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Synthesis of *(S)*-cEt-LNA

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To my family

Résumé

Nucleoside (*S*)-cEt-LNA a été synthétisé par trois voies différentes à partir de la 5-méthyluridine qui est commercialement disponible. Le chemin le plus court comprend une méthylation diastéréosélective d'un aldéhyde, et une cyclisation 5-exo-tet d'un éther par déplacement S_N2.

Mots-clés: (*S*)-cEt-LNA, technologie antisens, nucleoside.

Abstract

(*S*)-cEt-LNA nucleoside was synthesized by three different routes starting from commercially available 5-methyluridine. The shortest route includes a diastereoselective methylation of an aldehyde, and a 5-exo-tet cylization of an ether via S_N2 displacement.

Keywords: antisense technology, (*S*)-cEt-LNA, nucleoside.

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Abbreviations

ASO	Antisense Oligonucleosides
Bn	benzyl
calc'd	calculated
CSA	camphorsulfonic acid
d	doublet
DBU	1,8-diazabicycloundec-7-ene
DCM	dichloromethane
dd	doublet of doublets
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyiridine
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	dimethylsulfoxide
DMTr	4,4'-dimethoxytrityl
eq (equiv.)	equivalent
ESI	electrospray ionization
Et	ethyl

g	gram
h	hour
HMPA	hexamethylphosphoramide
HRMS	high resolution mass spectrometry
Hz	Hertz
ImH	imidazole
IR	Infrared spectroscopy
J	coupling constant
LRMS	Low resolution mass spectrometry
m	multiplet
Me	methyl
MeCN	acetonitrile
min	minutes
mL	mililiters
mmol	millimole
Ms	methanesulphonyl
Nap	naphthylidene
NMR	nuclear magnetic resonance
Piv	pivaloyl
ppm	parts per million
py	pyridine
q	quartet
r.t	room temperature

s	singlet
SDS	solvent delivery system
t	triplet
TBAF	tetra-*n*-butylammonium fluoride
$TBANO_2$	tetra-*n*-butylammonium nitrite
TBAOH	tetra-*n*-butylammonium hydroxide
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	2,2,6,6-tetramethylpiperidine
Tr	trityl
Ts	*p*-toluenesulfonyl
UV	ultraviolet

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1 Chapter One – Introduction

1.1 DNA and RNA structure

The 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 the nucleic acids is a pentose, which depending on the substitution at the position 2', can be D-ribose (-CH-OH) or D-deoxyribose (-CH_2-) (Figure 1a). In DNA the deoxyribose is the constituent sugar of the nucleic acid, while in RNA, ribose is the sugar present in the nucleic acid.

In both DNA and RNA, the pentose is substituted by a phosphate group 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 "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; part of this family are adenine and guanidine. On the other hand pyrimidines, such as cytosine, thymine and uracil, have a six-membered nitrogen ring. Adenine, guanidine and cytosine are found in both DNA and RNA. Uracil is found exclusively in RNA. 5-Methyl uracil, thymidine, is present only in DNA.

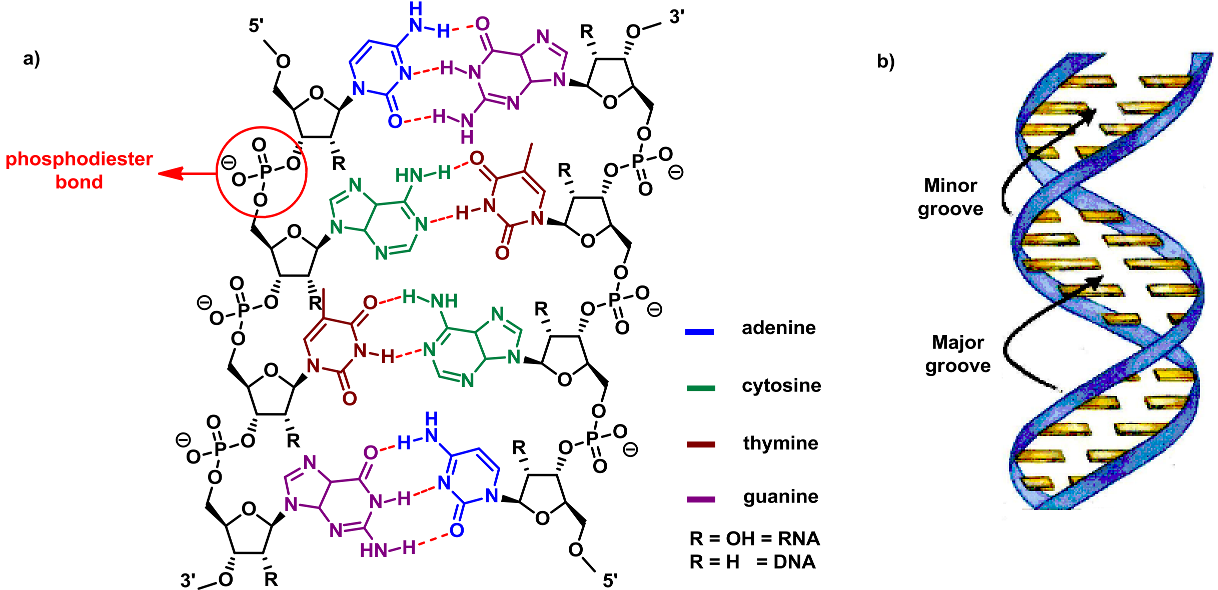


Figure 1. a) Generic nucleic acid double strand. b) Minor and major groove of helix formed by double strand nucleic acids.

Thanks to the X-ray diffraction pattern analysis data taken in the 50's[1] and to chemical analysis, it was demonstrated that the amount of adenine (A) is always the same as the amount of thymine (T). Similarly, the amount of guanine (G) is always equal to the amount of cytosine (C). It was concluded that DNA was a double strand, where the nucleobases of each chain forms hydrogen bonds with its other chain counterparts. Further studies on the helical structure of DNA concluded that exclusively the pairs cytosine-guanine and adenine-thymine were always present along the double strand. This complementary behavior dictates that each strand of the DNA is exactly like the other one but linked in an opposite direction.[1a]

The interactions between the different nucleobases are unique. The pair guanine-adenine forms three hydrogen bonds, while the pair cytosine-thymine only shows two hydrogen bonds. As result, DNA with higher percentage of G-A 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*)”. This value is an indicator for measuring the stability of DNA and its derivates. 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 thus increasing the absorption of light in a phenomena called hyperchomicity.

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 duplex present a difference in the size and shape of the grooves formed by the helix (Figure 1b). The DNA 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 DNA duplex.

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. RNAp 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, opening and closing the double strand on its way. The RNA synthesis starts inside this transcription bubble where 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) that forms a loop in the RNA that helps to dissociate the RNAp transcription complex, and releases the new RNA strand (primary transcript).

1.3 RNA processing

In eukaryotic cells, the RNA obtained from the transcription (primary transcript) stills needs to undergo 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. This is achieved by capping at the 5' position with 7-methylguanosine linked through a 5'-5' triphosphate bond (Figure 2).[2] 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 RNA.[3]

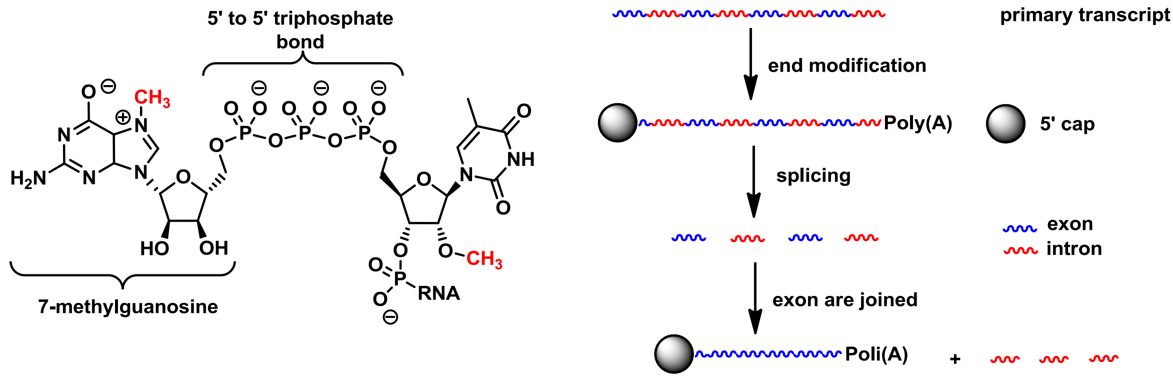


Figure 2. RNA processing

The next step is the splicing of the stabilized RNA (Figure 2) where some sequences, known as introns, are deleted. The amount of sequences removed as introns depends on each specie, but at the end all the remaining parts that codify for a gene (exons) are joined, forming the final mRNA responsible of translate the genetic message.

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 by both RNA and proteins. The amino acids that will form part of the 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. The way to interpret the information is by reading the sequences of nucleosides in groups of three (triads), which are called codons. Each codon is responsible of codifying for a specific amino acid, although one amino acid can be codified by different codons.

The translation process begins when the first molecule of tRNA interacts with the mRNA detecting the initiation codon and binding in presence of the ribosome (Figure 3). 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[4]. The process continues until a codon is reached which does not codify for an amino acid (stop codon). That last codon is recognized by a protein called "releasing factor", which helps the protein synthetized to be expelled from the complex finishing the translation process.

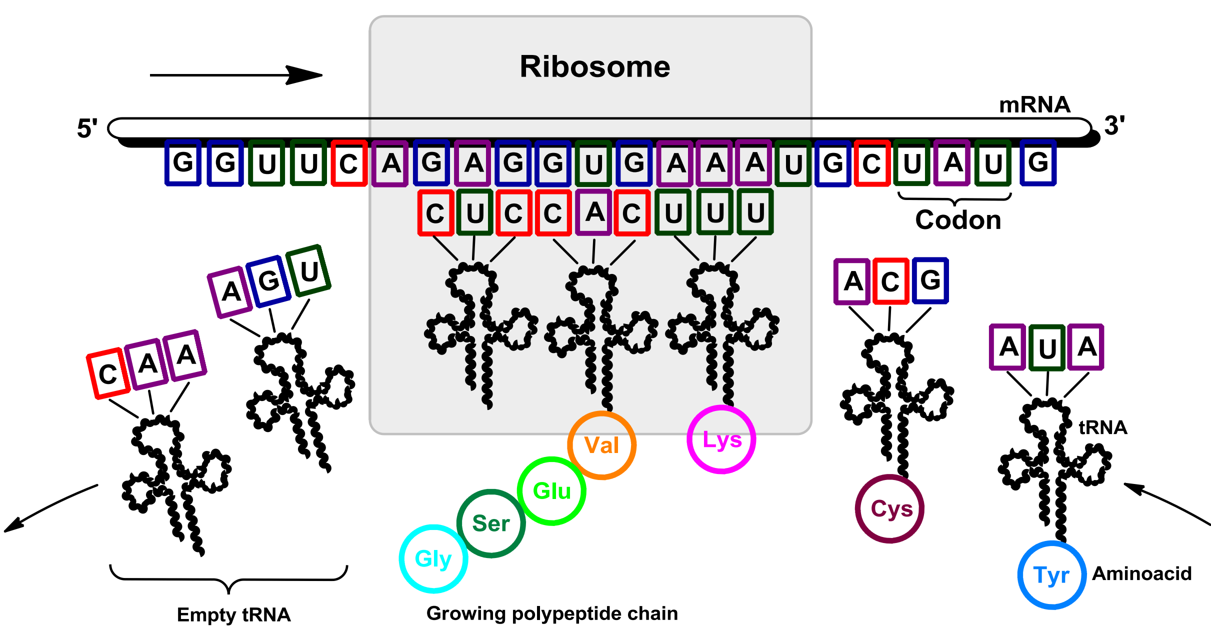


Figure 3. Translation process

2 Chapter Two – Antisense technology

2.1 History

In 1978 Stephenson and Zamecnik[5] 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 are identical sequences of nucleosides next to the 5' cap and poly(A) 3' end. The 21-nucleoside sequence was the target of the study applying a synthetically prepared ribonucleoside, complementary to a segment of the target sequence. The 13 nucleotide sequence d(AATGGTAAAATGG) was bonded to the target sequence, blocking the normal functioning of the virus by competitive hybridization.

Together with many subsequent studies, it was realized that using the RNA as a target, it would be possible to treat different diseases related to the malfunction of RNA, or to block the translation process in the cell. The complementary nucleotide chain is called “antisense” because it has a sequence of bases complementary to the sense of the message in RNA (5' to 3'). Binding to the target RNA is the general mechanism of action of all the antisense oligonucleotides (ASO).

2.2 Molecular mechanism of action

The multiple types of RNAs and their multiple roles in the cell have become a growing area of interest with therapeutic potential in recent years. This includes the historically important

mRNA, the small nuclear RNA (smRNA) that plays key roles in the splicing process and the less studied microRNA which seems to act as a natural gene regulator. Thus, the RNA molecule in general is a very attractive target in the development of new kinds of therapies for the treatment of diverse diseases.

2.2.1 RNA cleavage

The destruction of the RNA chain by a family of enzymes called RNase H is the most direct approach to inactivate its biological function. These enzymes found in mammals recognize the RNA-DNA duplex and hydrolyze the phosphodiester bond in the RNA and release the intact DNA strain.

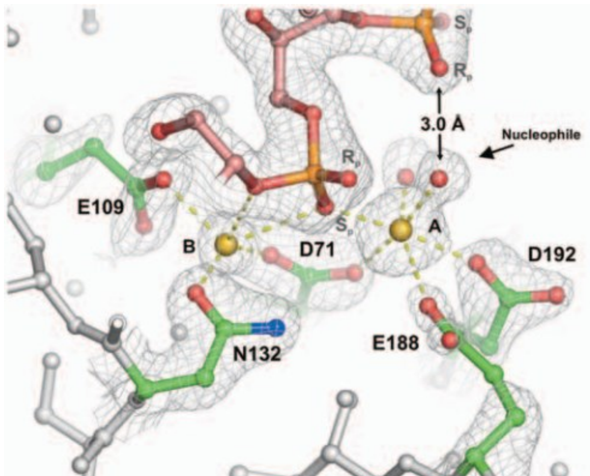


Figure 4. RNase H active site[6].The active site RNase in complex with the RNA/DNA hybrid. Active-site residues are shown in green; the RNA strand in pink, orange, and red; and the magnesium ions as yellow spheres. The water molecule positioned to attack the scissile phosphate is indicated. Metal-ion coordination is shown as dashed yellow lines.

This happens when the enzyme recognizes[7] both, the RNA and the DNA strands. The duplex fits in two grooves in the enzyme structure with the 2'-OH of five consecutive nucleotides of the RNA strand interacting with one groove in the active site of the enzyme.[6] Different active–site residues of the enzyme like carboxylate and amino groups form hydrogen bonds with the 3' and 5' sides of the phosphate bond to be cleaved (Figure 4). In the active site is also found two Mg^{2+} ions, 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 that orients the attack and serves as a general base for deprotonation. After the attack, the other 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 supress the function on the cell, has been the subject of hundreds of studies.[8] Depending on the structure of the nucleotides that form the ASO, the duplex ASO-RNA can support the RNase H activity resulting in the hydrolysis of the RNA target. Surprisingly, some modifications focussing on increasing the metabolic stability and increasing the affinity towards the target RNA, resulted in a loss of RNase H activity[9], thus forcing the use of ASO hybrids composed by sections that support the RNase activity and others sections that provide the affinity to the complementary RNA.

