Novel Synthetic Routes for the Synthesis of Nucleotide Active Pharmaceutical Ingredients

A Thesis

submitted to

Indian Institute of Science Education and Research Pune in partial fulfilment of the requirements for the BS-MS Dual Degree Programme

by

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April, 2023

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Certificate

This is to certify that this dissertation entitled '**Novel Synthetic Routes for the synthesis of Nucleotide Active Pharmaceutical Ingredients**' towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Anish Das** at Indian Institute of Science Education and Research under the supervision of Prof. Srinivas Hotha, Department of Chemistry, during the academic year 2022-2023.

Prof. Srinivas Hotha

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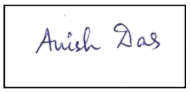
This thesis is dedicated to those who never give up.

Declaration

I hereby declare that the matter embodied in the report entitled '**Novel Synthetic Routes for the synthesis of Nucleotide Active Pharmaceutical Ingredients'** are the results of the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune, under the supervision of Prof. Srinivas Hotha and the same has not been submitted elsewhere for any other degree.



Anish Das

Acknowledgements

The success and execution of this project required significant support and guidance from a large number of people. I would like to express my sincere gratitude to all those who have supported and guided me throughout my master's thesis journey. Everything I've accomplished has been made possible due to their aid, counsel and direction.

To begin with, I express my gratitude towards Dr. Srinivas Hotha, who gave me the chance to work with him on my thesis. I am thankful for his priceless direction, backing, and motivation, which he provided during the research phase. His proficiency and positive critiques played a pivotal role in moulding the course of my thesis. Next, I would like to thank all senior and fellow lab members (Sumit Sen, Pratim Das, Saptashwa Chakraborty, Kameshwar Prasad, Pooja Joshi, Jayashree, Nitesh Kasdekar, Yogesh Sutar, Ganesh Shinde, Bodhayan Biswas, Prashant Kumar, Ankita Sharma, Dhanashree and Ananya Sharma) for assisting me and encouraging my interest in experimental work. They have been instrumental in guiding me and maintaining a cordial lab environment to thrive in. Without their help, I would not have been able to bring the project to a successful completion. I would like to express gratitude to all academic, technical and non-teaching personnel at IISER Pune for providing a conducive environment all throughout my stay.

I express my heartfelt gratitude and best wishes to all my friends and batchmates for their support, encouragement and making my stay here worthwhile at IISER Pune. Without the excellent scientific and learning environment offered by IISER Pune, none of this would have been possible.

Above all, I am indebted to my family and well-wishers who have always been by my side through thick and thin and have been an integral part of this journey.

Abstract

Glycosides are an important class of naturally occurring carbohydrate compounds that have immense significance in the genetic makeover of the body. Chemically, glycosides are often defined as compounds where carbohydrate molecules are covalently linked to an aglycone via a glycosidic bond. Glycosides can be linked by variety of glycosidic bonds leading to different classes of glycosides. N-glycosides or Glucosamines are glycosides where the anomeric linkage is a C-N linkage. Among other naturally occurring N-glycosides, nucleosides are the most relevant and commonly known N-glycosides since they are the fundamental building blocks of DNA and RNA. Besides, they play a key role in various other key cellular processes. This has led scientists to target this class of compounds to chemically modify and synthesize drugs that can mimic their physiological counterparts and exploit their mechanism to introduce therapeutic benefits. Substituted nucleoside analogues are hence seen as the key in medicinal chemistry for pioneering antiviral and anticancer therapies. Chemical synthesis and modification of nucleosides though has remained a challenging process owing to compatibility issues between basicity of nucleobases and acidic reaction protocols. Thus, novel strategies for successfully synthesizing nucleosides are a constantly evolving and challenging endeavour keeping all the limitations in mind.

In this project, I have aimed to synthesize two furanose nucleoside derivatives with different C-2' substituents in sugar moiety utilizing different protecting groups through a novel synthetic route developed by our group while working on N-glycosides. For this process, I have used adenine as the nucleobase acceptor and ribose sugar moiety as the donor. N-glycosylation was achieved through [Au/Ag] catalysis of the acceptor and ethynyl cyclohexyl carbonate ribose donor which had been previously established by the Hotha group. Various post glycosylation modifications were tried at the C-2' position of the sugar moiety to create a library of modified nucleoside analogues that could be further developed to examine therapeutic benefits. In a nutshell, an efficient and stable synthetic procedure to develop various modified nucleosides from furanose has been envisioned in this project.

Abbreviations

˚A	Angstrom
Ac	Acetate
AcCl	Acetyl chloride
AcBr	Acetyl Bromide
Bn	Benzyl
Bz	Benzoyl
BzCl	Benzoyl Chloride
cat.	Catalytic
$CDCl_3$	Chloroform-*d*
$CHCl_3$	Chloroform
DDQ	2,3-Dichloro-5,6-dicyano-1,4- benzoquinone
DCM	Dichloromethane
DEPT	Distortion less Enhancement by Polarization Transfer
DMAP	4-Dimethyl aminopyridine
DMF	N, N-Dimethyl formamide
eq.	equivalents
Et_3N	Triethyl amine

Et_2O	Diethyl Ether
EtoAc	Ethyl Acetate
g	gram
HRMS	High resonance Mass Spectroscopy
Hz	Hertz
J	Coupling Constant
MeI	Methyl Iodide
mg	milligram
min.	minutes
MHz	Megahertz
mL	millilitre
MS	Molecular Sieves
NaH	Sodium Hydride
NAP	2-methyl Naphthalene
NIS	N-Iodo Succinimide
NMR	Nuclear Magnetic Resonance
PMB	Para Methoxy Benzyl
PMBCl	Para Methoxy Benzyl Chloride
PTSA	p-Toluene sulfonic acid

ppm	Parts per million
rt	Room temperature
sat.	Saturated
TBAI	Tetra Butylammonium Iodide
TBAF	Tetra Butylammonium Fluoride
TfOH	Trifluoro methane sulfonic acid
TFA	Trifluoro acetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMSOTf	Trimethylsilyltrifluoromethanesulfonate

Contents

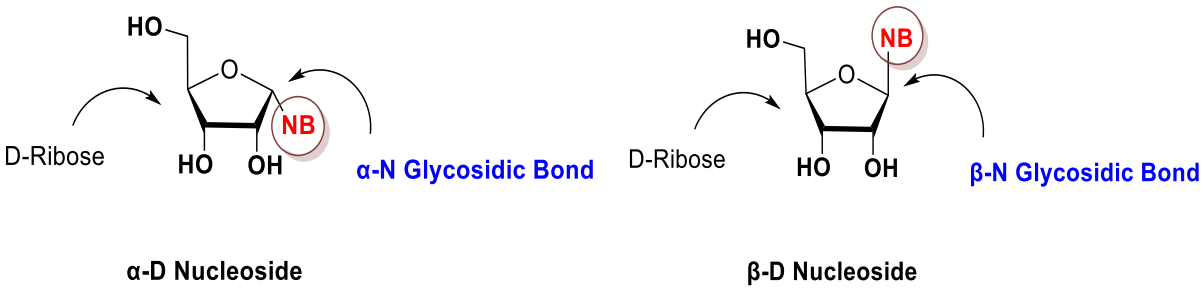
1. Introduction

Carbohydrates are one of the most abundant forms of biomolecules found in nature. They play a key role in the storage and utilization of energy besides serving as building blocks for complex molecules. Various deadly diseases such as cancer, hepatitis, HIV result from direct involvement of carbohydrate in cellular processes. Hence critical understanding of carbohydrates and their derivatives is required to synthesize effective resistant drugs that can tackle aforementioned diseases. A great majority of biological carbohydrates are present as glycoconjugates which are often overlooked due to their complex structures and intricate functions. Glycoconjugates are defined as carbohydrate molecules covalently linked with other functional groups like proteins(glycoproteins), lipids(glycolipids). Among glycoconjugates, N-glycosides are of particular importance as they are the building blocks of DNA and RNA.

Central Dogma of molecular biology explains how heredity information compiles in the form of genes in DNA and leads to functional proteins. However, mutated genes (DNA) transcripts into mutated mRNA which translates to disease associated proteins. Antibiotics were introduced to treat infectious diseases, however, rapid rise of antimicrobial resistance led to a search for alternate ways to tackle this problem. One approach was to target DNA or mRNA. The DNA locus of a gene can be altered by knockout technologies like CRISPR/Cas9 and homologous recombination, which can remove or replace mutated genes. This can be achieved by introducing a premature stop codon or removing the entire DNA locus.[9] Another way of approaching the problem was to incorporate modified nucleosides which share similar properties with DNA/RNA and could interfere in the process. Hence chemically synthesized N-glycosides with key modifications possess strong potential to mediate this problem. This is the area of our interest and we will discuss glycosylation and importance of N-glycosides and derived modifications in the subsequent sections.

1.1 N-Glycosides

N-Glycosides are chemically defined as compounds having a carbohydrate unit attached to an aglycone through an anomeric C-N linkage. The most well-known N-Glycosides are nucleosides where a sugar moiety is covalently linked with a nucleobase. Endogenous compounds called nucleosides are essential for various cellular processes, including DNA and RNA synthesis, enzyme regulation, and metabolic processes.[10] While there are several nucleosides that occur naturally, the commonly recognized ones are adenosine, guanosine, cytidine, thymidine, and uridine. These nucleosides can be found in biological processes, as well as in DNA and RNA.



α-D Nucleoside **β-D Nucleoside**

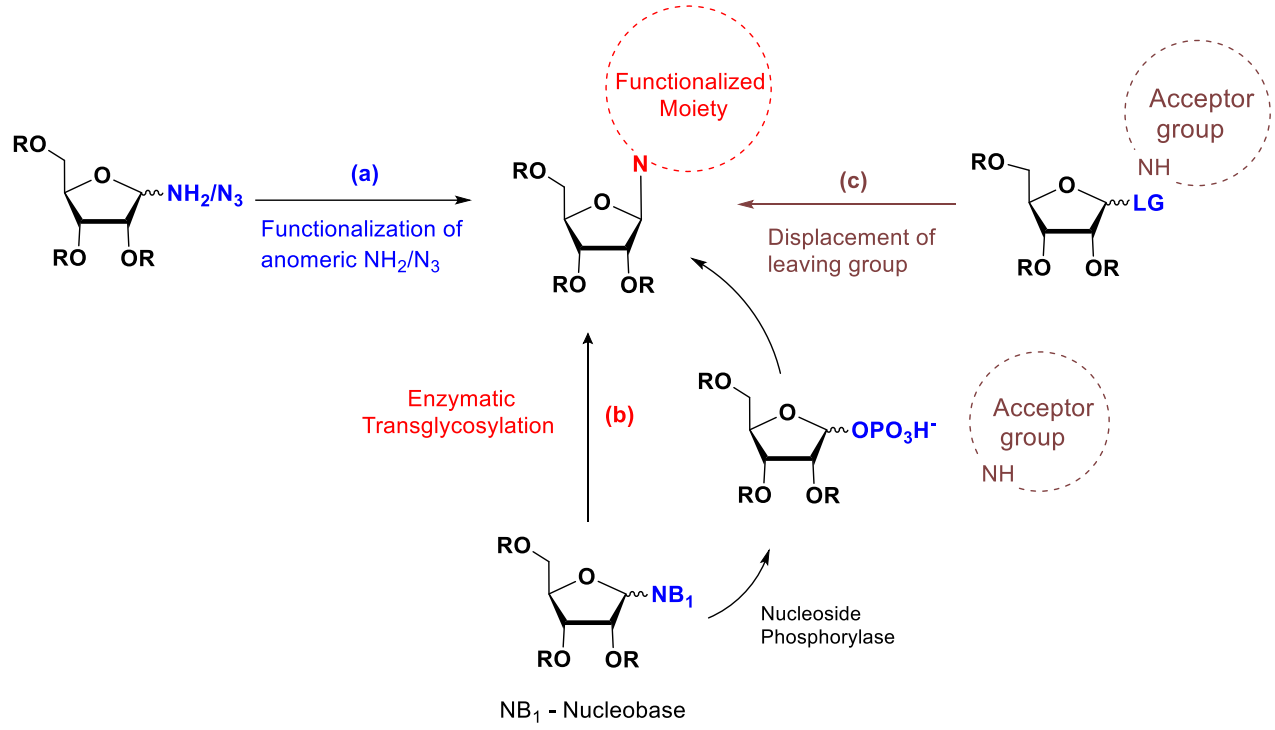
NB - Nitrogenous Base

Fig. 1.1: Configuration of nucleosides

Substituted N-Glycosides have tremendous scope in medicinal chemistry because of their occurrence in biomacromolecules and antibiotics. Among them, modified nucleoside compounds have displayed potential scope for antiviral and anticancer resistance. Nucleoside analogues are artificially altered chemicals designed to imitate natural nucleosides and be incorporated into DNA and RNA to inhibit their replication and cellular division.[7] In addition, these analogues can hinder important enzymes such as human and viral polymerases, kinases, and more. Modified nucleoside analogues have shown potency against numerous viruses. The stereoselective synthesis of nucleosides has become a significant area of focus in recent years due to the important role that nucleosides and their analogues play in all areas of life science.

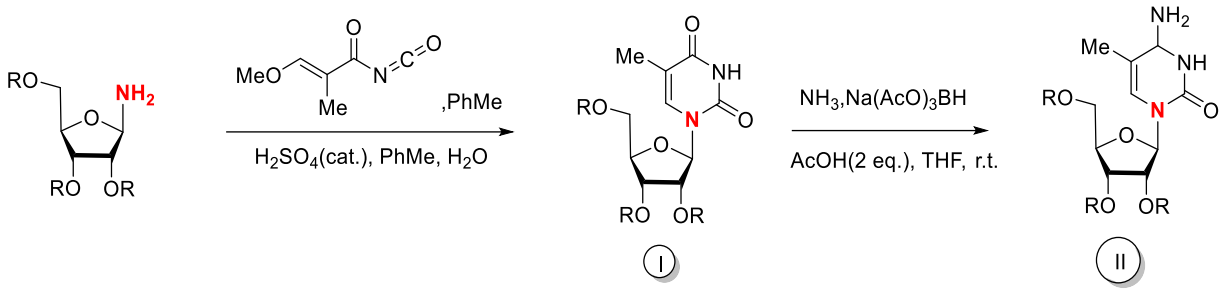
1.2. Strategies to synthesize Nucleoside

Currently, there are three notable synthetic strategies to approach the stereoselective synthesis of modified nucleosides. They are (a) Chemical functionalization of anomeric amine group (b) Enzymatic Trans glycosylation (c) Nucleophilic displacement of anomeric leaving group.

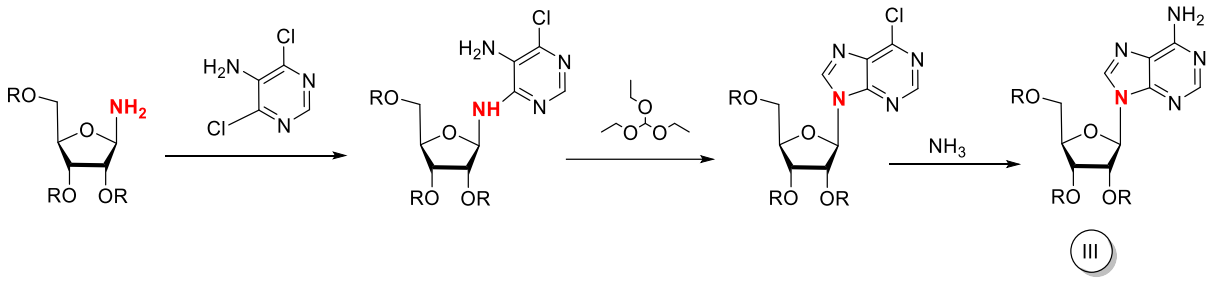


Scheme 1.1: Strategies for Nucleoside synthesis

(a) *Chemical functionalization of anomeric amine group:* Anomeric N-functionalization to synthesize nucleosides have been a well reported strategy to synthesize nucleosides in literature. The stereochemistry is determined by the nitrogen configuration on the anomeric carbon. This involves a coupling reaction between base precursor with the amine derivative. The ring closure generally takes place through Michael cyclization after the glycosylation reaction.[12] The reaction acts as a good alternative to the site selectivity concerns of other methodologies. This strategy is predominant to synthesize carbocyclic nucleosides. Some demonstrations of reported literature reactions through this methodology are given below.

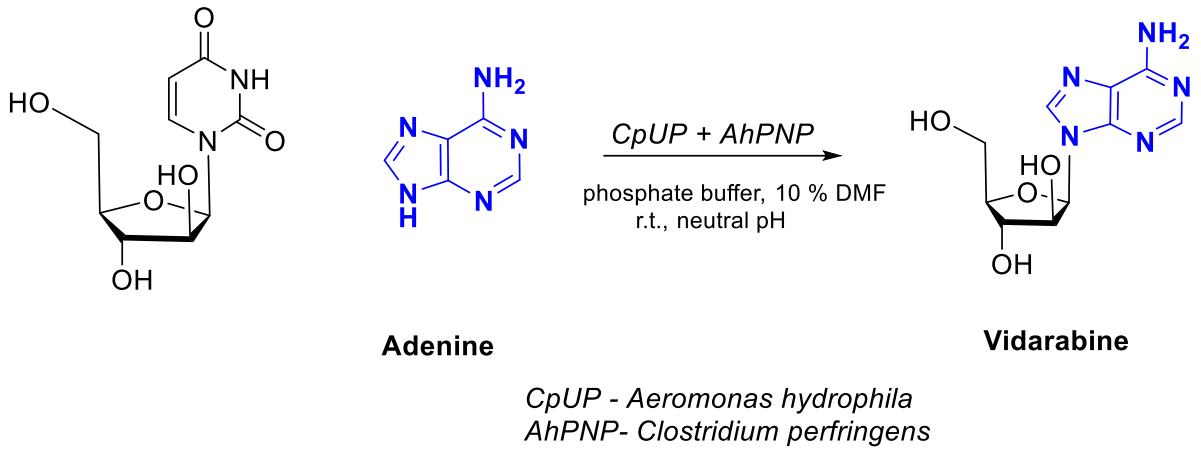


Scheme 1.2: Uridine(I) & Cytidine (II) Derivatives derived by N-functionalization[23]



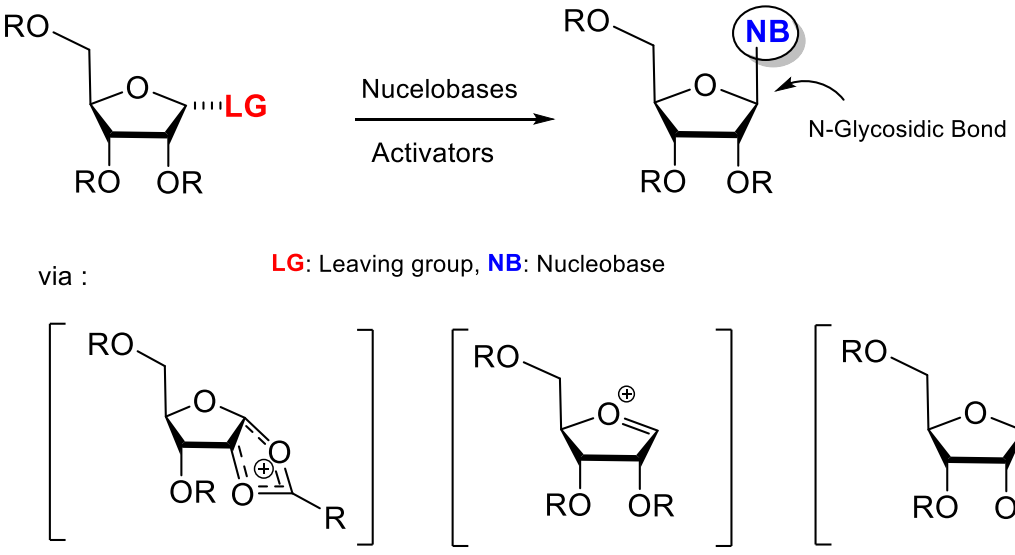
Scheme 1.3: Adenine derivative (III) derived by N-functionalization[23]

(b) Enzymatic trans glycosylation: Enzymatic trans glycosylation has emerged as an alternative to traditional methods to synthesize nucleosides with the help of enzyme engineering technologies, artificial synthesis. The benefit is that enzymatic trans glycosylation is completely kinetically controlled rather than thermodynamically controlled like other synthesis methods. The methodology is based on enzyme catalysis of an existing nucleoside forming a donor and then catalyses the glycosylation with nucleobase acceptor. The main enzymes used for catalysis are Nucleoside Phosphorylases (NP) and 2'-deoxyribosyltransferases (NDT). NDTs are exclusively used for deoxyribonucleosides. Based on substrate specificity, NDTs can be classified into two types based on purine and pyrimidine.[8] Nuclease phosphatases are a group of enzymes of family of transferases that catalyse reversible cleavage of N-Glycosidic bond of nucleoside in presence of an inorganic phosphatase. The drawbacks associated with this methodology is operational complication with usage of enzyme.



Scheme 1.4: Vidarabine derived from by enzymatic trans glycosylation from nucleoside[4]

(c)<u>*Nucleophilic displacement of anomeric leaving group:*</u> The most trivial method for synthesis of β-nucleoside analogues has been through linkage between the nucleobase acceptor and the sugar activated with a leaving group at the anomeric position. The reactions could occur by creating different intermediate products such as a 1'-2 dioxolenium ion or an oxocarbenium ion, or by directly being attacked by a nucleophile.[8] The stereospecificity of these reactions is controlled mainly by neighbouring group participation of C-2' protecting group. Vorbrüggen[13] pioneered the process by using glycosyl acetates with trimethylsilyl protected pyrimidine acceptors and strong Lewis's acids. Over the years, various glycosyl synthons have been explored like ester type[15], ether type[33], 1,2-orthoesters[32], glycosyl thioglycosides[28] etc.

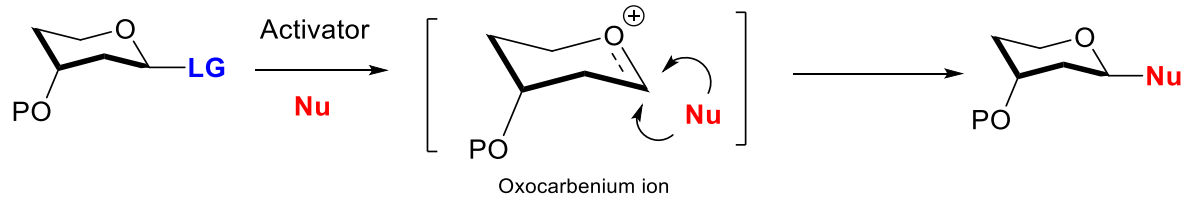


Scheme 1.5: N-Glycosylation techniques

1.3. Glycosylation

Glycosylation is a process by which carbohydrate molecules are covalently attached to a target molecule. This process is considered to be one of the most challenging, non-templated, heterogenous and crucial post-translational modification that has many structural and biophysical roles. Glycosylation is highly sensitive to altercations in cellular function thus any disruption in the process can cause a number of diseases. Glycosylation can be differentiated based on the type of linkage between carbohydrate and the aglycon molecule viz. O-glycoside (-O linkage), N-glycoside (-N linkage), C-glycoside (-C linkage). Glycosylation is a natural process controlled and optimized by enzymes. Chemically synthesizing such site-specific and stereoselective complex molecules is a challenging task.

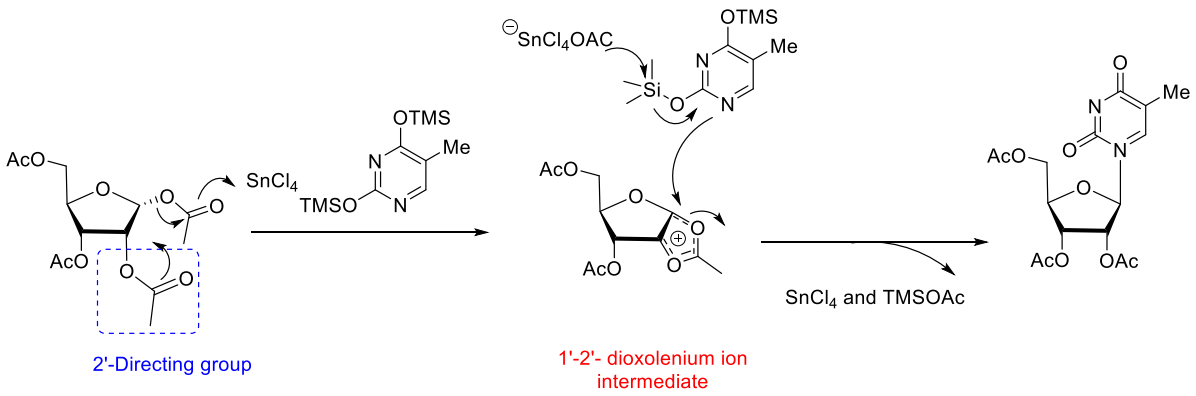
1.3.1. Chemical Glycosylation: A chemical glycosylation can be defined as a nucleophilic displacement reaction of a leaving group attached to a glycosyl donor in presence of activators by an acceptor which is our aglycon. The mechanism which it follows is forming an oxocarbenium ion intermediate which is attacked by a nucleophile acceptor to form a glycoconjugate with the acceptor. One of the biggest challenges faced is controlling the stereoselectivity of the forming acceptor bond. Various factors determine the outcome and stereoselectivity of the glycosylation. Some of them are *anomeric effect, structure of donor and acceptors, protecting groups, solvent effect etc.*[24] Hence, methodologies have been focused on developing better and efficient stereoselective glycosylation techniques.



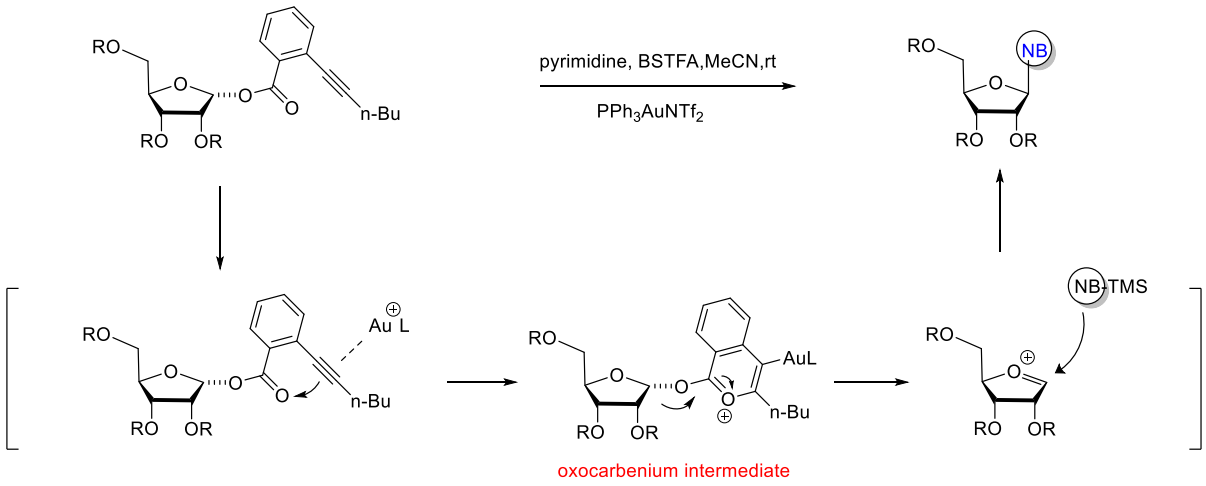
Nu - Nucleophile
LG - Leaving group

Scheme 1.6: Chemical glycosylation representation

1.3.2. N-Glycosylation: N-Glycosylation is the process by which sugar molecules are linked with target molecules through an anomeric C-N linkage. As N-glycosides play a crucial role in biomedical research, there is a continuous effort to investigate diverse glycosyl synthons that can react with different nucleobases in a stereo-specific manner to develop a mild and efficient N-glycosylation method. This research is an ongoing process. Traditionally, the most well-known procedure along these lines has been the Vorbrüggen reaction[13] which involves a glycosyl acetate donor in presence of strong Lewis's acids reacts with a nucleobase by forming a 1'-2' dioxolenium ion intermediate. Further modifications were identified and explored to pursue a mild and efficient synthesis process. Chen and He[14] developed a Pd-catalysed glycosylation by utilizing glycosyl chloride as the donor to form the β-nucleoside through an oxocarbenium ion. Further modifications included using thioglycoside donors[28] activated by (p-Tol)$_2$SO/Tf$_2$O. In 2011, Yu group reported gold-catalysed glycosylation by using glycosyl ortho-hexynyl benzoate as the donor activated by [Ph$_3$PAuNTf$_2$] by forming the glycosyl oxocarbenium intermediate.[15] Both purine and pyrimidine nucleosides could be stereo specifically synthesized using this method. Hotha group has worked upon gold catalysed activation of propargyl orthoester donor [6] for modified pyrimidine glycosides which is the basis of my work too. Some of the schemes are shown below:

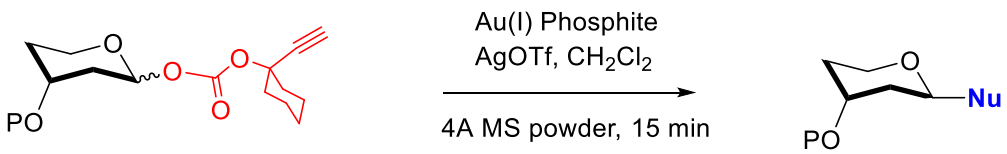


Scheme 1.7: Vorbrüggen Glycosylation[13]



Scheme 1.8: Yu Glycosylation[15]

1.3.3. Gold Catalysed Glycosylation: Inspired by the work done by Hashmi[22] on gold catalysed synthesis of arenes, Hotha and Kashyap had developed activation of anomeric propargyl glycosyl donor with gold catalysis first in 2006.[6] Prior to this, although use of other transition metals was reported, the use of gold catalysis in glycosylation was not explored much. The development of $AuCl_3$ mediated activation of glycosyl 1-2 propargyl orthoester as a donor by Hotha group was also a significant development in this field. Later it was identified that, 1-ethynylcyclohexanyl glycosyl donors were more reactive and easily activated than propargyl glycosides using $AuCl_3$ and AgOTf as catalysts.[5] Subsequently 1-ethynylcyclohexyl glycosyl carbonate donor catalysed by Au(I)/Ag(I) was established as a mild reactive glycosylation template by Mishra and co-workers.[21] Au(I) catalysed glycosylation are significant improvements over previous methods of glycosylation because of higher yield and milder reaction conditions.



