Design and synthesis of novel iminosugar analogues of biological relevance

By
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Arunkumar T.

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Abbrevations

µg	Microgram
µL	Microlitre
µM	Micromolar
^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
2D NMR	Two dimensional
Ac	Acetyl
aq	aqueous
Ac_2O	Acetic anhydride
AcOH	Acetic acid
$AlCl_3$	Aluminium chloride
$Ba(OH)_2$	Barium hydroxide
BBr_3	Boron tribromide
bd	broad doublet
$BF_3.OEt_2$	Boron trifluoride diethyl etherate
Boc	t-Butoxycarbonyl
$(Boc)_2O$	Di-tert-butyl dicarbonate
Bn	benzyl
Br_2	Bromine
brd	Broad doublet
brs	Broad singlet
°C	Degrees Celsius
calcd	Calculated
Cat.	Catalytic
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CH_3CN	Acetonitrile
$CHCl_3$	Chloroform
COSY	Correlation spectroscopy
CuI	Copper iodide
CuAAC	copper(I)-catalyzed alkyne-azide cycloaddition
$CuSO_4$	Copper sulfate
Cs_2CO_3	Cesium carbonate
d	doublet

D_2O	Deuterium oxide
DDQ	2,3-Dichloro 5,6-dicyano 1,4-benzoquinone
DCM	Dichloromethane
dd	doublet of doublets
ddd	Doblet of doublet of doublet
ddd	doublet of doublet of doublet
DAB-1	1,4-Dideoxy-1,4-imino-d-arabinitol
DEPT-135°	Distortionless Enhancement of Polarization Transfer using a 135 degree decoupler pulse
DEAD	Diethyl azodicarboxylate
DIPEA	N, N-Diisopropylethylamine
DMAP	4-(Dimethylamino) pyridine
DMDP	2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine
DMP	Dess-Martin periodinane
DMMP	Dimethyl methyl phosphonate
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6.	Deuterated Dimethyl sulfoxide
DNJ	Deoxynojirimycin
dq	Doublet of quartet
dt	doublet of triplet
E_2	elimination, bimolecular
eq	Equivalent
Equiv	equivalent
ESI	Electronspray ionization
Et	Ethyl
Et_2O	Diethyl ether
Et_3SiH	Triethylsilane
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and drug administration
Fmoc	Fluorenylmethyloxycarbonyl
g	Gram
GH	Glaser-Hay
Gal	Galactose
Glu	Glucose
h	Hour
^1H NMR	Proton nuclear magnetic resonance

H_2	Hydrogen gas
H_2O	Water
$H_{37}RV$	Mycobacterium tuberculosis strain
HCl	Hydrogen chloride
HCOOH	Formic acid
$Hg(OAc)_2$	Mercuric acetate
HNJ	Homonojirimycin
HMJ	Homonojirimycin
HeLa	human cervical cancer cells
HIV-1	Human immunodeficiency virus 1
HMBC	Heteronuclear multiple bond correlation spectroscopy
HR-ESI-MS	High resolution- Electron spray ionisation- mass spectrometry
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation spectroscopy
Hz	Hertz
IC_{50}	Inhibition concentration 50%
iPrOH	Isopropyl alcohol
IR	Infrared spectroscopy
IFN-γ	Interferon-gamma
IL-12	Interleukin 12
kg	Killogram
K_2CO_3	Potassium carbonate
KOtBu	Potassium t-butoxide
LA	Lewis acid
LC/ GC	Liquid chromatography/ Gas chromatography
LPS	Lipopolysaccharide
m	multiplet
M	Molar
m/z	Mass to charge ratio
MCF7	breast cancer
m-CPBA	*meta*-Chloroperoxybenzoic acid
Me	Methyl
MeOH	Methanol
mg	Milligram
MHz	Megahertz
MIC	minimum inhibitory concentration
mins	Minutes
mL	Millilitre
mmol	millimole

$MeNO_2$	Nitromethane
Mp	Melting point
$Me_2C(OMe)_2$	2,2,-Dimethoxypropane
min	Minute
n-BuLi	*n*-Butyl Lithium
$Na_2S_2O_3$	Sodium thiosulfate
Na_2SO_4	Sodium sulfate
NaH	Sodium hydride
$NaBH_4$	Sodium borohydride
$NaCNBH_3$	Sodium cyano borohydride
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
nM	Nanomolar
NMO	N-methylmorpholine N-oxide
NH_3	Ammonia
NIS	N-iodosuccinimide
NJ	Nojirimycin
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
Nu	Nucleophile
OsO_4	Osmium tetroxide
Pd/C	Palladium on carbon
Ph	Phenyl
pmb	*para*-methoxy benzyl
ppm	Parts per million
PP	3,5-dihydro-4H-pyrrolo[3,2-d]pyrimidin-4-one
PP-Cl	4-chloro-5H-pyrrolo[3,2-d]pyrimidine
PP-N_3	4-azido-5H-pyrrolo[3,2-d]pyrimidine
q	quartet
qd	quartet of doublet
Quant	quantitative
Rf	Retardation factor
rt	Room temperature
s	singlet
S_N2	Nucleophilic Substitution bimolecular
t	triplet
TBAI	Tetra-n-butylammonium iodide

TCC	Traditonal chinese medicine
td	Triplet of doublet
TfN_3	Trifluoromethanesulfonyl azide
THF	Tetrahydrofuran
TFAA	Trifluoroacetic anhydride
TLC	Thin layer chromatography
TNF-α	Tumour Necrosis Factor alpha
UV	Ultraviolet
Ph_3PCH_3Br	Methyl triphenylphosphonium bromide
PPh_3	Triphenylphosphine
α	alfa
β	beta
γ	gamma
δ	delta

Preface

Iminosugars are one of the leading carbohydrate mimics with excellent biological activity. They are structurally similar to the carbohydrates but with the endocyclic oxygen replaced by nitrogen. In addition to that, they are responsible for numerous biological properties exhibited by plants, in which iminosugars are the common component. They are promising drug candidates possessing several intrinsic properties. Mainly, natural imino sugars come under five classes-pyrrolidines, piperidines, indolizidines, pyrrolizidines, and nor-tropanes. Usually, imino sugars exist in nature in polyhydroxy form, even though varieties such as amides carboxylic acids are also available. Iminosugars exhibit high solubility, low molecular weight, polar nature, better inhibition, thus making them favourable to synthetic and biological research. However, iminosugars such as 1-deoxynojirimycin, Miglitol and Miglustat under clinical trials have already passed regulatory approval. Owing to their potent bio-efficacy, research groups around the world have invested heavily in synthesising iminosugars of monocyclic as well as bicyclic nature. Notable endeavours include fagomine, castanospermine, swainsonine, 1,4-dideoxy-1,4-iminod- arabinitol (DAB) and their analogues. Due to their growing interest in the biological domain, we decided to synthesise iminosugar variants like iminosugar N-alkyl C –glycoside and the bicyclic iminosugars.

Chapter 1 concerns the introduction of synthetically inspiring iminosugars and their importance in drug discovery, especially C-glycosides. The emerging research on iminosugars is a result of their ability to inhibit glycoprocessing enzymes. Inhibitors of these enzymes are the main therapeutic targets for debilitating diseases like cancer, diabetes, lysosomal storage disorders and rare genetic disorders.

The second chapter deals with the synthesis of various iminosugar C-glycosides with a few interesting transformations. Along with that, bicyclic iminosugars (aziridine) were also synthesized based on the medicinal chemistry relevance of bicyclic iminosugars. The isomers

(L and D) of bicyclic compounds were characterized via 1D-COSY and 1D NOE. All the compounds were unambiguously characterized by ^{1}H, ^{13}C, 2D NMR, HRMS and optical rotation.

The third chapter describes the synthesis of iminosugar appended Miltefosine analogues via click chemistry. Initial attempts on the phospholipid bromide led to an unsuccessful reaction because of its poor solubility in the polar aprotic solvents. Based on that observation, we designed and synthesized a triazole bridged, N-propargylated glucose and galactose variants of 1-deoxyiminosugar and phospholipid azide via click chemistry.

In the fourth chapter, three new classes of nojirimycin analogues viz. N-alkyl with C1-substituent (4-phenylbutyl), N-substituted 1-deoxynojirimycin and its congener δ-lactam, and a 4-phenylbutyl-β-C-glycoside were designed and synthesized for immunological studies. The resulting diverse compound library exhibited proliferation of B Cells and T cells induced by LPS and Con A, respectively. A deoxynojirimycin-triazole conjugate of phytosphingosine analogue was superior in the responses and exhibited nitric oxide response equal to LPS.

In the final chapter, we have demonstrated the limitation of CuAAC ‘click’ reaction with a 2- azidopyridine substrate, owing to its equilibrium with a tetrazole isomer, which is exploited herein for its utility in Glaser–Hay reaction. A catalytic combination of a 2-azidopyridine analogue, 4-azido-5Hpyrrolo[3,2-d]pyrimidine, and CuI afforded homocoupled products of terminal alkynes, without any trace of triazole product, under mild conditions with a broad substrate scope. Emphasis on carbohydrate-based substrates appended to a propargylic group led to 1,3-diynes in good to excellent yields.

Chapter 1

Introduction to Iminosugars

1.1. Abstract

Introduction of carbohydrate mimetics such as iminosugars, whose natural occurrence and chemical synthesis is well documented is the focus of the first chapter. The structural diversity of iminosugars by functionalized nitrogen and *C*-glycosidic linkage is described with the reported synthetic strategies. Additionally, the significance of iminosugars as approved drugs, in drug discovery and their biological relevance is described.

1.2. A brief Introduction to carbohydrates and their biological significance

Carbohydrates are abundant biomolecules associated with immense biological relevance. The structural diversity of carbohydrates underpins its relevance as a pivotal molecular scaffold in the field of drug discovery. There are natural and synthetic carbohydrates with potent bioactivity used for various ailments. Many antibiotics have carbohydrate origin, which have been featured in majority of the approved drugs. The ability to cope with biological processes for the functioning in the living cell and their receptors renders interesting properties to these carbohydrate biomolecules. Perhaps, these non-identical functions in biological systems further expand their importance in diet, medicine, etc. The pharmacokinetic properties such as hydrophilicity and reduced toxicity contributed by these molecular entities are exceptional. Iminosugars are one of the most potent mimetics of carbohydrates with interesting stability as well as biological properties.[1–4]

1.3. Introduction to Iminosugars

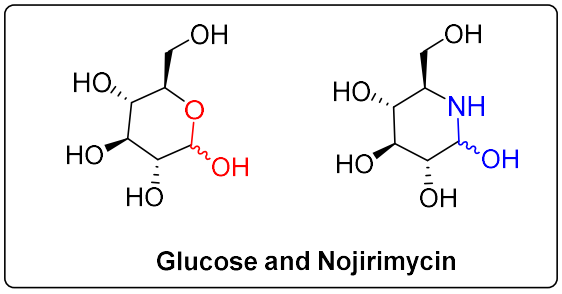


Figure 1.3.1a. Carbohydrate and its iminosugar mimic

Iminosugars are also called polyhydroxylated secondary and tertiary amines. The endocyclic oxygen in an iminosugar is replaced by nitrogen (figure 1.3.1a). They are one of the leading carbohydrate mimics with excellent biological activity. In addition to that, they also partake in numerous biological activities exhibited by plants, in which iminosugars are the common component, they are also present in microorganisms. However, these small polar molecules always come under the category of carbohydrates with distinct bio-profiling rather than small heterocyclic molecules. Their efficient uptake, biological and chemical stability provide them more prominence when compared to unstable small carbohydrate molecules. Iminosugars always find a perceptible place in the area of glycobiology due to their ability to interact with the biological system maintaining a most stable form. They are promising drug candidates possessing several intrinsic properties. Iminosugars exhibit high solubility, low molecular weight, polar nature, better inhibition, thus making them conducive to synthetic and biological research. The researchers who discovered the first-ever iminosugar did not opine about the hidden potential of this class of molecule. It came to the limelight after the emergence of Glycet, the first iminosugar-based medicine, in 1996. Many similar molecules had undergone clinical trials for various treatments, including cancer, viral diseases, genetic diseases, diabetics, etc. The rationale behind the ongoing biological investigations of these molecules is their ability to interact with the living

system efficiently. Despite the sheer number of molecules described, only a few molecules were tested for their biological effectiveness. Deoxynojirimycin (DNJ), swainsonine, and castanospermine are among the most examined compounds. These small molecules will always provide fresh insights for drug development. The first-generation iminosugars were initially at the center stage of cancer and anti-HIV research, even though the diversity and chemical heterogeneity were minuscule in the initial stages. The biological efficacy of this new class of carbohydrate mimic was well-studied due to their inhibition of glycosidases.[1,5–10]

1.4. Naturally occurring iminosugar and their biological significance

Pyrrolidines, piperidines, indolizidines, pyrrolizidines, and nor-tropanes are the five main class of natural iminosugars. Iminosugars are the most commonly found polyhydroxy forms. However, other types such as amides and carboxylic acids are also known.

1.4.1. Piperidines

In 1968, the first piperidine-based iminosugars called nojirimycin was isolated from the fermentation broths containing the strains of *Streptomyces.* This revolutionalized the fate of the carbohydrate family of bioactives. They were seen as forerunners in the fields of antibiotics and glycosidase inhibitors.[11] Furthermore, they exhibited anti-bacterial characteristics that were promising. More interestingly, the absolute configurations of these six-membered iminosugars featuring hydroxyl groups were similar to naturally occurring sugar molecules.[11–14] Later in 1984, another piperidine analogue, mannonojiromycin, was isolated from *Streptomyces lavandulae*. Galactostatin is a kind of piperidine identified in 1987 by Miyake *et al.* in *Streptomyces lydicus* PA-5725, it is a potent inhibitor of α- and β-galactosidases.[15]

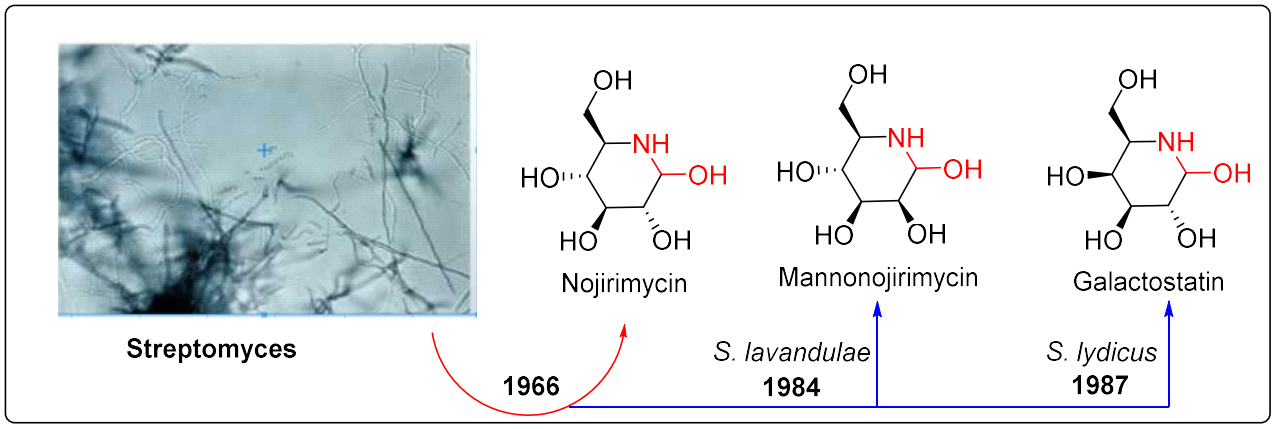


Figure 1.4.1.1a. Nojirimycin, mannonojirimycin and galactostatin

Later in 1976, Deoxynojirimycin (DNJ) was isolated from the roots of *Morus alba*, which was synthesized previously in 1967 by Paulsen *et al.* DNJ is an iminosugar that is more stable compared to NJ. The instability is due to its hemiaminal functionality arising due to the presence of an anomeric hydroxyl group.

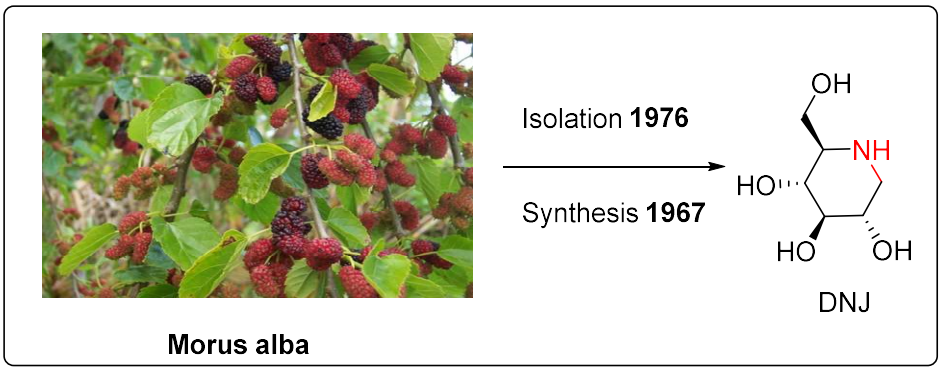


Figure 1.4.1.2a. Deoxynojirimycin (DNJ)

Fagomine is another interesting six-membered, piperidine-based iminosugar isolated in 1974 from Buck heat (*Fagopyrum esculentum*). It is a 1,2-dideoxy cognate of NJ with two of its epimers always present in nature. The molecule fagomine is also present in mulberry leaves, gogi roots, etc. Fagomine is also chemically and metabolically stable due to the lack of anomeric hydroxyl groups. Reports suggest the usage of D-fagomine in diet or functional food as it remove the additional *Enterobacteriales*, which are commonly associated with unhealthy food diet. In

1979, 1-deoxy-D-mannonojirimycin was isolated from *Lonchocarpus sericeus.* Yet again, it was shown to be a stable analogue of mannojirimycin.[16,17]

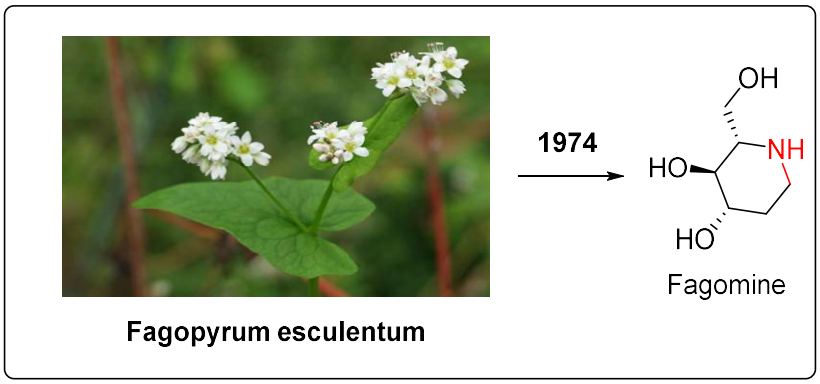


Figure 1.4.1.3a. Fagomine from *Fagopyrum esculentum*

1.4.2. Pyrrolidines

In 1976, for the first time, a five-membered iminosugar was introduced by Welter. Hence the molecule they isolated was named DMDP (2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine), which exhibited glycosidase inhibitor activity. Other members in this class CYB-3 (2-Hydroxymethyl-3-hydroxypyrrolidine) and DAB-1 (1,4-Dideoxy-1,4-imino-d-arabinitol) were isolated in 1985 from *Castanospermum australe* and *Angylocaiyx boutiqueanus*, respectively. In addition, 6-deoxy-DMDP, DGDP, nectrisine, homo-DMDP also fall under this class of molecule.[18,19]

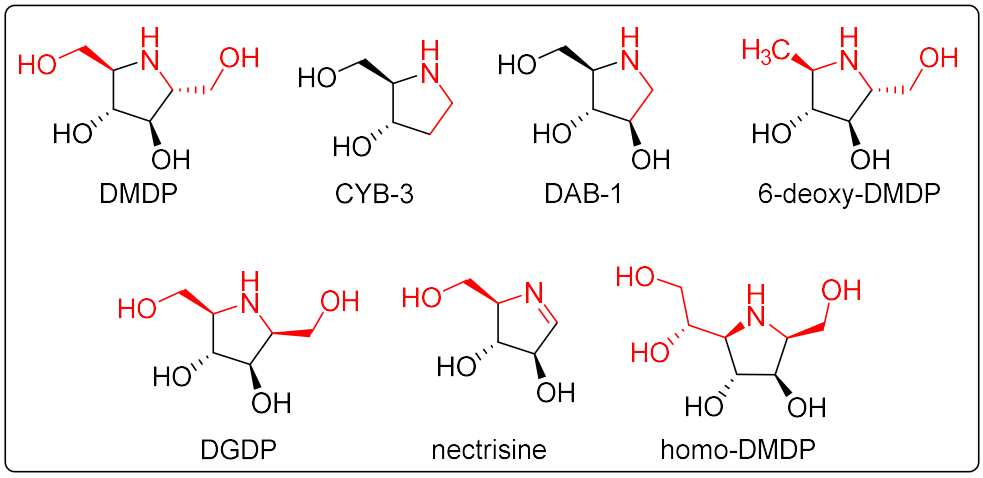


Figure 1.4.2.1a. Naturally occurring pyrrolidine compounds

1.4.3. Pyrrolizidines

The pyrrolizidine iminosugar incudes casuarine, alexine, australine, hyacinthacine, etc. in which they possess a fused pyrrolidine ring system. Among these molecules, the highly oxygenated one casuarina was isolated in 1994 from *Casuarina equisetifolia.* The molecules that fall under this class exhibit various inhibitory properties. Australine is an excellent glycosidase inhibitor isolated in 1988 from *Castanospermum australe*. In addition to this, the molecule hyacinthacines is known for their biological activity. However, pochonicine and broussonetine-N are part of this category of pyrrolizidine-based iminosugar.

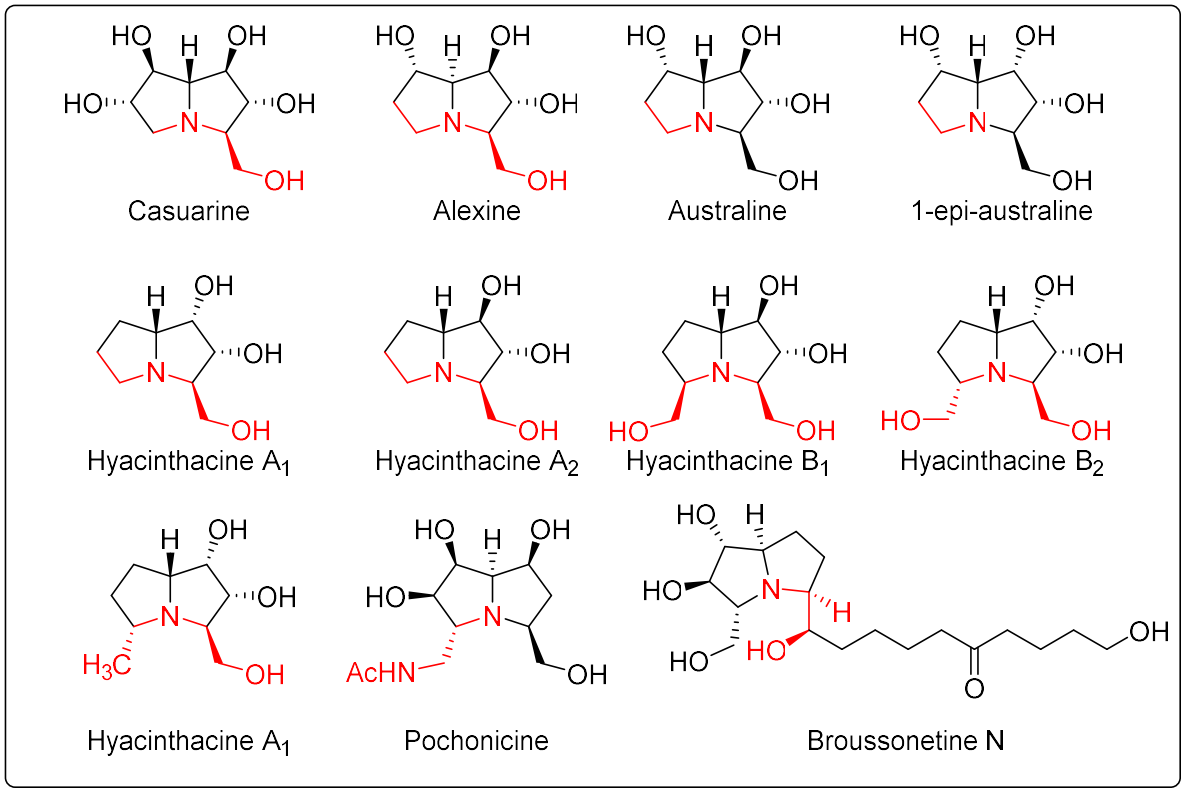


Figure 1.4.3.1a. Naturally occuring pyrrolizidine compounds

1.4.4. Indolizidines

Castanospermine, swainsonine, lentiginosine, and uniflorine are the molecules falling under indolizidine alkaloids. Castanospermine was isolated for the first time from *Castanosperm-*

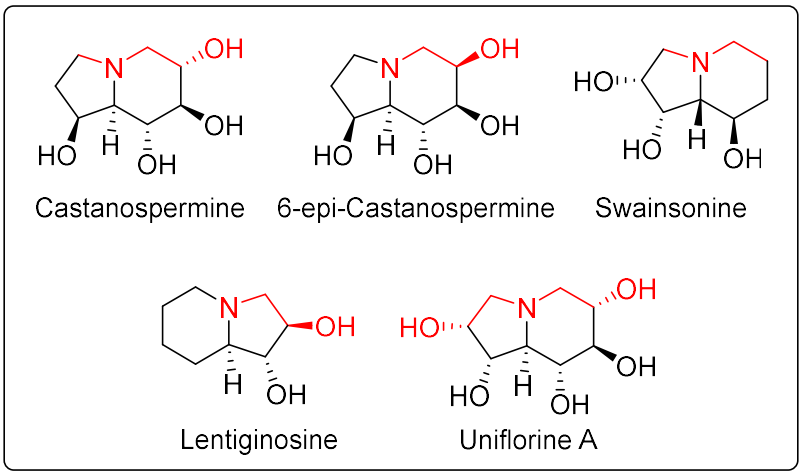


Figure 1.4.4.1a. Naturally occurring indolizidine compounds

um australe and swainsonine was isolated from *Swainsona canescens*. The synthetic efforts taken by scientists in this area are vast due to its excellent biological activities. In addition, the molecule under indolizidines, castanospermine is interesting for the treatment of autoimmune diseases and viral diseases. Apart from that, they are considered for treating cancer, diabetes, hepatitis C and HIV.[20–22]

1.4.5. Nortropanes

The nortropane iminosugars mainly comprise a group of calystegines and polyhydroxylated alkaloids. They are present in potatoes, tomatoes, cabbages, aubergines, and

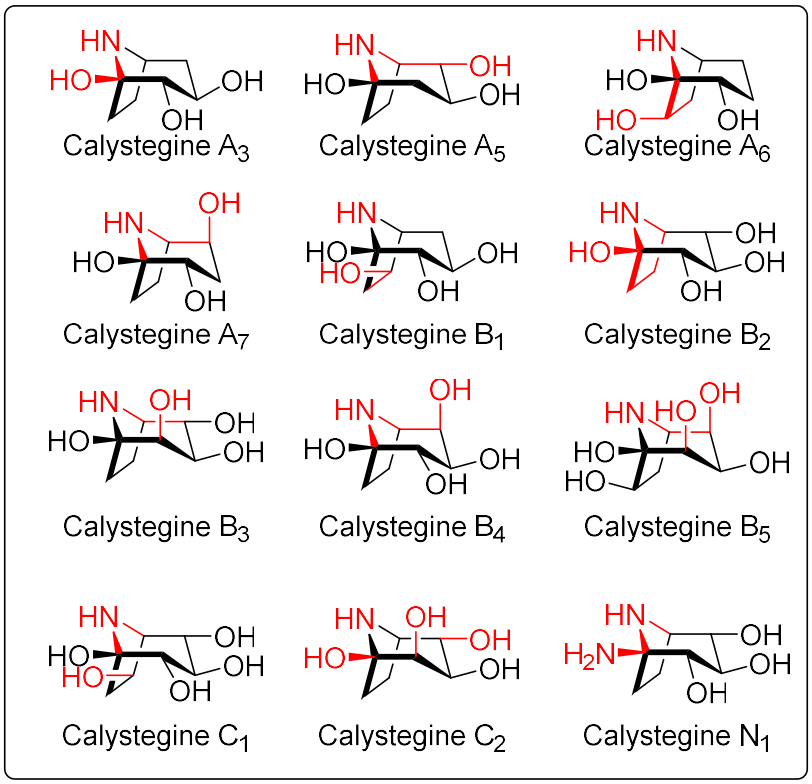


Figure 1.4.5.1a. Naturally occuring nortropane compounds

solanaceous food materials. They are known for their excellent glycosidase and biological activities similar to swainsonine. This important hydrophilic molecule was first isolated in 1988

from *Calystegia sepium*. Structurally calystegines are the molecules have similarity to alkaloid together with that they also possess three to five hydroxyl groups in it at different positions.[23–27]

1.5. Naturally occurring iminosugar C-glycosides

In contrast to carbohydrate analogues of nojirimycin-like molecules, *C*-glycosides are more stable molecules. Kite *et al.* reported the first known iminosugar *C*-glycoside compound, α-homonojirimycin, from *Omphalea diandra* in 1988.[28–32] In 1990, the same research group detected the α-HNJ in the *Urania fulgens* moth. This is the first time α-HNJ has been found in animals. Various plant species such as *Stemona tuberosa*, *Commelina communis*, and others were sources for isolation of α-HNJ and β-HNJ.[28–32] These compounds were proven to be more effective than nojirimycin and deoxynojirimycin.

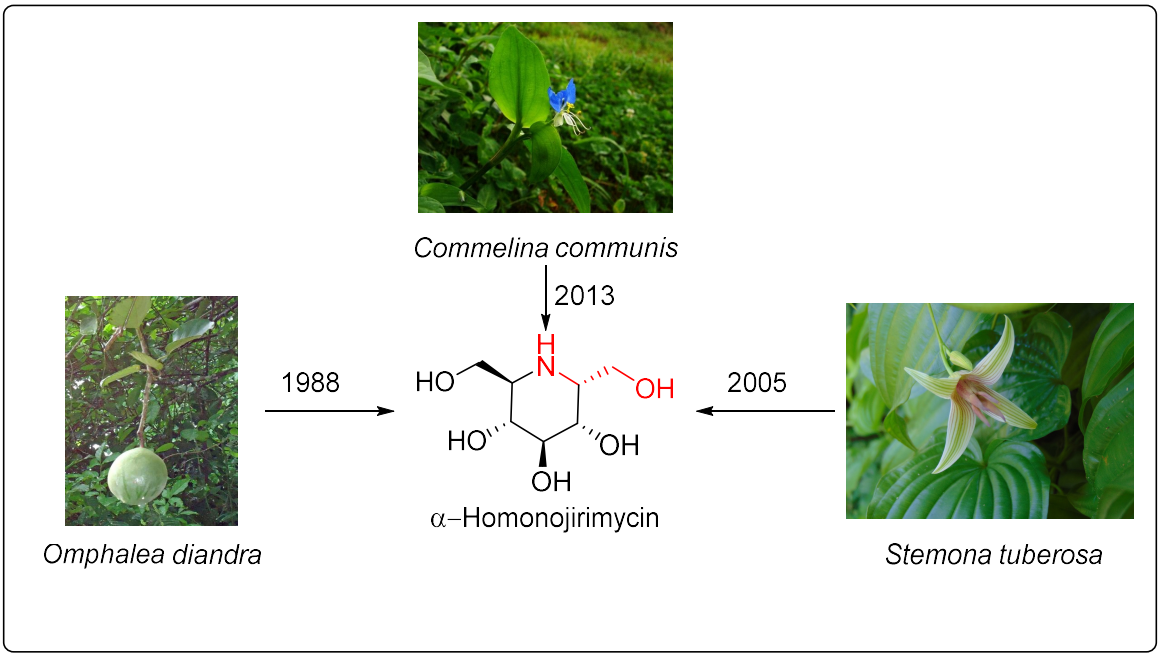


Figure 1.5.1a. α-Homonojirimycin

The iminosugar compound, α-homomannonojirimycin is also found in nature. α-HMJ is a *C*-2 epimeric compound of α-homonojirimycin. Asano *et al.* reported the α- and β-HMJ from the

plant species *Aglaonema trubii* in 1997. In 1998, the same group isolated α- and β-HMJ from *Hyacinthus orientalis*. These molecules were effective as glycosidase inhibitors.[33–35]

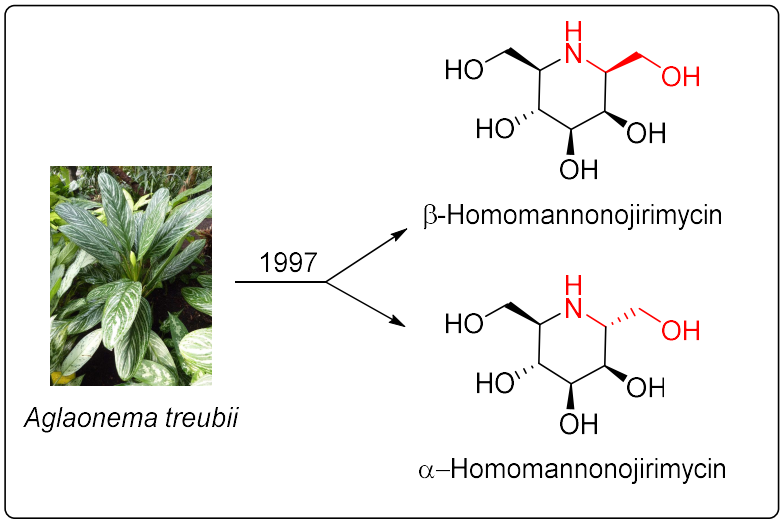


Figure 1.5.2a. *α*-Homomannojirimycin

Adenophorine is a unique molecule since it has an aliphatic substituent at its *C*-6 position. The molecule was isolated from *Adenophora triphylla* roots and aerial parts *Lobelia sessilifolia*. The molecule has shown moderate activity against α-glycosidase and was effective against α-galactosidase.[36,37]

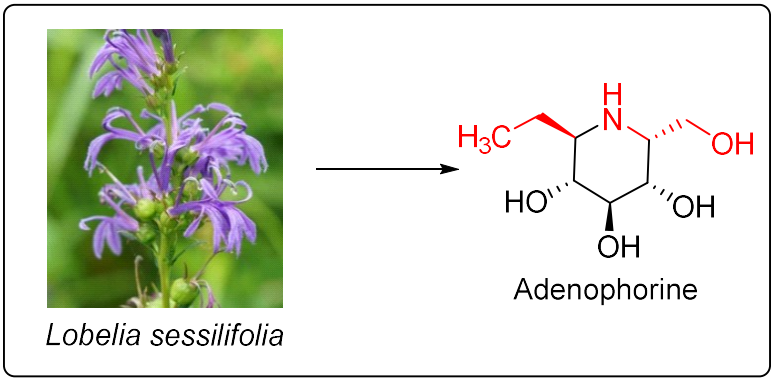


Figure 1.5.3a. Adenophorine

Batzellasides A, B, and C are the *C*-1 alkylated derivatives of piperidine iminosugars derived from marine sources. Crews *et al.* identified these iminosugars from *Batzella* sp. Sponge in west Madagascar in 2005.[38–42] With minimum inhibitory concentrations (MICs) of less than 6.3 g/mL, these compounds demonstrated anti-bacterial activity against *Staphylococcus epidermidis*.[42]

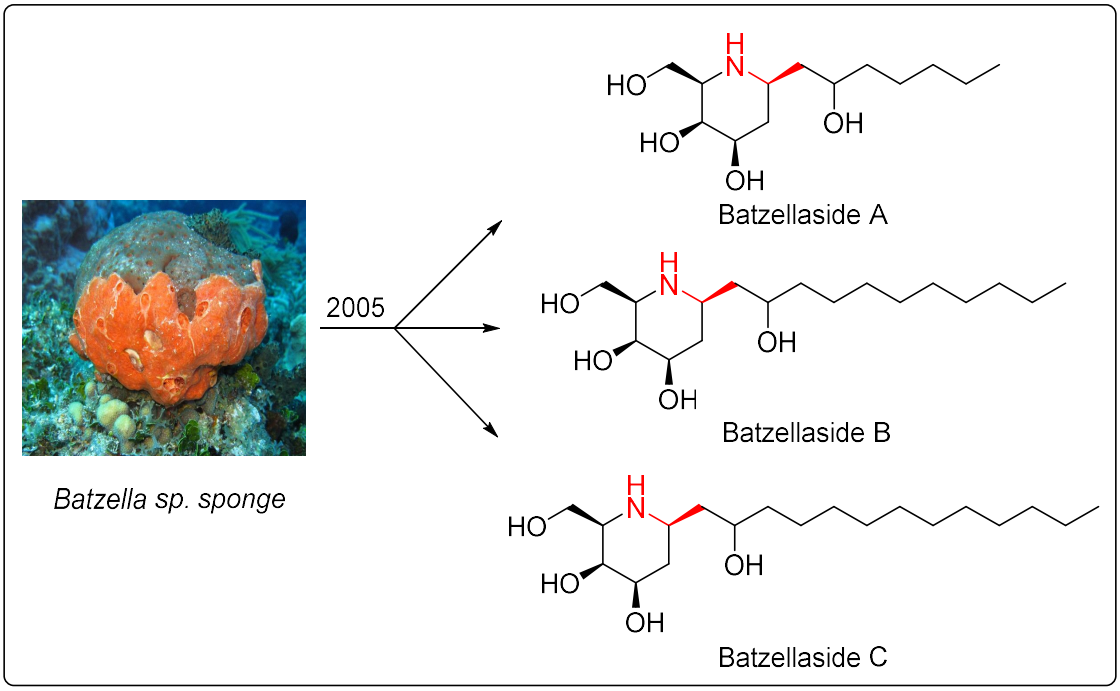


Figure 1.5.4a. Batzellasides from marine sponge

1.6. Importance of iminosugar C-glycosides

Bayers demonstrated the first synthesis of iminosugar *C*-glycoside in 1980, ten years before its first isolation. The iminosugar *O*-glycosides are unstable, hence, *C*-glycosides were the apparent choice for conjugation at *C*1 position. Its relevance grew after discovering the hidden biological significance and its superior performance in ameliorating different biological conditions. As a result of the rising interest, a large number of researchers focused on the synthesis of iminosugar, particularly, proposing innovative and cost-effective methodologies for synthesis of *C*-glycosides.

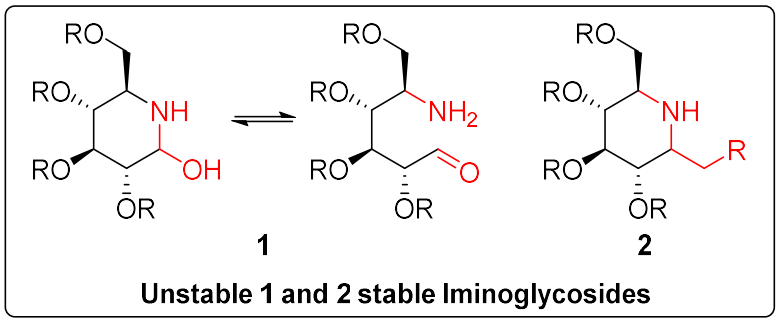


Figure 1.6.1a. Hemiaminal and *C*-glycoside

Enantiomerically pure iminosugar *C*-glycoside is synthetic challenge, still methods are being published for stereocontrol, after four decades of its initial synthesis. Furthermore, the structural difference associated with *C*-glycosides is minimal compared to *O*- and *N*-glycosides, rendering them resistant to enzymatic and acid degradation.[43,44]

1.7. Synthetic strategies for iminosugars and their C-glycosides:

Hitherto, many synthetic strategies for these small bioactive compounds have been described. There are two methods for synthesizing this tricky iminosugar synthesis: asymmetric synthesis, which require introduction of stereocenters or chiral pool, which begins with a chiral starting material that already possesses the required stereochemistry. Owing to their homogeneity and availability, carbohydrates have long been a common building block for iminosugar synthesis.

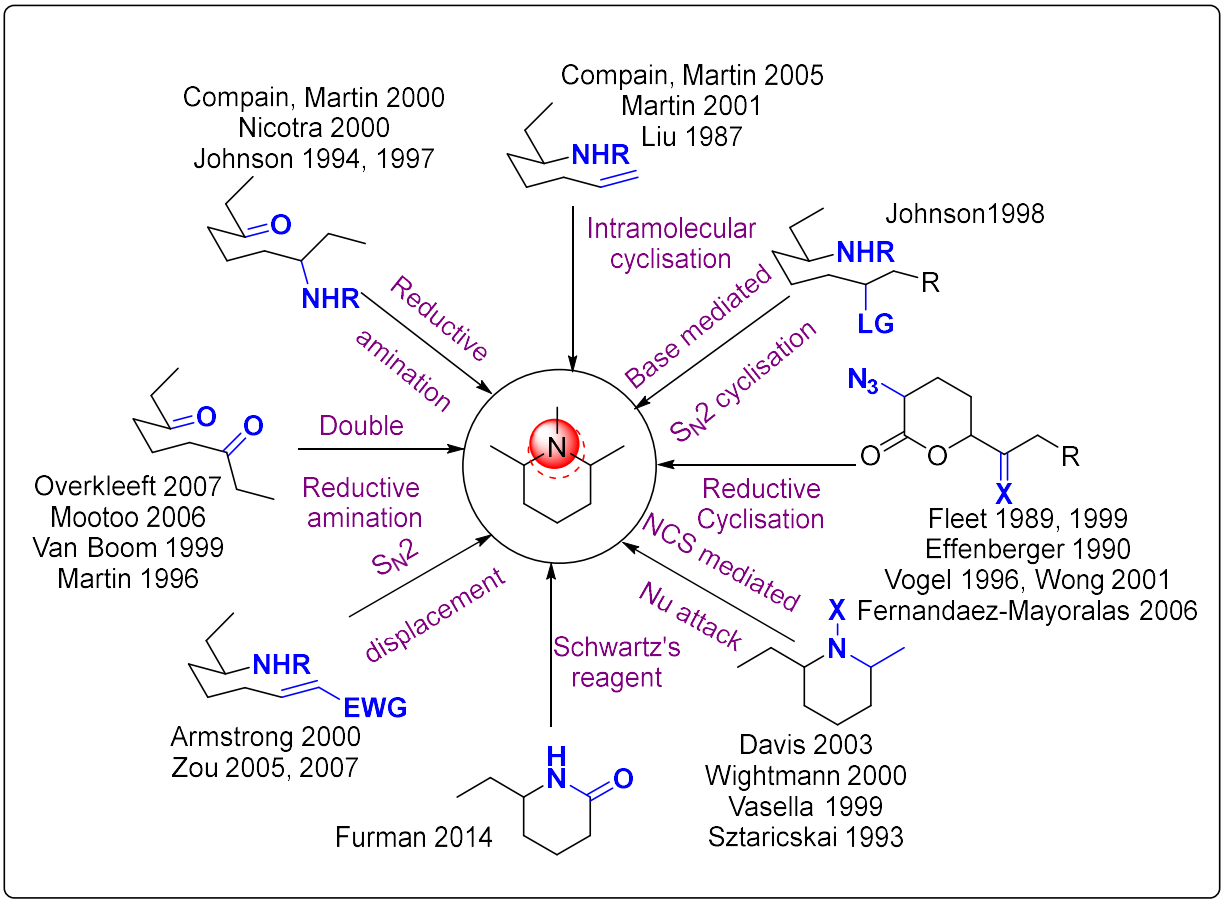


Figure 1.7.1a. Synthetic strategies for iminosugar *C*-glycosides

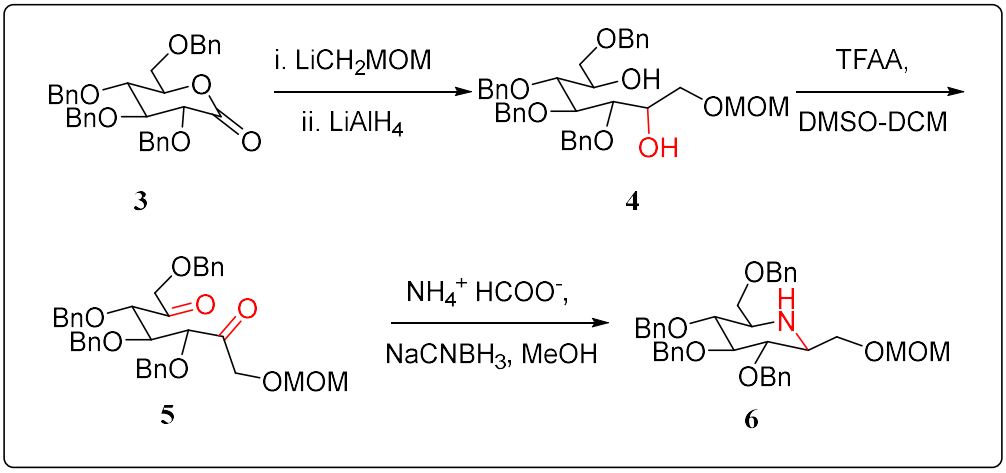
1.7.1. Reductive amination

1.7.1.1. Intermolecular (Double) reductive amination

In iminosugars, owing to the inherent reactivity of amines, the perfect protective group is pivotal. Chemists have used the reactivity of aza group to expand synthetic approaches that aren't pertinent in conventional *C*-glycosides. To date, the most common reaction for synthesizing iminosugar *C*-glycosides is reductive amination. This chemistry lets the advancement of the *C*5–*N* and/or *C*1–*N* bonds in expansion to the change of one or more stereogenic centers at an indistinguishable time. Reductive amination is additionally well suited with a colossal extent of utilitarian groups. The approach of inclination for the synthesis of iminosugar *C*-glycosides is theoretically two-fold reductive amination of dicarbonyl sugars. *C*5–*N* and *C*1–*N* bonds can be

stereoselectively created to collect the piperidine ring in a single synthetic process and intermolecular creation of amine capability offers concurrent entry to a whole lot of N-substituted iminosugar *C*-glycosides. However, literature has proven this technique to be the most effective application for the synthesis of iminosugar β-*C*-glycosides in good yields.[45,46]

In 1996, Martin and Saavedra disclosed the first double-reductive amination synthesis of six-membered iminosugar *C*-glycosides.[47] In two steps, the lactone compound derived from D-glucose was converted into a diol molecule. The diol was then oxidized under TFAA-DMSO conditions. Following the formation of the diketone, it was subjected to a reductive amination reaction in the presence of ammonium formate and sodium cyanoborohydride. The β-homonojirimycin compound was formed as a result of the final deprotection steps.

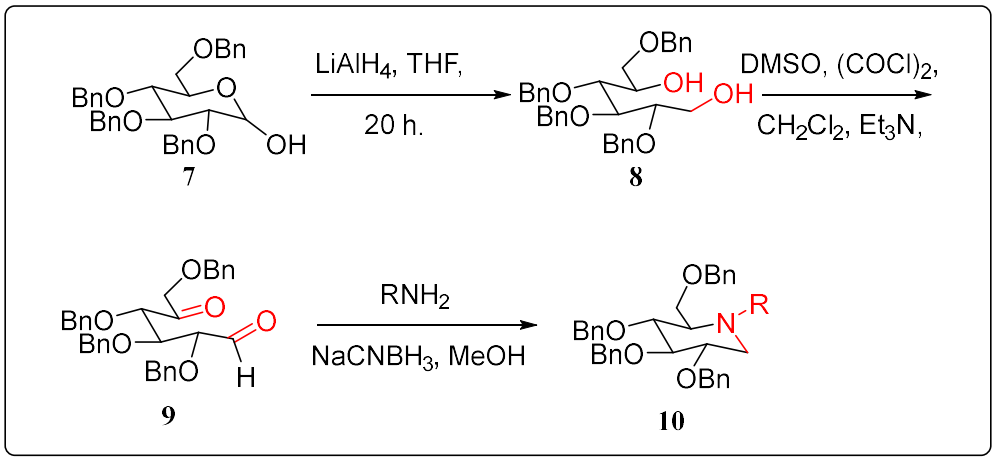


Scheme 1.7.1.1a. First double reductive amination method

Van Boom and Mootoo *et al.* reported the discovery of an amazing double reductive amination strategy. They described similar approaches for the synthesis of disaccharide mimetics using lactone derivatives. For the reductive amination, Van Boom *et al.* used three diketone substrates derived from lactones in the D-manno, D-gluco, and D-galacto series. In all cases, the reaction yielded only β-disaccharide products, which indicates that the configurations of *C*-2 or *C*-

4 seldom affect the stereochemistry of the amination reaction. With diketone derivatives shielded by various protective groups or substrates containing a lipophilic aglycon part, complete β-selectivity was observed. These results lend support to the generality of the double reductive amination strategy for obtaining piperidine series of iminosugar β-*C*-glycosides.[45,48–50]

Deoxy iminosugar and its *N*-alkyl iminosugar derivatives are also synthesized through double reductive amination. Using this approach, several reports were published, resulting in a variety of biologically active 1-deoxyiminosugar analogues. This strategy was applied by Overkleeft *et al.* in 2007 to synthesize *N*-alkyl-1-deoxyiminosugar molecules of biological interest. Swern oxidation of diol, which was synthesized from hemiacetal component of glucose, was used to create the diketo compound. Later, the diketo group was subjected to conventional double reductive amination with various amines, yielding the anticipated deoxy derivatives.[50]

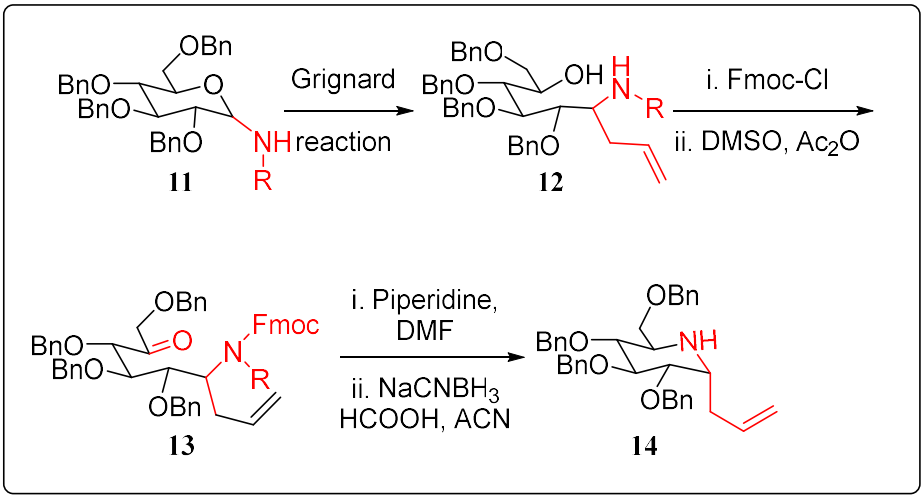


Scheme 1.7.1.2a. Reductive amination method for deoxyiminosugar

1.7.1.2. Intramolecular (Single) Reductive amination:

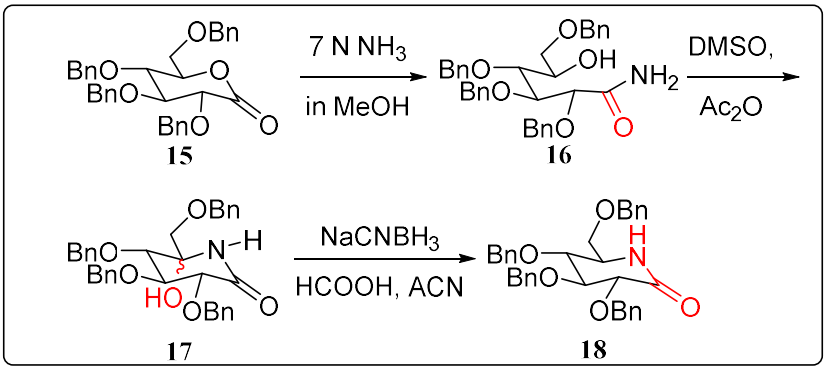
Intramolecular reductive amination reaction, like intermolecular reductive amination reaction, is an efficient technique used for synthesizing iminosugar compounds. Cipolla *et al.* produced one of the best reports on this topic in 2000. The synthesis of allyl-α-*C*-glycoside of nojirimycin was achieved in six steps using a tetrabenzyl glucose hemiacetal molecule. The

methodology for synthesizing *C*-glycoside derivatives described here is high yielding and stereoselective.[51]



Scheme 1.7.1.2.1a. Intramolecular reductive amination method for *C*-glycoside

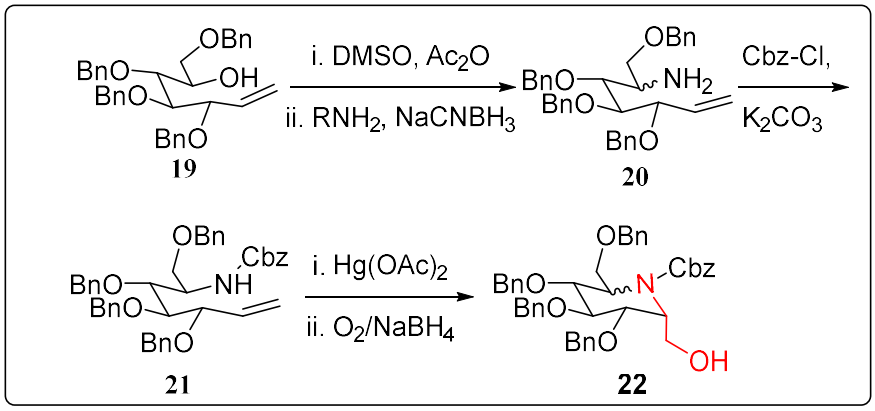
Lactam iminosugars, which may be transformed to iminosugar *C*-glycosides, were synthesized using the same approach. Overkleeft *et al.* used this approach in 1994 to synthesize lactam iminosugars from lactone in three stages. The essential steps, which includes addition of ammonia, oxidation of the *C*-5 hydroxy, and the subsequent reductive dehydroxylative amination reactions to yield a lactam derivative of the iminosugar.[51]



Scheme 1.7.1.2.2a. Intramolecular reductive amination method for lactam iminosugar

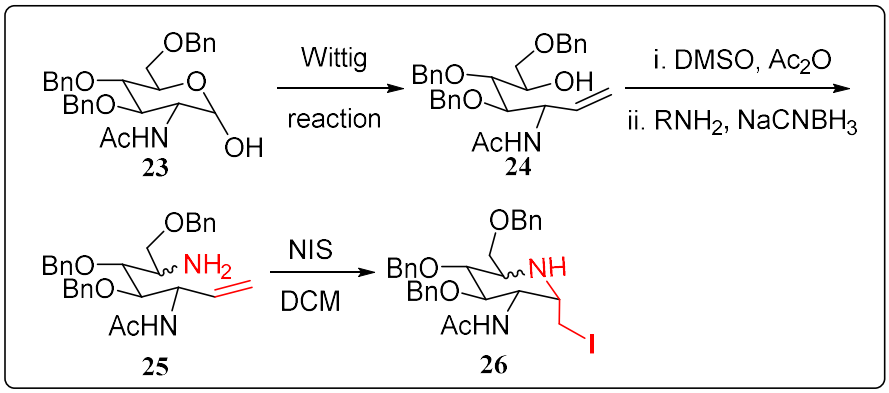
1.7.2. Intramolecular cyclization method

The intramolecular cyclization technique is a popular approach for synthesizing iminosugar *C*-glycosides from aminoalkene derivatives. It is also called the electrophile-induced cyclization tactic. The *C*-glycoside molecules are readily achieved by this aminoalkene cyclization. The technique employs mercuric acetate or triflate and iodo-mediated cyclization procedures. In 1986, Liu *et al.* described the synthesis of *C*-glycosides from aminoalkene molecules. Initially, the process was carried out via mercury-mediated cyclization, which resulted in the formation of a mercury adduct in the molecule. Later, mercury reduction followed by oxygenation with $NaBH_4$-DMF-O_2 resulted in the creation of a protected derivative of the homonojirimycin molecule. This method is one of the most easily achievable methods for producing iminosugar derivatives.[52]



Scheme 1.7.2.1a. Mercury mediated cyclisation method

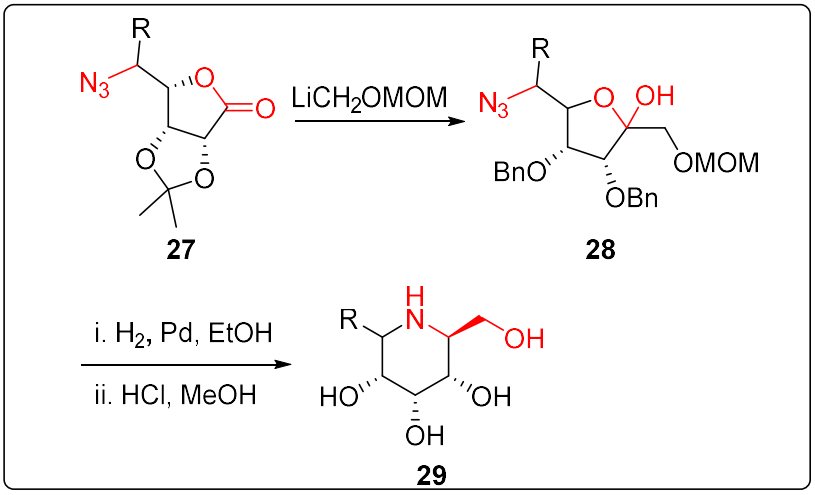
Iodine-mediated reaction is another intramolecular cyclization method. Hsu *et al.* published this method in 2014 for the synthesis of iminosugar *C*-glycoside analogues of α-D-GlcNA*C*-1-Phosphate. The crucial step in this entire synthesis process is the intramolecular iodoamination-cyclization reaction. The cyclization technique employs NIS as an iodine source.[52]



Scheme 1.7.2.2a. NIS mediated cyclisation strategy

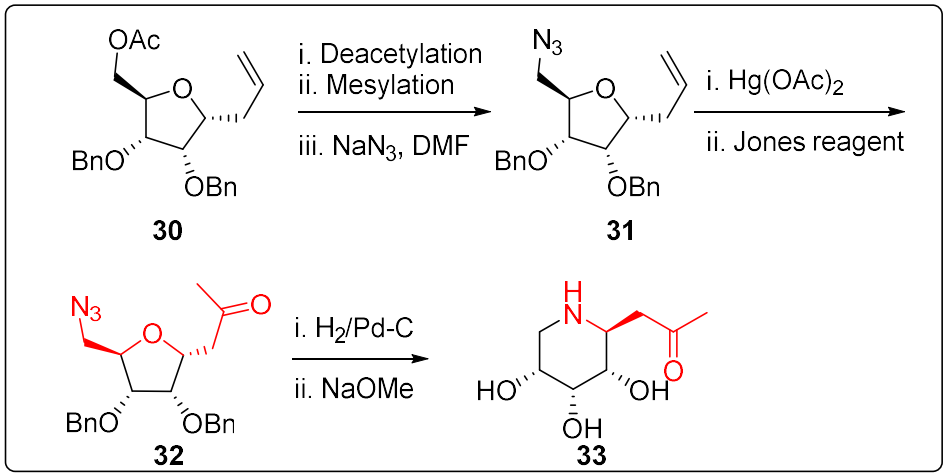
1.7.3. Reductive cyclization method

A reductive cyclization is an intriguing approach for producing iminosugar *C*-glycosides. Under specific circumstances, the azide in the molecule will be reduced to create an amine moiety, which then undergoes cyclization to generate *C*-glycosides. Many synthetic studies based on this approach were widely published. This method was used by Fleet *et al.* in 1999 to synthesize the *C*-glycosides of iminosugars. The reduction of azide in the presence of H_2/Pd-C was carried out, and subsequent treatment with HCl in methanol gave a significant amount of the corresponding molecule.[53,54]



Scheme 1.7.3.1a. Fleet method for reductive cyclisation

In 2005, Zou *et al.* described an intramolecular hetero-Michael addition method for synthesizing aza-*C*-glycosides from 1-*C*-(20-oxoalkyl)-5-amino-5-deoxy-*C*-glycofuranosides. Under normal conditions, simultaneous β-elimination and conjugate addition provide easy accessibility for the bioactive aza-*C*-glycosides derivatives.[55,56]

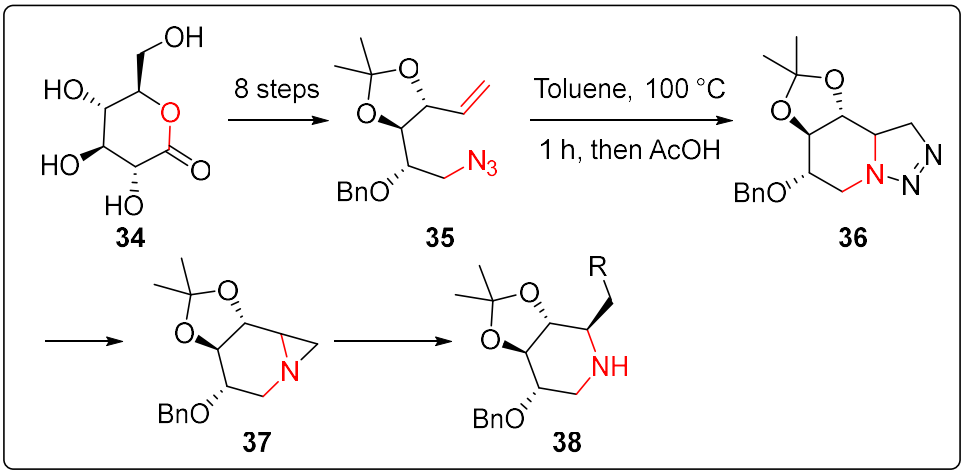


Scheme 1.7.3.2a. Zou method for reductive cyclisation

1.7.4. Miscellaneous methods

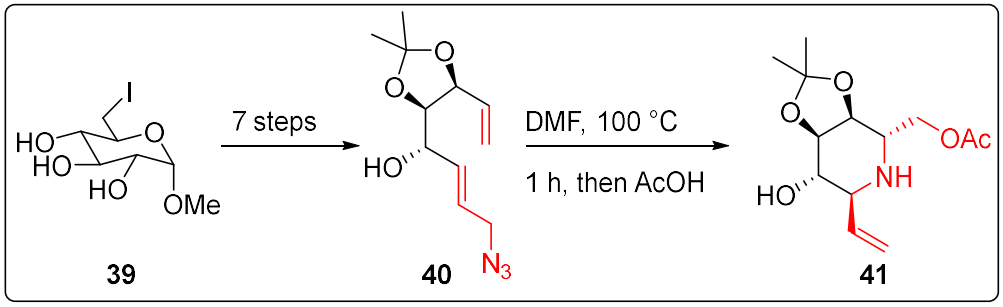
1.7.4.1. Tandem alkene and azide cyclisation method

In 2008, Murphy *et al.* reported Huisgen cycloaddition reaction of alkene and azide. The reaction produced deoxyiminosugars and iminosugar *C*-glycosides. The precursor for this reaction, alkene and azide moiety in the molecule, was generated from lactone in eight steps. The Huisgen cycloaddition reaction was carried out in toluene at 100 °C, followed by the addition of acetic acid to the reaction mixture. The thermally accelerated Huisgen 1,3-cycloaddition produces the triazoline molecule. The subsequent loss of nitrogen from the molecule leads to the formation of aziridine. Nucleophiles later open the aziridine ring to create deoxyiminosugar compounds in an excellent yield.[57]



Scheme 1.7.4.1.1a. Alkene azide cyclization strategy for DNJ

A similar procedure was employed to create iminosugar *C*-glycoside compounds. Murphy *et al.* used an allylic azide and a terminal alkene moiety to synthesize *C*-glycosides at this time. The triazoline molecule was made possible by the tandem allylic azide rearrangement and subsequent Huisgen cycloaddition. The triazoline molecule decomposed in the presence of nucleophiles, resulting in the production of the piperidine ring. They created two stereocenters in a controlled manner throughout this procedure.[58]

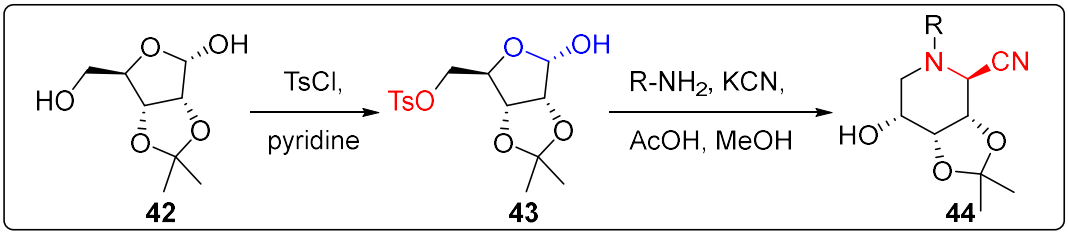


Scheme 1.7.4.1.2a. Alkene and allylic azide cyclization strategy for *C*-glycosides

1.7.4.2. Synthesis of six-membered piperidine compounds from five-membered sugar derivatives.

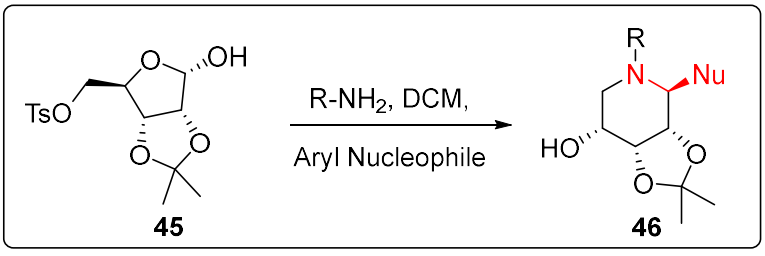
Only hexose carbohydrate molecules have so far been utilized in the production of piperidine *C*-glycosides. For the first time, five-membered sugar derivatives were deployed to

create six-membered iminosugar *C*-glycosides. Hu *et al.* (2014) proposed a collective synthesis strategy for iminosugar *C*-glycoside compounds that included a crucial step involving a surprisingly stable, well-defined ribose-derived iminium salt. A significant variety of iminosugar *C*-glycoside products with excellent stereocontrol were produced upon treating organometallic reagents to this vital intermediate.[59]



Scheme 1.7.4.2.1a. Hu's strategy for six membered *C*-glycosides

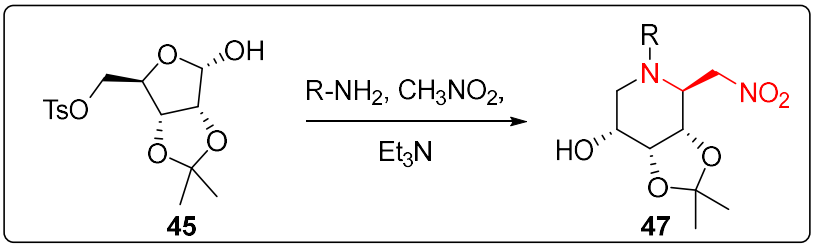
In the same year, Sundarababu *et al.* postulated a simple and effective one-pot technique for producing *C*-aryl glycosides in good yields. This one-pot technique is highly diastereoselective with numerous sensitive functional groups *viz.* –OH, –NH_2, ester, cyclopropyl, propargyl, and alkene. They were stable under the reaction conditions. At room temperature, the novel diversity-oriented method allows simple access to a large spectrum of iminosugar *C*-aryl glycosides. The domino synthesis of synthetically challenging iminosugar-based hybrid compounds highlights the synthetic promise of this method.[60]



Scheme 1.7.4.2.2a. Sundarababu's strategy for six-membered *C*-glycosides

In 2017, the same group published *C*-glycosides from five-membered sugar derivatives. However, the use of nitromethane as a nucleophile under normal conditions resulted in a high yield

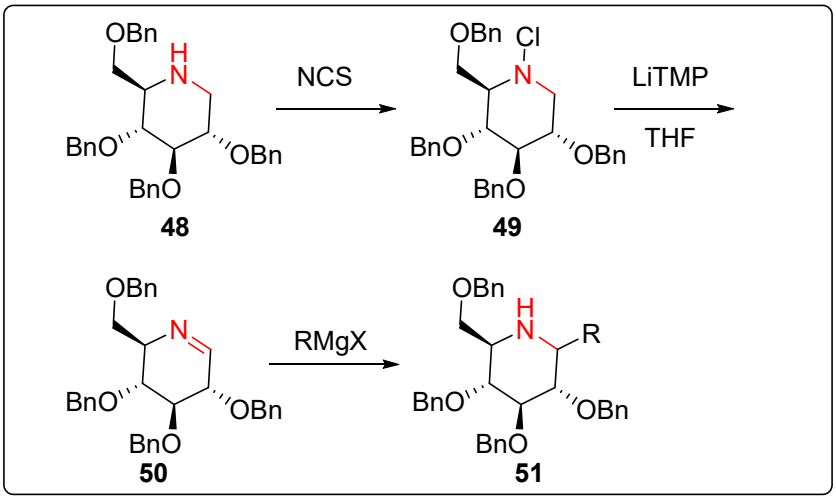
of β-*C*-nitromethyl glycoside. The inclusion of a nitromethyl group in the iminosugar Cglycosides provides these scaffolds a distinct synthetic relevance since the selective transformation of this nitromethyl group allows simple access to the synthesis of a diverse variety of functionalized bicyclic iminosugars.[61]



Scheme 1.7.4.2.3a. Sundarababu's strategy for six-membered *C*-glycosides using CH_3NO_2

1.7.4.3. Iminosugar C-glycosides from 1-deoxyiminosugar derivatives

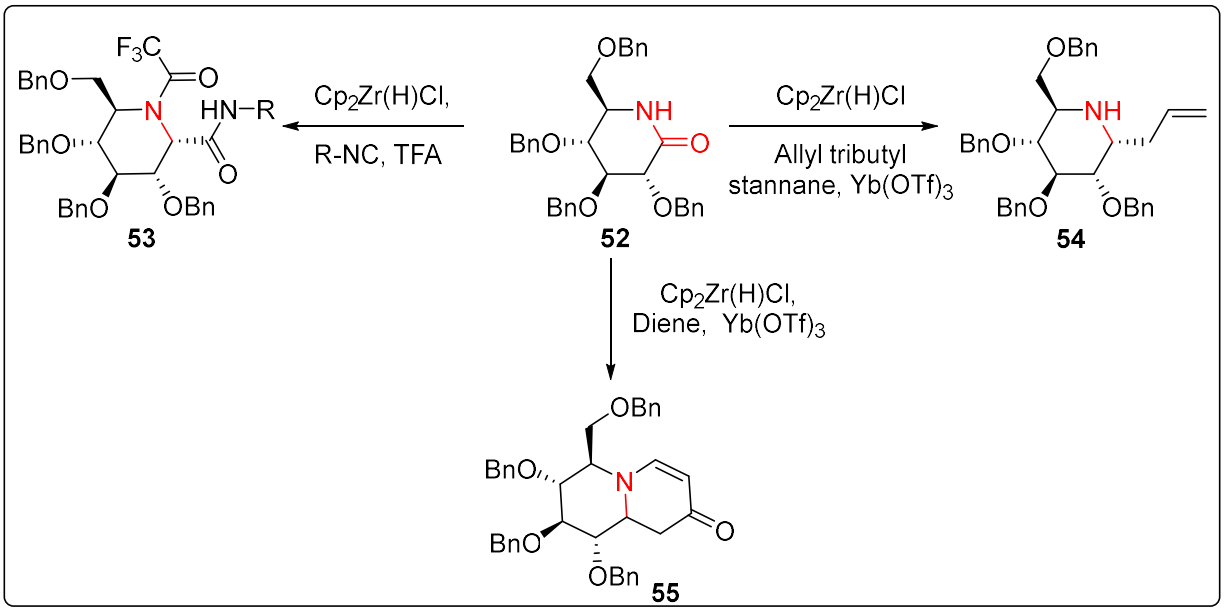
Upon trying various approaches for synthesizing iminosugar *C*-glycosides, this ploy proved to be significantly distinct. The use of iminosugar derivative as the starting material is the reason for the success. Davis *et al.* 2002 and 2003 used deoxyiminosugars to create a *C*-glycoside molecule. The described method begins with chlorination in the presence of NCS, followed by base treatment to produce a cyclic imine product. The cyclic imine was then treated with different Grignard reagents to produce a variety of functionalized iminosugar derivatives of *C*-glycosides. This method works for both pyrrolidine and piperidine deoxyiminosugars.[62–65]



Scheme 1.7.4.3.1a. Strategies using deoxyiminosugars

1.7.4.4. Lactam iminosugars in the synthesis of C-glycosides

This approach, like the synthesis of *C*-glycosides from deoxyiminosugars, is an intriguing way for *C*-glycoside synthesis. To obtain a cyclic imine for the nucleophilic addition reaction with deoxyiminosugars, the molecule must be *N*-chlorinated. In this scenario, however, the lactam molecule will be immediately converted into a cyclic imine product in a single step. In a typical reaction, the Schwartz reagent was employed to initiate the imine production process. Furman *et al.* used the same procedure to synthesize different *C*-glycoside derivatives of biological relevance. Initially, Furman used Zirconium as a catalyst in the imine formation process. Because of the effectiveness of the imine creation, they used it in several techniques for producing *C*-glycoside based on allytributylstanne addition and multi-component reaction.[66] Later, in 2014, the same cyclic imine was utilized to synthesize bicyclic iminosugar by using the appropriate diene as a nucleophile. Furman developed this approach for generating different iminosugar compounds of biological relevance.[67–69]



Scheme 1.7.4.4.1a. Strategies using lactam iminosugars

1.8. Therapeutic applications of iminosugars and their biological significance

Glycosidases, glycosyltransferases, glycogen phosphorylases, nucleoside-processing enzymes, a sugar nucleotide mutase, and metalloproteinases are mostly impeded by both natural and synthetic iminosugar compounds. These enzymes partake in several essential biological transformations, and their inhibition can interrupt oligosaccharide synthesis, interfering with all of these actions. Due to their biochemical characteristics, iminosugars are used to treat cancer, diabetes, viral diseases like HIV and hepatitis B and C, lysosomal storage disorders like Gaucher's disease, Fabry's disease, and genetic diseases like cystic fibrosis.[6,49,78–81,70–77]

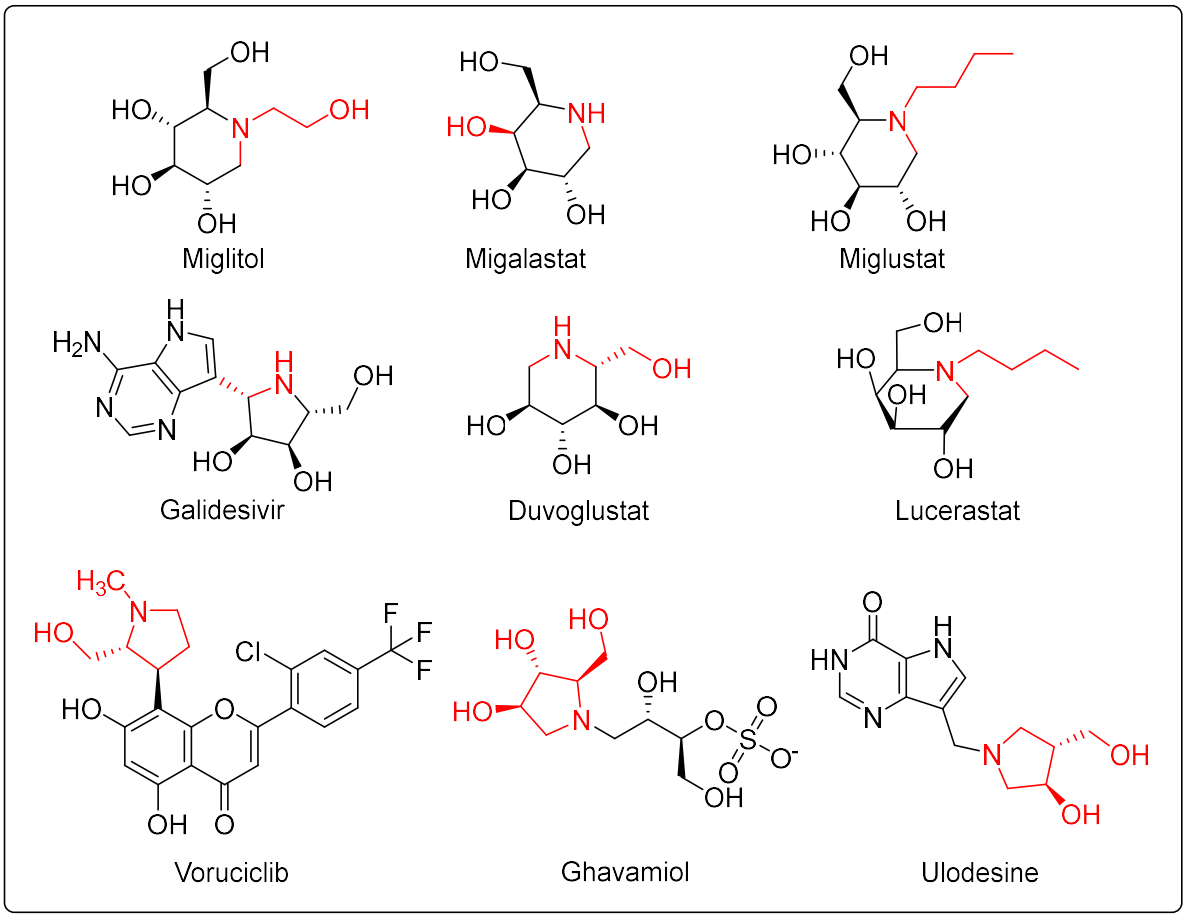


Figure 1.8.1a. Iminosugar drugs and molecuels in clinical trials

Because of the similarities between iminosugars and carbohydrates, they have been identified as receptors that interact with pathways without interfering with their intended functionality. Courtesy of their chemical and metabolic stability, as well as their water solubility, they can easily cross the blood-brain barrier. Even before its discovery, iminosugars were administered as medications for several illnesses. The medicinal formulations of mulberry leaves were used in China to cure diabetes in the 17th century. Iminosugars, including DNJ and its glycosides, are now known to be the primary components of Morus alba leaves. This discovery contributed to the growth of deoxynojirimycin derivatives in modern medicine as diabetes inhibitors with enhanced activity and the ultimate introduction of Miglitol as a commercial diabetic medication in 1996. Another *N*-alkyl deoxyiminosugar Miglustat got approval from FDA and was

prescribed for individuals with mild to moderate type I Gaucher disease who aren't suited to undergo complete enzyme replacement. Migalastat is an alpha-galactosidase inhibitor that is used to treat Fabry disease in people who have a GLA variation that is susceptible to it. In clinical studies, ulodesine was used to treat Gout, Arthritis, Hyperuricemia, and Joint Disease. Voruciclib, a five-membered iminosugar derivative linked to the chalcone is being investigated for the treatment with B-cell malignancies or acute myeloid leukemia in a phase I research. In clinical study NCT03425539, lucerastat is being tested for its effectiveness and safety on oral monotherapy in adult subjects with Fabry Disease. Galidesivir inhibited a wide range of RNA viruses, including coronaviruses, filoviruses, and arenaviruses. The drug's safety in humans is now under phase 1 clinical trials. It is seen as a potential COVID-19 treatment candidate due to its activity against other coronaviruses.

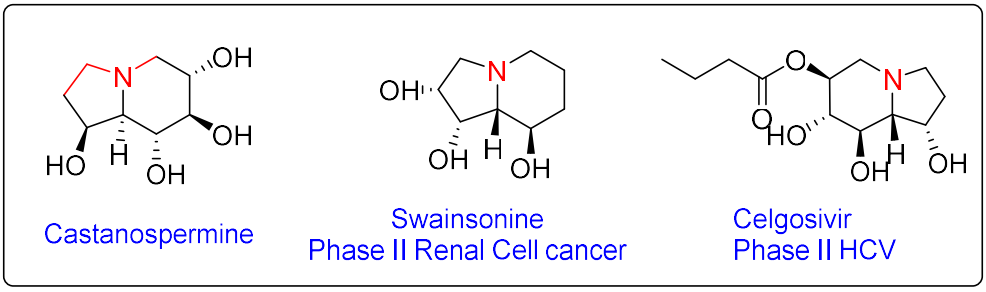


Figure 1.8.2a. Bicyclic iminosugars in Clinical trials

Bicyclic iminosugars, including piperidine iminosugars, have been proven to be more powerful therapeutic entities. Castanospermine, a bicyclic iminosugar, is a potent inhibitor of the dengue virus. Celgosivir, a derivative of castanospermine, is an antiviral candidate currently being scrutinized for the treatment of hepatitis C virus (HCV) infection.[82] Swainsonine, like celgosivir, is a bioactive bicyclic iminosugar that is in phase II clinical studies for renal cell carcinoma.

Despite promising developments, the fate of the iminosugars in clinical applications has been hindered by unpredictable activity and poor clinical selectivity, both of which result in

substantial side effects. Finding novel targets with enhanced activity and selectivity ensures better bioactivity. At the same time, the proposed approach should also address the challenges associated with synthesis and purification.

1.9. Conclusion and present work

Iminosugars are a class of carbohydrate mimics with excellent biological activity. They are highly water soluble compounds having a low molecular weight. Compared to other small carbohydrates, they stand out due to their efficient absorption and biological and chemical stability. Because of their capacity to interact with the biological system while remaining in the most stable state, they always find a noteworthy position in the field of glycobiology. In therapeutic applications, iminosugars have been hampered by variable action and poor clinical selectivity, both of which resulted in significant side effects. Seeking out new targets with improved activity and selectivity, guarantees that therapeutic efficacy is strengthened. In this context, different methods are utilised to synthesize strained aziridine bicyclic iminosugar, which is the topic of the chapter two, prepared for glucosidase inhibition. Because of miltefosine's significance in the biological domain as anti-cancer and anti-parasitic, the third chapter focuses on the synthesis of iminosugar miltefosine conjugate. The fourth chapter is about the synthesis of iminosugar *C*-glycosides using HWE reaction to create deoxynojirimycin analogues, which were used in immunomodulatory investigations alongside 1-deoxy-*N*-alkyliminosugars and *N*-alkyllactam iminosugars. The final chapter uses a simple strategy to demonstrate efficient 1,3-diyne coupling reactions on iminosugar terminal alkynes without the use of metals or bases.

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Chapter 2

Synthesis of novel iminosugar based aziridines

2.1. Abstract

The focus of this chapter is on the synthesis of novel analogues of iminosugars such as aziridines of biological relevance as glucosidase inhibitors. Attempts to accomplish the synthesis of methods for iminosugars were described, which include the conventional amination and novel strategies. During these attempts, several unexpected results were obtained.

2.2. Introduction

Iminosugars are potent inhibitors of glycosidases and glycosyltransferases, and are widely used for treating cancer, diabetes, lysosomal storage disorders, and viral diseases. The addition of alkyl or aryl substituents at the N-/C1-position, as well as stereochemical switching, can change the potency and specificity of monocyclic or bicyclic iminosugars.[1–7] The two N-alkyl derivatives of nojirimycin viz. Glyset® and Zavesca® are used as oral prescribed drugs for type II diabetes and Gaucher disease, respectively. Bicyclic iminosugars such as pyrrolizidines, indolizidines, and other bicyclic compounds have demonstrated inhibitory activity with several carbohydrate-processing enzymes to date. Natural bicyclic iminosugars are known for their excellent biological properties viz. swainsonine, castanospermine,[8] and its derivative celgosivir were reported for their antitumor and immunosuppressive activities.[9–11] Celgosivir is in phase II clinical trials for treating acute dengue fever, hepatitis C, and HIV.[12,13] Australine is a specific inhibitor of amyloglucosidase that possesses antiviral and anti-HIV activities.[14–16] Other iminosugars such as casuarine,[17] hyacinthacine A2,[16] and steviamine[18] are well known for their glycosidase inhibitory activity. The bio-efficacy of iminosugars has sparked immense interest in the synthetic community for

developing efficient and gentle synthetic strategies by the chiral pool or enantio- and diastereoselective methodologies.

Carbohydrate aziridines or epimines are formed when aziridine is fused to a pyranose or furanose ring or an exocyclic part of a carbohydrate.[19] Cyclophellitol, a naturally occurring substance derived from *Phellinus* sp. and β-glucosidase inhibitor, is a cyclitol mimic of glucose with an epoxide group.[20] Tatsuta *et al.* developed an aziridine derivative of cyclophellitol and demonstrated its strong glucosidase inhibitory action.[20] Following that, a number of *N*-functionalized cyclophellitol aziridine derivatives, analogues such as configurational cyclohexenol isomers, and deoxygenated cyclophellitol aziridines were synthesized and described as glycosidase inhibitors.[21–25] These aziridine derivatives, which are 7-azabicyclo[4.1.0]heptanes, are Type I fused-ring aziridines.

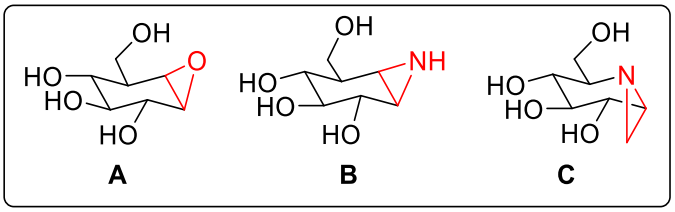
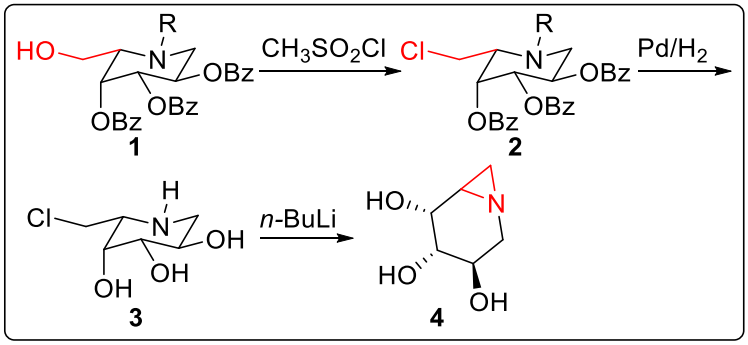


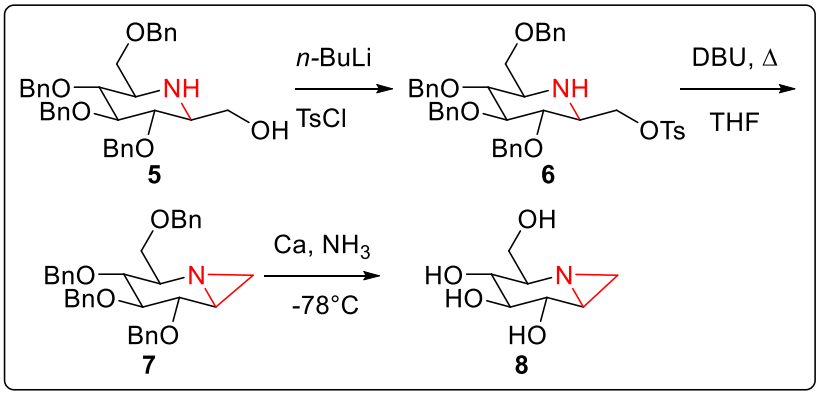
Figure 2.2.1a. Cyclophellitol (A) and its aziridine derivative (B), and bicyclic aziridine iminosugar (C)

Type II fused-ring aziridines, such as 1-aza-3,4,5-trihydroxybicyclo[4.1.0] heptane, are bicyclic aziridine iminosugars. One such aziridine was synthesized from 1-deoxygalactonojirimycin via intramolecular cyclization and has been reported by Tong *et al.* as a selective α-galactosidase inactivator.[26] Paulsen *et al.* reported a 1-deoxy-L-galactonojirimycin-derived aziridine iminosugar derivative in 1990. Conversion of *C*-6 hydroxy to chloride, followed by deprotection and base-mediated intramolecular cyclization are the main steps. The synthesized compound was found to be an inhibitor of alpha L-fucosidase.[26]



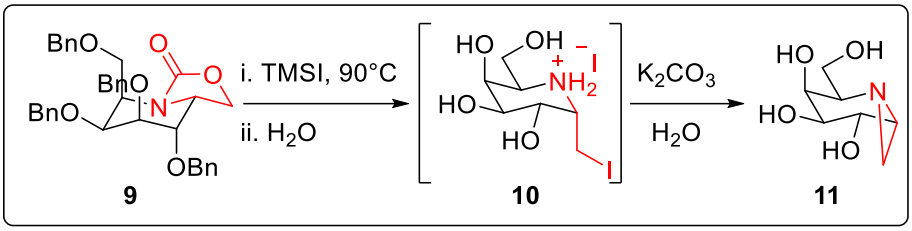
Scheme 2.2.2a. Synthesis of 1-deoxy-L-galactonojirimycin-derived aziridine iminosugar

Martin *et al.* reported the first chemical synthesis of homonojirimycin's β-anomer and its 1, *N*-anhydro derivative, a potential glucosidase inactivator, in 1995. They synthesized the β-homonojirimycin compound as a single isomer in a 50% yield using the double reductive amination strategy. The three-step synthesis of bicyclic aziridine iminosugar **8** from β-homonojirimycin **5** is also mentioned in the report. The formation of the aziridine ring was facilitated by the base-mediated intramolecular cyclization.[27]



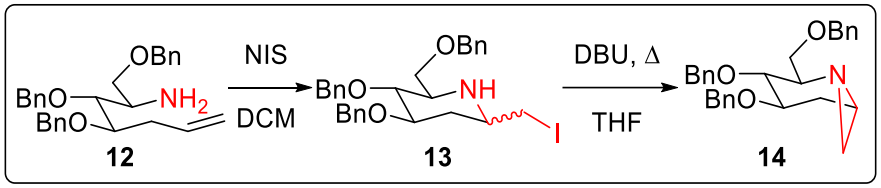
Scheme 2.2.3a. Synthesis of aziridine iminosugar from β-homonojirimycin

The following year, Martin *et al.* published their galactose-based synthesis of homogalactostatin and its 1, *N*-anhydro derivative. They used a TMSI-mediated debenzylation which resulted in the ring-opening of bicyclic sugar to form the iodo derivative intermediate, which was then treated with K_2CO_3-mediated cyclization to afford aziridine **11**.[28]



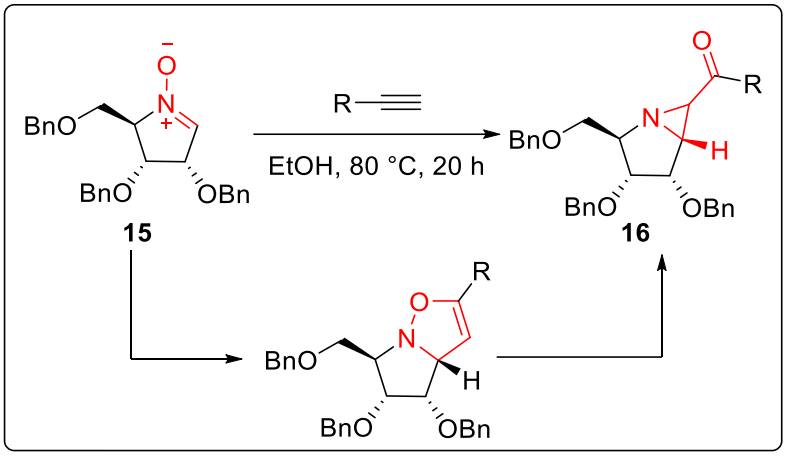
Scheme 2.2.4a. Synthesis of galactose derived aziridine iminosugar

Goujon *et al.* in 2005 reported the NIS-mediated cyclization of aminoalkene derivative and subsequent intramolecular cyclization leading to the formation of an aziridine molecule. They also showed that the aziridine obtained from iminosugar was an adaptable intermediate for synthesizing fagomine *C*-glycosides and congeners with a wide range of functional groups at the anomeric position.[28]



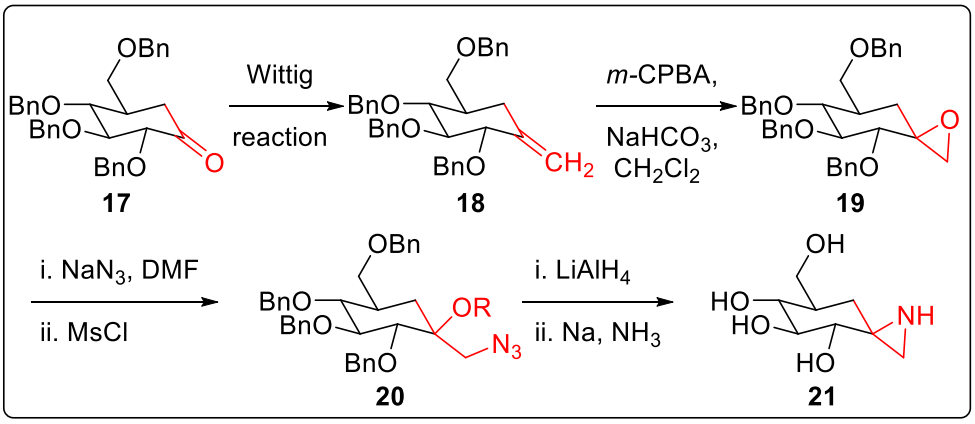
Scheme 2.2.5a. Synthesis of fagomine derived aziridine iminosugar

Tangara *et al.* demonstrated that carbohydrate-derived nitrone intermediates are effective in synthesizing aziridine iminosugars. The reported method employs Baldwin rearrangement of isoxazolines to produce a stable aziridine derivative. This strategy employs only carbohydrate-derived nitrones and alkynes in the reaction. The strategy allows the revelation of completely undiscovered 1-azabicyclo-hexanes and 1-azabicyclo-heptanes with an aziridine ring substituent.[29]



Scheme 2.2.6a. Synthesis of aziridine iminosugar from carbohydrate-derived nitrones

Spiroaziridines were developed by Vasella *et al.* as potential inhibitors of alpha and beta glucosidases. The reaction began with cyclohexanone derived from validoxylamine A. Wittig reaction, epoxidation, S_N2 sodium azide ring-opening, and reductive cyclization reactions are the important steps in the production of spiroaziridines.[30]



Scheme 2.2.7a. Synthesis of aziridine iminosugar from validoxylamine

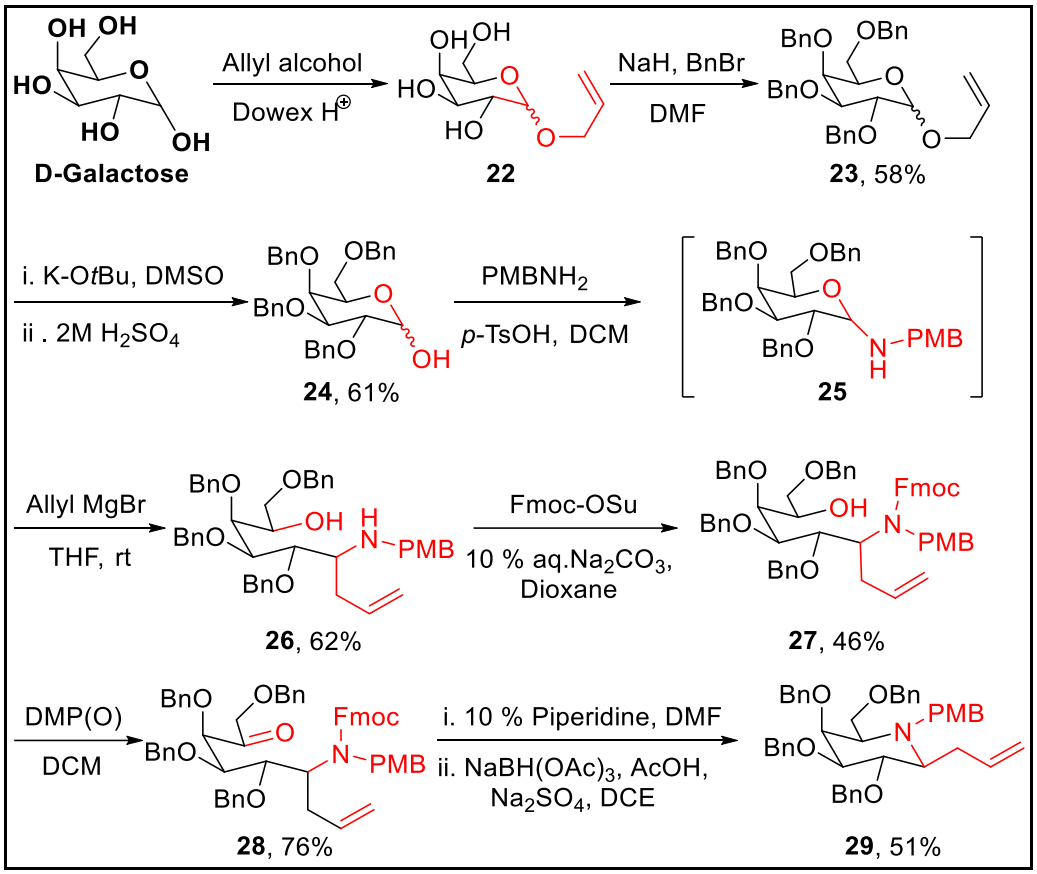
Due to their prominence in the biological domain and inherent synthetic challenges, a novel and feasible route were used for the synthesis of iminosugar *C*-glycosides and subsequent conversion of bicyclic iminosugar. This chapter discusses the attempts made to synthesize iminosugar *C*-glycosides and the successive creation of the bicyclic iminosugar aziridine.

2.3. Results and Discussion

The objective of this chapter is to synthesize aziridine based iminosugars with alteration of stereochemistry in order to obtain novel analogues. Hence, all the attempts for obtaining iminosugars were initiated with D-galactose as most of the existing iminosugars have D-glucose origin.

2.3.1. Attempt by intramolecular reductive amination

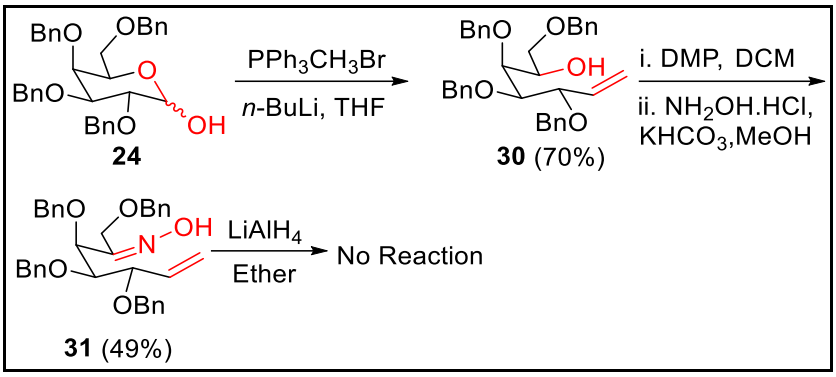
D-Galactose was converted in four steps to benzyl-protected hemiacetal **24** by following conventional methods. Hemiacetal **24** was treated with *p*-methoxybenzylamine and *p*-TsOH that resulted in compound **25**, which was subjected to Grignard reaction conditions, without purification, by using allyl magnesium bromide to obtain amine **26**. The secondary amine was protected using Fmoc group followed by oxidation of the secondary alcohol in the presence of Dess-Martin periodinane afforded a keto compound **28** that was subjected to intramolecular ring closing for obtaining iminosugar. Piperidine-mediated Fmoc deprotection, followed by dehydroxylative reductive amination processes yielded the aza-β-*C*-glycoside **29**. The intention was to create the α-*C*-glycoside analogue followed by functionalization of the olefin by double bond isomerization and intramolecular ring closing by treatment with NBS. As the method resulted in the formation of aza-β-*C*-glycoside, we sought an alternate route for iminosugar.



Scheme 2.3.1.1a. Synthesis of aza-β-*C*-glycoside **29**

2.3.2. Attempt by using oxime intermediate

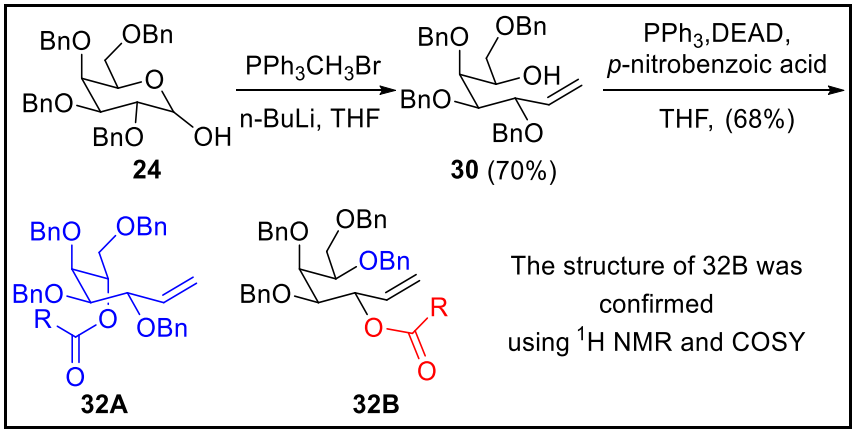
In the second attempt, hemiacetal **24** was subjected to Wittig reaction conditions to afford the alkenol **30** in 70% yield. Oxidation of the secondary alcohol with Dess-Martin periodinane and further treatment with hydroxylamine hydrochloride resulted in an oxime **31**. Attempts to reduce oxime to amine led to no reaction.



Scheme 2.3.2.1a. Synthesis of oxime **31**

2.3.3. Attempts by Mitsunobu reaction

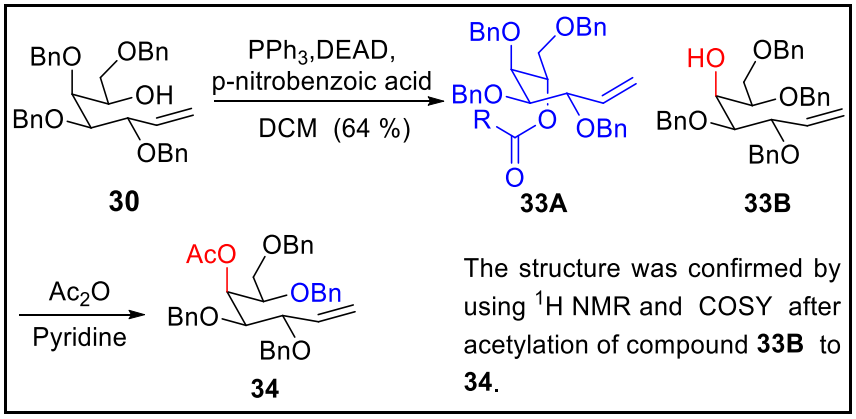
Initially, the intention was to apply a double Mitsunobu reaction method to synthesize the necessary *C*-5 amine stereoisomer. Accordingly, the Wittig product obtained from hemiacetal compound **24** was subjected to Mitsunobu reaction, using THF as the solvent.



Scheme 2.3.3.1a. Mitsunobu reaction on Wittig product **30**

The Mitsunobu reaction progressed well but afforded compound **32B** with the migration of benzyl from *C*-2 to *C*-5 position. The ester incorporation in the compound was confirmed using NMR spectroscopy. The migration of the benzyl and ester formation was confirmed using COSY correlations and HRMS. To obtain the expected Mitsunobu reaction product, the reaction was carried out by varying the solvents. The Mitsunobu reaction yielded a product with the same polarity as the starting material while using DCM as the solvent. The NMR analysis of the product

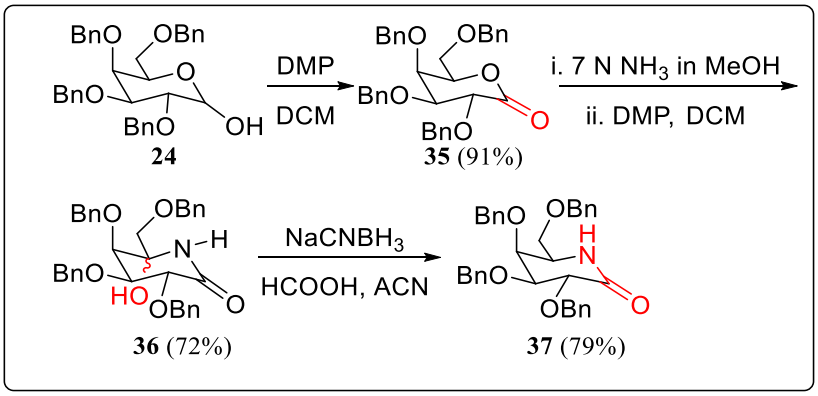
obtained in this reaction exhibited a peak at 2.88 ppm in ^{1}H NMR, which was a hydroxyl group. To confirm the structure of the compound, the product was subjected to acetylation reaction in the presence of Ac_2O and pyridine. The ^{1}H NMR and COSY correlations of the acetylated product revealed the migration of benzyl from *C*-4 to *C*-5 position with a hydroxyl on a *C*-4 position.



Scheme 2.3.3.2a. Mitsunobu reactions on Wittig product with solvent variation

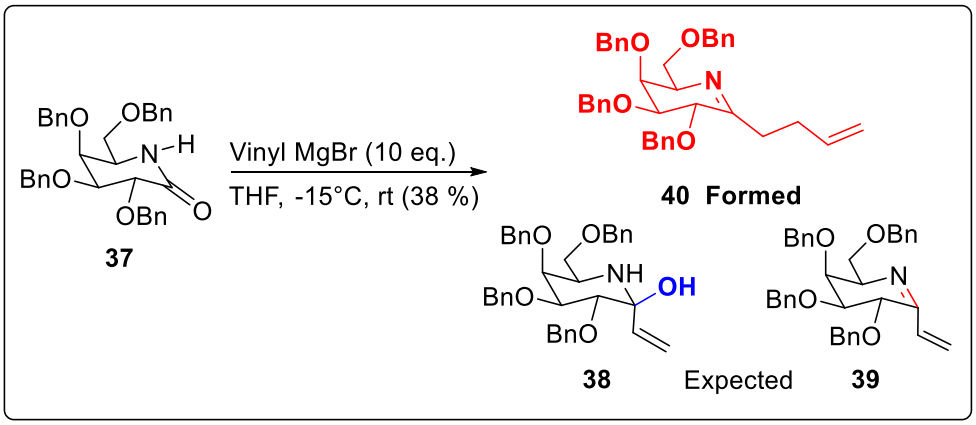
2.3.4. Attempt by using lactam iminosugar

As the oxime and Mitsunobu routes were not successful in synthesising relevant iminosugar intermediate, iminosugar lactam interdmediate was considered in our next attempt. Oxidation of hemiacetal **24** yielded the lactone **35**. Treatment with 7N ammonia in methanol and subsequent oxidation using Dess-Martin periodinane yielded hemiaminal compound **36** in 72% yield. Two methods were used to successfully carry out the dehydroxylative reductive amination. One method was the use of Kishi reduction conditions, while the other was the use of traditional $NaCNBH_3$ and formic acid. Both the reactions afforded respective lactam iminosugar **37**. The structure of the lactam **37** was confirmed by a comparison of spectral data with the reported literature.[31]



Scheme 2.3.4.1a. Synthesis of lactam iminosugar **37**

Initially, unprotected lactam 37 was treated vinyl and allyl magnesium bromides. However, the reaction proceeded only with vinylmagnesium bromide and not with allylmagnesium bromide. Even though the outcome of this reaction was expected to be either **38** or **39**, formation of compound **40** was observed. Imine **40** was obtained by an initial Grignard addition followed by subsequent Michael addition of Grignard reagent. Structure of compound **40** was unambiguously confirmed using ^{1}H and ^{13}C NMR data.



Scheme 2.3.4.2a. Grignard reaction on lactam **37**

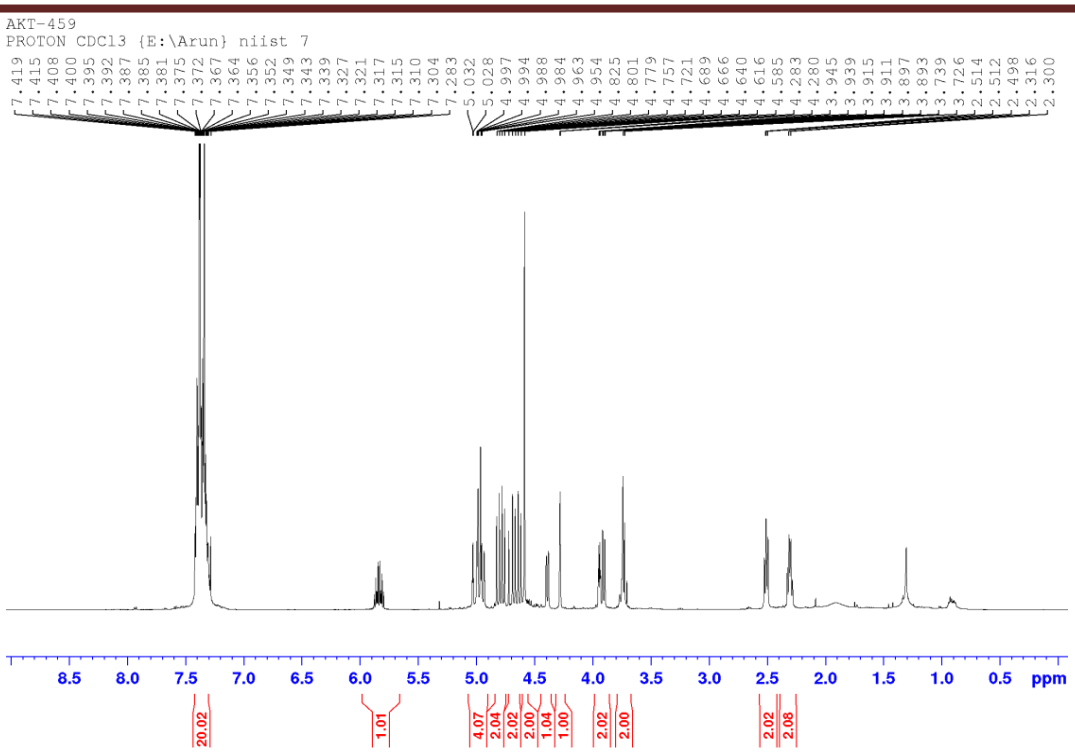


Figure 2.3.4.1a. ^{1}H NMR of compound **40**

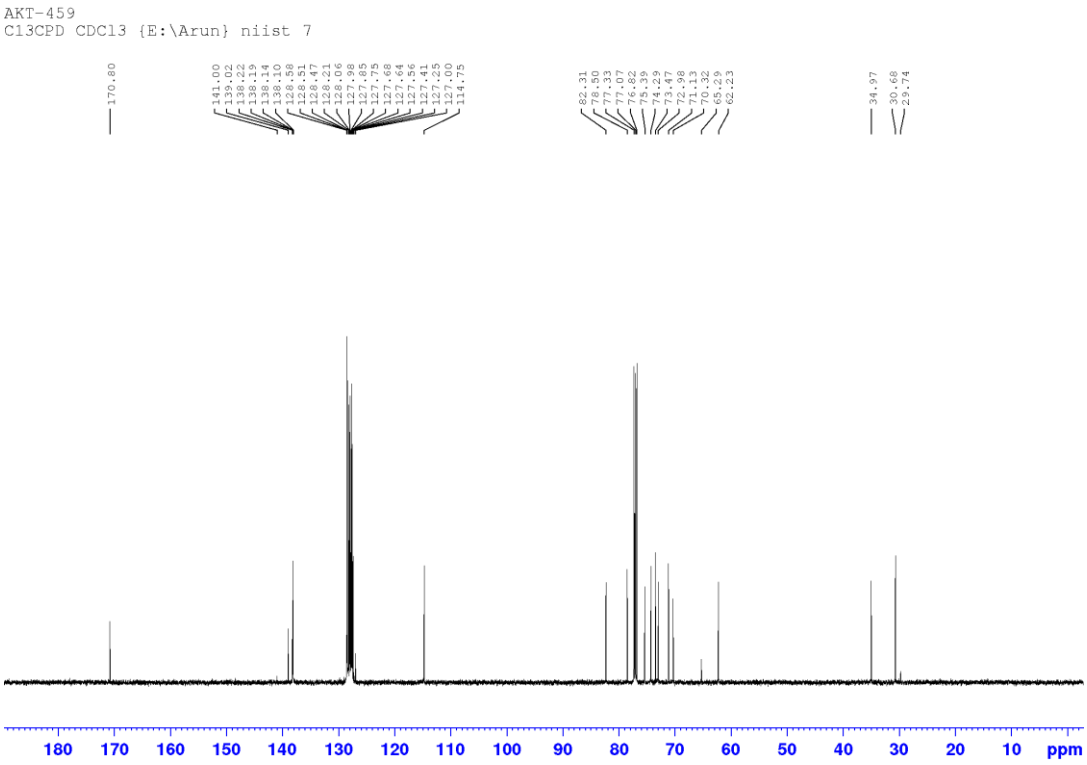
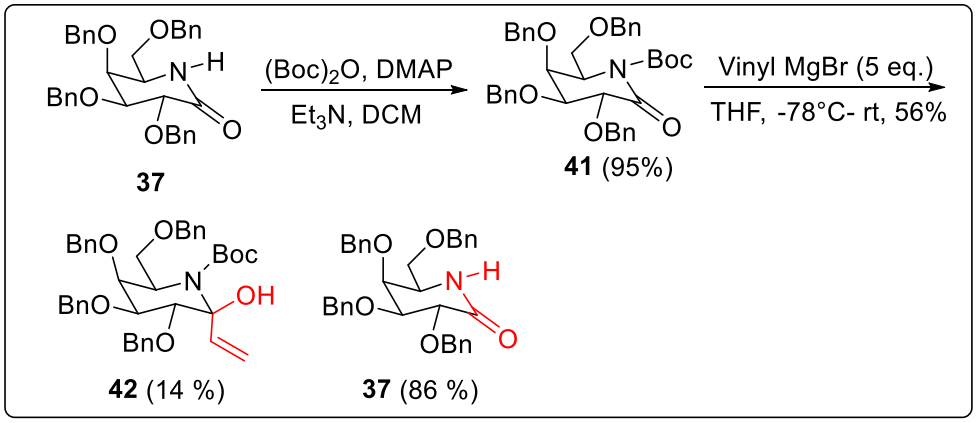


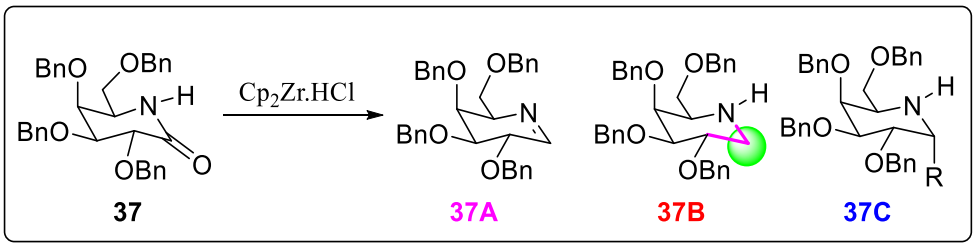
Figure 2.3.4.2a. ^{13}C NMR of compound **40**

As Grignard reaction with unprotected free NH lactam **37** did not yield a fruitful outcome, compound **37** was protected using $(Boc)_2O$ in the presence of DMAP and Et_3N, affording N-Boc lactam **41** in 95% yield. Grignard reaction was performed with vinylmagnesium bromide at -78 °C. The reaction proceeded well and resulted in two different products **42** and **37** in 14% and 86% yields, respectively. After isolation and detailed characterization, the major product **37** was identified as free NH lactam along with the expected product in a minor amount.



Scheme 2.3.4.3a. Grignard reaction on protected lactam **41**

Table: 2.3.4.1a. Addition reaction attempts on lactam 37 with Schwartz reagent.



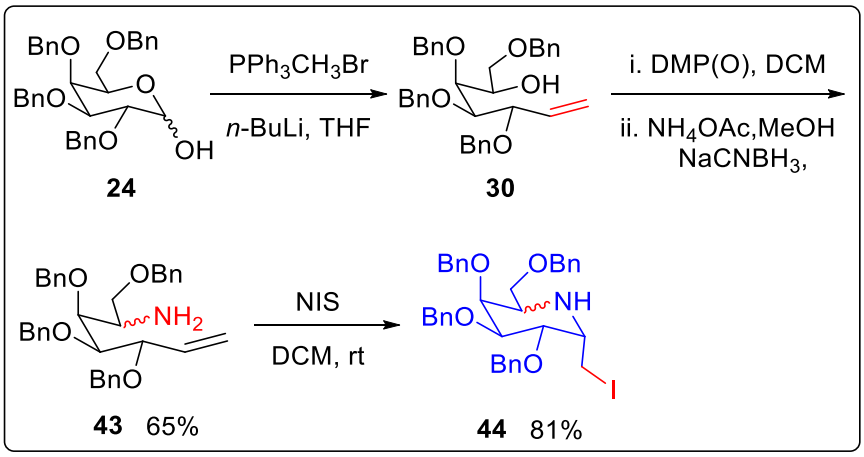
Entry	Lewis acid (1.5 equiv)	Second Reagent (3 equiv)	Temperature	Solvent	Product
1	$Yb(Otf)_3$	Allyltributylstannane	-10 °C		**B**
	-	PhMgBr	-25 °C	DCM	**B**
2	$Yb(Otf)_3$	Allyltributylstannane	-10 °C		**B**

	-	PhMgBr	-25 °C	THF	**B**
3	-	-	-10 °C		**B**
			-25 °C	Toluene	**B**
4	-	-	-10 °C		**B**
			-25 °C	Benzene	**B**

In 2014, Furman *et al*. reported a methodology of using Schwartz reagent for synthesis of iminosugars. The same protocol was adopted for our molecules by addition reaction on compound **37**. The reactions were carried out with variations in temperature, solvents, equivalents of the catalyst (Table: 2.3.4.1a). Unfortunately, the reaction produced a 1-deoxysugar in all the attempts.

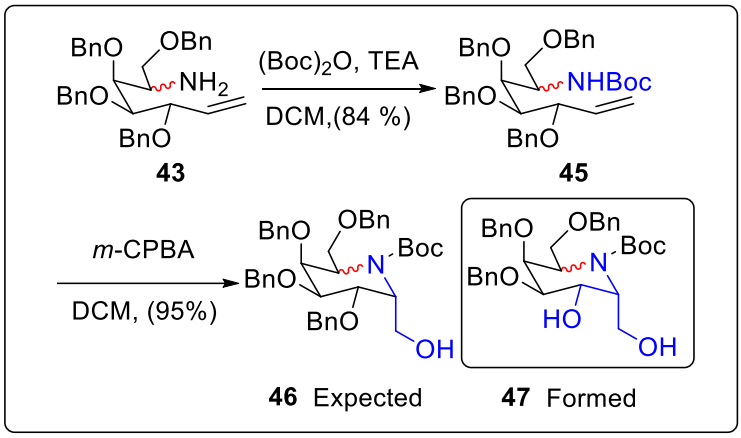
2.3.5. Attempt by using Wittig product

Initially, the Wittig product **30** was oxidized using Dess-Martin periodinane and then subjected to subsequent reductive amination reaction. This resulted in *C*-5 epimeric mixtures of compound **43** in a 65% yield. Furthermore, under NIS-mediated cyclization, amino alkene compound **43** was converted to compound **44** in an 81% yield.



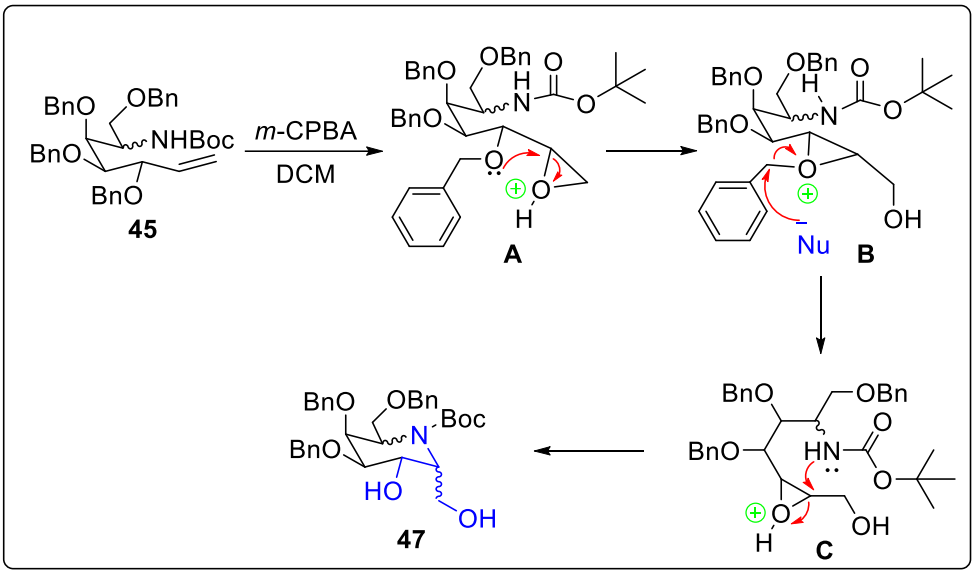
Scheme 2.3.5.1a. Synthesis of compound **44**

Cyclization strategies of amino alkene **43** were carried out before attempting any reactions on compound **44**. Accordingly, the possibility of using the *m*-CPBA epoxidation approach for the cyclization of the amino alkene **43** was explored. Initially, the amine of compound **43** was protected using $(Boc)_2O$, which resulted in compound **45** (84%). Subsequently, the product was subjected to *m*-CPBA epoxidation at a lower temperature. Surprisingly, the reaction gave rise to a 1,3-diol compound **47**, instead of mono hydroxyl compound **46**.



