Synthesis of Cyclo, Ring Expanded, and Backbone Extended Nucleosides

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SYNTHESIS OF CYCLO, RING EXPANDED, AND BACKBONE EXTENDED NUCLEOSIDES

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by

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Abstract

SYNTHESIS OF CYCLO, RING EXPANDED,
AND BACKBONE EXTENDED NUCLEOSIDES

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Under the guidance of Larry W. McLaughlin

Nucleic acids are responsible for maintaining the biological information responsible for the activities of all known living organisms. Research of nucleic acids provides opportunities to help understand, prevent, and cure disease in addition to allowing us to gain a greater appreciation for the wonders of nature.

This work presents the synthesis and properties of several modified nucleosides. Chapter 2 presents an improved synthesis of *R* and *S* 6,5'-cyclouridine, which are rigidified nucleosides locked in the *anti* conformation. This work helps to understand the properties of these interesting molecules and will allow scientists to synthesize large quantities of these monomers for future research.

Chapter 3 presents the synthesis of novel 6,6'-(*S*)-cyclo-2'-deoxyuridine. This work is highlighted by a zinc mediated cyclization to form a seven-membered ring; the first published reaction of its kind. The compound itself is a mimic of thymidine that also has the base locked in the *anti* position.

Lastly, Chapter 4 presents work on 6' extended backbone nucleosides. These molecules have the potential to form a new type of helical structure and will help us to gain a greater understanding of the properties and dynamics that contribute to duplex stability in DNA.

For my family, friends, and Akiko

Thank you for your support and love

Acknowledgements

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To my sister Lindsay, you are an amazing person and a wonderful role model. Thank you for your love, support, and advice through the years.

To my parents, without you none of what I have achieved would have been possible. You provided me with an excellent education and helped to foster my

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Lastly, I have to acknowledge my one and only, the love of my life, and my sweetie, Akiko. I truly can't wait to marry you. Your smile, companionship, and love provide inspiration for me every single day. I love you so much and look forward to our journey through life together.

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1.1

Abbreviations and Acronyms

A	adenosine
Ac	acetyl
ACN	Acetonitrile

AIBN	azobis(isobutyronitrile)
aq	aqueous
Bn	benzyl
Bz	benzoyl
Calcd	Calculated
CAN	ceric ammonium nitrate
CD	circular dichroism
d	doublet
dA	2'-deoxyadenosine
dC	2'-deoxycytidine
dG	2'-deoxyguanosine
DCM	dichloromethane/methylene chloride
DIPA	diisopropylamine
DIPEA	diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMTr	4,4'-dimethoxytrityl
DMSO	dimethylsulfoxide
DNA	β-D-2'-deoxyribonucleic acid
ether/Et_2O	diethyl ether
Et	ethyl
EtOAc	ethyl acetate

g	gram
G	guanosine
GNA	glycol nucleic acid
hrs	hour(s)
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrum
iPr	*iso*-propyl
L	liter
LNA	locked nucleic acid
m	milli
m	multiplet
M	molar
Me	methyl
min	minute(s)
NMR	nuclear magnetic resonance
PAGE	polyacrylamide gel electrophoresis
PNA	peptide nucleic acid
PPTS	pyridinium *p*-toluenesulfonate
pyr	pyridine
q	quartet
r.t.	room temperature
s	singlet
t	triplet

T	thymidine
TBAF	*n*-tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetic acid
TEA	triethylamine
THF	tetrahydrofuran
TFA	trifluoroacetic acid
TIPS	triisopropylsilyl
TLC	thin layer chromatography
Tm	thermal melting
TMS	trimethylsilyl/tetramethylsilane
TNA	threose nucleic acid
Tol	*para*-toluoyl
Ts	tosyl
RNA	β-D-ribonucleic acid
U	uridine
UV	ultraviolet
[α]	specific rotation
δ	chemical shift in parts per million
ε	extinction coefficient
μ	micro

Chapter 1

Introduction

1.1 An Introduction to Nucleic Acid Structure

Nucleic acids define all known forms of life. The biopolymer 2'-deoxyribonucleic acid (DNA) is responsible for maintaining the genetic code, holding the information essential for cellular functions and also allowing organisms to pass on their information to future generations[1, 2]. Ribonucleic acid (RNA) is largely responsible for transferring the information stored in the DNA through the processes of transcription. It then converts the information to amino acid based proteins through translation to make most of the machinery used by the cell[1]. Additionally, RNA is hypothesized to have carried out most cellular processes in early life before proteins and DNA became dominant[3]. Some vestiges of these ribozymes remain, most notably the RNA-protein structure of the ribosome, where translation of messenger RNA (mRNA) to protein takes place[4].

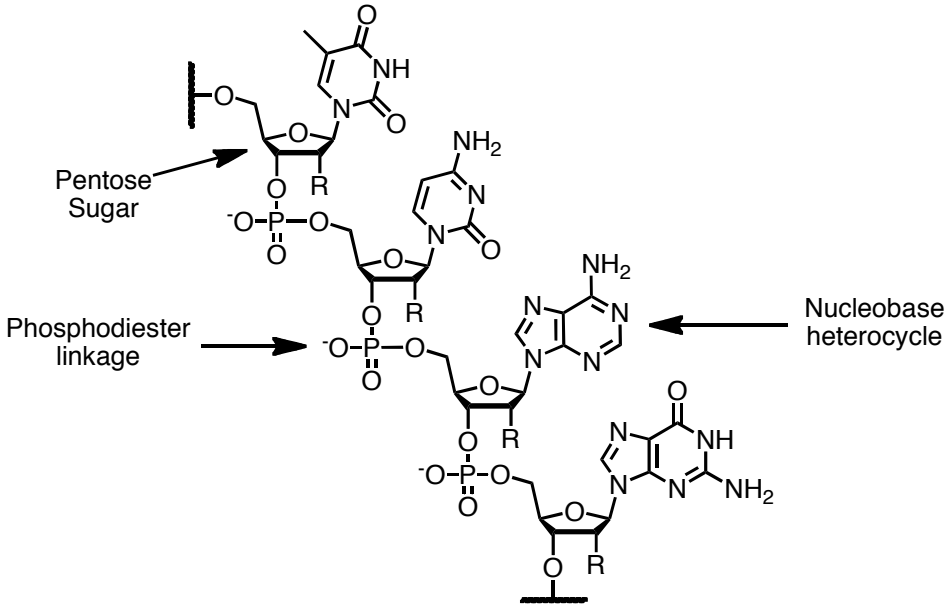

Figure 1: The three main components of DNA and RNA. In DNA R=H, while in RNA R=OH.

Nucleic acid polymers consist of three main parts (Figure 1)[5]. First are the heterocyclic bases adenine, guanine, cytosine, thymine, and uracil (Figure 2).

Adenine and guanine are purine bases, while cytosine, thymine, and uracil are pyrimidine bases. Second is a ribose-based sugar moeity. The sugar is of the same structure in DNA and RNA, except that in DNA the sugar lacks a secondary hydroxyl, hence "deoxy"ribo nucleic acid[6]. Lastly the phosphodiester backbone links each monomer together in the DNA or RNA strand. A nucleoside is a compound consisting of just the heterocycle and sugar, while a nucleotide also features a phosphate group[1, 6].

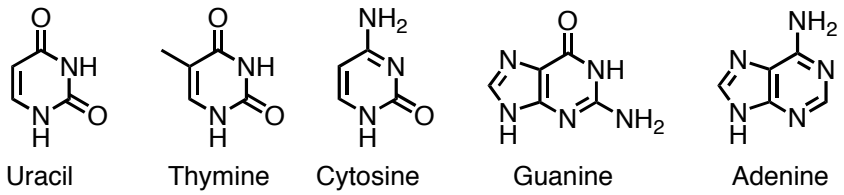

Figure 2: The five nucleobases

Before we address nucleic acid structure further, it is essential to understand the basic numbering of nucleosides. Pyrimidine bases are numbered starting at the nitrogen attached to the sugar (N1) and proceed in a counter-clockwise manner. Purine bases begin at the top-right nitrogen atom on the six-membered ring and are numbered in a clockwise pattern[1].

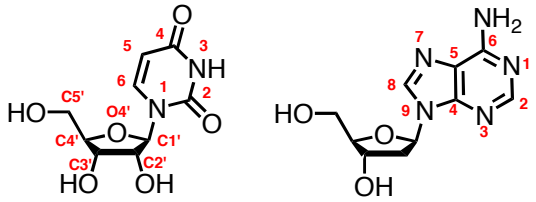

Figure 3: Pyrimidine and purine numbering illustrated on uridine (left) and 2'-deoxyadenosine (right)

The remaining three atoms on the five-membered ring are numbered sequentially with the 9 position attached to the sugar. The sugar ring positions are designated with a prime symbol. The carbon attached to the base is the C1' and numbering

proceeds as is standard for carbohydrates. The oxygen atoms are designated based on the carbon to which they are attached, such as the 5' OH.

RNA features the full ribose sugar and all of the heterocyclic bases except thymine. DNA lacks the hydroxyl in the 2' position and contains thymine instead of uracil[1, 6]. The phosphate linkages in both polymers are between the 5' and 3' hydroxyls of adjacent nucleotides. The nucleosides for the five bases are cytidine (C), thymidine (T), guanosine (G), adenosine (A), and uridine (U). To indicate a 2'-deoxynucleoside a "d" is placed before the letter (i.e. dG) although T is usually written without the "d" as it inherently lacks the 2' OH.

The base on a nucleoside or nucleotide can be positioned in either a *syn* or and *anti* conformation[6]. In the *anti* position the base points away from the sugar ring and is placed over the ring in the *syn* position upon rotation about the glycosidic bond (Figure 4). As you might expect, the *anti* conformation is dominant in nucleosides due to steric interactions caused by rotating the base into proximity with the sugar. However, bulky substituents on the 8 position of modified purines and on the 6 position of modified pyrimidines can cause the steric effect to be reversed in favor the *syn* conformation[7]. Hydration conditions, salt concentrations, and enzymatic activity can also cause the base to be rotated into the *syn* conformer[6, 8, 9].

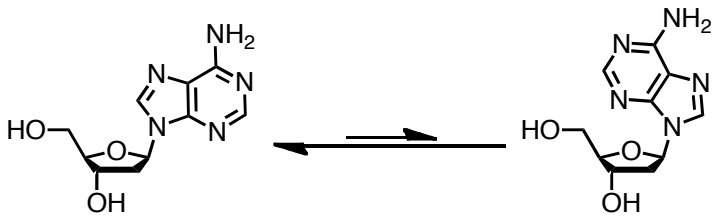

Figure 4: The *anti* (left) and *syn* (right) conformers of 2'-deoxyadenosine

The sugar portion of the ring can adopt many different conformations, but the two most important are the C2'-*endo* and the C3'-*endo* families[1]. The term *endo* defines whether the carbon atoms sit on the same side of the C4'-O4'-C1' plane as the C5' carbon. The C2'-*endo* position is dominant in DNA, while RNA prefers the C3'-*endo* position[1]. The structural implications of these conformations will be discussed shortly.

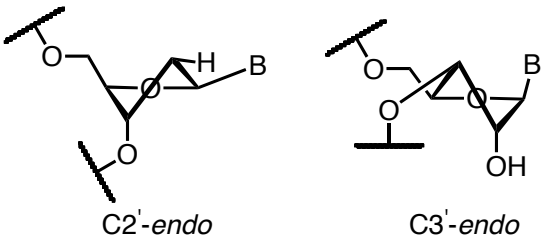

Figure 5: The dominant sugar pucker conformations. As drawn DNA prefers the C2'-*endo* pucker while RNA prefers the C3'-*endo* position.

The phosphodiester linkage between nucleotides is a critically important structural piece of a nucleic acid oligomer[10, 11]. Phosphate groups have both properties of stability and reactivity. They are not easily hydrolyzed by water or attacked by other common nucleophiles, such as chloride, found in the cytoplasm[11]. However, they can be broken apart by specialized enzymes, which help to maintain and repair DNA[12, 13]. The negatively charges at the phosphodiester sites also help to contain the oligomers inside of the cell, as they are unable to penetrate the hydrophobic cellular membrane[11].

Complementary DNA oligomers can partner together to form a double helical structure[5]. In the double helix the strands are anti-parallel, meaning one runs 5' to 3' while its complement runs 3' to 5'. The structure and stability of the helix is

determined by many factors, most important of which are base pairing, base stacking, hydration effects, and charge-charge repulsion.

Hydrogen bonding pairs between nucleobases on opposing strands are formed through specific hydrogen bonding interactions. The standard hydrogen bonding interactions are called Watson and Crick base pairing, after the scientists involved in the discovery of the structure of DNA[5]. The two canonical base pairs which can be formed are A-T and G-C. The A-T base pair features two hydrogen-bonding interactions; one is between the N6 of A and the O4 of T, while the other is between the N1 of A and the N3 of T. G-C base pairs feature three hydrogen-bonding interactions between the O6, N1, and N3 positions of G and the N4, N3, and O2 positions of C respecitvely, making them stronger base pairs than A-T[1].

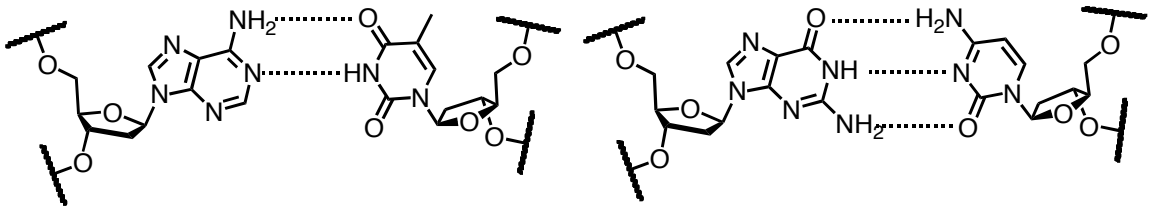

Figure 6: dA-T and dG-dC base pairs

While Watson and Crick base pairs dominate the interactions seen in DNA duplexes, other types of bonding can occur, such as Hoogsteen pairing or wobble base pairing. Hoogsteen base pairs occur when the purine base is rotated into the *syn* position of a DNA duplex[14]. They are rarely observed in duplex interactions, but play an important role in other DNA structures such as triplexes, where a third strand of nucleic acids binds the duplex[15]. They are also found in structures called G quadruplexes. These structures help to stabilize the terminus of a chromosome, which is single stranded and lacks the positive interactions found in a duplex

structure[15, 16]. Similarly, wobble pairings are seldom found in standard duplexes. However, they do play an important role in RNA structures called transfer RNA (tRNA); a critical molecule involved in the translation process of mRNA protein[17, 18]. Many wobble pairings on tRNA molecules use the base inosine, which is not found in the chromosomal DNA. A three nucleotide segment of messenger RNA (mRNA), the product of transcription, is called a codon and binds to a three nucleotide segment of tRNA called an anticodon[17, 19]. Wobble base pairs at the 5' end of an anticodon allow the tRNA to bind multiple codons[20]. For example inosine can bind C through Watson and Crick base pairing or U and A through wobble pairing[21].

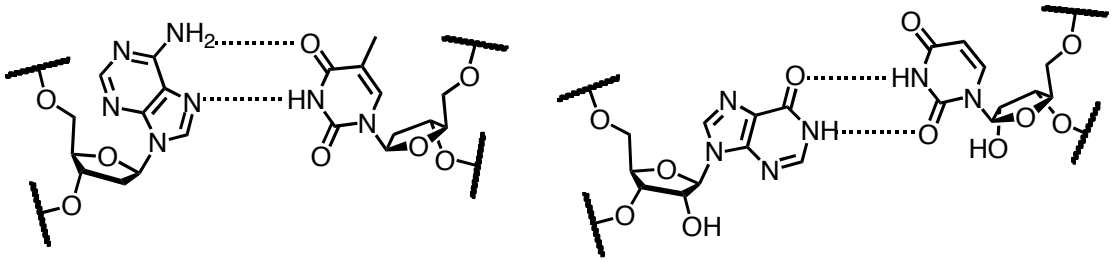

Figure 7: A dA-T Hoogsteen base pair and an I-U wobble base pair

Base stacking interactions are the dominant force holding the duplex together, and actually contribute more to stability than base pairing[22]. The dominant forces at work are London dispersion forces, hydrophobic interactions, and the π-π electrostatic forces between adjacent bases[23, 24]. The longer the polymer, the stronger the base stacking effect will be[25]. Although stacking contributes more to stability than base pairing, it should be noted that a base pair mismatch could cause disturbances in the stacking ability of the pair[26]. If the base rotates to avoid negative hydrogen bonding interactions, it could create bulges or other deformations that disrupt the π-π interactions. In order to maximize both

hydrogen bonding and base stacking interactions the nucleobases can adjust their individual three-dimensional conformation within the duplex the interactions twist, roll, tilt, and propeller twist (Figure 8)[1].

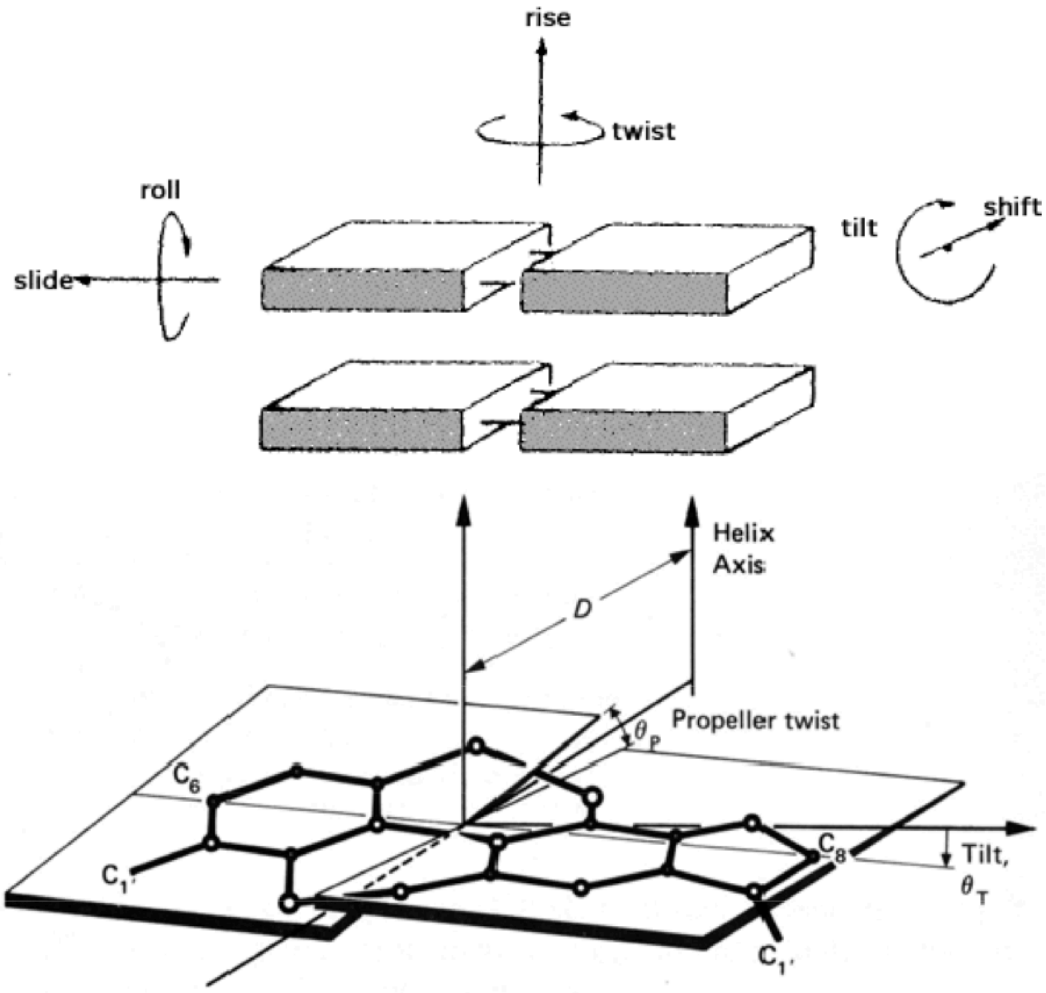

Figure 8: Two figures showing conformational adjustments to maximize duplex stabilizing interactions (Images from Saenger)[1].

Hydration energy is a central driving force governing the type of duplex that is formed. DNA homoduplexes are found in a B-form helix under physiological conditions, where each sugar is in the C2'-*endo* conformation (Figure 9)[27]. However, DNA-RNA heteroduplexes and RNA-RNA homoduplexes are found in A-form helices,

where the sugar is in the C3'-*endo* conformation. The hydration packing of the 2'-OH on RNA causes the C3'-*endo* sugar puckering to maximize entropic effects[1]. Dehydrated DNA-DNA duplexes, such as those used by Rosalind Franklin in her X-ray diffractions that led to the discovery of the structure of DNA, also form A-type helices[28].

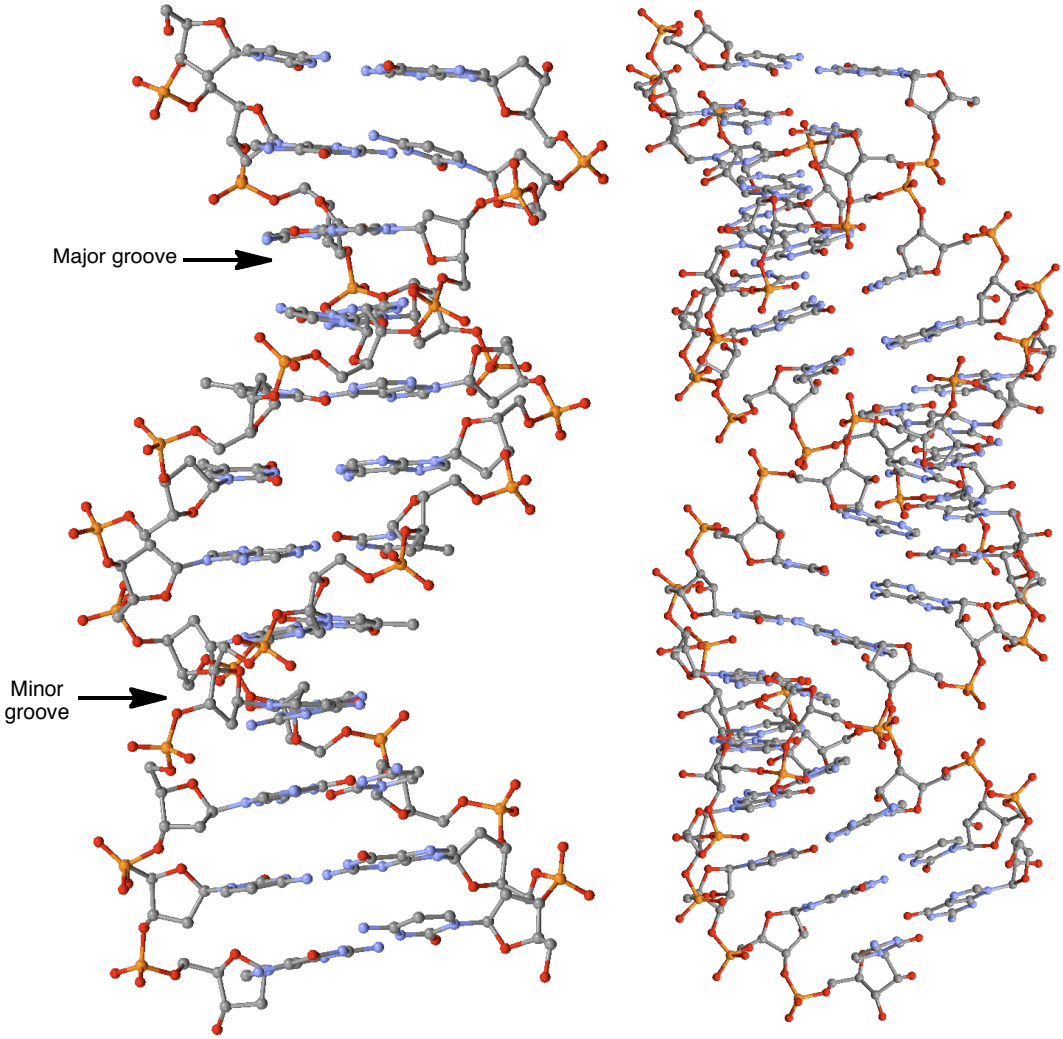

Figure 9: B-form and A-form duplex structures[27, 29]

Hydration can influence the structure of a helix in many other ways than just sugar puckering. For example it has been shown that in poly A-T segments a "spine

of hydration" occurs in the minor groove of B-form DNA, which is the narrower groove seen in Figure 9[30]. This is a highly ordered pattern of water molecules, which form hydrogen bonds between neighboring adenine and thymine base pairs at the N3 and O2 positions (Figure 10)[1, 30].

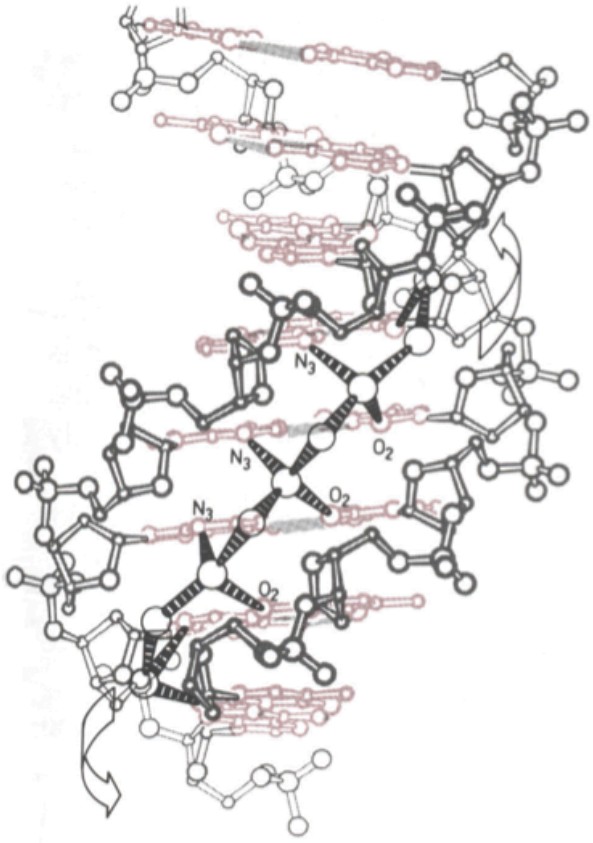

Figure 10: The spine of hydration in the minor grove of a poly A-T region (Image from Saenger)[1].

The last major feature influencing DNA structure is charge-charge repulsion. At physiological pH the phosphates along the backbone carry a negative charge. The charges must be distributed in order to avoid repulsion between adjacent phosphates. Cations such as magnesium and potassium mitigate these repulsions and increase the stability of the duplex against thermal melting[31, 32].

1.2 Nucleoside Modifications

Manipulation of nucleosides and nucleotides serves many different purposes. Modifications can be used to create pharmaceutical drugs or to create tools to enhance our understanding of the biological roles of nucleic acids. Changes can be made to the nucleobase, the sugar, the phosphate backbone, or combinations of any of the above.

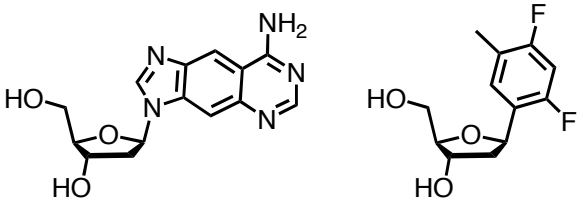

Figure 11: Expanded and non-hydrogen bonding nucleosides by Kool et al.[33, 34].

Base modifications have the potential to enhance stabilizing interactions in oligonucleotide strands. For example, expanded nucleobases feature the addition of phenyl rings or other π conjugated systems. This can help to enhance base stacking interactions between adjacent base pairs (Figure 11)[34]. Even non-expanded systems lacking hydrogen bonding faces can be incorporated into duplexes, helping to prove that base stacking is a primary driving force of helix formation[22, 33].

Hydrogen bonding interactions can be manipulated in modified nucleobases. 2,6-diaminopurine is used by cyanophage S-2L in living systems and is utilized by scientists to increase binding affinity with T[35]. Other non traditional heterocycles can also form hydrogen bonding interactions (Figure 12)[36].

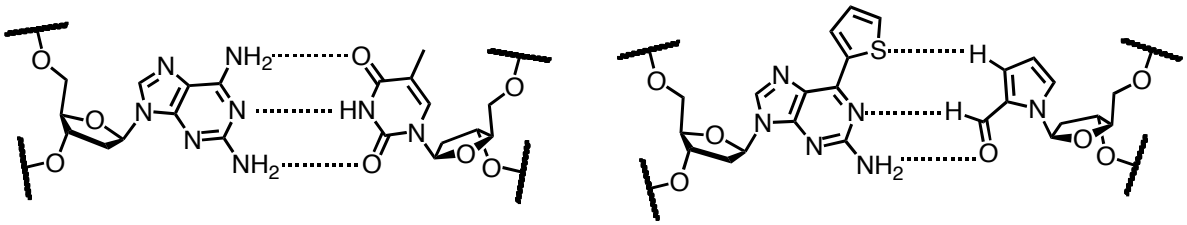

Figure 12: 2,6-diaminopurine partnering with thymine and 2-amino-6-(2-thienyl)purine pairing with pyrrole-2-carbaldehyde.

Modifications to the (deoxy)ribose can be facilitated by changing the size of the ring, the sugar's stereochemistry, or by replacing native atoms with non-traditional heteroatoms. Hexose sugars, as seen in Figure 13, can inhibit enzyme machinery due to the increased ring size. However, they are not as stable in duplex formation, helping to answer the question of why pentose sugars evolved in native oligonucleotides[37-39]. One or all of the stereocenters on the sugar can be inverted, for example the enantiomeric L-deoxyribose nucleosides[40]. Lastly, the atoms on the sugar itself can be altered, with substitutions such as sulfur or carbon in the O4' position. This can affect properties such as cytotoxicity and C1' anomerization[41, 42].

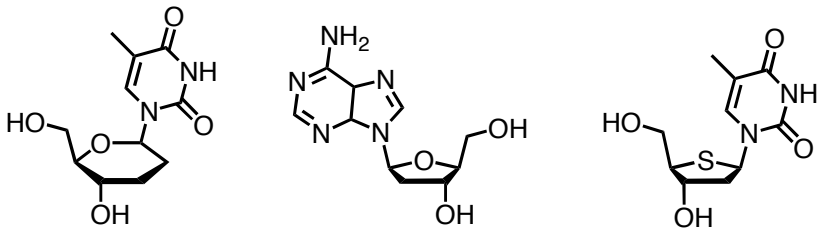

Figure 13: A hexose thymidine, L-2'-deoxyadenosine, and 4'-thionucleoside illustrating various sugar modifications.

Backbone modifications can include alteration of the phosphodiester linkage or the creation of an entirely different backbone. Phosphate modifications have largely revolved around sulfur or selenium substitution in place of the oxygen atoms, although other substitutions can be made[43, 44]. A phosphorothiolate linkage slows degradation and creates a distinct chiral center, which allows for study of

stereochemically specific interactions along the backbone[43]. A heavy oxygen isotope substitution also permits stereospecific monitoring[45].

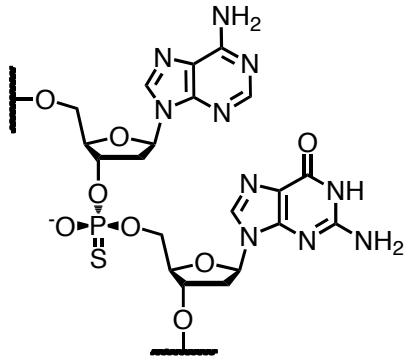

Figure 14: An *S* stereospecific phosphorothiolate coupling

The most famous example of a completely modified backbone system is that of peptide nucleic acids, or PNA[46, 47]. The name is a misnomer, as the backbone does not contain any phosphodiester linkages, rather a pseudo-peptide linkage of "nucleosides". The backbone consists of repeating N-(2-aminoethyl)-glycine units, with the bases attached by a carbonyl methylene linker. This number of atoms per repeating unit maintains the spacing observed in native DNA and RNA polymers.

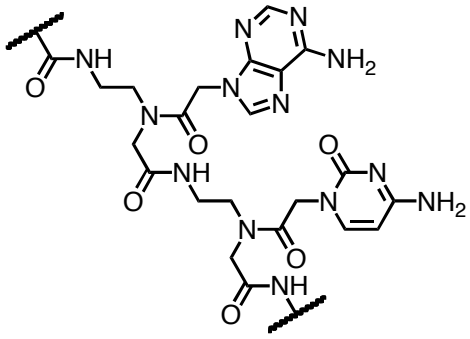

Figure 15: Peptide nucleic acid

PNA lacks negative charges along its backbone, eliminating charge-charge repulsion effects seen in DNA and RNA. PNA is known to form duplexes with itself as well as DNA and RNA strands[48, 49]. The lack of phosphate groups causes the PNA

to be more hydrophobic, but solubility in aqueous solutions can be improved by addition of polar substituents, such as lysine, at the α-position[50].

1.3 Introduction to Chapters 2, 3, and 4

By studying nucleic acid structure, we are not only able to develop pharmaceutical drugs to treat illness, but also gain a greater appreciation for the biology involved in making life possible. This thesis presents syntheses of several modified nucleosides. Chapter 2 presents an improved synthesis of 5,6'-cyclouridine, a rigid monomer locked in the *anti* conformation. Chapter 3 demonstrates the synthesis and properties of ring expanded 6,6'-cyclo-2'-deoxyuridine, a novel compound whose structure mimics that of thymidine. Lastly, Chapter 4 presents work on 6' backbone extended thymidine and 2'-deoxyadenosine. These molecules may allow access to a novel helical structure and will allow us to probe various interactions within oligomers strands.

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Chapter 2

Synthesis of 6,5'-(*S*)- and 6,5'-(*R*)–Cyclouridine

2.1.1 Introduction to Rigidified and Cyclonucleosides

Rigidified nucleic acids feature an additional linkage or bridge on the sugar or between the sugar and the nucleobase. Arguably the most famous examples of rigid nucleosides are those found in locked nucleic acids (LNA). The U and C monomers were synthesized by the Imanishi group in Japan and independently synthesized by the Wengel group in Denmark along with A,T,G, and 5-methylcytosine[1, 2]. These compounds incorporate a methylene group between the O2' and C4' positions "underneath" the pentose ring (Figure 1). The bridge "locks" the sugar into the C3'-*endo* pucker conformation. This reduces any entropic costs associated with the sugar flipping between the C2'-*endo* and C3'-*endo* conformations.

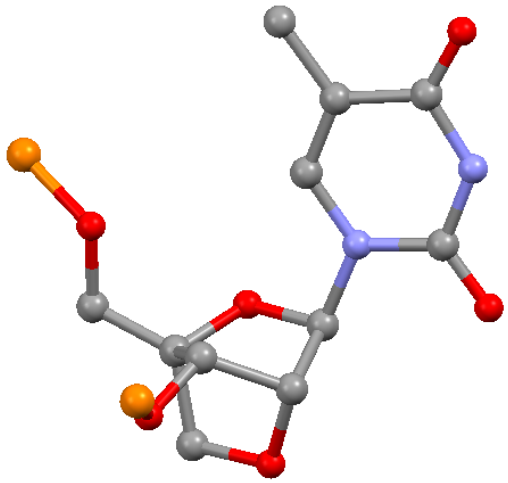


Figure 1: A locked thymidine nucleotide excised from an LNA duplex crystal structure[3].

LNA is able to bind to itself, DNA, and RNA to form stable duplex structures[4-7]. Due to the rigid C3'-*endo* sugar conformation, LNA containing duplexes form A-type helical structures[7]. LNA incorporation significantly increases the stability of the duplex as measured by thermal melting (Tm) studies. For example the Tm of a

9-mer DNA duplex increased from 33 ˚C to 49 ˚C upon the replacement of three nucleotides with LNA monomers[7]. The Tm for a 9-mer DNA/RNA heteroduplex increased from 28 ˚C to 58 ˚C upon replacement of three DNA nucleotides[7].

Several analogues of LNA have been made (Figure 2). The oxygen at the 2' position can be replaced with another heteroatom, such as sulfur or nitrogen[8]. Additionally an α-L-LNA sugar can be synthesized, which forms B-type helices[7, 9]. These α-L-LNA nucleotides also cause an increase in Tm stability of approximately 3 ˚C per modification when partnering with DNA[9].

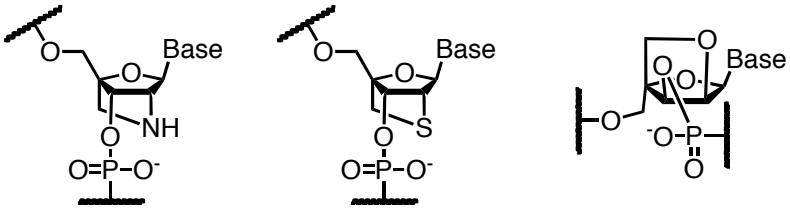


Figure 2: 2' modified LNA and α-L-LNA

Cyclonucleosides are another class of rigid nucleosides. They feature a linkage between the C5' position on the sugar and either the C8 position of purines or the C6 position of pyrimidines (Figure 3). This locks the base in the *anti* conformation and prevents rotation along the gylcosidic bond. At the C5' position the cyclonucleoside can adopt *R* or *S* stereochemistry in relation to the hydroxyl.

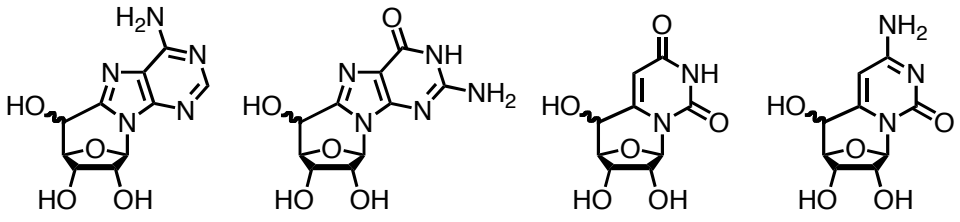


Figure 3: The 8,5'-cyclopurines and 6,5'-cyclopyrimidines

Cyclonucleosides, especially 8,5'-cycloadenosine and its 2'-deoxy version, have been observed as products of oxidative damage under hypoxic conditions[10-14]. Most of these lesions are formed when a hydroxyl radical abstracts a proton at the C5' position[15]. The resulting carbon radical cyclizes to the C8 of the purine base and subsequent removal of the H8 hydrogen forms the product[16]. The two linkages between the base and the sugar prevent base excision repair mechanisms and the cell must use nucleoside excision repair to remove the lesion. The glycosidic bond of *S*-cyclo-dA is approximately 40 times more resistant to acidic depurination than native dA[17].

There have been many reported biological effects of the 8,5'-cyclo-2'-deoxyadenosine lesion in DNA. The TATA binding protein (TBP) reads a TATA promoter region of DNA in order to begin the transcription process. Cyclo-dA lesions prevent the attachment of the TBP to the promoter and can lead to a 75% reduction in gene expression at the lesion sites, equivalent to a base-pair mismatch[18]. Cyclo-dA has been shown to affect many other transcription factors and can cause over 90% of protein association to the DNA oligomer to be disrupted [19]. If the cyclo-dA occurs in the segment of the gene being transcribed, it can cause RNA polymerase II to stall and skip 7, 13, or 21 subsequent nucleotides on the template before resuming transcription[20]. The *R* and *S* diastereomers of 8,6'-cyclo-2'-deoxyadenosine have been observed in human urine samples and are potential bio-markers for oxidative damage caused by carcinogenesis[21].

Cyclo-2'-deoxyguanosine, while not as extensively studied as cyclo-dA, exhibits many similar disruptions. It is formed in a similar manner from radiation

induced oxidative damage and produces the *S* diastereomer over the *R* in a 3:1 ratio[22-24]. In *E. coli, S*-cyclo-dG causes severe mutagenesis, is repaired slowly, and can disrupt DNA replication[25]. Cell mutations of the genes BRAC1 and BRAC2, which cause susceptibility to breast and ovarian cancer, show a deficiency in repairing both cyclo-dA and cyclo-dG compared to control genes and can cause futher mutagenesis[26].

Cyclopyrimidines have been studied less than their purine counterparts. Like the cyclopurines, they are formed as products from gamma radiation[27-29]. However, upon radiation the dihydro nucleosides are produced instead of the standard cyclopyrimidines (Figure 4).

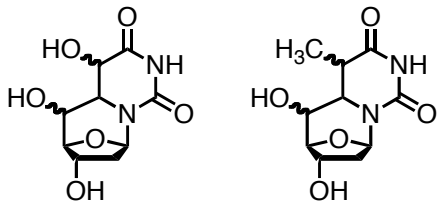


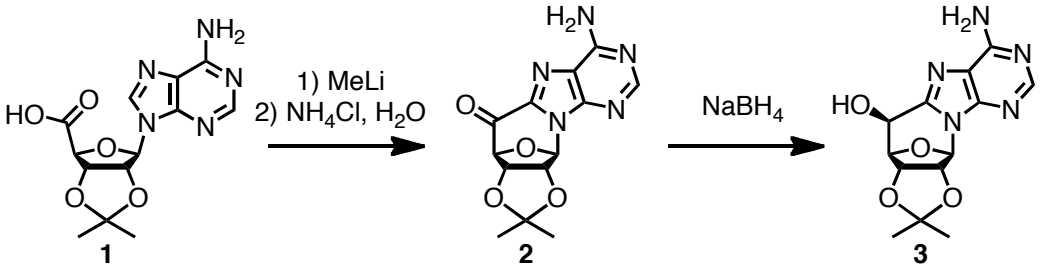
Figure 4: The cyclo lesions formed from gamma radiation of dC and T[27-29].

Uridine phosphorylase (UrdPase), an enzyme involved in the production and catabolism of pyrimidines is often upregulated by cancer cells[30-32]. UrdPase is unable to bind cyclo-U nucleosides since it is locked in the *anti* conformation. *Syn* locked nucleosides have shown to be potent inhibitors of UrdPase[30-32]. Ribonucleases are enzymes that degrade single stranded RNA and can have cytotoxic effects on cancer cells, especially the homolog Ranpirnase, which is isolated from frogs and shares a similar active site to the commonly studied RNase A superfamily[33]. Cyclouridine has been shown to greatly inhibit the hydrolysis mechanism of Ribonuclease A[34].

2.1.2 Previous Cyclonucleoside Syntheses:

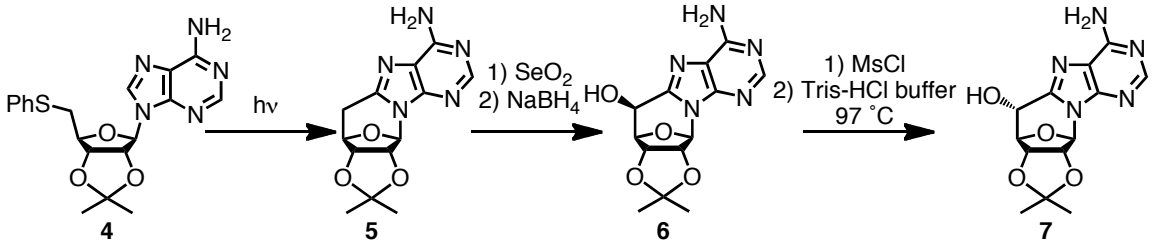
Cyclonucleosides present a unique set of challenges in organic synthesis. The high degree of functionality on the nucleobase requires various protections and deprotection steps. The issue of selectivity between the 2',5', and in the case of ribonucleosides, 3', hydroxyls must be addressed. Steric constraints, always present in nucleoside chemistry, are amplified by the cyclo C-C bridge.

The first reported cyclo-A synthesis was reported by Harper and Hampton in 1972 (Scheme 1)[35]. They begin with the 5' carboxylic acid of 2',3' isopropylidene protected adenosine. Reaction with CH_3Li affords the ketone compound **2** in approximately 50% yield, which can be reduced to the alcohol **3** with $NaBH_4$.



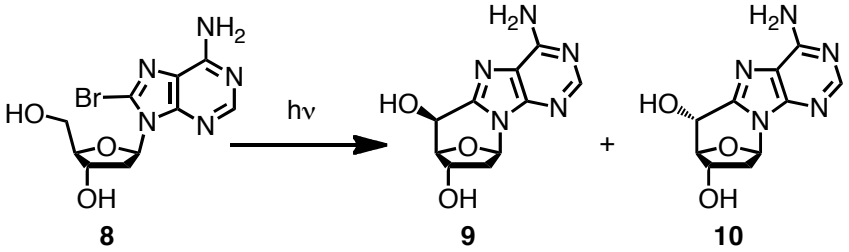
Scheme 1: Harper and Hampton's synthesis of cyclo-A[35].

Ueda and coworkers released several papers in the 1970's on the synthesis of cyclo-A[36-38]. Their syntheses began by irradiating 5'-phenylthioadenosine to form the 8,5'-cyclic product (Scheme 2). Oxidation with SeO_2 and reduction with $NaBH_4$ provides the *S* diastereomer. Isomerization in buffer with heat allows access to the *R* diastereomer **7**. If the phenylthio group is placed on the 8 position of the base, both isomers are formed upon irradiation, although yields are low[38].



Scheme 2: Cyclo-A synthesis by Ueda et al[37].

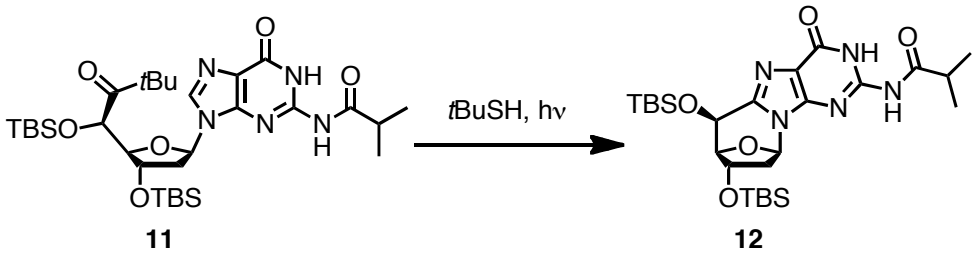
More recent synthetic efforts follow many of the general themes used by Ueda. Romieu, Cadet, and coworkers follow Ueda's scheme to access the 2'-deoxygenated cycloadenosine compound[39]. Barton et al. accomplish their radical cyclization by placing an aryltelluride group at the 5' position, instead of the phenylthio functionality, before irradition to form cyclo-A[40]. Jimenez et al. improved upon Ueda's 8 position radical generation by using 8-bromoadenine as their base (Scheme 3)[41]. After optimization they were able to obtain an approximately 2:1 *R* to *S* ratio of cyclo-dA in 65% total yield. Jimenez also demonstrated that the *S* diastereomer could be photoisomerized to the *R* diastereomer upon sunlight irradiation[42].



Scheme 3: One step access to the *S* and *R* diastereomers by Jimenez et al[41].

Romieu et al. were able to follow Ueda's plan to quickly form cyclo-dG, in just two steps, although they did encounter some difficulty with the solubility of their compounds[43]. Manetto et al. induce radical formation at the C5' after attachment of

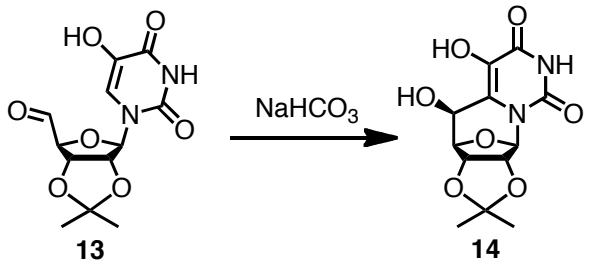
a ketone in a novel method to form cyclo-dG[44]. However, this method is low yielding and did not work in their attempts towards the cyclization of thymine[44].



11 **12**

Scheme 4: Manetto's synthesis of cyclo-dG[44].

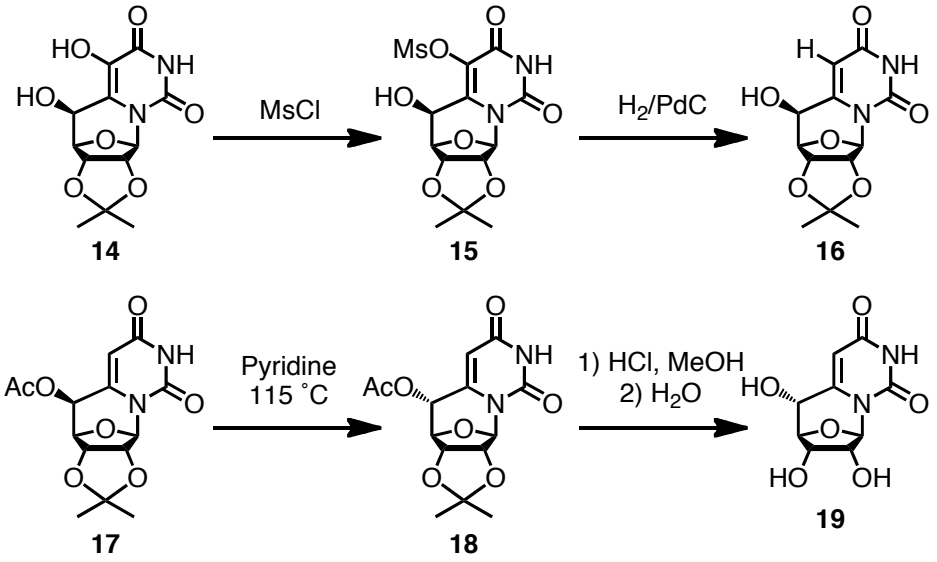
The earliest synthesis of a cyclouridine derivative was carried out by Rabi and Fox[45]. Their nucleobase contains a hydroxyl group at the 5 position. The key step in the synthesis is base catalyzed cyclization after conversion of the 5' OH to an aldehyde (Scheme 5).



13 **14**

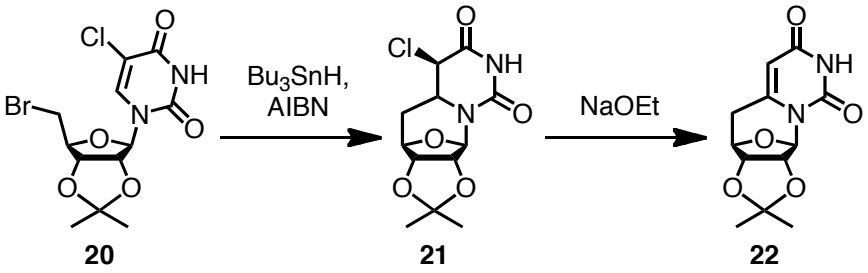
Scheme 5: The first cyclopyrimidine synthesis by Rabi and Fox[45].

In a follow up paper, Otter and Fox remove the OH at the 5 position (Scheme 6)[46]. They selectively form the methanesulfonyl ester at the 5 position and hydrogenate to the uracil base of **16**. The paper also provides the only previous synthesis of the *R* diastereomer **19** other than the work presented in the next section. The 5' OH is acetylated and a 24 hr epimerization provides an *S* to *R* ratio of 2:1, although purification of the mixture of diastereomers proved difficult.



Scheme 6: Removal of the OH at the 5 position and *S* to *R* converson by Otter and Fox[46].

Ueda et al. also worked on syntheses of cyclo-U and cyclo-dU using a strategy of generating a radical at the C5' that was used for their cyclopurine syntheses[47, 48]. They brominate the 5' position and use tributyltin hydride and AIBN to facilitate radical generation. The 5' radical attacks the double bond of the base at the C6 position to form **21**. In order to regenerate the double bond, Ueda installed a Cl on the C5 of the base. Sodium ethoxide facilitates an elimination reaction by removing the H6 hydrogen to form the alkene of **22**. This scheme was also employed to synthesize the cyclo-2'-deoxyuridine compound, and was used as a model for the synthesis presented in this thesis[49, 50]. Ueda did not reinstall the 5' hydroxyl in his work.



Scheme 7: Ueda's synthesis of 6,5'-cyclouridine[47, 48].

Several recent papers have focused on the preparation of the dihydro-cyclopyrimidines, which are observed as products of gamma radiation[27, 29, 51]. These syntheses use a 5' aldehyde cyclization, similar to that used by Fox. However, they use a Bu_3SnH/AIBN radical reaction instead of a base catalyzed cyclization. These compounds lack a leaving group at the 5 position to regenerate aromaticity in the base.

2.1.3 Introduction to Our Work on 6,5'-cyclouridine

Our goal was to improve the synthesis of the *S* and *R* diastereomers of 6,5'-cyclouridine. With only one previous and largely inefficient synthesis reported, we especially sought to improve access to the *R* diastereomer. The synthesis of large amounts of 6,5'-cyclouridine will allow scientists to carry out further biological studies on how the cyclo conformation affects the function of nucleosides and/or nucleotides. We also sought to synthesize the 5' triphosphates of cyclo-U, which will be presented in section 2.3, with the goal of performing polymerase studies.

Our synthesis builds off of the previous work above, especially Ueda's cyclouridine synthesis. We improved upon his steps before installing the 5' OH, which he never accomplished. A selenium dioxide/t-BuOOH oxidation was used to access both diastereomers, which will allow production of these unique monomers in good yield for scientists to further study.

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2.2.1

An Efficient Synthetic Approach to 6,5' (*S*) and 6,5' (*R*) – Cyclouridine*

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Note: Compound, scheme, and reference numbers reset in this section. References listed at the end of the section.

Here we present new routes for the efficient syntheses of 6,5'-(*S*)- and 6,5'-(*R*)-cyclouridine. The syntheses utilize readily accessible uridine as a starting material. This route to the *R* diastereomer is significantly more efficient than previous synthetic efforts, allowing us to obtain large amounts of pure material for future biological testing.

Cyclonucleosides have been interesting targets of the nucleoside community due to their rigid geometry. A second linkage between the 5'-carbon of the sugar and the nucleobase fixes the base in the *anti* conformation. Such a linkage results in two unique diastereomers at the 5'-position in regards to the 5'-OH (**Figure 1**).