Nu - Nucleophile

Scheme 1.9: Au/Ag catalysed glycosylation of 1-ethyl cyclohexyl carbonate donor[5]

1.4. Modified Nucleosides as Therapeutic agents

Nucleoside and nucleotide analogs have proven to be valuable in the field of medicinal chemistry. Following the FDA's approval of cytarabine in 1969, numerous nucleoside analogs have been created and tested for their anticancer and antiviral properties. Naturally occurring nucleosides are particularly useful as a foundation for drug development because of their prevalence in biological processes. Currently, over 25 nucleoside and nucleotide analogs have been approved for clinical use as antiviral and anticancer agents.

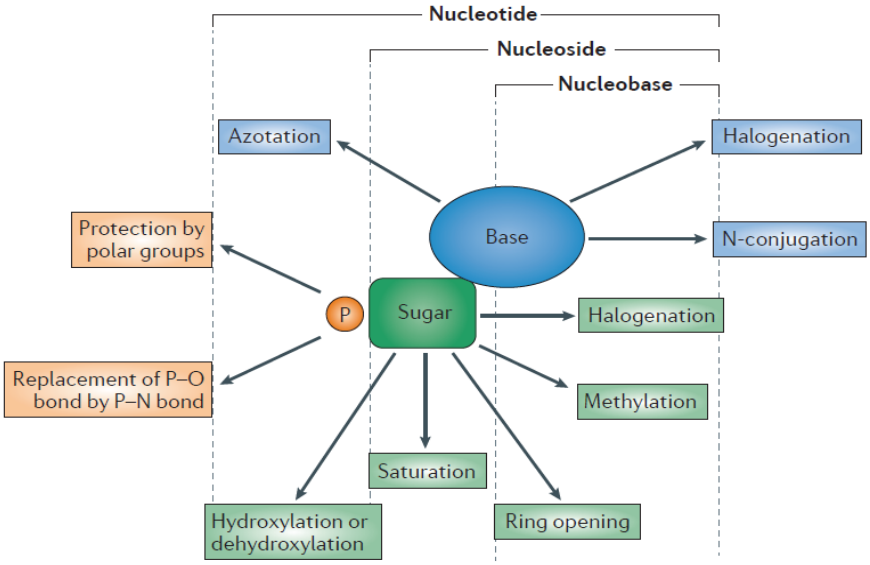


Fig.1.2.: Chemical modification sites of nucleoside and nucleotide analogues[7]

Because these artificial copies are similar to their natural equivalents, they can be identified by enzymes found in viruses or cells. However, because of the changes made to their structure, they can disrupt replication and other biological processes. As a result, they may be incorporated into viral or cellular RNA or DNA, interfering with the replication process. Enzymes recognize substances based on factors such as shape, size, and bonding interactions, allowing for the creation of specific substrates to target particular biological components. Chemical modifications have been attempted over the years, with the primary modifications to nucleoside analogues occurring in the nucleobase, sugar ring, phosphate backbone, or a combination of these areas.

1.5. Mechanism of modified nucleosides[7]

Synthetically produced analogues use the same metabolic pathways and naturally available nucleosides. They enter the cell through designated nucleoside transporters. Once inside, the drugs are phosphorylated multiple times as shown in the below scheme. These phosphorylation steps are carried out by different enzymes. After the third phosphorylation step, triphosphorylated nucleosides are formed. With accumulation of triphosphorylated nucleosides in the cell, they start competing with their physiological counterparts and can inhibit nucleic acid synthesis by disrupting key enzymes required for chain elongation like polymerases.

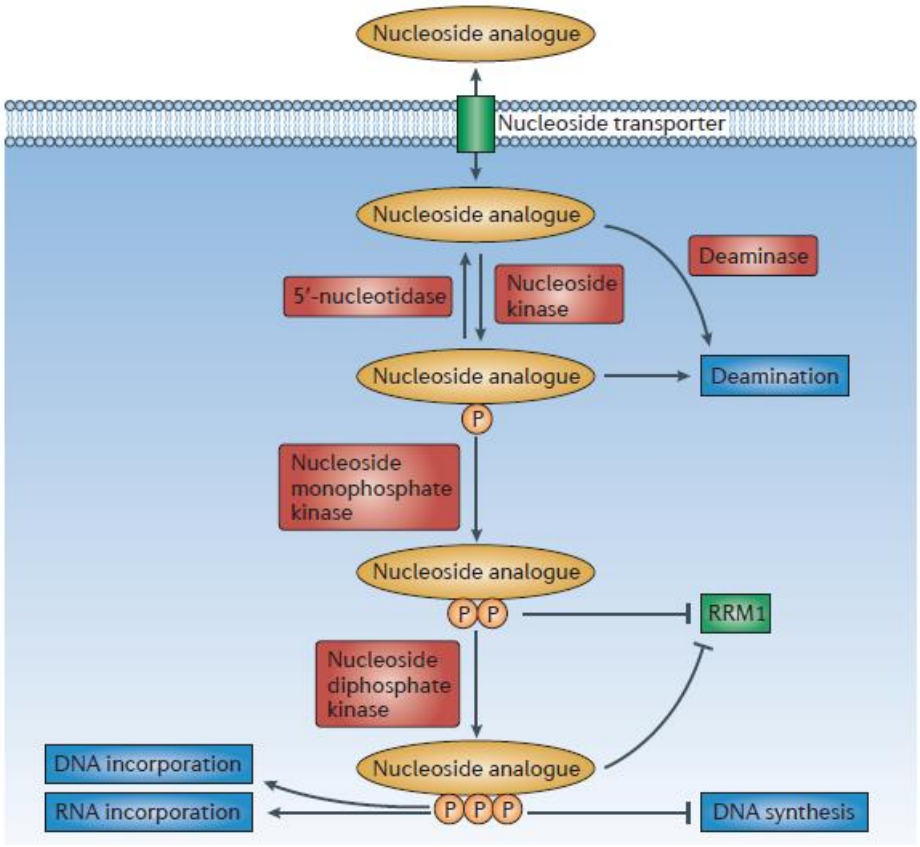


Fig 1.3: Mode of action of nucleoside analogues[7]

1.6. Modifications in nucleoside analogues

1.4.1. Sugar Modifications: In an attempt to create nucleoside drugs, scientists made early modifications to the sugar framework. The resulting analogues provided valuable information on nucleoside interactions and their potential medicinal applications. Reports of modification at sugar ring by replacing -OH or -H group has already been established. Among them, the first analogues to show therapeutic benefits are those having -OH modifications at C-2' of the sugar moiety like *Cytarabine* and *Vidarabine*. The 2'-OH being a key distinguishing feature between DNA and RNA has led to significant research on substitution of different moieties in place of -OH. Some of the first modifications was halogenation. Fluorine is often used an isosteric replacement of hydrogen due to similar size and also similar in electronegativity to hydroxyl group in ribose nucleosides.[11] Coupled with the fact that fluorine showed increase stability towards enzymatic cleavage of glycosidic bond, fluorine became a common modification in drug design. Other notable modifications like alkylation, ring opening and hydroxylation were also studied. With the success of initial modifications, various other modifications were carried out in the sugar ring to give rise to acyclic, carbocyclic nucleosides.

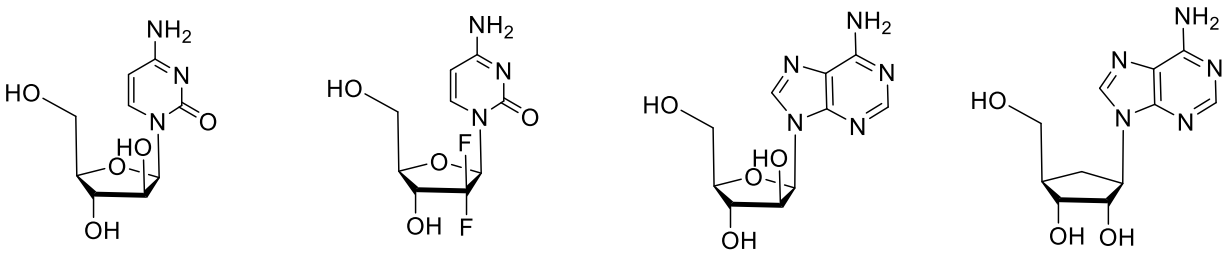
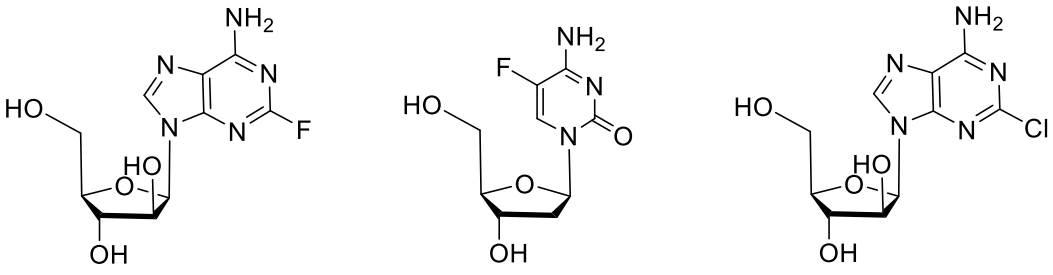


Fig 1.4: Sugar modified nucleoside drugs

Recent advances in nucleoside drug design have also focused on the stereochemistry of the modification and have led to the discovery of many unnatural nucleosides. Modified nucleosides are found to have many biotechnological applications and can be helpful in solving problems of first-generation drugs. Some of the well-known and approved nucleoside drugs with sugar modification have been listed.

1.4.2. Nucleobase modifications: Similar to the sugar modification, various synthetic approaches were developed for nucleobase modifications both to the purine and pyrimidine bases of the nucleoside. Key factors that played a role in deciding nucleobase modifications were sugar-base interactions, enzyme-ligand recognition, interaction between two strands of DNA or RNA. This translated to strategies like modifying the steric, hydrogen bonding interactions, electronics associated with the base and studying subsequent biological activity. One of the earliest modifications have been adding substituents to C-5' of the pyrimidine ring which influenced the interactions between enzyme binding site and nucleoside analogue. Halogenation, mainly fluorination also found good use as a bio isosteric replacement for hydrogen in the nucleobase.[11] Other notable modifications explored over the years are alkylation, azotation and conjugation of N-centre of bases.[30] Further, expanded nucleobases can enhance stacking between two base pairs.



Fludarabine
Anti-cancer

Floxuridine
Anti cancer

Clofarabine
Anti cancer

Fig 1.5: Nucleobase modified nucleoside drugs

1.4.3. Phosphate Backbone modification: Another potential site of modification that has been explored upon has been the phosphate backbone. Recent developments and modifications include replacement of P-O bond with P-N bond and protection of phosphate groups with more polar groups.[31] This allows alteration in structure and functionality of the phosphodiester linkage which induces biological activity.

1.6. Current work

Having already discussed the techniques and importance of synthesizing nucleoside analogues, currently through the tenure of my project I have tried to synthesize two different modified nucleoside analogues starting from the cheap commercially available ribose having post glycosylation modifications at 2' in sugar ring. For this we have used two different protecting groups for each nucleoside at the C-3' and 5' position to compare their stability and reactivity. The aim is to synthesize these modified nucleoside analogues in a mild, efficient and quantitative manner which can pave the way to build a library of nucleoside analogues that can further be used as active pharmaceutical ingredients in development of nucleoside drugs. Previously similar work has been carried out in our group in synthesizing nucleosides with other protecting groups.[24] We are further optimizing the process and using easily removable protecting groups for ease in later stages.

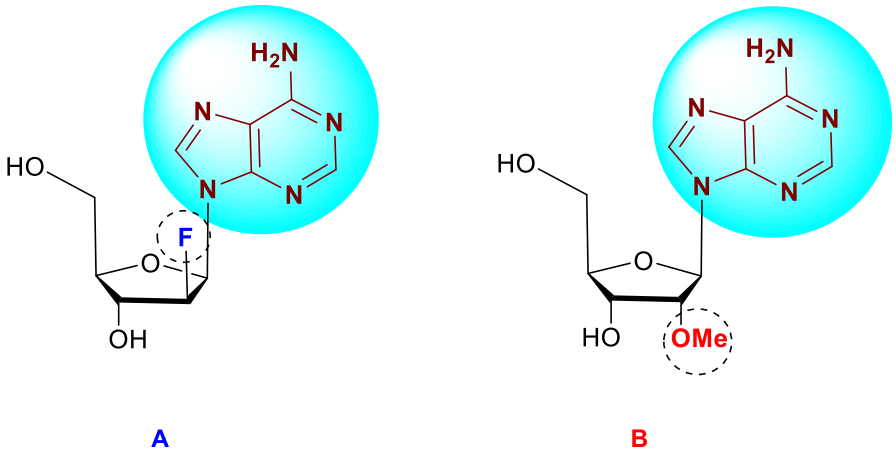
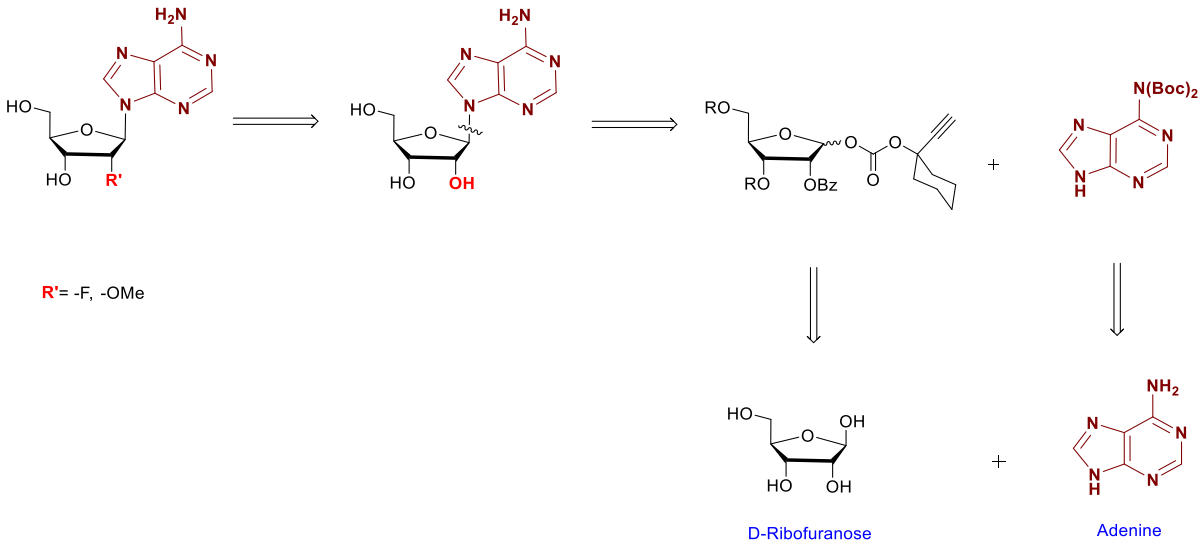


Fig 1.6: Target molecules

1.7. Retrosynthesis

The target molecule can be broken down retro synthetically into donor and acceptor given in the following scheme. The ethynyl cyclohexyl ribofuranosyl donor was synthesized from D-Ribose. The role of -OBz at C-2' is important to direct the N-Glycoside as a participating group to form trans 1,2-trans nucleosidic linkage and for post glycosylation modification at 2' position. Prior to that; 1,2-Orthoester was formed to form the benzoate at 2' position. The C-3' and C-5' has to be protected throughout. The adenine derivative was Boc-protected to increase its solubility and suppress reactivity at N-9 position.



Scheme 1.9: Retrosynthetic model

The various synthetic pathways undertaken are discussed in the following chapter.

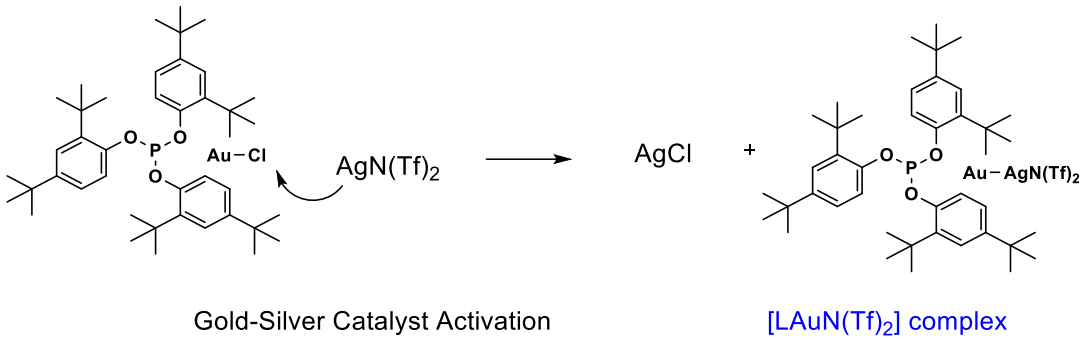
2. Results and Discussion

2.1 Key Reaction Mechanisms

I will first discuss the reaction mechanism involving the key step of N-Glycosylation by Au/Ag catalysation in brief as it is a key step in synthesising the nucleoside. Our choice of donor is the 1-ethynyl cyclohexyl carbonate donor developed by Hotha group which undergoes nucleophilic displacement in presence of Au/Ag catalysts. The plausible mechanism given by Mishra et. al.[21] is described below with schematics:

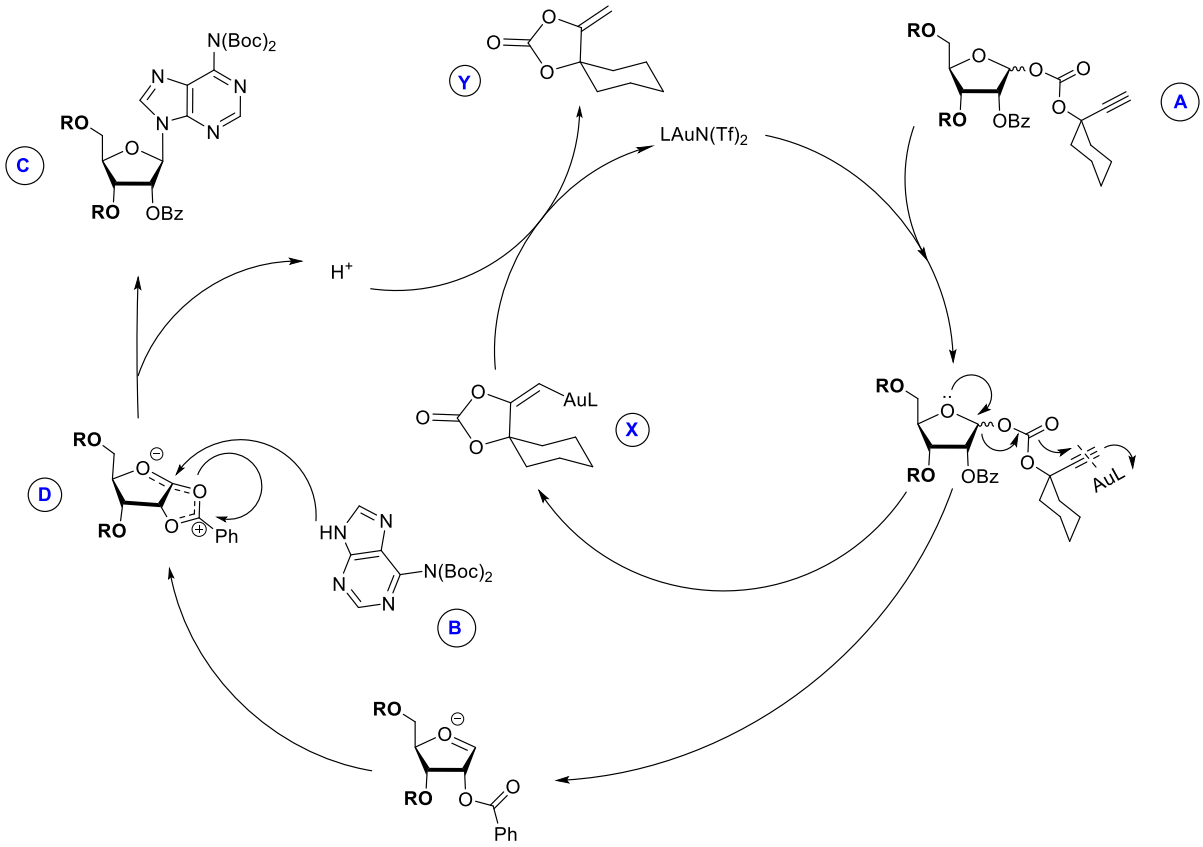
The silver ion Ag^+ of the $AgN(Tf)_2$ acts as a chloride scavenger when the gold catalyst reacts with it to form the active Au-Ag complex ($LAuN(Tf)_2$). This complex easily co-ordinates with the alkyne group of the donor to synthesize a gold-alkyne complex.

The triple bond on the donor **A** on activation ensures cyclization of the 1-ethynyl cyclohexyl carbonate donor due to the presence of lone pair on the endocyclic Oxygen moiety which pushes electron towards the electrophilic centre. Due to presence of -OBz on C-2', a trioxolenium ion **D** is formed which stabilizes the intermediate. The free N-H of the nucleobase derivative **B** acts as an acceptor and attacks the electrophilic anomeric centre forming the nucleoside **C**. Vinyl gold cyclic carbonate **X** captures the proton thus forming the spirocyclic compound **Y** as a by-product and thereby regenerating the catalyst.



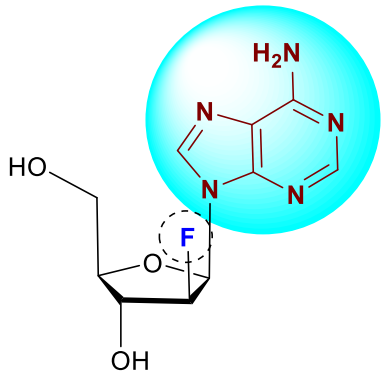
Gold-Silver Catalyst Activation

[LAuN(Tf)$_2$] complex



Scheme 2 : Plausible mechanism for Gold catalysed glycosylation

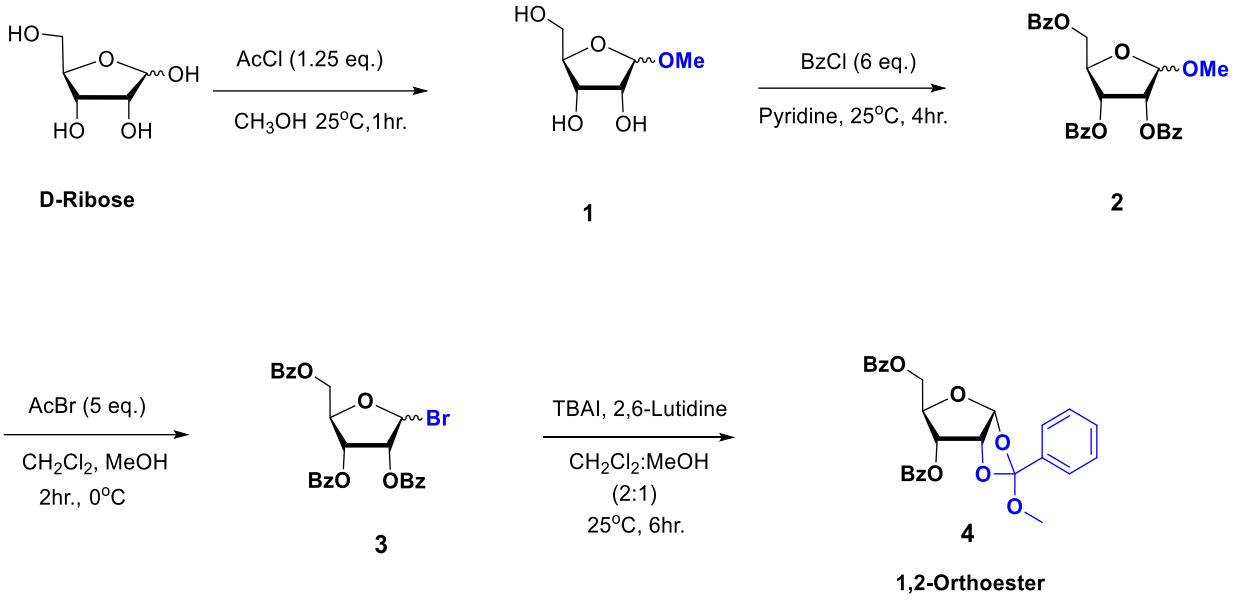
2.2 Synthesis of Compound A



As we have seen in the retrosynthetic pathway, the above compound is envisioned to be synthesised from commercially available D-ribose and adenine. The pathway undertaken is synthesis of glycosyl 1-ethynyl cyclohexyl carbonate donor through a 1,2- orthoester derivative which provides the 2'-Bz on ring opening which is necessary to direct the oncoming glycoside into a trans 1,2-linkage exclusively. The Bromosyl ribofuranoside is synthesised from D-ribose in 3 steps.

2.2.1 Synthesis of 1,2-Orthoester (5)

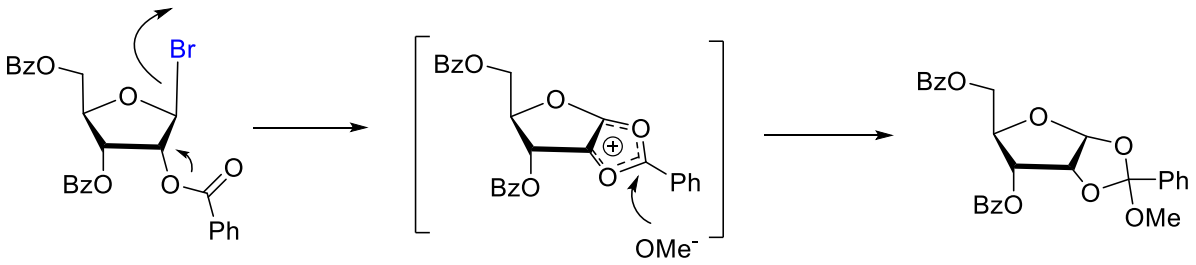
Synthesis of bromosyl furanoside requires conformation locking to ensure bromination at anomeric carbon. First, D-Ribose was converted into methyl ribofuranoside using Fischer's glycosidation reaction followed by global Benzoyl protection of the free -OH groups. This is followed by bromination using Acetyl Bromide to synthesise bromosyl ribofuranoside. The bromoside was reacted with Methanol which reacts in presence of TBAI with the dioxolenium intermediate to form the 1,2-orthoester. The orthoester being thermally labile is required to be treated in low temperature so as to not form direct glycosides. The step of formation of orthoester produces many side products giving sub-optimal yield. The formation of orthoester was verified by ^{1}H,^{13}C NMR and Mass spectrometric techniques.