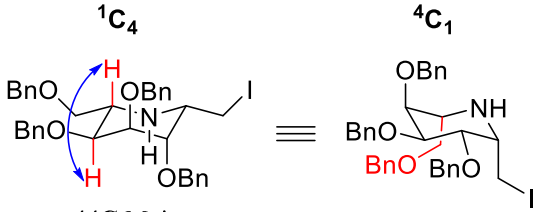
Scheme 2.3.5.2a. Cyclization reaction using *m*-CPBA

The plausible mechanistic pathway behind formation of compound **47** is by mediation of byproduct under *m*-CPBA conditions that initiates the rearrangement process in this reaction by protonating the epoxide. *C*-2 oxygen opens the activated epoxide to form **B** with concomitant nucleophilic attack on benzylic carbon to generate the internal epoxide **C**. Subsequent protonation and epoxide ring-opening by nitrogen led to the formation of 1,3-diol compound **47**.



Scheme 2.3.5.3a. Plausible mechanism in *m*-CPBA reaction in formation of compound **47**

At this stage, we decided to go back to intermediate **44** but the exact stereoisomer of the *C*-5 chiral centre remains unknown. Hence, ^{1}H NMR of **44C** and **44D** epimers were taken in $CDCl_3$ for solving the structure. In $CDCl_3$, NMR was not so clear to facilitate the respective peak identification using COSY. However, the change of NMR solvents from $CDCl_3$ to acetone-d6 gave an excellent NMR splitting pattern which assisted in solving the structure of the respective compound. The 1D COSY helped to identify the *C*-4 proton of **44C**, which showed a coupling constant of 10.5 *Hz*, indicating a $^{1}C_4$ configuration instead of a $^{4}C_1$ configuration.



44C Major

The reductive amination reaction afforded an L- and D-isomer as major and minor compounds, respectively. Compound **44C** revealed that the L-isomer of the compound only underwent *m*-CPBA epoxidation followed by rearrangement. The D-isomer was unreactive in the *m*-CPBA

epoxidation conditions. These results prompted us to focus on iodo derivatives for further transformations.

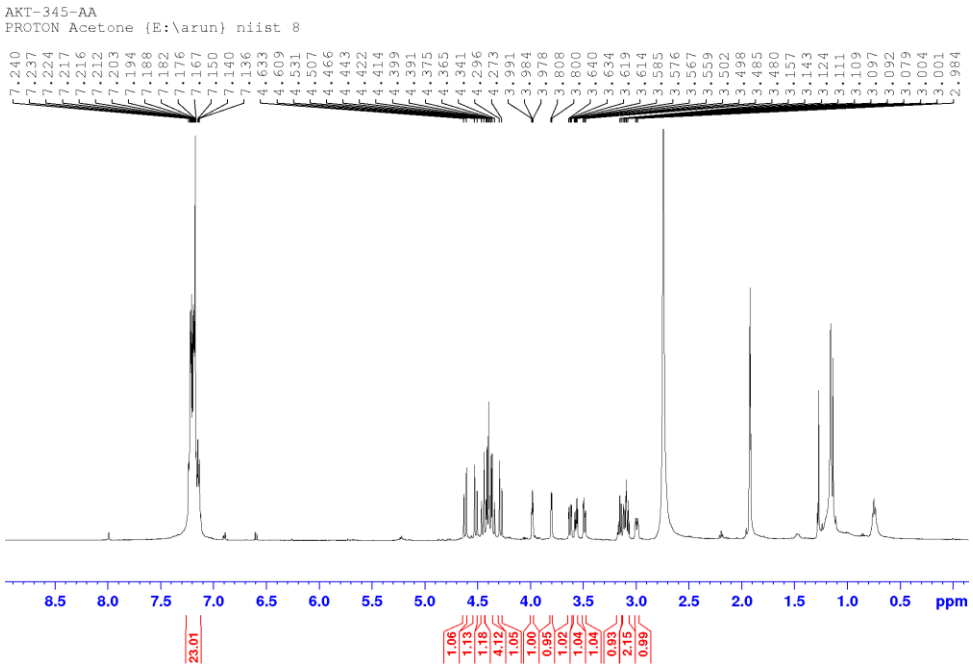


Figure 2.3.5.1a. ^{1}H NMR of compound **44C** in acetone-d_6

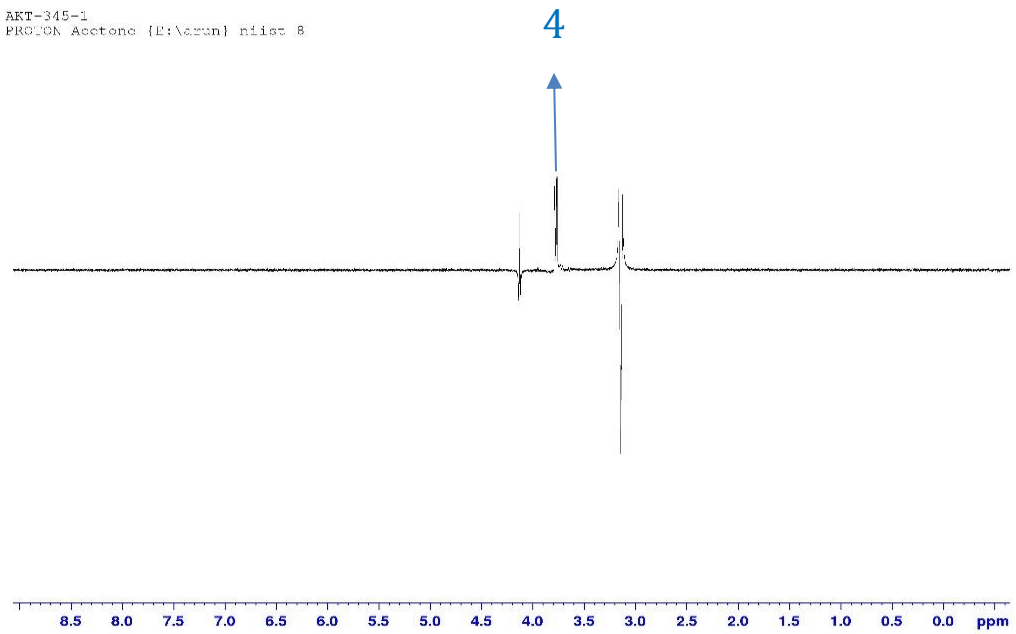
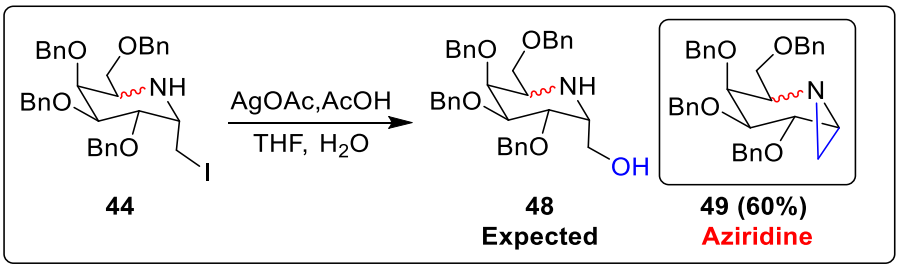


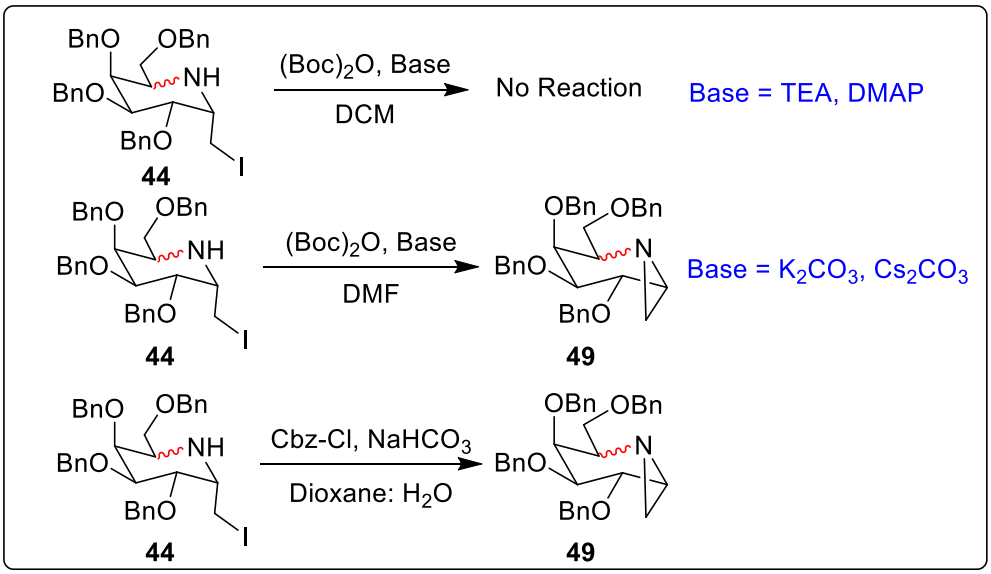
Figure 2.3.5.2a. 1D COSY NMR of compound **44C** in acetone-d_6

The iodo isomer was taken for further reactions. A simple and feasible method from a literature report outlined the conversion of iodo to hydroxyl molecules. The same protocol was adopted in the present scenario. The starting materials were treated with silver acetate and acetic acid in THF/H_2O solvent system. As per previous reports, conversion of iodo to a hydroxyl group was expected, however, a cyclization reaction afforded aziridine.



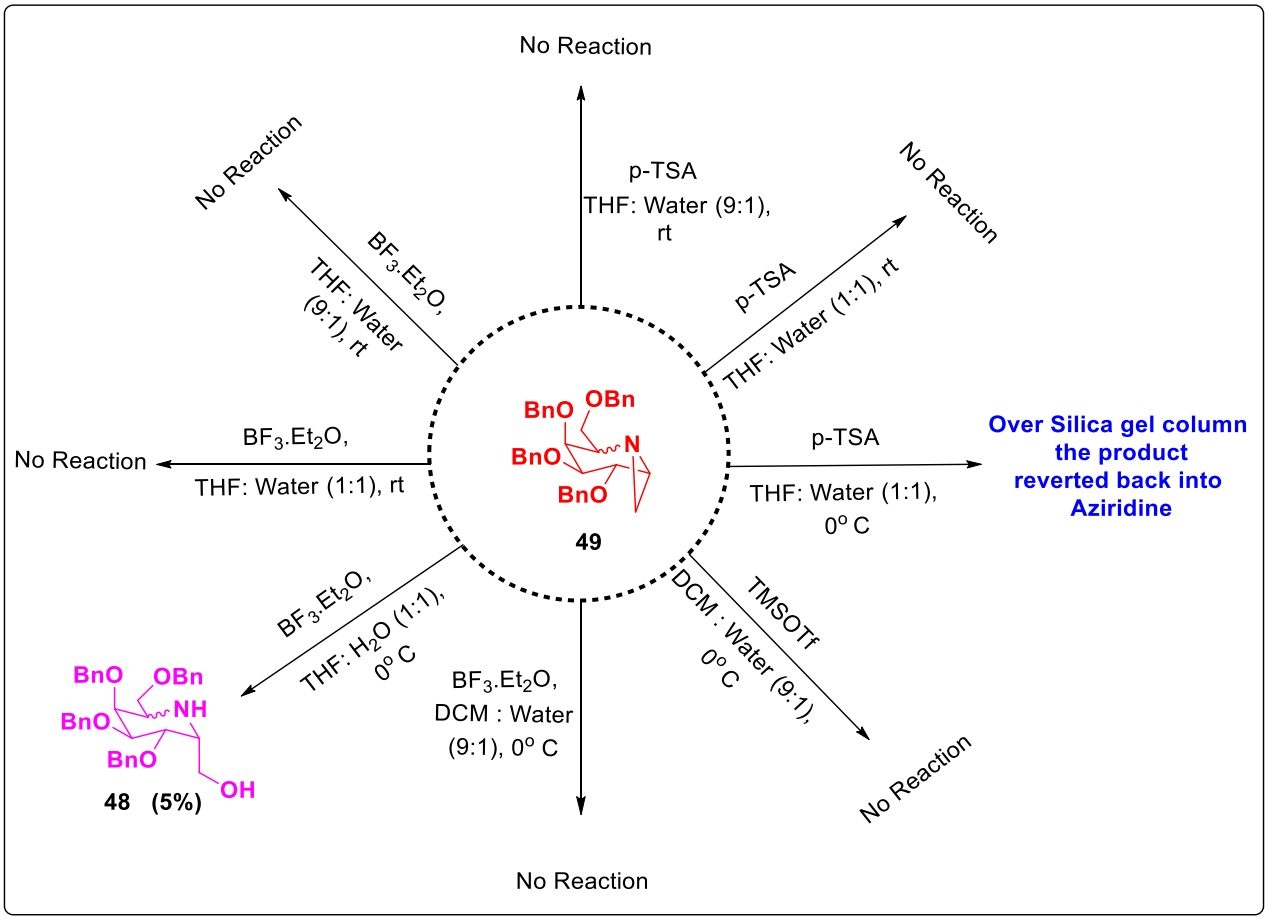
Scheme 2.3.5.4a. Synthesis of aziridine **49**

The aziridine molecule was characterized using NMR and HRMS techniques. At first, due to the presence of the free NH group, a cyclization reaction was expected. Protecting the nitrogen atom with different protecting groups and varying the bases also afforded aziridines.



Scheme 2.3.5.5a. Attempts of NH protection of compound **44** leading to aziridines

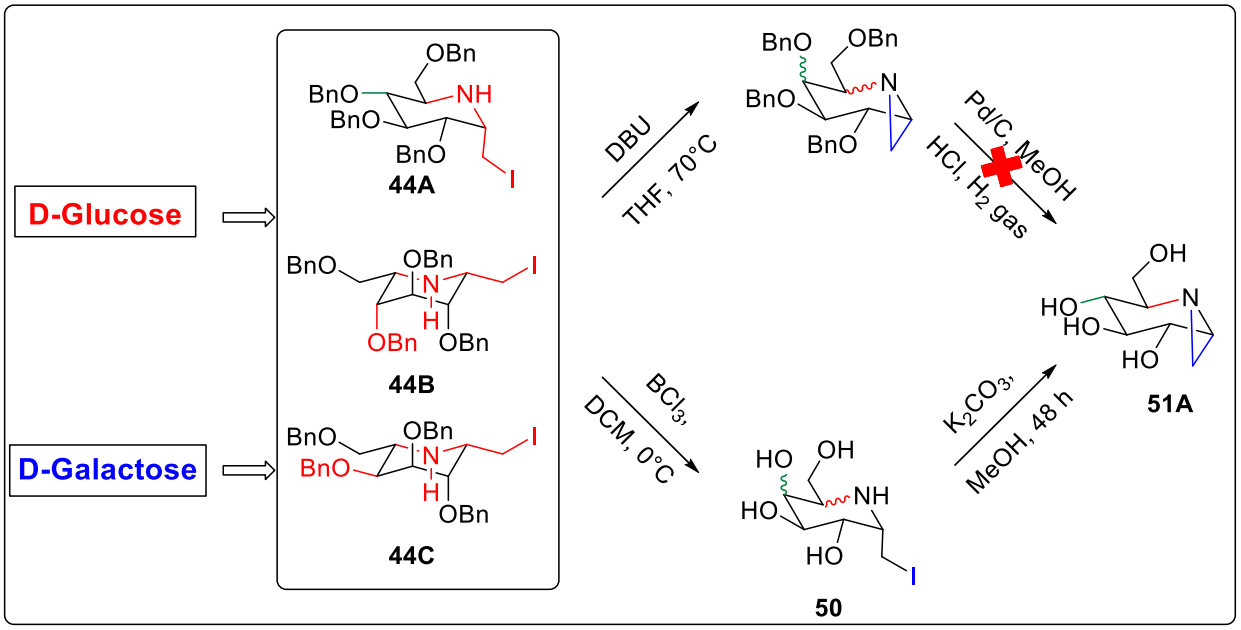
Instead of converting the iodo functionality into a hydroxyl group, ring-opening reactions of aziridine were attempted with support from literature on ring-opening reactions of aziridine moieties with different nucleophiles. Attempts were made by using different types of solvents along with a combination of water under low temperatures and also by varying the acids. Though many of the trials were unsuccessful, two attempts yielded positive outcome. The reaction was conducted using THF/H_2O, in the presence of $BF_3.Et_2O$, which led to the formation of a hydroxyl molecule in a trace amount. Reaction using *p*-TsOH under the same solvent combinations afforded a product, unfortunately, attempts to purify the product using silica gel chromatography reverted to the starting material aziridine.



Scheme 2.3.5.6a. Ring-opening reaction attempts in the presence of water

Since ring-opening reactions using water failed to produce any favourable outcome, other nucleophiles were attempted in opening the aziridine ring. Attempts with different nucleophiles and various acids and bases were also probed. Unfortunately, none of the reactions produced the aziridine ring-opened products.

So far, only D-galactose has been investigated in all the attempts. However, the formation of aziridine **49** prompted us to explore D-glucose as well. Initially, D-Glucose and D-Galactose were converted into an iodo compound via previously mentioned methods.[32] The plan was to synthesize benzylated aziridine derivatives and global debenzylation to afford expected molecules. The iodo compounds were converted into bicyclic aziridine compounds, using DBU under reflux conditions. However, attempts of debenzylation reaction using Pd/C gave rise to a complex mixture in ^{1}H NMR. Hence, the scheme was reversed to an initial debenzylation and final stage cyclization reaction to obtain aziridine. Debenzylation was carried out using BCl_3 at lower temperature that resulted in iodo derivatives **50 A-C**, which was further subjected to a cyclization reaction in the presence of K_2CO_3 in MeOH. Only the D-glucose iodo derivative **50A** afforded the intended aziridine molecule **51A**, however, the molecule was found to be unstable, as evidenced by complex ^{1}H NMR taken after few weeks. Although the debenzylation of L-iodo derivatives from both D-Glucose and D-Galactose were effective, the cyclization process employing K_2CO_3 produced no cyclized product.



Scheme 2.3.5.7a. Synthesis of aziridine **51A**

2.4. Conclusion

Synthesis of iminosugars of biological relevance as glucosidase inhibitors is the focus of the present chapter. Several attempts to accomplish the synthesis of iminosugars were made, which include the conventional intramolecular double reductive amination strategies. Attempts were made with an oxime intermediate, Mitsunobu double inversion, and reduction on lactam using Schwartz reagent. However, through a conventional lactam route, aziridine synthesis was accomplished from D-glucose.

2.5. General information

2.5.1. General experimental conditions:

^{1}H and ^{13}C NMR were recorded on a Bruker ASCEND^{TM}-500 spectrometer at 500 and 125 MHz, respectively using $CDCl_3$, acetone-d_6, and CD_3OD solvents. NMR data are reported as follows: chemical shifts in ppm (δ) with integration, coupling constant in Hz and multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, etc.). HR-ESI-MS analysis was recorded on a Thermo Scientific Exactive-LCMS instrument by electrospray ionization method

with ions given in m/z using Orbitrap analyzer. Reactions were monitored by silica gel G-60 F_{254} aluminium TLC plates and compounds were visualized by short wavelength lamp and by charring the TLC plate after spraying with 15% sulfuric acid in ethanol. Chromatographic separations were carried out by conventional column chromatography on silica gel (100 - 200 mesh). Reagents were purchased at the highest commercial quality and used without further purification.

(2S,3S,4R,5S,6R)-2-allyl-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(4-methoxybenzyl) piperidine (**29**): To a solution of compound **27** (115 mg, 0.12 mmol) in DCM was added Dess–Martin periodinane (106 mg, 0.24 mmol) and kept stirring at room temperature. After 2 h, quenched with sat. aq. $Na_2S_2O_3$ (5 mL) and vigorously stirred for 10 min. The mixture was diluted with DCM (50 mL) and added sat. aq. $NaHCO_3$ (5 mL), then washed with water (2 x 15 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude mixture was passed through silica and subjected to next reaction. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{60}H_{59}NNaO_8$ 944.4140; Found 944.4133. To the crude mixture in DMF was added piperidine (50 µL, 0.24 mmol) at 0 °C. After stirring for 30 mins at 0 °C, the reaction mixture was diluted with Et_2O (50 mL) and washed with water (2 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in dry DCE (10 mL), and the mixture was cooled to –35 °C, added AcOH (28 µL) followed by the successive addition of Na_2SO_4 (273 mg, 2 mmol) and $NaCNBH_3$ (65 mg, 0.3 mmol). The reaction mixture was stirred at –20 °C under argon for 16 h. The mixture was diluted with EtOAc (50 mL) and washed with sat. aq. $NaHCO_3$ (50 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by silica gel column chromatography to provide **29** (22 mg) in 51% yield as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.15 (m, 22H), 6.80 (d, J =8.7 Hz, 2H), 5.76–5.67 (m, 1H), 5.02–4.92 (m, 3H), 4.90 (d, J = 10.7 Hz, 1H), 4.80 (d, J = 10.9 Hz, 1H), 4.58 (d, J = 10.7 Hz, 1H), 4.51–4.46 (m, 2H),

4.38–4.34 (m, 2H), 3.95 (d, J = 13.9 Hz, 1H), 3.84 (dd, J = 4.9, 10.4 Hz, 1H), 3.81–3.69 (m, 8H), 3.07–3.04 (m, 2H), 2.42–2.33 (m, 2H). HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{45}H_{50}NO_5$ 684.3679; Found 684.3684.

1,3,4,5-tetrakis(benzyloxy)hept-6-en-2-ol (**30**): A solution of methyltriphenylphosphonium bromide (10.1 g, 28.3 mmol, 3 equiv) in dry toluene (50 mL) was stirred for 10 min at room temperature, and then a solution of *n*-BuLi in hexane (20 mL, 30.5 mmol, 3.5 equiv) was slowly added at room temperature under nitrogen atmosphere. The resulting solution was stirred for 2 h at same temperature, and then a solution of compound **24** (5.1 g, 9.4 mmol, 1 equiv) in dry toluene (20 mL) was transferred to the reaction mixture using cannula. After 12 h stirring, the reaction mixture was quenched with sat. aq. NH_4Cl (50 mL), and extracted with EtOAc (100 mL x 2). The organic layer was washed with excess water, dried over anhydrous Na_2SO_4, concentrated, and purified by flash chromatography using hexane/EtOAc, which afforded compound **30** (3.55 g, 70%) as a pale-yellow viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.2-7.4 (20H, m), 5.92 (1H, m), 5.37(2H, m), 4.4-4.9 (8H, m), 3.9 (2H, m), 3.5 (2H, m), 3.1 (1H, d, J=5.5Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.2, 138.19, 138.15, 138.0, 135.7, 128.3, 128.1, 128.0, 127.75, 127.73, 127.69, 127.60, 119.2, 82.1, 80.7, 76.5, 75.2, 73.1, 73.1, 71.2, 70.3, 69.6. HRMS-ESI (m/z) calc. for $C_{35}H_{38}NaO_5$ [M + Na+]: 561.2617; Found: 561.2603.

(E)-1,3,4,5-tetrakis(benzyloxy)hept-6-en-2-one oxime **(31):** To a solution of compound **30** (1.3 g, 2.4 mmol, 1 equiv) in DCM (30 ml) was added Dess–Martin periodinane (3.07 g, 7.2 mmol, 3 equiv) at room temperature and kept for stirring. After 2 hours, the reaction mixture was quenched using sat. aq. $Na_2S_2O_3$ and allowed to stir for 10 more minutes, then added sat. aq. $NaHCO_3$, and extracted with DCM (100 mL x 2), washed, dried and concentrated. The crude mixture obtained was then dissolved in methanol and were added potassium bicarbonate (795 mg, 7.2 mmol, 3

equiv) and hydroxyl amine hydrochloride (452 mg, 10.8 mmol, 4.5 equiv) at room temperature. The reaction mixture was then moved into reflux condition and allowed to stir for 2 hours. The reaction stopped after 2 hours and the reaction mixture is filtered through a sintered tube to avoid the precipitate formed. The filtrate is then diluted with EtOAc, added 1N HCl (20 mL), then added sat. aq. $NaHCO_3$. It is then extracted using EtOAc (100 mL x 2), washed, dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc afforded oxime **31** (650 mg) in 49% yield as colorless semisolid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.15-7.35 (20H, m), 5.84 (1H, m), 5.20 (2H, m), 4.4-4.8 (8H, m), 4.22 (1H, d, J=12 Hz), 4.16 (1H, dd, J=4.5 Hz, 11 Hz), 4.07 (2H, m), 3.79 (1H, m). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 157.0, 138.5, 138.42, 137.9, 137.7, 136.0, 128.3, 128.28, 128.24, 128.22, 128.1, 128.0, 127.97, 127.93, 127.8, 127.78, 127.73, 127.70, 127.66, 127.62, 127.56, 127.54, 127.51, 127.46, 127.42, 118.3, 82.7, 81.5, 77.9, 74.5, 73.6, 71.3, 70.6, 63.3. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{35}H_{37}NNaO_5$ 574.2569; Found 574.2583.

4,5,6,7-tetrakis(benzyloxy)hept-1-en-3-yl 4-nitrobenzoate (**32B**): To a solution of compound **30** (1.47 g, 2.7 mmol, 1 equiv), triphenyl phosphine (2.15 g, 8.1 mmol, 3 equiv) and 4-nitrobenzoic acid (1.82 g, 10.9 mmol, 4 equiv), DEAD (1.7 ml, 10.9 mmol, 4 equiv) was added at 0 °C and slowly brought to room temperature and allowed to stir for overnight. After completion of the starting material, the reaction was stopped and the solvents were evaporated. The crude mixture was purified by flash column chromatography using hexane:EtOAc to afford compound **32B** (1.3 g) in 68% yield as colorless viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.25 (2H, d, J=8.90 Hz), 8.08 (2H, d, J=8.85 Hz), 7.33 (20H, m), 6.09 (1H, m), 5.97 (1H, dd, J=2.5, 7.5 Hz), 5.32 (2H, m), 4.80 (3H, m), 4.67 (3H, m), 4.50 (2H, q, J=11 Hz), 4.09 (2H, m), 3.86 (1H, m), 3.81 (1H, m), 3.75 (1H, m). HRMS (ESI) m/z: [M+H]+ Calcd. $C_{42}H_{41}NNaO_8$ 710.2730; Found 710.2626.

1,2,4,5-tetrakis(benzyloxy)hept-6-en-3-ol (**33B**): To a solution of compound **30** (135 mg, 0.25 mmol, 1 equiv), triphenyl phosphine (99 mg, 0.37 mmol, 1.5 equiv) and 4-nitrobenzoic acid (84 mg, 0.5 mmol, 2 equiv), DEAD (80 µL, 0.5 mmol, 2 equiv) was added at 0 °C and slowly brought to room temperature and allowed to stir for 8 h. After the completion of the starting material, the reaction was stopped and the solvents were evaporated. The crude mixture was purified by flash column chromatography using hexane:EtOAc to afford compound **33B** (87 mg) in 64% yield as colorless viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.15-7.35 (20H, m), 5.91 (1H, m), 5.25 (2H, m), 4.50 (8H, m), 4.1 (1H, dd, J= 3.5Hz, 7.5Hz), 3.83 (2H, m), 3.68 (2H, dd, J=1.5Hz, 4.5Hz), 3.61 (1H, dd, J=3.5Hz,8.5Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 138.7, 138.2, 138.1, 138.0, 135.3, 130.7, 128.38, 128.36, 128.30, 127.9, 127.89, 127.84, 127.66, 127.65, 127.61, 127.5, 118.6, 80.1, 79.9, 76.4, 73.6, 73.4, 73.3, 72.4, 71.4, 70.9. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{35}H_{38}NaO_5$ 561.2617; Found 561.2640.

1,2,4,5-tetrakis(benzyloxy)hept-6-en-3-yl acetate (**34**): To a solution of compound **33B** (20 mg) in pyridine (1 mL) was added acetic anhydride (0.5 mL) at room temperature. After 3 h, the reaction mixture was quenched with excess sat. aq. $NaHCO_3$ and extracted with EtOAc (20 mL x 2), washed with distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using hexane:EtOAc to afford compound **34** as colorless viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.20 (20H, m), 5.87 (1H, m), 5.31 (3H, m), 4.56 (3H, m), 4.38 (4H, m), 4.27 (1H, d, J=11 Hz), 4.07 (1H, d, J=3.5 Hz), 3.92 (1H, d, J=2.5 Hz), 3.61 (1H, m), 3.56 (1H, m), 3.49 (1H, m), 1.92 (3H, s). HRMS (ESI) m/z: [M+H]+ Calcd. $C_{37}H_{40}NaO_6$ 603.2723; Found 603.2734.

(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)piperidin-2-one (**37**): The lactam compound was synthesized using Overkleeft protocol[31] and confirmed by comparison of the NMR

data. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (2H, m), 7.22 (16H, m), 7.11 (2H, m), 6.27 (1H, s), 5.09 (1H, d, J = 11 Hz), 4.85 (1H, d, J = 11 Hz), 4.73 (2H, m), 4.62 (1H, d, J = 11 Hz), 4.49 (1H, d, J = 11 Hz), 4.42 (3H, m), 4.30 (1H, m), 4.26 (1H, dd, J = 2, 18 Hz), 3.84 (1H, t, J = 1.5 Hz), 3.46 (1H, d, J = 8.5 Hz), 3.35 (1H, d, J = 8.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 171.8, 138.4, 138.2, 137.7, 136.7, 128.7, 128.3, 128.27, 128.18, 128.10, 127.9, 127.65, 127.62, 127.60, 82.1, 78.0, 77.6, 76.7, 75.2, 74.6, 73.9, 73.4, 73.1. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{34}H_{36}NO_5$ 538.2593; Found 538.2612.

(2R,3S,4S,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(but-3-en-1-yl)-2,3,4,5-tetra hydropyridine (**40**): To a solution of compound **37** (1.2 g, 2.23 mmol) in dry THF (20 mL) was added vinyl magnesium bromide (23 mL, 22.3 mmol) at 0 °C. After 8 h, the reaction mixture was quenched with excess sat. aq. NH_4Cl and extracted with EtOAc (75 mL x 2), washed with distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using hexane:EtOAc to afford compound **40** (402 mg) in 38% (pale yellow viscous liquid). ^{1}H-NMR ($CDCl_3$) 7.36 (20H, m), 5.84 (1H, m), 4.98 (2H, m), 4.79 (2H, m), 4.69 (1H, d, J=11 Hz), 4.63 (1H, d, J=12 Hz), 4.59 (2H, s), 4.39 (1H, dd, J=2, 9 Hz), 4.28 (1H, d, J=1.5 Hz), 3.94 (1H, q, J=3 Hz), 3.90 (1H, dd, J=2, 9 Hz), 3.73 (2H, m), 2.51 (2H, q, J=5 Hz), 2.31 (2H, m). 170.8, 139.0, 138.2, 138.19, 138.14, 138.10, 128.5, 128.40, 128.2, 128.1, 128.0, 127.8, 127.7, 127.60, 127.5, 127.4, 114.7, 82.3, 78.5, 75.4, 74.3, 73.5, 73.0, 71.1, 70.3, 62.2, 35.0, 30.7. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{38}H_{42}NO_4$ 576.3114; Found 576.3116.

(2R,3S,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidine (**37B**): A solution of lactam **37** (200 mg, 0.4 mmol) in THF (5 mL) was added dropwise to suspension of $Cp_2Zr(H)Cl$ (142 mg, 0.55 mmol, 1.5 equiv) in THF (5 mL) at -25 °C under argon atmosphere. The reaction

mixture was brough slowly to room temperature and stirred until white suspension disappeared and clear solution appeared. Then the reaction mixtre was cooled to -25 °C and $Yb(OTf)_3$ was added (248 mg, 0.4 mmol, 1.0 equiv). After 10 min, allyltributylstannane (375 ml, 1.5 mmol, 3 equiv) was added dropwise. The mixture was allowed to attain room temperature and stirred overnight. Solvent was evaporated using rotavapor and the residue was diluted with MeCN (20 mL), and washed with hexanes (15 mL). The MeCN layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using hexane/EtOAc (7:3) to afford lactam and compound **37B** (85 g, 43%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.32 (18H, m), 7.22 (2H, d, *J* = 6 Hz), 5.00 (1H, d, *J* = 11 Hz), 4.87 (2H, t, *J* = 11 Hz), 4.71 (2H, q, *J* = 9.5 Hz), 4.48 (3H, m), 3.69 (1H, m), 3.55 (3H, m), 3.38 (1H, t, *J* = 9 Hz), 3.27 (1H, dd, *J* = 4.5, 12 Hz), 2.75 (1H, m), 2.53 (1H, t, *J* = 12 Hz). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 138.9, 138.5, 138.4, 138.0, 128.4, 128.4, 128.4, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 87.3, 80.7, 80.1, 75.7, 75.2, 73.4, 72.8, 70.3, 59.8, 48.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{34}H_{38}NO_4$ 524.2801; Found 524.2812.

tert-butyl (2R,3S,4R,5S)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-hydroxy-6-(hydroxy methyl)piperidine-1-carboxylate (**47**): To a solution of compound **45** (307 mg, 0.5 mmol) in DCM (8 mL) was added *m*-CPBA (208 mg, 1.2 mmol) at 0 °C. After 8 h, the reaction mixture was quenched with excess sat. aq. $NaHCO_3$ and extracted with EtOAc (50 mL x 2), washed with distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using hexane:EtOAc to afford compound **47** (257 mg) in 95% yield as colorless viscous liquid. ^{1}H-NMR ($CDCl_3$) 7.33 (24H, m), 5.17 (1H, m), 4.75 (1H, d, J=11 Hz), 4.52 (6H, m), 4.37 (1H, d, J=11 Hz), 4.28 (1H, s), 4.16 (2H, m), 4.02 (3H,

m), 3.87 (4H, m), 3.69 (1H, s), 3.56 (2H, m), 1.45 (14H, s). HRMS (ESI) m/z: [M+H]+ Calcd. $C_{33}H_{41}NNaO_7$ 586.2781; Found 586.2749.

***2.5.2. General procedure NIS mediated cyclisation**:*

To a solution of aminoalkene (1 equiv) in distilled CH_2Cl_2 was added NIS (1 equiv.) and the mixture was stirred for 2 h at room temperature under an argon atmosphere in dark. The reaction mixture was then quenched with sat. aq. $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic extract was dried over anhydrous Na_2SO_4, and concentrated. Separation of diastereomeric mixture **44 A-C** by column chromatography (230-400 mesh silica gel) was carried out using hexane/EtOAc 19/1 and hexane/CH_2Cl_2 18.5/1.5 as mobile phase from the crude mixtures derived from D-galactose and D-glucose, respectively.

(2R,3R,4S,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(iodomethyl)piperidine **(44A)**

(Pale yellow viscous liquid)**:** ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.18 (20H, m), 4.83 (1H, d, J = 11 Hz), 4.75 (1H, d, J = 11 Hz), 4.69 (1H, d, J = 10.5 Hz), 4.59 (2H, m), 4.51 (1H, d, J = 12 Hz), 4.38 (2H, t, J = 10 Hz), 3.67 (1H, m), 3.59 (3H, m), 3.40 (1H, m), 3.33 (1H, m), 3.25 (1H, m), 2.82 (1H, t, J = 7 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.7, 138.2, 138.1, 138.0, 128.5, 128.4, 128.3, 127.9, 127.88, 127.85, 127.79, 127.72, 127.6, 83.0, 81.0, 80.3, 75.6, 75.1, 73.1, 72.9, 70.4, 56.3, 52.1, 8.1. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{35}H_{38}INNaO_4$ 686.1743; Found 686.3749.

(2S,3R,4S,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(iodomethyl)piperidine (**44B**)

(Pale yellow viscous liquid): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.31 (20H, m), 4.60 (1H, m), 4.55 (1H, m), 4.49 (2H, m), 4.45 (1H, s), 4.42 (2H, m), 4.38 (1H, m), 3.73 (1H, s), 3.67 (1H, s), 3.62 (1H, t, J = 7 Hz), 3.47 (1H, t, J = 7 Hz), 3.41 (1H, s), 3.33 (1H, t, J = 7 Hz), 3.27 (1H, t, J = 7 Hz), 3.23 (1H, d, J = 9 Hz), 3.18 (1H, t, J = 7 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.3, 138.0,

137.9, 128.48, 128.47, 128.3, 128.28, 128.25, 127.9, 127.85, 127.83, 127.80, 127.7, 127.6, 73.6, 73.3, 72.7, 72.5, 72.4, 72.1, 70.9, 57.7, 55.8, 6.9. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{35}H_{39}INO_4$ 664.1924; Found 664.1928.

(2S,3S,4S,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(iodomethyl)piperidine (**44C**) (Pale yellow viscous liquid): ^{1}H NMR (Acetone-d6, 500 MHz): δ 7.33 (20H, m), 4.77 (1H, d, J = 12 Hz), 4.67 (1H, d, J = 12 Hz), 4.55 (1H, m, J = 11 Hz), 4.43 (1H, d, J = 11 Hz), 4.13 (1H, t, J = 3 Hz), 3.96 (1H, d, J = 3 Hz), 3.78 (1H, dd, J = 2.5, 10.5 Hz), 3.73 (1H, dd, J = 4.5, 9 Hz), 3.65 (1H, dd, J = 2, 9 Hz), 3.27 (1H, m, J = 6 Hz), 3.15 (1H, td, J = 3, 10 Hz). ^{13}C{^{1}H} NMR (Acetone-d6, 125 MHz): δ 139.1, 138.9, 138.86, 138.7, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.38, 127.30, 76.4, 74.7, 72.8, 72.7, 72.6, 72.3, 71.0, 70.1, 56.1, 54.6, 6.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{35}H_{39}INO_4$ 664.1924; Found 664.1972.

2.5.3. General procedure for debenzylation:

To a solution of compound **44 A-C** (1 equiv) in distilled CH_2Cl_2 (6 mL) was added 1M BCl_3 (9 equiv.) in CH_2Cl_2 at -10 °C and the mixture was stirred at room temperature under an argon atmosphere. After 6 h, an additional 1M BCl_3 (6 equiv.) in CH_2Cl_2 was added at the same temperature. After 3 h, the reaction mixture was allowed to attain room temperature, MeOH (5 mL) was added and concentrated. The resulting solid was precipitated over CH_2Cl_2 to obtain compounds **50 A-C**.

(2R,3R,4S,5S)-2-(hydroxymethyl)-6-(iodomethyl)piperidine-3,4,5-triol (**50A**) (colorless semi solid): ^{1}H NMR (CD_3OD, 500 MHz): δ 3.91 (1H, m), 3.73 (3H, m), 3.60 (1H, m), 3.52 (2H, m), 3.40 (1H, m), 3.37 (1H, m). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 70.4, 68.7, 67.6, 59.6, 56.8, 54.3, -4.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_7H_{15}INO_4$ 302.9968; Found 304.0052.

(2S,3R,4S,5S)-2-(hydroxymethyl)-6-(iodomethyl)piperidine-3,4,5-triol (**50B**) (colorless semi solid): ^{1}H NMR (CD_3OD, 500 MHz): δ 4.14 (1H, s), 3.96 (1H, t, *J* = 3.5 Hz), 3.78 (3H, m), 3.55 (1H, m), 3.48 (1H, m), 3.44 (2H, m). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 67.7, 67.4, 66.8, 58.9, 57.9, 56.6, -4.5.

(2S,3S,4S,5S)-2-(hydroxymethyl)-6-(iodomethyl)piperidine-3,4,5-triol **(50C)** (colorless semi solid)**:** ^{1}H NMR (CD_3OD, 500 MHz): δ 4.30 (1H, d, *J* = 3 Hz), 4.00 (3H, m), 3.81 (H, dd, *J* = 6.5, 12 Hz), 3.70 (1H, t, *J* = 7 Hz), 3.49 (2H, m), 3.38 (1H, m). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 69.5, 67.1, 63.3, 58.3, 56.8, 55.7, -4.6.

(2R,3R,4R,5S,6R)-2-(hydroxymethyl)-1-azabicyclo[4.1.0]heptane-3,4,5-triol (**51**): To a solution of compound **50A** (100 mg, 0.33 mmol, 1 equiv.) in distilled water (5 mL) was added K_2CO_3 (174 mg, 1.32 mmol, 4 equiv.) and the mixture was stirred at room temperature. After 2 days, the reaction mixture was concentrated and purified by reverse phase preparative HPLC to afford aziridine iminosugar **51** as colorless semi solid. ^{1}H NMR (D_2O, 500 MHz): δ 4.08 (1H, t, *J* = 7 Hz), 3.81 (1H, d, *J* = 10 Hz), 3.67 (1H, m), 3.28 (1H, t, *J* = 7 Hz), 2.63 (1H, s), 2.36 (1H, m), 1.99 (1H, d, *J* = 6 Hz), 1.73 (1H, d, *J* = 4.5 Hz). $^{13}C\{^{1}H\}$ NMR (D_2O, 125 MHz): δ 73.4, 72.1, 65.7, 63.2, 36.3, 33.5, 29.3, 22.9.

Figure 2.5.2.1: NMR and HRMS spectras of compound **29**

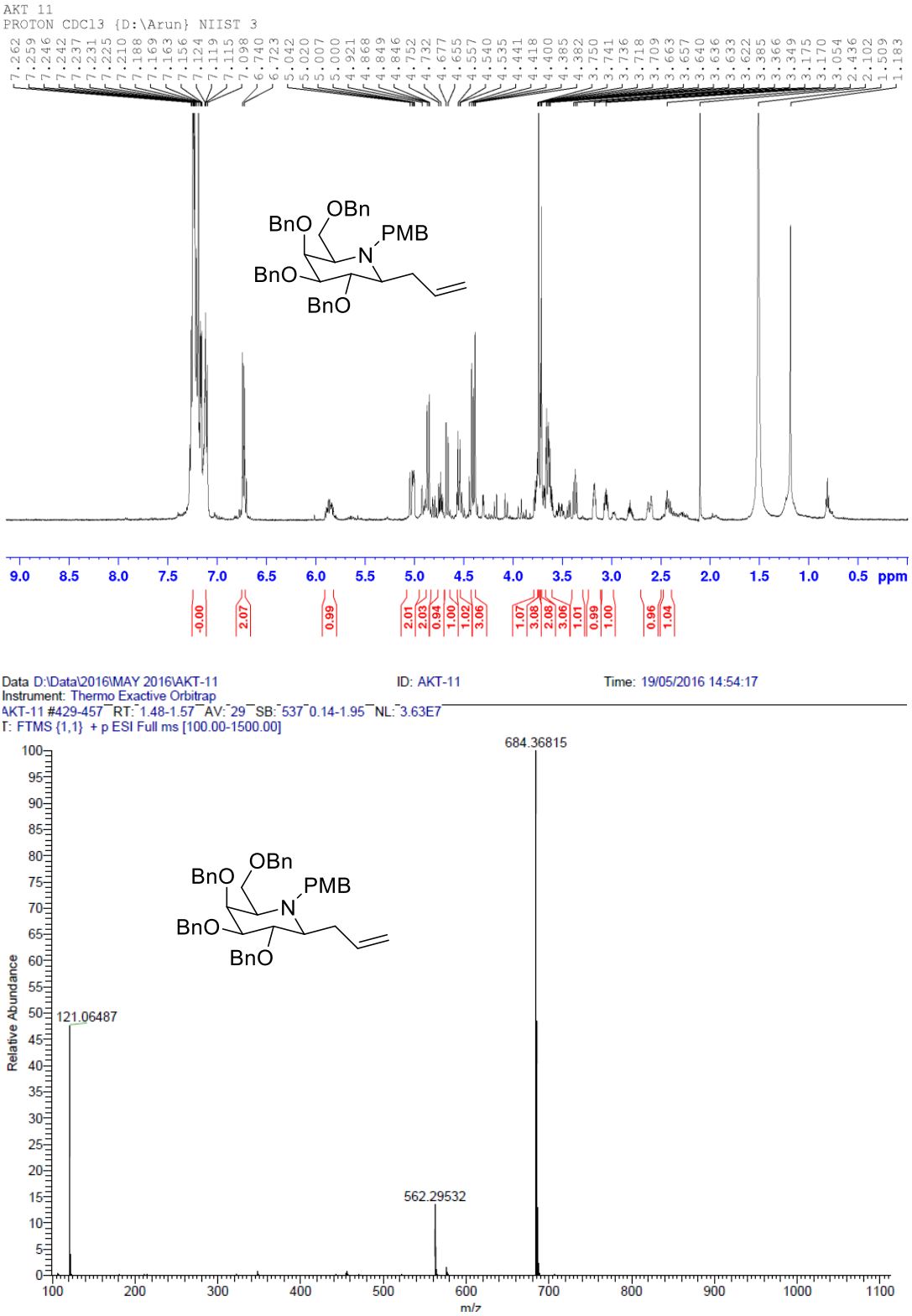


Figure 2.5.2.2: NMR spectras of compound **30**

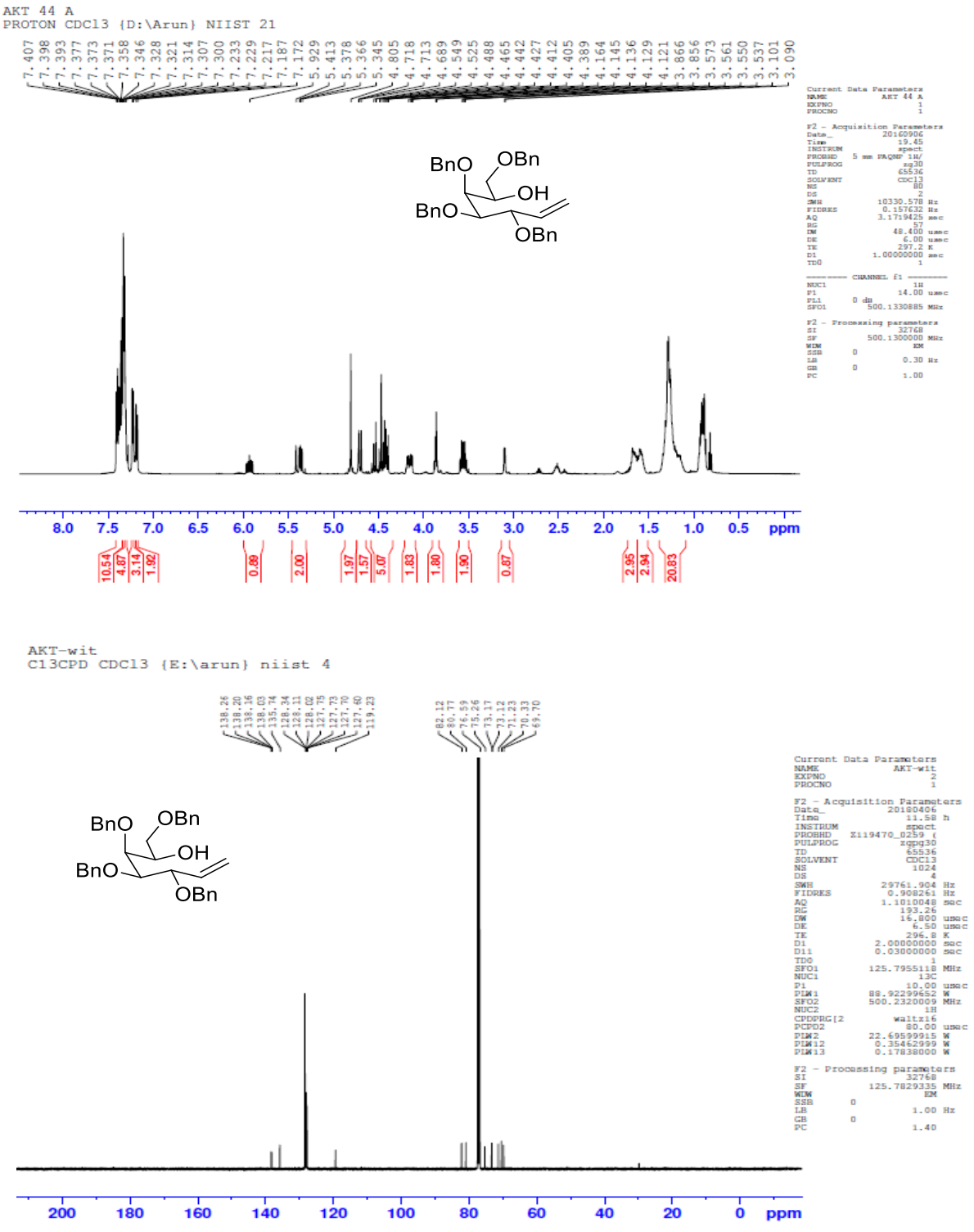
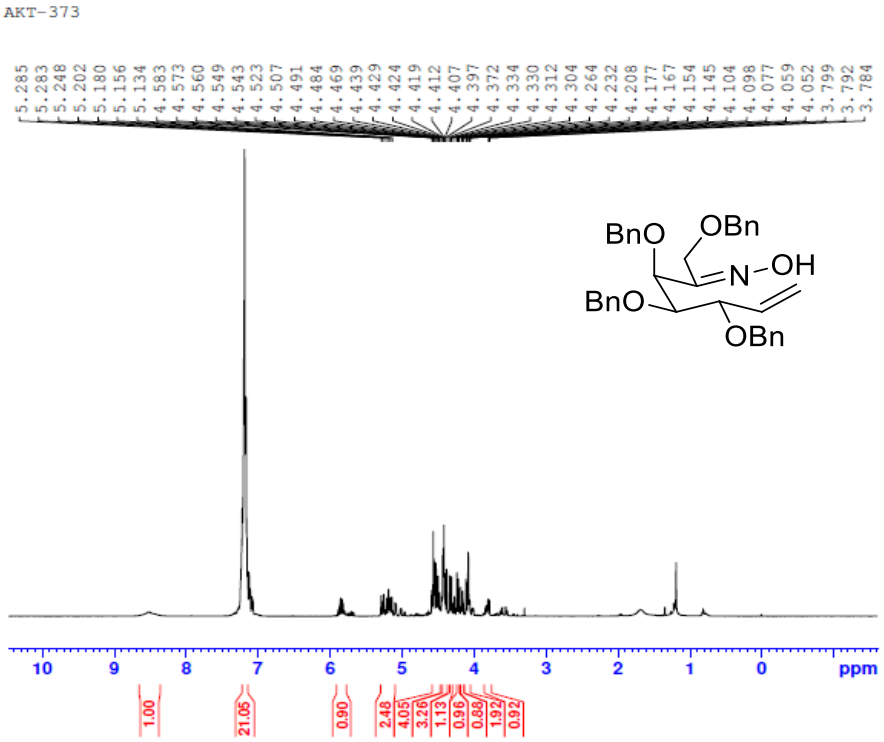


Figure 2.5.2.3: NMR spectras of compound **31**

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PROCNO                      1

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DE                     6.50 usec
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TD0                         1

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PLW1               20.69000053 W

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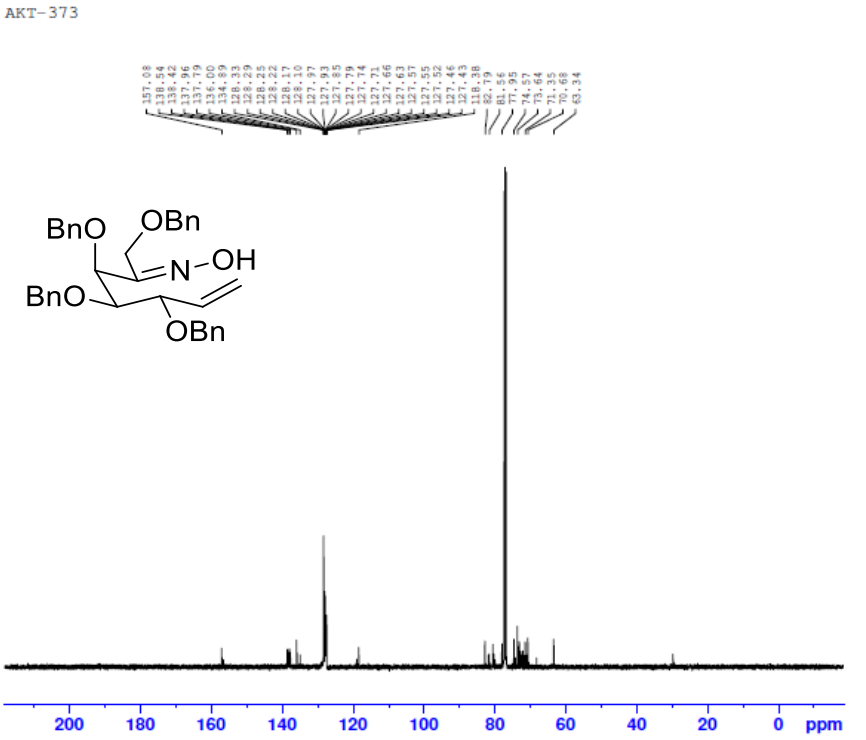


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NUC2                       1H
CPDPRG[2                waltz16
PCPD2                   80.00 usec
PLW2               20.69000053 W
PLW12               0.63362998 W
PLW13               0.40551999 W

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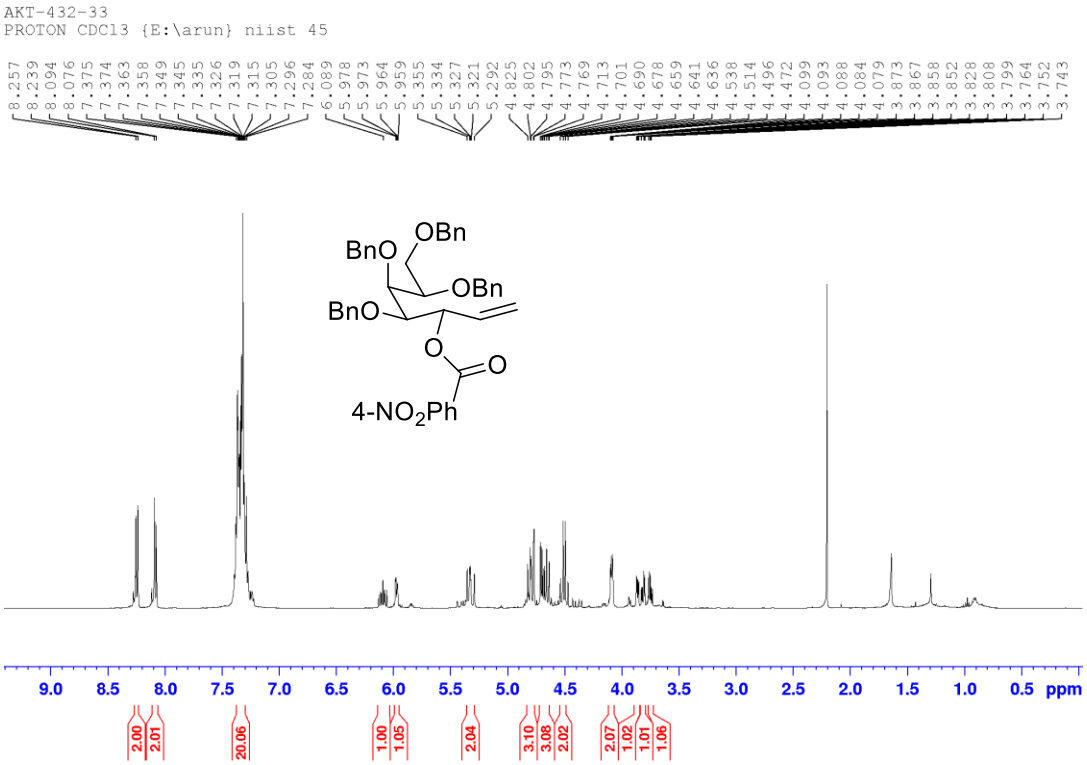
Figure 2.5.2.4: NMR spectras of compound **32B**



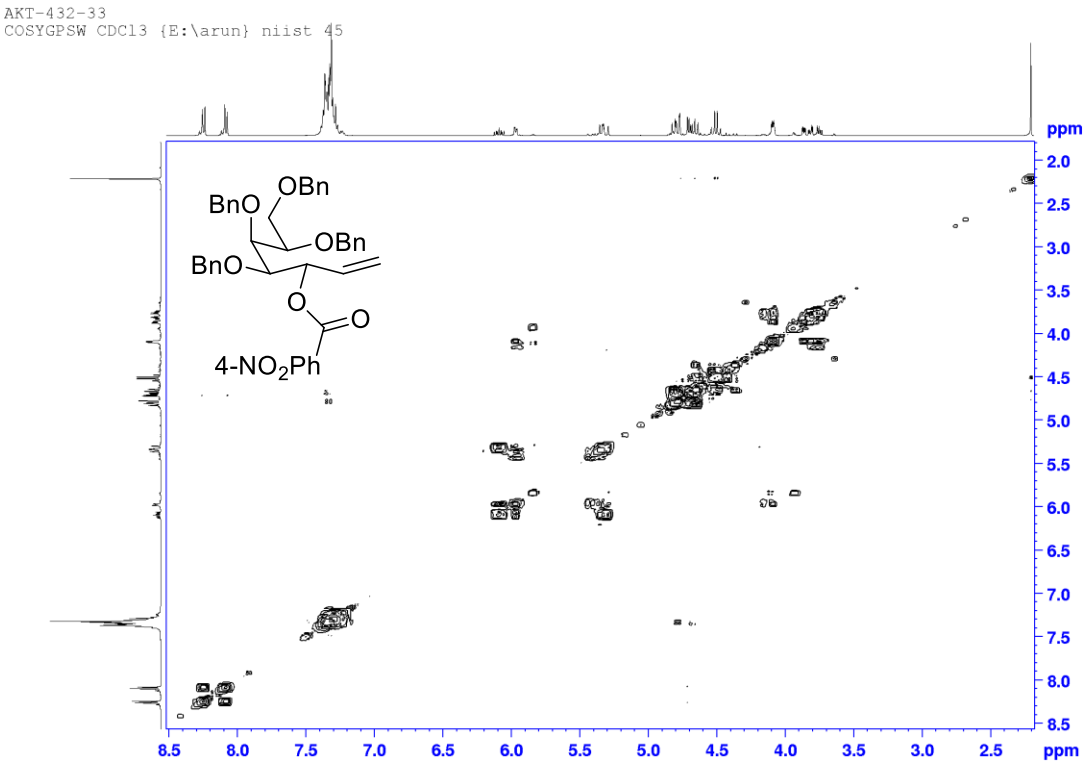
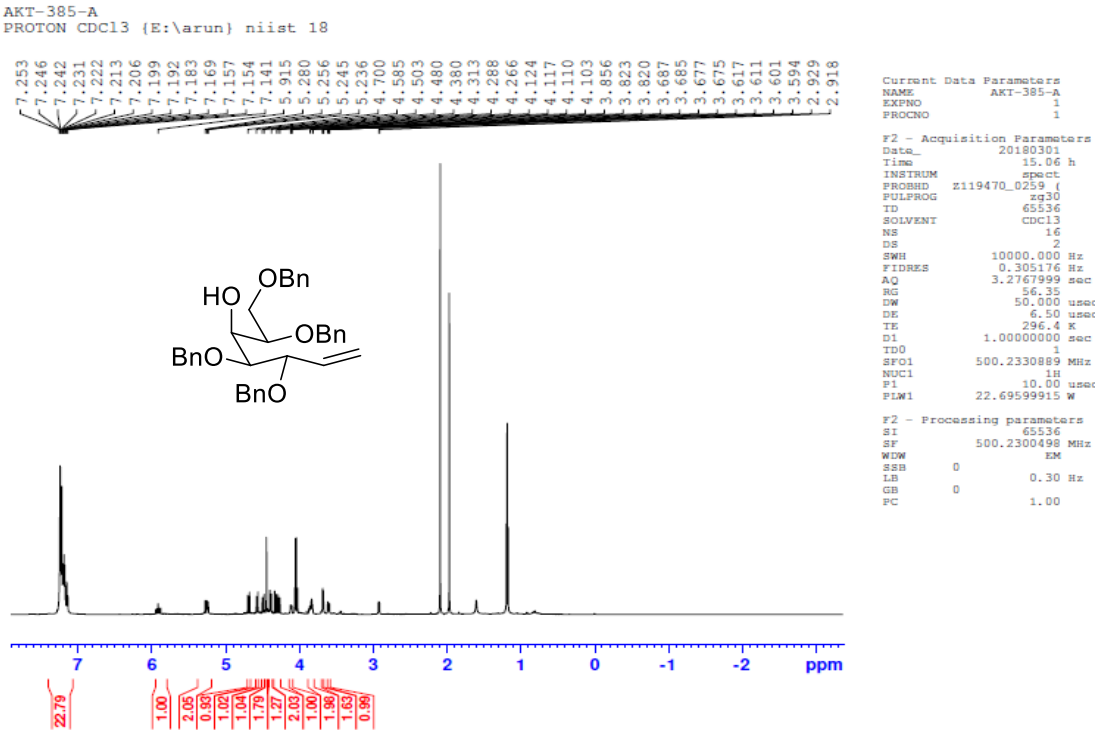


Figure 2.5.2.5: NMR spectras of compound **33B**



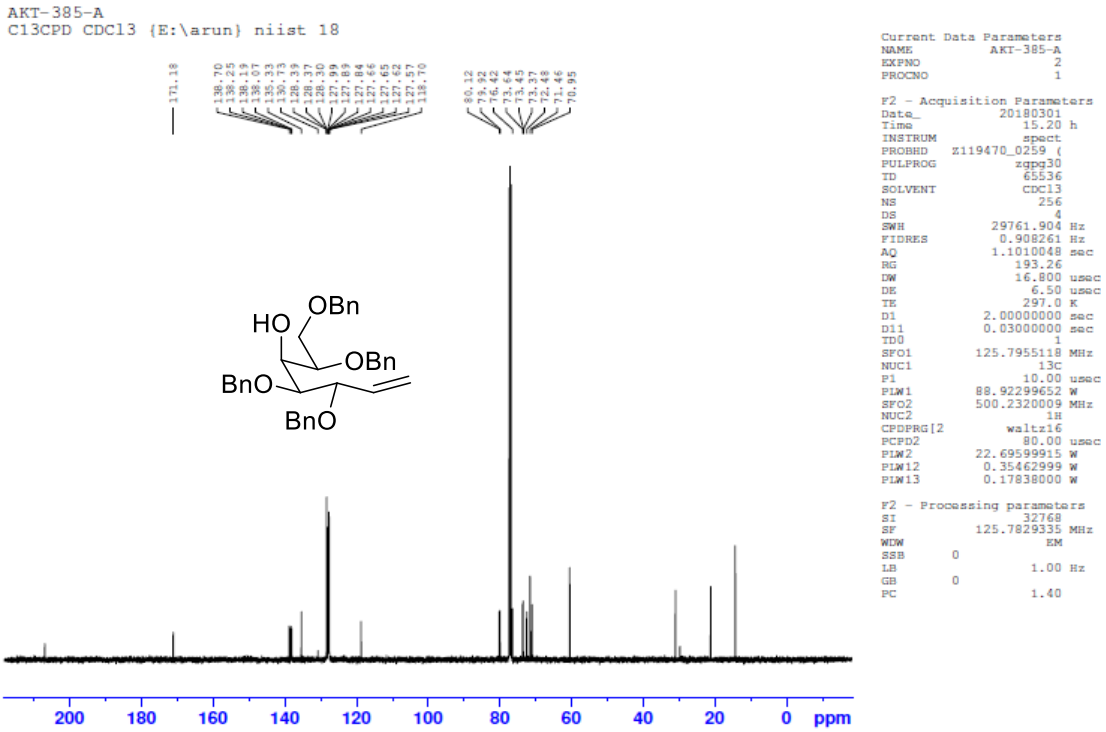
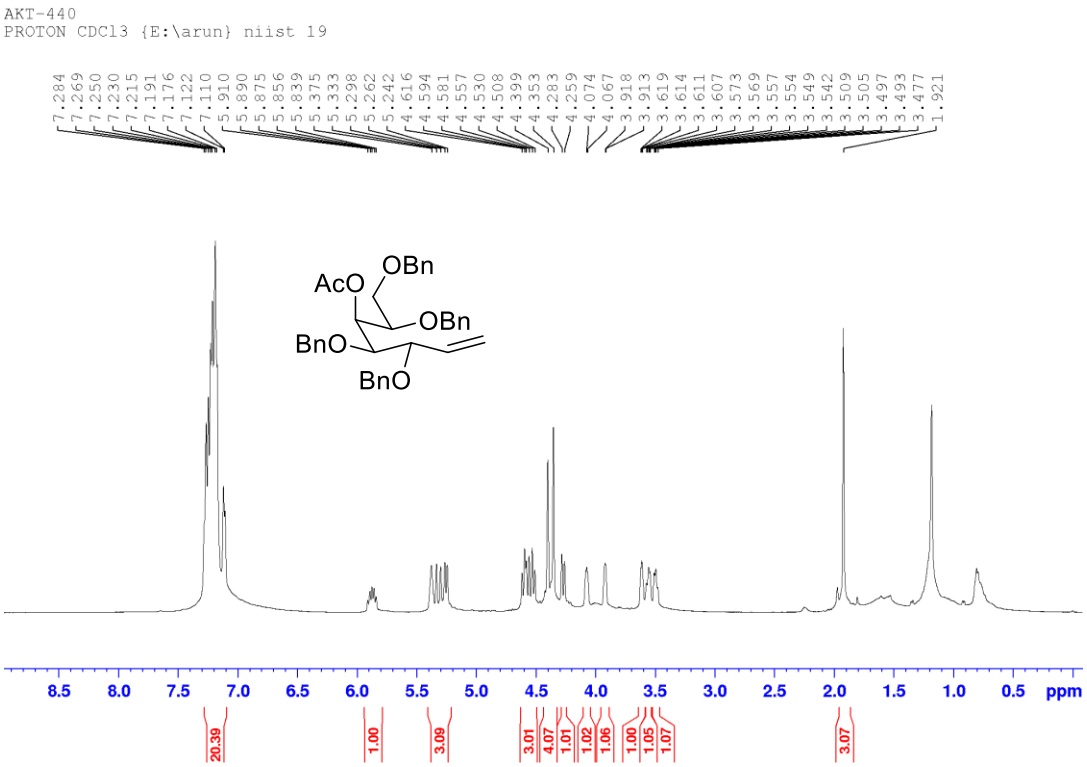


Figure 2.5.2.6: NMR spectras of compound **34**



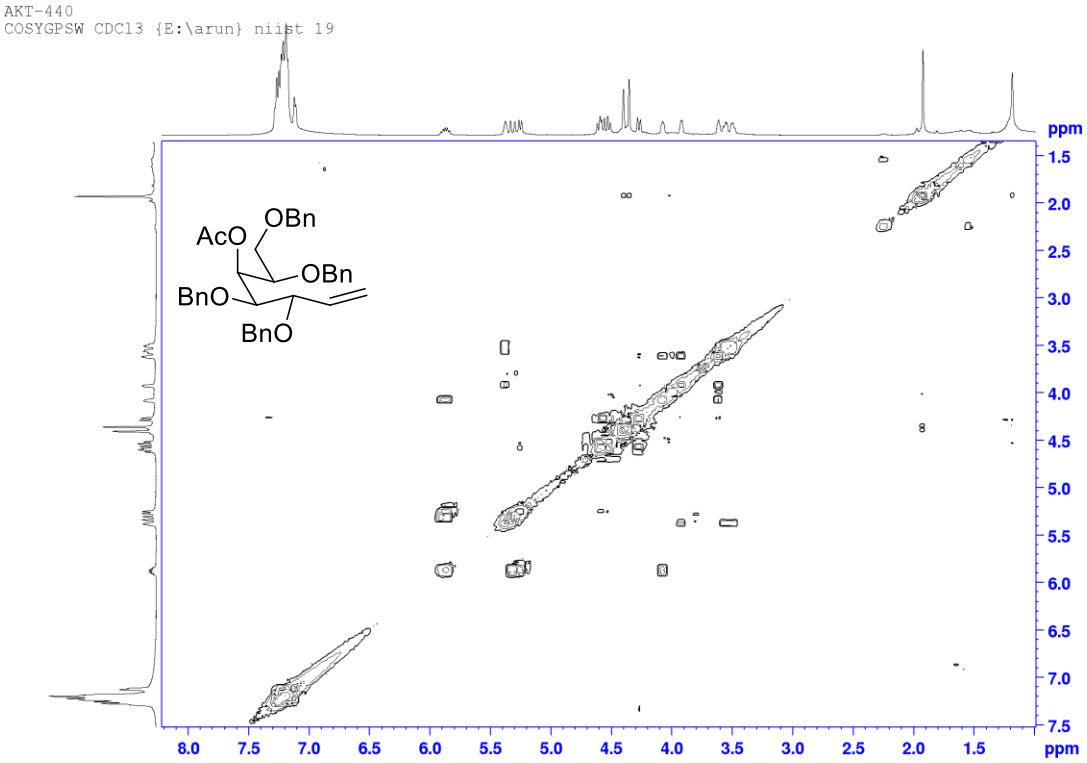


Figure 2.5.2.7. NMR spectras of compound **37**

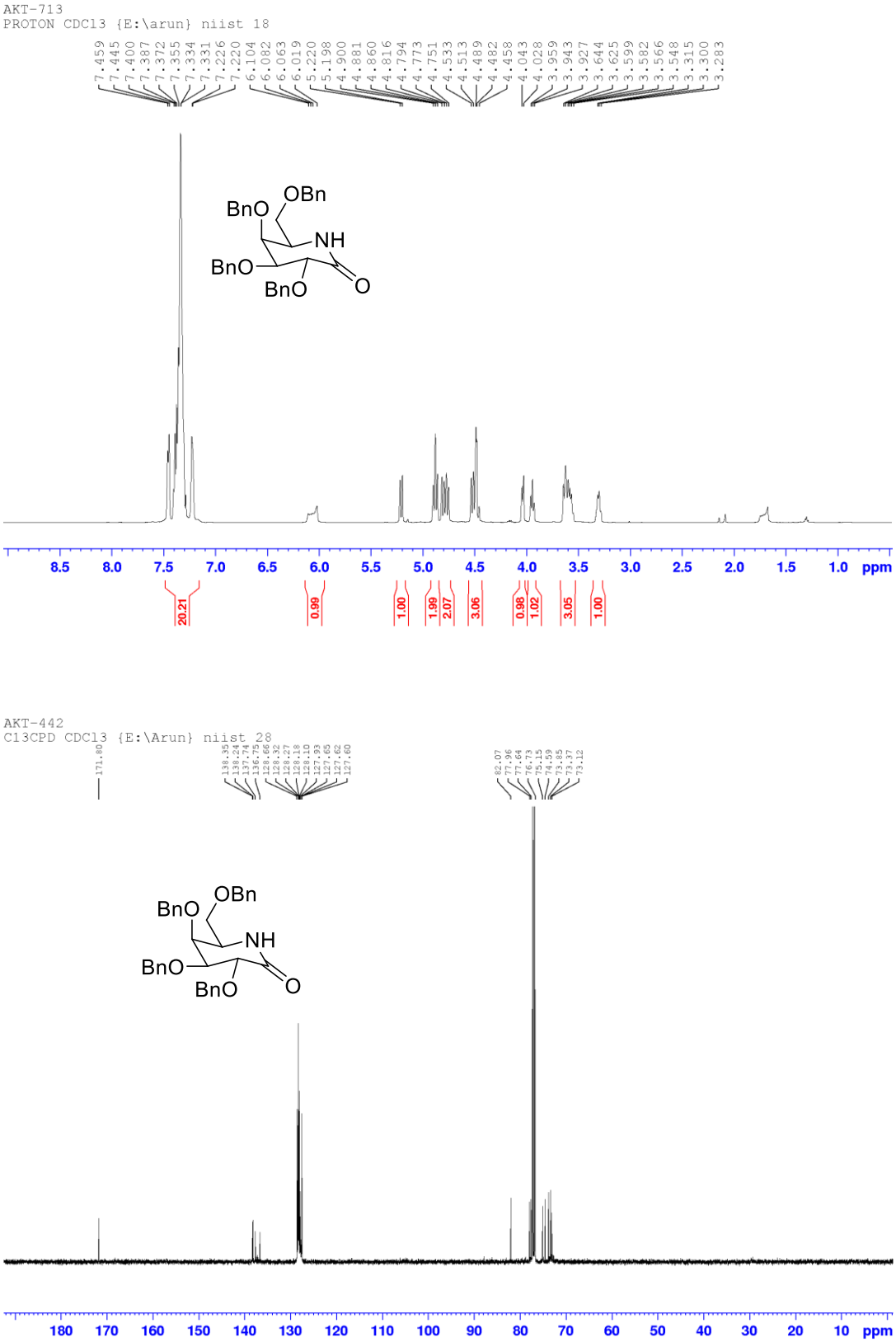


Figure 2.5.2.8: NMR spectras of compound **40**

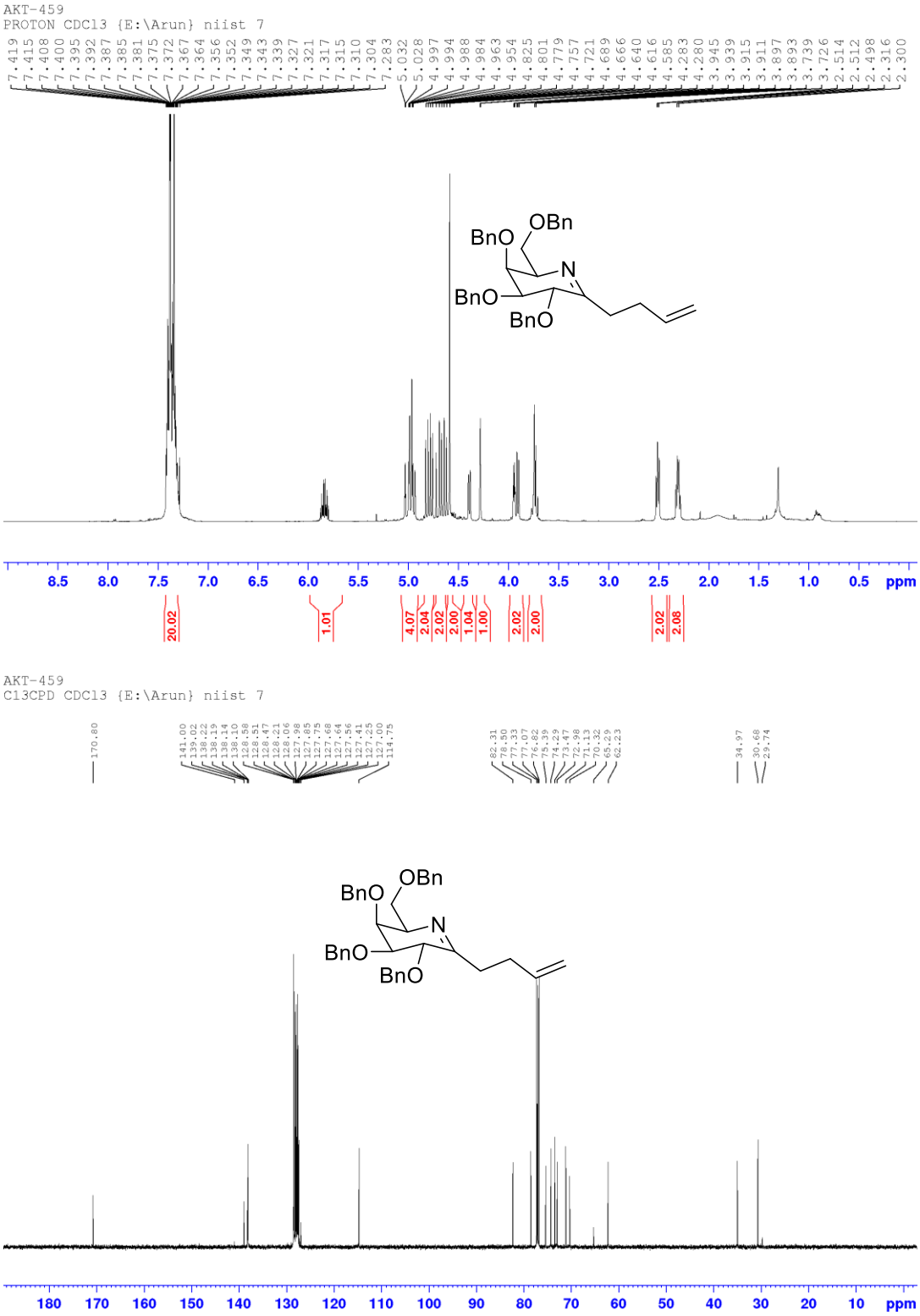


Figure 2.5.2.9. NMR spectras of compound **37B**

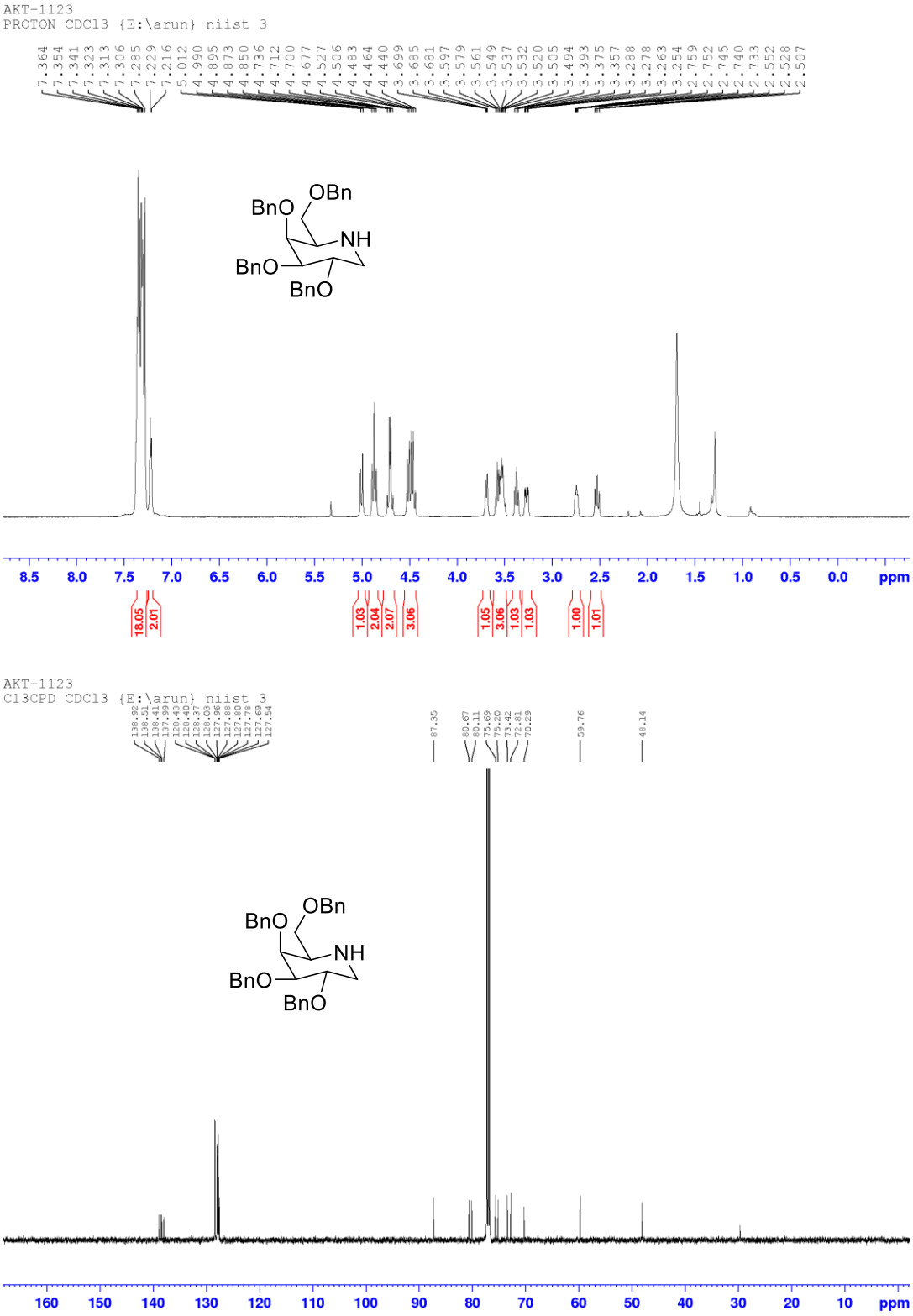
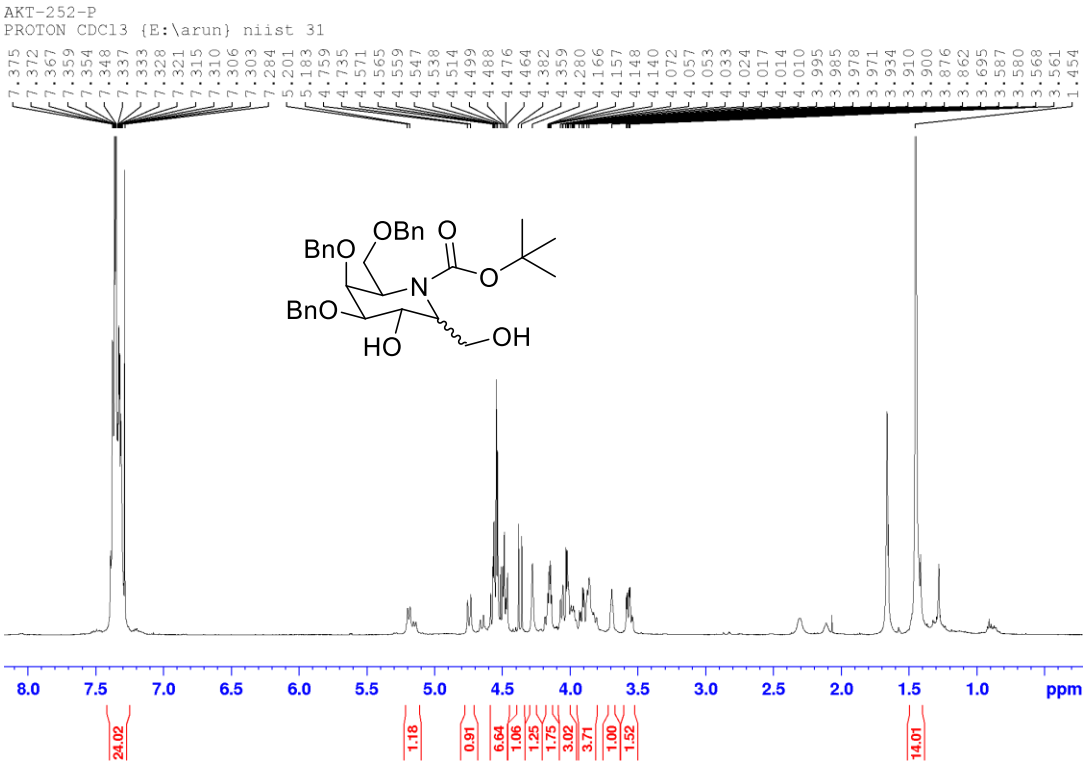


Figure 2.5.2.10: NMR spectras of compound **47**



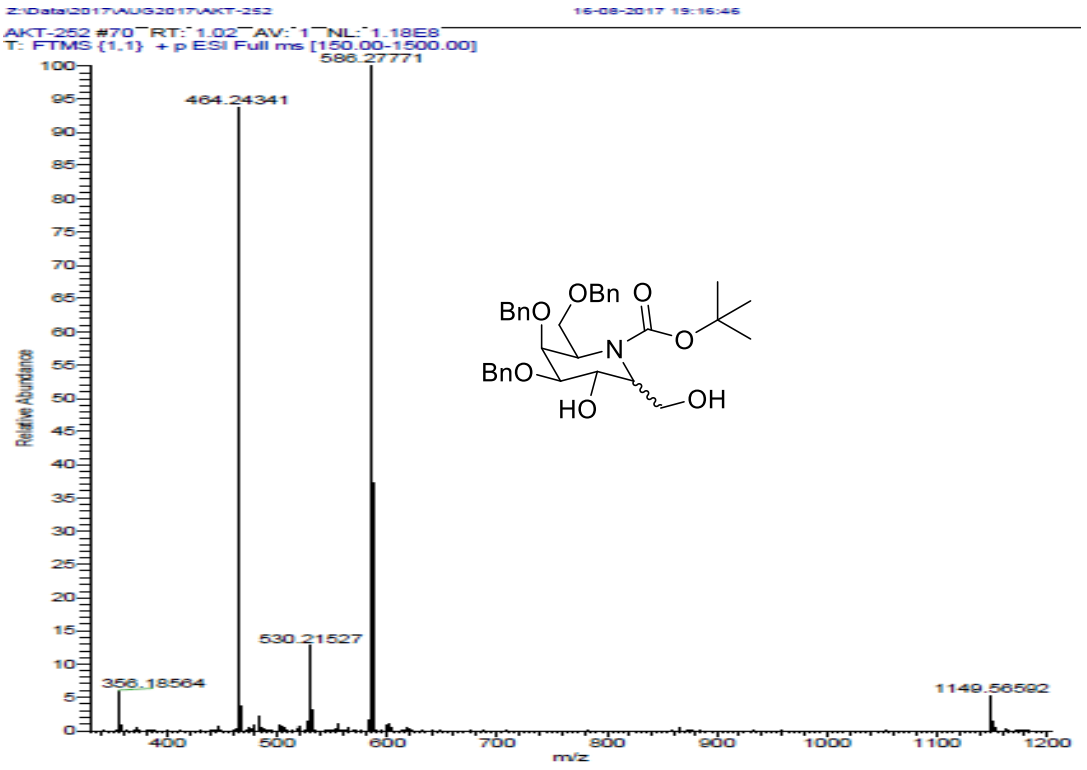
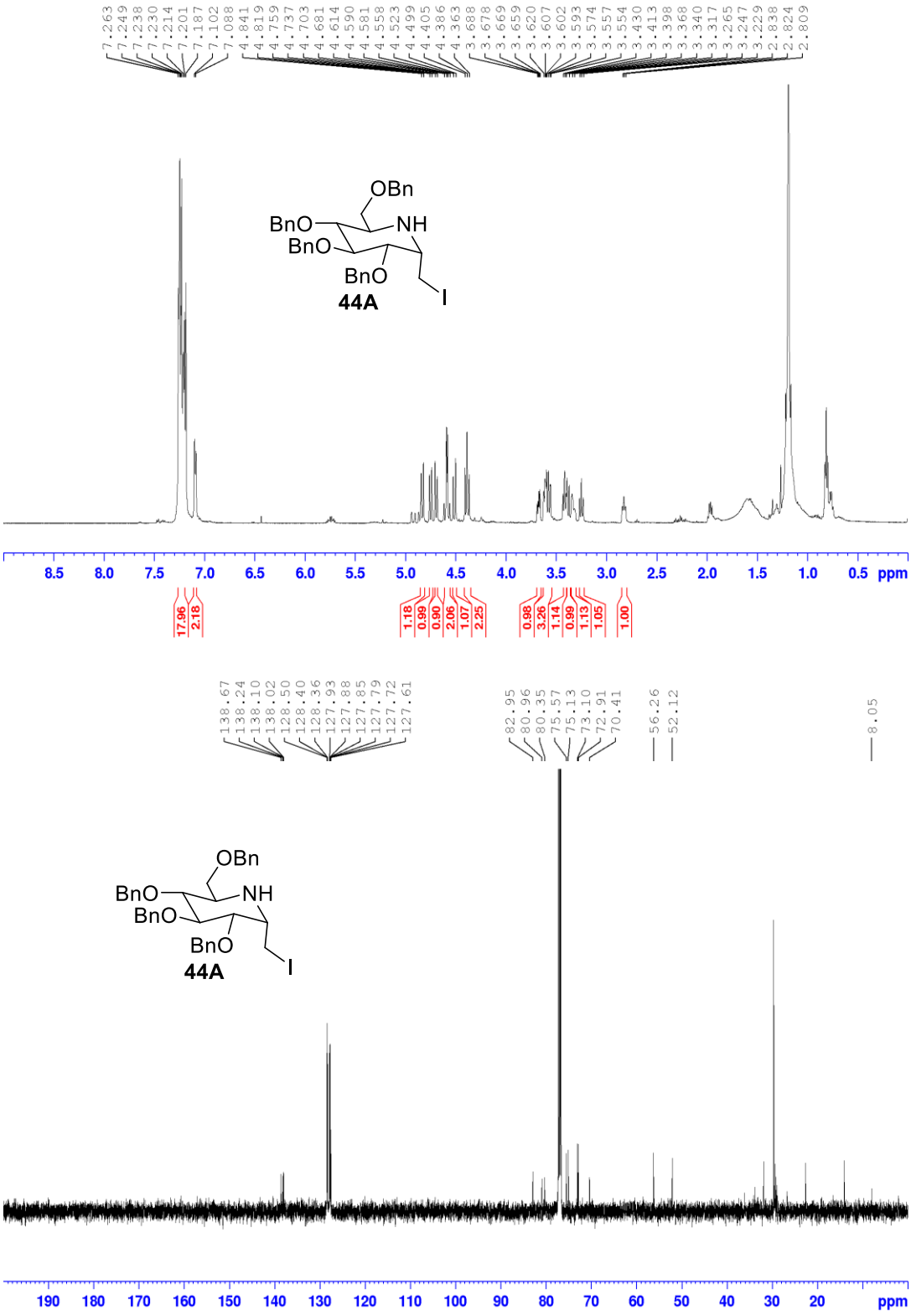
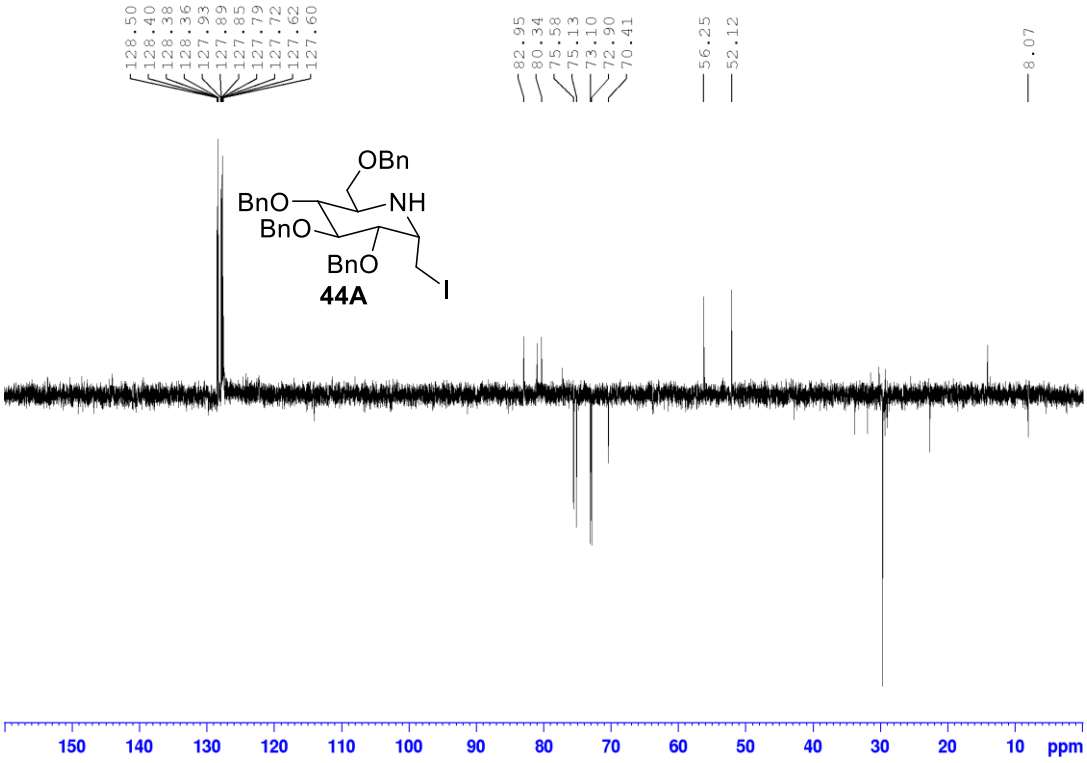


Figure 2.5.2.11: NMR spectras of compound **44A**

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73.10
72.90
70.41
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OBn
BnO
NH
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I
44A
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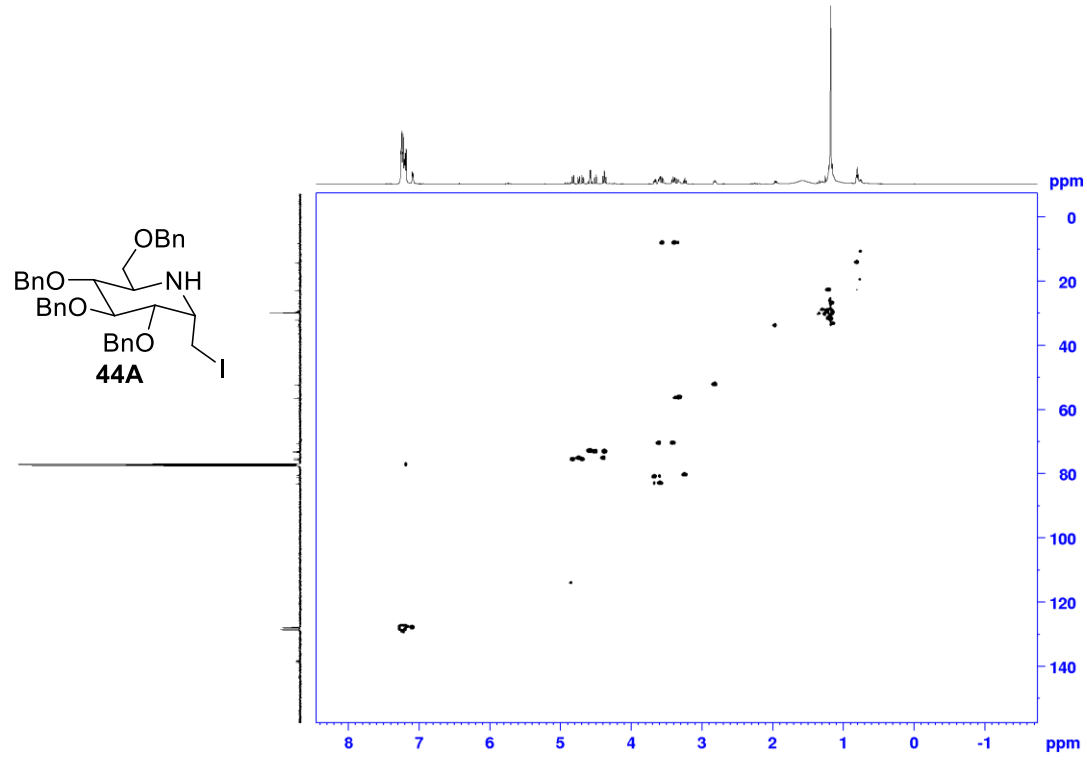
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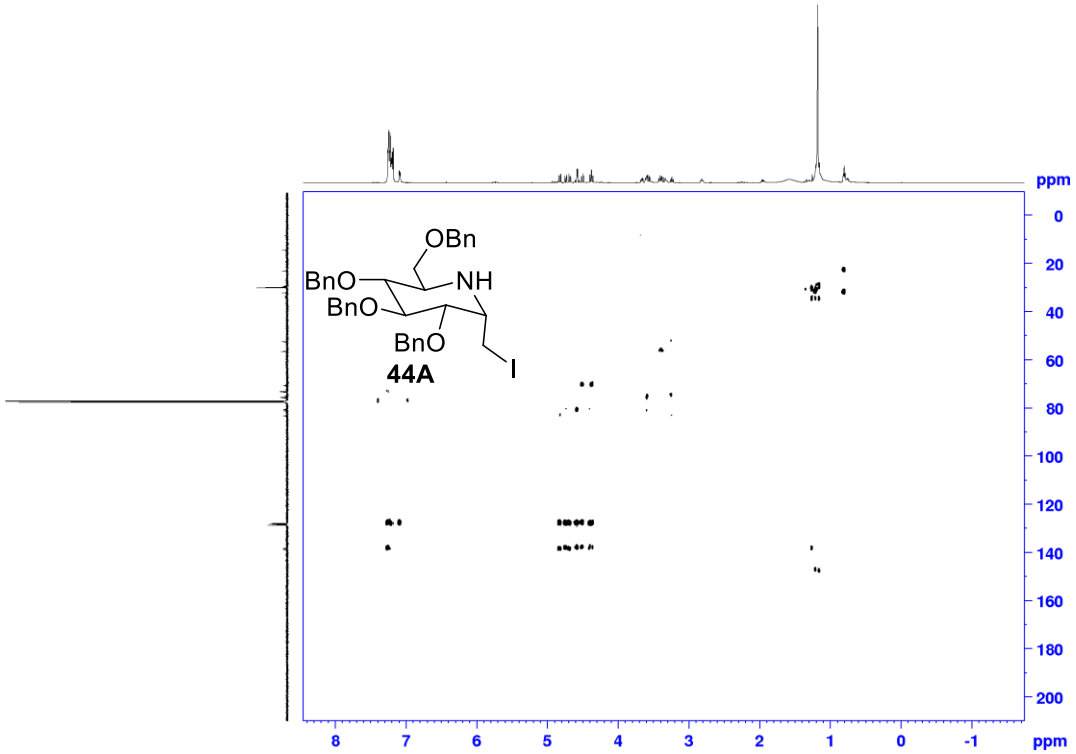
8 7 6 5 4 3 2 1 0 -1 ppm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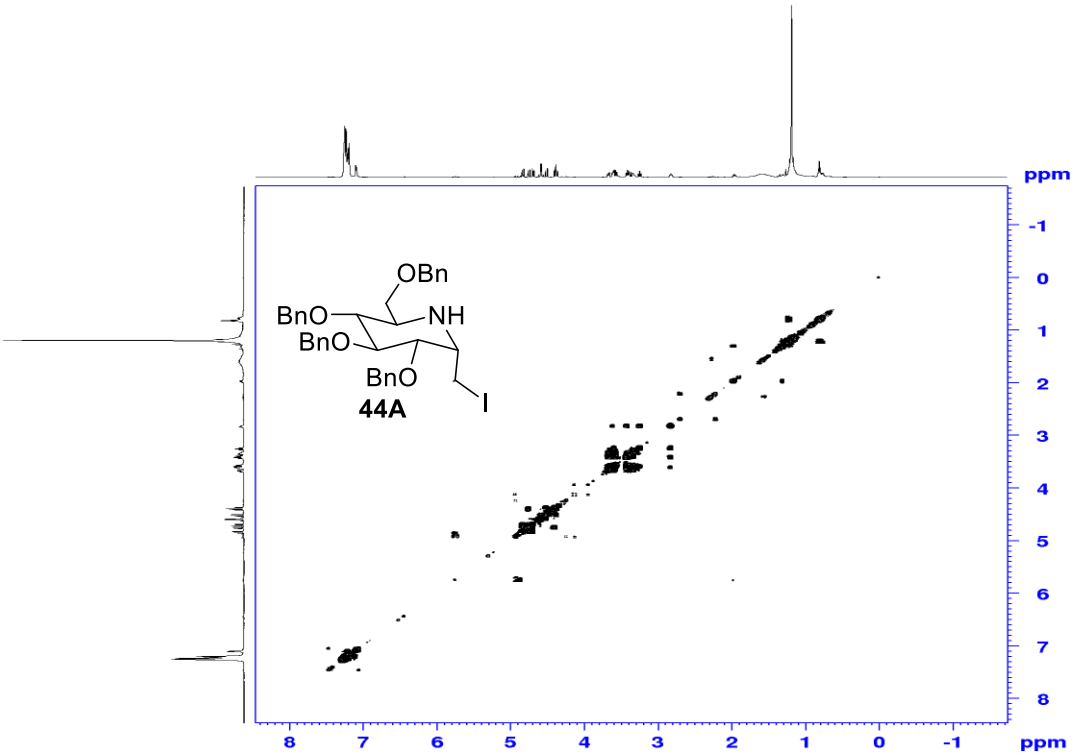
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Figure 2.5.2.12: Compound **44A** 2D NMR assignment

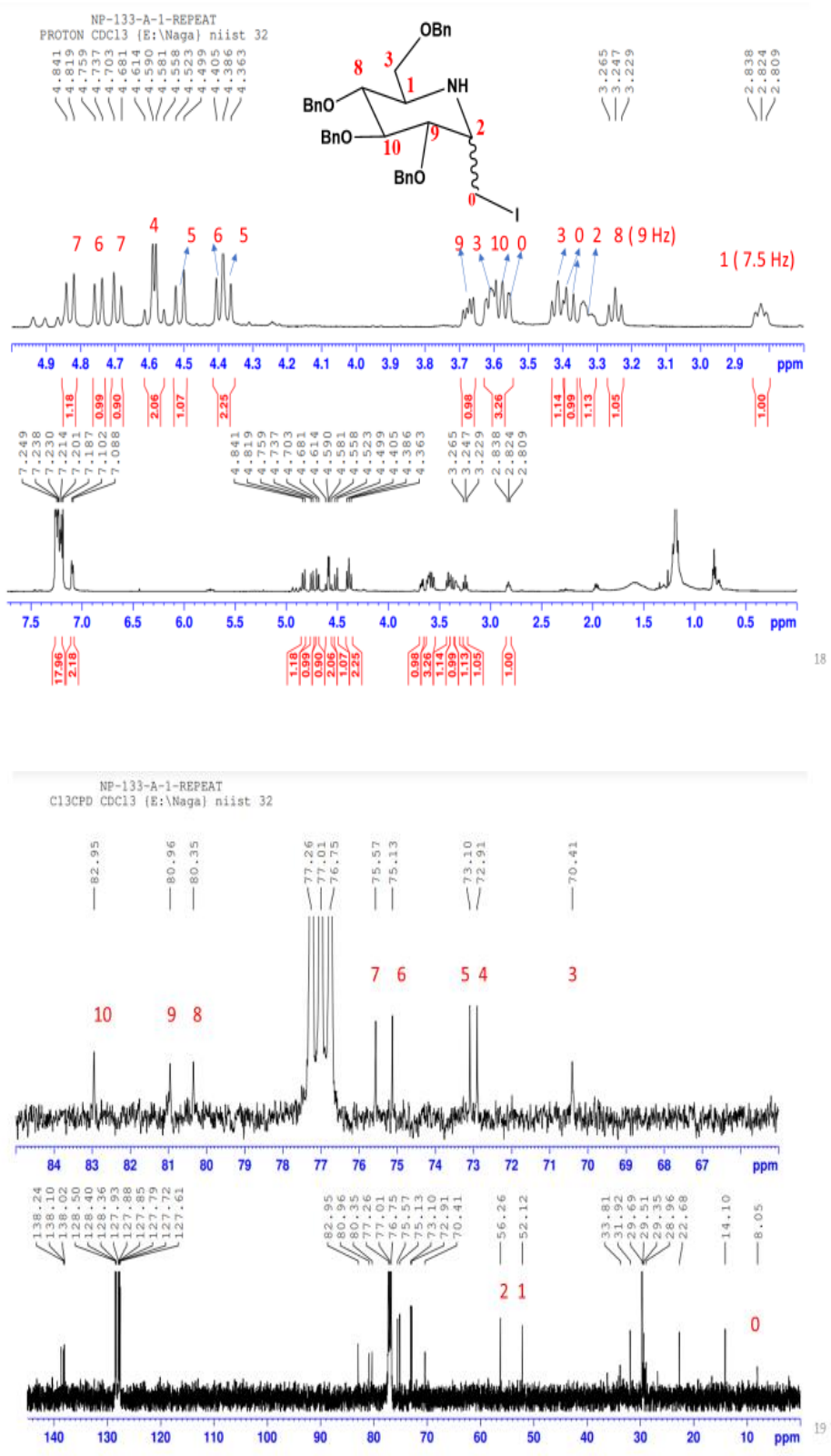
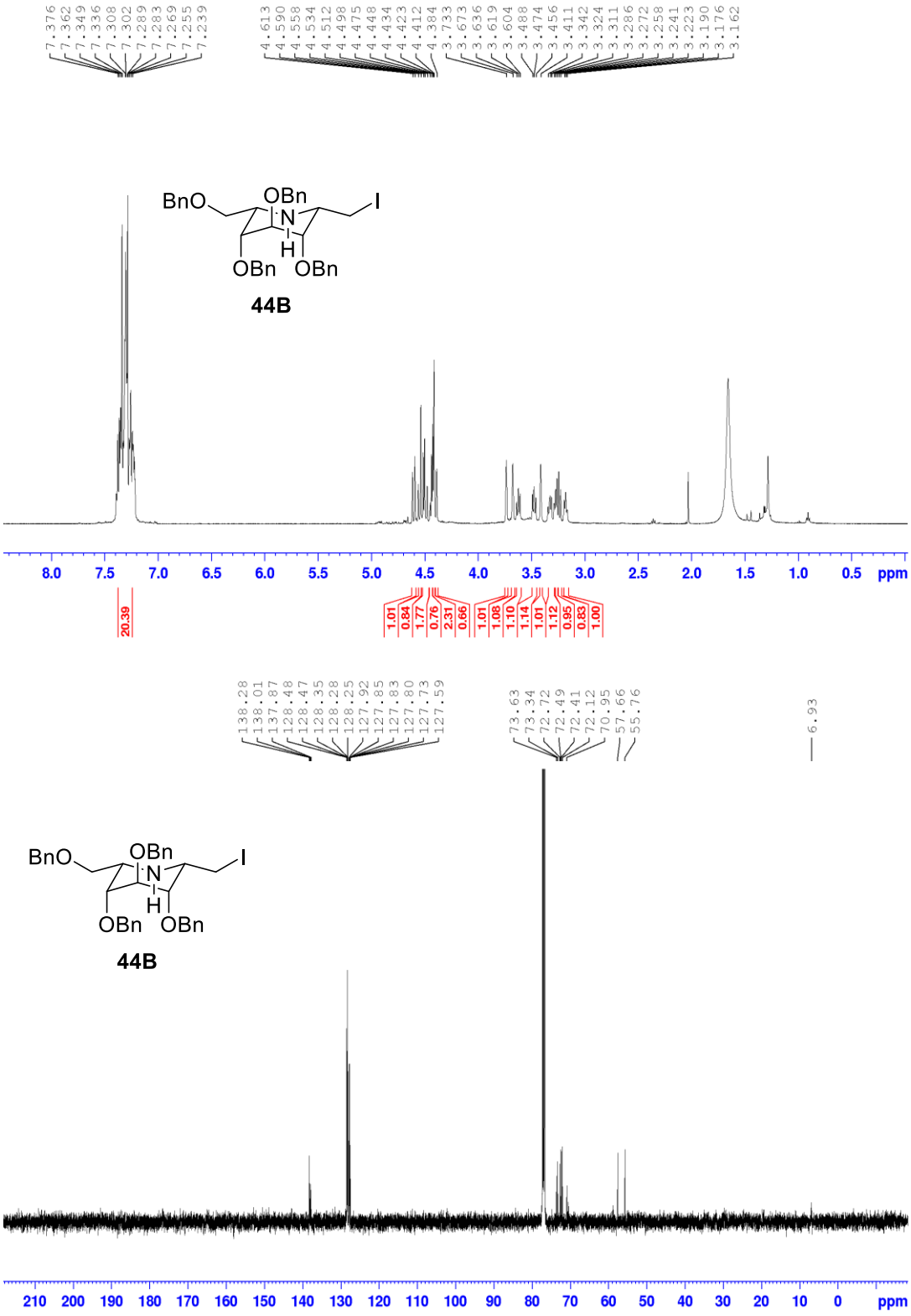
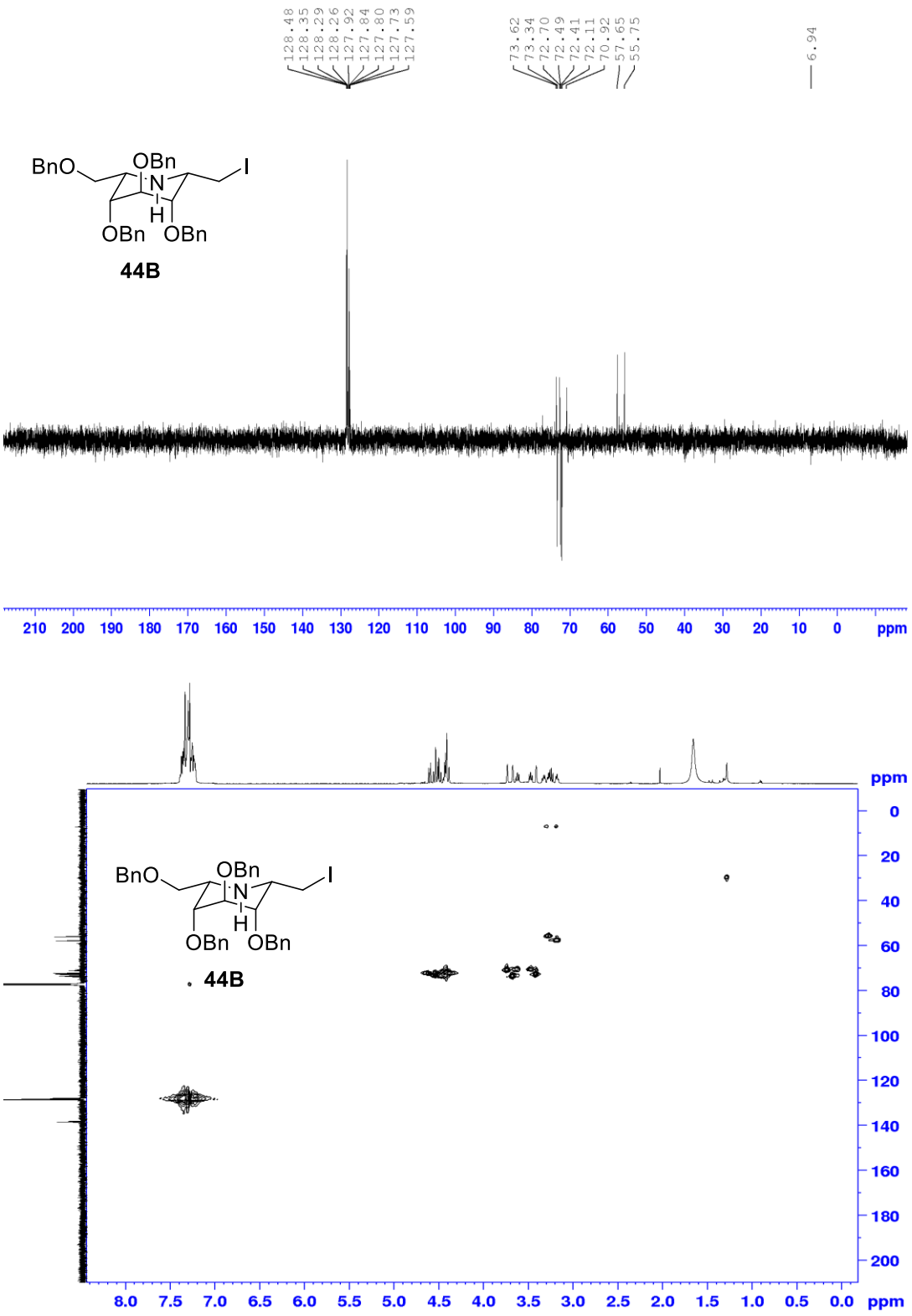


Figure 2.5.2.13: NMR spectras of compound **44B**

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55.75
6.94
BnO
OBn
N
H
I
OBn
OBn
44B
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
ppm
0
20
40
60
80
100
120
140
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200
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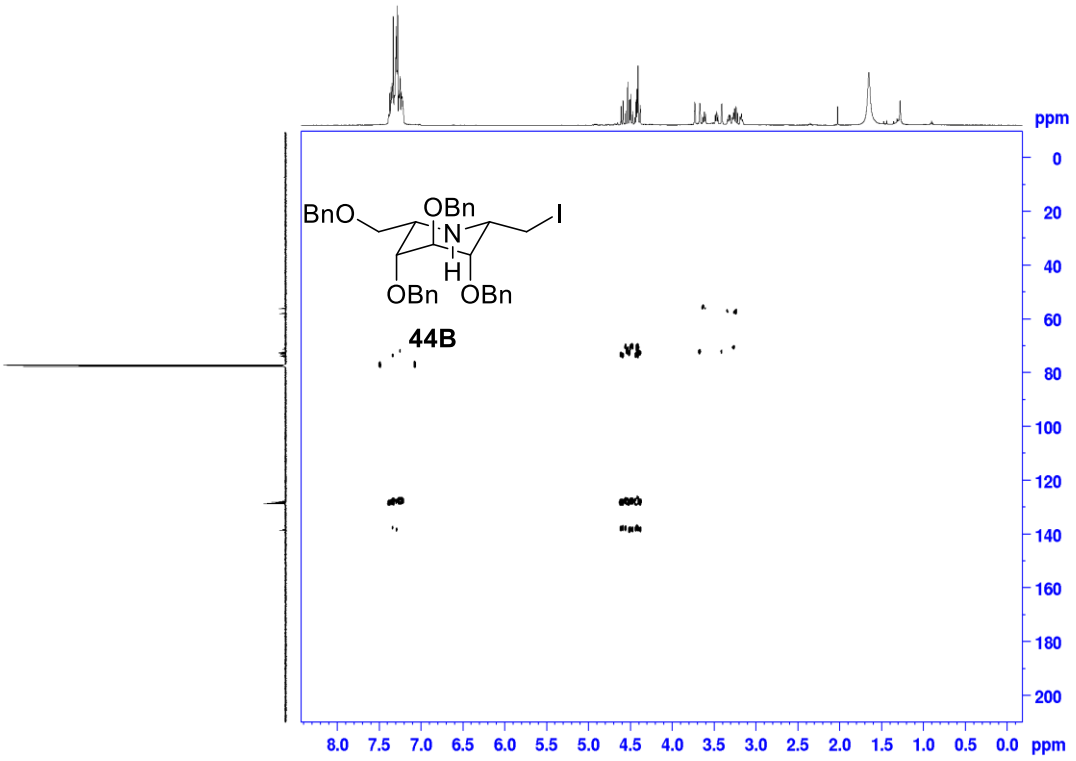
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200
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7.5
7.0
6.5
6.0
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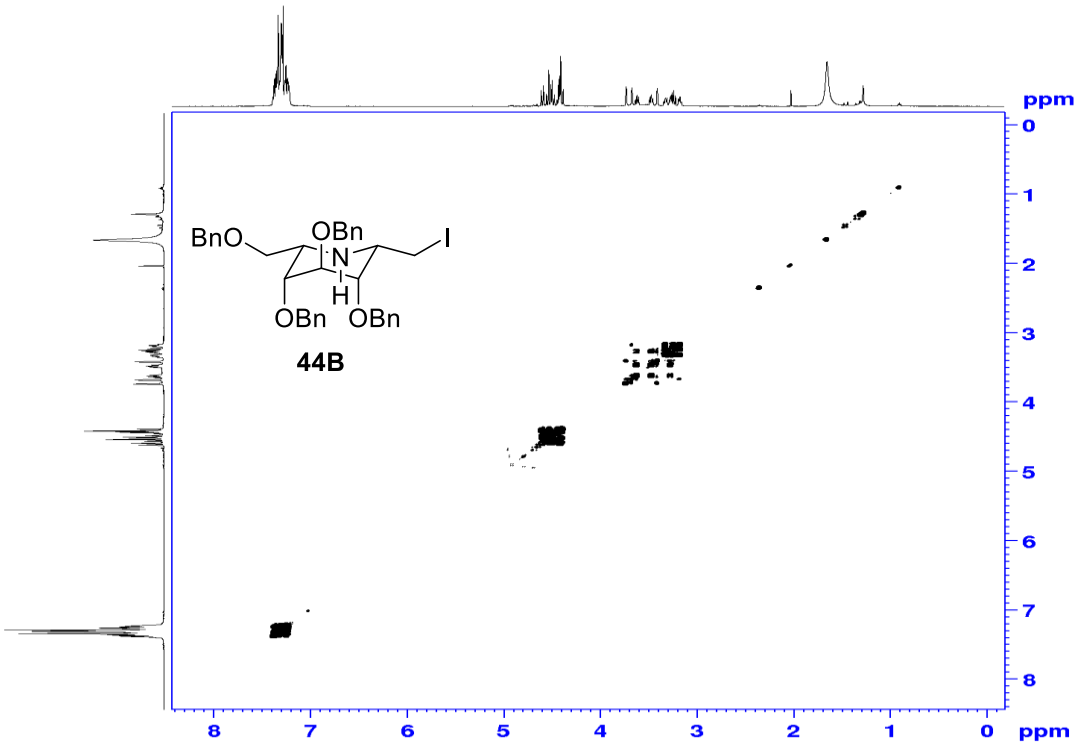
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44B
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2
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ppm