2.2.2 Non RNA cleavage

Antisense oligonucleosides (ASOs) can be designed to work as competitive antagonists, binding to specific sequences in RNA and interrupting the normal interactions with proteins, enzymes, or other factors in the cell. One of the processes that can be affected is the maturation of 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. However, to date there is no report of this approach in antisense technology. Another fundamental step in RNA processing is the splicing. Therefore, if an ASO is bound to a sequence in RNA that had to be spliced, a barrier will be imposed and the splicing suppressed. Promising approaches focussed on interrupting or modifying the RNA splicing process have been reported, for example, the use of ASOs by Kole, proved to be efficient in the correction of an aberrant splicing in mutated RNA, restoring the correct splicing site.[10] ASOs designed to bind with high affinity to the 3'-splice sites in murine pre mRNA exhibit good activity in deletion of specific exons, resulting in the inhibition of mRNA production and suppression of protein synthesis.[11]

2.3 ASO modification

Obtaining a higher resistance against the degradative action of nucleases was the first challenge in the ASO design. Replacing a non-bridging oxygen in the phosphodiester linkage by a sulfur atom gave as result a high resistance of the new modified nucleotides[12] toward action of hydrolases. This new type of nucleosides received the name of phosphothioate (PS) nucleosides (Figure 5a). When the RNA-PS nucleotide duplex is formed, attack of the RNase occurs, which hydrolyzes the RNA strand and releases the PS nucleotide, blocking the production of the protein. The first use of an PS oligonucleotide was reported by Matsukura,[13] where oligonucleotides containing the phosphotioate modification showed inhibition *in vitro* against human immunodeficiency virus (HIV). Although PS oligonucleotides present a slightly similar

affinity for the RNA compared to natural nucleic acids,[14] they exhibit tendency towards non-specific interaction with certain proteins[15] causing undesired effects in the body.

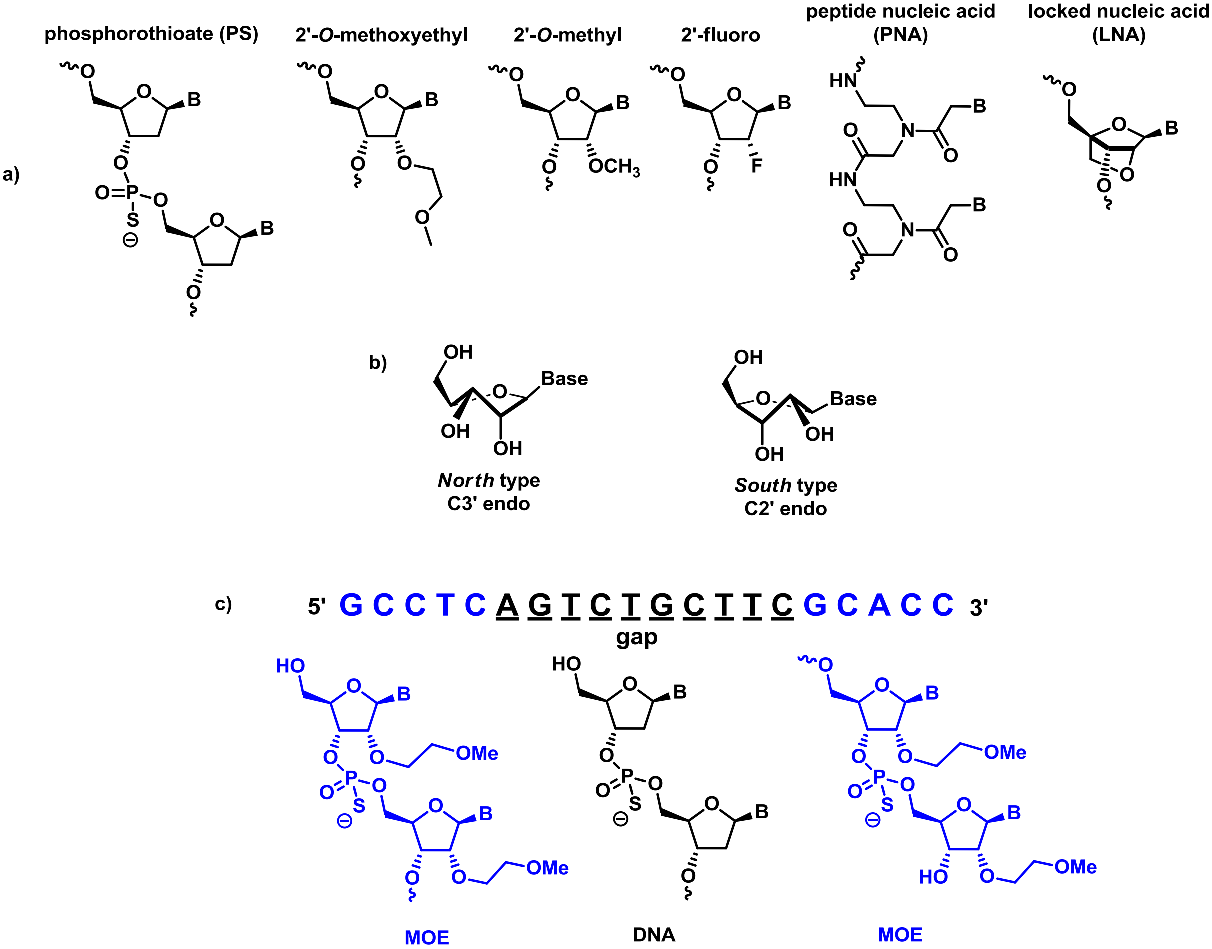


Figure 5. a) Generations of ASOs. b) North and South type conformation. c) Example of Gap design oligonucleotide with MOE.

The next generation of ASOs was designed to increase both the affinity with the RNA, and the nuclease resistance. Between all kinds of reported modifications, two types of ASOs have been widely studied. Methylation of the 2'-OH of pentose gives rise to the 2'-*O*-methyl nucleoside (Figure 5a). This modification blocks the possibility of self-hydrolysis of the phosphodiester

linkage by attack of the 2'-OH. The 2'-*O*-methoxyethyl (MOE) nucleosides together with the PS backbone modification established the most studied second generation of ASOs.[16] The methoxyethyl group assumes a rigid conformation due to the *gauche* effect between the two oxygen atoms. The MOE group also forms hydrogen bonds with a molecule of water that acts as a protecting shield of the phosphodiester bond which results in an increased resistance against nucleases.[17]

Together with the 2'-fluoro modification (Figure 5a), the 2'-*O*-alkyl modification confers to the nucleoside a north type conformation (Figure 5b) that shortens the distance between the 3'-5' phosphate bonds, generating, a more compact structure that assumes a type A helix conformation, increasing the affinity of the ASO to the RNA.

The second generation modifications resulted in a decrease in the toxicity associated with the first generation of ASOs,[18] but also presented some difficulties in the design of new oligonucleotides, being incompatible with the action of the RNase and the RNA cleavage mechanism. This limitation was overcome by the use of a "gap" design[9] (Figure 5c), which uses 2'-*O*-alkyl nucleosides in the 3' and 5' end of the chain, and 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, a medicine for the treatment of homozygous familial hypercholesterolemia.[19]

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) (Figure 5a). Designed

with a pseudopeptide backbone, consisting of *N*-(2-aminoethyl)-glycine, this PNA shows great affinity to RNA and DNA. Due to its pseudopeptidic structure, PNA is resistant to degradation of nucleases and proteases, but they are not compatible with the RNase mechanism of action. This stability makes the PNA a good candidate for its use in the disruption of RNA processing, by the translation inhibition[20] and splicing modulation mechanisms.[21]

Locked nucleic acid (LNA)[22] is a restricted analog of 2'-OMe nucleoside, where the 2' hydroxyl group methyl is tethered to the 4'-carbon (Figure 5a). The resulting bicyclic structure confers to the molecule a north type conformation that presents an improved stacking between the nucleobases compared to the RNA duplex.[23] 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 cleavage.[24] Further studies have shown the successful use of a LNA-gap design.[25]

3 Chapter Three – Synthesis of (*S*)-cEt-LNA

3.1 Original synthesis of (*S*)-cEt-LNA

In 2010 Seth et al. reported the synthesis of a new generation of modified nucleosides.[26] The 2',4'-constrained MOE (cMOE) (Figure 6) was designed to combine the reduced toxicity of MOE nucleotides, with the great affinity showed by LNA nucleosides. To achieve the desired properties, the methoxyethyl group found in MOE was restricted covalently to the 4' position forming a bicyclic structure as in LNA. Both (*R)* and (*S)*-cMOE isomers, showed high resistance against nuclease attack but when tested *in vivo,* cMOE ASOs exhibited a considerably diminution in activity compared to LNA ASOs.[27] The reduced activity was attributed to the steric hindrance generated by the terminal *O*-methyl group, so it was hypothesized that the removal of the methoxy end, will restore the biological activity.

Figure 6. cMOE and cEt-LNA nucleosides

Given the previous results, both isomers of the 2',4'-constrained ethyl LNA (cEt-LNA) (Figure 6) were synthetized an tested. The cEt-LNA can be seen as a 6'-methylated analog of LNA, or a cMOE without the methoxy chain at 6' position. When introduced in ASOs, they presented no hepatotoxicity in mice expressed as a measure of the transaminases level. The thermal and nuclease stability of ASOs containing (*S*)-cEt-LNA were also studied. The results showed that oligonucleotides containing (*S*)-cEt-LNA present higher stability than those containing LNA.[28] 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.

The original synthesis of (*S*)-cEt-LNA started from the commercially available diacetone D-allofuranose (Scheme 1). Protection of the free alcohol with 2-naphthyl bromide provided **1** in quantitative yield. Hydrolysis of the *exo*-acetal in **1**, cleavage of the diol to furnish the aldehyde, and aldol reaction with formaldehyde afforded **2** in 66% yield over 3 steps. Selective protection of the diol moiety in **2,** gave **3** in addition to bis silylated products. Oxidation of the primary alcohol under the Swern conditions, and treatment of the resulting aldehyde with MeMgBr, produced **4** in a ratio of 10:1 of the desired (*S*)-alcohol. A sequential oxidation/reduction led to **5** as a single isomer. Mesylation of the secondary alcohol in **5** followed by acetolysis furnished **6** in 90% yield over two steps**.** Vorbrüggen reaction of the diacetate **6** installed the 5-methyluridine moiety at the anomeric position of the sugar. Cleavage of the 2'-OAc, and intramolecular displacement of the mesylate group gave bicyclic nucleoside **7**. Finally, cleavage of the 3'-ONap protecting group gave the 5'-TBDPS*O*-(*S*)-cEt-LNA in 14 steps and 22% overall yield.

Scheme 1. Original synthesis of (*S*)-cEt-LNA.

3.2 Retrosynthetic analysis of (*S*)-cEt-LNA

It was envisaged that the 2',6'-ether bridge in (*S*)-cEt-LNA can be formed via S_N2 displacement of an appropriate leaving group by the 2' alcohol of the nucleoside **A** (Scheme 2). This approach has been successfully applied in the synthesis of complex nucleosides bearing the protected 3'-OH.[29] In our case, we face a 4-*exo-tet* vs. 5-*exo-tet* cyclization both favored by Baldwin rules,[30] but the formation of the 4-member ring was expected to be less favored due to the ring strain generated in the oxetane product. With this strategy in mind, the selective addition of a methyl group to the corresponding aldehyde to obtaining the (*R*)-alcohol **B**, required particular attention. The desired configuration would be achieved during the inversion in the S_N2 displacement.

Scheme 2. Retrosynthetic analysis of (*S*)-cEt-LNA

The free alcohol on the α-face of the nucleoside can be obtained via protection-deprotection sequence on **C**. The compound **C** can be easily obtained from 5-methyluridine.

As the main objective of the project was to develop a synthetic route that provides (*S*)-cEt-LNA in large scale with a reduced number of synthetic steps, and an improved overall yield compared to the original route, we decided to use 5-methyluridine ($4.33 per mmol) as starting material vs. diacetone D-allofuranose ($16.8 per mmol). Furthermore this approach avoids the introduction of the 5-methyluracil moiety late in the synthesis.

3.3 First Generation Synthesis

The first objective towards (*S*)-cEt-LNA consisted in obtaining the key intermediate **5** (Scheme 3), which takes advantage of the inherent functionality of the molecule in order to avoid the use of multiple protecting groups. The formation of a 2-5' anhydronucleoside as a key intermediate gives a dual protection to the 5' OH and the 3-NH of the nucleobase.

4-*exo vs.* 5-*exo*-tet cyclization

Key Intermediate

Scheme 3. Retrosynthetic analysis of anhydronucleoside key intermediate **5**.

Starting from 5-methyluridine, protection of the *cis*-2',3' diol as a cyclohexylidene acetal provided **1** in 96% yield (Scheme 4). Oxidation of the primary alcohol using Dess-Martin periodinane[31] followed by cross-aldol reaction with formaldehyde and one-pot reduction following the Moffatt protocol[32] gave diol **2** in 51% yield. Treatment of the diol with triflic anhydride in pyridine afforded bistriflate nucleoside that was subsequently treated with DBU, resulting in the displacement of the 5'-OTf, to give anhydronucleoside triflate **3**. Different conditions were used such as H_2O/THF, LiOH/H_2O_2, TBAOH, NaOH/THF, focused on displacing the remaining triflate in order to obtain the alcohol, the acetate or directly the aldehyde via a Korblum oxidation.[33] They proved to be unsuccessful leading to the opening of

the anhydro bridge. A known reaction in carbohydrate chemistry consists in the displacement of triflates by nitrite salts forming an alcohol with an inverted configuration.[34] In our system, the use of $NaNO_2$ in DMF provided the corresponding alcohol **4** in low yield. However, when the nitrite source was replaced by $TBANO_2$ in THF, alcohol **4** was obtained in 85% yield. Oxidation with DMP gave aldehyde **5** in 85 % yield.

Scheme 4. Synthesis of key intermediate **5**.

An alternative route to the key intermediate **5** was performed through the treatment of **2** with TBDPSCl affording the protected of the β-5'-alcohol in 68% yield. The regioselective protection of the α-5'-hydroxymethyl vs. the β-5'-hydroxymethyl could also be achieved by using bulky such as Tosyl, TBS, TBDPS and Tr.

With **6** in hands, we proceeded to install a triflate on the remaining 5'-hydroxymethyl group, followed by a DBU mediated displacement to yield **7**. Silyl ether cleavage by TBAF and DMP oxidation afforded the key intermediate **5**. Interestingly, when the mesyl group is used instead of the triflate as leaving group in **6**, the displacement to produce **7** did not work.

With compound **5** in hand, we tried adding different methyl sources like MeMgBr, MeLi, Me_2CuLi and $AlMe_3$, but they all gave low yields and poor diastereoselectivity. Additionally, the opening of the anhydro bridge led to by-products, showing signs of its labile character. Analyzing the conformation of the molecule it was suspected that due to the anhydro bridge the molecule will be in a non-favorable conformation for the desired attack from the 2'-OH in a 5-*exo-tet* cyclization, leading us to abandon this route.

3.4 Second Generation Synthesis

The second approach consisted of a protection-deprotection sequence to differentiate between the α and β-5'-hydroxymethyl groups in **2** (Scheme 5). This is based on the higher reactivity of the hydroxymethyl on the α-face of the sugar due to favorable steric reasons. The first attempt to protect the α-hydroxymethyl alcohol was using standard benzylation conditions (BnBr, NaH), but the basic conditions produced mixtures of the desired product and a side-products derived from the benzylation at N-3 of the nucleobase. A better selection of the protecting group led to the use of trityl chloride to obtain **8** in 70% yield. Introduction of TBDPS group for the protection of the remaining alcohol gave compound **9.** Cleavage of the trityl group under acidic conditions, was followed by oxidation with Dess-Martin periodinane (DMP) to the corresponding aldehyde. Diasteroselective methylation with $AlMe_3$ in toluene gave **11** in 68% yield as a single isomer. The relative configuration of compound **11** could not be fully established at that point. However, by comparison with known C5'-OPiv analog it was thought to be the (*S*)-alcohol. In order to invert the configuration of the alcohol we used an oxidation-reduction sequence of the ketone, starting with the use of DMP on nucleoside **11**, then reduction of the crude mixture with $LiAlH_4$ at -78 °C to afford **12** as a single isomer.

Scheme 5. Synthesis of intermediate alcohol **12**.

The observed selectivity in the methylation to obtain **11** can be explained using the Cram chelate model, where the addition of $AlMe_3$ takes place through a chelated state between the oxygen of the aldehyde and the oxygen of the furanose ring (Figure 7). Due to the steric hindrance created by the 5'-*O*TBDPS it would be expected that the methyl group would be delivered on the *si* face of the aldehyde, via a $AlMe_3$-dimer.[35] In the case of the oxidation-reduction sequence with $LiAlH_4$ to form **12,** the same steric assistance and chelate formation would provide the selective transfer of the hydride on the *si* face of the methyl ketone.

