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Synthesis of Constrained Nucleosides

par

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To my family

Résumé

La thérapie antisens, sous sa forme la plus basique, implique la liaison d'une séquence oligonucléotidique courte à un acide ribonucléique messager complémentaire (ARNm), ce qui peut finalement entraîner une prévention de la maladie génétique en stoppant la production de protéines pathogènes. Les oligonucléotides composés d'acides nucléiques naturels sont capables d'une reconnaissance de liaison à haute affinité à des séquences complémentaires d'ARN et d'ADN; cependant, ils subissent rapidement une digestion intracellulaire par l'action de nucléases et ne conviennent donc pas à la thérapie antisens. La présente thèse présente et détaille la synthèse de quatre nouveaux analogues d'acides nucléiques pouvant être utilisés comme agents antisens potentiels et dans des applications thérapeutiques associées.

Basé sur une stratégie à double contrainte, le chapitre trois explore la synthèse de TriNA 2, un analogue nucléosidique qui étend la structure bicyclique de LNA en un noyau tricyclique, limitant la rotation autour de l'angle de torsion γ.

Dans le quatrième chapitre, deux voies de synthèse pour la synthèse d'un analogue de nucléoside pipéridino bicyclique sont discutées; l'une basée sur un nucléoside comme point de départ et la seconde étant une synthèse qui utilise un sucre comme point de départ.

Au chapitre cinq, la conception et la synthèse d'un nouvel analogue de nucléoside phosphonate oxabicyclique sont discutées. La conception de ce nucléoside comporte un noyau perhydrofuropyranne qui limite la rotation autour des angles ɣ, δ et ε d'un nucléoside naturel.

Le dernier chapitre décrit différentes approches de la synthèse de nucléotides à squelette macrocyclique basés sur des unités liées aux phosphonates. Notre première stratégie a utilisé

des H-phosphonates diastéréopure et leur alkylation stéréorétensive correspondante pour la construction du premier trinucléotide macrocyclique à cycle à 11 chaînons. Une approche de phosphoramidite complémentaire a fourni une voie complémentaire pour la synthèse du macrocycle à cycle à 11 chaînons et s'est avéré être un outil précieux pour la synthèse de macrocyles de différentes tailles.

Mots-clé : Thérapie antisens, acides nucléiques tricycliques, acides nucléiques bicycliques, acides nucléiques bloqués, restriction conformationelle, nucléosides, oligonucléotides, acides nucléiques, , la stabilité thermique des duplex

Abstract

In its basic form, antisense technology involves binding of a short oligonucleotide sequence to a complementary messenger ribonucleic acid (mRNA), which can ultimately result in prevention of genetic diseases prevention by stopping the production of pathogenic proteins. Oligonucleotides composed of natural nucleic acids are capable of high-affinity binding recognition to complementary RNA and DNA sequences; however, they rapidly undergo intracellular digestion through the action of nucleases and are thus unsuitable for antisense-based therapeutics. The present thesis reports and details the synthesis of four new nucleic acid analogues that can be used as potential antisense agents and in related therapeutic applications.

Based on a *double-constrain strategy*, chapter three explores the synthesis of **TriNA 2**, a nucleoside analog that extends the bicyclic structure of LNA into a tricyclic core, restricting rotation around torsion angle γ.

In chapter four, two synthetic routes towards the synthesis of a bicyclic piperidino nucleoside analog are discussed; one based on a nucleoside as starting point and the second one being a carbohydrate-based synthesis.

In chapter five the conception and synthesis of a novel oxabicyclic nucleoside phosphonate analog is discussed. The design of this nucleoside features a perhydrofuropyran core which restricts rotation around angles ɣ, δ and ε of a natural nucleoside.

The last chapter describes different approaches toward the synthesis of macrocyclic backbone constrained nucleotides based on phosphonate linked units. Our first strategy used diastereopure H-phosphonates and their corresponding stereoretentive alkylation for the construction of the first 11-membered ring macrocyclic trinucleotide. A complementary phosphoramidite approach

provided a complementary route for the synthesis of the 11-membered ring macrocycle and showed a valuable tool for the synthesis of different size macrocyles.

Keywords : Antisense therapy, tricyclic nucleic acids, bicyclic nucleic acids, locked nucleic acids, conformational restriction, nucleosides, oligonucleotides, nucleic acids, duplex thermal stability.

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List of symbols and abbreviations

Å	Ångström
Ac	acetyl
Alloc	allyl chloroformate
ASO	Antisense Oligonucleosides
aq	aqueous
B	general placeholder for a nucleobase
Bn	benzyl
BCNA	backbone constrained nucleic acids
BOM	benzyloxymethyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BRSM	based on recovered starting material
BSA	bis(trimethylsilyl)acetamide
BusCl	*tert*-butylsulfonyl chloride
CAN	ceric ammonium nitrate
CSA	camphorsulfonic acid
d	doublet
DBU	1,8-diazabicycloundec-7-ene
DCC	N, N'-dicyclohexylcarbodiimide
DCE	dichloroethane (e.g., 1,2-DCE = 1,2-dichloroethane)
DCM	dichloromethane
dd	doublet of doublets
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3H)-one
DIAD	diisopropyl azodicarboxylate
DIBAL	diisodbutylaluminium hydride
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide

DMI	1,3-dimethyl-2-imidazolidinone
DMP	Dess-Martin periodinane
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethyl sulfoxide
DMT	4,4'-dimethoxytrityl
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq (equiv.)	equivalent
ESI	electrospray ionization
Et	ethyl
g	gram
h	hour
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoramide
HRMS	high resolution mass spectrometry
Hz	Hertz
i	iso (as in *i*-Pr)
IBX	2-iodoxybenzoic acid
ImH	imidazole
IR	infrared spectroscopy
J	coupling constant
Lev	levulinyl
LICA	ligand-conjugated antisense
LNA	locked nucleic acid
LRMS	low resolution mass spectrometry
m	multiplet
M	**m**olar (mol/L)
Me	methyl
min	minutes
mL	milliliters

mmol	millimole
MOE	2'-*O*-methoxyethyl
MOP	methoxypropyl
Ms	methanesulfonyl
NaHMDS	Sodium bis(trimethylsilyl)amide
Nap	2-naphthylmethyl
NIS	N-iodosuccinimide
NMO	N-methylmorpholine N-oxide
NMR	nuclear magnetic resonance
%	percentage
PDC	pyridinium dichromate
Piv	pivaloyl
PMB	*p*-methoxybenzyl
PNA	peptide nucleic acid
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
PS	phosphorothioate
py	pyridine
q	quartet
RCM	ring-closing metathesis
ROESY	Rotating-frame Overhauser effect spectroscopy
r.t	room temperature
s	singlet
SDS	solvent delivery system
t	triplet
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TES	chlorotriethylsilane
TFA	trifluoroacetic acid

TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Tr	trityl
Ts	*p*-toluenesulfonyl
UV	ultraviolet
v/v	volume per volume

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My beloved wife Lore, te amo con todo mi corazón. Mua ….

1 Chapter One – Introduction

1.1 DNA and RNA structure

Nucleotides are the monomers of the polymeric macromolecules called nucleic acids. Each nucleotide is composed of three parts: the sugar backbone, the nitrogenous base and a phosphate residue. The sugar present in natural nucleic acids is a pentose, which depending on the substitution at the position 2', can be D-ribose (-CH-OH) or D-2-deoxyribose ($-CH_2-$) (a in Figure 1). In DNA the deoxyribose is the constituent sugar of the nucleic acid. In RNA, ribose is the sugar present in the nucleic acid.

In both DNA and RNA, the pentose is attached to a phosphate group which acts as a linker to form a chain that constitutes the backbone of the nucleic acid. The phosphate bond is formed between the 3'-OH of one pentose to the 5'-OH of another pentose, thus forming a phosphodiester bond. In the anomeric position of each sugar (1'), there is a nitrogenated heterocycle called the "nucleobase", which is *cis* (β face) to the hydroxymethyl group at the 5' position. The nucleobases are classified as purines and pyrimidines. Purines consist of a five-membered and a six-membered nitrogen-containing ring, fused together; Such as adenine and guanidine. On the other hand, pyrimidines such as cytosine, thymine and uracil, have a six-membered nitrogen containing ring. Adenine, guanine and cytosine are found in both DNA and RNA. Uracil is found exclusively in RNA. 5-Methyluracil (thymine) is present only in DNA.

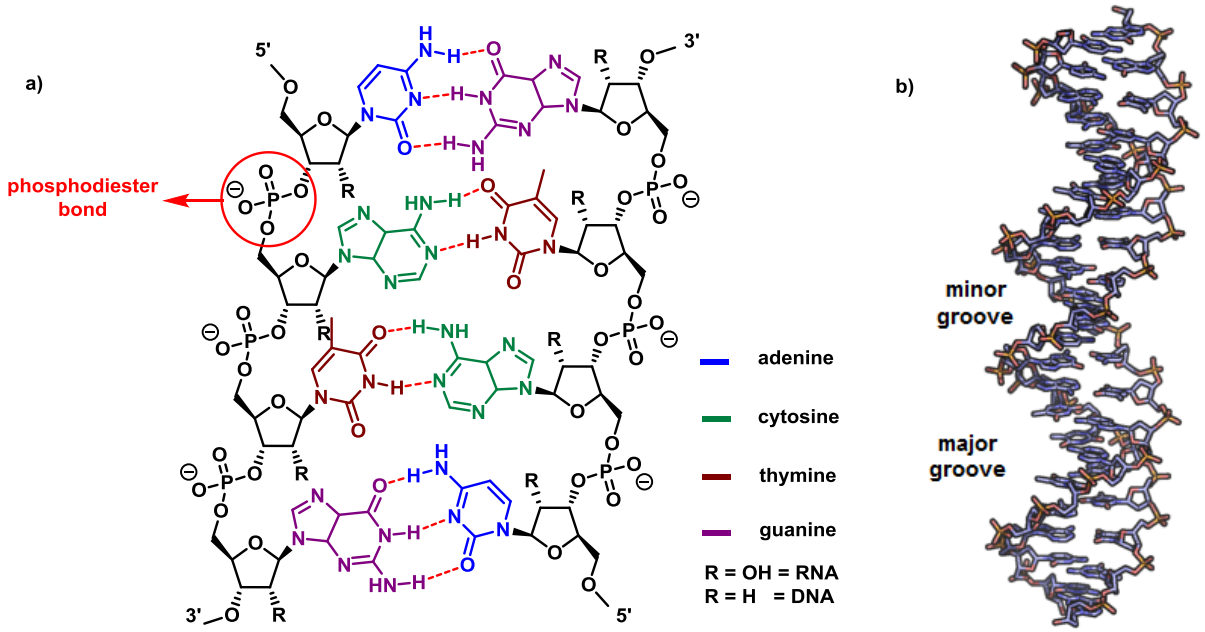


Figure 1. a) Generic nucleic acid double strand. b) Minor and major grooves of helix formed by double strand nucleic acids[1]

Thanks to the X-ray diffraction pattern analysis data taken in the 50's[2,3] and to chemical analysis, it was demonstrated that the number of adenines (A) is always the same as the number of thymines (T). Similarly, the number of guanines (G) is always equal to the number of cytosines (C). It was concluded that DNA was a double strand, where the nucleobases of each chain form hydrogen bonds with its other chain counterparts. Further studies on the helical structure of DNA concluded that the pairs cytosine-guanine and adenine-thymine were exclusively always present along the double strand. This complementary behavior dictates that each strand of the DNA is exactly like the complementary strand but linked in an opposite direction.[2]

The interactions between the different nucleobases are unique. The pair guanine-adenine forms three hydrogen bonds, while the pair cytosine-thymine only forms two hydrogen bonds. As a result, DNA with a higher percentage of G-C becomes more stable. As the hydrogen bond is a

non-covalent bond, it is possible to separate the double strand. This process is reversible in solution; however, it is necessary to heat the DNA. The temperature at which fifty percent of the molecules of DNA are separated in 2 single strands, is called “the melting temperature (T*m*)”. The T*m* value is an indicator for measuring the stability of DNA and its derivatives. Several techniques have been developed for the measurement of the T*m*. One of the most common is based on measuring the absorption of UV light by the nucleobases present in DNA, which exhibit a maximal absorption at 260 nm. Complementary pairs of nucleobases interact with each other pair of nucleobases by stacking of their pi-cloud electrons and by formation of hydrogen bonds. When the DNA is unwound to form two single strands, the stacking is weakened, increasing the absorption of light in a phenomenon called hyperchromicity.

The duplex formed by two strands of DNA or two strands of RNA assumes a right-handed helix where the nucleobases are stacked in the inner part of the helix and the sugar-phosphate polymer is installed in the exterior of the helix. The RNA and DNA duplexes present a difference in the size and shape of the grooves formed by the helix (b in Figure 1). The DNA duplex forms a type B helix where the major groove is wider than the minor groove. The RNA duplex shows a more compact and stable helical structure, with a major groove smaller than in a DNA duplex.

The conformation of a DNA strand is highly affected by the sugar-phosphate structural features. The most common way to describe the orientation of the sugar-phosphate backbone into the space is by the torsion angles which are defined by the rotation about each chemical bond (Figure 2). The standard nomenclature takes as a departing point the bond formed between the 5’-oxygen and the corresponding phosphorus, defined by the Greek letter α, followed by the bond O5’-C5’ which is defined as β, and continues through the nucleoside backbone until the bond formed between the oxygen at the 3’-oxygen and the phosphorus of the next nucleoside is

defined as ζ. Torsion angle X describes the rotation around the anomeric carbon-nitrogen bond which in turn describes the position of the nucleobase. The nucleobases can be positioned over the furanose core in a *syn*-conformation, or in an *anti*-conformation with the nucleobases pointing away from the sugar as in Figure 2.

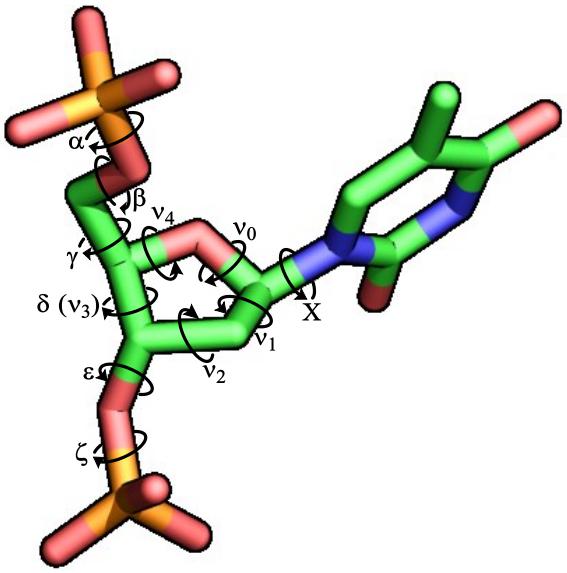


Figure 2. Torsion angles in nucleic acids[4]

The angles defining the furanose ring are named from ν_0 to ν_4 and they help to describe the distribution of the atoms in the furanose core, being commonly known as the sugar puckering. As the furanose ring is a cyclic system, it is not possible to rotate freely, so a pseudorotation process takes place which helps to minimize the steric congestion of eclipsed groups in the sugar In nucleotides, the furanose ring can be classified into one of the possible conformations which are represented in the pseudorotation circle in Figure 3. Each conformation can be classified into the circle where **E** (envelop) and **T** (twist) nomenclatures are used to describe the furanose puckering. The superscript value describes which atom is in an *endo* conformation and the subscript value describes the atom located in an *exo* conformation.

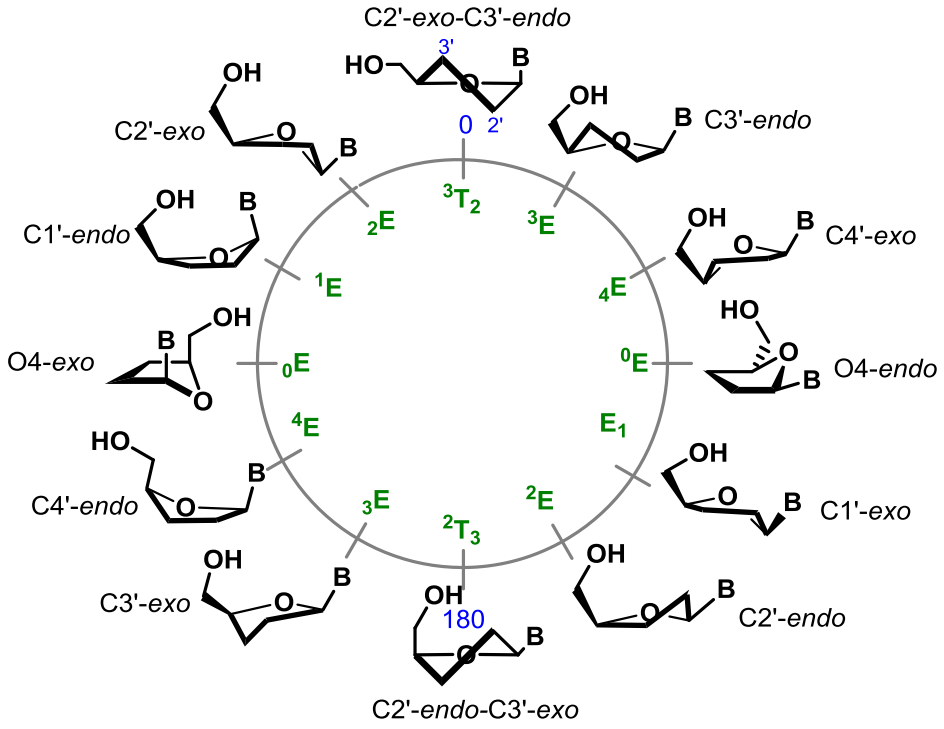


Figure 3. Sugar pucker of the furanose ring[4]

When one of the atoms of the furanose ring is pointing above the reference plane in the same direction as the nucleobase, it is said that it is in an *endo* conformation as shown in Figure 3; if such atom is pointing in the opposite direction, it is in the *exo* conformation.

There are two main conformers with the lower energy profiles, C2'-*endo*-C3'-*exo* (also known as S-type) and C2'-*exo*-C3'-*endo* (N-type).[5] The former one is encountered in DNA and the latter in RNA. The energy required to change between the different possible conformers is dependent on the substituents on the furanose sugar core but in most of the cases is ~2 KJ/mol.[6-9]

The predominant sugar puckering in natural nucleic acids (N and S-type) affects the global helicity of the nucleic acid. For deoxyribose, as in DNA, the S-type conformation is lower in energy (≈ 0.6 Kcal/mol) over the N-type giving to DNA a so-called B-form, which is the most

common form *in vivo*. In the B-form, the predominant S-type conformation keeps adjacent phosphates at a distance of ~7 Å (Figure 4) and the base pairs are effectively perpendicular to and centered over the helical axis. In the ribose ring, the N-type conformation is predominant, giving DNA-RNA and RNA-RNA duplexes an A-type helix. The C2'-exo conformation forces adjacent phosphates to become close (~5.9 Å) forming a denser helix with a displacement of the bases away from the central helix.

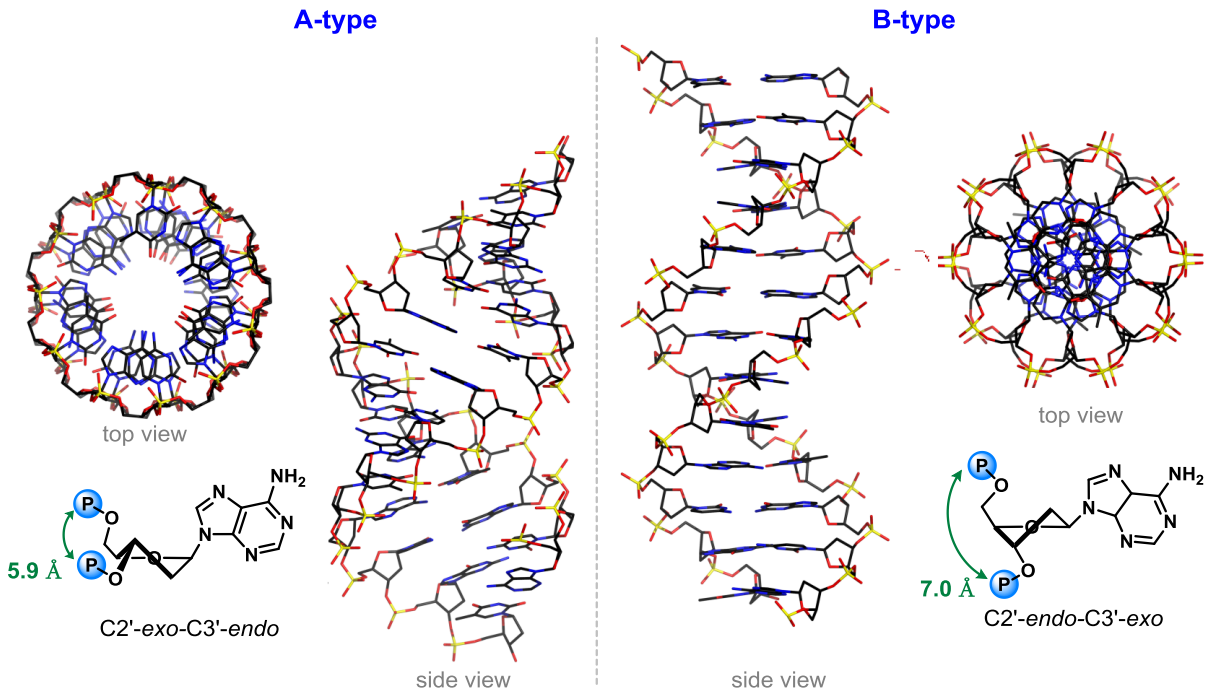


Figure 4. Structures of A-type and B-type double stranded nucleic acids

1.2 DNA transcription

Transcription is the process of copying the genetic information found in the double-stranded DNA into a single-stranded RNA, which is complementary to the segment of DNA from which it was copied. In order to begin the transcription process, it is necessary to have the four 5'-

triphosphate nucleosides (C, U, G, A), magnesium (II) and RNA polymerase (RNAp). RNAp is an enzymatic complex formed by several enzyme subunits which recognizes specific sequences in DNA (promoters) to start the transcription process. When the RNAp binds to the double-stranded DNA, it is necessary to unwrap a sequence consisting of about 14 base pairs. The gap formed will move through the DNA, as RNA opens and closes the double strand on its way through the DNA (Figure 5). RNA synthesis starts inside this transcription bubble in which each new nucleoside that joins the chain is chosen following the complementary base on DNA. The process of RNA synthesis continues until the DNA encodes a sequence (transcriptor terminator) and forms a loop in the RNA that helps to dissociate the RNAp transcription complex, and releases the new RNA strand (primary transcript).

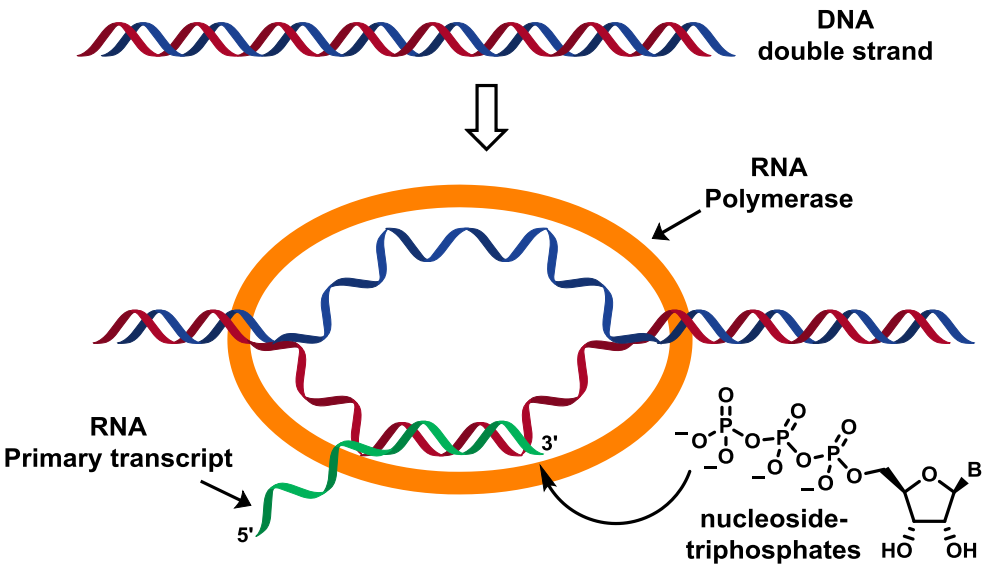


Figure 5. General scheme for DNA transcription[10]

1.3 RNA processing

In eukaryotic cells, the RNA obtained from the transcription process (primary transcript) goes through some changes in its structure in order to be able to transmit the stored genetic information. The first transformation of the primary transcript is addressed by imparting stability of the newly synthesized RNA strand towards nucleases, which are enzymes that cleave the phosphodiester bond. Such transformation is achieved by capping at the 5' position with 7-methylguanosine linked through a 5'-5' triphosphate bond (a in Figure 6).[11] Other modifications include the methylation of the 2'-OH of the first and second ribose units in the strand, and the addition of a long tail (between 100 and 200) of adenine ribonucleotides known as poly(A) at the 3' end of the RNA.[12]

The next step is the splicing of the stabilized RNA when some sequences, known as introns, are deleted (b in Figure 6). The number of sequences removed as introns depends on each species, but at the end all the remaining parts that codify for a gene (exons) are joined, forming the final mRNA responsible for translation of the genetic message.

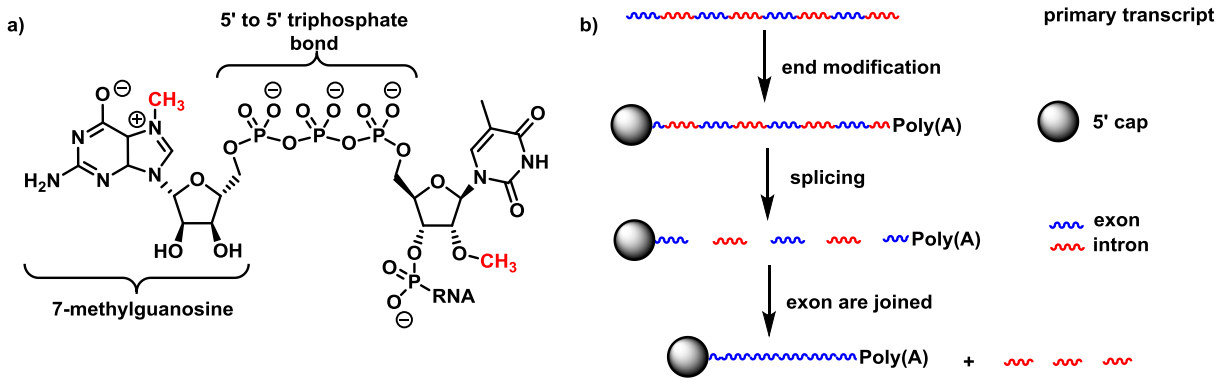


Figure 6. RNA processing

1.4 Protein synthesis – translation

The synthesis of proteins (translation) is carried out in the rough endoplasmic reticulum (RER) and performed within the ribosome, which is a large macromolecular complex made of both RNA and protein.[13] The amino acids that will form part of the newly synthesized protein are carried to the ribosome by transfer RNA (tRNA), a kind of RNA that is bonded to an amino acid and is able to recognize sequences of mRNA. Information is read from the sequences of nucleosides in groups of three (triads), which are called codons. Each codon is responsible for codifying a specific amino acid. One amino acid can be codified by different codons. Translation begins when the first molecule of tRNA interacts with the mRNA detecting the initiation codon and binding in the presence of the ribosome (Figure 7). With the ribosome reading the information and moving through the mRNA from the 5' to the 3' end, a second molecule of tRNA recognizes the next codon and brings the corresponding amino acid to the complex. The two amino acids react by forming a peptide bond, while the ribosome moves to the next codon, adding more amino acids and releasing the now empty tRNA. The rate of protein synthesis in eukaryotes can reach up to 14 to 18 amino acids/second per ribosome[14]. The process continues until a codon is reached which does not codify for an amino acid (stop codon). The last codon is recognized by a protein called “releasing factor”, which helps the synthetized protein to be expelled from the complex completing the translation process.[15]

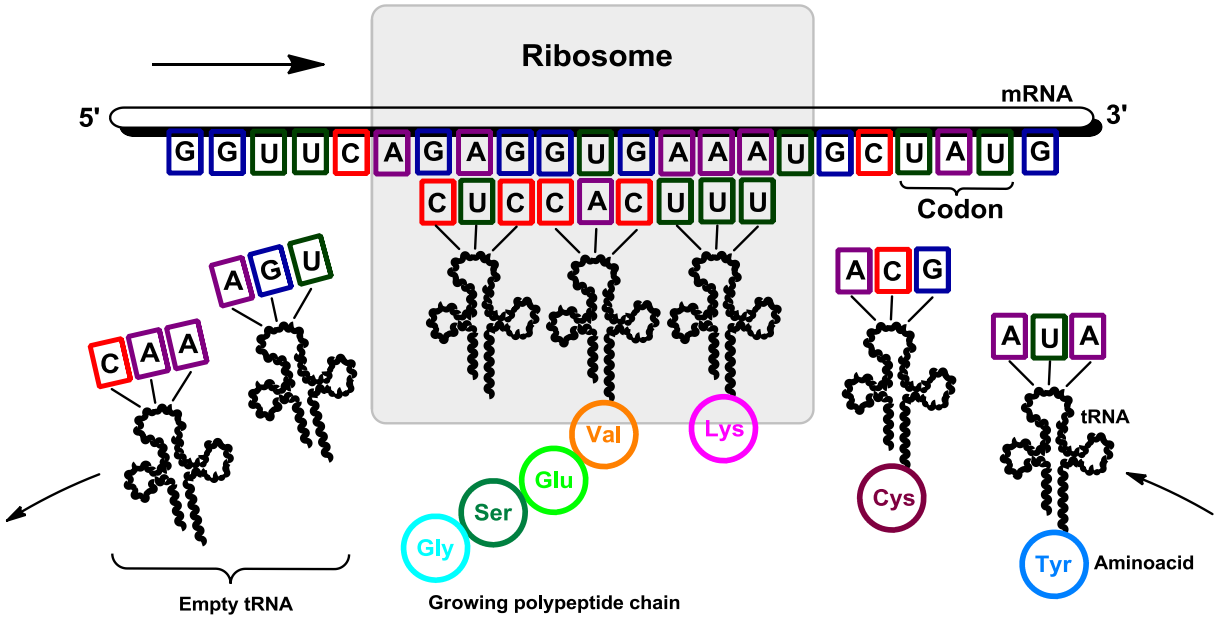


Figure 7. Translation process[10]

2 Chapter Two – Antisense technology

2.1 History

In 1978, Stephenson and Zamecnik,[16] working with chick embryo fibroblast cells infected with Rous sarcoma virus (RVS), achieved the inhibition of the production of the virus with a synthesized oligodeoxynucleotide. In the primary structure of the RVS, there were identical sequences of nucleotides next to the 5' cap and poly(A) 3' end. A 21-nucleoside sequence was the target of the study. A synthetic ribonucleoside was prepared complementary to a segment of the target sequence. The 13 nucleotide sequence d(AATGGTAAAATGG) bound to the target sequence, blocking the normal functioning of the virus by competitive hybridization.

Together with many subsequent studies, the idea of use RNA as a target was realized. The potential to treat different diseases was envisioned to be possible through malfunction of RNA, or blocking the translation process in the cell by introducing a complementary synthetic oligonucleotide that could hybridize with the mRNA. The complementary nucleotide chain is called the “antisense” chain. Binding to the target mRNA is a common mechanism of action for all antisense oligonucleotides (ASO).

2.2 Molecular mechanism of action

The multiple types of RNA and their multiple roles in the cell have become a growing area of interest with therapeutic potential in recent years. Historically important RNA include, mRNA, the small nuclear RNA (smRNA)[17] that plays key roles in the splicing process, and the less

studied microRNA[18] which seems to act as a natural gene regulator. Thus, the RNA molecule in general is a very attractive target for the development of new kinds of therapies for the treatment of diverse diseases, basically by preventing harmful proteins from being produced at the genetic level.

2.2.1 RNA cleavage

The destruction of the RNA chain by a family of enzymes called RNase H (ribonuclease H) is among direct approaches to inactivate its biological function. The RNase H enzymes found in mammals recognize the RNA-DNA duplex, hydrolyze the phosphodiester bond in the RNA and release the intact DNA strand.

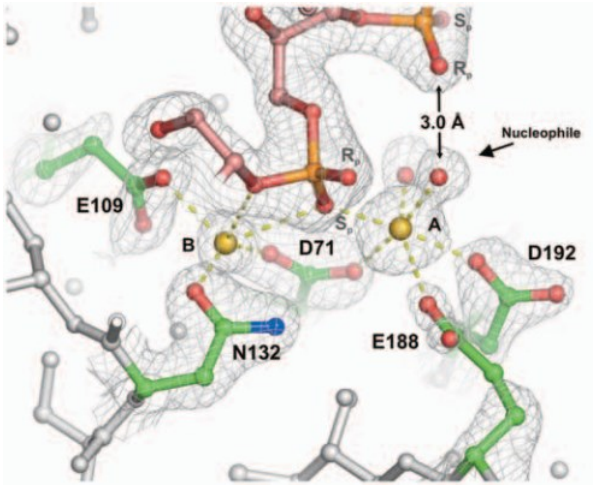


Figure 8. RNase H active site[19].The active site RNase in complex with the RNA/DNA hybrid. Active-site residues are shown in green; the RNA strand in pink, orange, and red; and the magnesium ions as yellow spheres. The water molecule (nucleophile) positioned to attack the scissile phosphate bond is indicated. Metal-ion coordination is shown as dashed yellow lines.

The cleavage necessitates enzyme recognition[20] of both the RNA and DNA strands. The duplex fits in two grooves at the enzyme structure. The 2'-OH of five consecutive nucleotides of the RNA strand interacts with one groove in the active site of the enzyme.[19] Different active–site residues of the enzyme, such as carboxylate and amino groups, form hydrogen bonds with the 3' and 5' sides of the phosphate bond to be cleaved (Figure 8). In the active site, two Mg^{2+} ions are chelated by four carboxylate groups and two molecules of water. One of the molecules of water attacks the phosphate linkage, assisted by one oxygen of the phosphate group which orients the attack and serves as a general base for deprotonation. After the attack, another metallic ion stabilizes the 3'-OH group from the cleaved phosphodiester bond.

The use of a designed ASO that binds a RNA target in order to destroy and therefore suppress the cellular function has been the subject of hundreds of studies.[21,22] Depending on the structure of the nucleotides that form the ASO, the duplex ASO-RNA can facilitate RNase H activity resulting in the hydrolysis of the RNA target. Modifications focussing on increasing the metabolic stability and increasing the affinity towards the target RNA, have often resulted in a loss of RNase H activity.[23] As an alternative, ASO hybrids have been developed composed of sections that support the RNase H activity and provide affinity to the complementary RNA (*quod vide*).

2.2.2 Alternative mechanisms involving non-RNA cleavage

Antisense oligonucleotides (ASOs) can be designed to work as competitive antagonists that bind to specific sequences in RNA and interrupt normal interactions with proteins, enzymes or other factors in the cell. One process that can be affected is maturation of the RNA primary transcript,

a fundamental step to obtain different functional RNAs such as mRNA and tRNA. Inhibition of 5' capping on RNA may be performed by designing an ASO capable of binding to the 3' end of the primary transcript. Another fundamental step in RNA processing is splicing. An ASO that binds to a sequence in RNA to be spliced, could create a barrier and supress splicing. Promising approaches of interrupting or modifying the RNA splicing process have been reported. For example, the use of ASOs by Kole[24] corrected of an aberrant splicing in mutated RNA and restored the natural splicing site. ASOs designed to bind with high affinity to the 3'-splice sites in murine pre-mRNA have exhibited activity in deletion of specific exons, resulting in the inhibition of mRNA production and suppression of protein synthesis.[25] In December 2016, the FDA approved Spinraza®, a medication used to treat spinal muscular atrophy (SMA). Spinal muscular atrophy is caused primarily by a genetic defect in the *SMN1* gene which encodes a defective SMN protein, a protein required for the survival of motor neurons in the spinal cord. Spinraza® is an oligonucleotide that binds the pre-mRNA and promotes the inclusion of exon 7 in the final protein.[26]

2.3 ASO modification

Obtaining a higher resistance against the degradative action of nucleases was the first challenge in ASO design. Replacing a non-bridging oxygen atom in the phosphodiester linkage by a sulfur atom resulted in modified nucleotides[27] which conferred resistance towards hydrolases. The new nucleotides received the name of phosphothioate (PS) nucleotides **2.1** (a in Figure 9). Upon RNA-PS nucleotide duplex formation, RNase H hydrolyzes the RNA strand and releases the PS nucleotide, blocking the production of the protein. Matsukura[28] was the first to report that

oligonucleotides containing the phosphothioate modification could inhibit human immunodeficiency virus (HIV) *in vitro*.

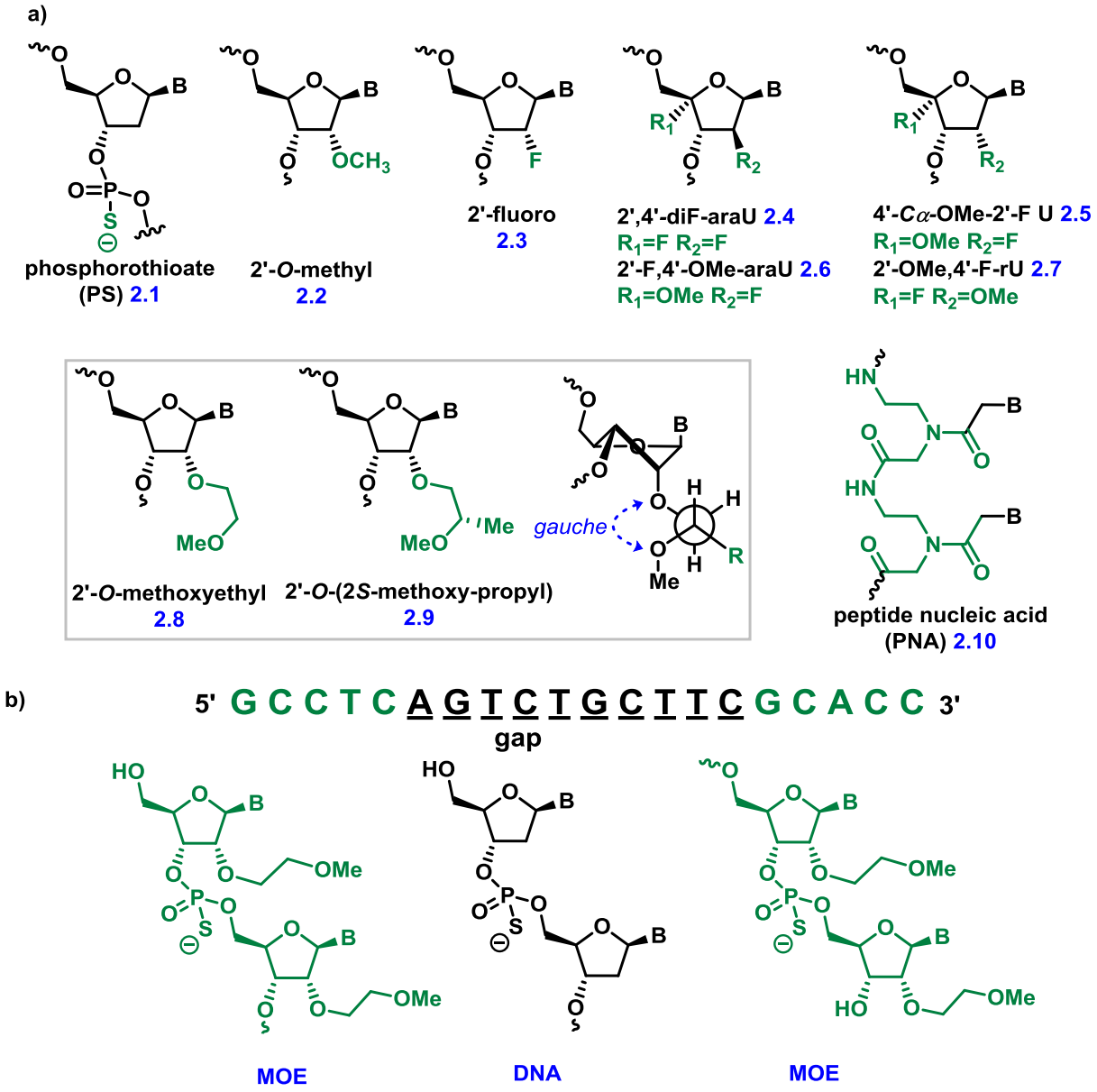


Figure 9. a) Generations of ASOs. b) Example of gap design oligonucleotide with MOE

PS oligonucleotides present a similar affinity for RNA compared to natural nucleic acids (ΔT_m ≈ -2°C per modification).[29] They promote binding to plasma proteins, favoring the distribution

in the body. Overall, PS-ASOs are among the most common modifications in antisense technology, because they combine hydrolytic stability of the backbone with affinity for the complementary RNA strand.

The next generation of ASOs was designed to increase the affinity with the RNA and nuclease resistance. Methylation of the 2'-OH of the furanose gave rise to the 2'-*O*-methyl nucleoside (**2.2** in Figure 9), which blocks the possibility of self-hydrolysis of the phosphodiester linkage by attack of the 2'-OH, and increase affinity *vs.* RNA ($\Delta T_m \approx 1°C$ per modification). The 2'-fluoro modification **2.3** (a in Figure 9), like the 2'-*O*-alkyl modifications, confers to the nucleoside a N- type conformation that shortens the distance between the 3'-5' phosphate bonds, generating a more compact structure that assumes a type A helix conformation, which increases the affinity of the ASO to the RNA.[30]

A series of studies by Damha and coworkers have shown that introducing fluorine and methoxy groups at specific positions of the furanose ring, renders the puckering into an N-type conformation. Introduction of two fluorine atoms at the 2' and 4'-position of furanose ring rendered nucleoside 2',4'-diF-araU **2.4**[31] into a N-type conformation, as a result of the anomeric effect between the antibonding orbital of C4'-F4'and the lone pair at oxygen of the furanose ring. The 4'-Cα-OMe-2'-F U nucleoside **2.5** also adopted an N-type conformation. When inserted into oligonucleotide sequences, **2.5** showed small changes in thermal affinity but gave high resistance towards the action of nucleases.[32] Parenting compounds 2'-F,4'-OMe-araU **2.6** and 2'-OMe,4'-F-rU **2.7** presented high percentages in the N-type conformation as measured by ^{1}H NMR spectrospcopy.[33] It was suggested that the predominant effect responsible for N-type conformation in nucleoside **2.8** is the $\sigma_{C3'H3'} \rightarrow \sigma^*_{C4'OMe}$ hyperconjugation effect. Introduction

of **2.6** or **2.7** into DNA:DNA duplexes is destabilizing but it showed a slight improvement in thermal affinity when introduced into RNA:DNA duplexes.

The 2'-*O*-methoxyethyl (MOE) nucleosides **2.8** together with PS backbone modifications represent the most studied second generation of ASOs.[34-37] The methoxyethyl group assumes a rigid conformation due to the *gauche* effect between the two oxygen atoms giving the furanose ring a N-type conformation. The MOE group is also suggested to form hydrogen bonds with water to protect the phosphodiester bond and increase resistance against nucleases[38] by increasing hydration in the minor groove reducing interaction of the double strand with nucleases. In an attempt to enhance the RNA affinity and metabolic stability, scientists at Ionis Pharmaceuticals prepared oligonucleotides containing the 2'-*O*-(2*S*-methoxypropyl) modification (2*S*-MOP).[39] The 2*S*-MOP modification (**2.9** in Figure 9) was designed to further reinforce the gauche conformation and increase the hydrophobicity in the minor groove, however, the 2*S*-MOP modification did not improve the activity compared to the MOE ASOs.

Such second generation of modifications resulted in a decrease in the toxicity associated with the first generation of ASOs,[40] however, they presented some difficulties in the design of new oligonucleotides, due to incompatibility with the action of the RNase and the RNA cleavage mechanism. The former limitation was overcome by the use of a "gap" design[23] (b in Figure 9), which combines 2'-*O*-alkyl nucleosides at the 3' and 5' end of the chain, with a central DNA region of PS nucleosides. In the gap design, the modified nucleosides located at the ends of the ASO increase the affinity for the RNA, and the PS internal chain supports the action of the RNase H for the cleavage of the target RNA. The success of the second generation of ASOs was shown by the approval in January 2013 of the gap designed oligonucleotide KYNAMRO™ by the FDA as a drug for the treatment of homozygous familial hypercholesterolemia.[41]

The third generation of ASOs contain different modifications on the carbon backbone. One of the more drastic changes was found in the peptide nucleic acid (PNA) **2.10** (Figure 9). Designed with a pseudopeptide backbone, consisting of *N*-(2-aminoethyl) glycine, PNA shows great affinity to RNA and DNA. Due to its pseudopeptidic structure, PNA is resistant to degradation by nucleases and proteases; however, it is not compatible with the RNase H mechanism of action. Such stability makes PNA a good candidate for use in the disruption of RNA processing by translation inhibition[42] and splicing modulation mechanisms.[43]

2.4 Constrained nucleosides

Over the last 20 years, three distinct strategies have used covalent bonds to incorporate a conformational constraint into different nucleic acids. The first strategy is based on restriction of the furanose ring mobility by a bridge between C2' and C4', that locks the ring into an N-type conformation as exemplified in LNA and α-L-LNA. Locked nucleic acid (LNA)[44] **2.7** is a restricted analog of 2'-OMe nucleoside, in which the 2' hydroxyl group methyl is tethered to the 4'-carbon (Figure 10). The resulting bicyclic structure confers a N-type conformation that presents an improved stacking between the nucleobases compared to that of the RNA duplex.[45] ASO strands containing LNA modified nucleosides present a pronounced increase in duplex stability relative to the DNA-RNA duplex.[46] The studies modifying the position of LNA in gap designed oligonucleotides concluded that a gap of at least 8 DNA nucleosides is necessary to induce efficient RNase H cleavage.[47] Further studies have shown the successful use of a LNA-gap design.[48]

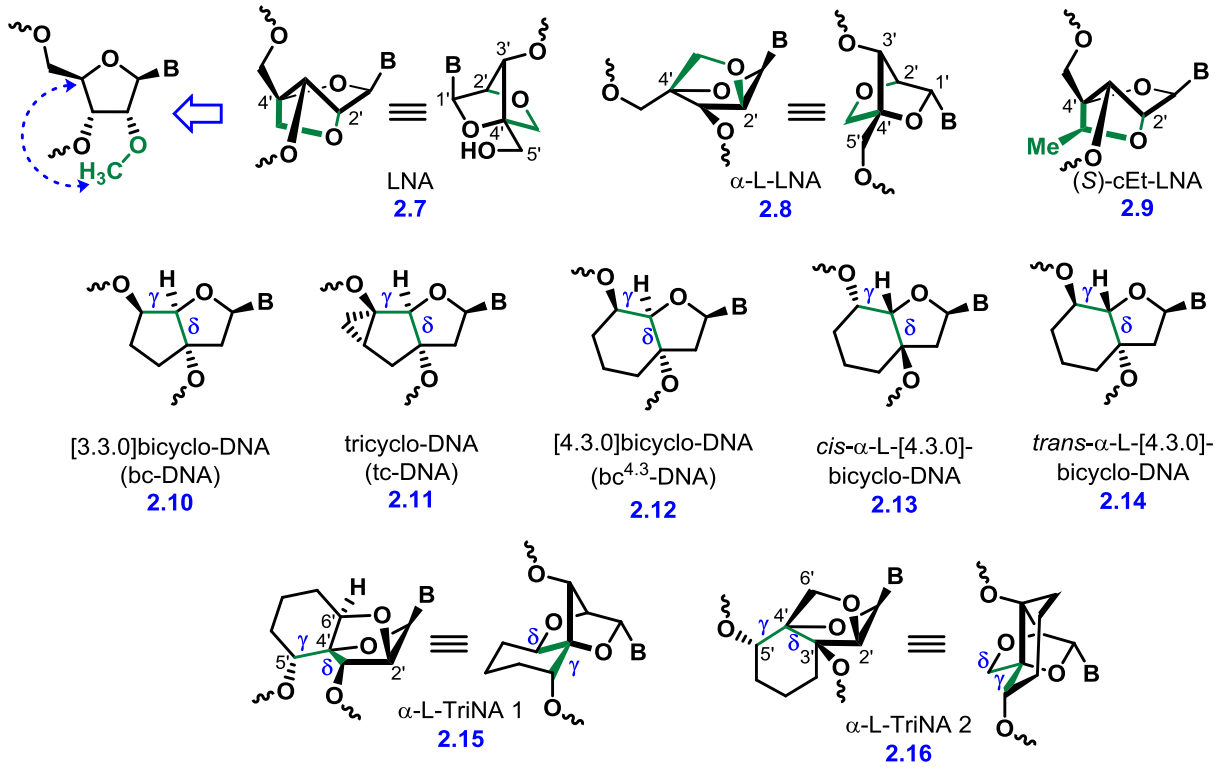


Figure 10. Constraint of torsion angles ɣ and δ in selected nucleosides.

Reported by Wengel, the hybridization properties of the eight isomers of LNA were investigated.[49] It was found that α-L-LNA (**2.8** in Figure 10) with the 2',4'-bridge on the same side as the nucleobases and the 3'-hydroxy group inverted, presented comparable affinity for RNA as does LNA. Further studies showed that α-L-LNA assumed an S-type conformation when introduced into an ASO paired with RNA which produced a duplex with an intermediate character between A and B-type helix.[50,51] As part of a continuing effort at Ionis Pharmaceuticals, a 6'-methylated analog of LNA was synthesised. The resulting constrained analog (*S*)-cEt-LNA (**2.9** in Figure 10) increased the stability of duplex compared to those containing LNA.[52] Exposure of a 10-mer poly T DNA oligomer with two (S)-cEt-LNA modifications at the 3' end to snake venom phosphodiesterase, revealed that the LNA oligomer

was completely hydrolyzed in 1290 min while the (S)-cEt-LNA modified chains were >70% intact.[53]

The second strategy comprises the restriction of the torsion angles ɣ and δ and has been widely studied since reports by Leumann of the [3.3.0]bicyclo-DNA (bc-DNA) **2.10.**[54-56] In bc-DNA (Figure 10) the carbocyclic ring formed between the 3'and the 5'-positions of the furanose ring forced torsion angle ɣ into non-natural positions[57] resulting in a poor affinity toward complementary DNA and RNA. A further constraint to bc-DNA was applied by introducing a cyclopropyl ring, forming tricyclo-DNA[58] (tc-DNA) **2.11** which showed an in increased RNA affinity. Tc-DNA Duplex comprised exclusively of poly-A and poly-T strands, presented a highly stable self-pairing.[59,60] X-ray crystallographic analysis of tc-DNA containing duplexes[61] showed a change in torsion angle ɣ and β compared to bc-DNA forcing the furanose into a C2'-*exo* conformation.

The six-membered ring constrained nucleoside [4.3.0]bicyclo-DNA **2.12** was synthesized and introduced into ASOs presenting similar thermal stability compared to natural DNA and RNA.[62]

With a similar strategy, scientists at Ionis Pharmaceuticals, in collaboration with the Hanessian group synthesized the bicyclic constrained nucleosides: *cis*-**2.13** and *trans*-bicyclo-DNA **2.14**, isomers of the previously synthesized bc$^{4.3}$-DNA (Figure 10).[63] Only incorporation of the monomer **2.13** into oligonucleotide sequences was possible, with the resulting ASOs presenting a slightly destabilizing effect upon duplex formation.

Combining the two approaches, the Hanessian group used a dual conformational restriction strategy featuring tricyclic nucleosides α-L-TriNA 1[64] **2.15** and α-L-TriNA 2[65] **2.16**. In α-L-TriNA 1, a spiro-annulation around carbon C4' restricts the torsion angle ɣ and locks the

furanose ring into a N-type conformation. In the α-L-TriNA 2, the N-type conformation is achieved as a result of a bridge between C2' and C4' and a fused 6-membered ring. Restriction of the torsion angles ɣ and δ is almost identical in both tricyclic nucleosides. α-L-TriNA 1 showed unprecedented duplex stabilizing properties versus DNA and RNA complements (8.3 °C/mod for RNA). In the case of oligonucleotide sequences containing α-L-TriNA 2, only a slight stabilizing effect was measured against RNA.

The third strategy of constraint was developed by Escudier, by restricting backbone torsion angles α and β (Figure 11). The dioxaphosphorinane-constrained nucleic acid[66,67] (α,β-D-CNA in Figure 11) in which torsion angle α is found in a in a non-canonical value (+*sc*), promote the bend in a single-stranded DNA, preorganizing it into a looplike structure. Such a structure was used to act as a chain terminator for proof reading DNA polymerases.[68] The synthesis of both P-isomers of phostone-constrained nucleic acids (P-CNA in Figure 11)[69] features the formation of a six-membered ring cycle locked in a chair conformation which provides a restrain on the α and β torsion angles giving them atypical values. The dinucleotide containing LNA/ α,β-D-CAN (Figure 11) with the α,β-D-CAN in a (*R*)-C5',R_P configuration, forced the dimer into a A-type duplex as determined by NMR studies.[70]

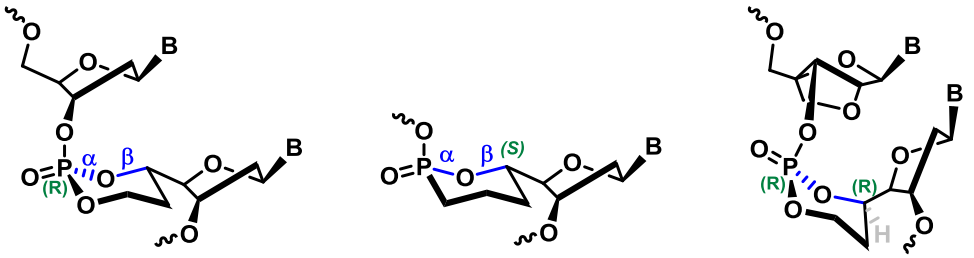
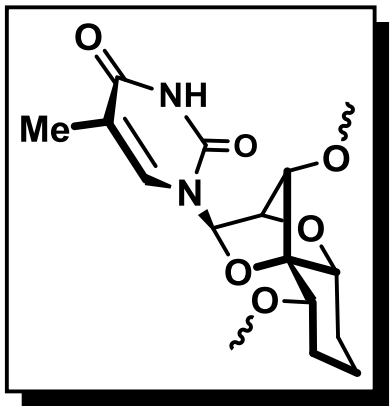


Figure 11. Restriction of torsion angles α and β in selected nucleosides.

3 Chapter Three

Synthesis of TriNA 2



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3.1 Design of TriNA 2

Starting from LNA as a template, a further restriction was applied by scientists at Ionis Pharmaceuticals, who introduced a methyl group into the C6' position of LNA (Figure 12), to reduce rotation of the C4'-C5' bond.[52,53] Two isomers were obtained: 6'-(*S*)-Me-LNA and 6'-(*R*)-Me-LNA (Figure 12). After introduction into different sequences of oligonucleotides, the thermal stability values obtained for the ASOs were comparable to the values presented by LNA containing uracil as nucleobase (ΔTm = 4.5 °C/mod.). Replacement of uracil by thymine improved affinity of the (*S*)-isomer.

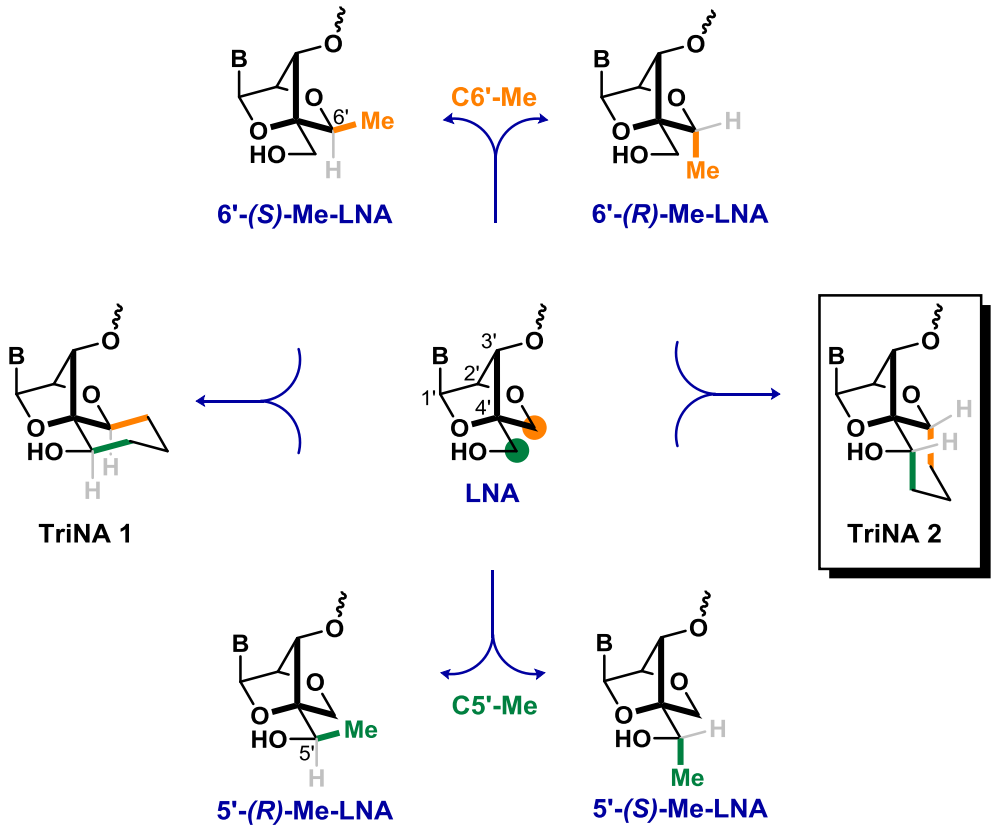


Figure 12. Introduction of a methyl group at different positions of the LNA core.

In a subsequent study, the effect of methyl group introduction at the C5' position of LNA was studied.[71,72] Oligonucleotides with a single modification containing 5'-(*S*)-Me-LNA (Figure 12) presented a similar thermal stability vs. RNA (ΔTm = 4.5 °C/mod). In contrast, 5'-(*R*)-Me-LNA completely reduced the stabilizing effect on duplex formation vs. both DNA and RNA.

These studies showed that introduction of methyl groups into the C5' and C6' positions were well tolerated, and a non-detrimental effects on thermal affinity was observed depending on the position and special orientation of the methyl group. To expand the constraining strategy, two dual-locked analogs were designed: TriNA 1 and TriNA 2 (**Tri**cyclic **N**ucleic **A**cid) (Figure 12). Merging two of the methyl modifications into a single nucleoside with a new carbocyclic core, TriNA 1 was synthesized by Dr. Robert Giacometti in the Hanessian group and will not be discussed in detail in the present document.[73]

The synthetic approach used for the synthesis of TriNA 2 will be presented in this chapter.

3.2 Retrosynthetic analysis of TriNA 2

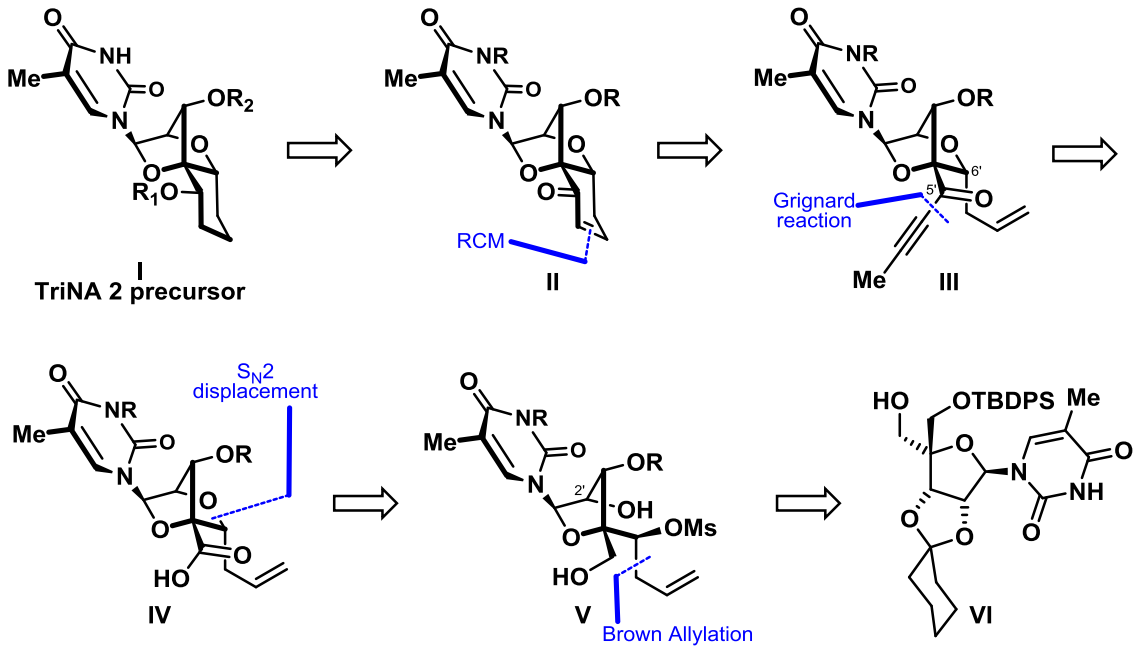


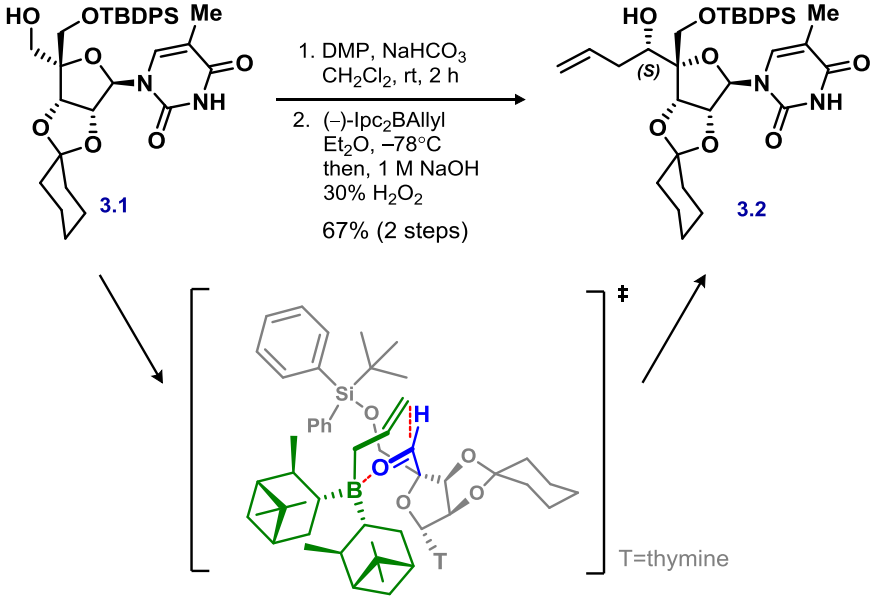
Figure 13. Retrosynthetic analysis for the synthesis of TriNA 2.

The core of the TriNA 2 nucleoside **I**, is formed by locking the furanose ring of a LNA type bicyclic structure by a 6-membered ring. The synthesis of orthogonally protected nucleoside TriNA 2 precursor **I** was envisaged to originate from the selective reduction of enone **II**. Enone **II** would be synthesized by a ring-closing metathesis reaction between two appropriate partners at the C5' and C6' positions of bicycle **III**. Propargyl ketone **III** could be prepared by the addition of a Grignard reagent onto a Weinreb amide derived from acid **IV**. An intramolecular S_N2 displacement involving the C2'-alcohol onto the 6' carbon containing an appropriate leaving group would result in the formation of the constrained bicyclic nucleoside **IV**. At this point the configuration of the carbon bearing leaving group at 6' would play an essential role in the synthetic strategy with a planned inversion. The formation of homoallylic mesylate **V**, could be achieved by the stereocontrolled addition of an allyl group to appropriate aldehyde at the C6'

position. Finally, we chose as a starting point an α-OTBDPS protected bis-hydroxymethyl thymidine **VI** which could be obtained in several steps from commercially available thymidine.

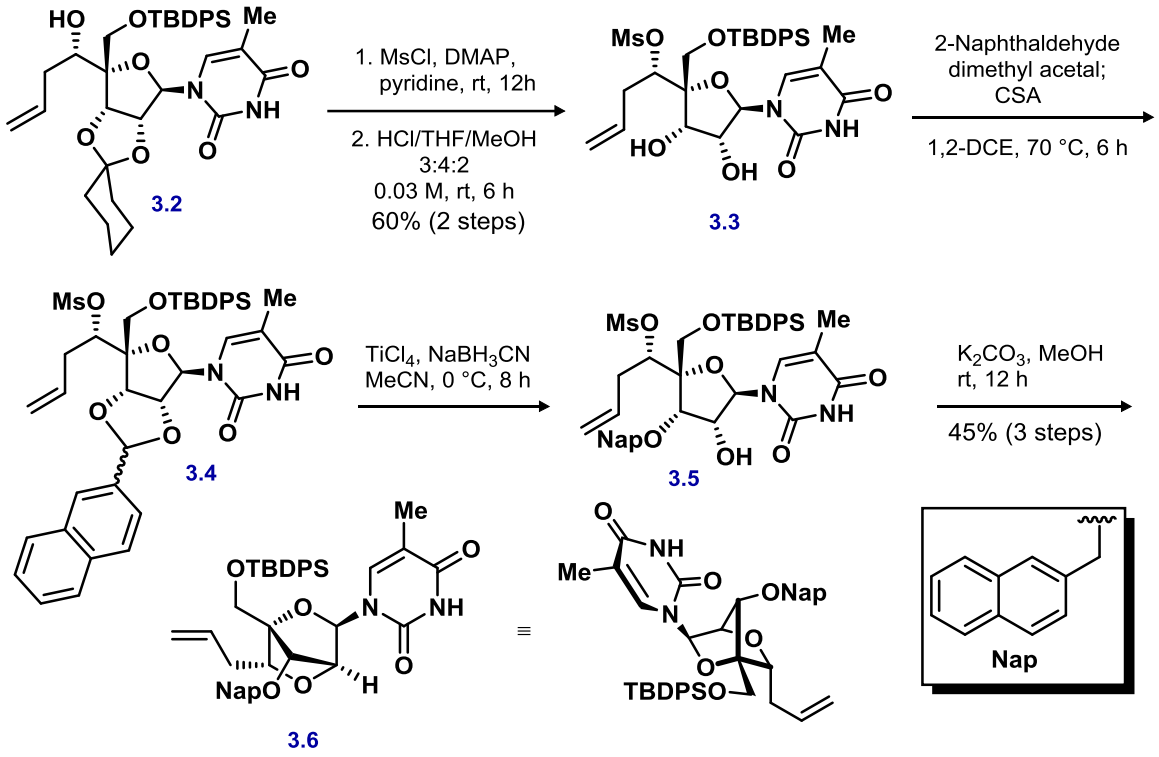
3.3 Synthesis of TriNA 2

The synthesis of TriNA 2 started from the previously prepared nucleoside **3.1**, which had been used in the synthesis of (S)-cEt-BNA nucleoside.[74] Accordingly, the required homoallylic alcohol (Scheme 1), was introduced through a two-step sequence comprising oxidation with Dess–Martin periodinane,[75] followed by a Brown allylation reaction with (−)-Ipc_2B(allyl).[76] The required (*S*)-alcohol **3.2** was obtained as a single diastereomer, with the selectivity arising from the attack of the allyl group in a chair-like transition state‡ onto the aldehyde. In the six-membered transition state‡, the facial selectivity is determined by minimization of the steric interactions between the aldehyde and the methyl groups in the Ipc ligand (Scheme 1).



Scheme 1. Synthesis of homoallylic alcohol **3.2**.

With alcohol **3.2** in hand, the corresponding methanesulfonate was synthesized and the cyclohexylidene acetal moiety was removed under acidic conditions to furnish diol **3.3** (Scheme 2).



Scheme 2. Synthesis of bicyclic nucleoside **3.6**.

The formation of bicyclic core **3.6** from diol **3.3** required the selective attack of the 2' instead of 3' hydroxyl group. In principle, the formation of the tetrahydrofuran ring is favored over the oxetane ring; however, previous experience with the cyclization of similar substrates[74] suggested protection of the C3'–OH of **3.3**. The desired C3'-O-naphthylmethyl ether was successfully installed using a two-step sequence that involved protection of the diol moiety as a diastereoisomeric mixture of naphthylidene acetals **3.4**, followed by selective deprotection of

the C2'-hydroxy group in the presence of $TiCl_4/NaBH_3CN$[77] to furnish alcohol **3.5**.* Base-promoted intramolecular displacement of the mesylate provided access to the bicyclic framework of **3.6** in 45% yield over three steps.

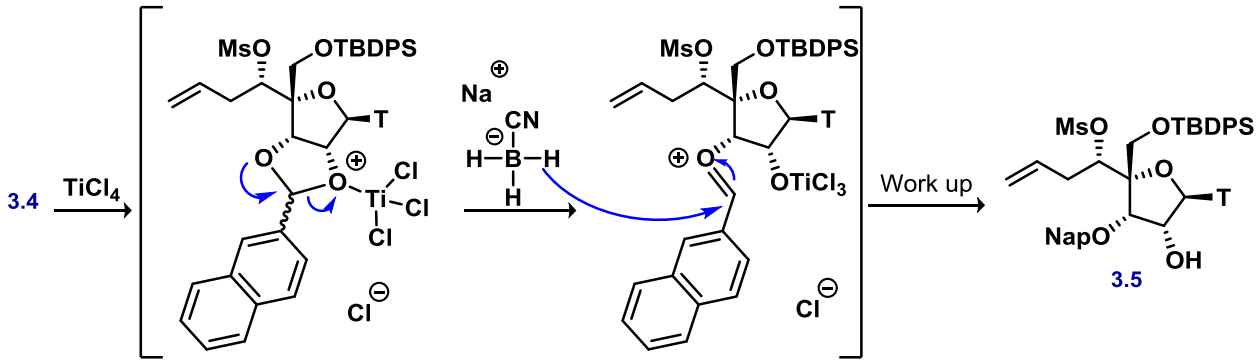
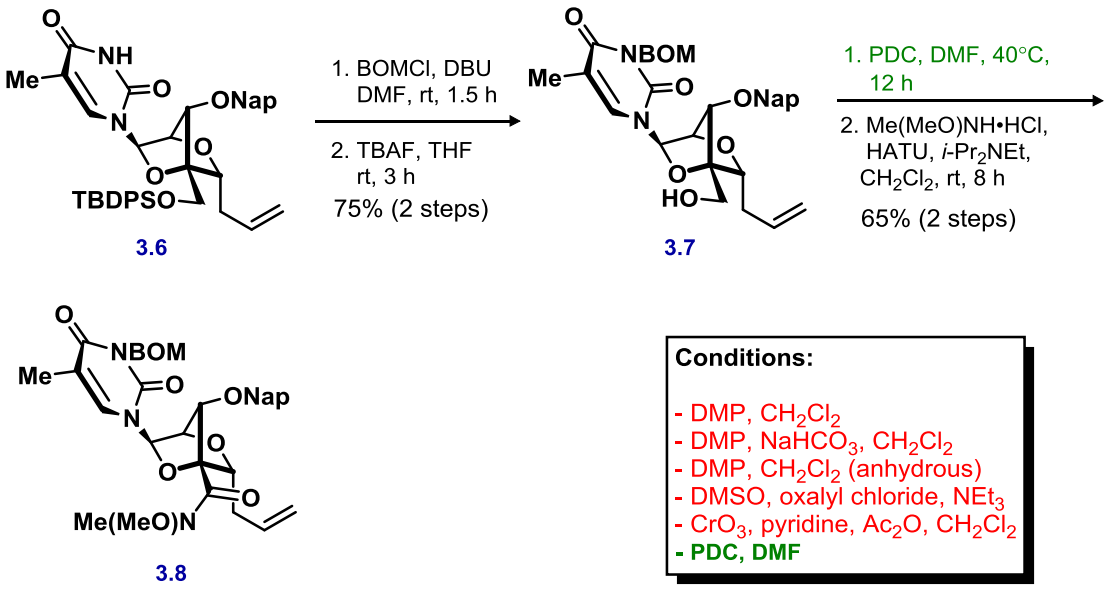


Figure 14. Regioselective reductive cleavage of acetal **3.4**.

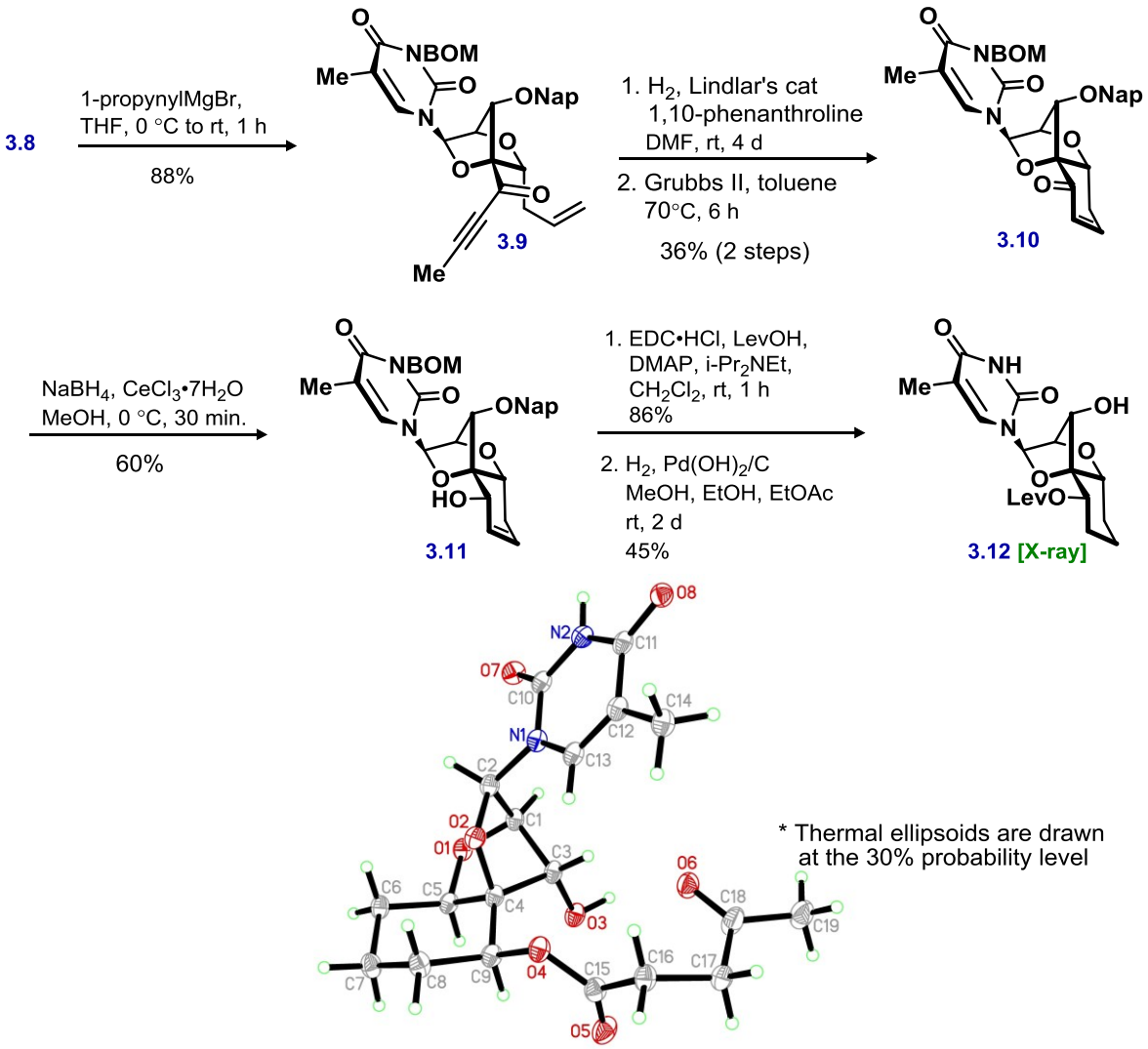
In the regioselective reductive cleavage of the naphthylidene acetal (Figure 14), titanium chloride (IV) acted as a Lewis acid that coordinates the less hindered oxygen of the acetal. Subsequent reduction of the generated oxocarbenium ion by sodium borohydride gave the 3'-Nap ether **3.5**.

* Heating diol **3.3** with 2-naphthaldehyde in a Dean-Stark apparatus gave a low conversion to acetal **3.4**. Instead, naphthaldehyde dimethyl acetal was used to improve conversion to acetal **3.4**



Scheme 3. Synthesis of Weinreb amide **3.8**

The N3-position of the nucleobase was protected as an N-benzyloxymethyl derivate to avoid potential side-reactions in the next steps (Scheme 3). Subsequent cleavage of the TBDPS group with TBAF provided alcohol **3.7** in 75% over two steps. As part of our strategy to extend the carbon chain at position C5', we attempted to oxidize alcohol **3.7** into the corresponding aldehyde. To this end, several different side-products were obtained when the reaction was carried out in the presence of Dess–Martin periodinane, Swern conditions, or with a mixture of TEMPO and PIDA,[78] a method developed for the synthesis of nucleoside-5'-carboxylic acids. To overcome the difficulties isolating the aldehyde, we opted to directly oxidize the primary alcohol to a carboxylic acid, before converting it to Weinreb amide **3.8**. The oxidation was successfully accomplished under Corey-Schmidt conditions (PDC in DMF).[79] HATU mediated coupling of the carboxylic acid with N,O-dimethylhydroxylamine hydrochloride afforded Weinreb amide **3.8** in good yield.



Scheme 4**.** Synthesis of TriNA 2.

Addition of 1-propynylmagnesium bromide to Weinreb amide **3.8** furnished propargyl ketone **3.9**, which we hoped to selectively reduce to the enone. Since ring-closing metathesis reaction has several precedents in nucleoside synthesis,[80-84] we decided to form the tricyclic core of TriNA 2 (Scheme 4) using such a strategy. Partial hydrogenation of **3.9** using Lindlar's catalyst[85,86] in the presence of 1,10-phenanthroline as a bidentate ligand minimized over-reduction.[87] The α,ß-unsaturated ketone was converted to enone **3.10** using Grubbs' II

generation catalyst, subsequent reduction of the enone under Luche conditions[88] favoured formation of the desired (*S*)-alcohol **3.11** over the corresponding epimer, with a ratio of 2.1:1. Use of (*R*)-CBS gave the desired (*S*)-alcohol albeit in low yields (≈10%). Formation of the levulinate ester and subsequent olefin hydrogenation with concomitant Nap hydrogenolysis provided alcohol **3.12**, which crystallized. Definitive evidence for the structure of TriNA 2 was obtained using X-ray crystallographic analysis. Transformation of **3.12** into the corresponding phosphoramidite and introduction into oligonucleotides sequences was performed at Ionis Pharmaceuticals.

3.4 Duplex thermal stability measurements

The duplex stabilizing properties of TriNA 1 and TriNA 2 versus complementary RNA were measured by scientists at Ionis Pharmaceuticals using a previously described oligonucleotide sequence.[62] Modified nucleotides were incorporated into DNA oligonucleotides at four different locations to provide a position and sequence context for the Tm studies (Table 1). Moreover, the duplex–stabilizing properties of LNA, (*S)*-cEt, and *(R)*-cEt modified oligonucleotides were measured as additional controls. Relative to an unmodified DNA control, incorporation of TriNA 1 produced an average increase in duplex thermal stability of +4.4 °C/mod. TriNA 2 was found to be more stabilizing, producing an average increase in Tm of +6.2 °C/mod. By comparison, LNA, *S*-cEt, and *R*-cEt produced average Tm increases of +6 °C/mod. Consequently, TriNA 1, which has the 5′-(*R*),6′-(*S*) configuration stabilized less the duplex than TriNA 2 containing the 5′-(*S*),6′-(*R*) configuration.

Table I. Duplex thermal stability of TriNA nucleotides.

Sequence (5' to 3')	ΔT/mod (°C)				
	LNA	*S*-cEt	*R*-cEt	TriNA 1	TriNA 2
GGA**T**GTTCTCGA	6.3	6.3	6.6	5.3	6.8
GGATG**T**TCTCGA	5.6	5.1	5.6	4.0	6.1
GGATGT**T**CTCGA	7.0	6.9	7.0	4.5	6.4
GGATGTTC**T**CGA	5.0	4.7	4.6	3.6	5.5
Average ΔTm	6.0	5.8	6.0	4.4	6.2

To further understand the origins of the differential duplex stabilizing properties of TriNA 1 and TriNA 2, a structural model was developed based on an overlap with the crystal structure of a *S*-cEt-modified A-form DNA duplex (Figure 15).[89] Both TriNA analogues were well accommodated within the duplex, with the six-membered carbocyclic rings projecting into the minor groove for TriNA 1 and towards the edge of the major groove for TriNA 2. Visual analysis of the structures suggested that the (*R*)-5′-methylene group of the carbocyclic ring in TriNA 1 may experience a tight contact (2.7 Å) with one of the non-bridging oxygen atoms of the 5′-phosphodiester linkage. In contrast, the analogous distance for TriNA 2 is 3.2 Å, and the tightest contact (2.9 Å) is likely between the (S)-5′-methylene group and the 3′-oxygen atom of the 3′-adjacent nucleotide. This suggested that the incorporation of TriNA 1 into the duplex might produce subtle changes around torsion angles α and/or β, or enhance conformational mobility of the phosphodiester backbone, in order to relieve this tight spacing, and consequently lead to a smaller enhancement in duplex stability.