Figure 2.5.2.14: Compound **44B** 2D NMR assignment

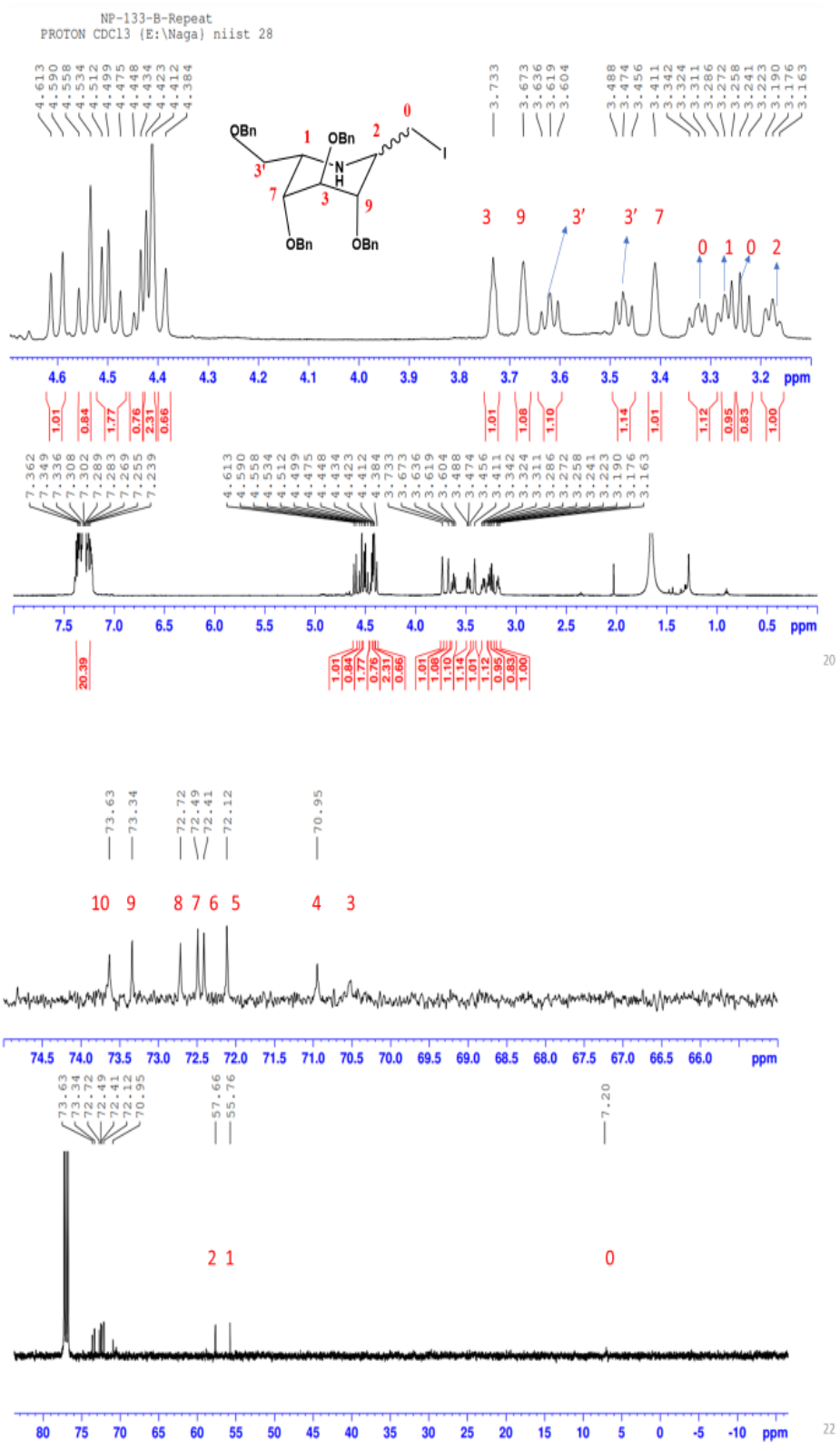
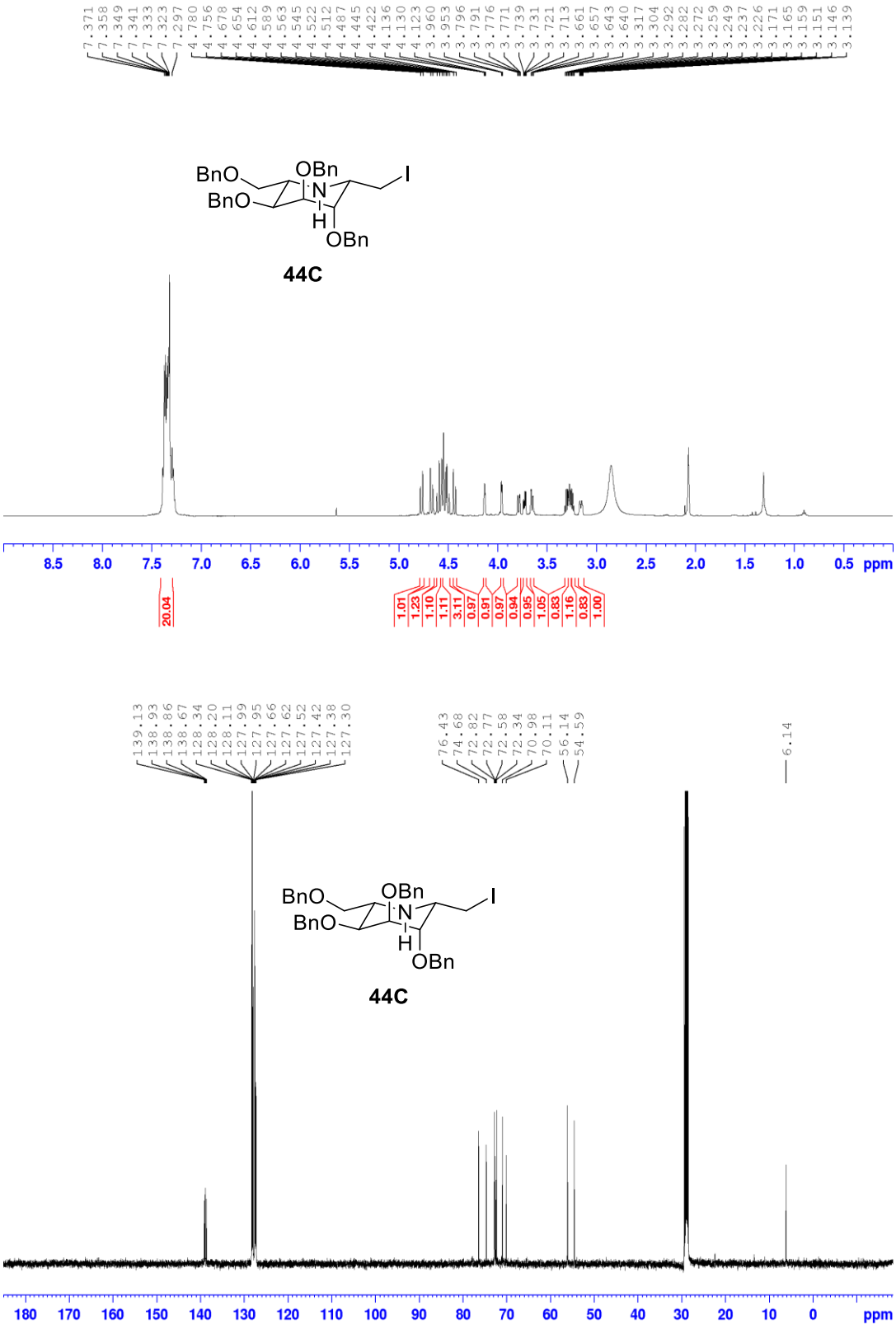
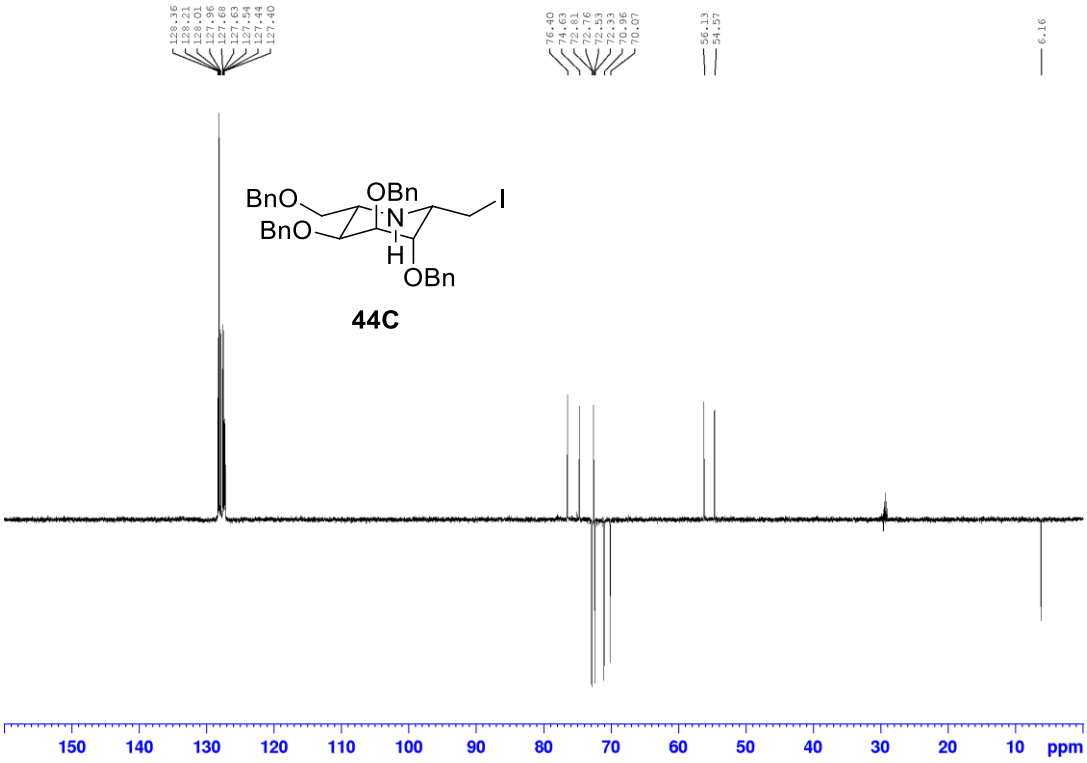


Figure 2.5.2.15: NMR spectras of compound **44C**

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76.40
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72.76
72.53
72.33
71.96
70.07
56.13
54.57
6.16
BnO
OBn
BnO
N
H
I
OBn
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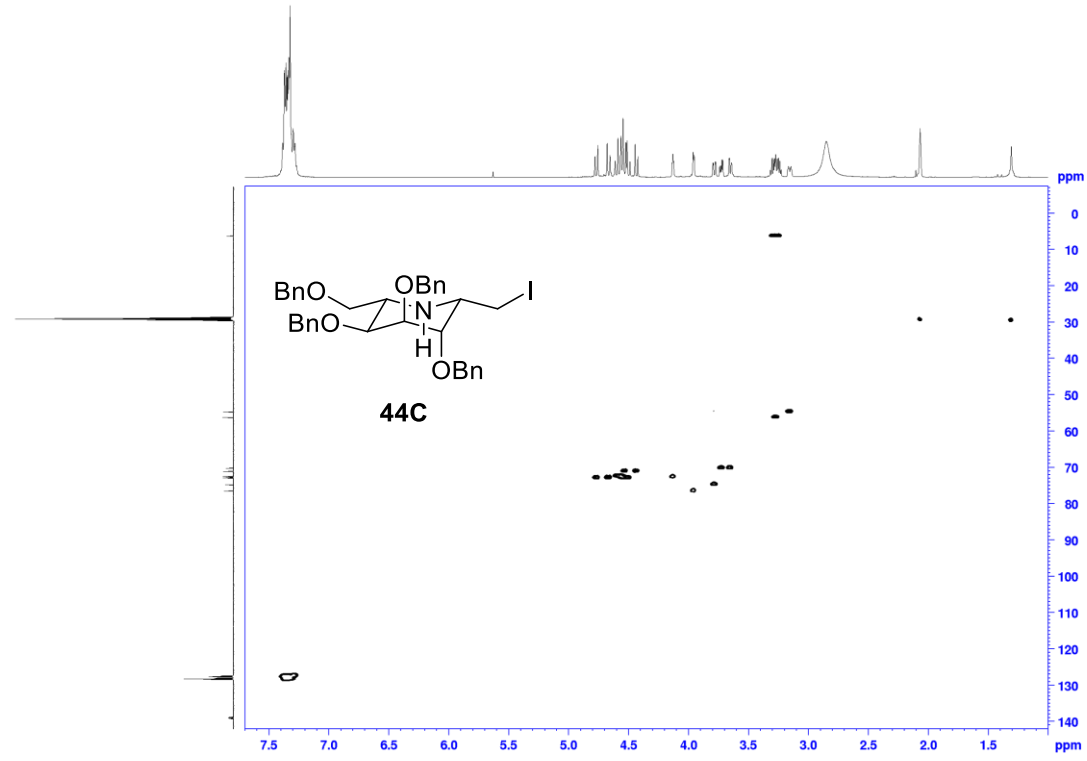
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

44C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7.0
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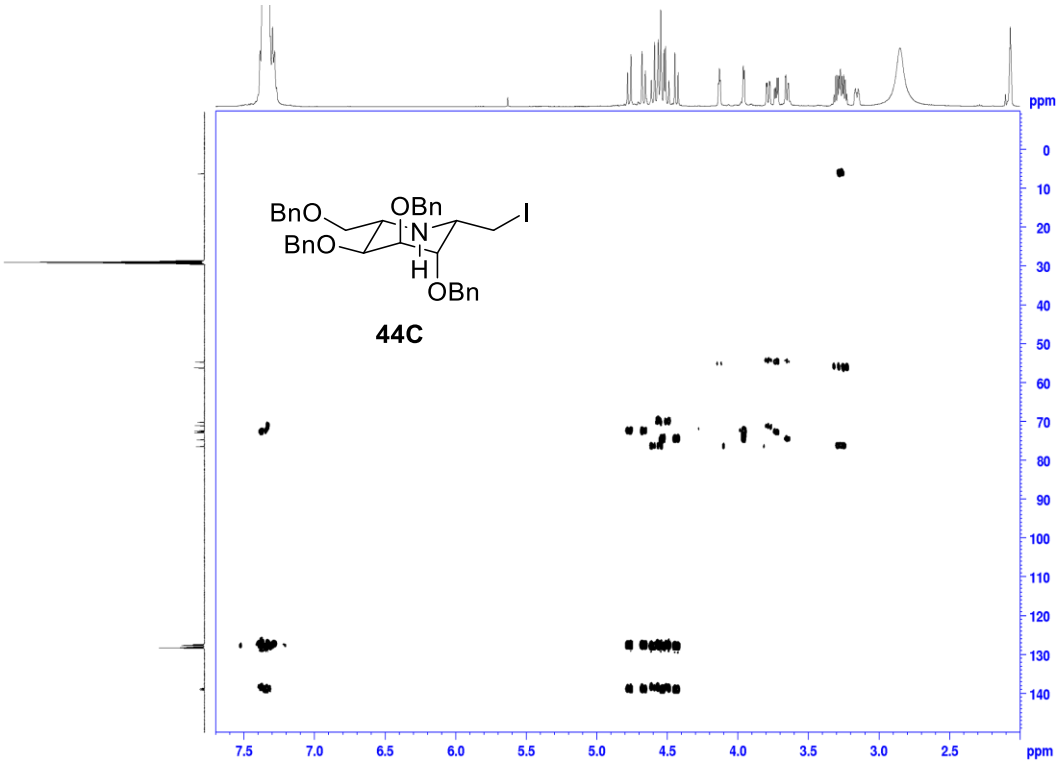
3.0
2.5
2.0
1.5
ppm

44C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OBn
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130
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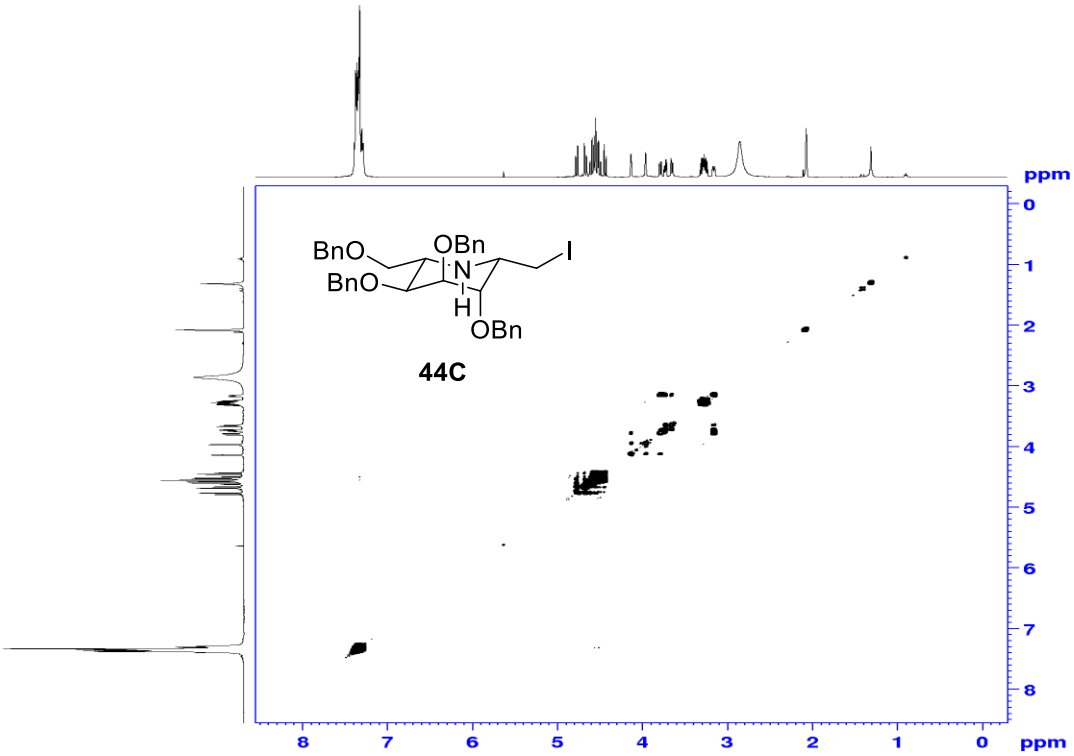
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I
OBn
44C
ppm
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2
3
4
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6
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7
6
5
4
3
2
1
0
ppm

Figure 2.5.2.16: Compound **44C** 2D NMR assignment

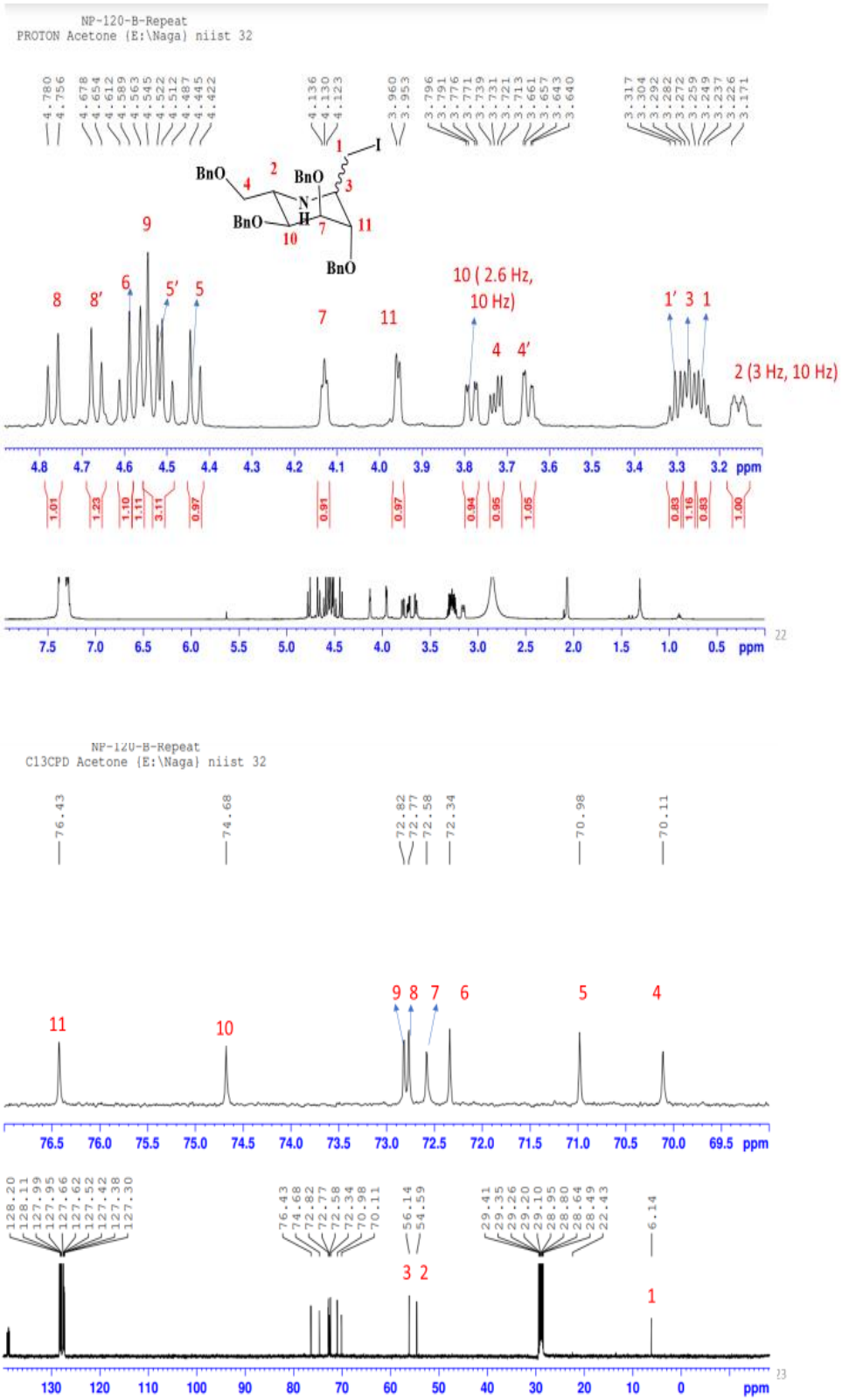


Figure 2.5.2.17: NMR spectras of compound **50A**

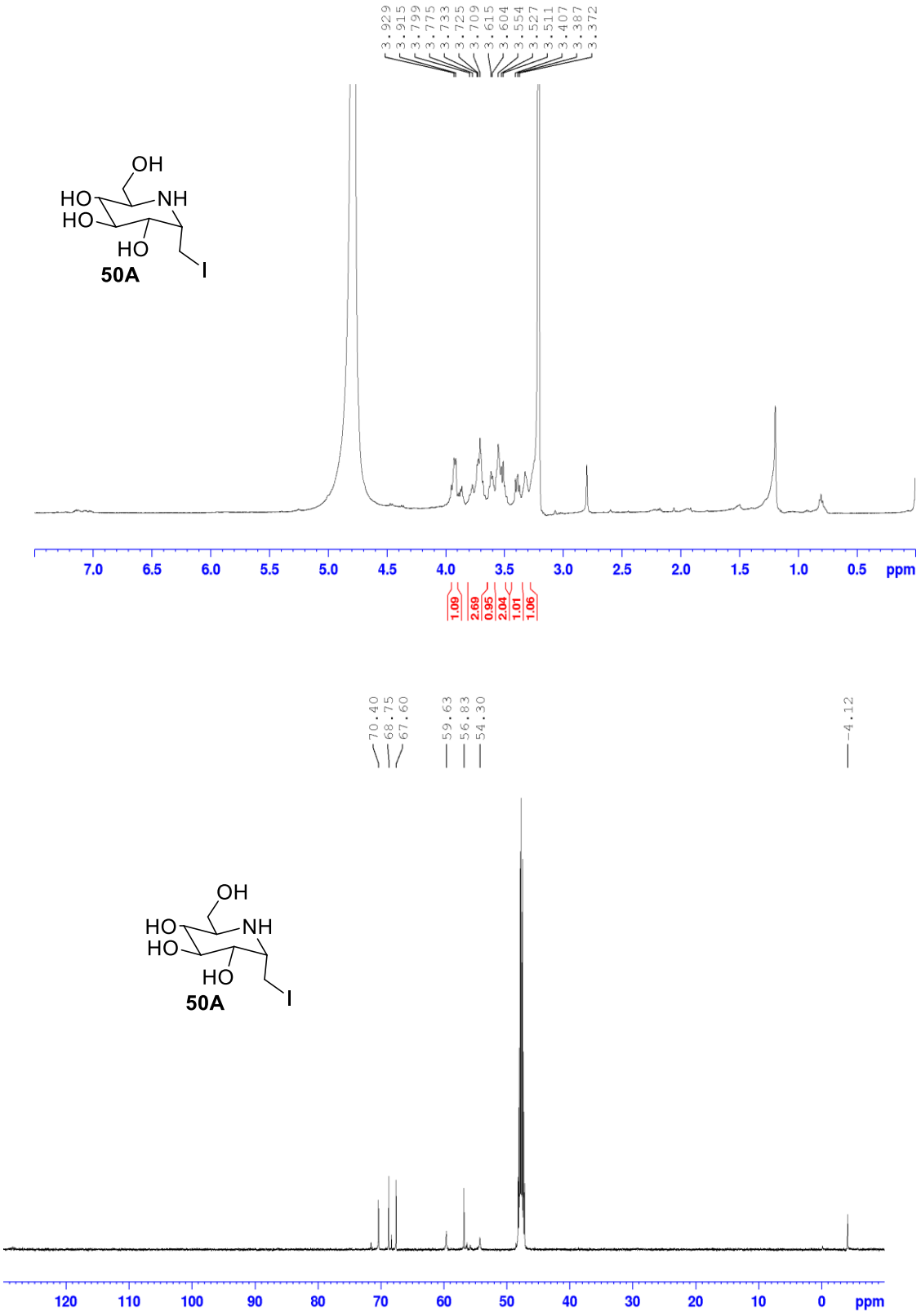


Figure 2.5.2.18: NMR spectras of compound **50B**

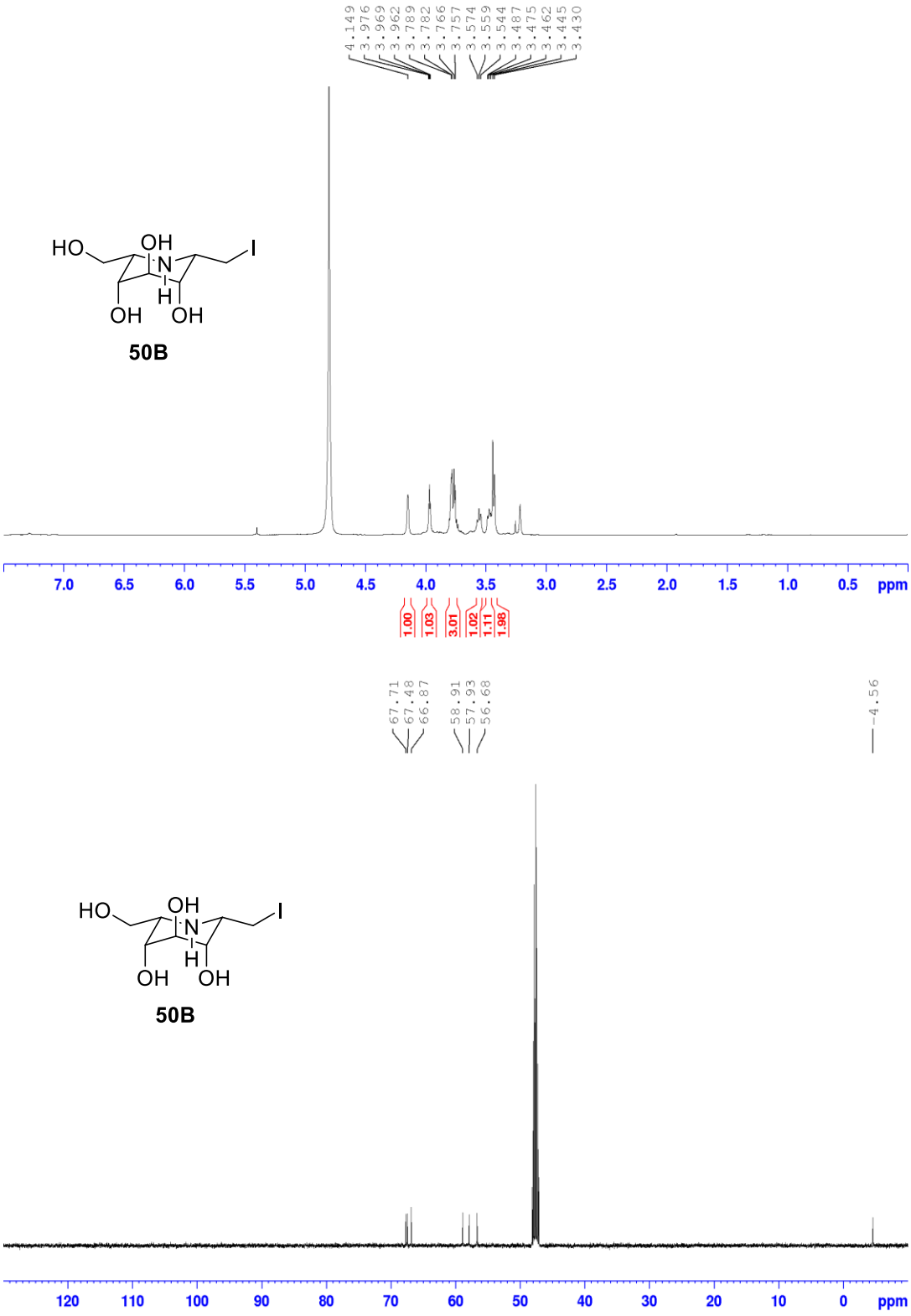


Figure 2.5.2.19: NMR spectras of compound **50C**

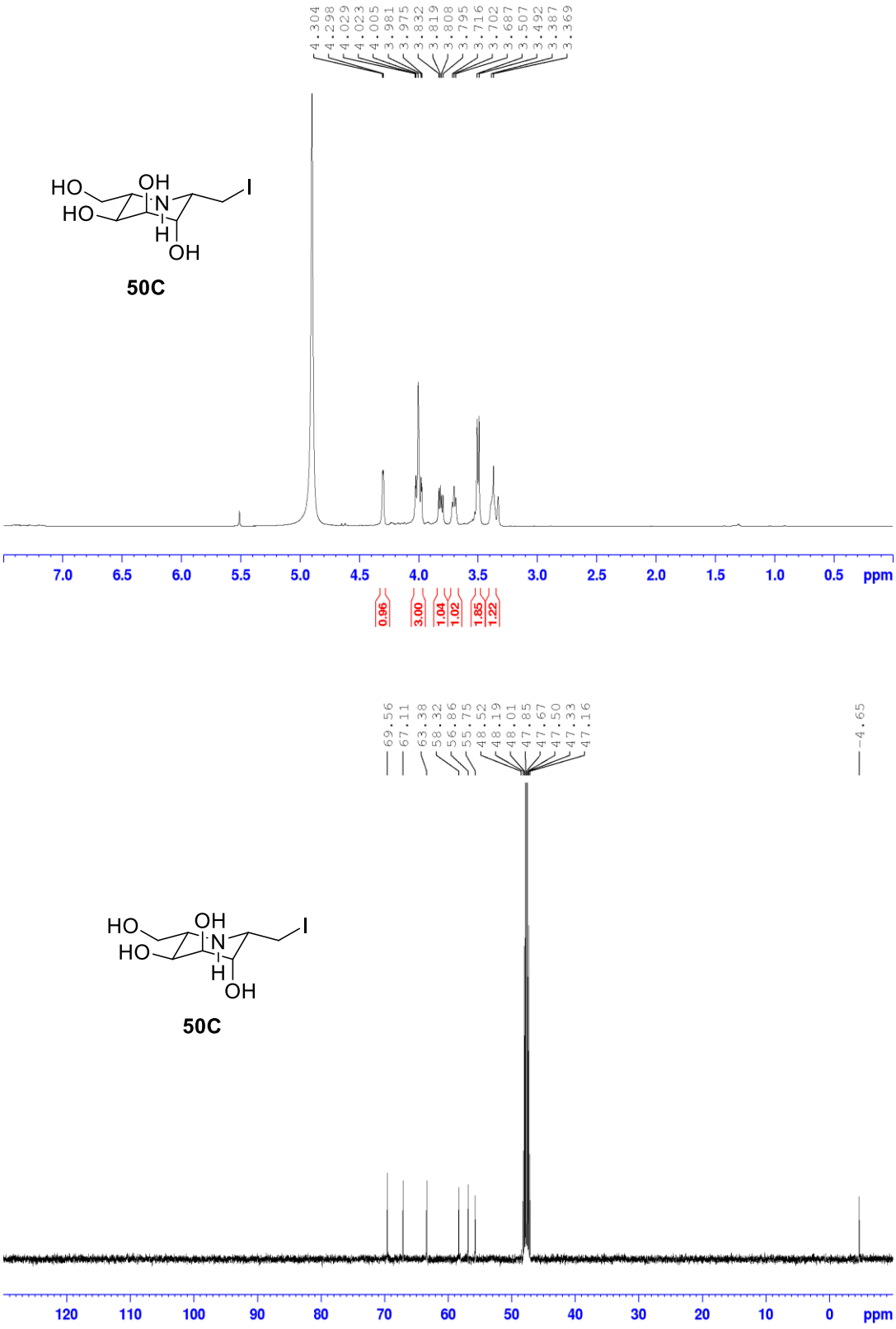
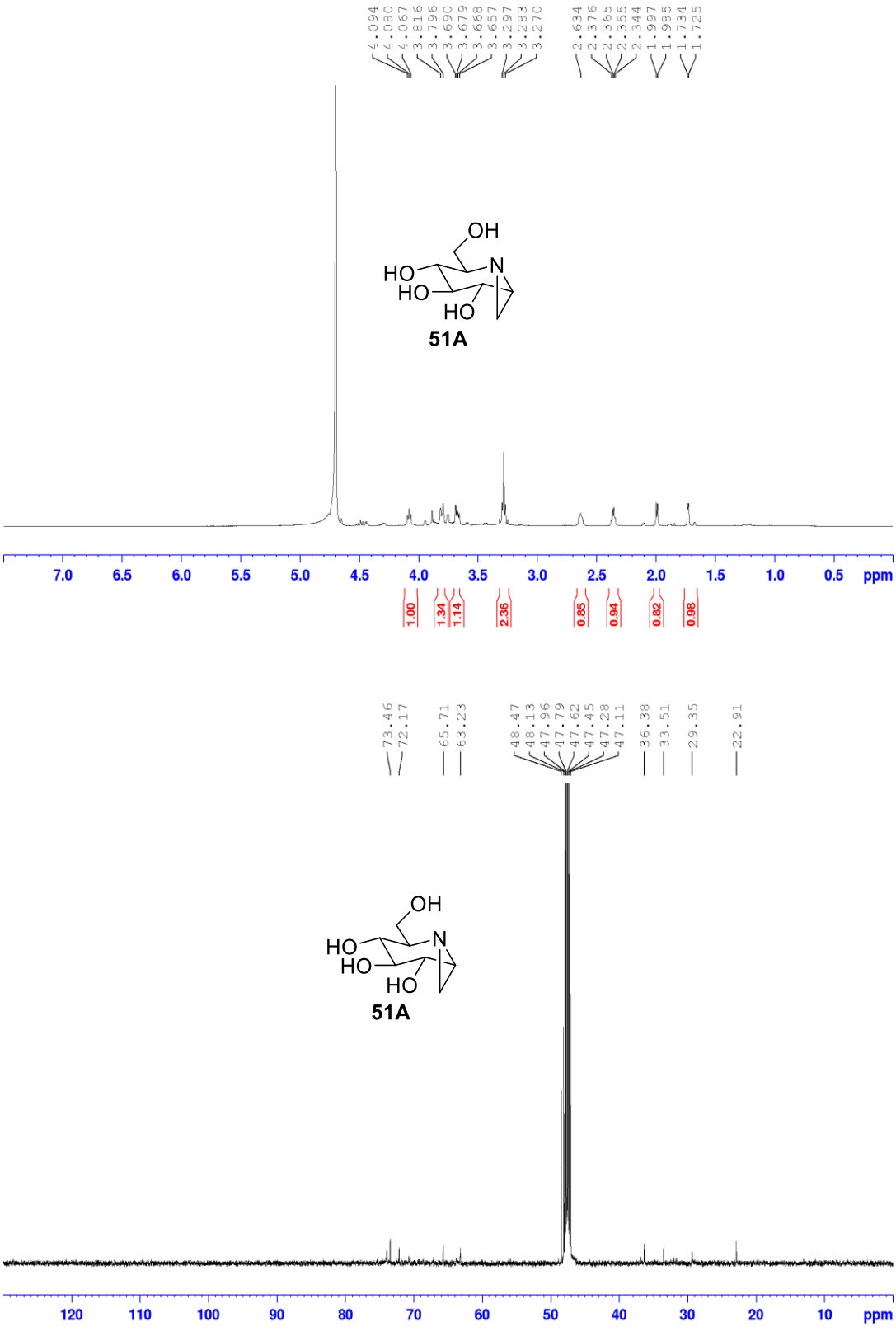


Figure 2.5.2.20: NMR spectras of compound **51A**



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Chapter 3

Synthesis of Iminosugar Appended Miltefosine Analogue

3.1. Abstract

The focus of this chapter is on the synthesis of iminosugar-appended miltefosine analogue of bilogical relevance. Using click chemistry, the coupling process between the iminosugar derivative and the phospholipid was achieved, with an objective to explore their antifungal and anticancer studies.

3.2. Introduction

Alkylphosphocholines are phospholipids that are well known for their pharmacological relevance. As a result, several synthetic procedures are available for synthesis of novel alkylphosphocholines for drug discovery. The variations in phosphocholine ester synthesis have been reported on aliphatic alcohols, chain length, unsaturation, and a shift in the location of the cis-double bond of the aliphatic linkage. In the 1970s, the first lysophospholipid analogues were synthesized, and their toxicity in cancer cells indicated their anticancer potency. Later, the essential phospholipid molecules were synthesized as lysophosphatidylcholine counterparts.[1,2] Because alkylphosphocholines have been intimately correlated to anticancer effects. Hilgard's research in 1993 disclosed to the scientific realm that the glycerol component is not necessary for phosphocholines.[3]

Miltefosine, a hexadecylphosphocholine molecule, has shown significant anticancer activity against a variety of human cell lines, including (breast [MDA-MB-231], prostate [PC-3], colon [KM12], lung [HOP-92], melanoma [M14]) and others. Initially, miltefosine proved to be

an anticancer and sedative drug,[4] but in 1980, it was repurposed for the first time for treatment of visceral leishmaniasis.[5–10] Other notable synthetic phospholipids known for their biological properties besides miltefosine include erufosine,[11,12] erucylphosphocholine,[13–15] perifosine,[16] edelfosine,[17,18] and ilmofosine.[19,20] Majority of these phospholipids are well-known for their anticancer properties and most of them are at various stages of clinical trials.

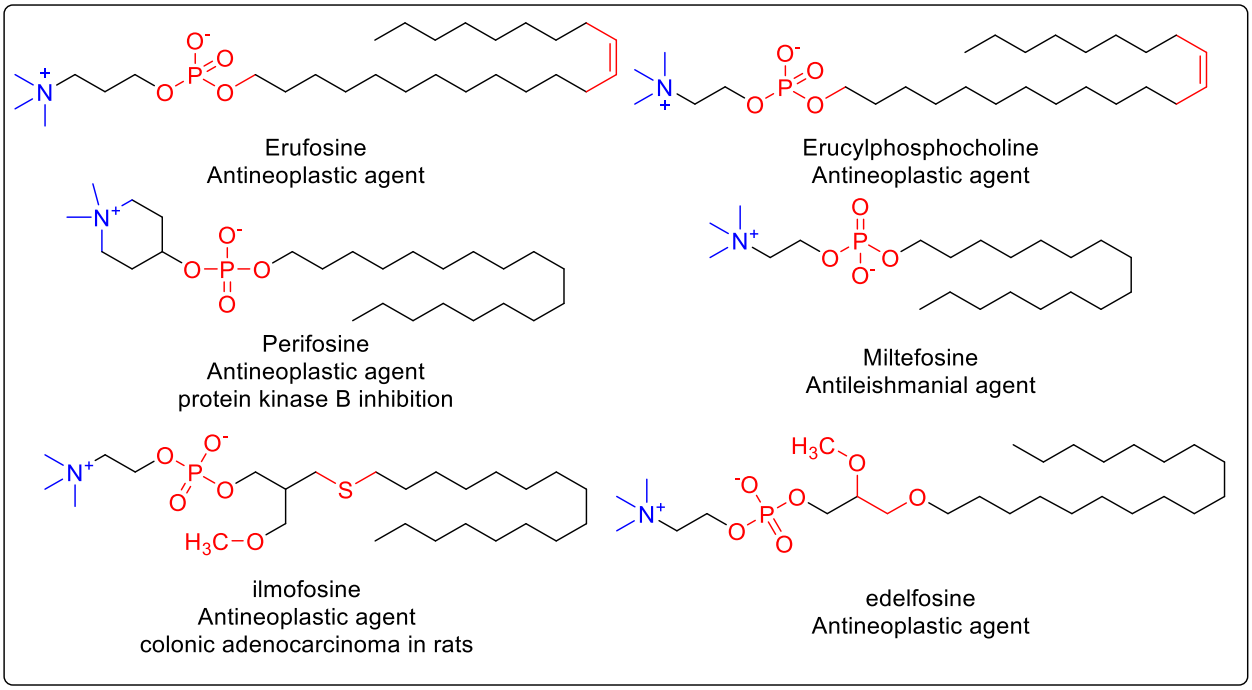
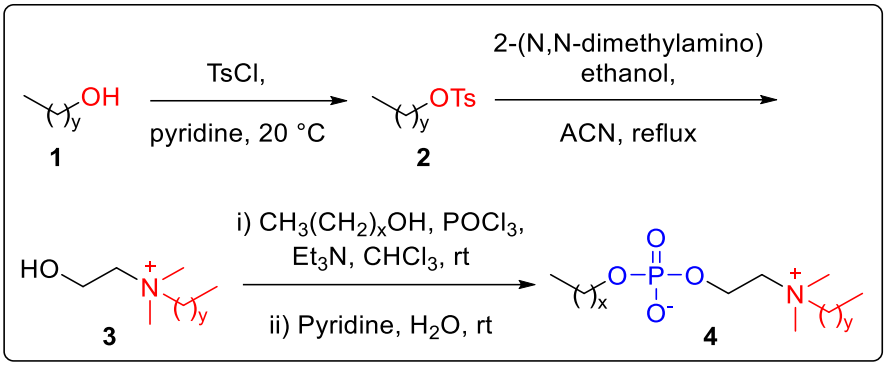


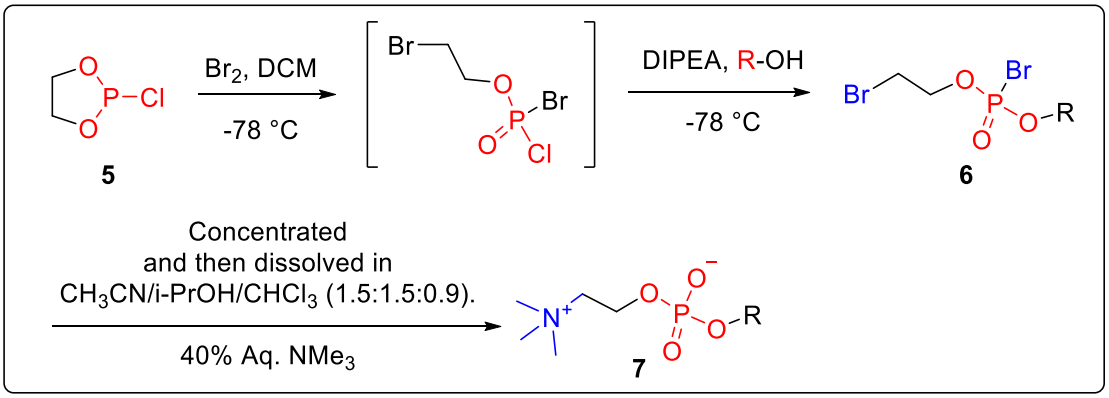
Figure 3.2.1a. Bioactive alkylphosphocholines

The reported instances of oral cure, usefulness against visceral leishmaniasis, and bio-active features such as anti-bacterial, anti-fungal, and anti-HIV encouraged researchers to devise innovative synthetic routes for the synthesis of Miltefosine. In 2005, Jurgen *et al.* reported the synthesis of novel alkyl phospholipid derivatives for treating various diseases in mammals caused by microorganisms, specifically parasites, protozoa, bacteria, and viruses. The study detailed a multistep synthesis of phospholipid derivatives that included the generation of quaternary ammonium attached hydroxyl moieties, followed by $POCl_3$ phosphorylation. The procedure involved varying the N-alkyls and lipid moieties in phospholipid derivatives.[21]



Scheme 3.2.2a. Synthesis of novel alkyl phospholipids using $POCl_3$

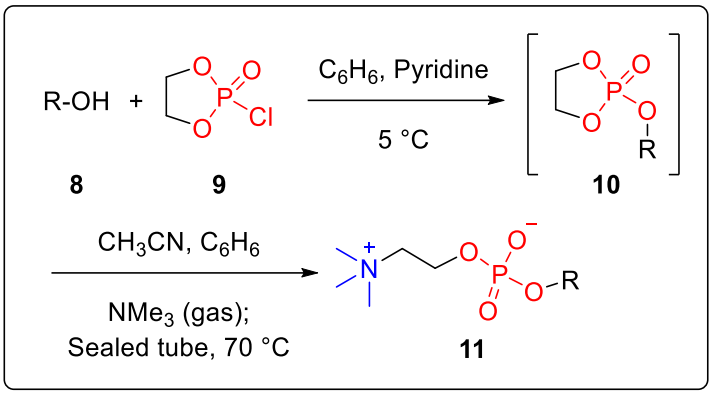
Bittman *et al.* detailed the synthesis of miltefosine and similar phospholipid derivatives that utilize five linear steps from 2-chloro-dioxophospholane. It is a one-pot strategy for five consecutive reactions. The initial step was ring-opening of the five-membered phosphocycle using bromine and subsequent primary alcohol coupling in the presence of a base. The final step is the transformation of alkyl bromide into quaternary ammonium salts utilizing aqueous trimethylamine in a combination of three solvents CH_3CN, *i*PrOH and $CHCl_3$.[22]



Scheme 3.2.3a. Synthesis of alkylphosphocholines using 2-chloro-dioxophospholane

Lankalapalli *et al.* detailed the first chemical synthesis of a rare analogue of plasmalogen bearing a trans-O-vinyl connected glycerol phospholipid compound. The main steps in the synthesis are the *E*-stereoselective enol ether arrangement through iridium(I)-mediated olefin

isomerization of O-allyl ether and phosphorylation on glycerol followed by ring-opening reaction with trimethylamine gas.[23]



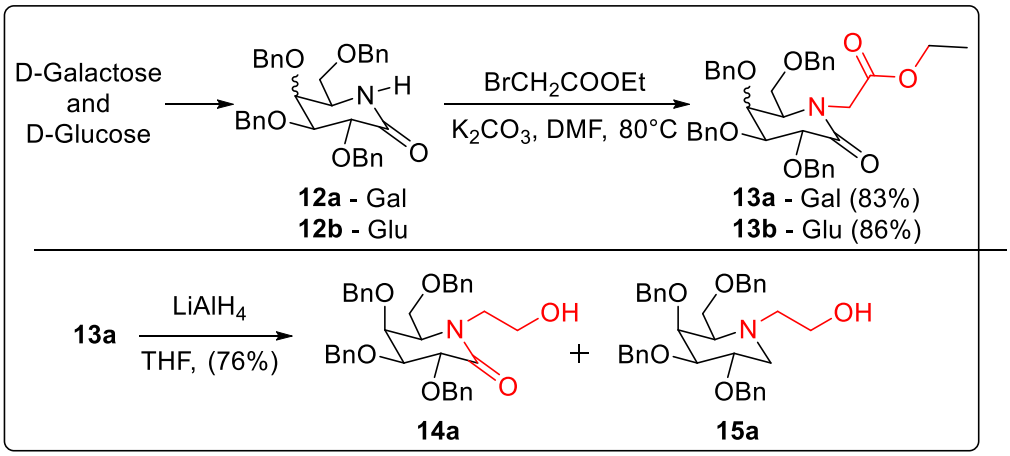
Scheme 3.2.4a. Synthesis of plasmalogen analogues

In the biological sphere, however, iminosugars are the prominent bioactives. These compounds are well-known for their glycosidase, anti-viral, canticancer properties, and the ability to treat rare genetic diseases such as Gaucher disease and Fabry disorders. Miglitol and miglustat are FDA-approved iminosugar compounds. With more iminosugar molecules in the pipeline for clinical studies, their scope is widening day by day.[24–27] Previous reports on the therapeutic potential of iminosugars and miltefosine molecules motivates us to investigate a conjugate of these two molecules.

3.3. Results and Discussion

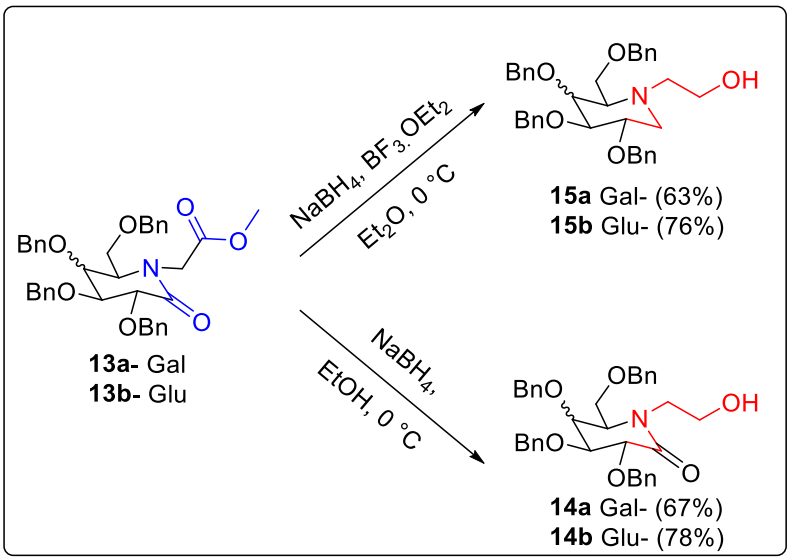
Phosphoramidite methodology could be an efficient route for synthesis of iminosugar-linked miltefosine analogues. Firstly, the synthesis of iminosugar primary alcohol derivative was carried out via multistep synthesis from D-galactose, which afforded lactam iminosugar over eight steps. The lactam NH was treated with bromomethyl acetate, which afforded the amide ester compound **13a**. The plan was to reduce the amide ester into the deoxy ethanol derivative of compound **15a**. Likewise**,** similar endeavours were in place for the reduction reaction.

Surprisingly, a combination of compounds such as totally reduced and ester alone reduced molecules **14a** and **15a** in 76 % yield was observed.

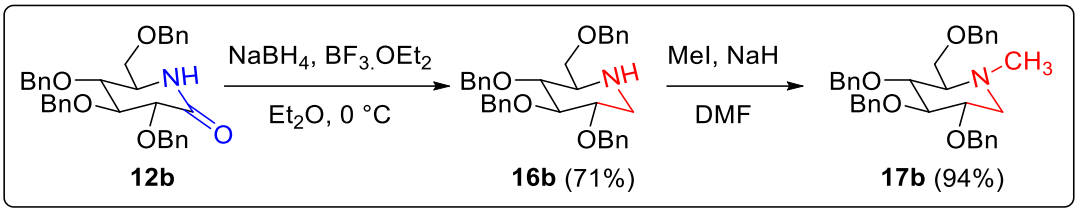


Scheme 3.3.1a. Synthesis of compounds **14a** and **15a**

$NaBH_4$ and $BF_3.OEt_2$ in diethyl ether at 0 °C permitted the total reduction of lactam ester to afford **15a-b**. However, reduction by utilizing $NaBH_4$ in ethanol at 0 °C resulted in compound **14a-b**. Furthermore, we reduced lactam **12b** to produce the *N*-methyl deoxynojirimycin (DNJ) derivative **17b** via compound **16b**. Compound **17b** can serve as a precursor for preparation of quaternary ammonium analog of miltefosine.

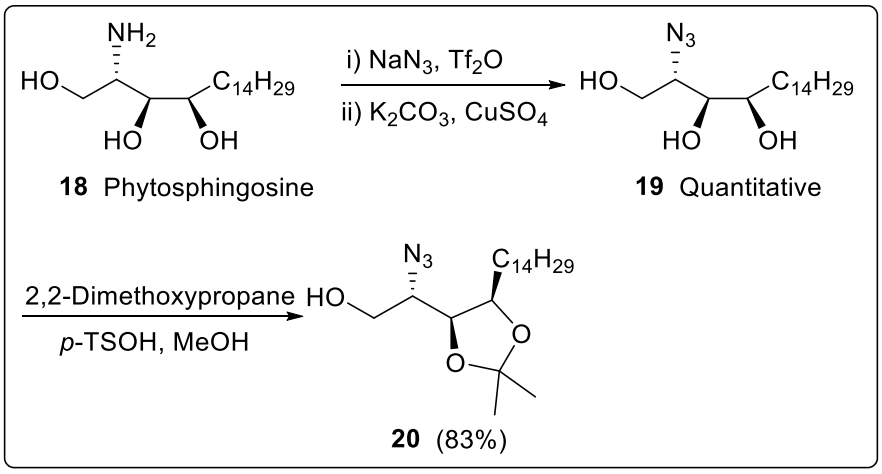


Scheme 3.3.2a. Reduction of the lactam sugars using $NaBH_4$



Scheme 3.3.3a. Synthesis of 1-DNJ **16b** and *N*-methyl DNJ **17b**

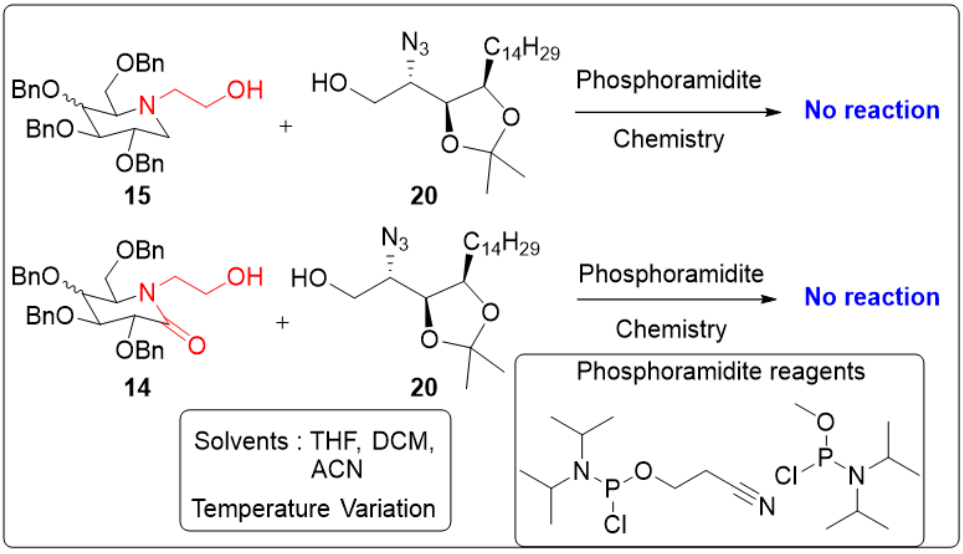
This led to the synthesis of one of the coupling partner for the phosphoramidite chemistry reaction. After the successful synthesis of iminosugar molecule, the focus turned towards the preparation of another coupling partner, namely lipid primary alcohol. For the lipid partner, the phytosphingosine molecule was chosen in order to produce a sphingosine analogue of milteofsine.



Scheme 3.3.4a. Synthesis of azido-alcohol compound **20**

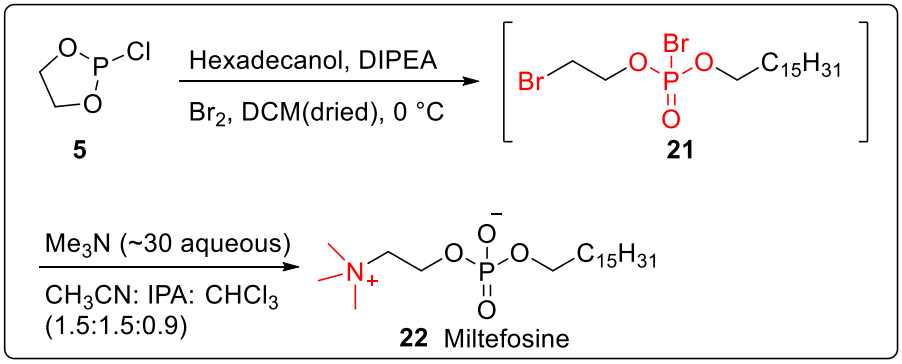
Synthesis began by reacting NaN_3 and Tf_2O to make a triflic azide intermediate which was treated with phytosphingosine **18** in the presence of K_2CO_3 and $CuSO_4$. The reaction went smoothly and resulted in a phytosphingosine azido triol. Vicinal diol was protected using 2,2-dimethoxy propane under the 1,2-diol protection procedure to afford **20** in a good yield. With the primary alcohol in hand, attempt to utilize the phosphoramidite reaction to couple these compounds made.

The base-mediated coupling reaction started with the iminosugar primary alcohol as the primary step. The reaction was attempted with both 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and *N*,*N*-Diisopropylmethylphosphonamidic chloride phosphoramidite reagents. Both the attempts were unsuccessful in coupling with the primary alcohol under basic conditions.

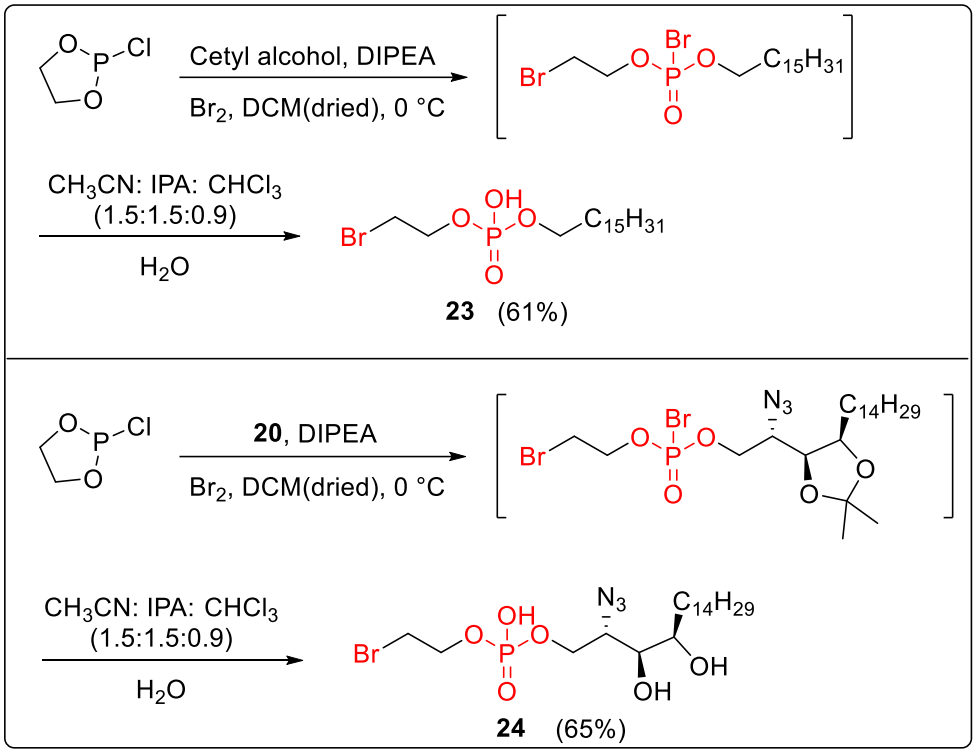


Scheme 3.3.5a. Attempts on phosphoramidite reaction

A simple cetyl alchol was considered instead of compound **20** for phosphoramidite chemistry, which was also unsuccessful. Attempt to couple with iminosugar was made with cetyl alcohol by variation of phosphorous reagent to 2-chloro-1,3,2-dioxophospholane **5**. Cetyl alcohol and *N*,*N*-diisopropylethylamine were treated with chloro reagent **5** at 0 °C, which led to the formation of bromo intermediate **21**. The Bromo intermediate was treated with ~30 % trimethylamine in water in a combination of solvents viz. chloroform/acetonitrile/isopropyl alcohol for 24 hours as an attempt to check the feasibility of miltefosine preparation method and indeed the outcome was a success and miltefosine **22** was obtained.

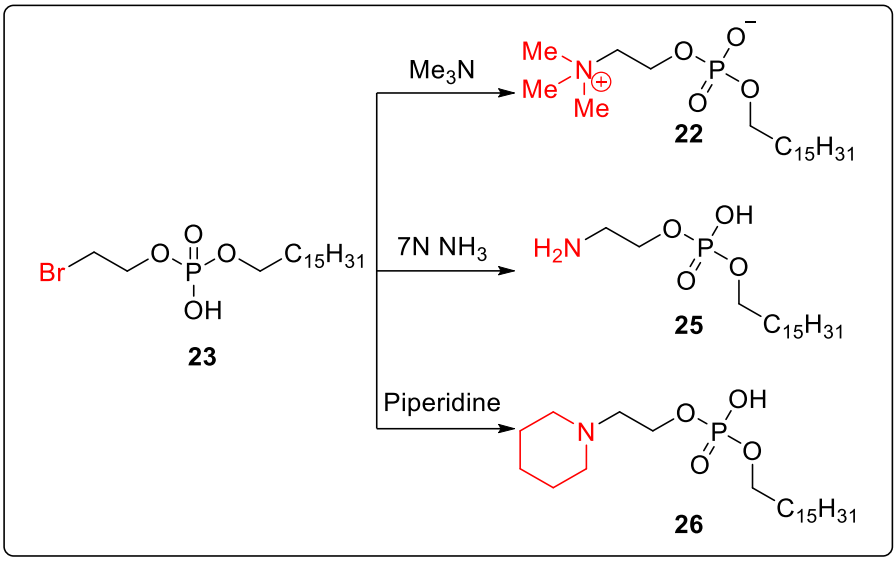


Scheme 3.3.6a. Synthesis of miltefosine **22**



Scheme 3.3.7a. Synthesis of bromides **23** and **24**

By following the same protocol, the synthesis of alkyl halide derivatives of phospholipids was undertaken and by conversion of P-Br to P-OH moiety. Indeed compounds **23** and **24** were synthesized by variation of aliphatic chain and producing a phosphate with alkyl halide handle for further functionalization.



Scheme 3.3.8a. S_N2 reaction attempts on alkyl bromide **23**

Compound **23** was treated with ~ 30 % trimethylamine in water, ammonia in methanol, and piperidine. With all the amines, the S_N2 reaction was successful to afford the expected compounds **22**, **25**, and **26**, confirmed by HRMS. The S_N2 reaction, hence, was extended to iminosugar **16** with bromide **23**. However, attempts of conditions by variation of solvents, temperature and base resulted in no reaction (Table 3.3.1a).

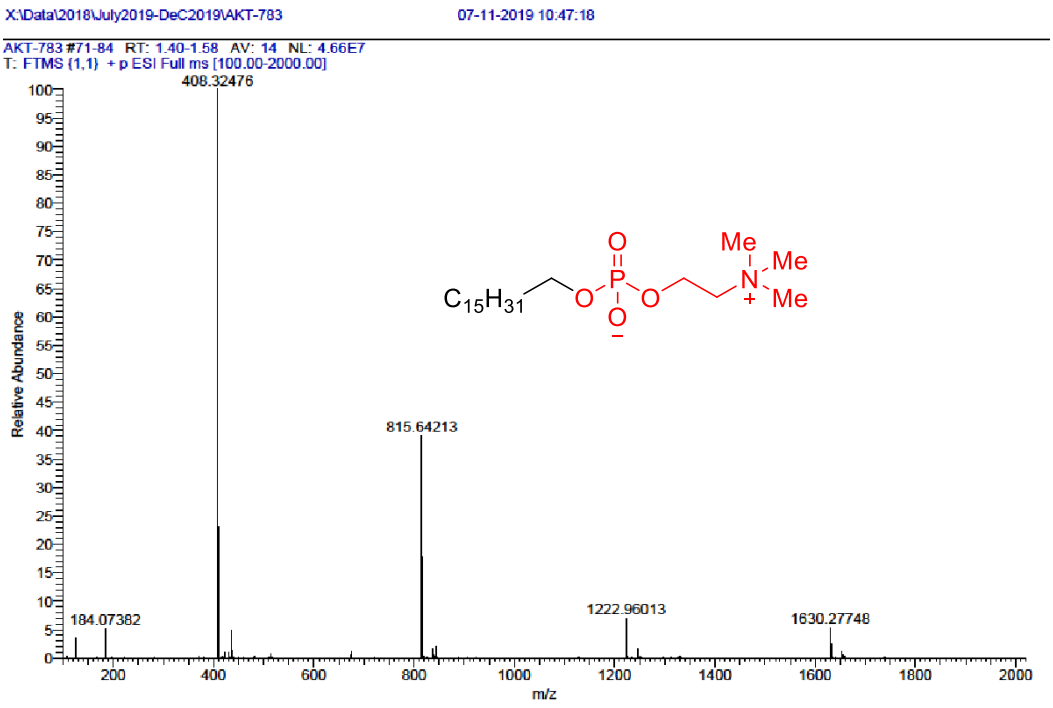


Figure 3.3.1a. HRMS of Miltefosine

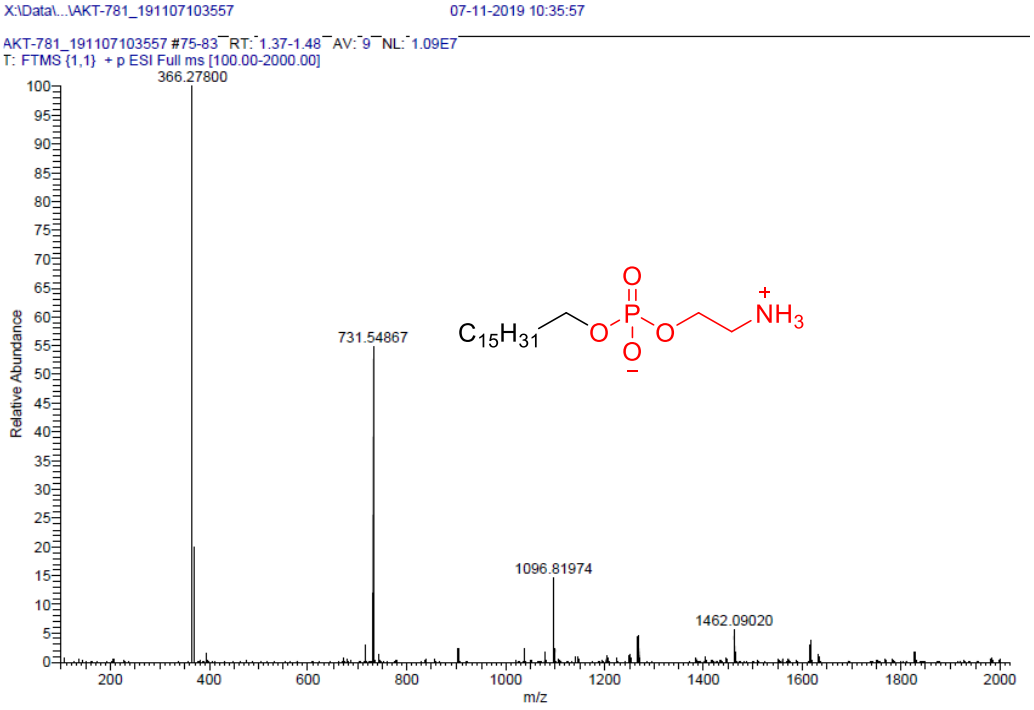


Figure 3.3.2a. HRMS of compound **25**

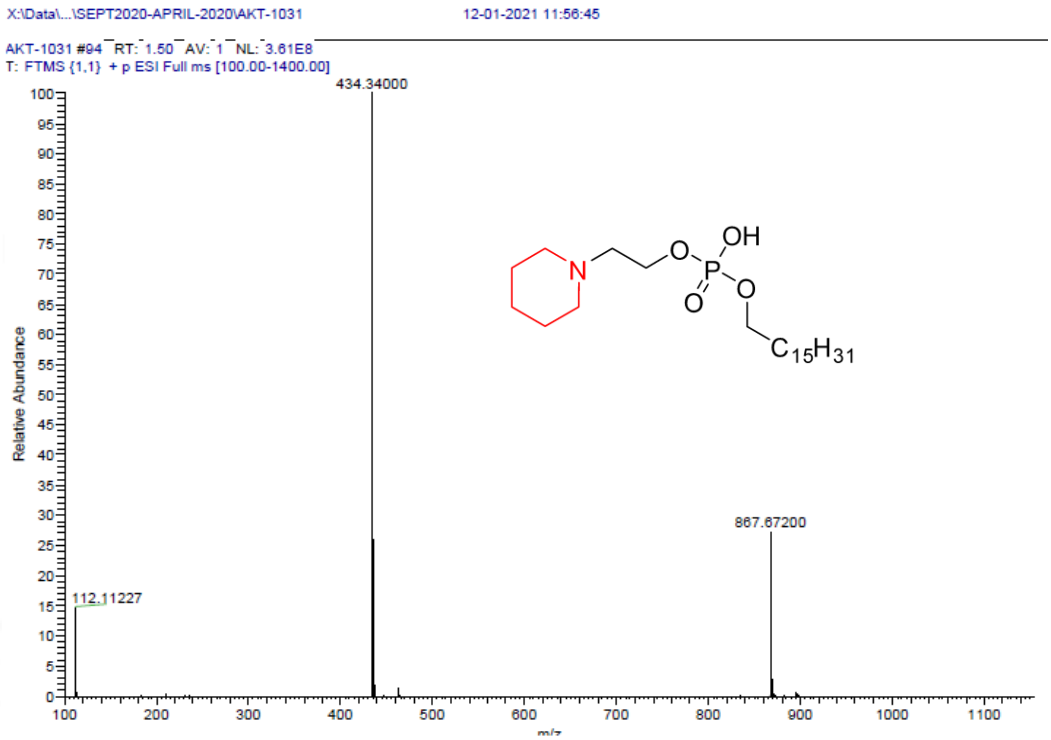
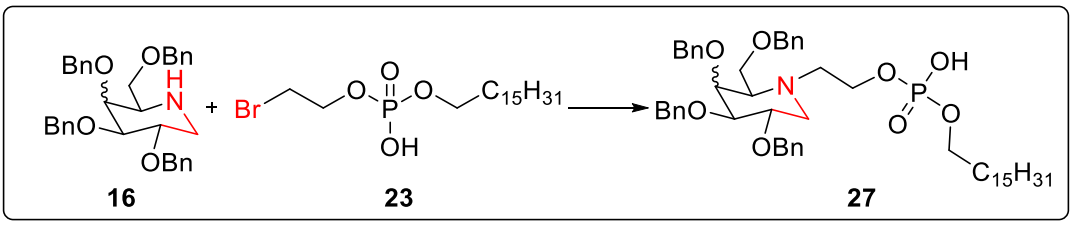


Figure 3.3.3a. HRMS of compound **26**

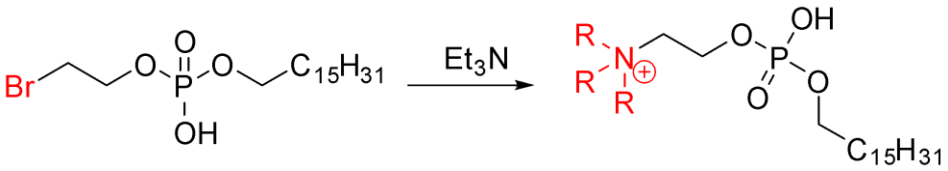
Table 3.3.1a. Attempts on S_N2 reactions of iminosugar **16** with bromide **23**



Entry	Temperature	Base (2 equiv)	Solvent	Product
1	Reflux	-	Toluene	No reaction
2	80 °C	-	DMF	No reaction
3	Reflux	-	DCM	No reaction
4	Reflux	TEA	DCM	No reaction
5	Reflux	K_2CO_3	DCM	No reaction
6	80 °C	K_2CO_3	DMF	No reaction

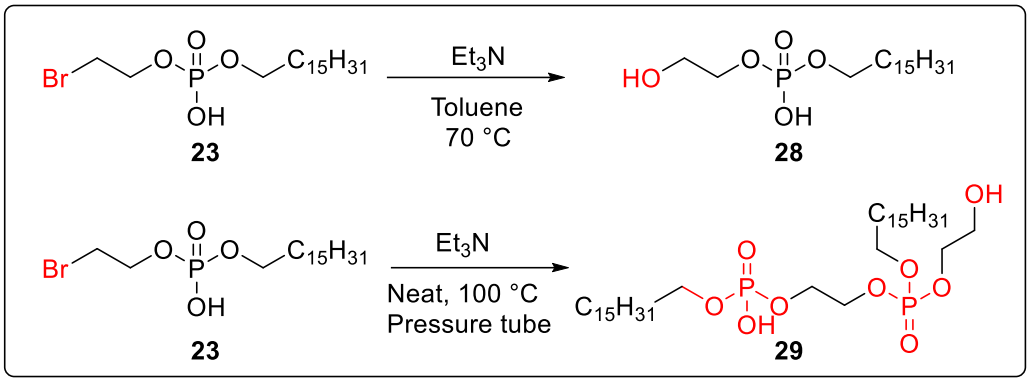
Quaternization of phospholipids for preparing miltefosine is a thoroughly investigated process. The same methodology was planned for coupling the iminosugar moiety with the phospholipids. But this time, rather than specifically attempting with the iminosugar, triethylamine was used for quaternization reaction. The reaction was attempted with single, dual, and tri solvent combinations by variation of temperature (Table 3.3.2a). However, the expected quaternization reaction did not take place and there was no reaction.

Table 3.3.2a. S_N2 reactions attempts on compound **23** with Et_3N



Entry	Temperature	Solvent	Product
1	rt	ACN: IPA: $CHCl_3$ (5:5:3)	No reaction
2	rt	(ACN: IPA: $CHCl_3$): H_2O (1:1)	No reaction
3	80 °C	DCM: ACN	No reaction
4	80 °C	DCM: DMF	No reaction
5	Reflux	$CHCl_3$	No reaction
6	rt	DMF	No reaction
7	rt	ACN	No reaction
8	45 °C	DCM	No reaction

However, reaction conducted in toluene at 70 °C led to substitution of bromide with hydroxyl group, affording compound **28**, confirmed by HRMS. Interestingly, an attempt under neat reaction conditions by heating at 100 °C with triethylamine led to the formation of biphosphate compound **29**, confirmed by HRMS.



Scheme: 3.3.9a. Attempts of Et_3N with compound **23**

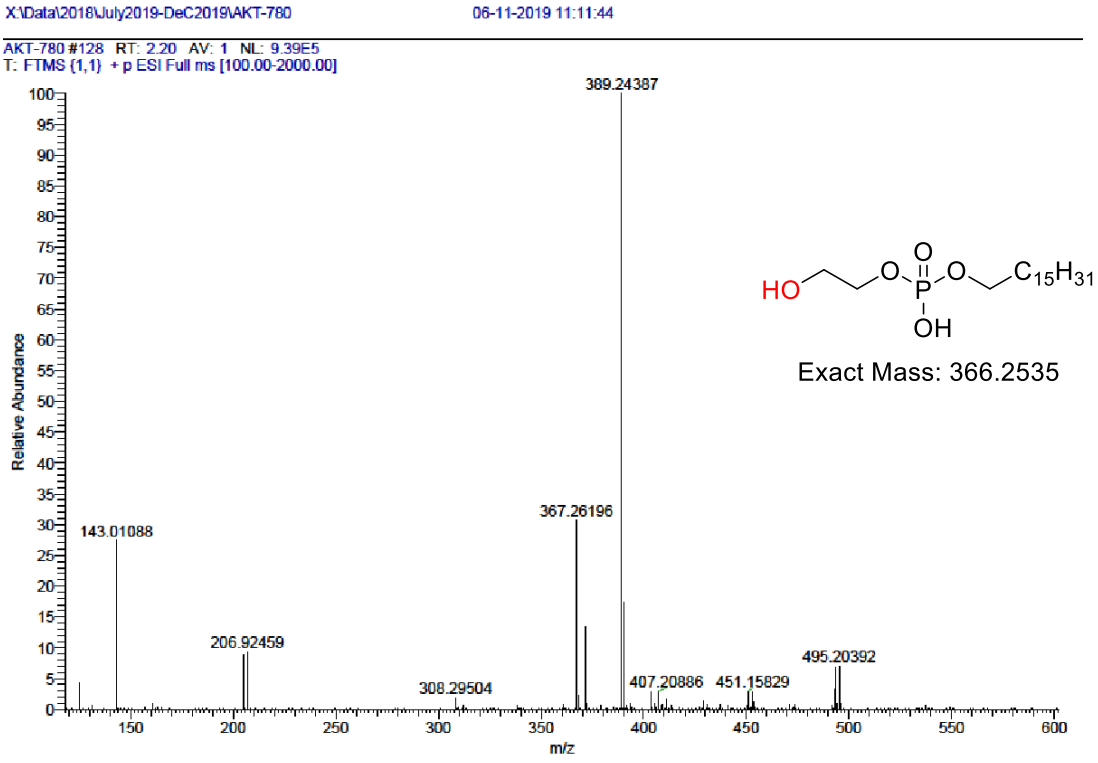


Figure 3.3.4a. HRMS of compound **28**

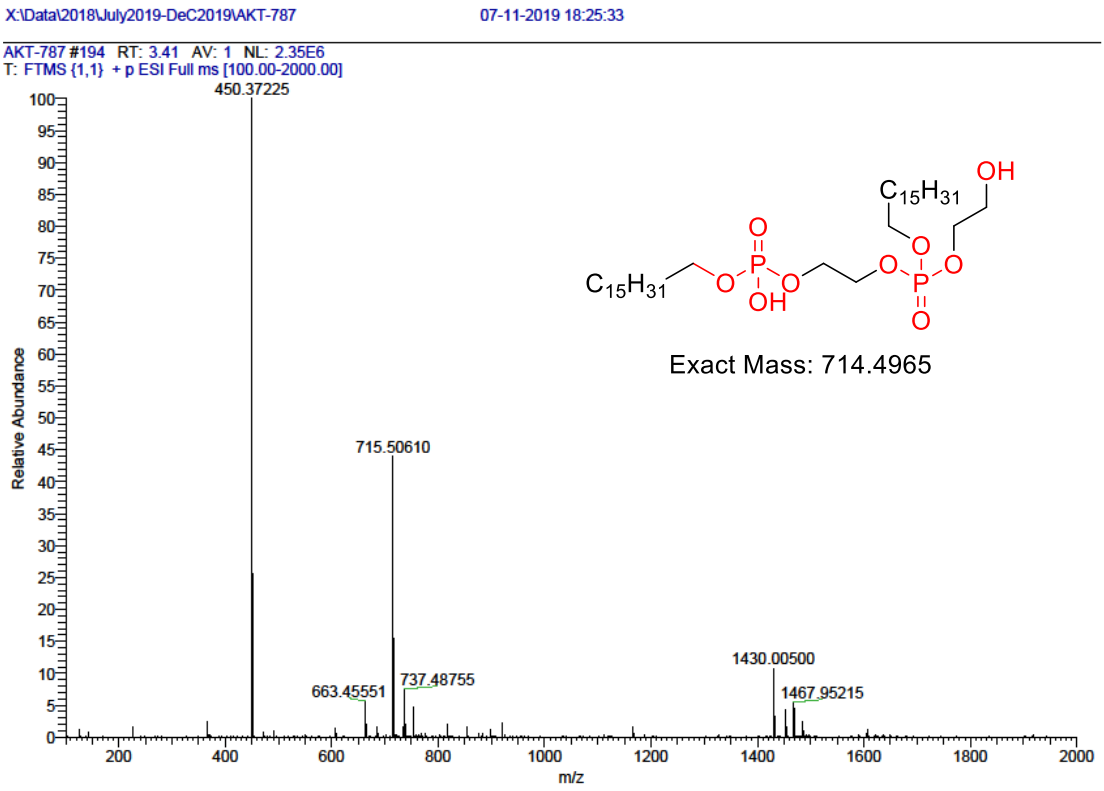
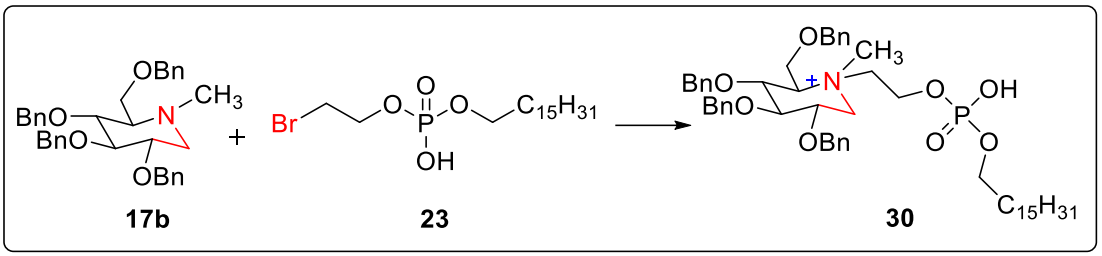


Figure 3.3.5a. HRMS of compound **29**

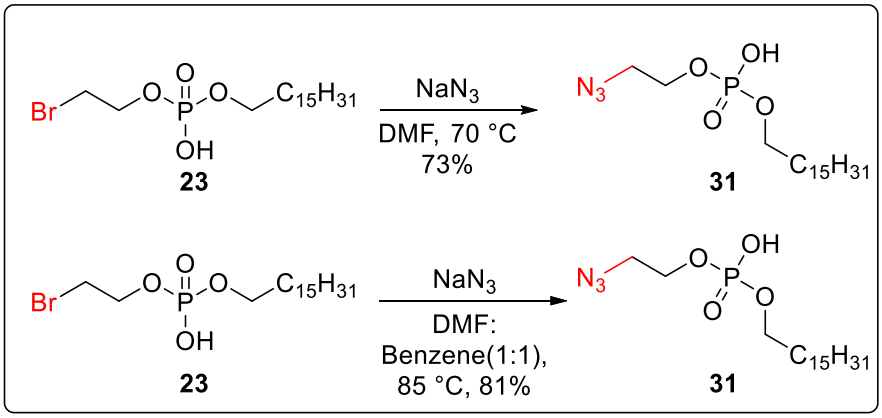
Attempts with direct utilization of the iminosugar **17b** for quaternization reaction with bromide **23** was also attempted, which led to no desirable outcome.

Table 3.3.3a. Quaternization attempts using iminosugar **17b** with bromide **23**

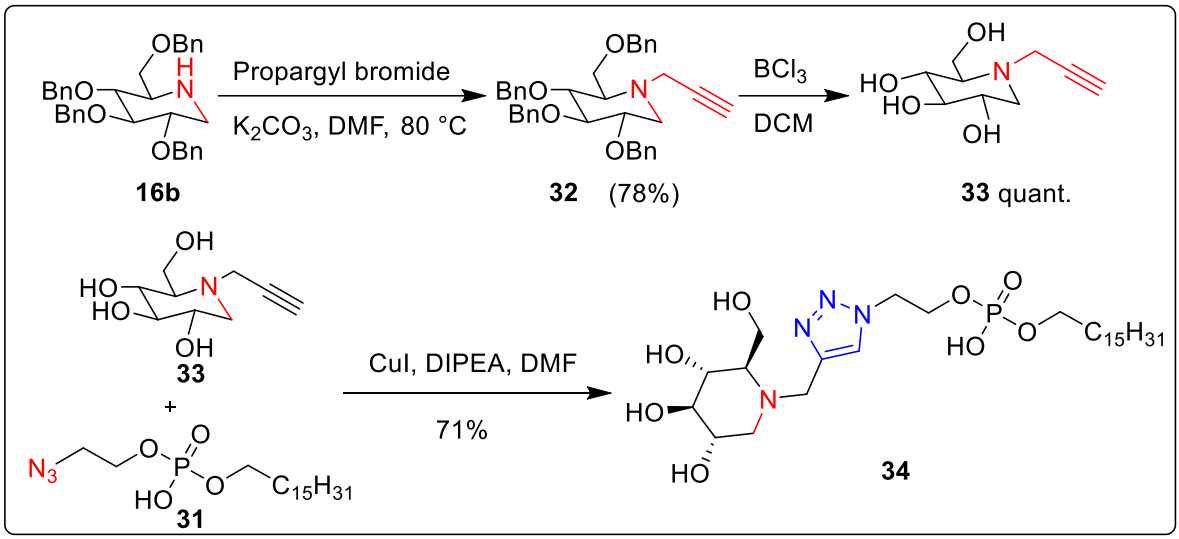


Entry	Temperature	Solvent	Product
1	Reflux	ACN: IPA: $CHCl_3$ (5:5:3)	No reaction
2	80 °C	ACN	No reaction
3	Reflux	Toluene	No reaction
4	Reflux	neat	No reaction
5	Reflux	ACN: IPA: DMF (5:5:3)	No reaction

The failure to synthesize iminosugar appended phospholipids prompted a shift in the focus by adopting 'Click chemistry' approach for cross-coupling. The transformation of bromide **23** to azide **31** was attempted under two conditions. Attempt with sodium azide in DMF at 70 °C, and in DMF/benzene (1:1) at 85 °C led to decent yields, confirmed by HRMS. However, a similar attempt to convert the bromide **24** did not afford the desired azide. With the azide **31** in hand, a propargylic group appended to iminosugar was the next requirement for 'Click chemistry'. The 1-deoxy nojirimycin **16b** was treated with the propargyl bromide in the presence of K_2CO_3 in DMF, affording compound **32** in 89 % yield. Compound **32** was subjected to global debenzylation using BCl_3 in dry DCM at -78 °C, which yielded compound **33** in an excellent yield, which set the stage for the 'click' reaction. Finally, coupling of compounds **31** and **33** was accomplished with copper iodide and *N*,*N*-diisopropylethylamine in DMF at 0 °C to yield the target compound **34** in 75%.



Scheme 3.3.10a. Synthesis of phospholipid azide derivative **31**



Scheme 3.3.11a. Synthesis of triazole compound **34**

3.4. Conclusion

In this chapter, a novel synthesis of iminosugar appended miltefosine analogue was attempted. Initial attempts involving S_N2 reactions on a phosphate bromide, appended to an aliphatic chain (cetyl and phytosphingosine) were unsuccessful. However, by adopting 'click chemistry' approach, the iminosugar and the phospholipid were appended as a triazole derivative. Currently, this novel iminosugar miltefosine analogue is being studied for anticancer activity against various

cancer cell lines in comparison with anticancer molecule miltefosine, and based on the results further mechanistic studies will be planned.

3.5. General information

3.5.1. General experimental conditions:

^{1}H and ^{13}C NMR were recorded on a Bruker ASCEND^{TM}-500 spectrometer at 500 and 125 MHz, respectively using $CDCl_3$, and CD_3OD solvents. NMR data are reported as follows: chemical shifts in ppm (δ) with integration, coupling constant in Hz and multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, etc.). HR-ESI-MS analysis was recorded on a Thermo Scientific Exactive-LCMS instrument by electrospray ionization method with ions given in m/z using Orbitrap analyzer. Reactions were monitored by silica gel G-60 F^{254} aluminum TLC plates and by charring the TLC plate after spraying with 15% sulfuric acid in ethanol. Chromatographic separations were carried out by conventional column chromatography on silica gel (100 × 200 mesh). Reagents were purchased at the highest commercial quality and used without further purification.

3.5.2. General procedure

(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)piperidin-2-one **(12a)**: The lactam compound was synthesized using Overkleeft protocol[29] and matched NMR datas with the literature. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (2H, d, J=8 Hz), 7.24 (16H, m), 7.15 (2H, d, J=7 Hz), 5.85 (1H, s), 5.15 (1H, d, J=11 Hz), 4.83 (1H, d, J=11 Hz), 4.73 (2H, q, J=9.5 Hz), 4.61 (1H, d, J=12 Hz), 4.49 (1H, d, J=11.5 Hz), 4.41 (1H, d, J=11.5 Hz), 4.35 (1H, d, J=12 Hz), 4.27 (1H, d, J=9 Hz), 3.90 (1H, s), 3.76 (1H, d, J=9 Hz), 3.48 (2H, m), 3.36 (1H, dd, J=3, 8.5 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 171.2, 138.2, 138.1, 137.8, 137.3, 128.5, 128.4, 128.39, 128.30, 128.0,

127.9, 127.89, 127.85, 127.80, 127.7, 127.68, 127.60, 80.6, 77.3, 75.4, 74.1, 73.5, 73.1, 73.0, 60.4, 53.5. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{34}H_{36}NO_5$ 538.2593; Found 538.2605.

(3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)piperidin-2-one **(12b)**: The lactam compound was synthesized using Overkleeft protocol[29] and matched NMR datas with the literature. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (2H, m), 7.22 (16H, m), 7.11 (2H, m), 6.27 (1H, s), 5.09 (1H, d, *J* = 11 Hz), 4.85 (1H, d, *J* = 11 Hz), 4.73 (2H, m), 4.62 (1H, d, *J* = 11 Hz), 4.49 (1H, d, *J* = 11 Hz), 4.42 (3H, m), 4.30 (1H, m), 4.26 (1H, dd, *J* = 2, 18 Hz), 3.84 (1H, t, *J* = 1.5 Hz), 3.46 (1H, d, *J* = 8.5 Hz), 3.35 (1H, d, *J* = 8.5 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 171.8, 138.4, 138.2, 137.7, 136.7, 128.7, 128.3, 128.27, 128.18, 128.10, 127.9, 127.65, 127.62, 127.60, 82.1, 78.0, 77.6, 76.7, 75.2, 74.6, 73.9, 73.4, 73.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{34}H_{36}NO_5$ 538.2593; Found 538.2612.

ethyl 2-((2R,3S,4S,5R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-oxopiperidin-1-yl) acetate (**13a**): To a solution of compound **12a** (1.5 g, 2.8 mmol) in DMF (20 mL) was added K_2CO_3 (2.3 g, 16.7 mmol), bromo ethyl aetate (1.9 mL, 16.7 mmol) and TBAI (270 mg, 0.83 mmol) at 80 °C and then heated to 80 °C for 24 h. After completion of the starting material, the reaction mixture was quenched with cold water (25 mL), then extracted with EtOAc (3 x 100 mL) and washed with excess of water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified using silica gel chromatography to afford compound **13a** (1.45 g, 83%) as colorless viscous liquid. ^{1}H NMR (Acetone-d_6, 500 MHz): δ 7.39 (20H, m), 5.10 (1H, d, J=11.5 Hz), 4.92 (1H, d, J=11 Hz), 4.81 (3H, m), 4.73 (1H, m), 4.49 (2H, m), 4.44 (1H, s), 4.30 (1H, d, J=7 Hz), 4.20 (2H, m), 4.10 (3H, m), 4.03 (1H, s), 3.97 (2H, m), 1.19 (3H, t, J=7 Hz). $^{13}C\{^1H\}$ NMR (Acetone-d_6, 125 MHz): δ 174.1, 174.0, 144.0, 143.9, 143.7, 143.5, 133.5, 133.4, 133.3,

133.2, 133.1, 133.0, 132.8, 132.7, 84.0, 81.8, 79.5, 78.9, 78.3, 77.9, 77.6, 77.3, 65.5, 64.4, 51.6, 18.8. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{38}H_{42}NO_7$ 624.2961; Found 624.2970.

ethyl 2-((2R,3R,4S,5R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-oxopiperidin-1-yl) acetate (**13b**): A similar procedure used for preparation of compound **13a** was followed to afford compound **13b** (1 g, 86%) as colorless viscous liquid. ^{1}H NMR (Acetone-d_6, 500 MHz): δ 7.38 (20H, m), 5.11 (1H, d, J=11.5 Hz), 4.88 (1H, d, J=11 Hz), 4.76 (2H, m), 4.65 (2H, t, J=12.5 Hz), 4.51 (2H, q, J=9.5 Hz), 4.36 (1H, d, J=17 Hz), 4.21 (1H, d, J=8 Hz), 4.10 (3H, m), 3.94 (2H, m), 3.81 (1H, bs), 3.76 (1H, m), 3.70 (1H, m), 1.21 (3H, t, J=7.5 Hz). ^{13}C{^{1}H} NMR (Acetone-d_6, 125 MHz): δ 169.5, 168.9, 138.8, 138.70, 138.5, 138.2, 128.3, 128.2, 128.16, 128.10, 127.94, 127.90, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 81.3, 78.2, 77.5, 73.5, 73.4, 72.8, 72.6, 69.0, 61.0, 60.5, 46.6, 13.6. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{38}H_{42}NO_7$ 624.2961; Found 624.2976.

(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(2-hydroxyethyl)piperidin-2-one (**14a**): To a solution of compound **13a** (1.25 g, 2.0 mmol) in dry EtOH (15 mL) was added $NaBH_4$ (455 mg, 12.0 mmol) at 0 °C and allowed to stir at same temperature for 6 hours. After completion of the starting material, the reaction mixture was quenched with sat. aq. NH_4Cl (25 mL), then extracted with EtOAc (2 x 100 mL) and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude mixture was purified using silica gel chromatography to afford compound **14a** (780 mg, 67%) as a viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33 (20H, m), 5.04 (1H, d, J=11 Hz), 4.73 (3H, m), 4.62 (3H, m), 4.48 (2H, m), 4.14 (2H, m), 3.98 (1H, d, J=9 Hz), 3.85 (1H, m), 3.76 (5H, m), 3.69 (1H, m), 3.58 (1H, t, J=11.5 Hz), 2.12 (1H, t, J=6.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.2, 137.9, 137.8, 137.75, 137.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.89, 127.86, 127.79, 127.74, 127.6, 77.8, 74.4, 73.7, 73.4,

72.8, 72.8, 71.8, 62.4, 60.5, 52.2, 50.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{36}H_{40}NO_6$ 582.2856; Found 582.2861.

(3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(2-hydroxyethyl)piperidin-2-one (**14b**): A similar procedure applied for synthesis of compound **14a** is followed to afford compound **14b** (988 mg, 78%) as a viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.47 (2H, d, J=6 Hz), 7.32 (18H, m), 5.16 (1H, d, J=115 Hz), 4.76 (1H, d, J=11.5 Hz), 4.66 (3H, m), 4.48 (3H, m), 4.15 (1H, d, J=7 Hz), 3.90 (5H, m), 3.73 (1H, m), 3.61 (1H, m), 3.55 (1H, t, J=7.5 Hz), 3.33 (1H, m). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.3, 138.0, 137.9, 137.4, 137.0, 128.6, 128.5, 128.45, 128.40, 128.3, 128.2, 128.1, 128.04, 128.02, 127.9, 127.79, 127.78, 81.6, 78.8, 76.8, 74.4, 73.4, 73.3, 72.1, 68.3, 62.6, 61.5, 50.5. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{36}H_{40}NO_6$ 582.2856; Found 582.2866.

2-((2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)ethan-1-ol (**15b**): To a solution of compound **13b** (270 mg, 0.4 mmol) in dry Et_2O (5 mL) was added $NaBH_4$ (98 mg, 2.6 mmol) and $BF_3.OEt_2$ (74 µL, 0.26 mmol) at 0 °C and allowed to stir at same temperature for 3 hours. After completion of the starting material, the reaction mixture was quenched with sat. aq. NH_4Cl (20 mL), then extracted with EtOAc (2 x 50 mL) and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude mixture was purified using silica gel chromatography to afford compound **15b** (188 mg, 76%) as viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22 (18H, m), 7.08 (2H, d, J=7 Hz), 4.88 (1H, d, J=11 Hz), 4.81 (1H, d, J=11 Hz), 4.74 (1H, d, J=11 Hz), 4.60 (2H, q, J=12 Hz), 4.36 (3H, m), 3.62 (2H, s), 3.48 (5H, m), 3.07 (1H, dd, J=4.5, 11.5 Hz), 2.93 (1H, m), 2.40 (2H, m), 2.17 (1H, t, J=10 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 138.9, 138.4, 137.6, 128.4, 128.38, 128.36, 128.2, 127.9, 127.86, 127.85, 127.80, 127.7, 127.6, 127.5, 87.0, 78.5, 78.3, 75.3, 75.2, 73.3, 72.9, 66.4, 65.0, 59.3, 54.6, 53.2. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{36}H_{42}NO_5$ 568.3063; Found 568.3053.

(2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidine (**16b**): A similar procedure followed for synthesis of compound **15b** was followed to afford compound **16b** (3.32 g, 71%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.32 (18H, m), 7.22 (2H, d, *J* = 6 Hz), 5.00 (1H, d, *J* = 11 Hz), 4.87 (2H, t, *J* = 11 Hz), 4.71 (2H, q, *J* = 9.5 Hz), 4.48 (3H, m), 3.69 (1H, m), 3.55 (3H, m), 3.38 (1H, t, *J* = 9 Hz), 3.27 (1H, dd, *J* = 4.5, 12 Hz), 2.75 (1H, m), 2.53 (1H, t, *J* = 12 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 138.9, 138.5, 138.4, 138.0, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 87.3, 80.7, 80.1, 75.7, 75.2, 73.4, 72.8, 70.3, 59.8, 48.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{34}H_{38}NO_4$ 524.2801; Found 524.2812.

(2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-methylpiperidine (**17b**): To a solution of compound **16b** (2.3 g, 4.4 mmol) in DMF (25 mL) was added K_2CO_3 (1.52 g, 11 mmol) and methyl iodide (822 µL, 13.2 mmol) at 0 °C and allowed to stir at same temperature for 1 hour. After completion of the starting material, the reaction mixture was quenched with cold water (30 mL), then extracted with EtOAc (3 x 100 mL) and washed with excess of water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude was purified using silica gel chromatography to afford compound **17b** (2.25 g, 94%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22 (18H, m), 7.04 (2H, d, J=8 Hz), 4.88 (1H, d, J=11 Hz), 4.79 (1H, d, J=10.5 Hz), 4.74 (1H, d, J=11 Hz), 4.59 (2H, q, J=10 Hz), 4.41 (2H, q, J=11 Hz), 4.31 (1H, d, J=11 Hz), 3.62 (2H, m), 3.54 (1H, t, J=9 Hz), 3.49 (1H, dd, J=2.5, 10.5 Hz), 3.40 (1H, t, J=9 Hz), 3.00 (1H, dd, J=4.5, 11 Hz), 2.24 (3H, s), 2.02 (1H, t, J=11 Hz), 1.88 (1H, d, J=9.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 139.0, 138.54, 138.50, 137.8, 128.5, 128.42, 128.36, 128.35, 128.0, 127.88, 127.87, 127.7, 127.6, 127.5, 87.3, 78.2, 75.4, 75.2, 73.6, 72.8, 67.2, 65.3, 59.0, 42.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{35}H_{40}NO_4$ 538.2957; Found 538.2967.

(S)-2-azido-2-((4S,5S)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethan-1-ol (**20**): To a solution of phytosphingosine azido triol **19** (1 g, 2.9 mmol) in dimethoxy propane (18 mL) was added *p*-toluene sulfonic acid (40 mg, 0.29 mmol) at 0 °C and slowly warmed up to room temperature. After 2 h of stirring, the reaction mixture was diluted with MeOH (30 mL), then allowed to stir again for one more hour. Later, the solvents were evaporated, and the residue was purified using silica gel chromatography to afford compound **20** (928 mg) in 83% yield. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.20 (1H, m), 4.00 (2H, m), 3.89 (1H, m), 3.49 (1H, m), 2.21 (1H, s), 1.59 (2H, m), 1.45 (3H, s), 1.32 (27H, m), 0.90 (3H, t, J=7 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 108.4, 77.8, 76.7, 63.9, 61.2, 31.9, 29.7, 29.66, 29.61, 29.59, 29.54, 29.4, 29.3, 28.0, 26.5, 25.6, 22.7, 14.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{21}H_{41}N_3NaO_3$ 406.3046; Found 406.3053.

(2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidine (**32**): To a solution of 1-deoxyglucose iminosugar **16b** (100 mg, 0.19 mmol, 1 equiv) in DMF (3 mL) was added K_2CO_3 (66 mg, 0.47 mmol, 2.5 equiv) and propargyl bromide (43 µL, 0.57 mmol, 3 equiv) at room temperature and then allowed stir for 1 h at 80 °C. After formation of the non-polar product as indicated by TLC, the reaction mixture was quenched with H_2O (5 mL) and extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (9:1) as the eluent to afford compound **32** as a colorless viscous liquid (84 mg, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (18H, m), 7.17 (2H, d, *J* = 7 Hz), 5.03 (1H, d, *J* = 11 Hz), 4.93 (1H, d, *J* = 11 Hz), 4.88 (1H, d, *J* = 11 Hz), 4.73 (2H, s), 4.60 (1H, d, *J* = 12 Hz), 4.48 (1H, d, *J* = 12 Hz), 4.41 (1H, d, *J* = 11 Hz), 3.78 (3H, m), 3.70 (1H, t, *J* = 9 Hz), 3.63 (1H, d, *J* = 11 Hz), 3.55 (1H, t, *J* = 9 Hz), 3.44 (1H, m), 3.02 (1H, dd, *J* = 4.5, 10.5 Hz), 2.60 (1H, t, *J* = 11 Hz), 2.50 (1H, d, *J* = 10 Hz), 2.26 (1H, s); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 139.0, 138.6,

138.5, 137.7, 128.5, 128.45, 128.47, 128.45, 128.42, 128.1, 128.0, 127.9, 127.9, 127.7, 127.67, 127.60, 87.2, 78.2, 78.1, 75.5, 75.2, 74.2, 73.6, 72.8, 64.8, 62.2, 55.0, 42.3; HRMS (ESI) m/z: [M+H]+ Calcd. $C_{37}H_{40}NO_4$ 562.2957; Found 562.3070.

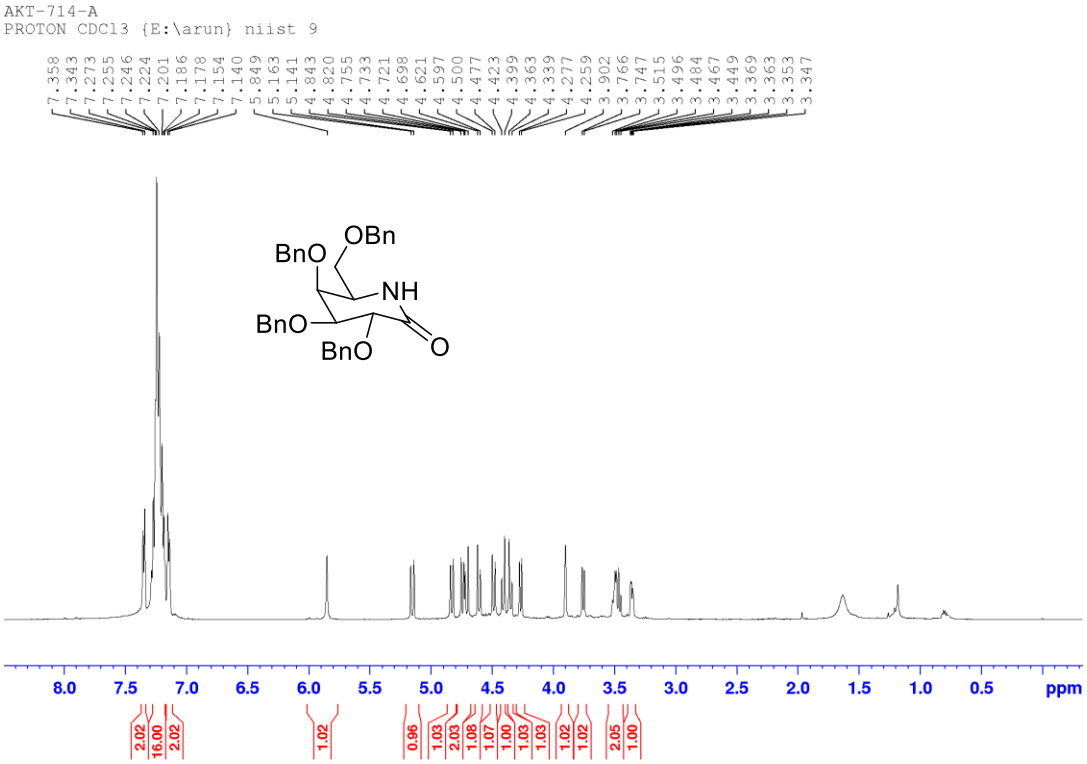
(2R,3R,4R,5S)-2-(hydroxymethyl)-1-(prop-2-yn-1-yl)piperidine-3,4,5-triol (**33**): To a solution of compound **32** (100 mg, 0.17 mmol) in distilled CH_2Cl_2 (5 mL) was added 1M BCl_3 (1.7 mL, 1.78 mmol, 10 equiv.) in CH_2Cl_2 at -10 °C and the mixture was stirred at same temperature under argon atmosphere. After 30 mins, 1M BCl_3 (1.81 mL, 1.81 mmol, 5 equiv.) in CH_2Cl_2 was added once again at the same temperature. After 30 mins, the reaction mixture was allowed to attain room temperature, MeOH (5 mL) was added and concentrated. The compound was precipitated using DCM to get colorless sticky solid **33** (35 mg) in quantitative yield. ^{1}H NMR (D_2O, 500 MHz): δ 4.12 (2H, s), 3.99 (1H, d, *J* = 13 Hz), 3.85 (1H, d, *J* = 13 Hz), 3.70 (1H, m), 3.56 (2H, m), 3.40 (1H, t, *J* = 9 Hz), 3.19 (2H, m), 3.09 (1H, s). ^{13}C{^{1}H} NMR (D_2O, 125 MHz): δ 81.2, 75.7,70.2, 66.8, 65.9, 64.6, 53.6, 53.5, 42.8.

2-bromoethyl hexadecyl hydrogen phosphate (**23**): Hexadecanol (1.92 g, 7.9 mmol, 1 equiv) and DIPEA (1.41 mL, 7.9 mmol, 1 equiv) in dry DCM (20 mL) was added 2-Chloro-1,3,2-dioxaphospholane (0.71 mL, 7.9 mmol, 1 equiv) by dropwise and the resulting mixture was stirred at 0° C under argon atmosphere. After 20 minutes of post stirring, Br_2 was added dropwise to the reaction mixture at 0° C under argon atmosphere. After 2 hours of stirring the reaction was stopped and the solvents were evaporated using rotary evaporator. The resulting residue was dissolved in a combination of solvents CH_3CN: IPA: $CHCl_3$ (1.5:1.5:0.9) 15 mL and then added 15 mL of water at room temperature. After 2 hours of stirring, the reaction was stopped and the solvents were evaporated using rotary evaporator again. The crude mixture was then purified by column chromatography using chloroform/MeOH (8:2) as the eluent to afford compound **23** as a colorless

solid (2.15 g, 61%). ^{1}H NMR ($CDCl_3$ and CD_3OD {1:1}, 500 MHz): δ 4.16 (2H, d, *J* = 6.5 Hz), 3.93 (2H, d, *J* = 6 Hz), 3.46 (2H, t, *J* = 6 Hz), 1.60 (2H, t, *J* = 6.5 Hz), 1.19 (26H, m), 0.80 (3H, t, *J* = 7 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$ and CD_3OD {1:1}, 125 MHz): δ 67.6, 66.0, 63.4, 31.9, 30.2, 30.1, 29.6, 29.54, 29.53, 29.4, 29.3, 29.1, 25.4, 22.6, 14.0. ^{31}P NMR ($CDCl_3$ and CD_3OD {1:1}, 202 MHz) δ -1.16: HRMS (ESI) m/z: [M+H]+ Calcd. $C_{18}H_{38}BrNaO_4P$ 451.1589; Found 451.1593.

hexadecyl (2-(4-(((2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl) hydrogen phosphate (**34**): A solution of bromo **23** (100 mg, 0.23 mmol, 1 equiv) in DMF (2 mL) was transferred into a pressure tube and then added NaN_3 (45 mg, 0.69 mmol, 3 equiv). The resulting mixture was stirred at 80 °C for overnight. After complete consumption of the starting material as indicated by TLC (hexane/EtOAc 7:3), the reaction mixture was quenched with H_2O (25 mL) and extracted with chloroform (2 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Without purification, the azide compound **31** was subjected to click reaction. To a solution of compound **33** (20 mg, 0.09 mmol, 1 equiv) and **31** (2.5 equiv) in DMF (10 mL) at 0 °C were added CuI (38 mg, 0.2 mmol, 2 equiv) and DIPEA (52 µL, 0.3 mmol, 3 equiv). The resulting mixture was stirred for 1 h under inert atmosphere and after complete consumption of both the starting materials, as indicated by TLC, the reaction was stopped. The reaction mixture was filtered and evaporated using methanol. The crude was precipitated using EtOAc to yield triazole **34** as a pale yellow solid (28 mg) in 71%. ^{1}H NMR (CD_3OD, 500 MHz): δ 8.41 (1H, s), 4.60-4.68 (3H, m), 4.02-4.31 (5H, m), 3.80-3.92 (3H, m), 3.60 (2H, m), 3.36 (1H, m), 2.90 (2H, bs), 1.53 (2H, m), 1.19 (26H, m), 0.80 (3H, m). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 138.7, 131.2, 80.5, 79.0, 75.6, 71.4, 70.3, 69.2, 69.0, 63.4, 57.8, 57.5, 37.2, 35.6, 33.33, 33.30, 33.0, 29.2, 26.3, 17.0. ^{31}P NMR (CD_3OD, 202 MHz) δ -0.38:

Figure 3.5.3.1. NMR spectras of compound **12a**



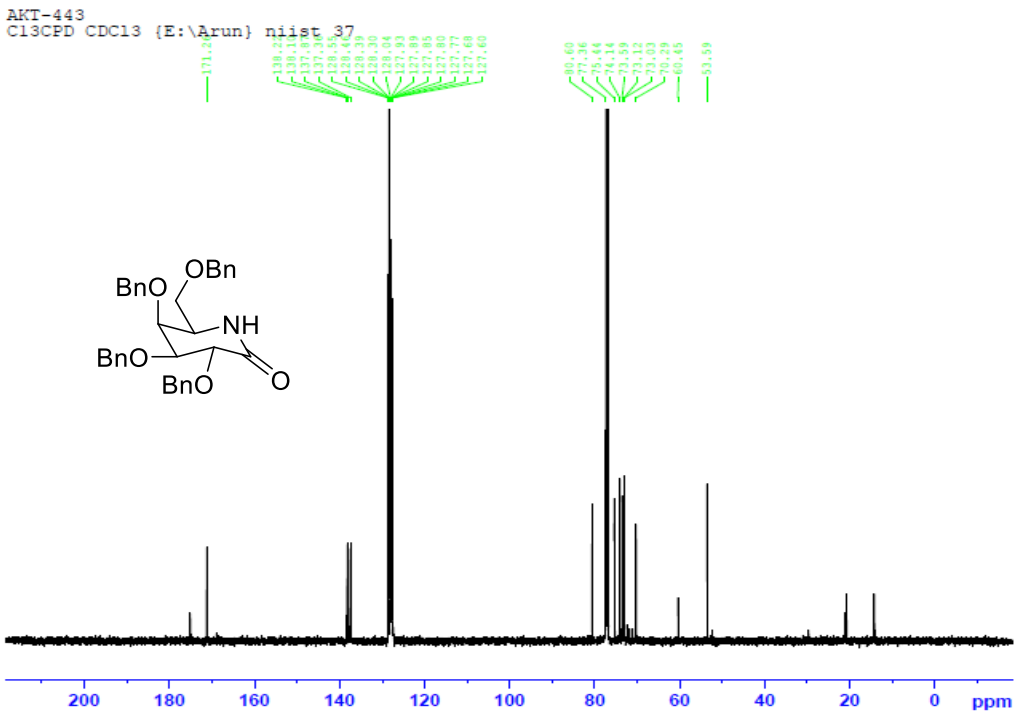


Figure 3.5.3.2. NMR spectras of compound **12b**

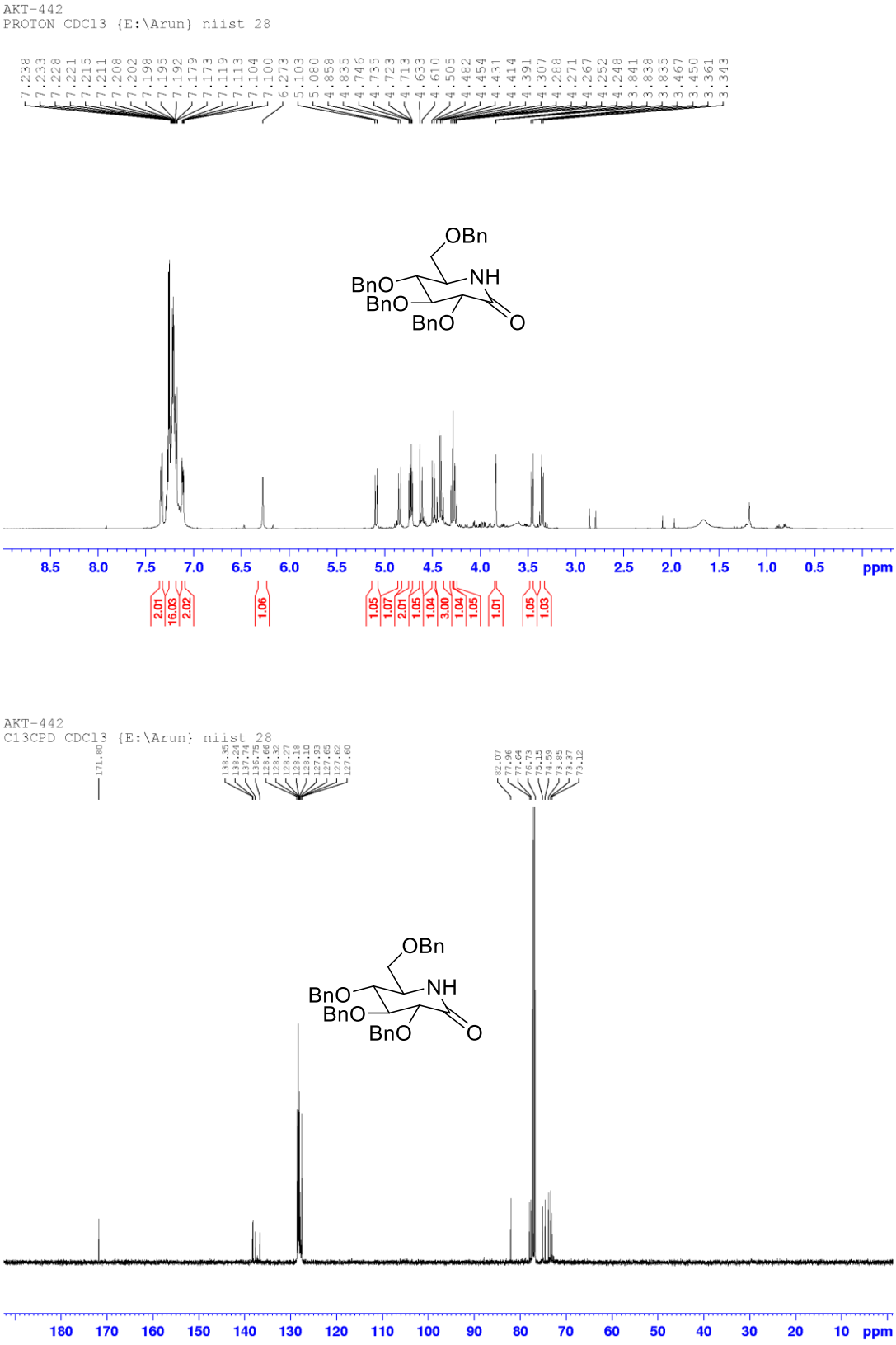
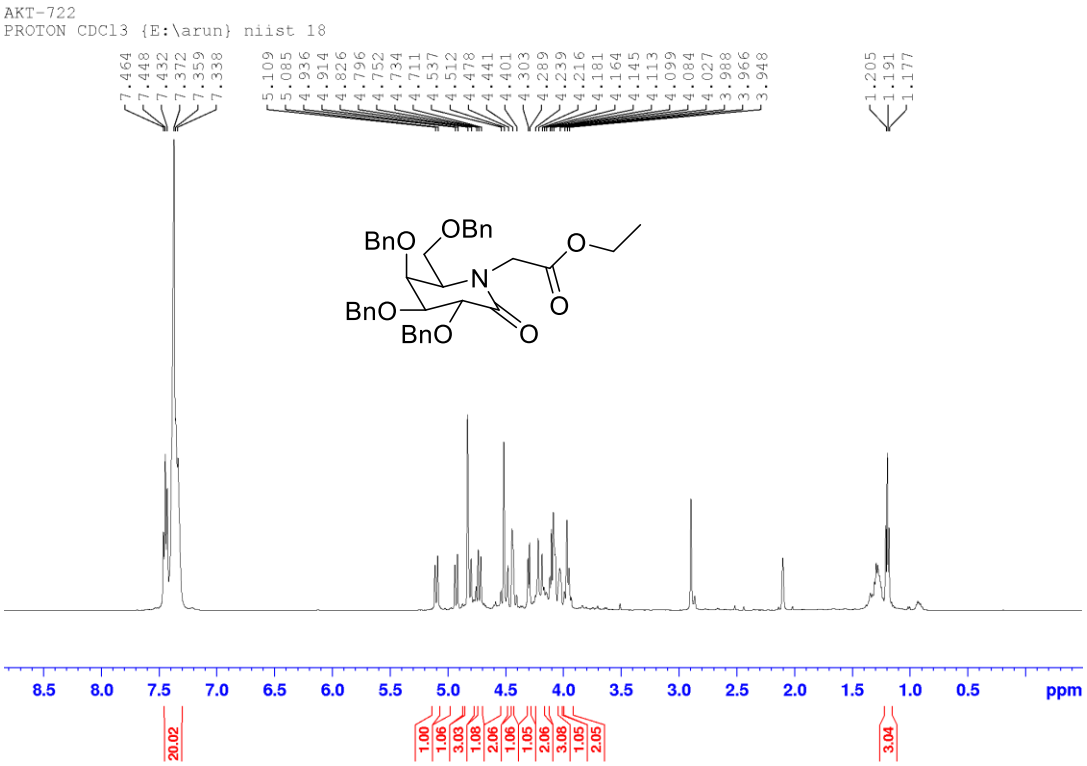


Figure 3.5.3.3. NMR spectras of compound **13a**



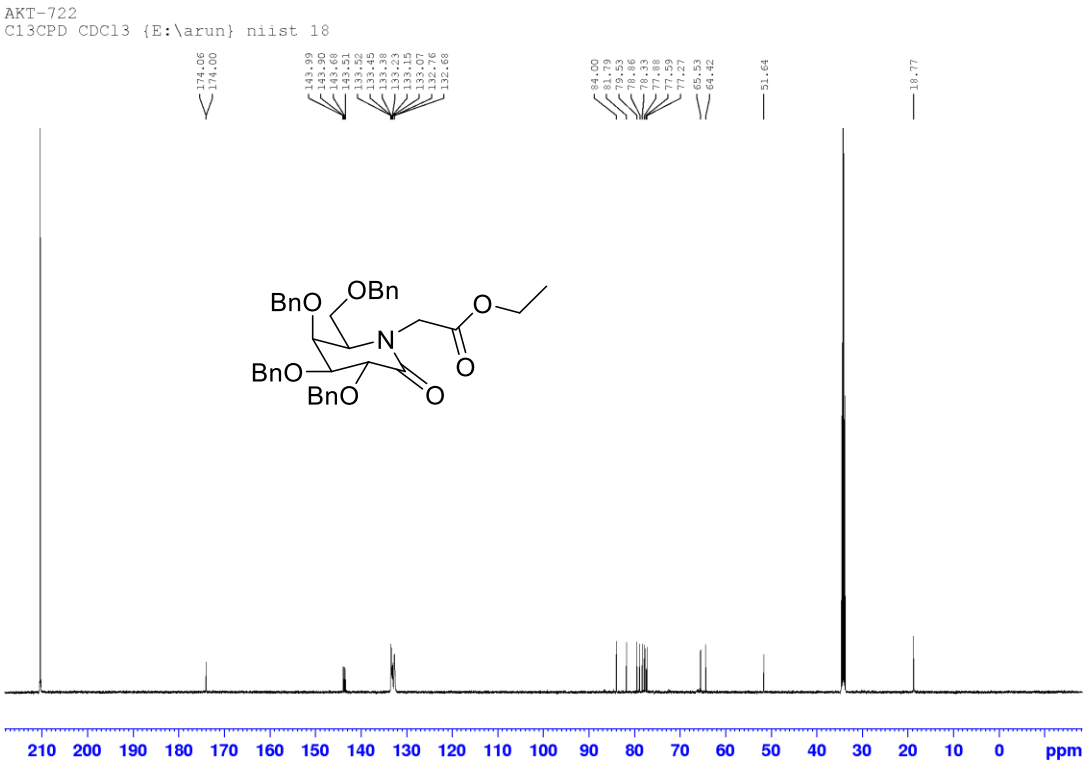
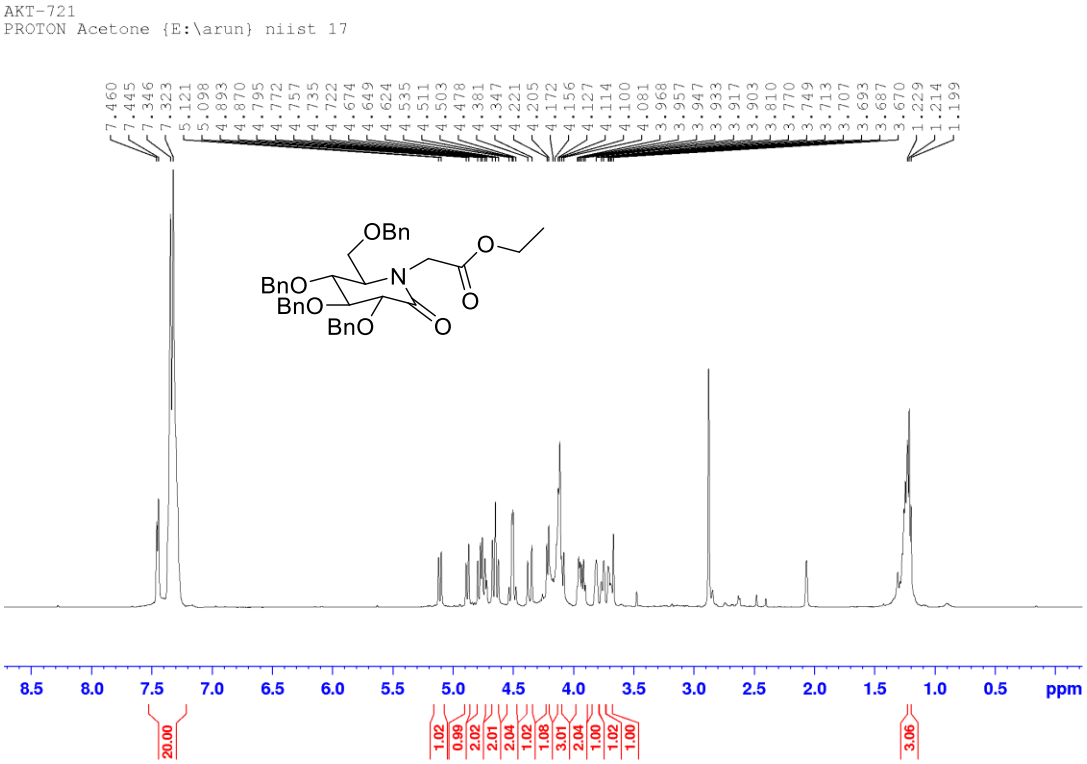


