BIOMIMETIC REACTIONS RELEVANT TO THE STUDY OF ABASIC SITES IN DNA

A Dissertation

presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

CHRISTOPHER A. NEL

Dr. Kent S. Gates, Dissertation Supervisor

May 2020

The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

BIOMIMETIC REACTIONS RELEVANT TO THE STUDY OF ABASIC SITES IN DNA

Presented by Christopher A. Nel

a candidate for the degree of Doctor of Philosophy

and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Kent Gates

Professor Susan Lever

Professor Silvia Jurisson

Professor Anand Chandrasekhar

ACKNOWLEDGEMENTS

I would like to begin by thanking the two most important people in my life; my wife, Julie, and my mom, Sue. Their support through this process is beyond words, and my love for them knows no depths.

Behind every well-oiled research group, there is an advisor derailing group meeting with topics that allow us to grow as scientists and as people. I am thankful for Dr. Gates for fostering a cohesive team in which I had the opportunity be a part. While I may not have always been the easiest graduate student to coach, I sincerely appreciate all his time and effort in order to mold me into being a better scientist and a better person. Our lengthy conversations are invaluable to me.

To that, I am thankful for being a part of this research group, and thankful to all Gates members past and present for helping me through some of my research challenges and for their input regarding this work. I would like to personally thank Kurt Housh and Tuhin Haldar. In these two, I sought to make friends, and ending up finding a family.

A very special thanks to Rev. Mike Harmata, PhD for helping me gain a firm understanding of organic chemistry through the courses he taught. I am also beyond thankful because he has been a personal mentor to both myself and Julie at the start of our marriage. He helped coach us through to the wedding day, and then proceeded to give a wonderful sermon. Julie and I are beyond grateful that he is a part of our life.

I would also like to thank Drs. Lever, Jurisson, and Chandrasekhar for accepting to be a part of my thesis committee and ask me questions throughout my tenure.

Table of Contents

LIST OF SCHEMES

Chapter One Schemes

Chapter Two Schemes

Chapter Three Schemes

LIST OF FIGURES

Chapter One Figures

Chapter Two Figures

Chapter Three Figures

Chapter Four Figures

LIST OF TABLES

BIOMIMETIC REACTIONS RELEVANT TO THE STUDY OF ABASIC SITES IN DNA

Christopher A. Nel

Dr. Kent Gates, Dissertation Advisor

ABSTRACT

Abasic (Ap) sites represent the most common form of DNA damage in mammalian cells. With steady state levels of 10,000 sites existing in each cell, there is a dire need to study these noxious biological electrophiles. While much work has been done to study these lesions using small biomolecules, such as oligonucleotides, there is still much to be learned regarding the fundamental organic chemistry. This thesis attempts to use the tools of basic organic chemistry to understand the complex reactions, structure, and equilibria of the abasic site.

Chapter One: An introduction to relevant Ap site chemistry

1.1 Introduction

DNA represents the genetic code within all living organisms. The sequence of DNA is composed of four nitrogenous bases (Figure 1.1.1) held together via hydrogen bonding and hydrophobic interactions giving DNA it's helical structure (Figure 1.1.2).[1,2] The faithful reading and replication of the double helix is central to biological function (Scheme 1.1.1).[3] Damage to this process, either by endogenous or exogenous means, may have detrimental consequences to the well-being of a cell. The cell has various methods by which to respond to these damages, including altering gene expression, preventing cell division, or inducing apoptosis.[4,5] A comprehensive review of the chemistry regarding DNA damage was written by Gates.[6] This chapter will attempt to lay the foundation for the topics of this thesis. I will describe some of the fundamental chemistry that an Ap site can perform, describe a potential degradation which can occur with an Ap site, and briefly discuss a potential pathway to avoid the degradation pathway.

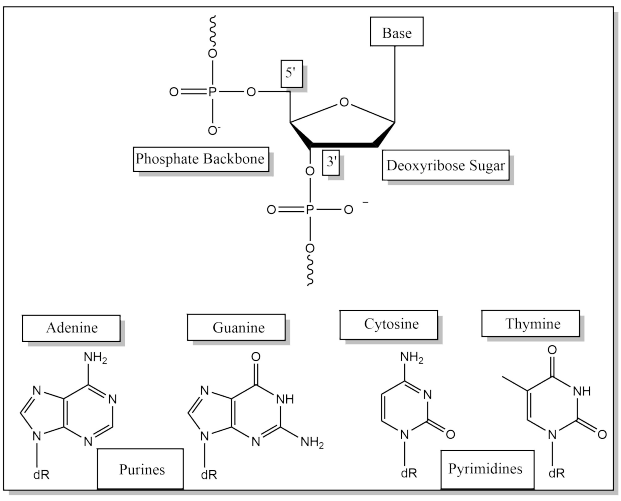


Figure 1.1.1 Structure of nitrogenous DNA bases.

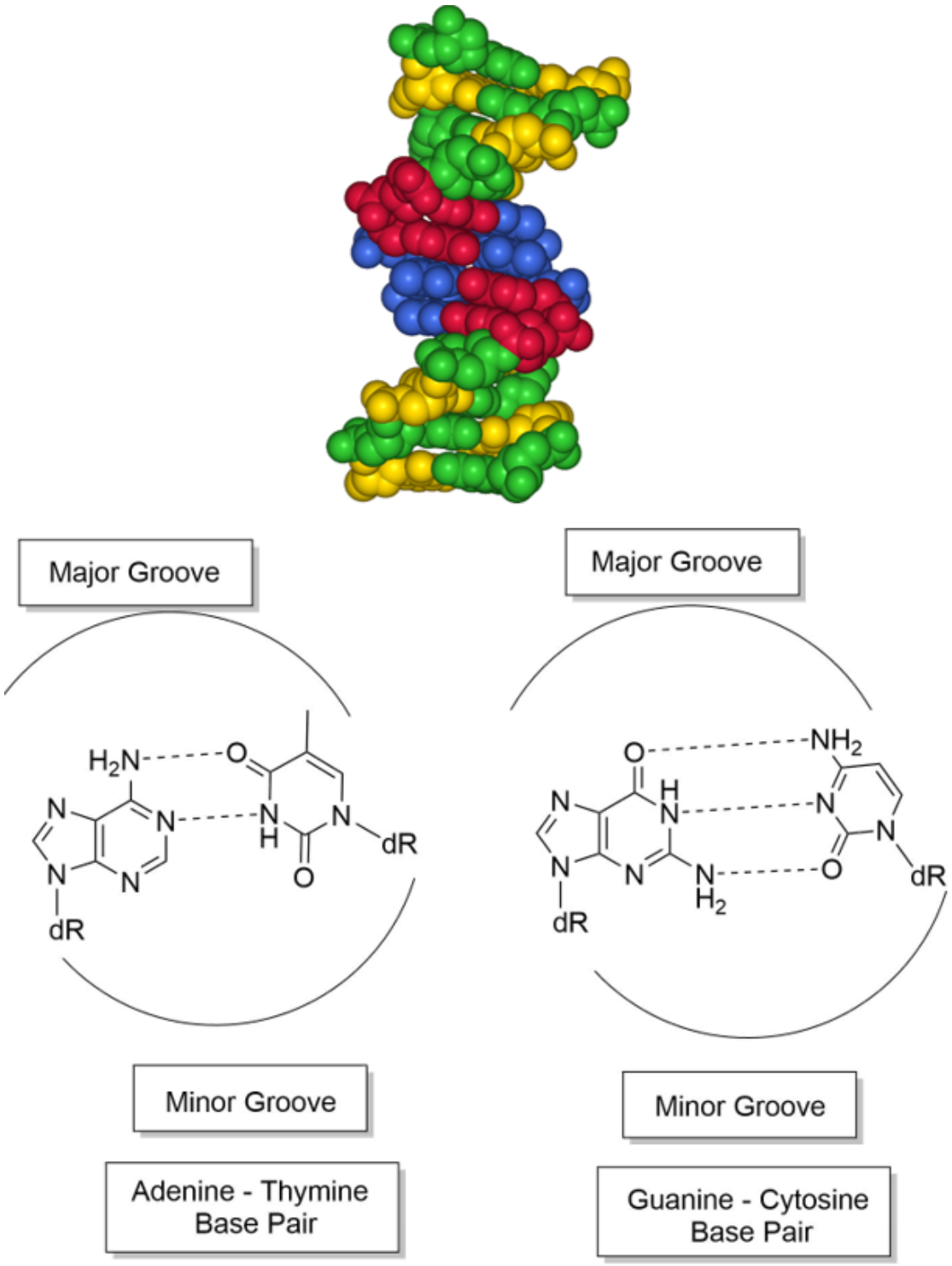
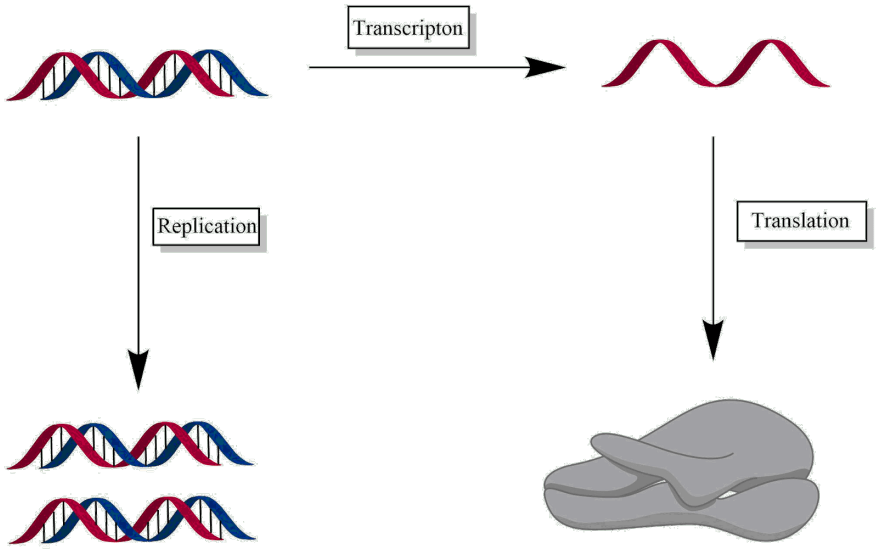


Figure 1.1.2 Structure of DNA. PBD code:1BDNA[7]

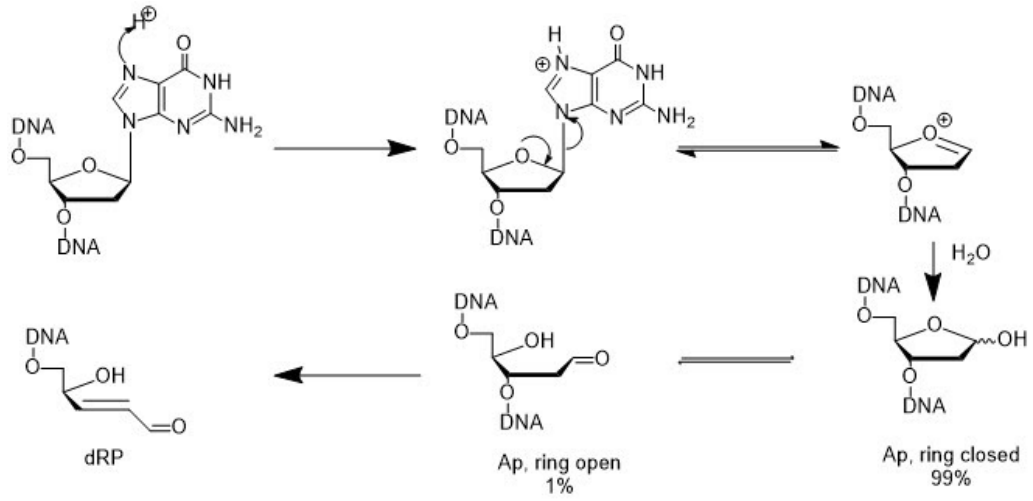
Scheme 1.1.1 Central dogma of biology.



1.2 The abasic site

The most common lesions within DNA are abasic (Ap) sites. Ap sites are generated at a rate of 10,000 sites per cell per day,[8] with approximately 50,000[9,10] sites detected within cells. Ap sites are generated via cleavage of the aminoglycosidic bond to generate a highly electrophilic oxocarbenium ion, which is readily hydrolyzed to generate the hemiacetal Ap site (Scheme 1.2.1). While the ring-closed hemiacetal is the major tautomer (99%) of the Ap site,[11] it has been proposed that much of the activity stems from the ring-open aldehyde (1%). These ring open aldehydes serve as active electrophiles able to form covalent crosslinks between DNA bases,[12,13] proteins,[14,15] interact with drugs,[16,17] be oxidized to other noxious lesions[18,19], or be cleaved to generate the bis-electrophilic unsaturated aldehyde [20,21] (dRP). While there are some repair pathways[22,23] which lessen the damage caused by these lesions, some lesions evade repair leading to consequences such as neurodegeneration,[24, 25] aging,[26, 27] cancer,[28,29] or mutagenesis.[30,31]

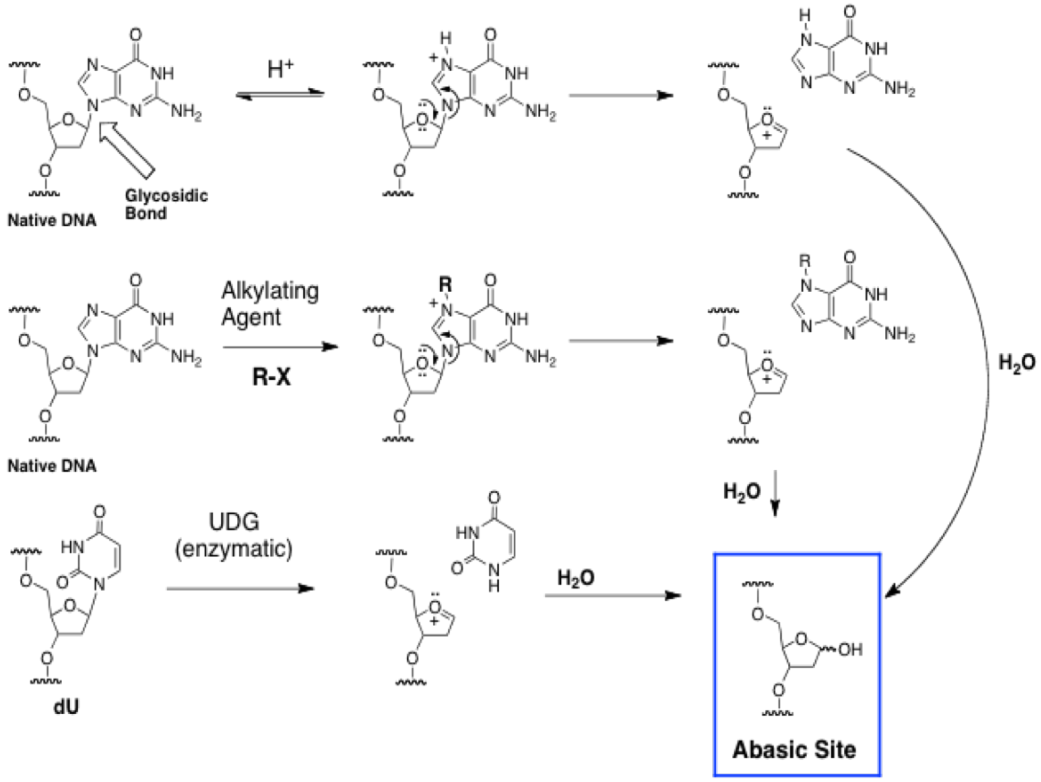
Scheme 1.2.1 Generation of Ap sites via depurination.



1.3 Ap sites can be generated via chemical or enzymatic cleavage

Ap sites can be generated either through a chemical or enzymatic route, generally through the excision of a damaged base through depurination. There are specific sites (Figure 1.3.1) in DNA which are deemed as hot spots for depurination[32,33] (Scheme 1.2.1) upon chemical modification. Early work by Verly[34] utilized the ability to generate Ap sites in order to describe their ability to generate interstrand cross-links with an opposing exocyclic amine in the opposing strand of DNA (Scheme 1.4.1). Ap sites can also be generated via base excision enzymes, such as Uracil DNA-glycosylase (UDG) (Scheme 1.3.1). This process has been exploited for research purposes to generate site-specific Ap sites within an oligonucleotide sequence.[35] Once these damaged bases are excised from the double helix, the electrophilic Ap site is able to generate covalent linkages with various biological nucleophiles.

Scheme 1.3.1 Overview of Ap site formation via both chemical and enzymatic methods.



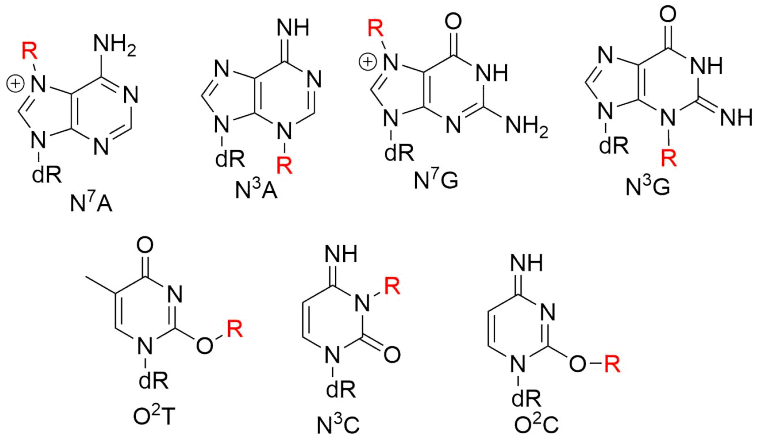
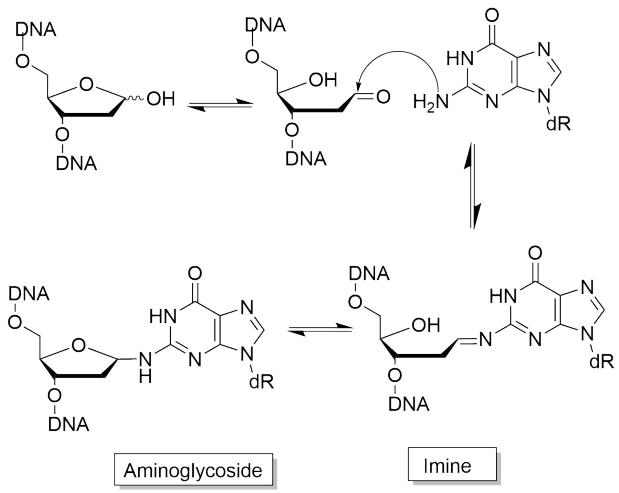


Figure 1.3.1 Alkylation of nucleophilic hot spots prone to depurinate.

1.4 Ap sites have the ability to generate interstrand cross-links with exocyclic amines

One classic[34] reaction of the Ap site is its ability to generate a Schiff base and subsequent aminoglycoside with an opposing exocyclic amine. (Scheme 1.4.1). While the early work was instrumental in determining an Ap site's ability to generate these covalent linkages, it left many questions left to be answered regarding sequence specificity, stability, ability to be repaired, and possible secondary lesions. Work performed since[12, 36,37,38] has made strides in assessing some of these questions.

Scheme 1.4.1 Generation of a cross-link using deoxyguanosine (dG) and an Ap site.



It is critical to address the biological consequences of the DNA interstrand cross-links stemming from the reaction between an Ap site and an exocyclic amine. Covalently holding the strands of DNA together can be cytotoxic, as it prevents strand separation during replication[39], and it can be mutagenic if upon repair an incorrect base is installed.[40] While there are methods[41] by which these cross-links can be repaired, overwhelming these processes can lead to profound biological consequences. The generation of interstrand cross-links has been utilized intentionally by anticancer agents to induce cell apoptosis in cancerous cells[42,43] to either stall or overwhelm these enzymatic repair pathways. It is plausible that unrepaired cross-links between an Ap site and an exocyclic amine may pose similar, unintentional biological consequences.

1.5 DNA interstrand cross-linking in a duplex is driven by sterics

One aspect of DNA interstrand cross-linking which is still not well understood is the reason as to why some nucleosides in duplex DNA are able to generate cross-links at higher yields or faster rates than others. A consideration for this may be one of simple electronics; for example, guanine may not be able to generate cross-links at a high yield compared to adenine in similar sequences[13,36] because the nucleophilicity of the exocyclic amine is conjugated with a carbonyl (Figure 1.5.1). This may suggest that the nucleophilicity of the exocyclic amine is reduced to that of an extended amide rather than a true amine. If this were the sole reason, then reactions of the nucleosides in solution would reflect that conclusion.

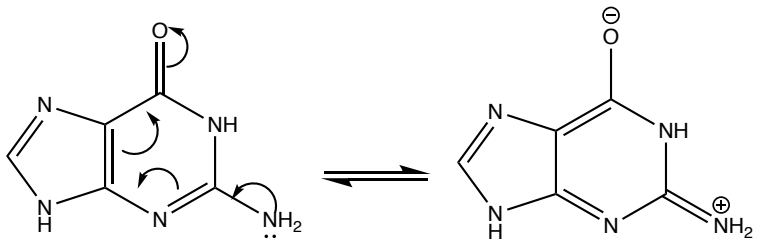


Figure 1.5.1. Resonance structure of guanine showing conjugation with the carbonyl.

Work performed by Michael Catalano suggests that nucleosides containing an exocyclic amine are capable of generating an aminoglycosidic bond with a model Ap site with similar yields. Research within this study show that the non-native nucleoside, 2'aminopurine is able to generate significantly higher cross-linking yields than that native

bases. As is represented in Figure 1.5.2, the structure of 2'aminopurine is a hybrid of both deoxyadenosine and deoxyguanine.[44,45,46] Work done in our group show that a reduced cross-link between d2AP and an Ap site is able to be generated in high yields in duplex DNA.[47]

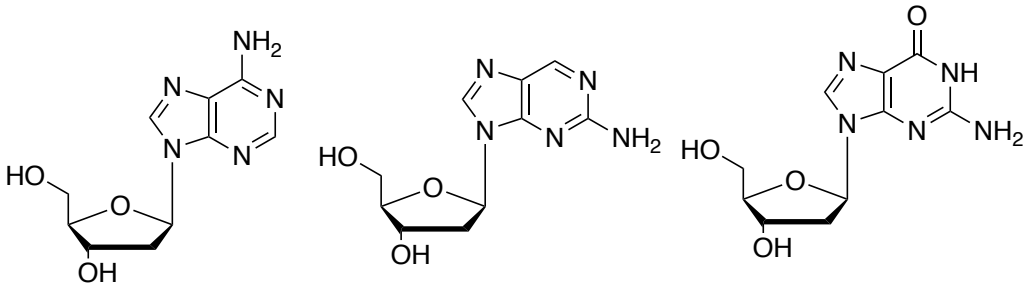


Figure 1.5.2. Structures of 2'deoxyadenosine, 2'deoxyaminopurine, and 2'deoxyguanosine respectively.

The other major factor in considering DNA crosslinking with exocyclic amines involves the sterics of the double helix. When comparing deoxyadenonsine to deoxyguanine in similar sequences, deoxyadenosine generally gives higher yielding cross-links. This may be due to the placement of the exocyclic amine of deoxyadenosine compared to that of deoxyguanine. While the difference between deoxyguanine and deoxyadenosine being able to generate a crosslink with a model Ap site is marginal (Figure 1.5.2), that difference is widened in duplex DNA. Typical dG-Ap crosslinking yields occur on the order of 2-5%,[12] whereas dA-Ap crosslinks occur on the order of 15-85%.[13] Research performed by Dumont[48] addresses this by proposing a structure between an Ap site and an exocyclic amine from an opposing nucleoside (Figure 1.5.3). Their work shows that the cross-link between an Ap site and a deoxyguanine has a greater

degree of distortion within the helix than that of an Ap site and deoxyadenosine. This strengthens the argument that cross-linking between exocyclic amines and Ap sites in duplexed DNA is driven by the location of the exocyclic amine in duplex DNA.

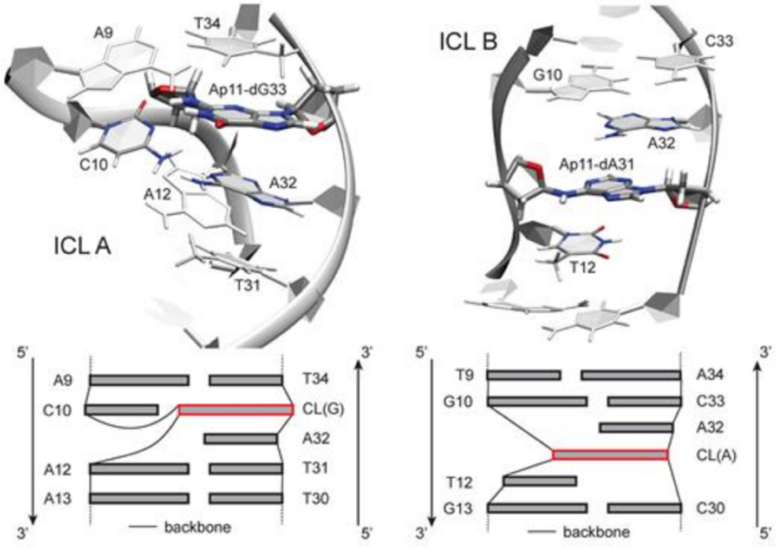


Figure 1.5.3. From Dumont[48] showing distortion in the double helix upon cross-linking of an Ap site with either deoxyadenosine or deoxyguanine.

In order to fully understand the extent to which sterics plays a role with DNA cross-linking within a double helix, I sought to generate an unreduced cross-link between 2'aminopurine in a helix to determine if it's cross-linking ability compared to that of deoxyguanine. Sequences **1.1** and **1.2** contained 2'aminopurine and deoxyguanine respectively (Table 1.5.1). From our data (Figure 1.5.4), I see that the unreduced cross-link between an Ap site and a 2'aminopurine residue is able to generate at a higher yield, and higher rate than the comparative deoxyguanine-Ap cross-link.

Comparing the nucleoside studies performed by Catalano, it would appear that 2'aminopurine would be the most capable of generating a cross-link with an Ap site.

While 2'aminopurine does generate significantly more cross-link than deoxyguanine in a comparable sequence, it is not able to generate cross-links at the 85% yield seen by deoxyadenosine.[31] This guides us to believe that steric effects, more so than electronic effects, are the determining factor in the generation of cross-links between an exocyclic amine and an opposing Ap site in duplex DNA.

The role of Ap sites as a bio-electrophile is not limited to generating cross-links within DNA. It also maintains the capacity to have covalent interactions with other nucleophilic moieties in the body, such as thiols, alcohols, or aliphatic amines. In subsequent sections of this chapter, I will explore some of the fundamental chemistries of each of these lesions.

Table 1.5.1. Sequences used to compare cross-linking capabilities of dG and 2'aminopurine. 2'aminopurine is denoted as P in 1.1.

1.1 5′-ATC ATG CAT AGA TGA ATU AAG ACA TAT AGC CAT AC-3′ 3′-TAG TAC GTA TCT CAT TPA TTC TGT ATA TCG GTA TG-5′
1.2 5′-ATC ATG CAT AGA TGA ACU AAG ACA TAT AGC CAT AC-3′ 3′-TAG TAC GTA TCT ACT TGA TTC TGT ATA TCG GTA TG-5′

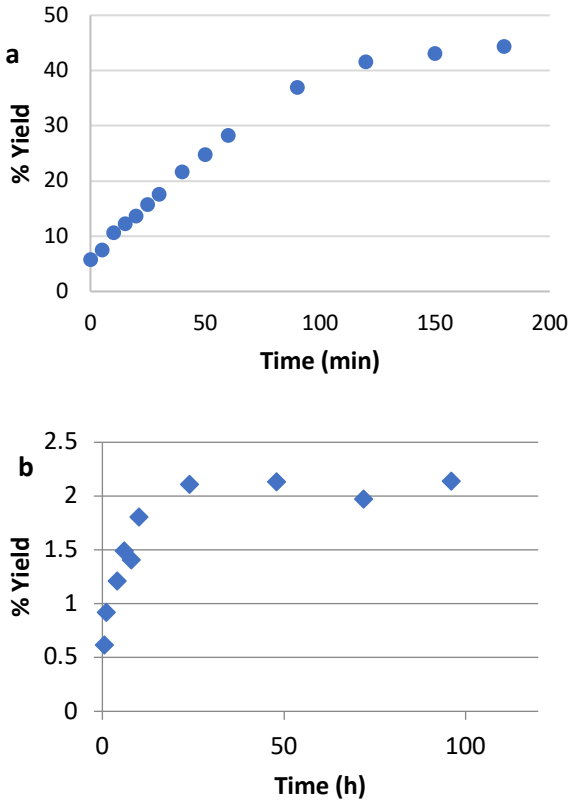
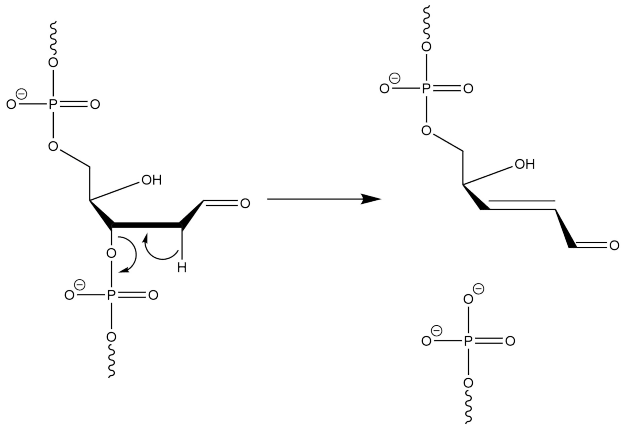


Figure 1.5.4 Cross-linking between a duplex containing an Ap site and either 2'aminopurine (a) and deoxyguanine (b).

1.6 Ap sites can be degraded to a highly reactive species

One major secondary lesion of the Ap site occurs upon strand cleavage stemming from beta-elimination of the 3'phosphate residue (Scheme 1.6.1).[49,50,51] The alkenal (dRP) afforded from this elimination has been detected within cells,[52,53] and is potentially more noxious than the parent lesion.[54]

Scheme 1.6.1 Beta-elimination to generate dRP from an abasic site.



This field of chemistry has been explored recently[20] showing the alkenals ability to generate interstrand cross-links, and proposes a structure (Figure 1.6.1) and mechanism for the formation of the structure.

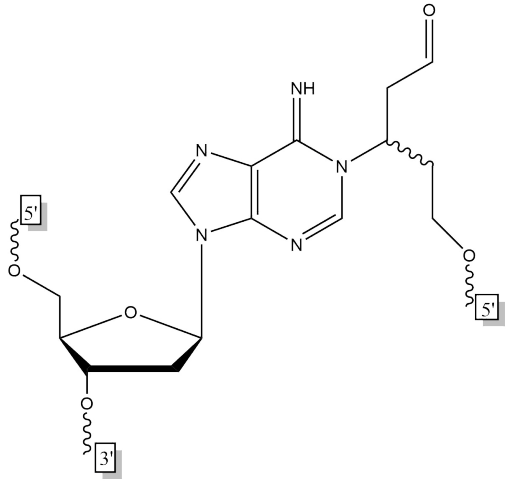


Figure 1.6.1 Proposed product of the reaction between dRP and dA.

The chemistry of biological unsaturated aldehydes has been studied using the biologically relevant 4-hydroxy-2(E)-nonenal (HNE). HNE is generated as a product of lipid peroxidation in conditions of high oxidative stress[55] and has been shown to react with various nucleophiles in the body, such as DNA bases[56,57], glutathione[58] (GSH), or

lysine residues[59] (Figure 1.6.2). Due to the structural similarities of dRP to that of HNE, one can hypothesize that their routes of reactivity may be similar. In Chapter 2 of this thesis, I will further explore the chemistries of the dRP end group.

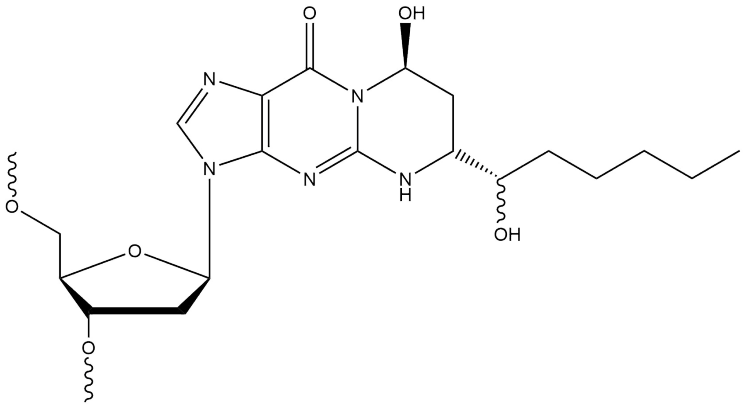


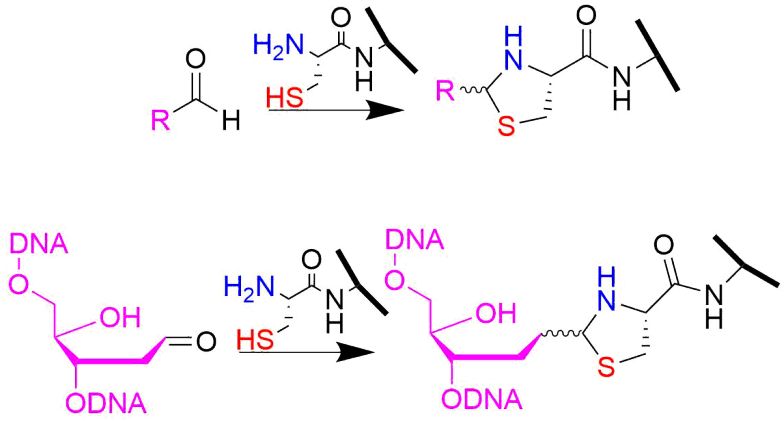
Figure 1.6.2 Cyclized structure of the dG-HNE adduct.

1.7 Thiols present an important, yet not well understand, potential to react with Ap sites

As will be re-discussed in Chapter 3 of this thesis, the fundamental chemistry of thiols reacting with Ap sites is not well understood. There is a significant shortage of literature regarding chemistry relevant to Ap sites with biologically relevant thiols, such as glutathione or cysteine residues in proteins. Recent literature suggests that HMCES is able to generate a semi-stable lesion with an Ap site to inhibit degradation.[14,60,61] The chemical face of this reaction occurs with an N-terminal cysteine being able to generate a stable thiazolidine with the Ap site. There is literature precedent[62] showing that the formation of thiazolidines with N-terminal cysteines is a thermodynamically favorable

reaction (Scheme 1.7.1), however until recently, there was not a link showing this was possible with an Ap site.

Scheme 1.7.1 Reaction of N-terminal cysteine with a sample aldehyde and the proposed reaction with an Ap site in DNA.



From a materials perspective, the generation of thiazolidines and thioglycosides is particularly interesting. Thioglycosides have been explored for the potency as antibiotics[63] however the conditions in which these compounds are formed tend to require strong Lewis acids, and often in stoichiometric excess.[64] This has afforded the opportunity to develop a mild, high yielding process by which thioglycosides can be generated. There is also a need to study the formation and stability of thioglycosides within a duplex in order to understand their biological relevance.

1.8 Conclusions

Studying DNA is a basic science fundamental to all fields of science. In order to understand how essential faithfully replicated DNA is, it is important to address what occurs when DNA is damaged. The most common form of damaged DNA is the Ap site. While there are studies showing some of the chemistries of the Ap site, whether it be strand cleavage, DNA interstrand cross-link formation and repair, or generation of other covalent adducts, there still is much to be answered. This thesis will attempt to give a chemical face to the structures and reactions which are occurring in the human body, whether it be studying DNA interstrand cross-links, the chemistry of strand cleavage in DNA, or how Ap sites may be protected by a semi-stable linkage with a protein.

[1] Todd, A. R. (1954) Chemical structure of the nucleic acids. Proc. Natl. Acad. Sci. U.S.A. 40, 748–755.

[2] Watson, J. D., and Crick, F. H. C. (1953) A structure for deoxyribose nucleic acid. Nature 171, 737–738.

[3] Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002) Molecular Biology of the Cell, 4th ed., Garland Science, New York

[4] Zhou, B.-B. S., and Elledge, S. J. (2000) The DNA damage response: Putting checkpoints in perspective. Nature 408, 433–439.

[5] Norbury, C. J., and Hickson, I. D. (2001) Cellular responses to DNA damage. Annu. ReV. Pharmacol. Toxicol. 41, 367–401.

[6] Gates, K (2009) An Overview of Chemical Processes That Damage Cellular DNA: Spontaneous Hydrolysis, Alkylation, and Reactions with Radicals. Chem. Res. Toxicol. 22, 1747-1760.

[7] Drew, H.R.; Wing, R.M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R.E.; (1981) Structure of a B-DNA dodecamer: conformation and dynamics. Proc. Natl. Acad. Sci. 78, 2179-2183.

[8] Lindahl, T., and Nyberg, B. (1972) Rate of depurination of native deoxyribonucleic acid. Biochemistry 11, 3610–3618.

[9] Nakamura, J., Walker, V. E., Upton, P. B., Chiang, S. Y., Kow, Y. W., and Swenberg, J. A. (1998) Highly sensitive apurinic/apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. Cancer Res. 58, 222–225.

[10] Nakamura, J., and Swenberg, J. A. (1999) Endogenous apurinic/ apyrimidinic sites in genomic DNA of mammalian tissues. Cancer Res. 59, 2522–2526.

[11] Wilde, J. A., Bolton, P. H., Mazumdar, A., Manoharan, M., and Gerlt, J. A. (1989) Characterization of the equilibrating forms of the abasic site in duplex DNA using 17O-NMR. J. Am. Chem. Soc. 111, 1894– 1896.

[12] Dutta, S.; Chowhury, G.; Gates, K.S. (2007) Interstrand Cross-links Generated by Abasic Sites in Duplex DNA. J. Am. Soc. 129, 1852-1853.

[13] Price, N.; Johnson, K.M.; Wang, J.; Fekry, M.I.; Wang, Y.; Gates, K.S. (2014) Interstrand DNA-DNA cross-link formation between adenine residues and abasic sites in duplex DNA. J. Am. Chem, 136, 3483-3490.

[14] Mohni, K.N.; Wessel, S.R.; Zhao, R.; Wojciechowski, A.C.; Luzwick, J.W.; Layden, H.; Eichman, B.F.; Thompson, P.S.; Mehta, K.P.M.; Cortez, D. (2019) HMCES maintains genome integrity by shielding abasic sites in single-strand DNA. Cell. 176, 144-153.

[15] Sczepanski JT, Wong RS, McKnight JN, Bowman GD, Greenberg MM (2010). Rapid DNA-protein cross-linking and strand scission by an abasic site in a nucleosome core particle. Proc Natl Acad Sci 107,22475-80.

[16] Imani-Nejad, M.; Johnson, K.M.; Price, N.E.; Gates, K.S. (2016) A new cross-link for an old cross-linking drug: the nitrogen mustard anticancer agent mechloroethamine generates cross-links derived from abasic sites in addition to the expected drug-bridged cross-links. Biochemistry, 55, 7033-7041.

[17] Melton, D.; Lewis, C.; Price, N.E.; Gates, K.S. (2014) Covalent adduct formation between the antihypertensive drug hydralazine and abasic sites in double- and single-stranded DNA. Chem. Res. Toxicol. 27, 2113-2118.

[18] Guan, L.; Greenburg, M.M.; (2009) DNA interstrand cross-link formation by the 1,4-dioxibutane abasic lesion. J. Am. Chem. Soc; 131, 15225-15231.

[19] Guan, L.; Greenburg, M.M. (2011) An oxidized abasic lesion as an intramolecular source of DNA adducts Aust. J. Chem, 64, 438-442.

[20] Yang, Z.; Price, N. E.; Johnson, K. M.; Wang, Y.; Gates, K. S. (2017) Interstrand cross-links arising from strand breaks at true abasic sites in duplex DNA. Nucleic Acids Res. 45, 6275-6283.

[21] Bailly V; Verly WG; (1988) Possible routes of beta-elimination and delta-elimination reactions in the repair of DNA containing AP (apurinic/apyrimidinic) sites in mammalian cells. Biochem J. 253,553-559.

[22] Semlow DR, Zhang J, Budzowska M, Drohat AC, Walter JC (2016) Replication-dependent unhooking of DNA interstrand cross-links by the NEIL 3 glycosylase. Cell. 167:498-511.

[23] Sczepanski, J. T.; Jacobs, A. C.; Van Houten, B.; Greenberg, M. M.(2009) Double-Strand Break Formation During Nucleotide Excision Repair of a DNA Interstrand Cross-link.Biochemistry 48, 7565-7567.

[24] McKinnon, P.J.; (2009) DNA repair deficiency and neurological disease Nat. Rev. Neurosci. 10, 100-112.

[25] Madabhushi R., Pan L., Tsai L.-H. (2014) DNA damage and its links to neurodegeneration. Neuron. 83:266–282.

[26] Borgesius N.Z., de Waard M., van der Pluijm I., Omrani Z., Zondag G.C.M., ver der Horst G.T.J., Melton D.W., Hoeijmakers J.H.J., Jaarsma D., Elgersma Y. (2011) Accelerated age-related cognitive decline and neurodegeneration, caused by deficient DNA repair. J. Neurosci.; 31:12543–12553.

[27] Schumacher B., Garinis G.A., Hoeijmakers J.H.J. (2007) Age to survive: DNA damage and aging. Trends Genet. 24:77–85.

[28] Menck C.F.M., Munford V. (2014) DNA repair diseases: what do they tell us about cancer and aging? Genet. Mol. Biol. 2014; 37:220–233.

[29] Hoeijmakers J.H.J. DNA damage, aging, and cancer. (2009). J. Engl. J. Med. 361:1475–1485.

[30] Friedberg E.C., McDaniel L.D., Schultz R.A (2004). The role of endogenous and exogenous DNA damage and mutagenesis. Curr. Opin. Genet. Dev; 14, 5–10.

[31] Cline S.D. (2012). Mitochondrial DNA damage and its consequences for mitochondrial gene expression. Biochim. Biophys. Acta. 1819:979–991.

[32] Lawley, P. D., and Brookes, P. (1963) Further studies on the alkylation of nucleic acids and their constituent nucleotides. Biochem. J. 89, 127–138.

[33]Gates, K. S., Nooner, T., and Dutta, S. (2004) Biologically relevant chemical reactions of N7-alkyl-2′-deoxyguanosine adducts in DNA. Chem. Res. Toxicol. 17, 839–856.

