give **3.21** (282 mg, 88%) as a white

solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H, H-2), 8.21–8.04 (m, 3H, H-8, Ph), 8.00–7.74 (m, 4H, Ph), 7.65–7.53 (m, 1H, Ph), 7.51–7.27 (m, 14H, Ph), 5.36 (td, *J* = 4.2, 1.5 Hz, 1H, H-2′), 5.15 (tt, *J* = 11.7, 4.3 Hz, 1H, H-4′), 5.01 (d, *J* = 1.3 Hz, 1H, H-1′), 4.86 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.64 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.24 (appt, *J* = 10.9 Hz, 1H, H-5′), 4.03 (ddd, *J* = 10.4, 5.1, 1.8 Hz 1H, H-5′), 3.01 (td, *J* = 13.3, 3.1 Hz, 1H, H-3′), 2.44 (dt, *J* = 13.2, 5.2 Hz, 1H, H-3′); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2 (Ph*C*O), 165.4 (Ph*C*O), 153.1 (C-4), 152.0 (C-2), 152.0 (C-6), 142.8 (C-8), 136.8, 134.1, 133.5, 132.9, 129.8, 129.4, 128.7, 128.6, 128.5, 128.1, 127.9(Ph), 127.4 (C-5), 95.1 (C-1′), 69.5 (*C*H$_2$Ph), 68.9 (C-2′), 61.5 (C-5′), 47.8 (C-4′), 29.0 (C-3′); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{38}H_{32}N_5O_6$ 654.2346; Found 654.2358.

2-*O*-Benzoyl-4-(*N*^{6}-benzoyladenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranose (3.22). To a stirred solution of **3.21** (140 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (4 mL) was added 1 M BCl_3 (0.76 mL, 0.76 mmol) at -78 °C. Then, the reaction mixture was slowly warmed to 0 °C over 2.5 h and stirred at this temperature for 30 min. After completion of the reaction, the mixture was cooled to -20 °C and quenched by dropwise addition of saturated aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layer was washed with brine (10 mL) and dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was filtered through a pad of silica gel to give crude **3.22** as a colourless oil, which was used as such in the following step. HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{24}H_{22}N_5O_5$ 460.1615; Found 460.1617.

Acetyl 2-*O*-benzoyl-4-(*N*^{6}-benzoyladenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.23). Compound **3.23** was prepared using a similar procedure as that described for **2.26c** starting from **3.22** (117 mg, 0.25 mmol), pyridine (5 mL), and acetic anhydride (0.048 mL, 0.5 mmol), and obtained after silica gel column chromatography (gradient hexane/EtOAc, 8:1, v/v; 3:1, v/v;) as a colorless sticky oil (99 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 (s, 1H, H-2), 8.19–8.05 (m, 3H, H-8, Ph), 8.02 (d, *J* = 7.4 Hz, 2H, Ph), 7.63–7.53 (m, 2H, Ph), 7.49 (td, *J* = 7.9, 2.1 Hz, 4H, Ph), 6.19 (d, *J* = 1.8 Hz, 1H, H-1′), 5.33 (td, *J* = 5.3, 1.8 Hz, 1H, H-2′), 5.14 (tt, *J* = 11.0, 4.3 Hz, 1H, H-4′), 4.34 (appt, *J* = 10.8 Hz, 1H, H-5′), 4.12 (ddd, *J* = 11.1, 4.7, 1.8 Hz, 1H, H-5″), 3.04 (ddd, *J* = 14.3, 12.1, 3.2 Hz, 1H, , H-3′), 2.51 (dt, *J* = 14.2, 4.7 Hz, 1H, H-3″), 2.24 (s, 3H, C*H*$_3$CO); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8 (CH$_3$*C*O), 165.3 (Ph*C*O), 152.6 (C-2), 152.2 (C-4), 150.0 (C-6),

141.3(C-8), 133.8, 133.6, 132.9, 123.0, 129.2, 128.9, 128.7, 128.1 (Ph), 123.5 (C-5), 89.6 (C-1′), 68.0 (C-2′), 63.3 (C-5′), 47.9 (C-4′), 29.3 (C-3′), 21.1 (*C*H_3CO); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{26}H_{24}N_5O_6$ 502.1721; Found 502.1723.

Benzyl 3,4-dideoxy-4-trifluoroacetylamino-*α*-D-*threo*-pentopyranoside (3.25). To a mixture of **3.18** (1 g, 4.48 mmol) and triethylamine (1.87 mL, 13.44mmol) in methanol (50 mL), ethyl trifluoroacetate (1.07 mL, 8.96 mmol) was added dropwise. The mixture was stirred at room temperature for 2 h. After removal of all the volatiles under reduced pressure, the resulting residue was purified by silica gel column chromatography (hexane/EtOAc, 3:1) to give **3.25** (1.3g, 91%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55–7.16 (m, 5H, Ph), 6.91 (d, *J* = 7.9 Hz, 1H, NH), 4.80 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 4.53 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 4.45 (d, *J* = 4.8 Hz, 1H, H-1), 4.27 (m, 1H, H-2), 3.80–3.61 (m, 3H, H-5, H-5′ and OH), 3.01 (d, *J* = 3.8 Hz, 1H, H-4), 2.17–1.98 (ddd, *J* = 13.5, 6.7, 4.0 Hz, 1H, H-3), 1.88–1.69 (ddd, *J* = 13.5, 7.4, 4.1 Hz, 1H, H-3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.0 (q, $^2J_{F,C}$ = 37.8 Hz, *C*OCF_3), 137.0, 128.64, 128.20 (Ph), 115.8 (q, $^1J_{F,C}$ = 288.9 Hz, *C*F_3), 101.3 (C-1), 70.3 (C-2), 66.6 (*C*H_2Ph), 64.6 (C-5), 44.4 (C-4), 32.8 (C-3); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{14}H_{16}F_3N_1O_4Na$ 342.0924; Found 342.0916.

3,4-dideoxy-4-trifluoroacetylamino-*α*/*β*-D-*threo*-pentopyranose (3.26). To a solution of **3.25** (1.00 g, 3.13 mmol) in methanol (50 mL) was added 10% Pd/C (1 g, 0.94 mmol), and evacuation-replacement cycles were carried out using a hydrogen baloon (3×). The reaction mixture was stirred at room temperature for 20 h under an atmospheric pressure of hydrogen. After completion of the reaction, the catalyst was removed by filtration through a pad of Celite and washed with methanol. The filtrate was concentrated under reduced pressure to afford **3.26** (590 mg, 82%) as a colorless foam without further purification.^{1}H NMR (600 MHz, DMSO) δ 9.32 (d, *J* = 6.7 Hz, 0.16 H, NH*β*), 9.26 (d, *J* = 7.9 Hz, 1H, NH*α*), 6.34 (d, *J* = 4.0 Hz, 1H, OH-1*α*), 6.29 (d, *J* = 4.3 Hz, 0.16 H, OH-1*β*), , 4.94 (bs, 1H, OH-2*α*), 4.85–4.77 (m, 0.22H, OH-2*β*), 4.69 (dd, *J* = 4.8, 2.0 Hz, 0.18 H, H-1*β*), 4.68 (dd, *J* = 3.3, 2.2 Hz, 1H, H-1*α*), 4.17–4.08 (m, 1H, H-4*α*), 4.07–4.00 (m, 0.2 H, H-4*β*), 3.80 (ddd, *J* = 11.5, 3.6, 0.9 Hz, 0.18 H, H-5*β*), 3.66 (t, *J* = 10.2 Hz, 1H, H-5*α*), 3.56 (bs, 1.14 H, H-2*α*), 3.43 (ddd, *J* = 10.6, 4.6, 1.6 Hz, 1 H, H-5′*α* and H-2*β*), 3.30 (dd, *J* = 11.1, 6.6 Hz, 0.3 H, H-5′*β*), 1.96 (ddd, *J* = 13.1, 10.9, 3.2 Hz, 1H, H-3*α*), 1.88 (dddd, *J* = 13.3, 7.3, 4.3, 0.9 Hz, 0.2 H, H-3*β*),

1.65 (dtd, *J* = 13.1, 4.8, 1.4 Hz, 1H, H-3′*α*), 1.42 (ddd, *J* = 13.3, 7.8, 3.7 Hz, 0.14 H, H-3′*β*); ^{13}C NMR (151 MHz, DMSO) δ 156.2 (q, $^{2}J_{F,C}$ = 38 Hz, *C*OCF$_3$), 115.9 (q, $^{1}J_{F,C}$ = 289 Hz, *C*F$_3$), 93.7 (C-1*β*), 93.4 (C-1*α*), 68.1(C-2*β*), 66.4 (C-2*α*), 62.8 (C-5*β*), 60.8 (C-5*α*), 44.3 (C-4*β*), 42.6 (C-4*α*), 32.2 (C-3*β*), 31.3 (C-3*α*); HRMS (ESI-TOF) m/z: [M+Na]$^+$ calcd for $C_7H_{10}F_3N_1O_4Na$ 252.0454; Found 252.0448.

1,2-Di-*O*-acetyl-3,4-dideoxy-4-trifluoroacetylamino-*α*-D-*threo*-pentopyranoside (3.27). 1*O*,2*O*-Diacetylated compound **3.27** was prepared using a similar procedure as that described for **2.26c** starting from **3.26** (500 mg, 2.18 mmol), Ac_2O (0.62 mL, 6.55 mmol), and pyridine (25 mL), and obtained after silica gel column chromatography (gradient hexane/EtOAc, 6:1, v/v; 2:1, v/v) as a colorless sticky oil (550 mg, 80%), ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.78 (d, *J* = 7.9 Hz, 1H, NH), 5.88 (d, *J* = 2.8 Hz, 1H, H-1), 4.93 (td, *J* = 6.1, 3.8 Hz, 1H, H-2), 4.37 (dtt, *J* = 17.6, 7.9, 4.6 Hz, 1H, H-4), 3.88 (ddd, *J* = 11.4, 4.2, 1.5 Hz,1H, H-5), 3.66 (dd, *J* = 11.0, 9.7 Hz, 1H, H-5′), 2.17–2.09 (m, 8H, C*H*$_3$CO, C*H*$_3$CO, H-3, H-3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0 (CH$_3$*C*O), 168.9 (CH$_3$*C*O), 157.0 (q, $^{2}J_{F,C}$ = 38 Hz, *C*OCF$_3$), 115.5 (q, $^{1}J_{F,C}$ = 289 Hz, *C*F$_3$), 89.8 (C-1), 67.1 (C-2), 63.4 (C-5), 42.5 (C-4), 29.5 (C-3), 21.0 (*C*H$_3$CO), 20.9 (*C*H$_3$CO); HRMS (ESI-TOF) m/z: [M+Na]$^+$ calcd for $C_{11}H_{14}F_3N_1O_6Na$ 336.0666; Found 336.0660.

Diisopropylphosphonomethyl 2-*O*-acetyl-3,4-dideoxy-4-trifluoroacetylamino-*α*-D-*threo*-pentopyranoside (3.28). Phosphonomethylated compound **3.28** was prepared using a similar procedure as that described for **2.27** starting from **3.27** (300 mg, 0.96 mmol), TMSOTf (0.35 mL, 1.92 mmol), diisopropylphosphonomethanol (564 mg, 2.87 mmol) in dry DCM (15 mL). Purification by silica gel column chromatography (gradient CH_2Cl_2/MeOH, 99:1, v/v; 49:1, v/v; 24:1, v/v) afforded **3.28** as a colorless sticky oil, which was still contaminated with diisopropylphosphonomethanol. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.3 Hz, 1H, NH), 4.91 (td, *J* = 4.7, 2.2 Hz, 1H, H-2), 4.83–4.69 (m, 2H, [C*H*(CH$_3$)$_2$]), 4.69 (d, *J* = 1.8 Hz, 1H, H-1), 4.45–4.34 (m, 1H，H-4), 3.92 (dd, *J* = 13.2, 10.0 Hz, 1H, PC*H*$_2$), 3.79–3.72 (ddd, *J* = 10.9, 5.1, 1.7 Hz, 1H, H-5), 3.72–3.66 (m, 1H, PC*H*$_2$), 3.64 (appt, *J* = 10.7 Hz, 1H, H-5′), 2.12 (s, 3H, C*H*$_3$CO), 2.11–2.08 (m, 1H, H-3), 2.07–1.99 (dt, *J* = 13.7, 4.2 Hz, 1H, H-3′), 1.38–1.30 (m, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.2 (CH$_3$*C*O), 157.1 (q, $^{2}J_{F,C}$ = 37.0 Hz, *C*OCF$_3$), 115.8 (q, $^{1}J_{F,C}$ =

288.9 Hz, *C*F_3), 96.4 (d, $^3J_{P,C}$ = 11.1Hz, C-1), 71.8, 71.7 (d, $^2J_{P,C}$ = 6.2 Hz, [*C*H(CH_3)$_2$]), 68.0 (C-2), 60.9 (d, $^1J_{P,C}$ = 171.7 Hz, P*C*H_2), 61.4 (C-5), 41.8 (C-4), 29.0 (C-3), 24.0 [CH(*C*H_3)$_2$]; ^{31}P NMR (121 MHz, $CDCl_3$) δ 19.0; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{16}H_{28}F_3N_1O_8P_1$ 450.1499; Found 450.1497.

