Total synthesis of glycolipids of biological significance containing aza-sugars and structure elucidation of Uttroside B

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Dedicated to my beloved parents and teachers

18th January, 2017

DECLARATION

I hereby declare that the Ph.D. thesis entitled "**Total synthesis of glycolipids of biological significance containing aza-sugars and structure elucidation of Uttroside B"** is an independent work carried out by me under the supervision of **Dr. Ravi Shankar Lankalapalli** at the Organic chemistry Section, CSTD, CSIR-NIIST, Thiruvananthapuram and it has not been submitted anywhere else for any other degree or diploma.

In keeping the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigators.

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CERTIFICATE

This is to certify that the work incorporated in this Ph.D. thesis entitled "**Total synthesis of glycolipids of biological significance containing aza-sugars and structure elucidation of Uttroside B"** submitted by **Mr. Gorantla Jaggaiah Naidu** to Academy of Scientific and Innovative Research (AcSIR) in fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in Chemical Sciences, embodies original research work under my supervision/guidance. I further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table, etc. used in the thesis from other sources have been duly cited and acknowledged.

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Jaggaiah Naidu G.

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ABBREVIATIONS

Å	:	Angstrom
Ac	:	Acetyl
Ac_2O	:	Acetic anhydride
AcOH	:	Acetic acid
α-GalCer	:	α-Galactosyl ceramide
$AgClO_4$	:	Silver perchlorate
aq	:	aqueous
APCs	:	Antigen presenting cells
app t	:	apparent triplet
$Ba(OH)_2$	:	Barium hydroxide
$BF_3.OEt_2$	:	Boron trifluoride diethyl etherate
BODIPY^{TM}	:	Boron dipyrromethane
Bn	:	Benzyl
BnBr	:	Benzylbromide
Boc	:	t-Butoxycarbonyl
$(Boc)_2O$	:	Di-tert-butyl dicarbonate
Bu_2SnO	:	Dibutyl tinoxide
°C	:	Degrees Celsius
^{13}C NMR	:	Carbon-13 nuclear magnetic resonance
calcd	:	Calculated

cat	:	Catalytic
CCl_3CN	:	Trichloroacetonitrile
CD1	:	Cluster of Differentiation 1
C_5D_5N	:	Deuterated pyridine
$CDCl_3$	:	Deuterated chloroform
CD_3OD	:	Deuterated methanol
CH_2Cl_2	:	Dichloromethane
CH_3CN	:	Acetonitrile
COSY	:	Correlation spectroscopy
CuI	:	Copper iodide
$CuSO_4$	:	Copper sulfate
$Cu(OTf)_2$	:	Copper (II) trifluoromethanesulfonate
$CeCl_3$	:	Cerium chloride (III)
Cs_2CO_3	:	Cesium carbonate
d	:	doublet
DC	:	Dendritic cell
dd	:	doublet of doublets
ddd	:	doublet of doublet of doublet
DDQ	:	2,3-Dichloro 5,6-dicyano 1,4-benzoquinone
DEPT-135°	:	Distortionless Enhancement of Polarization Transfer using a 135 degree decoupler pulse
DIPEA	:	N,N-Diisopropylethylamine
DMAP	:	4-(Dimethylamino) pyridine

DMF	:	Dimethylformamide
DMP (O)	:	Dess-Martin periodinane oxidation
DMSO	:	Dimethyl sulfoxide
DNA	:	Deoxyribonucleic acid
DNJ	:	1-deoxynojirimycin
D_2O	:	Deuterium oxide
Eq	:	equivalent
ESI	:	Electron spray microscopy
Et_3N	:	Triethylamine
Et_3SiH	:	Triethylsilane
Et_2O	:	Diethyl ether
EtOAc	:	Ethyl acetate
EtOH	:	Ethanol
FDA	:	Food and drug administration
Gal	:	Galactose
Glc	:	Glucose
GSL	:	Glycosphingolipid
gpl20	:	Glycoprotein120
^1H NMR	:	Proton nuclear magnetic resonance
H_2	:	Hydrogen gas
HCl	:	Hydrochloric acid
HCOOH	:	Formic acid
HIV	:	Human immune virus

HMBC	:	Heteronuclear multiple bond correlation spectroscopy
HSQC	:	Heteronuclear single quantum correlation spectroscopy
HRMS	:	High resolution mass spectrometry
hr	:	Hour
HWE	:	Horner-Wadsworth-Emmons
Hz	:	Hertz
H_2PtCl_6	:	Chloroplatinic acid
HPLC	:	High performance liquid chromatography
H5N1	:	Hemagglutinin Type 5 and Neuraminidase Type 1 (Avian Influenza A)
IC50	:	Inhibition concentration 50%
IFN-γ	:	Interferon-gamma
IgG	:	Immunoglobulin G
IgM	:	Immunoglobulin M
IL-4	:	Interleukin-4
Im_2CO	:	Carbonyldiimidazole
iNKTcells	:	Invariant natural killer T cells
Ki	:	Inhibitory constant
K_2CO_3	:	Potassium carbonate
KOH	:	Potassium hydroxide
KOtBu	:	Potassium t-butoxide
LacCer	:	Lactosylceramide
LiBr	:	Lithium bromide

LiHMDS	:	Lithium bis (trimethylsliyl) amide (or) Lithium Hexamethyldisilazide
LPS	:	Lipopolysaccharide
LTB4DH	:	Leukotriene B4 12-hydroxydehydrogenase (antibody)
m-CPBA	:	meta-Chloroperoxybenzoic acid
mM	:	Millimolar
µL	:	Microliter
µM	:	Micromolar
µmol	:	Micromol
$MeNO_2$	:	Nitromethane
MeOH	:	Methanol
$Me_2C(OMe)_2$	:	2,2,-Dimethoxypropane
Me_3Al	:	Trimethylaluminum
MMP2	:	Matrix metalloproteinase-2
MMP9	:	Matrix metalloproteinase-9
$Me_3N.SO3$	:	Trimethylamine sulfur trioxide
$MgCl_2$	:	Magnesium chloride
$MgSO_4$	:	Magnesium sulfate
min	:	Minute
MPM	:	4-methoxybenzyl
MS	:	Molecular sieves
MsCl	:	Methanesulfonyl chloride
MTT	:	3-(4,5-Dimethylthiazol-2-yl)-2,5-

		Diphenyltetrazolium Bromide
m/*z*	:	Mass to charge ratio
n-BuLi	:	*n*-Butyl Lithium
$NaBH_4$	:	Sodium borohydride
NaCl	:	Sodium chloride
$NaCNBH_3$	:	Sodium cyanoborohydride
NaH	:	Sodium hydride
$NaHCO_3$	:	Sodium bicarbonate
$NaHSO_3$	:	Sodium bisulfite
NMM	:	N-methylmorpholine
NMO	:	N-methylmorpholine N-oxide
NaN_3	:	Sodium azide
$NaIO_4$	:	Sodium periodate
NaOH	:	Sodium hydroxide
NaOMe	:	Sodium methoxide
Na_2SO_4	:	Sodium sulfate
$Na_2S_2O_3$	:	Sodium thiosulfate
NBS	:	N-Bromosuccinimide
NH_4	:	Ammonium
NH_4HCO_3	:	Ammonium bicarbonate
NH_4Cl	:	Ammonium chloride
NIS	:	N-iodosuccinimide
NKT	:	Natural Killer T

nM	:	Nanomolar
NMR	:	Nuclear magnetic resonance
NSCLC	:	Non small cell lung cancer
NOESY	:	Nuclear Overhauser effect spectroscopy
OsO_4	:	Osmium tetroxide
pH	:	Potential hydrogen
Pd/C	:	Palladium on carbon
ppm	:	Parts per million
Quant	:	quantitative
RCM	:	Ring closing metathesis
rt	:	Room temperature
R_f	:	Retention factors
s	:	singlet
SAR	:	Structure Activity Relationship
sat	:	Saturated
SV40	:	Simian virus 40
$SnCl_2$	:	Stannous chloride
t	:	triplet
TBAF	:	Tetra-n-butylammonium fluoride
TBAI	:	Tetra-n-butylammonium iodide
TBDMS	:	tert-Butyldimethylsilyl
TBDMSCl	:	tert-Butyldimethylsilane chloride
TBDPS	:	tert-Butyldiphenylsilyl

TBSOTf	:	tert-Butyldimethylsilyl trifluoromethanesulfonate
TCR	:	T-cell receptor
TMS	:	Trimethylsilyl
TMSOTf	:	Trimethylsilyl trifluoromethane sulfonate
Tf Triflate	:	Trifluoromethanesulfonate
TFA	:	Trifluoroacetic acid
TfN_3	:	Trifluoromethanesulfonyl azide
Tf_2O	:	Trifluoromethanesulfonic anhydride
Th1	:	Type helper 1
Th2	:	Type helper 2
THF	:	Tetrahydrofuran
TLC	:	Thin layer chromatography
TMSOTf	:	Trimethylsilyl trifluoromethanesulfonate
TOF	:	Time of flight
TOCSY	:	Total correlation spectroscopy
p-TsOH	:	*p*-Toluenesulfonic acid
Ph_3CCl	:	Tritylchloride
Ph_3PCH_3Br	:	Methy triphenylphosphonium bromide
PPh_3	:	Triphenylphosphine
$PdCl_2(PPh_3)_2$	:	Dichlorobis (triphenylphosphine) palladium(II)
ROESY	:	Rotating frame nuclear overhauser effect spectroscopy
Xyl	:	Xylose

Abstract

The KRN7000 (α-galactosylceramide) is a structure-activity derived synthetic glycosphingolipid (GSL) analog of a naturally occurring agelasphin isolated from *Agelas mauritianus* in 1993. KRN7000 is one of the well-studied glycolipid which elicits cytokine response upon complexation with iNKT cells resulting in immunoregulatory responses of therapeutic interest. Several variants of KRN7000 for SAR studies and their impact on cytokine profile production are presented by researches for the past two decades because simultaneous cytokine secretion of Th1 (IFN-γ) and Th2 (IL4) type reduces an effective immune response to treat a particular pathological condition. Therefore, the task of selective or biased secretion of cytokines is required by considering variations of KRN7000 lipid. Variants of KRN7000 with variations in the chain length of amide linkage, sphingosine backbone, 3- and 6- positions of galactose, glycosidic linkage and ring oxygen variants (Figure 1a) are described in the first chapter. Aza-sugars are carbohydrates produced by replacement of ring oxygen with nitrogen and these molecules exhibit a broad spectrum of pharmacological activities. Aza-sugars are also present in some branded drugs such as Glyset and Zavesca. The objective of chapter 2 and chapter 3 involves design and synthesis of analogs of KRN7000 by variation in the sugar portion with aza-sugar and the glycosidic linkage.

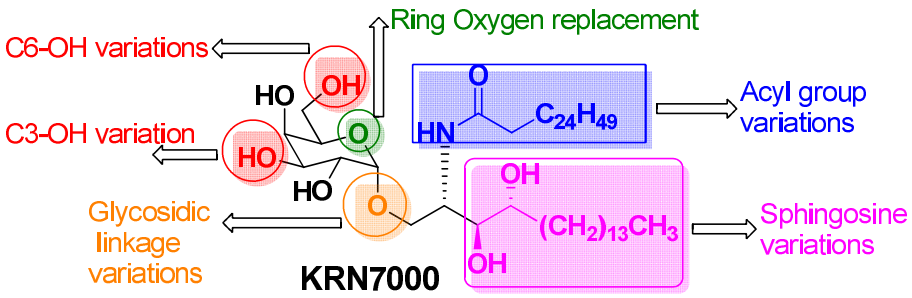

Figure 1a: All structural variations in KRN7000

The second chapter demonstrates an attempt made towards synthesis of aza-*O*-GalCer by conventional O-glycosylation conditions using phytosphingosine **2** as the acceptor and N-Boc protected piperidin-2-ol **1** as the donor (Figure 2a). Compound **1** was synthesized from D-galactose in nine steps, and phytosphingosine-1-ol **2** was synthesized from phytosphingosine in four steps. Unfortunately, during deprotective conditions the glycosylated product **3** cleaved to individual starting materials. This result demonstrated the labile nature of aza-*O*-GalCer **3**; therefore, we focused our attempts for C-glycosidic linkage using Horner-Wadsworth-Emmons (HWE) reaction.

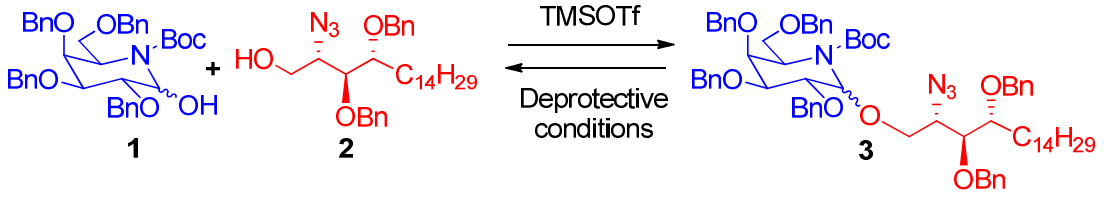

Figure 2a: Attempt towards synthesis of aza-*O*-GalCer

HWE reaction strategy was applied towards total synthesis of *β*-*C*-GalCer **6** and its aza-variant aza-*β*-*C*-GalCer **7**. A common intermediate β-ketophosphonate **4** underwent C-C bond formation with phytosphingosine-1-al **5** under HWE reaction conditions (Figure 2b). Other attempts made towards this C-C bond formation were also described. This novel KRN7000 analog **7** with aza-sugar offers a new entry to the repertoire of GSLs that is being currently evaluated for its immunological activity.

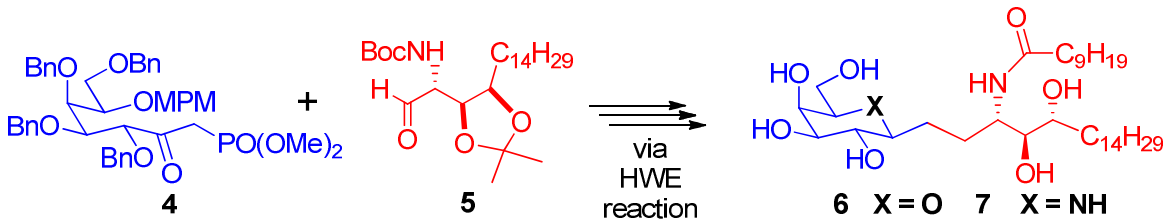

Figure 2b: Synthesis of *β-C*-GalCer and its aza-variant from D-galactose and phytosphingosine

During our multistep organic synthesis, we have observed the formation of 2-pyridone **8** from D-galactose in a serendipitous manner. 2-Pyridone **8** was efficiently converted to a pharmaceutically relevant 3,5-dihydroxy pyridine derivatives (**9a-f**) to demonstrate its synthetic utility (Figure 2c). These derivatives served as inhibitors of matrix metalloproteinases MMP2 and MMP9. This result is the first report for 2-pyridone synthesis from a carbohydrate, and also first of its kind for a novel 3,5-dihydroxypyridine.

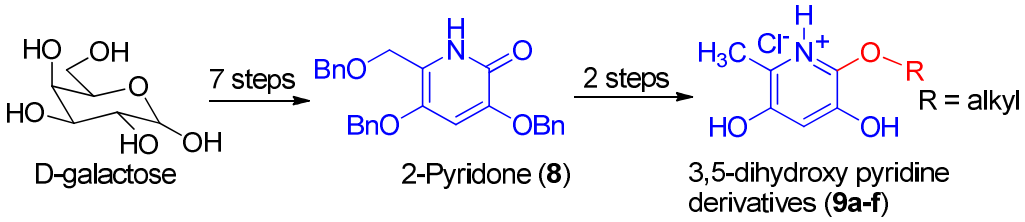

Figure 2c: Synthesis of 3,5-dihydroxypyridine derivatives from D-galactose

Third chapter describes the synthesis of polyhydroxy 2-pyrrolidinone azasugar (**10**) from D-galactose which is appended to simple aliphatic chains resulting in N-alkyl 2-pyrrolidinone derivatives (**11a-e**). 2-Pyrrolidinone was further appended to phytoceramide via a 1,2,3-triazole linker to afford GSL analogues **12a-d**. These novel GSL analogues **12a-d** (Figure 3a) are also being explored for their immunological activity.

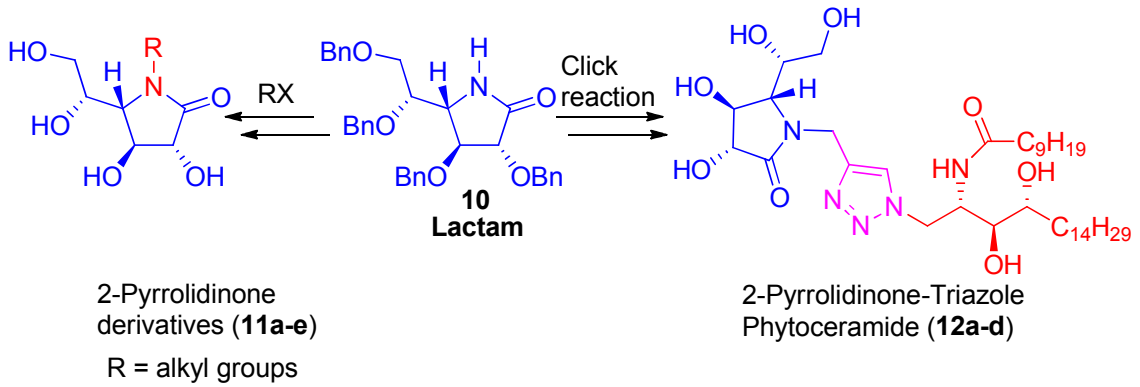

Figure 3a: Synthesis of N-alkyl and 1,2,3-triazole linked 2-pyrrolidinone derivatives from D-galactose.

In the fourth chapter a complete structural characterization of steroidal saponin uttroside B by 1D- and 2D- NMR techniques were explained. Using reverse phase HPLC the steroidal saponin was isolated from the methanolic extract of leaves of *Solanum nigrum*. The ^{1}H-NMR of this saponin presented a complex pattern of signals in CD_3OD and pyridine-d5. To reduce the complexity in the spectra, the saponin was derivatized to peracetylated saponin which resulted in a well resolved ^{1}H-NMR spectra in 700 MHz NMR followed by sequential 1D, 2D NMR, HRESIMS and MS-MS experiments helped us to solve the complete structure of uttroside B (Figure 4a).

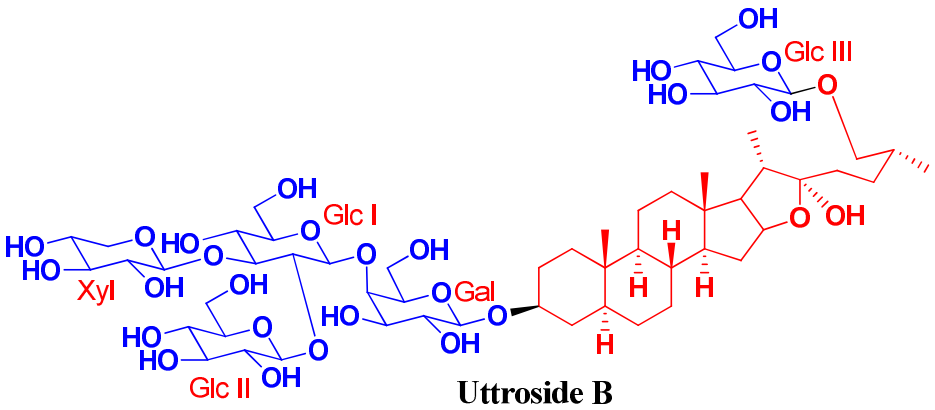

Figure 4a: Structure of uttroside B

(Key references, which covers the thesis)

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8. Evaluation of uttroside B, a saponin from *Solanum nigrum* Linn, as a promising chemotherapeutic agent against hepatocellular carcinoma. Lekshmi R. Nath[δ], Jaggaiah N. Gorantla[δ], Arunkumar T. Thulasidasan, Vinod Vijayakurup, Shabna Shah, Shabna Anwer, Sophia M. Joseph, Jayesh Antony, Kollery Suresh Veena, Sankar Sundaram,

Udaya K. Marelli, Ravi S. Lankalapalli,* Ruby John Anto.* *Sci. Rep.* **2016,** *6,* 36318**.δ –**

Contributed equally.

Chapter 1

Introduction of Glycolipids and Aza-sugars

1.1. Definition and function of glycolipids:

Glycolipids are generally defined as carbohydrate derivatives of their corresponding lipids such as acylglycerols, ceramides, sphingolipids attached by a glycosidic or ether bond.[1,2] They are amphiphilic components of the cell membrane, composed of a hydrophilic polar sugar head group appended to a hydrophobic non-polar lipid moiety which anchors the molecule in the cell membrane (Figure 1.1A). The sugar portion is projected on the outer leaflet of the lipid membrane which enables them to serve as cell surface receptors, recognition sites for cell to cell interactions, and they can modulate the immune response.