[34] Goffin, C.; Verly, W. G. Interstrand (1983) DNA crosslinks due to AP (apurinic/apyrimidinic) sites. FEBS. 161, 140-144.

[35] Imani Nejad, M.; Guo, X.; Housh, K.; Nel, C.; Yang, Z.; Price, N.E.; Gates, K.S. (2019) Preparation and purification of oligodeoxynucleotide duplexes containing a site-specific, reduced, chemically stable covalent interstrand cross-link between a guanine residue and an abasic site. Non-Natural Nucleic Acids. Methods in Molecular Biology, vol 1973. 163-175.

[36] Catalano, M. J.; Liu, S.; Anderson, N.; Yang, Z.; Johnson, K. M.; Price, N. E.; Wang. Y. and Gates, K. S. (2015) Chemical structure and properties of interstrand cross-links formed by reaction of guanine residues with abasic sites in duplex DNA. J. Am. Chem. Soc. 137, 3933-3945.

[37] Price, N. E.; Catalano, M. J.; Liu, S.; Wang, Y. and Gates, K. S. (2015) Chemical and structural characterization of cross-links formed between abasic sites and adenine residues in duplex DNA. Nucleic Acids Res. 43, 3434-3441.

[38] K. M. Johnson, N. E. Price, J. Wang, M. I. Fekry, S. Dutta, D. R. Seiner, Y. Wang, and K. S. Gates. (2013) On the formation and properties of interstrand DNA-DNA cross-links forged by reaction of an abasic site with the opposing guanine residue of 5'Cap sequences in duplex DNA. J. Am. Chem. Soc. 135, 1015-1025.

[39] Osawa T; Davies, D; Hartley, JA (2011) Mechanism of cell death resulting from DNA interstrand cross-links in mammalian cells. Cell Death and Disease, 2, 1-11.

[40] Shen, X.; Li, L Mutagenic repair of interstrand cross-links. (2010) Env. Mol. Mutagen. 51, 493-499.

[41] McVey, M. (2010) Strategies for DNA interstrand cross-link repair: insights from worms, flies, frogs, and slime molds. Env. Mol. Mutagen, 51, 646—658.

[42] Lawley, P.D.; Phillips, D.H.; (1996) DNA adducts from chemotherapeutic agents. Mutat Res. 355, 13-40.

[43] Scharer, O.D.; (2005) DNA interstrand crosslinks: natural and drug-induced DNA adducts that induce unique cellular responses. Chembiochem 6, 27-32.

[44] Dallmann, A., Dehmel, L., Peters, T., Mügge, C., Griesinger, C., Tuma, J., and Ernsting, N. P. (2010) 2-Aminopurine incorporation perturbs the dynamics and structure of DNA. Angew. Chem., Int. Ed. 49, 5989−5992.

[45] Law, S. M., Eritja, R., Goodman, M. F., and Breslauer, K. J. (1996) Spectroscopic and colorimetric characterization of DNA duplexes containing 2-aminopurine. Biochemistry 35, 12329−12337.

[46] Fagan,P.A.,Fabrega,C.,Eritja,R.,Goodman,M.F.,and Wemmer, D. E. (1996) NMR study of the conformation of the 2- aminopurine:cytosine mismatch in DNA. Biochemistry 35, 4026− 4033.

[47] Imani, Nejad; M.; Price, N.E.; Haldar, T.; Lewis, C.; Wang, Y.; Gates, K.S; (2019) Interstrand DNA cross-links derived from reaction of a 2-aminopurine residue with an abasic site. ACS Chem Biol, 14, 1481-1489.

[48] Bignon, E.; Drsata, T.; Morell, C.; Lankas, F.; Dumont, E.; (2016) Interstrand cross-linking implies contrasting structural consequences for DNA: insights from molecular dynamics. Nucleic Acids Res, 45, 2188-2195.

[49] Bayley, C.R.; Brammer, K.W.; Jones, A.S.; (1961) The nucleotide sequence in deoxyribonucleic acids. Part V. The alkaline degradation of apurinic acids. Journ Chem Soc 1, 1903-1907.

[50] Crine, P.; Verly, W.G. (1976) A study of DNA spontaneous degradation. BBA 2, 50-57.

[51] Lindahl, T.; Andersson, A.; (1972). Rate of chain beakage at apurinic sites in double-stranded deoxyribonucleic acid. Biochemistry, 11, 3618-3623.

[52] Ma, W.; Westmoreland, J.W.; Gordenin, D.A.; Resnick, M.A.; (2011) Alkylation base damage is converted into repairable double-strand breaks and complex intermediates in G2 cells lacking AP endonuclease. PLOS Genetics, 4, 1-15.

[53] Guillet, M.; Boiteux, S.; (2003) Origin of endogenous DNA abasic sites in Saccharamoyces cerevisiae Mol Cell Biol 22, 8386-8394.

[54] Boiteux, S.; Guillet, M; (20014) Abasic sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. DNA Repair, 1, 1-12.

[55] Zhong, H.; Yin, H.; (2015) Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. Redox Biology 4, 193-199.

[56] Wada, T; Tanji, N; Ozawa, A; Wang, J; Shimamoto, K; Yokoyama, M; (2006). Mitochondrial DNA mutations and 8-hydroxy-2'deoxyguanosine content in Japanese patients with urinary bladder and renal cancers. Anticancer Res, 26, 3403-3408.

[57] Chung, F.L.; Nath, R.G.; Nath, J.; Nishikawa, O.A.; Zhang, L.; (2000) Deoxyguanosine adducts of t-4-hydroxynonenal are endogenous DNA lesions in rodents and humans: detection and potential sources. Cancer Res, 60, 1507-1511.

[58] Voelkel, W.; Alvarez-Sanches, R.; Weick, I.; Mally, A.; Dekant, W.; Paehler, A. (2005) Glutathione conjugates of 4-hydroxy-2(E)-nonenal as biomarkers of hepatic oxidative stress-induced peroxidation in rats. Free Radic Biol Med. 11, 1526-1536.

[59] Jacobs, A.T.; Marnett, L.J.; (2010) Systems analysis of protein modifications and cellular responses induced by electrophile stress. Acc. Chem. Res, 5, 673-678.

[60] Shukla, V.; Halabelian, L.; Balagere, S.; Samaniego-Castruita, D.; Feldman, D.E.; Arrowsmith, C.H.; Rao, A.; Aravind, L.; (2020) HMCES functions in the alternative end-joining pathway of the DNA DSB repair during class switch recombination in B cells. Mol Cell. 2, 384-394.

[61] Halabelian, l; Ravichandran, M.; Li, Y.; Zeng, H.; Rao, A.; Aravind, L.; Arrowsmith, C.H.; (2019) Structural basis of HMCES interactions with abasic DNA and multivalent substrate recognition. Nat Struct Mol Biol, 26, 607-612.

[62] Carrico, I.S. (2008). Chemoselective modification of proteins: hitting the target. Chem. Soc. Rev. 37, 1423-131.

[63] Manabe, S.; Ishii, K; Ito, Y; (2007) Synthesis of a natural oligosaccharide antibiotic active against *Helicobacter pylori* J. Org. Chem. 16, 6107-6115.

[64] Peng, P.; Xiong, D.C; Ye X-S (2014) *ortho*-Methylphenylthioglycosides as glycosyl building blocks for preactivation-based oligosaccharaide synthesis. Carbohydrate research, 384, 1-8.

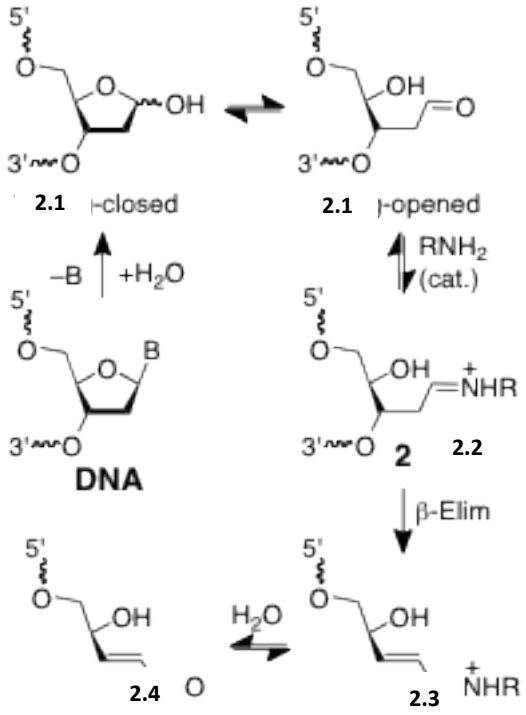
Chapter 2: Biomimetic model reactions suggest that amine-catalyzed strand cleavage at abasic sites in DNA has the potential to generate a complex mixture of interconverting products (a dynamic product mixture of previously unrecognized complexity)

2.1 Introduction

Abasic sites (**2.1**, Scheme 2.1.1) are generated by spontaneous[1,2],chemically-induced[3] and enzyme-catalyzed[4,5,6,7] hydrolysis of the glycosidic bonds that connect the nucleobases (**B** in Scheme 2.1.1) to the deoxyribose backbone of DNA. These may be the most common unavoidable lesions found in the genetic material of living cells and it is important to understand their reactivity.[8,9,10,11] Abasic sites exist as an equilibrium mixture of the ring-closed hemiacetal alongside small amounts (~1%) of the ring-opened aldehyde (Scheme 2.1.1).[12,13] The electrophilic nature of the abasic aldehyde residue leads to the generation of secondary lesions including interstrand DNA-DNA cross-links,[14,15,16,17] DNA-protein cross-links,[18,19,20,21] and strand breaks.[22,23,24]

Strand cleavage at abasic sites in DNA involves β-elimination of the 3'-phosphate residue to generate a break with a phosphoryl residue on the 5'-side and a reactive α,β-unsaturated alkenal sugar remnant on the 3'-side (Scheme 2.1.1).[22,23,24] The 3'-alkenal sugar remnant has been detected in cellular DNA[25, 26] and there is evidence from studies involving dysregulated base excision repair that these lesions are more toxic than the parent abasic site.[27,28,29]

Scheme 2.1.1. Amine-catalyzed strand cleavage at an abasic site in DNA.



In the analysis of DNA damage and repair, β-elimination at abasic sites often is induced by heat, NaOH, or amines.[8,24,30] In cells, however, most strand cleavage at abasic sites probably is catalyzed by amine groups in small molecules and proteins. The nucleus is rich in low molecular weight polyamines such as spermine that can catalyze β-elimination at abasic sites.[24,31,32,33,34,35] Amine residues in peptides, histones, and other nuclear proteins also can catalyze strand cleavage at abasic sites.[36,37,38,39,40,41,42,43] In enzymology, the ability to catalyze this reaction is termed a β-lyase activity.[44] Amine-catalyzed strand cleavage at abasic sites proceeds via iminium ion intermediates **2.2** to generate the α,β-unsaturated iminium products **2.3** (Scheme 2.1.1) For example, the pK_a

values of *N*-alkyliminium ions derived from benzaldehydes are approximately 5-7, the pK_a of the iminium ion derived from acetone and glycine is 7.6, and the pK_a of the *N*-alkyl-α,β-unsaturated iminium ion derived from crotonaldehyde is 8.6.[45,46,47] The β-elimination reaction at abasic sites is accelerated by iminium ion formation because the α-protons in iminium ions are at least 7 orders of magnitude more acidic than those in the corresponding parent aldehyde. Early work by Bender describes the early characterization of this effect.[46,48] Amine-catalyzed strand cleavage by a Lys-Trp-Lys peptide[49] and the enzyme T4 pyrimidine dimer glycosylase (T4-pdg or T4 Endo V) was shown to generate the *trans*-alkenal **2.4** as the major product (Scheme 2.1.1).[41,50,51,52] Heating a DNA abasic site at 90 ˚C in pH 7 phosphate buffer also generates predominantly the *trans*-alkenal **2.4** as the initial product.[30]

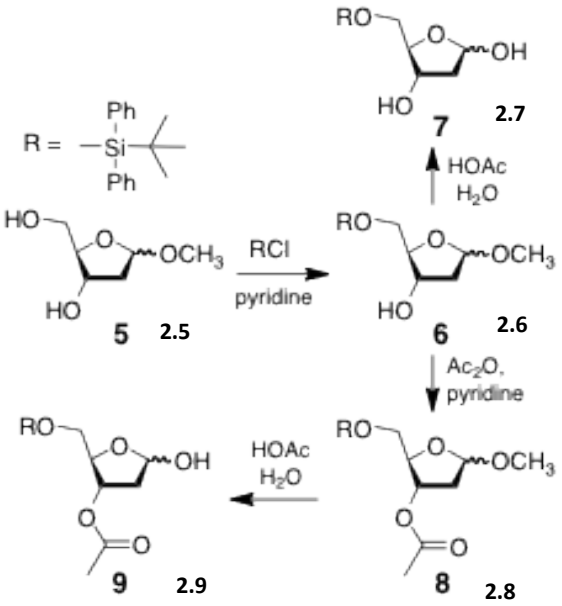
Amine-catalyzed cleavage of abasic sites in DNA is important in biology and biochemistry. During the course of some recent studies in which I employed spermine to induce strand cleavage at abasic sites in DNA,[53] I came to suspect that the products generated in this reaction are more complex than commonly thought. This inspired me to develop a low molecular weight, biomimetic system that enabled us to model the formation and properties of products arising from amine-catalyzed β-elimination at an abasic site in the DNA backbone. The results of my studies suggest that α,β-unsaturated iminium ion intermediates are at the center of an equilibrating mixture of products of previously unrecognized complexity.

2.2 Design, synthesis, and validation of a low molecular weight compound that models amine-catalyzed strand cleavage at abasic sites in DNA.

My approach involved preparation of a 2-deoxyribose derivative bearing a moderately active leaving group at the 3-position that would be resistant to spontaneous elimination reactions in aqueous buffer, while readily undergoing amine-catalyzed elimination via an iminium ion intermediate. I anticipated that an acetyl group might provide the desired balance of stability and reactivity. My design also incorporated a silyl protecting group on the 5-OH to lock the sugar into the 5-membered, furanose form found in DNA and to facilitate analysis by TLC, organic separations, and chromatographic separations on silica gel.

I converted 2-deoxy-D-ribose to the corresponding methyl acetal **2.5**,[54] followed by reaction with *t*-butyldiphenylsilyl chloride in pyridine to obtain the 5-silylated derivative **2.6** as a mixture of the β and α anomers.[55] Treatment of **2.6** with acetic anhydride in pyridine gave the acetylated product **2.8** as a mixture of the β and α anomers.[54] Removal of the methyl acetal protecting group in acid provided the desired compound **2.9** in good yield. (Scheme 2.2.1)

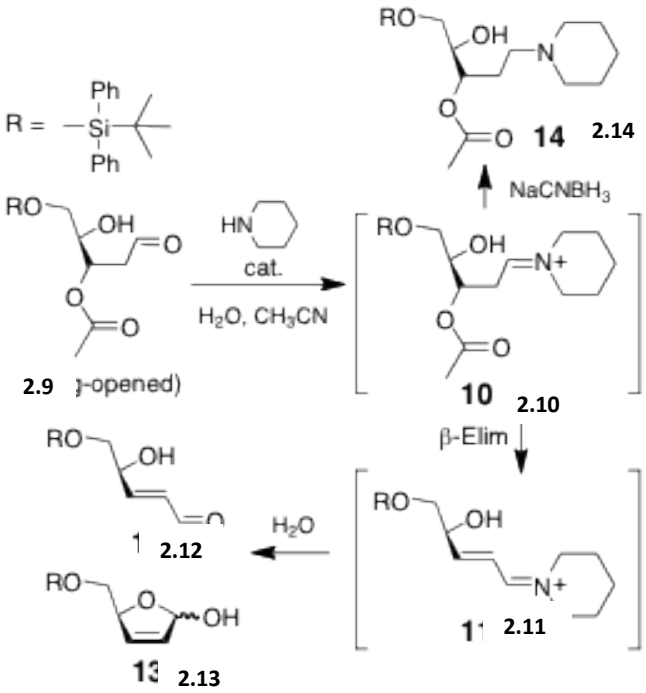
Scheme 2.2.1. Synthesis of the DNA abasic site model compound 2.9.



As hoped, compound **2.9** (25 mM) was stable for 2 h in a solvent mixture composed of 1:1 acetonitrile/buffer (50 mM sodium phosphate, pH 7, 24 h, 24 ˚C). Extended incubation of **2.9** at 37°C for 7 d gave an approximately 30% yield of 9:1 mixture of the **2.12** and **2.13**. On the other hand, **2.9** (25 mM) completely decomposed within 2 h when catalytic amounts (0.1-10 mol%) of an amine catalyst such as *N*,*N*'-dimethylethylenediamine (DMEDA) or piperidine was added to the reaction mixture. I expected the amine-catalyzed decomposition of **2.9** to proceed via an iminium ion intermediate **2.14**. I and others have previously used hydride reagents to trap iminium ion intermediates generated in the reactions of low molecular weight amines with Ap sites in DNA.[53, 42, 56,57,58,59] Work performed by Jay Jha in the group showed that incubation of **2.9** with piperidine in the presence of the hydride reagent $NaCNBH_3$, followed by LC-MS analysis revealed a major signal in the HPLC-UV and ion current chromatograms with an

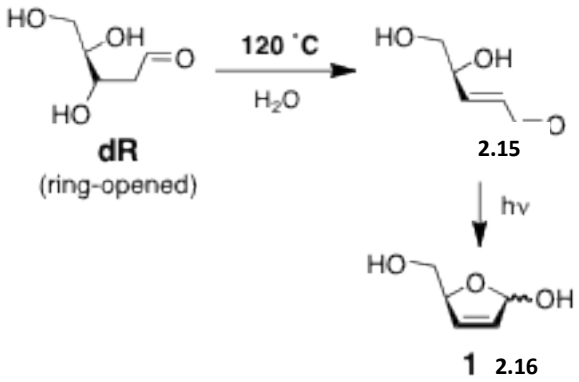
m/*z* of 484, corresponding to the $[M+H]^+$ ion of reduced iminium ion **2.14** (Scheme 2.2.2, Figure S2.1).

Scheme 2.2.2. Amine catalyzed generation of alkenal products from precursor 2.9.



The elimination reactions considered here have the potential to generate either the *cis*- or *trans*-alkenal products (Scheme 2.2.2). The vinylic region in the proton NMR is diagnostic for these compounds.[12,60,61] For spectroscopic comparison, I prepared the *trans*-alkenal **2.15** by thermolysis of 2-deoxyribose (dR, Scheme 2.2.3).[60] The crude *cis*-alkenal **2.16** was prepared by photolysis of the *trans* isomer **2.15** (Scheme 2.2.3).[12]

Scheme 2.2.3 Preparation of *cis*- and *trans*-alkenals 2.15 and 2.16.



When **2.9** (25 mM) was treated with piperidine (10 mol%) at 24 ˚C in the standard solvent mixture composed of 1:1 acetonitrile/buffer (50 mM sodium phosphate, pH 7) and the reaction extracted with ethyl acetate at a very early stage (5 min), I observed a product mixture displaying NMR signals consistent with a 9:1 mixture of the *trans*-alkenal **2.12** and the *cis*-alkenal **2.13** (Scheme 2.2.2 Figure S3.2). The ^{1}H-NMR resonances of these products closely matched those observed for the synthetic standards described above. When the amine-catalyzed decomposition of **2.9** was allowed to proceed to completion (1 h) in the absence of an added nucleophile to intercept the α,β-unsaturated iminium ion intermediate, I observed low yields of **2.12** and **2.13** embedded in a complex mixture of decomposition products.

When **2.9** (15 mM) was treated with piperidine (122 mM) at 24 ˚C in a solvent mixture composed of 1:1 acetonitrile/buffer containing HCl (12 mM), the reaction cleanly generates **2.12** within 15 min (Figure S2.2). After 1 h, the products evolved to a 77:23 mixture of **2.12** and **2.13** that could be isolated by column chromatography in 70% combined yield. A control reaction showed that there was no significant decomposition of **2.9** under these conditions in the absence of piperidine. After 3 h, the mixture contained a 46:55 ratio of **2.12** and **2.13**, with little decomposition. These reaction conditions may

circumvent oligomerization of the α,β-unsaturated aldehyde and the corresponding iminium ion.[62] The results suggest that the *trans*-alkenal **2.12** isomerizes to the *cis*-alkenal **2.13** in aqueous buffered solution, in the presence of an amine catalyst. Literature precedents describing amine-catalyzed *cis*-*trans* isomerization of α,β-unsaturated carbonyl compounds provide a reasonable mechanistic basis for the conversion of the *trans*-alkenal **2.12** to the *cis* isomer **2.13** (Scheme 2.2.3).[63,64]

Overall, the results provide evidence that the amine-catalyzed elimination of acetate from **2.9** proceeds via iminium ion intermediates to generate the *trans*-alkenal **2.4** that is analogous to the characteristic product of amine-catalyzed strand cleavage in DNA.[41,50,51] In addition, our results provide evidence that the *trans*-alkenal product can convert to the *cis*-alkenal in the presence of an amine.

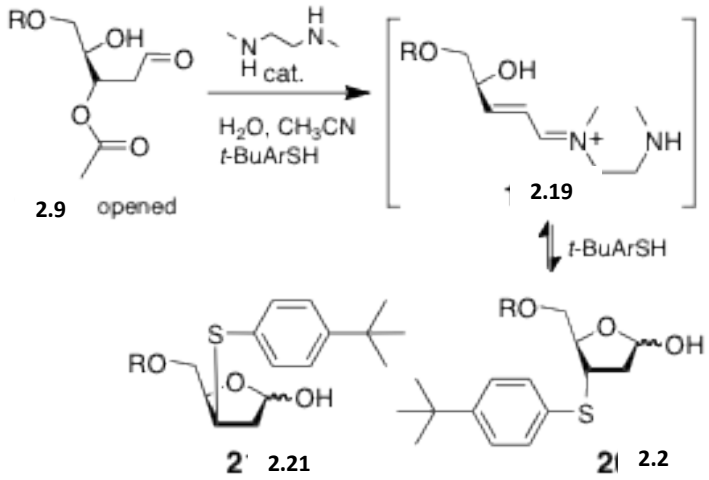
2.3 Evidence for reversible conjugate addition of thiol-containing compounds to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from 2.9.

No significant reaction was observed when **2.9** (25 mM) was stirred with *t*-butylbenzenethiol (10 equiv) in the absence of an amine catalyst in the standard solvent mixture for 24 h at 24 ˚C. On the other hand, when **2.9** (25 mM) was mixed with the amine catalyst DMEDA (10 mol%) and *t*-butylbenzenethiol (10 equiv), all starting material was consumed in less than 5 min. From this reaction, I obtained an 85% yield of thiol-addition products displaying NMR resonances consistent with a mixture of the C3-epimers **2.20** and **2.21**, envisioned to arise from conjugate addition of the thiol to the α,β-unsaturated iminium ion **2.19** (Scheme 2.3.1). Further examination of this reaction revealed that the

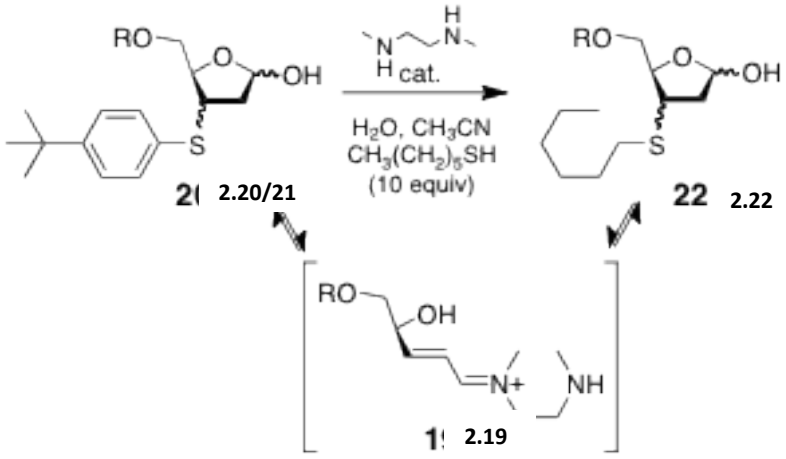
3:2 mixture of **2.20** and **2.21** observed at 5 min gave way to a >20:1 mixture of **2.20** and **2.21** upon extended incubation (30 min), suggestive of an initial kinetic mixture evolving to a thermodynamic mixture of products. Each compound exists as a mixture of β and α anomers. Similar mixtures of adducts were obtained for a variety of structurally diverse thiols (Supporting Information). DMEDA, piperidine, and spermine all catalyzed decomposition of **2.9**, with concomitant generation of the thiol adducts **2.20** and **2.21** within 5 min. Reactions induced by lysine were somewhat slower (about 2 h for complete consumption of starting material), while reactions triggered by valine and tryptophan and threonine were sluggish, with most starting material remaining intact after 24 h. Incubation of **2.9** with glutathione (20 mM), in the standard solvent mixture and in the absence of amine catalyst, did not yield significant reaction over the course of 24 h.

Incubation of the *t*-butylbenzenethiol adducts **2.20** and **2.21** in the presence of the amine catalyst DMEDA (10 mol%) and an excess (10 equiv) of hexanethiol led to an 81% yield of a stereoisomeric mixture of the thiol exchange products **2.22** within 5 min (Scheme 2.3.2). On the other hand, in the absence of the amine catalyst, **2.20** and **2.21** did not undergo thiol-exchange over the course of 1 h. Overall, the results provide evidence for reversible addition of thiols to the α,β-unsaturated iminium ion **2.19**.

Scheme 2.3.1. Amine-catalyzed generation of thiol adducts from 2.9.



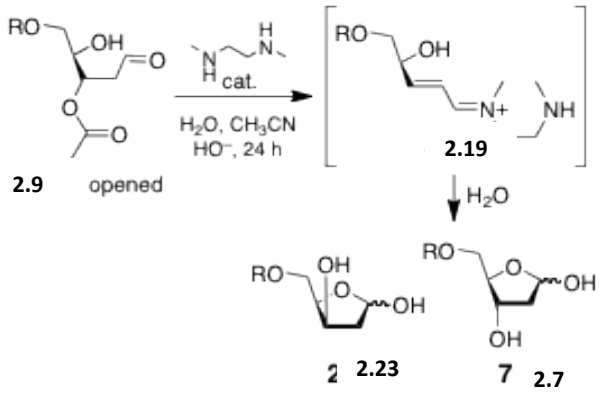
Scheme 2.3.2. Thiol exchange reactions catalyzed by DMEDA.



2.4 Evidence for reversible conjugate addition of water to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from 2.9.

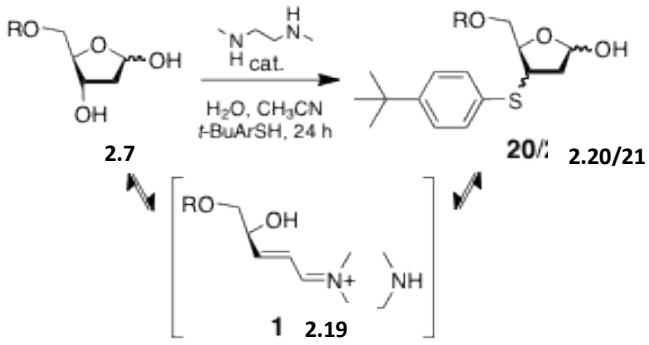
Treatment of **2.9** (25 mM) with DMEDA (10 mol%) for 12 h in a solvent composed of 1:1 acetonitrile and aqueous NaOH (10 mM), produced an 85% yield of a mixture composed of the 2-deoxyribose derivative **2.7** alongside traces of the 2-deoxyxylose isomer **2.23** (Scheme 2.4.1). The biological amine, spermine (10 mol%), catalyzes formation of the same products from **2.9** under these conditions. The structure of the major isomer **2.7** was confirmed by comparison of the ^{1}H-NMR data to that of an authentic standard prepared by treatment of the methyl acetal **2.2** with mild aqueous acid (Scheme 2.2.1). Several control experiments suggest that **2.7** arises via conjugate addition of water to **2.19** rather than by hydrolysis of the acetyl group in **2.9**. First, **2.7** was not generated from **2.9** in the absence of the amine catalyst, consistent with the idea that the iminium ion **2.19** is an obligate intermediate in the generation of **2.7**. Second, incubation of the methyl acetal **2.8** with the amine catalyst DMEDA (10 mol%) in the solvent composed of a mixture of 1:1 acetonitrile and aqueous NaOH did not lead to hydrolysis of the acetyl group at the C3-OH group, simultaneously providing evidence that the reaction conditions do not support hydrolysis of the acetyl group at C3 and that the C1-aldehyde is required.

Scheme 2.4.1. Conjugate addition of water.



Incubation of **2.7** with DMEDA and *t*-butylbenzene thiol (10 equiv), generated the corresponding thiol adducts **2.20** and **2.21** in modest yield (23%) after 48 h at 24 ˚C (Scheme 2.4.2). This reaction is much less effective than the amine-catalyzed transthiolation described above (Scheme 2.3.2). Overall, the evidence is consistent reversible conjugate addition of water to the α,β-unsaturated iminium ion **2.19** generated by amine-catalyzed elimination of acetate from **2.9**.

Scheme 2.4.2. Amine-catalyzed water-thiol exchange reaction from 2.7.



2.5 Discussion

Amine-catalyzed strand cleavage at abasic sites in DNA is a biologically important reaction[24,31-40,43] and it is important to fully characterize the products of these processes. The general expectation based on literature precedents[12,41,50] is that amine-catalyzed strand cleavage at abasic sites generates a nick with a 3'-*trans*-α,β-unsaturated aldehyde and 5'-phosphoryl end groups (**2.4**, Schemes 2.1.1)[12,22-24,33] Some catalysts, higher temperatures, or longer reaction times, can generate a single-nucleotide gap flanked by 5'-phosphoryl and 3'-phosphoryl end groups via sequential α,β and γ,δ-elimination reactions.[12,22-24,33]

Here I developed a low molecular weight, biomimetic system that models the formation and properties of products arising from amine-catalyzed β-elimination at abasic sites in DNA. Use of a low molecular weight model system enabled high-resolution characterization of the chemical structures and properties of the products generated in the reactions. Amine-catalyzed elimination of acetate from my model compound **2.9** proceeds via iminium ion intermediates **2.10** and **2.11** to generate the *trans*-alkenal **2.12** that is analogous to the characteristic product **2.4** arising from amine-catalyzed strand cleavage in DNA.[12,41,50,51] In addition, under some conditions, I observed conversion of the initially-formed *trans*-alkenal **2.12** to the *cis*-alkenal **2.13** in the amine-catalyzed elimination of acetate from **2.9**. This is a striking observation, as I have found only two reports mentioning formation of the *cis*-alkenal **2.25** in DNA (Scheme 2.6.1). The *cis*-alkenal end group was seen in the crystallographic analysis of the repair enzyme 8-oxoguanine glycosylase (OGG) complexed with an abasic duplex[65] and low yields of this product were generated by heating a DNA abasic site at 90 ˚C in pH 7 phosphate buffer.[66] Our results suggest that, once generated, the *cis*-alkenal is relatively stable. The occurrence of this

product in the amine-catalyzed cleavage of abasic sites in DNA deserves further investigation.

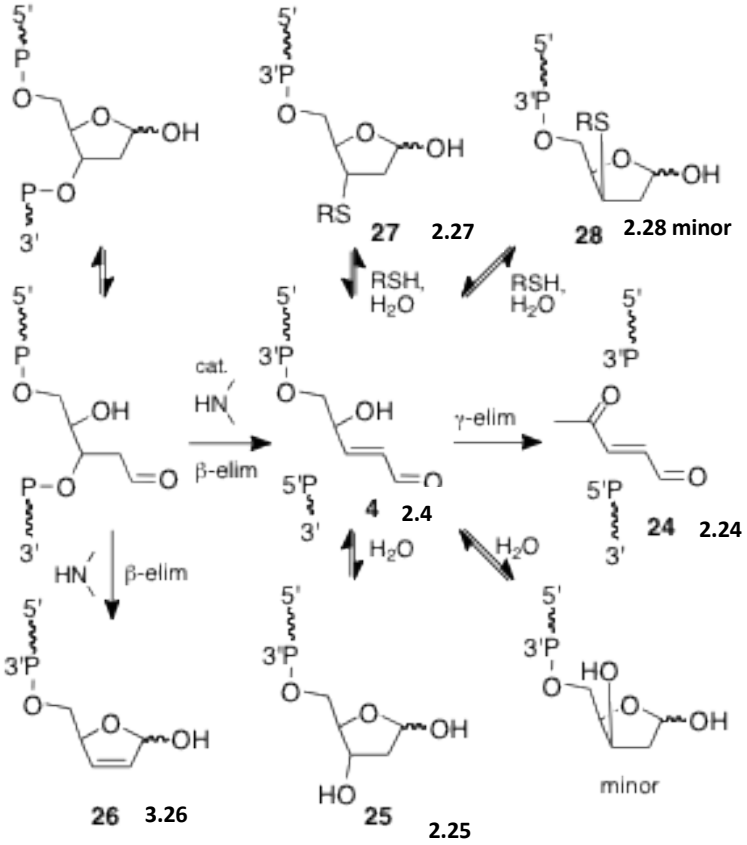
Early researchers recognized that thiols can add to the 3'-alkenal group generated by amine-catalyzed strand cleavage but the precise chemical structure(s) and reactivity of the resulting products has not been characterized previously.[11,21,67] In general, the conjugate addition of thiols to low molecular weight α,β-unsaturated aldehydes under biological conditions has ample precedent.[60,68,69,70] I find that thiol-containing compounds undergo rapid conjugate addition to the *trans*-α,β-unsaturated aldehyde **2.12** and the corresponding iminium ion **2.11**. The addition of thiols to the *trans*-α,β-unsaturated iminium ion intermediate **2.11** is noticeably faster than reaction with the α,β-unsaturated aldehyde **2.12**. These reactions generate mixtures of epimeric products (**2.20** and **2.21**, Scheme 2.3.1). The thiol adducts **2.20** and **2.21** are stable in the absence of amines but readily undergo stereochemical equilibration and thiol exchange reactions in the presence of an amine catalyst (Scheme 2.3.2). Clearly, both conjugate addition of nucleophiles and the reverse reaction involving elimination of the nucleophile are accelerated by iminium ion formation. Cells contain millimolar concentrations of thiols such as glutathione[71] and I expect that thiol adducts such as **2.26** and **2.27** (Scheme 2.6.1) should be major products arising from β-elimination at abasic sites in DNA in biological environments. This possibility was recognized by Bailly and Verly in 1988,[21] but the existence of such products in cellular DNA has yet to be established.

I also observed reversible conjugate addition of water to the α,β-unsaturated iminium ion intermediate **2.11**, under mildly basic conditions (Scheme 2.4.1). In contrast, water does *not* add to the parent α,β-unsaturated aldehyde **2.12** under these conditions. This result

clearly illustrates the amplified reactivity of the α,β-unsaturated iminium ion **2.11** relative to the corresponding α,β-unsaturated aldehyde **2.12**. The 3'-deoxyribose end group **2.28** (Scheme 2.6.1) arising from the addition of water to the *trans*-alkenal **2.4** has not been observed previously in the cleavage of DNA abasic sites catalyzed by low molecular weight amines; however, there is evidence consistent with the generation of this product by the lyase action of the base excision repair glycosylase, Endo III.[72,73] The 3'-deoxyribose end group was identified as a minor product arising from thermal cleavage of an abasic site in a DNA dinucleotide at 90 ˚C in pH 7 phosphate buffer.[66] In a broader chemical context, my result is consistent with early literature describing piperidine- and sarcosine-catalyzed conjugate addition of water to crotonaldehyde[74] and highlights the amplified reactivity of the *trans*-α,β-unsaturated iminium ion **2.11** compared to the α,β-unsaturated aldehyde **2.12**, with respect to conjugate addition processes. This effect that has been exploited by synthetic chemists working in the area of iminium ion organocatalysis.[75,76,77] Another possible precedent illustrating the amplified reactivity of α,β-unsaturated iminium ion intermediates may be found in the arginine and histone-catalyzed reactions of 4-hydroxy-2-nonenal (HNE) with the nucleoside 2'-deoxyguanosine.[78,79] Thus, the results presented here, alongside previous precedents, suggest that iminium ion intermediates could be important in the generation of interstrand cross-links arising from amine-catalyzed strand cleavage at abasic sites in duplex DNA.[20] Overall, my results suggest that amine-catalyzed strand cleavage at abasic sites in DNA has the potential to generate dynamic product mixtures of previously unrecognized complexity. My results forecast at least eleven distinct products arising from amine-catalyzed cleavage of an abasic site, when diastereomers originating from the existence of

α- and β-anomers are considered (Scheme 2.5.1). A number of these products and processes have not been characterized previously. To the extent that my reactions model the cellular environment, it is important to recognize that each of these strand cleavage products represents a distinct 3'-blocking group that must be removed or "trimmed", in order to complete the gap-filling and ligation steps in base excision repair or single-strand break repair pathways.[80,81,82] Finally, each of these strand cleavage products presents equilibrium amounts of an electrophilic aldehyde or the corresponding iminium ion group and, as a result, has the potential to generate chemically distinct DNA-DNA[20] or DNA-protein cross-links[83,84] that could be important in biology. A collaborative project within the group not shown within this thesis describes the results of a parallel study characterizing the products formed by strand cleavage at abasic sites in DNA oligonucleotides. The electrophilic nature of the abasic aldehyde residue leads to the generation of secondary lesions including interstrand DNA-DNA cross-links,[17,19,35,] DNA-protein cross-links,[14,19,21,85] and strand breaks.[22-24]

Scheme 2.5.1. My results suggest that α,β-unsaturated iminium ion intermediates are at the center of a dynamic, equilibrating mixture of products of previously unrecognized complexity.



Material and Methods.

All commercial materials were used as received unless otherwise noted. Commercially available materials were obtained from Sigma-Aldrich, TCI, and Alfa Aesar. Flash column chromatography was performed using 230-400 mesh silica gel as a stationary phase. Thin layer chromatography was performed on silica gel plates from Sigma Chemical Co. Deuterated solvents were purchased from Cambridge Isotope Laboratories. ^{1}H NMR was recorded on a 500 MHz or a 600 MHz spectrometer while ^{13}C NMR spectra was obtained on the same instruments at 126 or 151 MHz. The chemical shift values (δ) are reported in ppm (residual chloroform δ = 7.26 ppm and 77.16 ppm for ^{1}H and ^{13}C respectively). The ^{1}H spectra are reported as follows δ (multiplicity, coupling

constant *J*, number of protons). HPLC analysis were performed on a modular system equipped with a 168-diode array detector and 32 KARAT software. The HPLC system was coupled with an ion-trap mass spectrometer. Beta-basic C18, 150 Å, 0.46 cm × 15 cm column was used for analysis. Mobile phase for the run were water and acetonitrile each containing 0.1% TFA.

Experimental Procedures

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (2.6). The solution of 2'-deoxyribose (1.84 g, 13.72 mmol), methanol (60 mL) and HCl (1 mL, 2 M) was stirred for 1 h at 24 ˚C. The reaction was quenched by the addition of pyridine (5 mL) and the crude mixture containing **2.5** was concentrated *in vacuo*. The residue was dissolved in pyridine (20 mL) and t-butyldiphenylsilyl chloride (3.74 mL, 14.4 mmol) was added, followed by stirring at 24 ˚C under an atmosphere of nitrogen gas for 20 h. The reaction was quenched by addition of water (20 mL) and then extracted with ethyl acetate (3 x 40 mL). The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **2.6** (3.70 g, 70% yield) as a colorless oil: Rf = 0.3 (1:4 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.70 – 7.37 (m, 10H), 5.11 (d, *J* = 4.6 Hz, 0.6H), 5.05 (dd, *J* = 5.4, 2.1 Hz, 0.4H), 4.51 (td, *J* = 6.7, 4.4 Hz, 0.4H), 4.30 (dd, *J* = 10.3, 6.0 Hz, 0.6H), 4.16 (ddd, *J* = 5.0, 3.5, 1.5 Hz, 0.6H), 3.98 – 3.91 (m, 0.4H), 3.82 (dd, *J* = 10.2, 5.2 Hz, 0.4H), 3.75 (dd, *J* = 11.0, 3.6 Hz, 0.6H), 3.66 (dd, *J* = 10.2, 7.6 Hz, 0.4H), 3.61 (dd, *J* = 11.0, 4.9 Hz, 0.6H), 3.38 (s, 1.8H), 3.27 (s, 1.2H), 2.85 (d, *J* = 10.7 Hz, 1H),

2.24 – 2.15 (m, 1.2H), 2.03 (d, J = 1.1 Hz, 0.8H), 1.08 (s, 3.6H), 1.05 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.8(135.7), 133.4, 130.0(129.9), 127.9(127.8), 105.8(105.1), 88.0(85.8), 73.5(73.4), 65.5(64.5), 55.2(54.9), 41.3(41.2), 27.0(26.9), 19.4.; HRMS (ESI, $[M+Na]^+$) *m/z* calcd for $C_{22}H_{30}SiO_4Na$: 409.1811; found 409.1807.

