Synthesis of Oxepane Nucleosides by Ring Expansion and Ring Closing Routes

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February, 2011

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Abstract

Modified nucleosides form an important class of antiviral and anticancer agents, and nucleic acids bearing modified nucleosides hold great promise as therapeutic agents. Oxepane nucleosides and nucleic acids are members of this important class of compounds. Oxepane nucleosides have a seven-membered carbohydrate ring instead of the canonical five-membered pentafuranose ring. This thesis describes work in the development of new synthetic approaches and routes in the synthesis of oxepane nucleosides. In chapter 2, the synthesis of oxepane nucleosides based on the ring expansion approach is described. Building on previous work in the Damha lab, studies in the synthesis of oxepane nucleosides by a ring expansion approach were carried out. In particular, oxepane nucleosides were synthesized using thioglycoside derivatives. Routes in the modification of the oxepane scaffold were explored, but later abandoned in favor of new synthetic approaches to oxepane nucleosides.

In chapter 3, work towards the development of new routes in the synthesis of oxepane nucleosides by ring-closing metathesis is described. This new approach to the synthesis of oxepane nucleosides allows for the use of commercially-available nucleosides and circumvents the difficulties observed in glycosylation reaction employed in the previous approach. In chapter 3, various synthetic routes are examined, and work towards the development of viable routes is described. In particular, the direct addition of Grigard reagents to nucleosides dialdehydes, followed by Grubbs II mediated ring closing metathesis serves as a proof-of-principle of the synthesis of oxepane nucleosides by a ring closing route.

Abrégé

Les nucléosides modifiés sont des agents thérapeutiques très efficaces contre le cancer et contre des virus. Les oxepanes font partie de ce groupe important. Au lieu d'avoir un cycle de 5 atomes, les oxepanes ont un cycle modifié de 7 atomes. Cette thèse décrit la recherche sur le développement de nouvelles méthodes pour la synthèse des oxepanes. Dans le deuxième chapitre, la synthèse des oxepanes par la méthode d'expansion de cycle est traitée. Notamment, la synthèse des oxepanes par le biais des hétérosides contenant des thiols est décrite. Dans le troisième chapitre, de nouvelles méthodes pour la synthèse des oxepanes sont étudiées. Toutes ces approches ont pour but ultime d'utiliser la métathèse d'alcènes comme l'étape clé. Notamment, l'addition directe des réactifs de Grignard à des nucléosides contenant deux aldéhydes, suivi d'une métathèse d'alcène, constitue une démonstration de faisabilité de cette approche synthétique.

Acknowledgments

First, I would like to thank Professor Damha for welcoming me into his group first as an undergraduate student and later as a graduate student. I would like to thank him for his support, motivation and encouragement. Professor Damha gave me the opportunity to pursue a project I found interesting and challenging. While in his lab, I learned a great deal and for that I am truly grateful to Professor Damha.

I would also like to thank members of the Damha lab for their help and friendship. First, I would like to thank Jeremy Lackey for introducing me to organic synthesis as an undergraduate student. I would like to thank Dilip for all his help with my project and for being a great presence in the lab. I would like to thank Alex, Adam, Glen, Rob and Richard for making the lab an enjoyable place to work and for always being ready for lunch at the Eaton's centre. I would also like to thank Robert and Richard for being helpful for discussing chemistry and research in general. I am grateful to Maryam and Feresteh for teaching me some Persian and for their generous laughter. I would also like to thank Malik for my knowledge of essential Telegu and for always brightening up my day. Finally, I would also like to thank everyone else in the Damha lab I met: David, Debbie, Paul, Siarra, Jon, Julia, Paula, Matt, Jovanka, Nerea and Kim. It was a pleasure working with such great people.

McGill's staff has also been extremely helpful during my time as a student. For all her help with registration and administrative issues, I would like to thank Chantal. For his help with NMR, I thank Fred Morin. For helping me have a great time working as a teaching assistant, I would like to acknowledge Jean-Marc. Finally, I would like to thank Nadim for his incredible help with MS.

Table of Contents

List of Figures

List of Schemes

Abbreviations

Å	Angstrom
4Å-MS	4 Angstrom molecular sieves
Ac	acetyl
9-BBN	9-borabicyclo[3.3.1]nonane
BSA	*N, O*-bis(trimethylsilyl)acetamide
DCI	4,5-diacynoimidazole
DCM	dichloromethane
DMF	*N,N*-Dimethylformamide
DNA	2'-deoxyribonucleic acid
ESI-MS	electrospray ionization mass spectrometry
HMDS	hexamethyldisilazane
HRMS	high-resolution mass spectrometry
Imid	imidazole
J	scalar coupling constant (in Hz)
KOtBu	potassium *tert*-butoxide
Ms	mesyl
MMTr	4,4'-dimethoxytrityl
mRNA	messenger ribonucleic acid
NBS	*N*-bromosuccinimide
MeCN	acetonitrile
NMI	N-methylimidazole
NMR	nuclear magnetic resonance

ONA	oxepane nucleic acids
PMB	p-methoxybenzyl
RCM	ring-closing metathesis
Rf	retention factor
RISC	RNA-induced silencing complex
RNA	ribonucleic acic
RNAi	RNA interference
siRNA	short interfering RNA
TBAI	tetrabutylammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenyl silyl
t-BuLi	t-butyl lithium
TCA	trichloroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TMSSPh	trimethylsilylthiophenol
tRNA	tranfer ribonucleic acid
Ts	tosyl

Chapter 1 Introduction

1.1 Structure and Function of Nucleic Acids

1.1.1 Discovery of Nucleic Acids

Nucleic acids were first discovered by Friedrich Miescher, a Swiss physician, in 1868. While this new substance was initially found in human leukocytes that were taken from used surgical bandages,[1] nucleic acids were later found in other cell types. Indeed nucleic acids are essential components found in the cells of all organisms on Earth. Almost a century would pass, however, before the biological role and significance of nucleic acids would be understood. Up until the beginning of the 1940s, the seeming simplicity of nucleic acids led most biologists to assume that they played only a relatively minor in the body. However, in 1944 Oswald Avery, in a seminal publication, laid the foundations for our understanding of DNA as the carrier of genetic information.[2] Within a decade of this publication, the double helical structure of DNA[3] was elucidated by Watson and Crick with the indispensable help of Rosalind Franklin and Maurice Wilkins. [3,4]

1.1.2 Structure of Nucleic Acids

DNA is a linear polymer comprised of four basic building blocks, which are shown in figure 1.1. Deoxyadenosine, **1.1**, and deoxyguanosine, **1.2**, are the purine nucleosides; deoxycytidine, **1.3**, and thymidine (or deoxythymidine), **1.4**, are the pyridine nucleosides. RNA is constituted of similar subunits, also shown in figure 1.1. In fact, the key structural difference is the sugar component. Instead of D-2-deoxyribose, RNA incorporates D-ribose as its sugar moiety. This subtle structural feature has profound implications on the chemical

properties and biological function of RNA. The nucleobases of adenosine, **1.5**, guanosine, **1.6**, and cytidine, **1.7** are identical to their DNA counterparts. Only the nucleobase of uridine, **1.8**, is different from its DNA analogue, lacking a methyl group at the C5-position (figure 1.1).

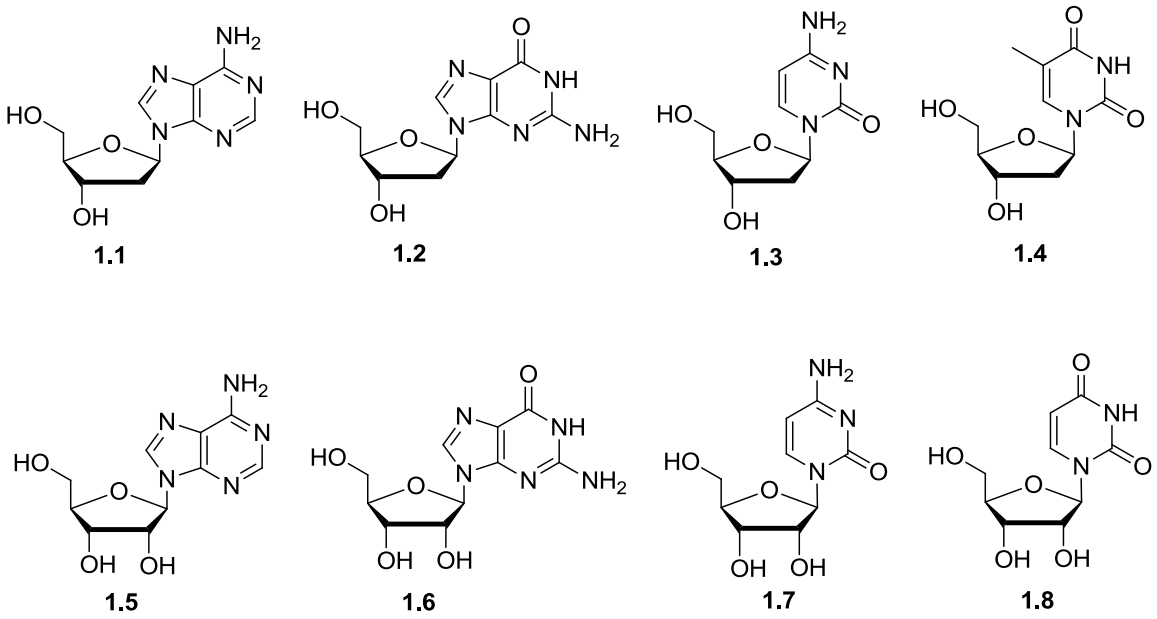


Figure 1.1 Structures of Nucleosides. Structures 1.1-1.4 are the basic building blocks of DNA. Structures 1.5-1.8 are the basic building blocks of RNA.

To facilitate discussion of structure, nucleosides are assigned a standard numbering system. For all nucleosides, the carbon atoms of the pentose sugar moiety are numbered using primed numbers, shown in red in figure 1.2. This numbering starts at the carbon bearing the glycosidic linkage, known as the anomeric carbon, and increases in a clockwise fashion. Numbering of the nitrogenous base is more complex and depends on the class of the nucleobase. For the bicyclic purines, as in the case of **1.5**, numbering begins at one of the nitrogen atoms in the six-membered, shown in blue, and proceeds until all the atoms have been numbered. In this system, the nitrogen atom directly attached to the sugar moiety is

labeled as atom 9. For the monocyclic pyrimidines, as in the case of **1.8**, numbering begins at the nitrogen involved in the glycosidic bond and proceeds in a counterclockwise fashion around the ring.

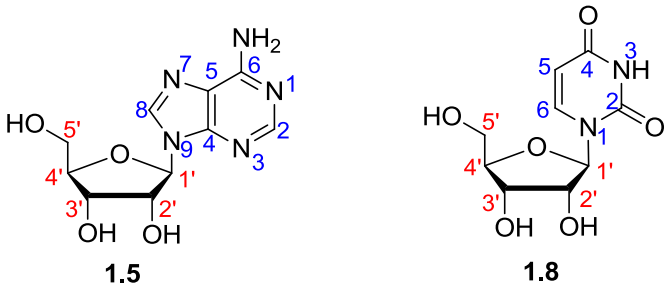
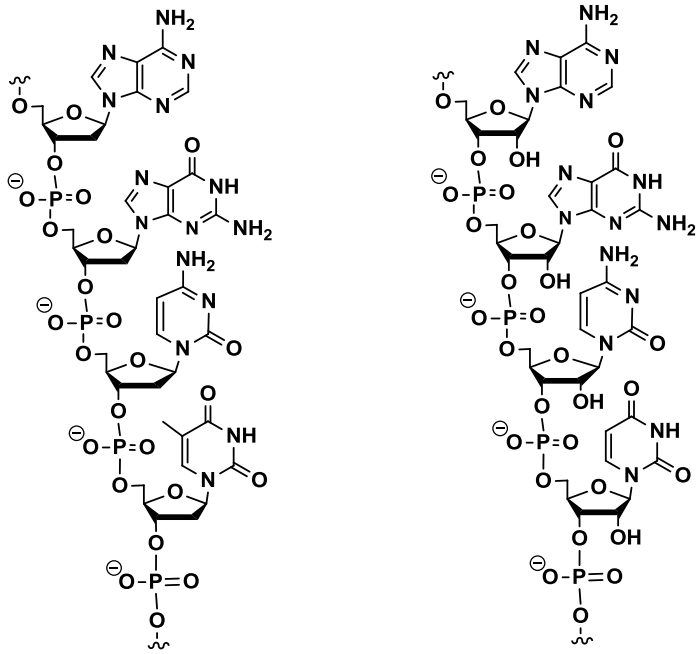


Figure 1.2 Numbering of purine and pyrimidine nucleosides. Adenosine, **1.5**, is shown as representative purine. Uridine, **1.8**, is shown as a representative pyrimidine.

When a phosphate group is attached to the 5' or 3' oxygen, the compound is referred to as a nucleotide. These compounds are the repeating units, or monomers, of DNA and RNA. Polymers of nucleic acids are formed by a phosphodiester linkage between adjacent nucleotide subunits. In both DNA, **1.9**, and RNA, **1.10**, this linkage is formed using the 5' hydroxyl of one nucleotide and the 3' hydroxyl of another nucleotide, as shown in figure 1.3. The sequence of the nucleotides in a strand of DNA or RNA is referred to as the primary structure. Complimentary strands of nucleic acids self-assemble to form the iconic double helix, which is the secondary structure. Hydrogen bonding between complementary nucleobases and hydrophobic stacking of the bases drive this spontaneous process. Watson-Crick base pairing of the nucleobases follows a consistent pattern that was initially discovered by Watson based on ratios observed by Chargaff. Adenine pairs with thymine, cytosine pairs with guanine.[5] In the case of RNA, thymine is replaced by uracil and the base pairing relationships are the same. This complementary base pairing, as was famously noted

by Watson and Crick, is the molecular basis for the storing and copying of genetic information.[3,6]

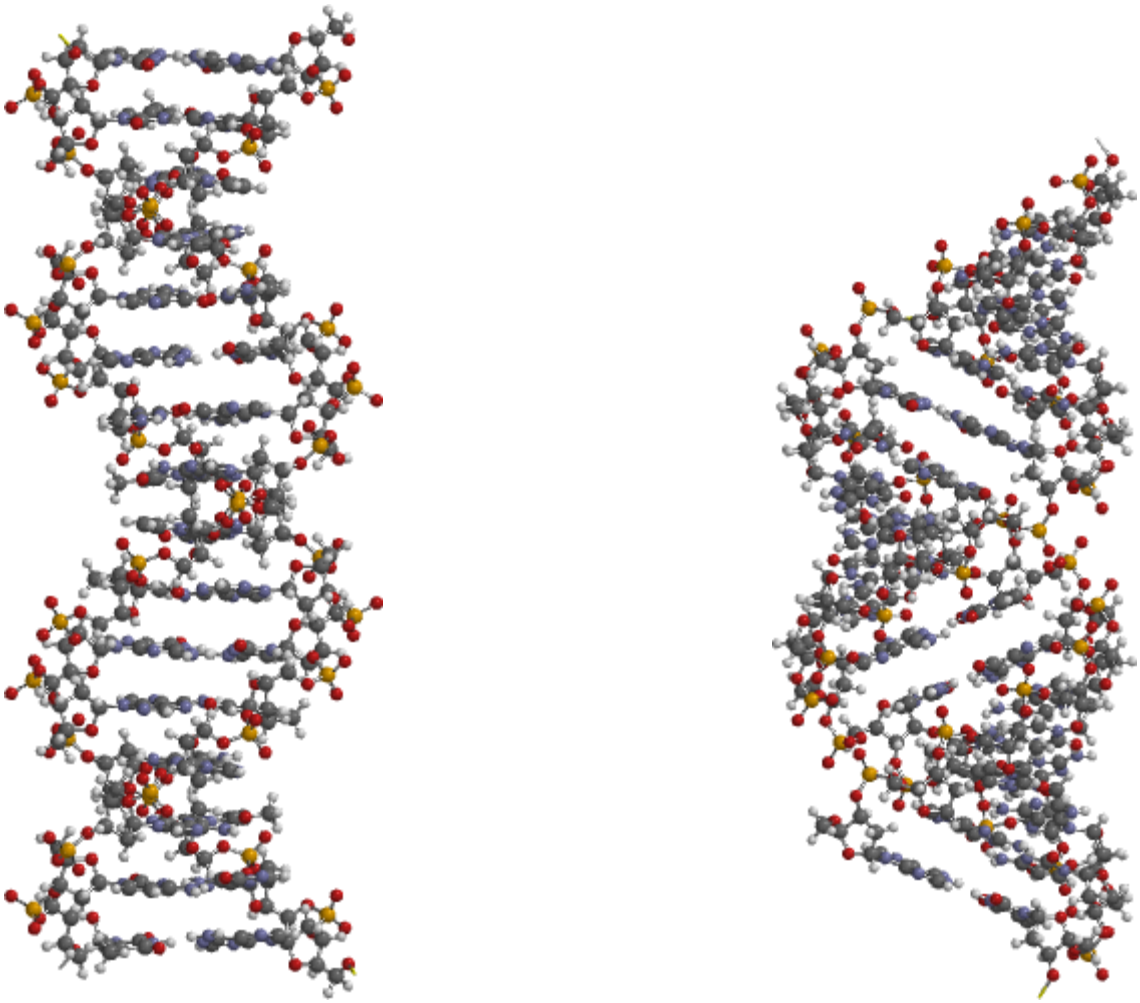


1.9 1.10

Figure 1.3 Primary structure of DNA, **1.9**, and RNA, **1.10**. The 2' hydroxy groups in **1.10** greatly alter the chemical and biological properties of RNA.

While DNA's secondary structure is the famous double helix, the actual shape, structure and parameters of the helix vary depending on conditions. Under low salt concentration and under high humidity, a form known as B-DNA, in figure 1.4, the most commonly found form of double stranded DNA, is observed. In contrast, under high salt and low humidity, A-DNA, in figure 1.4, is favoured. Both B-DNA and A-DNA are right-handed helices, though they differ in other helical parameters. For example, B-DNA has 10 residues per turn, while A-DNA has 11. Differences in the widths of the major and minor grooves are also observed.[7] For example, alternating GC duplexes of B-DNA have wide and shallow major grooves (width of 11.4 Å and a depth of 4.0 Å) and a narrow and deep minor groove (width of 5.9 Å and a depth of 5.5 Å). In contrast, alternating GC duplexes of A-

DNA have narrow and deep major grooves (width of 2.2 Å and a depth of 9.5 Å) and a wide and shallow minor groove (width of 10.9 Å and no defined depth).[8] The major groove parameters of nucleic acids are important since most DNA binding proteins interact through this structural feature. A third double helical conformation, called Z-DNA, is known to occur. Z-DNA is distinguished by its left-handed helical twist and exists only as a transient conformation in biological systems.[9]



B-DNA **A-DNA**

Figure 1.4 Double helical structures of DNA. B-DNA is the most commonly observed form of DNA found in biological systems. Both structures are 16 nucleotides in length. Images generated with Spartan '08 Version 1.2.0.

1.1.3 Function of Nucleic Acids

In living organisms, the genetic code essential for the transmission of hereditary information is stored in DNA. Base pairing between nucleobases allows for the template-directed replication of nucleic acids by polymerases.[10,11] Genetic information flows from DNA to mRNA to protein, a process referred to as the central dogma of molecular biology, which was first described by Crick in 1958.[12] The biosynthesis of proteins, the cell's molecular machines, is directed by the sequence of nucleotides found in genes. This process is initiated by the transcription of DNA, found in the nucleus in eukaryotic cells, into mRNA, which is then exported into the cytoplasm. Ribosomes, large complexes of protein and RNA, translate the mRNA into a nascent polypeptide with the aid of tRNA molecules.[13]

While the storage of genetic information is ostensibly the most important role nucleic acids play, they have many other functions. For example, mRNA acts as a liaison between the DNA, located in the nucleus, and the ribosomes located in the cytoplasm. RNA in the ribosome[14] is responsible for the catalytic activity of this organelle. Moreover, t-RNA molecules act as adapters, allowing the genetic code to be translated into proteins.

1.2 Synthesis of Nucleic Acids

1.2.1 Biochemical Synthesis of Nucleic Acids

In living systems, nucleic acids are synthesized by a class of enzymes known as polymerases in a semiconservative process. Cells achieve this process by the template-mediate addition, catalyzed by polymerases, of nucleoside triphosphate monomers to the growing DNA strands. DNA polymerases add nucleotides in a 5' to 3' direction. Since the two strands in duplex DNA are antiparallel, this means that replication proceeds differently

on the two strands. On the leading strands, replication proceeds continuously in the direction of replication fork movement. On the lagging strand, however, replication proceeds discontinuously as Okazaki fragments,[15] which are eventually joined together by the action of DNA ligase enzymes.

1.2.2 Chemical Synthesis of Nucleic Acids

Chemical synthesis of nucleic acids in the laboratory started with humble beginnings: In the 1950s, the first dinucleotide was synthesized by A.M Michelson and Sir Alexander R. Todd (University of Cambridge).[16] By the 1970s the first total synthesis of a yeast tRNA gene, by the phosphodiester method, had been reported by H.G. Khorana.[17,18] Modern synthesis of nucleic acids involves the use of phosphoramidite chemistry[19] and solid-phase synthesis[20], shown below in figure 1.5.

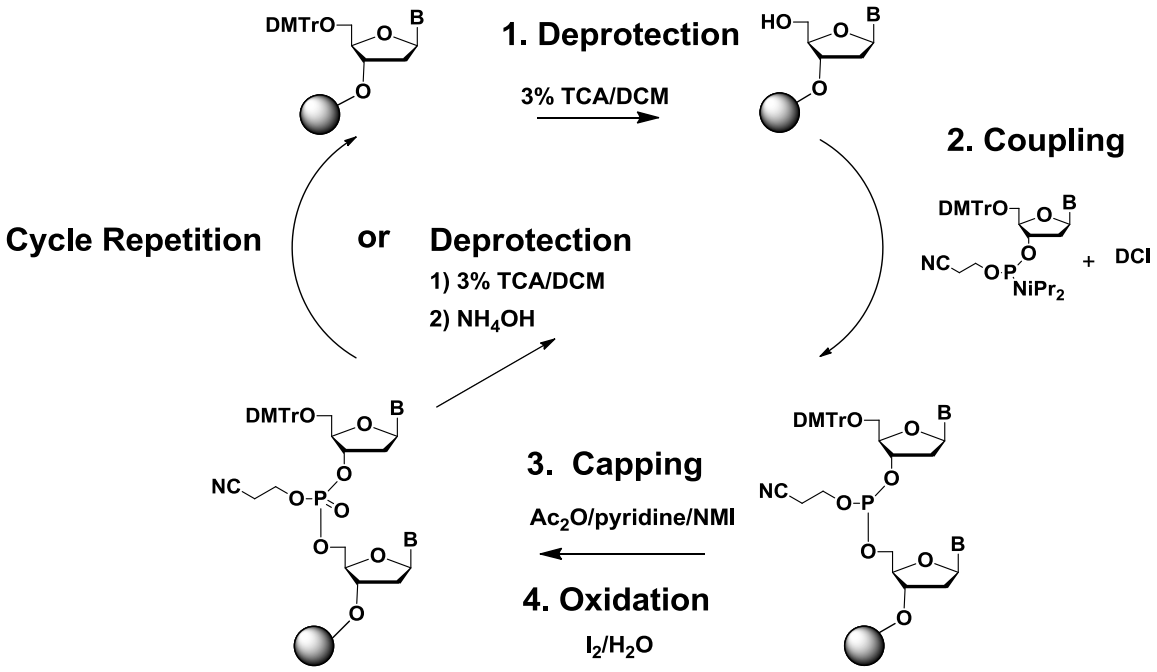


Figure 1.5 Solid-phase Synthesis Cycle.