Diisopropylphosphonomethyl 4-amino-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.29). Compound **3.29** was prepared using a similar procedure as that described for **2.28** starting from crude **3.28** and 7 M NH_3 in MeOH (15 mL), and obtained after silica gel column chromatography (gradient CH_2Cl_2/MeOH, 50:1, v/v; 25:1, v/v; 6:1, v/v) as a colorless sticky oil (121 mg, 40% over two steps), ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.81–4.67 (m, 2H, [C*H*(CH_3)$_2$]), 4.54 (d, *J* = 3.0 Hz, 1H, H-1), 3.99 (dd, *J* = 13.6, 9.1 Hz, 1H, PC*H*$_2$), 3.91 (m, 1H, H-2), 3.76–3.68 (m, 2H, H-5 and PC*H*$_2$), 3.65 (appt, *J* = 9.5 Hz, 1H, H-5′), 3.49–3.42 (m, 1H, H-4), 2.06–2.01 (m, 1H, H-3), 1.96 (td, *J* = 11.2, 3.2 Hz, 1H, H-3′), 1.37–1.29 (m, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (151 MHz, $CDCl_3$) δ 101.2 (d, $^3J_{P,C}$ = 10.4 Hz, C-1′), 71.7, 71.5 (d, $^2J_{P,C}$ = 5.6 Hz, [*C*H(CH_3)$_2$]), 65.7 (C-2), 64.7 (C-5), 61.6 (d, $^1J_{P,C}$ = 170.9 Hz, P*C*H_2), 43.6 (C-4), 33.6 (C-3), 24.0 [CH(*C*H_3)$_2$]; ^{31}P NMR (121 MHz, $CDCl_3$) δ 19.3; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{12}H_{27}N_1O_6P_1$ 312.1570; Found 312.1570.

Diisopropylphosphonomethyl 4-(6-chloropurin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.30). Compound **3.30** was prepared using a similar procedure as that described for **3.19** starting from **3.29** (120 mg, 0.39 mmol), 4,6-dichloro-5-formamidopyrimidine (96 mg, 0.5 mmol), and DIPEA (0.2 mL, 1.16 mmol) in n-butanol (5 mL), and obtained after silica gel column chromatography (gradient CH_2Cl_2/MeOH, 99:1, v/v; 49:1, v/v; 24:1, v/v) as a colorless sticky oil (130 mg, 75%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.75 (s, 1H, H-2), 8.37 (s, 1H, H-8), 5.14 (tt, *J* = 12.6, 4.1 Hz, 1H, H-4′), 4.80 (tt, *J* = 12.4, 6.2 Hz, 2H, [C*H*(CH_3)$_2$]), 4.74 (d, *J* = 3.8 Hz, 1H, H-1′), 4.29 (dd, *J* = 11.5, 7.7 Hz, 1H, H-5′), 4.09 (dd, *J* = 13.8, 9.4 Hz, 1H, PC*H*$_2$), 3.98 (dd, *J* = 11.7, 3.8 Hz, 1H, H-5′), 3.98 (td, *J* = 7.0, 3.8 Hz, 1H, H-2′), 3.84 (dd, *J* = 13.8, 8.3 Hz, 1H, PC*H*$_2$), 2.71 (ddd, *J* = 13.0, 9.1, 3.6 Hz, 1H, H-3′), 2.20 (dt, *J* = 13.2, 4.8 Hz, 1H, H-3′), 1.40–1.32 (m, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.0 (C-2), 151.9 (C-4), 151.4 (C-6), 144.0 (C-8), 131.8 (C-5), 102.2 (d, $^3J_{P,C}$ = 11.1 Hz, C-1′), 71.9, 71.7 (d, $^2J_{P,C}$ = 6.2 Hz, [*C*H(CH_3)$_2$]), 66.0 (C-2′), 63.8 (C-5′), 62.1 (d, $^1J_{P,C}$ = 171.1 Hz, P*C*H_2), 48.8 (C-4′), 32.7 (C-3′), 24.2 [CH(*C*H_3)$_2$]; ^{31}P NMR

(121 MHz, $CDCl_3$) δ 19.6; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{27}Cl_1N_4O_6P_1$ 449.1351; Found 449.1349.

Diisopropylphosphonomethyl 4-(adenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.31). Compound **3.31** was prepared using a similar procedure as that described for **3.20** starting from **3.30** (100 mg, 0.22 mmol) in a mixture of aq. NH_3 (25%)/1,4-dioxane,1:1, v/v; (10mL), and obtained after silica gel column chromatography (gradient CH_2Cl_2/MeOH, 50:1, v/v; 25:1, v/v; 15:1, v/v) as a colorless sticky oil (88 mg, 92%). ^{1}H NMR (600 MHz, MeOD) δ 8.23 (d, *J* = 7.7 Hz, 1H, H-8), 8.19 (s, 1H, H-2), 5.08–4.97 (tt, *J* = 10.7, 3.7 Hz,1H, H-4′), 4.82–4.75 (m, 2H, [C*H*$(CH_3)_2$]), 4.74 (d, *J* = 2.3 Hz, 1H, H-1′), 4.14 (appt, *J* = 10.5 Hz, 1H, H-5′), 4.10–4.05 (dd, *J* = 14.0, 9.2 Hz, 1H, PC*H*$_2$), 3.95–3.89 (m, 2H, H-2′ and H-5″), 3.88 (dd, *J* = 14.0, 9.2 Hz, 1H, PC*H*$_2$), 2.70 (ddd, *J* = 14.6, 11.1, 3.0 Hz,1H, H-3′), 2.19–2.14 (m, 1H, H-3′), 1.40–1.34 (dd, *J* = 6.2, 1.9 Hz, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (151 MHz, MeOD) δ 157.3 (C-6), 153.7 (C-2), 150.8 (C-4), 140.9 (C-8), 120.1 (C-5), 101.7 (d, $^3J_{P,C}$ = 11.6 Hz, C-1′), 73.4, 73.3 [*C*H$(CH_3)_2$], 67.2 (C-2′), 63.6 (C-5′), 62.2 (d, $^1J_{P,C}$ = 170.9 Hz, P*C*H$_2$), 48.5 (C-4′), 32.9 (C-3′), 24.3 [CH(*C*H$_3$)$_2$]; ^{31}P NMR (121 MHz, MeOD) δ 20.0; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{29}N_5O_6P_1$ 430.1850; Found 430.1848.

Phosphonomethyl 4-(adenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (3.32). Compound **3.32** was prepared using a similar procedure as that described for **2.29** starting from **3.31** (70 mg, 0.16 mmol), 2,6-lutidine (0.15 mL, 1.3 mmol), and TMSBr (0.17 mL, 0.16 mmol) in dry acetonitrile (2 mL) and obtained as a colorless sticky oil (22 mg, 40%). ^{1}H NMR (600 MHz, D_2O) δ 8.22 (s, 1H, H-8), 8.05 (s, 1H, H-2), 4.72 (m, 1H, H-4′), 4.71 (d, *J* = 3.3 Hz, 1H, H-1′), 4.09 (dd, *J* = 11.4, 8.7 Hz, 1H, H-5′), 3.97 (td, *J* = 4.8, 3.3 Hz, 1H, H-2′), 3.92 (dd, *J* = 11.4, 4.0 Hz, 1H, H-5′), 3.90 (dd, *J* = 13.2, 9.3 Hz, 1H, PC*H*$_2$), 3.64 (dd, *J* = 13.0, 9.5 Hz, 1H, PC*H*$_2$), 2.56 (ddd, *J* = 13.4, 10.1, 3.4 Hz, 1H, H-3′), 2.15 (dt, *J* = 13.3, 4.3 Hz, 1H, H-3″); ^{13}C NMR (151 MHz, D_2O) δ 156.5(C-6), 153.3(C-2), 149.8 (C-4), 141.7 (C-8), 119.4 (C-5), 102.4 (d, $^3J_{P,C}$ = 11.8 Hz, C-1′), 67.1 (C-2′), 65.3 (d, $^1J_{P,C}$ = 158.5 Hz, P*C*H$_2$), 64.1 (C-5′), 48.7 (C-4′), 32.6 (C-3′); ^{31}P NMR (121 MHz, D_2O) δ 15.2; HRMS (ESI-TOF) m/z: $[M-H]^+$ calcd for $C_{11}H_{15}N_5O_6P_1$ 344.0765; Found 344.0760.

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18. The formation of the β isomer was also observed. However isolation by column chromatography provided only trace amounts of material insufficiently pure for complete characterisation.

Chapter 4. Expansion of the synthetic routes and biological/biochemical evaluation

Abstract: Uracil and hypoxanthine 1´-*O*-phosphonomethyl nucleoside phosphonates are synthesised by following the strategies developed for the thymine and adenine analogues. The solution conformation of these phosphonates, carrying A, T, U and H, were studied by NMR and found to exhibit preferential a 1,4-syn relationship of bulky substituents with the equatorial orientation of the nucleobase. Adenine and thymine nucleoside phosphonate were tested against several herpesviruses, which included HSV, VZV and HCMV. However, both of them are devoid of activity. The diphosphate of the adenine nucleoside phosphate was synthesised and tested against engineered polymerases. The test showed that these polymerases were able to recognise the modified six-membered diphosphate analogue and a maximum of two phosphonate nucleosides could be added to the primer strand.

4.1 Introduction

XNAs are synthetic nucleic acids alternative who has different backbone motifs than in natural nucleic acids and are aimed as alternative genetic polymers.[1] The building blocks of XNA do not occur in nature thus have to be synthesised and supplied to cells which will need evolved polymerase enzymes for XNA synthesis. Polymerases are key in the process of the replication and transcription of XNAs. For the expansion of the genetic codes, the base modified nucleic acid have been developed, which also needs the engineering of xenobiotic polymerases for its replication. Advances in directed polymerases engineering have been successful in demonstrating the replication and *in vitro* evolution of HNA[2], as well as XNAzyme[3].

Significant progress is made in the development of sugar[4] and nucleobase[5] modified XNAs. In order to achieve orthogonality, the development of XNAs that do not interact and affect the natural biology is still in its infancy and is a hurdle for their *in vivo* applications. One of the most promising phosphate modification strategies is the use of nucleoside phosphonates, and this principle is applied to develop potent antiviral compounds, but not yet in the XNA field. (see **Chapter 1, Section 1.2.1** and **1.4**) As an example, Therminator polymerase has proven to recognise diphosphate of *threosyl* nucleoside phosphonate to form phosphonate oligonucleotide mimetics.[6] The synthesis and study of other backbone modified nucleoside phosphonates as substrates of engineered polymerases will be fundamental and vital for the discovery of the orthogonal XNAs. The phosphonate bond has the additional advantages that it endows the XNA with higher enzymatic stability.

Except for hexitol nucleosides, most the synthesised six-membered pyranosyl nucleosides are devoid of antiviral activity.[7] The conformational differences between these inactive nucleosides and hexitol nucleosides are thought to account for this. These inactive nucleosides adopt conformations in which nucleobases are oriented equatorially, whereas in hexitol nucleosides the nucleobase is in axial orientation and the hydroxymethyl group is in equatorially oriented.[8]

The hexitol nucleoside can be considered as a mimic of a furanose nucleoside with an *N*-type

conformation as found in RNA, and this may explain the initial success of the evaluation of HNA as potential XNA.[7-8] However, it is clear that HNA is not enough orthogonal to RNA to consider it for further development in the field of synthetic biology.

The phosphonate mimetics of hexitol nucleoside phosphates, which have been synthesised in this thesis, may adopt similar or different conformation as hexitol nucleosides in HNA. Their study as an antiviral (relates to kinases recognition) and application as substrates in XNA field is of high relevance and results could be correlated to the observed conformational preferences of the six-membered ring.[8] If the nucleobase is oriented equatorially instead of axial, the XNA, derived from the proposed phosphonate nucleosides would be more orthogonal than HNA.

In this chapter, we describe the following experiments: 1) the synthetic experience obtained during the synthesis of thymine and adenine nucleoside phosphonate was utilised for the synthesis uracil and hypoxanthine congeners of these nucleoside phosphonates. 2) NMR studies of these nucleoside phosphonates were conducted thoroughly to gain information on the conformational preference of this novel structure. 3) a bioactivity test of thymine and adenine nucleoside phosphonate has been carried out using herpesviruses, which include HSV-1, HSV-2, HCMV and VZV. 4) the diphosphate of adenine nucleoside phosphonate was synthesised and tested as substrate of several polymerases.

4.2 Results and Discussion

For the evolution of XNAs *in vivo*, it is usually necessary to synthesise the nucleotide analogues with all four bases (A, C, T, G). In this way fully, modified XNA's can be synthesized from each codon. However, this is a hard burden for the organic chemist to synthesise all four protected nucleoside phosphoramidites for the oligonucleotide synthesis. Therefore, we envisaged the use of only two bases to code the various amino acids in the active site of an enzyme (a binary genetic cassette). The enzyme that is used to evaluate the viability of XNA's *in vivo* is thymidylate synthase, and its active site (Ala-Pro-Cys-His) is coded by GCG CCG TGC CAT.[9] However this enzyme may serve the same function when the amino residue are Val-Val-Cys-Gly in its active site, coded by the sequence GTT GTT TGT GGT. In this way, only two (G/T) protected nucleoside phosphoramidites

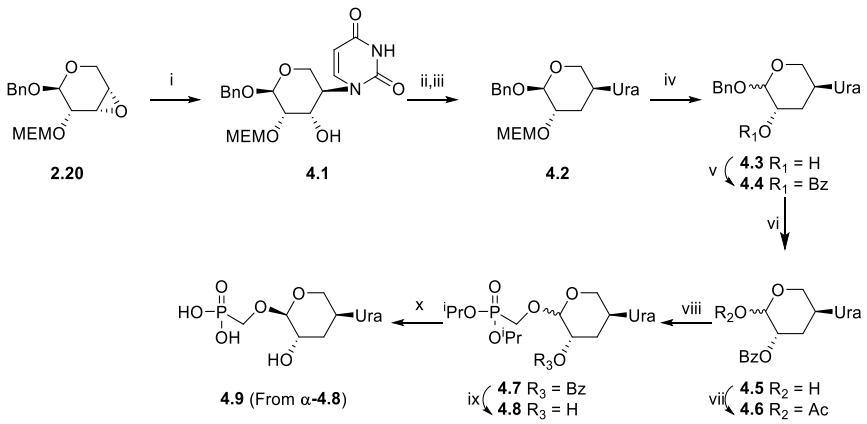
are needed to be synthesised for oligonucleotide preparation to evaluate the XNA at the level of four codons fully. However,disadvantage in this system is that G nucleosides are more difficult to synthesise as compared with the A, C and T nucleosides. In addition, G nucleobase moiety requires protecting group during the synthesis of oligonucleotide. This is not the case for a hypoxanthine base (Hx). Hx forms base-pairing with C, as it has close resemblance with guanine and can form two hydrogen bonds with C. Thus the researchers have decided to replace G by Hx in the binary genetic cassette. It is known that hypoxanthine results in more mismatch formation[10-11] than guanine base. However, this is not so important in this case, as only viable clones are isolated to evaluate the potential of backbone modified XNAs *in vivo*. A further chemical simplification is the alteration of the thymine base into uracil base. Indeed, the uracil base could be chemically converted into cytosine base using efficient available amination reactions.[12-14] So that it is not necessary to couple two different bases (T and C) on the same modified sugar to have two informative pyrimidine bases available (this, of course, also holds for T and ^{Me}C). In this way, initial evaluation of new backbone modified XNA's can be carried out with minor chemical efforts. For this reason, we started the synthesis of the phosphonate pyranosyl nucleosides with Hx and U as base moieties (**4.11** in **Scheme 4-2** and **4.9** in **Scheme 4-1)**.

	amino acid Sequence	oligonucleotide Sequence
active site in thymidylate synthetase	Ala-Pro-Cys-His	GCG CCG TGC CAT
simplified active site with binary codons G/T	Val-Val-Cys-Gly	GTT GTT TGT GGT
simplified Hx/U binary code	Val-Val-Cys-Gly	HxUU HxUU UHxU HxHxU

Figure 4-1: A binary genetic cassette consisting of Hx and U base to simplify the chemical efforts for the evaluation of XNA *in vivo*.