Synthesis of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (2.7). The solution of **2.6** (1.0 g, 2.6 mmol) in acetone (20 mL), water (10 mL), and acetic acid (60 mL) was heated in an oil bath at 65 ˚C with stirring for 8 h. The mixture was then diluted with diethyl ether (100 mL) and saturated sodium bicarbonate was added in 20 mL aliquots until the bubbling ceased. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (3:7) gave **2.7** (0.78 g, 84% yield) as a colorless oil: Rf = 0.3 (3:7 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.67 (dddt, J = 11.8, 6.7, 3.8, 1.5 Hz, 4H), 7.48 – 7.34 (m, 6H), 5.59 (s, 0.4H), 5.58 (s, 0.6H), 4.39 (dt, J = 5.7, 1.2 Hz, 0.4H), 4.30 (ddd, J = 5.0, 3.7, 1.2 Hz, 0.6H), 4.03 – 3.79 (m, 0.6H), 3.79 – 3.75 (m, 0.4H), 3.71 (dd, J = 11.0, 3.8 Hz, 1.2H), 3.56 (dd, J = 10.9, 5.1 Hz, 0.8H), 2.23 – 2.08 (m, 1.2H), 2.07 – 2.01 (m, 0.8H), 1.12 – 1.07 (m, 3.6H), 1.05 (d, J = 1.6 Hz, 5.4H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.7(135.6), 133.3(133.2), 123.0(129.9), 127.9(127.8), 99.6(99.0), 87.9(86.4), 73.6(73.0), 65.2(64.5), 41.6, 27.0(26.9), 19.3; HRMS (ESI $[M +Na]^+$) *m/z* calcd for $C_{21}H_{28}SiO_4Na$: 395.1655; found 395.1652.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (2.8). The solution of **2.6** (2.0 g, 5.2 mmol) in pyridine (10 mL), and acetic anhydride (2 mL, 21.2 mmol) was stirred for 2 h at 24 ˚C. The reaction was quenched by addition of methanol (3 mL) and the crude mixture was concentrated *in vacuo*. The residue was mixed with water (30 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:9) gave **2.8** (2.19 g, 99% yield) as a colorless oil: Rf = 0.8 (1:9 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.69 (ddt, *J* = 8.1, 5.0, 1.8 Hz, 4H), 7.48 – 7.36 (m, 6H), 5.35 (ddd, *J* = 6.9, 4.3, 2.8 Hz, 0.4H), 5.27 (ddd, *J* = 8.0, 3.3, 1.8 Hz, 0.6H), 5.12 (dt, *J* = 5.5, 1.7 Hz, 1H), 4.23 – 4.08 (m, 1H), 3.82 (ddd, *J* = 47.1, 11.0, 3.6 Hz, 1.2H), 3.76 – 3.67 (m, 0.8H), 3.40 (s, 1.8H), 3.30 (s, 1.2H), 2.44 (ddd, *J* = 14.6, 8.0, 5.5 Hz, 0.6H), 2.32 (ddd, *J* = 14.1, 6.9, 3.1 Hz, 0.4H), 2.13 – 1.98 (m, 4H), 1.06 (s, 3.6H), 1.05 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 171.1(170.50), 135.8(135.7), 133.4(133.3), 129.8(129.8), 127.8(127.8), 105.6(105.5), 84.2(84.1), 75.5(74.8), 64.9(64.3), 55.4(55.2), 39.6(39.2), 26.9, 21.3(21.2), 19.4(19.3); HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{24}H_{32}SiO_5Na$: 451.1916; found 451.1911.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (2.9). The solution of **2.8** (1.0 g, 2.33 mmol) in acetone (20 mL), water (10 mL), and acetic acid (60 mL) was heated in an oil bath at 65 ˚C with stirring for 8 h. The mixture was then diluted with diethyl ether (100 mL) and

saturated sodium bicarbonate was added in 20 mL aliquots until the bubbling ceased. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (2:3) afforded **2.9** (0.8 g, 84% yield) as a colorless oil: Rf = 0.3 (2:3 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.74 – 7.61 (m, 4H), 7.49 – 7.33 (m, 6H), 5.63 (d, *J* = 4.9 Hz, 0.6H), 5.59 (dd, *J* = 5.5, 2.7 Hz, 0.4H), 5.41 (ddd, *J* = 7.0, 4.2, 2.6 Hz, 0.4H), 5.37 (ddd, *J* = 7.1, 2.2, 1.2 Hz, 0.6H), 4.32 (td, *J* = 3.5, 2.1 Hz, 0.4H), 4.16 – 4.08 (m, 0.6H), 3.86 – 3.79 (m, 1.2H), 3.72 (dd, *J* = 11.1, 3.7 Hz, 0.8H), 2.50 – 2.32 (m, 0.6H), 2.24 (ddd, *J* = 14.2, 5.5, 4.1 Hz, 0.4H), 2.17 (s, 1.2H), 2.09 (s, 1.8H), 2.07 (s, 0.4H), 2.05 (d, *J* = 5.6 Hz, 0.6H), 1.09 (s, 3.6H), 1.05 (s, 5.4H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 170.8, 135.9(135.7), 133.2(133.1), 130.2(129.9), 128.0(127.8), 99.4, 84.9(84.8), 75.5(75.4), 65.2(64.3), 41.6(40.2), 27.0(26.9), 21.3(21.1), 19.31; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{23}H_{30}SiO_5Na$: 437.1760; found 437.1757.

Synthesis of (2R,3R)-1-((tert-butyldiphenylsilyl)oxy)-2-hydroxy-5-(piperidin-1-yl)pentan-3-yl acetate (2.14) as an evidence for the generation of iminium ion intermediate 3.10 in the amine-catalyzed elimination of 2.9. To a solution of **2.9** (0.04 g, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 24 ˚C, piperidine (0.01 g, 0.12 mmol) and $NaBH_3CN$ (25 mg, 0.4 mmol) was added. The reaction mixture was stirred for 3 h followed by quenching by addition of water (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The

crude product was analyzed *via* LC-MS which revealed a major signal in UV-chromatogram with an *m/z* of 484 corresponding to the $[M+H]^+$ ion (Figure S2.1).

Synthesis of (S,E)-4,5-dihydroxypent-2-enal (2.15). The solution of 2'-deoxyribose (0.50 g, 3.73 mmol) in water (20 mL) was heated at 120 ˚C for 2 h in an autoclave. The reaction mixture was concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (9:1) afforded **2.15** (0.08 g, 18% yield) as a pale-yellow oil: Rf = 0.3 (ethyl acetate): ^{1}H NMR (CD_3CN, 600 MHz) δ 9.54 (d, *J* = 8.0 Hz, 1H), 6.93 (dd, *J* = 15.7, 4.2 Hz, 1H), 6.26 (ddd, *J* = 15.7, 8.0, 1.8 Hz, 1H), 4.54 – 4.21 (m, 1H), 3.76 (bs, 1H), 3.60 (dd, *J* = 11.2, 4.6 Hz, 1H), 3.49 (dd, *J* = 11.2, 6.4 Hz, 1H), 3.34 (bs, 1H); ^{13}C NMR (CD_3CN, 151 MHz) δ 194.9, 158.1, 132.1, 72.3, 65.7.; HRMS (ESI $[M-H_2O]^+$) *m/z* calcd for $C_5H_6O_2$: 98.0367; found 98.0361.

Synthesis of (5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-ol (2.16). The solution of **2.15** (5.0 mg, 0.043 mmol) in $CDCl_3$ (0.5 mL) in a borosilicate NMR tube was placed under UV-light (115 V, 60 Hz, 0.2 amps, 254 nm) for 6 h. The solution containing **2.16** was directly used for NMR characterization. The Proton NMR showed resonances for the alkene and C-1 protons consistent with the presence of the **2.16**: ^{1}H NMR (D_2O, 500 MHz) δ 6.22 (dd, J = 6.1, 1.4 Hz, 0.6H), 6.03 – 5.95 (m, 0.4H), 5.63 (t, J = 4.7 Hz, 1H), 5.58 (dt, J = 5.5, 1.5 Hz, 1H).

Evidence for the generation of alkenal products 2.12 and 2.13 from the amine catalyzed elimination of acetate from 2.9. To a solution of **2.9** (0.04 g, 0.1 mmol) in 4

mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 24 ˚C, piperidine (1.0 mg, 0.015 mmol) was added. The reaction mixture was stirred for 5 min followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The crude proton NMR showed resonances in the alkene region consistent with the presence of the trans and cis alkenal products **2.12** and **2.13**: The crude NMR showed resonances for the alkene and C-1 protons consistent with the prescence of the trans and cis alkenal products **2.12** and **2.13**: ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.69 (dd, J = 15.7, 4.2 Hz, 1H), 6.36 (ddd, J = 15.7, 7.9, 1.8 Hz, 1H), 6.19 (dt, J = 6.0, 1.3 Hz, 0.6H), 6.08 (d, J = 4.2 Hz, 0.4H), 6.03 (dt, J = 6.0, 1.6 Hz, 1H), 5.99 – 5.96 (m, 1H).

Evidence for the isomerization of *trans*-alkenal 2.12 to *cis*-alkenal 2.13 by the amine catalyzed elimination of acetate from 2.9. To a solution of **2.9** (25 mg, 0.06 mmol) in 4 mL of a 1:1 mixture of acetonitrile and water at 24 ˚C, piperidine (50 µL, 0.5 mmol) and HCl (50 µL, 1 M) were added. The reaction mixture was stirred for 3 h and then diluted by the addition of water (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The crude proton NMR showed resonances for the alkene and C-1 protons consistent with the presence of the trans and cis alkenal products **2.12** and **2.13** (see above).

Synthesis of (R)-4-((4-(tert-butyl)phenyl)thio)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetra hydrofuran-2-ol (2.20/21) as an evidence for reversible conjugate addition of thiol-containing compounds to the *α,β*-unsaturated iminium ion. To a solution of **2.9** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and 4-*tert*-butyl benzenethiol (0.17 g, 1.0 mmol) were added. The reaction mixture was stirred for 0.5 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **3.20/21** (44 mg, 85% yield) as a colorless oil: Rf = 0.4 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.69 – 7.27 (m, 14H), 5.68 – 5.41 (m, 1H), 4.58 – 3.08 (m, 4H), 2.84 – 1.87 (m, 2H), 1.30 (d, *J* = 2.1 Hz, 9H), 1.14 – 0.98 (m, 9H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.9(135.8), 132.4, 131.9, 130.1(130.0), 129.9, 128.0(127.9), 126.4(126.3), 99.6(98.5), 85.7(84.6), 64.7(64.5), 45.4, 43.7, 42.9, 31.4, 27.1(27.0), 19.4; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{31}H_{40}SiSO_3Na$: 543.2365; found 543.2362.

Thiol exchange reaction of 2.20/21 to synthesize (R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(hexylthio)tetrahydrofuran-2-ol (2.22). To a solution of **2.20**/**21** (50 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and hexanethiol (0.12 mg, 1.0 mmol) was added. The reaction mixture was stirred for 5 min followed by

quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **2.22** (38 mg, 81% yield) as a colorless oil: Rf = 0.4 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.87 – 7.32 (m, 10H), 5.68 – 5.39 (m, 1H), 4.49 – 2.91 (m, 4H), 2.68 – 2.44 (m, 2H), 2.42 – 1.89 (m, 2H), 1.62 – 1.20 (m, 8H), 1.16 – 1.03 (m, 9H), 0.89 (tdd, *J* = 7.2, 2.1, 1.2 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 136.1(135.9, 135.8, 135.7), 133.6(133.5, 132.8, 132.7), 130.2(130.1, 129.9, 129.7), 128.0(127.9, 127.8, 127.7), 99.4(99.1, 98.6, 97.8), 86.2(84.9, 81.9, 81.3), 64.7(64.6), 44.7(44.2, 43.4), 42.2(42.1, 40.6, 40.5), 33.3(32.8, 32.0), 31.9(31.7, 31.6, 31.5), 30.2(29.9, 29.8, 29.5), 28.7(28.6), 27.1(27.0, 26.9), 22.8(22.7), 19.4(19.3), 14.3(14.2); HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{27}H_{40}SiSO_3Na$: 495.2365; found 495.2359.

Conjugate addition of water to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from 2.9. The solution of **2.9** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and NaOH (10 mM), and DMEDA (0.88 mg, 0.01 mmol) was stirred for 12 h at 37 ˚C. The reaction was quenched by the addition of saturated ammonium chloride (2 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (3:7) afforded **2.7** (32 mg, 85% yield) with spectroscopic properties matching the same material synthesized by hydrolysis of **2.6** (see above).

Amine-catalyzed water-thiol exchange reaction of 2.7 to synthesize 2.20/21. To a solution of **2.7** (80 mg, 0.22 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (1.94 mg, 0.022 mmol) and 4-*tert*-butyl benzenethiol (0.37 g, 2.20 mmol) were added. The reaction mixture was stirred for 24 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **2.20/21** (32 mg, 28% yield) with spectroscopic properties matching the same material synthesized by thiolation of **2.9** (see above).

Conjugate addition of thiol to the *trans*-alkenal (2.12) for the synthesis of 2.20/21. The solution of **2.12** (35 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7), and 4-*tert*-butyl benzenethiol (0.17 g, 1.0 mmol) was stirred for 1.5 h at 37 ˚C. The reaction was quenched by the addition of water (4 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:6) afforded **2.20/21** (37 mg, 71% yield) with spectroscopic properties matching the same material synthesized by thiolation of **2.9** (see above).

Synthesis of (5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(phenylthio)tetrahydrofuran-2-ol (Compound S2.1)

To a solution of **2.9** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and benzenethiol (50 uL, 0.5 mmol) were added. The reaction mixture was stirred for 0.5 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:9) gave the title compounds as a mixture of diastereomers. The title compounds were isolated as light yellow oils (39 mg, 87% yield). Rf = 0.5 (1:9 ethyl acetate: hexanes). ^{1}H-NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.67-7.27 (m, 15H), 5.52-5.47 (m, 1H), 4.48-3.95 (m, 1H), 3.90-3.72 (m, 1H), 3.70-3.56 (m, 1H), 2.63-2.58 (m, 1H), 2.15-2.03 (m, 1H) 1.06 (s, 9H). ^{13}C-NMR (CDCl3, 151 MHz): δ 135.9, 135.8, 135.7, 135.6, 132.0, 131.6, 130.1, 129.9, 129.3, 128, 127.9, 127.5, 127.4, 99.5, 98.6, 85.8, 84.6, 64.7, 64.5, 45.0, 43.5, 42.9, 40.7, 27.0, 19.4; HRMS (ESI [M+Na]) *m/z* calcd C27H32SiSO3Na 487.173915; actual mass 487.17351.

Synthesis of (5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(naphthalen-2-ylthio)tetra hydrofuran-2-ol (Compound S2.2)

To a solution of **2.9** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and

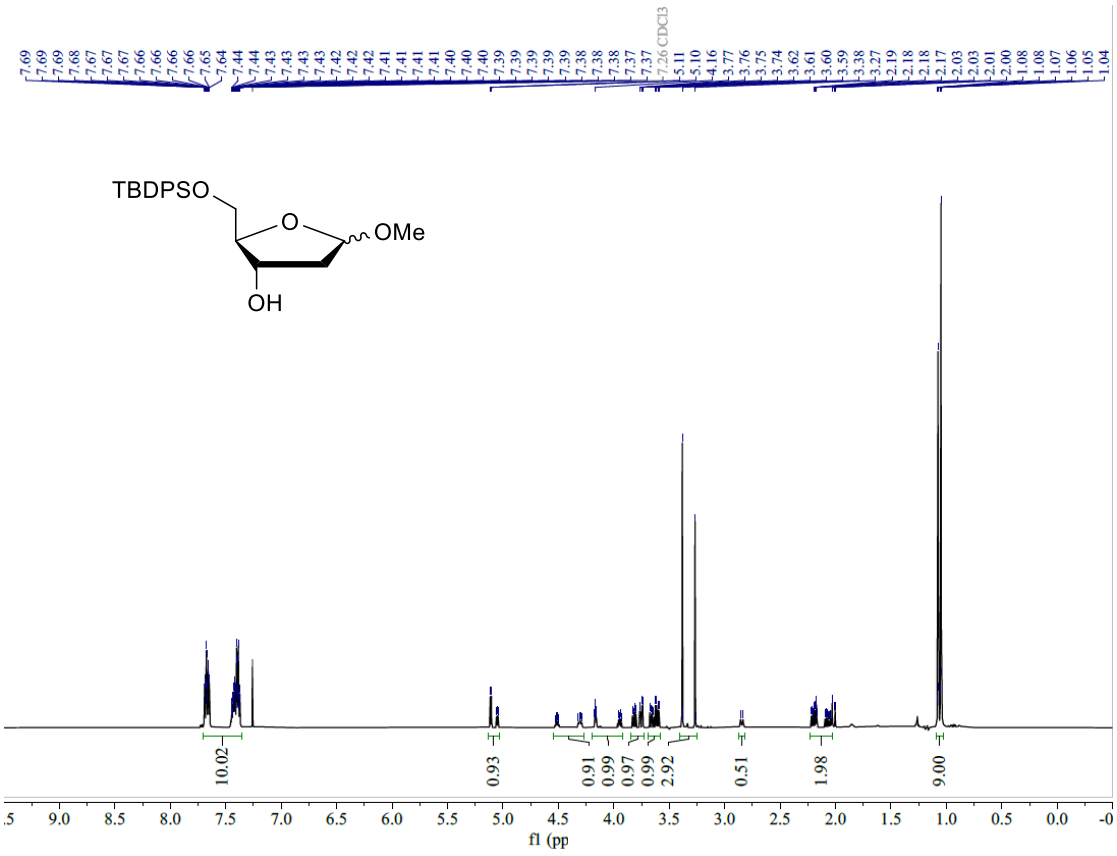
2’napthalenethiol (50 mg, 0.3 mmol) were added. The reaction mixture was stirred for 0.5 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (10:90) gave the title compounds as a mixture of diastereomers as light yellow oils (43 mg, 88% yield). Rf = 0.8 (1:9 ethyl acetate: hexanes) ^{1}H-NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.85-7.31 (m, 17H), 5.52-5.48 (m, 1H), 4.48-4.41 (m, 1H), 4.05-3.71 (m, 1H), 3.68-3.51 (m, 1H), 2.61-2.48 (m, 1H), 2.15-2.01 (m, 1H), 1.02 (s, 9H)). ^{13}C-NMR (CDCl3, 151 MHz): δ 135.9, 135.8, 135.7, 132.6, 130.8, 130.5, 130.1, 129.9, 129.4, 128.9, 128.0, 127.9 127.8 127.6, 126.9 126.5, 99.5, 98.6, 64.6, 45.1, 43.6, 43.0, 27.0 26.9, 19.4;HRMS (ESI [M+Na]) *m/z* calcd C31H34SiSO3Na 537.189566; actual mass 537.18891.

Synthesis of (5R)-4-(benzylthio)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2-ol (Compound S2.3)

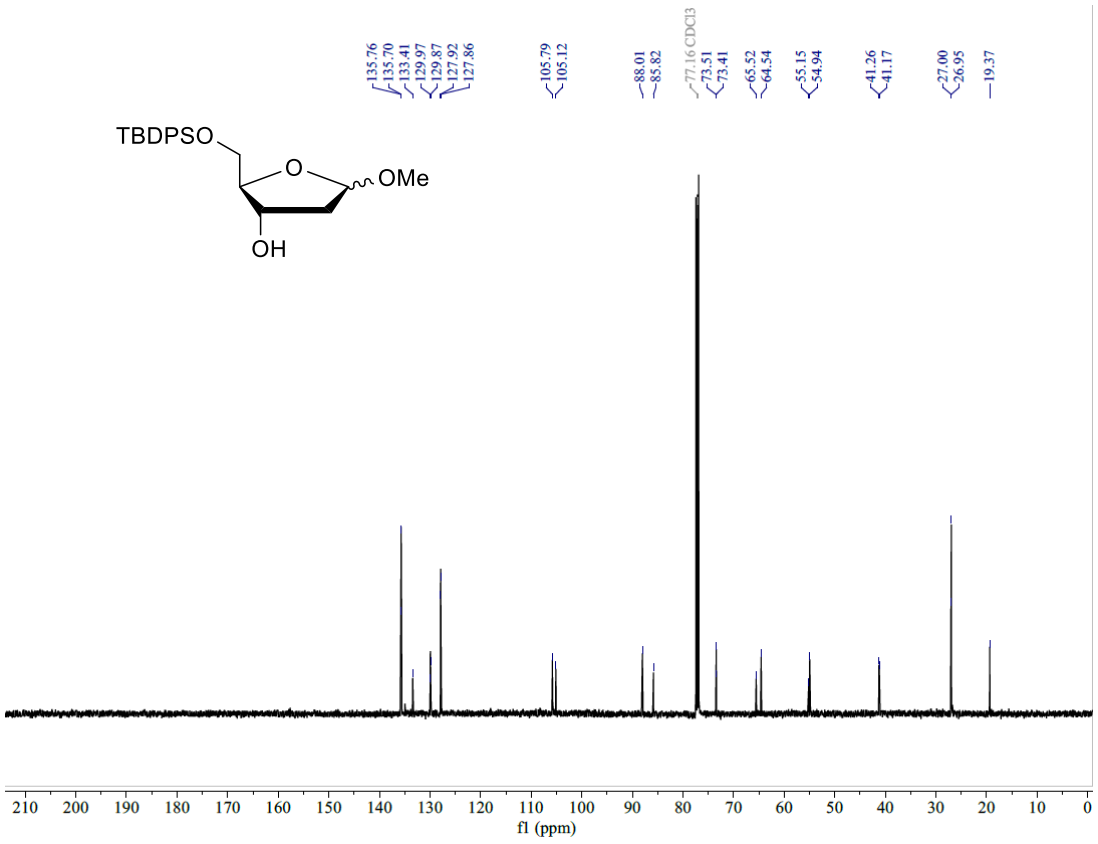
To a solution of **2.9** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and benzyl mercaptan (50 uL, 0.4 mmol) were added. The reaction mixture was stirred for 0.5 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The

mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:9) gave the title compounds as a mixture of diastereomers. The title compounds were isolated as light yellow oils (41 mg, 90% yield). Rf = 0.5 (1:9 ethyl acetate: hexanes). ^{1}H-NMR ($CDCl_3$, 600 MHz): δ (diastereomers) 7.65-7.24 (m, 15H), 5.62-5.13 (m, 1H), 4.41-4.13 (m, 1H), 3.85-3.62 (m, 1H), 3.72-3.41 (m, 1H), 2.50-2.41 (m, 1H), 2.40 (m, 1H), 2.10-2.00 (m, 2H), 1.06 (s, 9H). ^{13}C-NMR (CDCl3, 151 MHz): δ 135.9, 135.8, 133.7, 132.6, 130.2, 130.1, 129.9, 129.0 128.8, 128.0, 127.9, 127.4, 105.7, 99.6, 98.7, 85.6, 84.8, 82.0, 75.2, 65.0, 74.4, 55.4, 43.7, 41.8, 40.8, 40.3, 39.2, 36.6, 27.2, 27.0, 26.9, 21.1, 21.0, 19.3; HRMS (ESI [M+Na]) *m/z* calcd C28H34SiSO3Na 501.189565; actual mass 501.18997.

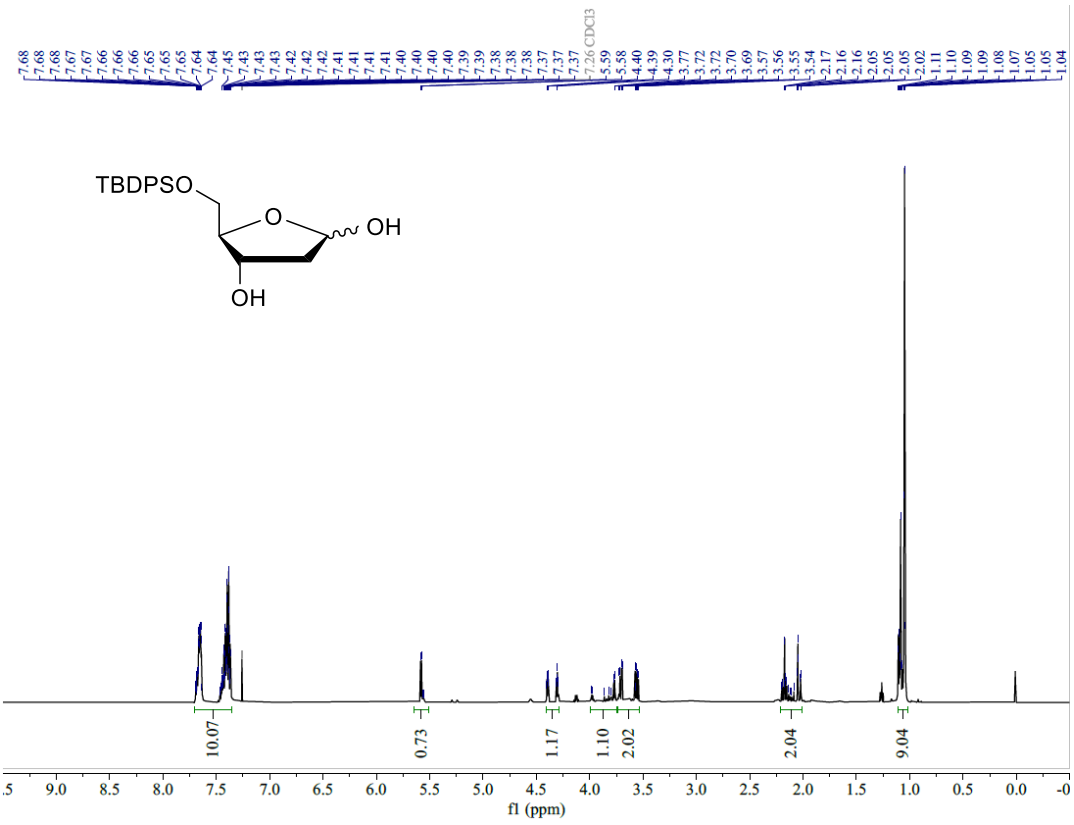
NMRs and Supplemental Information



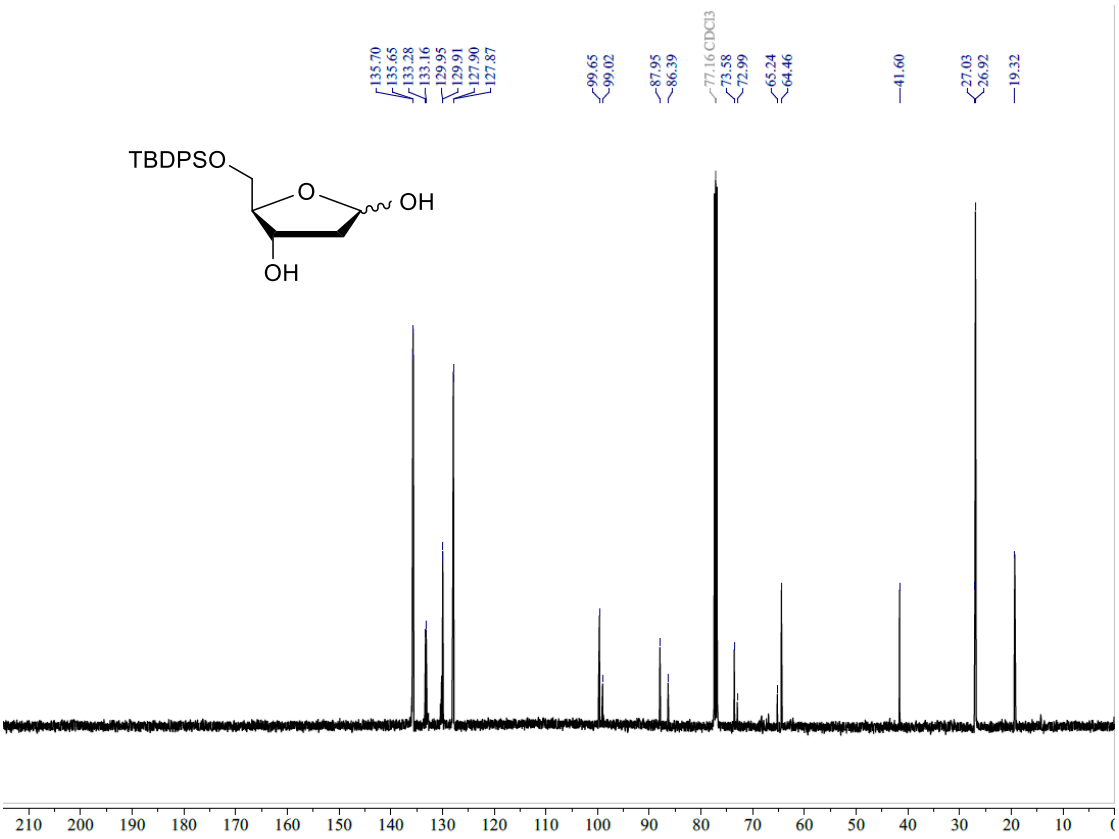
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (2.6).



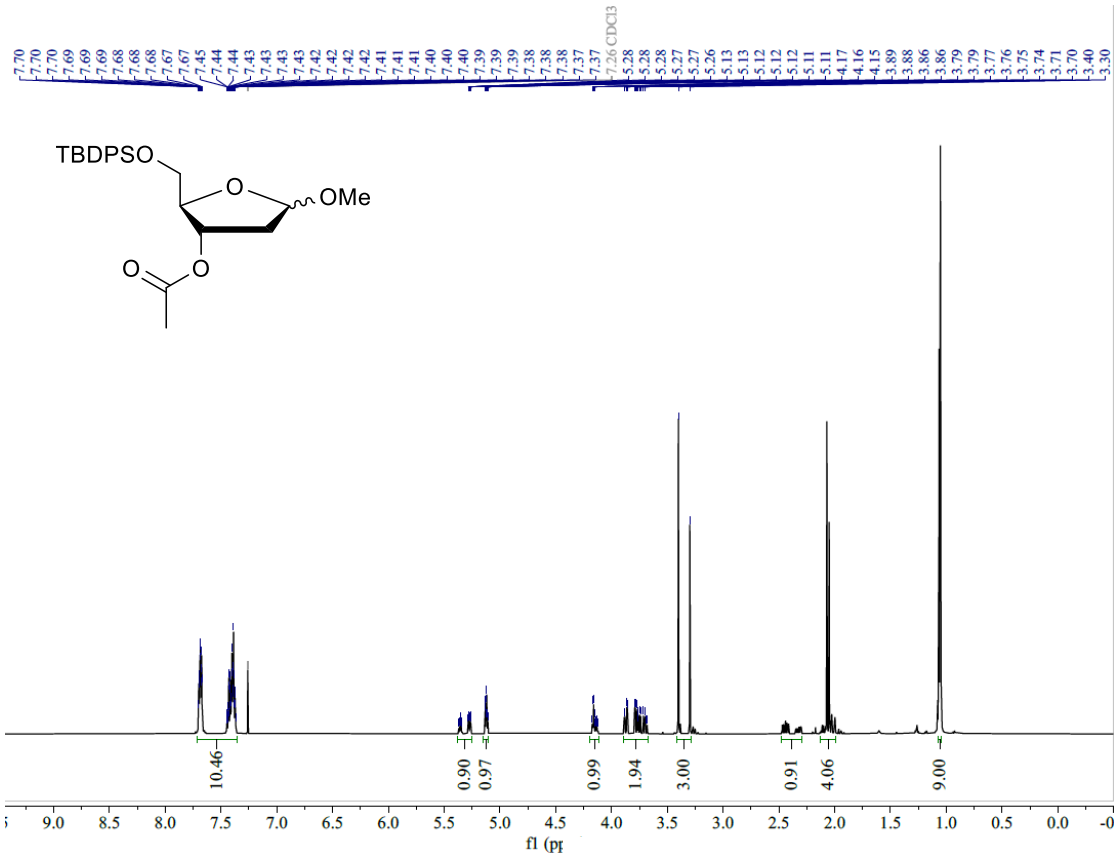
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (2.6).



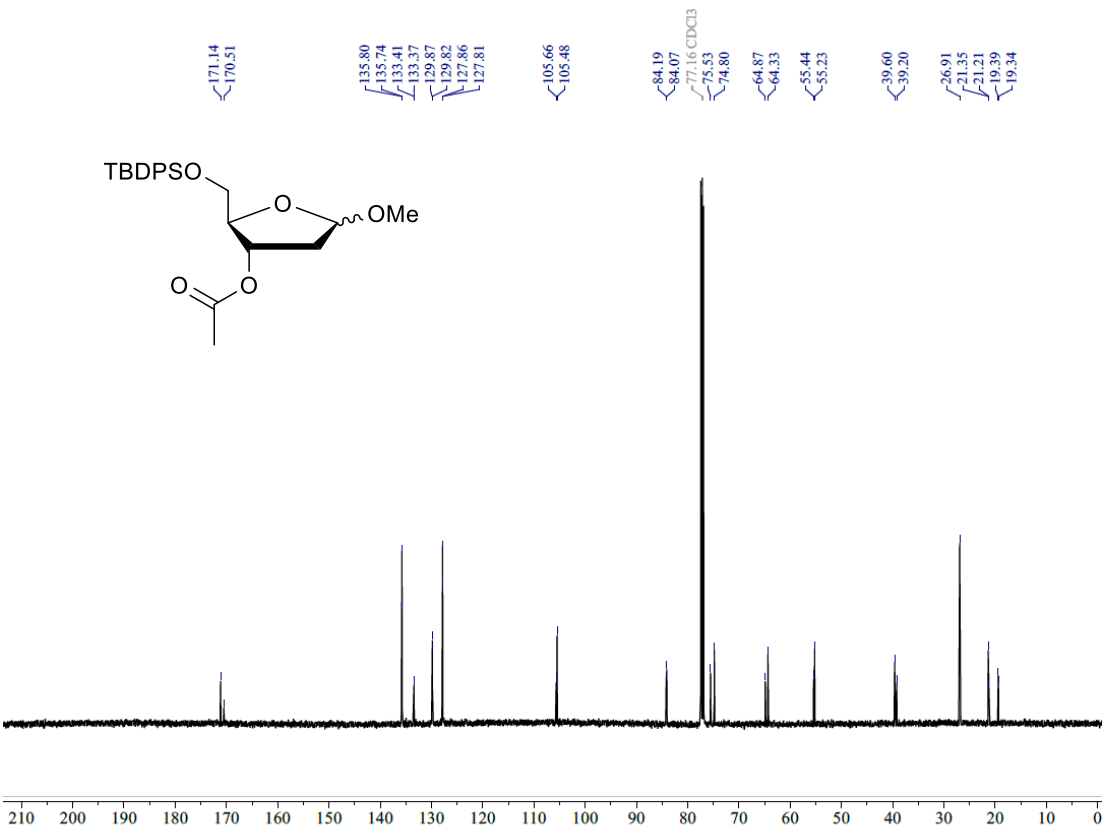
^{1}H-NMR (600 MHz) of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (2.7).



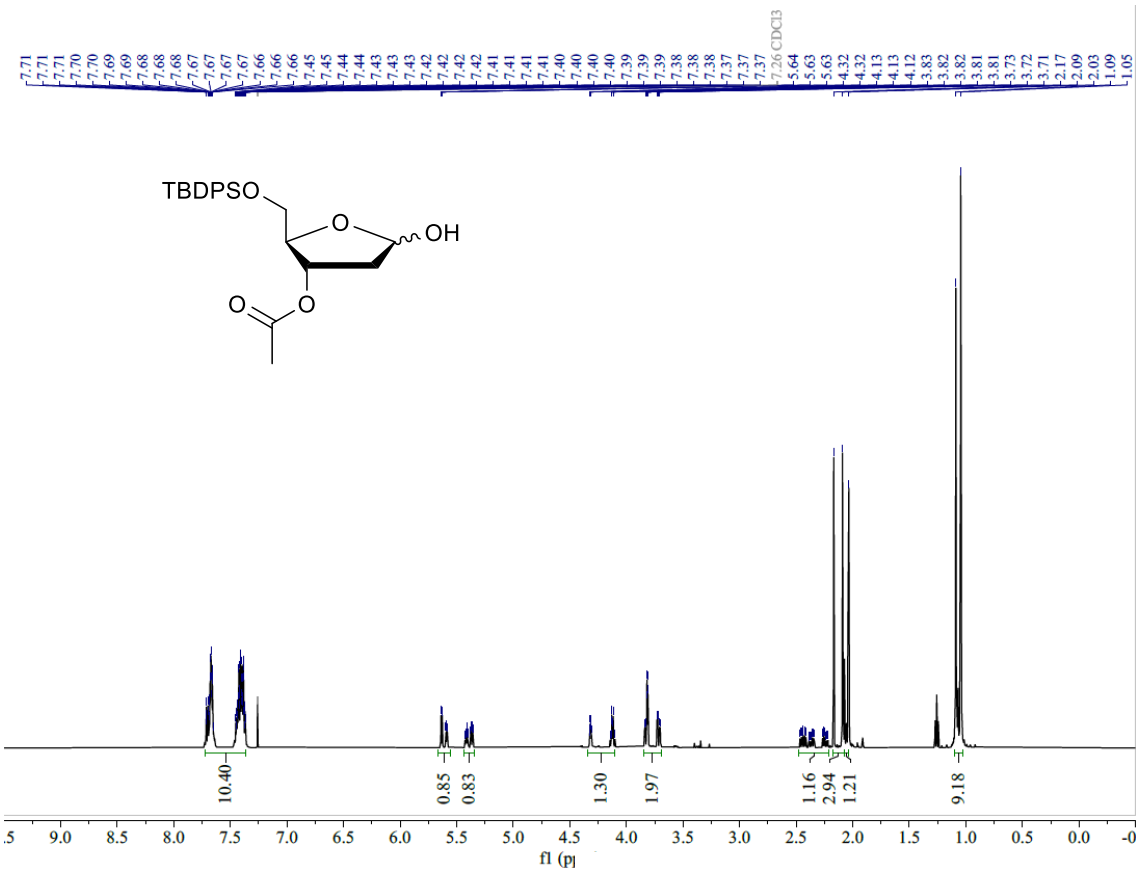
^{13}C-NMR (151 MHz) of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (2.7).



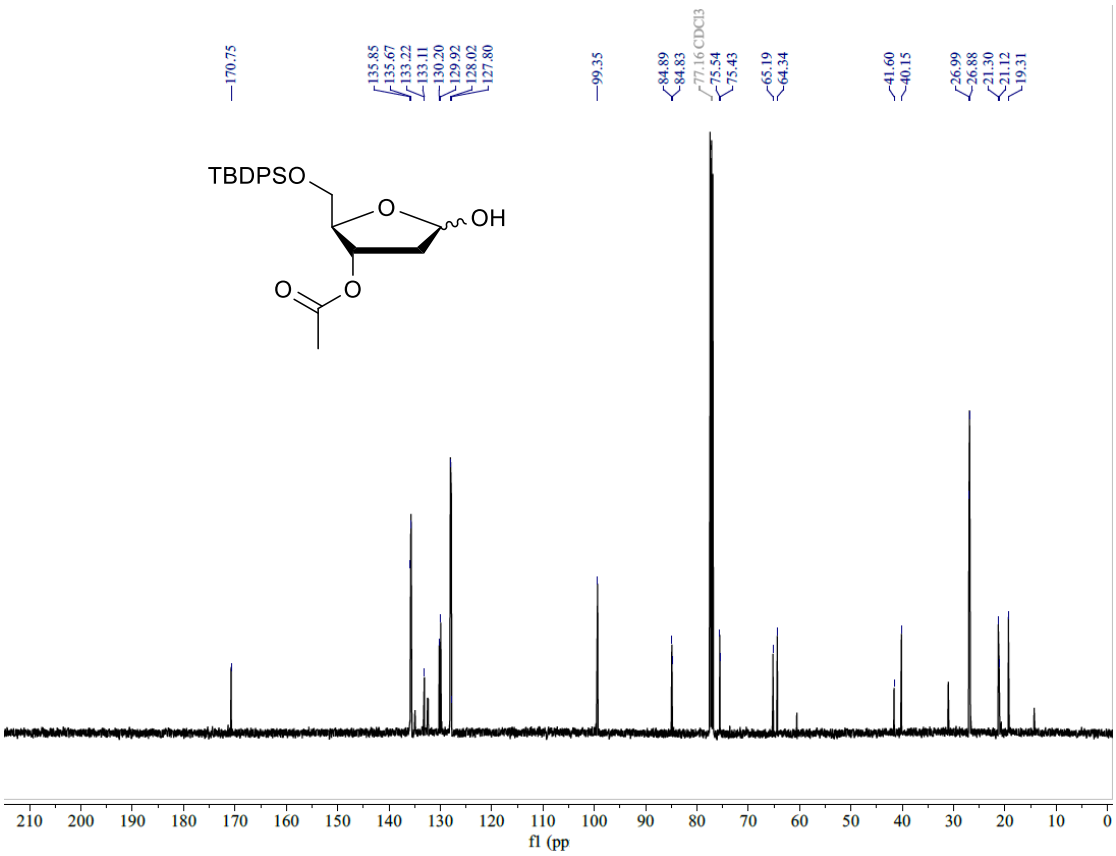
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (2.8).



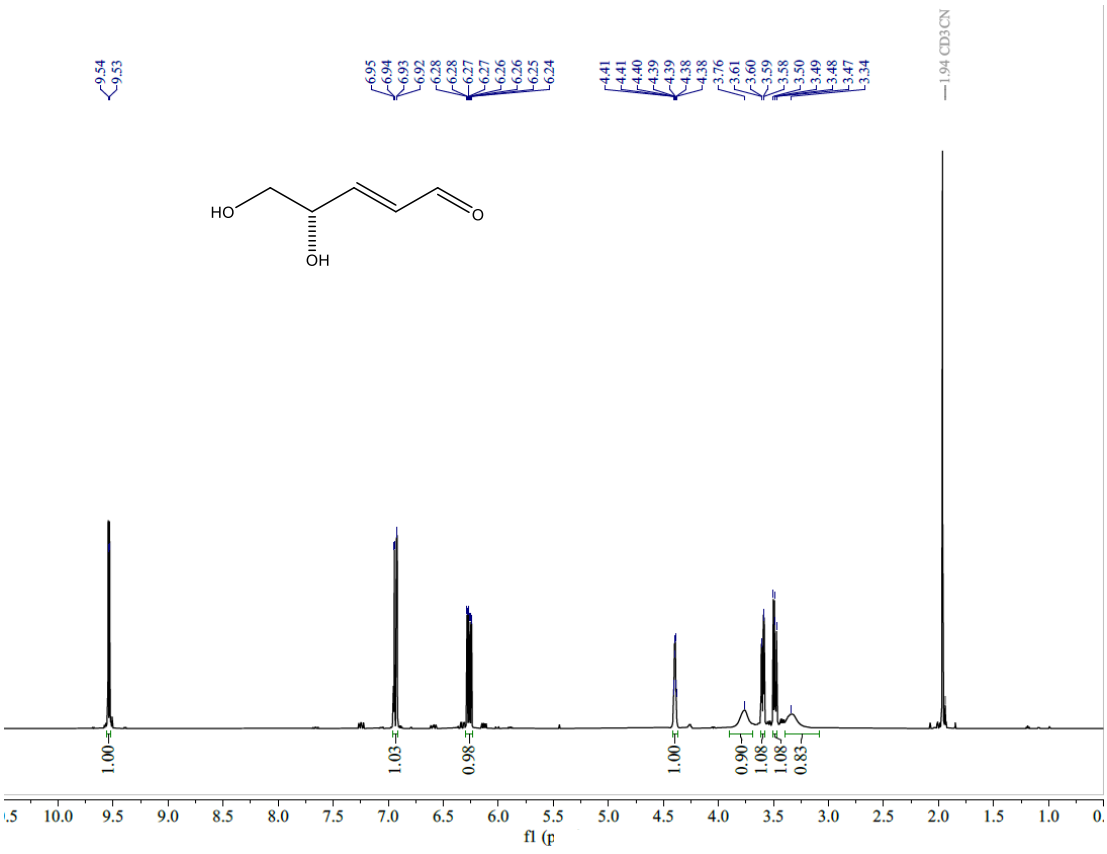
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (2.8).