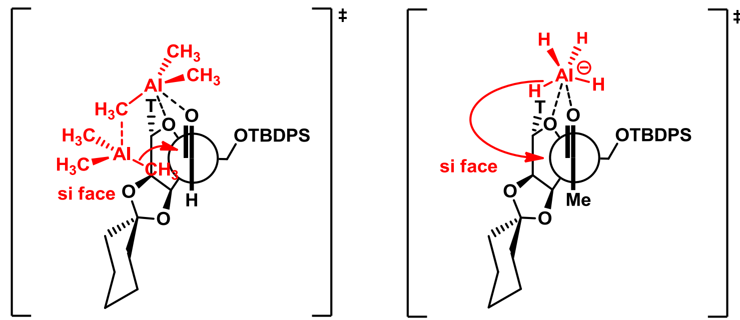


Figure 7. Proposed Cram chelate model for the diastereoselective methyl and hydride additions.

The next alternative was to invert the 6’-OH group via the mesylate **13** (Scheme 6). Cleavage of the cyclohexylidene acetal under acidic conditions gave the diol **13** in 52% yield over two steps (Scheme 6). At this point, the first attempt to directly cyclize diol **13** to obtain the (*S*)-cEt-LNA, led to the four membered oxetane ring **14** from the 4-*exo-tet* cyclization mode in 90% yield. The structure of **14** was correlated with the 5'-OH pivaloyl analog provided by Isis Pharmaceuticals. With the aim to complete the synthesis, it was decided to use a detour by protecting the diol moiety in **13** as a benzylidene acetal. Selective reductive cleavage[36] of the diastereomeric mixture of acetals in **15** was achieved by treatment with $TiCl_4$/$NaBH_3CN$ to furnish the 2'-OH free nucleoside, which then, readily cyclized under basic conditions to give **16**. Different attempts to cleave of the benzyl ether on **16** via hydrogenolysis were unsuccessful ($Pd(OH)_2$, Pd/C). When harsher conditions were employed ($Pd(OH)_2$, ammonium formate), the nucleobase underwent double bond reduction as reported previously.[37]

OTBDPS
Me
HO
O
N
Me
O
NH
O
O
O
12

1. MsCl, pyridine
2. HCl/MeOH/THF (3:4:2)
52% (2 steps)

13

K_2CO_3, MeOH, 80 °C
4-*exo*-tet cyclization
90%

14
correlated with C5'-OPiv analogue from ISIS

$PhCH(OMe)_2$
CSA, 1,2-DCE
82%

15

1. $TiCl_4$, MeCN
$NaCNBH_3$
2. K_2CO_3, MeOH
73% (2 steps)

16

Scheme 6. Synthesis of 3'-benzyl-(*S*)-cEt-LNA **16** and oxetane **14**.

3.5 Third Generation Synthesis

Due to the challenges presented with the removal of the benzyl group in **16**, it was decided to use a similar strategy by protecting the 2',3' diol with a more labile protecting group. The chosen group was the naphthylidene acetal, which in principle should show the same regioselectivity during the reductive cleavage just as the benzylidene acetal did. First, protection of the vicinal diol in 5-methyluridine with commercially available 2-naphthaldehyde, resulted in low yield and poor conversion. Therefore, it was decided to use the more reactive naphthaldehyde dimethyl acetal. The naphthylidene dimethyl acetal is not commercially available, so it was prepared starting from 2-naphthaldehyde[38] and used without further purification. The reaction of 5-methyluridine with naphthaldehyde dimethyl acetal catalyzed by CSA proceeded smoothly in 85% yield furnishing a mixture of diasteroisomers at the acetal carbon atom of **17** (Scheme 7). Unfortunately, the sequence of oxidation/aldol reaction/ reduction to produce **18** was not only low yielding but also had solubility issues.

Scheme 7. Synthesis of diol **18**.

Reductive cleavage of the acetal at the 2' position in **17** gave **19** in 94% yield (Scheme 8). Double protection of the primary and secondary alcohols in **19** with TBSCl and subsequent cleavage of

the silyl group on 5'-OH gave nucleoside **20**. The use of the same sequence for the synthesis of **2**, was employed on **20** furnishing the 6'-hydroxymethyl analog **21** in 48% yield over two steps. This time, the use of trityl chloride proved to be effective to get compound **22,** which then followed the same sequence of protecting groups used during the first approach, affording compound **24** in 54% over three steps. Transformation of compound **22** into **24** can be done in a one pot procedure in 69% yield.

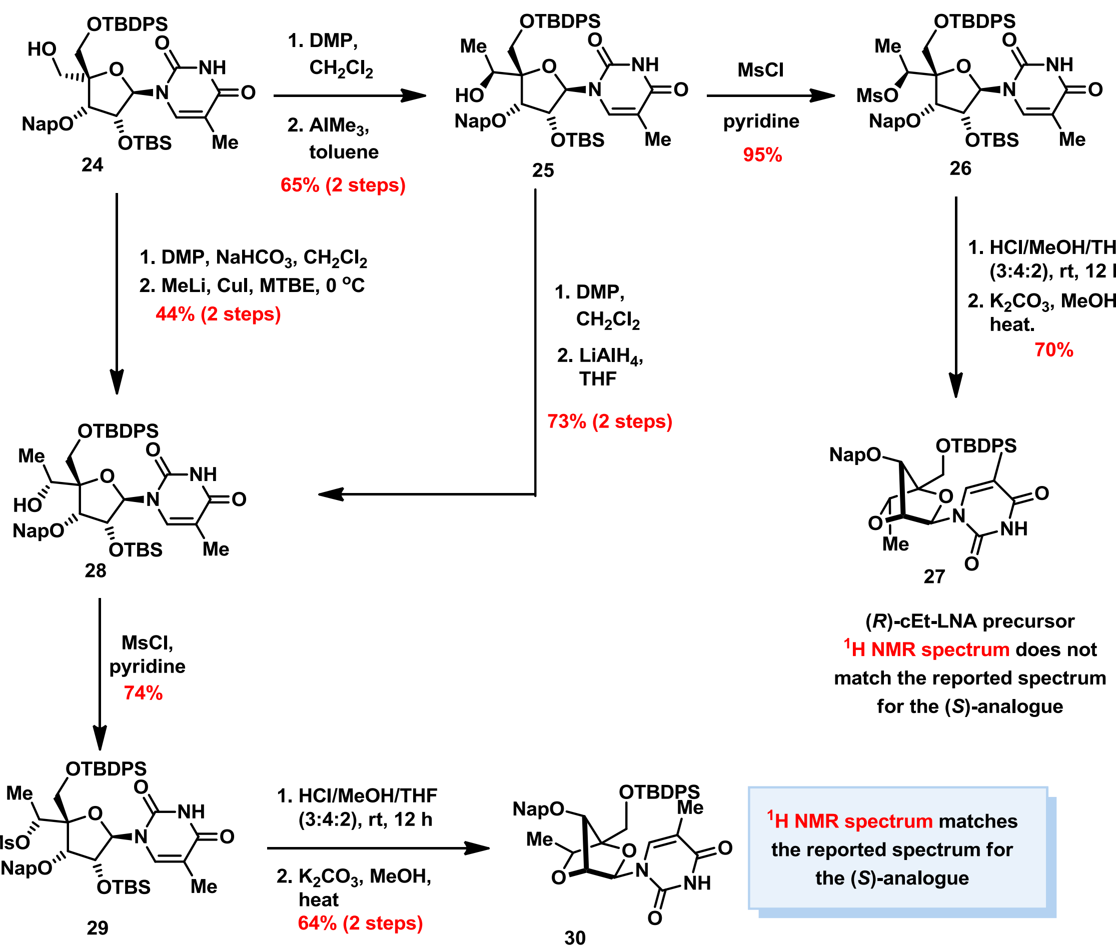
17 → (TiCl$_4$, NaBH$_3$CN, MeCN; 94%) → 19 → (1. TBSCl, DMAP, pyridine; 2. TFA, AcOH; 52% (2 steps)) → 20 → (1. DMP, CH$_2$Cl$_2$; 2. NaOH, CH$_2$O, then NaBH$_4$; 48% (2 steps)) → 21 → (TrCl, DMAP, NEt$_3$, DMF; 70% (brsm)) → 22 → (TBDPSCl, ImH, CH$_2$Cl$_2$; 80%) → 23 → (TFA, CH$_2$Cl$_2$; 97%) → 24

22 → (TBDPSCl, ImH, CH$_2$Cl$_2$ then TFA; 69%) → 24

Scheme 8. Synthesis of intermediate **24**.

The aldehyde obtained from oxidation of **24** was treated with $AlMe_3$ to give the secondary alcohol **25** which was readily mesylated to obtain nucleoside **26** (Scheme 9). Deprotection of the 2'-OH and cyclization under basic conditions furnished compound **27**, whose ^1H NMR did not correspond to the desired product. From this result it was clear that the addition of the methyl

group on **25** gave the undesired (*S*)-isomer at 6'-position. Therefore, it was necessary to oxidize to the corresponding ketone with DMP and reduce with $LiAlH_4$ to obtain the (*R*)-isomer. In the study of the fourth generation synthesis, we found that the use of cuprates instead of $AlMe_3$ provided the (*R*)-alcohol as major product. Applying these conditions to the aldehyde obtained from oxidation of **24**, led to formation of alcohol **28** in 44% yield over two steps. With compound **28** in hand, mesylate **29** was prepared, then displaced by the 2'-alcohol after cleavage of the 2'-OTBS in **29** and treated with K_2CO_3 in MeOH. The ^1H and ^{13}C NMR for compound **30** matched with the one reported by Isis Pharmaceuticals.[37]



Scheme 9. Synthesis of 3'-naphthyl-(*S*)-cEt-LNA **30**.

3.6 Fourth Generation Synthesis

The last generation synthesis was focussed on optimizing and reducing the number of total steps, in addition to improving the selectivity in the key steps of the synthesis using the knowledge obtained from the previous syntheses. During the first generation approach, the protection of the diol in 5-methyluridine was accomplished by the use of cyclohexanone as solvent which forced us to purify the product by flash chromatography in order to remove large excess of unreacted ketone. In order to improve the efficiency of this process, it was changed to cyclohexanone dimethyl acetal in 1,2-dichloroethane. This reaction furnished protected 5-methyluridine with a purity up to 95%, allowing its use in the subsequent step without further purification. Oxidation of the crude alcohol with DMP, followed by aldol condensation with formaldehyde and reduction with sodium borohydride afforded **2** in 62% yield (Scheme 10).

Scheme 10. Synthesis of compound **32**.

Transformation of **2** into **10** was previously accomplished in three steps with a purification between each step (Scheme 5). In the current approach, the number of purifications was reduced without significantly sacrificing the yield, adopting a one-pot procedure to protect the α-5'-hydroxymethyl group with TBSCl at 0 °C followed by adding an excess of TBDPSCl. The reaction mixture containing the TBS/TBDPS-nucleoside and other silylated products was then diluted with methanol and treated with TFA to cleave the more acid sensitive TBS group, thus giving **10** as product after purification by flash chromatography. All the remaining fractions containing other silylated products were concentrated, dissolved in anhydrous THF, and treated with TBAF recovering **2** as product that was eventually recycled.

In the search to obtain alcohol **12** without the oxidation/reduction sequence, it was found that the system MeLi/CuI in Et_2O affords the desired (*R*)-alcohol as a major product. The change of solvent (Scheme 10) from Et_2O to the safer MTBE, led surprisingly to increased selectivity, giving (*R*)-alcohol in a ratio up to 8:1 in 51% yield over 2 steps. Standard conditions for *O*-tosylation of **12,** provided nucleoside **31** which was then treated with a mixture of HCl/MeOH/THF to remove the cyclohexylidene acetal affording diol **32** in good yield.

With diol **32** in hand, several conditions to favor the formation of the 5-membered ring tetrahydrofuran over the 4-membered ring oxetane were studied (Table I). In entry 2-6, the use of pyridine showed a selectivity towards the five-membered ring, albeit requiring long reaction times or a sealed tube to complete the reaction. The use of magnesium bromide or water as co-solvent decreased the selectivity of the reaction. Driven by the promising results obtained with pyridine, different compounds bearing the pyridine scaffold such as 2,2'-bipyridine, quinoline, phenanthridine, and 1,10-phenanthroline were screened (entry 7-11), obtaining the best ratio with the latter (entry 11).

Employing common amine bases such as DBU, NEt_3, DIPEA, imidazole or TMP (entry 12-18) gave low selectivity towards the 5-*exo-tet* cyclization product **33**. Bicyclic guanidines (entry 19-23), that in principle present dual activation through hydrogen bonding[39], were also tested providing the oxetane **14** as product. The five and six-member ring cyclic amidines in entry 24 and 25 were not effective in performing the desired selective cyclization producing a complex mixture in the case of entry 24. Similarly, bases such BEMP and N-methylmorpholine produced **14** as major product (entry 26 and 27). Other less conventional conditions including salts, basic alumina, molecular sieves, and silica gel did not give satisfactory results with exception of the last, where a reasonable ratio of the desired compound was obtained albeit with low conversion.

Table I. Screening conditions for 5-*exo-tet*-cylization

Entry	Condition	Ratio 14 : 33 : SM[a]
1	Potassium carbonate, MeCN	10 : 1 : 0
2	Pyridine, 80 °C, 3 days.	1 : 1.7 : 0
3	Pyridine, $MgBr_2$ (2 equiv.), 80 °C,	3.7 : 1 : 0
4	Pyridine anhydrous, 80 °C, 24 h	1 : 1.8 : 12.5* 1 : 2.1 : 10*
5	Pyridine/H_2O 10:1, 80 °C, 20 h	1 : 1 : 1.2

6	Pyridine anhydrous, sealed tube, 120 °C, 20 h	1 : 1.6 : 0
7	2,6-lutidine (3 equiv.), toluene reflux, 24 h	2 : 1 : 0
8	(2 equiv.), toluene, 80 °C, 20 h	1 : 1 : 22
9	(2 equiv.), toluene, 80 °C, 20 h	1 : 0.9 : 5.6
10	(2 equiv.), toluene, 80 °C, 20 h	1 : 1.5 : 5.1
11	(2 equiv.), toluene, 80 °C, 20 h	1 : 2 : 2.6
12	DBU, dioxane	3.8 : 1 : 0
13	DBU (2 equiv.), toluene, 80 °C, 24 h	3.5 : 1 : 5
14	Triethylamine (2 equiv.), DMSO, 80 °C, 24 h	1.3 : 1 : 0.3
15	DIPEA (5 equiv.), toluene, 80 °C, 48 h	5 : 1 : 1
16	Imidazole (2 equiv.), toluene, 80 °C, 24 h	1 : 1 : 0
17	1-methylimidazole (2 equiv.), toluene, 80 °C, 24 h	3 : 1 : 10

18	(2 equiv.), toluene reflux, 48 h	2 : 1 : 0
19	(2 equiv.), toluene, 80 °C, 24 h	5 : 1 : 0
20	(2 equiv.), toluene, 80 °C, 24 h	6.6 : 1 : 0
21	(2 equiv.), toluene, 80 °C, 24 h	6.6 : 1 : 0
22	(2 equiv.), toluene, 80 °C, 24 h	1.2 : 1 : 1.3
23	(2 equiv.), toluene, 80 °C, 24 h	2.3 : 1 : 12.5
24	(1.5 equiv.), toluene, 80 °C, 24 h	Decomposition
25	(1.5 equiv.), toluene, 80 °C, 24 h	1 : 1 : 8.5

26	, Toluene, rt, 36 h	>20 : 1 : 0
27	(2 equiv.), toluene, 80 °C, 20 h	7 : 1 : 0
28	$MgBr_2$ (2 equiv.), toluene, 80 °C, 20 h	6 : 1 : 0.8
29	LiBr (2 equiv.), toluene, 80 °C, 20 h	6.2 : 1 : 1.6
30	TBAOAc (2 equiv.), toluene, 80 °C, 20 h	3.4 : 1 : 0
31	Molecular sieves, toluene, 80 °C, 24 h	8.5 : 1 : 14*
32	Basic alumina, toluene, 80 °C, 20 h	1 : 1 : 4
33	Silica gel, toluene, 80 °C, 20 h	1 : 2.5 : 30*
34	DMF, 80 °C, 24 h	1.8 : 1 : 7.4*
35	, toluene, 80 °C, 24 h	No Rxn
36	, 80 °C, 24 h	1 : 2.1 :11*
37	DMSO, 80 °C, 24 h	1 : 2.7 : 1 1 : 2.6 : 5.7 (duplicate)
38	(2 equiv.), toluene, 80 °C, 20 h	No Rxn

39	(2 equiv.), toluene, 80 °C, 20 h	No Rxn
40	(2 equiv.), toluene, 80 °C, 20 h	No Rxn
41	(2 equiv.), toluene, 80 °C, 20 h	No Rxn
42	100 °C, 20 h	1 : 1 : 14*
43	HMPA, 100 °C, 20 h	1 : 3.5 : 0 1 : 3.6 : 0 (duplicate)
44	DMPU, 100 °C, 20 h	1 : 1.1 : 0
45	DMI, 100 °C, 20 h	1 : 0.78 : 0
46	Water, 90 °C, 20 h	1 : 2.4 : 4.7
47	Pyridine, 110 °C, 36 h[b]	1 : 1 : 0

All ratios were measured by ^{1}H NMR. **SM** refers to the amount of starting material unreacted measured by ^{1}H NMR. [a]2-naphthylsulfonate was installed as leaving group instead of *p*-toluenesulfonyl. (*) = low conversion.