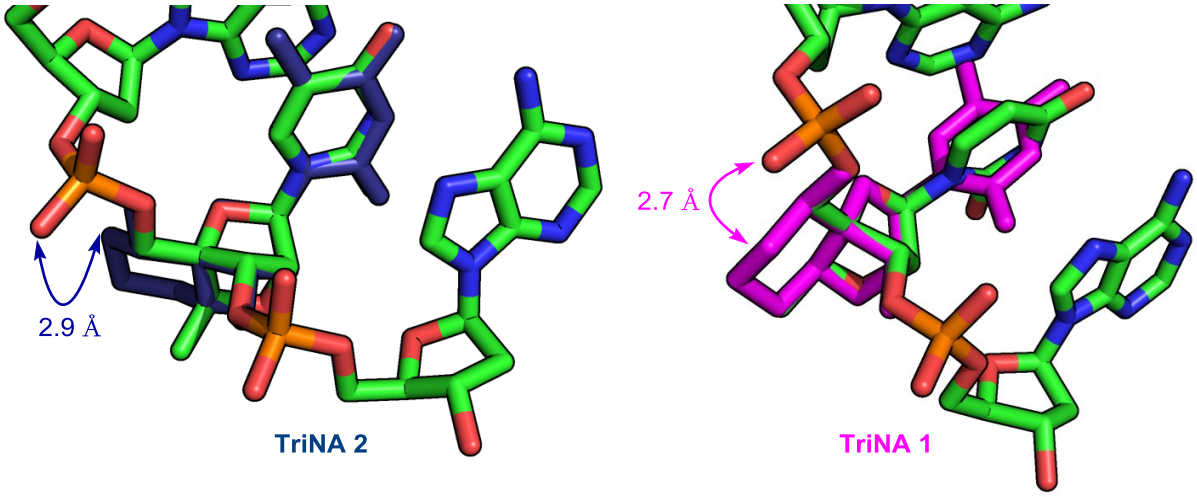


Figure 15. Structural models of tricyclic nucleosides overlaid on oligonucleotide duplexes containing the corresponding (*S*)-cEt bicyclic modification.

3.5 Crystallographic and conformational analysis

X-ray quality crystals analysis of ester **3.12** confirmed the absolute configuration of the molecule and provided parameters for the furanose sugar puckering and pseudorotation angle (P).[5] In nucleotides, the pseudorotation angle value can be calculated from the values for the torsion angles of the furanose using the formula in Figure 16. Depending on the value obtained, the furanose can be classified into one of the possible conformations which are represented in the pseudorotation circle in Figure 16. For nucleoside **3.12,** the value of P was 17°, which corresponded to a ^{2}E C2-*endo* conformation. For comparison, LNA had a P value of 17°,[90] confirming that TriNA 2 is constrained into a N-type conformation. The pucker amplitude (ν_{max}) is defined as the amount by which the fifth atom is displaced from the plane defined by the remaining four atoms. In LNA the pucker amplitude is ≈60°[91] and the measured value for nucleoside **3.12** using equation shown in Figure 16 was 59°.

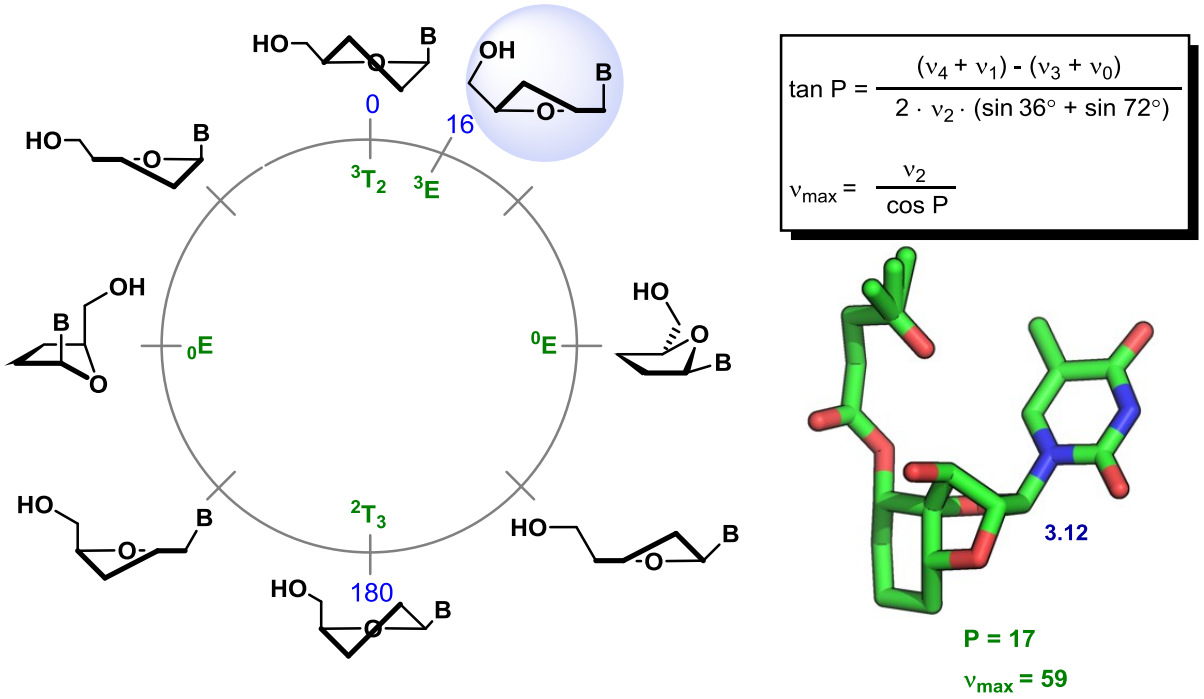


Figure 16. Pseudorotation pathway of the furanose ring

The dual constraint affected the torsion angle γ which has an average value[92] of 54° for double-stranded RNA-DNA duplexes. The value obtained from the X-ray crystallographic analysis for nucleoside **3.12** was 59.1°, and that obtained for LNA[93] was ≈50°, similar to the canonical value.

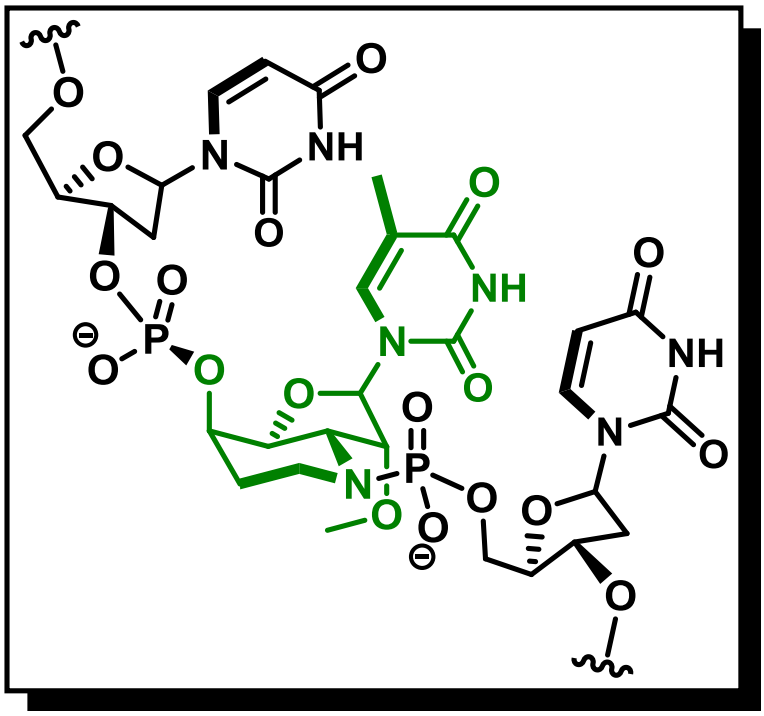
3.6 Conclusions

When introduced into different positions of oligonucleotides constructs, TriNA 2 produced an average increase in the T*m* value of +6.2 °C/mod, being more stabilizing than LNA and (*S*)-cEt-LNA.

The synthesis of compound **3.12** was successfully achieved in 17 synthetic steps and its absolute configuration was confirmed by X-ray analysis.

4 Chapter Four

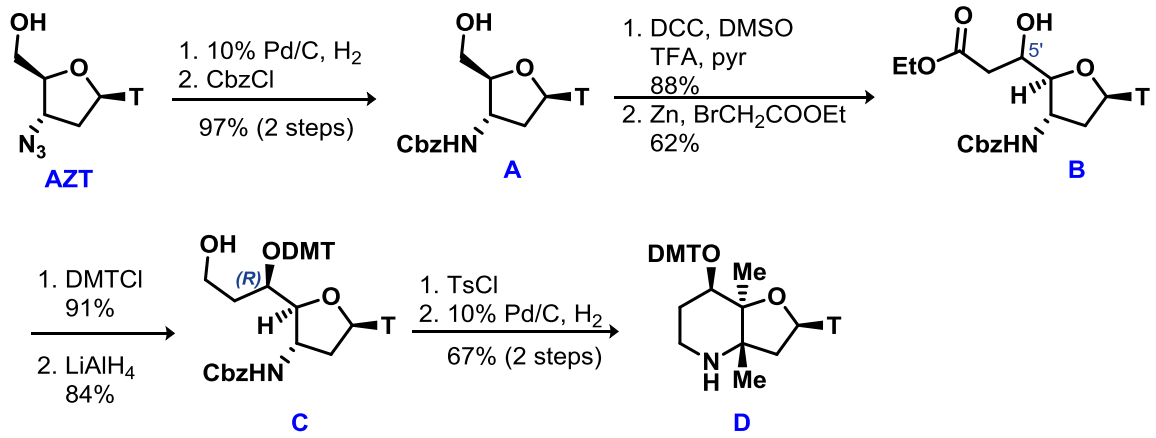
Synthesis of an azabicyclic nucleoside



Manuscript in preparation

4.1 Previous syntheses of azabicyclic nucleosides

In 1999, Wang[94] reported the synthesis of nucleoside **D** using azidothymidine (AZT) as starting point (Scheme 5). The synthesis took advantage the preinstalled azido functionality on AZT to obtain the protected 3'-amino thymidine **A,** which was oxidized under Moffatt conditions[95] and reacted in a Reformatsky reaction.[96] to afford a mixture of (*R*) and (*S*) isomers at 5'-OH. After O-tritylation and ester reduction, diols were separated and the (*R*)-alcohol **C** was tosylated. Intramolecular cyclization took place after removal of the benzyl carbamate group from the 3'nitrogen to give bicyclic nucleoside **D**.

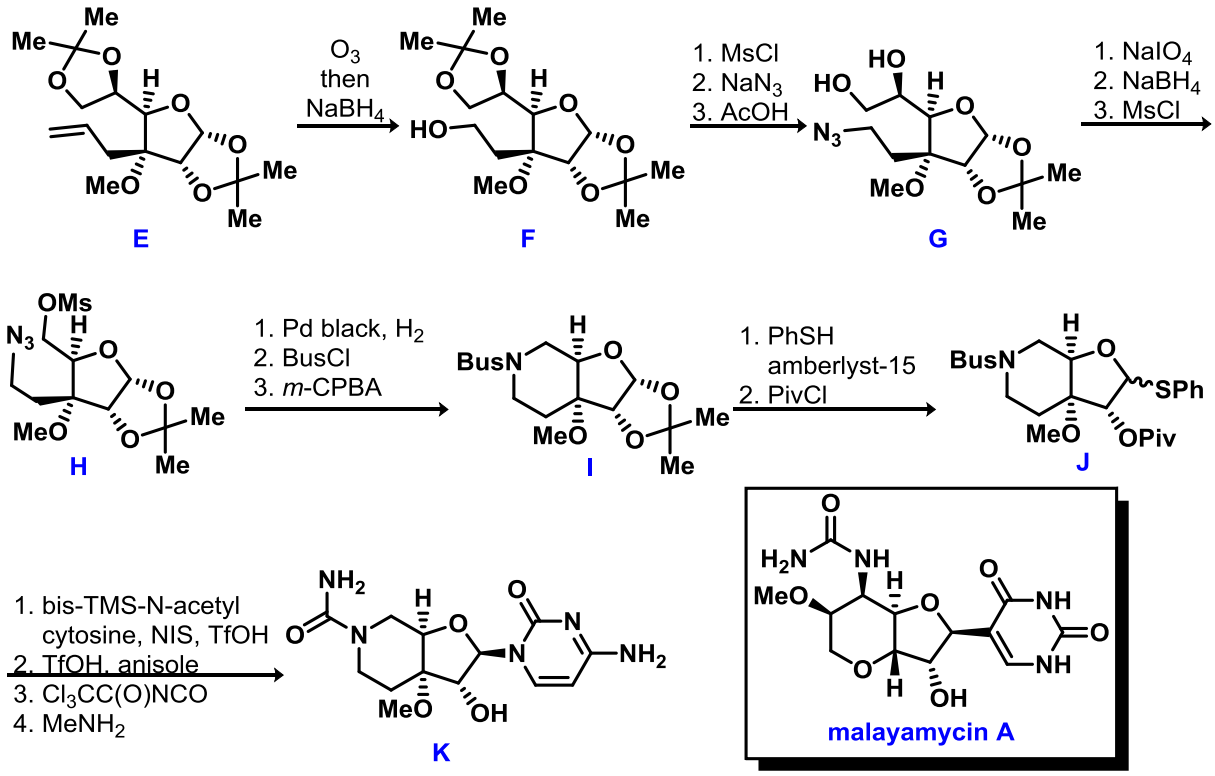


Scheme 5. The Wang synthesis of nucleoside **D**

In a later publication,[97] nucleoside **D** was introduced at two positions of a oligonucleotide, the duplex forming affinity of which was decreased against both DNA and RNA. No further exploration of the effect of the insertion of nucleoside **D** for other positions of oligonucleotide sequences was reported.

A related N-bicyclic nucleoside **K** was synthesized by Hanessian and coworkers as a ring-modified analog of malayamycin A (Scheme 6).[98] The known olefin **E**[99] was transformed into

alcohol **F** by oxidative cleavage and reduction of the aldehyde intermediate. The primary alcohol in **F** was mesylated and transformed to the corresponding azide. Subsequent hydrolysis of the 5,6-isopropylidene acetal gave diol **G** which was cleaved with sodium periodate. The resulting aldehyde was reduced to the alcohol which was mesylated to give **H**. Upon hydrogenation, cyclization took place to give piperidine **I** after protection of the nitrogen with a N-Bus[100] (N-*tert*-butylsulfonyl) group. Acid mediated thioglycoside formation with thiophenol was followed by protection as a pivaloyl ester. Treatment of **J** with a mixture of NIS/TfOH[101] as source of iodonium ion promoted a stereoselective installation of the cytosine nucleobase. After a series of deprotections and introduction of the urea group, nucleoside **K** was obtained, but unlike malayamycin A did not exhibit any fungicidal activity.



Scheme 6. The Hanessian synthesis of a malayamycin analog **K**

4.2 Design of an azabicyclic nucleoside

The ability to restrict rotation around torsion angles ɣ, δ and ε, while maintaining canonical bond-geometries and sugar pucker, presents challenges in the design and synthesis of novel nucleoside analogs. Scientists at Ionis Pharmaceuticals proposed an azabicyclic nucleoside **I** in which the orientation of the 3'-phosphate simulated the A-form of RNA (Figure 17). Restriction of rotation around the ε torsion angle was achieved by forming a piperidine ring with an appended equatorial phosphate group. Previously used in a related compound (Scheme 5), the strategy was amended by incorporation of a 2'-OMe to provide an extra degree of constraint to assume the RNA-like C3'-endo sugar pucker.

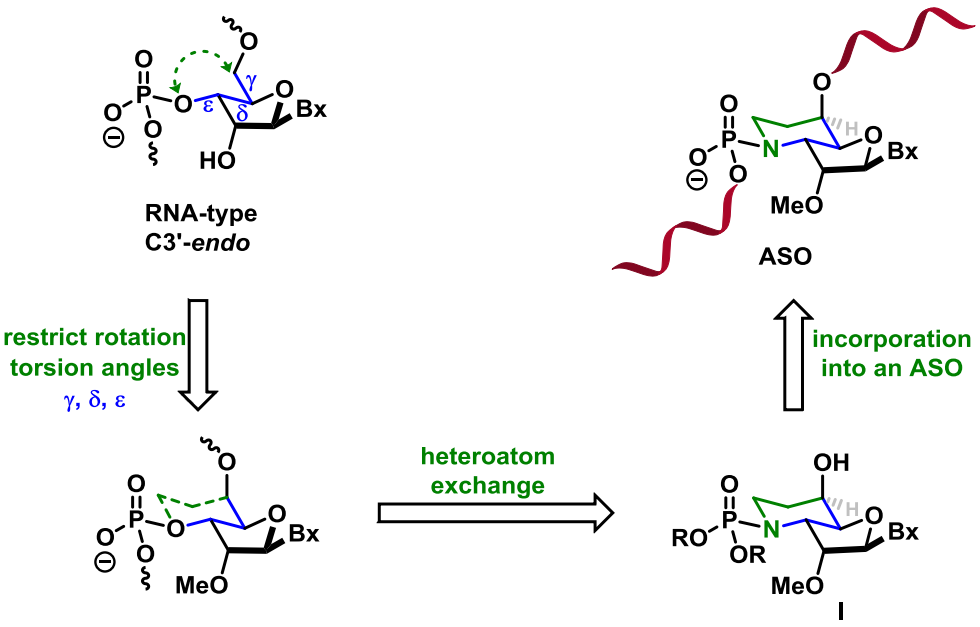


Figure 17. Design rationale of the azabicyclic nucleoside

The introduction of the azabicyclic nucleoside into an ASO and measurement of the thermal affinity in complexation with RNA and DNA strands would provide information about the relationship between the structure of a modified nucleoside and its capacity to hybridize with complementary nucleic acids.

4.3 Retrosynthetic analysis based on a nucleoside

After a general analysis of the structure of the intended nucleoside **I**, the piperidine moiety was envisioned to arise from an intramolecular aza-Wittig reaction (Figure 18). The required partners for such a reaction could come from an iminophosphorane formed between the 3'-azido group in aldehyde **II.** Aldehyde **II** could arise from a diastereoselective allylation of aldehyde **III** to the (*R*)-homoallylic alcohol followed by oxidative cleavage. The secondary alcohol at the 5'-position would have the (*R*)-configuration. Core unit **III** would originate from uridine containing the 3'-azide with an α-orientation. Introduction of a nitrogen at the 3'-position of the furanose ring has usually required lengthy steps.[102] A well-known strategy for the formation of azide **III** involves the regioselective opening of 2',3'-epoxide **IV** by an inorganic azide. The required epoxide **IV** could be obtained by a multistep manipulation of a protected uridine.

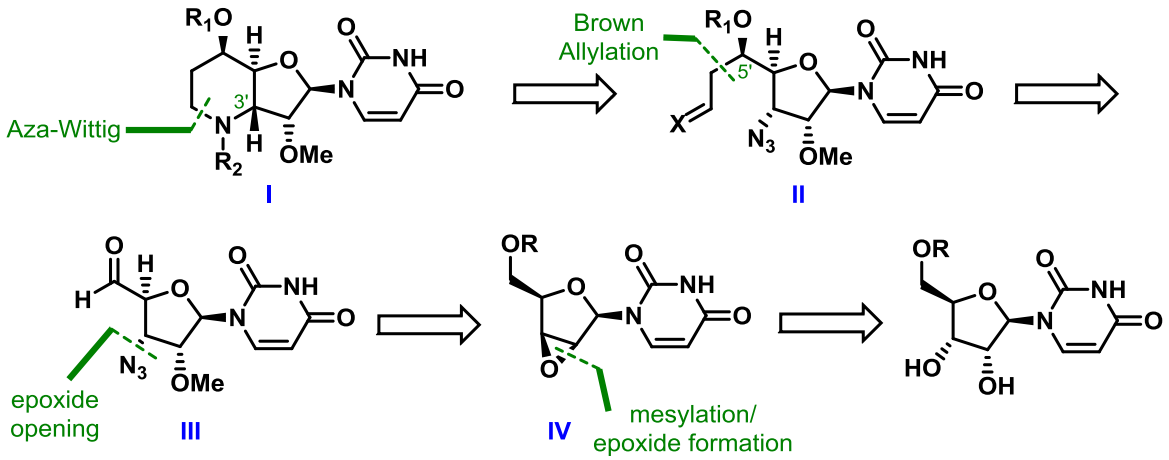
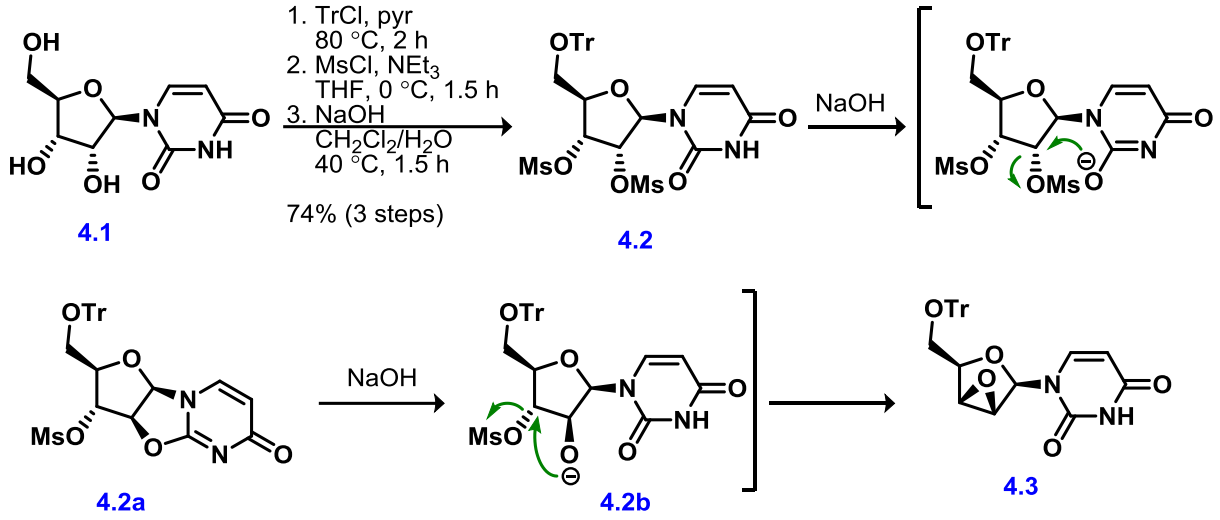


Figure 18. Retrosynthetic analysis of azabicyclic nucleoside **I**

4.4 Synthesis based on a nucleoside precursor

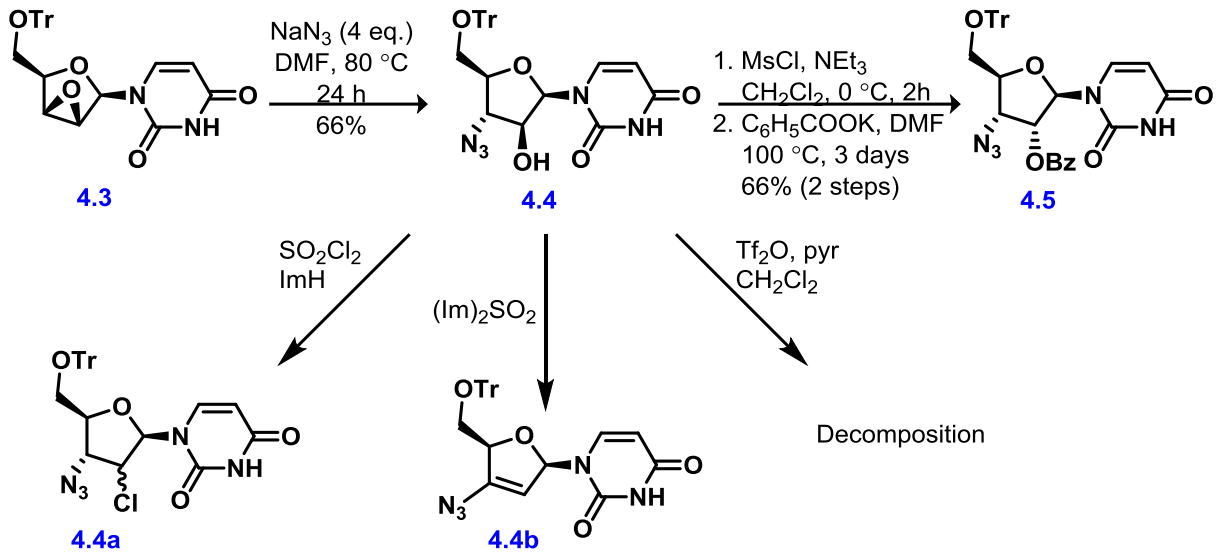
Starting with uridine **4.1**, the 2',3'-epoxide was prepared by a reported sequence[103-105] in which tritylation of the 5'-alcohol was followed by formation of 2',3'-bis-mesylated **4.2** (Scheme 7)**.** Treatment of the latter with aqueous NaOH triggered formation of the 2',2-anhydro bridge as in

4.2a which underwent rapid hydrolysis to reveal alkoxide **4.2b** followed by formation of epoxide **4.3**.



Scheme 7**.** Synthesis of epoxide **4.3**.

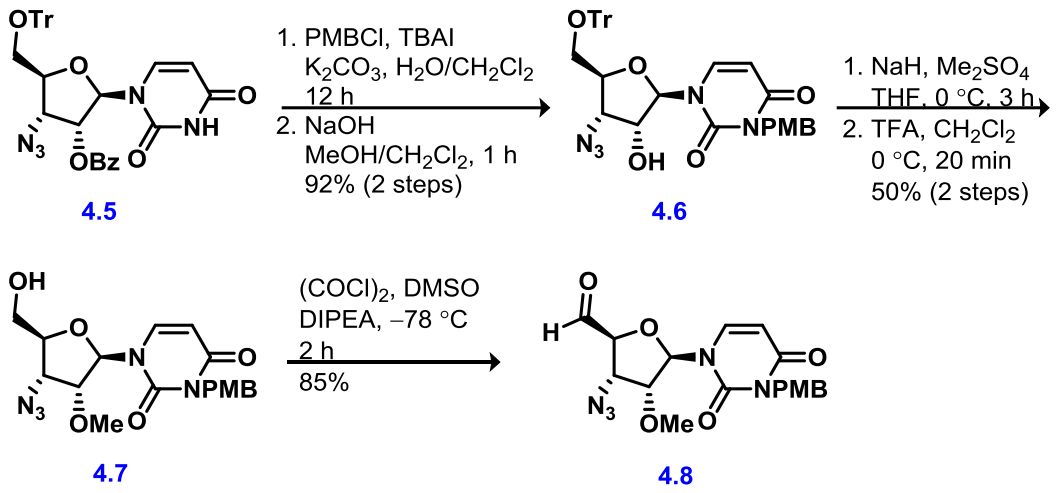
Treatment of **4.3** with NaN_3 gave azide **4.4** as the major regioisomer (3:1 ratio) as a result of epoxide opening at the 3'-position (Scheme 8).[106,107] The ring opening step is believed to proceed by activation of the epoxide in a S_N2 like transition state where excess azide salt increases the ionic strength of the solution.[102] Inversion of configuration at the 2'-OH was planned by displacement of a leaving group with an O-nucleophile, however, attempts using the corresponding triflate led to decomposition. Imidazylate (imidazole-1-sulfonate),[108,109] a leaving group with similar nucleofugal properties as a triflate group developed in our group, was next investigated. Alcohol **4.4** reacted with sulfuryl chloride in the presence of imidazole, to provide chloride **4.4a** in 86% yield. Treatment of alcohol **4.4** with N,N'-sulfuryldiimidazole resulted in elimination to form vinylazide **4.4b**. After some experimentation, mesylation of the 2'-alcohol followed by treatment with KOBz was found to give ester **4.5** in good yield.[105]



Scheme 8. Synthesis of nucleoside **4.5**

Installation of a methyl group at the 2'-OH position required the protection of the N3 position of the nucleobase by reaction with *p*-methoxybenzyl chloride (Scheme 9). After protection of nucleoside **4.5,** the benzoate ester was cleaved under basic conditions to provide alcohol **4.6** in 92% yield over two steps. Treatment with dimethyl sulfate led to the 2'-O-methyl ether which was converted to primary alcohol **4.7** and oxidized under Swern conditions to give aldehyde **4.8** in good yield[†].

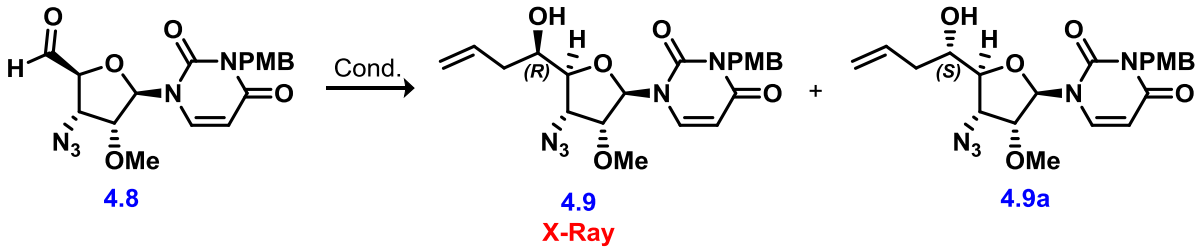
[†] Use of Dess-Martin periodinane for the oxidation of **4.7** gave lower yield of **4.8** (≈40%)

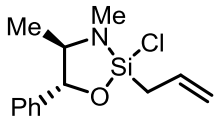
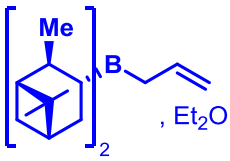


Scheme 9. Synthesis of aldehyde **4.8**.

With aldehyde **4.8** in hand, conditions were screened to favor formation of the *(R)*-homoallylic alcohol **4.9**. Lewis acid mediated allylation with allyltrimethyltin gave different ratios of the desired isomer, with variable selectivities (Table II). Initial attempts favored the undesired (*S*)-isomer **4.9a**, or gave a 1:1 mixture of alcohols. Employing $MgBr_2 \cdot Et_2O$ led to a mixture of alcohols slightly enriched in the desired (*R*)-alcohol. Using the strained silacycle developed by Leighton,[110,111] an almost equal mixture of alcohols was obtained. Gratifyingly, the use of Brown conditions,[76] (+)-Ipc_2BAllyl, furnished the (*R*)-isomer as a single diastereomer in 90% yield after oxidative work-up. The absolute configuration of alcohol **4.9** was established by X-ray crystallographic analysis (Figure 19). The diastereoselectivity may be explained by a chair-like transition state (**a** in Figure 19) with the furanose ring positioned in an equatorial position to minimize a steric clash between the allyl chain and the methyl groups from the isopinocamphene auxiliary.

Table II. Diastereoselective allylation of aldehyde **4.7**.



Conditions	Ratio *R*/*S*
$SnMe_3$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, –78 °C	1 : 2
$SnMe_3$, $TiCl_4$, , CH_2Cl_2, –78 °C	1 : 1
$SnMe_3$, $MgBr_2{\cdot}Et_2O$, CH_2Cl_2, –78 °C	1.5 : 1
 , toluene, –4 °C	1.2 : 1
 , Et_2O, –78 °C, then NaOH, H_2O_2.	Only **4.9**

Formation of piperidine **4.11** (Scheme 10) was envisaged by oxidative cleavage of terminal alkene **4.9**. With the homoallylic alcohol in hand, we planned on an oxidative cleavage to the corresponding aldehyde, to be followed by an intramolecular aza-Wittig ciclization to yield **4.11**. However numerous conditions such as ozonolysis, oxidations with OsO_4, RuO_3 and $KMnO_4$ to obtain the corresponding aldehyde where not successful. Protection of the allylic alcohol did not change the course of the oxidation, such that we were unable to obtain aldehyde **4.10** for cyclization.

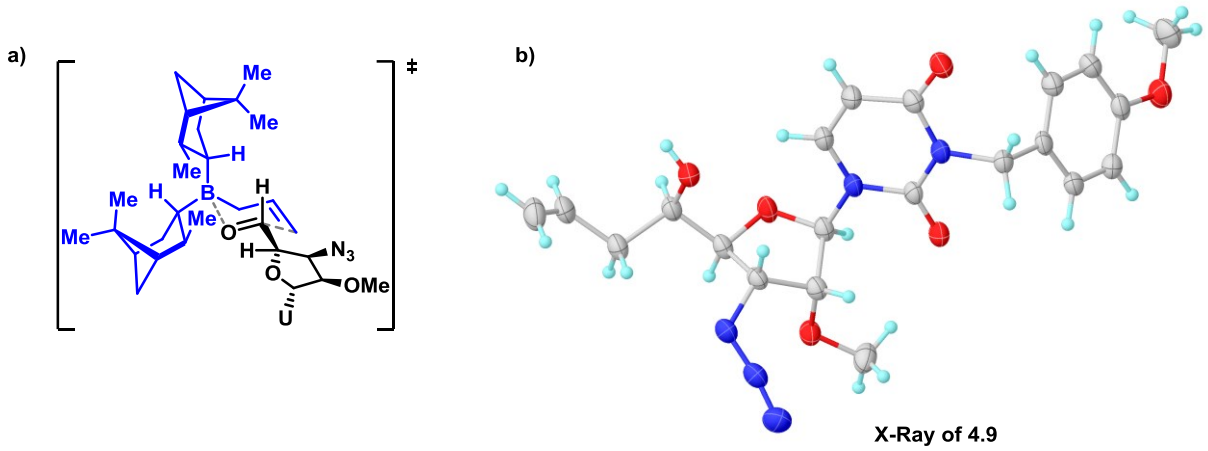
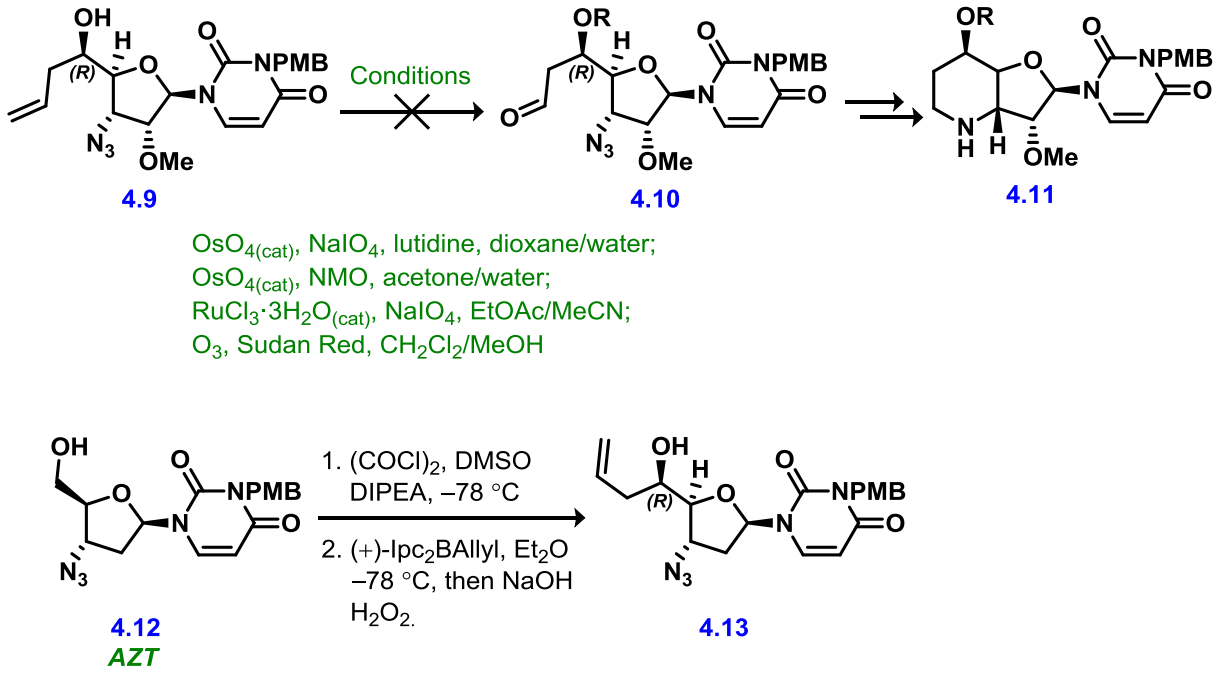


Figure 19. a) Proposed transition state for the diastereoselective allylation of compound **4.8**. b) X-Ray crystallographic analysis of compound **4.9**.

In order to study the oxidation of homoallylic alcohols, we used AZT (azidothymidine) as model (Scheme 10). Oxidation of AZT and Brown allylation **4.12** under previously optimized conditions gave homoallylic alcohol **4.13** with good selectivity. Once again attempts to oxidize the terminal alkene in nucleoside **4.13** or its 5'-OTBS protected version failed using various conditions: $OsO_{4(cat)}$, $NaIO_4$, lutidine, dioxane/water; $OsO_{4(cat)}$, NMO, acetone/water; $RuCl_3{\cdot}3H_2O_{(cat)}$, $NaIO_4$, EtOAc/MeCN; O_3, Sudan Red as indicator, CH_2Cl_2/MeOH.



Scheme 10. Allylation of AZT

Despite good results in the establishment of the stereogenic center at the 5'-position and successful introduction of the azido functionality as in **4.9**, the failed oxidation attempts led us to explore an alternative route using a carbohydrate as starting material.

4.5 Retrosynthetic analysis based on a carbohydrate precursor

Difficulties in our first approach evoked a new synthetic route using a carbohydrate as starting material (Figure 20). With the previously mentioned remarks in mind, our first retrosynthetic disconnection towards bicycle **I** could be installation of nucleobase at the 1'-position using Vorbrüggen glycosidation.[112] Stereocontrol was expected to arise from the neighbouring group participation of the 2'-OAc from the α-face of the furanose ring. Formation of the piperidine moiety could be achieved by an intramolecular Mitsunobu reaction between an alcohol at the 7'-position and an amine at the 3'-position.

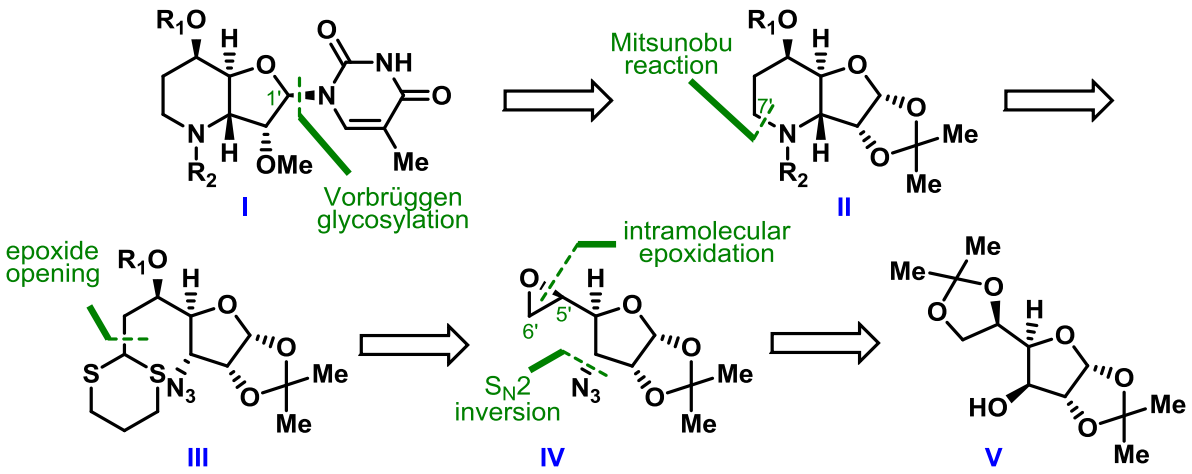
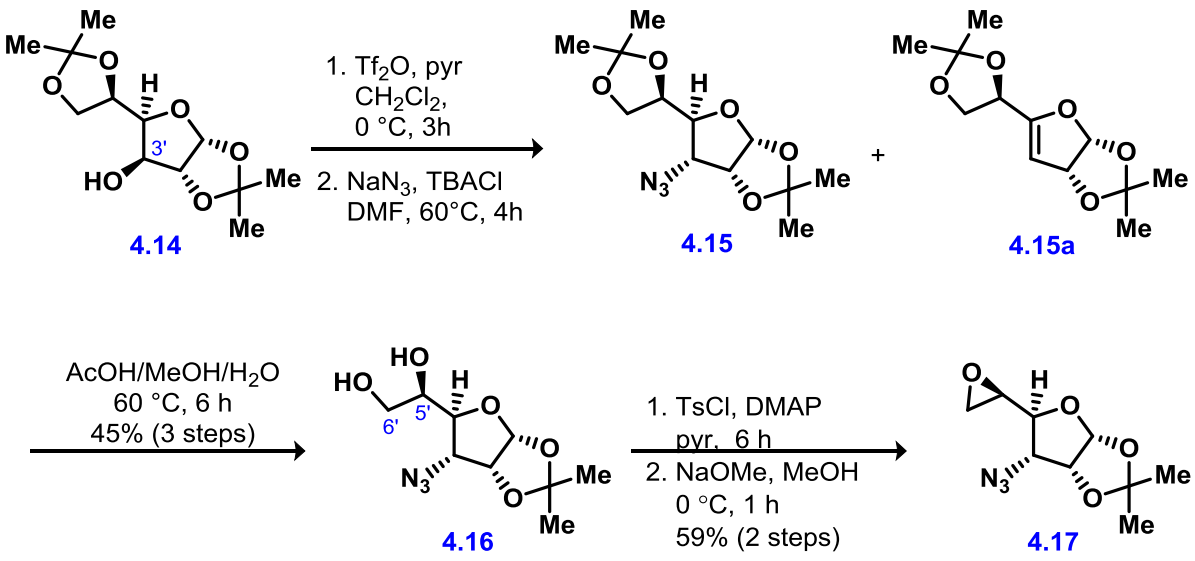


Figure 20. Retrosynthetic analysis of azabicyclic nucleoside **I**

The required alcohol at the 7'-position would be obtained from 1,3-dithioacetal precursor as in **III**. The dithioacetal would provide the required extra carbon for the formation of the piperidine core and could be prepared by the nucleophilic opening of epoxide **IV**. The aforementioned 5',6-epoxide could be formed from 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose **V**.

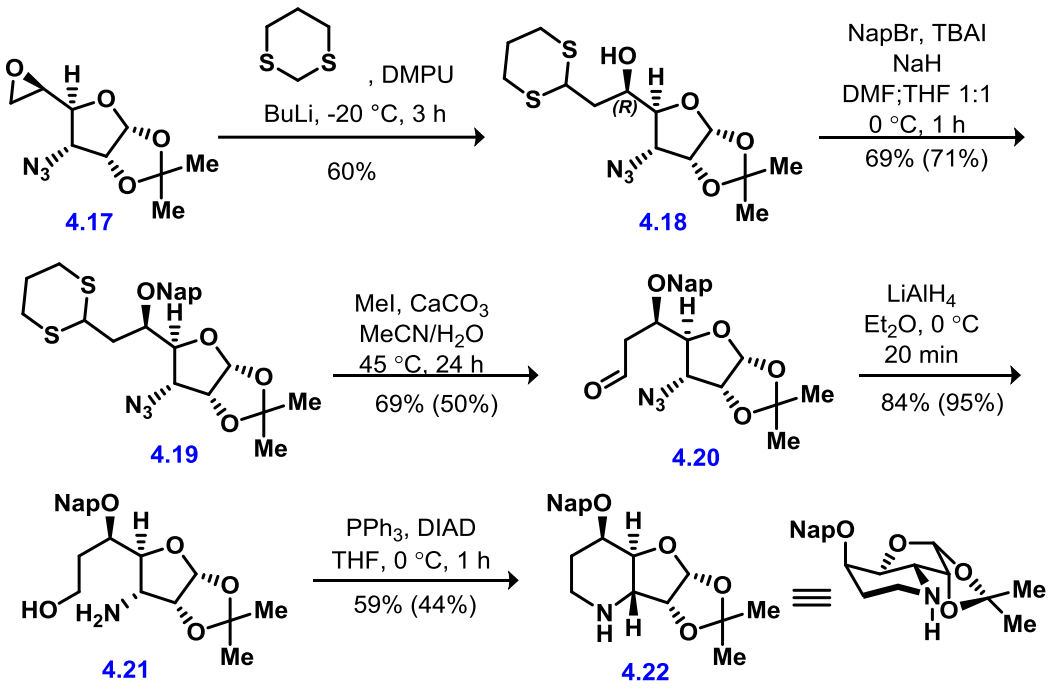
4.6 Synthesis based on a carbohydrate precursor

The starting material for the new route was the commercially available 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose **4.14**. The first step consisted in the introduction of an appropriate nitrogen nucleophile by inversion at the 3'-OH position (Scheme 11). The activation was successfully achieved by treatment with triflic anhydride and subsequent treatment of the crude triflate with NaN_3.[113,114] Azide **4.15** was obtained along with substantial quantities of elimination product **4.15a** as previously reported.[115] As an alternative, the formation of the 3'-O-imidazole sulfonate was achieved successfully and subsequently displaced with sodium azide. The amount of elimination product was comparable with the triflate method, but the latter was chosen due to the practicality of scale-up. To facilitate the isolation of azide **4.15** from elimination product **4.15a**, the mixture was treated with an acetic acid/methanol/water mixture to cleave selectively the 5',6'-isopropylidene group and provide a mixture separable by flash column chromatography. Selective tosylation of the primary alcohol of **4.16** followed by treatment with base promoted formation of terminal epoxide **4.17** in which the 5'-carbon maintained its original stereochemistry.



Scheme 11. Synthesis of epoxide **4.17**.

Our strategy envisaged epoxide opening with a nucleophile to extend the chain length by one carbon. For such transformation, we chose to treat epoxide **4.17** with lithiated 1,3-dithiane to provide alcohol **4.18** containing the extra carbon masked as a protected aldehyde (Scheme 12). Such a strategy has been previously studied in the synthesis of functionalized carbohydrates.[116-119] At this point in the synthesis, we had to choose a protecting group for the secondary alcohol in **4.18.** Such a protecting group had to be compatible enough to endure the subsequent steps in the synthesis and to be removed in the last steps. We decided to use a benzyl ether as protecting group and proceeded to complete the synthesis. Later, when we tried to remove the benzyl group in the final stages of the synthesis we encountered numerous problems. It was decided to scale up the synthesis using a 2-naphthylmethyl ether which had been used in previous nucleoside syntheses in our group.[74] Protection of alcohol **4.18** with 2-bromomethylnaphthalene gave naphthylmethyl ether **4.19** in 69% yield. It should be noted that the yields in parentheses (Scheme 12) refer to the yields using benzyl as protecting group.

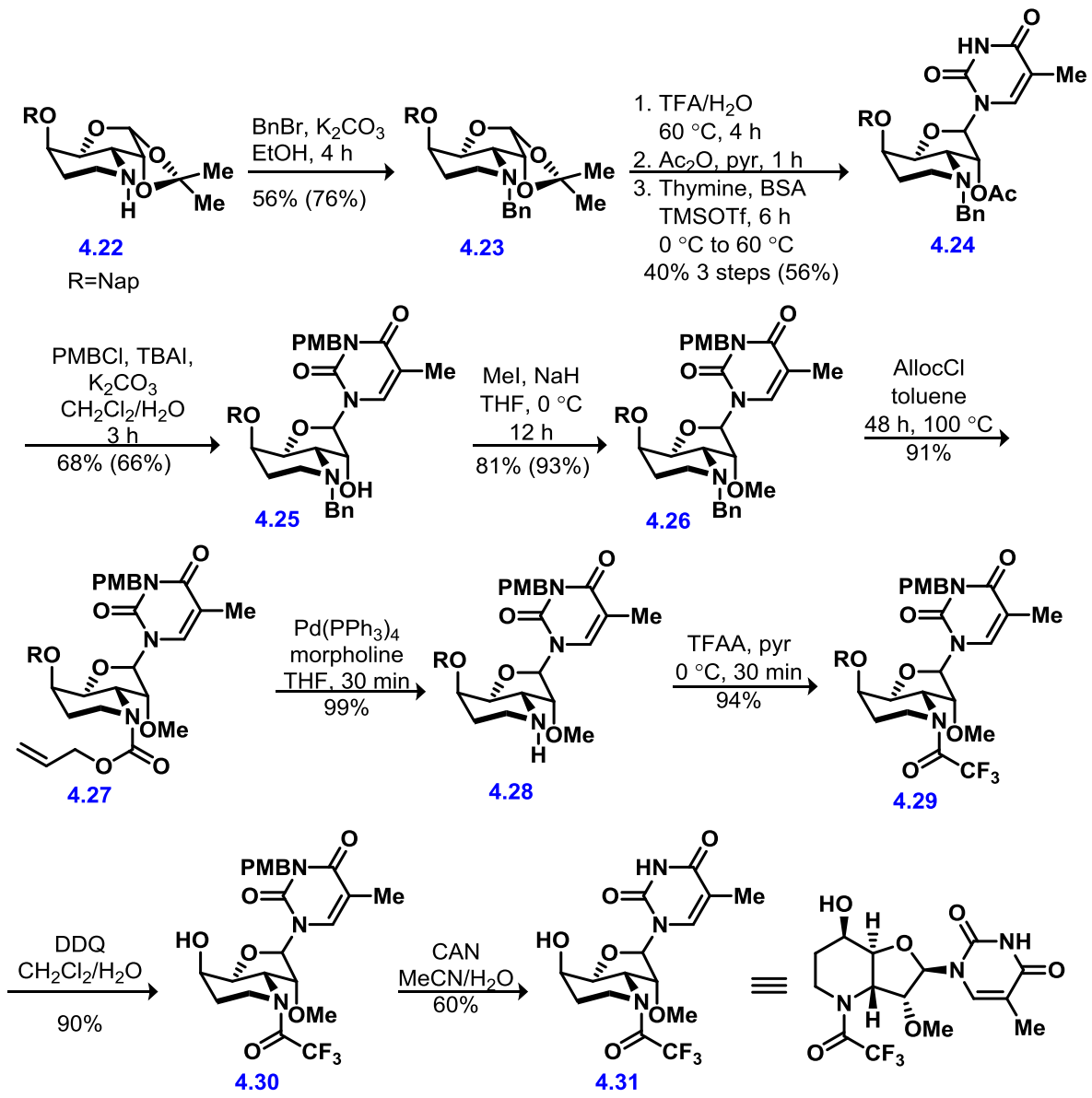


Scheme 12. Synthesis of bicyclic sugar **4.22**

Liberating aldehyde **4.20** from dithiane **4.19** was achieved in acceptable yields (Scheme 12) by treatment with MeI to form a sulfonium salt and for subsequent hydrolysis. Alternative conditions for the deprotection were problematic. For example, Dess-Martin periodinane gave only starting material,[120] treatment with periodic acid[121] led to decomposition, and bis((trifluoroacetoxy)iodo)benzene,[122] and dye-sensitized mediated photolysis[123] with visible light gave low yields.

With azido aldehyde **4.20** in hand, we tried to form the piperidine core in one synthetic operation. The desired sequence involved the treatment of **4.20** with a phosphine in anhydrous conditions to form an iminophosphorane which would cyclize with the aldehyde as in an aza-Wittig cyclization.[124] Subsequent reduction of the iminium ion with sodium cyanoborohydride would render the expected piperidine core **4.22**. However using phosphines (PPh_3, PMe_3 and PBu_3), were not successful forcing us to investigate alternative paths.

There are only few reports of the formation of azacycles by a Mitsunobu reaction of an amino alcohol.[125,126] This is potentially a direct method of intramolecular cyclization without prior activation of the amino group (as a more nucleophilic sulfonamide for example), and the alcohol as a sulfonate ester. We were therefore pleased that treatment of **4.21** with triphenylphosphine and diisopropylazodicarboxylate (DIAD), let to the desired piperidine in 59% yield. Protection of the nitrogen in **4.22** was imperative for the installation of the nucleobase (Scheme 13). Some exploratory work was done at this point with the N-Fmoc and the N-trifluoroacetamide derivatives of **4.22**, but neither resisted the conditions used to cleave the isopropylidene group. We found that benzylation of **4.22** with benzyl bromide[127] led to the N-benzylpiperidine **4.23** in 56% yield. The thymine nucleobase was successfully introduced by a three-step sequence involving TFA[128]-mediated cleavage of the 1,2-isopropylidene group, acetylation of the anomeric mixture of alcohols and Vorbrüggen glycosylation to give nucleoside **4.24** in 56% overall yield for three steps.



Scheme 13. Synthesis of nucleoside **4.31**

Protection of nucleobase **4.24** under phase transfer conditions resulted in installation of the PMB group on N3 with concomitant cleavage of the 2'-acetate group to afford alcohol **4.25**. Methylation gave the 2'-O methyl ether nucleoside **4.26**. Removal of the N-benzyl group was problematic under several hydrogenolysis conditions, but exchange of the benzyl group for an Alloc was successfully achieved by heating piperidine **4.26** at reflux with allyl chloroformate in

toluene for 2 days to give N-Alloc protected nucleoside **4.27**. The reaction proceeds by acylation of the tertiary amine to form a quaternary ammonium salt which upon attack by chloride at the benzylic carbon releases benzyl chloride. Palladium-mediated cleavage of the Alloc group in presence of morpholine, provided amine **4.28,** which was protected as the trifluoroacetamide **4.29** suitable for the synthesis of the ASOs.

The final sequence involved cleavage of the 5'-ONap from ether **4.29** by treatment with DDQ to give alcohol **4.30**, followed by CAN-mediated cleavage of the PMB group to yield thymidine **4.31**. The introduction of nucleoside **4.31** into a series of ASOs and the measurement of their corresponding Tm values is in progress at Ionis Pharmaceuticals.

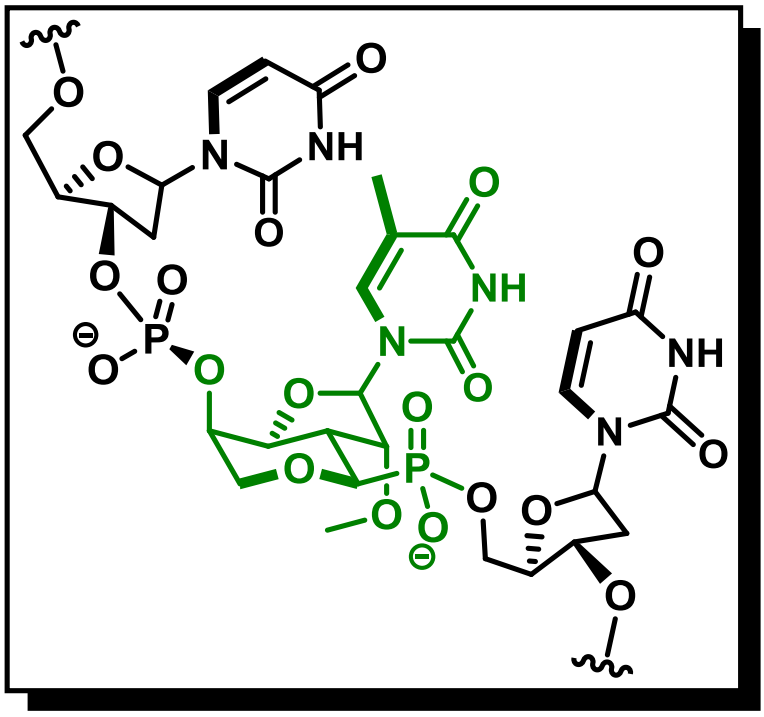
4.7 Conclusions

Nucleotide **4.31** was synthesized from 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose in 21 synthetic steps. The route features a dithiane one-carbon homologation and piperidine formation by way on a Mitsunobu reaction from an amino alcohol.

We have developed an alternative synthesis of azabicyclo nucleosides incorporating a piperidine ring similar to the Wang nucleoside **D** (Scheme 5). However, one synthesis involves the 2'-O-methyl ether as in **4.31** which should be a better nucleoside to be incorporated into an ASO based on previous experience.

5 Chapter Five

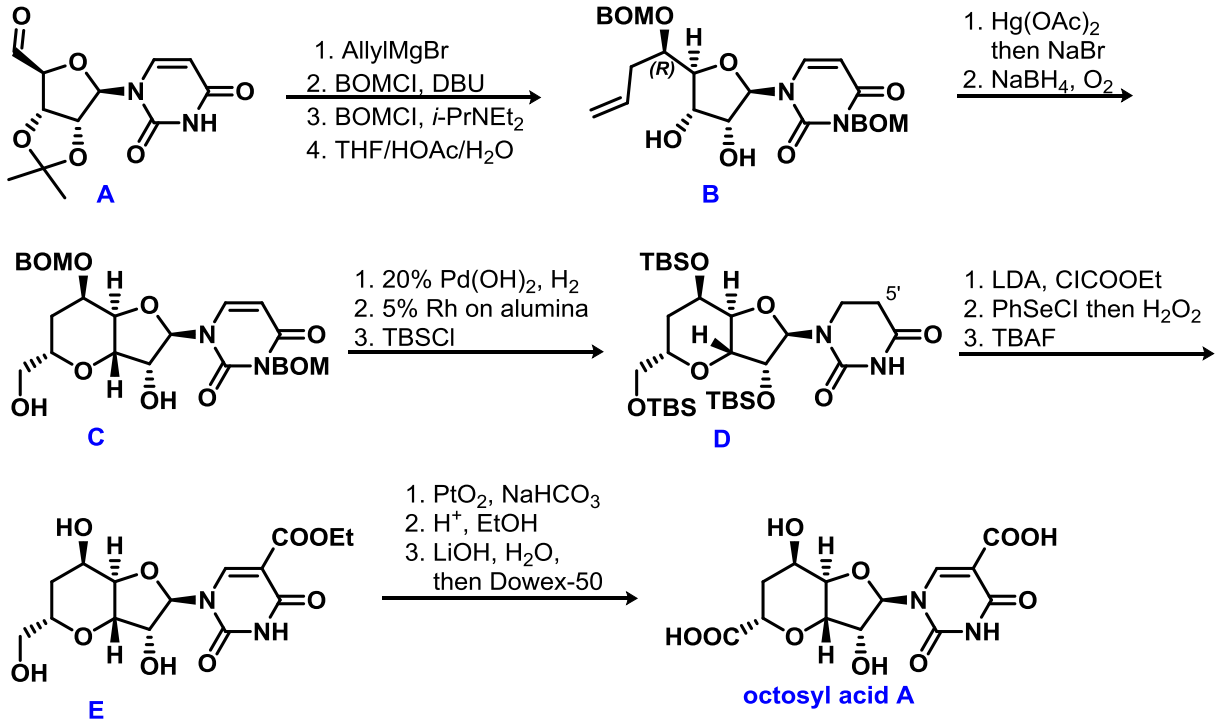
Synthesis of a novel oxabicyclic nucleoside phosphonate



Org Lett, **2018**, 20, 5296

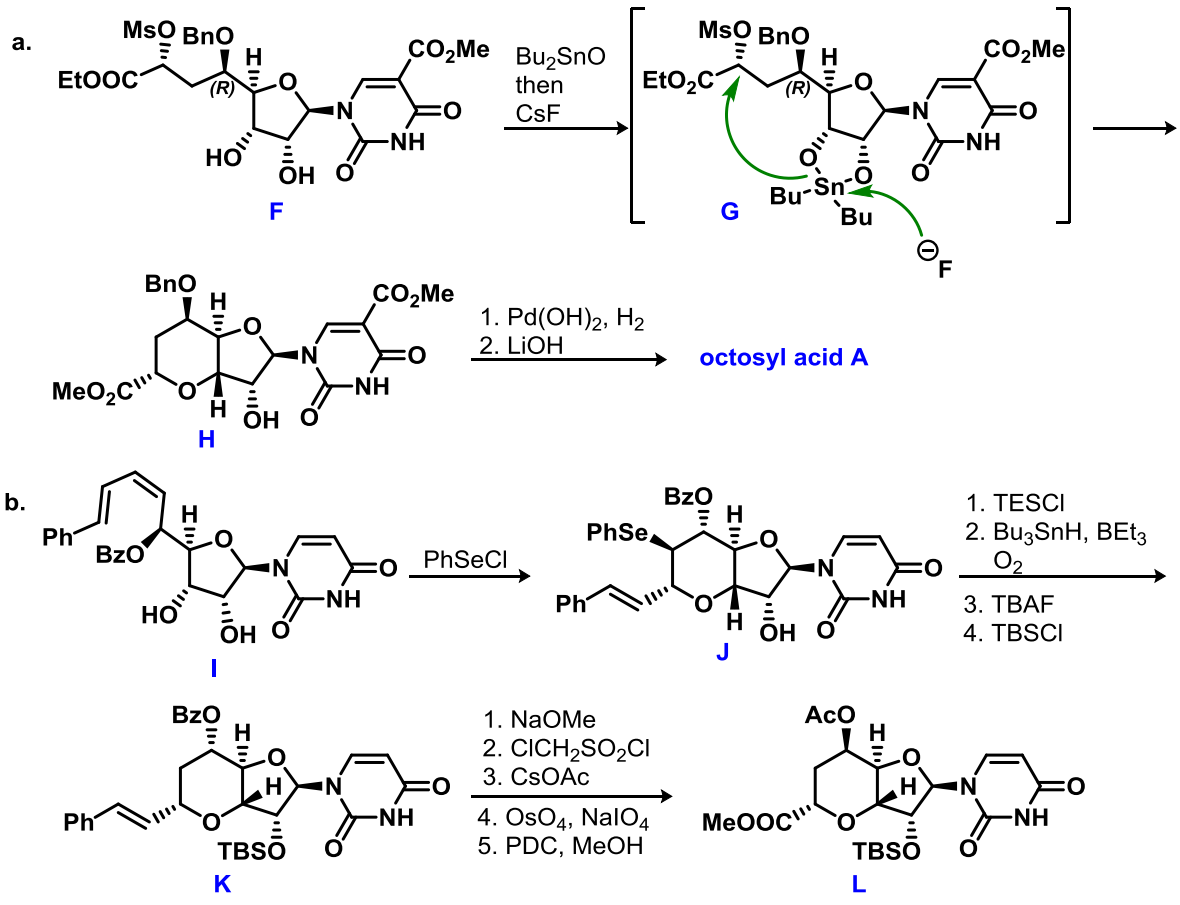
5.1 Previous syntheses of bicyclic perhydrofuropyran nucleosides

Octosyl acid A was isolated from *Streptomyces cacaoi* var. asoerisis by Isono[129] in 1975 and its total synthesis has been reported by several groups since then.[130-136] Its structure features a hexahydro-2H-furo[3,2-b]pyran core with a modified uracil nucleobase. The first total synthesis was reported by Hanessian[130] using as starting point the known aldehyde **A**,[137] readily prepared from uridine (Scheme 14). (*R*)-Homoallylic alcohol **B** was obtained as a major product (16:1) after treatment with allylmagnesium bromide followed by a protection sequence. A key step involved the formation of the perhydrofuropyran core by an intramolecular oxymercuration followed by reductive removal of the organomercury substituent with sodium borohydride[138] to give bicyclic nucleoside **C**.



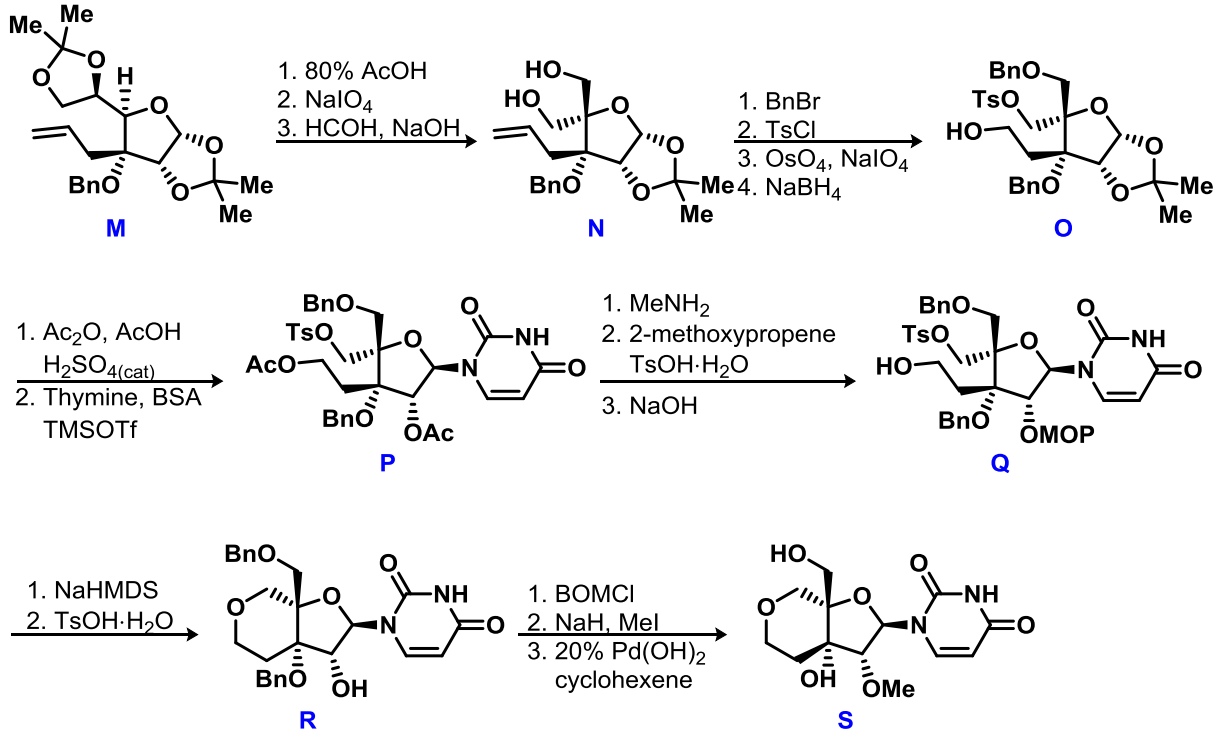
Scheme 14. The Hanessian synthesis of octosyl acid A

Reduction of the uracil base in **C** and persilylation gave pyrimidine **D,** the 5'-position of which was carboxyethylated and the 5,6-unsaturation was restored by a sequence involving the formation of an α-phenylselenide with subsequent oxidation and elimination.[139] Primary alcohol **E** was oxidized to the carboxylic acid using catalytic PtO_2, which upon saponification of the 5'-ester gave octosyl acid A. Other approaches have been used for the synthesis of the tetrahydropyran moiety. For example, Danishefsky[131] used the advanced intermediate **F** (**a** in Scheme 15) to form cyclic stannylene derivative **G,** which after nucleophilic attack of a fluoride anion, underwent an intramolecular cyclization to give bicyclic perhydrofuropyran nucleoside **H**. Removal of the protective groups afforded octosyl acid A.



Scheme 15. Key steps in the synthesis of octosyl acid A by different groups

In 1998 Miyasaka[134] reported an oxyselenation of conjugated diene **I** (**b** in Scheme 15) to form bicyclic nucleoside **J** as single isomer, by a *6-endo-trig* cyclization. Removal of the phenyl selenium group by treatment with Bu_3SnH/BEt_3 in the presence of dry oxygen. Cleavage of the benzoate ester was followed by chloromethylsulfonylation and inversion with CsOAc.[140] Finally, the alkene was cleaved under Lemieux-Johnson[141] conditions and the aldehyde was subsequently oxidized to give octosyl acid precursor **L**.



Scheme 16. Synthesis of bridged nucleic acid **S**

Imanishi[142,143] and Nielsen[144] independently reported the synthesis of bicyclic perhydrofuropyran nucleoside **S** (Scheme 16) with a hexahydro-2H-furo[2,3-c]pyran central core structure restricted in a south-type conformation. Imanishi's synthesis used known intermediate **M**[145] to produce diol **N** through an aldol/Cannizzaro reaction sequence (Scheme 16).[146] Protection of the hydroxymethyl group on the β-side of the furanose ring with BnBr and

tosylation of the remaining α-hydroxymethyl group enabled the subsequent oxidative cleavage/reduction sequence to produce furanose **O**. Installation of the nucleobase using a Vorbrüggen reaction gave nucleoside **P**, which underwent a series of reactions to exchange the protecting group at the 2'-OH for a MOP group and to subsequently cleave the primary acetate. The resulting nucleoside **Q** was treated with NaHMDS to trigger the formation of the tetrahydropyran core. Cleavage of the 2'-O protecting group gave bicyclic nucleoside **R**. Final steps required nucleobase protection, methylation of the 2'-OH group and deprotection to give oxabicyclic nucleoside **S**. Formation of the required phosphoramidite derivative at the 3'-position of **S** was however not possible which precluded introduction into oligonucleotides.

Notably, no reports of bicyclic nucleoside phosphonates possessing a hexahydro-4H-furo[3,2-c]pyran cores such as our proposed target nucleoside have been reported to date (**I** in Figure 22).

5.2 Design of a oxabicyclic nucleoside phosphonate surrogate of a phosphate

The design of an oxabicyclic nucleoside phosphonate followed the same design principle as that used for the N-bicyclic piperidine nucleoside, including the restriction about the torsion angles ɣ, δ and ε, to maintain canonical bond-geometries and sugar pucker. The dioxaperhydrofuropyran phosphonic acid **I** was intended to mimic the C3'-*endo* conformation of RNA (Figure 21). Although restriction of rotation around ε is not possible in DNA or RNA because it would entail a trivalent 3'-oxygen atom, in an oxabicyclic phosphonate nucleoside, the desired geometry may be achieved by forming a six-membered tetrahydropyran ring

possessing an appended equatorial phosphonate group. The presence of a 2'-*O*-Me group could further enforce the RNA-like C3'-*endo* sugar pucker.

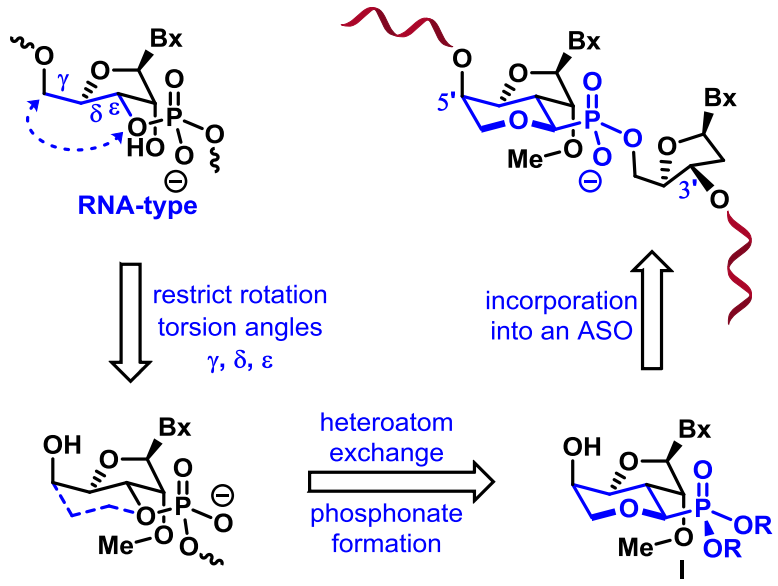


Figure 21. Design rationale for the P-oxabicyclic nucleoside.

The introduction of the oxabicyclic nucleoside into an ASO and measurement of the thermal affinity versus RNA and DNA strands would provide valuable information about the relationship between the structure of a modified nucleoside and its effect on the ability to hybridize with complementary nucleic acids.

5.3 Retrosynthetic analysis

The general structure of nucleoside **I** features an oxabicyclic perhydrofuropyran moiety with an appending phosphonate (Figure 22). In our strategy, we chose to install the nucleobase of the P-oxabicyclic nucleoside **I** in the last steps of the synthesis by a Vorbrüggen glycosidation on the isopropylidene diol **II**. Tetrahydropyran ring in **II** could be obtained by a Williamson ether

formation featuring nucleophilic displacement of an appropriate leaving group at carbon 6' by an alcohol. The alcohol responsible for the aforementioned attack must contain an α-phosphonate group with the (*R*) configuration as in **III**. Synthesis of the α-hydroxyphosphonate can be achieved by the attack of an alkylphosphite anion onto an aldehyde, using the so-called Pudovik-Abramov reaction.[147,148] Aldehyde **IV** could be easily obtained from commercially available 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose **V** by a sequence involving a chain extension at C3'.

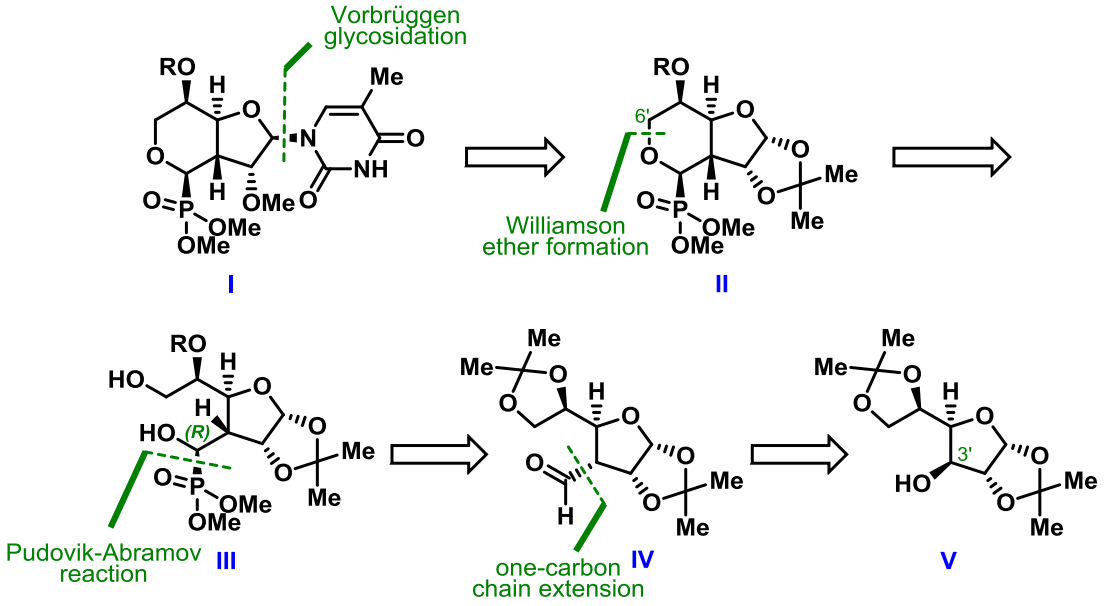


Figure 22. Retrosynthetic analysis of nucleoside **I**

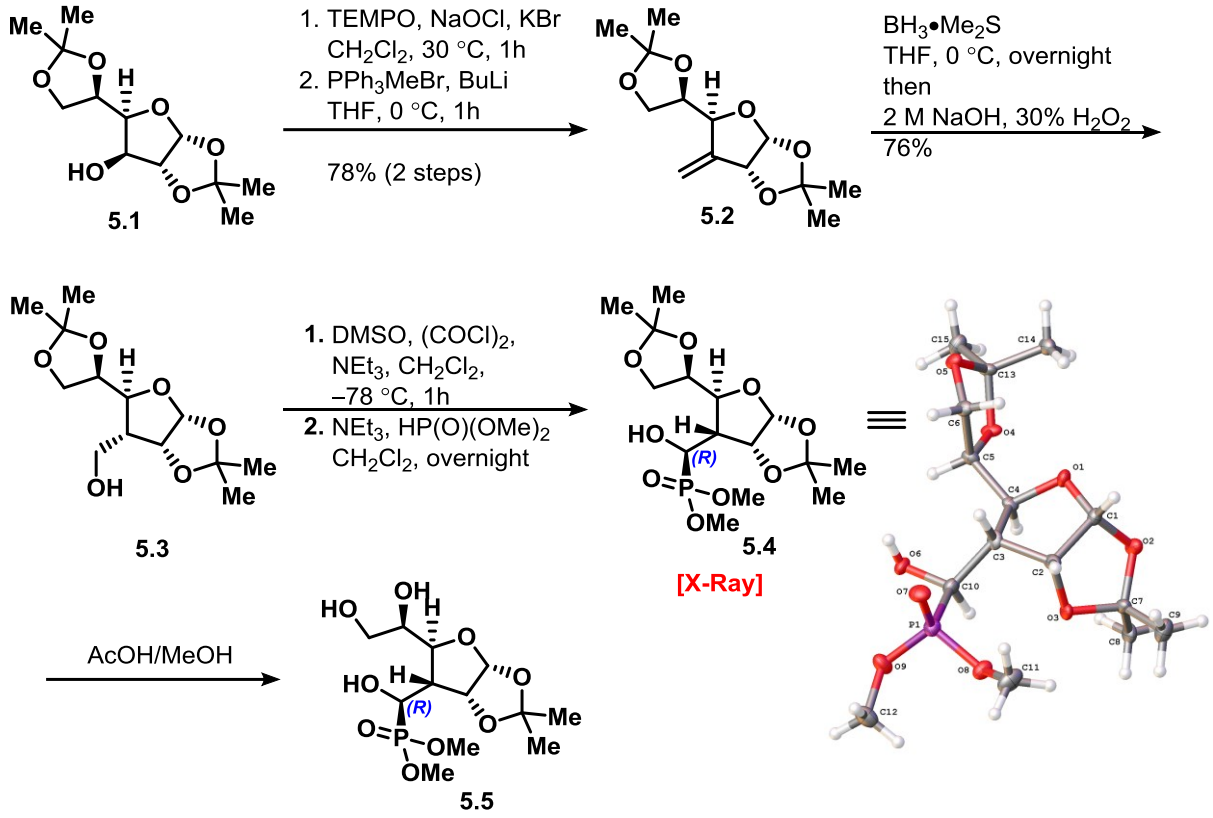
5.4 First synthetic route

The synthesis started with the transformation of the commercially available 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose **5.1** into alkene **5.2** (Scheme 17). The TEMPO-mediated oxidation[149] was performed on multi-gram scale followed by a Wittig reaction of the crude

ketone to afford terminal alkene **5.2** in good yield[‡]. Hydroboration/oxidation[150] sequence produced primary alcohol **5.3**, with the hydride being delivered to the β-face of the furanose ring in **5.2.**[151] In our first attempt to form α-hydroxyphosphonate **5.4**, alcohol **5.2** was oxidized under Swern conditions and the resulting aldehyde was treated with a mixture of dimethyl phosphite and triethylamine. Using ^{31}P and ^{1}H NMR, we detected three compounds in an 18:2:1 ratio. X-ray quality crystals were obtained from the major component of the mixture which revealed the required (*R*) stereochemistry of the α-hydroxyphosphonate **5.4**. In order to facilitate the separation of the desired product, we proceeded to treat the mixture of the three compounds with AcOH/MeOH/H_2O, which provided triol **5.5** as a single isomer after chromatography.

Initial attempts to form the tetrahydropyran core focused on formation of the cyclic sulfate **5.6** by treatment of triol **5.5** with thionyl chloride to form the cyclic sulfite[152] (Scheme 18). However, no desired product was obtained upon subsequent oxidation with $RuCl_3/NaIO_4$. A more direct approach was attempted by treating **5.5** with sulfuryl chloride but cyclic sulfate **5.6** was not obtained.

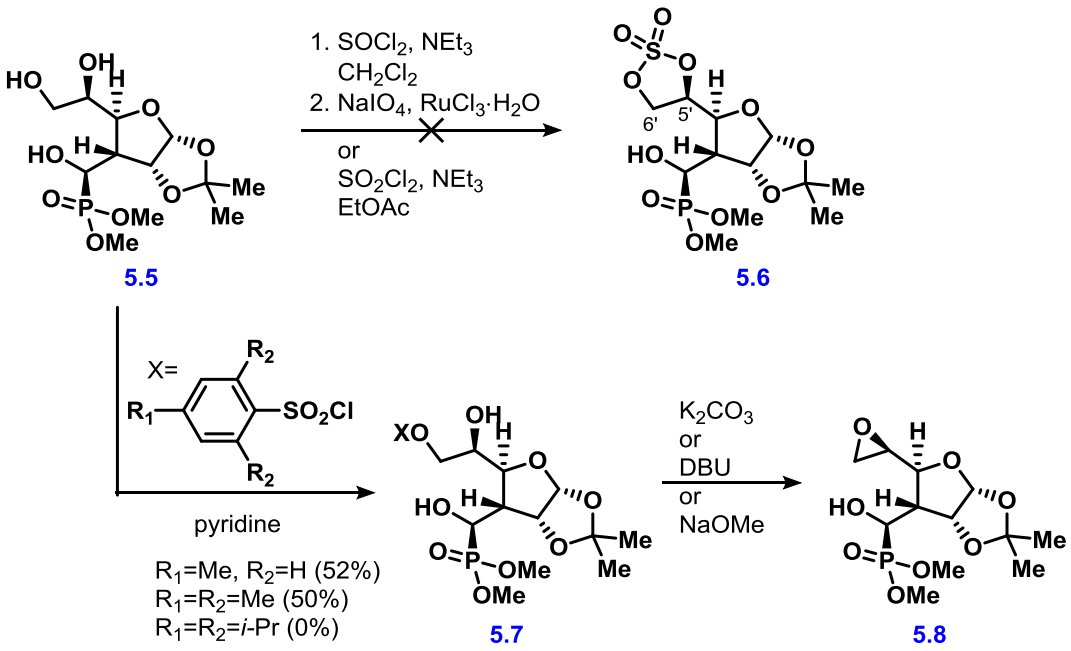
[‡] Originally we performed the transformation of **5.1** into **5.2** by oxidizing with CrO_3/pyridine/Ac_2O followed by Wittig reaction with PPh_3MeBr/BuLi. The reaction was performed in 20 g scale to obtain a 72% yield over two steps. Due to the toxicity of CrO_3, the TEMPO-mediated oxidation was preferred.



Scheme 17. Synthesis of α-hydroxyphosphonate **5.5**.