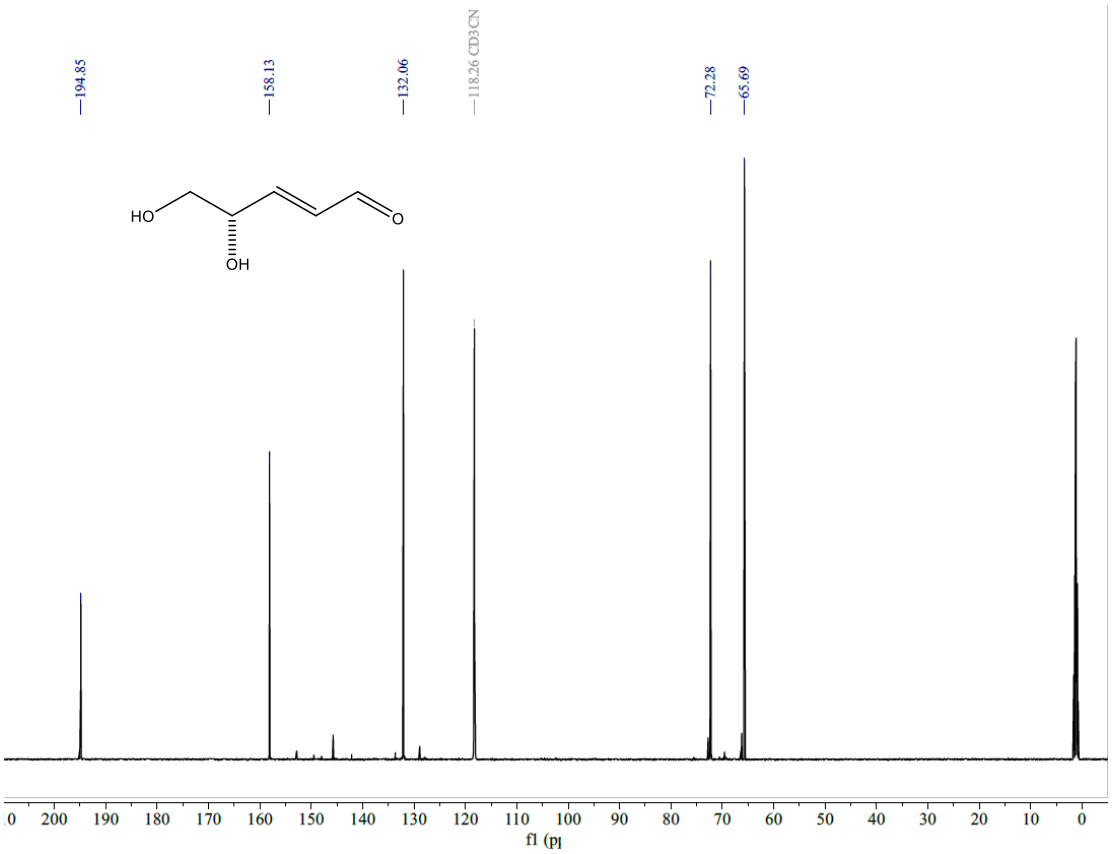
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (2.9).



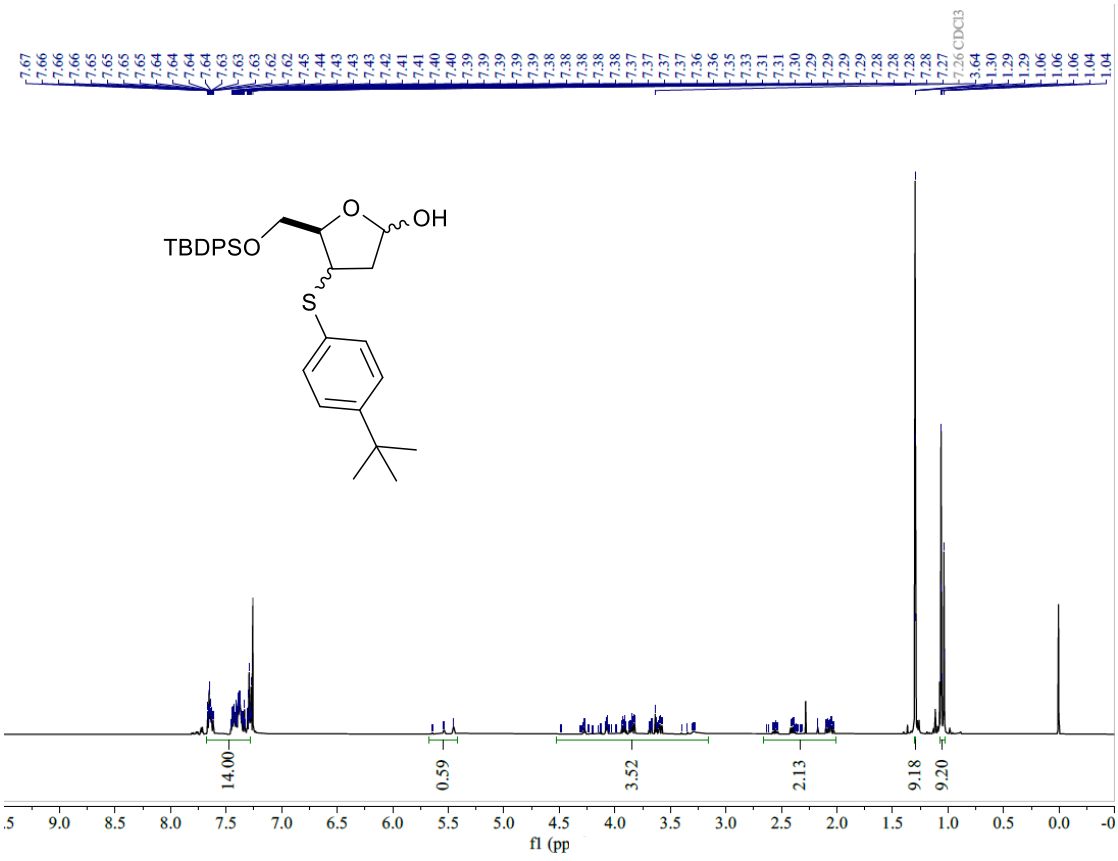
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (2.9).



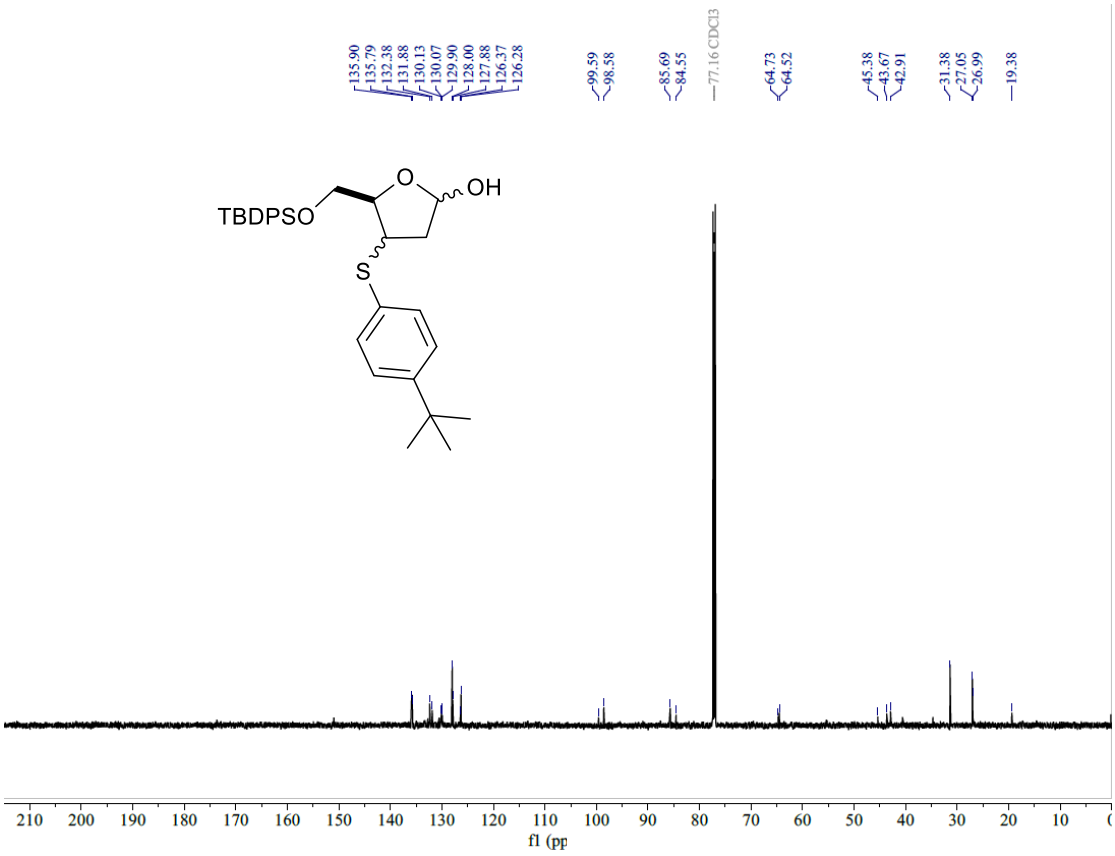
^{1}H-NMR (600 MHz) of (S,E)-4,5-dihydroxypent-2-enal (2.15).



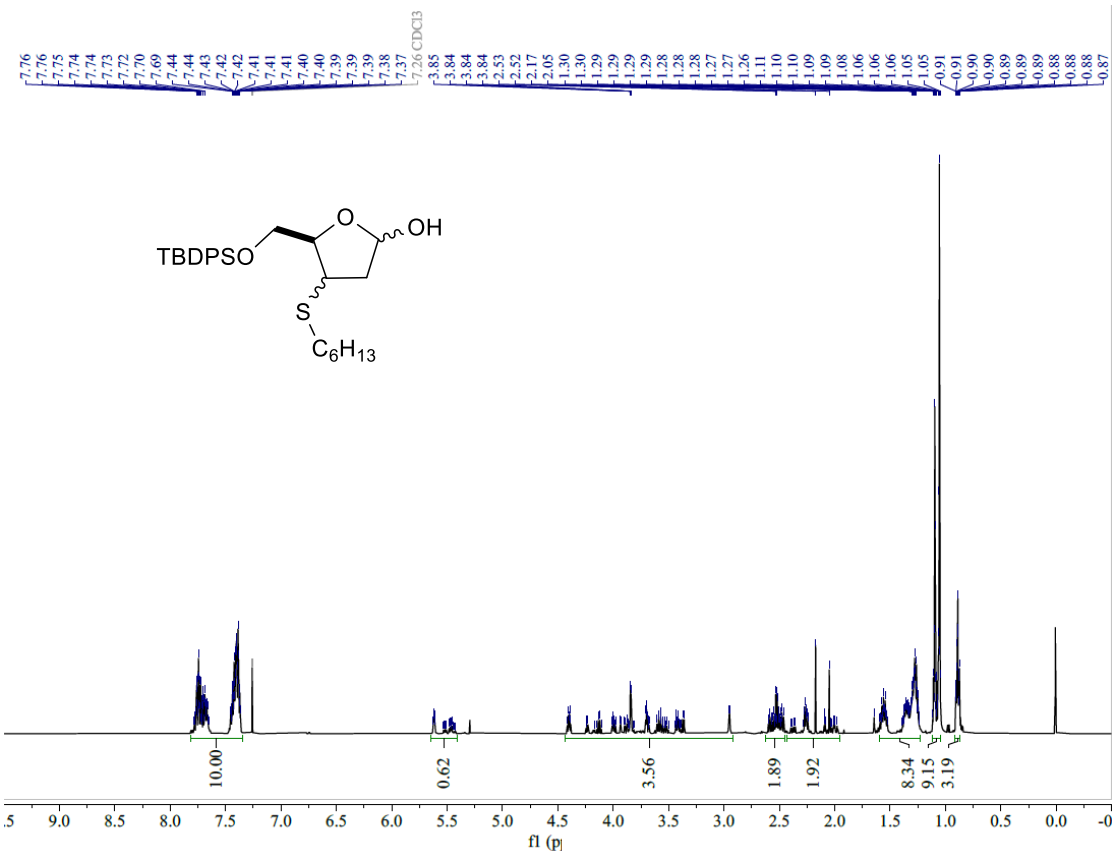
^{13}C-NMR (151 MHz) of (S,E)-4,5-dihydroxypent-2-enal (2.15)



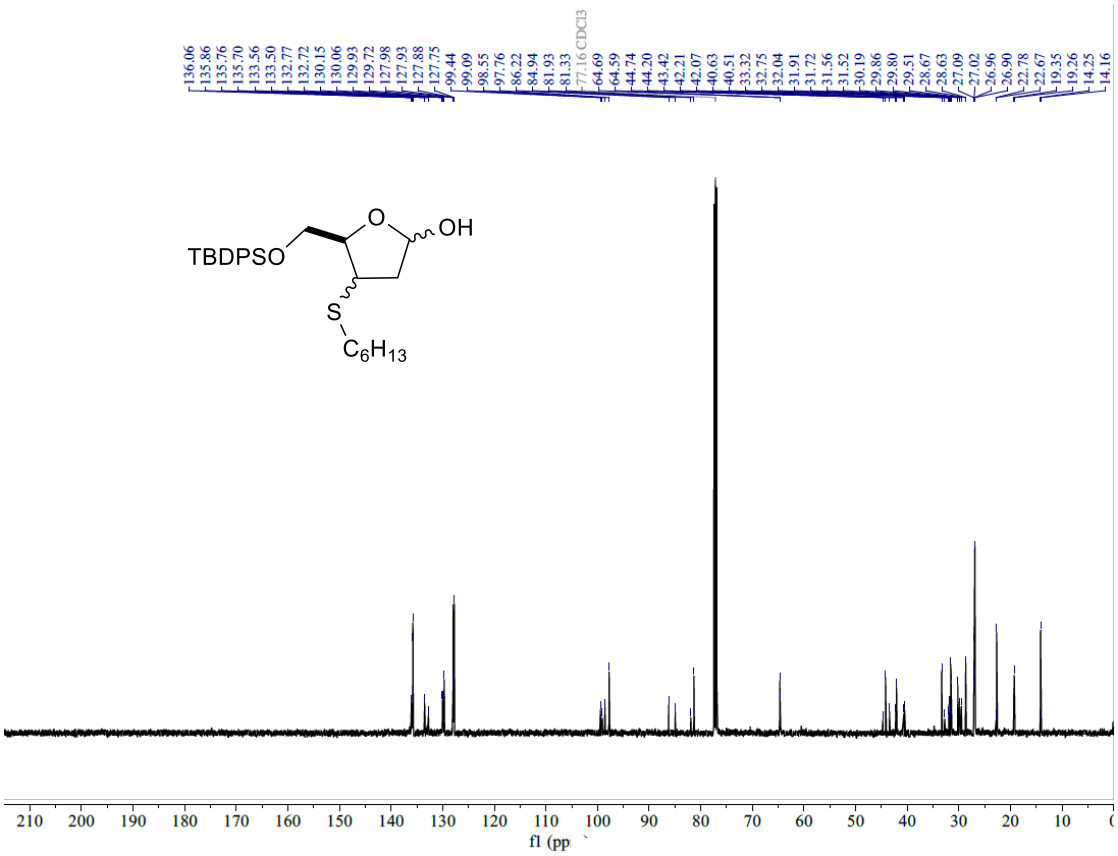
^{1}H-NMR (600 MHz) of (R)-4-((4-(tert-butyl)phenyl)thio)-5-(((tert-butyldiphenyl silyl)oxy)methyl) tetrahydrofuran-2-ol (2.20/21).



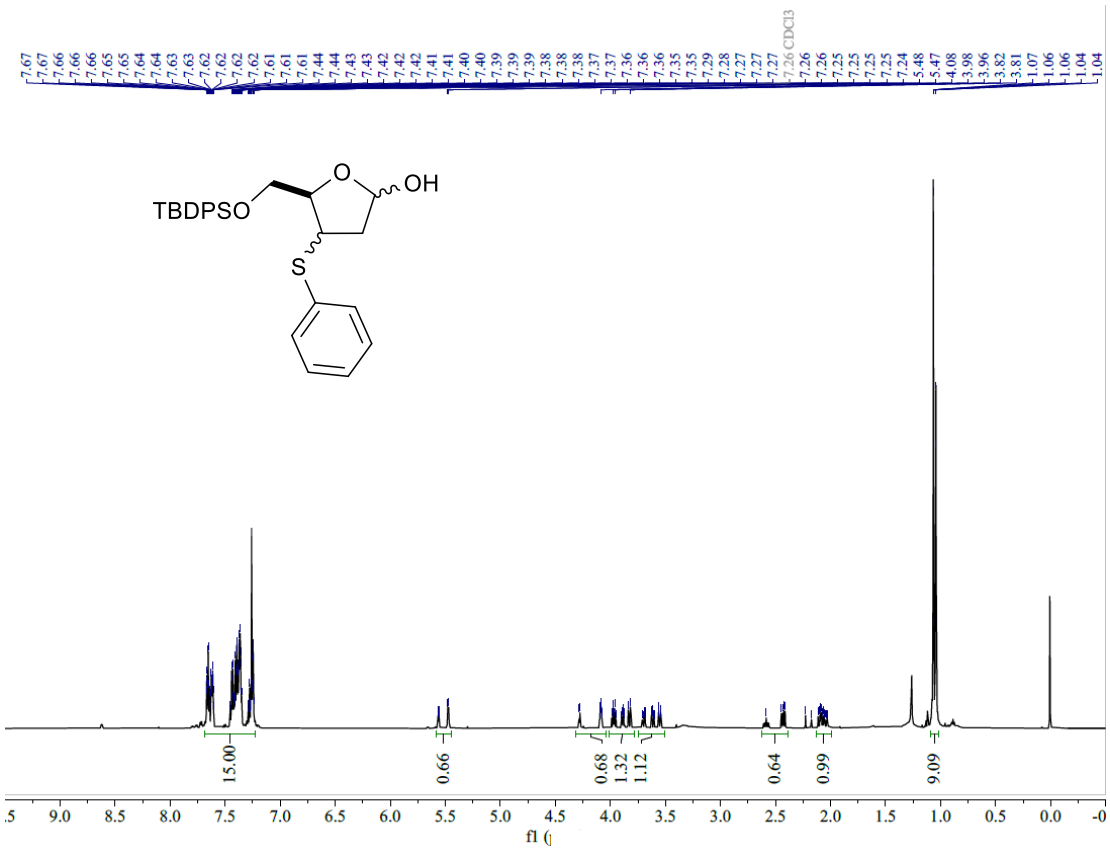
^{13}C-NMR (151 MHz) of (R)-4-((4-(tert-butyl)phenyl)thio)-5-(((tert-butyldiphenylsilyl)oxy)methyl) tetrahydrofuran-2-ol (2.20/21)



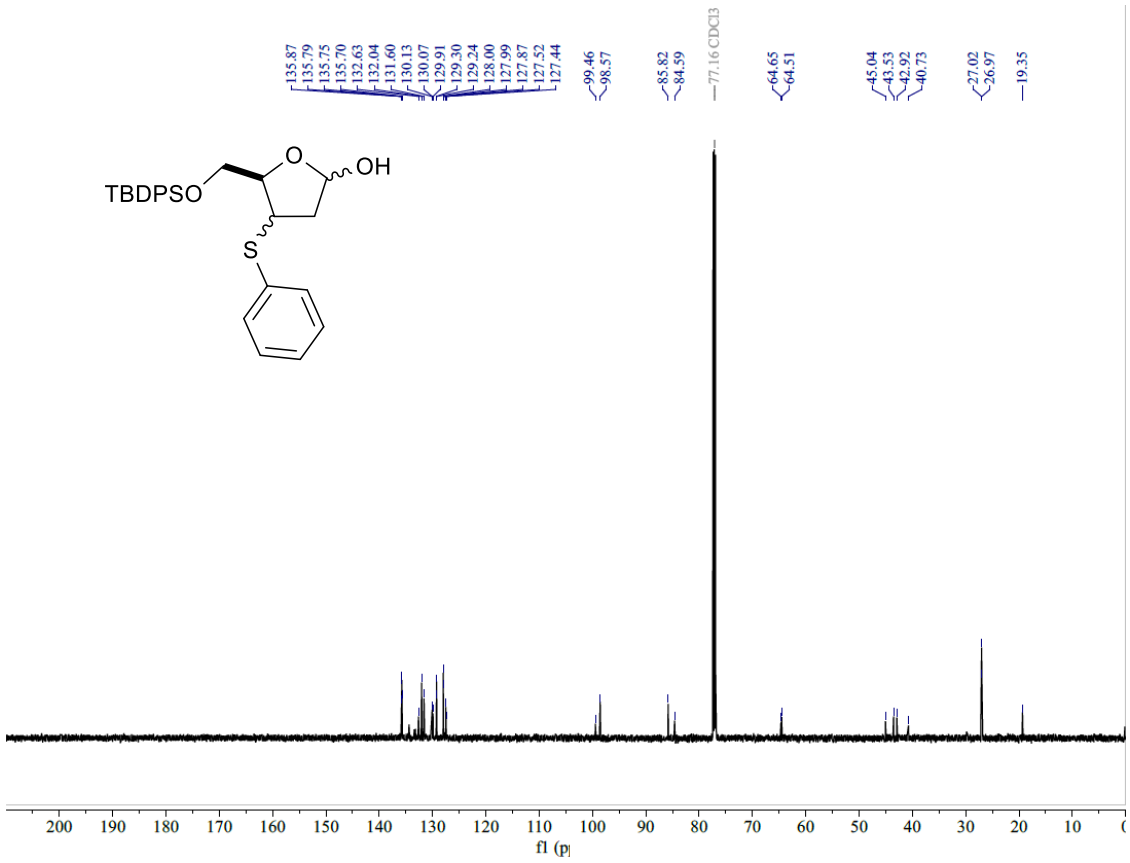
^{1}H-NMR (600 MHz) of (R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(hexylthio)tetrahydrofuran-2-ol (2.22)



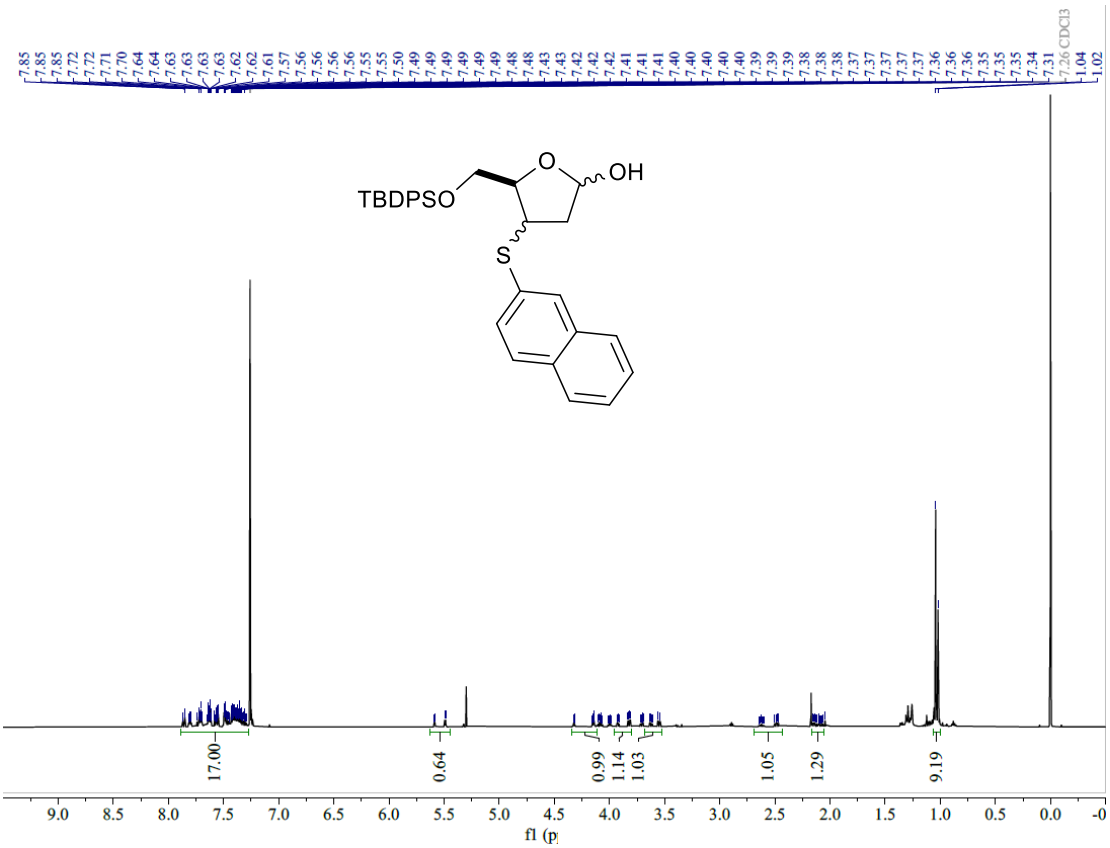
^{13}C-NMR (151 MHz) of (R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(hexylthio)tetrahydrofuran-2-ol (2.22)



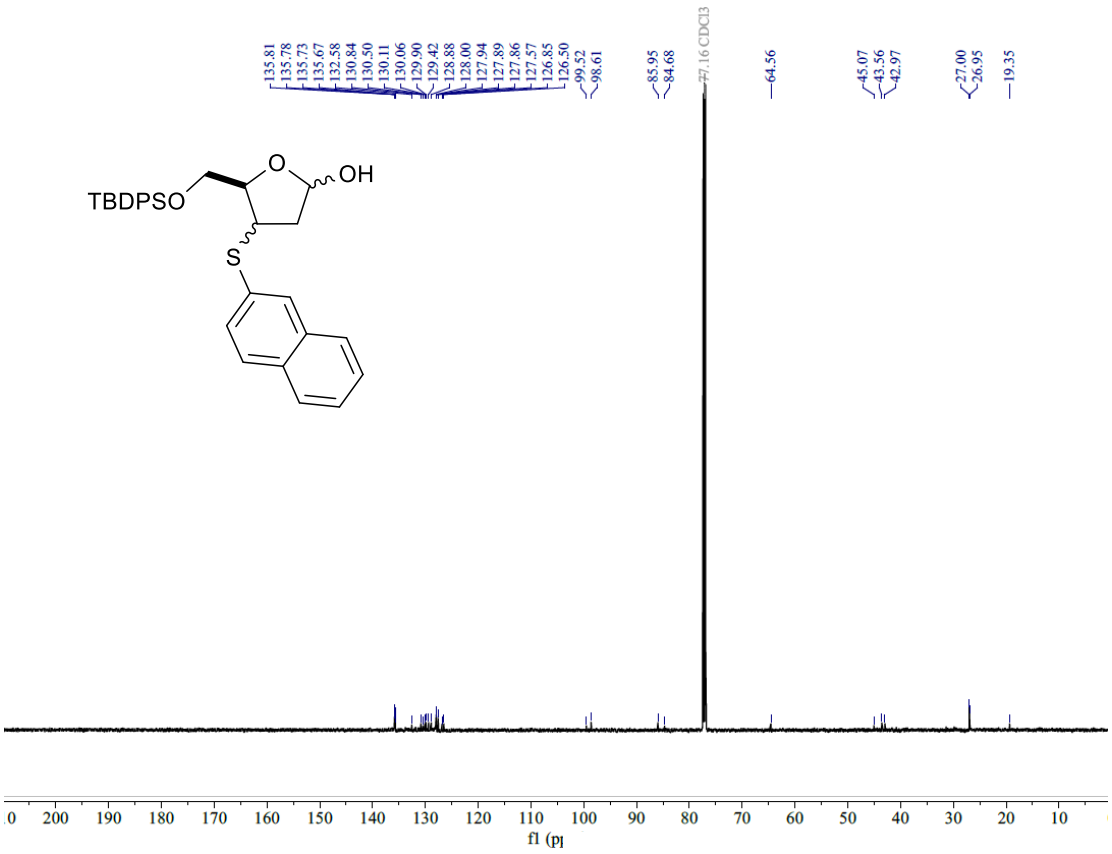
^{1}H-NMR (600 MHz) of (5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(phenylthio)tetrahydrofuran-2-ol (Compound S2.1)



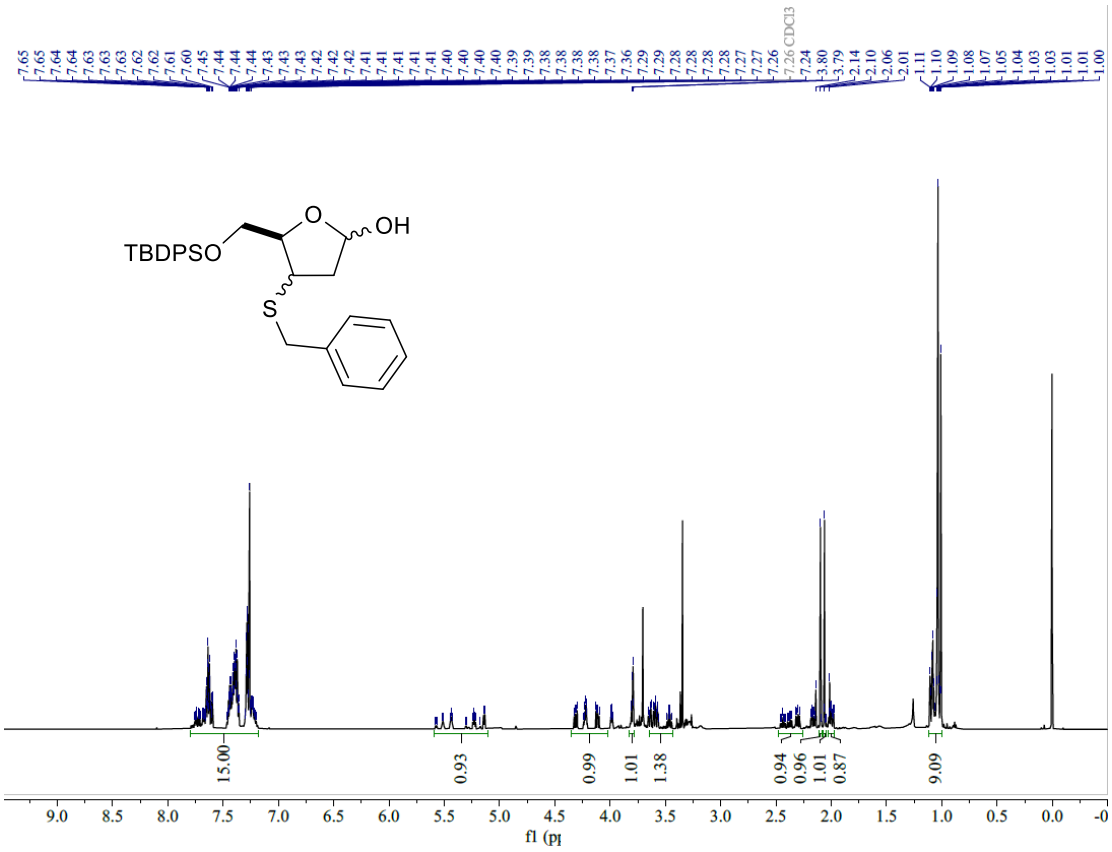
^{13}C-NMR (151 MHz) of (5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(phenylthio)tetrahydrofuran-2-ol (Compound S2.1)



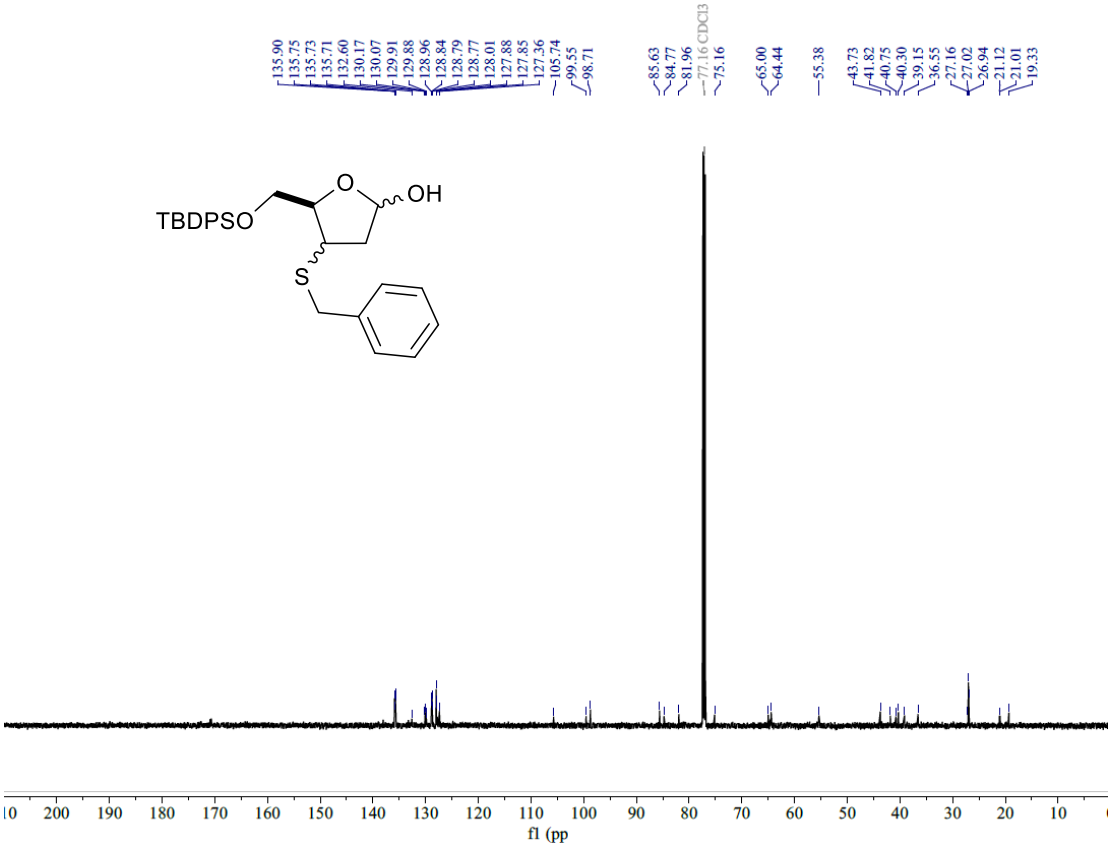
^{1}H-NMR (600 MHz) of (5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(naphthalen-2-ylthio)tetrahydrofuran-2-ol (Compound S2.2)



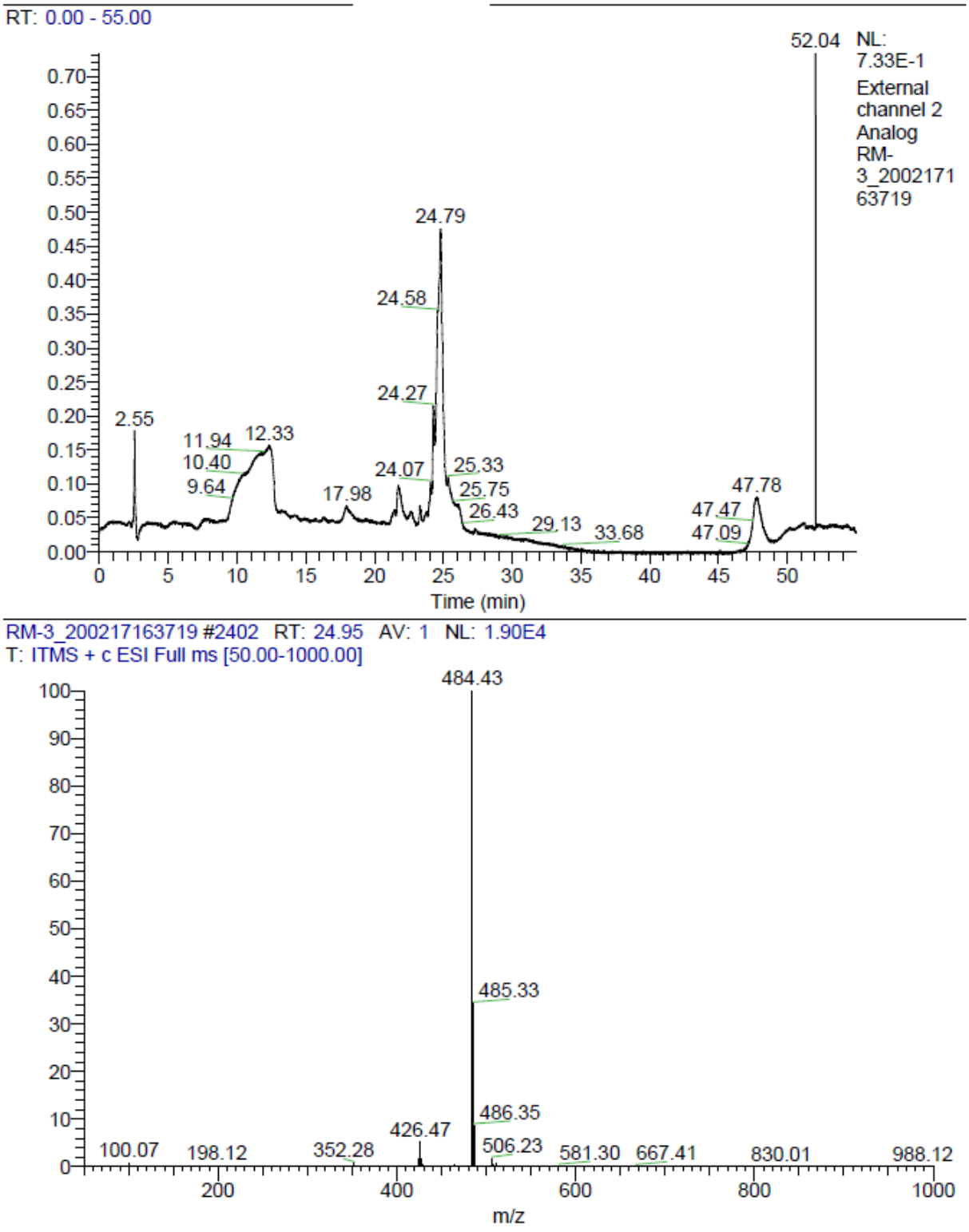
^{13}C-NMR (151 MHz) of (5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(naphthalen-2-ylthio)tetrahydrofuran-2-ol (Compound S2.2)



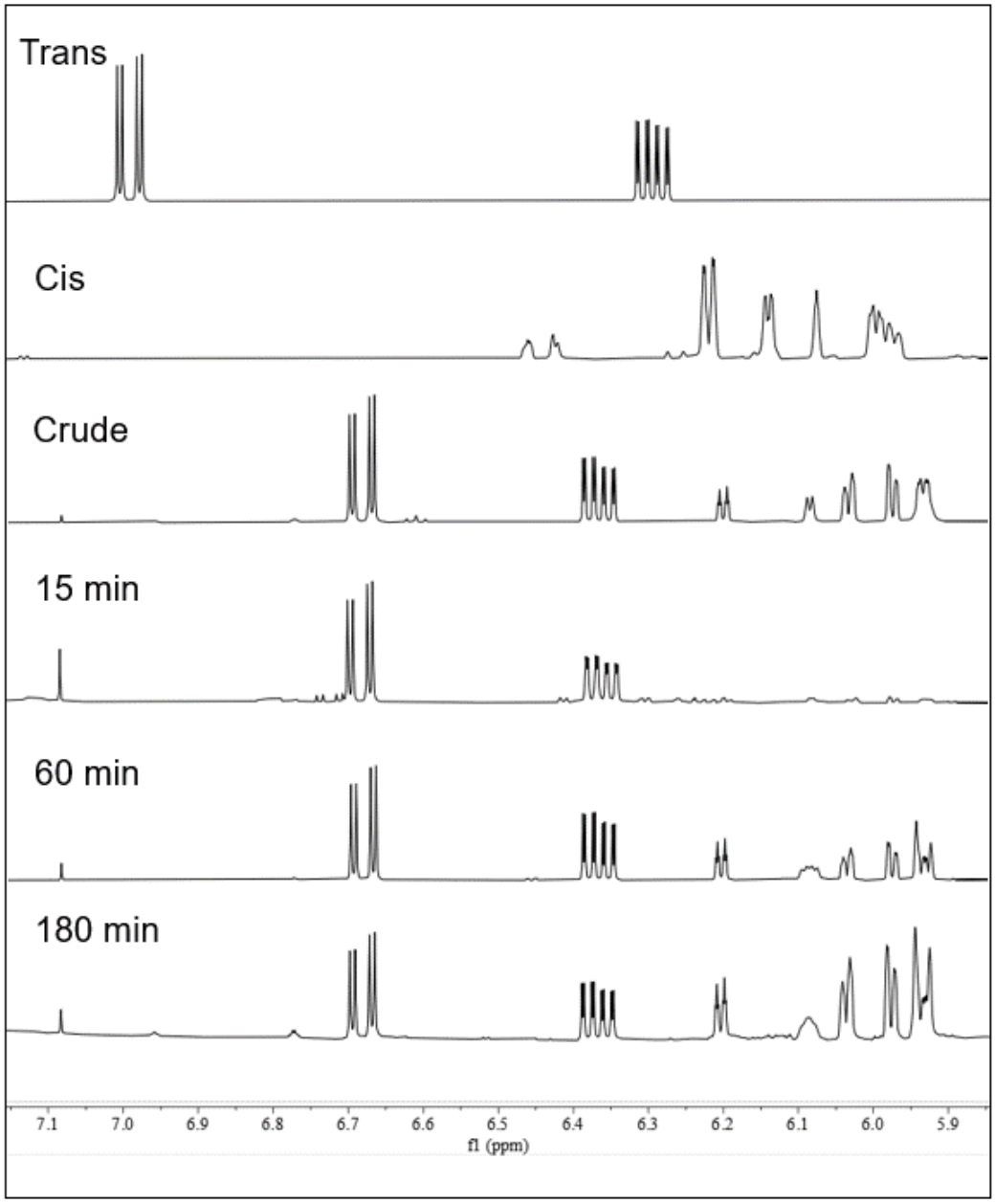
^{1}H-NMR (600 MHz) of (5R)-4-(benzylthio)-5-(((tert-butyl diphenylsilyl)oxy)methyl)tetrahydrofuran-2-ol (Compound S2.3)



^{13}C-NMR (151 MHz) of (5R)-4-(benzylthio)-5-(((tert-butyl diphenylsilyl)oxy)methyl)tetrahydrofuran-2-ol (Compound S2.3)



LC-MS spectrum of the crude products containing 2.14 (Figure S2.1). Data collected by Jay Jha.



Comparison of section of ^{1}H-NMR of *trans*- 2.12 and *cis*-alkenal 2.13 to demonstrate trans-cis isomerization. Trans and cis represent compounds 2.15 and 2.16 respectively. (Figure S2.2).