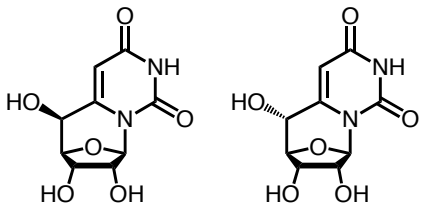


Figure 1. 6,5'-(*S*)- and 6,5'-(*R*)-cyclouridine

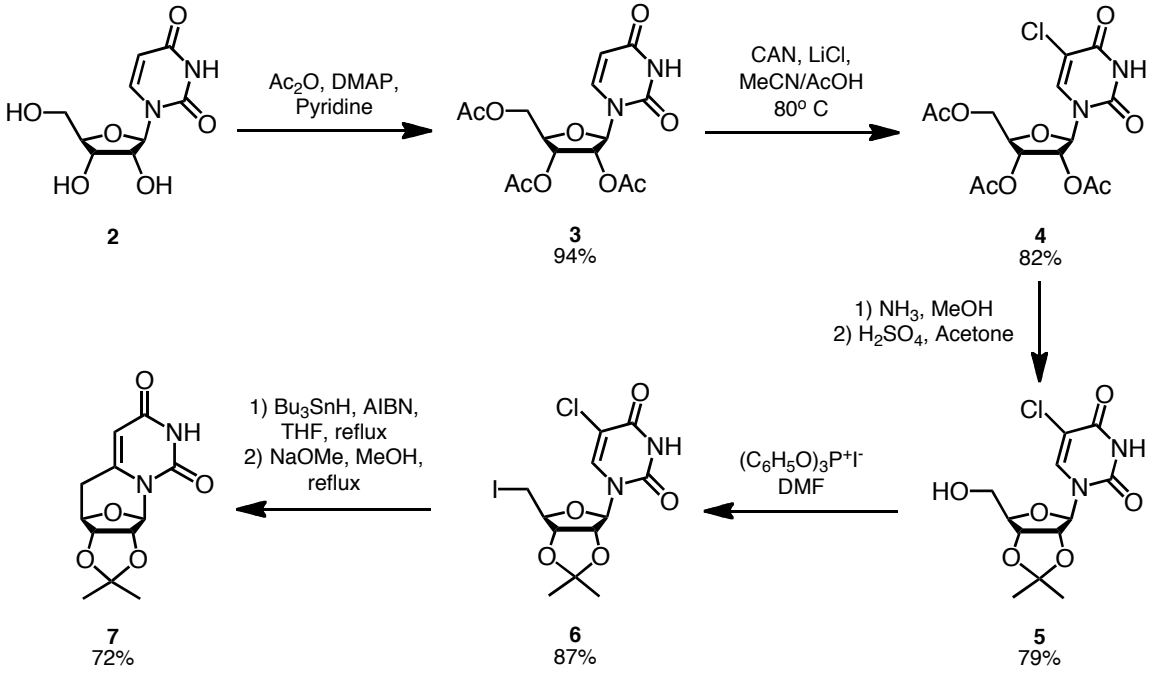
6,5'-cyclouridine (1*S* and 1*R*) and similar compounds are unable to bind uridine phosphorylase (UrdPase), an enzyme involved in the anabolism and catabolism of pyrimidines, that has been shown to be upregulated in multiple human cancer cell lines [1-3]. Nucleosides locked in a *syn* conformation are known to be potent inhibitors of UrdPase. Cyclouridine has been shown to reduce the rate of hydrolysis by Ribonuclease A [4]. Ribonucleases degrade unprotected single stranded RNA and some homologs, most notably Ranpirnase, have been shown to have cytotoxic effects on cancer cells [5]. To date, information relating to the specific

binding events of cyclouridine is sparse, due to low amounts of pure, stereometrically defined, samples. An efficient synthesis of the *S* and *R* diastereomers of 6,5'-cyclouridine will allow for further investigation of the interactions that these enzymes have with cyclouridine, and allow for stability studies to be performed with oligonucleotide strands. Cyclonucleosides also provide unique opportunities for template studies, where the cyclonucleoside could be used in the template strand or as a substrate to test the promiscuity of polymerases.

Several syntheses of 6,5'-cyclo-5'-deoxyuridine have been undertaken, but access to the 5'-OH derivative has been problematic [6-10]. The *R* 5'-OH derivative has been particularly difficult to make, with only one previously reported synthesis, which relied on the epimerization of the *S* diastereomer and resulted in an unfavorable 2:1 ratio of *S* to *R* after a 24 hr reaction [6].

Our synthesis is a modification of the path established by Ueda et. al to obtain the protected 6,5'-cyclo-5'-deoxyuridine compound, **7**. We have been able to achieve higher yields with fewer steps while synthesizing compound **7**. Our synthesis begins by protecting the 2', 3', and 5' OH positions on uridine with acetate groups (**Scheme 1**). The 5 position on the uridine base is then chlorinated with CAN and LiCl using Asakura's method [11]. Deprotection of the acetates and subsequent acetonide protection of the 2' and 3' hydroxyls yields **5**. At this point we perform a one-step iodination of the 5' position using Moffatt's chemistry, instead of the two-step procedure previously utilized [9, 12]. Radical cyclization with AIBN and Bu_3SnH and dehydrohalogenation with $NaOCH_3$ produces **7** in very good yield.

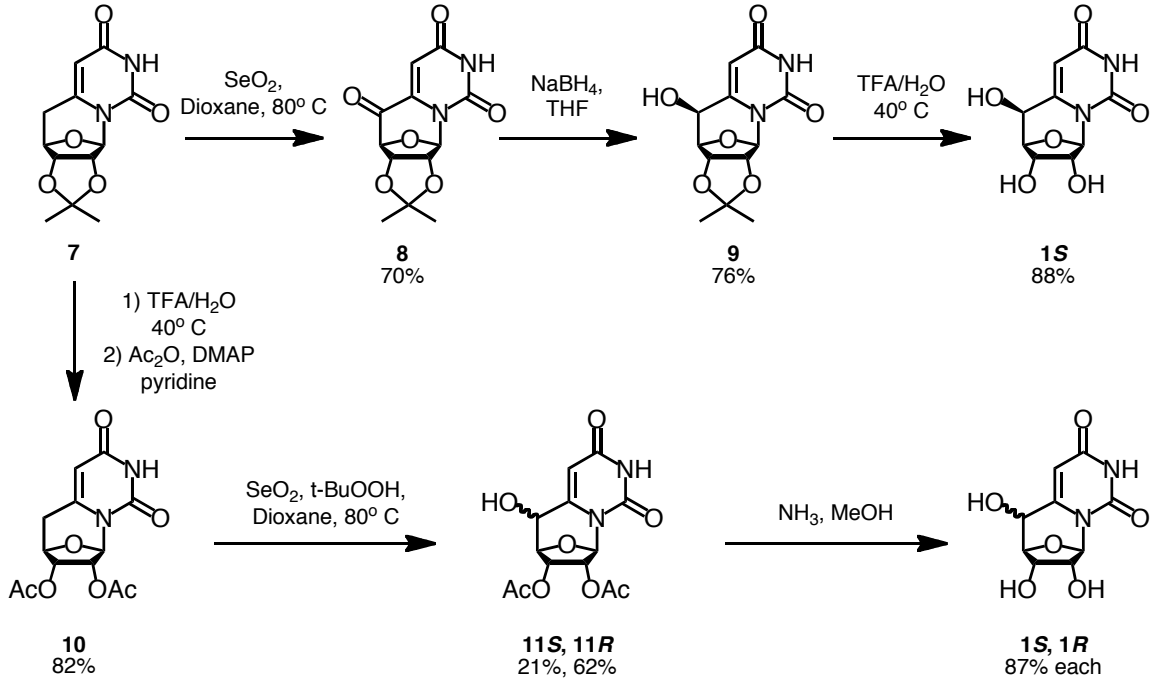
Compound **7** can be directly oxidized to ketone, **8**, via an allylic oxidation with SeO_2 (**Scheme 2**). Reduction with $NaBH_4$ yields exclusively the *S* diastereomer at the 5' position. Acidic removal of the acetonide gives 6,5'-(*S*)-cyclouridine, **1*S***. This represents the most efficient pathway that has been reported to obtain the *S* diastereomer.



Scheme 1. The synthesis of 2', 3'-isopropylidine 6-5'-cyclo-5'-deoxyuridine

Meanwhile the acetonide of **7** can be replaced with acetates to give **10**. Oxidation with SeO_2 and *t*-BuOOH, using methods established by Sharpless, affords a mixture of the *R* and *S* products, with the *R* diastereomer being the dominant product in a better than 3/1 ratio [13]. The two diastereomers can be separated with purification by column chromatography. A justification for this reactivity is that when *t*-BuOOH is added during SeO_2 oxidation of **7**, it results in a mixture of the *S* diastereomer and starting material. Likely, the rigid nature of the acetonide protected sugar prevents the *R* diastereomer from forming, while the acetates allow

the sugar to be more flexible during the oxidation and the *R* diastereomer can be obtained. The acetates on **11*S*** and **11*R*** were removed with 7N NH_3 in MeOH to yield the cyclonucleosides.



Scheme 2. Synthesis of *R* and *S* diastereomers of 6,5'-cyclouridine

Crystal structures of compounds **9** and **11*R*** were obtained to confirm the stereochemistry at the 5' position (**Figure 2** and Supplementary data). We had difficulty obtaining crystal structures of the fully deprotected products; however, the crystal of **11*R*** is a good approximation of **1*R*** when the acetate protecting groups are not shown. When overlaying the crystal of **11*R*** and a uridine nucleotide whose structural parameters were obtained from a crystal of an RNA dodecamer, several differences are observed between the cyclo and native nucleosides [14]. The additional bond between the 5' carbon and the 6 carbon causes the U base to be "pulled" back quite dramatically. This may explain why the rate of hydroysis in RNase A is slowed, since the shape and conformation of the nucleoside is distorted.

Pulling the base away from the Watson-Crick interactions could be detrimental to interstrand base pairing. Studies relating to the strength of base pairing interactions are ongoing in the lab.

One beneficial characteristic of the compounds was seen with regards to the glycosidic torsion angle, χ, of **11*R***. The angle is -148.80°, very close to ribonucleosides, which have a χ value near -160°. Cyclouridine provides an effective substrate for studies requiring a nucleoside in the *anti* conformation.

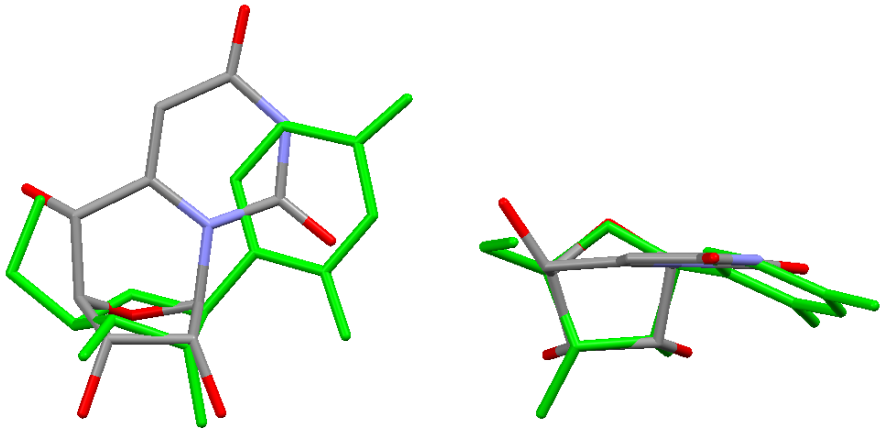


Figure 2. A front and overhead view of the crystal structure of **11*R*** with the acetates removed overlaid with a crystal of uridine, in green, excised from an RNA strand [14]

The sugar pucker of the cyclouridine resides in the envelope configuration rather than the C_3'-*endo* half chair adopted by the native nucleoside. This change in the sugar conformation has implications for the structure of an oligomer of cyclonucleotides, as the 3' OH is shifted further down from the *endo* position. Further investigation is needed to understand if this is the preferred sugar pucker that is adopted when cyclouridine is inserted into an oligonucleotide strand.

The 5' OH in **11*R*** is in a *gauche, trans* position in relation to the O_4' and the C_3', while the *S* diastereomer is locked in a *trans, gauche* position. Native

nucleosides usually adopt the *gauche, gauche* position, where the 5' OH points back towards the furanose ring, as seen in the crystal structure overlay. The implication of the sugar pucker will be taken into consideration in our continued investigations.

Conclusions

In summary a new and improved route to the *S* and *R* diastereomers of 6,5'-cyclouridine is presented. A key oxidation step with SeO_2 and *t*-BuOOH affords the *R* diastereomer in a 3:1 ratio compared with the *S* diastereomer, greatly increasing the yield and lowering the reaction time previously reported. These syntheses will allow for the production of large quantities of each diastereomer of cyclouridine to be obtained for further biological studies. Additionally, the crystal structures of cyclouridine have helped to elucidate the unique structural features caused by the introduction of the C6-C5' bond.

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2.2.2 Relevant Notes on the Synthesis of 6,5'-Cyclouridine*

Some of the early protection and deprotection steps in the synthesis of 6,5'-cyclouridine may seem unnecessary at first; however, they were essential for the production of the final product. An initial triacetate protection was performed on

* Sections **2.2.2** and **2.2.3** were not part of the *Chemical Communications* manuscript and is an addition for this thesis.

uridine before the chlorination of the 5 position on the base. After this chlorination, the acetates were removed and the 2',3' position was protected with an acetonide group. If the acetonide protection was done before the chlorination, the harsh acidic reaction conditions cleaved the acetonide and no product was produced. The paper from which we obtained the chlorination conditions, reports that the chlorination can be achieved on the free nucleoside, which in their case was 2'-deoxyuridine[11]. We were unable to achieve a similar result with uridine, and opted to use the acid stable acetate group to protect the 2',3', and 5' OH's.

We also used the less common methyltriphenoxyphosphonium iodide reagent to facilitate the 5' iodination reaction instead of the more standard two step tosylation and iodination procedure, which was preformed in our lab for the synthesis of the 2'-deoxy cyclonucleosides[12]. The two step reaction scheme produced erratic results on the uridine monomer. Although these steps occasionally produced excellent yields, often the tosyl displacement with sodium iodide did not occur, even after 24 hrs. The one-step methyltriphenoxyphosphonium iodide method proceeds in 30 mins compared to over 24 hrs for the 2-step procedure, and cuts down on purification time.

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2.2.3 6,5'-Cyclouridine Follow Up*

Cyclonucleoside Triphosphates

In addition to synthesizing the *R* and *S* diastereomers of 5,6'-cyclouridine, we also hoped to convert them to their 5'-triphosphates and further utilize them for enzymatic template and kinetic studies. However, all of our synthetic efforts did not yield the triphosphate products. This section serves to briefly discuss our methods and attempts to synthesize these monomers.

The two most commonly utilized methods use 2-chloro-1,3,2-benzodioxaphosphorin-4-one (Eckstein's method) or $POCl_3$ to synthesize triphosphates[1-3]. Both of these reactions involve activating the 5' OH with a reactive phosphorous compound. Pyrophosphate is then added to the reaction and a cyclic intermediate is formed that is oxidized to the final product (Figures 1,2).

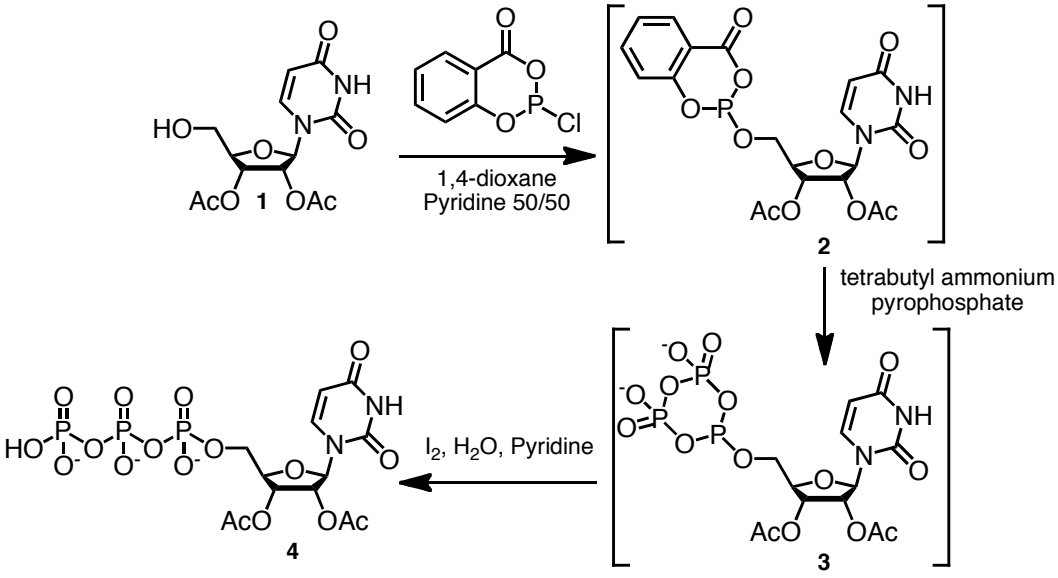


Figure 1: An example of the Eckstein Method to synthesize triphosphates

* ***Note:*** Compound, scheme, and reference numbers reset in this section. References listed at the end of the section.

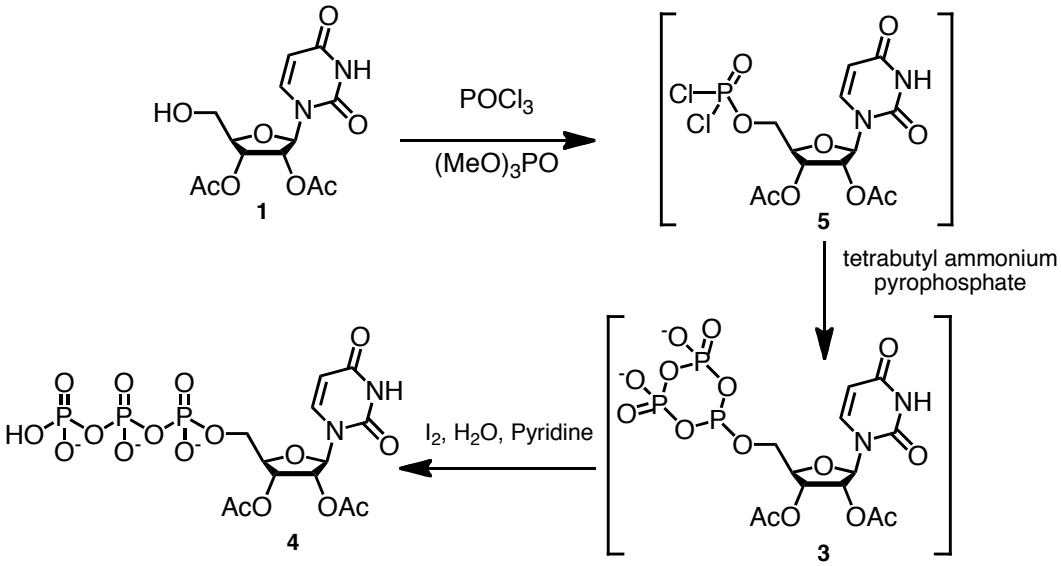


Figure 2: The $POCl_3$ method to synthesize triphosphates

Unfortunately both the Eckstein and $POCl_3$ methods failed to produce cyclonucleoside triphosphates despite trying many variations of the reactions. Our theory is that the extremely confined sterics of 5,6'-cyclouridine either do not allow nucleophilic attack by pyrophosphate or the sterics prevent the cyclic triphosphates from forming (Figure 3). HPLC and phosphorous NMR traces primarily showed monophosphate formation along with unreacted starting material.

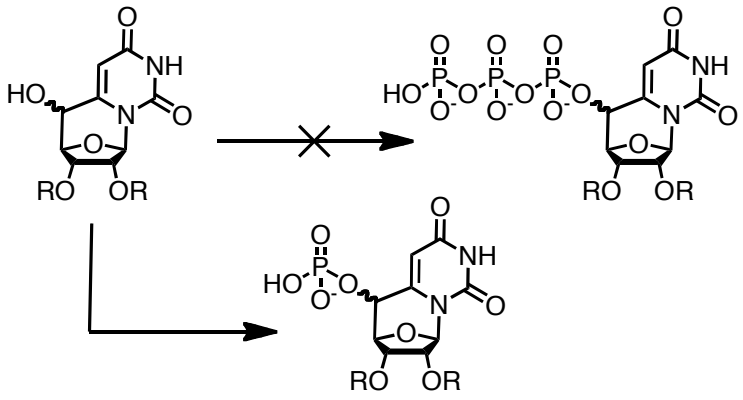


Figure 3: Failed cyclo-triphosphate synthesis.

Work done by Han Yueh and Hongchuan Yu in our group on 8,5'-cyclo-2'-deoxyadenosine and 6,5'-cyclo-2'-deoxyuridine also demonstrated that the 5' OH on

cyclonucleosides is very hindered[4]. They made the phosphoramidites of the cyclo-2'-deoxynucleosides for use on a DNA synthesizer. The standard 5' OH protecting group, dimethoxytrityl chloride, proved too bulky to be added to the monomer, and a 1-ethoxyethyl protecting group was used in its place (Figure 4).

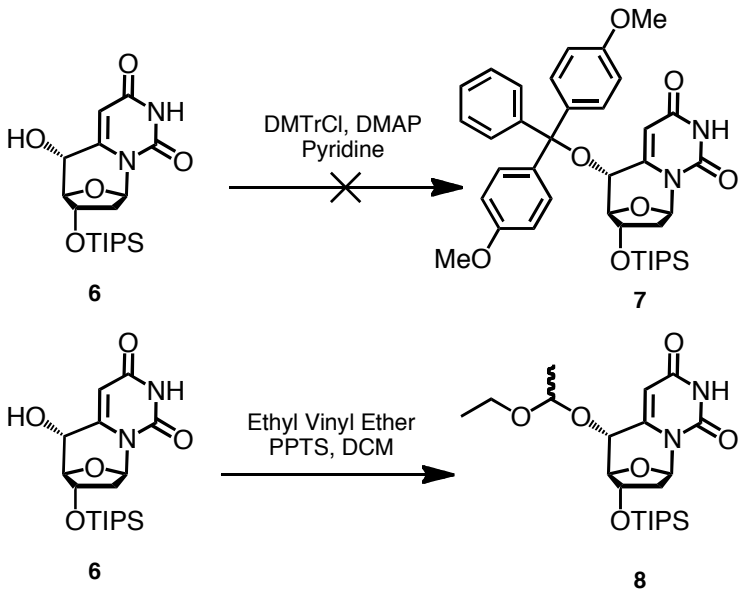


Figure 4: Work by Yueh and Yu demonstrating the steric constraints of the 5'OH group on cyclonucleosides.

Several other less common triphosphate procedures were also attempted in addition to variations of the Eckstein procedure. Most of these involved activation of the monophosphate and addition of pyrophosphate to displace the reactive group[5, 6]. These methods unfortunately did not yield the desired triphosphate either, despite not containing the cyclic intermediate found in the Eckstein and $POCl_3$ reactions.

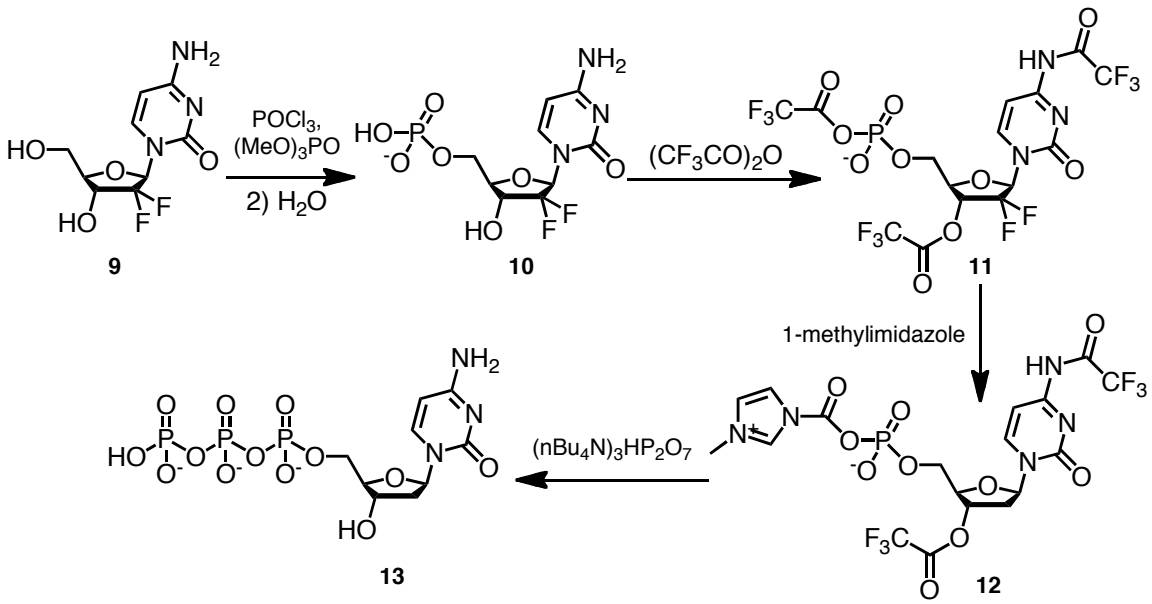


Figure 5: An example of activation of a monophosphate to yield triphosphate by Risbood et al. This and similar activations failed on cyclouridine.

1. Ludwig, J.; Eckstein, F., Rapid and Efficient Synthesis of Nucleoside 5'-O-(1-Thiotriphosphates), 5'-Triphosphates and 2',3'-Cyclophosphorothioates Using 2-Chloro-4h-1,3,2-Benzodioxaphosphorin-4-One. *Journal of Organic Chemistry* **1989,** *54* (3), 631-635.
2. Yoshikaw.M; Kato, T.; Takenish.T, Studies of Phosphorylation .3. Selective Phosphorylation of Unprotected Nucleosides. *Bulletin of the Chemical Society of Japan* **1969,** *42* (12), 3505-&.
3. Yoshikawa, M.; Kato, T.; Takenishi, T., A Novel Method for Phosphorylation of Nucleosides to 5'-Nucleotides. *Tetrahedron Letters* **1967,** (50), 5065-5068.
4. Yueh, H.; Yu, H.; Theile, C. S.; Pal, A.; Horhota, A.; Greco, N. J.; McLaughlin, L. W., Synthesis and Properties of DNA Containing Cyclonucleosides. *Nucleosides, Nucleotides and Nucleic Acids* **2012,** *Advanced Article*.
5. Risbood, P. A.; Kane, C. T.; Hossain, M. T.; Vadapalli, S.; Chadda, S. K., Synthesis of gemcitabine triphosphate (dFdCTP) as a tris(triethylammonium) salt. *Bioorganic & Medicinal Chemistry Letters* **2008,** *18* (9), 2957-2958.
6. Hoard, D. E.; Ott, D. G., Conversion of Mono- and Oligodeoxyribonucleotides to 5'-Triphosphates. *Journal of the American Chemical Society* **1965,** *87* (8), 1785-&.

2.3 Supporting Information

General Information

Chemicals used were of ACS grade and purchased from Sigma-Aldrich, Fisher, Acros, Oakwood, and Chem-Impex. Pyridine, DMF, acetonitrile, and THF were HPLC grade and further purified in a solvent system containing drying columns. 1,4-dioxane was purchased in a sure-seal bottle and the dry MeOH was distilled over magnesium and iodine.

2.3.1 Experimental Proceedures

Compound **3**: Uridine (10.0 g, 40.5 mmol) and DMAP (100 mg, 0.819 mmol) were dissolved in 250 mL of pyridine and acetic anhydride (13.2 mL, 139.2 mmol) was added. The reaction was stirred for 4 hrs at 25^{o} C before being quenched with water. The solvent was removed be rotary evaporation and coevaporated twice with toluene. The crude compound was purified by flash chromatography (ethyl acetate) to yield **3** as a white solid (14.1 g, 38.07 mmol, 94%). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 2.054 (s, 3H), 2.087 (s, 3H), 2.091 (s, 3H), 4.352 (m, 3H), 5.427 (dd, *J*=6.0, 4.5 Hz, 1H), 5.507 (t, *J*=5.5 Hz, 1H), 5.709 (d, *J*=8.0 Hz, 1H), 6.021 (d, *J*=5.0 Hz, 1H), 7.700 (d, *J*=8.5 Hz, 1H), 10.390 (s, 1H). ^{13}C NMR (125 MHz, $(CD_3)_2CO$), δ 20.87, 20.97, 20.18, 64.46, 71.61, 73.93, 81.23, 89.53, 103.96, 141.95, 151.77, 163.87, 170.60, 170.62, 171.17. HRMS (DART) Calcd for $C_{15}H_{18}N_2O_9$ 371.10905; Found, 371.10835

Compound **4**: **3** (920 mg, 2.10 mmol), ceric ammonium nitrate (2.30 g, 4.20 mmol) and LiCl (110 mg, 2.52 mmol) were dissolved in 40 mL of acetic acid and 40 mL of

acetonitrile. The reaction was heated to 80° C for 8 hrs before being cooled to ambient temperature and quenched with water. The solvents were removed under vacuum and coevaporated several times with ethanol. The crude product was dissolved in ethyl acetate and washed with water, saturated $NaHCO_3$ and brine. The solvent was removed under vacuum and the compound was purified by column chromatography (3:1 ethyl acetate to hexanes) to yield **4,** as a white foam (815 mg, 1,73 mmol, 82%). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 2.06 (s, 3H), 2.09 (2, 3H), 2.13 (s, 3H), 4.40 (m, 3H), 5.44, (dd, *J*=6.0, 5.0 Hz, 1H), 5.53 (dd, *J*=6.5, 5.5 Hz, 1H), 6.05 (d, *J*=5.0 Hz, 1H), 8.02 (s, 1H), 10.64 (s, 1H). ^{13}C NMR (125 MHz, $(CD_3)_2CO$), δ 20.81, 20.91, 21.26, 64.18, 71.22, 74.22, 81.45, 89.54, 110.06, 138.71, 150.83, 159.73, 170.50, 170.51, 171.05. HRMS (DART) Calcd for $C_{15}H_{18}ClN_2O_9$ 405.07008; Found, 405.07108.

Compound **5**: Compound **4** (3.38 g, 8.34 mmol) was dissolved in 150 mL of 7N NH_3 in MeOH and stirred for 5 hrs. The solvent was removed by rotary evaporation and the crude compound was dried on vacuum. The material was dissolved in 200 mL of dry acetone and 200 μL of H_2SO_4 was added dropwise. The reaction was stirred for 7 hrs before being slowly quenched with saturated $NaHCO_3$. The acetone was removed by rotary evaporation and the compound was extracted from the aqueous layer with ethyl acetate. The organic layer was washed with brine and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (4:1 ethyl acetate to hexanes) to yield 5 as a white solid (2.10 g,

6.59 mmol, 79%). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.33 (s, 3H), 1.52 (s, 3H), 3.83 (m, 2H), 4.25 (q, *J*=3.2 Hz, 1H), 4.94 (dq, *J*=6.4, 4.0 Hz, 1H) 5.97 (d, *J*=2.8 Hz, 1H), 8.26 (s, 1H), 10.51 (s, 1H). ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ 26.05, 28.07, 63.21, 81.98, 86.10, 88.44, 93.44, 109.44, 114.80, 140.02. HRMS (DART) Calcd for $C_{12}H_{16}ClN_2O_6$ 319.06969; Found, 319.06913.

Compound **6**: **5** (994 mg, 3.12 mmol) was dissolved in 20 mL of DMF and a solution of Methyltriphenoxyphosphonium iodide (2.12 g, 4.68 mmol) in 30 mL of DMF was added. The reaction was stirred for 30 mins before being quenched with saturated $Na_2S_2O_3$. The solvent was removed by rotary evaporation and the crude material was dissolved in ethyl acetate and washed with saturated $Na_2S_2O_3$ and water. The ethyl acetate was evaporated and the product recrystallized from chloroform and hexanes to yield **6** as a white solid (1.16 g, 2.71 mmol, 87%). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.33 (s, 3H), 1.51 (s, 3H), 4.45 (dd, *J*=10.0, 6.0 Hz, 1H), 3.58 (dd, *J*=10.0, 7.2 Hz, 1H), 4.23 (dq, *J*=6.0, 4.0 Hz, 1H), 4.83 (dd, *J*=6.4, 3.6 Hz, 1H), 5.20 (dd, *J*=6.4, 2.0 Hz, 1H), 5.84 (d, *J*=2.0 Hz, 1H), 8.05 (s, 1H), 10.61 (s, 1H). ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ 6.55, 25.96, 27.84, 85.40, 85.91, 88.56, 95.75, 109.78, 115.34, 141.45. HRMS (DART) Calcd for $C_{12}H_{15}ClIN_2O_5$ 428.97142; Found, 428.97090.

Compound **7**: **6** (1.01 g, 2.36 mmol) was dissolved in 50 mL of THF and brought to a reflux. A solution of Bu_3SnH (0.720 mL, 2.71 mmol) and AIBN (12.0 mg, 73.1 μmol) in 10 mL of THF was added via syringe pump over two hours. The reaction was

continued for another two hours at reflux before being cooled to ambient temperature and the solvent removed by rotary evaporation. The crude material was dissolved in MeOH and washed three times with hexanes. The MeOH was evaporated and the material was dried under vacuum. The intermediate was then dissolved in 40 mL of dry MeOH and NaOMe (0.92mL of 25 wt% in MeOH, 11.8 mmol) was added. The mixture was heated to reflux for three hours. The reaction was cooled to ambient temperature and the excess NaOMe was neutralized with 0.1 M HCl. The solvent was removed and the crude material was dry packed onto silica gel (4.00 g) and purified by column chromatography (95:5 DCM to MeOH) to yield **7** as a white solid (452 mg, 1.70 mmol, 72%). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.30 (s, 3H), 1.43 (s, 3H), 2.76 (d, *J*=15.2 Hz, 1H), 3.19 (ddd, *J*=18.8, 6.8, 2.0 Hz, 1H), 4.59 (d, *J*=6.8 Hz, 1H), 4.81 (d, *J*=2.4 Hz, 2H), 5.41 (t, *J*=1.6 Hz, 1H), 6.01 (s, 1H), 9.98 (s, 1H). ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ 25.55, 26.83, 80.82, 81.60, 84.04, 86.03, 86.87, 102.05, 114.19, 150.12. HRMS (DART) Calcd for $C_{12}H_{15}N_2O_5$ 267.09810; Found, 267.09842.

Compound **8**: The acetonide protected cyclized product **7** (165 mg, 0.619 mmol) and selenium dioxide (206 mg, 1.86 mmol) were dissolved in 1,4-dioxane (15 mL). The reaction mixture was heated to 85^{o} C for 24 hours. The solvent was removed by rotary evaporation and the crude material was dry packed on silica gel before being purified by column chromatography (95:5 DCM to MeOH) to yield **8** as a white solid (122 mg, 0.436 mmol, 70%). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 1.34 (s, 3H), 1.49 (s, 3H), 4.86 (s, 1H), 5.08 (m, 2H), 6.17 (s, 1H), 6.18 (s, 1H), 10.47 (s, 1H). ^{13}C NMR (125

MHz, $(CD_3)_2CO$), δ 25.61, 26.77, 81.85, 85.02, 88.76, 88.84, 104.29, 115.82, 142.93, 150.20, 163.32, 186.59. HRMS (DART) Calcd for $C_{12}H_{13}N_2O_6$ 281.07736; Found, 281.07694.

Compound **9**: Compound **8** (400 mg, 1.429 mmol) was dissolved THF (12 mL) and $NaBH_4$ (65.0 mg, 1.714 mmol) was slowly added. The reaction was stirred for 2 hrs and the solvent was removed by rotary evaporation. The crude material was dry packed on silica gel (1.2 g) and purified by column chromatography (95:5 DCM to isopropanol) to yield **9** as a white solid (306 mg, 1.09 mmol, 76%) ^{1}H NMR (500 MHz, Pyridine-D5), δ 1.38 (s, 3H), 1.58 (s, 3H), 4.91 (d, *J*=6.5 Hz, 1H), 4.94 (d, *J*=6.0 Hz), 5.18 (d, *J*=6.0 Hz, 1H), 5.45 (d, *J*=5.5 Hz, 1H), 6.37 (s, 1H), 6.60 (s, 1H), 13.44 (s, 1H). ^{13}C NMR (125 MHz, Pyridine-D5), δ 25.31, 26.58, 63.31, 78.91, 84.58, 85.17, 87.17, 101.90, 113.79, 151.09, 154.91, 164.42. HRMS (DART) Calcd for $C_{12}H_{15}N_2O_6$ 283.09301; Found, 283.09301.

Compound **10:** The acetonide protected **7** (250 mg, 0.938 mmol) was dissolved in a 50/50 mixture of water and trifluoroacetic acid (7 mL each) and stirred at ambient temperature for 10 hours. The solvent was removed by rotary evaporation and coevaporated three times with ethanol. The crude deprotected product was coevaporated with pyridine twice before being dissolved in pyridine with DMAP (12.0 mg, 93.8 μmol). Acetic anhydride (354 μL, 3.75 mmol) was added dropwise and the mixture was stirred for 6 hours. The reaction was quenched with water and

the solvent was removed by rotary evaporation and coevaporated twice with toluene. The crude product was dry packed onto silica gel (1.25 g) and purified by flash chromatography (95:5 DCM to isopropanol) to yield **10** as a white solid (239 mg, 0.796 mmol, 82%) ^{1}H NMR (500 MHz, Pyridine-D5) δ 2.03 (s, 3H), 2.06 (s, 3H), 2.92 (d, *J*=18.5 Hz, 1H), 3.28 (ddd, *J*=18.5, 6.5, 2.0 Hz, 1H), 4.88 (d, *J*=7.0 Hz, 1H), 5.65 (d, *J*=6.0 Hz, 1H), 5.67 (s, 1H), 5.98 (d, *J*=6.0 Hz, 1H), 6.72 (s, 1H), 13.42 (s, 1H). ^{13}C NMR (125 MHz, Pyridine-D5), δ 20.60, 20.68, 30.84, 75.75, 77.21, 80.40, 86.78, 102.60, 148.31, 151.30, 163.89, 169.87, 170.47. HRMS (DART) Calcd for $C_{13}H_{15}N_2O_7$ 311.08793; Found, 311.08679.

Compounds **11*S*** and **11*R***: The diacetate protected **10** (965 mg, 3.11 mmol), selenium dioxide (345 mg, 3.11 mmol), and tertbuoxide (4.31 mL of a 70 wt% solution in H_2O, 31.10 mmol) was dissolved in 60 mL of 1,4-dioxane and heated to 90^{o} C for 10 hours. The reaction mixture was cooled and 3.00 g of activated charcoal was added to absorb the stench. The mixture was filtered through Celite and the solvent was removed by rotary evaporation. The crude material dry packed onto silica gel (4.00 g) and was purified by flash chromatography (95:5 DCM to isopropanol) to yield each diastereomer as a white solid (**11*S***: 209 mg, 0.64 mmol, 21%; **11*R***: 629 mg, 1.93 mmol, 62%). Compound **11*S***: ^{1}H NMR (500 MHz, Pyridine-D5) δ 2.00 (s, 3H), 2.04 (s, 3H), 5.09 (d, *J*=6.5 Hz, 1H), 5.29 (dd, *J*=6.0, 1.5 Hz, 1H), 6.10 (d, *J*=6.0 Hz, 1H), 6.34 (d, *J*=6.3 Hz, 1H), 6.385 (s, 1H), 6.72 (s, 1H), 13.49 (s, 1H). ^{13}C NMR (125 MHz, Pyridine-D5), δ 20.66, 20.68, 64.32, 71.09, 76.55, 83.85, 87.78, 102.54, 151.04, 154.41, 164.43, 169.72, 169.99. HRMS (DART) Calcd for $C_{13}H_{15}N_2O_8$

327.08284; Found, 327.08230. Compound **11*R***: ^{1}H NMR (500 MHz, Pyridine-D5) δ 2.05 (s, 3H), 2.09 (s, 3H), 5.01 (s, 1H), 5.16 (s, 1H), 5.71 (d, *J*=6.0 Hz, 1H), 6.01 (d, *J*=6.0 Hz, 1H), 6.28 (s, 1H), 6.77 (s, 1H), 13.52 (s, 1H). ^{13}C NMR (125 MHz, Pyridine-D5), δ 20.66, 20.69, 66.47, 72.82, 75.83, 87.01, 87.33, 105.08, 151.14, 152.18, 164.46, 169.96, 170.65. HRMS (DART) Calcd for $C_{13}H_{15}N_2O_8$ 327.08284; Found 327.08248.

Compounds **1*S*** and **1*R***: Compound **1*S*** can be prepared from either **11*S*** or from compound **9**. If compound **9** is used as the starting material, it (142 mg, 0.504 mmol) is dissolved in a mixture of water and trifluoroacetic acid (8 mL each). The reaction is stirred at room temperature for 4 hrs before the solvent is removed by rotary evaporation. The material is coevaporated twice with water before being dry packed on silica gel (500 mg) and purified by column chromatography (94:6 ethyl acetate to ethanol) to yield **1*S*** as a white solid (107 mg, 0.442 mmol, 88%). If starting with **11*S***, it (32 mg, 0.098 mmol) is dissolved in 7N NH_3 in MeOH (10 mL) and stirred for 5 hrs. The solvent was removed by rotary evaporation and the crude material was dry packed on silica gel (100 mg) before being purified using column chromatography (94:6 ethyl acetate to ethanol) to yield **1*S*** as a white solid (21 mg, 0.085 mmol, 87%). ^{1}H NMR (500 MHz, Pyridine-D5) δ 4.74 (d, *J*=6.0 Hz, 1H), 4.99 (d, *J*=5.5 Hz, 1H), 5.23 (m, 2H), 6.42 (d, *J*=1.5 Hz, 1H), 6.69 (s, 1H). ^{13}C NMR (125 MHz, Pyridine-D5) δ 65.03, 69.76, 76.48, 87.18, 90.66, 101.58, 151.45, 155.89, 164.67. HRMS (DART) Calcd for $C_9H_{11}N_2O_6$ 243.06171; Found 243.06314.

1*R* was prepared from **11*R***. **11*R*** (62 mg, 0.19 mmol) was dissolved in 7N NH_3 in MeOH (13 mL) and stirred for 5 hrs. The solvent was removed by rotary

evaporation and the crude material was dry packed on silica gel (250 mg) before being purified by column chromatography (94:6 ethyl acetate to ethanol) to yield **1*R*** as a white solid (40 mg, 0.16 mmol, 87%). ^{1}H NMR (500 MHz, Pyridine-D5) δ 4.66 (s, 2H), 4.79 (t, *J*=1.0 Hz, 1H), 5.11 (s, 1H), 6.24 (s, 1H), 6.71 (s, 1H). ^{13}C NMR (125 MHz, Pyridine-D5) δ 67.21, 71.80, 75.76, 90.16, 90.50, 104.65, 151.65, 153.43, 164.91. HRMS (DART) Calcd for $C_9H_{11}N_2O_6$ 243.06171; Found 243.06228.

2.3.2 Crystal Structures:

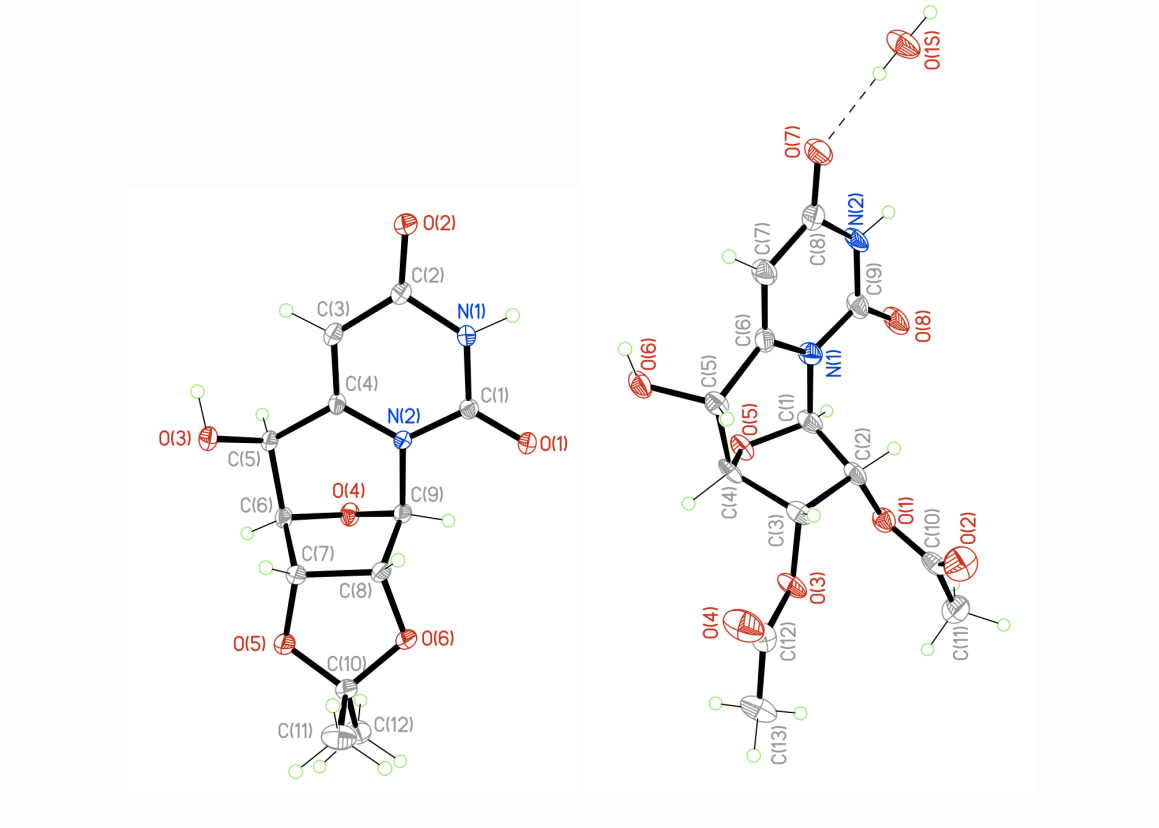
Crystal structures of compounds **9** and **11*R*** were obtained by the slow evaporation of a solution of the compounds in methanol. Ellipsoid plots are shown below. Cif files are available online.

Crystal Data Collection Procedure*

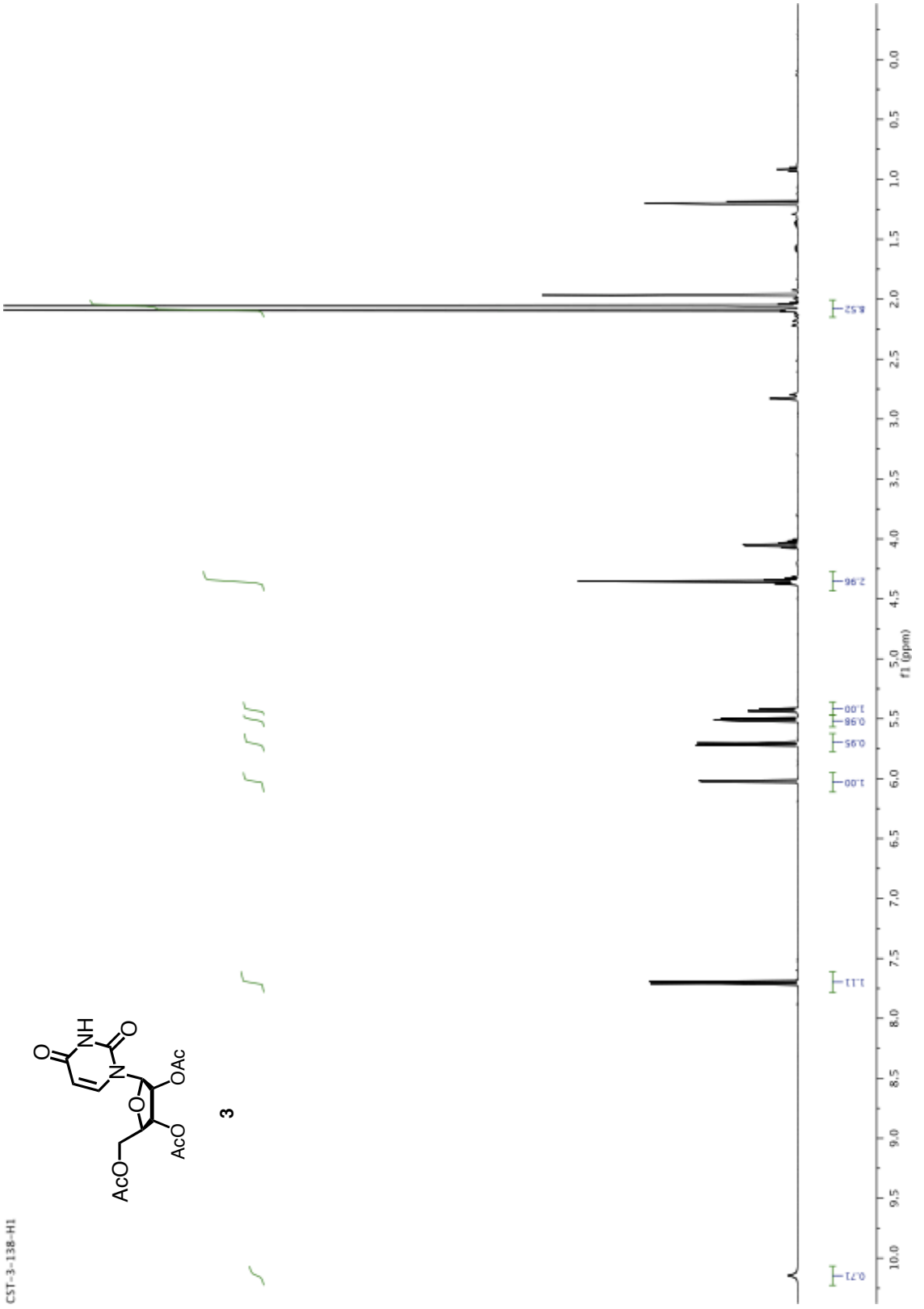
Selected single crystals suitable for X-ray crystallographic analysis were used for structural determination. The X-ray intensity data were measured at 100(2) K (Oxford Cryostream 700) on a Bruker Kappa APEX Duo diffractometer system equipped with a sealed Mo-target X-ray tube (λ = 0.71073 Å) and a high brightness IμS copper source (λ = 1.54178 Å). The crystals were mounted on a goniometer head with paratone oil. The detector was placed at a distance of 6.000 cm from the crystal. For each experiment, data collection strategy was determined by APEX software package and all frames were collected with a scan width of 0.5° in ω and ϕ with an exposure time of 10 or 20 s/frame.

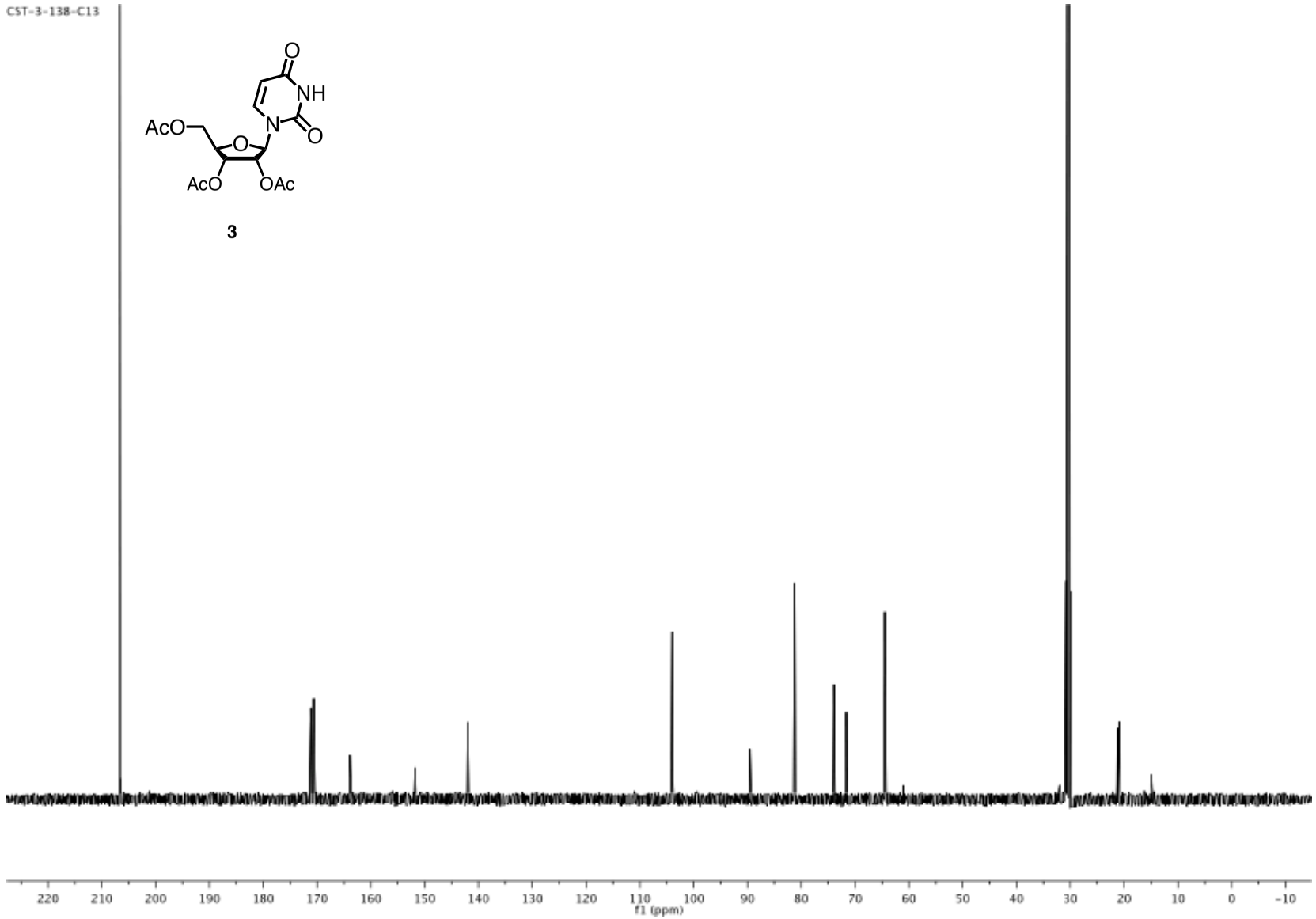
* This procedure is authored by Dr. Bo Li at the Boston College X-ray Crystallography Center.

The frames were integrated with the Bruker SAINT Software package using a narrow- frame integration algorithm to a maximum 2θ angle of 56.54° (0.75 Å resolution) for Mo data. The final cell constants are based upon the refinement of the XYZ-centroids of several thousand reflections above 20 $\sigma(I)$. Analysis of the data showed negligible decay during data collection. Data were corrected for absorption effects using the empirical method (SADABS). The structures were solved and refined by full-matrix least squares procedures on $|F^2|$ using the Bruker SHELXTL (version 6.12) software package. All hydrogen atoms were included in idealized positions for structure factor calculations except for those forming hydrogen bonds or on a chiral center. Anisotropic displacement parameters were assigned to all non-hydrogen atoms, except those disordered.