With the current results, it was realized that the use of base would not produce selectively the desired compound. Even if the 5-membered ring product was the most thermodynamically favorable. Therefore, it was necessary to find new conditions that would allow a conformational change in the molecule from the *S*-type to an *N*-type. (Figure 8)

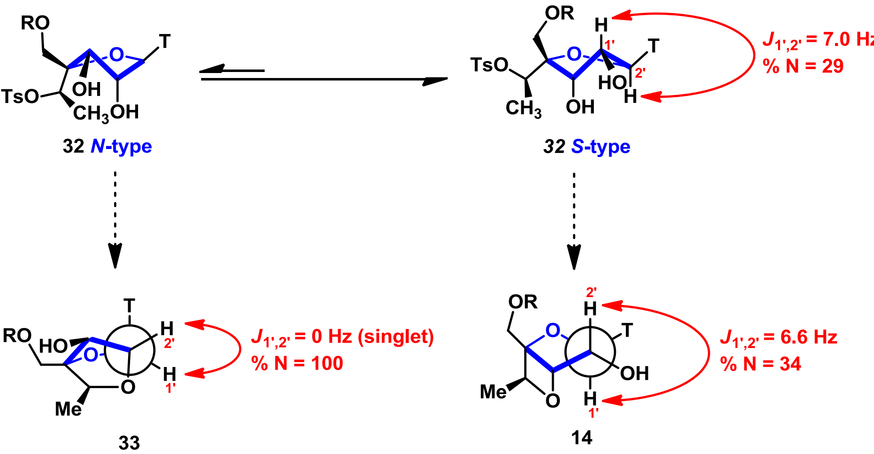


Figure 8. Conformational equilibrium. Coupling constant $J_{1'2'}$ and percentage of north-type conformation of selected nucleosides.

Calculating the coupling constants $J_{1'2'}$ of compounds **32, 14,** and **33,** it was possible to determine the conformation expressed as a percentage of the north type conformation[40] in solution ($CDCl_3$). In the cEt-LNA (**33**), the anomeric signal appears as a singlet, which means that 100 % of the population of **33** molecule is in a northern type conformation (Figure 8). The starting material of the cyclization (**32**) is in a 29% of *N*-type which compared with the 34% of the undesired oxetane **14,** showed how the conformational equilibrium in Figure 8 is strongly displaced towards *S*-type conformation. In order to try to change the natural conformation of the molecule and favor the *N*-type conformation, it was decided to try the cyclization in solvents with different polarities that could influence the conformational equilibrium. Experimentation began with the use of non-protic polar solvents such as DME and DMF. The first solvent gave no conversion, and DMF favored the formation of the oxetane product (Table 1, entries 34 and 35). When 1-methyl-2-pyrrolidinone and DMSO were employed as solvents (entry 36 and 37), a better selectivity was achieved without the use of any base. However, when other solvents such as sulfoxides and sulfones (entry 38 to 42), no improvement in the reaction selectivity was

obtained. The use of HMPA as solvent, afforded a **3.6 : 1** ratio favouring the desired product. Other common solvents such as DMPU and DMI did not show improvement compared to the result obtained with HMPA (entry 44 and 45). Applying the better conditions for the cyclization found in entry 43 to a larger scale, produce **33** in 50% isolated yield.

Another option that was worth studying is the work developed by Tan where 5'-protected nucleosides can be either silylated at 2' or 3'-alcohol by using the appropriate catalyst.[41] Following Tan's conditions, selective protection of the 3'-OH in **14** to subsequently cyclize to obtain the 3'-OTES-(*S*)-cEt-LNA was tried (scheme 11). Independent of the conditions used, only the 2'-OTES protected nucleoside **34** was obtained. Surprisingly, when **14** was treated with an excess of TESOTf, **34** was obtained as sole product. Different conditions were tried to introduce a TBS group in the remaining 3'-OH, but probably due to the steric hindrance, it was not possible to obtain the product forcing us to abandon this approach.

TESCl, DIPEA, DIPEA·HCl, THF, Catalyst 1 → Obtained / Expected

TESCl, DIPEA, DIPEA·HCl, THF, Catalyst 2 → Obtained / Expected

TESOTf (4 eq), 2,6-lutidine, 0 °C, CH_2Cl_2 → Obtained / Expected

Scheme 11. Selective protection of nucleoside **32**

3.7 Synopsis

The comparative yields obtained for each one of the reported synthesis are shown in Table II. The first generation did not afford the final product, instead, **5** was obtained in 7 steps and 26% yield. Alkylation of aldehyde **5** proved to be difficult giving a complex mixture under different conditions, forcing us to abandon this approach.

The second generation synthesis produced 3'-benzyl-(*S*)-cEt-LNA **16** in 4% yield. The need of an oxidation/reduction sequence in order to obtain the desired isomer **12** as a single product reduced the efficiency of the route. Also, the protection of the 3'-alcohol proved to be necessary to avoid the formation of the undesired oxetane product.

The third generation synthesis produced 3'-naphthyl-(S)-cEt-LNA **30** in 2% yield. The use of a cuprate as a methyl source provides the alcohol **28** with the desired configuration. However, it was still required to protect the 3'-alcohol as a 2-methylnaphthyl ether, which not only allowed the exclusive formation of the 5-membered ring, but also gave an easily cleavable protecting group compared to the benzyl ether in **16**. This approach involves 13 steps and warrants further improvement in overall yield.

The fourth generation affords the desired product in 7% yield in 9 linear steps. The key step of this route is the cyclization of **32** in HMPA that affords the (*S*)-cEt-LNA with the 3'-OH free. The use of inexpensive reagents in the synthesis and the reduced number of steps, make the fourth generation synthesis in an interesting option for a future scalable production.

Table II. Number of steps, yields and final products of the four synthesis.

	Number of Steps	Yield	Product
First generation	7	26%	5
Second generation	15	4%	16
Third generation	13	2%	30
Fourth generation	9	7%	33
Literature Reference[a]	14	22%	33

[a] Diacetone D-allofuranose as starting material.

4 Conclusions

- The synthesis of 5’-O-TBDPS-(*S*)-cEt-LNA was achieved in 9 steps with 7 % overall yield.
- The combined use of 5-methyluridine as starting material and readily available and reasonably priced reagents provides an option for a scalable route to (*S*)-cEt-LNA.

5 Experimental Procedures

5.1 General Experimental

All non-aqueous reactions were run 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 these solvents through activated columns of alumina, while 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[42] 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}.

5.2 Experimental Section

5.2.1 First Generation Synthesis

CSA (5 mol%)
cyclohexanone
1,2-dichloroethane
60 °C, 4 h

(1-((3a'R,4'R,6'R,6a'R)-4'-(hydroxymethyl)tetrahydrospiro[cyclohexane-1,2'-furo[3,4-d]-[1,3]dioxol]-6'-yl)-5-methylpyrimidine-2,4(1H,3H)-dione) (1). 5-Methylurdine (5.01 g, 19.4 mmol) was added to a stirred solution of cyclohexanone (30 mL) and *p*-toluenesulfonic acid (0.51 g, 2.5 mmol). The reaction mixture was heated to 50 °C for 40 h, cooled to room temperature, then directly purified by flash chromatography (20 × 5.5 cm, 1:1 EtOAc/hexanes) to give nucleoside **1** as a white foam (6.37 g, 96 %) R_f = 0.10 (EtOAc). ^{1}H and ^{13}C NMR data match with previously reported data.[43]

1. DMP, $NaHCO_3$, CH_2Cl_2, 23 °C, 1 h
2. CH_2O, NaOH, THF/H_2O (1:1), 23 °C, 16 h, then $NaBH_4$.

1-((3a'S,6'R,6a'R)-4',4'-bis(hydroxymethyl)tetrahydrospiro[cyclohexane-1,2'-furo[3,4-d]-[1,3]dioxol]-6'-yl)-5-methylpyrimidine-2,4(1H,3H)-dione) (2). Dess-Martin periodinane (1.8 g, 4.3 mmol) and sodium bicarbonate (1.00 g, 11.9 mmol) were added to a stirred solution of **1** (1.12 g, 3.30 mmol) in dichloromethane (15 mL). After stirring at room temperature for 3 h, ethyl acetate (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 EtOAc (4 × 5 mL). The combined organic extracts were concentrated under reduced pressure. The residue was dissolved in THF (15 mL), and stirred with a 37 % aqueous solution of formaldehyde (4.0 mL, 54 mmol), and 1 M NaOH (6 mL, 6 mmol). After 12 h, the reaction mixture was cooled to 0 °C and sodium borohydride (0.50 g, 13 mmol) was added. The cooling bath was removed and the reaction was allowed to warm to room temperature over a 3 h period. The reaction mixture was neutralized via the addition of 1 M HCl, and the resulting solution was extracted with dichloromethane (5 × 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 (15 × 3.5 cm, 9:1 EtOAc/hexanes) to give nucleoside **2** as a white foam (620 mg, 51% over 2 steps) R_f = 0.20 (4:1 EtOAc/hexanes). ^{1}H and ^{13}C NMR data match with previously reported data.[43]

((3aS,4S,12R,12aR)-9-methyl-8-oxo-3a,4,5,8,12,12a-hexahydrospiro[4,12-epoxy[1,3]dioxolo[4,5-e]pyrimido[2,1-b][1,3]oxazocine-2,1'-cyclohexan]-4-yl)methyl trifluoromethanesulfonate (3). Pyridine (1.40 mL, 17.2 mmol) and trifluoromethanesulfonic anhydride (1.70 mL, 10.1 mmol) were added to stirred solution of **2** (1.55 g, 4.21 mmol) in dichloromethane (22 mL) at 0 °C. After 1 h, water (5 mL) was added and the resulting mixture was stirred for 10 min. The layers were separated and the organic layer was washed with 0.5 M HCl (3 × 20 mL) and water (10 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (14 × 5 cm, 2:8 EtOAc/hexanes) to give bistriflate nucleoside as a white foam (2.39 g, 90%); R_f = 0.87 (EtOAc). DBU (0.38 mL, 2.5 mmol) was added to a stirred solution of bistriflate nucleoside (0.53 mg, 0.84 mmol) in dry MeCN (6 mL) at room temperature. After stirring for 10 min; dichloromethane (15 mL) was added and the organic solution was washed with 0.5 M HCl (5 mL) and water (5 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (8 × 3 cm, EtOAc) to give nucleoside **3** as a white foam (340 mg, 83 %) R_f = 0.28 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 1H), 5.42 (s, 1H), 5.03 – 4.93 (m, 2H), 4.81 (d, J = 11.2 Hz, 1H), 4.68 (d, J = 11.1 Hz, 1H), 4.55 (d, J = 12.6 Hz, 1H), 4.20 (d, J = 12.8 Hz, 1H), 2.00 (s, 3H), 1.86 – 1.33 (m, 10H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 137.2, 115.6, 98.3, 88.2, 86.1, 81.9, 74.9, 72.9, 35.8, 34.2, 24.9, 23.9, 23.7, 13.6.

(3aS,4R,12R,12aR)-4-(hydroxymethyl)-9-methyl-4,5,12,12a-tetrahydrospiro[4,12-epoxy-[1,3]dioxolo[4,5-e]pyrimido[2,1-b][1,3]oxazocine-2,1'-cyclohexan]-8(3aH)-one (4). Tetrabutylammonium nitrite (400 mg, 1.38 mmol) was added to a stirred solution of **3** (0.11 g, 0.24 mmol) in THF (5 mL) at room temperature. After 20 h, water (10 mL) and dichloromethane (5 mL) were added, the layers were separated and the resulting solution was extracted with dichloromethane (5 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (14 × 2 cm, 5 % MeOH/EtOAc) to give nucleoside **4** as a white foam (71 mg, 85%) R_f = 0.10 5% MeOH/EtOAc); ^{1}H NMR (300 MHz, 1:1 MeOH-d_4/$CDCl_3$) δ 7.19 (s, 1H), 5.36 (s, 1H), 4.93 (q, J = 5.6 Hz, 2H), 4.66 (d, J = 12.9 Hz, 1H), 4.18 (d, J = 12.9 Hz, 1H), 4.00 (d, J = 12.1 Hz, 1H), 3.85 (d, J = 12.1 Hz, 1H), 3.01 (s, 1H), 1.98 (s, 3H), 1.79 – 1.32 (m, 10H); ^{13}C NMR (75 MHz, 1:1 MeOH-d_4/$CDCl_3$) δ 172.8, 157.1, 139.0, 119.3, 114.0, 97.87, 90.6, 85.5, 81.3, 76.8, 59.9, 35.4, 33.8, 24.5, 23.4, 23.2, 12.4. HRMS (ESI) calc'd for $C_{17}H_{23}N_2O_6$ $[M+H]^+$ m/z = 351.1551, found 351.1556; calc'd for $C_{17}H_{22}N_2NaO_6$ $[M+Na]^+$ m/z = 373.137, found 373.136.

DMP, $NaHCO_3$, CH_2Cl_2

(3aS,4R,12R,12aR)-9-methyl-8-oxo-3a,4,5,8,12,12a-hexahydrospiro[4,12-epoxy[1,3]di-oxolo[4,5-e]pyrimido[2,1-b][1,3]oxazocine-2,1'-cyclohexane]-4-carbaldehyde (5). Dess Martin periodinane (60 mg, 0.14 mmol) and sodium bicarbonate (20 mg, 0.23 mmol) were added to a stirred solution of nucleoside **4** (32 mg, 0.091 mmol) in dichloromethane (1 mL). After 2 h, ethyl acetate (2 mL) and 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 EtOAc (4 × 2 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (8 × 1 cm, 1:1 acetone/dichloromethane) to give nucleoside **5** as a white powder (27 mg, 85% over 2 steps) R_f = 0.17 (1:1 acetone/dichloromethane); ^{1}H NMR (300 MHz, 1:1 MeOH-d_4/$CDCl_3$) δ 5.31 (d, *J* = 4.3 Hz, 1H), 4.78 – 4.64 (m, *J* = 5.7 Hz, 3H), 4.58 – 4.41 (m, *J* = 18.4, 12.8 Hz, 1H), 4.22 (s, 4H), 4.10 – 3.96 (m, 1H), 3.23 (s, 1H), 3.15 (d, *J* = 5.6 Hz, 2H), 3.13 – 3.07 (m, 1H), 1.74 (s, 3H), 1.57 – 0.94 (m, 10H). ^{13}C NMR (75 MHz, 1:1 MeOH-d_4/$CDCl_3$) δ 172.83, 172.80, 157.14, 138.85, 119.46, 119.40, 113.99, 113.86, 98.20, 98.07, 96.30, 96.11, 91.67, 91.52, 85.47, 85.18, 81.95, 81.85, 81.61, 77.16, 74.54, 74.42, 55.31, 54.73, 35.61, 35.56, 33.86, 33.75, 24.60, 23.70, 23.57, 23.39, 23.29, 12.62.