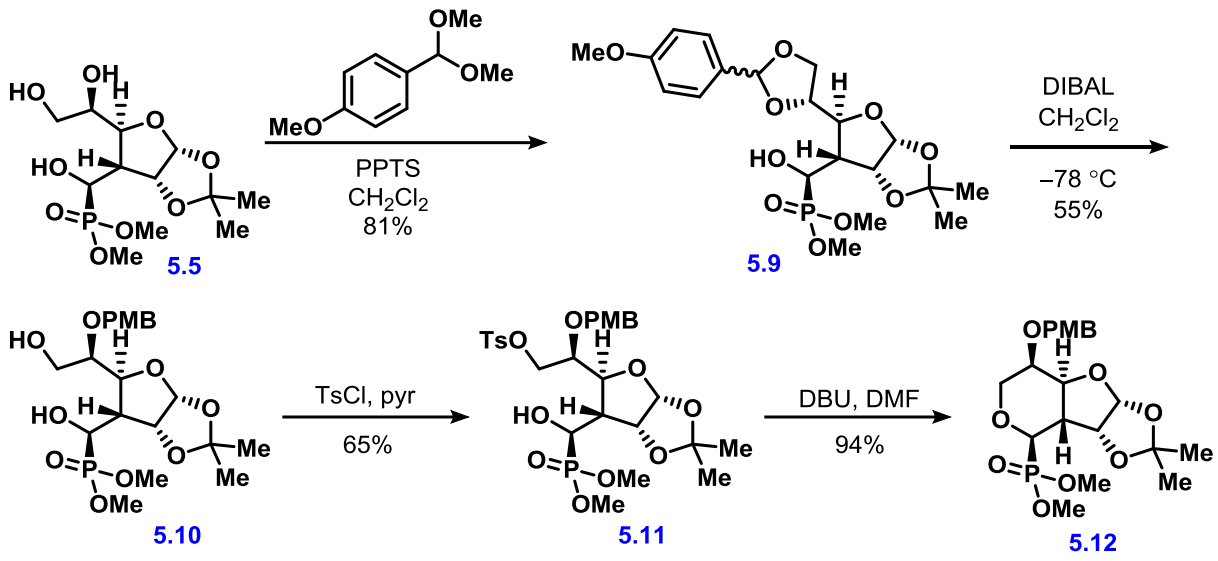
In view of own failure to activate the vicinal diol for the planned intramolecular cyclic ether formation, we attempted to convert primary alcohol **5.5** into the corresponding tosylate (Scheme 18) to form the perhydrofuropyran by nucleophilic attack of the α-hydroxy phosphonate. Tosylation was accompanied by several by-products that were difficult to separate. To increase the selectivity for the primary alcohol, the bulkier 2,4,6-trimethylbenzenesulfonyl chloride and 2,4,6-triisopropylbenzenesulfonyl chloride were explored. The "trimethyl" reagent reacted in a similar way as tosyl chloride did but the "triisopropyl" reagent did not react with alcohol **5.5**. Treatment of the primary sulfonates with K_2CO_3 gave a small amount (≈20%) of an unknown compound which did not exhibit ^{31}P signals. Complete decomposition was observed using NaOMe, and DBU gave small amounts of epoxide **5.8** (≈20%) without detectable sign of the

tetrahydropyran moiety. Faced with discouraging results, we decided to switch to an alternative cyclization strategy.



Scheme 18. Attempts to form the tetrahydropyran core.

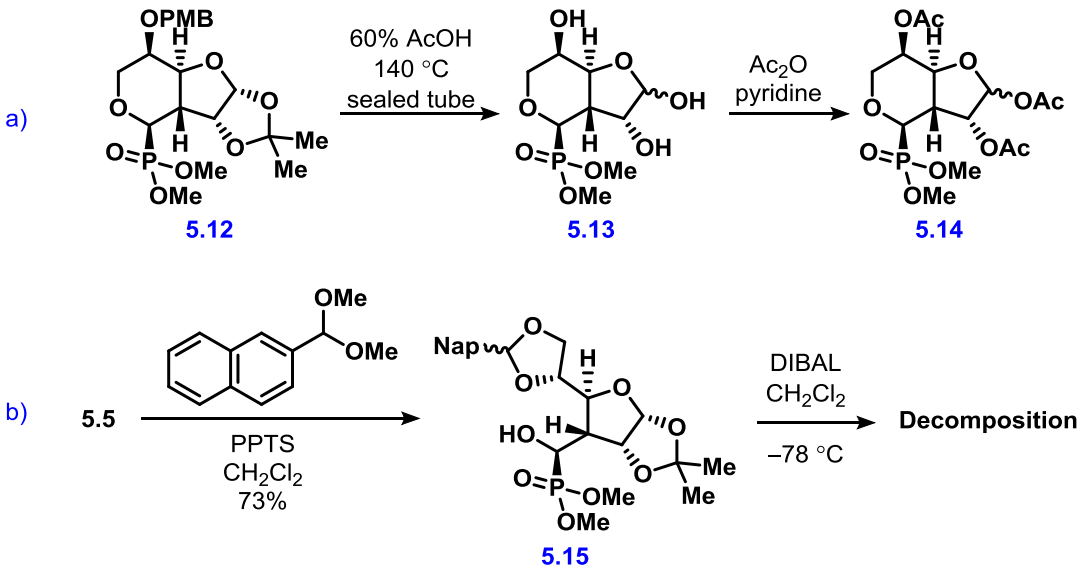
Trying to avoid the previously mentioned problems, we decided to use a similar strategy as that employed in the synthesis of nucleoside **3.5** during synthesis of TriNA 2 (Chapter 3, Scheme 2). Vicinal diol **5.5** was protected as a *p*-methoxybenzylidene acetal in 81% yield (Scheme 19). The 1:1 mixture of diastereomers, **5.9** was treated with an excess of DIBAL to obtain ether **5.10** together with its regioisomer (not shown) in a 7:1 ratio. The mixture of regioisomers was dissolved in pyridine and allowed to react with TsCl. Isomer **5.10** reacted exclusively to afford monotosyl **5.11**, which cyclized cleanly into tetrapyran **5.12** using DBU as a base.



Scheme 19. Synthesis of oxabicyclic sugar **5.12**

With oxabicyclic sugar **5.12** in hand, the next task involved cleavage of the 1,2-isopropylidene acetal and installation of the nucleobase at the anomeric position. Several standard conditions were tried for such a transformation ($AcOH/Ac_2O/H_2SO_4$(cat), Dowex 50W-8X[63], 80% TFA[128], 20% oxalic acid[153], $AcOH/BF_3{\cdot}Et_2O$[154], MeOH/TMSCl) but only decomposition occured. For example, heating **5.12** in presence of $FeCl_3{\cdot}6H_2O$[155] in acetone in a sealed tube caused cleavage of the PMB group.

In one of the more promising conditions, oxabicyclic sugar **5.12** was dissolved in 60% acetic acid (**a** in Scheme 20), and heated at 140 °C for 1 h, to afford triol **5.13**. The mixture was dissolved in pyridine and acetylated to give **5.14** which was submitted to Vorbrüggen glycosidation conditions. Unfortunately no desired product was formed.



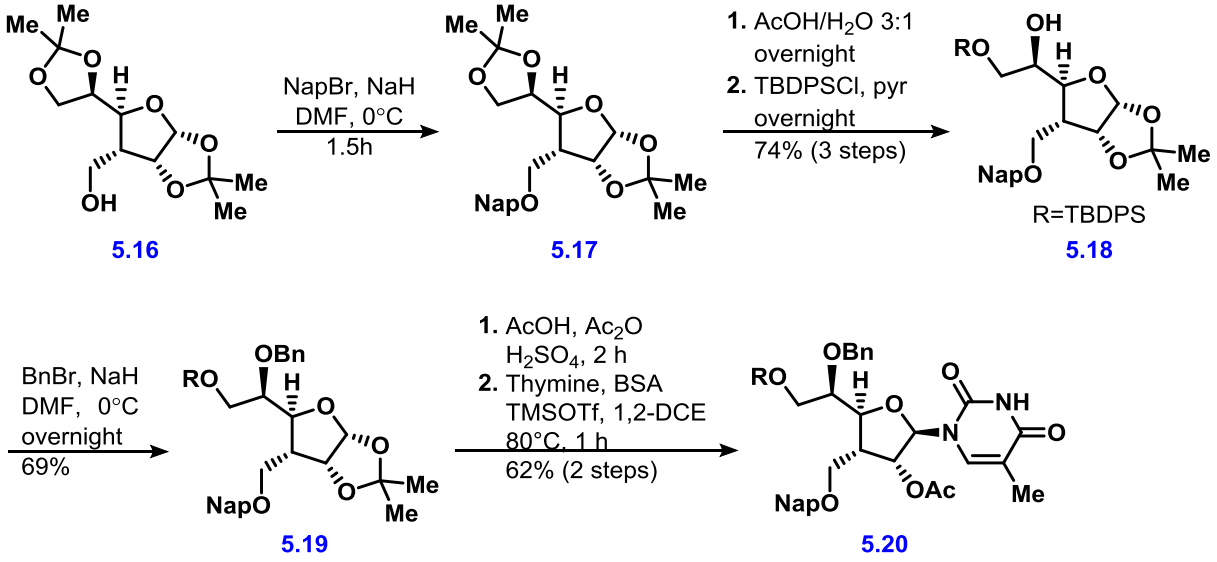
Scheme 20. a) Synthesis of peracetylated sugar **5.14**. b) Synthesis of naphthylidene acetal **5.15**.

To advance the synthesis, an attempt was made to install a Nap protecting group on the 5' hydroxyl group (**b** in Scheme 20). Although treatment of diol **5.5** with 2-naphthylcarboxaldehyde dimethylacetal produced a 1:1 mixture of diastereoisomers **5.15**, attempts to reductively open the naphthylidene acetal selectively failed.

Having encountered numerous problems with this first approach, we chose to change to a new approach where the nucleobase at the anomeric position was installed before formation of the α-hydroxyphosphonate.

5.5 Second synthetic route

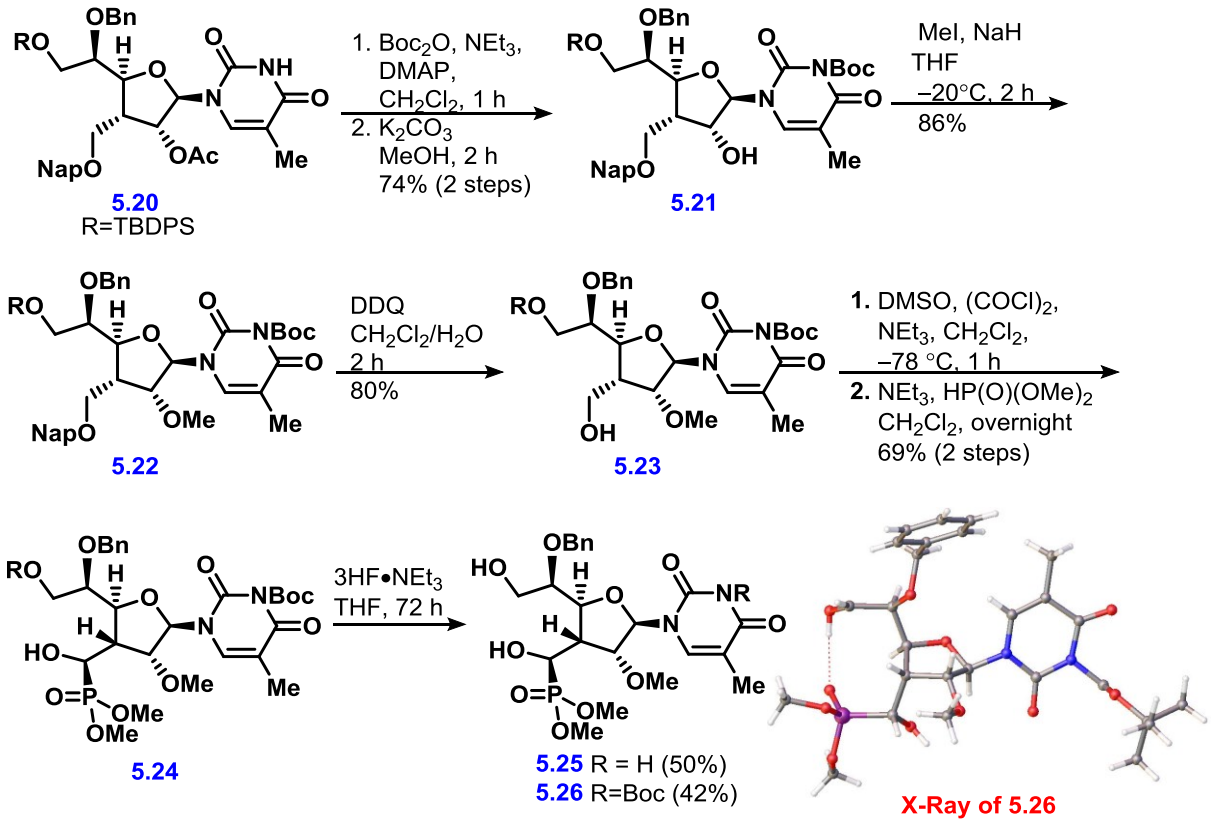
Our second route started with a series of orthogonal protections using previously prepared alcohol **5.16** (Scheme 21). Alkylation of primary alcohol **5.16** with 2-bromomethylnaphthalene gave ether **5.17,** which was used without further purification, in an acid mediated cleavage of the 5',6'-isopropylidene acetal. Selective protection of the primary alcohol with TBDPSCl gave **5.18**, which was benzylated to give the fully protected intermediate **5.19**.



Scheme 21. Synthesis of nucleoside **5.20**.

Cleavage of 1,2-isopropylidene acetal in **5.19** using acetolysis in a mixture of AcOH/Ac_2O containing a catalytic amount of sulfuric acid,[156] followed by a Vorbrüggen reaction[112] led to the nucleoside **5.20** in good overall yield. Cleavage of the isopropylidene acetal required screening several conditions. For example, after 4 days at room temperature, treatment of acetal **5.17** with $FeCl_3 \cdot 6H_2O$ or 80% AcOH resulted in recovered starting material. Interestingly, the use of AcOH/Ac_2O/H_2SO_4 while successful also required optimization. The use of more than

5% mol of sulfuric acid produced several by-products including some from cleavage of the naphthylmethyl ether and acetylation.



Scheme 22. Synthesis of diols **5.25** and **5.26**.

Direct alkylation of the 2'-OH of an unprotected nucleoside is a difficult task[157]. Protection of thymine base at N-3 was examined using a t-butyl-carbamate[158] (Scheme 22). Subsequent base-mediated cleavage of the 2'-acetate gave alcohol **5.21** in 74% yield over 2 steps. Methylation using NaH and iodomethane provided ether **5.22.** Orthogonal cleavage of the 2-naphthylmethyl ether with DDQ[159] afforded primary alcohol **5.23** in excellent overall yield.

Swern oxidation followed by a Pudovik-Abramov[147,148] reaction with dimethyl-H-phosphonate gave α-hydroxyphosphonate **5.24** as a single isomer. The selectivity in the addition of the

dimethoxy H-phosphonate anion to the aldehyde may be controlled by the 2'-methoxy group of the furanose ring since in analogy with the reaction on related substrate **5.4** (Figure 23)

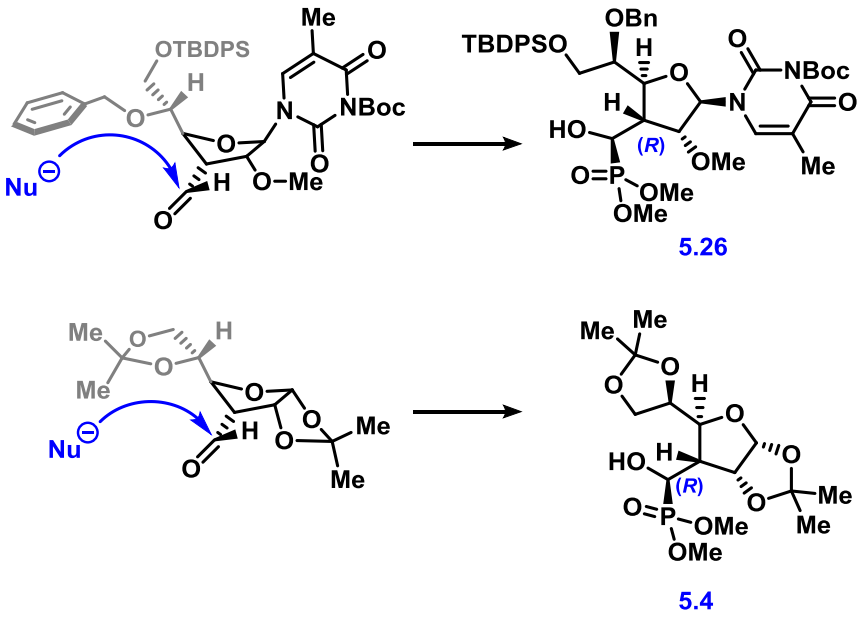
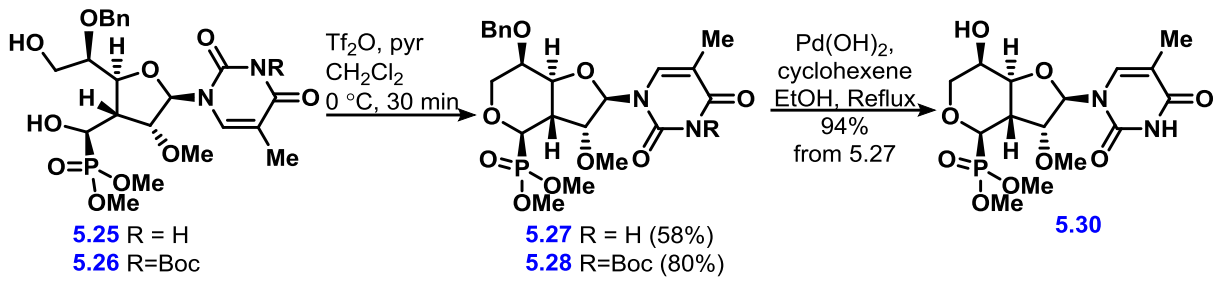


Figure 23. Models for selective addition in the formation of phosphonates **5.4** and **5.26**

Cleavage of the silyl ether **5.24** with 3HF•NEt_3 was accompanied with loss of the Boc group giving a mixture of alcohols **5.25** and **5.26** depending on the quality of the reagent[§]. The configuration of the newly formed stereogenic center in the (*R*)-α-hydroxyphosphonate **5.24** was based on analogy with the single crystal X-Ray analysis of alcohol **5.26** (Scheme 22). Attempts to form the perhydrofuropyran (Scheme 23) via mesylation or tosylation of primary alcohols **5.25** and **5.26,** followed by DBU mediated cyclization led to low cyclization yields. Gratifyingly, the desired oxabicyclic compounds **5.27** and **5.28** were obtained in good yields using triflic anhydride in pyridine.

[§] Original bottle used in the project produced compound **5.26** in 70% yield as single product. A second used bottle gave a mixture of **5.25** and **5.26**. Use of TBAF gave a low yield of the desired compound.

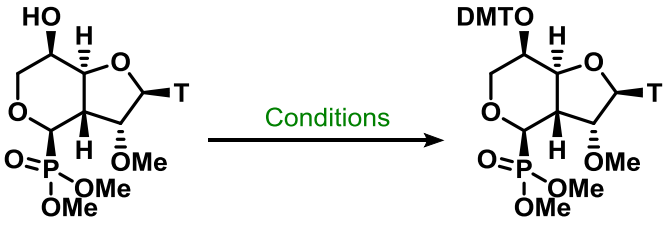


Scheme 23**.** Synthesis of nucleoside **5.30**.

Having successfully formed the pyran, we proceeded to deprotect bicycle **5.28** under catalytic transfer hydrogenation conditions.[160] The use of $Pd(OH)_2$ and cyclohexene in ethanol at reflux, not only cleaved the benzyl ether but also led to the cleavage of the N-Boc protection, probably by thermal decomposition[161,162] furnishing nucleoside **5.30.** The latter was sent to Ionis Pharmaceuticals for incorporation into an oligonucleotide sequence and to the measurement of the melting temperature values.

The standard procedure for the introduction of a nucleoside into an oligonucleotide requires the installation of an easily cleavable protecting group on the 5'-alcohol. The most commonly used group is the venerable 4',4-dimethoxytrityl (DMT) protection, an acid labile group which releases a characteristically orange color once cleaved. However, attempts to install the DMT group on **5.30** by Ionis Pharmaceuticals scientists failed (Table III).

Table III. Failed attempts of DMT group installation on alcohol **5.30** by Ionis Pharmaceuticals.

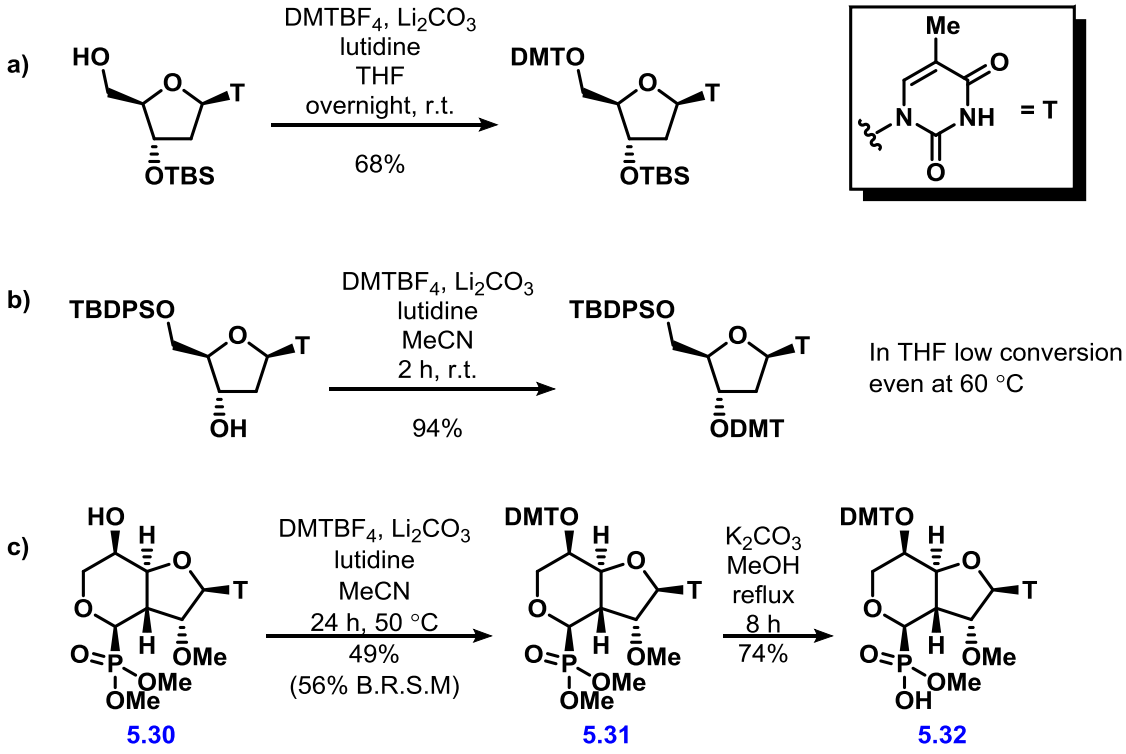


5.30 **5.31**

Reaction #	Amount of **5.30** (mg)	DMTCl (eq.)	Base	Solvent	Notes
1	10	2.8	pyridine	pyridine	No reaction overnight at r.t Add 200 µl 2,6 lutidine. Still no rxn. Heating to 45 °C did not help
2	0.5	5	pyridine	pyridine	Room temp → 45 °C → 55 °C, no rxn
3	0.5	5	Imidazole (3eq.)	DCM	New orange spot by TLC, but cannot ID as product. Still SM spot.
4	0.5	5	DMAP (3 eq.)	pyridine	No rxn.
5	0.5	5	DIPEA (3 eq.)	DCM	No product by LCMS
6	0.5	3	Lutidine (3 eq.)	pyridine	55 °C overnight, no rxn
7	0.5	1.2	DBU (3 eq.)	DCM	SM consumed. LCMS shows traces of product

In spite of the different bases and solvents tried, no significant amount of the desired compound was obtained. We decided independently to use $DMTBF_4$, a reagent used in challenging protections of nucleosides.[163-165] Freshly prepared $DMTBF_4$ reagent was reacted with a series of substrates to find optimum conditions. Using 3'OTBS-thymidine under the reported conditions, we isolated the 5'-ODMT nucleoside in 68% yield (**a** in Scheme 24). In THF, the protection of

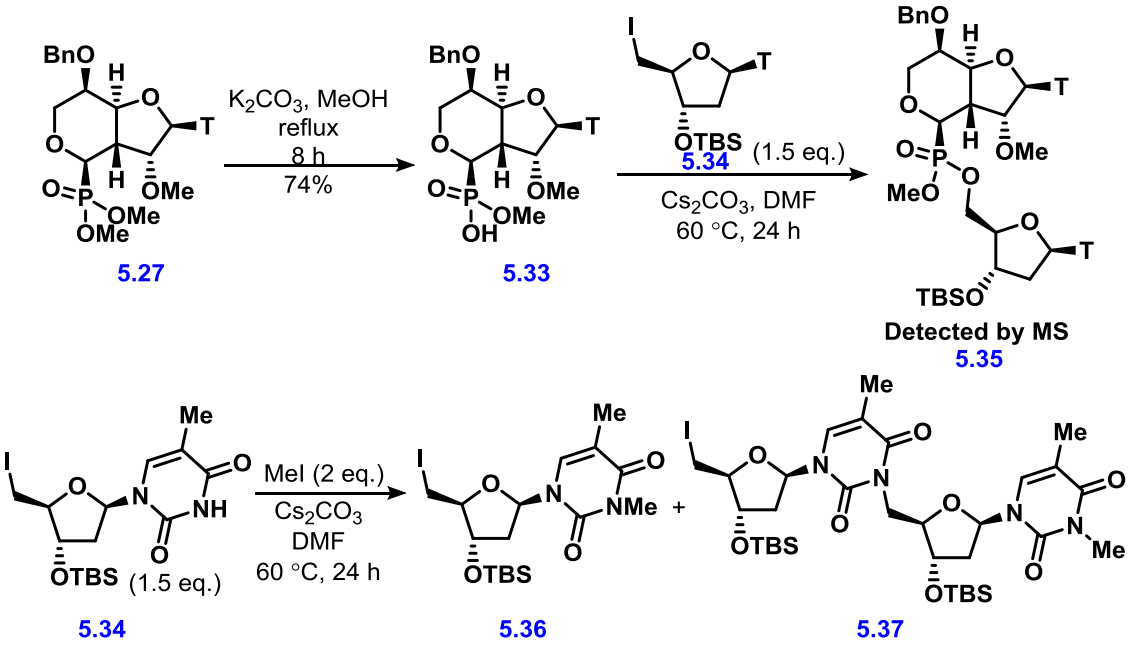
the secondary alcohol of 5'-OTBDPS thymidine was unsuccessful at room temperature, even by heating at 60 °C for several hours (**b** in Scheme 24); however, switching to acetonitrile as solvent, the reaction was completed at room temperature in 2 h to afford DMT-ether in excellent yield. Under optimized conditions (**c** in Scheme 24), alcohol **5.30** did not react at room temperature, but on heating at 50 °C for 24 h a good yield of DMT-ether **5.31** was obtained. Cleavage of one of the methyl phosphate groups was next achieved by treatment with K_2CO_3 in methanol at reflux to give acid **5.32**.



Scheme 24. Synthesis of nucleoside **5.32**

After overcoming the difficulties encountered in the introduction of the DMT group onto alcohol **5.30**, we focussed on coupling of nucleoside **5.32** with another 5'-OH nucleoside to form a P-O linkage. Various conditions to make phosphonates (e.g. Mitsunobu conditions,[166-168] and coupling conditions with BOP[169], HATU[170] and DEPBT[171,172]) gave however no desired

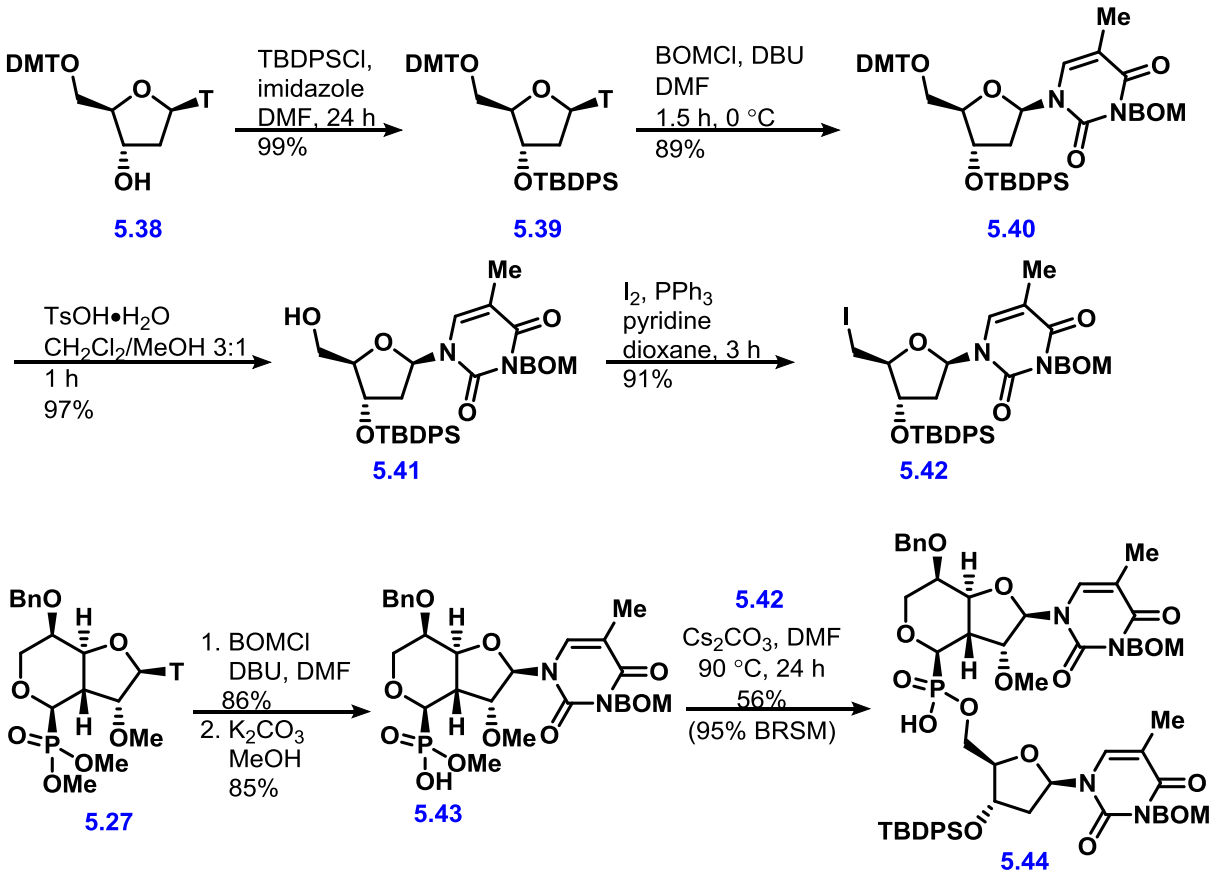
product, instead recovered starting material and several unidentifiable by-products from DMT cleavage were detected by TLC analysis. The previous results together with the need to prepare more nucleoside **5.31**, led us to use the more available material **5.27** to continue the optimization process with the benzyl group considering the higher stability compared to the DMT group. Phosphonate **5.27** was submitted to basic conditions to cleave one of the methyl ester groups to give acid **5.33** in good yield (Scheme 25).



Scheme 25. Attempts to form nucleotide dimer **5.35**

As an alternative coupling approach, we reasoned that nucleotide **5.35** could be obtained by reacting hemi-phosphonic ester **5.33** and 5'-activated nucleoside **5.34** as the nucleofugal partner. Inspired by a recent report[173], we prepared iodonucleoside electrophile **5.34** in three steps from thymidine. After heating a mixture of **5.33** and **5.34** and Cs_2CO_3 in DMF, nucleotide **5.35** was observed by MS analysis accompanied by several by-products. We suspected that under the previous conditions, the nucleobases at both nucleosides **5.33** and **5.34** could react with the

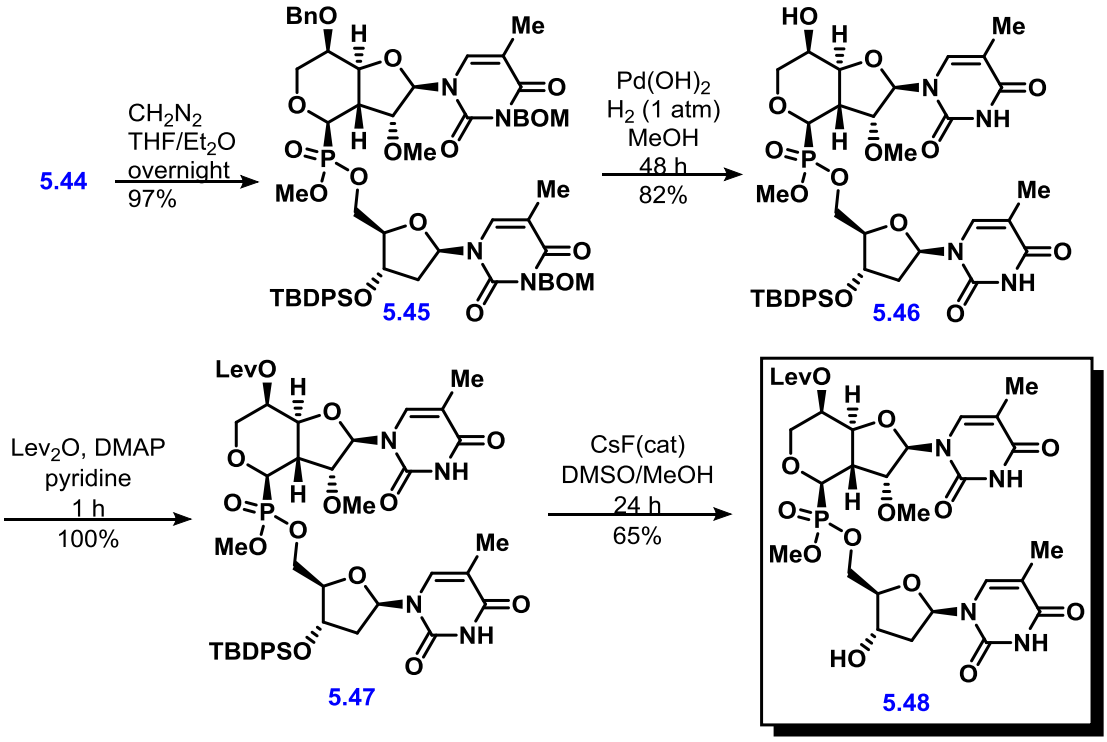
iodonucleoside resulting in N3-alkylation at the nucleobases. A control reaction, using **5.34** and methyl iodide under the previously mentioned conditions (Scheme 25) gave as major products the N3-methyl thymidine **5.36** and dimer **5.37**, confirming the reactivity of the nucleobases under the conditions used. We therefore decided to protect the nucleobase to reduce the N-alkylation by-products. Attempts to use N-Boc protection on both the iodonucleoside and the bicyclic phosphonate **5.33** during the alkylation gave a mixture of products which after isolation revealed N-alkylation occurred with cleavage of the N-Boc group.



Scheme 26. Synthesis of nucleotide **5.44**

All the previous experiments led us to wisely choose the protecting groups used in the nucleotide synthesis. Starting from commercially available 5'-ODMT thymidine **5.38**, we used a sequence

involving protection of the 3'-OH with TBDPSCl due to the higher stability *vs*. TBS protecting group (Scheme 26). For the nucleobases, we chose an N-BOM protection[64] in **5.39** to give **5.40**. Cleavage of DMT ether **5.40** gave alcohol **5.41.** Primary iodide **5.42** was prepared under Appel conditions.[174] Nucleoside **5.43** was made in two steps, first by treatment of the nucleobase in **5.27** with BOMCl followed by mono-hydrolysis of methylphosphonate. Finally, when a solution of **5.27**, **5.42** and cesium carbonate in DMF was heated for 24 h, compound **5.44** was obtained in good yield (56%) with some recovered starting material that could be recycled.



Scheme 27. Synthesis of nucleotide **5.48**.

Esterification of nucleotide **5.44** with freshly prepared diazomethane gave **5.45,** an inseparable 1:1 mixture of *R*- and *S*- phosphonate isomers in excellent yield (Scheme 27). Extensive hydrogenolysis of **5.45** in the presence of Pearlman's catalyst[175] produced dimer **5.46** as a 1:1 mixture of P-isomers. Considering the difficult installation of the DMT group in the bicyclic

core, the 5'-OH group was protected as a levulinic ester, an alternative group used in oligonucleotide synthesis.[176] Treatment of alcohol **5.46** was treated with levulinic anhydride[177] gave ester **5.47**. The final removal of the silyl ether in **5.47** was achieved by using catalytic amounts of CsF[178] in a mixture of DMSO/MeOH under neutral conditions to afford alcohol **5.48** in 65 % yield. Transformation of **5.48** into the corresponding phosphoramidite and introduction into oligonucleotides sequences was performed at Ionis Pharmaceuticals.

5.6 Duplex thermal stability measurements

The dimer **5.48** was incorporated at Ionis Pharmaceuticals into a DNA oligonucleotide to determine its ability to stabilize duplexes. The oligonucleotide modified with **5.48** was then paired with matched and mismatched RNA (Table IV), where it showed a modest enhancement in duplex thermal stability and a similar ability to discriminate mismatches as natural DNA.

Table IV. Duplex stabilizing and mismatch discrimination properties of compound **5.48**

	*T*m °C (ΔTm/mod)			
Sequence (5'-3')	RNA Y=A	RNA Y=C	RNA Y=G	RNA Y=U
GGATGTTCTCGA	49.7	34.5	44.0	35.8
GGATGTTCTCGA	50.2	34.9	46.7	35.8

Tm values were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA; RNA complement 3'-CCUACYAGAGCU-5'.

The results show that the dioxaperhydrofuropyran ring in **A** (Figure 24) can mimic conformational preferences around γ, δ and ε in nucleic acid duplexes but does not significantly enhance duplex stability. To help understand these observations, we created a structural model

which depicts the preferred conformation around the inter-nucleosidic phosphodiester linkage observed in RNA duplexes (Figure 24).

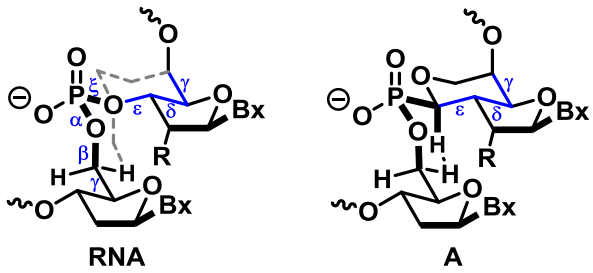


Figure 24. Conformational analysis of RNA versus nucleotide **A**

The O3'-P-O5'-C5'-H bonds form the outline of a six-membered chair and this conformation is stabilized by anomeric effects through the phosphorus atom.[179] Presumably, these non-bonding interactions serve to pre-organize the backbone in nucleic acid duplexes such that further conformational restriction around ε does not provide additional benefits.

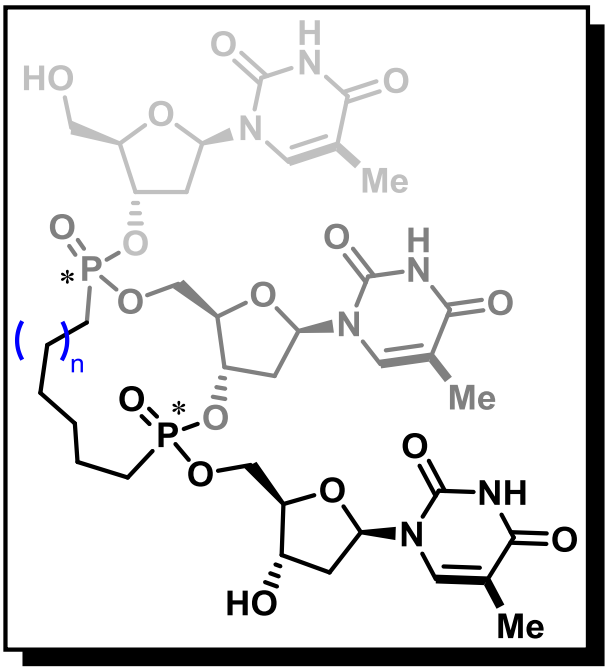
5.7 Conclusions

Nucleotide **5.48** was synthesized in 24 steps using a chiron approach with 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose as starting point. The route featured a diastereoselective α-hydroxyphosphonate formation and a Tf_2O-mediated perhydrofuropyran formation.

Duplexes containing nucleotide **5.48** presented a slight increase in thermal affinity studies when compared with the corresponding unmodified sequences.

6 Chapter six

Synthesis of backbone constrained nucleic acids



6.1 Macrocyclic nucleotides

In previous chapters, we presented several strategies to constrain nucleosides by forming covalent bonds between different parts of its structure. Once the constrained nucleosides are introduced into an oligonucleotide, the conformation of the neighbouring nucleotides is modified,[180,181] affecting the overall conformation of the strand and also the affinity towards complementary nucleic acid strands.

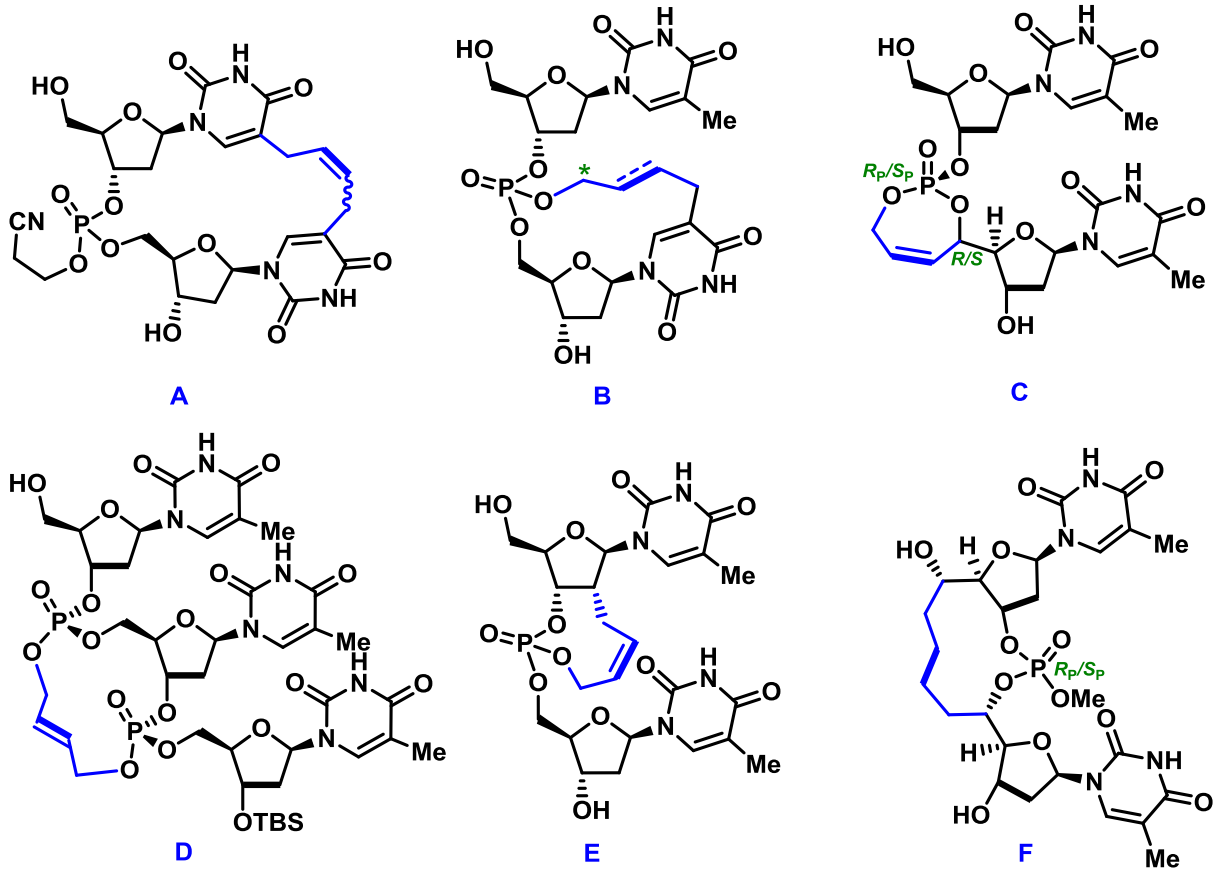


Figure 25. Macrocyclic nucleoside phosphates developed by Nielsen. The bold bond indicates the junction point between the terminal olefin precursors. *site of cleavage with ammonia.

A less studied strategy to constrain oligonucleotides involves the joining of two different nucleotides within an ASO by a chain external to the backbone, thus forming a macrocycle. The

first macrocyclic oligonucleotides joined by phosphate ester bonds were developed by Nielsen[182-187] who used RCM to build the macrocycle (Figure 25). The resulting di- and tri-nucleotides had constrained backbones which could result into a diminished flexibility that could be used to mimic nucleic acid secondary structures. For example, macrocyle **A** (Figure 25) was prepared by RCM using 5 mol% second generation Grubbs catalyst, affording an optimum yield of 23% for the cyclization step.[188] In the same publication, cyclic phosphate **B** was synthesized, but was cleaved at the indicated position (*) when treated with 32% aqueous ammonia during standard automated oligonucleotide synthesis conditions. The four possible isomers of the seven-membered cyclic phosphotriester **C** were also synthesized. The conformational behaviour and the configuration at the P-center of the isomers of **C** were studied by NMR spectroscopy and molecular dynamics simulations,[182,187] which showed the (*R*, S_P) isomer to favor nucleobase stacking suggesting high affinity for complementary nucleic acids.

The synthesis of macrocycle **D** was briefly described by Nielsen.[184] The key RCM step did not proceed using first generation Grubbs catalyst; instead, second generation Grubbs catalyst in refluxing dichloromethane resulted in cyclization with concomitant cleavage of the DMT group from the 5'-position. The mixture of phosphorus and olefin isomers of **D** included 8 possible diastereomers which were not separated nor individually characterized; instead, the synthesis probed feasibility of the cyclization reaction.

Finally, macrocycles **E** and **F** were prepared by RCM and introduced into oligonucleotides.[183,185] Hybridization measured against complementary DNA and RNA sequences, showed significant drop in duplex stability which was attributed to an expected distortion from the natural nucleic acid structure as a result of the constrained macrocycle.

6.2 General design of backbone constrained nucleic acids

In an effort to probe the effect of different modifications in ASOs on DNA and RNA duplex stability, scientists at Ionis Pharmaceuticals in collaboration with the Hanessian Lab initiated a study to assess the effect of constraining the backbone of an ASO by incorporating a macrocyclic nucleoside trimer linked by two phosphonate bonds (Figure 26). As the introduction of a carbon-phosphorus bond in place of an oxygen in the phosphodiester linkage renders the phosphorus atom chiral, we decided to study also the influence of the R_P and S_P phosphorus centers in the Backbone Constrained Nucleic Acids (BCNA).

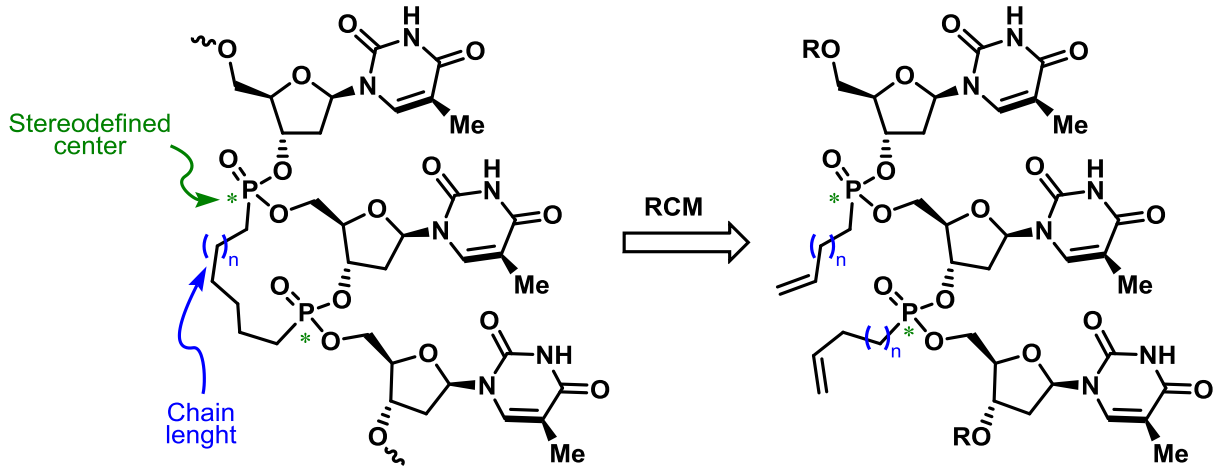


Figure 26. General design of the backbone constrained nucleic acids

We envisaged that RCM would provide the macrocycles from trimers 2'-deoxythymidines containing stereodefined alkenyl phosphonate ester appendages (Figure 26). In principle, the stereochemistry of the phosphorus center could be secured by stereoselective synthesis and separation of the R_P and S_P diastereomers. These novel carbon chain linked macrocyclic phosphonates could be considered as surrogates for the corresponding phosphates. However the isolation of stereodefined P-diastereomers present a major problem in isolation and characterization of individual R_P/S_P and combination of isomers.

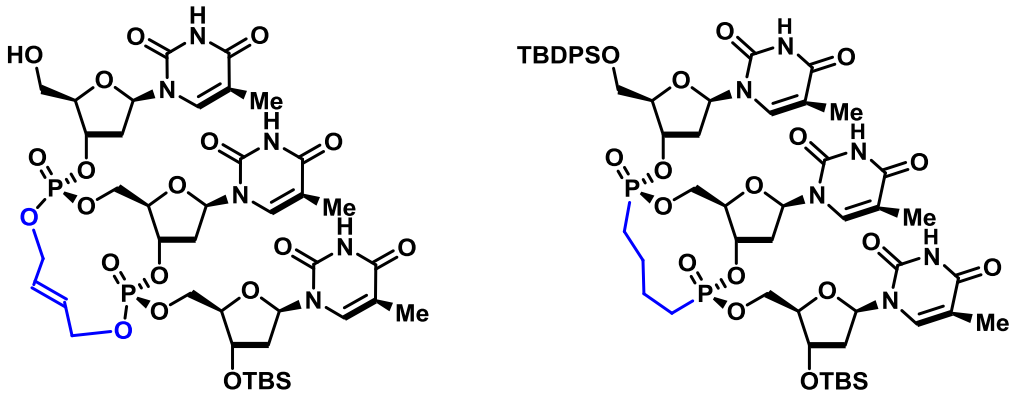


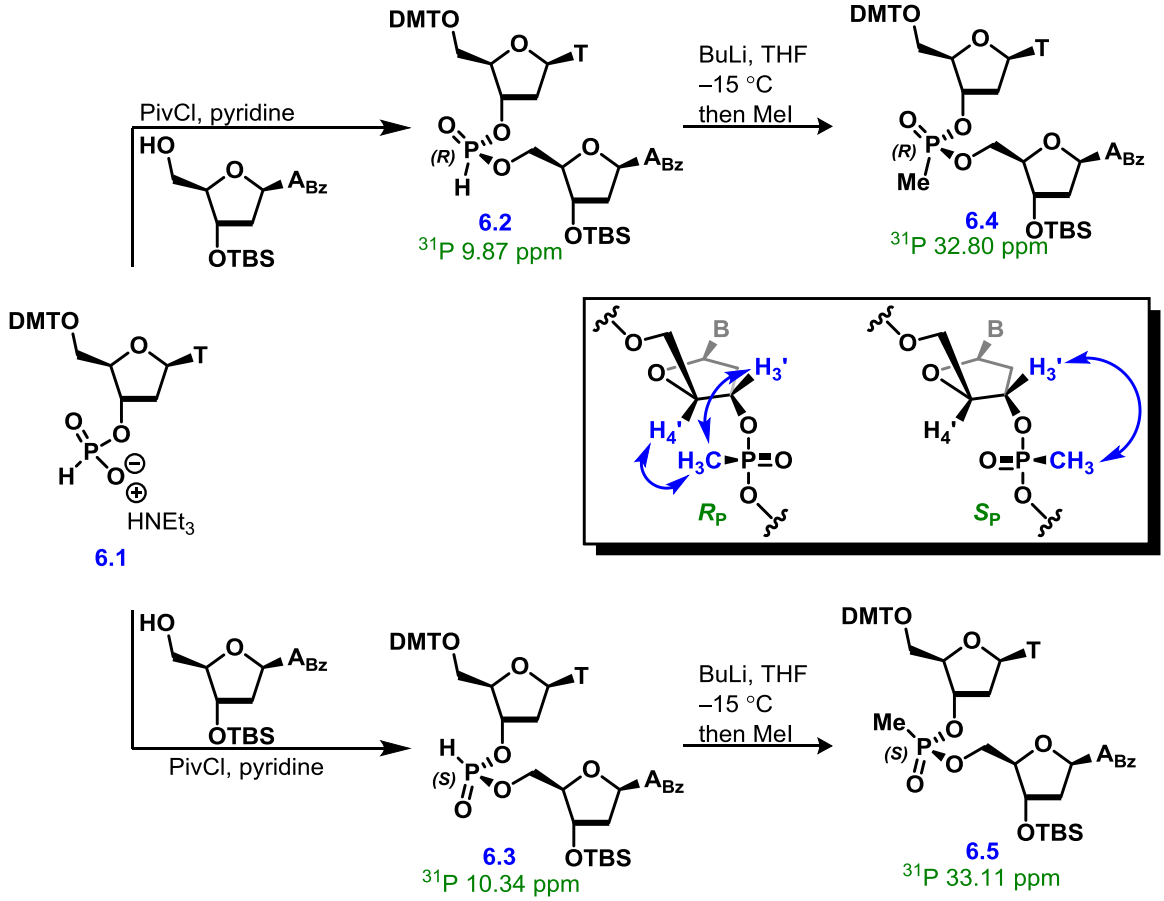
Figure 27. Comparison of macrocyclic phosphate synthesized by Nielsen and our proposed macrocyclic phosphonate

The main objective was to synthesize the phosphonate equivalent of Nielsen macrocyclic diphosphate trimeric thymidine nucleosides as stereochemically defined single isomers at both P-sites (Figure 27).

6.3 H-Phosphonate strategy

The lack of methods for the stereoselective synthesis of alkyl phosphonates led us to search for an alternative path to obtain the required compounds. In 1991, Seela and coworkers reported the synthesis of diastereomerically pure methyl phosphonates by direct alkylation of H-phosphonates[189] (Scheme 28). Their synthesis started with the coupling of the H-phosphonate salt **6.1** with 3'-OTBS deoxyadenosine to produce a mixture of *R*p and *S*p H-phosphonates **6.2** and **6.3**, which were separated by flash column chromatography. Interestingly, the isomers migrating at higher R_f on TLC exhibited ^{31}P NMR signals upfield from those having lower R_f values. Treatment of diastereomers **6.2** and **6.3** with BuLi followed by MeI, gave phosphonates with *retention* of configuration. The absolute configuration of diastereomers **6.4** and **6.5** was

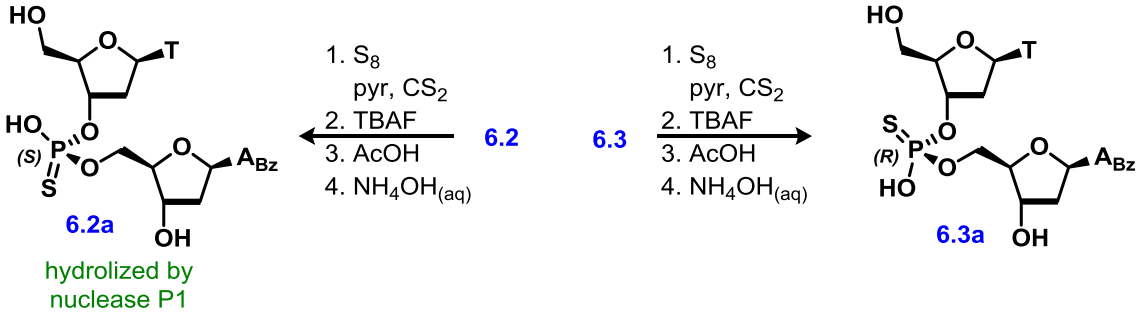
assigned by 2D NMR ROESY.[190] Assuming that dimeric nucleosides **6.2** and **6.3** are found in a nearly undistorted conformation similar to unmodified nucleic acids,[191] the P-methyl group may be found in two spatial orientations (Scheme 28). The R_P-phosphorus configuration would give rise to two ROESY interactions between the P-methyl group and the H3' and H4' atoms of the top nucleoside in the dimer. Similarly, the S_P-phosphorus configuration would exhibit a ROESY interaction between the P-methyl group and the H3' atom of the top nucleoside in the dimer. Such measurements together with statistical analyses enabled Engels to predict with high probability the configuration of several methylphosphonates.[192]



Scheme 28. Stereoretentive alkylation of H-phosphonates.

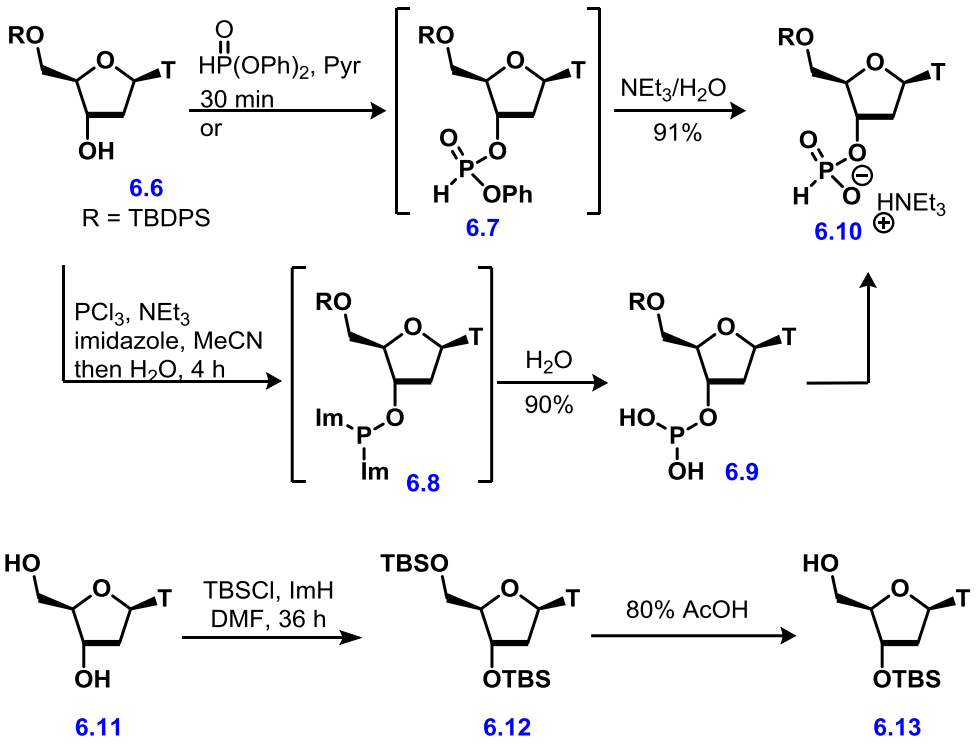
The faster migrating compound on TLC (**6.4** in Scheme 28) was considered to be the R_P isomer and exhibited a ^{31}P signal upfield of that of the S_P isomer **6.5**. It is important to mention that under the basic conditions used, Seela did not observe alkylation of the thymine or adenine nucleobases.[189]

To further confirm the configurations of H-phosphonates **6.2** and **6.3**, each compound was subjected to a sulfurization reaction, known to be stereospecific (Scheme 29).[193] The obtained compounds were fully deprotected and the resulting phosphorothioates **6.2a** and **6.3a** were digested with nuclease P1, an enzyme that stereoselectively hydrolyses S_P phosphorothioates.[194] Only (R_P) phosphothioate **6.2a** was hydrolyzed.



Scheme 29. Sulfurization of H-phosphonates

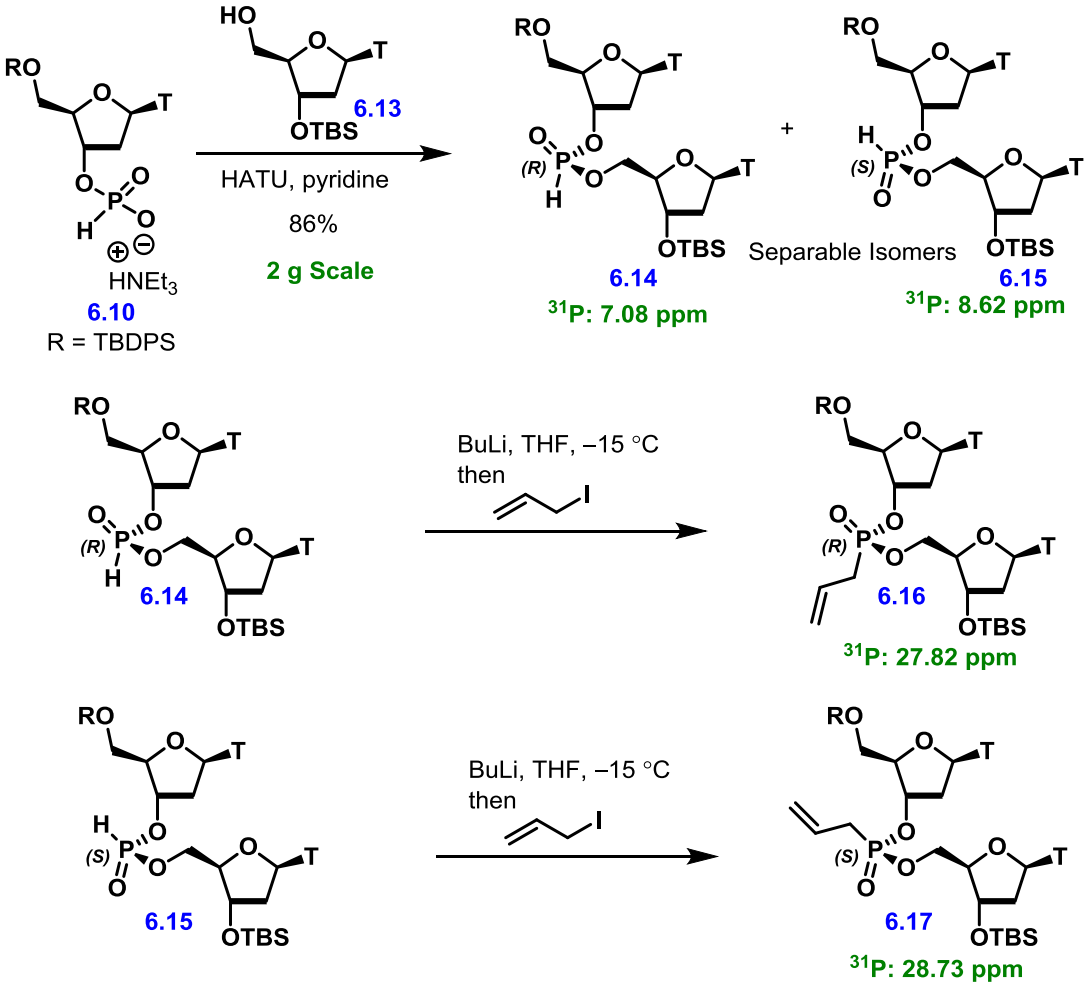
Following the same strategy used by Seela, starting materials for the formation of the dimers were prepared from thymidine. A TBDPS group was installed at the 5'-position to give silyl ether **6.6** (Scheme 30). The triethylamine salt **6.10** was next prepared by two different routes.



Scheme 30. Synthesis of precursors **6.10** and **6.13**

The first route used diphenyl phosphite[195] to form a mixed phosphite **6.7**, which was hydrolyzed on treatment with a mixture of NEt_3/water to give salt **6.10** (Scheme 30). The second approach used PCl_3[196] as a source of phosphorus by way of bis-imidazolium **6.8** which upon hydrolysis formed dihydroxy phosphite intermediate **6.9** that was quickly transformed into the corresponding H-phosphonate.[197] Nucleoside **6.13** was prepared by treating thymidine **6.11** with an excess of TBSCl to obtain the bis-silyl ether **6.12** and subsequent cleavage of the primary TBS group under acidic conditions.[198] Attempts to form dimeric H-phosphonate nucleoside **6.14** by way of the mixed anhydride using pivaloyl chloride as coupling agent led to several by-products. In an effort to identify a more convenient activating agent, we found a complete study performed by Wada[170] where he identified N,N-bis(2-oxo-3-oxazolidin-l-yl)phosphonic chloride (BOPCl) as an excellent activating agent for the synthesis of dimeric nucleoside H-

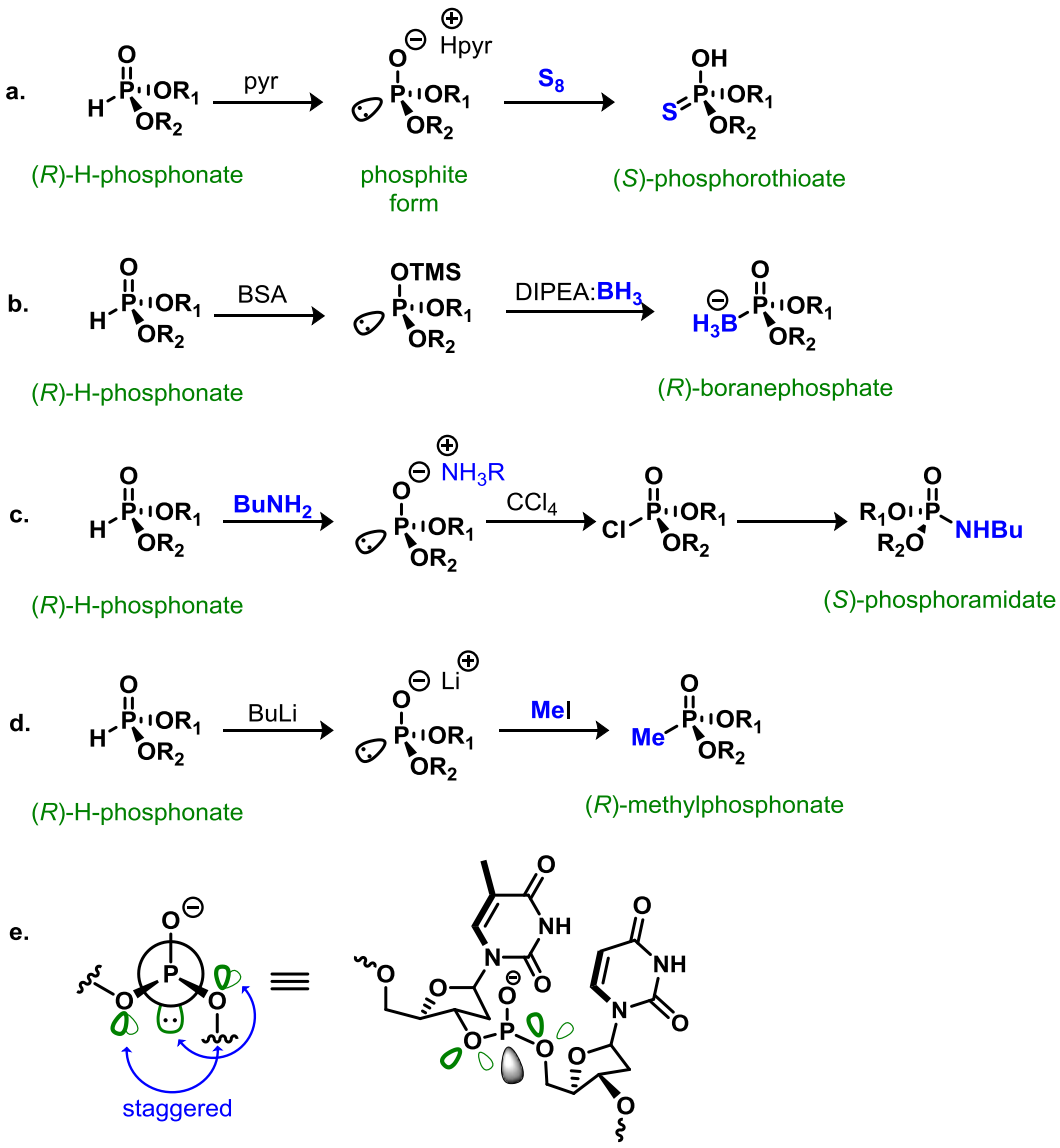
phosphonates. In the same study it was also shown that HATU[199] could be used as an efficient alternative to BOPCl. After some optimization, we found that coupling of 3'-OTBS thymidine **6.13** with the nucleoside **6.10** using HATU in pyridine gave a mixture of H-phosphonates **6.14** and **6.15** in excellent yield (Scheme 31). The separation of **6.14** and **6.15** by flash chromatography was difficult, leading to the (R_P)-pure isomer, significant amounts of (R_P) + (S_P) compounds and some fractions of pure (S_P)-isomer. In some cases, a second short chromatographic purification was required to remove the hydrolysis by-products formed during prolonged residence time of phosphonates (R_P) and (S_P) on silica gel.



Scheme 31. Synthesis of R_P and S_P allyl phosphonates

With (R_P) H-phosphonate **6.14** in hand, we proceeded to allylate using the conditions reported by Seela (Scheme 31). The reaction proceeded cleanly with full consumption of the H-phosphonate in 60% yield giving a single isomer as ascertained by ^{31}P NMR spectroscopy. Allylation was assumed to proceed with retention of the stereoconfiguration as in the case of the methyl phosphonates **6.4** and **6.5**. Attempts to perform a ROESY experiment to assign configuration as was done for the methylphosphonate were however hampered by signal overlap of the 3'-hydrogen of the upper nucleoside unit with the terminal vinylic hydrogens of the allyl chain.

A rationale to account for the stereoretentive alkylation of H-phosphonates may be proposed from a study of other reactions in which diastereopure H-phosphonate nucleosides reacted stereospecifically (Scheme 32).[200]

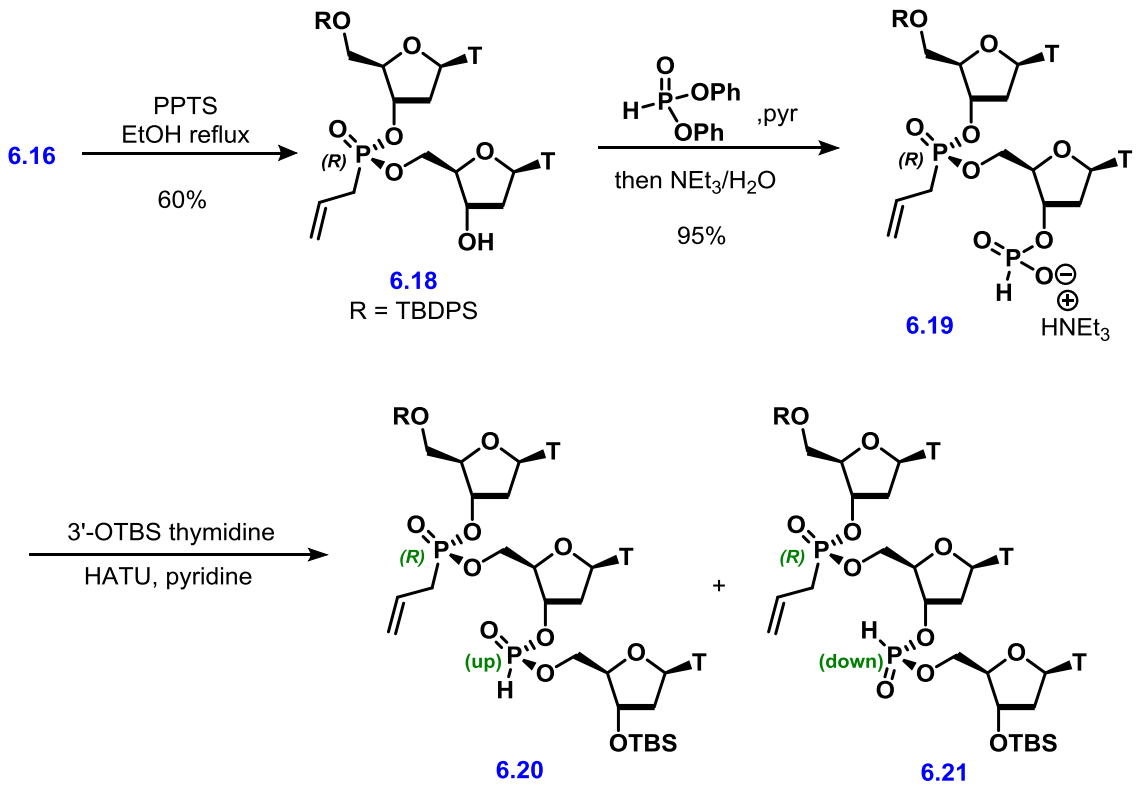


Scheme 32. Stereospecific reactions of H-phosphonates

For example, formation of phosphorothioates is known to proceed with retention of the configuration (**a** in Scheme 32).[189,201] The starting H-phosphonate is believed to be deprotonated by a base, forming an intermediate phosphite anion that reacts with elemental sulfur to give the corresponding phosphothioate. After treatment with BSA, H-phosphonates are converted into O-silylphosphites, that react with DIPEA:BH_3 complex to give borane phosphates with retention of configuration (**b** in Scheme 32).[202,203] In the Atherton-Todd reaction,[204] chiral H-

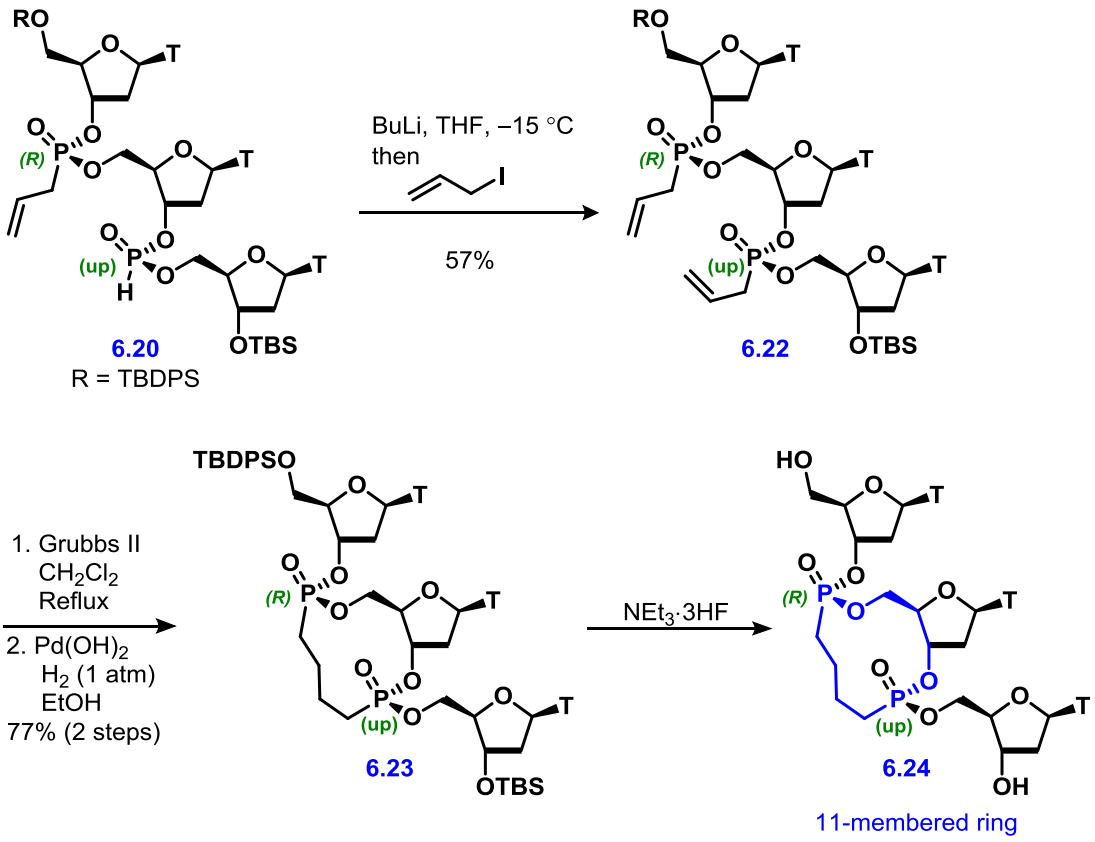
phosphonates are transformed into phosphoramidates with complete stereoinversion, due to the intermediacy of a phosphoric diester chloridate (**c** in Scheme 32).[205]

In the examples mentioned above, the intermediate phosphite is configurationally stable. The same stability may be responsible for retention of the R_P configuration during alkylation with methyl iodide and allyl iodide (**d** in Scheme 32). For a phosphite anion to epimerize, the pyramidal geometry must interconvert through a planar transition state, a process that has a reported inversion barrier energy value of 33 Kcal/mol[206] for a nucleosidic acyclic phosphite, similar to the value for a phosphine. Based on stereoelectronic considerations, it is reasonable to assume that the R_P-H-phosphonate, hence the intermediate deprotonated phosphite (**e** in Scheme 32), adopts a conformation in which repulsive forces between lone pairs on oxygen are minimized (lone pairs staggered) such that the stereochemistry after allylation or methylation is retained.[207] The hypothetical planar transition state for inversion would require surmounting a high energy barrier created by repulsion between the lone pairs of the oxygen and phosphorus atoms.[208]



Scheme 33. Synthesis of trimeric nucleosides **6.19** and **6.20**.

With the R_P isomer **6.16** in hand, we proceeded to cleave selectively the 3'OTBS group by using PPTS in refluxing ethanol to afford alcohol **6.18** (Scheme 33). The triethylammonium salt **6.19** was obtained using the diphenyl phosphite method[195] because conditions involving PCl_3/imidazole[196] gave by-products. Using HATU as activating agent, we prepared trimeric nucleosides **6.20** and **6.21** which after a difficult separation by flash chromatography, gave only the P-isomer pure which eluted first from the column. A recovered mixture of trimer **6.20** and **6.21** was set aside.



Scheme 34. Synthesis of 11-membered ring macrocycle **6.24**.

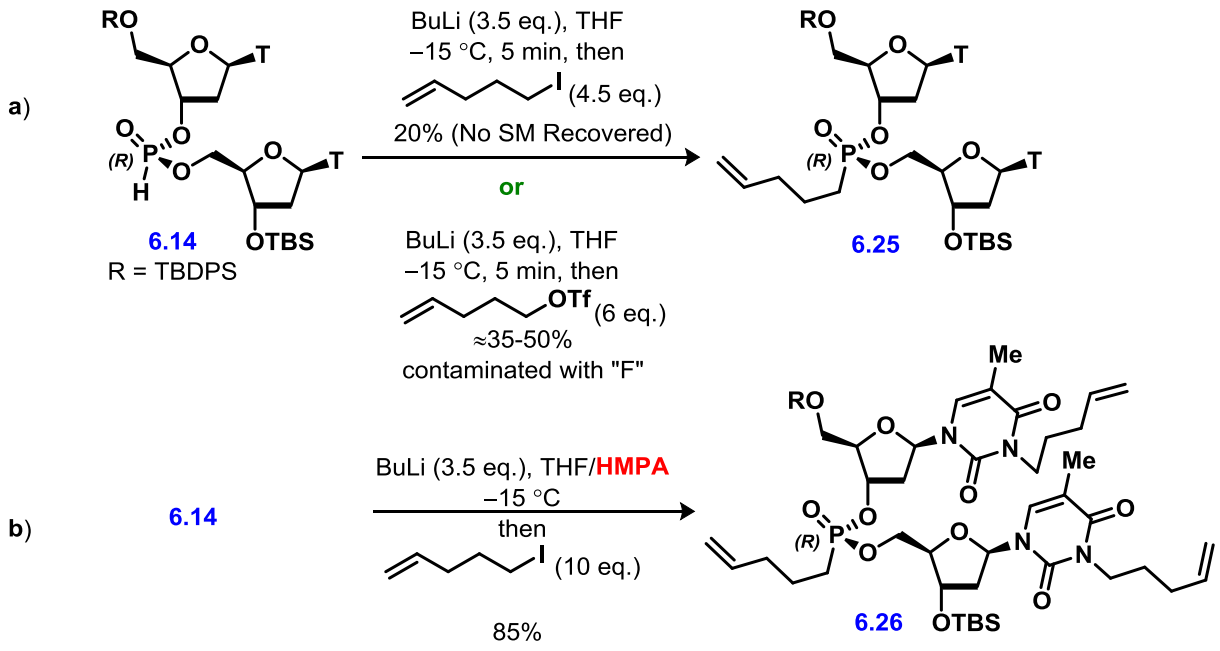
The isomer with the higher R_f value in the **6.20** trimer cannot be assumed to have the (R_P) configuration at the phosphorus because the previous comparison applied to dimers. For simplicity, we shall the trimer **6.20** as the (R_P),(*up*) isomer and the trimer **6.21**, (R_P),(*down*) isomer. We chose to proceed with the trimeric nucleoside **6.20,** and the isomeric (R_P),(*down*) trimer was set aside.

Treatment of the H-phosphonate trimer **6.20** with 4.5 eq of BuLi and trapping the P-anion with excess allyl iodide afforded trimer **6.22** in good yield (Scheme 34). In spite using excess BuLi and allyl iodide, we did not detect any N-alkylation of the nucleobases. With the bis-allyl compound **6.22** in hand, we tested the key intramolecular cross metathesis reaction of the terminal alkenes to form the corresponding macrocycle. Using the second-generation Grubbs

catalyst,[209] diene **6.22** readily produced macrocycles (not shown) as inseparable mixtures of *cis*/*trans* isomers as detected by ^{31}P NMR spectroscopy. Hydrogenation of the mixture with $Pd(OH)_2$ led to 11-membered ring macrocycle **6.23** in 77% yield over two steps. Final removal of the silyl groups with TBAF resulted however in complete decomposition of the starting material. Treatment of macrocycle **6.23** with $NEt_3 \cdot 3HF$ gave the fully deprotected cyclic trimer **6.24**.

In summary, the synthesis of macrocyclic phosphonate linked nucleotide **6.24** proceeded in a 5'→3' fashion (north to south) by sequential coupling of H-phosphonates followed by allylation. The synthesis of macrocycle **6.24** proved the viability of the metathesis strategy for an 11-membered ring macrocycle, which may be extended to larger rings. The remaining question concerning the configuration of the second phosphorus atom could not be fully assessed at this point of the synthesis. Instead, an alternative route presented in the section **6.6** provided complementary information for the assignment of the P-configuration of the second phosphonate (see Scheme 43).