Scheme 2.1: Synthesis of 1,2-Orthoester

2.2.2 Mechanism of formation of Ortho-ester

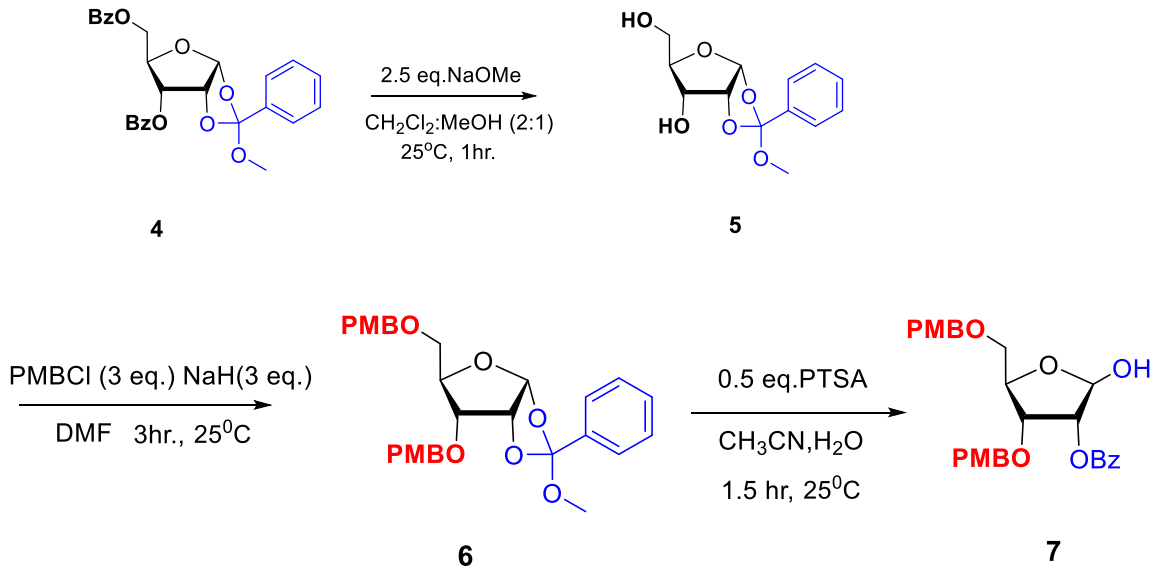
In presence of activator Br^- is displace by attack of carbonyl from Benzoyl group at C-2'. This forms a 1,2- dioxolenium intermediate which is stabilised by methoxy anion subsequently locking the conformation into a 1,2-orthoester which can later be decomposed into C-2' -OBz and hemiacetal for synthesis of donor.

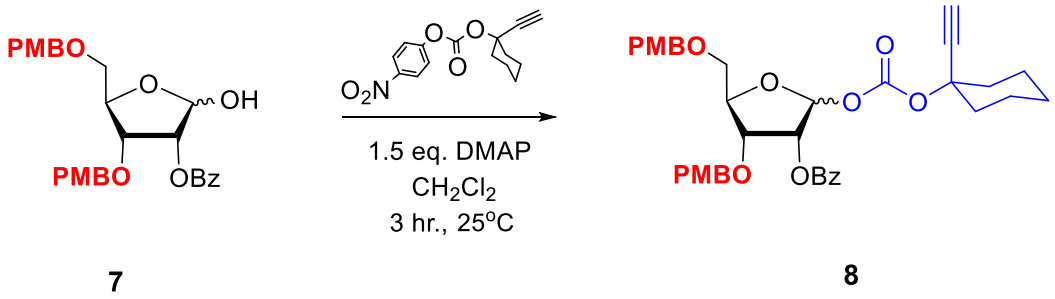


Scheme 2.2: Mechanism of formation of Orthoester

2.2.3 Synthesis of 1-ethynyl cyclohexyl carbonate donor

We have tried to use different functional groups on C-3' and 5' moieties keeping in mind the post- glycosylation modification at C-2' position. For Compound A, I have used 4-methoxy Benzyl ester as the protecting group. Starting from the 1,2-Orthoester (4), deprotection of Benzoyl group is carried out by Sodium Methoxide in Methanol. The resultant diol orthoester is protected in presence of para-methoxy benzyl chloride. As I have mentioned previously, the orthoester is susceptible to acidity and high temperature and to prevent it from degrading into a direct glycoside, all chromatography techniques have to be done by quenching in triethylamine and under low temperature. After the protection of C-3' and C-5' position by p-methoxy benzyl, the orthoester is broken down into hemiacetal and 2'-OBz in presence of a weak acid like p-Toluene Sulfonic Acid. Care has to be taken during this step due to the competition of methoxy ion and hydroxyl ion which might lead to formation of direct glycoside and loss in yield. The hemiacetal (**7**) is used for reaction with ethynyl cyclohexyl (4-nitrophenyl) carbonate reagent prepared separately in presence of DMAP to synthesize the desired donor (**8**) which would be used for Au/Ag catalysed glycosylation. The donor was well characterized by spectrometric techniques as an α/β mixture.



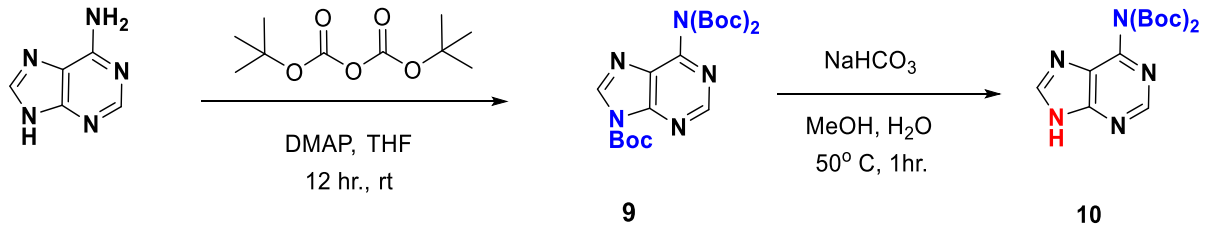


7 8

Scheme 2.3: Synthesis of PMB-protected Ribose Donor

2.2.4 Synthesis of Adenine Acceptor

Commercially available adenine was used to synthesize our nucleobase acceptor. Due to multiple reactive sites in Adenine, selective protection was required to ensure glycosylation at target N-group. So, adenine was first globally protected with tert-butuloxycarbonyl group in presence of DMAP. After global protection was achieved, selective deprotection was carried out at more reactive N-9 position which would be our reactive centre for glycosylation. This was achieved by refluxing in methanol in presence of sodium bicarbonate and water. This step has to be done carefully with careful optimized addition of sodium bicarbonate due to the tendency for other Boc-groups to be deprotected in the process leading to lower yield and unsuitable conversion. The derived di-Boc adenine (**10**) was further used for glycosylation with the donors to produce the desired glycosides.

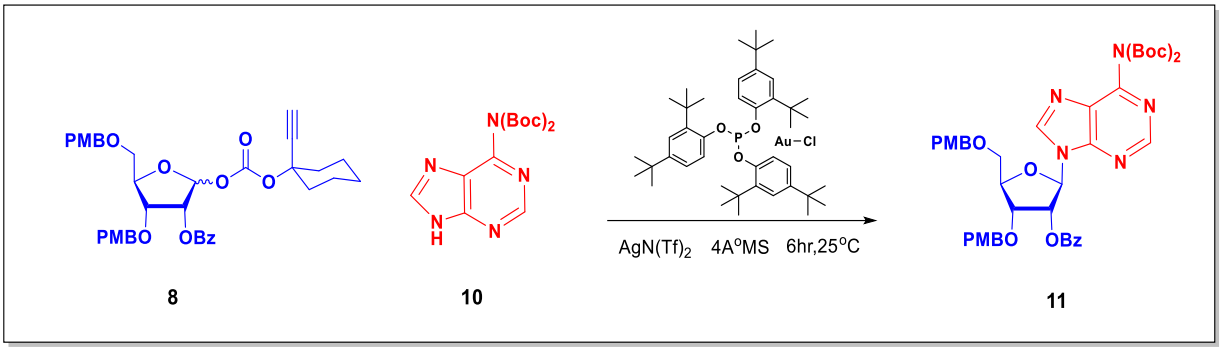


9 10

Scheme 2.4: Synthesis of Adenine Acceptor[17]

2.2.5 Glycosylation of PMB-protected Ribose Donor (8) and Acceptor (10)

Once the PMB-protected Ribose Donor and Adenine derived acceptor is synthesised, Au/Ag catalysed glycosylation is performed in presence of gold-phosphite catalyst, co-catalysed by $AgNTf_2$. To minimize side product formation of hemiacetal, dry conditions and dry solvent had to be used. Use of molecular sieves further capture any moisture present. The reaction is well reported and reproduced for synthesis of N-Glycosides. It is postulated that the reaction proceeds by forming an oxocarbenium ion stabilised by the oxygen lone pair of endocyclic ring oxygen upon detachment of the ethynyl cyclohexyl carbonyl group which is a good leaving group. The adenine acceptor acts as the nucleophile at N-9 position to attack the corresponding cation to produce the required nucleoside in quantitative yield. (**11**)

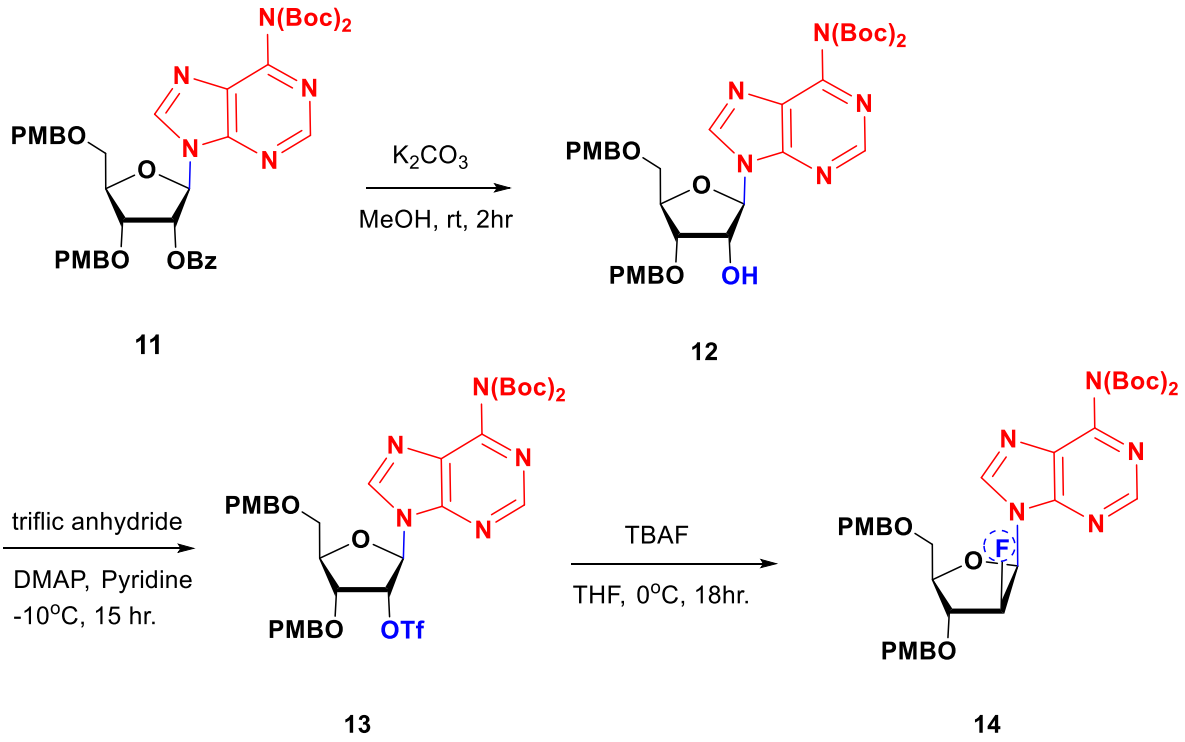


Scheme 2.5: Glycosylation Reaction

2.2.6 Post-glycosylation modifications

The nucleoside thus obtained was envisioned to undergo C-2' modification. Here the role of installing -OBz moiety at 2' position comes into play as it can be selectively deprotected in presence of -PMB protecting groups and be further modified. The plan was to install a trans fluorine moiety by nucleophilic displacement at 2'. This required 3 intermediate steps which are discussed below.

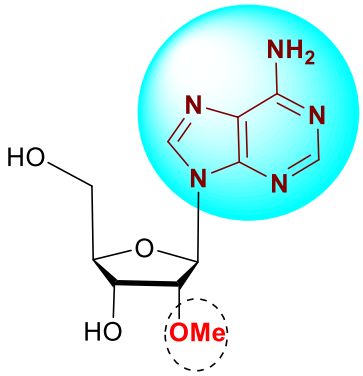
First, the -OBz moiety was deprotected to obtain a lactol which could then be converted into a triflate which acts as a good leaving group. The triflate participates in SN2 nucleophilic displacement in presence of a fluoride ion source like Tetra butyl ammonium fluoride which would then give us the trans fluoro modified nucleoside. While the triflate was synthesised with quantitative yield and ease, the fluorination displacement reaction although was performed, there was problem in purifying the desired compound due to many side products and very less quantitative amount of the product, the pure compound couldn't be optimally separated and hence there is a need to further optimize the process in future.



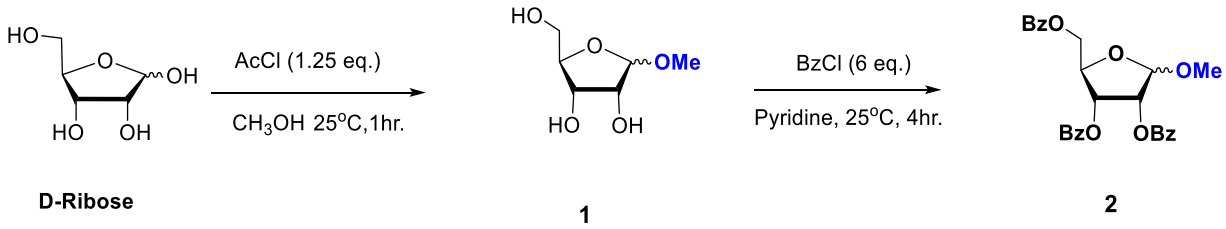
Scheme 2.6: Fluorination at C-2'

On successful synthesis of fluorinated compound (**14**), it can be further globally deprotected in a single step to obtain the target molecule in presence of TFA and trifluoromethanesulfonic acid.

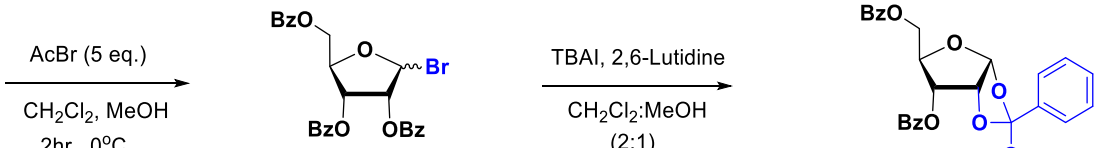
2.3 Synthesis of Compound B



For the synthesis of the above compound, similar reaction pathway has been followed from D-ribose and adenine. But in this case, I have used 2-methyl naphthalene as the protecting group in place of para methoxy benzyl as PMB group is susceptible to acids and naphthalene being a more stable group suited better. The 1-ethynyl cyclohexyl carbonate donor was formed in a similar manner but we faced an inconvenience in the formation of orthoester so we had to take an alternate route which I will be discussing later. The adenine derivative that was synthesised above was used. Subsequently glycosylation was carried out.



D-Ribose **1** **2**

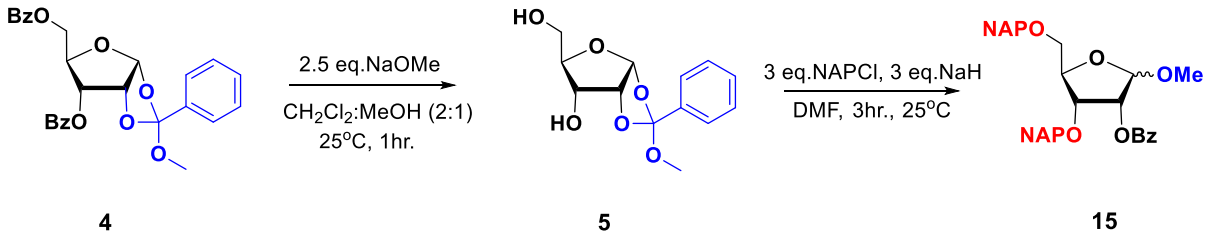


3 **4**

1,2-Orthoester

2.3.1 Synthesis of direct glycoside

Due to some error, direct glycoside was formed which was identified using spectrometric techniques during some step during Naphthalene protection. This was a problem as -OMe is very stable and we tried various acids to deprotect the methoxy group but it was not possible. Hence, we had to synthesize the desired hemiacetal through an intermediary pentenyl derivative.

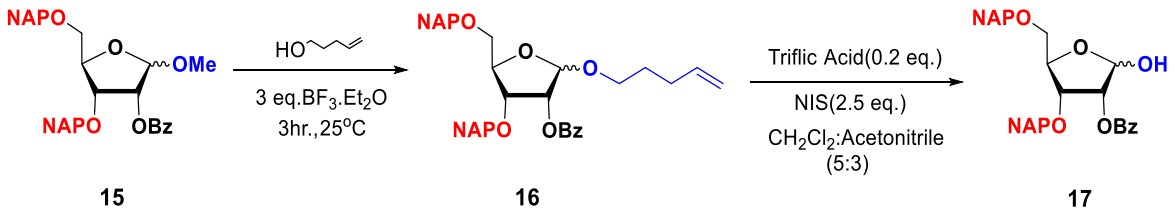


4 5 15

Scheme 2.7: Synthesis of direct glycoside (error)

2.3.2 Synthesis of hemiacetal

The direct glycoside was converted into pentenyl substrate by reacting with 4-penten-1-ol in presence of BF_3.Etherate as an activator. The pentenyl being highly reactive displaces the -OMe group. Once synthesised, the pentenyl group is deprotected under NIS and Triflic Acid to afford the desired hemiacetal which can be converted into the donor.

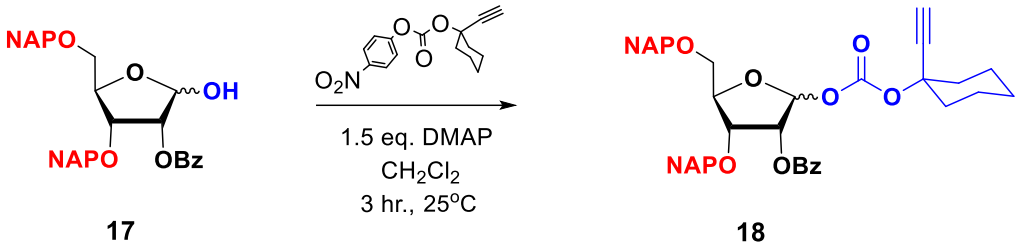


15 16 17

Scheme 2.8: Synthesis of hemiacetal

2.3.3 Synthesis of Nap - protected Donor

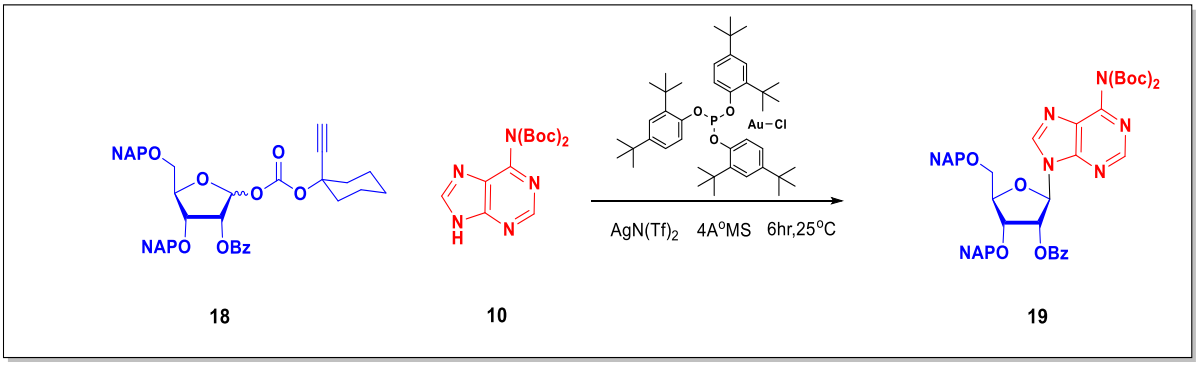
Following the already mentioned process, the hemiacetal is converted using the 1-ethynyl cyclohexyl (4-nitrophenyl) carbonate reagent in presence of DMAP into the ethynyl cyclohexyl carbonate donor which was obtained in an enantiomeric mixture. This is further used for glycosylation.



Scheme 2.9: Synthesis of Nap-Donor

2.3.3 Glycosylation of Nap-Donor

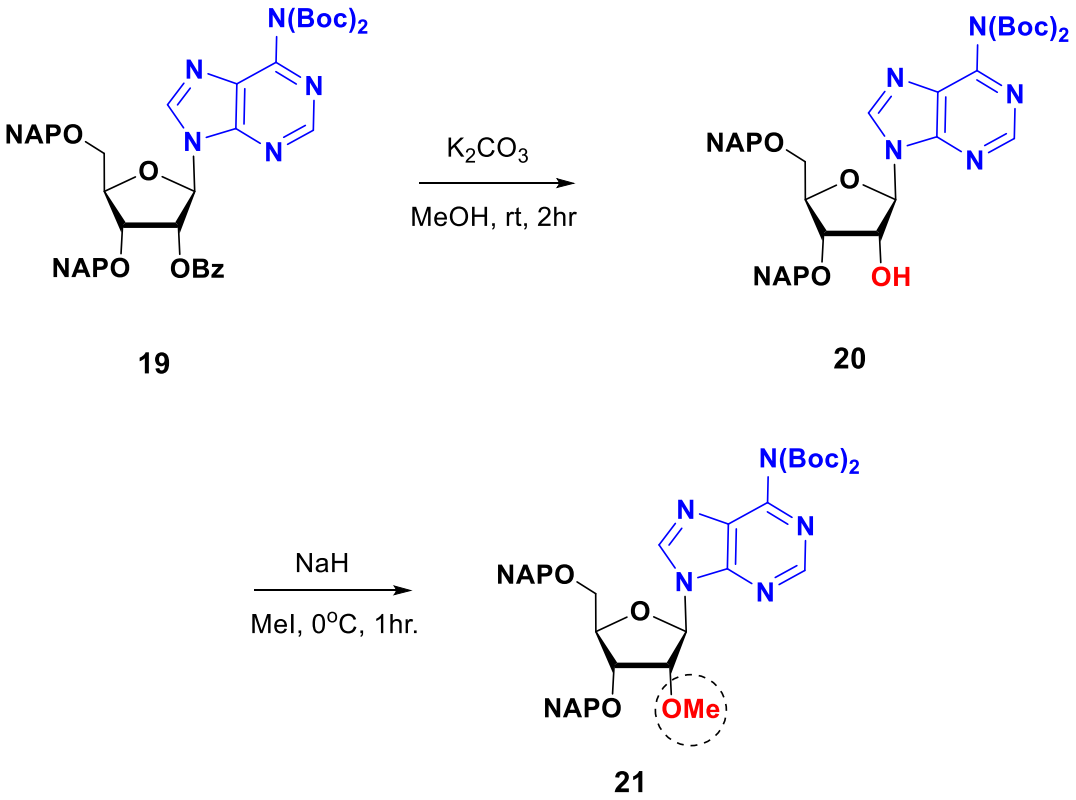
Glycosylation was carried out in a similar method between donor **18** and acceptor **10** by Au/Ag catalysis as mentioned above in presence of molecular sieves. The N-glycoside product formed was well characterized by spectrometric techniques and can be used for further post glycosylation modifications.



Scheme 2.9: Synthesis of Nap-nucleoside

2.3.3 Post-Glycosylation modification

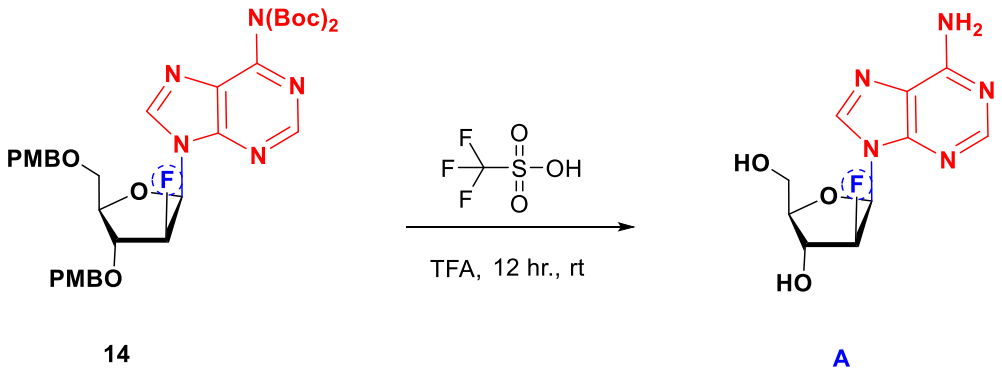
The modification we have envisioned for this molecule is methylation at 2' position. Again, the presence of -OBz moiety allows better control and specificity over the modification at that position. Methylation is a simple 2-step process involving deprotection of the -OBz group followed by methylation of the hemiacetal. This gives us the compound **21.** This has to be followed by global deprotection which would give us the target molecule **B.** Currently the deprotection of Boc group is being optimized and further deprotection of Naphthalene group needs to be carried out.



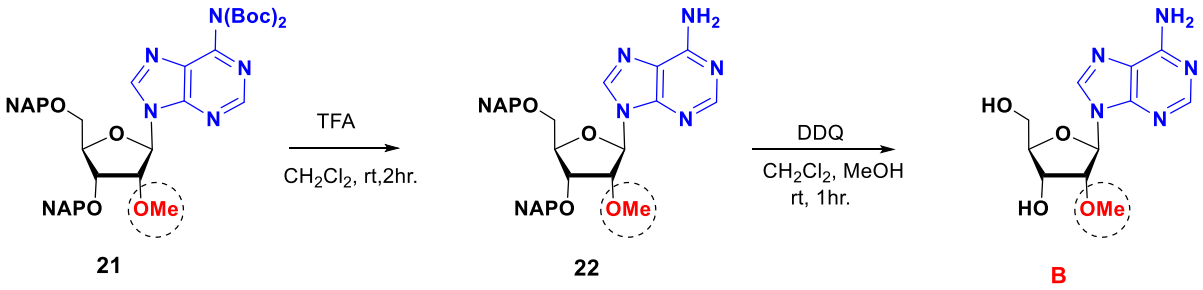
Scheme 2.10: Methylation at C-2'

2.4 Future proposal

On the successful synthesis of compound **14**, one step deprotection of -PMB and -Boc groups can be carried out in presence of TFA and trifluoromethanesulfonic acid. This gives us the target molecule **A**. Further, from compound **12**, we can perform subsequent oxidation and reduction on the hemiacetal derivative to enhance the modifications stereo selectively. Following the same route of synthesising triflate and then fluorination gives us the cis fluorine. Similarly, compound **21** can be globally deprotected to afford molecule **B.** Thus, utilising this method, we can successfully incorporate stereo specific post-glycosylation modifications in the sugar ring. In the future, various other derivatives can be explored in place of fluorine like azides, alkylating groups.