1-((3a'S,4'S,6'R,6a'R)-4'-(((tert-butyldiphenylsilyl)oxy)methyl)-4'-(hydroxymethyl)tetra-hydrospiro[cyclohexane-1,2'-furo[3,4-d][1,3]dioxol]-6'-yl)-5-methylpyrimidine-2,4-(1H,3H)-dione (6): *tert*-Butyldiphenylsilyl chloride (0.24 mL, 0.92 mmol) and imidazole (62 mg, 0.92 mmol) were added to a stirred, 0 °C solution of nucleoside **2** (0.30 g, 0.83 mmol) in DMF (4 mL). After 6 h, 1 M HCl (10 mL) was added to the reaction mixture, the layers were separated and the resulting solution was extracted with Et_2O (3 × 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue

was purified by flash chromatography (14 × 3 cm, 3:7 EtOAc/hexanes) to give nucleoside **6** as a white foam (343 mg, 68%) R_f = 0.34 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.74 – 7.62 (m, 4H), 7.48 – 7.34 (m, 6H), 7.09 (s, 1H), 5.25 – 5.16 (m, 2H), 4.96 (d, *J* = 5.9 Hz, 1H), 4.01 – 3.90 (m, 3H), 3.68 (d, *J* = 10.7 Hz, 1H), 3.37 (dd, *J* = 7.8, 4.0 Hz, 1H), 1.92 (s, 3H), 1.73 – 1.31 (m, 10H), 1.06 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 150.5, 139.5, 135.8, 133.3, 129.9, 127.8, 127.8, 115.0, 111.2, 96.6, 89.2, 82.0, 81.1, 65.8, 63.80, 36.9, 34.5, 26.9, 25.0, 24.0, 23.5, 19.4, 12.5; LRMS (ESI) calc'd for $C_{33}H_{42}N_2O_7NaSi$ $[M+Na]^+$ *m/z* = 629.27, found 629.3.

(3aS,4S,12R,12aR)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-9-methyl-4,5,12,12a-tetrahydrospiro[4,12-epoxy[1,3]dioxolo[4,5-e]pyrimido[2,1-b][1,3]oxazocine-2,1'-cyclohexan]-8-(3aH)-one) (7). Pyridine (0.80 mL, 10 mmol) and trifluoromethanesulfonic anhydride (0.90 mL, 5.3 mmol) were added to a stirred solution of **6** (0.29 g, 0.47 mmol) in dichloromethane (4 mL) at 0 °C. After 40 min, water (2 mL) and dichloromethane (10 mL) were added and stirred for 10 min. The layers were separated and the organic layer was washed with 0.5 M HCl (3 × 5 mL) and water (5 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10 × 3 cm, 2:8 EtOAc/hexanes) to give nucleoside **6a** as a white foam (329 mg, 93%) R_f = 0.53 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 7.77 – 7.63 (m, 4H), 7.51 – 7.35

(m, 6H), 6.97 (s, 1H), 5.32 (d, J = 2.6 Hz, 1H), 5.17 (dd, J = 6.4, 2.6 Hz, 1H), 4.97 (d, J = 9.8 Hz, 1H), 4.90 (d, J = 5.3 Hz, 1H), 4.83 (d, J = 9.8 Hz, 1H), 1.94 (s, 3H), 1.75 – 1.25 (m, 12H), 1.08 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.1, 150.6, 138.7, 135.9, 135.8, 133.1, 132.9, 129.9, 127.9, 127.8, 115.5, 111.8, 94.4, 87.7, 83.7, 81.4, 75.3, 62.5, 35.7, 33.9, 26.9, 25.0, 23.9, 23.5, 19.3, 12.3; LRMS (ESI) calc'd for $C_{34}H_{41}F_3N_2O_9NaSSi$ $[M+Na]^+$ m/z = 761.22, found 761.1. DBU (0.13 mL, 0.87 mmol) was added to a stirred solution of **6a** (0.33 g, 0.44 mmol) in dry MeCN (2 mL) at room temperature. After 10 min, dichloromethane (5 mL) was added and the resulting solution was washed with 0.5 M HCl (2 × 2 mL) and water (2 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10 × 3 cm, EtOAc) to give nucleoside **7** as a white foam (260 mg, 99%) R_f = 0.32 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.59 (m, 4H), 7.49 – 7.35 (m, 6H), 7.16 (s, 1H), 5.29 (s, 1H), 4.93 (dd, J = 14.4, 5.6 Hz, 2H), 4.72 (d, J = 12.7 Hz, 1H), 4.18 (d, J = 12.7 Hz, 1H), 4.07 (d, J = 10.7 Hz, 1H), 3.73 (d, J = 10.7 Hz, 1H), 1.99 (s, 3H), 1.68 – 1.47 (m, 10H), 1.05 (s, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 156.8, 137.5, 135.7, 135.6, 132.6, 132.5, 130.1, 128.0, 127.9, 120.3, 114.5, 98.4, 91.0, 85.9, 81.8, 62.0, 36.0, 34.5, 26.8, 25.0, 23.9, 23.8, 19.3, 13.6; LRMS (ESI) calc'd for $C_{33}H_{40}N_2O_6Si$ $[M+H]^+$ m/z = 589.27, found 589.3.

5.2.2 Second Generation Synthesis

1-((3a'S,4'S,6'R,6a'R)-4'-(hydroxymethyl)-4'-((trityloxy)methyl)tetrahydrospiro[cyclo-hexane-1,2'-furo[3,4-d][1,3]dioxol]-6'-yl)-5-me-thylpyrimidine-2,4(1H,3H)-dione (8). Trityl chloride (4.53 g, 16.2 mmol), 4-dimethylaminopyridine (0.14 g, 1.1 mmol) and triethylamine (3.00 mL, 21.5 mmol) were added to a stirred solution of nucleoside **2** (3.98 g, 10.8 mmol) in anhydrous DMF (55 mL). After 12 h, EtOAc (50 mL) and 1 M HCl (50 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (3 × 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 (20 × 4 cm, 4:6 EtOAc/hexanes) to give nucleoside **8** as a white foam (4.69 g, 71%) R_f = 0.20 (2:3 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.55 – 7.43 (m, 6H), 7.34 – 7.27 (m, J = 8.4, 6.4 Hz, 6H), 7.25 – 7.15 (m, J = 7.2 Hz, 3H), 5.38 (d, J = 4.4 Hz, 1H), 5.20 – 5.13 (m, J = 6.3, 4.3 Hz, 1H), 5.01 (d, J = 6.3 Hz, 1H), 3.93 – 3.82 (m, 2H), 3.47 – 3.35 (m, 2H), 3.20 (d, J = 9.9 Hz, 1H), 1.90 (s, 3H), 1.69 – 1.29 (m, 10H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 150.7, 143.8, 139.2, 128.8, 127.9, 127.2, 115.0, 111.2, 95.6, 88.9, 87.1, 82.4, 81.1, 65.6, 63.3, 36.6, 34.4, 25.0, 23.9, 23.6, 12.4; $[\alpha]_D^{25}$ = -2 (10 mg/mL, CH_2Cl_2).

1-((3a'S,4'R,6'R,6a'R)-4'-(((tert-butyldiphenylsilyl)oxy)methyl)-4'-((trityloxy)methyl)-tetrahydrospiro[cyclohexane-1,2'-furo[3,4-d][1,3]dioxol]-6'-yl)-5-ethylpyrimidine-2,4-(1H,3H)-dione (9). *tert*-Butyldiphenylsilyl chloride (6.2 mL, 24 mmol) and imidazole (1.51 g, 22.2 mmol) were added to a stirred solution of nucleoside **8** (10.4 g, 17.1 mmol) in anhydrous dichloromethane (150 mL). After 24 h, 1 M HCl (50 mL) was added to the reaction, the layers were separated and the resulting solution was extracted with dichloromethane (3 × 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 (15 × 4 cm, 1:4 EtOAc/hexanes) to give nucleoside **9** as a white foam (12.5 g, 90%), R_f = 0.60 (2:3 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.72 – 7.63 (m, 4H), 7.54 – 7.24 (m, 21H), 6.02 (s, 1H), 4.85 (s, 2H), 4.12 (d, J = 11.1 Hz, 1H), 3.78 (d, J = 11.0 Hz, 1H), 3.53 (d, J = 9.7 Hz, 1H), 3.15 (d, J = 9.7 Hz, 1H), 1.78 – 1.33 (m, 13H), 1.14 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.8, 150.3, 143.8, 135.9, 135.7, 135.5, 132.9, 132.4, 130.2, 130.2, 128.7, 128.1, 128.1, 127.9, 127.1, 114.9, 111.3, 89.8, 88.1, 86.9, 83.9, 81.5, 67.2, 62.4, 36.9, 34.9, 27.2, 25.0, 23.9, 23.7, 19.5, 12.1; HRMS (ESI) calc'd for $C_{52}H_{56}N_2O_7Si$ $[M^+H]^+$ m/z = 871.3749, found 871.37506.

1-((3a'S,4'R,6'R,6a'R)-4'-(((tert-butyldiphenylsilyl)oxy)methyl)-4'-(hydroxymethyl)tetra-hydrospiro[cyclohexane-1,2'-furo[3,4-d][1,3]dioxol]-6'-yl)-5-methylpyrimidine-2,4-(1H,3H)-dione (10). Trifluoroacetic acid (0.83 mL, 11 mmol) was added in three portions, with 1 minute stirring between additions them to a stirred solution of nucleoside **9** (0.91 mg, 1.1 mmol) in dichloromethane (7.5 mL). After 10 min, the reaction mixture was neutralized via addition of a saturated sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with dichloromethane (4 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (16 × 3 cm, 3:7 EtOAc:hexanes) to give nucleoside **10** as a white foam (625 mg, 95% based on recovered starting material). R_f = 0.35 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 7.68 – 7.60 (m, 4H), 7.46 – 7.32 (m, 6H), 7.28 (s, 1H), 6.02 (s, 1H), 4.87 (s, 2H), 4.01 (d, *J* = 11.2 Hz, 1H), 3.95 (d, *J* = 11.1 Hz, 1H), 3.82 (d, *J* = 12.1 Hz, 1H), 3.73 (d, *J* = 12.0 Hz, 1H), 2.35 (s, 1H), 1.89 – 1.81 (m, 2H), 1.71 – 1.51 (m, 9H), 1.45 – 1.35 (m, 2H), 1.10 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 150.4, 136.2, 135.6, 135.3, 132.9, 132.4, 130.1, 130.0, 127.9, 127.9, 115.7, 111.4, 90.4, 88.3, 84.1, 81.4, 66.2, 62.8, 36.4, 34.3, 27.1, 24.9, 24.1, 23.6, 19.4, 11.9; HRMS (ESI) calc'd for $C_{33}H_{43}N_2O_7Si$ $[M+H]^+$ m/z = 607.2834, found 607.28448.

OTBDPS HO Me N O NH O O O 10 — 1. DMP, CH_2Cl_2 2. $AlMe_3$, toluene → OTBDPS Me (S) HO Me N O NH O O O 11

1-((3a'S,4'S,6'R,6a'R)-4'-(((tert-butyldiphenylsilyl)oxy)methyl)-4'-((S)-1-hydroxyethyl)-tetrahydrospiro[cyclohexane-1,2'-furo[3,4-d][1,3]dioxol]-6'-yl)-5-methylpyrimidine-2,4-(1H,3H)-dione (11). Dess-Martin periodinane (0.68 g, 1.6 mmol) and sodium bicarbonate (0.5 g, 6 mmol) were added to a stirred solution of nucleoside **10** (0.75 g, 1.2 mmol) in anhydrous dichloromethane (7.0 mL). After 1 h, dichloromethane (7 mL) and a 10 % (w/v) aqueous 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 dichloromethane (4 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in dry toluene (6.0 mL), cooled to 0 °C and mixed with 2 M $AlMe_3$ in hexanes (1.9 mL, 3.8 mmol). After 1 h, the reaction mixture was diluted with 1 M HCl, then the layers were separated and the aqueous layer was extracted with dichloromethane (4 × 5 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 × 3 cm, 3:7 EtOAc/hexanes) to give nucleoside **11** as a white foam (521 mg, 68%) R_f = 0.20 (2 % MeOH in dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.69 – 7.58 (m, J = 15.4, 6.5, 1.6 Hz, 4H), 7.49 – 7.35 (m, 7H), 6.14 (d, J = 5.3 Hz, 1H), 4.95 (d, J = 6.3 Hz, 1H), 4.79 (dd, J = 6.3, 5.4 Hz, 1H), 4.25 – 4.16 (m, 1H), 4.13 (d, J = 11.2 Hz, 1H), 3.96 (d, J = 11.2 Hz, 1H), 2.72 (s, 1H), 1.92 – 1.23 (m, 16H), 1.13 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 150.4, 135.6, 135.4, 135.3, 133.0, 132.2, 130.4, 130.3, 128.3, 128.2, 115.9, 111.7, 89.3, 89.1, 84.1, 81.5, 68.1, 64.4, 37.0, 34.8, 27.3, 25.0, 24.2, 23.8, 19.6, 16.4, 11.93; HRMS (ESI) calc'd for $C_{34}H_{45}N_2O_7Si$ $[M+H]^+$ *m/z* = 621.29905, found 621.30039; calc'd for $C_{34}H_{44}N_2NaO_7Si$ $[M+Na]^+$ *m/z* = 643.281, found 643.28199. $[\alpha]_D^{25}$ = -1 (10 mg/mL, CH_2Cl_2)

1-((3a'S,4'S,6'R,6a'R)-4'-(((tert-butyldiphenylsilyl)oxy)methyl)-4'-((R)-1-hydroxyethyl)-tetrahydrospiro[cyclohexane-1,2'-furo[3,4-d][1,3]dioxol]-6'-yl)-5-methylpyrimidine-2,4-(1H,3H)-dione (12). Dess-Martin periodinane (0.46 g, 1.1 mmol) and sodium bicarbonate (0.45 g 5.3 mmol) were added to a stirred solution of nucleoside **11** (0.52 g, 0.84 mmol) in anhydrous dichloromethane (4.5 mL). After 1 h, dichloromethane (5 mL) and a 10 % (w/v) aqueous 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 dichloromethane (4 × 4 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in dry THF (4.5 mL) and stirred with lithium aluminum hydride (108 mg, 2.81 mmol) at -78 °C. After 1 h, the reaction mixture was diluted with ethyl acetate (5 mL) and HCl 1 M (5 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 4 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 × 3 cm, 4:6 EtOAc/hexanes) to give nucleoside **12** as a white foam (520 mg, quantitative), R_f = 0.42 (2 % MeOH in dichlorometane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.67 – 7.59 (m, J = 8.0, 7.3, 1.5 Hz, 4H), 7.48 – 7.33 (m, 7H), 6.12 (d, J = 5.5 Hz, 1H), 4.83 (t, J = 5.7 Hz, 1H), 4.73 (d, J = 6.0 Hz, 1H), 4.15 (d, J = 6.5 Hz, 1H), 3.99 (d, J = 11.3 Hz, 1H), 3.91 (d, J = 11.3 Hz, 1H), 1.89 – 1.30 (m, J = 116.3, 3.7 Hz, 14H), 1.17 (d, J = 6.5 Hz, 3H), 1.12 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 150.5, 135.8, 135.6, 135.4, 132.8,

132.1, 130.4, 130.3, 128.2, 128.1, 115.4, 111.5, 89.8, 89.8, 83.7, 81.7, 69.1, 65.5, 37.1, 34.6, 27.3, 25.0, 24.1, 23.7, 19.5, 17.6, 11.9; HRMS (ESI) calc'd for $C_{34}H_{45}N_2O_7Si$ $[M+H]^+$ *m/z* = 621.29905, found 621.29981; calc'd for $C_{34}H_{44}N_2NaO_7Si$ $[M+Na]^+$ *m/z* = 643.281, found 643.28169; $[\alpha]_D^{25}$ = -8° (10 mg/mL, CH_2Cl_2)