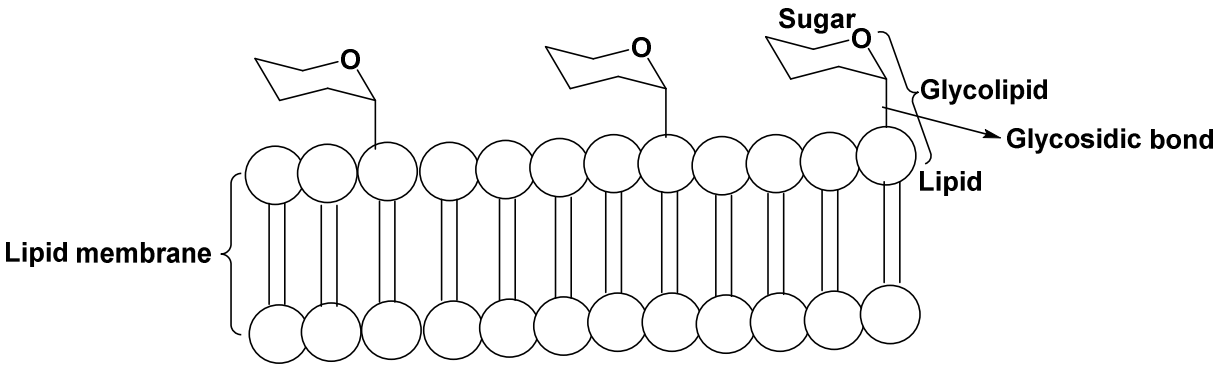


Figure 1.1A: Pictorial representation of a glycolipid

1.2. Glycolipid drugs approved by FDA:

Glycolipids are not just mere structural components of the cell membranes, they are involved in various physiological functions and there are certain glycolipids which served as life saving drug

molecules. Few of the glycolipid drugs that have been approved by FDA includes: (a) A natural product mifamurtide which is a derivatized muramyl dipeptide approved in 2010 for the treatment of osteosarcoma (malignant tumor of bone in which the proliferating spindle cells produce osteoid or immature bone), (b) Cytarabine ocfosfate which is another anticancer agent is used against leukemia, (c) Mupirocin is an antibiotic which was approved in 1985 and it is used to treat certain skin infections such as impetigo, and (d) Enocitabine is used as neoplastic agent which is a substance that inhibits or prevents the proliferation of neoplasms. The structures of these glycolipid drugs and their therapeutic use are shown in figure 1.2A.[3]

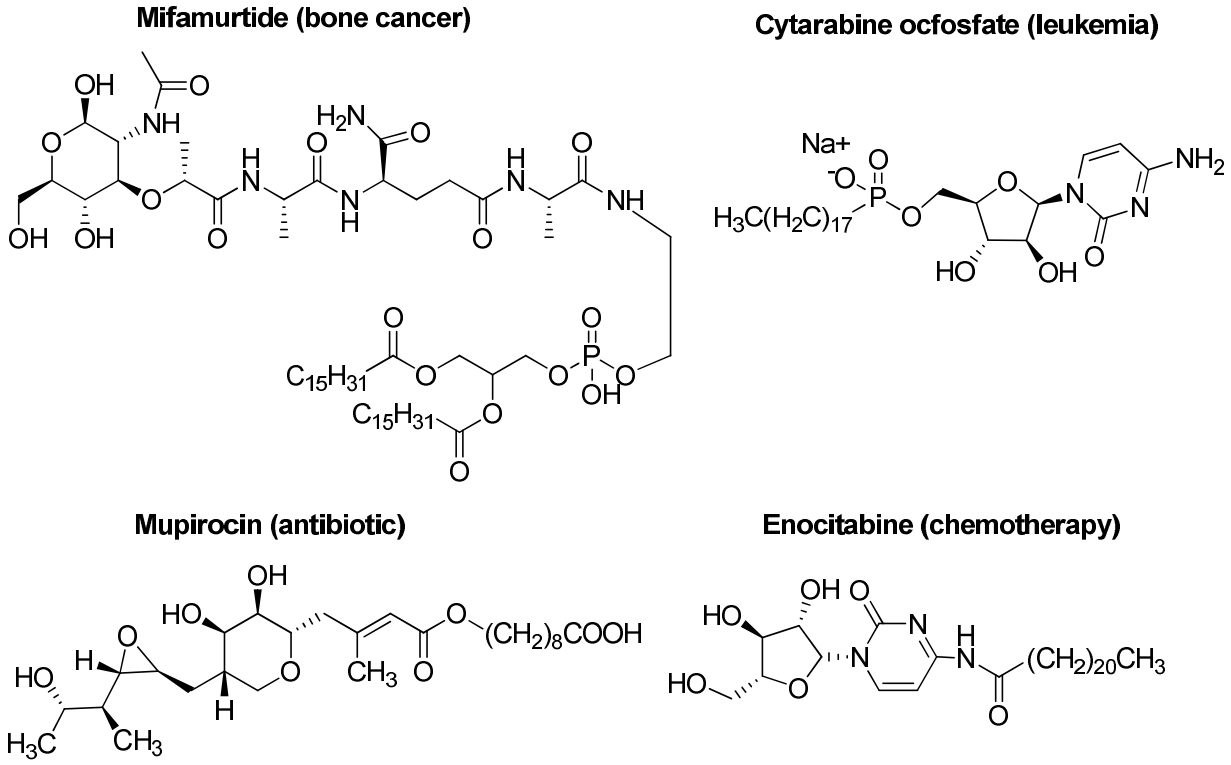


Figure 1.2A: Structures of glycolipid drugs approved by FDA

1.3. Glycosphingolipids (GSLs):

The term sphingolipids was coined by J. L. W. Thudichum (1828-1901) after the mythological Sphinx owing to their enigmatic properties. Glycosphingolipids (GSLs), an important class of glycolipids, are present in the outer leaflet of the plasma membrane.[4] Structurally GSL is a sphingosine or ceramide appended through its primary alcohol to different sugars through a glycosidic bond (Figure 1.3A). GSLs serve as primary binding sites for various pathogens including viruses, bacteria, fungi, and parasites. GSLs such as β-glucosylceramide is commonly found in fungal, mammal and plant sources.[5a] The β-xylosylceramide was isolated from the salt gland of the herring gull bird.[5b] α-Fucosylceramide was found to be present in human colon tumors,[5c] and β-galactosylceramide presence can be seen in fungal and mammal cell membranes except for plant sources (Figure 1.3B).[5a]

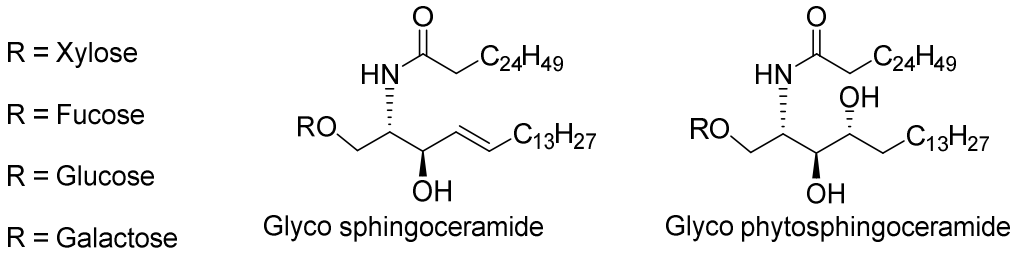


Figure 1.3A: Representative structures of glycosphingolipids (GSLs)

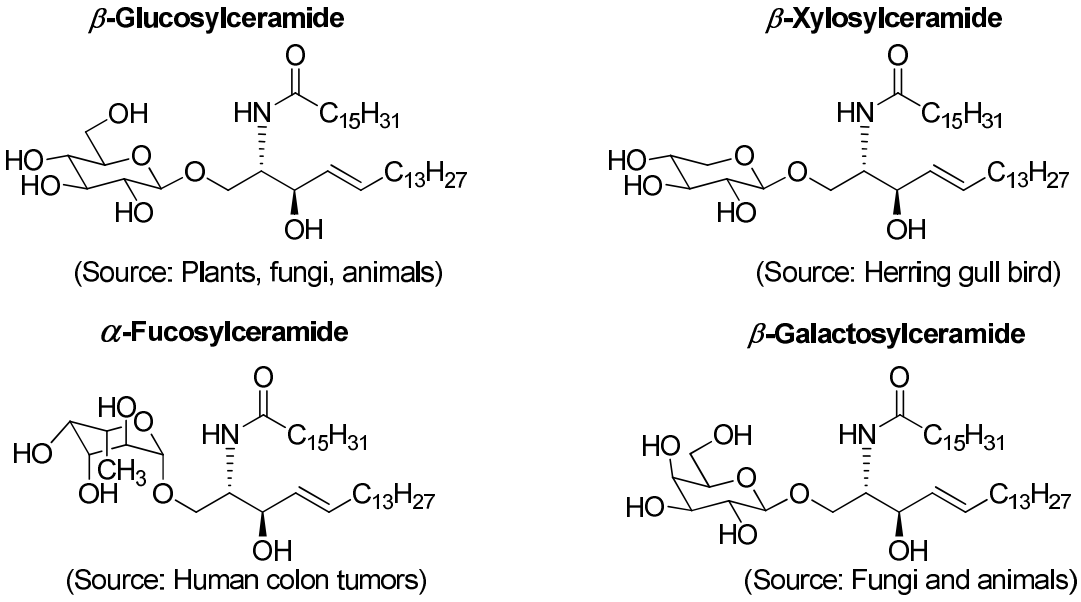


Figure 1.3B: Structures of natural glycosphingolipids (GSLs)

1.4. Origin, therapeutic importance and synthesis of KRN7000 (1):

In a related family of GSLs, one of the most important and well-explored glycosphingolipid is KRN7000. It is a structure-activity derived synthetic GSL analogue of a naturally occurring agelasphin which demonstrated antitumor properties from an extract of a marine sponge *Agelas mauritianus* in 1993. Later in 1995, various analogues of agelasphin (Figure 1.4A) were synthesized in search for lead analogue for clinical applications.[7] KRN7000 (**1**, Figure 1.4A) is a variant of agelasphin in that the variation is in the amide linkage.[6]

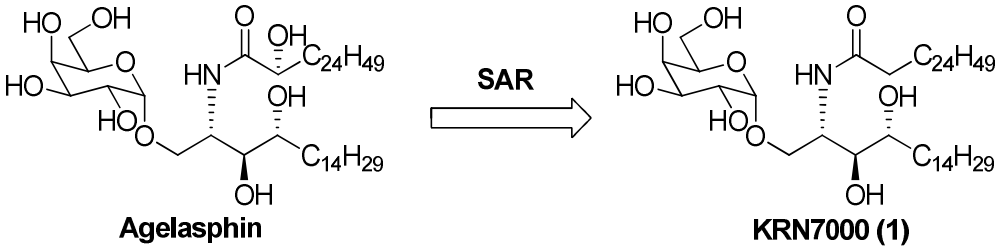
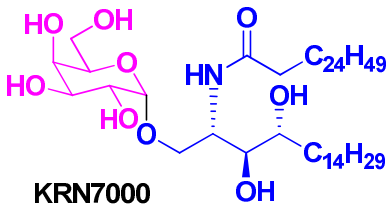


Figure 1.4A: Structure of agelasphin and KRN7000 (**1**)

Since its inception, KRN7000 has been well explored for its broad range of biological activities against malaria, tuberculosis, fungal pathogens, inflammation, and autoimmune diseases like lupus and diabetes mellitus. As a result, KRN7000 has been present at different levels of clinical trials for anti-tumor, anti-viral, and adjuvant properties. KRN7000 was evaluated for the anti-tumor activity by examination of the effect of NKT cell activation against hepatoma cells and compared the activation of NKT and NK cells between liver and extrahepatic sites. The KRN7000 administration completely abolished growth of hepatoma cells in the liver but did not exhibit an anti-tumor effect when hepatoma cells were subcutaneously injected (Figure 1.4B).[8a] KRN7000 was tested at phase-I and phase-II levels for its anti-viral activity against hepatitis B, and found that it has no significant effect in treating hepatitis.[8b] In the search of identifying biomarkers for predicting the immunological response to KRN7000-pulsed DC therapy in patients with NSCLC (non small cell lung cancer), two genes were identified namely LTB4DH and DPYSL3 (antibody blood proteins) which are thus considered to be potentially useful biomarkers.[8c] The administration of KRN7000-pulsed APCs into the nasal submucosa was found to be safe and its anti-tumor activity in patients was reported based on phase-I clinical studies (Figure 1.4B).[8d] The sphingosine back bone ($-C_{14}H_{29}$) was varied with *p*-fluorophenyl short chains ($-C_4H_8$-Ph-*p*-F) and examined the adjuvant effects of one of the most potent analogue on consensus hemagglutinin based DNA vaccine (pCHA5) for H5N1 virus.[9e] Similarly the *p*-fluoro phenyl containing KRN7000 stimulated the immune system to produce IgG instead of IgM which could serve as a promising adjuvant for the Globo H (GH) vaccine.[8f]

Anti-tumor activity	**Anti-viral activity**	**Anti-viral activity**
Liver	**Hepatitis B**	**Lung**
Int. J. Cancer	*Antivir Ther*	*Cancer Sci.*
2003, *106,* 81	**2009**, *14,* 809	**2010**, *101,* 2333

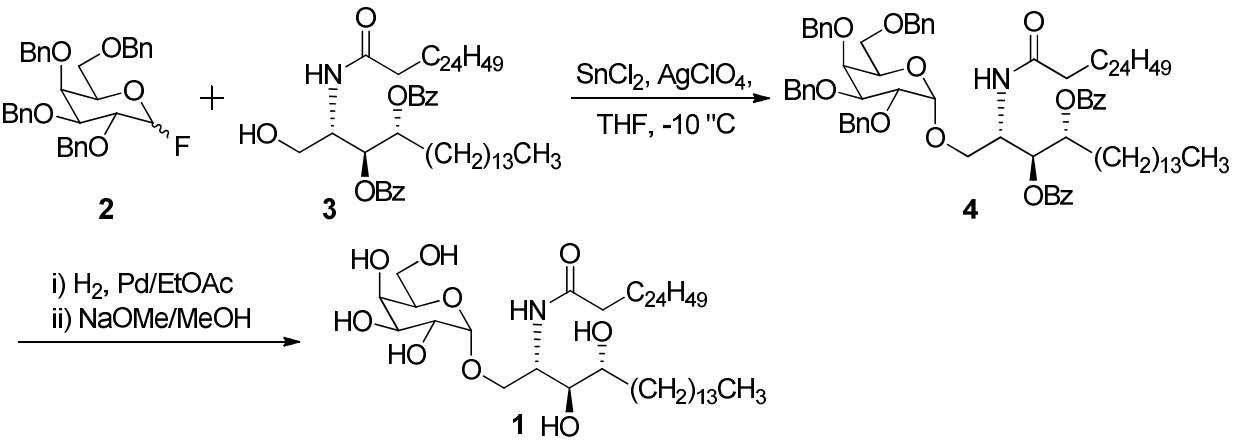


KRN7000

Adjuvant for	**Adjuvant for**	**Anti-tumor activity**
Breast cancer vaccine	**Hepatitis B vaccine**	**Head and Neck**
PNAS	*Vaccine*	*Cancer Immunol Immunother*
2013, *110,* 2517	**2014**, *32,* 6178	**2008**, *57,* 337

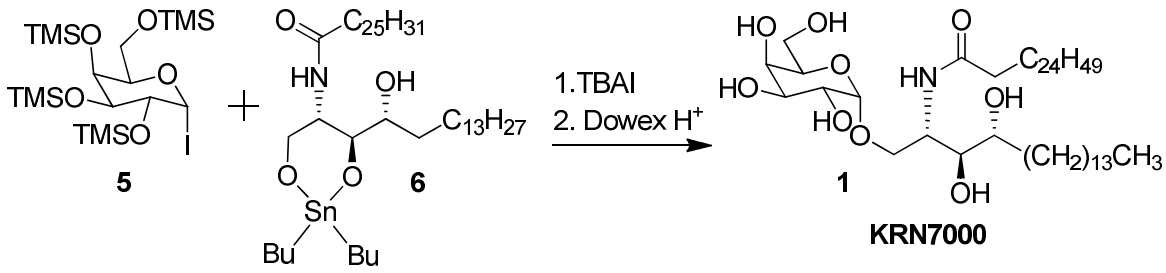
Figure 1.4B: Structure of KRN7000 and its biological activities

KRN7000 was synthesized by standard convergent approaches traditionally followed in synthetic carbohydrate chemistry. As depicted in Scheme 1.1 phytosphingosine-1-ol (**3**) and benzylgalactosyl fluoride **2** were treated with $SnCl_2$, silver perchlorate which resulted in a glycosylated product **4**. Deprotection of benzyl and benzyloxy groups gave rise to KRN7000.[7]



Scheme 1.1: Synthesis of KRN7000 from compound **2** and **3**

The reaction of the stannyl derivative of ceramide **6** with glycosyl donor **5** was performed under optimized reaction conditions. Hydrolysis of protecting groups in presence of acidic resin afforded the final product KRN 7000 (**1**) with complete α-selectivity (Scheme 1.2).[9]



Scheme 1.2: Synthesis of KRN7000

1.4.1. Mechanism of action of KRN7000:

The mechanism of action of KRN7000 involves initial binding to the CD1d protein on dendritic cells. The resulting dimeric complex is in turn recognized by T-cell receptor on natural killer T-cells (iNKTcells) and forms a trimeric complex. The trimeric complex secretes Th1 type (IFN-γ) and Th2 type (IL-4) cytokines. It was found that the IFN-γ cytokine is responsible for anti-tumor, anti-viral and adjuvant properties. IL-4 cytokine was responsible for treating auto-immune diseases like lupus, diabetes mellitus. The trimeric complex of KRN7000 with CD1d protein and iNKT cells was proven by X-ray analysis in 2007 (Figure 1.4.1A).[10]

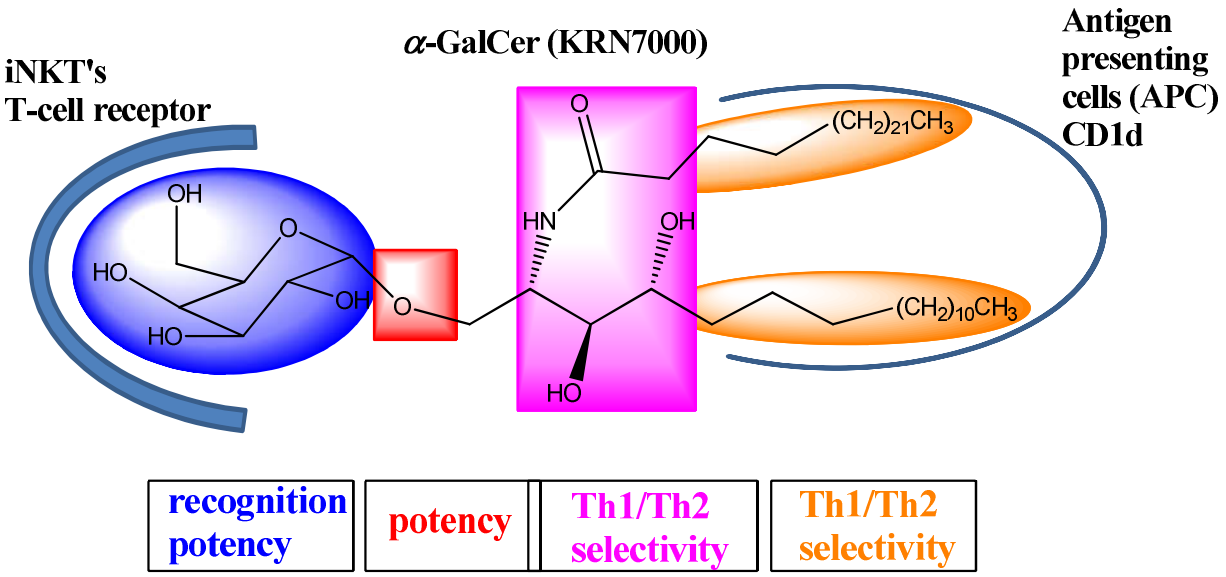


Figure 1.4.1A: Pictorial representation of Trimeric complex

However, the simultaneous secretion of cytokines Th1 (IFN-γ) and Th2 (IL-4) has suppressed the immunological potency of KRN7000 due to their reciprocal action resulting in no net benefit. Therefore, the task of selective or biased secretion of cytokines is required which can be envisaged by considering variations of KRN7000 lipid antigen. Several variants of KRN7000 across the length and breadth of the molecule for selective cytokine secretion were pursued by researches in synthetic community for the past two decades.

1.5. Variations in KRN7000 for its selective cytokine response:

Selective or biased cytokine response can be produced by structural variations in KRN7000 which include varying the amide linkage and the sphingosine backbone with different chain lengths, glycosidic linkage with carbon and other hetero atoms, C3 and C6 hydroxyl groups in galactose varied with sulfate and amide functionalities, and also the ring oxygen with sulfur and carbon (Figure 1.5A).

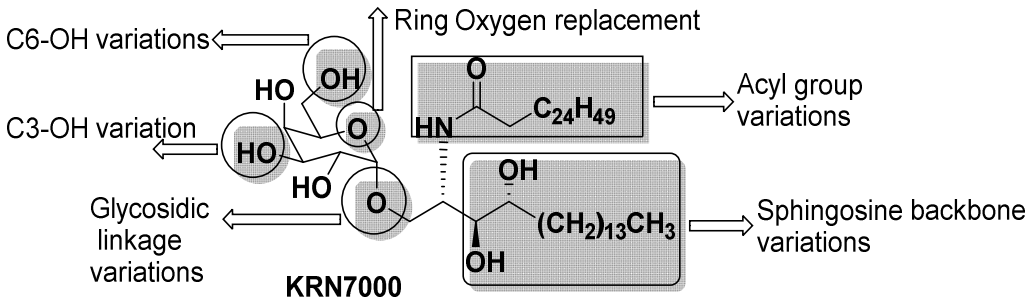


Figure 1.5A: All structural variations in KRN7000

1.5.1. Variations of the amide linkage:

The aliphatic chain ($-C_{25}H_{51}$) through the amide bond was replaced with a short chain ($-C_7H_{15}$) that resulted in a biased IL-4 cytokine response. Further substitutions with short chains terminating with an aromatic group have shown selective IFN-γ secretion. Unsaturated fatty acyl chain substitution and complete replacement of the azide functionality with triazoles led to no biased cytokine response (Figure 1.5.1.A). A series of KRN7000 analogues were synthesized by varying the lipid chain lengths, and studied their responses on NKT cells. It was found that further truncation of the phytosphingosine lipid chain increases the IL-4 vs IFN-γ bias of released cytokines. In similar fashion, the length of the acyl chain in KRN7000 (**7b**) influences IL-4 cytokine release profiles and may prove useful in altering immune responses and in the treatment of certain autoimmune diseases.[11a] Introduction of an aromatic group to the fatty acyl chain greatly enhances IFN-γ/IL-4 secretion and enables the tuning of Th1/Th2 cytokine profile, possibly through alteration of glycolipid/CD1d complex stability. Compound **7d** represent the first examples of NKT cell agonists which are more potent than KRN7000 and also exhibit a stronger Th1 cytokine response.[11b] The acyl group varied with short or polyunsaturated (**7c**) lipid variants of the NKT cell antigen KRN7000 exhibit decreased potency and a Th2 bias *in vivo* even though sealed T-cell receptor contact residues and stable binding to CD1d at neutral and

acidic pH.[11c] Recently synthesis and immunological screening of a focused library of benzyloxyalkyl-substituted 1,2,3-triazolyl (**7a**) of KRN7000 analogues are reported. The amide moiety in the structure of α-GalCer was replaced with a 1,2,3-triazole linker which led to elicit a biased response toward Th2 immunity, and KRN7000 containing an aromatic ring in the terminus of their acyl or phytosphingosine structural component exhibited an enhanced Th1 immune response.[11d]

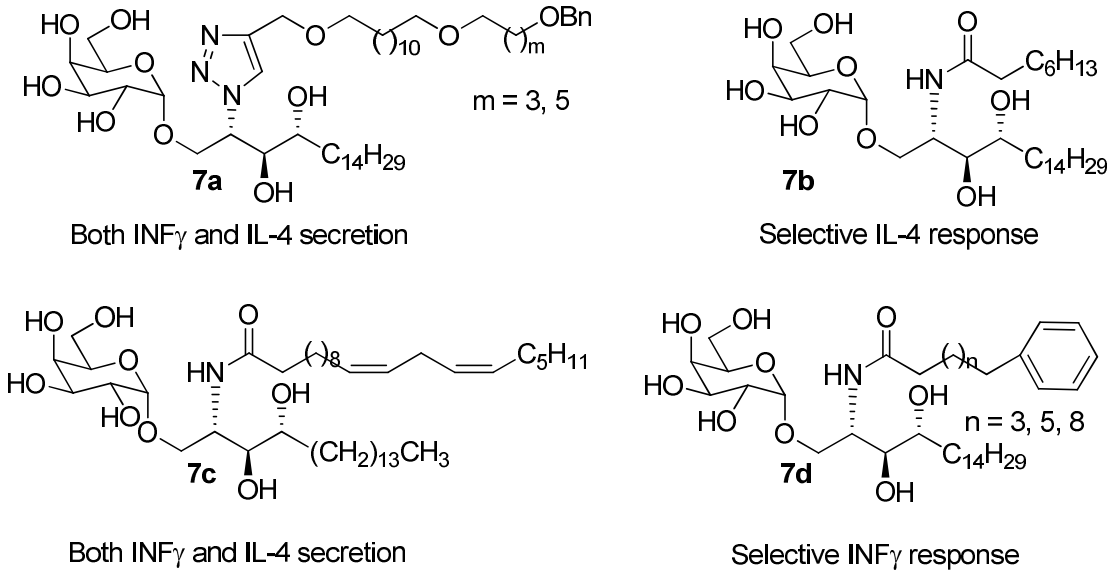


Figure 1.5.1A: Structures of KRN7000 analogues with variations across the amide bond