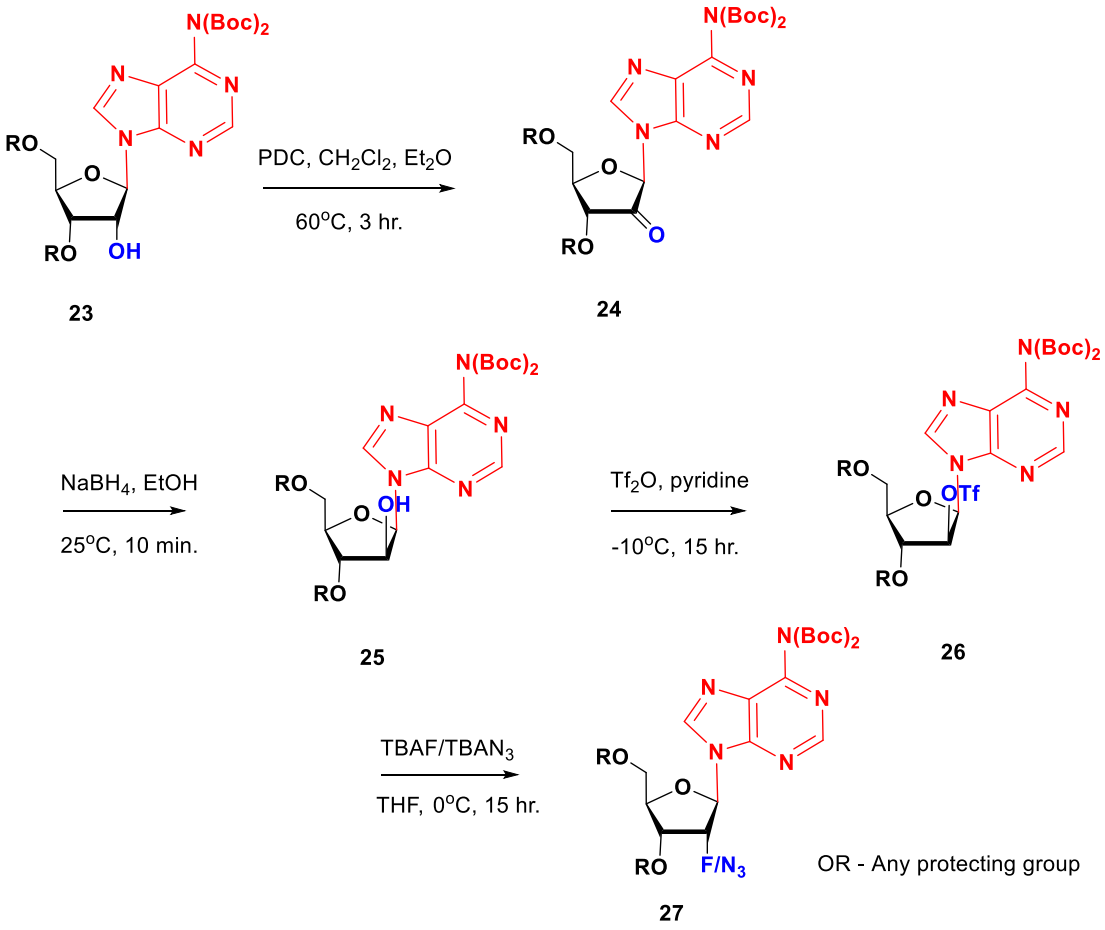


Scheme 2.11: Synthesis of molecule A



Scheme 2.12: Synthesis of molecule B

Likewise, we can try out various other protecting groups in place of -PMB and -NAP and compare their stability and ease of deprotection in final stages. The acquired molecules A and B can also be further modified based on theoretical understanding and need. Other nucleobases can also be incorporated using this glycosylation process. Ensuring better optimization and yield is a major hurdle towards developing this novel process of synthesising modified nucleosides.



Scheme 2.12: Further stereoselective modifications to be tried out

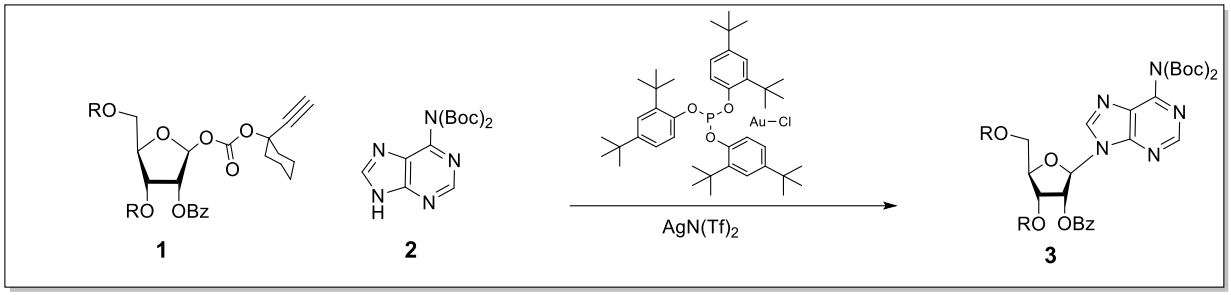
Following the schemes underlined above, various modifications can be carried out at C-2' post glycosylation. Different sugar groups and nucleobases can also be explored leading to a wide combination of modified nucleosides. This can serve as an efficient way to synthesise a library of building blocks for further modification into nucleoside drugs.

3. Materials and Methods

- The NMR spectra were captured using Bruker 400 MHz, JEOL ECX 400, and Bruker 600 MHz machines. To establish an internal reference, tetramethyl silane (TMS) was utilized. Chemical shifts were measured in ppm units, relative to the downshift of TMS. All synthesis chemicals are acquired from Sigma-Aldrich, TCI or Spectrochem.
- High Resolution Mass Spectroscopy was conducted on a Waters Synapt G2 or a MALDI-TOF spectrometer.
- All reactions were monitored using Thin Layer Chromatography on E-Merck silica plates (0.25mm, F-254) that had been pre-coated. The plates were visualized using UV light and subjected to chemical staining using anisaldehyde stain.
- Silica Gel column chromatography was conducted in 100-200 mesh silica gel.
- All reactions were carried out in an inert argon or nitrogenous environment with anhydrous solvents acquired from Merck and Finar.
- All evaporations were carried out using a Heidolph rotary evaporators under temperature below 50^0C.
- Multinational commercial suppliers provided with all gold and transitional metal salts.

3.1. General procedures

3.1.1. Glycosylation reaction



AgOTf (0.1eq.) and Au-phosphite (0.1eq.) were added to a solution of Donor (1) (1eq) and acceptor (2) (1.2 eq.) in anhydrous CH_2Cl_2 containing 4Å MS powder and stirred at rt. for 6 hr, concentrated under reduced pressure, and resulting residue was purified by silica gel column chromatography (n-hexane/EtOAc) to afford pure nucleoside.

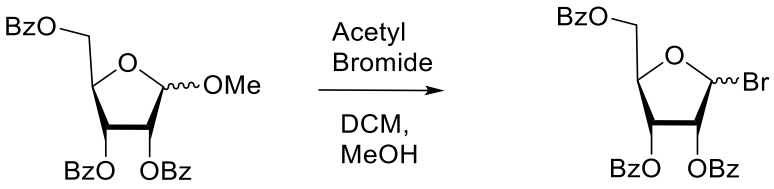
3.1.2 Methoxy protection of anomeric carbon

The process involved mixing a solution of sugar (at a quantity of 1 equivalent) in methanol with a separate solution of acetyl chloride (at a quantity of 1.25 equivalents) in methanol, which was then stirred at a temperature of 0°C. The reaction mixture was later stirred at room temperature for an hour. Upon completion, the reaction was halted by introducing pyridine. Pyridine was eliminated via rotary evaporation and high vacuum treatment, leading to the formation of crude product.

3.1.3 Benzoyl protection

To initiate the reaction, the reactant is dissolved in pyridine at a very low temperature. Benzoyl Chloride (at a quantity of 6 equivalents) is gradually added to the reaction mixture at ice-cold temperature. and then stirred at rt. for 4 hours. When the reaction is complete, 1N HCL is added to quench the reaction. The organic layer is extracted twice using Ethyl Acetate followed by washing twice with aqueous 1N HCl and brine solution, and finally dried over anhydrous Na_2SO_4. The mixture is thereby concentrated under reduced pressure and purified using column chromatography.

3.1.4 Bromination



The methyl per-O-benzoyl ribofuranoside, which was prepared earlier, was dissolved in CH_2Cl_2 and cooled to 0 ºC. Acetyl bromide was added to the mixture, followed by the slow addition of MeOH while continuously stirring at 0 ºC. The reaction mixture was stirred at 0 ºC for 1 hour before being diluted with CH_2Cl_2. The mixture was then poured into an ice-water mixture, and the resulting aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with cold saturated sodium bicarbonate solution, dried over sodium sulphate, and concentrated under high vacuum. This produced ribofuranosyl bromide which was used in the next step.

3.1.5 Ortho – ester protection

The initial step involved dissolving the unrefined ribofuranosyl bromide in a mixture of anhydrous CH_2Cl_2, methanol, and 2,6-lutidine. Tetra ^{n}-butyl ammonium iodide was then introduced into the reaction mixture, which was stirred for 6 hours at room temperature. The resulting mixture was further diluted with CH_2Cl_2 and water, and the aqueous layer was separated and extracted with CH_2Cl_2. The organic extract was washed with saturated oxalic acid and saturated sodium bicarbonate solutions. After drying the organic phase with sodium sulphate, it was concentrated. The crude residue was then purified via silica gel column chromatography, using a mixture of ethyl acetate and petroleum ether ultimately yielding a white solid product.

3.1.6 Benzoyl deprotection

The procedure involved dissolving the reagent (at a quantity of 1 equivalent) in a mixture of CH_2Cl_2 and MeOH (at a ratio of 1:2) and stirring. NaOMe (at a quantity of 2.5 equivalents) was added and the mixture was stirred at room temperature for an hour. Once the reaction was complete, the reaction mixture was concentrated under vacuum and re-dissolved in a combination of water and EtOAc, which was then extracted with EtOAc. The resulting extract was dried using sodium sulphate and then concentrated. The crude residue was subsequently mixed with pet ether (to eliminate methyl benzoate) and stirred for 10 minutes. The pet ether layer was then separated to obtain the pure product.

3.1.7 Hemiacetal formation from ortho-ester

The reactant is dissolved in Acetonitrile followed by a drop of water and p-Toluene sulfonic acid (0.5 eq.). The reaction was stopped using triethylamine after using up the initial materials. The mixture resulting from the reaction was concentrated under vacuum, and the organic layer was separated using CH_2Cl_2, washed with a solution of $NaHCO_3$, and then evaporated. The product was subsequently purified using silica gel column chromatography with a mixture of hexane and EtOAc.

3.1.8 Carbonate Donor preparation

An ice-cooled solution of reagent (I) in dry CH_2Cl_2 was combined with 1.5 equivalents of DMAP and stirred for 20 minutes. A solution of compound (1.5 equivalents) in dry CH_2Cl_2 (4mL) was then added dropwise and stirred at 0°C for 30 minutes. The reaction mixture was then stirred at 25°C and after 2 hours, the reaction was concentrated under vacuum to produce an oily residue. This residue was purified using silica gel column chromatography with a hexane/EtOAc mixture to obtain the final purified product.

3.1.9 4-Penten-1-ol addition at anomeric position

The sugar compound was dissolved in anhydrous CH_2Cl_2 and 1.5 equivalents of 4-Penten-1-ol was added dropwise to the solution at 0°C. The mixture was stirred for 30 minutes, after which $BF_3.Et_2O$ was added dropwise and left to stir for 3 hours. Once the reactant was fully consumed, the reaction was stopped by adding triethylamine. The reaction mixture was then concentrated under vacuum and further purified using silica gel column chromatography with a mixture of petroleum ether and EtOAc to obtain the desired compound.

3.1.10 Pentenyl deprotection

The reactant is dissolved in 5:3 (CH_2Cl_2: Acetonitrile), Triflic acid (0.2eq) and a drop of water is added followed by *N*-Iodosuccinimide(2.5eq). The reaction was stopped after 1.5 hours by adding a solution of $NaHCO_3$. The resulting mixture was then washed two times with a solution of sodium bicarbonate, followed by two washes with brine solution. The product was purified using silica gel column chromatography with a mixture of hexane and EtOAc.

3.1.11 Boc-protection

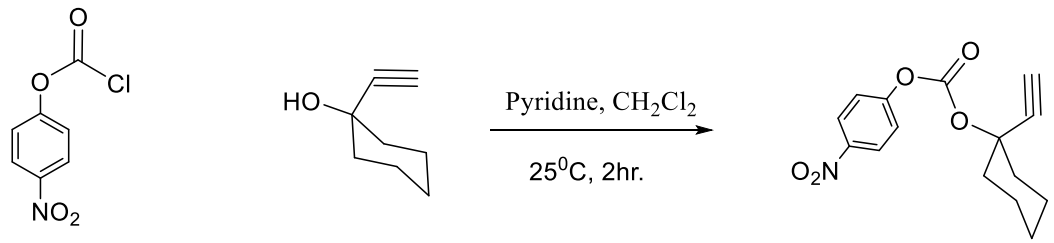
To a solution of reactant (1 eq.) is dissolved in dry THF, DMAP and tert-butyloxycarbonyl (4 eq.) was added. Under nitrogenous conditions, the reaction was stirred for 12 hours in room temperature. Upon clean conversion, excess THF was evaporated under vacuo and column chromatography was performed in EtoAc/Hexane to separate the desired compound.

3.1.12 Selective Boc Deprotection

The tris-Boc Adenine compound was dissolved in ethyl acetate and then washed with 1N HCl and brine. Upon evaporation of the organic layer, a colorless oil was obtained. This oil was added to a solution of MeOH and a saturated bicarbonate solution (30%) was added. The resulting solution became cloudy and was refluxed at 50°C for 1 hour until it was converted to di-Boc derivative. MeOH was then evaporated and water was added to the mixture. The aqueous layer was extracted using CH2Cl2, and the organic layer was evaporated to yield a white solid. The crude material was further purified using a silica gel column to obtain the final pure product.

3.1.13 Synthesis of 1-Ethynylcyclohexyl 4-nitrophenyl carbonate

The tris-Boc Adenine compound was diluted in ethyl acetate and then washed with 1N HCl and brine. Upon evaporation of the organic layer, a colorless oil was obtained. This oil was added to a solution of MeOH and a saturated bicarbonate solution (30%) was added. The resulting solution became cloudy and was refluxed at 50°C for 1 hour until it was converted to di-Boc. MeOH was then evaporated and water was added to the mixture. The aqueous layer was extracted using CH_2Cl_2, and the organic layer was evaporated to yield a white solid. The crude material was further purified using a silica gel column to obtain the final pure product.



(I) **(II)** **(III)**

3.1.14 Triflate protection

The purified product was obtained by adding a reagent (1 equivalent) to anhydrous CH_2Cl_2 and stirring it with pyridine and DMAP (3 equivalents) at rt for 15 mins. Trifluoromethanesulfonic acid (2 equivalents) was added to the reaction mixture at ice cold temperature and allowed to stir at -10 degrees Celsius for 8 hours until the reaction was complete. After completion of the reaction, the reaction mixture was concentrated in a vacuum, extracted with CH_2Cl_2, and purified using silica gel column chromatography with n-hexane/EtoAc.

3.1.15 Methylation

To an ice-cooled solution of reagent (1 eq.) in CH_2Cl_2, Sodium hydride (3 eq.) was added batch-wise followed by dropwise addition of Methyl Iodide (2 eq.). The reaction was allowed to stir at room temperature for 2 hours until the starting material was consumed. Upon completion, the reaction was quenched with addition of ice-cold water and extracted with ethyl acetate (2x) followed by a brine wash and dried over sodium sulfate to afford the crude product.

3.1.16 4-Methoxy Benzyl protection

In a DMF solution, a reagent (1 eq.) was combined with NaH (3 eq.) in an ice-cooled environment, followed by the addition of 4-Methoxy Benzyl Chloride (3 eq.) in batches. The reaction mixture was stirred at room temperature for three hours. Once the reaction was complete, it was quenched with ice-cold water and extracted with ethyl acetate. The organic layer was washed with a brine solution and dried using sodium sulphate.

3.1.17 Naphthalene protection

The reagent (1 eq.) was added to a DMF solution along with NaH (3 eq.) in an ice-cooled environment, followed by the addition of Bromomethyl naphthalene (3 equivalents) in batches. The reaction mixture was stirred at room temperature for 3 hours. Once the reaction was complete, it was quenched by adding ice-cold water and then extracted with ethyl acetate. The resulting organic layer was washed with a brine solution and dried with Na_2SO_4.

3.1.18 Fluorination

The sugar (1 eq.) was added to an anhydrous THF solution and stirred while Tetra Butyl ammonium fluoride (1.5 eq.) was added. The reaction mixture was then stirred at 0^0 Celsius for 18 hours until the reaction was complete. After the reaction was complete, the reaction mixture was concentrated under reduced pressure and purified using silica gel column chromatography with n-hexane/EtoAc as the solvent to obtain the desired product.

3.1.19 Naphthalene deprotection

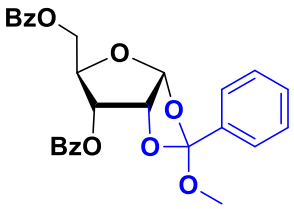
A solution of reaction mixture in CH_2Cl_2: MeOH (1:2) was treated with DDQ (2 eq.) at room temperature for an hour. After completion of reaction, the reaction mixture was concentrated in vacuo and diluted in CH_2Cl_2 and washed over sodium bicarbonate and brine solution. Thereafter, the organic residue was concentrated in vacuo and purified by silica gel chromatography.

3.1.20 Boc deprotection

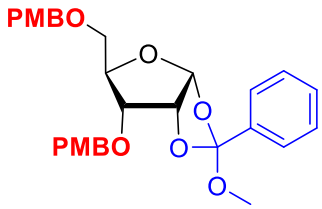
The reaction mixture was dissolved in CH_2Cl_2 and treated with Trifluoroacetic acid (25% in CH_2Cl_2) at room temperature for two hours. Once the reaction was complete, the reaction mixture was concentrated in a vacuum. The resulting residue was diluted in EtOAc, washed with $NaHCO_3$ solution and brine. The organic layer was dried using Na_2SO_4 and then concentrated to obtain the desired product.

4. Characterization Data

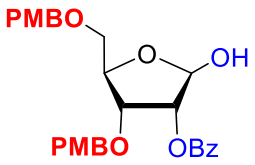
Compound 4 - ^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.00 (d, *J* = 0.6 Hz, 1H), 7.98 – 7.97 (m, 2H), 7.96 – 7.95 (m, 1H), 7.64 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.46 – 7.42 (m, 2H), 7.39 – 7.34 (m, 5H), 6.21 (d, *J* = 4.1 Hz, 1H), 5.30 – 5.26 (m, 1H), 5.03 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.59 (dd, *J* = 12.3, 3.3 Hz, 1H), 4.39 – 4.33 (m, 1H), 4.16 – 4.10 (m, 1H), 3.24 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*): δ 166.14, 165.70, 137.21, 133.72, 133.31, 130.04, 129.81, 129.59, 129.42, 128.96, 128.59, 128.46, 128.26, 126.21, 124.40, 104.59, 76.25, 73.19, 62.63, 50.29. HRMS (ESI/MS) : m/z Calculated : 476.15 Found : 476.19



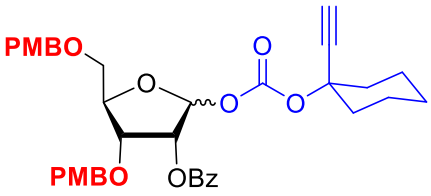
Compound 6 - ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.69 (s, 2H), 7.35 – 7.32 (m, 3H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.87 (s, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 6.04 (d, *J* = 4.1 Hz, 1H), 4.79 (t, *J* = 4.3 Hz, 1H), 4.67 (d, *J* = 11.3 Hz, 1H), 4.48 (d, *J* = 7.4 Hz, 1H), 4.45 (d, *J* = 7.9 Hz, 1H), 4.35 (d, *J* = 11.8 Hz, 1H), 3.85 (dd, *J* = 9.2, 4.6 Hz, 1H), 3.79 (dt, *J* = 4.6, 1.3 Hz, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.62 (d, *J* = 11.4 Hz, 1H), 3.44 (dd, *J* = 11.4, 3.7 Hz, 1H), 3.23 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.56, 159.53, 159.28, 136.95, 136.92, 130.06, 129.71, 129.44, 129.19, 128.18, 126.35, 124.01, 113.87, 113.78, 104.55, 78.42, 77.99, 77.08, 73.04, 71.86, 67.19, 55.30, 55.27, 50.44. HRMS (ESI/MS) : m/z Calculated : 508.21 Found : 508.24



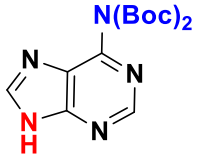
Compound 7: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.12 – 8.06 (m, 2H), 7.62 – 7.54 (m, 1H), 7.49 – 7.41 (m, 1H), 7.24 – 7.18 (m, 1H), 7.13 (dd, *J* = 8.5, 5.5 Hz, 1H), 6.89 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.77 (dd, *J* = 8.6, 3.9 Hz, 1H), 5.42 (d, *J* = 4.5 Hz, 1H), 4.59 – 4.50 (m, 1H), 4.54 – 4.47 (m, 1H), 4.41 (d, *J* = 6.0 Hz, 1H), 4.32 (d, *J* = 11.3 Hz, 1H), 4.29 – 4.23 (m, 1H), 3.80 (s, 3H), 3.77 (dt, 1H), 3.75 (s, 3H), 3.64 (d, *J* = 2.5 Hz, 1H), 3.47 (d, *J* = 4.2 Hz, 1H), 3.45 – 3.41 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.73, 159.50, 159.43, 133.45, 133.25, 129.91, 129.80, 129.54, 129.32, 128.53, 128.42, 113.96, 113.87, 113.84, 113.80, 100.54, 96.26, 81.55, 81.11, 77.05, 75.81, 73.24, 72.92, 72.74, 69.53, 68.62, 55.29, 55.26. HRMS (ESI/MS) : m/z Calculated : 494.19 Found : 494.31



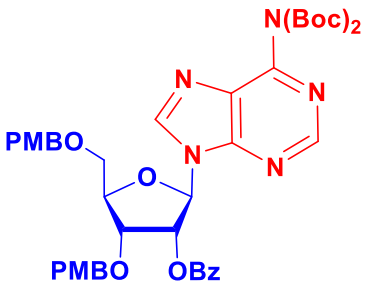
Compound 8: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.12 – 8.05 (m, 2H), 7.59 – 7.54 (m, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.25 – 7.19 (m, 2H), 7.17 – 7.11 (m, 2H), 6.90 – 6.87 (m, 2H), 6.76 – 6.73 (m, 1H), 6.43 (d, *J* = 4.6 Hz, 1H), 5.38 (dd, *J* = 6.6, 4.6 Hz, 1H), 4.58 (d, *J* = 11.8 Hz, 1H), 4.49 (d, *J* = 11.8 Hz, 1H), 4.42 (d, *J* = 2.3 Hz, 1H), 4.40 (d, *J* = 3.8 Hz, 1H), 4.39 (d, *J* = 2.1 Hz, 1H), 4.21 (dd, *J* = 6.7, 4.1 Hz, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.51 (dd, *J* = 10.8, 3.5 Hz, 1H), 3.38 (dd, *J* = 10.7, 3.7 Hz, 1H), 2.48 (s, 1H), 2.19 – 2.06 (m, 2H), 1.85 (t, *J* = 10.3 Hz, 2H), 1.66 – 1.51 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.61, 159.31, 159.26, 151.53, 133.45, 133.29, 130.12, 129.87, 129.85, 129.49, 129.46, 129.35, 128.37, 113.86, 113.68, 97.95, 84.11, 82.79, 77.84, 74.89, 73.17, 72.77, 71.95, 68.66, 55.30, 55.27, 55.23, 36.92, 36.78, 36.72, 36.55, 29.71, 24.98, 22.59, 22.49. HRMS (ESI/MS) : m/z Calculated : 644.26 Found : 644.25



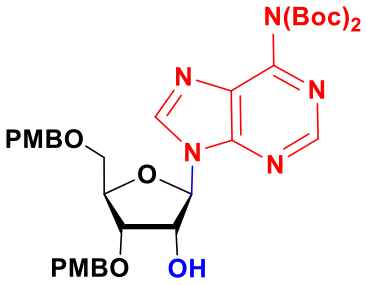
Compound 10: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.92 (s, 1H), 8.58 (s, 1H), 1.46 (s, 18H). HRMS (ESI/MS) : m/z Calculated : 335.16 Found : 335.16



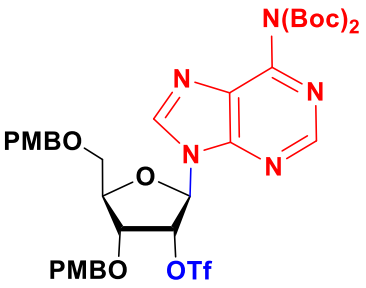
Compound 11: ^{1}H NMR (400 MHz, Chloroform-d) δ 8.84 (s, 1H), 8.51 (s, 1H), 8.08 – 8.05 (m, 2H), 7.62 – 7.57 (m, 1H), 7.46 (t, J = 7.8 Hz, 2H), 7.25 – 7.23 (m, 2H), 7.14 – 7.10 (m, 2H), 6.91 – 6.88 (m, 2H), 6.78 – 6.75 (m, 2H), 6.51 (d, J = 3.7 Hz, 1H), 5.91 (dd, J = 4.9, 3.7 Hz, 1H), 4.60 (dd, J = 5.9, 4.9 Hz, 1H), 4.54 (d, J = 11.9 Hz, 2H), 4.45 (d, J = 11.7 Hz, 1H), 4.39 (dt, J = 8.8, 2.7 Hz, 2H), 3.81 (s, 3H), 3.79 (d, J = 2.6 Hz, 1H), 3.76 (s, 3H), 3.56 (dd, J = 10.8, 3.2 Hz, 1H), 1.45 (s, 18 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.42, 159.50, 159.47, 152.81, 152.22, 150.49, 150.35, 143.39, 133.61, 129.97, 129.75, 129.55, 129.39, 129.25, 129.20, 129.05, 128.53, 114.00, 113.81, 87.10, 83.83, 82.50, 75.89, 75.32, 73.22, 72.93, 68.13, 55.28, 55.24, 27.82. HRMS (ESI/MS) : m/z Calculated : 811.34 Found : 811.82



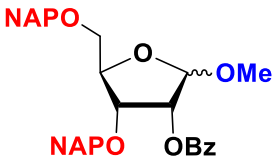
Compound 12: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.80 (s, 1H), 8.38 (s, 1H), 7.23 – 7.17 (m, 4H), 6.86 (ddd, *J* = 7.5, 2.5, 1.4 Hz, 4H), 6.13 (d, *J* = 4.5 Hz, 1H), 4.61 – 4.56 (m, 1H), 4.54 (d, 2H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.41 (d, *J* = 11.6 Hz, 1H), 4.28 (dt, *J* = 7.6, 3.9 Hz, 2H), 3.78 (s, 6H), 3.71 (dd, *J* = 10.6, 2.7 Hz, 1H), 3.51 (dd, *J* = 10.8, 2.7 Hz, 1H), 1.43 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.85, 159.55, 153.06, 152.12, 150.60, 150.35, 143.54, 129.95, 129.63, 129.45, 129.37, 128.92, 128.82, 114.16, 114.05, 89.43, 83.90, 81.96, 74.43, 73.33, 72.66, 68.87, 55.38, 55.35, 27.89. HRMS (ESI/MS) : m/z Calculated : 707.32 Found : 707.35



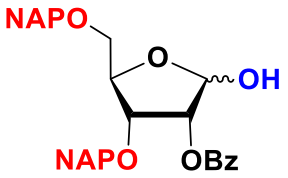
Compound 13: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.85 (s, 1H), 8.44 (s, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.90 (dd, *J* = 8.6, 1.7 Hz, 4H), 6.47 (d, *J* = 3.5 Hz, 1H), 5.77 (t, *J* = 4.0 Hz, 1H), 4.73 (d, *J* = 11.3 Hz, 1H), 4.53 (dd, *J* = 5.8, 4.6 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.45 (d, *J* = 11.3 Hz, 1H), 4.41 (d, *J* = 11.5 Hz, 1H), 4.38 – 4.34 (m, 1H), 3.83 (s, 6H), 3.79 (d, *J* = 2.4 Hz, 1H), 3.51 (dd, *J* = 11.0, 2.6 Hz, 1H), 1.47 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.81, 159.60, 152.52, 152.42, 150.57, 150.37, 142.81, 130.04, 129.98, 129.56, 129.14, 128.97, 128.38, 119.98, 114.04, 114.02, 85.97, 85.45, 83.92, 81.90, 74.97, 73.23, 73.16, 67.30, 55.30, 55.28, 27.77. HRMS (ESI/MS) : m/z Calculated : 839.27 Found : 839.53



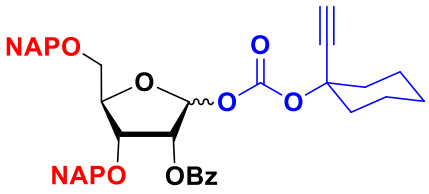
Compound 15: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 7.1 Hz, 2H), 7.83 (dd, *J* = 6.1, 3.5 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.74 (d, *J* = 6.7 Hz, 2H), 7.61 (d, *J* = 2.2 Hz, 2H), 7.61 – 7.54 (m, 2H), 7.51 – 7.42 (m, 2H), 7.43 (d, *J* = 6.5 Hz, 2H), 7.45 – 7.39 (m, 2H), 7.42 – 7.31 (m, 2H), 5.50 (d, *J* = 4.4 Hz, 1H), 5.06 (s, 1H), 4.78 (d, *J* = 11.8 Hz, 2H), 4.71 (d, *J* = 5.1 Hz, 2H), 4.59 (d, *J* = 11.8 Hz, 1H), 4.41 (td, *J* = 8.2, 7.2, 3.4 Hz, 1H), 4.29 (dd, *J* = 7.7, 4.4 Hz, 1H), 3.70 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.56 (dd, *J* = 10.5, 6.1 Hz, 1H), 3.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.73, 135.68, 134.90, 133.33, 133.28, 133.12, 133.00, 129.95, 129.69, 128.47, 128.14, 128.10, 127.90, 127.87, 127.72, 127.65, 127.60, 126.88, 126.35, 126.09, 126.01, 125.90, 125.85, 125.77, 125.68, 106.41, 80.62, 77.90, 77.04, 74.37, 73.36, 73.03, 71.27, 55.13. HRMS (ESI/MS) : m/z Calculated : 548.22 Found : 548.23