(R)-1-((2R,3S,4R,5R)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)ethyl methanesulfonate (13). Methanesulfonyl chloride (50 µL, 0.18 mmol) and 4-dimethylaminopyridine (10 mg, 0.082 mmol) were added to a stirred solution of nucleoside **12** (0.25 g, 0.084 mmol) in anhydrous pyridine (2 mL) at 0 °C. The reaction was stirred at room temperature for 12 h, and then diluted with 1 M HCl (3 mL) and EtOAc (3 mL). The layers were separated and the aqueous layer was extracted with EtOAc (4 × 4 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (3:7 EtOAc/hexanes) to give nucleoside **12a** (240 mg, 85%), R_f = 0.60 (15 × 2.5 cm, 5 % MeOH in dichlorometane); ^1H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.67 – 7.57 (m, 4H), 7.50 – 7.36 (m, 7H), 6.13 (d, *J* = 5.6 Hz, 1H), 5.00 (q, *J* = 6.5 Hz, 1H), 4.87 – 4.82 (m, 1H), 4.58 (d, *J* = 5.5 Hz, 1H), 3.99 (s, 2H), 2.98 (s, 3H), 1.87 – 1.33 (m, 17H), 1.12 (s, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 150.5, 135.7, 135.4, 135.3, 130.7, 130.5, 128.4, 128.3, 115.4, 111.8, 89.5, 88.9, 83.4, 81.0, 80.8, 64.3, 38.4, 37.2, 34.8, 27.3, 24.9, 24.1, 23.7, 19.5, 18.2, 12.1;

HRMS (ESI) calc'd for $C_{35}H_{47}N_2O_9SSi$ $[M+H]^+$ *m/z* = 699.2766, found 699.27731; calc'd for $C_{35}H_{46}N_2NaO_9SSi$ $[M+Na]^+$ *m/z* = 721.25855, found 721.25913; $[\alpha]_D^{25}$ = -16 (10 mg/mL, CH_2Cl_2). A 37 % aqueous solution of HCl (1.50 mL, 17.5 mmol) was added to a stirred solution of nucleoside **12a** (0.15 g, 0.22 mmol) in THF (1 mL) and MeOH (2 mL). After 12 h, the reaction was neutralized via addition of saturated sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with dichloromethane (5 × 4 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (2:1 EtOAc/hexanes) to give nucleoside **13** as a white foam (86 mg, 63%), R_f = 0.28 (15 × 1.5 cm, 4:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.69 – 7.57 (m, 4H), 7.49 – 7.32 (m, 7H), 6.12 (d, J = 7.5 Hz, 1H), 5.37 – 5.14 (m, J = 6.4 Hz, 2H), 4.58 (dd, J = 7.6, 5.4 Hz, 1H), 4.43 (d, J = 5.3 Hz, 1H), 3.98 – 3.87 (m, J = 7.4 Hz, 2H), 3.62 (s, 1H), 3.01 (s, 3H), 1.40 (d, J = 6.5 Hz, 3H), 1.35 (s, 3H), 1.10 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8, 152.1, 136.0, 135.6, 135.3, 132.8, 131.86, 130.6, 130.3, 128.3, 128.2, 111.4, 89.6, 81.5, 74.7, 72.6, 64.1, 38.3, 29.8, 27.3, 19.6, 18.3, 11.9; HRMS (ESI) calc'd for $C_{29}H_{39}N_2O_9SSi$ $[M+H]^+$ *m/z* = 619.214, found 619.21509; calc'd for $C_{29}H_{38}N_2NaO_9SSi$ $[M+Na]^+$ *m/z* = 641.19595, found 641.19717.

OTBDPS Me Me MsO HO OH O N O NH O 13 — K_2CO_3, MeOH, 80 °C → OTBDPS Me Me O O H OH N O NH O 14

1-((1S,3R,4R,5S,7S)-1-(((tert-butyldiphenylsilyl)oxy)methyl)-4-hydroxy-7-methyl-2,6-dioxabicyclo[3.2.0]heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (14). Potassium

carbonate (3 mg, 0.02 mmol) was added to a stirred solution of **13** (9.2 mg, 0.014 mmol) in methanol (2 mL). After stirring at 70 °C for 24 h, the solution was concentrated under reduced pressure and the solid residue was dissolved in MeOH:CH_2Cl_2 (5 %). The solution was passed through a silica gel pad and eluted with MeOH:CH_2Cl_2 (5%). The obtained solution was concentrated under reduced pressure. The residue did not required further purification and provided nucleoside **8** as a white foam (7 mg, 90%), R_f = 0.21 (3:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.73 – 7.57 (m, 4H), 7.52 – 7.34 (m, 6H), 7.19 (s, 1H), 6.35 (d, J = 6.7 Hz, 1H), 4.96 (d, J = 4.8 Hz, 1H), 4.77 (q, J = 6.6 Hz, 1H), 4.14 – 4.03 (m, 1H), 3.96 (q, J = 11.6 Hz, 2H), 3.03 (d, J = 10.1 Hz, 1H), 1.66 (s, 3H), 1.36 (d, J = 6.7 Hz, 3H), 1.08 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.3, 150.8, 135.6, 135.4, 132.9, 132.3, 130.4, 130.3, 128.2, 128.1, 112.1, 89.0, 88.0, 86.4, 81.7, 76.1, 61.5, 27.0, 19.5, 18.1, 12.2; HRMS (ESI) calc'd for $C_{28}H_{35}N_2O_6Si$ $[M+H]^+$ *m/z* = 523.22589, found 523.22523; calc'd for $C_{28}H_{34}N_2NaO_6Si$ $[M+Na]^+$ *m/z* = 545.20783, found 545.2074.

(1R)-1-((3aS,4R,6R,6aR)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-phenyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)ethyl methanesulfonate (15). Camphorsulfonic acid (2 mg, 0.008 mmol) and benzaldehyde dimethyl acetal (0.1 mL, 0.6 mmol) were added to a stirred solution of **13** (38 mg, 0.062 mmol) in 1,2-dichloroethane (0.3 mL). After refluxing for 1 h, the mixture was cooled to room temperature and diluted with dichloromethane (1 mL). The crude mixture was pre-adsorbed on silica gel

and purified by flash chromatography (14 × 1 cm, 3:7 EtOAc/hexanes) to give an 1:1 mixture of diastereomers **15,** based on the integration of the signals at 5.97 and 6,19 ppm, as a white foam (36 mg, 82%), R_f = 0.46 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.29 (m, 15H), 7.06 (d, *J* = 6.7 Hz, 1H), 6.19 (d, *J* = 4.9 Hz, 1H), 5.97 (d, *J* = 6.5 Hz, 1H), 5.10 – 4.92 (m, 2H), 4.82 (d, *J* = 6.1 Hz, 1H), 4.73 (d, *J* = 6.0 Hz, 1H), 4.07 – 3.92 (m, 2H), 3.55 (d, *J* = 4.9 Hz, 3H), 1.62 (d, 3H), 1.36 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 7.2 Hz, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1, 137.9, 135.7, 135.7, 135.5, 135.4, 135.2, 134.9, 134.2, 132.3, 132.1, 131.7, 130.7, 130.6, 130.3, 130.2, 128.9, 128.8, 128.4, 128.3, 128.3, 128.3, 128.1, 127. 9, 127.8, 127.0, 127.0, 125.9, 125.7, 108.2, 107.9, 89.8, 84.8, 83.5, 83.5, 80.9, 80.7, 64.3, 64.2, 57.7, 38.4, 38.1, 27.3, 27.3, 19.5, 19.4, 18.2, 18.1, 13.0, 13.0; HRMS (ESI) calc'd for $C_{36}H_{43}N_2O_9SSi$ $[M+H]^+$ m/z = 707.2453, found 707.24234.

1-((1S,3R,4R,6S,7S)-7-(benzyloxy)-1-(((tert-butyldiphenylsilyl)oxy)methyl)-6-methyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (16). Titanium chloride (28 μL, 0.25 mmol) was added dropwise to a stirred solution of sodium cyanoborohydride (16 mg, 0.25 mmol) and nucleoside **15** (36 mg, 0.051 mmol) in dry acetonitrile (0.5 mL) at 0 °C. After 1 h, the reaction mixture was diluted with saturated sodium bicarbonate (1 mL) and dichloromethane (1 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (4 × 1 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude solid was dissolved

in dichloromethane and passed through a silica gel pad and eluted with 5% MeOH:CH_2Cl_2. The obtained solution was concentrated under reduced pressure. The crude solid was dissolved in MeOH (1 mL) and mixed with K_2CO_3 (6 mg, 0.04 mmol). After refluxing for 2 h, the solution was concentrated under reduced pressure and the solid residue was dissolved in 5% MeOH:CH_2Cl_2. The solution was passed through a silica gel pad and eluted with 5% MeOH:CH_2Cl_2. The obtained solution was concentrated under reduced pressure. The residue did not required further purification and provide nucleoside **16** as a white foam (23 mg, 73% over 2 steps), R_f = 0.48 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.74 – 7.65 (m, 4H), 7.49 – 7.26 (m, 13H), 5.64 (s, 1H), 4.67 (d, J = 11.3 Hz, 1H), 4.64 (s, 1H), 4.49 (d, J = 11.3 Hz, 1H), 4.15 – 3.95 (m, 4H), 1.68 (s, 3H), 1.25 (d, J = 6.6 Hz, 4H), 1.10 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7, 149.7, 136.9, 135.7, 135.5, 134.6, 132.9, 132.4, 130.2, 128.6, 128.2, 128.1, 128.0, 127.9, 110.4, 89.6, 87.4, 81.2, 72.5, 59.3, 27.0, 26.7, 19.6, 16.5, 12.4.

5.2.3 Third Generation Synthesis

1-((3aR,4R,6R,6aR)-6-(hydroxymethyl)-2-(naphthalen-2-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (17). Naphthaldehyde dimethyl acetal (250

mg, 1.23 mmol) and camphorsulphonic acid (12 mg, 0.05 mmol) were added to a stirred solution of 5-methyluridine (120 mg, 0.465 mmol) in anhydrous 1,2-dichloroethane (2 mL). The reaction was heated to reflux for 2 h, cooled to room temperature, and mixed with solid sodium bicarbonate (50 mg). The filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography (15 × 1.5 cm, 7:3 EtOAc:hexanes) to give **17** as a mixture of diastereomers (156 mg, 85%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 (s, 2H), 8.00 – 7.78 (m, 7H), 7.66 – 7.46 (m, 5H), 7.16 – 7.07 (m, 2H), 6.27 (s, 1H), 6.17 (s, 1H), 5.69 – 5.62 (m, 2H), 5.33 – 5.27 (m, 1H), 5.26 – 5.13 (m, 3H), 4.50 (q, J = 3.2 Hz, 1H), 4.37 (q, J = 3.5 Hz, 1H), 4.07 – 3.82 (m, 3H), 3.25 (s, 2H), 1.97 – 1.85 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.9, 163.9, 150.7, 150.7, 139.5, 139.0, 134.2, 134.1, 133.3, 133.2, 133.0, 132.9, 128.7, 128.7, 128.5, 128.5, 127.9, 127.9, 127.0, 126.9, 126.7, 126.6, 126.6, 126.6, 123.6, 111.6, 111.5, 108.1, 104.5, 96.5, 95.7, 86.9, 85.3, 84.2, 83.6, 81.7, 80.2, 62.9, 62.8, 51.0, 12.5, 12.4; HRMS (ESI) calc'd for $C_{21}H_{21}N_2O_6$ $[M+H]^+$ m/z = 397.13941, found 397.13953; calc'd for $C_{21}H_{20}N_2NaO_6$ $[M+Na]^+$ m/z = 419.12136, found 419.1215.

1-((3aR,4R,6aS)-6,6-bis(hydroxymethyl)-2-(naphthalen-2-yl)tetrahydrofuro[3,4-d][1,3]-dioxol-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (18). Dess-Martin periodinane (0.18 g, 0.43 mmol) and sodium bicarbonate (80 mg, 0.95 mmol) were added to a stirred solution of **17**

(0.13 g, 0.32 mmol) in dichloromethane (2 mL). After stirring at room temperature for 2 h, ethyl acetate (2 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 EtOAc (4 × 2 mL). The combined organic extracts were concentrated under reduced pressure. The residue was dissolved in THF (2 mL), and stirred with a 37 % aqueous solution of formaldehyde (0.28 mL, 3.8 mmol), and 1 M NaOH (0.5 mL, 0.5 mmol). After 6 h, the reaction mixture was cooled to 0°C and sodium borohydride (41 mg, 1.1 mmol) was added. The cooling bath was removed and stirring was continued for 10 h at room temperature. The reaction mixture was neutralized via the addition of 1 M HCl, and the resulting solution was extracted with dichloromethane (5 × 4 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 × 1.5 cm, 9:1 EtOAc/hexanes) to give nucleoside **18** as a white foam (21 mg, 15 % over 2 steps) R_f = 0.20 (4:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.06 – 7.77 (m, 4H), 7.70 – 7.42 (m, 3H), 7.19 (s, 1H), 6.16 (s, 1H), 5.72 (d, *J* = 3.7 Hz, 1H), 5.47 – 5.37 (m, 1H), 5.21 (d, *J* = 7.0 Hz, 1H), 3.97 – 3.73 (m, 4H), 1.90 (s, 3H). HRMS (ESI) calc'd for $C_{22}H_{23}N_2O_7$ $[M+H]^+$ *m/z* = 427.14998, found 427.15169; calc'd for $C_{22}H_{22}N_2NaO_7$ $[M+Na]^+$ *m/z* = 449.13192, found 449.13346.

OH O NH N O O O O Me 17 TiCl4, NaBH3CN, MeCN OH O NH N O NapO OH Me 19

1-((2R,3R,4S,5R)-3-hydroxy-5-(hydroxymethyl)-4-(naphthalen-2-ylmethoxy)tetrahydro-furan-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (19). Titanium chloride (2.55 mL, 23.2 mmol) was added dropwise to a stirred solution of sodium cyanoborohydride (1.46 g, 23.2 mmol) and nucleoside **17** (1.84 g, 4.63 mmol) in dry acetonitrile (100 mL) at 0 °C. After 2 h, the reaction was diluted with saturated sodium bicarbonate (30 mL) and dichloromethane (30 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (4 × 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 (15 × 3 cm, 4:1 EtOAc/hexanes) to give nucleoside **19** as a white foam (1.74 g, 94%), R_f = 0.37 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.87 – 7.71 (m, 4H), 7.51 – 7.38 (m, 3H), 7.28 (s, 1H), 5.65 (d, J = 5.1 Hz, 1H), 4.87 – 4.72 (m, 2H), 4.55 – 4.45 (m, 1H), 4.28 – 4.20 (m, 1H), 4.20 – 4.13 (m, 1H), 3.92 – 3.81 (m, 1H), 3.70 – 3.58 (m, 1H), 3.41 (s, 1H), 1.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.2, 151.0, 138.0, 134.6, 133.3, 133.2, 128.6, 128.0, 127.8, 127.1, 126.5, 126.3, 125.8, 111.0, 93.0, 83.4, 77.4, 73.1, 72.9, 62.2, 12.4; HRMS (ESI) calc'd for $C_{21}H_{23}N_2O_6$ $[M+H]^+$ *m/z* = 399.15506, found 399.15546; calc'd for $C_{21}H_{22}N_2NaO_6$ $[M+Na]^+$ *m/z* = 421.13701, found 421.13759; $[\alpha]_D^{25}$: -20 (10 mg/mL, CH_2Cl_2).