1.5.2. Variations of the sphingosine backbone:

Variations were also carried out on the sphingosine backbone by replacing the hydroxyl groups with fluorine which produced selective IFN-γ secretion but replacement with hydrogen atoms provides no selectivity. The backbone long chain (-$C_{14}H_{29}$) was also replaced with heteroaromatic substituted long chains resulting in selective IL-4 cytokine response (Figure 1.5.2A). The 4-deoxy-KRN7000 derivative **8b** induced potent cytokine responses, comparable to those of KRN7000, both from human iNKT cells *in vitro* and from their murine counterpart *in*

vivo.[12a] The 3,4-dideoxy-3,3-difluoro KRN7000 analogue **8a** restores the stability of the ternary CD1d/GalCer/TCR complex both *in vivo* in mice and *in vitro* in human iNKT cells. The 3,4-dideoxy-3,3-difluoro KRN7000 analogue **8a** delivered a suitable cytokine secretion while maintaining Th1 polarization, may pave the way toward candidates for anticancer immunotherapy.[12b] Variation of the sphingosine back bone with a pyrazole moiety and a phenyl group along the aliphatic chain produced analogue **8c** (Figure 1.5.2A) that induced greater polarization toward Th2 and greater secretion of the immunomodulatory cytokine IL-4.[12c]

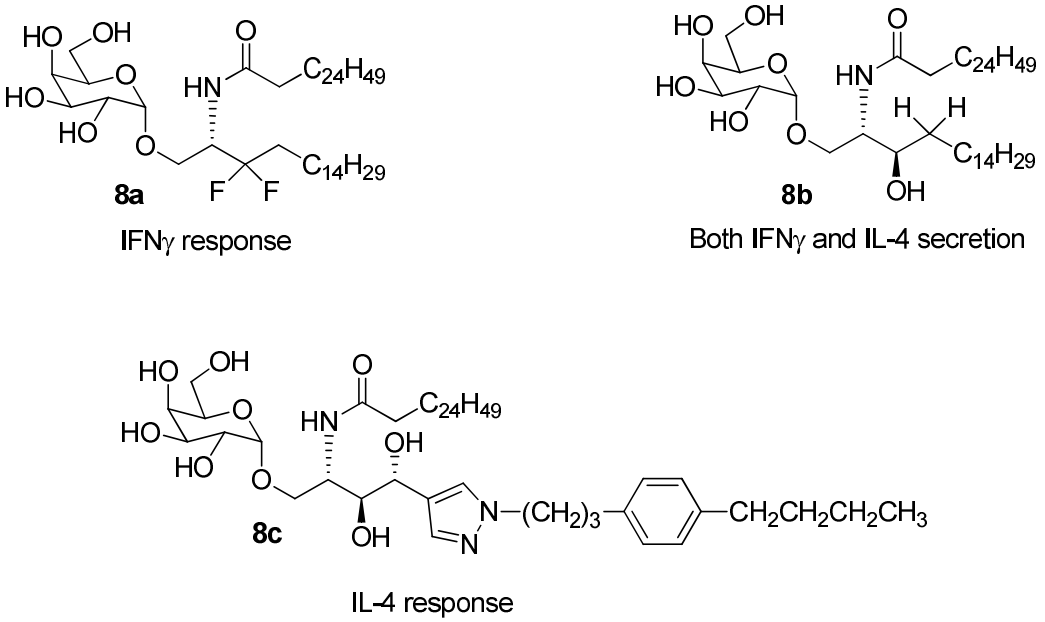


Figure 1.5.2A: Variations of the sphingosine backbone of KRN7000

1.5.3. Variations of the galactose moiety:

The C-6 hydroxyl group was replaced with amide and methyl functionalities, and the C-3 hydroxyl group was substituted with sulfate that resulted in no selective cytokine response (Figure 1.5.3A). *a*-L-Fucosylceramide was synthesized by incorporating the methyl group at 6-

position of the galactose moiety of KRN7000, to examine their bioactivity for mouse lymphocytes. Bioassay of compound **9b** showed more potent stimulatory effect on mouse lymphocytes than KRN7000 to induce the production of a large amount of IFN-γ *in vivo*.[13a-b] Sulfatide analogue of KRN7000 **9c** produced activities similar to KRN7000 suggesting that modification of the C3-OH position of the galactose moiety with sulfate has no significant effect on NKT cell stimulation.[13c] The 2′-OH, 3′-OH, and 4′-OH of the galactose ring form hydrogen bonds with amino acid residues of the invariant TCR α-chain. This mode of binding is consistent with the specificity the NKT TCR exhibits for KRN7000 versus closely related analogues modified at the sugar part. Interestingly, the Gal 6′-OH is the only sugar alcohol not involved in H-bond formation, suggesting the possibility of introducing modifications at that position. As it was envisioned that extra interactions might be established between CD1d and a 6′-OH modified with 3-CF_3, 4-ClPh urea functionality (**9d**) in galactose moiety of KRN7000. The compound **9d**, containing a 3-CF_3,4-Cl benzamide substituent, emerged as the most promising Th1 polarizing agent, since it induced IFN-γ levels comparable to KRN7000 and only low levels of IL-4.[13d] 1-Aminophytosphingosine and 6-aminogalactosyl phytosphingosine analogues of KRN7000, were prepared and the compound **9a** (Figure 1.5.3A) able to produce both the IFN-γ and IL-4 cytokines.[13e]

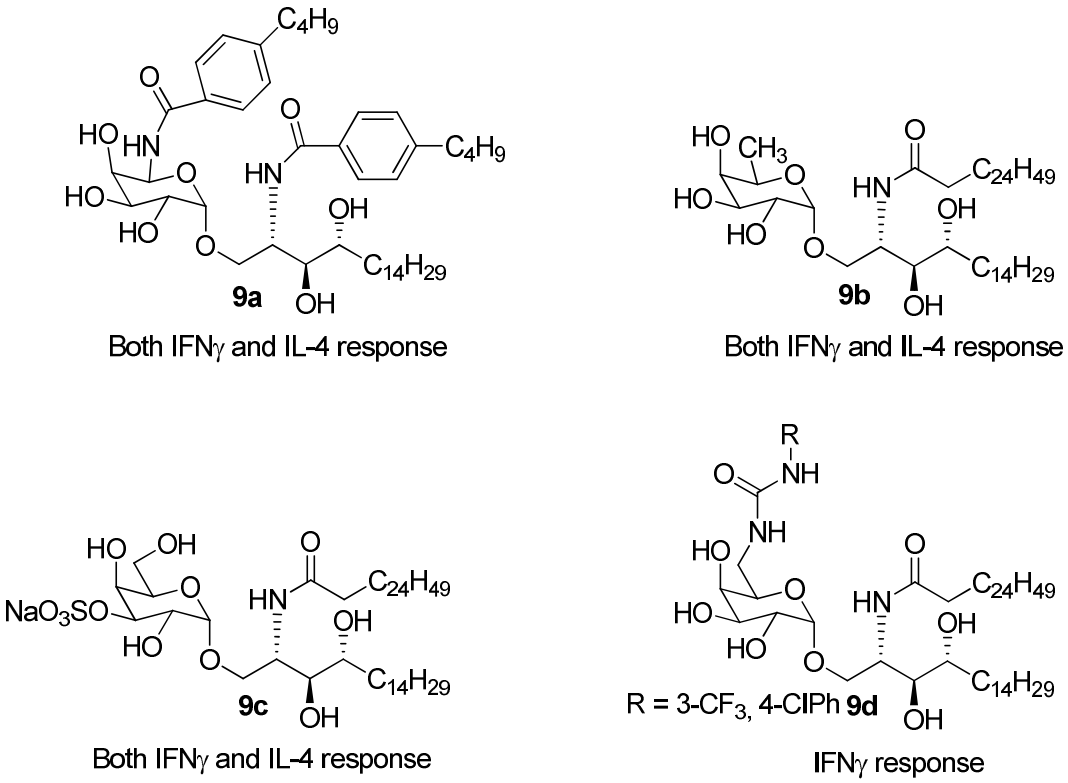


Figure 1.5.3A: Variations at C3, C6 hydroxyl groups of galactose moiety

Galactose ring oxygen varied with carbon and sulfur atoms produced carba and thio sugar analogues of KRN7000, respectively, which showed selectivity in IFNγ cytokine production. The carbasugar analogue (**9e**) have the hydrophobic CH_2 group instead of the hydrophilic pyranose O atom, therefore, their solubility are lower than that of KRN7000 and it was found that **9e** could make a more stable complex with CD1d than KRN7000. The compound **9e** the carba-α-D-galactose analogue was found to be the remarkably potent inducer of Th1-biased cytokine production in mice in vivo.[14a] The 5-thio-KRN7000 (**9f**) stimulated higher IFN-γ production of iNKT cells than that of KRN7000. Compared to KRN7000, S-analogue **9f** presented a similar tendency and ability to induce the release of key immunomediators IFN-γ and IL-4 in mice (Figure 1.5.3B).[14b]

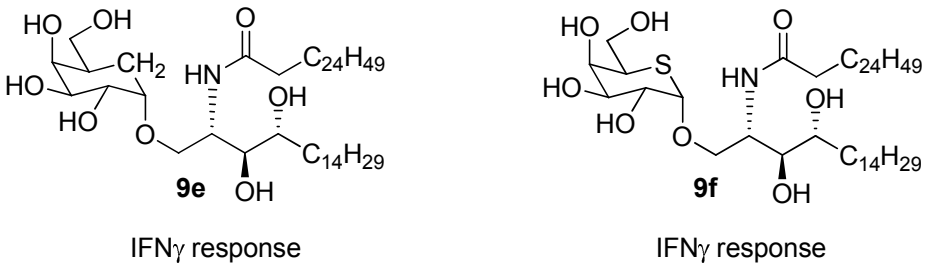
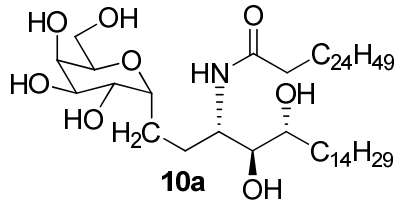


Figure 1.5.3B: Variations of the galactose ring oxygen

1.5.4. Variations at the glycosidic linkage:

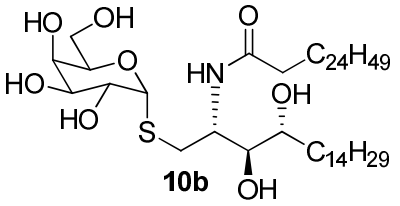
Variations carried out at the glycosidic linkage by replacing the glycosidic oxygen with selenium, nitrogen, sulfur, carbon produced KRN7000 analogues responded for selective IFN-γ cytokine secretion. Interestingly, the C-glycoside analogue of KRN7000 was found to be more potent than KRN7000 and selective towards the production of IFN-γ cytokine response (Figure 1.5.4A). The C-glycoside (**10a**) was tested in a mouse malaria model, whereby mice were treated with galactosyl ceramide **10a**, after an interval the animals were sacrificed and their livers were assayed for the sporozoite stage of malaria. Both the O-glycoside and the C-glycoside **1** and **10a**, were very effective at reducing sporozoite levels at a dosage level of 1 mg per mouse relative to the control. However, the C-glycoside **10a** (Figure 1.5.4A) continued to show excellent activity at the 1-ng level. Thus, **10a** is approximately 1000 times more protective than the O-glycoside (**1**). Melanoma challenge in C57BL/6 mice was treated with either the C-glycoside (or) the O-glycoside. The C-glycoside **10a** at the 10 ng dosage level was far more effective than the O-glycoside at the same dose. In fact, detailed dose response experiments showed that the C-glycoside **10a** was 100 times more effective than the O-glycoside **1** (KRN7000).[15a] The synthesis and evaluation for iNKT stimulation of α-S-galactosylceramide (**10b**) was reported, this analogue of the KRN7000, did not stimulate iNKT cells either *in vitro* or *in vivo*.[15b] The

aminocyclitol analogue of KRN7000 (Figure 1.5.4A, **10d**) selectively induced a very strong production of IFN-γ indicative of a potent Th1 cytokine profile in mice, these results confirm the agonist activity of KRN7000 lipid analogues having charged amino-substituted polar head group and their capacity to modulate the response arising from iNKT cell activation *in vivo*.[15c] Recently, McDonagh *et al* reported the synthesis of Se-glycoside analogue of KRN7000 and the biological activity of this 1-Se-GalCer (**10c**) yet to be tested.[15d]



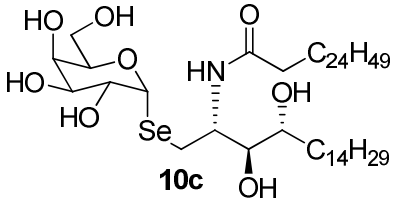
10a

IFNγ response (anti-tumor activity 100 times potent than KRN7000)



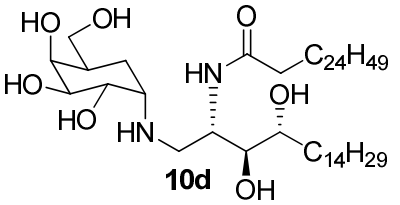
10b

No activity



10c

Activity yet to be tested



10d

IFNγ response

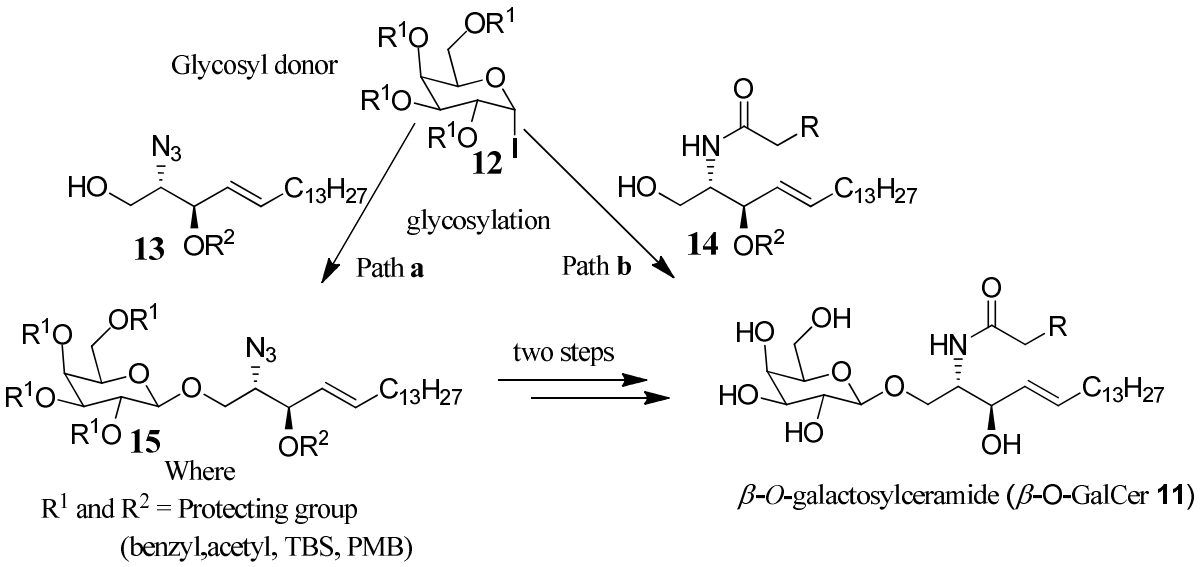
Figure 1.5.4A: Variations at the glycosidic oxygen with Se, N, S, C atoms

1.6. Synthesis and biological importance of *β-O*-galactosylceramide:

β-O-Galactosylceramide inhibited viral internalization and infection in two CD-negative cell lines derived from the nervous system. Furthermore, recombinant HIV surface glycoprotein gp120 bound to galactosylceramide but no to any other glycolipids. These results suggest a role for galactosylceramide or a highly related molecule in HIV entry into neural cells.[16] In 1991,

Bhat S. *et al* reported results demonstrates that galactosyl ceramide (GalCer) or a molecule derived from it may serve as an alternative receptor for human immunodeficiency virus in the nervous system. Recombinant gpl20, an envelope glycoprotein of human immunodeficiency virus type 1, specifically binds to GalCer and its derivatives. This specificity was studied by inhibiting binding of radioiodinated gpl20 to GalCer with antibodies to GalCer, antibodies to gpl20, and an excess of unlabeled gpl20. Binding activity was also removed by absorbing gpl20 with liposomes containing GalCer. In addition, studies using natural and semisynthetic lipids indicate that the linkage between galactose and ceramide is essential for binding. The significance of an alternative receptor for human immunodeficiency virus in the nervous system was discussed.[17] Effective biological activity and scarcity in the availability of GSLs from the natural sources, chemists developed new synthetic routes for synthesis of GSLs. The key steps in these synthetic protocols involve the construction of a glycosidic bond between protected carbohydrates and sphingosine or azido-sphingosine.[18a]

β-Galactosylceramide and glycolipid analogues were prepared in high yield with complete chemo and stereoselectivity by reaction of *a*-iodo glycosides with stannyl ceramides formed *in situ*. TBAI was used to activate both the iodogalactose and the stannyl ether. One of the drawbacks in the synthesis of GSLs is that the yield in the direct glycosylation of ceramides is low. The reason attributed is low nucleophilicity of ceramide **14** which was avoided by using azido-sphingosine **13** (Scheme 1.3). This strategy affords high yields in the glycosylation step but increases the number of steps; this issue was avoided by applying the simple and very efficient procedure for the glycosylation of *β*-amidoalcohols and ceramides by using glycosyl iodides as glycosyl donors to afford glycosylated product (**15**), which was then converted to *β*-galactosylceramide (**11,** Scheme 1.3).[18b]



Scheme 1.3: Synthesis of *β-O*-galactosylceramide from azido sphingosine

1.7. Synthesis of *C*-glycosides:

Replacement of the glycosidic oxygen atom with a methylene group (-CH_2) resulting in *C*-glycosides. The natural *O*-glycosides are prone to enzymatic hydrolysis which has implications in the use of carbohydrate derived molecules as drugs thus serving as a limiting point. This problem can be circumvented by replacement of the glycosidic oxygen atom with a methylene group (-CH_2), which results in a stable C-C bond between carbohydrate (glycone) and aglycone moieties. Several synthetic chemists are focusing the attention towards development of stable mimics such as *C*-glycosides which are inert under acid and enzymatic hydrolysis. Numerous methods have been developed for the synthesis of *C*-glycosides as mimics for *O*-glycosides. The synthesis and biological activities of alkyl, aryl, phenolic, peptide, oligosaccharide, and sphingolipid *C*-glycosides have been extensively reported. The strategies for *C*-glycoside synthesis includes Wittig olefination, Horner-Wadsworth-Emmons (HWE),[19] ring closing cross metathesis,[20] cross metathesis followed by cyclization,[21] Ramberg-Backlund (RB) reaction,[22]

metal mediated *C*-glycosylation,[23] nucleophilic additions,[24] Mitsunobu reaction,[25] addition reaction on glycal,[26] Hantzsch-type approach,[27] and other reactions.[28] Among the *C*-glycosides, α & β-*C*-galactosylceramides (Figure 1.7A), have been extensively reported for their immunostimulation, immunosuppression, and antiviral activities. A compelling biological activity of galactosylceramides has motivated synthetic chemists for the development of new multistep synthetic routes via linear and convergent approaches.

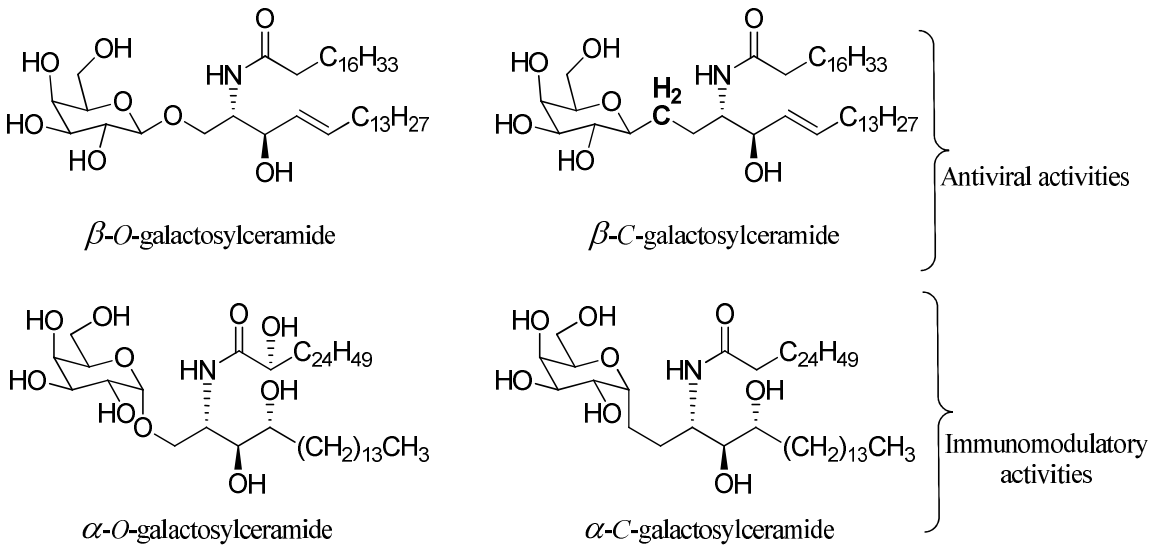
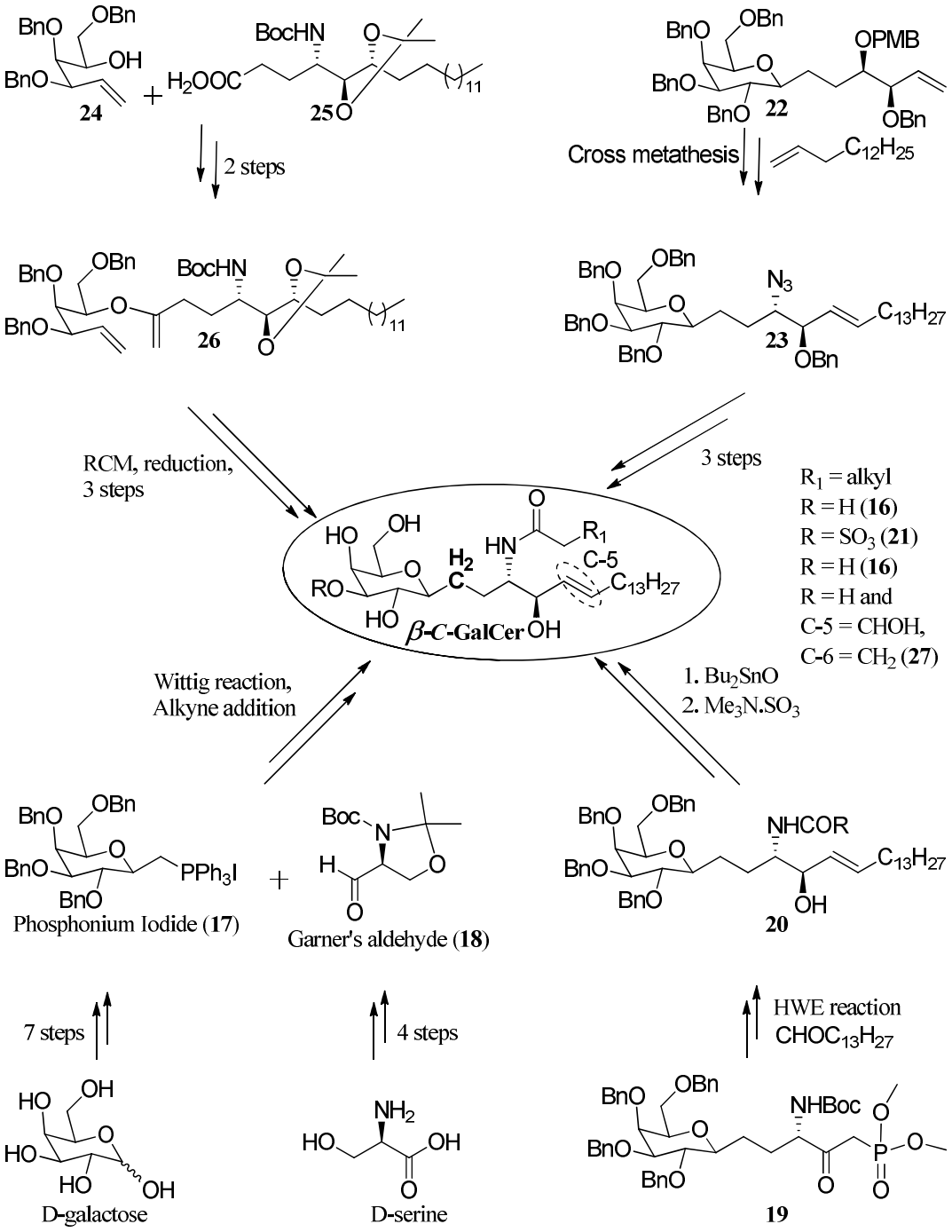