Solid-phase synthesis of nucleic acids starts with a 5' protected nucleoside tethered to a solid support. Deprotection of the 5' hydroxyl group with 3% trichloroacetic acid (TCA) in dichloromethane (DCM) initiates the cycle. Coupling of the tethered nucleoside and an incoming phosphoramidite, catalyzed by 4,5-dicyanoimidazole (DCI), is then carried out. Next a capping step, employing acetic anhydride, pyridine and N-methylimidazole (NMI) is carried out to block the hydroxyl groups of any unreacted components in the mixture. Once capping has been carried out, an Arbuzov-type oxidation of the phosphorous is effected with an aqueous solution of iodine, altering the oxidation state of the phosphorous from a P(III) to a P(V) species. Finally, the cycle can be repeated in an iterative fashion to grow oligonucleotides of a desired length. Alternatively, sequential deprotection of the 5' hydroxyl, with TCA in DCM, and the phosphate protecting groups, with an ammonium hydroxide solution can be carried out at this point. Treatment with ammonium hydroxide also cleaves the nascent oligonucleotide from the solid support, thus ending the solid-phase synthesis cycle. Synthesis of RNA oligonucleotide can also be achieved by adding additional protection/deprotection steps. Protection of RNA's 2' hydroxyl group is commonly effected by the use of the *tert*-butyldimethylsilyl (TBDMS) protecting group. The TBDMS protecting group was first introduced in 1972 by E.J. Corey[21] as a general protection method for hydroxyl groups, and later it was employed as a specific protection method in the context of oligonucleotide synthesis by K.K. Ogilvie.[22] In the case of both DNA and RNA oligonucleotide synthesis, cytidine, adenosine and guanosine also need to be protected. To protect exocyclic amine groups of cytidine and adenosine, the benzoyl protecting group is used. To protect the exocylic amine of guanosine, the isobutyryl group is used.[23] Under normal solid-phase synthesis conditions, the N3 imido functions of uridine and thymidine do not require protection.

1.3 Nucleic Acids and Nucleoside Analogues as Therapeutics

1.3.1 Antisense Therapeutics

The potential of nucleic acids as therapeutic agents was first discovered by Paul Zamecnik in 1978 when he observed that replication of the Rous sarcoma virus could be inhibited by the use of an antisense oligodeoxynucleotide.[24] Antisense therapeutics involves targeting the expression of aberrant gene products with a complementary oligonucleotide. The antisense pathway is show below in figure 1.6.

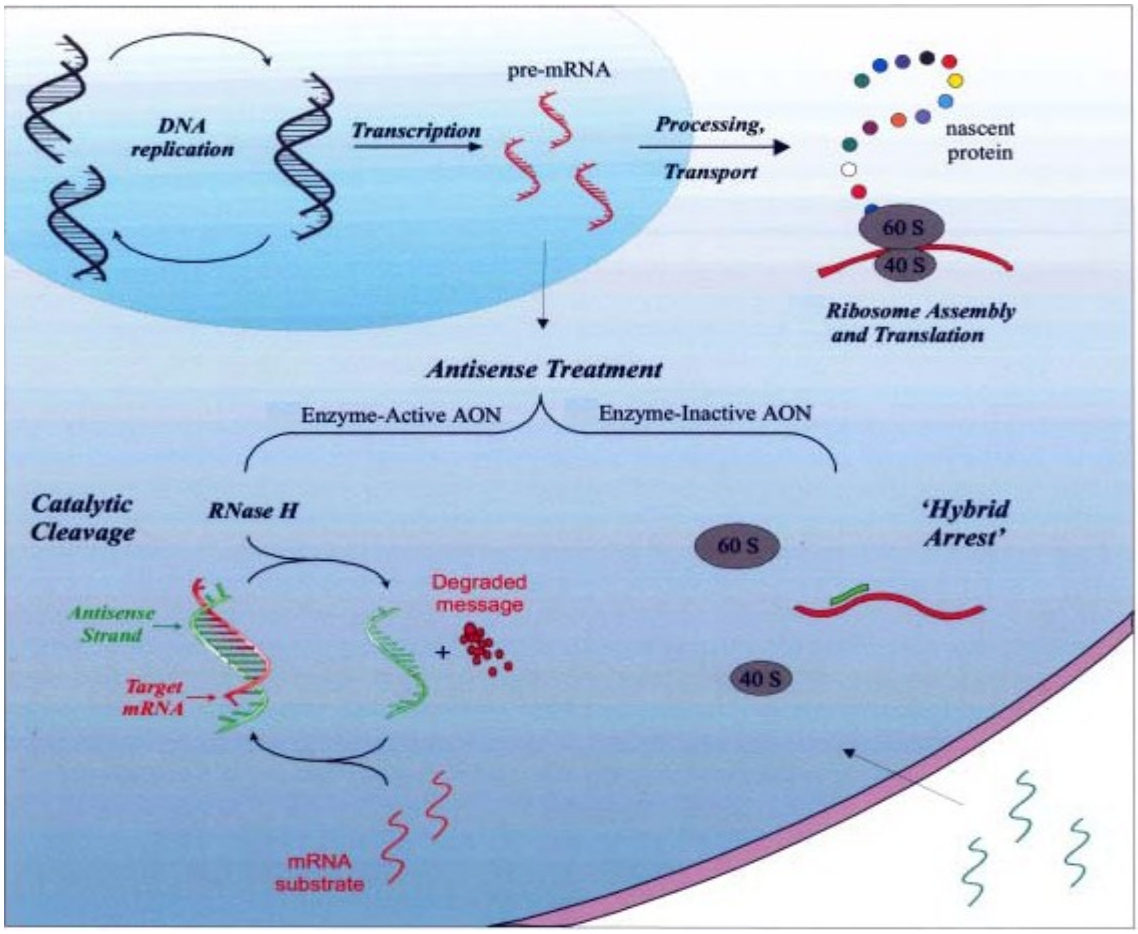


Figure 1.6 Antisense pathway of gene silencing. Antisense oligonucleotides can silence genes by activating RNase H or by acting as a steric block to translation. Figure adapted from.[25]

Once exported to the cytoplasm, mRNAs are normally translated into proteins. For normal gene products, this process is crucial for the production of essential gene products, such as enzymes, receptors and structural proteins. In the case of aberrant gene products, however, the expression (or overexpression) can lead to disease. The antisense approach to therapeutics involves attacking deleterious mRNAs before they can be translated into disease-causing proteins. In general, the antisense pathway can block translation by two mechanisms. In the enzyme-active mechanism, the antisense oligonucleotide binds to the target mRNA and recruits RNase H, which degrades the RNA strand in the heteroduplex. The antisense strand does not get cleaved by the RNase H enzyme, and is freed up upon RNA degradation. This allows the antisense strand to bind to another mRNA strand, and hence promote the cleavage of several target mRNA molecules. In the enzyme-inactive mechanism, the antisense oligonucleotide binds to the target mRNA and acts as a steric block to translation. While antisense therapeutics is an elegant approach to silencing genes, it has thus far led to the development of only one approved drug. In 1998, the FDA approved Vitravene, a phosphorotioate DNA antisense, for the treatment of cytomegalovirus infections.[26] Despite this modest success, antisense oligonucleotides still hold great potential as therapeutic agents. Currently, there are many ongoing clinical trials involving oligonucleotides that underscore this potential. For example, Isis Pharmaceuticals has developed an antisense drug, mipomersen, that is currently in Phase III clinical trials.[27] Mipomersen is an anti-cholesterol drug that targets apolipoprotein B (ApoB).

1.3.2 RNA Interference

While working with the nematode worm *caenorhabditis elegans,* Fire and Mello discovered the phenomenon of RNA interference.[28] RNAi is a natural gene silencing pathway that is triggered by double-stranded RNA, and is shown in figure 1.7. Long double-stranded RNA is processed into duplexes of between 21 to 23 nucleotides in length by an enzyme called dicer. These shorter double-stranded duplexes, called short interfering RNA (siRNA), then recruit the RNA-induced silencing complex (RISC), a larger protein complex. Cleavage of the target then ensues by the action of argonaute 2 (ago2), an enzyme found in the RISC complex.[29]

Currently, there are several clinical trials employing RNAi drugs, and some of the candidates have advanced to phases of human clinical trials. For example, bevasiranib, a drug targeting VEGF-A, is currently in phase III and could eventually make it to the clinic, where it would help those suffering from age-related macular degeneration.[30] RNA interference holds great potential as a therapeutic tool, but it faces several hurdles. Stability of RNA oligonucleotides introduced into systemic circulation is problematic, and so a host of modification have been introduced, such as FANA, 2'OMe-RNA, and LNA.[27] However, the greatest obstacle to RNA interference is undoubtedly delivery. The phosphodiester backbone of oligonucleotides is negatively charged, a fact that makes the cellular uptake of RNAi drugs difficult. To overcome this problem a number of delivery vehicles have been developed, which often involve lipid nanoparticles.[27,31] While RNA interference does not yet have the wide applicability that traditional small-molecule drugs enjoy, RNAi therapeutics has the potential to revolutionize medicine.

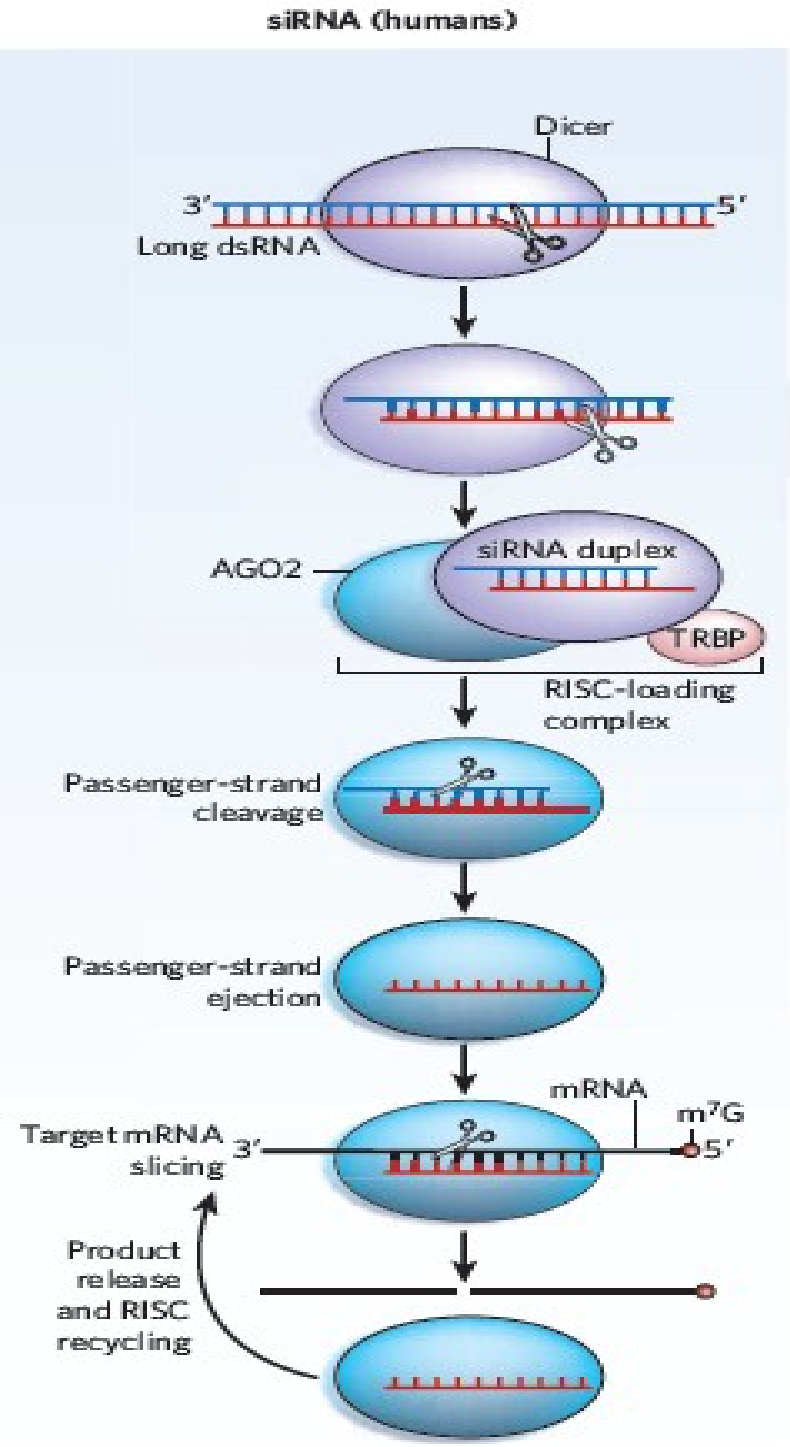


Figure 1.7 RNA interference silencing pathway. Double stranded RNA is processed by an enzyme called dicer. The resulting siRNA is loaded into the RISC complex where the action of argonaute 2 cleaves the mRNA. Figure adapted from.[29]

1.3.3 Nucleoside Analogues as Therapeutic Agents

Nucleoside analogues have been studied intensely since the 1960s and have been a very successful class of antiviral compounds. During this time, many structural modifications have been examined and explored in the search for new medicinal compounds. Even though this area has been studied for so long, there is still great potential for the development of new therapeutic agents based on modified nucleoside analogues.

Differences in cellular division activity between healthy and diseased cells allow for the therapeutic targeting by nucleoside analogues. Most cells in the body are in the G zero period of the cell cycle, a phase of quiescence and inactivity with respect to DNA replication.[32] In contrast, cancer cells and viruses are continually replicating and can thus be targeted by chemotherapeutic agents. Once inside the cell, nucleoside analogues are phosphorylated and converted into 5' triphosphate derivatives. Incorporation of these analogues into nascent nucleic acid strands leads to chain termination. Because viral polymerases discriminate less effectively between phosphorylated nucleoside analogues and their natural counterparts, nucleoside analogues are particularly effective as antiviral agents. A range of nucleoside analogues are used in the treatment of diseases caused by HCMV, HSV, HBV, HCV and HIV infections.[33] Figure 1.8 shows nucleoside analogues that have been approved as antiviral therapeutic agents. As can be seen in figure 1.8, nucleoside analogues come in many varieties. Some nucleoside analogues are acyclic (i.e., devoid of a cyclic sugar moiety), such as acyclovir and ganciclovir. Others have carbocyclic rings, such as abacavir and entecavir. Another interesting class of nucleoside analogues employs L-nucleosides, instead of the natural D-nucleosides. Successful antiviral compounds exhibiting this unnatural configuration include lamivudine (3TC) and emtricitabine (FTC).

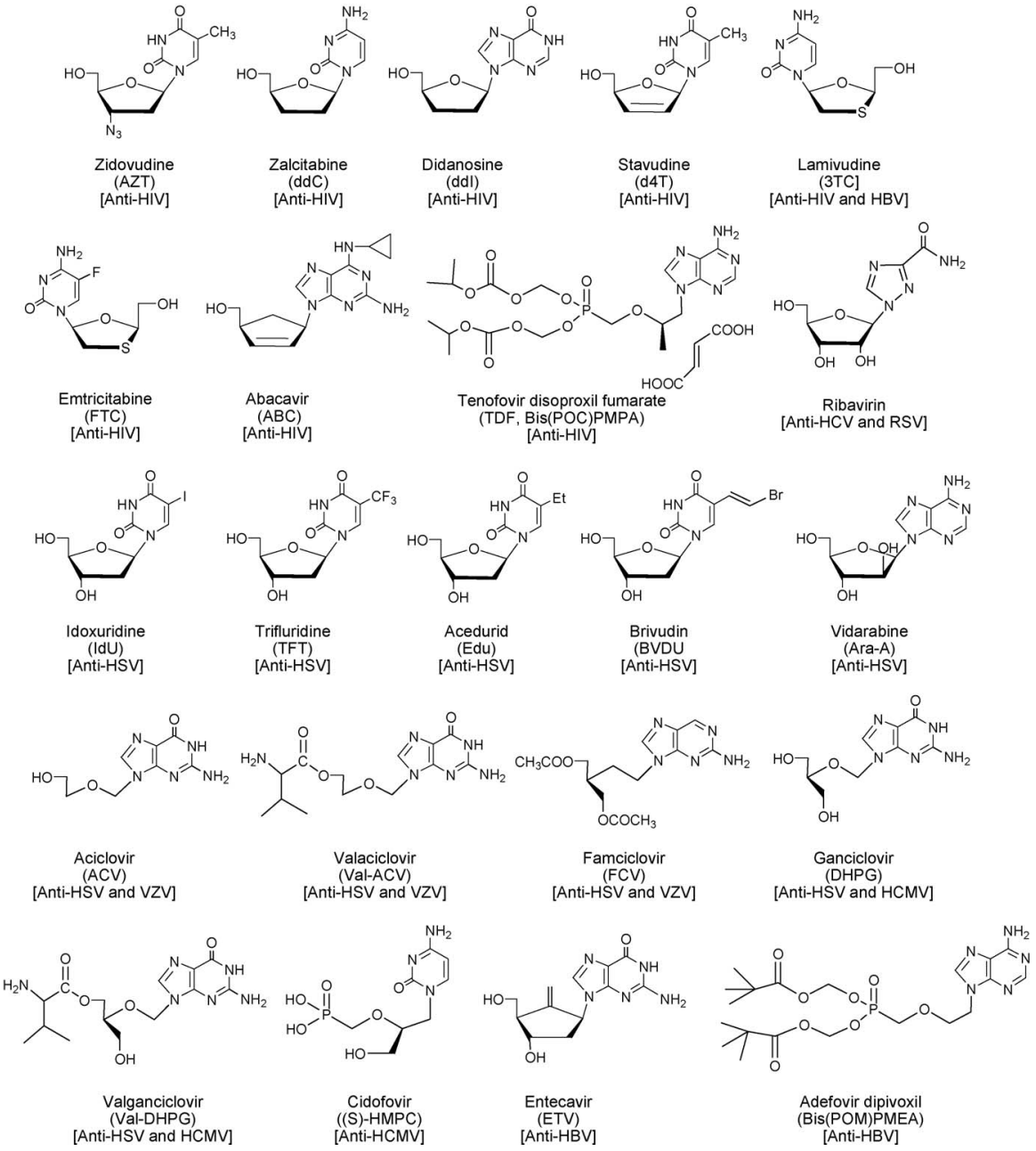
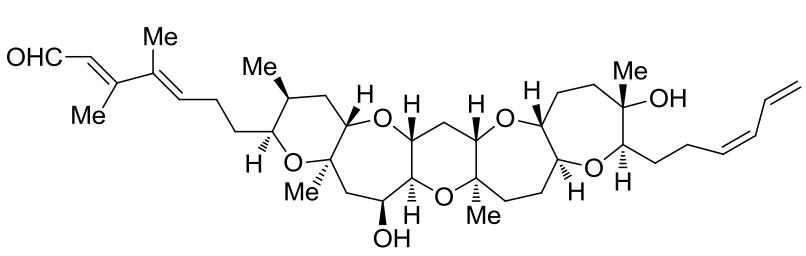


Figure 1.8 Nucleoside Analogues used in the treatment of viral infections. Figure adapted from[33]

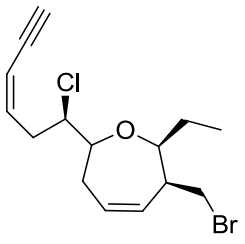
Chapter 2 Synthesis of Oxepanes by Ring Expansion

2.1 Introduction

Oxepanes and oxepenes, seven-membered oxacycles, are found in a number of natural products such as zoapatanol,[34,35] rogiolenyne,[36,37] ciguatoxin[38] and brevenal.[39] In nature, however, the sugar components of nucleic acids are comprised exclusively of ribose and deoxyribose, five-membered oxacycles. These observations inspire us to ask interesting questions: Can nucleosides with larger sugar rings be synthesized? If they could be synthesized efficiently, what properties would they have? In turn, these questions motivate the study of ring expansion in nucleic acids, and the subsequent effects on the biophysical and medicinal properties of these nucleoside analogues. In this chapter, the synthesis of oxepane nucleic acids via ring expansion of cyclopropanated glycals is discussed.



Brevenal



Rogiolenyne

Figure 2.1 Even though five and six membered rings are more common in nature, there are instances of compounds containing an oxepane moiety. Brevenal and rogiolenyne are two examples of natural products containing seven-membered oxacyclic rings.

2.1.1 Early Work on Oxepane Nucleic Acids

Oxepane nucleic acids were first synthesized by Dr. David Sabatino, a former member of the Damha group. Earlier work in the Damha group indicated that the increased flexibility of the oligonucleotide backbone led to an increase in RNase H activity and a consequent increase in antisense gene silencing.[25,40] Based on these findings, a logical (though not obvious) approach to the development of antisense therapeutics would involve the synthesis of ring-expanded nucleoside analogues, which would be unconstrained and thus able to elicit RNase H activity. Since six-membered nucleosides had already been synthesized by Eschemoser,[41] and their cyclohexane-like structure was less flexible relative to furanose nucleosides, the next logical choice for ring expansion was a seven-membered ring. Following this logical progression, Sabatino synthesized oxepane nucleic acid analogues of thymidine and adenosine, shown below in figure 2.2, and indeed found that oxepane nucleic acids induced RNase H activity.[42] In particular, duplexes comprised of oligomers of oxepane **2.2** hybridized to oligomers of riboadenosine elicited RNase H cleavage of the RNA strand, albeit at a slower rate than thymidine oligomers. Promisingly, it was also noted that oligomers of both oxepanes **2.2** and **2.4** were much more resistant to nuclease degradation than DNA oligomers.

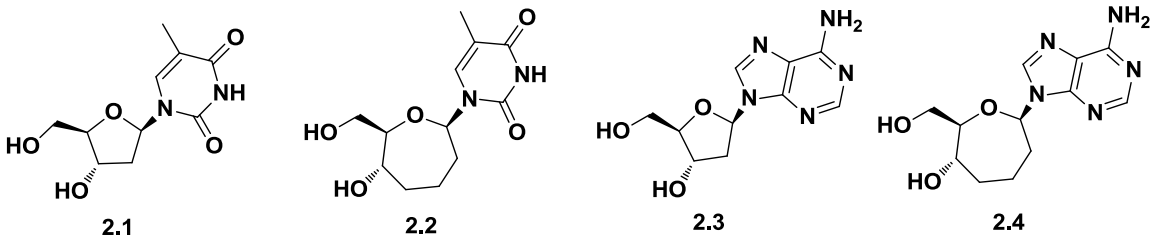


Figure 2.2 Structures of ring expanded analogues to natural DNA. Oligomers derived from oxepanes **2.2** and **2.4** were shown to be more nuclease resistant than their natural DNA counterparts. When elaborated into oligonucleotides, oxepane **2.2** was shown to elicit RNase H activity.

Even though the ring-expanded oxepanes elicited RNase H activity and were nuclease resistant, they suffered from low binding affinity to their complementary oligoribonucleotide strands. For example, the melting temperatures of oxepane **2.2**/riboadenosine duplexes (15mers) were found to be 13 ºC. In contrast, the analogous DNA/RNA duplexes had melting temperatures of 37 ºC. Since the normal human body temperature of 37 ºC is much higher than the melting temperature of the oxepane/RNA, improvement in the duplex stability is critical in the development of oxepane nucleic acids (ONAs) as antisense gene silencing agents.

2.1.2 Proposed Modifications to Location of the Phosphodiester Backbone

One factor that could impact the melting temperatures of ONA/DNA duplexes is the location of the internucleotide linkage, shown below in figure 2.3. Shifting the location of the phosphodiester backbone, we surmised, could lead to an increase in duplex stability.

The initial thrust of the project was to synthesize the series of dihydroxylated series of oxepane analogues shown below in figure 2.4 and study their biophysical and medicinal properties. Along the way, however, the project became more focused on developing novel strategies and routes for the synthesis of oxepane nucleic acids in general. Because many of the intermediates in later synthetic schemes (Chapter 3) are novel compounds, it should be noted that oxepane nucleic acids hold promise not only as antisense gene silencing agents, but also as potential antiviral and anticancer agents. From this vantage point, the development of synthetic routes for oxepane nucleic acids can be seen to be of central importance in the exploration of their therapeutic potential.