4.2.1 Synthesis of Phosphonomethyl 3,4-dideoxy-4-(uridin-1-yl)-*α*-D-*threo*-pentopyranoside 4.9

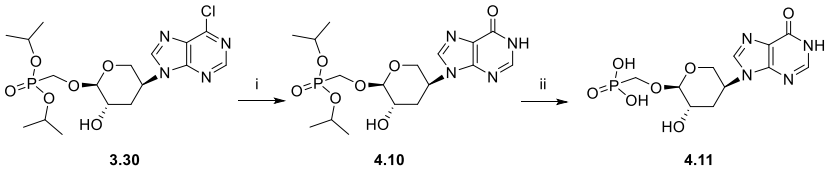
The synthesis of uracil nucleoside phosphonate follows a similar strategy (**Chapter 1**, **Scheme 2-2**) as that of thymine nucleoside phosphonate with the overall yield of 0.94 %, using epoxide **2.20** as the key intermediate. Epoxide ring opening by uracil underwent smoothly followed by similar deoxygenation and deprotection steps as described previously for thymine nucleoside. Epimeric mixture at the anomeric centre was also obtained during acid-mediated cleavage of MEM protection. Noteworthy, no reduction of the pyrimidine ring was observed during debenzylation using cyclohexene as the hydrogen source and palladium on carbon as a catalyst. During the phosphonylation reaction, a slightly better yield was obtained compared to the result obtained with the thymine base. Again, it proved to be a troublesome synthetic pathway. Upmost anhydrous condition and careful control of the reaction parameters are needed for a productive outcome. The overall synthetic route is shown in **Scheme 4-1.**



Scheme 4-1. Synthesis of uracil nucleoside phosphonate **4.9.** *Reagents, conditions and yields*: (i) uracil, DBU, DMF, 85%; (ii) TCDI, DMAP, DCM; (iii) Bu_3SnH, AIBN, toluene, 80% over two steps; (iv) 1M HCl in dioxane, 75% for ***α*-4.3** and 6% for ***β*-4.3**; (v) BzCl, pyridine, 0 °C, 90%; (vi) Pd/C, cyclohexene, ethanol, 88%; (vii) Ac_2O, pyridine, 92%; (viii) TMSOTf, hydroxymethyl phosphonic acid diisopropyl ester, CH_2Cl_2; (ix) 7 M NH_3 in methanol, 25% over two steps for ***α*-4.8**; (x) TMSBr, 2,6-lutidine, acetonitrile, 40%.

4.2.2 Synthesis of Phosphonomethyl 3,4-dideoxy-4-(hypoxanthin-9-yl)-*α*-D-*threo*-pentopyranoside 4.11

The conversion of 6-Cl purine nucleoside phosphonate **3.30** to its hypoxanthine congeners **4.11** (overall yield 1.1%) was successful, although in moderate yield, using 2-mercaptoethanol and sodium methoxide.



Scheme 4-2. Synthesis of hypoxanthine nucleoside phosphonate **4.11**. *Reagents, conditions and yields*: (i) 2-mercaptoethanol, NaOMe, MeOH, reflux, 20 h, 73%; (ii) TMSBr, CH_3CN, 40%.

4.2.3 Conformational study

A detailed solution conformational analysis of the final phosphonate products **2.29**, **3.32**, **4.9** and **4.11** was carried out using NMR spectroscopy. A preference for a 1,4-*syn* substitution pattern (**Figure 4-2**) was observed and established for both pyrimidine and purine derivatives following complete assignment using 1D and 2D NMR experiments and based on the values of the coupling constants observed in the ^{1}H NMR spectra. **Table 4-1** summarises the proton NMR spectroscopic data acquired for thymine containing nucleoside phosphonate **2.29**.

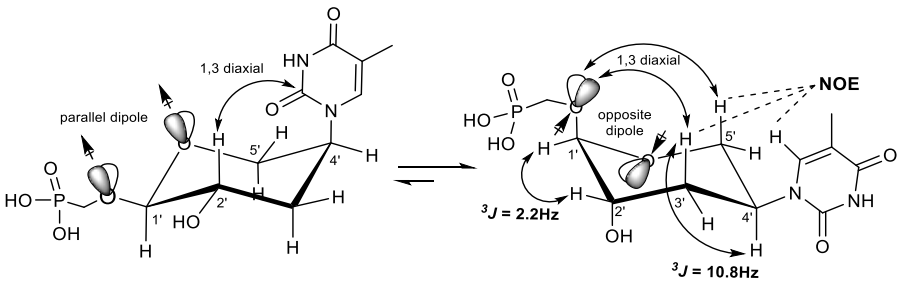


Figure 4-2. Schematic representation of the solution phase preferential conformation of 1′-phosphonate D-*threo*-pentopyranose nucleoside bearing thymine as a base (**2.29**) as determined by NMR studies. Dashed

lines indicate the observed NOE interaction between the H6 of thymine and the axially positioned H3′ and H5′.

As shown in the ^{1}H NMR data of **2.29** (**Table 4-1**), H1′ (δ 4.68) and H2′ (δ 4.04) appear as a doublet and triplet of doublets, respectively, with a small $^{3}J_{1',2'}$ diequatorial coupling constant, which is consistent with a chair conformation with both phosphonomethyl group and 2′-OH occupying an axial orientation. The td pattern of H2′ with the largest splitting equal to 3.6 Hz is characteristic of an equatorial position of this proton. Furthermore, the H4′ signal at δ 4.79 consists of a triplet of triplets arising from the coupling with the protons at C3′ and C5′; notably, the observed $^{3}J_{4',3'}$ and $^{3}J_{4',5'}$ values of 12.1 and 10.8 Hz between H4′ and H3′a and H5′a, respectively, support an equatorial orientation of the nucleobase at the 4′-position. This chair conformation of the pyranose ring was further confirmed by the presence of a clear NOE correlation between the H6 of thymine and the axially positioned H3′ and H5′.

Such 1,4-*syn*-relationship is similar to that previously reported between the heterocyclic base and either the 5′-hydroxymethyl to be phosphorylated in 1,5-anhydrohexitol nucleosides[15] or the 4′-*O*-phosphonomethyl moiety in 2′,3′-dideoxy-2′,3′-didehydro-pentopyranosyl[16] nucleoside phosphonates. However, nucleoside phosphonates **2.29**, **3.32**, **4.9** and **4.11** differ in their conformational behaviour from their hexitol nucleoside phosphate counterpart by the orientation of the nucleobase, which is equatorial rather than axial, with both the hydroxymethyl and secondary OH groups at the 1′- and 2′-positions respectively are axially positioned. Thus, it can be assumed that the preferred conformation in **2.29**, **3.32**, **4.9** and **4.11** is sterically enforced in order to avoid the 1,3-diaxial steric interactions among bulky substituents and/or between the lone pairs of the ring oxygen and the anomeric oxygen, along with the favourable opposite direction of the dipole moment.

Table 4-1. ^{1}H NMR Analysis of the Sugar Skeleton of Thymine D-*Threo*-pentopyranoside Nucleoside Phosphonate 2.29 (600 MHz, D_2O).

bond	orientation[a]	δ (ppm)	multiplicity	*J* (Hz) (D_2O)	^{3}J connection
C-H1′	e	4.68	d	**2.2**	H1′e-H2′e
C-H2′	e	4.04	td	2.2–3.0	H2′e-H1′e
					H2′e-H3′e
				3.6	H2′e-H3′a
C-H3′	a	2.38	ddd	3.0	H3′a-H2′e
				12.1	H3′a-H4′a
				13.8	H3′a-H3′e
C-H3″	e	1.93	dtd	1.2	H3′e-H2′e
				3.9	H3′e-H4′a
				13.1	H3′e-H3′a
C-H4′	a	4.79	tt	**10.8**	H4′a-H3′a
					H4′a-H5′a
				4.8	H4′a-H3′e
					H4′a-H5′e
C-H5′	a	3.91	t	10.7	H5′a-H5′e
					H5′a-H4′a
C-H5″	e	3.73	ddd	1.4	H5′e-H3′e[b]
				4.7	H5′e-H4′a
				11.2	H5′e-H5′a

[a]e stands for equatorial, a for axial. [b]W proton-proton coupling (^{4}JHH).

For comparison, the β-anomer of compound **2.29** (***β*-2.29**) was obtained upon deprotection of ***β*-2.28** and fully characterized by 1D and 2D NMR experiments (**Table 4-2** and **Figure 4-3**). Accordingly, the analysis of these NMR data indicated that the preferred chair conformation of the pyranose ring in ***β*-2.29** adopts to a 1,4-*trans*-diaxial orientation of the bulky substituents.

It is noteworthy that the conformation of such nucleoside analogues is expected to easily adapt to external conditions such as those required by enzyme binding[17] or insertion within a nucleic acid chain,[18] thus influencing oligonucleotides properties in a variable way.

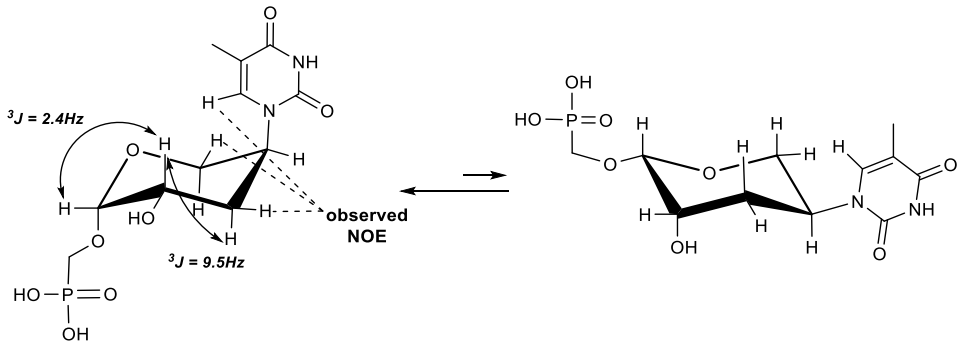


Figure 4-3. Preferential conformation of 1′-phosphonate β-D-*threo*-pentopyranose nucleoside bearing thymine as base (**β-2.29**) as determined by NMR studies. Dashed lines indicate observed NOE interaction between H6 of thymine and the equatorially positioned H3′ and H5′.

Table 4-2. ^{1}H NMR Analysis of the Sugar Skeleton of *β*-anomer of Thymine D-*Threo*-pentopyranoside Nucleoside Phosphonate β-2.29 (600 MHz, D2O).

bond	orientation[a]	δ (ppm)	multiplicity	*J* (Hz) (D_2O)	^3J connection
C-H1′	e	4.86	d	**2.4**	H1′e-H2′a
C-H2′	a	3.98	ddd	2.6	H2′a-H1′e
				4.0	H2′a-H3′e
				9.5	H2′a-H3′a
C-H3′	a	2.21	ddd	9.6	H3′a-H2′a
				4.6	H3′a-H4′a
				13.6	H3′a-H3′e
C-H3′′	e	2.10	dt	4.8	H3′e-H2′a
					H3′e-H4′a
				13.6	H3′e-H3′a
C-H4′	e	4.66	q	**4.5**	H4′e-H3′a
					H4′e-H5′a

					H4′e-H3′e H4′e-H5′e
C-H5′	a	4.16	dd	12.8 4.2	H5′a-H5′e H5′a-H4′a
C-H5″	e	3.86	dd	4.2 12.9	H5′e-H4′a H5′e-H5′a

[a] e stands for equatorial, a for axial.

In the **Table 4-3, Table 4-4 and Table 4-5** summarize the proton NMR spectroscopic data acquired for nucleoside phosphonates **3.32** (adenine), **4.09** (uracil), and **4.11** (hypoxanthine) respectively.