Attempting to extend the present strategy, different olefins were studied to lead to cycles of larger sizes. Alkylation of H-phosphonate **6.14** using freshly prepared 5-iodo-1-pentene (**a** in Scheme 35) gave however olefin **6.25** in only 20% yield in spite several attempts to improve yield. 5-Bromo-1-pentene (not shown) gave no product. Successful alkylations with methyl iodide and allyl iodide suggests that activated electrophiles are required to react effectively with the formed P-lithium anion.



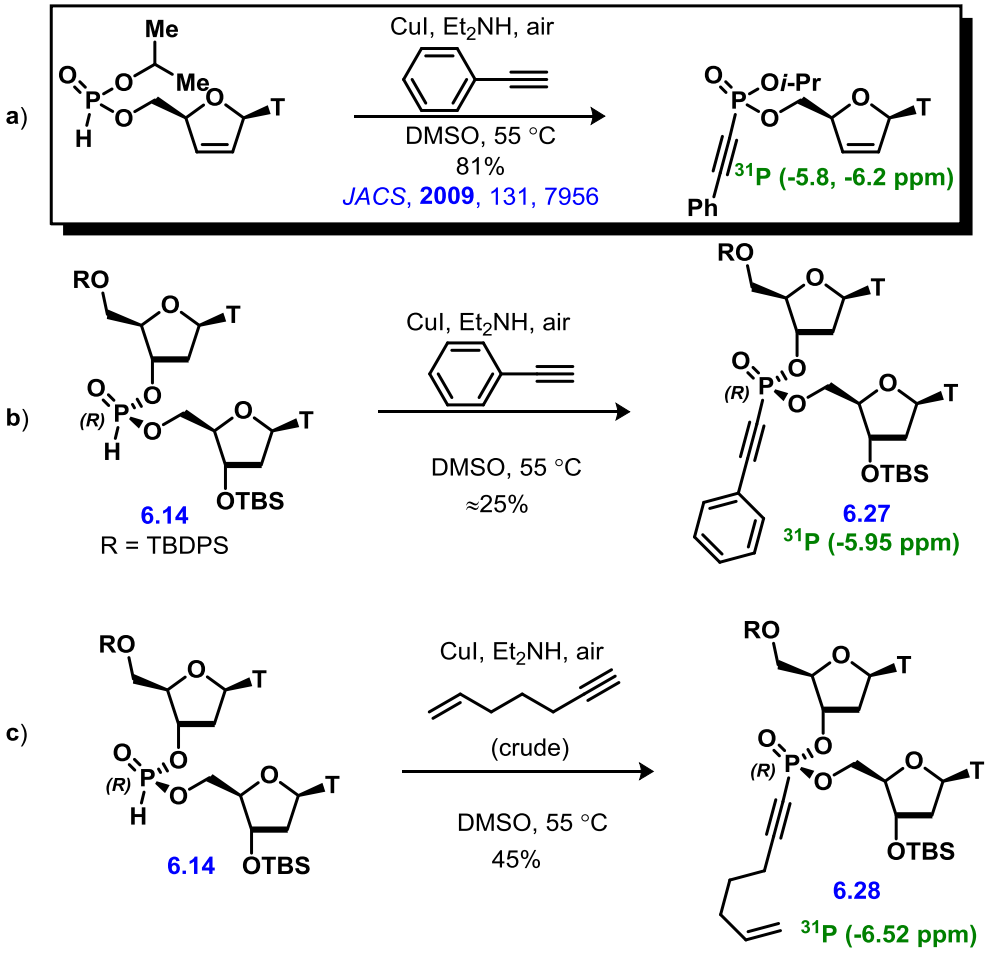
Scheme 35. Synthesis of alkyl phosphonate **6.23**.

Employment of an electrophile with higher reactivity, such as a triflate, was considered because is known to be 10^6 times more active than iodide.[210] The corresponding alkenyl triflate was however sensitive to light and heat.[211] With precautions, we succeeded to use pent-4-en-1-yl trifluoromethanesulfonate in the alkylation of H-phosphonate **6.14** (**a** in Scheme 35) but phosphonate **6.25** was contaminated with an unknown impurity containing fluorine as detected by ^{19}F NMR spectroscopy. Removal of the impurity was not possible by chromatography, but it was reduced using less of alkenyl triflate (6 eq). The mass recovery of the reaction varied from 35 to 50% including the fluorinated impurity which could not be quantified. Trying to avoid the fluorine-containing impurity, we attempted to increase the reactivity of the P-anion by coordinting the Li cation using HMPA as co-solvent (**b** in Scheme 35); however, peralkylated nucleoside **6.26** was isolated as the only product. Varying the amounts of HMPA did not

produce reproducible results and the use of alternative DMPU[212] gave only a low yield of product **6.25**.

6.4 Copper-mediated coupling of H-phosphonates with alkynes

In search of alternative methods to functionalize H-phosphonate **6.14**, we investigated a copper-mediated coupling of terminal alkynes. Research published by Han[213] presented a single example of the reaction of an H-phosphonate nucleoside to give the corresponding alkynyl phosphate as a mixture of P-isomers as observed in the ^{31}P NMR spectrum (**a** in Scheme 36). It was not clear from the Han paper if the starting H-phosphonate was diastereopure, so first, we studied the outcome of the reaction using pure H-phosphonate **6.14**. Coupling of the (R_P) H-phosphonate **6.14** with phenylacetylene (**b** in Scheme 36) gave alkynyl phosphonate **6.27** in 25% yield (unoptimized conditions) as a single isomer according to ^{1}H and ^{31}P NMR spectroscopy. Motivated by this result, we prepared hept-1-en-6-yne using 5-bromo-1-pentene and the commercially available lithium acetylide·ethylenediamine complex for the use in the Copper-mediated coupling.



Scheme 36. Copper-catalyzed coupling of a terminal alkyne with H-phosphonates.

Coupling phosphonate **6.14** and hept-1-en-6-yne using CuI produced alkyne **6.28** as a single isomer (**c** in Scheme 36) in 45% yield. However, further attempts to run the reaction in larger scale (≈100 mg) resulted in variable yields, mainly because of the volatility of the hept-1-en-6-yne and the need to run the reaction in open air flasks.

Concerning the mechanism of the reaction, Han proposed the pathway shown in Figure 28. Intermediate **A** is formed by base-mediated insertion of the Cu (I) into the position 1 of the alkyne. The formation of copper acetylide was suggested by the formation of a precipitate at the beginning of the reaction, which disappears once the reaction is complete. Although it is known

that the equilibrium between pentavalent H-phosphonate **B** and trivalent phosphite **C** is fully displaced toward the former,[214,215] the proposed mechanism claims that the phosphite form **C** coordinates to the copper center to give intermediate **D,** which then produces alkynylphosphonate **E** and active catalyst **A** with aid of molecular oxygen. We believe that H-phosphonate **F** is involved in the coordination with the metallic center, because of literature precedent for the formation of compounds like **G**.[216] The coordination of the H-phosphonate has been also proposed by Stawinski and coworkers during the palladium-mediated coupling of H-phosphonates with aryl halides.[217,218]

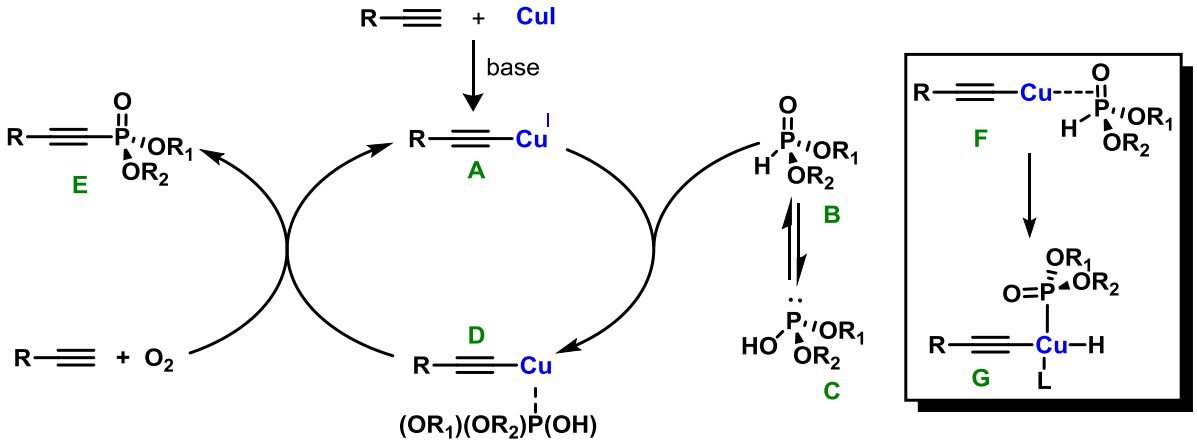


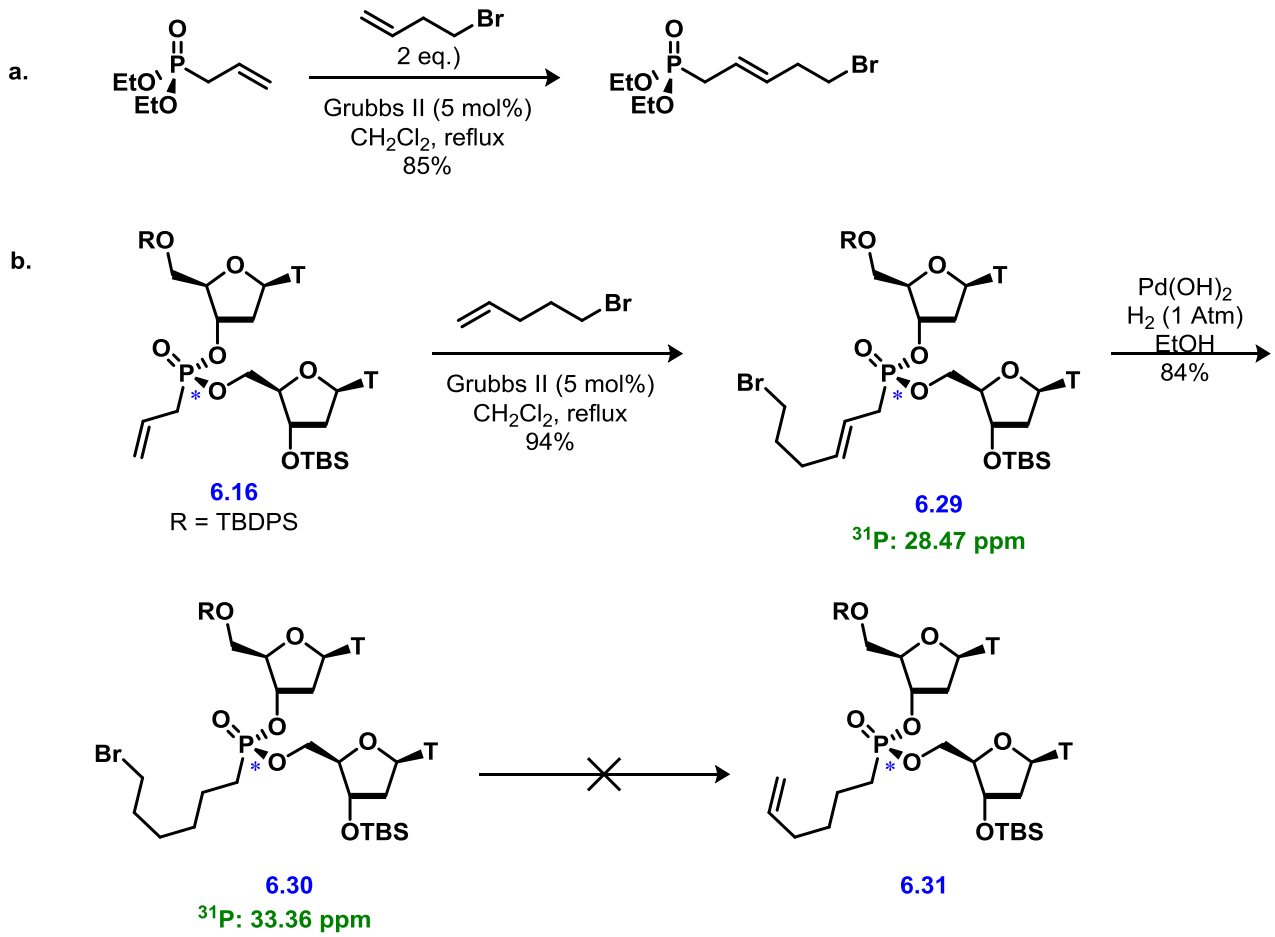
Figure 28. Mechanism proposed by Han for the copper-mediated formation of alkynylphosphonates

Further exploration of this strategy may be merited to provide alkyne phosphonates with retention in the stereochemistry.

6.5 Chain extension by cross-metathesis

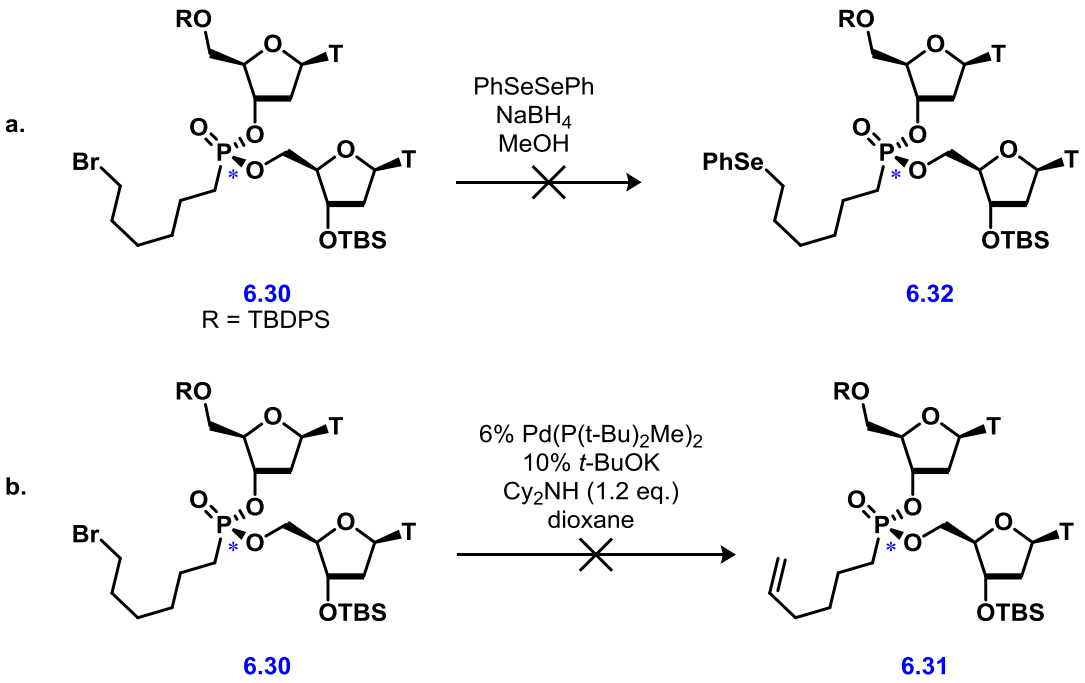
After gaining access to pure allyl phosphonates **6.16** and **6.17** (Scheme 31), we turned our attention to the use of alkene cross-metathesis to extend the chain of the allyl phosphates. In 2001, Grubbs[219] reported the cross-metathesis of diethyl allylphosphonate with terminal alkenes containing different functionalities (**a** in Scheme 37). When we carried out the reaction of compound **6.16** with 5-bromo-1-pentene and second generation Grubbs catalyst, *trans*-alkene **6.29** was obtained as a single isomer (**b** in Scheme 37). After successful elongation of the carbon chain, the next critical step was to hydrogenate the internal double bond without cleavage of the halogen-carbon bond and reduction of the unsaturation at the nucleobases. By using $Pd(OH)_2$ we managed to avoid completely the reduction of the bromide, but careful monitoring of the reaction by MS was required to minimize the reduction of nucleobase in **6.30**.

The successful synthesis of alkyl bromide **6.30** opened the door to different synthetic options. The most useful transformation for our purposes was the elimination of the primary bromide to give the corresponding terminal alkene **6.31**. Use of K_2CO_3 gave no product, and bases such as DBU and *t*BuOK led to hydrolysis of the dimer. The presence of the acidic N3 protons (pKa ≈ 9.8)[220] on the nucleobases and the α-hydrogens to the phosphonate (which are more acidic than the β-hydrogens to the bromide) demanded the use of an excess of base, which apparently was responsible for hydrolysis of the dimer.



Scheme 37. Synthesis of bromide **6.30**

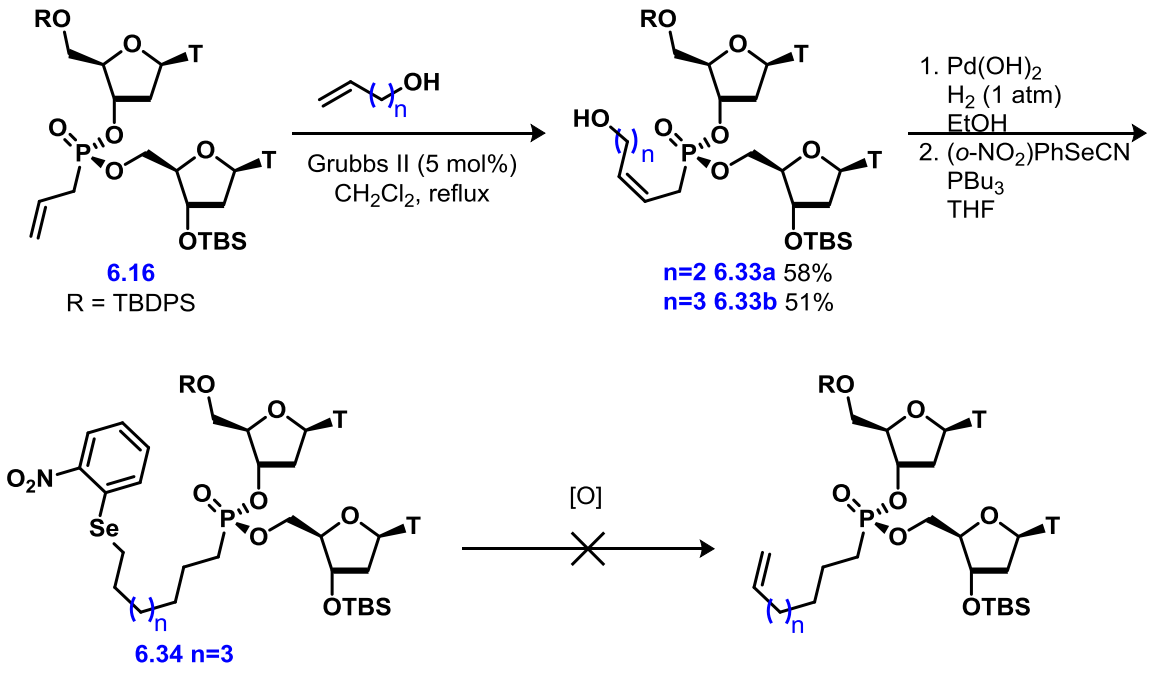
As an alternative to the halide elimination, we attempted to form phenylselenide **6.32** (**a** in Scheme 38), which after oxidation could eliminate to terminal alkene **6.31**. The use of *in situ* generated phenylselenide anion[221] to displace the primary bromide was however unsuccessful and we recovered starting material.



Scheme 38. Attempts to functionalize compound **6.31**

In 2012 Fu[222] developed a palladium-mediated dehalogenation of alkyl bromides to form terminal alkenes. The method takes advantage of the tendency of alkylpalladium complexes to decompose rapidly through a β-hydride elimination pathway forming an alkene. Several attempts under the reported conditions failed to convert primary bromoalkane **6.30** to olefin **6.31** (**b** in Scheme 38)**.**

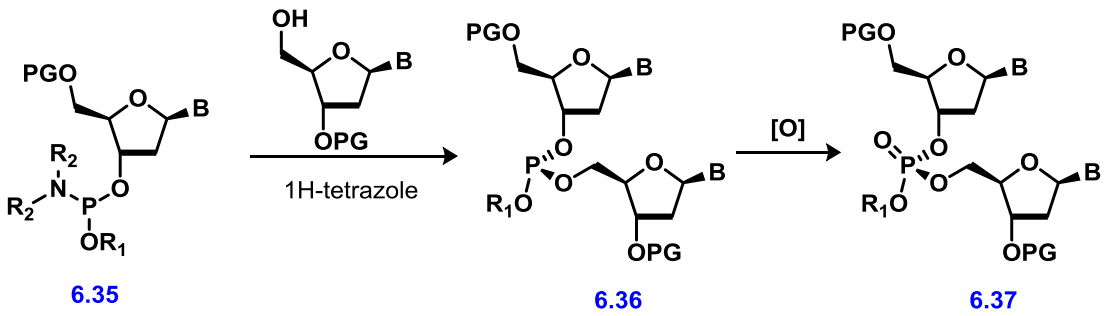
In a final attempt to use the intermolecular cross-metathesis strategy, we synthesized alcohols **6.33a** and **6.33b** using olefin **6.16** and the corresponding alkenols (Scheme 39). Hydrogenation of the alkenols took place, followed by formation of a (*o*-NO_2)phenylselenide using Grieco's conditions.[223] Attempts to oxidize nitrophenyl selenide **6.34** led to partial hydrolysis of the dimer but no trace of the alkene. Due to the toxicity of the reagents used to obtain **6.34** and the lack success in the synthesis of alkenes, we decided to explore alternative methods to obtain extended alkenylphosphonates.



Scheme 39. Attempted Grieco elimination of primary alcohols.

6.6 Phosphoramidite-Arbuzov method

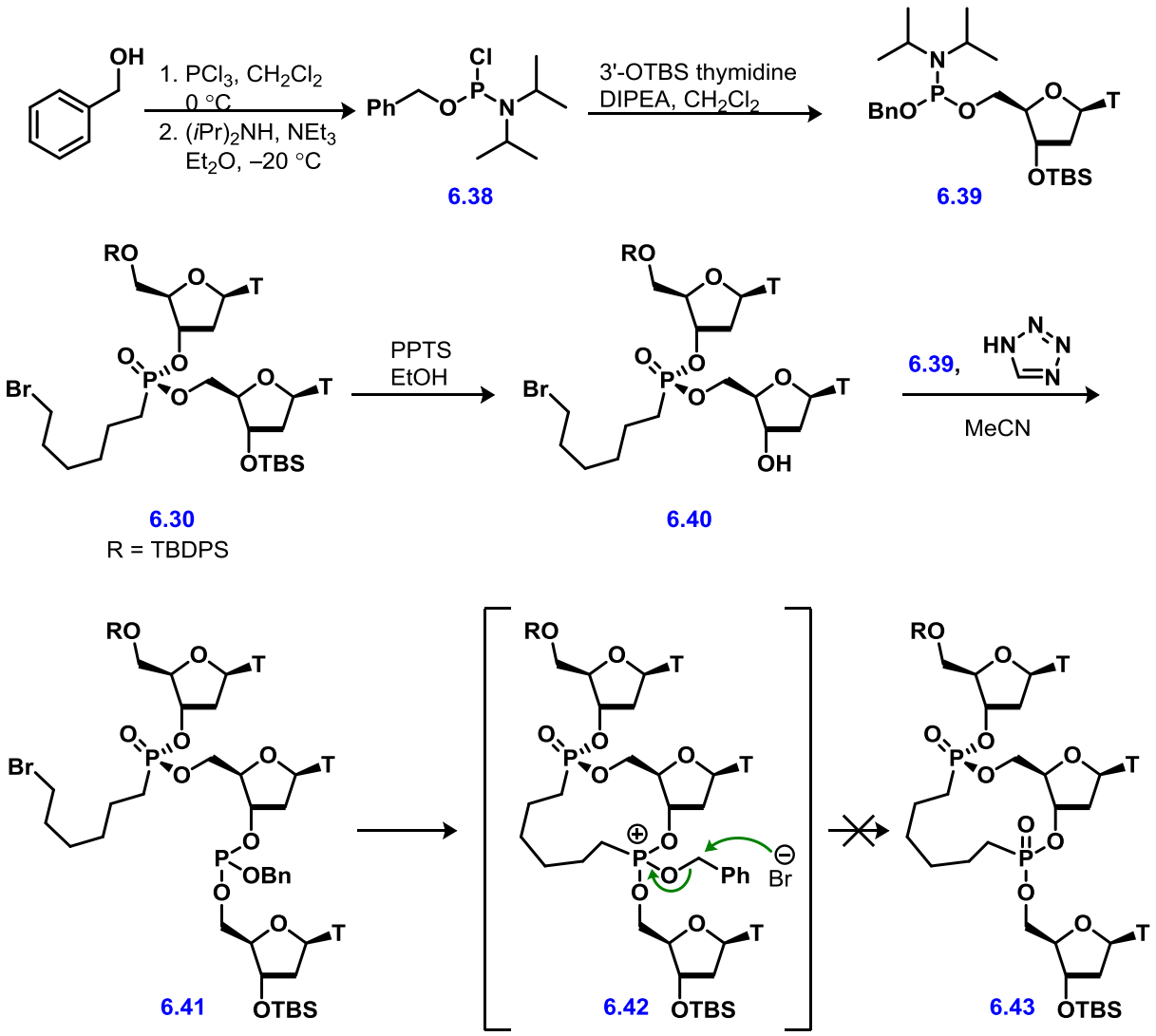
Trying to find a suitable alternative to obtain larger macrocycles, we turned to phosphoramidite chemistry, which is a common strategy in oligonucleotide nucleotide synthesis. Modern oligonucleotide synthesis employs phosphoramidite chemistry developed by Beaucage and Caruthers.[224] In their original publication, the preparation and use of N,N-dimethylaminophosphoramidites **6.35** (R_1 = R_2 = Me) was reported to readily react with an appropriate 3'-protected nucleoside in the presence of an activating agent such as 1H-tetrazole to form phosphites **6.36** (Scheme 40). Phosphites like **6.36** can be isolated or oxidized *in situ* to produce the corresponding phosphates **6.37**; additionally, further investigation found that N,N-diisopropylphosphoramidites (R_2 = *i*Pr, R_1 = Me) are more stable than their dimethyl counterparts.[225]



Scheme 40. Phosphoramidite method for the synthesis of oligonucleotides

With the aim to synthesize macrocycle **6.43**, phosphoramidite chemistry was used to obtain a suitable precursor for an intramolecular Arbuzov reaction, similar to the strategy reported by Escudier[69] in the synthesis of α,β-P-CAN (Figure 11). To facilitate the Arbuzov reaction, O-benzylphosphoramidite (**6.41** in Scheme 41) was employed to form phosphonate nucleotide **6.43** by the attack of halide ion at the benzylic position.

O-Benzyl *N*,*N*-diisopropylchlorophosphoramidite **6.38** was prepared by condensation of anhydrous benzyl alcohol with PCl_3 and diisopropylamine followed by coupling with 3'-OTBS thymidine to produce 5'-phosphoramidite **6.39** (Scheme 41). Dimer **6.30** was deprotected with PPTS, and alcohol **6.40** was coupled with phosphoramidite **6.39** using 1H-tetrazole as activator to afford trimer **6.41**.



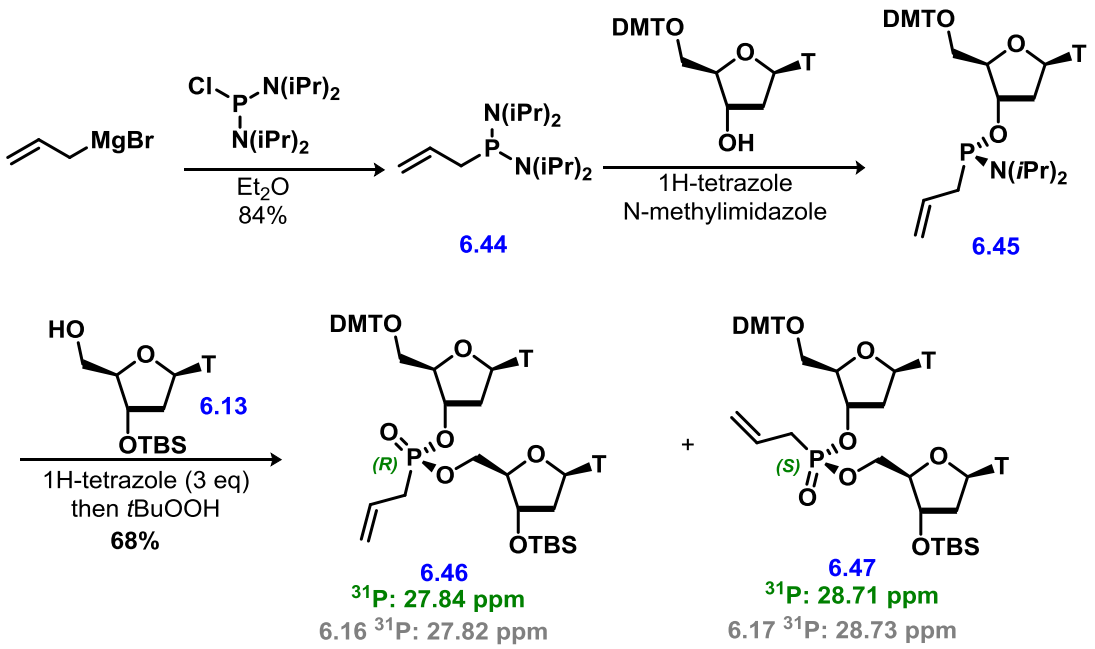
Scheme 41. Attempted Arbuzov reaction of dimer **6.41**.

Heating a solution of bromide **6.41** in acetonitrile at reflux for several days or under microwave conditions at 90 °C for 4 h gave no detectable macrocycle **6.43**. In both cases, solid LiBr was added as additive assuming that the formation of intermediate **6.42** is favored, making the attack of the bromide into the benzylic position the rate-determining step of the reaction as reported by Escudier.[69] Although the key cyclization was not achieved in the previous strategy, we realized that the phosphoramidite chemistry could be successfully applied for our purposes.

6.7 Phosphoramidite method

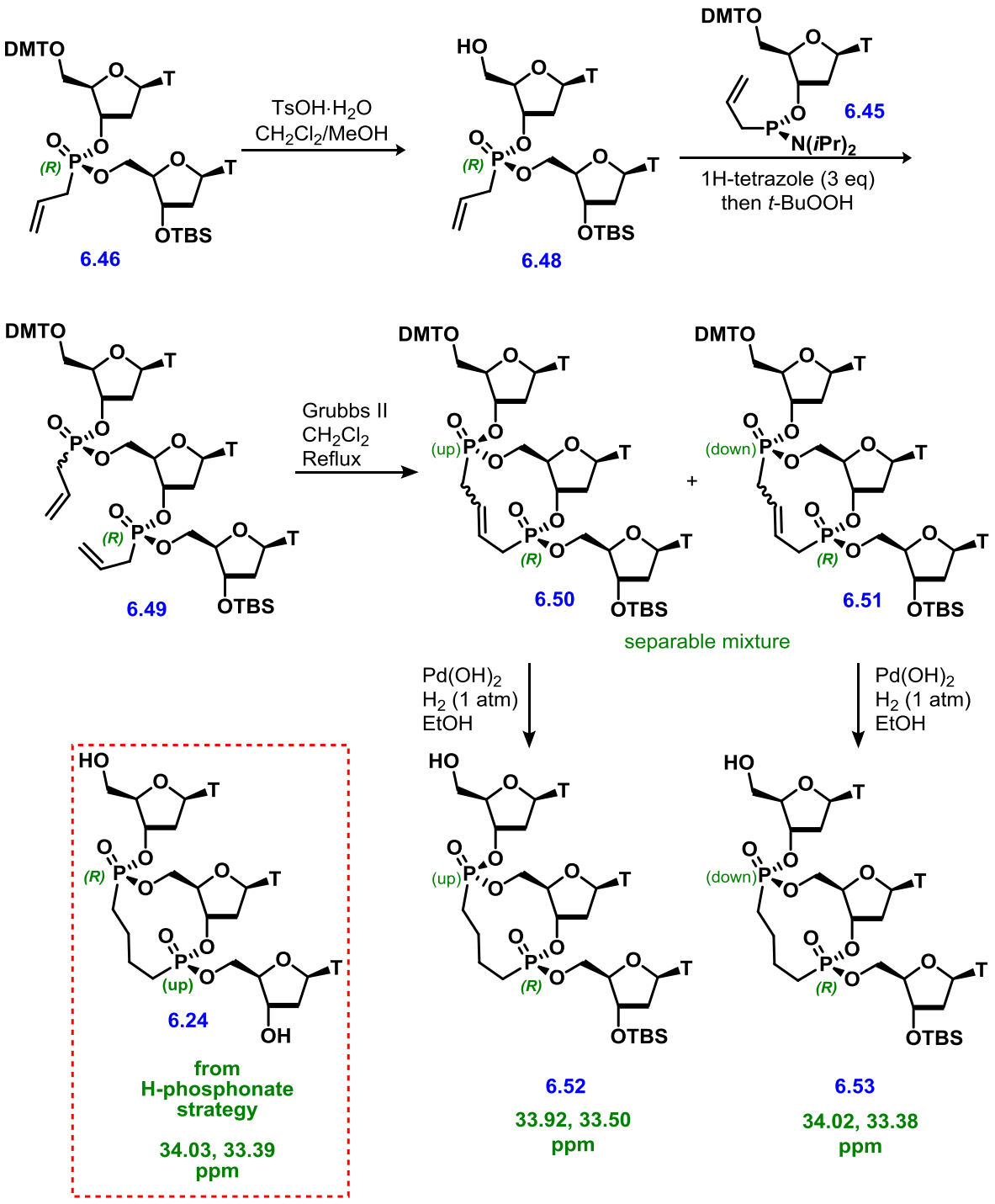
A new strategy to build trimers was explored using sequential addition of appropriately protected nucleosides to phosphoramidites containing alkyl chains.[226,227] Similar strategies have been successful in the synthesis of trimeric nucleotides.[228]

Allylmagnesium bromide was reacted with bis(diisopropylamino)chlorophosphine to give olefin **6.44** (Scheme 42), which was isolated and coupled with 5-ODMT thymidine using 1H-tetrazole as activating agent to give phosphoramidite **6.45**. Thymidine **6.45** was purified by flash chromatography and stored at -20 °C under argon without significant decomposition. Phosphonate **6.46** was prepared as a diastereomeric mixture by coupling phosphoramidite **6.45** and nucleoside **6.13**, followed by *in situ* oxidation with tBuOOH. We correlated the mobility on silica gel and the ^{31}P chemical shifts of the diastereomeric phophonates **6.46** and **6.47** *versus* allyl phosphonates **6.16** and **6.17**. Higher R_f values were consistent with chemical shifts at higher field (^{31}P **6.16** = 27.82 ppm, **6.46** = 27.84 ppm) *vs* lower R_f values (^{31}P **6.17** = 28.73 ppm, **6.47** = 28.71 ppm) (Scheme 42). Based on previous results, we designated compound **6.46** as the (R_P)-phosphonate and **6.47** as the (S_P)-phosphonate.



Scheme 42. Synthesis of compounds **6.46** and **6.47**

After removal of the DMT group from **6.46** under acidic conditions, alcohol **6.48** was coupled to allylphosphoramidite **6.45,** and oxidation with *t*BuOOH afforded an inseparable mixture of diastereomers in **6.49** (Scheme 43). Fortunately when the mixture of compounds **6.49** was cyclized using the second-generation Grubbs catalyst, we obtained a separable mixture of products **6.50** (up) and **6.51** (down), each one containing a mixture of *cis/trans* isomers. Separately, each of the products containing the *cis/trans* mixture (**6.50** and **6.51**) was hydrogenated, a process that cleaved the DMT group and produced a single compound. The ^{31}P NMR signals of macrocycle **6.24** obtained from the H-phosphonate route (See Scheme 34) differed from **6.52** but correlated with **6.53** resulting from the phosphoramidite route (Scheme 43).



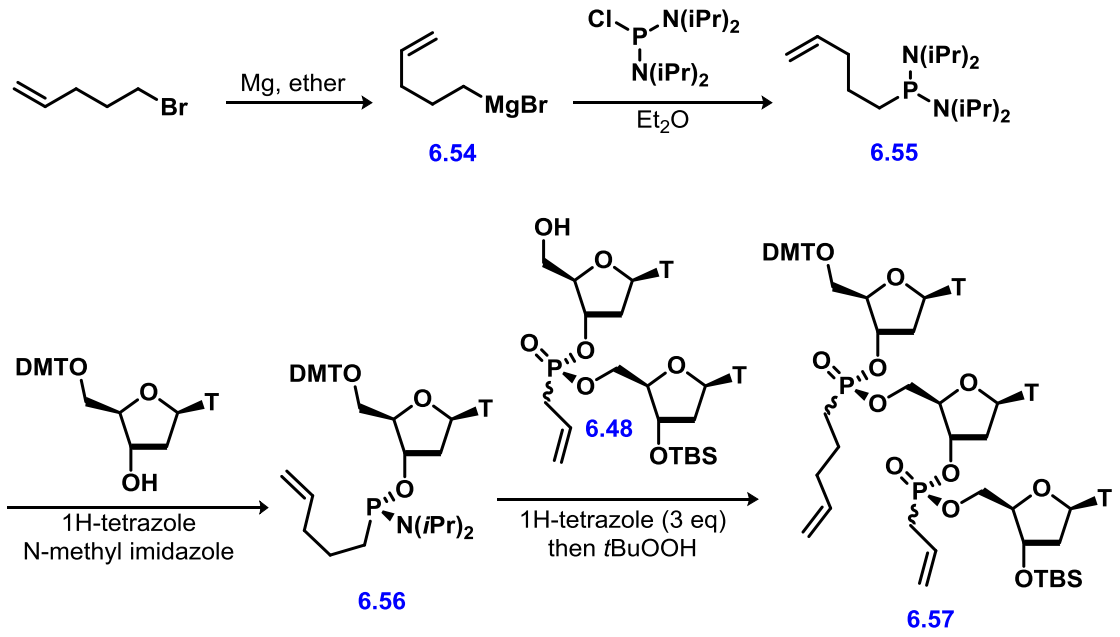
Scheme 43. Synthesis of macrocycles **6.53** and **6.55**

In considering how **6.24** was obtained, we need to remember that the phosphorus atom located between the first and the second nucleoside (5' to 3' direction) has an (R_P)-configuration and

the configuration of the other phosphonate was unassigned. In the case of macrocycles **6.52** and **6.53**, the second phosphorus atom (5' to 3' direction) was established to have an (R_P)-configuration, as they both were prepared from alcohol **6.48**. However, the configuration at the first phosphorus atom (5' to 3' direction) was not established. The fact that **6.24** and **6.53** had almost identical signals suggests that they are the same compound having the (R_P,R_P) configuration.

To summarize, this strategy allowed the synthesis of two P-isomeric macrocycles **6.52** and **6.53** using alcohol **6.48** as the key intermediate for the coupling of the third nucleoside at the 5'-position (south to north). The same strategy can be applied for the formation of the other pair of isomers using S_P nucleoside **6.47**. Future work must focus on the synthesis of the four possible P-isomers of the 11-membered ring macrocycle and their full characterization to assess the absolute configuration of the phosphorus stereogenic centers.

Preliminary results were also obtained for the synthesis of larger macrocycles. By starting from 5-bromo-1-pentene, we prepared the Grignard reagent **6.54** which was used immediately to prepare compound **6.55** (Scheme 44). Formation of phosphoramidite **6.56** proceeded cleanly, and the obtained compound was stable at -20 °C under argon for several weeks without significant signs of decomposition. Compound **6.48** (isomeric mixture at P-center) was prepared to determine if the separation of an isomeric mixture was easier with the 5'-position deprotected; however, the mixture was inseparable. As a test reaction, we decided to couple compound **6.48** with phosphoramidite **6.56** to give a mixture of four inseparable compounds in **6.57**. Although we did not proceed further with the route, we proved that the preparation of phosphoramidites with longer chains is feasible and opened the door to different size macrocycles.



Scheme 44. Exploratory work towards the synthesis of different size macrocycles

The preparation of 11-membered ring macrocycles and other related macrocycles in large quantities (≈100 mg) is an ongoing task in Hanessian group and the measurement of thermal affinity values, once introduced into ASOs, will be measured by Ionis Pharmaceuticals.

6.8 Conclusions

We accomplished the synthesis of 11-membered ring macrocycle by two different synthetic routes.

The configuration at the phosphorus atoms in macrocycles **6.52** and **6.53** (Scheme 43) was established by comparison with the macrocycle obtained using H-phosphonate intermediates (**6.24**).

The phosphoramidite route can be adapted for the synthesis of different sized macrocycles as exemplified in the synthesis of compound **6.57**.

7 Future Perspective

The unique mode of action of ASO in targeting complementary RNAs distinguishes this approach to drug development from traditional ones where treatment takes place once the disease is diagnosed (ex. inhibition of enzymes or interaction with receptors). ASOs target production of harmful proteins before they act on physiological processes that eventually lead to a disease. Even with the recent success of antisense technology over the last years with the approval of KYNAMRO® and SPINRAZA® (Figure 29) by the FDA for the treatment of homozygous familial hypercholesterolemia and spinal muscular atrophy, respectively, there is still much work to be done in the field. The previously mentioned drugs belong to the "Gen 2+" antisense technology with ED_{50} (median effective dose) of ≈ 150 mg of ASOs per week. The so-called 2.5 generation of antisense uses (*S*)-cEt-BNA nucleoside as a building block, which translates into a ED_{50} of 15 mg/wk, reducing significantly the amount of ASOs to be used and the corresponding toxicities associated with high doses of the drug. The successful identification of tissues where the ASOs are accumulated has led to a more specific selection of targets, especially those located in the liver where a large number of proteins of interest are synthesized.[229,230] New technology developed by Ionis Pharmaceuticals uses **Li**gand-**C**onjugated **A**ntisense (LICA) to further enhance the potency of Gen2+ and Gen2.5 ASOs, reaching levels of ED_{50} up to 1 mg/wk. The LICA contains the ASOs conjugated to N-acetylgalactosamine (GalNAc), a moiety that presents high affinity for the asialoglycoprotein (ASGPR) receptor which is highly expressed in hepatocytes.[231] The development of new LICAs that improve potency by selective target delivery will extend the used of antisense technology to many other therapeutic areas.

The synthesis of new macrocyclic nucleic acid trimers such as the ones described in this thesis and their incorporation in a series of oligonucleotides with predetermined sequences may lead to improved binding affinity for complementary RNA strands. This could enhance the pharmacokinetic properties of the corresponding ASOs, hopefully contributing to improved antisense technology for the future.

* = 2'-O-(2-methoxyethyl)
m = 5-methyl
d = 2'-deoxy

5'—mU*—mC*—A*—mC*—mU*—mU*—mU*—mC*—A*—mU*—A*—A*—mU*—G*—mC*—mU*—G*—G*—**3'**

5'—G*—mC*—mC*—mU*—mC*—dA—dG—dT—dmC—dT—dG—dmC—dT—dT—dmC—G*—mC*—A*—mC*—mC*—**3'**

Figure 29. Spinraza and Kynamro with their corresponding nucleotide sequences

The synthesis of the first phosphate-linked macrocyclic trimeric nucleosides by Nielsen showed the compatibility of the Grubbs RCM reaction with highly polar substrates. However the individual P-isomers were not separated. In this thesis, we have created what amounts to phosphonate surrogates of Nielsen's macrocycles and separated the P-diastereomers. Their incorporation into ASOs will allow to show the difference of P-stereochemistry on the ability to hybridize with complementary RNA and DNA sequences.

8 Experimental Procedures

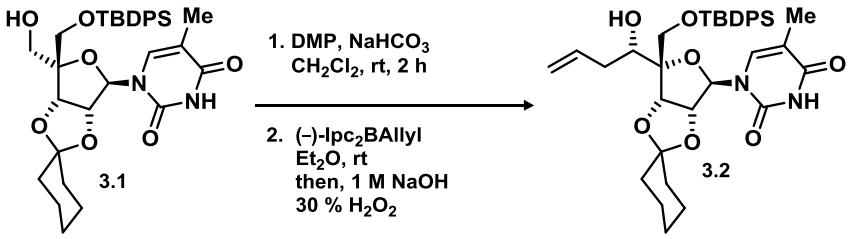
8.1 General experimental

All non-aqueous reactions were performed in oven (120 °C) or flame-dried glassware under a positive pressure of argon, with exclusion of moisture from reagents and glassware, using standard techniques for manipulating air-sensitive compounds, unless otherwise stated. Anhydrous tetrahydrofuran, diethyl ether, toluene, and dichloromethane were obtained by passing through activated columns of alumina. All other solvents were used as received from chemical suppliers. Reagents were purchased and used without further purification. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous material, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica plates (SIL 60, G-25, UV254) that were visualized using a UV lamp (254 nm) and developed with an aqueous solution of ceric ammonium molybdate, or an ethanolic solution of *p*-anisaldehyde. Flash chromatography[232] was performed using SiliaFlash® P60 40-63 μm (230-400 mesh) silica gel and all column dimensions are reported as height × diameter in centimeters. NMR spectra were recorded on Bruker AV-300, ARX-400, or AV-400 instruments, calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$, δ = 7.26 ppm), and reported in parts per million relative to tetramethylsilane (TMS δ = 0.00 ppm) as follows: chemical shift (multiplicity, coupling constant (Hz), integration). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets. High resolution mass spectra (HRMS) were recorded at the Centre Régional de Spectrométrie de Masse de l'Université de Montréal on an Agilent LC-MSD TOF mass spectrometer by electrospray ionization time of

flight reflectron experiments. Specific rotation measurements were recorded on a Perkin-Elmer 343 Polarimeter and are reported in units of deg·cm^3·g^{-1}·dm^{-1}.

8.2 Experimental section

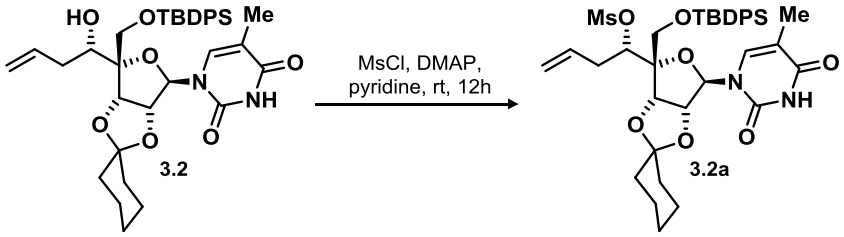
8.2.1 Synthesis of TriNA 2



Compound 3.2

Dess-Martin periodinane (0.481 g, 1.13 mmol) and sodium bicarbonate (200 mg, 2.38 mmol) were added to a stirred solution of alcohol **3.1** (530 mg, 0.873 mmol) in CH_2Cl_2 (5 mL). After stirring at room temperature for 2 h, CH_2Cl_2 (10 mL) and a 10 % (w/v) solution of $Na_2S_2O_3$ were added and stirred until a clear organic phase was obtained. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtrered and concentrated under reduced pressure. The residue was passed through a short silica pad to give the corresponding aldehyde (437 mg). A solution of (–)-Ipc_2BAllyl borane in pentanes (1 M 1.08 mL, 1.08 mmol) was added to a stirred solution of the aldehyde (437 mg) in diethyl ether (12 mL) at -78 °C. After stirring overnight at the same temperature, 1 M NaOH (1.9 mL, 1.9 mmol) was added dropwise to the reaction mixture, followed by 30% H_2O_2 (0.93 mL), and the mixture was heated to reflux for 1 h. The mixture was cooled and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (4 × 5

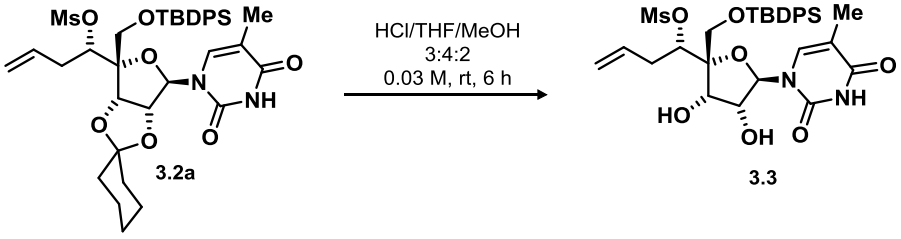
mL). The combined organic extracts were concentrated under reduced pressure. The residue was purified by column chromatography (3:7 EtOAc/Hex) to afford homoallylic alcohol **3.2** as white foam (380 mg, 67% yield over two steps): R_f 0.65 (3% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 7.76 – 7.58 (m, 4H), 7.52 – 7.33 (m, 7H), 6.17 (d, *J* = 6.6 Hz, 1H), 5.93 – 5.78 (m, 1H), 5.20 – 5.01 (m, 2H), 4.95 (d, *J* = 6.1 Hz, 1H), 4.81 (t, *J* = 5.8 Hz, 1H), 4.16 (d, *J* = 11.2 Hz, 1H), 4.10 – 4.02 (m, 1H), 3.96 (d, *J* = 11.2 Hz, 1H), 2.70 (d, *J* = 2.9 Hz, 1H), 2.46 – 2.32 (m, 1H), 1.92 – 1.36 (m, 14H), 1.10 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.76, 150.46, 135.34, 135.31, 135.02, 132.74, 131.83, 130.14, 130.00, 127.98, 127.88, 117.00, 115.43, 111.52, 88.85, 88.82, 83.69, 81.26, 71.48, 64.35, 36.70, 34.67, 34.59, 27.01, 24.66, 23.89, 23.48, 19.28, 11.60; HRMS (ESI) calculated for $C_{33}H_{43}N_2O_7Si$ $[M+H]^+$ *m/z* = 607.2834, found 607.2845.



Compound 3.2a

Methanesulfonyl chloride (0.71 mL, 9.2 mmol) and 4-dimethylaminopyridine (60 mg, 0.46 mmol) were added to a stirred solution of nucleoside **3.2** (2.96 g, 4.58 mmol) in anhydrous pyridine (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 h, and then diluted with 1 M HCl (60 mL) and EtOAc (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (4 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used directly

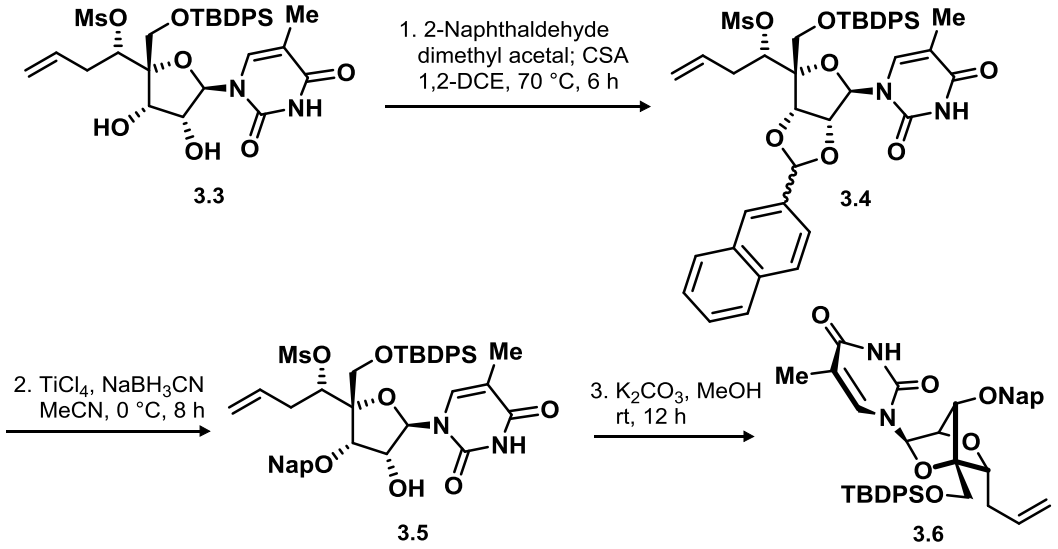
in the next step without further purification. A portion of the residue was purified by flash chromatography (3:7 EtOAc/hexanes) to give mesylate **3.2a** (240 mg, 85%), R_f 0.65 (3% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.70 – 7.56 (m, 4H), 7.52 – 7.32 (m, 7H), 6.10 (d, J = 6.3 Hz, 1H), 5.94 – 5.78 (m, 1H), 5.22 (dd, J = 9.4, 2.8 Hz, 1H), 5.18 – 5.04 (m, 2H), 4.92 (t, J = 5.4 Hz, 1H), 4.66 (d, J = 5.8 Hz, 1H), 4.10 – 3.93 (m, 2H), 2.92 (s, 3H), 2.73 – 2.59 (m, 1H), 2.55 – 2.40 (m, 1H), 1.96 – 1.30 (m, 13H), 1.11 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.03, 150.58, 136.07, 135.64, 135.36, 133.78, 132.49, 131.86, 130.34, 130.30, 128.09, 118.72, 115.52, 111.46, 91.01, 88.76, 83.67, 81.23, 80.66, 65.83, 39.24, 36.89, 35.41, 34.71, 27.11, 24.89, 23.75, 23.48, 19.35, 12.00; HRMS (ESI) calculated for $C_{37}H_{49}N_2O_9SSi$ $[M+H]^+$ *m/z* = 725.29225, found 725.29264; calculated for $C_{37}H_{48}N_2NaO_9SSi$ $[M+Na]^+$ *m/z* = 747.2742, found 747.27566.



Compound 3.3

A 37% aqueous solution of HCl (45 mL) was added to a stirred solution of crude mesylate **3.2a** (3.20 g, 0.22 mmol) in THF (30 mL) and MeOH (60 mL). After 6 h, the reaction mixture was neutralized via addition of saturated aqueous solution of $NaHCO_3$ (40 mL) followed by careful addition of solid $NaHCO_3$ until the solution reached pH 7. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (5 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified

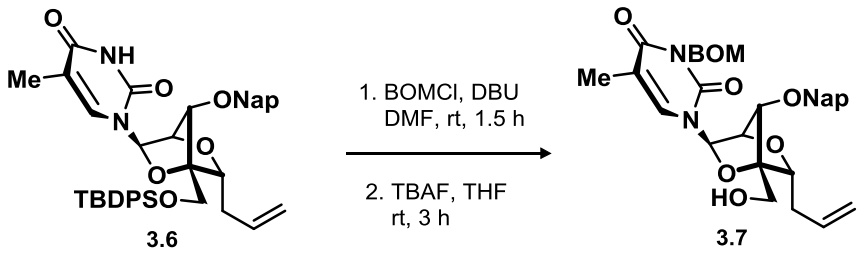
by flash chromatography (3% MeOH in CH_2Cl_2) to give diol **3.3** as a white foam (1.78 g, 60% over two steps), R_f 0.25 (3% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.94 (s, 1H), 7.75 – 7.60 (m, 4H), 7.54 – 7.38 (m, 7H), 6.27 (d, *J* = 7.8 Hz, 1H), 5.89 – 5.74 (m, 1H), 5.18 (dd, *J* = 9.8, 2.6 Hz, 1H), 5.13 – 5.02 (m, 2H), 4.93 (d, *J* = 4.4 Hz, 1H), 4.77 – 4.67 (m, 1H), 4.41 – 4.33 (m, 1H), 3.94 (s, 2H), 3.79 (d, *J* = 2.3 Hz, 1H), 2.98 (s, 3H), 2.67 – 2.56 (m, 1H), 2.38 – 2.17 (m, 1H), 1.56 (s, 3H), 1.14 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.94, 152.30, 135.82, 135.38, 133.64, 132.47, 131.62, 130.60, 130.35, 128.30, 128.25, 118.77, 111.71, 88.17, 87.15, 80.51, 74.57, 72.14, 65.40, 39.19, 35.06, 27.23, 19.39, 12.25; HRMS (ESI) calculated for $C_{31}H_{41}N_2O_9SSi$ $[M+H]^+$ *m/z* = 645.22965, found 645.23047; calculated for $C_{31}H_{40}N_2NaO_9SSi$ $[M+Na]^+$ *m/z* = 667.2116, found 667.21242.



Compound 3.6

Naphthaldehyde dimethyl acetal (0.87 g, 4.30 mmol) and camphorsulphonic acid (50 mg, 0.22 mmol) were added to a stirred solution of diol **3.3** (1.38 g, 2.14 mmol) in anhydrous 1,2-dichloroethane (11 mL). The reaction mixture was heated at 70 °C for 6 h, cooled to room

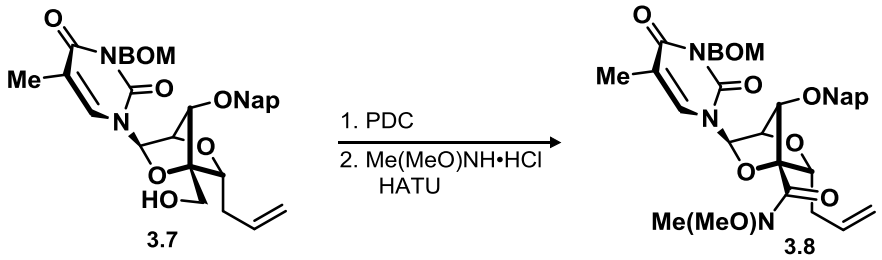
temperature, and mixed with silica gel. The solvent was removed under reduced pressure and the crude mixture was passed through a short silica column (2% MeOH in CH_2Cl_2) affording 1.527 g of acetal **3.4**. The product was dissolved in acetonitrile (50 mL). The solution was cooled to 0 °C and a 1 M solution of $NaBH_3CN$ in THF (9.75 mL, 9.75 mmol) was added, followed by a 1 M solution of $TiCl_4$ in CH_2Cl_2 (9.75 mL, 9.75 mmol). After 8 h at 0 °C, the reaction mixture was slowly quenched by the addition of a saturated aqueous solution of $NaHCO_3$ (50 mL) and CH_2Cl_2 (100 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give crude alcohol **3.5**. The crude residue was dissolved in methanol (50 mL), mixed with K_2CO_3 (1.08 g, 7.82 mmol) and heated at 50 °C for 12 h. The reaction mixture was cooled to room temperature and mixed with silica gel. The solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography (4:6 EtOAc/hexanes) to give bicycle **3.6** as a white foam (723 g, 45% over three steps), R_f 0.51 (4:6 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.92 – 7.56 (m, 9H), 7.55 – 7.27 (m, 9H), 5.85 – 5.70 (m, 1H), 5.68 (s, 1H), 5.19 – 4.96 (m, 2H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.73 (d, *J* = 11.4 Hz, 1H), 4.60 (s, 1H), 4.33 – 4.19 (m, 2H), 4.03 (d, *J* = 12.0 Hz, 1H), 3.90 (d, *J* = 12.0 Hz, 1H), 2.56 – 2.40 (m, 1H), 2.32 – 2.16 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.93, 149.97, 135.57, 135.40, 134.36, 134.30, 133.78, 133.24, 133.22, 132.82, 132.38, 130.22, 130.18, 128.52, 128.10, 127.97, 127.84, 126.99, 126.50, 126.35, 125.86, 118.00, 110.57, 89.31, 86.94, 80.09, 76.58, 72.62, 58.93, 33.89, 27.09, 19.56, 12.18; HRMS (ESI) calculated for $C_{41}H_{45}N_2O_6Si$ $[M+H]^+$ *m/z* = 689,30414, found 689.30352; calculated for $C_{41}H_{44}N_2NaO_6Si$ $[M+Na]^+$ *m/z* = 711.28608, found 711.28599.



Compound 3.7

1,8-diazabicyclo[5.4.0]undec-7-ene (190 μL, 1.26 mmol) and a 60% technical grade solution of benzyl chloromethyl ether (0.44 mL, 1,9 mmol) were sequentially added to a stirred 0 °C solution of bicycle **3.6** (723 mg, 1.05 mmol) in *N*,*N*-dimethylformamide (21 mL). After 1.5 h the reaction mixture was partitioned between diethyl ether (20 mL) and water (100 mL). The layers were separated and the aqueous portion was extracted with diethyl ether (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford an oil, which was used directly in the next step without purification. Tetrabutylammonium fluoride (1 M in THF, 1.6 mL, 1.6 mmol) was added to a stirred solution of crude oil (599 mg, theor. 1.05 mmol) in THF (10 mL). The mixture was stirred at room temperature for 3 h before the volatiles were removed under reduced pressure. The residue was reconstituted in CH_2Cl_2 (30 mL) and partitioned with water (20 mL). The layers were separated and the aqueous portion was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (2:8 EtOAc/hexanes) to give alcohol **3.7** as a white foam (450 mg, 75% over two steps), R_f 0.16 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.70 (m, 4H), 7.26 (s, 10H), 5.94 – 5.76 (m, 1H), 5.62 (s, 1H), 5.46 (d, J = 9.6 Hz, 1H), 5.41 (d, J = 9.6 Hz, 1H), 5.23 – 5.10 (m, 2H), 4.83 – 4.77 (m, 1H), 4.71 (d, J = 9.9 Hz, 3H), 4.46 (s, 1H), 4.32 – 4.24 (m, 1H), 4.11 (s, 1H), 3.97 (d, J = 12.4 Hz, 1H), 3.85 (d, J = 12.5 Hz,

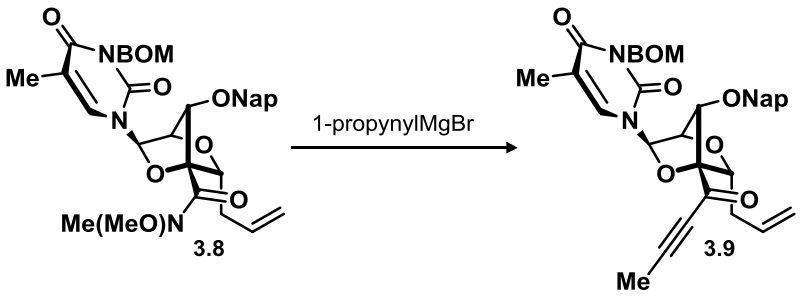
1H), 2.65 – 2.44 (m, 2H), 2.38 – 2.27 (m, 1H), 1.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.35, 150.43, 137.87, 134.39, 133.77, 133.59, 133.10, 133.07, 128.44, 128.35, 127.82, 127.78, 127.76, 127.68, 126.78, 126.44, 126.30, 125.57, 117.98, 109.59, 88.52, 86.96, 79.85, 77.36, 76.68, 72.53, 72.36, 70.39, 57.21, 33.60, 13.40; HRMS (ESI) calculated for $C_{33}H_{34}N_2O_7$ $[M+H]^+$ *m/z* = 571,24388, found 571,24608; calculated for $C_{33}H_{34}N_2NaO_7$ $[M+Na]^+$ *m/z* = 593,22582, found 593,22769.



Compound 3.8

Pyridinium dichromate (2.08 g, 5.53 mmol) was added to a stirred solution of alcohol **3.7** (450 mg, 0.717 mmol) in *N*,*N*-dimethylformamide (12 mL). After stirring at 40 °C for 12 h, the reaction mixture was partitioned with a saturated aqueous solution of ammonium chloride (50 mL). The layers were separated and the aqueous portion was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to afford the crude carboxylic acid, which was used directly in the next step without purification. *N*,*O*-dimethylmethylamine hydrochloride (230 mg, 2.6 mmol), *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (360 mg, 0.949 mmol), and *N*,*N*,-diisopropylethylamine (0.69 mL, 3.96 mmol) were added sequentially to a solution of crude carboxylic acid (461 mg, theor. 0.717 mmol) in CH_2Cl_2 (12 mL). The mixture was stirred at room temperature for 8 h, before aqueous 1 M HCl (10 mL) was added to the flask. The layers were separated and the aqueous portion was extracted with

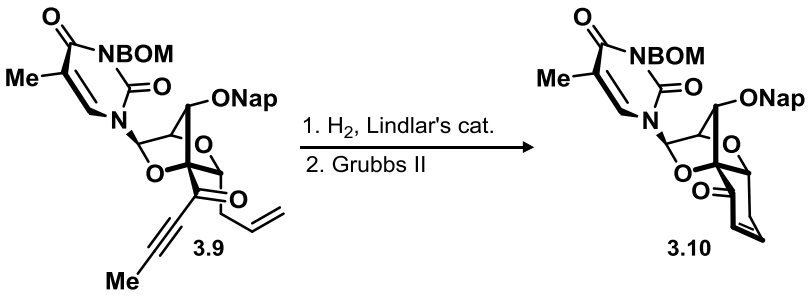
CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (4:6 ethyl acetate/hexanes) to afford amide **3.8** as a colorless oil (322 mg, 65% over two steps), R_f 0.21 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.68 (m, 4H), 7.57 (s, 1H), 7.52 – 7.21 (m, 9H), 6.03 – 5.89 (m, 1H), 5.72 (s, 1H), 5.44 (q, *J* = 9.6 Hz, 2H), 5.25 – 5.08 (m, 2H), 4.93 (dd, *J* = 9.4, 3.8 Hz, 1H), 4.84 – 4.66 (m, 4H), 4.53 (s, 1H), 4.29 (s, 1H), 3.52 (s, 3H), 3.25 (s, 3H), 2.64 – 2.55 (m, 1H), 2.53 – 2.42 (m, 1H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.22, 163.18, 150.51, 138.03, 134.60, 133.97, 133.87, 133.26, 133.17, 128.60, 128.42, 127.93, 127.84, 127.82, 127.76, 127.35, 126.58, 126.50, 125.93, 117.41, 110.05, 88.55, 86.45, 82.05, 81.02, 77.82, 72.82, 72.45, 70.50, 61.86, 34.05, 33.61, 13.24; HRMS (ESI) calculated for $C_{35}H_{37}N_3O_8$ $[M+H]^+$ *m/z* = 628,26534, found 628,26737; calculated for $C_{35}H_{37}N_3NaO_8$ $[M+Na]^+$ *m/z* = 650,24729, found 650,24943.



Compound 3.9

A commercially available 0.5 M solution of 1-propynylmagnesium bromide (1.5 mL, 1.5 mmol) in THF was added dropwise to a stirred, 0 °C solution of Weinreb amide **3.8** (322 mg, 0.513 mmol) in THF (20 mL). The reaction was warmed to room temperature and stirred until near complete consumption of the starting material by TLC analysis (*ca*. 1 h). The reaction mixture

was poured into a 0 °C, aqueous solution of 1 M HCl (100 mL), warmed to room temperature, and subsequently diluted with ethyl acetate (20 mL). The layers were separated and the aqueous portion was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (3:7 ethyl acetate;hexanes) to afford propargyl ketone **3.9** as a colorless oil (271 mg, 88%); R_f 0.29 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 3H), 7.70 (s, 1H), 7.58 – 7.27 (m, 9H), 5.93 – 5.75 (m, 1H), 5.65 (s, 1H), 5.50 – 5.36 (m, 2H), 5.26 – 5.08 (m, 2H), 5.01 – 4.91 (m, 1H), 4.86 – 4.66 (m, 4H), 4.60 (s, 1H), 4.10 (s, 1H), 2.61 – 2.35 (m, 2H), 1.95 (s, 3H), 1.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.13, 162.92, 150.23, 137.87, 133.59, 133.29, 133.09, 133.01, 132.85, 128.47, 128.28, 127.77, 127.70, 127.61, 126.97, 126.42, 126.33, 125.59, 118.25, 109.79, 95.61, 90.16, 88.00, 81.52, 80.94, 78.76, 78.74, 72.86, 72.31, 70.35, 33.51, 13.47, 4.21; HRMS (ESI) calculated for $C_{36}H_{34}N_2O_7$ $[M+H]^+$ *m/z* = 607,24388, found 607,24601; calculated for $C_{36}H_{34}N_2NaO_7$ $[M+Na]^+$ *m/z* = 629,22582, found 629,22829.

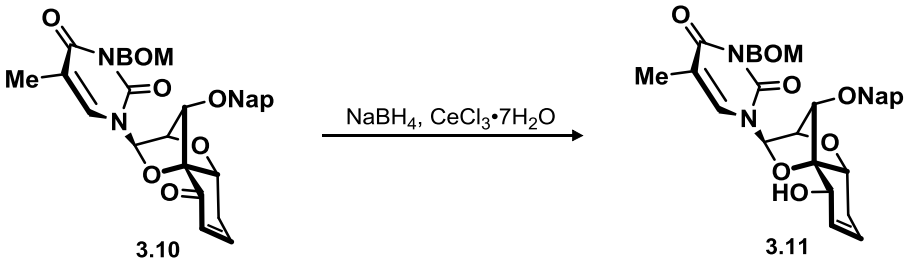


Compound 3.10

1,10-phenanthroline (49 mg, 0.27 mmol) and 5% (w/w) palladium on calcium carbonate poisoned with lead (20 mg, 0.0094 mmol) were added sequentially to a stirred solution of ketone **3.9** (137 mg, 0.225 mmol) in *N,N*-dimethylformamide (2.4 mL). The suspension was purged

with hydrogen gas and maintained under an atmosphere of hydrogen gas with a hydrogen-filled balloon. After 3 d, the reaction mixture was filtered through a pad of Celite®, the filter cake was washed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure. The residue was reconstituted in diethyl ether (10 mL) and partitioned with aqueous water (10 mL). The layers were separated and the aqueous portion was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. Crude reaction mixture was dissolved in *N,N*-dimethylformamide (2.4 mL) and mixed with 1,10-phenanthroline (49 mg, 0.27 mmol) and 5% (w/w) palladium on calcium carbonate poisoned with lead (40 mg, 0.019 mmol). The suspension was purged with hydrogen gas and maintained under an atmosphere of hydrogen gas with a hydrogen-filled balloon. After 1 d, the reaction mixture was filtered through a pad of Celite®, the filter cake was washed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure. The residue was reconstituted in diethyl ether (10 mL) and partitioned with aqueous water (10 mL). The layers were separated and the aqueous portion was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure to afford the crude alkene, which was used directly in the next step without purification. Grubbs' second generation catalyst (7 mg, 0.008 mmol) was added to a stirred solution of the crude alkene (137 mg, theor. 0.225 mmol) in anhydrous toluene (16 mL). The solution was stirred at 70 °C for 6 h before the volatiles were removed under reduced pressure. The residue was purified by flash column chromatography on silica gel (1:1 ethyl acetate/hexanes) to afford ketone **3.10** (45.6 mg, 36% yield over two steps) as a colorless oil; Rf 0.38 (1:1 ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.55 – 7.20 (m, 9H), 7.17 – 7.05 (m, 2H), 6.33 – 6.21 (m, 1H), 5.62 (s, J = 16.0 Hz, 1H), 5.49 – 5.35 (m, 2H), 4.82 (s, 2H), 4.75 – 4.62 (m, 3H), 4.57 (s, 1H),

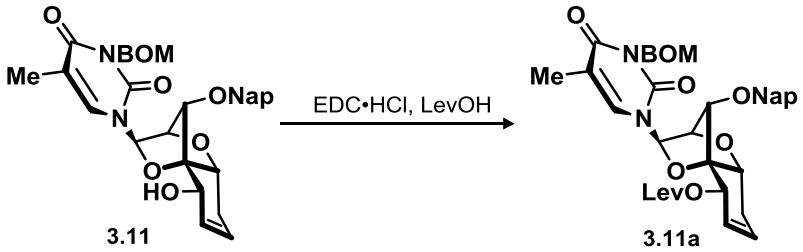
4.28 (s, 1H), 3.03 – 2.87 (m, 1H), 2.81 – 2.66 (m, 1H), 1.85 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.06, 163.12, 150.42, 149.90, 138.05, 134.15, 133.24, 132.37, 130.51, 128.55, 128.41, 128.00, 127.83, 127.75, 126.99, 126.48, 126.38, 125.70, 110.44, 87.82, 83.62, 78.78, 77.25, 75.28, 73.12, 72.48, 70.52, 29.09, 13.45; HRMS (ESI) calculated for $C_{33}H_{30}N_2O_7$ $[M+H]^+$ *m/z* = 567,21258, found 567,21402; calculated for $C_{33}H_{30}N_2NaO_7$ $[M+Na]^+$ *m/z* = 589,19452, found 589,19601.



Compound 3.11

Cerium (III) chloride heptahydrate (26 mg, 0.069 mmol) was added at 0 °C to a stirred solution of enone **3.10** (26 mg, 0.046 mmol) in methanol (1 mL). The resulting solution was stirred for 10 min. before sodium borohydride (5.2 mg, 0.14 mmol) was added in portionwise fashion. Upon complete consumption of the starting material by TLC analysis (*ca*. 30 min.), acetone (2 mL) was added and the mixture was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL), washed with 1 M HCl (5 mL), then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1% MeOH in CH_2Cl_2) to afford alcohol **3.11** as a white foam, (15.6 mg, 60% yield): Rf 0.14 (1% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.71 (m, 4H), 7.52 – 7.27 (m, 9H), 5.86 – 5.79 (m, 1H), 5.73 – 5.64 (m, 2H), 5.46 (d, *J* = 9.6 Hz, 1H), 5.41 (d, *J* = 9.6 Hz, 1H), 4.84 (d, *J* = 11.5 Hz, 1H), 4.76 (d, *J* = 11.5 Hz, 1H), 4.72 – 4.65 (m, 3H), 4.58 (s, 1H), 4.44 – 4.35 (m, 1H), 4.08 (s, 1H), 2.66 – 2.51 (m, 1H), 2.38 – 2.25

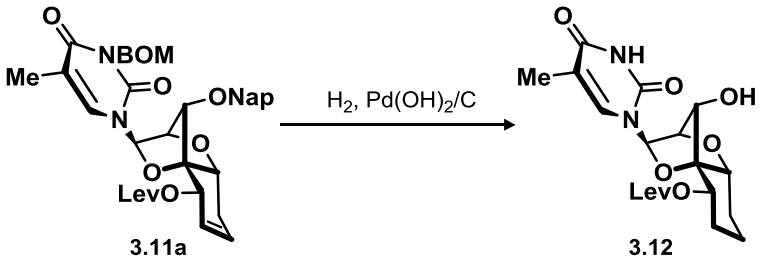
(m, 1H), 1.85 (d, J = 1.0 Hz, 3H), 1.63 (s, J = 20.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.28, 150.58, 138.03, 134.31, 133.25, 133.08, 128.61, 128.46, 127.97, 127.88, 127.84, 127.52, 126.94, 126.57, 126.44, 125.70, 110.06, 86.99, 84.62, 77.58, 76.52, 75.45, 72.85, 72.49, 70.51, 64.29, 27.81, 13.55; HRMS (ESI) calculated for $C_{33}H_{33}N_2O_7$ $[M+H]^+$ m/z = 569,22823, found 569,22853; calculated for $C_{33}H_{32}N_2NaO_7$ $[M+Na]^+$ m/z = 591,21017, found 591,21017.



Compound 3.11a

Levulinic acid (4.0 μL, 0.038 mmol), *N*-(3-dimethyl-aminopropyl)-*N*'-ethylcarbodiimide hydrochloride (5.5 mg, 0.028 mmol), 4-(dimethylamino)pyridine (1.2 mg, 0.009 mmol), and *N*,*N*-diisopropylethylamine (10.0 μL, 0.057 mmol) were added sequentially to a stirred solution of alcohol **3.11** (10.8 mg, 0.019 mmol) in CH_2Cl_2 (1.0 mL). After 1 h, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with 1 M HCl (2 mL × 2), a saturated aqueous solution of $NaHCO_3$ (2 mL), then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1% MeOH in CH_2Cl_2) to afford ester **3.11a** as a white foam (10.9 mg, 86% yield); Rf 0.23 (1% MeOH in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 4H), 7.59 – 7.27 (m, 9H), 6.03 – 5.96 (m, 1H), 5.95 – 5.87 (m, 1H), 5.67 – 5.57 (m, 2H), 5.44 (d, J = 9.6 Hz, 1H), 5.35 (d, J = 9.6 Hz, 1H), 4.79 – 4.65 (m, 4H), 4.54 – 4.40 (m, 2H), 4.09 (s, 1H), 3.03 – 2.86 (m, 1H), 2.82 – 2.51 (m, 4H), 2.48 – 2.29 (m, 1H), 2.16 (s, 3H), 1.80 (d, J = 1.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.51, 172.02, 163.40, 150.56, 138.02, 134.53, 133.57, 133.25, 133.21, 128.85, 128.46, 128.02,

127.88, 127.83, 127.09, 126.41, 126.31, 125.96, 124.73, 109.52, 86.90, 83.50, 77.85, 76.42, 75.51, 73.04, 72.44, 70.44, 65.48, 38.19, 29.78, 28.19, 28.06, 13.45; HRMS (ESI) calculated for $C_{38}H_{39}N_2O_9$ $[M+H]^+$ *m/z* = 667,26501, found 667,226; calculated for $C_{38}H_{38}N_2NaO_9$ $[M+Na]^+$ *m/z* = 689,24695, found 689,24821.

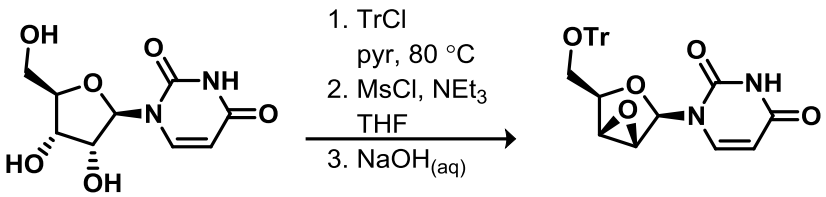


Compound 3.12

20% (w/w) palladium hydroxide on carbon (2.3 mg, 4.3 μmol) was added to a stirred solution of ester **3.11a** (10.9 mg, 0.016 mmol) in 2:2:1 methanol–ethanol–ethyl acetate (0.4 mL). The suspension was purged with hydrogen gas and maintained under an atmosphere of hydrogen gas with a hydrogen filled balloon. After 24 h, 20% (w/w) palladium hydroxide on carbon (2.3 mg, 4.3 μmol) was added was added and the mixture stirred for an additional 24 h. The reaction mixture was filtered through a pad of Celite®, the filter cake washed with methanol, and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1:22 methanol:CH_2Cl_2) to afford alcohol **3.12** as a colorless solid (2.7 mg, 45% yield); Rf 0.11 (3% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.58 (s, 1H), 5.57 (s, 1H), 4.91 (dd, *J* = 11.5, 5.6 Hz, 1H), 4.44 (s, 1H), 4.14 – 4.11 (m, 1H), 4.09 (s, 1H), 3.04 – 2.89 (m, 2H), 2.75 – 2.62 (m, 2H), 2.58 – 2.48 (m, 1H), 2.18 (s, 3H), 2.02 (s, 1H), 1.98 – 1.83 (m, 6H), 1.76 – 1.61 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.85, 174.12, 163.73, 149.81, 135.12, 110.02, 86.28, 85.90, 79.86, 78.69, 69.97, 68.54, 37.97, 29.69, 28.16, 26.02, 24.86, 20.24, 12.87; HRMS (ESI) calculated for $C_{19}H_{25}N_2O_8$ $[M+H]^+$ *m/z* =

409,16054, found 409,15919; calculated for $C_{19}H_{24}N_2NaO_8$ $[M+Na]^+$ *m/z* = 431,14249, found 431,14283.

8.2.2 Synthesis of a N-bicyclic nucleoside

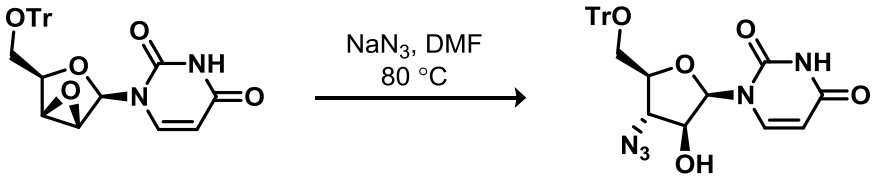


4.1 **4.3**

Compound 4.3

Trityl chloride (8.77 g, 31.4 mmol) was added to a stirred solution of uridine **4.1** (8.00 g, 32.7 mmol) in pyridine (16 mL). The reaction mixture was heated to 80 °C for 2 h, cooled to room temperature then diluted with 1 M HCl (50 mL) and CH_2Cl_2 (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in dry THF (48 mL), mixed at 0 °C with triethylamine (15.0 mL, 107 mmol), and treated dropwise with methanesulfonyl chloride (6.0 mL, 77 mmol). After stirring for 1.5 h at room temperature, the reaction mixture was diluted with 1 M HCl (30 mL) and water (20 mL). The mixture was extracted with CH_2Cl_2 (4 × 40 mL). The combined organic extracts were mixed with 4 M NaOH (40 mL, 0.16 mol) and heated at 40 °C for 1.5 h. The reaction mixture was neutralized via addition of 1 M HCl, and the resulting solution was extracted with CH_2Cl_2 (4 × 40 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (18 x 5.5 cm, 3:2 EtOAc/hexanes) to give nucleoside **4.3** as a yellow foam (11.3 g, 74% over 3 steps) Rf = 0.23

(3:2 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.53 – 7.45 (m, 5H), 7.37 – 7.23 (m, 10H), 6.23 (s, 1H), 5.69 (dd, *J* = 8.2, 0.8 Hz, 1H), 4.20 (t, *J* = 5.7 Hz, 1H), 3.95 (d, *J* = 3.0 Hz, 1H), 3.87 (d, *J* = 3.2 Hz, 1H), 3.50 (dd, *J* = 9.7, 6.1 Hz, 1H), 3.38 (dd, *J* = 9.7, 5.5 Hz, 1H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.47, 150.73, 143.56, 141.45, 128.72, 128.06, 127.37, 102.60, 87.24, 81.85, 62.33, 56.31, 56.12; HRMS (ESI) calculated for $C_{28}H_{25}N_2O_5$ $[M+H]^+$ *m/z* = 469.1758, found 469.17649; calculated for $C_{28}H_{24}N_2NaO_5$ $[M+Na]^+$ *m/z* = 491.15774, found 491.15846.