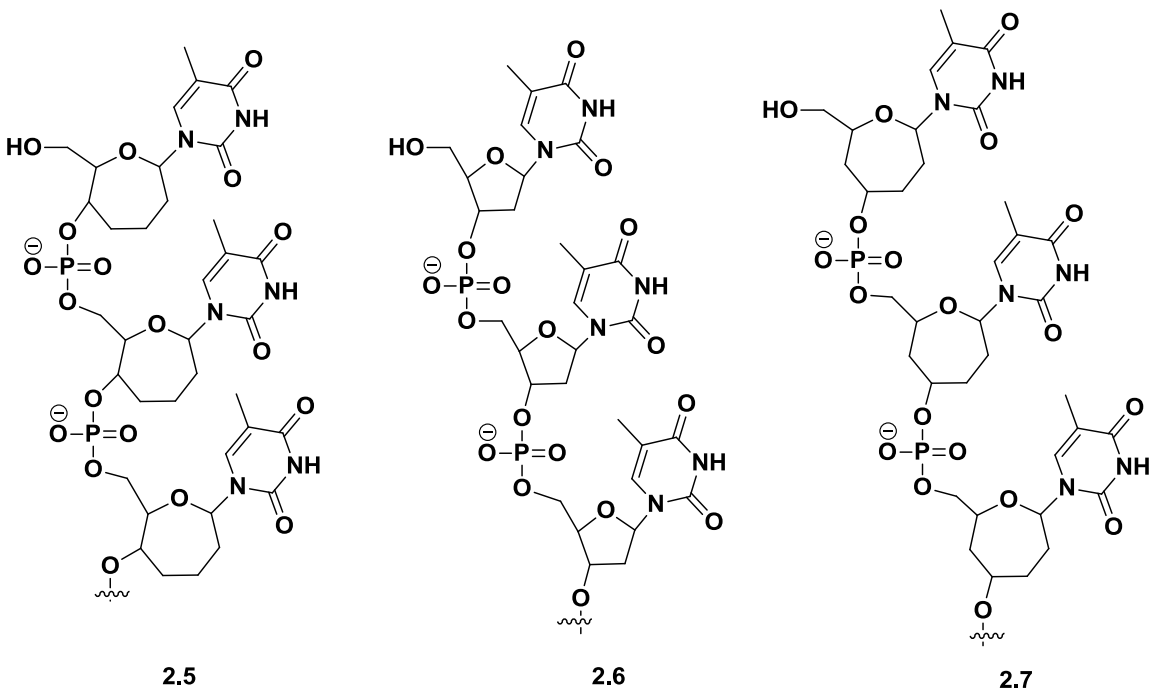


Figure 2.3 Schematic showing the location of the phosphodiester backbone in oxepanes and natural DNA oligonucleotides. In the modified ONA **2.5**, the location of the phosphodiester backbone, in relation to the nucleobase, differs from natural DNA, as shown in **2.6**. Moving the location of the internucleotide linkage, as in **2.7**, could lead to ONA:RNA duplexes of increased stability.

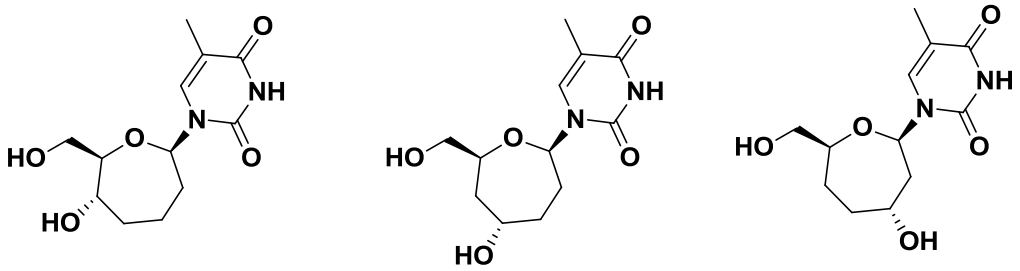


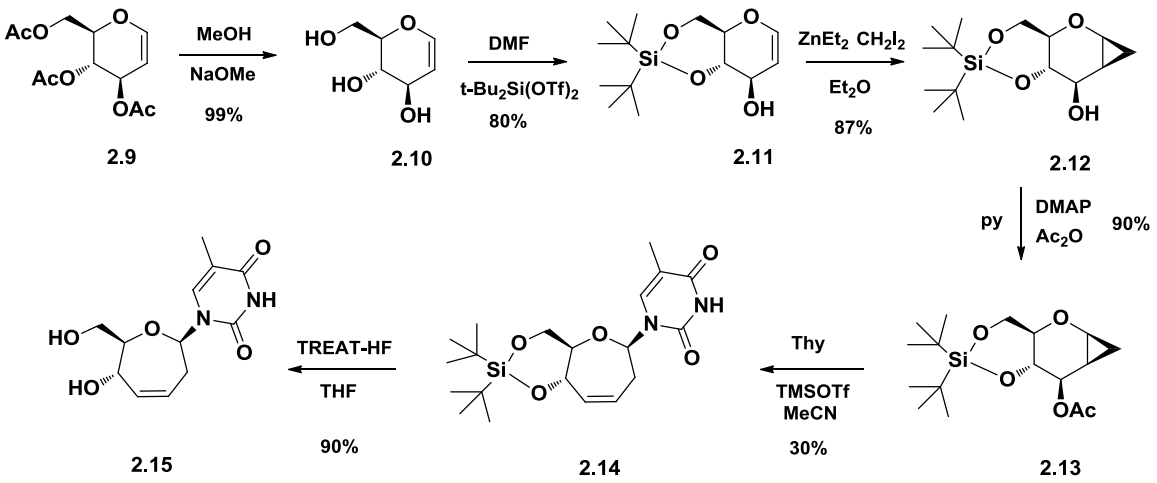
Figure 2.4 Initial synthetic targets of the research project. By varying the location of the secondary hydroxyls systematically, the effects on duplex stability of ONA/RNA hybrids could be studied.

2.2 Synthesis of ONA via Ring Expansion of Cyclopropanated Glycals

2.2.1 Overview of ONA Synthesis Using Cyclopropanated Glycals

The synthesis of compounds containing an oxepane moiety via ring expansion of cyclopropanated glycal derivatives was first carried out by John O. Hoberg, a carbohydrate chemist at the University of Wyoming, in the late 1990s.[43] Years later this approach was first exploited by the Damha group to synthesize oxepane nucleosides, as shown below in scheme 2.1. [42]

Starting from peracetylated D-glucal, the oxepine monomer **2.15** can be synthesized in six steps. The peracetylated D-glucal starting material, **2.9**, is deprotected in a simple transesterification reaction employing sodium methoxide in methanol, yielding D-glucal, **2.10**, in 99% yield. In turn, D-glucal is protected with the di-*tert*-butylsilyl group[44,45] to yield the protected glycal **2.11.** Installation of the protecting group using the ditriflate reagent provided the best results; attempts using the analogous dichloro reagent led to lower yields. It should also be noted that other silyl ether protecting groups, such as the *tert*-butyldiphenyl silyl (TBDPS) group, were also examined in the selective protection for the 1º alcohol, but they were found to interfere with the subsequent cyclopropanation step. Once the silyl ether protecting group was installed, a substrate-directed[46] Simmons-Smith cyclopropanation[47-49] was employed to generate compound **2.12**. Acetylation of **2.12** was facile and yielded the ONA precursor **2.13** in 90% yields.



Scheme 2.1 Synthesis of oxepane nucleosides via ring expansion of cyclopropanated glycal derivatives.[42] Starting from a convenient precursor, **2.9**, the oxepine monomer **2.15** can be synthesized in six steps. All steps are high yielding, except for the key glycosylation reaction that generates **2.14.**

From the cyclopropanated glycal derivative **2.13**, nucleoside **2.14** can be formed in a glycosylation reaction. Because of the generation of a side-product (discussed below in the next section), and because of the modest diastereoselectivity, this reaction is low-yielding and represents the least efficient step in this synthetic pathway. Finally, the deprotected nucleoside **2.15** can be obtained by treating **2.14** with fluoride (TREAT-HF in THF).

2.2.2 Problems Associated with the Glycosylation Reaction

The glycosylation reaction shown above in scheme 2.1 is not a simple glycosylation reaction. In fact, it involves a Ferrier-type rearrangement[50,51] that yields an oxonium intermediate[43] (shown below in figure 2.5), which then reacts with a nucleophile. Overall, the result is a ring-expansion of the glycal derivative **2.13** and the formation of a covalent bond with the incoming nucleophile, as can be seen in scheme 2.2.

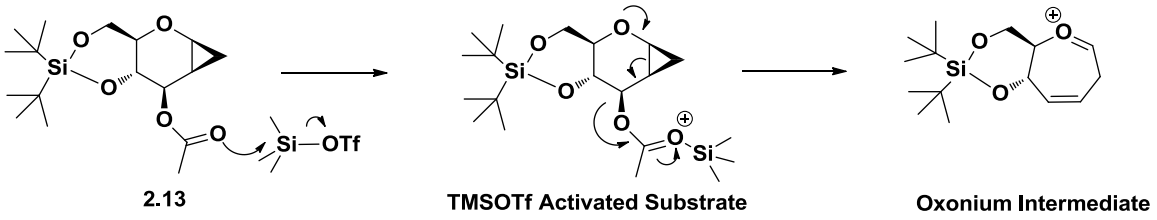
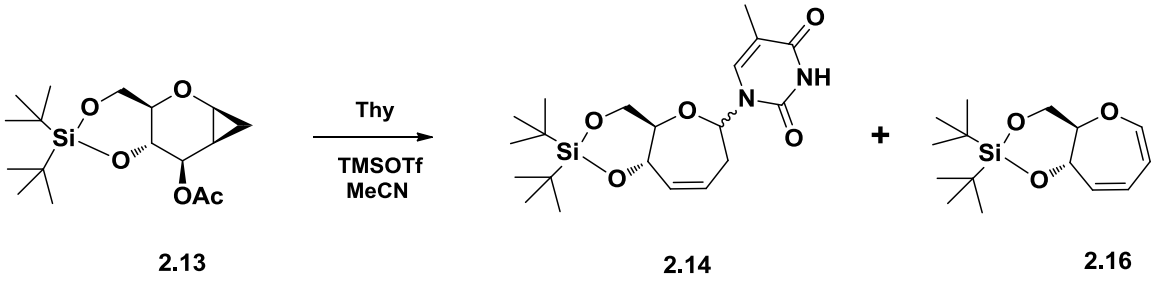


Figure 2.5 TMSOTf catalyzed ring expansion of the cyclopropanated glycal derivative. Once the substrate has been activated by TMSOTf, a Ferrier-type rearrangement generates the ring expanded oxonium intermediate.

In Hoberg's original paper, it was noted that along with the desired ring-expanded product, a diene side product **2.16** (shown below in scheme 2.2) was also generated, in varying amounts that depended on the nucleophile employed. Thus it was no surprise when this side product was also observed in the synthesis discussed in scheme 2.1. When the silylated nucleobase was employed as a nucleophile, however, the amount of this diene side product was much higher than initially expected. For example, in the original Hoberg paper, the amount of the generated diene side product ranged between 0% to 12%;[43] in contrast, Sabatino and Damha found that the amount of side product observed when a silylated nucleobase acts as a nucleophile ranged between 15% to 40%.[42] This along with the modest diastereoselectivity (10:1 for thymine and only 2:1 for adenosine[42]) and difficulties encountered in the purification account for the overall low yield of this key step.



Scheme 2.2 Glycosylation of cyclopropanated glycal **2.13** generates an α/β mixture of diastereomers along with a diene side product **2.16.**

2.2.3 Initial Work on Improving the Glycosylation Reaction

Because the key glycosylation reaction shown in scheme 2.1 was low yielding, some attempts at improving this step were carried out. The diene side product **2.16** is thought to occur through an isomerization mechanism shown in figure 2.6. When a potent nucleophile and low temperatures are used, as in the original Hoberg study, the amount of this side product is low. However, silylated nucleobases are relatively weak nucleophiles and so the original procedure developed by Sabatino and Damha involved refluxing the reaction mixture overnight at 81° C. To test whether heat promoted the isomerization, the reaction was ran at slightly lower temperatures, but no improvement in the yield of the desired nucleoside was noted.

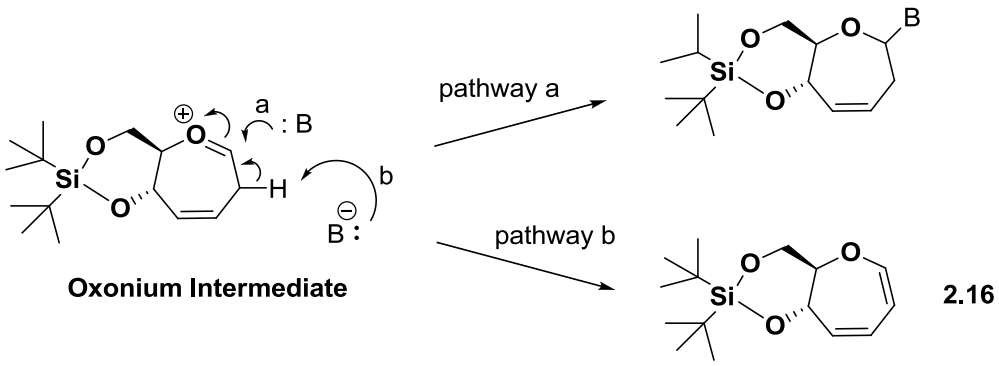
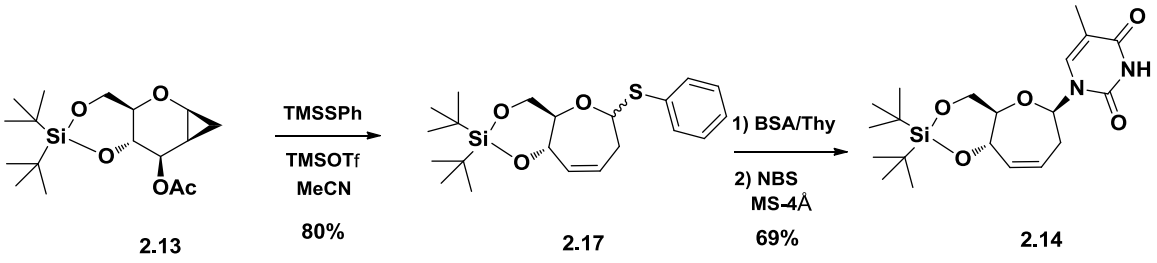


Figure 2.6 Proposed mechanisms of diene side product generation and glycosylation reaction. The diene **2.16** is generated in an isomerization reaction involving the oxonium intermediate (pathway b).

Silylation of the nucleobase was carried out *in situ* with hexamethyldisilazane (HMDS) in the original procedure. The *in situ* silylation of the nucleobase was also carried out with *N, O*-Bis(trimethylsilyl)acetamide (BSA), a typically much more efficient silylating agent, but no improvement on the overall glycosylation reaction was observed under these conditions.

2.2.4 Improvement of the Glycosylation Reaction Using Thioglycosides

As mentioned in previous sections, the glycosylation reaction was actually a Ferrier-type rearrangement followed by an *in situ* glycosylation reaction. In an effort to improve yields, we separated these two processes into distinct steps. This was achieved by opening the ring with trimethylsilylthiophenol (TMSSPh), and then using the resulting thioglycoside, **2.17**, as a glycosyl donor[52-56] in a subsequent glycosylation reaction, shown below in scheme 2.3.

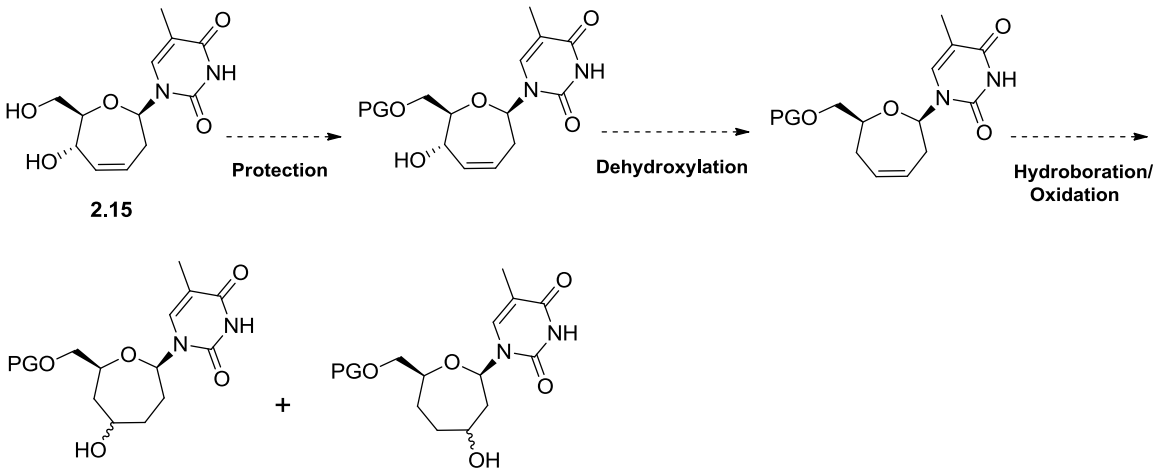


Scheme 2.3 Synthesis of ONA via thioglycoside intermediates. Breaking the ring expansion and glycosylation reactions into two distinct steps using thioglycosides improves the overall yield. Thioglycoside intermediate **2.17** is activated *in situ* using NBS.

Ring expansion using TMSSPh as the nucleophile generates a 2:1 diastereomeric mixture of anomers, as measured by integration of *tert*-butyl methyl protons, in 80% yield. In the next step, the thioglycoside is activated using *N*-bromosuccinimide (NBS), a mild activation method developed by Hanessian[57], and treated with silylated thymine to afford ONA **2.14** in 55% overall yield. As in the case of the original synthetic scheme, this approach generated a mixture of α/β anomers that were separated by column chromatography. While this yield represents an improvement over the original, direct method, the conditions have not been optimized and so there exists potential for further improvement. For example, experimental parameters such as reaction time, temperature, solvent and *in situ* activating agent for the glycosyl donor could be modified in an effort to improve the reaction sequence.

2.3 Initial Work Towards the Dihydroxylated Series

Under ideal circumstances, the desired dihydroxylated series shown in figure 2.4 could be synthesized in a three-step process, starting from oxepine **2.15**, as shown below in scheme 2.4. Because of the inherent difficulty of deoxygenating allylic systems, things are not as simple as in this generalized scheme. Attempting to deoxygenate the protected derivative of oxepine **2.15** via a Barton deoxygenation[58] or even a Myers deoxygenation[59] would lead to rearrangement of the double bond as both reactions involve radical intermediates (shown in figure 2.7). The net effect of this rearrangement would be the generation of two deoxygenated products, which would limit the efficiency and simplicity of the overall process. Because of this complication, the actual devised scheme involved additional protection/deprotection steps.



Scheme 2.4 Generalized scheme showing the idealized synthesis of the dihydroxylated series. Because of difficulties in deoxygenating allylic systems, actual synthetic scheme is much longer, involving protection/deprotection steps.

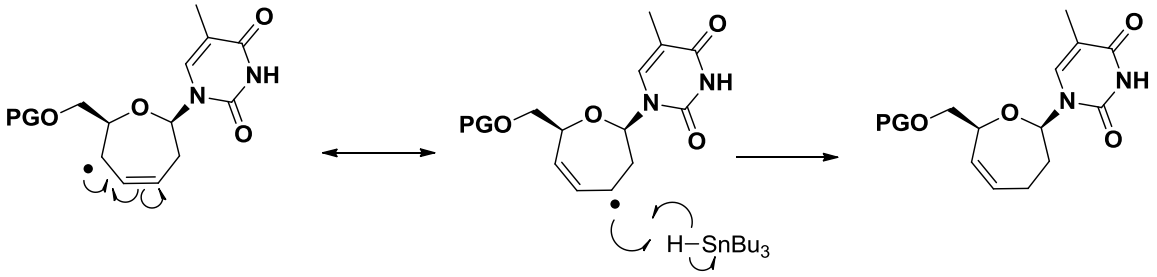
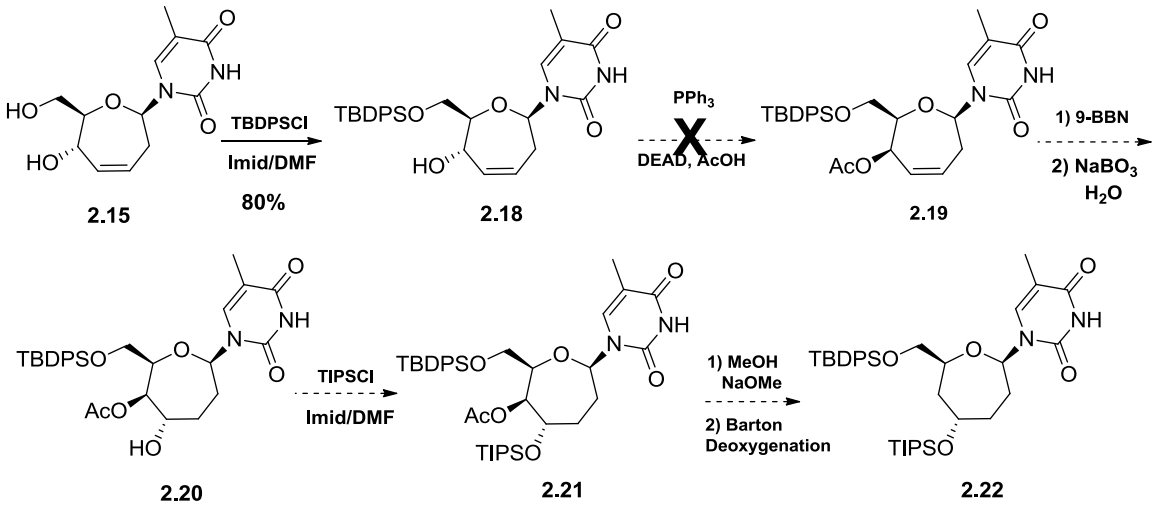


Figure 2.7 Rearrangement of the double bond under radical deoxygenation conditions. In either the Myers or Barton deoxygenation, a radical intermediate is generated. This radical can either react directly with tributyl tinhydride (not shown), or can react through its resonance form. Overall, two deoxygenated products are expected, and the process would not be very efficient.

Since the allylic hydroxyl could not be directly deoxygenated in an efficient manner, a synthetic scheme employing the stereodirecting effect of the hydroxyl group was devised, and is shown in scheme 2.5. In outline, synthetic scheme 2.5 was designed to exploit the stereodirecting effect of cyclic allylic alcohols. When cyclic allylic alcohols are hydroborated with 9-BBN, the main product is typically a 1, 2-anti diol.[60] It was hypothesized that the combined bulk of the nucleobase and the protecting group at the primary hydroxyl group would favor the formation of diastereomers below the ring. In an effort to put the stereodirecting effects of the cyclic allylic alcohol and the steric bulk in the same direction, the Mitsunobu step was introduced before the hydroboration/oxidation step. In practice, the Mitsunobu inversion[61-64] step to generate **2.19** proved to be problematic. Initial attempts used acetic acid as the acidic component, but led to little conversion. Later experiments used 4-nitrobenzoic acid, a commonly used acidic pronucleophile in the inversion of secondary centers,[65] but also failed to generate the desired intermediate **2.19**. At this point, the bulk of the TBDPS protecting group was suspected to interfere with the Mitsunobu step. Changing the 1° protecting group to something less bulky, such as the *tert*-butyldimethylsilyl (TBDMS)

group was considered. But when the total number of steps to get to the desired dihydroxylated product was evaluated, a search for a new synthetic scheme began. Starting from peracetylated D-glucal, synthesis of the dihydroyxlated targets from figure 2.4 would require completing twelve steps. Synthesis of the phosphoramidites, required for oligonucleotide synthesis, would necessitate two additional steps, bringing the total to fourteen steps. Clearly, a shorter, more efficient synthesis was desirable, and the search for a better route was the motivation for the studies carried out in the next chapter.



Scheme 2.5 Initial synthetic scheme for the dihydroxylated targets. The synthetic scheme exploits the stereodirecting effect of cyclic allylic systems.

2.4 Future Work

2.4.1 Glycosylation Reaction

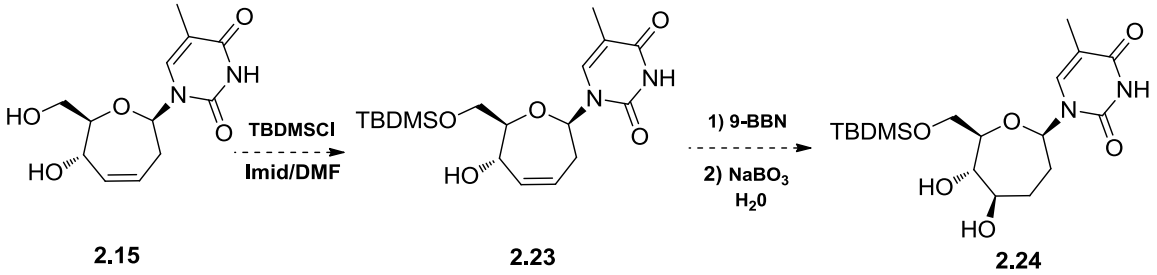
Even though the thioglycoside approach was an improvement over the original synthesis, more work needs to be done. The overall yield over the ring opening and glycosylation steps is 55%, and so this two-step process could be optimized, in an effort to increase the overall yield. While this could be achieved by several approaches, a logical starting point would involve screening for activator and solvent effects on the yield and diastereoselectivity. However, predicting the effects of changing the solvent, activator, or even the protecting group on the glycosyl donor on the outcome of a glycosylation reaction can be difficult, if not impossible.[54,66,67] Thus, it will require some additional experimentation to find optimal conditions.