Table 4-3. ^{1}H NMR Analysis of the Sugar Skeleton of Adenine D-*Threo*-pentopyranoside Nucleoside Phosphonate 3.32 (600 MHz, D_2O).

bond	orientation[a]	δ (ppm)	multiplicity	*J* (Hz) (D_2O)	^{3}J connection
C-H1′	e	4.71	d	**3.3**	H1′e-H2′e
C-H2′	e	3.97	td	**3.3** 4.8	H2′e-H1′e H2′e-H3′e H2′e-H3′a
C-H3′	a	2.50	ddd	3.4 **10.1** 13.4	H3′a-H2′e H3′a-H4′a H3′a-H3′e
C-H3″	e	2.15	dt	4.3 13.3	H3′e-H2′e H3′e-H4′a H3′e-H3′a
C-H4′	a	4.72	Overlapping with D_2O		H4′a-H3′a H4′a-H5′a H4′a-H3′e H4′a-H5′e
C-H5′	a	4.09	dd	8.7 11.4	H5′a-H5′e H5′a-H4′a

C-H5″	e	3.92	dd	4.0 11.4	H5′e-H4′a H5′e-H5′a

[a]e stands for equatorial, a for axial.

Table 4-4. ^{1}H NMR Analysis of the Sugar Skeleton of Uracil D-*Threo*-pentopyranoside Nucleoside Phosphonate 4.09 (600 MHz, D_2O).

bond	orientation[a]	δ (ppm)	multiplicity	*J* (Hz) (D_2O)	^3J connection
C-H1′	e	4.65	d	**2.2**	H1′e-H2′e
C-H2′	e	3.98	td	**2.9** **3.6**	H2′e-H1′e H2′e-H3′e H2′e-H3′a
C-H3′	a	2.35	ddd	3.0 **10.7** 13.5	H3′a-H2′e H3′a-H4′a H3′a-H3′e
C-H3″	e	1.93	dtd	1.2 4.0 13.2	H3′e-H2′e H3′e-H4′a H3′e-H3′a
C-H4′	a	4.85	tt	**10.8** 4.8	H4′a-H3′a H4′a-H5′a H4′a-H3′e H4′a-H5′e
C-H5′	a	3.94	t	10.7	H5′a-H5′e H5′a-H4′a
C-H5″	e	3.75	ddd	1.0 4.7 10.8	H5′e-H3′e[b] H5′e-H4′a H5′e-H5′a

[a]e stands for equatorial, a for axial. [b]W proton-proton coupling (^4JHH).

Table 4-5. ^{1}H NMR Analysis of the Sugar Skeleton of Hypoxanthine D-*Threo*-pentopyranoside

Nucleoside Phosphonate 4.11 (600 MHz, D_2O).

bond	orientation[a]	δ (ppm)	multiplicity	*J* (Hz) (D_2O)	^3J connection
C-H1′	e	4.74	Overlapping with D_2O		H1′e-H2′e
C-H2′	e	4.02	dt	**3.5** **5.4**	H2′e-H1′e H2′e-H3′e H2′e-H3′a
C-H3′	a	2.65	ddd	3.4 **10.0** 13.4	H3′a-H2′e H3′a-H4′a H3′a-H3′e
C-H3″	e	2.21	dt	4.2 13.5	H3′e-H2′e H3′e-H4′a H3′e-H3′a
C-H4′	a	4.97	tt	**9.5** 4.3	H4′a-H3′a H4′a-H5′a H4′a-H3′e H4′a-H5′e
C-H5′	a	4.18	dd	8.9 11.4	H5′a-H5′e H5′a-H4′a
C-H5″	e	3.98	dd	4.3 11.6	H5′e-H4′a H5′e-H5′a

[a] e stands for equatorial, a for axial.

4.2.4 Anti-herpes viruses activity

In order to define whether D-*threo*-pentopyranoside nucleoside phosphonates are still recognised by enzymes involved in nucleoside and nucleotide metabolism, compounds **2.29** and **3.32** were at first evaluated for their antiviral activity against different herpesviruses (varicella-zoster virus (VZV), HSV-1, and HSV-2). Contrary to the results obtained for hexitol nucleoside[15] as well as

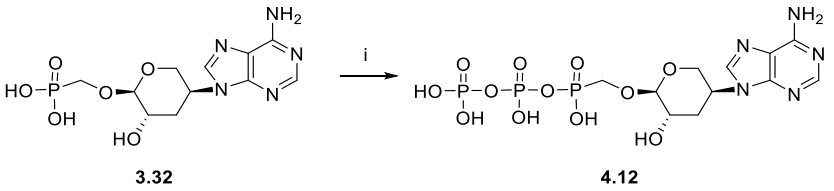
known furanose nucleoside phosphonates, [19] these studies revealed that **2.29** and **3.32** exerted very poor inhibitory activity of DNA viral replication. This could be due to a lack of cell penetration (the negatively charged phosphonate moiety is very polar) as well as inefficient intracellular enzymatic phosphorylation. Specifically, the very high EC_{50} values determined for **2.29** and **3.32** (**Table 4-6**) suggest that these issues may not be overcome by just synthesising the corresponding prodrugs. As discussed before (**Chapter 1, Section 1.2.7**), except hexitol nucleoside, other pyranose nucleosides adopt conformations with equatorial nucleobase, which make them poor substrates for intercellular phosphorylation kinase. This might also be the reason for the observed poor antiviral activity for **2.29** and **3.32.**

Table 4-6. Antiviral Results of Adenine and Thymine D-*Threo*-pentopyranoside Nucleoside Phosphonates 3.32 and 2.29

Compound	Antiviral activity EC_{50} (µM)							Cytotoxicity[e] (µM)
	HCMV[a]		VZV[b]		HSV[b]			
	AD-169 strain	Davis strain	TK^+ strain	TK^- strain	HSV-1 KOS strain	HSV-2 G strain	HSV-1 KOS ACV^r strain	
2.29	163	228	163	125	>298	>298	>298	>298
3.32	>58.1	>290	38.9	66.5	290	290	159	>291
Acyclovir	ND	ND	5.46	64.4	0.7	0.49	>88.8	>444
Penciclovir	ND	ND	ND	ND	0.63	1.07	>79.0	>79.0
Brivudin	ND	ND	0.04	6.03	0.03	30.0	>30.0	>300
Ganciclovir	2.08	2.27	ND	ND	0.01	0.01	17.5	>39.2
Foscarnet	ND	ND	ND	ND	173	126	317	>1587
Cidofovir	0.57	0.57	ND	ND	2.18	2.87	8.38	>71.6
Adefovir	ND	ND	ND	ND	46.4	38.3	358	732

[a]Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). [b]Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). [c]Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [d]ND = Not determined. [e]Minimum concentration required to reduce a microscopically detectable alteration of cell morphology.

4.2.5 Synthesis of diphosphate and chain elongation experiments



Scheme 4-3. Phosphorylation of adenine *α*-D-*threo*-pentopyranoside phosphonate **3.32**. *Reagents, conditions and yields*: (i) (a) 1,1′-carbonyldiimidazole, DMF, (b) $(HNBu_3)_2H_2P_2O_7$, (c) TEAB buffer, 30%.

To evaluate the potential utility of these synthetic analogues for the selection of artificial information systems, the adenine containing nucleoside phosphonate **3.32** was converted to its corresponding diphosphate **4.12** using a known phosphorylation procedure using 1,1′-carbonyl diimidazole (CDI) as the coupling reagent.[20] (**Scheme 4-3**). Compound **4.12** was assayed for its ability to be accepted as a substrate by DNA polymerases in an enzymatic primer extension assay where a primer-template duplex with a seven 5′-dT overhang was used. Enzymes lacking 3′,5′-proofreading activity such as Klenow fragment (*exo*-) and Vent (*exo*-) were tested in the presence or absence of Mn^{2+} ions and shown to be able to use **4.12** as a substrate, although only the formation of P+1 or P+2 products was detected, as exemplified in **Figure 4-4** to **Figure 4-6**.

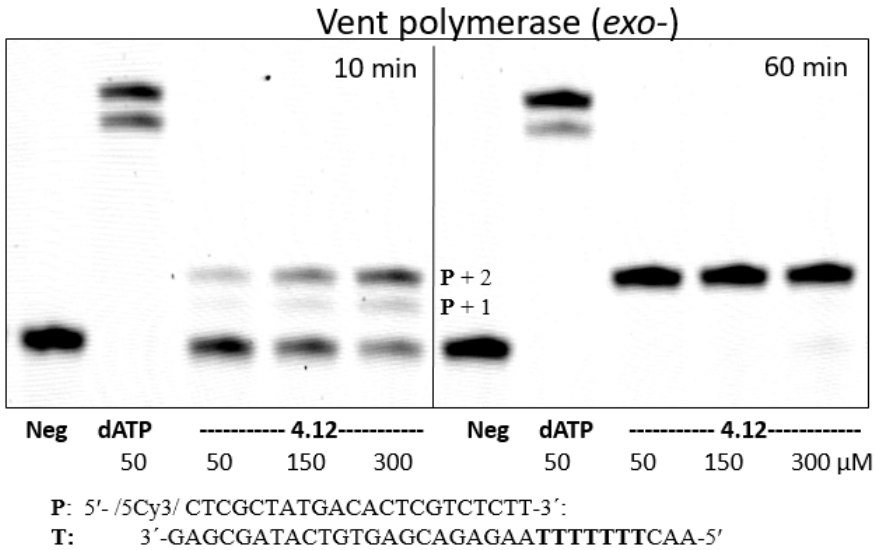


Figure 4-4. Primer extension of compound **4.12** in the presence of Vent polymerase (exo-) and Mn^{2+} ions. The positions where **4.12** has to be incorporated opposite the template oligonucleotide are in bold. dATP stands for 2′-deoxyadenosine triphosphate (positive control), while Neg for negative control.

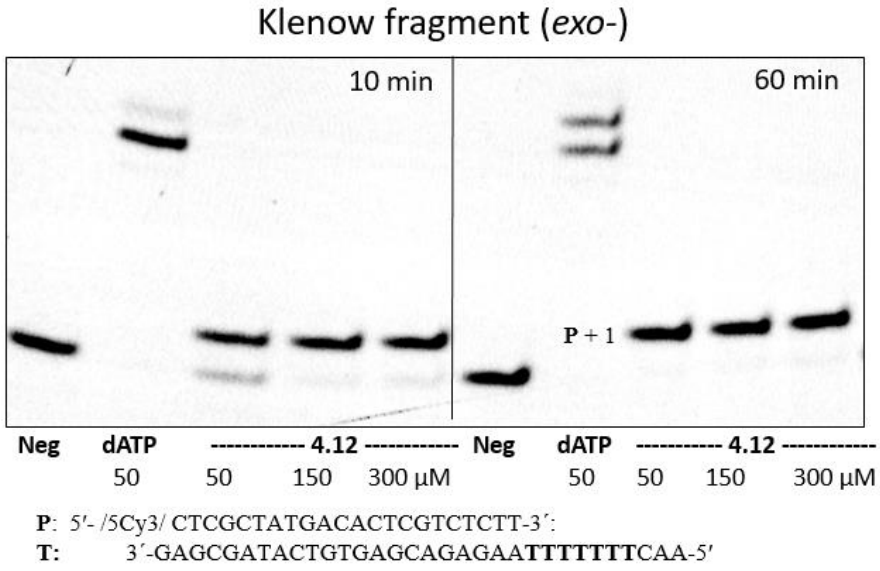


Figure 4-5. Primer extension of compound **4.12** in the presence of Klenow (-*exo*) polymerase and Mn^{2+} ions. Abbreviations: Neg = Negative control, dATP = 2′-deoxyadenosine triphosphate.

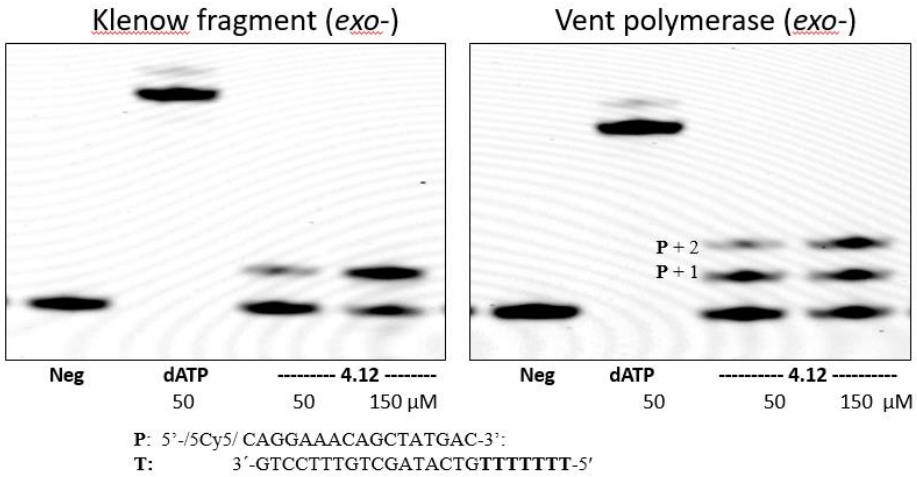


Figure 4-6. Primer extension of compound **4.12** in the presence of Klenow (-*exo*) and Vent (-*exo*) polymerase, in the absence of Mn^{2+} ions. Abbreviations: Neg = Negative control, dA = 2′-deoxyadenosine triphosphate.

4.3 Conclusion

Two more D-*threo*-pentopyranoside nucleoside analogues, with uracil and hypoxanthine respectively, bearing anomeric phosphonate functionality have been synthesised. The synthetic routes are similar to the work discussed in **Chapter 2** (**Scheme 2-2**) and **Chapter 3** (**Scheme 3-5**). According to NMR studies, the solution conformation of these pentopyranoside nucleoside phosphonates shows a preference for an equatorial orientated nucleobase of the base moiety circumventing unfavourable 1,3-diaxial interactions. Adenine and thymine nucleoside phosphonates were submitted to anti-herpesviruses (HSV, HCMV and VZV) tests. However, only very low activity has been detected in the adenine analogues, while the thymine analogue was devoid of activity. Preliminary primer incorporation reactions using a mesophilic and thermophilic polymerase revealed that the compounds synthesised by these pathways constitute potentially useful monomers for biotechnological applications.