2.3.3 ^{1}H and ^{13}C NMR Spectra

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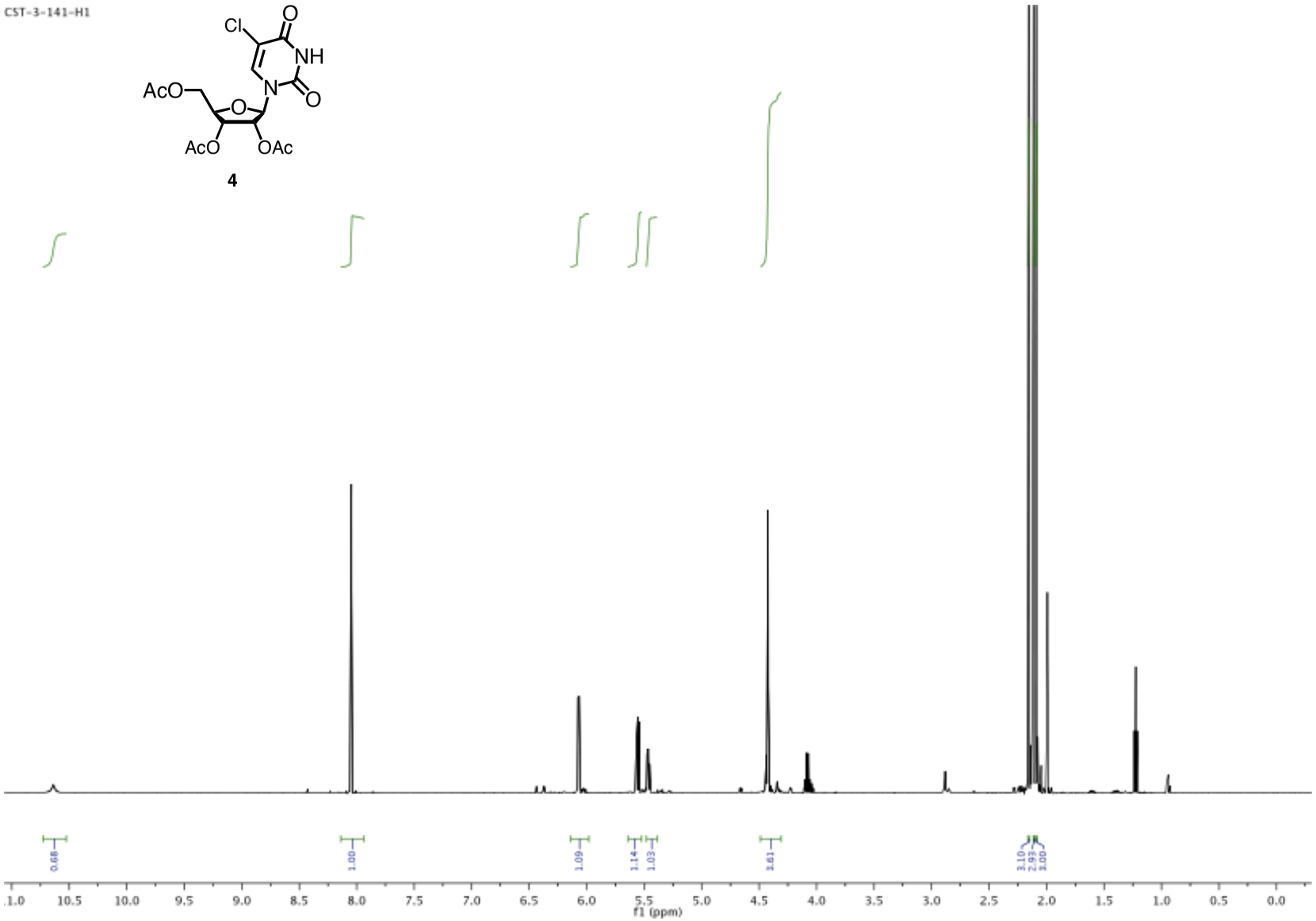
f1 (ppm)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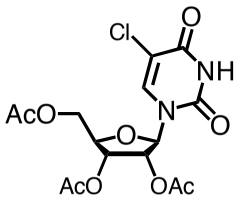

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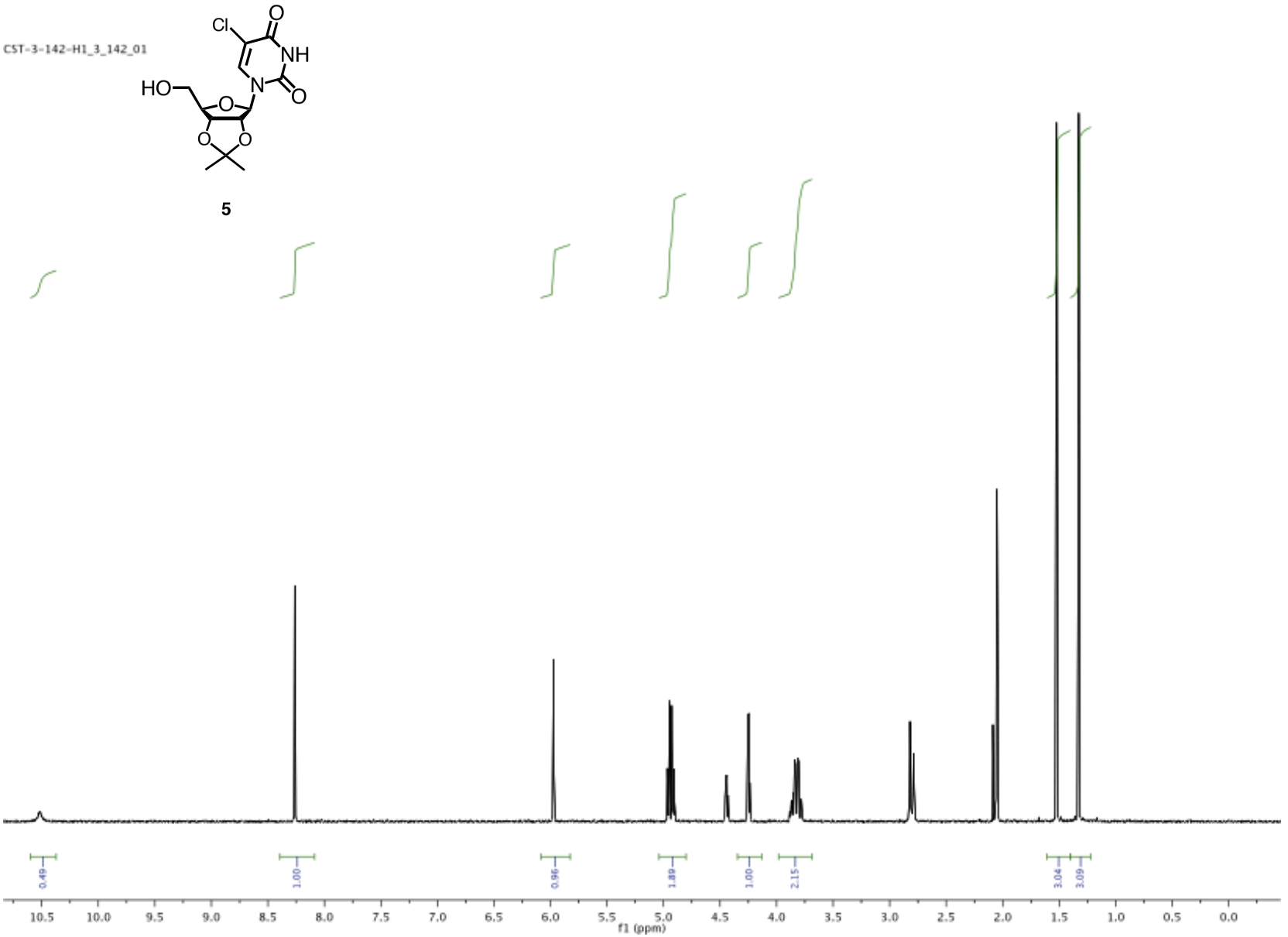
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1.00
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10.0
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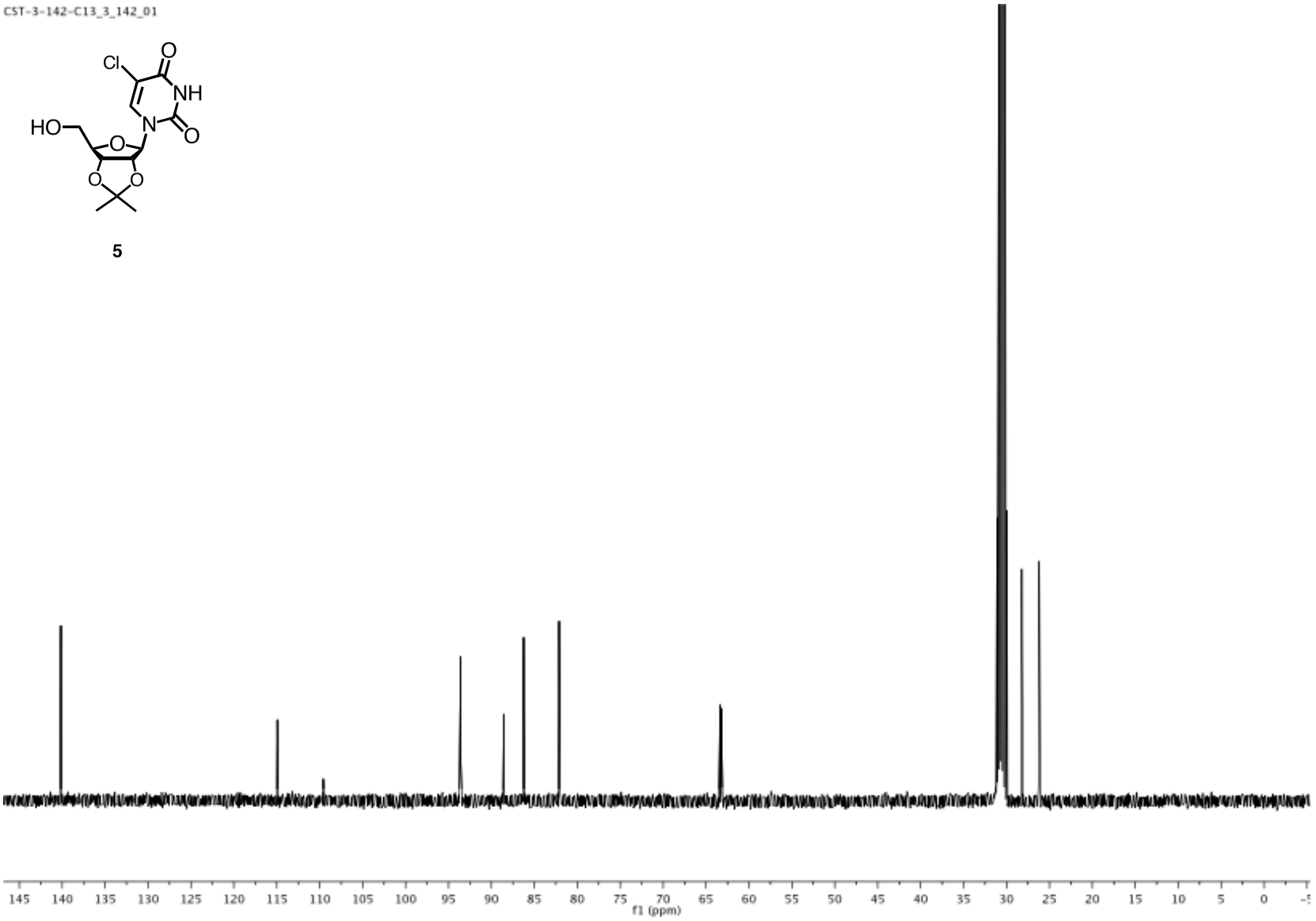
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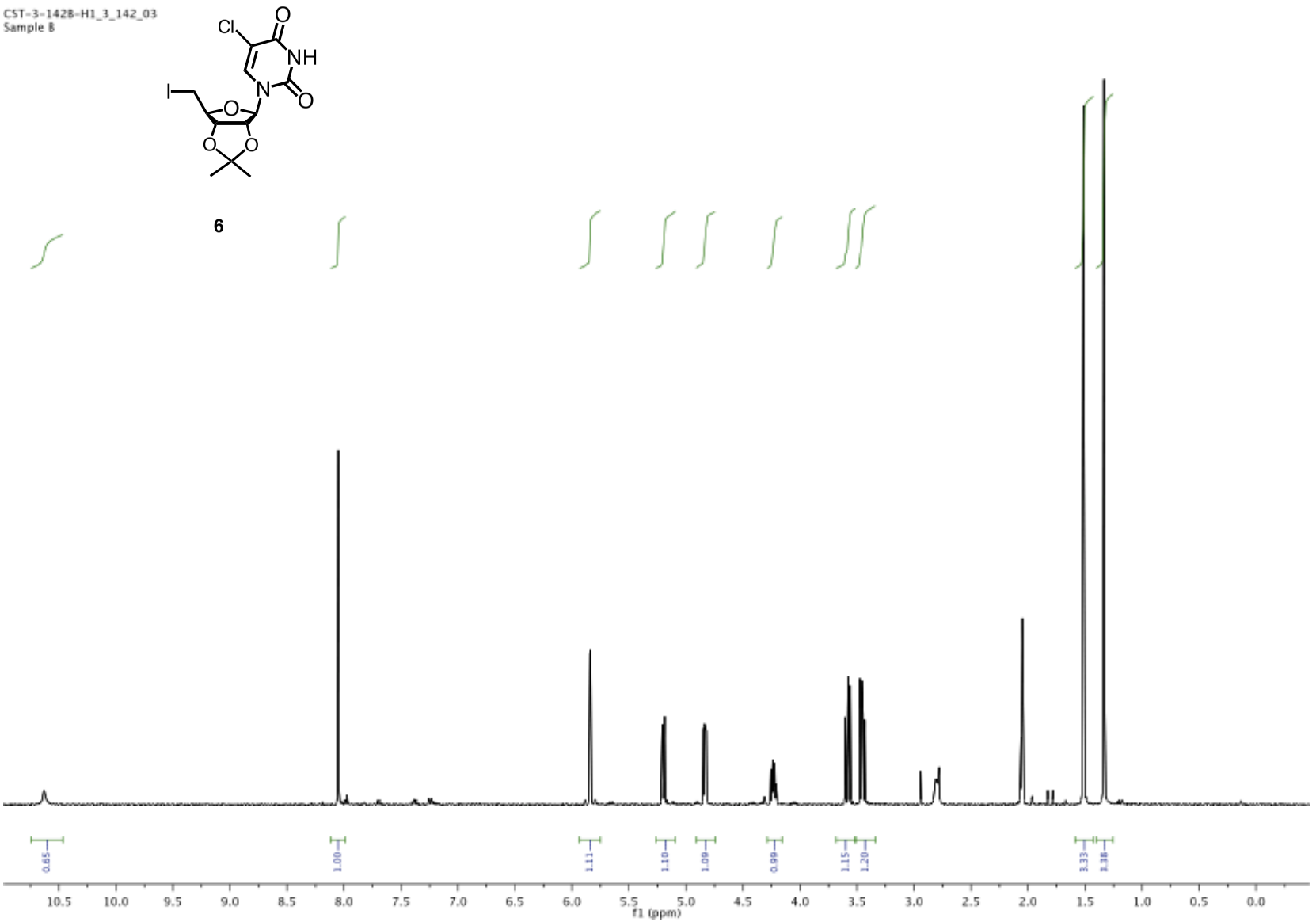
f1 (ppm)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NH
N
O
I
O
O
O
6
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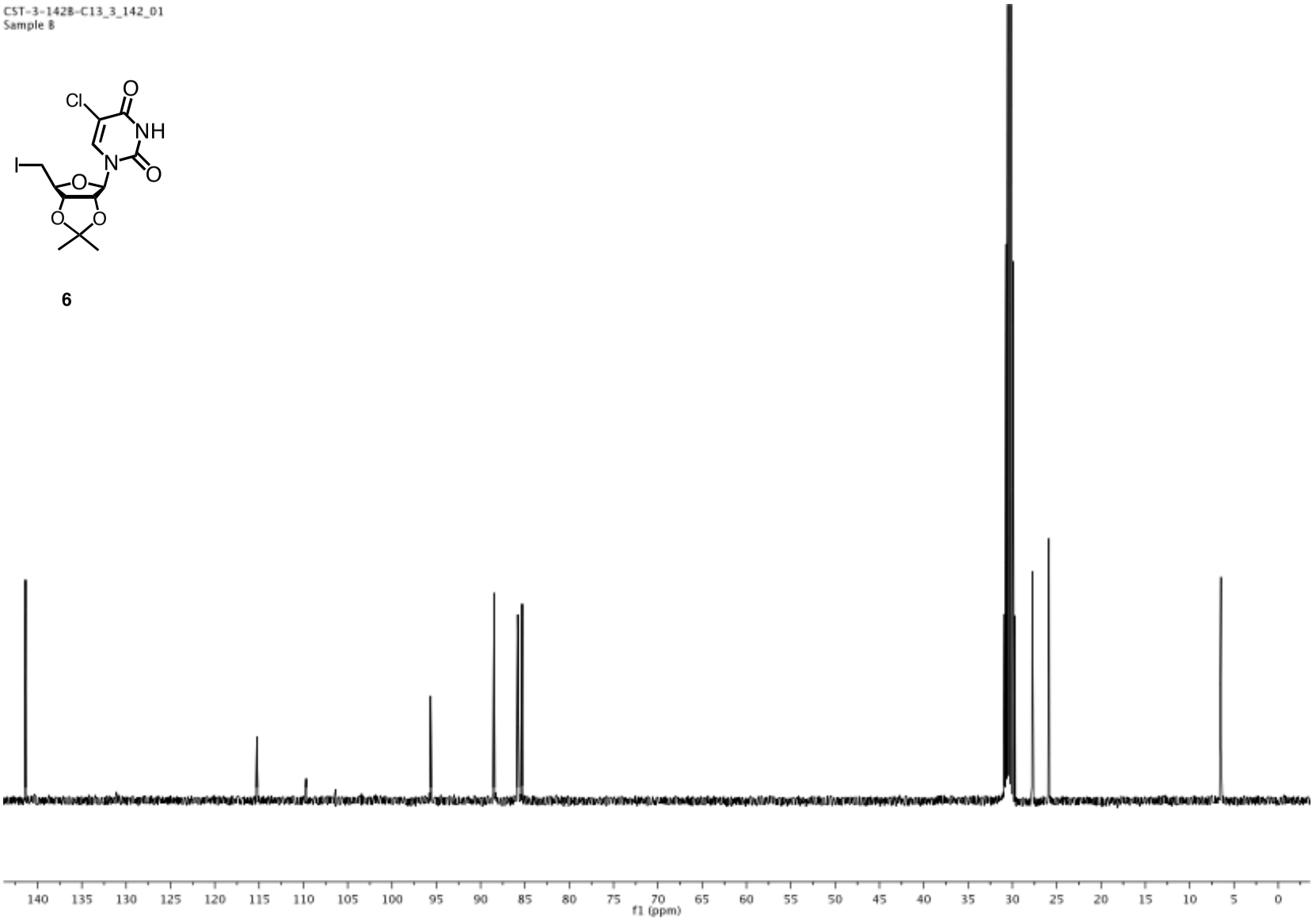
1.00
1.11
1.10
1.09
0.99
1.15
1.20
3.33
3.38
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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NH
N
O
I
O
O
O
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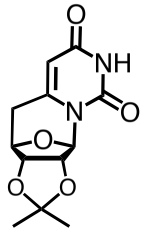
f1 (ppm)

6

CST-3-142C-H1_3_142_01
Sample B



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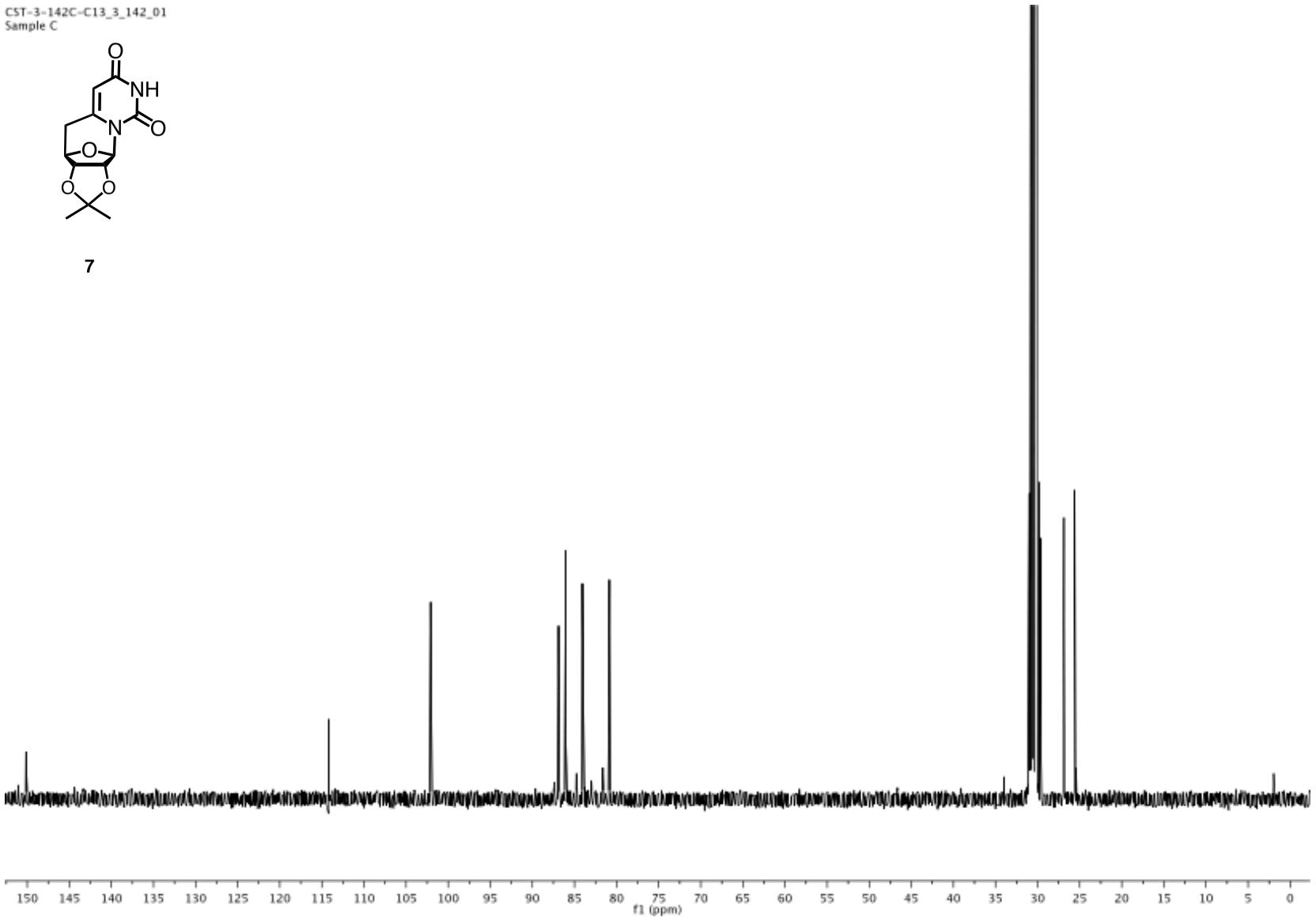
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Sample C
O
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N
O
O
O
O
7
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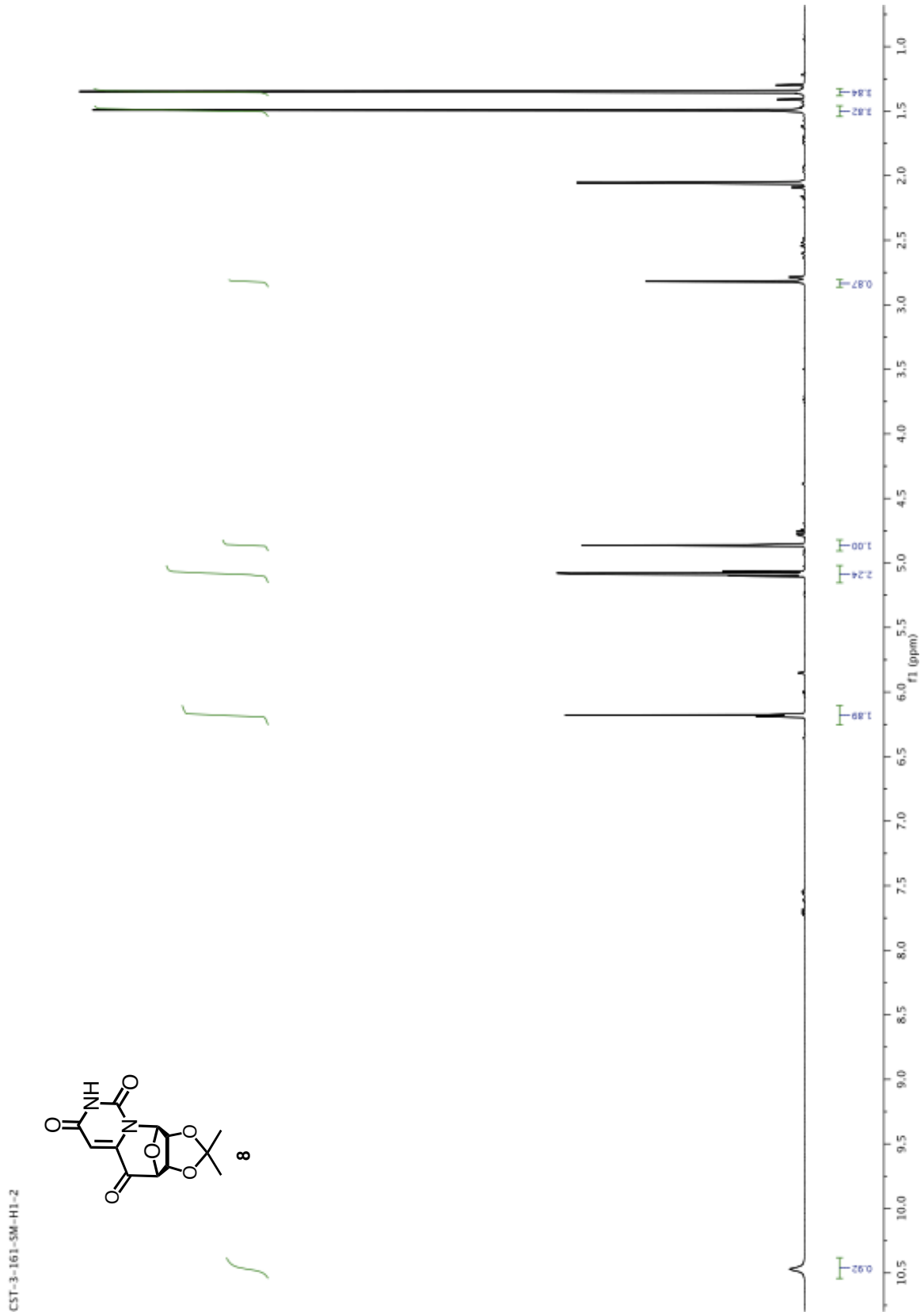
f1 (ppm)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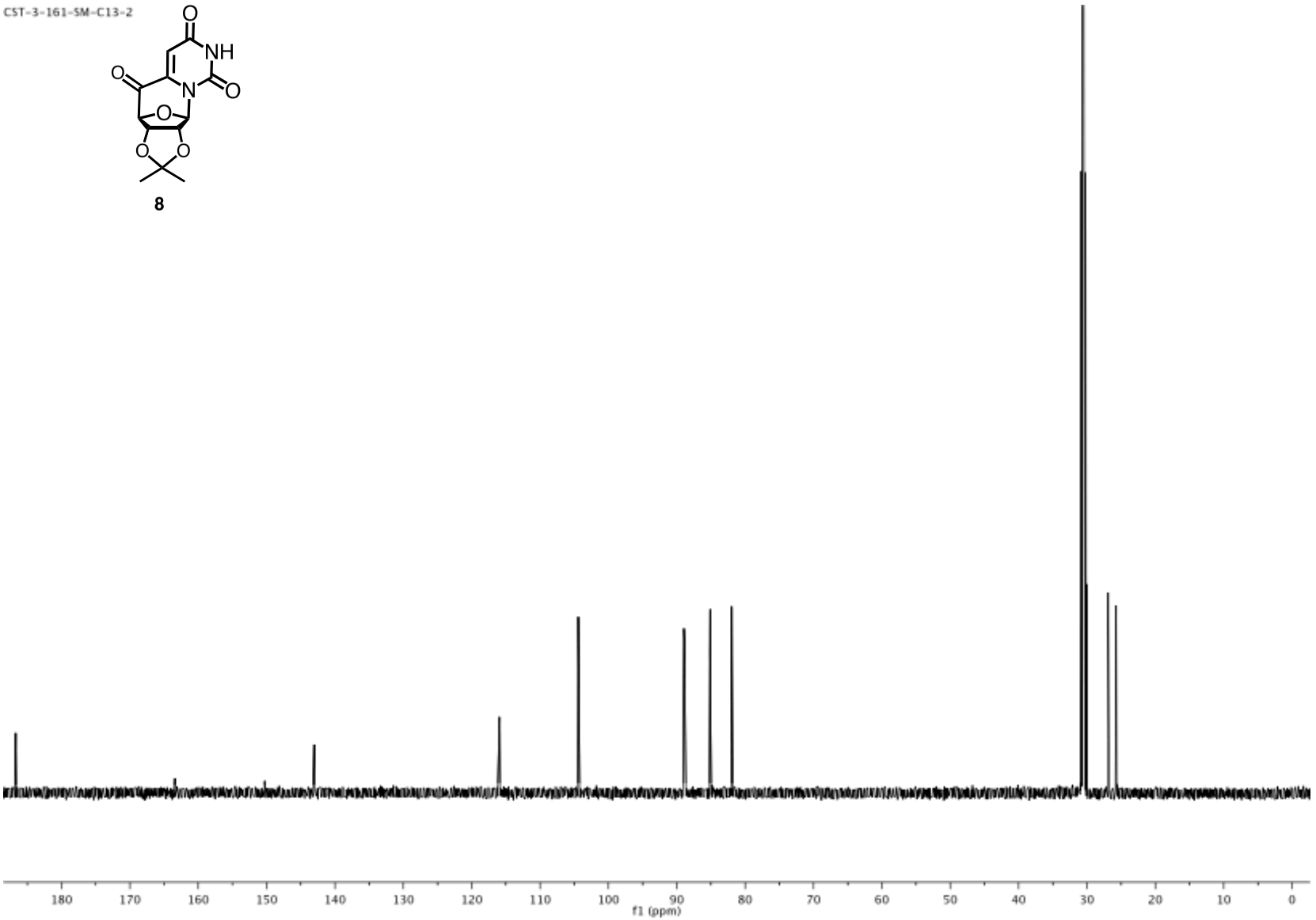
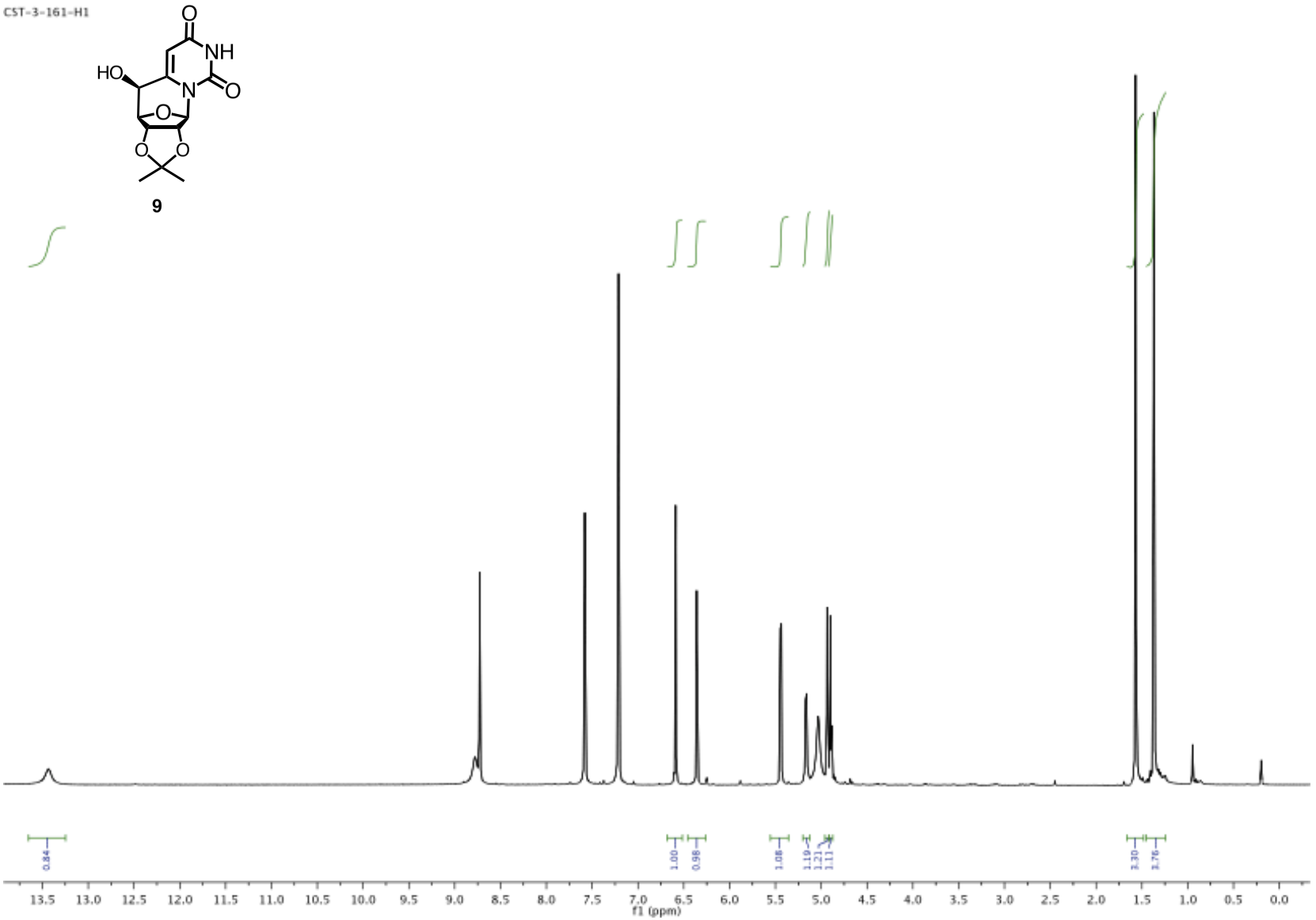
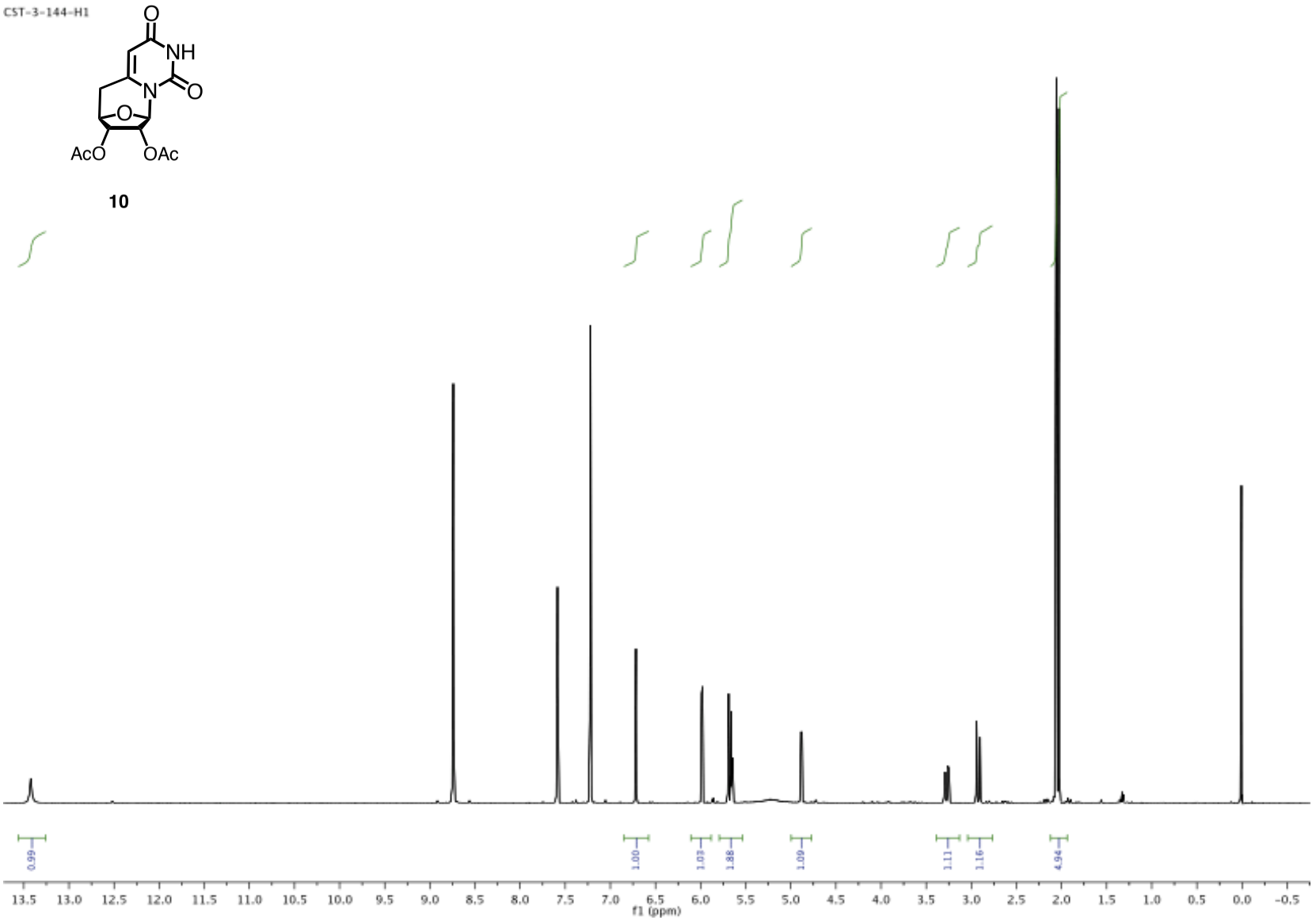
1.00
2.24
1.89
0.92
f1 (ppm)


CST-3-161-SM-C13-2
O
NH
O
N
O
O
O
O
8
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


CST-3-161-H1
HO
NH
O
N
O
O
O
O
9
0.84
1.00
0.98
1.08
1.19
1.21
1.11
3.30
2.76
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

CST-3-161-C13
9
f1 (ppm)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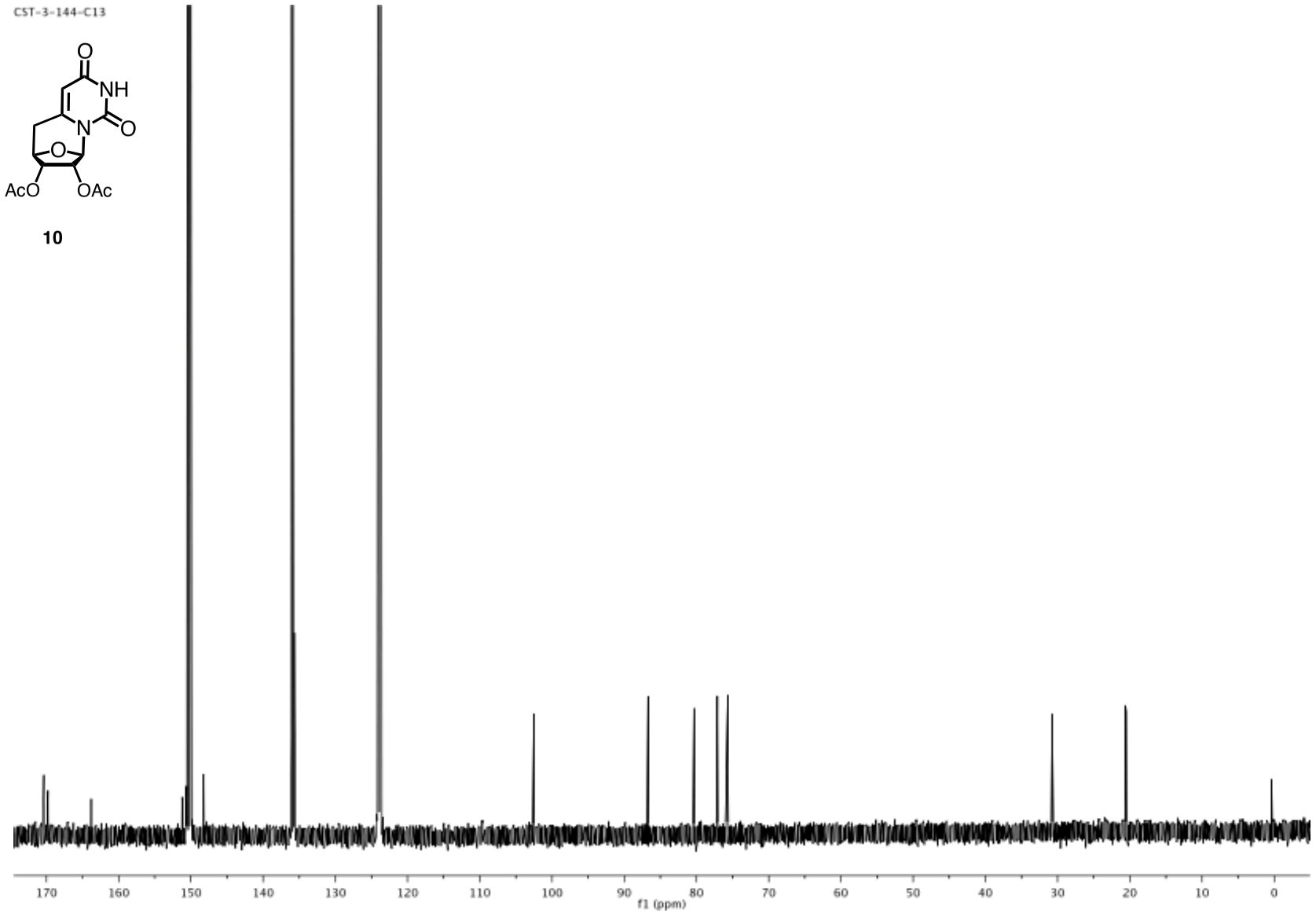
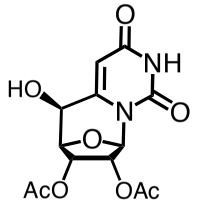
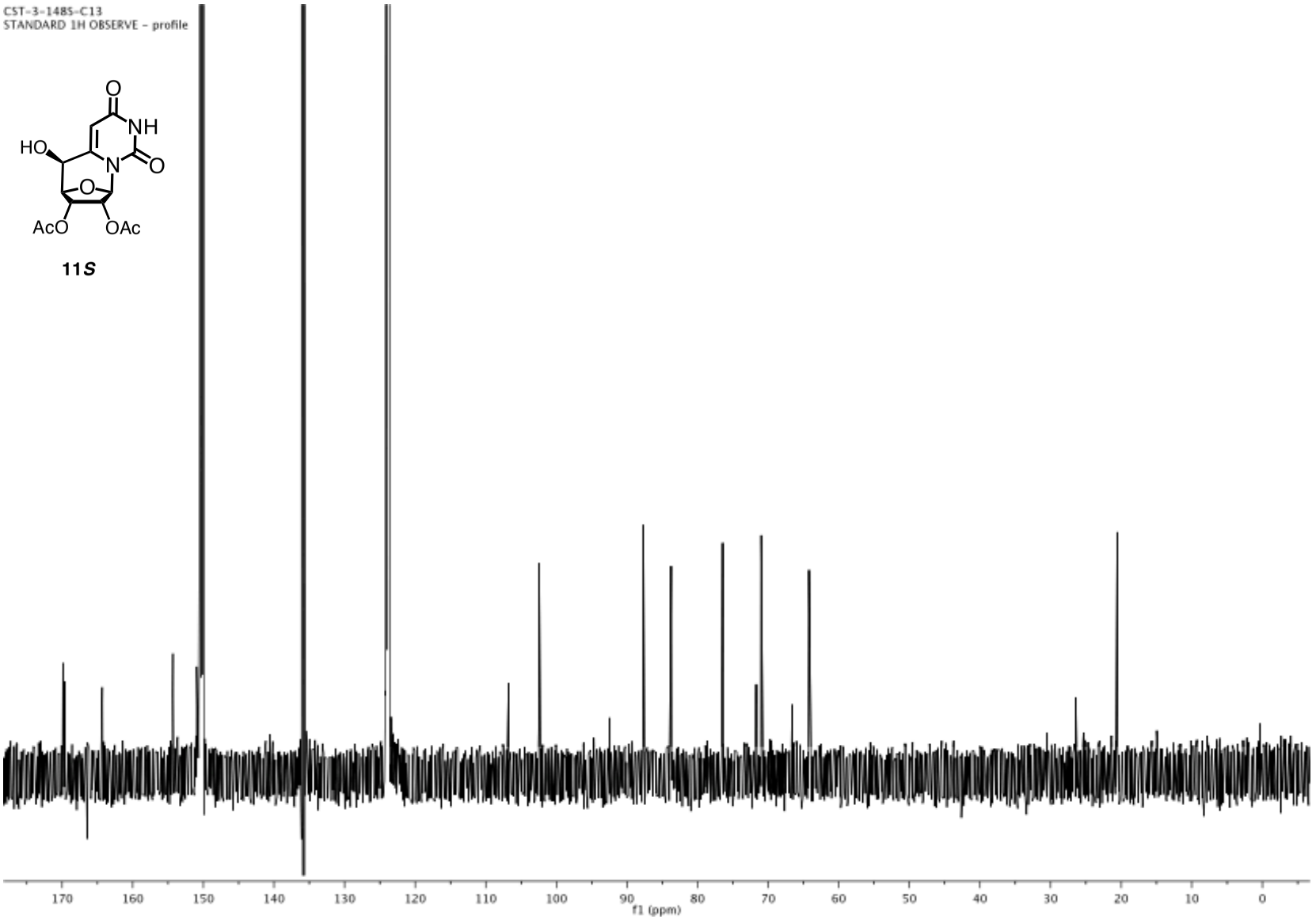
O
O
AcO
OAc
10
0.99
1.00
1.03
1.88
1.09
1.11
1.16
4.94
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

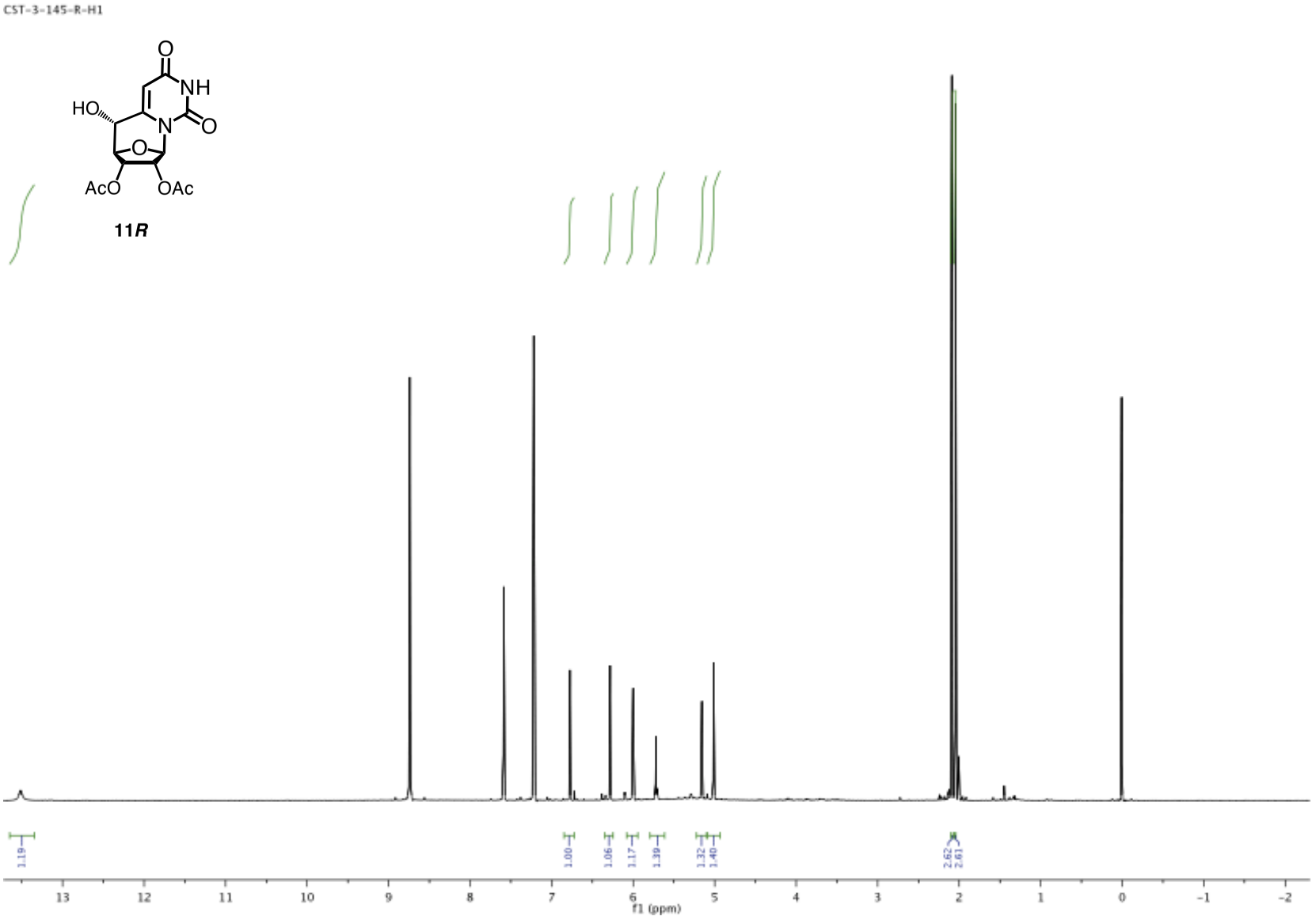

CST-3-144-C13
O
NH
N
O
O
AcO
OAc
10
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

CST-3-148S-H1
STANDARD 1H OBSERVE - profile



11*S*


CST-3-148S-C13
STANDARD 1H OBSERVE - profile
HO
O
NH
N
O
O
AcO
OAc
11S
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


CST-3-145-R-H1
O
NH
HO
N
O
O
AcO
OAc
11R
1.19
1.00
1.06
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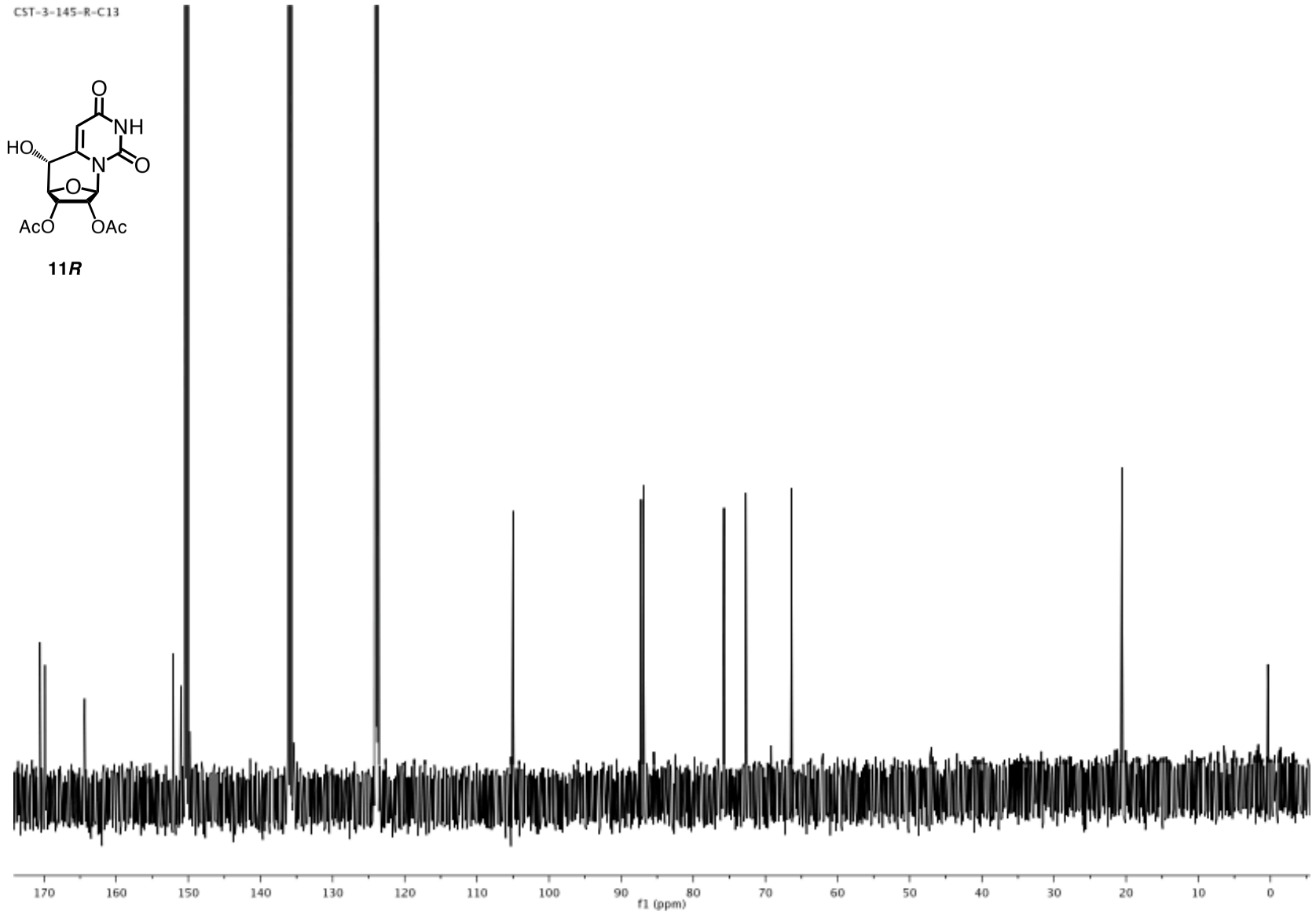
1.39
1.32
1.40
2.62
2.61
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
f1 (ppm)