Figure 1.7A: Representative structures of α, β-*C*-galactosylceramides

1.8. Synthesis and biological importance of β-*C*-galactosylceramide:

The interaction between HIV gp120 and galactose-containing cell surface glycolipids such as GalCer is known to assist HIV binding to both CD4+ as well as CD4− cells. In that regards to develop small molecule HIV-1 entry inhibitors with improved solubility and efficacy, a series of C-glycoside analogues of GalCer were synthesized and tested for their anti HIV-1 activity.[29a] Non-isosteric water soluble *C*-glycoside of galactosphingolipid and its derivatives were first

synthesized and it was shown that compound **16** (Scheme 1.4) binds specifically to HIV-1 gp120. Replacement of *C*-glycoside confers resistance to both chemical and enzymatic deglycosylation which is important for *in vivo* applications.[29b] The C3-galactosulfatide analogue of *β-C*-GalCer **21** showed less immunogenic properties than natural *β*-GalCer **11**, but induces a preferential secretion of the proinflammatory cytokine IFN-γ.[30] The first total synthesis of *β-C*-GalCer was reported using witting reaction between phosphonium iodide **17** (Scheme 1.4) and protected aldehyde **18**. The phosphonium salt **17** was prepared from the formyl *C*-galactoside, while the aldehyde **18** was obtained from D-serine. Coupling of these intermediates by Wittig reaction followed by addition of alkyne gave rise to *β-C*-GalCer (**16**).[19a] Sulfatide analogue of *β-C*-GalCer (**21**) was synthesized by coupling the galactose derived ketophosphonate (**19**) and 1-butadecenal under Horner-Wadsworth-Emmons olefination reaction. Reduction of ketone and global debenzylation and selective C-3 sulfatide formation yielded sulfatide analogue **21**.[30] Recently *β-C*-GalCer (**16**) synthesis was reported using the key cross metathesis reaction between a galactose derived alkene (**22**) and 1-pentadecene, Sharpless AD dihydroxylation reaction and stereoselective azidation reactions.[31] Alternatively, the *β-C*-GalCer was synthesized using ester formation between a galactose derived enetol (**24**) and homo phytosphingosine carboxylic acid (**25**) followed by conversion of ester carbonyl into alkene (**26**) gave rise to coupled diene product. Subsequently, Grubb's ring closing metathesis reaction followed by hydroboration of double bond, amidation and global debenzylation afforded *β-C*-GalCer analogue **27**.[32]



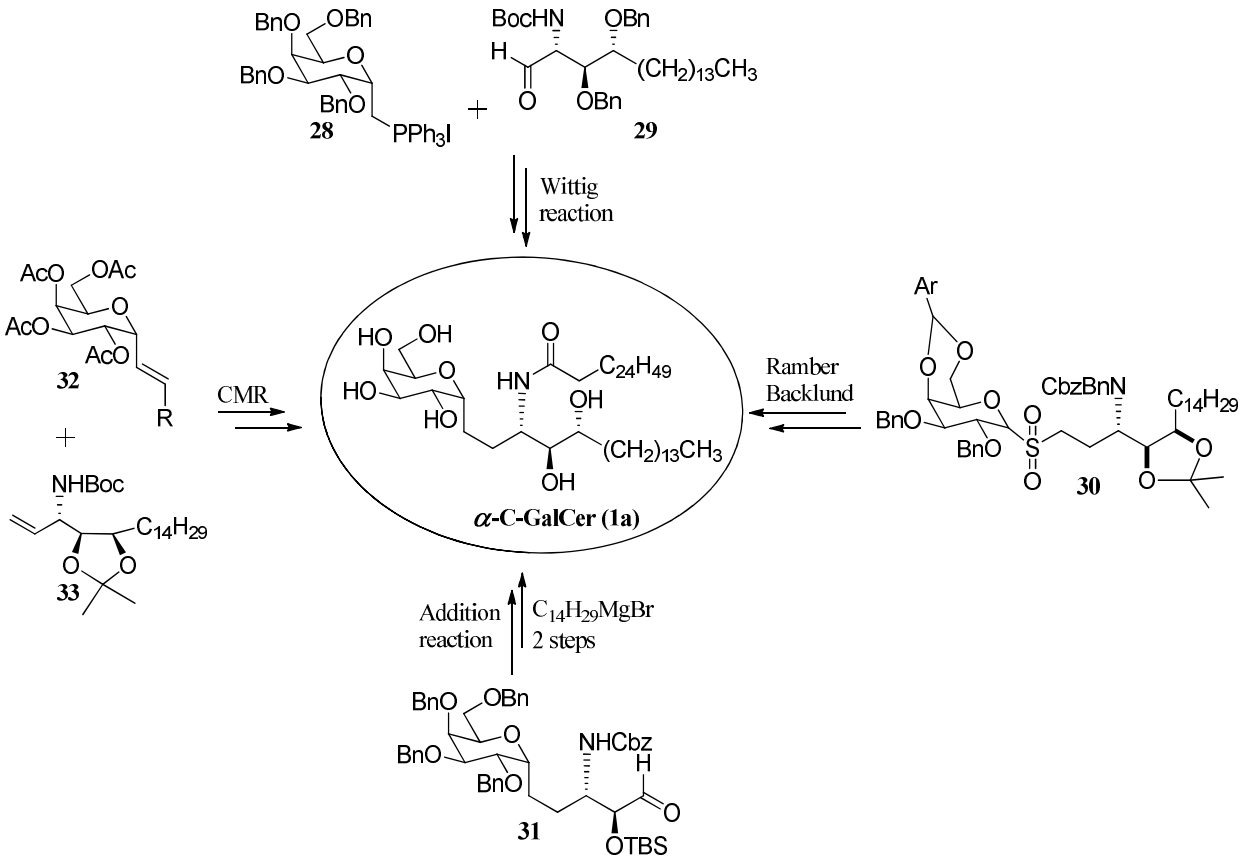
Scheme 1.4: Synthesis of *β-C*-GalCer and its analogues

1.9. Synthesis and biological importance of *α-C*-galactosylceramide:

In 2003, Schmieg J. *et al.* reported the synthesis of C-glycoside analogue *α-C*-GalCer [Scheme 1.5, (**1a**)] which acts as natural killer T cell ligand *in vivo*, and stimulates an enhanced Th1-type response in mice. In two disease models requiring Th1-type responses for control, namely malaria and melanoma metastases, *α-C*-GalCer (**1a**) exhibited a 1,000-fold more potent antimalaria activity and a 100-fold more potent antimetastatic activity than KRN7000. Moreover, *α-C*-GalCer consistently stimulated prolonged production of the Th1 cytokines IFN-γ and IL-12, and decreased production of the Th2 cytokine IL-4 compared with KRN7000. Finally, *α-C*-GalCer enhanced therapeutic activity required the presence of IL-12, which was needed to stimulate natural killer cells for optimal IFN-γ production but did not affect IL-4.[33] These results suggest that *α-C*-GalCer can afford a selective immune response toward Th1 or Th2 cytokines. Therefore, synthesis of *α-C*-GalCer analogues has been an attractive approach for immune based therapeutic approaches.

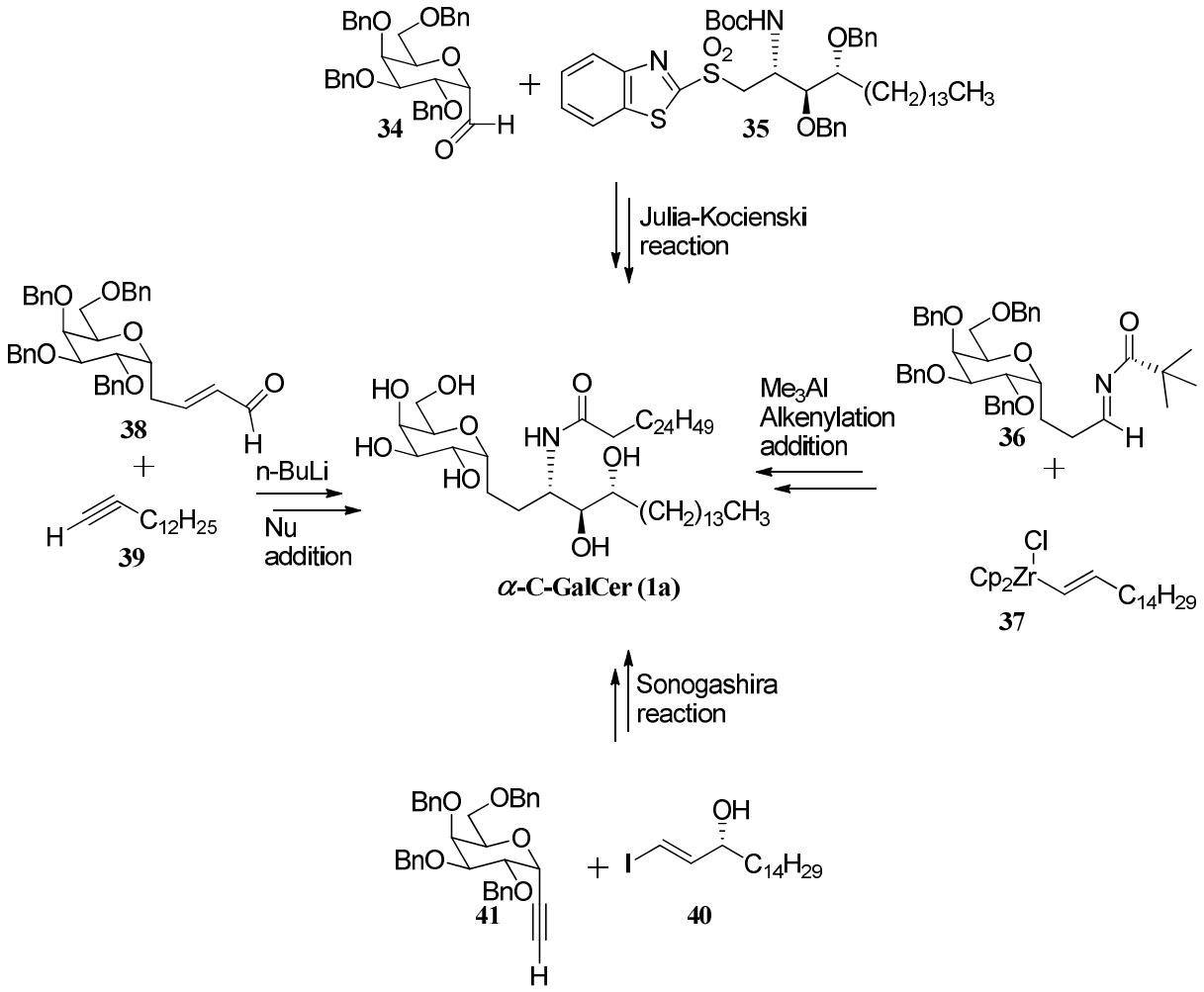
The first synthesis of *α-C*-GalCer (**1a**) was reported in the patent literature by Kotobuki Pharma in 2002. The key Wittig reaction was employed between the D-galactosyl triphenyl phosphonium ylide (**28**) and the aldehyde (**29**) derived from phytosphingosine to yield *α-C*-GalCer in low yield.[34] Later it was noted that the Wittig conditions lead to the epimerization at the amine bearing carbon at the aldehyde precursor stage resulting in an epimeric mixture of compounds. In 2004 Franck *et al.* solved the epimerization problem by applying a Ramberg-Backlund reaction conditions on sulfone (**30**) as the key C-C bond forming step to afford olefin which was reduced with chlorotrimethyl silane in methanol. The *C*-glycosidic bond was generated via the stereo selective introduction of an anomeric hydrogen delivery from the *β*-face of the exo-glycal to form an *α-C*-glycoside bond.[15b] Alternatively, Grignard reagent addition on protected amino

hydroxy aldehyde (**31**) afforded C-4 epimeric mixture of two alcohols; the undesired major isomer, desired minor isomer were then separated and then the epimers matched with the reported original *α*-*C*-GalCer. Finally the minor isomer was characterized as the desired *α*-*C*-GalCer.[35] Cross metathesis reaction strategy was employed for the synthesis of *α*-*C*-GalCer, initially the sphingosine alkene **33** was synthesized from the commercially available starting material phytosphingosine using protection and deprotection strategy, and the tetraacetyl alkene **32** was easily obtained in two steps from pentaacetyl galactose. Coupling of both the alkene partners **32** and **33** in the presence of Grubbs catalyst resulted in the coupled product which was converted into compound **1a**.[36]



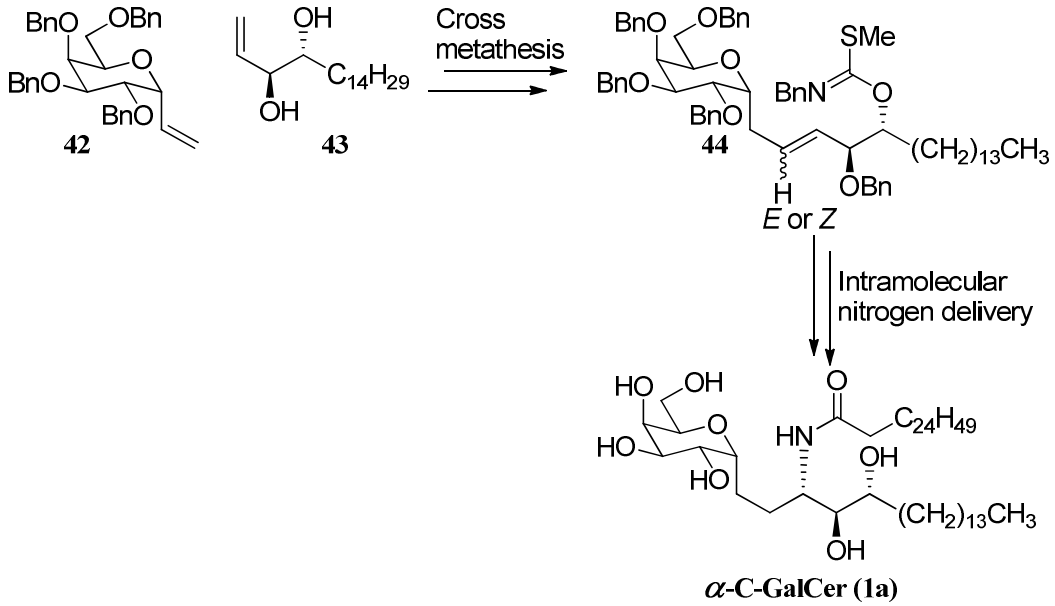
Scheme 1.5: Synthesis of *α*-*C*-GalCer (**1a**)

In 2006, *a-C*-GalCer was produced using the key Julia-Kocienski reaction by linking the galactosyl aldehyde (**34**) which was obtained from the methyl galactoside in five steps, and from the pentaacetyl galactoside in seven steps. The Julia precursor phytosphingosine sulfone (**35**) was synthesized from a commercially available starting material phytosphingosine in six steps. Fortunately the aldehyde **34** was stable under the basic conditions of the condensation reaction without any epimerization or elimination (Scheme 1.6).[37] The α-C-galactosylceramide analogue of KRN7000 synthesis was reported using key reactions include hydrozirconation of 1-hexadecyne to generate alkenylzirconocene **37** followed by a diastereoselective alkenylalane addition to an *N*-tert-butanesulfinyl imine (**36**) in presence of aluminum transmetalation/imine addition. Convenient *in situ* deprotection of the labile sulfinyl protecting group with aqueous HCl afforded the desired allylic amine which was subjected to epoxidation/carbamate ring opening sequence to establish the aminodiol and final deprotections gave rise to compound **1a**.[38] The galactose derived α,β-unsaturated aldehyde **38** was synthesized from a pentaacetyl galactose in seven steps, and then the tetradecynyl (**39**) nucleophilic addition on unsaturated aldehyde (**38**) in presence of *n*-BuLi yielded addition product which was subsequently converted into *a-C*-GalCer.[39] Sonogashira coupling of alkyne (**41**) with lipid vinyl iodide (**40**) followed by Sharpless epoxidation and opening of epoxide via intramolecular nitrogen delivery followed by amidation and global deprotection afforded *a-C*-GalCer **1a** (Scheme 1.6).[40]



Scheme 1.6: Synthetic routes for α-*C*-GalCer (**1a**)

Recently α-*C*-GalCer synthesis was reported using cross metathesis and intramolecular nitrogen delivery as the key reactions. The tetrabenzyl-α-vinyl galactose (**42**) and lipid alkene (**43**) intermediates were synthesized in several steps later coupled under Grubbs cross metathesis reaction conditions and then the resulting coupled intermediate (**44**) were subjected to intramolecular nitrogen delivery (Scheme 1.7) and then converted into α-*C*-GalCer (**1a**).[41] Miscellaneous synthetic methods were reported for the synthesis of α-*C*-GalCer, and their biological activities were explored against cancer and viral diseases.[42]



Scheme 1.7: Synthesis of α-*C*-GalCer (**1a**) via an intramolecular nitrogen delivery

1.10. Structural features and biological importance of azasugars:

Azasugars are carbohydrates where the endocyclic oxygen is replaced with basic nitrogen atom. The origin and therapeutic use of azasugars goes back to ancient times and traditional Chinese phytomedicines. Haarlem oil was the first medication recommended for the treatment of diabetes and for whitening the skin.[43a] One of the major constituents of Haarlem oil was an extract from leaves of *Morus alba* (white mulberry) is a rich source of azasugars.[43b] In 1966, the first synthesis of 1-deoxynojirimycin (DNJ) was reported; later Inouye *et al.* isolated nojirimycin from bacteria (*Streptomyces*) and found its antibiotic properties. The first interest of azasugars came from the isolation of DNJ from natural sources and the finding of its biological activity as a α-glucosidase inhibitor by Bayer chemists in 1976. This discovery triggered an enormous amount of interest in imino analogues of carbohydrates.[43c]

The diversity of enzymes inhibited by azasugars promises a new generation of drugs in a broad range of diseases such as diabetes, viral infections, lysosomal storage disorders or tumour

metastasis. Various structures are currently involved in clinical trials, *N*-ethanol-deoxynojiromycin (or) Glyset™ was approved by FDA (Figure 1.10A, **46**) in 1996 for the treatment of complications associated with type II diabetes. *N*-Butyl-deoxynojiromycin (or) Zavesca™ (**47**) in 2003, was used for the oral treatment of severe lysosomal storage disorders (Gaucher disease). Commercialization of the first azasugar based drug Glyset (**46**) in 1996 sparked interest in the synthetic community in utilizing their derivatives for therapeutic benefit.[43a]

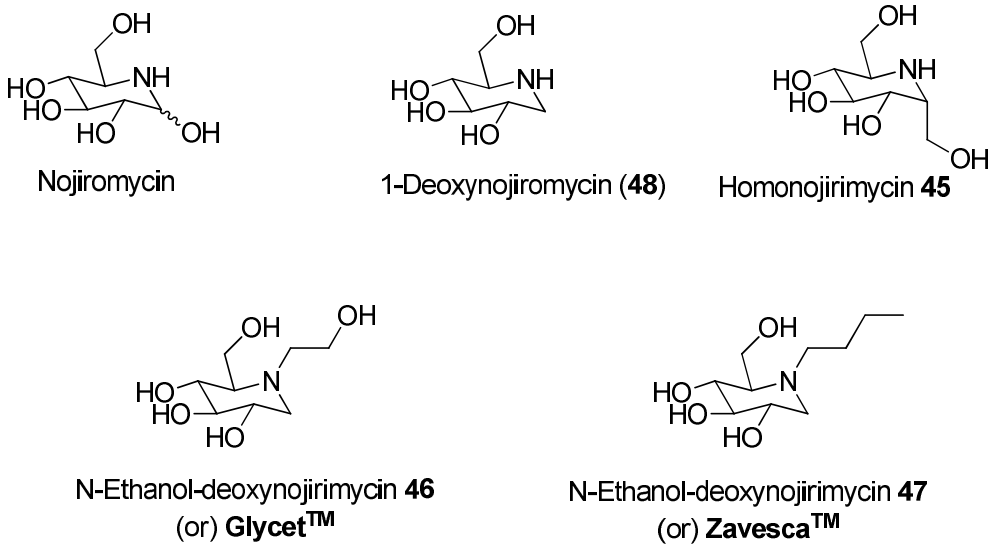


Figure 1.10A: Structures of Glycet (**46**) and Zavesca (**47**)

One of the major drawbacks associated with imino-analogues of glycosides is their instability caused by the lability of the hemiaminal or N,O-acetal function (Figure 1.10B) under enzymatic and acid hydrolysis conditions which limited their use as biological probes or drug candidates. By similarity with C-glycosides, the replacement of the glycosidic oxygen atom of the N,O-acetal function by a methylene group to form a stable C–C bond at C-1 has been a frequently used strategy to generate stable analogues of glycoconjugates. The first synthesis of iminosugar C-glycosides was performed by Bayer's chemists in the early 1980.[44]

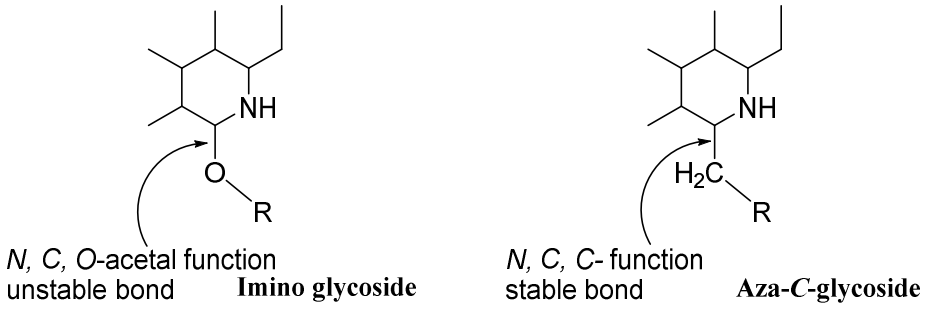
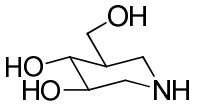
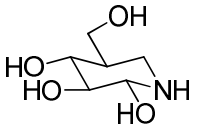
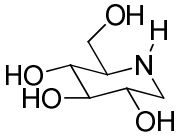
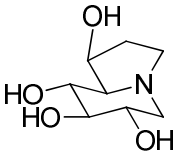
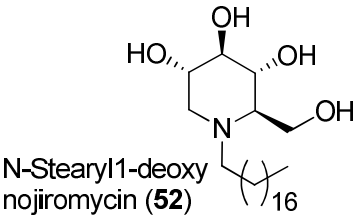
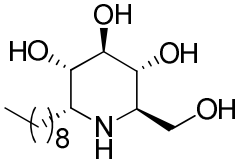
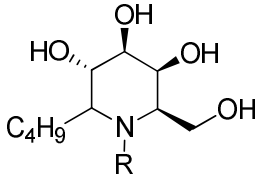