OH
NapO
OH
Me
19
1. TBSCl, DMAP
pyridine
2. TFA, AcOH
OH
NapO
OTBS
Me
20

1-((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-4-(naphthalen-2-ylmethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (20). Imidazole (720 mg, 10.6 mmol) and TBSCl (1.58 g, 10.5 mmol) were added to a solution of nucleoside **19**

in anhydrous DMF (12 mL). After stirring for 15 hours, EtOH (10 mL) and 1 M HCl (10 mL) were added. The layers were separated and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain a yellow oil. TFA (0.86 mL, 11 mmol) and 80 % (w/v) AcOH (20 mL) were added to a stirred solution of the crude oil in THF (2 mL). After stirring at room temperature for 6 h, the reaction was neutralized via addition of a saturated solution of sodium bicarbonate. The layers were separated and the aqueous layer was extracted with dichloromethane (4 × 5 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 × 3 cm, 1:1 EtOAc/hexanes) to give nucleoside **20** (1.16 g, 52% over two steps), R_f = 0.3 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.90 – 7.72 (m, J = 16.4, 10.4 Hz, 4H), 7.55 – 7.28 (m, 4H), 5.53 (d, J = 5.6 Hz, 1H), 4.94 (d, J = 11.9 Hz, 1H), 4.82 – 4.61 (m, 2H), 4.33 – 4.21 (m, 1H), 4.10 – 3.99 (m, J = 11.9, 7.5 Hz, 1H), 3.97 – 3.86 (m, 1H), 3.74 – 3.60 (m, J = 20.0, 9.4 Hz, 1H), 3.22 (s, 1H), 1.89 (s, 3H), 0.92 (s, 9H), 0.11 (d, J = 17.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 150.5, 139.3, 135.4, 133.4, 133.3, 128.6, 128.1, 128.0, 126.7, 126.5, 126.3, 125.8, 111.0, 95.0, 84.5, 73.2, 73.1, 62.6, 26.0, 25.9, 18.3, 12.5, -4.5, -4.8; HRMS (ESI) calc'd for $C_{27}H_{37}N_2O_6Si$ $[M+H]^+$ *m/z* = 513.24154, found 513.24233; calc'd for $C_{27}H_{36}N_2NaO_6Si$ $[M+Na]^+$ *m/z* = 535.22348, found 535.2241.

20 → 21: 1. DMP, CH_2Cl_2; 2. NaOH, CH_2O, then $NaBH_4$

1-((2R,3R,4S)-3-((tert-butyldimethylsilyl)oxy)-5,5-bis(hydroxymethyl)-4-(naphthalen-2-ylmethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (21). Dess-Martin Periodinane (1.2 g, 2.9 mmol) and sodium bicarbonate (1.02 g, 11.9 mmol) were added to a stirred solution of nucleoside **20** (1.16 g, 2.26 mmol) in anhydrous dichloromethane (12 mL) at room temperature, stirred for 1.5 h. EtOAc (10 mL), treated with a 10 % (w/v) aqueous solution of $Na_2S_2O_3$ and stirred until a clear organic phase was obtained. The layers were separated and the aqueous layer was extracted with EtOAc (4 × 8 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in THF (18 mL), and stirred with a 37 % aqueous solution of formaldehyde (2.4 mL, 32 mmol), and 1 M NaOH (4.5 mL, 4.5 mmol). After 1 h, the reaction mixture was cooled to 0°C and sodium borohydride (440 mg, 11.6 mmol) was added. The cooling bath was removed and stirring was continued for 3 h at room temperature. The reaction mixture was neutralized via the addition of 1 M HCl, and the resulting solution was extracted with dichloromethane (5 × 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 **21** (594 mg, 48% over 2 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.90 – 7.79 (m, 3H), 7.76 (s, 1H), 7.53 – 7.41 (m, 3H), 7.19 (s, 1H), 5.53 (d, *J* = 5.4 Hz, 1H), 5.07 (d, *J* = 11.5 Hz, 1H), 4.98 – 4.89 (m, 1H), 4.67 (d, *J* = 11.5 Hz, 1H), 4.40 (d, *J* = 5.7 Hz, 1H), 3.78 (d, *J* = 12.0 Hz, 1H), 3.73 – 3.54 (m, 3H), 3.29 (s, 1H), 2.76 (s, 1H), 1.91 (s, 3H), 0.91 (s, 9H), 0.14 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.8, 150.6, 139.8, 134.9, 133.3, 133.2, 128.7, 128.0, 127.9, 126.8, 126.5, 126.4, 125. 6, 111.2, 96.4, 88.9, 79.1, 74.5, 73.7, 64.7, 64.0, 25.8, 18.05, 12.4, -4.6, -4.8; HRMS (ESI) calc'd for $C_{28}H_{38}N_2NaO_7Si_2$ $[M+Na]^+$ *m/z* = 565.23405, found 565.23545.

1-((2R,3R,4S,5S)-3-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-4-(naphthalen-2-ylmethoxy)-5-((trityloxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (22). Trityl chloride (594 mg, 1.65 mmol), DMAP (14 mg, 0.12 mmol) and triethylamine (0.31 mL, 2.2 mmol) were added to a stirred solution of nucleoside **21** (0.59 g, 1.1 mmol) in anhydrous DMF (5.5 mL). After stirring at room temperature for 12 h, EtOAc (5 mL) and 1 M HCl (5 mL) were added. The layers were separated and the aqueous layer was extracted with Et_2O (5 × 5 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 × 2 cm, 4:6 EtOAc/hexanes) to give nucleoside **22** as a white foam (464 mg, 70% based on recovered starting material (133 mg)); R_f = 0.72 (6:4 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.91 – 7.22 (m, 23H), 5.56 (d, *J* = 6.1 Hz, 1H), 4.98 (d, *J* = 11.5 Hz, 1H), 4.95 – 4.89 (m, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.22 (d, *J* = 5.1 Hz, 1H), 4.14 (dd, *J* = 12.2, 3.5 Hz, 1H), 3.79 – 3.70 (m, 1H), 3.67 (d, *J* = 10.4 Hz, 1H), 3.27 (d, *J* = 10.3 Hz, 1H), 3.17 (dd, *J* = 8.7, 3.5 Hz, 1H), 1.99 (s, 3H), 0.85 (s, 9H), 0.11 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9, 149.9, 143.3, 138.7, 134.8, 132.7, 132.5, 128.3, 127.6, 127.5, 127.4, 127.3, 126.6, 125.9, 125.7, 125.6, 125.2, 110.6, 93.7, 88.0, 86.4, 78.5, 77.0, 73.3, 73.2, 65.0, 64.0, 25.2, 17.5, 12.0, 5.2, 5.4; HRMS (ESI) calc'd for $C_{47}H_{52}N_2NaO_7Si$ $[M+Na]^+$ *m/z* = 807.3436, found 807.34422.

1-((2R,3R,4S,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(naphthalen-2-ylmethoxy)-5-((trityloxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (23). *tert*-Butyldiphenylsilyl chloride (0.2 mL, 0.8 mmol) and imidazole (53 mg, 0.77 mmol) were added to a stirred solution of nucleoside **22** (464 mg, 0.591 mmol) in anhydrous dichloromethane (5 mL). After stirring at room temperature for 18 h, 1 M HCl (5 mL) was added to the reaction mixture, the layers were separated and the resulting solution was extracted with dichloromethane (3 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (16 × 2.5 cm, 1:4 EtOAc/hexanes) to give nucleoside **23** as a white foam (485 g, 80%), R_f = 0.44 (1:4 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 7.97 – 7.17 (m, 32H), 6.31 (d, J = 6.6 Hz, 1H), 5.06 (d, J = 11.7 Hz, 1H), 4.67 (d, J = 11.7 Hz, 1H), 4.62 – 4.55 (m, 1H), 4.25 (d, J = 5.1 Hz, 1H), 4.13 (d, J = 11.2 Hz, 1H), 3.92 (d, J = 11.3 Hz, 1H), 3.68 (d, J = 10.3 Hz, 1H), 3.25 (d, J = 10.3 Hz, 1H), 1.73 (s, 3H), 1.15 (s, 9H), 0.94 (s, 9H), 0.18 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 150.7, 143.8, 135.7, 135.5, 135.4, 135.3, 133.2, 133.0, 132.9, 132.2, 130.2, 130.1, 128.7, 128.2, 128.0, 128.04, 127.8, 127.7, 126.9, 126.4, 126.1, 125.9, 125.7, 114.3, 111.4, 87.5, 87.0, 86.7, 78.4, 75.8, 74.0, 66.8, 64.1, 27.2, 25.6, 19.5, 17.9, 12.0, -4.6, -4.8; HRMS (ESI) calc'd for $C_{63}H_{70}N_2NaO_7Si_2$ $[M+Na]^+$ *m/z* = 1045.46138, found 1045.46023; $[\alpha]_D^{25}$=+15 (10 mg/mL, CH_2Cl_2).

1-((2R,3R,4S,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-5-(hydroxymethyl)-4-(naphthalen-2-ylmethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (24). Trifluoroacetic acid (0.35 mL, 4.6 mmol) was added in three portions, with 1 minute difference between them to a stirred solution of nucleoside **23** (0.46 g, 0.45 mmol) in dry dichloromethane (4.0 mL) at -45 °C. After 10 min, the reaction was neutralized via addition of a saturated sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted dichloromethane (4 × 5 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 × 2.5 cm, 3:7 EtOAc/hexanes) to give nucleoside **24** as a white foam (343 mg, 97%), R_f = 0.17 (1:4 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.91 – 7.21 (m, 18H), 6.18 (d, J = 6.3 Hz, 1H), 5.13 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 11.7 Hz, 1H), 4.59 – 4.51 (m, 1H), 4.30 (d, J = 5.5 Hz, 1H), 3.85 (d, J = 11.3 Hz, 1H), 3.76 (d, J = 11.3 Hz, 1H), 3.71 (s, 2H), 2.49 (s, 1H), 1.61 (s, 3H), 1.10 (s, 9H), 0.94 (s, 9H), 0.17 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.8, 150.6, 135.9, 135.5, 135.3, 134.8, 133.3, 133.2, 132.8, 132.0, 130.2, 130.2, 128.8, 128.1, 128.1, 128.1, 127.9, 126.9, 126.5, 126.3, 125.7, 111.5, 88.4, 87.5, 78.8, 75.8, 74.4, 66.5, 63.7, 27.2, 25.7, 19.5, 18.0, 12.0, -4.5, -4.7; HRMS (ESI) calc'd for $C_{44}H_{56}N_2NaO_7Si_2$ $[M+Na]^+$ m/z = 803.35183, found 803.3521; $[\alpha]_D^{25}$: +38 (10 mg/mL, CH_2Cl_2).

1-((2R,3R,4S,5S)-3-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-5-((S)-1-hydroxyethyl)-4-(naphthalen-2-ylmethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (25). Dess-Martin periodinane (0.34 g, 0.80 mmol) and sodium bicarbonate (0.20 g, 2.4 mmol) were added to a stirred solution of nucleoside **24** (0.34 g, 0.44 mmol) in anhydrous dichloromethane (5 mL). After stirring at room temperature for 1 h, dichloromethane (5 mL) and a 10 % (w/v) aqueous 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 dichloromethane (4 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in dry toluene (3.0 mL) at 0 °C and stirred with 2 M $AlMe_3$ in hexanes (0.61 mL, 1.2 mmol). After 1 h, the reaction mixture was diluted with 1 M HCl (5 mL), the layers were separated and the aqueous layer was extracted with dichloromethane (4 × 5 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 × 2.5 cm, 4 % MeOH in dichloromethane) to give nucleoside **25** as a white foam (189 mg, 60% over two steps), R_f = 0.54 (2:3 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.92 – 7.24 (m, 18H), 6.19 (d, J = 7.2 Hz, 1H), 5.23 (d, J = 11.4 Hz, 1H), 4.59 (d, J = 11.4 Hz, 1H), 4.56 – 4.50 (m, 1H), 4.32 (d, J = 5.2 Hz, 1H), 4.26 – 4.17 (m, 1H), 4.01 (d, J = 11.3 Hz, 1H), 3.89 (d, J = 11.3 Hz, 1H), 2.64 (S, 1H), 1.62 (s, 3H), 1.15 (s, 9H), 1.01 (d, J = 6.7 Hz, 3H), 0.92 (s, 9H), 0.16 (s, 3H), -0.04 (s, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 163.5, 150.6, 135.6, 135.6, 135.4, 134.6, 133.4, 133.3, 132.9, 132.0, 130.4,

130.3, 129.2, 128.2, 128.1, 128.0, 127.3, 126.6, 126.5, 125.8, 114.4, 111.7, 88.2, 86.7, 79.2, 76.4, 74.9, 67.7, 64.3, 27.3, 25.7, 19.6, 17.9, 16.2, 12.0, -4.3, -4.8; HRMS (ESI) calc'd for $C_{45}H_{58}N_2NaO_7Si_2$ $[M+Na]^+$ *m/z* = 817.36748, found 817.36887; $[\alpha]_D^{25}$: +14 (10 mg/mL, CH_2Cl_2).

(S)-1-((2R,3S,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(naphthalen-2-ylmethoxy)-tetrahydrofuran-2-yl)ethyl methanesulfonate (26). Methanesulfonyl chloride (4 µL, 0.05 nmol) and 4-dimethylaminopyridine (1 mg, 0.02 mmol) were added to a stirred solution of nucleoside **25** (21 mg, 0.020 mmol) in anhydrous pyridine (0.3 mL) at 0 °C. The ice bath was removed and the reaction mixture was stirred at room temperature for 10 h, and then diluted with HCl 1 M (1 mL) and EtOAc (1 mL). The layers were separated and the aqueous layer was extracted with EtOAc (4 × 1 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 × 1.5 cm, 3:7 EtOAc/hexanes) to give nucleoside **26** (22 mg, 95%), R_f = 0.44 (4:6 EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.91 – 7.78 (m, 4H), 7.68 – 7.27 (m, 14H), 6.21 (d, *J* = 7.7 Hz, 1H), 5.18 – 5.06 (m, 2H), 4.63 (d, *J* = 11.5 Hz, 1H), 4.55 (dd, *J* = 7.6, 5.5 Hz, 1H), 4.21 (d, *J* = 5.3 Hz, 1H), 3.89 (d, *J* = 11.3 Hz, 1H), 3.74 (d, *J* =

11.3 Hz, 1H), 2.61 (s, 3H), 1.67 – 1.47 (m, 6H), 1.11 (s, 9H), 0.89 (s, 9H), 0.09 (s, 3H), -0.08 (s, 3H).

1-((1S,3R,4R,6R,7S)-1-(((tert-butyldiphenylsilyl)oxy)methyl)-6-methyl-7-(naphthalen-2-ylmethoxy)-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (27). Thirty seven percent (w/v) HCl (0.10 mL, 1.2 mmol) was added to a stirred solution of nucleoside **26** (10 mg, 0.011 mmol) in THF (0.07 mL) and MeOH (0.13 mL). After heating at reflux for 4 h, the reaction mixture was cooled and neutralized via addition of saturated sodium bicarbonate solution. Dichloromethane (2 mL) was added, then the layers were separated and the aqueous layer was extracted with dichloromethane (4 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue R_f = 0.19 (1:1 EtOAc/hexanes), was dissolved in MeOH (1 mL) and mixed with potassium carbonate (10 mg, 0.071 mmol). After heating to reflux for 1 h the reaction was diluted with dichloromethane, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography (6 × 1 cm, 1:1 EtOAc:hexanes) to give nucleoside **27** (5.3 mg, 70%) R_f = 0.84 (6:4 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.88 – 7.27 (m, 18H), 5.63 (s, 1H), 4.84 (s, 1H), 4.74 (d, J = 11.5 Hz, 1H), 4.52 (s, 1H), 4.32 (d, J = 6.5 Hz, 1H), 4.21 (s, 1H), 3.92 (s, 2H), 1.57 (s, 3H), 1.20 (d, J = 6.4 Hz, 3H), 1.10 (s, 9H).