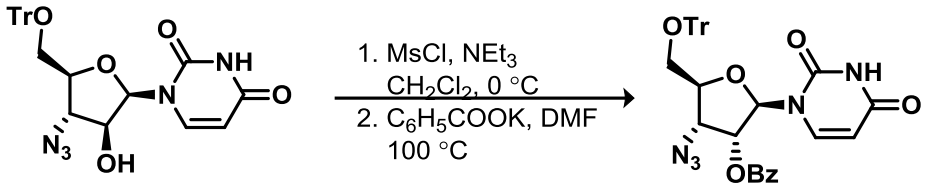


4.3 **4.4**

Compound 4.4

Sodium azide (530 mg, 8.15 mmol) was added to a stirred solution of nucleoside **4.3** (964 mg, 2.05 mmol) in anhydrous DMF (20 mL). The reaction was carried in a sealed tube and heated at 80 °C for 24 h, cooled, and treated with EtOAc (20 mL) and water (20 mL). The layers were separated. The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (20 x 2.5 cm, 3% MeOH in CH_2Cl_2) to give nucleoside **4.4** as a white foam (690 mg, 66 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.52 – 7.21 (m, 15H), 6.14 (d, *J* = 5.5 Hz, 1H), 5.40 (d, *J* = 8.1 Hz, 1H), 5.15 (s, 1H), 4.61 (t, *J* = 5.7 Hz, 1H), 4.25 (t, *J* = 7.4 Hz, 1H), 3.86 – 3.75 (m, 1H), 3.58 (dd, *J* = 11.1, 2.7 Hz, 1H), 3.46 (dd, *J* = 11.1, 3.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.78, 151.33,

143.27, 141.97, 128.81, 128.24, 127.61, 101.72, 87.86, 85.15, 79.73, 75.84, 63.76, 61.50.; HRMS (ESI) calculated for $C_{28}H_{26}N_5O_5$ $[M+H]^+$ *m/z* = 512.19285, found 512.19239; calculated for $C_{28}H_{25}N_5NaO_5$ $[M+Na]^+$ *m/z* = 534.17479, found 534.17504; FT-IR (azide) = 2117.0 cm^{-1}.

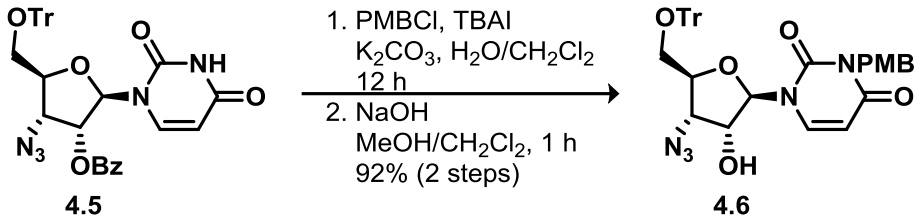


4.4 **4.5**

Compound 4.5

Methanesulfonyl chloride (0.94 mL, 12 mmol) was added to a stirred solution of nucleoside **4.4** (4.14 g, 8.09 mmol) and triethylamine (1.7 mL, 12 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After 2 h, water (20 mL) and 1 M HCl (20 mL) were added, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in dry DMF (50 mL) and mixed with potassium benzoate (3.10 g, 19.3 mmol) in a sealed tube. After heating for 3 days at 100 °C, the reaction mixture was diluted with water (250 mL), and the mixture was extracted with EtOAc (4 × 50 mL). The organic combined phase was washed with 1 M HCl (2 × 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (17 x 3.5 cm, 3:7 EtOAc/hexanes) to give nucleoside **4.5** as a white foam (3.28 g, 66% over 2 steps); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.52 – 7.26 (m, 20H), 6.21 (d, *J* = 4.6 Hz, 1H), 5.78 (dd, *J* = 5.8, 4.6 Hz, 1H), 5.44 (dd, *J* = 8.2, 2.2 Hz, 1H), 4.52 (t, *J* = 5.7 Hz, 1H), 4.21 – 4.14 (m, 1H), 3.63 (dd, *J* = 11.1, 2.6 Hz, 1H), 3.49 (dd, *J* = 11.2, 2.8 Hz, 1H); ^{13}C NMR (75

MHz, $CDCl_3$) δ 165.56, 162.69, 150.04, 143.03, 140.03, 134.09, 130.29, 128.81, 128.72, 128.47, 128.34, 127.78, 103.06, 88.15, 87.56, 81.94, 75.72, 64.94, 62.58, 61.95, 60.53; HRMS (ESI) calculated for $C_{35}H_{30}N_5O_6$ $[M+H]^+$ *m/z* = 616.21906, found 616.21837; calculated for $C_{35}H_{29}N_5NaO_6$ $[M+Na]^+$ *m/z* = 638.201, found 638.20107.

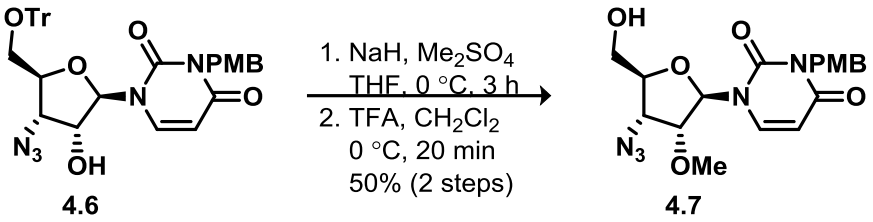


4.5 **4.6**

Compound 4.6

4-methoxybenzyl chloride (40 μL, 0.29 mmol), tetrabutylammonium iodide (15 mg, 0.04 mmol), and a solution of potassium carbonate (140 mg, 1.01 mmol) in water (1 mL), were added to a stirred solution of nucleoside **4.5** (125 mg, 0.203 mmol) in CH_2Cl_2 (1 mL). The biphasic reaction mixture was stirred vigorously for 12 h, the organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (4 × 1 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The oily residue was dissolved in methanol/ CH_2Cl_2 (6 mL, 9:1) and mixed sodium hydroxide (25 mg, 0.62 mmol). After 1 h, the solvent was removed under reduced pressure. The residue was purified by flash chromatography (15 x 1.5 cm, 3:7 EtOAc/hexanes) to give nucleoside **4.6** as a white foam (118 mg, 92% over 2 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.1 Hz, 1H), 7.47 – 7.26 (m, 17H), 6.82 (d, *J* = 8.7 Hz, 2H), 5.80 (d, *J* = 2.3 Hz, 1H), 5.49 (d, *J* = 8.1 Hz, 1H), 5.03 (q, *J* = 13.6 Hz, 2H), 4.45 (t, *J* = 5.6 Hz, 1H), 4.25 (dt, *J* = 7.4, 2.3 Hz, 1H), 4.16 – 4.09 (m, 1H), 3.98 (d, *J* = 3.9 Hz, 1H), 3.76 (s, *J* = 5.4 Hz, 3H), 3.62 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.43 (dd, *J* = 11.4,

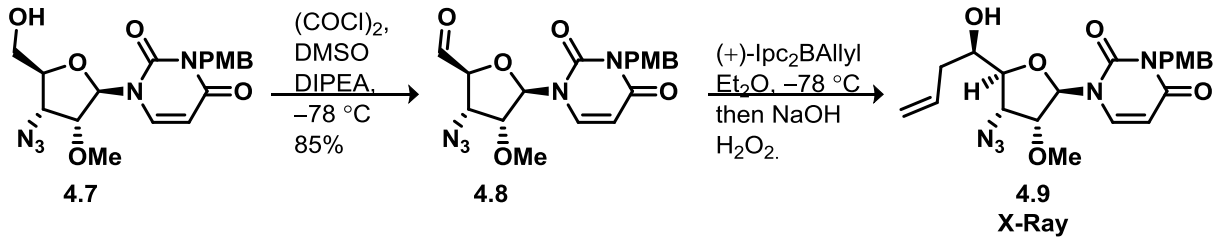
2.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.56, 159.26, 151.39, 143.05, 137.62, 130.90, 128.82, 128.72, 128.66, 128.23, 127.64, 113.83, 102.03, 91.68, 87.84, 81.31, 76.60, 61.60, 60.27, 55.32, 43.69; HRMS (ESI) calculated for $C_{36}H_{34}N_5O_6$ $[M+H]^+$ *m/z* = 632.25036, found 632.25229; calculated for $C_{36}H_{33}N_5NaO_6$ $[M+Na]^+$ *m/z* = 654.2323, found 654.2346.



Compound 4.7

A 60 % dispersion of sodium hydride (210 mg, 5.25 mmol) was added to a stirred solution of nucleoside **4.6** (2.2 g, 3.5 mmol) in THF (40 mL) at 0 °C. After 20 min, dimethyl sulphate (0.33 mL, 2.6 mmol) was added dropwise to the reaction mixture and stirred for 2 h, then methanol (10 mL) and water (50 mL) were added. The mixture was extracted with CH_2Cl_2 (4 × 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL) and cooled to 0 °C, then trifluoroacetic acid (2.7 mL, 35 mmol) was added in three equal portions with 5 minutes between each one. The reaction mixture was neutralized via addition of a saturated aqueous solution of $NaHCO_3$, then the organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (4 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 EtOAc/hexanes) to give nucleoside **4.7** as a white foam (675 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.1 Hz, 1H), 7.44 – 7.39 (m, 2H), 6.85 – 6.79 (m, 2H), 5.76 (d, *J* = 2.6 Hz, 1H), 5.72 (d, *J* = 8.1

Hz, 1H), 5.09 – 4.95 (m, 2H), 4.19 – 4.14 (m, 1H), 4.10 (dd, *J* = 5.2, 2.7 Hz, 1H), 4.07 – 4.01 (m, 1H), 3.99 (dd, *J* = 7.5, 5.2 Hz, 1H), 3.83 – 3.77 (m, 1H), 3.76 (s, 3H), 3.59 (s, 3H), 3.48 – 3.43 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.03, 159.22, 150.80, 139.08, 130.71, 128.71, 113.82, 101.73, 90.36, 84.00, 82.25, 60.51, 58.99, 57.97, 55.31, 43.68.

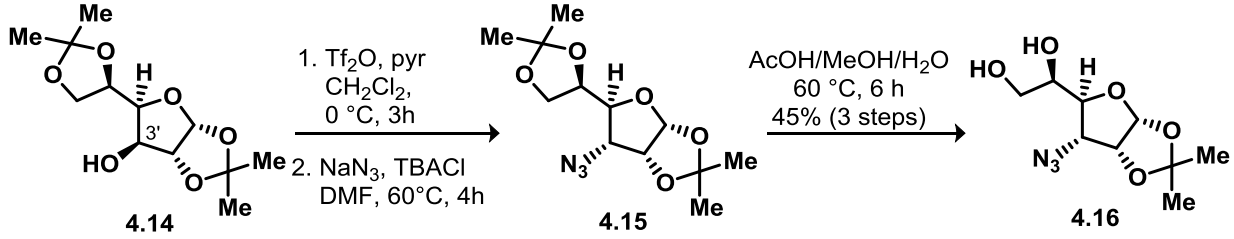


4.7 **4.8** **4.9**
X-Ray

Compound 4.9

A solution of DMSO (0.43 mL, 6.06 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a stirred solution of oxalyl chloride (0.26 mL, 3.03 mmol) in CH_2Cl_2 (7 mL) at -78 °C. After 20 min, a solution of nucleoside **4.7** (800 mg, 1.98 mmol) in CH_2Cl_2 (7 mL) was added dropwise and stirred at the same temperature for 1 h. DIPEA (1.75 mL, 10.04 mmol) was added to the reaction mixture, then the cooling bath was removed and stirred for 1 h at r.t. 1 M HCl (20 mL) was added to the reaction mixture, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude aldehyde **4.8** (680 mg, 85%). The crude aldehyde was pure enough to use in the next reaction without further purification (assessed by ^{1}H NMR). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 7.44 – 7.38 (m, 2H), 6.95 (d, *J* = 8.1 Hz, 1H), 6.84 – 6.80 (m, 2H), 6.26 (dd, *J* = 9.9, 3.0 Hz, 2H), 5.79 (d, *J* = 8.0 Hz, 1H), 5.10 – 4.93 (m, 3H), 4.85 (t, *J* = 3.0 Hz, 1H), 3.79 – 3.75 (m, 3H), 3.46 (s, 3H). The crude aldehyde **4.8** (680 mg, 1.69 mmol) was dissolved in diethyl ether (38 mL) and CH_2Cl_2

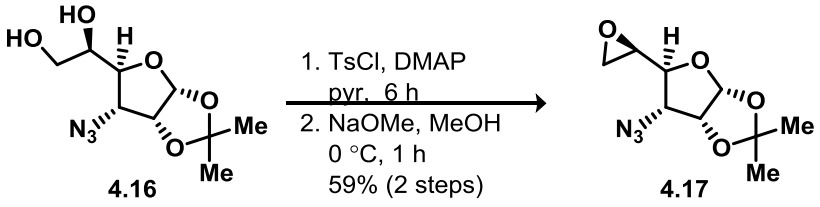
(5 mL) to fully solubilize the sample. The resulting solution was cooled down to -78 °C and a 1 M solution of (+)-Ipc_2BAllyl borane in pentane (2.6 mL, 2.6 mmol) was added. After stirring overnight at the same temperature, 1 M solution of NaOH (4 mL) and 30% H_2O_2 (1.6 mL) were added carefully to the reaction mixture and the resulting mixture was heated to reflux for 1 h. The reaction mixture was cooled down and extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (6:4 EtOAc/hexanes) to give nucleoside **4.9** as a white foam (657 mg, 88%); ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 8.1 Hz, 1H), 7.48 – 7.40 (m, 2H), 6.87 – 6.78 (m, 2H), 5.90 – 5.73 (m, 2H), 5.62 (d, *J* = 4.3 Hz, 1H), 5.29 – 5.16 (m, 2H), 5.12 – 4.95 (m, 2H), 4.28 (dd, *J* = 5.3, 4.3 Hz, 1H), 4.18 – 3.97 (m, 3H), 3.78 (s, 3H), 3.55 (s, 3H), 2.45 – 2.25 (m, 2H).



Compound 4.16

Pyridine (38 mL, 0.473 mol) was added to a stirred solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **4.14** (50.0 g, 0.192 mol) in CH_2Cl_2 (800 mL) at 0 °C. Trifluoromethanesulfonic anhydride (33 mL, 0.196 mol) was added to the reaction mixture over a period of 30 min keeping the temperature under 5°C. The coldbath was removed and the reaction mixture was stirred for 3 h. The organic phase was washed with 1 M HCl (2 × 100 mL), dried over Na_2SO_4, evaporated under reduced pressure, and coevaporated with toluene (2 × 50 mL) to furnish the corresponding

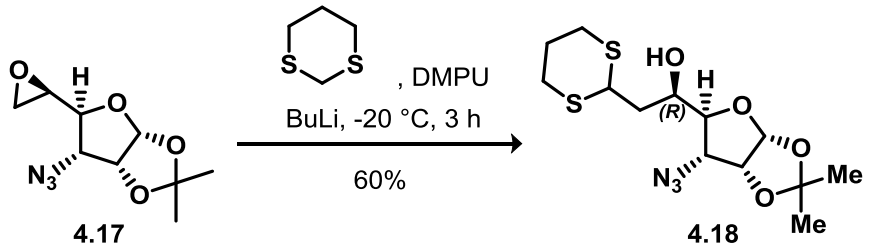
triflate. The residue was dissolved in anhydrous DMF (250 mL), mixed subsequently with tetrabutyl ammonium chloride (270 mg, 0.97 mmol) and sodium azide (25 g, 0.38 mol), heated at 60 °C for 4 h and then cooled. The organic phase was partitioned with the addition of water (750 mL) and extracted with diethyl ether (4 × 100 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give azide **4.15**. A mixture of methanol (210 mL), water (260 mL), acetic acid (160 mL) was added to the crude azide **4.15** and warmed to 60 °C for 6 h, then cooled. The mixture was neutralized via addition of solid sodium bicarbonate followed by extraction with ethyl acetate (3 × 200 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (7:3 ethyl acetate/hexanes) to give sugar **4.16** as an oil (18.6 g, 40% over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (d, J = 3.7 Hz, 1H), 4.72 – 4.67 (m, 1H), 4.00 (dd, J = 9.3, 4.1 Hz, 1H), 3.92 (dd, J = 7.2, 3.7 Hz, 1H), 3.65 (dd, J = 10.3, 5.4 Hz, 2H), 3.56 (dd, J = 9.3, 4.8 Hz, 1H), 1.51 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 113.22, 104.04, 80.57, 77.84, 71.57, 62.96, 60.26, 26.41, 26.38; HRMS (ESI) calculated for $C_9H_{15}N_3NaO_5$ $[M+Na]^+$ *m/z* = 268.09039, found = 268.08939; FT-IR (azide) = 2109.0 cm^{-1}.



Compound 4.17

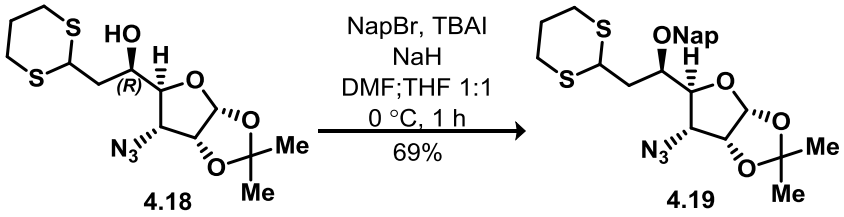
4-(dimethylamino)pyridine (930 mg, 7.58 mmol), and p-toluensulfonyl chloride (17.5 g, 90.3 mmol) were added sequentially to an stirred solution of diol **4.16** (18.6 g, 75.8 mmol) in pyridine

(200 mL). After 6 h the reaction was quenched with 1 M HCl (300 mL) and the resulting solution was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude tosylate was dissolved in methanol (300 mL) and cooled down to 0 °C. A 0.5 M solution of sodium methoxide (150 mmol, 300 mL) was added to the previous solution and let to warm up to room temperature for 1 h. The reaction was carefully quenched with water and the methanol was removed under reduced pressure. The remaining aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (15:85 ethyl acetate/hexanes) to give epoxide **4.17** as an oil (8.9 g, 52% over 2 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (d, *J* = 3.7 Hz, 1H), 4.71 (t, *J* = 4.2 Hz, 1H), 4.14 (dd, *J* = 9.5, 3.6 Hz, 1H), 3.29 (dd, *J* = 9.5, 4.7 Hz, 1H), 3.21 (dd, *J* = 6.7, 3.6 Hz, 1H), 2.84 (t, *J* = 4.5 Hz, 1H), 2.79 – 2.75 (m, 1H), 1.54 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 113.41, 104.33, 80.44, 77.61, 60.64, 50.47, 44.74, 26.59, 26.56; HRMS (ESI) calculated for $C_9H_{13}N_3NaO_4$ $[M+Na]^+$ *m/z* = 250.07983, found = 250.08086, calculated for $C_9H_{17}N_4O_4$ $[M+NH_4]^+$ *m/z* = 245.12443, found = 245.12549; FT-IR (azide) = 2105.5 cm^{-1}.



Compound 4.18

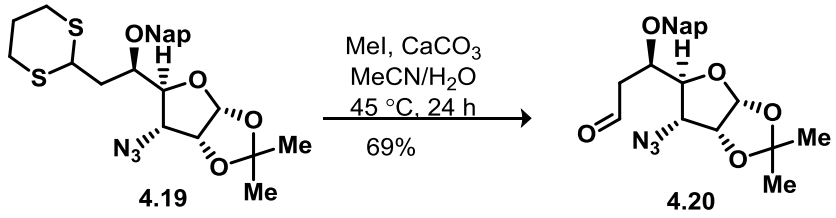
To a cooled (−20 °C) solution of 1,3-dithiane (6.25 g, 50.9 mmol) in THF (200 mL), was added dropwise a 1.6 M solution of *n*-butyllithium (31.8 mL, 50.9 mmol), then stirred at the same temperature for 0.5 h. A previously prepared solution of epoxide **4.17** (8.9 g, 39.2 mmol) in THF (200 mL) was added dropwise keeping the temperature at - 20 °C. The reaction mixture was allowed to warm to 0 °C and then DMPU (24.0 mL, 198 mmol) was added slowly. After stirring for 3 h at room temperature, the reaction mixture was quenched with NH_4Cl saturated and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 ethyl acetate/hexanes) to give alcohol **4.18** as an oil (6.8 g, 50%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (d, *J* = 3.6 Hz, 1H), 4.78 – 4.71 (m, 1H), 4.27 (dd, *J* = 9.4, 4.9 Hz, 2H), 4.01 (dd, *J* = 9.3, 3.4 Hz, 1H), 3.57 (dd, *J* = 9.3, 4.8 Hz, 1H), 2.96 – 2.82 (m, 4H), 2.43 (s, 1H), 2.18 – 1.83 (m, 4H), 1.58 (s, 3H), 1.36 (s, *J* = 11.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 113.39, 104.11, 80.98, 80.12, 67.46, 59.50, 43.75, 37.65, 30.17, 29.79, 26.63, 26.61, 25.99; HRMS (ESI) calculated for $C_{13}H_{22}N_3O_4S_2$ $[M+H]^+$ *m/z* = 348.10462, found = 348.10428; FT-IR (azide) = 2102.0 cm^{-1}.



Compound 4.19

Sodium hydride (1.17 g, 29.4 mmol) was added to a stirred solution of alcohol **4.18** (6.8 g, 19.6 mmol) in DMF (100 mL) at -20 °C. After stirring at the same temperature for 20 min, tetrabutylamonium iodide (730 mg, 1.96 mmol) and 2-(bromomethyl)-naphthalene (6.62 g, 29.4

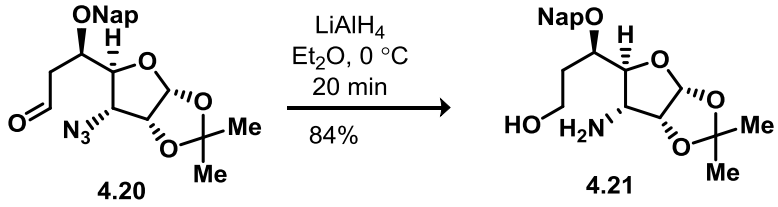
mmol) were added sequentially. The reaction mixture was warmed to 0°C and stirred for 1 h. The reaction was carefully quenched with water (400 mL) and the aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (1:9 ethyl acetate/hexanes) to give azide **4.19** as an oil (7.7 g, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.78 (m, 4H), 7.53 – 7.44 (m, 3H), 5.76 (d, *J* = 3.7 Hz, 1H), 4.93 (d, *J* = 11.8 Hz, 1H), 4.83 (d, *J* = 11.8 Hz, 1H), 4.72 – 4.67 (m, 1H), 4.28 – 4.14 (m, 2H), 4.07 (dd, *J* = 10.2, 4.2 Hz, 1H), 3.62 (dd, *J* = 9.3, 4.8 Hz, 1H), 2.86 – 2.62 (m, 3H), 2.55 – 2.44 (m, 1H), 2.23 – 2.11 (m, 1H), 2.07 – 1.88 (m, 2H), 1.88 – 1.72 (m, 1H), 1.59 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.96, 133.24, 132.92, 128.07, 127.91, 127.63, 126.71, 126.13, 126.07, 125.93, 113.06, 103.82, 80.66, 80.42, 74.52, 74.44, 59.63, 43.66, 37.04, 30.18, 29.53, 26.51, 26.47, 25.82; HRMS (ESI) calculated for $C_{24}H_{30}N_3O_4S_2$ $[M+H]^+$ *m/z* = 488.16722, found = 488.165960; FT-IR (azide) = 2105.0 cm^{-1}



Compound 4.20

Iodomethane (10.0 mL, 157.9 mmol) and calcium carbonate (7.9 g, 78.9 mmol) were added sequentially to a stirred solution of azide **4.19** (7.7g, 15.8 mmol) in 10:1 acetonitrile/water (350 mL). The resulting mixture was warmed to 45 °C for 24 h. The reaction mixture was partitioned with the addition of water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure.

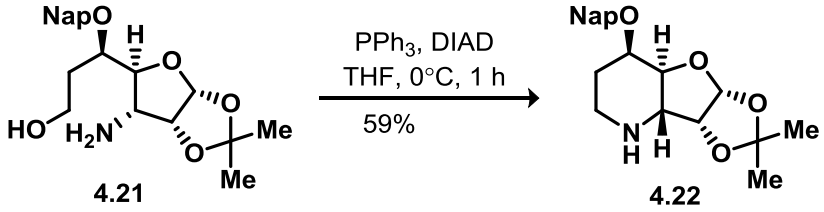
The residue was purified by flash chromatography (2:8 ethyl acetate/hexanes) to give aldehyde **4.20** as an oil (3.8 g, 61%) R_f = 0.36 (3:7 ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ δ 9.76 (s, 1H), 7.88 – 7.79 (m, 3H), 7.76 (s, 1H), 7.54 – 7.39 (m, 3H), 5.78 (d, *J* = 3.6 Hz, 1H), 4.91 – 4.80 (m, 2H), 4.78 – 4.70 (m, 1H), 4.39 (dt, *J* = 7.8, 3.8 Hz, 1H), 4.19 (dd, *J* = 9.4, 3.2 Hz, 1H), 3.58 (dd, *J* = 9.4, 4.7 Hz, 1H), 2.89 (ddd, *J* = 17.4, 8.3, 1.9 Hz, 1H), 2.75 – 2.64 (m, 1H), 1.58 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.76, 135.40, 133.33, 133.17, 128.40, 128.06, 127.83, 126.89, 126.33, 126.17, 126.00, 113.46, 104.00, 80.79, 79.81, 74.09, 73.03, 60.51, 45.53, 26.65, 26.61; HRMS (ESI) calculated for $C_{21}H_{23}N_3O_5Na$ $[M+Na]^+$ *m/z* = 420.15299, found = 420.15313; FT-IR (azide) = 2107.4 cm^{-1}



Compound 4.21

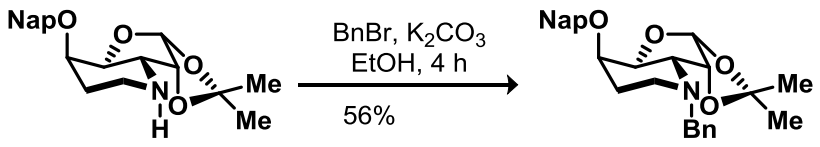
Lithium aluminium hydride (1.45 g, 38.3 mmol) was added portionwise to a stirred solution of aldehyde **4.20** (3.8 g, 9.6 mmol) in diethyl ether (160 mL) at 0 °C. After stirring for 20 min at the same temperature, the reaction mixture was carefully quenched with water and subsequently treated with 1 M NaOH (100 mL). The formed solid was filtered through a Celite® pad, the organig layer was separated and the remaining aqueous layer was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2 neat to 6 % methanol in CH_2Cl_2) to give alcohol **4.21** as an oil (3.5 g, 98%) R_f = 0.34 (6 % methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.75 (m, 8H), 7.52 – 7.42 (m, 6H), 5.77 (d, *J* =

3.7 Hz, 2H), 4.81 (s, 4H), 4.50 (dd, J = 4.9, 3.8 Hz, 2H), 3.95 – 3.87 (m, 4H), 3.84 – 3.67 (m, 4H), 3.37 – 3.29 (m, 2H), 2.23 – 1.86 (m, 11H), 1.53 (s, 6H), 1.35 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.75, 133.38, 133.14, 128.46, 128.05, 127.84, 126.95, 126.33, 126.14, 126.07, 112.23, 104.08, 83.22, 81.32, 78.24, 73.11, 59.51, 55.71, 34.02, 26.95, 26.68; HRMS (ESI) calculated for $C_{21}H_{28}N_1O_5$ $[M+H]^+$ m/z = 374.1962, found = 374.1967;



Compound 4.22

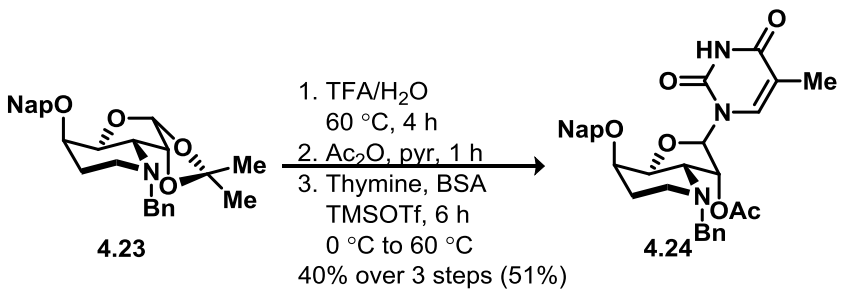
To a solution of triphenylphosphine (4.92g, 18.7 mmol) and alcohol **4.21** (3.5 g, 9.4 mmol) in CH_2Cl_2 (500 mL), was added slowly diisopropyl azodicarboxylate (3.7 mL, 18.7 mmol) at 0 °C. The cold bath was removed and the reaction mixture was stirred for 1 h. The reaction mixture was pre-adsorbed in silica and purified by flash chromatography (20 % acetone in CH_2Cl_2) to give amine **4.22** as an oil (2.06 g, 62%) R_f = 0.51 (6 % methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.54 – 7.39 (m, 3H), 5.84 (d, J = 3.4 Hz, 1H), 4.91 (d, J = 12.5 Hz, 1H), 4.77 (d, J = 12.5 Hz, 1H), 4.60 (t, J = 3.6 Hz, 1H), 4.27 (s, 1H), 3.70 (dd, J = 10.1, 2.0 Hz, 1H), 3.17 (dd, J = 10.1, 4.2 Hz, 1H), 3.04 (td, J = 13.1, 2.6 Hz, 1H), 2.91 (dd, J = 13.3, 4.3 Hz, 1H), 1.89 (d, J = 14.2 Hz, 1H), 1.54 (s, J = 9.3 Hz, 3H), 1.50 – 1.41 (m, 1H), 1.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.32, 133.27, 132.91, 128.08, 127.88, 127.69, 126.08, 126.05, 125.79, 125.61, 112.60, 104.62, 78.71, 78.07, 72.22, 57.34, 41.57, 30.50, 26.28, 26.06; HRMS (ESI) calculated for $C_{21}H_{26}NO_4$ $[M+H]^+$ m/z = 356.185635, found = 356.18594.



4.22 **4.23**

Compound 4.23

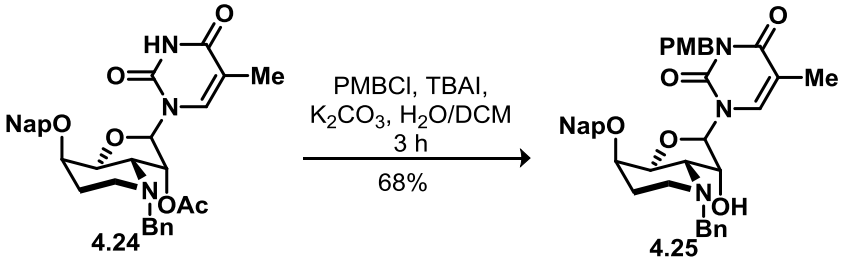
Benzyl bromide (0.76 mL, 6.4 mmol) and K_2CO_3 (0.88 g, 6.4 mmol) were added sequentially to a stirred solution of amine **4.22** (2.06 g, 5.80 mmol) in ethanol (23 mL) at room temperature. After stirring for 4 h, the solvent was removed under reduced pressure, and the residue dissolved in water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 Ethyl acetate/hexane) to give amine **4.23** as an oil (1.45 g, 56%) R_f = 0.24 (2:8 Ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.77 (m, 4H), 7.54 – 7.44 (m, 3H), 7.42 – 7.27 (m, 5H), 5.90 (d, *J* = 3.5 Hz, 1H), 4.94 (d, *J* = 12.6 Hz, 1H), 4.85 – 4.73 (m, 2H), 4.20 – 4.13 (m, 2H), 4.08 (dd, *J* = 9.9, 2.4 Hz, 1H), 3.39 (d, *J* = 12.7 Hz, 1H), 2.76 – 2.63 (m, 2H), 2.41 – 2.28 (m, 1H), 1.87 (dd, *J* = 14.5, 2.5 Hz, 1H), 1.71 – 1.57 (m, 4H), 1.40 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.47, 136.52, 133.28, 132.89, 129.78, 128.19, 128.02, 127.89, 127.68, 127.20, 126.01, 125.72, 125.63, 112.49, 104.73, 78.88, 77.54, 71.97, 71.38, 63.86, 59.59, 47.42, 29.01, 26.53, 26.03; HRMS (ESI) calculated for $C_{28}H_{31}NO_4$ $[M+H]^+$ *m/z* = 446.23258, found = 446,23335.



Compound 4.24

Amine **4.23** (1.45 g, 3.32 mmol) was dissolved in a 3:2 mixture of trifluoroacetic acid/water (20 mL) and heated to 60 °C for 4 h, then the solvent was removed under reduced pressure and co-evaporated with toluene. The crude residue was dissolved in pyridine (20 mL) followed by slow addition of acetic anhydride (3 mL). The resulting solution was stirred for 1 h, the solvent was removed under reduced pressure, co-evaporated with toluene and the crude diacetate was dissolved in anhydrous 1,2-dichloroethane (5 mL). N,*O*-bis(trimethylsilyl)acetamide (8.2 mL, 33.3 mmol) was added to an stirred solution of thymine (1.26 g, 9.99 mmol) in 1,2-dichloroethane (17 mL) and the resulting suspension was heated at 80 °C. After 1 h, the reaction mixture was cooled down to 0 °C and the previously prepared solution of the diacetate was transferred via cannula followed by addition of trimethylsilyl trifluoromethanesulfonate (1.81 mL, 9.99 mmol). The resulting solution was heated to 60 °C for 2 h, then quenched via addition of saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Ethyl acetate/hexane) to give nucleoside **4.24** as an oil (917 mg, 51%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H), 7.90 – 7.76 (m, 4H), 7.62 (d, *J* = 1.1 Hz, 1H), 7.53 – 7.43 (m, 3H), 7.35 – 7.20 (m, 7H), 5.95 (s, 1H), 5.59 (d, *J* = 4.3 Hz, 1H), 4.86 (d, *J* = 11.6 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.31 (s, 1H), 4.15 (dd, *J* = 10.5, 2.5 Hz, 1H), 4.06 (d, *J* = 13.0 Hz, 1H), 3.15 (d, *J* = 12.9

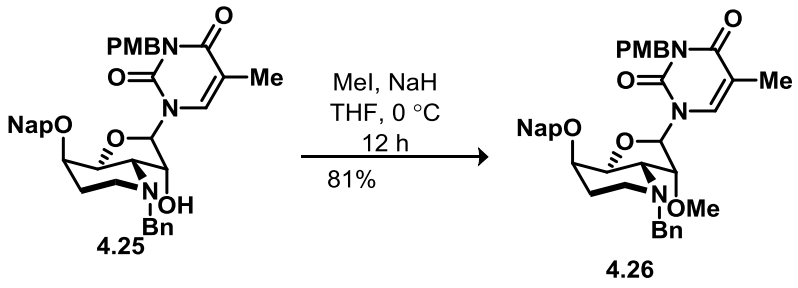
Hz, 1H), 2.88 (dd, J = 10.5, 4.3 Hz, 1H), 2.76 – 2.68 (m, 1H), 2.36 – 2.26 (m, 1H), 2.20 (s, 3H), 2.07 – 1.97 (m, 1H), 1.67 – 1.51 (m, 1H), 1.23 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.28, 164.03, 150.03, 137.81, 135.43, 135.27, 133.29, 133.11, 128.89, 128.53, 128.34, 127.80, 127.75, 127.26, 126.56, 126.42, 126.20, 125.63, 110.63, 89.57, 81.14, 74.26, 72.21, 71.49, 60.76, 60.15, 47.69, 27.32, 20.93, 11.80; HRMS (ESI) calculated for $C_{32}H_{34}N_3O_6$ $[M+H]^+$ m/z = 556.244212, found = 556.24490.



Compound **4.25**

Tetrabutylammonium iodide (49 mg, 0.13 mmol) and *p*-methoxybenzyl chloride (0.12 mL, 0.86 mmol) were added to a stirred solution of nucleoside **4.24** (367 mg, 0.66 mmol) in CH_2Cl_2 (3.3 mL). To the previous solution was added K_2CO_3 (460 mg, 3.3 mmol) in water (3.3 mL), and the resulting biphasic mixture was stirred vigorously for 3 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Ethyl acetate/hexane) to give nucleoside **4.25** as an oil (270 mg, 60%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.77 (m, 4H), 7.67 (d, J = 1.0 Hz, 1H), 7.55 – 7.42 (m, 5H), 7.37 – 7.27 (m, 5H), 6.85 – 6.77 (m, 2H), 5.91 (s, 1H), 5.03 (q, J = 13.5 Hz, 2H), 4.87 (d, J = 11.7 Hz, 1H), 4.73 (d, J = 11.7 Hz, 1H), 4.34 – 4.26 (m, 2H), 4.20 (dd, J = 10.4, 2.4 Hz, 1H), 4.00 (d, J = 12.9 Hz, 1H),

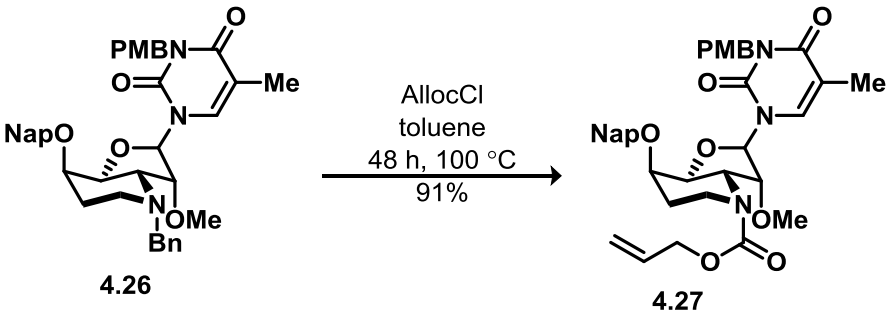
3.75 (s, 3H), 3.31 – 3.14 (m, 2H), 2.83 – 2.72 (m, 2H), 2.42 – 2.29 (m, 1H), 2.08 – 1.97 (m, 1H), 1.66 – 1.51 (m, 1H), 1.26 (d, J = 0.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.46, 159.14, 150.88, 137.46, 135.55, 133.68, 133.35, 133.16, 131.04, 129.31, 129.11, 128.59, 127.87, 127.81, 127.62, 126.50, 126.46, 126.23, 125.59, 113.74, 109.58, 92.07, 80.73, 74.18, 72.47, 71.39, 61.59, 59.98, 55.30, 47.78, 43.76, 27.34, 12.62; HRMS (ESI) calculated for $C_{38}H_{40}N_3O_6$ $[M+H]^+$ m/z = 634.29116, found = 634.29024.



Compound 4.26

To a stirred solution of nucleoside **4.25** (270 mg, 0.426 mmol) in THF (2.1 mL) at 0 °C was added 60% sodium hydride (26 mg, 0.85 mmol). After stirring at 0 °C for 30 min, methyl iodide (54 μL, 0.85 mmol) was added and the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was quenched with water (10 mL) and the resulting mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 Ethyl acetate/hexane) to give nucleoside **4.26** as a white foam (260 mg, 94%) R_f = 0.58 (1:1 Ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.73 (m, 5H), 7.54 – 7.27 (m, 11H), 6.84 (d, J = 8.4 Hz, 2H), 6.03 (s, 1H), 5.11 (d, J = 13.6 Hz, 1H), 5.01 (d, J = 13.6 Hz, 1H), 4.86 (d, J = 11.6 Hz, 1H), 4.70 (d, J = 11.6 Hz, 1H), 4.33 – 4.19 (m, 2H), 3.95 (d, J = 12.6 Hz, 1H), 3.84 (d, J = 4.1 Hz, 1H), 3.77 (s, 3H), 3.64 (s, 3H), 3.27 (d, J = 12.6

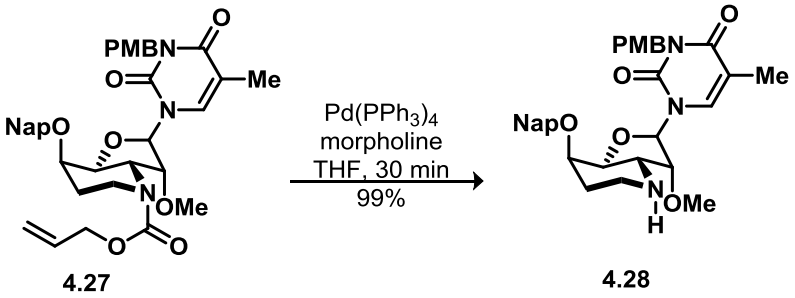
Hz, 1H), 2.80 – 2.60 (m, 2H), 2.32 (dd, J = 23.5, 12.4 Hz, 1H), 2.04 (d, J = 16.2 Hz, 1H), 1.69 – 1.56 (m, 1H), 1.18 (s, J = 11.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.41, 159.10, 150.70, 137.43, 135.49, 133.77, 133.31, 133.13, 130.77, 129.54, 129.32, 128.55, 128.33, 127.82, 127.76, 127.40, 126.58, 126.41, 126.18, 125.64, 113.73, 109.50, 88.71, 83.22, 80.97, 72.59, 71.18, 60.91, 60.06, 58.13, 55.26, 47.55, 43.62, 27.12, 12.45; HRMS (ESI) calculated for $C_{39}H_{42}N_3O_6$ $[M+H]^+$ m/z = 648.30681, found = 648.30851.



Compound 4.27

Allyl chloroformate (2 mL, 28.3 mmol) was added to a stirred solution of nucleoside **4.26** (260 mg, 0.40 mmol) in toluene (10 mL) and the resulting solution was stirred for 2 days at 100 °C. After the reaction was complete, the solvent was removed under reduced pressure and the crude residue was passed through a silica pad eluted with ethyl acetate to give nucleoside **4.27** (184 mg, 72%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.76 (m, 4H), 7.63 (d, J = 1.1 Hz, 1H), 7.52 – 7.38 (m, 5H), 6.85 – 6.78 (m, 2H), 5.99 – 5.88 (m, 2H), 5.37 – 5.19 (m, 2H), 5.07 (d, J = 13.6 Hz, 1H), 4.97 (d, J = 13.6 Hz, 1H), 4.87 (d, J = 11.5 Hz, 1H), 4.74 (d, J = 11.5 Hz, 1H), 4.69 – 4.61 (m, 1H), 4.56 (ddt, J = 13.2, 5.7, 1.3 Hz, 1H), 4.32 (dd, J = 17.7, 3.1 Hz, 2H), 4.16 (dd, J = 11.0, 2.6 Hz, 1H), 3.93 (ddd, J = 13.0, 4.9, 3.0 Hz, 1H), 3.76 (s, J = 3.6 Hz, 3H), 3.63 (s, 3H), 3.36 (dd, J = 11.0, 4.1 Hz, 1H), 3.26 (td, J = 12.7, 3.5 Hz, 1H), 2.13 (dq, J = 14.6, 3.2 Hz, 1H),

1.82 – 1.70 (m, 1H), 1.16 (d, J = 1.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.29, 159.05, 156.51, 150.60, 135.03, 133.28, 133.27, 133.14, 132.56, 130.64, 129.20, 128.62, 127.79, 127.75, 126.62, 126.48, 126.27, 125.52, 118.28, 113.71, 109.52, 88.77, 84.06, 80.11, 77.10, 71.56, 71.54, 66.55, 57.92, 55.23, 53.30, 43.60, 40.89, 26.83, 12.44; HRMS (ESI) calculated for $C_{36}H_{40}N_3O_8$ $[M+H]^+$ m/z = 642.28099, found = 642.28125

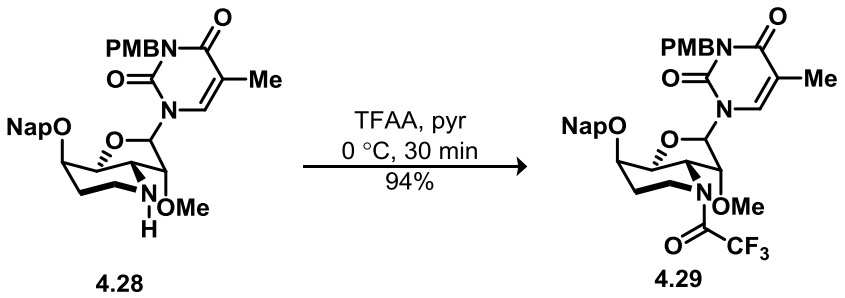


4.27 4.28

Compound 4.28

Morpholine (27 μL, 0.31 mmol) and $Pd(PPh_3)_4$ (9 mg, 7.8 μmol) were added sequentially to an stirred solution of nucleoside **4.27** (98 mg, 0.15 mmol) in THF (1 mL). After 30 min, the reaction mixture was diluted with CH_2Cl_2 and silica gel was added to the reaction mixture. The solvent was removed under reduced pressure and the dry residue was purified by flash chromatography (0% to 5% MeOH in CH_2Cl_2) to give nucleoside **4.28** as a white foam (87 mg, 99%) R_f = 0.43 (6% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.76 (m, 4H), 7.65 (d, J = 1.0 Hz, 1H), 7.52 – 7.36 (m, 5H), 6.80 (d, J = 8.7 Hz, 2H), 5.93 (s, 1H), 5.07 (d, J = 13.6 Hz, 1H), 4.97 (d, J = 13.6 Hz, 1H), 4.88 (d, J = 11.6 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 4.41 (s, 1H), 3.87 (dd, J = 10.8, 2.5 Hz, 1H), 3.76 (s, 3H), 3.74 – 3.70 (m, 2H), 3.65 (d, J = 4.6 Hz, 1H), 3.61 (s, 3H), 3.19 (dd, J = 10.8, 4.6 Hz, 1H), 2.07 (d, J = 13.8 Hz, 1H), 1.53 – 1.41 (m, 1H), 1.15 (d, J = 0.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.40, 159.10, 150.70, 135.54, 134.55, 133.66,

133.36, 133.17, 130.66, 129.30, 128.59, 127.85, 127.80, 126.55, 126.47, 126.22, 125.63, 118.53, 113.77, 109.64, 88.43, 84.19, 81.41, 73.84, 71.59, 67.03, 62.23, 58.24, 55.31, 54.80, 53.63, 43.66, 41.47, 29.06, 12.47; HRMS (ESI) calculated for $C_{32}H_{36}N_3O_6$ $[M+H]^+$ *m/z* = 558.25986, found = 558.26053

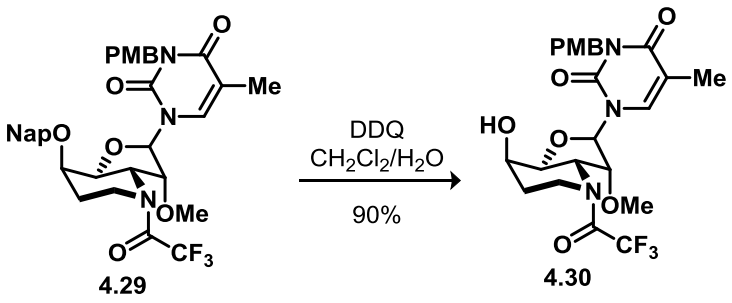


4.28 4.29

Compound 4.29

Triethylamine (27 μL, 0.187 mmol) and trifluoroacetic anhydride (27 μL, 0.187 mmol) were added to an stirred solution of nucleoside **4.28** (87 mg, 0.156 mmol) in a 1:1 mixture of Et_2O/CH_2Cl_2 (1 mL) at 0 °C. After stirring during 30 min at the same temperature, the reaction mixture was quenched with an saturated aqueous solution of $NaHCO_3$ and the resulting mixture was extracted with CH_2Cl_2 (5 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (3:7 Ethyl acetate/hexane) to give nucleoside **4.29** as a white foam (96 mg, 94%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.77 (m, 4H), 7.58 (d, *J* = 0.8 Hz, 1H), 7.55 – 7.39 (m, 5H), 6.82 (d, *J* = 8.7 Hz, 2H), 5.97 (s, 1H), 5.09 (d, *J* = 13.6 Hz, 1H), 4.98 (d, *J* = 13.6 Hz, 1H), 4.88 (d, *J* = 11.5 Hz, 1H), 4.79 (d, *J* = 11.6 Hz, 1H), 4.45 (d, *J* = 4.0 Hz, 1H), 4.41 (d, *J* = 2.8 Hz, 1H), 4.22 (dd, *J* = 11.2, 2.6 Hz, 1H), 3.89 – 3.79 (m, 1H), 3.77 (s, 3H), 3.59 (s, *J* = 7.8 Hz, 3H), 3.54 – 3.44 (m, 2H), 2.20 – 2.09 (m, 1H), 1.96 – 1.82 (m, 1H), 1.22 (s, 3H); ^{13}C NMR

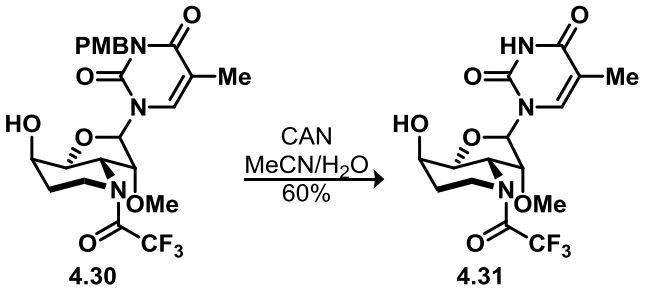
(101 MHz, $CDCl_3$) δ 163.27, 159.16, 157.55, 157.19, 150.63, 134.72, 133.32, 133.26, 132.97, 130.73, 129.18, 128.81, 127.87, 127.84, 126.77, 126.65, 126.46, 125.50, 113.81, 109.87, 89.17, 82.70, 79.13, 72.10, 70.94, 57.93, 55.29, 53.82, 43.73, 41.12, 29.79, 27.59, 12.62; HRMS (ESI) calculated for $C_{34}H_{35}F_3N_3O_7$ $[M+H]^+$ *m/z* = 654.24216, found = 654.24315.



Compound 4.30

To an stirred solution of nucleoside **4.29** (96 mg, 0.146 mmol) in a 9:1 mixture of CH_2Cl_2/water (1 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (100 mg, 0.44 mmol). The resulting mixture was stirred at room temperature for 3 h, followed by addition of aqueous 10% $NaHSO_3$. The resulting mixture was stirred for 10 minutes followed by addition of CH_2Cl_2. The layers were separated, then the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 Ethyl acetate/hexane) to give nucleoside **4.30** as an oil (68.3 mg, 90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.41 (d, *J* = 8.7 Hz, 2H), 6.86 – 6.78 (m, 2H), 5.93 (s, 1H), 5.10 (d, *J* = 13.7 Hz, 1H), 5.01 (d, *J* = 13.7 Hz, 1H), 4.57 (s, 1H), 4.45 (d, *J* = 4.1 Hz, 1H), 3.81 – 3.71 (m, 4H), 3.55 (s, 3H), 3.53 – 3.42 (m, 2H), 2.96 (t, *J* = 16.0 Hz, 1H), 2.03 – 1.89 (m, 2H), 1.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.64, 159.21, 157.62, 157.26, 150.61, 133.73, 133.70, 130.63, 129.02, 117.69,

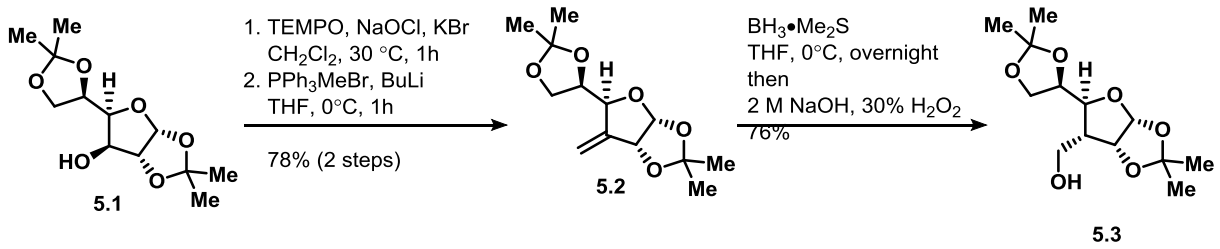
114.82, 113.87, 109.71, 89.48, 82.73, 79.27, 63.40, 57.95, 55.34, 53.05, 43.95, 40.88, 40.84, 31.09, 13.55.; HRMS (ESI) calculated for $C_{23}H_{27}F_3N_3O_7$ $[M+H]^+$ *m/z* = 514.17956, found = 514.17967.



Compound 4.31

Cerium ammonium nitrate (218 mg, 0.39 mmol) was added to and stirred solution of nucleoside **4.30** (68.3 mg, 0.13 mmol) in a 10:1 mixture of acetonitrile/water (3 mL). After stirring for 2 h, water and EtOAc were added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (6:4 Ethyl acetate/hexane) to give nucleoside **4.31** as an oil (31.5 mg, 60%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.59 (t, *J* = 1.3 Hz, 1H), 5.95 (s, 1H), 4.64 (d, *J* = 4.3 Hz, 1H), 4.48 (d, *J* = 4.2 Hz, 1H), 4.14 (dd, *J* = 11.2, 2.7 Hz, 1H), 3.78 (d, *J* = 13.4 Hz, 1H), 3.52 (s, 5H), 2.38 (s, 1H), 2.15 – 1.98 (m, 1H), 1.90 (d, *J* = 1.2 Hz, 3H). HRMS (ESI) calculated for $C_{15}H_{19}F_3N_3O_6$ $[M+H]^+$ *m/z* = 394.12205, found = 394.12257.

8.2.3 Synthesis of a oxabicyclic nucleoside phosphonate

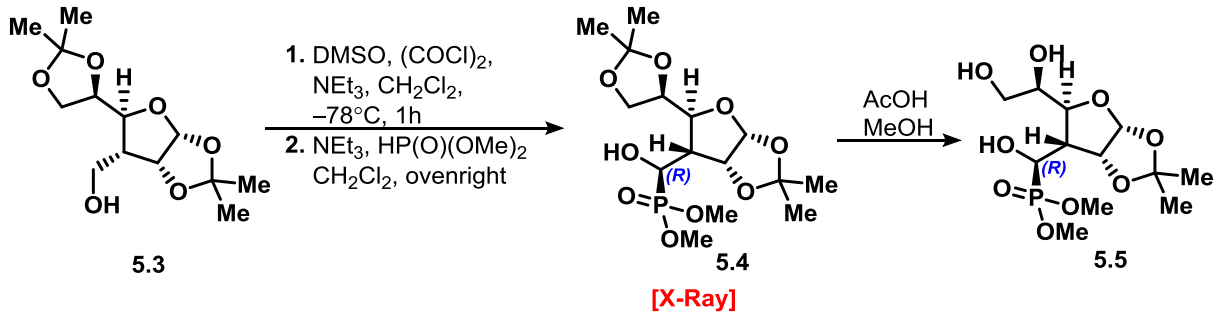


Compound 5.3

To a stirred solution of sugar **5.1** (25.5 g, 97.9 mmol) in CH_2Cl_2 (140 mL) was added potassium bromide (1.17 g, 9.83 mmol) and TEMPO (468 mg, 2.99 mmol). The resulting solution was heated at 30 °C and then a 10% solution of NaOCl (102 mL, 166.5 mmol) was added during 1 h (syringe pump). The layers were separated, washed sequentially with a solution of NaI (1.5 g) in 1 M HCl (50 mL), a solution of $Na_2S_2O_3$ (100g/mL, 50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was coevaporated with toluene (3 x 20 mL) and the crude ketone was dissolved in THF (250 mL). In a separated flask, a solution of PPh_3MeBr (41.16g, 0.115 mol) in THF (380 mL) at 0 °C was combined with a 2.5 M solution of BuLi (44.17 mL, 110.42 mmol) added during 10 min. The ice bath was removed and the reaction was stirred for 30 min. The solution was cooled down again to 0 °C and it was canulated to the ketone flask. The resulting solution was stirred for 1 h, filtered in a sintered glass filter and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (2:8 EtOAc/hex) to give alkene **5.2** (19.2 g, 78%).

Alkene **5.2** (19.2 g, 74.9 mmol) was dissolved in THF (350 mL) and a 10 M solution of $BH_3 \cdot Me_2S$ (11.24 mL, 112.4 mmol) was added and stirred overnight. The solution was cooled down to 0 °C and a 2 M solution of NaOH (224 mL, 448 mmol) was added carefully followed

by addition of a 30% solution of H_2O_2 (137 mL, 1.34 mol). After stirring for 1 h, the resulting solution was extracted with EtOAc (4 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 EtOAc/hexanes) to give alcohol **2** as a white foam (15.74 g, 76%) R*f* = 0.43 (4:6 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (d, *J* = 3.7 Hz, 1H), 4.75 – 4.65 (m, 1H), 4.14 – 4.03 (m, 1H), 4.01 – 3.90 (m, 2H), 3.90 – 3.74 (m, 3H), 3.21 (dd, *J* = 9.2, 3.5 Hz, 1H), 2.11 – 2.00 (m, 1H), 1.46 (s, 3H), 1.39 (s, 3H), 1.31 (s, *J* = 20.0 Hz, 3H), 1.26 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 112.3, 109.9, 104.9, 82.5, 81.50, 77.2, 67.9, 59.5, 51.4, 26.7, 26.5, 26.2, 25.2.

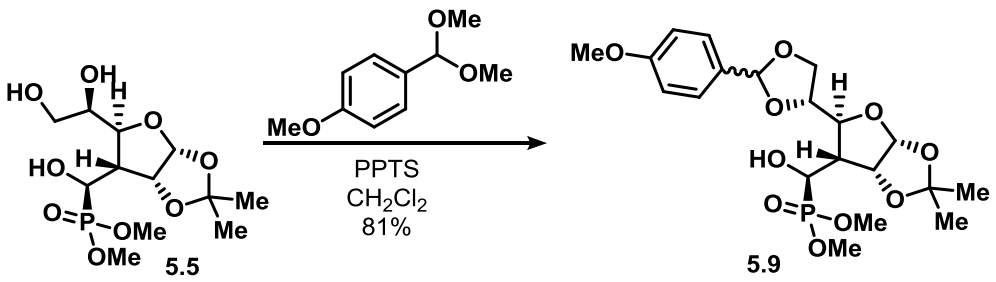


Compound 5.5

A solution of DMSO (4.56 mL, 64.20 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a stirred solution of oxalyl chloride (1.75 mL, 41.69 mmol) in CH_2Cl_2 (80 mL) at -78 °C. After 20 min, a solution of alcohol **5.3** (8.8 g, 32.08 mmol) in CH_2Cl_2 (55 mL) was added dropwise to the reaction mixture which was stirred at -78 °C for 1 h. Triethylamine (22.4 mL, 160.7 mmol) was added to the reaction mixture and the cooling bath was removed. The mixture was stirred for 1 h at r.t, treated with 1 M HCl (100 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine (30

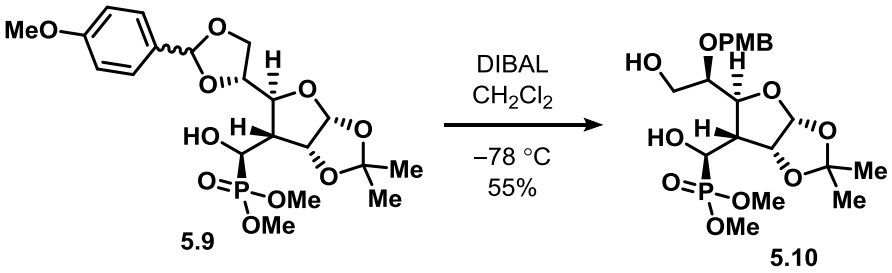
mL), dried over Na_2SO_4, filtered, and concentrated to a volume of approximately 80 mL. To the previous solution, triethylamine (18.0 mL, 129.13 mmol) and dimethyl phosphite (12.0 mL, 130.9 mmol) were added and stirred for 12 h. The reaction was quenched with H_2O (200 mL), the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude α-hydroxy-phosphonate **5.4**, which was used directly in the next step without purification (R*f* = 0.16 7:3 EtOAc/hexanes). A portion of the residue was purified by flash column chromatoghaphy on silica gel (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (d, *J* = 3.3 Hz, 1H), 4.88 – 4.76 (m, 2H), 4.34 – 4.25 (m, 1H), 4.23 – 4.14 (m, 1H), 4.09 – 3.93 (m, 3H), 3.87 (d, *J* = 10.3 Hz, 3H), 3.81 (d, *J* = 10.5 Hz, 3H), 2.46 – 2.32 (m, 1H), 1.51 (s, 3H), 1.44 (s, 3H), 1.35 (s, 3H), 1.30 (s, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 112.62, 110.65, 104.74, 82.36, 82.17, 81.88, 76.89, 68.39, 65.96, 64.28, 54.18, 54.12, 53.15, 53.07, 51.07, 27.09, 26.35, 26.34, 25.31; ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.71.

A solution of the α-hydroxyphosphonate **5.4** in AcOH/MeOH/H_2O 4:5:6 (110 mL) was stirred at 60 °C for 3 h, then the solvent was removed under reduced pressure. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give compound **5.5** (6.4 g, 58% over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.19 (s, 1H), 5.72 (d, *J* = 3.4 Hz, 1H), 5.62 (s, 1H), 4.65 (t, *J* = 3.9 Hz, 1H), 4.33 – 4.22 (m, 1H), 4.09 (t, *J* = 8.2 Hz, 1H), 3.84 – 3.70 (m, 8H), 3.67 – 3.55 (m, 2H), 3.12 (s, 1H), 2.49 – 2.33 (m, 1H), 1.47 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 112.30, 104.72, 81.47, 81.41, 81.23, 73.71, 65.40, 64.27, 63.75, 54.38, 54.31, 53.35, 53.27, 49.60, 49.58, 26.97, 26.40; ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.52.



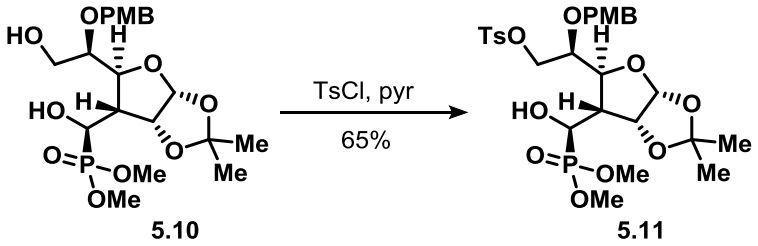
Compound 5.9

Pyridinium p-toluensulphonate (42 mg, 0.167 mmol) and p-methoxybenzaldehyde dimethyl acetal (0.427 mL, 2,51 mmol) were added sequentially to a stirred solution of diol **5.5** (572 mg, 1.67 mmol) in CH_2Cl_2 (17 mL) at 0 °C. After 3 h, the reaction mixture was partitioned between a saturated aqueous solution of $NaHCO_3$ and CH_2Cl_2. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (3% MeOH in CH_2Cl_2) to give nucleoside **5.9** as an isomeric mixture (600 mg, 78%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.30 (m, 2H), 6.91 – 6.82 (m, 2H), 5.90 (s, 0.5H), 5.77 (dd, J = 9.0, 3.4 Hz, 1H), 5.74 (s, 0.5H), 4.78 (dt, J = 5.8, 3.8 Hz, 1H), 4.40 – 4.19 (m, 4H), 4.16 – 3.95 (m, 1H), 3.88 – 3.74 (m, 9H), 2.43 – 2.25 (m, 1H), 1.52 (d, J = 4.2 Hz, 3H), 1.31 (d, J = 2.9 Hz, 3H).;^{31}P NMR (162 MHz, $CDCl_3$) δ 24.94, 24.90.



Compound 5.10

To a stirred solution of compound **5.9** (600 mg, 1.30 mmol) in CH_2Cl_2 (13 mL) at -78 °C was added a 1 M solution of DIBAL (10.4 mL, 10.4 mmol) during a period of 30 min (syringe pump). After the reaction is complete, CH_2Cl_2 and Rochelle salt were added and stirred for 1 h. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (5 x 20 mL), the combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (3% MeOH in CH_2Cl_2) to give nucleoside **5.10** (336.6 mg, 78%) in a 7:1 ratio of inseparable regioisomers; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.27 (m, 2H), 6.91 – 6.84 (m, 2H), 5.78 (d, *J* = 3.4 Hz, 1H), 4.71 (dd, *J* = 4.9, 3.4 Hz, 1H), 4.63 (d, *J* = 2.6 Hz, 1H), 4.53 – 4.47 (m, 1H), 4.31 (td, *J* = 9.9, 6.6 Hz, 1H), 3.89 – 3.71 (m, 11H), 3.63 – 3.55 (m, 1H), 2.72 – 2.57 (m, 1H), 1.31 (t, *J* = 1.0 Hz, 3H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.77, 130.21, 129.66, 129.08, 114.12, 114.01, 112.77, 105.18, 104.70, 82.02, 80.73, 80.60, 72.35, 66.26, 64.07, 61.21, 55.41, 54.38, 54.29, 53.10, 53.00, 47.25, 27.35, 26.67.

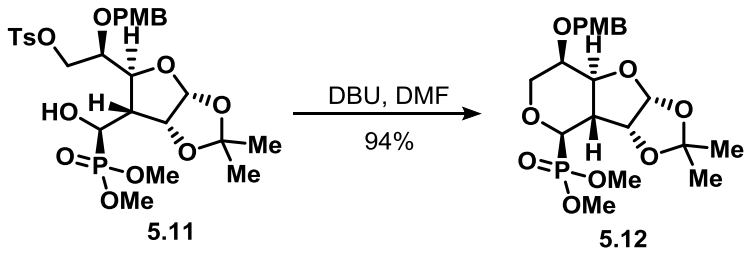


5.10 **5.11**

Compound 5.11

To a stirred solution of compound **5.10** (336.6 mg, 0.727 mmol) in pyridine (3.5 mL) p-toluenesulfonyl chloride (204.8 mg, 1.07 mmol) was added. After stirring for 24 h, a 1 M solution of HCl was added to the mixture followed by extractions with CH_2Cl_2 (3 x 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced

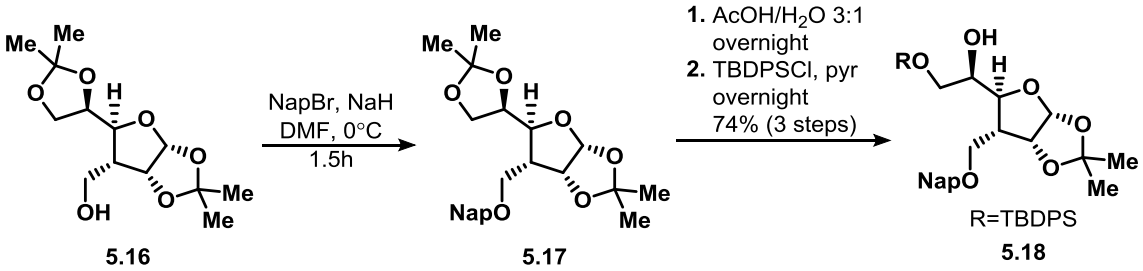
pressure. The residue was purified by flash chromatography (3% MeOH in CH_2Cl_2) to give nucleoside **5.11** (289 mg, 65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.3 Hz, 2H), 7.35 – 7.26 (m, 4H), 6.87 – 6.81 (m, 2H), 5.67 (d, *J* = 3.4 Hz, 1H), 4.69 – 4.62 (m, 2H), 4.53 (d, *J* = 10.9 Hz, 1H), 4.39 (dd, *J* = 10.8, 3.0 Hz, 1H), 4.25 (dd, *J* = 9.4, 5.7 Hz, 3H), 4.10 (dd, *J* = 10.8, 6.1 Hz, 1H), 3.81 – 3.76 (m, 7H), 2.57 – 2.46 (m, 1H), 2.43 (s, 3H), 1.45 (s, 3H), 1.28 (s, 3H).;^{13}C NMR (101 MHz, $CDCl_3$) δ 159.73, 145.03, 132.84, 130.42, 130.00, 129.60, 128.62, 128.11, 113.96, 113.84, 112.63, 105.12, 81.61, 79.90, 79.73, 78.26, 72.98, 69.71, 65.85, 64.23, 55.37, 54.11, 54.04, 53.26, 53.19, 47.87, 27.25, 26.60, 21.76;^{31}P NMR (162 MHz, $CDCl_3$) δ 25.26.



Compound 5.12

To a stirred solution of nucleoside **5.11** (289 mg, 0.469 mmol) in DMF (3 mL) was added DBU (0.14 mL, 0.93 mmol). After stirring for 3 h, a 1 M solution of HCl was added followed by extractions with EtOAc (3 x 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/hex 8:2) to give nucleoside **5.11** (196 mg, 94%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 6.5 Hz, 2H), 6.88 – 6.82 (m, 2H), 5.85 (d, *J* = 3.4 Hz, 1H), 4.79 (t, *J* = 3.7 Hz, 1H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.12 (dt, *J* = 12.6, 2.2 Hz, 1H), 3.94 (dt, *J* = 10.9, 8.3 Hz, 3H), 3.86 (d, *J* = 0.6 Hz, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.37 (d, *J* = 12.4 Hz, 1H), 2.51 – 2.41 (m, 1H), 1.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.28, 130.46,

129.32, 113.87, 112.36, 105.15, 79.17, 78.42, 78.25, 73.60, 71.86, 71.62, 69.80, 69.65, 55.40, 53.84, 53.78, 53.52, 53.45, 41.43, 26.35, 26.03; ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.06.

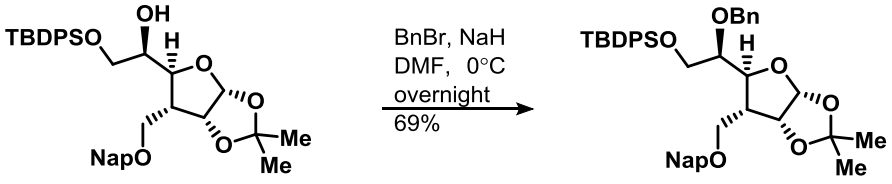


5.16 5.17 5.18

Compound 5.18

A solution of **5.16** (13.9 g, 50.8 mmol) in DMF (210 mL) was cooled to 0 °C and mixed with 60% NaH (3.05 g, 76.2 mmol). After stirring at 0 °C for 30 min, 2-(bromomethyl)naphthalene (16.8 g, 76.2 mmol) was added and then the cold bath was removed. After 1.5 h, the reaction was carefully quenched with water (300 mL) and extracted with diethyl ether (5 × 60 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The product **5.17** was used for the next step without further purification. For characterization purposes, a portion of the product was purified by flash chromatography (3:7 EtOAc/hexanes) R*f* = 0.42 (3:7 EtOAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.9 Hz, 4H), 7.53 – 7.41 (m, 3H), 5.76 (d, *J* = 3.5 Hz, 1H), 4.78 – 4.73 (m, 1H), 4.69 (s, 2H), 4.13 – 3.99 (m, 2H), 3.96 – 3.90 (m, 1H), 3.89 – 3.79 (m, 3H), 2.30 – 2.16 (m, 1H), 1.52 (s, 3H), 1.38 (s, 3H), 1.33 (d, *J* = 4.3 Hz, 6H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7, 133.0, 132.7, 127.7, 127.5, 127.4, 125.9, 125.7, 125.5, 125.4, 111.6, 109.1, 104.9, 81.0, 79.3, 77.35, 77.33, 72.9, 66.7, 65.8, 48.5, 26.6, 26.25, 26.2, 25.0; HRMS (ESI) calculated for $C_{24}H_{30}NaO_6$ $[M+Na]^+$ *m/z* = 437.1935,

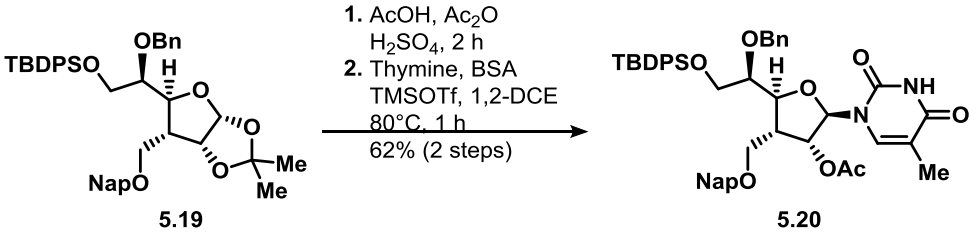
found 437.1919. Crude **5.17** was dissolved in a mixture of acetic acid/water 3:1 (190 mL) and the resulting solution was stirred overnight at room temperature. The reaction mixture was then concentrated under reduced pressure, mixed with EtOAc (100 mL), water (100 mL) and solid KOH until the pH of the solution was >12. The organic layer was sequentially washed with a saturated aqueous solution of $NaHCO_3$, brine, and dried over Na_2SO_4. The crude residue was co-evaporated with toluene (2 × 50 mL) obtaining a colorless oil. The crude product was dissolved in pyridine (200 mL) and then mixed with *tert*-butyl(chloro)diphenylsilane (13.5 mL, 52.1 mmol). The reaction mixture was stirred overnight at room temperature, then the solvent was removed under reduced pressure and purified by flash chromatography (2:8 EtOAc/hexanes) to give sugar **5.18** as a white foam (22.9 g, 73.6% over three steps) R*f* = 0.27 (2:8 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.74 (m, 8H), 7.60 – 7.38 (m, 9H), 5.82 (d, *J* = 3.6 Hz, 1H), 4.90 – 4.70 (m, 3H), 4.13 (dd, *J* = 9.8, 7.0 Hz, 1H), 4.03 – 3.86 (m, 4H), 3.86 – 3.76 (m, 1H), 3.48 (d, *J* = 3.6 Hz, 1H), 2.46 – 2.34 (m, 1H), 1.56 (s, *J* = 11.1 Hz, 3H), 1.40 (s, *J* = 14.1 Hz, 3H), 1.17 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 135.6, 135.3, 133.5, 133.31, 133.30, 133.1, 129.7, 128.2, 127.9, 127.7, 126.6, 126.1, 126.0, 125.7, 111.9, 104.8, 81.8, 79.9, 73.9, 73.5, 67.0, 65.3, 48.4, 26.9, 26.8, 26.6, 19.3; HRMS (ESI) calculated for $C_{37}H_{44}O_6SiNa$ $[M+Na]^+$ *m/z* = 635.2799, found 635.2799.



5.18 **5.19**

Compound 5.19

A solution of sugar **5.18** (22.9 g, 37.4 mmol) in DMF (150 mL) was cooled to 0 °C and mixed with 60 % sodium hydride (1.94g, 48.5 mmol). After stirring for 30 min at 0 °C, benzyl bromide (5.3 mL, 44.8 mmol) was added and the cold bath was removed. The reaction mixture was stirred overnight, then water was added carefully (500 mL) and the aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (1:9 EtOAc/hexanes) to give sugar **5.19** as a white foam (18.9 g, 69%) R*f* = 0.43 (2:8 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.87 (m, 8H), 7.72 – 7.36 (m, 14H), 5.97 (d, *J* = 3.5 Hz, 1H), 5.02 – 4.93 (m, 2H), 4.90 – 4.74 (m, 3H), 4.43 (dd, *J* = 9.9, 4.7 Hz, 1H), 4.18 – 4.03 (m, 3H), 4.01 – 3.94 (m, 2H), 2.73 (tt, *J* = 9.6, 4.9 Hz, 1H), 1.73 (s, 3H), 1.56 (s, *J* = 5.7 Hz, 3H), 1.31 (s, *J* = 13.5 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 136.0, 135.53, 135.52, 133.3, 133.2, 133.18, 132.9, 129.6, 128.2, 128.1, 127.9, 127.7, 127.65, 127.62, 127.4, 126.0, 125.9, 125.6, 125.5, 111.6, 104.8, 81.5, 81.0, 78.9, 77.4, 77.2, 73.1, 73.0, 66.7, 63.5, 46.6, 26.9, 26.8, 26.6, 19.1; HRMS (ESI) calculated for $C_{44}H_{50}NaO_6Si$ $[M+Na]^+$ *m/z* = 725.3285, found 725.3269.

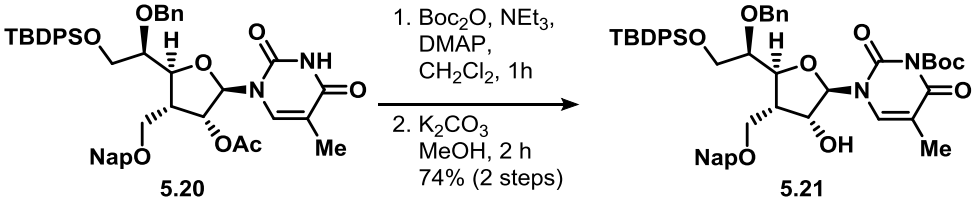


5.19 **5.20**

Compound 5.20

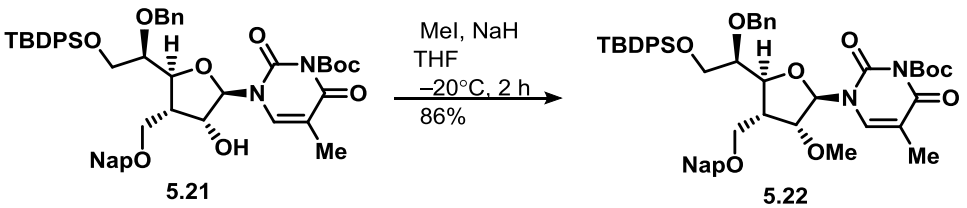
Sugar **5.19** (15.9 g, 22.6 mmol) was treated with acetic anhydride (10.7 mL, 113.2 mmol), glacial acetic acid (46.6 mL, 0.815 mol) and concentrated sulfuric acid (61 µL, 1.1 mmol). The resulting solution was stirred at room temperature for 2 h, then diluted with EtOAc (600 mL)

and mixed carefully with a saturated aqueous solution of $NaHCO_3$ (400 mL). The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to a residue (mixture of anomeric acetates) which was co-evaporated with toluene (3 × 50 mL) and dissolved in 1,2-dichloroethane (200 mL). In another flask, thymine (8.56 g, 67.90 mmol) was dissolved in 1,2-dichloroethane (500 mL), treated with N,O-bis(trimethylsilyl)acetamide (55.3 mL, 0.23 mol), heated to 80 °C for 1 h, cooled to 0 °C and treated with the solution of the preceding diacetylated product via cannula, followed by TMSOTf (12.3 mL, 67.9 mmol). The cooling bath was removed, and the reaction mixture was heated to 60 °C. After 2 h, the reaction mixture was mixed with a solution of saturated $NaHCO_3$ (200 mL) and extracted with CH_2Cl_2 (3 × 100 mL).The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 EtOAc/hexanes) to give nucleoside **5.20** as a white foam (11.5 g, 62%) R*f* = 0.14 (4:6 EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.90 – 7.79 (m, 4H), 7.72 (d, *J* = 7.3 Hz, 4H), 7.57 – 7.37 (m, 13H), 7.24 (s, 1H), 6.35 (d, *J* = 6.1 Hz, 1H), 5.56 – 5.46 (m, 1H), 4.82 (d, *J* = 12.2 Hz, 1H), 4.75 – 4.65 (m, 2H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.53 (dd, *J* = 4.6, 2.4 Hz, 1H), 4.00 – 3.83 (m, 3H), 3.65 (d, *J* = 5.2 Hz, 2H), 3.19 (dq, *J* = 10.4, 5.2 Hz, 1H), 1.98 (s, 3H), 1.57 (s, 3H), 1.13 (s, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 163.9, 150.8, 138.0, 135.6, 135.5, 135.2, 133.2, 133.0, 132.9, 132.8, 129.8, 128.5, 128.2, 127.9, 127. 8, 127.7, 127.6, 127.5, 126.5, 126.5, 126.1, 125.8, 125.7, 111.5, 86.4, 81.2, 80.9, 77.4, 77.2, 75.4, 73.5, 72.0, 67.0, 62.7, 40.0, 26.8, 20.5, 19.1, 11.9; HRMS (ESI) calculated for $C_{48}H_{53}N_2O_8Si$ $[M+H]^+$ *m/z* = 813.3566, found 813.3581.



Compound 5.21

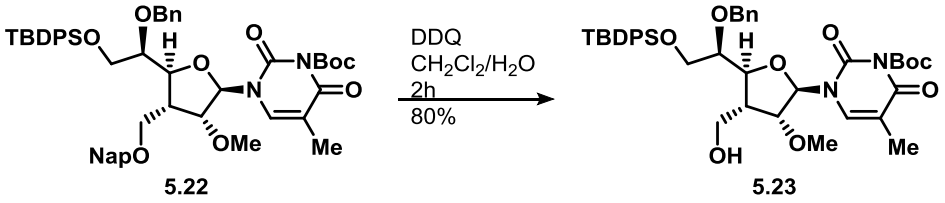
Triethylamine (2.53 mL, 18.15 mmol), di-*tert*-butyl carbonate (2.8 mL, 12.2 mmol) and 4-(dimethylamino)pyridine (0.37 g, 3.02 mmol) were added sequentially to a stirred solution of **5.20** (4.91 g, 6.04 mmol) in CH_2Cl_2 (38 mL). After 1 h, the reaction was quenched via addition of 1 M HCl (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in methanol (50 mL) and mixed with potassium carbonate (1.67 g, 12.10 mmol). After stirring for 2 h, the reaction mixture was diluted with water (20 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 EtOAc/hexanes) to give nucleoside **5.21** as a white foam (3.87 g, 74% over two steps) R*f* = 0.25 (1% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.79 (m, 4H), 7.75 – 7.70 (m, 4H), 7.54 – 7.30 (m, 15H), 6.03 (d, *J* = 4.4 Hz, 1H), 4.80 (d, *J* = 12.1 Hz, 1H), 4.70 (s, 2H), 4.60 – 4.50 (m, 3H), 3.98 – 3.76 (m, 5H), 3.61 (d, *J* = 5.8 Hz, 1H), 2.88 (p, *J* = 5.9 Hz, 1H), 1.67 (s, 9H), 1.58 (s, 3H), 1.14 (s, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.1, 148.9, 147.8, 137.9, 135.4, 134.9, 134.8, 133.1, 132.9, 132.8, 132.7, 129.8, 128.4, 128.3, 127.9, 127.7, 127.6, 127.5, 126.6, 126.4, 126.1, 125.9, 125.4, 110.3, 90.7, 86.4, 81.7, 80.6, 77.4, 77.2, 76.5, 73.4, 72.1, 67.4, 62.8, 41.4, 27.3, 26.7, 19.0, 11.9; HRMS (ESI) calculated for $C_{51}H_{58}N_2O_9SiNa$ $[M+Na]^+$ *m/z* = 893.3804, found 893.3810.