[1] Lindahl, T.; Karlstrom, O.; (1973) Heat-induced depyrimidation of deoxyribonucleic acid in neutral solution. Biochemistry. 25, 5151-5154.

[2] Lindahl, T; Nyberg, B; (1972) Rate of depurination of native deoxyribonucleic acid. Biochemistry, 19, 3610-3618.

[3] Gates, K.S.; Nooner, T.; Dutta, S.; (2004) Biologically relevant chemical reactions of N7-alkylguanine residues in DNA. Chem. Res. Toxicol. 17, 839-856.

[4] David, S.S.; Williams, S.D. (1998) Chemistry of glycosylases and endonucleases involved in base-excision repair. Chem Rev. 3, 1221-1262.

[5] Stivers, J.T; Jiang, Y.L.; (2003) A mechanistic perspective on the chemistry of DNA repair glycosylases. Chem. Rev. 7, 2729-2759.

[6] Brooks, S.C.; Adhikary, S.; Rubinson, E.H.; Eichman, B.F.; (2013) Recent advances in the structural mechanisms of DNA glycosylases. Biochim Biophys Acta., 1, 247-271.

[7] Imai, K.; Slupphaug, G.; Lee, W.I.; Revy, P.; Nonoyama, S.; Catalan, N.; Yei, L.; Forveille, M.; Kavli, B.; Krokan, H.E.; Ochs, H.D.; Fischer, A.; Durandy, A.; (2003) Human uracil-DNA glycosylace deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat. Immunol. 10, 1023-1028.

[8] Gates, K.S.; (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. Chem. Res. Toxicol. 22, 1747-1760.

[9] Nakamura, J.; Walker, V.E.; Upton, P.B.; Chiang, S.Y.; Kow, Y. W.; Swenberg, J.A.; (1998) Highly sensitive apurinic,apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. Chem. Res. 2, 222-225.

[10] De Bont, R.; van Larebeke, N.; (2004) Endogenous DNA damage in humans: review of quantitative data. Mutagenesis, 3, 169-185.

[11] Swenberg, J.A.; Lu, K; Moeller, B.C.; Gao, L.; Upton, P.B.; Nakamura, J.; Starr, T.B; (2011) Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology, and risk assessment. Toxicol Sci. 1, 130-145.

[12] Wilde, J.A.; Bolton, P.H; Mazumder, A.; Muthia, M.; Gerlt, J.A.; (1989) Characterization of the equilibrating forms of the aldehydic abasic site in duplex DNA by oxygen-17 NMR. J. Am. Chem. Soc. 5, 1894-1896.

[13] Overend, W.G.; Stacey, M.; (1950) Structure and synthesis in the group of deoxy-sugars. 1, 1027-1029.

[14] Imani-Nejad, M.; Price, N.E.; Haldar, T.; Lewis, C.; Wang, Y.; Gates, K.S.; (2019) Interstrand DNA cross-links derived from reaction of a 2-aminopurine residue with an abasic site. ACS Chem. Biol. 7, 1481-1489.

[15] Gamboa Varela, J; Gates, K.S; (2015) A simple high-yielding synthesis of DNA duplexes containing a covalent, thermally cleavable interstrand cross-link at a defined location. Ange. Chem., 26, 7666-7669.

[16] Johnson, K.M.; Price, N.E.; Wang, J.; Fekry, M.I.; Dutta, S.; Seiner, D.; Wang, Y.; Gates, K.S. (2013) On the formation and properties of interstrand DNA-DNA cross-links forged by reaction of an abasic site with the opposing guanine residue of 5'-Cap sequences in duplex DNA. J Am Chem Soc 3, 1015-1025.

[17] Price. N.E.; Johnson, K.M.; Wang, J.; Fekry, M.I.; Wang, Y.; Gates, K.S. (2014) Interstrand DNA-DNA cross-link formation between adenine residues and abasic sites in duplex DNA. J Am Chem Soc J Am Chem Soc 136, 3483-3490.

[18] Mohni, K.N.; Wessel, S.R.; Zhao, R.; Wojciechowski, A.C.; Luzwick, J.W; Layden, H.; Eichman, B.F.; Thompson, P.S.; Mehta, K.P.M.; Cortz, D.; (2019) HMCES maintains genome integrity by shielding abasic sites in single-strand DNA. Cell, 176, 144-153.

[19] Thompson, P.S.; Amidon, K.M.; Mohni, K.N.; Cortez, D; Eichman. B.F.; (2019) Protection of absic sites during DNA replication by a stable thiazolidine protein-DNA cross-link. Nat Struct Mol Biol, 7, 613-618.

[20] Shukla, V.; Halabelian, L.; Balagere, S.; Samaniego-Castruita, D.; Feldman, D.E.; Arrowsmith, C.H.; Rao, A.; Aravind, L.; (2020) HMCES functions in the alternative end-joining pathway of the DNA DSB repair during class switch recombination in B cells. Mol Cell. 2, 384-394.

[21] Chan, W.; Ham, Y.H.; Jin, L.; Chan, H.W.; Wong, Y.L.; Chan, C.K.; Chung, P.Y. (2019) Quantification of a Novel DNA-Protein Cross-Link Product Formed by Reacting Apurinic/Apyrimidinic Sites in DNA with Cysteine Residues in Protein by Liquid Chromatography-Tandem Mass Spectrometry Coupled with the Stable Isotope-Dilution Method. Anal Chem, 91, 4987-4994.

[22] Bayley, C.R.; Brammer, K.W.; Jones, A.S.; (1961) The nucleotide sequence in deoxyribonucleic acids. Part V. The alkaline degradation of apurinic acids. Journ Chem Soc 1, 1903-1907.

[23] Crine, P.; Verly, W.G.; (1976) A study of DNA spontaneous degradation. Biochim Biophys Acta 1, 50-57.

[24] Lindahl, T.; Andersson, A.; (1972) Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. Biochemistry, 19, 3618-1623.

[25] Rahimoff, R.; Kosmatchev, O.; Kirchner, A.; Pfaffeneder, T.; Spada, F.; Brantl, V.; Muller, M.; Carell,T.; (2017) 5-formyl- and 5-carboxydeoxycytidines do not cause

accumulation of harmful repair intermediates in stem cells. J Am Chem Soc 139, 10359-10364.

[26] Luke, A.M.; Chastain, P.D.; Pachkowski, B.F.; Afonin, V.; Takeda, S.; Kaufman, D.G.; Swenberg, J.A.; Nakamura, J.; (2010) Accumulation of true single strand breaks and AP sites in base excision repair deficient cells. 694, 65-71.

[27] Ma, W.; Westmoreland, J.W.; Gordenin, D.A.; Resnick, M.A.; (2011) Alkylation base damage is converted into repairable double-strand breaks and complex intermediates in G2 cells lacking AP endonuclease. PLOS Genetics, 4, 1-15.

[28] Guillet, M.; Boiteux, S.; (2003) Origin of endogenous DNA abasic sites in Saccharamoyces cerevisiae Mol Cell Biol 22, 8386-8394.

[29] Boiteux, S.; Guillet, M; (20014) Abasic sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. DNA Repair, 1, 1-12.

[30] Sugyiama, H.; Fijiwara, T.; Atsushi, U.; Tashiro, T.; Koji, Y,; Kawanishi, S.; Saito, I.; (1994) Chemistry of Thermal Degradation of Abasic Sites in DNA. Mechanistic Investigation on Thermal DNA Strand Cleavage of Alkylated DNA. Chem Res. Toxicol. 5, 673-683.

[31] Sarhan, S.; Seiler, N.; (1989) On the subcellular localization of the polyamines. Biol Chem Hoppe Seyler. 12, 1279-1284.

[32] Male, R.; Fosse, V.M.: Kleppe, K.; (1982) Polyamine-induced hydrolysis of apurinic sites in DNA and nucleosomes. Nucleic Acids Res. 20, 6305-6318.

[33] McHugh, P.J.; Knowland, J.; (1995) Novel reagents for chemical cleavage at abasic sites and UV photoproducts in DNA. Nucleic Acids Res. 23, 1664-1670.

[34] Raspaud, E.; Chaperon, I.; Leforestier, A.; Livolant, F.; (1999) Spermine-induced aggregation of DNA, nucleosome, and chromatin. Biophys J. 3, 1547-1555.

[35] Wallace, H.M.; Fraser, A.V.; Hughes, A.; (2003) A perspective of polyamine metabolism. Biochem J. 376, 1-14.

[36] Sczepanski, J.T.; Wong, R.S.; McKnight, J.N.; Bowman, G.D.; Greenberg, M.M.; (2010) Rapid DNA-protein cross-linking and strand scission by an abasic site in a nucleosome core particle. PNAS 1-6.

[37] Sczepanski, J.T.; Zhou, C.: Greenberg, M.M.; (2013) Nucleosome core particle-catalyzed strand scission at abasic sites. Biochemistry, 52, 2157-2164.

[38] Pruss, D.; Gavin, I.M.; Melnik, S.; Bayvkin, S.G.; (1999) DNA-protein cross-linking applications for chromatin studies in vitro and in vivo. Methods Enzymol. 304, 516-533.

[39] Zhou, C.; Greenberg, M.M. (2012) Histone-catalyzed cleavage of nucleosomal DNA containing 2-deoxyribonolactone. J Am Chem Soc 134 8090-8093.

[40] Stande,N.T., Carvajal,J., Hallett,R.A., Waters,C.A., Roberts,S.A., Strom,C., Kuhlman,B. and Ramsden,D.A. (2014) Requirements for 5'-dRP/AP lyase activity in Ku. Nucleic Acids Res., 42, 11136–1114.

[41] Mazumder, A.; Gerlt, J.A.; Rabow, L.; Absalon, M.J.; Stubee, J.; Bolton, P.H. (1989) UV endonuclease B from bacteriophage T4 catalyzes DNA strand cleavage at aldehydic abasic sites by a syn beta-elimination reaction. J Am Chem Soc. 20, 8029-8030.

[42] Kurtz, A.J.; Dodson, M.L.; Lloyd, R.S.; (2002) Evidence for Multiple Imino Intermediates and Identification of Reactive Nucleophiles in Peptide-Catalyzed β-Elimination at Abasic Sites. Biochemistry, 22, 7054-7064.

[43] Maher, R.L; Wallace, S.S.; Pederson, D.S; (2019) The lyase activity of bifunctional DNA glycosylases and the 3'-diesterase activity of APE1 contribute to the repair of oxidized bases in nucleosomes. Nucleic Acids Res. 47, 2922-2931.

[44] Cornish-Bowden, A.; (2014) Current IUBMB recommendations on enzyme nomenclature and kinetics. Perspectives in Science. 1, 74-87.

[45] Cordes, E.H.; Jencks, W.P.; (1963) The mechanism of hydrolysis of Schiff bases derived from aliphatic amines. J Am Chem Soc. 18, 2843-2848.

[46] Bender, M.L.; Williams, A.; (1966) Ketimine intermediates in amine-catalyzed enolization of acetone. J Am Chem Soc, 11, 2502-2508.

[47] Kosower, E.M.; Sorensen, T.S.; (1963) Some unsaturated imines. J Org Chem, 3, 692-695.

[48] Rios, A.; Crugeiras, J.; Amyes, T.L.; Richard, J.P.; (2001) Glycine enolates: the large effect of iminium ion formation on alpha-amino carbon acidity. J Am Chem Soc. 32, 7949-7950.

[49] Behmoaras, T.; Toulme J.J.; Helene, C.; (1981) A tryptophan-containing peptide recognizes and cleaves DNA at apurinic sites. Nature, 292, 858-859.

[50] Mazumder, A.; Gerlt, J.A.; Absalon, M.J.; Stubbe, J.; Cunningham R.P.; Withka, J.; Bolton, P.H.; (1991) Stereochemical studies of the beta-elimination reactions at aldehydic abasic sites in DNA: endonuclease III from Escherichia coli, sodium hydroxide, and Lys-Trp-Lys. Biochemistry, 30, 1119-1126.

[51] Withka, J.M.; Wilde, J.A.; Bolton, P.H.; Mazumder, A.; Gerlt, J.A.; (1991) Characterization of conformational features of DNA heteroduplexes containing aldehydic abasic sites. Biochemistry, 30, 9931-9940.

[52] Golan, G.; Zharkov, D.O.; Grollman, A.P.; Dodson, M.L; McCullough, A.K.; Lloyd, R.S.; Shoham G.; (2006) Structure of T4 pyrimidine dimer glycosylase in a reduced imine covalent complex with abasic site-containing DNA. Joun Molec Biol 2, 241-258.

[53] Yang, Z.; Price, N.E.; Johnson, K.M.; Wang, Y.; Gates, K.S. (2017) Interstrand cross-links arising from strand breaks at true abasic sites in duplex DNA. Nucleic Acids Res. 11, 6275-6283.

[54] Rosenthal, A.; Richards, C.M.; (1974) Branched-chain N-sugar nucleosides: Part III alpha and beta nucleosides of branched-chain 3-C-(cyanomethyl)-, 3-C-(2-aminoethyl)-, and 3-C-(N,N,dimethylcarbmoylmethyl)-2,3,dideoxy-D-ribo-hexapyranoses. Carb Res, 32, 67-77.

[55] Lagerwall, T.; Heinonen, P.; Oivanen, M.; (2003) Temperature controlled stereoselectivity in the synthesis of 5-halo-2'-deoxyuridine derivatives. Tet Lett 43, 5950-5953.

[56] Minko, I.G.; Jacobs, A.C.; de Leon, A. R.; Guppi, F.; Donley, N.; Harris, T.M.; Rizzo, C.J.; McCullough, A.K.; Lloyd, R.S.; (2016) Catalysts of DNA strand cleavage at apurinic/apyrimidinic sites. Scientific Reports, 6, 1-9.

[57] Marchand, C.; Krajewski, K.; Lee, H.F.; Antony, S; Johnson, A.A.; Amin, R.; Roller, P.; Kvaratskhelia, M.; Pommier, Y.; (2006) Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites. Nucleic Acid Res. 18, 5157-5165.

[58] Sun, B.; Latham, K.A.; Dodson, M.L.; Lloyd, R.S. (1995) Studies on the catalytic mechanism of five DNA glycosylases probing for enzyme-DNA imino intermediates. J Bio Chem. 33, 19501-19508.

[59] Fromme, J.C.; Verdine, G.L.; (2003) Structure of a trapped endonuclease III-DNA covalent intermediate. EMBO J. 13, 3461-3471.

[60] Esterbauer, H.; Zollner, H.; Scholz N.; (1975) Reaction of glutathione with conjugated carbonyls. Z Naturforsch C Biosci 4, 466-473.

[61] Motawia, M.S.; Nawwar, G.A.M.; Andreassen, E.S.; Jacobsen, J.P.; Pedersen, E.B.; (1987) Coupling of unprotected 2-deoxy-D-ribose at C-3 as a new route to 2,3-dideoxy-3-phthalimido-D-pentoses. Liebigs Annalen der Chemie, 12, 1111-1114.

[62] Tsutsui, A.; Imamaki, R.; Kitazume, S.; Hanashima, S.; Yamaguchi, Y.; Kaneda, M.; Oishi, S.; Fijii, N.; Kurbangalieva, A.; Taniguchi, N.; Tanaka, K.; (2014) Polyamine modification by acrolein exclusively produces 1,5-diazacyclooctanes: a previously unrecognized mechanism for acrolein-mediated oxidative stress. Org. Biomol. Chem, 12, 5151-5157.

[63] Nozaki, K. (1941) cis-trans isomerizations. II. The mechanism of the amine catalyzed isomerization of diethyl maleate. J. Am. Chem. Soc. 10, 2681-2683.

[64] Grunbaum, Z.; Patai, S.; Rappoport, Z.; (1966) Nucleophilic attacks on carbon-carbon double bonds. Part X. Nucleophile-catalysed trans isomerization of cis-4-nitrochalcone and of diethyl maleate in 95% ethanol. J Chem Soc B. 0, 1133-1137.

[65] Chung, S. J.; Verdine, G. L. (2004) Structures of end products resulting from lesion processing by a DNA glycosylase/lyase, Chem. Biol., 11, 1643–1649,

[66] Sugiyama, H.; Fijiwara, T.; Ura, A.; Tashior, T.; Yamamoto, K.; Kawanishi, I.; Saito, I.; (1994) Chemistry of thermal degradation of abasic sites in DNA. Mechanistic investigation on thermal DNA strand cleavage of alkylated DNA. Chem Res Toxicol, 5, 673-683.

[67] Bricteaux-Gregoire, S.; Verly, W.G.; (1989) The use of thioglycolate to distinguish between 3'AP (apurinic/apyrimidinic) endonucleases and AP lyases. Nucleic Acids Res. 17, 6269-6282.

[68] Seiner, D.R.; LaButti, J.N.; Gates, K.S.; (2007) Kinetics and mechanism of protein tyrosine phosphatase 1B inactivation by acrolein. Chem Res Toxicol. 9, 1315-1320.

[69] Esterbauer, H.; Ertl, A.; Scholz, N.; (1976) The reaction of cysteine with a,b unsaturated aldehydes. Tetrahedron, 2, 285-289.

[70] Wakita, C.; Maeshima, T.; Yamazaki, A.; Shibata, T.; Ito, S.; Akagawa, M.; Ohika, M.; Yoddol, J.; Uchida, K.; (2009(Stereochemical configuration of 4-hydroxy-2-nonenal-cystein adducts and their stereoselective formation in a redox-regulated protein. J Biol Chem, 42, 28810-28822.

[71] Meister, A.; (1983) Selective modification of glutathione metabolism. Science, 220, 472-277.

[72] Darwanto, A.; Farrel, A.; Rogstad, D.K.; Sowers, L.C.; (2009) Characterization of DNA glycosylase activity by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal Biochem, 394, 13-23.

[73] D'Ham, C.; Romieu, A.; Jaquinod, M.; Gasparutto, D.; Cadet, J.; (1999) Excision of 5,6-dihydroxy-5,6-dihydrothymine, 5,6-dihydrothymine, and 5-hydroxycytosine from defined sequence oligonucleotides by *Escherichia coli* endonuclease III and Fpg proteins: kinetic and mechanistic aspects. Biochemistry, 11, 3335-3344.

[74] Langebeck, W.; Sauerbier, R. (1937) *Chem. Ber.*, 70, 1540.

[75] Appel, R.; Chelli, S.; Tokuyasu, T.; Troshim, K.; Mayr, H.; (2013) Electrophilicities of benzaldehyde-derived iminium ions: quantification of the electrophilic activation of aldehydes by iminium formation. J Am Chem Soc 135, 6579-6587.

[76] Erkkila, A.; Pihko, P.M.; Clarke, M-R.; (2007) Simple primary anilines as iminium catalysts for the epoxidation of a-substituted acroleins. Adv. Synth and Catalysis, 6, 802-806.

[77] Lakhdar, S.; Tokuyasu, T.; Mayr, H.; (2008) Electrophilic reactivities of alpha,beta-unsaturated iminium ions. Angew Chem Int Ed Eng. 45, 8723-8726.

[78] Sako, M.; Yaekura, I.; (2002) A convenient preparative method for the 1, N2-cyclic adducts of guanine nucleosides and nucleotides with crotonaldehyde. Tetrahedron, 42, 8413-8416.

[79] Sako, M.; Inagaki, S.; Esaka, Y.; Deyashiki, Y.; (2003) Histones accelerate the cyclic 1, N2-propanoguanine adduct-formation of DNA by the primary metabolite of alcohol and carcinogenic crotonaldehyde. Bioorg Med Chem Lett, 20, 3497-3498.

[80] Fortini, P.; Dogliotti, E.; (2007) Base damage and single-strand break repair: mechanisms and functional significanc of short- and long-patch repair subpathways. DNA Repair, 6, 398-409.

[81] Andres, S.N.; Appel, C.D.; Westmoreland, J.W.; Williams, J.S.; Nguyen, Y.; Robertson, P.Dd.; Resnick, M.A.; Williams, R.S.; (2015) Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. Nat Struct Mol Biol , 2, 156-166.

[82] Abbotts, R.; Willson, D.M.; (2017) Coordination of DNA single strand break repair. Free Radic Biol Med. 107, 228-244.

[83] Mueller, I.; Merk, B.; Voss, K.O.; Averbeck, N.; Jakob, B.; Durante, M.; Taucher-Sholz, G.; (2013) Species conserved DNA damage response at the inactive human X chromosome. Mutat Res. 756, 30-36.

[84] Prasadd, R.; Horton, J.K.; Chastain, P.D.; Gassman, N.R.; Freudenthal, B.D.; Hou, E.W.; Wilson, S.H.; (2014) Suicidal cross-linking of PARP-1 to AP site intermediates in cells undergoing base excision repair. Nucleic Acids Res. 42, 6337-6351.

[85] Halabelian, l; Ravichandran, M.; Li, Y.; Zeng, H.; Rao, A.; Aravind, L.; Arrowsmith, C.H.; (2019) Structural basis of HMCES interactions with abasic DNA and multivalent substrate recognition. Nat Struct Mol Biol, 26, 607-612.

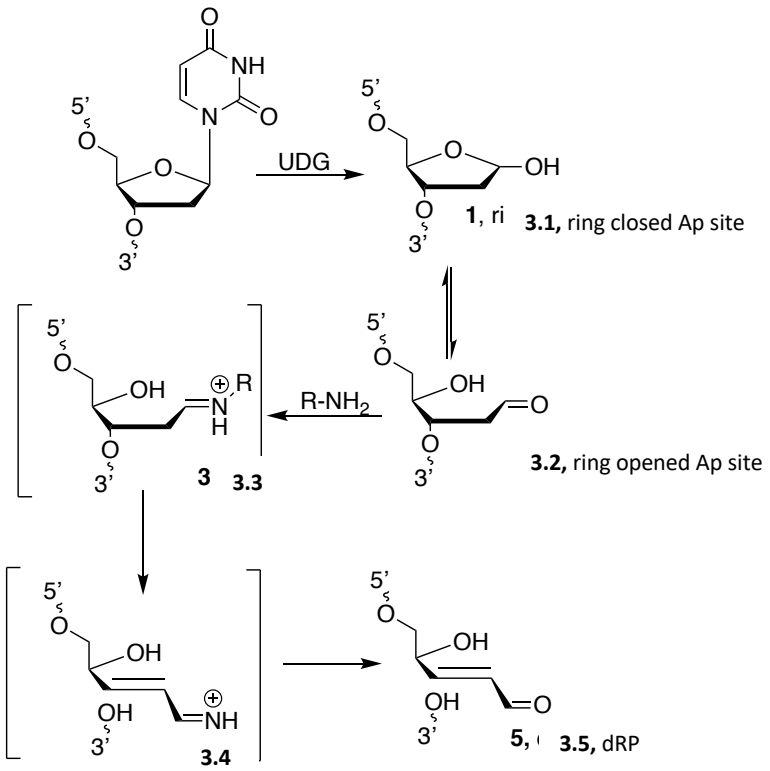
Chapter Three: Synthesis and study of the properties of a class of thioglycosides representative of Ap shielding agents in DNA

3.1 Introduction

As I have discussed throughout this thesis, abasic (Ap) sites represent the most common form of DNA lesion within the body,[1,2,3,4] occurring with steady state levels of 50,000 per cell. This has driven a need to fundamentally understand the chemistry of the electrophilic Ap site. As described previously, Ap sites have the ability to generate cross-links with opposing nucleobases in the double helix[5,6,7,8] and have the ability to undergo elimination to generate an unsaturated alkenal.[9,10,11]

These pathways may lead to profound biological consequences. It is therefore extremely important to understand the repair of Ap sites and the subsequent damages from which they stem. Base excision enzymes, such as NEIL3[12,13,14,] or Endo III[15,16,17], generate labile DNA-protein cross-links and enzymatically induce strand cleavage (**3.5,** Scheme 3.1.1). These reactions stem from a nucleophilic nitrogen within the enzymes, whether it be an N-terminal amine, or an available lysine residue.[12-17] APE1 is also able to repair Ap sites, but via a different mechanism that avoids iminium ion formation.[18,19] While it is generally accepted that the electrophilic nature of the Ap site is prone to reaction with amines, whether enzymatically as described above, or with polyamines, such as spermine,[20,21] it is important to note that these are not the only nucleophiles to which Ap sites are sensitive.

Scheme 3.1.1. Amine-induced strand cleavage to generate the dRP end group. Elimination occurs via the generation of an iminium ion 3.3.



Recent publications describe HMCES being able to generate a bond with an Ap site via a cysteine residue to generate a thiazolidine[22,23,24] (Figure 3.1.1). The thiazolidine impairs the ability for either enzymes or aliphatic polyamines to induce strand cleavage to form the dRP end group **3.5.** In light of this discovery, I ask whether thiols, in general, are able to generate linkages with an Ap site. The synthesis and stability of a small library of thioglycosides with a model Ap site described herein proposes that thiols, as a class of nucleophiles, represent an important, yet largely overlooked, source of reactivity with Ap sites. It is my belief that this study will assist in a wide range of scientists, from synthetic

organic chemists to nucleic acids researchers, by showing the ability to generate thioglycosides from a 2-deoxy-D-ribose model Ap site and study the properties surrounding their formation and stability.

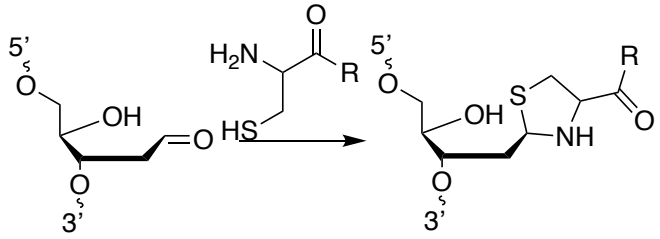


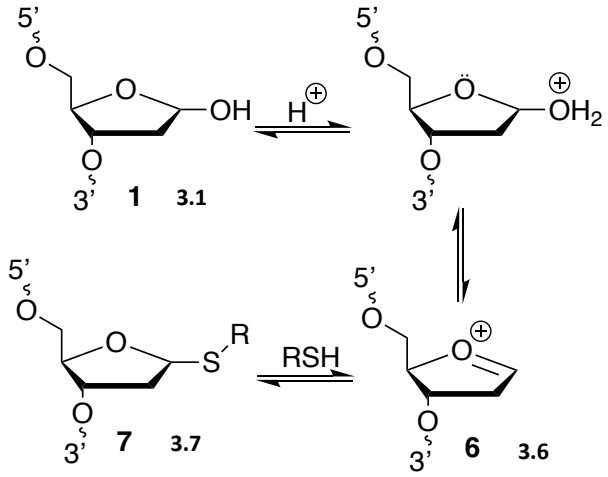
Figure 3.1.1. Reaction of an Ap site with an N-terminal cysteine, as is seen with HMCES, to generate a thiazolidine.

The most abundant thiol in the body is glutathione, existing at a steady state concentration of between 1 to 10 mM.[25,26] While glutathione has been shown the react with unsaturated aldehydes, such as HNE,[27,28] there has not been any research showing glutathione's ability to react with an Ap site to inhibit repair of the lesion. Recent work by Wilson[29] show that glutathione can inhibit APE1's ability to excise Ap sites from DNA, but the proposed mechanism comes from S-glutathionylation of a cysteine residue on APE1, not from direct reactivity with the Ap site within DNA.

The research here describes a realm of fundamental chemistry not yet explored. I show the reactivity of a model Ap site with various thiols and assess the stability of the adducts. I also show the generation of a DNA adduct with glutathione, and show its ability to withstand the base excision repair enzymes Fpg[30,31] and APE1. This chapter will serve to describe a separate form of DNA-protein cross-link that can occur as a

potential method to shield Ap sites from forming DNA-DNA cross-links or undergoing elimination. Herein I describe the formation of a class of semi-stable thioglycosides representative of a cysteine residue reacting with an Ap site to generate a labile DNA-protein cross-links (Scheme 3.1.2).

Scheme 3.1.2. Generation of a thioglycoside 3.7 from 3.1. Reaction proceeds via the generation of an electrophilic oxocarbenium ion, 3.6.

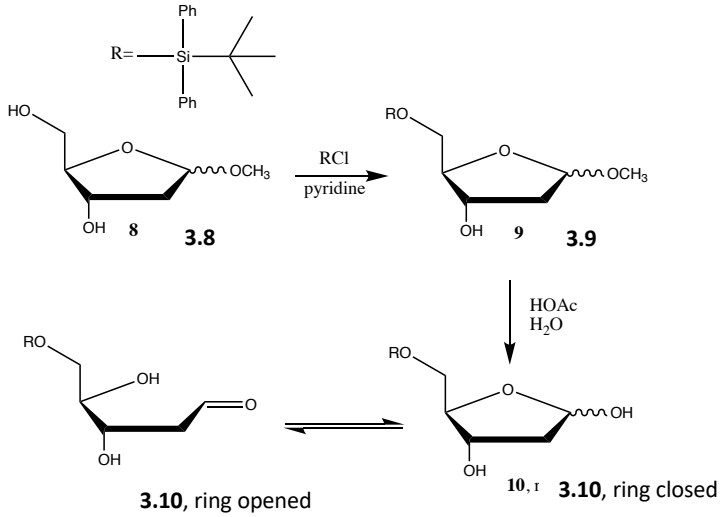


3.2 Generation of an Ap model capable of generating thioglycosides

In order to study thioglycoside formation, it is important to generate a model Ap site. Herein I use a 2-deoxy-D-ribose model with a 5'protecting group to prevent furanose/puranose equilibria. The silyl group installed on the 5'OH is relatively stable and affords the compounds to be easily purified on silica. I converted 2-deoxy-D-ribose to the corresponding methyl acetal,[32] followed by reaction with t-butyldiphenylsilyl

chloride in pyridine to obtain the 5-silylated derivative as a mixture of the anomers.[33] From there, the methyl acetal was removed in acidic conditions to afford the model Ap site (Scheme 3.2.1).

Scheme 3.2.1 Synthesis of precursor able to mimic Ap site 3.10.



3.3 Thioglycosides can readily be generated in a buffered solution.

The generation of thioglycosides is important to more than just the field of nucleic acid chemistry. Synthesis of thioglycosides have relevance in fields ranging from synthetic organic chemistry[34,35,36] to medicinal chemistry[37,38] to biochemistry.[39] While there are protocols for the generation of thioglycosides,[40,41,42] there is yet to be a complete study of their stability and reactivity under semi-physiological conditions as a means of understanding DNA-protein cross-links.

I envision the mechanism of formation of these compounds to proceed via an oxocarbenium ion intermediate (Scheme 3.1.2, **3.6**). The excision of water from the

anomeric carbon affords a charged aldehyde susceptible to nucleophilic attack of a thiol. While this reaction is reversible, excess concentration of thiol assists in driving the formation of the thioglycoside.

The incubation of model Ap site **3.10** in a solution consisting of acidic water and acetonitrile in the presence of excess thiol afforded good yields of thioglycoside. The overall yield of the thioglycoside produced was notably lower as the concentration of water was increased compared to acetonitrile. This is presumably because the higher aqueous conditions dampen the nucleophilicity of thiols as opposed to in higher organic solvent amounts. A small class of thioglycosides were synthesized and purified as a proof of concept (Figure 3.3.1).

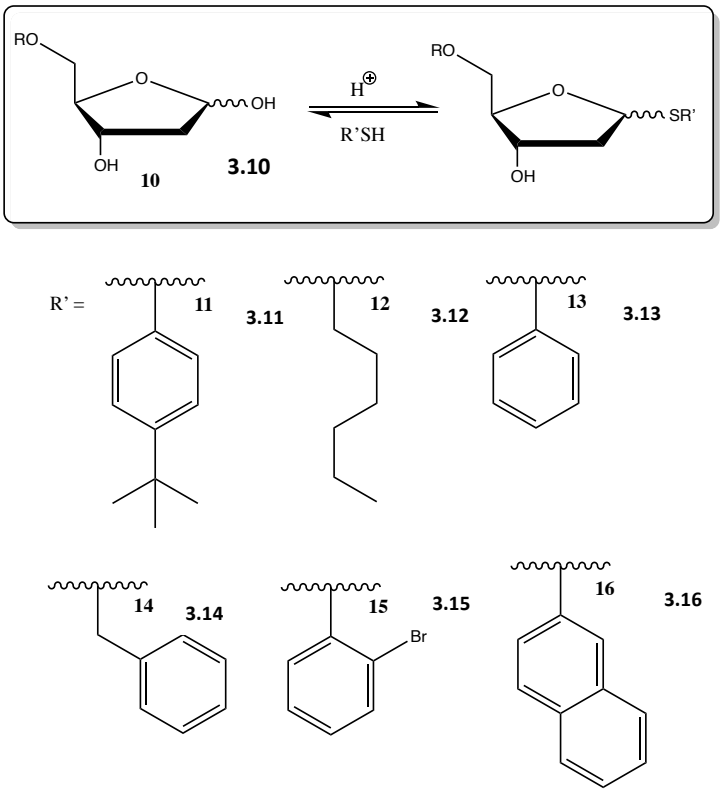
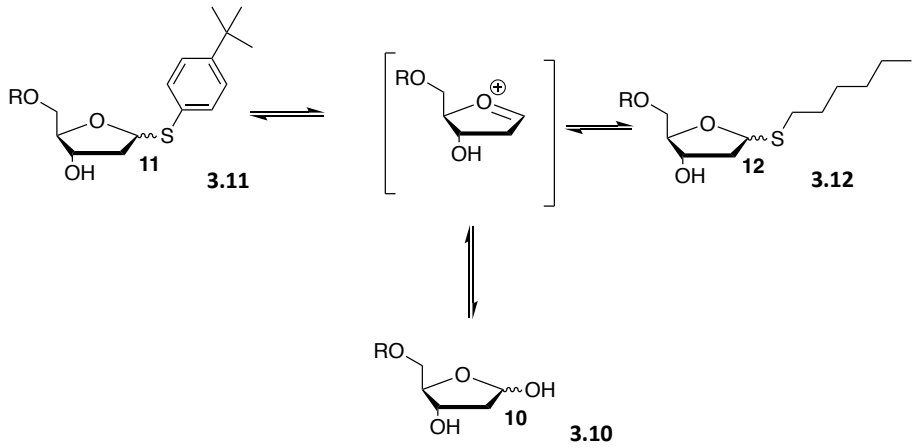


Figure 3.3.1. Generation of a small library of thioglycosides stemming from model Ap site 3.10.

3.4 The formation of thioglycosides is a reversible process.

Literature suggests that the generation of thioglycosides is a reversible process. Research performed with HMCES[22-24] show that the adduct is able to inhibit degradation, however the adduct is presumably excised under some conditions for subsequent repair of the Ap site to occur. To model this, purified thioglycoside **3.11** was incubated in an acidic water and acetonitrile solution to monitor the slow decomposition to the model Ap site **3.10**. Thioglycoside **3.11** showed no signs of decomposition to **3.10** in acetonitrile at 37°C. However, when incubated in a mixed solvent system consisting of 50:50 acetonitrile and water with catalytic acid, there was a decomposition to **3.11** (31%) over the course of 24 hours (Figure 3.4.1). The purified thioglycoside **3.11** was also able to be exchanged with an excess hexanethiol (>10 fold) to generate **3.12** when placed in the same solvent conditions that afforded hydrolysis (Scheme 3.4.1). There was no trace of **3.10** by ^{1}H-NMR, due to the enhanced nucleophilicity of thiols compared to that of alcohols. This is especially true in situations where the thiol is in great excess compared to that of the starting thioglycoside. I can imagine this situation existing in the active site of an enzyme, where there may be a large effective molarity of a cysteine residue.

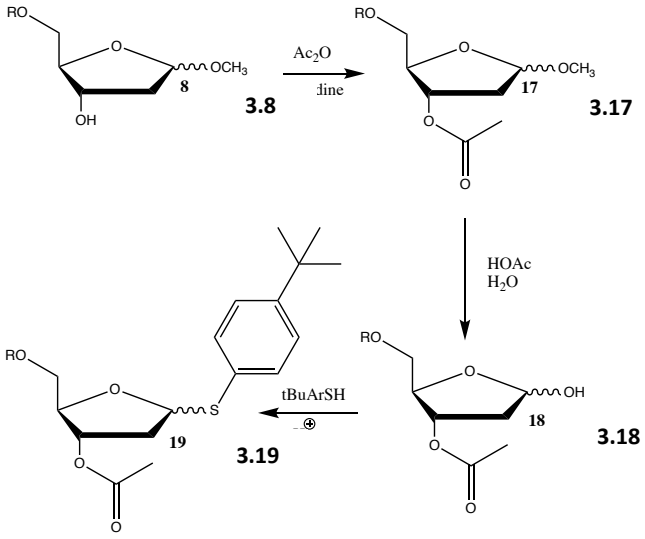
Scheme 3.4.1. Interconversion of 1'thioglycosides in acidic conditions. 3.12 is generated when there is excess hexanethiol present, while 3.10 is generated in the presence of acidic water.



3.5 Thioglycosides can form and be excised without inducing strand cleavage.

As suggested by studies with HMCES[22-24], the generation of a cysteine-based DNA-protein crosslink prevents strand cleavage. In order to test this hypothesis with the model, I synthesized a thioglycoside with a leaving group on the 3' end of the sugar. As hoped, the thioglycoside **3.19** is able to be generated without inducing strand cleavage in the model (Scheme 3.5.1).

Scheme 3.5.1 Generation of a thioglycoside 3.19 from an Ap model with a 3' leaving group.



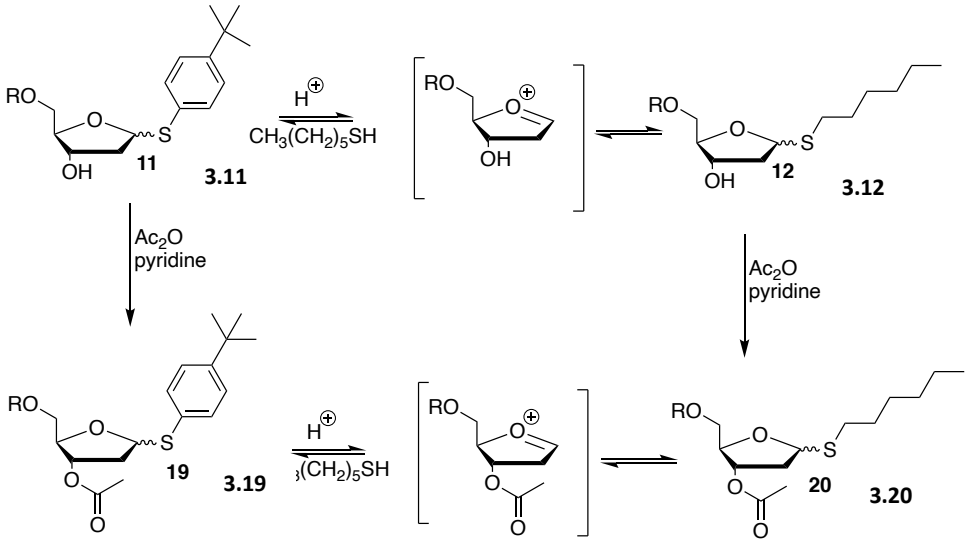
Thioglycoside **3.19** was stable from hydrolysis in the standard solvent system in the absence of HCl. However, upon addition of catalytic HCl, there was a low yielding dissociation of thiol over the course of 24 hours to regenerate **3.8** similar to that seen previously. There was no detection of the loss of the acetyl group on the 3' end of the sugar in these conditions. Interestingly, when **3.19** was incubated with piperidine in the

standard solvent system in the absence of HCl, there was no generation of an unsaturated alkenal. As shown in Chapter 2 of this thesis, piperidine is able to generate an unsaturated alkenal through an irreversible elimination process when there is a C1 aldehyde present. While Section 3.4 of this Chapter suggests that an acid catalyzed process by which the thioglycoside formation is reversible to regenerate **3.18**, this guides us to conclude that there is no appreciable amount of hemiacetal present in the absence of acidic conditions. This is presumably due to the fact that these reactions flow through an oxocarbenium ion as opposed to a direct nucleophilic displacement.

In conditions where there is an excess of thiol, an exchange of thiols is observed with a similar yield to that of hydrolysis. In the standard solvent system, incubation of **3.19** with an excess of hexanethiol (>10 fold) saw a low yielding transfer of thiols to generate **3.20** with no amount of alkenal generated from the reaction (Scheme 3.5.2). This supports reports that thiol addition to Ap sites inhibits spontaneous strand cleavage.

Alternatively, synthetic standards of the 3' acetylated thioglycosides **3.19** and **3.21** were synthesized from direct acetylation of the standard thioglycosides **3.11** and **3.12** respectively in the presence of acetic anhydride and pyridine (Scheme 3.5.2).

Scheme 3.5.2. Generation of 3'acetylated thioglycosides 3.19 and 3.20 either through transthiolation or direct acetylation of thioglycosides 3.11 and 3.12 respectively.



3.6 Discussion

While the field of thioglycosides is not new, the reactions relevant to that of Ap sites are largely undiscovered. While the prototypical Ap reaction with a protein involves a reaction with an amine, such as that with lysine or with an N-terminal amine of a protein,[12-14,16-17, 20-21,43] there is little research regarding the reaction of a cysteine thiol being able to form a covalent adduct with the Ap site (Scheme 3.1.2).[22-24] The studies with HMCES involve a bis-nucleophilic section of the protein where both a cysteine residue as well as an N-terminal amine are used to generate the thiazolidine residue (Figure 3.1.1). The purpose of my research in this chapter is to describe the ability of the thiol solely being able to generate a semi-stable DNA-protein adduct.

Understanding the fundamentals of DNA-protein crosslinks is of profound importance. The generation of an irreversible adduct between a protein and an Ap site has effects detrimental to cellular function. While there are mechanisms by which these crosslinks can be repaired (Figure 3.7.1), these crosslinks are overall extremely noxious. Among the pathways to repair DNA-protein cross-links involve a protease-dependent pathway in which a protease, such as SPRTN,[44,45,46] degrades the protein to a small sequence of amino acids which can be subsequently repaired via other pathways. It is also possible to directly hydrolyze the bond between between DNA and the protein using a phosphodieseterase such as TDP1 or TDP2.[47,48] It is also possible to cleave the DNA component of the strand opposing that of the DNA-protein cross-link via nuclease-dependent repair.[49,50] There is a review describing at length some of the mechanisms by which these cross-links can be repaired as well as some of the biological outcomes which may occur if these lesions fail to be repaired.[51] It is unclear whether these repair pathways are required to excise HMCES being bound to an Ap site.