Purification of the generated α/β mixture in the synthesis of **2.14** (in both the direct and thioglycoside routes) remains a problem. Differing only in one stereocenter, anomers have similar physical properties. Their *Rf* values, in particular, are extremely close to one another, complicating routine separation by column chromatography as in the synthesis of **2.14** (Schemes 2.2 and 2.3). During the purification of these compounds, there is often mixing of fractions, which lowers the recovered yield of the desired β anomer. Work towards the use of lipases, such as *Pseudomonas cepacia* lipase, in the purification of α/β mixtures could also be carried out. Lipases have been shown to assists in the purification of anomeric mixtures of nucleosides by selectively acylating (or hydrolyzing) one anomer over the other, allowing for simple subsequent separation.[68] Of course, the enzymes may have difficulty

recognizing the seven-membered ring, and so this methodology may be difficult to apply to oxepane nucleoside derivatives.

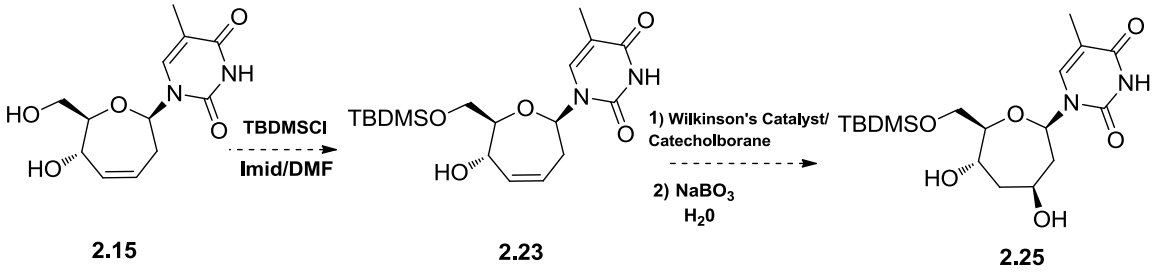
2.4.2 Trihydroxylated Monomer Series

By modifying scheme 2.5 slightly, a trihydroxylated series of monomer could be generated. Direct hydroboration/oxidation of protected **2.23** with 9-BBN should give the anti-1,2 diol product **2.24** (as shown in scheme 2.6) as the major product. By inserting a Mitsunobu step, the opposite stereochemistry could also be synthesized.



Scheme 2.6 Direct synthesis of trihydroxylated monomer. Stereoelectronic bias should generate the anti-1,2 diol as the major product.

By modifying the hydroborating reagent, the other regioisomer could also be synthesized. Evans has shown that hydroboration/oxidation of cyclic allylic systems with catecholborane and Wilkinson's catalyst generates an anti-1,3 diol as the major product. [60,69-71] This outcome can be exploited to generate the trihydroxylated monomer shown in scheme 2.7.



Scheme 2.7 Direct synthesis of other trihydroxylated monomer. By using Wilkinson's catalyst and catecholborane, the anti-1,3 diol product could be generated .

By using schemes 2.6 and 2.7, along with additional Mitsunobu steps, all four trihydroxylated monomers, shown below in figure 2.8, could be synthesized. Once the trihydroxylated monomers are synthesized and protected, they could be elaborated into oligonucleotides, and their biophysical and medicinal properties could be studied.

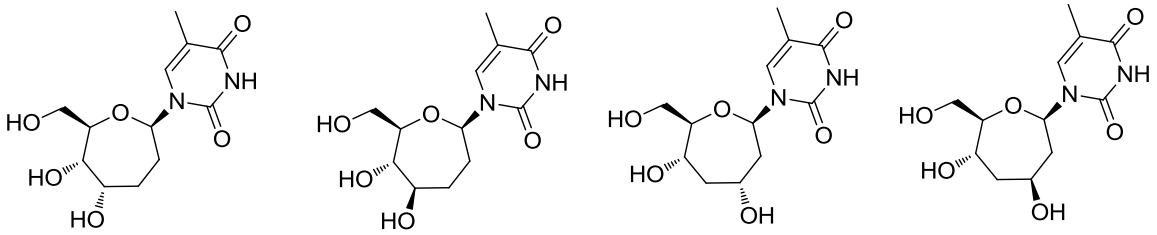


Figure 2.8 Trihydroxylated monomer series. These monomers could be synthesized using schemes 2.6 and 2.7, and by adding a Mitsunobu inversion step.

Chapter 3 Synthesis of Oxepanes by Ring Closing

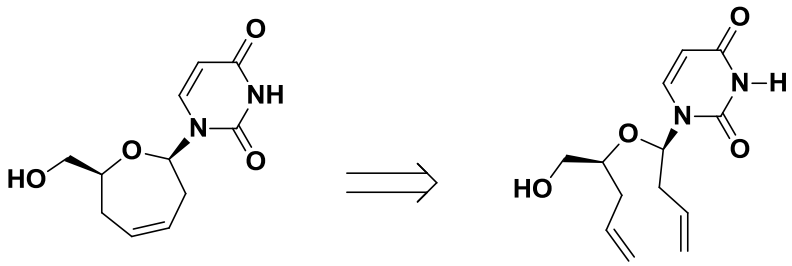
3.1 Introduction

While the ring expansion methodology discussed in the previous chapter was effective at synthesizing ONA, it had some drawbacks. The key glycosylation step was tricky and low yielding, even after the improvement using thioglycosides as glycosyl donors, and would need to be optimized for every desired nucleobase. Synthesis of the initial dihydroxylated monomer targets would necessitate multiple transformations, between 12 to 14 steps, and would likely have to be tuned for each nucleobase. When these difficulties were considered, it became clear that new synthetic routes had to be devised and explored. This chapter describes the work toward developing new routes in the synthesis of oxepane nucleosides.

3.1.1 Synthesis of Oxepane Nucleosides via Ring Closing Metathesis (RCM)

Early in the search for new synthetic routes, it was reasoned that oxepane nucleosides could potentially be synthesized via an RCM reaction, shown below in figure 3.1. Ring formation via olefin metathesis[72-74] is a powerful and synthetically useful transformation in the generation of small, medium and even large macrocyclic ring structures. For example, an RCM reaction was employed in the total synthesis of (-)-griseoviridin[75], a streptogramin antibiotic containing a 23-membered unsatured ring, and in the total synthesis of (+)-prelaureating,[76] a natural product containing an eight-membered ring. Similarly, RCM methodology has been employed in nucleic acid chemistry, and in particular by Poul Nielsen in the synthesis of novel nucleotides containing bridged structures between the sugar moiety

and the phosphate backbone.[77-79] Based on literature precedents, a simple RCM reaction generating an oxepane monomer **3.1** from an acyclic diene seemed like a potentially effective route to oxepane nucleosides. The diene precursor **3.2** could in principle be synthesized by synthetic transformations of the open-chain nucleosides, alternatively called seconucleosides[80-83] or unlocked nucleic acids.[84] Seconucleosides in turn can be synthesized from the corresponding ribonucleoside via sodium periodate cleavage followed by *in situ* reduction with sodium borohydride.[85,86]



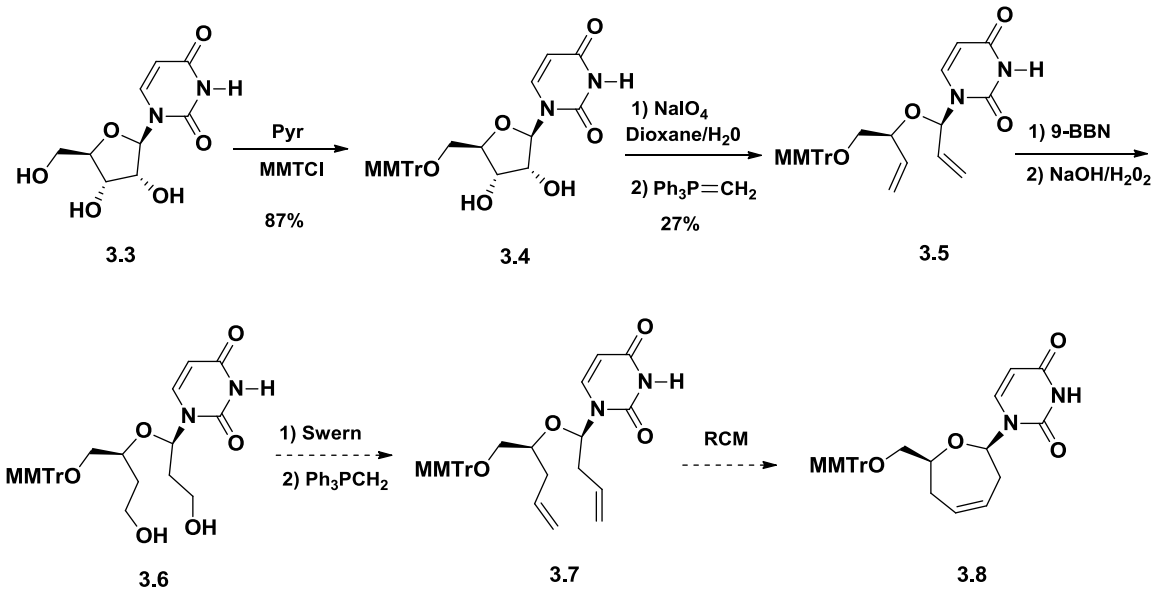
3.1 **3.2**

Figure 3.1 Key retrosynthetic disconnect in the proposed synthesis of oxepane nucleosides via an RCM approach.

Since the precursor **3.2** could be synthesized from a seconucleoside derivative, this synthetic approach would circumvent the glycosylation reaction and all the associated difficulties. Once an oxepane ring structure, such as in **3.1**, would be synthesized, it could be derivatized to generate modified ONA monomers. Because this approach started from commercially-available ribonucleosides, and because it had the potential to be a much quicker synthesis than the original ring expansion synthesis, this approach was pursued by several different synthetic routes.

3.2 Wittig Chemistry Synthetic Route

An initial approach towards synthesizing the RCM-derived ONA monomer **3.8** was a one-carbon homologation sequence employing the Wittig olefination[87] as the main carbon-carbon bond forming reaction (shown below in scheme 3.1).



Scheme 3.1 Wittig-based approach to synthesizing ONA monomer **3.8**. Strategy involves sequential one carbon homologation reactions using Wittig chemistry.

This approach allows for the use of commercially available nucleosides, uridine **3.3** in this case, as a starting material, thus obviating the need for a glycosylation reaction as in the previous synthetic routes. For protection of the 5' OH group, the monomethoxytrityl group (MMTr) was chosen because of its stability, and also for its compatibility with solid-phase chemistry. Once the protected nucleoside **3.4** was synthesized the subsequent synthetic strategy involved a carbon-carbon bond step formation via the Wittig reaction, generating dialkene nucleoside[88,89] **3.5**, and finally a hydroboration-oxidation to generate **3.6**. Once the homologated seconucleoside **3.6** was generated, the idea was to oxidize the diol via a Swern oxidation, and to extend the carbon chain by one more unit on each side with a Wittig step,

generating the RCM precursor **3.7**. However, as discussed below, difficulties were encountered in the generation of **3.5** and **3.6**.

3.2.1 Solution Structure of Nucleoside Dialdehydes

Initial attempts to synthesize dialkene nucleoside derivative **3.5** were surprisingly difficult. Starting from the protected nucleoside **3.4**, it involved the formation of an intermediate nucleoside dialdehyde, followed by a one-carbon extension using a methylene phosphorane reagent. Generation of the dialdehyde involved sodium periodate cleavage, and then subsequent use of the product without purification. But in all cases, attempts at Wittig methylenation failed. Examination of the ^{1}H NMR spectrum of the intermediate dialdehyde led to the surprising realization that the nucleoside dialdehyde was not one compound, but was actually a mixture of compounds having identical Rf values on TLC. Examination of older literature on nucleoside dialdehydes revealed that in solution uridine "dialdehydes" are actually a series of hydrated dioxane nucleosides, shown below in figure 3.2, in equilibrium with a very small amount of actual dialdehyde.[90]

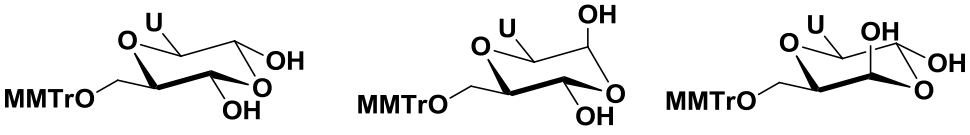


Figure 3.2 Solution structure of uridine "dialdehydes." Upon oxidative cleavage of uridine, the dialdehyde forms stable hydrated dioxane nucleosides, shown here in the chair conformation. Although there are four possible diastereomers, only the three shown are expected to be favored. Having the two hydroxyl groups axial incurs a high energetic penalty, and so this may explain why a fourth diastereomer is not observed.

3.2.2 Dehydration of Dioxane Nucleosides

After learning that treating nucleosides with sodium periodate led to the formation of stable, isolable dioxane nucleosides, it was decided that dehydration of the cyclic acetal structures to the dialdehyde needed to be explored. The dehydration reaction should essentially be the reverse of the initial hydration of the actual dialdehyde and is shown in figure 3.3.

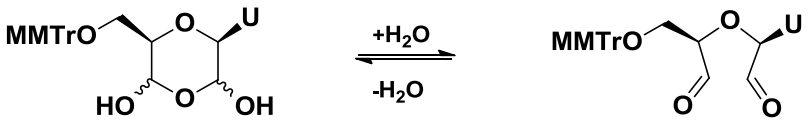


Figure 3.3 Dehydration of dioxane nucleosides and hydration of the dialdehyde (reverse reaction).

To effect this dehydration, the dioxane nucleoside mixture was dissolved in benzene and refluxed. With the aid of a Dean-Stark trap, the water was removed over a 2 hour period, and then the solvent condensed down under reduced pressure. Several subsequent azeotropic condensations from benzene were carried out, and the resulting spectra (shown in figures 3.4 and 3.5) were contrasted. Later trials simply employing azeotropic condensations from benzene were used, though the extent of dehydration seemed less. In either case, however, the dehydrated nucleosides were more reactive in the next reaction step, the Wittig methylenation, which will be discussed in the following section.

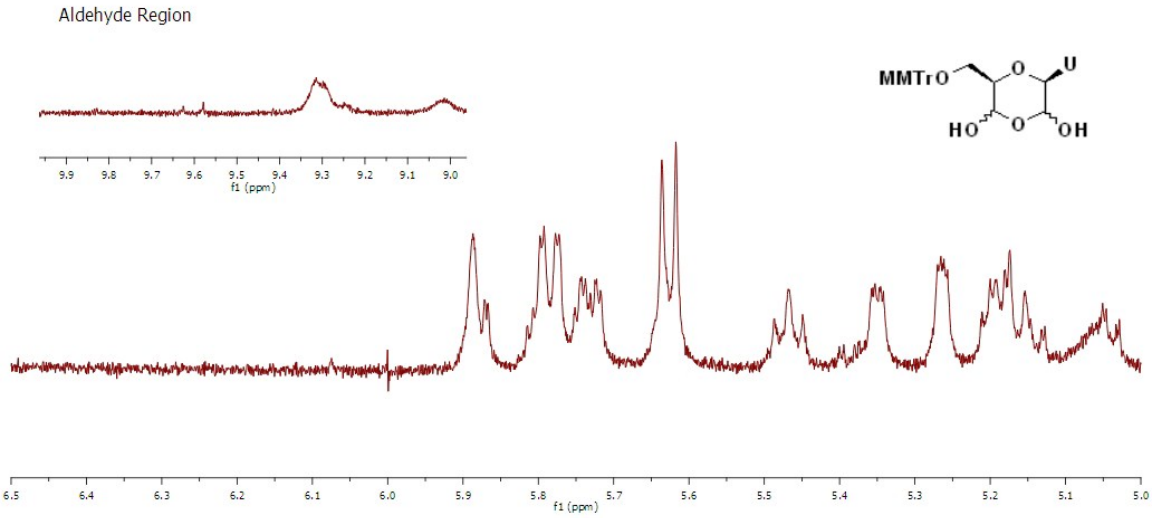


Figure 3.4 ^{1}H spectrum of the dioxane nucleoside mixture. In the 5.0-6.0 δ range a multiplicity of signals can be seen. In the aldehyde regions, shown in the inset, no sharp signals are observed.

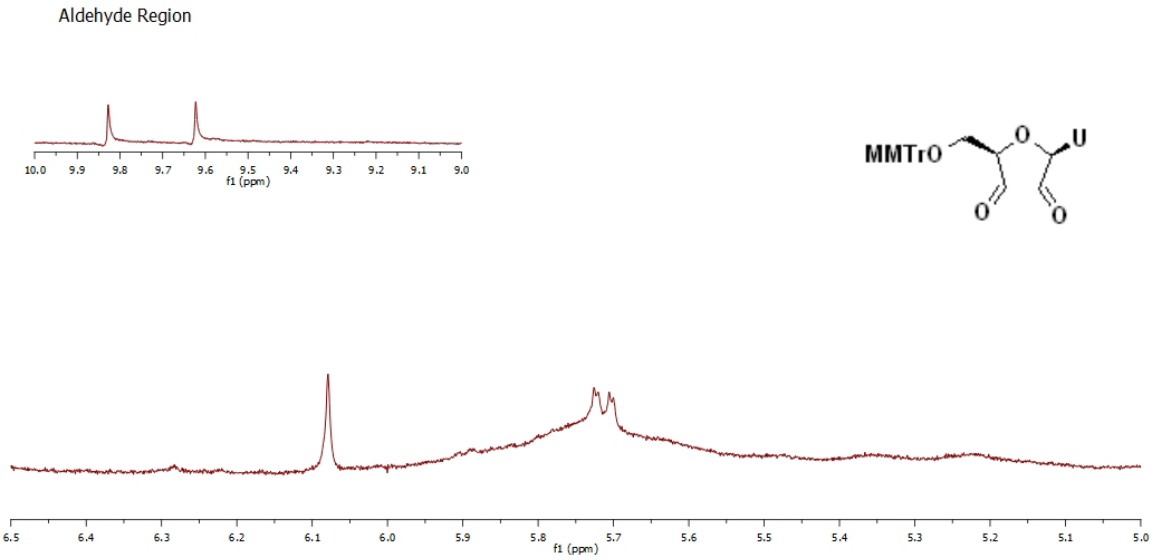


Figure 3.5 ^{1}H spectrum of the dehydrated nucleoside. In the 5.0-6.0 δ range, only two main signals can be seen, corresponding to the anomeric and H5 (nucleobase) protons. In the aldehyde region, shown in the inset, two sharp signals are clearly visible.

As can be seen in the above spectra, there are striking differences between the resulting compounds. In the case of the dioxane nucleoside diastereomers, there is a multiplicity of signals in the 5.0-6.0 δ range, but there is only a broad singlet in the aldehyde region, likely being the N3 imido proton of the nucleobase. In stark contrast, the dehydrated nucleoside only has two signals in the 5.0-6.0 δ, corresponding to the anomeric and H5 (nucleobase) protons, though there are some weaker signals buried under the H5 region. Conspicuously and importantly, however, there are two sharp aldehyde signals in the 9.6 to 9.9 δ range, indicating that the dioxane ring had effectively been dehydrated.

3.2.3 Synthesis of Dialkene Nucleoside

With the dioxane ring dehydrated to the corresponding dialdehyde, the Wittig reaction was repeated. Initial attempts using butyl lithium as the base led to long reaction times and little conversion to the desired dialkene nucleoside. However, switching the base to potassium *tert*-butoxide (KOtBu) resulted in much quicker reaction times. As had been previously noted in the literature, there seems to be a lithium salt effect that slows the decomposition of the oxaphosphetane intermediate into the alkene and triphenylphosphine oxide.[91] Yet even when using KOtBu as the base, the yields were low, around 27%, and a non-polar side product, later identified to be tritanol, was observed. Since the MMTr protecting group is stable to basic conditions, this detritylation was unexpected and puzzling. However, consideration of the intermediate dialdehyde and the potential for enolization has led to a proposed mechanism, which will be discussed below in the next section.

3.2.4 Proposed Mechanism of Detritylation

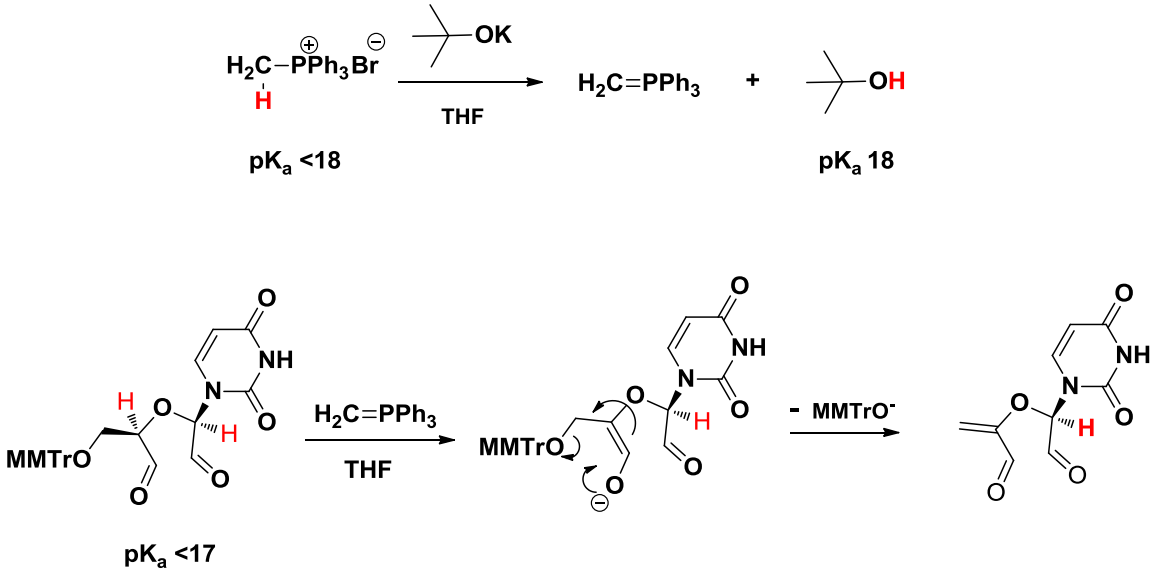


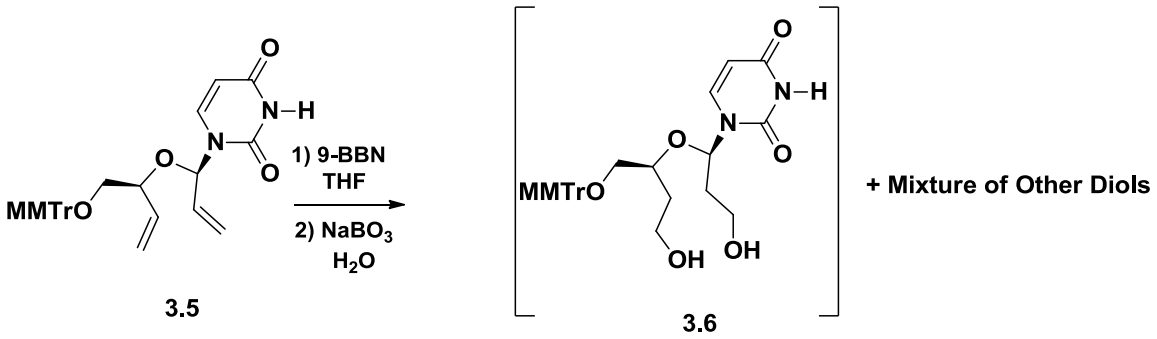
Figure 3.6 Proposed mechanism of detritylation. Since the α protons of the dialdehyde nucleoside, shown in red, are relatively acidic, the aldehyde has the potential to enolize. Enolization could be followed by E1cB elimination.

Because it was unusual to observed detritylation under basic conditions, some thought was put into proposing a plausible mechanism. For this discussion, aqueous pKa values are used, though the actual magnitudes in organic solvents are of course different. Formation of the ylide employs KOtBu as the base, and so the p*K*a of the phosphonium salt must be lower than 18, likely in the 15 to 16 range for quantitative formation of the ylide to occur. In the dialdehyde nucleoside, the p*K*a values of the two protons alpha to the aldehydes, shown in red, must be below 17, as the p*K*a values of an α proton in an alkyl aldehyde is near 17. Because of the inductive effects of the adjacent heteroatoms, the p*K*a values of both α proton in the dialdehyde nucleoside must be below 17, possibly 16 or so. From this analysis, it seems plausible that the aldehydes have the potential to enolize under these conditions. Once the enol forms, it may have the potential to undergo an E1cB type

elimination, forming an α/β unsaturated system and eliminating the MMTr protecting group as a tritanol derivative.