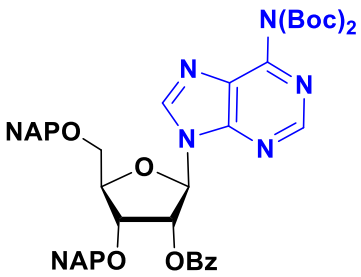
Compound 17: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.10 (dt, *J* = 8.4, 1.4 Hz, 2H), 7.83 – 7.78 (m, 2H), 7.78 – 7.74 (m, 3H), 7.73 (d, *J* = 3.7 Hz, 1H), 7.69 – 7.66 (m, 1H), 7.65 – 7.62 (m, 1H), 7.57 – 7.55 (m, 1H), 7.48 (d, *J* = 3.2 Hz, 1H), 7.46 (d, *J* = 1.8 Hz, 2H), 7.45 – 7.43 (m, 2H), 7.42 (d, *J* = 2.0 Hz, 1H), 7.41 – 7.39 (m, 1H), 7.39 (d, *J* = 1.4 Hz, 1H), 5.50 (d, *J* = 4.5 Hz, 1H), 4.76 (d, *J* = 1.8 Hz, 1H), 4.75 – 4.62 (m, 2H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.53 – 4.48 (m, 2H), 4.43 – 4.30 (m, 2H), 3.72 (ddd, *J* = 9.9, 7.8, 3.5 Hz, 1H), 3.48 (dd, *J* = 10.4, 3.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.77, 135.21, 134.87, 134.47, 133.52, 133.31, 133.23, 133.13, 133.07, 129.92, 128.58, 128.48, 128.33, 128.16, 127.95, 127.92, 127.75, 127.66, 127.16, 126.90, 126.78, 126.53, 126.24, 126.17, 126.13, 126.07, 125.94, 125.65, 125.58, 100.57, 96.34, 81.50, 81.07, 75.77, 73.68, 73.16, 69.87, 69.03. HRMS (ESI/MS) : m/z Calculated : 534.20 Found : 534.22



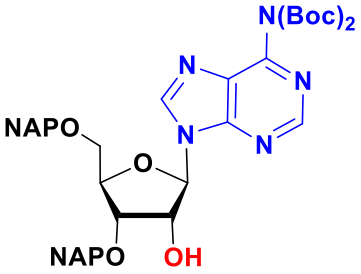
Compound 18: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.10 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.88 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.77 (td, *J* = 5.4, 4.7, 2.9 Hz, 2H), 7.75 – 7.72 (m, 2H), 7.67 – 7.65 (m, 1H), 7.64 – 7.61 (m, 2H), 7.60 – 7.57 (m, 1H), 7.48 – 7.46 (m, 2H), 7.45 (d, *J* = 3.4 Hz, 2H), 7.44 – 7.43 (m, 1H), 7.41 (dd, *J* = 2.3, 1.2 Hz, 1H), 7.40 (q, *J* = 1.3 Hz, 1H), 7.27 (dt, *J* = 7.9, 1.2 Hz, 2H), 6.27 (d, *J* = 20.5 Hz, 1H), 5.71 – 5.59 (m, 1H), 4.99 (d, *J* = 12.3 Hz, 1H), 4.81 (t, *J* = 5.9 Hz, 1H), 4.71 – 4.63 (m, 2H), 4.60 – 4.47 (m, 2H), 3.73 – 3.61 (m, 2H), 2.62 (d, *J* = 16.1 Hz, 1H), 2.15 (q, *J* = 18.0, 16.9 Hz, 2H), 2.03 – 1.74 (m, 2H), 1.68 – 1.50 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.43, 151.16, 135.73, 135.33, 134.94, 134.79, 133.57, 133.26, 133.10, 130.08, 129.83, 129.42, 129.04, 128.62, 128.50, 128.34, 128.23, 128.07, 127.98, 127.73, 127.02, 126.61, 126.36, 126.18, 126.05, 125.95, 125.83, 125.73, 102.90, 84.51, 82.13, 81.26, 78.51, 78.45, 75.41, 73.64, 73.33, 72.45, 68.97, 36.99, 36.79, 36.54, 25.06, 24.93, 22.72, 22.64. HRMS (ESI/MS) : m/z Calculated : 684.27 Found : 684.29



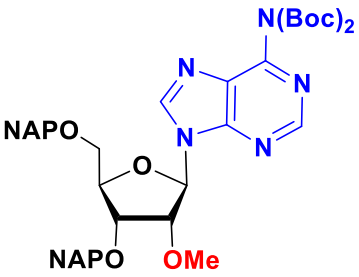
Compound 19: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.76 (d, *J* = 24.4 Hz, 1H), 8.46 (d, *J* = 7.8 Hz, 1H), 7.77 – 7.56 (m, 10H), 7.46 – 7.19 (m, 9H), 6.49 – 6.46 (m, 1H), 6.02 – 5.93 (m, 1H), 4.86 – 4.75 (m, 1H), 4.70 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.66 – 4.60 (m, 1H), 4.59 (d, *J* = 2.3 Hz, 1H), 4.52 (dd, *J* = 11.9, 4.7 Hz, 1H), 4.41 – 4.30 (m, 1H), 3.72 – 3.49 (m, 2H), 1.35 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.49, 153.03, 152.85, 152.33, 152.27, 150.50, 150.37, 143.54, 143.42, 134.96, 134.71, 134.50, 134.05, 133.77, 133.69, 133.25, 133.18, 133.17, 133.15, 133.14, 133.11, 133.06, 130.01, 129.74, 129.31, 129.03, 128.97, 128.59, 128.53, 128.49, 128.33, 128.23, 127.99, 127.96, 127.90, 127.88, 127.77, 127.74, 127.66, 127.19, 127.05, 126.84, 126.68, 126.30, 126.22, 126.13, 126.06, 125.76, 125.63, 125.58, 88.37, 87.19, 85.25, 83.82, 80.85, 76.29, 75.31, 73.70, 72.64, 69.18, 27.81. HRMS (ESI/MS) : m/z Calculated : 851.35 Found : 851.36



Compound 20: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.79 (s, 1H), 8.43 (s, 1H), 7.82 – 7.80 (m, 2H), 7.79 – 7.76 (m, 4H), 7.72 – 7.69 (m, 2H), 7.48 (dd, *J* = 3.0, 0.9 Hz, 2H), 7.47 – 7.46 (m, 2H), 7.40 – 7.33 (m, 2H), 6.18 (d, *J* = 4.4 Hz, 1H), 4.79 – 4.76 (m, 2H), 4.70 (d, *J* = 4.7 Hz, 2H), 4.66 – 4.61 (m, 1H), 4.58 (d, *J* = 12.1 Hz, 1H), 4.43 – 4.37 (m, 1H), 3.79 – 3.76 (m, 1H), 3.57 (dd, *J* = 10.7, 3.2 Hz, 1H), 1.43 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.01, 152.15, 152.08, 150.61, 150.53, 150.45, 143.54, 134.77, 134.22, 134.13, 134.04, 133.29, 133.21, 129.36, 128.74, 128.66, 128.61, 128.02, 127.83, 127.31, 126.99, 126.56, 126.50, 126.42, 126.32, 126.25, 125.77, 125.73, 125.65, 125.60, 89.61, 86.02, 84.55, 83.89, 81.97, 79.31, 74.38, 74.17, 73.82, 73.15, 72.55, 70.00, 69.10, 27.90, 27.87. HRMS (ESI/MS) : m/z Calculated : 747.33 Found : 747.35



Compound 21: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.76 (d, *J* = 29.4 Hz, 1H), 8.39 (s, 1H), 7.84 – 7.66 (m, 8H), 7.50 (td, *J* = 5.7, 5.2, 3.1 Hz, 4H), 7.43 – 7.32 (m, 2H), 6.42 (d, *J* = 19.0 Hz, 1H), 5.82 – 5.71 (m, 1H), 4.96 – 4.74 (m, 2H), 4.73 – 4.68 (m, 2H), 4.68 – 4.53 (m, 1H), 4.39 (ddd, *J* = 17.2, 5.4, 2.8 Hz, 1H), 3.86 – 3.59 (m, 2H), 3.53 (d, *J* = 3.8 Hz, 3H), 1.51 – 1.43 (m, 18H).



5. Conclusions

The synthetic pathway to efficiently produce modified nucleosides from cheap starting material in a sustainable way with reproducible and scalable steps has been a challenge for several years. The therapeutic benefits of some of the drugs from this family has already been explored and hence the ever increasing need to synthesise this assembly of drugs. Our technique invokes simple scalable and sustainable principles and uses a flexible donor in form of 1-ethynyl cyclohexyl carbonate which undergoes N-glycosylation conveniently in presence of Au/Ag catalysis. The main challenges faced are in the steps concerning orthoester where the moiety is susceptible to acids and high temperature. The orthoester formation is important for the presence of 2' Benzoate moiety which allows post-glycosylation modification. The Boc-group also being susceptible to acids should be treated in presence of basic conditions to ensure no side-products are formed. Although the final compound could not be separated due to quantitative difficulties further optimization is necessary for greater yield. Nevertheless, the synthetic pathway described can be utilized to build a library of nucleoside analogues with variety of modifications and different combinations of nucleobases and sugars which can serve as building blocks for nucleoside drug synthesis.