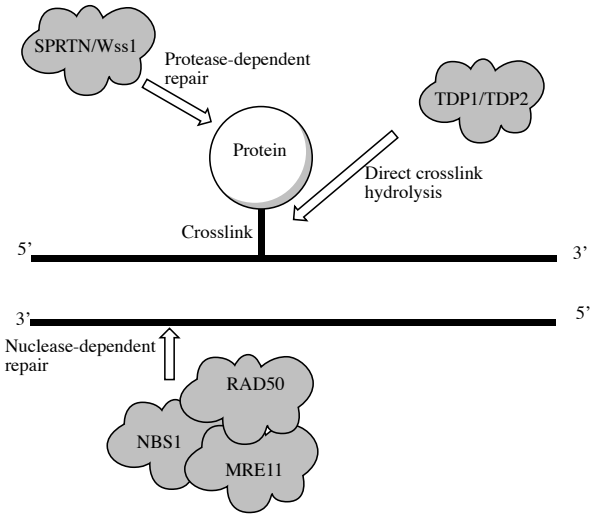


Figure 3.6.1. Various mechanisms by which a DNA-protein cross-link can be repaired.

The underlying hypothesis from this chapter is that there is a DNA-protein cross-link which can be generated as a method to inhibit further DNA degradation. The HMCES[22-24] reaction with an Ap site occurs with an Ap site on a single stranded section of the duplex. This reaction is key because degradation of the Ap site by methods described in Chapter Three of this thesis would lead to error prone repair. In duplex DNA, if strand cleavage were to occur, the information required to replicate the fragment lost during elimination still exists on the opposing strand. If this were to occur in single stranded DNA, there is no genetic information that would afford an enzyme to install the appropriate base lost upon elimination.

In the research described in this chapter, I describe the formation of a stable DNA-protein cross-link which would not require a bis-nucleophilic moiety. Thiols, by their own merit, are able to generate alkaline and amine stable adducts with an Ap model. While this reaction is reversible (Schemes 3.4.1 and 3.4.2), a relatively high concentration of thiol, as I would imagine being possible within the active site of an enzyme, would afford generation of the stable thioglycosidic bond.

While there is still much to be learned about the reversibility of this process, this project has given much headway into the reactions between a thiol and a model Ap site in hopes of mimicking a cysteine reaction with an Ap site in DNA. If this somewhat stable lesion is to be a guardian of the duplex, there must be some way in which the reaction is

reverted back to the Ap site. While the models suggest that it could be simple hydrolysis of the thioglycosidic bond in acidic conditions, there still leaves the possibility that an enzymatic cleavage or protease may be required in a purely biological system to degrade the cross-link.

NOTE: Compounds **3.9**, **3.10**, **3.17**, and **3.18** are the same as Compounds **2.6**, **2.7**, **2.8**, and **2.9** respectively. Experimental data and NMRs were carried over for ease of reading and publication purposes.

Materials and methods

All commercial materials were used as received unless otherwise noted. Commercially available materials were obtained from Sigma-Aldrich, TCI, and Alfa Aesar. Flash column chromatography was performed using 230-400 mesh silica gel as a stationary phase. Thin layer chromatography was performed on silica gel plates from Sigma Chemical Co. Deuterated solvents were purchased from Cambridge Isotope Laboratories. ^{1}H NMR was recorded on a 500 MHz or a 600 MHz spectrometer while ^{13}C NMR spectra was obtained on the same instruments at 126 or 151 MHz. The chemical shift values (δ) are reported in ppm (residual chloroform δ = 7.26 ppm and 77.16 ppm for ^{1}H and ^{13}C respectively). The ^{1}H spectra are reported as follows δ (multiplicity, coupling constant *J*, number of protons).

Experimental Procedure

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (3.9). The solution of 2'-deoxyribose (1.84 g, 13.72 mmol), methanol (60 mL) and HCl (1 mL, 2 M) was stirred for 1 h at 24 ˚C. The reaction was quenched by the addition of pyridine (5 mL) and the crude mixture containing **3.9** was concentrated *in vacuo*. The residue was dissolved in pyridine (20 mL) and t-butyldiphenylsilyl chloride (3.74 mL, 14.4 mmol) was added, followed by stirring at 24 ˚C under an atmosphere of nitrogen gas for 20 h. The reaction was quenched by addition of water (20 mL) and then extracted with ethyl acetate (3 x 40 mL). The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **3.9** (3.70 g, 70% yield) as a colorless oil: Rf = 0.3 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.72-7.67 (m, 7H), 7.47-7.39 (m, 11H), 5.13 (d, *J* = 4.5 Hz, 1H), 5.07 (dd, *J* = 7.5, 3.5, 0.7H), 4.55 (m, 0.7H), 4.32 (m, 1H), 4.19 (m, 1H), 3.96 (m, 0.7H), 3.86-3.77 (m, 1H), 3.69-3.62 (m, 1.8H), 3.41 (s, 3H), 3.29 (s, 1.9H), 2.24-2.20 (m, 1.8H), 2.06-2.03 (m, 2.2H) 1.10 (s, 7H), 1.07 (s, 9H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.6, 135.5, 133.2. 129.8, 129.7, 127.8, 127.7, 105.6, 105.0, 87.8, 85.7, 73.3, 65.4, 64.4, 55.0, 54.8, 41.1, 41.0, 36.8, 26.8, 19.2; HRMS (ESI, $[M+Na]^+$) *m/z* calcd for $C_{22}H_{30}SiO_4Na$: 409.1811; found 409.1807.

Synthesis of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (3.10). The solution of **3.9** (1.0 g, 2.6 mmol) in acetone (20 mL), water (10 mL), and acetic acid (60 mL) was heated in an oil bath at 65 ˚C with stirring for 8 h. The mixture

was then diluted with diethyl ether (100 mL) and saturated sodium bicarbonate was added in 20 mL aliquots until the bubbling ceased. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (3:7) gave **3.7** (0.78 g, 84% yield) as a colorless oil: Rf = 0.3 (3:7 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.75-7.69 (m, 8.6H), 7.66-7.39 (m, 12.8H), 5.61 (d, *J* = 4.5Hz, 1H), 5.43 (dd, *J* = 5.5, 8Hz, 0.22H), 5.10 (d, *J* = 4.5Hz, 0.03H), 5.08 (dd, *J* = 5.5, 8 Hz, 0.04H), 4.65 (m, 0.2H), 4.52 (m, 0.02H), 4.40 (m, 1H), 4.33 (m, 1H), 3.79 (m, 0.5H), 3.73 (m, 1.1H), 3.60 (m, 1.1H), 2.40 (m, 0.3H), 2.20-2.10 (m, 2.3H), 1.10 (s, 5.1H), 1.07 (s, 10.7H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.6, 135.5, 134.8, 129.8, 129.6, 127.9, 127.7, 127.6, 99.6, 87.9, 73.5, 64.3, 41.5, 26.9, 26.8, 26.5, 19.2; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{21}H_{28}SiO_4Na$: 395.1655; found 395.1652.

Synthesis of (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl) tetrahydrofuran-3-ol (3.11). To a solution of **3.10** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, HCl (1M, 50 uL) and 4-*tert*-butyl benzenethiol (0.17 g, 1.0 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **3.11** (41 mg, 78% yield) as a colorless oil: Rf = 0.3 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): (diastereomers) δ 7.69-7.67 (m,

6.2H), 7.47-7.29 (m, 14.5H), 5.72 (d, J=4.5 Hz, 1H), 5.53 (m, 0.2H), 4.65 (m, 0.2H), 4.34 (m, 1H), 4.25 (m, 1.2H), 3.85 (m, 1.4H), 3.69 (m, 1.1H), 2.75 (m, 1H), 2.48-2.05 (m, 3.1H), 1.43 (s, 8.9H), 1.41 (s, 2.6H), 1.08 (s, 2.5H), 1.06 (s, 9.1H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ150.5, 135.7, 135.6, 133.1, 131.6, 131.2, 129.9, 127.8, 127.7, 126.0, 125.9, 87.5, 86.8, 73.5, 64.3, 41.7, 34.6, 31.3, 26.9, 26.8, 19.2; HRMS (ESI $[M+Na-H]^+$) *m/z* calcd for $C_{31}H_{40}SiSO_3Na$: 543.23651; found 543.23511.

Synthesis of (2R,3S)-2-((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(hexylthio)tetrahydrofuran-3-ol (3.12)

To a solution of **3.10** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, HCl (1M, 50 uL) and hexanethiol (0.1 g, 0.8 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:2) afforded **3.12** (37 mg, 77% yield) as a colorless oil: Rf = 0.3 (1:2 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): (diastereomers) δ 7.72-7.68 (m, 9.7H), 7.48-7.40 (m, 13.7H), 5.53 (d, J=4.5Hz, 1H), 5.38 (t, J=4.5, 9 Hz, 0.9H), 4.53 (m, 0.9H), 4.48 (m, 0.9H), 4.23 (m, 1H), 4.00 (m, 0.9H), 3.78 (m, 2.4H), 3.48 (m, 1.9H), 2.68-2.53 (m, 6.8H), 2.37 (m, 0.9H), 2.23 (m, 1H), 1.98 (m, 0.5H), 1.72-1.53 (m, 4.7), 1.48-1.28 (m, 14.6H), 1.06 (s, 8.9H), 1.04 (s, 9H), 0.88 (m, 7.6H); ^{13}C-NMR ($CDCl_3$, 126 MHz): (diastereomers) δ135.6, 135.6, 134.8, 133.2, 133.1, 129.8, 129.7, 127.9, 127.7, 87.2, 86.5, 83.6, 83.5, 74.0, 73.7, 64.8, 64.3, 41.9, 41.1, 31.4, 31.2, 30.0, 29.6, 28.6, 26.9, 26.6.

22.6, 19.3, 19.2, 14.1; HRMS (ESI $[M+Na-H]^+$) *m/z* calcd for $C_{27}H_{39}SiSO_3Na$: 494.22869; found 494.22801.

Synthesis of (2R,3S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(phenylthio)tetrahydrofuran -3-ol (3.13)

To a solution of **3.10** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, HCl (1M, 50 uL) and benzenethiol (0.17 g, 1.5 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **3.13** (44 mg, 85% yield) as a colorless oil: Rf = 0.2 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): (diastereomers) δ 7.68 (m, 4.6H), 7.55 (m, 2.2H), 7.45 (m, 2.5H), 7.43 (m, 5.3H), 7.39-7.34 (m, 2.3H), 5.77 (m, 1H), 4.43 (m, 1.1 H), 4.34 (m, 1.1H), 3.89 (m, 1H), 3.45 (m, 1.1H), 2.75 (m, 1.1H), 2.37 (m, 1H), 2.23-2.05 (m, 1H), 1.06 (s, 9H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ135.6, 135.1 133.1, 133.0, 131.3, 129.9, 129.8, 128.9, 127.8, 127.2, 86.6, 86.1, 73.4, 64.2, 41.7, 26.8, 19.2; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{27}H_{31}SiSO_3Na$: 486.16609; found 486.16595.

Synthesis of (2R,3S)-5-(benzylthio)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran -3-ol (3.14)

To a solution of **3.10** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, HCl (1M, 50 uL) and benzyl mercaptan (0.2 g, 1.6 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:3) afforded **3.14** (42 mg, 84% yield) as a colorless oil: Rf = 0.3 (1:3 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): (diastereomers) δ 7.73-7.70 (m, 7.6H), 7.47-7.41 (m, 12.8H), 7.38-7.31 (m, 8.1H), 5.38 (m, 1H), 5.28 (m, 0.7H), 4.53 (m, 0.7H), 4.32 (m, 1H), 4.22 (m, 1.1H), 4.20 (m, 0.5H), 4.00 (0.8H), 3.87-3.82 (m, 3.6H), 3.81-3.77 (m, 3.9H), 2.65 (1.5H), 2.21 (m, 1.1H), 2.18 (m, 0.9H), 2.05 (m, 0.9H), 1.08 (s, 6H), 1.04 (s, 9H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ138.0, 135.7, 135.6, 133.2, 133.1, 129.8, 129.0, 128.5, 127.8, 127.7, 127.0, 126.9, 87.1, 86.6, 82.2, 81.9, 74.1, 73.7, 64.9, 64.3, 41.4, 40.6, 35.1, 35.0, 26.9, 26.8, 19.3; HRMS (ESI $[M+Na-H]^+$) *m/z* calcd for $C_{23}H_{33}SiSO_3Na$: 500.18174; found 500.18161.

Synthesis of (2R,3S)-5-((2-bromophenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)tetra hydrofuran-3-ol (3.15)

To a solution of **3.10** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, HCl (1M, 50 uL) and 2-bromothiophenol (0.2 g, 0.7 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:5) afforded **3.15** (49 mg, 89% yield) as a colorless oil: Rf = 0.3 (1:5 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): (diastereomers) δ7.70-7.67 (m, 8.7H), 7.59 (m, 1.1H), 7.46-7.39 (m, 12.8H), 7.11 (m, 1.1H), 5.85 (m, 1H), 4.43 (m, 1.2H), 4.34 (m, 1.1H), 4.12 (m, 0.8H), 3.83 (m, 1.1H), 3.42 (m, 1.4H), 2.20 (m, 2.3H), 1.08 (s, 2.5H), 1.07 (s, 9H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ135.6, 133.1, 132.9, 131.0, 129.8, 127.9, 127.8, 127.7, 86.2, 85.4, 73.2, 64.1, 41.7, 26.9, 19.3; HRMS (ESI $[M+Na-H]^+$) *m/z* calcd for $C_{27}H_{30}SiSBrO_3Na$: 564.07660; found 564.07637.

Synthesis of (2R,3S)-2-(2-(*tert*-butyldiphenylsilyl)ethyl)-5-(napthalen-2-ylthio)tetrahydro furan-3-ol (3.16)

To a solution of **3.10** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, HCl (1M, 50 uL) and 2-napthalenethiol (0.2 g, 1.25 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:5) afforded **3.16** (49 mg, 89% yield) as a colorless oil: Rf =

0.3 (1:5 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): (diastereomers) δ8.02 (m, 1.1H), 7.80-7.68 (m, 11.7H), 7.60-7.38 (m, 14.9H), 5.88 (m, 1H), 5.62 (m, 0.2H), 4.43 (m, 1.3H), 4.33 (m, 0.3H), 3.90 (m, 1.1H), 3.76 (m, 1.3H), 2.85 (m, 1H), 2.44-2.13 (m, 2.5H), 1.08 (s, 9H), 1.06 (s, 3H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ135.6, 133.7, 133.0, 132.6, 132.3, 129.9, 129.8, 129.7, 128.7, 128.4, 127.9, 127.8, 127.7, 126.5, 126.1, 86.7, 86.2, 73.4, 64.2, 41.8, 41.5, 26.9, 26.8, 19.3, 19.2; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{31}H_{33}SiSO_3Na$: 536.18174; found 536.18088.

Generation of 3.12 via the purified thioglycoside 3.11 in acidic conditions as evidence for the reversibility of thioglycoside formation.

To a solution of **3.11** (27 mg, 0.052 mmol) dissolved in water (1 mL) and acetonitrile (1 mL) has HCl (1 M, 50 uL) and hexanethiol (100 uL, 0.70 mmol) added. The reaction is stirred at 37 ˚C for 1 h before being quenched with the addition of saturated ammonium chloride (1 mL). The reaction was extracted with ethyl acetate (3 x 5 mL) and combined organic extracts were washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. **3.12** was isolated as described above with a yield of 8 mg (0.017 mmol, 32% yield). Spectral data matched that described above.

Hydrolysis of thioglycoside 3.11 in acidic conditions to generate the model Ap site 3.10.

To a solution of **3.11** (25 mg, 0.050 mmol) dissolved in water (1 mL) and acetonitrile (1 mL) has HCl (1 M, 50 uL) added. The reaction is stirred 37 ˚C for 1 h before being

quenched with the addition of saturated ammonium chloride (1 mL). The reaction was extracted with ethyl acetate (3 x 5 mL) and combined organic extracts were washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. **3.10** was isolated as described above with a yield of 7 mg (0.019 mmol, 38% yield). Spectral data matched that described above.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (3.17). The solution of **3.8** (2.0 g, 5.2 mmol) in pyridine (10 mL), and acetic anhydride (2 mL, 21.2 mmol) was stirred for 2 h at 24 ˚C. The reaction was quenched by addition of methanol (3 mL) and the crude mixture was concentrated *in vacuo*. The residue was mixed with water (30 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:9) gave **3.17** (2.19 g, 99% yield) as a colorless oil: Rf = 0.8 (1:9 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.71-7.70 (m, 7.2H), 7.42-7.28 (m, 11H), 5.38-5.34 (m, 0.6H), 5.30-5.29 (m, 1H), 5.15-5.13 (m, 1.7H), 4.19-4.17 (m, 1.7H), 3.90-3.80 (m, 1H), 3.78-3.72 (m, 2.4H), 3.42 (s, 3H), 3.32 (s, 1.9H), 2.37-2.35 (m, 1H), 2.36-3.32 (m, 0.6H), 2.09 (s, 3.3H), 2.07 (s, 2.1H) 1.08 (s, 4.9H), 1.07 (s, 9.6H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 171.0, 170.4, 135.6, 133.4, 133.3, 133.2, 129.7, 127.7, 105.5, 105.3, 84.0, 83.9, 75.4, 74.6, 64.7, 64.2, 55.3, 55.1, 39.4, 39.0, 26.7, 21.2, 21.1, 19.2; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{24}H_{32}SiO_5Na$: 451.1916; found 451.1911.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (3.18). The solution of **3.17** (1.0 g, 2.33 mmol) in acetone (20 mL), water (10 mL), and acetic acid (60 mL) was heated in an oil bath at 65 ˚C with stirring for 8 h. The mixture was then diluted with diethyl ether (100 mL) and saturated sodium bicarbonate was added in 20 mL aliquots until the bubbling ceased. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (2:3) afforded **3.18** (0.8 g, 84% yield) as a colorless oil: Rf = 0.3 (2:3 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.74-7.67 (m, 8.7H), 7.45-7.28 (m, 13.4H), 5.57 (d, *J* = 5.0 Hz, 1H), 5.62-5.61 (m, 1H), 5.44-5.40 (m, 0.8H), 5.39-5.38 (m, 0.8H), 4.34-4.33 (m, 1H) 4.16-4.14 (m, 0.9H), 3.86-3.84 (m, 2.9H), 3.74-3.72 (m, 1.4H), 2.49-2.41 (m, 1H), 2.40-2.36 (m, 0.9H), 2.30-2.25 (m, 0.9H), 2.11 (s, 5.1H), 2.06 (s, 2.9H), 1.11 (s, 9H), 1.07 (s, 11.4H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 170.6, 135.7, 135.6, 135.5, 134.8, 133.1, 133.0, 132.3, 132.2, 130.1, 130.0, 129.8, 129.7, 127.9, 127.8, 127.7, 99.2, 84.8, 74.7, 75.4, 75.3, 65.1, 64.2, 60.4, 41.5, 40.0, 26.9, 26.8, 21.2, 21.0, 19.2, 14.9; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{23}H_{30}SiO_5Na$: 437.1760; found 437.1757.

Synthesis of (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl) tetrahydrofuran-3-yl acetate (3.19) generated from 3.18.

To a solution of **3.18** (40 mg, 0.10 mmol) dissolved in acetonitrile (2 mL) and water (2 mL) has HCl (1 M, 50 uL) and tert-butylbenzenethiol (0.17g, 1.0 mmol) added. The

reaction is stirred at 37 ˚C for 1 h before being quenched by the addition of saturated ammonium chloride (2 mL) and being extracted with ethyl acetate (3 x 5mL). The organic extract was washed brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography on silica gel eluted with ethyl acetate and hexanes (1:9 ethyl acetate/hexanes) afforded **3.19** (43 mg, 76% yield) as a colorless oil: Rf = 0.8 (1:9 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): (diastereomers) δ 7.70-7.68 (m, 6.4H), 7.48-7.34 (m, 18.7H), 5.75 (d, J=4.5Hz, 1H), 5.53 (m, 0.2H), 5.48 (m, 0.3), 5.37 (m, 1.1H), 4.43 (m, 1.1H), 4.28 (m, 0.7H), 3.95 (m, 1H), 3.82 (m, 1.5H), 2.85 (m, 0.9H), 2.20 (s, 3H), 2.15 (s, 1.8H), 1.37 (s, 9H), 1.32 (s, 3.3H), 1.21 (s, 2.7H), 1.19 (s, 9H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ170.8, 150.2, 135.8, 135.7, 135.6, 134.8, 133.2, 132.2, 131.7, 131.1, 129.8, 129.7, 128.0, 127.8, 127.7, 126.0, 87.7, 83.8, 74.7, 64.1, 64.0, 39.8, 34.5, 31.3, 29.7, 26.9, 26.8, 26.6, 21.2, 19.3; HRMS (ESI $[M+Na-H]^+$) *m/z* calcd for $C_{33}H_{41}SiSO_4Na$: 584.23925; found 584.24004

Alternative synthesis of (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl) oxy)methyl) tetrahydrofuran-3-yl acetate (3.19) from the 3' acetylation of thioglycoside 3.11. To a solution of **3.11** (52 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, acetic anhydride (0.5mL, 5.3mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of methanol (1 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with

ethyl acetate and hexanes (1:9) afforded **3.19** (54 mg, 95% yield). Spectral data matched that described above.

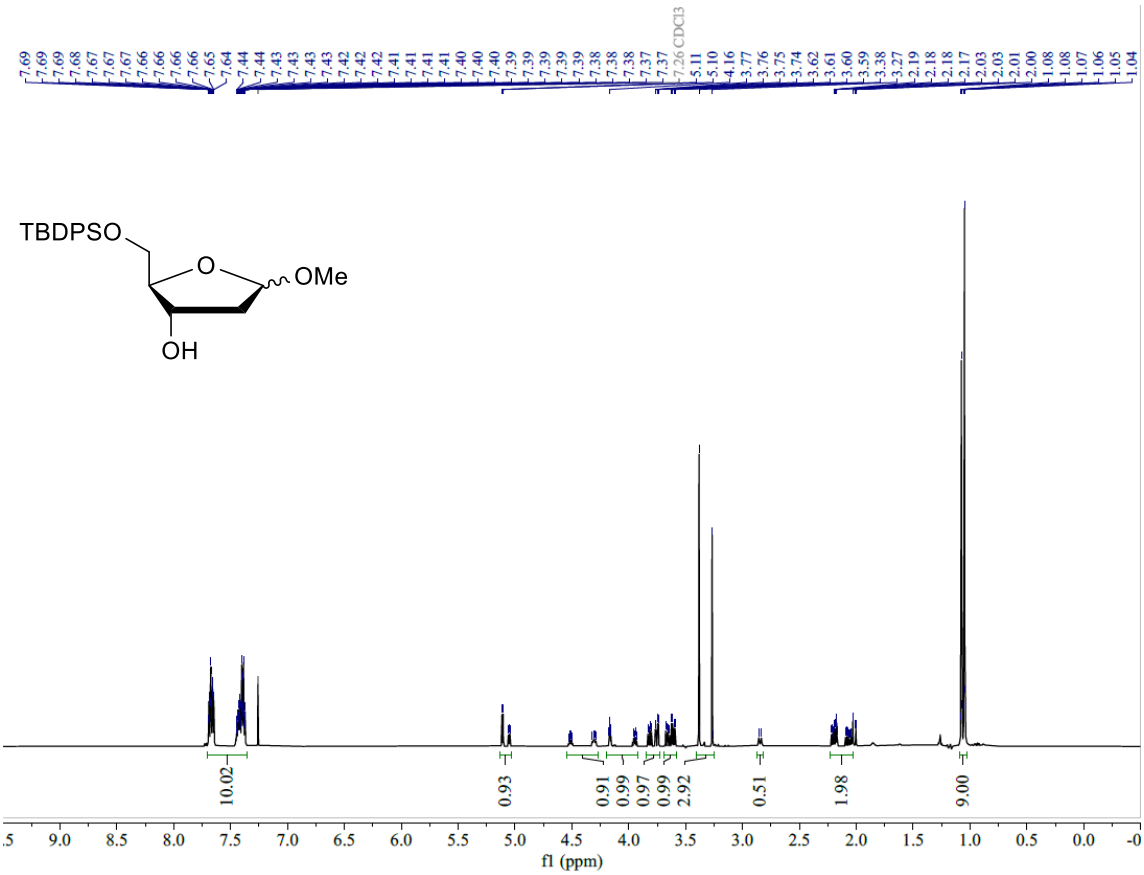
Synthesis of (2R,3S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(hexylthio)tetrahydrofuran-3-yl acetate (3.20) from the 3' acetylation of thioglycoside 3.12

To a solution of **3.12** (50 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile (2 mL) and water (2 mL) at 37 ˚C, acetic anhydride (0.5mL, 5.3mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of methanol (1 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography on silica gel of the residue eluted with ethyl acetate and hexanes (1:9) afforded **3.20** (51 mg, 95% yield) as a colorless oil: Rf = 0.7 (1:9 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 600 MHz): (diastereomers) δ7.76-7.69 (m, 9.4H), 7.47-7.40 (14.7H), 5.52 (d, J=4.5Hz, 1H), 5.48, (m, 0.8H), 5.43 (m, 0.8H), 5.40 (m, 1H), 4.43 (m, 1H), 4.31 (m, 0.8H), 4.12 (m, 1H), 3.87 (m, 1.9H), 3.65 (m, 1.3H), 2.85 (m, 0.7H), 2.78-2.65 (m, 4.4H), 2.38-2.12 (m, 1.8H), 2.08 (s, 6H), 1.63-1.58 (m, 4H), 1.49-1.1.28 (m, (14H), 1.22-1.15 (m, 6.1H), 1.08 (s, 9H), 0.88 (m, 7.9H); ^{13}C-NMR ($CDCl_3$, 151 MHz): (diastereomers) δ170.8, 170.4, 135.8, 135.6, 134.8, 133.2, 129.8, 129.7, 127.7, 85.6, 84.8, 83.8, 83.3, 75.1, 74.7, 64.2, 64.0, 39.8, 39.0, 31.9, 31.4, 30.1, 30.0, 28.7, 26.9, 26.8, 26.7, 22.6, 21.2, 21.1, 19.3, 19.2; HRMS (ESI [M+Na]-H^+) *m/z* calcd for $C_{29}H_{41}SiSO_4Na$: 536.23925; found 536.23898.

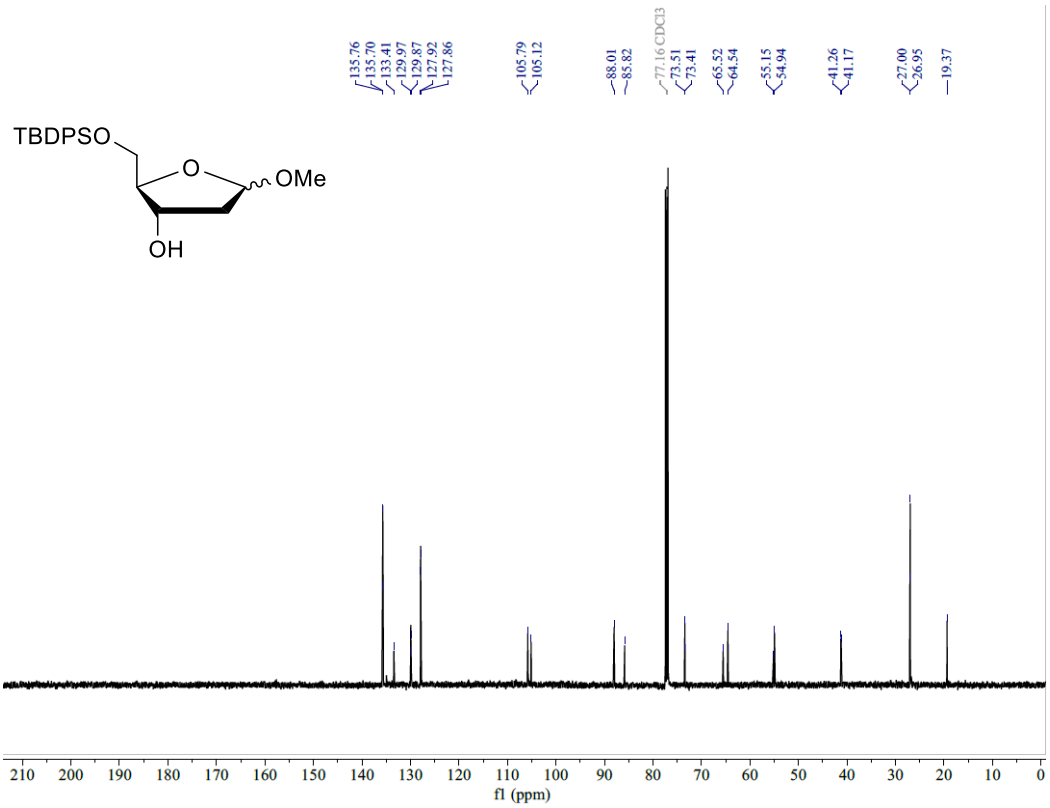
Generation of 3.20 formed by thiol exchange with the 3' acetylated thioglycoside 3.19.

To a solution of **3.19** (28 mg, 0.05 mmol) dissolved in water (2 mL) and acetonitrile (2 mL) has HCl (1 M, 50 ul) and hexanthiol (70 mg, 0.60 mmol) added. The reaction is stirred for 1 h at 37 ˚C before being quenched by the addition of ammonium chloride (2 mL) and being extracted with ethyl acetate (3 x 5 mL). The organic extract is washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography on silica gel of the residue eluted with ethyl acetate and hexanes (1:9) afforded **3.20** (9 mg, 33% yield). Spectral data matched that described above.

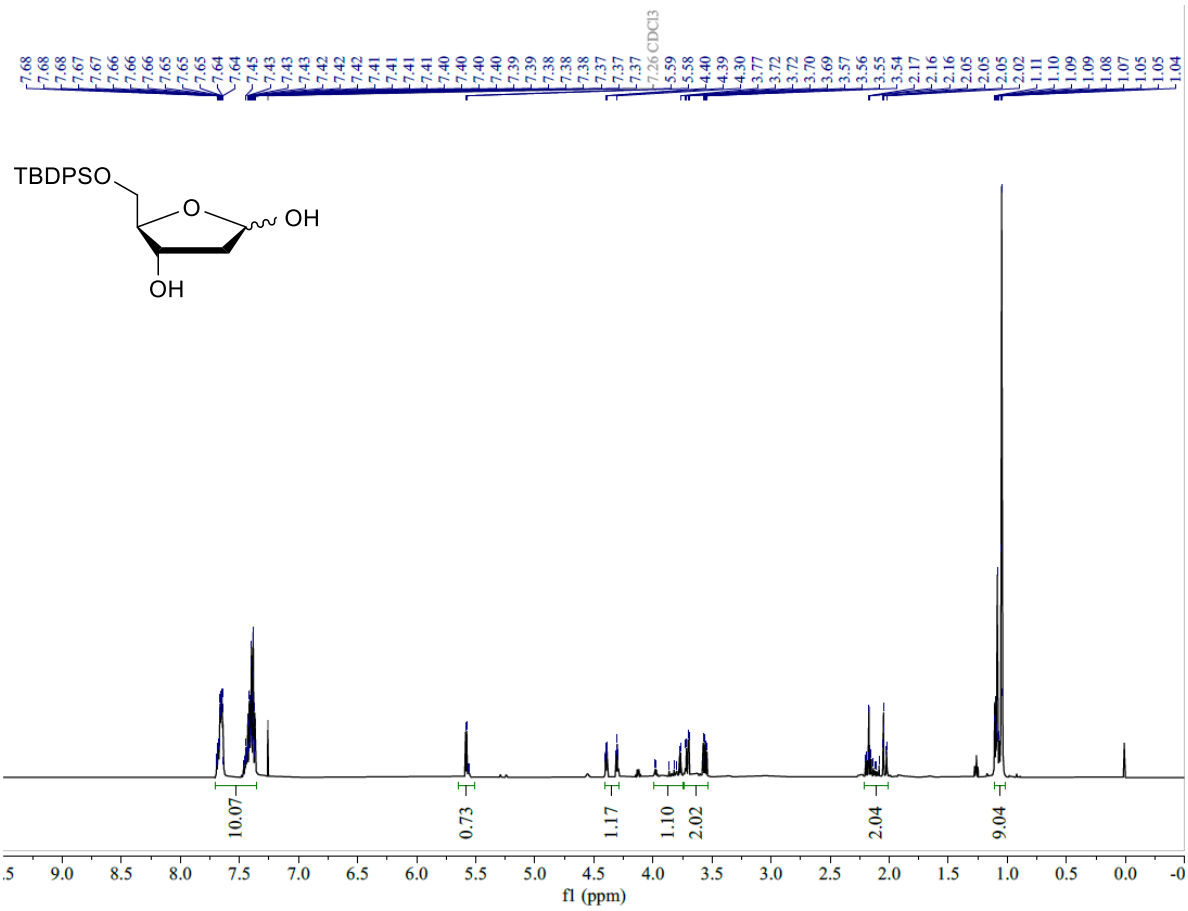
NMRs



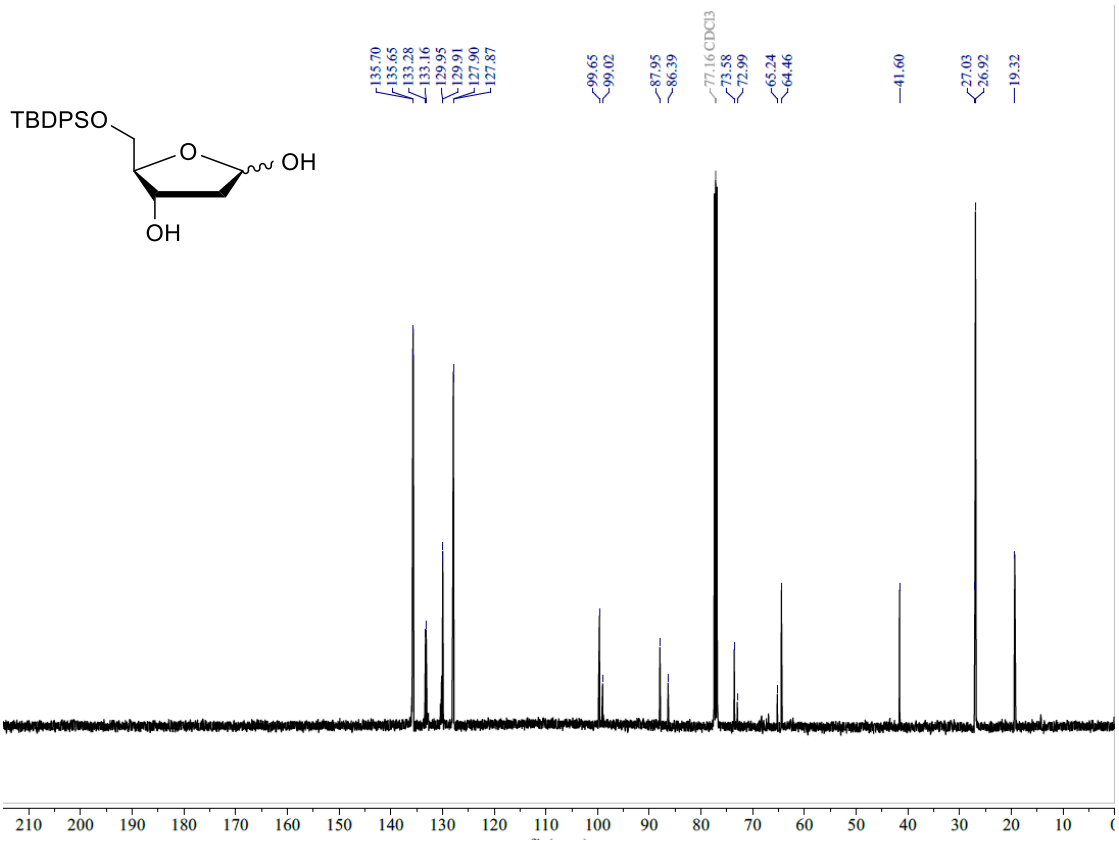
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (3.9).



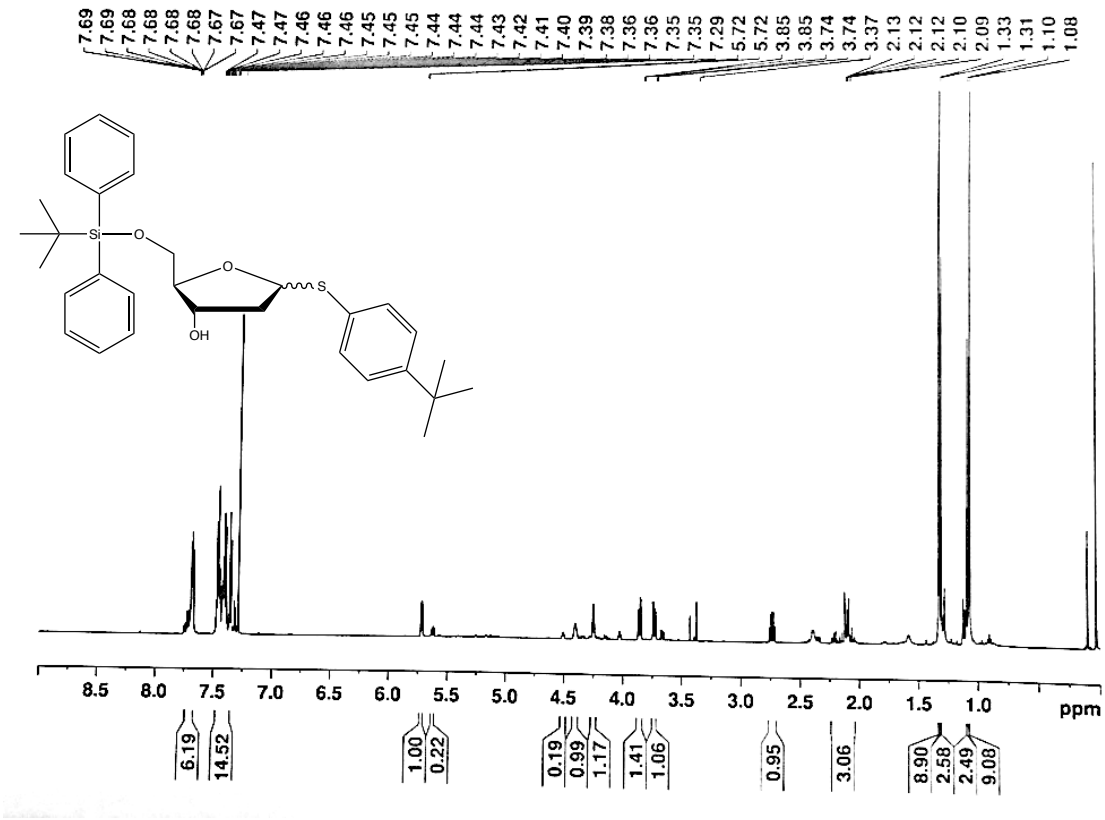
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (3.9).



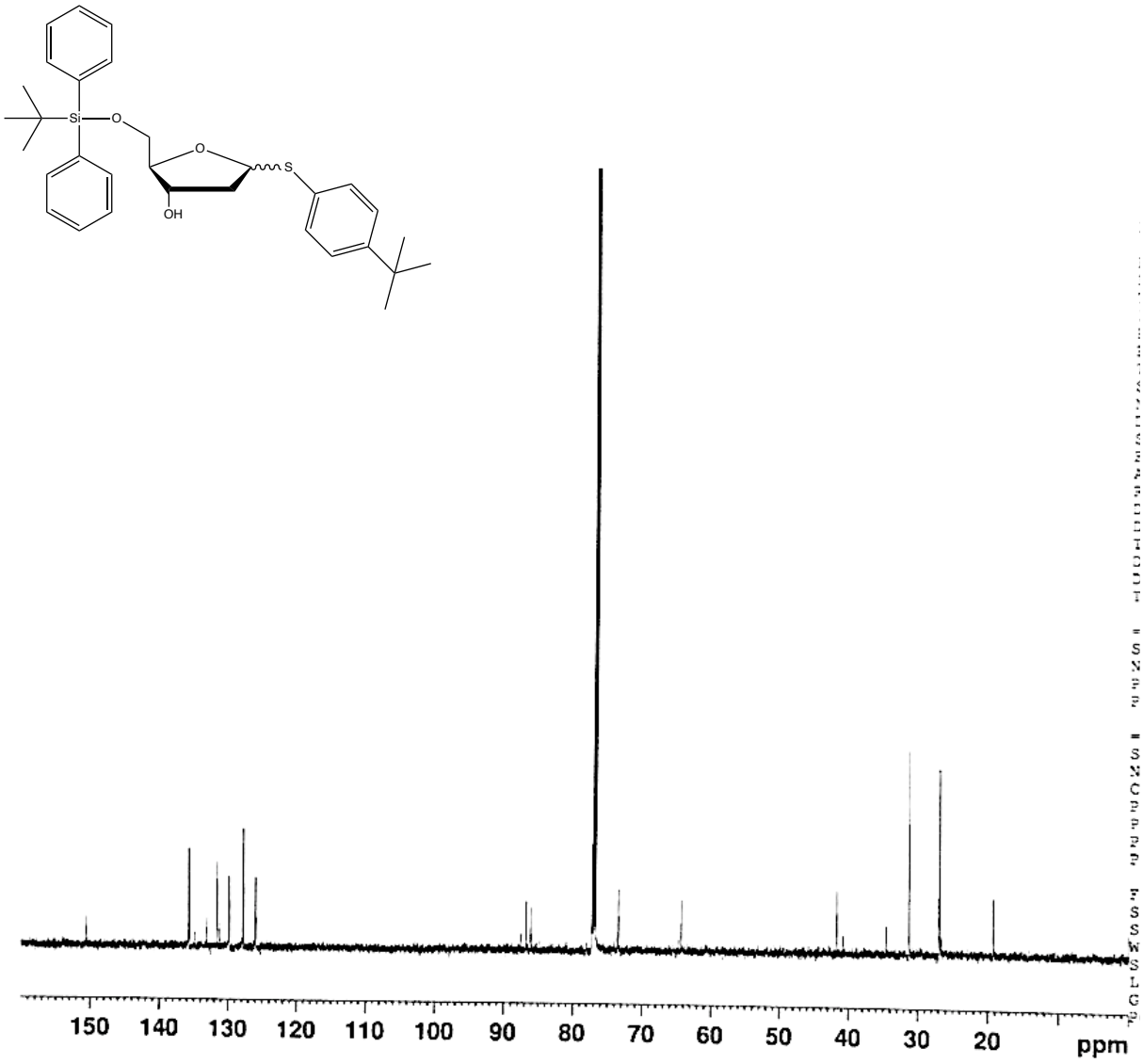
^{1}H-NMR (600 MHz) of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (3.10).