3.2.5 Hydroboration-Oxidation of Dialkene Nucleosides

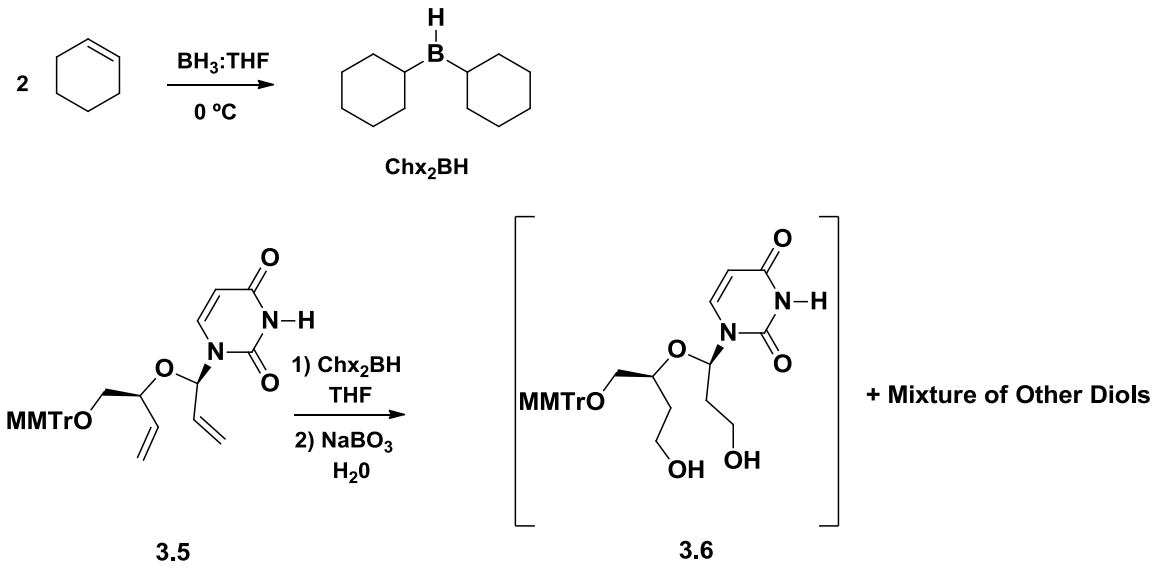
Once the dialkene nucleoside **3.5** was synthesized a hydroboration-oxidation reaction was carried out, and is shown below in scheme 3.2.



Scheme 3.2 Hydroboration-oxidation of dialkene nucleoside using 9-BBN. Reaction generated a mixture of diols.

For the hydroboration step, 9-BBN, a commercially-available reagent was used. For the oxidation step, sodium perborate, in lieu of the classical alkaline hydrogen peroxide system, was used. In comparative studies, sodium perborate has been found to be at least as good (and often better) than hydrogen peroxide-based oxidation.[92] Using these conditions, a mixture of diols, as evidenced by five spots on TLC, was observed after workup. Hydroborations of simple terminal alkenes typically give the terminal alcohol when 9-BBN and standard times and conditions are used.[93] But in this system there are also electronic factors, as well as the simpler steric effects to consider. Even though 9-BBN is often highly regioselective for the formation of terminal organoboranes,[94] it is also sensitive to electronic

factors from neighboring functional groups, such as electron-withdrawing alkoxy groups.[95] To see if another hydroborating reagent could lead to better selectivity, dicyclohexylborane, Chx_2BH, was assayed. Because Chx_2BH is not commercially available from commonly used chemical suppliers, it was made *in situ* and used immediately, as shown below in scheme 3.3.



Scheme 3.3 Hydroboration-oxidation using dicyclohexylborane, Chx_2BH. The hydroborating reagent was made *in situ* then used. Hydroboration-oxidation led to a mixture of diol products.

Similar to the reactions carried out with 9-BBN, hydroboration-oxidation with Chx_2BH lead to a mixture of products. At this point, hydroborations with other reagents, such as disiamylborane, were considered, as well as changing reaction conditions, such as temperature and hydroboration time. But when the low yield of the previous Wittig step was considered, along with the sheer number of possible isomers potentially generated in the hydroboration-oxidation reaction, shown below in figure 3.7, it was decided that new synthetic routes should be examined.

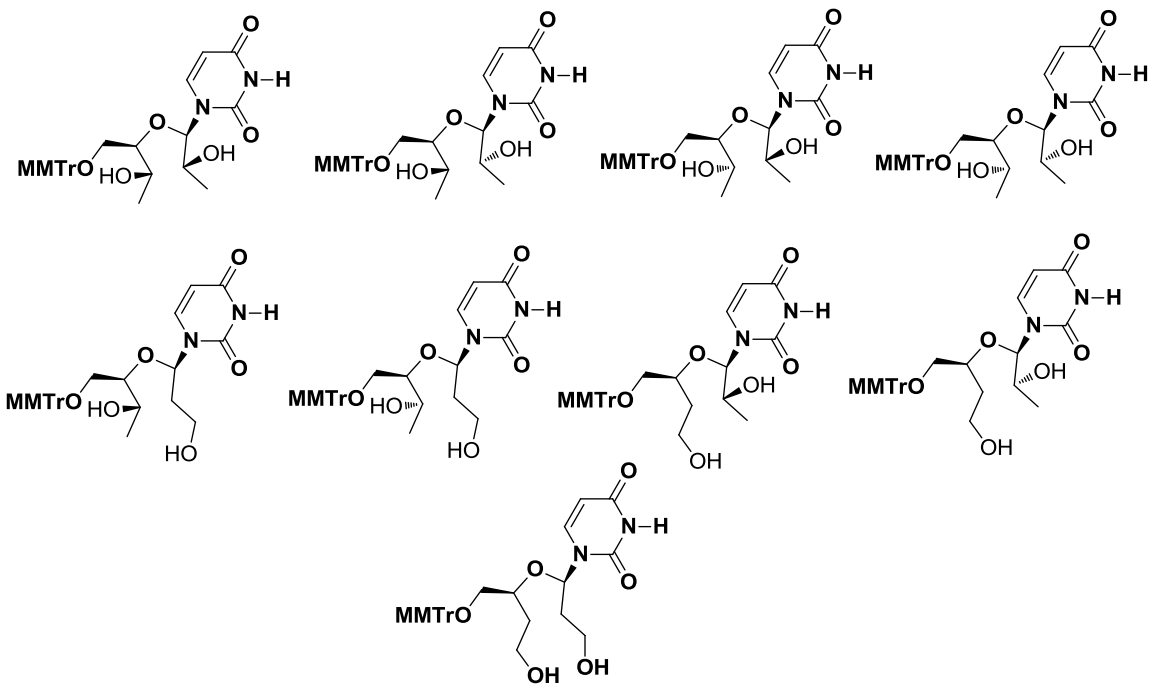
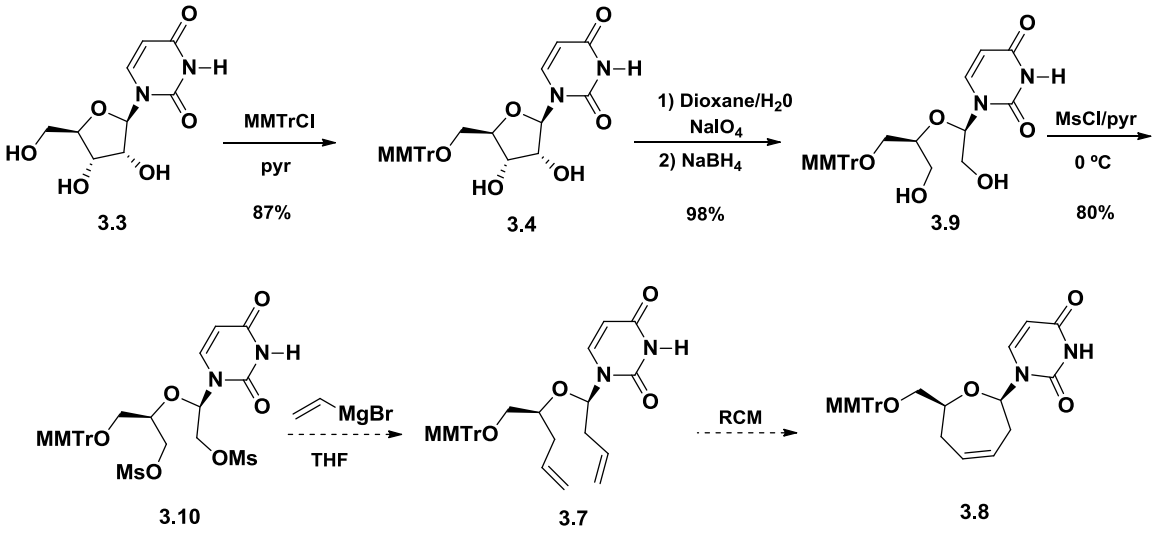


Figure 3.7 All possible isomers potentially generated in the hydroboration-oxidation reaction. Because of the electronic effects of the alkoxy groups and of the nucleobase, and because the transformation involves two alkenes, there are nine possible isomers, a factor greatly complicating a seemingly simple reaction.

3.3 Grignard Chemistry Synthetic Route

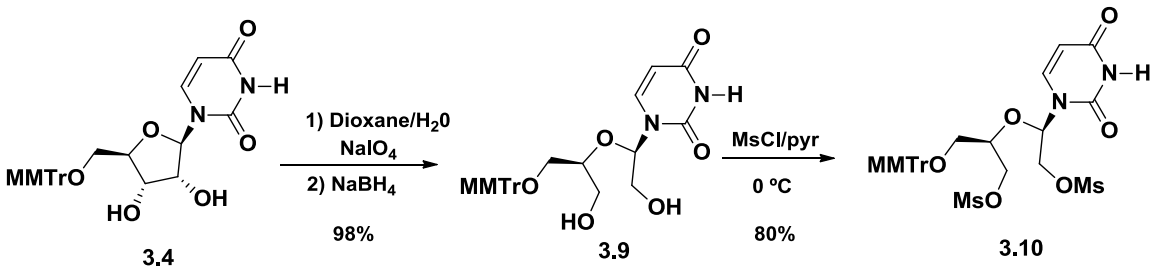
Because of the difficulties encountered in the Wittig chemistry scheme, a new approach based on the addition of an organometallic reagent, vinylmagnesium bromide,[96] was devised. Addition reactions of Grignard reagents[97] to mesylates and alkyl iodides had been seen in the literature,[98] and it was felt that if this synthetic scheme were successful, it would be much more elegant and streamlined than the previous approach. Instead of adding carbons one at a time, in a stepwise manner, the two carbons needed for the RCM precursor, **3.7** in scheme 3.4, could be added in one step, dispensing with the need for additional steps to adjust the oxidation state.



Scheme 3.4 Grignard chemistry synthetic scheme. The approach involves the addition of two carbon units in one reaction step.

3.3.1 Synthesis of Dimesylate

Starting from protected ribouridine, **3.4** the dimesylate **3.10** can be synthesized in two steps, as seen below in scheme 3.5. To synthesize seconucleoside **3.9**, oxidative cleavage with sodium periodate, followed by *in situ* reduction with sodium borohydride[86] is carried out and affords the desired acyclic nucleoside in near quantitative yields. Opening of the ribose ring produces a characteristic spectral signature of triplet resonances, as seen below in figure 3.8. In deuterated DMSO, the anomeric proton as well as the 2' OH and 3' OH appear as sharp, distinct triplets. Once seconucleoside **3.9** is worked up, it can be used in the next step without further purification. Formation of the dimesylate **3.10** is carried out by treating the nucleoside in pyridine with mesyl chloride,[99] and is fairly high yielding, with yields in the 80% range.



Scheme 3.5 Synthesis of dimesylate **3.10** from protected uridine. Oxidative cleavage followed by reduction forms the seconucleoside **3.9**, which then reacts with mesyl chloride in pyridine to form the dimesylate **3.10**.

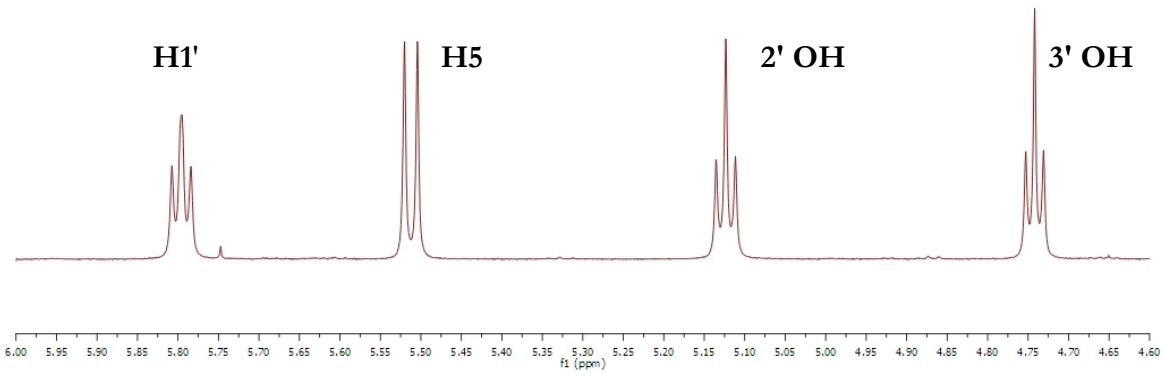
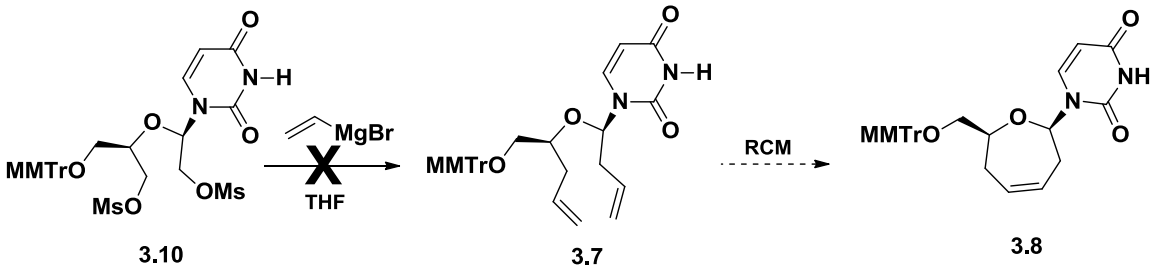


Figure 3.8 Expansion of ^{1}H NMR (DMSO-*d6)* of seconucleoside **3.9**. Opening of the ribose ring generates a pattern of triplets. The anomeric proton, H1', appears as a downfield triplet. In between the sugar protons, the H5 signal of the nucleobase appears as a sharply resolved doublet. Finally, the two hydroxyl protons appear as sharp triplets.

3.3.2 Vinylation Reaction with Vinylmagnesium Bromide

With the dimesylate **3.10** synthesized, attention was turned to attempting a coupling using vinylmagnesium bromide, and is shown below in scheme 3.6. Initial attempts were carried out at 0 ºC, but no reaction was observed. Only starting material was recovered.



Scheme 3.6 Vinylation reaction with vinylmagnesium bromide. Trials at low temperatures lead to no reactions; trials at ambient temperature led to an inseparable mixture of products.

Raising the temperature to ambient temperature in the presence of additives, such as catalytic CuI or TBAI, however, led to observed reactions. But in this case, the substrate was too reactive, and multiple spots were observed on TLC. Purification by column chromatography revealed that within the most non-polar spot, there were multiple, inseparable compounds, as evidenced by the multiplicity of signals observed in the H6 (nucleobase) and anomeric regions, shown below in figure 3.9. In a pure seconucleoside, the anomeric proton, H1', should appear as a triplet, and the H6 nucleobase proton should appear as a doublet. Estimating from these ^{1}H NMR regions, there appeared to be at least four inseparable compounds in this one spot on TLC. Clearly, a more chemoselective approach was needed, and so our attention turned to stoichiometric organocopper chemistry.

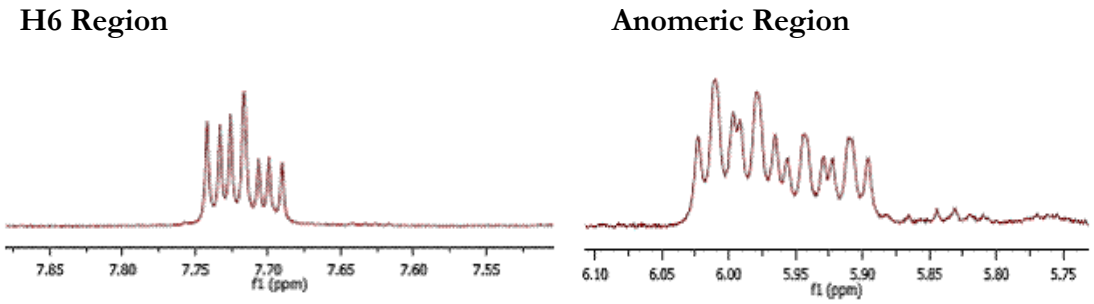


Figure 3.9 Expansions of the H6 and anomeric regions of the mixture isolated from the conversion of 3.10 to 3.7 (scheme 3.10). As seen in the multiplicity of signals in both these regions, the reaction generated a mixture of inseparable products.

3.4 Organocopper Chemistry Synthetic Route

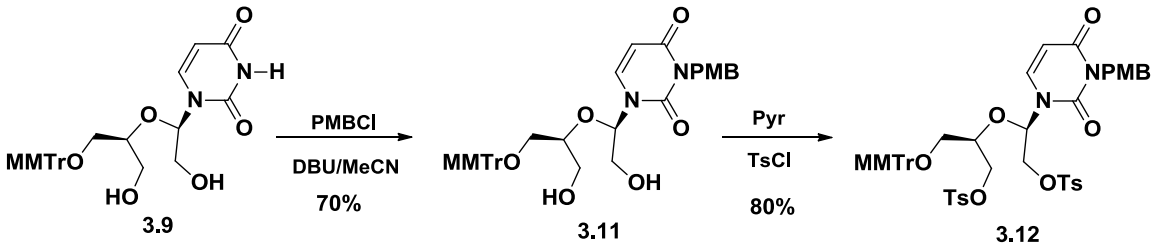
Because of the chemoselectivity problems observed with Grignard reagents, it was necessary to change the nucleophilic component in the coupling step in scheme 3.4. Since it was desirable to maintain a stepwise and streamlined approach, a vinyl-based organocopper species was required. Organocopper reagents, such as Gilman reagents, are well-established in the literature and are typically more effective at coupling with saturated electrophiles than Grignard reagents.[100-103] However, organocopper chemistry can be challenging technically. In practice, a myriad of factors affect the stability and reactivity of cuprate regents, including nature and purity of the copper (I) salt; presence and concentration of halide salts; temperature; oxygen content of the solvents; composition of solvent systems; and additives such as ligands and HMPA.

3.4.1 Synthesis of Ditosylate Nucleoside

When the initial organocopper coupling experiments were designed, copper iodide, the most common copper (I) salt, was chosen. Reports in the literature indicated that tosylates[104,105] were the best leaving groups for reactions with Gilman reagents, and so a ditosylate seconucleoside was selected as the substrate. Initial attempts at forming a ditosylate nucleoside were meet with some difficulties; and an examination of the literature indicated that the N3 imido function needed to be protected.[80] While many protecting groups[106-108] were available to protect the nucleobase's N3 imido nitrogen, the p-methoxybenzyl group (PMB) was chosen. The PMB protecting group can be installed selectively on the N3 imido nitrogen, even in the presence of free hydroxyl groups, and can be removed under relatively mild conditions.[109] Outside of nucleoside chemistry, the PMB group has also been employed

in the context of a total synthesis of tunicamycins[110] by Danishefsky, illustrating the wide applicability of this protecting group.

Shown below in scheme 3.7 is the synthesis of protected ditosylate nucleoside **3.12**. Starting from protected ribouridine **3.9**, the desired ditosylate **3.12** was synthesized in two steps. Selective protection of N3 imido nitrogen with the PMB group was facile, even in the presence of the two primary hydroxyl groups. Subsequent ditosylation[81,111] required longer reaction times than dimesylation, but yielded the desired substrate in good yields



Scheme 3.7 Synthesis of ditosylate nucleoside from protected ribouridine.

Installation of the PMB group produces a classical AB quartet in the ^{1}H NMR spectrum, and is shown in figure 3.10. This signal is associated with the diastereotopic methylene hydrogens.

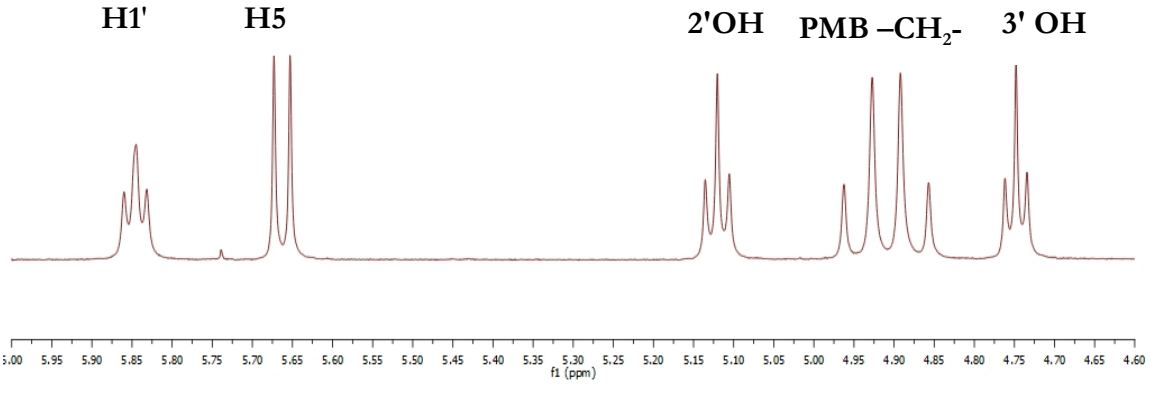
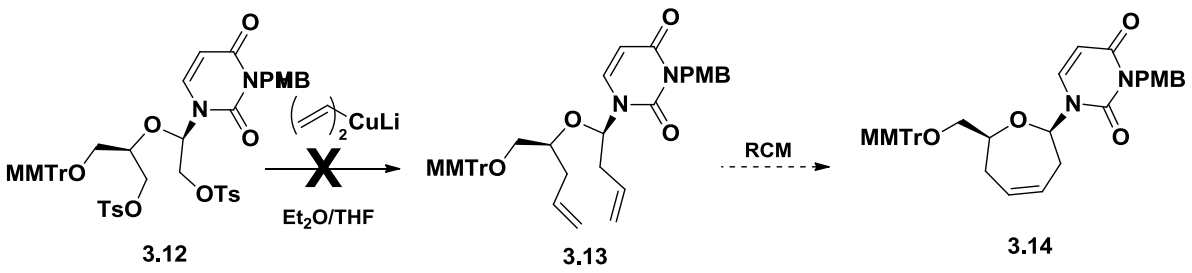


Figure 3.10 Expansion of ^{1}H NMR (DMSO-*d6)* of seconucleoside **3.11.** Installation of the PMB group produces a characteristic AB quartet, due to the PMB's group methylene protons.

3.4.2 Vinylation Reaction with Lithium Divinyl Cuprate

Once the ditosylate substrate **3.12** was synthesized, the coupling reaction was attempted (shown below in scheme 3.8) using lithium divinyl cuprate, a Gilman reagent. When the reaction was carried out at -40 °C, no reaction was observed. When the reaction was allowed to warm to 0 °C, color changes were observed, indicating that the Gilman reagent was decomposing, and unreacted starting material was recovered.



Scheme 3.8 Vinylation reaction with lithium divinylcuprate. Trials at -40 °C to 0°C did not produce the desired divinyl seconucleoside.

Instead of immediately abandoning this route, some attempts were made to troubleshoot this reaction. Since the lithium divinyl cuprate Gilman reagent was made *in situ,* as shown below in figure 3.11, the concentration of t-BuLi solution needed to be determined by titration. Many indicators for organolithium titrations[112-115] are available, but 1,3-diphenyl-2-propanone tosylhydrazone was selected as the preferred indicator because of its sharp and easily observable endpoint.[116]

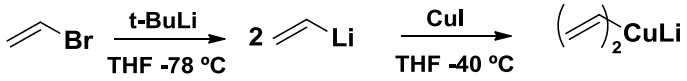


Figure 3.11 Formation of lithium divinyl cuprate. The Gilman reagent was formed *in situ* by a two-step process. First, vinyl bromide was converted to vinyl lithium via a lithium-halogen exchange reaction; then the Gilman reagent was formed by reacting the organolithium with copper iodide.