Figure 3.5.3.4. NMR spectras of compound **13b**



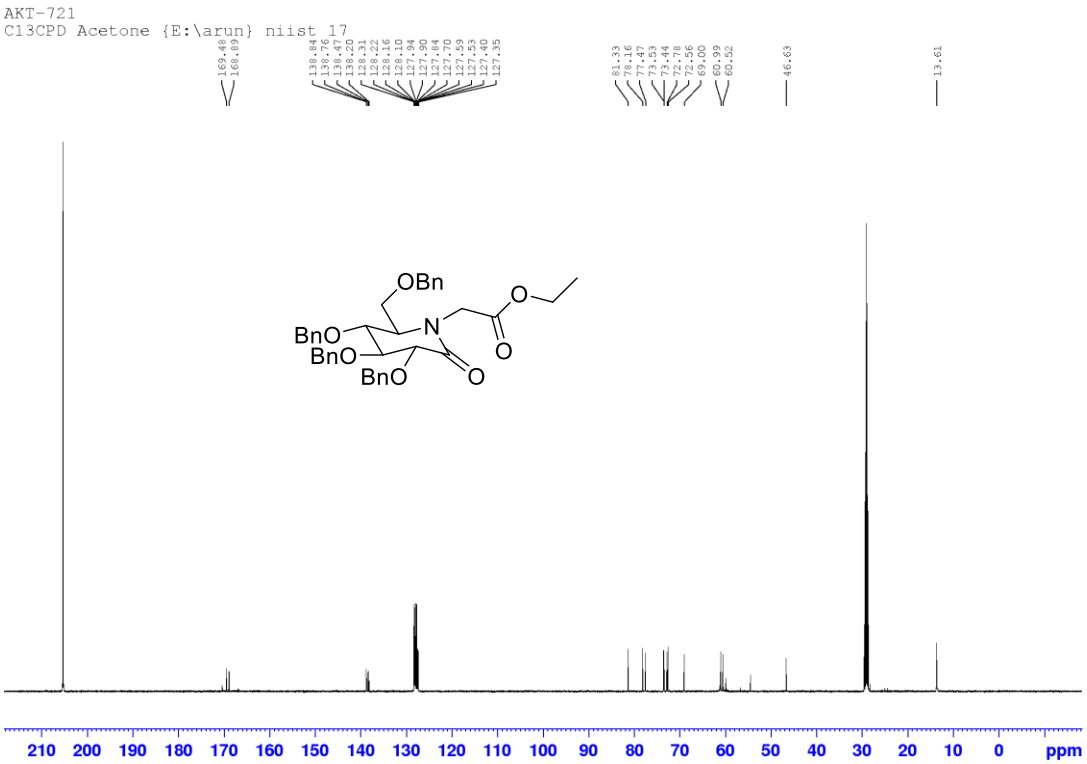
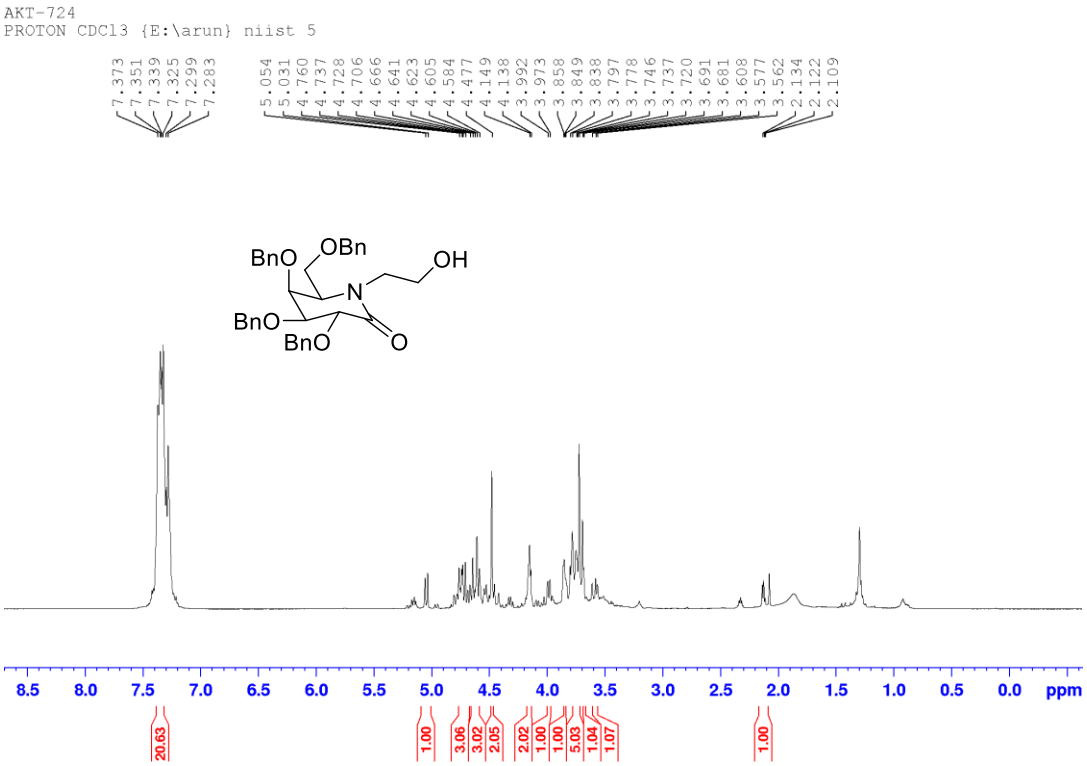


Figure 3.5.3.5. NMR spectras of compound **14a**



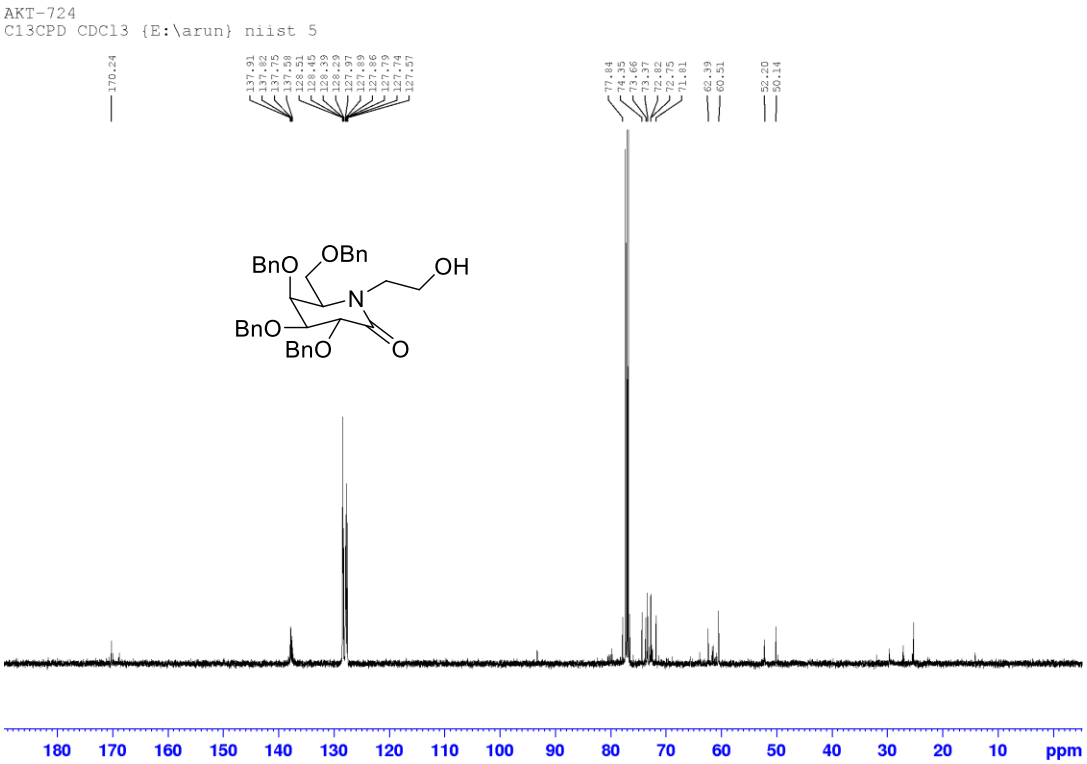
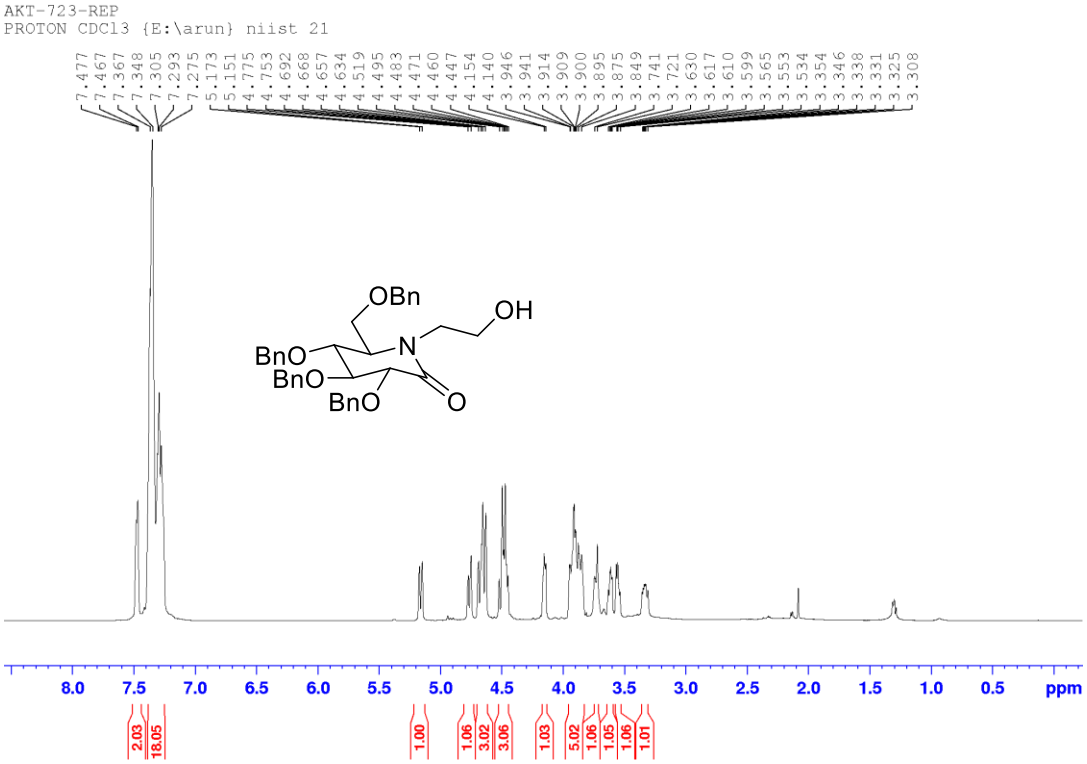


Figure 3.5.3.6. NMR spectras of compound **14b**



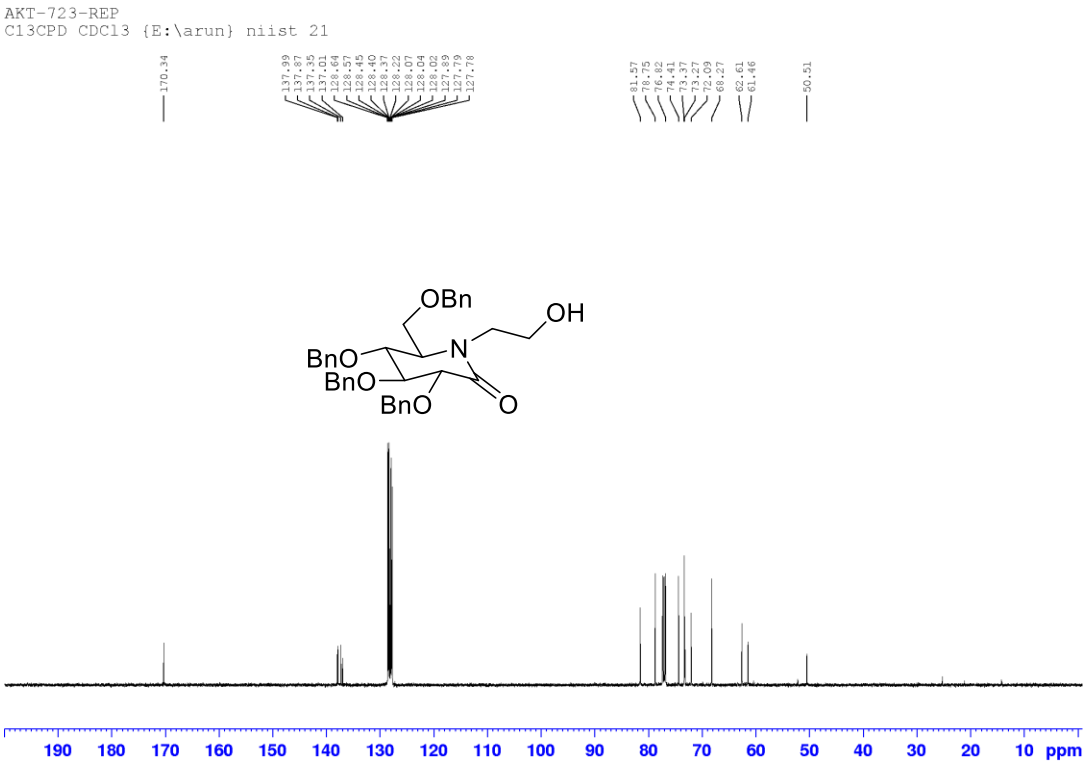
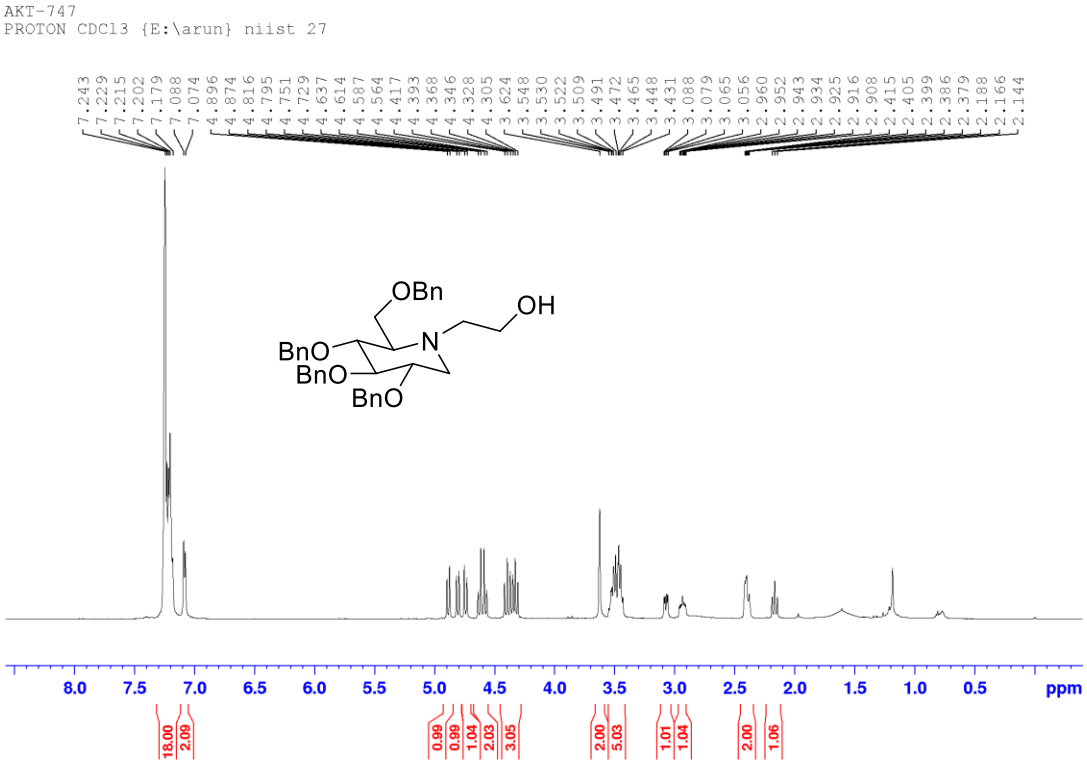


Figure 3.5.3.7. NMR spectras of compound **15b**



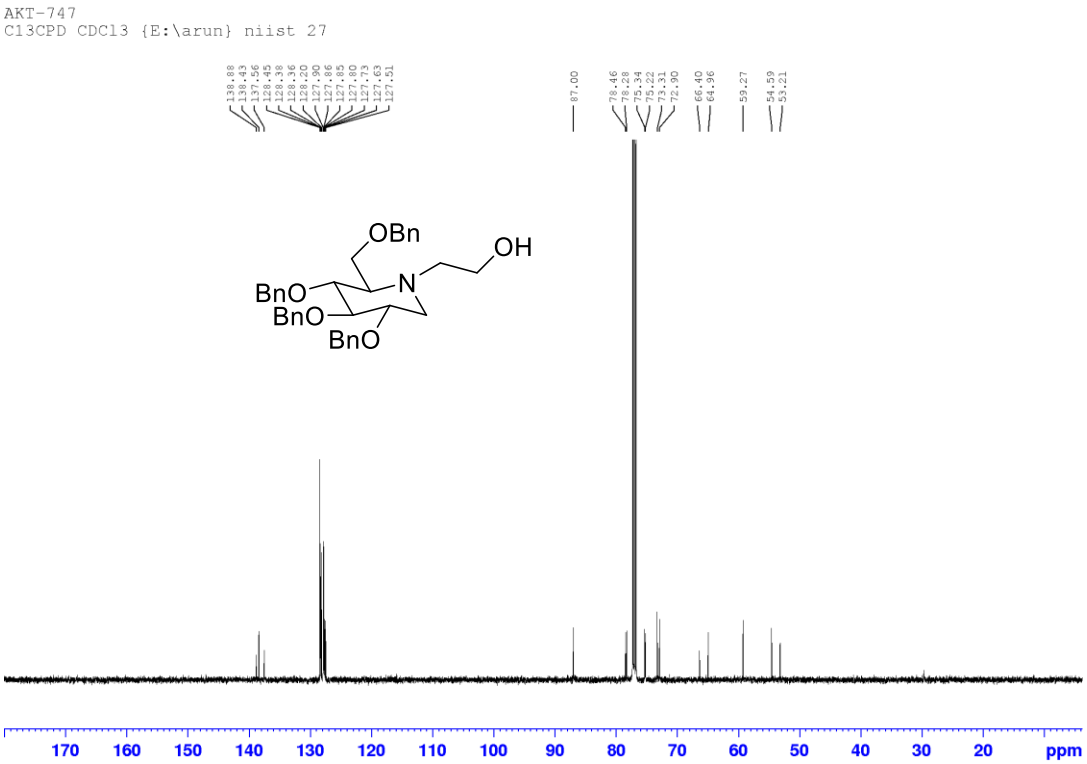


Figure 3.5.3.8. NMR spectras of compound **16b**

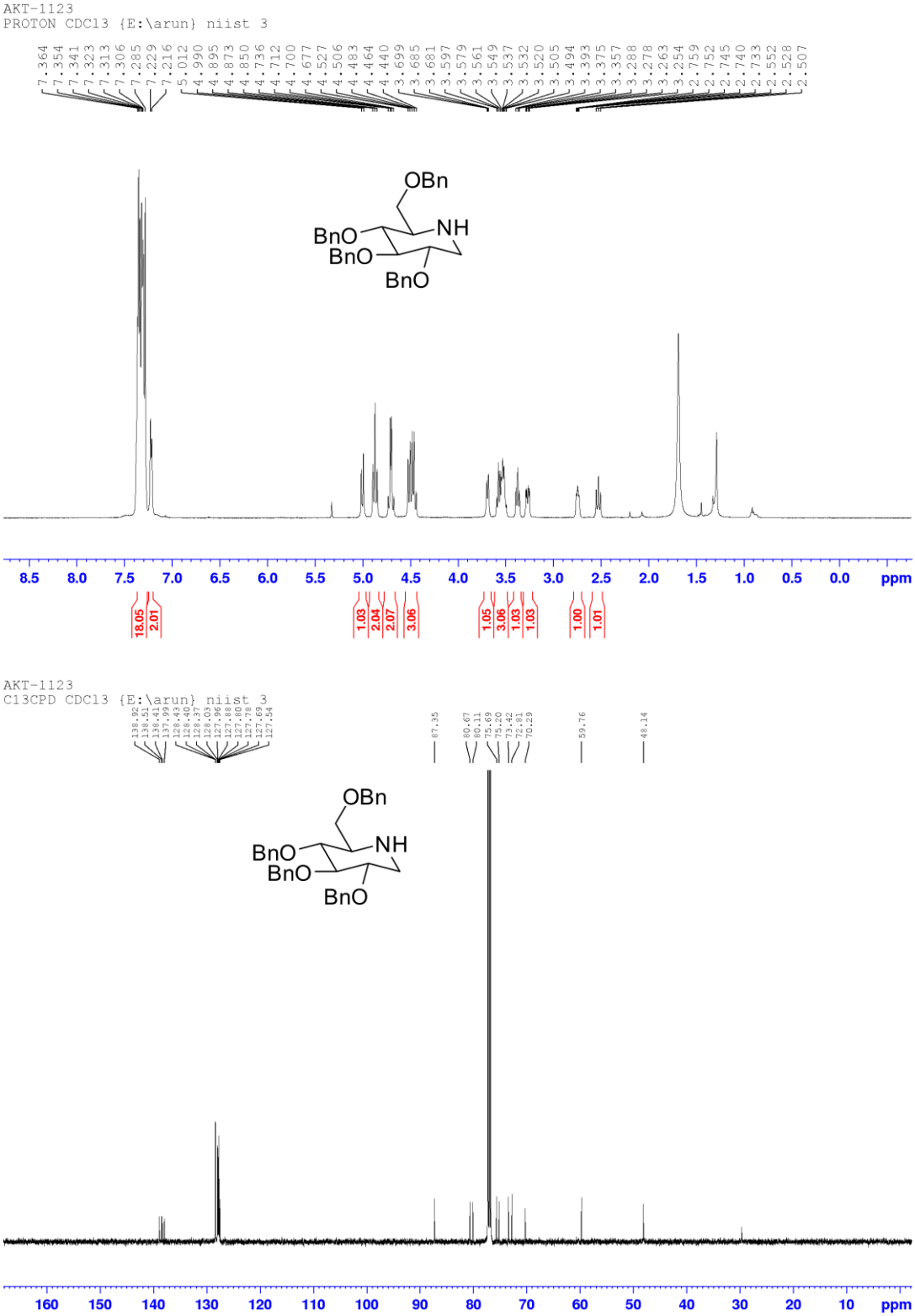


Figure 3.5.3.9. NMR spectras of compound **17b**

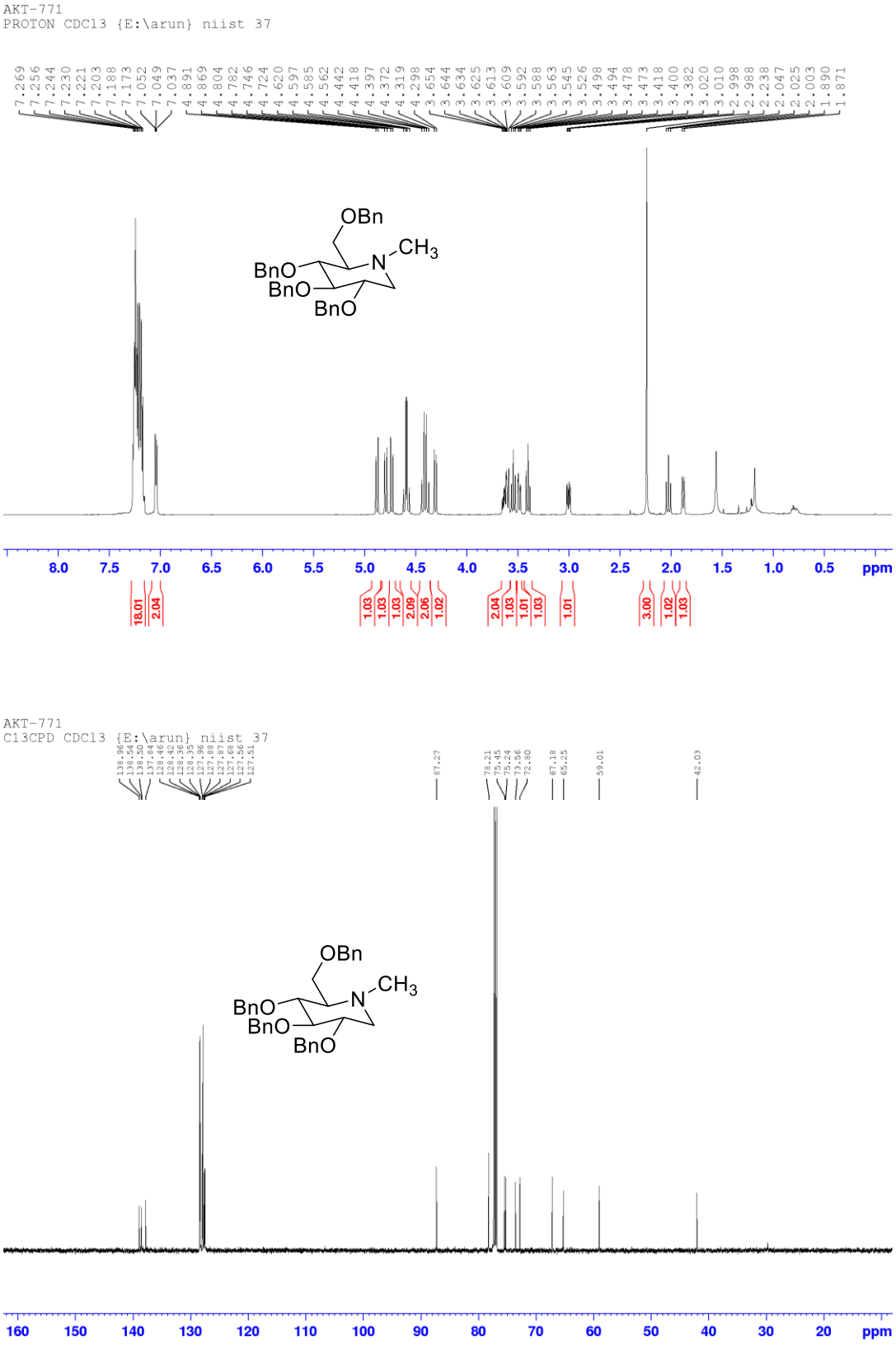
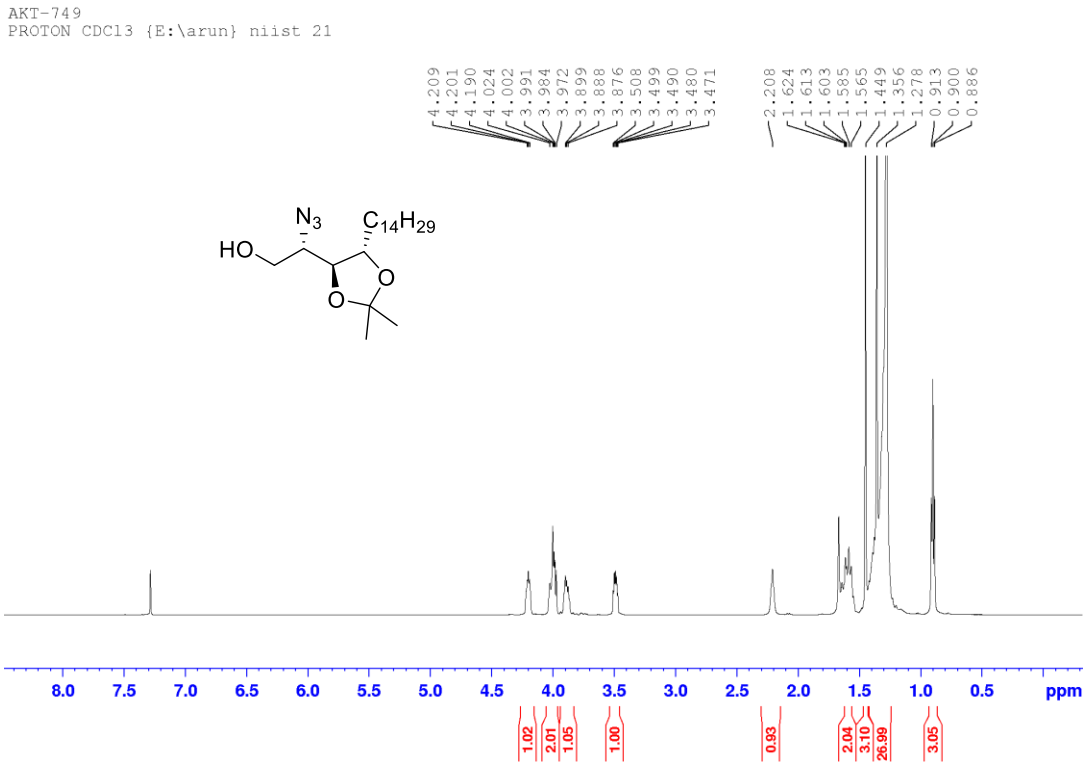


Figure 3.5.3.10. NMR spectras of compound **20**



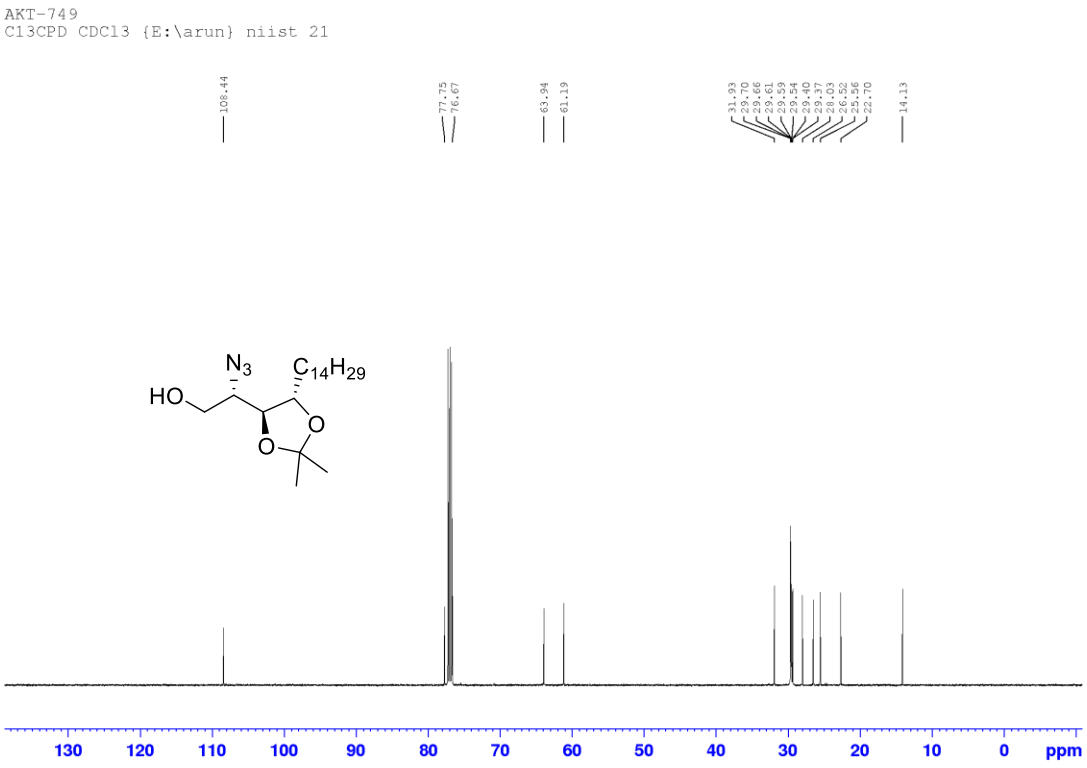
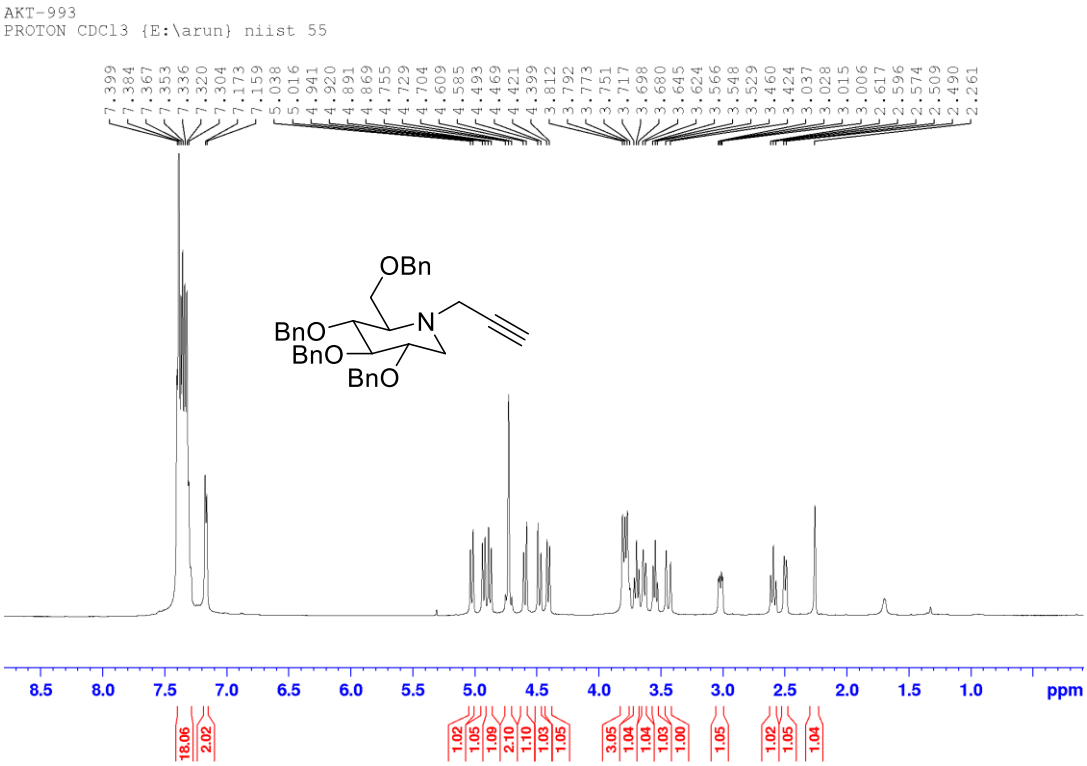


Figure 3.5.3.11. NMR spectras of compound **32**



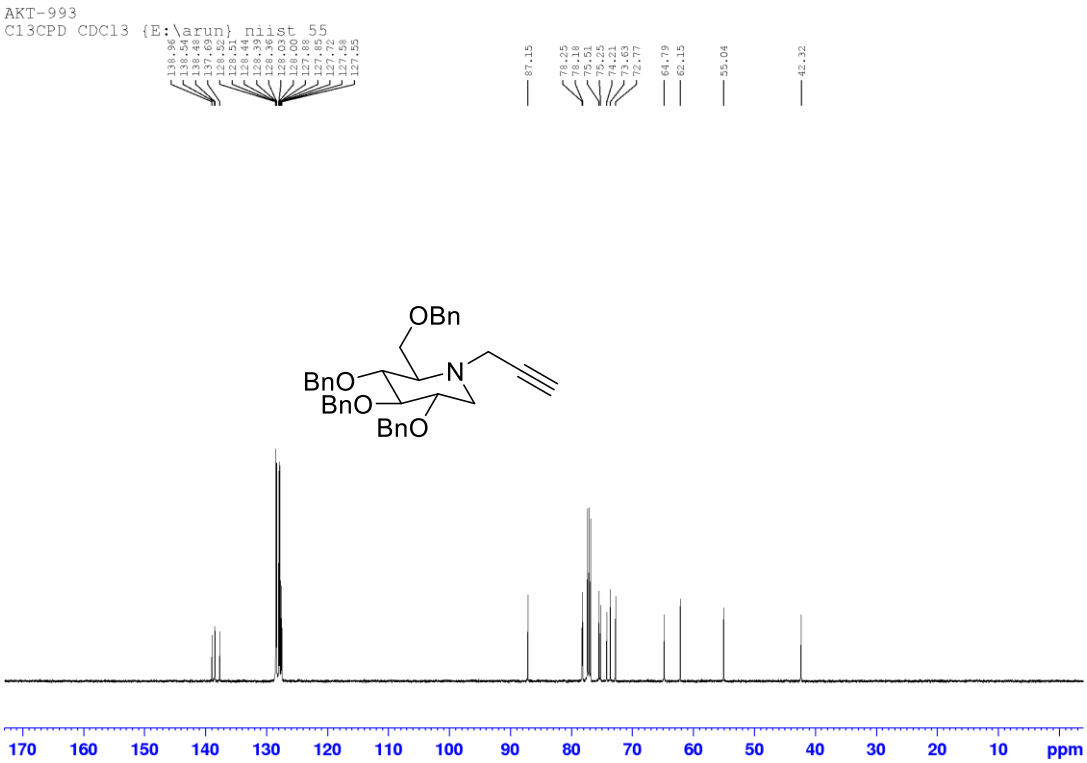
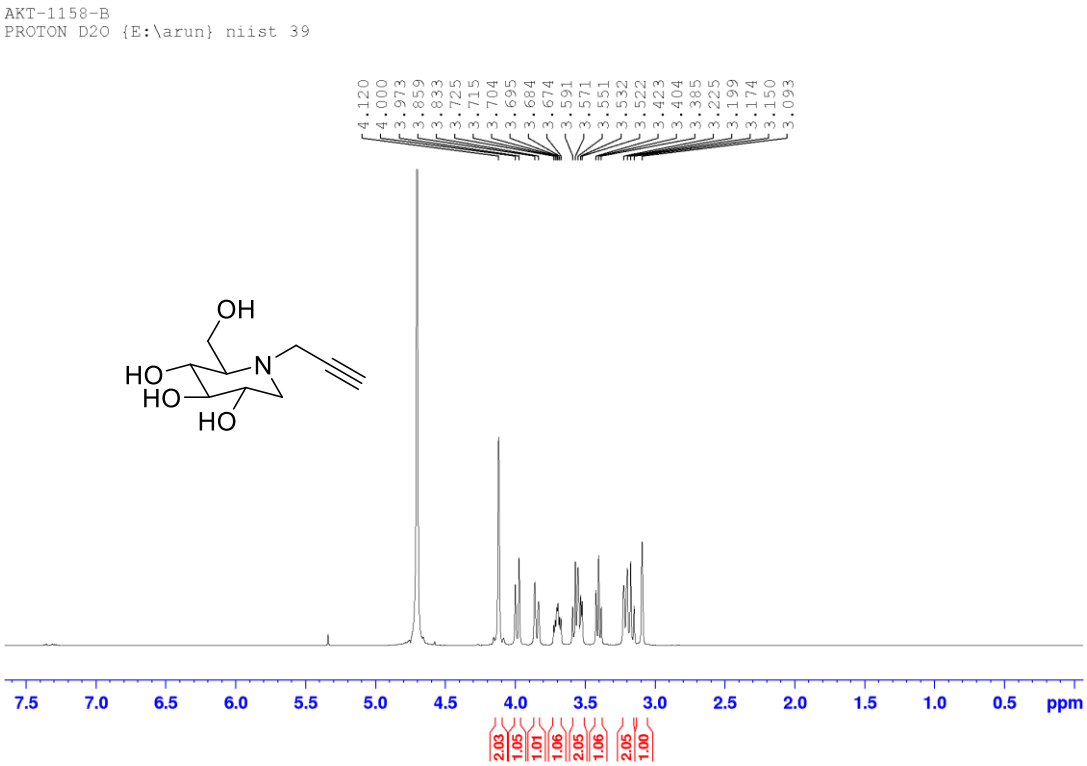


Figure 3.5.3.12. NMR spectras of compound **33**



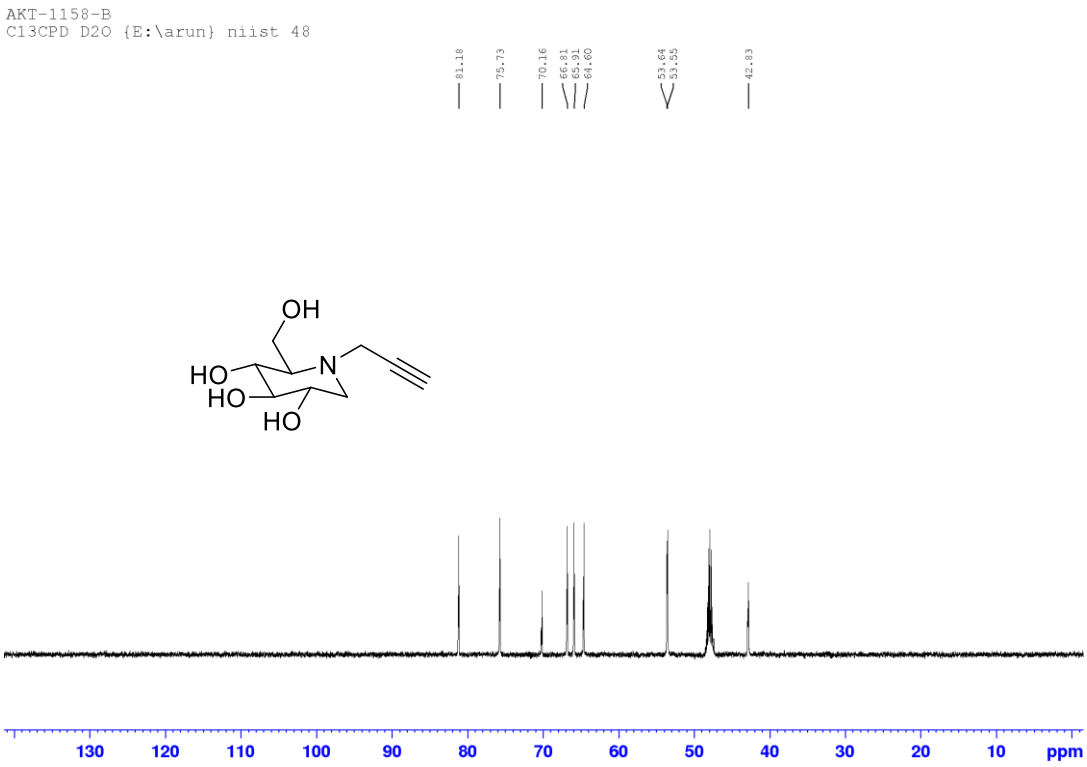
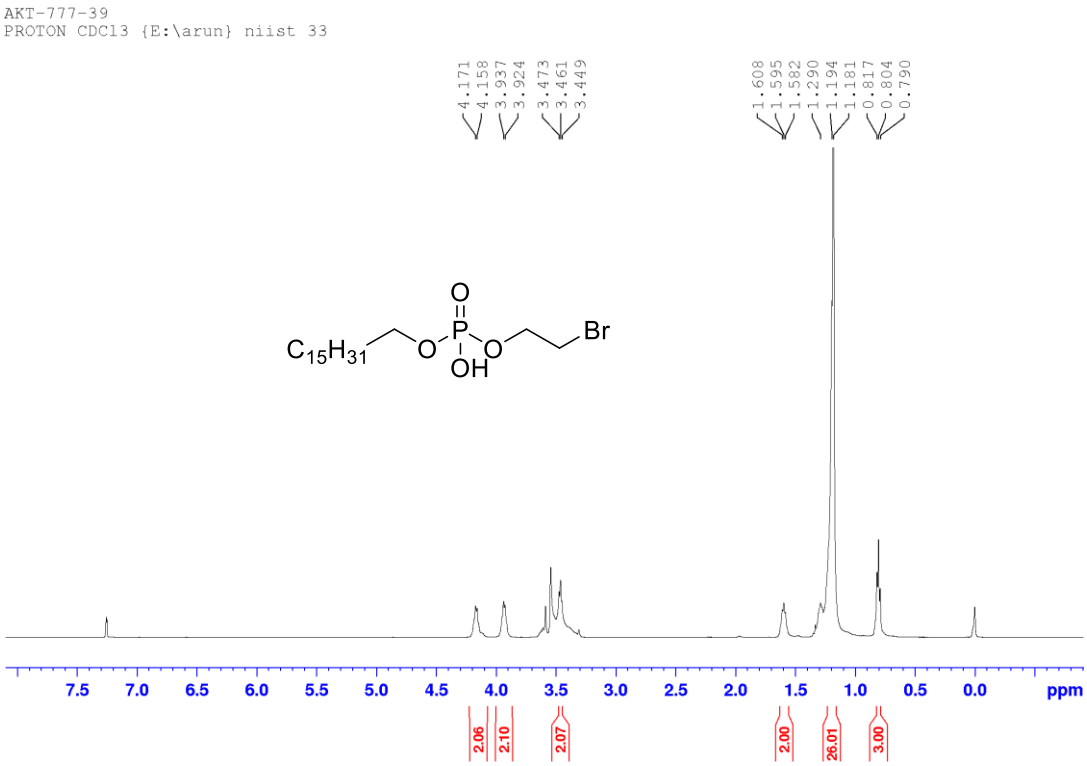
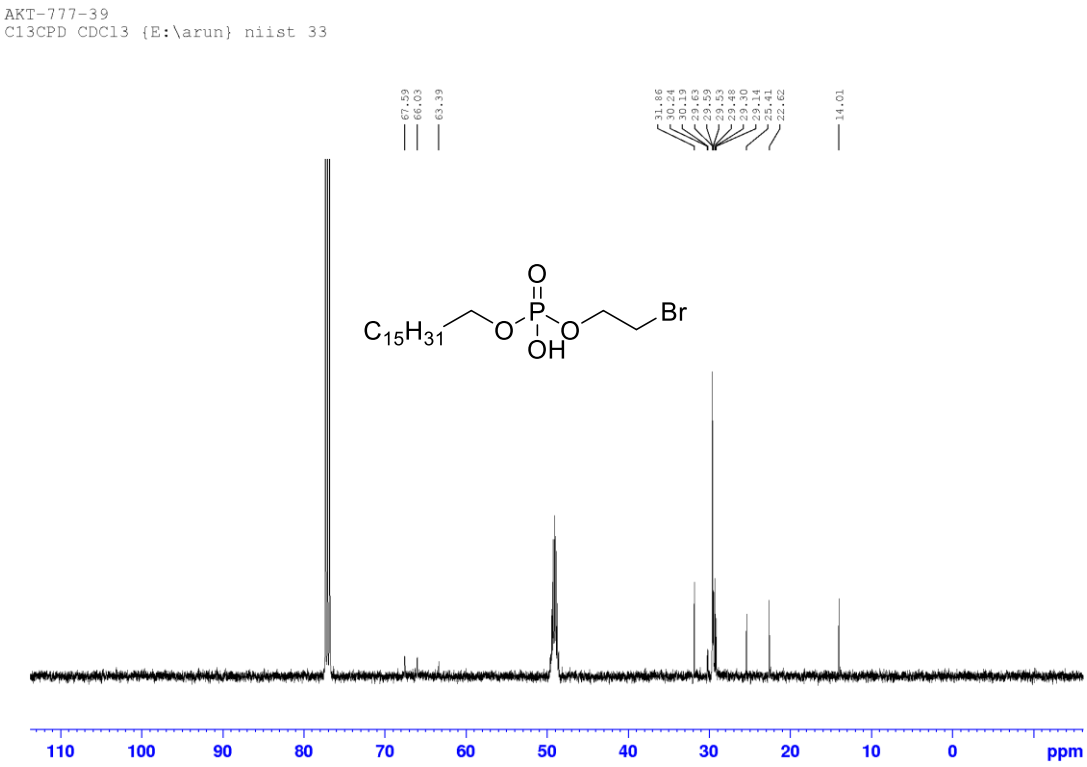


Figure 3.5.3.13. NMR spectras of compound **23**





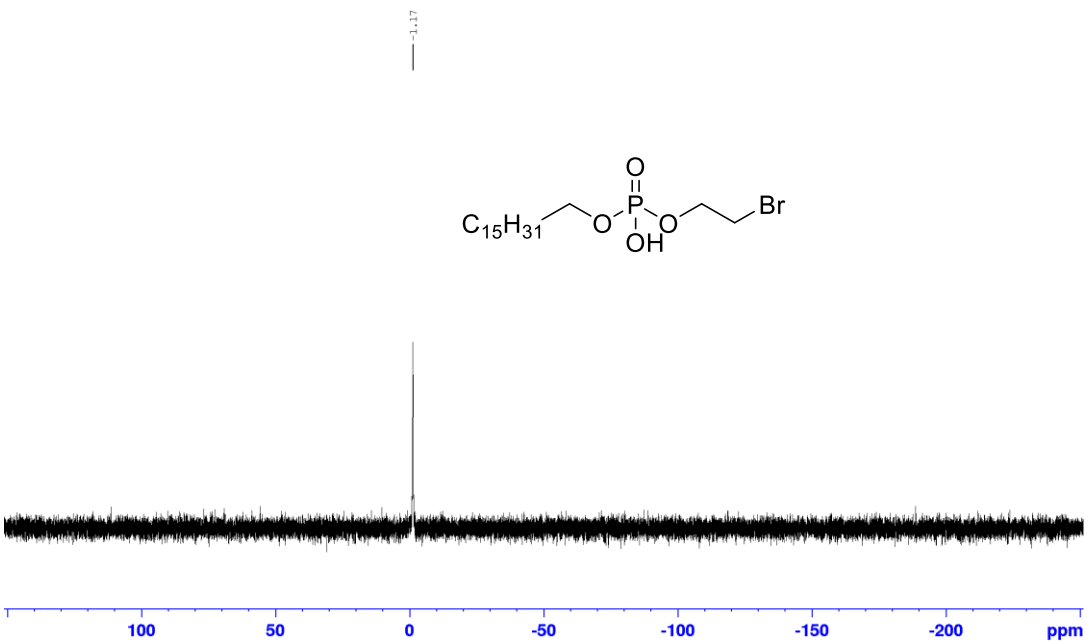
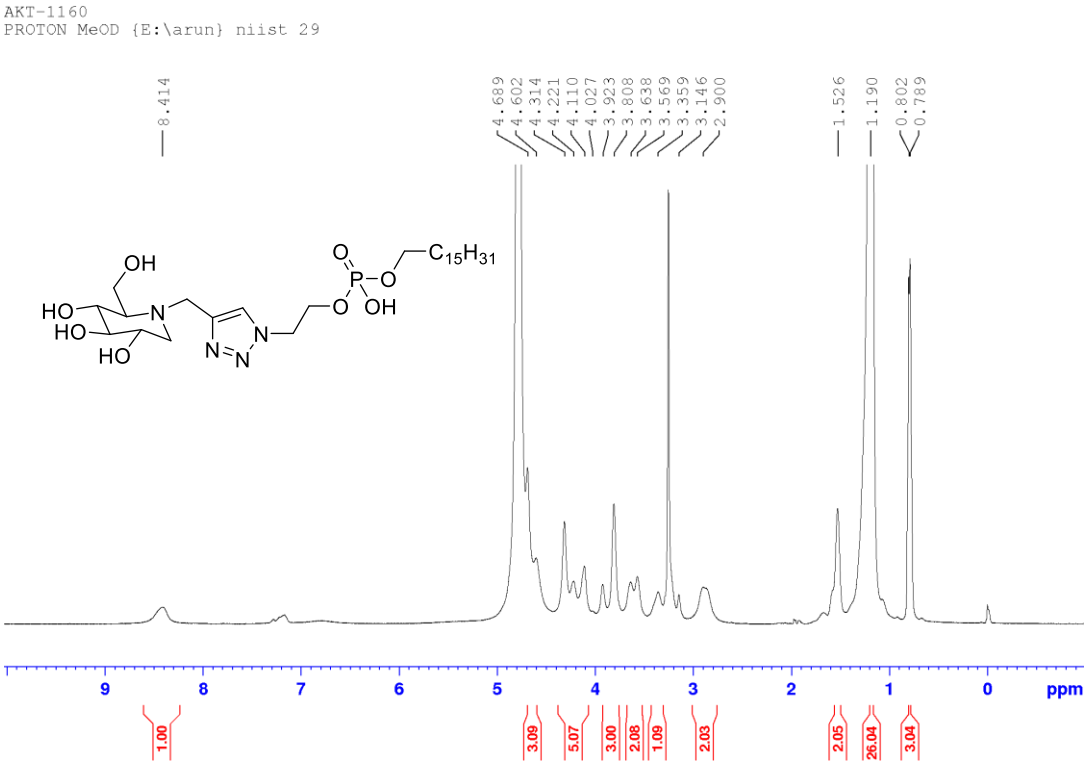


Figure 3.5.3.14. NMR spectras of compound **34**



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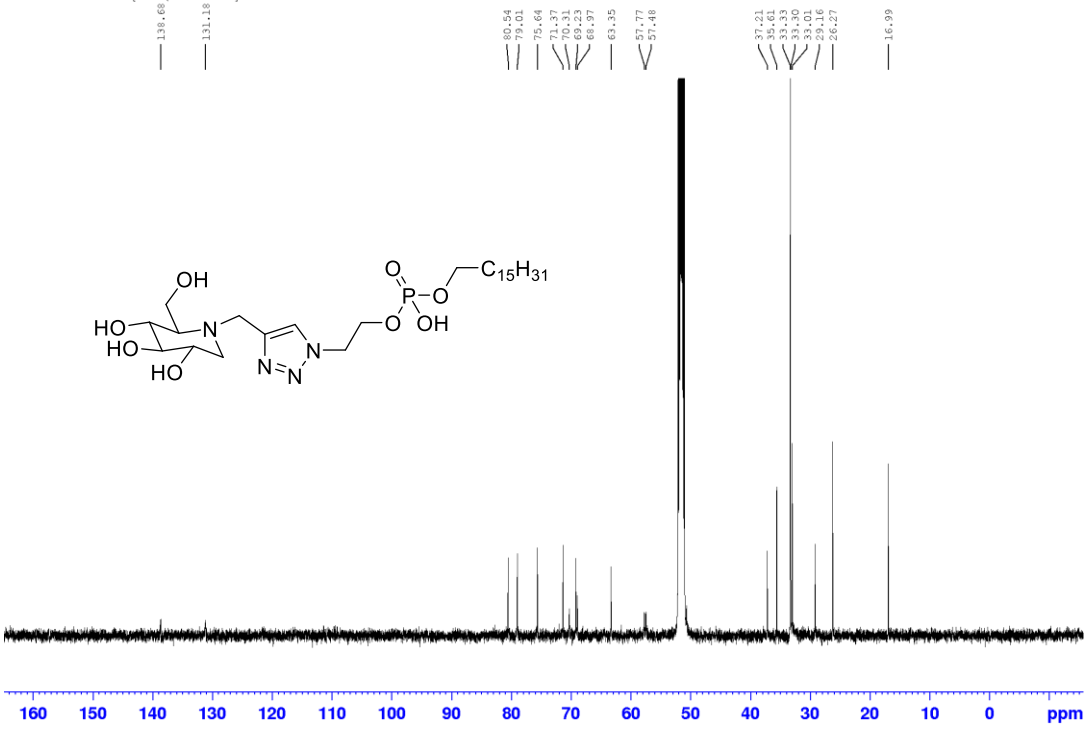
C13CPD MeOD {D:\Bruker} nmr 29



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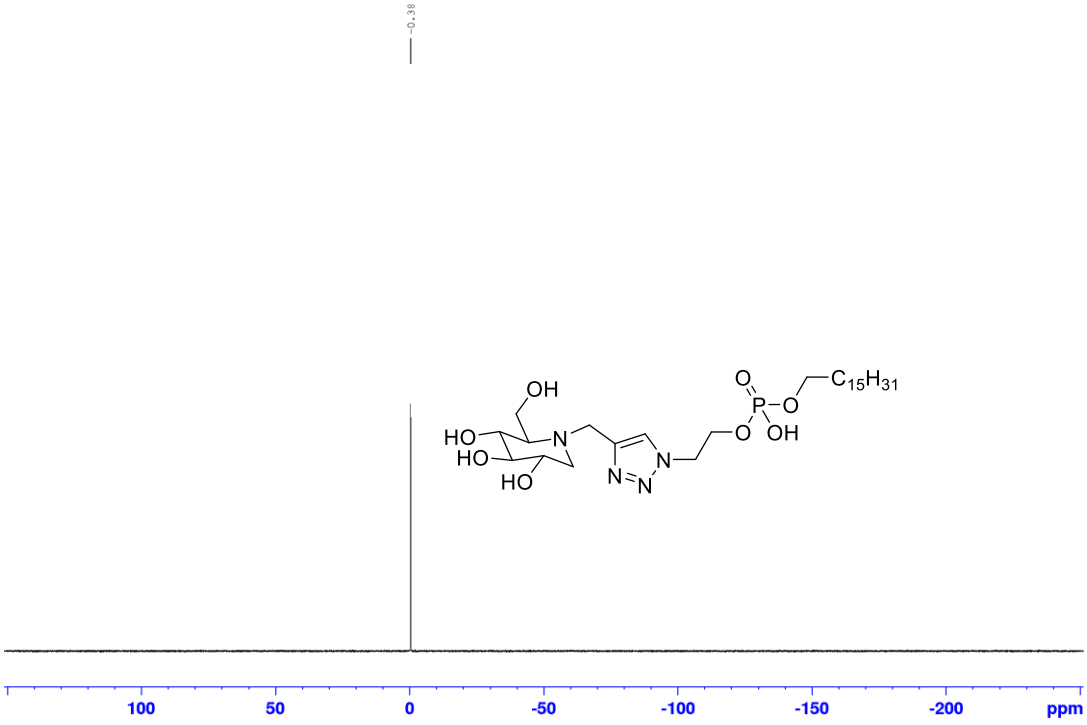

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Chapter 4

Synthesis of Novel Analogues of Nojirimycin

4.1. Abstract

The focus of this chapter is synthesis of various novel iminosguar analogues of interest for immunological studies. Three novel classes of nojirimycin analogues were synthesized: N-alkyl with C1-substituent (4-phenylbutyl), N-substituted 1-deoxynojirimycin and its congener δ-lactam, and a 4-phenylbutyl-β-*C*-glycoside. Early immunological studies suggested that the new nojirimycin analogues have an immunopotentiating effect when compared to findings on its congeners with immunosuppressive activity.

4.2. Introduction

Due to various inherent glycosidase inhibitory actions, plant and microbial iminosugars have various therapeutic advantages, prompting considerable efforts to create synthetic analogues of iminosugar nojirimycin (Figure 4.2.1a).[1,2] Glyset® and Zavesca®, synthetic iminosugars, support the core skeleton's medicinal potential.[3] Several iminosugar analogues with aliphatic/aromatic appendages on the nitrogen atom, C1 functionalized piperidine skeletons, and deoxy modifications around the piperidine skeleton were developed and showed a broad range of biological effects.[4] However, the immunomodulatory potential of iminosugar monocyclic compounds has been confined to immunosuppressive activity.[5,6] The emphasis on immunity at COVID-19 prompted renewed interest in natural products that have been demonstrated to improve immunity. To perform immunological research, medicinal chemists created a wide range of synthetic analogues of iminosugar 1-deoxynojirimycin (Figure 4.2.1a). In most investigations, N-alkylated iminosugars surpassed parent piperidines in terms of immunosuppressive effect.

Our group discovered that N-alkyl-2-pyrrolidinone-based iminosugars exhibit immunostimulant effects.[7] The synthesis of new nojirimycin analogues with N-alkyl appendages and C1 substituents such as carbonyl and 4-phenylbutyl substituents (Figure 1) is described here; the new analogues exhibited unprecedented immunopotentiating activity, which we attribute to structural differences between these novel iminosugars and previously reported ones. According to one study, the congener of bicyclic castanospermine enhanced the production of both IL-6 and IFN-γ and had a little inhibitory effect on IL-4 cytokines.[8] In contrast to the monocyclic analogues developed in this study, castanospermine is a bicyclic sugar.

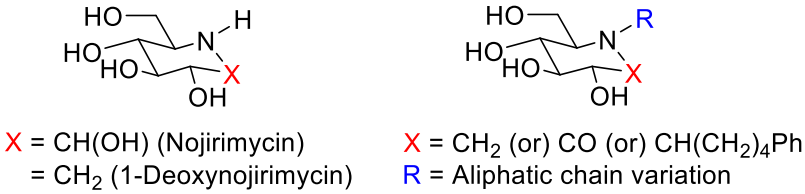


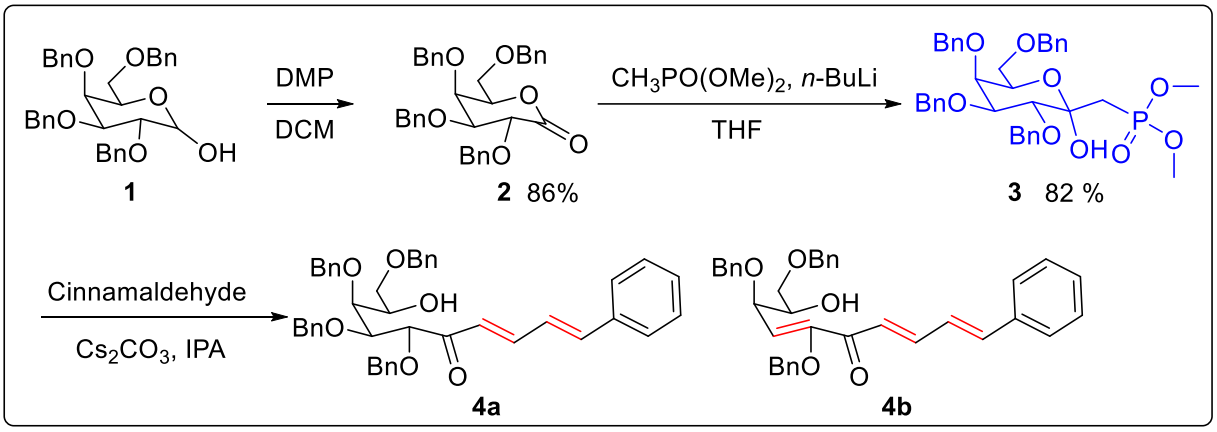
Figure 4.2.1a.: Structures of nojirimycins and designed analogues

4.3. Results and Discussion

4.3.1. Synthesis

The Horner-Wadsworth-Emmons (HWE) reaction, along with the β-keto phosphonate precursor of D-galactose and aliphatic aldehydes, has been reported as a novel technique for β-*C*-glycoside synthesis.[9] Because there have been numerous publications on *N*-alkyl variations of iminosugars for immunomodulatory actions, we generated a C1-alkyl variation of iminosugars via HWE reaction in the current study. KRN7000, a novel immunostimulant with a functionalized lipid chain terminating in a phenyl group, showed a substantial cytokine bias.[10] As a result, cinnamaldehyde was chosen as the aldehyde precursor for the HWE reaction because it permits C1 modification, which contains an aliphatic appendage with a terminal phenyl group.

The synthesis of β-keto phosphonate is a multistep synthetic endeavour demonstrated from our lab. Hence, synthesis of *C*-glycosides with masked β-keto phosphonate **3** to reduce the number of steps was planned that was synthesized from lactol **1** in two steps. After the preparation of masked keto phosphonate, the HWE reaction with cinnamaldehyde and Cs_2CO_3 as a base in isopropanol was attempted to obtain compound **4a**. Unfortunately, the reaction produced an unanticipated elimination product **4b**.



Scheme 4.3.1.1a. Synthesis of compound **3** and HWE reaction

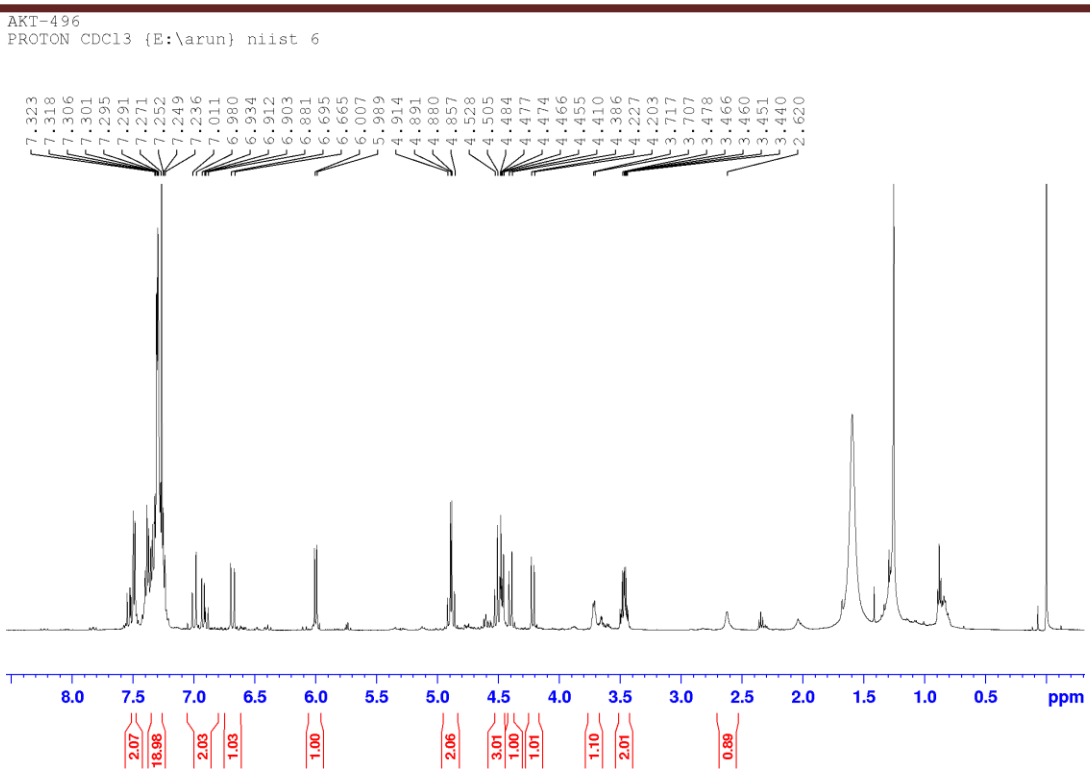


Figure 4.3.1.1a. ^{1}H NMR of compound **4b**

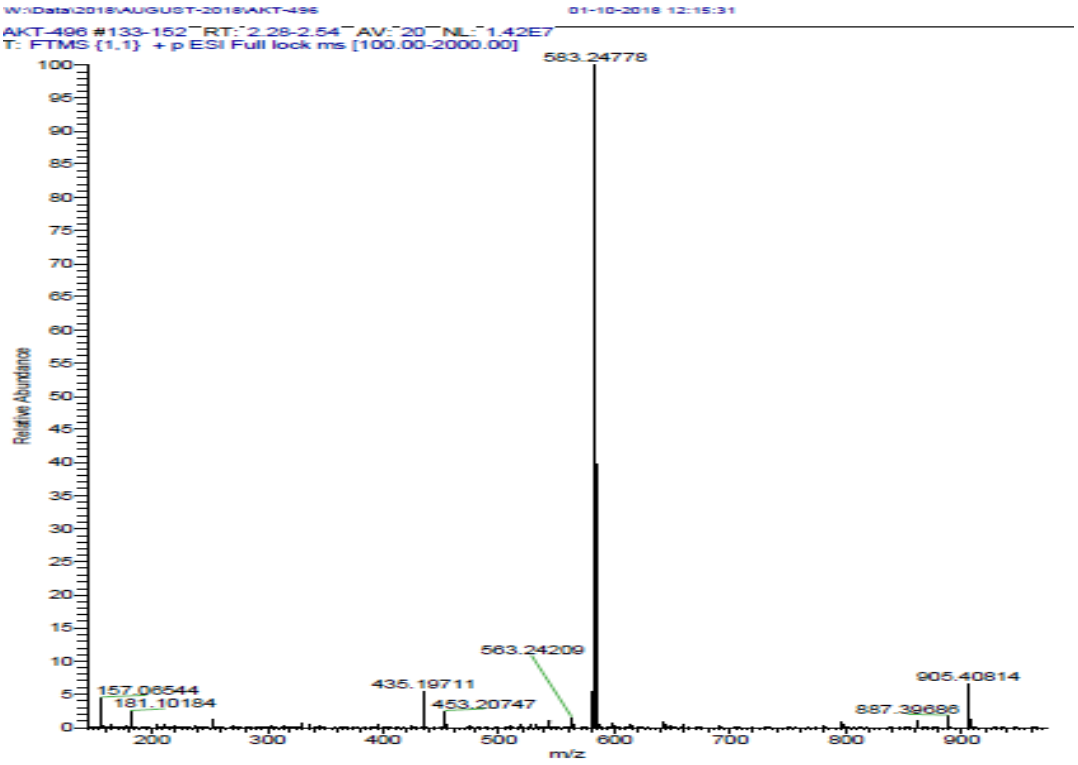
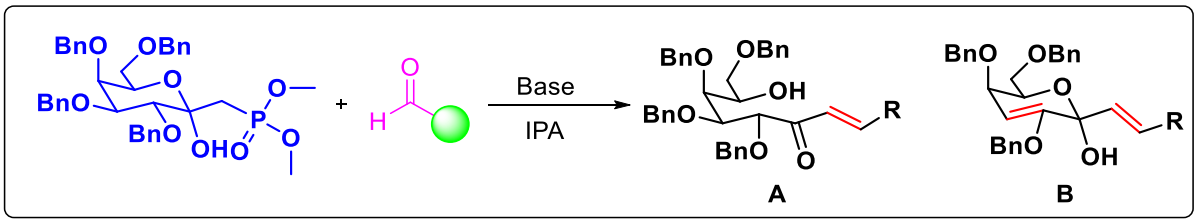


Figure 4.3.1.2a. HRMS of compound **4b**

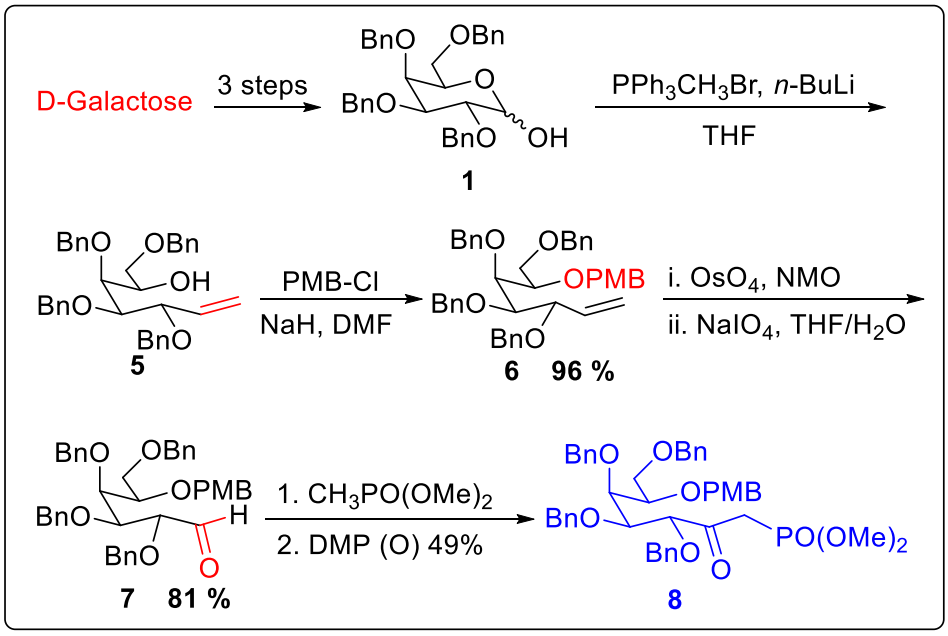
HWE reaction conditions by variation in base, temperature, solvent and aldehyde in isopropanol were conducted (Table 4.3.1.1a) to effect a desirable outcome but all the conditions led to undesired elimination product B.

Table 4.3.1.1a. Optimization of HWE reaction

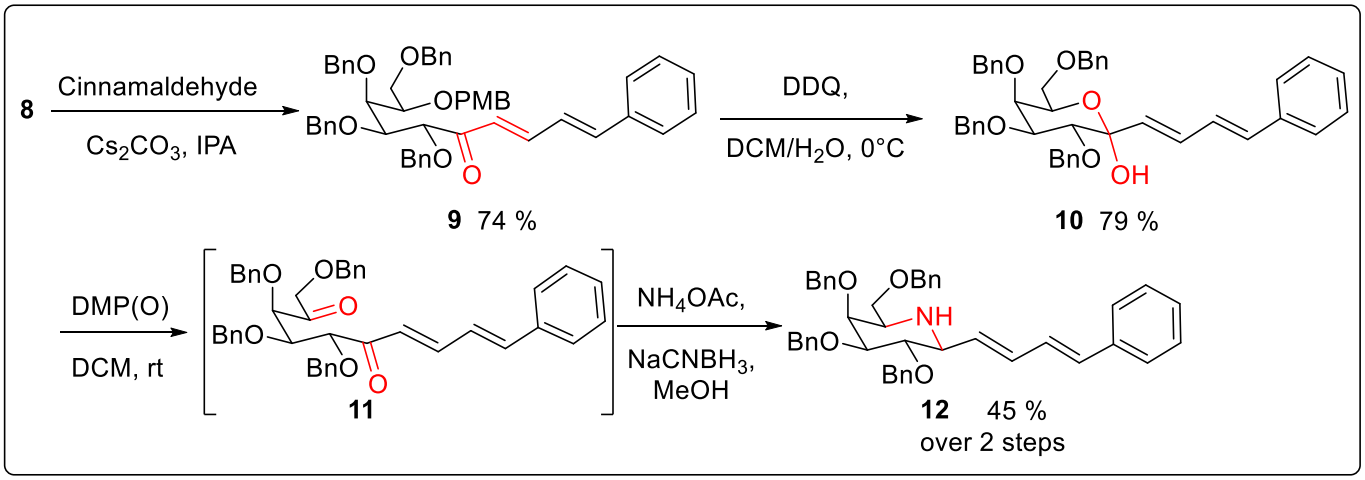


Entry	Aldehyde	Base (2 equiv)	Temperature	Solvent	Result
1	Octanal	$Ba(OH)_2$	rt	THF	**B**
		DIPEA		ACN	No reaction
		TEA		DCM	No reaction
		K-*t*BuO		Toluene	No reaction
		Na_2CO_3		THF	No reaction
		Cs_2CO_3		IPA	**B**
2	Benzaldehyde	$Ba(OH)_2$	rt	IPA	**B**
		DIPEA		THF	No reaction
		TEA		ACN	No reaction
		K-tBuO	rt	DCM	**B**
			-10 °C		No reaction
		Na_2CO_3	rt	Toluene	No reaction
		Cs_2CO_3		THF	**B**

Hence, the previous method of preparation of β-keto phosphonate was employed from D-galactose. The resulting β-keto phosphonate **8** was subjected to HWE reaction with cinnamaldehyde and Cs_2CO_3. Subsequent PMB deprotection, followed by oxidation and double reductive amination as per our previous procedure afforded compound **12**.

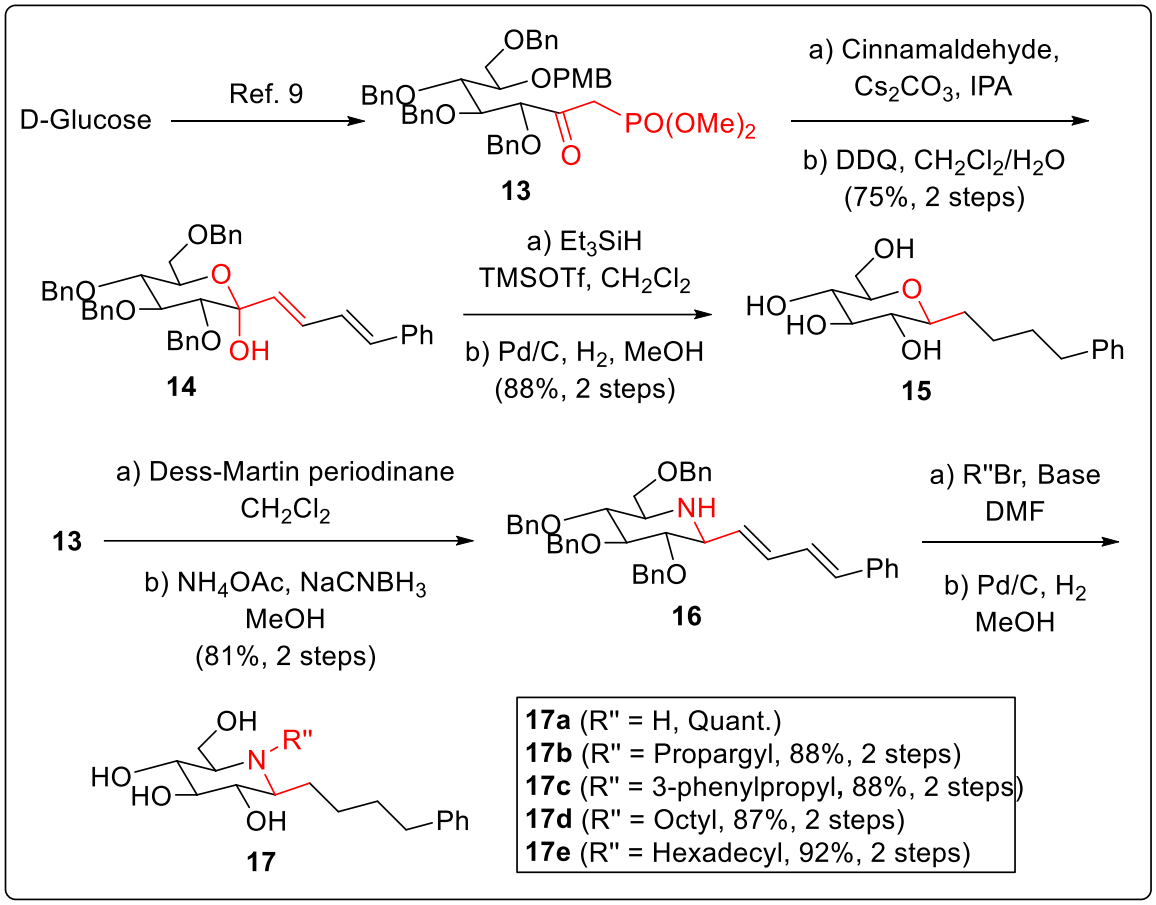


Scheme 4.3.1.2a. Synthesis of β-keto phosphonate **8**



Scheme 4.3.1.3a. Synthesis of iminosugar β-*C*-glycoside **12**

Because nojirimycin is a natural carbohydrate mimic of D-glucose, we commenced our synthesis of β-keto phosphonate **13** with D-glucose. The cross-coupled product is generated in good yields by HWE reaction with C(sp^2)-CHO precursors, while compound **14** was synthesized in 75% yield in two steps by HWE reaction in the presence of Cs_2CO_3 followed by PMB deprotection in the presence of DDQ. Although we are currently interested in iminosugar analogues, compound **14** provides access to the β-*C*-glycoside of D-glucose, allowing us to synthesize compound **15** with C1 aliphatic variation terminating with phenyl group in 88% yield by Kishi reduction of the 3° hydroxy group followed by global debenzylation using 10% Pd/C in the presence of H_2 under atmospheric pressure. Dess-Martin periodinane oxidation of compound **14** yielded the diketone, which followed by a double reductive amination reaction with ammonium acetate produced piperidine **16** in 81% yield. In the presence of NaH at 0 °C with propargyl and cinnamyl bromides and K_2CO_3 at 80 °C with octyl and hexadecyl bromides compounds **17a-e** were produced. Uunder Pd/C conditions, the propargyl group of compound **17b** remained unaffected.

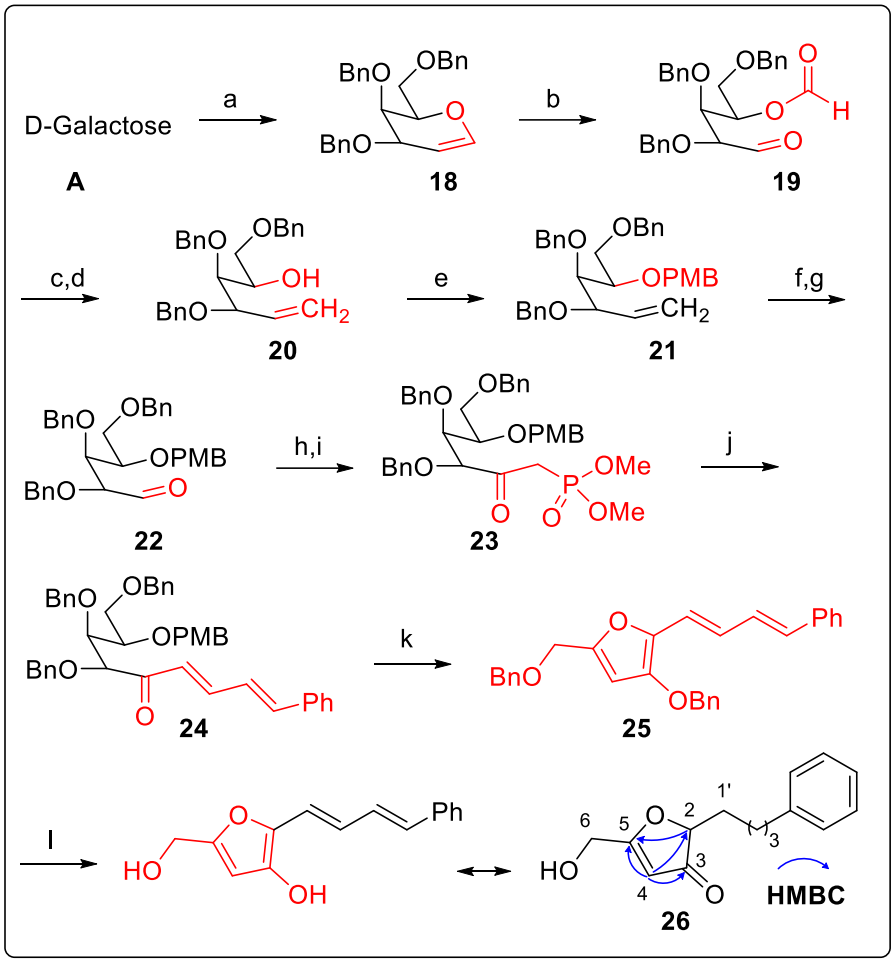


Scheme 4.3.1.4a. Synthesis of glucosyl-β-*C*-glycoside (**15**) and corresponding aza-β-*C*-glycosides (**17a-e**)

In a similar attempt, we envisaged the formation of furanoid *C*-glycosides utilizing the HWE reaction of β-keto phosphonate generated in multi-step synthesis from a glycal derived from D-galactose.[11] The synthesis commenced with the preparation of glycal **18** from D-galactose following the reported procedure. Oxidative cleavage of glycal **18** using OsO_4 and $NaIO_4$ produced formate **19** in 74% yield (Scheme 1). Methanolysis of formate **19** using NaOMe followed by Wittig reaction afforded the respective alkene **20**. Subsequent protection of the 2° alcohol with para-methoxybenzyl (PMB) group afforded PMB-protected alkene **21** in 98% yield. Osmylation in the presence of stoichiometric oxidant NMO, followed by oxidative cleavage of the diol product using aqueous $NaIO_4$ in THF, produced aldehyde **22** in 81% yield over two

steps. Nucleophilic addition of the anion generated from dimethyl methyl phosphonate and *n*-BuLi to aldehyde **22** and subsequent oxidation using Dess−Martin periodinane affording β-keto phosphonate **23** in 42% yield over two steps. We envisaged utilizing β-keto phosphonate **23** in the synthesis of *C*-glycosides with unsaturated aliphatic chains under HWE conditions by using pertinent aldehydes. Accordingly, the HWE reaction between cinnamaldehyde and β-keto phosphonate **23** in presence of Cs_2CO_3 afforded the desired HWE product **24** in 74% yield. Subsequent deprotection of PMB group in presence of DDQ should afford the hemiketal product which under reductive conditions affords the desired *C*-glycoside. However, treatment of HWE product **24** under DDQ mediated deprotection conditions afforded a product that exhibited a ^{1}H NMR wherein benzylic protons exhibited distinct singlets, reminiscent of our earlier observation, which helped in understanding the outcome of this reaction as the formation of trisubstituted furan **25** arising out of double elimination reactions. Pd/C hydrogenative conditions facilitated deprotection of benzylic group which undergoes tautomerization for facile synthesis of 3(2*H*)-furanone **26**. HMBC correlations from H-4 (δH 5.62) to *C*-2 (δC 86.6), *C*-5 (δC 191.4), *C*-3 (δC 204.3); correlations from H-2 (δH 4.41) to *C*-5; COSY correlations between H-2 and H-1′ (δH 1.66, 1.90, 2H) along with splitting pattern vividly confirm the structure of 3(2*H*)-furanone **26**.

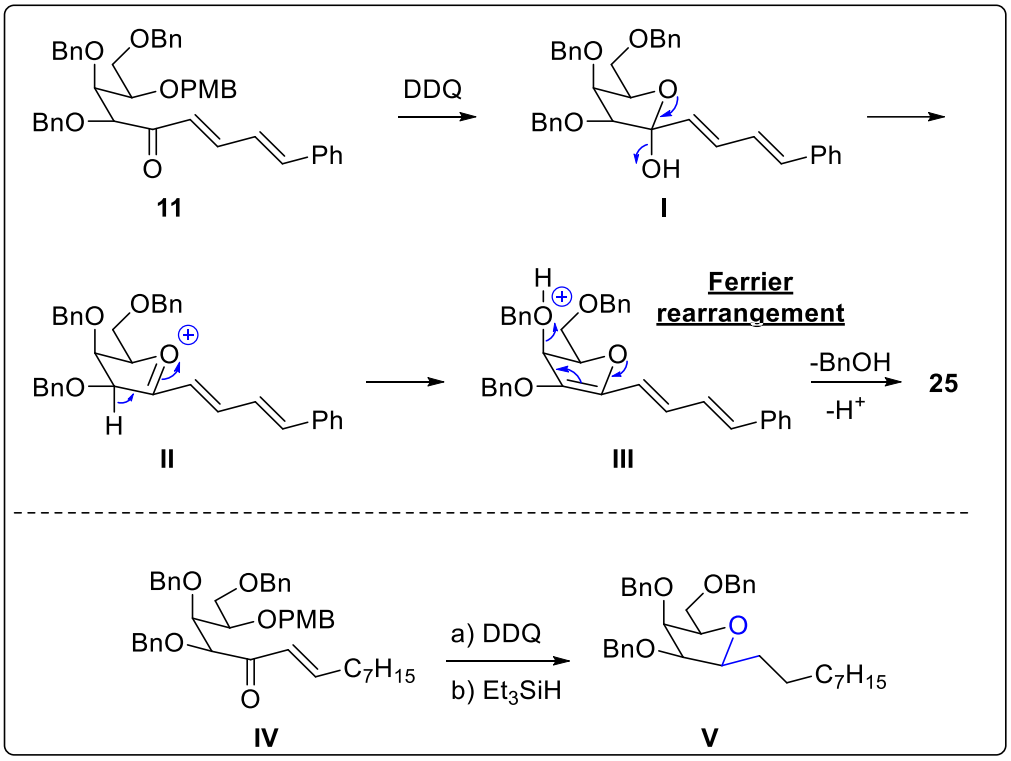
Scheme 4.3.1.5a. Synthesis of furanone **26**



Scheme 1. Reagents and conditions: a) Ref. 14; b) OsO_4, $NaIO_4$, THF:H_2O (2:1), rt, 6 h, (74%); c) NaOMe, MeOH, 0 °C, 10 min, (90%); d) PPh_3CH_3Br, *n*-BuLi, THF, (55%); e) NaH, PMBCl, DMF, (98%); f) OsO_4 (cat.), NMO, acetone:H_2O (4:1), rt, 8 h; g) $NaIO_4$, THF, 0°C to rt, 3 h, (81%, 2 steps); h) $CH_3PO(OMe)_2$, *n*-BuLi, THF, -78 °C; i) DMP, CH_2Cl_2, 1 h, rt, (42%, 2 steps); j) Cinnamaldehyde, Cs_2CO_3, *i*-PrOH, overnight, rt, (74%); k) DDQ, CH_2Cl_2:H_2O (10:1), rt, 3 h, (53%); l) Pd/C, H_2, EtOH, rt, 1 h (54 %).

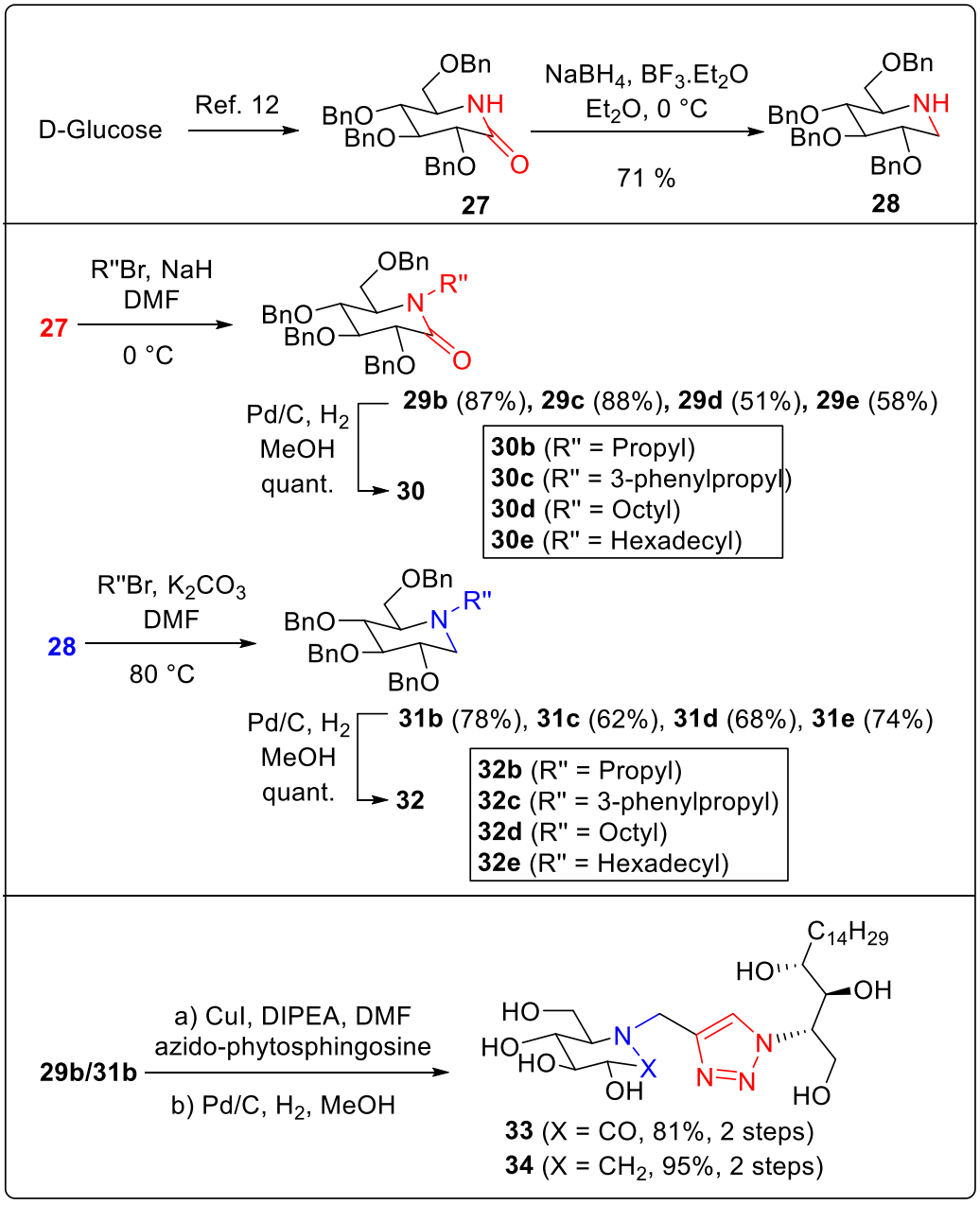
The plausible mechanistic pathway for the formation of furan **25** is depicted in Scheme 4.3.1.6a. Conventional DDQ mediated PMB deprotection conditions affords hemiketal **I** which undergoes spontaneous elimination of hydroxyl group to afford intermediate **II** and the driving

force for this step is extended π-conjugation. Subsequent stabilization of oxocarbenium ion **II** affords glycal **III** which sets the stage for Ferrier rearrangement which again is driven by extended π-conjugation and concomitant aromatization affords furan **25**. To substantiate the proposed mechanism behind the driving force for hydroxyl elimination of intermediate **I**, compound **IV** was synthesized from octanal under HWE conditions. DDQ mediated deprotection conditions afforded the expected hemiketal which was stable enough to be carried over to the next step involving Et_3SiH reduction to afford tetrahydrofuran **V**.



Scheme 4.3.1.6a. Mechanistic pathway for the formation of furan **25**

The design for immunomodulatory investigations includes analogues of 1-deoxynojirimycin with carbonyl and N-alkyl appendages in the C1 position. As a result, the stated conditions were used to synthesize lactam iminosugar **27** (Scheme 4.3.1.7a) from D-glucose.[12] Lactam **27** was N-alkylated with four distinct aliphatic bromides of various lengths in the presence



Scheme 4.3.1.7a Synthesis of novel nojirimycin analogues **30b-e**, **33** and **32b-e**, **34**

of NaH using the S_N2 procedure, yielding compounds **29b-e**, which were subsequently debenzylated to produce lactam analogues **30b-e**. $NaBH_4$ reduction of lactam **27**, followed by N-alkylation with the same aliphatic bromides in the presence of K_2CO_3, generated 1-deoxynojirimycin analogues **31b-e**, and debenzylation yielded 1-deoxynojirimycin analogues **32b-e**. Additionally, the propargylated compounds **29b** and **31b** were subjected to 'Click

chemistry' conditions with azido phytosphingosine, followed by debenzylation, producing the triazole attached analogues **33** and **34**. (Scheme 2).

The biological studies of the synthesized compounds were conducted by Dr. Sampath Kumar H. M. of CSIR-IICT. The novel analogues were subjected to various cell-based studies to determine their immunopotentiating effects. Compound **34** was found to stimulate the production of IL-12 cytokine in murine dendritic cells, and showed a remarkable TNF-α expression in murine peritoneal macrophages along with potent nitric oxide response. The observed immunopotentiation compared to immunosuppressive effects of nojirimycin analogues is attributed to minor to substantial structural changes in the analogues, in contrast to the earlier reports. The preliminary *in vitro* results of immunopotentiating activity of these novel iminosugar analogues warrants a detailed investigation.

4.4. Conclusion

Finally, three different classes of iminosugar 1-deoxynojirimycin were synthesized for immunological studies: 4-phenylbutyl substituted-*C*1 and *N*-alkyl variants **17a**-**e**; δ-lactams **30b**-**e**, **33**; and 1-deoxynojirimycin with *N*-alkyl variations **32b**-**e**, **34**, along with a β-*C*-glycoside **15**. The current work is the first to show various variants of iminosugar analogues with a focused library exhibiting immunopotentiating effects. It would not be possible to derive a structure-activity relationship since the majority of the analogues in the current study had almost equal magnitudes of reactions and as the library of analogues is diverse with three separate classes of iminosugars. Compound **34** was shown to be very efficient in promoting the production of IL-12 in murine dendritic cells, as well as substantial TNF-α expression by murine peritoneal macrophages and a strong nitric oxide response. The synthesis of new iminosugars in this work

adds to the repertoire of iminosugars and embodies the adage "structure dictates function," implying that they can be used as immunomodulators.

4.5. General experimental conditions:

Optical rotation was measured on a JASCO P-2000 polarimeter. ^{1}H and ^{13}C NMR were recorded on a Bruker ASCEND^{TM}-500 spectrometer at 500 and 125 MHz, respectively using $CDCl_3$, D_2O and CD_3OD solvents. NMR data are reported as follows: chemical shifts in ppm (δ) with integration, coupling constant in Hz and multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, etc.). HR-ESI-MS analysis was recorded on a Thermo Scientific Exactive-LCMS instrument by electrospray ionization method with ions given in *m/z* using Orbitrap analyzer. Reactions were monitored by silica gel G-60 F_{254} aluminum TLC and compounds were visualized by short wavelength lamp and by charring the TLC plate after spraying with 15% sulfuric acid in ethanol. Chromatographic separations were carried out by conventional column chromatography on silica gel (100× 200 mesh). Reagents were purchased at the highest commercial quality and used without further purification.

4.5.1. General procedure

4.5.1.2. General N-alkylation reaction procedure I

To a solution of compound **28** (1 equiv) in DMF (3 mL) was added K_2CO_3 (2.5 equiv) and R″Br (3 equiv) at room temperature and then allowed stir for 1 h at 80 °C. After the formation of the non-polar product, as indicated by TLC, the reaction mixture was quenched with H_2O (10 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc to afford the respective N-alkylated compounds **31b-e**.

4.5.1.3. General N-alkylation reaction procedure II

To a solution of compound **27** (1 equiv) in DMF (5 mL) was added 60% NaH (2.5 equiv) at 0 °C under nitrogen atmosphere. R″Br (3 equiv) was added at 0 °C and the reaction mixture was allowed to stir for 15-25 mins. After the formation of the non-polar product, as indicated by TLC, the reaction mixture was quenched with H_2O (25 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc to afford the respective N-alkylated compounds **29b-e**.

4.5.1.4. General debenzylation procedure I

To a solution of benzyl protected intermediate in MeOH (5 mL) was added Pd/C, a few drops of conc. HCl and purged H_2 gas for 2 min. The reaction mixture was stirred overnight under H_2 atmosphere. The reaction mixture was diluted with MeOH and filtered through a Celite® pad. The filtrate was concentrated and precipitated in dichloromethane, which afforded the respective product.

4.5.1.5. General debenzylation procedure II

To a solution of benzyl protected intermediate in MeOH (5 mL) was added Pd/C, a few drops of conc. HCl and purged H_2 gas for 2 min. The reaction mixture was stirred for one hour under H_2 atmosphere. The reaction mixture was diluted with MeOH and filtered through a Celite® pad. The filtrate was concentrated and precipitated in dichloromethane, which afforded the respective product.

(2R,3S,4R,5S,6S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-((1E,3E)-4-phenylbuta-1,3-dien-1-yl)piperidine (**12**): To a solution of hemiketal **10** (122 mg, 0.2 mmol, 1 equiv) in CH_2Cl_2 (5 mL) was added Dess−Martin periodinane (156 mg, 0.4 mmol, 2 equiv), and then the reaction mixture was stirred for 2 h at rt under an argon atmosphere. The reaction mixture was quenched

with saturated aqueous $Na_2S_2O_3$ (10 mL), and saturated aqueous $NaHCO_3$ (10 mL), and extracted with CH_2Cl_2 (3 × 25 mL), and the organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90/10 to 85/15 afforded diketone **11** as a colorless viscous solid. To a solution of diketone (67 mg, 0.1 mmol, 1 equiv) in MeOH (5 mL) was added ammonium acetate (78 mg, 1 mmol, 10 equiv) and $NaCNBH_3$ (31 mg, 0.5 mmol, 5 equiv), and then the reaction mixture was stirred overnight at 50 °C under an argon atmosphere. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (2 × 25 mL), and the organic layers were washed with H_2O (2 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated. Purification by flash chromatography using hexane/EtOAc 80/20 afforded compound **12** (54 mg, 45% over two steps) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33 (25H, m), 6.73 (1H, t, J=10 Hz), 6.47 (2H, m), 5.81 (1H, t, J=8 Hz), 4.97 (1H, d, J=11 Hz), 4.83 (1H, m), 4.77 (2H, s), 4.63 (2H, m), 4.45 (2H, m), 3.95 (1H, s), 3.71 (2H, m), 3.53 (2H, m), 3.31 (1H, bs), 3.22 (1H, bs), 2.88 (1H, s). 138.8, 138.5, 133.2, 132.6, 132.2, 128.7, 128.6, 128.5, 128.45, 128.42, 128.3, 128.2, 128.0, 127.8, 127.6, 127.6, 127.4, 126.4, 85.6, 80.9, 75.4, 74.4, 74.2, 73.5, 72.8, 70.4, 62.4, 57.6. HRMS (ESI) m/z: $[M+H]^+$ Calcd. for $C_{44}H_{46}NO_4$ 652.3427; Found 652.3440.

Dimethyl (3,4,5,7-tetrakis(benzyloxy)-6-((4-methoxybenzyl)oxy)-2-oxoheptyl)phosphonate (**13**): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33 (22H, m), 6.86 (2H, d, *J* = 8 Hz), 4.86 (1H, d, *J* = 12 Hz), 4.70-4.39 (10H, m), 4.01 (1H, m), 3.98 (1H, m), 3.91 (1H, m), 3.81 (4H, m), 3.72-3.59 (8H, m), 2.76 (1H, dd, *J* = 4, 11.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 200.2, 159.1, 138.2, 138.2, 137.7, 137.5, 130.6, 129.2, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.7, 127.5, 113.7, 81.2, 81.0, 78.5, 77.3, 73.6, 73.4, 73.4, 72.8, 71.5, 69.2, 55.3, 53.5, 52.9, 52.8, 52.7, 52.7, 39.4, 38.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{45}H_{51}NaO_{10}P$ 805.3118; Found 805.3123.

(2S,3R,4S,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-2-((1E,3E)-4-phenylbuta-1,3-dien-1-yl)tetrahydro-2H-pyran-2-ol (**14**): To a solution of compound **13** (2.4 g, 3.0 mmol, 1 equiv) in IPA (20 mL) was added Cs_2CO_3 (1 g, 3.0 mmol, 1 equiv), and then the mixture was stirred for 30 min at rt under an argon atmosphere. Cinnamaldehyde (700 µL, 6.1 mmol, 2 equiv) was added directly to the reaction mixture, and this mixture was stirred at rt under an argon atmosphere. After the mixture was stirred overnight, the reaction was stopped and the solvents were evaporated; the crude mixture was directly subjected to purification by flash chromatography using hexane/EtOAc 90/10, affording unsaturated ketone intermediate. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.48 (2H, d, *J* = 8 Hz), 7.28-7.42 (19H, m), 7.21 (7H, m), 6.92 (1H, d, *J* = 15.5 Hz), 6.84 (2H, d, *J* = 8.5 Hz), 6.74 (1H, dd, *J* = 11, 15.5 Hz), 6.60 (1H, d, *J* = 15.5 Hz), 4.76 (2H, s), 4.70 (2H, dd, *J* = 4.5, 11.5 Hz), 4.53 (4H, d, *J* = 9 Hz), 4.42 (2H, dd, *J* = 12, 14.5 Hz), 4.26 (1H, d, *J* = 2 Hz), 4.10 (2H, m), 3.89 (1H, dd, *J* = 4, 10 Hz), 3.79 (3H, s), 3.75 (1H, m), 3.69 (1H, m). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 199.7, 159.0, 143.0, 142.0, 138.9, 138.3, 138.1, 137.3, 136.1, 130.7, 129.2, 129.1, 128.9, 128.5, 128.44, 128.40, 128.36, 128.2, 128.1, 127.97, 127.92, 127.7, 127.6, 127.5, 127.3, 127.29, 129.0, 125.8, 113.7, 84.6, 80.7, 79.4, 78.9, 75.3, 74.7, 73.4, 72.9, 71.6, 69.6, 55.2. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{52}H_{52}NaO_7$ 811.3611; Found 811.3631. To a solution of unsaturated ketone (2.1 g, 2.6 mmol, 1 equiv) in CH_2Cl_2/H_2O (10:1, 30 mL) was added DDQ (1.52 g, 5.3 mmol, 2 equiv) at 0 °C, and the resulting brown colored reaction mixture was stirred for 2.5 h at rt under argon atmosphere. The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (3 × 50 mL), and the extracts were washed with water (2 × 250 mL), dried over anhydrous Na_2SO_4, and concentrated. Purification by flash chromatography using hexane/EtOAc (90/10) afforded hemiketal **14** (1.12 g, 75% over two steps) as a pale yellow viscous solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (45H, m), 6.77 (4H, m), 5.86 (1H, m), 4.89 (4H, m),

4.61 (9H, m), 4.34 (0.6H, d, *J* = 4.20 Hz), 4.07 (2H, m), 3.82 (1H, m), 3.74 (2H, m), 3.64 (1H, m), 3.48 (1H, m), 2.85 (1H, m). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 200.0, 143.4, 142.3, 138.7, 138.3, 138.1, 137.7, 137.6, 137.2, 137.0, 136.1, 134.4, 133.3, 132.4, 129.3, 128.9, 128.7, 128.6, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.3, 127.0, 126.5, 96.8, 83.7, 83.5, 82.9, 80.4, 78.2, 77.6, 75.8, 75.7, 75.0, 74.7, 73.8, 73.5, 73.4, 73.1, 71.7, 71.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{44}H_{44}NaO_6$ 691.3036; Found 691.3053.

(2R,3S,4R,5R,6S)-2-(Hydroxymethyl)-6-(4-phenylbutyl)tetrahydro-2H-pyran-3,4,5-triol (**15**): To a solution of compound **14** (270 mg, 0.4 mmol, 1 equiv) in CH_2Cl_2 (6 mL) was added Et_3SiH (646 µL, 4.0 mmol, 10 equiv) and TMSOTf (219 µL, 1.2 mmol, 3 equiv), at 0 °C, and the mixture was stirred for 10 min. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (2 × 30 mL), and the extracts were washed with brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated to afford the *C*-glycoside intermediate. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (2H, d, *J* = 7.5 Hz), 7.18-7.27 (21H, m), 7.08 (2H, m), 6.68 (1H, dd, *J* = 10.5, 15.5 Hz), 6.42-6.5 (2H, m), 5.74 (1H, dd, *J* = 7, 15 Hz), 4.87 (1H, d, *J* = 11 Hz), 4.81 (1H, d, *J* = 11 Hz), 4.76 (1H, d, *J* = 10.5 Hz), 4.68 (1H, d, *J* = 10.5 Hz), 4.55 (2H, m), 4.48 (2H, m), 3.79 (1H, dd, *J* = 7.5, 14.5 Hz, H-1), 3.65 (3H, m, H-3, H-4, H-6a), 3.59 (1H, t, *J* = 14.5 Hz, H-6b), 3.42 (1H, m, H-5), 3.29 (1H, t, *J* = 9 Hz, H-2). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.7, 137.9, 137.2, 133.7, 133.2, 130.4, 128.6, 128.46, 128.43, 128.39, 128.31, 127.98, 127.92, 127.86, 127.81, 127.7, 127.6, 126.5, 86.8, 82.5, 80.0, 78.7, 78.2, 75.7, 75.2, 75.0, 73.5, 68.9. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{44}H_{44}NaO_5$ 675.3086; Found 675.3089. The *C*-glycoside intermediate was debenzylated by using General debenzylation method I, which afforded **15** as an off-white semisolid (30 mg, 88% over two steps). $[\alpha]^{25}_D$ +9.9° ($CHCl_3$; *c* 0.75). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.08 (5H, m), 3.73 (1H, d, *J* = 11 Hz), 3.54 (1H, d, *J* = 8 Hz), 3.22-2.95 (4H, m), 2.51

(2H, s), 1.78 (1H, m), 1.54 (3H, m), 1.33 (2H, m), 1.16 (1H, m). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 142.6, 128.0, 127.9, 125.2, 80.1, 79.4, 78.5, 74.1, 70.7, 61.8, 35.5, 31.5, 31.3, 24.8. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{16}H_{24}NaO_5$ 319.1521; Found 319.1513.

(2R,3R,4R,5S,6S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-6-((1E,3E)-4-phenylbuta-1,3-dien-1-yl)piperidine (**16**): To a solution of hemiketal **14** (825 mg, 1.2 mmol, 1 equiv) in CH_2Cl_2 (15 mL) was added Dess−Martin periodinane (1.0 g, 2.4 mmol, 2 equiv), and then the reaction mixture was stirred for 1 h at rt under an argon atmosphere. The reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ (30 mL), and saturated aqueous $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2 (3 × 50 mL), and the organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90/10 to 85/15 afforded diketone as a colorless viscous solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.25-7.50 (25H, m), 7.17 (2H, m), 6.95 (1H, d, *J* = 15.5 Hz), 6.83 (1H, dd, *J* = 11, 15.5 Hz), 6.69 (1H, d, *J* = 15.5 Hz), 4.47-4.61 (6H, m), 4.41 (2H, s), 4.31 (1H, d, *J* = 4.5 Hz), 4.25 (2H, s), 4.20 (1H, d, *J* = 4.5 Hz), 4.16 (1H, t, *J* = 4.5 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 206.5, 199.6, 143.7, 142.6, 137.4, 137.09, 137.05, 136.0, 129.4, 128.9, 128.6, 128.5, 128.49, 128.43, 128.41, 128.36, 128.32, 128.07, 128.02, 127.9, 127.8, 127.3, 126.9, 125.4, 83.1, 81.1, 81.0, 74.7, 74.4, 73.8, 73.5, 73.2. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{44}H_{42}NaO_6$ 689.2879; Found 689.2861. To a solution of diketone (600 mg, 0.9 mmol, 1 equiv) in MeOH (10 mL) was added ammonium acetate (701 mg, 9.0 mmol, 10 equiv) and $NaCNBH_3$ (280 mg, 4.5 mmol, 5 equiv), and then the reaction mixture was stirred overnight at 50 °C under an argon atmosphere. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with EtOAc (2 × 80 mL), and the organic layers were washed with H_2O (2 × 70 mL), dried over anhydrous Na_2SO_4, and concentrated. Purification by flash chromatography using hexane/EtOAc 80/20 afforded compound **16** (519 mg, 81% over two steps)

as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): 7.33 (2H, d, *J* = 8 Hz), 7.11-7.27 (23H, m), 6.65 (1H, dd, *J* = 11, 15.5 Hz), 6.45 (1H, d, *J* = 13.5 Hz), 6.37 (1H, dd, *J* = 11, 15 Hz), 5.65(1H, dd, *J* = 6.5, 15 Hz), 4.87 (1H, d, *J* = 11 Hz), 4.78 (2H, t, *J* = 11.5 Hz), 4.68 (1H, d, *J* = 10.5 Hz), 4.55 (1H, d, *J* = 11 Hz), 4.43 (1H, d, *J* = 11 Hz), 4.39 (2H, s), 3.75 (1H, m), 3.66 (1H, d, *J* = 9 Hz), 3.55 (2H, t, *J* = 8.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 138.8, 138.3, 138.1, 137.9, 137.3, 133.1, 133.0, 132.5, 128.6, 128.5, 128.46, 128.42, 128.3, 128.1, 127.9, 127.83, 127.81, 127.76, 127.73, 127.6, 126.4, 87.8, 84.0, 80.4, 75.8, 75.3, 75.1, 73.5, 70.7, 62.1, 58.9. HRMS (ESI) m/z: $[M+H]^+$ Calcd. for $C_{44}H_{46}NO_4$ 652.3427; Found 652.3402.

(2R,3R,4R,5S,6S)-2-(Hydroxymethyl)-6-(4-phenylbutyl)piperidine-3,4,5-triol (**17a**): The conditions employed for the preparation of this compound were those described in General debenzylation method I, which afforded **17a** as an off-white semi solid (44 mg, quant.). $[\alpha]^{25}_{D}$ +34.7° (MeOH; *c* 1). ^{1}H NMR (D_2O, 500 MHz): δ 7.23 (5H, m), 3.80 (1H, d, *J* = 9.5 Hz), 3.46 (1H, dd, *J* = 7, 11 Hz), 3.23 (1H, t, *J* = 9 Hz), 3.12 (1H, t, *J* = 9.5 Hz), 3.00 (1H, t, *J* = 9.5 Hz), 2.55 (3H, m), 2.41 (1H, t, *J* = 7 Hz), 1.75 (1H, m), 1.57 (2H, m), 1.38 (1H, m), 1.25 (2H, m). ^{13}C{^{1}H} NMR (D_2O, 125 MHz): δ 142.9, 128.6, 125.9, 76.0, 71.1, 67.2, 59.9, 58.9, 57.2, 34.5, 30.5, 28.9, 24.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. for $C_{16}H_{26}NO_4$ 296.1862; Found 296.1882.

(2R,3R,4R,5S,6S)-2-(Hydroxymethyl)-6-(4-phenylbutyl)-1-(prop-2-yn-1-yl)piperidine-3,4,5-triol (**17b**): The conditions employed for the preparation of this compound were those described in General N-alkylation method II, which afforded N-propargylated compound as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33 (21H, m), 7.23 (1H, m), 7.15 (4H, m), 6.18 (1H, d, *J* = 9.5 Hz), 5.73 (1H, d, *J* = 9.5 Hz), 5.46 (1H, s), 4.98 (3H, m), 4.90 (1H, d, *J* = 10 Hz), 4.82 (1H, d, *J* = 11 Hz), 4.54 (3H, m), 3.90 (1H, t, *J* = 13.5 Hz, H-6b), 3.80 (1H, t, *J* = 9 Hz, H-3), 3.70 (2H, m, H-6a, H-2), 3.55 (1H, m, H-1′a), 3.11 (1H, d, *J* = 13.5 Hz, H-1′b), 3.03 (1H, t, *J* = 10 Hz, H-1),

2.36 (1H, d, *J* = 9.5 Hz, H-5), 2.24 (1H, t, *J* = 9 Hz, H-3′). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.8, 138.4, 138.2, 137.8, 137.7, 131.4, 128.5, 128.44, 128.42, 128.40, 128.0, 127.9, 127.7, 127.5, 126.3, 125.7, 120.6, 88.4, 83.5, 79.2, 75.6, 75.3, 74.8, 73.5, 69.2, 66.8, 65.1, 52.9, 44.5, 43.2. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{47}H_{48}NO_4$ 690.3583; Found 690.3612. The N-propargylated compound was debenzylated using General debenzylation method I, which gave **17b** as an off-white semi-solid (29 mg, 88% over two steps). $[\alpha]^{25}_D$ +10.3° (MeOH; *c* 1.01). ^1H NMR (CD_3OD, 500 MHz): δ 7.17 (4H, s), 7.06 (1H, s), 3.96 (1H, m), 3.82 (2H, m), 3.61 (2H, m), 3.41 (2H, m), 3.13 (1H, bs), 2.96 (1H, bs), 2.55 (2H, bs), 2.32 (2H, bs), 1.83 (2H, s), 1.63 (3H, m). 13C{1H} NMR (CD_3OD, 125 MHz): δ 147.0, 128.1, 126.5, 125.8, 78.0, 71.7, 68.0, 67.1, 66.6, 56.1, 55.4, 41.3, 39.6, 36.2, 35.6, 28.4, 24.2. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{19}H_{28}NO_4$ 334.2018; Found 334.2018.

(2R,3R,4R,5S,6S)-2-(Hydroxymethyl)-6-(4-phenylbutyl)-1-(3-phenylpropyl)piperidine-3,4,5-triol (**17c**): The conditions employed for the preparation of this compound were those described in General N-alkylation method II, which gave N-cinnamyl compound as a white solid. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.36 (2H, d, *J* = 8 Hz), 7.23 (26H, m), 7.08 (2H, d, *J* = 7 Hz), 6.75 (1H, dd, *J* = 10.5, 15.5 Hz), 6.43 (2H, m), 6.13 (2H, m), 5.69 (1H, dd, *J* = 9.5, 15.5 Hz), 4.81 (3H, m), 4.63 (1H, d, *J* = 10 Hz), 4.50 (2H, t, *J* = 12 Hz), 4.40 (2H, m), 3.63 (4H, m, H-6b, H-6a, H-4, H-3), 3.46 (2H, m, H-1′a, H-1′b), 3.34 (1H, t, *J* = 9 Hz, H-2), 3.12 (1H, t, *J* = 9 Hz, H-1), 2.48 (1H, d, *J* = 9 Hz, H-5). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.8, 138.5, 138.0, 137.7, 137.2, 136.9, 134.8, 133.9, 133.3, 132.4, 128.8, 128.7, 128.58, 128.54, 128.43, 128.41, 128.3, 128.0, 127.87, 127.84, 127.7, 127.6, 127.53, 127.50, 126.4, 126.2, 123.1, 87.2, 81.9, 78.3, 75.5, 75.3, 75.1, 73.6, 67.6, 65.0, 62.3, 50.5. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{53}H_{54}NO_4$ 768.4053; Found 768.4058. The N-cinnamyl compound was debenzylated by using General debenzylation method I, which gave **17c**

as an off-white semi-solid (41 mg, 88% over two steps). $[\alpha]^{24}_{D}$ -19.2° (MeOH; *c* 0.84). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.13 (10H, m), 3.83 (2H, bs), 3.48 (2H, bs), 2.97 (3H, m), 2.51 (4H, m), 1.95 (2H, m), 1.67-1.35 (8H, m). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 142.0, 128.6, 128.4, 128.1, 128.05, 128.02, 127.9, 126.3, 125.4, 125.3, 76.9, 71.8, 70.1, 67.5, 66.9, 60.8, 59.4, 57.3, 35.2, 35.0, 31.3, 29.6, 25.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{25}H_{36}NO_4$ 414.2644; Found 414.2644.

(2R,3R,4R,5S,6S)-2-(Hydroxymethyl)-1-octyl-6-(4-phenylbutyl)piperidine-3,4,5-triol (**17d**): The conditions employed for the preparation of this compound were those described in General N-alkylation method I, which afforded N-octyl compound as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.45 (2H, d, *J* = 7.5 Hz), 7.34 (16H, m), 7.22 (5H, s), 7.17 (2H, d, *J* = 7 Hz), 6.82 (1H, dd, *J* = 10.5, 15.5 Hz), 6.54 (1H, d, *J* = 15.5 Hz), 6.46 (1H, dd, *J* = 10.5, 15 Hz), 5.75 (1H, dd, *J* = 9.5, 15 Hz), 4.91 (3H, m), 4.70 (1H, d, *J* = 10 Hz), 4.58 (2H, m), 4.50 (2H, t, *J* = 12 Hz), 3.75 (1H, d, *J* = 10 Hz, H-6b), 3.70 (1H, t, *J* = 9 Hz, H-3), 3.53 (2H, m, H-6a, H-2), 3.40 (1H, t, *J* = 9 Hz, H-4), 3.08 (1H, t, *J* = 9 Hz, H-1), 2.87 - 2.70 (2H, m, H-5, H-1′a), 2.50 (1H, d, *J* = 9.5 Hz, H-1′b), 1.22 (10H, m), 0.98 (2H, m), 0.85 (3H, t, *J* = 7 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.9, 138.6, 138.0, 137.6, 137.3, 134.0, 133.3, 132.1, 128.8, 128.7, 128.64, 128.60, 128.45, 128.43, 128.3, 128.0, 127.9, 127.8, 127.7, 127.56, 127.54, 126.4, 87.4, 82.0, 78.5, 75.5, 75.3, 75.1, 73.6, 67.4, 65.2, 61.9, 53.4, 48.3, 31.8, 30.9, 29.3, 29.2, 27.0, 22.7, 19.8, 14.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{52}H_{62}NO_4$ 764.4679; Found 764.4683. The N-octyl compound was debenzylated using General debenzylation method I, which afforded **17d** as an off-white semi-solid (36 mg, 87% over two steps). $[\alpha]^{23}_{D}$ -3.3° (MeOH; *c* 0.75). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.21 (5H, m), 4.15 (1H, m), 3.94 (1H, m), 3.79 (1H, m), 3.65 (1H, m), 3.44 (1H, m), 3.17 (2H, m), 3.03 (1H, m), 2.77 (1H, m), 2.68 (1H, m), 2.56 (1H, m), 1.95 (1H, m), 1.75-1.65 (3H, m), 1.32 (14H, m), 0.91 (3H, m).$^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 148.7, 128.2, 128.0, 125.5, 76.5, 70.7, 66.9, 65.8,

54.6, 48.2, 34.9, 31.6, 30.9, 29.3, 29.1, 29.0, 28.7, 25.9, 25.5, 22.3, 21.2, 13.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{24}H_{42}NO_4$ 408.3114; Found 408.3108.

(2R,3R,4R,5S,6S)-1-Hexadecyl-2-(hydroxymethyl)-6-(4-phenylbutyl)piperidine-3,4,5-triol (**17e**): The conditions employed for the preparation of this compound were those described in General N-alkylation method I, which gave N-cetyl compound as a white solid. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.24 (25H, m), 6.73 (1H, dd, *J* = 10.5, 15.5 Hz), 6.40 (2H, m), 5.65 (1H, dd, *J* = 9.5, 15 Hz), 4.81 (3H, m), 4.61 (1H, d, *J* = 10 Hz), 4.48 (2H, d, *J* = 10 Hz), 4.40 (2H, t, *J* = 13 Hz), 3.66 (1H, d, *J* = 10 Hz, H-6a), 3.61 (1H, t, *J* = 9 Hz, H-3), 3.44 (2H, m, H-6b, H-2), 3.31 (1H, t, *J* = 9 Hz, H-4), 2.99 (1H, t, *J* = 9 Hz, H-1), 2.70 (2H, m, H-5, H-1′b), 2.41 (1H, d, *J* = 9.5 Hz, H-1′a), 2.26 (1H, t, *J* = 7.5 Hz), 1.56 (1H, m), 1.18 (24H, m), 0.89 (1H, m), 0.81 (3H, t, *J* = 6.5 Hz). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 138.0, 137.7, 137.3, 134.1, 133.1, 132.1, 128.9, 128.69, 128.66, 128.61, 128.47, 128.45, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 126.4, 87.4, 81.9, 78.4, 75.5, 75.3, 75.1, 73.6, 67.4, 65.2, 61.9, 48.2, 33.9, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 29.1, 27.0, 24.8, 22.7, 19.7, 14.1. HRMS (ESI) m/z: $[M+Na]^+$ Calcd $C_{60}H_{78}NO_4$ 876.5931; Found 876.5925. The N-cetyl compound was debenzylated using General debenzylation method I, which gave **17e** as an off-white semi-solid (34 mg, 92% over two steps). $[\alpha]^{23}_D$ -12.0° (MeOH; *c* 0.72). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.20 (5H, m), 4.13 (1H, m), 3.85 (2H, m), 3.62 (2H, m), 3.43 (2H, m), 3.15 (2H, m), 2.61 (2H, m), 1.98 (1H, m), 1.62 (6H, m), 1.28 (27H, m), 0.89 (3H, s). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 145.8, 132.1, 132.0, 129.5, 80.5, 74.6, 70.9, 69.8, 68.6, 58.9, 57.6, 52.9, 50.7, 38.9, 35.6, 35.0, 33.4, 33.4, 33.2, 33.0, 32.8, 31.6, 30.0, 29.6, 26.8, 26.3, 17.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{32}H_{58}NO_4$ 520.4366; Found 520.4357.