CST-3-145-R-C13
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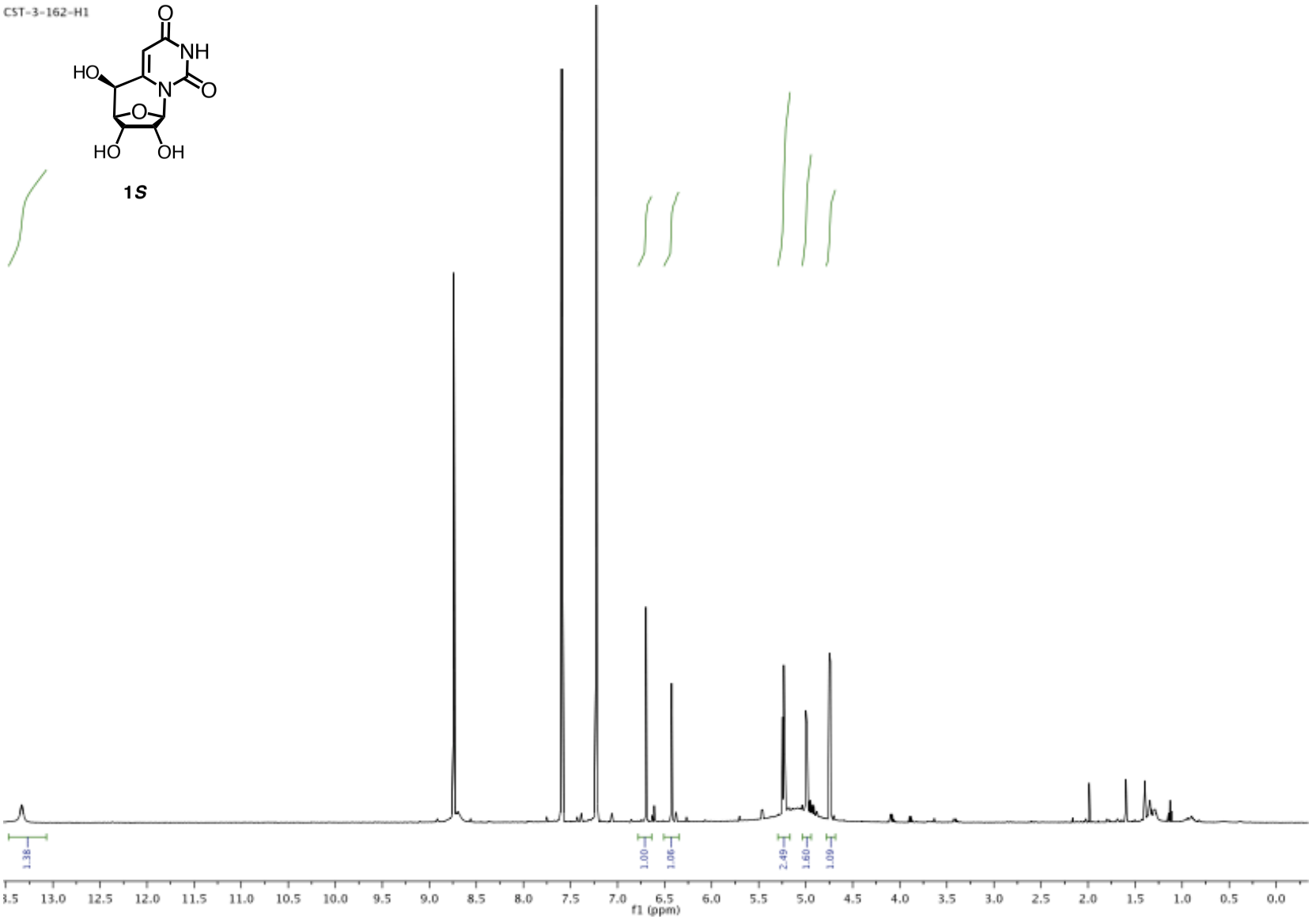
NH
HO
N
O
O
AcO
OAc
11R
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


CST-3-162-H1
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NH
HO
N
O
O
HO
OH
1S
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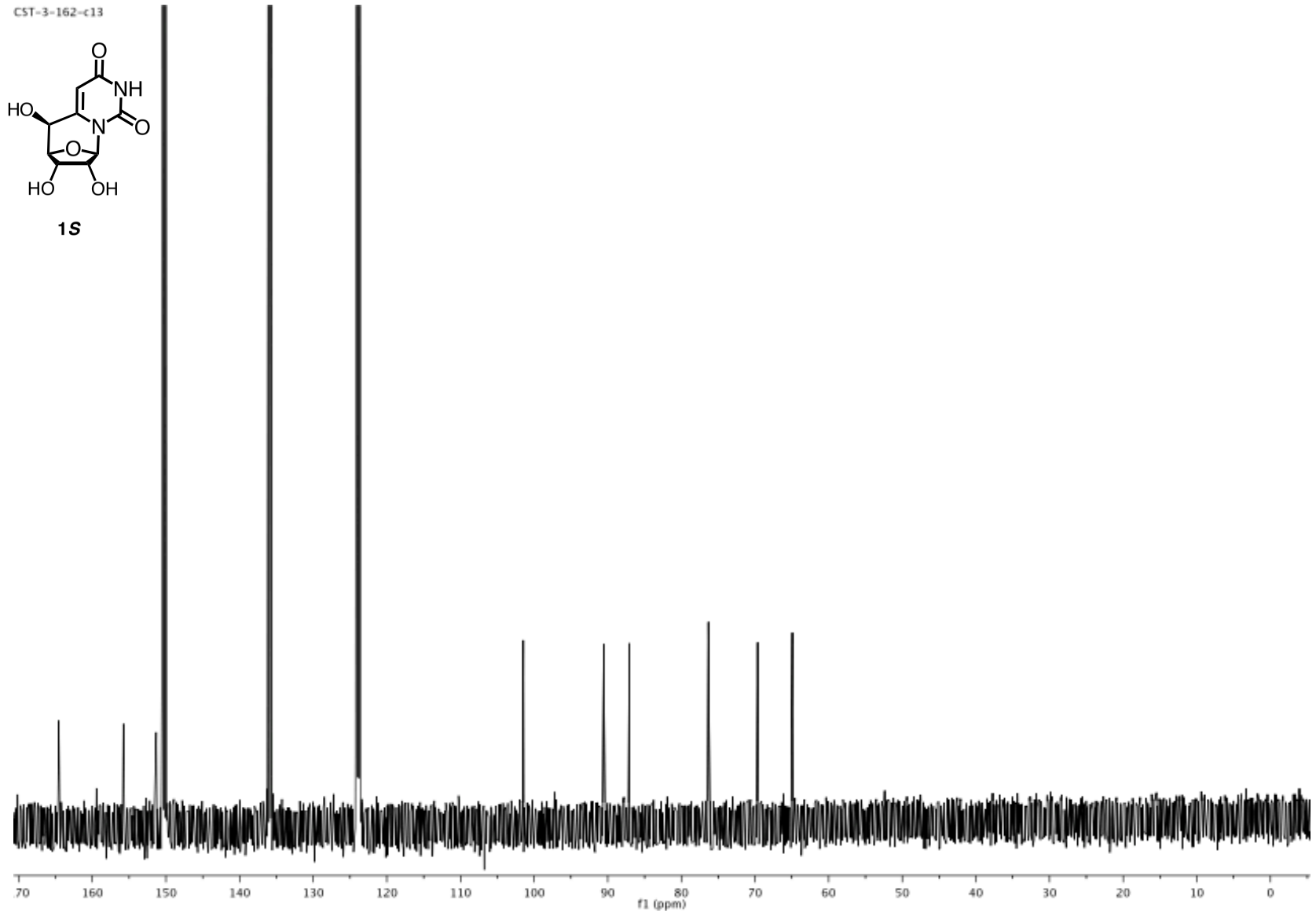
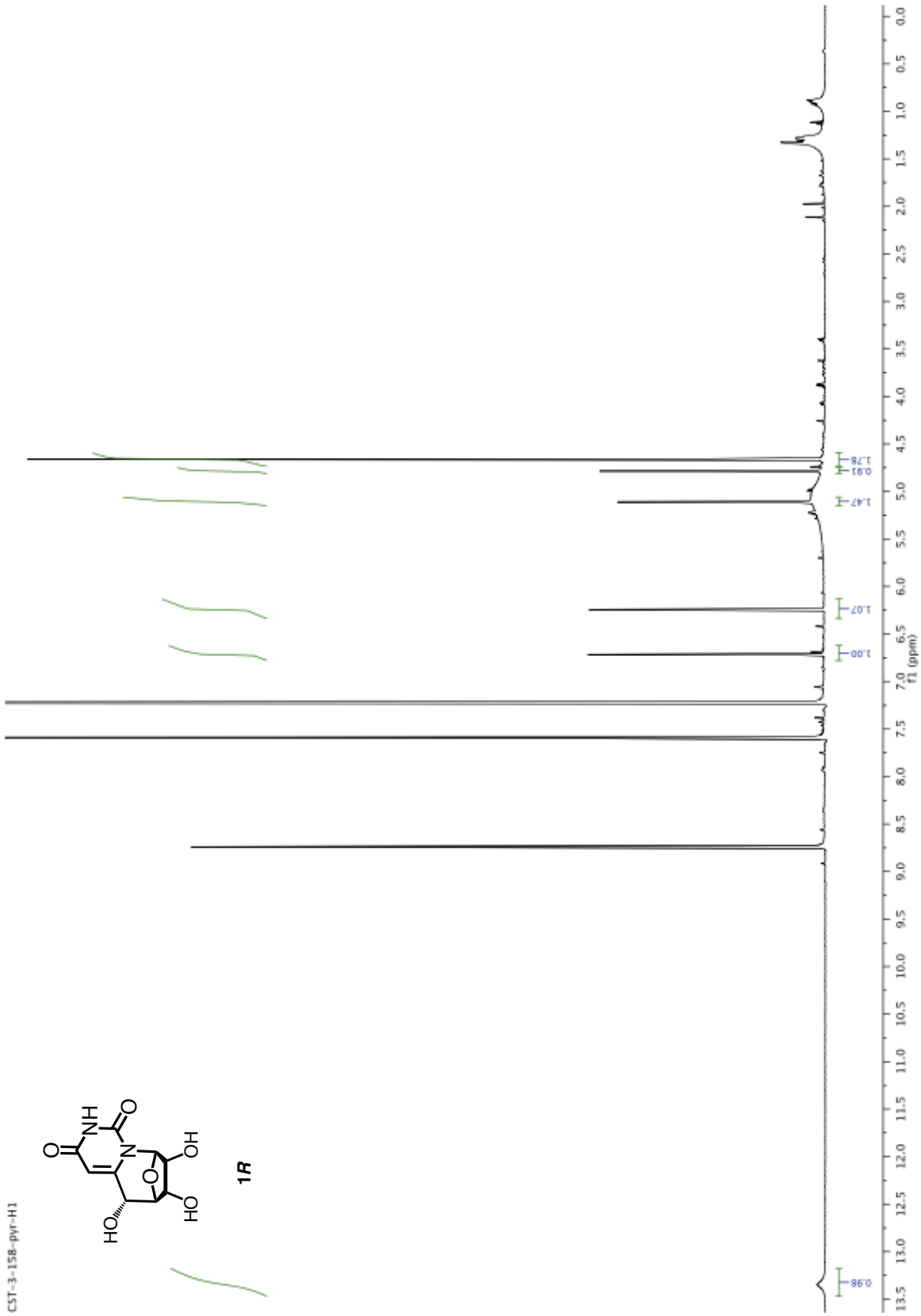
1.00
1.06
2.49
1.60
1.09
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


CST-3-162-c13
O
NH
HO
N
O
O
HO
OH
1S
70
160
150
140
130
120
110
100
90
80
f1 (ppm)
70
60
50
40
30
20
10
0


CST-3-158-pyr-H1
1R
f1 (ppm)

CST-3-158-pyr-C13
NH
O
O
N
O
OH
HO
HO
1R
f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160

Chapter 3

Synthesis and Properties of 6,6'-cyclo-2'-deoxyuridine

3.1 Introduction to Ring Expanded Cyclonucleosides

Another series of rigid nucleosides are the ring expanded cyclonucleosides. Compared to the cyclonucleosides featured in the previous chapter, they contain an additional atom between the C5' and the nucleobase. Either a new C6' carbon can be inserted in between the C5' and the base or the 5'O can be used to form the linkage (Figure 1).

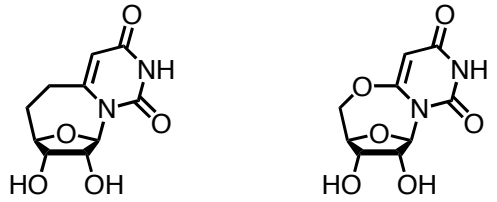


Figure 1: C6' carbon and O5' oxygen linked ring expanded cyclouridine

The 5'O linked cyclonucleosides have been observed as enzyme and ribozyme inhibitors. Rhodopsin kinase is a serine/threonine specific kinase, which is activated upon a light induced conformational change[1]. 8,5'O-cycloadenosine demonstrates high inhibition of this enzyme (K_i=27 μM) likely due to being locked in the *anti* conformation[1]. 8,5'O-cycloguanosine can be accepted by a ribozyme isolated from the protozoa *Tetrahymena* although it is processed at a rate only 6% of native guanosine[2]. While not well accepted by the ribozyme, this study confirmed that the base must be *anti* relative to the sugar in order to be processed by the ribozyme, as *syn* nucleosides were not accepted at all. 8,5'O-cycloadenosine also showed limited inhibition (10%) of platelet aggregation in rabbit plasma[3].

The antitumor agent gemcitabine is a 2'-deoxycytidine analogue that disrupts DNA synthesis and contains two fluorines at the 2' position[4]. In a study to measure the stability of gemcitabine, a solution of the drug was heated at 70 ˚C at pH 3.2 over

four days[4]. One minor isolated degradation product was the expanded 6,5'O uridine derivative (Figure 2). The H5 can be eliminated to kick off the 5'O as a leaving group to reform the 5-6 double bond on the base and break the cyclo ring.

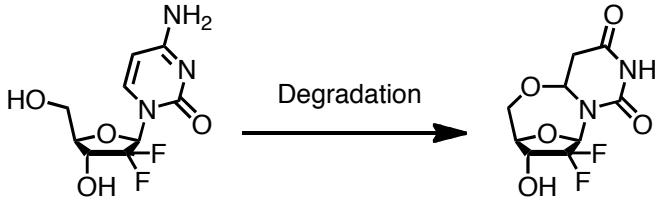
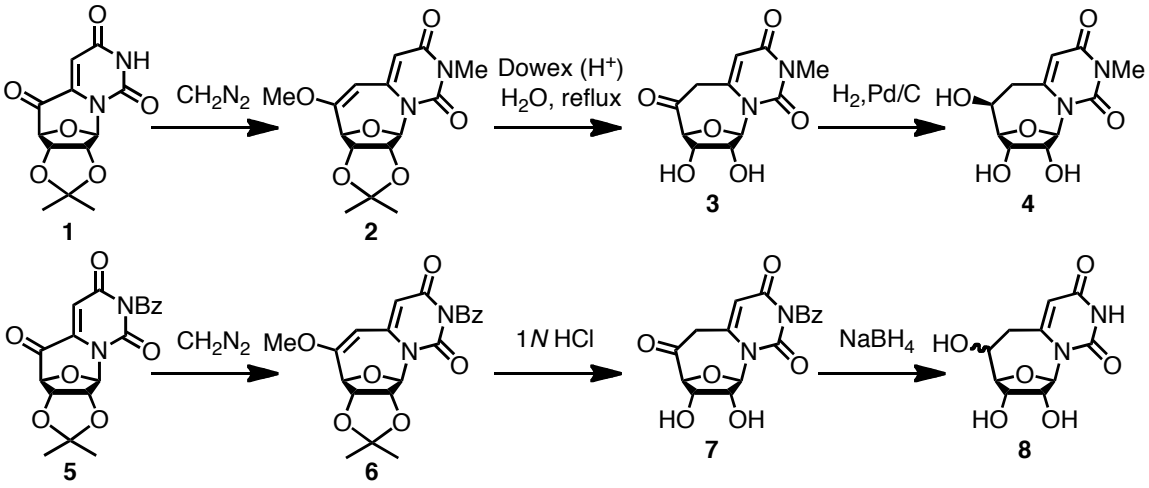


Figure 2: Degradation of gemcitabine

Although the above studies demonstrate some of the biological significance of 5'O linked cyclonucleosides, these compounds are unable to have a 5' OH without forming a less stable hemiacetal and therefore unlikely to form a 5' to 3' linked oligomer. For these reasons C6' linked nucleosides are of more interest to our group.

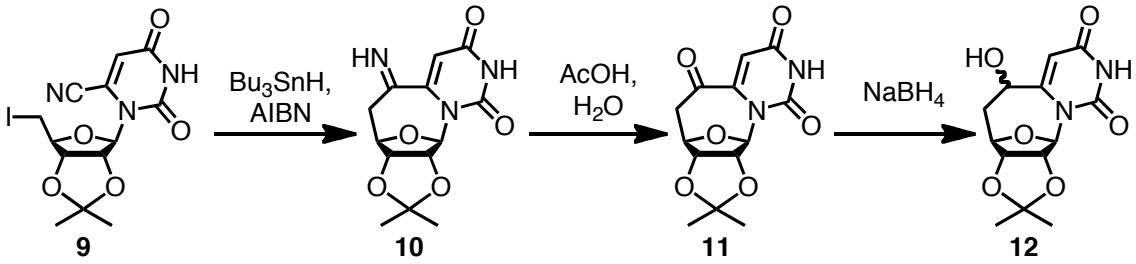
No biological studies have been performed on 8,6'-cyclopurines and 6,6'-cyclopyrimidines although several syntheses have been carried out. Otter and Falco took the C5' ketone of cyclouridine and facilitated a diazomethane ring expansion (Scheme 1)[5]. Reflux with acidic resin removed the acetonide protecting group and formed a 5' ketone, which was reduced to the *S* diastereomer **4**. This synthesis has several problems. The diazomethane step primarily forms an epoxide product and only a 20% yield of the desired ring expanded product is obtained. Similar ring expansion attempts in our lab yielded only the epoxide product and none of the ring expansion product. The N3 of the base is also methylated, eliminating the hydrogen bonding capabilities of the base. The acidic resin and reduction steps also have yields of 61% and 50% respectively. In a follow up paper, Sasson and Otter retain

the NH functionality of the base, but the synthesis is again hampered by low yields (12% for the expansion and 49% for conversion to *R* and *S* alcohols) along with extra steps related to protection of the N3 with a benzoyl group[6]. Interestingly, reduction with $NaBH_4$ yielded both diastereomers at the 5' position (Compound 8).



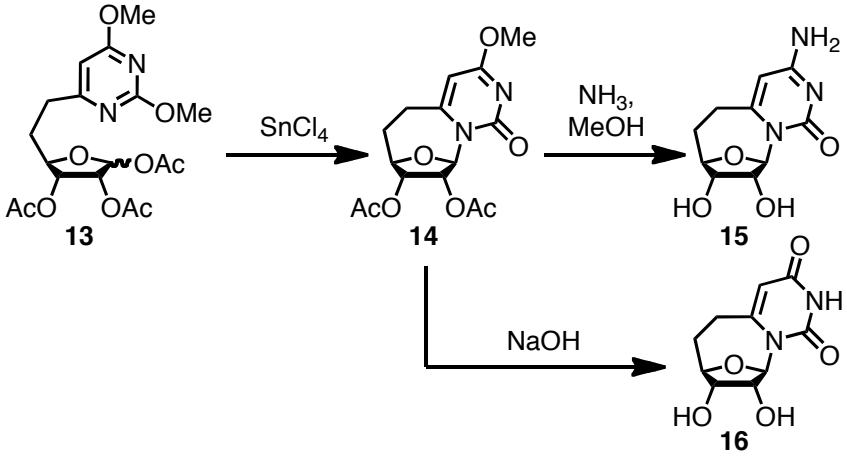
Scheme 1: Work by Otter, Falco, and Sasson on ring expanded uridine

Ueda et al. also worked on the synthesis of ring expanded cyclonucleosides. For their 6,6'-cyclouridine synthesis, they use radical cyclization conditions similar to those from their synthesis of 6,5'-cyclo-U and 6,5'-cyclo-dU. However, they installed a cyano group at the C6 position, which acts as the electrophile during the radical cyclization instead of the C5-C6 double bond (Scheme 2)[7, 8]. This creates a ketamine, which is hydrolyzed to the C6' ketone **11**. Reduction with $NaBH_4$ yields the C6' alcohol **12**. Further steps can access the C5'-C6' alkene or alkane. The drawbacks to this work are that the key cyclization step is performed in only a 35% yield and there is no installation of a hydroxyl at the 5' position.



Scheme 2: Synthesis by Ueda and coworkers

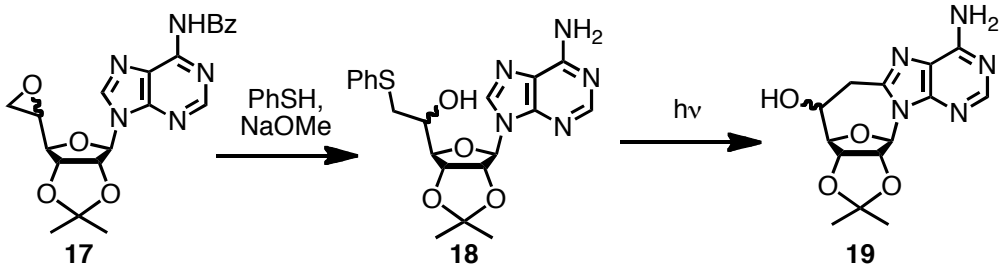
Ueda et al. designed a second synthesis for ring expanded cyclopyrimidines, which allows for access to both the uridine and cytidine monomers (Scheme 3). They tether a 2,4-dimethyoxypyrimidine base to the C6' position and then use a Lewis acid to catalyze glycosylation at the C1'. Removal of the acetate protecting groups and the C4 methoxy allow both 6,6'-cyclo-C (**15**) and 6,6'-cyclo-U (**16**) to be synthesized.



Scheme 3: Ueda and coworkers synthesis of expanded U and C derivatives

Lastly, Matsuda and Ueda synthesized a ring expanded cycloadenosine compound using a phenylthio compound similar to those presented in the previous chapter (Scheme 4)[9]. They installed the phenylthio group by opening the epoxide **17** at the C6' position. Light induced cyclization yields both diastereomers of the

product **19**, although yields were below 20% for each isomer with uncyclized byproduct dominating.



Scheme 4: *R*- and *S*-8,6'-cycloadenosine synthesis

Our goal was to create a rapid and efficient route to synthesize 6,6'-cyclo-2'-deoxyuridine. All previous work on ring expanded nucleosides was performed with the ribose sugar, with no reported syntheses of ring expanded 2'-deoxy compounds. We also wanted to prepare the 6,6'-cyclo-dU phosphoramidite for use on a DNA synthesizer and to insert the expanded nucleoside into DNA oligomers.

Based on the results presented in the previous chapter and work by Yu and Yueh in our lab, we determined that the hydrogen bonding capabilities of 6,5'-cyclopyrimidines and 8,5'-cyclopurines are severely compromised[10, 11]. Our hypothesis is that the C6' carbon would "push" the nucleobase back towards its native position to allow hydrogen bonding interactions. We also sought to test whether the fixed nature of the base would reduce entropic costs associated with gylcosidic bond rotation, similar to how LNA reduces the entropy involved in sugar pucker flipping.

1. Palczewski, K.; Kahn, N.; Hargrave, P. A., Nucleoside Inhibitors of Rhodopsin Kinase. *Biochemistry* **1990,** *29* (26), 6276-6282.
2. Lin, C. W.; Hanna, M.; Szostak, J. W., Evidence That the Guanosine Substrate of the Tetrahymena Ribozyme Is Bound in the Anti Conformation and That N7 Contributes to Binding. *Biochemistry* **1994,** *33* (9), 2703-2707.
3. Kikugawa, K.; Ichino, M.; Iizuka, K.; Higuchi, Y.; Hirayama, H., Platelet Aggregation Inhibitors .2. Inhibition of Platelet Aggregation by 5'-Substituted, 2-Substituted, 6-Substituted, and 8-Substituted Adenosines. *Journal of Medicinal Chemistry* **1972,** *15* (4), 387-&.
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6. Sasson, I. M.; Otter, B. A., The Synthesis of 6,6'-Cyclo-6'-Deoxyhexofuranosyluracils Via a Diazomethane-Promoted Ring-Enlargement Reaction. *Journal of Heterocyclic Chemistry* **1987,** *24* (5), 1439-1444.
7. Sano, T.; Inoue, H.; Ueda, T., Nucleosides and Nucleotides .54. Synthesis of 6,6'-Cyclo-5',6'-Dideoxyhexofuranosyluracils Via Radical Cyclization of a 6-Cyanouridine. *Chemical & Pharmaceutical Bulletin* **1985,** *33* (5), 1856-1860.
8. Ueda, T.; Shuto, S.; Sano, T.; Usui, H.; Inoue, H., Synthesis of carbon-bridged pyrimidine cyclonucleosides. *Nucleic Acids Symp. Ser.* **1982,** *11* (Symp. Nucleic Acids Chem., 10th, 1982), 5-8.
9. Matsuda, A.; Ueda, T., Nucleosides and Nucleotides .66. Synthesis of 8,6'-Cyclo-6'-Deoxyhexofuranosyladenines - Adenosines Fixed in an Anti-Conformation. *Chemical & Pharmaceutical Bulletin* **1986,** *34* (4), 1573-1578.
10. Yueh, H.; Yu, H.; Theile, C. S.; Pal, A.; Horhota, A.; Greco, N. J.; McLaughlin, L. W., Synthesis and Properties of DNA Containing Cyclonucleosides. *Nucleosides, Nucleotides and Nucleic Acids* **2012,** *Advanced Article*.
11. Theile, C. S.; McLaughlin, L. W., An efficient synthetic approach to 6,5 '-(S)- and 6,5 '-(R)-cyclouridine. *Chemical Communications* **2012,** *48* (45), 5587-5589.

3.2

Synthesis of *S*-6,6'-cyclo-2'-deoxyuridine Featuring a Unique Barbier Style Cyclization*

Christopher Stone Theile and Larry W. McLaughlin

Department of Chemistry, Merkert Chemistry Center, Boston College,

Chestnut Hill, MA 02467

* Theile, C.S. and McLaughlin, L.W. *RSC Advances,* **2012**, Accepted
Note: Compound, scheme, and reference numbers reset in this section. References listed at the end of section 3.3.

Here we present the synthesis of the novel nucleoside 6,6'-(*S*)-cyclo-2'-deoxyuridine (1). This efficient synthesis is carried out starting from 2'-deoxyuridine and reaches the deprotected product in few steps and high yield. Crystal structures of the product indicate that it is a very good structural mimic of thymidine as found in DNA duplexes. This rigidified nucleoside locks the uracil base in the *anti* conformation, positioning the nucleobase for Watson-Crick duplex formation at significant entropic savings. The structure of 6,6'-(*S*)-cyclo-2'-deoxyuridine maps more favorably onto naturally occurring nucleosides than previous cyclo nucleosides. The synthesis features a unique Barbier style cyclization, expanding on Luche and Sarandeses allyl zinc additions, to create a 7-membered ring in good yield.

Due to their complex structural motifs and high functionalization, non-natural nucleosides present opportunities for the development of novel chemical strategies that can benefit the synthetic community at large. Cyclonucleosides have been targets of the nucleoside community due to their rigid geometry. A second linkage between the 5'-carbon on the sugar to the nucleobase fixes the base in the *anti* conformation, lowering the entropy associated with rotation of the base. Previous work on cyclo-uridine and cyclo-2'-deoxyuridine has featured a direct linkage between the 5' position of the sugar and the C6 position of the pyrimidine base[1-8]. This bond "pulls" the base back from its native Watson and Crick bonding position, so that it can no longer effectively hydrogen bond in a duplex[1]. The torsion angles are also not optimized for 6,5'-cyclo-2'-deoxyuridine[2]. The χ angle, which

relates the position of the base to the C1'-O5' bond, adopts a position closer to that of a ribonucleoside.

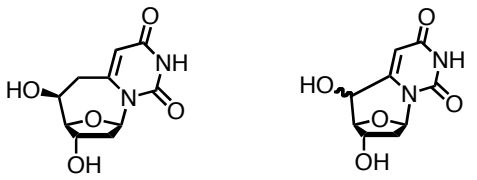
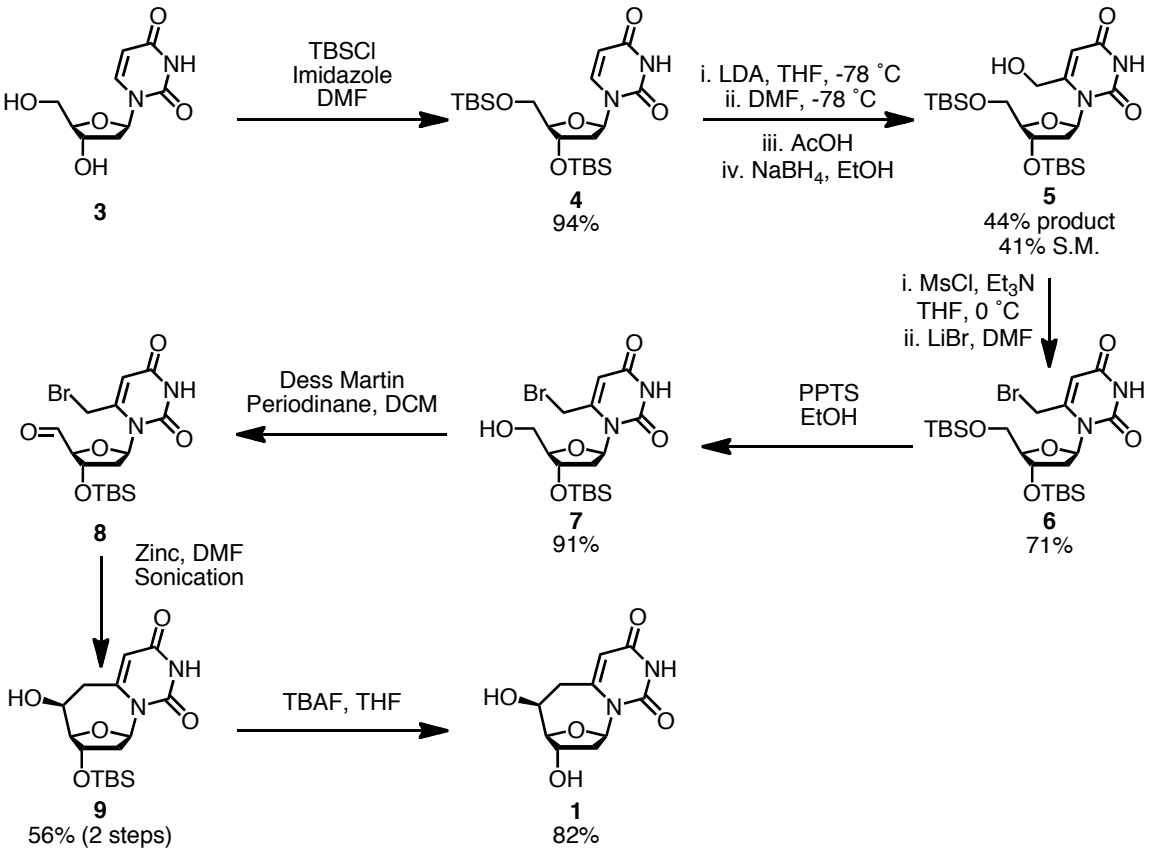


Figure 1: 6,6'-(*S*)-cyclo-2'-deoxyuridine and 6,5'-cyclo-2'-deoxyuridine

We predicted that by inserting a methylene group into the linkage, we could effectively "push" the base back toward its preferred position. The spacer would allow for a minimal amount of flexibility so the base can adopt a χ angle similar to what is observed in B-form DNA[9]. The base will still be locked into the *anti* position. When inserted into oligonucleotide strands, we predict that preorganization of the base into its Watson and Crick conformation will lower the entropic costs associated with rotation around the glycosidic bond.

Here we present the first synthesis of 6,6'-(*S*)-cyclo-2'-deoxyuridine. Previous work from Ueda et al. produced the ribonucleoside, 6,6'-cyclouridine, lacking a 5' OH and 8,6'-cyclo-adenosine[10-13]. Otter et al. was able to produce a modified, ribose variant of 6,6'-cyclouridine with a 5' OH, but the work relies on the use of diazomethane, which results in methylation of the N3 position, making studies related to duplex formation impossible[10-12, 14-16]. Eventually they were able to make the 6,6'-cyclouridine, but the synthesis was long and suffered from low yields[16]. Our synthesis of 6,6'-(*S*)-cyclo-2'-deoxyuridine takes only seven steps, highlighted by a cyclization, yielding the newly formed 7-membered ring. Due to synthetic limitations the uracil base was utilized instead of the naturally occurring

thymidine base, which prevents access to the cyclo bridge. Previous work has shown that deoxy-uridine is a sufficient mimic for thymidine[17-19].



Scheme 1: The synthesis of 6,6'-(*S*)-cyclo-2'-deoxyuridine

Our synthesis begins with a protection of the 3' and 5' hydroxyls with TBS chloride. We then carry out a one-pot multistep reaction to install a methyl-hydroxyl linkage on the C6 position of the base of **4**[20]. LDA is used to deprotonate the C6 position, which subsequently adds to dimethylformamide. After neutralization with acid, the aldehyde is reduced to the alcohol with $NaBH_4$ to form **5**. The resulting alcohol is then brominated in a two-step, one-pot reaction. The 5' TBS group is removed under mild conditions with PPTS to make **7**[21]. This allows for the 5' OH to be selectively oxidized with Dess Martin periodinane, yielding aldehyde

8. This product decomposes on silica, so the crude material is carried forward for the cyclization reaction.

To form the 7-membered ring **9** in good yield and excellent purity we modified Luche and Sarandeses' zinc allylation Barbier-style reaction[22, 23]. This chemistry has exclusively been used to perform intermolecular addition of allyl halides to aldehydes. Applying this chemistry towards an intramolecular cyclization significantly increases the scope of the reaction.

Seven membered rings have often created challenges in syntheses, and this reaction allows for an expansion of the toolbox to tackle molecules featuring this molecular framework.

A sonicator is employed to activate the zinc powder, allowing the cyclization to occur under mild conditions and with inexpensive reagents. Although the 56% 2-step yield is moderate, we hypothesize that the cyclization step is high yielding and the lower yield is due to the poor stability of compound **8**. Wittig reactions on other 5' aldehyde nucleosides in our lab have proceeded in approximately 50% yields over the 2-step procedure, reinforcing these results (unpublished results). TBAF deprotection of the 3' position results in the desired product **1**. Only a single diastereomer was observed by NMR and LC/MS analyses.

We were able to obtain a crystal structure of the final deprotected product, **1**. This confirmed that the stereochemistry of the 5' OH was in the S configuration, which was formed exclusively during the Barbier cyclization. Presumably the selectivity is controlled by zinc chelation; however, it is unclear which functional groups interact with the zinc metal to give the observed product, but further studies

are being done in our lab to test the selectivity of this reaction on other substrates. Several attempts were made to invert the hydroxyl to give the *R* stereochemistry, but they either gave elimination products or starting material.

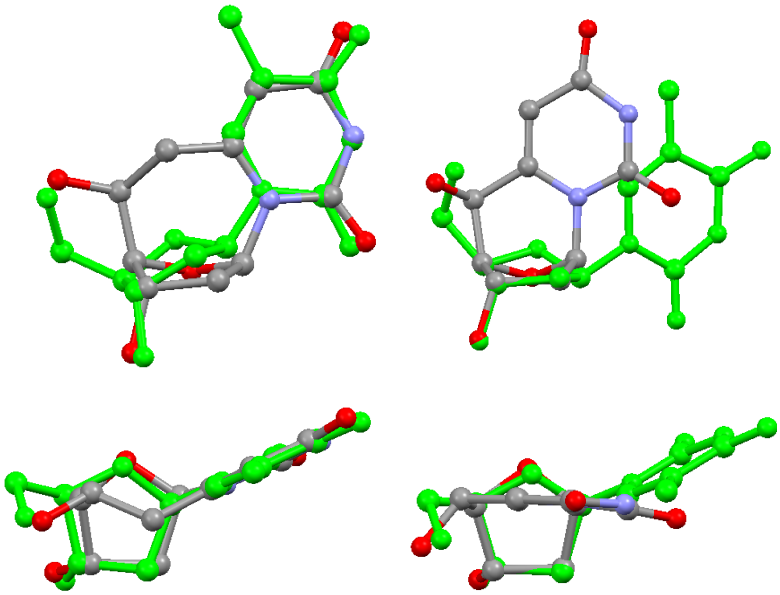


Figure 2: A front and top view of 6,6'-(*S*)-cyclo-2'-deoxyuridine, shown on the left, compared to 6,5'-(*S*)-cyclo-2'-deoxyuridine on the right. Both are overlayed with thymidine.

When the atoms on the nucleobase, as well as the 3' and 5' oxygen atoms of **1** were overlaid with the corresponding atoms from a crystal structure of thymidine that was cut from a Dickerson dodecamer B-form double helix, our product matches quite favorably (Figure 2)[24]. Conveniently the *S* diastereomer compares more favorably than the unobserved *R*, as the *R* configuration would cause the 5' OH to be in front of the C4'-O bond of the sugar ring. The root mean squared deviation of the relevant atoms averaged 0.293 Å. The χ angle of our crystal is -112.59°, which is in the middle of the range usually adopted by deoxynucleotides of about -95° to -125°. Comparatively, the previously synthesized *R* and *S* diastereomers of 6,5'-cyclo-2'-deoxyuridine monomers were very poor mimics[1]. Since the base is "pulled"

backwards from the Watson and Crick bonding position, either the position of the backbone hydroxyls or the base is compromised to the extent that it cannot form stable duplexes. The χ angle is also -152.11°, which is closer to a ribonucleoside than a deoxynucleoside.

Conclusions

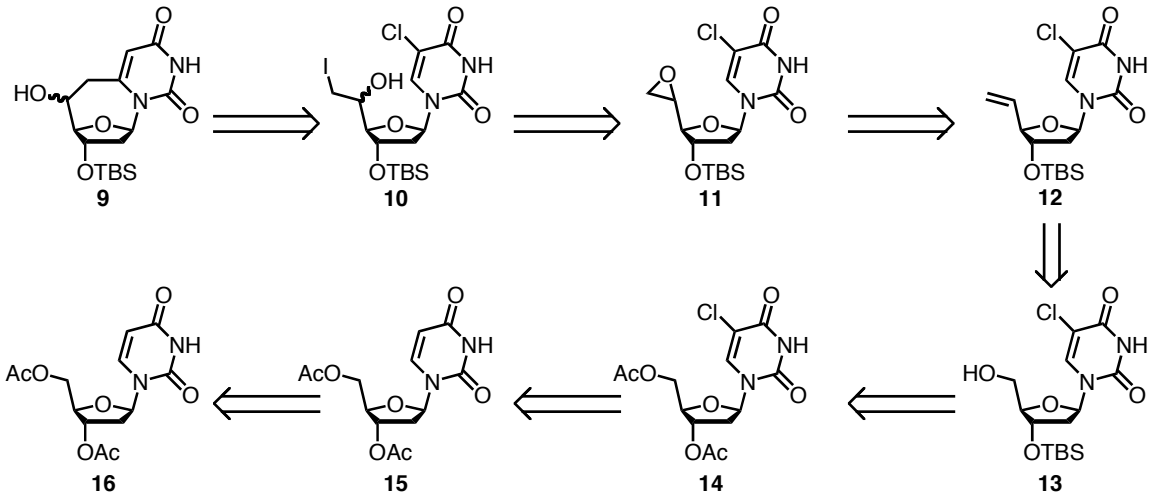
In summary, we present a rapid and high yielding synthesis of 6,6'-(*S*)-cyclo-2'-deoxyuridine. This molecule locks the base in the *anti* position while maintaining proper positions of the hydrogen bonding face and the 3' and 5' hydroxyls. The synthesis featured a unique Barbier cyclization, expanding the scope of Luche and Sarandeses' zinc allylation chemistry beyond intermolecular additions to an intramolecular cyclization of a medium sized ring. The cyclization is carried out in high yield using low cost reagents and mild conditions without affecting other regions of complex functionality. Ongoing investigations in our lab will indicate how the ring expanded cyclo linkage will affect the stability of an oligonucleotide strand. We are also currently exploring what features influence the selectivity observed during the cyclization. This molecule will allow us to probe enzymes associated with pyrimidine synthesis, such as thymidylate synthase, after monophosphorylation of the 5' OH. Ring expanded cyclo-dU also has great potential to test the promiscuity of bacterial and eurkaryotic DNA polymerase enzymes after incorporation into the template strand, or as a triphosphate monomer.

Notes and references

We would like to thank Dr. Bo Li and Marek Domin for their assistance with the crystal structure and mass spectroscopy respectively, Christopher Pace and Azade Hosseini for LC/MS analysis, and Tyler Mann for assistance with optical rotation measurements. We would also like to acknowledge Dr. Carl Christianson and Dr. Mark Schlegel for providing useful insight for the preparation of the manuscript. Lastly, we would also like to thank the NSF for funding this research.

3.3 Previous Work Towards 6,6'-cyclo-2'-deoxyuridine*

The original plan to synthesize 6,6'-cyclo-2'-deoxyuridine was to build out the 6' carbon from the C5' position and cyclize onto the base, as had been done in our lab's synthesis of 6,5'-cyclouridine and 6,5'-cyclo-2'-deoxyuridine, and shown in the retrosynthetic analysis below.

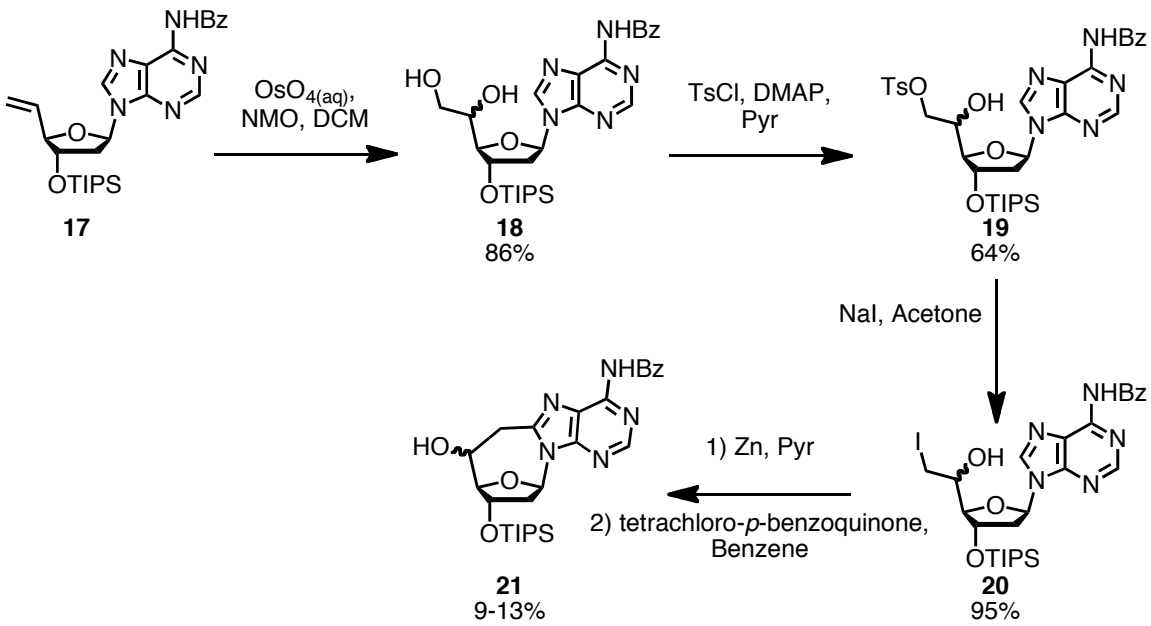


Scheme 2: Original retrosynthesis plan for 6,6'-cyclo-2'-deoxyuridine

* ***Note:*** Sections 3.3 and 3.4 were not part of the *RSC Advances* manuscript. References for the manuscript are listed after these sections.

The synthesis used a combination of reactions, which had been previously employed in our lab to reach **12**. Compound **14** was easily obtained by a global acetate protection followed by chlorination using CAN and LiCl[1, 2]. Simple protecting group chemistry yielded compound **13** from **14** over four steps. The acetates were removed, the 5' OH was selectively protected with DMTr, the 3' OH was silated, and the DMTr was removed under acidic conditions. Compound **12** was obtained in two-steps. First the 5' OH was oxidized to an aldehyde, which was followed by a Wittig reaction to obtain the olefin.

We successfully synthesized epoxide **11** using a mCPBA oxidation. However, all attempts to open the epoxide to form the 6' iodinated product failed. During the reaction, a new spot was observed by TLC, leading us to believe that product was forming. Yet upon workup and purification only starting material was recovered. We hypothesize the epoxide was opening in the reaction mixture, but upon workup, the 5' OH collapsed back on the 6' carbon to reform the epoxide and kick off the iodine as a leaving group. Several different iodination procedures were attempted but no product was ever obtained. We also tried to trap the 5' OH in the reaction mixture by adding acetic anhydride to the reaction after the disappearance of starting material was observed by TLC. This method was unsuccessful, possibly due to the steric crowding at the 5' position post iodination.



Scheme 3: Synthesis by Yueh et al. of 8,6'-cyclo-2'-deoxyadenosine

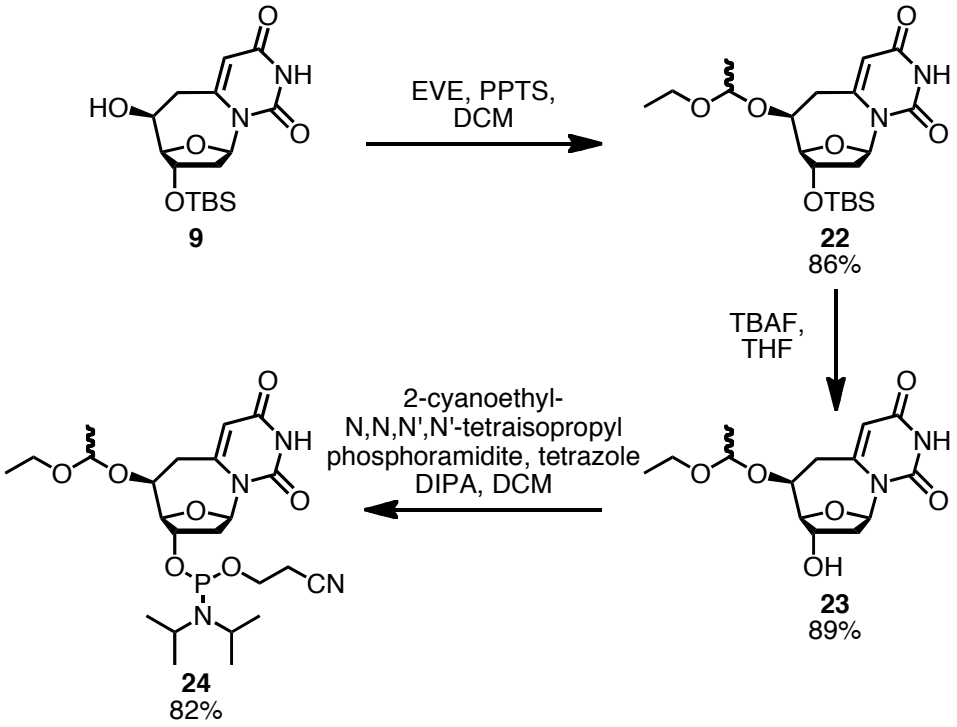
A lengthier synthesis to 8,6'-cyclo-2'-deoxyadenosine has been employed in our lab by Yueh et al. using the general theme of extending the 6' terminus and cyclizing onto the base (Scheme 3)[25]. Instead of forming an epoxide on the 5'-6' alkene, they used an OsO_4 mediated dihydroxylation. Tosylation and iodination of the 6' position allow for a radical cyclization onto the base, as had been employed in the synthesis of 8,5'-cyclo-2'-deoxyadenosine[1]. One advantage of this method is that it allows for the production of both the *R* and *S* diastereomers of the product. However, these are very difficult to separate. The tosylation step forms a notable amount of ditosylated species and to date the cyclization reaction has yields below 15%.

Although this route may be feasible for 6,6'-cyclo-2'-deoxyuridine, several challenges remain. First it is likely that post 6' tosylation, the 5' OH would also need to be protected, to prevent possible collapse to the 5'-6' epoxide. Steric contraints

would make the radical cyclization more difficult than the reactions used for the 6,5'-cyclo-U and dU compounds. This route is also much longer than the synthesis we employed to reach 6,6'-cyclo-2'-deoxyuridine via the zinc mediated Barbier cyclization.

3.4 6,6'-cyclo-2'-deoxyuridine Follow Up

As noted in the previous section, 6,6'-cyclo-2'-deoxyuridine overlays with thymidine better than 6,5'-cyclo-2'-deoxyuridine. We sought to synthesize the phosphoramidite of the ring-expanded compound and insert it into various oligo nucleotides to test if the rigidity caused by fixing the base increases stability.



Scheme 4: Synthesis of the 6,6'-cyclo-2'-deoxyuridine phosphoramidite

The synthesis of the phosphoramidite begins by taking the 3' OTBS protected cyclized compound and protecting the 5' OH with a 1-ethoxyethyl protecting group, which is formed by reacting the compound with ethyl vinyl ether under mildly

acidic conditions (Scheme 4). Similar to the work done by Yueh and Yu in our lab, the standard 5' OH protecting group, dimethoxytrityl chloride, proved to be too bulky to attach at the secondary OH of **9**[1].

The silyl protecting group was removed with TBAF, freeing the 3' OH for phosphitilation. Using standard protocol the phosphoramidite was synthesized. Normally phosphoramidites can be precipitated from a DCM solution with slow addition of hexanes. Due to the oily nature of compound **24** we relied on flash chromatography and trituration with hexanes for purification.

Three different 12-mer oligonucleotides containing 6,6'-cyclo-2'-deoxyuridine were synthesized and partnered to strands containing native 2-deoxyadenosine as the partner base. All of the oligomers were based on the Dickerson dodecamer (d(CGCGAATTCGCG)), but the C,G ends were scrambled so the strands were no longer self complementary. This prevented possible formation of hairpin strands.

Strand	Sequence	Mass Calcd	Mass Found
1	5'-d(CCGGAAU*TCGCC)-3'	3604	3763
2	5'-d(GGCGAATTCCGG)-3'	3686	3688
3	5'-d(CCGGAATTCGCC)-3'	3606	3607
4	5'-d(CCGGAU*AU*CGCC)-3'	3602	2996, 3300
5	5'-d(GGCGATATCCGG)-3'	3686	3687
6	5'-d(CCGGATATCGCC)-3'	3606	3607
7	5'-d(GGCGU*U*U*U*CCGG)-3'	3660	3661
8	5'-d(CCGGAAAACGCC)-3'	3624	3623
9	5'-d(GGCGTTTTCCGG)-3'	3668	3054, 3357

Table 1: Oligomers used for Tm studies. U* stands for *S*-6,6'-cyclo-2'-deoxyuridine

Unfortunately the data collected was either inconclusive or does not support our hypothesis that the expanded cyclo-bridge would help to stabilize the duplex. Thermal melting (Tm) studies of strands 1-2 and 4-5 showed non-typical melting

curves (Figure 1). The native duplexes had Tm values ranging from 50-60 ˚C depending on salt concentrations and sequence. Likely the DNA in the modified strands were not of sufficient purity, causing the gradual slope change observed compared to the sharp transition seen for the native strands. The masses (MALDI) of these samples do not match the found data either (Table 1). Unfortunately, due to time constraints and results from strands 7-9 below, these experiments were not repeated with gel purified or resynthesized DNA. The slight dual-transitions could possibly be indicative of higher order structures or alternative base pair arrangements as well.

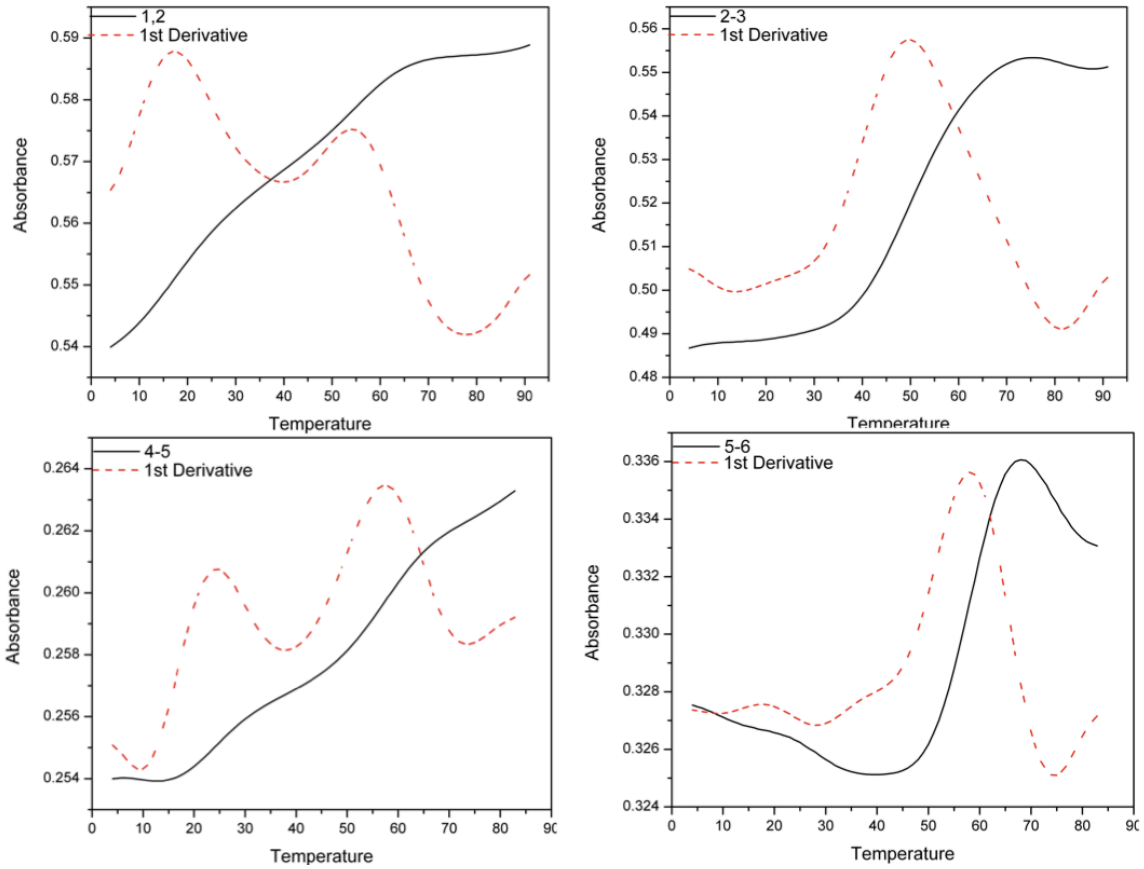


Figure 3: Tm plots for strands 1-2, 2-3, 4-5, and 5-6. All samples contained 500 mM NaCl and 20 mM pH 7 phosphate buffer. Note the irregular curves for the modified strands.

Perhaps the most telling Tm values came from the sequences featuring the tetra U* core. The native 7-9 pairing had a Tm value of 49 ˚C (500mM NaCl and 20 mM pH 7 phosphate buffer), while the modified strand was not able to form duplex (Figure 2). This was confirmed by running a native gel, which only showed single stranded DNA for the 7-8 pairing (Figure 3).