Once the quality of the *t*-BuLi had been ascertained, the copper iodide was purified by an existing protocol; purity of the copper salt is often critical to the success of coupling reactions.[117] Finally, the Gilman test,[118] using Michler's ketone was carried out, and is shown in figure 3.12. This qualitative test, first devised by Henry Gilman, allows for the detection of organolithium species. Since Michler's ketone does not react with organocopper reagents, the Gilman test allows for the indirect monitoring of Gilman reagent formation. Though useful, the Gilman test, it must be noted, cannot detect decomposition of the newly-formed Gilman reagents and so has limitations.

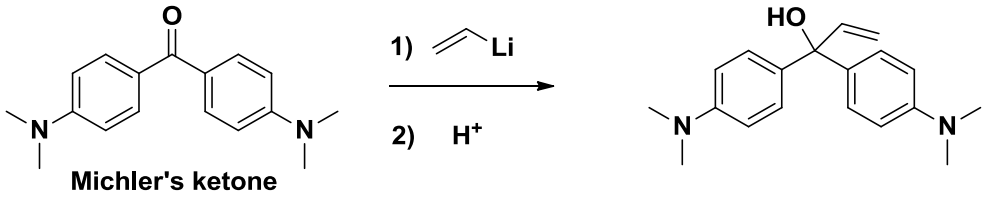


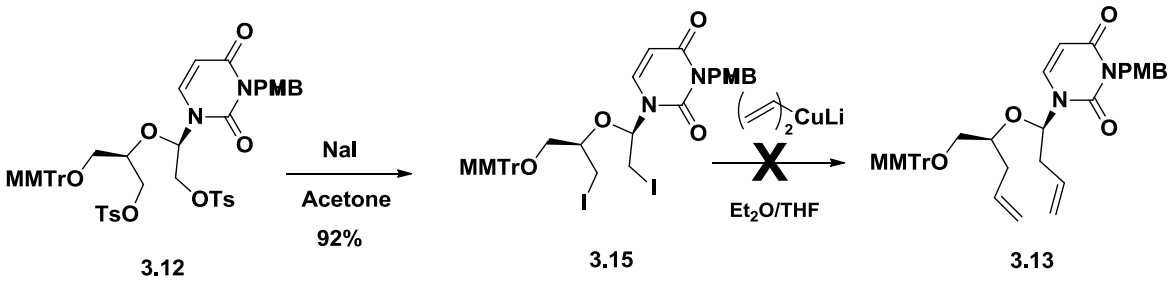
Figure 3.12 Gilman test for the presence of organolithium reagents. Treating an organolithium reagent with Michler's ketone produces a color change. Organocopper reagents do not produce a color change.

Even after taking these additional steps, attempts at nucleophilic coupling with lithium divinyl cuprate still did not yield the desired RCM precursor **3.13**. Accounts in the literature indicated that vinylic cuprates tend to be unstable and prone to thermal and oxidative decomposition[119,120]. Moreover, vinylic cuprates tend to be less reactive than alkyl –

or even aryl – cuprates, further complicating the chemistry of this crucial step.[103] At this point, it seemed like the organocopper route was a synthetic dead-end, but before abandoning it, a few more alternatives were explored. These are discussed in the following section.

3.4.3 Alternative Organocopper Approaches

To see if changing the leaving group would have an effect, the ditosylate **3.12** was converted to the di-iodo derivative **3.15** via the Finkelstein reaction,[121] and is shown below in scheme 3.9. Formation of the di-iodo required reflux conditions, as the Finkelstein reaction often does, but yielded the desired nucleoside in 92% yield. However, subjecting the di-iodo nucleoside to the same nucleophilic coupling conditions did not led to any observable product formation.



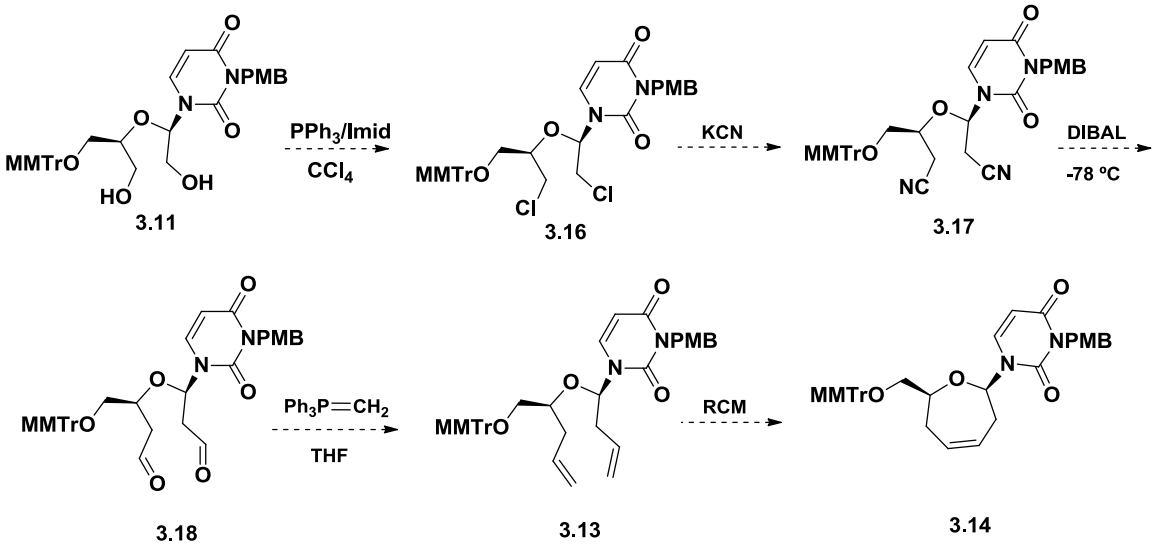
Scheme 3.9 Vinylation reaction attempt with di-iodo nucleoside. Di-iodo nucleoside **3.15** was formed via the Finkelstein reaction.

To enhance the stability of the Gilman reagent, phosphine ligands[100,122] were also assayed, but were not found to have an effect on the coupling reaction. Alternative copper salts, such as copper bromide[102,123] and copper cyanide[124,125] were also examined, but did not help this critical step. Other additives and conditions could have been assayed, but it was felt that the organocopper route was indeed a synthetic dead-end and that other approaches

should be developed. It was surmised that the lithium divinyl cuprate reagent was too sluggish and unstable to effectively undergo the coupling reaction. However, formation of the di-iodo nucleoside **3.15**, via the Finkelstein reaction, illustrated that nucleophilic coupling was indeed possible with a seconucleoside substrate.

3.5 Nitrile Chemistry Approach

Because of the difficulties encountered in the Grignard and organocopper routes, it was necessary to devise another approach. Formation of the di-iodo nucleoside **3.15** demonstrated that simple nucleophilic coupling could be used to generate seconucleoside derivatives. Building on this fact, a new synthetic scheme involving nitrile chemistry was formulated, and is shown below in scheme 3.10**.**



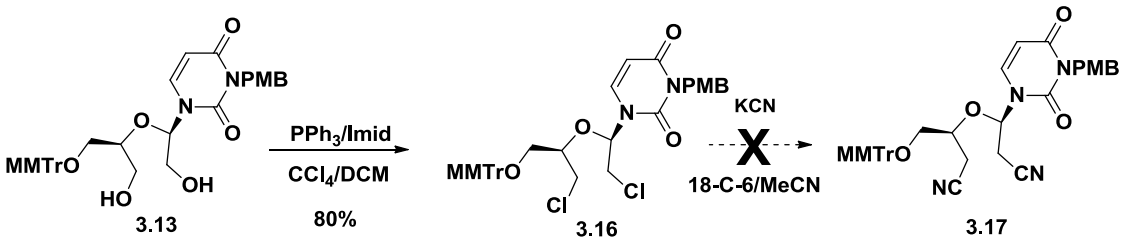
Scheme 3.10 Nitrile chemistry synthetic route. Formation of desired RCM precursor **3.13** is planned by adding one-carbon units at a time, and involves nitrile and Wittig chemistry.

In this approach, a dihalide, like dichloro **3.16**, is formed via a direct halogenation reaction from the doubly-protected seconucleoside **3.11.** Nucleophilic displacement with

cyanide would extent the carbon framework by one unit, generating dinitrile **3.17**. From this intermediate, a reduction with DIBAL would generate a homologated dialdehyde, which when subjected to a Wittig methyleneation could generate the desired RCM precursor **3.13**. Since the dialdehyde intermediate **3.18** contained extra carbons, it was surmised that the earlier problems of cyclization (section 3.2.1) and multiple hydroborations (section 3.2.5) would not be as significant, and so this could be a viable route. However, when this synthetic route was explored, problems were encountered in the formation of the dinitrile intermediate, and are discussed below.

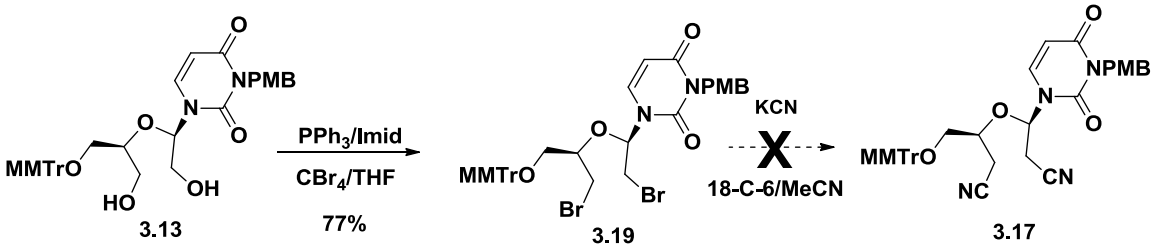
3.5.1 Acetonitrile-Crown Ether System for Nitrile Route

Nucleophilic substitution of halides by cyanide can be accomplished under various conditions, and as a starting point an acetonitrile-crown ether system was assayed. These conditions have been reported to be more efficient than ethanol-water solvent systems, and have been shown to work well even with bromides and chlorides in simple model systems, such as 1-chlorohexane and 1-bromohexane.[126] Synthesis of the dichloronucleoside **3.16**, shown below in scheme 3.11, proceeded smoothly, and in good yields, via a direct halogenation reaction employing triphenylphosphine and carbon tetrachloride in DCM.[127] However, treating this chlorinated substrate with KCN and the 18-C-6-MeCN system did not lead to any reaction, even with extended reaction times and even under reflux conditions.



Scheme 3.11 Nitrile synthetic route with dichlorides. Synthesis of dichloronucleoside **3.16** was accomplished via a direct halogenation reaction. The resulting dichloronucleoside was unreactive in the subsequent step.

Since the dichloronucleoside **3.16** proved to be too unreactive, a dibromonucleoside, **3.19** shown below in scheme 3.12, was synthesized. Formation of the brominated nucleoside was accomplished via a direct bromination reaction.[128-130] However, subjecting this more reactive substrate to the same 18-C-6-MeCN system did not lead to any observable reaction; only starting material was recovered.

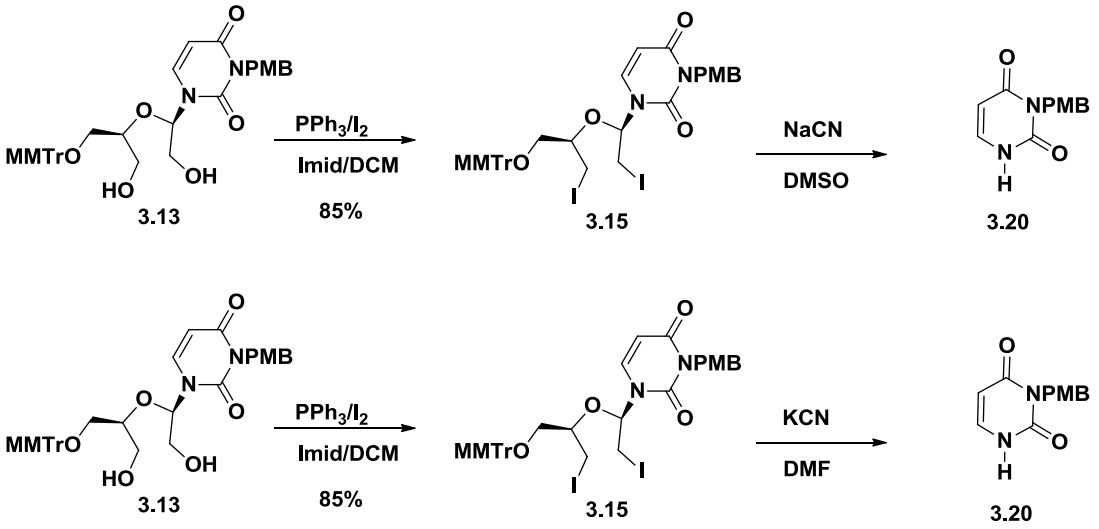


Scheme 3.12 Nitrile approach route with dibromides. Synthesis of dibromonucleoside **3.19** was accomplished via a direct halogenation reaction. The resulting brominated nucleoside was unreactive in the subsequent step.

3.5.2 DMSO and DMF Solvent Systems for Nitrile Route

Because of the lack of reactivity observed in the previous system, alternative conditions needed to be found. Examination of the literature showed many cases where halides were displaced by cyanide in DMSO or DMF, even in the absence of crown

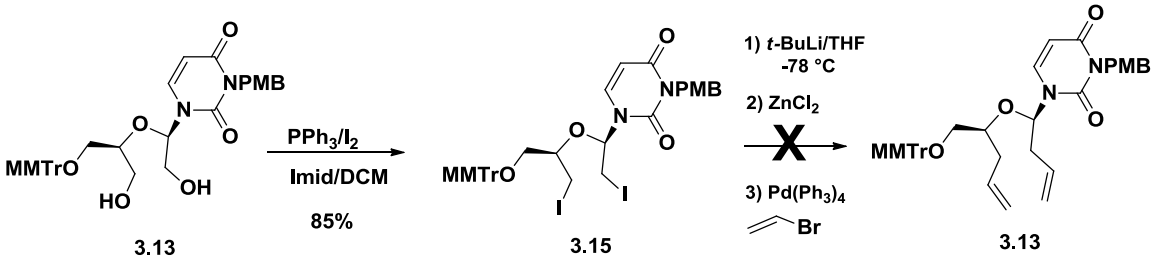
ethers.[131-133] To test these conditions, di-iodonucleoside **3.15** was synthesized via a direct iodination[134,135] reaction and used as the substrate. When this substrate was reacted with NaCN in DMSO at 40°C, the main recovered product was PMB-protected uracil, **3.20**, and is shown below in scheme 3.13. At room temperature, no reaction was observed. DMSO is known to be a reactive, nucleophilic solvent,[136] and so to rule out the possibility of a solvent effect, the reaction was repeated with DMF as the solvent, at 80 °C, and with KCN as the cyanide source. However, when the reaction was repeated under these conditions, the protected nucleobase, **3.20**, was again the main recovered product (at room temperature, no reaction was observed.). The exact mechanism for the decomposition of the nucleoside is not known, but may involve a substitution of the 2' iodine, followed by an elimination reaction. Direct nucleophilic substitution of the protected nucleobase by cyanide seems like an implausible reaction pathway, though it is of course possible. Regardless of the mechanism involved, these results indicated that a new synthetic route needed to be found.



Scheme 3.13 Nitrile approach route with iodides.. When this iodinated nucleoside was reacted with cyanide in DMSO or DMF, displacement of the protected nucleobase, **3.20**, was observed.

3.6 Negishi Coupling Approach

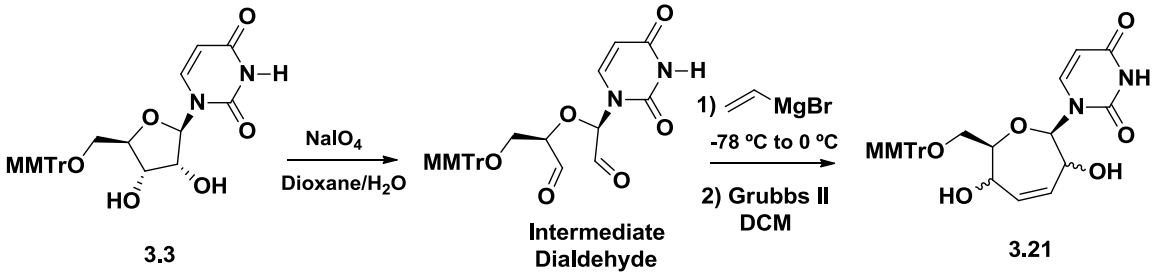
Up till this point, all of the synthetic approaches studied relied on nucleophilic cross-coupling, so the logical next step was to attempt a metal-catalyzed cross coupling reaction. The Negishi reaction, in particular, is a palladium (or nickel) catalyzed cross-coupling reaction that allows for carbon-carbon bond formation between saturated and unsaturated components.[137,138] In this approach, di-iodo nucleoside **3.15** was transformed into a diorganozinc halide *in situ*, and then reacted with vinyl bromide in the presence of palladium (0). When this reaction was initially carried out, it looked promising because complete consumption of starting material, as monitored by TLC, was observed within 3.5 hours. Inspection of ^{1}H NMR of the product indicated that coupling had indeed occurred, as evidenced by the appearance of signals in the olefinic region of the spectrum. However, this occurred with concomitant loss of the nucleobase through some unknown reaction pathway. Since the reaction conditions had been modeled on previous reports on the coupling of a homoallylic iodide, a fairly labile substrate, with a vinyl iodide fragment, this result was surprising.[139] Repeating this reaction, however, led to the same outcome and so this approach was abandoned.



Scheme 3.14 Negishi coupling approach using iodinated seconucleoside. Reaction led to some coupling with concomitant loss of the nucleobase.

3.7 Addition of Grignard Reagents to Dialdehyde Nucleosides

Even though synthetic roadblocks had been encountered in the previous attempts, the search for a synthetic method that would enable us to synthesize ONA monomers via an RCM approach was pursued. Few new methods, it seemed, were left to try, but the direct addition of a Grignard reagent to a nucleoside dialdehyde substrate was still unexplored. While designing this experiment, it was decided to leave the nucleoside unprotected at the N3 position to limit the amount of potential elimination product. In the presence of strong bases, such as Grignard regents, the N3 imido proton becomes deprotonated, generating an anion. For a subsequent elimination to occur, the nucleobase would have to leave as a dianion, which would be energetically unfavourable. To test this approach, a dialdehyde was generated *in situ* by the methods discussed in section 3.2. Subjecting this unstable intermediate to a Grignard addition with vinylmagnesium bromide, followed by a Grubbs II mediated RCM led to an inseparable mixture of ONA diastereomers, **3.21**, as shown in figure 3.15. Mass spectral analysis of the mixture showed peaks in the negative mode corresponding to the expected molecular weight. HRMS confirmed the elemental composition of **3.21** as being $C_{31}H_{29}N_2O_7$ (for M-H^+ in the negative mode).

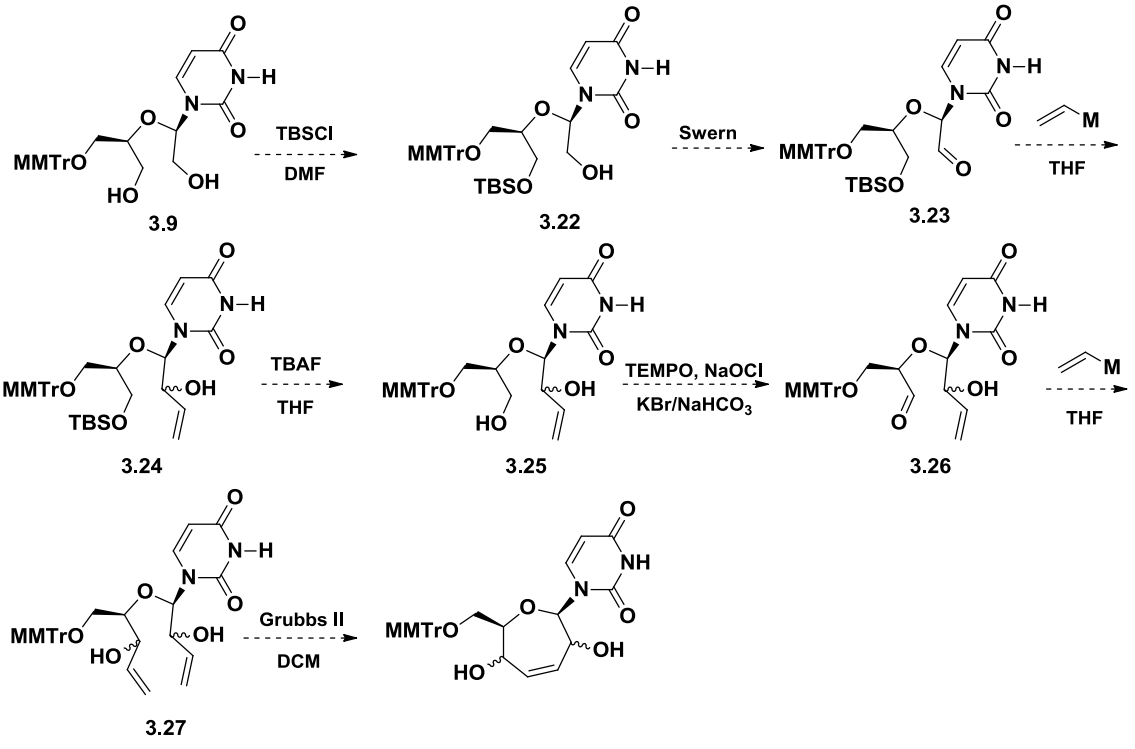


Scheme 3.15 Direct addition of vinylmagnesium bromide to an intermediate dialdehyde followed by RCM with Grubbs II led to an inseperable diastereomeric mixture **3.21**.

ONA mixture **3.21** was subsequently subjected to acetylation with acetic acid and pyridine, in an effort to alter the R_f values of the diastereomers and allow for chromatographic separation. However, the resulting diacetylated ONA mixture proved to be inseparable. HRMS analysis of the product of this reaction confirmed the molecular weight and elemental composition of the diacetylated ONA derivative.

3.8 Future Work

While the previous approach was a proof-of-principle for the synthesis of ONA via a ring-closing metathesis approach, it generated an inseparable mixture of diastereomers and introduced an unstable dialdehyde intermediate, factors which greatly limit the synthetic utility of this scheme. In order to fully explore this new approach to synthesizing ONA, a new scheme, shown below, is proposed.



Scheme 3.16 Proposed scheme for the synthesis of ONA via a ring closing approach.

To avoid the formation of the dialdehyde and to avoid the diastereoselectivity problem, a stepwise approach is suggested where only one stereocenter is set at a time. Since this synthesis would start with seconucleoside **3.9**, the intermediate dialdehyde would be avoided altogether. Monoprotection[86] of **3.9** with TBSCl, followed by separation of the monoprotected products would generate compound **3.22.** The subsequent steps involve performing Grignard additions sequentially. Key to this approach is the judicious use of the metal to tune the Felkin-Ahn or Cram Chelate selectivity in the Grignard addition steps used to generate intermediates **3.24** and **3.27**. Metals components, labeled M in scheme 3.16, ranging from $Ti(OiPr)_3$, $TiCl_3$, $Zr(OiPr)_3$, to $AlMe_3$, could be assayed to maximize the stereoselectivity of the Grignard addition reaction.[140] To limit the protection/deprotection steps, a selective oxidation (employing TEMPO/NaOCl as the oxidant) of the primary alcohol over the secondary alcohol is proposed to generate structure **3.26**.[141] Once the RCM precursor **3.27** is synthesized, the desired oxepene monomer could be made via a ring closing metathesis. By tuning the stereoselectivity of the Grignard addition steps, four different diastereomers could in principle be generate by this method.

Chapter 4 Experimental

4.1 General Methods

4.1.1 Solvents and Reagents

Anhydrous tetrahydofuran, dicholoromethane and acetonitrile were dried using an Mbraun solvent purification system. Anhydrous DMF and DMSO were obtained from sequential drying over molecular sieves. Anhydrous benzene was obtained by drying over activated alumina, Brockmann activity I. Pyridine was dried by distillation over CaH_2. Acetone was dried by stirring over 3 Å molecular sieves for 4 hours or by alternatively by stirring over boric anhydride followed by distillation. Sodium iodide was dried by heating (with stirring) *in vacuo* overnight in the dark. Methyltriphenylphosphonium bromide, MMTrCl, nucleosides and other reagents were dried by azeotropic condensations from benzene, toluene or pyridine. Molecular sieves were activated by heating under reduced pressure to drive off water. Other anhydrous solvents and reagents were purchased from Sigma-Aldrich. All anhydrous reactions were carried under an atmosphere of nitrogen gas in flame-dried glassware.