***1,3,4-tris(benzyloxy)-5-oxopentan-2-yl formate (19)*:** To a solution of compound **18** (40.0 g, 96.1 mmol, 1 equiv) in THF/H_2O (2:1, 750 mL) was added a pinch of OsO_4 and $NaIO_4$ (71.6 g, 336.3

mmol, 3.5 equiv) and the resulting mixture was stirred at room temperature under nitrogen atmosphere. After 6 h, the reaction mixture was quenched with saturated aqueous $NaHSO_3$, washed with water (2 × 400 mL), and extracted with EtOAc (2 × 300 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc (85/15 to 80/20) afforded aldehyde **19** (32 g, 74 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.60 (d, *J* = 1.5 Hz, 1H), 7.99 (s, 1H), 7.23–7.35 (m, 15H), 5.39 (q, *J* = 5 Hz, 1H), 4.52−4.66 (m, 4H), 4.43 (d, *J* = 11.5 Hz, 2H), 4.12 (t, *J* = 4.5 Hz, 1H), 3.97 (dd, *J* = 1.5, 4.5 Hz, 1H), 3.61–3.68 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.9, 160.3, 137.3, 137.1, 136.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 82.5, 77.5, 74.2, 73.2, 73.0, 71.7, 67.0; HR-ESI-MS $[M + MeOH + Na]^+$ $C_{28}H_{32}O_7Na$ calcd. for m/z 503.2046, found 503.2059.

1,3,4-tris(benzyloxy)hex-5-en-2-ol (20): To a solution of aldehyde **19** (9.2 g) in MeOH was added excess NaOMe in MeOH (3 mL) at 0 °C. After 10 min, the reaction mixture was diluted with MeOH (100 mL) and concentrated under reduced pressure. Purification by column chromatography using hexane/EtOAc (80/20) afforded hemiacetal **19a** (7.8 g, 90 %). HR-ESI-MS $[M + Na]^+$ $C_{26}H_{28}O_5Na$ calcd. for m/z 443.1834, found 443.1802.

A solution of methyltriphenylphosphonium bromide (19.94 g, 55.8 mmol, 3.5 equiv) in dry THF (70 mL) was stirred for 10 min at room temperature, and then a 2 M solution of *n*-BuLi in cyclohexane (32 mL, 55.8 mmol, 3.5 equiv) was slowly added at 0 °C under nitrogen atmosphere. The resulting yellow colored solution was stirred for 25 min at the same temperature. To the above reaction mixture a solution of hemiacetal **19a** (6.7 g, 15.9 mmol, 1 equiv) in dry THF (40 mL) was added at 0 °C. The resulting mixture was stirred for 30 min at 60 °C. After completion of the starting material as indicated by TLC, the reaction mixture was allowed to attain room temperature and quenched with saturated aqueous NH_4Cl (50 mL), extracted with Et_2O (2 × 200 mL). The

organic layer was washed with excess water, dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using hexane/EtOAc 95/5 affording compound **20** (3.64 g, 55 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.23–7.35 (m, 15H), 5.84–5.82 (m, 1H), 5.32–5.36 (m, 2H), 4.61–4.69 (m, 2H), 4.36−4.51 (m, 4H), 4.04–4.08 (m, 2H), 3.60 (dd, *J* = 3, 6 Hz, 1H), 3.50 (d, *J* = 5.5 Hz, 2H), 2.87 (d, *J* = 6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.1, 138.0, 135.5, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.65, 127.63, 126.9, 119.4, 80.5, 80.0, 73.9, 73.3, 71.0, 70.7, 69.7; HR-ESI-MS $[M + Na]^+$ $C_{27}H_{30}O_4Na$ calcd for m/z 441.2042, found 441.2027.

***1,3,4-tris(benzyloxy)-2-(4-methoxybenzyl)oxy)-hex-5-ene (21)*:** To a solution of compound **20** (3.34 g, 8.0 mmol, 1 equiv) in DMF (30 mL) at 0 °C was added NaH 60% suspension in mineral oil (736 mg, 18.4 mmol, 2.3 equiv), and the resulting mixture was stirred for 30 min. At the same temperature 4-methoxybenzyl chloride (1.85 mL, 13.2 mmol, 1.7 equiv) was added dropwise and then the reaction mixture was warmed to room temperature slowly. After 2 h, the reaction mixture was quenched with ice water (250 mL) at 0 °C and extracted with EtOAc (2 × 300 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc 95/5 to 90/10 afford compound **21** (4.27 g, 98 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25–7.26 (m, 15H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 9 Hz, 2H), 5.90–5.97 (m, 1H), 5.35 (m, 2H), 4.71 (d, *J* = 11 Hz, 1H), 4.48–4.57 (m, 4H), 4.41 (s, 1H), 4.19 (d, *J* = 11.5 Hz, 1H), 4.05 (t, *J* = 6.5 Hz, 1H), 3.86–3.90 (m, 1H), 3.75 (s, 3H), 3.72 (dd, *J* = 4, 6.5 Hz, 1H), 3.54 (dd, *J* = 1.5, 5.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1, 138.6, 138.2, 136.2, 130.9, 129.6, 129.4, 128.34, 128.32, 128.1, 127.7, 127.6, 127.5, 127.5, 127.4, 119.3, 113.8, 113.64, 81.2, 80.1, 77.4, 74.6, 73.3, 72.9, 70.0, 55.3, 55.2; HR-ESI-MS $[M + Na]^+$ $C_{35}H_{38}O_5Na$ calcd. for m/z 561.2617, found 561.2630.

2,3,5-tris(benzyloxy)-4-((4-methoxybenzyl)oxy)pentanal (**22**): To a solution of compound **21** (2.72 g, 2.72 mmol, 1 equiv) in acetone:H_2O (4:1, 20 mL) was added NMO (0.541 g, 4.64 mmol, 1.7 equiv) and a catalytic amount of OsO_4. The reaction mixture was stirred for 8 h at room temperature under nitrogen atmosphere. The reaction mixture was then quenched with saturated aqueous $NaHSO_3$, washed with water (2 × 300 mL), and extracted with EtOAc (2 × 50 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc 50/50 afforded the diol **21a** as a colorless viscous solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.21−7.35 (m, 17H), 6.83 (d, *J* = 8.5 Hz, 2H), 4.64−4.73 (m, 8H), 3.98 (q, *J* = 3, 1H), 3.82–3.86 (m, 2H), 3.77 (s, 3H), 3.62–3.76 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3, 149.3, 138.1, 138.0, 137.8, 136.4, 130.0, 129.8, 129.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.85, 127.83, 127.7, 123.91, 113.8, 80.2, 80.1, 78.6, 74.3, 73.4, 73.2, 72.9, 71.3, 70.1, 63.8, 55.2; HR-ESI-MS $[M + Na]^+$ $C_{35}H_{40}O_7Na$ calcd. for m/z 595.2672, found 595.2683.

To a solution of the diol **21a** (2.69 g, 4.7 mmol, 1 equiv) in THF (45 mL) was added a solution of aqueous $NaIO_4$ (2.0 g, 9.405 mmol, 2 equiv, 5 mL) at 0 °C, and the resulting mixture was stirred at room temperature under nitrogen atmosphere. After 3 h, the reaction mixture was quenched with brine (20 mL) and extracted with EtOAc (2 × 100 mL). The organic extracts were washed with water (2 × 300 mL), dried over anhydrous Na_2SO_4, concentrated and passed through silica plug resulting in aldehyde **22** (2.23 g) as a colorless viscous solid (81% over 2 steps). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.64 (d, *J* = 1.5 Hz, 1H), 7.24–7.32 (m, 15H), 7.18 (d, *J* = 9 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 4.41–4.64 (m, 8H), 4.02 (dd, *J* = 1.5, 3.5 Hz, 1H), 4.0 (t, *J* = 4 Hz, 1H), 3.84 (q, *J* = 5 Hz, 1H), 3.77 (s, 3H), 3.70 (d, *J* = 5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 201.4, 159.2, 138.1, 137.7, 137.3, 130.1, 129.7, 128.4, 128.34, 128.30, 128.1, 127.9, 127.83, 127.81,

127.7, 127.6, 113.7, 83.8, 79.9, 77.8, 73.6, 73.2, 72.7, 72.6, 69.9, 55.2; HR-ESI-MS [M + MeOH + Na]$^+$ $C_{35}H_{40}O_7Na$ calcd for m/z 595.2672, found 595.2682.

Dimethyl (3,4,6-tris(benzyloxy)-5-((4-methoxybenzyl)oxy)-2-oxohexyl)phosphonate (**23**)**:** To a solution of dimethyl methylphosphonate (0.395 mL, 3.5 mmol, 3 equiv) in THF (5 mL) at −78 °C was slowly added *n*-BuLi (2.07 mL, 4.1 mmol, 3.5 equiv), and the resulting yellow colored solution was stirred for 20 min at the same temperature under nitrogen atmosphere. A solution of compound **22** (640 mg, 1.2 mmol, 1 equiv) in THF (6 mL) was added to the above mixture and the resulting mixture was stirred for 1 h at −78 °C. The reaction mixture was then quenched with saturated aqueous NH_4Cl (20 mL) and extracted with EtOAc (2 × 70 mL). The organic extracts were washed with water (3 × 100 mL), dried over anhydrous Na_2SO_4, and concentrated. Purification by column chromatography using hexane/EtOAc 60/40 to 50/50 afforded the β-hydroxy phosphonate **22a**. HR-ESI-MS [M + Na]$^+$ $C_{37}H_{45}O_9PNa$ calcd. for m/z 687.2699, found 687.2711.

To a solution of the β-hydroxy phosphonate **22a** (410 g, 0.6 mmol, 1 equiv) in CH_2Cl_2 (15 mL) was added Dess−Martin periodinane (523 mg, 1.2 mmol, 2 equiv), and the resulting mixture was stirred at room temperature under nitrogen atmosphere. After 1 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ (75 mL) and saturated aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic extracts were washed with water (2 × 100 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc 60/40 afforded compound **23** (330 mg, 42% over two steps) as a pale yellow viscous solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.24–7.29 (m, 15H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 9 Hz, 2H), 4.40–4.64 (m, 7H), 4.28 (d, *J* = 4 Hz, 1H), 4.04 (dd, *J* = 4, 5 Hz, 1H), 3.80 (q, *J* = 5 Hz, 1H), 3.77 (s, 3H), 3.64–3.69 (m, 8H), 3.42 (dd, *J* = 14.5, 20 Hz, 1H), 3.02 (dd, *J* = 15,

22 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.8, 159.2, 138.1, 137.7, 137.5, 130.2, 129.7, 128.3, 128.0, 127.9, 127.74, 127.71, 127.6, 113.6, 83.8, 80.7, 78.1, 74.0, 73.1, 72.9, 72.7, 69.8, 55.2, 52.8, 38.3, 37.3; HR-ESI-MS $[M + Na]^+$ $C_{37}H_{43}O_9PNa$ calcd. for m/z 685.2542, found 685.2549.

(1E,3E)-6,7,9-tris(benzyloxy)-8-((4-methoxybenzyl)oxy)-1-phenylnona-1,3-dien-5-one (**24**)**:** To a solution of compound **23** (243 mg, 0.4 mmol, 1 equiv) in *i*-PrOH (5 mL) was added Cs_2CO_3 (78 mg, 0.7 mmol, 2 equiv) and the resulting mixture was stirred at room temperature under nitrogen atmosphere. After 30 min, cinnamaldehyde (139 µL, 1.1 mmol, 3 equiv) was added directly to the reaction mixture, and the reaction mixture was stirred at room temperature under nitrogen atmosphere. After the reaction mixture was stirred overnight, solvent was evaporated and the crude mixture was directly subjected to purification by column chromatography using hexane/EtOAc 95/5 which afforded compound **24** (180 mg, 74%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.22–7.49 (m, 24H), 6.71–6.91 (m, 4H), 4.33–4.61 (m, 7H), 4.07 (dd, *J* = 4.5, 5.5 Hz, 1H), 3.93 (q, *J* = 4.5, 1H), 3.77 (s, 3H), 3.67– 3.72 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.9, 159.1, 143.0, 142.0, 138.2, 137.5, 136.1, 130.8, 129.2, 128.4, 128.36, 128.34, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 127.0, 126.5, 113.6, 82.9, 80.2, 77.7, 74.3, 73.2, 72.8, 72.2, 69.8, 55.2; HR-ESI-MS $[M + Na]^+$ $C_{44}H_{44}O_6Na$ calcd. for m/z 691.3036, found 691.2923.

3-(benzyloxy)-5-((benzyloxy)methyl)-2-((1E,3E)-4-phenylbuta-1,3-dien-1-yl)furan (**25**): To a solution of compound **24** (260 mg, 0.4 mmol, 1 equiv) in CH_2Cl_2/H_2O 10:1 (10 mL) was added DDQ (223 mg, 0.8 mmol, 2 equiv) at room temperature and the resulting brownish reaction mixture was stirred at room temperature under nitrogen atmosphere. After 3 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic extracts were washed with water (2 × 200 mL), dried over anhydrous Na_2SO_4, and concentrated. Purification by column chromatography using hexane/ EtOAc 95/5 to 90/10 afforded

compound **25** (87 mg, 53%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.20–7.36 (m, 15H), 7.09–7.16 (m, 1H), 6.43–6.90 (m, 3H), 6.16 (s, 1H), 4.94 (s, 2H), 4.47 (s, 2H), 4.32 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.8, 145.3, 138.8, 137.7, 137.7, 136.8, 131.3, 129.6, 128.6, 128.4, 128.1, 128.0, 127.8, 127.6, 127.5, 127.1, 126.2, 126.0, 125.0, 117.7, 103.7, 73.7, 72.1, 64.4; HR-ESI-MS $[M + Na]^+$ $C_{29}H_{26}O_3Na$ calcd. for m/z 445.1780, found 445.1714.

5-(hydroxymethyl)-2-(4-phenylbutyl)-3(2H)- furanone (**26**): To a solution of compound **25** (40.0 mg, 0.02 mmol, 1 equiv) in EtOH (3 mL) was added 10% Pd/C (23.0 mg) and one drop of concentrated HCl. The reaction mixture was purged with H_2 gas for 1 minute and stirred at room temperature under H_2 atmosphere (balloon) for 1 h. The reaction mixture was then diluted with MeOH (10 mL) and filtered through a celite pad, washed with MeOH (15 mL) and the combined filtrates were concentrated. The resulting residue was purified by column chromatography using hexane/EtOAc (1:1) affording the final product **26** (12.6 mg, 54%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.08–7.22 (m, 5H, Ph), 5.62 (s, 1H, H-4), 4.42 (s, 2H, H-6), 4.41 (m, 1H, H-2), 2.54 (t, *J* = 7.5 Hz, 2H, H-4′), 1.87–1.97 (m, 2H, H-1′,2′); 1.65 (m, 1H, H-1′), 1.57–1.59 (m, 2H, H-3′), 1.40–1.46 (m, 1H, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.3, 191.4, 142.2, 128.6, 128.5, 128.4, 128.3, 125.7, 102.8, 86.6, 59.8, 35.6, 31.0, 30.9, 24.3; HR-ESI-MS $[M + Na]^+$ $C_{15}H_{18}O_3Na$ calcd. for m/z 269.1154, found 269.1112.

***(E)-1,3,4-tris(benzyloxy)-2-((4-methoxybenzyl)oxy)tetradeC-6-en-5-one (IV)*:** To a solution of compound **23** (220 mg, 0.33 mmol, 1 equiv) in THF (8 mL) was added $Ba(OH)_2.8H_2O$ (131 mg, 0.41 mmol, 1.25 equiv) and the resulting mixture was stirred at room temperature under nitrogen atmosphere. After 30 min, octanal (259 μL, 1.66 mmol, 2 equiv) was added directly and the reaction mixture was stirred at room temperature under nitrogen atmosphere. After the reaction mixture was stirred overnight, solvent was evaporated and the crude mixture was directly subjected

to purification by column chromatography using hexane/EtOAc 95/5 which afforded compound **IV** (153 mg, 69%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.19–7.34 (m, 19H), 6.96 (dt, *J* = 7, 15.5 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.52 (d, *J* = 15.5 Hz, 1H), 4.42–4.55 (m, 7H), 4.28 (d, *J* = 5.5 Hz, 1H), 4.27 (s, 1H), 4.00 (dd, *J* = 4, 7 Hz 1H), 3.88 (q, *J* = 5 Hz, 3H), 3.77 (s, 3H), 3.63 (m, 2H), 2.13 (m, 2H), 1.38 (m, 2H), 1.24–1.31 (m, 8H), 0.89 (t, *J* = 7 Hz, 3H); HR-ESI-MS $[M + Na]^+$ $C_{43}H_{52}O_6Na$ calcd. for m/z 687.3662, found 687.3564.

***(2R,3S,4R,5R)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-nonyltetrahydrofuran (V)*:** To a solution of compound **IV** (153 mg, 0.23 mmol, 1 equiv) in CH_2Cl_2/H_2O 10:1 (10 mL) was added DDQ (105 mg, 0.46 mmol, 2 equiv) at room temperature and the resulting brownish reaction mixture was stirred at room temperature under nitrogen atmosphere. After 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic extracts were washed with water (2 × 100 mL), dried over anhydrous Na_2SO_4, and concentrated. Purification by column chromatography using hexane/ EtOAc 80/20 afforded the desired hemiketal (94 mg, 74%). HR-ESI-MS $[M + Na]^+$ $C_{35}H_{44}O_5Na$ calcd. for m/z 567.3086, found 567.3074.

To a solution of hemiketal (27 mg, 0.05 mmol, 1 equiv) in CH_3CN (5 mL) were added Et_3SiH (79 μL, 0.49 mmol, 10 equiv) and TMSOTf (27 μL, 0.15 mmol, 3 equiv) at 0 °C. After stirring for 10 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (2 × 20 mL). The organic extracts were washed with water (2 x 50 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/ EtOAc 90/10 afforded compound **V** (20 mg, 76%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.18–7.25 (m, 15H), 4.36–4.64 (m, 6H), 4.10–4.15 (m, 1H), 4.05 (t, *J* = 4.5 Hz, 1H), 3.92–3.96 (m, 1H), 3.68 (dd, *J* = 6, 9.5 Hz, 1H), 3.60 (dd, *J* = 3.5, 10 Hz, 1H), 3.56 (dd, *J* = 4, 7 Hz, 1H), 1.17–1.48

(m, 16H), 0.80 (t, *J* = 6.5 Hz, 3H); HR-ESI-MS $[M + Na]^+$ $C_{35}H_{46}O_4Na$ calcd. for m/z 553.3294, found 553.3295.

(2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidine (**28**): To a solution of lactam **27** (4.8 g, 9.9 mmol, 1 equiv) in dry Et_2O (40 mL) was added $NaBH_4$ (2.0 g, 53.6 mmol, 6 equiv) and $BF_3.Et_2O$ (2.5 mL, 8.9 mmol, 1 equiv) at 0 °C and then allowed to stir for 2 h under argon atmosphere. After formation of the polar product, as indicated by TLC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with EtOAc (2 × 50 mL). The organic extract was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using hexane/EtOAc (7:3) to afford compound **28** (3.32 g, 71%) as a white solid. ^1H NMR (CD_3OD, 500 MHz): δ 7.32 (18H, m), 7.22 (2H, d, *J* = 6 Hz), 5.00 (1H, d, *J* = 11 Hz), 4.87 (2H, t, *J* = 11 Hz), 4.71 (2H, q, *J* = 9.5 Hz), 4.48 (3H, m), 3.69 (1H, m), 3.55 (3H, m), 3.38 (1H, t, *J* = 9 Hz), 3.27 (1H, dd, *J* = 4.5, 12 Hz), 2.75 (1H, m), 2.53 (1H, t, *J* = 12 Hz). $^{13}C\{^1H\}$ NMR (CD_3OD, 125 MHz): δ 138.9, 138.5, 138.4, 138.0, 128.47, 128.43, 128.40, 128.06, 128.02, 127.9, 127.88, 127.84, 127.7, 127.5, 87.3, 80.7, 80.1, 75.7, 75.2, 73.4, 72.8, 70.3, 59.8, 48.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{34}H_{38}NO_4$ 524.2801; Found 524.2812.

(3R,4S,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidin-2-one (**29b**): The conditions employed for the preparation of this compound were those described in General N-alkylation method II, which gave **29b** as a colorless viscous liquid (94 mg, 87%). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.35 (2H, d, *J* = 7 Hz), 7.20 (18H, m), 5.10 (1H, d, *J* = 11 Hz), 4.78 (1H, d, *J* = 11 Hz), 4.68 (1H, d, *J* = 11 Hz), 4.60 (3H, m), 4.29-4.44 (3H, m, H-5, CH_2Ph), 3.99 (1H, d, *J* = 9.00 Hz, H-4), 3.77 (3H, m, H-3, H-1′a, H-1′b), 3.67 (1H, s, H-2), 3.59 (1H, m, H-6a), 3.44 (1H, d, *J* = 10 Hz, H-6b), 2.10 (1H, s, H-3′). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 169.7, 138.2, 138.1, 137.8, 137.5, 128.5, 128.4, 128.4, 128.3, 128.04, 128.01, 127.9, 127.7, 127.7, 81.4,

78.6, 78.4, 74.8, 74.5, 73.5, 73.2, 72.5, 67.1, 33.8, 29.7. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{37}H_{38}NO_5$ 576.2750; Found 576.2762.

(3R,4S,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-1-cinnamylpiperidin-2-one (**29c**): The conditions employed for the preparation of this compound were those described in General N-alkylation method II, which gave **29c** as a white solid (108 mg, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.36 (2H, d, *J* = 7 Hz), 7.18 (21H, m), 7.07 (2H, d, *J* = 7 Hz), 6.40 (1H, d, *J* = 15.5 Hz), 6.05 (1H, m), 5.09 (1H, d, *J* = 11 Hz), 4.73 (1H, d, *J* = 11 Hz), 4.57 (4H, m), 4.35 (3H, m, H-5, CH_2Ph), 4.04 (1H, d, *J* = 8 Hz, H-4), 3.80 (2H, m, H-3, H-1′a), 3.67 (1H, dd, *J* = 8, 15.5 Hz, H-1′b), 3.52 (2H, m, H-6a, H-2), 3.38 (1H, dd, *J* = 4, 9.5 Hz, H-6b). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 169.6, 138.2, 138.2, 137.7, 137.5, 136.5, 133.1, 128.6, 128.5, 128.4, 128.4, 128.3, 128.0, 128.0, 127.9, 127.8, 127.75, 127.73, 126.5, 124.6, 82.0, 78.5, 77.1, 74.5, 73.9, 73.3, 72.6, 68.1, 59.6, 47.5. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{43}H_{43}NNaO_5$ 676.3039; Found 676.3066.

(3R,4S,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-1-octylpiperidin-2-one (**29d**): The conditions employed for the preparation of this compound were those described in General N-alkylation method II, which gave **29d** as a white solid (41 mg, 51%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (2H, d, *J* = 7.5 Hz), 7.14-7.25 (18H, m), 5.07 (1H, d, *J* = 11 Hz), 4.75 (1H, d, *J* = 11 Hz), 4.57 (3H, m), 4.44 (1H, d, *J* = 11.5 Hz), 4.34 (2H, m), 3.98 (1H, d, *J* = 9 Hz, H-4), 3.80 (1H, m, H-5), 3.67-3.77 (2H, m, H-3, H-1′a), 3.44 (2H, m, H-6b, H-2), 3.34 (1H, m, H-6b), 2.81 (1H, m, H-1′b), 1.44 (2H, m), 1.18 (10H, m), 0.79 (3H, t, *J* = 6.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 169.4, 138.26, 138.24, 137.9, 137.5, 128.5, 128.4, 128.3, 127.99, 127.94, 127.87, 127.80, 127.7, 12767, 127.61, 81.9, 78.3, 77.5, 74.4, 74.0, 73.3, 72.6, 68.3, 60.5, 45.8, 31.9, 31.8, 29.7, 29.4, 29.2, 27.5, 26.9, 22.7, 22.6, 14.13, 14.12. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{42}H_{52}NO_5$ 650.3845; Found 650.3852.

(3R,4S,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-1-hexadecylpiperidin-2-one (**29e**): The conditions employed for the preparation of this compound were those described in General N-alkylation method II, which gave **29e** as a white solid (51 mg, 58%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (2H, d, *J* = 7.5 Hz), 7.14-7.26 (18H, m), 5.07 (1H, d, *J* = 11.5 Hz), 4.76 (1H, d, *J* = 11.5 Hz), 4.57 (3H, m), 4.44 (1H, d, *J* = 11.5 Hz), 4.34 (2H, m), 3.98 (1H, d, *J* = 9 Hz, H-4), 3.80 (1H, m, H-5), 3.65-3.78 (2H, m, H-3, H-1′a), 3.44 (2H, m, H-6b, H-2), 3.34 (1H, m, H-6b), 2.81 (1H, m, H-1′b), 1.44 (2H, m), 1.18 (26H, m), 0.80 (3H, t, *J* = 6 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 169.4, 138.26, 138.24, 137.9, 137.5, 128.5, 128.4, 128.3, 128.2, 127.99, 127.94, 127.9, 127.79, 127.75, 127.67, 127.60, 81.9, 78.3, 77.5, 74.4, 74.0, 73.3, 72.6, 68.3, 60.5, 45.8, 31.9, 29.7, 29.67, 29.64, 29.5, 29.4, 29.3, 27.5, 26.9, 22.7, 22.7, 14.1. HRMS (ESI) m/z: [M+H]+ Calcd. $C_{50}H_{68}NO_5$ 762.5097; Found 762.5109.

(3R,4S,5R,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-propylpiperidin-2-one (**30b**): The conditions employed for the preparation of this compound were those described in General debenzylation method II, which afforded **30b** as an off-white semi-solid (20 mg, quant.). $[\alpha]^{23}_{D}$ +34.6° (MeOH; *c* 0.4). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.27-4.07 (2H, m), 3.98-3.44 (5H, m), 2.96 (1H, m), 1.63 (2H, m), 0.93 (3H, s). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 171.6, 69.5, 68.6, 64.2, 60.4, 59.9, 53.5, 19.9, 10.1. HRMS (ESI) m/z: [M+Na]+ Calcd. $C_9H_{17}NNaO_5$ 242.1004; Found 242.0998.

(3R,4S,5R,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-(3-phenylpropyl)piperidin-2-one (**30c**): The conditions employed for the preparation of this compound were those described in General debenzylation method II, which afforded **30c** as an off-white semi-solid (31 mg, quant.). $[\alpha]^{23}_{D}$ +16.9° (MeOH; *c* 0.36). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.19 (5H, m), 4.46-3.56 (7H, m), 3.04 (1H, s), 2.63 (2H, s), 1.90 (2H, m). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 170.4, 141.6, 128.0,

125.5, 72.7, 69.6, 66.2, 64.3, 60.9, 60.5, 60.3, 45.4, 32.6, 28.5. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{15}H_{22}NO_5$ 296.1498; Found 296.1491.

(3R,4S,5R,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-octylpiperidin-2-one (**30d**): The conditions employed for the preparation of this compound were those described in General debenzylation method II, which afforded **30d** as an off-white semi-solid (17 mg, quant.). $[\alpha]^{23}_D$ +16.4° (MeOH; *c* 0.5). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.27-4.07 (2H, m), 3.90-3.45 (5H, m), 3.00 (1H, s), 1.59 (2H, m), 1.32 (10H, m), 0.90 (3H, s). $^{13}C\{^1H\}$ NMR (CD_3OD, 125 MHz): δ 171.5, 69.4, 66.1, 64.0, 60.3, 56.5, 45.4, 31.6, 29.1, 29.0, 26.5, 22.3, 13.1. HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{14}H_{27}NNaO_5$ 312.1787; Found 312.1786.

(3R,4S,5R,6R)-1-Hexadecyl-3,4,5-trihydroxy-6-(hydroxymethyl)piperidin-2-one (**30e**): The conditions employed for the preparation of this compound were those described in General debenzylation method II, which afforded **30e** as an off-white semi-solid (26 mg, quant.). $[\alpha]^{23}_D$ +3.3° (MeOH; *c* 0.4). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.30-4.07 (2H, m), 3.93-3.55 (5H, m), 3.02 (1H, s), 1.60 (2H, m), 1.29 (26H, m), 0.91 (3H, m). $^{13}C\{^1H\}$ NMR (CD_3OD, 125 MHz): δ 171.4, 69.6, 69.3, 64.0, 60.4, 56.1, 45.4, 31.6, 29.4, 29.3, 29.3, 29.3, 29.1, 29.0, 26.6, 26.5, 22.3, 13.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{22}H_{44}NO_5$ 402.3219; Found 402.3211.

(2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidine (**31b**): The conditions employed for the preparation of this compound were those described in General N-alkylation method I, which afforded **31b** as a colorless viscous liquid (84 mg, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (18H, m), 7.17 (2H, d, *J* = 7 Hz), 5.03 (1H, d, *J* = 11 Hz), 4.93 (1H, d, *J* = 11 Hz), 4.88 (1H, d, *J* = 11 Hz), 4.73 (2H, s), 4.60 (1H, d, *J* = 12 Hz), 4.48 (1H, d, *J* = 12 Hz), 4.41 (1H, d, *J* = 11 Hz), 3.78 (3H, m, H-2, H-1′a, H-6a), 3.70 (1H, t, *J* = 9 Hz, H-4), 3.63 (1H, d, *J* = 11 Hz, H-6b), 3.55 (1H, t, *J* = 9 Hz, H-3), 3.44 (1H, m, H-1′b), 3.02 (1H, dd, *J* = 4.5, 10.5 Hz,

H-1), 2.60 (1H, t, *J* = 11 Hz, H-1), 2.50 (1H, d, *J* = 10 Hz, H-5), 2.26 (1H, s, H-3′). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 139.0, 138.6, 138.5, 137.7, 128.6, 128.5, 128.1, 128.47, 128.43, 128.05, 128.01, 127.9, 127.8, 127.7, 127.6, 127.6, 87.2, 78.2, 78.2, 75.5, 75.2, 74.2, 73.6, 72.8, 64.8, 62.2, 55.0, 42.3. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{37}H_{40}NO_4$ 562.2957; Found 562.2952.

(2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-cinnamylpiperidine (**31c**): The conditions employed for the preparation of this compound were those described in General N-alkylation method I, which afforded **31c** as a white solid (115 mg, 62%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22 (23H, m). 7.06 (2H, d, *J* = 7 Hz), 6.30 (1H, d, *J* = 16 Hz), 6.18 (1H, m), 4.88 (1H, d, *J* = 11 Hz), 4.81 (1H, d, *J* = 11 Hz), 4.73 (1H, d, *J* = 11 Hz), 4.58 (2H, q, *J* = 12 Hz), 4.42 (2H, q, *J* = 12 Hz), 4.34 (1H, d, *J* = 10.5 Hz), 3.60 (3H, m, H-2, H-6a, H-6b), 3.54 (1H, t, *J* = 9.5 Hz, H-4), 3.46 (1H, dd, *J* = 5, 14 Hz, H-1b), 3.40 (1H, t, *J* = 9 Hz, H-3), 3.22 (1H, m, H-5), 3.09 (1H, dd, *J* = 4, 11 Hz, H-1a), 2.27 (1H, d, *J* = 9.5 Hz), 2.16 (1H, t, *J* = 11 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.6, 138.5, 137.8, 136.9, 133.5, 128.67, 128.64, 128.5, 128.4, 128.3, 128.0, 127.92, 127.90, 127.8, 127.66, 127.63, 127.5, 126.3, 125.0, 87.3, 78.6, 78.4, 75.4, 75.2, 73.5, 72.8, 65.2, 64.1, 55.0, 54.9. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{43}H_{46}NO_4$ 640.3427; Found 640.3439.

(2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-octylpiperidine (**31d**): The conditions employed for the preparation of this compound were those described in General N-alkylation method I, which afforded **31d** as a white solid. (92 mg, 68%) ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.20 (18H, m), 7.05 (1H, d, *J* = 7 Hz), 4.88 (1H, d, *J* = 11 Hz), 4.80 (1H, d, *J* = 11 Hz), 4.74 (1H, d, *J* = 11 Hz), 4.59 (2H, q, *J* = 10 Hz), 4.36 (3H, m), 3.58 (2H, m, H-4, H-6a), 3.52 (1H, t, *J* = 9 Hz, H-2), 3.46 (1H, d, *J* = 10 Hz, H-6b), 3.38 (1H, t, *J* = 9 Hz, H-3), 3.01 (1H, dd, *J* = 4.5, 11 Hz, H-1), 2.58 (1H, m, H-1), 2.48 (1H, m, H-5), 2.22 (1H, d, *J* = 9.5 Hz), 2.15 (1H, t, *J* = 11Hz), 1.31 (1H, m), 1.19 (10H, m), 1.07 (1H, m), 0.81 (3H, t, *J* = 7 Hz). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$,

125 MHz): δ 139.1, 138.7, 138.6, 137.9, 128.5, 128.48, 128.45, 128.3, 127.95, 127.91, 127.7, 127.6, 127.5, 87.4, 78.7, 78.6, 75.3, 75.2, 73.5, 72.8, 65.3, 63.7, 54.5, 52.5, 31.9, 29.5, 29.3, 27.6, 23.6, 22.7, 14.2. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{42}H_{54}NO_4$ 636.4053; Found 636.3998.

(2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-hexadecylpiperidine (**31e**): The conditions employed for the preparation of this compound were those described in General N-alkylation method I, which afforded **31e** as a white solid (106 mg, 74%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22 (18H, m), 7.06 (2H, d, *J* = 7 Hz), 4.88 (1H, d, *J* = 11 Hz), 4.80 (1H, d, *J* = 11 Hz), 4.74 (1H, d, *J* = 11 Hz), 4.60 (2H, q, *J* = 11.5 Hz), 4.37 (2H, m), 4.35 (1H, d, *J* = 10.5 Hz), 3.59 (2H, m, H-4, H-6a), 3.52 (1H, t, *J* = 9 Hz, H-2), 3.46 (1H, d, *J* = 10 Hz, H-6b), 3.38 (1H, t, *J* = 9 Hz, H-3), 3.02 (1H, dd, *J* = 4, 11 Hz, H-1a), 2.59 (1H, m, H-1b), 2.49 (1H, m, H-5), 2.22 (1H, d, *J* = 9.5 Hz), 2.15 (1H, t, *J* = 11 Hz), 1.31 (1H, m), 1.19 (25H, m), 1.08 (2H, m), 0.81 (3H, t, *J* = 7 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 139.1, 138.6, 138.5, 137.8, 128.5, 128.4, 128.33, 128.30, 127.8, 127.6, 127.5, 127.4, 87.4, 78.6, 78.6, 75.3, 75.2, 73.5, 72.8, 65.3, 63.7, 54.5, 52.4, 31.9, 29.7, 29.7, 29.7, 29.6, 29.6, 29.4, 27.5, 23.5, 22.7, 14.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{50}H_{70}NO_4$ 748.5305; Found 748.5291.

(2R,3R,4R,5S)-2-(Hydroxymethyl)-1-propylpiperidine-3,4,5-triol (**32b**): The conditions employed for the preparation of this compound were those described in General debenzylation method I, which afforded **32b** as an off-white semi-solid (21 mg, quant.). $[\alpha]^{23}_D$ -5.5° (MeOH; *c* 0.25). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.09 (1H, s), 3.90 (2H, m), 3.77 (1H, m), 3.59 (2H, m), 3.42 (2H, m), 3.04-2.83 (2H, m), 1.78 (2H, m), 1.02 (3H, s). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 76.9, 76.7, 68.1, 67.5, 67.2, 66.4, 66.2, 60.6, 57.8, 54.7, 53.8, 53.7, 46.3, 16.4, 10.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_9H_{20}NO_4$ 206.1392; Found 206.1391.

(2R,3R,4R,5S)-2-(Hydroxymethyl)-1-(3-phenylpropyl)piperidine-3,4,5-triol (**32c**): The conditions employed for the preparation of this compound were those described in General debenzylation method I, which gave **32c** as an off-white semi-solid (35 mg, quant.). $[\alpha]^{23}_{D}$ -1.8° (MeOH; *c* 0.2). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.24 (5H, m), 4.07 (1H, s), 3.93-3.75 (3H, m), 3.60 (2H, m), 3.42 (4H, m), 3.10 (2H, m), 2.82 (2H, m), 2.12 (1H, bs). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 140.2, 128.4, 126.2, 76.9, 76.6, 68.1, 67.5, 67.2, 66.4, 66.3, 60.6, 57.8, 54.1, 54.1, 53.0,46.4, 32.5, 24.8. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{15}H_{24}NO_4$ 282.1705; Found 282.1699.

(2R,3R,4R,5S)-2-(Hydroxymethyl)-1-octylpiperidine-3,4,5-triol (**32d**): The conditions employed for the preparation of this compound were those described in General debenzylation method I, which afforded **32d** as an off-white semi-solid (21 mg, quant.). $[\alpha]^{23}_{D}$ -3.96° (MeOH; *c* 0.27). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.02 (1H, d, *J* = 10.5 Hz), 3.82 (1H, d, *J* = 11 Hz), 3.62 (1H, s), 3.52 (1H, s), 3.36 (1H, s), 3.29 (2H, m), 3.11 (1H, bs), 2.94 (2H, m), 1.66 (2H, m), 1.27 (10H, m), 0.81 (3H, d, *J* = 6 Hz). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 76.7, 67.4, 66.4, 66.2, 53.7, 53.7, 53.2, 31.5, 28.9, 28.9, 26.4, 22.9, 22.3, 13.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{14}H_{30}NO_4$ 276.2175; Found 276.2170.

(2R,3R,4R,5S)-1-Hexadecyl-2-(hydroxymethyl)piperidine-3,4,5-triol (**32e**): The conditions employed for the preparation of this compound were those described in General debenzylation method I, which afforded **32e** as an off-white semi-solid (35 mg, quant.). $[\alpha]^{23}_{D}$ -3.0° (MeOH; *c* 0.3). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.02 (1H, d, *J* = 9 Hz), 3.81 (1H, d, *J* = 9 Hz), 3.62 (1H, bs), 3.52 (1H, bs), 3.32 (3H, m), 3.11 (1H, s), 2.93 (2H, m), 1.65 (2H, m), 1.31-1.18 (26H, m), 0.80 (1H, t, *J* = 6 Hz). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 76.8, 67.4, 66.4, 66.2, 53.7, 53.2, 31.7, 29.4, 29.4, 29.3, 29.2, 29.1, 28.9, 26.4, 22.9, 22.3, 13.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{22}H_{46}NO_4$ 388.3427; Found 388.3420.

(3R,4S,5R,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-((1-((2S,3S,4R)-1,3,4-trihydroxyocta decan-2-yl)-1H-1,2,3-triazol-4-yl)methyl)piperidin-2-one (**33**): To a solution of compound **29b** (300 mg, 0.5 mmol, 1 equiv) and azido phytospingosine (184 mg, 0.5 mmol, 1 equiv) in DMF (10 mL) at 0 °C were added CuI (204 mg, 1.1 mmol, 2 equiv) and DIPEA (280 µL, 1.6 mmol, 3 equiv). The resulting mixture was stirred for 1 h under nitrogen atmosphere and after complete consumption of both the starting materials, as indicated by TLC, the reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc 50:50 yielded triazole as a colorless viscous liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.92 (1H, s), 7.39 (2H, d, *J* = 7 Hz), 7.28 (12H, m), 7.22 (4H, m), 7.14 (2H, m), 5.05 (2H, m), 4.79 (1H, m), 4.67 (2H, m), 4.53 (2H, m), 4.36 (4H, m), 4.11 (1H, d, *J* = 9 Hz), 4.05 (1H, dd, *J* = 5, 12 Hz), 3.96 (1H, dd, *J* = 4.5, 11.5 Hz), 3.84 (2H, m), 3.78-3.68 (3H, m), 3.49 (2H, m), 2.42 (3H, bs), 1.53 (1H, m), 1.41 (2H, m), 1.24 (23H, m), 0.88 (1H, t, *J* = 6.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.1, 143.3, 138.0, 137.8, 137.56, 137.55, 128.4, 128.3, 128.2, 128.0, 127.98, 127.94, 127.90, 127.8, 127.7, 124.1, 82.0, 78.3, 76.6, 75.1, 74.5, 73.8, 73.1, 72.5, 72.4, 67.7, 63.0, 61.0, 60.1, 40.9, 36.5, 32.7, 31.9, 31.5, 29.4, 25.8, 22.7, 14.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd $C_{55}H_{75}N_4O_8$ 919.5585; Found 919.5577. The conditions employed for subsequent debenzylation were those described in General debenzylation method II, which afforded **33** as an off-white semi-solid (65 mg, 81% over two steps). $[\alpha]^{23}_D$ +19.0° (MeOH; *c* 0.4). ^{1}H NMR (CD_3OD, 500 MHz): δ 8.19 (1H, s), 5.07 (1H, m), 4.59-4.30 (2H, m), 4.16-3.71 (8H, m), 3.47 (2H, m), 1.71 (1H, bs), 1.54 (1H, bs), 1.29 (24H, m), 0.90 (3H, t, J=6.5 Hz). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 173.2, 128.2, 126.4, 75.2, 74.9, 72.7, 71.7, 71.6, 67.4, 67.0, 65.3, 59.6,

59.0, 58.8, 33.4, 33.0, 31.7, 29.4, 29.3, 29.0, 25.3, 22.3 13.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{27}H_{51}N_4O_8$ 559.3707; Found 559.3703.

(2R,3R,4R,5S)-2-(Hydroxymethyl)-1-((1-((2S,3S,4R)-1,3,4-trihydroxyoctadecan-2-yl)-1H-1,2,3-triazol-4-yl)methyl)piperidine-3,4,5-triol (**34**): To a solution of compound **31b** (250 mg, 0.4 mmol, 1 equiv) and azido phytospingosine (152 mg, 0.4 mmol, 1 equiv) in DMF (10 mL) at 0 °C were added CuI (169 mg, 0.8 mmol, 2 equiv) and DIPEA (233 µL, 1.3 mmol, 3 equiv). The resulting mixture was stirred for 1 h under nitrogen atmosphere and after complete consumption of both the starting materials, as indicated by TLC, the reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc 60:40 yielded triazole as a colorless sticky solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.52 (1H, s), 7.28 (2H, d, *J* = 7.5 Hz), 7.22 (16H, m), 7.02 (2H, m), 4.84 (1H, d, *J* = 11 Hz), 4.78 (1H, d, *J* = 11 Hz), 4.69 (1H, d, *J* = 11 Hz), 4.57 (2H, s), 4.49 (1H, d, *J* = 12 Hz), 4.39 (1H, d, *J* = 12 Hz), 4.28 (1H, d, *J* = 11 Hz), 4.08 (2H, m), 3.89 (4H, m), 3.75 (1H, bs), 3.67 (1H, dd, *J* = 2.5, 10.5 Hz), 3.59 (2H, m), 3.48 (1H, t, *J* = 9 Hz), 3.29 (1H, t, *J* = 9 Hz), 3.08 (1H, dd, *J* = 5, 11.5 Hz), 2.71 (1H, bs), 2.19 (1H, d, *J* = 9.5 Hz), 2.09 (1H, m), 1.53 (1H, m), 1.38 (2H, m), 1.17 (23H, m), 0.81 (3H, t, *J* = 7 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 141.8, 138.8, 138.36, 138.33, 137.7, 128.56, 128.52, 128.4, 128.3, 128.0, 127.97, 127.95, 127.92,127.7, 127.6, 127.5, 124.0, 87.0, 78.4, 78.2, 75.4, 75.3, 75.2, 73.6, 72.7, 72.6, 66.4, 62.8, 62.6, 61.3, 54.5, 47.2, 32.8, 31.9, 29.7, 29.7, 29.7, 29.6 29.6, 29.4, 25.8, 22.7, 14.1. HRMS (ESI) m/z: $[M+H]^+$ Calcd $C_{55}H_{77}N_4O_7$ 905.5792; Found 905.5778. The conditions employed subsequent debenzylation were those described in General debenzylation method I, which afforded **34** as an off-white sticky solid. (58 mg, 95% over two steps) $[\alpha]^{23}_D$ +5.3° (MeOH; *c* 0.6). ^{1}H NMR (CD_3OD,

500 MHz): δ 8.41 (1H, s), 5.11 (1H, d, *J* = 3.5 Hz), 4.69 (2H, m), 4.33 (1H, d, *J* = 12 Hz), 4.21 (3H, m), 3.71 (3H, m), 3.47 (2H, m), 3.29 (1H, m), 2.99 (2H, m), 1.73 (1H, t, *J* = 9 Hz), 1.56 (2H, m), 1.31 (13H, m), 0.92 (1H, t, *J* = 6.5 Hz). $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz): δ 138.7, 131.2, 80.5, 79.0, 75.6, 71.4, 70.3, 69.2, 69.0, 63.4, 57.8, 57.5, 50.7, 37.2, 35.6, 33.3, 33.3, 33.0, 29.2, 26.3, 17.0. HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{27}H_{53}N_4O_7$ 545.3914; Found 545.3909.