Figure 1.10B: Structural representation of imino glycoside and aza-*C*-glycoside

Azasugars showed potent inhibitory activities against a number of enzymes of medicinal interest including glycosidases.[45] Type-2 diabetes causing target is glycogen phosphorylase (GP), the main regulatory enzyme in the liver responsible for the control of blood glucose levels. The glucose derivatives used as active site inhibitors to control the action of GP. Aza-sugars like isofagomine **49** (IC_{50} = 0.7 mM) and neuromycin **50** (IC_{50} = 4 mM) also bind strongly to the active site of GP, however, substitution on the nitrogens makes the binding weaker.[46a] Glycoprotein gp120 binds to the CD4 antigen and transmembrane glycoprotein gp41 anchors the envelope to the viral membrane. These glycoproteins play key roles in the early stages of viral infection. Consequently, compounds which can interfere with correct glycoprotein glycosylation can prevent binding and penetration. In a Moloney murine leukemia virus assay which served as a model for HIV, it was reported that glucosidase inhibitors 1-deoxynojirimycin (**48**) and castanospermine (**51**) were active at concentrations of 1-2 μg/ml.[46b] N-stearyl-1-deoxynojirimycin (**52**) was found to be most potent binding to gp120 and its activity and specificity matched that of β-GalCer.[47] The inhibitory potency of α-1-C-alkyl-1-deoxynojirimycin (**53**) derivatives increased with the length of the alkyl chain and reached a dissociation constant of a complex Ki value of 200 nm for α-1-C-nonyl-1-deoxynojirimycin (**54**)

which can help in treatment of Gaucher's disease.[48] β-1-C-butyl-deoxygalactonojirimycin at 100 μm in culture medium of Fabry lymphoblast's increased the intracellular α-Gal activity.[49]

Table 1.1A: Structures of various azasugars and their activity

Structure of azasugars	Activity
 Isofogamine (**49**)  Neuromycin (**50**)	Type 2 diabetes Ref (46a)
 1-deoxynojirimycin (**48**)  Castanospermine (**51**)	Anti-HIV activity Ref (46b)
 N-Stearyl1-deoxy nojiromycin (**52**)	Anti-HIV activity Ref (47)
 **54**  β-1-C-butyl-deoxygalacto nojirimycin (**53**)	Gaucher and Fabry disease Ref (48-49)

1.11. Methodologies for the synthesis of azasugars:

Synthetic methodologies for the synthesis of stable aza-*C*-glycosides are shown in figure 1.11A wherein the key step involves C5-N cyclization. The intrinsic reactivity of amines is always an issue that has to be considered in a synthetic scheme, and the choice of a suitable protecting

group is often critical. The challenge is to develop specific synthetic strategies that cannot be applicable to classical *C*-glycosides. Reductive amination has been the most popular reaction for the synthesis of *C*-glycosides of azasugars to date. This chemistry allows the formation of the C5–N bond with the concomitant generation of one or two stereogenic centers. In addition, reductive amination is compatible with a broad array of functional groups. Double reductive amination of dicarbonyl sugars is theoretically the method of choice for the synthesis of iminosugar *C*-glycosides (Path a), other methods include mercuric diacetate mediated cyclizations (Path b), intramolecular nucleophilic substitution (Path c), and reductive amination reactions (Path d).[44]

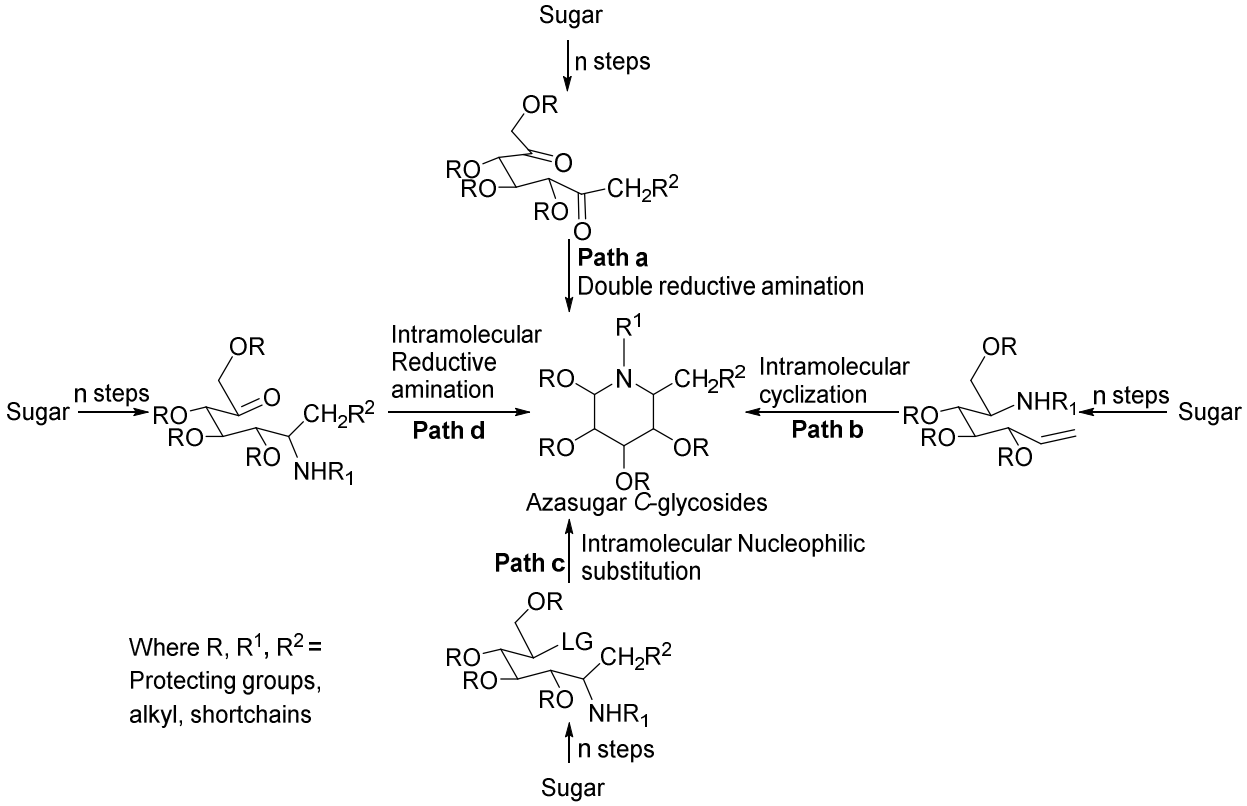


Figure 1.11A: The intramolecular cyclization approaches for the synthesis of azasugars

1.12. Conclusion and present work:

Among the glycosphingolipids (GSLs) the *β*-galactosylceramide has been explored for its anti-viral activity. The most significant and well known GSLs is *α*-*O*-galactosylceramide (KRN7000) is prone to have a wide range of biological activities due to its unique nature of secretion of Th1 and Th2 cytokine by activating iNKT cells. For a selective cytokine profile, KRN7000 and its structural variant analogues has been well explored for a broad range of biological activities against fungal pathogens, tuberculosis, malaria, inflammation, autoimmune diseases like lupus, diabetes mellitus, anti-tumor, anti-viral and adjuvant properties. Therefore, our interest was to bring a modification in KRN7000 by varying the ring oxygen with nitrogen atom to produce aza-sugar and also the glycosidic oxygen with carbon methylene group to generate azasugar analogues of KRN7000 bearing the *β*-configuration with an intention to screen for cytokine profile. In the present work we have developed multistep synthetic routes for the synthesis of GSLs, *β*-C-galactosylceramides and its new aza-variants. The synthetic strategies adopted for their synthesis were presented in Chapter 2 and 3.

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Chapter 2

Synthesis of aza-*β*-*C*-galactosylceramide

2.1. Abstract

In our initial attempt, we planned to synthesize of aza-*O*-GalCer as an aza-variant of KRN7000. The sugar portion with an N-Boc protected piperidin-2-ol **3** and phytosphingosine-1-ol **4** were coupled under glycosylation reaction which was performed using TMSOTf as the promoter in THF at -10 °C affording the desired glycosylated product **5**. Unfortunately, during deprotective conditions the glycosylated product **5** cleaved to afford back sugar **3** and lipid alcohol **4**, this result indicates the labile nature of hemiaminal ether linkage under acidic conditions (Figure 2.1A).

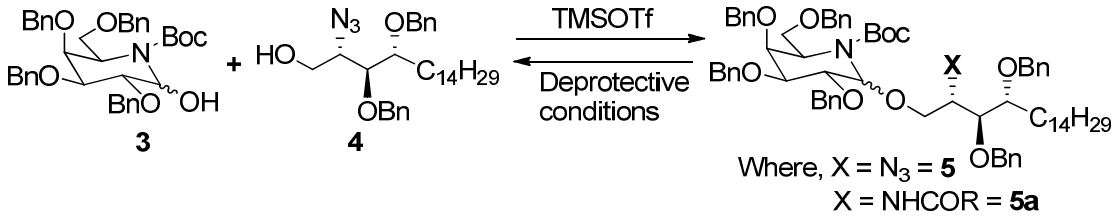


Figure 2.1A: Attempt towards synthesis of aza-*O*-GalCer

The labile nature of hemiaminal ether linkage was circumvented by combining the sugar and lipid moieties as a C-glycoside using HWE reaction. A general strategy towards the synthesis of *β*-C-glycosides is described using Horner-Wadsworth-Emmons reaction as the key step in combining the sugar and aglycone portions with C-glycosidic linkage. This strategy was successfully employed in total synthesis of *β*-C-galactosyl ceramide (GalCer **1**) and an unprecedented aza-variant of *β*-C-GalCer **2** (Figure 2.1B).

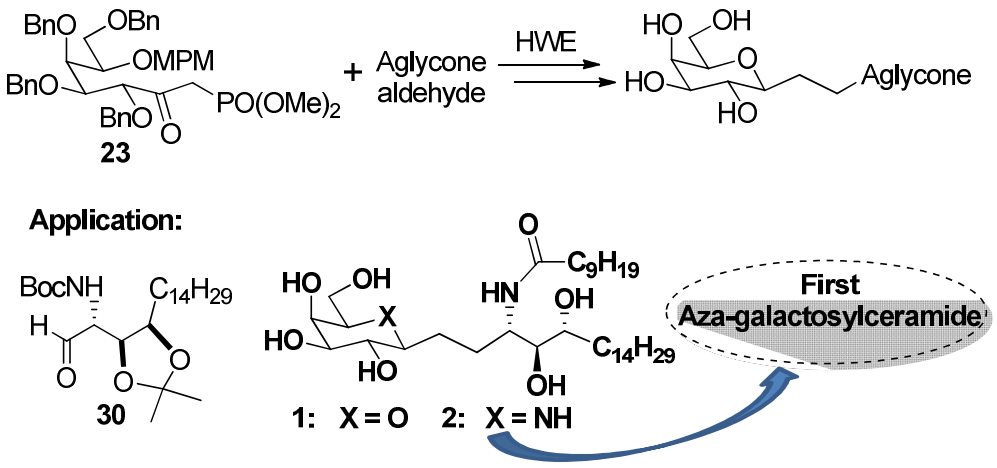


Figure 2.1B: Synthesis of *β-C*-GalCer (**1**) and aza-*β-C*-GalCer (**2**)

An unusual aromatization to perbenzylated 3,5-dihydroxy-6-(hydroxymethyl)-2-pyridone was demonstrated utilizing sugar lactam, under strong basic conditions, derived from D-Galactose. 2-Pyridone was efficiently converted to a pharmaceutically relevant 3,5-dihydroxy pyridine salt to demonstrate its synthetic utility. This result is the first report for 2-pyridone **40** synthesis from a carbohydrate, and also first of its kind for a novel 3,5-dihydroxypyridine derivatives **42a-g** (Figure 2.1C).

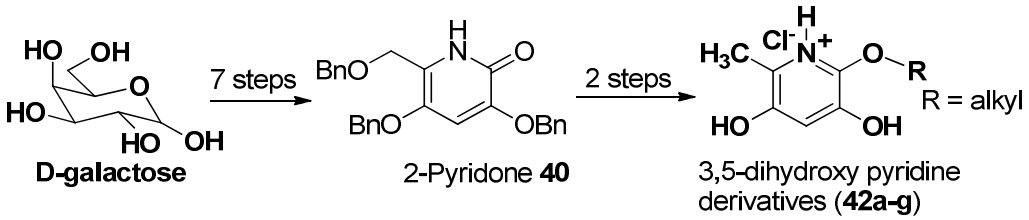


Figure 2.1C: Synthesis of 3,5-dihydroxypyridine derivatives from D-galactose

2.2. Introduction:

Mammalian glycosphingolipids (GSLs) are essential components of cellular membranes which play a critical role in a variety of biochemical functions.[1] A simple GSL comprises of a *β*-glycosidic linkage of a carbohydrate attached to the primary hydroxyl group of ceramide. The

carbohydrate portion is present in the outer leaflet of the plasma membrane serving as a receptor for various pathogens.[2] An important initial event behind HIV infection is interaction of cell surface expressed GSL galactosylceramide (GalCer) with V3 loop region of HIV gp120.[3] This and other findings enabled exploration of GalCer analogues, with *β*-anomeric configuration, as potential inhibitors for HIV infection.[4] In a different design for *β*-GalCer mimetics, derivatives with simplified aliphatic chains in place of ceramide and by replacement of galactose with 1-deoxynojirimycin have displayed a potent affinity towards gp120.[5] The C-glycoside analogues of *β*-GalCer were also synthesized, as these methylene isosteres renders chemical and enzymatic stability to the analogues.[6]

In a related family of GSLs KRN7000 a synthetic glycolipid derived from structure-activity studies on naturally occurring agelasphin discovered from marine sponge *Agelas mauritianus*, is well studied due to its potent immunomodulatory properties.[7] KRN7000 also referred as *α*-GalCer contains phytosphingosine base which upon complexation with an antigen presenting glycoprotein CD1d and subsequent complexation with an iNKT cell receptor results in the release of cytokines offering protection against several pathologies.[8] The C-glycoside analogue of *α*-GalCer displayed a remarkable immunostimulatory activity than the corresponding O-glycoside.[9] Postema et. al. reported the first synthesis of *β*-C-GalCer analogue which exhibited anti-solid tumor activity.[10]

A careful scrutiny of the reported *β*-GalCer analogues, *vide supra*, led us to design a piperidine aza-sugar coupled to phytosphingosine derived ceramide. As aza-sugar C-glycosides offer promising biological and therapeutic properties,[11] we expeditiously set our goal towards development of a methodology for synthesis of aza-*β*-C-GalCer (**2**, Figure 2.1B). In this pursuit, we report a general strategy for synthesis of *β*-C-glycosides in a convergent manner using the

Horner-Wadsworth-Emmons (HWE) reaction of *β*-keto phosphonate **23** (Scheme 2.4.1) derived from D-galactose and aglycone aldehyde (Figure 2.1B). Reports towards synthesis of *β*-C-GalCer for the most part relied on the stereoselective synthesis of the sphingosine backbone starting from a functionalized carbohydrate at the anomeric position.[10,12] Attempts concerning application of HWE methodology for C-glycoside synthesis have involved the presence of *β*-keto phosphonate in the aglycone portion.[13] In the present work, the HWE strategy was applied towards total synthesis of *β*-C-GalCer **1** and its variant aza-*β*-C-GalCer **2** from a common intermediate **32** (Scheme 2.4.4) derived from HWE reaction using phytosphingosine-1-al **30** (Scheme 2.4.3) as the aglycone component. The present work offers a new entry to the repertoire of GSLs with a piperidine aza-sugar which could serve as a potential inhibitor for HIV infection, and which may also have potential immunogenic properties.

In our endeavor to synthesize aza-sugars involving D-Galactose, a key intermediate lactam **14** (Scheme 2.5.1) was envisaged to undergo E2 eliminations under strong basic conditions. Surprisingly, treatment of lactam **14** with potassium hydroxide under reflux conditions resulted in a stable aromatized structure 2-pyridone **40 (**Scheme 2.5.2**)**. Thus, in the present work we have developed a new method for an expedient synthesis of polyhydroxylated 2-pyridone **40**, from a carbohydrate as the starting material and utilizing it we have also synthesized a novel 3,5-dihydroxypyridine derivatives **42a-g** (Scheme 2.5.3).

2.3. Results and Discussion:

In our initial attempt, we sought a direct synthesis of aza-*O*-GalCer by extending the precedented O-glycosidation of phytosphingosine by a galactose donor to the N-Boc azagalactose analog **5** (Figure 2.3A).The sugar portion with an N-Boc protected piperidin-2-ol **3** was synthesized from

D-galactose in nine steps,[14] and phytosphingosine-1-ol **4** from phytosphingosine in four steps.[15] The glycosylation reaction was performed using TMSOTf as the promoter in THF at -10 °C which afforded the desired glycosylated product **5**. Unfortunately, during deprotective conditions the glycosylated product cleaved to individual starting materials as evident by TLC as well as mass spectral data of the crude reaction mixture (Figure 2.3.4A). This result demonstrated the labile nature of aza-O-GalCer **5a**.

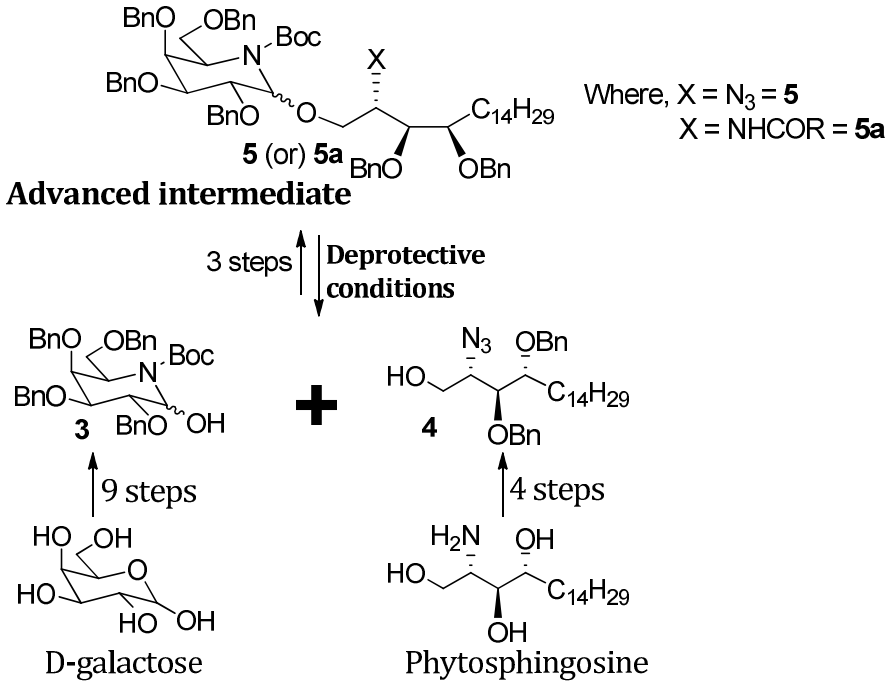
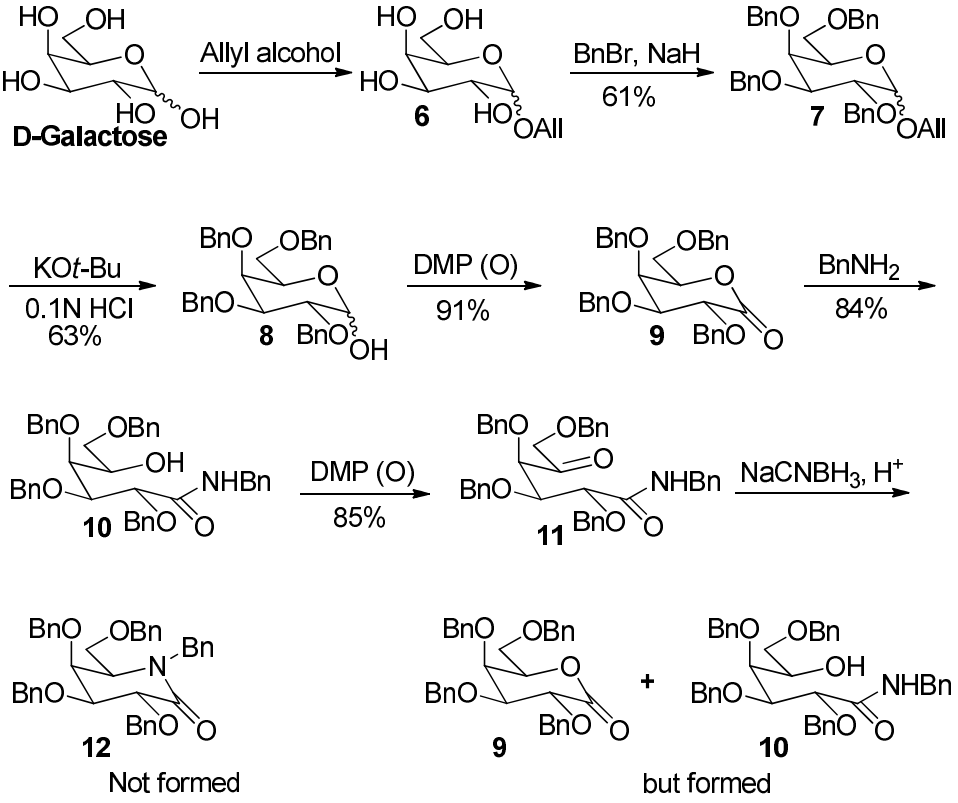


Figure 2.3A: Synthesis of advanced intermediate aza-*O*-GalCer

2.3.1. Initial attempt for the synthesis of aza-sugar by reductive amination:

Initially the anomeric hydroxyl group of D-galactose was selectively protected with allyl group to afford 1-*O*-allyl- D-galactose (**6**), which was then converted to compound **7** using benzylbromide in presence of NaH. The allyl group was removed by treating compound **7** with KO*t*-Bu under reflux followed by treatment with 0.1N HCl resulting in a lactol **8** which was subsequently

treated with Dess-Martin periodinane to afford lactone **9**. Ring opening of lactone **9** with benzylamine yielded 5-hydroxy-N-benzyl carboxamide (**10**). Compound **10** was oxidized into keto-carboxamide **11** which was then subjected to reductive amination conditions. Surprisingly, the expected product N-benzyl lactam **12** didn't form due to the reduction of keto group followed by intramolecular cyclization gave rise to the starting materials **9** and **10** (Scheme 2.1).