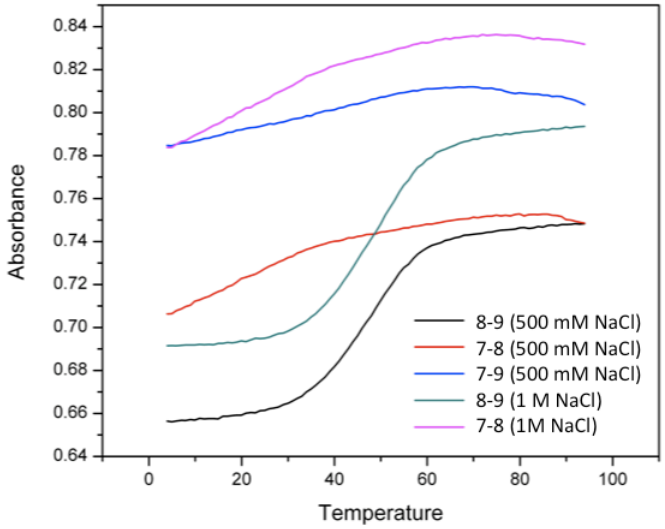


Figure 2: Tm curves for strands 7-9 with 500 mM or 1 M NaCl. Note how the mismatched 7-9 pairing has a similar curve as the matched strands containing the U*U*U*U* core.

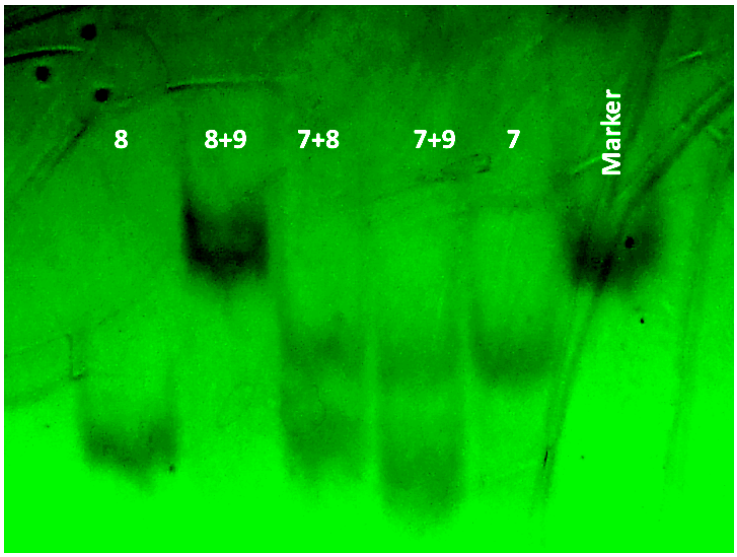


Figure 3: Native gel with strands 7-9. Lane 2 is the native duplex and lane 3 is the modified paired with native.

From the crystal structure analysis in the previous section we observed that the modified nucleoside's base and 3',5' hydroxyls overlap very well with native thymidine. However, there is an important structural difference between these monomers that may cause backbone perturbation, inhibiting the ring expanded cyclonucleoside from forming stable duplex structures. An overhead view of thymidine shows that the 5' OH points back over the sugar ring. In *S*-6,6'-cyclo-2'-deoxyuridine this space is occupied by the C-C bond between the C5' and C6' carbons (Figure 4). Instead the OH group is positioned where a hydrogen atom is located on the native nucleoside. While further structural analysis must be undertaken to study if this conformation change causes destabilization, it could be a relevant factor.

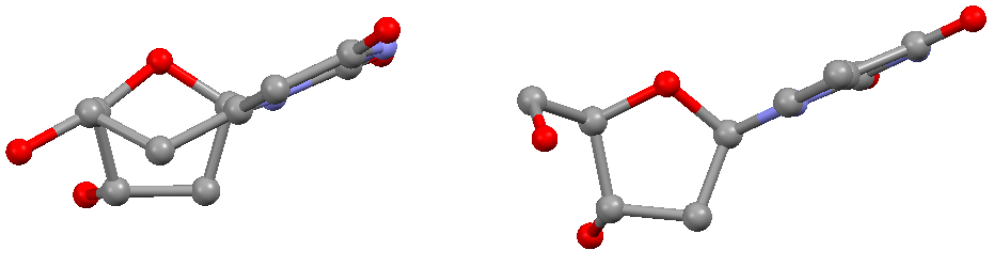


Figure 4: 6,6'-cyclo-2'-deoxyuridine on the left and thymidine cut from a Dickerson dodecamer crystal structure on the right[24].

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3.5.1 Nucleoside Synthesis Procedures

Compound 4: 2'-deoxyuridine (10.01 g, 43.82 mmol), tert-butyldimethylsilyl chloride (15.83 g, 105.2 mmol), and imidazole (7.150 g, 105.2 mmol) were dissolved in 300 mL of DMF and stirred for 12 hrs. The reaction mixture was partitioned between brine and ethyl acetate. The organic layer was washed again with brine and then dried over $NaSO_4$. This crude material was of sufficient purity to use in further reactions. For standardization and for characterization the crude was purified by flash chromatography (3:2 hexanes to ethyl acetate) to yield **4** as a white solid (18.81 g, 41.19 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.06 (s,6H), 0.87 (s, 9H), 0.90 (s, 9H), 2.04 (p, *J*=6.4 Hz, 1H), 2.31 (m, 1H), 3.74 (dd, *J*=11.2, 1.6 Hz, 1H), 3.89 (m, 3H), 4.39 (m, 1H), 5.66 (d, *J*=8.4 Hz, 1H), 6.26 (t, *J*=6.0 Hz, 1H), 7.88 (d, *J*=8.0 Hz, 1H), 8.33 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ -4.65, -4.41, 18.19, 18.57, 25.93, 26.08, 42.07, 62.65, 71.42, 85.41, 88.00, 102.34, 140.41, 150.23, 163.14. HRMS (DART) Calcd for $C_{21}H_{41}N_2O_5Si_2$ 457.25540; Found, 457.25651.

Compound 5: Diisopropyl amine (14.8 mL, 104 mmol) was added to 200 mL of THF and the mixture was cooled to -78 ^{o}C. A solution of *n*-BuLi (2.6M in hexanes, 39.6 mL, 104 mmol) was slowly added and the mixture was allowed to stir at 0 ^{o}C for 20 min. The flask was cooled back to -78 ^{o}C and a solution of **2** (9.45 g, 20.7 mmol) in 200 mL of THF was slowly added. After 2.5 hrs, DMF (40.1 mL, 517 mmol) was added and the reaction was stirred for 2.5 hrs. Acetic acid (15.5, 271 mmol) was added and the reaction was warmed to ambient temperature. The mixture was diluted with EtOH (200 mL) and $NaBH_4$ was slowly added (2.34 g, 61.9 mmol). The

reaction was stirred for 30 min before the solvents were removed by rotary evaporation. The crude mixture was dissolved in EtOAc and washed twice with brine before being dried over $NaSO_4$. The material was purified by flash chromatography (3:2 ethyl acetate to hexanes) to yield a mixture of the starting material, **4** (3.89 g, 8.49 mmol, 41%), and the product, **5** (4.43 g, 9.12 mmol, 44%), as a clear oil/white foam. If impurities remain, the material can be "recrysallized" from an ethanol water mixture to a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.07 (s, 6H), 0.86 (s, 6H), 0.88 (s, 6H), 2.14 (m, 1H), 2.65 (m, 1H), 3.79 (m, 2H), 3.86 (m, 1H), 4.86 (m, 1H), 5.77 (s, 1H), 6.19 (t, *J*=6.5 Hz, 1H), 9.34 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$), δ -4.91, -4.65, 17.83, 18.45, 25.67, 25.92, 39.32, 61.40, 62.82, 71.05, 85.49, 87.39, 102.59, 150.40, 155.47, 163.15. HRMS (DART) Calcd for $C_{22}H_{43}N_2O_6Si_2$ 487.26596; Found, 487.26819.

Compound 6: *N,N*-Diisopropylethylamine (1.05 mL, 6.01 mmol) and **3** (1.46 g, 3.00 mmol) were dissolved in THF (40 mL) and cooled to 0 ^{o}C. Mesyl chloride (0.785 mL, 4.51 mmol) was added and the reaction was stirred for 1.5 hrs. A solution of LiBr (522 mg, 6.01 mmol) in DMF (5.60 mL) was added drop wise and the mixture was allowed to warm to ambient temperature. After 3 hrs the reaction was quenched with sat. $NaHCO_3$. Ethyl acetate was added to help partition the mixture. The organic layer was washed with brine and the combined aqueous layers were back extracted with ethyl acetate. The product was purified by flash chromatography (2:1 hexanes to ethyl acetate) to yield the product as a tan oil (1.16 g, 2.12 mmol, 71%). This compound has some stability issues and we recommend immediately

setting up the following reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.07 (s, 6H), 0.88 (s, 18 H), 2.15 (m, 1H), 2.95 (p, *J*=5.5 Hz, 1H), 3.73 (dd, *J*=12.0, 7.0 Hz, 1H), 3.80 (m, 1H), 4.26 (q, *J*=11.5 Hz, 1H), 4.51 (m, 1H), 5.74 (s, 1H), 6.09 (t, *J*=7.0 Hz, 1H), 8.74 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ -5.11, -4.75, 18.06, 18.68, 25.91, 26.19, 38.60, 41.69, 43.42, 63.38, 71.81, 86.12, 87.98, 105.30, 150.29, 151.54, 162.65. HRMS (DART) Calcd for $C_{22}H_{42}BrN_2O_5Si_2$ 549.18156; Found, 549.18261.

Compound 7: **6** (1.16 g, 2.12 mmol) and PPTS (150 mg, 0.597 mmol) were dissolved in ethanol (25 mL) and stirred for 12 hrs. The reaction was quenched with saturated $NaHCO_3$ and the ethanol was removed by rotary evaporation. The crude product was extracted with ethyl acetate and washed with brine. The combined aqueous layers were back extracted with ethyl acetate and the organic layers were dried over $NaSO_4$. The material was purified by flash chromatography (7:3 ethyl acetate to hexanes) to yield **7** as a white solid (840 mg, 1.93 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.07 (s, 6H), 0.87 (s, 9H), 2.13 (m, 1H), 3.00 (p, *J*=7.0 Hz, 1H), 3.45 (dd, *J*=9.0, 2.5 Hz, 1H), 3.70 (dt, *J*=9.5, 3.0 Hz, 1H), 3.84 (dt, *J*=12.0, 2.5 Hz, 1H), 3.94 (q, *J*=3.5 Hz, 1H), 4.26 (d, *J*=13.0 Hz, 1H), 4.46 (d, *J*=13.0 Hz), 4.66 (m, 1H), 5.76 (s, 1H), 5.98 (t, *J*=7.0Hz, 1H) 9.20 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$), δ -4.51, 18.13, 25.94, 38.94, 42.05, 62.59, 72.27, 87.54, 88.95, 105.43, 150.76, 150.85, 162.06. HRMS (DART) Calcd for $C_{16}H_{28}BrN_2O_5Si$ 435.09509; Found, 435.09683.

Compound 9: Compound **5** (526 mg, 1.21 mmol) and Dess-Martin Periodinane (1.28 g, 3.02 mmol) were dissolved in dichloromethane (35 mL) and stirred for 2.5

hrs. The reaction was quenched with equal parts saturated $Na_2S_2O_3$ and saturated $NaHCO_3$. The organic layer was washed with brine and the combined aqueous layers were washed with ethyl acetate. The solvent was removed by rotary evaporation and the crude material was carried on to the next reaction as it decomposes on silica.

The crude aldehyde and zinc (1.97 g, 30.2 mmol) were dissolved in DMF (100 mL) and sonicated for 3 hrs. The reaction mixture was filtered through Celite and the Celite was rinsed several times with ethyl acetate. The mixture was partitioned between brine and ethyl acetate. The ethyl acetate layer was washed a second time with brine and the combined aqueous layers were back extracted with ethyl acetate. The organic layers were dried over $NaSO_4$ and the solvent was removed by rotary evaporation. The crude material was purified by column chromatography (65:35 ethyl acetate to hexane) to yield **9** as a white solid (559 mg, 1.58 mmol, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.09 (s, 3H), 0.10 (s, 3H), 0.88 (s, 9H), 2.37 (ddd, *J*=15.5, 6.5, 2.5 Hz, 1H), 2.56 (dd, *J*=15.5, 9.0 Hz, 1H), 2.71 (m, 2H), 4.02 (m, 1H), 4.25 (d, *J*=4.0 Hz, 1H), 4.70 (d, *J*=6.0 Hz, 1H), 5.60 (s, 1H), 7.02 (dd, *J*=9.0, 3.0 Hz, 1H), 8.52 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ -4.55, 18.25, 25.97, 39.30, 43.72, 67.80, 72.03, 79.35, 85.10, 90.29, 105.24, 151.08, 162.39. HRMS (DART) Calcd for $C_{16}H_{27}N_2O_5Si$ 355.16892; Found, 355.16813. $[\alpha]_D^{23}$ -151.36˚ (*c*= 1.33, Acetone).

Compound 1: The cyclized product, **9** (35.0 mg, 98.7 μmol), was dissolved in THF (5.00 mL). TBAF (495 μL of 1M solution in THF, 495 μmol) was added and the

reaction was stirred for 1.5 hrs. The reaction was quenched with water. The mixture was partitioned between water and ethyl acetate. The organic layer was washed three times with water and the aqueous layer was concentrated by rotary evaporation. The crude material was purified by flash chromatography (1:1 acetone to ethyl acetate) to yield **7** as a white solid (19.4 mg, 80.9 μmol, 82%). ^{1}H NMR (MHz, D_5-Pyridine) δ 2.76 (ddd, *J*=15.5, 7.0, 3.0 Hz, 1H), 2.92 (dd, *J*=15.5, 9.0 Hz, 1H), 3.12 (m, 2H), 4.38 (m, 1H), 4.94 (d, *J*=3.5 Hz), 5.35 (d, *J*=6.5 Hz, 1H), 5.90 (s, 1H), 7.61 (dd, *J*=9.0, 2.5 Hz), 13.32 (s, 1H). ^{13}C NMR (125 MHz, D_5-Pyridine), δ 40.30, 43.69, 68.67, 71.75, 85.45, 92.05, 105.69, 152.23, 153.14, 163.97. HRMS (DART) Calcd for $C_{10}H_{13}N_2O_5$ 241.08245; Found, 241.08255. $[\alpha]_D^{23}$ -7.50° (*c*= 0.33, H_2O).

Compound 22:

3' OTBS protected 6,6'-cyclo-2'-deoxyuridine (252 mg, 0.711 mmol) and PPTS (32.2 mg, 0.142 mmol) were dissolved in DCM (15 mL) and ethyl vinyl ether (1.36 mL, 14.22 mmol) was added. The reaction was stirred for 3 hrs before being washed with sat. $NaHCO_3$ and brine solution. The organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (75% EtOAc, 25% hexanes) to yield the product as a white solid (156 mg, 0.366 mmol). Note the product is a mixture of diastereomers in relation to the 1-ethoxyethyl protecting group. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H), 7.02 (t, *J*=2.5 Hz, 0.5H), 7.00 (t, *J*=2.5 Hz, 0.5H), 5.58 (s, 1H), 4.86-4.72 (m, 1H), 4.67 (dt, *J* = 11.3, 5.5 Hz, 1H), 4.29 (d, *J* =4.0 Hz, 0.5H), 4.21 (d, *J*=4.0 Hz, 0.5H), 3.93-3.76 (m, 1H), 3.65-3.53 (m, 1H), 3.53-3.43 (m, 1H), 2.90-2.77 (m, 1H), 2.72-2.57 (m,

2H), 2.53 (ddd, *J* = 15.5, 9.2, 2.4 Hz, 1H), 2.40-2.29 (m, 1H), 1.30 (s, 1.5H), 1.29 (s, 1.5H), 1.24-1.16 (m, 3H), 0.87 (s, 4.5H), 0.87 (s, 4.5H), 0.08 (s, 1.5H), 0.07 (s, 1.5H), 0.07 (s, 1.5H), 0.06 (s, 1.5H). HRMS (DART) Calcd for $C_{20}H_{35}N_2O_6Si_1$ 427.2264; Found, 427.2284.

Compound 23

Compound **22** (167 mg, 0.391 mmol) was dissolved in THF (5 mL) and a TBAF solution was added dropwise (979 μL of a 1M solution in THF, 0.979 mmol). The reaction was stirred for 1.5 hrs before being quenched with MeOH. The solvent was removed by rotary evaporation and the crude material was directly purified by flash chromatography (60% EtOAc, 40% acetone) to yield the product as a white solid(109 mg, 0.348 mmol, 89%). Note the product is a mixture of diastereomers in relation to the 1-ethoxyethyl protecting group. ^{1}H NMR (500 MHz, acetone) δ 9.94 (s, 1H), 6.92 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.61 (d, *J* = 9.0 Hz, 1H), 4.98-4.85 (m, 1H), 4.73 (dd, *J* = 28.0, 6.1 Hz, 1H), 4.35-4.24 (m, 1H), 3.90-3.77 (m, 1H), 3.77-3.59 (m, 1H), 3.59-3.47 (m, 1H), 2.91-2.87 (m, 2H), 2.72-2.55 (m, 1H), 2.50 (dd, *J* = 15.7, 9.0 Hz, 1H), 1.36-1.29 (t, *J*= 5.5 Hz 3H), 1.23-1.10 (m, 3H). HRMS (DART) Calcd for $C_{14}H_{21}N_2O_6$ 313.1400; Found, 131.1400.

Compound 24

Compound **23** (55.0 mg, 0.176 mmol) and diisopropyl amine (50.0 μL, 0.352 mmol) were dissolved in DCM (10 mL). 2-cyanoethyl-N,N,N',N'-tetraisopropyl phosphoramidite (111 μL, 0.352 mmol) and tetrazole (782 μL of a 0.45 M solution in acetonitrile, 0.352 mmol) were added and the reaction was stirred for 3 hrs at 0 ˚C. The solvent was removed by rotary evaporation and the crude material was purified

by column chromatography (74% EtOAc, 25% hexanes, 1% TEA). The resulting oil was triturated with hexanes to yield the product as a white foam upon drying under vacuum (74.0 mg, 0.144 mmol, 82%). ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.63, 148.55, 148.43, 148.14. HRMS (DART) Calcd for $C_{23}H_{38}N_4O_7P_1$ 513.2478; Found, 513.2485.

DNA Preparation

All native strands were made on an Applied Biosystems DNA/RNA synthesizer with 2 minute couplings. For the modified strands, couplings involving the addition cyclouridine phosphoramidite or the couplings after a modified base were increased to 15 minutes. The 5' terminus DMTr group was kept on.

The strands were purified by HPLC on an oligo-R3 column using a gradient (Buffer A: 50 mM TEAA 5% acetonitrile, Buffer B: 50 mM TEAA, 70% acetonitrile) of 0-60% Buffer B over 12 minutes, followed by flushing the column with Buffer B for 5 minutes. The solvent of the product containing fractions was removed by rotary evaporation.

The DMTr group was removed by stirring the oligomer in an 80% acetic acid solution for 30 minutes. Upon removal of the solvent by rotary evaporation, the DNA was desalted using NAP 10 columns.

DNA concentrations were measured using the absorbance from a UV spectrophotometer and an extinction coefficient from Integrated DNA Technologies' website using the formula $A=\varepsilon bc$ where A is the absorbance, ε is the extinction coefficient, b is the path length, and c is the concentration. A 50 μL sample of stock

DNA was diluted 20x for the absorbance measurements. For the 6,6'-cyclo-2'-deoxyuridine containing strands, an approximate extinction coefficient calculated from thymidine containing strands was used. The values for A and ε are listed below in Table 2. The cuvettes used to measure the absorbance had a path length of 1 cm, therefore b=1 for all the equations.

Strand	ε (L/mmol•cm)	Absorbance	Conc. (mM)
1	107.9	0.2373	0.0440
2	114.9	0.7110	0.1238
3	107.9	0.3566	0.0661
4	109.9	0.4798	0.0873
5	116.9	1.534	0.2625
6	109.9	0.4798	0.0873
7	106.5	0.5432	0.1020
8	114.7	0.6075	0.1059
9	106.5	0.7662	0.1439

3.5.2 Crystal Structure of Compound 1

Crystals of **1** were obtained by dissolving the purified compound in hot isopropanol and letting the solvent slowly evaporate over a period of two weeks.

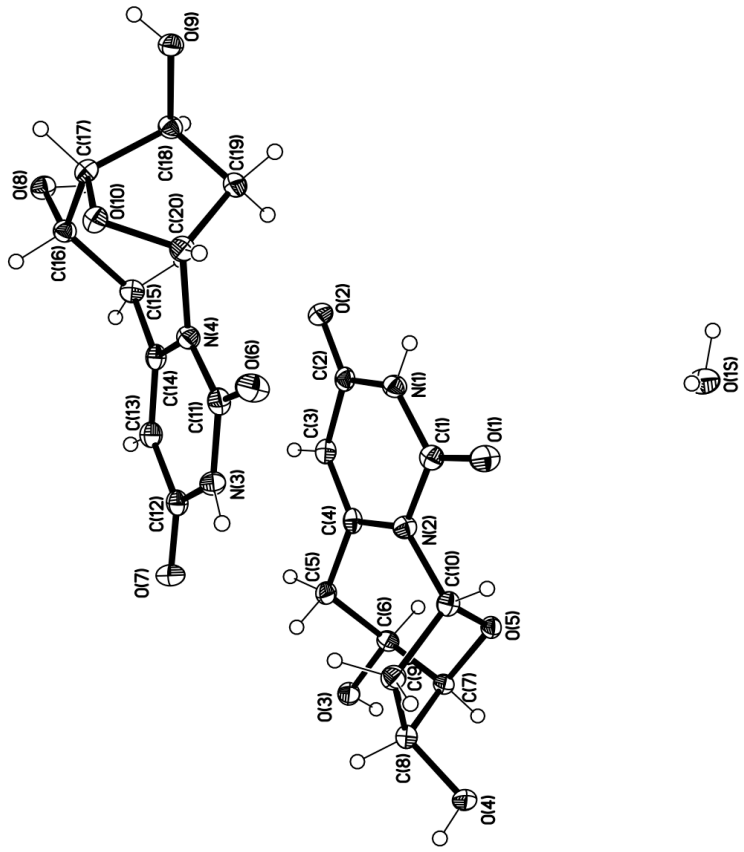
X-ray Crytallography Experimental Conditions*

Selected single crystals suitable for X-ray crystallographic analysis were used for structural determination. The X-ray intensity data were measured at 100(2) K (Oxford Cryostream 700) on a Bruker Kappa APEX Duo diffractometer system equipped with a sealed Mo-target X-ray tube (λ = 0.71073 Å) and a high brightness IμS copper source (λ = 1.54178 Å). The crystals were mounted on a goniometer head with paratone oil. The detector was placed at a distance of 6.000 cm from the

* The experimental conditions section is authored by Dr. Bo Li at the Boston College X-ray Crystallography Center.

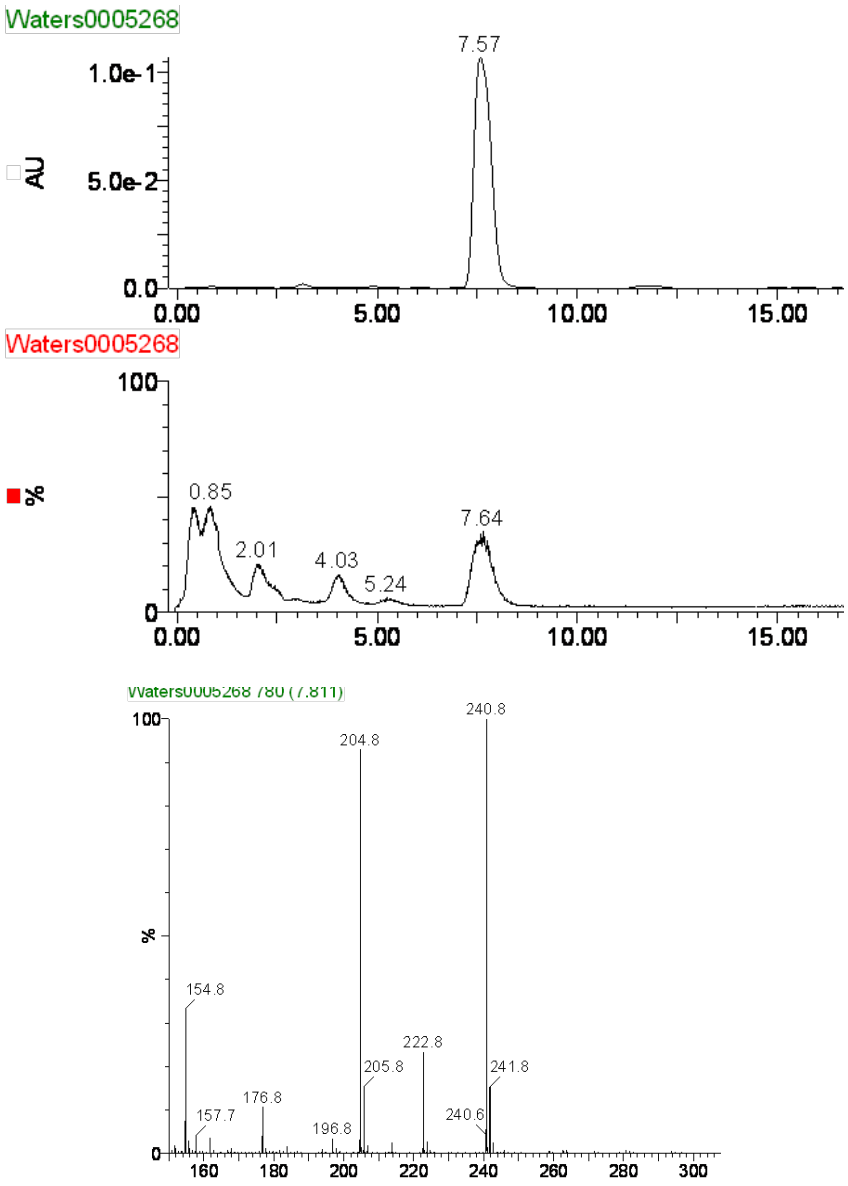
crystal. For each experiment, data collection strategy was determined by APEX software package and all frames were collected with a scan width of 0.5° in ω and ϕ with an exposure time of 10 or 20 s/frame.

The frames were integrated with the Bruker SAINT Software package using a narrow- frame integration algorithm to a maximum 2θ angle of 56.54° (0.75 Å resolution) for Mo data. The final cell constants are based upon the refinement of the XYZ-centroids of several thousand reflections above 20 $\sigma(I)$. Analysis of the data showed negligible decay during data collection. Data were corrected for absorption effects using the empirical method (SADABS). The structures were solved and refined by full-matrix least squares procedures on $|F^2|$ using the Bruker SHELXTL (version 6.12) software package. All hydrogen atoms were included in idealized positions for structure factor calculations except for those forming hydrogen bonds or on a chiral center. Anisotropic displacement parameters were assigned to all non-hydrogen atoms, except those disordered.



3.5.3 LC/MS Analysis of Compound 1

The top chromatogram shows absorbance detection at 260 nm, while the bottom chromatogram shows mass detection. The compound was eluted with water using a C_{18} analytical column. The spectrum below the chromatograms shows a peak at 240.8, corresponding to compound **1**. None of other minor peaks in the chromatogram had masses corresponding to the desired product.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0.00
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10.00
15.00
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100
%
0.85
2.01
4.03
5.24
7.64
0
0.00
5.00
10.00
15.00
Waters0005268 780 (7.811)
100
%
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204.8
154.8
222.8
205.8
241.8
176.8
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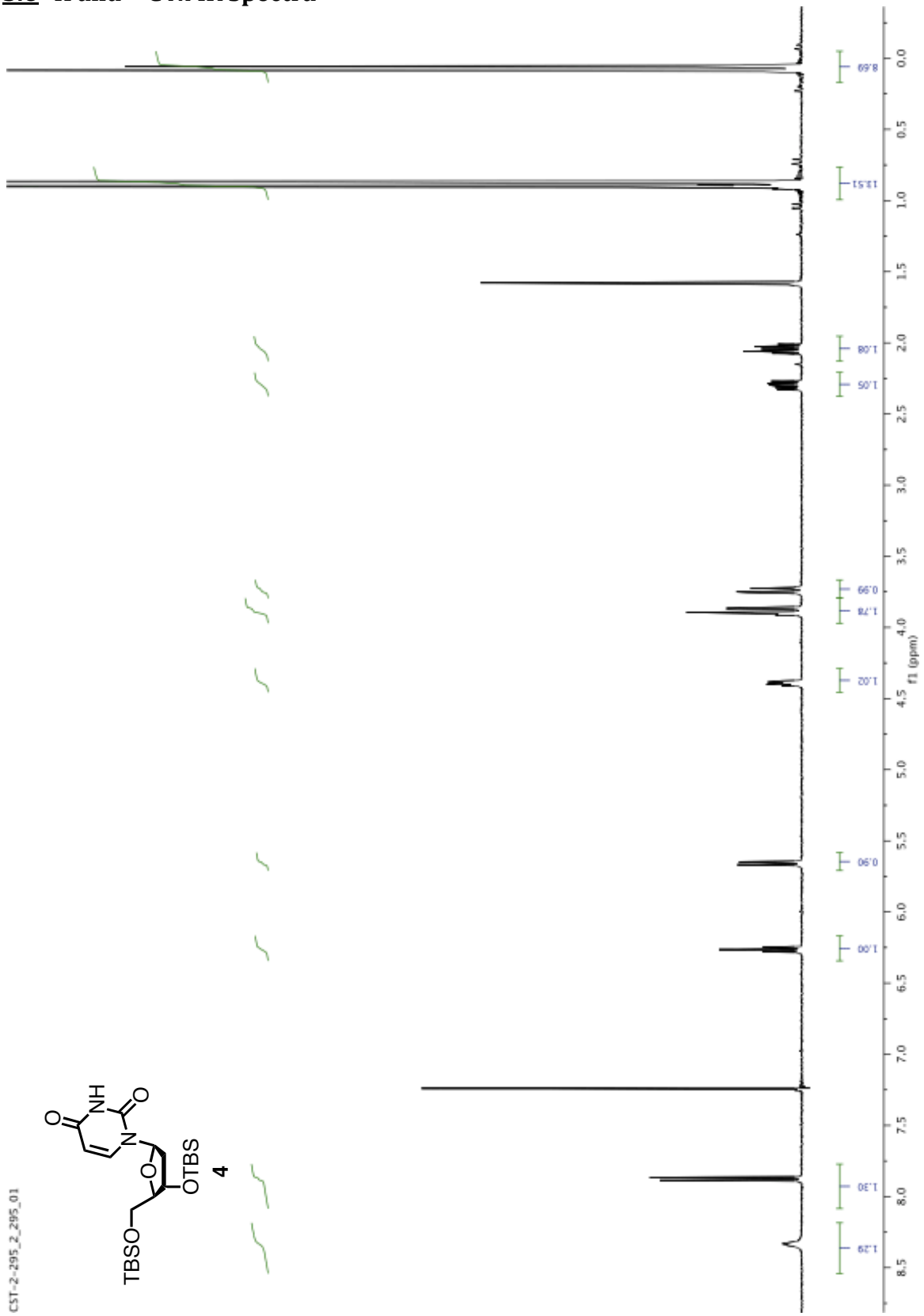
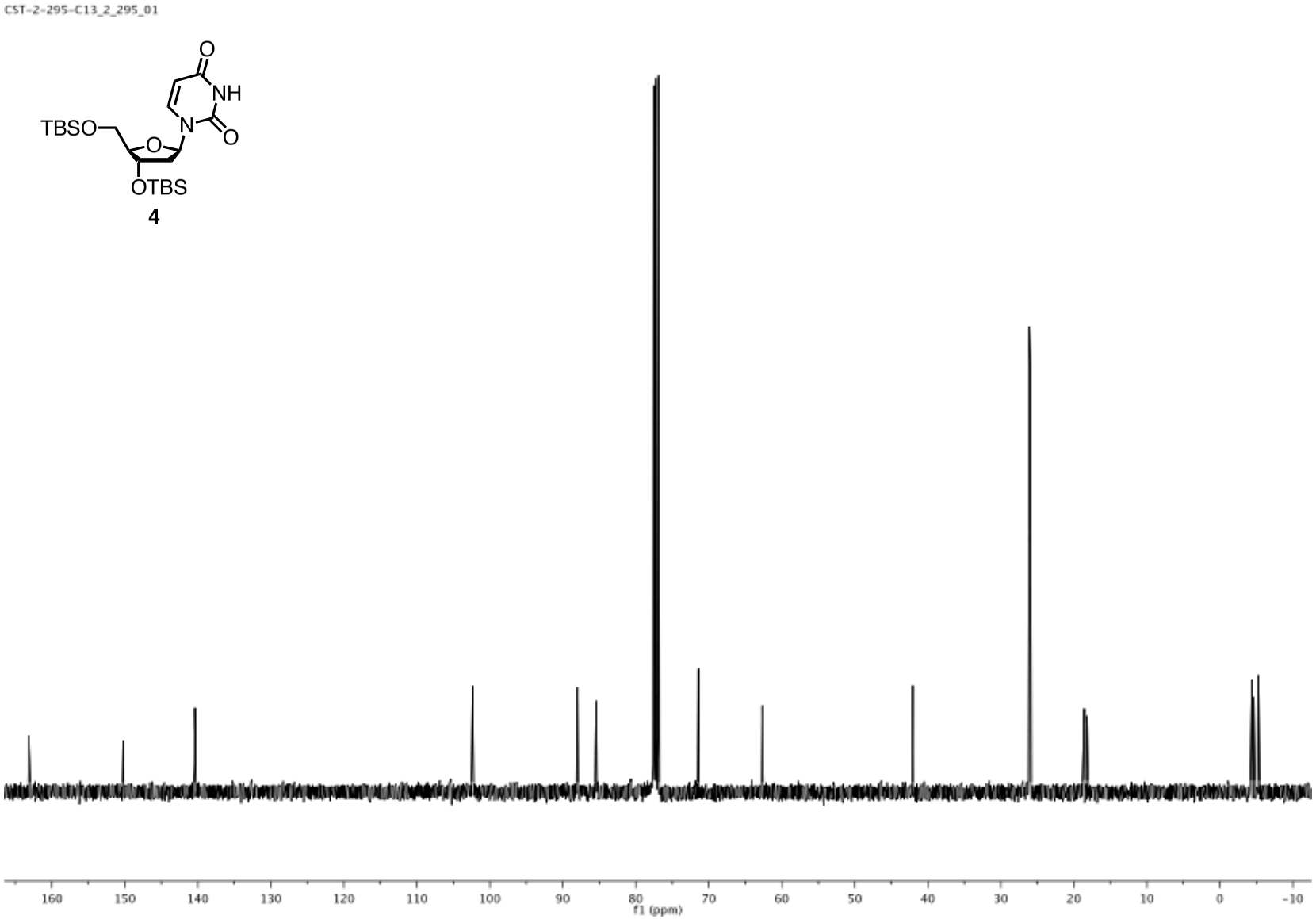
180
200
220
240
260
280
300

3.6 ^{1}H and ^{13}C NMR Spectra

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O
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OTBS
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30
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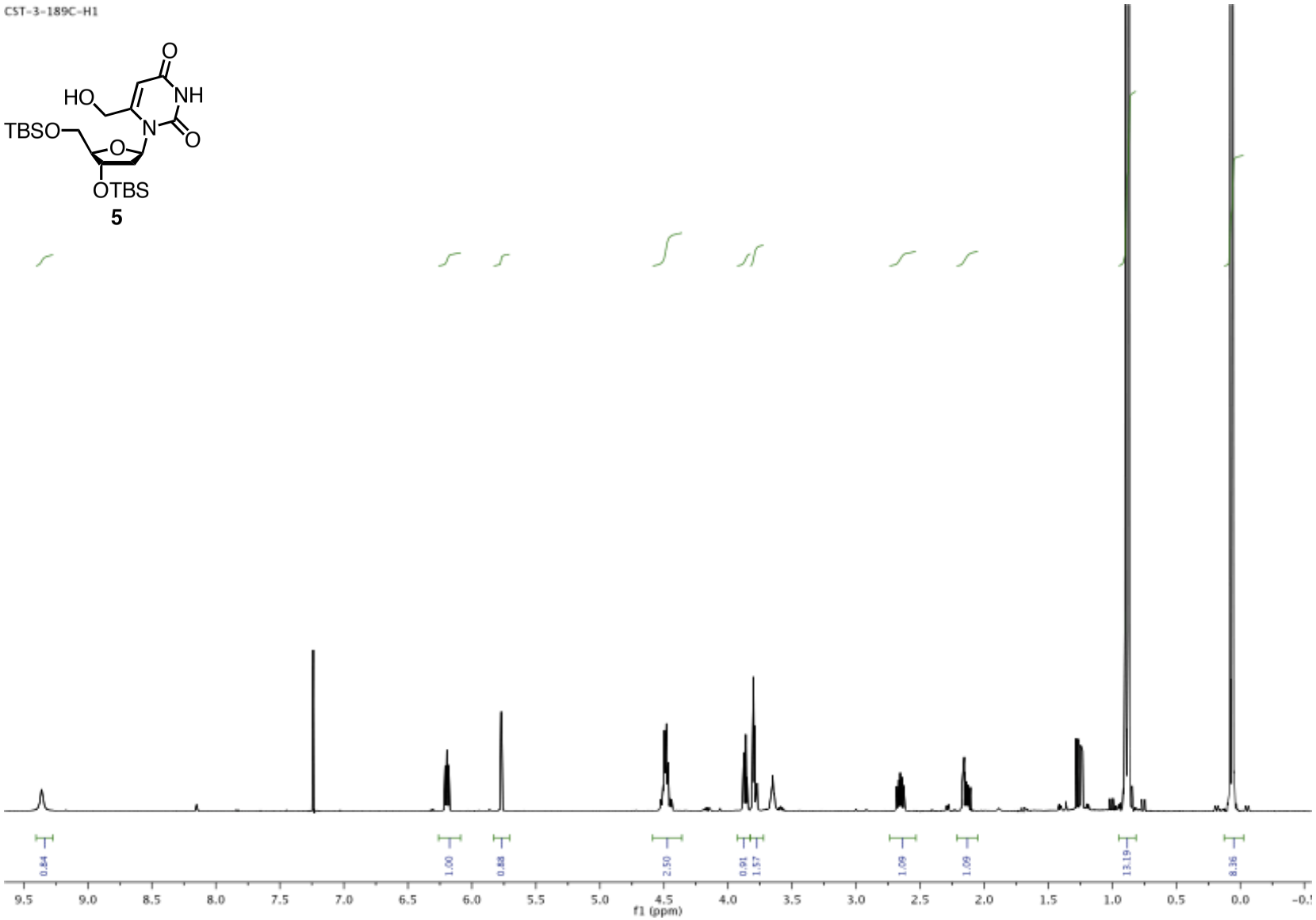
f1 (ppm)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1.09
1.09
13.19
8.36
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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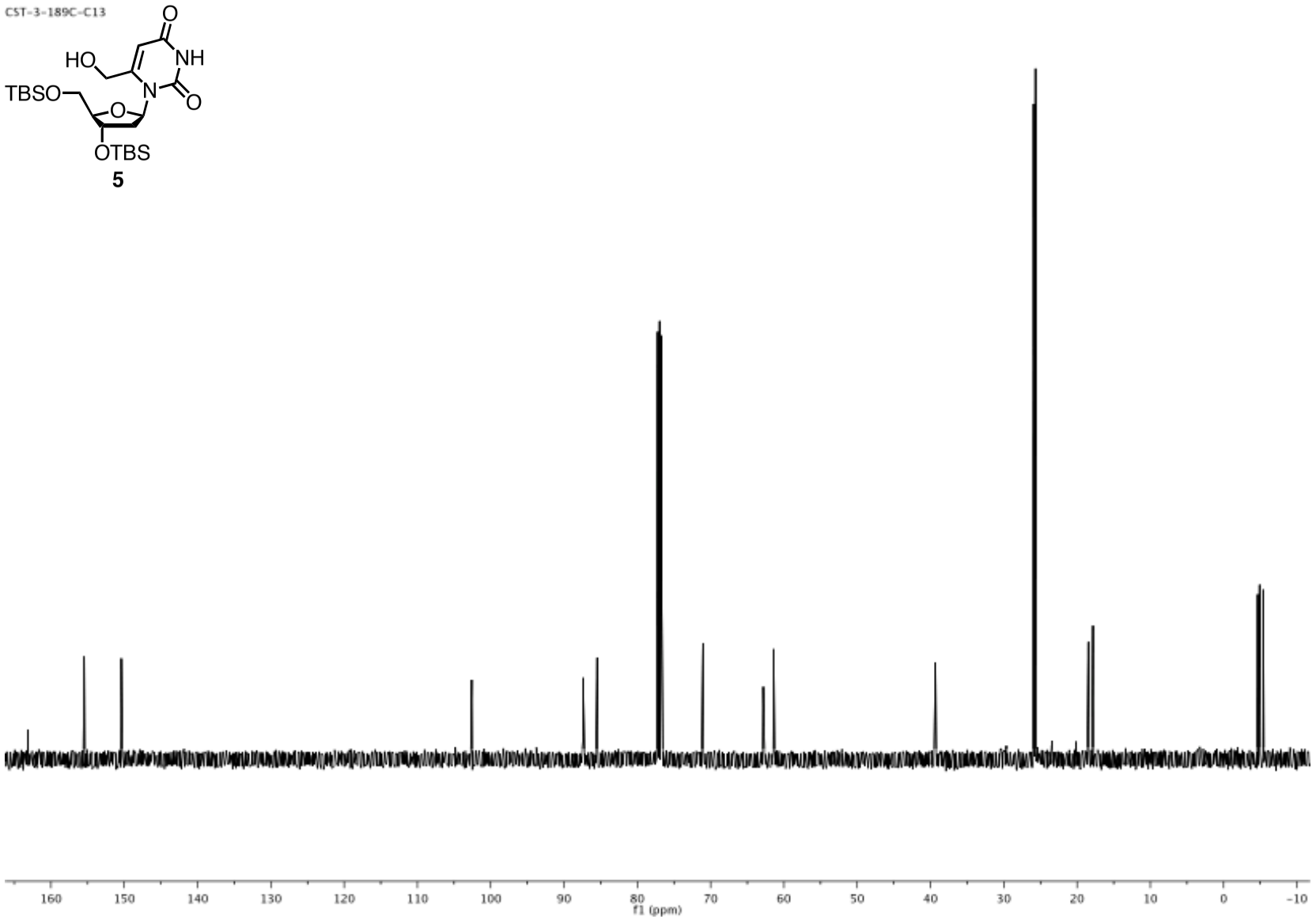
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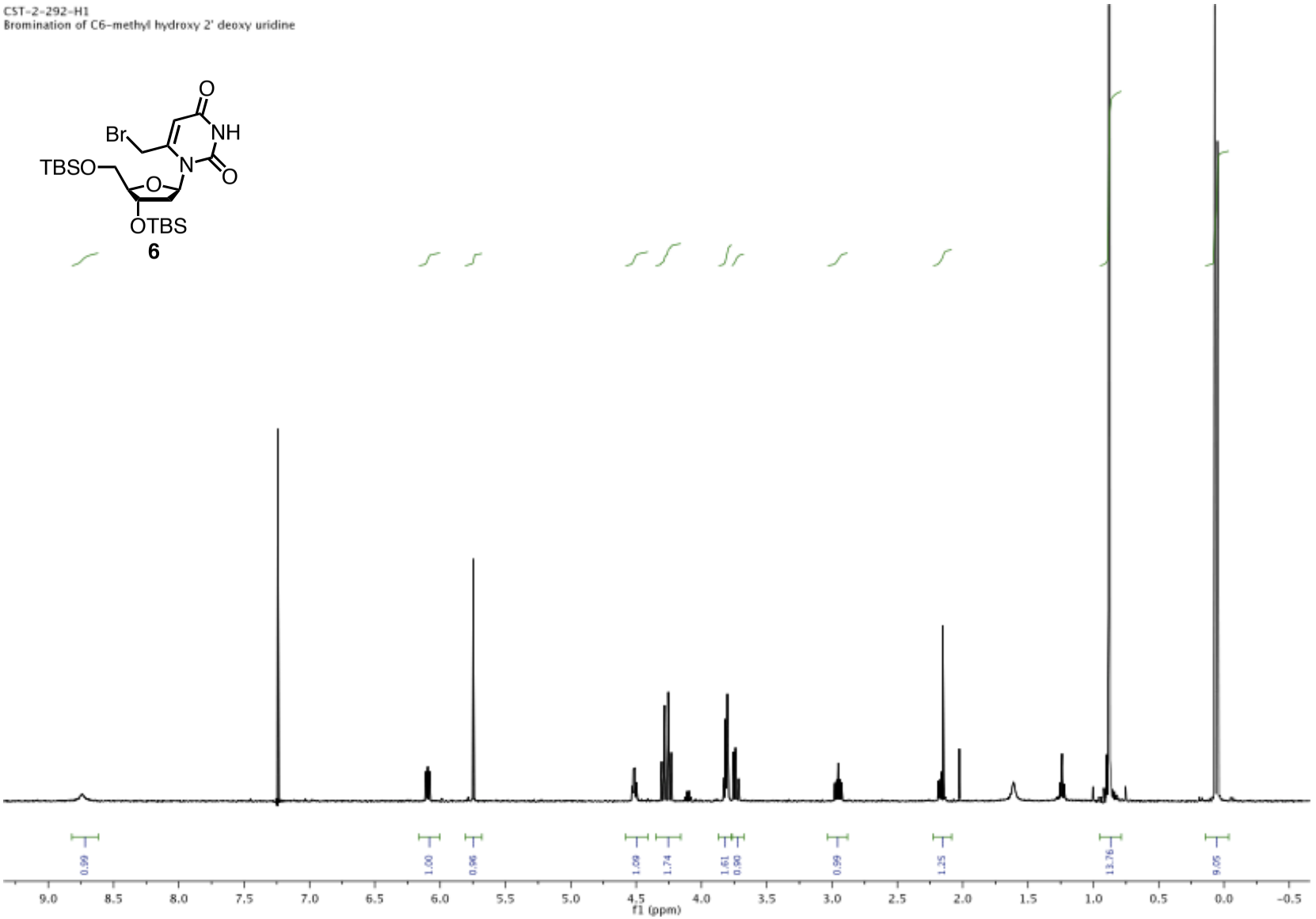
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1.74
1.61
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0.99
1.25
13.76
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9.0
8.5
8.0
7.5
7.0
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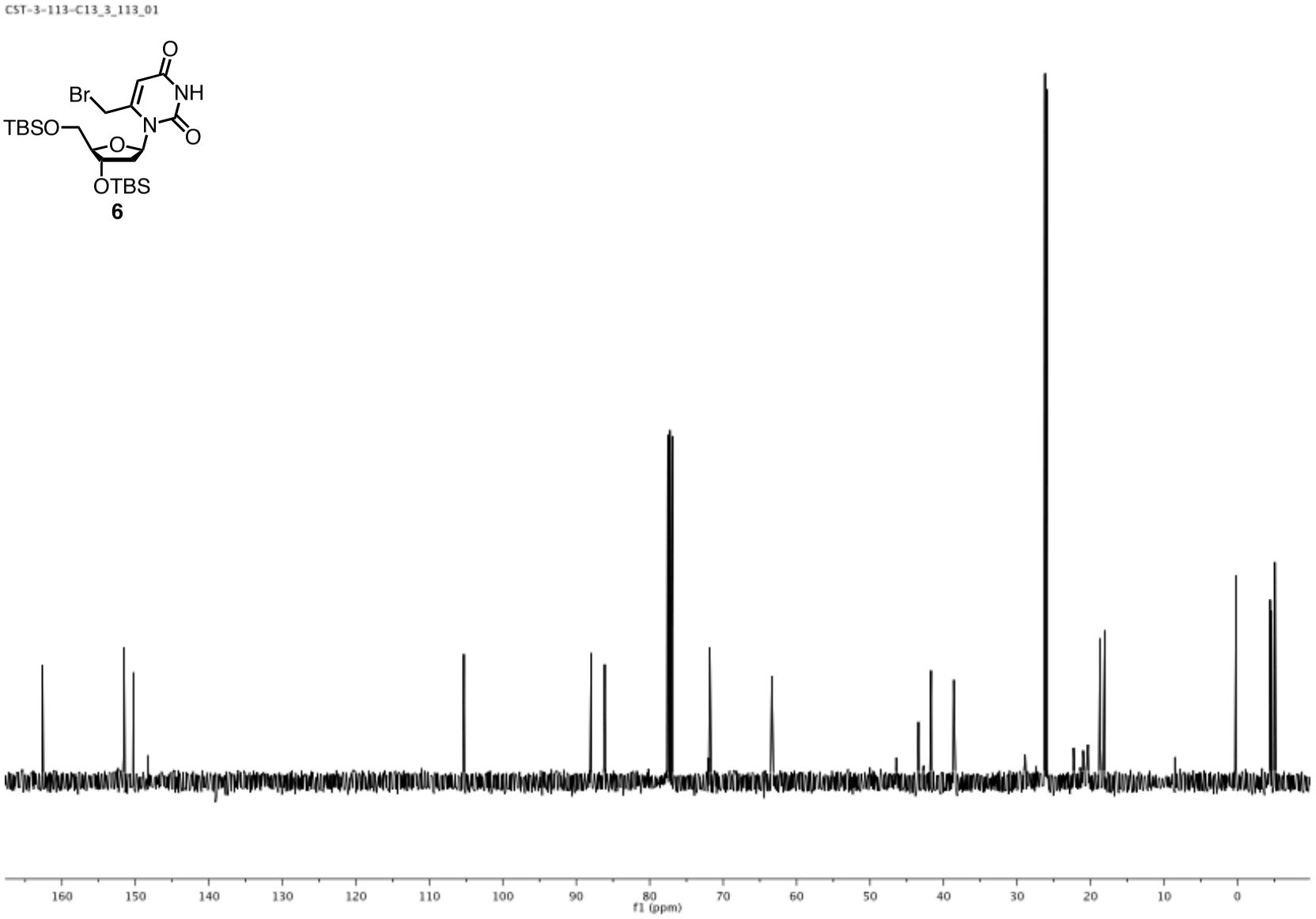
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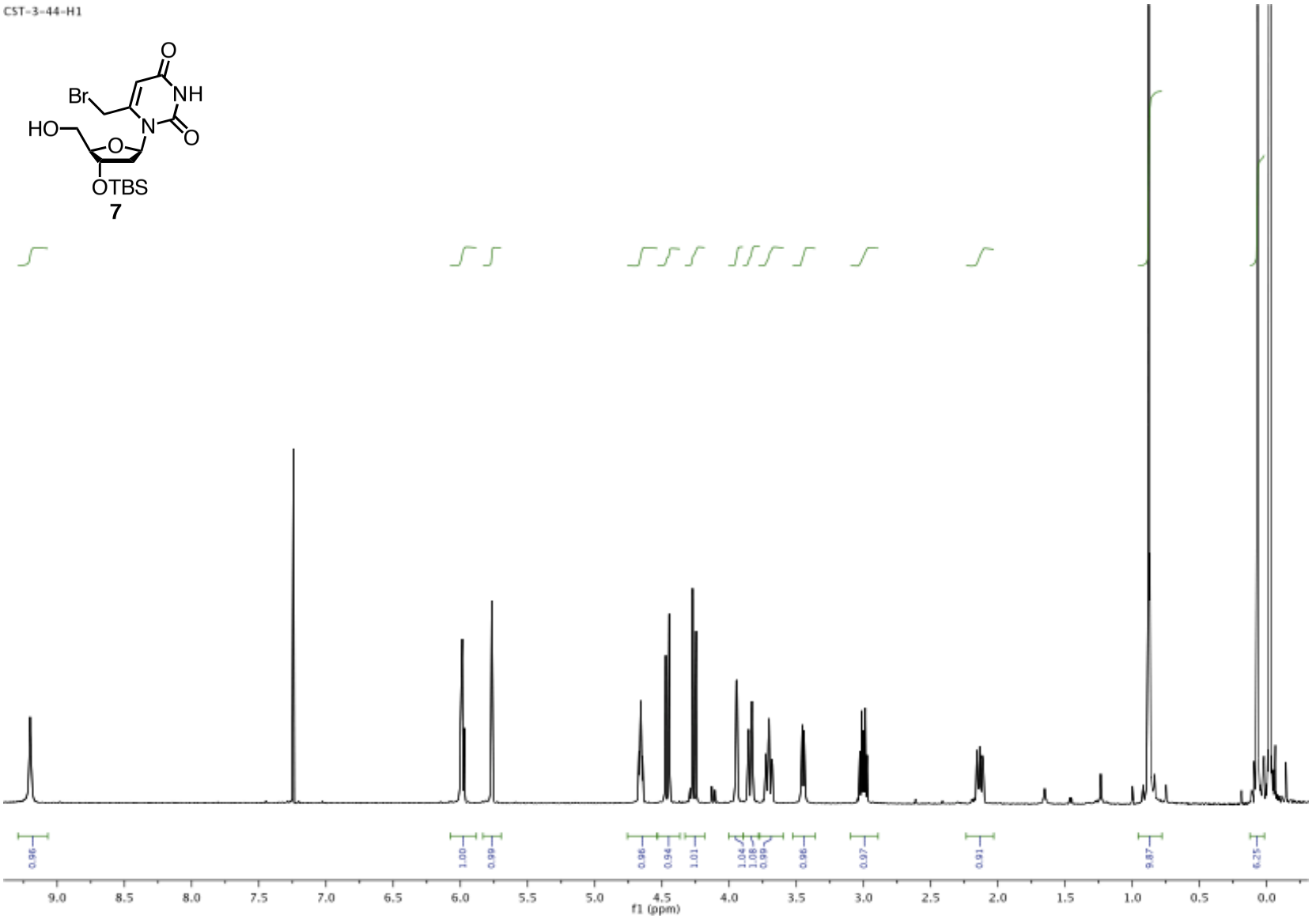
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1.08
0.99
0.96
0.97
0.91
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8.5
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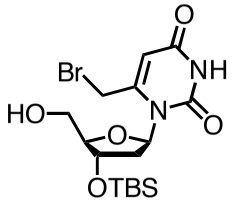
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Br
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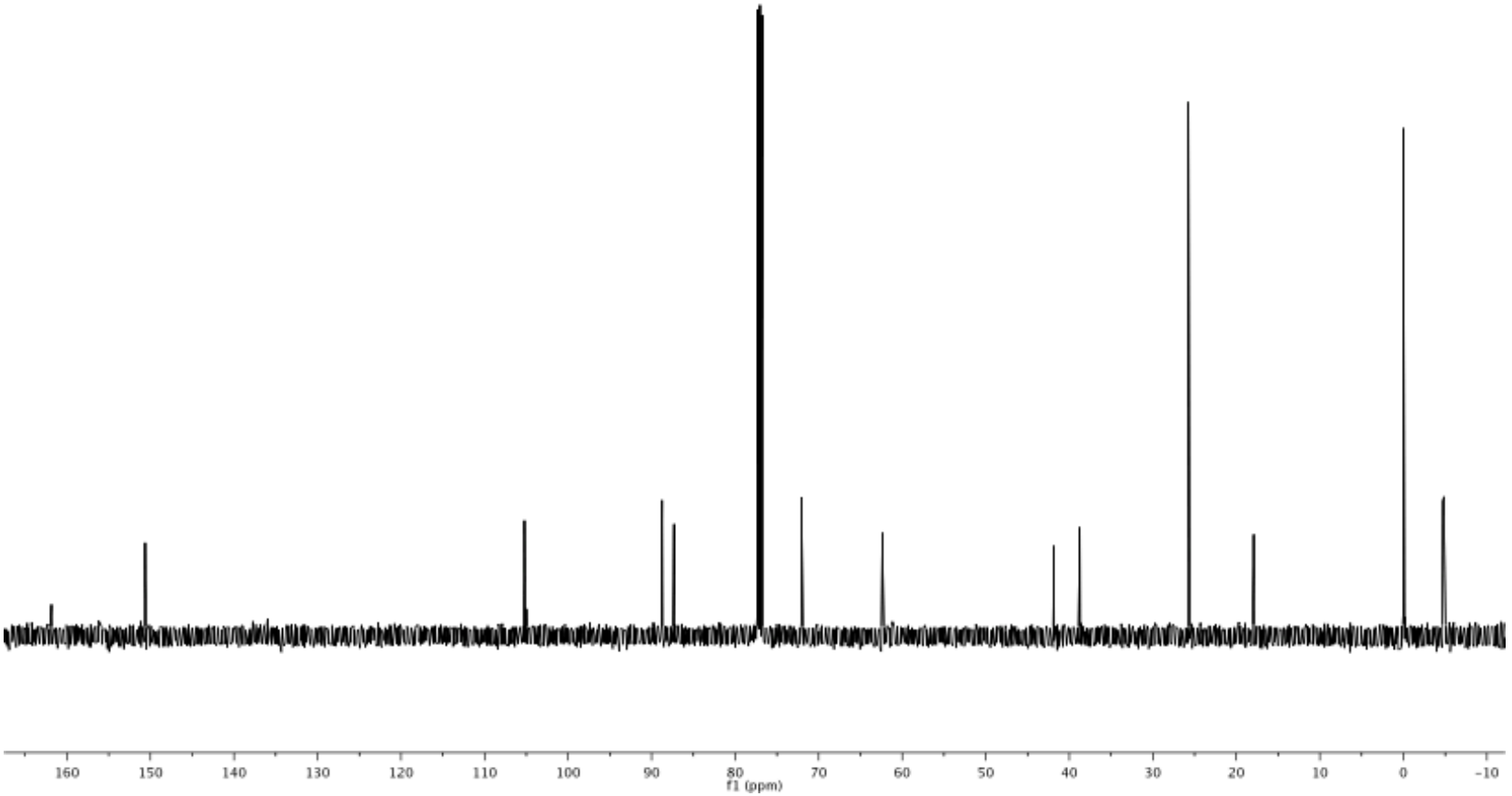
OTBS