5.21 5.22

Compound 5.22

Nucleoside **5.21** (1.35g, 1.54 mmol) was dissolved in DMF (10 mL) and cooled to -20 °C. 60% NaH (0.12 g, 3.07 mmol) was added to the previous solution and stirred at -20 °C for 30 min followed by addition of methyl iodide (0.24 mL, 3.85 mmol). After 2h at -20 °C, the reaction mixture was quenched with a saturated aqueous solution of NH_4Cl (40 mL) and the resulted aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 EtOAc/hexanes) to give nucleoside **5.22** as a white foam (1.18 g, 86%) R*f* = 0.63 (3:7 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.71 (m, 4H), 7.66 – 7.58 (m, 4H), 7.50 – 7.25 (m, 15H), 5.96 (d, *J* = 2.3 Hz, 1H), 4.75 (d, *J* = 12.1 Hz, 1H), 4.63 – 4.52 (m, 3H), 4.33 (dd, *J* = 8.6, 2.9 Hz, 1H), 3.98 – 3.90 (m, 2H), 3.89 – 3.66 (m, 3H), 3.60 (dd, *J* = 9.1, 5.6 Hz, 1H), 3.49 (s, 3H), 2.70 (p, *J* = 7.5, 6.7 Hz, 1H), 1.61 (s, 9H), 1.44 (s, 3H), 1.04 (s, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.48, 148.49, 148.14, 138.11, 135.7, 134.8, 133.4, 133.1, 133.0, 130.0, 128.6, 128.3, 128.0, 127.9, 127.9, 127.8, 127.0, 126.5, 126.2, 126.0, 125.8, 110.3, 88.1, 86.8, 85.5, 82.2, 80.3, 77.2, 73.6, 72.4, 66.3, 63.2, 58.7, 41.7, 27.6, 26.9, 19.2, 12.1; HRMS (ESI) calculated for $C_{52}H_{60}N_2O_9SiNa$ $[M+Na]^+$ *m/z* =907.3960, found 907.3980.

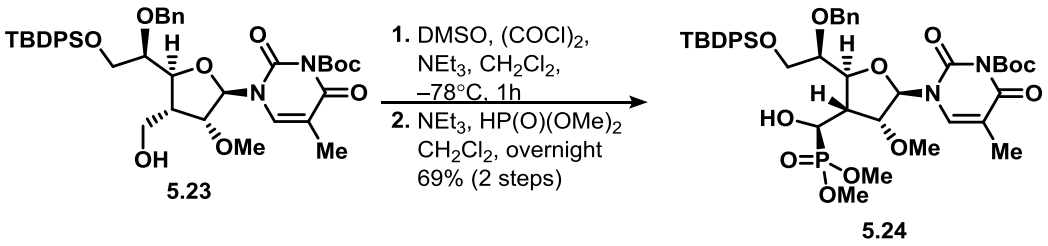


5.22 5.23

Compound 5.23

Nucleoside **5.22** (1.3924 g, 1.57 mmol) was dissolved in a 8:1 mixture of CH_2Cl_2/water (20 mL) and mixed with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (0.45 g, 1.96 mmol). After stirring for 2 h, the reaction was quenched with a 10% solution of $NaHSO_3$ (20 mL) and the resulting aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were

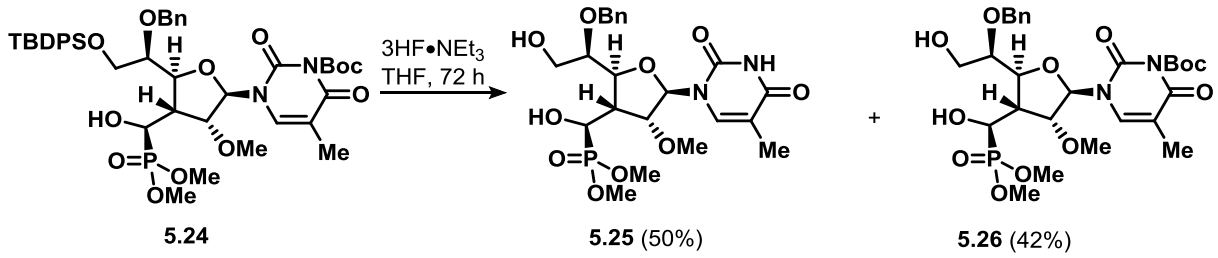
dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 EtOAc/hexanes) to give nucleoside **5.23** as a white foam (943 mg, 80%) R*f* = 0.10 (3:7 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 6.7, 0.7 Hz, 4H), 7.49 – 7.22 (m, 13H), 5.94 (d, *J* = 2.6 Hz, 1H), 4.77 (d, *J* = 12.0 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.42 (dd, *J* = 8.5, 3.0 Hz, 1H), 3.96 (dd, *J* = 6.6, 2.6 Hz, 1H), 3.92 – 3.80 (m, 4H), 3.80 – 3.71 (m, 1H), 3.51 (s, 3H), 2.64 – 2.54 (m, 1H), 2.45 (s, 1H), 1.60 (s, 9H), 1.49 (d, *J* = 1.0 Hz, 3H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.3, 148.4, 148.0, 137.9, 135.7, 135.7, 134.6, 133.0, 133.0, 130.1, 128.7, 128.0, 127.9, 127.1, 110.7, 88.1, 87.3, 86.9, 81.3, 80.3, 77.2, 72.3, 63.1, 59.9, 58.8, 43.4, 27.6, 27.0, 19.3, 12.2; HRMS (ESI) calculated for $C_{41}H_{52}N_2O_9SiNa$ $[M+Na]^+$ *m/z* =767.3334, found 767.3349.



Compound 5.24

A solution of anhydrous DMSO (0.12 mL, 1.74 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise to a -78 °C cooled solution of 2 M oxalyl chloride (0.57 mL, 1.14 mmol) in CH_2Cl_2 (3 mL). After stirring for 20 min at -78 °C, a solution of nucleoside **5.23** (653 mg, 0.876 mmol) in CH_2Cl_2 (2 mL) was added dropwise via cannula to the previous solution. The reaction mixture was stirred for 1 h at -78 °C and then triethylamine (0.61 mL, 4.38 mmol) was added. The reaction mixture was allowed to reach 0 °C and stirred at the same temperature for 1h. The reaction mixture was diluted with water (10 mL) and the aqueous layer was extracted with

CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude aldehyde was dissolved in CH_2Cl_2 (10 mL) and combined with triethylamine (0.25 mL, 1.8 mmol) and dimethyl phosphite (0.16 mL, 1.74 mmol). The resulting solution was stirred at room temperature overnight, then diluted with CH_2Cl_2 (50 mL) and washed sequentially with water (5 mL) and brine (5 mL). The organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (8:2 EtOAc/hexanes) to give nucleoside **5.24** as a white foam (515 mg, 69% over two steps) R*f* = 0.20 (8:2 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.63 (m, 4H), 7.47 – 7.21 (m, 12H), 7.07 (s, 1H), 6.01 (d, *J* = 4.9 Hz, 1H), 5.01 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.56 – 4.51 (m, 1H), 4.46 – 4.34 (m, 2H), 4.08 – 4.01 (m, 1H), 4.00 – 3.81 (m, 3H), 3.61 (dd, *J* = 10.4, 4.8 Hz, 6H), 3.38 (s, 3H), 2.85 – 2.73 (m, 1H), 1.58 (s, 8H), 1.50 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.3, 148.7, 147.9, 137.9, 135.6, 135.6, 134.5, 133.03, 133.01, 123.0, 129.9, 128.6, 127.9, 127.87, 127.82, 126.8, 110.8, 87.7, 86.6, 83.8, 83.7, 82.0, 79.8, 79.7, 77.2, 72.5, 64.3, 62.6, 58.6, 53.6, 53.5, 53.2, 53.17, 42.6, 27.4, 26.9, 19.2, 12.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.5; HRMS (ESI) calculated for $C_{43}H_{58}N_2O_{12}PSi$ $[M+H]^+$ *m/z* = 853.3491, found 853.3520.

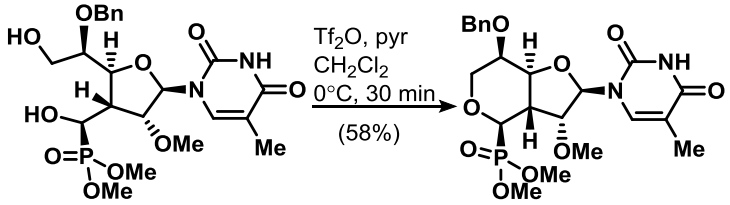


5.24 **5.25** (50%) **5.26** (42%)

Compounds 5.25 and 5.26

Triethylamine trihydrofluoride (3.6 mL, 22.0 mmol) was added to a stirred solution of nucleoside **5.24** (3.68 g, 4.31 mmol) in THF (43 mL). After 3 days, the reaction mixture was

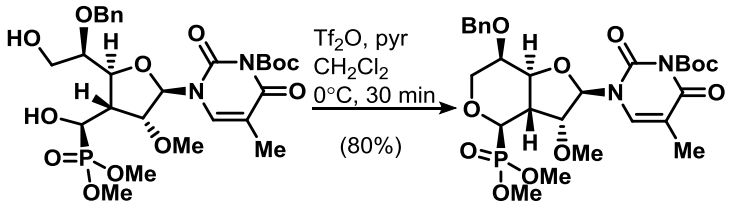
diluted with EtOAc (200 mL) and the resulting solution was sequentially washed with water (30 mL) and brine (30 mL). The organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (100% EtOAc) to give nucleoside **5.26** as a white foam (1.0982 g, 42%) R*f* = 0.34 (8:2 100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 6H), 6.11 (d, *J* = 6.7 Hz, 1H), 4.94 – 4.83 (m, 2H), 4.64 (d, *J* = 12.2 Hz, 1H), 4.48 (d, *J* = 10.2 Hz, 2H), 4.10 – 4.01 (m, 1H), 4.00 – 3.75 (m, 8H), 3.68 – 3.57 (m, 1H), 3.51 – 3.35 (m, 4H), 3.08 – 2.95 (m, 1H), 1.57 (s, 9H), 1.46 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.3, 149.0, 148.0, 138.0, 134.7, 128.8, 128.0, 126.5, 111.4, 87.1, 86.7, 83.5, 83.4, 81.4, 78.8, 78.8, 77.2, 71.5, 64.5, 62.8, 59.2, 59.1, 55.3, 55.3, 52.7, 52.6, 40.1, 27.5, 12.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.2; HRMS (ESI) calculated for $C_{27}H_{40}N_2O_{12}P$ $[M+H]^+$ *m/z* = 615.2313, found 615.2316.. Compound **5.25** was isolated as a white foam (1.1100 g, 50%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 7.36 – 7.28 (m, 6H), 6.15 (d, *J* = 7.0 Hz, 1H), 4.87 (d, *J* = 12.6 Hz, 2H), 4.65 (d, *J* = 12.2 Hz, 1H), 4.46 (dd, *J* = 17.7, 9.4 Hz, 2H), 4.05 (s, 1H), 3.92 (d, *J* = 10.3 Hz, 3H), 3.85 (d, *J* = 16.2 Hz, 2H), 3.79 (d, *J* = 10.5 Hz, 4H), 3.59 (s, 1H), 3.41 (s, 1H), 3.36 (s, 3H), 3.00 (s, 1H), 1.46 (s, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 151.0, 138.1, 135.4, 128.8, 127.9, 126.6, 111.7, 86.6, 83.4, 83.3, 81.5, 78.5, 77.2, 71.6, 59.3, 58.9, 55.4, 52.6, 27.5, 12.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.4. HRMS (ESI) calculated for $C_{22}H_{32}N_2O_{10}P$ $[M+H]^+$ *m/z* = 515.1789, found 515.1792.



5.25 **5.27**

Compound 5.25

Pyridine (0.38 mL, 4.68 mmol) and trifluoromethanesulfonic anhydride (0.38 ml, 2.34 mmol) were added to a stirred solution of nucleoside **5.25** (1.11 g, 2.23 mmol) in CH_2Cl_2 (15 mL) at 0 °C. After 20 min at the same temperature, the reaction was quenched with 1 M HCl (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (3% MeOH in CH_2Cl_2) to give nucleoside **5.27** as a white foam (628 mg, 58%) R*f* = 0.50 (6% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 3H), 7.66 (d, *J* = 1.1 Hz, 3H), 7.45 – 7.27 (m, 15H), 5.89 (s, 3H), 5.29 (s, 3H), 4.81 (d, *J* = 11.5 Hz, 3H), 4.59 (d, *J* = 11.5 Hz, 3H), 4.40 – 4.30 (m, 3H), 4.24 – 4.12 (m, 6H), 4.02 (dd, *J* = 10.4, 8.5 Hz, 3H), 3.84 (d, *J* = 10.6 Hz, 19H), 3.54 (s, 9H), 3.37 (d, *J* = 13.0 Hz, 3H), 2.46 – 2.34 (m, 3H), 2.16 (s, 1H), 1.34 (d, *J* = 0.9 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 150.2, 137.6, 135.5, 128.8, 128.3, 127.8, 110.4, 88.5, 84.4, 81.4, 81.2, 77.2, 73.8, 71.9, 71.3, 67.6, 67.4, 58.1, 53.9, 53.8, 53.6, 53.5, 38.4, 11.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.81; HRMS (ESI) calculated for $C_{22}H_{33}N_3O_9P$ $[M+NH_4]^+$ *m/z* = 514.1949, found 514.1959.

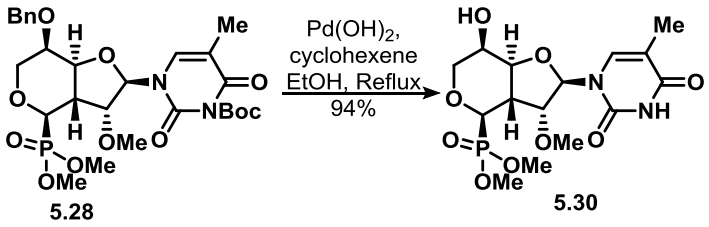


5.25 **5.28**

Compound 5.28

Pyridine (60 μL, 0.74 mmol) and trifluoromethanesulfonic anhydride (59.4 μL, 0.353 mmol) were added to an stirred solution of nucleoside **5.25** (207 mg, 0.337 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 20 min at the same temperature, the reaction was quenched with 1 M HCl (2 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts

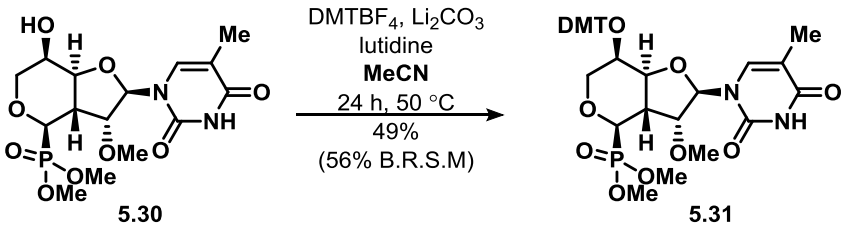
were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (3% MeOH in CH_2Cl_2) to give nucleoside **5.28** as a white foam (161 mg, 80%) R*f* = 0.47 (3% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 1.1 Hz, 1H), 7.41 – 7.27 (m, 5H), 5.87 (s, 1H), 4.82 (d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.36 (d, *J* = 13.1 Hz, 1H), 4.23 – 4.12 (m, 2H), 4.06 – 3.98 (m, 1H), 3.90 (d, *J* = 4.2 Hz, 1H), 3.86 (dd, *J* = 10.6, 4.0 Hz, 6H), 3.54 (s, 3H), 3.37 (d, *J* = 13.0 Hz, 1H), 2.44 – 2.31 (m, 1H), 1.61 (s, 10H), 1.33 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.5, 148.3, 148.2, 137.6, 134.9, 128.8, 128.3, 127.8, 110.1, 88.7, 87.0, 84.2, 81.5, 81.3, 77.2, 73.9, 73.7, 72.0, 71.2, 67.5, 67.4, 58.1, 53.8, 53.6, 38.4, 27.6, 12.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.81; HRMS (ESI) calculated for $C_{27}H_{37}N_2O_{11}PNa$ $[M+Na]^+$ *m/z* = 619.2027, found 619.2043.



Compound 5.30

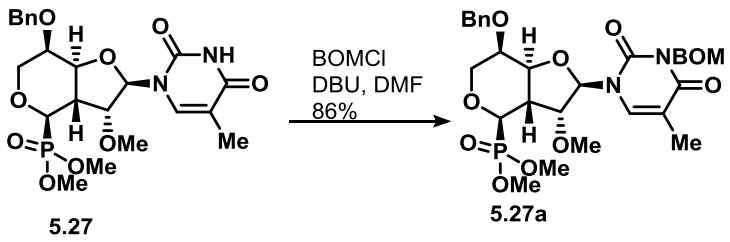
To a stirred solution of compound **5.28** (161 mg, 0.269 mmol) in ethanol (5 mL) was added 20% $Pd(OH)_2$ (75.8 mg, 0.108 mmol) and cyclohexene (1.1 mL, 10.9 mmol). The resulting mixture was heated at reflux overnight, then the reaction mixture was filtered through a Celite® pad and the solution was evaporated under reduced pressure. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give nucleoside **5.30** as a white foam (103 mg, 94%) R*f* = 0.40 (8% MeOH in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.83 (s, 1H), 5.86 (s, 1H), 4.35 (s, 1H), 4.09 (d, *J* = 11.9 Hz, 2H), 3.96 (dd, *J* = 10.2, 8.7 Hz, 1H), 3.88 (d, *J* = 4.2

Hz, 1H), 3.86 – 3.76 (m, 6H), 3.52 (d, J = 10.4 Hz, 4H), 2.41 – 2.26 (m, 1H), 1.84 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.48, 150.40, 135.92, 110.25, 88.51, 84.24, 81.73, 81.56, 73.66, 72.17, 72.01, 71.90, 66.84, 57.98, 54.23, 54.16, 53.36, 53.30, 37.77, 12.67; ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.04.



Compound 5.31

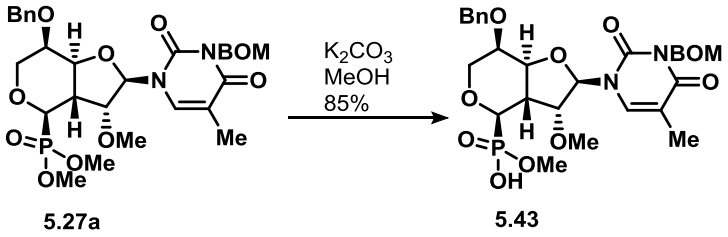
In a round-bottom flask equipped with a septum, compound **5.30** (111 mg, 0.273 mmol), $DMTBF_4$ (213 mg, 0.546 mmol), Li_2CO_3 (60 mg, 0.812 mmol) were added and the flask was then flushed with argon for 10 min. Acetonitrile (2 mL) and lutidine (0.16 mL, 1.38 mmol) were added and the reaction mixture was heated at 50 °C for 24 h. The reaction mixture was then cooled down, diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (4% MeOH in CH_2Cl_2) to give nucleoside **5.31** as a white (95 mg, 49%) and 14 mg of **5.30** recovered. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 7.70 (d, J = 1.5 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.46 – 7.33 (m, 4H), 7.32 – 7.22 (m, 5H), 6.87 – 6.77 (m, 4H), 5.98 (s, 1H), 4.41 – 4.35 (m, 1H), 4.06 (dd, J = 11.6, 2.8 Hz, 1H), 3.97 (dd, J = 10.6, 7.7 Hz, 1H), 3.87 (dd, J = 17.7, 10.5 Hz, 7H), 3.77 (d, J = 3.3 Hz, 6H), 3.54 (s, 3H), 2.75 (d, J = 12.9 Hz, 1H), 2.70 – 2.57 (m, 1H), 2.54 – 2.44 (m, 1H), 0.97 (d, J = 1.2 Hz, 3H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.24.



5.27 5.27a

Compound 5.27a

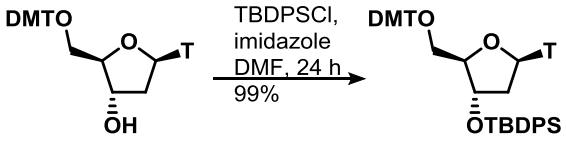
DBU (58 μL) and 70 % benzyl chloromethyl ether (113 μL) were added sequentially to a stirred solution of **5.27** (158 mg) in DMF (1.6 mL) at 0°C. After 1h at the same temperature, the reaction was diluted with water (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (100% EtOAc) to give nucleoside **5.27a** as a white foam (158 mg, 80%) R*f* = 0.41 (2% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, *J* = 10.2 Hz, 1H), 7.46 – 7.25 (m, 11H), 5.92 (d, *J* = 1.0 Hz, 1H), 5.49 (td, *J* = 10.1, 0.9 Hz, 2H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.72 (s, 2H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.37 (d, *J* = 13.0 Hz, 1H), 4.21 (d, *J* = 12.5 Hz, 2H), 4.05 (t, *J* = 9.3 Hz, 1H), 3.94 – 3.83 (m, 7H), 3.60 (d, *J* = 0.9 Hz, 3H), 3.40 (d, *J* = 13.2 Hz, 1H), 2.48 – 2.36 (m, 1H), 1.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 150.7, 138.0, 137.5, 134.2, 128.7, 128.3, 128.2, 127.8, 127.74, 127.70, 109.6, 88.9, 84.2, 81.3, 81.1, 77.2, 73.7, 73.6, 72.3, 71.8, 71.1, 70.4, 67.4, 67.3, 57.9, 53.8, 53.8, 53.45 53.4, 38.3, 12.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.82; HRMS (ESI) calculated for $C_{30}H_{38}N_2O_{10}P$ $[M+H]^+$ *m/z* = 617.2259, found 617.2269.



5.27a 5.43

Compound 5.43

Potassium carbonate (53 mg) was added to a stirred solution of **5.27a** (117 mg) in methanol (1 mL). The resulting suspension was heated at 80°C for 24 h. The reaction mixture was cooled down and pre-adsorbed in silica gel. The residue was purified by flash chromatography (10% MeOH in CH_2Cl_2) to give nucleoside **5.43** as a white foam (96.7 mg, 85%) R*f* = 0.20 (8% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.24 (m, 11H), 5.87 (s, 1H), 5.42 (dd, *J* = 29.3, 9.6 Hz, 2H), 4.75 (d, *J* = 11.6 Hz, 1H), 4.68 (s, 2H), 4.61 (d, *J* = 11.6 Hz, 1H), 4.32 – 4.02 (m, 5H), 3.94 (d, *J* = 3.1 Hz, 1H), 3.81 (t, *J* = 8.8 Hz, 1H), 3.60 (d, *J* = 9.9 Hz, 3H), 3.53 (s, 3H), 3.32 (d, *J* = 12.7 Hz, 1H), 2.25 (s, 1H), 1.45 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 150.8, 138.0, 137.3, 134.4, 128.9, 128.4, 128.3, 127.8, 127.8, 127.8, 109.6, 89.0, 84.5, 81.2, 81.0, 77.2, 74.1, 72.3, 71.8, 70.4, 67.6, 67.5, 57.6, 52.8, 39.4, 12.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.88; HRMS (ESI) calculated for $C_{29}H_{36}N_2O_{10}P$ $[M+H]^+$ *m/z* = 603.2102, found 603.2111.

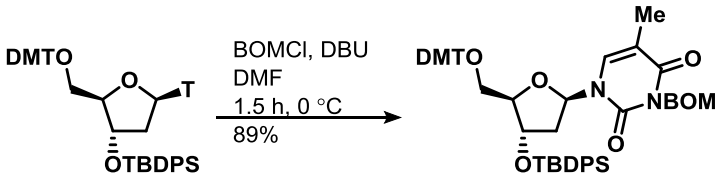


5.38 5.39

Compound 5.39

Commercially available **5.38** (1.0 g, 1.8 mmol) was dissolved in DMF (1 mL) and then sequentially were added imidazole (312 mg, 4.58 mmol) and TBDPSCl (0.54 mL, 2.07 mmol).

The reaction mixture was stirred for 24 h, then water (5 mL) was added and the resulting slurry was extracted with ether (4 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (1:1 EtOAc/hexanes) to give nucleoside **5.39** as a white foam (1.42 g, 99%). ^1H and ^{13}C matches with the reported data[**]; HRMS (ESI) calculated for $C_{47}H_{50}N_2O_7SiNa$ $[M+Na]^+$ $m/z = 805.3279$, found 805.3284.



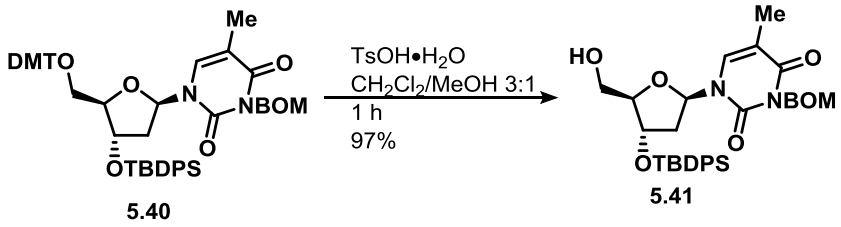
5.39 5.40

Compound 5.40

DBU (75.0 µL, 0501 mmol) and 70 % benzyl chloromethyl ether (115 µL, 0.578 mmol) were added sequentially to a stirred solution of **5.39** (326 mg, 0.416 mmol) in DMF (6 mL) at 0°C. After 1.5 h at the same temperature, the reaction was diluted with water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 EtOAc/hexanes) to give nucleoside **5.40** as a white foam (335 mg, 89%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 – 7.61 (m, 4H), 7.57 (s, 1H), 7.46 – 7.18 (m, 23H), 6.82 (dd, J = 8.9, 7.2 Hz, 4H), 6.62 (dd, J = 8.1, 5.7 Hz, 1H), 5.54 (s, 2H), 4.76 (s, 2H), 4.68 (s, 1H), 4.66 – 4.61 (m, 1H), 4.17 (d, J = 2.3 Hz, 1H), 3.80 (d, J = 1.3 Hz, 6H), 3.33 (dd, J = 10.5, 2.7 Hz, 1H), 3.00 (dd, J = 10.5, 2.7 Hz, 1H), 2.47 (ddd, J = 13.1, 5.6, 2.1 Hz, 1H), 2.19 – 2.10 (m, 1H), 1.46 (s, 3H), 1.13 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.4, 158.5, 158.5, 150.9, 144.2, 141.1, 138.0, 135.6,

[**] *J. Am. Chem. Soc.*, **2000**, *122* (27), 6512–6513.

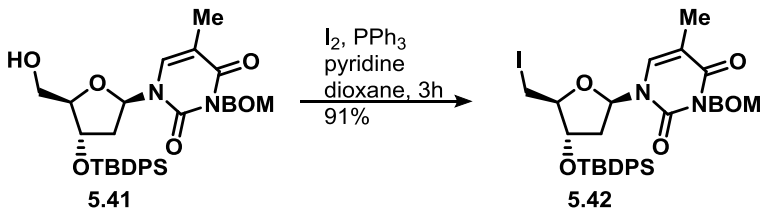
135.5, 135.2, 135.2, 134.4, 133.0, 133.0, 130.0, 129.9, 128.3, 128.2, 128.0, 127.8, 127.8, 127.5, 127.5, 127.3, 126.9, 126.8, 113.1, 110.2, 86.7, 85.5, 77.2, 73.8, 72.1, 70.5, 64.9, 63.2, 60.3, 55.1, 41.1, 26.8, 20.9, 18.9, 14.1, 12.3; HRMS (ESI) calculated for $C_{55}H_{58}N_2O_8SiNa$ $[M+Na]^+$ *m/z* = 925.3855, found 925.3849.



5.40 5.41

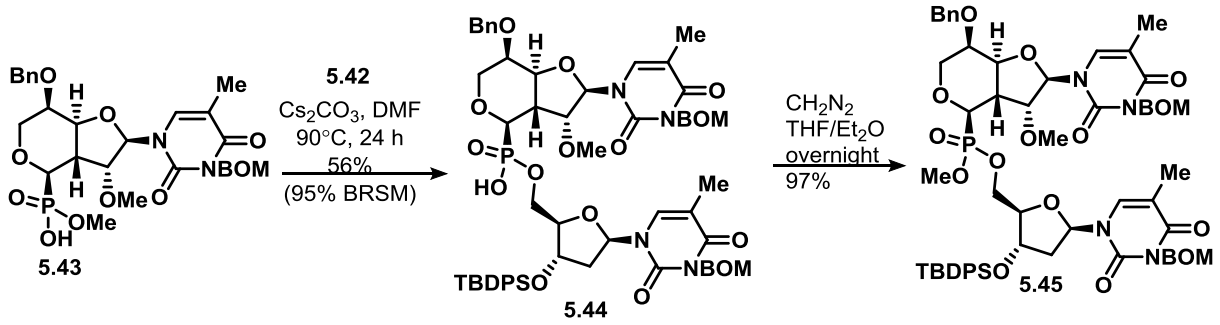
Compound 5.41

To a stirred solution of **5.40** (335 mg, 0.371 mmol) in 3:1 CH_2Cl_2/MeOH (30 mL) was added $TsOH \cdot H_2O$ (105 mg, 0.551 mmol). After 40 min at the same temperature, the reaction was quenched with solid Na_2CO_3 until the orange color disappeared. Water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (4 x 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 EtOAc/hexanes) to give nucleoside **5.41** as a white foam (217 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 – 8.08 (m, 4H), 7.94 – 7.70 (m, 12H), 6.75 (dd, *J* = 7.7, 6.1 Hz, 1H), 5.93 (s, 2H), 5.14 (s, 2H), 4.95 – 4.87 (m, 1H), 4.45 (d, *J* = 2.7 Hz, 1H), 4.09 (dd, *J* = 11.9, 2.4 Hz, 1H), 3.72 (dd, *J* = 11.9, 2.8 Hz, 1H), 2.75 (ddd, *J* = 13.3, 6.0, 2.9 Hz, 1H), 2.66 – 2.47 (m, 2H), 2.31 (d, *J* = 1.0 Hz, 3H), 1.56 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 151.1, 138.0, 135.8, 135.8, 135.5, 133.3, 133.3, 130.2, 130.2, 128.3, 128.0, 127.7, 127.7, 110.3, 87.7, 87.3, 77.2, 73.1, 72.3, 70.6, 62.2, 40.5, 27.0, 19.1, 13.3; HRMS (ESI) calculated for $C_{34}H_{41}N_2O_6Si$ $[M+H]^+$ *m/z* = 601.2728, found 601.2738.



Compound 5.42

To a stirred solution of nucleoside **5.41** (217 mg, 0.361 mmol) in 1,4-dioxane (2 mL) was added iodine (138 mg, 0.543 mmol), triphenylphosphine (142 mg, 0.541 mmol) and pyridine (60 µL, 0.75 mmol). After stirring for 3 h, methanol (3 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (20 mL), and a white insoluble solid was removed by filtration and discarted. The organic phase was washed sequentially with water (5 mL), 10% $Na_2S_2O_3$ (5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 EtOAc/hexanes) to give nucleoside **5.42** as a white foam (233 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 – 7.64 (m, 4H), 7.52 – 7.22 (m, 13H), 6.43 (dd, *J* = 8.1, 5.9 Hz, 1H), 5.50 (s, 2H), 4.71 (s, 2H), 4.25 – 4.20 (m, 1H), 3.77 (dd, *J* = 7.2, 3.9 Hz, 1H), 3.13 (dd, *J* = 11.0, 4.0 Hz, 1H), 2.73 (dd, *J* = 11.0, 4.1 Hz, 1H), 2.41 – 2.35 (m, 1H), 1.99 – 1.92 (m, 1H), 1.91 (d, *J* = 0.9 Hz, 3H), 1.12 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.3, 150.9, 138.0, 135.8, 135.7, 134.5, 133.0, 132.8, 130.3, 130.2, 128.3, 128.1, 128.0, 127.7, 127.6, 110.5, 84.9, 84.5, 77.2, 76.2, 72.2, 70.6, 40.5, 26.9, 19.0, 13.3, 7.4; HRMS (ESI) calculated for $C_{34}H_{39}N_2O_5ISi$ $[M+H]^+$ *m/z* = 711.1746, found 711.1750; HRMS (ESI) calculated for $C_{34}H_{38}N_2O_5ISiNa$ $[M+Na]^+$ *m/z* = 733.1565, found 733.1574.



Compound 5.45

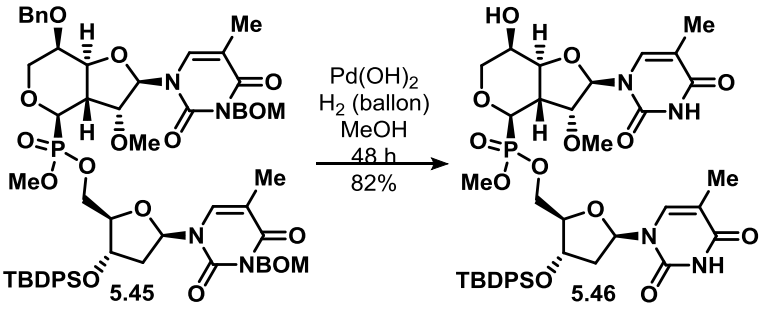
To a stirred solution of **5.43** (146.2 mg, 0.242 mmol) in DMF (2 mL) was added nucleoside **5.42** (224 mg, 0.315 mmol) and cesium carbonate (118 mg, 0.363 mmol). The resulting suspension was heated at 90 °C for 24 h. Water (10 mL) and 1 M HCl was added until pH was around 2, then the resulting mixture was extracted with EtOAc (4 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (6% MeOH in CH_2Cl_2 + 1% NH_4OH to 12% MeOH in CH_2Cl_2 + 1% NH_4OH) to give nucleoside **5.44** as a white foam (166 mg, 57%) and starting material **10** recovered (57 mg). A solution of **5.44** (44 mg, 0.073 mmol) in THF (1 mL) was mixed a previously prepared solution of diazomethane in ether (see below). The reaction mixture was allowed to react overnight, then methanol (10 mL) was added and stirred for 10 min. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (1% to 4% MeOH in CH_2Cl_2) to give nucleoside **5.45** as a white foam (43 mg, 97%). **1:1 mixture of P-Isomers**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.62 (m, 4H), 7.49 – 7.26 (m, 23H), 6.49 (dt, *J* = 9.5, 5.0 Hz, 1H), 5.88 (d, *J* = 2.0 Hz, 1H), 5.52 – 5.41 (m, 4H), 4.79 – 4.71 (m, 1H), 4.71 – 4.67 (m, 4H), 4.54 (dd, *J* = 11.7, 6.3 Hz, 1H), 4.41 – 4.31 (m, 1H), 4.17 – 3.96 (m, 5H), 3.85 (dd, *J* = 17.3, 4.6 Hz, 2H), 3.70 (dd, *J* = 25.6, 10.7 Hz, 4H), 3.50 (d, *J* = 13.2 Hz, 3H), 3.16 (dd, *J* = 19.8, 12.9 Hz, 1H), 2.43 – 2.24 (m, 2H), 1.88 (d, *J* = 12.1 Hz, 3H), 1.83 – 1.73 (m, 2H), 1.37

(s, 3H), 1.09 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 163.4, 163.38, 163.3, 151.1, 151.0, 150.8, 150.7, 139.9, 138.1, 138.0, 137.3, 135.8, 134.2, 134.1, 134.0, 134.0, 133.0, 132.8, 130.4, 130.3, 130.3, 128.8, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.7, 127.7, 110.7, 110.5, 109.8, 109.6, 88.9, 88.8, 85.9, 85.86, 85.7, 85.6, 85.59, 85.53, 84.0, 83.99, 81.1, 77.2, 73.7, 73.6, 73.59, 73.53, 73.27, 72.35, 72.29, 71.8, 71.3, 70.7, 70.68, 70.5, 70.4, 67.4, 67.4, 67.3, 67.2, 65.8, 58.0, 57.9, 54.0, 52.9, 40.7, 40.5, 38.3, 26.9, 19.1, 13.0, 12.99, 12.6; ^{31}P NMR (202 MHz, $CDCl_3$) δ 20.38, 20.02.

Freshly prepared diazomethane: N-nitroso-N-methylurea (NMU) (230 mg, 2.23 mmol) was deposited in a small erlenmeyer, mixed with diethyl ether (3 mL) and cooled down in an ice bath. To the previous suspension, an aqueous solution of 40% KOH (6 mL) was added dropwise with the help of an addition funnel with constant stirring of the erlenmeyer without removing it from the ice bath (the stirring is provided by a gentle circular movement of the Erlenmeyer with the hand). Once all the KOH solution was added, no solid NMU should remain in the erlenmeyer, otherwise more 40% KOH solution can be added. After complete reaction of the NMU, the content of the erlenmeyer was allowed to stand for 1 min approx. in the ice bath to facilitate the separation of the biphasic system (top yellow layer contains the ethereal solution of diazomethane). The ice bath is then quickly replaced for an acetone/dry ice bath (-78°C) which freeze the bottom aqueous layer (1 min approx.). The organic layer can now be transferred to an appropriate recipient and storaged at -20°C. In our case, the diazomethane solution was used completely as an excess (approx. 60 equivalents of NMU with respect of the substrate to be methylated) and was not titrated in order to avoid extra manipulations).

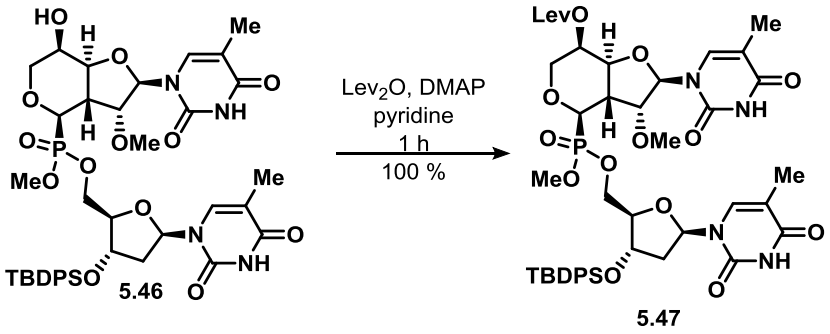
Notes: 1) NMU is carcinogen, mutagen, and teratogen, handle carefully. **2)** Diazomethane is toxic by inhalation or by contact with the skin or eyes. CH_2N_2 may explode in contact with sharp

edges, such as ground-glass joints, even scratches in glassware. **3)** Perform all the procedure in a fumehood.



Compound 5.46

A solution of **5.45** (97.2 mg, 0.082 mmol) in methanol (12 mL) was mixed with 20% $Pd(OH)_2$ (57 mg, 0.082 mmol). The flask was equipped with a three-way valve connected to a vacuum line and to a balloon filled with hydrogen. The flask was placed under vacuum until some bubbling was detected, then re-filled with hydrogen. The same procedure was performed three times. After stirring for 2 days, the reaction mixture was filtered through a Celite® pad and washed with methanol. The solvent was removed under reduced pressure and purified by flash chromatography (1% to 4% MeOH in CH_2Cl_2) to give nucleoside **5.46** (mixture of isomers) as a white foam (57.1 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (d, J = 54.4 Hz, 1H), 8.79 (d, J = 64.6 Hz, 1H), 7.75 – 7.54 (m, 5H), 7.54 – 7.29 (m, 7H), 6.49 – 6.33 (m, 1H), 5.81 (d, J = 19.7 Hz, 1H), 4.44 – 4.31 (m, 1H), 4.25 (d, J = 27.4 Hz, 1H), 4.15 – 3.91 (m, 3H), 3.91 – 3.57 (m, 7H), 3.54 – 3.39 (m, 3H), 3.32 (dd, J = 17.2, 8.6 Hz, 1H), 2.36 – 2.05 (m, 2H), 1.91 – 1.75 (m, 7H), 1.67 (s, 4H), 1.08 (s, 9H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.87, 19.95; HRMS (ESI) calculated for $C_{40}H_{52}N_4O_{13}PSi$ $[M+H]^+$ m/z = 855.3032, found 855.3044; HRMS (ESI) calculated for $C_{40}H_{51}N_4O_{13}PSiNa$ $[M+Na]^+$ m/z = 877.2852, found 877.2859.

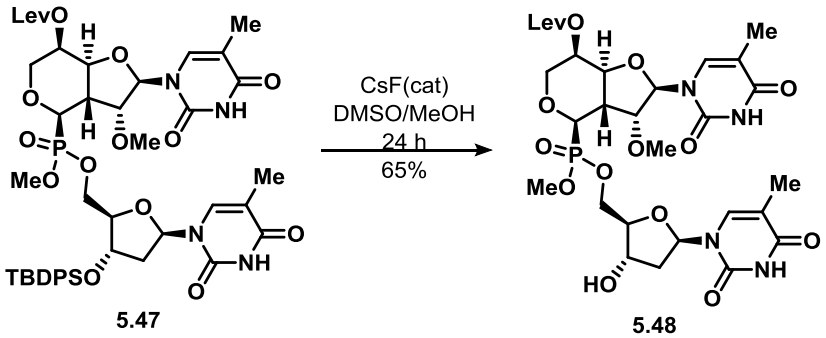


Compound 5.47

Preparation levulinic anhydride (Lev_2O): Levulinic acid (0.2 mL, 1.963 mmol) was added to a solution of DCC (197 mg, 0.954 mmol) in ether (2 mL) at r.t. After 3 h, the reaction mixture was filtered through a sintered glass filter under argon. The remaining solid was washed with ether (3 mL) giving a solution of Lev_2O (≈ 0.19 M).

To a solution of **5.46** (97.2 mg, 0.133 mmol) in pyridine (0.3 mL) was added sequentially DMAP (1 mg, 0.008 mmol) and 0.19 M Lev_2O (0.7 mL, 0.076 mmol). After stirring for 1 h, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2. The crude solution was washed with sat $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (MeOH 1% to 5% CH_2Cl_2 using Fluorosil as stationary phase) to give nucleoside **5.47** (mixture of isomers) as a white foam (53.3 mg, 100%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.07 – 8.51 (m, 2H), 7.66 – 7.60 (m, 4H), 7.49 – 7.37 (m, 7H), 7.37 – 7.27 (m, 1H), 6.47 – 6.40 (m, 1H), 5.86 – 5.77 (m, 1H), 5.34 (s, 1H), 4.34 – 4.24 (m, 1H), 4.18 – 4.05 (m, 3H), 4.01 – 3.95 (m, 1H), 3.92 – 3.88 (m, 1H), 3.87 – 3.81 (m, 1H), 3.78 – 3.64 (m, 3H), 3.55 – 3.43 (m, 3H), 3.34 – 3.29 (m, 1H), 2.90 – 2.70 (m, 2H), 2.66 – 2.58 (m, 2H), 2.37 – 2.15 (m, 5H), 1.92 – 1.81 (m, 7H), 1.08 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.2, 172.2, 163.7, 163.6, 163.58, 150.3, 150.0, 135.9, 135.8, 135.5, 135.3, 134.7, 134.68, 133.0, 132.9, 130.4, 130.4, 130.36, 128.2, 128.19, 128.16, 111.4,

111.3, 110.6, 110.5, 89.0, 88.9, 85.5, 85.4, 85.3, 83.8, 83.5, 77.2, 73.6, 73.3, 71.9, 68.8, 68.7, 66.7, 65.7, 65.6, 58.0, 57.9, 40.5, 40.3, 39.2, 39.1, 37.8, 29.9, 29.8, 28.3, 26.9, 19.1, 12.8, 12.4, 12.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.85, 19.79. HRMS (ESI) calculated for $C_{45}H_{58}N_4O_{15}PSi$ $[M+H]^+$ *m/z* = 953.3400, found 953.3414; HRMS (ESI) calculated for $C_{45}H_{57}N_4O_{15}PSiNa$ $[M+H]^+$ *m/z* = 975.3220, found 975.3235.



5.47 5.48

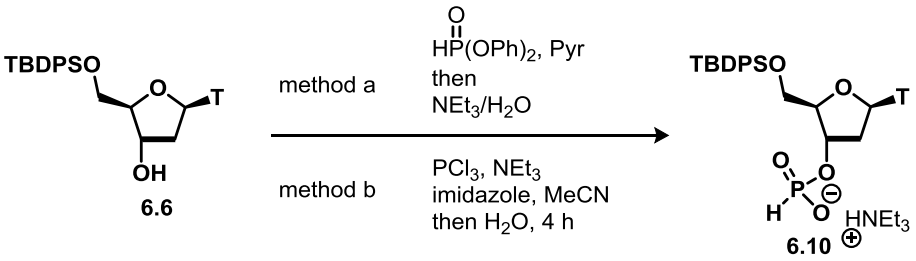
Compound 5.48

Preparation of ≈ 0.71 M CsF solution: CsF (19.6 mg, 0.129 mmol) was mixed with anhydrous DMSO (60 μL) and the remaining mixture was sonicated for 15 min. MeOH (120 μL) was added to the previous solution and sonicated again for 5 min.

A solution of **5.47** (45.4 mg, 0.0635 mmol) in DMSO (1 mL) was mixed with the ≈ 0.71 M CsF solution (5 μL, 0.00635 mmol). After stirring for 24h, the reaction mixture was diluted with water (3 mL) and extracted with EtOAc (5 x 5 mL). The combined organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (8% MeOH in CH_2Cl_2) to give nucleoside **5.48** (mixture of isomers) as a white foam (22 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 – 7.28 (m, 2H), 6.17 – 6.12 (m, 1H), 5.73 (d, *J* = 12.2 Hz, 1H), 5.29 (s, 1H), 4.37 – 4.20 (m, 4H), 4.17 – 4.09 (m, 2H), 4.05 – 3.94 (m, 2H), 3.94 – 3.82 (m, 2H), 3.81 – 3.71 (m, 3H), 3.66 (dd, *J* = 12.1, 3.2 Hz, 1H), 3.44 (s, 3H), 3.30 – 3.20 (m, 1H), 2.75 (q, *J* = 6.8, 6.2 Hz, 2H), 2.66 – 2.50 (m, 2H),

2.27 – 2.10 (m, 6H), 1.82 – 1.78 (m, 7H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.2, 172.2, 164.6, 164.4, 164.39, 150.8, 150.6, 150.3, 136.7, 135.7, 135.6, 135.0, 134.9, 111.1, 111.0, 110.8, 110.4, 110.3, 89.0, 89.0, 87.1, 85.4, 85.4, 85.0, 84.8, 83.5, 83.5, 78.8, 78.7, 77.2, 73.2, 72.9, 71.7, 70.7, 70.6, 70.1, 68.8, 68.6, 68.5, 61.6, 57.8, 57.6, 54.1, 53.3, 53.3, 40.2, 39.9, 39.8, 39.1, 37.7, 29.6, 28.0, 12.4, 12.2, 12.1; ^{31}P NMR (202 MHz, $CDCl_3$) δ 20.78, 20.33; HRMS (ESI) calculated for $C_{29}H_{40}N_4O_{15}P$ $[M+H]^+$ *m/z* = 715.2222, found 715.2214.

8.2.4 Synthesis of backbone constrained macrocycles



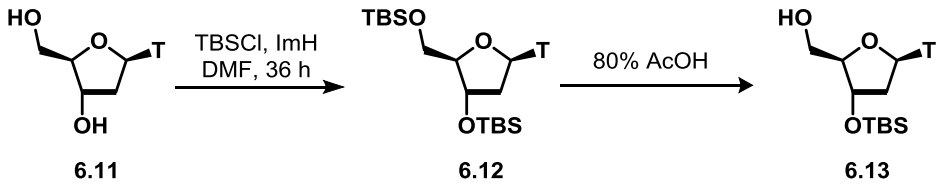
Compound 6.10

Method a

To a stirred solution of nucleoside **6.6** (1.3065 g, 2.71 mmol) in pyridine (14 mL) was added diphenyl phosphite (3.6 mL, 18.8 mmol). After 15 min, a 1:1 mixture of triethylamine:water (6 mL) was added and the resulting mixture was stirred for 15 min after which the solvent was removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (150 mL) and washed with aqueous 5% $NaHCO_3$ (3 x 60 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10 % MeOH in CH_2Cl_2 + 1% NEt_3) to give nucleoside **6.10** (1.423 mg, 81%) Rf = 0.31 (10 % MeOH in CH_2Cl_2 + 1% NEt_3).

Method b

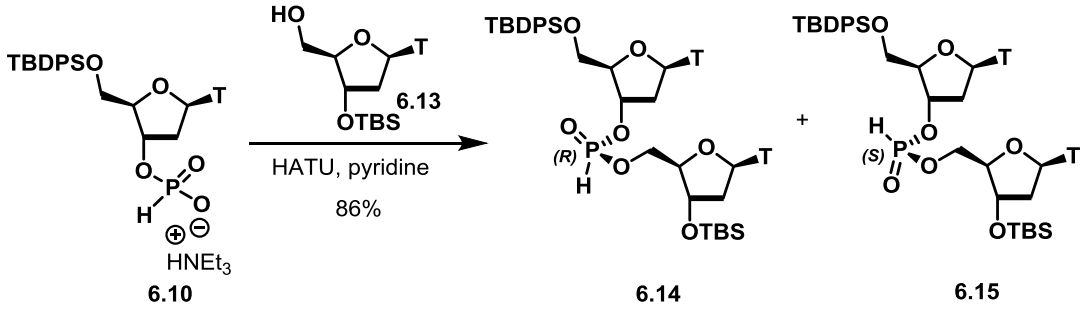
Phosphorus trichloride (0.289 mL, 3.31 mmol) and triethylamine (1.38 mL, 9.92 mmol) were added sequentially to a stirred solution of imidazole (675 mg, 9.92 mmol) in acetonitrile (20 mL) at 0 °C. After stirring for 30 min, a solution of nucleoside **6.6** (318 mg, 0.661 mmol) in acetonitrile (15 mL) was added, the ice bath was removed and the reaction mixture was allowed to react at room temperature for 3 h. Water (6.2 mL) was added to the reaction mixture and stirred for 30 min then the solvent was removed under reduced pressure. The crude residue was dissolved in a mixture containing pyridine (5 mL) and triethylamine (1 mL), and then evaporated to dryness. The dry residue was partitioned between water and CH_2Cl_2, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10 % MeOH in CH_2Cl_2 + 1% NEt_3) to give nucleoside **6.10** (385 mg, 83%) Rf = 0.31 (10 % MeOH in CH_2Cl_2 + 1% NEt_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H, *J* = 617.2 Hz, H-P), 7.60 (td, *J* = 7.6, 7.0, 1.7 Hz, 4H), 7.42 – 7.26 (m, 7H), 6.39 (dd, *J* = 8.8, 5.3 Hz, 1H), 6.11 (s, *J* = 617.2 Hz, 1H H-P), 4.95 (td, *J* = 7.2, 2.3 Hz, 1H), 4.21 – 4.15 (m, 1H), 3.99 – 3.83 (m, 2H), 3.00 (q, *J* = 7.3 Hz, 7H), 2.50 (ddd, *J* = 13.3, 5.3, 1.9 Hz, 1H), 2.22 – 2.12 (m, 1H), 1.40 (s, 3H), 1.26 (t, *J* = 7.3 Hz, 11H), 1.02 (s, 9H). ; ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.53; ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.13, 150.73, 135.50, 135.23, 135.19, 133.16, 132.33, 129.98, 129.87, 127.93, 127.85, 111.08, 86.16, 86.10, 84.30, 73.75, 73.71, 64.07, 45.54, 39.70, 39.67, 26.99, 19.34, 11.83, 8.62;^{31}P NMR (162 MHz, $CDCl_3$) δ 3.53.



6.11 6.12 6.13

Compound 6.13[198]

Imidazole (10.2 g, 0.15 mol) and TBSCl (17.8 mL, 0.125 mol) were added to a stirred solution of thymidine **6.11** (12.1 g, 0.05 mol) in pyridine (200 mL). After stirring overnight, pyridine was evaporated and the residue was diluted with ethyl acetate, washed with brine(x3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude **6.12**. The residue was dissolved in 80% AcOH (300 mL) and the resulting solution stood at room temperature for 4 days. Solvents were evaporated and the residue was passed through a short silica column (3:7 EtOAc/hex) to give 11.3 g (63 %) of **6.13** as a white solid. The ^1H and ^{13}C NMR matches with the reported data.[233]



6.10 6.14 6.15

Compounds 6.14 and 6.15

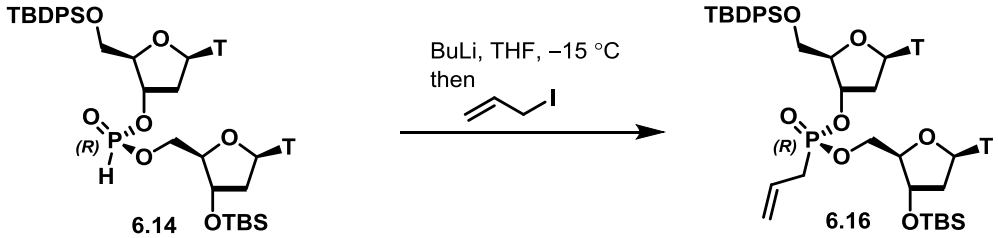
Compounds **6.10** (2.0 g, 3.1 mmol) and **6.13** (1.66 g, 4.65 mmol) were mixed in a flask with anhydrous pyridine (5 mL) and evaporated to dryness. The dry residue was dissolved in anhydrous pyridine (40 mL) and HATU (2.4 g, 6.19 mmol) was added. The reaction mixture

was stirred for 3 h then the solvent was removed under reduced pressure. The dry residue was dissolved in EtOAc (50 mL) and a white precipitate formed was filtered off. The organic later was washed with 5% LiCl (5 x 5 mL). The organic extract was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography () to give nucleoside **6.14** (1.2 g), **6.15** (0.50 g) and 0.5 g of a mixture containing **6.14** and **6.15**.

6.14: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.25 (s, 1H), 10.19 (s, 1H), 7.60 (t, *J* = 7.7 Hz, 5H), 7.43 – 7.29 (m, 9H), 6.38 (dd, *J* = 8.9, 5.5 Hz, 1H), 6.21 – 6.17 (m, 1H), 5.25 (t, *J* = 6.8 Hz, 1H), 4.44 – 4.31 (m, 2H), 4.27 – 4.13 (m, 2H), 4.00 – 3.90 (m, 2H), 3.90 – 3.82 (m, 1H), 2.75 (s, 2H), 2.58 (dd, *J* = 14.1, 5.4 Hz, 1H), 2.34 – 2.19 (m, 3H), 1.87 (s, 3H), 1.53 (s, 3H), 1.04 (s, 9H), 0.85 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.19, 164.04, 150.64, 150.48, 135.89, 135.43, 135.39, 135.16, 135.09, 134.60, 132.52, 131.85, 130.17, 130.04, 127.99, 127.92, 127.85, 127.81, 111.54, 111.10, 85.60, 85.25, 85.20, 84.64, 84.58, 84.06, 77.36, 71.12, 64.71, 64.66, 63.58, 40.19, 39.26, 38.50, 26.89, 25.64, 25.59, 19.25, 19.21, 17.77, 12.36, 11.95, 11.91, -4.74, -4.96; ^{31}P NMR (202 MHz, $CDCl_3$) δ 7.36; HRMS (ESI) calculated for $C_{42}H_{60}N_4O_{11}PSi_2$ $[M+H]^+$ *m/z* = 883.35292, found 833.35502.

6.15: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (d, *J* = 24.1 Hz, 2H), 7.84 (d, *J* = 2.4 Hz, 1H), 7.64 (td, *J* = 8.2, 1.6 Hz, 4H), 7.49 – 7.35 (m, 7H), 7.30 (s, 1H), 6.41 (dd, *J* = 9.1, 5.2 Hz, 1H), 6.22 (t, *J* = 6.6 Hz, 1H), 6.05 (s, 1H), 5.24 (t, *J* = 7.0 Hz, 1H), 4.38 – 4.31 (m, 1H), 4.30 – 4.14 (m, 3H), 4.04 – 3.88 (m, 3H), 2.56 (dd, *J* = 13.9, 5.3 Hz, 1H), 2.33 – 2.23 (m, 2H), 2.23 – 2.13 (m, 1H), 1.92 (d, *J* = 1.2 Hz, 3H), 1.57 (s, 3H), 1.09 (s, 9H), 0.88 (d, *J* = 2.8 Hz, 10H), 0.08 (t, *J* = 2.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.75, 150.57, 150.38, 135.83, 135.64, 135.34, 134.82, 132.75, 132.13, 130.44, 130.33, 128.26, 128.19, 111.84, 111.59, 85.86, 85.67, 85.02, 84.39, 71.35, 63.75, 40.58, 27.15, 25.79, 19.51, 18.02, 12.62, 12.12, -4.51, -4.73.;^{31}P NMR (162

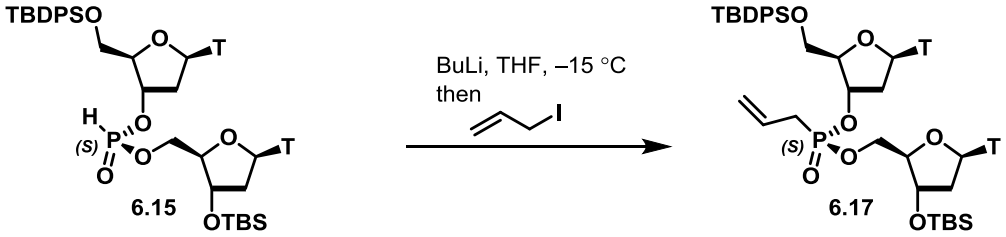
MHz, $CDCl_3$) δ 8.66; HRMS (ESI) calculated for $C_{42}H_{60}N_4O_{11}PSi_2$ $[M+H]^+$ *m/z* = 883.35292, found 833.35526.



Compound 6.16

To a stirred solution of compound **6.14** (800 mg, 0.906 mmol) in THF (150 mL) at -15 °C, a solution of 2.5 M BuLi in hexanes (1.3 mL, 3.25 mmol) was added dropwise. After 5 min, allyl iodide (0.38 mL, 4.15 mmol) was added dropwise to the reaction mixture, which was stirred at -15 °C for 30 min. The volatiles were evaporated on a rotavap system warmed at 35 °C. The residue was dissolved in EtOAc (200 mL), washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (8:2 ethyl acetate/hexane) to give compound **6.16** as a white foam (553 mg, 66%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.18 (s, 1H), 10.12 (s, 1H), 7.65 – 7.56 (m, 4H), 7.43 – 7.31 (m, 8H), 6.38 (dd, *J* = 9.1, 5.3 Hz, 1H), 6.21 (t, *J* = 6.7 Hz, 1H), 5.70 (ddq, *J* = 17.2, 10.1, 7.2 Hz, 1H), 5.23 – 5.09 (m, 3H), 4.36 (dt, *J* = 7.1, 3.7 Hz, 1H), 4.31 – 4.23 (m, 1H), 4.20 – 4.11 (m, 2H), 4.00 (q, *J* = 4.0 Hz, 1H), 3.94 (dd, *J* = 11.6, 2.6 Hz, 1H), 3.82 (dd, *J* = 11.7, 2.6 Hz, 1H), 2.69 – 2.59 (m, 2H), 2.54 (dd, *J* = 13.5, 5.3 Hz, 1H), 2.30 – 2.19 (m, 2H), 2.12 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.89 (d, *J* = 1.2 Hz, 3H), 1.53 (s, 3H), 1.04 (s, 9H), 0.85 (s, 10H), 0.05 (s, 6H).;^{13}C NMR (126 MHz, $CDCl_3$) δ 164.26, 164.07, 150.72, 150.53, 135.69, 135.46, 135.16, 134.67, 132.63, 131.95, 130.19, 130.08, 128.03, 127.96, 126.32, 126.23, 121.13, 121.01, 111.59, 111.10, 85.58, 85.53, 85.48, 85.07, 85.01, 84.13, 77.36, 77.09, 77.04, 71.48, 65.01, 64.96, 63.84, 40.47, 39.49, 32.39, 31.29, 26.94, 25.64, 19.28, 17.85, 12.38, 11.95, -4.68, -4.88;

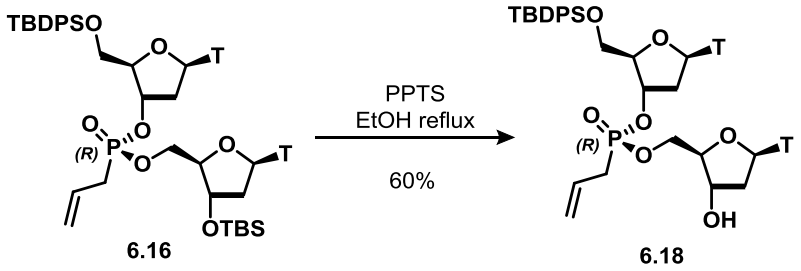
^{31}P NMR (202 MHz, $CDCl_3$) δ 27.88; HRMS (ESI) calculated for $C_{45}H_{64}N_4O_{11}PSi_2$ $[M+H]^+$ *m/z* = 923.38422, found 923.38536.



Compound 6.17

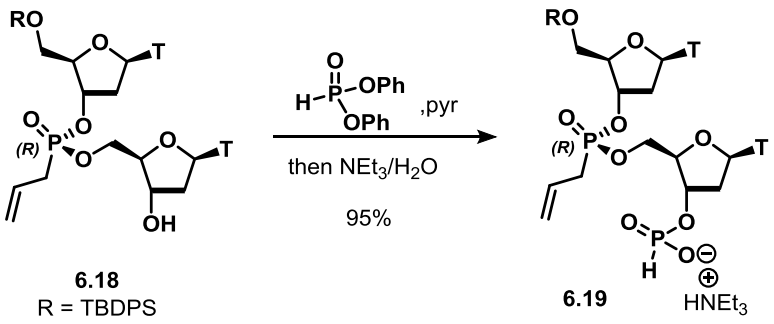
To a stirred solution of compound **6.15** (100 mg, 0.113 mmol) in THF (20 mL) at -15 °C was added dropwise a solution of 2.5 M BuLi in hexanes (0.16 mL, 0.39 mmol). After 5 min, allyl iodide (50 µL, 0.50 mmol) was added dropwise and the reaction mixture was kept at -15 °C for 30 min, followed by removal of the solvent aided by a rotavap system warmed at 35 °C. The crude residue was dissolved in EtOAc (20 mL), washed with water and brine. The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (8:2 ethyl acetate/hexane) to give compound **6.17** as a white foam (65 mg, %); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 15.4 Hz, 2H), 7.69 – 7.60 (m, 4H), 7.49 – 7.36 (m, 8H), 7.30 – 7.27 (m, 1H), 6.38 (dd, *J* = 9.2, 5.2 Hz, 1H), 6.20 (t, *J* = 6.7 Hz, 1H), 5.89 – 5.67 (m, 1H), 5.33 – 5.14 (m, 3H), 4.30 (dt, *J* = 6.7, 3.5 Hz, 1H), 4.26 – 4.10 (m, 2H), 4.10 – 3.81 (m, 4H), 2.69 (ddd, *J* = 22.0, 7.5, 1.4 Hz, 2H), 2.52 (dd, *J* = 13.7, 5.3 Hz, 1H), 2.34 – 2.17 (m, 2H), 2.17 – 2.00 (m, 1H), 1.99 – 1.89 (m, 3H), 1.57 (d, *J* = 1.2 Hz, 3H), 1.09 (s, 9H), 0.88 (s, 9H), 0.08 – 0.04 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.84, 163.80, 150.58, 150.34, 135.66, 135.54, 135.35, 134.86, 132.77, 132.19, 130.38, 130.30, 128.22, 128.17, 126.44, 126.33, 121.46, 121.31, 111.73, 111.37, 86.00, 85.96, 85.62, 85.38, 85.32, 84.41, 77.05, 71.68, 65.10, 65.04, 63.93, 60.51, 40.75, 39.57, 39.52, 32.74, 31.36, 27.14,

25.79, 21.16, 19.51, 18.02, 14.32, 12.69, 12.64, 12.12, -4.53, -4.55, -4.72, -4.75; ^{31}P NMR (121 MHz, $CDCl_3$) δ 28.65.



Compound 6.18

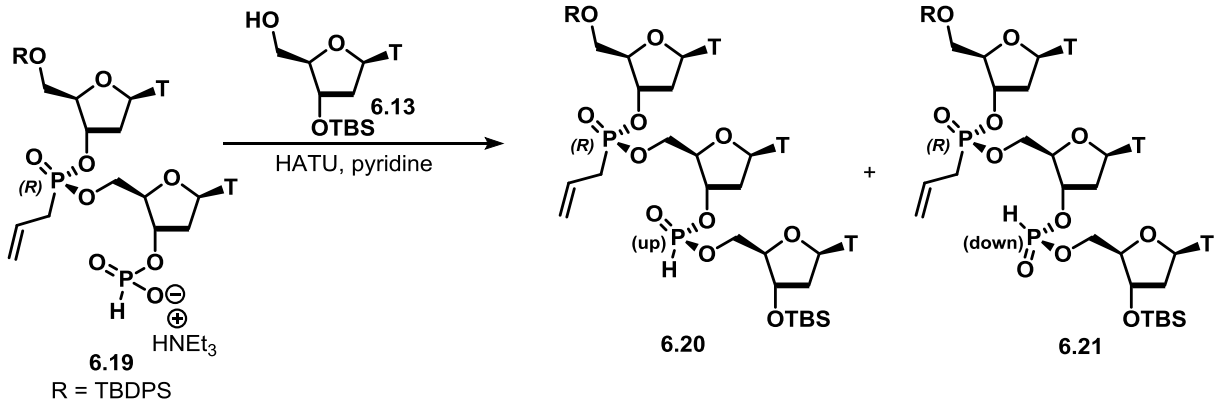
To a stirred solution of compound **6.16** (553 mg, 0.599 mmol) in ethanol (3 mL) was added PPTS (301 mg, 1.19 mmol) and the resulting solution was heated to reflux for 36 h. The reaction mixture was cooled to room temperature and combined with $NaHCO_{3\ (sat)}$ and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2 neat to 6% MeOH in CH_2Cl_2) to give compound **6.18** as a white foam (290 mg, 60%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (s, 1H), 9.96 (s, 1H), 7.70 – 7.59 (m, 4H), 7.50 – 7.34 (m, 8H), 6.34 (dd, *J* = 9.4, 4.9 Hz, 1H), 6.25 (t, *J* = 6.6 Hz, 1H), 5.70 (ddt, *J* = 17.0, 9.9, 7.2 Hz, 1H), 5.26 – 5.07 (m, 3H), 4.64 – 4.22 (m, 4H), 4.19 – 4.08 (m, 2H), 3.94 (dd, *J* = 11.8, 2.8 Hz, 1H), 3.79 (dd, *J* = 11.8, 2.6 Hz, 1H), 2.75 – 2.55 (m, 3H), 2.51 – 2.40 (m, 1H), 2.36 – 2.11 (m, 3H), 1.90 (s, 3H), 1.59 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.33, 164.08, 151.32, 150.79, 135.92, 135.66, 135.38, 134.69, 132.65, 132.08, 130.43, 130.32, 128.24, 128.18, 126.40, 126.29, 121.41, 121.26, 112.07, 111.16, 85.66, 84.91, 84.59, 78.04, 71.05, 65.52, 64.12, 40.29, 40.08, 32.80, 31.42, 27.11, 19.42, 12.64, 12.20; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.28. HRMS (ESI) calculated for $C_{39}H_{50}N_4O_{11}PSi$ $[M+H]^+$ *m/z* = 809.2977, found 809.2984.



Compound 6.19

To a stirred solution of compound **6.18** (290 mg, 0.358 mmol) in pyridine (1.8 mL) was added diphenyl phosphite (0.48 mL, 2.50 mmol). After 15 min, a 1:1 mixture of triethylamine:water (2 mL) was added and the resulting mixture was stirred for 15 min after which the solvent was removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with aqueous 5% $NaHCO_3$ (3 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10 % MeOH in CH_2Cl_2 + 1% NEt_3) to give nucleoside **6.19** (330 mg, 95%) Rf = (10 % MeOH in CH_2Cl_2 + 1% NEt_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (td, *J* = 8.0, 1.6 Hz, 4H), 7.45 – 7.34 (m, 8H), 6.40 – 6.23 (m, 2H), 5.70 (ddq, *J* = 17.1, 9.9, 7.2 Hz, 1H), 5.23 – 5.08 (m, 3H), 4.86 (s, 1H), 4.32 (q, *J* = 9.8 Hz, 3H), 4.12 (q, *J* = 2.3 Hz, 1H), 3.94 (dd, *J* = 11.6, 2.6 Hz, 1H), 3.81 (dd, *J* = 11.8, 2.7 Hz, 1H), 3.55 (qd, *J* = 9.4, 8.3, 4.8 Hz, 2H), 3.07 (q, *J* = 7.3 Hz, 8H), 2.70 – 2.45 (m, 4H), 2.31 – 2.08 (m, 2H), 1.92 (s, 3H), 1.50 (s, 4H), 1.34 (t, *J* = 7.3 Hz, 16H), 1.07 (s, 9H).;^{13}C NMR (126 MHz, $CDCl_3$) δ 164.08, 163.97, 150.50, 150.42, 135.21, 134.93, 134.53, 132.44, 131.77, 129.93, 129.80, 129.00, 128.71, 127.91, 127.78, 127.70, 126.14, 126.05, 124.98, 122.88, 120.86, 120.75, 120.41, 120.37, 111.08, 110.90, 85.15, 85.10, 84.59, 83.81, 83.72, 77.36, 72.51, 65.34, 65.28, 63.49, 52.39, 45.41, 39.04, 38.86, 34.01, 32.09, 30.99, 26.70, 21.58, 21.16, 19.02,

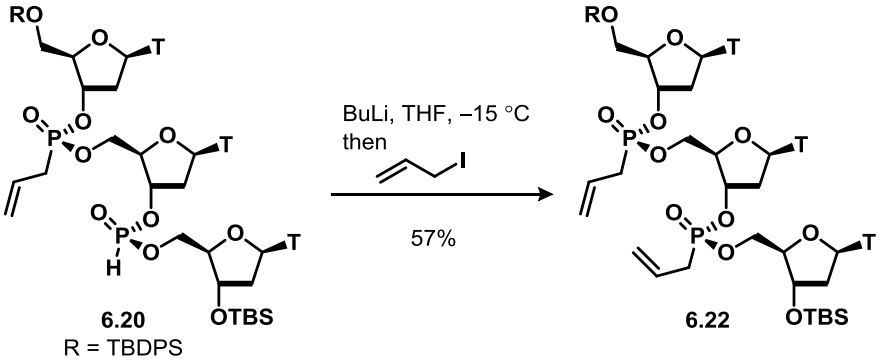
14.50, 12.13, 11.74, 8.36, 7.48; ^{31}P NMR (202 MHz, $CDCl_3$) δ 27.82, 2.82, 0.39. HRMS (ESI) calculated for $C_{39}H_{51}N_4O_{13}P_2Si$ $[M+H]^+$ *m/z* = 873.2692, found 873.2687.



Compounds 6.20 and 6.21

Compounds **6.19** (330 mg, 0.34 mmol) and **6.13** (181 mg, 0.50 mmol) were mixed in a flask with anhydrous pyridine (1 mL) and evaporated to dryness. The dry residue was dissolved in anhydrous pyridine (1.7 mL) and HATU (260 mg) was added. The reaction mixture was stirred for 3.5 h then the solvent was removed under reduced pressure. The dry residue was dissolved in EtOAc (50 mL) and a white precipitate formed was filtered off. The organic later was washed with 5% LiCl (5 x 5 mL). The organic extract was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (0% to 5% MeOH in CH_2Cl_2) to give nucleoside **6.20** (83 mg, 20%), and a mixture of **6.20** and **6.21** (117 mg, 28%). **6.20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 9.39 (s, 1H), 9.02 (s, 1H), (7.85 and 5.30, d, 1021.2 Hz), 7.64 (ddd, *J* = 8.0, 5.1, 1.6 Hz, 4H), 7.50 – 7.31 (m, 8H), 6.33 (dd, *J* = 9.4, 4.9 Hz, 1H), 6.20 (dd, *J* = 7.7, 6.1 Hz, 1H), 6.09 – 6.01 (m, 1H), 5.79 – 5.65 (m, 1H), 5.25 – 5.10 (m, 4H), 4.49 – 4.19 (m, 6H), 4.14 (d, *J* = 4.0 Hz, 1H), 4.01 (q, *J* = 4.3 Hz, 1H), 3.94 (dd, *J* = 11.6, 2.9 Hz, 1H), 3.81 (ddd, *J* = 11.7, 8.8, 2.0 Hz, 1H), 2.76 – 2.14 (m, 8H), 1.96 – 1.86

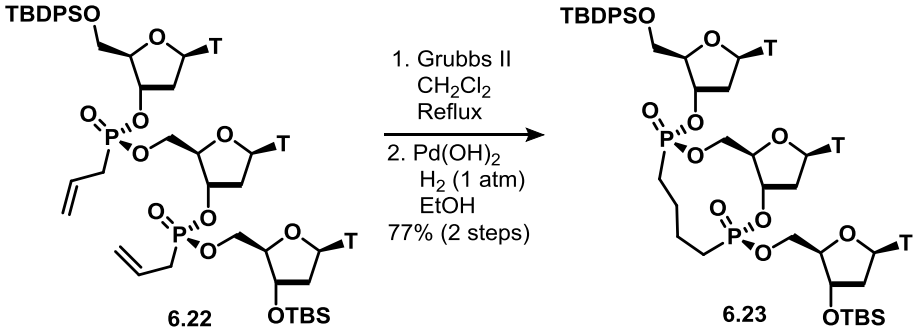
(m, 6H), 1.63 – 1.57 (m, 3H), 1.08 (s, 9H), 0.89 (d, J = 1.9 Hz, 10H), 0.09 (s, 6H).;^{31}P NMR (162 MHz, $CDCl_3$) δ 27.95, 7.48. **6.21**: ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.70, 8.27.



Compound 6.22

To a stirred solution of compound **6.20** (83 mg, 68 μmol) in THF (2 mL) at -15 °C was added dropwise a solution of 2.5 M BuLi in hexanes (0.13 mL, 0.325 mmol). After 5 min, allyl iodide (30 μL, 0.33 mmol) was added dropwise and the reaction mixture was kept at -15 °C for 30 min, followed by removal of the solvent aided by a rotavap system warmed at 35 °C. The crude residue was dissolved in EtOAc (20 mL), washed with water and brine. The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (8:2 ethyl acetate/hexane) to give compound **6.22** as a white foam (49 mg, 57%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 – 9.57 (m, 3H), 7.64 (ddd, J = 8.0, 6.3, 1.7 Hz, 4H), 7.48 – 7.34 (m, 9H), 6.34 (dd, J = 9.2, 5.1 Hz, 1H), 6.20 (t, J = 6.8 Hz, 2H), 5.84 – 5.61 (m, 2H), 5.32 – 5.05 (m, 6H), 4.43 – 4.08 (m, 7H), 4.01 (dd, J = 5.3, 3.4 Hz, 1H), 3.93 (dd, J = 11.7, 2.8 Hz, 1H), 3.80 (dd, J = 11.6, 2.8 Hz, 1H), 2.76 – 2.45 (m, 6H), 2.24 (dt, J = 12.5, 6.7 Hz, 4H), 1.97 – 1.84 (m, 6H), 1.07 (s, 9H), 0.87 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.16, 164.02, 163.96, 150.73, 150.67, 150.54, 136.36, 135.78, 135.66, 135.38, 134.82, 132.76, 132.18, 130.36, 130.25, 128.20, 128.14, 126.42, 126.36, 121.48, 121.33, 121.25, 111.67, 111.61, 111.42, 86.20, 85.83, 85.65, 85.24, 84.42, 83.86, 75.79, 71.72,

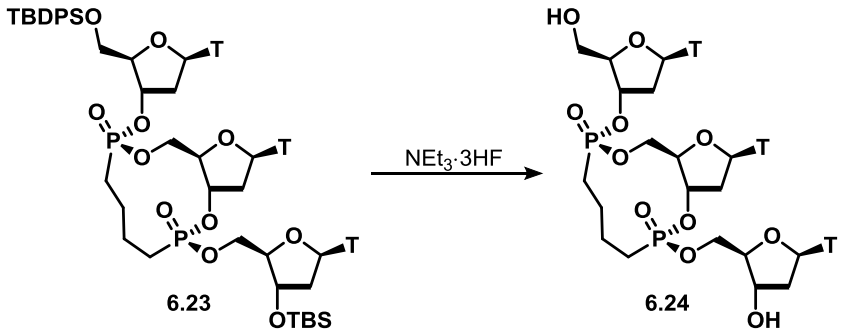
65.71, 65.06, 64.02, 40.15, 39.66, 32.75, 31.37, 27.11, 25.80, 19.44, 18.02, 12.47, 12.15, -4.53, -4.73; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.09, 27.85; HRMS (ESI) calculated for $C_{58}H_{81}N_6O_{17}P_2Si_2$ $[M+H]^+$ *m/z* = 1251.4666, found 1251.46610.



Compound 6.23

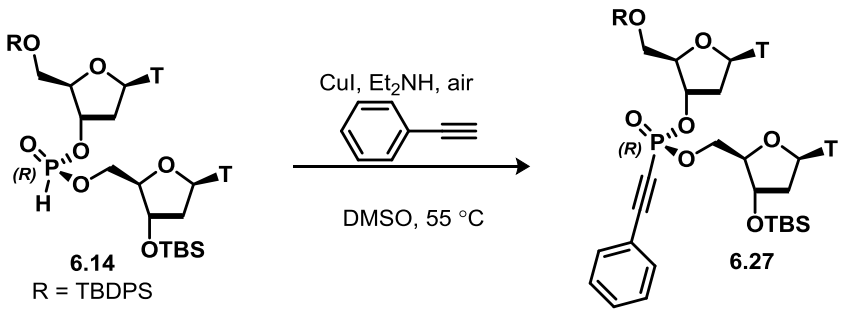
Second generation Grubbs catalyst (3.3 mg, 3.9 μmol) was added to a stirred solution of compound **6.22** (49 mg, 39 μmol) in CH_2Cl_2 (2 mL). After heating the reaction to reflux for 5 h, silica gel was added to the mixture and the solvent was removed under reduced pressure. The dry residue was purified through a short chromatographic column using 8% MeOH in CH_2Cl_2 obtaining an inseparable *cis/trans* mixture of alkenes (41 mg). The olefin mixture was dissolved in ethanol (0.5 mL) and mixed with $Pd(OH)_2$. (2.3 mg, 3.3 μmol) The flask was degassed under vacuum and refilled with hydrogen (x3). A balloon filled with hydrogen was connected to the flask and the reaction was stirred for 24 h. The reaction was diluted with ethanol, silica gel was added to the mixture and the solvent was removed under reduced pressure. The dry residue was purified through a short chromatographic column using 8% MeOH in CH_2Cl_2. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give compound **6.23** as a white foam (33.7 mg, 70% over 2 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 – 9.52 (m, 3H), 7.69 – 7.59 (m, 4H), 7.48 – 7.33 (m, 8H), 7.02 (s, 1H), 6.39 (dd, *J* = 9.0, 5.3 Hz, 1H), 6.17 (t, *J* = 6.7

Hz, 2H), 5.23 – 5.12 (m, 2H), 4.40 (dt, *J* = 7.5, 3.8 Hz, 1H), 4.35 – 4.20 (m, 3H), 4.20 – 4.09 (m, 3H), 4.02 (q, *J* = 4.0 Hz, 1H), 3.97 (dd, *J* = 11.4, 2.7 Hz, 1H), 3.87 (dd, *J* = 11.6, 2.5 Hz, 1H), 2.60 – 2.46 (m, 3H), 2.36 – 2.16 (m, 3H), 2.07 – 1.71 (m, 13H), 1.25 (s, 1H), 1.08 (s, 9H), 0.88 (s, 9H), 0.08 (s, 6H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 33.48, 33.17; HRMS (ESI) calculated for $C_{56}H_{79}N_6O_{17}P_2Si_2$ $[M+H]^+$ *m/z* = 1225.451, found 1225.45396.



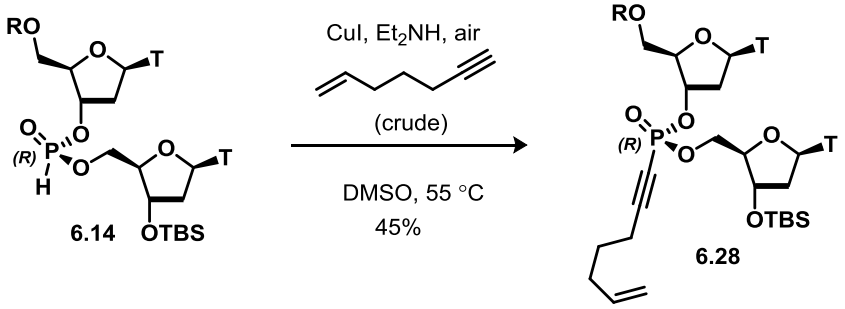
Compound 6.24

To a stirred solution of compound **6.23** (33 mg, 27 μmol) in THF (0.3 mL) was added $NEt_3 \cdot 3HF$ (27 μL, 165 μmol). After stirring for 24 h at room temperature, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (10% MeOH in CH_2Cl_2) to give compound **6.24** as a white foam (5 mg, 21%); ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.81 (d, *J* = 1.3 Hz, 1H), 7.57 (d, *J* = 1.3 Hz, 1H), 7.43 (d, *J* = 1.3 Hz, 1H), 6.34 – 6.24 (m, 2H), 6.14 (t, *J* = 6.5 Hz, 1H), 5.33 – 5.13 (m, 2H), 4.50 – 4.15 (m, 9H), 4.12 (d, *J* = 7.1 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.82 (d, *J* = 3.4 Hz, 2H), 2.79 – 2.38 (m, 4H), 2.36 – 2.05 (m, 5H), 1.91 (dd, *J* = 5.2, 1.2 Hz, 16H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 34.15, 33.63.