25

1. DMP, CH_2Cl_2
2. $LiAlH_4$, THF

28

1-((2R,3R,4S,5S)-3-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-5-((R)-1-hydroxyethyl)-4-(naphthalen-2-ylmethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (28). Dess-Martin Periodinane (92 mg, 0.21 mmol) and sodium bicarbonate (0.10 g 1.2 mmol) were added to a stirred solution of nucleoside **25** (0.13 g, 0.17 mmol) in anhydrous dichloromethane (1.0 mL). After stirring at room temperature for 1.5 h, dichloromethane (1 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 dichloromethane (4 × 2 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in dry THF (1.0 mL) and stirred with lithium aluminum hydride (24 mg, 0.63 mmol) at -78 °C. After 1 h, the reaction was diluted with ethyl acetate (3 mL) and HCl 1 M (3 mL), then extracted with dichloromethane (4 × 4 mL). The organic extractions were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (15 × 2 cm, 3:7 EtOAc/hexanes) to give nucleoside **28** as a white foam (97 mg, 73% over two steps), R_f = 0.19 (5% acetone in dichlorometane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 11.1 Hz, 1H), 7.92 – 7.17 (m, 18H), 6.22 (d, J = 6.6 Hz, 1H), 5.13 (d, J = 11.5 Hz, 1H), 4.65 (d, J = 11.5 Hz, 1H), 4.60 – 4.47 (m, 1H), 4.43 – 4.30 (m, 1H), 4.18 – 4.04 (m, 1H), 3.79 (d, J = 10.9 Hz, 1H), 3.59 (d, J = 11.1 Hz, 1H), 3.31 (s, 1H), 1.64 (s, 3H), 1.16 – 0.98 (m, 12H), 0.91 (s, 9H), 0.12 (s, 3H), -0.02 (s, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 163.7, 150.4, 136.2, 135.6, 135.4, 134.8, 133.3, 133.2, 132.7, 132.0, 130.3, 130.27, 128.6, 128.2, 128.1, 128.0, 127.9, 126.8, 126.4,

126.3, 125.7, 111.5, 89.3, 88.9, 79.6, 75.7, 74.5, 68.6, 65.8, 27.3, 27.2, 25.7, 19.5, 17.9, 16.6, 12.0, -4.4, -4.7; HRMS (ESI) calc'd for $C_{45}H_{59}N_2O_7Si_2$ $[M+H]^+$ *m/z* = 795.38553, found 795.3851; calc'd for $C_{45}H_{58}N_2NaO_7Si_2$ $[M+Na]^+$ *m/z* = 817.36748, found 817.36765; $[\alpha]_D^{25}$: +30 (10 mg/mL, CH_2Cl_2).

(R)-1-((2R,3S,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-2-(((tert-butyldiphenylsilyl)oxy)-methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(naphthalen-2-ylmeth-oxy)tetrahydrofuran-2-yl)ethyl methanesulfonate (29). Methanesulfonyl chloride (15 µL, 0.64 mmol) and 4-dimethylaminopyridine (3 mg, 0.02 mmol) were added to a stirred solution of nucleoside **28** (97 mg, 0.12 mmol) in anhydrous pyridine (1 mL) at 0 °C. The reaction was stirred at room temperature for 12 h, and then diluted with HCl 1 M (2 mL) and EtOAc (2 mL). The layers were separated and the aqueous layer was extracted with EtOAc (4 × 2 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 × 15 cm, 1:4 EtOAc/hexanes) to give nucleoside **29** (78 mg, 74%), R_f = 0.89 (1:1 EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.91 – 7.28 (m, 18H), 6.26 (d, J = 7.0 Hz, 1H), 5.20 – 5.08 (m, 2H), 4.69 – 4.60 (m, 1H), 4.54 (d, J = 11.3 Hz, 1H), 4.16 (d, J = 4.8 Hz, 1H), 3.95 (d, J = 11.5 Hz, 1H), 3.88 (d, J = 11.5 Hz, 1H), 2.94 (s, 3H), 1.64 (s, 3H), 1.22 – 1.11 (m, 12H), 0.90 (s, 9H), 0.14 (s, 3H), -0.02 (s, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 163.5, 150.6, 135.6, 135.5, 135.4, 134.4, 133.2,

133.2, 132.3, 131.7, 130.6, 130.5, 128.5, 128.3, 128.1, 127.9, 127.0, 126.5, 126.3, 126.0, 111.9, 87.9, 87.0, 81.0, 78.7, 75.8, 74.7, 63.9, 38.2, 27.3, 25.7, 19.6, 18.0, 17.9, 12.0, -4.4, -4.7; HRMS (ESI) calc'd for $C_{46}H_{61}N_2O_9SSi_2$ $[M+H]^+$ *m/z* = 873.36308, found 873.36274; calc'd for $C_{46}H_{60}N_2NaO_9SSi_2$ $[M+Na]^+$ *m/z* = 895.34503, found 895.34561; $[\alpha]_D^{25}$: -14 (10 mg/mL, CH_2Cl_2).

1-((1S,3R,4R,6S,7S)-1-(((tert-butyldiphenylsilyl)oxy)methyl)-6-methyl-7-(naphthalen-2-ylmethoxy)-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (30). 37 % (w/v) HCl (0.5 mL) was added to a stirred solution of nucleoside **29** (50 mg, 0.058 mmol) in THF (0.35 mL) and MeOH (0.5 mL). After 12 h, the reaction mixture was neutralized via addition of saturated sodium bicarbonate solution. Dichloromethane (2 mL) was added, then the layers were separated and the aqueous layer was extracted with dichloromethane (4 × 2 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 × 15 cm, 1:1 EtOAc/hexanes) to give 2'-OH free nucleoside (30 mg, 70%). R_f = 0.25 (1:1 EtOAc/hexanes). Potassium carbonate (30 mg, 0.21 mmol) was added to a stirred solution of nucleoside 2'-OH free nucleoside (30 mg, 0.04 mmol) in MeOH (1.5 mL). After heating to reflux for 1 h the reaction was diluted with dichloromethane, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography (8 × 1 cm, 3:7 EtOAc:hexanes) to give

nucleoside **30** (24 mg, 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.86 – 7.64 (m, 8H), 7.52 – 7.28 (m, 10H), 5.64 (s, 1H), 4.83 (d, *J* = 11.4 Hz, 1H), 4.70 – 4.62 (m, 2H), 4.16 – 4.06 (m, 2H), 4.01 (d, *J* = 12.6 Hz, 2H), 1.59 (s, 3H), 1.33 – 1.24 (m, 3H), 1.09 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 149.7, 135.7, 135.5, 134.4, 134.2, 133.2, 133.2, 132.9, 132.5, 130.2, 130.2, 128.6, 128.1, 128.0, 127.9, 127.8, 127.0, 126.6, 126.4, 125.9, 110.4, 89.6, 87.3, 81.2, 72.7, 59.3, 27.0, 19.6, 16.5, 12.3; HRMS (ESI) calc'd for $C_{39}H_{43}N_2O_6Si$ $[M+H]^+$ *m/z* = 663.2849, found 663.28852; calc'd for $C_{39}H_{42}N_2NaO_6Si$ $[M+Na]^+$ *m/z* = 685.270433, found 685.27056; $[\alpha]_D^{25}$: +24 (10 mg/mL, CH_2Cl_2).

5.2.4 Fourth Generation Synthesis

Nucleoside 2: Camphorsulfonic acid (1.41 g, 6.10 mmol) and cyclohexanone dimethyl acetal (18.5 mL, 0.122 mol) were added to a stirred solution of 5-methyluridine (15.7 g, 60.7 mmol) in 1,2-dichloroethane (230 mL). After refluxing for 2 h, the mixture was cooled to room temperature and diluted with dichloromethane (100 mL) and saturated sodium bicarbonate solution (100 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude foam was dissolved in dichloromethane (200 mL) and mixed with Dess-Martin periodinane (31.0 g, 73 mmol) and sodium bicarbonate (10 g, 12 mmol). After stirring at room temperature for 3 h, ethyl acetate (100 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 EtOAc (4 × 50 mL). The

combined organic extracts were concentrated under reduced pressure. The residue was dissolved in THF (300 mL), and stirred with a 37 % aqueous solution of formaldehyde (90 mL, 1.2 mol), 1 M NaOH (117 mL, 117 mmol). After 12 h, the reaction mixture was cooled to 0 °C and sodium borohydride (11.5 g, 0.30 mmol) was added slowly. The cooling bath was removed and the reaction was allowed to warm to room temperature over a 3 h period. The reaction mixture was neutralized via the addition of 1 M HCl, and the resulting solution was extracted with dichloromethane (5 × 80 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography to give nucleoside **2** as a white foam (14 g, 62%).

Nucleoside 10: *tert*-Butyldimethylsilyl chloride (2.86 mL, 18.9 mmol) and imidazole (1.29 g, 18.9 mmol) were added to a stirred solution of nucleoside **2** (5.37 g, 14.5 mmol) in anhydrous DMF (70 mL). After 4 h at 0 °C, the reaction mixture is warmed to room temperature and mixed with imidazole (2.00 g, 2.94 mmol) and *tert*-butyldiphenylsilyl chloride (7.6 mL, 29 mmol). After 18 h, the reaction mixture was diluted with methanol (70 mL), then mixed with trifluoroacetic acid (11.2 mL, 65.9 mmol) added in three portions with 30 min of difference between each one. After 48 h, the reaction mixture was neutralized via addition of saturated sodium bicarbonate solution and water (300 mL). The reaction mixture was extracted with Et_2O/EtOAc 1:1 (4 × 50 mL), the combined organic extracts were washed with 1 M HCl (100 mL). The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (17 × 5 cm, 3:7 EtOAc/hexanes) to give nucleoside **10** (3.58 g, 40%). The column was flushed with EtOAc and the extract was mixed with all the non-combined fractions. The mixture of silylated products was concentrated under reduced pressure, dissolved in THF (100 mL) and mixed with 1 M TBAF in THF (50 mL,

50 mmol). After 12 h, water (50 mL) was added and the mixture was extracted with dichloromethane (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 to give nucleoside **2** as a white foam (1.81 g, 61% of nucleoside **10** brsm).

Nucleoside 12: Dess-Martin Periodinane (303 mg, 0.714 mmol) and sodium bicarbonate (200 mg, 2.38 mmol) were added to a stirred solution of nucleoside **10** (334 mg, 0.551 mmol) in anhydrous dichloromethane (3 mL). After 2 h, dichloromethane (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 dichloromethane (4 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Methyl lithium (1.6 M in diethyl ether) (3.4 mL, 5.44 mmol) was added dropwise to a 0 °C solution of copper iodide (526 mg, 2.76 mmol) in MTBE (16 mL). After 30 min, the solution of the crude aldehyde in MTBE (10 mL) was cannulised quickly to the cuprate suspension. The reaction mixture was allowed to reach room temperature in a period of 3 h, then saturated ammonium chloride (50 mL) was added and stirred for 10 min. The organic layer was separated and the aqueous layer was extracted with dichloromethane (4 × 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 (12 × 3 cm, 4:6 EtOAc/hexanes) to give nucleoside **12** (175 mg, 51% over two steps). (38 mg of the undesired (*S*)-alcohol were isolated.)

TsCl, DMAP, pyridine, 70 °C

(R)-1-((3a'S,4'R,6'R,6a'R)-4'-(((tert-butyldiphenylsilyl)oxy)methyl)-6'-(5-methyl-2,4-di-oxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrospiro[cyclohexane-1,2'-furo[3,4-d][1,3]di-oxol]-4'-yl)ethyl 4-methylbenzenesulfonate (31). *p*-toluenesulfonic chloride (270 mg, 1.42 mmol) and 4-dimethylaminopyridine (12 mg, 0.10 mmol) were added to a stirred solution of nucleoside **12** (0.29 g, 0.46 mmol) in anhydrous pyridine (2.5 mL). The reaction mixture was stirred at 70 °C for 12 h, and then diluted with HCl 1 M (10 mL) and EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (5 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (12 × 3 cm, 4:6 EtOAc/hexanes) to give nucleoside **31** (260 mg, 76%) R_f = 0.35 (4:6 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.72 (d, J = 8.3 Hz, 2H), 7.65 – 7.52 (m, 4H), 7.51 – 7.33 (m, 7H), 7.22 (d, J = 8.1 Hz, 2H), 7.16 (s, 1H), 5.51 (d, J = 5.5 Hz, 1H), 4.93 – 4.81 (m, 1H), 4.63 (t, J = 5.5 Hz, 1H), 4.42 (d, J = 5.5 Hz, 1H), 3.91 (s, 2H), 2.35 (s, 3H), 1.81 – 1.19 (m, 16H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7, 149.9, 144.7, 135.7, 135.4, 135.3, 134.1, 132.4, 131.9, 130.6, 130.5, 129.3, 128.3, 128.2, 128.1, 115.1, 110.8, 89.3, 88.7, 84.3, 80.9, 80.4, 64.1, 37.3, 34.9, 27.2, 24.9, 24.1, 23.7, 21.7, 19.4, 17.7, 12.0; LRMS calc'd for $C_{41}H_{50}N_2NaO_9SSi$ $[M+Na]^+$ *m/z* = 797.29, found 797.1.

(R)-1-((2R,3S,4R,5R)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate (32). 37 % (w/v) HCl (0.3 mL) was added to a stirred solution of nucleoside **31** (20 mg, 0.026 mmol) in THF (0.2 mL) and MeOH (0.15 mL). After heating at 50 °C for 9 h, the reaction mixture was cooled and neutralized via addition of saturated sodium bicarbonate. Dichloromethane (3 mL) was added, then the layers were separated and the aqueous layer was extracted with dichloromethane (4 × 2 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 × 1 cm, 6:4 EtOAc/hexanes) to give nucleoside **32** (17 mg, 94%). R_f = 0.14 (6:4 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 1H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.64 – 7.52 (m, 4H), 7.52 – 7.33 (m, 6H), 7.24 – 7.15 (m, 3H), 5.58 (d, *J* = 7.2 Hz, 1H), 5.09 (q, *J* = 6.5 Hz, 1H), 4.46 – 4.34 (m, 1H), 4.24 (d, *J* = 5.1 Hz, 1H), 3.84 (s, 2H), 3.50 (bs, 2H), 2.33 (s, 3H), 1.57 (s, 3H), 1.32 (d, *J* = 6.6 Hz, 3H), 1.03 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8, 151.7, 144.4, 135.6, 135.6, 135.4, 134.7, 132.4, 131.8, 130.6, 130.4, 129.3, 128.3, 128.2, 128.1, 110.98, 90.0, 88.5, 81.2, 76.2, 72.9, 64.0, 27.1, 21.7, 19.4, 17.8, 12.2; HRMS (ESI) calc'd for $C_{35}H_{43}N_2O_9SSi$ $[M+H]^+$ *m/z* = 695.2453, found 695.24669; calc'd for $C_{35}H_{42}N_2NaO_9SSi$ $[M+Na]^+$ *m/z* = 717.22725, found 717.22889.

1-((1R,3R,4R,6S,7S)-1-(((tert-butyldiphenylsilyl)oxy)methyl)-7-hydroxy-6-methyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (33). A solution of nucleoside **32** (50 mg, 72 μmol) in HMPA (0.5 mL) was heated at 150 °C for 1 h. The mixture was allowed to cool to room temperature and diluted with water (3 mL). Extractions with Et_2O (3 × 1 mL) followed by washing of the combined organic extracts with water (2 × 1 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 × 8 cm, 1:1 EtOAc:hexanes) to give nucleoside **32** (19 mg, 50%). R_f = 0.12 (1:1 EtOAc:hexanes); ^{1}H and ^{13}C NMR data match with previously reported data[22].

(R)-1-((2R,3S,4R,5R)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-((triethylsilyl)oxy)tetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate (34). Triethylsilyl trifluoromethanesulfonate (40 μL, 0.17 mmol) was added dropwise to a stirred solution of 2,6-lutidine (40 μL, 0.34 mmol) and **32** (30 mg, 0.040 mmol) in dichloromethane (0.5 mL) at 0 °C. After stirring for 0.5 h, the reaction mixture was diluted with water (1 mL) and stirred for 10 min. Dichloromethane (2 mL) was added, then the layers were separated and the aqueous layer was extracted with dichloromethane (4 × 2 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 × 1 cm, 3:7 EtOAc/hexanes) to give nucleoside **34** (32 mg, 92%). $R_f = 0.15$ (3:7 EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.70 (d, J = 8.2 Hz, 2H), 7.63 – 7.52 (m, 4H), 7.52 – 7.33 (m, 6H), 7.24 – 7.11 (m, 3H), 5.65 (d, J = 7.7 Hz, 1H), 4.95 (q, J = 6.5 Hz, 1H), 4.35 – 4.25 (m, 1H), 4.06 (d, J = 5.1 Hz, 1H), 3.90 (dd, 2H), 2.96 (s, 1H), 2.34 (s, 3H), 1.45 (s, 3H), 1.35 (d, J = 6.5 Hz, 3H), 1.11 (s, 9H), 0.85 (t, J = 7.9 Hz, 9H), 0.56 – 0.38 (m, 6H); HRMS (ESI) calc'd for $C_{41}H_{57}N_2O_9SSi_2$ $[M+H]^+$ m/z = 809.33178, found 809.32779; calc'd for $C_{41}H_{60}N_3O_9SSi_2$ $[M+NH_4]^+$ m/z = 826.35833, found 826.36833.

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