Figure 4.5.2.1. NMR spectras of compound **12**

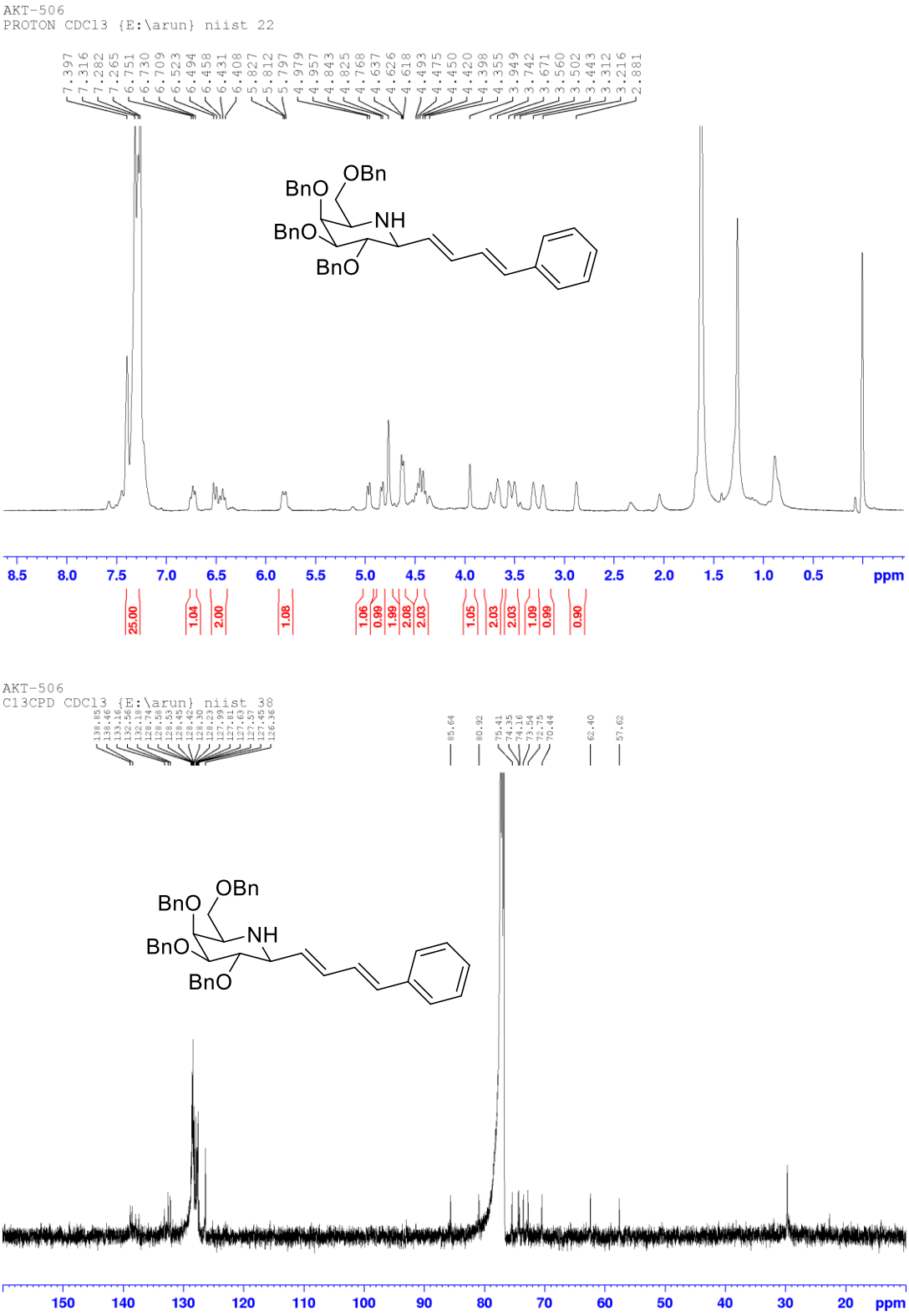


Figure 4.5.2.2. NMR spectras of compound **13**

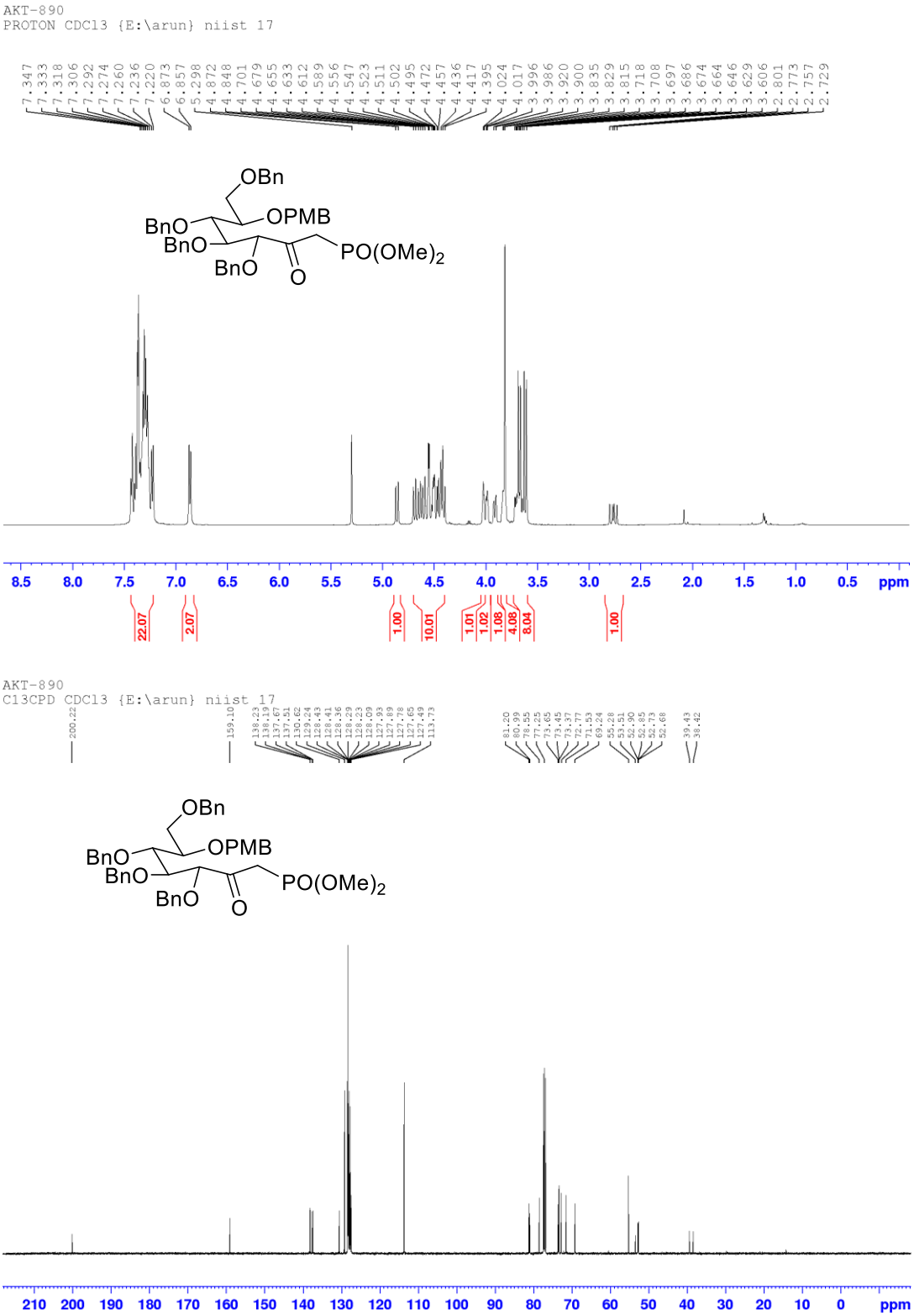


Figure 4.5.2.3. NMR spectras for precursor intermediate of compound **14**

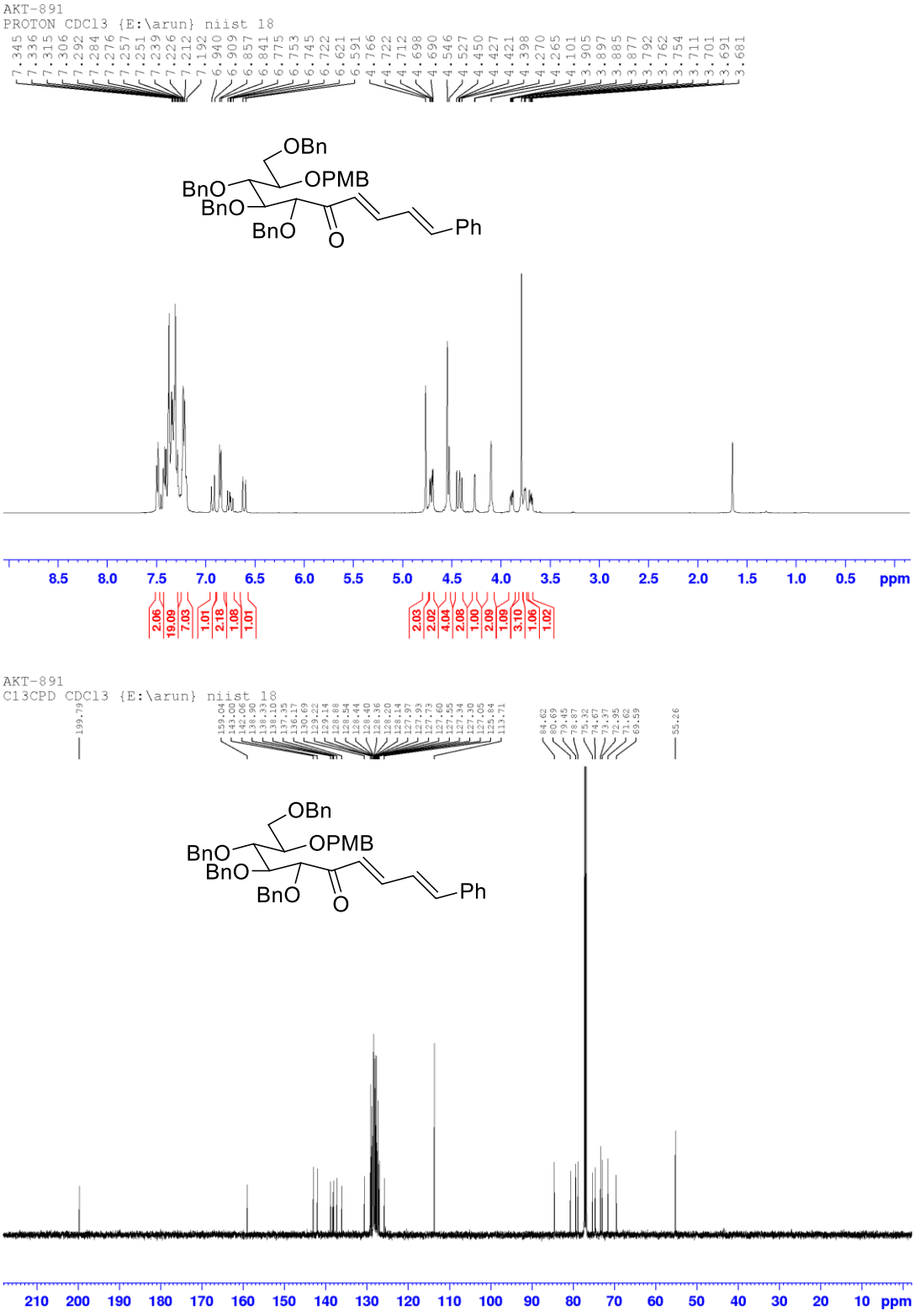


Figure 4.5.2.4. NMR spectras of compound **14**

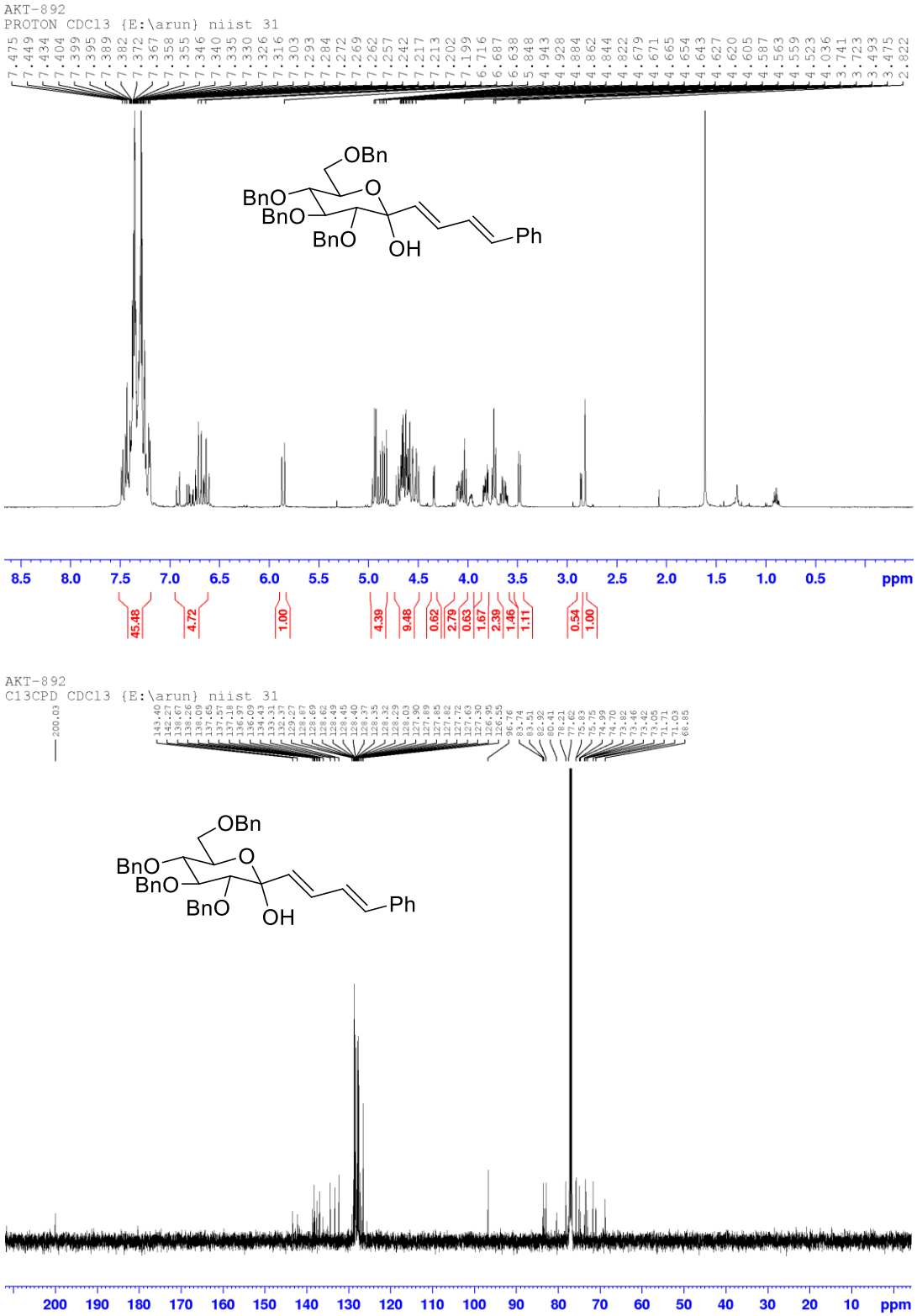


Figure 4.5.2.5. NMR spectras for precursor intermediate of compound **15**

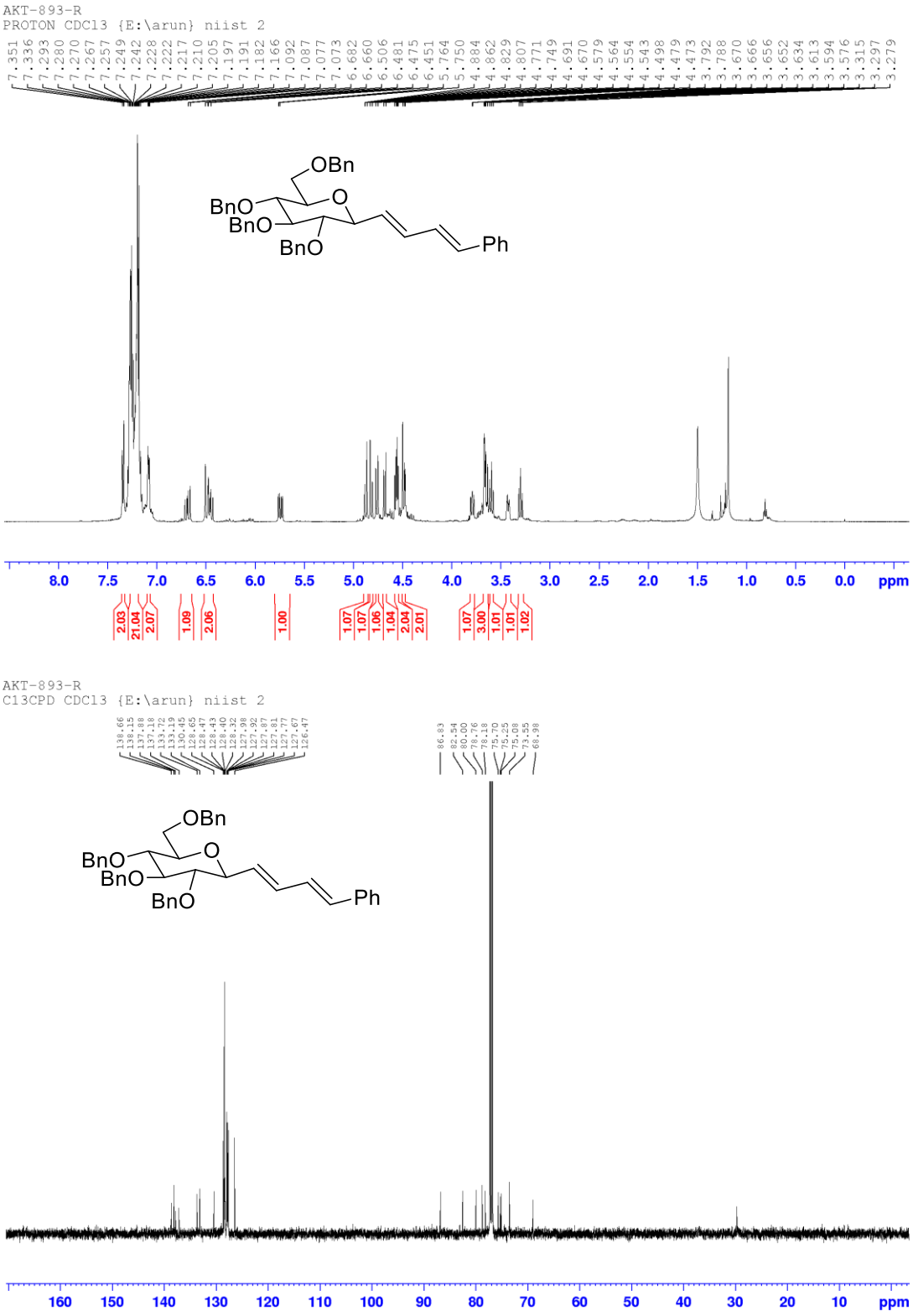


Figure 4.5.2.6. NMR spectras of compound **15**

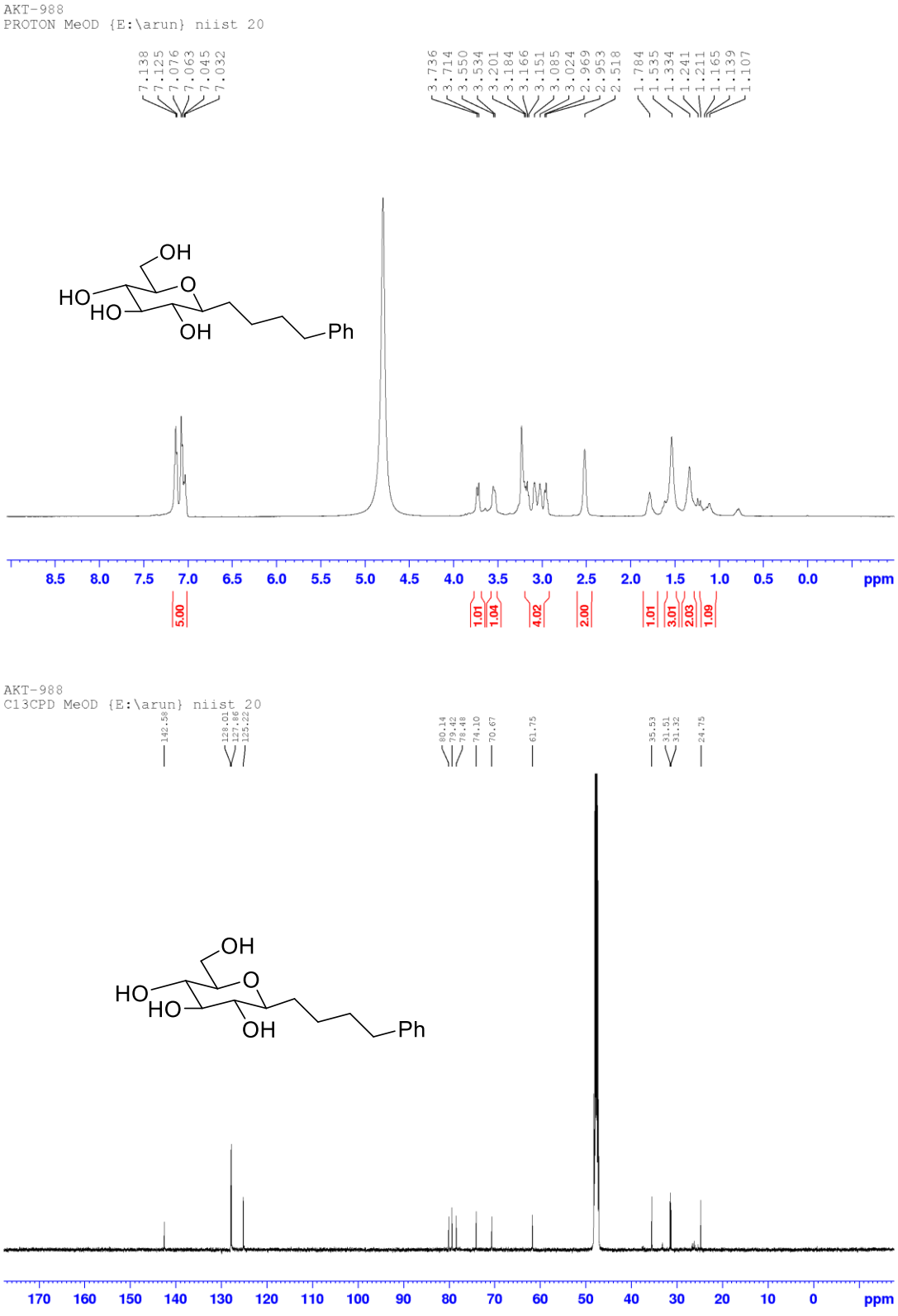


Figure 4.5.2.7. NMR spectras for precursor intermediate of compound **16**

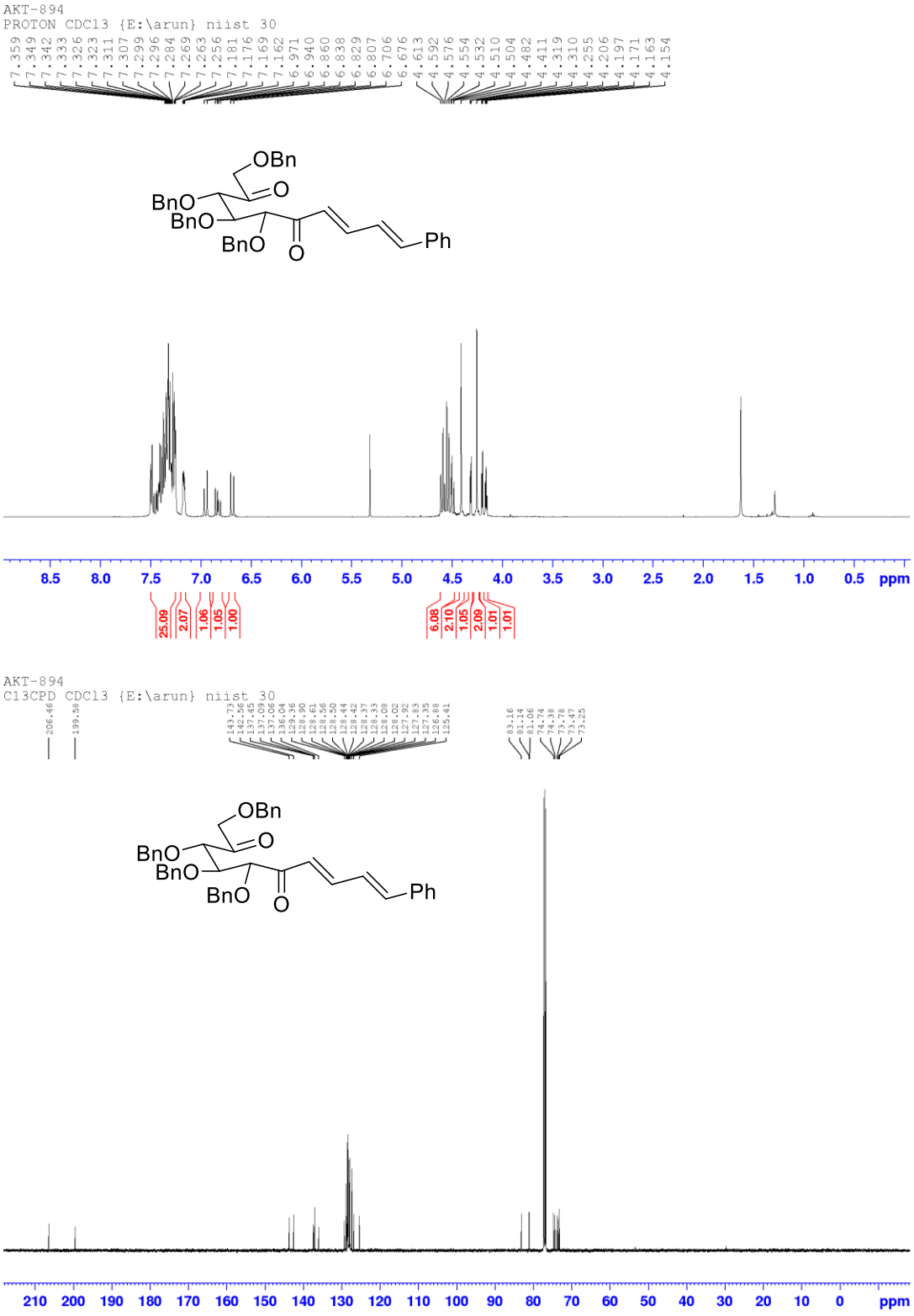


Figure 4.5.2.8. NMR spectras of compound **16**

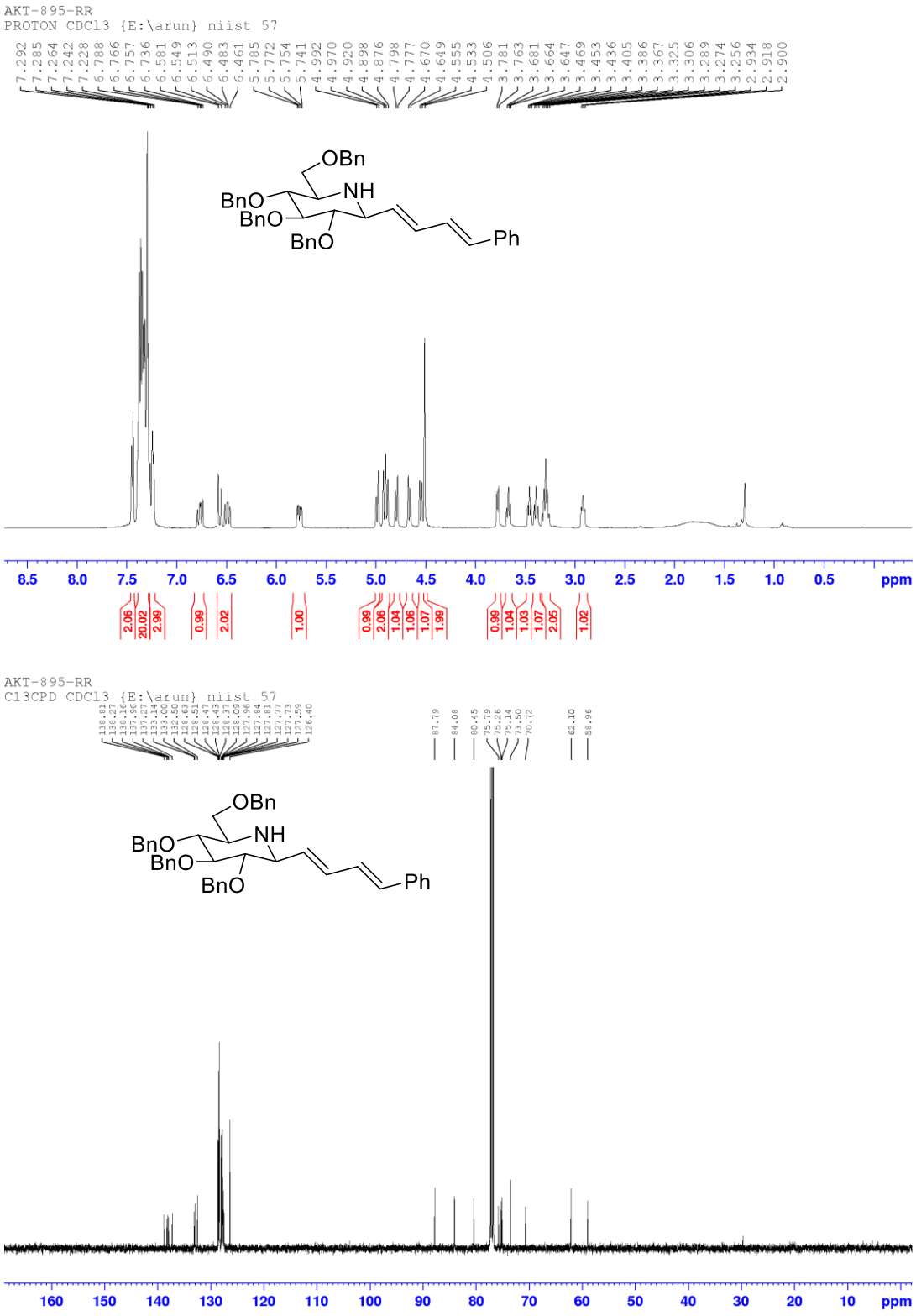


Figure 4.5.2.9. NMR spectras of compound **17a**

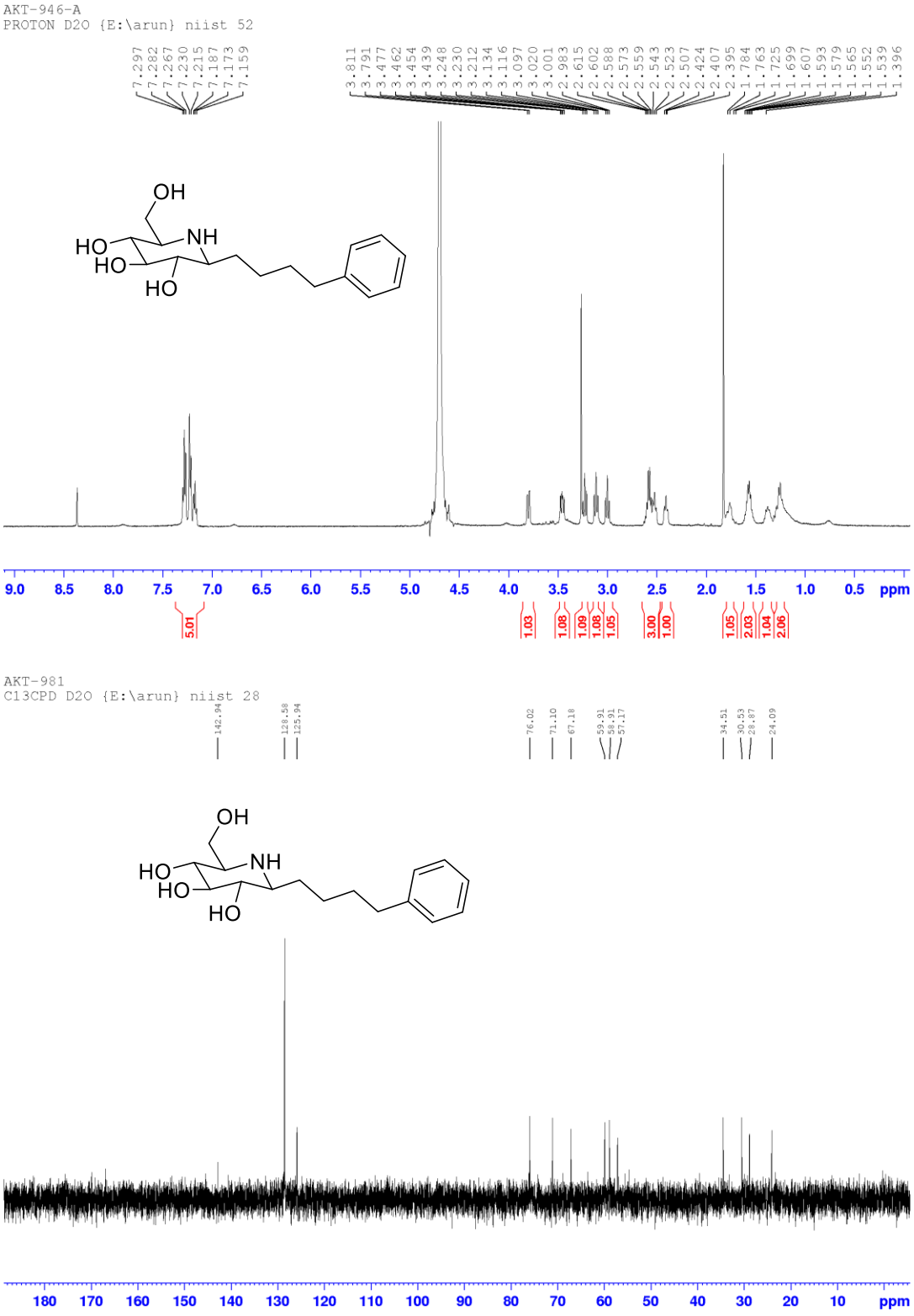


Figure 4.5.2.10. NMR spectras for precursor intermediate of compound **17b**

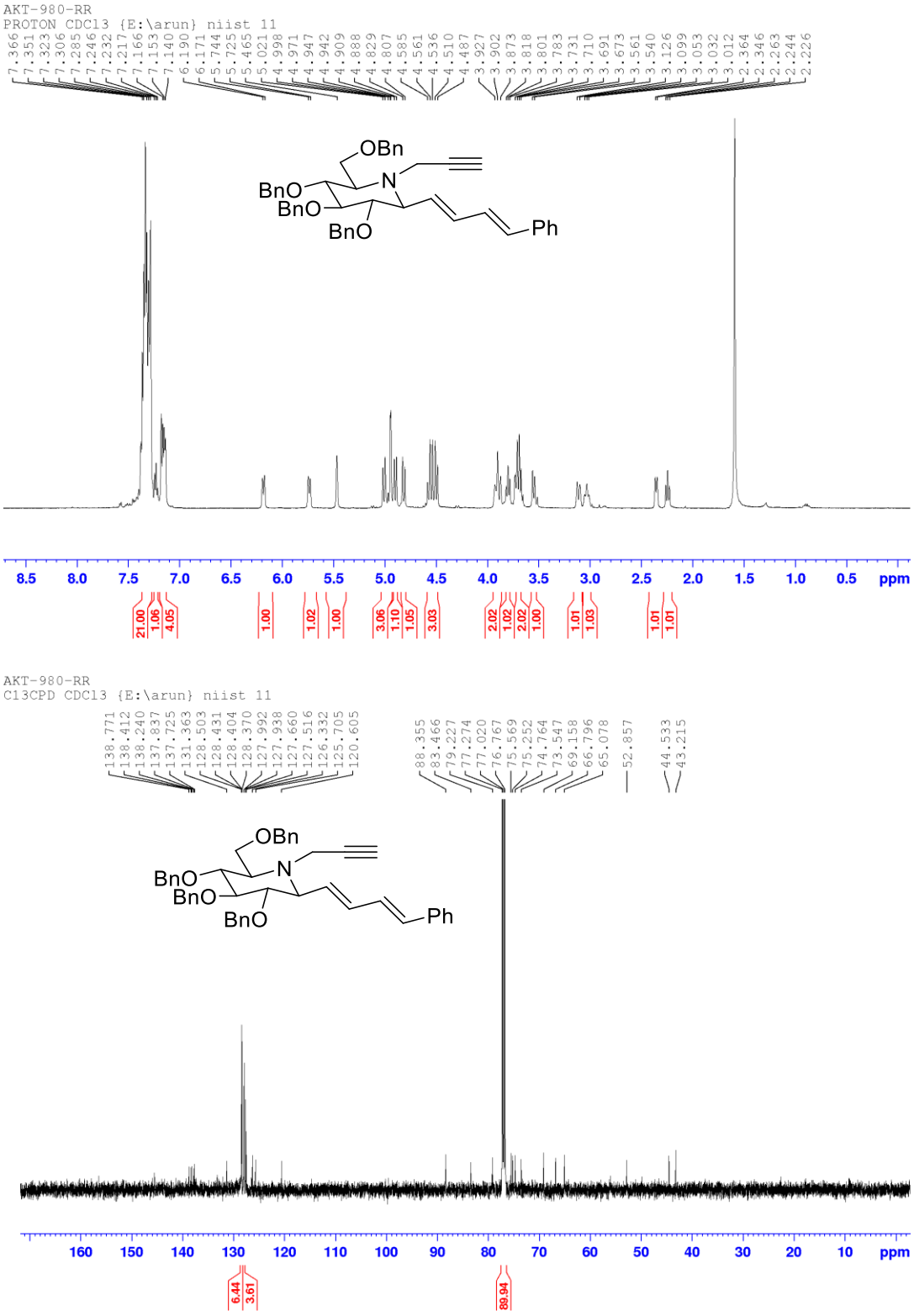


Figure 4.5.2.11. NMR spectras of compound **17b**

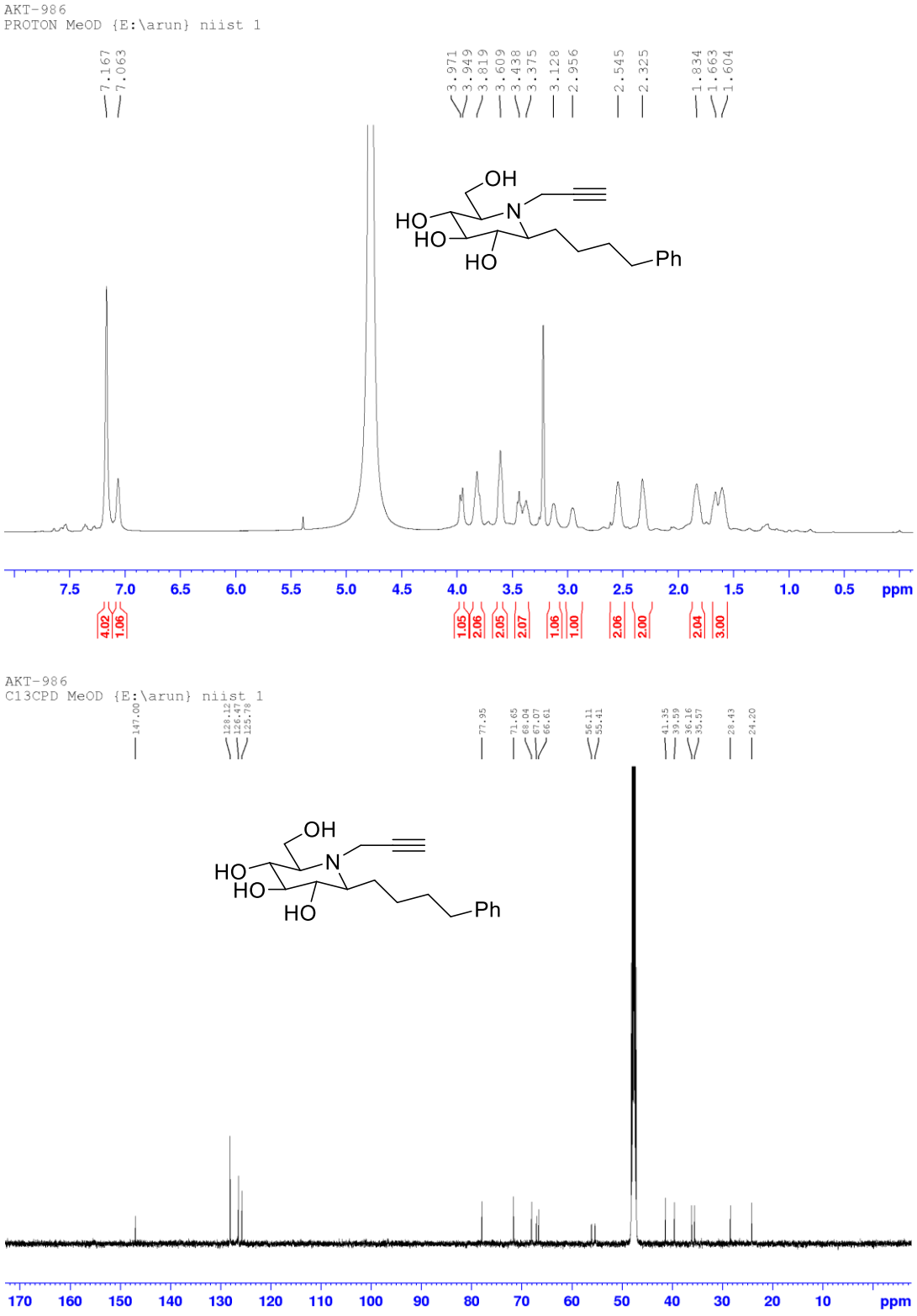


Figure 4.5.2.12. NMR spectras for precursor intermediate of compound **17c**

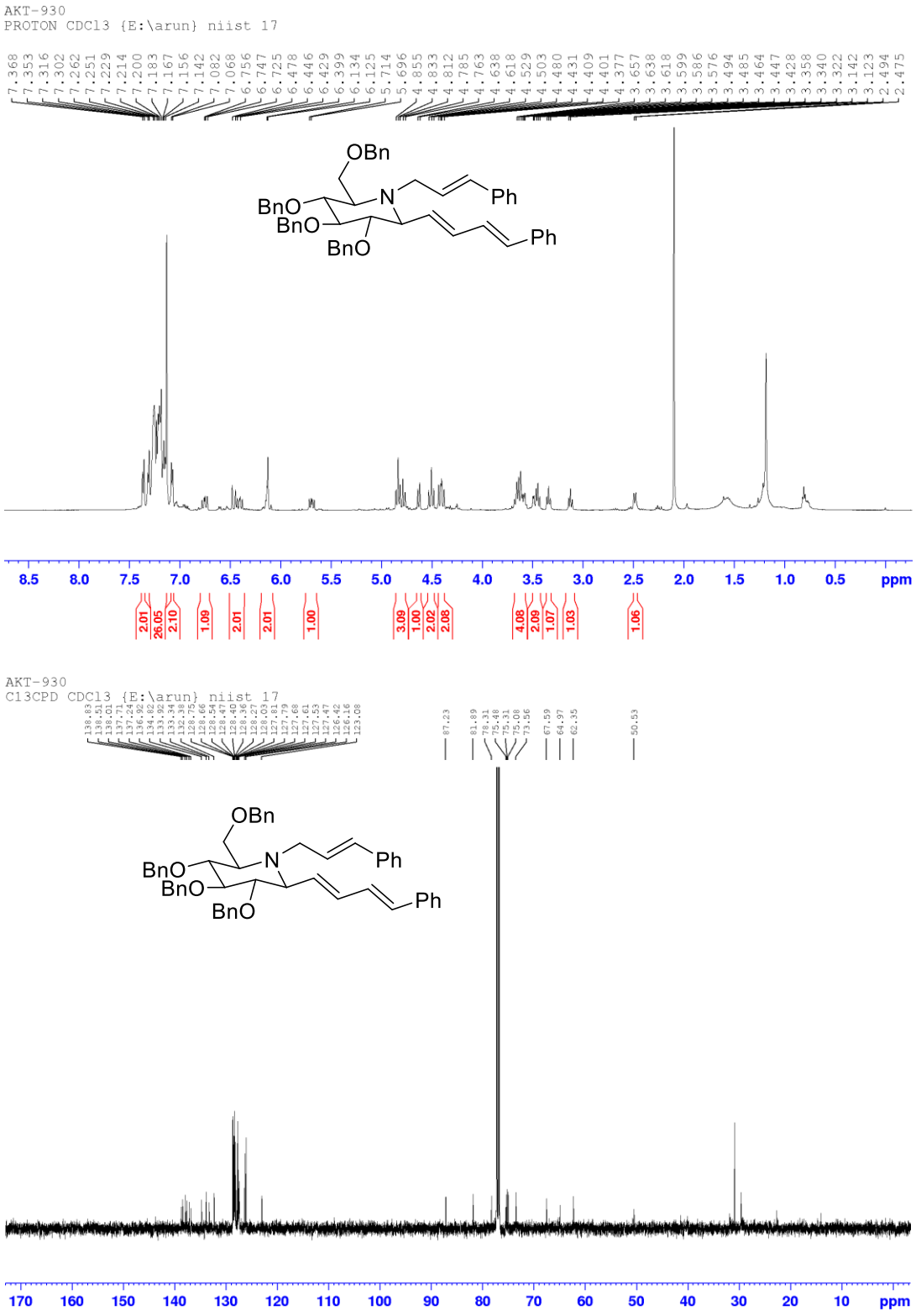


Figure 4.5.2.13. NMR spectras of compound **17c**

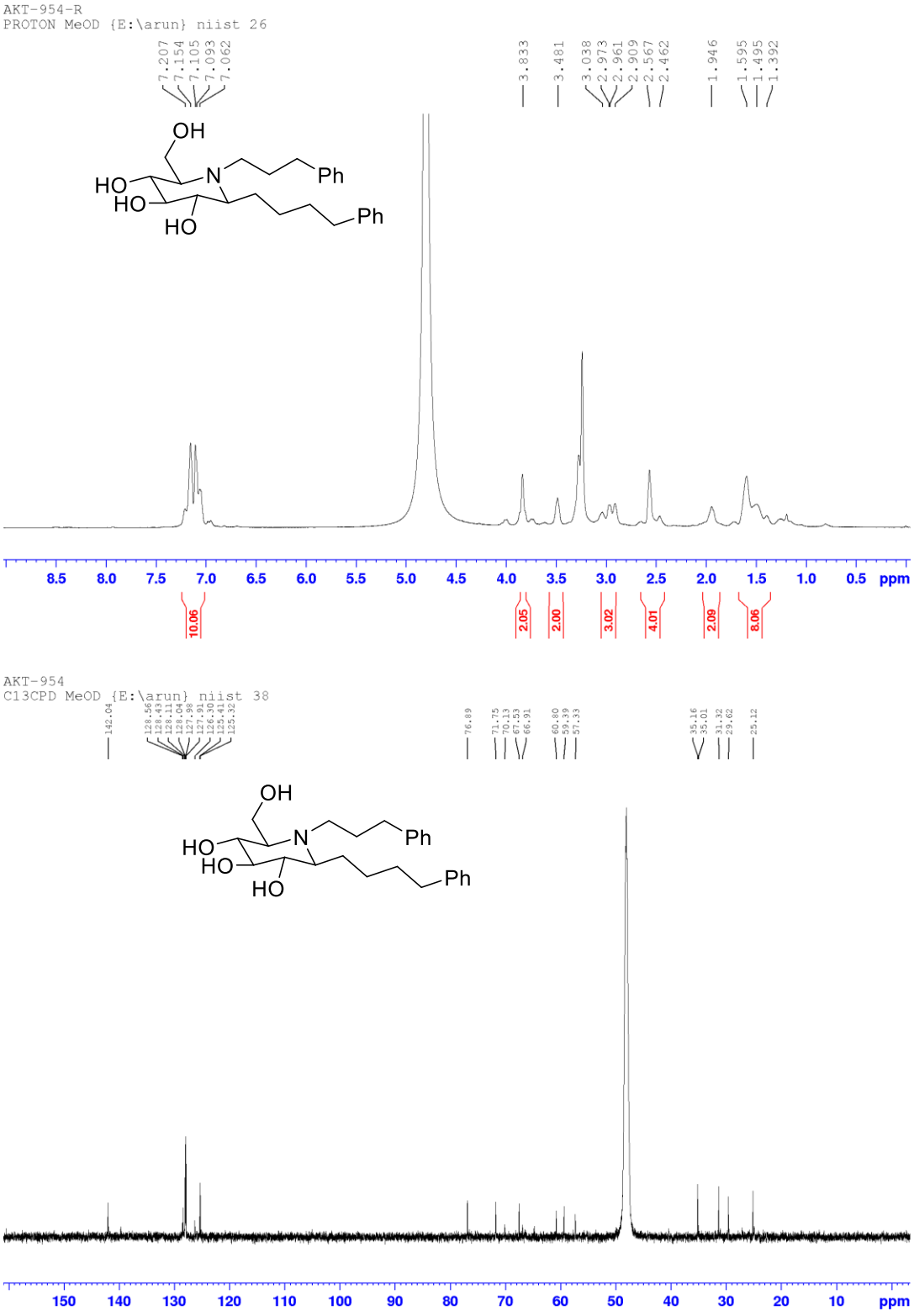


Figure 4.5.2.14. NMR spectras for precursor intermediate of compound **17d**

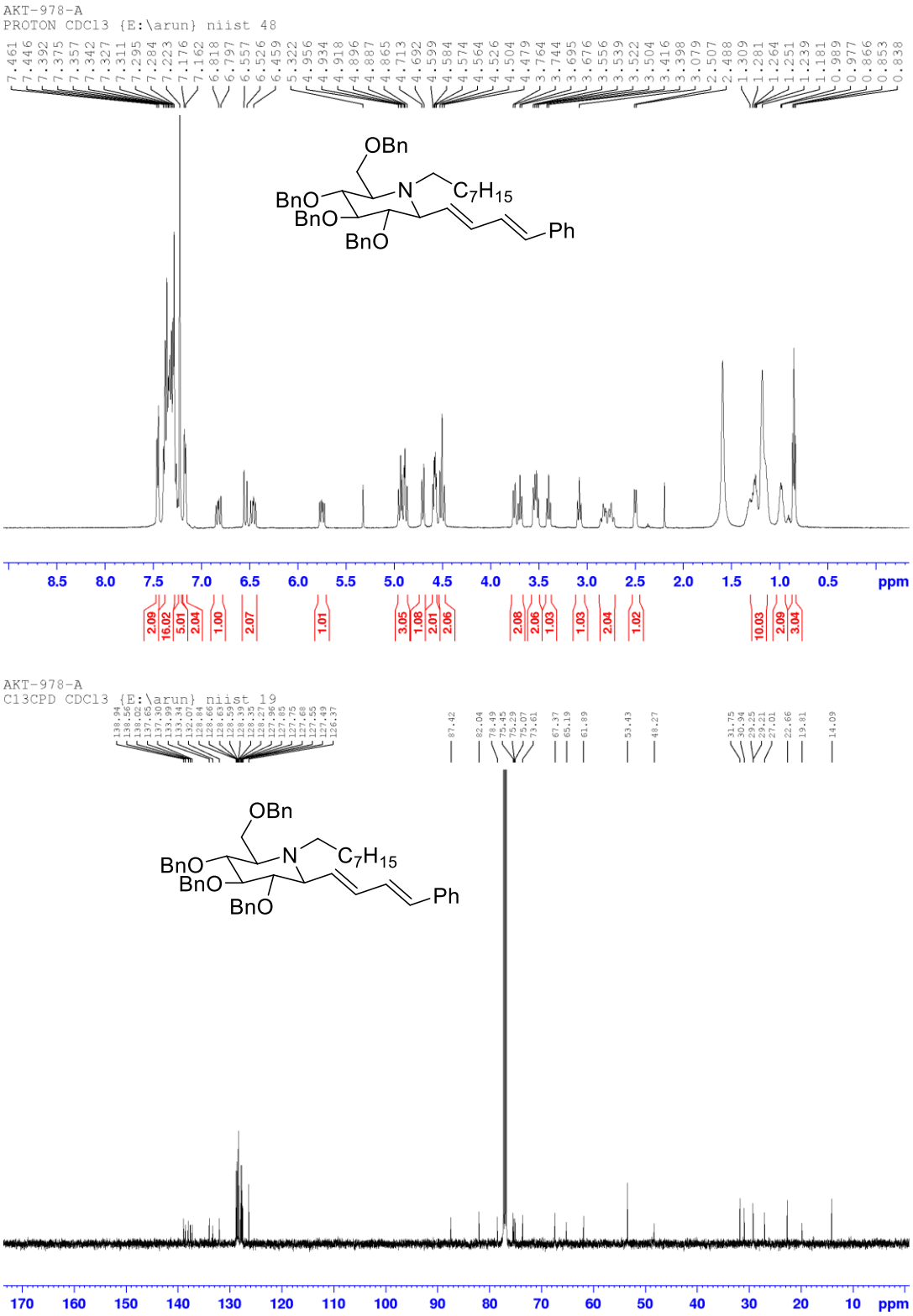


Figure 4.5.2.15. NMR spectras of compound **17d**

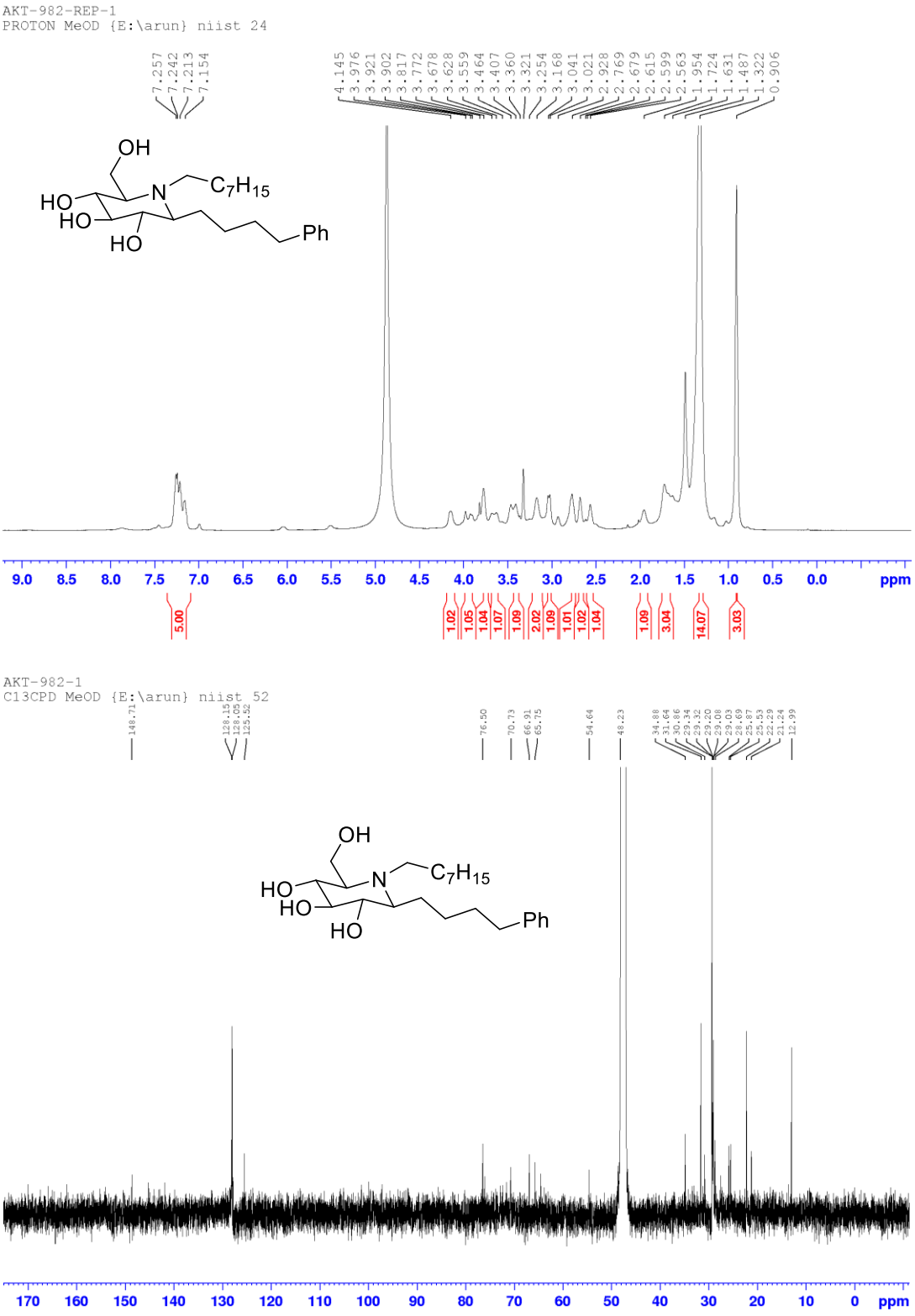


Figure 4.5.2.16. NMR spectras for precursor intermediate of compound **17e**

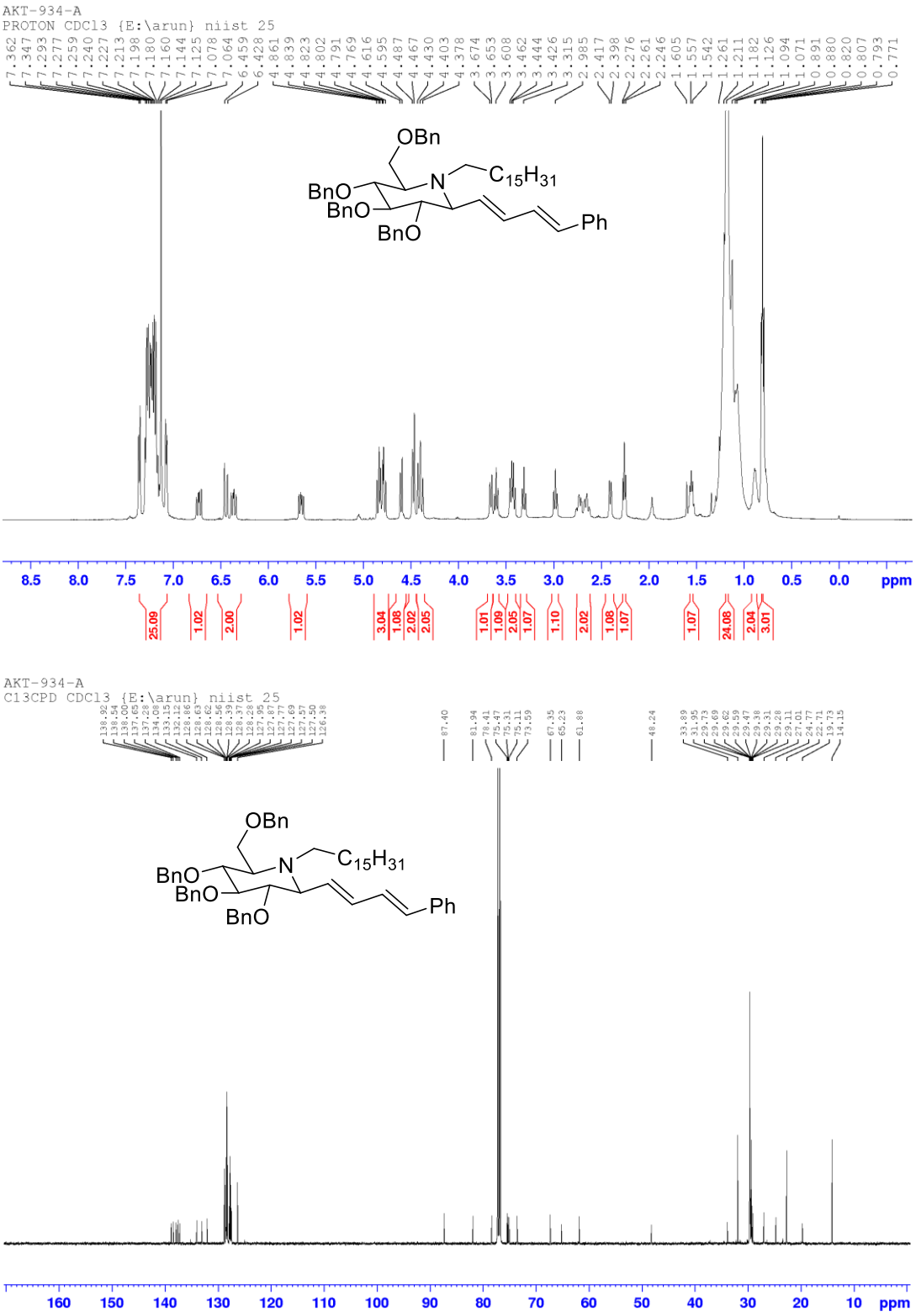


Figure 4.5.2.17. NMR spectras of compound **17e**

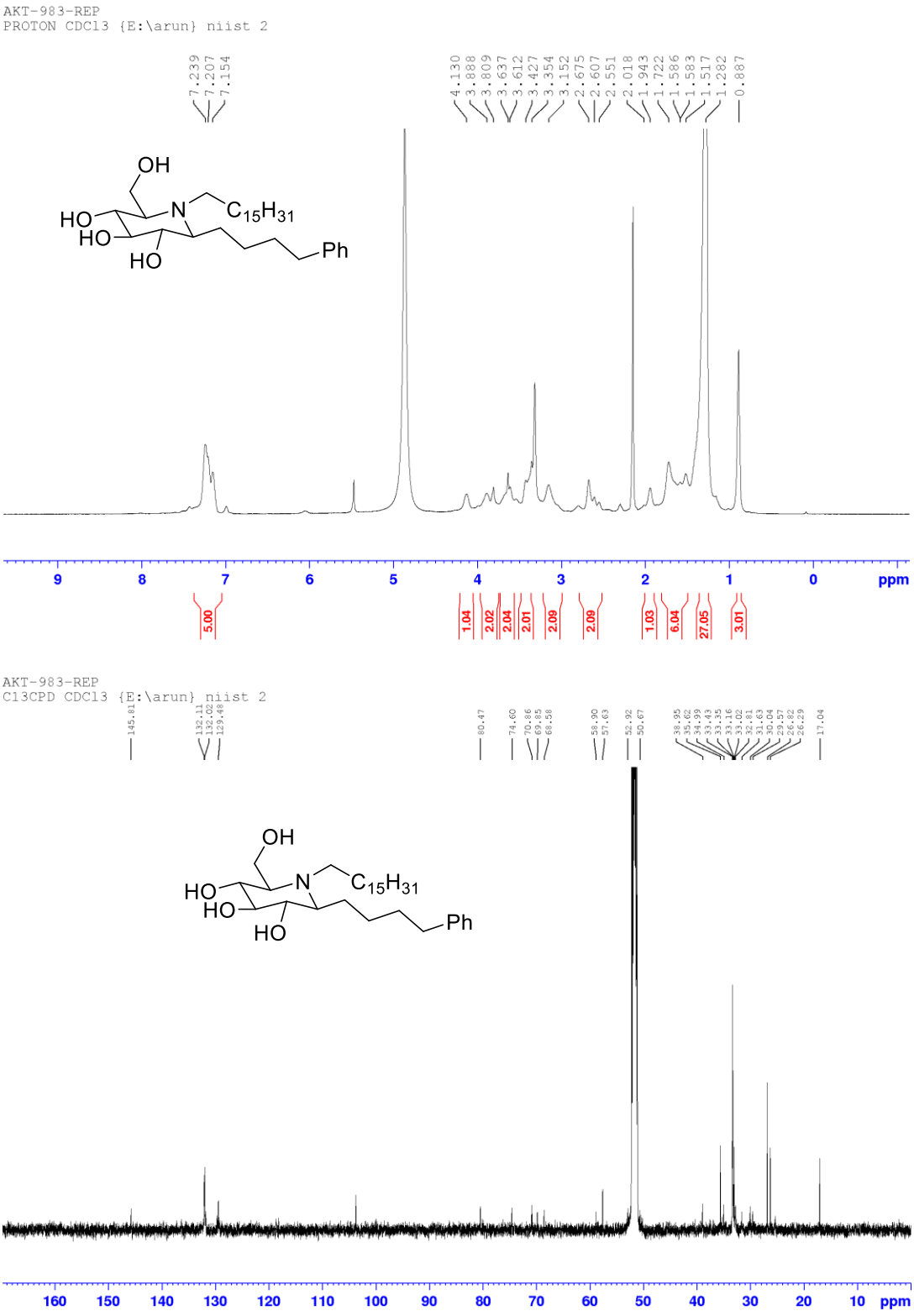


Figure 4.5.2.18. NMR spectras of compound **19**

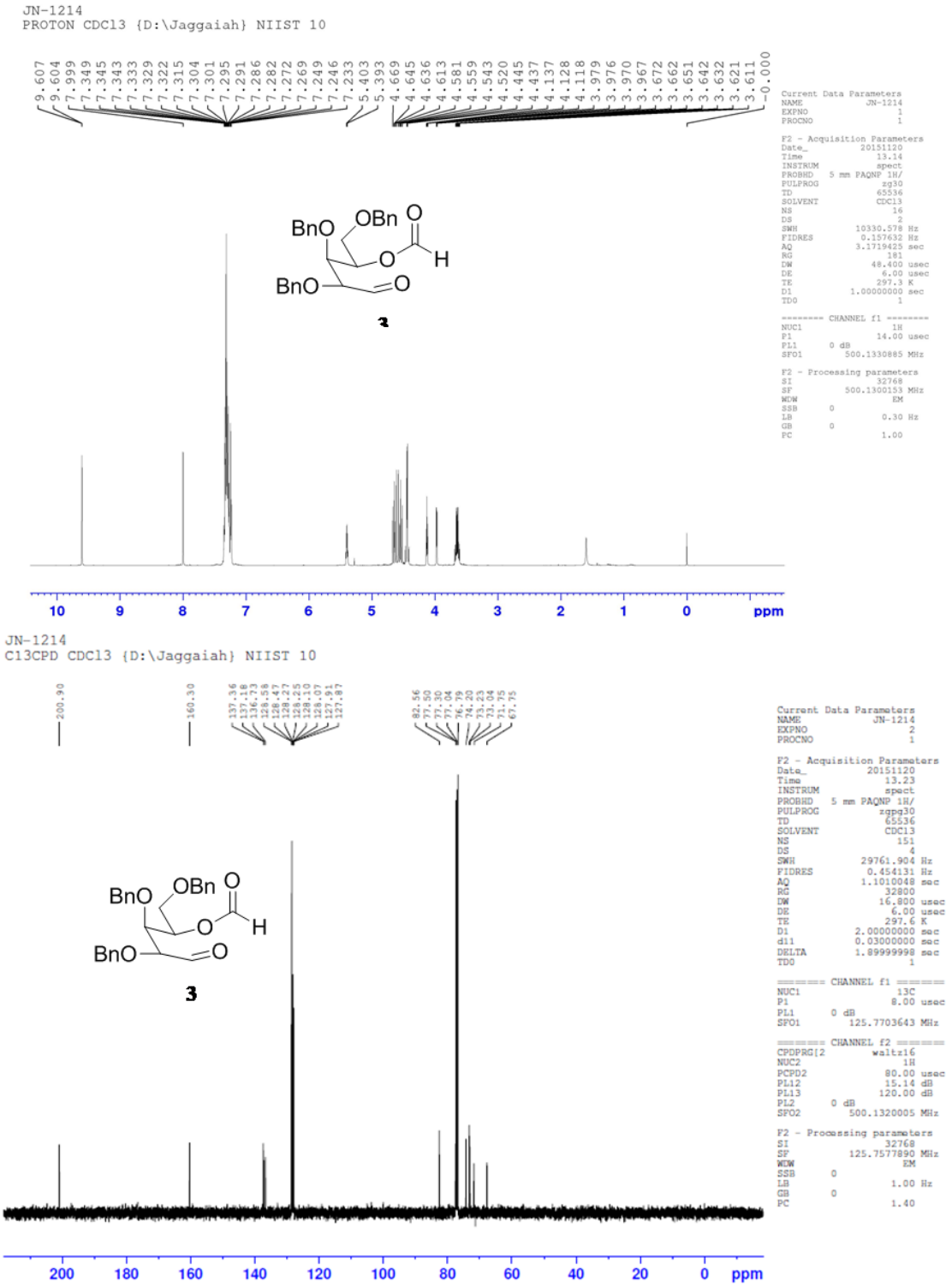


Figure 4.5.2.19. NMR spectras of compound **20**

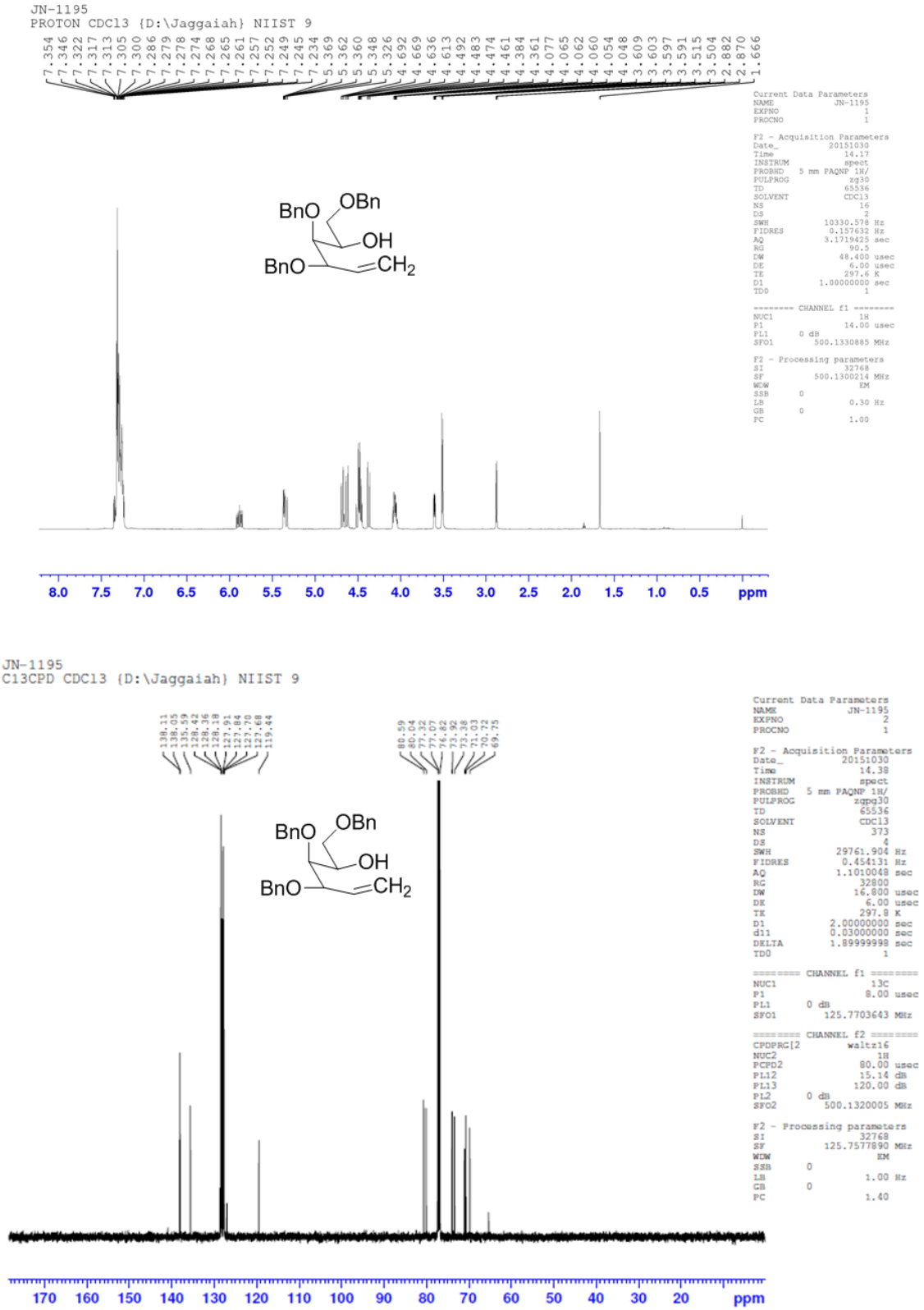


Figure 4.5.2.20. NMR spectras of compound **21**

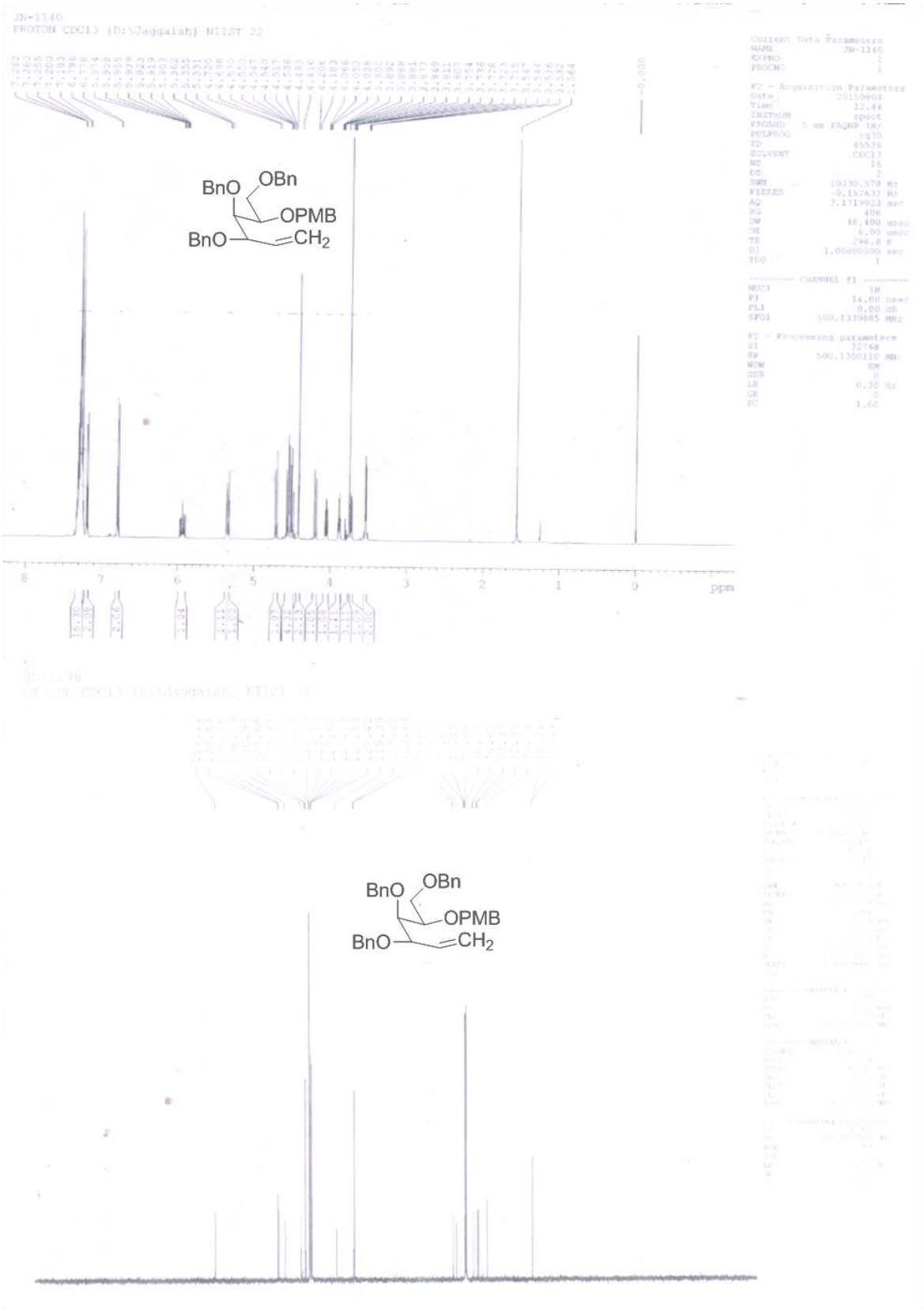


Figure 4.5.2.21. NMR spectras of compound **21a**

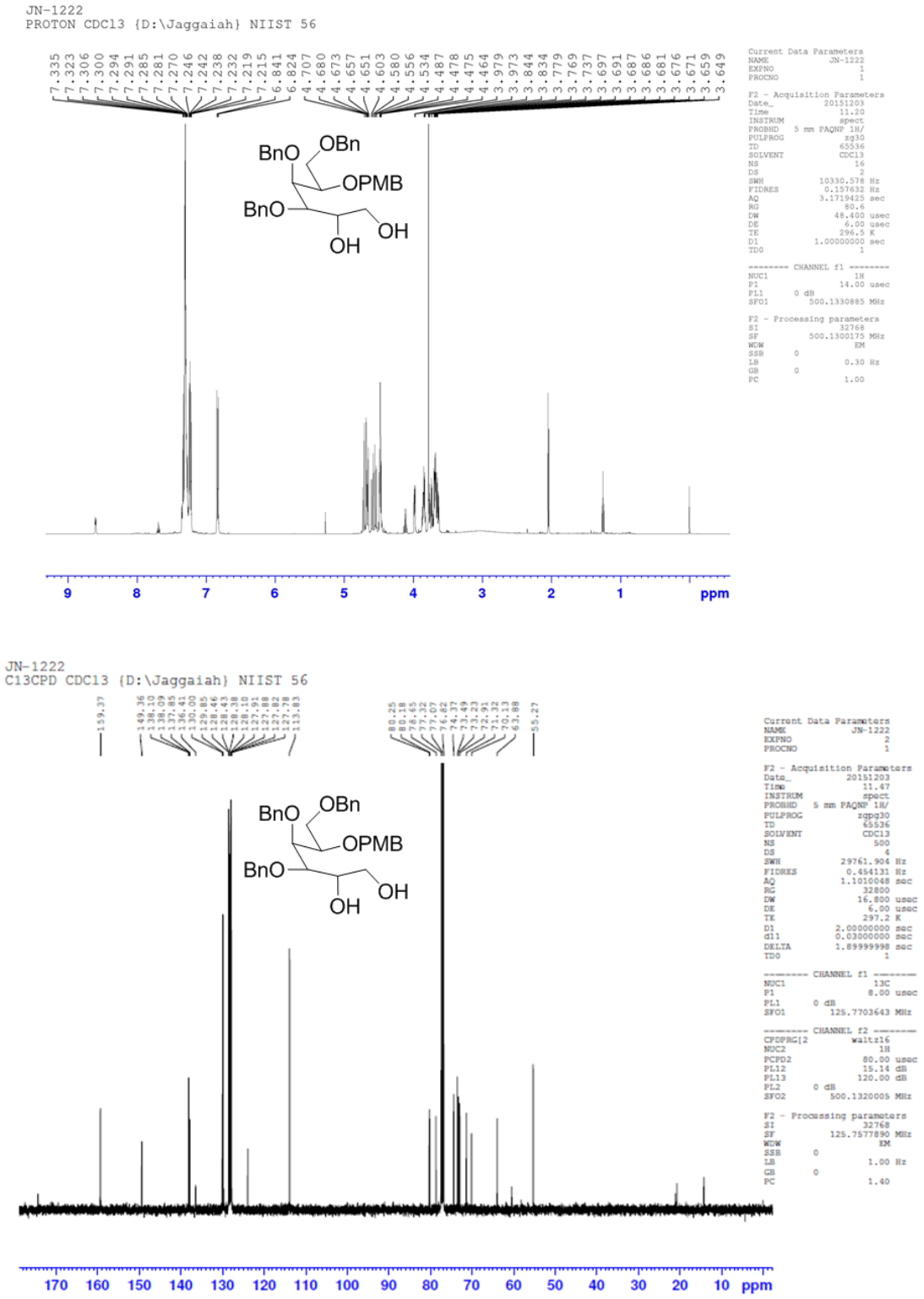


Figure 4.5.2.22. NMR spectras of compound **22**

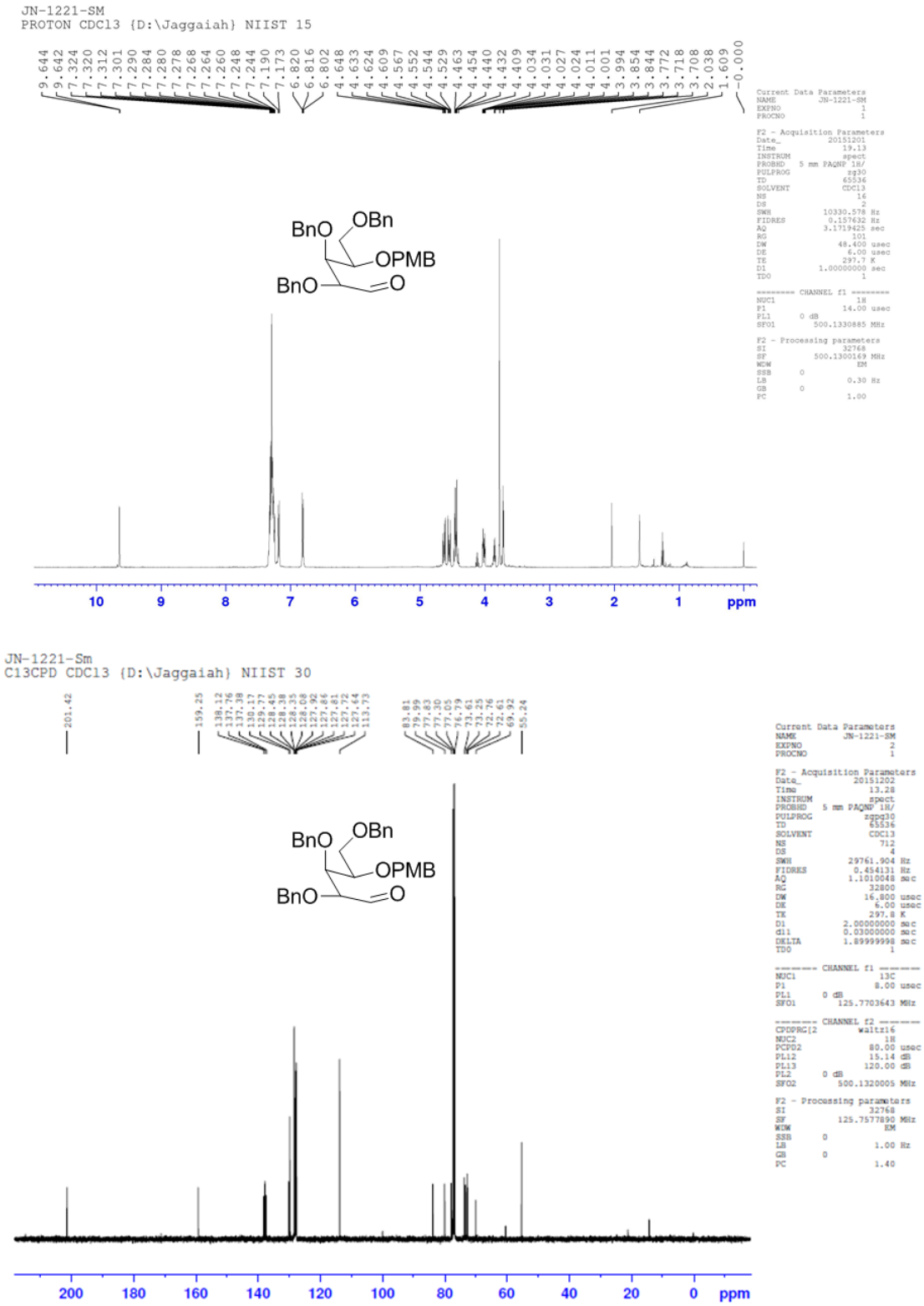


Figure 4.5.2.23. NMR spectras of compound **22a**

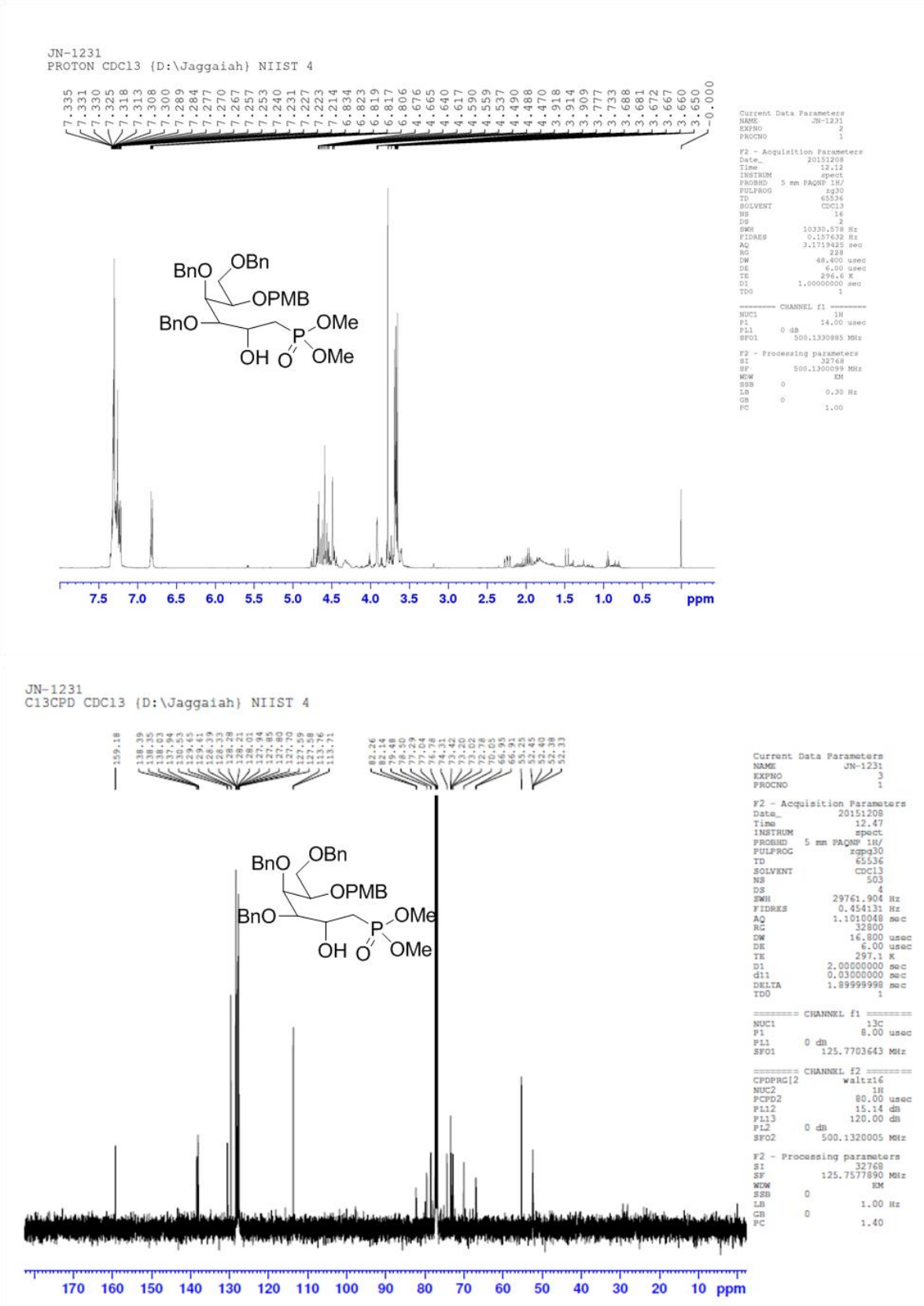


Figure 4.5.2.24. NMR spectras of compound **23**

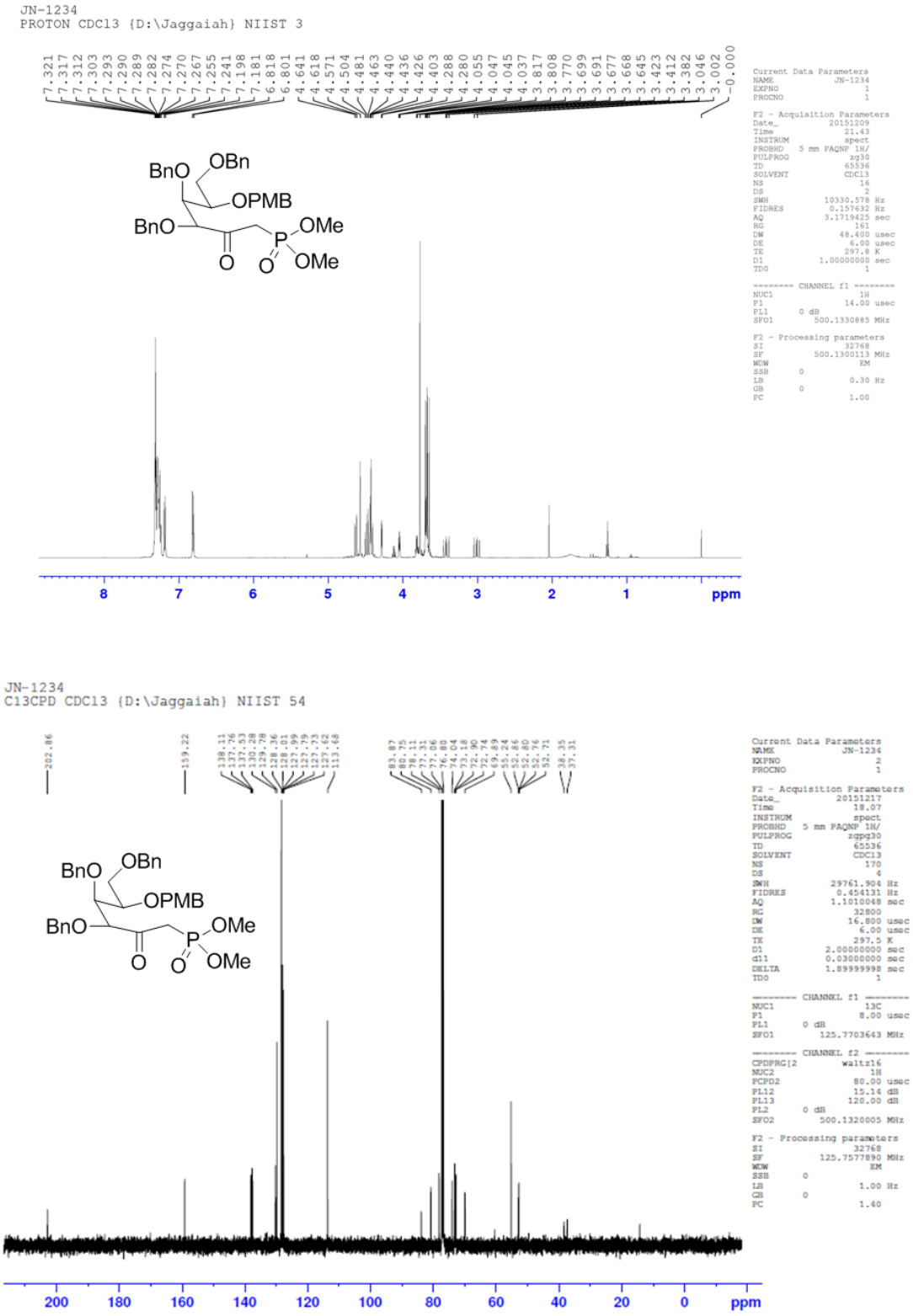


Figure 4.5.2.25. NMR spectras of compound **24**

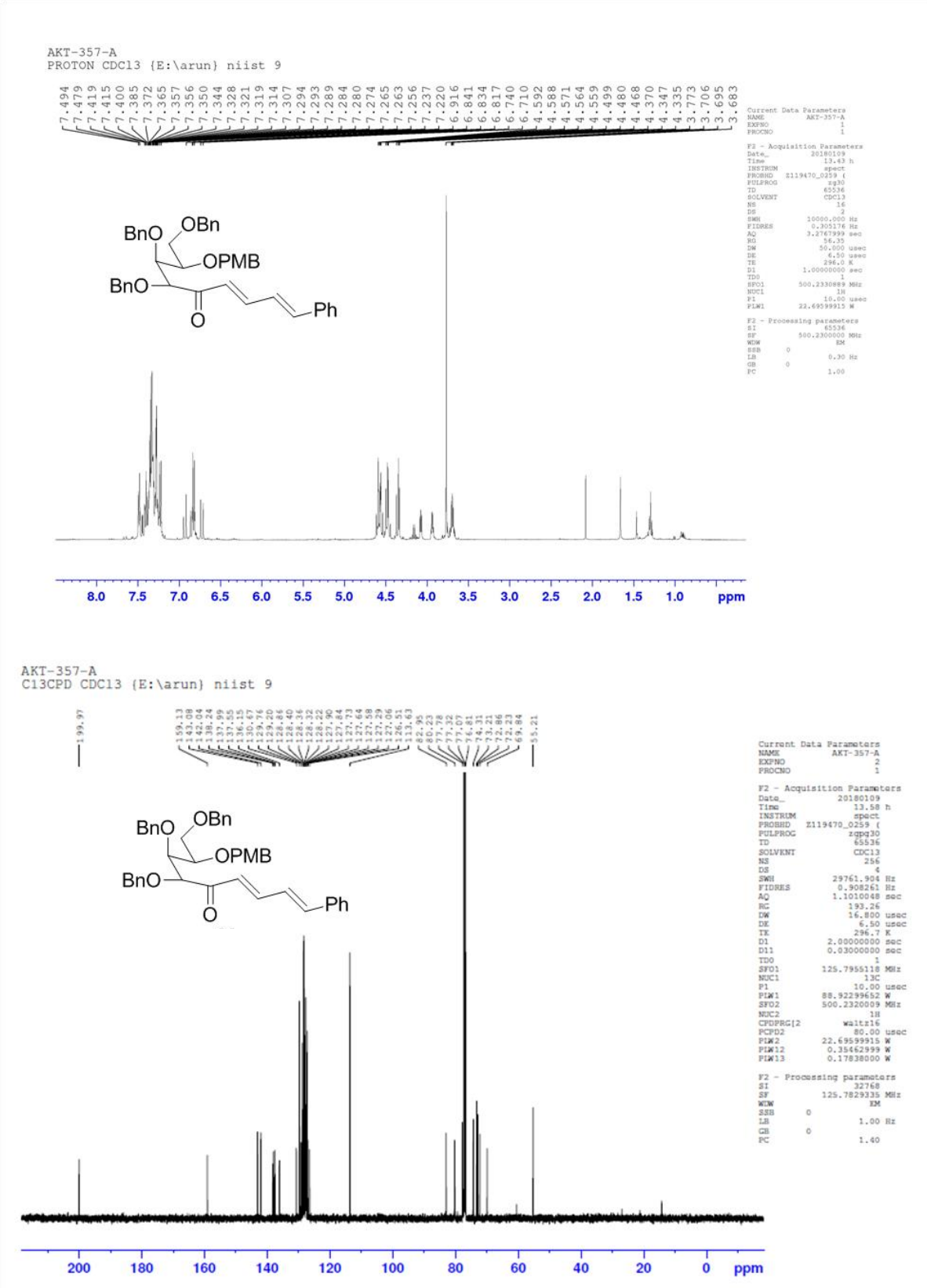


Figure 4.5.2.26. NMR spectras of compound **25**

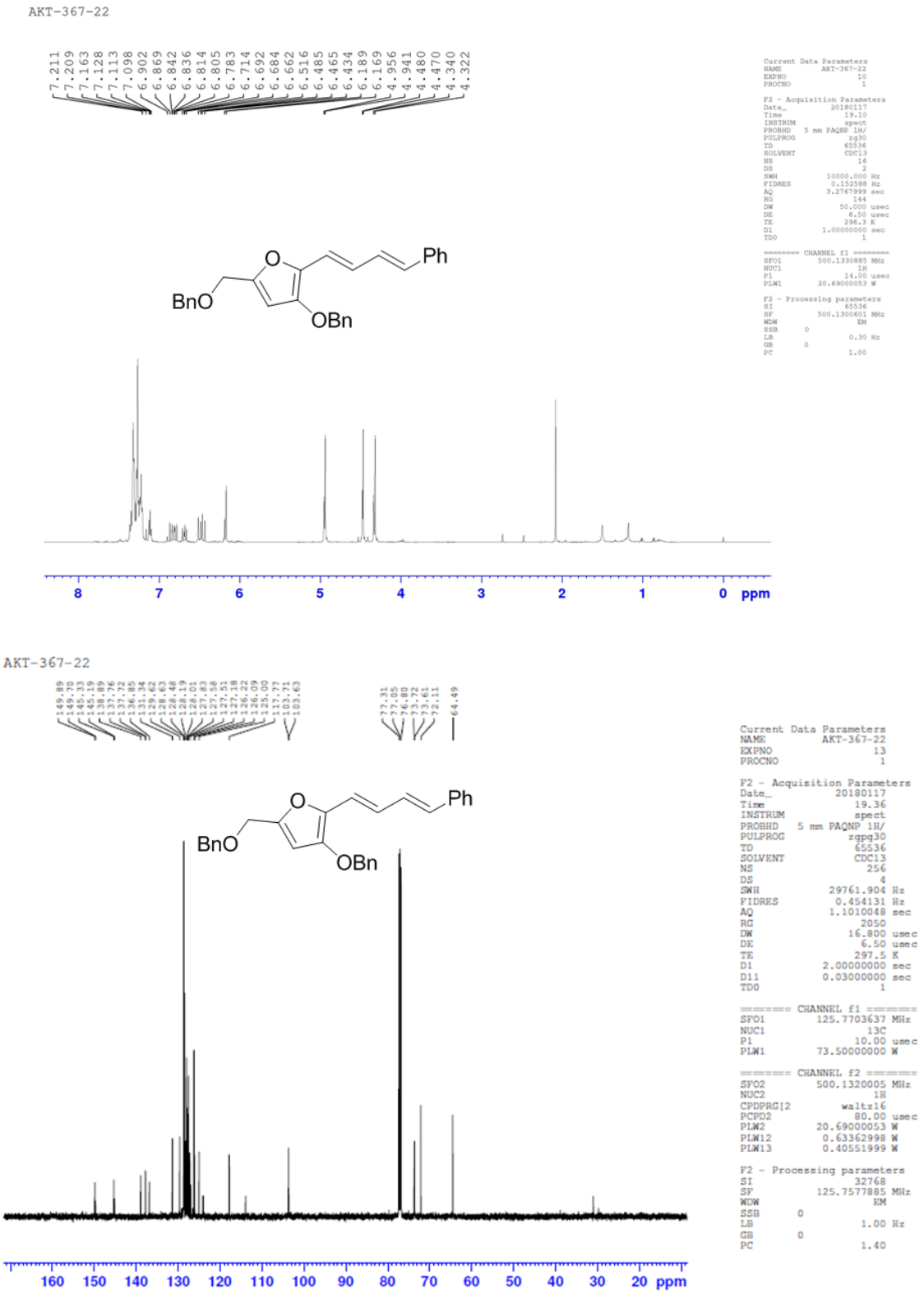
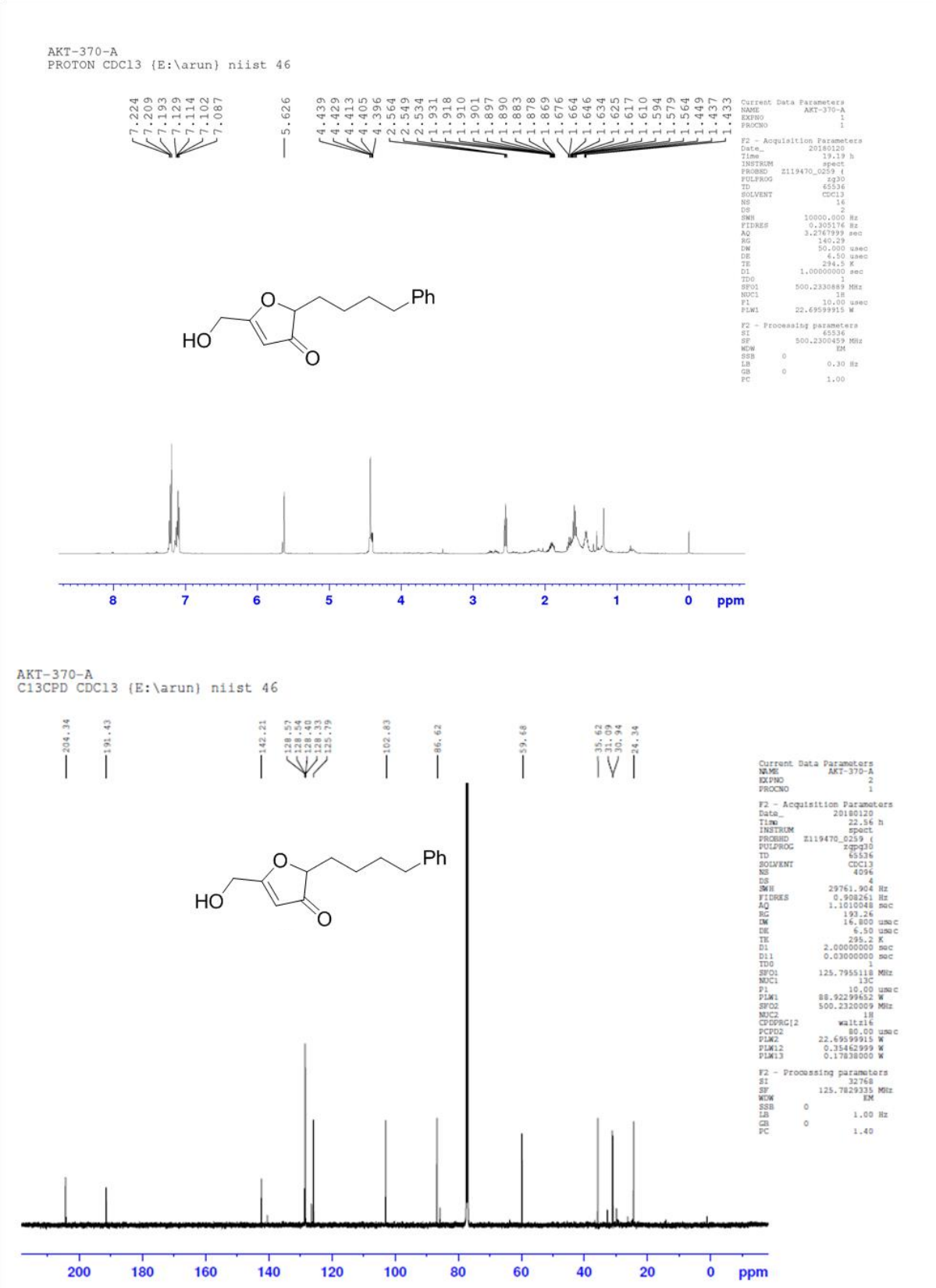
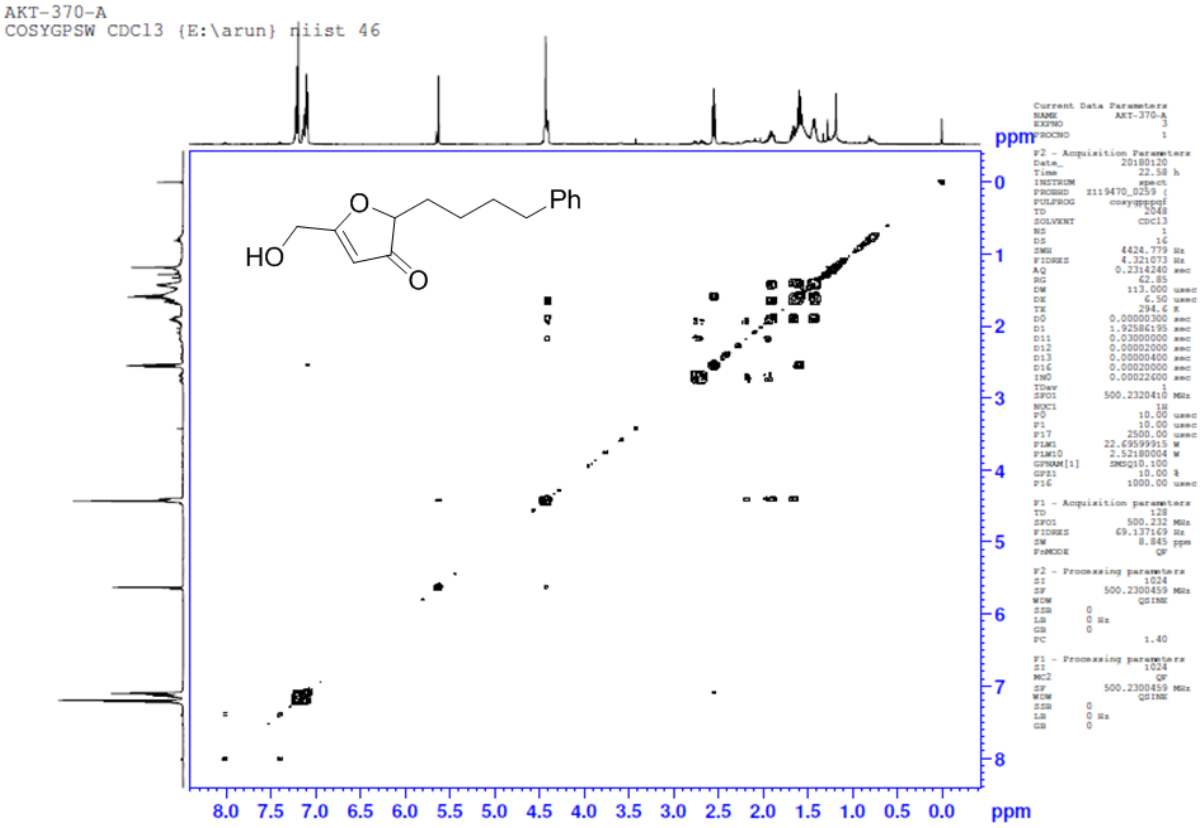


Figure 4.5.2.27. NMR spectras of compound **26**




AKT-370-A
COSYGPSW CDCl3 {E:\arun} niist 46
HO
O
Ph
O
ppm
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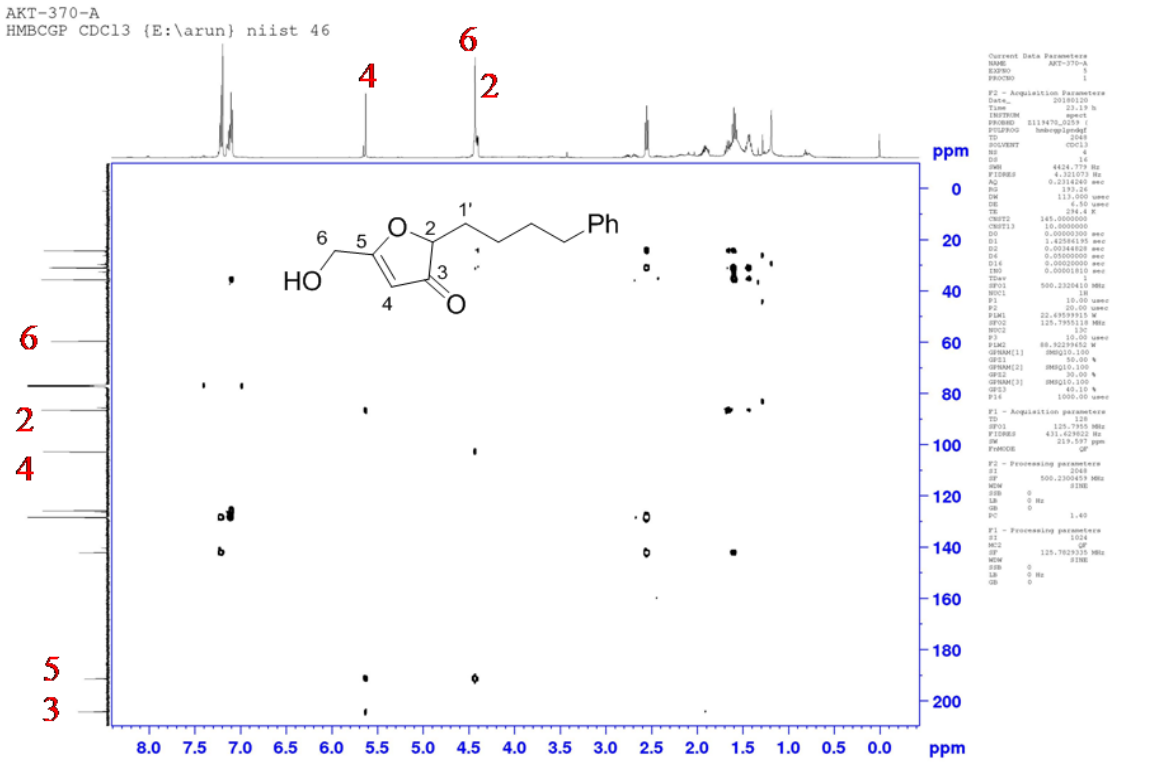

AKT-370-A
HMBCGP CDCl3 {E:\arun} niist 46
6
4
2
HO
O
Ph
ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

Figure 4.5.2.28. NMR spectras of compound I**V and V**

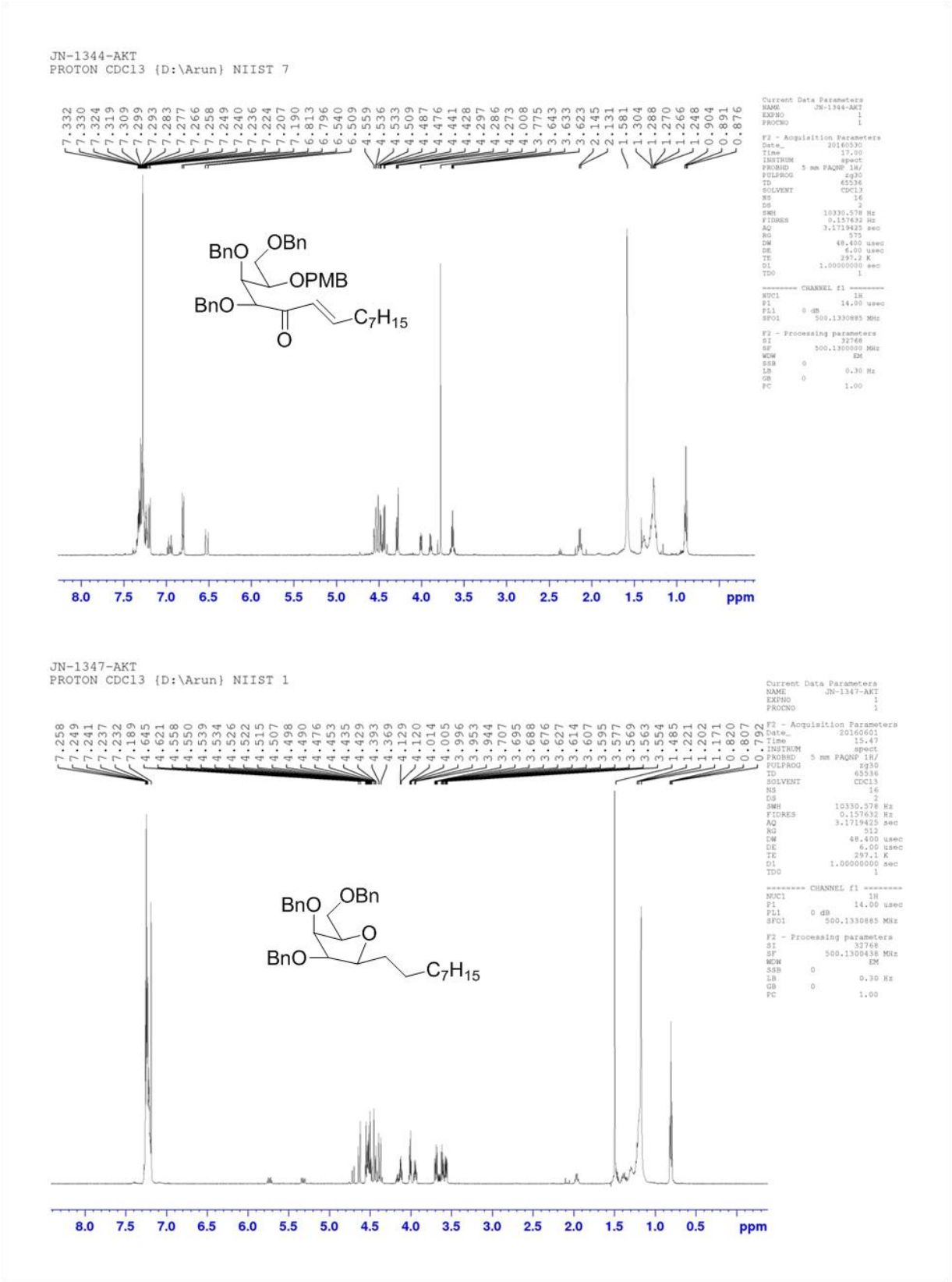


Figure 4.5.2.29. NMR spectras of compound **28**

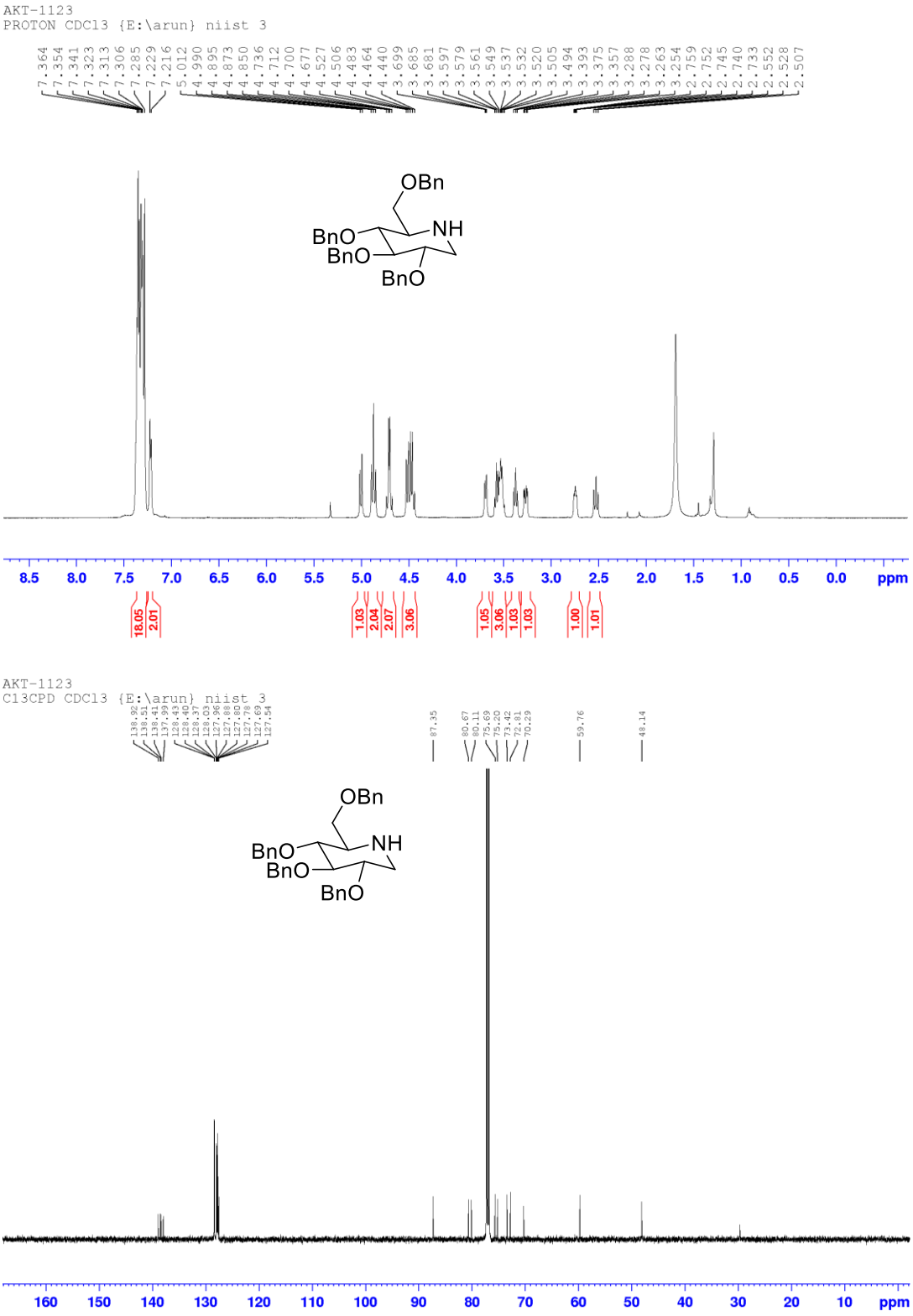


Figure 4.5.2.30. NMR spectras of compound **29b**

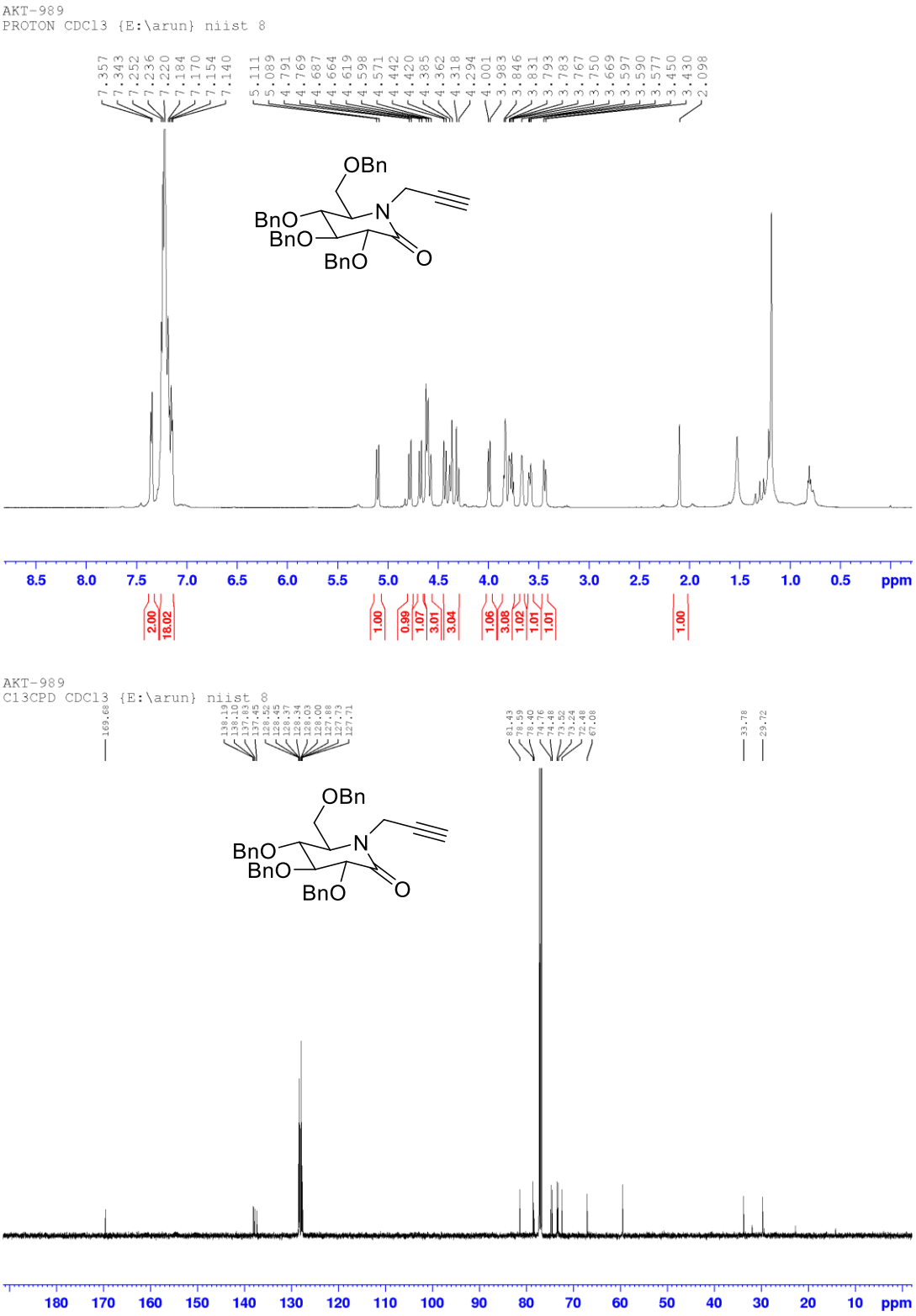


Figure 4.5.2.31. NMR spectras of compound **29c**

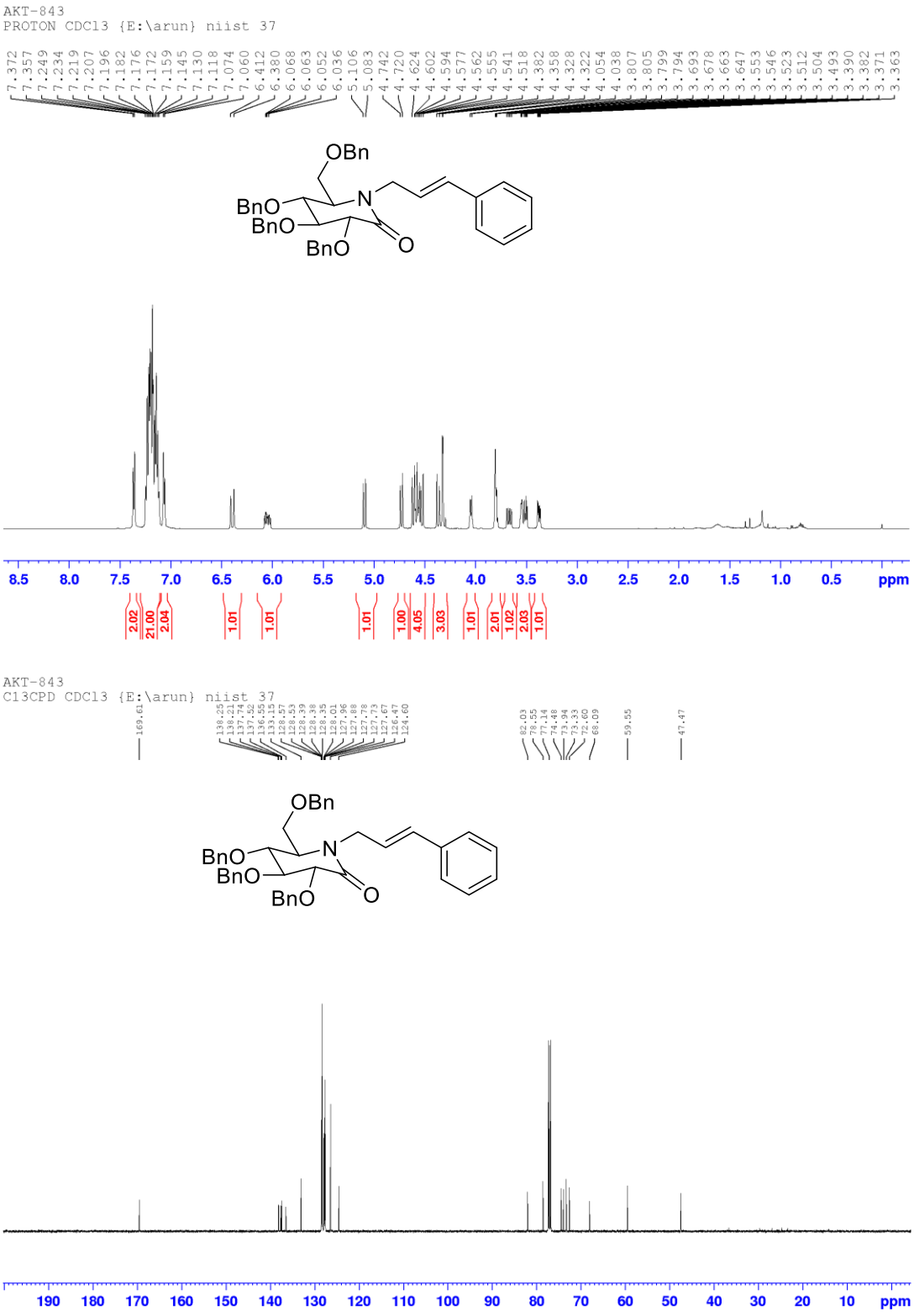


Figure 4.5.2.32. NMR spectras of compound **29d**

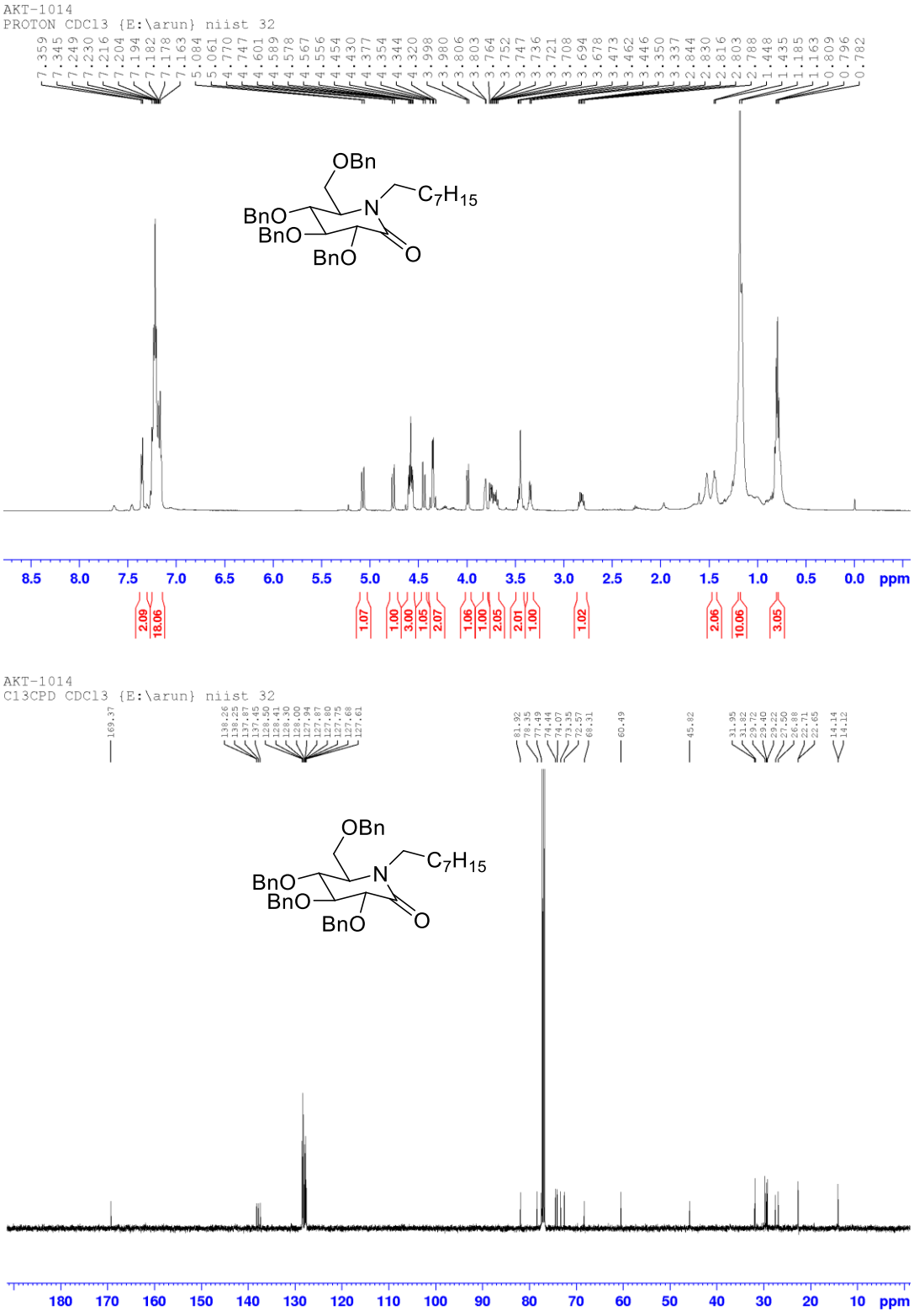


Figure 4.5.2.33. NMR spectras of compound **29e**

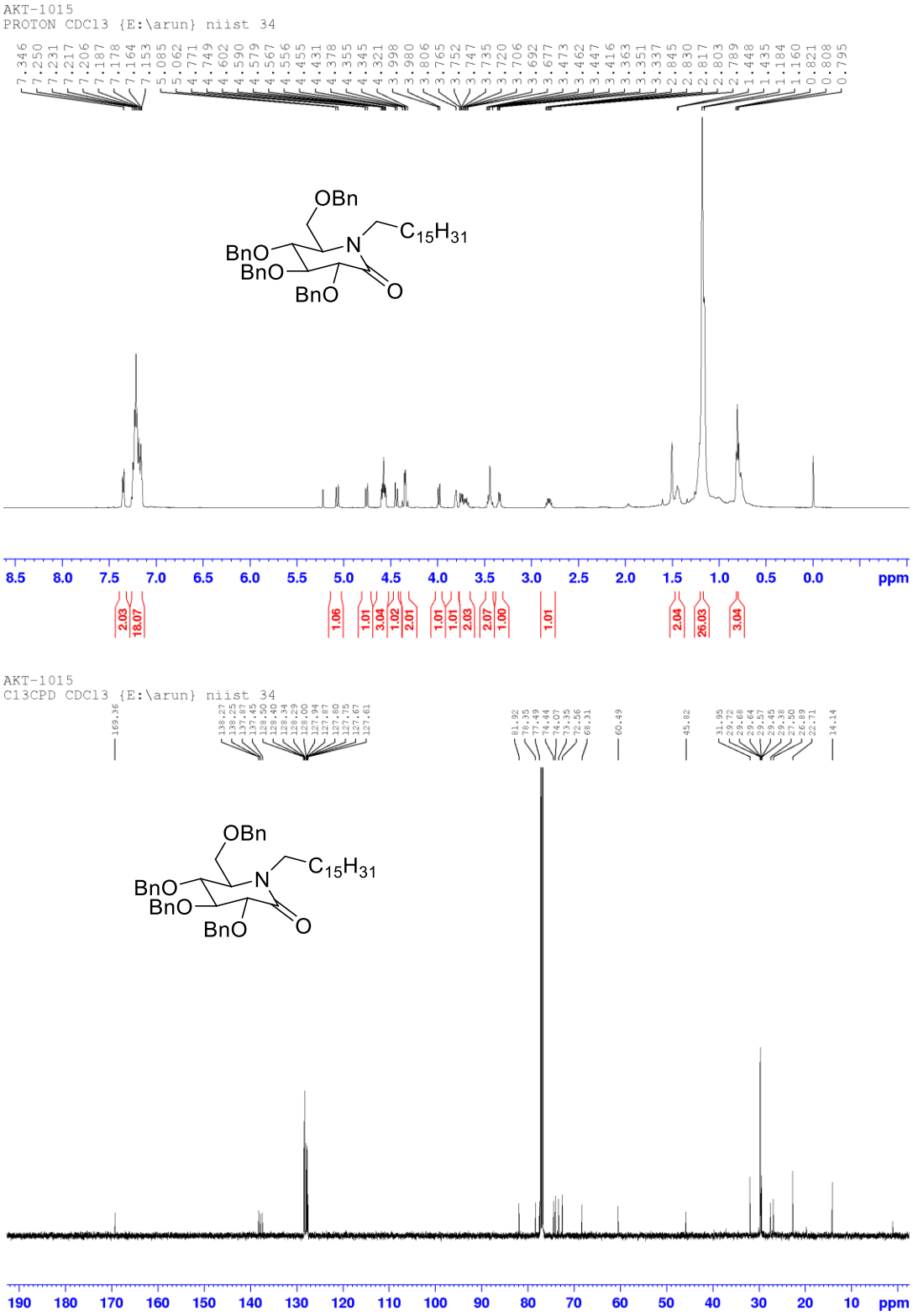
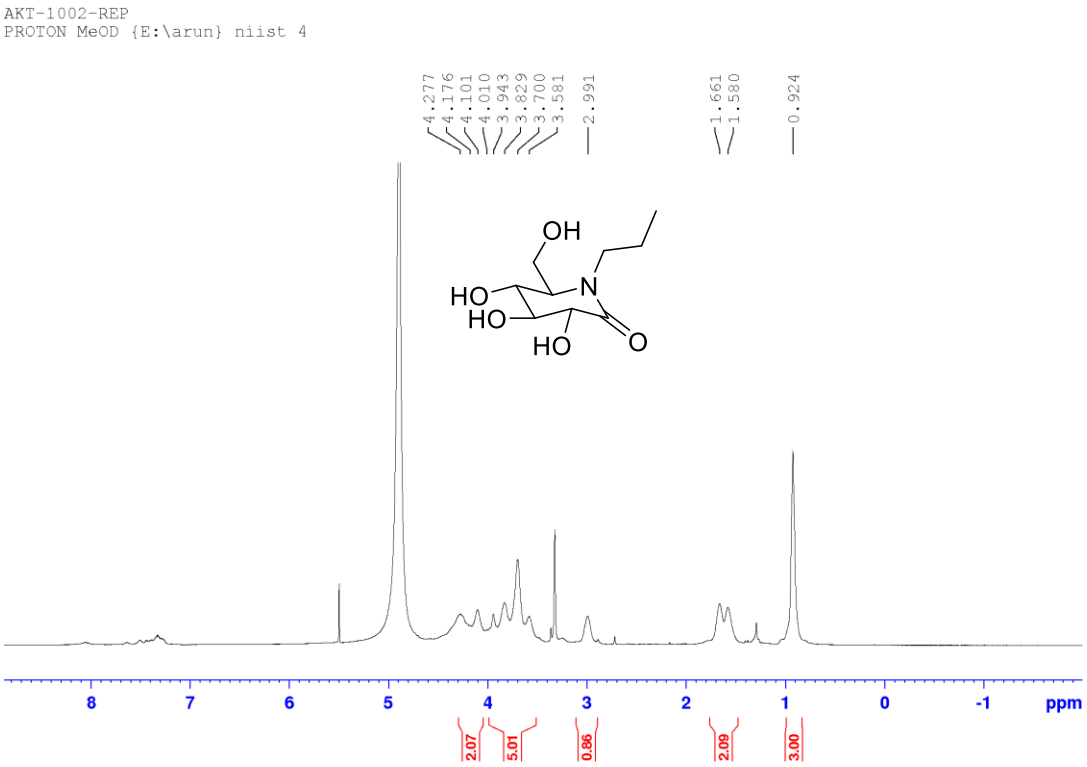


Figure 4.5.2.34. NMR spectras of compound **30b**



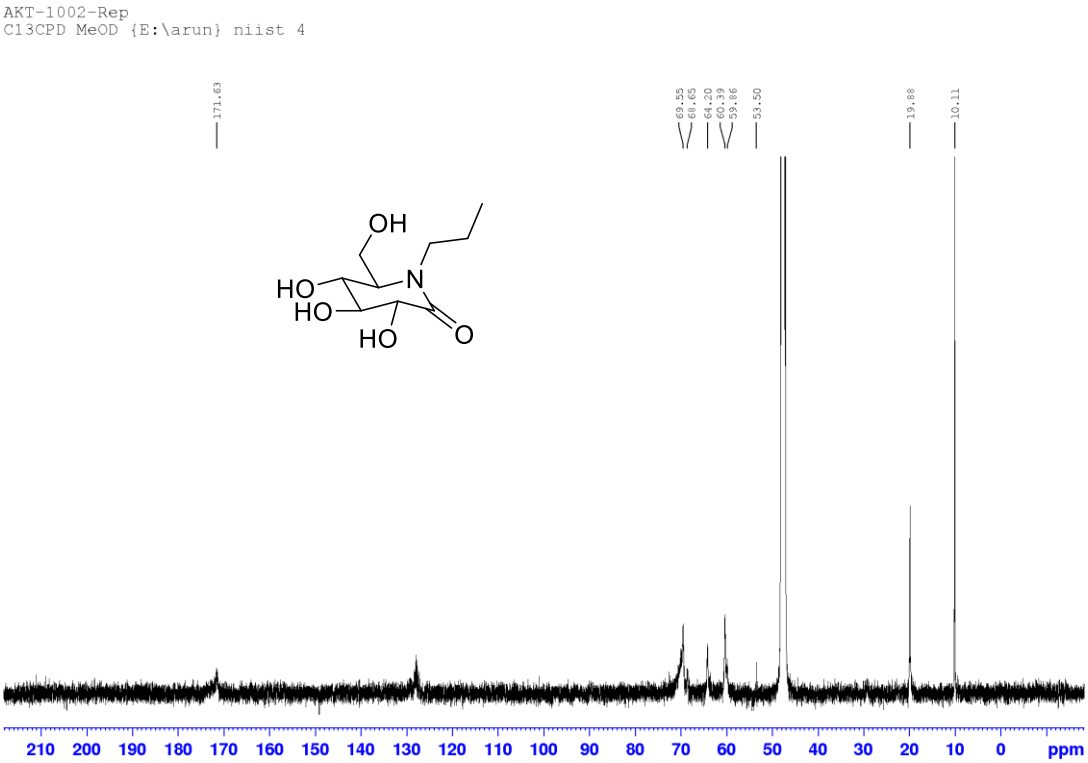


Figure 4.5.2.35. NMR spectras of compound **30c**

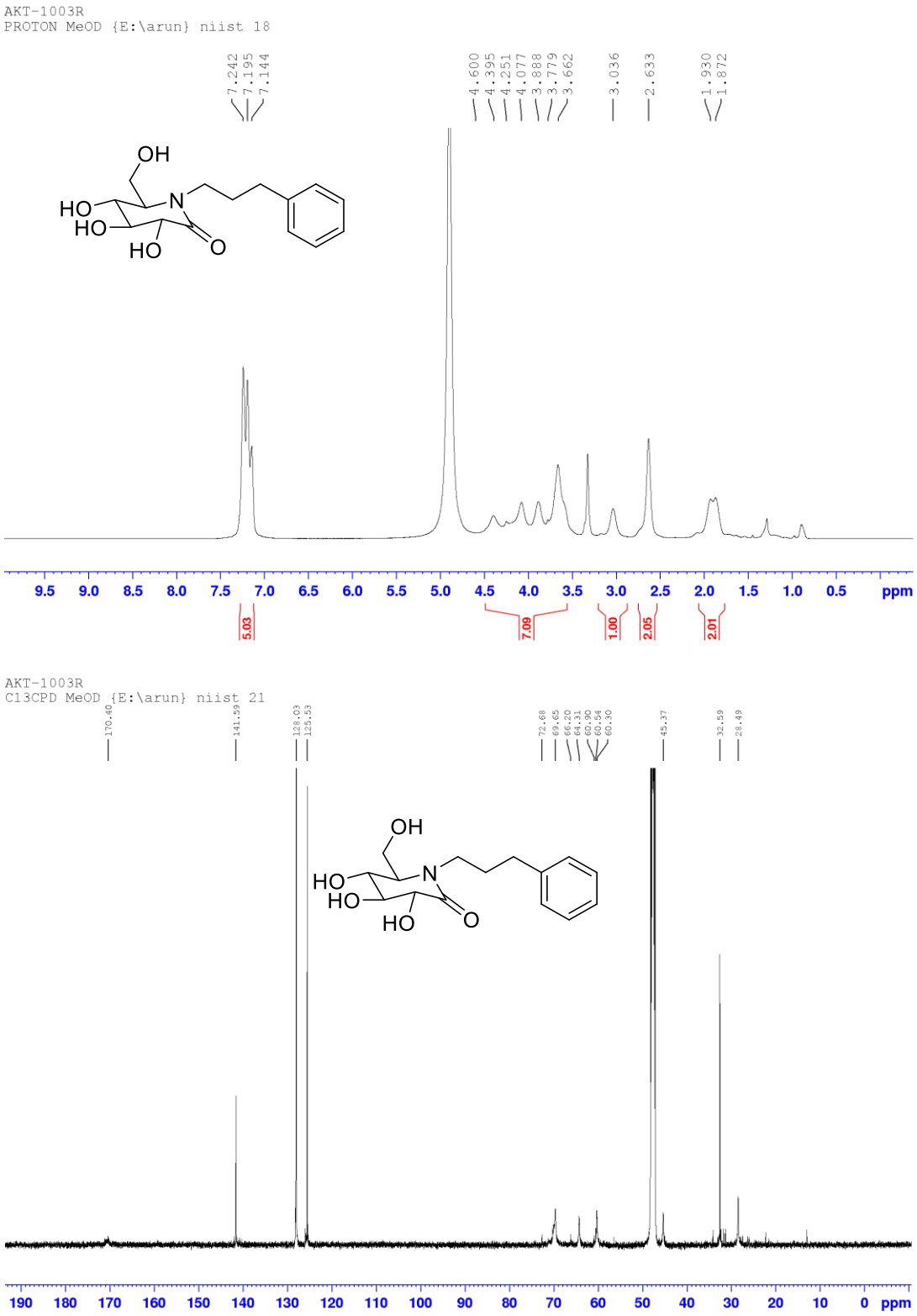


Figure 4.5.2.36. NMR spectras of compound **30d**

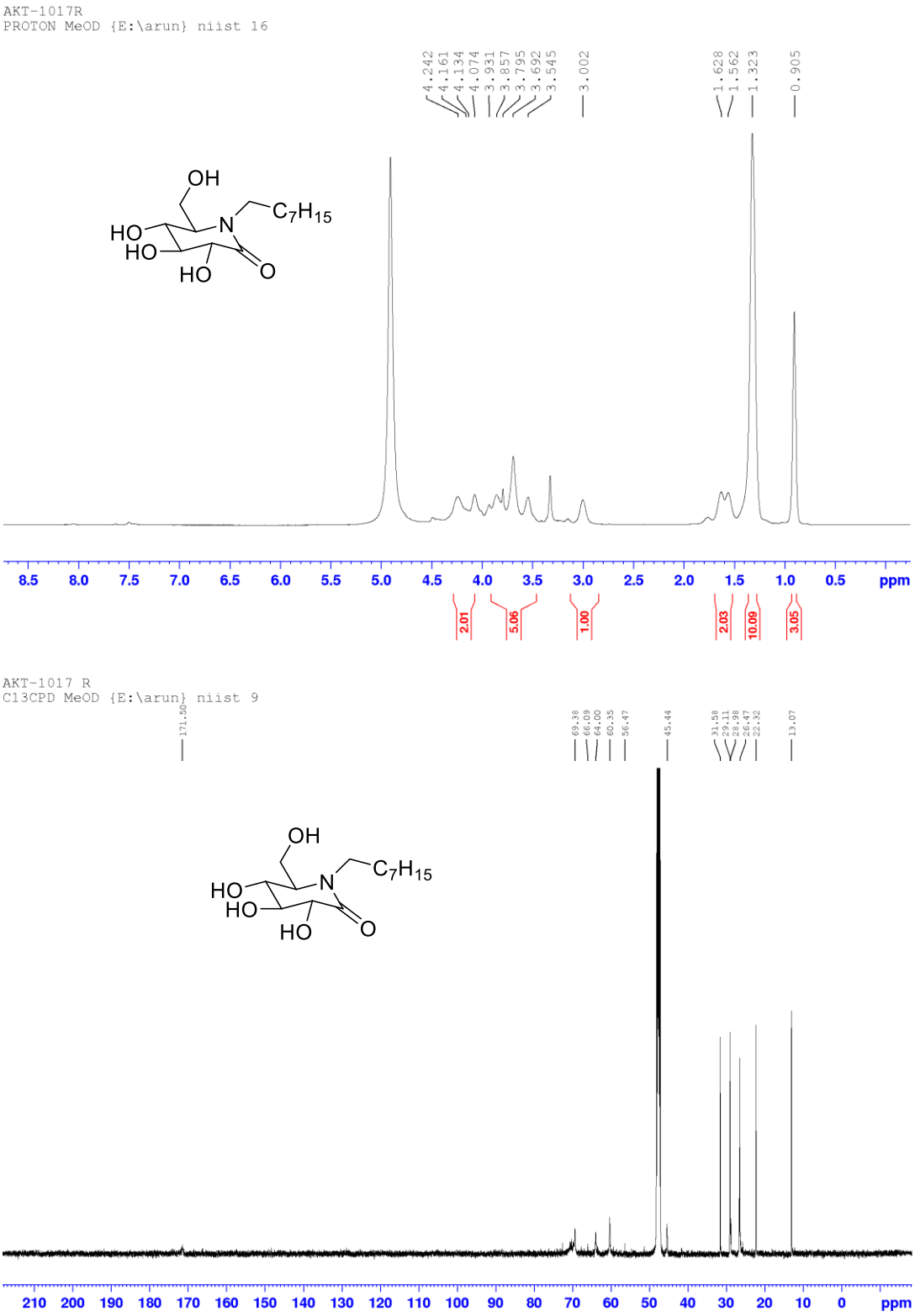


Figure 4.5.2.37. NMR spectras of compound **30e**

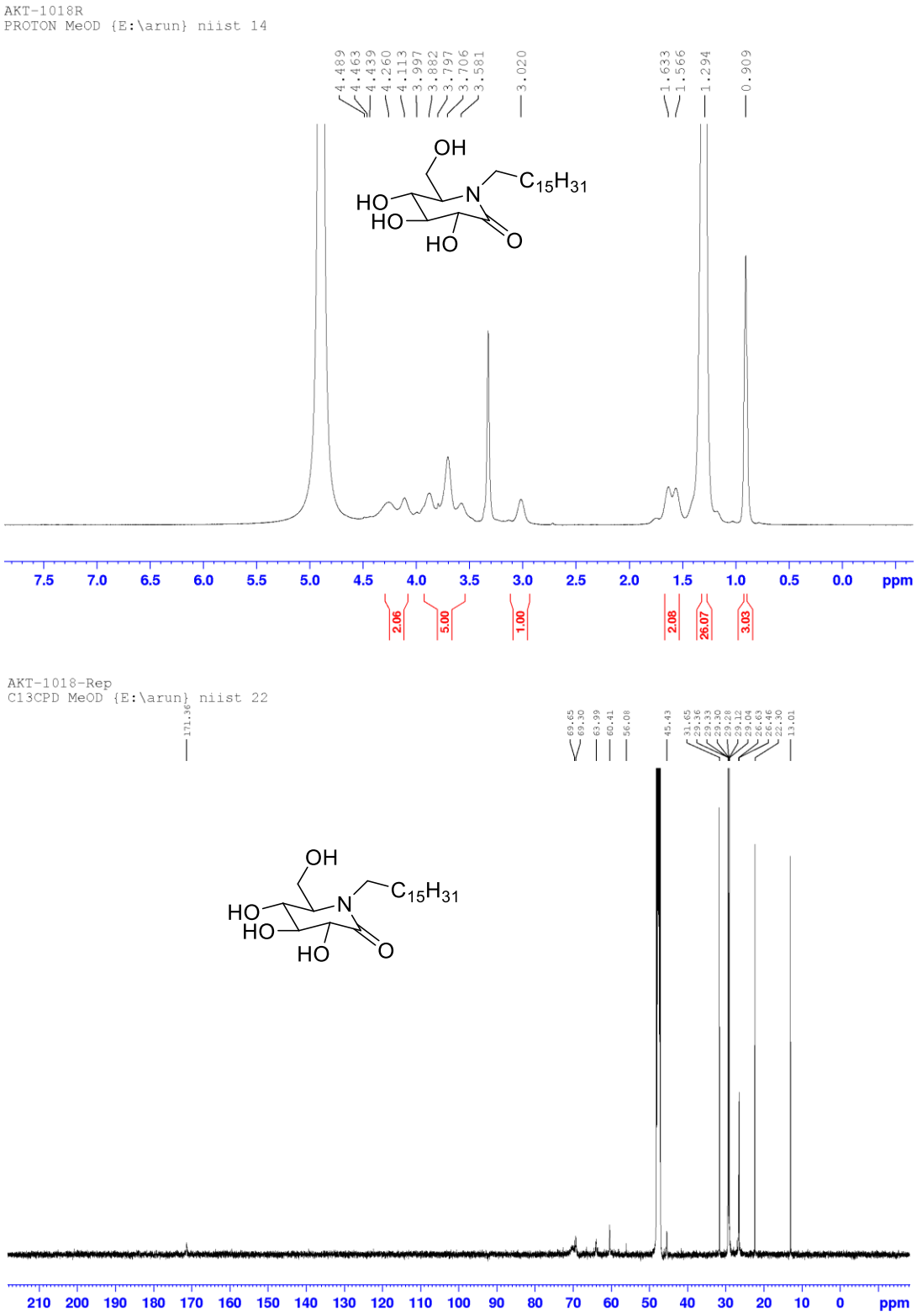


Figure 4.5.2.38. NMR spectras of compound **31b**

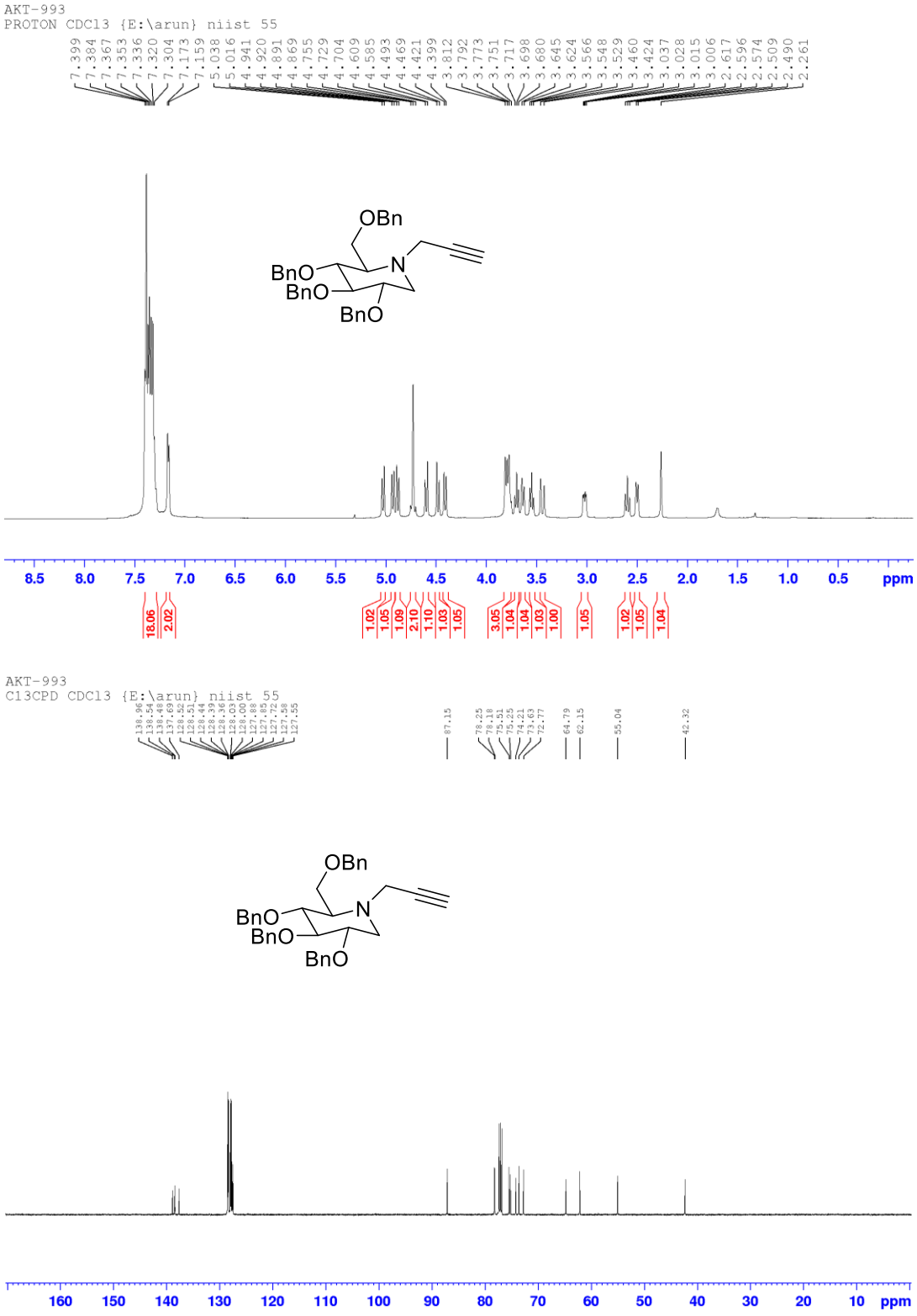


Figure 4.5.2.39. NMR spectras of compound **31c**

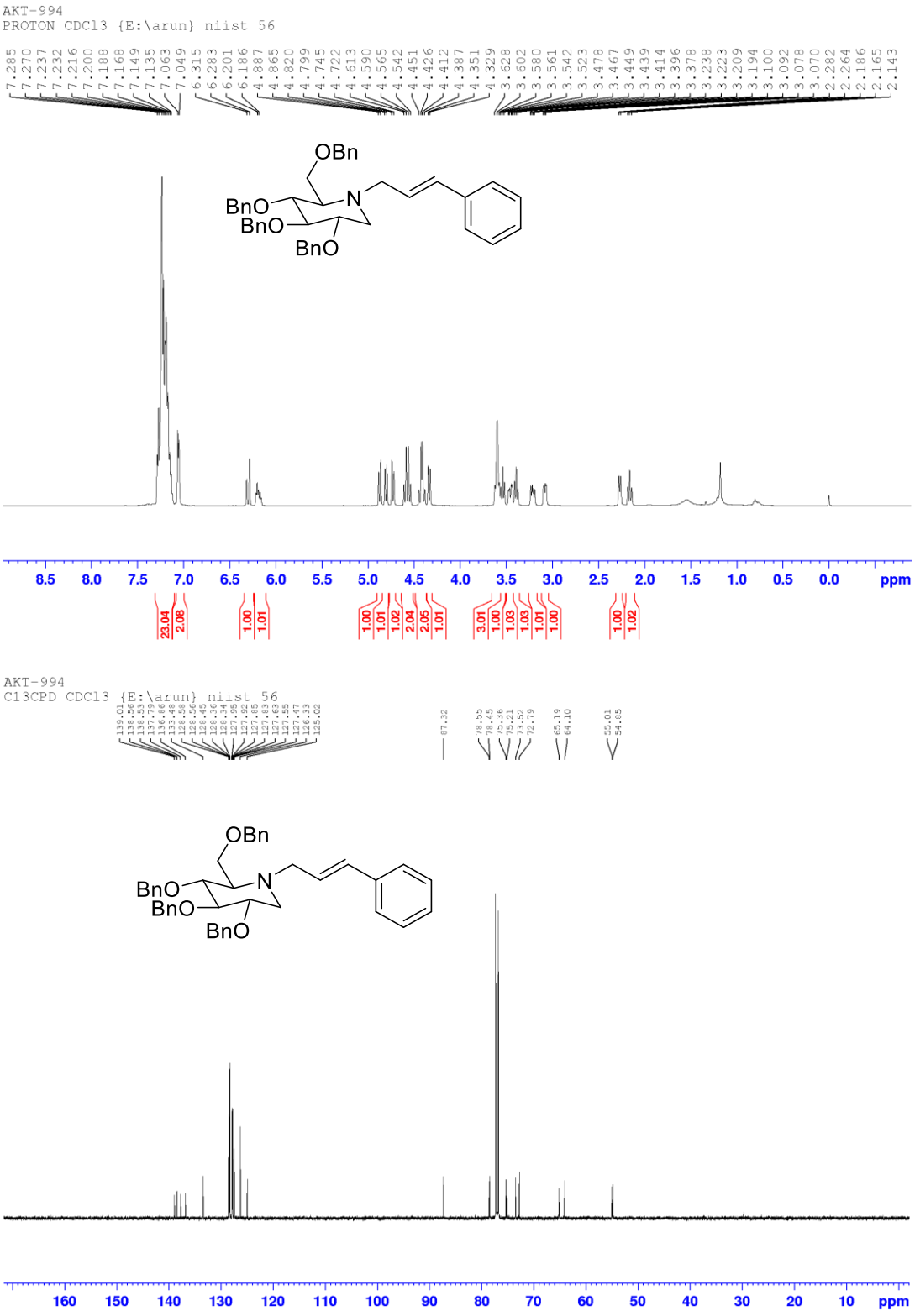


Figure 4.5.2.40. NMR spectras of compound **31d**

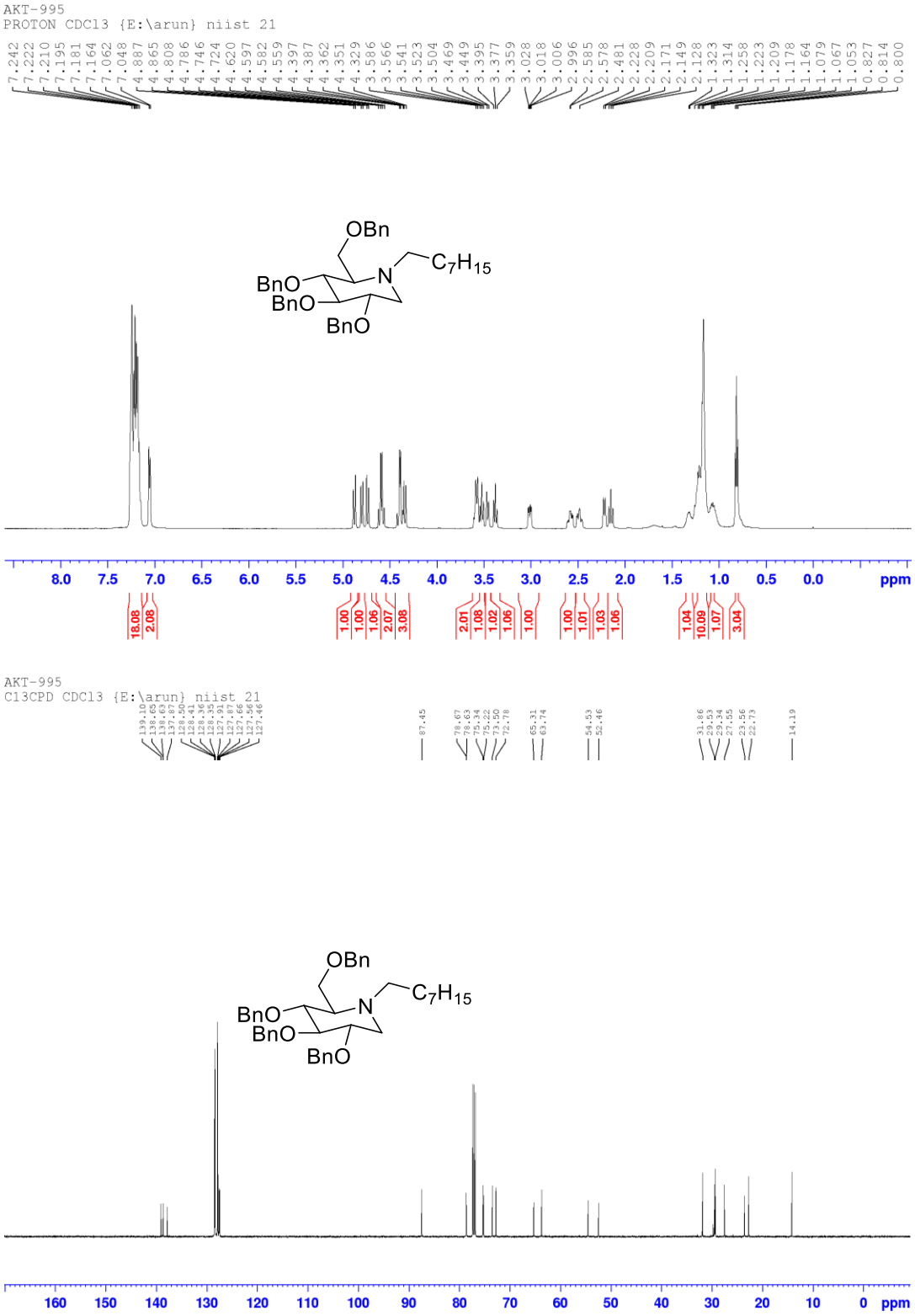


Figure 4.5.2.41. NMR spectras of compound **31e**

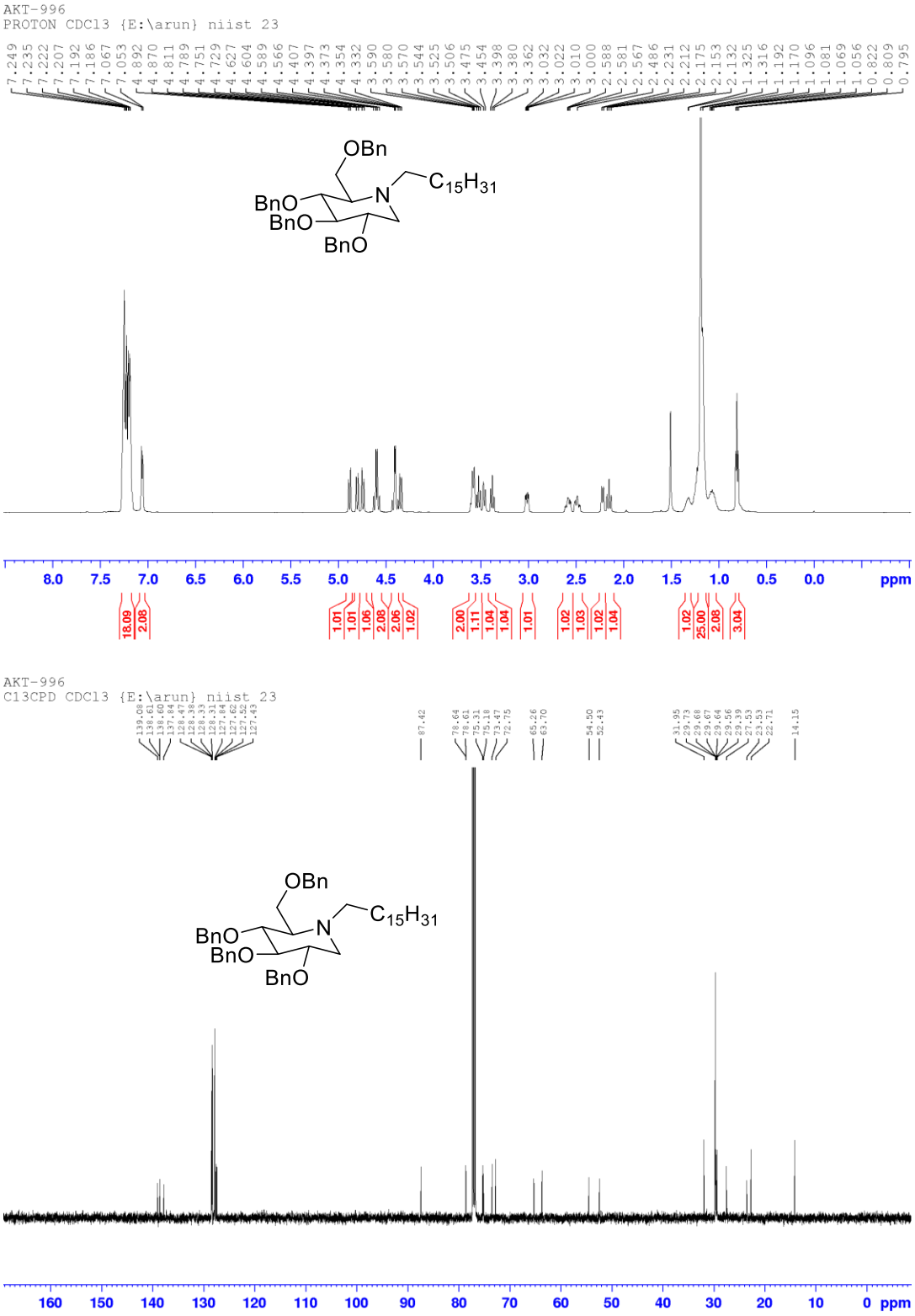


Figure 4.5.2.42. NMR spectras of compound **32b**

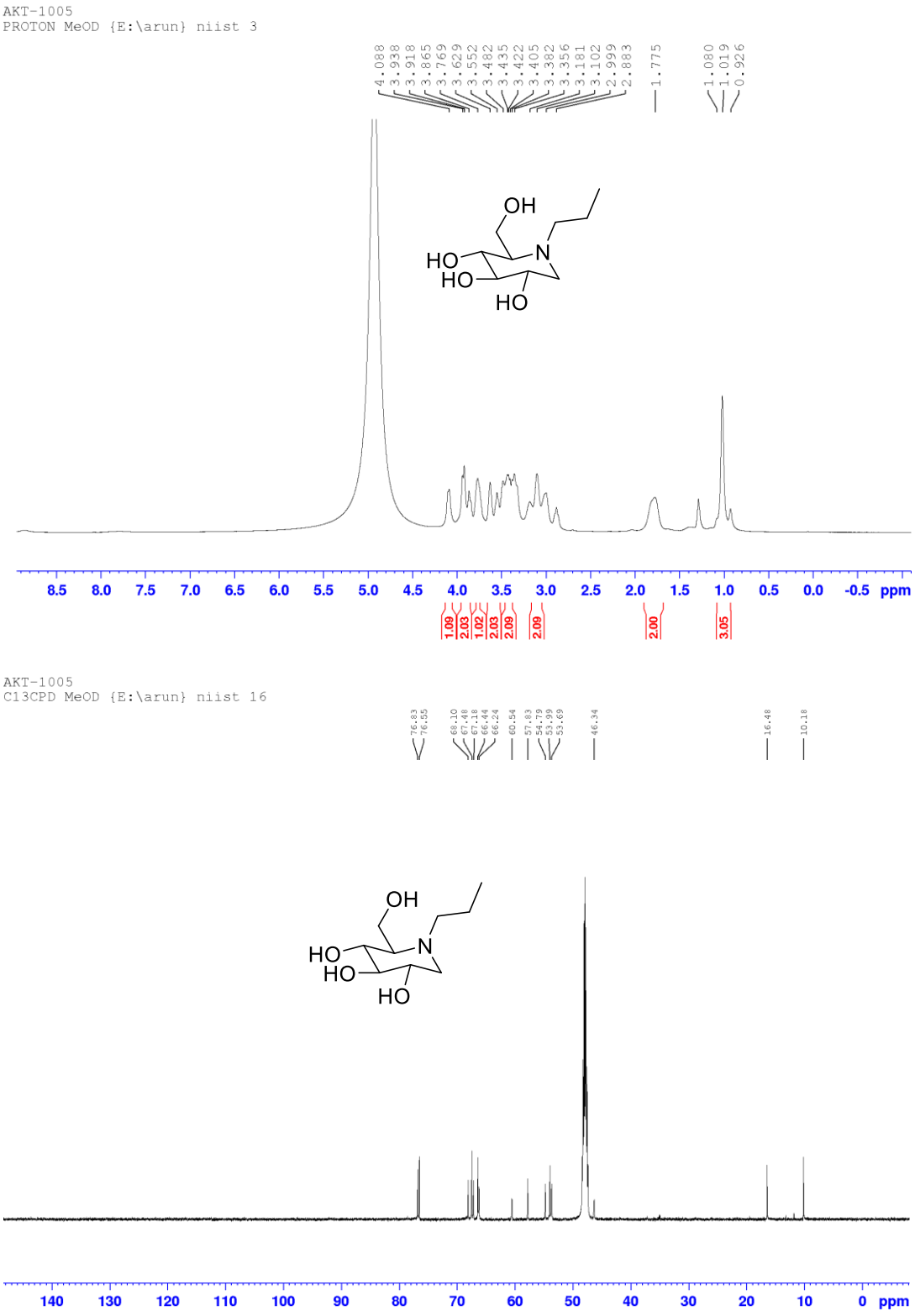


Figure 4.5.2.43. NMR spectras of compound **32c**

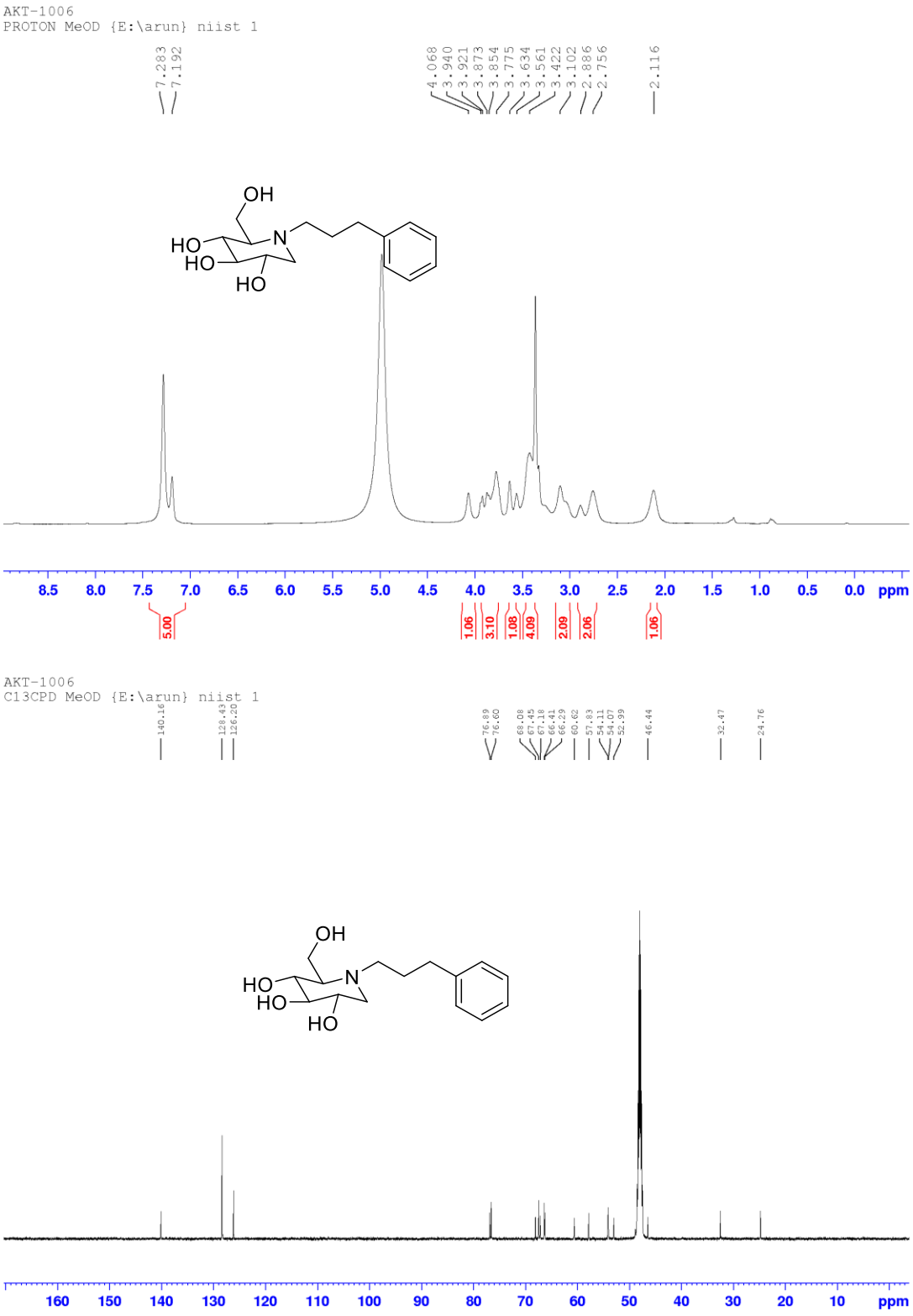


Figure 4.5.2.44. NMR spectras of compound **32d**

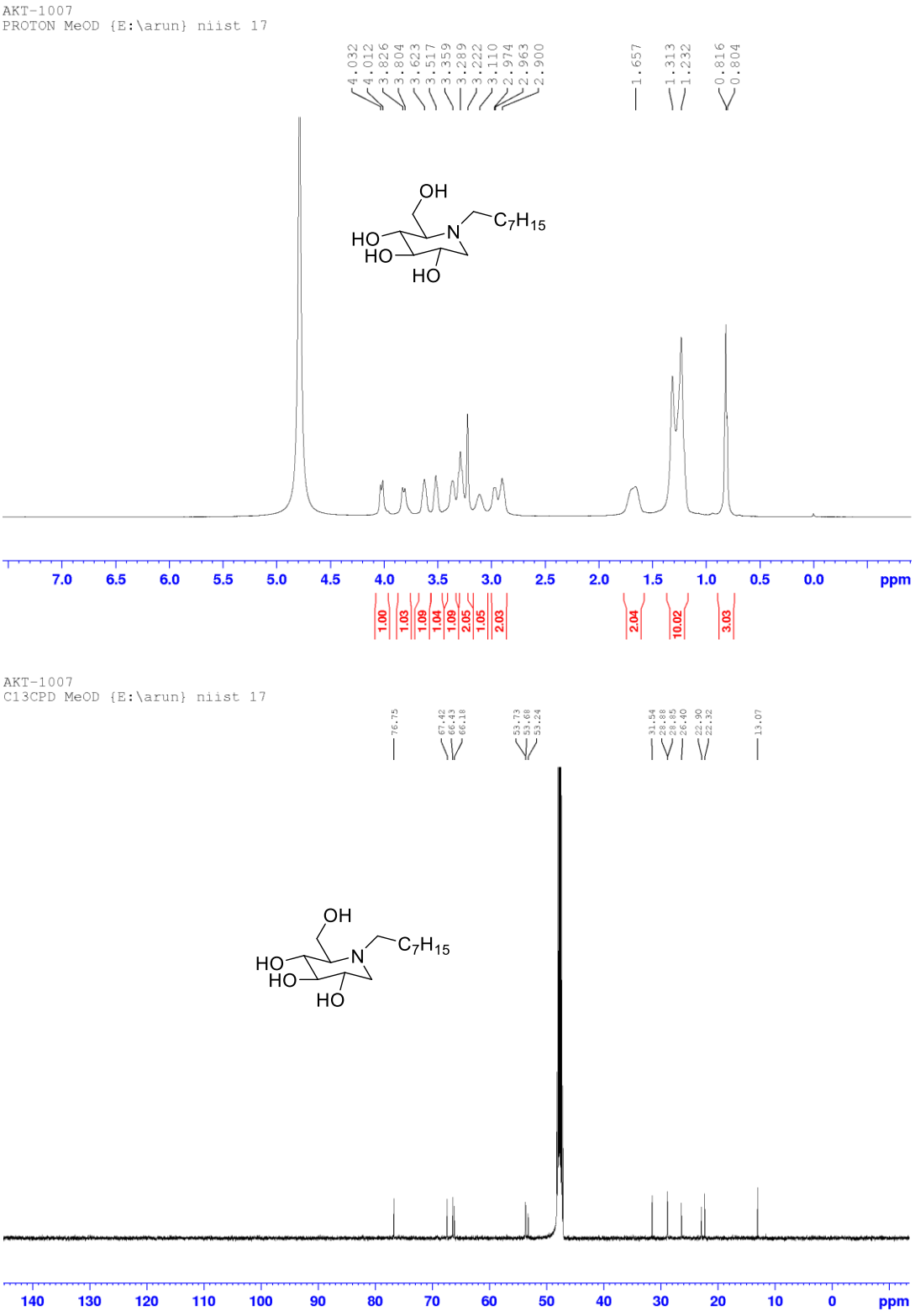


Figure 4.5.2.45. NMR spectras of compound **33e**

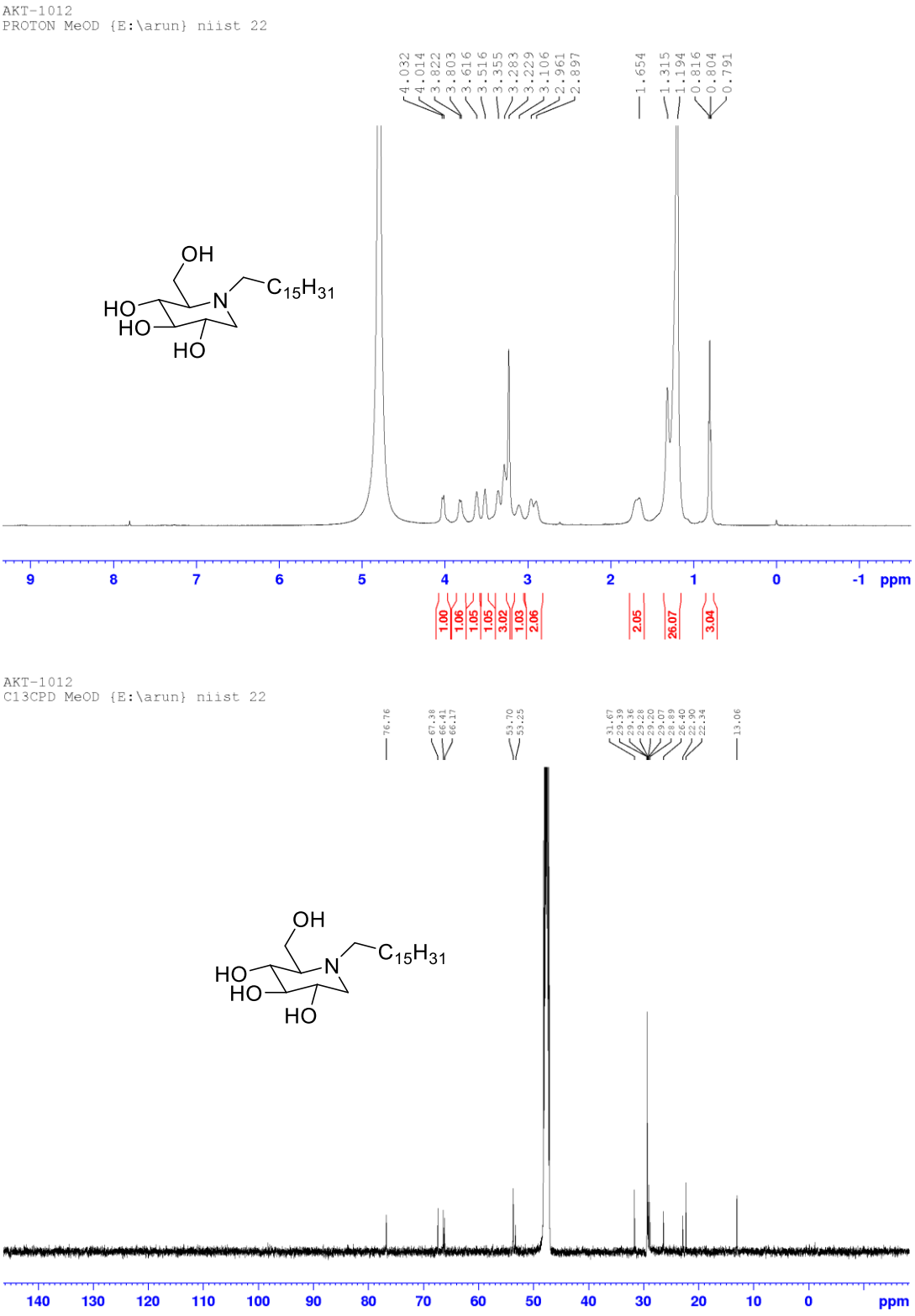


Figure 4.5.2.46. NMR spectras for precursor intermediate of compound **33**

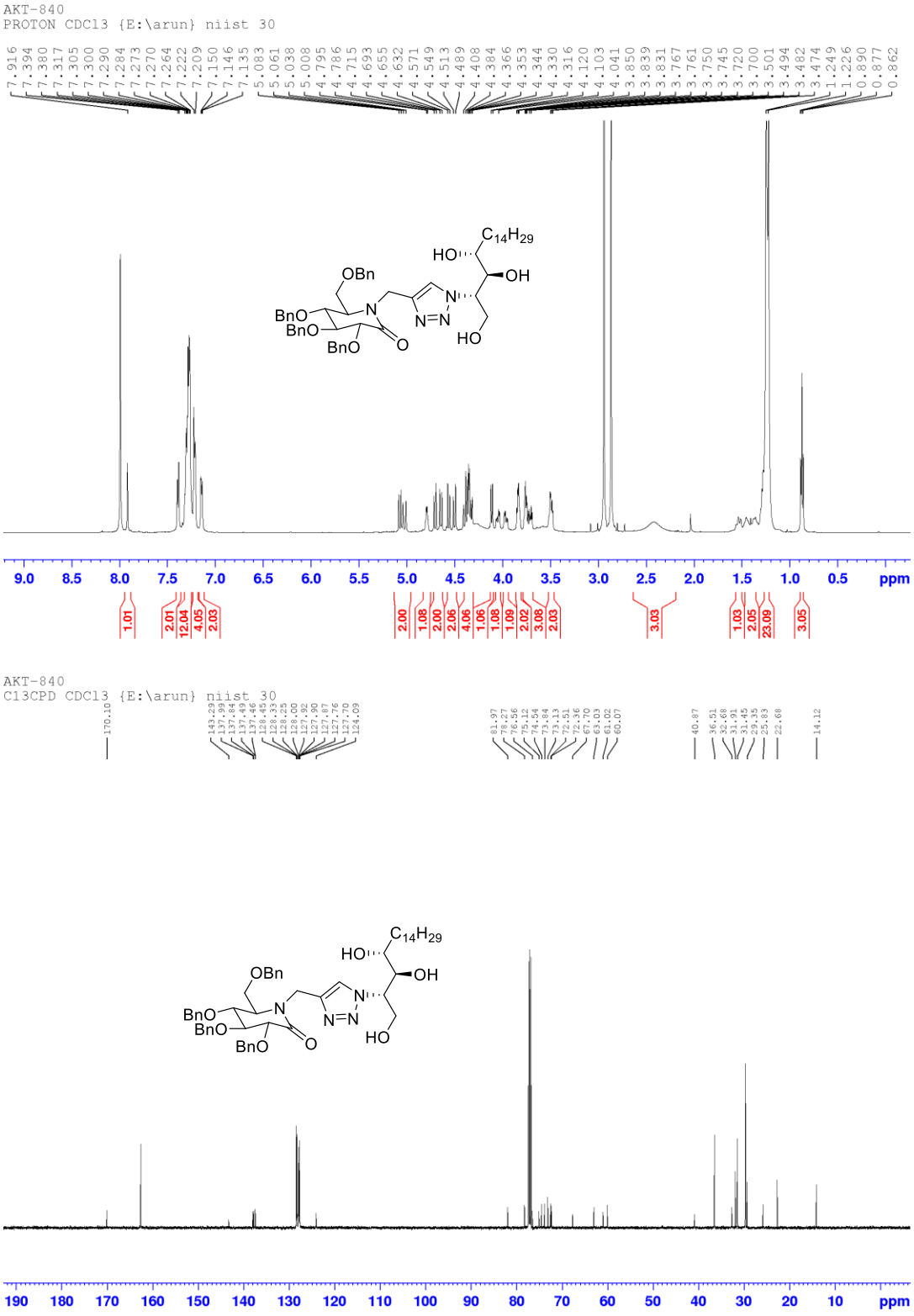


Figure 4.5.2.47. NMR spectras of compound **33**

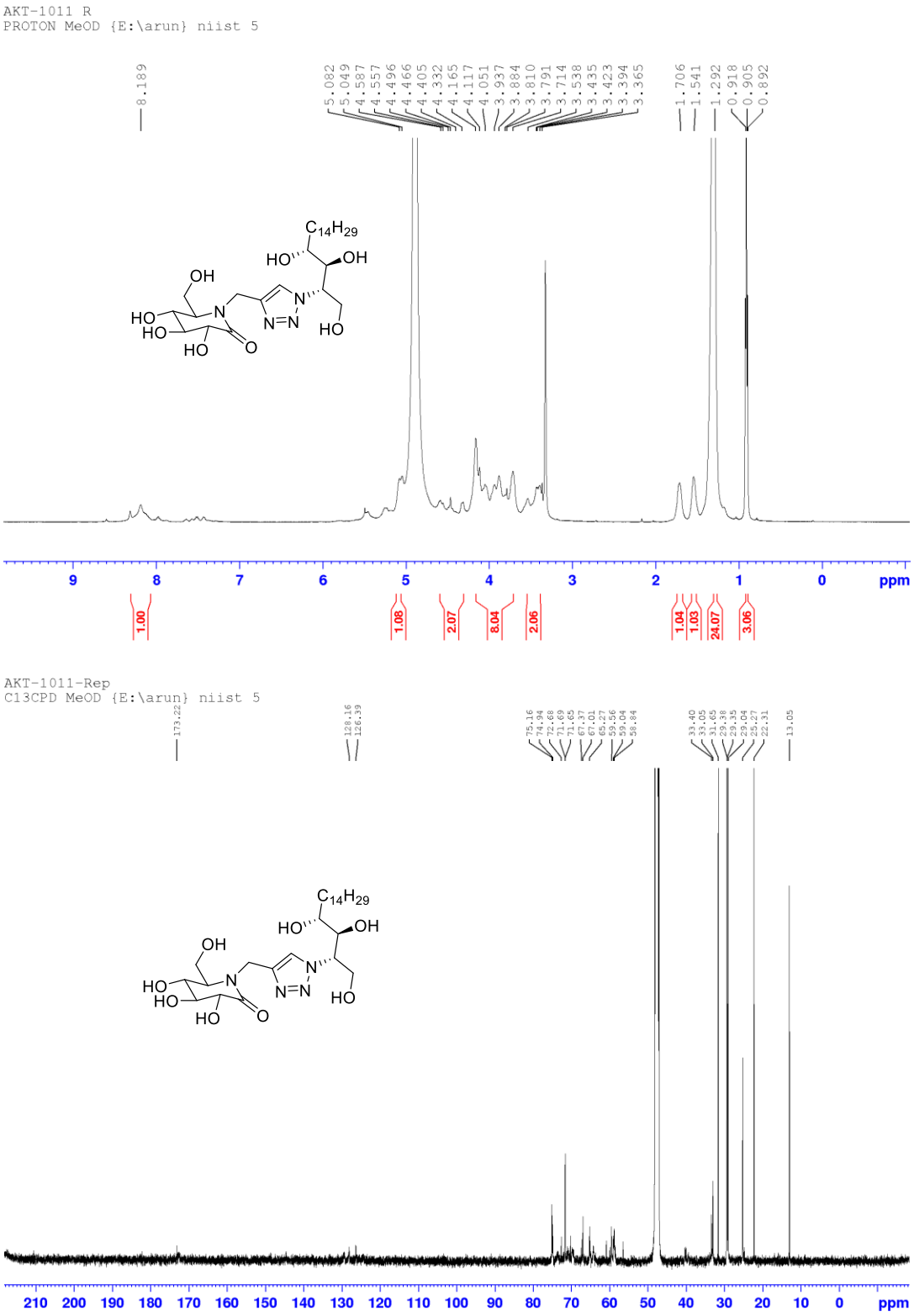


Figure 4.5.2.48. NMR spectras for precursor intermediate of compound **34**

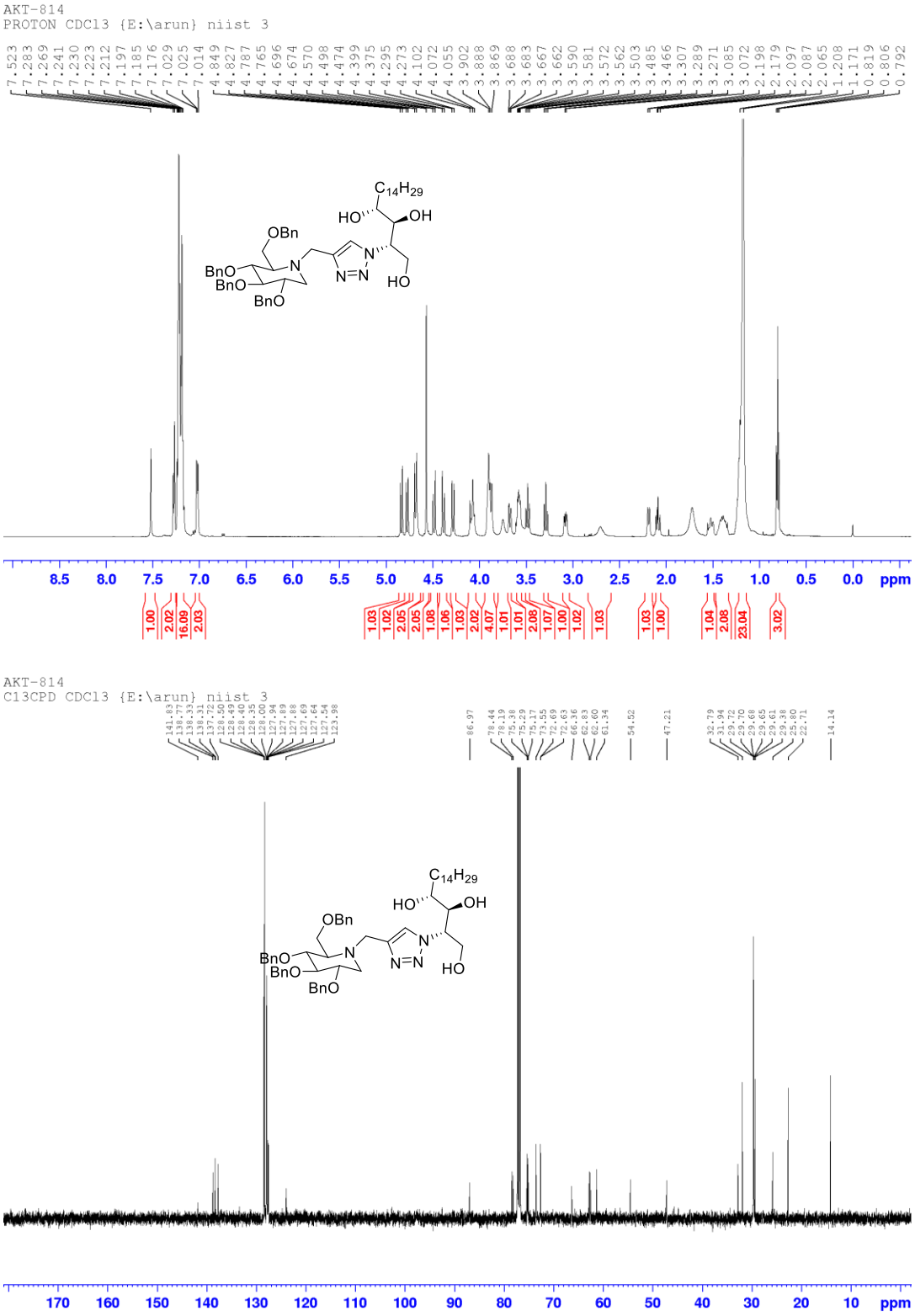
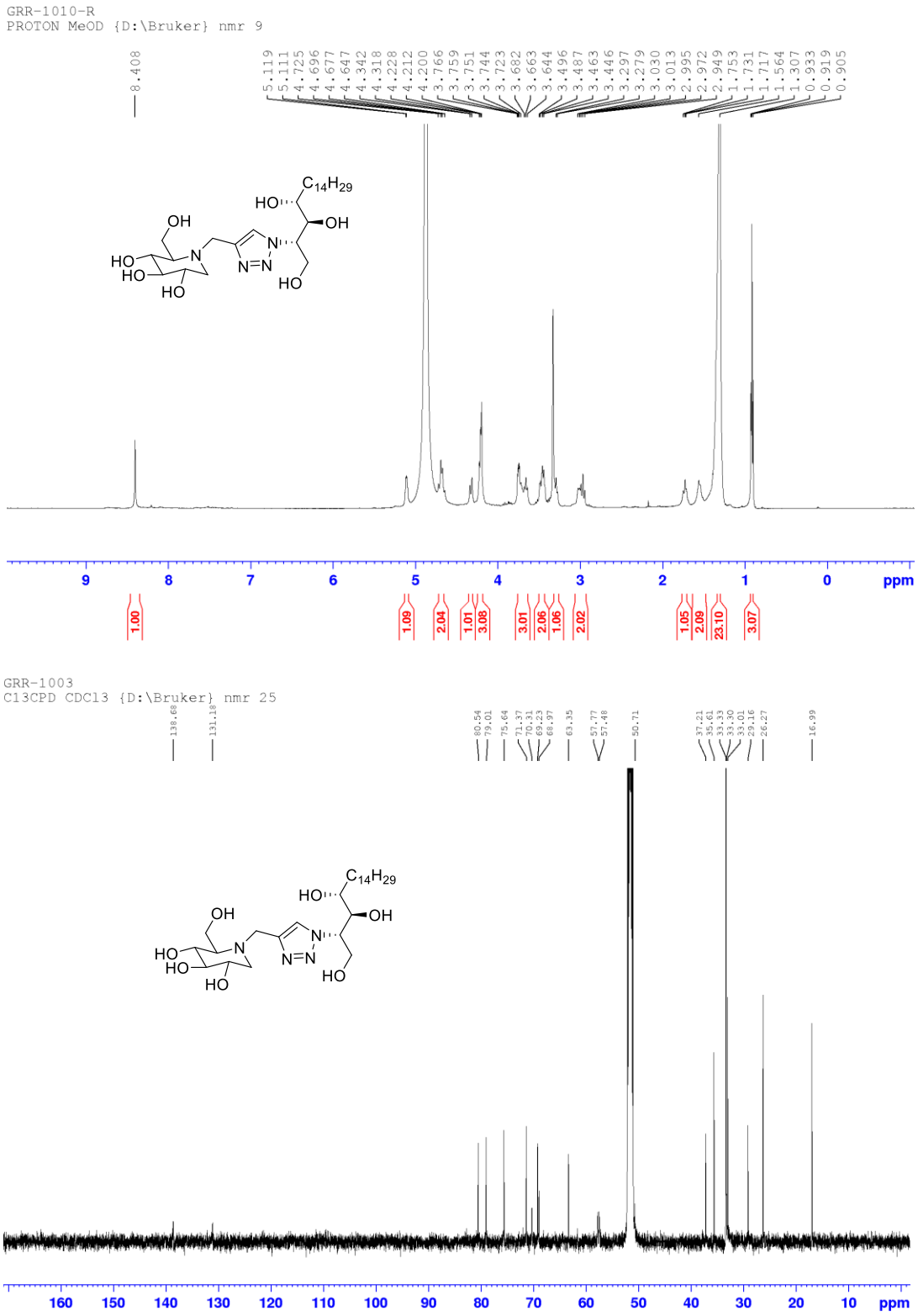


Figure 4.5.2.49. NMR spectras of compound **34**



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Azido-pyrrolopyrimidine: A recalcitrant substrate in CuAAC 'click' reaction, an efficient catalyst in Glaser-Hay reaction

5.1. Abstract

The focus of this chapter is homocoupling of terminal alkynes using 4-azido-5*H*-pyrrolo[3,2-*d*]pyrimidine as a catalyst. CuAAC's 'click' reaction with a 2-azidopyridine substrate is restricted by equilibrium with a tetrazole isomer, which is successfully used in the Glaser–Hay reaction. A catalytic combination of a 2-azidopyridine analogue, 4-azido-5*H*-pyrrolo[3,2-*d*]pyrimidine, and CuI produced homocoupled terminal alkynes without any trace of triazole product under mild conditions with a broad substrate scope. The utilization of iminosugar-based substrates in this chapter for homocoupling yielded good to excellent yields of 1,3-diynes.

5.2. Introduction

CuAAC, or copper(I)-catalyzed azide-alkyne cycloaddition, produces 1,4-disubstituted triazoles, which have several applications in chemistry, biology, and materials science.[1–5] Another interesting copper(I)-catalyzed reaction with technologically relevant applications in the field of materials research is the Glaser–Hay (GH) reaction, which takes place in the presence of *N,N,N′,N′* tetramethylethylenediamine/air and converts terminal acetylenes to symmetric 1,3-diynes via homo-coupling.[6–8] CuAAC and GH reactions are catalytic, necessitating the coordination of alkynes to Cu(I) species before the formation of electrophilic Cu(I)-acetylides.[1,6] Because of the similarities between the CuAAC and GH reactions, it is unclear if these two reactions will battle for the terminal alkyne. A work that exemplifies this possibility used excess alkyne with azide in

the presence of excess CuI and DIPEA in DMF, with the CuAAC product formed immediately by azide consumption and the residual alkyne undergoing a prolonged GH reaction.[9] This result proves that the CuAAC reaction is significantly faster than the GH reaction.