Compound 6.27

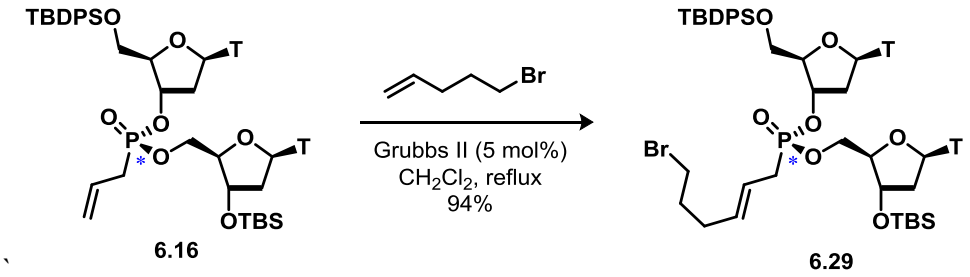
To a stirred solution of compound **6.14** (12.4 mg, 0.0126 mmol) in DMSO (50 µL) was added phenylacetylene (2 µL, 0.0182 mmol), diethylamine (3 µL, 0.0290 mmol) and copper iodide (I) (0.3 mg, 1.5 µmol). The flask was equipped with a trap containing drierite and the reaction was warmed to 55 °C. After stirring for 20 h the solvent was removed under reduced pressure, the dry residue was dissolved in methanol, silica gel was added and the slurry was dried under reduced pressure. The residue was purified by flash chromatography (8:2 ethyl acetate/hexane) to give compound **6.27** as a white foam (2.5 mg, 18%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 11.0 Hz, 3H), 7.71 – 7.60 (m, 6H), 7.60 – 7.33 (m, 18H), 6.48 (dd, J = 9.3, 5.1 Hz, 1H), 6.30 (t, J = 6.8 Hz, 1H), 5.41 – 5.34 (m, 1H), 4.50 (dt, J = 6.7, 3.5 Hz, 1H), 4.39 – 4.29 (m, 3H), 4.12 – 4.03 (m, 1H), 4.00 (dd, J = 6.7, 2.2 Hz, 2H), 2.44 – 2.10 (m, 3H), 1.91 (d, J = 1.2 Hz, 3H), 1.55 (d, J = 1.2 Hz, 3H), 1.10 (s, 9H), 0.90 (s, 11H), 0.10 (s, 6H); ^{31}P NMR (121 MHz, $CDCl_3$) δ -5.94. LRMS calculated for $C_{50}H_{63}N_4O_{11}PSi_2$ $[M+H]^+$ m/z = 983.4, found 983.6.



Compound 6.28

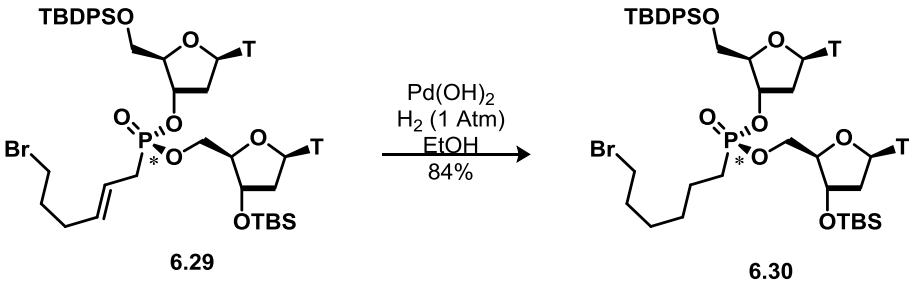
Hept-1-en-6-yne: To a stirred solution of commercially available 90% lithium-acetylide ethylenediamine complex (1.6 g, 15.6 mmol) in anhydrous DMSO (3.8 mL) at 0 °C, 5-bromo-1-pentene (1.0 mL, 8.44 mmol) was added dropwise over a period of 30 min and the mixture was stirred for 20 min at 0 °C. The reaction was carefully quenched via addition of water (10 mL), then extracted with pentanes (3 x 10 mL). The combined organics were washed with water, filtered through a Celite® pad and evaporated under reduced pressure without heating. The product can be used without further purification or distilled (bulb to bulb). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 5.09 – 4.95 (m, 2H), 2.24 – 2.13 (m, 4H), 1.95 (t, *J* = 2.7 Hz, 1H), 1.63 (p, *J* = 7.2 Hz, 2H).

To a stirred solution of compound **6.14** (22 mg, 34.4 μmol) in DMSO (0.1 mL) was treated with **hept-1-en-6-yne** (10 mg, 106 μmol), diethylamine (5.2 μL, 50.3 μmol) and copper iodide (I) (0.5 mg, 2.7 μmol). The flask was equipped with a trap comtaining drierite® and the reaction was warmed to 55 °C. After stirring for 20 h the solvent was removed under reduced pressure, the dry residue was dissolved in methanol, silica gel was added and the slurry was dried under reduced pressure. The residue was purified by flash chromatography (8:2 ethyl acetate/hexane) to give compound **6.28** as a white foam (11 mg, 45%); ;^{31}P NMR (162 MHz, $CDCl_3$) δ -6.52. HRMS (ESI) calculated for $C_{49}H_{68}N_4O_{11}PSi_2$ $[M+H]^+$ *m/z* = 975.4155, found 975.4183.



Compound 6.29

Second generation Grubbs catalyst (7 mg, 7.3 μmol) and 5-bromo-1-pentene (35 μL, 0.295 mmol) were added to a stirred solution of **6.16** (135 mg, 0.146 mmol) in CH_2Cl_2 (0.8 mL). The resulting mixture was heated to reflux for 24 h, then the reaction mixture was diluted with CH_2Cl_2 and adsorbed on silica gel. The residue was purified by flash chromatography (8:2 EtOAc/hexanes) to give nucleoside **6.29** as a white foam (143.5 mg, 94%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.59 (m, 4H), 7.46 – 7.34 (m, 8H), 6.38 (dd, *J* = 9.3, 5.1 Hz, 1H), 6.21 – 6.13 (m, 1H), 5.61 – 5.49 (m, 1H), 5.47 – 5.33 (m, 1H), 5.20 (t, *J* = 6.2 Hz, 1H), 4.38 (dt, *J* = 6.2, 3.8 Hz, 1H), 4.30 – 4.11 (m, 3H), 4.06 – 3.92 (m, 2H), 3.89 – 3.80 (m, 1H), 3.42 – 3.29 (m, 2H), 2.67 – 2.50 (m, 3H), 2.35 – 2.05 (m, 5H), 1.93 (d, *J* = 1.2 Hz, 3H), 1.91 – 1.81 (m, 2H), 1.55 (d, *J* = 1.2 Hz, 3H), 1.08 (s, 9H), 0.89 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.17, 164.18, 163.99, 150.69, 150.47, 135.89, 135.55, 135.25, 135.09, 134.76, 132.73, 132.04, 130.29, 130.18, 128.14, 128.06, 119.09, 118.98, 111.68, 111.17, 85.82, 85.20, 84.30, 71.58, 65.16, 63.96, 60.41, 40.51, 39.49, 33.17, 32.89, 31.69, 31.66, 31.37, 30.75, 29.98, 27.03, 25.72, 21.06, 19.38, 17.93, 14.22, 12.47, 12.01, -4.59, -4.78.; ^{31}P NMR (121 MHz, $CDCl_3$) δ 28.47.

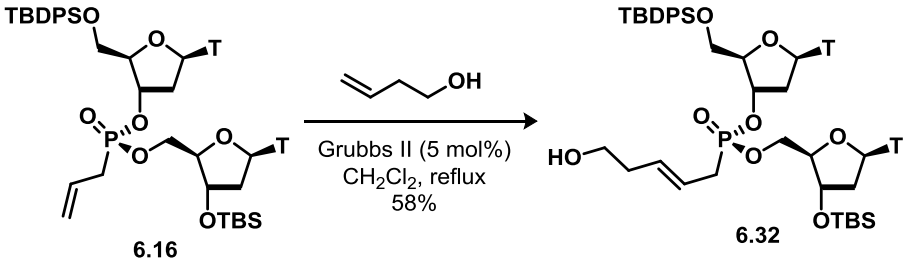


6.29 **6.30**

Compound 6.30

To an stirred solution of **6.29** (143 mg, 0.179 mmol) in ethanol (3 mL) was added 20% $Pd(OH)_2$ (10 mg, 0.018 mmol). The flask was degassed under vacuum and refilled with hydrogen (x3).

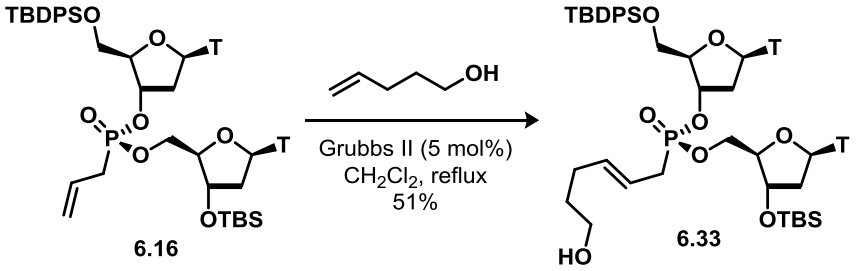
A balloon filled with hydrogen was connected to the flask and the reaction was stirred for 2 h. The advance of the reaction was followed by MS analysis in order to detect any sign of overreduction in the nucleobases. Once all the starting material was consumed, the reaction mixture was diluted with ethanol and filtered through a Celite® pad rinsing with more ethanol. The resulting solution was purified by flash chromatography (8:2 EtOAc/hexanes) to give nucleoside **6.30** as a white foam (120.9 mg, 84%) Rf = (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.49 (s, 2H), 7.71 – 7.57 (m, 4H), 7.51 – 7.32 (m, 9H), 6.38 (dd, *J* = 8.9, 4.9 Hz, 1H), 6.18 (t, *J* = 6.1 Hz, 1H), 5.21 (s, 1H), 4.54 – 3.75 (m, 7H), 3.36 (t, *J* = 6.6 Hz, 1H), 2.58 (d, *J* = 9.6 Hz, 1H), 2.34 – 2.11 (m, 2H), 1.92 (s, 3H), 1.88 – 1.68 (m, 3H), 1.66 – 1.57 (m, 1H), 1.48 – 1.29 (m, 3H), 1.07 (s, 9H), 0.88 (s, 9H), 0.08 (s, 6H); ^{31}P NMR (121 MHz, $CDCl_3$) δ 33.25.



Compound 6.32

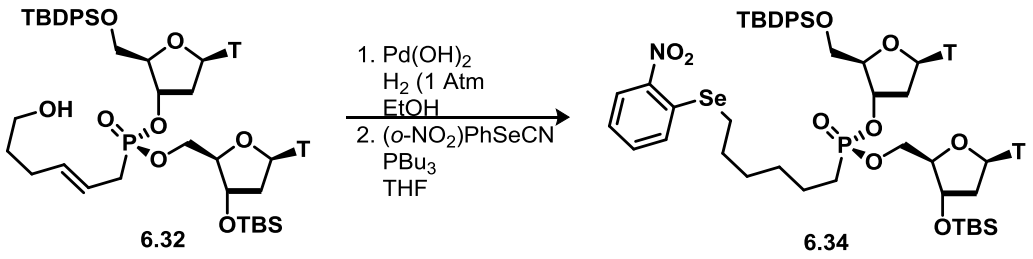
Second generation Grubbs catalyst (1.2 mg, 1.4 μmol) and 3-buten-1-ol (6 μL, 69 μmol) were added to a stirred solution of **6.16** (27 mg, 41.8 μmol) in CH_2Cl_2 (0.2 mL). The resulting mixture was heated to reflux for 24 h, then the reaction mixture was diluted with CH_2Cl_2 and adsorbed on silica gel. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give nucleoside **6.32** as a white foam (mg, 94%) Rf = (EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (d, *J* = 18.1 Hz, 2H), 7.63 (tt, *J* = 7.9, 1.9 Hz, 4H), 7.48 – 7.35 (m, 8H), 6.37 (dd, *J* = 9.3, 5.1 Hz, 1H), 6.13 (dt, *J* = 9.9, 6.6 Hz, 1H), 5.62 (dq, *J* = 12.6, 6.4 Hz, 1H), 5.36 (dt, *J* = 14.9,

7.1 Hz, 1H), 5.20 (t, *J* = 6.2 Hz, 1H), 4.39 (dt, *J* = 6.5, 4.0 Hz, 1H), 4.32 – 4.11 (m, 3H), 4.07 – 3.92 (m, 2H), 3.91 – 3.82 (m, 1H), 3.59 (t, *J* = 6.4 Hz, 2H), 2.75 – 2.52 (m, 3H), 2.32 – 2.20 (m, 3H), 2.06 (t, *J* = 6.8 Hz, 1H), 1.97 – 1.77 (m, 4H), 1.69 – 1.49 (m, 5H), 1.08 (s, 9H), 0.89 (s, 10H), 0.09 (s, 6H);^{31}P NMR (162 MHz, $CDCl_3$) δ 28.85, 28.71;



Compound 6.33

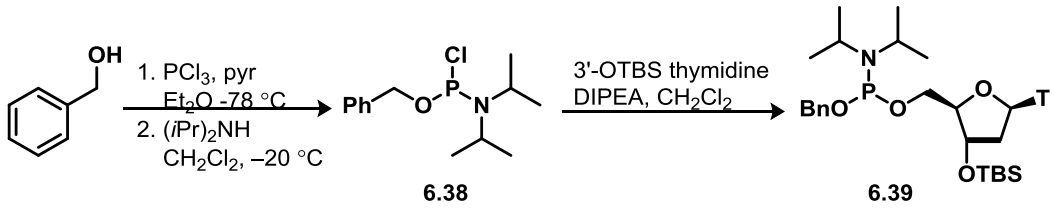
Second generation Grubbs catalyst (1.2 mg, 1.4 μmol) and pent-4-en-1-ol (6 μL, 58.2 μmol) were added to a stirred solution of **6.16** (25 mg, 27.1 μmol) in CH_2Cl_2 (0.2 mL). The resulting mixture was heated to reflux for 24 h, then the reaction mixture was diluted with CH_2Cl_2 and adsorbed on silica gel. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give nucleoside **6.33** (13.5 mg, 51%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 18.3 Hz, 2H), 7.64 (td, *J* = 7.9, 1.6 Hz, 4H), 7.51 – 7.33 (m, 8H), 6.38 (dd, *J* = 9.4, 5.0 Hz, 1H), 6.14 (td, *J* = 6.7, 3.9 Hz, 1H), 5.73 – 5.38 (m, 2H), 5.21 (q, *J* = 6.6 Hz, 1H), 4.44 – 4.35 (m, 1H), 4.32 – 4.10 (m, 3H), 4.08 – 3.93 (m, 2H), 3.92 – 3.82 (m, 1H), 3.73 – 3.55 (m, 2H), 2.78 – 2.53 (m, 3H), 2.37 – 2.15 (m, 5H), 1.93 (dd, *J* = 2.8, 1.2 Hz, 3H), 1.53 (d, *J* = 1.2 Hz, 3H), 1.08 (s, 9H), 0.89 (s, 9H), 0.09 (s, 6H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.86, 28.38; LRMS calculated for $C_{50}H_{63}N_4O_{11}PSi_2$ $[M+H]^+$ *m/z* = 983.4, found 983.6.



Compound 6.34

To an stirred solution of **6.32** (13.5mg, 13.7 μmol) in ethanol (0.5 mL) was added 20% $Pd(OH)_2$ (1.2mg). The flask was degassed under vacuum and refilled with hydrogen (x3). A balloon filled with hydrogen was connected to the flask and the reaction was stirred for 6 h. Once all the starting material was consumed, the reaction mixture was diluted with ethanol and filtered through a Celite® pad rinsing with more ethanol. The crude residue was used without further purification**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (d, *J* = 54.3 Hz, 1H), 7.64 (t, *J* = 6.5 Hz, 4H), 7.47 – 7.34 (m, 8H), 6.38 (dd, *J* = 9.0, 4.9 Hz, 1H), 6.13 (t, *J* = 6.6 Hz, 1H), 5.27 – 5.14 (m, 1H), 4.47 – 4.37 (m, 1H), 4.29 – 4.11 (m, 3H), 4.05 – 3.83 (m, 3H), 3.61 (t, *J* = 6.4 Hz, 1H), 2.59 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.37 – 2.15 (m, 3H), 1.92 (s, 3H), 1.77 (dt, *J* = 17.5, 8.4 Hz, 3H), 1.49 (s, 7H), 1.45 – 1.23 (m, 5H), 1.08 (s, 9H), 0.89 (s, 9H), 0.09 (d, *J* = 1.8 Hz, 6H).;^{31}P NMR (162 MHz, $CDCl_3$) δ 33.52. Crude alcohol (17.5 mg, 17.8 μmol) was dissolved in THF (0.2 mL) and cooled to 0 °C. 2-nitrophenyl selenocyanide (5 mg, 22 μmol) and tributylphosphine (6 μL, 0.024 μmol). After 12 h, water was added and the resulting mixture was extracted with EtOAc (3 x 1 mL). The combined organic extracts were combines, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (4% MeOH in CH_2Cl_2) to give compound **6.34** (13.9 mg, impure with $O{=}PBu_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 0H), 8.64 (s, 0H), 8.27 (dd, *J* = 8.0, 1.2 Hz, 0H), 7.63 (ddt, *J* = 7.8, 6.5, 1.5 Hz, 1H), 7.54 – 7.32 (m, 2H), 7.29 (ddd, *J* = 8.4, 6.3, 2.2 Hz, 0H), 6.38 (dd, *J* = 9.3, 5.1 Hz, 0H), 6.14 (t, *J* = 6.6 Hz, 0H), 5.25 – 5.18 (m, 0H), 4.39 (dt, *J* =

6.6, 4.0 Hz, 0H), 4.23 (ddd, *J* = 11.1, 6.5, 3.4 Hz, 0H), 4.18 – 4.11 (m, 0H), 4.05 – 3.84 (m, 1H), 2.88 (t, *J* = 7.4 Hz, 0H), 2.62 – 2.54 (m, 0H), 2.31 – 2.18 (m, 0H), 1.92 (d, *J* = 1.2 Hz, 1H), 1.25 (s, 0H), 1.08 (d, *J* = 2.0 Hz, 2H), 0.88 (s, 1H); ^{31}P NMR (202 MHz, $CDCl_3$) δ 33.16.

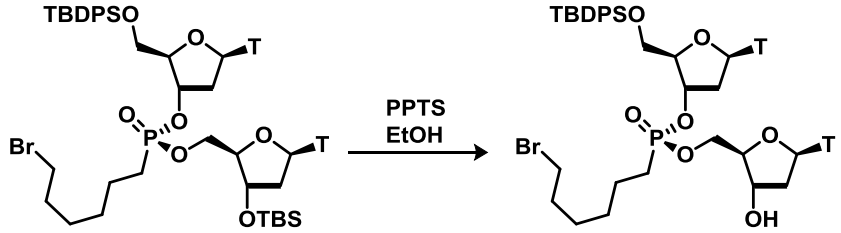


6.38 **6.39**

Compound 6.39

To a stirred solution of PCl_3 (4.22 mL, 48.3 mmol) in Et_2O (50 mL) at -78 °C, was added pyridine (3.89 mL, 48.3 mmol). A solution of benzyl alcohol (5 mL, 48.3 mmol) in Et_2O (25 mL) was added dropwise over a period of 90 min. The formed precipitate was filtered under argon, rinsed with Et_2O and the solvent was then removed under reduced pressure. The residue was distilled (bulb to bulb) to give (benzyloxy)dichlorophosphine[234] (3.38 g) as a colorless oil. A solution of diisopropylamine (4.53 mL, 32.3 mmol) in CH_2Cl_2 (5 mL) was added dropwise over a period of 30 min to a solution containing distilled chlorophosphite (3.38 g, 16.17 mmol) in CH_2Cl_2 (10 mL) at -20 °C. The icebath was removed and the reaction was allowed to react for 1.5 h. Diisopropylammonium chloride was removed by filtration and washed with CH_2Cl_2 (2 x 3 mL). The combined filtrates were evaporated, the resulting yellow oil was dissolved in dry ether (25 mL), and the additional ammonium salt that precipitated was removed by filtration. Evaporation of the solvent gave the salt-free phosphine product **6.38**.[235] The product was used without further purification. DIPEA (34 μL, 0.19 mmol) and compound **6.38** (77 mg, 0.28 mmol) were added sequentially to a stirred solution of 3'-OTBS thymidine (57.2 mg, 0.16 mmol) in CH_2Cl_2 (3 mL). After 40 min, the reaction mixture was diluted with CH_2Cl_2 and water. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4,

filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2 % MeOH in CH_2Cl_2) to give compound **6.39** as a mixture of isomers (^{31}P 3:1 ratio) (19.5 mg, 21%); ^1H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.70 (q, *J* = 1.2 Hz, 1H), 7.39 – 7.23 (m, 5H), 6.37 – 6.28 (m, 1H), 4.81 – 4.62 (m, 2H), 4.43 (dt, *J* = 6.1, 3.0 Hz, 1H), 4.02 (dq, *J* = 21.9, 2.5 Hz, 1H), 3.90 – 3.75 (m, 2H), 3.70 – 3.58 (m, 2H), 2.27 – 2.15 (m, 1H), 2.06 – 1.94 (m, 1H), 1.81 (dd, *J* = 19.3, 1.2 Hz, 3H), 1.21 (dd, *J* = 11.0, 6.7 Hz, 12H), 0.89 (s, 9H), 0.10 – 0.04 (m, 6H).; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.56, 148.32.

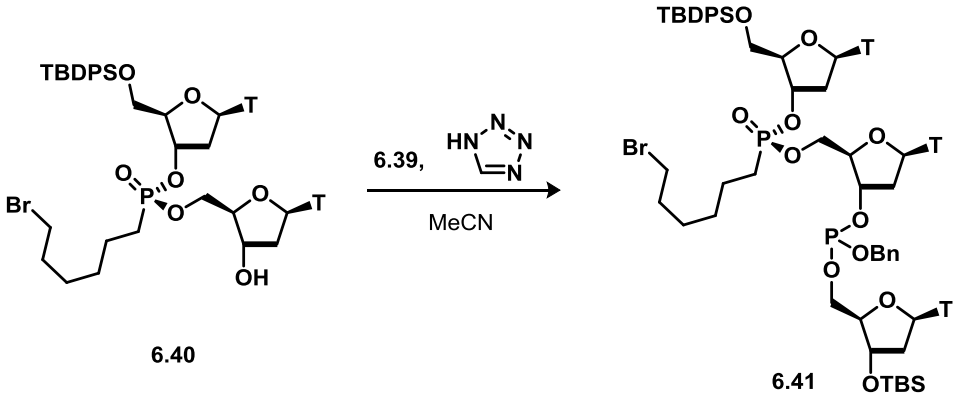


6.30 6.40

Compound 6.40

To a stirred solution of compound **6.30** (38.6 mg, 0.0369 mmol) in ethanol (3 mL) was added PPTS (19 mg, 0.076 mmol) and the resulting solution was heated to reflux for 36 h. The reaction mixture was cooled to room temperature and combined with aq. $NaHCO_{3\,(sat)}$ and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (6% MeOH in CH_2Cl_2) to give compound **6.40** as a white foam (25.2 mg, 73%); ^1H NMR (500 MHz, $CDCl_3$) δ 10.17 (s, 1H), 9.74 (s, 1H), 7.70 – 7.62 (m, 4H), 7.52 – 7.35 (m, 8H), 6.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 6.25 (t, *J* = 6.5 Hz, 1H), 5.21 (t, *J* = 5.7 Hz, 1H), 4.53 (s, 1H), 4.41 (d, *J* = 4.3 Hz, 1H), 4.37 – 4.29 (m, 2H), 4.22 – 4.13 (m, 2H), 3.98 (dd, *J* = 11.7, 3.1 Hz, 1H), 3.85 (dd, *J* = 11.7, 2.7 Hz, 1H), 3.39 (t, *J* = 6.7 Hz, 2H), 2.77 – 2.66 (m, 1H), 2.53 – 2.42 (m, 1H), 2.34 – 2.20 (m, 2H), 1.98 (s, 2H), 1.93 (d, *J* = 1.2 Hz, 3H), 1.89 – 1.73 (m,

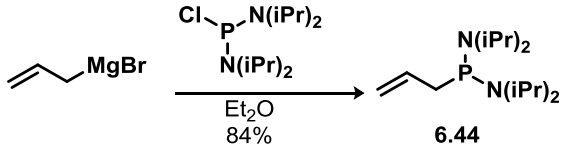
4H), 1.61 (d, J = 11.0 Hz, 6H), 1.49 – 1.31 (m, 4H), 1.31 – 1.17 (m, 2H), 1.10 (s, 10H), 1.00 – 0.80 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.05, 163.87, 151.18, 150.55, 135.86, 135.55, 135.28, 134.58, 132.54, 131.99, 130.32, 130.23, 128.15, 128.09, 111.99, 111.06, 84.61, 70.84, 63.97, 33.59, 33.18, 32.31, 29.65, 29.51, 27.51, 27.01, 26.57, 22.12, 19.34, 12.58, 12.11; ^{31}P NMR (202 MHz, $CDCl_3$) δ 33.65.



Compound 6.41

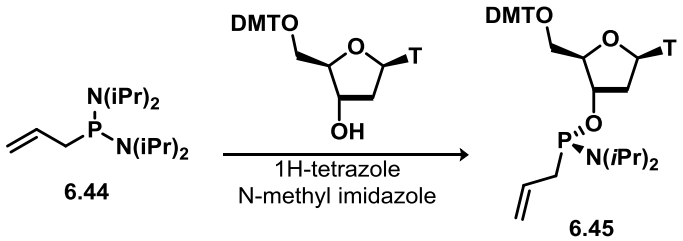
Compound **6.39** (32 mg, 0.054 mmol) and a 0.45 M solution of 1H-tetrazole (0.15 mL, 0.067 mmol) in MeCN were added to a solution of compound **6.40** (21 mg, 0.023 mmol) in MeCN (0.6 mL). The resulting solution was stirred overnight, diluted with EtOAc and washed with a 10% solution of Na_2CO_3. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4% MeOH in CH_2Cl_2) to give compound **6.41** as a white foam (23.6 mg, 73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 – 9.22 (m, 3H), 7.64 (ddd, J = 7.8, 3.8, 1.7 Hz, 4H), 7.50 – 7.27 (m, 15H), 6.35 (dd, J = 9.1, 5.0 Hz, 1H), 6.17 (dt, J = 19.0, 6.5 Hz, 2H), 5.18 (t, J = 6.2 Hz, 1H), 4.91 (dd, J = 8.8, 2.4 Hz, 2H), 4.82 (dd, J = 17.0, 8.2 Hz, 1H), 4.37 (s, 1H), 4.27 – 3.88 (m, 8H), 3.83 (dd, J = 11.5, 2.7 Hz, 1H), 3.41 – 3.31 (m, 1H), 2.63 – 2.53 (m, 1H), 2.46 – 2.36 (m, 1H), 2.31 – 2.14 (m, 4H), 1.95 – 1.66 (m, 12H), 1.57 (d, J = 2.3 Hz, 5H), 1.47 – 1.18 (m, 7H), 1.07 (s, 10H), 0.88 (d, J =

1.0 Hz, 10H), 0.06 (t, J = 1.3 Hz, 6H); ^{31}P NMR (121 MHz, $CDCl_3$) δ 140.05, 139.82, 33.20, 33.13.



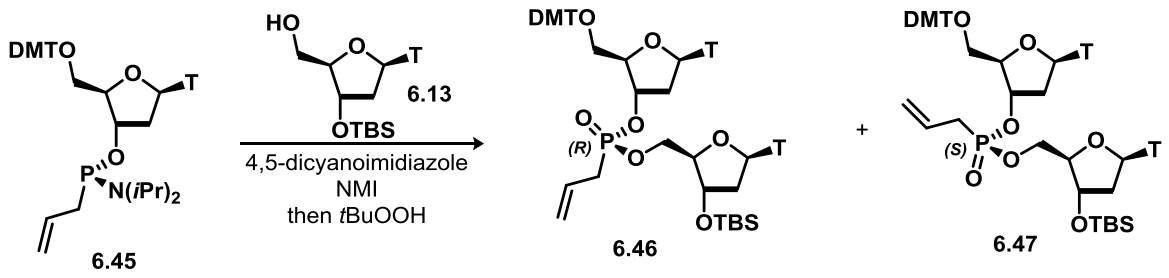
Compound 6.44

Bis(diisopropylamino)chlorophosphine (1.0 g, 3.56 mmol) was suspended in diethyl ether (12 mL), and was cooled to 0 °C in an ice bath with stirring. A 1 M commercial solution of allylmagnesium bromide (4 mL, 4 mmol) in ether was transferred to the chlorophosphine suspension. The reaction was allowed to come to room temperature and was stirred for one hour. The reaction mixture was filtered through a plug of Celite®, and the solids were rinsed with Et_2O. The filtrates were pooled, and the solvent was removed under reduced pressure. The residue was suspend in dry MeCN (10 ml). The reaction mixture was transferred to a separatory funnel and was extracted with hexanes (30 mL). The hexanes layer was washed with MeCN (2 x 15 ml). The hexanes layer was collected and passed thru a plug of cotton to remove particulates. The filtrate was concentrated under reduced pressure to give **6.44** (815 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.94 – 5.68 (m, 1H), 5.11 – 4.92 (m, 2H), 3.39 (dhept, J = 10.5, 6.7 Hz, 4H), 2.53 (ddt, J = 7.5, 2.9, 1.3 Hz, 2H), 1.19 (d, J = 6.7 Hz, 12H), 1.09 (d, J = 6.6 Hz, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.82, 135.58, 115.56, 115.40, 46.63, 46.50, 35.23, 35.13, 24.51, 24.42, 24.20, 24.16, 24.12; ^{31}P NMR (121 MHz, $CDCl_3$) δ 48.75.



Compound 6.45

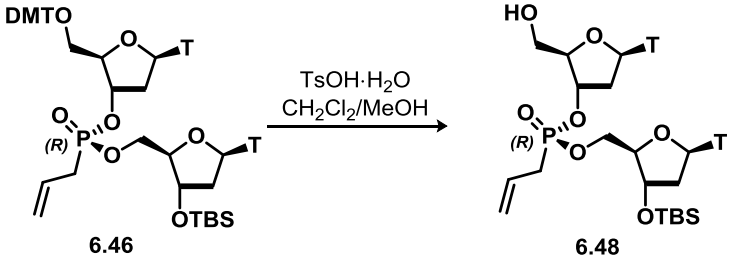
5'-ODMT thymidine (100 mg, 0.186 mmol) was coevaporated with toluene (x 2) until complete dryness, then the flask was connected to the high vacuum pump for 20 min. Dry 5'-ODMT thymidine was dissolved in dry DMF (1.9 mL). In a second flask, a solution of 4,5-dicyanoimidazole (20.8 mg, 0.176 mmol) in acetonitrile (1.6 mL) was combined with 1-methyl imidazole (7 μL, 88 μmol). In a third flask a solution of **6.44** (75 mg, 0.27 mmol) in DMF (0.3 mL) was prepared. The solution containing 4,5-dicyanoimidazole and 1-methyl imidazole was added dropwise (via cannula) to the solution of 5'-ODMT thymidine followed by the dropwise addition of the solution of compound **6.44** (via cannula). Each flask was rinsed with a minimum amount of the corresponding solvent. The reaction was allow to stir at room temperature for approx. 24 h then the solvent was removed under reduced pressure (keeping temp under 35 °C). The residue was purified by flash column chromatography (3:7 EtOAc/hex + 1%NEt_3) to give compound **6.45** as a mixture of P-diastereosiomers (112 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.63 (d, *J* = 16.4 Hz, 1H), 7.43 – 7.32 (m, 2H), 7.33 – 7.18 (m, 8H), 6.86 – 6.74 (m, 4H), 6.38 (dt, *J* = 8.3, 6.1 Hz, 1H), 5.80 – 5.55 (m, 1H), 5.17 – 4.92 (m, 2H), 4.53 (dd, *J* = 11.3, 5.9 Hz, 1H), 3.79 (s, 6H), 3.63 – 3.38 (m, 3H), 3.31 (ddd, *J* = 17.8, 10.5, 2.7 Hz, 1H), 2.59 – 2.36 (m, 2H), 2.27 (td, *J* = 13.1, 12.5, 5.8 Hz, 2H), 1.40 (s, 3H), 1.19 – 0.97 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.28, 158.65, 150.69, 144.33, 144.21, 135.63, 135.46, 135.36, 135.31, 135.19, 132.53, 132.41, 132.28, 130.09, 130.04, 128.13, 128.09, 127.90, 127.06, 127.03, 117.50, 117.38, 117.17, 117.06, 111.22, 111.12, 86.87, 85.87, 84.90, 84.61, 63.70, 63.58, 60.30, 44.43, 44.34, 43.03, 40.33, 40.10, 37.93, 37.82, 24.53, 24.09, 20.95, 14.15, 11.66; ^{31}P NMR (162 MHz, $CDCl_3$) δ 124.71, 124.27; HRMS (ESI) calculated for $C_{40}H_{51}N_3O_7P$ $[M+H]^+$ *m/z* = 716.3459, found 716.3452;



Compounds 6.46 and 6.47

Previous to the reaction, nucleoside **6.13** was coevaporated with toluene to dryness (x2) and connected to the high vacuum pump for 15 min. Nucleoside **6.13** (404 mg, 1.13 mmol) was dissolved in anhydrous acetonitrile (12 mL). A solution of phosphoramidite **6.45** (1.0749 g, 1.50 mmol) in acetonitrile (15 mL) was added dropwise via cannula, followed by addition of a solution containing 4,5-dicyanoimidazole (199 mg, 1.68 mmol) and N-methylimidazole (13.4 µL, 0.168 mmol) in acetonitrile (17 mL) dropwise via cannula. The progress of the reaction was followed by TLC analysis (≈1 h) and MS. Upon completion, 5M *t*BuOOH in decanes (0.67 mL, 3.37 mmol) was added dropwise and the reaction mixture was stirred for 45 min. The reaction mixture was then diluted with EtOAc, cooled down to 0 °C and washed with a 10% $NaHSO_3$(aq) solution (1 mL) and a saturated aqueous solution of $NaHCO_3$ (5 mL). The aqueous solution was extracted with EtOAc and the combined organic layers were dried over Na_2SO4, filtered, and concentrated under reduced pressure. The residue was adsorbed in silica and purified by flash chromatography using a Teledyne combiflash system (120 g cartridge) using a gradient hexanes/CH_2Cl_2 7:2 with an increasing percentage of EtOH from 0% to 10%. Nucleoside **6.46** (308 mg, 27%) and **6.47** (281 mg, 25%) were obtained as white foams. **6.46:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.26 – 9.93 (m, 2H), 7.51 (s, 1H), 7.43 – 7.29 (m, 3H), 7.26 – 7.11 (m, 7H), 6.80 (d, J = 8.5 Hz, 4H), 6.41 (dd, J = 8.4, 5.5 Hz, 1H), 6.26 – 6.10 (m, 1H), 5.95 – 5.51 (m, 1H), 5.41 – 5.01 (m, 3H), 4.65 – 3.83 (m, 5H), 3.74 (s, 6H), 3.48 (d, J = 10.7 Hz, 1H), 3.31 (d,

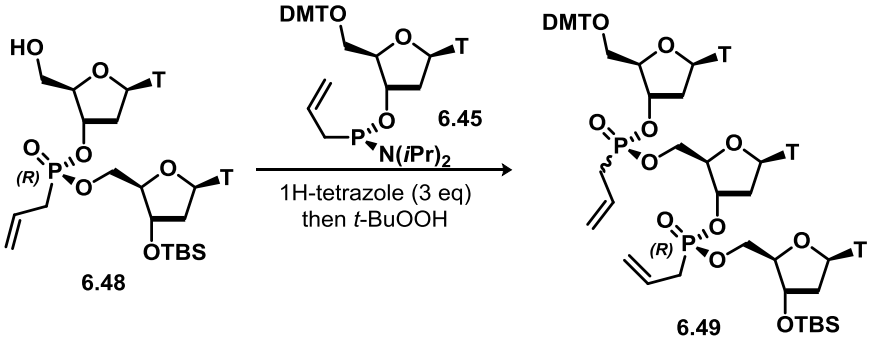
J = 9.0 Hz, 1H), 2.71 – 2.49 (m, 3H), 2.48 – 2.31 (m, 1H), 2.30 – 2.17 (m, 1H), 2.11 (dt, J = 13.3, 6.7 Hz, 1H), 1.85 (s, 3H), 1.36 (s, 3H), 0.85 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.20, 164.07, 158.70, 150.74, 150.49, 143.93, 135.71, 135.00, 134.93, 131.72, 130.02, 128.07, 127.95, 127.18, 126.27, 126.11, 121.14, 120.95, 119.05, 113.25, 111.65, 111.06, 110.77, 87.13, 85.48, 85.04, 84.95, 84.55, 84.14, 71.45, 68.72, 64.96, 63.17, 55.16, 40.40, 39.46, 32.68, 30.85, 25.66, 25.62, 17.82, 12.41, 12.32, 11.63, -4.71, -4.77, -4.92**;** ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.84; HRMS (ESI) calculated for $C_{50}H_{64}N_4O_{13}PSi$ $[M+H]^+$ *m/z* = 1009.3791, found 1009.3796. **6.47**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.18 (d, J = 38.1 Hz, 2H), 7.63 (s, 2H), 7.36 (dd, J = 8.5, 6.9 Hz, 2H), 7.27 – 7.17 (m, 7H), 6.85 – 6.79 (m, 5H), 6.42 – 6.33 (m, 1H), 6.21 – 6.07 (m, 1H), 5.86 – 5.60 (m, 1H), 5.33 – 5.19 (m, 2H), 4.42 – 3.85 (m, 5H), 3.77 (s, 6H), 3.56 – 3.31 (m, 2H), 2.74 (dd, J = 22.0, 7.3 Hz, 2H), 2.64 – 2.08 (m, 4H), 1.87 (s, 3H), 1.41 – 1.33 (s, 3H), 0.86 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.77, 164.69, 158.88, 158.78, 151.13, 150.91, 150.47, 144.33, 144.06, 140.33, 136.50, 135.86, 135.40, 135.32, 135.07, 134.98, 131.12, 130.15, 129.36, 129.22, 128.17, 127.40, 127.25, 126.02, 125.87, 121.79, 121.60, 116.07, 113.98, 113.41, 113.36, 113.22, 111.79, 111.50, 111.14, 110.49, 87.37, 87.07, 85.96, 84.90, 84.53, 72.64, 71.36, 63.74, 63.19, 55.35, 32.73, 25.71, 17.93, 12.60, 11.73, -4.60, -4.82;^{31}P NMR (162 MHz, $CDCl_3$) δ 28.56; HRMS (ESI) calculated for $C_{50}H_{64}N_4O_{13}PSi$ $[M+H]^+$ *m/z* = 1009.3791, found 1009.3801.



6.46 6.48

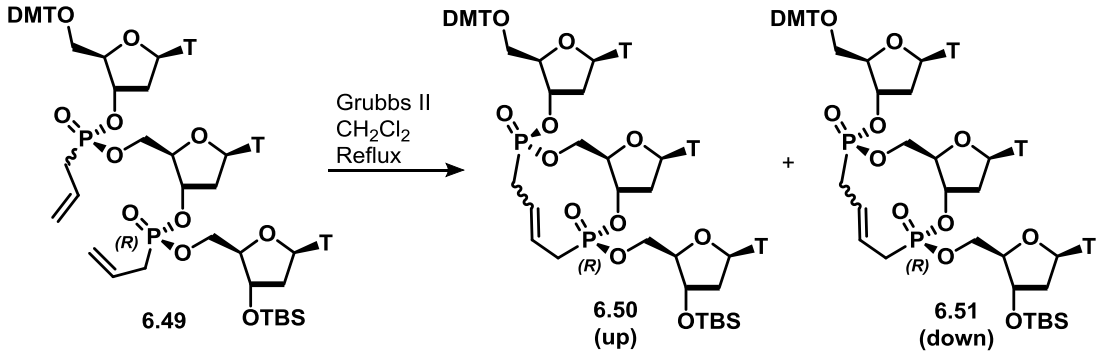
Compound 6.48

To a stirred solution of **6.46** (72 mg, 0.73 mmol) in 3:1 CH_2Cl_2/MeOH (7.3 mL) was added *p*-toluenesulfonic acid monohydrate (16.6 mg, 0.876 mmol) at 0 °C for 1 h. Once the reaction was completed, solid Na_2CO_3 was added and the mixture was stirred until the orange color disappeared. Water (2 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (4 x 3 mL). The organic extractions were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (0% to 5% MeOH in CH_2Cl_2) to give nucleoside **6.48** (42.2 mg, 83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.18 (s, 1H), 9.98 (s, 1H), 7.56 (s, 1H), 7.39 (s, 1H), 6.26 – 6.16 (m, 2H), 5.74 (ddq, *J* = 17.1, 10.0, 7.2 Hz, 1H), 5.28 – 5.15 (m, 3H), 4.39 – 4.28 (m, 1H), 4.24 – 4.08 (m, 3H), 4.00 – 3.93 (m, 2H), 3.76 (q, *J* = 15.2, 14.2 Hz, 2H), 2.68 (dd, *J* = 22.2, 7.4 Hz, 2H), 2.52 – 2.38 (m, 1H), 2.37 – 2.12 (m, 3H), 1.88 (s, 3H), 1.82 (s, 3H), 0.85 (s, 9H), 0.04 (s, 6H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.21; ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.65, 164.46, 150.74, 136.58, 136.31, 126.35, 126.24, 121.35, 121.20, 111.45, 111.14, 86.11, 85.60, 85.12, 71.49, 65.79, 61.92, 40.19, 39.07, 32.56, 31.17, 25.73, 17.94, 12.52, 12.39, -4.61, -4.82; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.25; HRMS (ESI) calculated for $C_{29}H_{46}N_4O_{11}PSi$ $[M+H]^+$ *m/z* = 685.2664, found 685.2697.



Compound 6.49

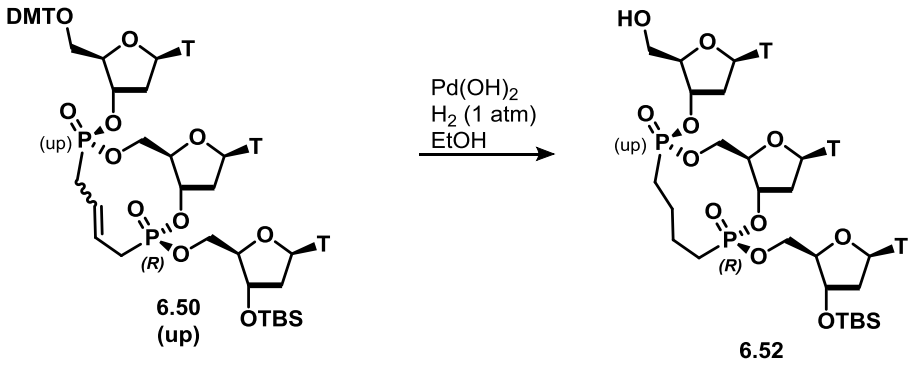
To a stirred solution of **6.48** (40 mg, 0.0584 mmol) in MeCN (0.7 mL) was added sequentially a solution of phosphoramidite **6.45** (137.8 mg, 0.192 mmol) in MeCN (2 mL), a 0.45 M solution of 1H-tetrazole (0.2 mL, 0.09 mmol) and N-methylimidazole (5 μL, 0.062 mmol). The reaction mixture was stirred for 2 h, then a 5 M solution of *t*BuOOH in decanes (0.07 mL, 0.35 mmol) was added dropwise and stirred for additional 30 min. The reaction mixture was diluted with EtOAc, cooled down to 0 °C and combined with a sat solution of $NaHCO_3$ (2 mL) and a 10% solution of $NaHSO_3$ (1 mL). The biphasic mixture was stirred for 5 min, then the layers were separated and the aqueous layer was extracted with EtOAc. The organic combined were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (0% to 6% MeOH in CH_2Cl_2) to give nucleoside **6.49** as an inseparable mixture of isomers (75 mg, 91%). ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.62, 28.18, 28.13, 28.00; HRMS (ESI) calculated for $C_{63}H_{80}N_6O_{19}P_2Si$ $[M+Na]^+$ *m/z* = 1337.4615, found 1337.4635.



Compounds 6.50 and 6.51

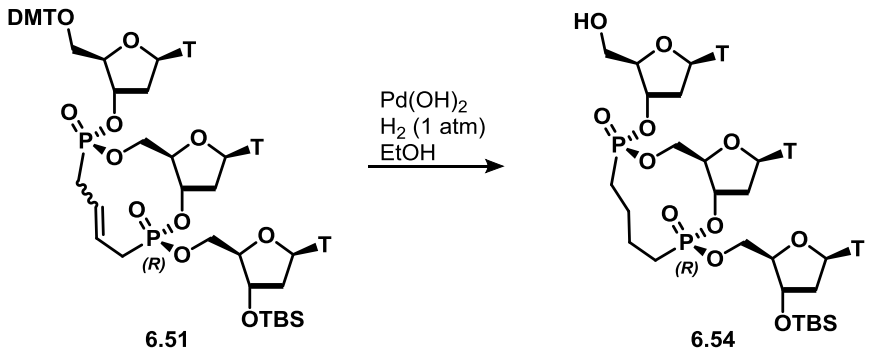
To a stirred solution of **6.49** (32 mg, 24.3 μmol) in CH_2Cl_2 (1 mL) was added second generation Grubbs catalyst (2 mg, 2.3 μmol) in a sealed tube. The reaction mixture was heated at 45 °C for 24 h, then the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography (4% to 8% *i*PrOH in CH_2Cl_2) to give **6.50** (6.7 mg, 32%) and **6.51** (6.3

mg, 31%). Both isolated products contained an inseparable mixture of *cis/trans* isomers. Compound **6.50**: ^{31}P NMR (202 MHz, $CDCl_3$) δ 28.06, 27.94, 27.81, 27.69. Compound **6.51**: ^{31}P NMR (202 MHz, $CDCl_3$) δ 27.16, 27.03, 26.67, 26.54.



Compound 6.52

To an stirred solution of **6.50** (6 mg, 4.6 μmol) in ethanol (0.5 mL), 20% $Pd(OH)_2$ (≈2 mg) was added. The flask was degassed under vacuum and refilled with hydrogen (x3). The reaction was stirred for 24 h under a balloon filled with hydrogen that was connected to the flask. The reaction mixture was diluted with ethanol and filtered through a Celite® pad rinsing with more ethanol. The resulting solution was purified by flash chromatography (0% to 9% MeOH in CH_2Cl_2) to give nucleoside **6.52** (2.5 mg, 54%); ^{1}H NMR (400 MHz, Methanol-d4) δ 7.80 (s, 1H), 7.54 (s, 1H), 7.41 (s, 1H), 6.37 – 6.29 (m, 1H), 6.22 (t, J = 6.8 Hz, 1H), 6.09 (t, J = 6.7 Hz, 1H), 5.31 (dd, J = 8.1, 4.1 Hz, 1H), 5.13 (s, 1H), 4.57 – 4.49 (m, 2H), 4.37 – 4.08 (m, 2H), 4.08 – 4.01 (m, 1H), 3.98 – 3.90 (m, 1H), 3.83 – 3.75 (m, 2H), 2.74 – 2.66 (m, 1H), 2.59 – 2.51 (m, 1H), 2.61 – 2.36 (m, 3H), 2.30 – 2.25 (m, 1H), 2.20 – 2.09 (m, 2H), 2.09 – 1.97 (m, 2H), 1.96 – 1.75 (m, 11H), 1.16 (d, J = 6.1 Hz, 4H), 0.94 (s, 9H), 0.22 – 0.01 (s, 6H); ^{31}P NMR (162 MHz, MeOD) δ 33.92, 33.50; HRMS (ESI) calculated for $C_{40}H_{61}N_6O_{17}P_2Si$ $[M+H]^+$ *m/z* = 987.3332, found 987.3343.



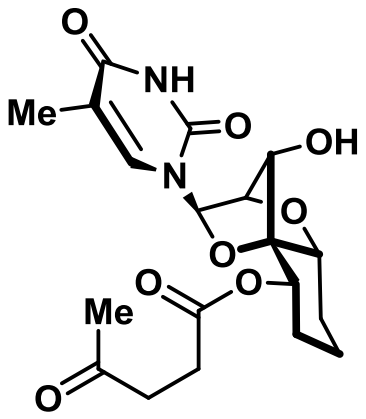
Compound 6.54

To an stirred solution of **6.51** (6 mg, 4.6 μmol) in ethanol (0.5 mL), 20% $Pd(OH)_2$ (≈2 mg) was added. The flask was degassed under vacuum and refilled with hydrogen (x3). The reaction was stirred for 24 h under a balloon filled with hydrogen that was connected to the flask. The reaction mixture was diluted with ethanol and filtered through a Celite® pad rinsing with more ethanol. The resulting solution was purified by flash chromatography (0% to 9% MeOH in CH_2Cl_2) to give nucleoside **6.54** (2.4 mg, 52%); ^{1}H NMR (400 MHz, Methanol-d4) δ 7.80 (s, 1H), 7.54 (s, 1H), 7.42 (s, 1H), 6.33 – 6.28 (m, 1H), 6.23 (t, J = 6.8 Hz, 1H), 6.16 – 6.10 (m, 1H), 5.30 – 5.21 (m, 1H), 5.19 – 5.11 (m, 1H), 4.57 – 4.50 (m, 1H), 4.41 – 4.14 (m, 3H), 4.11 – 3.77 (m, 4H), 2.76 – 2.66 (m, 2H), 2.65 – 2.40 (m, 4H), 2.27 (t, J = 5.7 Hz, 2H), 2.19 – 2.00 (m, 5H), 1.95 – 1.79 (m, 11H), 1.00 (d, J = 6.7 Hz, 4H), 0.93 (s, 9H), 0.14 (s, 3H); ^{31}P NMR (162 MHz, MeOD) δ 34.02, 33.38.; HRMS (ESI) calculated for $C_{40}H_{61}N_6O_{17}P_2Si$ $[M+H]^+$ *m/z* = 987.3332, found 987.3326.

9 X-Ray crystallographic data

9.1 Crystal and molecular structure of compound $C_{19}H_{24}N_2O_8$ (HAN489)

Hanessian Group
Département de chimie, Université de Montréal,
C.P. 6128, Succ. Centre-Ville, Montréal, Québec, H3C 3J7 (Canada)



3.12 [X-ray]

Structure solved and refined in the laboratory of
X-ray diffraction (Université de Montréal) by Michel Simard.

Table 1 Crystal data and structure refinement for han489.

Identification code	han489
Empirical formula	$C_{19.35}H_{24.7}N_2O_8$
Formula weight	413.31
Temperature/K	105
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.1213(3)
b/Å	8.9288(3)
c/Å	29.9909(12)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	1906.96(13)
Z	4
ρ_{calc}g/cm^3	1.440
μ/mm^{-1}	0.610
F(000)	875.0
Crystal size/mm^3	0.24 × 0.16 × 0.04
Radiation	GaKα (λ = 1.34139)
2Θ range for data collection/°	5.126 to 121.198
Index ranges	$-7 \leq h \leq 9$, $-11 \leq k \leq 10$, $-37 \leq l \leq 38$
Reflections collected	27667
Independent reflections	4360 [R_{int} = 0.0520, R_{sigma} = 0.0339]
Data/restraints/parameters	4360/48/301
Goodness-of-fit on F^2	1.091
Final R indexes [I>=2σ (I)]	R_1 = 0.0407, wR_2 = 0.1041
Final R indexes [all data]	R_1 = 0.0421, wR_2 = 0.1052
Largest diff. peak/hole / e Å^{-3}	0.40/-0.28
Flack parameter	0.04(5)

Table 2 Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for han489. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
C1	6244(3)	5399.0(19)	5941.4(6)	31.6(4)
C2	8011(3)	4543.0(19)	5808.4(6)	31.5(4)
C3	5319(3)	4241.6(19)	6250.1(6)	31.4(4)
C4	7091(3)	4186.0(19)	6540.0(6)	29.9(4)
C5	7341(3)	5842.4(18)	6653.5(6)	30.5(4)

C6	9320(3)	6151(2)	6818.7(6)	33.4(4)
C7	9694(3)	5145(2)	7225.6(7)	37.9(4)
C8	9326(3)	3479(2)	7130.9(7)	37.5(4)
C9	7352(3)	3236.3(18)	6946.9(6)	33.1(4)
C10	7688(3)	3975(2)	5024.2(6)	32.4(4)
C11	7417(3)	1367(2)	4751.1(6)	31.3(4)
C12	7427(3)	850.3(19)	5211.7(6)	32.1(4)
C13	7582(3)	1889.2(19)	5536.3(6)	31.9(4)
C14	7289(3)	-807(2)	5301.0(7)	37.7(4)
C15	5586(3)	962(2)	6986.3(6)	34.4(4)
C16	5560(3)	-654(2)	6840.2(7)	35.5(4)
C17	3601(3)	-1172(2)	6704.7(7)	35.3(4)
C18	2837(3)	-301(3)	6313.8(8)	47.7(5)
C19	970(30)	-758(19)	6138(5)	58(4)
C20	1300(30)	-1190(20)	6028(7)	64(5)
C21	2204(12)	-2509(7)	5752(2)	50.1(16)
C22	616(17)	-96(12)	6348(5)	50(2)
N1	7712(2)	3410.4(17)	5453.4(5)	31.4(3)
N2	7573(2)	2901.1(17)	4694.5(5)	33.4(3)
O1	6882(2)	6575.1(13)	6234.9(4)	32.6(3)
O2	8519.3(19)	3793.9(14)	6210.9(4)	31.0(3)
O3	3730(2)	4753.9(15)	6481.4(5)	37.4(3)
O4	7126(2)	1664.7(14)	6832.7(4)	34.7(3)
O5	4420(3)	1533.7(16)	7222.3(5)	47.9(4)
O6	3664(2)	735.6(16)	6149.8(5)	41.0(3)
O7	7750(2)	5309.5(15)	4946.0(5)	39.5(3)
O8	7279(2)	540.4(15)	4422.7(4)	36.5(3)

Table 3 Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for han489. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	27.5(9)	26.0(7)	41.3(9)	-0.8(7)	-1.9(7)	-0.5(7)
C2	29.3(9)	26.8(7)	38.3(8)	0.6(6)	-1.5(7)	-1.2(7)
C3	24.8(9)	25.5(7)	43.9(9)	-2.1(7)	0.1(7)	0.1(6)
C4	25.2(9)	25.7(7)	38.8(8)	-1.2(6)	1.2(7)	0.1(6)
C5	28.7(9)	24.4(7)	38.5(8)	0.7(6)	0.6(7)	-0.5(7)
C6	31.8(9)	25.1(7)	43.5(9)	-0.3(6)	-2.7(8)	-3.7(7)
C7	39.9(11)	29.3(8)	44.4(10)	0.7(7)	-9.1(8)	-4.7(8)
C8	41.3(11)	27.3(8)	44.0(9)	1.9(7)	-9.3(8)	-1.3(8)

C9	36.8(10)	22.5(7)	40.1(8)	0.2(6)	0.3(8)	-3.4(7)
C10	25.3(8)	31.1(8)	40.9(9)	2.3(7)	2.6(7)	0.6(7)
C11	20.3(8)	31.5(8)	41.9(9)	-0.4(7)	2.3(7)	1.4(7)
C12	24.9(9)	28.7(7)	42.6(9)	1.7(6)	0.4(7)	1.7(7)
C13	26.2(9)	28.0(8)	41.5(9)	2.5(6)	0.7(8)	0.1(7)
C14	37.2(10)	28.5(8)	47.3(10)	1.1(7)	-1.3(9)	-0.2(8)
C15	37.8(10)	25.8(8)	39.6(9)	1.5(6)	-3.4(8)	-2.0(8)
C16	35.8(10)	25.0(8)	45.7(9)	1.2(7)	-2.9(8)	-1.1(8)
C17	34.1(10)	28.5(8)	43.3(9)	2.3(7)	0.2(8)	-2.8(7)
C18	34.5(11)	52.4(12)	56.3(11)	18.1(10)	-6.8(9)	-12.3(10)
C19	49(7)	71(9)	55(9)	25(6)	-18(6)	-23(7)
C20	56(10)	87(12)	50(7)	18(6)	-18(6)	-50(9)
C21	70(5)	33(3)	48(3)	0(2)	-3(3)	-1(3)
C22	42(5)	40(5)	67(7)	11(4)	-2(5)	-3(4)
N1	28.6(8)	27.0(6)	38.4(7)	-0.1(5)	0.2(6)	0.6(6)
N2	30.6(8)	31.7(7)	38.0(8)	2.1(6)	2.1(7)	1.7(7)
O1	33.0(7)	24.3(5)	40.5(6)	1.0(5)	-2.9(5)	-0.2(5)
O2	26.3(7)	29.4(6)	37.4(6)	1.2(5)	-0.1(5)	3.0(5)
O3	25.8(7)	36.6(7)	49.7(8)	-1.9(6)	3.4(6)	1.7(6)
O4	35.3(7)	24.0(5)	44.7(7)	-1.3(5)	0.3(6)	-2.3(5)
O5	49.9(10)	35.6(7)	58.0(9)	-9.0(6)	14.6(8)	-9.2(7)
O6	42.6(8)	35.5(6)	44.8(7)	5.5(6)	-4.5(6)	-8.3(6)
O7	44.0(8)	31.2(6)	43.3(7)	4.5(5)	1.8(6)	1.7(6)
O8	30.5(7)	37.6(6)	41.4(6)	-4.5(5)	2.2(6)	0.8(6)

Table 4 Bond Lengths for han489.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1	C2	1.525(3)	C10	O7	1.215(2)
C1	C3	1.536(3)	C11	C12	1.457(3)
C1	O1	1.444(2)	C11	N2	1.384(2)
C2	N1	1.484(2)	C11	O8	1.235(2)
C2	O2	1.427(2)	C12	C13	1.349(3)
C3	C4	1.533(3)	C12	C14	1.507(2)
C3	O3	1.404(2)	C13	N1	1.384(2)
C4	C5	1.528(2)	C15	C16	1.508(2)
C4	C9	1.498(2)	C15	O4	1.345(3)
C4	O2	1.460(2)	C15	O5	1.205(3)
C5	C6	1.519(3)	C16	C17	1.525(3)
C5	O1	1.453(2)	C17	C18	1.508(3)

C6	C7	1.538(3)	C18	C19	1.486(19)
C7	C8	1.537(2)	C18	C20	1.60(2)
C8	C9	1.526(3)	C18	C22	1.596(12)
C9	O4	1.4534(19)	C18	O6	1.202(3)
C10	N1	1.383(2)	C20	C21	1.576(19)
C10	N2	1.380(2)			

Table 5 Bond Angles for han489.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C2	C1	C3	100.03(14)	N2	C11	C12	115.43(16)
O1	C1	C2	105.33(14)	O8	C11	C12	124.56(16)
O1	C1	C3	104.88(14)	O8	C11	N2	120.01(16)
N1	C2	C1	114.26(15)	C11	C12	C14	118.65(16)
O2	C2	C1	102.88(14)	C13	C12	C11	117.83(16)
O2	C2	N1	108.91(13)	C13	C12	C14	123.52(17)
C4	C3	C1	90.62(14)	C12	C13	N1	123.40(17)
O3	C3	C1	115.11(15)	O4	C15	C16	110.91(17)
O3	C3	C4	113.19(15)	O5	C15	C16	124.58(19)
C5	C4	C3	101.00(14)	O5	C15	O4	124.45(17)
C9	C4	C3	125.63(16)	C15	C16	C17	112.26(17)
C9	C4	C5	110.60(14)	C18	C17	C16	112.38(17)
O2	C4	C3	101.41(14)	C17	C18	C20	114.0(9)
O2	C4	C5	107.56(14)	C17	C18	C22	111.5(5)
O2	C4	C9	109.20(14)	C19	C18	C17	117.2(7)
C6	C5	C4	110.85(15)	O6	C18	C17	122.6(2)
O1	C5	C4	102.54(14)	O6	C18	C19	120.2(7)
O1	C5	C6	114.13(15)	O6	C18	C20	119.8(8)
C5	C6	C7	108.27(16)	O6	C18	C22	115.0(4)
C8	C7	C6	112.89(16)	C21	C20	C18	112.1(15)
C9	C8	C7	111.19(16)	C10	N1	C2	114.91(14)
C4	C9	C8	109.18(15)	C10	N1	C13	121.64(15)
O4	C9	C4	109.92(14)	C13	N1	C2	123.33(15)
O4	C9	C8	108.93(15)	C10	N2	C11	127.20(16)
N2	C10	N1	114.46(15)	C1	O1	C5	105.67(12)
O7	C10	N1	122.47(17)	C2	O2	C4	106.43(13)
O7	C10	N2	123.06(17)	C15	O4	C9	117.39(15)

Table 6 Hydrogen Bonds for han489.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O3	H3A	O8[1]	0.91(4)	2.16(3)	2.914(2)	140(3)

[1]-1/2+X,1/2-Y,1-Z

Table 7 Torsion Angles for han489.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
C1	C2	N1	C10	81.5(2)	C15	C16	C17	C18	61.6(2)
C1	C2	N1	C13	-102.4(2)	C16	C15	O4	C9	-179.93(15)
C1	C2	O2	C4	1.35(17)	C16	C17	C18	C19	177.1(7)
C1	C3	C4	C5	54.58(15)	C16	C17	C18	C20	154.6(8)
C1	C3	C4	C9	-179.94(16)	C16	C17	C18	C22	-146.2(5)
C1	C3	C4	O2	-56.07(14)	C16	C17	C18	O6	-3.9(3)
C2	C1	C3	C4	56.13(14)	C17	C18	C20	C21	-71.1(14)
C2	C1	C3	O3	172.24(15)	N1	C2	O2	C4	-120.24(15)
C2	C1	O1	C5	-73.96(17)	N1	C10	N2	C11	2.4(3)
C3	C1	C2	N1	79.79(17)	N2	C10	N1	C2	174.65(16)
C3	C1	C2	O2	-38.09(16)	N2	C10	N1	C13	-1.5(3)
C3	C1	O1	C5	31.09(18)	N2	C11	C12	C13	0.1(3)
C3	C4	C5	C6	-162.47(14)	N2	C11	C12	C14	-179.16(18)
C3	C4	C5	O1	-40.27(17)	O1	C1	C2	N1	-171.61(14)
C3	C4	C9	C8	178.56(16)	O1	C1	C2	O2	70.51(16)
C3	C4	C9	O4	59.1(2)	O1	C1	C3	C4	-52.82(15)
C3	C4	O2	C2	36.09(16)	O1	C1	C3	O3	63.28(19)
C4	C5	C6	C7	-57.28(19)	O1	C5	C6	C7	-172.44(14)
C4	C5	O1	C1	5.70(18)	O2	C2	N1	C10	-164.16(16)
C4	C9	O4	C15	-110.01(18)	O2	C2	N1	C13	12.0(2)
C5	C4	C9	C8	-60.09(19)	O2	C4	C5	C6	-56.63(19)
C5	C4	C9	O4	-179.52(15)	O2	C4	C5	O1	65.56(17)
C5	C4	O2	C2	-69.45(17)	O2	C4	C9	C8	58.09(18)
C5	C6	C7	C8	53.9(2)	O2	C4	C9	O4	-61.34(19)
C6	C5	O1	C1	125.64(16)	O3	C3	C4	C5	-63.21(18)
C6	C7	C8	C9	-54.1(2)	O3	C3	C4	C9	62.3(2)
C7	C8	C9	C4	55.8(2)	O3	C3	C4	O2	-173.86(13)
C7	C8	C9	O4	175.84(16)	O4	C15	C16	C17	-139.86(17)
C8	C9	O4	C15	130.40(17)	O5	C15	C16	C17	42.6(3)
C9	C4	C5	C6	62.5(2)	O5	C15	O4	C9	-2.4(3)
C9	C4	C5	O1	-175.27(14)	O6	C18	C20	C21	88.1(15)
C9	C4	O2	C2	170.48(14)	O7	C10	N1	C2	-5.8(3)
C11	C12	C13	N1	0.7(3)	O7	C10	N1	C13	178.0(2)

C12	C11	N2	C10	-1.7(3)	O7	C10	N2	C11	-177.1(2)
C12	C13	N1	C2	-175.75(18)	O8	C11	C12	C13	-179.83(18)
C12	C13	N1	C10	0.1(3)	O8	C11	C12	C14	0.9(3)
C14	C12	C13	N1	179.85(19)	O8	C11	N2	C10	178.18(18)

Table 8 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for han489.

Atom	*x*	*y*	*z*	U(eq)
H2A	7690(40)	3200(30)	4414(10)	48(7)
H3A	2760(60)	4650(40)	6285(11)	72(10)
H1	5446	5741	5687	38
H2	9023	5258	5718	38
H3	5081	3269	6095	38
H5	6412	6141	6887	37
H6A	9447	7218	6903	40
H6B	10240	5930	6580	40
H7A	11016	5271	7320	45
H7B	8881	5472	7475	45
H8A	10259	3107	6913	45
H8B	9478	2898	7410	45
H9	6404	3523	7178	40
H13	7603	1559	5837	38
H14A	6165	-1209	5155	56
H14B	7208	-979	5623	56
H14C	8406	-1310	5183	56
H16A	6020	-1291	7087	43
H16B	6425	-783	6585	43
H17A	3646	-2248	6626	42
H17B	2739	-1056	6962	42
H19A	1002	-1821	6057	87
H19B	670	-159	5873	87
H19C	12	-593	6367	87
H20A	339	-1603	6232	77
H20B	675	-486	5822	77
H21A	2494	-2158	5450	75
H21B	1315	-3345	5735	75
H21C	3361	-2843	5898	75
H22A	-8	-1020	6252	74
H22B	221	734	6156	74

H22C	270	125	6658	74

Table 9 Atomic Occupancy for han489.

Atom	*Occupancy*	Atom	*Occupancy*	Atom	*Occupancy*
C19	0.4	H19A	0.4	H19B	0.4
H19C	0.4	C20	0.35	H20A	0.35
H20B	0.35	C21	0.35	H21A	0.35
H21B	0.35	H21C	0.35	C22	0.25
H22A	0.25	H22B	0.25	H22C	0.25

Experimental

Single crystals of $C_{19.35}H_{24.7}N_2O_8$ **[han489]** was selected and mounted on a mylar loop on a **Bruker Venture Metaljet** diffractometer. The crystal was kept at 105 K during data collection. Using Olex2 [1], the structure was solved with the XT [2] structure solution program using Direct Methods and refined with the XL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Sheldrick, G.M. (2015). Acta Cryst. A71, 3-8.
3. Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.

Crystal structure determination of [han489]

Crystal Data for $C_{19.35}H_{24.7}N_2O_8$ (M =413.31 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 7.1213(3) Å, b = 8.9288(3) Å, c = 29.9909(12) Å, V = 1906.96(13) Å^3, Z = 4, T = 105 K, μ(GaKα) = 0.610 mm^{-1}, *Dcalc* = 1.440 g/cm^3, 27667 reflections measured (5.126° ≤ 2Θ ≤ 121.198°), 4360 unique (R_{int} = 0.0520, R_{sigma} = 0.0339) which were used in all calculations. The final R_1 was 0.0407 (I > 2σ(I)) and wR_2 was 0.1052 (all data).

Refinement model description

Number of restraints - 48, number of constraints - unknown.
Details:
1. Fixed Uiso
At 1.2 times of:
All C(H) groups, All C(H,H) groups
At 1.5 times of:
All C(H,H,H) groups
2. Uiso/Uaniso restraints and constraints
C19 ≈ C20 ≈ C21 ≈ C22: within 1.7A with sigma of 0.04 and sigma for terminal atoms of 0.08
Uanis(C19) ≈ Ueq, Uanis(C20) ≈ Ueq, Uanis(C21) ≈ Ueq, Uanis(C22) ≈ Ueq: with sigma of 0.1 and sigma for terminal atoms of 0.2
3. Others

Fixed Sof: C19(0.4) H19A(0.4) H19B(0.4) H19C(0.4) C20(0.35) H20A(0.35) H20B(0.35) C21(0.35) H21A(0.35) H21B(0.35) H21C(0.35) C22(0.25) H22A(0.25) H22B(0.25) H22C(0.25)

4.a Ternary CH refined with riding coordinates:
C1(H1), C2(H2), C3(H3), C5(H5), C9(H9)

4.b Secondary CH2 refined with riding coordinates:
C6(H6A,H6B), C7(H7A,H7B), C8(H8A,H8B), C16(H16A,H16B), C17(H17A,H17B), C20(H20A,H20B)

4.c Aromatic/amide H refined with riding coordinates:
C13(H13)

4.d Idealised Me refined as rotating group:
C14(H14A,H14B,H14C), C19(H19A,H19B,H19C), C21(H21A,H21B,H21C), C22(H22A,H22B, H22C)

9.2 Crystal and molecular structure of compound $C_{21}H_{25}N_5O_6$ (ROBE39)

Hanessian Group
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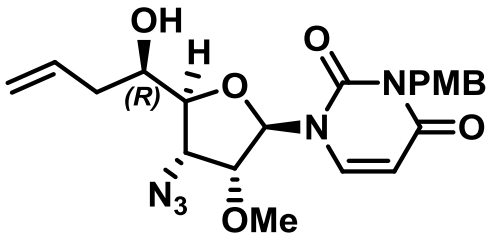

4.9
X-Ray

Structure solved and refined in the laboratory of
X-ray diffraction (Université de Montréal) by Michel Simard and Robert Giacometti

Table 1 Crystal data and structure refinement for robe39.

Identification code	robe39
Empirical formula	$C_{21}H_{25}N_5O_6$
Formula weight	443.46
Temperature/K	100
Crystal system	monoclinic
Space group	$P2_1$
a/Å	14.3027(5)
b/Å	4.5978(2)
c/Å	16.0670(5)
α/°	90
β/°	94.209(2)
γ/°	90
Volume/Å^3	1053.73(7)
Z	2
ρ_{calc}g/cm^3	1.398
μ/mm^{-1}	0.872
F(000)	468.0
Crystal size/mm^3	0.2 × 0.04 × 0.02
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	5.516 to 142.788
Index ranges	-17 ≤ h ≤ 17, -5 ≤ k ≤ 5, -19 ≤ l ≤ 19
Reflections collected	14385
Independent reflections	4022 [R_{int} = 0.0527, R_{sigma} = 0.0468]
Data/restraints/parameters	4022/1/293
Goodness-of-fit on F^2	1.030
Final R indexes [I>=2σ (I)]	R_1 = 0.0437, wR_2 = 0.1125
Final R indexes [all data]	R_1 = 0.0470, wR_2 = 0.1155
Largest diff. peak/hole / e Å^{-3}	0.27/-0.18
Flack parameter	0.3(3)

Table 2 Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for robe39. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O_1	9186.9(13)	3859(5)	1681.6(11)	35.0(4)
O_2	11311.8(13)	-1702(5)	3240.3(12)	40.4(5)
O_3	6840.5(13)	6851(5)	2261.0(11)	33.8(4)
O_4	8164.6(13)	7073(5)	3787.0(12)	33.8(4)
O_5	7813.7(13)	2961(5)	5114.2(11)	33.5(4)

O_6	13128.4(14)	7340(5)	188.7(13)	42.9(5)
N_1	10222.1(15)	944(5)	2446.8(14)	30.7(5)
N_2	9116.3(15)	3808(6)	3091.2(13)	30.3(5)
N_3	6005.0(15)	2907(6)	3372.0(14)	36.6(5)
N_4	5635.2(16)	2533(6)	2670.2(15)	38.2(5)
N_5	5198.4(19)	2146(8)	2058.1(17)	51.8(7)
C_1	9494.7(17)	2951(6)	2353.6(15)	28.8(5)
C_2	10636.6(18)	-27(7)	3214.2(17)	34.1(6)
C_3	10198(2)	1010(9)	3933.9(17)	42.1(7)
C_4	9462.9(18)	2809(8)	3851.4(16)	37.1(6)
C_5	8345.1(17)	5983(6)	2997.5(16)	30.1(5)
C_6	7424.0(17)	4639(6)	2626.8(16)	30.7(5)
C_7	7013.4(16)	3610(6)	3429.3(16)	29.5(5)
C_8	7242.4(18)	6154(6)	4017.8(16)	31.1(6)
C_9	7295.2(18)	5569(7)	4948.7(16)	32.4(6)
C_{10}	6307(2)	5371(7)	5262.3(18)	38.9(6)
C_{11}	6345(2)	5022(8)	6196.5(19)	42.4(7)
C_{12}	5948(2)	2872(9)	6581(2)	51.6(8)
C_{13}	6987(2)	7302(8)	1399.0(18)	45.8(7)
C_{14}	10585.1(18)	-184(6)	1663.6(16)	31.3(5)
C_{15}	11258.8(17)	1877(6)	1286.8(15)	30.4(5)
C_{16}	12150.7(19)	2257(7)	1671.0(16)	36.8(6)
C_{17}	12794.7(19)	4091(7)	1327.0(18)	38.7(6)
C_{18}	12543.4(19)	5538(7)	590.7(17)	34.5(6)
C_{19}	11645.6(19)	5203(7)	200.6(16)	35.2(6)
C_{20}	11013.7(18)	3374(6)	554.9(16)	32.9(6)
C_{21}	14031(2)	7853(10)	592(2)	52.7(8)

Table 3 Anisotropic Displacement Parameters ($Å^2×10^3$) for robe39. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O_1	30.9(9)	45.6(11)	29.3(9)	2.7(8)	8.7(7)	2.3(8)
O_2	31.5(9)	53.7(13)	37.0(10)	6.6(10)	8.3(7)	7.2(9)
O_3	33.0(9)	38.9(10)	30.4(9)	3.7(8)	8.1(7)	4.7(8)
O_4	28.9(9)	38.6(10)	35.5(9)	-5.4(8)	12.4(7)	-6.1(8)
O_5	30.2(9)	40.4(10)	30.5(8)	-0.3(8)	6.0(7)	2.4(8)
O_6	37.4(10)	53.6(13)	39.3(10)	0.5(10)	12.8(8)	-9.5(10)
N_1	25.9(10)	38.7(11)	28.6(10)	0.3(9)	8.7(8)	-3.0(9)
N_2	23.9(9)	39.7(11)	28.2(10)	-1.1(9)	8.5(7)	-3.0(9)

N_3	25.2(10)	49.3(14)	36.0(11)	0.2(11)	7.3(8)	-3.3(10)
N_4	25.8(10)	46.2(14)	43.2(13)	0.2(11)	7.3(9)	-2.1(10)
N_5	34.8(12)	74(2)	46.1(14)	-1.1(15)	-0.3(11)	-8.4(13)
C_1	23.9(11)	36.4(12)	26.9(11)	0.6(10)	8.2(8)	-5.1(10)
C_2	25.4(12)	44.8(15)	32.9(13)	3.3(12)	7.4(10)	-2.0(11)
C_3	30.3(13)	69(2)	27.5(12)	5.8(14)	8.0(10)	5.2(13)
C_4	26.9(12)	56.5(17)	29.0(11)	-3.1(12)	8.6(9)	0.0(12)
C_5	26.9(12)	32.6(12)	31.9(12)	-2.0(10)	9.0(9)	-2.3(10)
C_6	26.6(12)	34.5(13)	31.8(12)	0.7(11)	6.6(9)	-0.7(10)
C_7	22.3(11)	34.7(13)	32.1(12)	2.5(11)	6.7(9)	-0.5(10)
C_8	24.7(11)	34.4(13)	35.5(13)	-1.0(11)	10.4(9)	-0.7(10)
C_9	26.8(12)	38.4(14)	32.8(13)	-3.8(11)	8.2(10)	-0.1(10)
C_{10}	29.7(13)	50.9(17)	37.4(14)	1.2(13)	12.1(10)	2.7(12)
C_{11}	32.4(14)	56.8(19)	39.4(15)	-3.3(14)	12.3(11)	9.2(13)
C_{12}	50.4(18)	63(2)	43.3(15)	6.7(17)	20.9(13)	9.2(16)
C_{13}	54.4(17)	52.5(19)	31.7(13)	5.4(14)	11.0(12)	11.5(15)
C_{14}	28.3(12)	38.7(13)	27.5(11)	-2.7(11)	7.0(9)	-0.7(10)
C_{15}	26.3(11)	36.3(13)	29.7(12)	-6.2(11)	10.2(9)	1.7(10)
C_{16}	30.3(13)	48.5(16)	32.3(12)	4.0(13)	6.5(10)	2.7(12)
C_{17}	26.6(12)	53.5(17)	36.8(13)	-1.1(13)	7.3(10)	-1.0(12)
C_{18}	30.3(12)	40.8(14)	34.0(13)	-4.9(12)	12.9(10)	-0.9(11)
C_{19}	34.2(13)	43.5(15)	28.6(12)	-0.5(12)	7.5(10)	2.2(12)
C_{20}	27.1(12)	42.1(15)	30.4(12)	-4.9(11)	7.2(9)	0.8(11)
C_{21}	34.8(15)	68(2)	56.3(18)	6.9(18)	12.2(13)	-11.1(16)

Table 4 Bond Lengths for robe39.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O_1	C_1	1.210(3)	N_4	N_5	1.140(4)
O_2	C_2	1.233(4)	C_2	C_3	1.437(4)
O_3	C_6	1.416(3)	C_3	C_4	1.337(4)
O_3	C_{13}	1.431(3)	C_5	C_6	1.535(3)
O_4	C_5	1.405(3)	C_6	C_7	1.531(3)
O_4	C_8	1.459(3)	C_7	C_8	1.525(4)
O_5	C_9	1.425(3)	C_8	C_9	1.516(4)
O_6	C_{18}	1.372(3)	C_9	C_{10}	1.538(3)
O_6	C_{21}	1.421(4)	C_{10}	C_{11}	1.506(4)
N_1	C_1	1.390(4)	C_{11}	C_{12}	1.316(5)
N_1	C_2	1.401(4)	C_{14}	C_{15}	1.509(4)
N_1	C_{14}	1.489(3)	C_{15}	C_{16}	1.387(4)

N_2	C_1	1.395(3)	C_{15}	C_{20}	1.385(4)
N_2	C_4	1.364(4)	C_{16}	C_{17}	1.393(4)
N_2	C_5	1.488(3)	C_{17}	C_{18}	1.382(4)
N_3	N_4	1.222(3)	C_{18}	C_{19}	1.395(4)
N_3	C_7	1.474(3)	C_{19}	C_{20}	1.387(4)

Table 5 Bond Angles for robe39.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C_6	O_3	C_{13}	112.5(2)	C_7	C_6	C_5	99.7(2)
C_5	O_4	C_8	110.8(2)	N_3	C_7	C_6	116.7(2)
C_{18}	O_6	C_{21}	116.7(2)	N_3	C_7	C_8	111.8(2)
C_1	N_1	C_2	124.8(2)	C_8	C_7	C_6	101.9(2)
C_1	N_1	C_{14}	116.4(2)	O_4	C_8	C_7	102.81(19)
C_2	N_1	C_{14}	118.8(2)	O_4	C_8	C_9	108.8(2)
C_1	N_2	C_5	115.8(2)	C_9	C_8	C_7	117.9(2)
C_4	N_2	C_1	121.8(2)	O_5	C_9	C_8	108.7(2)
C_4	N_2	C_5	122.3(2)	O_5	C_9	C_{10}	111.6(2)
N_4	N_3	C_7	116.3(2)	C_8	C_9	C_{10}	110.7(2)
N_5	N_4	N_3	172.3(3)	C_{11}	C_{10}	C_9	111.5(2)
O_1	C_1	N_1	123.1(2)	C_{12}	C_{11}	C_{10}	124.2(3)
O_1	C_1	N_2	121.5(2)	N_1	C_{14}	C_{15}	113.1(2)
N_1	C_1	N_2	115.4(2)	C_{16}	C_{15}	C_{14}	119.6(2)
O_2	C_2	N_1	120.6(2)	C_{20}	C_{15}	C_{14}	121.5(2)
O_2	C_2	C_3	124.5(3)	C_{20}	C_{15}	C_{16}	118.8(3)
N_1	C_2	C_3	114.9(2)	C_{15}	C_{16}	C_{17}	120.9(3)
C_4	C_3	C_2	120.8(3)	C_{18}	C_{17}	C_{16}	119.6(3)
C_3	C_4	N_2	121.9(2)	O_6	C_{18}	C_{17}	124.4(3)
O_4	C_5	N_2	109.4(2)	O_6	C_{18}	C_{19}	115.3(3)
O_4	C_5	C_6	106.5(2)	C_{17}	C_{18}	C_{19}	120.3(3)
N_2	C_5	C_6	112.2(2)	C_{20}	C_{19}	C_{18}	119.2(3)
O_3	C_6	C_5	109.6(2)	C_{15}	C_{20}	C_{19}	121.2(2)
O_3	C_6	C_7	108.7(2)				

Table 6 Hydrogen Bonds for robe39.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O_5	H_5	O_2^1	0.84	2.01	2.845(3)	169.6

[1]2-X,1/2+Y,1-Z

Table 7 Torsion Angles for robe39.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
O_2	C_2	C_3	C_4	-179.5(3)	C_5	O_4	C_8	C_7	18.0(3)
O_3	C_6	C_7	N_3	48.8(3)	C_5	O_4	C_8	C_9	143.7(2)
O_3	C_6	C_7	C_8	-73.3(2)	C_5	N_2	C_1	O_1	-2.5(4)
O_4	C_5	C_6	O_3	82.4(3)	C_5	N_2	C_1	N_1	179.3(2)
O_4	C_5	C_6	C_7	-31.5(3)	C_5	N_2	C_4	C_3	-175.3(3)
O_4	C_8	C_9	O_5	-71.8(3)	C_5	C_6	C_7	N_3	163.5(2)
O_4	C_8	C_9	C_{10}	165.3(2)	C_5	C_6	C_7	C_8	41.4(2)
O_5	C_9	C_{10}	C_{11}	63.2(3)	C_6	C_7	C_8	O_4	-37.2(2)
O_6	C_{18}	C_{19}	C_{20}	178.7(3)	C_6	C_7	C_8	C_9	-156.8(2)
N_1	C_2	C_3	C_4	-1.2(5)	C_7	C_8	C_9	O_5	44.6(3)
N_1	C_{14}	C_{15}	C_{16}	72.0(3)	C_7	C_8	C_9	C_{10}	-78.2(3)
N_1	C_{14}	C_{15}	C_{20}	-109.1(3)	C_8	O_4	C_5	N_2	-112.6(2)
N_2	C_5	C_6	O_3	-157.9(2)	C_8	O_4	C_5	C_6	8.9(3)
N_2	C_5	C_6	C_7	88.2(2)	C_8	C_9	C_{10}	C_{11}	-175.6(3)
N_3	C_7	C_8	O_4	-162.6(2)	C_9	C_{10}	C_{11}	C_{12}	-124.1(3)
N_3	C_7	C_8	C_9	77.8(3)	C_{13}	O_3	C_6	C_5	90.4(3)
N_4	N_3	C_7	C_6	16.8(4)	C_{13}	O_3	C_6	C_7	-161.5(2)
N_4	N_3	C_7	C_8	133.6(3)	C_{14}	N_1	C_1	O_1	-3.0(4)
C_1	N_1	C_2	O_2	-176.3(3)	C_{14}	N_1	C_1	N_2	175.2(2)
C_1	N_1	C_2	C_3	5.4(4)	C_{14}	N_1	C_2	O_2	2.6(4)
C_1	N_1	C_{14}	C_{15}	80.2(3)	C_{14}	N_1	C_2	C_3	-175.7(3)
C_1	N_2	C_4	C_3	1.6(5)	C_{14}	C_{15}	C_{16}	C_{17}	178.5(3)
C_1	N_2	C_5	O_4	-168.2(2)	C_{14}	C_{15}	C_{20}	C_{19}	-178.2(3)
C_1	N_2	C_5	C_6	73.9(3)	C_{15}	C_{16}	C_{17}	C_{18}	-0.4(5)
C_2	N_1	C_1	O_1	175.9(3)	C_{16}	C_{15}	C_{20}	C_{19}	0.7(4)
C_2	N_1	C_1	N_2	-5.9(4)	C_{16}	C_{17}	C_{18}	O_6	-178.4(3)
C_2	N_1	C_{14}	C_{15}	-98.8(3)	C_{16}	C_{17}	C_{18}	C_{19}	1.0(5)
C_2	C_3	C_4	N_2	-2.1(5)	C_{17}	C_{18}	C_{19}	C_{20}	-0.8(4)
C_4	N_2	C_1	O_1	-179.6(3)	C_{18}	C_{19}	C_{20}	C_{15}	-0.1(4)
C_4	N_2	C_1	N_1	2.2(4)	C_{20}	C_{15}	C_{16}	C_{17}	-0.5(4)
C_4	N_2	C_5	O_4	8.9(3)	C_{21}	O_6	C_{18}	C_{17}	-3.8(4)
C_4	N_2	C_5	C_6	-109.1(3)	C_{21}	O_6	C_{18}	C_{19}	176.7(3)

Table 8 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for robe39.