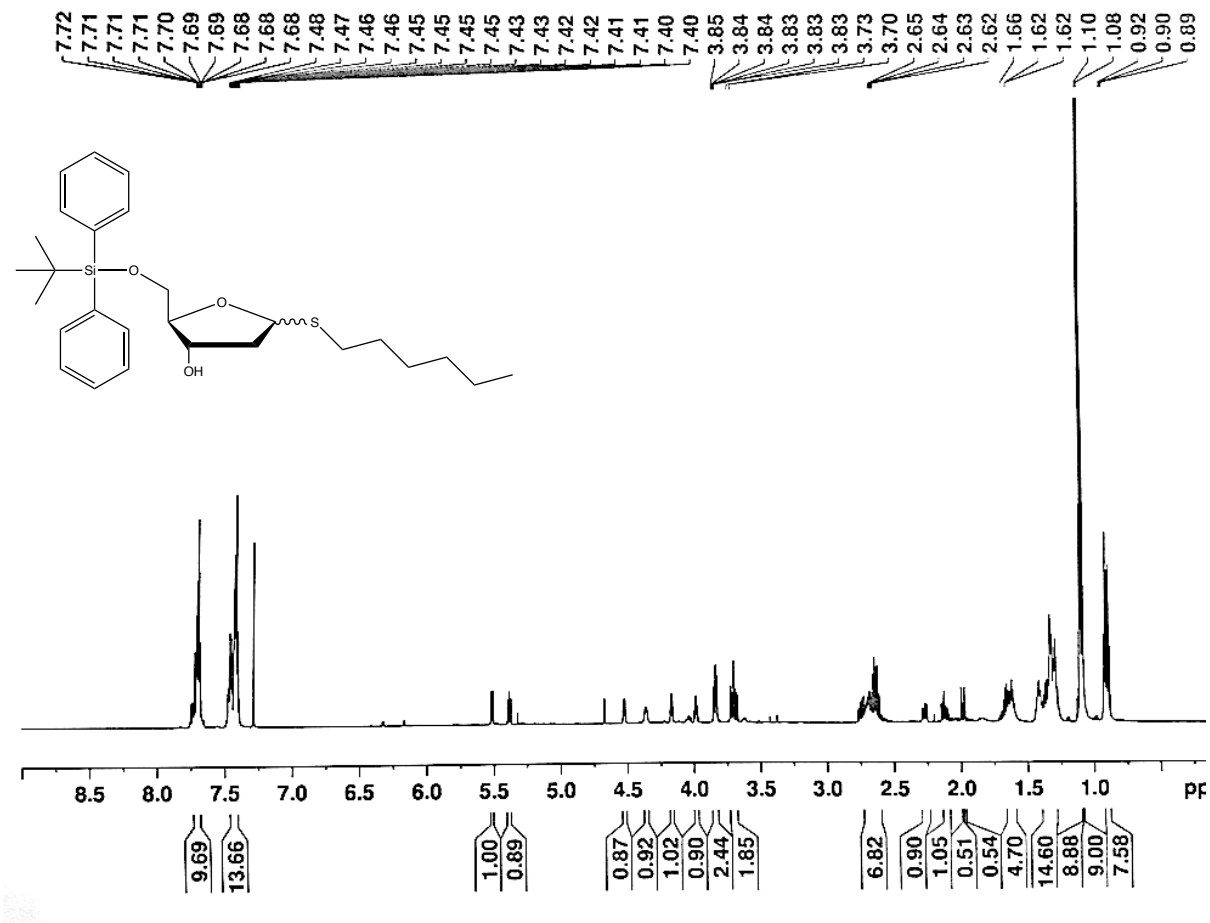
^{13}C-NMR (151 MHz) of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (3.10).



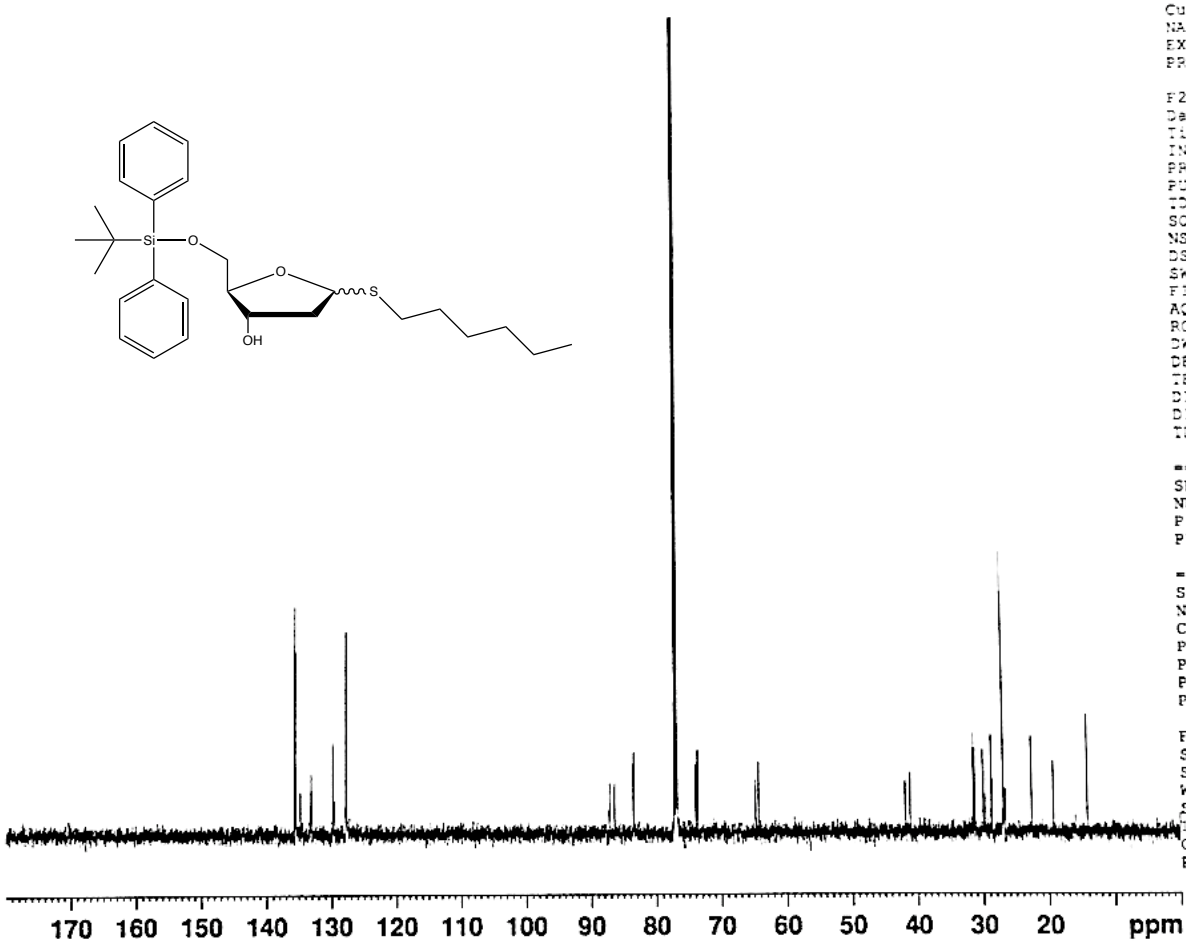
^{1}H-NMR (600 MHz) (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy) methyl)tetrahydrofuran-3-ol (3.11).



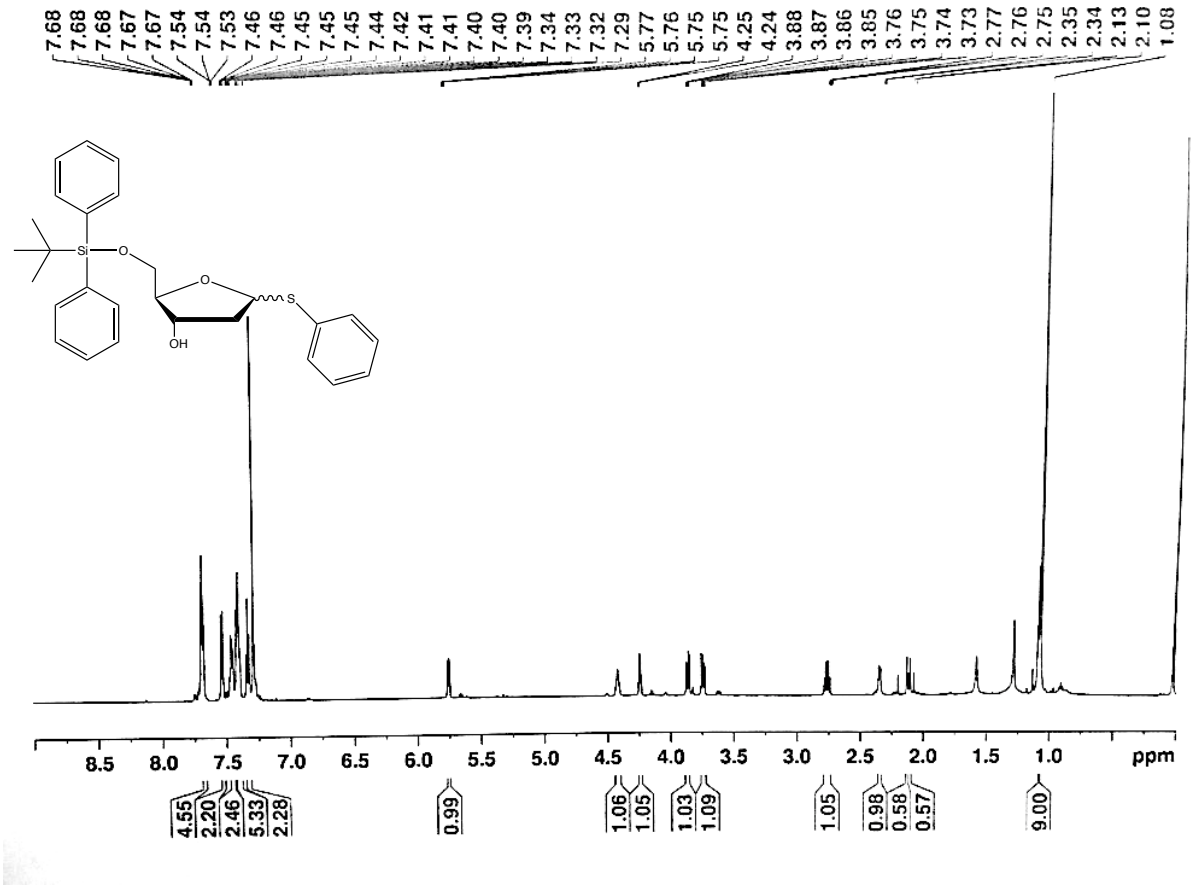
^{13}C-NMR (151 MHz) (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy) methyl)tetrahydrofuran-3-ol (3.11).



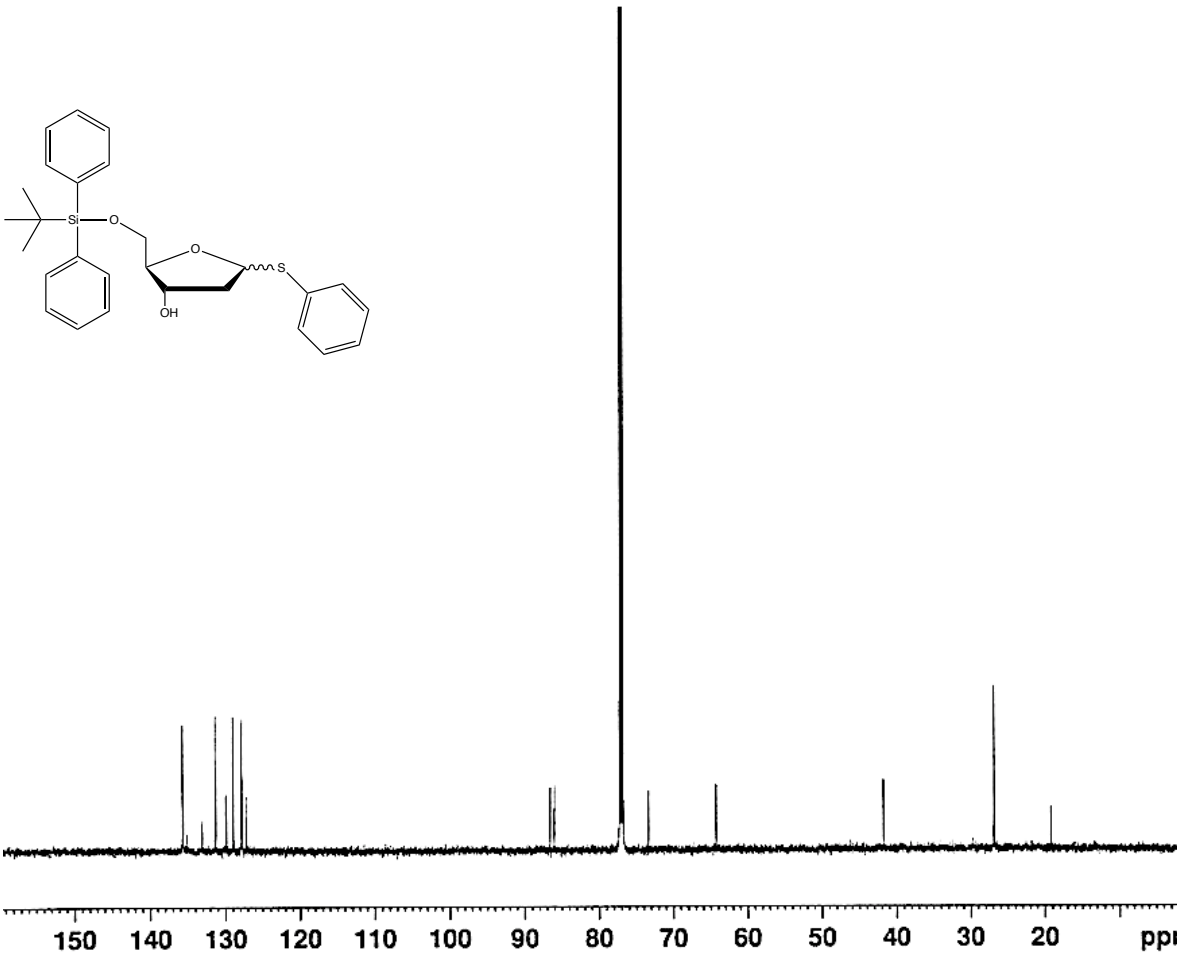
^{1}H-NMR (600 MHz) of (2R,3S)-2-((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(hexylthio)tetra hydrofuran-3-ol (3.12).



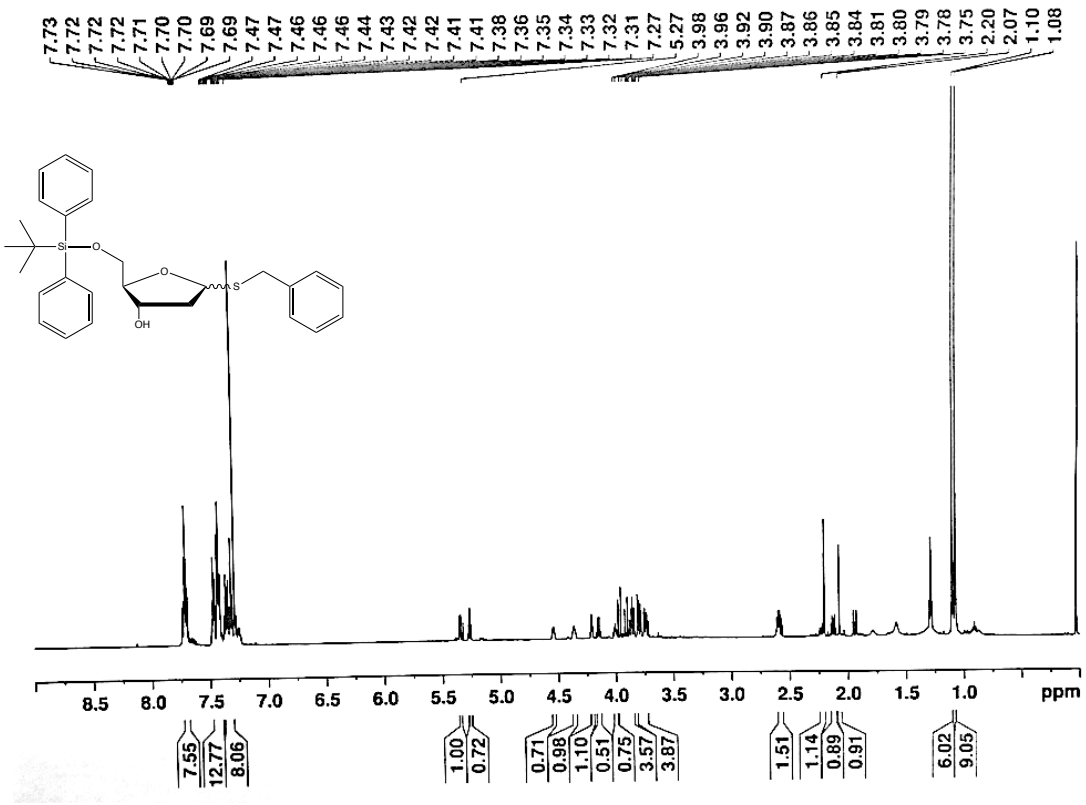
^{13}C-NMR (151 MHz) of (2R,3S)-2-((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(hexylthio)tetra hydrofuran-3-ol (3.12).



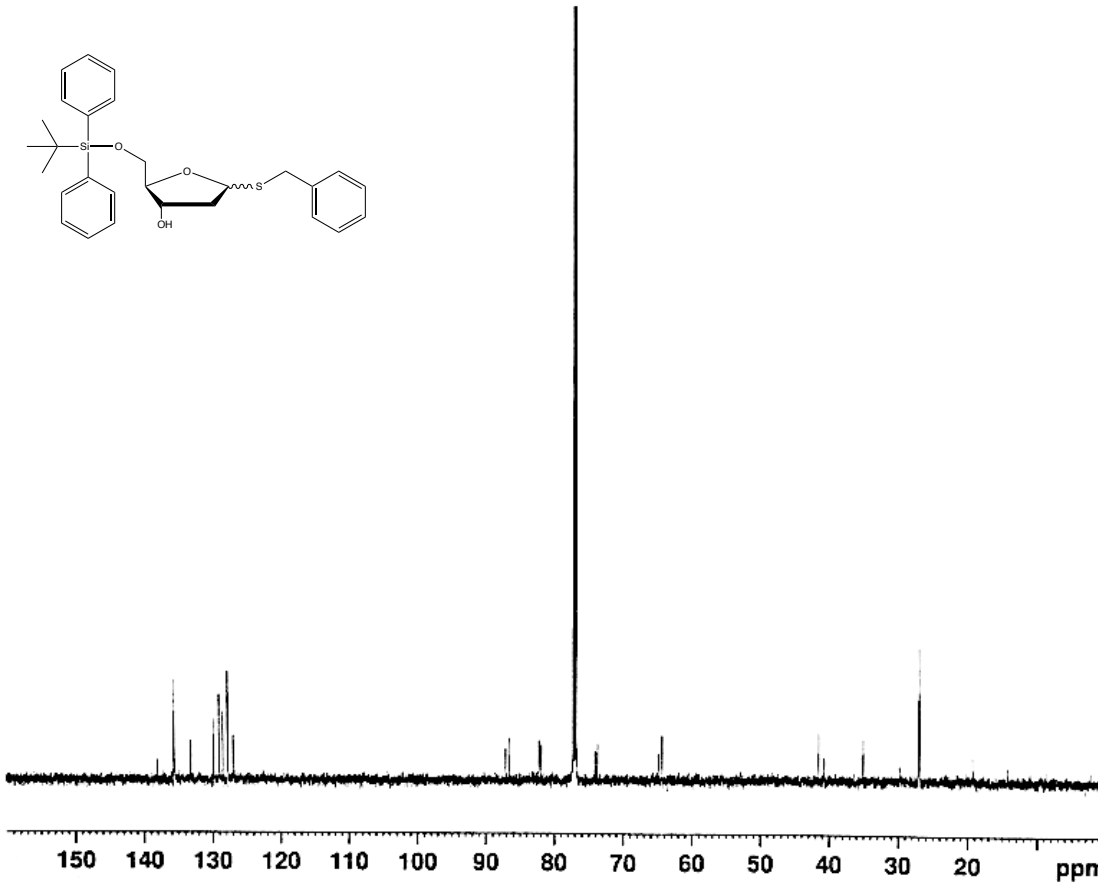
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(phenylthio) tetrahydrofuran-3-ol (3.13).



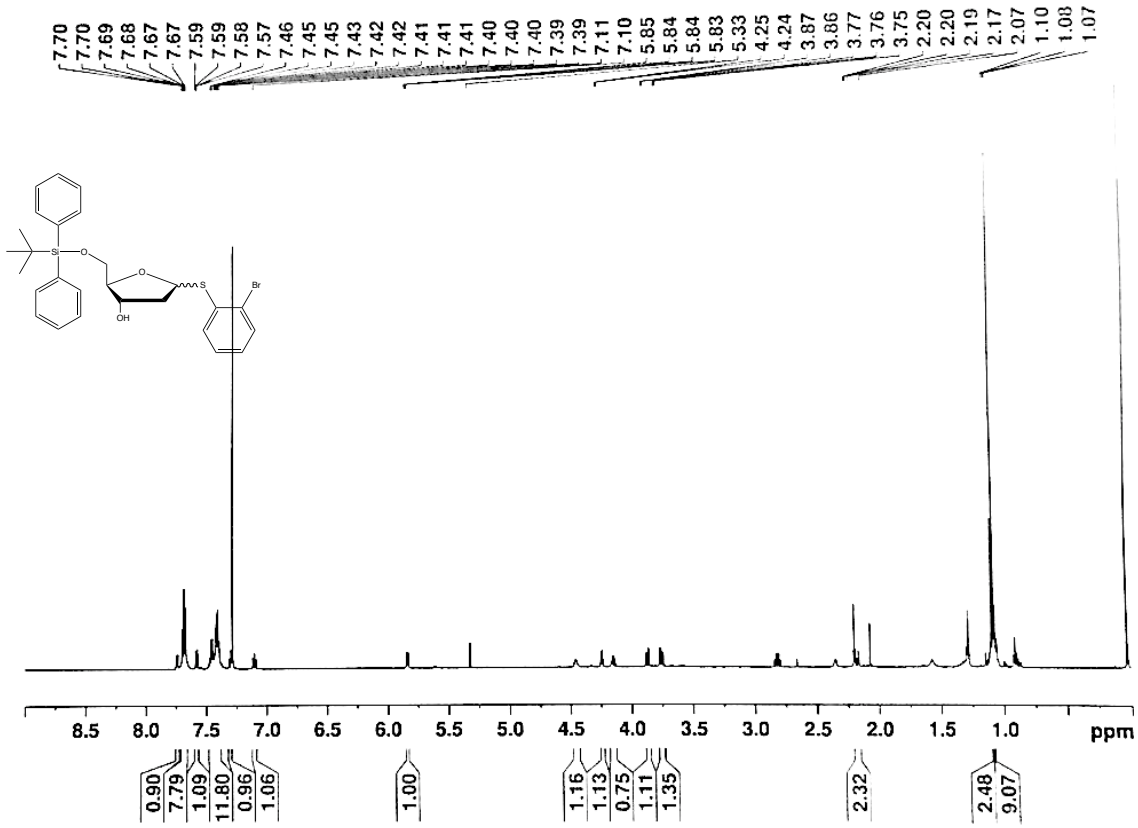
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(phenylthio) tetrahydrofuran-3-ol (3.13).



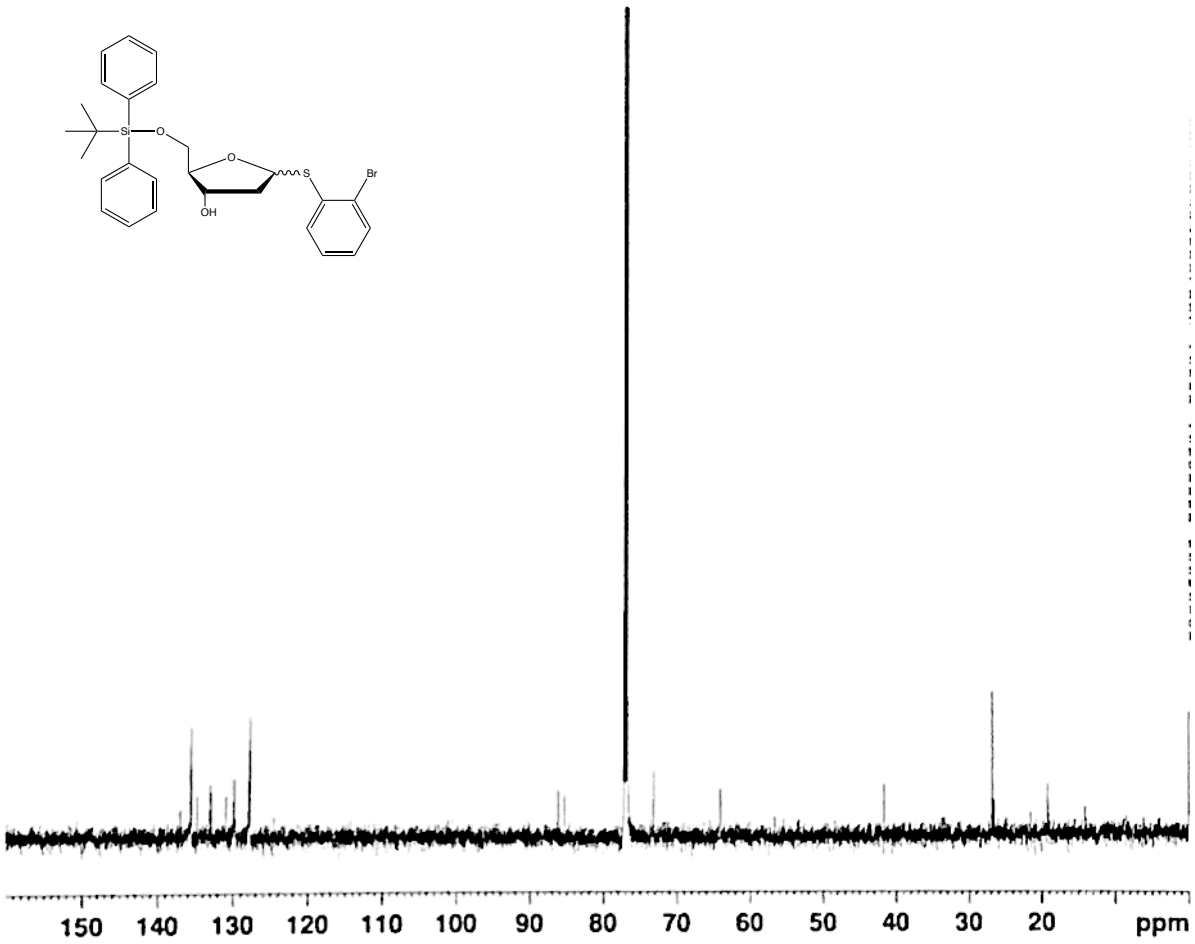
^{1}H-NMR (600 MHz) of (2R,3S)-5-(benzylthio)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl) tetrahydrofuran-3-ol (3.14).



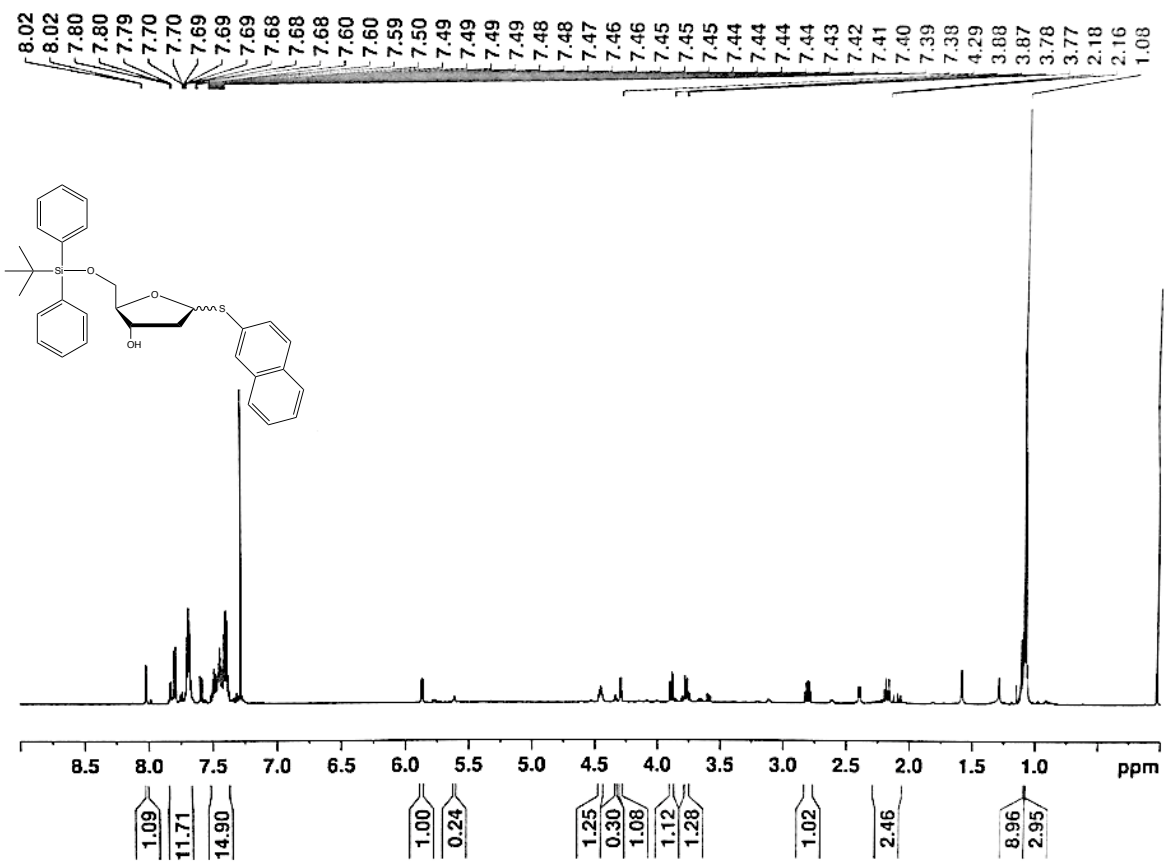
^{13}C-NMR (151 MHz) of (2R,3S)-5-(benzylthio)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl) tetrahydrofuran-3-ol (3.14).



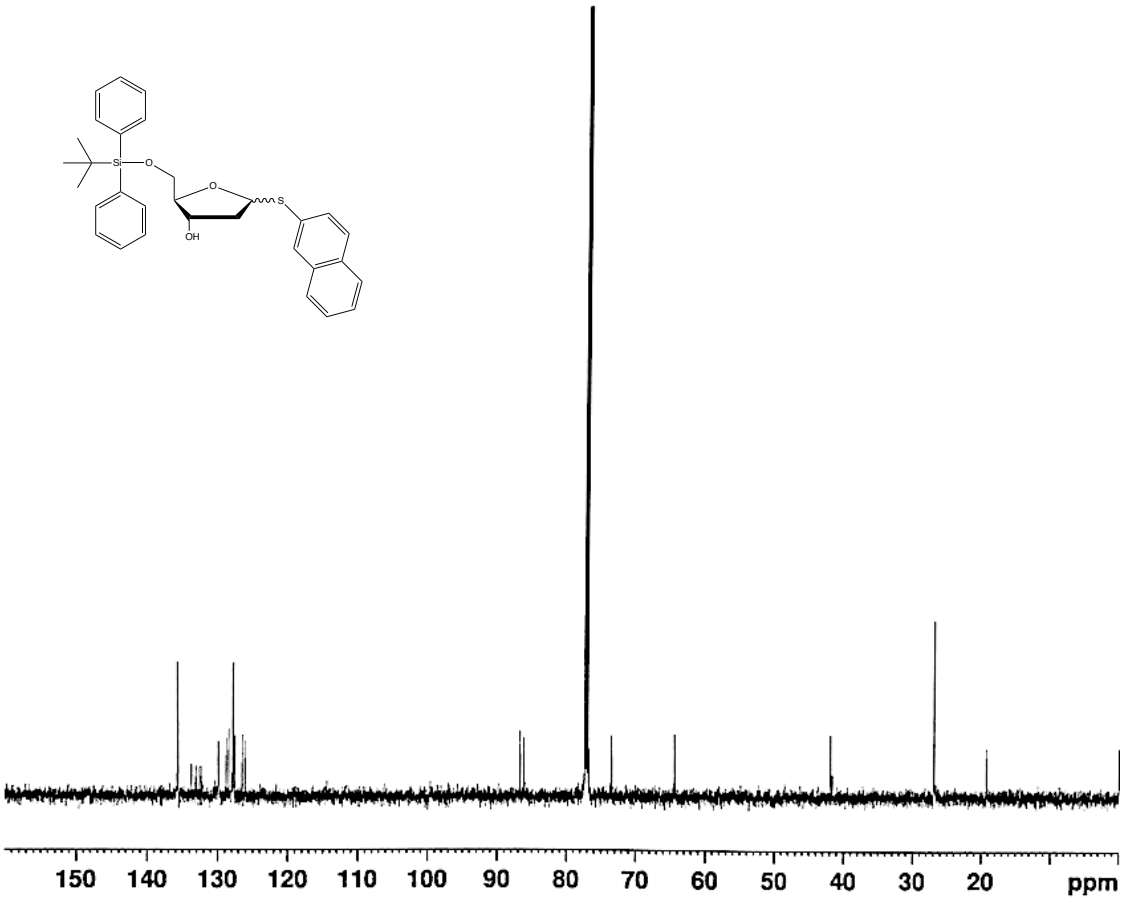
^{1}H-NMR (600 MHz) of (2R,3S)-5-((2-bromophenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy) methyl)tetrahydrofuran-3-ol (3.15).



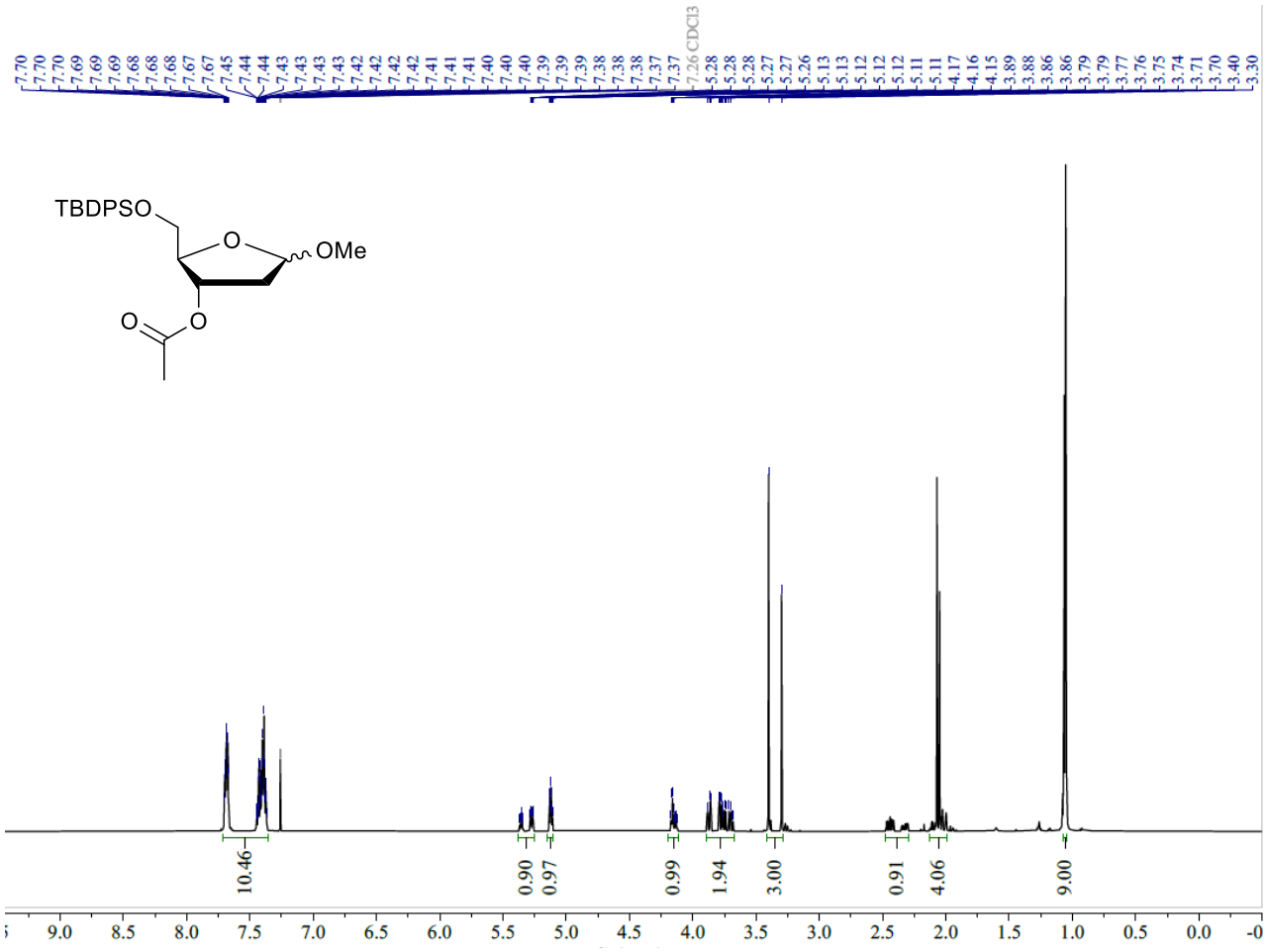
^{13}C-NMR (151 MHz) of (2R,3S)-5-((2-bromophenyl)thio)-2-(((*tert*-butyldiphenylsilyl)oxy) methyl)tetrahydrofuran-3-ol (3.15).



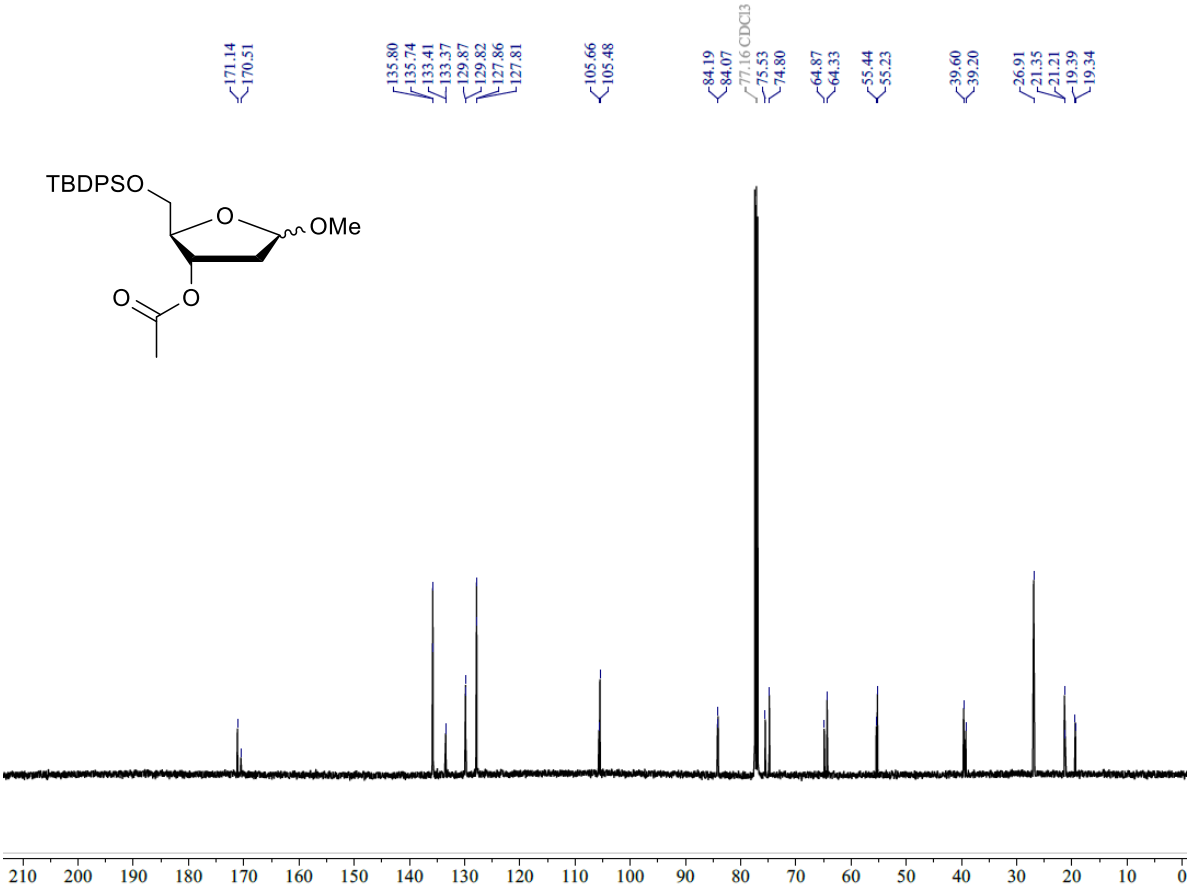
^{1}H-NMR (600 MHz) of (2R,3S)-2-(2-(*tert*-butyldiphenylsilyl)ethyl)-5-(napthalen-2-ylthio) tetrahydrofuran-3-ol (3.16).