Despite the CuAAC reaction's versatility, which can 'click' any scaffold with a wide range of functional group endurance, 2-azidopyridine is an exception. Because 2-azidopyridine and tetrazolo[1,5-*a*]pyridine are in equilibrium at ambient temperature, the CuAAC reaction has poor efficiency, especially under polar solvents.[10,11] There was no result from a CuAAC reaction of a tetrazolo[1,5-*c*]quinazoline with phenylacetylene.[12] When reaction conditions that could change the equilibrium to azido form, such as high temperature, non-polar solvents, or extended reaction times, were adjusted, triazole was generated.[13–18] Bolje *et al.* identified more by-products such as homocoupled 1,3-diyne and 1,5-disubstituted 1,2,3-triazole derivatives associated with prolonged warming in toluene with 2-azidopyridine substrates in the CuAAC process.[16] In other words, in the presence of Cu(I), a 2-azidopyridine in equilibrium with its tetrazole isomer can undergo the GH reaction instead of the CuAAC 'click' reaction. There are sophisticated GH approaches for the synthesis of 1,3-diynes from terminal alkynes catalyzed by CuI with broad substrate scope in good to excellent yields by changing the ligands and bases and operating under mild conditions.[19–21] Several GH methods demonstrate the synthesis of 1,3-diynes with carbohydrate substrates coupled to terminal alkynes, however, the substrate scope expansion is lacking.[20,22–24] The current study establishes the utilization of a 2-azidopyrimidine analogue, 4-azido-5*H*-pyrrolo[3,2-*d*]pyridine (PP-N_3, Figure 5.2.1a), in the presence of a catalytic CuI as a good strategy for GH reaction with emphasis on iminosugar-based substrates.

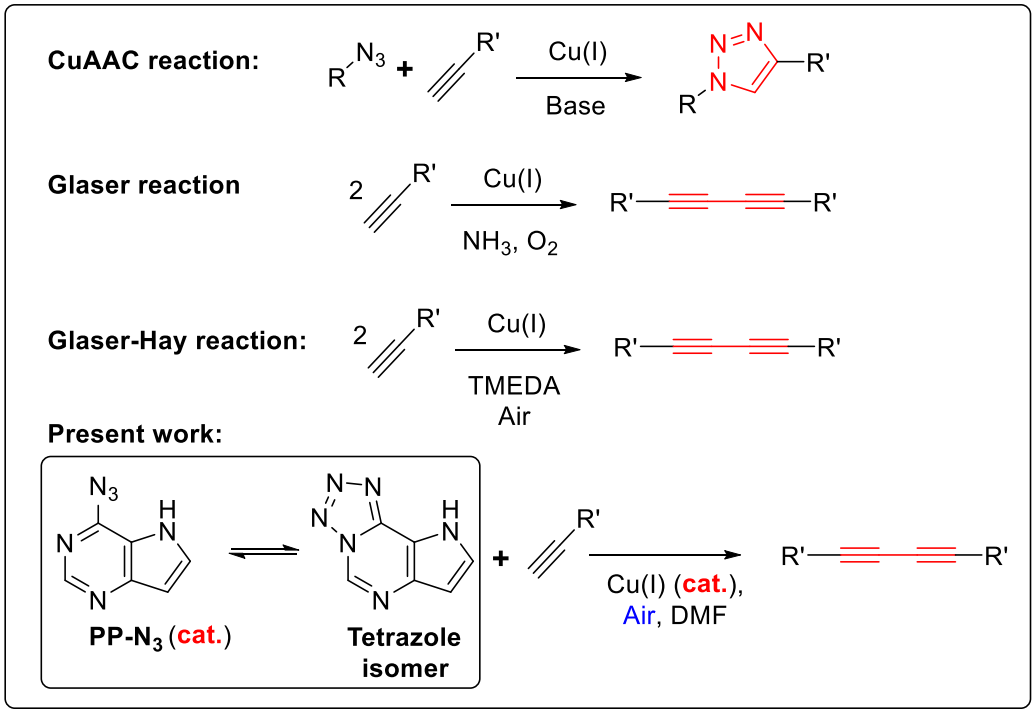


Figure 5.2.1a. Cu(I)-catalysed CuAAC reaction, Glaser reaction, Glaser-Hay reaction and present work.

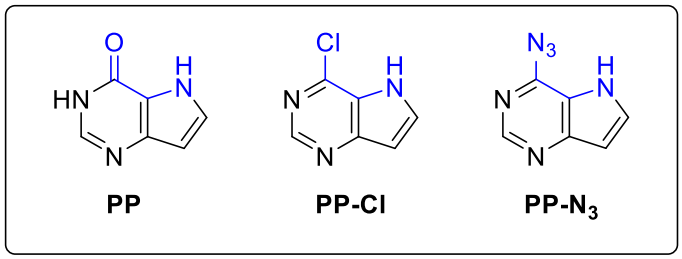
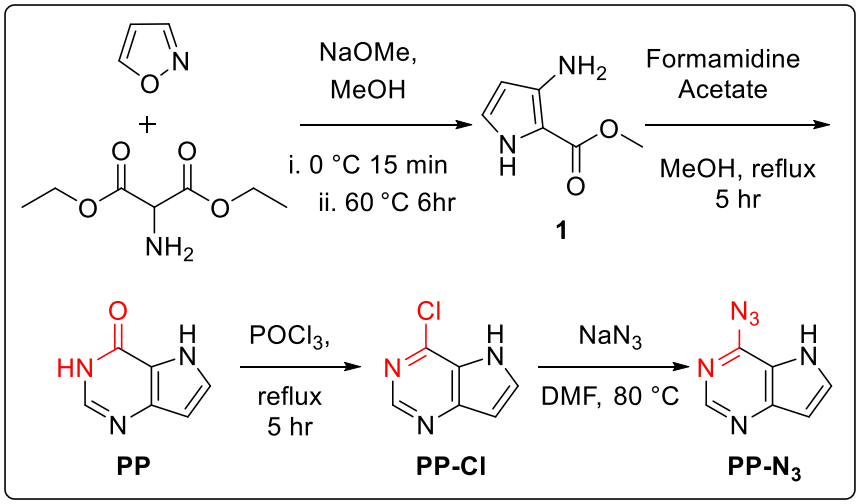


Figure 5.2.2a. Pyrrolopyrimidines used in the present study.

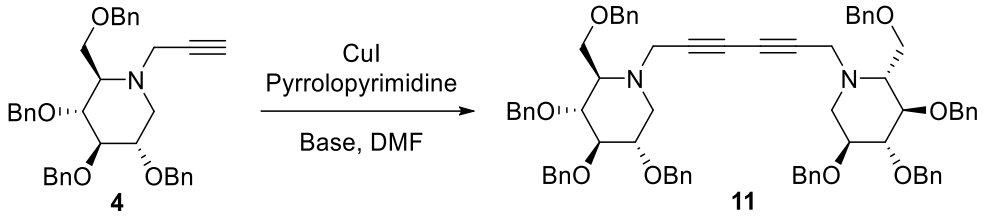
5.3. Results and Discussion

Although the typical CuAAC reaction uses a mixture of $CuSO_4$ and sodium ascorbate, the presence of the reducing agent ascorbate with oxygen fuels reactive oxygen species.[1] As a result, in our first reaction, we employed a CuAAC condition with excess CuI and DIPEA, which our lab has previously used to synthesise a phytosphingosine conjugate of 2-pyrrolidinone triazole product.[25] We chose DMF as the solvent but designed our first attempt at 0 °C after learning that warming a phenylethynylcopper(I) intermediate in DMF at reflux for 24 hours generated 1,3-diyne in the literature.[26] The use of N-propargylated deoxynojirimycin **4** (Table 5.3.1a) in the GH reaction with **PP-N$_3$** was driven by our interest in the synthesis of iminosugar derivatives. In a different study, **PP-N$_3$** was designed and synthesized of immucillins BCX-1777 and BCX-4430. Standard procedures were used to synthesise **PP-N$_3$** from 9-deazahypoxanthine (**PP**), with **PP-Cl** acting as an intermediate along the route (Scheme 5.3.1a).[27,28]



Scheme 5.3.1a. Synthesis of **PP**, **PP-Cl,** and **PP-N$_3$**.

Table 5.3.1a. Reaction optimization.[a]



Entry	Catalyst (equiv)	Pyrrolopyrimidine (equiv)	Base (equiv)	Temp (°C)	Yield **11** (%)[f]
1	CuI (2)	**PP-N$_3$** (1)	DIPEA (3)	0	74
2	CuI (2)	**PP** (1)	DIPEA (3)	0	12
3	CuI (2)	**PP-Cl** (1)	DIPEA (3)	0	11
4	CuI (2)	---	DIPEA (3)	0	trace
5	CuI (2)	**PP-N$_3$** (0.5)	DIPEA (3)	0	79
6	CuI (2)	**PP-N$_3$** (0.1)	DIPEA (3)	0	79
7	CuI (2)	**PP-N$_3$** (0.1)	-	0	81
8	CuI (0.2)	**PP-N$_3$** (0.1)	-	0	83
9	CuI (0.2)	**PP-N$_3$** (0.1)	-	rt	86
10	CuI (0.2)	**PP-N$_3$** (0.1)	-	100	85
11[b]	CuI (0.2)	**PP-N$_3$** (0.1)	-	rt	82
12[c]	CuI (0.2)	**PP-N$_3$** (0.1)	-	rt	0

13[d]	CuI (0.2)	**PP-N_3** (0.1)	-	rt	0
14[e]	CuI (0.2)	**PP-N_3** (0.1)	-	rt	trace

[a] Reaction conditions: To a solution of alkyne compound **4** (30-50 mg) in DMF (1 mL), CuI and **PP-N_3** were added successively and the reaction takes 5-6 hrs for complete consumption of the starting material. [b] Open air. [c] Solvent – MeOH. [d] Solvent – CH_2Cl_2. [e] Solvent – CH_3CN. [f] Isolated yields.

In our initial GH reaction, a clean transformation to a homocoupled symmetric 1,3-diyne product **11** in 74% yield was detected over TLC as a polar product, with no trace of any triazole adduct (entry 1, Table 5.3.1a) (entry 1, Table 5.3.1a). The absence of PP-N_3 and the use of the other two pyrrolopyrimidines, PP and PP-Cl, resulted in a considerable decrease in reaction yield (entries 2-4), with a large amount of unreacted terminal alkyne; hence, PP-N_3 plays a key part in this homocoupling process. By lowering the equivalents of PP-N_3 to catalytic levels, the yield of compound **11** was slightly increased (entries 5-6). Compound **11** was produced in 81-83% yield in the absence of DIPEA (entry 7-8), while compound **11** was obtained in 86 and 85% yields, respectively, by utilising catalytic amounts of CuI and PP-N_3 and repeating the reaction at room temperature or 100 °C (entry 9-10). All of the above-mentioned reactions were carried out with no strict controls, i.e. by just halting the RB and using undistilled solvents; an open-air condition generated compound **11** in an 82% yield (entry 11). However, the process was delayed in the solvents methanol, dichloromethane, and acetonitrile (entries 12-14). Accordingly, in the absence of a base and under open-air circumstances, the homocoupling of terminal alkyne **4** in DMF with catalytic amounts of CuI and PP-N_3 may proceed at room temperature, showing the method's mildness (entry 11). Because reactions with a lower mole percentage of catalysts take longer to

complete, entry 11 is considered as the best reaction condition for demonstrating the reaction's feasibility with multiple substrates.

The lack of PP-N_3 causes low yields (entries 2-4), in the GH reaction (entries 2-4), suggesting that Cu(I) and PP-N_3 complexation is the driving force in the GH reaction. Change from stoichiometric to catalytic PP-N_3 does not affect the reaction, indicating that PP-N_3 can function as a ligand similar to TMEDA in the conventional GH reaction. The fact that DMF and acetonitrile have the same capacity to coordinate with transition metals,[29] (entry 14) suggests that the variation in reactivity is related to solvation. Acetonitrile has high solvation of Cu(I) and preferential solvation with co-solvent DMF,[30] thus limiting reactivity outside its solvation shell, particularly when CuI is employed in catalytic amounts. The involvement of PP-N_3 as a ligand in the GH reaction is obvious after studying reaction optimization efforts. We infer that PP-N_3 exists in the tetrazole form in DMF, promoting GH reaction while inhibiting CuAAC reaction, because we were unable to identify the triazole product in all of our attempts. The pyrimidine proton of PP-N_3 was discovered in ^{1}H NMR at H 9.81 ppm, which matched the H 9.80 ppm of identical tetrazolo[4,5-c]pyrimido[5,4-b]indole.[31]

The 1,5-disubstituted tetrazole moiety of PP-N_3 can function as a multidentate coordinating[32] ligand, as well as a base, due to its nitrogen electron-donating atoms. The pyrimidine nitrogen or the tetrazole moiety, on the other hand, forms monodentate coordination complexes with copper.[33,34] As a consequence, as anticipated by Bohlmann, PP-N_3 produces a dimeric intermediate (Figure 5.3.1a) with CuI, which takes the constructive path to 1,3-diyne and rejuvenates the catalyst.

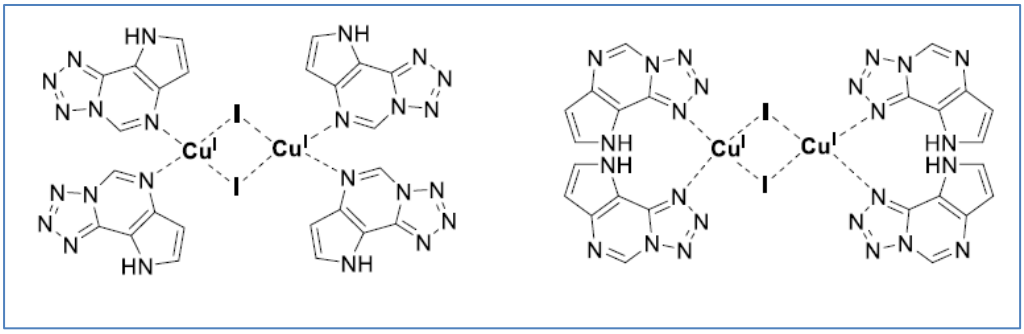
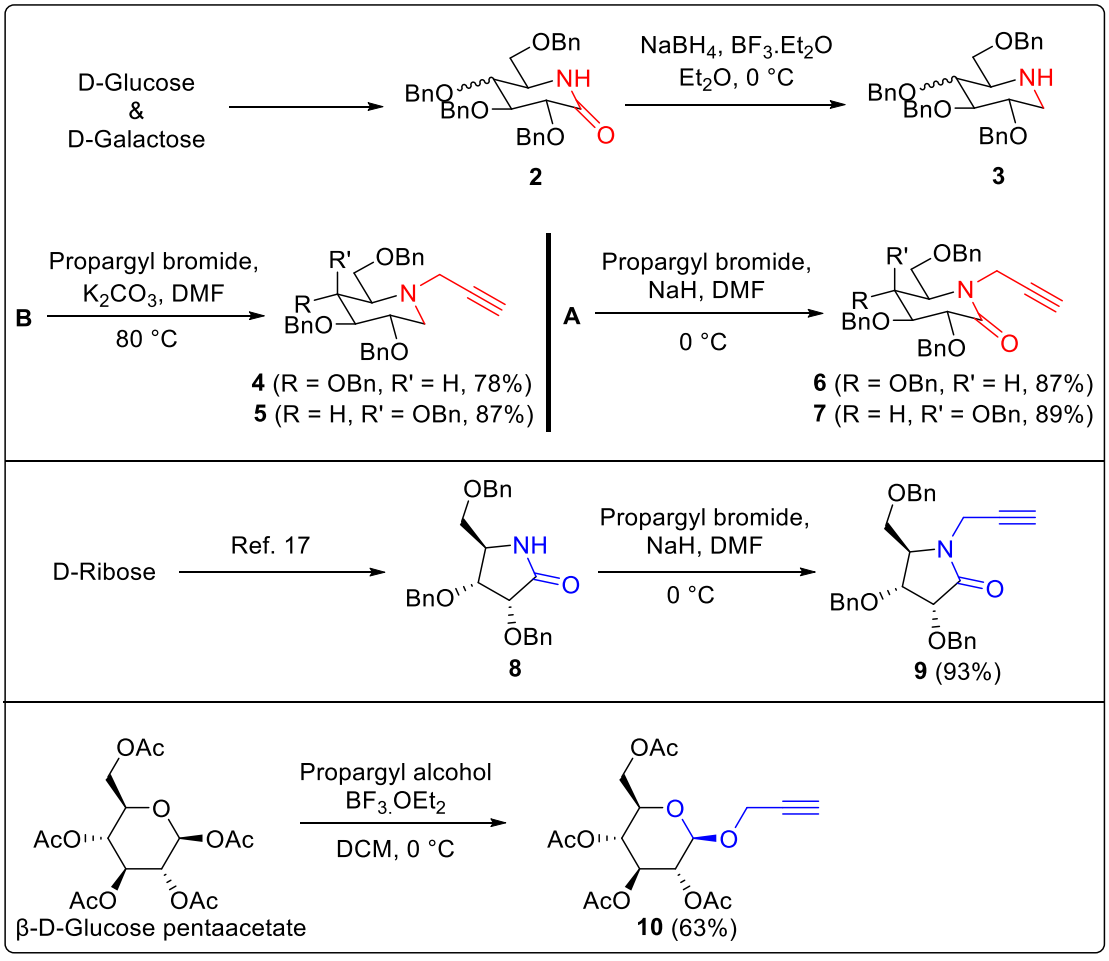


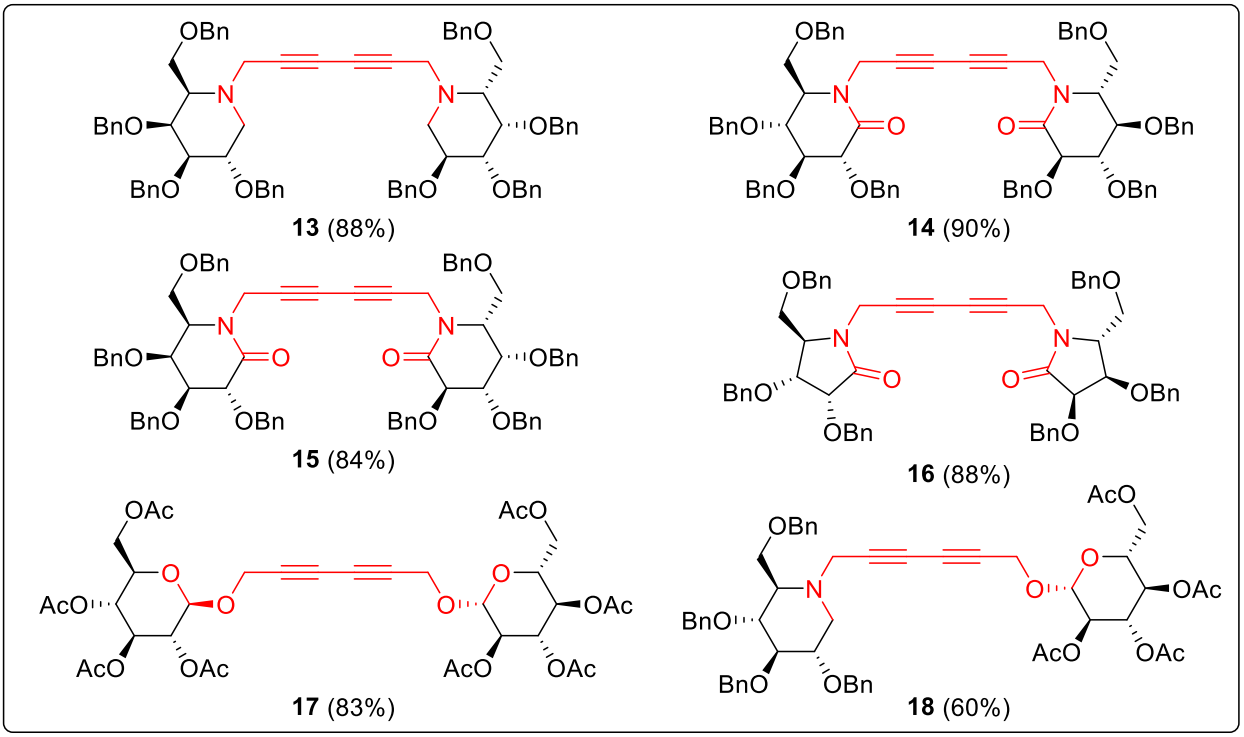
Figure 5.3.1a. Probable complexes with CuI and tetrazole isomer of **PPN**$_3$.



Scheme 5.3.2a. Synthesis of carbohydrate-based terminal alkynes.

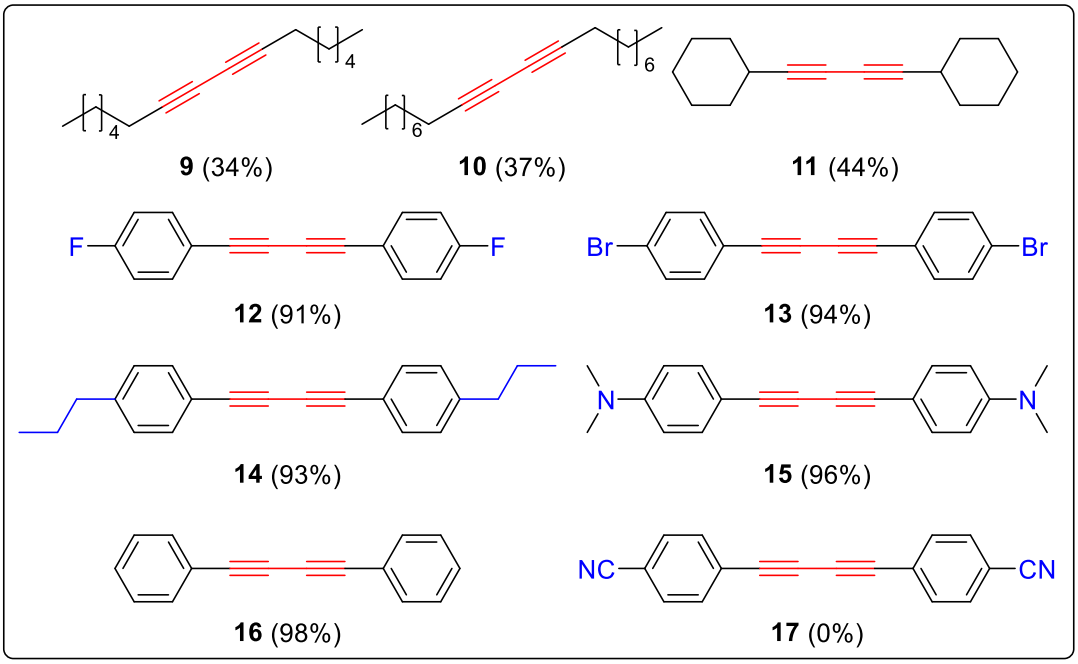
Compounds **4** to **7** were synthesized from D-glucose and D-galactose, **9** from D-ribose, and **10** from β-D-glucose pentaacetate,[35] using conventional synthetic carbohydrate chemistry

techniques to examine the efficacy of the current approach with additional carbohydrate-based substrates (Scheme 5.3.2a). Compound **13** was synthesized from compound **5**, which was obtained from D-galactose, with an 88% yield. Homocoupling of the lactams **6** to **9** went easily as well, yielding compounds **14** to **16** in excellent quantities. Compound **17** was easily obtained by homocoupling 1-*O*-propargylated glucose pentaacetate **10**. In an attempt to get unsymmetric 1,3-diynes, compound **4** and **10** were treated in a 1:1 ratio under reaction conditions, yielding heterocoupled product **18** in 60%.



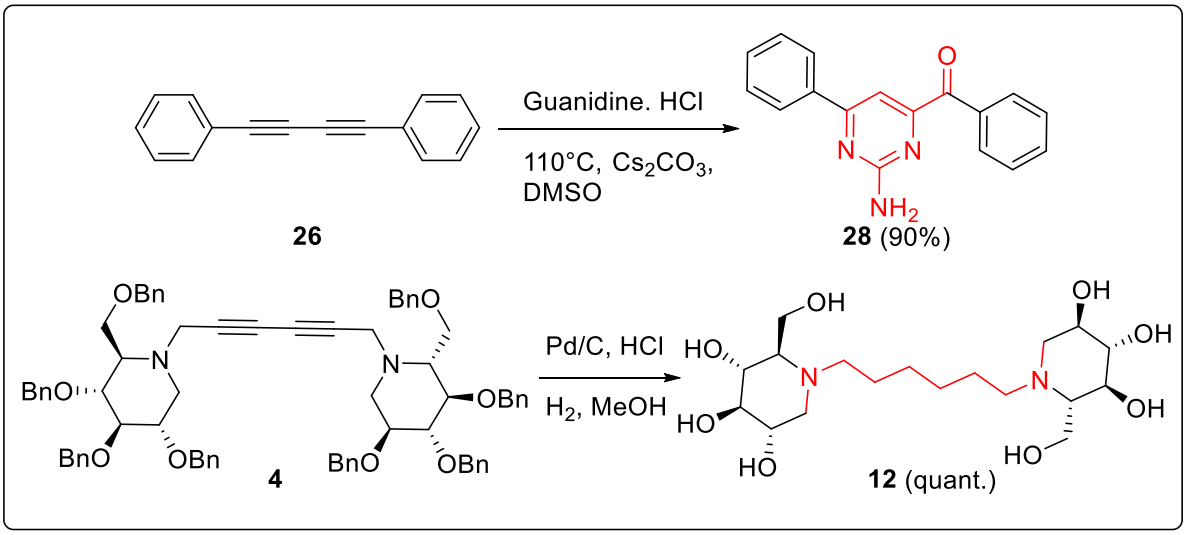
Scheme 5.3.3a. Carbohydrate based substrate scope

Using terminal alkynes with aliphatic groups resulted in a poor yield of homocoupled products **19** to **21**. Compounds **22-26** were produced in high yields from terminal alkynes with aromatic groups, but no reaction occurred when the substrate was replaced with para-cyano.



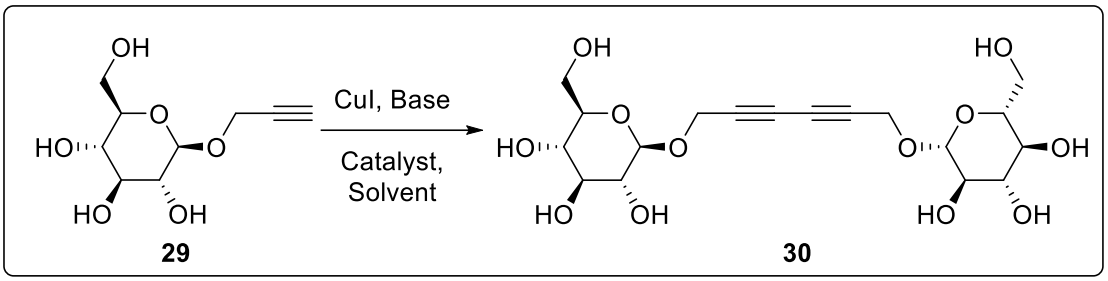
Scheme 5.3.4a. Aliphatic and aromatic terminal alkynes based substrate scope

Compound **11** was debenzylated using Pd/C to provide a novel DNJ-dimer **12** (Scheme 5.3.5a) with an aliphatic linker, which would have pharmacological relevance. Finally, compound **26** was converted to 2-amino pyrimidine **28**, which can be easily converted to azide, yielding another pyrimidine-based catalyst capable of participating in the GH process.[36]



Scheme 5.3.5a. Synthesis of compounds **28** and **12**

Table 5.3.2a. Investigation of PP-N_3 versus reported GH reaction



Entry	Base	Catalyst	Solvent	Yield (%)
1	DMAP	-	ACN	No reaction
2	DMAP	-	DMF	No reaction
3	-	**PP-N_3**	DMF	75 %

Attempts were undertaken with carbohydrate-based substrates in the current work to investigate the relevance of **PP-N_3** in GH reaction versus known ligands/bases in GH reactions. For a protected glycosylated alkyne, the GH reaction with a benzotriazole ligand generated only 15% 1,3-diyne and using benzotriazole instead of **PP-N_3** with our carbohydrate terminal alkynes yielded even lower yields. In contrast, a CuI and DMAP in acetonitrile catalytic system reported under open air conditions with a protected glycosylated alkyne gave 1,3-diyne in excellent yield. As a result, we evaluated the possibility of accomplishing a GH reaction with DMAP using a glycosylated alkyne that had been deprotected. A fully deacetylated terminal alkyne of compound 10 was tried for GH reaction in the presence of DMAP, but no reaction occurred in acetonitrile and DMF. Using **PP-N_3**, however, a straightforward GH reaction occurred, generating 1,3-diyne in a 75% yield. Homocoupled glycosylated alkynes are treated to traditional deprotection of acetyl groups under Zemplén conditions in GH reactions with TMEDA, while PP-N_3 has the capability of running effectively with deprotected carbohydrate substrates. These results indicate that PP-N_3

and carbohydrate substrates have a mutual connection for a successful GH reaction, implying that the current approach might be applied in glycobiology.

5.4. Conclusion

Finally, a resistant 2-azidopyridine towards CuAAC 'click' reaction is proposed for use as a ligand for the Glaser-Hay coupling reaction of terminal alkynes to 1,3-diynes due to its equilibrium with the tetrazole isomer. 4-azido-5*H*-pyrrolo[3,2-*d*]pyrimidine was examined as a model in the presence of CuI and terminal alkynes, giving 1,3-diynes with no indication of triazole 'click' product. The new approach is the first to employ azidopyrimidine as a ligand in a catalytic amount in conjunction with catalytic CuI, without any base, and with a broad substrate range, adding to a long list of exquisite GH techniques. The reaction's applicability in the production of diverse carbohydrate-based dimers encourages further diversification and investigation for biological studies.

5.5. General information

5.5.1. General experimental conditions:

^{1}H and ^{13}C NMR were recorded on a Bruker ASCEND^{TM}-500 spectrometer at 500 and 125 MHz, respectively using $CDCl_3$, DMSO-d6 and CD_3OD solvents. NMR data are reported as follows: chemical shifts in ppm (δ) with integration, coupling constant in Hz and multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, etc.). HR-ESI-MS analysis was recorded on a Thermo Scientific Exactive-LCMS instrument by electrospray ionization method with ions given in *m/z* using Orbitrap analyzer. Reactions were monitored by silica gel G-60 F_{254} aluminum TLC and compounds were visualized by short wavelength lamp and by charring the TLC plate after spraying with 15% sulfuric acid in ethanol. Chromatographic separations were carried out by conventional column chromatography on silica gel (100× 200 mesh). Reagents were purchased at the highest commercial quality and used without further purification.

5.5.2. General Procedure for synthesis of homocoupled product 1,3-diyne:

To a stirred solution of terminal alkyne (1 mmol) in DMF (3 mL), CuI (20 mol%) and PP-N_3 (10 mol%) were added successively and allowed to stir at room temperature for 5 hours. The reaction progress was monitored by TLC. After complete consumption of the starting material, the reaction mixture was diluted with EtOAc (4 mL) and saturated aqueous NH_4Cl solution (4 mL) was added. The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using hexane/EtOAc as eluent to afford the corresponding 1,3-diyne.

3,5-dihydro-4H-pyrrolo[3,2-d]pyrimidin-4-one **(PP):** ^{1}H NMR (DMSO-d6, 500 MHz): δ 11.97 (1H, bs), 7.80 (1H, s), 7.37 (1H, d, J=3 Hz), 6.37 (1H, d, J=2.5 Hz); $^{13}C\{^1H\}$ NMR (DMSO-d6, 125 MHz): δ 154.2, 145.1, 142.0, 127.9, 118.2, 103.4.

4-chloro-5H-pyrrolo[3,2-d]pyrimidine **(PP-Cl):** ^{1}H NMR (DMSO-d6, 500 MHz): δ 12.47 (1H, s), 8.64 (1H, s), 7.99 (1H, s), 6.75 (1H, s); $^{13}C\{^1H\}$ NMR (DMSO-d6, 125 MHz): δ 151.4, 149.7, 142.2, 135.0, 124.4, 102.8.

4-azido-5H-pyrrolo[3,2-d]pyrimidine **(PP-N_3):** ^{1}H NMR (DMSO-d6, 500 MHz): δ 13.26 (1H, s), 9.82 (1H, s), 7.82 (1H, s), 6.90 (1H, s); $^{13}C\{^1H\}$ NMR (DMSO-d6, 125 MHz): δ 142.2, 140.0, 132.8, 129.6, 111.4, 105.0.

5.5.3. General procedure for synthesis of compound **(3):**

To a solution of lactam **2** (1 equiv) in dry Et_2O (5 mL) was added $NaBH_4$ (6 equiv) and $BF_3.Et_2O$ (1 equiv) at 0 °C and then allowed stir for 2 h under argon atmosphere. After formation of the polar product as indicated by TLC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (2 × 25 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (7:3) as the eluent to afford compound **3**.

(2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidine (**4**)**:** To a solution of 1-deoxyglucose iminosugar **3** (100 mg, 0.19 mmol, 1 equiv) in DMF (3 mL) was added K_2CO_3 (66 mg, 0.47 mmol, 2.5 equiv) and propargyl bromide (43 µL, 0.57 mmol, 3 equiv) at room temperature and then allowed stir for 1 h at 80 °C. After formation of the non-polar product as indicated by TLC, the reaction mixture was quenched with H_2O (5 mL) and extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (9:1) as the eluent to afford compound **4** as a colorless viscous liquid (84 mg, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (18H, m), 7.17 (2H, d, J=7 Hz), 5.03 (1H, d, J=11 Hz), 4.93 (1H, d, J=11 Hz), 4.88 (1H, d, J=11 Hz), 4.73 (2H, s), 4.60 (1H, d, J=12 Hz), 4.48 (1H, d, J=12 Hz), 4.41 (1H, d, J=11 Hz), 3.78 (3H, m), 3.70 (1H, t, J=9 Hz), 3.63 (1H, d, J=11 Hz), 3.55 (1H, t, J=9 Hz), 3.44 (1H, m), 3.02 (1H, dd, J=4.5, 10.5 Hz), 2.60 (1H, t, J=11 Hz), 2.50 (1H, d, J=10 Hz), 2.26 (1H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 139.0, 138.5, 138.5, 137.7, 128.5, 128.5, 128.1, 128.4, 128.4, 128.0, 128.0, 127.9, 127.9, 127.7, 127.6, 127.6, 87.2, 78.2, 78.2, 75.5, 75.2, 74.2, 73.6, 72.8, 64.8, 62.2, 55.0, 42.3; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{37}H_{40}NO_4$ 562.2957; Found 562.3070.

(2R,3S,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidine (**5**)**:** To a solution of 1-deoxygalactose iminosugar (649 mg, 1.24 mmol, 1 equiv) in DMF (8 mL) was added K_2CO_3 (428 mg, 3.1 mmol, 2.5 equiv) and propargyl bromide (299 µL, 3.72 mmol, 3 equiv) at room temperature and then allowed stir for 1 h at 80 °C. After formation of the non-polar product as indicated by TLC, the reaction mixture was quenched with H_2O (10 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (9:1) as the eluent to afford compound **5** as a colorless viscous liquid (610 mg, 87%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (20H, m), 4.96 (1H, d, J=11Hz), 4.81 (3H, m), 4.68 (2H, m), 4.44 (3H, m), 4.13 (1H, s), 3.67 (1H, m), 3.58 (1H, dd, J=2, 18 Hz), 3.48 (2H, m), 3.36 (1H, dd, J=2, 18 Hz), 2.98 (1H, dd, J=4.5, 11 Hz), 2.75 (1H, m), 2.58 (1H, t, J=10.5 Hz), 2.26 (1H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 138.8, 138.8, 138.7, 138.0, 128.7, 128.4, 128.1, 128.3, 128.3, 128.2,

127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 75.6, 75.3, 73.3, 73.0, 72.3, 71.9, 71.5, 66.4, 60.3, 51.4, 43.1; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{37}H_{40}NO_4$ 562.2957; Found 562.2952.

(3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidin-2-one **(6):** To a solution of glucose-derived lactam **2** (1056 mg, 1.96 mmol, 1 equiv) in DMF (10 mL) was added 60% NaH (196 mg, 4.9 mmol, 2.5 equiv) at 0 °C under nitrogen atmosphere. Propargyl bromide (447 µL, 5.9 mmol, 3 equiv) was then added at 0 °C to the reaction mixture and allowed stir for 25 mins. After formation of the non-polar product, as indicated by TLC, the reaction mixture was quenched with H_2O (25 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (9:1) as the eluent to afford compound **6** as a white solid (988 mg, 87%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (2H, d, J=7 Hz), 7.20 (18H, m), 5.10 (1H, d, J=11 Hz), 4.78 (1H, d, J=11 Hz), 4.68 (1H, d, J=11 Hz), 4.60 (3H, m), 4.29-4.44 (3H, m), 3.99 (1H, d, J=9.00 Hz), 3.77 (3H, m), 3.67 (1H, s), 3.59 (1H, m), 3.44 (1H, d, J=10 Hz), 2.10 (1H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 169.7, 138.2, 138.1, 137.8, 137.5, 128.5, 128.4, 128.4, 128.3, 128.0, 128.0, 127.9, 127.7, 127.7, 81.4, 78.6, 78.4, 74.8, 74.5, 73.5, 73.2, 72.5, 67.1, 33.8, 29.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{37}H_{38}NO_5$ 576.2750; Found 576.2770.

(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)piperidin-2-one **(7):** To a solution of galactose-derived lactam **2** (500 mg, 0.93 mmol, 1 equiv) in DMF (10 mL) was added 60% NaH (93 mg, 2.32 mmol, 2.5 equiv) at 0 °C under nitrogen atmosphere. Propargyl bromide (211 µL, 2.79 mmol, 3 equiv) was then added at 0 °C to the reaction mixture and allowed to stir for 25 mins. After formation of the non-polar product, as indicated by TLC, the reaction mixture was quenched with H_2O (20 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (9:1) as the eluent to afford compound **7** as a colorless viscous liquid (480 mg, 89%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (20H, m), 5.16 (1H, d, J=11 Hz), 4.79 (1H, d, J=11 Hz), 4.56-4.68 (5H, m), 4.44 (2H, m), 4.32 (1H, d, J=8 Hz), 4.09 (1H, s), 3.95 (2H, m), 3.86 (1H, d, J=18 Hz), 3.60 (1H, dd, J=3.5, 10.5 Hz), 3.49 (1H, m), 2.21 (1H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 169.0, 138.3, 138.1, 137.7, 137.4, 128.5, 128.4, 128.4,

128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 77.5, 75.4, 74.8, 73.3, 73.2, 72.4, 72.0, 68.3, 68.0, 57.9, 34.8; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{37}H_{38}NO_5$ 576.2750; Found 576.2758.

(3R,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-1-(prop-2-yn-1-yl)pyrrolidin-2-one **(9):** To a solution of ribose-derived lactam **8** (195 mg, 0.46 mmol, 1 equiv) in DMF (5 mL) was added 60% NaH (47 mg, 1.16 mmol, 2.5 equiv) at 0 °C under nitrogen atmosphere. Propargyl bromide (106 µL, 1.4 mmol, 3 equiv) was then added at 0 °C to the reaction mixture and allowed stir for 1 h. After formation of the non-polar product, as indicated by TLC, the reaction mixture was quenched with H_2O (5 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was then purified by column chromatography using hexane/EtOAc (9:1) as the eluent to afford compound **9** as an off-white solid (198 mg, 93%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.32 (2H, d, J=7 Hz), 7.22 (11H, m), 7.11 (2H, d, J=7 Hz), 4.87 (1H, d, J=12Hz), 4.71 (1H, d, J=12 Hz), 4.57 (1H, d, J=12 Hz), 4.40 (4H, m), 4.12 (1H, d, J=5 Hz), 3.96 (1H, t, J=2 Hz), 3.80 (1H, s), 3.68 (1H, d, J=18 Hz), 3.57 (1H, d, J=10 Hz), 3.45 (1H, d, J=10 Hz), 2.11 (1H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 171.0, 137.7, 137.4, 128.5, 128.4, 128.4, 128.3, 128.0, 128.0, 127.9, 127.8, 127.7, 77.4, 74.8, 74.5, 73.3, 72.5, 72.4, 71.9, 66.8, 61.0, 30.3; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{29}H_{30}NO_4$ 456.2175; Found 456.2163.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate **(10):** D-Glucose pentaacetate (2 g, 5.13 mmol) was dissolved in dry DCM (20 mL) under nitrogen and cooled to 0 °C. Propargyl alcohol (355 µL, 6.16 mmol) was added, followed by dropwise addition of borontrifluoride diethyletherate (2.13 mL, 7.70 mmol). After warming to room temperature, the reaction mixture was stirred for 6 hours. Saturated aqueous sodium bicarbonate (100 mL) was added to quench the reaction and stirred for 20 min and then extracted with DCM (100 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography using hexane/EtOAc (1:1) as the eluent to afford compound **10** as white crystals (1.25 g, 63%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.26 (1H, t, J=9.5 Hz), 5.12 (1H, t, J=10 Hz), 5.03 (1H, t, J=9 Hz), 4.80 (1H, d, J=8 Hz), 4.39 (2H, s), 4.29 (1H, m), 4.16 (1H, m), 3.75 (1H, m), 2.49 (1H, d, J=2 Hz), 2.11 (3H, s), 2.08 (3H, s), 2.04 (6H, d, J=9 Hz); ^{13}C{^{1}H}

NMR ($CDCl_3$, 125 MHz): δ 170.4, 170.0, 169.3, 169.2, 98.0, 78.1, 75.6, 72.6, 71.7, 70.8, 68.2, 61.6, 55.8, 20.5, 20.5, 20.4; HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{17}H_{22}NaO_{10}$ 409.1111; Found 409.1107.

1-((2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)-6-((2S,3S,4S, 5R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)hexa-2,4-diyne **(11):** Off-white semi-solid (43 mg, 86%, entry 9, Table 1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.27-7.38 (m, 18H), 7.12 (m, 2H), 4.99 (d, 1H, *J* = 11 Hz), 4.86 (dd, 2H, *J* = 11, 21 Hz), 4.69 (d, 2H, *J* = 2Hz), 4.56 (d, 1H, *J* = 12.5 Hz), 4.44 (d, 1H, *J* = 12 Hz), 4.34 (d, 1H, *J* = 11 Hz), 3.68-3.86 (m, 3H), 3.64 (t, 1H, *J* = 9 Hz), 3.51-3.60 (m, 2H), 3.44 (d, 1H, *J* = 18.5 Hz), 2.96 (d, 1H, *J* = 6.5 Hz), 2.58 (t, 1H, *J* = 11 Hz), 2.47 (d, 1H, *J* = 8Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.9, 138.4, 137.6, 128.53, 128.50, 128.4, 128.36, 128.33, 128.0, 128.9, 127.8, 127.7, 127.5, 87.0, 78.0, 75.5, 75.3, 73.6, 72.8, 64.7, 62.1, 55.2, 42.9, 29.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{74}H_{77}N_2O_8$ 1121.5680; Found 1121.5699.

(2R,2'R,3R,3'R,4R,4'R,5S,5'S)-1,1'-(hexane-1,6-diyl)bis(2-(hydroxymethyl)piperidine-3,4, 5-triol) **(12):** To a solution of compound **11** (35 mg, 0.05 mmol, 1 equiv) in EtOH (5 mL) was added 20 mg of 10% Pd/C and 2 drops of concentrated HCl. Hydrogen gas was purged into the reaction mixture for 2 min, and the mixture was stirred overnight under hydrogen atmosphere using a balloon. The reaction mixture was then diluted with MeOH (50 mL), filtered through a Celite® pad, and concentrated to afford the desired compound **12** as a colorless oil in quantitative yield. ^{1}H-NMR (CD_3OD, 500 MHz) δ 2.78-4.07 (10H, m), 1.75 (1H, m), 1.36 (2H, m), 0.89 (1H, m); $^{13}C\{^1H\}$ NMR (CD_3OD, 125 MHz): δ 68.1, 67.5, 67.2, 66.4, 60.6 ,57.9, 46.5, 31.2, 26.3, 23.1, 22.3, 13.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{18}H_{37}N_2O_8$ 409.2550; Found 409.2541.

1-((2R,3S,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)-6-((2S,3R,4S,5 R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)hexa-2,4-diyne **(13):** Off-white semi-solid (44 mg, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (20H, m), 4.95 (1H, d, J=10.5 Hz), 4.80 (3H, m), 4.68 (2H, m), 4.44 (2H, m), 4.10 (1H, s), 3.67 (3H, m), 3.47 (3H, m), 3.00 (1H, m), 2.77 (1H, m), 2.61 (1H, t, J=10.5 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 138.8, 138.8, 138.7, 138.4, 138.0, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 75.6, 75.5, 74.9, 73.3, 73.1, 72.9, 72.3,

71.9, 71.6, 66.4, 60.5, 43.9, 29.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{74}H_{77}N_2O_8$ 1121.5680; Found 1121.5656.

(3S,4R,5S,6S)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(6-((2R,3R,4S,5R)-3,4,5-tris (benzyloxy)-2-((benzyloxy)methyl)-6-oxopiperidin-1-yl)hexa-2,4-diyn-1-yl)piperidin-2-one **(14):** Yellow semi-solid (45 mg, 90%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33 (d, 2H, *J* = 6.5Hz), 7.11-7.24 (m, 18H), 5.07 (d, 1H, *J* = 11 Hz), 4.74 (d, 1H, *J* = 11 Hz), 4.53-4.69 (m, 4H), 4.24-4.42 (m, 3H), 3.95 (d, 1H, *J* = 9Hz), 3.86 (d, 1H, *J* = 18 Hz), 3.70-3.80 (m, 2H), 3.48-3.61 (m, 2H), 3.40 (dd, 1H, *J* = 3, 10Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ169.6, 138.1, 138.0, 137.8, 137.4, 128.5, 128.4, 128.36, 128.34, 128.33, 128.04, 128.01, 127.9, 127.8, 127.72, 127.70, 81.4, 78.6, 76.5, 74.8, 74.5, 73.6, 73.3, 68.4, 66.9, 59.7, 34.3, 29.7; HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{74}H_{72}NaN_2O_{10}$ 1171.5085; Found 1171.5075.

(3S,4R,5R,6S)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-1-(6-((2R,3S,4S,5R)-3,4,5-tris (benzyloxy)-2-((benzyloxy)methyl)-6-oxopiperidin-1-yl)hexa-2,4-diyn-1-yl)piperidin-2-one **(15):** Colourless viscous liquid (126 mg, 84%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.40 (2H, d, J=6.90 Hz), 7.29 (18H, m), 5.12 (1H, d, J=11 Hz), 4.73 (2H, m), 4.62 (2H, t, J=12 Hz), 4.54 (2H, m), 4.39 (2H, m), 4.28 (1H, d, J=7 Hz), 4.02 (1H, s), 3.94 (2H, m), 3.86 (1H, m), 3.52 (1H, m), 3.44 (1H, m); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 169.0, 138.3, 138.1, 137.7, 137.4, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 77.5, 75.4, 74.8, 73.3, 73.2, 72.4, 72.0, 68.3, 68.0, 57.9, 34.8; HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{74}H_{72}NaN_2O_{10}$ 1171.5085; Found 1171.5080.

(3S,4S,5S)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-1-(6-((2R,3R,4R)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-oxopyrrolidin-1-yl)hexa-2,4-diyn-1-yl)pyrrolidin-2-one **(16):** Off-white semi-solid (71 mg, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.17-7.31 (m, 13H), 7.08 (d, 2H, *J* = 6 Hz), 4.84 (d, 1H, *J* = 12 Hz), 4.68 (d, 2H, *J* = 11.5 Hz), 4.53 (d, 1H, *J* = 11.5 Hz), 4.27-4.43 (m, 4H), 4.08 (d, 1H, *J* = 14.5Hz), 3.90 (t, 1H, *J* = 2.5 Hz), 3.68-3.81 (m, 2H), 3.51 (d, 1H, *J* = 10.5 Hz), 3.40 (d, 1H, *J* = 10.5 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.9, 137.6, 137.5, 137.3, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 74.7, 74.5, 73.3, 72.5, 72.4, 71.9, 68.2, 66.8, 61.3, 30.9; HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{58}H_{56}NaN_2O_8$ 931.3934; Found 931.3910.

***(2S,3S,4R,5S,6S)-2-(acetoxymethyl)-6-((6-(((2R,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxy methyl)tetra hydro-2H-pyran-2-yl)oxy)hexa-2,4-diyn-1-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate* (17):** White solid (166 mg, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.26 (t, 1H, *J* = 9.5Hz), 5.12 (t, 1H, *J* = 9.5Hz), 5.03 (m, 1H) 4.74 (d, 1H, *J* = 7.5Hz), 4.47 (s, 2H), 4.29 (dd, 1H, *J* = 4.5, 12.5Hz), 4.17 (dd, 1H, *J* = 1.5, 12Hz), 3.77 (m, 1H), 2.10 (d, 6H, *J* = 11Hz), 2.04 (d, 6H, *J* = 9Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.7, 170.3, 169.4, 169.3, 98.4, 74.2, 72.7, 72.0, 70.96, 70.92, 68.3, 68.2, 61.7, 56.4, 20.73, 20.70, 20.6; HRMS (ESI) m/z: $[M+Na]^+$ Calcd. $C_{34}H_{42}NaO_{20}$ 793.2167; Found 793.2149.

(2S,3S,4R,5S,6S)-2-(acetoxymethyl)-6-((6-((2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzylo xy)methyl) piperidin-1-yl)hexa-2,4-diyn-1-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**18**)**:** To a stirred solution of compound **4** (50 mg, 1 equiv) and compound **10** (34 mg, 1 equiv) in DMF (3 ml), CuI (20 mol%) and PP-N_3 (10 mol%) were added successively and allowed to stir at room temperature for 5 hours. Progress of the reaction was monitored by TLC. After complete consumption of starting material, the reaction mixture was diluted with EtOAc (4 mL) and added saturated aqueous NH_4Cl solution (4 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using 30% to 50% hexane/EtOAc as eluent to afford the corresponding 1,3-diynes **11** (30%) and **18** (60%), respectively. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.31 (18H, m, J=4.93 Hz), 7.13 (2H, d, J=6.5 Hz), 5.28 (1H, t, J=9.5 Hz), 5.13 (1H, t, J=10 Hz), 4.98-5.05 (2H, m), 4.87 (2H, m), 4.77 (1H, d, J=7.5 Hz), 4.69 (2H, m), 4.55 (1H, m), 4.46 (2H, m), 4.36 (1H, m), 4.29 (1H, m), 4.16 (2H, m), 3.69- 3.86 (4H, m), 3.64 (1H, t, J=9.5 Hz), 3.59 (1H, m), 3.50 (2H, m), 2.99 (1H, dd, J=4.5, 10.5 Hz), 2.57 (1H, t, J=10.5 Hz), 2.48 (1H, d, J=9.5 Hz), 2.11 (3H, s), 2.09 (3H, s), 2.05 (3H, s), 2.03 (3H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.2, 169.5, 169.4, 138.8, 138.3, 138.3, 137.5, 128.5, 128.5, 128.4, 128.4, 128.1, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 98.1, 87.0, 78.1, 78.0, 75.5, 75.3, 74.8, 73.6, 72.8, 72.8, 72.0, 72.0, 71.4, 71.0, 68.2, 64.8, 62.3, 61.8, 61.7, 61.5, 60.4, 56.3, 55.2, 43.0, 29.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{54}H_{60}NO_{14}$ 946.4014; Found 946.3997.

***Hexadeca-7,9-diyne* (19):** Pale brown oil (34 mg, 34%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.26 (t, 2H, *J* = 7 Hz), 1.53 (m, 2H), 1.40 (m, 2H), 1.26-1.34 (m, 4H), 0.90 (t, 3H, *J* = 6.5 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 77.5, 65.2, 31.3, 28.5, 28.3, 22.5, 19.2, 14.0.

***Icosa-9,11-diyne* (20):** Brown viscous liquid (37 mg, 37%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.17 (2H, t, J=7 Hz), 1.44 (2H, m), 1.30 (2H, m), 1.20 (8H, m), 0.81 (1H, t, J=6 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 84.8, 68.0, 31.8, 29.2, 29.1, 28.8, 28.5, 22.7, 18.4, 14.1.

***1,4-dicyclohexylbuta-1,3-diyne* (21):** Pale yellow solid (44 mg, 44%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.45 (1H, s), 1.81 (1H, d, J=11.61 Hz), 1.72 (1H, d, J=4.10 Hz), 1.48 (1H, m, J=9.09 Hz), 1.30 (1H, q, J=7.94 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 81.9, 65.1, 32.3, 29.5, 25.8, 24.8.

***1,4-bis(4-fluorophenyl)buta-1,3-diyne* (22):** White solid (91 mg, 91%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44 (2H, m), 6.96 (1H, t, J=8.5 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 164.1, 162.1, 134.6, 134.5, 117.8, 117.8, 116.0, 115.8, 80.4, 73.5.

***1,4-bis(4-bromophenyl)buta-1,3-diyne* (23):** Pale yellow solid (94 mg, 94%,). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.41 (2H, d, J=8 Hz), 7.31 (2H, d, J=8 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 133.6, 131.6, 123.2, 121.1, 82.6, 78.4.

***1,4-bis(4-propylphenyl)buta-1,3-diyne* (24):** White solid (93 mg, 93%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.47 (2H, d, J=8 Hz), 7.17 (2H, d, J=7.5 Hz), 2.63 (2H, t, J=7.5 Hz), 1.67 (2H, q, J=7.5 Hz), 0.97 (3H, t, J=7 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 144.2, 132.4, 128.6, 119.1, 81.6, 73.5, 38.1, 24.3, 13.8.

***4,4'-(buta-1,3-diyne-1,4-diyl)bis(N,N-dimethylaniline)* (25):** Pale brown solid (96 mg, 96%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.29 (2H, d, J=9 Hz), 6.54 (2H, d, J=9 Hz), 2.90 (6H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 150.4, 133.2, 111.7, 108.8, 84.9, 74.8, 40.2.

***1,4-diphenyl buta-1,3-diyne* (26):** White solid (98 mg, 98%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.46 (d, 2H, *J* = 7 Hz), 7.22-7.33 (m, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 132.5, 129.2, 128.5, 121.8, 81.6, 73.9.

***(2-amino-6-phenylpyrimidin-4-yl)(phenyl)methanone* (28):** Compound **26** (0.12 mmol), guanidine hydrochloride (13 mg, 0.13 mmol), Cs_2CO_3 (80 mg, 0.24 mmol), were dissolved in DMSO (2 mL) in a sealed tube, and the resulting mixture was stirred at 120 °C. After 12 h of stirring, the reaction mixture was

diluted with NaCl solution (4 mL). After extraction with EtOAc (4 mL), the organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography using hexane/EtOAc (9/1), affording compound **28** (90%) as a white solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.10 (4H, m), 7.66 (1H, t, J=7.4 Hz), 7.53 (6H, m), 5.35 (2H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 193.5, 167.3, 163.7, 162.8, 136.8, 135.2, 133.7, 131.1, 130.9, 128.9, 128.4, 127.2, 106.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd. $C_{17}H_{14}N_3O$ 276.1137; Found 276.1128.

(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran-3,4,5-triol (**29**): To a solution of compound **10** (108 mg, 0.05 mmol, 1 equiv) in MeOH (5 mL) was added 100 µL of NaOMe at 0 °C and allowed to stir for 5 mins. The reaction mixture was then diluted with MeOH (20 mL), passed through a Dowex H^+ resin, and concentrated to afford the desired compound **29** as Off-white solid in quantitative yield. ^{1}H NMR (CD_3OD, 500 MHz): δ 4.48 (1H, d, *J* = 8 Hz), 4.44 (2H, dd, *J* = 2.5, 5.5 Hz), 3.89 (1H, m), 3.69 (1H, dd, *J* = 5.5, 12 Hz), 3.40 (1H, t, *J* = 8.5 Hz), 3.32 (2H, m), 3.22 (1H, t, *J* = 8 Hz), 2.89 (1H, t, *J* = 2 Hz). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 100.7, 78.7, 76.6, 76.6, 74.9, 73.4, 70.2, 61.3, 55.2.

(2R,2'R,3S,3'S,4S,4'S,5R,5'R,6R,6'R)-6,6'-(hexa-2,4-diyne-1,6-diylbis(oxy))bis(2-(hydroxy methyl)tetrahydro-2H-pyran-3,4,5-triol) (**30**): Off white solid (18 mg, 75%) ^{1}H NMR (CD_3OD, 500 MHz): δ 4.44 (2H, m), 4.32 (1H, d, *J* = 8 Hz), 3.77 (1H, m), 3.56 (1H, dd, *J* = 4, 11.5 Hz), 3.26 (1H, m), 3.18 (2H, m), 3.09 (1H, t, *J* = 9 Hz). ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz): δ 101.0, 76.7, 76.6, 74.7, 73.5, 70.2, 69.6, 61.3, 55.5.

Figure 5.5.3.1. NMR spectras of **PP**

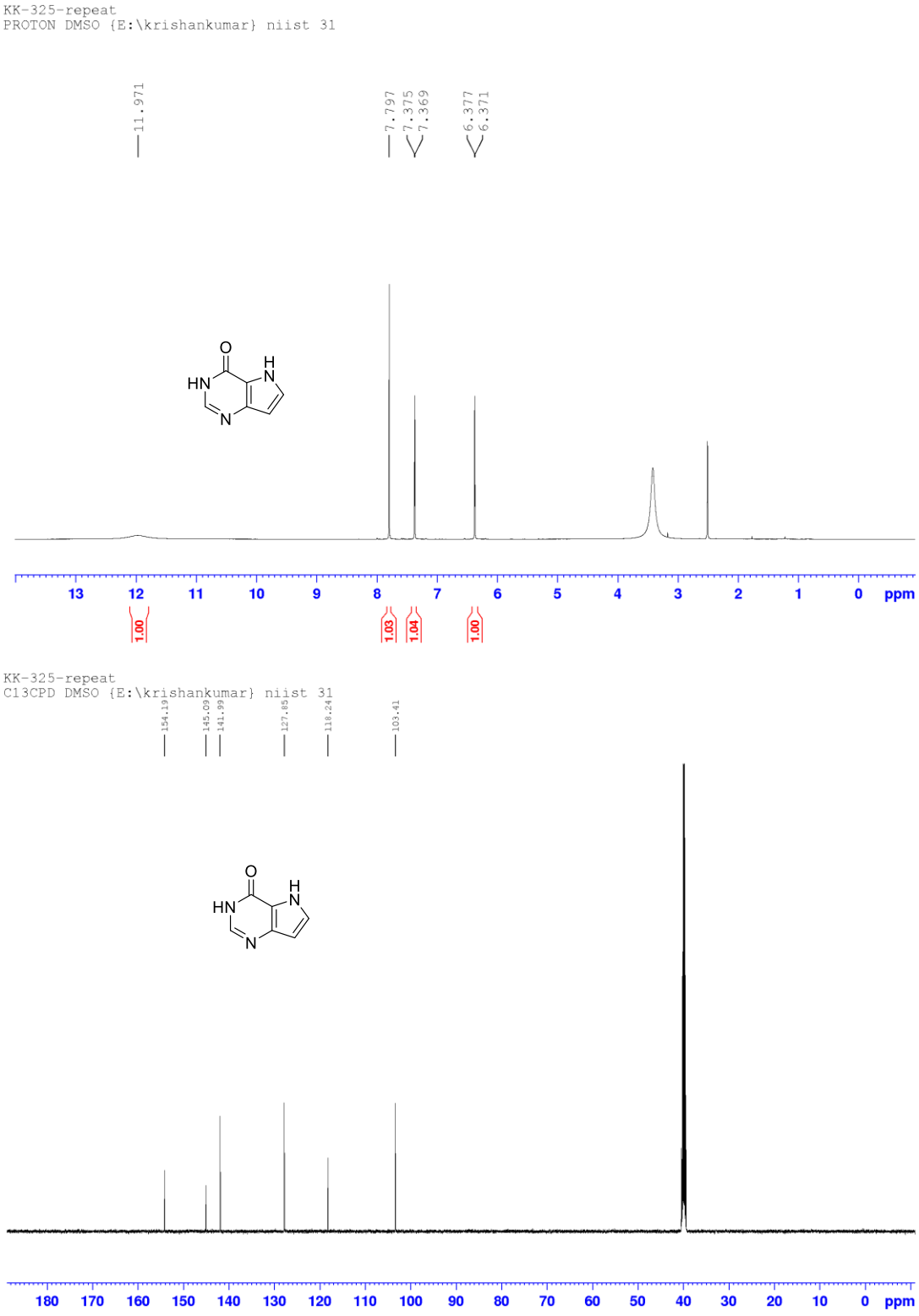


Figure 5.5.3.2. NMR spectras of **PP-Cl**

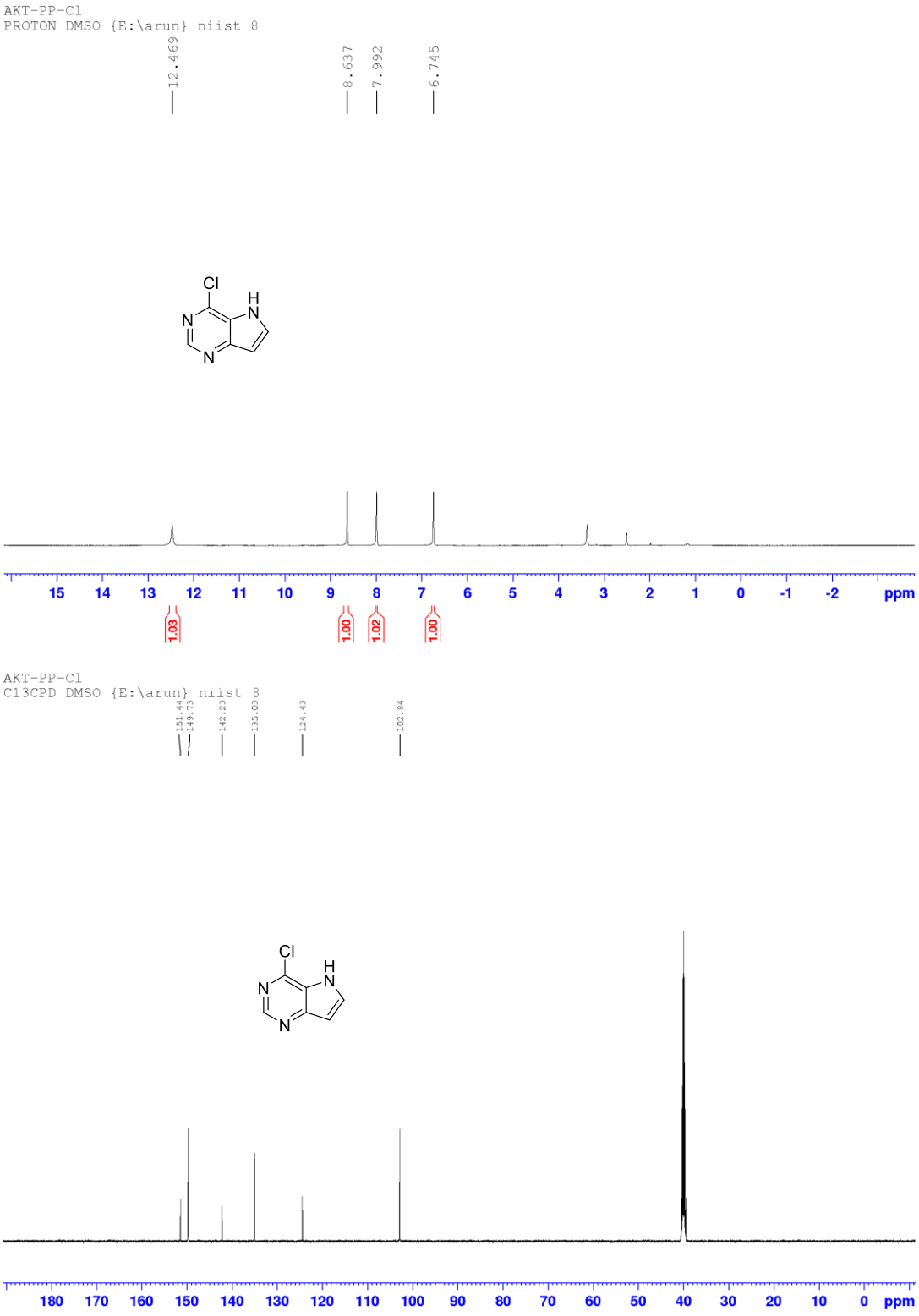


Figure 5.5.3.3. NMR spectras of **PP-N_3**

Figure 5.5.3.4. NMR spectras of compound **4**

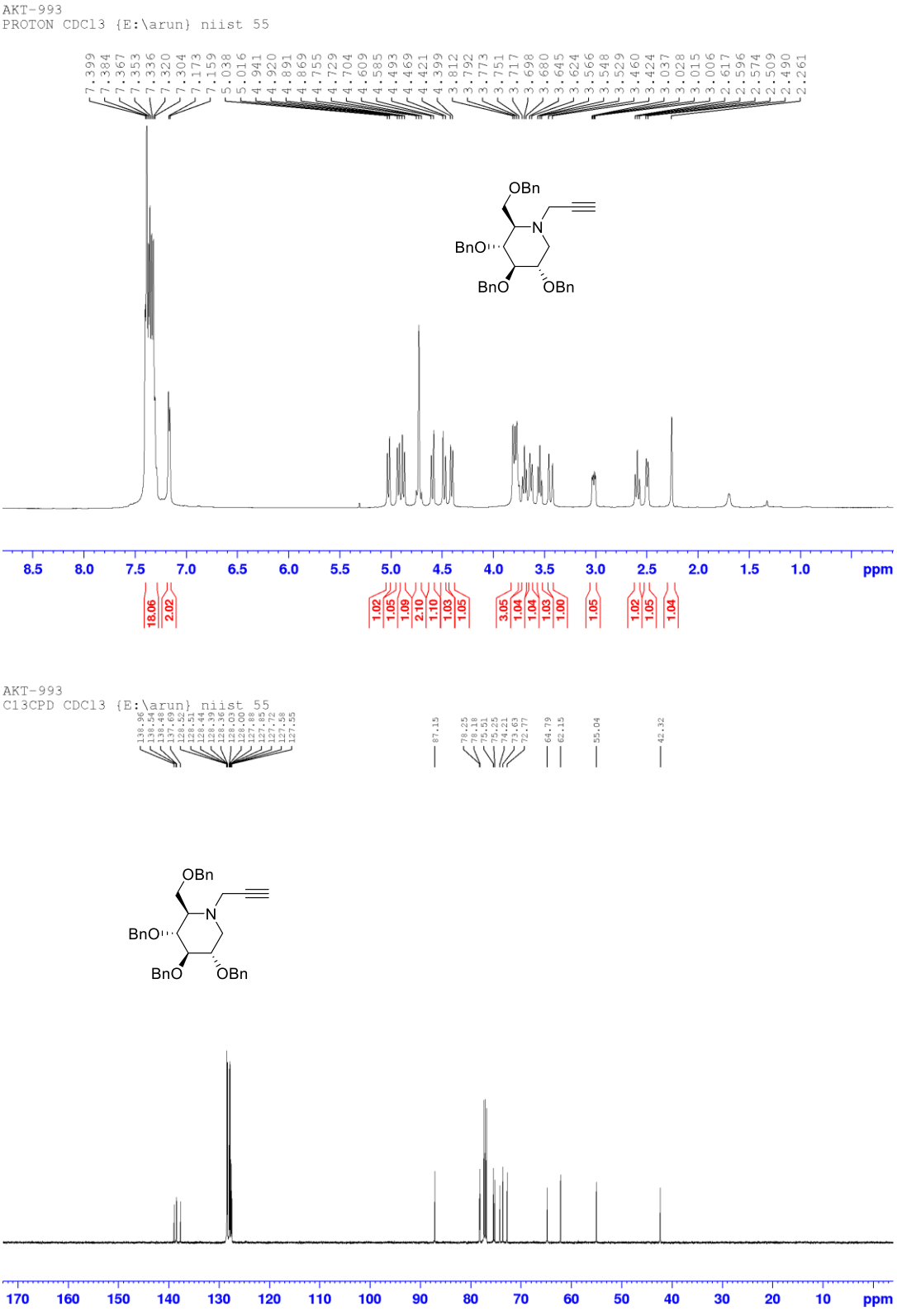
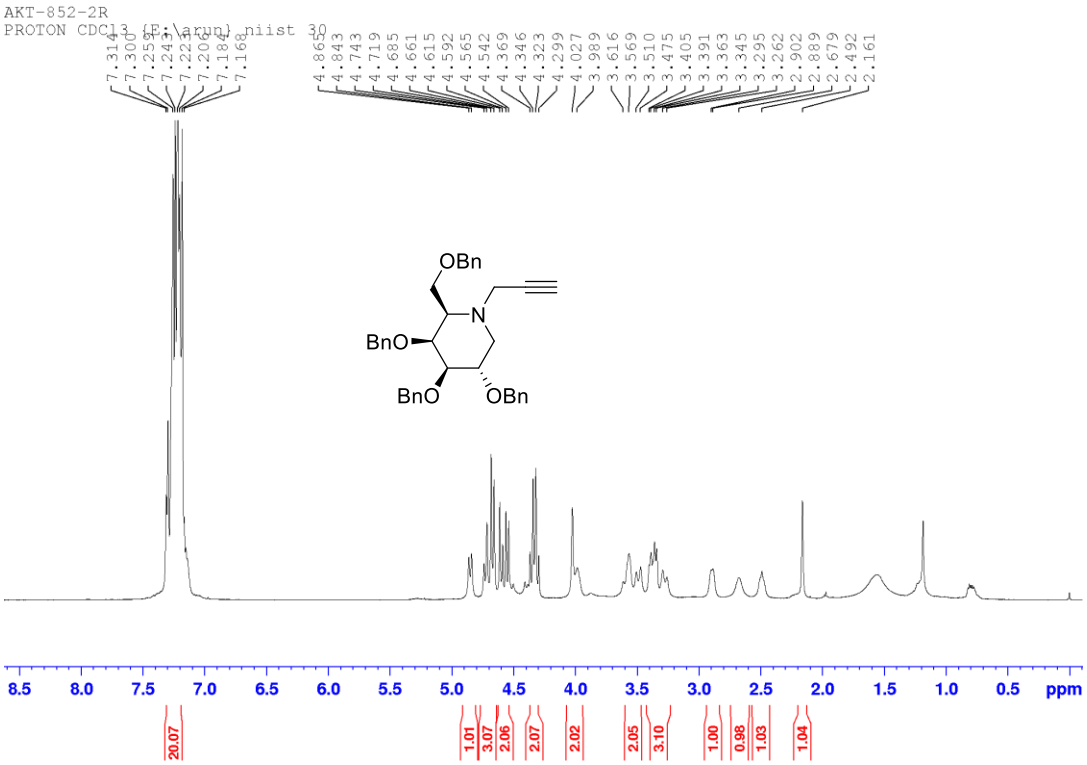


Figure 5.5.3.5. NMR spectras of compound **5**



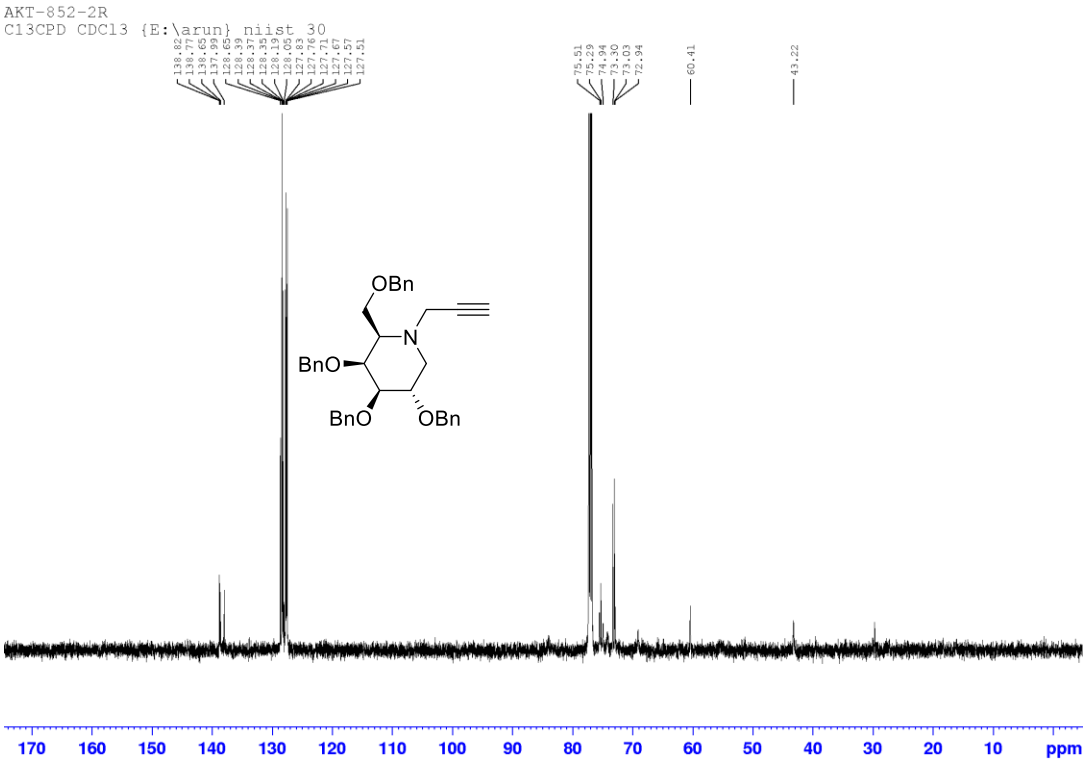
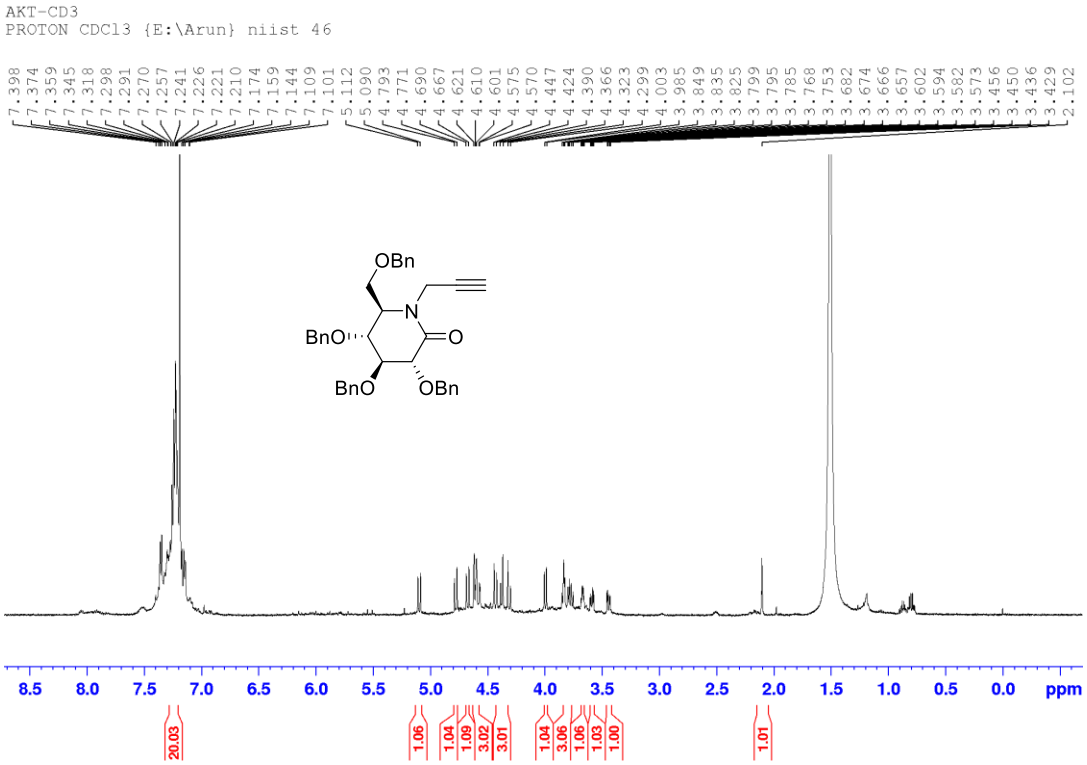


Figure 5.5.3.6. NMR spectras of compound **6**



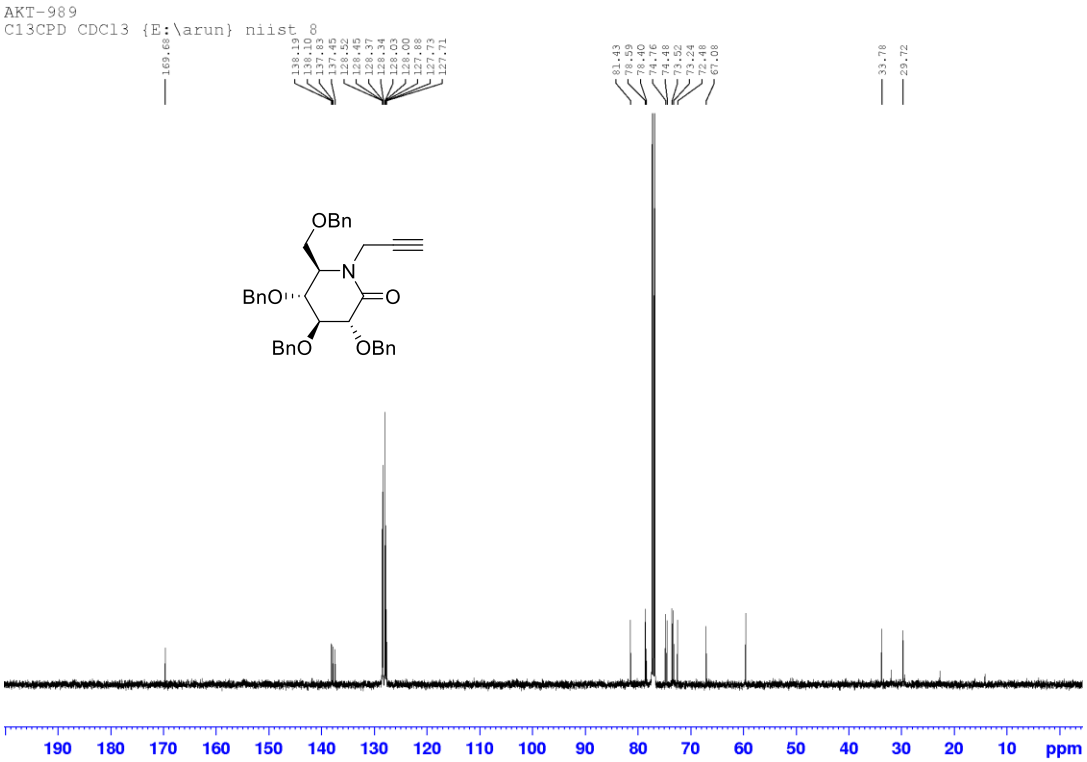
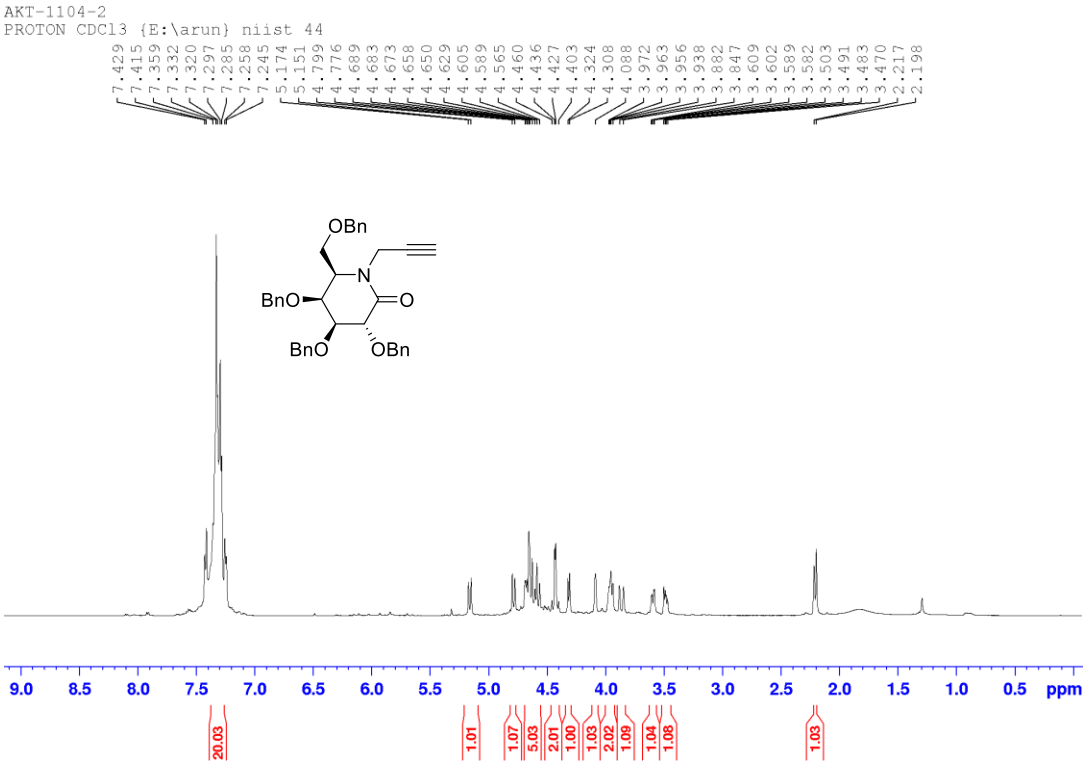


Figure 5.5.3.7. NMR spectras of compound **7**



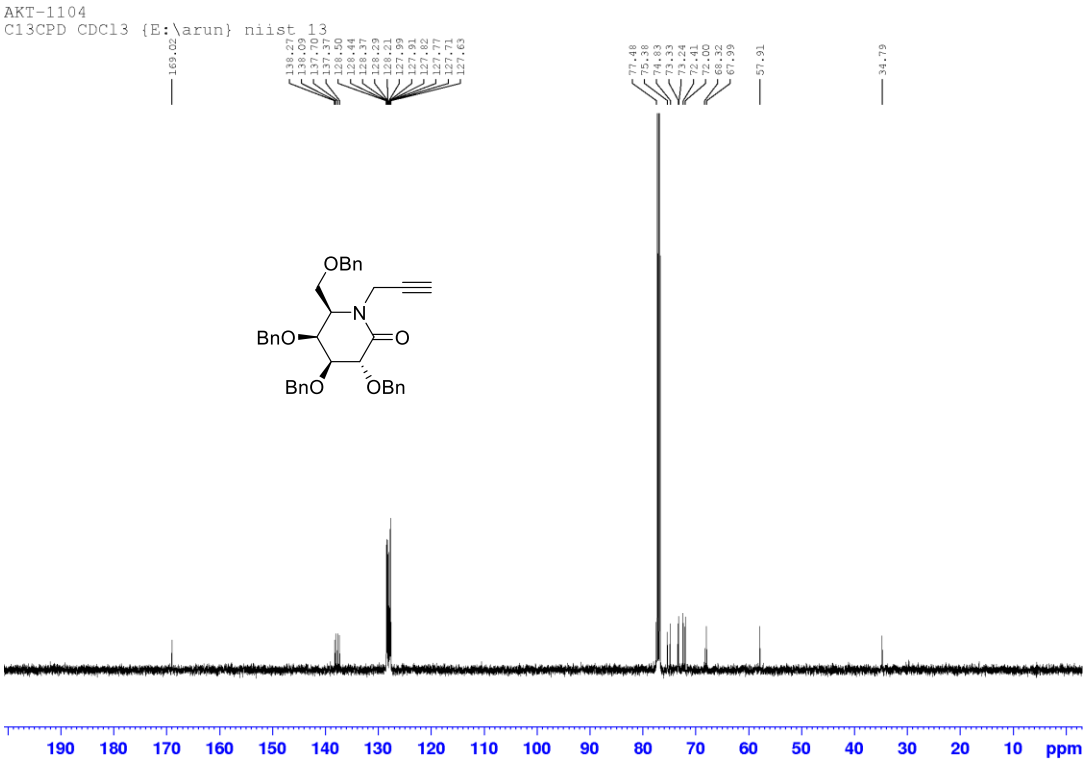
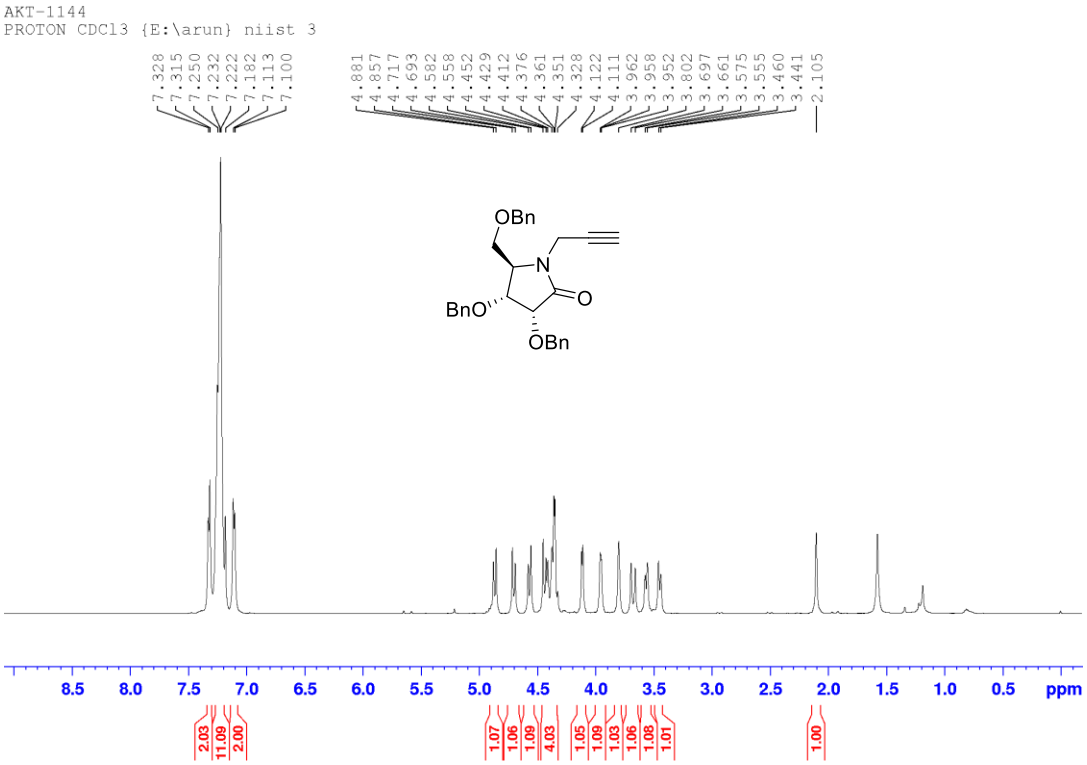


Figure 5.5.3.8. NMR spectras of compound **9**



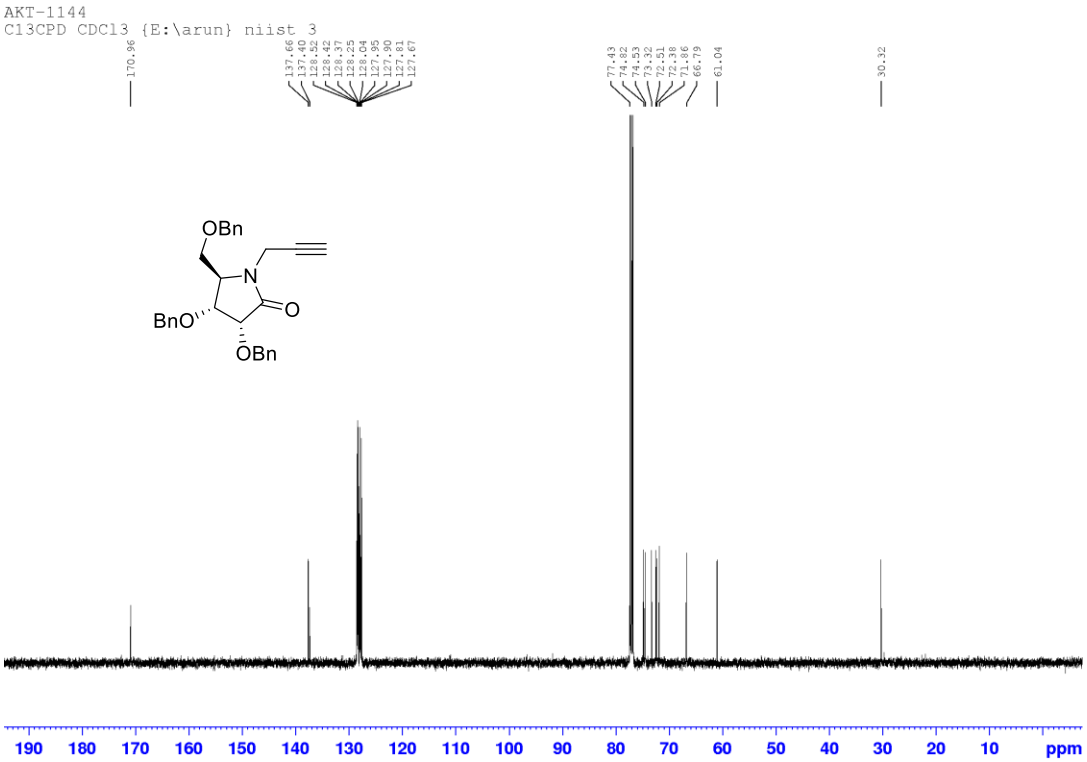
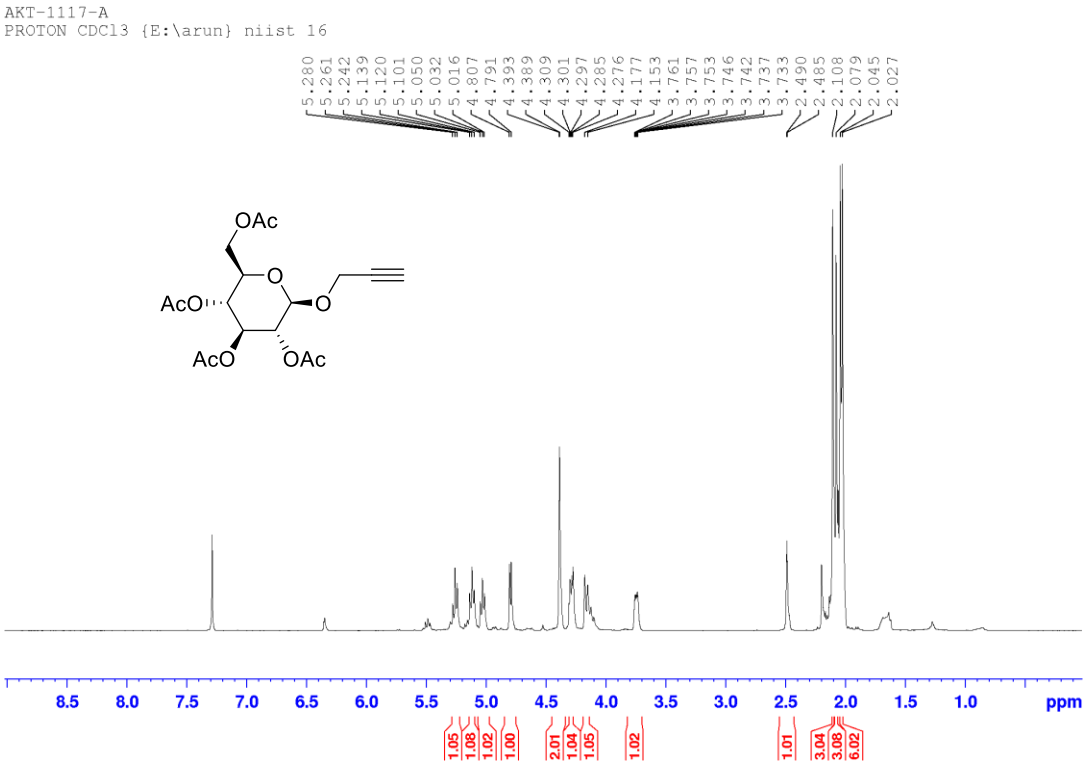


Figure 5.5.3.9. NMR spectras of compound **10**



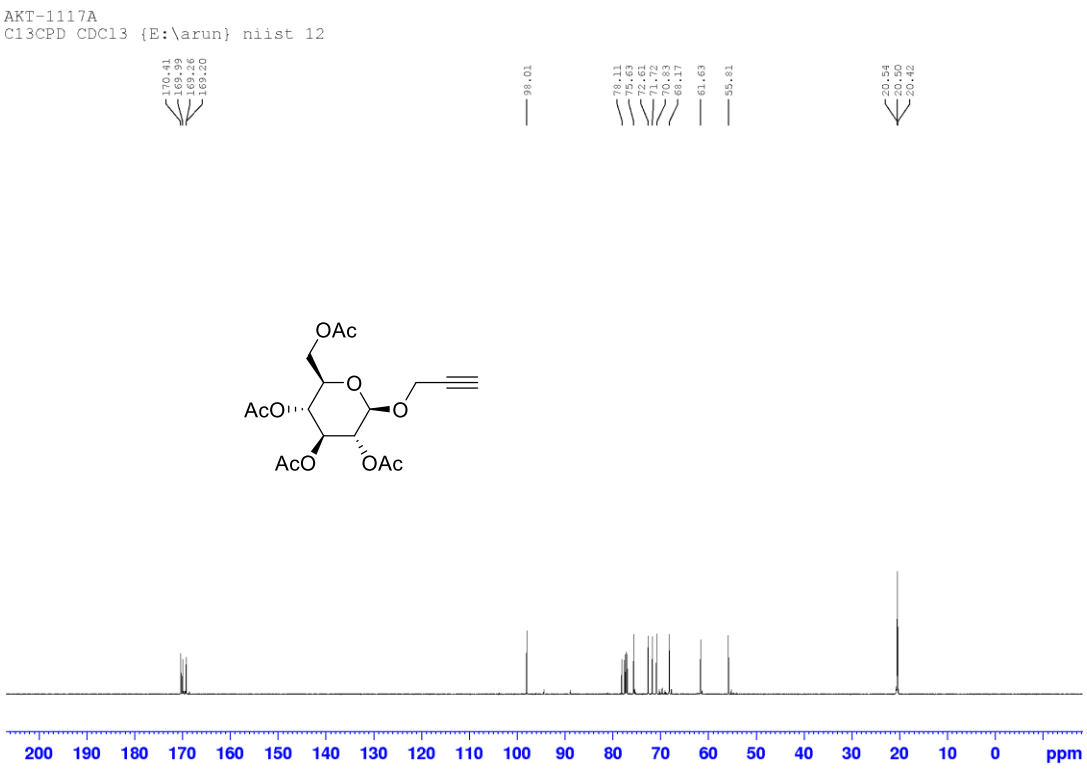


Figure 5.5.3.10. NMR spectras of compound **11**

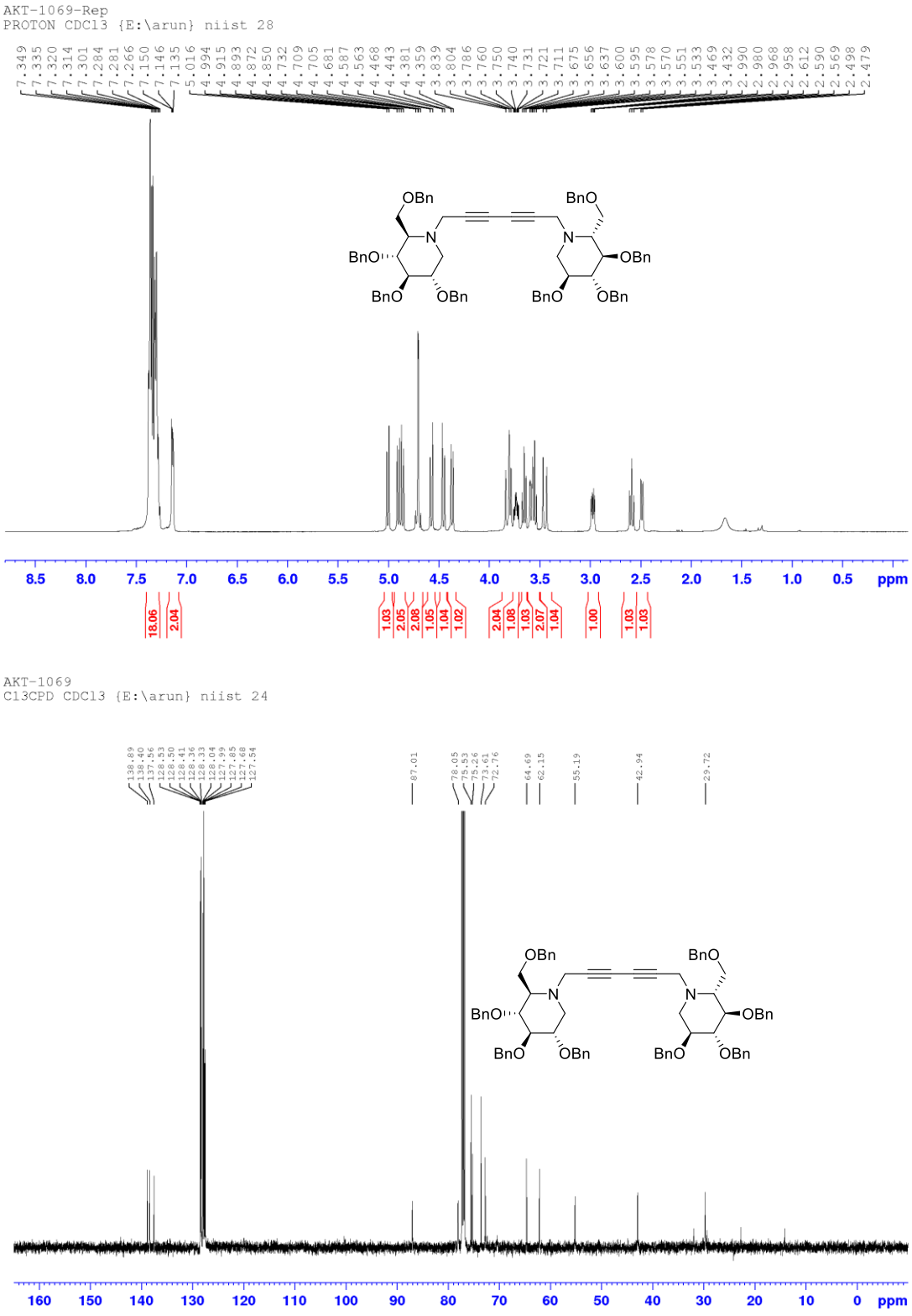


Figure 5.5.3.11. NMR spectras of compound **13**

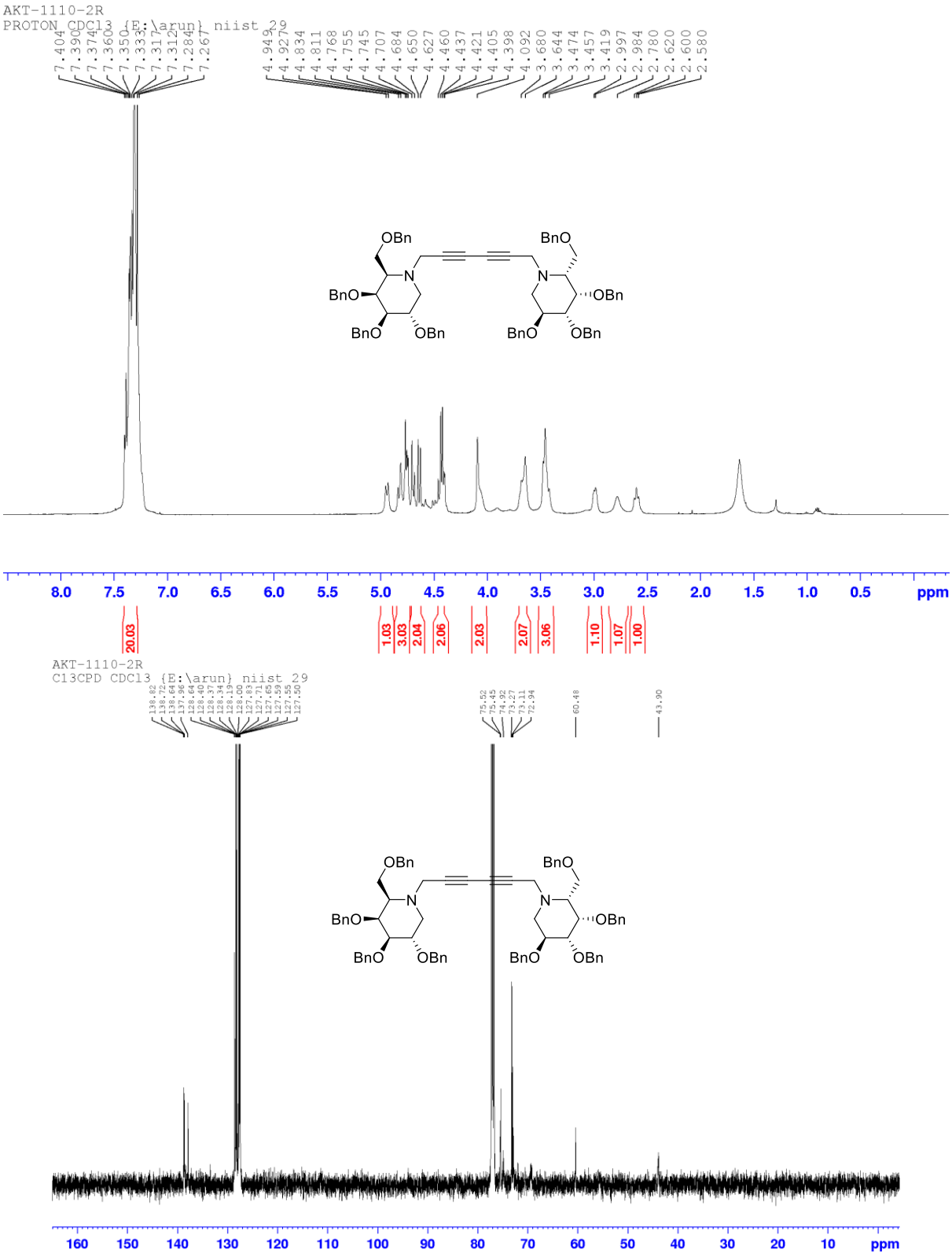


Figure 5.5.3.12. NMR spectras of compound **14**

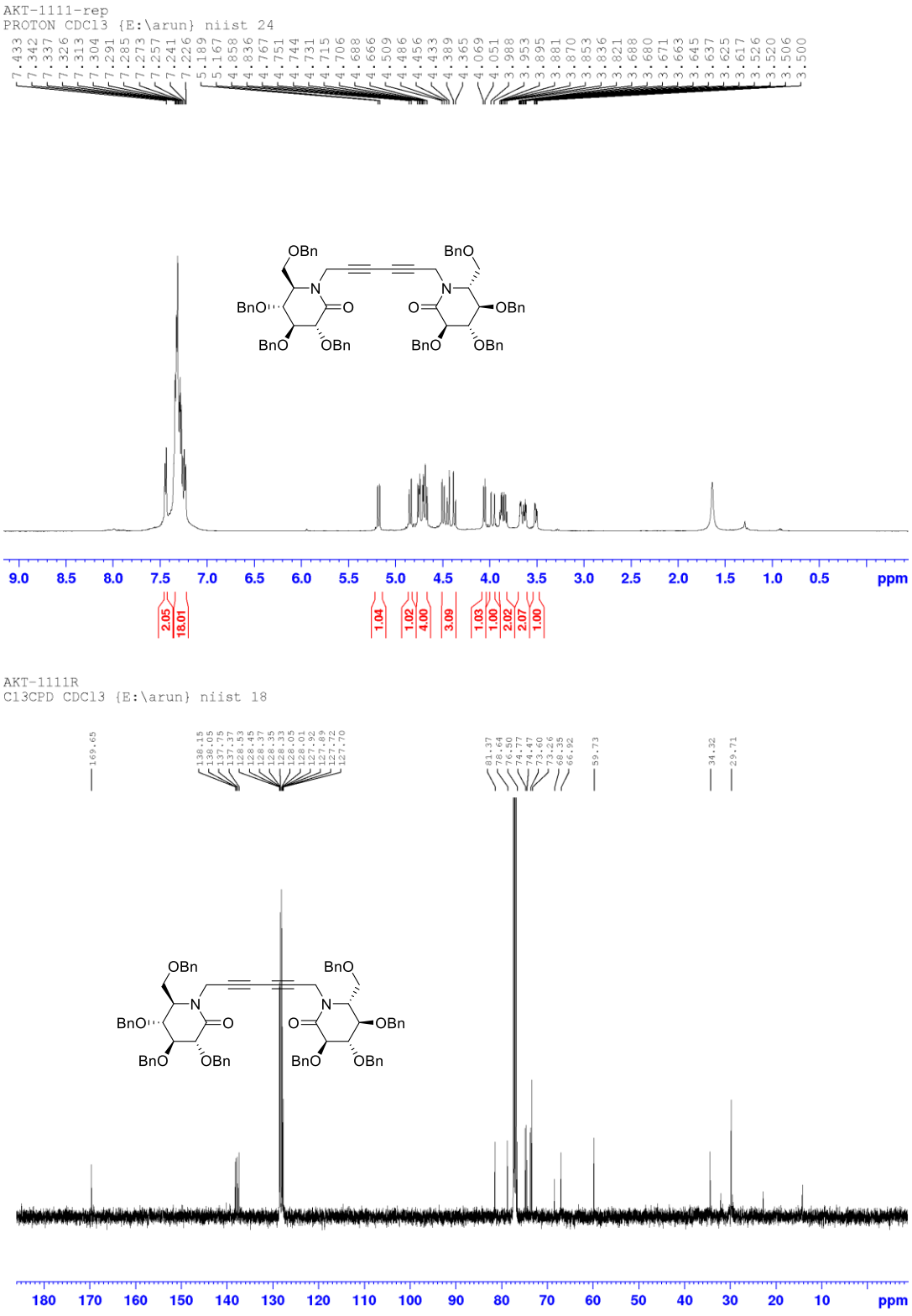


Figure 5.5.3.13. NMR spectras of compound **15**

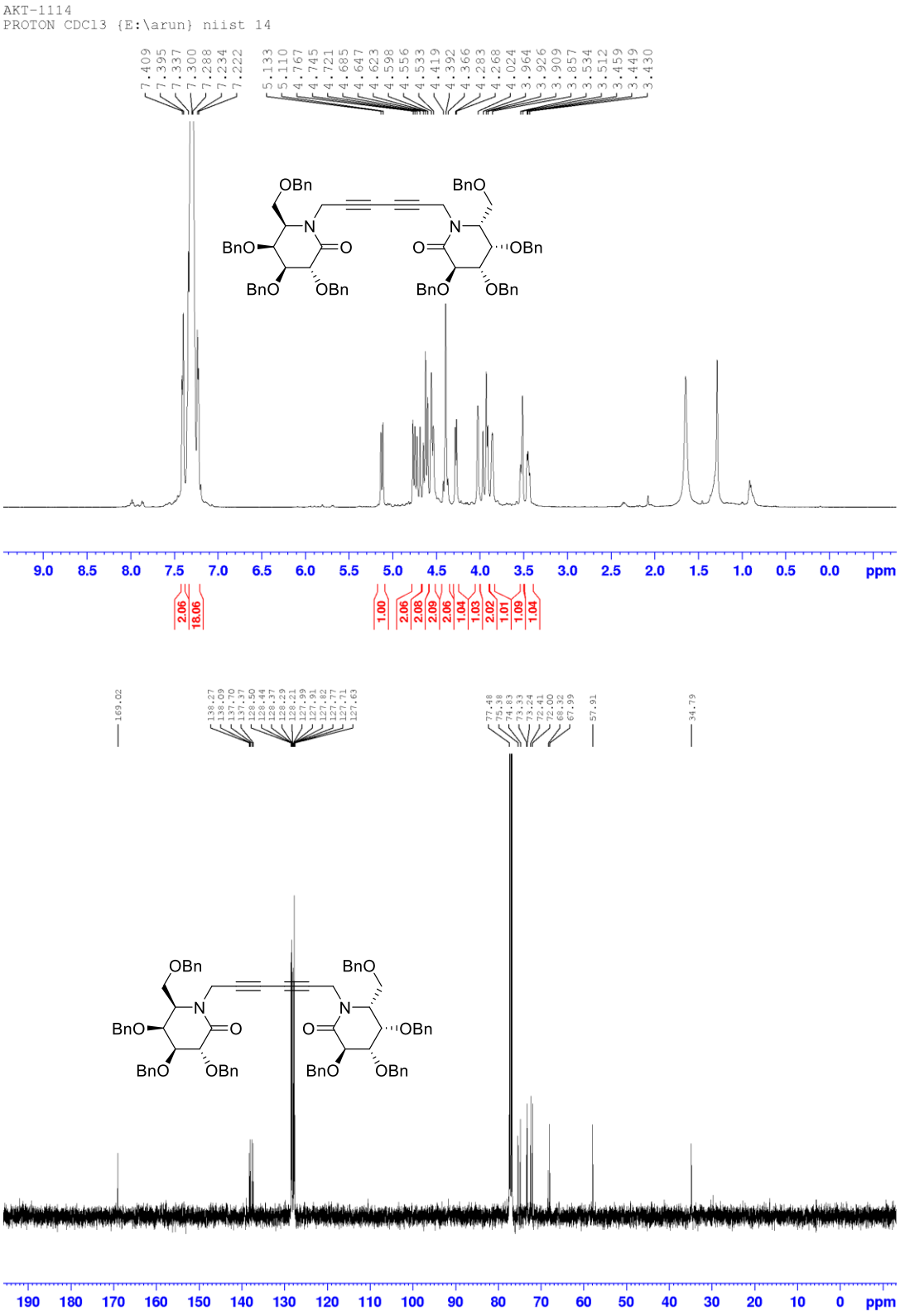


Figure 5.5.3.14. NMR spectras of compound **16**

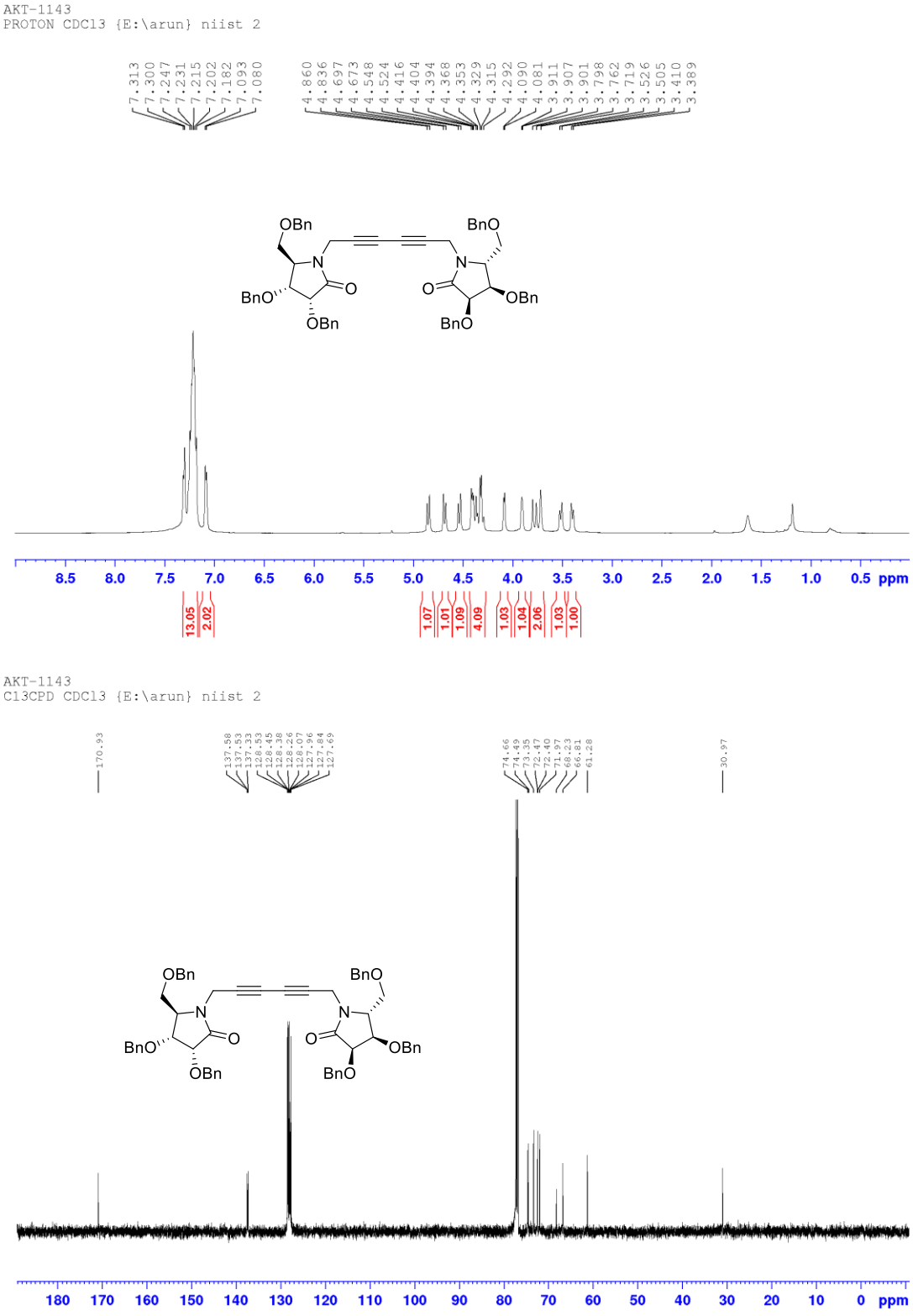
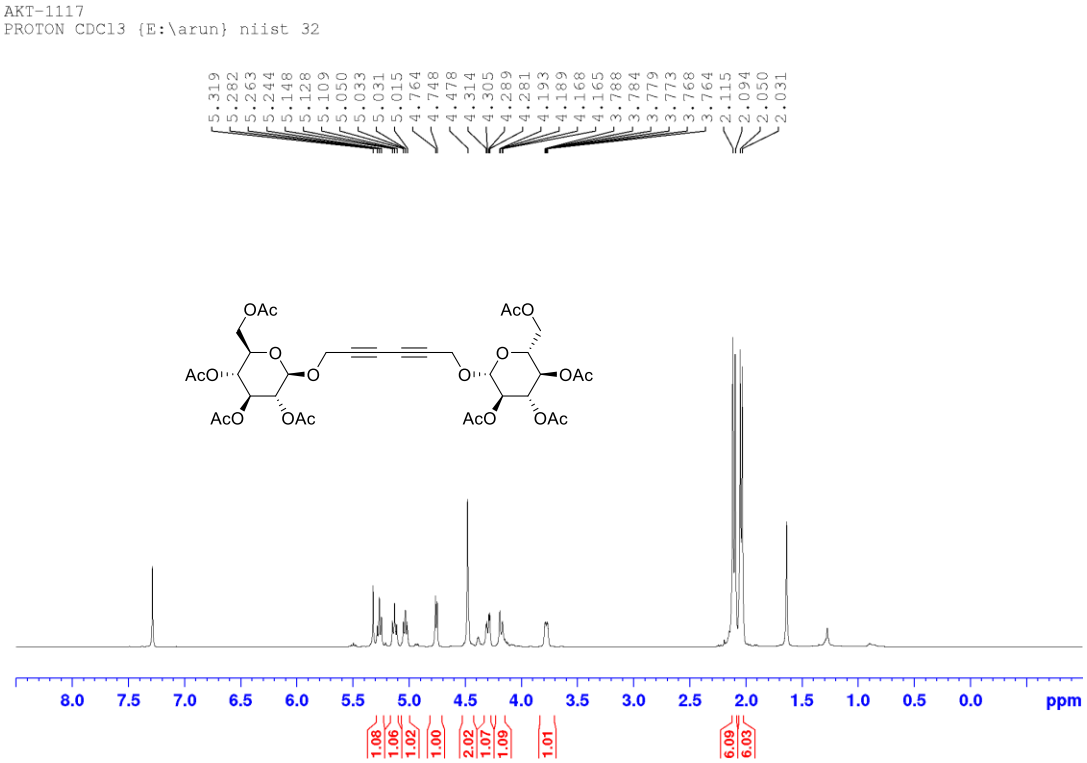


Figure 5.5.3.15. NMR spectras of compound **17**



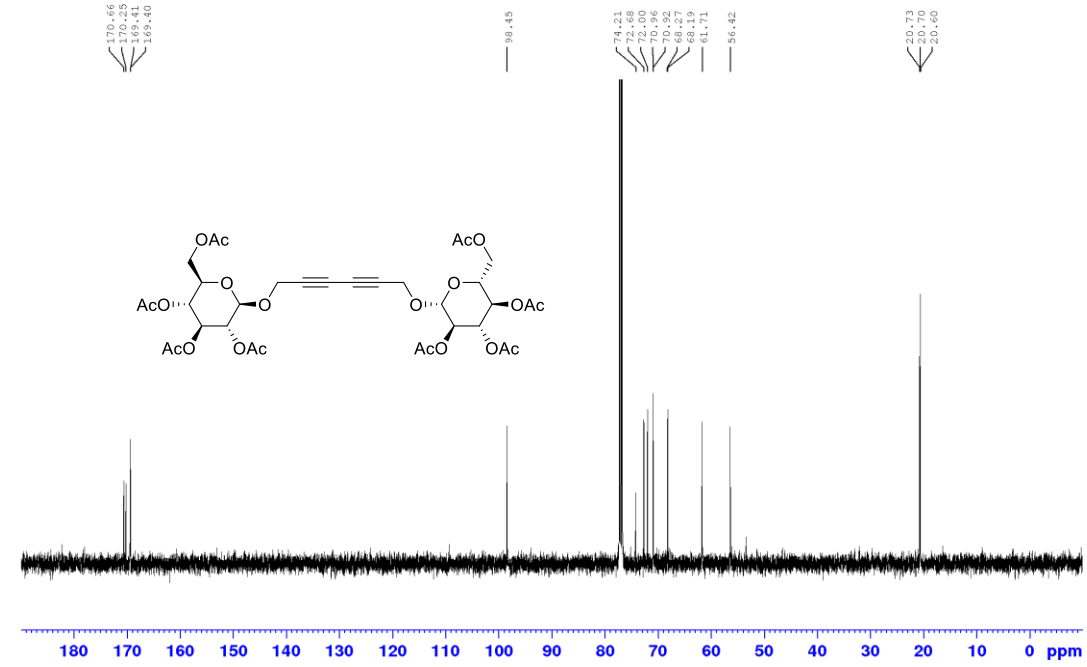


Figure 5.5.3.16. NMR spectras of compound **18**

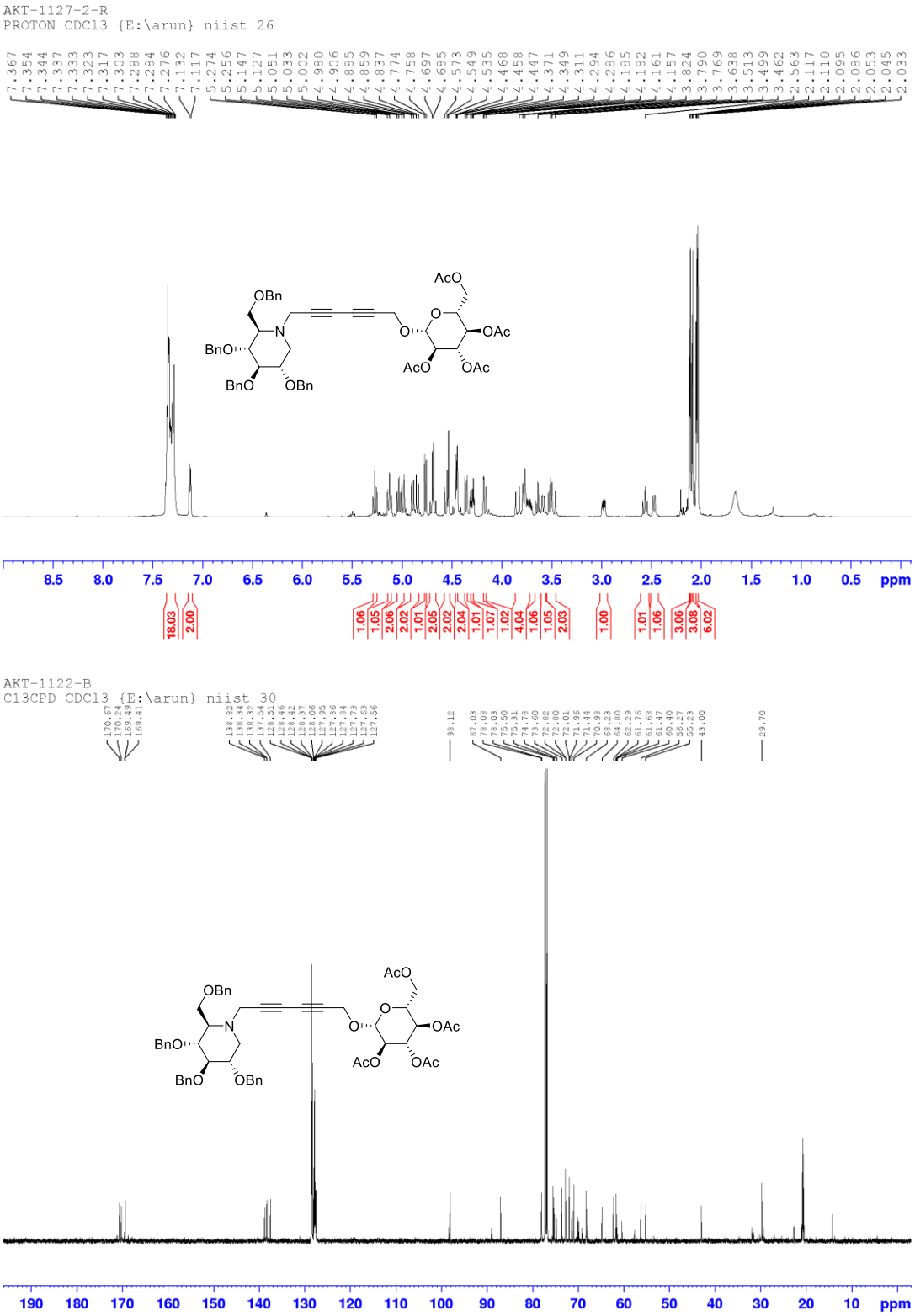
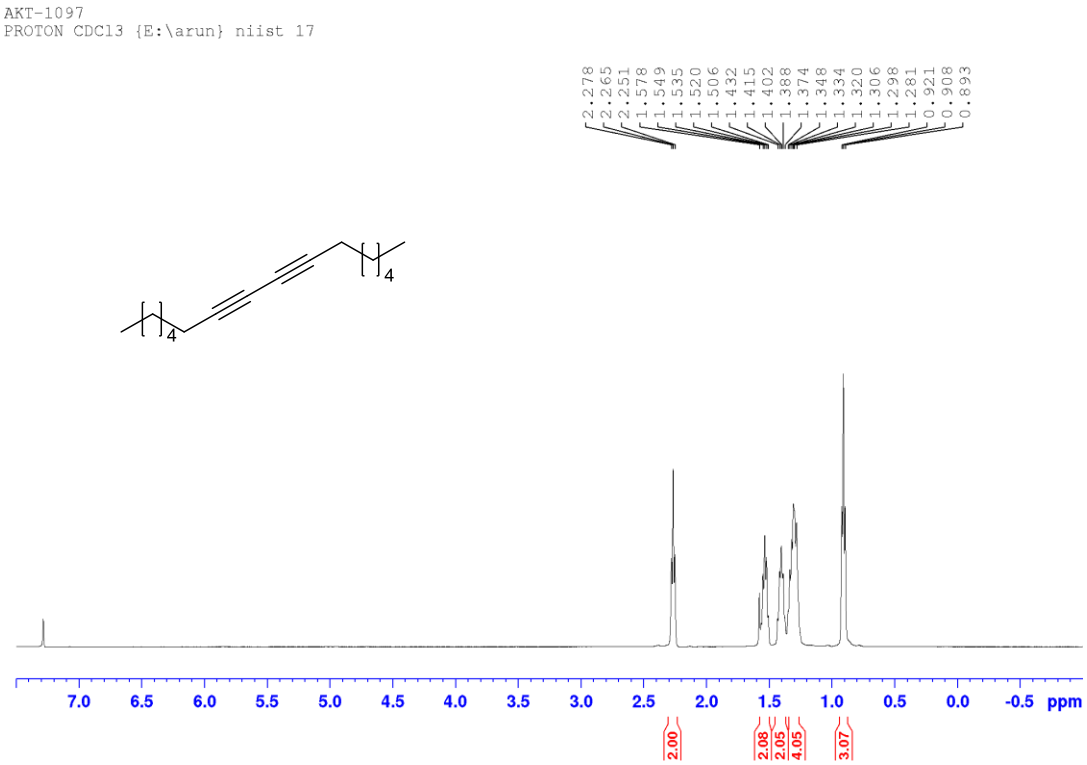