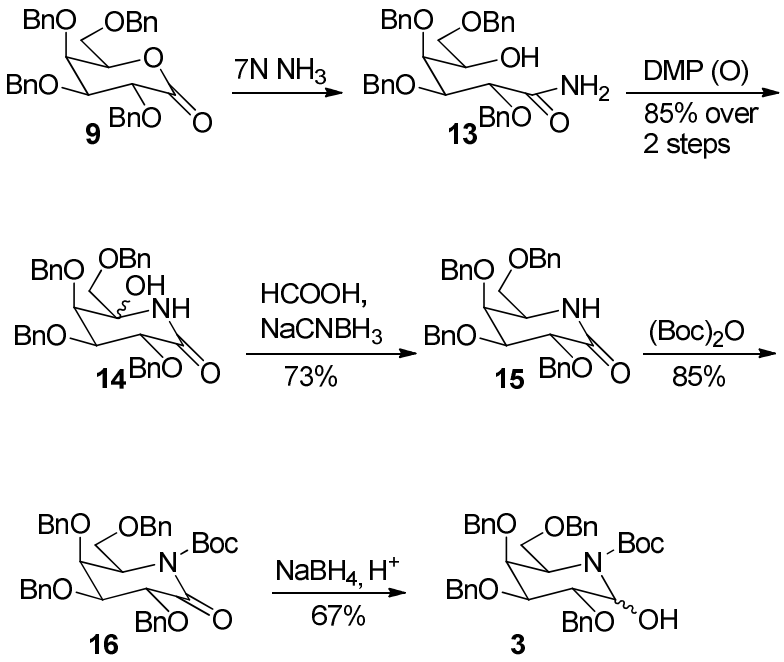


Scheme 2.1: Attempted synthetic route for lactam (**12**) from D-galactose

2.3.2. Synthesis of aza-sugar (3) via N-Boc protection:

Aza-sugar (**3**) synthesis commenced from lactone **9** which was treated with 7N NH_3 in methanol affording hydroxy-carboxamide **13**. Oxidation of compound **13** under Dess-Martin periodinane

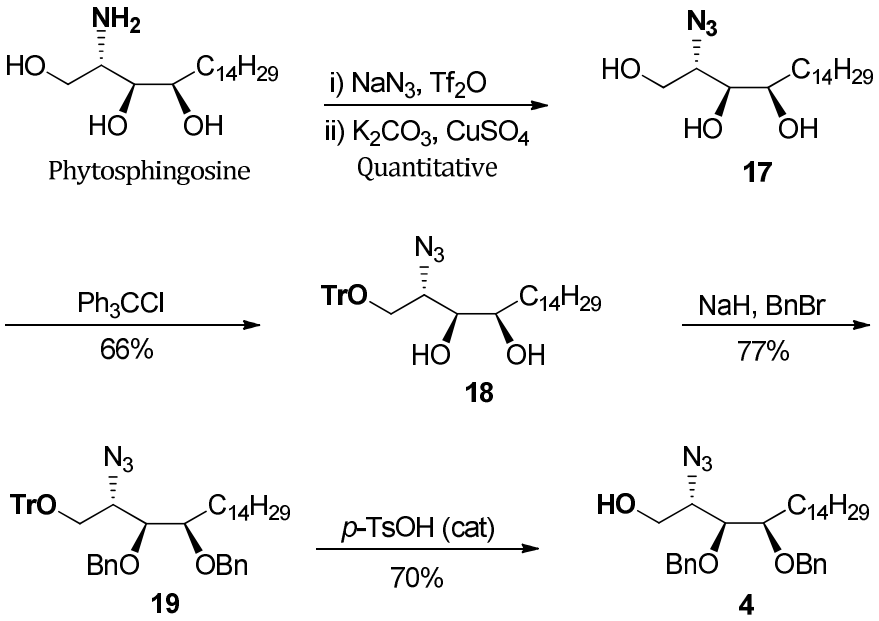
conditions resulted in lactam epimers (**14**) which was treated under reductive amination conditions yielding lactam **15**. Subsequent protection with $(Boc)_2O$ afforded N-Boc protected lactam (**16**), followed by reduction with $NaBH_4$ yielded aza-sugar **3** (Scheme 2.2)



Scheme 2.2: Synthesis of aza-sugar (**3**) from lactone (**9**)

2.3.3. Synthesis of phytosphingosine-1-ol (4) from phytosphingosine:

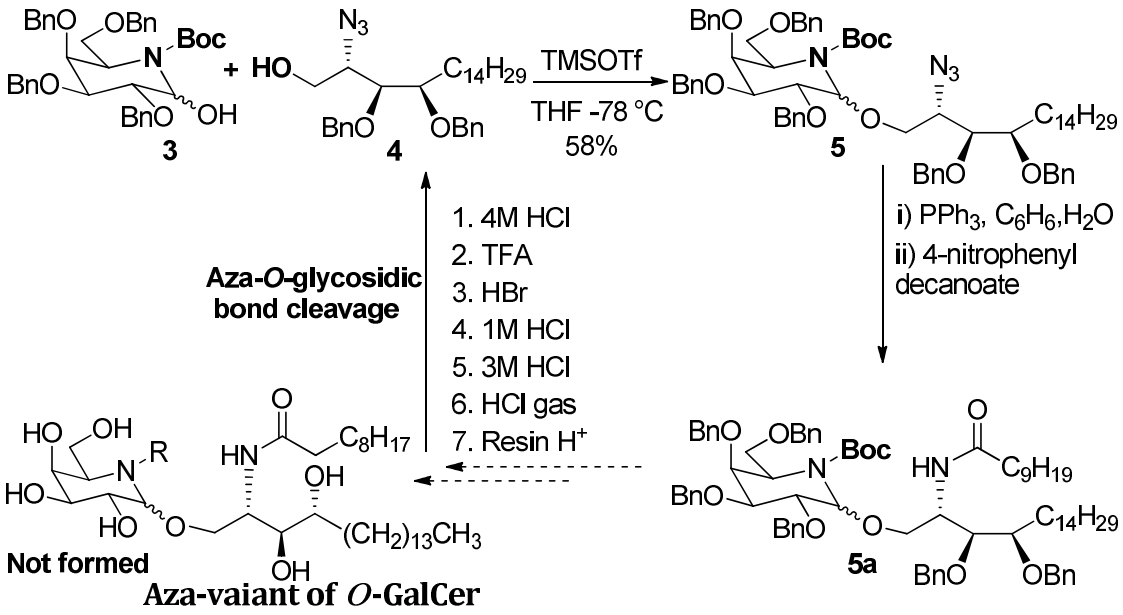
Synthesis of phytosphingosine-1-ol (**4**) commenced from commercially available phytosphingosine which was initially treated with *in situ* generated TfN_3 giving rise to azido phytosphingosine (**17**). The primary alcohol selectively blocked with trityl group to afford compound **18**, subsequent protection of secondary alcohols with benzyl group afforded compound **19**. Removal of trityl group removal from compound **19** using *p*-TsOH afforded phytosphingosine-1-ol **4** (Scheme 2.3).



Scheme 2.3: Synthesis of phytosphingosine-1-ol (**4**) from phytosphingosine

2.3.4. Synthesis of advanced intermediate aza-O-galactosylceramide (5a):

Compounds **3** and **4** were successfully coupled under the glycosylation conditions using TMSOTf as promoter to afford the glycosylated product **5**. Staudinger reaction of compound 5 afforded the amine which was then subsequently reacted with *p*-nitrophenyl ester in presence of K_2CO_3 to afforded aza-*O*-galactosylceramide **5a** (Scheme 2.3.4). The hemiaminal ether glycosidic link cleavage in advanced intermediate (**5**) was proven by HRMS analysis (Figure 2.3.4A). However, final deprotection of Boc and benzyl groups under different conditions as shown in scheme 2.4 led to the cleavage of the hemiaminal ether linkage giving back the starting materials aza-sugar (**3**) and lipid moiety (**4**). This result demonstrated the labile nature of **5** and aza-*O*-GalCer **5a;** therefore, we focused our attempts towards synthesis of C-glycoside of the aza-sugar (Figure 2.3.5).



Scheme 2.3.4: Synthesis of advanced intermediate aza-*O*-GalCer (**5a**)

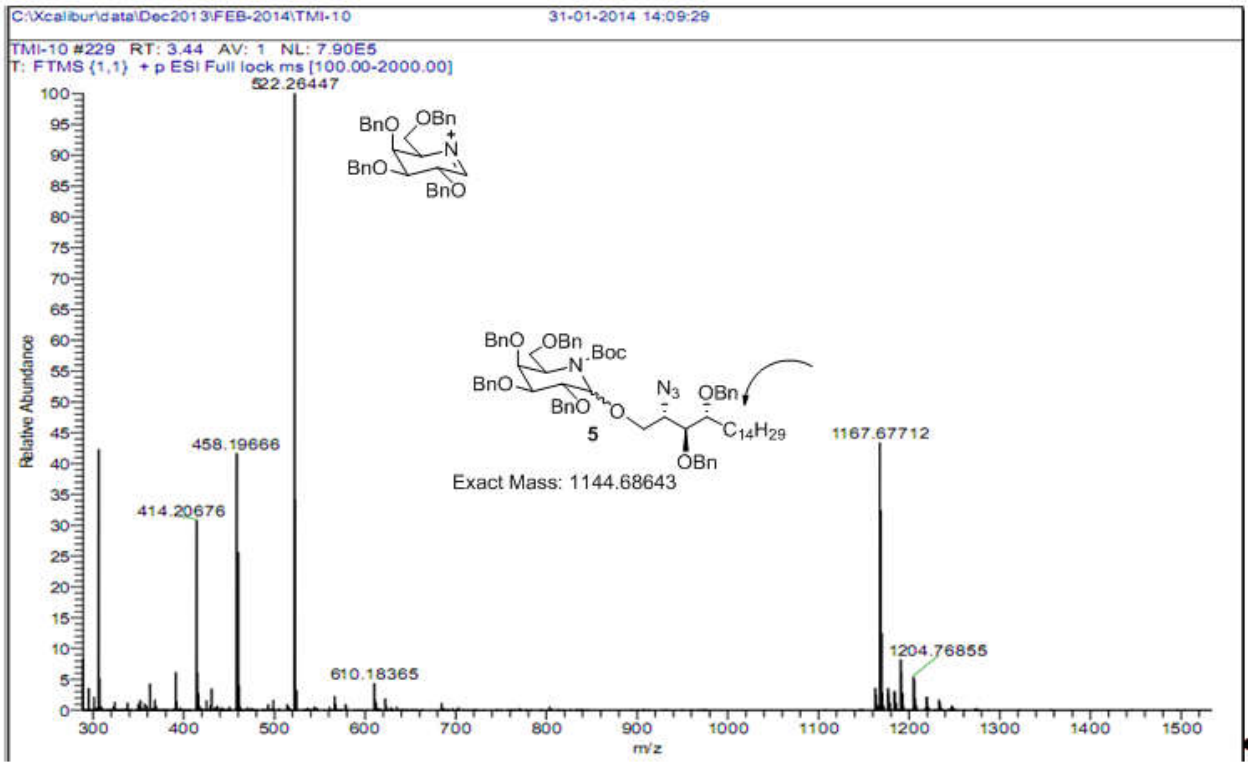


Figure 2.3.4A: Mass spectra of hemiaminal ether link cleavage in advanced intermediate

2.3.5. Modified design target aza-C-galactosylceramide (2):

To circumvent the problem of labile nature of hemiaminal ether linkage, the glycosidic oxygen was replaced with methylene group to generate the C-C bond between sugar and lipid moieties (Figure 2.3.5). C-C bond makes the molecule stable towards acid and enzymatic hydrolysis.

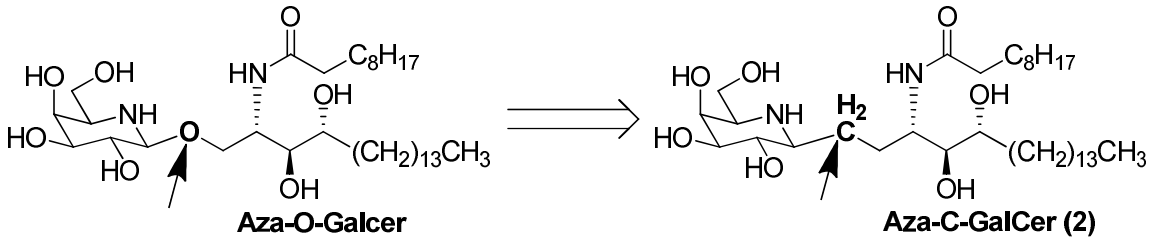
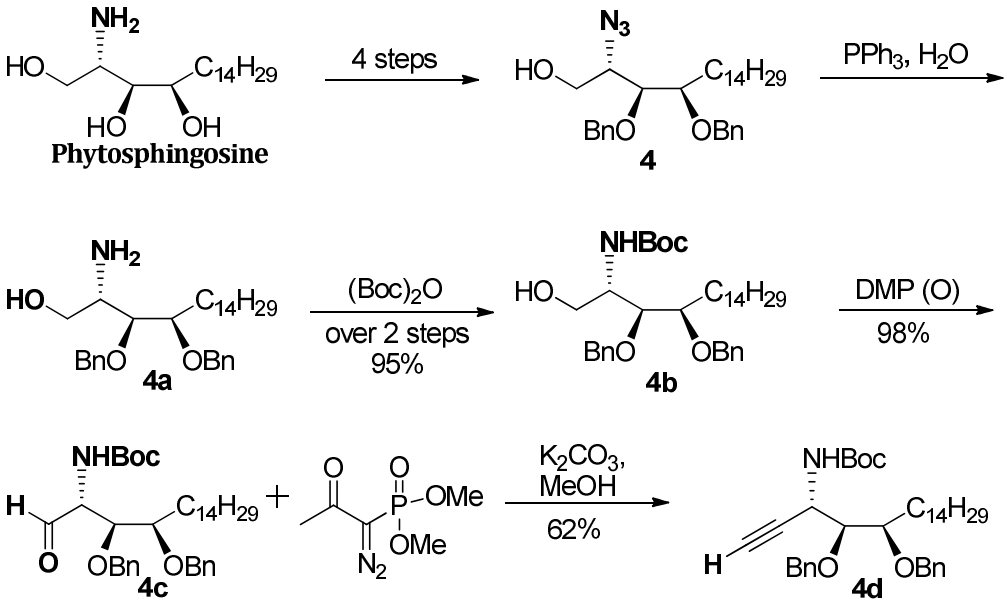


Figure 2.3.5: Designed target aza-*C*-galactosylceramide

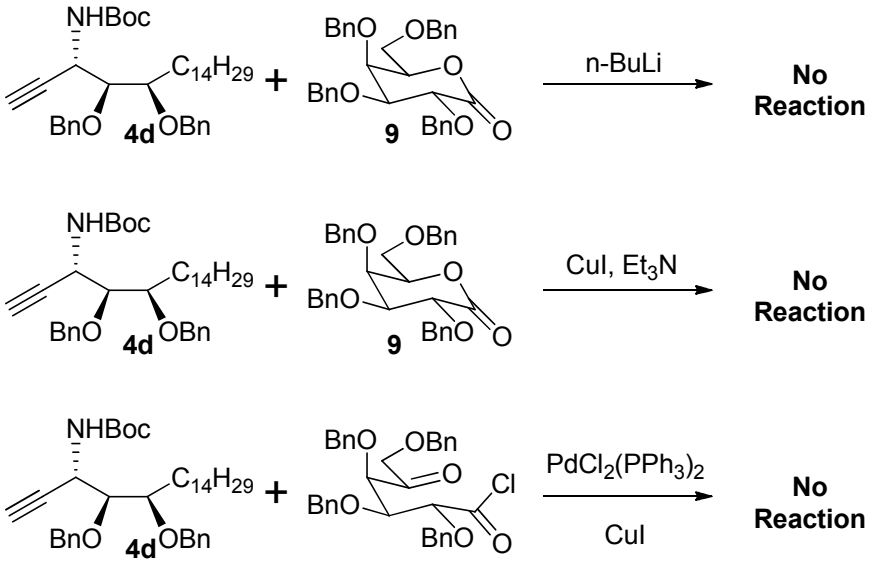
2.3.6. Attempts towards synthesis of *β*-C-GalCer via alkyne addition and coupling reactions:

The synthesis of alkyne **4d** (Scheme 2.3.6) commenced from a commercially available phytosphingosine which was converted to azido alcohol **4** in four steps (Scheme 2.3). The azide was treated under Staudinger reaction conditions to afford amine **4a** which was subsequently protected with Boc group. Oxidation of primary alcohol **4b** under Dess-Martin periodinane conditions afforded aldehyde **4c**. Aldehyde **4c** was subjected to Seyferth-Gilbert homologation reaction using Ohira-Bestmann reagent in presence of K_2CO_3 in methanol yielding alkyne (**4d**) in 62% yield (Scheme 2.3.6).



Scheme 2.3.6: Synthesis of alkyne **4d** from phytosphingosine

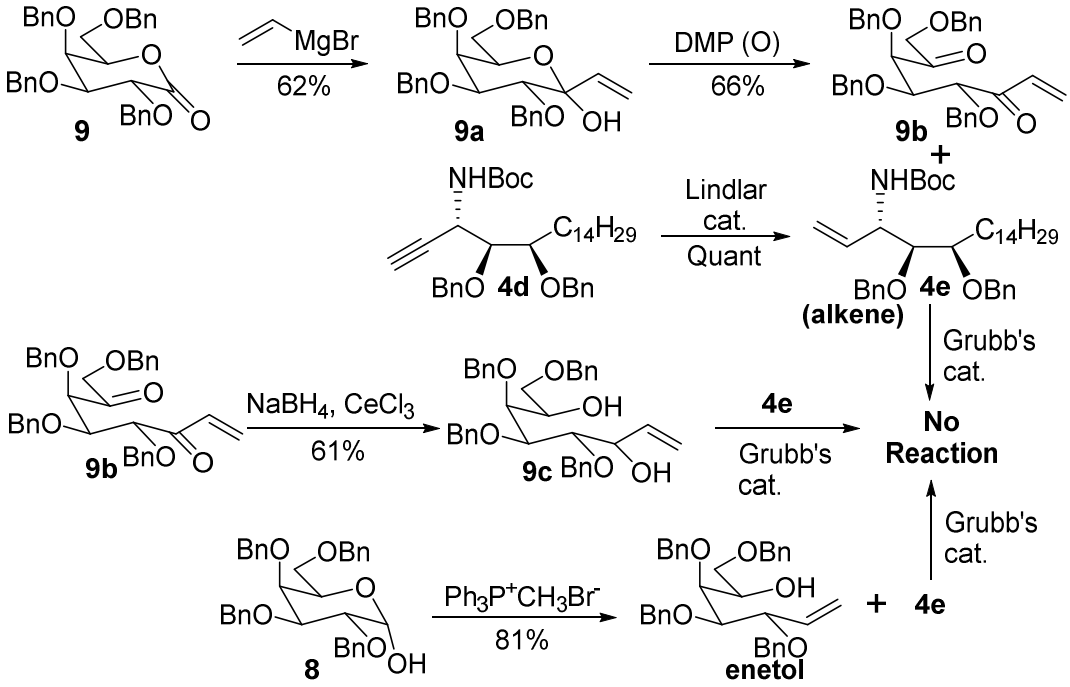
Alkyne **4d** was treated with *n*-BuLi in THF and then lactone **9** was added to accomplish C-C bond formation but there was no reaction. Changing the reaction conditions for the same step under metal mediated coupling reaction conditions also could not afford the C-C bond formation (Scheme 2.3.6A).



Scheme 2.3.6A: Unsuccessful attempts via addition and coupling reactions

2.3.7. Attempts towards C-C formation bond via cross metathesis reaction:

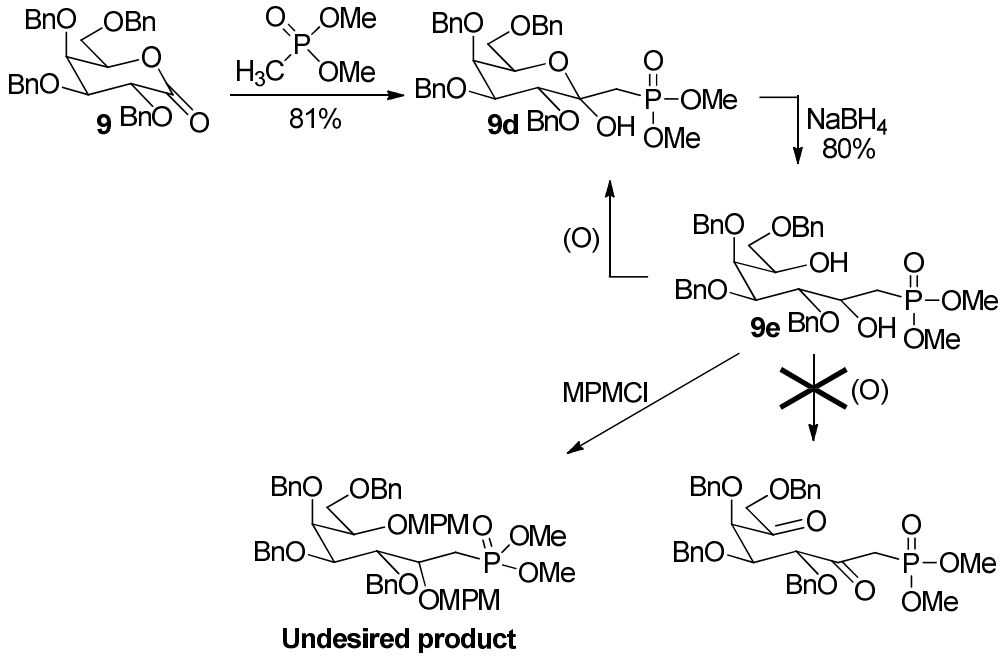
As the alkyne chemistry didn't work well in connecting the sugar and lipid moieties, thus, we shifted towards olefin cross metathesis reaction. Alkene **9b** (Scheme 2.3.7) was obtained from lactone **9** in two steps *viz.* a) treatment with vinyl magnesium bromide to afford addition product **9a**, and b) Dess-Martin periodinane (DMP) oxidation to α,β-unsaturated diketone (**9b**). Lipid alkene **4e** was obtained by reducing alkyne **4d** in presence of the Lindlar catalyst (Scheme 2.3.7). The two alkene partners, α,β-unsaturated diketone (**9b**) and lipid alkene **4e**, were treated under Grubb's cross metathesis reaction conditions which resulted in no reaction. The α,β-unsaturated diketone (**9b**) was reduced to allylic alcohol **9c** under Luche reduction conditions, and then tried reacting with lipid alkene **4e** under Grubb's cross metathesis reaction conditions which afforded no reaction. Our final cross metathesis attempt between simple enetol and lipid alkene **4e** also resulted in no reaction (Scheme 2.3.7).



Scheme 2.3.7: Unsuccessful attempts via cross metathesis reaction

2.3.8. Attempt for the synthesis of *β*-keto phosphonate:

Synthesis of beta-keto phosphonate was attempted with an aim to connect both the sugar and aglycone moieties under Horner-Wadsworth-Emmons (HWE) reaction conditions. Initially, treatment of lactone **9** with dimethyl methylphosphonate resulted in an addition product **9d** in 81% yield, subsequent reduction afforded diol **9e** (Scheme 2.3.8). However, Dess-Martin periodinane oxidation of compound **9e** gave back the starting material **9d**. Finally, we have attempted protection strategy on diol using *p*-methoxybenzyl chloride which afforded the undesired double MPM protected product (Scheme 2.3.8).



Scheme 2.3.8: Attempts towards synthesis of *β*-keto phosphonate

Horner-Wadsworth-Emmons (HWE):

The reaction of aldehydes (or) ketones with stabilized phosphonate carbanions lead to olefin bond formation. The HWE reaction mechanism is depicted below (Figure 2.3.8A).