6. NMR Spectral Charts

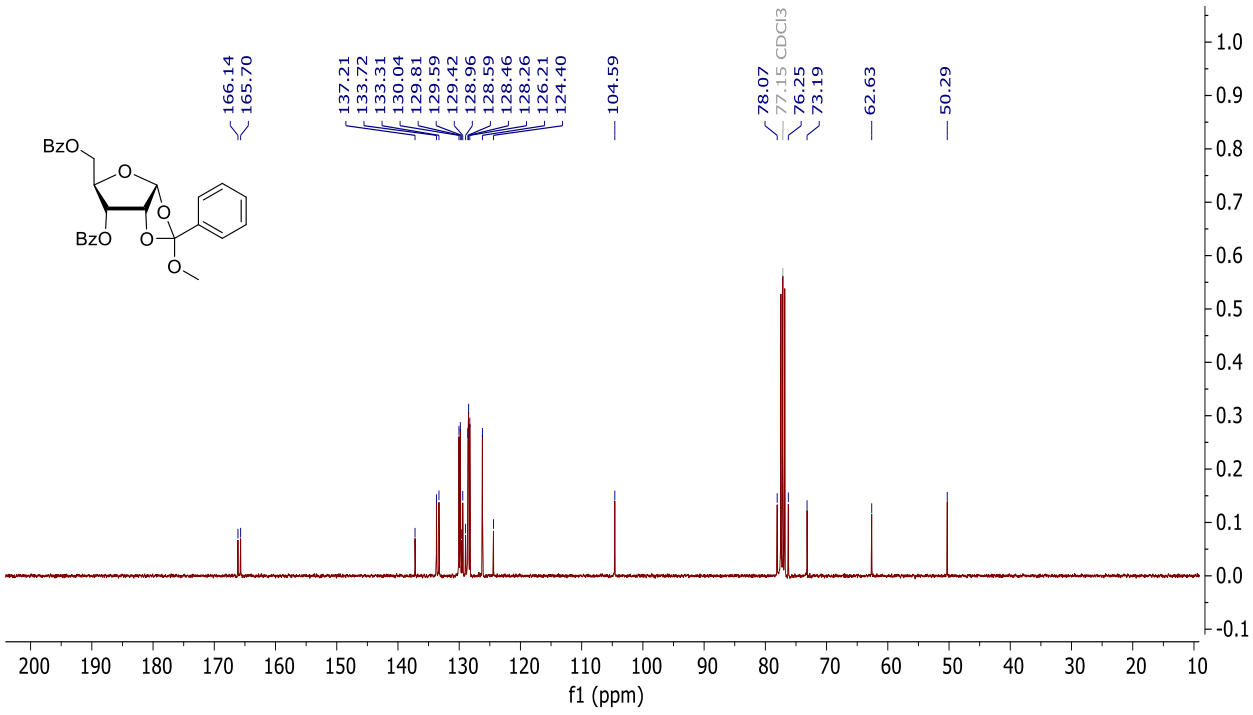


Figure 6.1: ^{13}C NMR Spectrum of **compound 4**

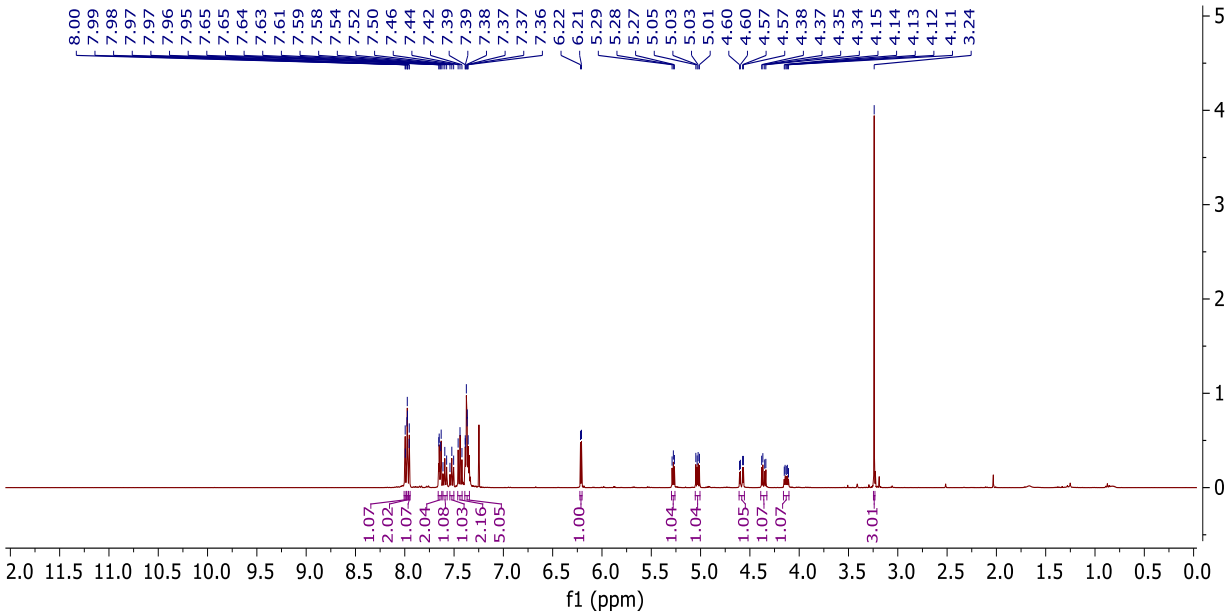


Figure 6.2: ^{1}H NMR Spectrum of **compound 4**

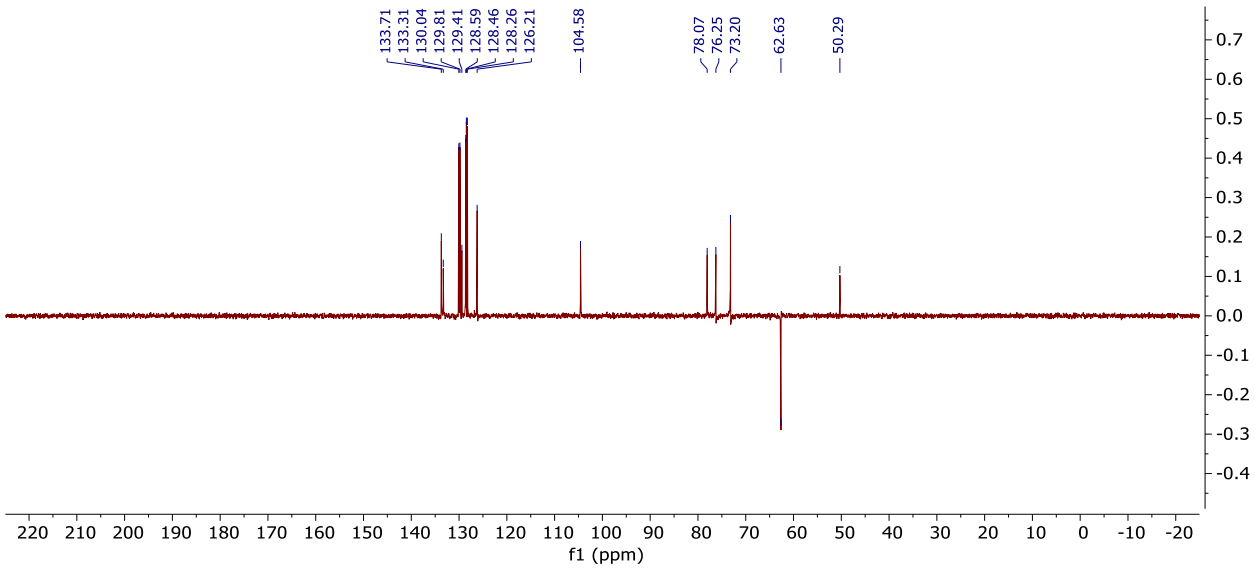


Figure 6.3: DEPT NMR Spectrum of **compound 4**

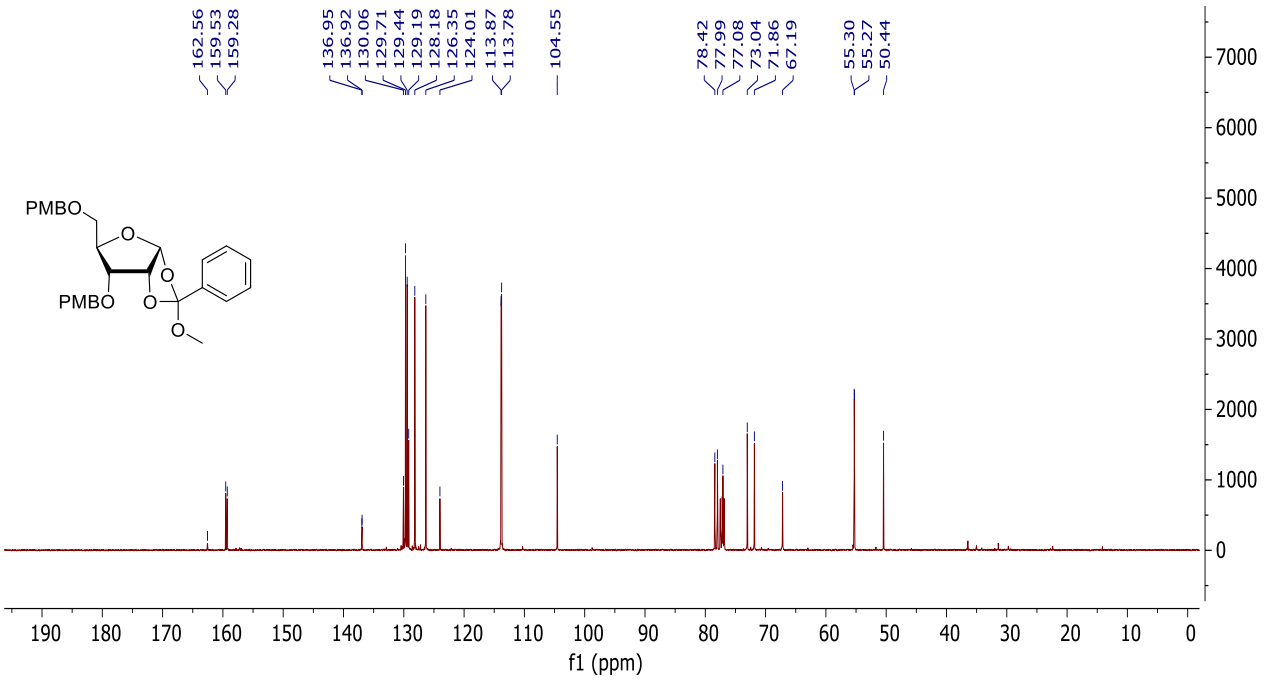


Figure 6.4: ^{13}C NMR Spectrum of **compound 6**

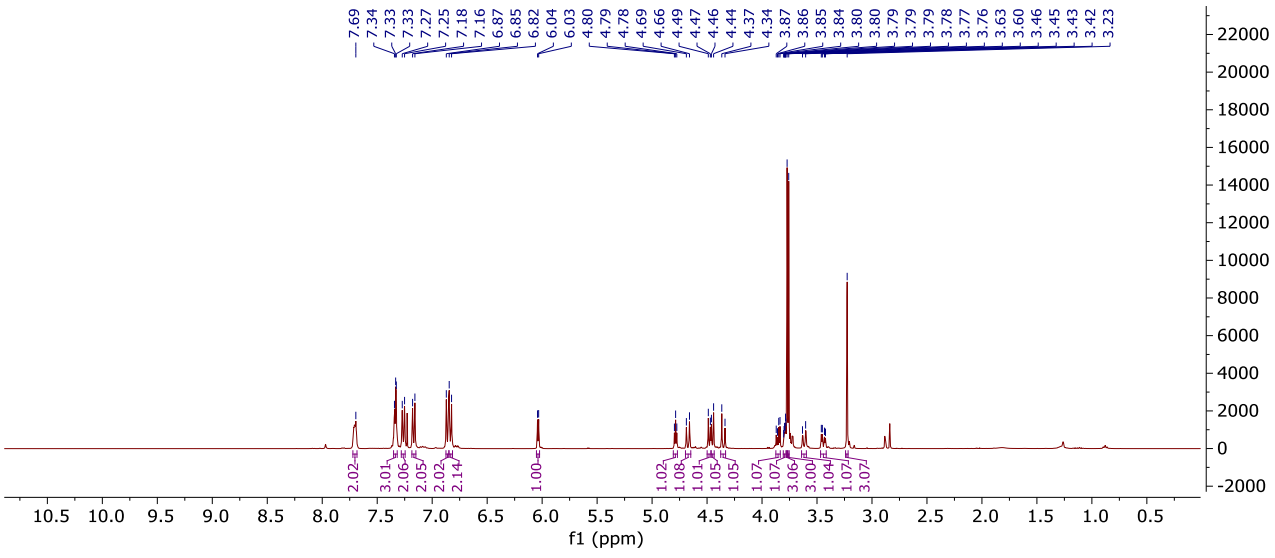


Figure 6.5: ^{1}H NMR Spectrum of **compound 6**

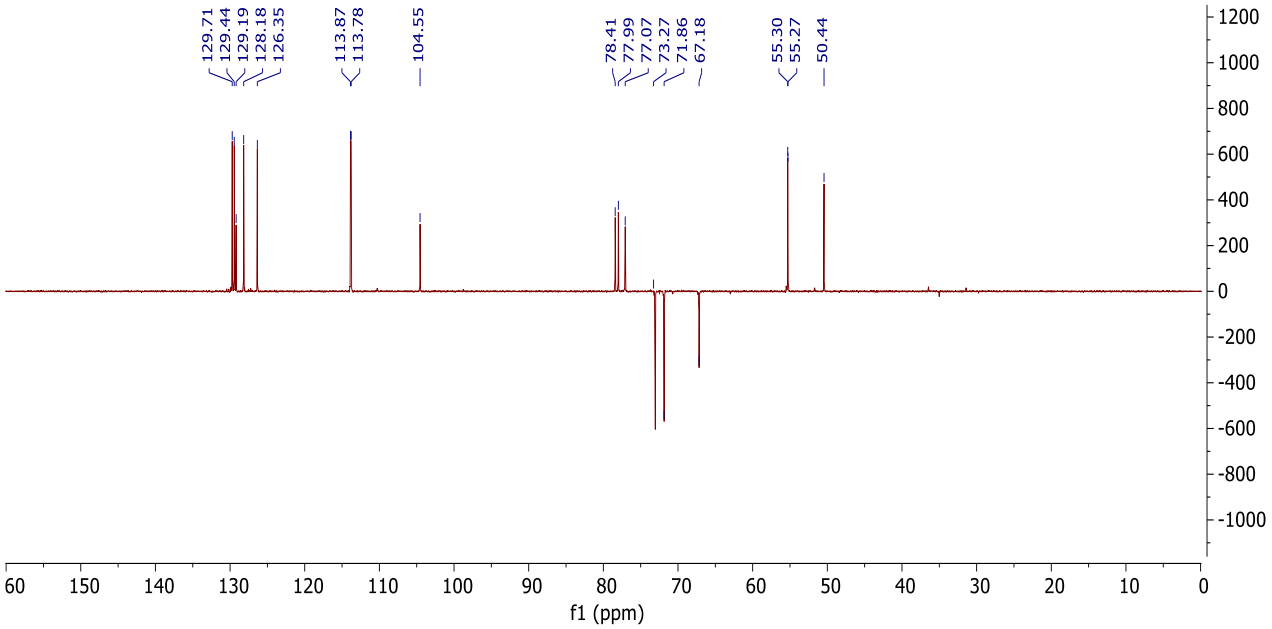


Figure 6.6: DEPT NMR Spectrum of **compound 6**

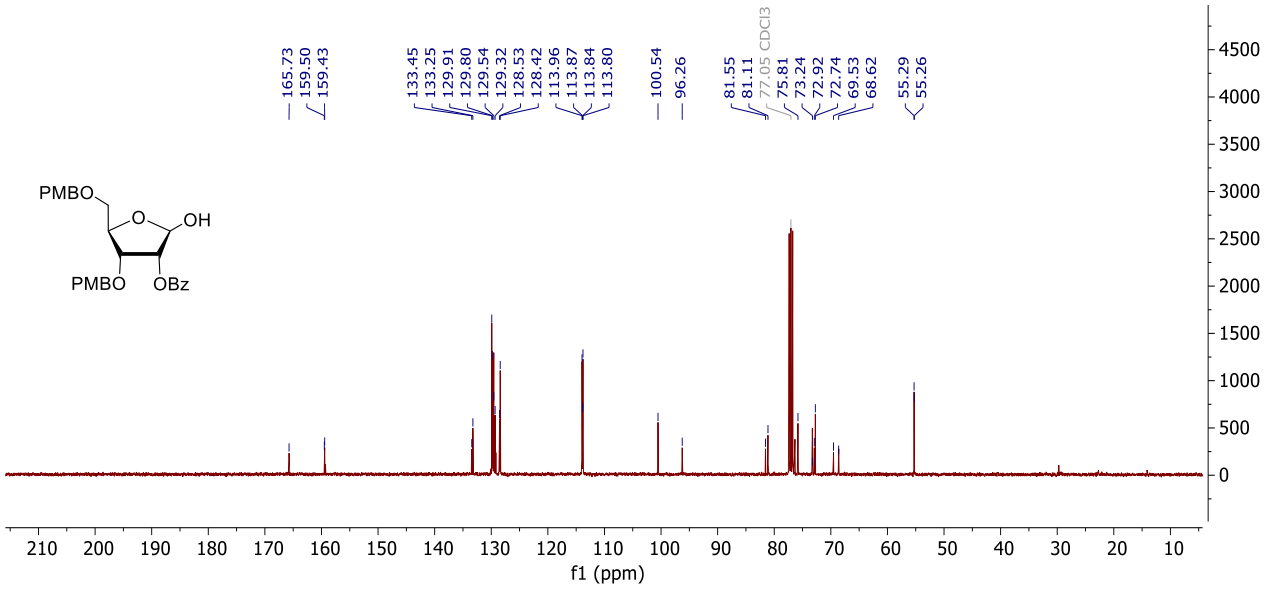


Figure 6.7: ^{13}C NMR Spectrum of **compound 7**

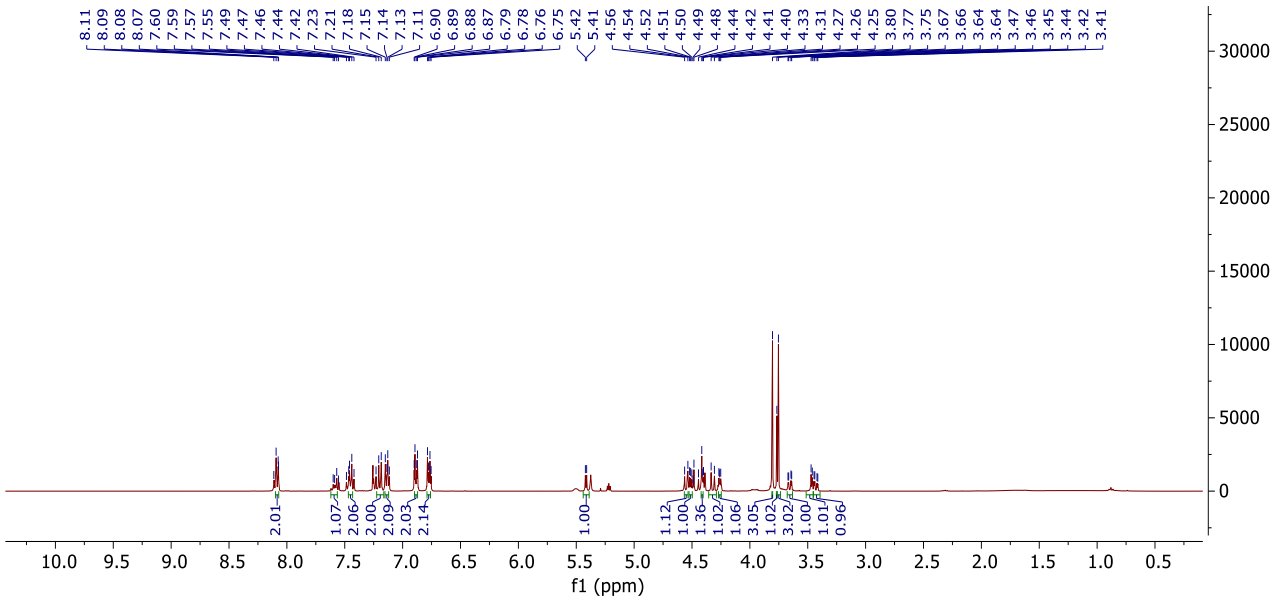


Figure 6.8: ^{1}H NMR Spectrum of **compound 7**

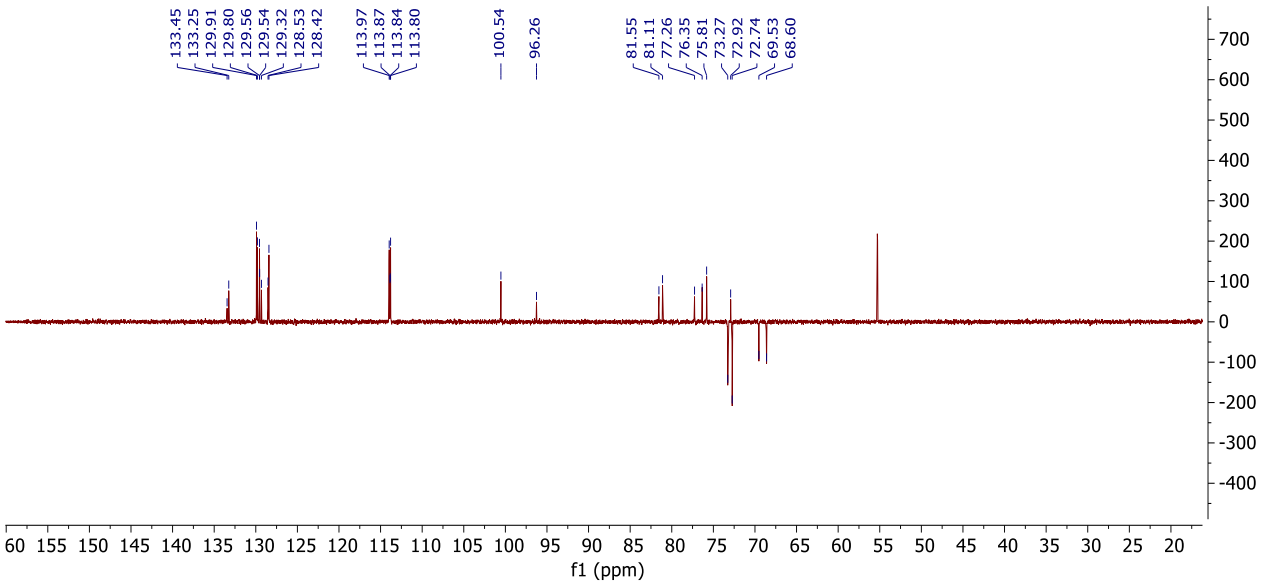


Figure 6.9: DEPT Spectrum of **compound 7**

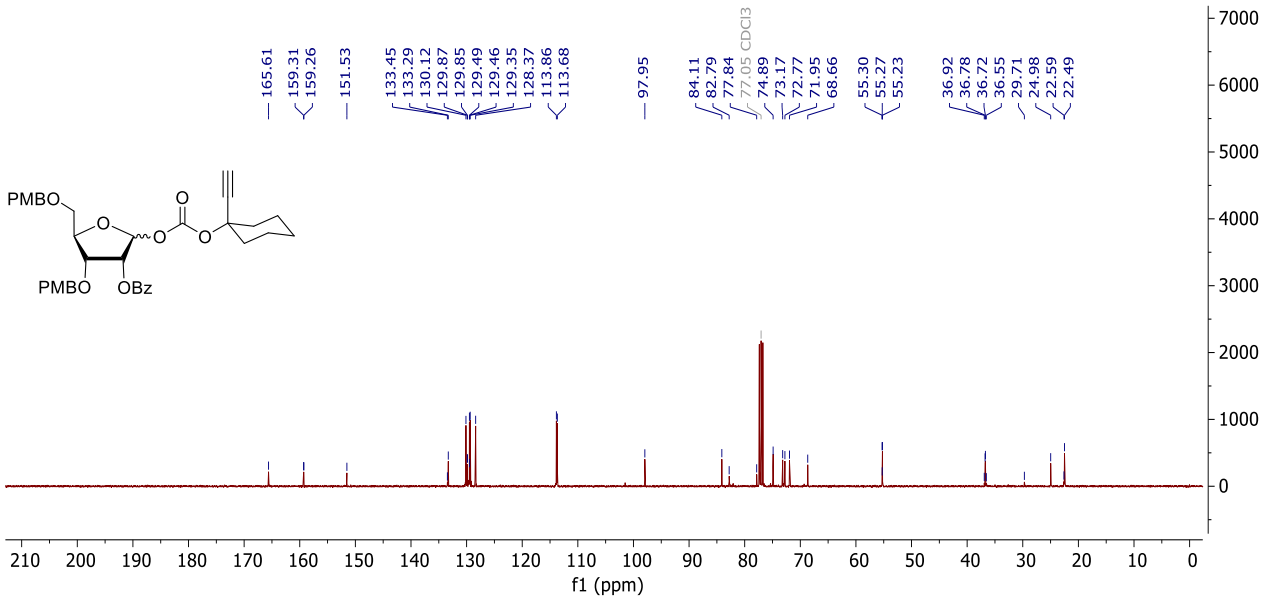


Figure 6.10: ^{13}C NMR Spectrum of **compound 8**

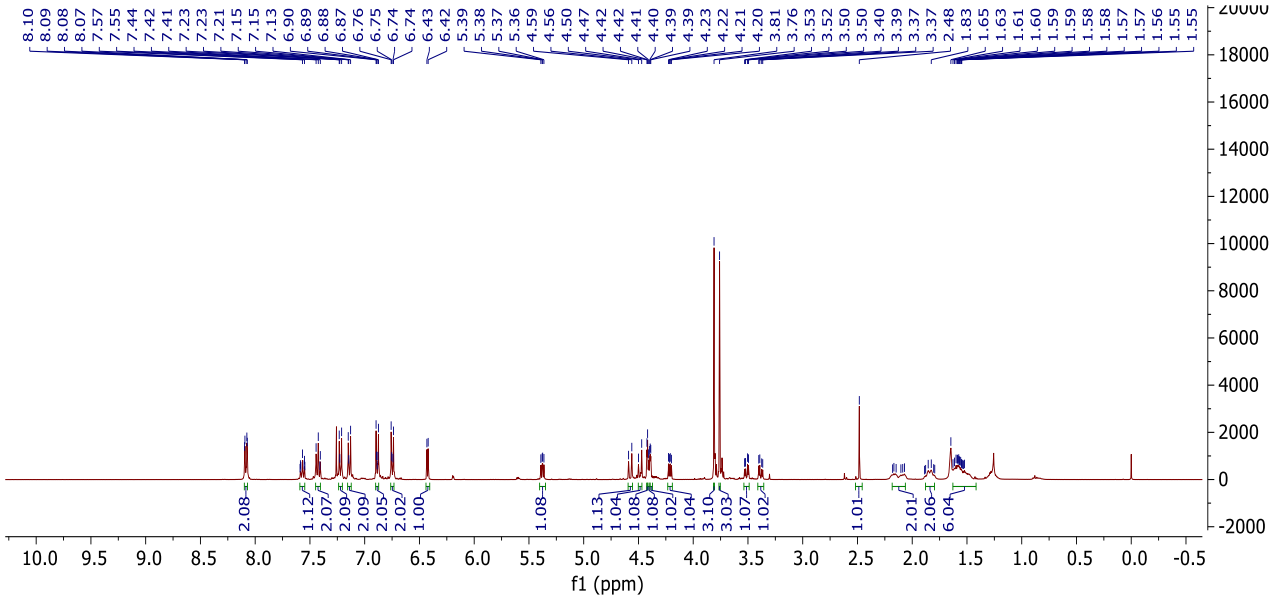


Figure 6.11: ^{1}H NMR Spectrum of **compound 8**

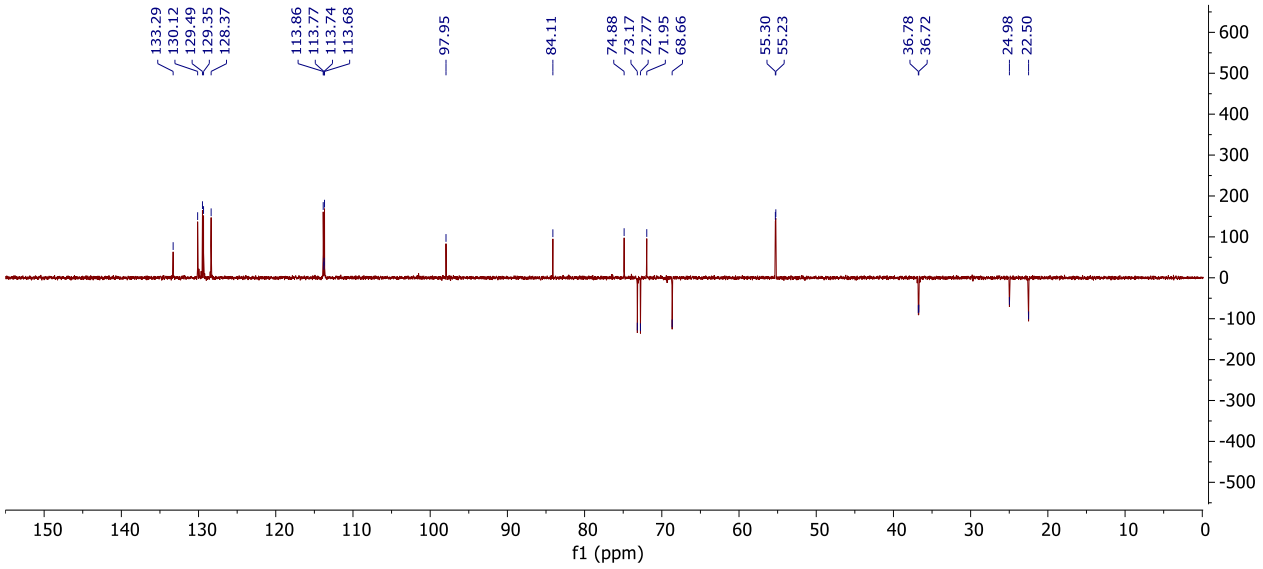


Figure 6.12: DEPT Spectrum of **compound 8**

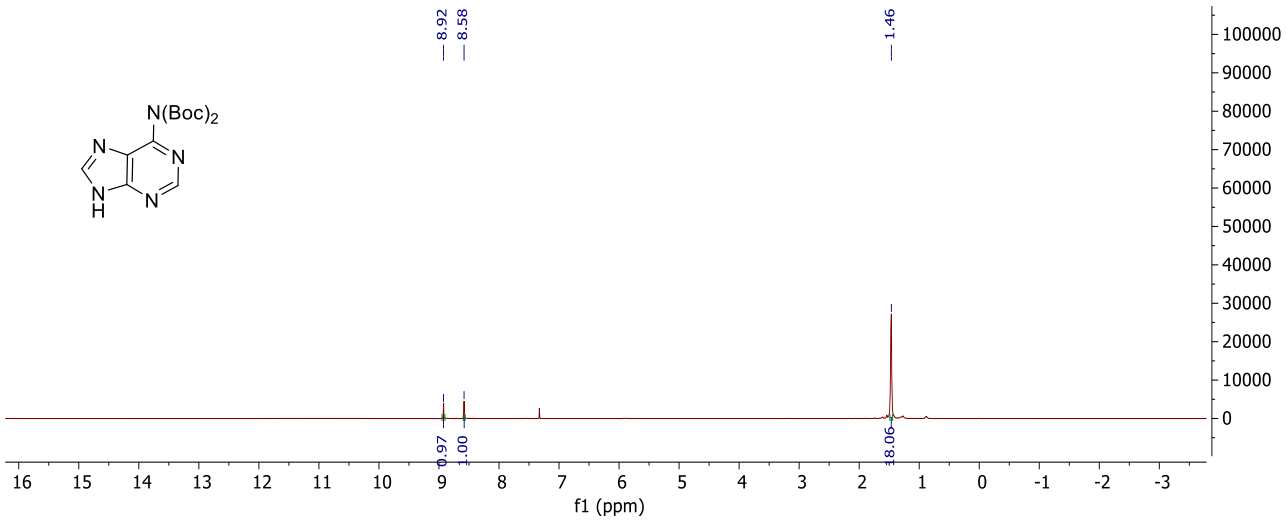


Figure 6.13: ^{1}H NMR Spectrum of **compound 10**

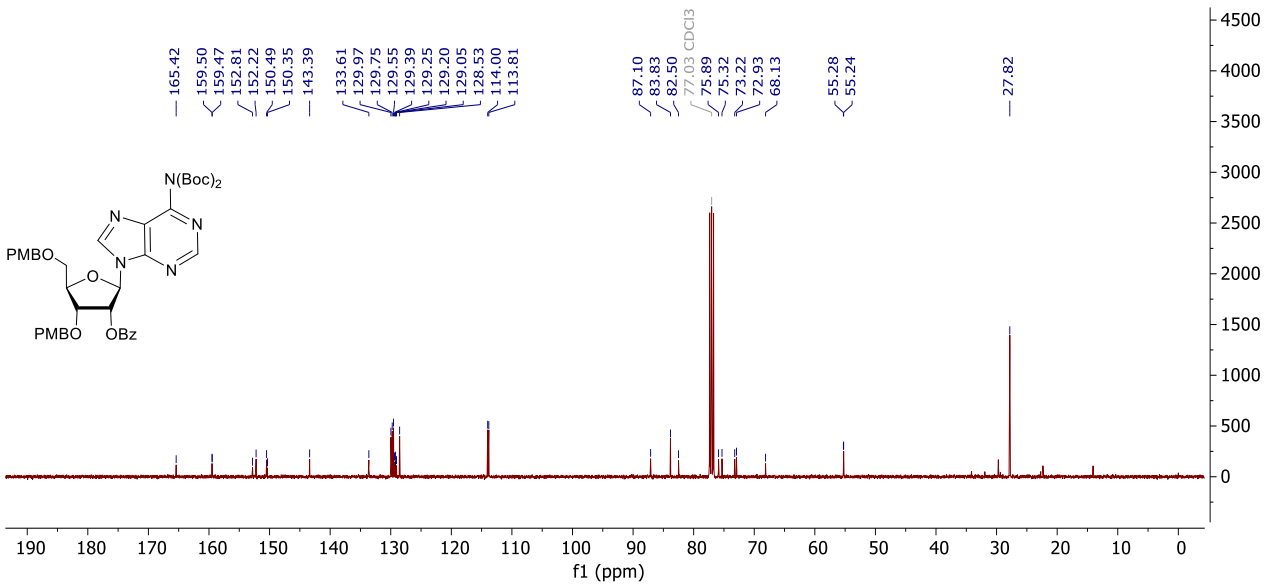


Figure 6.14: ^{13}C NMR Spectrum of **compound 11**

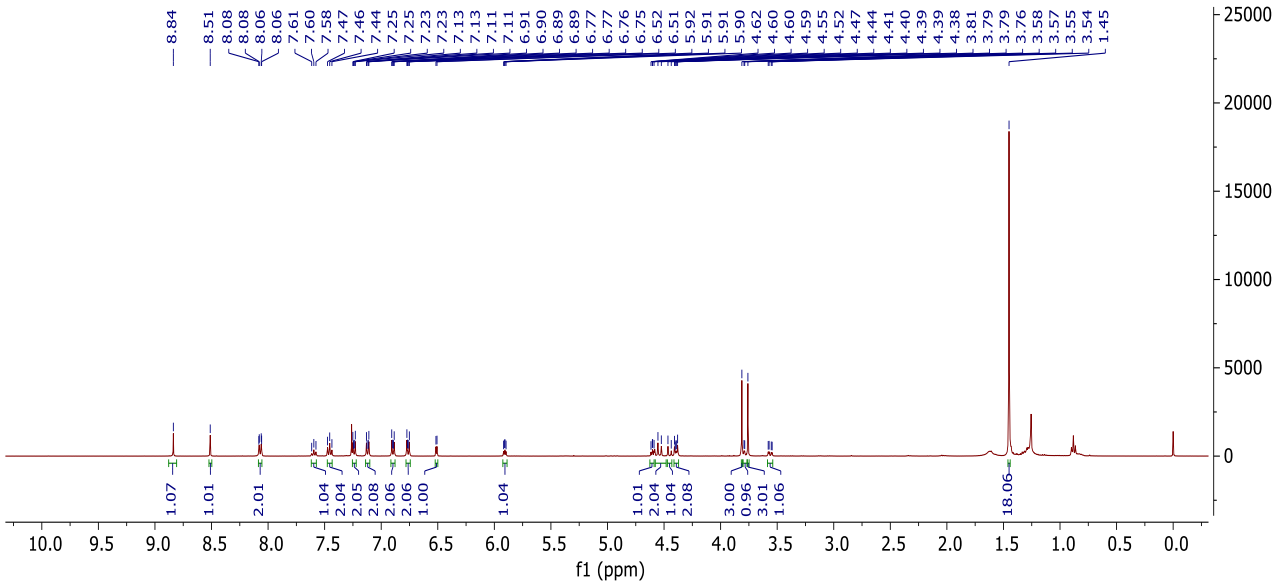


Figure 6.15: ^{1}H NMR Spectrum of **compound 11**

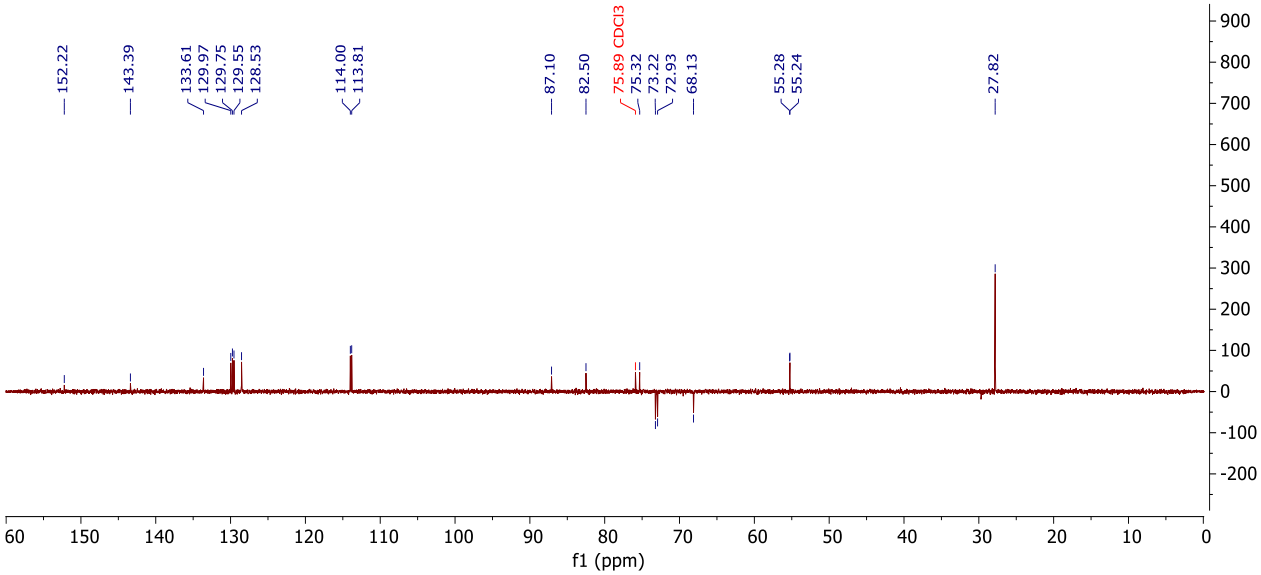


Figure 6.16: DEPT Spectrum of **compound 11**

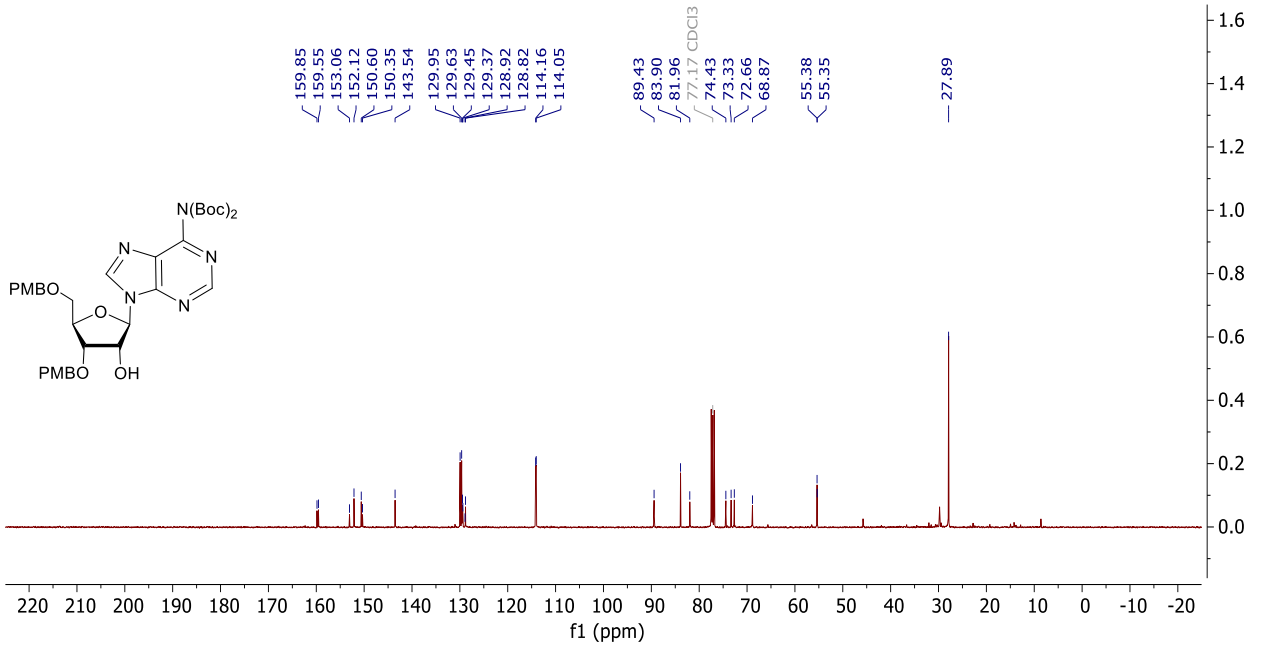


Figure 6.17: ^{13}C Spectrum of **compound 12**

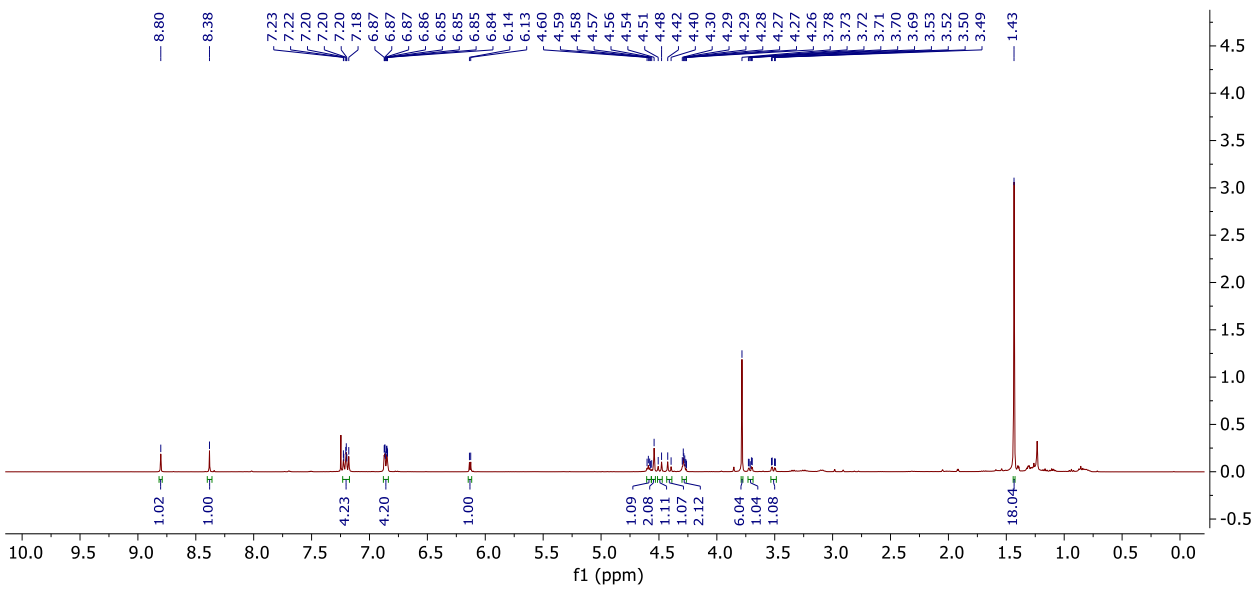


Figure 6.18: ^{1}H NMR Spectrum of **compound 12**

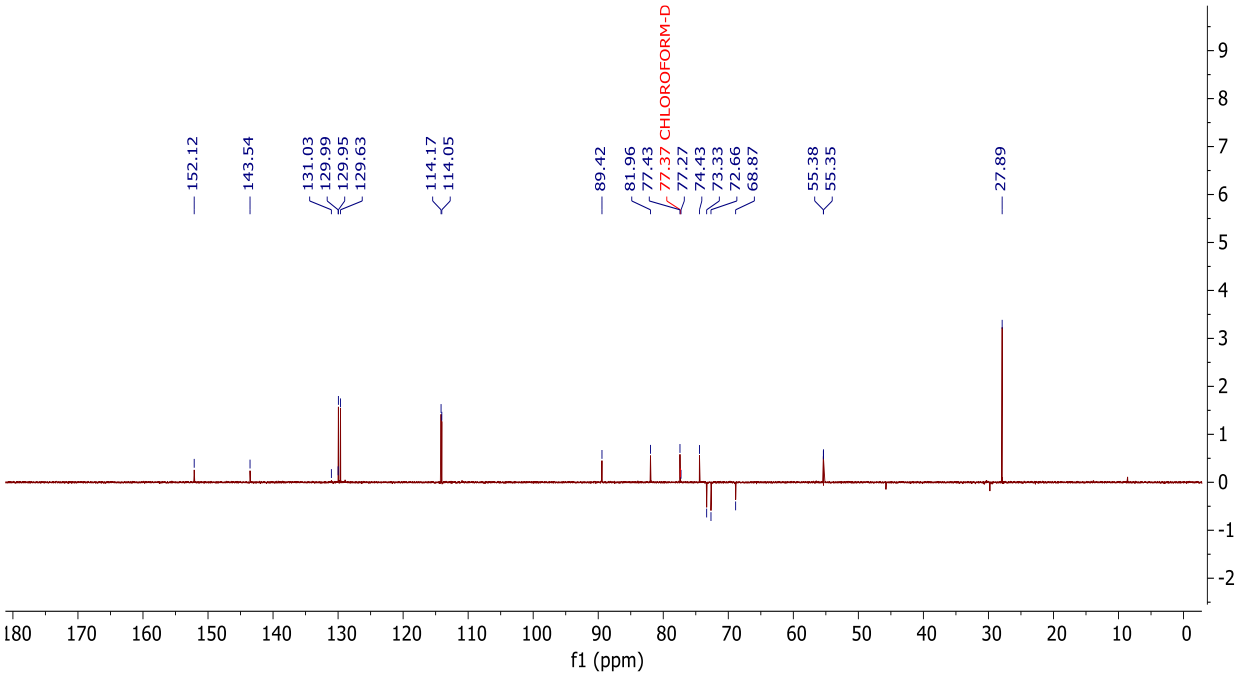


Figure 6.19: DEPT Spectrum of **compound 12**

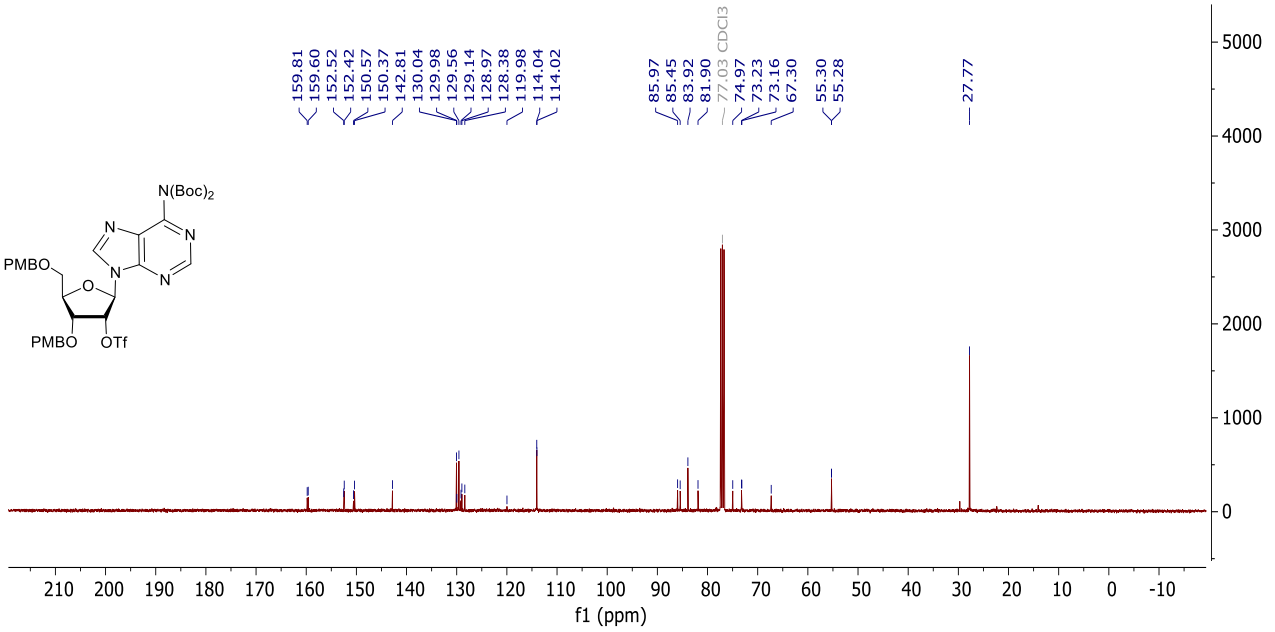


Figure 6.20: ^{13}C Spectrum of **compound 13**

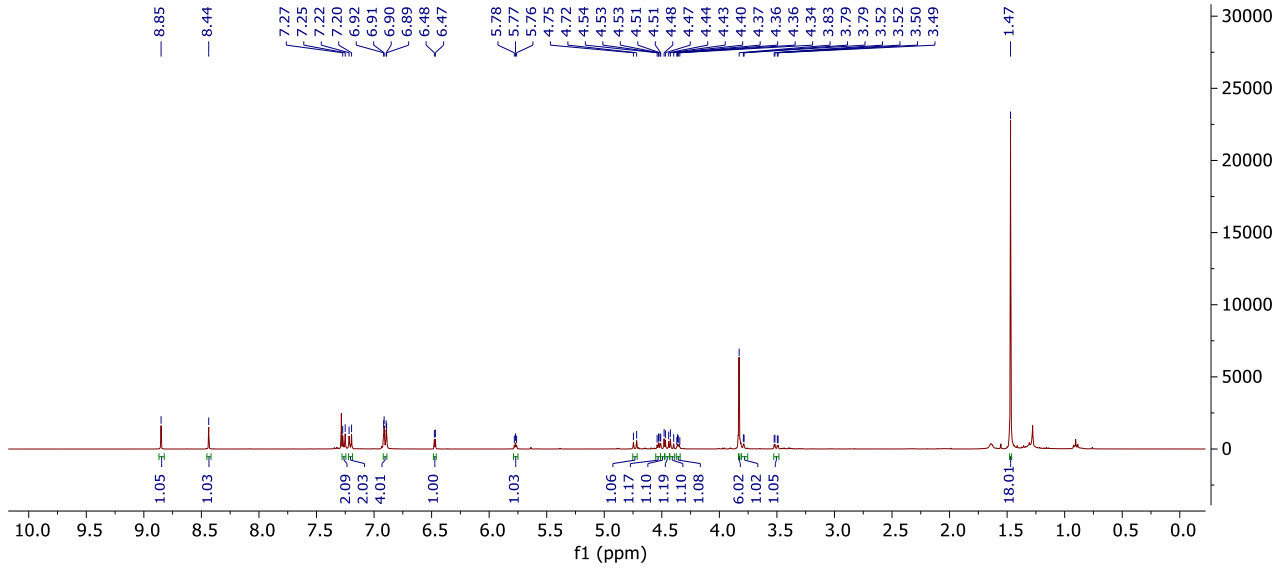


Figure 6.21: ^{1}H NMR Spectrum of **compound 13**

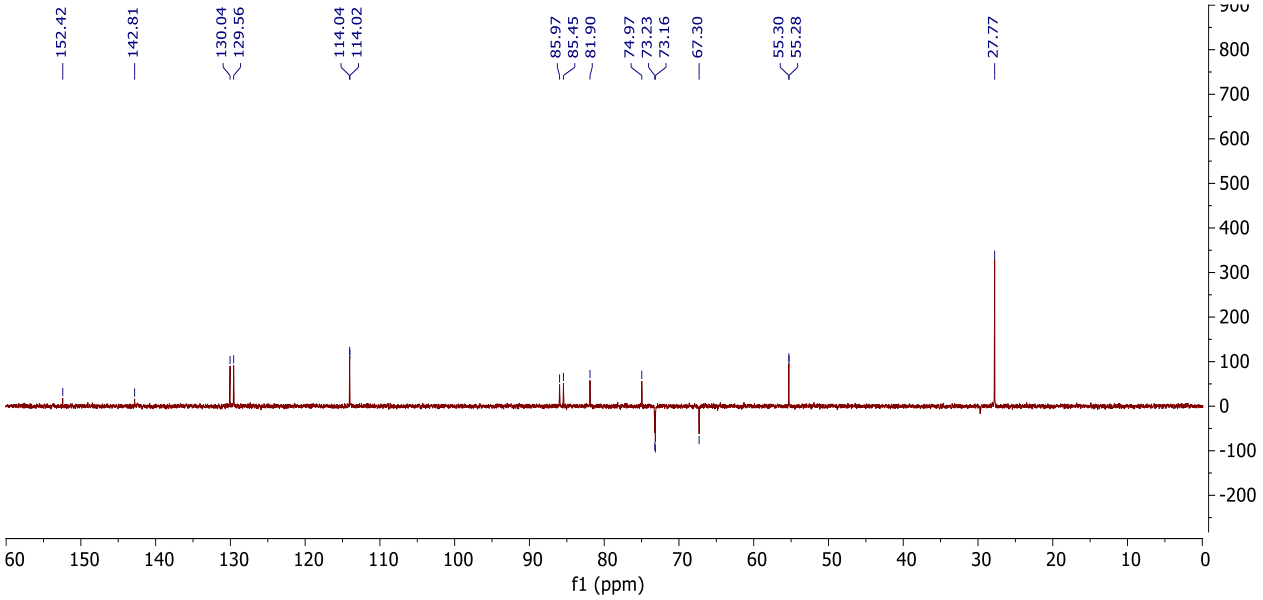


Figure 6.22: DEPT Spectrum of **compound 13**

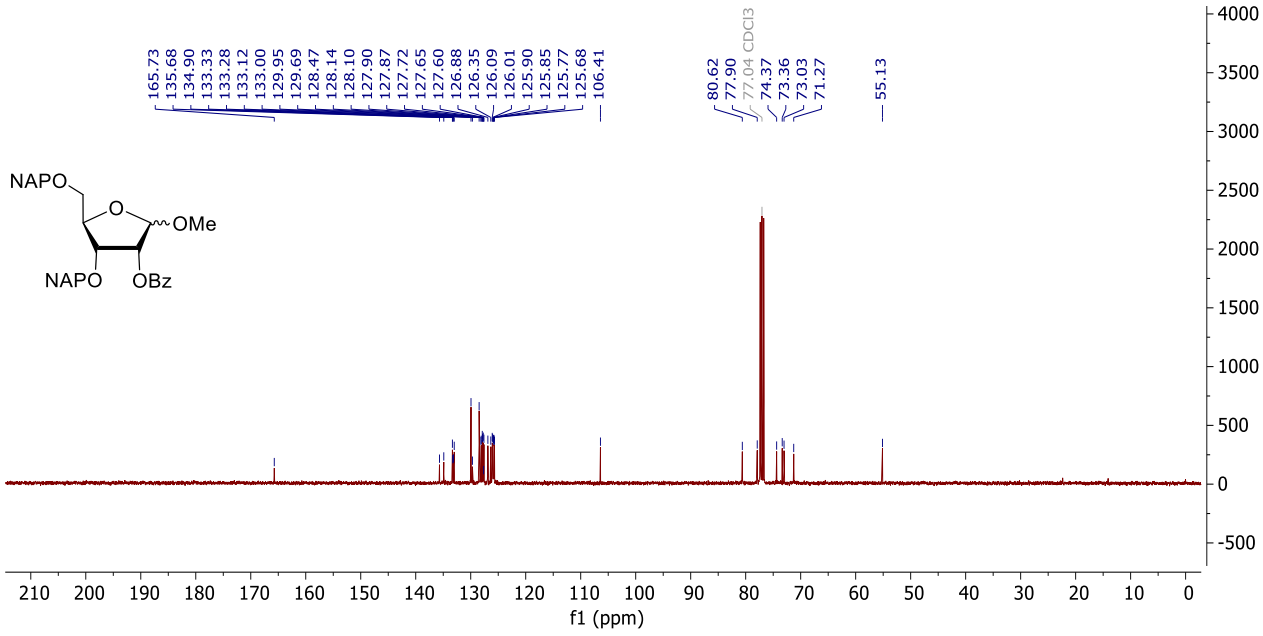


Figure 6.23: ^{13}C Spectrum of **compound 15**

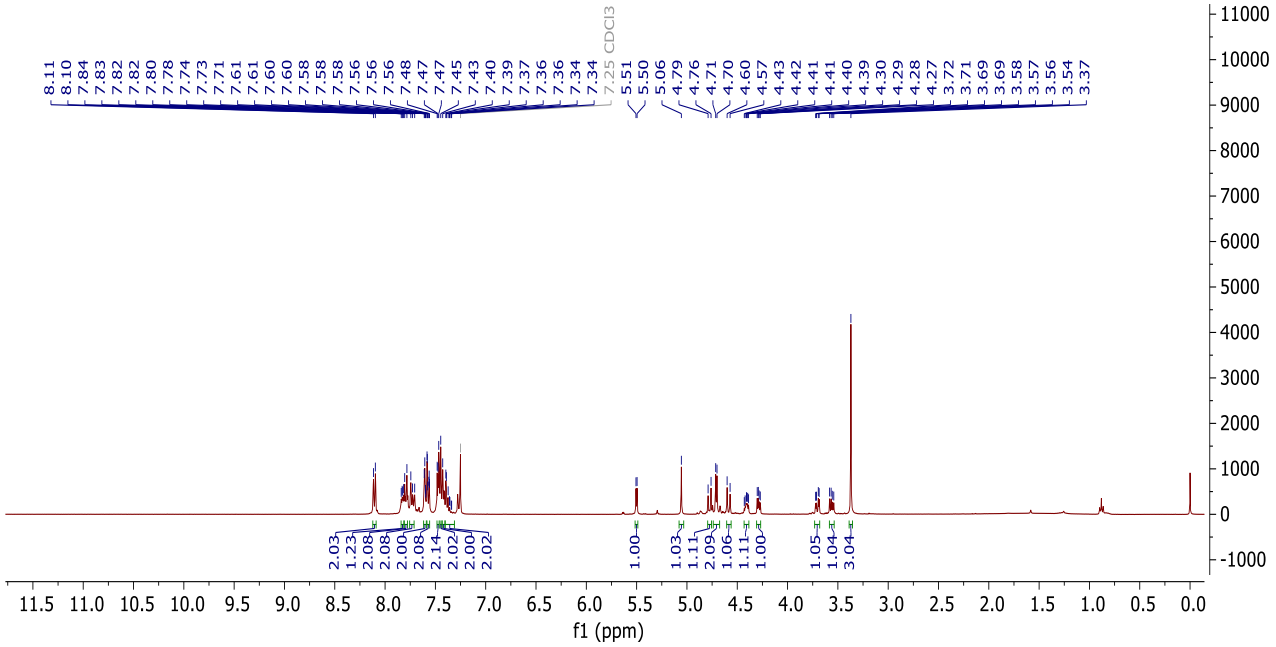


Figure 6.24: ^{1}H NMR Spectrum of **compound 15**

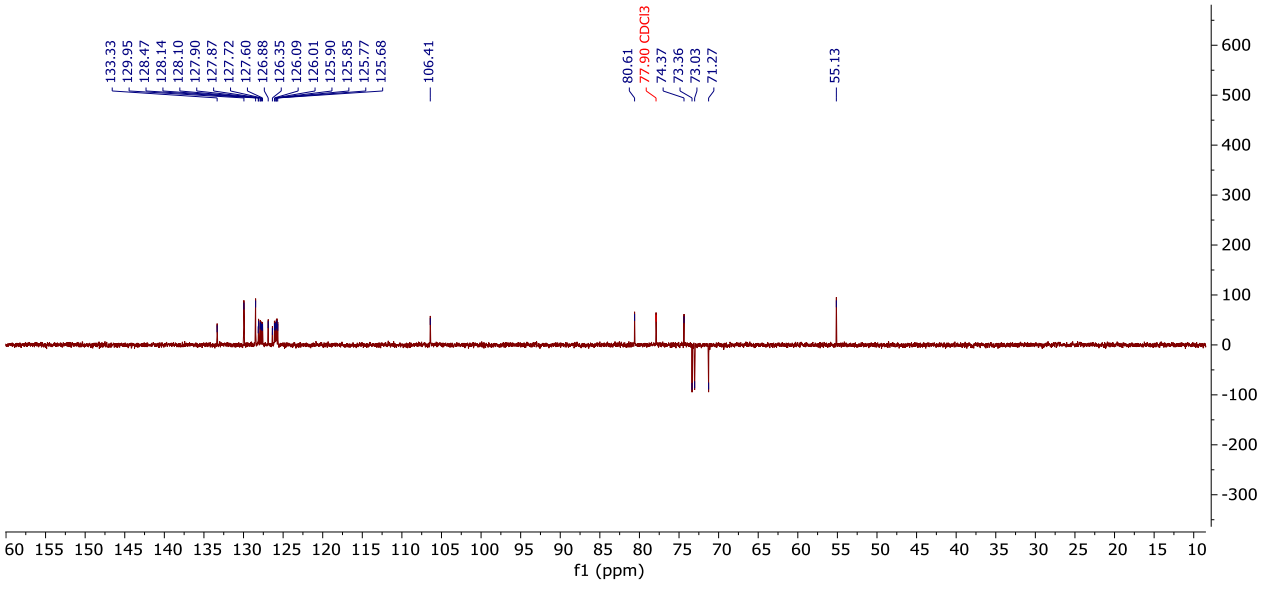


Figure 6.25: DEPT Spectrum of **compound 15**

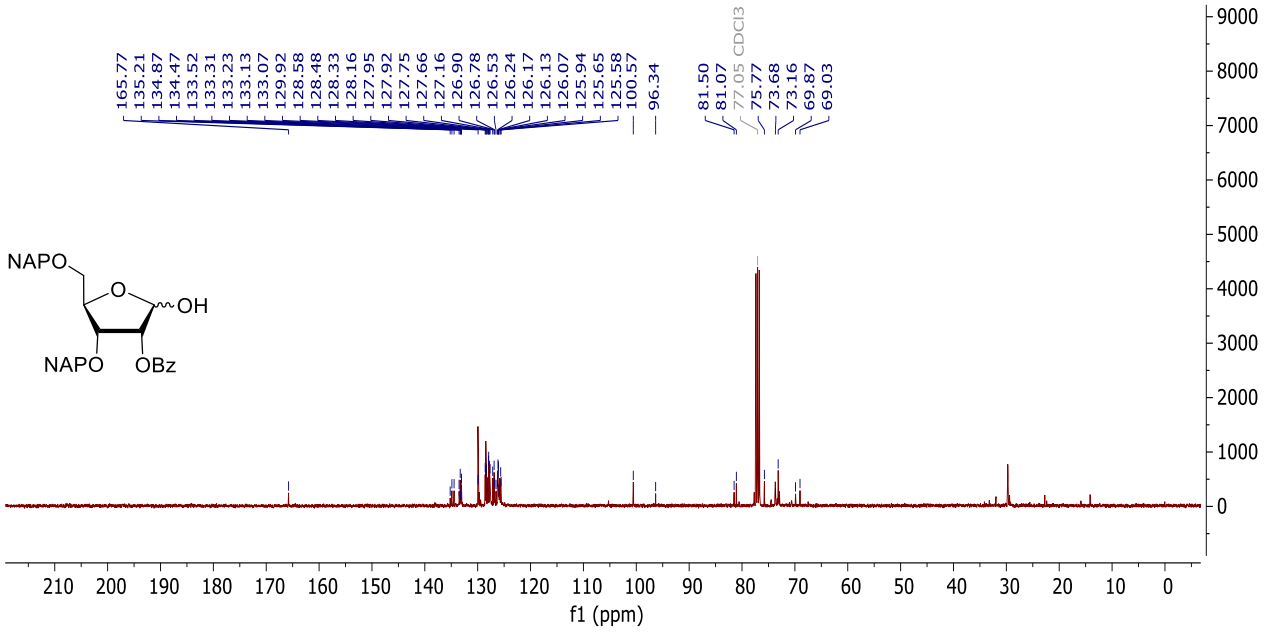


Figure 6.26: ^{13}C Spectrum of **compound 17**

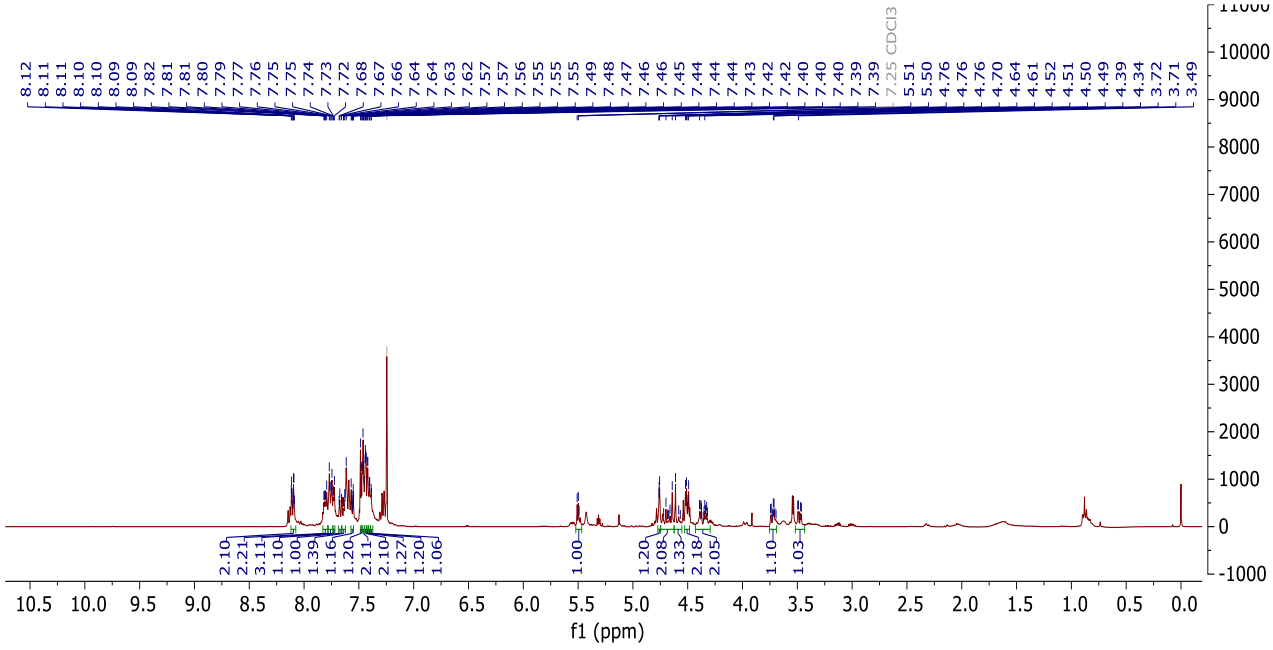


Figure 6.27: ^{1}H NMR Spectrum of **compound 17**

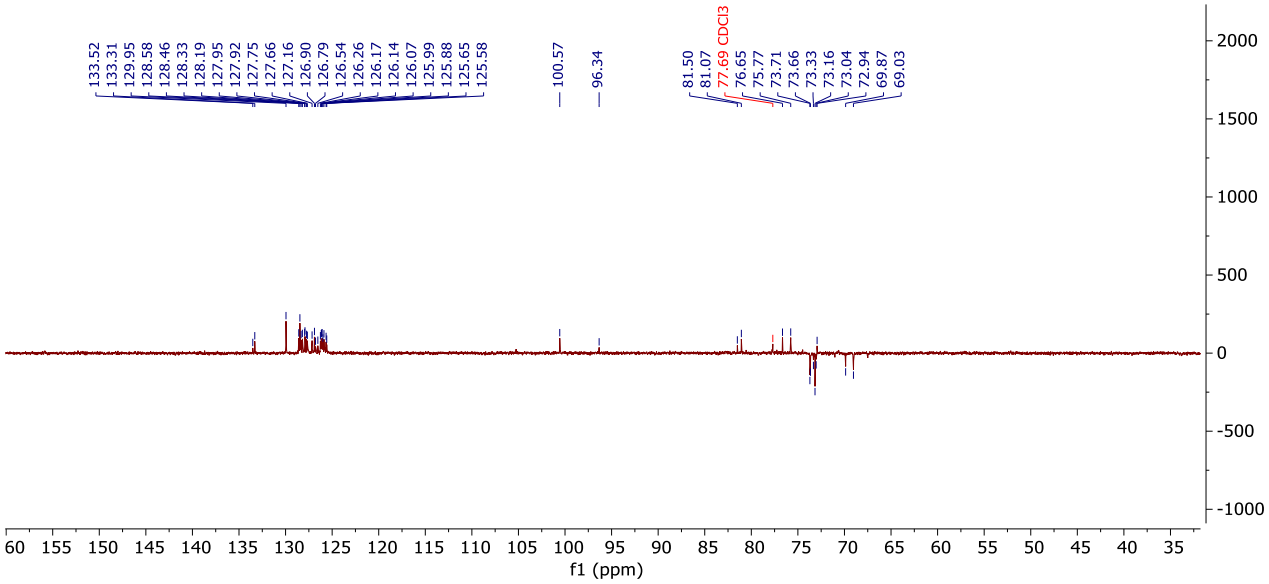


Figure 6.28: DEPT Spectrum of **compound 17**

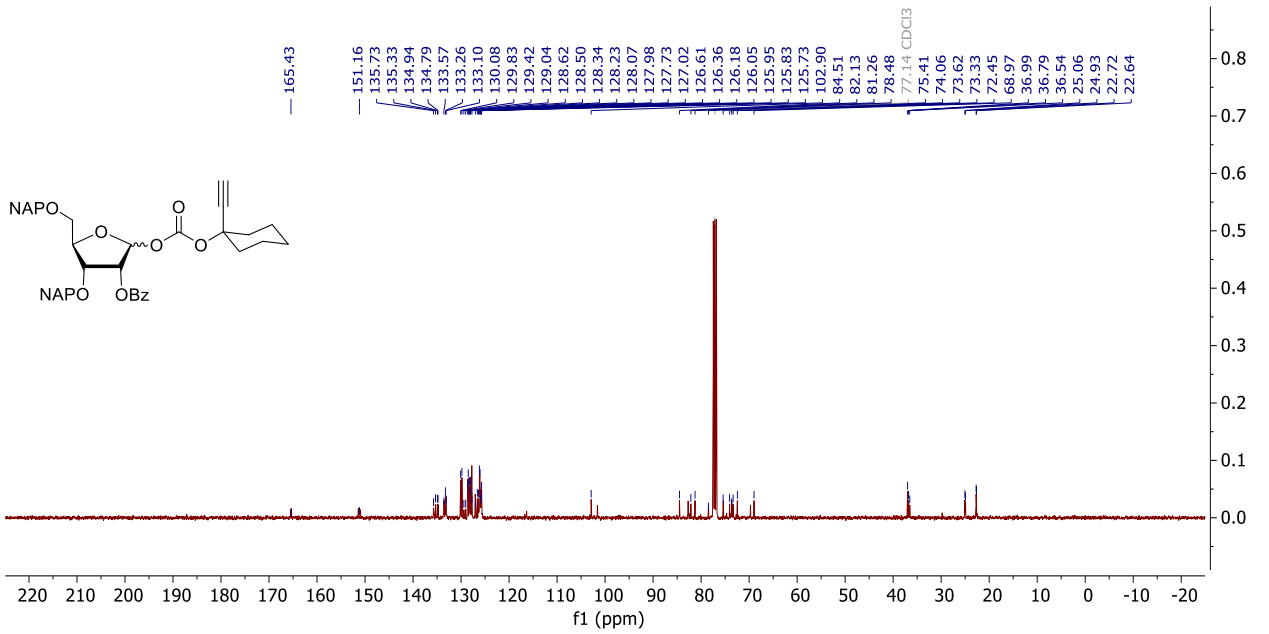


Figure 6.29: ^{13}C Spectrum of **compound 18**

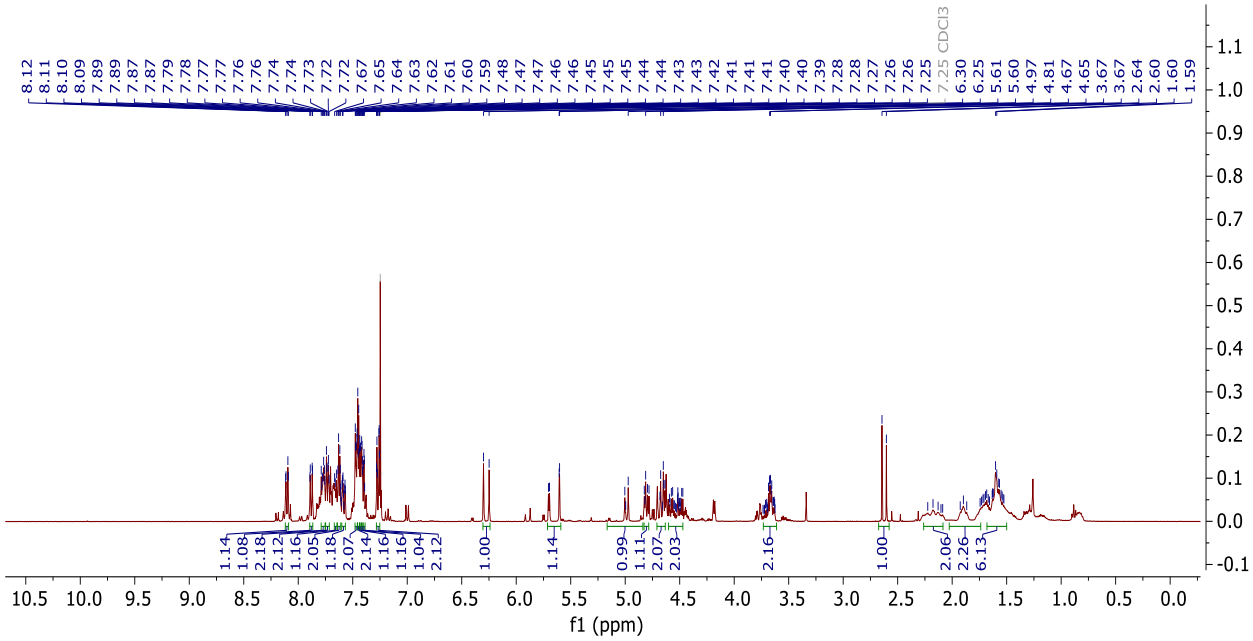


Figure 6.30: ^{1}H NMR Spectrum of **compound 18**

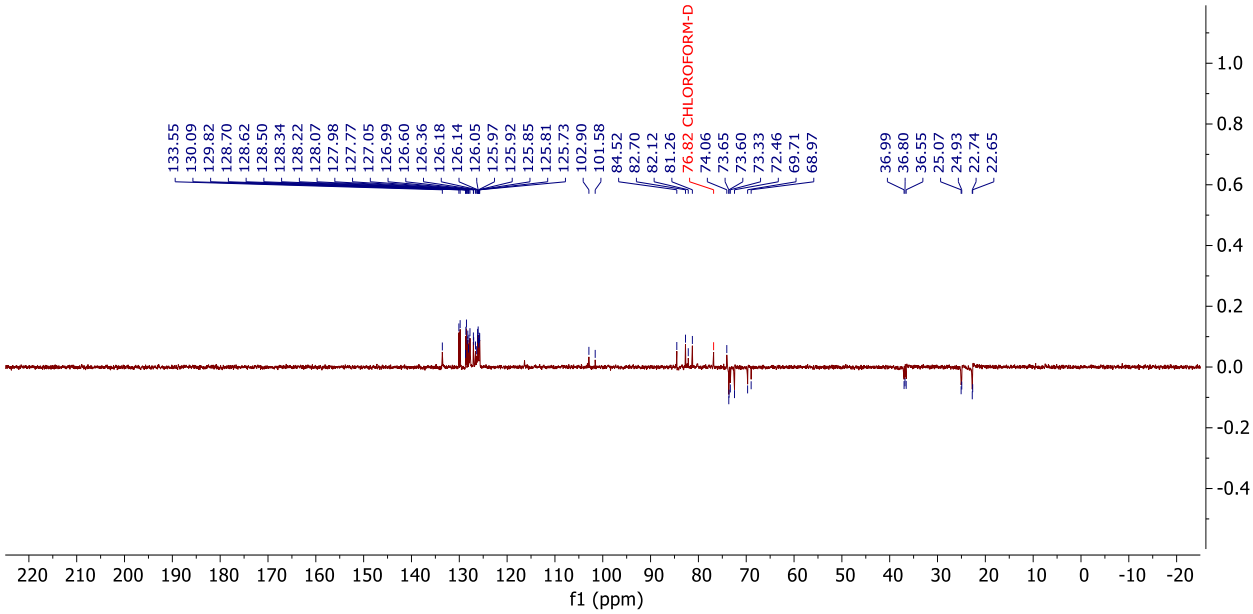


Figure 6.31: DEPT Spectrum of **compound 18**

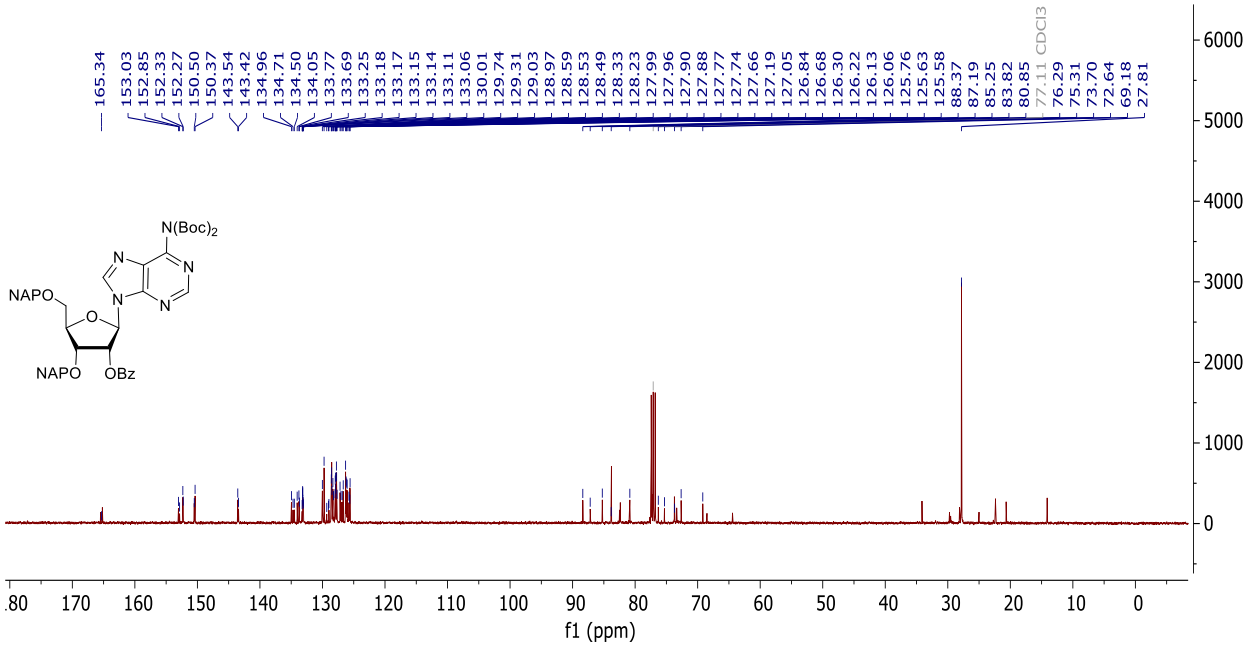


Figure 6.32: ^{13}C Spectrum of **compound 19**

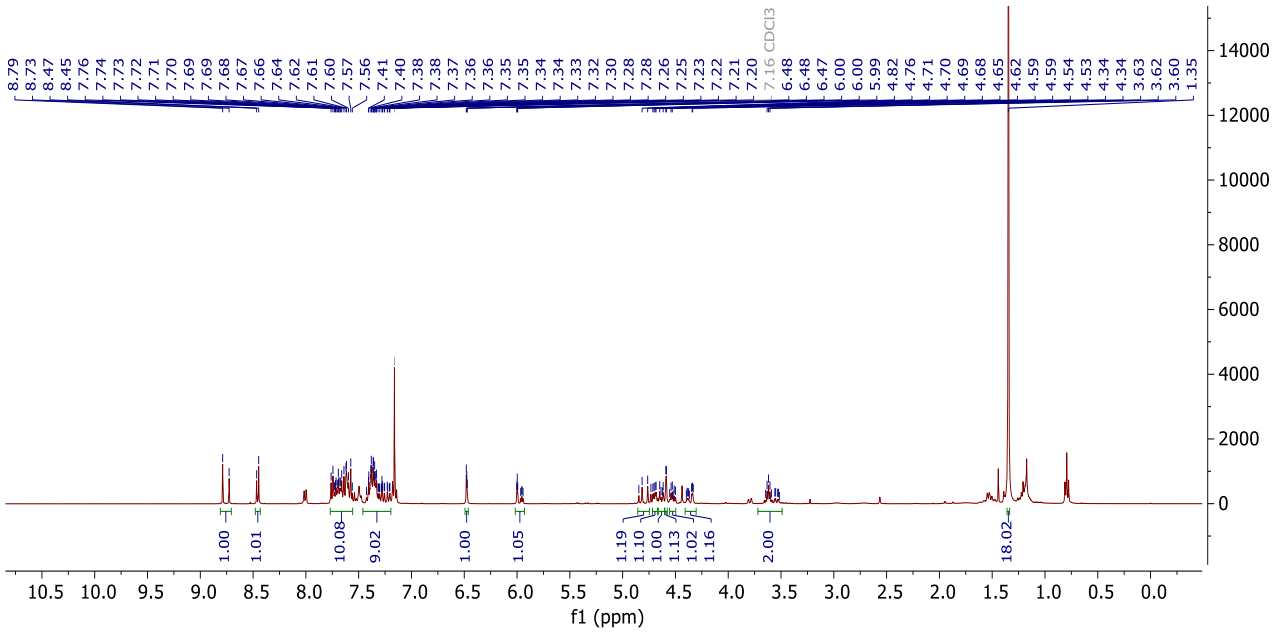


Figure 6.33: ^{1}H NMR Spectrum of **compound 19**

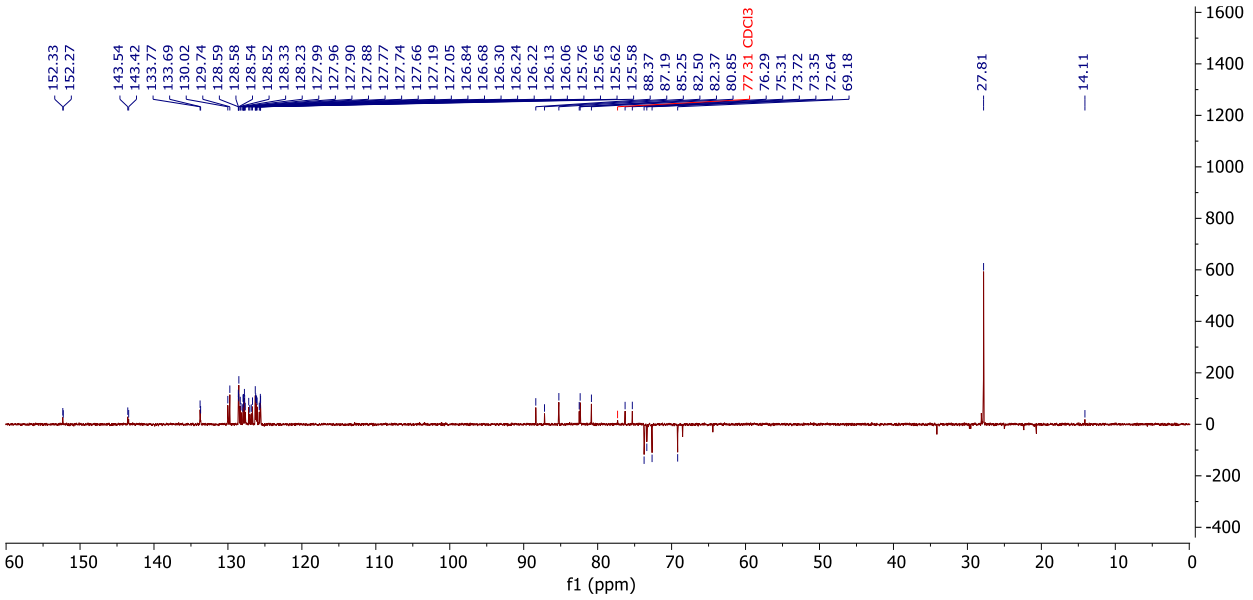


Figure 6.34: DEPT Spectrum of **compound 19**