4.4 Experimental section

Chemistry. All reagents and solvents were purchased from commercial sources and used as obtained. Moisture sensitive reactions were performed using oven-dried glassware under a nitrogen or argon atmosphere. NMR spectra were recorded on a Bruker Advance 300 MHz (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz; ^{31}P NMR, 121 MHz), 500 MHz (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz; ^{31}P NMR, 202 MHz), or 600 MHz (^{1}H NMR, 600 MHz; ^{13}C NMR, 150 MHz) spectrometer with tetramethylsilane as internal standard or referenced to the residual solvent signal, and 85% H_3PO_4 for ^{31}P NMR. All intermediates and final compounds were characterized by using 2D NMR (^{1}H-COSY, HSQC, NOESY, and HMBC) spectroscopic techniques. For NMR assignment of sugar protons and carbons, prime numbering is used. High-resolution mass spectra [HRMS (ESI)] were obtained on a quadruple orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min and spectra were obtained in positive (or in negative) ionization mode with a resolution of 15000 (fwhm) using leucine enkephalin as lock mass. Precoated aluminium sheets (254 nm) were used for TLC. Products were

purified by column chromatography on silica gel (60 Å, 0.035−0.070 mm, Acros Organics). Preparative RP-HPLC purifications were carried out on a Phenomenex Gemini 110A column (C18, 10 μm, 21.2 mm × 250 mm) using CH_3CN/0.05 M TEAB buffer or H_2O/ CH_3CN as eluent gradient.

Phosphonomethyl 3,4-dideoxy-4-(thymid-1-yl)-*β*-D-*threo*-pentopyranoside (*β*-2.29). Compound ***β*-2.29** was prepared using a similar procedure as that described for **2.29** starting from ***β*-2.28.** ^{1}H NMR (500 MHz, D_2O) δ 7.82 (s, 1H, H-6), 4.86 (d, *J* = 2.4 Hz, 1H, H-1′), 4.66 (q, *J* = 4.5 Hz, 1H, H-4′), 3.98 (ddd, *J* = 9.5, 4.0, 2.6 Hz, 1H, H-2′), 3.88 (dd, *J* = 12.6, 9.3 Hz, 1H, PC*H*$_2$), 3.86 (dd, *J* = 12.8, 3.9 Hz, 1H, H-5′), 3.74 (ddd, *J* = 12.9, 4.2, 0.8 Hz, 1H, H-5″), 3.61 (dd, *J* = 12.6, 9.3 Hz,1H, PC*H*$_2$), 2.21 (ddd, *J* = 13.6, 9.6, 4.6 Hz, 1H, H-3′), 2.10 (dt, *J* = 13.6, 4.8 Hz, 1H, H-3″), 1.87 (s, 3H, T C*H*$_3$); ^{13}C NMR (125 MHz, D_2O) δ 166.4 (C-4), 152.2 (C-2), 139.9 (C-6), 110.6 (C-5), 99.6 (d, $^3J_{P,C}$ = 12.4 Hz, C-1′), 66.3 (C-2′), 64.3 (d, $^1J_{P,C}$ = 155.4 Hz, P*C*H$_2$), 61.7 (C-5′), 50.4 (C-4′), 29.9 (C-3′), 11.5 (T *C*H$_3$); ^{31}P NMR (121 MHz, D_2O) δ 15.1

Benzyl 4-deoxy-2-*O*-(2-methoxyethoxymethyl)-4-(uridin-1-yl)-*α*-D-lyxopyranoside (4.1). To a stirred suspension of **2.20** (1.90 g, 6.12 mmol) and uracil (2.06 g, 18.4 mmol) in dry DMF (50 mL) at 0 °C was slowly added DBU (3 mL, 18.4 mmol). The suspension was stirred at room temperature for 30 min until all the solid dissolved and then heated at 80 °C for 8 h. After removal of all the volatiles under reduced pressure, the remaining syrup was dissolved in EtOAc and washed with saturated aq. $NaHCO_3$. The organic phase was collected, dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by silica gel column chromatography (100:2 DCM/Methanol) to afford **4.1** (2.20 g, 85% yield) as a colorless oil.^{1}H NMR (300 MHz, $CDCl_3$) δ 10.13 (s, 1H, NH), 7.45 – 7.18 (m, 6H, Ph and H-6), 5.68 (d, *J* = 8.0 Hz, 1H, H-5), 5.01 (s, 1H, H-1′), 4.90 – 4.65 (m, 4H, H-4′, C*H*$_2$Ph and OC*H*$_2$O), 4.52 (d, *J* = 11.9 Hz, 1H, , C*H*$_2$Ph), 4.26 (m, 2H, H-5′, H-5″), 3.99 (bs, 1H, H-2′), 3.77 (dd, *J* = 7.8, 3.4 Hz, 3H, H-3′, OC*H*$_2$CH$_2$O), 3.49 (dd, *J* = 8.2, 3.6 Hz, 2H, OC*H*$_2$CH$_2$O), 3.32 (s, 3H, OC*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7 (C-4), 151.6 (C-2), 141.8 (C-6), 136.9, 128.5, 127.9, 127.8 (Ph), 102.4 (C-5), 98.3 (C-1′), 96.5 (O*C*H$_2$O), 78.4 (C-2′), 71.4 (OCH$_2$*C*H$_2$O), 69.3 (*C*H$_2$Ph), 67.5 (O*C*H$_2$CH$_2$O), 65.7 (C-3′), 60.0 (C-5′), 58.8 (O*C*H$_3$), 55.1 (C-4′). HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{20}H_{26}N_2O_8Na$, 445.1582; found, 445.1580.

Benzyl 3,4-dideoxy-2-*O*-(2-methoxyethoxymethyl)-4-(uridin-1-yl)-*α*-D-*threo*-pentopyranoside (4.2). To a solution of **4.1** (1.50 g, 3.55 mmol) and DMAP (100 mg, 0.8 mmol) in anhydrous DCM (30 mL) was added TCDI (1.27g, 7.10 mmol) at room temperature. The reaction mixture was stirred at 40 °C overnight. It was then washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was dissolved in toluene (50 mL), and AIBN (204 mg, 1.32 mmol) was added followed by tributyltin hydride (1.43 mL, 5.32 mmol). The reaction mixture was refluxed for 1 h. After removal of all the volatiles under reduced pressure, the crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 100:0, v/v; 100:2, v/v; 30:1, v/v) to afford **4.2** (1.10 g, 83% yield over two steps) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.42 (s, 1H, NH), 7.46 – 7.26 (m, 5H, Ph), 7.28 – 7.18 (d, *J* = 8.1 Hz, 1H, H-6), 5.73 (d, *J* = 8.0 Hz, 1H, H-5), 5.08 – 4.89 (m, 1H, H-4′), 4.78 (m, 4H, OC*H*$_2$O, C*H*$_2$Ph and H-1′), 4.55 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 3.91 (bs, 1H, H-2′), 3.82 – 3.65 (m, 4H, H-5′, H-5′, OCH_2CH_2O), 3.50 (dd, *J* = 5.4, 3.77 Hz, 2H, OCH_2CH_2O), 3.34 (s, 3H, OC*H*$_3$), 2.39 – 2.17 (td, *J* = 12.8, 3.0 Hz, 1H, H-3′), 2.07 – 1.93 (dt, *J* = 12.8, 3.8 Hz, 1H, H-3″).^{13}C NMR (75 MHz, $CDCl_3$) δ 163.1 (C-4), 150.9 (C-2), 141.0 (C-6), 137.3, 128.6, 128.1, 128.0 (Ph), 102.6 (C-5), 96.8 (O*C*H$_2$O), 95.1 (C-1′), 72.8 (C-2′), 71.7 (OCH_2CH_2O), 69.5 (*C*H$_2$Ph), 67.4 (OCH_2CH_2O), 61.3 (C-5′), 59.0 (O*C*H$_3$), 47.9 (C-4′), 29.2 (C-3′). HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{20}H_{26}N_2O_7Na$, 429.1632; found, 429.1626.

Benzyl 3,4-dideoxy-4-(uridin-1-yl)-*α*/*β*-D-*threo*-pentopyranoside (4.3). Hydrochloric acid in 1,4-dioxane (4 M, 8 mL) was added dropwise to a solution of **4.2** (1.10 g, 2.71 mmol) in 1,4-dioxane (24 mL), and the reaction mixture was stirred at room temperature for 8 h. After removal of all the volatiles under reduced pressure, the remaining syrup was co-evaporated with triethylamine (5 mL) once and then subjected to column chromatography on silica gel to give *α*/*β*-**4.3**. Data for the *α*-**4.3**: (0.68 g, 79% yield**)**; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (s, 1H, NH), 7.55 – 7.15 (m, 6H, Ph and H-6), 5.71 (d, *J* = 8.1 Hz, 1H, H-5), 4.97 (m, 1H, H-4′), 4.79 (d, *J* = 12.3 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J* = 2.9 Hz, 1H, H-1′), 4.57 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 3.91 (bs, 1H, H-2′), 3.88 – 3.76 (m, 2H, H-5′ and H-5″), 3.17 (s, 1H, OH), 2.43 – 2.16 (m, 1H, H-3′), 2.10 – 1.88 (m, 1H, H-3″).^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4 (C-4), 151.4 (C-2), 141.4 (C-6), 137.2, 128.9, 128.4, 128.4 (Ph), 103.0

(C-5), 99.2 (C-1′), 70.1 (C-2′), 67.2 (*C*H_2Ph), 62.3 (C-5′), 48.0 (C-4′), 31.5 (C-3′); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{16}H_{19}N_2O_5$, 319.1288; found, 319.1286.

Data for the *β*-**4.3**: (34 mg, 4% yield**)**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.8 (bs, 1H, NH), 7.84 (d, *J* = 7.9 Hz, 1H, H-6), 7.51–7.10 (m, 5H, Ph), 5.69 (d, *J* = 7.9 Hz, 1H, H-5), 4.92 (d, *J* = 2.6 Hz, 1H, H-1′), 4.84 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.76–4.52 (m, 2H, C*H*$_2$Ph, H-4′), 4.13 (dd, *J* = 12.5, 3.0 Hz, 1H, H-5′), 3.85–3.73 (m, 2H, H-5′, H-2′), 2.48 (d, *J* = 8.3 Hz, 1H, OH), 2.21– 2.08 (m, 1H, H-3′), 2.00– 1.89 (m, 1H, H-3″); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.1 (C-4), 150.8 (C-2), 142.5 (C-6), 136.9, 128.8, 128.3, 128.2 (Ph), 102.4 (C-5), 97.5 (C-1′), 70.3 (C-2′), 64.6 (*C*H_2Ph), 61.1 (C-5′), 51.7 (C-4′), 31.3 (C-3′).

Benzyl 2-*O*-benzoyl-3,4-dideoxy-4-(uridin-1-yl)-*α*/*β*-D-*threo*-pentopyranoside (4.4). To a solution of *α*/*β*-**4.3** (400 mg, 1.26 mmol) in pyridine (15 mL) at 0 °C, benzoyl chloride (0.22 mL, 1.88 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h. After removal of all the volatiles under reduced pressure, the remaining residue was partitioned between DCM (30 mL) and saturated aq. $NaHCO_3$ (20 mL). The aqueous layer was extracted again with DCM (20 mL). The combined organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3:1 hexane/EtOAc) to afford *α*/*β*-**4.4** (438 mg, 83% yield) as a colourless oil.

Data for *α*-**4.4**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.33 (s, 1H, NH), 8.09 (m, 2H, Ph), 7.64 –7.30 (m, 8H, Ph), 7.23 (d, *J* = 8.2 Hz, 1H, H-6), 5.71 (dd, J = 8.2, 1.7 Hz, 1H, H-5), 5.30 (dt, *J* = 3.5, 3.2 Hz, 1H, H-2′), 5.09 (tt, *J* = 11.7, 4.6, 1H, H-4′), 4.92 (d, *J* = 1.6 Hz, 1H, H-1′), 4.82 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 4.62 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 3.87 – 3.81 (m, 2H, H-5′ and H-5″), 2.49 (td, *J* = 13.0, 3.2 Hz, 1H, H-3′), 2.26–2.04 (m, 1H, H-3″); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.5 (Ph*C*O), 162.9 (C-4), 150.7 (C-2), 140.4 (C-6), 136.7, 133.5, 129.8, 129.3, 128.6, 128.5, 128.1, 127.9 (Ph), 102.8 (C-5), 95.0 (C-1′), 69.6 (*C*H_2Ph), 69.0 (C-2′), 60.8 (C-5′), 47.3 (C-4′), 28.2 (C-3′); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{23}H_{23}N_2O_6$, 423.1551; found, 423.1544.

Data for *β*-**4.4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (s, 1H, NH), 8.08–7.72 (m, 2H, Ph), 7.59 – 6.95 (m, 9H, Ph and H-6), 5.68 (d, *J* = 7.7 Hz, 1H, H-5), 5.32 – 5.18 (m, 1H, H-2′), 4.97 – 4.84 (m, 1H,

H-4′), 4.78 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.56 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ph), 4.51 (bs, 1H, H-4′), 4.01 (dd, *J* = 3.0, 12.1 Hz, 1H, H-5′), 3.53 (appt, *J* = 11.9 Hz, 1H, H-5′′), 2.04–1.80 (m, 2H, , H-3′ and H-3′′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5 (Ph*C*O), 163.1 (C-4), 151.3 (C-2), 140.4 (C-6), 136.8, 133.6, 130.1, 128.5, 128.5, 127.99, 127.9 (Ph), 103.3(C-5), 100.7 (C-1′), 71.1 (C-2′), 70.5 (*C*H$_2$Ph), 62.6 (C-5′), 54.5 (C-4′), 30.9 (C-3′).