Figure 5.5.3.17. NMR spectras of compound **19**



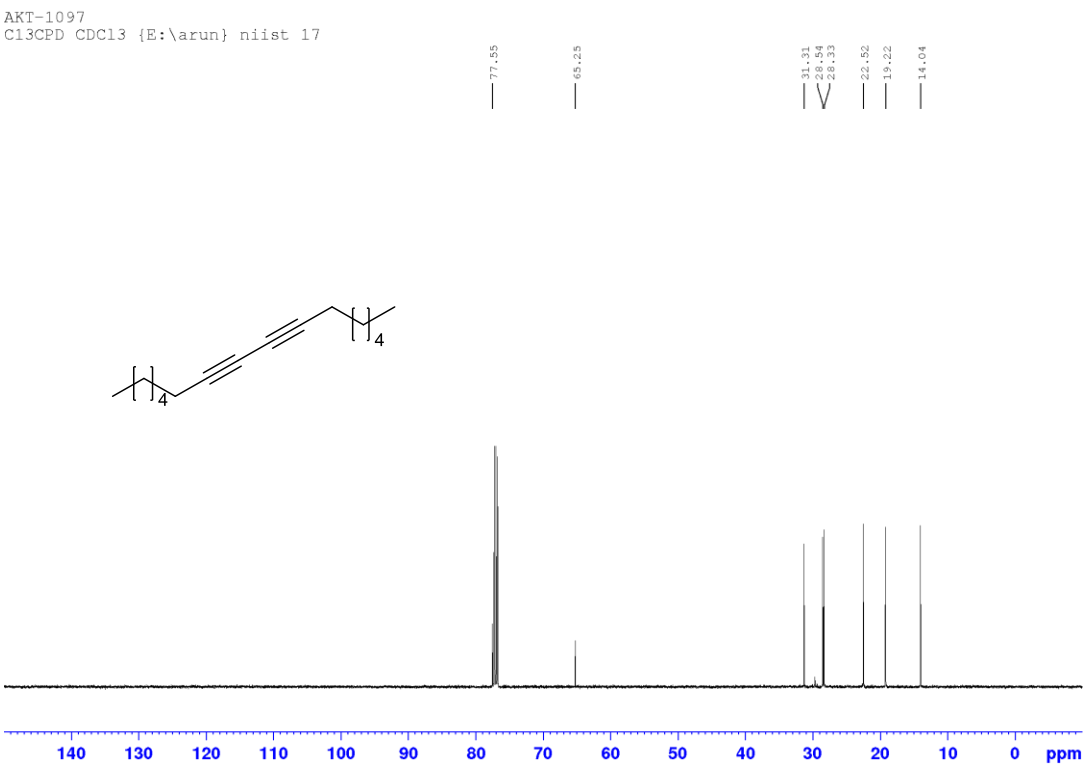


Figure 5.5.3.18. NMR spectras of compound **20**

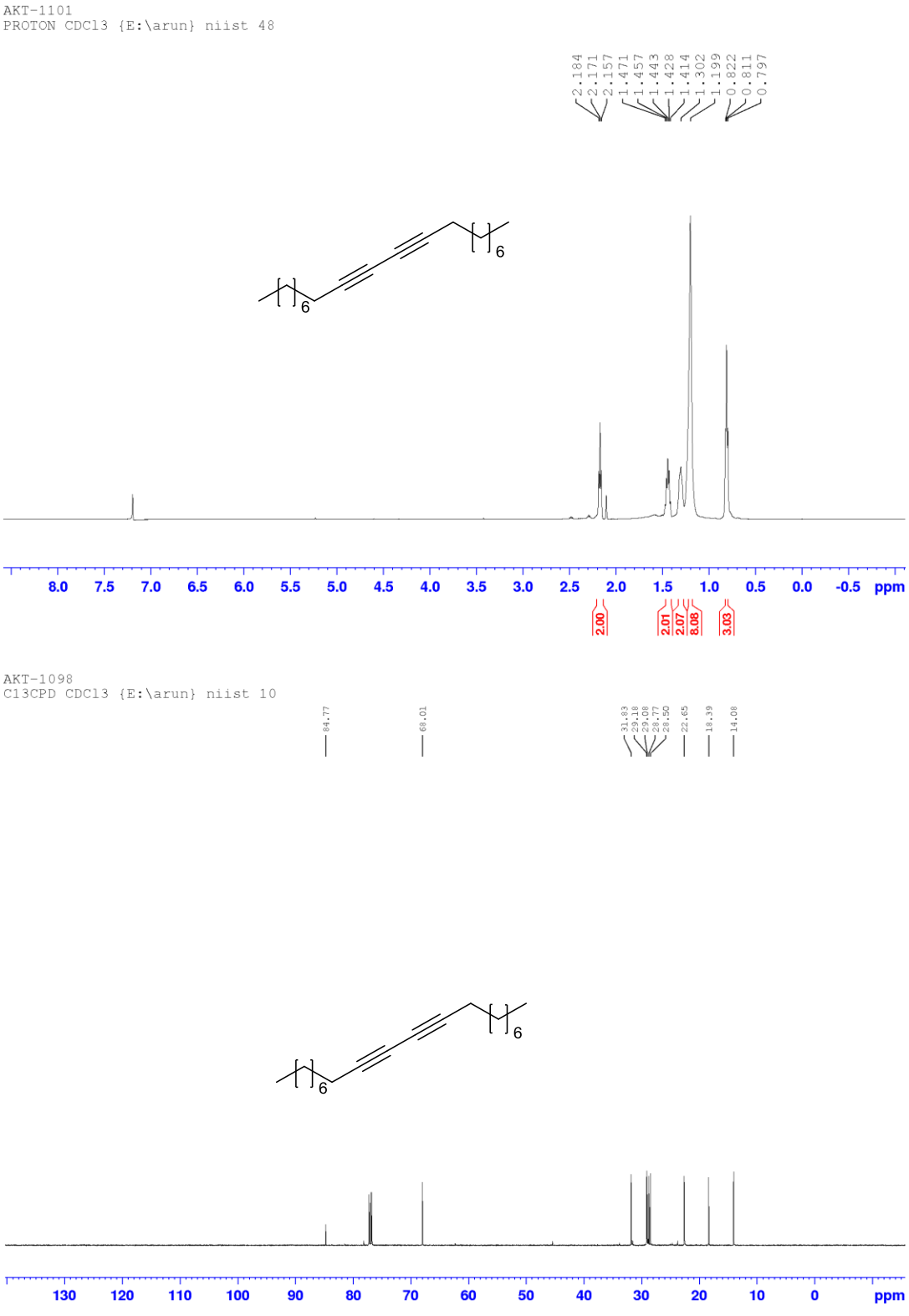


Figure 5.5.3.19. NMR spectras of compound **21**

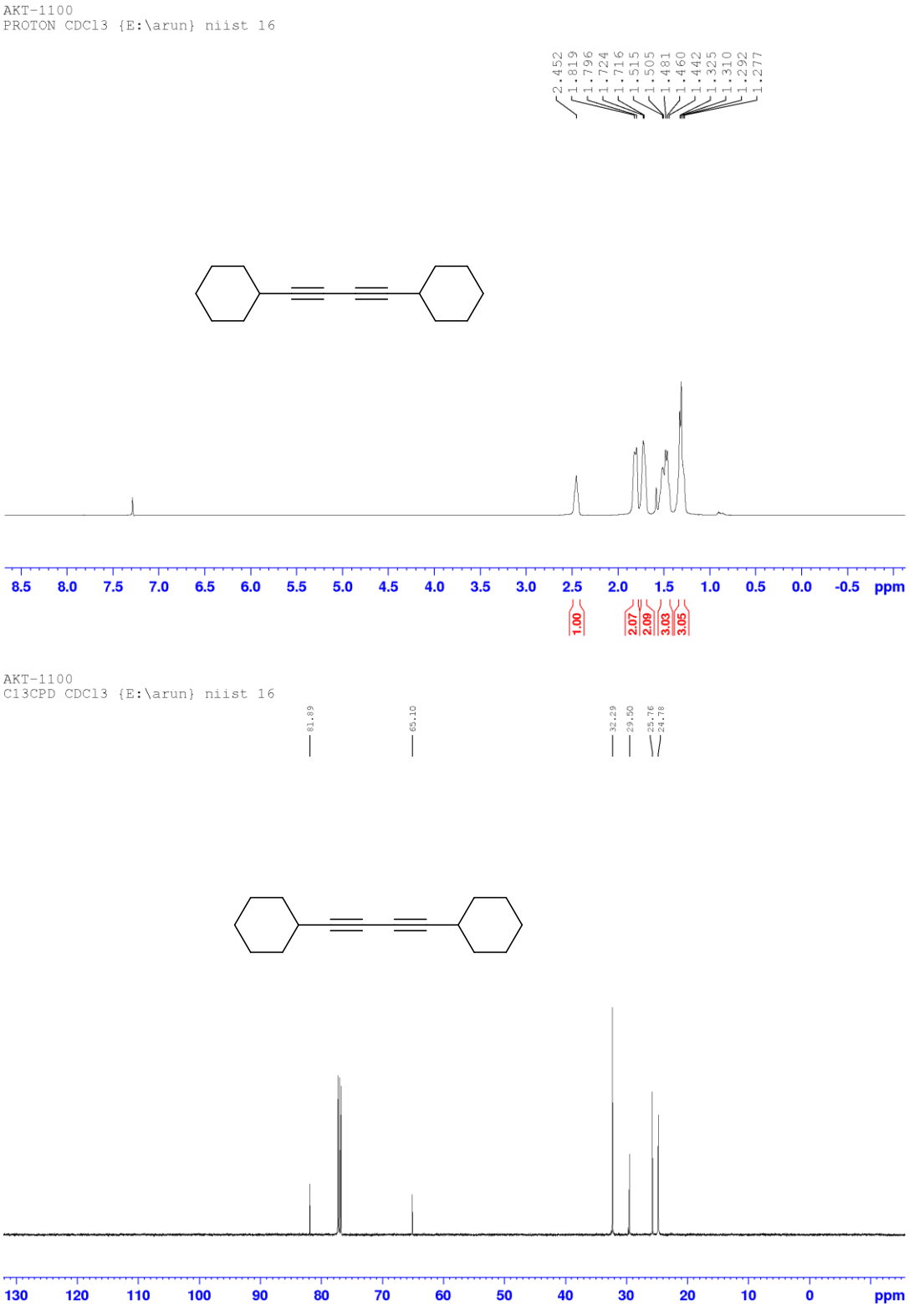


Figure 5.5.3.20. NMR spectras of compound **22**

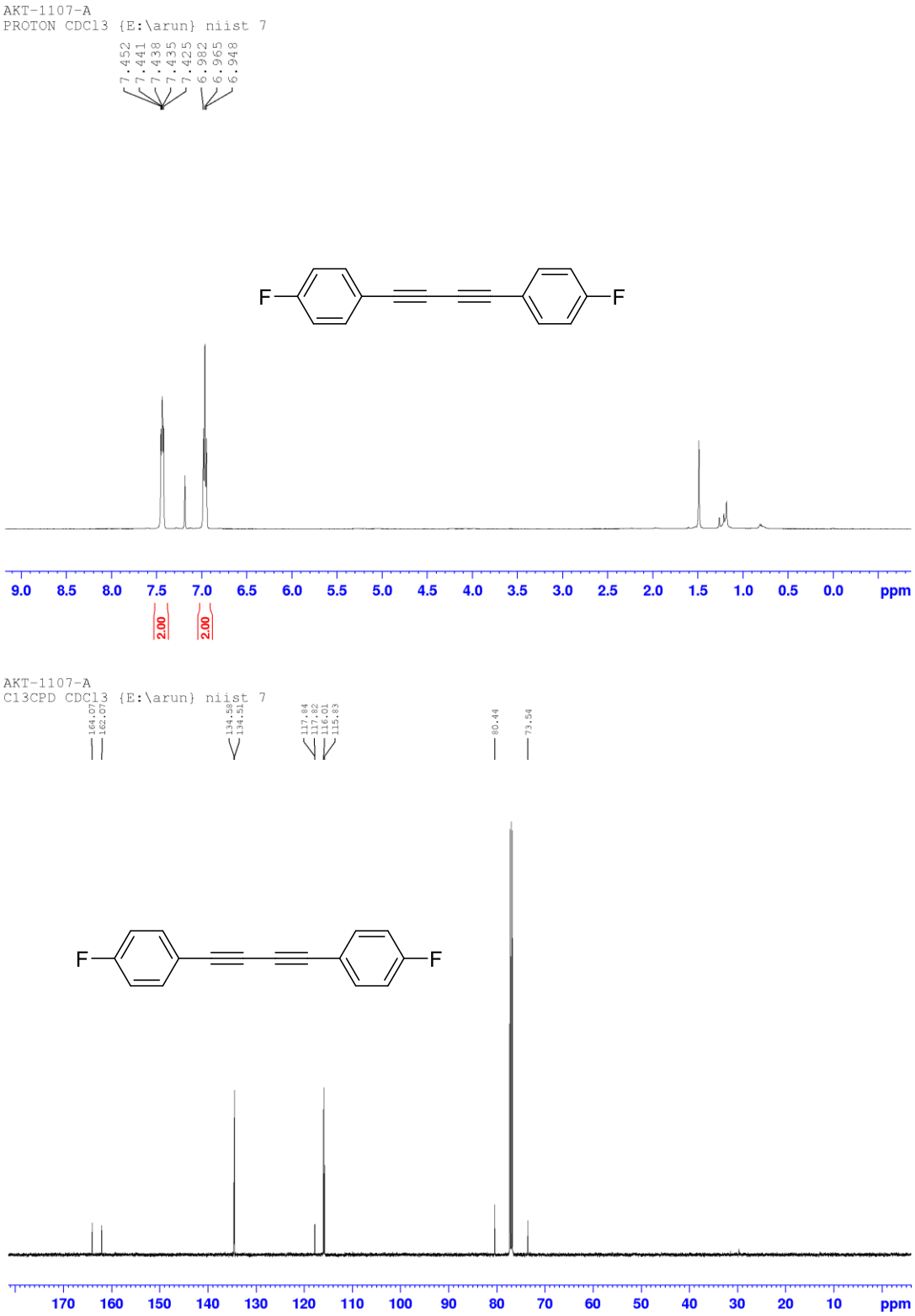
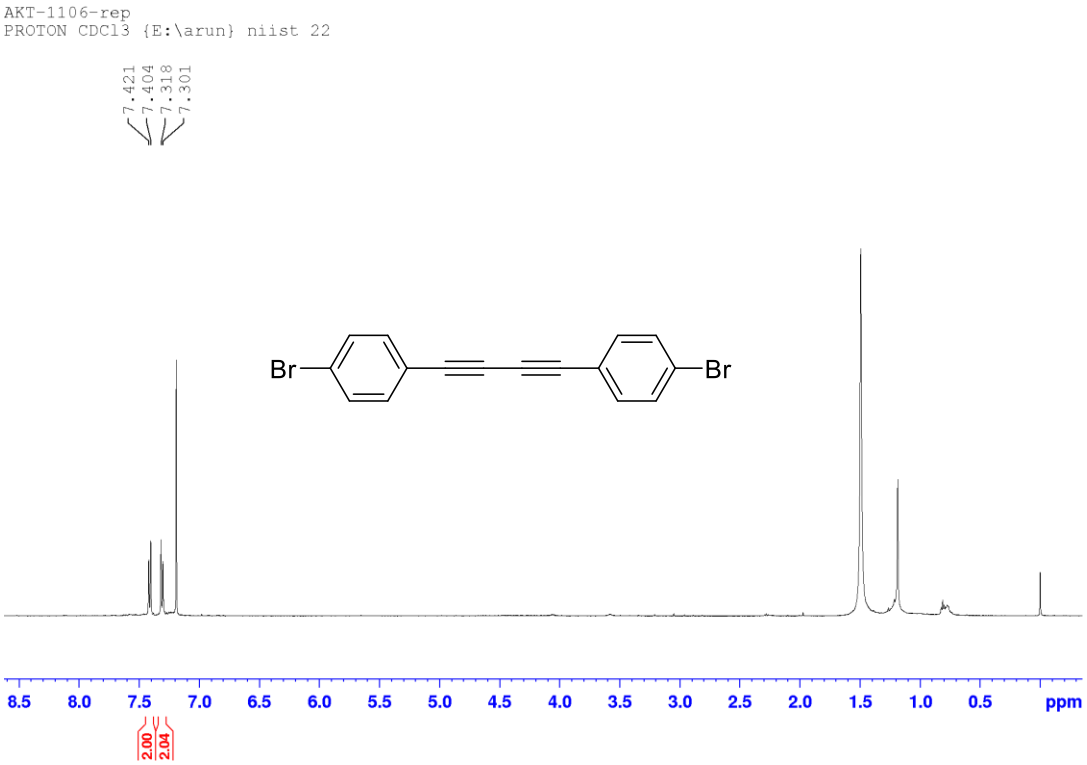


Figure 5.5.3.21. NMR spectras of compound **23**



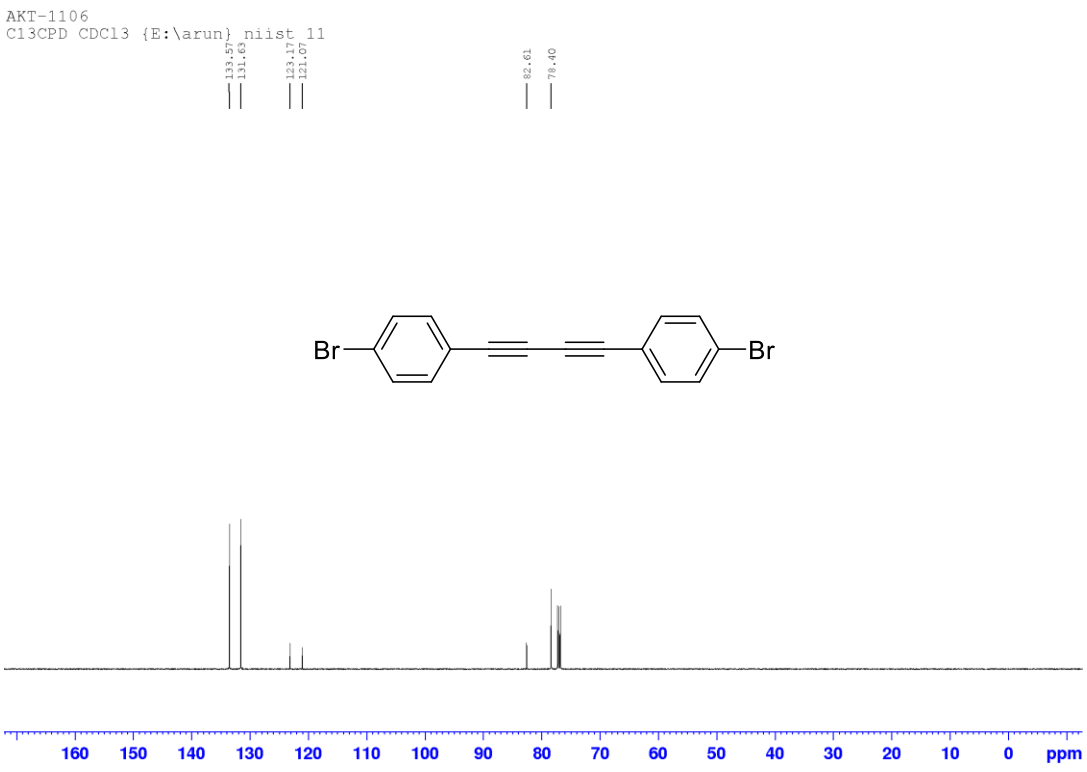


Figure 5.5.3.22. NMR spectras of compound **24**

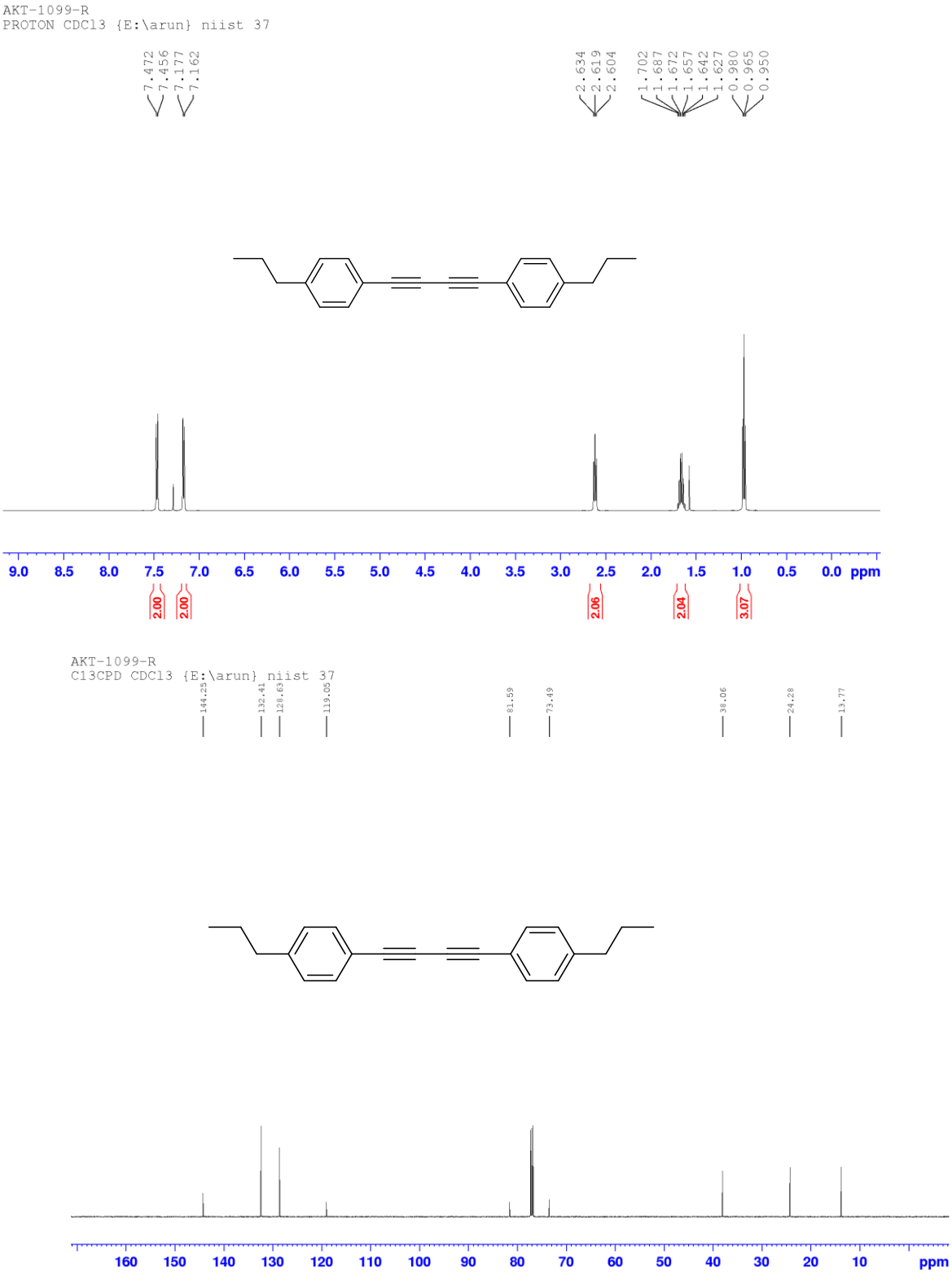
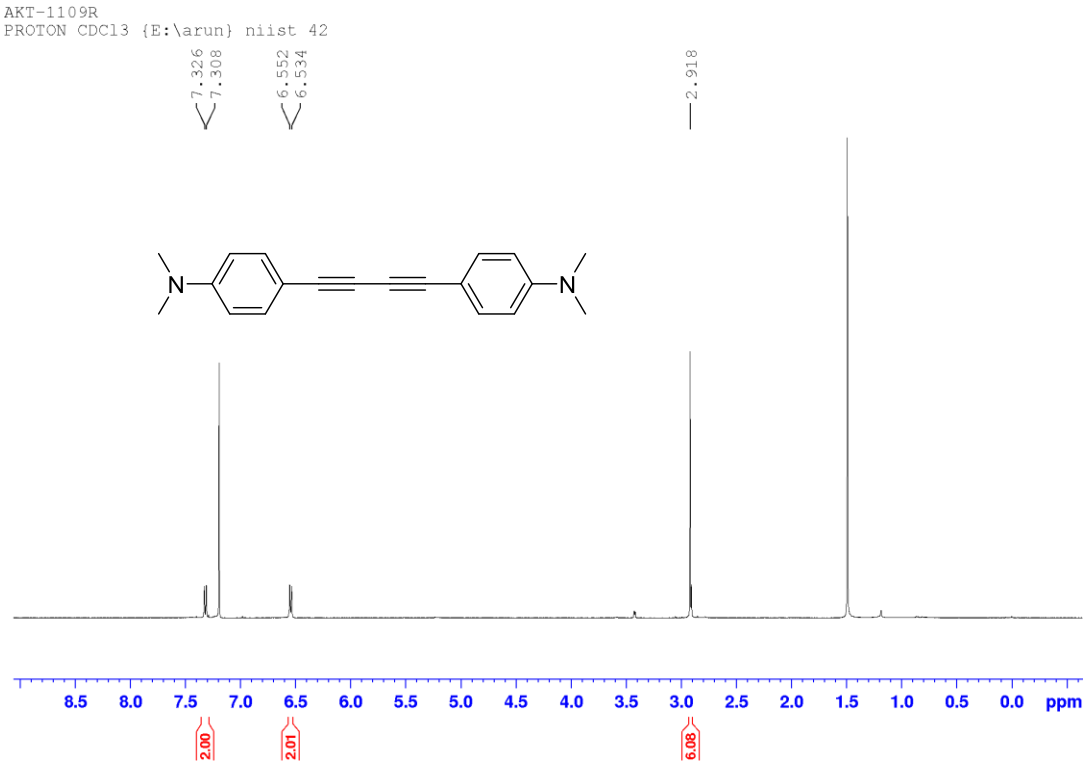


Figure 5.5.3.23. NMR spectras of compound **25**



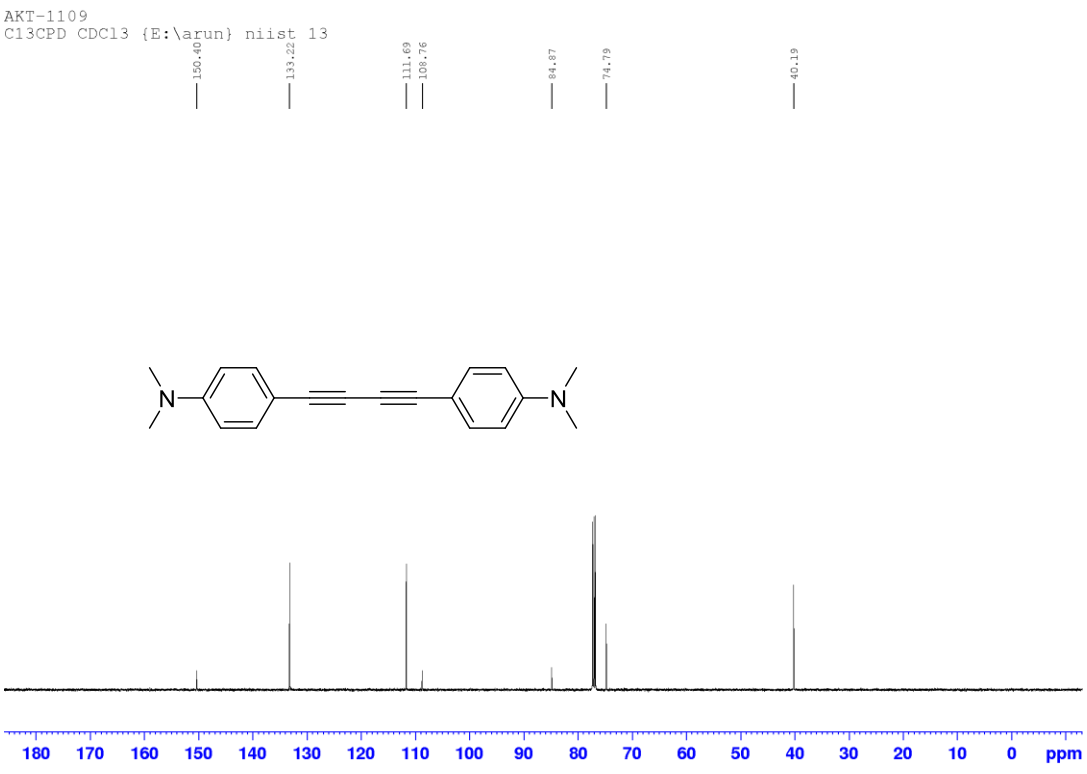
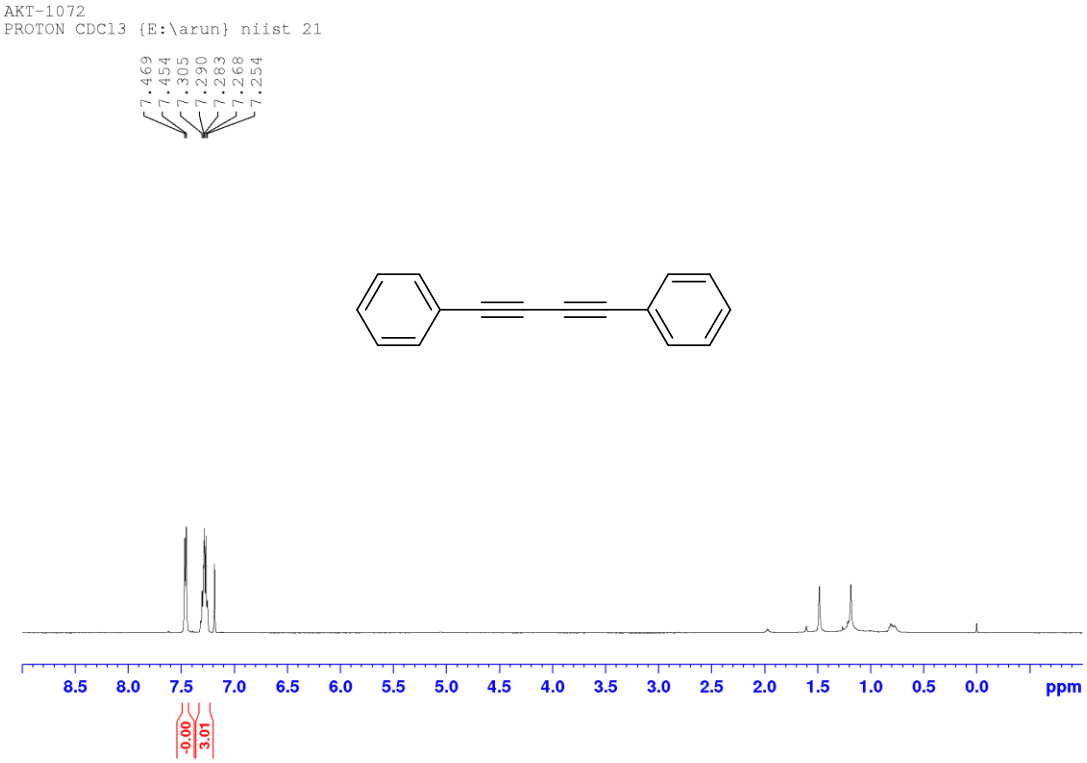


Figure 5.5.3.24. NMR spectras of compound **26**



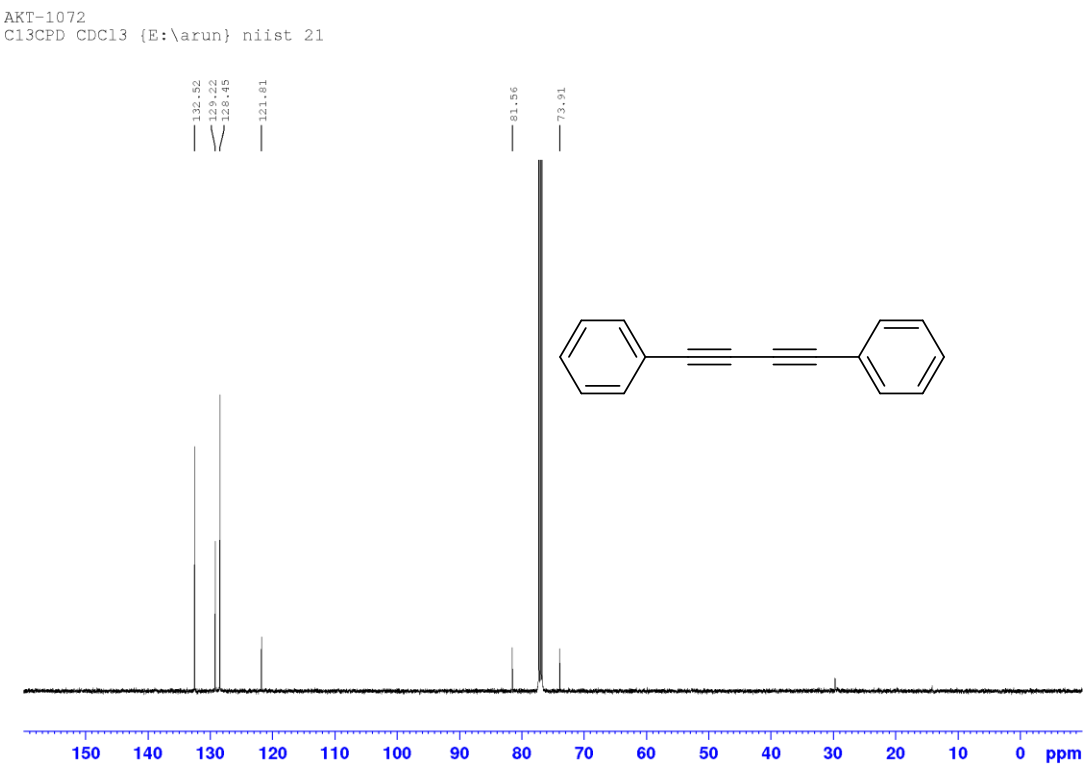
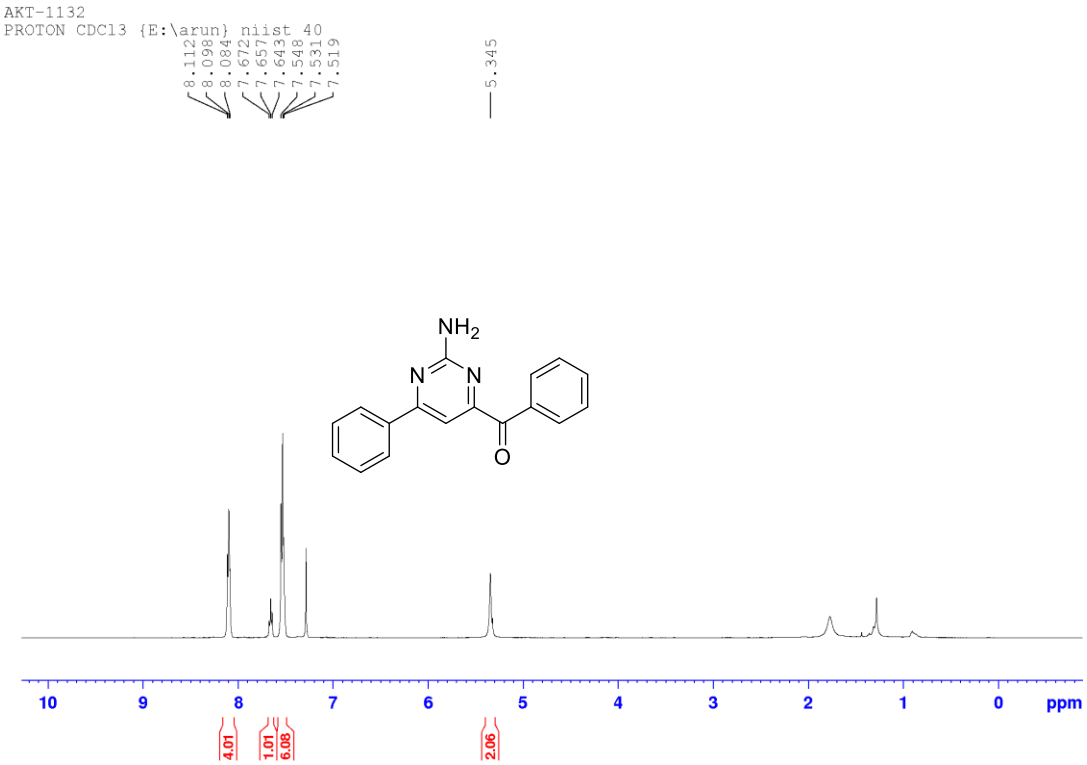


Figure 5.5.3.25. NMR spectras of compound **28**



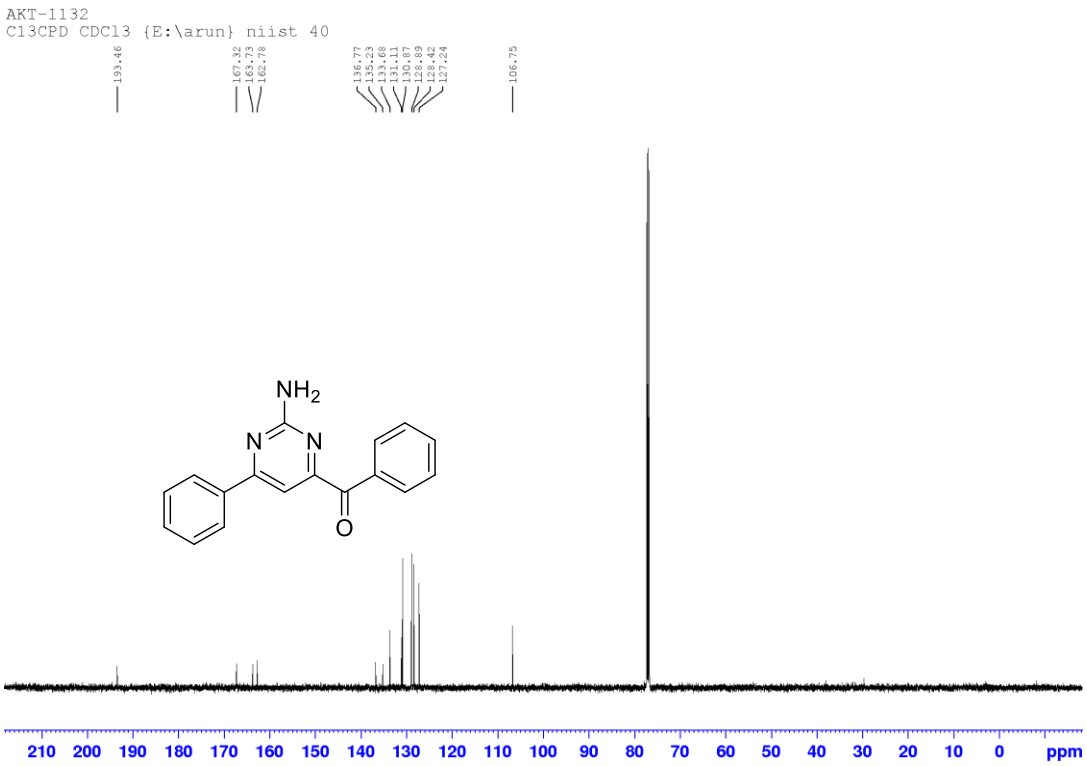
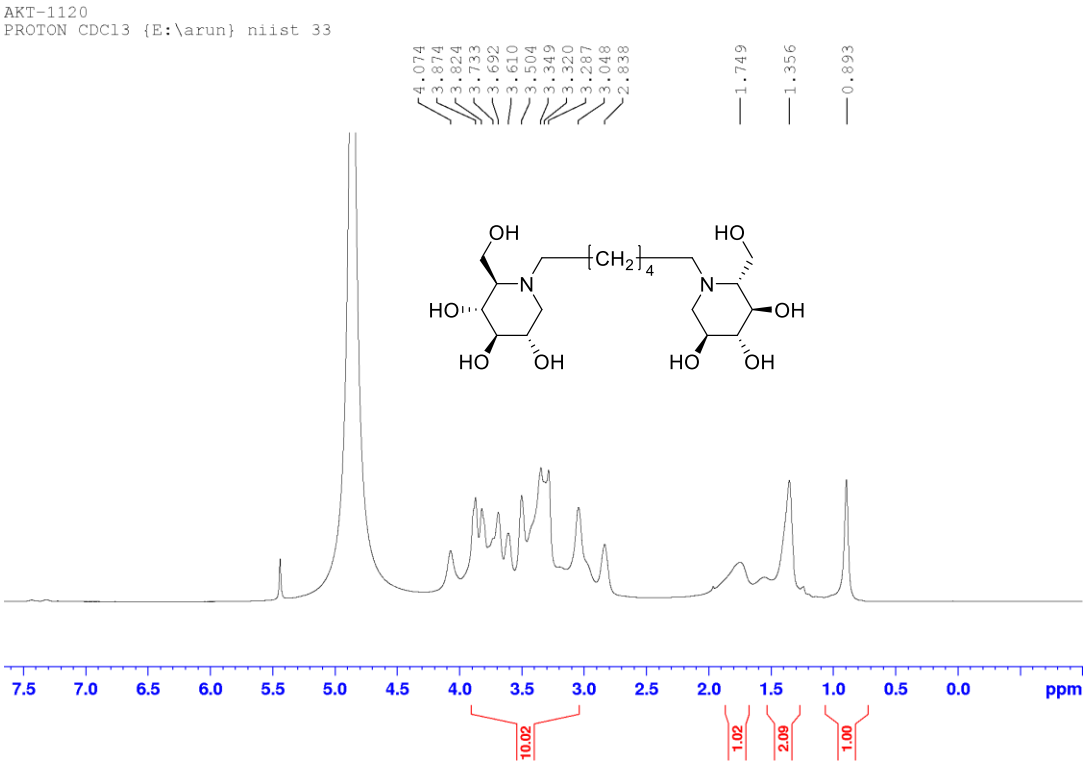


Figure 5.5.3.26. NMR spectras of compound **12**



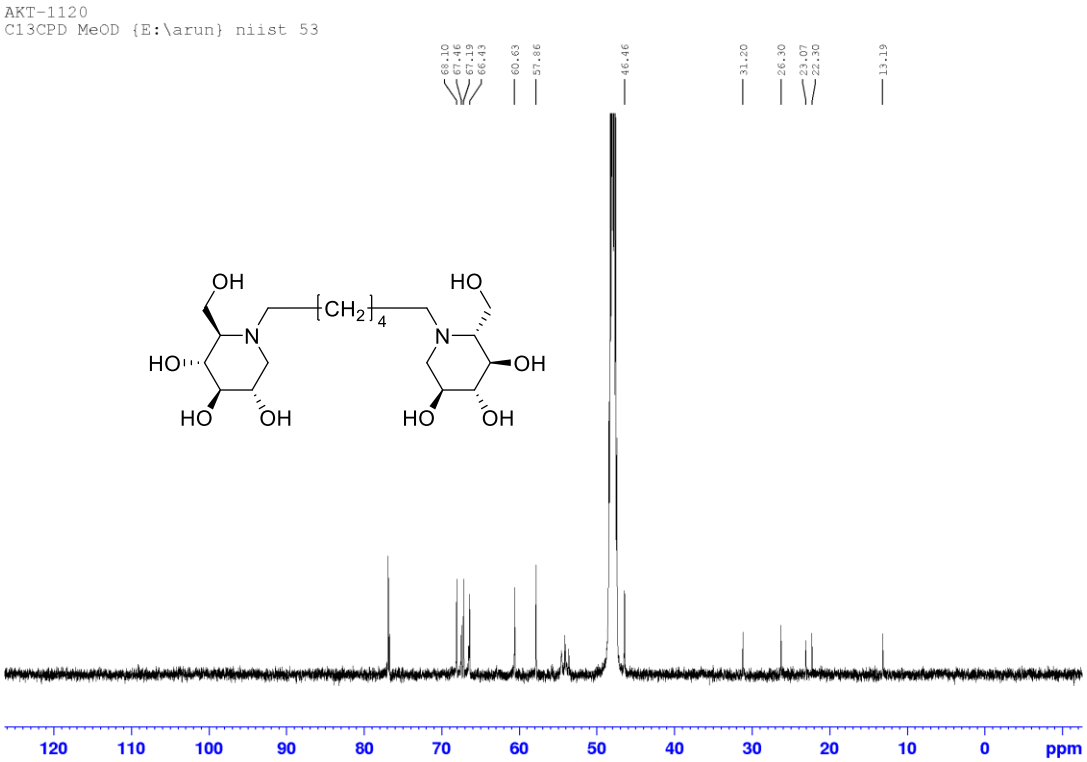
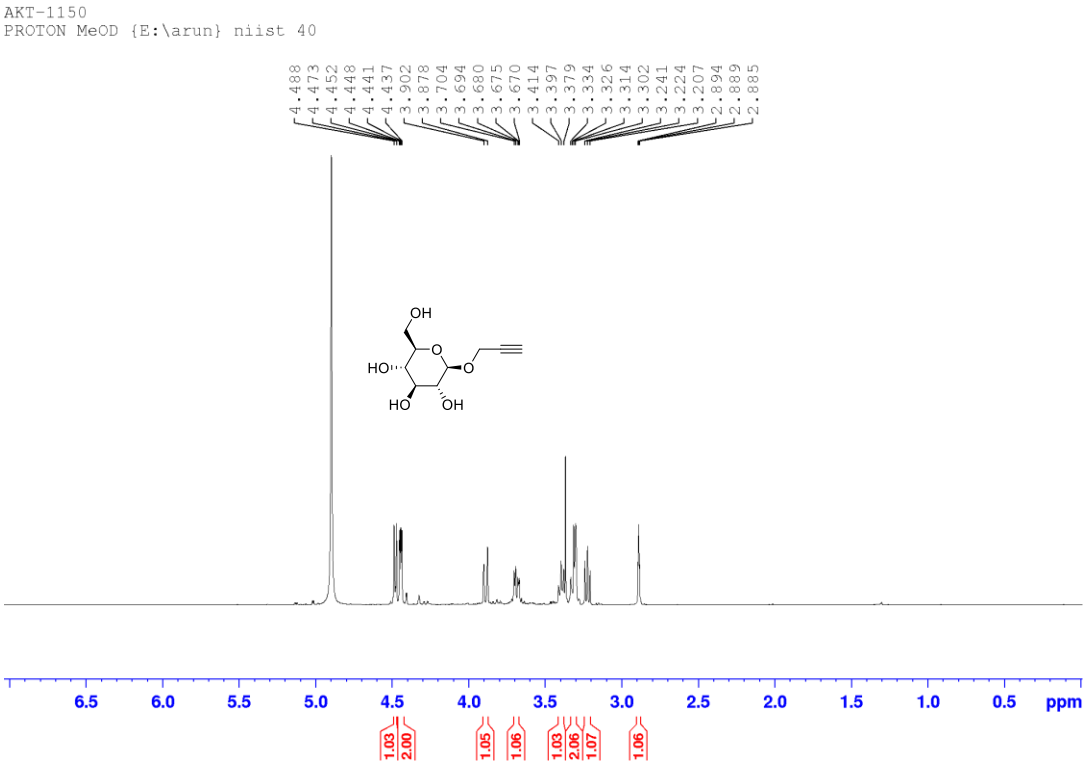


Figure 5.5.3.27. NMR spectras of compound **29**



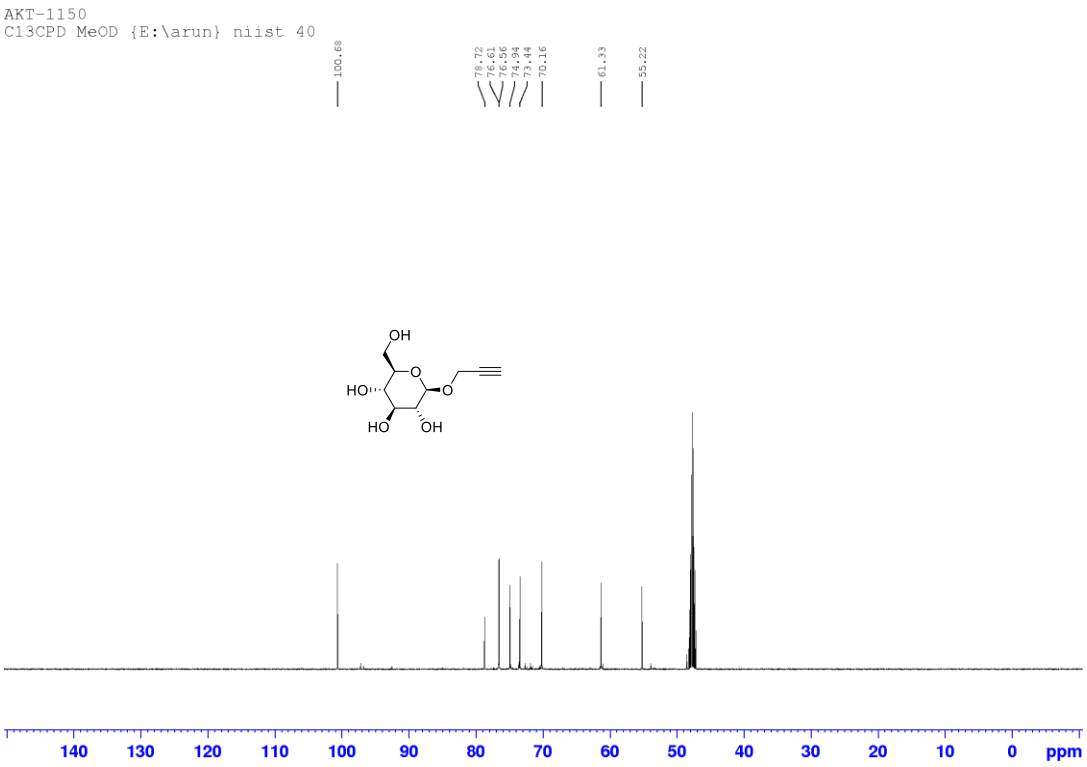
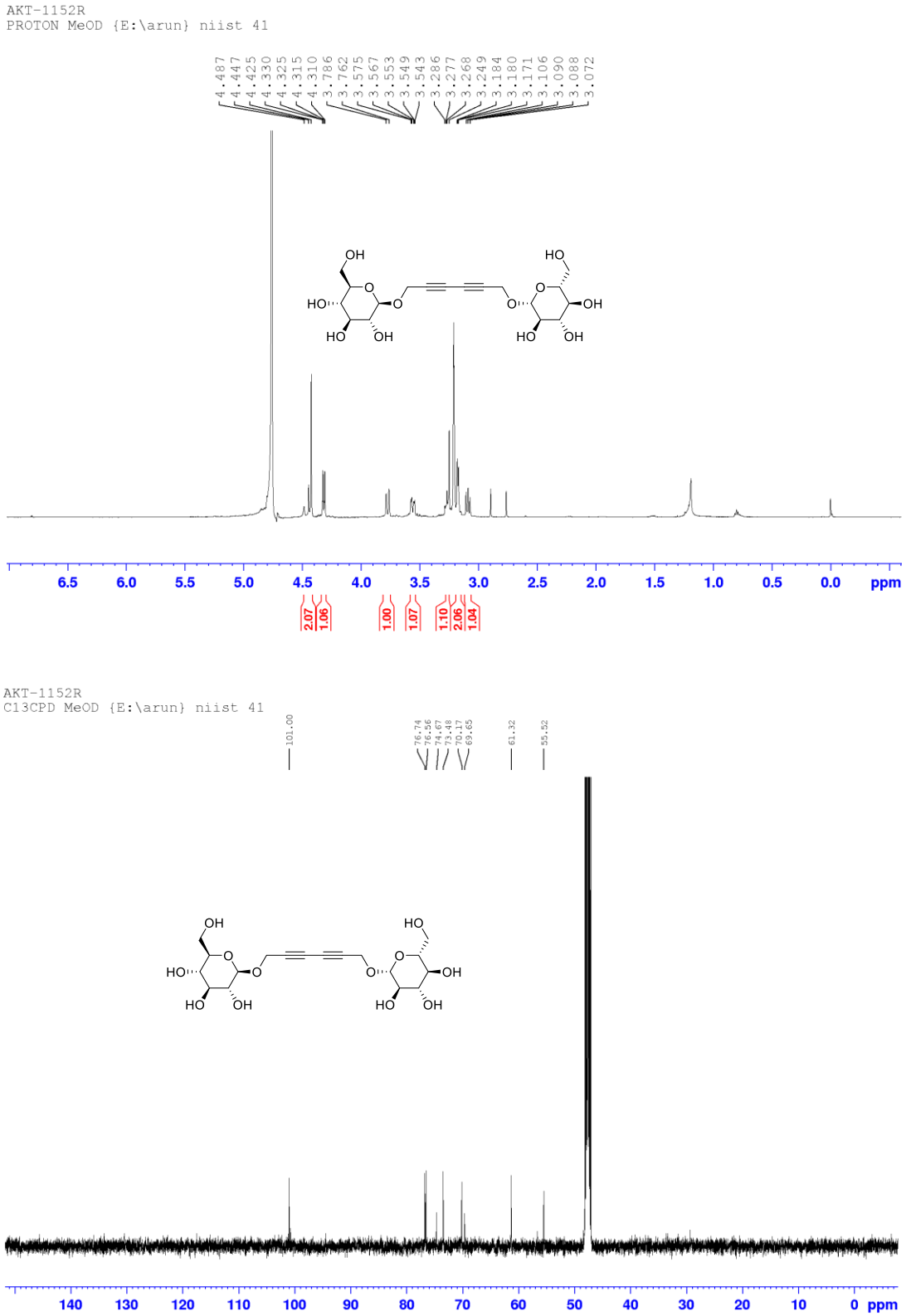


Figure 5.5.3.28. NMR spectras of compound **30**



5.6. References

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ABSTRACT

Name of the Student**:** **Mr. Arunkumar T.** Registration No**.:** 10CC15A39008
Faculty of Study**:** Chemical Sciences Year of Submission**:** 2021
AcSIR academic centre/CSIR Lab**:** CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST) Name of the Supervisor**:** Dr. Ravi Shankar Lankalapalli
Title of the thesis: **Design and synthesis of novel iminosugar analogues of biological relevance**

Iminosugars are one of the leading carbohydrate mimics with excellent biological activity. The **first chapter** concerns the introduction of synthetically inspiring iminosugars and their importance in drug discovery, especially C-glycosides. The emerging research on iminosugars is a result of their ability to inhibit glycoprocessing enzymes. Inhibitors of these enzymes are the main therapeutic targets for debilitating diseases like cancer, diabetes, lysosomal storage disorders and rare genetic disorders.

The **second chapter** deals with the synthesis of various iminosugar C-glycosides with a few interesting transformations. Along with that, bicyclic iminosugars (aziridine) were also synthesized based on the medicinal chemistry relevance of bicyclic iminosugars. The isomers (L and D) of bicyclic compounds were characterized via 1D-COSY and 1D NOE. All the compounds were unambiguously characterized by ^{1}H, ^{13}C, 2D NMR, HRMS and optical rotation.

The **third chapter** describes the synthesis of iminosugar appended Miltefosine analogues via click chemistry. Initial attempts on the phospholipid bromide led to an unsuccessful reaction because of its poor solubility in the polar aprotic solvents. Based on that observation, we designed and synthesized a triazole bridged, N-propargylated glucose and galactose variants of 1-deoxyiminosugar and phospholipid azide via click chemistry.

In the fourth chapter, three new classes of nojirimycin analogues *viz.* N-alkyl with C1-substituent (4-phenylbutyl), N-substituted 1-deoxynojirimycin and its congener δ-lactam, and a 4-phenylbutyl-β-C-glycoside were designed and synthesized for immunological studies. The resulting diverse compound library exhibited proliferation of B Cells and T cells induced by LPS and Con A, respectively. A deoxynojirimycin-triazole conjugate of phytosphingosine analogue was superior in the responses and exhibited nitric oxide response equal to LPS.

In the **final chapter**, we have demonstrated the limitation of CuAAC 'click' reaction with a 2-azidopyridine substrate, owing to its equilibrium with a tetrazole isomer, which is exploited herein for its utility in Glaser–Hay reaction. A catalytic combination of a 2-azidopyridine analogue, 4-azido-5Hpyrrolo[3,2-d]pyrimidine, and CuI afforded homocoupled products of terminal alkynes, without any trace of triazole product, under mild conditions with a broad substrate scope. Emphasis on carbohydrate-based substrates appended to a propargylic group led to 1,3-diynes in good to excellent yields.

List of publications related to Thesis

1. A Cu(I)-azidopyrrolo[3,2-d]pyrimidine catalyzed Glaser-Hay reaction under mild conditions. *ACS Organic & Inorganic AU*, 2021 (DOI: 10.1021/acsorginorgau.1c00015). **Arun K. Thangarasu**, Velickakathu O. Yadhukrishnan, K. A. Krishnakumar, Sanjay Suresh Varma, and Ravi S. Lankalapalli*.
2. Design, Synthesis, and Preliminary Immunopotentiating Activity of New Analogues of Nojirimycin. *Carbohydrate Research*, 2022, 511, 108479. **Arun K. Thangarasu**, Shainy Sambyal, Halmuthur Mahabalarao Sampath Kumar* and Ravi S. Lankalapalli.*

Papers Published from Other Related Works

1. Semi-synthetic diversification of Coronarin D, a Labdane Diterpene, under Ugi Reaction Conditions —*Nat. prod. res.* 2020, 34, 1-7. Kollery S. Veena, Murikkinthara Taniya, Jaice Ravindran, **Arun Kumar Thangarasu**, Sulochana Priya and Ravi Shankar Lankalapalli.*
2. Polyhydroxy-N-alkyl-2-pyrrolidinones as a new class of glycolipids with immune modulation potential. *J. Carbohydr. Chem.,* 2018, 37:1, 30-43. Srinivasa R Bonam, Jaggaiah N Gorantla, **Arun K. Thangarasu**, Ravi S. Lankalapalli* and H M Sampath Kumar*.
3. Uttroside B: An efficacious and non-haemolytic plant-derived saponin vaccine adjuvant. *Vaccines,* 2021 (Communicated). Srinivasa Reddy Bonam, **Arun Kumar Thangarasu**, Jaggaiah Naidu Gorantla, Ravi Shankar Lankalapalli and Halmuthur Mahabalarao Sampath Kumar.*
4. Blockade of Uttroside B-Induced Autophagic Pro-Survival Signals Augments its Chemotherapeutic Efficacy Against Hepatocellular Carcinoma. *Frontiers in Oncology*, 2021. Lekshmi R Nath*, Mundanattu Swetha*, Vinod Vijayakurup*, **Arun Kumar Thangarasu***, Nair Hariprasad Haritha, Sreekumar U Aiswarya, Tennyson P Rayginia1, C K Keerthana, Kalishwaralal Kalimuthu, Sankar Sundaram, Ravi Shankar Lankalapalli,

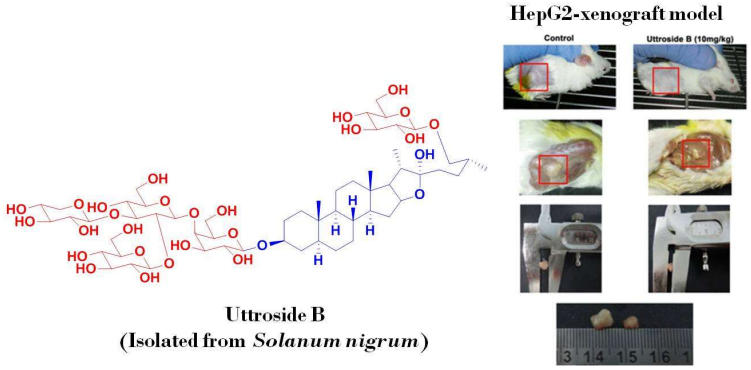
Sreekumar Pillai, Rheal Towner, Noah Isakov, and Ruby John Anto. (SSRN-id-3942642). *Equally contributed

5. Improved single vessel process for preparation and purification of alkylphosphocholines and miltefosine thereof. *Indian Pat. Appl*. (2020), IN *201911003844.* Lankalapalli, Ravi Shankar; **Thangarasu, Arun Kumar**; Ayyappanpillai, Ajayaghosh.
6. A process for production of uttroside from plants. Lankalapalli, Indian Patent Application No. 202211018238 (Date of filing: 28-03-2022). Ravi Shankar; **Thangarasu, Arun Kumar**; Induja DK, Reshma M V.
7. A process for the synthesis of BCX-1777 AND BCX-4430. Indian Patent Application No. 202111061664 (Date of filing: 29/12/2021). Lankalapalli Ravi Shankar, Karunakaran Anitha Krishnakumar, Suresh Sanjay Varma, Velickakathu Omanakuttan Yadhukrishnan, Kapiya Sangeeth, Chekrain Valappil Shihas Ahammed, Bernard Prabha, **Thangarasu Arun Kumar**, Doddramappa Doddamani Shridevi, John Jubi, Kokkuvayil Vasu Radhakrishnan, Ayyappanpillai Ajayaghosh.
8. Augmented efficacy of uttroside B over sorafenib in a murine model of human hepatocellular carcinoma. Swetha Mundanattu, C K Keerthana, Tennyson P Rayginia, Lekshmi R Nath, Haritha Hariprasad Nair, Anwar Shabna, Kalishwaralal Kalimuthu, **Arun Kumar Thangarasu**, Somaraj Jannet, Sreekumar Pillai, Kuzhuvelil B. Harikumar, Sankar Sundaram, Nikhil Ponnoor Anto, Dee Wu, Ravi S. Lankalapalli, Rheal Towner, Noah Isakov, Sathyaseelan S. Deepa, Ruby John Anto * (Communicated)

Contribution to academic conferences

1. Uttroside B, a potent chemotherapeutic agent, isolated and characterized from methanolic extract of leaves of Solanum nigrum. Poster presentation in “Indo-German workshop on Recent applications of Carbohydrates in Chemistry and Biology (RACCB-2017)" IIT-BHU Varanasi. **Arun K. Thangarasu**, Kollery S. Veena & Ravi S. Lankalapalli*. (Best poster Award)

Solanum nigrum, commonly known as black nightshade, is a medicinal plant member of Solanaceae family, widely used in many traditional systems of medicine. Two furostanol saponins, uttroside A and B have been reported from the stems and roots of *S. nigrum*. In the present study we describe the isolation and characterization of uttroside B from the leaves of *S. nigrum*. The methanolic extract was subjected to normal silica gel column chromatography followed by reverse phase HPLC purification which afforded uttroside B. Peracetylated uttroside B was characterized by extensive 2D NMR spectroscopic techniques like TOCSY, HSQC, HMBC & NOESY; further structural confirmation was done by tandem mass spectrometry. Drastic inhibition of tumour growth produced by uttroside B in NOD-SCID mice having human liver cancer xenografts illustrates and underscores the chemotherapeutic efficacy of uttroside B.

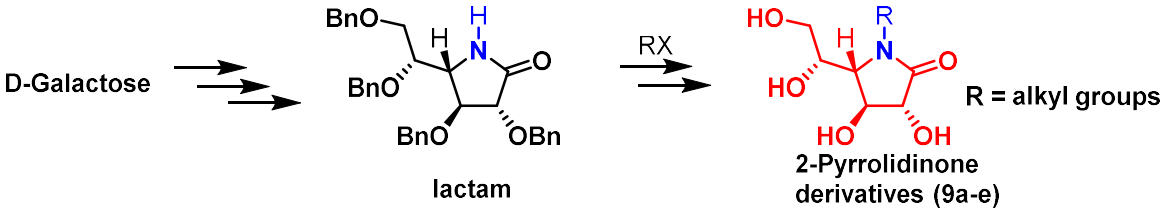


Uttroside B
(Isolated from *Solanum nigrum*)

2. Design and synthesis of glycoconjugates, their immunomodulatory and antimicrobial properties. Poster presentation in 30th KSC, 2018. **Arun K. Thangarasu**, & Ravi S. Lankalapalli*.

Polyhydroxy alkaloids such as iminosugars or azasugars have gained considerable interest in recent years owing to their biological potential, evident from commercial drugs Glyset ® (anti-diabetic) and Zavesca ® (treats type I Gaucher disease). Iminosugars are piperidine analogues wherein the oxygen in the ring of the sugar is replaced with nitrogen, thus, they serve as glycosidase inhibitors. This work explores the multistep synthesis of polyhydroxy pyrrolidinone motifs and iminoglycoconjugates from commercially available D-Galactose. All the intermediates were well characterised by using NMR and HRMS analysis. D-Galactose was converted in seven steps to lactam by following our recently reported procedure. Lactam was treated with different alkyl bromides in presence

of NaH in DMF to produce the respective N-alkyl-2-pyrrolidinone derivatives in good yields. Global debenzylation with 10% Pd/C under H 2 atmosphere afforded polyhydroxy-N-alkyl-2 pyrrolidinone derivatives in quantitative yields. These pyrrolidinone derivatives were screened for immunomodulatory activities. In addition, iminoglycoconjugates with aliphatic chains were synthesized from a HWE precursor that was screened for their antimicrobial activities. Novel polyhydroxy-N-alkyl-2-pyrrolidinones were identified as immunopotentiators. Compound 9e is found to be effective in stimulating the production of IL-12 in murine dendritic cells with significant expression of IFN-γ in human PBMCs. It also demonstrates their potential as immunostimulators which warrants further investigation of their potential utility as immunotherapeutic agents and vaccine adjuvants. In addition, iminoglycoconjugates with aliphatic chains were synthesized from a HWE precursor that was screened for their antimicrobial activities.



3. **National workshop on Applications of High field NMR in drug discovery**, from 24th to 26th of August, 2016, CDRI-Lucknow, India.

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Design, synthesis, and preliminary immunopotentiating activity of new analogues of nojirimycin

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ABSTRACT

Three new classes of nojirimycin analogues *viz.* N-alkyl with C1-substituent (4-phenylbutyl), N-substituted 1-deoxynojirimycin and its congener δ-lactam, and a 4-phenylbutyl-β-C-glycoside were designed and synthesized for immunological studies. The resulting diverse compound library exhibited proliferation of B Cells and T cells induced by LPS and Con A, respectively. The majority of the analogues augmented the secretion of IL-12 in dendritic cells and TNF-α secretion in murine peritoneal macrophages compared to LPS (10 μg/ml). A deoxynojirimycin-triazole conjugate of phytosphingosine analogue was superior in the responses mentioned above and exhibited nitric oxide response equal to LPS. In comparison to findings on its congeners with immunosuppressive action, early immunological tests show that the novel nojirimycin analogues have immunopotentiating effect. Hence, nojirimycin analogues offer tremendous potential in tuning the immunomodulatory activity of iminosugars by subtle to substantial structural variations.

1. Introduction

Plant and microbial iminosugars confer numerous therapeutic benefits due to their glycosidase inhibitory activity, which led to widespread efforts to develop synthetic analogues of iminosugar nojirimycin (Fig. 1) [1,2]. Synthetic iminosugar drugs Glyset® and Zavesca® underpin the pharmaceutical potential of its core skeleton [3]. Several iminosugar analogues with aliphatic/aromatic appendages on the nitrogen atom, C1 functionalized piperidine skeletons, and deoxy alterations surrounding the piperidine skeleton were synthesized, and they showed a wide spectrum of biological activity [4]. However, iminosugar monocyclic analogues' immunomodulatory potential has been limited to immunosuppressive action [5,6]. The focus on immunity during COVID-19 sparked fresh interest in natural products that have been shown to boost immunity. In order to conduct immunological research, medicinal chemists developed a significant variety of synthetic versions of iminosugar 1-deoxynojirimycin (Fig. 1). In most studies, *vide infra*, the N-alkylated iminosugars outperformed the parent piperidines in terms of immunosuppressive action.

Our team has recently demonstrated that N-alkyl-2-pyrrolidinone-based iminosugars have immunopotentiating properties [7]. Design and synthesis of new nojirimycin analogues with N-alkyl appendages and C1 variations such as carbonyl and 4-phenylbutyl substituents (Fig. 1) is described here. The new analogues exhibited unprecedented immunopotentiating activity, which attributes to structural differences between these novel iminosugars and previously reported iminosugar-based immunosuppressants. Reports of iminosugars with immunopotentiating activity are rare, a congener of bicyclic castanospermine increased the production of both IL-6 and IFN-γ, and had a modest inhibitory impact on IL-4 cytokines, according to one research, which also lays emphasis on minor structural/stereochemical alterations [8]. Castanospermine, on the other hand, is a bicyclic sugar, unlike the monocyclic analogues produced in this work.

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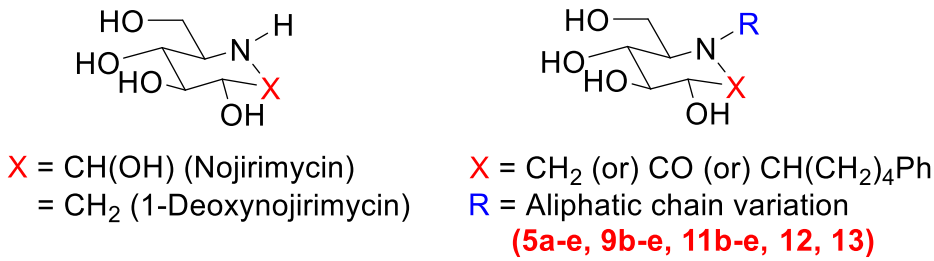
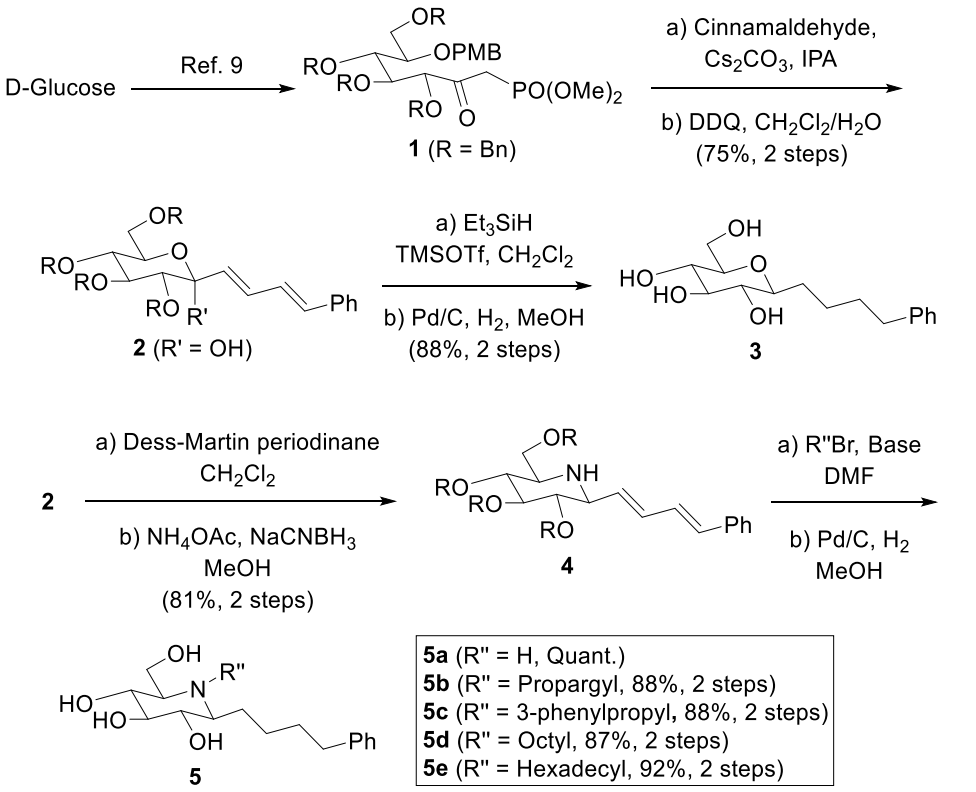
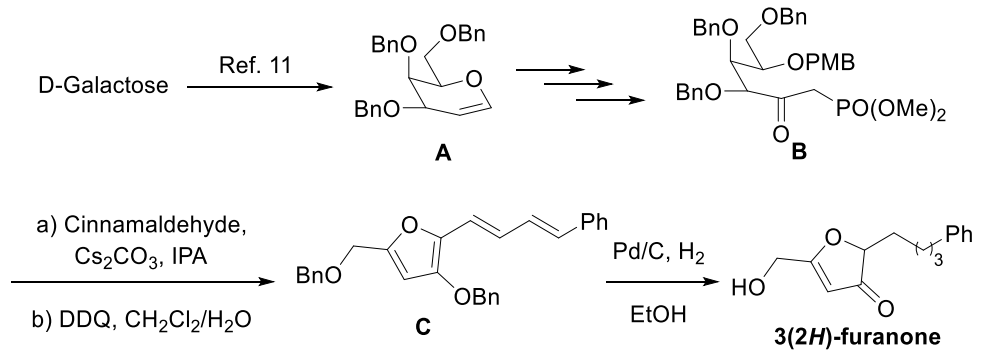


Fig. 1. Structures of nojirimycins and designed analogues.



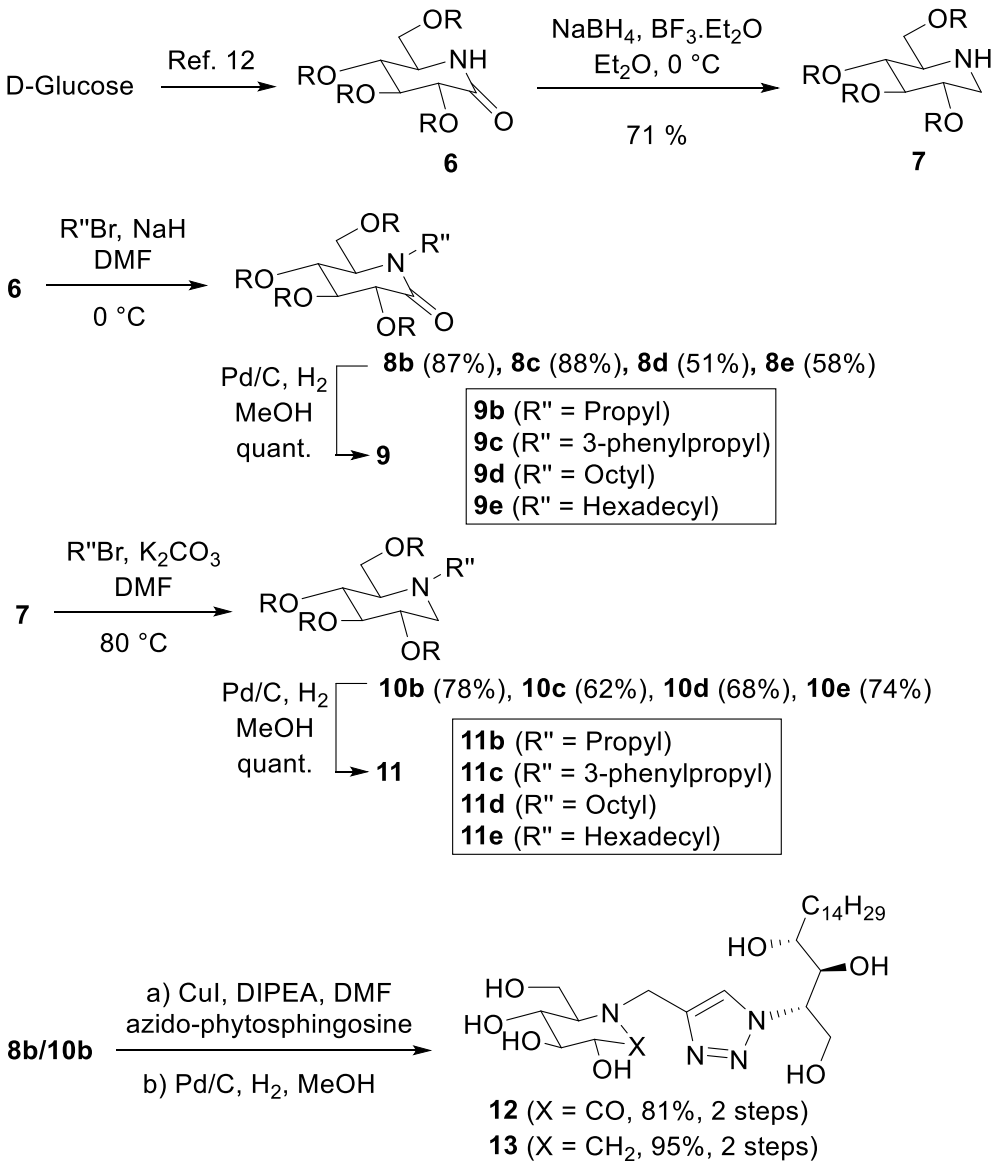


Scheme 1. Synthesis of glucosyl-β-C-glycoside (**3**) and corresponding aza-β-C-glycosides (**5a-e**); and failed attempt in synthesis of furanoid C-glycoside iminosugar.

2. Results and discussion

2.1. Chemistry

We have described a new method for β-C-glycoside synthesis using the Horner-Wadsworth-Emmons (HWE) reaction with the β-keto phosphonate precursor of D-galactose and aliphatic aldehydes [9]. In the current work, we developed a C1-alkyl variation of iminosugars by HWE reaction since there have been many reports on N-alkyl versions of iminosugars for immunomodulatory activities. Since nojirimycin is a natural carbohydrate mimic of D-glucose, our synthesis of β-keto phosphonate **1** (Scheme 1) initiated from D-glucose. In our previous report



Scheme 2. Synthesis of 2-piperidone (**9b-e**, **12**) and 1-deoxynojirimycin (**11b-e**, **13**) analogues.

[9], compound **1** was synthesized from D-galactose in 9 steps with an overall yield of 18%. In the present work, compound **1** was obtained in an overall yield of 23%, starting from D-glucose. KRN7000, an interesting immunostimulating compound with a functionalized lipid chain terminating in a phenyl group, elicited a significant cytokine bias [10]. As a result, cinnamaldehyde was chosen as the HWE reaction's aldehyde precursor because it allows for C1 modification, which results in an aliphatic appendage with a terminal phenyl group. HWE reaction with C (sp^2)-CHO precursors afford the cross-coupled product in good yields, and indeed compound **2** was produced in 75% yield over two steps by HWE reaction in the presence of Cs_2CO_3 followed by *p*-methoxybenzyl (PMB) deprotection in the presence of 2,3-dichloro-5,6-dicyano-1, 4-benzoquinone (DDQ). Although iminosugar analogues are our present interest, compound **2** provides access to β-C-glycoside of D-glucose, which enabled the synthesis of compound **3** with C1 aliphatic variation terminating with phenyl group in 88% yield by Kishi reduction of the 3° hydroxy group followed by global debenzylation using 10% Pd/C in the presence of H_2 under atmospheric pressure. Compound **2** serves as a common intermediate for the synthesis of the C1 variant of iminosugar by double reductive amination. Initially, Dess-Martin periodinane oxidation of compound **2** afforded the diketone, followed by double reductive amination reaction using ammonium acetate in the presence of $NaBH_4$ afforded piperidine **4** in 81% yield over two steps. N-alkylation of piperidine **4** by S_N2 reaction was affected by NaH at 0 °C with propargyl and cinnamyl bromides, and K_2CO_3 at 80 °C with octyl and hexadecyl bromides, followed by global denbenzylation afforded compounds **5a-e**. Surprisingly, the propargyl group of compound **5b** was unaffected under Pd/C reductive conditions, perhaps, due to steric reasons.

We also intended to synthesize furanoid C-glycoside iminosugars by HWE reaction of β-keto phosphonate from glycal generated from D-glucose and D-galactose [11], but instead got 3(2*H*)-furanone. Briefly, glycal **A** was converted in 8 steps to β-keto phosphonate **B** [9], which afforded the desired HWE product with cinnamaldehyde. However, treating the HWE product under DDQ mediated PMB deprotection conditions yielded an undesired trisubstituted furan **C**, by double elimination reactions, which displayed benzylic protons as distinct singlets in ^{1}H NMR. Finally, Pd/C deprotection conditions led to a tautomerized product 3(2*H*)-furanone.

Analogues of 1-deoxynojirimycin with carbonyl and N-alkyl appendages in the C1 position were also included in the design for immunomodulatory studies. Accordingly, lactam iminosugar **6** (Scheme

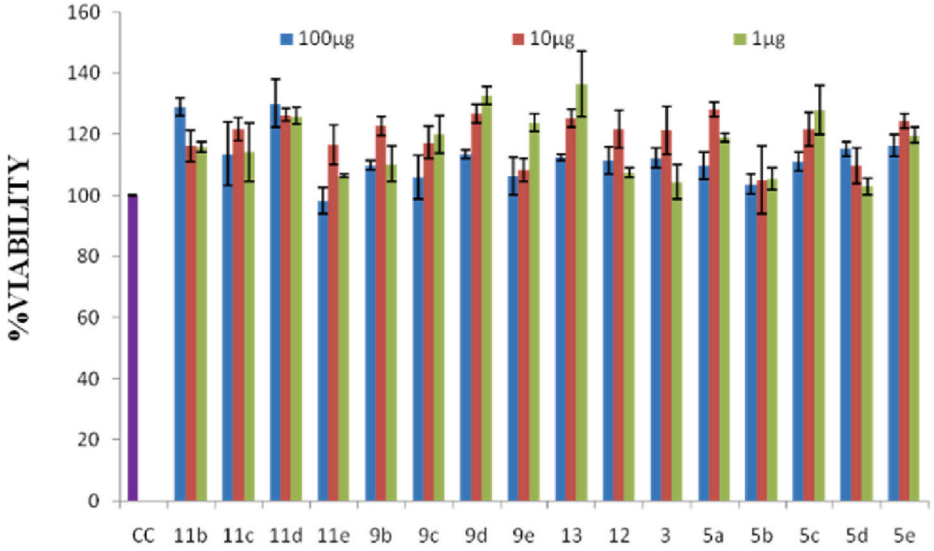


Fig. 2. Effect of novel analogues on splenocyte viability. Splenocytes were treated with analogues at 100, 10, and 1 μg/ml for 48 h, and viability was estimated by the MTT method. The optical density (OD) at 630 nm was measured, and the values were presented as mean ± SD (n = 3). CC (cell control), untreated cells subjected to the same conditions as the treated cells.

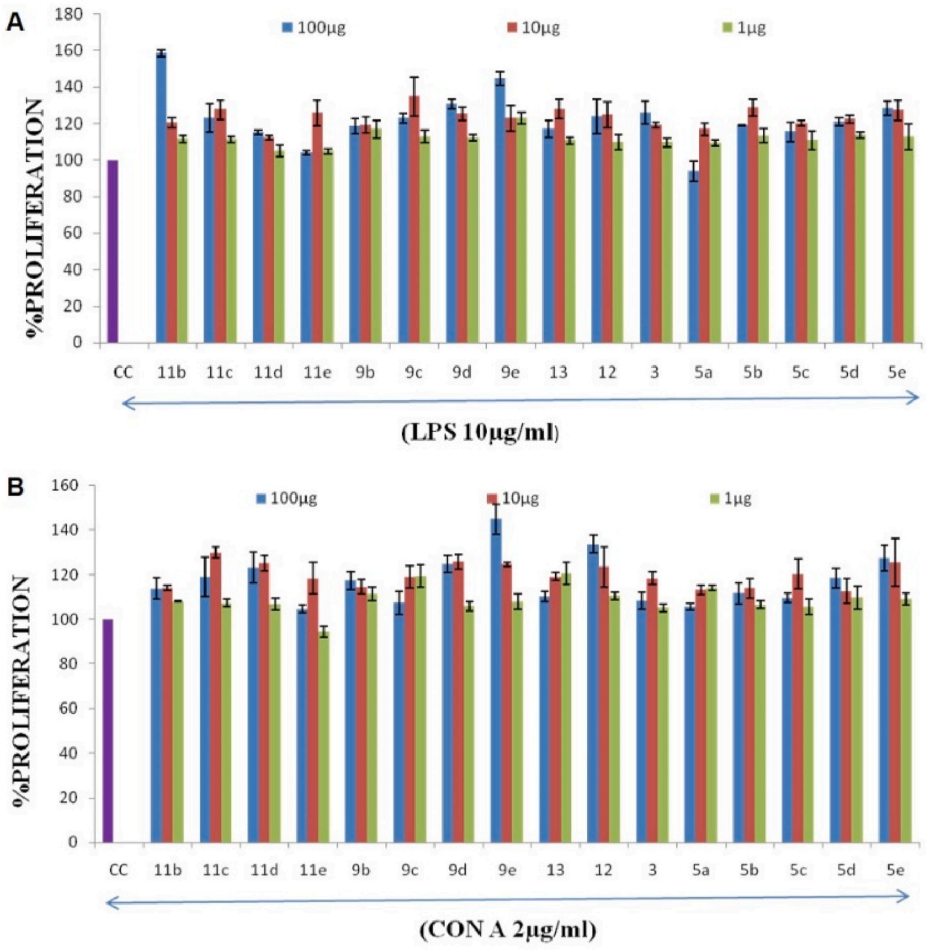


Fig. 3. Effect of analogues on splenocyte proliferation. Splenocytes were cultured in the presence of positive controls, LPS (10 μg/ml) and Con A (2 μg/ml) with or without analogues for 48 h. Splenocyte proliferation was assessed by MTT assay, and results were represented as mean ± SD (n = 3). CC (cell control), untreated cells subjected to the same conditions as the treated cells.

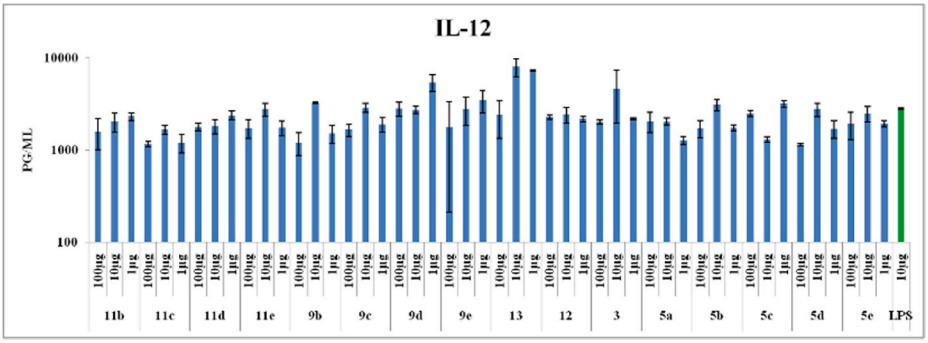


Fig. 4. Dendritic cell stimulation assay. Magnetically separated dendritic cells were stimulated with indicated analogues (100, 10, and 1 μg/ml) and positive control LPS (10 μg/ml) for 48 h. Levels of IL-12 released by DCs were quantified by ELISA. The data were analyzed with the Prism software (Graph- Pad, San Diego, CA, USA), expressed as mean ± SD.

2) was synthesized from D-glucose by reported conditions [12]. N-alkylation of lactam **6** with four different aliphatic bromides of varying lengths *viz.* propargyl, cinnamyl, octyl and hexadecyl, through the S_N2 reaction in the presence of NaH afforded compounds **8b-e**, which were then debenzylated to yield lactam analogues **9b-e**. 1-Deoxynojirimycin **7** was produced by $NaBH_4$ reduction of lactam **6**, followed by N-alkylation with propargyl, cinnamyl, octyl and hexadecyl bromides in the presence of K_2CO_3 afforded **10b-e** and debenzylation produced 1-deoxynojirimycin analogues **11b-e**. Furthermore, the propargylated compounds **8b** and **10b** were subjected to 'Click chemistry' conditions with azido phytosphingosine, followed by debenzylation, yielding the corresponding triazole appended analogues **12** and **13** in 81% and 95% yields, respectively (Scheme 2).

2.2. Biology

Initially, the effect of these novel analogues of iminosugar 1-deoxynojirimycin **5a-e**, **9b-e**, **11b-e**, **12**, **13** and β-C-glycoside **3** on splenocyte proliferation was assessed by MTT assay at doses of 100, 10, and 1 μg/ml for 48 h at 37 °C. The cell viability results show that the analogues were non-toxic to murine splenocytes even at 100 μg/ml (Fig. 2), and the compounds augmented the cell proliferation.

Next, we evaluated the effect of these analogues on mitogen-induced proliferation of splenocytes in the presence of positive controls at 10 μg/ml of LPS (B cell) and 2 μg/ml of Con A (T cell) (Fig. 3) [13]. The cells were exposed to analogues at concentrations of 100, 10, and 1 μg/ml for 48 h at 37 °C. The results show that the analogues were equally effective in mediating B cell proliferation caused by LPS (Fig. 3A) and T cell proliferation induced by Con A (Fig. 3B). Based on these results, we concluded that the analogues were not cytotoxic and promote B and T cell proliferation. In other words, the new analogues cause splenocyte growth without the need of mitogens.

Furthermore, in order to analyse the effect of these analogues on dendritic cell stimulation, mouse spleen derived dendritic cells were treated with analogues at varying concentrations of 100, 10, and 1 μg/ml. Overall, all the analogues produced a similar magnitude of secretion of IL-12 in par with LPS (10 μg/ml) (Fig. 4). However, analogues **3**, **9d** and **13** induced significant production of IL-12 when compared to positive control LPS.

According to published reports, N-substituted iminosugars inhibit splenocytes, indicating immunosuppressive effects. Iminosugar derivatives, D-galacto-*N*-biphenyl-lactam, *N*-alkylated 1,4-dideoxy-l,4-iminotetritol with terminal phenyl group, and *N*-alkyl dideoxy iminoalditols, displayed inhibitory effects in splenocyte proliferation assay [14–16]. *N*-octyl-5-fluorinated piperidine iminosugars inhibited human lymphocytes in the mixed lymphocyte reaction at IC_{50} 5–6 μM in T-cells [17]. *N*-didecyl phosphoryl iminosugar exhibited inhibition at IC_{50} 23 μM in Con A-induced proliferation of mouse splenocytes [18]. C1 functionalized *N*-nonyl iminosugar inhibited proliferation of the mouse splenocytes at IC_{50} 22 μM [19]. C1 functionalized L-altro iminosugar exhibited inhibitory effects on the secretion of cytokines [20].

In comparison to our new analogues of 1-deoxynojirimycin that exhibited splenocyte proliferation, structural aspects of the iminosugar derivatives in the reports, *vide supra*, showing inhibition of splenocytes, macrophages, lymphocytes, and suppression of cytokines, have subtle to significant structural differences, vindicating the role of iminosugar analogues as immunomodulators. Two reports with structural resemblance of deoxynojirimycin analogues **11b-e**, and **13** with variations in N-alkylation, *vide infra*, exhibited inhibition of cytokines. *N*-Pentafluorobenzyl-1-deoxynojirimycin strongly inhibited IL-4, a Th2 type

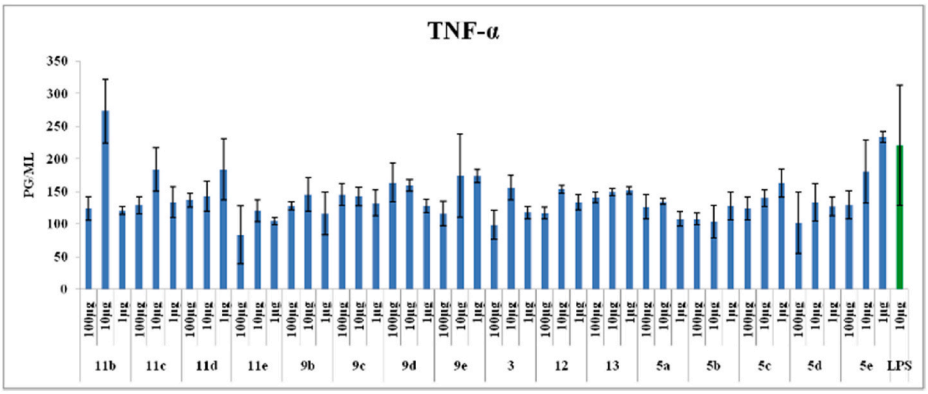


Fig. 5. Macrophage stimulation assay. Peritoneal macrophages were incubated with the analogues (100, 10, and 1 μg/ml) and positive control LPS (10 μg/ml) for 48 h. The supernatants were analyzed by ELISA for TNF-α production. The data were analyzed with the Prism software (Graph Pad, San Diego, CA, USA), expressed as mean ± SD.

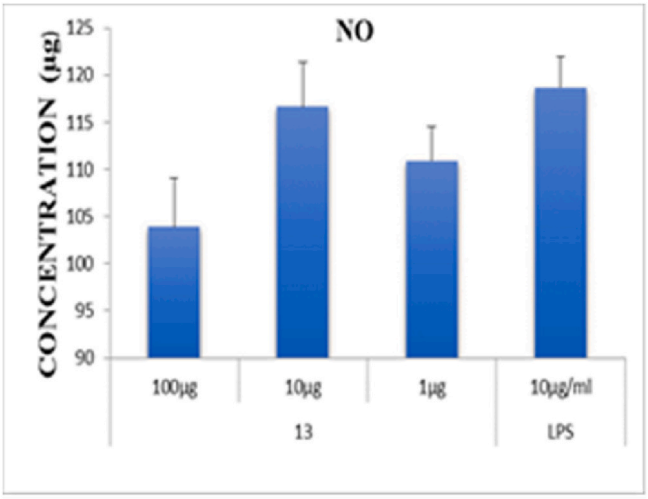


Fig. 6. Nitric oxide assay, peritoneal macrophages were incubated with analogue **13** (100, 10, and 1 µg/ml) and positive control LPS (10 µg/ml) for 24 h. The supernatants were analyzed by sodium nitrite. The data were analyzed with the Prism software (Graph Pad, San Diego, CA, USA) expressed as mean ± SD.

cytokine, production in splenocytes in *Schistosoma japonicum* infected mouse model [21]. *N*-(5-Adamantane-1-yl-methoxy-pentyl)-deoxynojirimycin attenuated intralesional IFN-γ and IL-18 cytokine production and also reduced anti-trinitrobenzene sulphonic acid and anti-oxazolone antibody responses *in vivo*, demonstrating immunosuppressive activity [22]. Interestingly, analogues **11b** and **13** with N-propyl and N–CH_2(triazole)-phytosphingosine appendages, respectively, induced production of IL-12.

Even though these analogues were active at specialized murine immune cells (dendritic cells), we were interested in determining their effect on murine peritoneal macrophages. Effect of these analogues on TNF-α secretion as tested on murine peritoneal macrophages at 100, 10, and 1 µg/ml showed that the analogues stimulated macrophages. Two analogues **11b** and **5e** showed TNF-α secretion in macrophages at doses 10 and 1 µg/ml, respectively, compared to LPS (10 µg/ml) (Fig. 5). In our earlier report, N-alkyl-2-pyrrolidinone-based iminosugars were found to be activators of dendritic cells but not macrophages [7]. Certain sp^2-iminosugar glycolipids elicited a selective cytokine secretion in a cell-context-dependent manner involving binding to allosteric lipid-binding site of p38 MAPK [23], and inhibited LPS-induced TNFα production, suppressed the activation of human monocyte-derived dendritic cells [24]. Iminosugar 4-epi-fagomine attenuated Th1 and Th2 type cytokines and suppressed LPS-mediated activation of Raw 264.7 macrophage cells, suggesting the reduction of immune cell-mediated beta cell destruction implicated in type 2 diabetes [25]. Stimulation of macrophages by analogues **11b** and **5e**, in particular, is again attributed to substantial structural variations from reports.

Additionally, nitric oxide assay was performed to determine the macrophage activation. Only analogue **13** was selected from the aforementioned responses, and it exhibited nearly equal response to standard LPS (Fig. 6). Previous study from our group on polyhydroxy-*N*-alkyl-2-pyrrolidinones and results from Vyavahare et al. on polyhydroxylated indolizidine alkaloids showed immunopotentiating activity, evidenced by the splenocyte proliferation, using LPS and Con A as positive controls [7,8]. Superior to these studies, the novel nojirimycin analogues induced higher levels of TNF-α by stimulated macrophages and promoted Con A mediated T cell proliferation effectively. Overall, the novel nojirimycin analogues exhibited potent immunostimulatory attributes.

3. Conclusions

In conclusion, three distinct classes of iminosugar nojirimycin novel analogues *viz.* 4-phenylbutyl substituted-C1 and N-alkyl variants **5a-e**; δ-lactams **9b-e**, **12**; 1-deoxynojirimycin with N-alkyl variants **11b-e**, **13**, including a β-C-glycoside **3** were synthesized for immunological studies. The current study is the first report demonstrating multiple variations of iminosugar analogues with a focussed library that exhibited an unprecedented immunopotentiating activities. Because the majority of the analogues in the current investigation had approximately identical magnitude of responses and as the library of analogues is heterogeneous with three distinct classes of iminosugars, it was not possible to derive a structure-activity link. However, compound **13**, in particular, was found to be effective in stimulating the production of IL-12 in murine dendritic cells with significant TNF-α expression in murine peritoneal macrophages along with potent nitric oxide response. The observed immunopotentiation is attributed to minor to substantial structural changes in the analogues, in contrast to the earlier iminosugars, which displayed immunosuppressive activity. The present study's synthesis of novel iminosugars marks their addition to the repertoire of iminosugars. The preliminary *in vitro* results of immunopotentiating ability of these new iminosugar analogues need a more thorough investigation to fully utilise the untapped potential of iminosugars for immunopotentiation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2021.108479.

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Letter

Cu(I)-azidopyrrolo[3,2-*d*]pyrimidine Catalyzed Glaser–Hay Reaction under Mild Conditions

Arun K. Thangarasu, Velickakathu O. Yadhukrishnan, K. A. Krishnakumar, Sanjay Suresh Varma, and Ravi S. Lankalapalli*

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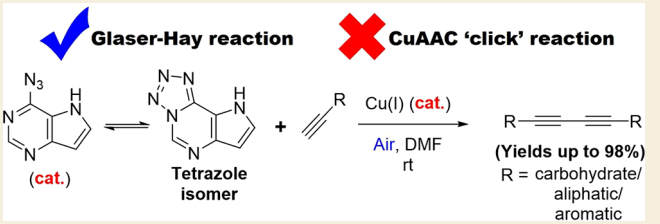
ABSTRACT: The limitation of the CuAAC "click" reaction with a 2-azidopyridine substrate, owing to its equilibrium with a tetrazole isomer, is exploited herein for its utility in the Glaser–Hay reaction. A catalytic combination of a 2-azidopyridine analogue, 4-azido-5*H*-pyrrolo[3,2-*d*]pyrimidine, and CuI afforded homocoupled products of terminal alkynes, without any trace of triazole product, under mild conditions with a broad substrate scope. Emphasis on carbohydrate-based substrates appended to a propargylic group led to 1,3-diynes in good to excellent yields.



KEYWORDS: *Glaser–Hay reaction, Homocoupling, CuAAC click reaction, 2-Azidopyridine, Pyrrolopyrimidine*

Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC, Figure 1) is a versatile "click" reaction that affords 1,4-disubstituted triazoles with a broad range of applications in the fields of chemistry, biology, and materials science.[1−5] The Glaser–Hay (GH, Figure 1) reaction is another interesting copper(I)-catalyzed reaction in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA)/air that converts terminal acetylenes, by homocoupling, to symmetric 1,3-diynes, which exhibit technologically relevant applications in the field of materials science.[6−8] Mechanistically, both CuAAC and GH reactions are truly catalytic that involve an initial π-coordination of alkynes to Cu(I) species, affording electrophilic Cu(I)-acetylides.[1,6]

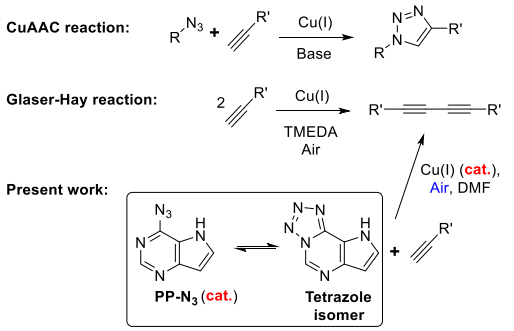


Figure 1. Cu(I)-catalyzed CuAAC reaction, Glaser–Hay reaction, and present work.

A report which demonstrates a competition between both CuAAC and GH reactions, conducted by the use of excess alkyne in the presence of azide and CuI, clearly showed an initial consumption of azide to form a CuAAC product and then the remaining alkyne underwent slower GH reaction.[9] In another report, Bolje et al. reported the observation of a homocoupled 1,3-diyne GH byproduct along with the intended CuAAC reaction with 2-azidopyridine substrate due to prolonged heating in toluene.[10] In both of these reports, the formation of the GH product during an intended CuAAC reaction indicates the commonalities in both reactions. In the later report, the 2-azidopyridine substrate is well-known as an exception to an otherwise versatile CuAAC reaction, with vast functional group tolerance, due to its equilibrium with tetrazolo[1,5-*a*]pyridine at room temperature, particularly in polar solvents.[11,12] However, alteration of the reaction conditions, which can shift the equilibrium to the azido form such as elevated temperature or nonpolar solvents or prolonged reaction times, resulted in triazole formation.[13−15] In other words, a 2-azidopyridine substrate that exists in equilibrium with its tetrazole isomer, in the presence of Cu(I), can form a CuAAC "click" product under harsh conditions but under mild conditions a GH reaction can operate wherein 2-azidopyridine takes the role of a ligand. In the present study, a mild method for a GH reaction catalyzed by a 2-azidopyrimidine analogue, 4-azido-5*H*-pyrrolo[3,2-*d*]-pyrimidine (PP-N_3, Figure 1), in the presence of a catalytic CuI is demonstrated.

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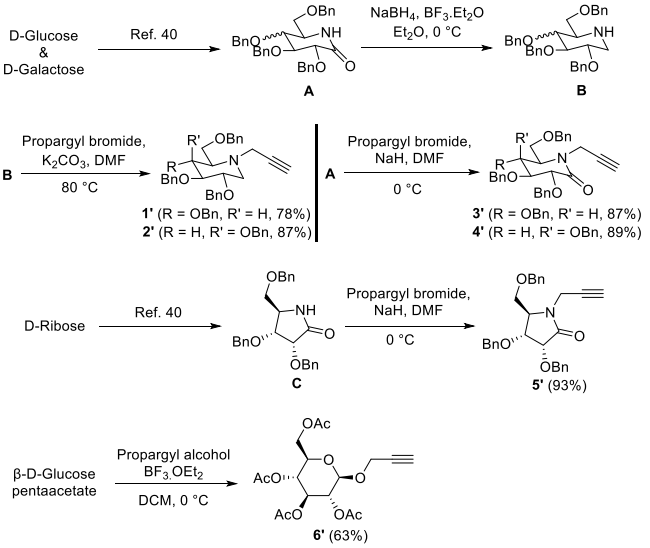
The GH reaction was given its name from the introduction of a catalytic amount of bidentate ligand TMEDA by Hay in the presence of copper(I) chloride (Figure 1).[16] Ever since, several GH methods were introduced for the synthesis of 1,3-diynes from terminal alkynes catalyzed by copper(I) by the variation of ligands and under ligand-free conditions by the use of base. Recently, a mild method was reported with 1*H*-benzotriazole as a ligand in the Cu-catalyzed GH reaction in the presence of potassium carbonate; the same group also demonstrated a GH reaction with D-glucosamine as the ligand.[17,18] L-Proline was used as a ligand along with excess pyrrolidine in the GH reaction of a natural product conjugate 9-ethynylnoscapine.[19] A carbazole-based NHC catalyst in combination with CuI in the presence of KO*t*Bu led to a GH reaction.[20] Under ligand-free conditions, the base itself serves as a ligand, albeit monodentate; e.g., DMAP, NBS/DIPEA, and ethyl lactate were reported in catalytic combination with CuI in promoting the GH reaction under aerobic conditions.[21−23] A nonafluorobutanesulfonyl azide was used as a reagent in the presence of a base in the GH reaction that afforded 1,3-diynes with a fast coupling rate.[24] Copper coordination complexes such as $[Cu_2(ophen)_2]$ and $[Cu_4(ophen)_4(tp)]$ and a $Cu_3(BTC)_2$-metal−organic framework were also used as catalysts in GH reactions.[25,26]

The choice of ligand is crucial in GH reactions with certain substrates; e.g., the choice of ligand 4,4′-bis(hydroxymethyl)-2,2′-bipyridine was decisive in biomolecule GH conjugation involving peptides, which further helped in sequestering Cu(II) species that are detrimental to peptides by oxidative damage.[27] To decipher the role of the ligand, the GH reaction was investigated by varying the amines. The use of triethylamine resulted in the precipitation of a Cu-acetylide complex; however, upon addition of TMEDA, the precipitated complex dissolved and the desired GH reaction took place.[28]

There are GH methods that demonstrate the synthesis of 1,3-diynes with carbohydrate substrates appended to terminal alkynes, but expansion of the substrate scope is missing.[17,21,22,29] α-D-Mannopyranosides appended with propargylic and phenylacetylene groups were homocoupled using copper(II)acetate in refluxing pyridine for 48 h.[29] Hence, in the present study, emphasis was given to carbohydrate based substrates to demonstrate the efficiency of the method.

A CuAAC condition with excess CuI and DIPEA that was utilized by our group for the synthesis of a phytosphingosine conjugate of 2-pyrrolidinone triazole product was applied in our initial reaction.[30] In a report, heating a phenylethynylcopper(I) intermediate in DMF at reflux for 24 h resulted in 1,3-diyne;[31] hence, we chose DMF as the solvent but planned our first attempt at 0 °C. Our interest in the area of synthesis of iminosugar derivatives prompted the application of *N*-propargylated deoxynojirimycin **1′** (Scheme 1) for the GH reaction with PP-N_3. In a separate study from our lab, PP-N_3 was intended for synthesis of immucillins BCX-1777 and BCX-4430. By following known procedures,[32,33] PP-N_3 was derived from 9-deazahypoxanthine (PP) and en route PP-Cl serves as an intermediate (Figure 2). A clean transformation took place in our initial GH reaction, observed over TLC as a polar product, to a homocoupled symmetric 1,3-diyne product **1** in 74% yield (entry 1, Table 1), with no trace of any triazole adduct. The use of the other two pyrrolopyrimidines PP and PP-Cl and the absence of PP-N_3 led to a drastic loss in the yield of the reaction (entries 2−4), with substantial amounts of unreacted terminal alkyne; hence, PP-N_3 has a major role in this homocoupling reaction. Reducing the equivalents of PP-N_3 to catalytic amounts led to a tad improvement in the yield of compound **1** (entries 5 and 6). Furthermore, in the absence of DIPEA, taking CuI and PP-N_3 in catalytic amounts afforded compound **1** in 81−83% yields (entries 7 and 8), and repeating the reaction at room temperature or at 100 °C afforded

Scheme 1. Synthesis of Propargylated Carbohydrate Based Substrates



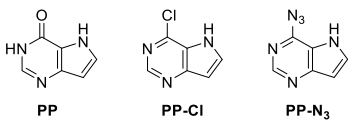
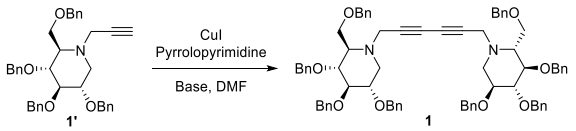


Figure 2. Pyrrolopyrimidines used in the present study.

Table 1. Reaction Optimization[a]



entry	catalyst (equiv)	pyrrolopyrimidine (equiv)	base (equiv)	temp (°C)	yield **1** (%)[f]
1	CuI (2)	PP-N_3 (1)	DIPEA (3)	0	74
2	CuI (2)	PP (1)	DIPEA (3)	0	12
3	CuI (2)	PP-Cl (1)	DIPEA (3)	0	11
4	CuI (2)		DIPEA (3)	0	trace
5	CuI (2)	PP-N_3 (0.5)	DIPEA (3)	0	79
6	CuI (2)	PP-N_3 (0.1)	DIPEA (3)	0	79
7	CuI (2)	PP-N_3 (0.1)		0	81
8	CuI (0.2)	PP-N_3 (0.1)		0	83
9	CuI (0.2)	PP-N_3 (0.1)		rt	86
10	CuI (0.2)	PP-N_3 (0.1)		100	85
11[b]	CuI (0.2)	PP-N_3 (0.1)		rt	82
12[c]	CuI (0.2)	PP-N_3 (0.1)		rt	0
13[d]	CuI (0.2)	PP-N_3 (0.1)		rt	0
14[e]	CuI (0.2)	PP-N_3 (0.1)		rt	trace

[a]Reaction conditions: To a stirred solution of alkyne **1′** (30−50 mg) in DMF (1 mL), CuI and PP-N_3 were added successively and the reaction takes 5−6 h for complete consumption of the starting material. [b]Open air. [c]Solvent: MeOH. [d]Solvent: CH_2Cl_2. [e]Solvent: CH_3CN. [f]Isolated yields.

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compound **1** in 86 and 85% yields, respectively (entries 9 and 10). All the aforementioned reactions were conducted without any stringent measures, i.e., by a mere stoppering of the round-bottom flask (RB) and using undistilled solvents; a reaction conducted under open-air atmosphere afforded compound **1** in 82% yield (entry 11). However, the reaction suffered a setback in the solvents methanol, dichloromethane, and acetonitrile (entries 12−14). Thus, the homocoupling of terminal alkyne **1′** can take place at room temperature in DMF with catalytic amounts of CuI and PP-N_3, in the absence of base and under open-air atmosphere, signifying the mildness of the present method (entry 11). Reactions with a further decrease in the mole percentage of catalysts lead to longer reaction times; hence, entry 11 is considered as the optimized reaction condition for demonstrating the feasibility of the reaction with different substrates.

Reaction optimization studies reveal that the GH reaction in the absence of PP-N_3 suffers from low yields (entries 2−4), suggesting Cu(I) and PP-N_3 complexation, which is the driving force for the GH reaction. The reaction is unaffected by the change from stoichiometric to catalytic PP-N_3, thus fulfilling the role of PP-N_3 as a ligand like TMEDA, used in standard GH reactions. A successful outcome in DMF against acetonitrile (entry 14), where both solvents have the same coordinating ability with transition metals,[34] prompts the difference in reactivity due to solvation. Acetonitrile exhibits strong solvation of Cu(I) and preferential solvation with cosolvent DMF;[35] hence, acetonitrile inhibits reactivity beyond its solvation shell, especially where CuI is used in catalytic amounts. Having considered the reaction optimization studies, the role of PP-N_3 as a ligand in the GH reaction is apparent. As we were unable to detect the triazole product in all of our attempts, we conclude that PP-N_3 exists in the tetrazole form in DMF and thus promotes the GH reaction and inhibits the CuAAC reaction. As further evidence, the pyrimidine proton of PP-N_3 appeared at δ_H 9.81 ppm in the ^{1}H NMR spectrum (Supporting Information) which matches with analogous tetrazolo[4,5-*c*]pyrimido[5,4-*b*]indole at δ_H 9.80 ppm.[36]

The tetrazole moiety of PP-N_3 is 1,5-disubstituted, and through its nitrogen electron-donating atoms it can exist as a multidentate coordinating ligand.[37] Additionally, tetrazole can serve as a base. However, the tetrazole moiety or the pyrimidine nitrogen complexes with copper in a monodentate coordination mode.[38,39] Accordingly, PP-N_3, complexes with CuI to yield a dimeric intermediate (Figure 3) proposed by Bohlmann,[6−8] which takes the productive route to 1,3-diyne and regenerates the catalysts.

In order to check the feasibility of the present method with other carbohydrate based substrates, compounds **1′−4′** were prepared from D-glucose and D-galactose, **5′** from D-ribose, and **6′** from *β*-D-glucose pentaacetate by standard synthetic carbohydrate chemistry methods (Scheme 1).[40,41] Initially, compound **1** was subjected to Pd/C debenzylation reduction to generate a novel DNJ-dimer **2** (Scheme 2) with an aliphatic linker, which can be of pharmacological relevance. Compound

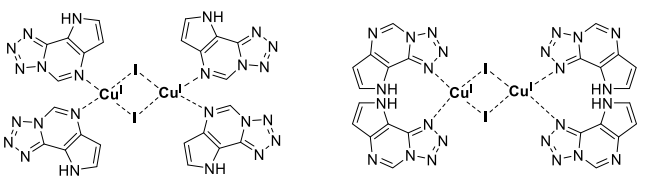
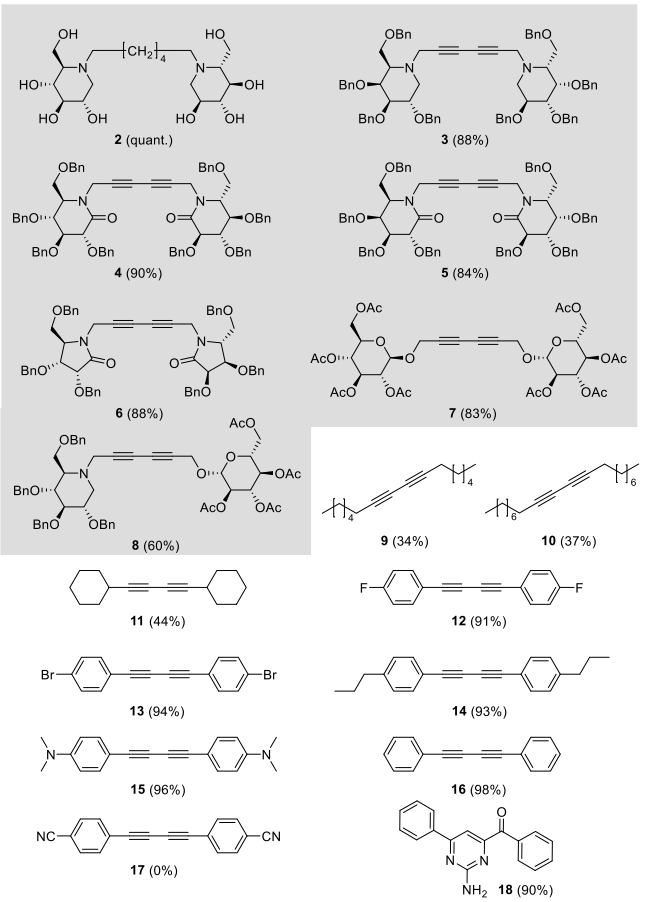


Figure 3. Probable complexes with CuI and tetrazole isomer of PP-N_3.

Scheme 2. Substrate scope



2′ derived from D-galactose underwent homocoupling to afford compound **3** in 88% yield. Homocoupling of the lactams **3′−5′** also proceeded smoothly to afford compounds **4−6** in good yields. 1-*O*-Propargylated glucose pentaacetate **6′** homocoupled conveniently to afford compound **7** in 83% yield. An attempt to afford unsymmetric 1,3-diynes led to the treatment of compounds **1′** and **6′** in a 1:1 ratio under the reaction conditions which resulted in heterocoupled product **8** in 60% yield. An attempt with terminal alkynes having aliphatic groups produced homocoupled products **9** to **11** in low yields. Terminal alkynes with aromatic groups gave rise to compounds **12−16** in excellent yields, but there was no reaction with the *para*-cyano substituted substrate. Finally, compound **16** was intentionally converted with guanidine in a known reaction[42] to 2-amino pyrimidine **18**, which can be easily converted to azide and results in another pyrimidine-based catalyst that can participate in the GH reaction.

To probe the significance of PP-N_3 in GH reaction against reported ligands/bases in GH reactions, attempts were made with carbohydrate based substrates in the present study (Scheme S1, Supporting Information). GH reaction with a benzotriazole ligand for a protected glycosylated alkyne afforded 1,3-diyne in only 15% yield,[17] and the use of benzoriaozle in place of PP-N_3 with our carbohydrate substrates led to poor yields indeed. On the other hand, a catalytic system comprising CuI and DMAP in acetonitrile

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reported under aerobic conditions with a protected glycosylated alkyne afforded 1,3-diyne in a good yield.[21] Hence, we investigated the feasibility of the GH reaction with DMAP using a deprotected glycosylated alkyne. A globally deacetylated alkyne of compound **6′** was attempted for the GH reaction, in the presence of DMAP, and there was no reaction in either acetonitrile or DMF. Remarkably, with PP-N_3, a facile GH reaction took place that afforded 1,3-diyne in 75% yield. Homocoupled glycosylated alkynes under GH reaction conditions using TMEDA are subjected to standard deprotection of acetyl groups under Zemplén conditions;[29] however, PP-N_3 provides an advantage of working directly with deprotected carbohydrate substrates. These results suggest a reciprocal relation between PP-N_3 and carbohydrate substrates for a fruitful GH reaction, which prompts the application of the present method in glycobiology.

In conclusion, a recalcitrant 2-azidopyridine toward CuAAC "click" reaction owing to its equilibrium with tetrazole isomer is hypothesized for use as a ligand for the Glaser−Hay coupling reaction of terminal alkynes to 1,3-diynes. As a model, 4-azido-5*H*-pyrrolo[3,2-*d*]pyrimidine was studied in the presence of CuI and terminal alkynes, which afforded 1,3-diynes without any trace of triazole "click" product. The present method is an add-on to the repertoire of GH methods and serves as the first report of the use of azidopyrimidine as a ligand in catalytic amounts along with catalytic CuI, without any base, that displays a broad substrate scope. The utility of the reaction in the synthesis of various carbohydrate based dimers prompts further diversification and exploration for biological studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsorginorgau.1c00015.

Experimental procedures and NMR spectral information (PDF)

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Notes

The authors declare no competing financial interest.

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