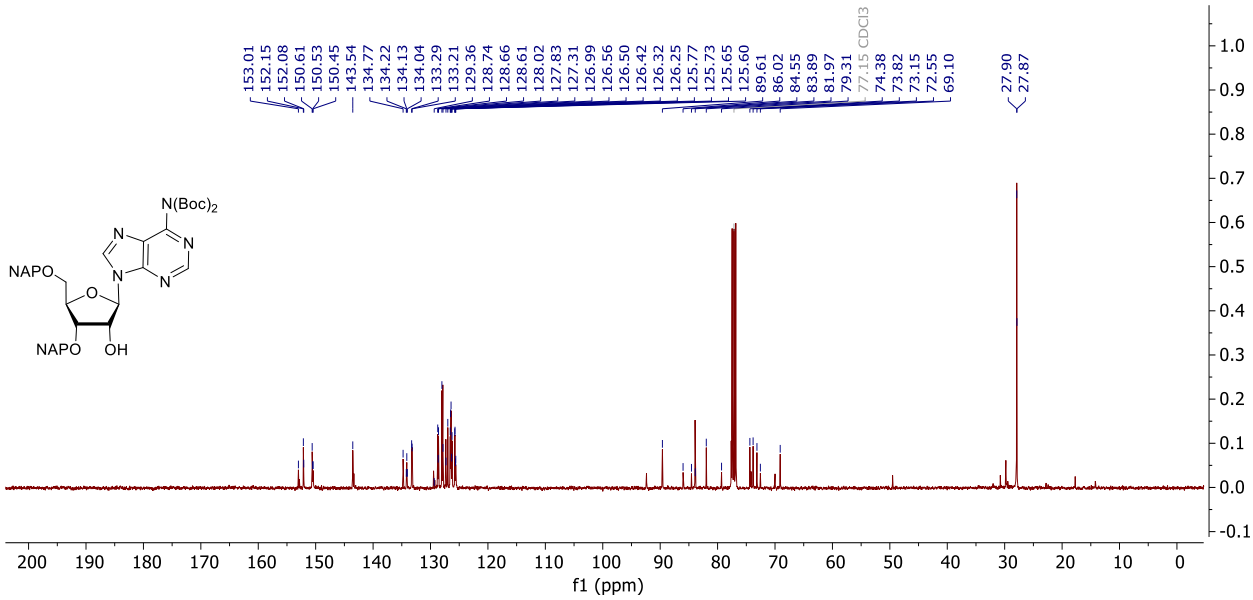


Figure 6.35: ^{13}C Spectrum of **compound 20**

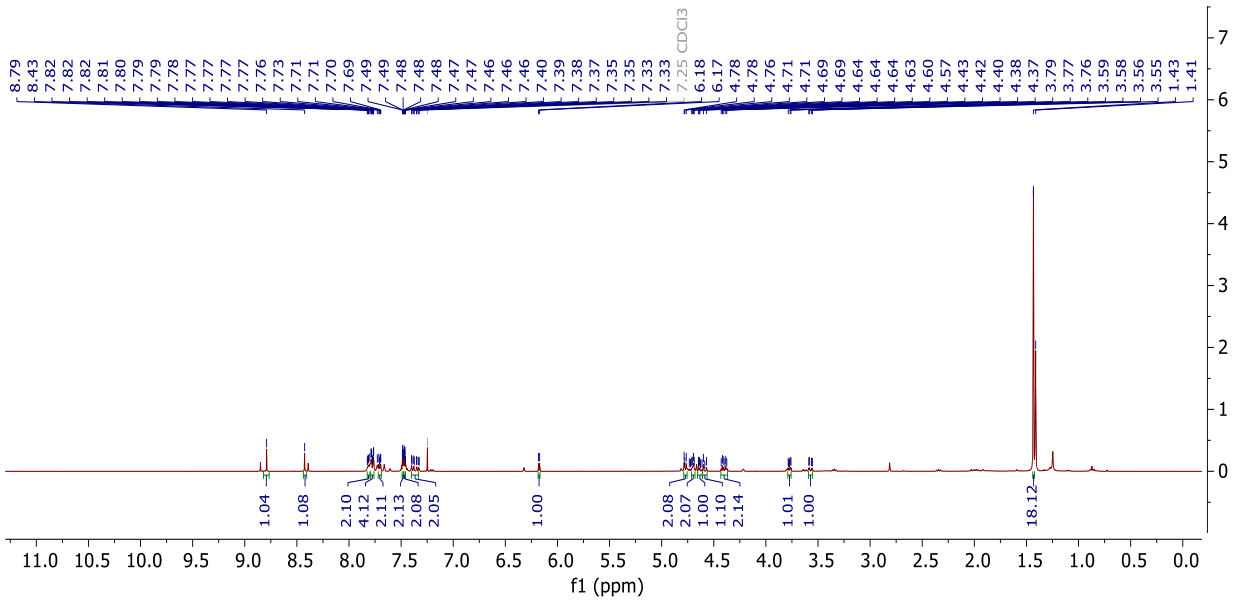


Figure 6.36: ^{1}H NMR Spectrum of **compound 20**

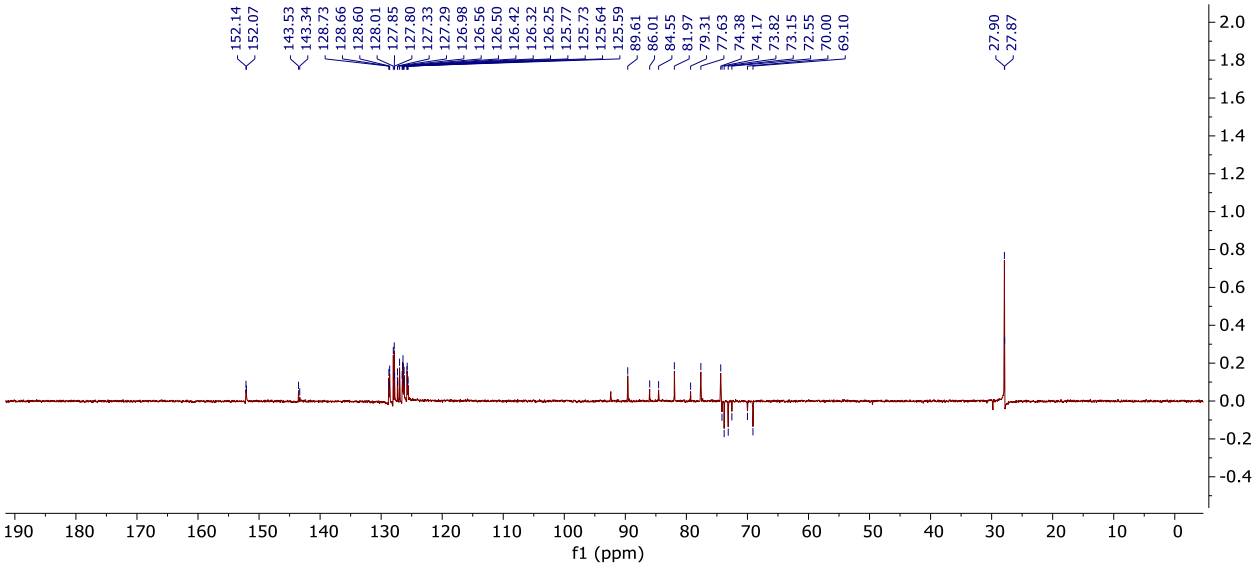


Figure 6.37: DEPT Spectrum of **compound 20**

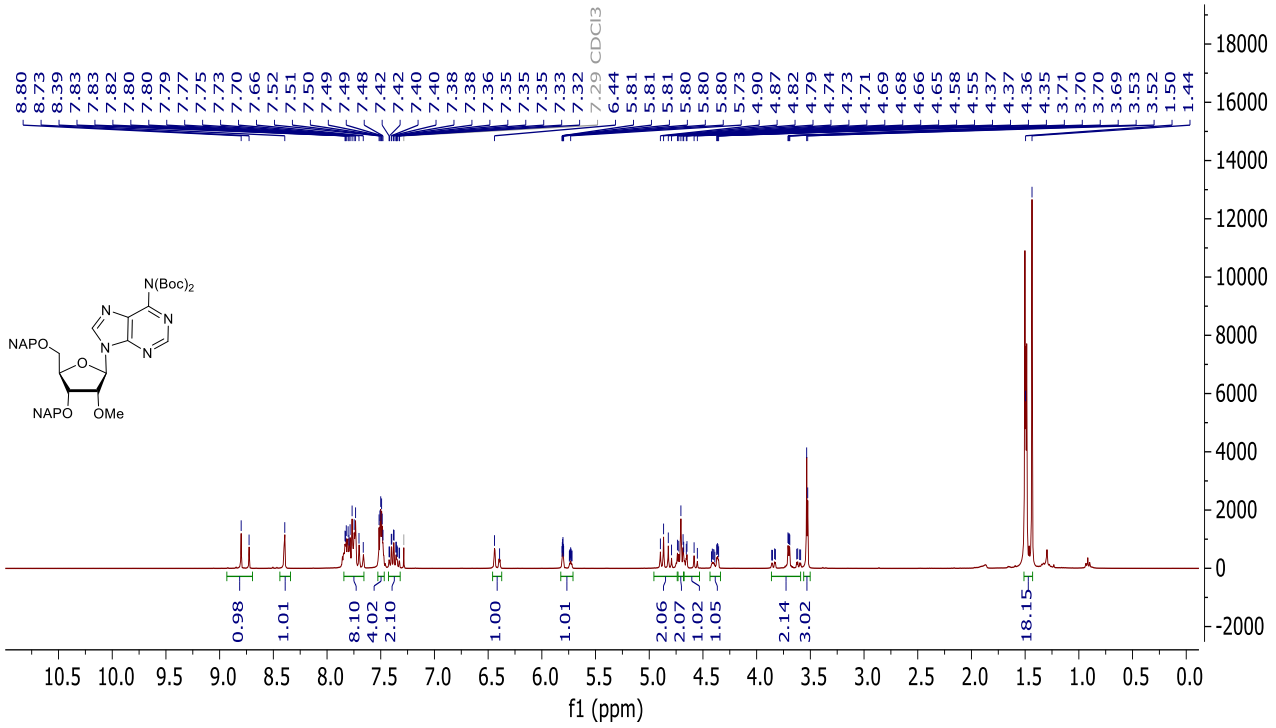


Figure 6.38: ^{1}H NMR Spectrum of **compound 21**

Bibliography

(1) Serpi M, Ferrari V, Pertusati F. Nucleoside Derived Antibiotics to Fight Microbial Drug Resistance: New Utilities for an Established Class of Drugs? J Med Chem. 2016 Dec 8;59(23):10343-10382. doi: 10.1021/acs.jmedchem.6b00325. Epub 2016 Sep 27. PMID: 27607900.

(2) Yates MK, Seley-Radtke KL. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. Antiviral Res. 2019 Feb;162:5-21. doi: 10.1016/j.antiviral.2018.11.016. Epub 2018 Dec 8. PMID: 30529089; PMCID: PMC6349489.

(3) Org. Biomol. Chem., 2015,13, 5094

(4) I. Serra, S. Daly, A. R. Alcantara, D. Bianchi, M. Terreni and D. Ubiali, Redesigning the synthesis of vidarabine via a multienzymatic reaction catalysed by immobilized nucleoside phosphorylases, RSC Adv., 2015, 5, 23569–23577.

(5) Mishra, B.; Neralkar, M.; Hotha, S. Stable Alkynyl Glycosyl Carbonates: Catalytic Anomeric Activation and Synthesis of a Tridecasaccharide Reminiscent of Mycobacterium Tuberculosis Cell Wall Lipoarabinomannan. *Angew. Chem Int. Ed..* **2016**, *128*, 7917–7922.

(6) B. V. Rao, S. Manmode and S. Hotha, Propargyl 1,2-orthoestersfor a catalytic and stereoselective synthesis of pyrimidine nucleosides, J. Org. Chem., 2015, 80, 1499–1505.

(7) Jordheim, L., Durantel, D., Zoulim, F. *et al.* Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat Rev Drug Discov* **12**, 447–464 (2013). https://doi.org/10.1038/nrd4010.

(8) Chemical and chemoenzymatic stereoselective synthesis of β-nucleosides and their analogues. Hui-Jing Wang, Yang-Yang Zhong, You-Cai Xiao and Fen-Er Chen.

(9) Pauli A, Montague TG, Lennox KA, Behlke MA, Schier AF. Antisense Oligonucleotide-Mediated Transcript Knockdown in Zebrafish. PLoS One. 2015 Oct 5;10(10):e0139504. doi: 10.1371/journal.pone.0139504. PMID: 26436892; PMCID: PMC4593562.

(10) María J. Lapponi, Cintia W. Rivero, María A. Zinni, Claudia N. Britos, Jorge A. Trelles,New developments in nucleoside analogues biosynthesis: A review, Journal of Molecular Catalysis B: Enzymatic, Volume 133,2016.

(11) Katherine L. Seley-Radtke, Mary K. Yates, The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part 1: Early structural modifications to the nucleoside scaffold, Antiviral Research, Volume 154,2018.

(12) (2007). N-Glycosides. In: Synthesis and Characterization of Glycosides. Springer, Boston, MA. https://doi.org/10.1007/978-0-387-70792-1_3.

(13) Vorbrüggen,H.;Ruh-Polenz, C. Org. React. 1999, 55, 1. doi:10.1002/0471264180.or055.01

(14) S. An, Q. Wang, W. Zhu, Q. Sun, G. He and G. Chen, Palladium-catalysed O- and N-glycosylation with glycosyl chlorides, CCS Chem., 2020, 2, 1821–1829.

(15) Q. Zhang, J. Sun, Y. Zhu, F. Zhang and B. Yu, An efficient approach to the synthesis of nucleosides: gold(I)-catalysed N-glycosylation of pyrimidines and purines with glycosyl ortho-alkynyl benzoates, Angew. Chem., Int. Ed., 2011, 50,4933–4936.