2-*O*-Benzoyl-3,4-dideoxy-4-(uridin-1-yl)-*α*-D-*threo*-pentopyranose (4.5). To a solution of *α*/*β*-**4.4** (250 mg, 0.59 mmol) in ethanol (10 mL), Pd/C (314 mg, 0.30 mmol) and cyclohexene (1.2 mL, 11.8 mmol) were added. The reaction mixture was stirred at 80 ºC for 6 h. It was then cooled and filtered through a pad of Celite to give **4.5** (154 mg, 78%) as a white solid. ^{1}H NMR (600 MHz, DMSO) δ 11.3 (s, 1H, NH), 8.02 (m, 2H, Ph), 7.76 (d, *J* = 7.0 Hz, 1H, H-6), 7.72–7.49 (m, 3H, Ph), 7.00 (d, *J* = 4.9 Hz, 1H, OH), 5.57 (d, *J* = 7.0 Hz, 1H, H-5), 5.04 (m, 1H, H-2′), 5.01 (dd, *J* = 4.7, 1.4 Hz, 1H, H-1′), 4.79 (tt, *J* = 10.7, 4.3 Hz, 1H, H-4′), 4.03 (appt, *J* = 10.8 Hz, 1H, H-5′), 3.59 (m, 1H, H-5′′), 2.59 (m, 1H, H-3′), 2.04 (dt, *J* = 13.4, 4.5 Hz, 1H, H-3′); ^{13}C NMR (151 MHz, DMSO) δ 165.3 (Ph*C*O), 163.2 (C-4), 151.1 (C-2), 142.6 (C-6), 138.0, 129.3, 128.9, 128.8 (Ph)**,** 101.8 (C-5), 90.0 (C-1′), 71.2 (C-2′), 60.1 (C-5′), 47.9 (C-4′), 27.6 (C-3′); HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{16}H_{17}N_2O_6$, 333.1081; found, 333.1084.

Acetyl 2-*O*-benzoyl-3,4-dideoxy-4-(uridin-1-yl)-*α*-D-*threo*-pentopyranoside (4.6). To a solution of **4.5** (120 mg, 0.36 mmol) in pyridine (5 mL) at 0 °C, acetic anhydride (0.068 mL, 0.72 mmol) was added dropwise. The reaction mixture was slowly warmed to room temperature and left stirring for 2 h. After removal of all the volatiles under reduced pressure, the remaining residue was taken up with DCM (10 mL), and 10 mL of saturated aq. $NaHCO_3$ was added. The aqueous layer was then extracted with DCM (2 × 10 mL). The combined organic layer was washed with saturated aq. $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2:1 hexane/EtOAc) to give **4.6** (110 mg, 81%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.53 (s, 1H, NH), 8.06 (dd, *J* = 16.7, 15.3 Hz, 2H, Ph), 7.72 – 7.55 (m, 1H, Ph), 7.47 (dd, *J* = 16.2, 8.7 Hz, 2H, Ph), 7.39 – 7.19 (m, 1H, H-6), 6.10 (d, *J* = 1.9 Hz, 1H, H-1′), 5.79 (dd, *J* = 10.3, 3.9 Hz, 1H, H-5), 5.28 (dt, *J* = 3.4, 1.9 Hz, 1H, H-2′), 4.97 (td, *J* = 9.6, 4.5 Hz, 1H, H-4′), 4.10 – 3.85 (m, 2H, H-5′ and H-5′′), 2.56 (td, *J* = 13.8, 3.1 Hz, 1H,

H-3′), 2.36 – 2.22 (dt, *J* = 13.1, 3.7 Hz, 1H, H-3″), 2.19 (s, 3H, C*H*$_3$CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4 (CH_3*C*O), 165.3 (Ph*C*O), 163.0 (C-4), 150.8 (C-2), 141.0 (C-6), 133.8, 130.0, 129.8, 128.7(Ph), 103.1 (C-5), 89.7 (C-1′), 68.2 (C-2′), 62.7 (C-5′), 48.8 (C-4′), 28.6 (C-3′), 21.0 (*C*H_3CO). HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{18}H_{18}N_2O_7Na$, 397.1006; found, 397.1001.

Diisopopylphosphonomethyl 2-*O*-benzoyl-3,4-dideoxy-4-(uridin-1-yl)-*α*-D-*threo*-pentopyranoside (4.7). To a stirred solution of **4.6** (100 mg, 0.27 mmol) and diisopropylphosphonomethanol (104 mg, 0.53 mmol) in dry DCM (5 mL) was added 4 Å molecular sieves, and the mixture was stirred for 30 min at room temperature. The reaction mixture was cooled to 0 ºC and TMSOTf (0.063 mL, 0.35 mmol) was added. The mixture was warmed to room temperature and stirred for 10 h. The reaction was quenched by addition of saturated aq. $NaHCO_3$ at 0 ºC. It was then diluted with DCM (10 mL) and saturated aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with DCM (2 × 10 mL). The combined organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford **4.7** as a mixture of α and *β* isomers, along with residual diisopropylphosphonomethanol. This material was used in the next step without further purification. HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{23}H_{31}N_2O_9P_1Na$, 533.1660; found, 533.1649.

Diisopopylphosphonomethyl 3,4-dideoxy-4-(uridin-1-yl)-*α*/*β*-D-*threo*-pentopyranoside (4.8). A solution of crude **4.7** in 7 N NH_3 in MeOH (5 mL) was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the resultant residue was purified by silica gel column chromatography (20:1 CH_2Cl_2/MeOH) to give *α*/*β*-**4.8** as a white foam.

Data for α-**4.8**: 28 mg, 25% over two steps; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.17 (s, 1H, NH), 7.48 (d, *J* = 8.0 Hz, 1H, H-6), 5.72 (d, *J* = 8.0 Hz, 1H, H-5), 4.96 (tt, *J* = 9.3, 4.7 Hz, 1H, H-4′), 4.84–4.73 (m, 2H, [C*H*(CH_3)$_2$]), 4.65 (d, *J* = 2.0 Hz, 1H, H-1′), 4.03–3.98 (dd, *J* = 13.6, 9.9 Hz, 1H, PC*H*$_2$), 3.96–3.94 (m, 1H, H-2′), 3.90 (appt, *J* = 9.8 Hz, 1H, H-5′),3.80–3.70 (m, 2H, H-5″ and PC*H*$_2$), 2.28 (ddd, *J* = 12.9, 10.4, 2.5 Hz, 1H, H-3′), 2.05–1.97 (dt, *J* = 13.2, 4.3 Hz,1H, H-3″), 1.41–1.29 (m, 12H, [CH(C*H*$_3$)$_2$]); ^{13}C NMR (151 MHz, $CDCl_3$) δ163.0 (C-4), 150.9 (C-2), 141.2 (C-6), 102.6 (C-5), 100.7 (d, $^3J_{P,C}$ = 10.8 Hz, C-1′), 71.5 [*C*H(CH_3)$_2$], 66.2 (C-2′), 62.2 (C-5′), 61.6

(d, $^{1}J_{P,C}$ = 171.9 Hz, P*C*H$_2$), 47.5 (C-4′), 31.1 (C-3′), 24.0 [CH(*C*H$_3$)$_2$]); ^{31}P NMR (121 MHz, CDCl$_3$) δ 20.0; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{16}H_{28}N_2O_8P_1$, 407.1578; found, 407.1582.

Data for *β*-**4.8**: 10 mg, 9% over two steps; ^{1}H NMR (600 MHz, CDCl$_3$) δ 9.04 (s, 1H, NH), 7.85 (d, *J* = 8.1 Hz, 1H, H-6), 5.70 (d, *J* = 8.1 Hz, 10H), 4.93 (d, *J* = 2.7 Hz, 1H, H-1′), 4.86 – 4.72 (m, 2H, [C*H*(CH$_3$)$_2$]), 4.70 – 4.56 (tt, *J* = 4.4, 3.7 Hz, 1H, H-4′), 4.20 (dd, *J* = 13.1, 3.3 Hz, 1H, H-5′), 4.02 (dd, *J* = 13.8, 9.4 Hz, 1H, PC*H*$_2$), 3.93 – 3.74 (m, 3H, H-5″, PC*H*$_2$ and H-2′), 2.20 (dt, *J* = 13.2, 5.2 Hz, 1H, H-3′), 2.16 – 2.09 (ddd, *J* = 14.2, 10.9, 4.2 Hz, 1H, H-3″), 1.34 (m, 12H, [CH(C*H*$_3$)$_2$]).^{13}C NMR (151 MHz, CDCl$_3$) δ 163.2 (C-4), 150.8 (C-2), 142.4 (C-6), 102.4 (C-5), 99.7 (d, $^{3}J_{P,C}$ = 10.1 Hz, C-1′), 71.8 (d, $^{2}J_{P,C}$ = 16.3 Hz, [*C*H(CH$_3$)$_2$]), 64.2 (C-2′), 62.7 (d, $^{1}J_{P,C}$ = 171.0Hz, P*C*H$_2$), 61.0 (C-5′), 51.5 (C-4′), 30.8 (C-3′), 24.2 [CH(*C*H$_3$)$_2$].; ^{31}P NMR (121 MHz, CDCl$_3$) δ 20.0.

Phosphonomethyl 3,4-dideoxy-4-(uridin-1-yl)-*α*-D-*threo*-pentopyranoside (4.9). To a solution of α-**4.8** (20 mg, 0.048 mmol) and 2,6-lutidine (0.045 mL, 0.39 mmol) in dry CH$_3$CN (2 mL) was added bromotrimethylsilane (0.045 mL, 0.39 mmol) at 0 °C. The reaction mixture was stirred at room tempearture overnight and quenched with 1.0 M TEAB solution (1 mL). After removal of all the volatiles under reduced pressure, the remaining residue was partitioned between water and EtOAc/ether (1:1) and the water layer was lyophilized. The crude residue was first purified by silica gel column chromatography (10:1:0 to 10:5:1, CH$_2$Cl$_2$/MeOH/1 M TEAB) and then by preparative reverse phase HPLC with a gradient of CH$_3$CN in 0.05 M TEAB ranging from 2 to 30% to give **4.9** (Et$_3$N salt, 8.1 mg, 40%) as a white foam. ^{1}H NMR (600 MHz, D$_2$O) δ 7.66 (d, *J* = 8.1 Hz, 1H, 1H, H-6), 5.70 (d, *J* = 8.1 Hz, 1H, H-5), 4.85 (tt, *J* = 10.8, 4.8 Hz, 1H, H-4′), 4.65 (d, *J* = 2.5 Hz, 1H, H-1′), 4.01 (td, *J* = 3.6, 2.9 Hz, 1H, H-2′), 3.94 (appt t, *J* = 11.2 Hz, 1H, H-5′), 3.87 (dd, *J* = 12.9, 9.5 Hz, 1H, PC*H*$_2$), 3.75 (ddd, *J* = 10.8, 4.5, 1.0 Hz, 1H, H-5″), 3.63 (dd, *J* = 13.6, 9.9 Hz,1H, PC*H*$_2$), 2.35 (ddd, *J* = 13.5, 10.7, 3.0 Hz, 1H, H-3′), 1.93 (dtd, *J* = 13.2, 4.0, 1.2 Hz, 1H, H-3′); ^{13}C NMR (151 MHz, D$_2$O) δ 163.2 (C-4), 150.9 (C-2), 141.6 (C-6), 102.5 (C-5), 100.7 (d, $^{3}J_{P,C}$ = 12.0 Hz, C-1′), 65.7 (C-2′), 62.9 (d, $^{1}J_{P,C}$ = 156.6 Hz, P*C*H$_2$), 60.4 (C-5′), 46.7 (C-4′), 29.2 (C-3′); ^{31}P NMR (121 MHz, D$_2$O) δ 15.7; HRMS (ESI-): $[M-H]^-$ calcd for $C_{10}H_{14}N_2O_8P_1$, 321.0493; found, 321.0499.

Diisopropylphosphonomethyl 3,4-dideoxy-4-(hypoxanthin-9-yl)-*α*-D-*threo*-pentopyranoside (4.10). To a solution of **3.30** (0.1 g, 0.22 mmol) in methanol (5 mL) were added 2-mercaptoethanol (0.07 g, 0.89 mmol) and sodium methoxide (1.78 ml, 0.89 mmol, 5.4 M 30% wt in methanol). The mixture was then refluxed for 20 h. It was cooled to room temperature and neutralized with 0.5M TEAB buffer. The residue was partitioned between water and EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to leave a residue that was purified by column chromatography (30:1, DCM/MeOH) to afford compound **4.10** (70 mg, 73% yield) as a white foam.^{1}H NMR (600 MHz, MeOD) δ 8.18 (s, 1H, H-8), 8.05 (s, 1H, H-2), 5.13 – 5.00 (m, 1H, H-4′), 4.82 – 4.73 (m, 2H, [C*H*$(CH_3)_2$]), 4.73 (d, *J* = 2.3 Hz, 1H, H-1′), 4.13 (appt, *J* = 10.5 Hz, 1H, H-5′), 4.06 (dd, *J* = 14.0, 9.2 Hz, 1H, PC*H*$_2$), 3.93 (dd, *J* = 6.5, 3.1 Hz, 1H, H-2′), 3.91 – 3.83 (m, 2H, H-5′ and PC*H*$_2$), 2.68 (ddd, *J* = 13.1, 11.8, 3.2 Hz, 1H, H-3′), 2.16 (dtd, *J* = 12.9, 4.1, 1.8 Hz, 1H, H-3′), 1.37 (dd, *J* = 6.2, 1.6 Hz, 12H, [CH(C*H*$_3)_2$]).^{13}C NMR (151 MHz, MeOD) δ 159.0 (C-6), 150.2 (C-4), 146.5 (C-2), 140.4 (C-8), 125.4 (C-5), 101.6 (d, $^3J_{P,C}$ = 11.5 Hz, C-1′), 73.4, 73.3 [*C*H$(CH_3)_2$], 67.2 (C-2′), 63.6(C-5′), 62.2 (d, $^1J_{P,C}$ = 167.5 Hz, P*C*H$_2$), 33.0 (C-3′), 24.4 [CH(*C*H$_3)_2$]; ^{31}P NMR (121 MHz, MeOD) δ 20.0; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{28}N_4O_7P_1$, 431.1690; found, 431.1696.