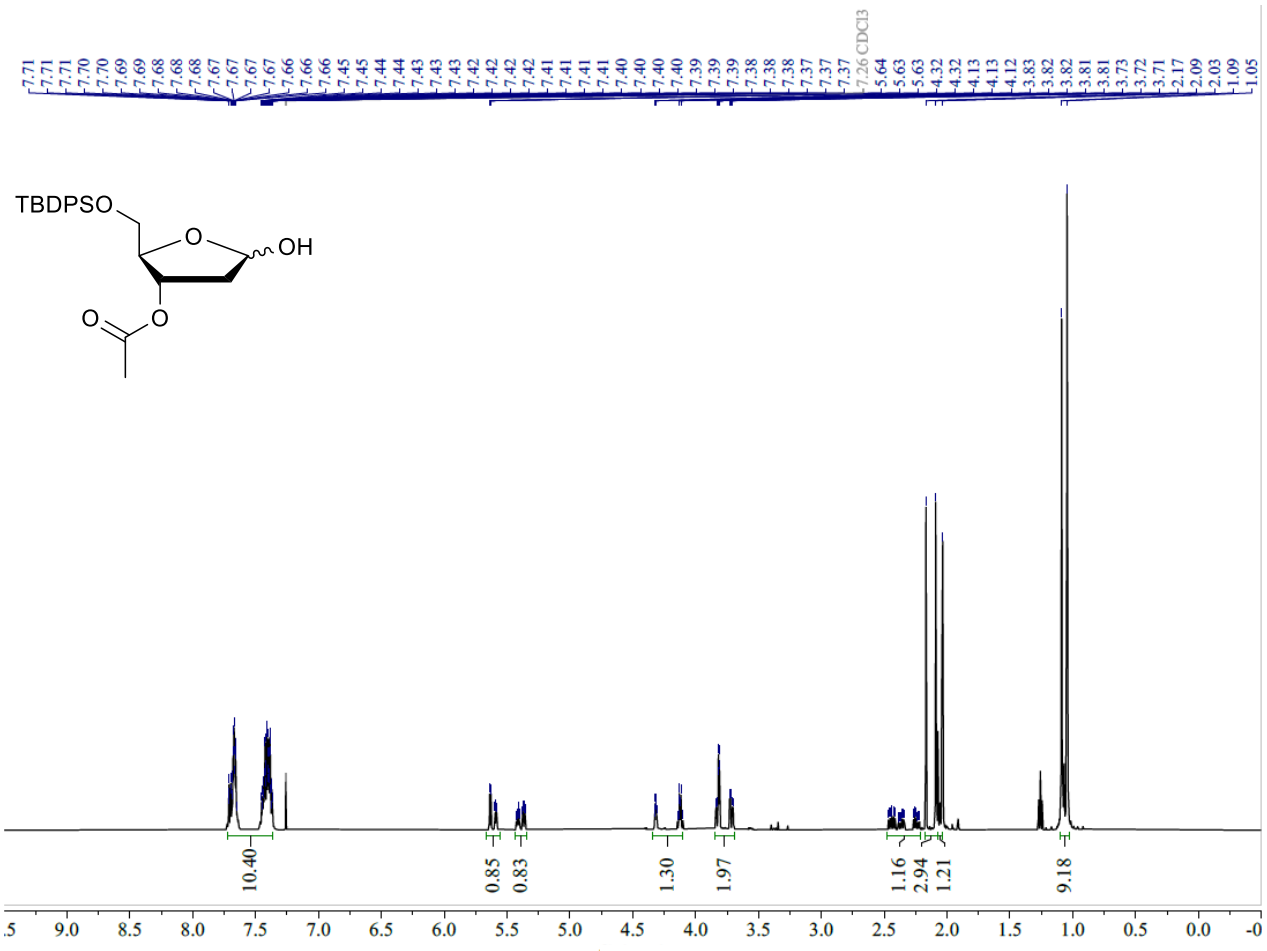
^{13}C-NMR (151MHz) of (2R,3S)-2-(2-(*tert*-butyldiphenylsilyl)ethyl)-5-(napthalen-2-ylthio) tetrahydrofuran-3-ol (3.16).



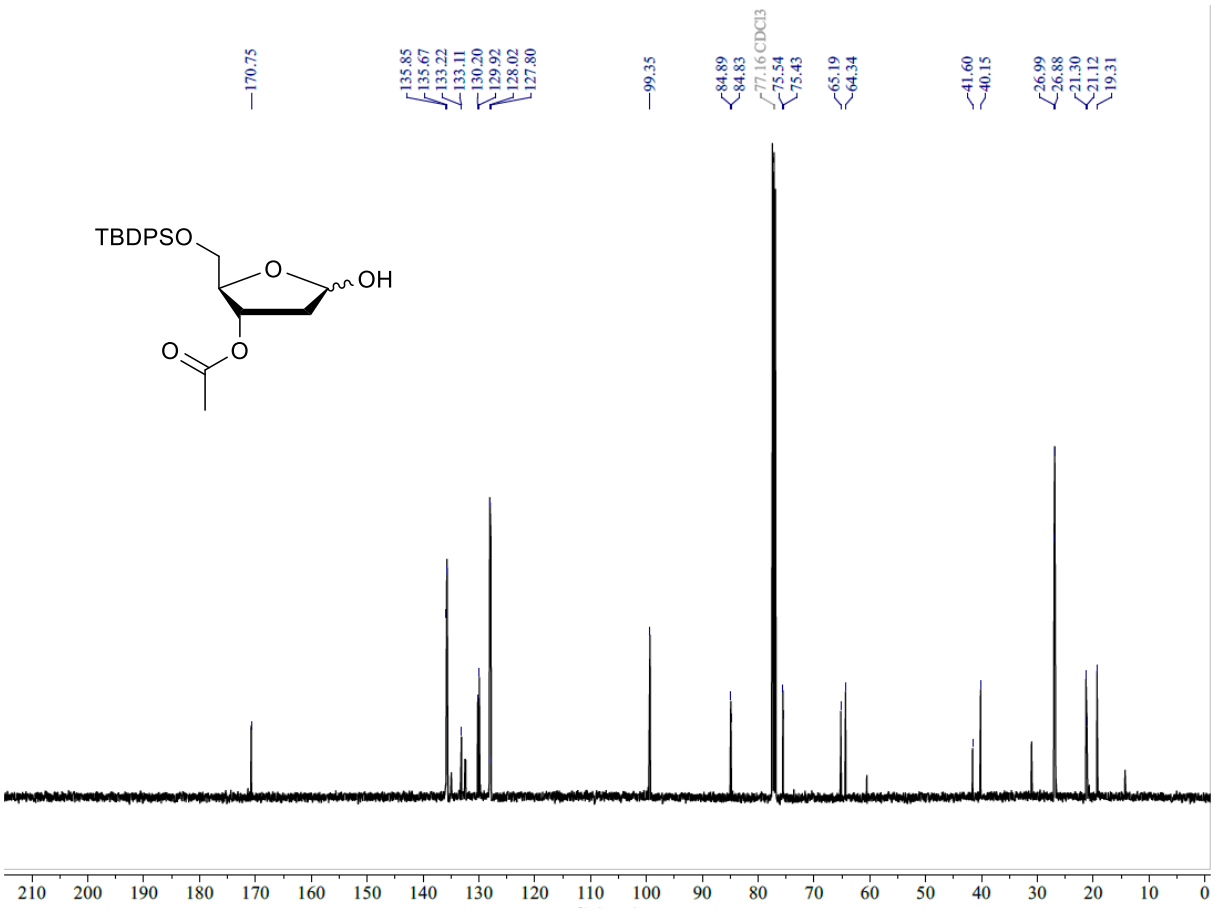
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (3.17).



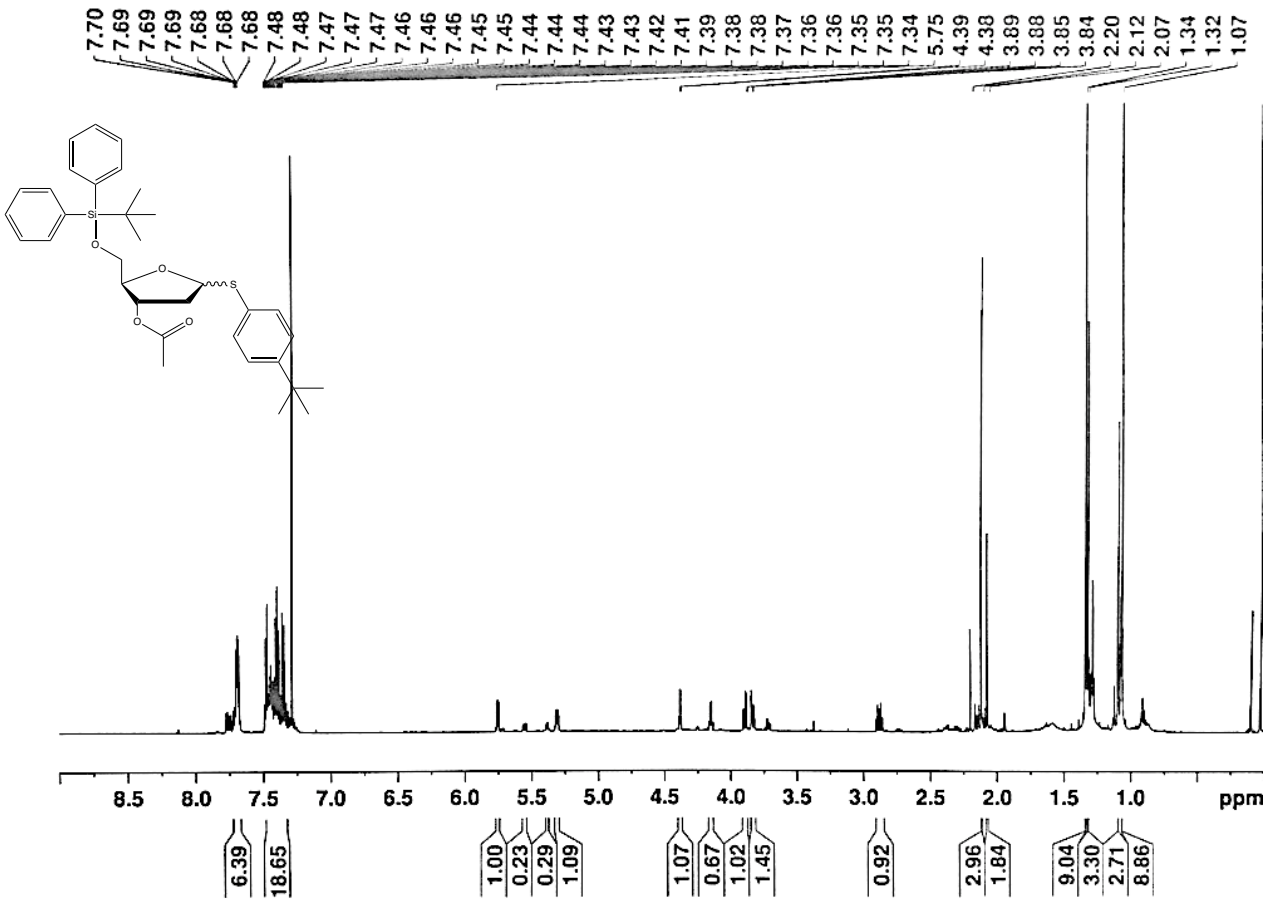
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (3.17).



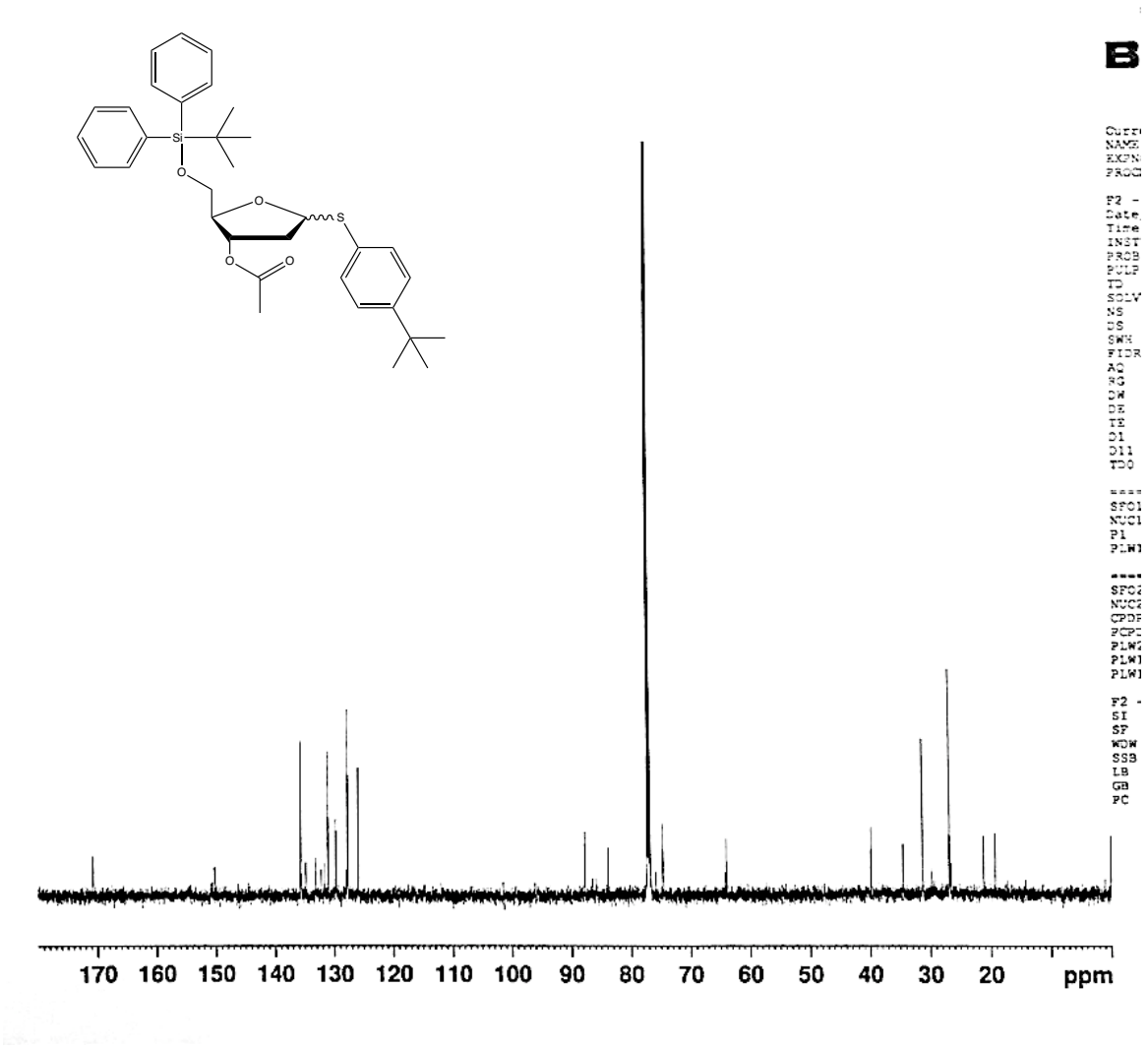
^{1}H-NMR (600 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (3.18).



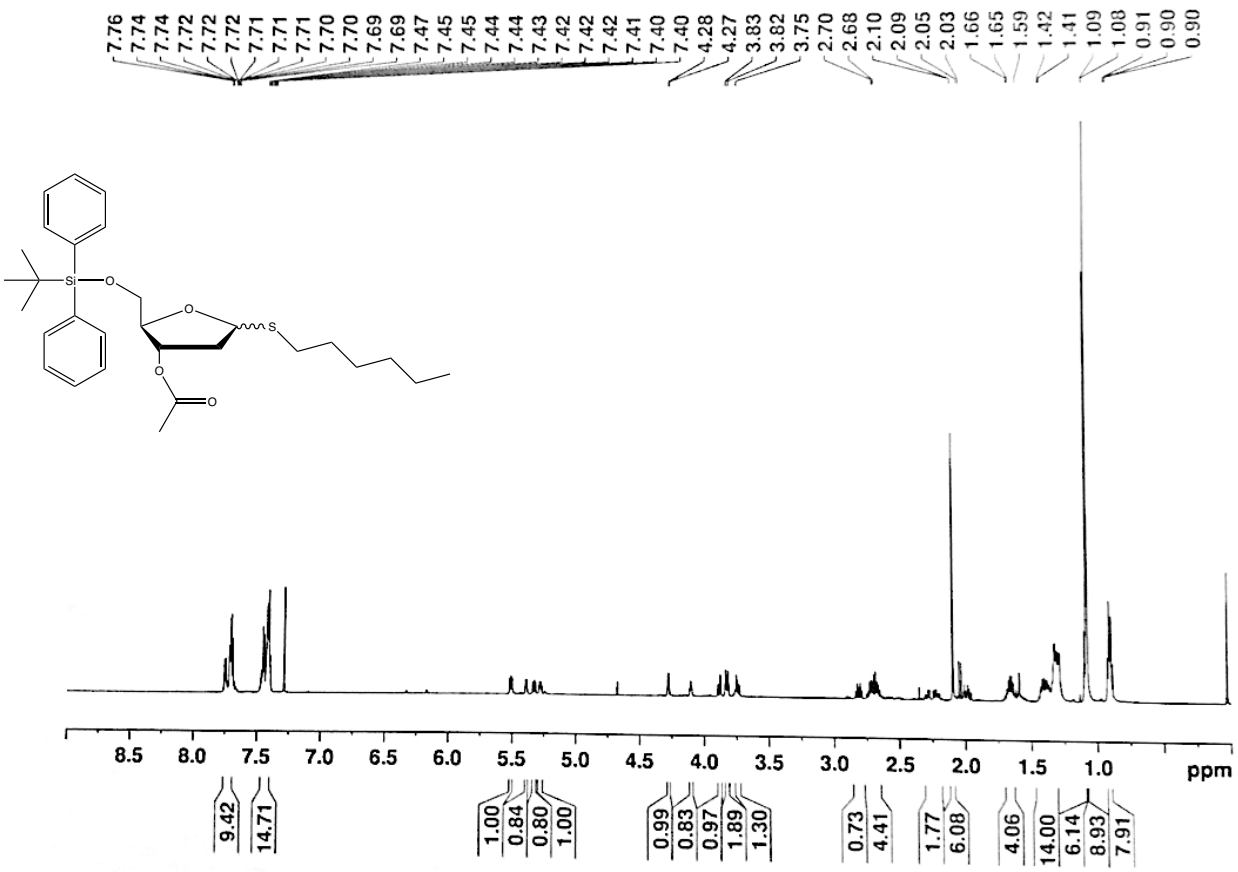
^{13}C-NMR (151 MHz) of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (3.18).



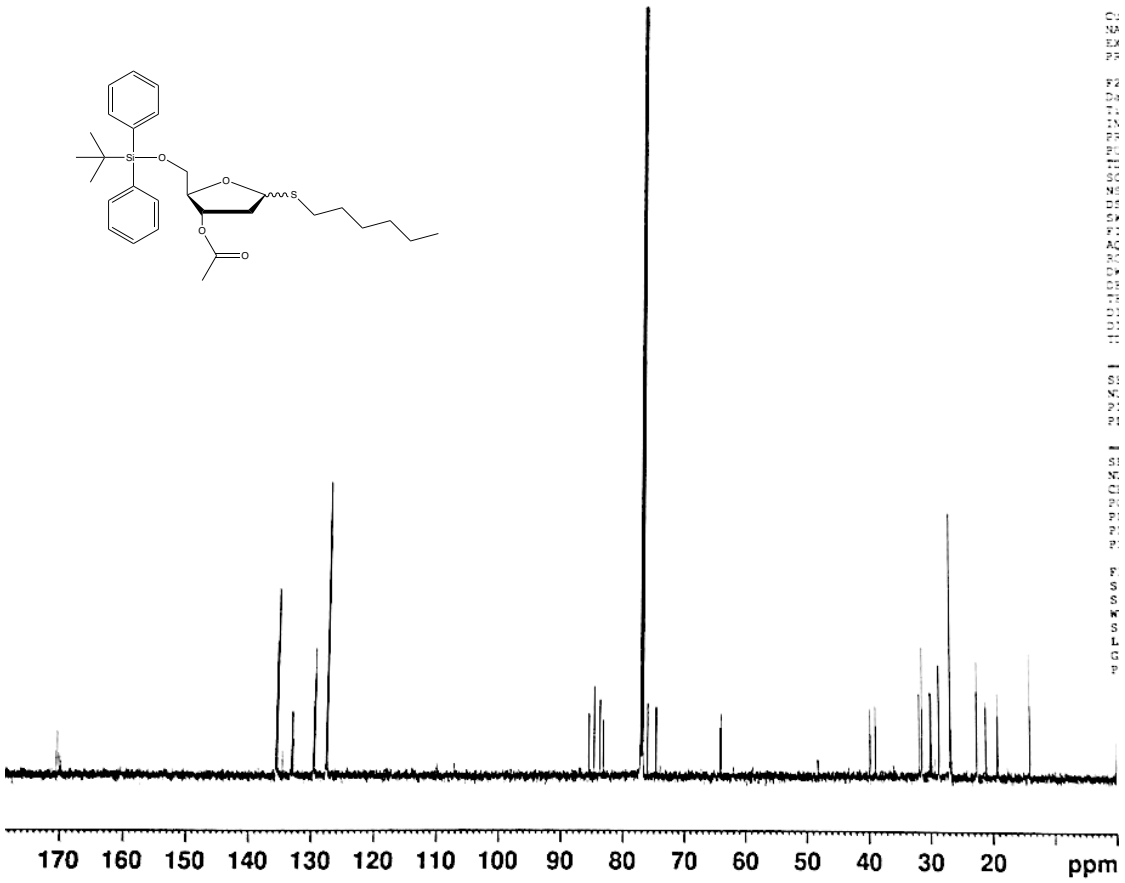
[1]H-NMR (600 MHz) of (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl) oxy)methyl)tetrahydrofuran-3-yl acetate (3.19).



^{13}C-NMR (151 MHz) of (2R,3S)-5-((4-(*tert*-butyl)phenyl)thio)-2-(((*tert*-butyldiphenylsilyl) oxy)methyl)tetrahydrofuran-3-yl acetate (3.19).



^{1}H-NMR (600 MHz) of (2R,3S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(hexylthio)tetra hydrofuran-3-yl acetate (3.20).



^{13}C-NMR (151 MHz) of (2R,3S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(hexylthio)tetra hydrofuran-3-yl acetate (3.20).

[1]Gates, K.S.; (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. Chem. Res. Toxicol. 22, 1747-1760.

[2] Nakamura, J.; Walker, V.E.; Upton, P.B.; Chiang, S.Y.; Kow, Y. W.; Swenberg, J.A.; (1998) Highly sensitive apurinic,apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. Chem. Res. 2, 222-225.

[3] De Bont, R.; van Larebeke, N.; (2004) Endogenous DNA damage in humans: review of quantitative data. Mutagenesis, 3, 169-185.

[4] Swenberg, J.A.; Lu, K; Moeller, B.C.; Gao, L.; Upton, P.B.; Nakamura, J.; Starr, T.B; (2011) Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology, and risk assessment. Toxicol Sci. 1, 130-145.

[5] Imani-Nejad, M.; Price, N.E.; Haldar, T.; Lewis, C.; Wang, Y.; Gates, K.S.; (2019) Interstrand DNA cross-links derived from reaction of a 2-aminopurine residue with an abasic site. ACS Chem. Biol. 7, 1481-1489.

[6] Gamboa Varela, J; Gates, K.S; (2015) A simple high-yielding synthesis of DNA duplexes containing a covalent, thermally cleavable interstrand cross-link at a defined location. Ange. Chem., 26, 7666-7669.

[7] Johnson, K.M.; Price, N.E.; Wang, J.; Fekry, M.I.; Dutta, S.; Seiner, D.; Wang, Y.; Gates, K.S. (2013) On the formation and properties of interstrand DNA-DNA cross-links forged by reaction of an abasic site with the opposing guanine residue of 5'-Cap sequences in duplex DNA. J Am Chem Soc 3, 1015-1025

[8] Price. N.E.; Johnson, K.M.; Wang, J.; Fekry, M.I.; Wang, Y.; Gates, K.S. (2014) Interstrand DNA-DNA cross-link formation between adenine residues and abasic sites in duplex DNA. J Am Chem Soc J Am Chem Soc 136, 3483-3490.

[9] Bayley, C.R.; Brammer, K.W.; Jones, A.S.; (1961) The nucleotide sequence in deoxyribonucleic acids. Part V. The alkaline degradation of apurinic acids. Journ Chem Soc 1, 1903-1907.

[10] Lindahl, T.; Andersson, A.; (1972) Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. Biochemistry, 19, 3618-1623.

[11] Crine, P.; Verly, W.G.; (1976) A study of DNA spontaneous degradation. Biochim Biophys Acta 1, 50-57.

[12] Yang, Z.; Nejad, M.I.; Gamboa Verla, J.; Price, N.E.; Wang, Y.; Gates, K.S. (2017) A role for the base excision repair enzyme NEIL3 in replication-dependent repair of interstrand DNA cross-links derived from psoralen and abasic sites. DNA Repair, 52, 1-11.

[13] Liu M, et al. (2010) The mouse ortholog of NEIL3 is a functional DNA glycosylase in vitro and in vivo. Proc Natl Acad Sci U S A. (11):4925–4930

[14] Liu M, Bandaru V, Holmes A, Averill AM, Cannan W, Wallace SS. (2012) Expression and purification of active mouse and human NEIL3 proteins. Protein Expr Purif. 1:130–139.

[15] Dianov, G.L.; Thybo, T.; Dianova, I.I.; Lipinski, L.J.; Bohr, V.A.; (2000) Single Nucleotide Patch Base Excision Repair Is the Major Pathway for Removal of Thymine Glycol from DNA in Human Cell Extracts. J Biol Chem, 275, 11809-11813.

[16] Fromme, J.C.; Verdine, G.L.; (2003) Structure of a trapped endonuclease III-DNA covalent intermediate. EMBO J, 13, 3461-3471.

[17] Xing, D.; Dorr, R.; Cunningham, R.P.; Scholes, C.P.; (1995) Endonuclease III Interactions with DNA Substrates. 2. The DNA Repair Enzyme Endonuclease III Binds Differently to Intact DNA and to Apyrimidinic/Apurinic DNA Substrates as Shown by Tryptophan Fluorescence Quenching. Biochemistry, 8, 2537-2544.

[18] Mundle, S.T.; Delaney, J.C.; Essignmann, J.M.; Strauss, P.R.; (2010) Enzymatic mechanism of human apurinic/apyrimidinic endonuclease againt against a THF AP site model substrate. Biochemistry, 48, 19-26.

[19] Whitaker, A.M.; Flynn, T.S.; Freudenthal, B.D. (2018) Molecular snapshots of APE1 proofreading mismatches and removing DNA damage. Nature Communications, 9, 1-11.

[20] Raspaud, E.; Chaperon, I.; Leforestier, A.; Livolant, F.; (1999) Spermine-induced aggregation of DNA, nucleosome, and chromatin. Biophys J. 3, 1547-1555.

[21] Wallace, H.M.; Fraser, A.V.; Hughes, A.; (2003) A perspective of polyamine metabolism. Biochem J. 376, 1-14.

[22] Mohni, K.N.; Wessel, S.R.; Zhao, R.; Wojciechowski, A.C.; Luzwick, J.W; Layden, H.; Eichman, B.F.; Thompson, P.S.; Mehta, K.P.M.; Cortz, D.; (2019) HMCES maintains genome integrity by shielding abasic sites in single-strand DNA. Cell, 176, 144-153.

[23] Shukla, V.; Halabelian, L.; Balagere, S.; Samaniego-Castruita, D.; Feldman, D.E.; Arrowsmith, C.H.; Rao, A.; Aravind, L.; (2020) HMCES functions in the alternative end-joining pathway of the DNA DSB repair during class switch recombination in B cells. Mol Cell. 2, 384-394.

[24] Halabelian, l; Ravichandran, M.; Li, Y.; Zeng, H.; Rao, A.; Aravind, L.; Arrowsmith, C.H.; (2019) Structural basis of HMCES interactions with abasic DNA and multivalent substrate recognition. Nat Struct Mol Biol, 26, 607-612.

[25] Maher, P.; (2005) The effects of stress and aging on glutathione metabolism. Ageing Research Reviews, 2, 288– 314.

[26] Halliewell, B.; Gutteridge, J.M.C.; (1989) Free Radicals in Biology and Medicine, Clarendon Press, Oxford, UK.

[27] Voelkel, W.; Alvaerz-Sanchez, R.; Weick, I.; Mally, A.; Dekant, W.; Paehler, A.; (2005) Glutathione conjugates of 4-hydroxy-2(E) – nonenal as biomarkers of hepatic oxidative stress-induced lipid peroxidation in rats. Free Radic Biol Med, 11, 1526-1536.

[28] Meister, A.; (1983) Selective modification of glutathione metabolism. Science, 220, 472-277.

[29] Kim, Y.J.; Kim, D.; Illuzzi, J.L.; Delaplane S.; Su, D.; Bernier, M.; Gross, M.L.; Georgiadis, M.M.; Wilson, D.M.; (2011) S-glutathionylation of cysteine-99 in the APE1 protein impairs abasic endonuclease activity. J Mol Biol. 3, 313-326.

[30] Morland I; Rolseth V; Luna L; Rognes T; Bjørås M; Seeberg E; Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. Nucleic Acids Res. 2002;30(22):4926–4936.

[31] Coste, F.; Ober, M.; Bihan, Y.L.; Izquierdo, M.A.; Hervouet, N.; Mueller, H.; Carell, T.; Castaing, B.; (2008) Bacterial Base Excision Repair Enzyme Fpg Recognizes Bulky N7-Substituted-FapydG Lesion via Unproductive Binding Mode. Chemistry & Biology, 7, 706-717.

[32] Rosenthal, A.; Richards, C.M.; (1974) Branched-chain N-sugar nucleosides: Part III alpha and beta nucleosides of branched-chain 3-C-(cyanomethyl)-, 3-C-(2-aminoethyl)-, and 3-C-(N,N,dimethylcarbmoylmethyl)-2,3,dideoxy-D-ribo-hexapyranoses. Carb Res, 32, 67-77.

[33] Lagerwall, T.; Heinonen, P.; Oivanen, M.; (2003) Temperature controlled stereoselectivity in the synthesis of 5-halo-2'-deoxyuridine derivatives. Tet Lett 43, 5950-5953.

[34] Wang, P.; Mo, Y.; Cui, X.; Ding, X.; Zhang, X.; Li, Z.; (2020) Hydrogen-bond-mediated aglycone delivery: synthesis of b-d-fructofuranosides. Organic Letters, ahead of print.

[35] Zhu, M.; Alami, M.; Messaoudi, S.; (2020) Electrochemical nickel-catalyze dMigita cross-coupling of 1-thiosugars with aryl, alkenyl and alkynyl bromides. Chem Comm, ahead of print.

[36] Herrera-Gonzalez, I.; Sanchez-Fernandez, E.; Sau,A.; Nativi, C.; Garcia Fernanddez, J.; Galan, M.; Ortiz Mellet, C.; (2020) J Org Chem, 85, 5038-5047.

[37] Abu-Zaiedd, M.; Loutfy, S.; Hassan, A.; Elgemeie (2019) Novel purine thioglycoside analogs: synthesis, nanoformulation and biological evaluation in *in vitro* human liver and breast cancer models. Drug Design, Devel. And Therapy, 13, 2437-2457.

[38] Tolan, H.; El-Sayed, W.; Tawfek, N.; Abdel-Megeid, F.; Kutkat, O.; (2019) Synthesis and anti-H5N1 virus activity of triazole- and oxadiazole-pyrimidine hybrids and their nucleoside analogs. Nucleosides, nucleotides, and nucleic acids. Ahead of print.

[39] Lee, R.; Lee, Y.; (1980) Preparation and some biochemical properties of neoglycoproteins produced by reductive amination of thioglycosides containing an w-aldehydoaglycon. Biochemistry, 19, 156-163.

[40] Doyle, L.; Meany, F.; Murphy, P.; (2019) Lewis acid promoted anomerization of alkyl O- and S-xylo-,arabino- and fucupyranosides. Carbohydrate Res. 471, 85-94.

[41] Salmasan, R.; Manabe, Y.; Kitawaki, Y.; Chang, T-C.; Fukase, K.; (2014) Efficient glycosylation using In(OTf)3 as a lewis acid: activation of n-phenyltrifluoroacetimidate or thioglycosides with halogenated reagents or PhIO. Chem Letters, 43, 956-958.

[42] Xiong, D-C.; Gao, C.; Li, W.; Wang, Y.; Li, Q.; Y, X-S.; (2014) Synthesis of 2-deoxy-C-glycosides via Lewis acid-mediated rearrangement of 2,3-anhydro-1-thiopyranosides. Organic Chemistry Frontiers, 7, 798-806.

[43] Yang, K.; Prasse, C.; Greenberg, M.; (2019) Effect of histone lysine methylation on DNA lesion reactivity in nucleosome core particles. Chem Res Toxicol (32) 910-916.

[44] Stingele, J. et al. Mechanism and regulation of DNA-protein crosslink repair by the DNA-dependent metalloprotease SPRTN. (2016). Mol. Cell 64, 688–703.

[45] Vaz, B. et al. Metalloprotease SPRTN/DVC1 orchestrates replication-coupled DNA-protein crosslink repair. (2016) Mol. Cell 64, 704–719.

[46] Lopez-Mosqueda, J. et al. (2016) SPRTN is a mammalian DNA-binding metalloprotease that resolves DNA-protein crosslinks. eLife 5, e21491.

[47] Pommier, Y. et al. (2014) Tyrosyl-DNA-phosphodiesterases (TDP1 and TDP2). DNA Repair (Amst.) 19, 114–129.

[48] Hudson, J. J., Chiang, S. C., Wells, O. S., Rookyard, C. & El-Khamisy, S. F. (2012) SUMO modification of the neuroprotective protein TDP1 facilitates chromosomal single-strand break repair. Nat. Commun. 3, 733.

[49] Hartsuiker, E. et al.(2009) Ctp1^{CtIP} and Rad32^{Mre11} nuclease 73. activity are required for Rec12^{Spo11} removal, but Rec12^{Spo11} removal is dispensable for other MRN-dependent meiotic functions. Mol. Cell. Biol. 29, 74. 1671–1681.

[50] Hartsuiker, E., Neale, M. J. & Carr, A. M. (2009) Distinct requirements for the Rad32^{Mre11} nuclease and Ctp1^{CtIP} in the removal of covalently bound topoisomerase I 75. and II from DNA. Mol. Cell 33, 117–123.

[51] Stingele, J.; Bellelli, R.; Boulton, S.; (2017) Mechanisms of DNA-protein crosslink repair. Nat Reviews Molec Cell Biol 18, 563-573.

Chapter Four: Conclusion and directionality of research

4.1 Introduction

The previous chapters of this thesis have involved projects which are connected by the fact that Ap sites within DNA are a source of biologically important electrophile. There is a need to develop models, either through small molecules or with known-sequenced oligonucleotides, to understand the fundamental chemistry of an Ap site. A firm understanding of the Ap site, from a chemical perspective, will help generate a link between the arrow-pushing mechanisms of the electrophile to the profound biological consequences which stem from it. This chapter will serve to discuss future directionality of each of these projects.

4.2 The dRP end group has the potential to generate adducts with glutathione or generate interstrand cross-links with exocyclic amines

Chapter Two shows the reaction between an Ap site and a polyamine gives rise to a highly charged unsaturated alkenal (Scheme 2.2.1). This bis-electrophile was shown to react with thiols, as well as with water to regenerate a deoxyribose structure. While this chapter was somewhat comprehensive regarding conditions required to generate the dRP group, there is still much to be learned about the chemistry surrounding it.

One key reaction which must still be discussed is the reaction between glutathione, the most biologically abundant thiol, and the dRP end group. There is literature precedent that glutathione is able to react with the unsaturated alkenal HNE (Figure 4.2.1).[1] The structure formed between HNE and glutathione has a stark similarity to those shown in Chapter Two.

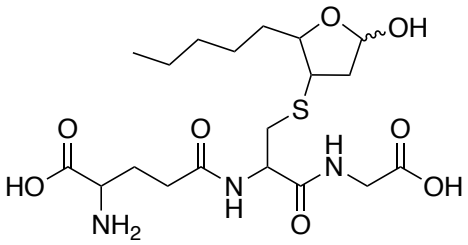


Figure 4.2.1. Structure of HNE-glutathione adduct

Due to this, there is a potential that the dRP end group has the ability to generate an adduct with glutathione. The reaction between the *trans*-alkenal **2.15** and excess glutathione did show the proposed adduct is present (Figure 4.2.2), but there still needs to be studies showing the structure and stability of the product.

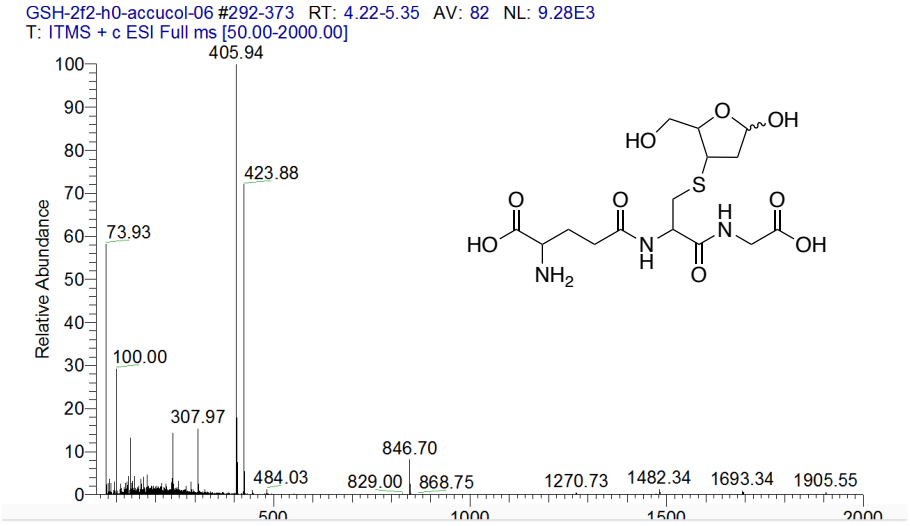


Figure 4.2.2. Reaction of 2.15 and glutathione showing the generation of the proposed adduct. M/z of proposed adduct is 423.

Beyond the reactivity with thiols, there is also a need to understand the dRP's ability to generate an interstrand cross-link with an exocyclic amine of a nucleoside. This reaction has been shown with oligonucleotides[2] however a rigid assignment of structure

has not been generated. Unpublished work by Deepak Ahire utilized **2.9** and generated the unsaturated iminium ion using conditions described in Chapter Two in the presence of 2'-deoxyguanosine. The product of this reaction shows a conjugate addition by the exocyclic amine of guanine followed by a ring-closing with the endocyclic amine. This structure is similar to that shown with HNE and guanosine (Figure 4.2.3).[3] Current efforts in the Gates group have been taken to make standards of the dRP-nucleoside adducts. Future work into the structure, generation, and repairability of these adducts are needed.

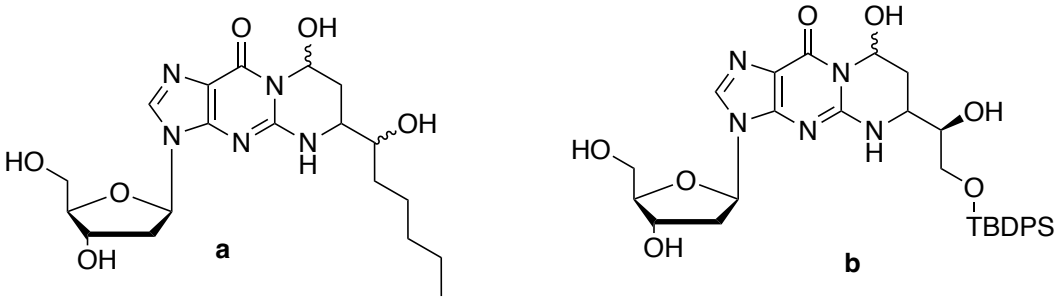


Figure 4.2.3. Structures of guanosine reaction with unsaturated alkenals. a) shows the reaction with HNE while b) shows the reaction with 2.9.

4.3 Thioglycosides generate a stable adduct when reacting with an Ap site and may play a role in medicinal chemistry

Chapter Three shows the ability of a thiol to react with an Ap site. This work was done in light of recent research done with HMCES. While Chapter Three gives insight into reactions between thiols and Ap sites, there is still research needed to determine the structure of an Ap site with a biological relevant thiol, such as glutathione. Research efforts within the group have shown that an Ap site in an oligonucleotide is able to react with glutathione, and be stable under various conditions. While this research suggests

these reactions are possible, it has yet to be detected within cells. To assist these efforts, a synthetic standard of glutathione and 2-D-deoxyribose must be generated. This will allow the detection and quantification of the adduct by mass spectrometry in cell extracts. A more comprehensive study into the repairability of these adducts is also required.

If thioglycosides, as a class of molecules, are able to withstand degradation by APE1, they may pose as interesting biologically active compounds. Methoxyamine is currently being tested as an APE1 inhibitor.[5] If thioglycosides are able to also inhibit APE1, they could perhaps be utilized as lead compounds for future generations of bioactive molecules.

4.4 Conclusions

Ap sites represent an abundant, yet not well understood, electrophilic lesion in DNA. If these lesions are not repaired, there are various pathways which become available. I also have the ability to degrade the Ap site to a highly active alkenal, and Chapter Two goes into some of the chemistry which stems from the generation of that product. I also have the ability to protect the Ap site with a reversible nucleophile. Chapter Three goes into some detail about a newfound type of chemistry involving an Ap site's ability to react with a thiol.

The research performed in this thesis has many intricacies, but it is all founded on the principle that Ap sites are electrophiles. It is my belief that I can use simple organic reactions to understand the complex reactions of the Ap site. There is beauty in that simplicity.

"How lucky I am to have something that makes saying goodbye so hard." – A.A. Milne

---

[1] Singhal, S.S.; Singh, S.P.; Singhal, P.; Horne, D.; Singhal, J.; Awasthi, S. (2015) Antioxidant role of glutathione S-transferases: 4-Hydroxynonenal, a key molecule in stress-mediated signaling. Toxicol. Appl. Pharmacol. 289, 361–370

[2] Yang, Z.; Price, N.E.; Johnson, K.M.; Wang, Y.; Gates, K.S.; (2017) Interstrand cross-links arising from strand breaks at true abasic sites in duplex DNA. Nucleic Acids Res. 45, 6275-6283.

[3] Chung, F.L.; Nath, R.G.; Nath, J.; Nishikawa, O.A.; Zhang, L.; (2000) Deoxyguanosine adducts of t-4-hydroxynonenal are endogenous DNA lesions in rodents and humans: detection and potential sources. Cancer Res, 60, 1507-1511.

VITA

Christopher A. Nel was born in Johannesburg, South Africa. He and his family immigrated to the United States in 1999, where they settled in the suburbs of Kansas City. He earned a bachelor's degree in chemistry from the University of Missouri-Columbia, during which he did undergraduate research in an inorganic chemistry lab. He gained a fascination with medicinal chemistry during this time. He joined the lab of Dr. Gates as a graduate student in 2016. As a graduate student, he gained knowledge of various organic techniques, including synthesis, NMR, analytical chromatography, and various other analytical techniques applying them toward investigating the reactions of abasic sites in DNA. He also had the ability to teach and mentor various undergraduates from freshmen chemistry to a senior level lab course. He obtained his Ph.D. in 2020, after which he took a position as a postdoctoral researcher for the drug company NUBAD, LLC in Greenville, SC.