Atom	*x*	*y*	*z*	U(eq)

H_5	8015.15	2925.5	5618.56	50
H_3	10434.15	411.11	4475.06	51
H_4	9173.3	3409.38	4336.51	45
H_{5A}	8532.8	7615.05	2633.76	36
H_6	7527.1	3007.68	2232.23	37
H_7	7371.84	1864.63	3644.15	35
H_8	6782.69	7756.77	3885.25	37
H_9	7636.76	7214.28	5242.63	39
H_{10A}	5951.42	7155.96	5100.15	47
H_{10B}	5971.5	3693.24	4994.4	47
H_{11}	6676.98	6444.01	6529.66	51
H_{12A}	5610.57	1413.78	6265.54	62
H_{12B}	5998.4	2778.28	7172.81	62
H_{13A}	6828.09	5522.13	1084.23	69
H_{13B}	7646.55	7792.52	1342.64	69
H_{13C}	6588.17	8899.96	1179.83	69
H_{14A}	10049.6	-553.98	1251.27	38
H_{14B}	10906.02	-2061.38	1783.45	38
H_{16}	12324.77	1252.57	2175.45	44
H_{17}	13402.66	4346	1596.81	46
H_{19}	11469.29	6216.25	-301.93	42
H_{20}	10401.76	3144.39	290.97	40
H_{21A}	14377.37	9201.81	257.09	79
H_{21B}	14373.18	6010.03	654.08	79
H_{21C}	13963.56	8699.3	1144.62	79

Experimental

A suitable single crystals of $C_{21}H_{25}N_5O_6$ robe39 was selected and mounted on a mylar loop on a Bruker Smart APEX diffractometer. The crystal was kept at 100 K during data collection. Using Olex2 [1], the structure was solved with the ShelXS [2] structure solution program using Direct Methods and refined with the XL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.
3. Sheldrick, G.M. (2015). Acta Cryst. C71, 3-8.

Crystal structure determination of robe39

Crystal Data for $C_{21}H_{25}N_5O_6$ (M =443.46 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 14.3027(5) Å, b = 4.5978(2) Å, c = 16.0670(5) Å, β = 94.209(2)°, V = 1053.73(7) Å^3, Z = 2, T = 100 K, μ(CuKα) = 0.872 mm^{-1}, $Dcalc$ = 1.398 g/cm^3, 14385 reflections measured (5.516°

≤ 2Θ ≤ 142.788°), 4022 unique (R_{int} = 0.0527, R_{sigma} = 0.0468) which were used in all calculations. The final R_1 was 0.0437 (I > 2σ(I)) and wR_2 was 0.1155 (all data).

Refinement model description

Number of restraints - 1, number of constraints - unknown.

Details:

1. Twinned data refinement
 Scales: 0.7(3)
 0.3(3)
2. Fixed Uiso
 At 1.2 times of:
  All C(H) groups, All C(H,H) groups
 At 1.5 times of:
  All C(H,H,H) groups, All O(H) groups
3.a Ternary CH refined with riding coordinates:
 C5(H5A), C6(H6), C7(H7), C8(H8), C9(H9)
3.b Secondary CH2 refined with riding coordinates:
 C10(H10A,H10B), C14(H14A,H14B)
3.c Aromatic/amide H refined with riding coordinates:
 C3(H3), C4(H4), C11(H11), C16(H16), C17(H17), C19(H19), C20(H20)
3.d X=CH2 refined with riding coordinates:
 C12(H12A,H12B)
3.e Idealised Me refined as rotating group:
 C13(H13A,H13B,H13C), C21(H21A,H21B,H21C)
3.f Idealised tetrahedral OH refined as rotating group:
 O5(H5)

9.3 Crystal and molecular structure of compound $C_{15}H_{27}O_9P$ (ROBE52)

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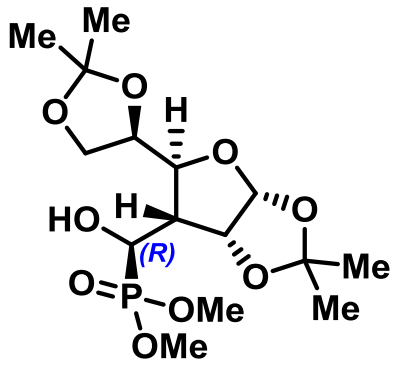

5.4

Structure solved and refined in the laboratory of
X-ray diffraction (Université de Montréal) by Michel Simard and Robert Giacometti

Table 1 Crystal data and structure refinement for robe52.

Identification code	robe52
Empirical formula	$C_{15}H_{27}O_9P$
Formula weight	382.33
Temperature/K	100
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	6.3865(2)
b/Å	16.3617(5)
c/Å	17.3897(5)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	1817.12(10)
Z	4
ρ_{calc}g/cm^3	1.398
μ/mm^{-1}	1.134
F(000)	816.0
Crystal size/mm^3	0.12 × 0.04 × 0.04
Radiation	GaKα (λ = 1.34139)
2Θ range for data collection/°	10.022 to 121.252
Index ranges	$-8 \le h \le 7$, $-21 \le k \le 20$, $-22 \le l \le 22$
Reflections collected	27079
Independent reflections	4144 [R_{int} = 0.0367, R_{sigma} = 0.0242]
Data/restraints/parameters	4144/0/235
Goodness-of-fit on F^2	1.068
Final R indexes [I>=2σ (I)]	R_1 = 0.0322, wR_2 = 0.0866
Final R indexes [all data]	R_1 = 0.0327, wR_2 = 0.0872
Largest diff. peak/hole / e Å^{-3}	0.45/-0.24
Flack parameter	0.020(5)

Table 2 Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for robe52. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
C_1	3348(3)	4770.3(12)	3347.4(11)	19.4(4)
C_2	3704(3)	4551.3(12)	4201.9(11)	18.3(4)

C_3	5617(3)	3983.1(11)	4188.5(11)	16.1(3)
C_4	6737(3)	4275.9(11)	3453.8(11)	16.8(4)
C_5	8205(3)	3675.5(12)	3068.0(10)	17.0(3)
C_6	7192(3)	2908.1(12)	2731.6(11)	18.7(4)
C_7	3413(3)	5957.6(12)	4080.9(11)	18.6(4)
C_8	4888(3)	6677.5(12)	4104.2(13)	23.7(4)
C_9	1197(3)	6168.1(14)	4334.2(13)	26.2(5)
C_{10}	9076(3)	3515.2(11)	1768.7(11)	16.7(4)
C_{11}	11249(3)	3436.8(14)	1430.6(13)	25.4(4)
C_{12}	7469(3)	3826.4(13)	1193.0(12)	23.0(4)
C_{13}	6979(3)	4036.4(12)	4915.3(11)	16.0(4)
C_{14}	1984(4)	4306.1(16)	6215.8(15)	32.0(5)
C_{15}	7016(4)	3721.9(17)	7194.0(12)	30.5(5)
O_1	3375(3)	5636.2(9)	3315.9(8)	23.4(3)
O_2	4270(2)	5318.2(8)	4541.8(8)	19.3(3)
O_4	5047(2)	4446.3(9)	2930.5(8)	20.9(3)
O_5	9142(2)	4066.0(8)	2411.9(8)	18.8(3)
O_6	8483(2)	2742.3(8)	2078.6(8)	18.9(3)
O_{13}	8958(2)	3651.4(10)	4834.3(8)	20.3(3)
O_{14}	4154(2)	4400.5(9)	6022.1(9)	23.0(3)
O_{15}	7440(3)	3689.6(12)	6380.5(9)	28.6(3)
O_{16}	4498(3)	2889.7(9)	5681.5(9)	27.8(3)
P_1	5618.0(8)	3670.0(3)	5772.7(3)	18.05(13)

Table 3 Anisotropic Displacement Parameters ($Å^2×10^3$) for robe52. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C_1	17.8(9)	24.0(9)	16.5(9)	-0.3(7)	-2.0(7)	-0.5(8)
C_2	17.6(8)	22.3(9)	14.9(8)	0.1(7)	-0.3(7)	-1.7(7)
C_3	17.6(8)	16.9(8)	13.7(8)	0.1(6)	-0.7(7)	-2.5(7)
C_4	19.3(9)	18.3(8)	12.9(8)	-0.4(7)	0.4(7)	-0.3(7)
C_5	19.3(8)	19.1(8)	12.7(8)	0.3(7)	1.0(6)	-0.7(7)
C_6	23.7(9)	16.2(8)	16.3(8)	1.2(7)	0.3(7)	-0.9(7)
C_7	20.0(9)	21.2(9)	14.6(9)	0.8(7)	-2.1(7)	3.5(7)
C_8	24.0(9)	22.9(10)	24.1(10)	2.8(8)	-3.1(8)	1.0(8)
C_9	20.6(9)	31.3(11)	26.7(11)	0.3(8)	1.7(8)	6.0(8)
C_{10}	18.7(8)	17.7(8)	13.5(8)	-0.8(6)	-0.1(7)	-0.5(7)
C_{11}	20.8(9)	29.7(11)	25.7(10)	-2.7(8)	5.2(8)	1.7(8)
C_{12}	25.4(10)	23.8(10)	19.9(9)	4.8(8)	-5.3(8)	-2.3(8)

C_{13}	17.3(8)	17.6(8)	13.0(8)	0.5(6)	1.4(7)	-0.8(7)
C_{14}	22.2(10)	36.0(12)	37.9(13)	-5.3(10)	6.1(9)	0.5(9)
C_{15}	30.7(11)	45.4(13)	15.5(9)	3.1(9)	-2.9(8)	-6.9(10)
O_1	33.1(8)	23.3(7)	13.9(6)	-0.7(5)	-1.8(6)	5.8(6)
O_2	24.2(7)	18.6(6)	15.2(6)	0.2(5)	-3.0(5)	3.6(6)
O_4	22.0(7)	27.4(7)	13.4(6)	-0.2(5)	-1.3(5)	5.3(6)
O_5	23.6(7)	18.8(6)	13.8(6)	-2.7(5)	3.1(5)	-5.3(6)
O_6	25.1(7)	15.6(6)	16.0(6)	-0.4(5)	0.8(6)	0.6(5)
O_{13}	18.4(6)	22.7(7)	19.9(7)	-2.9(6)	-1.0(5)	2.5(6)
O_{14}	22.0(7)	22.0(7)	24.9(7)	-1.2(6)	4.1(6)	-2.7(6)
O_{15}	23.3(7)	46.9(9)	15.5(7)	3.5(7)	-1.8(5)	-1.1(7)
O_{16}	34.5(8)	20.8(7)	28.1(8)	0.5(6)	5.6(7)	-7.2(6)
P_1	20.0(2)	20.3(2)	13.8(2)	1.64(17)	0.61(19)	-2.13(18)

Table 4 Bond Lengths for robe52.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C_1	C_2	1.545(3)	C_7	O_1	1.431(2)
C_1	O_1	1.418(2)	C_7	O_2	1.427(2)
C_1	O_4	1.409(2)	C_{10}	C_{11}	1.513(3)
C_2	C_3	1.535(3)	C_{10}	C_{12}	1.521(3)
C_2	O_2	1.433(2)	C_{10}	O_5	1.437(2)
C_3	C_4	1.541(3)	C_{10}	O_6	1.426(2)
C_3	C_{13}	1.537(3)	C_{13}	O_{13}	1.419(2)
C_4	C_5	1.515(3)	C_{13}	P_1	1.827(2)
C_4	O_4	1.439(2)	C_{14}	O_{14}	1.435(3)
C_5	C_6	1.529(3)	C_{15}	O_{15}	1.441(3)
C_5	O_5	1.438(2)	O_{14}	P_1	1.5783(16)
C_6	O_6	1.429(2)	O_{15}	P_1	1.5721(16)
C_7	C_8	1.509(3)	O_{16}	P_1	1.4720(15)
C_7	C_9	1.522(3)			

Table 5 Bond Angles for robe52.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
O_1	C_1	C_2	105.49(15)	C_{11}	C_{10}	C_{12}	113.04(17)
O_4	C_1	C_2	107.10(16)	O_5	C_{10}	C_{11}	109.19(15)
O_4	C_1	O_1	110.29(16)	O_5	C_{10}	C_{12}	108.79(15)
C_3	C_2	C_1	104.07(15)	O_6	C_{10}	C_{11}	108.37(16)
O_2	C_2	C_1	103.33(15)	O_6	C_{10}	C_{12}	111.51(15)

O_2	C_2	C_3	109.63(15)	O_6	C_{10}	O_5	105.65(14)
C_2	C_3	C_4	101.17(15)	C_3	C_{13}	P_1	112.54(13)
C_2	C_3	C_{13}	113.80(15)	O_{13}	C_{13}	C_3	113.42(15)
C_{13}	C_3	C_4	113.67(15)	O_{13}	C_{13}	P_1	111.03(13)
C_5	C_4	C_3	116.94(16)	C_1	O_1	C_7	109.36(15)
O_4	C_4	C_3	103.66(15)	C_7	O_2	C_2	108.27(14)
O_4	C_4	C_5	108.05(15)	C_1	O_4	C_4	108.98(14)
C_4	C_5	C_6	116.12(16)	C_{10}	O_5	C_5	109.06(14)
O_5	C_5	C_4	108.71(15)	C_{10}	O_6	C_6	106.57(13)
O_5	C_5	C_6	103.73(14)	C_{14}	O_{14}	P_1	123.73(15)
O_6	C_6	C_5	102.45(15)	C_{15}	O_{15}	P_1	121.45(15)
C_8	C_7	C_9	113.34(18)	O_{14}	P_1	C_{13}	104.93(8)
O_1	C_7	C_8	108.82(16)	O_{15}	P_1	C_{13}	100.96(9)
O_1	C_7	C_9	109.66(16)	O_{15}	P_1	O_{14}	103.77(9)
O_2	C_7	C_8	108.54(16)	O_{16}	P_1	C_{13}	115.33(9)
O_2	C_7	C_9	111.10(17)	O_{16}	P_1	O_{14}	113.49(9)
O_2	C_7	O_1	105.03(15)	O_{16}	P_1	O_{15}	116.72(10)

Table 6 Hydrogen Bonds for robe52.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O_{13}	H_{13A}	O_{16}[1]	0.82(3)	1.91(3)	2.698(2)	158(3)

[1]1/2+X,1/2-Y,1-Z

Table 7 Torsion Angles for robe52.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
C_1	C_2	C_3	C_4	26.98(18)	C_{12}	C_{10}	O_5	C_5	108.57(17)
C_1	C_2	C_3	C_{13}	149.27(16)	C_{12}	C_{10}	O_6	C_6	-87.79(19)
C_1	C_2	O_2	C_7	24.78(19)	C_{13}	C_3	C_4	C_5	80.8(2)
C_2	C_1	O_1	C_7	-8.2(2)	C_{13}	C_3	C_4	O_4	-160.48(15)
C_2	C_1	O_4	C_4	-18.1(2)	C_{14}	O_{14}	P_1	C_{13}	132.55(18)
C_2	C_3	C_4	C_5	-156.86(16)	C_{14}	O_{14}	P_1	O_{15}	-121.92(19)
C_2	C_3	C_4	O_4	-38.09(17)	C_{14}	O_{14}	P_1	O_{16}	5.8(2)
C_2	C_3	C_{13}	O_{13}	-167.12(15)	C_{15}	O_{15}	P_1	C_{13}	158.48(19)
C_2	C_3	C_{13}	P_1	65.78(18)	C_{15}	O_{15}	P_1	O_{14}	50.0(2)
C_3	C_2	O_2	C_7	135.26(16)	C_{15}	O_{15}	P_1	O_{16}	-75.7(2)
C_3	C_4	C_5	C_6	65.3(2)	O_1	C_1	C_2	C_3	-124.53(16)
C_3	C_4	C_5	O_5	-178.24(15)	O_1	C_1	C_2	O_2	-9.99(19)

C_3	C_4	O_4	C_1	35.90(19)	O_1	C_1	O_4 C_4	96.21(17)
C_3	C_{13}	P_1	O_{14}	-79.34(14)	O_1	C_7	O_2 C_2	-30.42(19)
C_3	C_{13}	P_1	O_{15}	173.04(14)	O_2	C_2	C_3 C_4	-83.01(17)
C_3	C_{13}	P_1	O_{16}	46.29(17)	O_2	C_2	C_3 C_{13}	39.3(2)
C_4	C_3	C_{13}	O_{13}	-52.0(2)	O_2	C_7	O_1 C_1	23.5(2)
C_4	C_3	C_{13}	P_1	-179.11(12)	O_4	C_1	C_2 C_3	-7.0(2)
C_4	C_5	C_6	O_6	147.22(15)	O_4	C_1	C_2 O_2	107.51(16)
C_4	C_5	O_5	C_{10}	-134.59(15)	O_4	C_1	O_1 C_7	-123.54(17)
C_5	C_4	O_4	C_1	160.62(15)	O_4	C_4	C_5 C_6	-51.0(2)
C_5	C_6	O_6	C_{10}	-35.96(18)	O_4	C_4	C_5 O_5	65.39(19)
C_6	C_5	O_5	C_{10}	-10.46(19)	O_5	C_5	C_6 O_6	28.04(18)
C_8	C_7	O_1	C_1	139.58(17)	O_5	C_{10}	O_6 C_6	30.25(18)
C_8	C_7	O_2	C_2	-146.67(16)	O_6	C_{10}	O_5 C_5	-11.26(19)
C_9	C_7	O_1	C_1	-95.94(19)	O_{13}	C_{13}	P_1 O_{14}	152.30(12)
C_9	C_7	O_2	C_2	88.07(19)	O_{13}	C_{13}	P_1 O_{15}	44.68(15)
C_{11}	C_{10}	O_5	C_5	-127.63(17)	O_{13}	C_{13}	P_1 O_{16}	-82.07(15)
C_{11}	C_{10}	O_6	C_6	147.17(16)				

Table 8 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for robe52.

Atom	*x*	*y*	*z*	U(eq)
H_1	1985.41	4547.39	3156.73	23
H_2	2450.02	4295.96	4448.34	22
H_3	5138.24	3406.27	4115.75	19
H_4	7510.63	4793.62	3566.45	20
H_5	9327.58	3512.68	3438.51	20
H_{6A}	7224.58	2449.99	3103.32	22
H_{6B}	5724.33	3012.61	2576.69	22
H_{8A}	4942.48	6897.1	4628.17	35
H_{8B}	4388.08	7102.13	3751.68	35
H_{8C}	6291.23	6501.04	3947.96	35
H_{9A}	350.34	5668.88	4351.16	39
H_{9B}	577.29	6553.77	3968.05	39
H_{9C}	1236.44	6417.09	4846.55	39
H_{11A}	12244.75	3298.91	1839.18	38
H_{11B}	11655.13	3956.2	1192.98	38
H_{11C}	11253.88	3004.4	1040.62	38
H_{12A}	7406.51	3453.39	752.64	35
H_{12B}	7875.04	4372.54	1016.1	35

H_{12C}	6090.84	3853.63	1439.39	35
H_{13}	7265.56	4630.11	5001.98	19
H_{14A}	1184.79	4767.25	6007.73	48
H_{14B}	1829.04	4291.73	6776.33	48
H_{14C}	1454.34	3794.91	5995.65	48
H_{15A}	6200.29	4213.88	7310.76	46
H_{15B}	8340.59	3737.25	7477.91	46
H_{15C}	6218.03	3236.54	7346.37	46
H_{13A}	8790(50)	3170(20)	4706(17)	31

Experimental

A suitable single crystals of $C_{15}H_{27}O_9P$ [robe52] was selected and mounted on a mylar loop on a Bruker Venture Metaljet diffractometer. The crystal was kept at 100 K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Intrinsic Phasing and refined with the XL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Sheldrick, G.M. (2015). Acta Cryst. A71, 3-8.
3. Sheldrick, G.M. (2015). Acta Cryst. C71, 3-8.

Crystal structure determination of robe52

Crystal Data for $C_{15}H_{27}O_9P$ (M =382.33 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 6.3865(2) Å, b = 16.3617(5) Å, c = 17.3897(5) Å, V = 1817.12(10) Å^3, Z = 4, T = 100 K, μ(GaKα) = 1.134 mm^{-1}, *Dcalc* = 1.398 g/cm^3, 27079 reflections measured (10.022° ≤ 2Θ ≤ 121.252°), 4144 unique (R_{int} = 0.0367, R_{sigma} = 0.0242) which were used in all calculations. The final R_1 was 0.0322 (I > 2σ(I)) and wR_2 was 0.0872 (all data).

Refinement model description

Number of restraints - 0, number of constraints - unknown.
Details:
1. Fixed Uiso
At 1.2 times of:
All C(H) groups, All C(H,H) groups
At 1.5 times of:
All C(H,H,H) groups, All O(H) groups
2.a Ternary CH refined with riding coordinates:
C1(H1), C2(H2), C3(H3), C4(H4), C5(H5), C13(H13)
2.b Secondary CH2 refined with riding coordinates:
C6(H6A,H6B)
2.c Idealised Me refined as rotating group:
C8(H8A,H8B,H8C), C9(H9A,H9B,H9C), C11(H11A,H11B,H11C), C12(H12A,H12B,H12C),
C14(H14A,H14B,H14C), C15(H15A,H15B,H15C)

9.4 Crystal and molecular structure of compound $C_{27}H_{39}N_2O_{12}P$ (HAN499)

Hanessian Group
Département de chimie, Université de Montréal,
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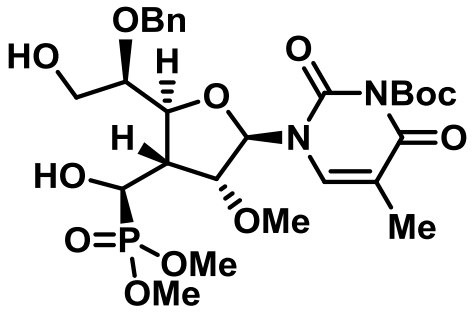

5.26

Structure solved and refined in the laboratory of
X-ray diffraction (Université de Montréal) by Michel Simard.

Table 1. Crystal data and structure refinement for han499.

Identification code	han499
Empirical formula	$C_{27}H_{39}N_2O_{12}P$
Formula weight	614.57
Temperature/K	100
Crystal system	monoclinic
Space group	$P2_1$
a/Å	18.8079(8)
b/Å	11.7857(5)
c/Å	20.6661(9)
α/°	90
β/°	97.314(2)
γ/°	90
Volume/Å^3	4543.7(3)
Z	6
ρ_{calc}g/cm^3	1.348
μ/mm^{-1}	0.879
F(000)	1956.0
Crystal size/mm^3	0.28 × 0.24 × 0.03
Radiation	GaKα (λ = 1.34139)
2Θ range for data collection/°	3.75 to 121.436
Index ranges	-24 ≤ h ≤ 24, -15 ≤ k ≤ 15, -26 ≤ l ≤ 26
Reflections collected	93733
Independent reflections	20828 [R_{int} = 0.0446, R_{sigma} = 0.0367]
Data/restraints/parameters	20828/1/1180
Goodness-of-fit on F^2	1.032
Final R indexes [I>=2σ (I)]	R_1 = 0.0378, wR_2 = 0.1007
Final R indexes [all data]	R_1 = 0.0387, wR_2 = 0.1017
Largest diff. peak/hole / e Å^{-3}	0.48/-0.23
Flack parameter	-0.008(7)

Table 2. Fractional Atomic Coordinates (×104) and Equivalent Isotropic Displacement Parameters (Å2×103) for han499. Ueq is defined as 1/3 of of the trace of the orthogonalised UIJ tensor.

Atom	*x*	*y*	*z*	U(eq)
P1	4534.6(3)	2239.3(5)	2740.6(3)	30.16(12)
O11	6907.5(9)	1070.8(14)	3196.3(7)	32.2(3)
O12	7101.5(9)	3037.8(14)	2378.9(8)	34.6(3)
O13	5612.2(11)	2817.0(17)	1586.7(8)	40.8(4)
O14	5318.2(9)	1027.6(15)	3667.8(8)	32.7(3)
O15	4611(1)	1548.9(16)	2154.7(8)	38.6(4)
O16	4451.5(10)	3522.1(14)	2535.7(8)	34.2(3)
O17	3875.5(9)	1929.0(16)	3111.9(8)	36.6(4)
O18	6351.2(9)	3071.5(13)	4316.3(7)	29.0(3)
O19	7324.3(9)	463.4(14)	5027.2(7)	33.1(3)
O110	9659(1)	1449(2)	5075.2(10)	49.5(5)
O111	8846.3(12)	-517.0(19)	5747.1(10)	49.8(5)
O112	8435.4(10)	1171.1(16)	6075.1(8)	38.4(4)
N11	7637.8(10)	1477.8(17)	4156.0(9)	30.8(4)
N12	8489.0(11)	923.1(19)	5008.0(9)	34.8(4)
C11	6899.9(12)	1511.7(19)	3833.8(10)	28.6(4)
C12	6585.5(12)	2695.5(19)	3732.7(10)	28.1(4)
C13	5992.8(12)	2509.1(18)	3143.9(10)	27.2(4)
C14	6350.8(13)	1598.0(19)	2748.6(10)	30.2(4)
C15	6722.4(14)	2020(2)	2180.3(10)	34.2(5)
C16	6227.6(15)	2138(2)	1544.5(11)	40.3(5)
C17	7778.1(15)	3119(2)	2134.9(12)	40.1(5)
C18	8166.8(14)	4180(2)	2387.6(12)	37.8(5)
C19	8682.0(16)	4651(3)	2038.7(13)	48.7(7)
C110	9057.1(17)	5609(3)	2263.6(15)	55.4(8)
C111	8935.4(16)	6120(3)	2839.4(17)	51.7(7)
C112	8430.9(17)	5661(3)	3193.4(17)	50.3(7)
C113	8048.6(16)	4698(2)	2966.0(14)	43.7(6)
C114	5285.9(12)	2129.4(18)	3383(1)	28.1(4)
C115	4247.7(16)	4401(2)	2971.6(13)	41.5(5)
C116	3156.1(15)	1929(3)	2764.1(14)	46.0(6)
C117	8186.2(14)	1968(2)	3863.9(11)	37.1(5)
C118	8876.5(14)	1983(3)	4125.7(13)	43.2(6)
C119	9070.8(13)	1460(2)	4759.1(12)	39.2(5)
C120	7773.5(12)	922.4(19)	4746.1(10)	29.9(4)

C121	9463.3(17)	2548(4)	3813.2(18)	63.6(10)
C122	8619.6(13)	421(2)	5658.1(12)	37.2(5)
C123	8442.0(16)	875(3)	6776.7(12)	44.9(6)
C124	9206.8(18)	649(4)	7067.5(15)	60.5(9)
C125	7946.8(17)	-126(3)	6836.5(15)	53.9(7)
C126	8140(2)	1949(3)	7051.1(14)	59.1(8)
C127	6238.3(14)	4271(2)	4321.1(11)	33.1(4)
P2	4368.8(3)	7860.7(5)	3678.6(3)	28.86(11)
O21	6696.1(9)	9017.1(14)	3867.2(7)	30.4(3)
O22	6892.1(9)	7183.2(15)	4789.1(7)	33.4(3)
O23	5348.9(10)	8590.5(17)	5366.5(9)	37.6(4)
O24	5167.6(9)	8946.1(14)	2945.0(8)	29.9(3)
O25	4401.3(9)	8696.9(15)	4213.0(8)	34.1(3)
O26	4331.2(9)	6582.7(14)	3877.6(8)	35.2(3)
O27	3696.2(9)	8022.1(16)	3145.6(9)	38.7(4)
O28	6273.7(9)	6959.6(13)	2620.7(7)	29.8(3)
O29	7162.2(9)	9199.5(15)	2056.4(7)	33.2(3)
O210	9530.0(9)	9377.4(18)	2826.5(9)	42.3(4)
O211	8475.1(11)	10529.0(16)	1619.0(9)	42.6(4)
O212	8643.0(9)	8634.2(15)	1519.1(7)	32.4(3)
N21	7500(1)	8690.8(16)	3124.9(8)	27.2(3)
N22	8341.2(10)	9279.2(17)	2467.3(9)	30.2(4)
C21	6749.2(12)	8573.8(18)	3239.2(10)	26.9(4)
C22	6469.3(11)	7362.7(18)	3259.7(9)	26.4(4)
C23	5836.3(11)	7518.0(17)	3664.3(10)	25.9(4)
C24	6111.2(12)	8475.8(18)	4142.5(10)	26.5(4)
C25	6391.1(12)	8087.0(19)	4829.2(10)	28.7(4)
C26	5788.8(13)	7681(2)	5210.7(11)	34.1(5)
C27	7466.5(14)	7220(2)	5320.7(12)	39.8(5)
C28	7850.3(13)	6102(2)	5353.5(11)	34.9(5)
C29	7524.0(16)	5142(3)	5568.8(13)	44.6(6)
C210	7881.1(19)	4106(3)	5608.3(16)	55.2(7)
C211	8567.1(19)	4034(3)	5438.5(16)	55.1(7)
C212	8888.2(16)	4979(3)	5213.3(15)	50.3(7)
C213	8535.6(14)	6013(2)	5175.1(12)	40.7(5)
C214	5138.5(11)	7840.8(19)	3229.5(10)	27.8(4)
C215	3858.9(14)	6244(2)	4352.9(12)	38.6(5)
C216	3428.8(16)	9149(3)	2983.8(14)	46.9(6)
C217	8044.7(13)	8578(2)	3633.5(10)	30.1(4)
C218	8736.0(13)	8803(2)	3583.0(11)	32.4(4)
C219	8928.9(13)	9170(2)	2953.3(11)	32.2(4)

C220	7621.3(12)	9066.3(19)	2516.4(10)	28.2(4)
C221	9332.8(14)	8674(3)	4133.1(12)	42.4(6)
C222	8495.0(12)	9577(2)	1812.1(11)	31.3(4)
C223	8813.6(13)	8649(2)	835.8(10)	34.9(5)
C224	9480.2(17)	9338(3)	791.9(17)	55.7(8)
C225	8169.4(19)	9074(4)	392.7(13)	56.2(8)
C226	8941.1(17)	7402(3)	708.6(13)	45.8(6)
C227	6138.4(14)	5774(2)	2608.4(12)	36.8(5)
P3	5752.8(3)	6099.0(5)	546.4(3)	29.70(11)
O31	3695.5(9)	6053.9(14)	1606.2(7)	31.0(3)
O32	3420.2(9)	8009.7(14)	770.8(8)	33.8(3)
O33	5212(1)	8632.9(15)	1663.9(8)	34.3(3)
O34	5051.8(10)	4822.5(16)	1320.7(9)	38.3(4)
O35	5880.8(9)	7149.8(16)	927.6(8)	36.3(3)
O36	5612.4(9)	6237.7(15)	-215.6(8)	34.3(3)
O37	6396.2(10)	5228.5(17)	659.3(10)	42.1(4)
O38	3590.2(9)	4479.0(13)	135.1(7)	30.2(3)
O39	2752.4(10)	3071.1(15)	1391.1(8)	36.5(4)
O310	541.7(10)	4672.4(18)	1277.8(9)	42.5(4)
O311	1095.4(12)	2249.8(19)	985.8(8)	47.0(5)
O312	1341.8(9)	2610.7(15)	2071.2(7)	34.3(3)
N31	2667(1)	5008.0(17)	1250.4(9)	29.8(4)
N32	1661.3(11)	3918.5(17)	1360.3(9)	32.1(4)
C31	3437.7(12)	5136.0(19)	1201.7(10)	28.8(4)
C32	3585.7(12)	5462.5(19)	516.6(10)	28.1(4)
C33	4304.7(11)	6116.8(19)	647(1)	28.2(4)
C34	4237.7(12)	6685.9(19)	1311.4(10)	29.5(4)
C35	4012.8(13)	7924(2)	1275.9(11)	32.1(4)
C36	4625.1(14)	8733(2)	1160.4(11)	34.4(5)
C37	3054.5(15)	9064(2)	736.3(13)	40.3(5)
C38	2337.3(14)	8939(2)	324.4(12)	36.2(5)
C39	1806.6(15)	9759(2)	375.1(13)	41.3(6)
C310	1142.3(15)	9652(3)	9.1(15)	46.3(6)
C311	982.9(15)	8750(3)	-404.5(14)	47.6(7)
C312	1511.4(15)	7937(3)	-466.9(12)	43.1(6)
C313	2185.4(14)	8039(2)	-108.4(12)	37.9(5)
C314	4957.4(12)	5311.3(19)	689.4(11)	31.2(4)
C315	6095.3(15)	6909(2)	-557.0(11)	37.7(5)
C316	7004.4(16)	5383(3)	1149.4(15)	53.9(7)
C317	2232.5(12)	5953(2)	1170.3(10)	31.1(4)
C318	1516.0(13)	5922(2)	1175.1(11)	33.9(5)

C319	1183.3(13)	4835(2)	1267.3(11)	35.4(5)
C320	2398.7(13)	3931(2)	1337.4(10)	30.3(4)
C321	1046.1(14)	6955(2)	1084.8(13)	41.9(5)
C322	1332.1(13)	2806(2)	1442.7(11)	35.6(5)
C323	914.7(14)	1636(2)	2294.6(12)	37.7(5)
C324	128.7(15)	1861(3)	2071.6(14)	45.2(6)
C325	1084.5(15)	1719(3)	3031.8(13)	45.3(6)
C326	1180.0(18)	515(3)	2049.3(15)	49.3(6)
C327	3607.1(14)	4728(2)	-541.7(11)	35.5(5)

Table 3 Anisotropic Displacement Parameters ($Å^2×10^3$) for han499. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
P1	36.5(3)	30.5(3)	22.7(2)	1.9(2)	0.90(19)	0.6(2)
O11	43.0(8)	31.5(7)	22.1(7)	-1.0(6)	4.5(6)	8.1(7)
O12	44.0(9)	32.6(8)	29.8(7)	-0.1(6)	14.6(7)	3.1(7)
O13	52.7(10)	39.4(9)	30.7(8)	5.0(7)	6.7(7)	5.7(8)
O14	44.0(9)	29.0(7)	24.4(7)	4.0(6)	1.7(6)	-0.6(7)
O15	48.4(10)	38.7(9)	27.4(8)	-2.4(7)	-0.6(7)	0.9(7)
O16	42.2(9)	31.8(8)	28.7(7)	4.4(6)	5.5(6)	4.4(7)
O17	36.3(8)	42.5(9)	30.1(8)	6.3(7)	0.3(6)	-3.7(7)
O18	37.6(8)	28.8(7)	21.4(6)	-1.9(5)	6.5(6)	0.5(6)
O19	38.8(8)	35.8(8)	24.7(7)	2.1(6)	4.2(6)	-1.2(7)
O110	33.7(9)	65.5(13)	48.8(10)	8.7(10)	2.7(8)	3.6(9)
O111	53.8(11)	48.9(11)	45.9(10)	8.6(9)	2.4(9)	15.2(9)
O112	45.3(9)	43.9(9)	25.0(7)	4.1(7)	1.1(6)	-1.0(8)
N11	34.0(9)	36.0(9)	23.2(8)	1.9(7)	6.1(7)	4.5(8)
N12	36(1)	41.7(11)	27.4(9)	3.1(8)	6.1(7)	5.1(8)
C11	33.8(10)	30.8(10)	21.3(9)	0.1(8)	4.3(8)	3.1(8)
C12	34.1(10)	29.6(10)	21.1(9)	-0.6(7)	5.6(8)	1.4(8)
C13	35.8(10)	25.6(9)	20.2(8)	-0.3(7)	4.0(8)	1.8(8)
C14	41.5(11)	28.1(10)	21.4(9)	-1.4(7)	4.6(8)	4.9(8)
C15	48.6(13)	31.7(11)	23.5(9)	-0.4(8)	9.3(9)	6.1(9)
C16	58.0(15)	39.9(13)	23.8(10)	-1.1(9)	8.2(10)	7.1(11)
C17	48.0(14)	44.4(13)	30.9(11)	-0.9(10)	16.4(10)	5.8(11)
C18	39.3(12)	42.1(13)	32.7(11)	9(1)	7.5(9)	7.1(10)
C19	44.5(14)	74(2)	28.1(11)	6.2(12)	7.2(10)	-4.5(14)
C110	44.5(15)	77(2)	44.9(15)	12.2(14)	5.9(12)	-13.3(14)
C111	43.0(14)	49.2(15)	63.1(18)	-0.7(14)	7.9(13)	-1.5(12)
C112	49.3(15)	46.2(14)	58.0(17)	-9.7(13)	17.6(13)	-0.2(12)

C113	48.1(14)	40.5(13)	46.2(14)	-0.5(11)	19.8(11)	2.8(11)
C114	36(1)	26.3(10)	21.4(8)	0.6(7)	1.5(7)	0.6(8)
C115	52.8(15)	34.8(12)	37.4(12)	0.7(10)	7.3(11)	7.7(11)
C116	40.9(13)	55.6(16)	39.7(13)	8.6(12)	-1.6(10)	-1.1(11)
C117	40.2(12)	44.7(13)	28.4(10)	4.4(9)	11.6(9)	5.4(10)
C118	37.1(12)	57.4(16)	37.4(12)	5.1(11)	13.7(10)	3.5(11)
C119	36.2(12)	47.3(13)	35.1(11)	1.5(10)	8.9(9)	5.4(10)
C120	38.0(11)	30.3(10)	21.7(9)	-2.9(8)	5.0(8)	3.6(8)
C121	40.5(15)	93(3)	60.3(18)	27.6(18)	19.3(14)	1.9(15)
C122	34.5(11)	43.6(13)	32.2(11)	7.5(10)	-0.9(9)	1.8(10)
C123	49.0(14)	60.6(17)	24.5(11)	5.6(11)	1.9(10)	1.7(12)
C124	48.0(16)	95(3)	35.8(13)	13.2(15)	-5.5(12)	-1.5(16)
C125	53.0(16)	64.7(19)	43.8(14)	22.5(14)	5.4(12)	0.2(14)
C126	75(2)	70(2)	32.5(13)	-2.7(13)	8.5(13)	5.7(17)
C127	42.7(12)	29.7(10)	28.1(10)	-3.4(8)	9.0(9)	-0.8(9)
P2	31.5(3)	29.8(2)	26.7(2)	-0.5(2)	9.4(2)	-1.1(2)
O21	36.5(8)	32.7(8)	24.6(7)	-5.6(6)	13.6(6)	-5.9(6)
O22	37.6(8)	37.6(8)	24.1(7)	-4.3(6)	0.0(6)	7.2(7)
O23	41.4(9)	45.8(10)	27.4(8)	-2.7(7)	11.2(7)	1.1(8)
O24	37.1(8)	30.7(8)	23.0(7)	2.4(6)	7.7(6)	0.9(6)
O25	39.8(8)	34.5(8)	30.2(7)	-3.5(6)	12.9(6)	-0.9(7)
O26	41.4(9)	31.0(8)	36.2(8)	0.0(6)	16.4(7)	-3.3(7)
O27	35.1(8)	44(1)	37.4(9)	-1.1(7)	5.6(7)	1.2(7)
O28	36.9(8)	31.9(8)	21.3(6)	-4.5(6)	6.9(6)	-0.1(6)
O29	34.8(8)	42.7(9)	22.7(7)	2.4(6)	6.4(6)	1.0(7)
O210	33.4(9)	56.4(11)	37.9(9)	3.8(8)	7.7(7)	-5.2(8)
O211	56.8(11)	36.2(9)	39.4(9)	4.8(7)	23.1(8)	2.1(8)
O212	38.1(8)	38.3(8)	22.2(7)	1.7(6)	9.2(6)	2.5(7)
N21	30.7(9)	31.7(9)	20.5(7)	-1.5(7)	7.8(6)	-1.0(7)
N22	33.7(9)	36.1(9)	22.1(8)	0.3(7)	9.0(7)	-1.3(7)
C21	32(1)	28.7(9)	21.7(9)	-1.8(7)	9.8(7)	-0.7(8)
C22	30.9(10)	28.5(10)	20.7(8)	-1.3(7)	6.4(7)	0.6(8)
C23	30.8(10)	26.9(9)	20.8(8)	-0.2(7)	7.1(7)	-0.8(7)
C24	31.1(10)	28.6(10)	21.3(9)	-0.4(7)	9.4(7)	-1.5(8)
C25	33.6(10)	31.6(10)	21.3(9)	-2.0(7)	5.5(8)	1.7(8)
C26	41.3(12)	39.3(12)	23.1(9)	0.7(8)	9.2(8)	-0.5(9)
C27	42.0(12)	45.5(13)	29.8(11)	-5.9(10)	-3.6(9)	5.1(11)
C28	37.3(11)	42.8(12)	23.1(9)	1.3(9)	-1.9(8)	2.2(10)
C29	42.4(13)	54.8(16)	35.7(12)	9.2(11)	1.9(10)	-3.4(12)
C210	62.5(19)	46.7(16)	52.8(16)	14.8(13)	-6.8(14)	-8.5(14)

C211	60.3(19)	44.7(15)	55.8(17)	3.2(13)	−10.3(14)	11.7(13)
C212	41.1(14)	61.2(18)	47.8(15)	2.0(13)	2.1(11)	10.2(13)
C213	39.9(12)	46.0(14)	35.3(11)	4.6(10)	1.2(9)	-2.8(11)
C214	32.1(10)	28.6(10)	23.7(9)	-0.5(8)	7.4(7)	-1.3(8)
C215	45.3(13)	35.8(12)	38.1(12)	3.4(9)	18.1(10)	-5.9(10)
C216	47.7(14)	50.7(15)	42.7(13)	4.3(12)	7.9(11)	14.0(12)
C217	37.3(11)	32.3(10)	21.5(9)	-0.6(8)	7.1(8)	0.7(8)
C218	35.7(11)	36.5(11)	24.9(9)	-1.8(8)	3.8(8)	-0.7(9)
C219	32.8(11)	36.2(11)	28.3(10)	-2.1(8)	6.2(8)	-1.2(9)
C220	33.3(10)	29.1(10)	23.9(9)	-2.3(8)	9.5(8)	0.3(8)
C221	38.3(12)	57.5(15)	30.3(11)	0.9(11)	0.1(9)	-3.1(11)
C222	32(1)	38.7(12)	24.8(9)	1.1(8)	9.7(8)	-0.5(9)
C223	38.3(12)	46.9(13)	21.1(9)	1.4(9)	10.2(8)	3.1(10)
C224	50.7(16)	63.7(19)	59.4(18)	-8.4(15)	32.6(14)	-8.6(14)
C225	59.6(18)	80(2)	28.2(12)	2.2(13)	0.8(11)	22.7(16)
C226	58.9(16)	50.5(15)	29.9(11)	-5.8(10)	13.3(11)	4.6(12)
C227	45.0(13)	32.8(11)	32.7(11)	-6.5(9)	4.8(9)	-0.2(9)
P3	31.8(3)	33.0(3)	24.6(2)	0.9(2)	4.94(19)	-0.2(2)
O31	37.3(8)	32.4(7)	24.2(7)	0.9(6)	6.7(6)	-3.2(6)
O32	37.9(8)	29.7(8)	33.4(8)	0.6(6)	3.4(6)	3.2(6)
O33	39.7(9)	35.5(8)	27.6(7)	-2.4(6)	4.6(6)	-0.2(7)
O34	44.2(10)	36.2(9)	35.4(9)	10.5(7)	8.6(7)	5.9(7)
O35	39.1(8)	40.4(9)	30.3(7)	-4.3(7)	8.5(6)	-5.3(7)
O36	39.9(8)	38.5(8)	24.8(7)	-1.2(6)	4.8(6)	-5.1(7)
O37	33.3(8)	45.9(10)	46.2(10)	2.3(8)	2.0(7)	5.3(7)
O38	38.7(8)	28.6(7)	23.9(7)	0.4(6)	6.4(6)	0.1(6)
O39	44.9(9)	32.2(8)	33.7(8)	4.8(6)	9.7(7)	-1.0(7)
O310	35.0(9)	54.8(11)	38.3(9)	-0.8(8)	6.1(7)	-7.2(8)
O311	66.3(12)	49.4(10)	25.8(8)	-4.6(8)	8.1(8)	−18.5(10)
O312	38.7(8)	42.3(9)	22.9(7)	0.0(6)	7.6(6)	-9.6(7)
N31	32.4(9)	32.1(9)	25.9(8)	3.5(7)	7.3(7)	-1.0(7)
N32	37.9(10)	35.9(10)	23.7(8)	0.3(7)	8.5(7)	-7.2(8)
C31	33.1(10)	29.5(10)	24.9(9)	2.6(8)	7.3(8)	0.0(8)
C32	33.5(10)	28.6(10)	23.2(9)	3.1(8)	6.9(8)	0.6(8)
C33	31.6(10)	27.8(9)	25.8(9)	2.8(8)	6.1(7)	1.1(8)
C34	34.4(10)	29.9(10)	24.8(9)	2.8(8)	6.0(8)	-0.2(8)
C35	38.7(11)	30.4(10)	27.3(10)	0.4(8)	5.1(8)	2.4(9)
C36	41.0(12)	32.4(11)	29.7(10)	1.9(8)	4.0(9)	-2.9(9)
C37	48.9(14)	31.7(11)	40.3(12)	0.6(10)	5.9(10)	6.7(10)

C38	42.6(13)	34.7(11)	33.1(11)	9.8(9)	12.7(9)	6.3(9)
C39	49.3(14)	37.2(12)	41.0(12)	10.3(10)	19.3(11)	9.9(11)
C310	44.6(14)	46.2(14)	51.7(15)	19.0(12)	20.5(12)	15.5(11)
C311	39.0(13)	63.7(18)	40.7(13)	18.3(13)	7.8(10)	10.0(12)
C312	46.7(14)	51.4(14)	31.6(11)	7.7(11)	6(1)	6.2(12)
C313	43.2(12)	41.5(13)	29.9(10)	6.5(9)	8.3(9)	11.4(10)
C314	32.2(10)	30.7(10)	31.2(10)	2.4(8)	6.0(8)	1.2(8)
C315	52.4(14)	34.3(11)	27.6(10)	0.4(9)	9.6(10)	-6.6(10)
C316	40.6(14)	77(2)	41.9(14)	1.5(14)	-2.1(11)	14.8(14)
C317	37.1(11)	32.4(11)	24.7(9)	3.2(8)	7.3(8)	0.3(9)
C318	36.0(11)	39.4(12)	27.3(10)	2.2(9)	7.7(8)	0.9(9)
C319	38.3(12)	45.4(13)	22.8(9)	-0.1(9)	5.4(8)	-1.9(10)
C320	37.6(11)	34.0(11)	20.0(8)	1.5(8)	7.2(8)	-3.5(9)
C321	38.3(12)	46.9(14)	42.2(13)	8.6(11)	11.1(10)	7.5(11)
C322	41.5(12)	40.5(12)	25.9(10)	0.5(9)	9.2(9)	-7.8(10)
C323	41.8(12)	41.4(13)	31.3(11)	5.0(9)	9.8(9)	-9.9(10)
C324	41.0(13)	52.2(15)	42.6(13)	6.7(11)	6.1(10)	-11.1(11)
C325	47.0(14)	60.5(17)	29.9(11)	9.0(11)	11.3(10)	-7.1(12)
C326	61.0(17)	42.9(14)	45.6(14)	3.4(11)	13.8(13)	-7.6(13)
C327	44.3(12)	38.8(12)	24.2(10)	3.1(9)	7.7(9)	3(1)

Table 4. Bond Lengths for han499.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
P1	O15	1.4809(18)	N22	C219	1.401(3)
P1	O16	1.5725(17)	N22	C220	1.394(3)
P1	O17	1.5817(18)	N22	C222	1.463(3)
P1	C114	1.816(2)	C21	C22	1.524(3)
O11	C11	1.418(2)	C22	C23	1.550(3)
O11	C14	1.446(3)	C23	C24	1.545(3)
O12	C15	1.429(3)	C23	C214	1.541(3)
O12	C17	1.431(3)	C24	C25	1.520(3)
O13	C16	1.419(3)	C25	C26	1.537(3)
O14	C114	1.424(3)	C27	C28	1.499(4)
O16	C115	1.456(3)	C28	C29	1.387(4)
O17	C116	1.449(3)	C28	C213	1.389(4)
O18	C12	1.407(2)	C29	C210	1.390(5)
O18	C127	1.430(3)	C210	C211	1.382(5)
O19	C120	1.212(3)	C211	C212	1.377(5)
O110	C119	1.211(3)	C212	C213	1.384(4)
O111	C122	1.191(3)	C217	C218	1.344(3)

O112	C122	1.311(3)	C218	C219	1.460(3)
O112	C123	1.490(3)	C218	C221	1.500(3)
N11	C11	1.461(3)	C223	C224	1.506(4)
N11	C117	1.386(3)	C223	C225	1.508(4)
N11	C120	1.379(3)	C223	C226	1.519(4)
N12	C119	1.416(3)	P3	O35	1.4711(18)
N12	C120	1.385(3)	P3	O36	1.5719(16)
N12	C122	1.460(3)	P3	O37	1.5810(19)
C11	C12	1.519(3)	P3	C314	1.817(2)
C12	C13	1.558(3)	O31	C31	1.415(3)
C13	C14	1.554(3)	O31	C34	1.457(3)
C13	C114	1.543(3)	O32	C35	1.430(3)
C14	C15	1.525(3)	O32	C37	1.418(3)
C15	C16	1.517(3)	O33	C36	1.422(3)
C17	C18	1.507(4)	O34	C314	1.417(3)
C18	C19	1.394(4)	O36	C315	1.453(3)
C18	C113	1.385(4)	O37	C316	1.440(3)
C19	C110	1.380(5)	O38	C32	1.402(3)
C110	C111	1.379(5)	O38	C327	1.433(3)
C111	C112	1.380(4)	O39	C320	1.210(3)
C112	C113	1.393(4)	O310	C319	1.225(3)
C117	C118	1.342(4)	O311	C322	1.188(3)
C118	C119	1.451(4)	O312	C322	1.317(3)
C118	C121	1.503(4)	O312	C323	1.508(3)
C123	C124	1.510(4)	N31	C31	1.474(3)
C123	C125	1.518(5)	N31	C317	1.379(3)
C123	C126	1.525(5)	N31	C320	1.386(3)
P2	O25	1.4757(17)	N32	C319	1.403(3)
P2	O26	1.5653(18)	N32	C320	1.394(3)
P2	O27	1.5796(19)	N32	C322	1.470(3)
P2	C214	1.816(2)	C31	C32	1.527(3)
O21	C21	1.414(2)	C32	C33	1.550(3)
O21	C24	1.449(2)	C33	C34	1.548(3)
O22	C25	1.432(3)	C33	C314	1.545(3)
O22	C27	1.440(3)	C34	C35	1.519(3)
O23	C26	1.416(3)	C35	C36	1.537(3)
O24	C214	1.433(3)	C37	C38	1.508(4)
O26	C215	1.461(3)	C38	C39	1.403(3)
O27	C216	1.444(3)	C38	C313	1.394(4)
O28	C22	1.408(2)	C39	C310	1.381(4)
O28	C227	1.420(3)	C310	C311	1.373(5)

O29	C220	1.210(3)	C311	C312	1.398(4)
O210	C219	1.217(3)	C312	C313	1.390(4)
O211	C222	1.190(3)	C317	C318	1.349(3)
O212	C222	1.312(3)	C318	C319	1.449(4)
O212	C223	1.488(2)	C318	C321	1.502(4)
N21	C21	1.468(3)	C323	C324	1.515(4)
N21	C217	1.377(3)	C323	C325	1.520(3)
N21	C220	1.379(3)	C323	C326	1.521(4)

Table 5. Bond Angles for han499.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
O15	P1	O16	108.95(10)	O23	C26	C25	111.80(19)
O15	P1	O17	115.67(11)	O22	C27	C28	108.6(2)
O15	P1	C114	114.27(10)	C29	C28	C27	120.1(2)
O16	P1	O17	107.20(10)	C29	C28	C213	119.1(3)
O16	P1	C114	107.88(10)	C213	C28	C27	120.8(2)
O17	P1	C114	102.34(9)	C28	C29	C210	120.3(3)
C11	O11	C14	110.17(16)	C211	C210	C29	119.9(3)
C15	O12	C17	112.89(18)	C212	C211	C210	120.0(3)
C115	O16	P1	122.84(15)	C211	C212	C213	120.3(3)
C116	O17	P1	119.96(16)	C212	C213	C28	120.3(3)
C12	O18	C127	112.36(16)	O24	C214	P2	105.81(14)
C122	O112	C123	120.8(2)	O24	C214	C23	112.92(17)
C117	N11	C11	120.42(19)	C23	C214	P2	112.35(14)
C120	N11	C11	118.29(19)	C218	C217	N21	123.69(19)
C120	N11	C117	121.3(2)	C217	C218	C219	118.7(2)
C119	N12	C122	117.9(2)	C217	C218	C221	124.0(2)
C120	N12	C119	128.0(2)	C219	C218	C221	117.3(2)
C120	N12	C122	113.61(19)	O210	C219	N22	119.8(2)
O11	C11	N11	106.98(17)	O210	C219	C218	126.5(2)
O11	C11	C12	104.99(16)	N22	C219	C218	113.77(19)
N11	C11	C12	114.72(19)	O29	C220	N21	125.0(2)
O18	C12	C11	109.28(17)	O29	C220	N22	121.47(19)
O18	C12	C13	116.30(17)	N21	C220	N22	113.52(19)
C11	C12	C13	101.84(17)	O211	C222	O212	130.0(2)
C14	C13	C12	101.15(17)	O211	C222	N22	122.3(2)
C114	C13	C12	110.54(16)	O212	C222	N22	107.62(19)
C114	C13	C14	114.63(18)	O212	C223	C224	110.2(2)
O11	C14	C13	106.71(16)	O212	C223	C225	109.1(2)

O11	C14	C15	105.51(18)	O212	C223	C226	102.15(19)
C15	C14	C13	116.83(18)	C224	C223	C225	113.3(3)
O12	C15	C14	108.27(17)	C224	C223	C226	111.1(2)
O12	C15	C16	113.6(2)	C225	C223	C226	110.4(3)
C16	C15	C14	114.0(2)	O35	P3	O36	116.37(10)
O13	C16	C15	114.45(19)	O35	P3	O37	113.41(11)
O12	C17	C18	110.3(2)	O35	P3	C314	115.16(10)
C19	C18	C17	119.3(2)	O36	P3	O37	104.07(10)
C113	C18	C17	122.7(2)	O36	P3	C314	100.33(10)
C113	C18	C19	118.0(3)	O37	P3	C314	105.97(11)
C110	C19	C18	120.7(3)	C31	O31	C34	110.44(15)
C111	C110	C19	120.9(3)	C37	O32	C35	115.23(19)
C110	C111	C112	119.2(3)	C315	O36	P3	120.49(15)
C111	C112	C113	120.0(3)	C316	O37	P3	123.1(2)
C18	C113	C112	121.2(3)	C32	O38	C327	112.45(17)
O14	C114	P1	110.83(15)	C322	O312	C323	119.60(19)
O14	C114	C13	113.81(18)	C317	N31	C31	118.83(18)
C13	C114	P1	111.54(14)	C317	N31	C320	122.37(19)
C118	C117	N11	124.4(2)	C320	N31	C31	118.70(19)
C117	C118	C119	118.5(2)	C319	N32	C322	115.5(2)
C117	C118	C121	124.0(3)	C320	N32	C319	127.6(2)
C119	C118	C121	117.5(3)	C320	N32	C322	116.7(2)
O110	C119	N12	119.6(2)	O31	C31	N31	107.70(17)
O110	C119	C118	126.8(2)	O31	C31	C32	105.43(17)
N12	C119	C118	113.6(2)	N31	C31	C32	112.73(18)
O19	C120	N11	125.0(2)	O38	C32	C31	109.14(17)
O19	C120	N12	121.0(2)	O38	C32	C33	116.19(18)
N11	C120	N12	114.0(2)	C31	C32	C33	102.84(17)
O111	C122	O112	130.1(2)	C34	C33	C32	101.66(16)
O111	C122	N12	122.2(2)	C314	C33	C32	111.92(18)
O112	C122	N12	107.7(2)	C314	C33	C34	111.70(18)
O112	C123	C124	108.5(2)	O31	C34	C33	106.84(17)
O112	C123	C125	109.4(2)	O31	C34	C35	107.82(18)
O112	C123	C126	102.1(2)	C35	C34	C33	115.29(18)
C124	C123	C125	113.2(3)	O32	C35	C34	106.80(18)
C124	C123	C126	112.3(3)	O32	C35	C36	111.90(18)
C125	C123	C126	110.7(3)	C34	C35	C36	113.19(19)
O25	P2	O26	116.41(10)	O33	C36	C35	111.24(18)
O25	P2	O27	113.24(10)	O32	C37	C38	109.6(2)
O25	P2	C214	115.55(10)	C39	C38	C37	119.0(2)
O26	P2	O27	103.90(10)	C313	C38	C37	122.2(2)

O26	P2	C214	100.80(10)	C313	C38	C39	118.9(3)
O27	P2	C214	105.36(10)	C310	C39	C38	119.9(3)
C21	O21	C24	110.09(16)	C311	C310	C39	121.4(2)
C25	O22	C27	111.64(17)	C310	C311	C312	119.1(3)
C215	O26	P2	119.39(15)	C313	C312	C311	120.2(3)
C216	O27	P2	119.78(18)	C312	C313	C38	120.4(2)
C22	O28	C227	111.95(17)	O34	C314	P3	110.39(16)
C222	O212	C223	120.79(18)	O34	C314	C33	107.91(18)
C217	N21	C21	120.36(17)	C33	C314	P3	109.97(15)
C217	N21	C220	122.24(19)	C318	C317	N31	123.7(2)
C220	N21	C21	116.81(18)	C317	C318	C319	118.2(2)
C219	N22	C222	117.03(18)	C317	C318	C321	123.3(2)
C220	N22	C219	128.05(18)	C319	C318	C321	118.4(2)
C220	N22	C222	114.77(18)	O310	C319	N32	119.5(2)
O21	C21	N21	107.15(17)	O310	C319	C318	125.7(3)
O21	C21	C22	104.80(16)	N32	C319	C318	114.8(2)
N21	C21	C22	115.81(17)	O39	C320	N31	125.0(2)
O28	C22	C21	109.88(16)	O39	C320	N32	121.7(2)
O28	C22	C23	114.76(17)	N31	C320	N32	113.2(2)
C21	C22	C23	101.32(16)	O311	C322	O312	130.1(2)
C24	C23	C22	102.34(16)	O311	C322	N32	121.4(2)
C214	C23	C22	111.73(16)	O312	C322	N32	108.47(19)
C214	C23	C24	112.54(17)	O312	C323	C324	108.1(2)
O21	C24	C23	106.23(15)	O312	C323	C325	101.89(19)
O21	C24	C25	108.01(17)	O312	C323	C326	110.4(2)
C25	C24	C23	115.13(17)	C324	C323	C325	111.5(2)
O22	C25	C24	108.81(16)	C324	C323	C326	113.6(2)
O22	C25	C26	109.37(18)	C325	C323	C326	110.7(2)
C24	C25	C26	112.49(18)				

Table 6 Hydrogen Bonds for han499.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O13	H13	O15	1.06(5)	1.73(5)	2.779(3)	169(4)
O14	H14	O24[1]	0.79(4)	2.14(4)	2.867(2)	153(3)
O23	H23	O25	0.81(4)	1.99(4)	2.792(3)	171(3)
O24	H24	O33	0.81(3)	1.88(3)	2.685(2)	178(3)
O33	H33	O35	0.90(4)	1.87(4)	2.728(2)	159(3)
O34	H34	O13	0.83(6)	1.84(6)	2.618(3)	156(6)

[1]+X,-1+Y,+Z

Table 7. Torsion Angles for han499.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
O11	C11	C12	O18	162.08(17)	C27	O22	C25	C26	-91.7(2)
O11	C11	C12	C13	38.5(2)	C27	C28	C29	C210	-179.2(2)
O11	C14	C15	O12	-77.3(2)	C27	C28	C213	C212	179.4(2)
O11	C14	C15	C16	155.21(19)	C28	C29	C210	C211	0.6(5)
O12	C15	C16	O13	-70.7(3)	C29	C28	C213	C212	-0.1(4)
O12	C17	C18	C19	-156.0(2)	C29	C210	C211	C212	-1.8(5)
O12	C17	C18	C113	25.8(3)	C210	C211	C212	C213	2.0(5)
O15	P1	O16	C115	169.53(19)	C211	C212	C213	C28	-1.0(4)
O15	P1	O17	C116	-55.7(2)	C213	C28	C29	C210	0.3(4)
O15	P1	C114	O14	-68.46(18)	C214	P2	O26	C215	169.04(18)
O15	P1	C114	C13	59.47(18)	C214	P2	O27	C216	-92.65(19)
O16	P1	O17	C116	66.0(2)	C214	C23	C24	O21	−102.25(19)
O16	P1	C114	O14	170.23(14)	C214	C23	C24	C25	138.27(18)
O16	P1	C114	C13	-61.85(17)	C217	N21	C21	O21	34.3(3)
O17	P1	O16	C115	43.7(2)	C217	N21	C21	C22	-82.2(2)
O17	P1	C114	O14	57.35(17)	C217	N21	C220	O29	-178.7(2)
O17	P1	C114	C13	−174.72(15)	C217	N21	C220	N22	1.9(3)
O18	C12	C13	C14	−153.40(18)	C217	C218	C219	O210	-178.1(3)
O18	C12	C13	C114	-31.6(2)	C217	C218	C219	N22	1.9(3)
N11	C11	C12	O18	-80.8(2)	C219	N22	C220	O29	179.7(2)
N11	C11	C12	C13	155.64(17)	C219	N22	C220	N21	-0.9(3)
N11	C117	C118	C119	-0.4(4)	C219	N22	C222	O211	-95.5(3)
N11	C117	C118	C121	-178.1(3)	C219	N22	C222	O212	84.7(2)
C11	O11	C14	C13	3.6(2)	C220	N21	C21	O21	−137.06(19)
C11	O11	C14	C15	128.56(18)	C220	N21	C21	C22	106.4(2)
C11	N11	C117	C118	179.6(3)	C220	N21	C217	C218	-1.1(3)
C11	N11	C120	O19	-0.8(3)	C220	N22	C219	O210	179.0(2)
C11	N11	C120	N12	179.15(19)	C220	N22	C219	C218	-1.0(3)
C11	C12	C13	C14	-34.72(19)	C220	N22	C222	O211	88.6(3)
C11	C12	C13	C114	87.12(19)	C220	N22	C222	O212	-91.3(2)
C12	C13	C14	O11	20.1(2)	C221	C218	C219	O210	0.5(4)
C12	C13	C14	C15	-97.6(2)	C221	C218	C219	N22	-179.5(2)
C12	C13	C114	P1	165.97(14)	C222	O212	C223	C224	62.0(3)
C12	C13	C114	O14	-67.7(2)	C222	O212	C223	C225	-63.0(3)
C13	C14	C15	O12	41.0(3)	C222	O212	C223	C226	-179.8(2)

C13	C14	C15	C16	-86.4(2)	C222	N22	C219	O210	3.6(3)
C14	O11	C11	N11	149.14(18)	C222	N22	C219	C218	-176.4(2)
C14	O11	C11	C12	-26.8(2)	C222	N22	C220	O29	-4.9(3)
C14	C13	C114	P1	-80.5(2)	C222	N22	C220	N21	174.58(19)
C14	C13	C114	O14	45.8(2)	C223	O212	C222	O211	-0.7(4)
C14	C15	C16	O13	54.0(3)	C223	O212	C222	N22	179.10(18)
C15	O12	C17	C18	177.22(19)	C227	O28	C22	C21	168.91(18)
C17	O12	C15	C14	141.8(2)	C227	O28	C22	C23	-77.7(2)
C17	O12	C15	C16	-90.5(2)	O31	C31	C32	O38	159.13(17)
C17	C18	C19	C110	-178.8(3)	O31	C31	C32	C33	35.2(2)
C17	C18	C113	C112	178.3(3)	O31	C34	C35	O32	-72.8(2)
C18	C19	C110	C111	0.4(5)	O31	C34	C35	C36	163.59(17)
C19	C18	C113	C112	0.1(4)	O32	C35	C36	O33	179.60(18)
C19	C110	C111	C112	0.1(5)	O32	C37	C38	C39	161.7(2)
C110	C111	C112	C113	-0.4(5)	O32	C37	C38	C313	-18.6(3)
C111	C112	C113	C18	0.3(5)	O35	P3	O36	C315	50.3(2)
C113	C18	C19	C110	-0.4(4)	O35	P3	O37	C316	7.7(3)
C114	P1	O16	C115	-65.9(2)	O35	P3	C314	O34	-69.89(18)
C114	P1	O17	C116	179.4(2)	O35	P3	C314	C33	49.07(19)
C114	C13	C14	O11	-98.8(2)	O36	P3	O37	C316	135.2(2)
C114	C13	C14	C15	143.5(2)	O36	P3	C314	O34	164.35(16)
C117	N11	C11	O11	53.0(3)	O36	P3	C314	C33	-76.69(16)
C117	N11	C11	C12	-62.9(3)	O37	P3	O36	C315	-75.29(19)
C117	N11	C120	O19	-179.2(2)	O37	P3	C314	O34	56.34(18)
C117	N11	C120	N12	0.8(3)	O37	P3	C314	C33	175.30(15)
C117	C118	C119	O110	-176.1(3)	O38	C32	C33	C34	151.83(17)
C117	C118	C119	N12	3.7(4)	O38	C32	C33	C314	-32.5(2)
C119	N12	C120	O19	-176.8(2)	N31	C31	C32	O38	-83.6(2)
C119	N12	C120	N11	3.2(3)	N31	C31	C32	C33	152.42(18)
C119	N12	C122	O111	-85.7(3)	N31	C317	C318	C319	-0.2(3)
C119	N12	C122	O112	95.2(3)	N31	C317	C318	C321	-179.8(2)
C120	N11	C11	O11	-125.3(2)	C31	O31	C34	C33	1.7(2)
C120	N11	C11	C12	118.7(2)	C31	O31	C34	C35	126.21(19)
C120	N11	C117	C118	-2.1(4)	C31	N31	C317	C318	176.8(2)
C120	N12	C119	O110	174.4(2)	C31	N31	C320	O39	1.6(3)
C120	N12	C119	C118	-5.4(4)	C31	N31	C320	N32	178.53(17)
C120	N12	C122	O111	101.7(3)	C31	C32	C33	C34	-32.7(2)

C120	N12	C122	O112	-77.5(3)	C31	C32	C33	C314	86.6(2)
C121	C118	C119	O110	1.8(5)	C32	C33	C34	O31	19.9(2)
C121	C118	C119	N12	-178.4(3)	C32	C33	C34	C35	-99.9(2)
C122	O112	C123	C124	65.5(3)	C32	C33	C314	P3	159.49(15)
C122	O112	C123	C125	-58.5(3)	C32	C33	C314	O34	-80.0(2)
C122	O112	C123	C126	-175.7(2)	C33	C34	C35	O32	46.4(2)
C122	N12	C119	O110	2.9(4)	C33	C34	C35	C36	-77.2(2)
C122	N12	C119	C118	-176.9(2)	C34	O31	C31	N31	−143.92(17)
C122	N12	C120	O19	-5.1(3)	C34	O31	C31	C32	-23.3(2)
C122	N12	C120	N11	174.9(2)	C34	C33	C314	P3	-87.27(19)
C123	O112	C122	O111	-4.7(4)	C34	C33	C314	O34	33.2(2)
C123	O112	C122	N12	174.3(2)	C34	C35	C36	O33	-58.9(2)
C127	O18	C12	C11	163.48(18)	C35	O32	C37	C38	−164.23(19)
C127	O18	C12	C13	-82.0(2)	C37	O32	C35	C34	169.65(19)
O21	C21	C22	O28	160.71(17)	C37	O32	C35	C36	-66.0(2)
O21	C21	C22	C23	38.9(2)	C37	C38	C39	C310	-179.1(2)
O21	C24	C25	O22	-68.3(2)	C37	C38	C313	C312	178.3(2)
O21	C24	C25	C26	170.36(17)	C38	C39	C310	C311	0.6(4)
O22	C25	C26	O23	169.50(18)	C39	C38	C313	C312	-2.1(4)
O22	C27	C28	C29	-71.6(3)	C39	C310	C311	C312	-1.5(4)
O22	C27	C28	C213	108.9(3)	C310	C311	C312	C313	0.7(4)
O25	P2	O26	C215	43.2(2)	C311	C312	C313	C38	1.1(4)
O25	P2	O27	C216	34.6(2)	C313	C38	C39	C310	1.3(4)
O25	P2	C214	O24	-61.25(17)	C314	P3	O36	C315	175.20(18)
O25	P2	C214	C23	62.40(18)	C314	P3	O37	C316	-119.5(2)
O26	P2	O27	C216	161.80(18)	C314	C33	C34	O31	-99.6(2)
O26	P2	C214	O24	172.37(14)	C314	C33	C34	C35	140.6(2)
O26	P2	C214	C23	-63.98(17)	C317	N31	C31	O31	49.7(2)
O27	P2	O26	C215	-81.99(19)	C317	N31	C31	C32	-66.2(2)
O27	P2	C214	O24	64.55(16)	C317	N31	C320	O39	177.8(2)
O27	P2	C214	C23	−171.80(15)	C317	N31	C320	N32	-2.4(3)
O28	C22	C23	C24	−151.99(17)	C317	C318	C319	O310	-179.4(2)
O28	C22	C23	C214	-31.4(2)	C317	C318	C319	N32	1.6(3)
N21	C21	C22	O28	-81.5(2)	C319	N32	C320	O39	-175.9(2)
N21	C21	C22	C23	156.75(17)	C319	N32	C320	N31	4.2(3)
N21	C217	C218	C219	-1.0(4)	C319	N32	C322	O311	84.2(3)
N21	C217	C218	C221	-179.5(2)	C319	N32	C322	O312	-94.1(2)

C21	O21	C24	C23	6.7(2)	C320	N31	C31	O31	−134.02(19)
C21	O21	C24	C25	130.72(18)	C320	N31	C31	C32	110.1(2)
C21	N21	C217	C218	−171.9(2)	C320	N31	C317	C318	0.6(3)
C21	N21	C220	O29	−7.5(3)	C320	N32	C319	O310	177.1(2)
C21	N21	C220	N22	173.12(18)	C320	N32	C319	C318	−3.9(3)
C21	C22	C23	C24	−33.69(19)	C320	N32	C322	O311	−91.4(3)
C21	C22	C23	C214	86.9(2)	C320	N32	C322	O312	90.3(2)
C22	C23	C24	O21	17.8(2)	C321	C318	C319	O310	0.1(4)
C22	C23	C24	C25	−101.65(19)	C321	C318	C319	N32	−178.8(2)
C22	C23	C214	P2	173.12(14)	C322	O312	C323	C324	−63.1(3)
C22	C23	C214	O24	−67.3(2)	C322	O312	C323	C325	179.4(2)
C23	C24	C25	O22	50.2(2)	C322	O312	C323	C326	61.7(3)
C23	C24	C25	C26	−71.2(2)	C322	N32	C319	O310	2.0(3)
C24	O21	C21	N21	−152.68(17)	C322	N32	C319	C318	−178.93(19)
C24	O21	C21	C22	−29.1(2)	C322	N32	C320	O39	−0.9(3)
C24	C23	C214	P2	−72.39(19)	C322	N32	C320	N31	179.20(18)
C24	C23	C214	O24	47.2(2)	C323	O312	C322	O311	−9.7(4)
C24	C25	C26	O23	−69.5(2)	C323	O312	C322	N32	168.4(2)
C25	O22	C27	C28	164.80(19)	C327	O38	C32	C31	170.30(18)
C27	O22	C25	C24	145.1(2)	C327	O38	C32	C33	−74.1(2)

Table 8. Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for han499.

Atom	*x*	*y*	*z*	U(eq)
H13	5230(30)	2270(40)	1760(20)	79(13)
H14	5291(18)	600(30)	3372(18)	41(9)
H11	6586	1034	4079	34
H12	6958	3226	3604	34
H13A	5915	3224	2884	33
H14A	5986	1013	2586	36
H15	7091	1439	2106	41
H16A	6502	2471	1213	48
H16B	6069	1372	1391	48
H17A	7702	3135	1652	48
H17B	8073	2446	2274	48
H19	8776	4308	1642	58
H110	9404	5920	2018	67

H111	9196	6779	2991	62
H112	8344	6003	3592	60
H113	7701	4391	3212	52
H114	5190	2673	3733	34
H11A	4156	5111	2729	62
H11B	4637	4515	3328	62
H11C	3813	4168	3151	62
H11D	3159	1560	2339	69
H11E	2988	2713	2699	69
H11F	2835	1515	3019	69
H117	8065	2317	3450	45
H12A	9816	1978	3720	95
H12B	9699	3121	4111	95
H12C	9258	2913	3406	95
H12D	9373	-58	6888	91
H12E	9231	580	7542	91
H12F	9512	1278	6961	91
H12G	7486	13	6570	81
H12H	7871	-220	7294	81
H12I	8164	-816	6684	81
H12J	8450	2594	6982	89
H12K	8119	1851	7519	89
H12L	7657	2090	6828	89
H12M	5851	4473	3977	50
H12N	6680	4661	4243	50
H12O	6107	4500	4746	50
H23	5077(19)	8700(30)	5037(18)	39(8)
H24	5183(16)	8870(30)	2560(16)	33(7)
H21	6435	9020	2905	32
H22	6843	6860	3498	32
H23A	5763	6807	3911	31
H24A	5718	9041	4165	32
H25	6646	8736	5070	34
H26A	5491	7114	4947	41
H26B	6002	7306	5618	41
H27A	7271	7365	5736	48
H27B	7803	7840	5250	48
H29	7054	5192	5690	53
H210	7654	3449	5752	66
H211	8817	3331	5477	66
H212	9354	4923	5084	60

H213	8764	6665	5026	49
H214	5049	7270	2870	33
H21A	3358	6338	4162	58
H21B	3954	6721	4743	58
H21C	3947	5448	4473	58
H21D	3642	9433	2607	70
H21E	3557	9654	3357	70
H21F	2906	9123	2878	70
H217	7925	8328	4043	36
H22A	9132	8618	4547	64
H22B	9651	9334	4145	64
H22C	9606	7984	4067	64
H22D	9378	10142	859	84
H22E	9634	9237	360	84
H22F	9862	9084	1128	84
H22G	7739	8681	496	84
H22H	8235	8924	-62	84
H22I	8116	9892	456	84
H22J	9348	7130	1012	69
H22K	9046	7303	259	69
H22L	8512	6966	772	69
H22M	5974	5536	2160	55
H22N	5768	5601	2887	55
H22O	6580	5367	2769	55
H33	5520(20)	8160(30)	1508(18)	51(9)
H34	5260(30)	4210(50)	1300(30)	98(18)
H31	3700	4424	1346	35
H32	3201	5987	316	34
H33A	4335	6705	303	34
H34A	4707	6619	1597	35
H35	3842	8134	1699	38
H36A	4789	8556	735	41
H36B	4447	9524	1144	41
H37A	3344	9645	543	48
H37B	2984	9317	1181	48
H39	1904	10387	661	50
H310	788	10216	44	56
H311	520	8679	-645	57
H312	1409	7313	-755	52
H313	2545	7492	-159	45
H314	4863	4699	355	37

H31A	6019	6724	-1023	56
H31B	6592	6739	-381	56
H31C	6000	7718	-497	56
H31D	7445	5195	965	81
H31E	6958	4885	1521	81
H31F	7025	6175	1295	81
H317	2450	6666	1108	37
H32A	809	7071	1475	63
H32B	683	6847	705	63
H32C	1339	7620	1015	63
H32D	4	2624	2209	68
H32E	-164	1299	2266	68
H32F	38	1807	1595	68
H32G	1604	1671	3156	68
H32H	850	1094	3235	68
H32I	909	2444	3180	68
H32J	1045	462	1576	74
H32K	963	-114	2264	74
H32L	1703	476	2150	74
H32M	3565	4021	-793	53
H32N	4061	5102	-597	53
H32O	3207	5231	-698	53

Experimental

Single crystals of $C_{27}H_{39}N_2O_{12}P$ **han499** were obtained from dissolution of compound in chloroform followed by careful addition of hexanes. A suitable crystal was selected and mounted on a loop fiber on a Bruker Venture Metaljet diffractometer. The crystal was kept at 100 K during data collection. Using Olex2 [1], the structure was solved with the XT [2] structure solution program using Direct Methods and refined with the XL [3] refinement package using Least Squares minimisation.

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Crystal structure determination of han499

Crystal Data for $C_{27}H_{39}N_2O_{12}P$ (M =614.57 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 18.8079(8) Å, b = 11.7857(5) Å, c = 20.6661(9) Å, β = 97.314(2)°, V = 4543.7(3) Å^3, Z = 6, T = 100 K, μ(GaKα) = 0.879 mm^{-1}, *Dcalc* = 1.348 g/cm^3, 93733 reflections measured (3.75° ≤ 2Θ ≤ 121.436°), 20828 unique (R_{int} = 0.0446, R_{sigma} = 0.0367) which were used in all calculations. The final R_1 was 0.0378 (I > 2σ(I)) and wR_2 was 0.1017 (all data).

Refinement model description

Number of restraints - 1, number of constraints - unknown.
Details:
1. Fixed Uiso
At 1.2 times of:
All C(H) groups, All C(H,H) groups
At 1.5 times of:
All C(H,H,H) groups
2.a Ternary CH refined with riding coordinates:
C11(H11), C12(H12), C13(H13A), C14(H14A), C15(H15), C114(H114), C21(H21), C22(H22), C23(H23A), C24(H24A), C25(H25), C214(H214), C31(H31), C32(H32), C33(H33A), C34(H34A), C35(H35), C314(H314)
2.b Secondary CH2 refined with riding coordinates:
C16(H16A,H16B), C17(H17A,H17B), C26(H26A,H26B), C27(H27A,H27B), C36(H36A, H36B), C37(H37A,H37B)
2.c Aromatic/amide H refined with riding coordinates:
C19(H19), C110(H110), C111(H111), C112(H112), C113(H113), C117(H117), C29(H29), C210(H210), C211(H211), C212(H212), C213(H213), C217(H217), C39(H39), C310(H310), C311(H311), C312(H312), C313(H313), C317(H317)
2.d Idealised Me refined as rotating group:
C115(H11A,H11B,H11C), C116(H11D,H11E,H11F), C121(H12A,H12B,H12C), C124(H12D, H12E,H12F), C125(H12G,H12H,H12I), C126(H12J,H12K,H12L), C127(H12M,H12N,H12O), C215(H21A,H21B,H21C), C216(H21D,H21E,H21F), C221(H22A,H22B,H22C), C224(H22D, H22E,H22F), C225(H22G,H22H,H22I), C226(H22J,H22K,H22L), C227(H22M,H22N,H22O), C315(H31A,H31B,H31C), C316(H31D,H31E,H31F), C321(H32A,H32B,H32C), C324(H32D, H32E,H32F), C325(H32G,H32H,H32I), C326(H32J,H32K,H32L), C327(H32M,H32N,H32O)

This report has been created with Olex2, compiled on 2016.02.19 svn.r3266 for OlexSys.

10 References

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