Phosphonomethyl 3,4-dideoxy-4-(hypoxanthin-9-yl)-*α*-D-*threo*-pentopyranoside (4.11). Compound **4.11** was prepared using a similar procedure as that described for **2.29** starting from **4.10** (20 mg, 0.046 mmol), 2,6-lutidine (0.04 mL, 0.37 mmol), and TMSBr (0.05 mL, 0.37 mmol) in dry acetonitrile (2 mL) and obtained as a colorless sticky oil (6 mg, 40%). ^{1}H NMR (600 MHz, D_2O) δ 8.35 (s, 1H, H-8), 8.19 (s, 1H, H-2), 4.97 (tt, *J* = 9.5, 4.3 Hz,1H, H-4′), 4.74 (m, 1H, H-1′), 4.18 (dd, *J* = 11.4, 8.9 Hz, 1H, H-5′), 4.02 (dt, *J* = 5.4, 3.5 Hz, 1H, H-2′), 3.98 (dd, *J* = 11.6, 4.3 Hz, 1H, H-5′), 3.93 (dd, *J* = 13.1, 9.3 Hz, 1H, PC*H*$_2$), 3.69 – 3.65 (dd, *J* = 13.1, 9.5 Hz, 1H, PC*H*$_2$), 2.65 (ddd, *J* = 13.4, 10.0, 3.4 Hz, 1H, H-3′), 2.21 (dt, *J* = 13.5, 4.2 Hz, 1H, H-3′). ^{13}C NMR (151 MHz, D_2O) δ 160.0 (C-6), 150.2 (C-4), 147.0 (C-2), 141.8 (C-8), 124.5 (C-5), 102.2 (d, $^3J_{P,C}$ = 10.7 Hz, C-1′), 67.1 (C-2′), 65.0 (d, $^1J_{P,C}$ = 163.2 Hz, P*C*H$_2$), 64.0 (C-5′), 48.0 (C-4′), 32.6 (C-3′); ^{31}P NMR (121 MHz, D_2O) δ 15.2; ^{31}P NMR (121 MHz, D_2O): *δ* 13.1. HRMS (ESI-TOF) m/z: $[M-H]^-$ calcd for $C_{11}H_{14}N_4O_7P$, 345.0606; found, 345.0597.

Diphosphorylphosphonomethyl 4-(adenin-9-yl)-3,4-dideoxy-*α*-D-*threo*-pentopyranoside (4.12). Adenine *α*-D-*threo*-pentopyranoside nucleoside phosphonic acid **3.32** (30 mg, 0.087 mmol) was dissolved in anhydrous DMF (1 mL) and carbonyldiimidazole (70.4 mg, 0.434 mmol) was added. The mixture was stirred for 30 min at room temperature, and then a solution of pyrophosphate (333 mg, 0.608 mmol) in dry DMF (2 ml) was added and the stirring was continued overnight. An excess of 25% aq. ammonia (1 mL) was added and the mixture was concentrated *in vacuo*. The resulting residue was purified by chromatography on a DEAE-cellulose column (gradient: 0.1 to 1 M TEAB (v/v); 1/0, 1/0.2, 1/0.5, 1/1). Further purification was performed by RP-HPLC to afford diphosphate(phosphonomethyl) 4-(adenin-9-yl)-3,4-dideoxy-α-D-threo-pentopyranoside triethylammonium salt **4.12** as a white solid (30% yield). ^{1}H NMR (600 MHz, D_2O): δ 8.38 (s, 1H, H-2), 8.23 (s, 1H, H-8), 4.91 (tt, J = 9.3, 4.1 Hz, 1H, H-4′), 4.82 (d, J = 3.2 Hz, 1H, H-1′), 4.19 (dd, J = 12.0, 8.6 Hz, 1H, H-5′), 4.08–4.03 (m, 2H, PCH_2 and H-2′), 3.99 (dd, J = 12.0, 4.1 Hz, 1H, H-5″), 4.19 (dd, J = 13.2, 9.8 Hz, 1H, PCH_2), 2.63 (ddd, J = 13.5, 9.9, 3.1 Hz, 1H, H-3′), 2.15 (dt, J = 13.2, 5.1 Hz, 1H, H-3″); ^{13}C NMR (150 MHz, D_2O): δ 157.0 (C-6), 153.7 (C-2), 150.3 (C-4), 142.1 (C-8), 119.8 (C-5), 102.6 (d, $^{3}J_{P,\,C}$ = 9.8 Hz, C-1′), 67.0 (C-2′), 65.2 (d, $^{1}J_{P,\,C}$ = 167.1 Hz, P*C*H$_2$), 60.3 (C-5′), 48.7 (C-4′), 32.6 (C-3′); ^{31}P NMR (121 MHz, D_2O): δ 7.6 (d, J = 23.1 Hz, P_α), -6.4 (d, J = 18.9 Hz, P_γ), -22.7 (t, J = 21.4 Hz, P_β); HRMS (ESI-TOF) m/z: $[M-H]^-$ calcd for $C_{11}H_{17}N_5O_{12}P_3$, 504.0092; found 504.0096.

Antiviral evaluation

The compounds were evaluated against the following viruses: herpes simplex virus 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) strain Oka, TK^- VZV strain 07−1, human cytomegalovirus (HCMV) strains AD-169 and Davis. The antiviral assays are based on inhibition of virus-induced cytopathicity (HSV and HCMV) or plaque formation (VZV) in human embryonic lung (HEL) fibroblasts. Confluent cell cultures in microtiter 96-well plates are inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (VZV). After 2 hours of adsorption, the viral inoculum is removed and the cultures are further incubated in the presence of varying concentrations of the test

compounds. Viral cytopathicity or plaque formation is recorded after 2-3 (HSV), 5 (VZV) or 6-7 (HCMV) days post-infection. Antiviral activity is expressed as the EC_{50} or compound concentration required inhibiting virus-induced cytopathicity or viral plaque formation by 50%.

The cytostatic activity measurements are based on the inhibition of cell growth. HEL cells are seeded at a rate of 5 x 10^3 cells/well into 96-well microtiter plates and allow proliferating for 24 hours. Then, the medium containing different concentrations of the test compounds is added. After 3 days of incubation at 37 °C, the cell number is determined with a Coulter counter. The cytostatic concentration is calculated as the CC_{50}, or the compound concentration required reducing cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values are estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity of the test compounds is expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.

Incorporation Assay Protocol.

Incorporation Assay Protocol. Fluorescent-labelled primer (IDT) was annealed with template oligonucleotide (IDT) in a 1:2 molar ratio by heating the mixture at 95 °C for 5 min, followed by slow cooling to room temperature. The reaction mixtures consisted of 125 nM primer-template complex, reaction buffer [1X ThermoPol® supplied with Vent (-*exo*) or Neb2 supplied with Klenow fragment (-*exo*)], with or without 1 mM $MnCl_2$, different concentrations of **4.12** (50, 150, 300 µM) and either 0.05 U/µl Klenow fragment (-*exo*) polymerase (New England Biolabs) or 0.02 U/µl Vent polymerase (New England Biolabs). In the positive control reaction, 50 µM of the dATP was used, while ultrapure water was employed in the negative control reaction. The reactions were performed at 37 and 75 °C for Klenow and Vent polymerase, respectively. Aliquots were taken after 10 and 60 min and quenched by addition of a double volume of gel loading buffer (90% formamide, 50 mM EDTA, and 0.05 % bromophenol blue). Samples were heated at 95 °C for 5 min prior to separation on a 1.0 mm 15% denaturing polyacrylamide gel, and gel bands were visualized using a Typhoon FLA 9500 phosphor imager (GE Healthcare Life Sciences). The images were processed using

ImageQuant TL v8.1.0.0 (GE Healthcare).

4.5 Reference

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Chapter 5. Summary of the Thesis and Perspectives

Significant interest in synthetic analogues, mimicking the function of natural DNA and RNA has marked the past three decades of research in the field of chemical biology. Synthetic nucleic acids which closely mimic the properties of natural genetic material have found invaluable applications in therapy[1], molecular biology, diagnostics, material science and computing.[2] It has been proven that all the three nucleotide moieties, sugar, base and phosphate of the nucleic acids can be modified to achieve desired functionalities with increased chemical and enzymatic stability.

Xenobiotic nucleic acids (XNAs) are synthetic analogues of natural nucleic acids that contain different backbones and/or nucleobases than in the DNA and RNA. Sugar backbone modified XNAs (HNA, CeNA, ANA, FANA, TNA, and LNA) can store genetic information and evolved polymerases can retrieve this information.[3] These polymerases were obtained by directed evolution to accept modified nucleotides.[3] Recently, several XNAzymes which can accept modified sugars (ANA, FANA, HNA, CeNA), and mimic natural catalytic activities: such as RNA endonuclease, RNA ligase, and XNA-XNA ligase activity have been described.[4] Notably, in the year 2011, Marliere *et al*. have developed a method for generating *E. coli* cells, which can survive without thymine in its chromosome by using three natural (A, C and G) and one non-natural nucleobase (5-chlorouracil).[5]

Although advances have been made in the field of applied genetic engineering in agriculture, medicine and molecular biology, concerns about the possible contamination of the natural eco-system with mutated genes remain.[6] XNA is proposed as one of the solutions to addresses these concerns using orthogonal synthetic nucleic acid analogues as informational polymers. Although several XNAs mentioned above are viable synthetic alternatives, more efforts are still needed to optimise the chemistry of these nucleic acids. This will require the structural and biological analysis of the new series of backbone modified nucleic acids.

One example of such modified nucleic acids is HNA which was found to be more resistant against

phosphodiesterase degradation than DNA.[7] However, this XNA has many drawbacks. The main drawback of using it *in vivo* is its strong hybridisation with RNA. In addition, while HNA oligonucleotides are resistant against cellular nucleases,[7] their phosphorylated nucleoside precursors might be dephosphorylated by phosphatases in a cellular environment. Another important issue is that HNA monomers are poor substrates for cellular kinases and are only phosphorylated in herpesvirus-infected cells. One potential approach to avoid these issues is to replace the natural P-O-C bond with a phosphonate (P-C-O) linkage.[8-9] The phosphonate moiety is a bioisostere of the phosphate group with similar polar and electronic properties. The improved stability of phosphonate mimics may also lead to a prolonged intracellular half-life of the modified nucleic acids.

In this project, we aimed at exploring the chemical and biological properties of phosphonate pyranose nucleosides and compared them with the properties of a hexitol nucleoside phosphate. In the first part of this thesis (**Chapter 2**), different strategies were explored for synthesising D-*threo*-pentopyranosides bearing a phosphonomethanol at the anomeric centre and a thymine nucleobase at the 4′ position. Mitsunobu coupling reaction was successfully used for the synthesis of hexitol nucleosides[7,10] However it was not applicable in the case of pentitol (pentopyranose) since only elimination product was isolated. During the sulfonate (mesylate and tosylate) substitution reaction, no desired product was formed, whereas the use of more active triflate leads to elimination product. Epoxide ring opening pathway proved to be suitable for this nucleoside synthesis. Notably, the stereochemical outcome of the Vorbrüggen glycosylation step utilised en route to the thymine analogue clearly suggests the absence of anchimeric assistance, as opposed to what is usually observed during nucleoside synthesis using protected furanose precursors. With the absence of neighbouring group assistance, the lability of the leaving group on the glycosyl donor influences the ratio of the α/β isomers. It can be postulated that at low temperature (-78 ºC) the presence of aglycone moieties with an increased leaving group ability to support the formation of β isomer (kinetic product), whereas in the case of **2.26c** bearing a poorer leaving group the reaction favours α isomer (thermodynamic product).

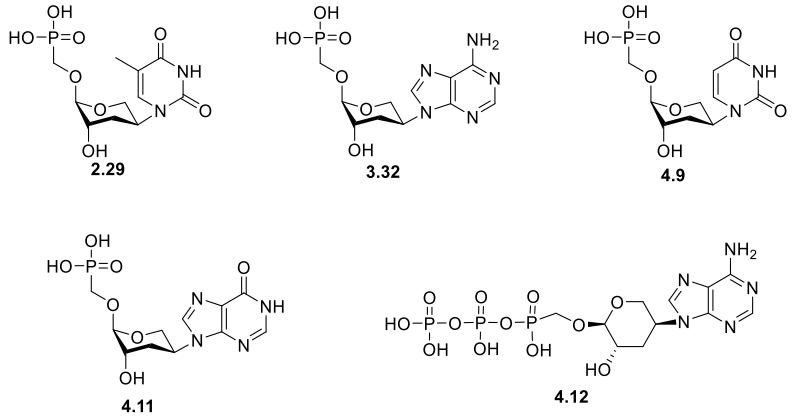


Figure 5-1. Synthesised nucleoside phosphonates and diphosphate.

In the second part of this thesis (**Chapter 3**), synthesis of D-*threo*-pentopyranoside phosphonate nucleoside with adenine as the nucleobase was explored. Sulfonate substitutions as used for the synthesis of purine-containing hexitol nucleosides, as well as the epoxide opening method used for the thymine nucleoside phosphonate, were not successful. The *in situ* construction of adenine from amino moiety at 4-position of pentopyranose was performed to afford the desired nucleoside. However, the Vorbrüggen glycosylation of adenine nucleoside failed to give the target phosphonate glycoside. Thus the strategy was revised, where phosphonomethyl glycoside was synthesised first followed by the construction of the purine nucleobase. It is worth mentioning that 1-*O*-diisopropylphosphonomethyl-3,4-dideoxy-4-amino-α-D-*threo*-pentopyranoside might constitute a versatile intermediate for the construction of a variety of analogues with different purine or pyrimidine nucleobases.[11]

In the third part of this thesis (**Chapter 4**), based on the results presented in **Chapter 2** and **Chapter 3**, D-*threo*-pentopyranoside phosphonates bearing uracil or hypoxanthine nucleoside synthesis was performed. Detailed solution conformational analyses of all the four final nucleoside phosphonate products were carried out using NMR spectroscopy. The observed 1,4-*syn* substitution pattern was established while these phosphonates differ in conformational behaviour from their phosphate counterpart (**Figure 5-2**). These phosphonates adopt a chair conformation with equatorial nucleobases while both the phosphonomethyl and the secondary OH groups at the 1′ and 2′ positions are axially positioned. This conformation is expected to be in favour for enzyme binding.[12] However, preliminary antiviral results for thymine and adenine nucleoside phosphonates showed their lack of

activity against herpesviruses, which may indicate these phosphonates are not recognised by enzymes involved in nucleotide metabolism.