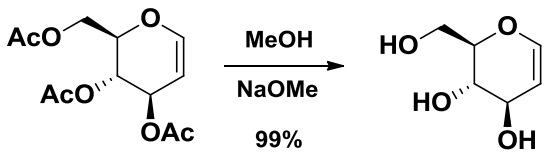
4.1.2 Purification and Characterization of Compounds

Compounds were purified column chromatography using 40-60 micron (230-400 mesh) silica gel from Silicycle. Reactions were monitored by thin-layer chromatography using Merck Kieselgel 60 F-254 aluminum backed silica gel sheets. Visualization was achieved using UV light and with 5 % solutions of H_2SO_4 in MeOH. NMR spectra were obtained using 500 MHz, 400 MHz and 300 MHz instruments. Solvent peaks of $CDCl_3$ and DMSO-*d6* were used as internal standards. Routine mass spectral data was acquired using low

resolution ESI-MS in both negative and positive modes. Elemental composition of certain compounds was obtained using HRMS analysis.

4.2 Chapter 2 Characterization

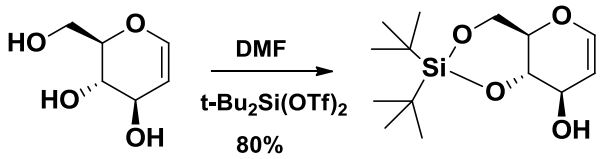
D-Glucal (2.10)



2.9 **2.10**

To a stirred solution of peracetylated D-glucal **2.9** (5.023g, 18.47 mmol) in 40 mL of MeOH was added 0.050 mL of 25% NaOMe/MeOH. After 2.5 hours at room temperature, the solvent was removed by rotary evaporation. Subjecting the crude mixture to column chromatography (9:1 DCM/MeOH) yielded 2.668 g of **2.10** as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 6.45 (d, *J* = 6.1, 1H), 4.84 (dd, *J* = 6.1, 2.4, 1H), 4.26 (d, *J* = 7.0, 1H), 3.98 – 3.86 (m, 3H), 3.75 – 3.64 (m, 1H). ESI-MS calcd. for $C_{14}H_{26}O_4Si$ [M+Na^+] 309.16, found 309.17.

4,6-*O*-Di-(tert-butyl)silanediyl-D-glucal (2.11)

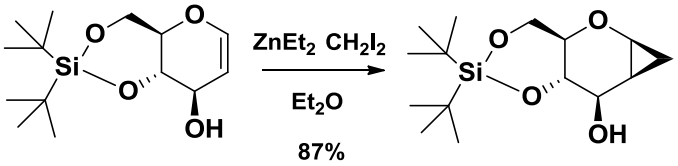


2.10 **2.11**

Dissolved **2.10** (2.612 g, 17.89 mmol, 1eq) in 70 mL of anhydrous DMF under constant N_2 flow. The resulting solution was then cooled to -40 °C. Added t-$Bu_2Si(OTf)_2$ dropwise (6.93 mL, 21.44 mmol, 1.2 eq) over a period of five minutes. Reaction was then

stirred at -40 °C for 50 minutes. Quenching of the reaction was achieved by adding 2 mL of pyridine (24.73 mmol, 1.4 eq) followed by stirring for an additional 15 minutes. Diluted reaction mixture with ethyl acetate; and organic layer was washed successively with 5% $NaHCO_3$ and brine solutions, dried with $MgSO_4$ and concentrated *in vacuo*. Purification of the crude product by column chromatography (25:1 to 5:1 gradient Hexanes/EtOAc) afforded 4.090 g of **2.11** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.25 (dd, J = 6.0, 1.8, 1H), 4.74 (dd, J = 6.1, 1.9, 1H), 4.28 (d, J = 6.0, 1H), 4.16 (dd, J = 10.2, 4.9, 1H), 3.98 – 3.77 (m, 3H), 2.39 (d, J = 2.0, 1H), 1.05 (s, 9H), 0.97 (s, 9H). ESI-MS calcd. for $C_{14}H_{26}O_4Si$ [M+Na^+] 309.16, found 309.17.

1,5-Anhydro-2-deoxy-1,2-C-methylene-4,6-*O*-(di-tertbutylsilanediyl)-D-*glycero*-D-hexitol (2.12)

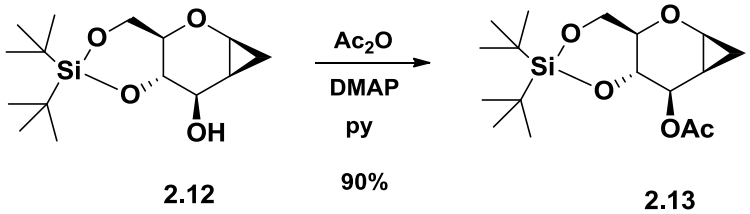


2.11 **2.12**

To a stirred solution of **2.11** (1.323 g, 4.62 mmol) in 20 mL of anhydrous diethyl ether at 0 °C was added 12.60 mL of 1.1 M diethyl zinc solution (13.86 mmol, 3 eq) drop wise followed by 1.12 mL of CH_2I_2 (13.86 mmol, 3 eq) under flow of N_2. When the reaction had gone to completion after 6 hour, 50 mL of saturated NH_4Cl solution was added as a quenching agent. The aqueous layer was extracted with ethyl acetate; and the organic extracts were washed with 5% $NaHCO_3$ and saturated brine solutions. Drying of the organic layer was effected by $MgSO_4$, and the solvent was removed by rotary evaporation. Subjecting the crude reaction mixture to flash column chromatography (10:1 to 5:1 gradient hexanes/EtOAc) afforded 1.219 g of **2.12**, which was collected as a white solid. ^{1}H NMR

(400 MHz, $CDCl_3$) δ 4.15 (t, *J* = 7.4, 1H), 4.07 (dd, *J* = 10.4, 4.9, 1H), 3.78 – 3.73 (m, 1H), 3.68 (t, *J* = 10.2, 1H), 3.48 (dd, *J* = 9.7, 8.0, 1H), 3.36 (td, *J* = 10.0, 5.0, 1H), 2.55 (bs, 1H), 1.48 – 1.39 (m, 1H), 1.03 (s, 9H), 0.97 (s, 9H), 0.79 – 0.71 (m, 2H). ESI-MS calcd. for $C_{15}H_{28}O_4Si$ [M+Na^+] 323.18, found 323.20.

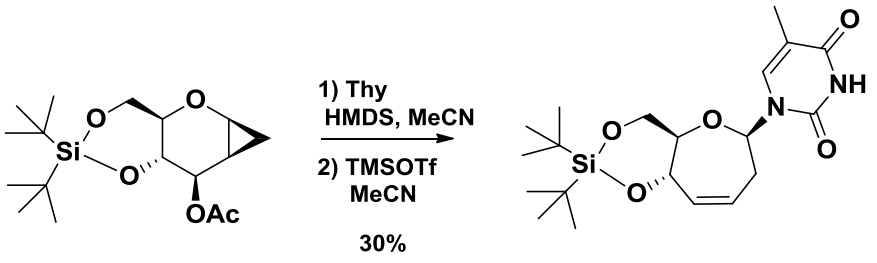
3-Acetyl-1,5-anhydro-2-deoxy-1,2-*C*-methylene-4,6-*O*-(di-tert-butylsilanediyl)-D-*glycero*-D-hexitol (2.13)



2.12 **2.13**

To a stirred solution of **2.12** (2.592 g, 7.57 mmol, 1 eq) and a catalytic amount of DMAP (90 mg) in 15 mL of anhydrous pyridine was added 3.258 mL of Ac_2O (34.47 mmol, 4.5 eq) dropwise at 0 °C and under an atmosphere of N_2. When the reaction had gone to completion, as monitored by TLC, the reaction mixture was diluted with diethyl ether. The organic layer was washed with water, dried with $MgSO_4$, and concentrated *in vacuo*. Purification by column chromatography (10:1 to 5:1 hexanes/EtOAc gradient) yielded 2.334 g of **2.13,** which was recovered as an oily substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 – 5.14 (m, 1H), 4.07 (dd, *J* = 10.4, 4.9, 1H), 3.77 – 3.57 (m, 3H), 3.40 (td, *J* = 10.0, 4.9, 1H), 2.11 (s, 3H), 1.61 – 1.52 (m, 1H), 0.99 (s, 9H), 0.94 (s, 9H), 0.69 (dd, *J* = 8.7, 4.3, 2H). ESI-MS calcd. for $C_{17}H_{30}O_5Si$ [M+Na^+] 365.19, found 365.13

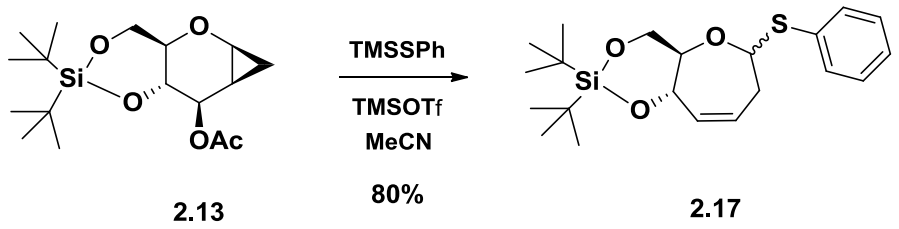
(1R)-1-[(2,3,4-Trideoxy-(5S,6R)-5,7-di-tert-butylsilanediyl)-β-oxepinyl]thymine (2.14)



2.13 **2.14**

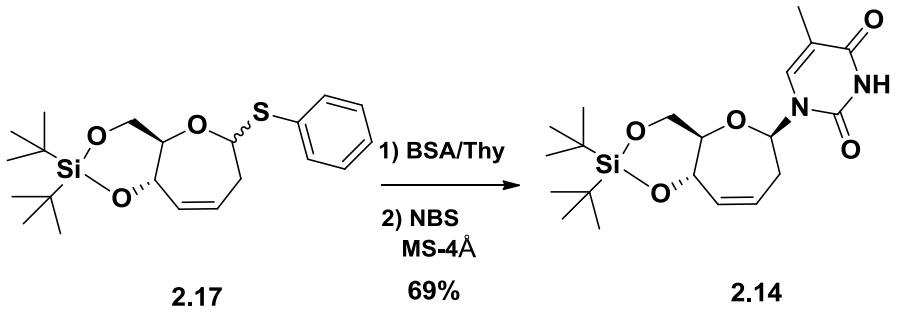
Refluxed 4.824 g of thymine (38.25 mmol, 5.1 eq), 12.59 ml of HMDS, and 527 mg of $(NH_4)_2SO_4$ in MeCN for four hours until solution became clear, which indicated that the nucleobase had been silylated. Removed solvent by rotary evaporation in a heated water bath. Transfer a solution of **2.13 (**2.342 g, 7.56 mmol, 1 eq) in 25 mL of MeCN via a cannula into the flask containing the recently silylated nucleobase under an atosphere of N_2. Once substrate **2.13** had been transferred, added 464 μL of TMSOTf dropwise. Reluxed reaction mixture overnight. When reaction had went to completion, as monitored by TLC, mixture was diluted with ethyl acetate. Addition of 5% $NaHCO_3$ caused a white precipitate to form, which was then filtered off. The aqueous layers was drawn off in a separatory funnel, and the organic layer was susquently washed with brine, dried with $MgSO_4$, and condensed down. The crude product was purified (20:1 to 2:1, hexanes/EtOAc gradient) by column chromatography and yielded 1.012 g of **2.14**, β anomer, R_f = 0.22 in 2:1 Hex:EtOAc. (α anomer, R_f = 0.20 in 2:1 Hex:EtOAc) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.19 (d, *J* = 1.0, 1H), 5.91 (dt, *J* = 12.2, 2.4, 1H), 5.73 (dd, *J* = 10.3, 1.6, 1H), 5.69 (dd, *J* = 7.4, 4.4, 1H), 4.59 (dd, *J* = 9.2, 2.2, 1H), 4.06 (dd, *J* = 10.6, 4.8, 1H), 3.85 (t, *J* = 10.4, 1H), 3.68 – 3.57 (m, 1H), 2.63 – 2.51 (m, 1H), 2.43 – 2.32 (m, 1H), 1.93 (d, *J* = 1.0, 3H), 1.05 (s, 9H), 0.98 (s, 9H). ESI-MS calcd. for $C_{20}H_{32}N_2O_5Si$ [M+Na$^+$] 431.21, found 431.18.

(1R,7S)-3-(Phenylthio)-9,9-di-tert-butyl-2,8,10-trioxa-9-silabicyclo[5.4.0]undec-5-ene (2.17)



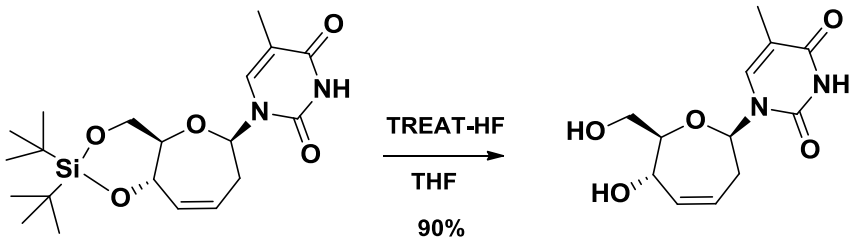
To a stirred solution of **2.13** (3.601 g, 10.50 mmol, 1 eq) in 30 mL of MeCN at 0 °C was added 4.96 mL of TMSSPh dropwise followed by 350 μL of TMSOTf under an atmosphere of N_2. Reaction was ran overnight and quenched by the addition of 5% $NaHCO_3$. Aqueous layer was extracted with ethyl acetate; organic extracts were washed with brine and dried over $MgSO_4$. Flash chromatography of the crude (250:1 to 50:1 gradient, hexanes/EtOAc) yielded 3.358 g of **2.17**, mixture of anomers, as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.41 (m, 4H), 7.36 – 7.21 (m, 6H), 5.88 – 5.81 (m, 1H), 5.79 (t, *J* = 2.2, 1H), 5.72 – 5.62 (m, 2H), 5.45 (dd, *J* = 6.2, 4.4, 1H), 4.87 (dd, *J* = 9.9, 2.6, 1H), 4.66 – 4.59 (m, 2H), 4.37 (td, *J* = 9.9, 4.9, 1H), 3.93 – 3.81 (m, 4H), 3.36 (dd, *J* = 16.2, 8.1, 1H), 2.85 – 2.52 (m, 4H), 1.04 (s, 9H), 1.03 (s, 9H), 1.01 (s, 9H), 0.95 (s, 9H). ESI-MS calcd. for $C_{21}H_{32}O_3SSi$ [M-H$^+$] 391.18, found 391.17.

(1R)-1-[(2,3,4-Trideoxy-(5S,6R)-5,7-di-tert-butylsilanediyl)-β-oxepinyl]thymine (2.14)



To a stirred solution of **2.17** (1.159 g, 2.95 mmol, 1 eq), thymine (0.744 g, 5.90 mmol, 2 eq), and 200 mg of $(NH_4)_2SO_4$ in 50 mL of DCM was added 0.793 mL of BSA dropwise under an atosphere of N_2. Refluxed until solution became clear, cooled solution to ambient temperature, then added 2 g of 4 Å molecular sieves and 578 mg NBS (3.25 mmol, 1.1 eq). Refluxed reaction overnight and then quenched with 5% $NaHCO_3$. Filtered reaction mixture over Celite filter aid. Extracted with ethyl acetate, washed organic extracts with brine and dried over $MgSO_4$; condensed organic layer under reduced pressure. The crude product was purified (20:1 to 2:1, hexanes/EtOAc gradient) by column chromatography and yielded 0.823 g of **2.14,** β anomer, R_f = 0.22 in 2:1 Hex:EtOAc. (α anomer, R_f = 0.20 in 2:1 Hex:EtOAc) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.19 (d, *J* = 1.0, 1H), 5.91 (dt, *J* = 12.2, 2.4, 1H), 5.73 (dd, *J* = 10.3, 1.6, 1H), 5.69 (dd, *J* = 7.4, 4.4, 1H), 4.59 (dd, *J* = 9.2, 2.2, 1H), 4.06 (dd, *J* = 10.6, 4.8, 1H), 3.85 (t, *J* = 10.4, 1H), 3.68 – 3.57 (m, 1H), 2.63 – 2.51 (m, 1H), 2.43 – 2.32 (m, 1H), 1.93 (d, *J* = 1.0, 3H), 1.05 (s, 9H), 0.98 (s, 9H). ESI-MS calcd. for $C_{20}H_{32}N_2O_5Si$ $[M+Na^+]$ 431.21, found 431.18.

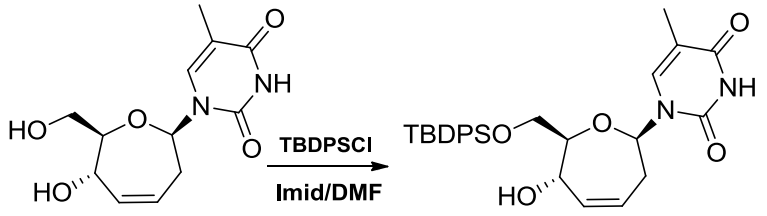
(1R)-1-[(2,3,4-Trideoxy-(5S,6R)-5-hydroxy-7-hydroxymethyl)-β-oxepinyl]thymine (2.15)



2.14 **2.15**

To a stirred solution of 767 mg of **2.14**(1.88 mmol, 1 eq) in 5 mL of THF was added 612 μL of TREAT-HF dropwise at 0 °C. After 3 hours, reaction was observed to go to completion. Solvent and residual TREAT-HF was removed by a rotary evaporator connected to an external vacuum pump. Purified crude product by flash chromatography (5% MeOH/DCM) and obtained 452 mg of **2.15**. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.31 (s, 1H), 7.60 (s, 1H), 5.77 – 5.71 (m, 1H), 5.67 – 5.54 (m, 2H), 4.03 (dd, *J* = 10.9, 4.6, 1H), 3.68 – 3.61 (m, 1H), 3.47 – 3.36 (m, 2H), 3.16 (d, *J* = 5.3, 1H), 2.74 – 2.64 (m, 1H), 2.34 (m, 1H), 1.78 (s, 3H), 1.56 (s, 1H). ESI-MS calcd. for $C_{12}H_{16}N_2O_5$ [M+Na$^+$] 291.11, found 291.14

(1R)-1-[(2,3,4-Trideoxy-(5S,6R)-5-hydroxy-7-*t*-butyldiphenylsilyl)-β-oxepinyl]thymine (2.18)



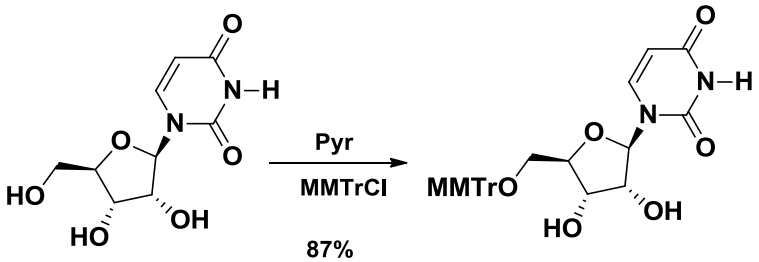
2.15 **2.18**

To a stirred solution of 203 mg of **2.15** (0.76 mmol, 1 eq) and 77 mg of imidazole (1.13 mmol, 1.5 eq) in 5 mL of DMF was added 217 μL of TBDPSCl dropwise (0.83 mmol, 1.1 eq) at 0 °C under an atmosphere of N_2. Reaction was run overnight at room temperature

and quenched with 5% $NaHCO_3$ solution. Extracted several times with ethyl acetate, washed organic extracts with brine, dried over $MgSO_4$ and removed solvent *in vacuo*. Purification by flash chromatography (1% to 5% MeOH/DCM gradient) yielded 307 mg of **2.15**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.68 – 7.60 (m, 4H), 7.45 – 7.32 (m, 6H), 7.18 (s, 1H), 5.85 (dt, *J* = 12.3, 2.5, 1H), 5.77 – 5.71 (m, 1H), 5.70 – 5.61 (m, 1H), 4.54 (dd, *J* = 8.9, 2.2, 1H), 3.97-3.82 (m, 3H), 3.65 (dt, *J* = 9.3, 4.7, 1H), 2.59 – 2.36 (m, 2H), 1.87 (d, *J* = 1.1, 3H), 1.04 (s, 9H). ESI-MS calcd. for $C_{28}H_{34}N_2O_5Si$ [M+Na^+] 529.22, found 529.21.

4.3 Chapter 3 Characterization

5'-*O*-Monomethoxytrityl-β-D-uridine (3.4)

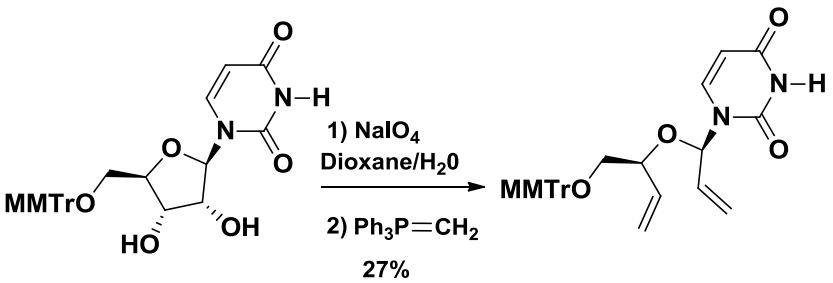


3.3 **3.4**

To a stirred solution of 3.022 g of **3.3** (12.38 mmol, 1 eq) in 60 mL of anhydrous pyridine was added (via cannula transfer) a solution of 4.185 g of MMTrCl (13.55 mmol, 1.09 eq) under an atmosphere of nitrogen. After running reaction overnight at room temperature, a 5% $NaHCO_3$ solution was added as a quench and reaction was stirred for an additional 15 minutes. Most of the pyridine was then removed by rotary evaporation; and the residue was dissolved in DCM. Organic layer was washed with water and brine, dried over $MgSO_4$ and finally condensed down. Purification by column chromatography (2% to 5% MeOH/DCM gradient) afforded 5.467 g of **3.4** as a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.38 (s, 1H), 7.72 (d, *J* = 8.0, 1H), 7.41 – 7.21 (m, 12H), 6.92 (d, *J* = 8.9, 2H), 5.74 (d, *J* = 3.1, 1H),

5.53 (d, J = 4.6, 1H), 5.31 (d, J = 8.0, 1H), 5.18 (d, J = 5.5, 1H), 4.12 – 4.04 (m, 2H), 3.95 (d, J = 2.7, 1H), 3.75 (s, 3H), 3.31 – 3.17 (m, 2H). ESI-MS calcd. for $C_{29}H_{28}N_2O_7$ [M+Na^+] 539.19, found 539.18.

1-{1-[1-(4,4'-Monomethoxytrityloxymethyl)prop-2-enyloxy]prop-2-enyl}uracil (3.5)



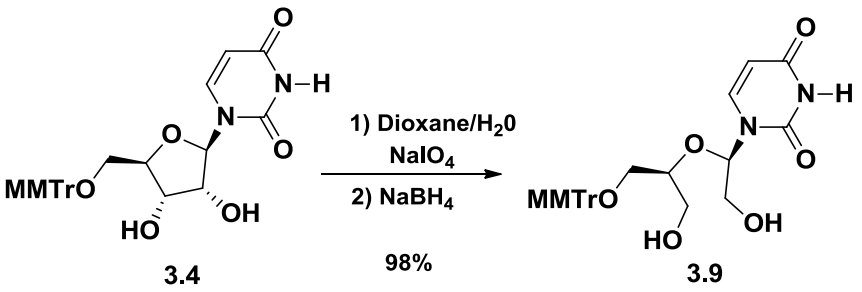
3.4 **3.5**

To a stirred solution of 582.6 mg of **3.4** (1.13 mmol, 1 eq) in 10 mL of dioxane was added 280.1 mg of $NaIO_4$ (suspended in 2 mL of H_2O) dropwise. Reaction proceeded at room temperature for 3 hours and then was diluted with 10 mL of dioxane. After filtering over Celite filter aid, the reaction was worked-up with DCM and brine, dried over $MgSO_4$ and condensed down. Residues were dried by azeotropic condensation from benzene and then dissolved in 60 mL of anhydrous benzene. Solution was then refluxed and 40 mL were removed via a Dean-Stark trap over a 2 hour period in order to dehydrate dioxane ring. Residual amount of benzene was removed by rotary evaporation, and the residue was subjected to high-vacuum until used in section below.

To a stirred suspension of 4.577 g of dry methyltriphenylphosphonium bromide in 13 mL of anhydrous THF was added 12 mL of 1M KOtBu solution dropwise at 0 °C. Reaction was allowed to stir for 1 hour at room temperature; resulting yellow solution was the 0.50 M ylide solution used below.