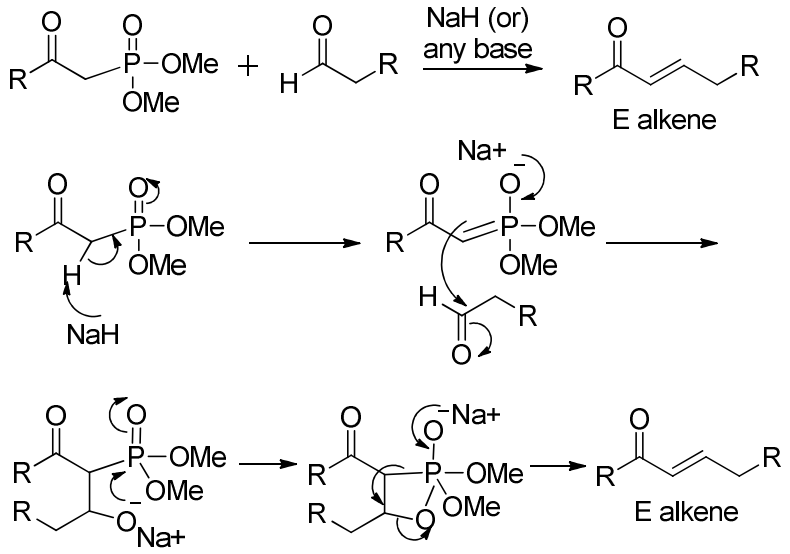


Figure 2.3.8A: Mechanism of Horner-Wadsworth-Emmons (HWE) reaction

2.4. Successful synthesis of *β*-C-galactosylceramide (1) and its new aza-variant (2) via Horner-Wadsworth-Emmons (HWE) reaction:

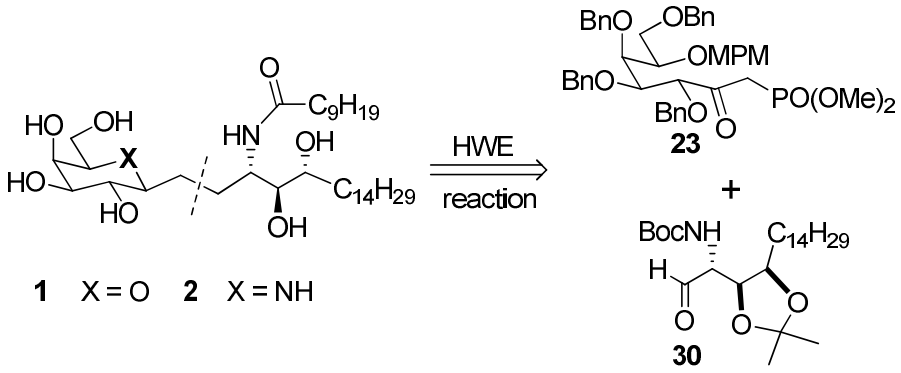
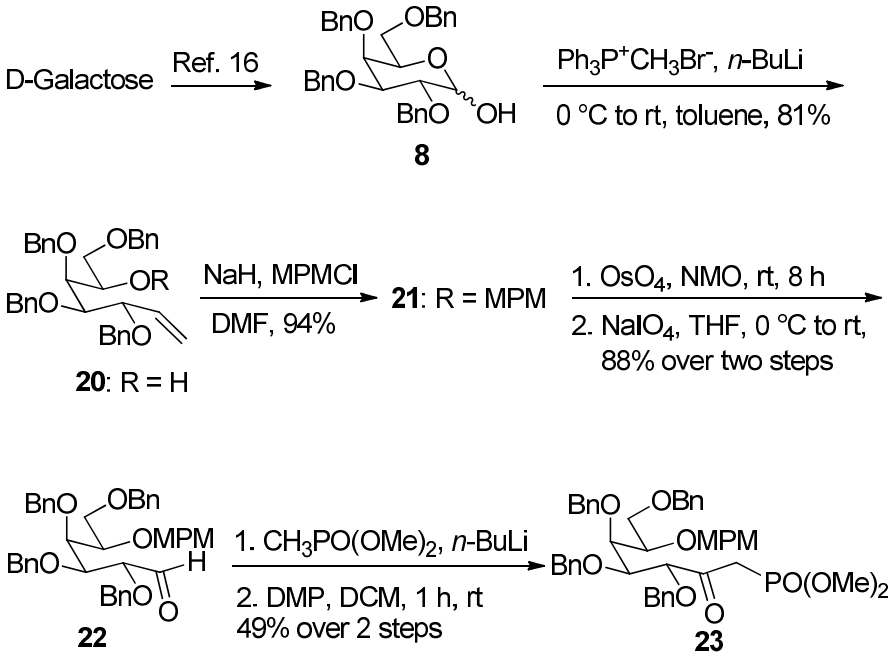


Figure: 2.4A. Retrosynthetic analysis for *β-C*-galactosylceramide and its new aza-variant

Synthesis of HWE precursor *β*-keto phosphonate **23** (Scheme 2.4.1) commenced from lactol **8** (Scheme 2.1), which was prepared in three steps from D-galactose.[16] Witting reaction of lactol **8** in dry THF led to the formation of diene product with elimination of C-3 benzyl group, however, changing the solvent to dry toluene produced the desired alkene **20** in 81% yield. Protection of 2° alcohol with MPM group afforded alkene **21** in 94% yield. Osmylation in presence of

stoichiometric oxidant NMO, followed by oxidative cleavage of the diol product using aqueous $NaIO_4$ in THF produced aldehyde **22** in 88% yield over two steps. Nucleophilic addition of the anion generated from dimethyl methylphosphonate and *n*-BuLi to aldehyde **22** afforded the 2° alcohol which was subsequently oxidized using Dess-Martin periodinane giving rise to galactosyl *β*-keto phosphonate **23** in 49% yield over 2 steps (Scheme 2.4.1).

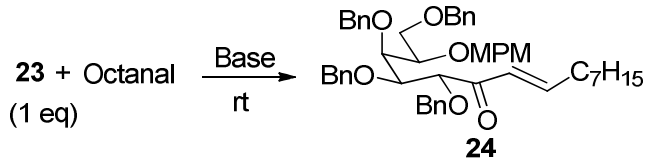


Scheme 2.4.1: Synthesis of *β*-keto phosphonate **23** from D-galactose

In an attempt to develop a generalized HWE methodology for *β*-C-glycosides, *β*-keto phosphonate **23** was subjected to HWE conditions with octanal by variation of base and solvent. In the entire trials (Table 2.1) compound **23** was pretreated with base for 30 minutes before addition of octanal. The initial attempt with NaH in THF afforded the HWE product **24** in 15% yield (entry 1). Usage of an organic base, DIPEA, gave rise to trace amount of product (entry 2). However, with K_2CO_3 in EtOH the reaction was complete within 3 hours affording the product in 28% yield (entry 3). There was a slight improvement in the yield with Cs_2CO_3 to 41% (entry

4). Interestingly, using $Ba(OH)_2.8H_2O$ (1.25 eq) the reaction was complete overnight with an improved yield of 65%. Finally, with *t*-BuOK (4 eq) the HWE product was formed in 47% yield.

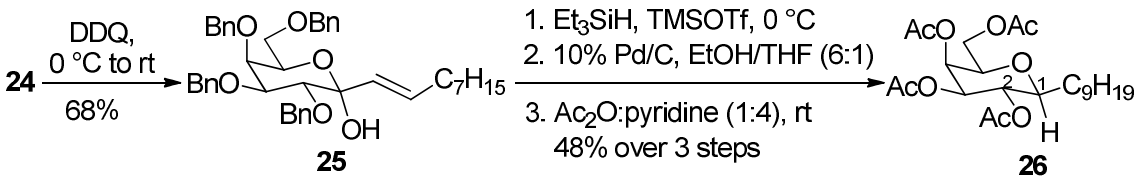
Table 2.1: Horner-Wadsworth-Emmons reaction optimization conditions



Entry	Base (eq)	Octanal (eq)	Solvent	Time (h)	Yield (%)[a]
1	NaH (2)	2	THF	Overnight	15
2	DIPEA (2)	3	CH_3CN	Overnight	Trace
3	K_2CO_3 (1.8)	3	EtOH	3	28
4	Cs_2CO_3 (2.1)	3	IPA	6	41
5	$Ba(OH)_2.8H_2O$ (1.25)	2	THF	Overnight	65
6	*t*-BuOK (4)	4	THF	Overnight	47

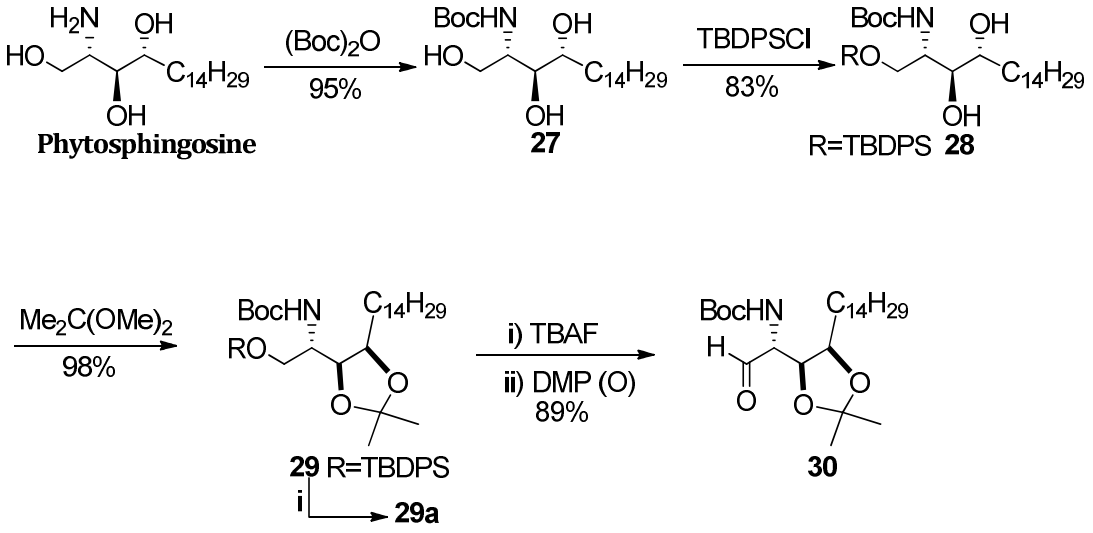
[a] Isolated yields

α,β-unsaturated ketone **24** was subjected to deprotective conditions, DDQ in DCM, to produce the hemiketal **25** (Scheme 2.4.2). The 3° hydroxy group was removed by reduction using Et_3SiH and TMSOTf, followed by alkene reduction and debenzylation using 10% Pd/C under H_2, and finally the resulting product was subjected to acetylation affording galactosyl-*β*-*C*-glycoside **26** in 48% yield over 3 steps (Scheme 2.4.2). Presence of COSY correlation between H-1 at δ_H3.36 (m, 1H) and H-2 at δ_H 5.08 (app t, J = 9.5 Hz, 1H) and trans-coupling constant helped to determine the *β*-configuration of C-glycoside **26** (see experimental section).



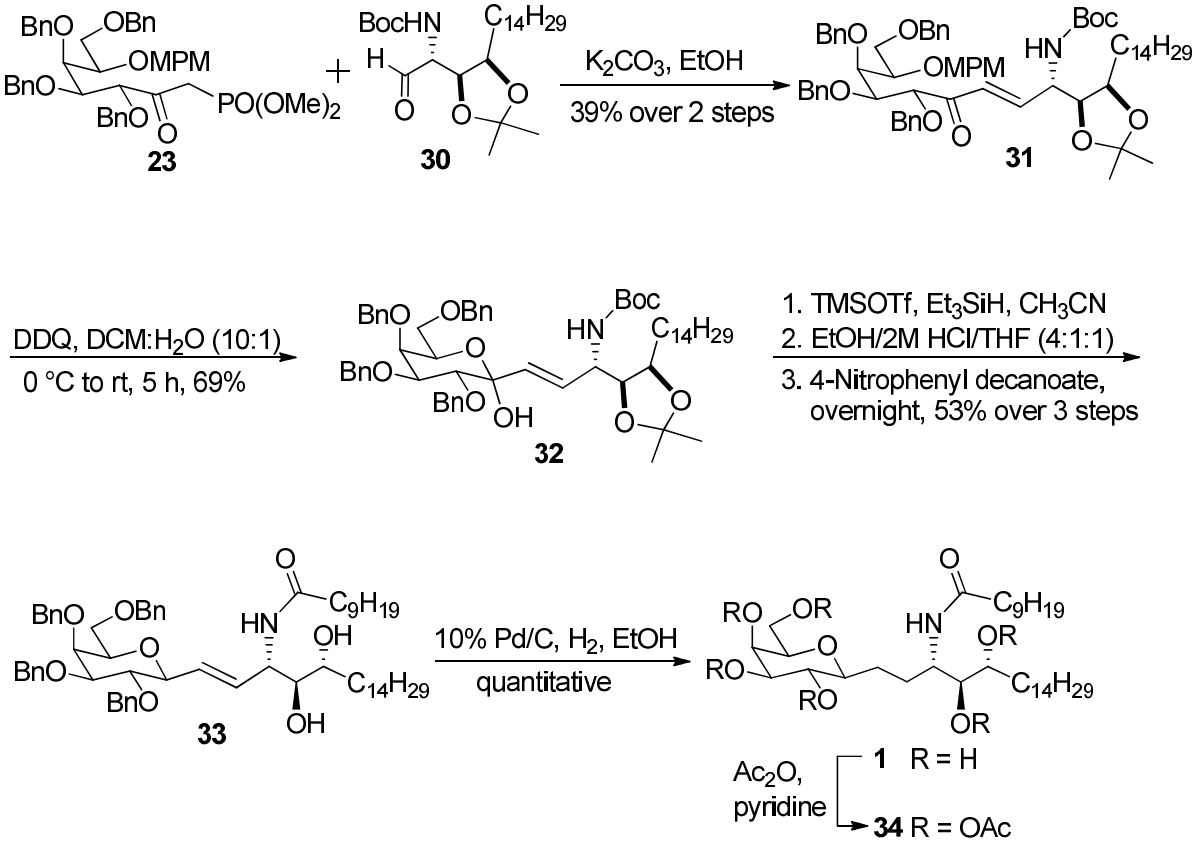
Scheme 2.4.2: Synthesis of *β*-C-glycoside **26**

Successful synthesis of *β*-C-glycoside **26** from keto-phosphonate **23** and octanal utilizing HWE reaction encouraged us to apply this generalized methodology towards total synthesis of biologically relevant structures *β*-C-GalCer **1** and its unprecedented variant aza-*β*-C-GalCer **2** from a common intermediate (**32**). In a convergent approach, firstly, the HWE aldehyde precursor of ceramide was synthesized from commercially available phytosphingosine following a known procedure to afford the protected phytosphingosine-1-ol **29a** (Scheme 2.4.3).[17]Alcohol **29a** was oxidized using Dess-Martin periodinane in presence of excess $NaHCO_3$ which resulted in phytosphingosine-1-al **30**.



Scheme 2.4.3: Synthesis of phytosphingosine-1-al (**30**) from phytosphingosine

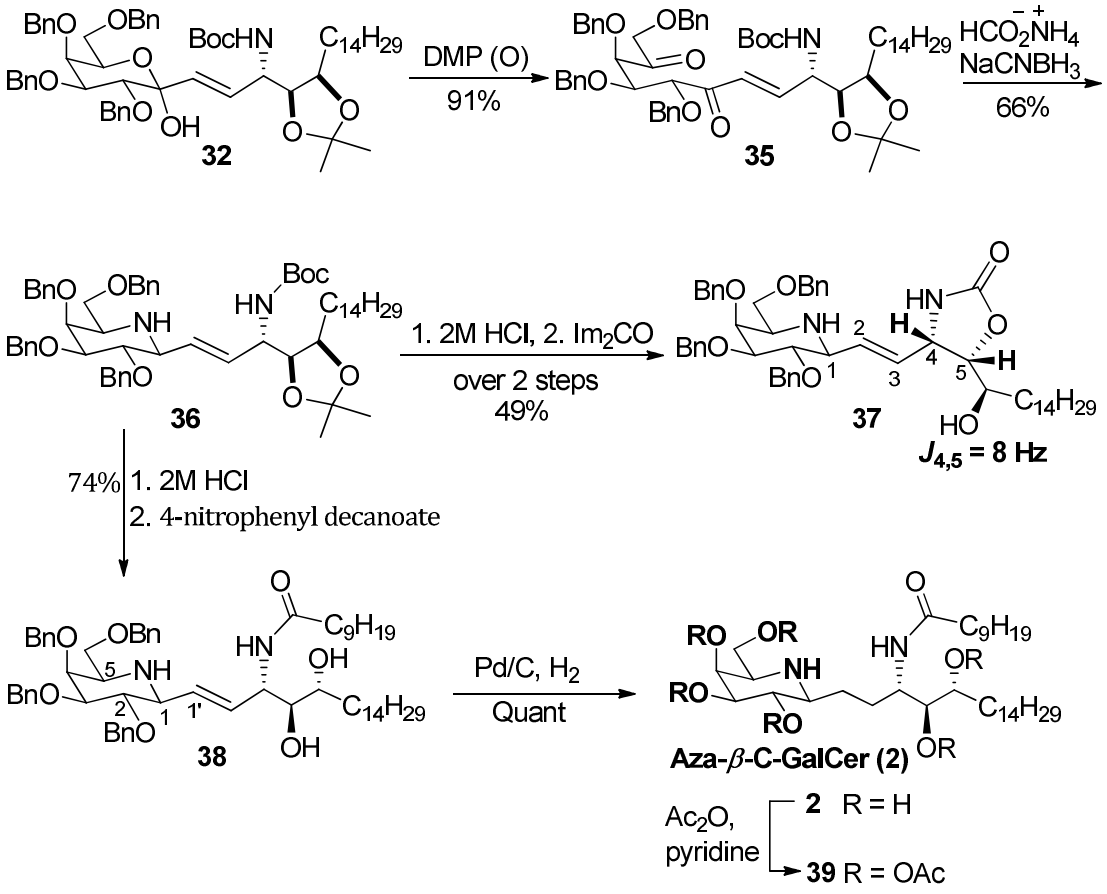
The initial HWE reaction between aldehyde **30** and β-keto phosphonate **23** using $Ba(OH)_2.8H_2O$ afforded the HWE adduct **31** in a poor yield. However, a change of base to K_2CO_3 afforded the desired product **31** in 39% yield over two steps. MPM deprotection using DDQ in DCM/H_2O (10:1) formed the hemiketal product **32** in 69% yield. Removal of 3° hydroxyl group under reductive conditions, deprotection of Boc and isopropylidene groups under acidic conditions, and the resulting free amine was subjected to amidation using *p*-nitrophenyl decanoate to afford the advanced intermediate **33** in 53% yield over three steps. Finally, debenzylation using 10% Pd/C under H_2 afforded *β*-C-GalCer **1** in a quantitative yield which was subjected to global acetylation affording the peracetylated *β*-C-GalCer **34**. In this context, the present work offers an alternative route to *β*-C-GalCer **1** (Scheme 2.4.4) prepared by Postema et. al.[10]



Scheme 2.4.4: Synthesis of *β*-C-GalCer **1**

Synthesis of aza-*β*-C-GalCer **2** was envisaged by utilizing the standard double reductive amination conditions usually employed for piperidine aza-D-sugar synthesis.[18] Initially, the hemiketal product **32** was oxidized with Dess-Martin periodinane which afforded diketone **35** (Scheme 2.4.5) in 91% yield. Double reductive amination reaction facilitated facile cyclization to form piperidine aza-D-sugar **36** in 66% yield. To address the concern of stereochemical integrity of C-2 stereocenter of the phytosphingosine aldehyde **30** under HWE reaction conditions, compound **36** was converted into an oxazolidinone **37**. Comparison of the H-4 and H-5 coupling constant value ($J_{4,5}$ = 8 Hz) with literature confirms that under HWE conditions the C-2 stereocenter did not undergo epimerization.[6,12] Dondoni et. al. reported epimerization of Garner aldehyde under Wittig reaction conditions using *n*-BuLi,[19] whereas, Compostella et. al. reported no such epimerization under HWE reaction conditions using a milder base such as K_2CO_3 even while stirring for 18 h.[12] Nevertheless, the present HWE reaction using K_2CO_3 facilitated consumption of phytosphingosine-1-al **30** within one hour. Deprotection of Boc and isopropylidene groups of compound **37** in EtOH: 2M HCl (4:1) at 70 °C produced the free amine which was subjected to amidation using *p*-nitrophenyl decanoate to afford ceramide **38** (Scheme 2.4.5) in 74% yield over two steps. Surprisingly, during the later amidation conditions only the sphingosine amine was affected leaving the piperidine aza-sugar unaltered, perhaps, due to steric hindrance. The indifference of 2° amine of piperidine aza-sugar towards amide bond formation was further substantiated by a previous report for N-benzoylation utilizing Grignard reagent for deprotonation.[20] Finally, global debenzylation with 10% Pd/C under H_2 afforded aza-*β*-C-GalCer **2** in a quantitative yield which was treated with Ac_2O/pyridine to produce the peracetylated aza-*β*-C-GalCer **39** (Scheme 2.4.5). Presence of COSY correlation in compound **39** between H-1 at δ_H3.14 (dd, *J* = 9, 7 Hz) with H-1' at δ_H 5.72 (dd, *J* = 16, 7 Hz) and H-2 at δ_H 3.63(app t, *J* = 9

Hz) helped to confirm the β-configuration by trans-coupling constant (see experimental section). Furthermore, presence of H-5 δ_H 2.85 which is upfield to H-1 indicates that H-1 and H-5 are neighboring to NH of the sugar. Presence of three COSY correlations for H-5 with δ_H 3.33, 3.48, and 3.95 further confirms the identity of H-5 in the sugar. This COSY analysis confirms amidation of sphingosine backbone leaving the aza-portion of the sugar unaffected.