The adenine containing nucleoside phosphonate was converted into its corresponding diphosphate and assayed for its ability to function as a substrate for variants of DNA polymerases. Klenow fragment (*exo*-) and Vent (*exo*-) were tested in the presence or absence of Mn^{2+} ions and shown to be able to use **4.12** as a substrate, although only the formation of P+1 or P+2 products was detected. This polymerases incorporation study are encouraging on the potential utility of these analogues for the selection of XNA systems.

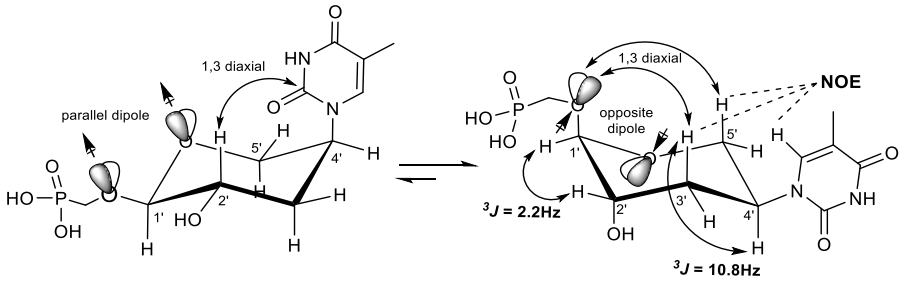


Figure 5-2. Preferential conformation of 1′-phosphonate D-*threo*-pentopyranose nucleoside bearing thymine as a base (**2.29**) as determined by NMR studies. Dashed lines indicate observed NOE interaction between H6 of thymine and the axially positioned H3′ and H5′.

Future perspectives

Structure-activity relationship study (SAR)

In this thesis, we have successfully developed strategies for the synthesis of four phosphonomethyl D-*threo* pentopyranoside nucleoside analogues **2.29, 3.32, 4.9** and **4.11** (**Figure 5-1**) bearing a 4′-nucleobase. However, preliminary antiviral tests of adenine **(3.32)** and thymine nucleoside phosphonates **(2.29)** did not yield promising results. More structural related candidates could be synthesised in the search for compounds with improved activity. As demonstrated during the synthesis of adenine nucleoside phosphonate in **Chapter 3**, a key intermediate, phosphonometylated 4-amino pentopyranoside synthon, could also be used for the construction of other natural (cytosine and guanine)[11] and unnatural nucleobases (position 5-modified pyrimidine; position 2, or 6 or 7 modified purine)[13-14] (**Figure 5-3**, **A**). Different strategies about heterocycle constructions could be investigated. In addition, C3-modification (counterpart in the place of C2 in

ribose) could be a possible strategy for the modulation of the biological activity. Substitutions such as N_3 and F have shown their importance in antiviral research (See **Chapter 1**, approved drugs). Whether these substitutions will change the conformational preference of these phosphonates is still to be answered. Furthermore, interesting properties have been described for 3′-F HNA[15] and L-HNA[10], and phosphonate mimics of these nucleic acids may also be considered (**Figure 5-3**, **B**).

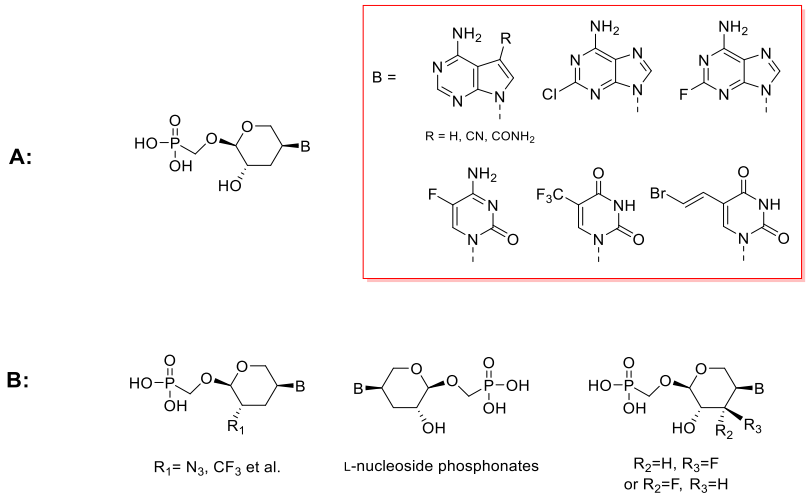
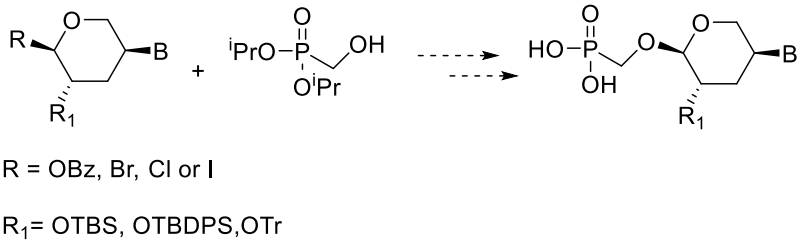


Figure 5-3. A: Proposed base-modified D-*threo*-pento-pyranoside nucleoside phosphonates to be investigated. **B**: Proposed sugar-modified D-*threo*-pento-pyranoside nucleoside phosphonates to be investigated.

The work presented in this doctoral thesis allows the preparation of small amounts (milligram scale) of the desired phosphonates. For more extensive studies and the development of new phosphonomethyl D-*threo*-pentopyranoside nucleoside analogues, and their use in oligonucleotides synthesis, more materials are needed, and a more efficient synthetic method is required. Herein, re-investigation or even re-design of the synthetic route will be required. The glycosylation reaction seems to be the most vital step to the overall yield. It was also observed that the outcome of the phosphonylation reaction is dependent on the properties and bulkyness of the C4 substituent like 4-thymine, 4-amino and 4-adenine. The stereoselectivity at the anomeric centre is influenced by the character of the different glycosyl donor and Lewis acid catalysts. All these chemical reactions need to be studied in more detail to be able to optimise the synthetic schemes.

In addition, whether a bulky group at C2 position will influence the conformation of the six membered ring thus affect the selectivity of α/β isomers remain to be futhur investigated. (**Scheme**

5-1).



R = OBz, Br, Cl or I

R_1= OTBS, OTBDPS,OTr

Scheme 5-1. Proposed strategies using different sugar scaffolds to affect the selectivity of glycosylation.

Triphosphate synthesis and enzymatic incorporation

The polymerase-mediated incorporation of nucleoside triphosphate analogues into oligonucleotides is a prerequisite for advancing their future applications in XNAs.[16] As shown in **Chapter 4**, commercially available Klenow (*exo*-) fragment and Vent (*exo*-) DNA polymerases were able to recognise diphosphate of adenine nucleoside phosphonate, however, only with 1 or 2 incorporations. For the further development of the phosphonate XNA, engineered polymerases that can mediate replication, transcription, and even reverse transcription processes of these XNAs with high efficiency and fidelity will be needed. Eventually, a replication cycle based on these novel pentopyranosyl nucleoside phophonates should be established, which can allow not only the storage but also the retrieve of the genetic information.

In the first instance and in order to advance the application of this type of nucleotide analogues into a functional artificial genetic system, it is necessary to engineer and identify potential polymerase candidates with improved recognition and incorporation capacities. This is possible with directed evolution of polymerases.[17] These evolved polymerases can be further used to test the potent of synthetic gentic polymers about the capability of heredity, evolution as well as acting as XNAzymes.[3,4]

Chemical synthesis of oligonucleotides

The availability of chemically synthesised oligonucleotides (XNA) is essential for the study of the physicochemical properties, to establish whether XNA could support duplex formation with a natural nucleic strand or a complementary XNA, and to select engineered polymerases that could

recognise and incorporate synthetic XNA precursors.[16]

Efficient phosphoramidates strategies are well established for the synthesis of sugar modified oligonucleotides[10,18]. However, the phosphoramidites method is not well applicable to the synthesis of phosphonate oligonucleotides (**Figure 5-4**). Recently, our group reported the first example of synthetic oligonucleotides with threose phosphonate modification using H-phosphonate chemistry, however, with limited yield.[6] Further research is still required to develop better synthetic methods suitable for XNA via H-phosphonate method (**Figure 5-5**), and to investigate proper coupling reagents and conditions.

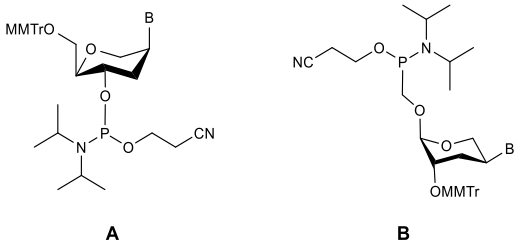


Figure 5-4. A: Hexitol nucleoside phosphoramidites. **B**: Proposed phosphonamidites as the building blocks of phosphonate oligonucleotides.

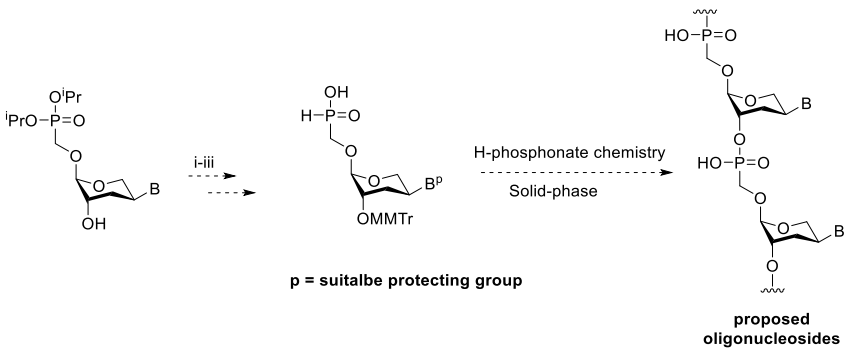


Figure 5-5. Proposed H-phosphonate precursors and solid-phase synthesis of oligonucleotides. Proposed reagents and conditions: (i) MMTrCl, $AgNO_3$, pyridine, THF; (ii) $LiAlH_4$, TMSCl, THF, –78 °C; (iii) (a) H_2O_2, THF/H_2O; (b) suitable protection strategies for different nucleobases.

So far, the nucleoside phosphonates obtained adopt different conformation as compared to their hexitol congeners. What kind of conformation will these six membered rings adopt during formation of the oligomers remains to be explored. In addition, it is essential to establish whether these synthesized oligonucleotides are capable of hybridizing with DNA/RNA, and self-pairing. Their physicochemical properties and the enzymatic stability of these oligomers are also important for further biological applications.

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Acknowledgement, Personal Contribution and Conflict of Interest Statements

1. Scientific acknowledgement

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2. Personal contribution

Prof. Graciela Andrei, Prof. Robert Snoeck and colleagues (Laboratory of Virology and Chemotherapy, Rega Institute) contributed to the antiviral activity evaluation which included in Chapter 4. Dr. Mikhail Abromov (Laboratory of Medicinal Chemistry, Rega Institute) contributed to synthesizing diphosphate of adenine nucleoside phosphonate which was used for incorporation study in Chapter 4. Dr. Hoai Nguyen (Laboratory of Medicinal Chemistry, Rega Institute) contributed to the incorporation study in Chapter 4.

3. Conflict of interest statement

No conflicts of interest are declared.

Curriculum Vitae

***Xiaochen Li** was born in Lingbao, China in 1990. He studied pharmaceutical science in the department of Pharmaceutical Sciences, Zhengzhou University, China and received a Bachelor of Science degree in 2011. Then he continued to study pharmaceutical science and obtained his Master of Science degree under the supervision of Prof. Hongmin Liu at the same university in 2013. Since September of 2013, he joined the Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, and started his PhD under the supervision of Prof. Piet Herdewijn.*

Publications

1. ***Xiaoche .li,** Shrinivas G. Dumbre, Eveline Lescrinier, Elisabetta Groaz, and Piet Herdewijn. Synthesis and Conformation of Pentopyranoside Nucleoside Phosphonates, J. Org. Chem. Accepted,* https://pubs.acs.org.ccindex.cn/doi/10.1021/acs.joc.8b03178.
2. *Duan, Ying-Chao, Yi-Chao Zheng, **Xiao-Chen Li**, Meng-Meng Wang, Xian-Wei Ye, Yuan-Yuan Guan, Gai-Zhi Liu, Jia-Xin Zheng, and Hong-Min Liu. Design, synthesis and antiproliferative activity studies of novel 1,2,3-triazole–dithiocarbamate–urea hybrids. Eur. J. Med. Chem. **2013**, 64, 99-110.*

Conferences/Symposiums Attended

1. *BOSS XVI, 16th Belgian Organic Synthesis Symposium, Brussels, Belgium, July 8-13, 2018. (Poster presentation)*
2. *Symposium on 'Tumor Immunology &Immunotherapy', Campus Gasthuisberg, Leuven, Belgium. 12-14th September, 2016.*
3. *3rd Spring Symposium "Synthetic Biology", Leuven, Belgium, 15th April, 2016.*