7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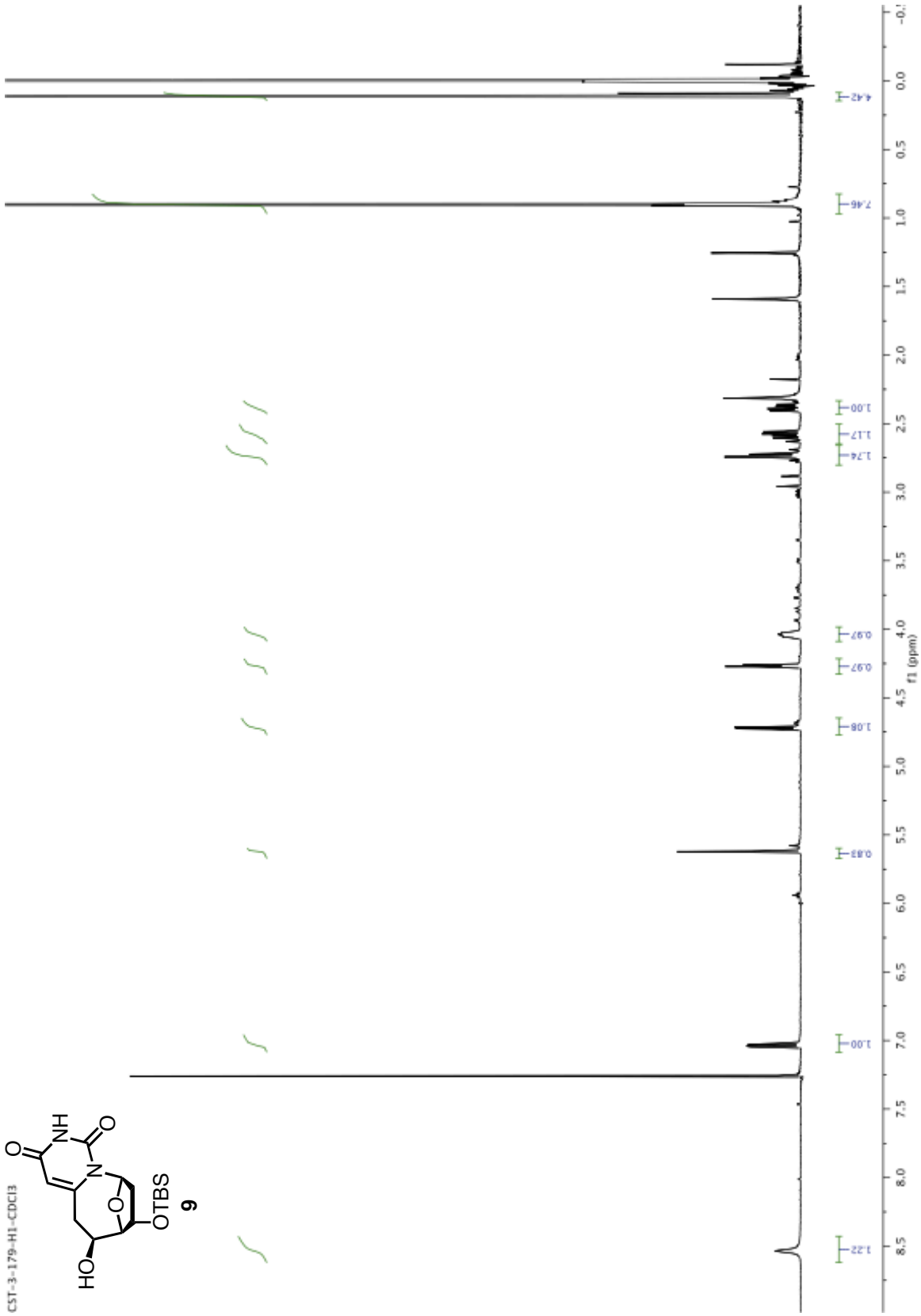
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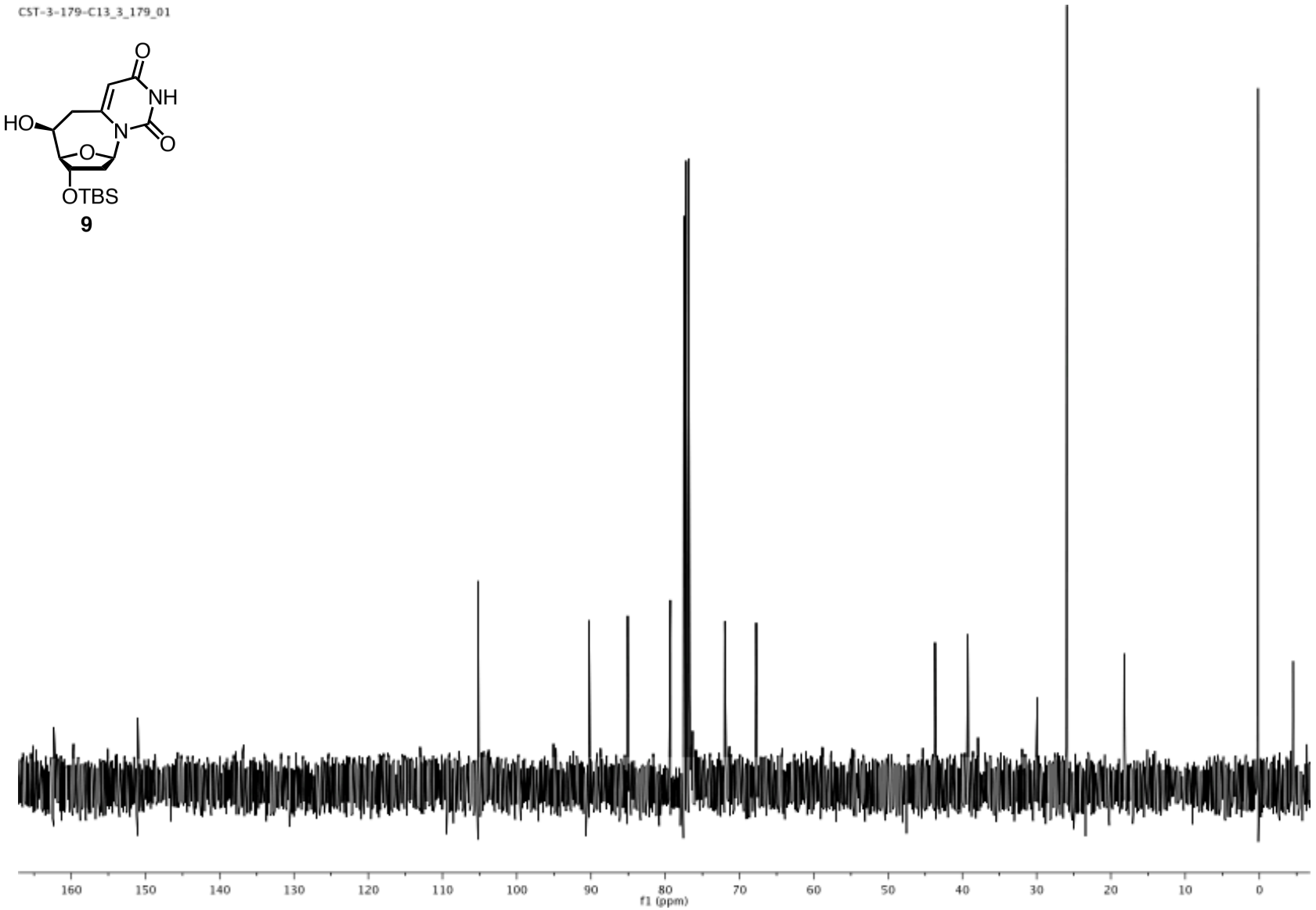
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O
O
OTBS
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160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
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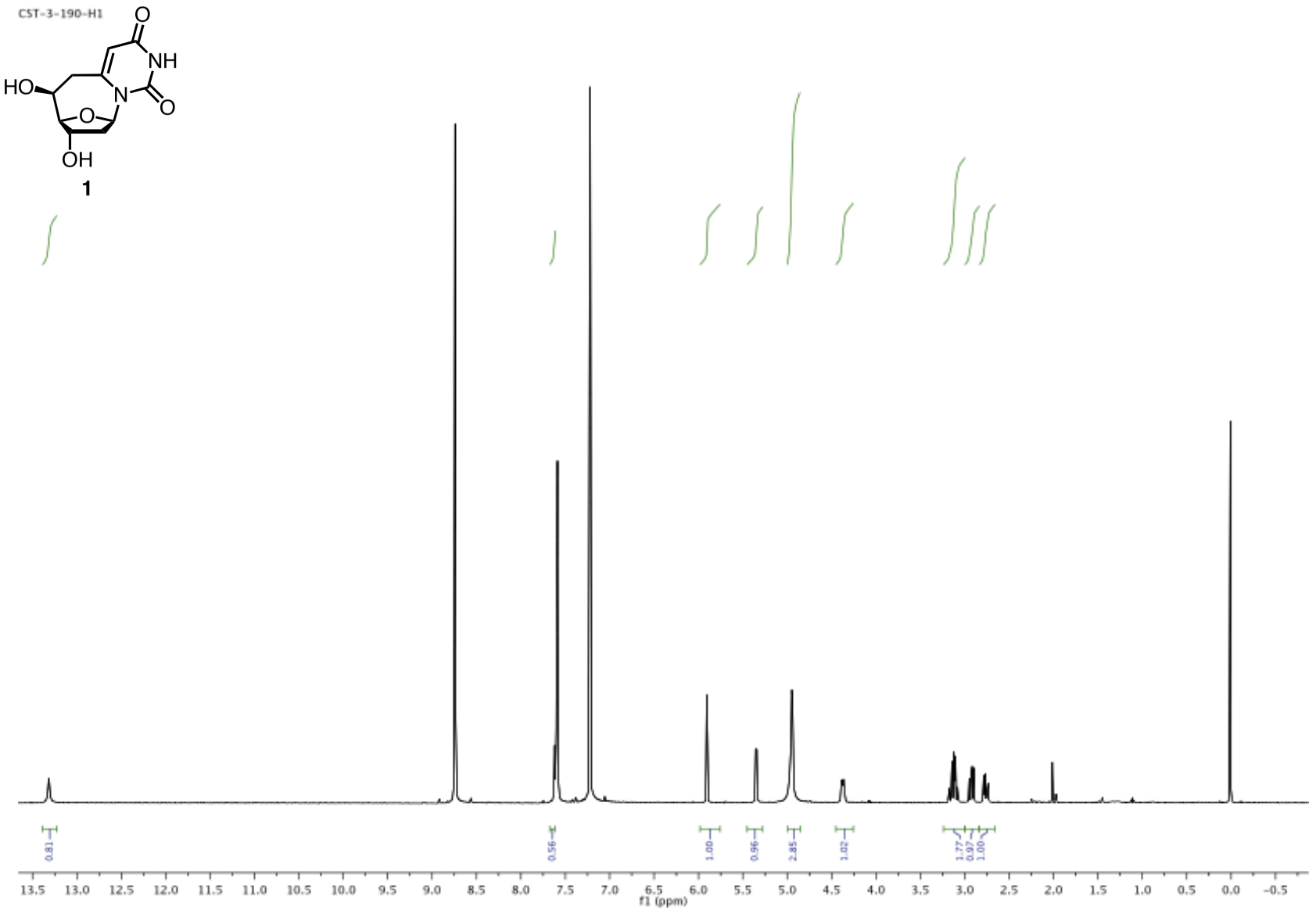
f1 (ppm)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OH
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1.00
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1.02
1.77
0.67
1.00
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
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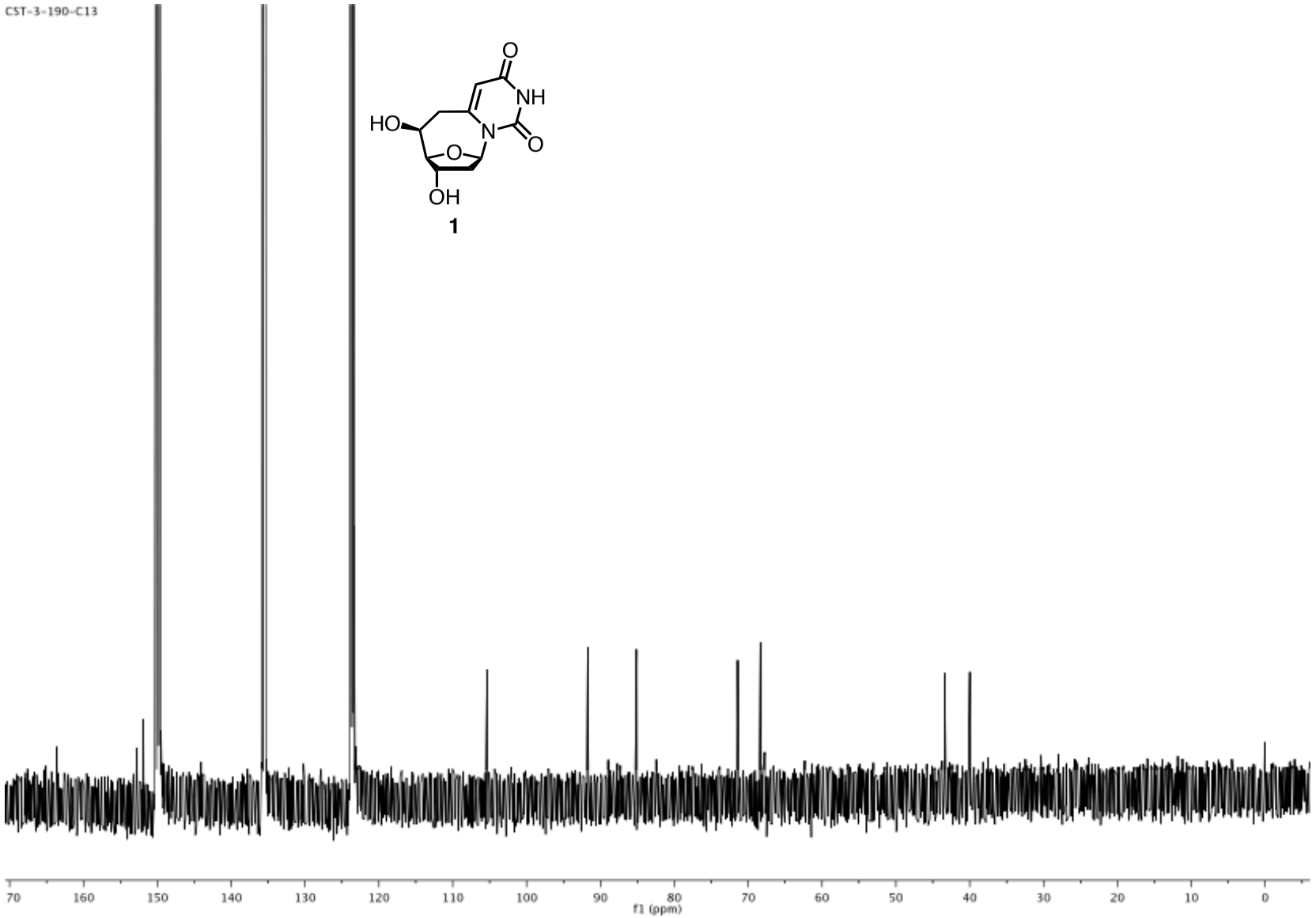
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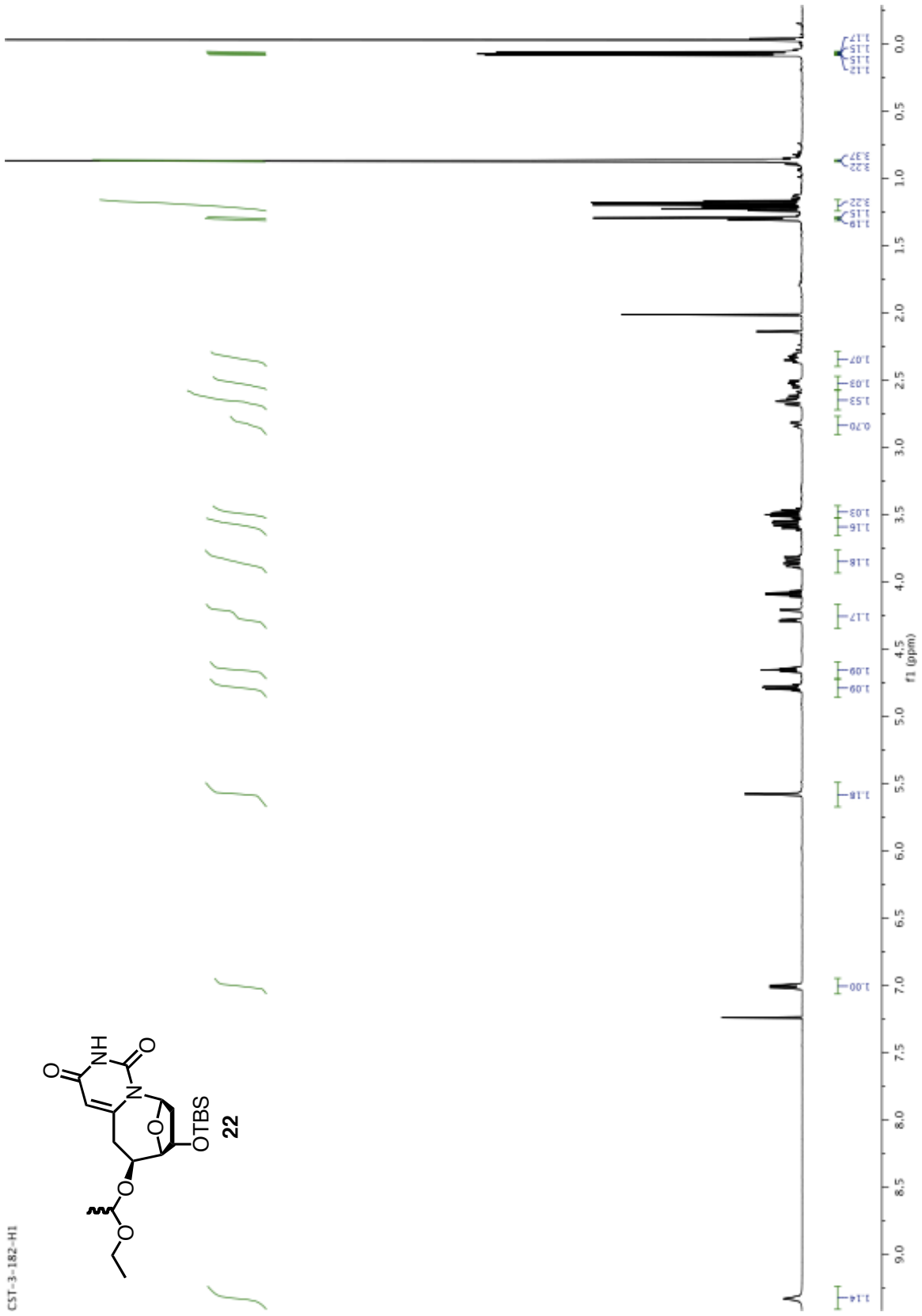
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O
OTBS
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O
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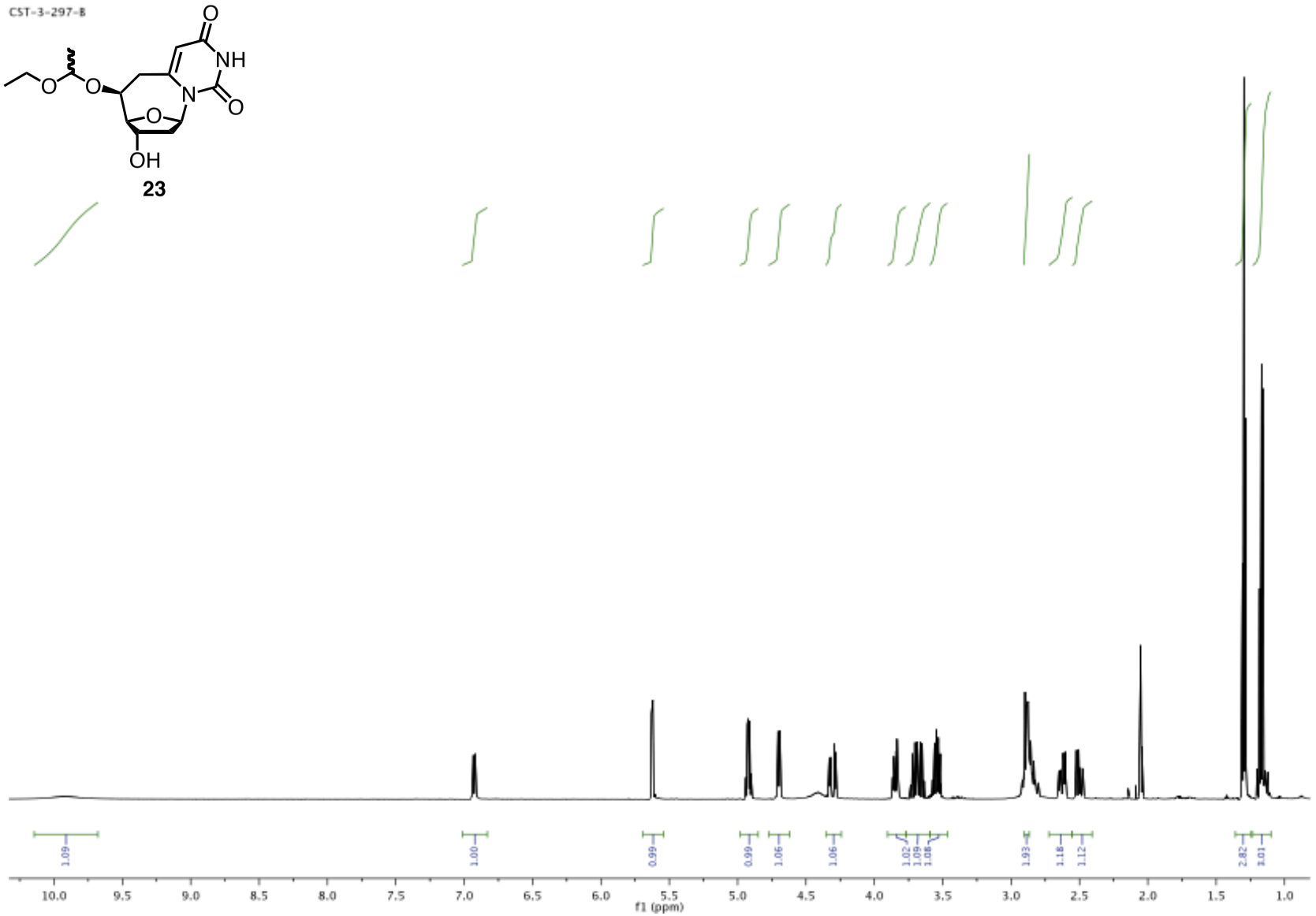
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O
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1.02
1.09
1.08
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1.12
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8.0
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2.0
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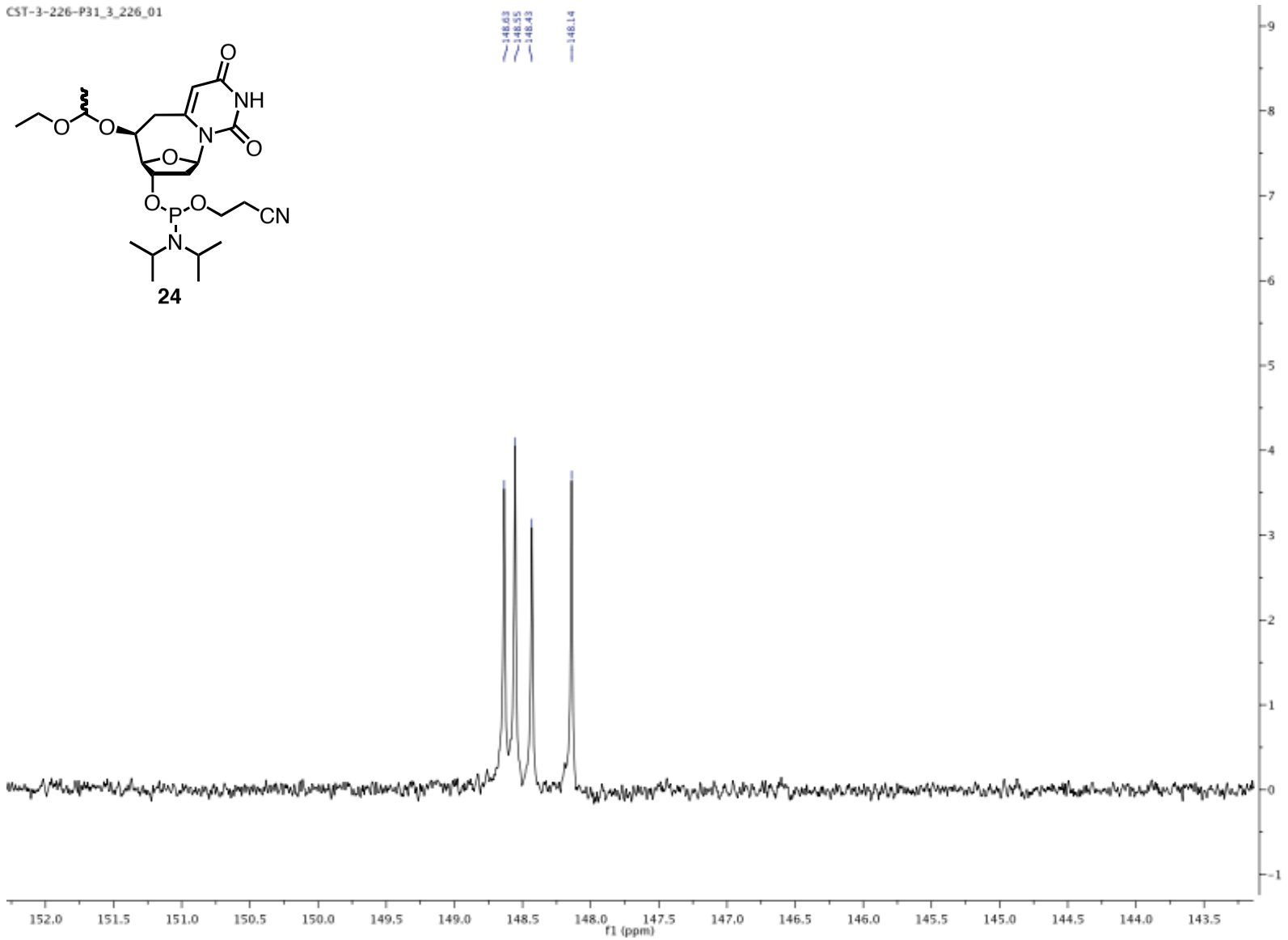
f1 (ppm)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P
O
CN
N
24
f1 (ppm)

Chapter 4

Synthesis and Properties of 6' Extended Backbone Nucleosides*

* ***Note:*** Compound, scheme, and reference numbers reset in this section. References listed at the end of section 4.5.

4.1.1 Introduction to 6' Nucleosides

DNA and RNA are the known naturally occurring nucleic acid based biopolymers. The role of DNA in maintaining the genetic code is responsible for all life as we know it. However, scientists have seen the importance of making modifications to the backbone of oligonucleotides. Previously our lab has studied modified polymers called threose nucleic acid (TNA) and glycol nucleic acids (GNA) amongst others[1-6].

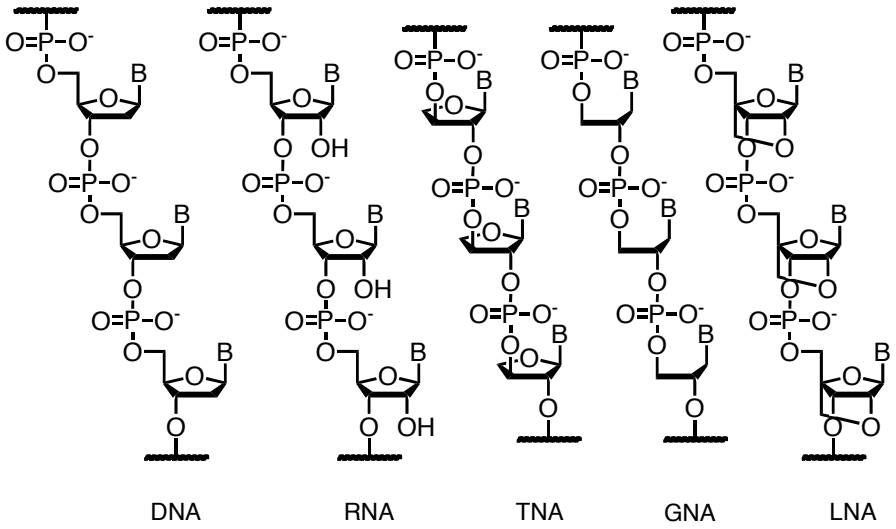


Figure 1: Various native and modified oligonucleotides

In contrast to the more complex LNA or the cyclonucleosides covered in the chapters 2 and 3, TNA and GNA are more simplified systems. TNA features a 2'-3' phosphate linkage, removing the stereo center at the C4' position. GNA is even more fundamental, lacking the pentose sugar ring. TNA can bind to DNA and form duplexes, while both TNA and GNA triphosphates can be accepted by DNA polymerase enzymes[1-3, 7]. Due to their simplistic nature both TNA and GNA have been proposed as possible predecessors to RNA[8]. It is unknown whether RNA arose directly out of the "primordial soup." Due to the complex nature of a ribose sugar

oligonucleotide, some scientists have predicted that more basic systems could have evolved first in a scenario dubbed, "the pre-RNA world"[8, 9].

While TNA and GNA feature shorter 2' to 3' linked backbones compared the 3' to 5' linkage of DNA, we are currently interested in extending the spacing between nucleosides. This section will address the preliminary research into 6' nucleosides, which feature an extra methylene unit at the 5' carbon.

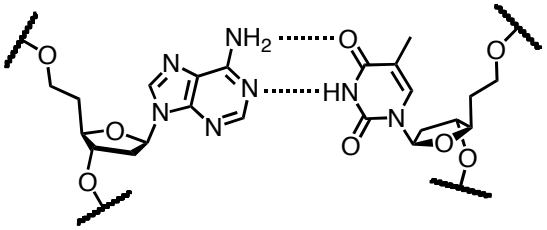


Figure 2: A 6'-dA and 6'-T base pair

We were curious to analyze how an extra carbon atom at the 5' position would affect the structure of an oligonucleotide strand. We hypothesized that a duplex featuring 6' nucleosides paired to each other would form a new type of helical structure. We predicted that the helix would either wind tighter in order to maintain base stacking or become more spaced out due to the increased steric bulk in the backbone, possibly changing the 3.4 Å distance between base pairs. The spacing and charge distribution of the phosphodiester groups are also likely to be a factor in new duplex formation.

We also want to understand how the extended backbone will affect stability when partnering with both native DNA and strands containing partnering 6'-nucleosides. Eventually we would like to synthesize 6' triphosphates and test them with a variety of DNA and RNA polymerase enzymes, such as the promiscuous Therminator, to see if they can be accepted by the enzyme's machinery.

Lastly, we are interested to see if we can make monomers that work in a non-enzymatic templated synthesis reaction. These systems help to understand the origins of life and represent attempts to create artificial life. Szostak et al. worked on a series of nucleosides featuring a 2' NH_2 to 5' OH phosphate linkage[10]. Rather than using the native triphosphate 5' terminus, with pyrophosphate as the leaving group, their monomers use a phosphoimidizolide. The imidazole leaving group, coupled with the more reactive 2'-amino nucleophile, enabled 5' to 2' template directed non-enzymatic polymerization (Figure 3).

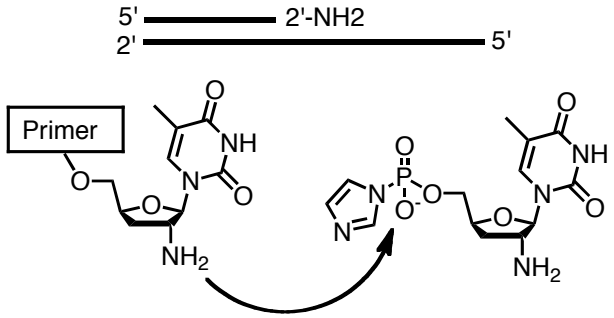


Figure 3: Non-enzymatic polymerization by Szostak et al.

Szostak's monomers use 2' amino nucleophiles as the 3' amines self-cyclized onto the 5' phosphate. The 2' to 5' linkage contains six atoms. 6' extended backbone nucleosides also contain a six atom linkage; therefore we are curious as to whether we could carry out a similar non-enzymatic polymerization.

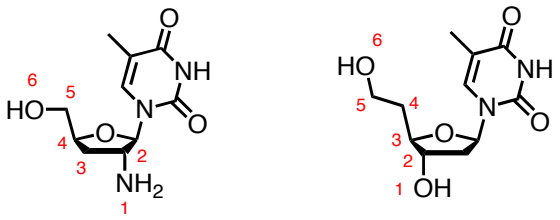
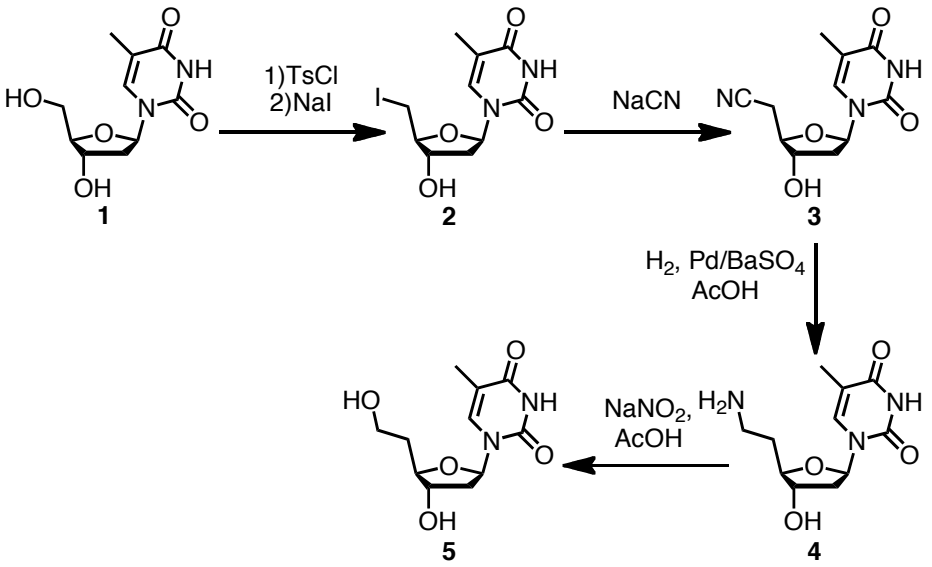


Figure 4: Comparison of backbone length between Szostak's monomers and 6' nucleosides

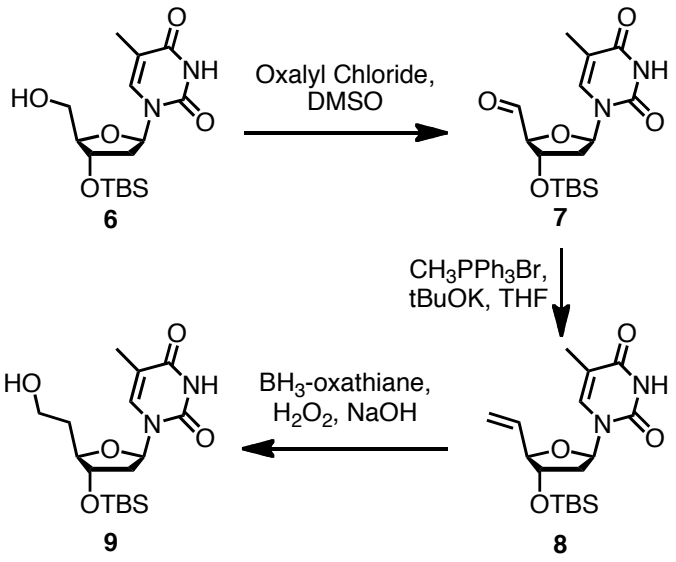
4.1.2 Previous 6' Nucleoside Syntheses

There have been a few previous syntheses of extended backbone nucleosides, beginning with a 6'-thymidine synthesis by Etzold et al. (Scheme 1)[11]. They start by converting the 5' OH to the nitrile **3** over three steps. Hydrogenation gives the 6' amine, which is transformed to the hydroxyl **5** by sodium nitrite under acidic conditions. Although short, several of the steps in this synthesis have mediocre yields and the compounds are poorly characterized.



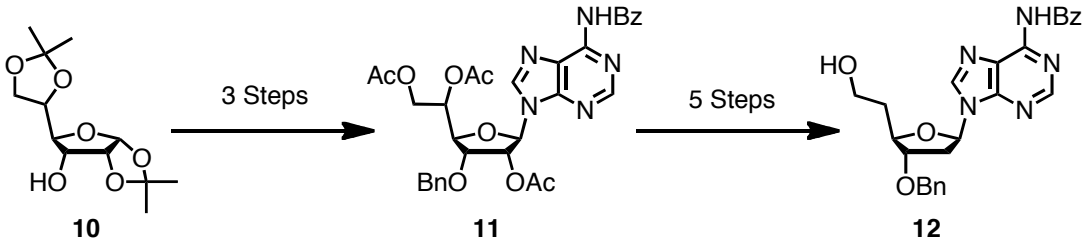
Scheme 1: Etzold et al.'s synthesis of 6'-thymidine[11].

A more recent synthesis by Kofoed and coworkers takes 3'-O silyl protected thymidine and oxidizes the 5' OH to the aldehyde using Swern conditions (Scheme 2)[12]. A Wittig reaction gives the terminal olefin **8**, which is extended to the 6' hydroxyl **9** with a BH_3 mediated hydroboration.



Scheme 2: 6' extension by Kofoed et al[12].

There have also been syntheses of 6' extended adenosine and 2'-deoxyadenosine. The only previous synthesis of 6'-dA was completed by Henningfeld et al. (Scheme 3)[13]. They began with di-acetonide protected α-D-allofuranose, which already features the 6' framework. After three protecting group steps, they installed the adenine base. Over five additional steps, they removed the unwanted 2' and 5' OH's to give the extended product. The main disadvantages of this scheme are its length and the deoxygenation that is performed using a Barton-McCombie procedure. Additionally, some of the reported yields are over 95% leading us to suspect their purity. Similar procedures have been utilized to access the ribose 6'-adenosine derivative[14, 15].



Scheme 3: The framework presented by Henningfeld and coworkers[13].

4.1.3 Biological Effects

In their paper containing their 6'-T synthesis, Kofoed et al. inserted a single modified residue into an oligonucleotide 14-mer[12]. They found that upon hybridization with a native strand the Tm value fell by 5 ˚C due to destabilization from the 6' extension. They also showed that a sequence with one modification could recognize a native sequence containing a hairpin or bulge motif. Interestingly, they only placed modified residues into one of the two oligonucleotide strands.

6' extended thymidine has been shown to be accepted by thymidine phosphorylase, an enzyme involved in nucleoside metabolism[16]. The modified monomer binds to the enzyme as well as thymidine. However, the turnover is less than 1% of native nucleotide.

4.1.4 Introduction to Our Research

The next sections of this thesis will show an improved synthesis of 6' extended thymidine phosphoramidite and how this molecule affects stability in a DNA duplex. In comparison to previous work, we have inserted the 6'-T monomers into both strands of the duplex.

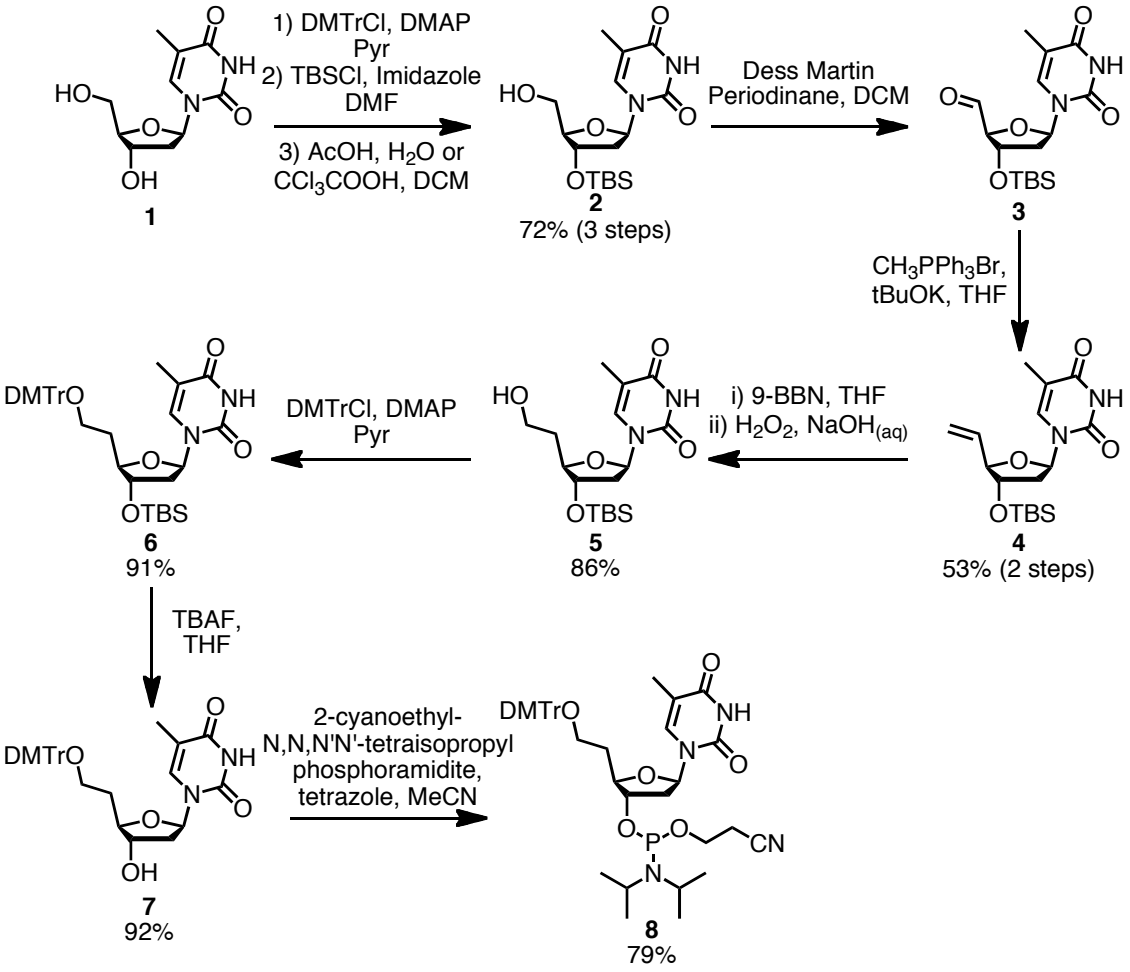
This thesis also presents preliminary work towards an improved 6' extended dA synthesis. This is a challenging molecule to make compared to 6'-T, due to its reactive.

4.2 Synthesis of 6' Extended Thymidine*

As mentioned in the introduction, our route towards 6'-T is based off of the work by Kofoed et al[12]. We begin with three protection group reactions (Scheme 4).

* ***Note:*** Compound numbers reset in this section.

First a DMTr group is placed on the 5' OH, which allows for silation of the 3' position with TBSCl. The DMTr group is then removed under acidic conditions to form **2**.



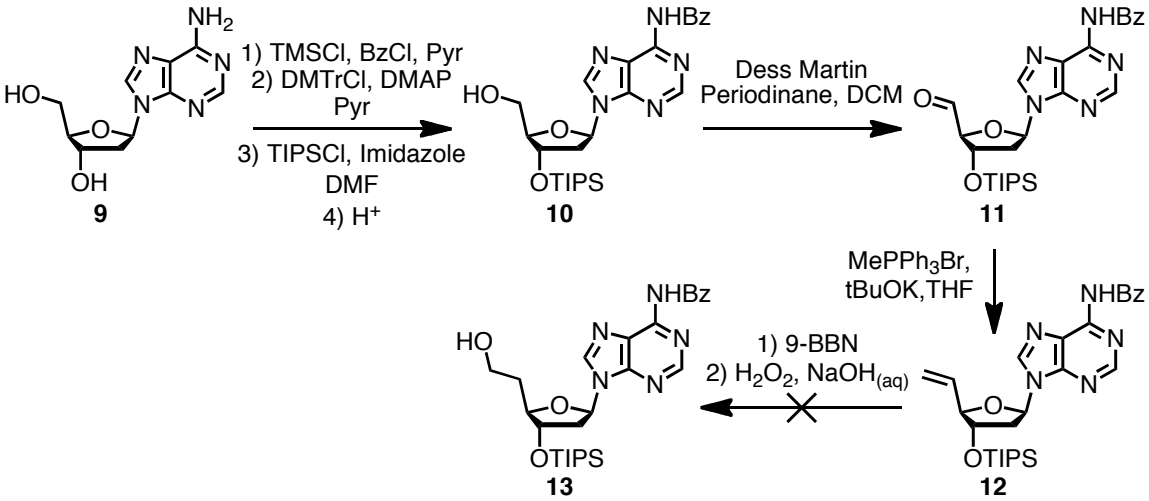
Scheme 4: Our synthesis of 6' Thymidine

The 5' OH was oxidized using Dess-Martin periodinane to yield the 5' aldehyde **3**. The aldehyde is not stable to column chromatography conditions and the crude material was carried on after an aqueous workup. A Wittig reaction gives the terminal olefin **4**. Kofoed's procedure used a Swern oxidation and had a two-step yield of 35%. Our two-step yield is 53%, representing a significant improvement, which also avoids the unpleasant thiols of the Swern oxidation.

Hydroboration with 9-BBN gave the 6' hydroxyl extension. This milder reagent produced the product in an 86% yield compared to the 61% achieved by Kofoed using BH_3. DMTr protection of the 6' position, silyl removal with TBAF, and phosphorylation produces the phosphoramidite **8** for use on a DNA synthesizer.

4.3 Work Towards the Synthesis of 6' Extended 2'-deoxyadenosine

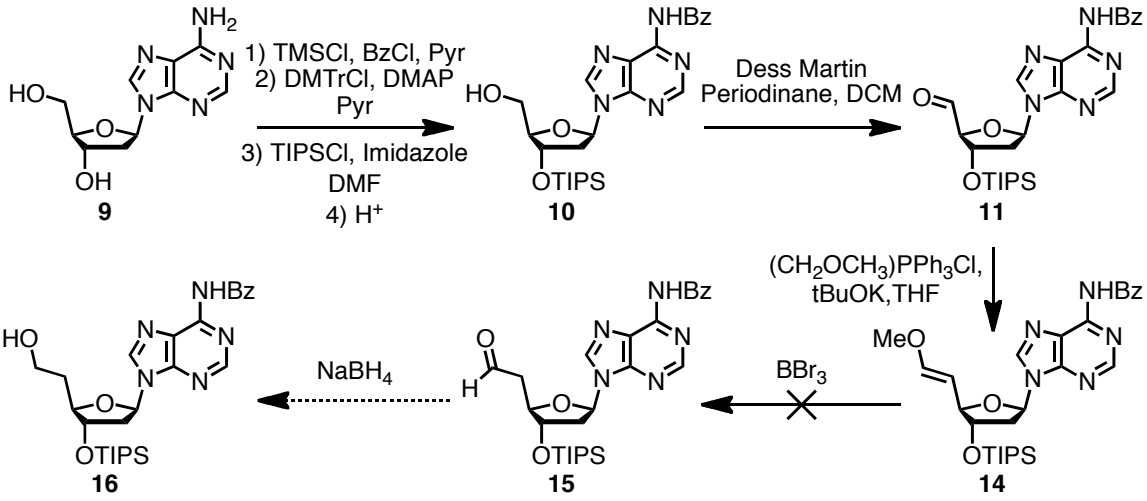
Our initial plan for 6' extended dA was to follow the same general route used in our synthesis of 6'-T. However, work by Eric Hardter in our group showed that the hydroboration did not proceed with adenine as the nucleobase. Our theory is that the exocyclic amine reacts with the boron complex instead of the olefin. Substituting BH_3 for 9-BBN and changing other aspects of the reaction failed to produce product.



Scheme 5: Work by Eric Hardter attempting a hydroboration with an adenosine based nucleoside

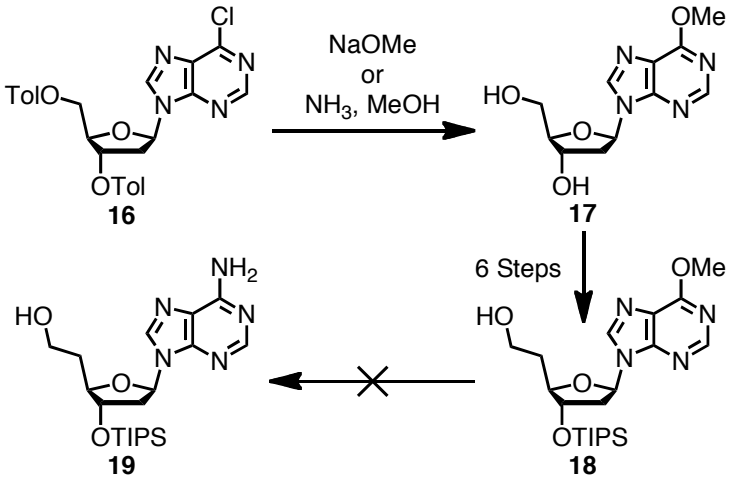
We then sought to try an alternative Wittig reagent, which would not require hydroboration to achieve the 6' OH. Using (methoxymethyl)triphenylphosphonium chloride, we obtained our desired Wittig product. However, deprotection of the

methoxy group to a terminal aldehyde uses a boron based Lewis acid, which caused depurination.



Scheme 6: Attempts to deprotect the methoxy methyl group by Eric Hardter

Lastly we tried to use 6-chloropurine as the heterocycle instead of adenine (Scheme 7). During the preparation of this monomer, we needed to remove 3' and 5' toluoyl protecting groups with sodium methoxide or NH_3 in MeOH. These reactions unfortunately displaced the chlorine at the 6 position with a methoxy group. Although we were able to complete the extension of the 6' position, we were unable to convert the methoxy to an amine.



Scheme 7: 6-methoxy attempted synthesis (with Eric Hardter)

Our current route uses 2'-deoxyinsosine as the nucleoside, which allows for the backbone extension. We begin our synthesis by converting 2'-deoxyadenosine to 2'-deoxyinosine using bovine adenosine-deaminase. We then follow the 6'-T route to the hydroboration product **24**. To improve solubility we substituted the 3' TBS protecting group for a TBDPS group. A Dess-Martin oxidation did not produce aldehyde product **22**, so we utilized a Corey-Kim oxidation, which proceeded in a 67% two-step yield with the Wittig reaction. This represents a significant improvement from the 6'-T synthesis, and in the future we plan to use this oxidation for thymidine as well. A hydroboration with 9-BBN allows us to access the 6' OH in good yield.