(16) Lin X, Liang C, Zou L, Yin Y, Wang J, Chen D, Lan W. Advance of structural modification of nucleosides scaffold. Eur J Med Chem. 2021 Mar 15;214:113233. doi: 10.1016/j.ejmech.2021.113233. Epub 2021 Jan 30. PMID: 33550179; PMCID: PMC7995807.

(17) *J. Org. Chem.* 2000, 65, 22, 7697–7699 https://doi.org/10.1021/jo000983i

(18) Brito-Arias, M. N-Glycosides. In *Synthesis and Characterization of Glycosides*; Brito-Arias, M., Ed.; Springer International Publishing: Cham, **2022**; pp 229–284.

(19) Chida, N. Total Synthesis of Nucleoside Antibiotics Possessing Novel N-Glycoside Structures. *J. Synth. Org. Chem.* **2008**, *66*, 1105–1115.

(20) Thadke, S. A.; Mishra, B.; Hotha, S. Facile Synthesis of β- and α-Arabinofuranosides and Application to Cell Wall Motifs of M. Tuberculosis. *Org. Lett.* **2013**, *15*, 2466–2469.

(21) Chakraborty, S.; Mishra, B.; Neralkar, M.; Hotha, S. Stable Benzylic (1-Ethynylcyclohexanyl) Carbonates Protect Hydroxyl Moieties by the Synergistic Action of [Au]/[Ag] Catalytic System. *J. Org. Chem.* **2019**, *84*, 6604–6611.

(22) Chem. Soc. Rev., 2012, **41**, 2448–2462

(23) Alan D Borthwick, Keith Biggadike, Synthesis of chiral carbocyclic nucleosides,Tetrahedron,Volume 48, Issue 4,1992.

(24) Chakraborty S, Mishra B, Kumar Das P, Pasari S, Hotha S. Synthesis of N-Glycosides by Silver-Assisted Gold Catalysis. Angew Chem Int Ed Engl. 2023 Feb 1;62(6):e202214167. doi: 10.1002/anie.202214167. Epub 2022 Dec 28. PMID: 36458817.

(25) Geraghty, R.J.; Aliota, M.T.; Bonnac, L.F. Broad-Spectrum Antiviral Strategies and Nucleoside Analogues. *Viruses* **2021**, *13*, 667. https://doi.org/10.3390/v13040667

(26) Katherine L. Seley-Radtke, Joy E. Thames, Charles D. Waters, Chapter Three - Broad spectrum antiviral nucleosides—Our best hope for the future, Annual Reports in Medicinal Chemistry, Academic Press, Volume 57,2021.

(27) Vincent Roy, Luigi A. Agrofoglio, Nucleosides and emerging viruses: A new story, Drug Discovery Today, Volume 27, Issue 7,2022.

(28) G. Mata and N. W. Luedtke, Stereoselective N-glycosylation of 2-deoxythioribosides for fluorescent nucleoside synthesis, J. Org. Chem., 2012, 77, 9006–9017.

(29) Davison, E.K., Petrone, D.A., Meanwell, M. *et al.* Practical and concise synthesis of nucleoside analogs. *Nat Protoc* **17**, 2008–2024 (2022). https://doi.org/10.1038/s41596-022-00705-7.

(30) Isobe, H.; Fujino, T.; Yamazaki, N.; Guillot-Nieckowski, M.; Nakamura, E. Triazole-Linked Analogue of Deoxyribonucleic Acid (TLDNA): Design, Synthesis, and Double-Strand Formation with Natural DNA. *Org. Lett.* **2008**, *10*, 3729–3732.

(31) Micklefield, J. Backbone Modification of Nucleic Acids: Synthesis, Structure and Therapeutic Applications. *Curr. Med. Chem.* **2001**, *8*, 1157–1179.

(32) B. Fraser-Reid, P. Ganney, C. V. S. Ramamurty, A. M. Gomez and J. C. Lopez, A reverse strategy for synthesis of nucleosides based on n-pentenyl orthoester donors, Chem. Commun., 2013, 49, 3251–3253.

(33) Z. Hu, Y. Tang and B. Yu, Glycosylation with 3,5-Dimethyl-4-(2-phenylethynylphenyl)phenyl (EPP) glycosides via a dearomative activation mechanism, J. Am. Chem. Soc., 2019, 141, 4806–4810