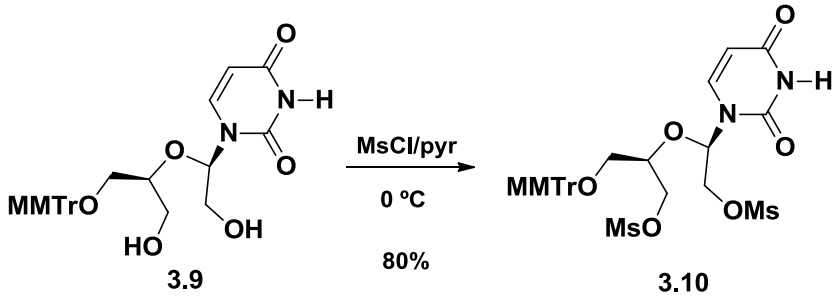
To a stirred solution of 1 mmol of dialdehyde intermediate (generated in first part) in 10 mL of THF was added 10 mL of 0.50 M ylide solution (5 mmol, 1 eq) dropwise at 0 °C. Reaction was stirred for 1 hour 15 minutes at room temperature, at which point complete consumption of starting material was observed. Water was added to reaction mixture using a Pasteur pipette until yellow color disappeared in order to quench excess ylide. Diluted reaction mixture with DCM and washed with saturated NH_4Cl solution and with brine. Extracted aqueous layers with DCM, dried pooled organic extracts over $MgSO_4$, and condensed down to an oil. Purification by flash chromatography (isocratic 3:2 hexanes/EtOAc) yielded 125 mg of **3.5**. It should be noted that under these conditions another compound co-elutes with the desired 3.5 and is possibly an isomerized epimer of 3.5. See discussion of potential enolization of dialdehyde in section 3.2.4. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.48 (d, *J* = 8.1, 1H), 7.42 – 7.35 (m, 4H), 7.29 – 7.24 (m, 7H), 7.21 (d, *J* = 2.3, 1H), 6.80 (d, *J* = 8.9, 2H), 6.23 – 6.20 (m, 1H), 5.69 (dd, *J* = 8.1, 2.3, 1H), 5.59 (dd, *J* = 17.2, 10.1, 3H), 5.41 (d, *J* = 10.5, 1H), 5.30 (d, *J* = 4.0, 1H), 5.26 (s, 1H), 3.87 (d, *J* = 2.8, 1H), 3.78 (s, 3H), 3.32 (dd, *J* = 10.4, 8.2, 1H), 3.03 (dd, *J* = 10.5, 3.1, 1H). ESI-MS calcd. for $C_{31}H_{30}N_2O_5$ [M+Na^+] 533.22, found 533.11. For an alternative procedure and for spectra of similar compounds, see synthesis of d4T.[88]

5'-*O*-Monomethoxytrityl-2',3'-seco-β-D-uridine (3.9)



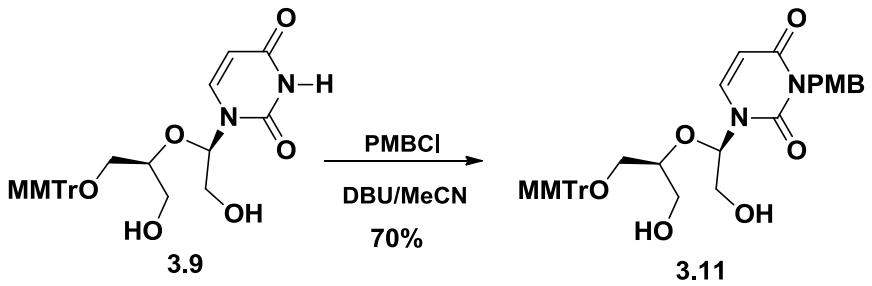
To a stirred solution of 2.759 g of **3.4** (5.35 mmol, 1 eq) in 25 mL of dioxane was added 1.2622 g of $NaIO_4$ (5.89 mmol, 1.1 eq) and 5 mL of water. When oxidative cleavage had gone to completion, as monitored by TLC, within 3 hours, the reaction mixture was diluted with an additional 25 mL of dioxane. Filtered diluted reaction mixture and added 228.5 mg (5.91 mmol, 1.1 eq) of $NaBH_4$. Reduction was allowed to proceed to completion, about 20 minutes, at room temperature. Acetone (10 mL) was added to quench excess reducing agent and reaction was stirred for an additional 10 minutes. A small amount of saturated NH_4Cl was added to neutralize reaction mixture, and solvent volume of reaction mixture was decreased to around 15 mL by rotary evaporation. (Alternatively, dioxane can be removed under reduced pressure and NH_4Cl neutralization can be done in work-up.) Reaction mixture was diluted with DCM, washed with water and brine, dried over $MgSO_4$ and condensed down. Recovered 2.713 g of **3.9** as a white foam; reaction proceeds cleanly and column chromatography is unnecessary. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.36 (s, 1H), 7.64 (d, *J* = 8.0, 1H), 7.34 – 7.19 (m, 10H), 7.17 (d, *J* = 8.9, 2H), 6.87 (d, *J* = 8.9, 2H), 5.81 (t, *J* = 5.8, 1H), 5.52 (d, *J* = 8.0, 1H), 5.13 (t, *J* = 5.9, 1H), 4.75 (t, *J* = 5.5, 1H), 3.74 (s, 3H), 3.71 – 3.52 (m, 3H), 3.40 (t, *J* = 5.2, 2H), 3.04 – 2.88 (m, 2H). ESI-MS calcd. for $C_{29}H_{30}N_2O_7$ [M+Na^+] 541.21, found 541.19.

5'-*O*-Monomethoxytrityl-2',3'-*O*-dimesyl-seco-β-D-uridine (3.10)



To a stirred solution of 303.1 mg of **3.9** (0.59 mmol, 1 eq) in 5 mL of pyridine at 0 °C was added 200 μL of MsCl. Reaction was stirred for 2.5 hours at 0 °C and then solvent was removed by rotary evaporation. Dissolved residue in DCM, washed with water and brine, dried over $MgSO_4$ and condensed down. Purification by column chromatography (0% to 5% MeOH/DCM gradient) yielded 319 mg of **3.10** as a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.49 (d, *J* = 1.9, 1H), 7.70 (d, *J* = 8.1, 1H), 7.36 – 7.15 (m, 12H), 6.91 – 6.85 (m, 2H), 6.05 (t, *J* = 5.7, 1H), 5.57 (dd, *J* = 8.0, 2.1, 1H), 4.49 (d, *J* = 5.6, 2H), 4.41 – 4.25 (m, 2H), 3.74 (s, 3H), 3.22 (s, 3H), 3.16 (s, 3H), 3.15 – 2.97 (m, 3H). ESI- MS calcd. for $C_{31}H_{34}N_2O_{11}S_2$ [M+Na$^+$] 697.16, found 697.05.

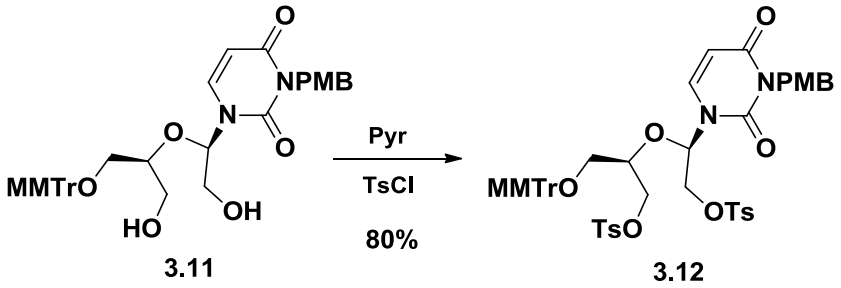
5'-*O*-Monomethoxytrityl-2',3'-seco-β-D-(*N*3-*p*-methoxybenzyl)-uridine (3.11)



To a stirred solution of 2.161 g of **3.9** (4.17 mmol, 1 eq) in 42 mL of acetonitrile was added 1.25 mL of DBU (8.35 mmol, 2 eq) dropwise followed by 1.02 mL of PMBCl (7.52

mmol, 1.8 eq) dropwise. Heated reaction mixture to 60 °C and stirred reaction for 1 hour. Diluted with DCM and washed organic layer with 5% NaHCO3 and brine. Dried organic layer over $MgSO_4$ and condensed. Neutralized silica gel with 1% NEt_3/DCM and purified crude reaction mixture by flash chromatography (1% to 3% MeOH/DCM gradient). Recovered 1.869 g of **3.11**, which was a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.67 (d, *J* = 8.0, 1H), 7.30 – 7.15 (m, 12H), 7.12 (d, *J* = 8.8, 2H), 6.81 (d, *J* = 8.9, 2H), 6.76 (d, *J* = 8.7, 2H), 5.85 (t, *J* = 5.7, 1H), 5.66 (d, *J* = 8.0, 1H), 5.12 (t, *J* = 6.0, 1H), 4.91 (abq, *J* = 14.1, 2H), 4.75 (t, *J* = 5.5, 1H), 3.71 (s, 3H), 3.64 (s, 3H), 3.69 – 3.50 (m, 3H), 3.40 (t, *J* = 5.3, 2H), 3.00 – 2.92 (m, 2H). ESI-MS calcd. for $C_{37}H_{38}N_2O_8$ [M+Na^+] 661.26, found 661.27.

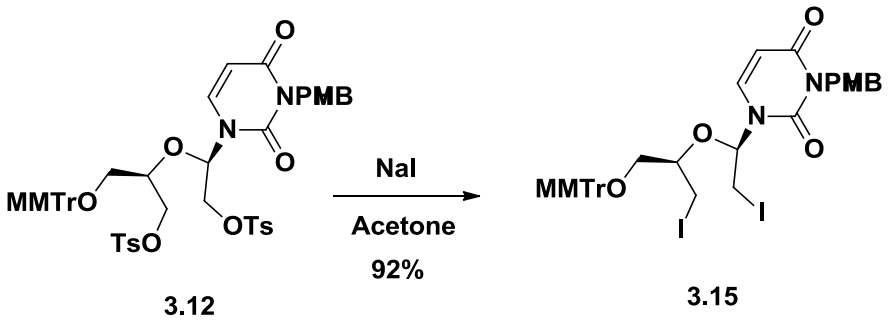
5'-*O*-Monomethoxytrityl-2',3'-*O*-ditosyl-seco-β-D-(*N*3-*p*-methoxybenzyl)-uridine (3.12)



To a stirred solution of 1.552 g of **3.11** (2.4 mmol, 1 eq) in 10 mL of pyridine added a second solution of 1.858 g of TsCl in 10 mL of pyridine (9.75 mmol, 4.06 eq) via cannula transfer under atmosphere of N_2 flow. Reaction was run overnight at room temperature. Diluted with DCM and worked up with 5% $NaHCO_3$ and brine solutions. (Alternatively, pyridine can be removed first and residue dissolved in DCM.) Organic layer was dried over $MgSO_4$ and condensed down. Purification by column chromatography (0.5% to 1% acetone/DCM) afforded 1.794 g of **3.12** as a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.71 (d, *J* = 8.3, 2H), 7.63 (d, *J* = 8.3, 2H), 7.45 – 7.39 (m, 3H), 7.32 (d, *J* = 8.1, 2H), 7.23 –

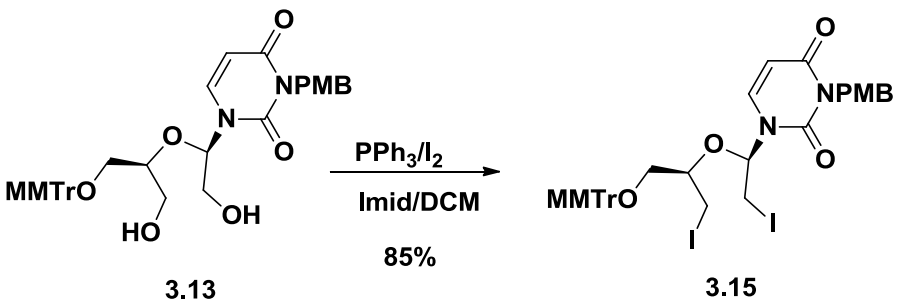
7.12 (m, 7H), 7.02 (dd, *J* = 8.9, 2.6, 4H), 6.78 (d, *J* = 8.7, 6H), 5.87 (t, *J* = 5.2, 1H), 5.50 (d, *J* = 8.1, 1H), 4.77 (abt, *j* = 16, 2H), 4.32 – 4.24 (m, 1H), 4.19 – 3.92 (m, 3H), 3.75-3.70 (m, 2H), 3.72 (s, 3H), 3.67 (s, 3H), 2.94 – 2.78 (m, 2H), 2.39 (s, 3H), 2.37 (s, 3H). ESI- MS calcd. for $C_{51}H_{50}N_2O_{12}S_2$ [M+Na^+] 969.28, found 969.13.

5'-*O*-Monomethoxytrityl-2',3'-*O*-di-iodo-seco-β-D-(*N*3-*p*-methoxybenzyl)-uridine (3.15)



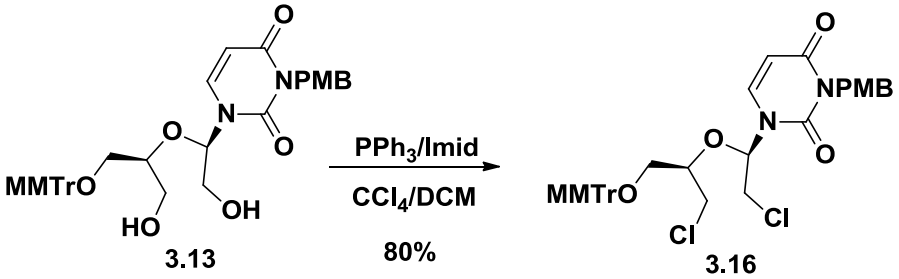
To a stirred solution of 1.173 g of **3.12** (1.24 mmol, 1 eq) in 25 mL of dry acetone was added 829.7 mg of previously dried NaI. Reaction was run under reflux conditions overnight. Reaction mixture was then diluted with DCM, and sodium tosylate precipitate was filtered off. Washed organic layer with brine; extracted aqueous layers with DCM. Dried organic extracts over $MgSO_4$, and condensed pooled organic fractions. Recoverd 982 mg of **3.15** as a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.76 (d, *J* = 8.1, 1H), 7.31 – 7.12 (m, 14H), 6.84 (d, *J* = 8.9, 2H), 6.79 (d, *J* = 8.7, 2H), 5.97 (t, *J* = 6.5, 1H), 5.76 (d, *J* = 8.0, 1H), 4.97 – 4.86 (m, 2H), 3.73 (s, 3H), 3.66 (s, 3H), 3.62 – 3.39 (m, 5H), 3.12 – 2.94 (m, 2H). ESI-MS calcd. for $C_{37}H_{36}I_2N_2O_6$ [M+Na^+] 881.07, found 881.03.

5'-*O*-Monomethoxytrityl-2',3'-*O*-di-iodo-seco-β-D-(*N*3-*p*-methoxybenzyl)-uridine (3.15)



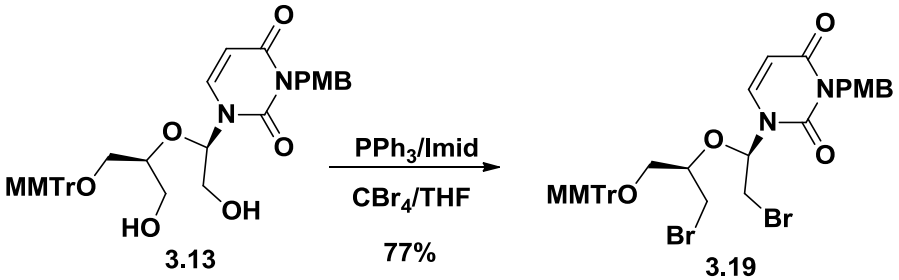
To a stirred solution of 1.09 g of **3.13** (1.71 mmol, 1 eq), 1.361 g of triphenylphosphine (5.14 mmol, 3.0 eq), and 1.170 g of imidazole (17.09 mmol, 10 eq) in 15 mL of DCM was added 1.3061 g of I_2 (5.05 mmol, 2.95 eq) at added at 0 °C. Reaction was allowed to react at room temperature for 2 hours; reaction was then cooled to 0 °C and another 2 equivalents of PPh_3 and I_2 were added. Reaction was then run overnight at room temperature and then diluted with DCM. Organic layer was quenched with aqueous sodium thiosulfate, washed with brine, dried over $MgSO_4$ and finally condensed down. Purification by column chromatography (4:1 to 7:3 hexane/EtOAc gradient) yielded 1.251 g of **3.15** as a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.76 (d, *J* = 8.1, 1H), 7.31 – 7.12 (m, 14H), 6.84 (d, *J* = 8.9, 2H), 6.79 (d, *J* = 8.7, 2H), 5.97 (t, *J* = 6.5, 1H), 5.76 (d, *J* = 8.0, 1H), 4.97 – 4.86 (m, 2H), 3.73 (s, 3H), 3.66 (s, 3H), 3.62 – 3.39 (m, 5H), 3.12 – 2.94 (m, 2H). ESI-MS calcd. for $C_{37}H_{36}I_2N_2O_6$ [M+Na^+] 881.07, found 881.03.

5'-*O*-Monomethoxytrityl-2',3'-*O*-dichloro-seco-β-D-(*N*3-*p*-methoxybenzyl)-uridine (3.16)



To a stirred solution of 1.869 g of **3.13** (2.93 mmol, 1 eq), 3.117 g of triphenylphosphine (11.8 mmol, 4.02 eq) and 404.8 mg (5.92 mmol, 2.02 eq) in 16 mL of DCM was added 3.4 mL of CCl_4 (35.23 mmol, 12.03 eq) dropwise at 0 °C under an atmosphere of N_2. Reaction was allowed to proceed at room temperature overnight and then condensed down to a white foam. Purification of the crude reaction mixture by column chromatography (10% to 30% EtOAc/hexanes gradient) afforded 1.583 g of **3.16** as a white foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.78 (d, *J* = 8.1, 1H), 7.31 – 7.18 (m, 12H), 7.15 (d, *J* = 8.8, 2H), 6.84 (d, *J* = 8.9, 2H), 6.78 (d, *J* = 8.7, 2H), 6.06 (t, *J* = 6.2, 1H), 5.76 (d, *J* = 8.1, 1H), 4.91 (abq, *J* = 14.2, 2H), 4.01 (qd, *J* = 12.0, 6.3, 2H), 3.89 – 3.79 (m, 3H), 3.73 (s, 3H), 3.67 (s, 3H), 3.15 – 3.00 (m, 2H). ESI-MS calcd. for $C_{37}H_{36}Cl_2N_2O_6$ [M+Na$^+$] 697.20, found 697.19.

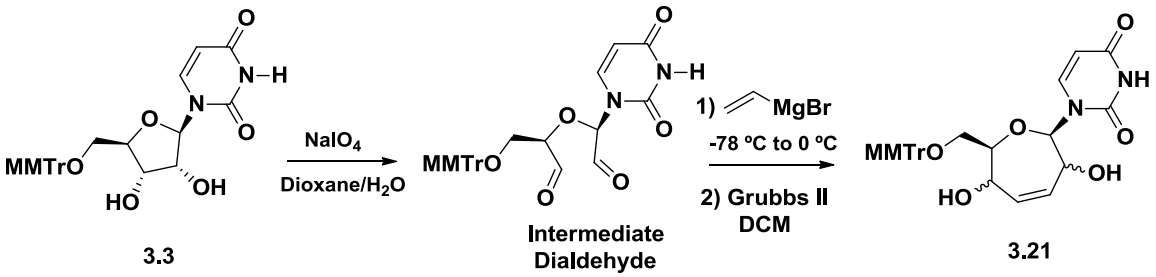
5'-*O*-Monomethoxytrityl-2',3'-*O*-dibromo-seco-β-D-(*N*3-*p*-methoxybenzyl)-uridine (3.19)



To a stirred solution of 1.218 g of **3.13** (1.90 mmol, 1 eq), 259 mg of imidazole (3.79 mmol, 2 eq), and 2.535 g of CBr_4 (7.56 mmol, 3.98 eq) in 20 mL of anhydrous THF was

added a second solution of 2.010 g PPh_3 (7.58 mmol, 4 eq) in 5 mL of THF dropwise at 0 °C. Reaction was stirred for 1.5 hour at room temperature then diluted with DCM. Organic layer was washed with brine, dried over $MgSO_4$ and condensed. Neutralized silica gel with 1% NEt_3/DCM and purified by column chromatography (9:1 to 4:1 hexanes/EtOAc gradient). Recovered 1.128 g of **3.19** as a foam. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.76 (d, *J* = 8.1, 1H), 7.29 – 7.17 (m, 12H), 7.12 (d, *J* = 8.9, 2H), 6.82 (d, *J* = 8.8, 2H), 6.76 (d, *J* = 8.6, 2H), 6.05 (s, 1H), 5.74 (d, *J* = 8.1, 1H), 4.95 – 4.83 (abq, 2H), 3.94 – 3.87 (m, 1H), 3.82 (dd, *J* = 10.6, 5.4, 2H), 3.71 (s, 3H), 3.68 (d, *J* = 4.4, 2H), 3.64 (s, 4H), 3.12 – 3.00 (m, 2H). ESI-MS calcd. For $C_{37}H_{36}Br_2N_2O_6$ [M+Na^+] 785.08, found 784.98.

7'-*O*-Monomethoxytrityl-2,5-dihydroxy- β-oxepinyl-uridine (3.21)



3.3 3.21

To a stirred solution of 1.1026 g of **3.3** (2.14 mmol, 1 eq) in 20 mL of dioxane was added 502.9 mg of $NaIO_4$ (suspended in 4 mL of H_2O) dropwise. Reaction proceeded at room temperature for 3 hours and then was diluted with 20 mL of dioxane. After filtering over Celite filter aid, the reaction was worked-up with DCM and brine, dried over $MgSO_4$ and condensed down. Residues were dried by azeotropic condensation from benzene and used in the next step without further purification.

To a stirred solution of 380.8 mg (0.74 mmol, 1 eq) of previously generated dialdehyde intermediate in 3 mL of THF was added 3.7 mL of 1 M vinylmagnesium bromide solution (3.7 mmol, 5 eq) dropwise at -78 °C under an atmosphere of N_2. Reaction was

stirred for 2 hours 40 minutes at -78 °C, and then it was allowed to warm to 0 °C. At 0 °C, the reaction mixture was quenched with saturated NH_4Cl solution, diluted with DCM. Organic layer was washed with brine, dried over $MgSO_4$ and condensed down. A filter column (2.5% to 7% gradient MeOH/DCM) was performed on the reaction mixture. The product was immediately used in the next step.

To a stirred solution 170 mg (0.3 mmol) of the previously obtained intermediate in 30 mL of anhydrous DCM was added 25 mg of Grubbs II (0.03 mmol, 10 mol %) Reaction was stirred at room temperature for 48 hours, diluted with DCM, worked up and purified. On TLC purified reaction product ran as one spot, but examination of ^1H NMR indicated that multiple compounds were present (the various diastereomers) precluding full spectral analysis. ESI-MS calcd. for $C_{31}H_{30}N_2O_7$ [M+Cl$^-$] 577.17, found 577.08; [M-H$^+$] 541.20, found 541.20. HRMS calcd. for $C_{31}H_{30}N_2O_7$ [M-H$^+$] 541.19693, found 541.19800, supporting elemental composition.

Contribution to Knowledge

Since nucleosides, nucleoside analogues and oligonucleotides have such great potential as therapeutic agents, their study is not only academically interesting but also directly tied to potential medicinal applications. This thesis describes work towards developing novel routes in the synthesis of seven-membered (oxepane) nucleosides. In chapter 2, studies on the synthesis of oxepane nucleosides by a ring-expansion route were described, building on previous work done in the Damha lab. While this approach was a viable route to the synthesis of oxepane nucleoside, it was low-yielding in the key glycosylation step. To improve this crucial step, thioglycoside intermediates were used in the synthesis of oxepane nucleosides. Initial work towards the synthesis of dihydroxylated monomers was carried, but was eventually set aside for the examination RCM-based routes. Grubbs catalyst mediated ring-closing metathesis is an effective synthetic methodology in the formation of rings, and so it was surmised that oxepane nucleosides could be made by this approach. In chapter 3, studies on the development of synthetic routes for oxepanes are described. Synthetic routes involving Wittig chemistry, Grignard chemistry, organocopper chemistry, nitrile chemistry were explored. In particular, the direct addition of vinyl magnesium bromide to nucleoside dialdehydes, followed by Grubbs II mediated ring metathesis served as a proof-of-principle for the synthesis of oxepane nucleosides by a ring closing approach. In the course of these syntheses, novel acyclic nucleosides, which could be assayed for antiviral and anticancer activity, were also synthesized.

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