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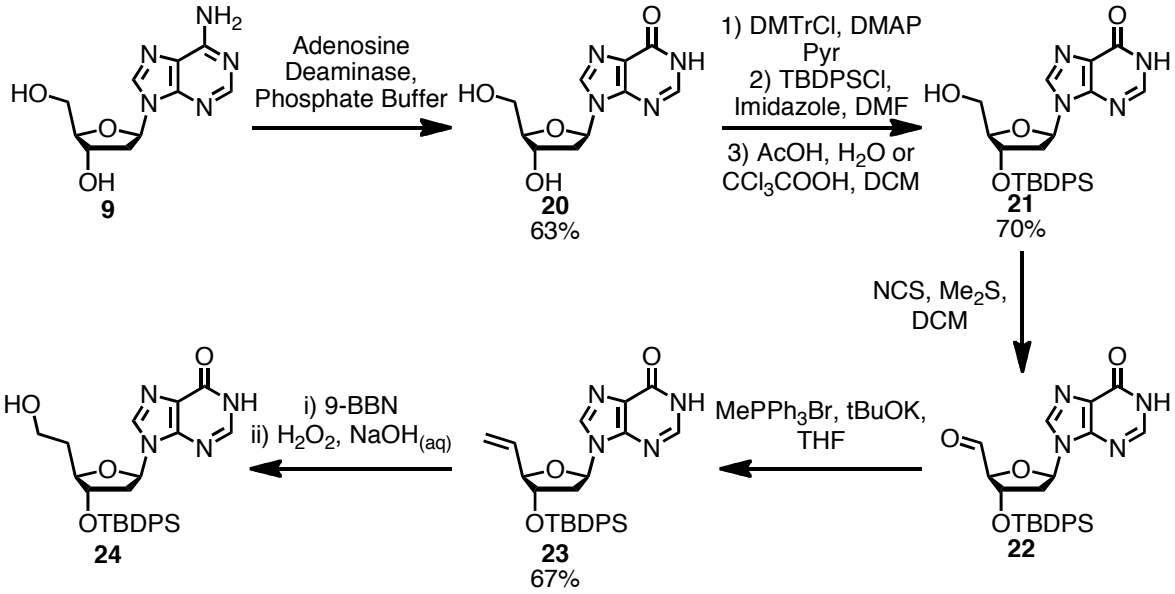
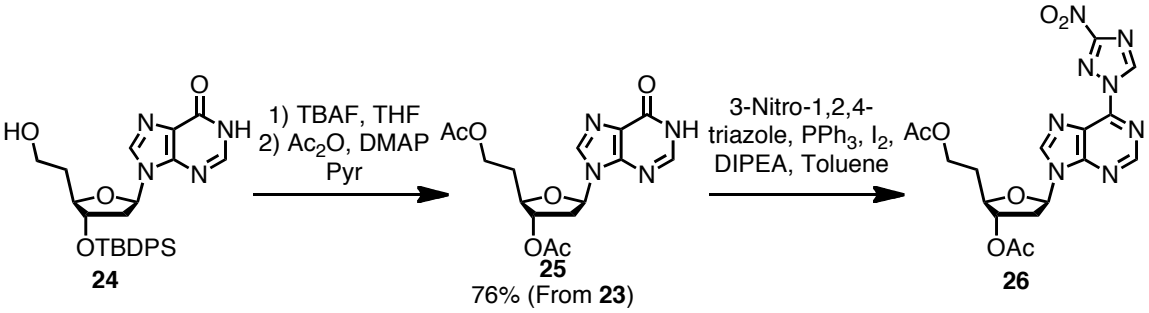


Scheme 8: 6' extension of 2'-deoxyinosine

At this point we need to convert the base back to adenine. Most literature references convert the base to 6-chloropurine by heating with thionyl chloride, $POCl_3$, or CCl_4[17-19]. First we removed the silyl group with TBAF and protected both

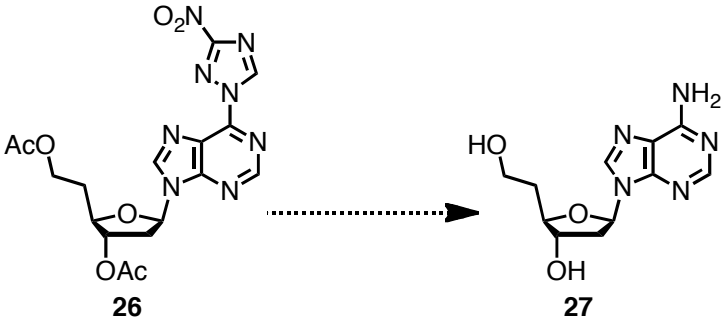
the 3' and 6' OH's with acid stable acetate groups to form **25**. However, we found the reactions with these chlorinating agents were too harsh and led to depurination.

Our new strategy uses a nitro-triazole functional group to access the adenine base. Again, both the 3' and 6' OH's are protected with acetates. The O6 on the base is activated with triphenylphosphine and iodine, before displacement with the nitro-triazole to form **26**.



Scheme 9: Installation of the nitro-triazole group

Our initial attempt to displace the triazole with 7N NH_3 in MeOH surprisingly installed a methoxy group at the 6 position. We are currently exploring whether an ammonia-dioxane solution will allow for displacement of the triazole moiety to install the amine.



Scheme 10: Remaining conversion to the exocyclic amine

4.4 Initial DNA Studies Involving 6' Extended Thymidine

Upon synthesis of the 6'-T phosphoramidite, we incorporated the modified monomer into various DNA oligomers. Our first test was to insert two modifications into the Dickerson dodecamer to form Strand 2 (Table 1). The Dickerson dodecamer is a self-complementary 12-mer sequence with an AATT core (Strand 1). These modifications would create a duplex with four total extended backbone nucleotides, with two on each strand. The Tm values we obtained for sequences containing the modification were around 70 ˚C, much higher than the 50-60 ˚C range seen with native Dickerson dodecamer duplexes.

Strand	Sequence
1	5'-d(CGCGAATTCGCG)-3'
2	5'-d(CGCGAAT*T*CGCG)-3'
3	5'-d(CCGGAATTCGCC)-3'
4	5'-d(GGCGAATTCCGG)-3'
5	5'-d(CCGGAAT*T*CGCC)-3'
6	5'-d(GGCGAAT*T*CCGG)-3'

Table 1: Native and 6'-T (T*) modified sequences

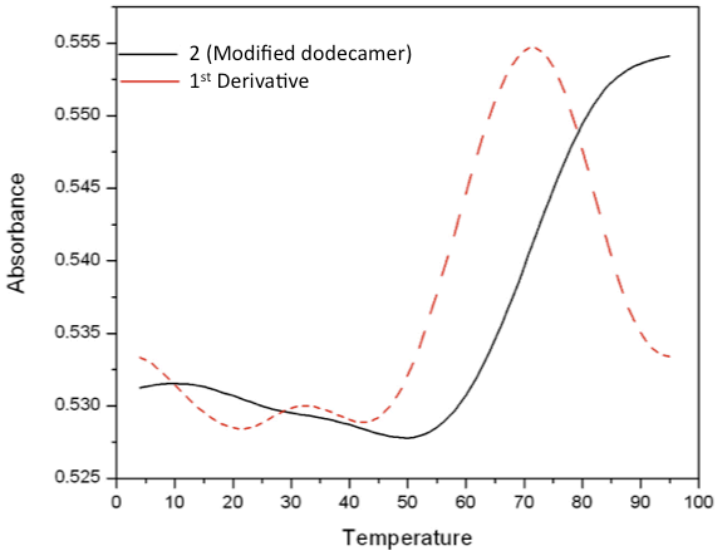


Figure 4: A Tm curve for strand 2, which contains an AAT*T* core (Tm=~70 ˚C).

However, upon running a native gel of strand 2 against the native strand 1, we observed that the modified dodecamer ran much faster than the native duplex (Figure 5). Our hypothesis is that the 6' extension may cause strand 2 to form a hairpin rather than a duplex. The hyperchromicity for the Tm curve of strand 2 is very weak at ~5%. This means that any helical structures or hairpins are likely only partially formed. Therefore we sought to synthesize analogous strands that are not self-complementary.

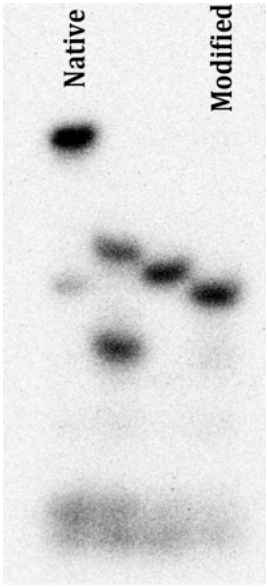


Figure 5: A ^{32}P labeled native gel of a native Dickerson dodecamer on the left and a 6'-T modified dodecamer on the right. The middle two lanes do not relate to this project.

Sequences 3-4 form a native duplex featuring a similar AATT core to that of the Dickerson dodecamer, but the C-G ends are scrambled to avoid self-complementary sequences. Strands 5-6 feature two modified 6'-T residues each. The Tm for the modified duplex was around 45 ˚C compared to near 60 ˚C for the native sequences (Figure 6). This shows that although the modifications are not severe enough to prevent duplex formation, they are destabilizing. The

hyperchromicity of the modified duplex is ~9.5% compared to ~13.5% for the native duplex. While not a particularly strong transition, it is more significant than observed for the self-complementary modified strand, lending futher support for duplex formation.

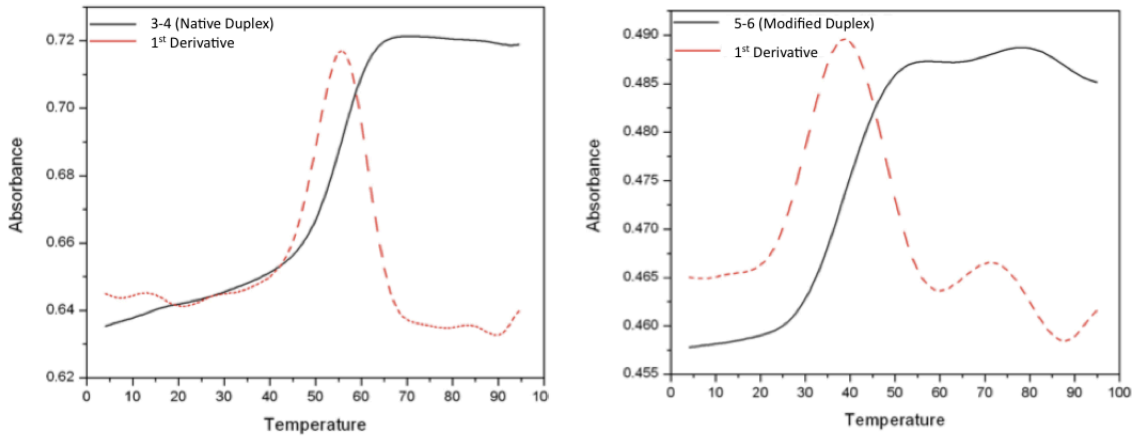


Figure 6: Native duplex Tm of strands 3-4 on the left and a Tm for modified strands 5-6 on the right. Both of these samples had 100 mM NaCl and 20 mM pH 7 phosphate buffer.

We also took circular dichroism (CD) measurements of the modified and native duplexes. The general shape of the modified is similar to that of the native, showing that the modified residues still maintain B-form like structure, although there are likely perturbations caused by the extra carbons.

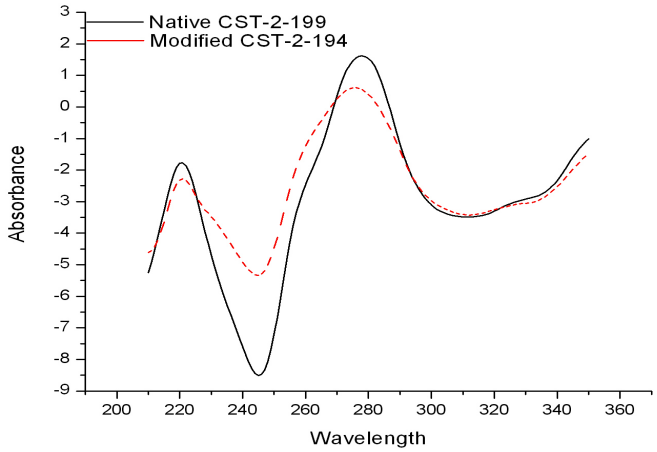


Figure 7: Circular dichroism measurements of native (black) and modified (red) duplexes containing an AATT or AAT*T* core.

4.5 Future Directions

Our most immediate goal is to finish the synthesis of 6' extended 2'-deoxyadenosine phosphoramidite. Our initial studies show that 6' monomers are destabilizing when paired with native nucleotides in duplexes. We would like to explore how 6' nucleosides interact when partnered with each other. Eventually we would like to make a duplex consisting entirely of 6'-T and 6'-dA monomers. We believe that as the core of modified T and dA residues is expanded they will become better tolerated and eventually will form a new helical structure.

One problem of inserting a 6' nucleotide core into a native duplex is that at the native-modified junctions, the spacing will be distorted. Since the strands are anti-parallel, even if each strand contains an equal number of 6' nucleotides, at each end of the modified core, a 6' "overhang" will exist. If we introduce a 3' extended monomer across from these 6' overhangs, it will eliminate the distortion at the junction. In Figure 8, the blue 3' nucleotides in the bottom duplex reposition the "crooked" base pairs caused by the 6' overhand in the top duplex.

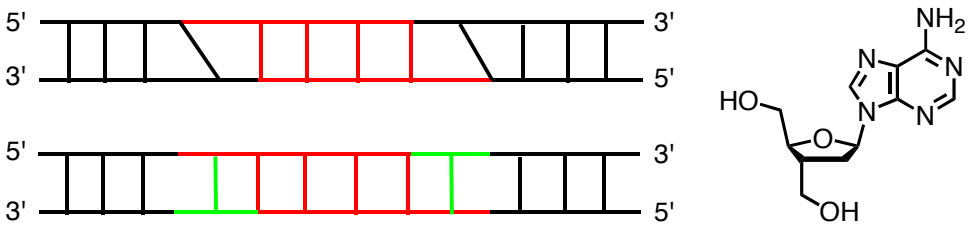
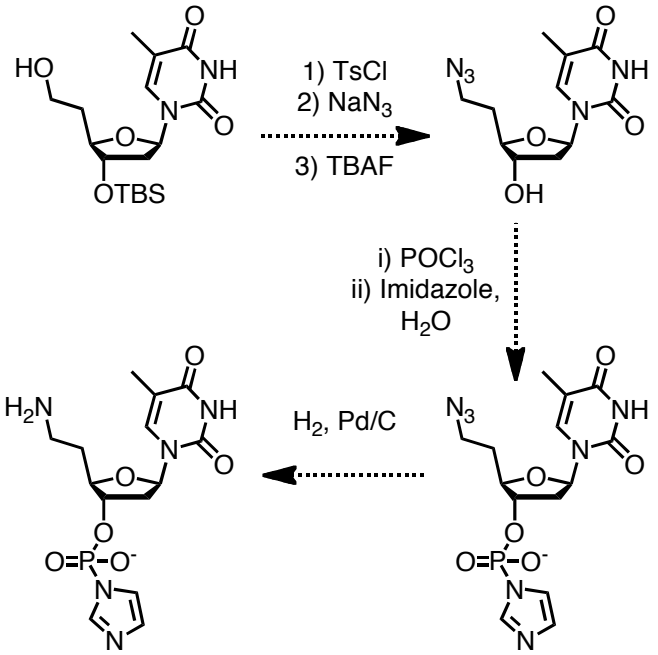


Figure 8: Duplexes diagrams showing native nucleotides in black, 6' extended in red, and 3' extended in green. To the right is an example of a 3' extended nucleoside.

Depending on the results achieved with the 6'-6' duplexes we are also curious to attempt non-enzymatic polymerization reactions akin to Szostak's, which

were presented in this chapter's introduction. 6' nucleosides contain the same number of bonds as Szostak's 2' NH_2-5' phosphoimidizolide monomers. For ease of synthesis, we would place the amino nucleophiles at the 6' position and the phosphoimidizolide at the 3' (Scheme 11).



Scheme 11: Planned synthesis of 6' monomers for non-enzymatic polymerization

Overall backbone extended nucleosides present a multitude of challenges and opportunities for synthesis. Our hope is that upon completion of the 6'-dA synthesis, we will be able to carry out many interesting biological studies related to duplex formation and stability.

1. Chen, J. J.; Tsai, C. H.; Cai, X.; Horhota, A. T.; McLaughlin, L. W.; Szostak, J. W., Enzymatic Primer-Extension with Glycerol-Nucleoside Triphosphates on DNA Templates. *Plos One* **2009,** *4* (3).
2. Horhota, A.; Zou, K. Y.; Ichida, J. K.; Yu, B.; McLaughlin, L. W.; Szostak, J. W.; Chaput, J. C., Kinetic analysis of an efficient DNA-dependent TNA polymerase. *Journal of the American Chemical Society* **2005,** *127* (20), 7427-7434.
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4.6 6' Extended Experimental Procedures

3' OTBS Protected Thymidine (2)

Thymidine (10.0 g, 41.3 mmol), dimethoxytrityl chloride (14.7 g, 43.4 mmol), and DMAP (100 mg, 0.820 mmol) were dissolved in pyridine (265 mg, 2.17 mmol) and stirred for 12 hrs. The reaction was quenched with methanol and the solvent was removed by rotary evaporation. After coevaporating with toluene, the crude material was dissolved in DMF with tertbutyldimethylsilyl chloride (9.29 g, 61.8 mmol) and imidazole (5.60 g, 82.56 mmol). The reaction was stirred for 12 hrs before being partitioned between brine and ethyl acetate. The ethyl acetate layer was washed an additional two times with brine and concentrated by rotary evaporation. The intermediate material was dissolved in 80% acetic acid and stirred for 2 hours before the solvent was removed by rotary evaporation and co-evaporation with ethanol. The crude product was purified by flash chromatography (80% EtOAc, 20% hexanes) to yield **2** as a white solid (11.13 g, 31.25 mmol, 72% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.15 (s, 1H), 6.01 (t, *J* = 6.8 Hz, 1H), 4.38 (dt, *J* = 6.8, 3.5 Hz, 1H), 3.81 (m, 2H), 3.64 (dd, *J* = 12.6, 3.6 Hz, 1H), 2.25 (dt, *J* = 13.6, 6.9 Hz, 1H), 2.10(m, 1H), 1.81 (s, 3H), 0.79 (s, 9H), -0.09 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.88, 150.58, 137.25, 111.18, 87.79, 87.13, 71.82, 62.26, 40.72, 25.93, 18.13, 12.87, -4.63. HRMS (DART) Calcd for $C_{16}H_{29}N_2O_5Si$ 357.1846; Found, 357.1859.

Thymidine Wittig (4)

3' OTBS thymidine (700 mg, 1.96 mmol) was dissolved in DCM (25 mL) and Dess-Martin Periodinane (6.14 mL of a 0.48 M solution in DCM, 2.95 mmol) was

added. The reaction was stirred for 3 hrs before being quenched with a 50 mL of sat. $NaHCO_3$ and 50 mL of sat. $Na_2S_2O_3$. The organic layer was further washed with brine and dried over Na_2SO_4 before the solvent was removed by rotary evaporation.

$MePPh_3Br$ (2.80 g, 7.84 mmol) and *t*-BuOK (770 mg, 6.86 mmol) were dissolved in THF (100 mL) and stirred at r.t. for 1 hr. A solution of the crude aldehyde in THF (50 mL) was added and stirring continued for 12 hrs. The reaction was quenched with saturated NH_4Cl solution and the organic layer was subsequently washed with brine solution. The organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (60% EtOAc, 40% hexanes) to yield **4** as a white solid (366 mg, 1.04 mmol, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.17 (s, 1H), 6.21 (t, *J* = 6.5 Hz, 1H), 5.87 (ddd, *J* = 17.1, 10.5, 6.6 Hz, 1H), 5.38 (d, *J* = 17.1 Hz, 1H), 5.28 (d, *J* = 10.5 Hz, 1H), 4.24 (m, 1H), 4.16 (dt, *J* = 6.5, 4.4 Hz, 1H), 2.30 (ddd, *J* = 13.5, 6.4, 4.4 Hz, 1H), 2.08 (dt, *J* = 13.3, 6.6 Hz, 1H), 1.92 (s, 3H), 0.87 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.49, 150.22, 135.32, 135.28, 118.40, 111.30, 94.82, 87.72, 85.42, 75.40, 40.75, 26.16, 18.33, 13.10, -4.33. HRMS (DART) Calcd for $C_{17}H_{29}N_2O_4Si$ 353.1897; Found, 353.1910.

Thymidine Hydroboration (5)

The thymidine Wittig product (1.05 g, 2.96 mmol) was dissolved in THF (30 mL) and a 0.5 M solution of 9-BBN in THF (23.5 mL, 11.83 mmol) was added. The reaction was stirred for three hours before being quenched with 1 M NaOH (46.8 mL) and 30% H_2O_2 (7.75 mL). The organic layer was further washed with brine and dried over Na_2SO_4. The crude material was purified by flash chromatography

(100% EtOAc) to yield the product as a white foam (940 mg, 2.55 mmol, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.12 (s, 1H), 6.15 (t, *J* = 6.6 Hz, 1H), 4.19-4.10 (m, 1H), 3.95-3.85 (m, 1H), 3.84-3.74 (m, 2H), 2.24 (ddd, *J* = 13.6, 6.9, 4.9 Hz, 1H), 2.12 (dt, *J* = 13.3, 6.4 Hz, 1H), 1.9s (s, 3H), 0.87 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.30, 155.04, 140.19, 116.11, 89.80, 79.69, 65.29, 45.15, 40.34, 30.51, 22.70, 17.49, 0.28. HRMS (DART) Calcd for $C_{17}H_{31}N_2O_5Si$ 371.2002; Found, 371.2006.

6' DMTr Protection (6)

The 6' OH thymidine product (896 mg, 2.42 mmol), DMTrCl (1.64 g, 4.84 mmol), and DMAP (15.0 mg, 0.121 mmol) were dissolved in 50 mL of pyridine and stirred for 12 hrs. The reaction was quenched with water and the solvents were removed by rotary evaporation and coevaporation with toluene. The material was dissolved in EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude material was purified by flash chromatography (60% EtOAc, 40% hexanes) to yield **6** as a light yellow foam (1.49 g, 2.21 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.44-7.34 (m, 2H), 7.34-7.23 (m, 6H), 7.19 (dd, *J* = 15.2, 8.0 Hz, 1H), 7.03 (s, 1H), 6.89-6.69 (m, 4H), 6.10 (t, *J* = 6.6 Hz, 1H), 4.15-4.03 (m, 1H), 3.95 (dt, *J* = 8.4, 4.2 Hz, 1H), 3.85-3.69 (m, 5H), 3.21 (t, *J* = 6.6 Hz, 2H), 2.24 (ddd, *J* = 13.5, 6.2, 3.8 Hz, 1H), 2.00-1.90 (m, 2H), 1.85 (s, 3H), 0.86 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.05, 163.23, 149.76, 141.03, 139.89, 134.73, 132.86, 132.55, 131.52, 117.83, 115.52, 90.97, 89.63, 89.07, 79.81, 65.12, 59.97, 45.31, 39.01, 30.47, 22.70, 17.49, 4.76, 0.16. HRMS (DART) Calcd for $C_{38}H_{48}N_2O_7Si$ 672.3231; Found, 672.3849.

3' Deprotection (7)

Compound **6** (1.49 g, 2.21 mmol, 91%) as dissolved in THF (30 mL) and TBAF (6.81 mL of a 1 M solution in THF) was added. The reaction was stirred for 1 hr and quenched with water. The THF was removed by rotary evaporation and EtOAc was added. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed by rotary evaporation and purified by flash chromatography (99% EtOAc, 1% Et_3N) to yield the product as a light yellow foam (1.14 g, 2.03 mmol, 92%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.48-7.36 (m, 2H), 7.35-7.30 (m, 5H), 7.26-7.21 (m, 1H), 7.10 (d, *J* = 1.2 Hz, 1H), 6.90-6.81 (m, 4H), 6.15 (dd, *J* = 7.3, 5.1 Hz, 1H), 4.27 (dd, *J* = 13.9, 6.4 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 4H), 3.79-3.74 (m, 1H), 3.48 (dt, *J* = 9.6, 4.6 Hz, 1H), 3.18 (td, *J* = 9.8, 3.5 Hz, 1H), 2.48-2.35 (m, 1H), 2.29-2.19 (m, 1H), 1.95 (s, 3H).

6' Thymidine Phosphoramidite (8)

The 3' deprotected product (1.11 g, 1.98 mmol) was dissolved in acetonitrile (25 mL). 2-cyanoethyl-N,N,N',N'-tetraisopropyl phosphoramidite (1.26 mL, 3.96 mL) and tetrazole (4.43 mL of a 0.45 M solution in acetonitrile) were added and the reaction was stirred for 3 hours. The reaction was quenched with MeOH and the solvents were removed by rotary evaporation. The crude material was purified by column chromatography (65% DCM, 33% EtOAc, 1% Et_3N) to yield the product as a white foam (1.18 g, 1.56 mmol, 79%). ^{31}P NMR (162 MHz, acetone) δ 146.34, 145.87.

2'-Deoxyinosine (10)

2'-deoxyadenosine (26.92 g, 100 mmol) was dissolved in 1.48 L of 0.02 M KH_2PO_4 pH 7 aqueous buffer. 90 units of bovine intestine adenosine deaminase was

added and the reaction was stirred for 24 hrs at 40 ˚C. The volume was reduced to 1.0 L by rotary evaporation and the suspension was filtered. The filtrate was further concentrated to 500 mL and filtered again. The combined solid was recrystallized from hot ethanol after cooling at 4 ˚C overnight to yield the product as white fluffy crystals (15.94 g, 63.2 mmol, 63%). ^{1}H NMR (500 MHz, pyridine) δ 8.78 (s, 1H), 8.37 (s, 1H), 6.94 (t, *J* = 6.7 Hz, 1H), 5.19 (m, 1H), 4.61 (q, *J* = 3.5 Hz, 1H), 4.21 (ddd, *J* = 41.9, 11.9, 3.6 Hz, 2H), 3.18-3.00 (m, 1H), 2.78 (ddd, *J* = 13.1, 6.2, 3.6 Hz, 1H). ^{13}C NMR (126 MHz, pyridine) δ 158.37, 149.24, 146.35, 139.17, 126.88, 90.07, 85.70, 72.28, 63.18, 42.20. HRMS (DART) Calcd for $C_{10}H_{13}N_4O_4$ 253.0937; Found, 253.0941.

3' OTBDPS 5' OH Inosine (11)

2'-deoxyinosine (1.55 g, 6.15 mmol), dimethoxytrityl chloride (2.18 g, 6.45 mmol), and DMAP (37.6 mg, 0.308 mmol) were dissolved in pyridine (80 mL) and stirred for 12 hrs at r.t. The reaction was quenched with MeOH and the solvent was removed by rotary evaporation.

The crude material, along with imidazole (833 mg, 12.3 mmol), and TBDPSCl (3.19 mL, 12.29 mmol) were dissolved in DMF (80 mL) and stirred for 8 hrs. The reaction was partitioned between brine and EtOAc. The EtOAc layer was washed two additional times with brine solution before being dried over Na_2SO_4.

This intermediate was dissolved in DCM (100 mL) and Cl_3COOH (5.02 g, 30.8 mmol) and stirred for 1 hr. The reaction was quenched with Et_3N and the solvents are removed by rotary evaporation. The material was redissolved in DCM and washed twice with brine. The organic layer is dried over Na_2SO_4, evaporated, and

purified by flash chromatography (15% isopropanol, 85% CH_2Cl_2) to yield **11** as a white foam (g, 4.31 mmol, 70% yield over 3 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.10 (s, 9H), 2.28 (dd, *J*=13.0, 5.5 Hz, 1H), 2.71 (m, 1H), 3.12 (d, *J*=12.0 Hz, 1H), 3.66 (dd, *J*=12.5, 1.5 Hz, 1H), 4.11 (s, 1H), 4.66 (d, *J*=4.5 Hz, 1H), 6.32 (m, 1H), 7.38 (m, 6H), 7.63 (m, 4H), 7.74 (s, 1H), 8.19 (s, 1H), 12.98 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$), δ 19.25, 27.14, 31.10, 41.89, 62.96, 74.97, 87.75, 90.11, 126.47, 128.13, 130.30, 130.33, 133.30, 133.44, 135.86, 135.90, 140.11, 145.51, 147.89, 158.65. HRMS (DART) Calcd for $C_{26}H_{30}N_4O_4NaSi$ 513.1929; Found, 513.1934.

Alternatively the DMTr group can be removed by dissolving the intermediate in 80% AcOH and stirred for 3 hrs. The solvent is removed by rotary evaporation and coevaporated with EtOH. The crude material is redissolved in DCM and washed with sat $NaHCO_3$ and brine. The remaining workup and purification follows as written above. Yields are comparable.

Inosine Wittig (13)

N-chlorosuccinimide (1.73 g, 13.0 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 0 ˚C. Dimethyl sulfide (8.88 mL, 64.9 mmol) was added to the mixture, which immediately formed a white precipitate. The mixture was stirred at -78 ˚C for 1 hr before a solution of ___ (1.07 g, 2.16 mmol) in CH_2Cl_2 (20 mL) was added. The reaction was stirred for 3 hrs before Et_3N (6.05 mL, 43.2 mmol) was added and stirring continued at -78 ˚C for an additional hour. The cooling bath was removed and the reaction was diluted with CH_2Cl_2 (20 mL) and MeOH (5 mL) after 15 mins.

The mixture was washed with 1% aqueous HCl and brine before being dried over Na_2SO_4 and concentrated by rotary evaporation to provide the crude 5' aldehyde.

$MePPh_3Br$ (3.09 g, 8.65 mmol) and t-BuOK (847 mg, 7.57 mmol) were dissolved in THF (30 mL) and stirred at r.t. for 1 hr. A solution of the crude aldehyde in THF (30 mL) was added and stirring continued for 12 hrs. The reaction was quenched with saturated NH_4Cl solution and the organic layer was subsequently washed with brine solution. The organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (15% isopropanol, 85% CH_2Cl_2) to yield the product as a white foam (701 mg, 1.44 mmol, 67% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 9H), 2.46 (m, 2H), 4.40 (dt, *J*=4.8, 3.2 Hz, 1H), 4.46 (m,1H), (tt, *J*=5.2, 1.2 Hz, 1H), 5.10 (t, *J*=1.6 Hz, 1H), 5.62 (m, 1H), 6.44 (t, *J*=6.8 Hz, 1H), 7.39 (m, 6H), 7.62 (m, 4H), 7.83 (s, 1H), 8.10 (s, 1H), 12.99 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 19.31, 27.12, 40.10, 84.81, 88.54, 117.90, 125.41, 128.10, 128.12, 130.33, 130.36, 133.18, 133.31, 135.45, 135.93, 136.04, 138.60, 145.04, 148.93, 159.33. HRMS (DART) Calcd for $C_{27}H_{31}N_4O_3Si$ 487.21654; Found, 487.21580.

Inosine Hydroboration (14)[*]

The inosine Wittig product (281 mg, 0.576 mmol) was dissolved in THF (30 mL) and a 0.5 M solution of 9-BBN in THF (23.5 mL, 11.83 mmol) was added. The reaction was stirred for three hours before being quenched with 1 M NaOH (46.8 mL) and 30% H_2O_2 (7.75 mL). The organic layer was further washed with brine and dried over Na_2SO_4. . The crude material was purified by flash chromatography (10 to

[*] Reaction performed with Yiran Li

20% isopropanol gradient in DCM) to yield **14** as a white foam (256 mg, 0.507 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.73 (s, 1H), 8.04 (s, 1H), 7.80 (s, 1H), 7.67-7.63 (m, 4H), 7.50-7.33 (m, 6H), 6.33 (t, *J* = 6.6 Hz, 1H), 4.42 (dt, *J* = 5.7, 3.7 Hz, 1H), 4.15 (td, *J* = 6.5, 3.5 Hz, 1H), 3.60-3.49 (m, 2H), 2.54-2.47 (m, 1H), 2.47-2.40 (m, 1H), 1.61 (q, *J* = 6.1 Hz, 2H), 1.09 (s, 9H). HRMS (DART) Calcd for $C_{27}H_{33}N_4O_4Si$ 505.2271; Found, 505.2255.

Inosine 3',6'-Diacetate Product(15)

The crude hydroboration product (254 mg, 0.502 mmol) was dissolved in THF (30 mL) and TBAF (7.90 mL of a 1 M solution in THF) was added dropwise. The reaction was stirred for 1 hr before 20 mL of water was added. The THF was removed by rotary evaporation and the water was washed with DCM twice. The water was removed by rotary evaporation and coevaporated twice with pyridine.

The crude deprotected material was dissolved in pyridine (30 mL) along with DMAP (15.0 mg, 0.125 mmol). Acetic anhydride (990 μL, 10.6 mmol) was added and the reaction was stirred for 12 hrs. The reaction was quenched with methanol and the solvent was removed by rotary evaporation and coevaporated with toluene. The crude material was purified by flash chromatography (85% DCM, 15% iPrOH) to yield the product as a white solid (153 mg, 0.437 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.81 (s, 1H), 8.18 (s, 1H), 8.02 (s, 1H), 6.30 (dd, *J* = 21.6, 13.6 Hz, 1H), 5.33-5.27 (m,1H), 4.27-4.18 (m, 2H), 4.18-4.06 (m, 1H), 3.01-2.93 (m, 1H), 2.52 (ddd, *J* =16.0, 8.0, 4.0 Hz, 1H), 2.13-2.06 (m, 2H), 2.12 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.10, 170.53, 159.19, 148.78, 145.18, 138.85, 125.76,

84.99, 82.51, 60.98, 37.23, 32.96, 21.22, 21.14. HRMS (DART) Calcd for $C_{15}H_{19}N_4O_6$ 351.1305; Found, 351.1307.

Nitrotriazole Reaction (16)

3',6'-diacetate-2'-deoxyinosine (95.0 mg, 0.271 mmol), 3-nitro-1,2,4,-1H-triazole (108 mg, 0.949 mmol), PPh_3 (178 mg, 0.678 mmol), iodine (138 mg, 0.542 mmol), and iPr_2NEt (237 μL, 1.36 mmol) were dissolved in 7 mL of toluene and heated to 95 ˚C for 1.5 hrs. The solvent was removed by rotary evaporation and the crude material was purified by flash chromatography (75% EtOAc, 25% Hexanes) to yield the product as a white foam (106 mg, 0.236 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 8.98 (s, 1H), 8.43 (s, 1H), 6.47 (dt, *J* = 14.6, 7.4 Hz, 1H), 5.37-5.34 (m, 1H), 4.39-4.18 (m, 2H), 4.15-4.08 (m, 1H), 3.23-2.98 (m, 1H), 2.67 (ddd, *J*=14.4, 6.0, 2.4 Hz, 1H), 2.24-2.09 (m, 2H), 2.14 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.62, 171.08, 170.49, 154.28, 152.53, 147.43, 145.75, 144.03, 124.01, 85.62, 82.84, 60.86, 37.03, 32.90, 21.20, 21.13. HRMS (DART) Calcd for $C_{17}H_{19}N_8O_7$ 447.1377; Found, 447.1390.

DNA Preparation

All native strands were made on an Applied Biosystems DNA/RNA synthesizer with standard 2 minute couplings. The 5' terminus DMTr group was kept on.

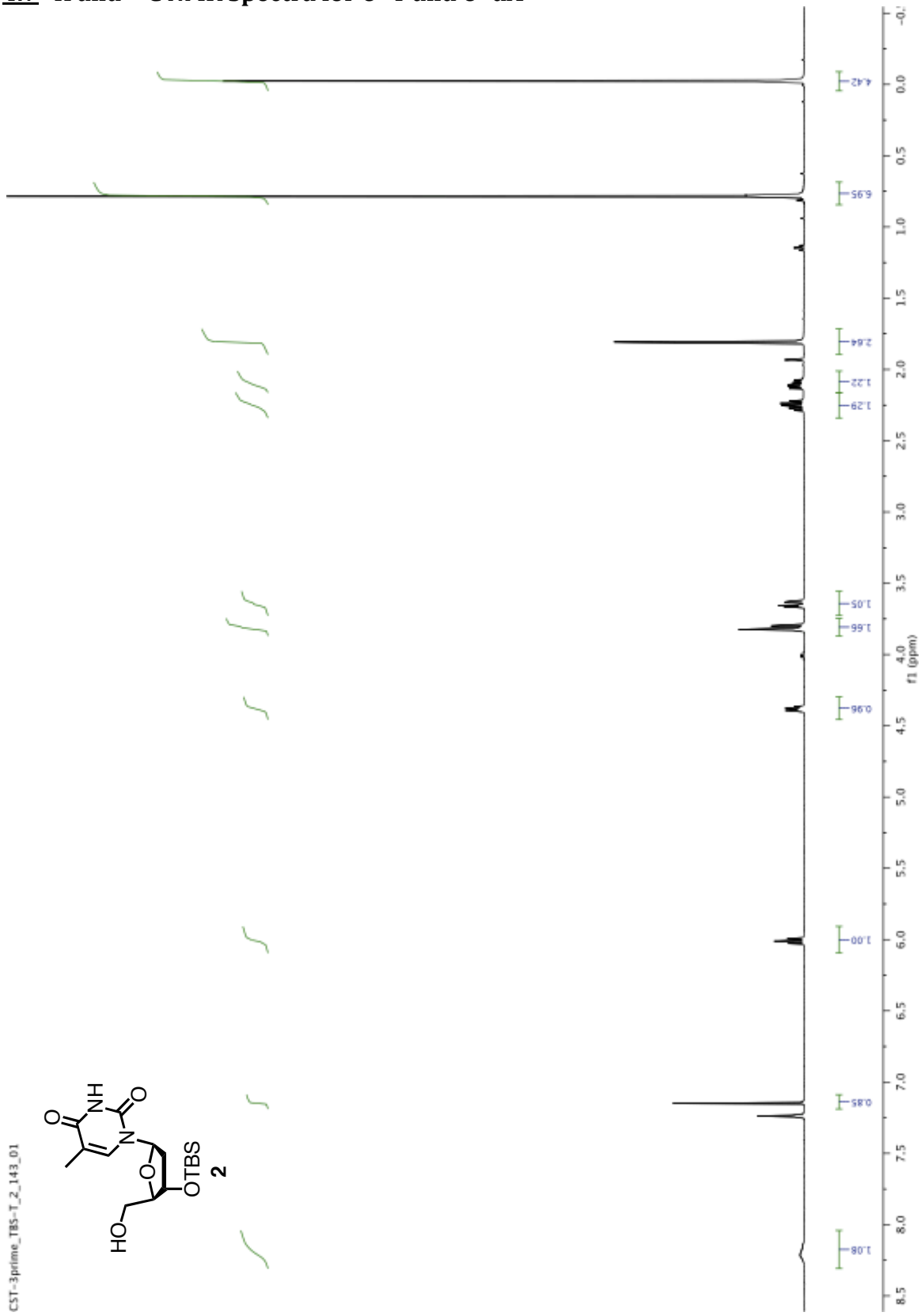
The strands were purified by HPLC on an oligo-R3 column using a gradient (Buffer A: 50 mM TEAA 5%, acetonitrile, Buffer B: 50 mM TEAA, 70% acetonitrile) of 0-60% Buffer B over 12 minutes, followed by flushing the column with Buffer B for 5

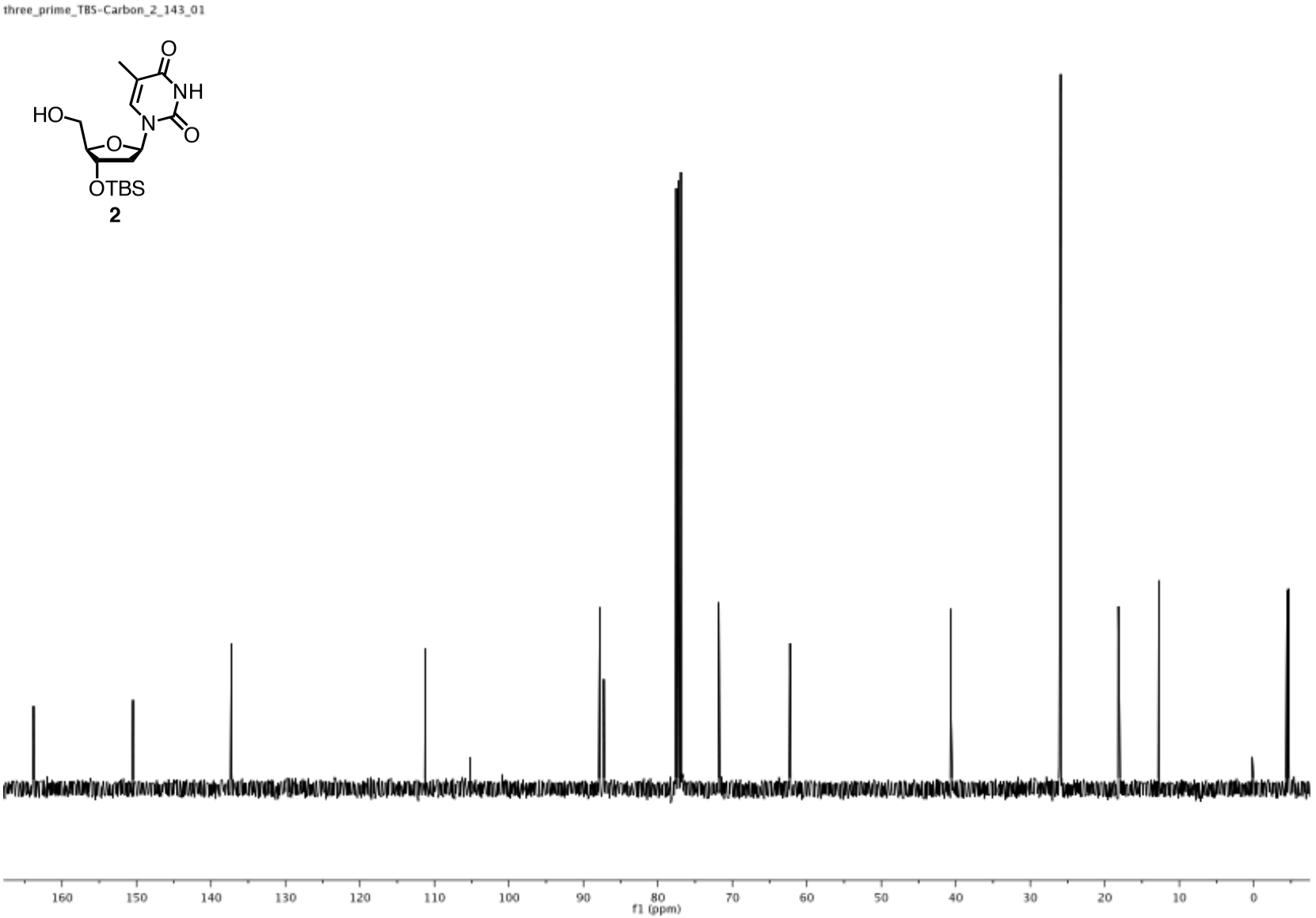
minutes. The solvent of the product containing fractions was removed by rotary evaporation.

The DMTr group was removed by stirring the oligomer in an 80% acetic acid solution for 30 minutes. Upon removal of the solvent by rotary evaporation, the DNA was desalted using NAP 10 columns.

DNA concentrations were measured using the absorbance from a UV spectrophotometer and an extinction coefficient from Integrated DNA Technologies' website. For the 6' extended thymidine containing strands, an approximate extinction coefficient calculated from thymidine containing strands was used.

4.7 ^{1}H and ^{13}C NMR Spectra for 6'-T and 6'-dA

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NH
HO
N
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O
OTBS
2
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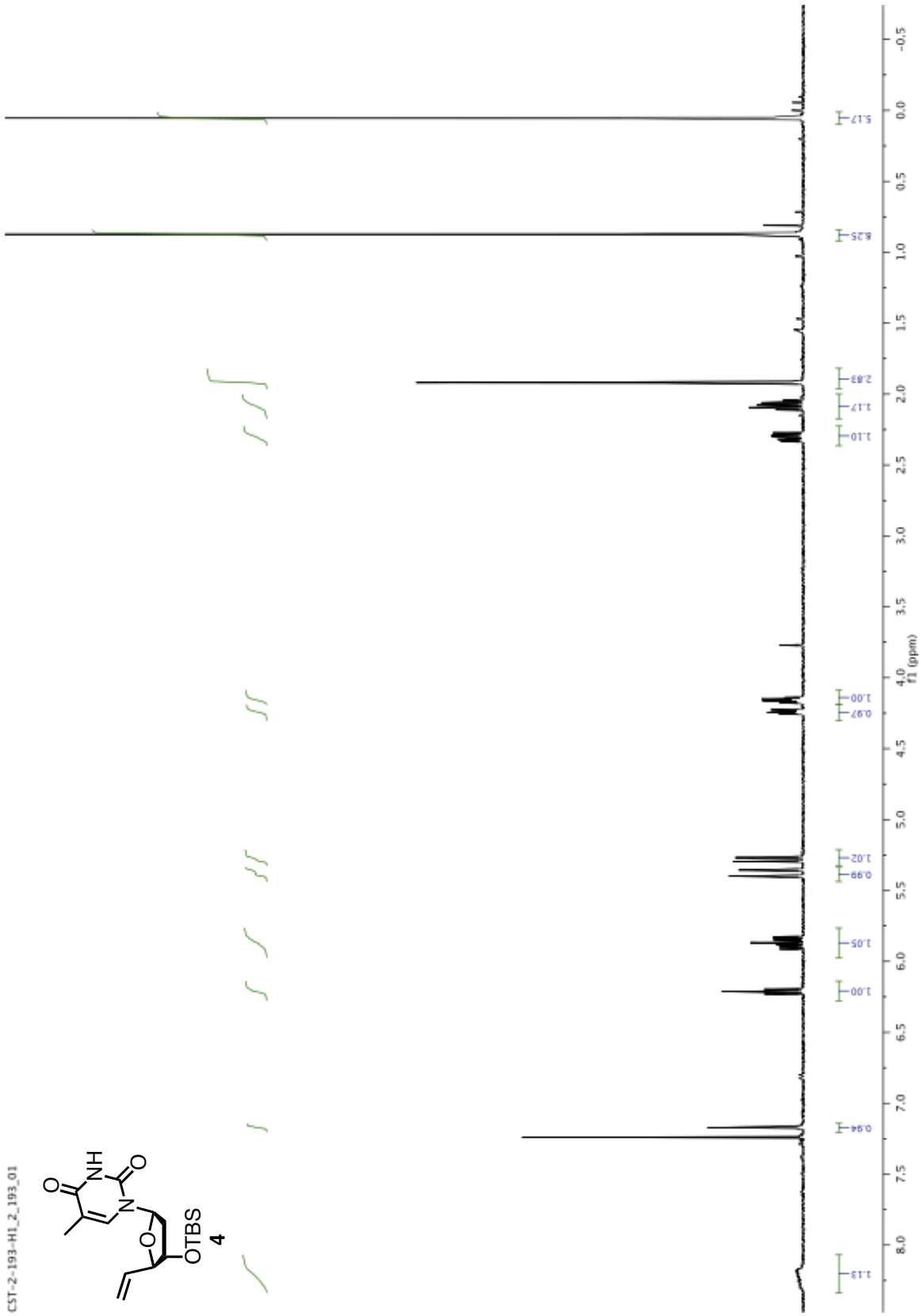
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4
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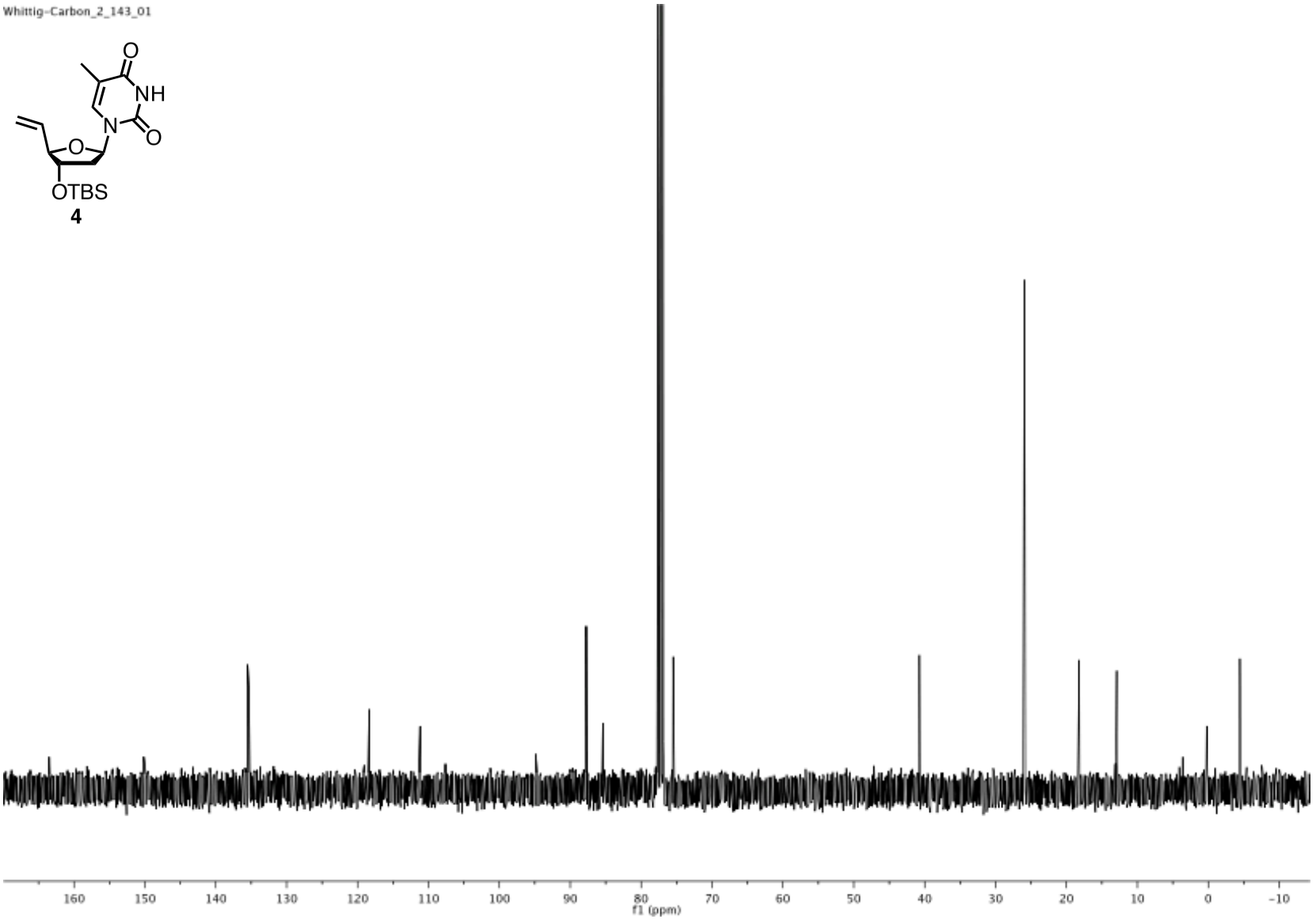
Whittig-Carbon_2_143_01
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NH
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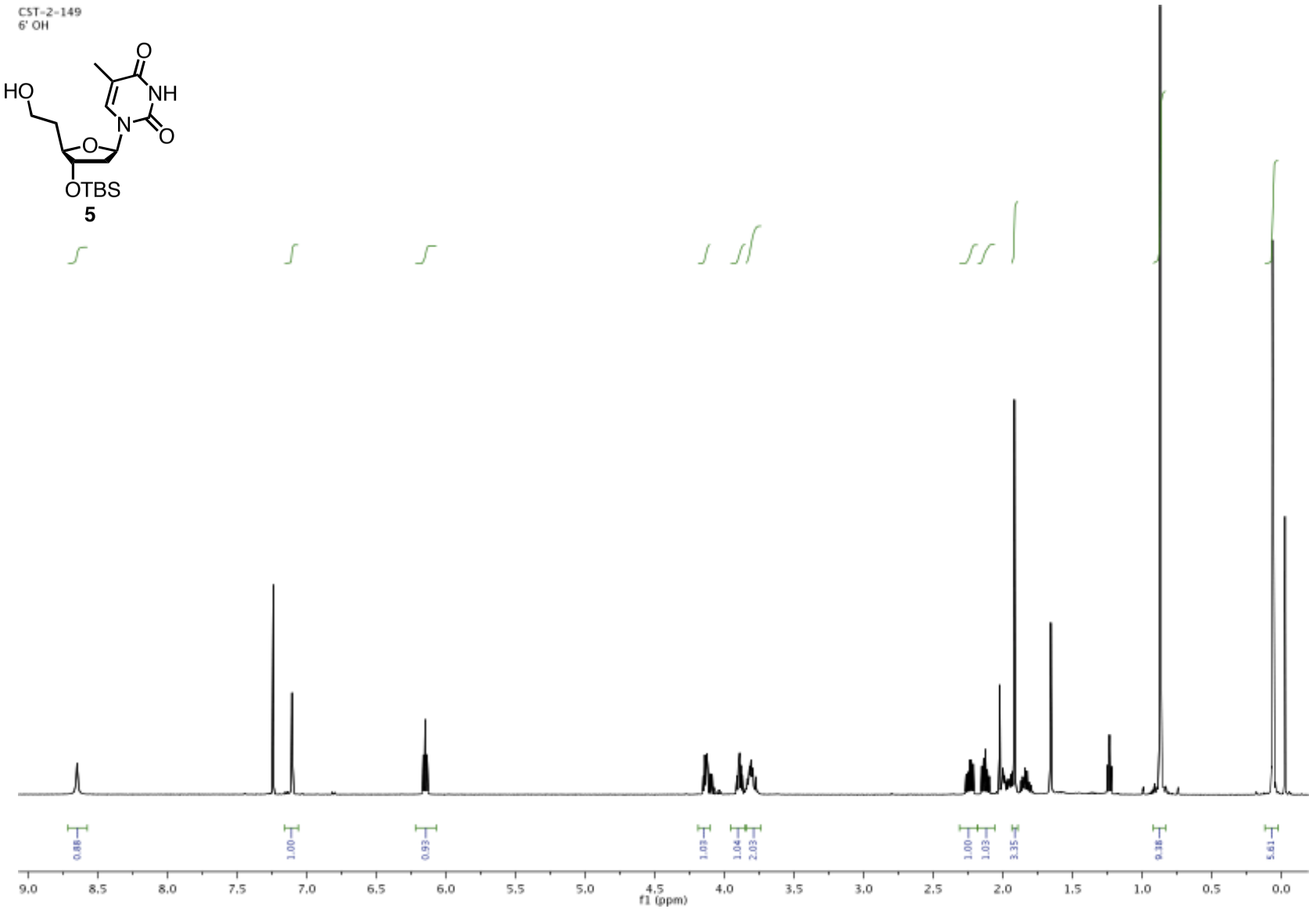
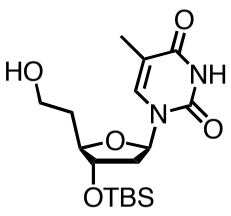
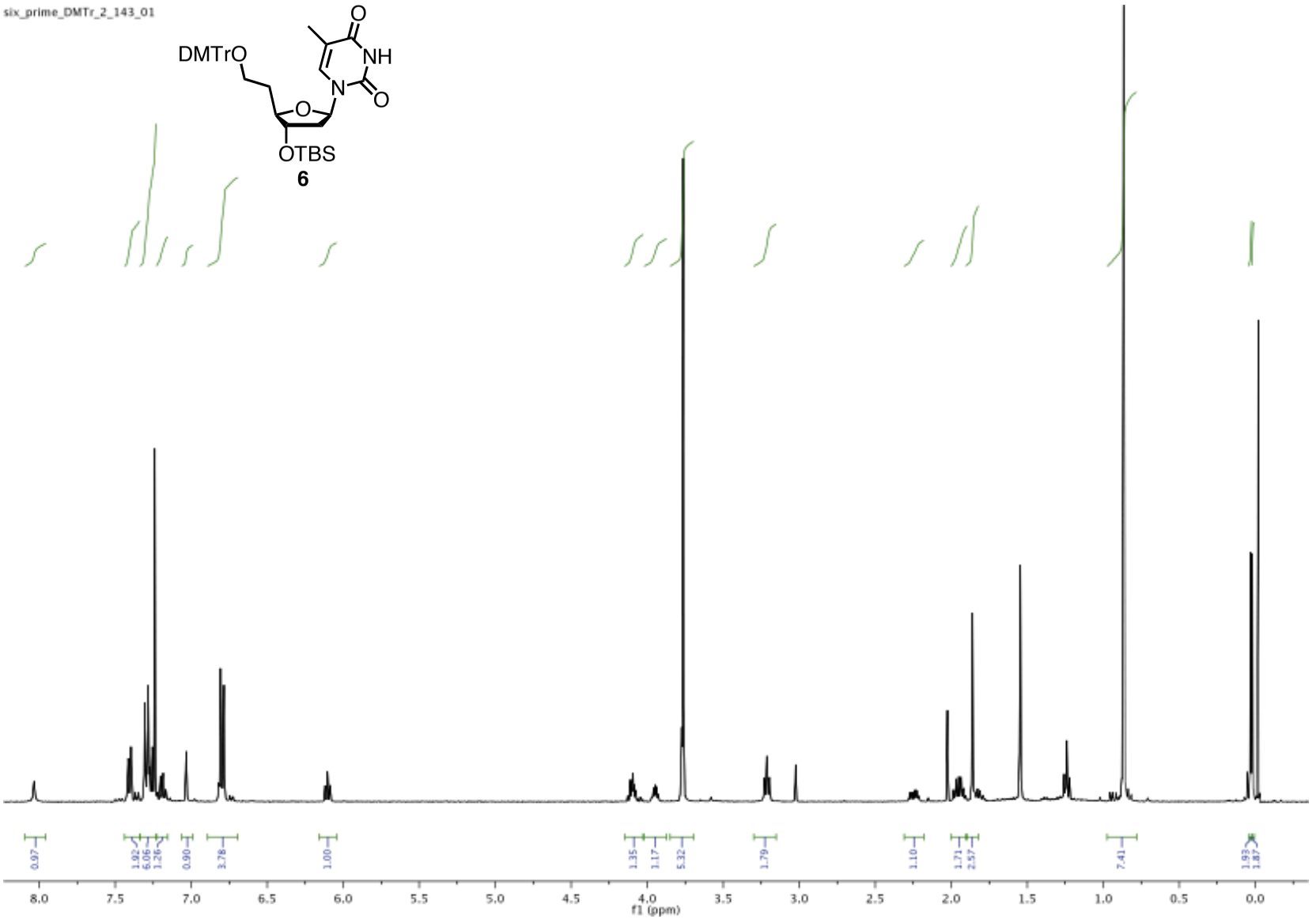
O
OTBS
4
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

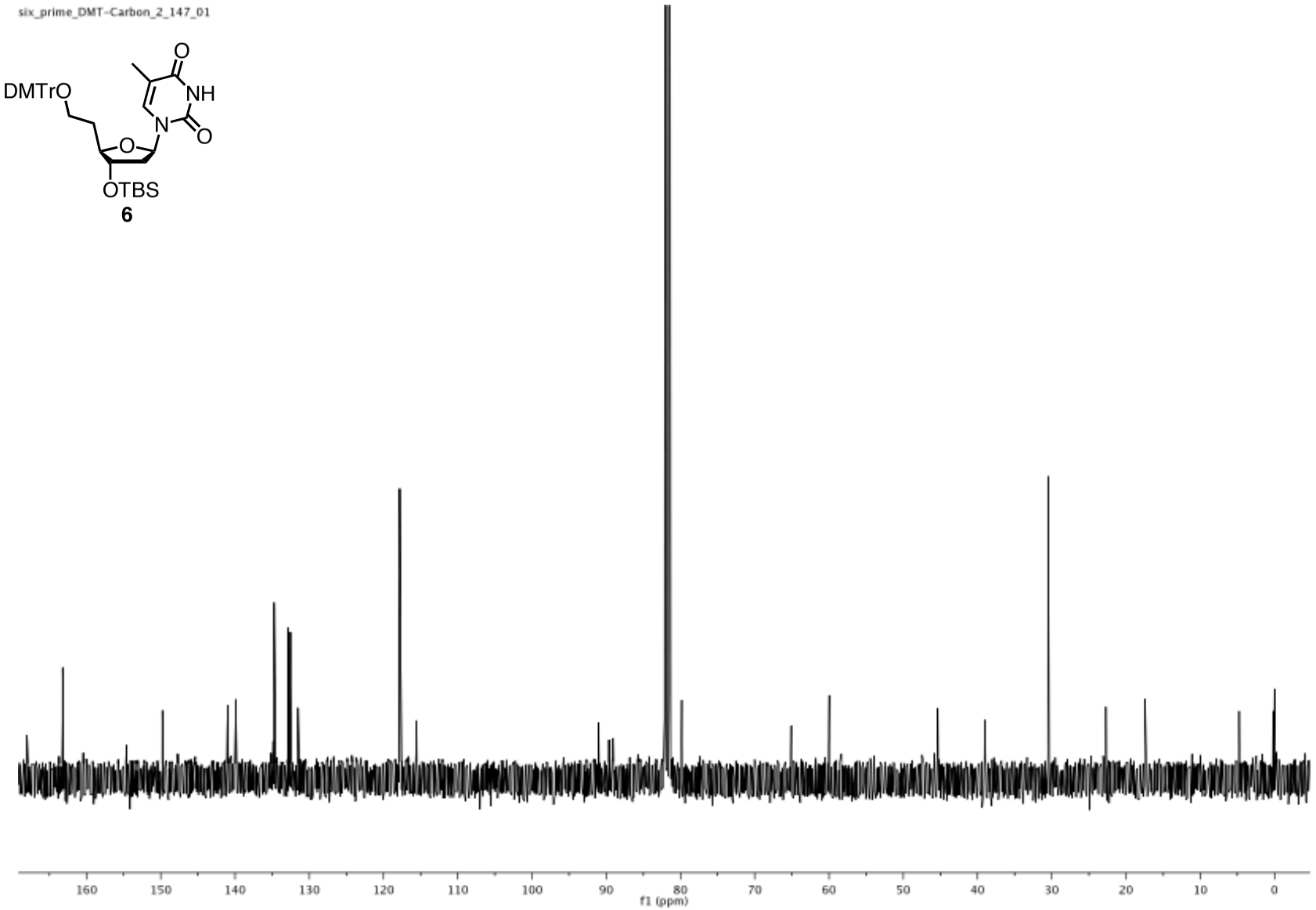

CST-2-149
6' OH
HO
O
NH
N
O
O
OTBS
5
0.88
1.00
0.93
1.03
1.04
2.03
1.00
1.03
3.35
9.38
5.61
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

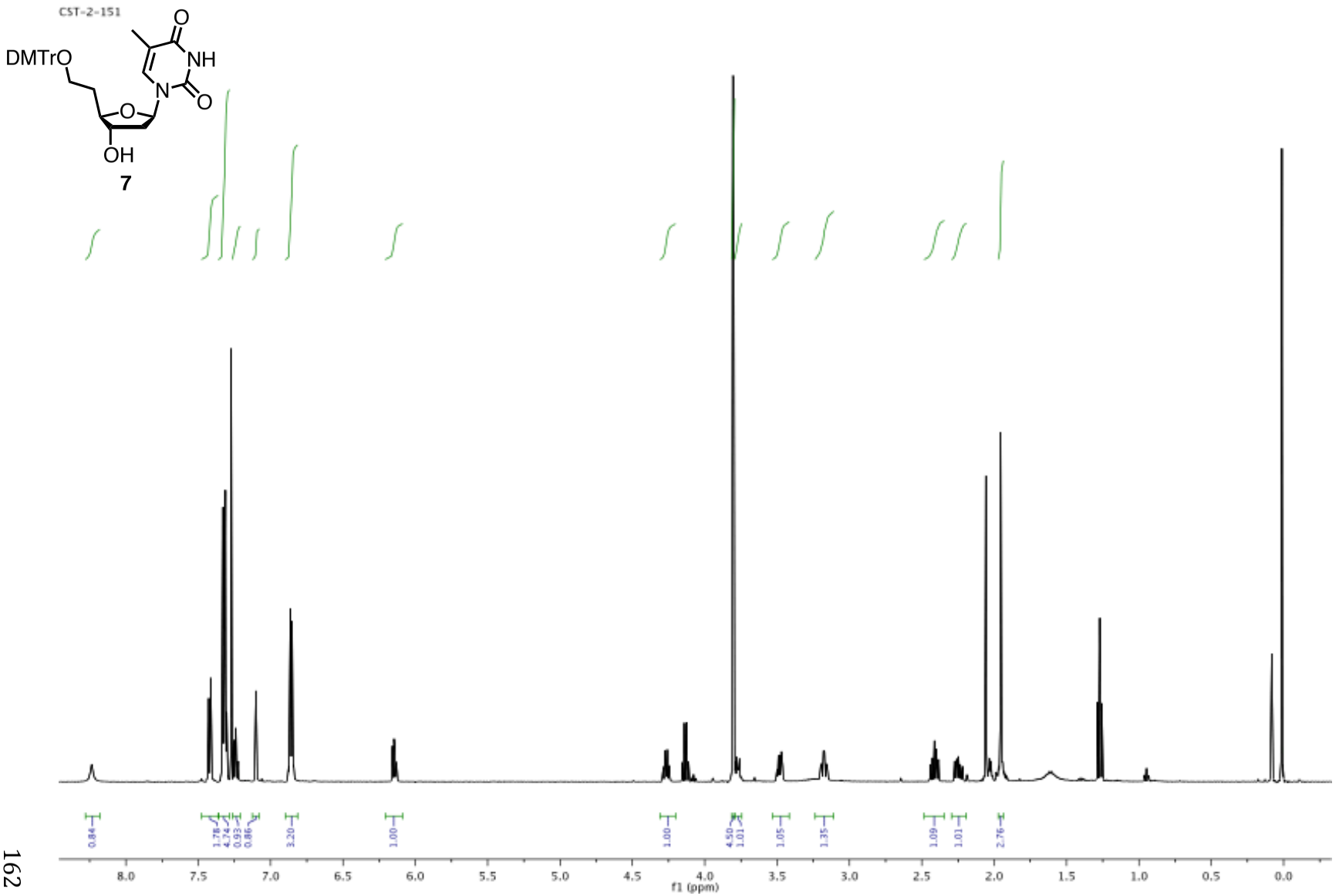
six_prime_OH_CARBON_2_149_01



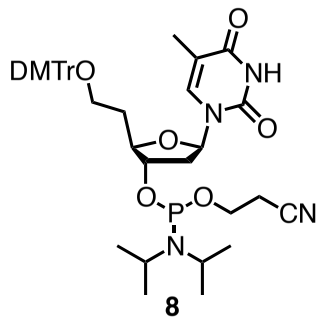
5


six_prime_DMTr_2_143_01
DMTrO
O
NH
N
O
O
OTBS
6
0.97
1.92
6.06
1.26
0.90
3.78
1.00
1.35
1.17
5.32
1.79
1.10
1.71
2.57
7.41
1.93
1.87
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

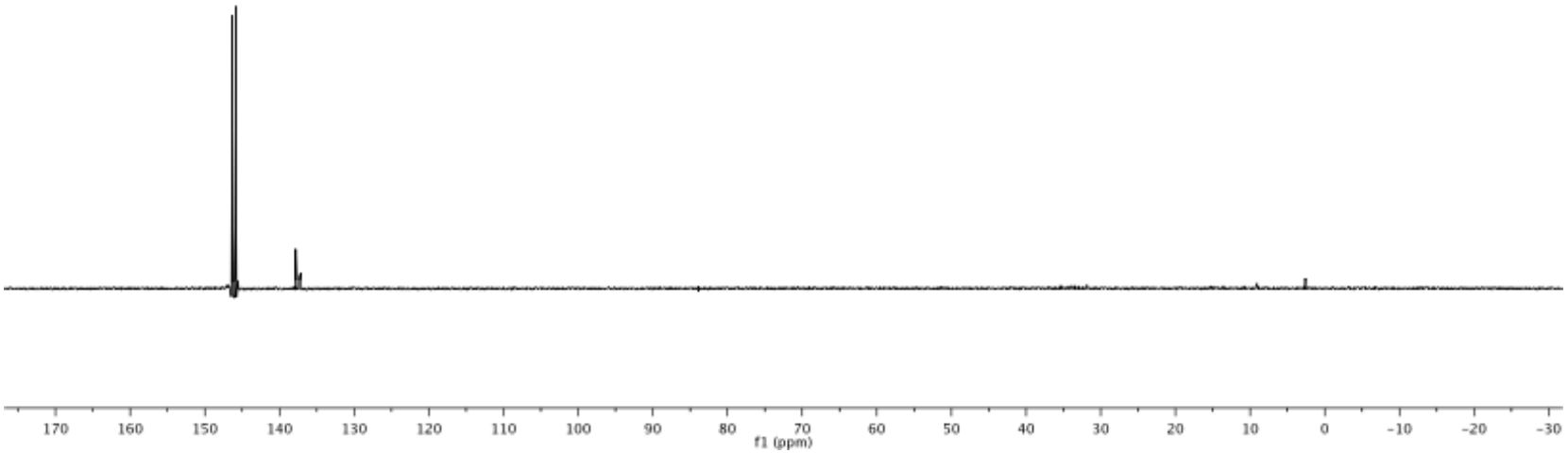

six_prime_DMT-Carbon_2_147_01
DMTrO
O
NH
N
O
O
OTBS
6
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

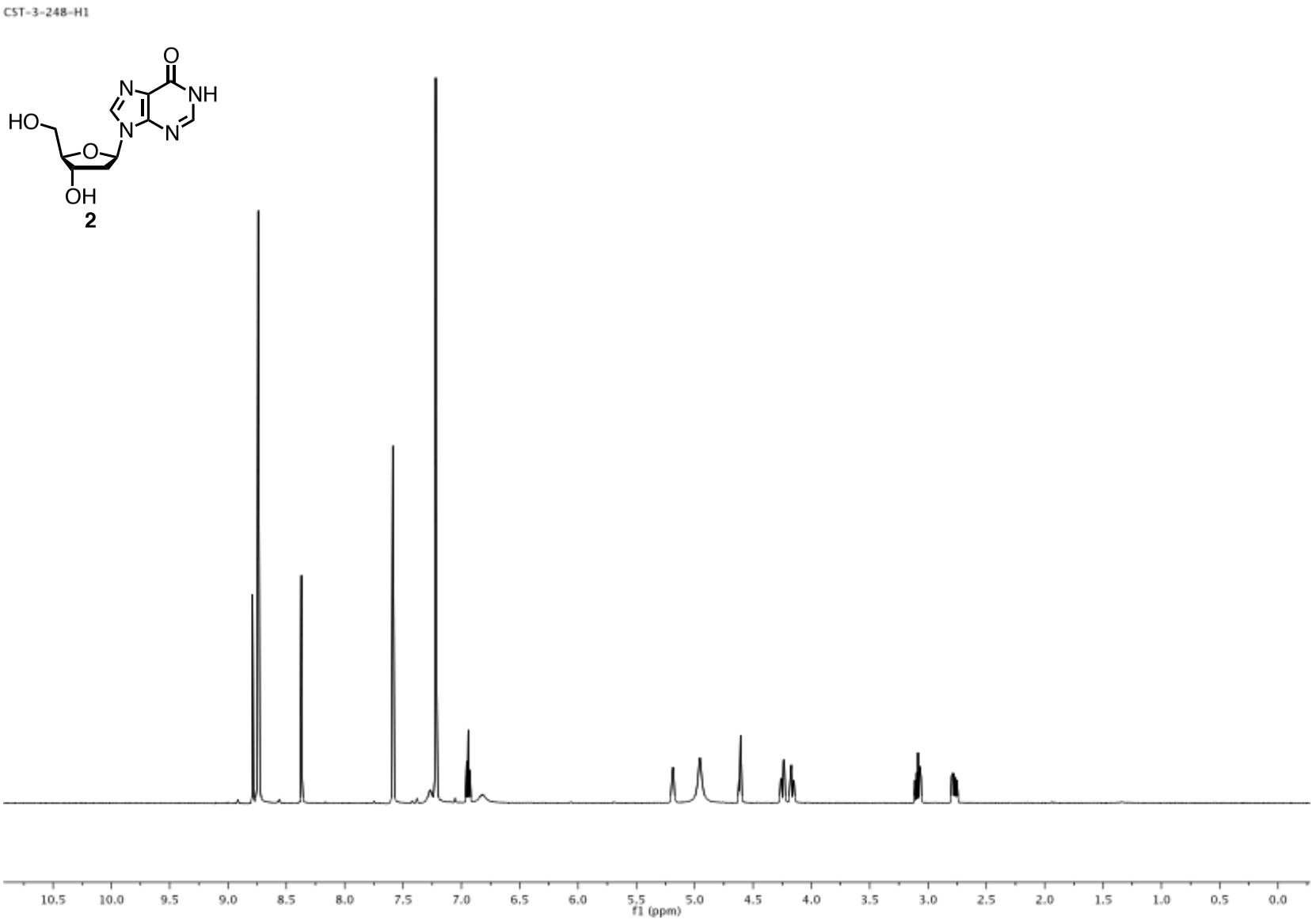

CST-2-151
DMTrO
O
NH
N
O
O
OH
7
0.84
1.78
4.74
0.93
0.86
3.20
1.00
1.00
4.50
1.01
1.05
1.35
1.09
1.01
2.76
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

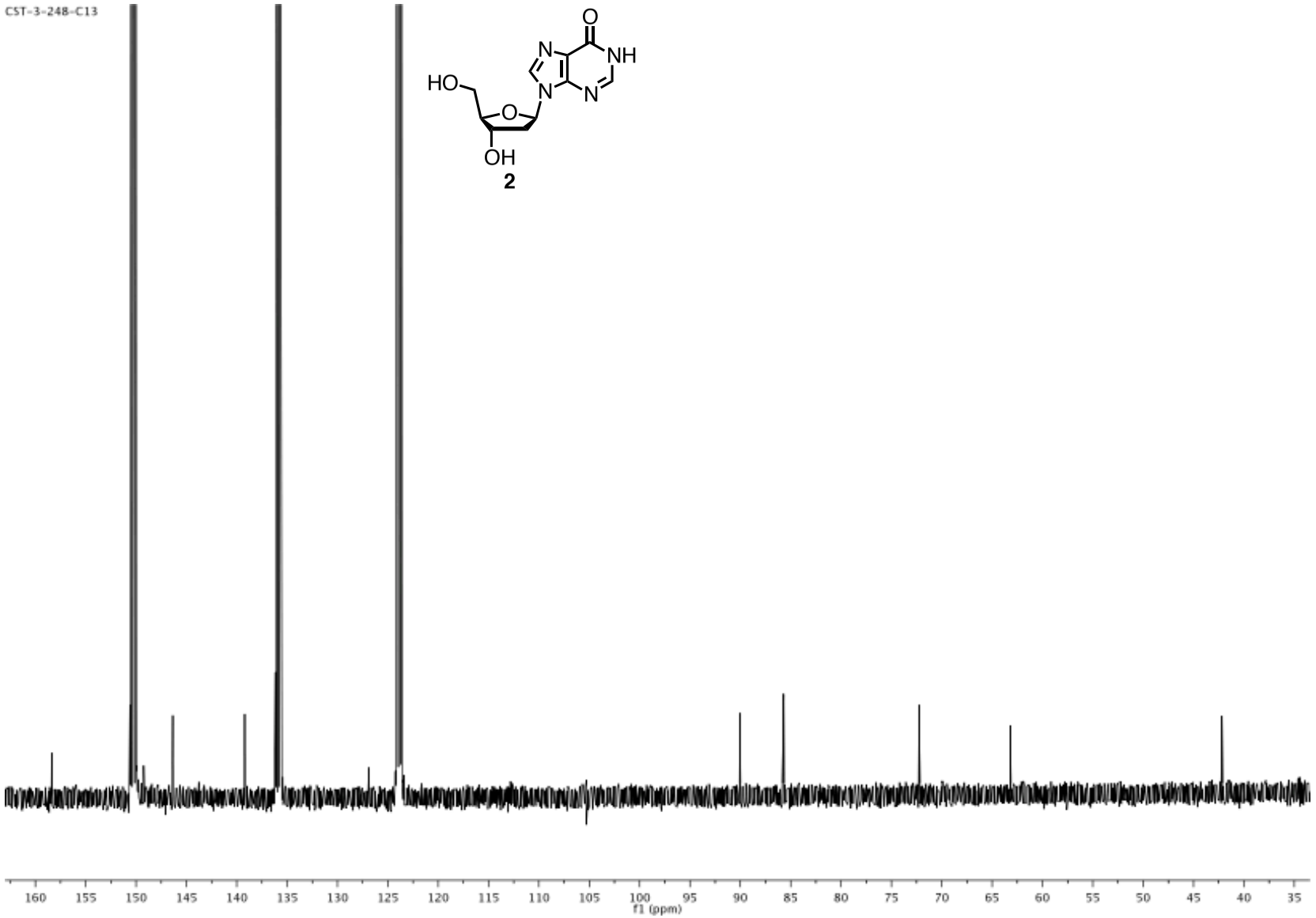
six_prime_T_phosphoramidite_2_139_02

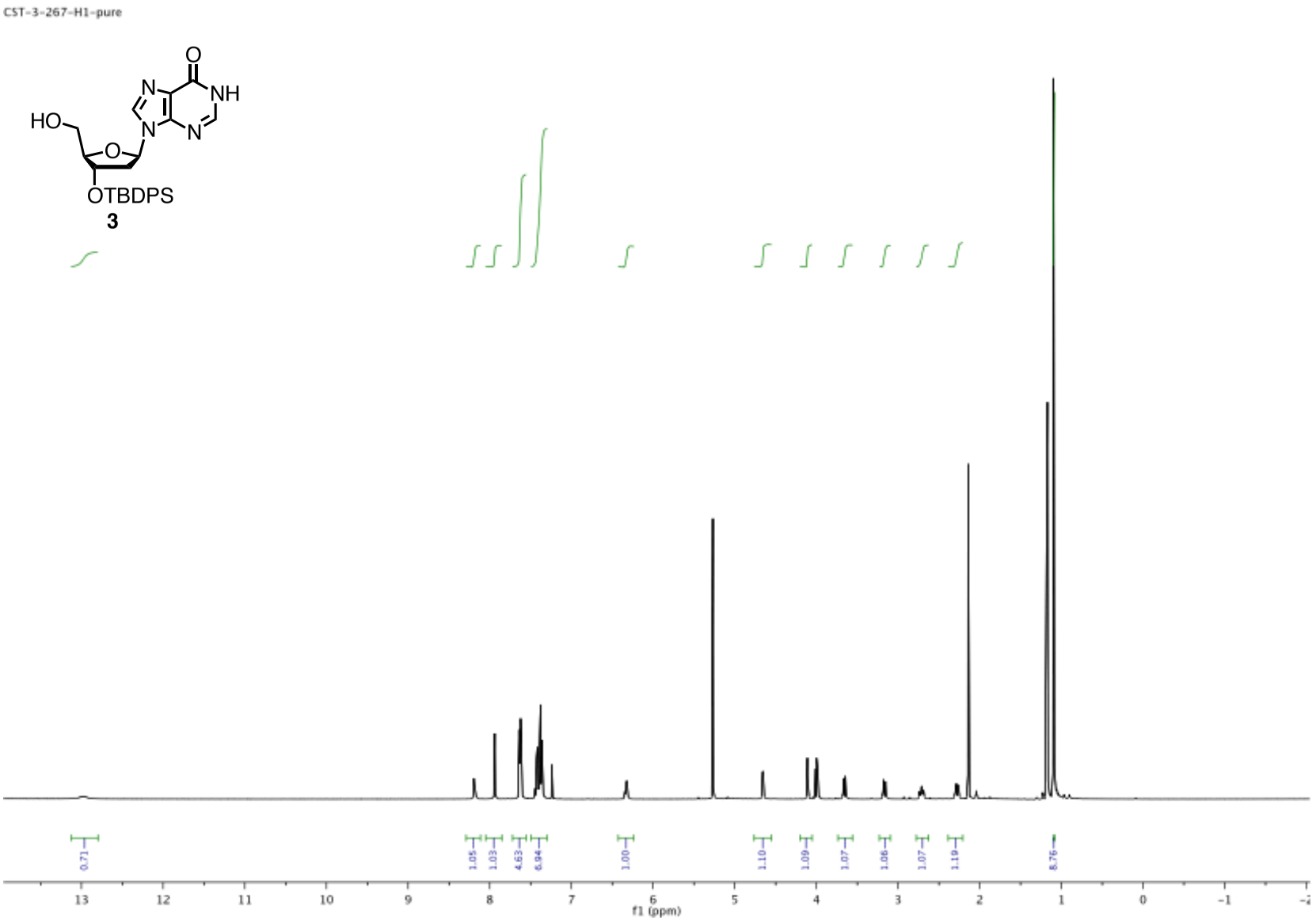


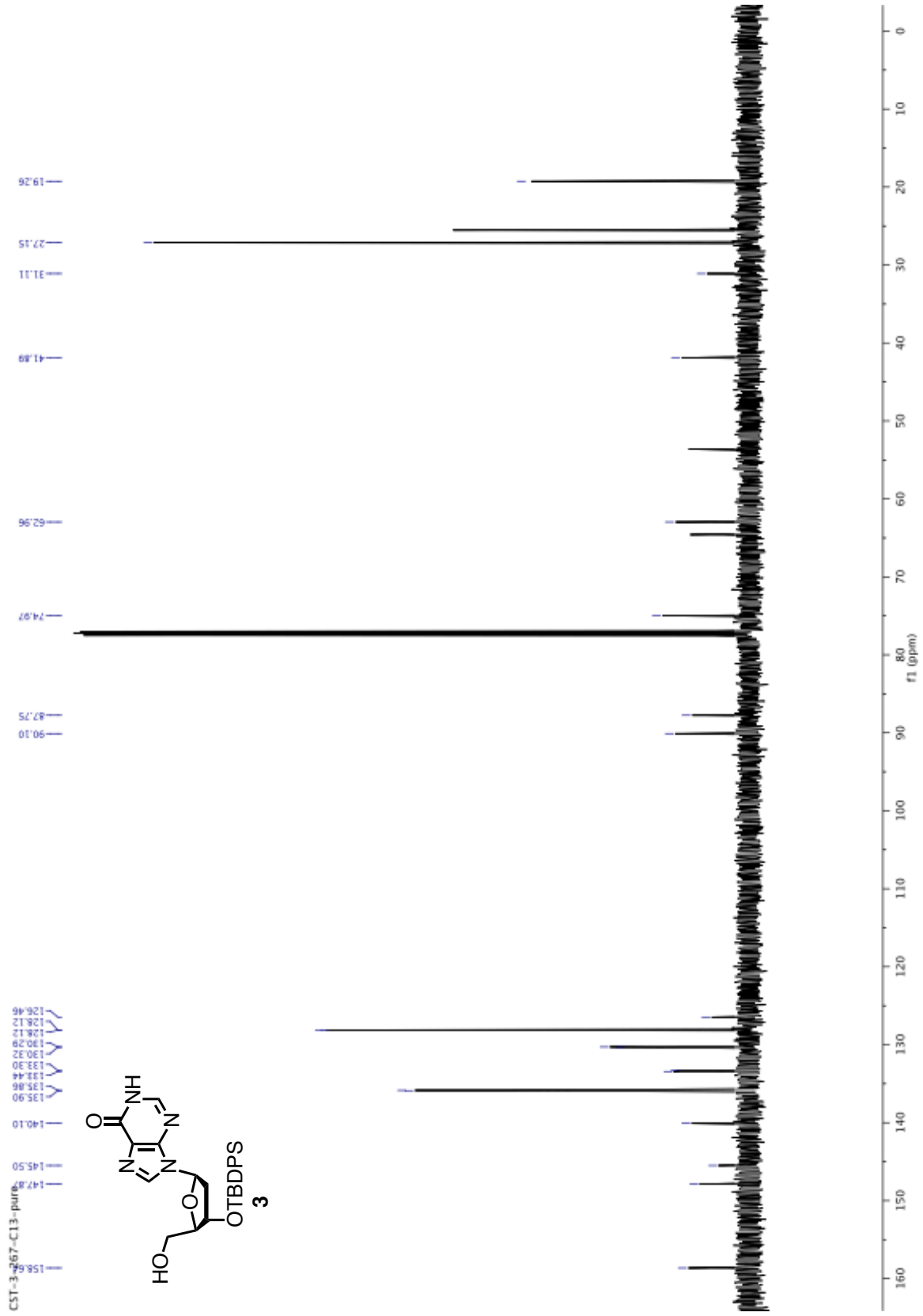
8

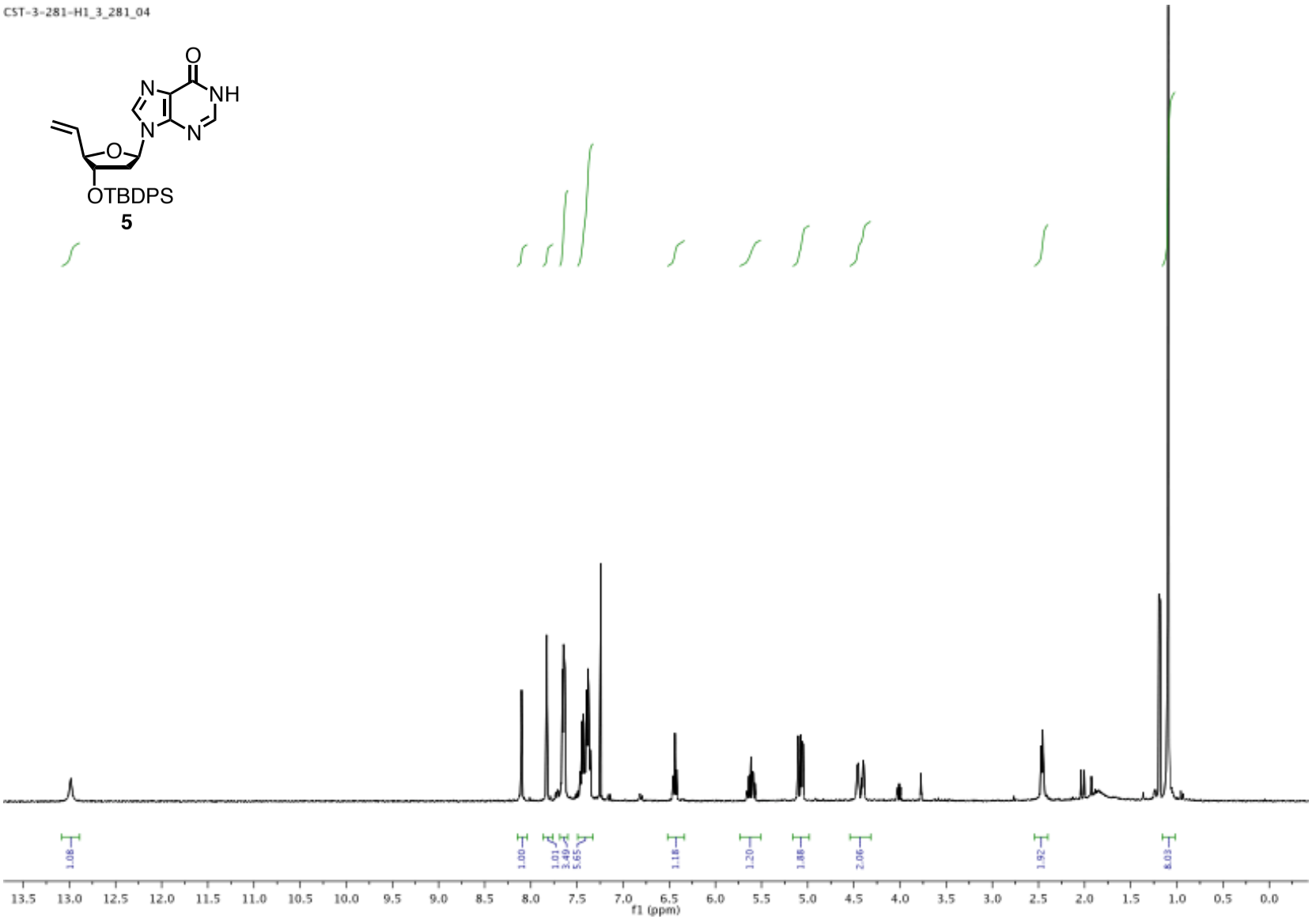



CST-3-248-H1
HO
O
OH
N
N
N
NH
O
2
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

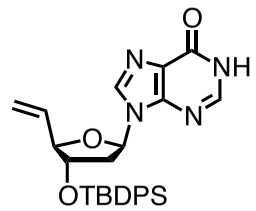

CST-3-248-C13
HO
O
N
NH
N
N
O
OH
2
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
f1 (ppm)


CST-3-267-H1-pure
HO
O
N
NH
N
N
O
OTBDPS
3
0.71
1.05
1.03
4.63
6.84
1.00
1.10
1.09
1.07
1.06
1.07
1.11
8.76
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
f1 (ppm)

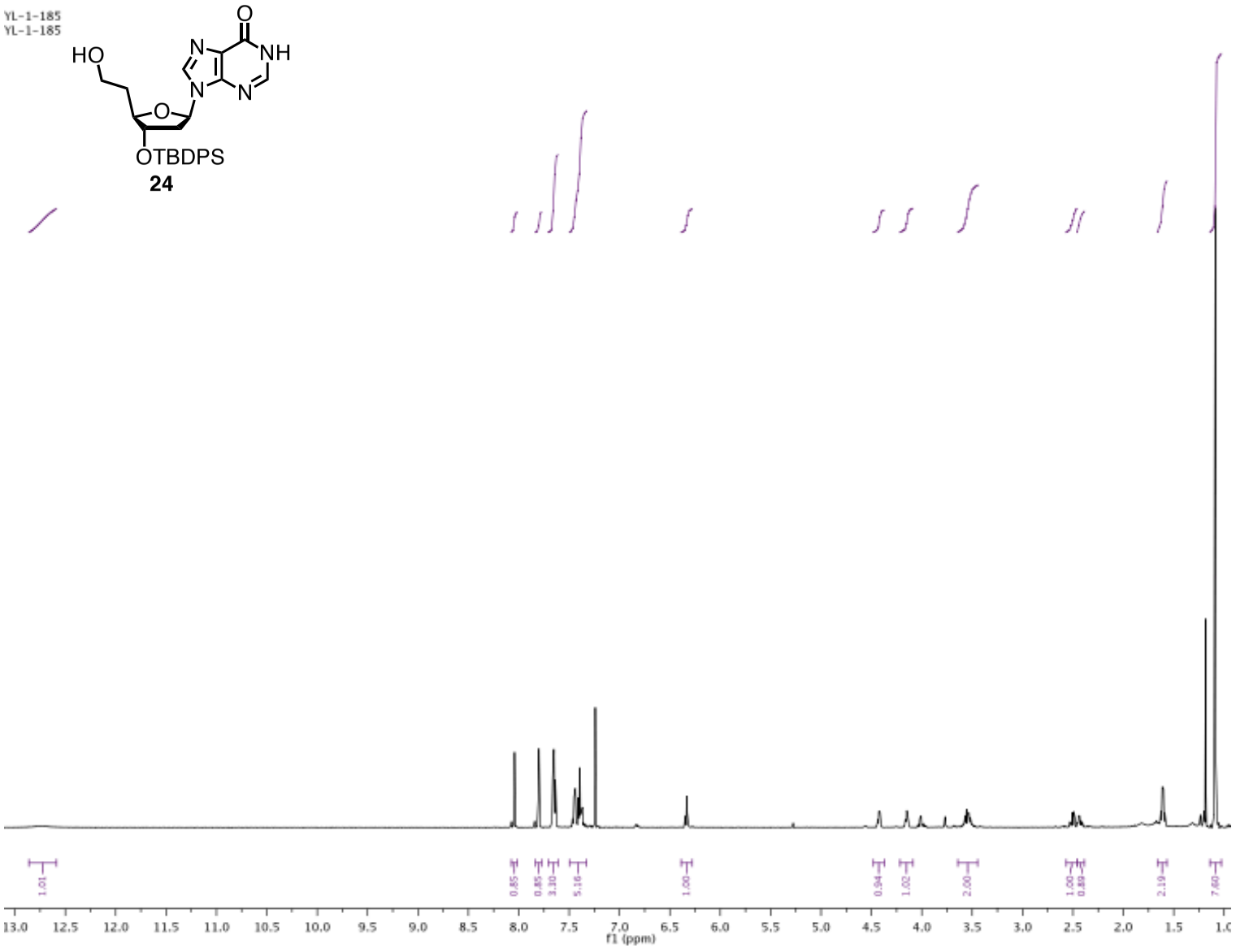

CST-3-267-C13-pure
19.26
27.15
31.11
41.89
62.96
74.97
83.75
90.10
126.46
128.12
128.12
130.29
130.32
133.30
133.44
135.86
135.90
140.10
145.50
147.87
158.64
HN
N
N
N
O
O
HO
OTBDPS
3
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
f1 (ppm)

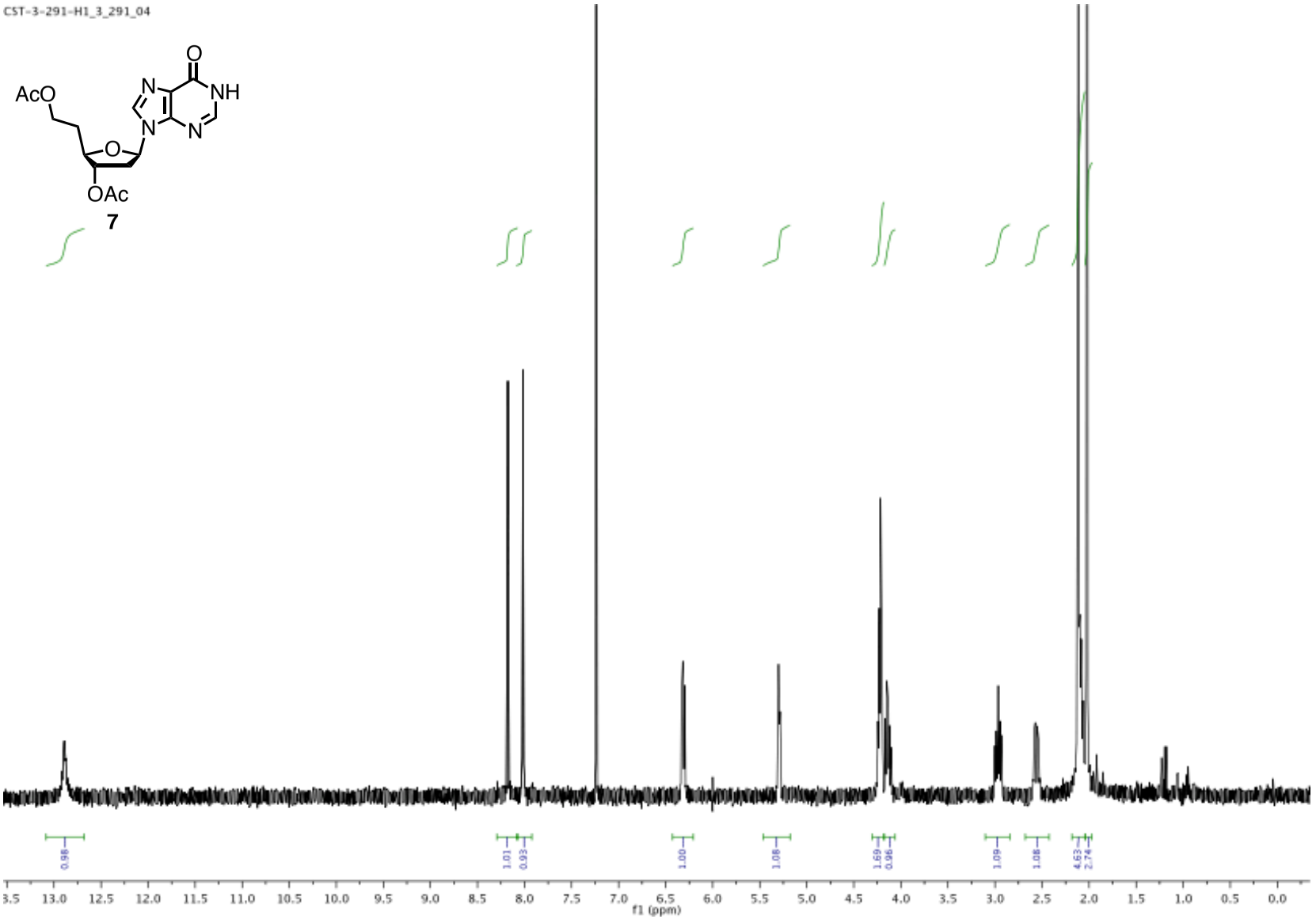

CST-3-281-H1_3_281_04
O
NH
N
N
N
O
OTBDPS
5
1.08
1.00
1.01
3.49
5.65
1.18
1.20
1.88
2.06
1.92
8.03
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

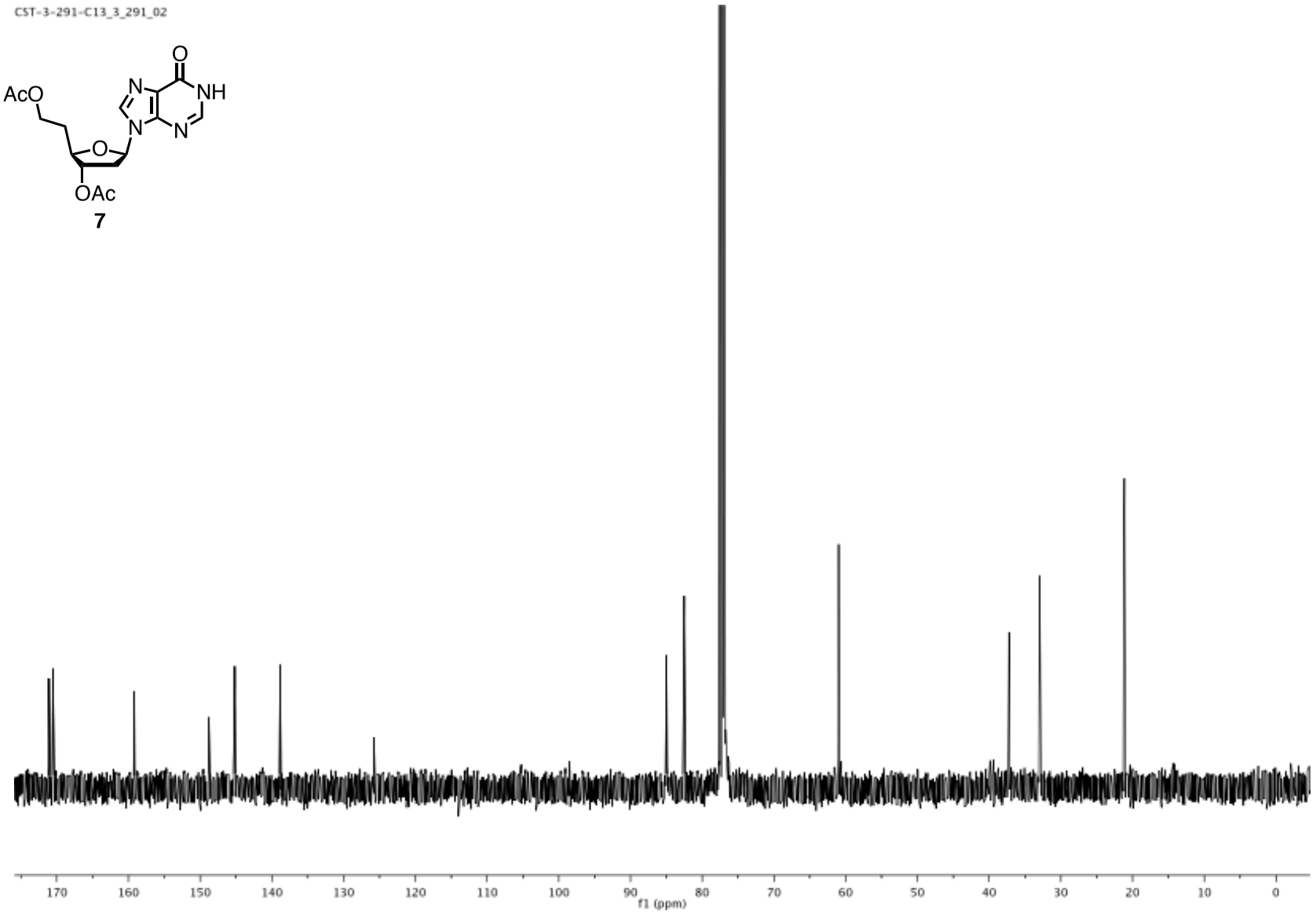
CST-3-281-C13_3_281_01

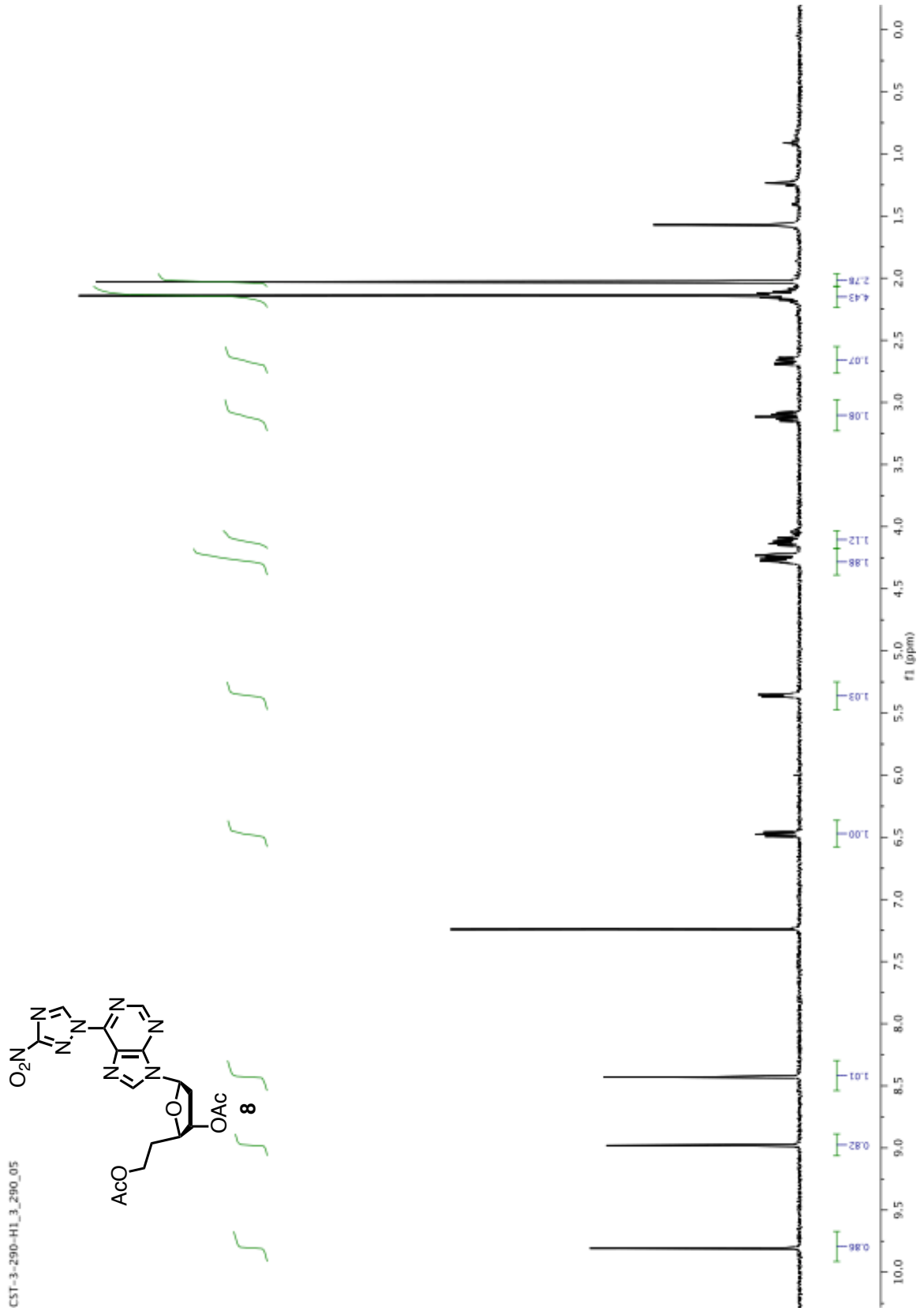


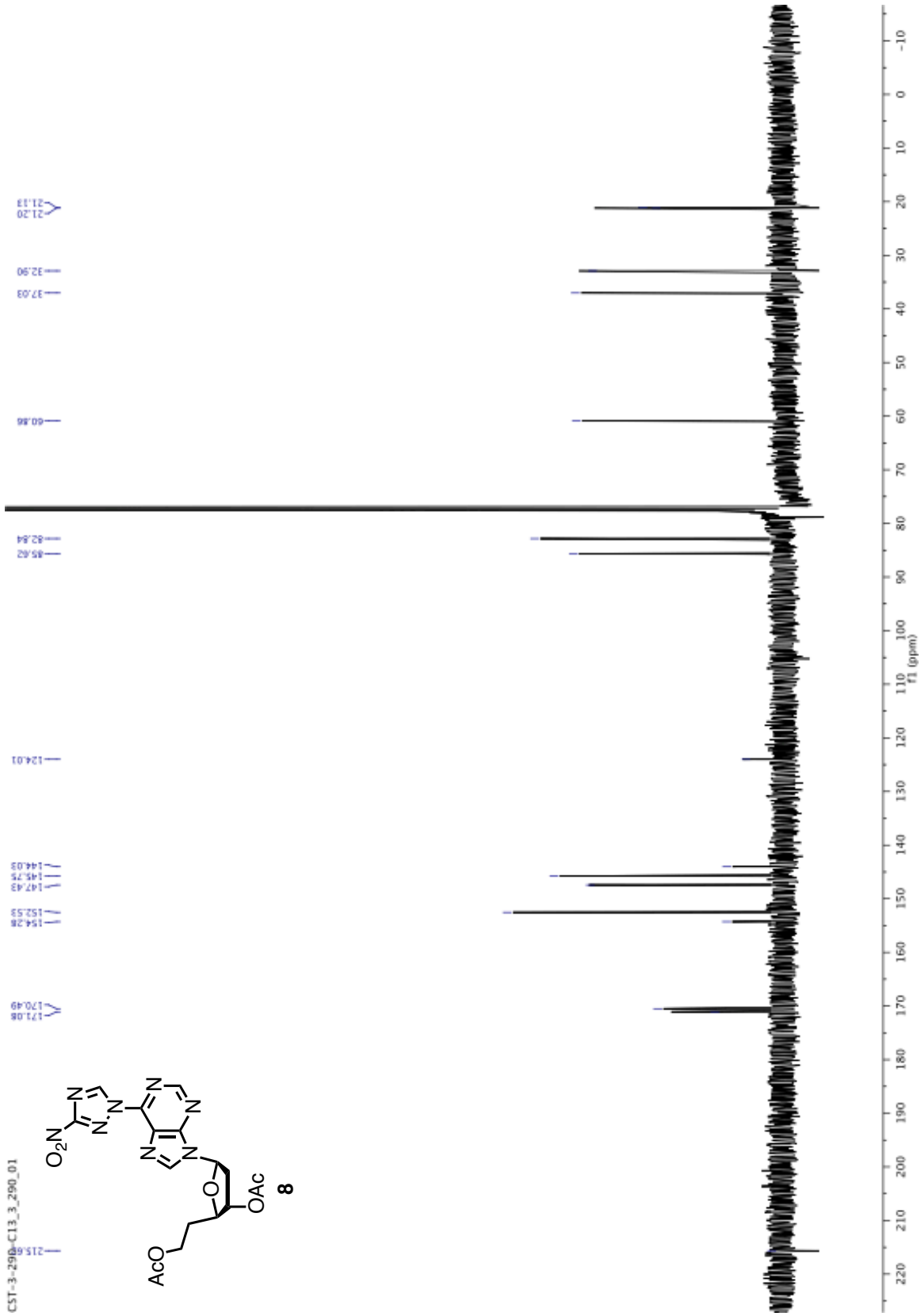
5

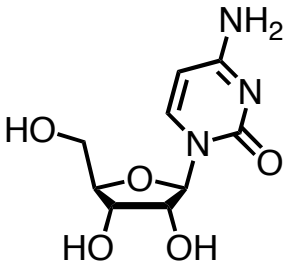

YL-1-185
YL-1-185
HO
O
N
NH
N
N
O
OTBDPS
24
1.01
0.85
0.85
3.30
5.16
1.00
0.94
1.02
2.00
1.00
0.89
2.19
7.60
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


CST-3-291-H1_3_291_04
AcO
O
N
NH
N
N
O
OAc
7
0.98
1.01
0.93
1.00
1.08
1.69
0.96
1.09
1.08
4.63
2.74
f1 (ppm)

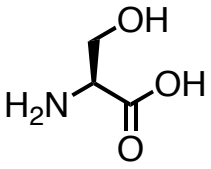

CST-3-291-C13_3_291_02
AcO
O
N
NH
N
N
O
OAc
7
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


CST-3-290-H1_3_290_05
8
AcO
OAc
O
N
O2N
f1 (ppm)

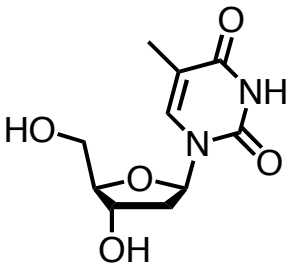

CST-3-290-C13_3_290_01
21.13
21.20
32.90
37.03
60.86
82.84
85.62
124.01
144.03
145.75
147.43
152.53
154.28
170.49
173.08
215.68
f1 (ppm)
8
AcO
OAc
O
N
O2N



C



S



T