Scheme 2.4.5: Synthesis of aza-β-C-GalCer **2**

2.5. Synthesis of 3,5-dihydroxy pyridine and its derivatives from D-galactose:

Among the heteroaromatic rings 2-pyridone plays a pivotal role as a privileged scaffold in drug design due to its inherent amide functionality which serves as a peptidomimetic backbone. Several derivatives of 2-pyridone are associated with diverse biological activities.[21-27] 2-Pyridone is a core structure in the well-known anticancer agent camptothecin (Figure 2.5A) isolated from Chinese plant *Camptotheca acuminate*.[28] Structural modification of camptothecin resulted in derivatives irinotecan and topotecan which are used in treating colorectal and ovarian cancer.[29] To date, several synthetic strategies have been developed to construct 2-pyridone scaffold.[24, 30-34] An equally important scaffold is 3-hydroxypyridine which is present in diverse bioactive substances like pyridoxin (vitamin B6), orellanine,[35] fumonisin[36] (Figure 2.5A). Few of the recent significant contributions involving development of novel methods in 3-hydroxypyridine synthesis includes usage of *α,α*-dicyanoalkenes in a hetero Diels-Alder reaction with 1-aza-dienes,[37] from *δ*-diazo oxime ethers using dirhodium complex.[38]

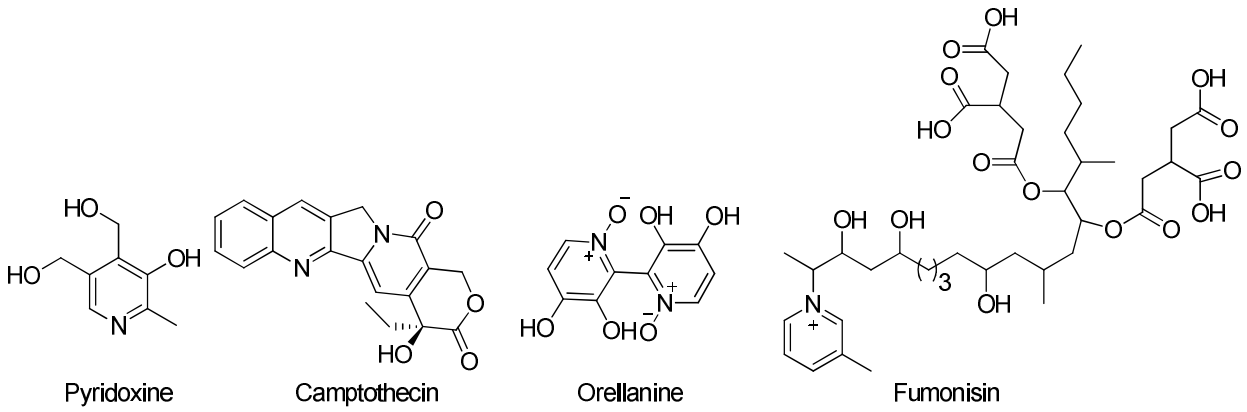
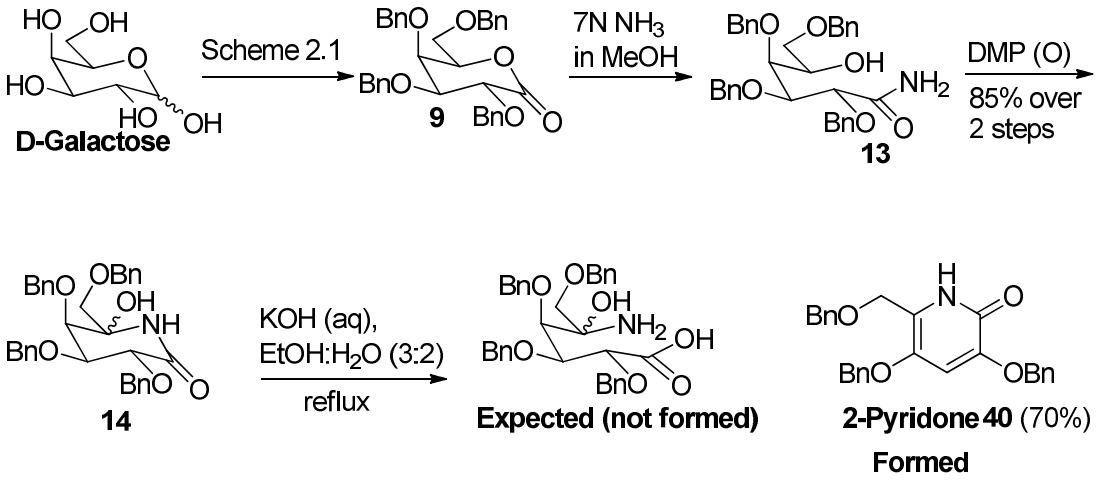


Figure 2.5A: Bioactive natural substances containing 3-hydroxy pyridine rings

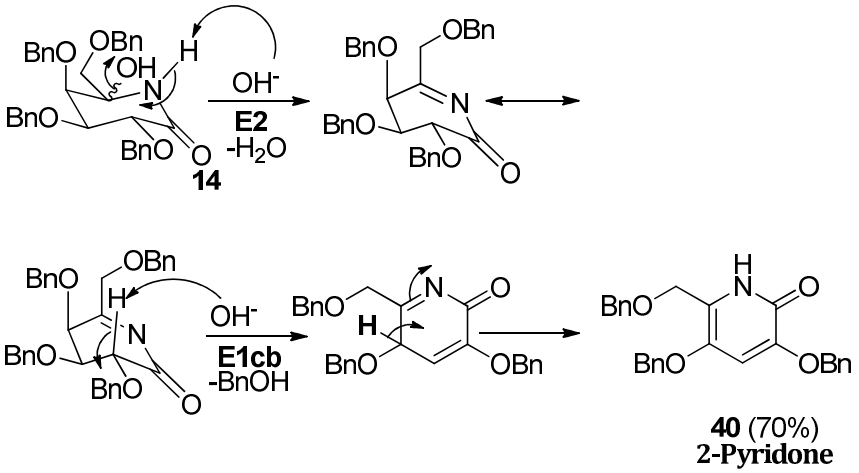
2.5.1: Synthesis of 2-pyridone (40) from D-galactose:

D-Galactose was initially treated with allyl alcohol in presence of an acidic resin and the resulting 1-*O*-allyl galactose **6** was perbenzylated using benzyl bromide to afford protected D-galactose **7** (Scheme 2.1). Deprotection of allyl group using *t*-BuOK afforded lactol **8** which was converted to lactone **9** (Scheme 2.1) under Dess-Martin periodinane conditions. Lactone **9** was conveniently converted to lactam **14** (Scheme 2.5.1) using conditions described by Overkleeft *et al*.[39] Treatment of lactone **9** with 7 N NH_3 in MeOH afforded the ring-opening product **13** which was oxidized without purification under Dess-Martin conditions to afford the ketone which instantaneously cyclizes to an epimeric mixture of lactam **14**. Lactam epimers **14** were then treated under reflux in presence of potassium hydroxide to afford 2-pyridone **40** (Scheme 2.5.1).



Scheme 2.5.1: Synthesis of 2-pyridone **40** from D-galactose

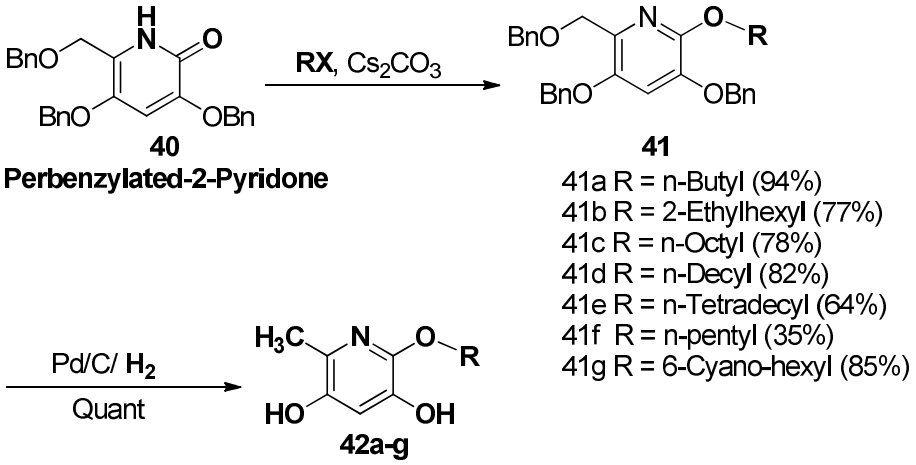
The mechanistic rationale behind 2-pyridone formation involves E2, E1cb eliminations (Scheme 2.5.2). Initial E2 elimination reaction affords dihydro-2-pyridone which undergoes facile E1cb elimination due to the presence of C3 α-acidic proton and also owing to attainment of ring stability driven by aromatization providing 2-pyridone (**40**).



Scheme 2.5.2: Mechanism of 2-pyridone (**40**) formation from lactam epimers (**14**)

A clear distinction can be observed in the ^{1}H-NMR spectra by comparison of lactam epimers **14** with complex signal pattern to reduced complexity in 2-pyridone **40** (See experimental section). The appearance of benzylic protons as distinct singlets in **40** is indicative of the flat structure of the molecule. To demonstrate the synthetic utility of 2-pyridone **40**, it was converted to a pyridine derivative conveniently resulting in 2-alkoxy-3,5-dihydroxy pyridine salt **42** (Scheme 2.5.3). Treatment of **40** with butyl bromide in presence of cesium carbonate afforded 2-*O*-alkylated product **41a** as verified from ^{13}C NMR which indicates disappearance of carbonyl peak at δ157 ppm in compound **40**. This result is in contrast to the thermodynamically more stable *N*-substituted pyridone product.[40] Attempts to remove benzyl protective groups under Pd/H_2 in ethanol resulted in a complex mixture with no trace of the expected pyridine derivative. Surprisingly, when the same reaction was conducted by addition of a drop conc. HCl it resulted in cleavage of benzyl protections along with a loss of the primary hydroxyl group to afford 3,5-dihydroxypyridine salt **42a**. This is the first report towards synthesis of 3,5-dihydroxypyridine providing access to a second hydroxyl group to the well known privileged structural motif 3-hydroxypyridine. To demonstrate the substrate scope of this transformation different alkyl

halides were converted to pyridine derivatives **41b-g** in moderate to good yields. Subsequently, they were converted to 3,5 dihydroxypyridine salts **42b-g** in a quantitative yield.



Scheme 2.5.3: Synthesis of 3,5-dihydroxypyridine derivatives (**42a-g**) from 2-pyridine (**40**)

2.6. Conclusion:

In summary, we have attempted the synthesis of aza-O-GalCer and demonstrated the labile nature of hemiaminal ether linkage present in aza-O-GalCer. To conquer the stability issues pertaining to the aza-O-GalCer we modified the design to aza-C-GalCer. We have successfully demonstrated Horner-Wadsworth-Emmons reaction in *β*-C-glycoside synthesis using *β*-keto phosphonate generated from a sugar and an aglycone aldehyde. This methodology was successfully employed in total synthesis of biologically relevant stable glycosphingolipids, *β*-C-galactosylceramide **1** (GalCer) and its unprecedented variant aza-*β*-C-GalCer (**2**). In the light of importance of *β*-GalCer derivatives as potential inhibitors for HIV infection as well as possessing immunogenic properties, aza-*β*-C-GalCer marks a novel entry in the glycolipid related therapeutic approaches. To the best of our knowledge, this is the first report for glycosphingolipid with a piperidine aza-sugar. Aza-substitution of a sugar also opens a new diversity element which can be further functionalized to prepare a library of therapeutically

relevant molecules. Currently β-C-GalCer and its new aza-variant derivatives are being screened for their immunomodulatory activity.

We have also developed a new route demonstrating synthesis of pyridine derivatives (**42a-g**) from a carbohydrate. This is also the first report typifying synthesis of 3,5-dihydroxypyridine. Among the derivatives the neutral form of 2-O-tetradeayl 3,5-dihydroxy pyridine (**42e**) showed good inhibition against MMP2 and MMP9 enzymes which are implicated in cancer.[41]

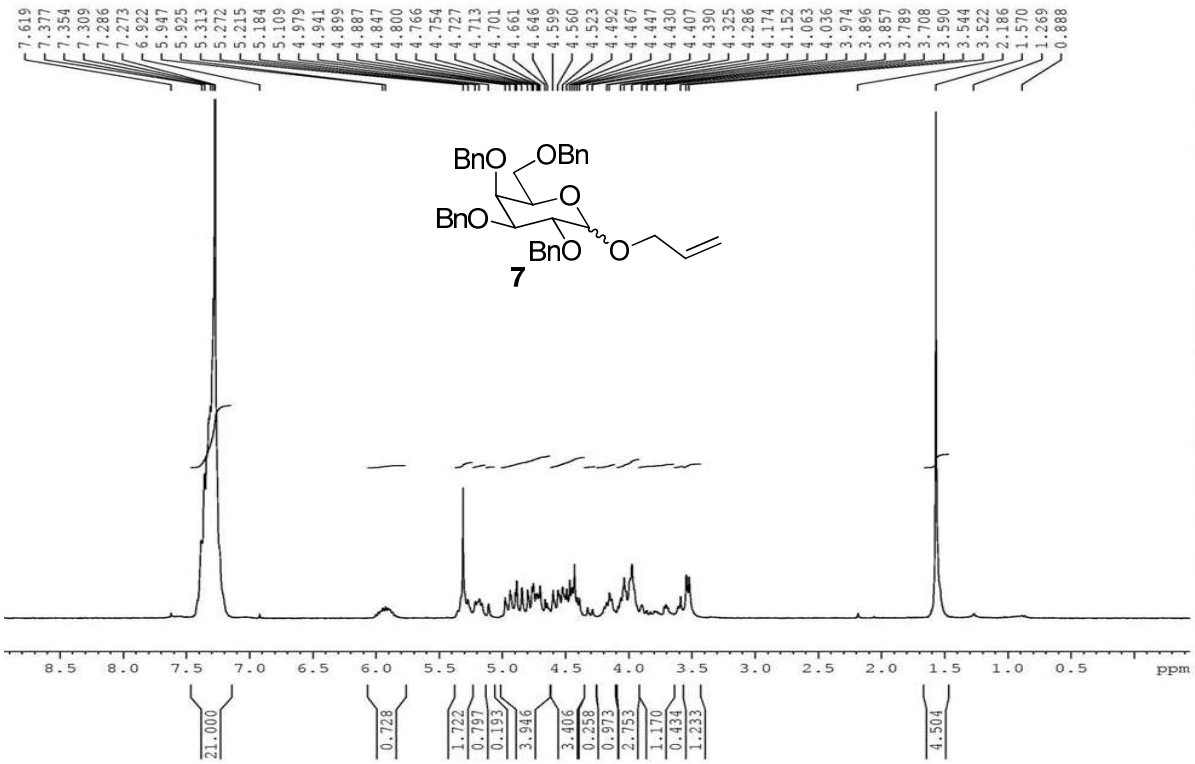
2.7. Experimental Section:

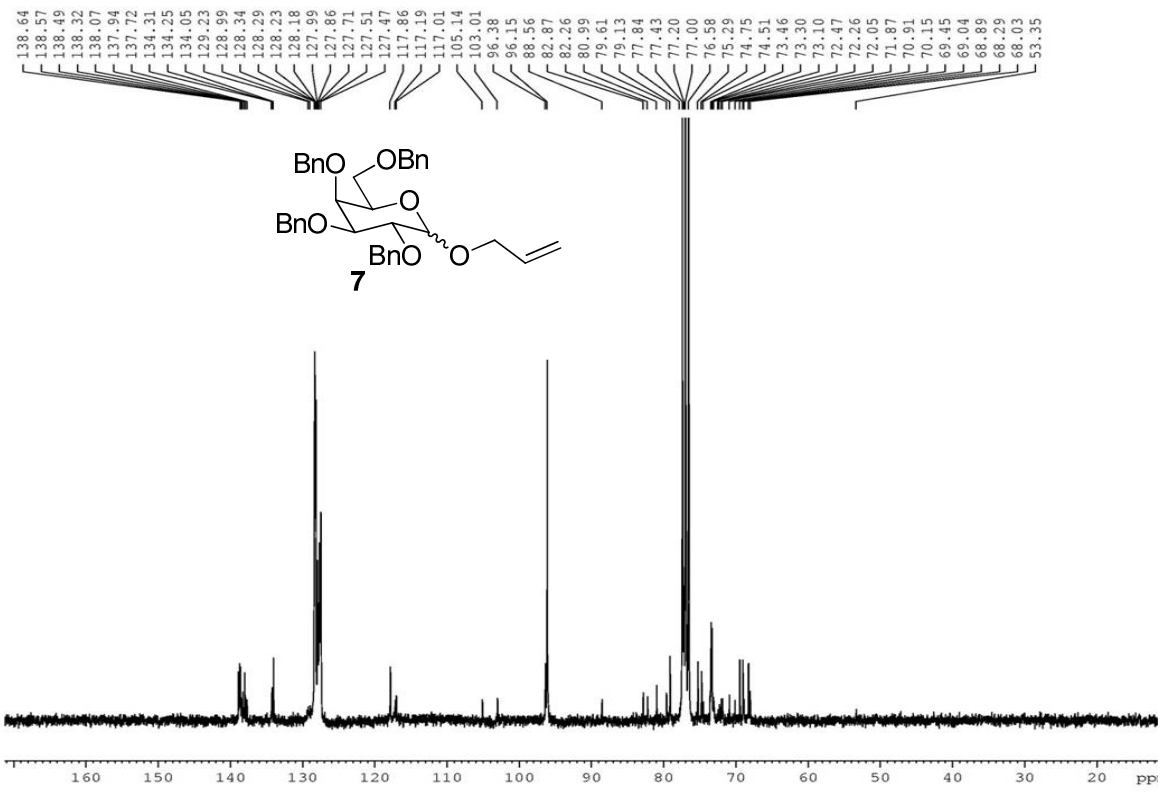
The solvents were dried as follows: THF and toluene were heated at reflux over sodium, CH_3CN and CH_2Cl_2 were distilled over calcium hydride, toluene was dried over molecular sieves, EtOH was dried over magnesium turnings, and EtOAc was dried over K_2CO_3. All reactions were carried out under argon atmosphere using oven dried glassware. Silica gel G-60 F_{254} aluminum TLC plates were used to monitor the reactions with short wavelength ultraviolet light to visualize the spots, and by charring the TLC plate after spraying with 15% sulfuric acid. Flash column chromatography was performed on silica gel 120-200 and 230-400 mesh. ^{1}H NMR spectra were recorded at 500 MHz, chemical shifts are given in parts per million and coupling constant in hertz. ^{13}C NMR spectra were recorded at 125 MHz. HR-ESI-MS analysis was performed on a Thermo Scientific Exactive Mass Spectrometer with ions given in m/z.

α,β-Allyloxy-2,3,4,6-tetra-*O*-benzyl-D-galactose (7). To a suspension of D-galactose (8.1 g, 45 mmol) in allyl alcohol **6** (65 mL), Dowex 50 x 8 (H^+) (3 g) was added and the heterogeneous reaction mixture was heated to reflux. After 6 h, the reaction mixture became homogenous and was filtered. The filtrate was diluted with MeOH (100 mL), concentrated and applied vacuum. The crude residue was dissolved in DMF (100 mL) and at 0 °C NaH 60% suspension in mineral oil (14.4 g, 360 mmol) was added in one portion and the reaction mixture was stirred for 30 min.

At the same temperature benzyl bromide (32.1 mL, 270 mmol) was added dropwise and let the reaction mixture attain room temperature slowly. After 7 h, to the reaction mixture ice water (250 mL) was added at 0 °C slowly and extracted with ethyl acetate (3 x 100 mL), dried (Na_2SO_4) and concentrated. Purification by flash chromatography (hexane/EtOAc 95:5) afforded compound **7** (16 g, 61% two steps) as brown viscous solid. R_f 0.5 (hexane/EtOAc 2:1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.54-3.91 (m, 3H), 3.99-4.34 (m, 4H), 4.42-4.61 (m, 4H), 4.66-4.78 (m, 4H), 4.81-4.99 (m, 2H), 5.13-5.35 (m, 2H), 5.94 (m, 1H), 7.27-7.37 (m, 20H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 68.2, 69.0, 69.4, 73.3, 73.4, 75.2, 76.5, 79.3, 96.15, 96.3, 117.8, 127.5, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 134.0, 137.7, 137.9, 138.3, 138.5, 138.6; FAB $[M + Na]^+$ m/z 604.58

^{1}H and ^{13}C-NMR of compound (**7**)

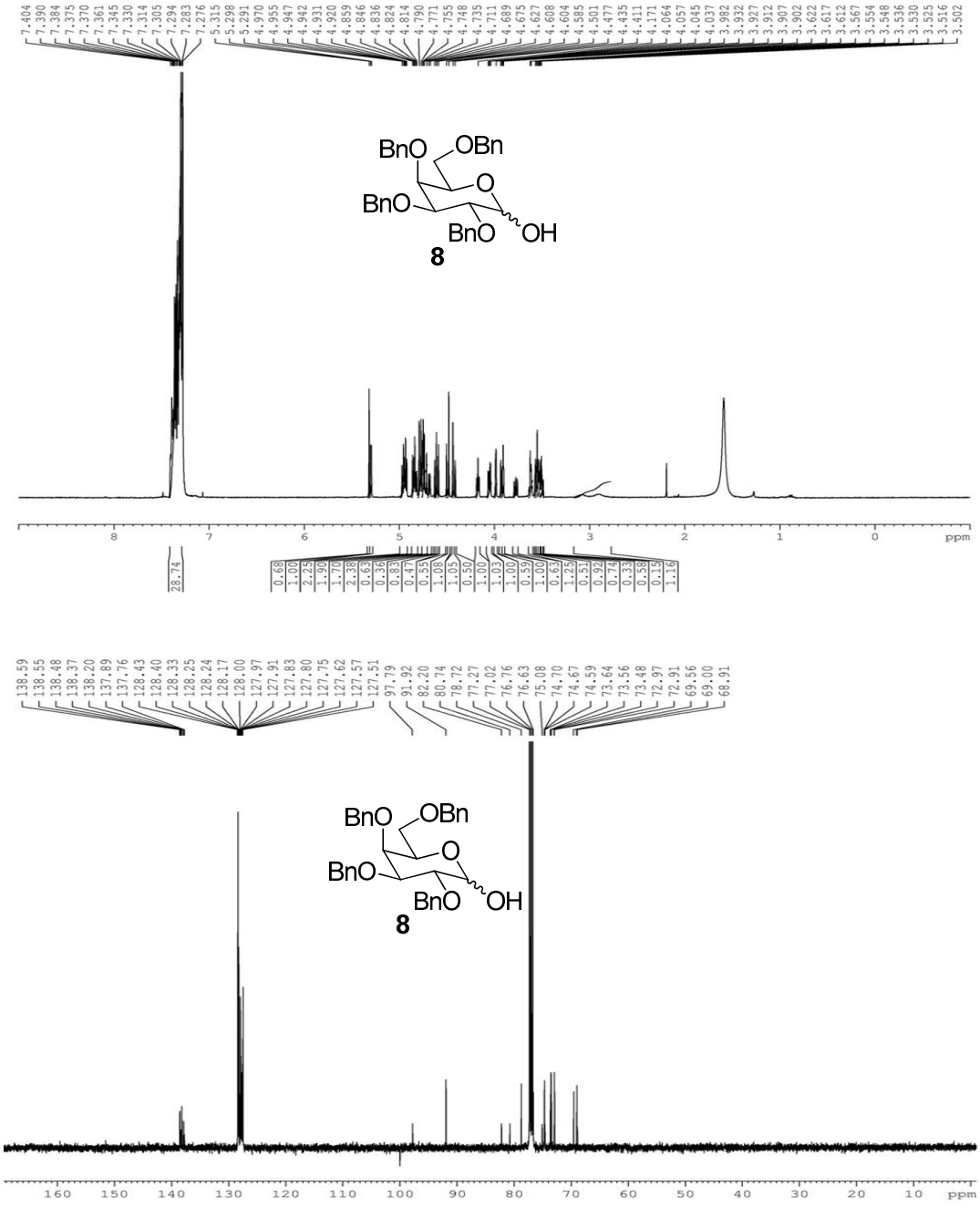




α,β-Hydroxy-2,3,4,6-tetra-*O*-benzyl-D-galactose (8). To a solution of compound **7** (14.9 g, 25.7 mmol) in DMF (50 mL) was added *t*-BuOK (4.3 g, 38.6 mmol) at room temperature, and the reaction mixture was heated at 70 °C. After 2 hrs, the reaction was quenched with ice water (100 mL) and 6 N HCl (6.4 mL, 38.6 mmol), and stirred for 5 min. The aqueous layer was washed with diethyl ether (4 × 100mL), dried (Na_2SO_4), and concentrated. The resulting crude residue was dissolved in acetone (80 mL) and 0.1 N HCl (11.2 mL, 1.12 mmol) was added and heated to 70 °C. After 4 h, the reaction was stopped and evaporated in vacuum. The resulting residue was treated with H_2O (200 mL) and extracted with ethyl acetate (3 x 150mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (hexane/EtOAc 4:1) afforded compound **8** (8.7 g, 63%) as reddish brown viscous solid. R_f 0.3 (hexane/EtOAc 2:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.48-3.51(m, 1H), 3.52-3.57 (m, 1H), 3.60-3.62 (m, 1H), 3.75-.79 (m, 1H), 3.90-3.93 (m, 1H), 3.98 (s, 1H), 4.05 (dd, 1H, J = 3.5, 9.5 Hz), 4.17 (t, 1H, J = 6.5 Hz), 4.41-

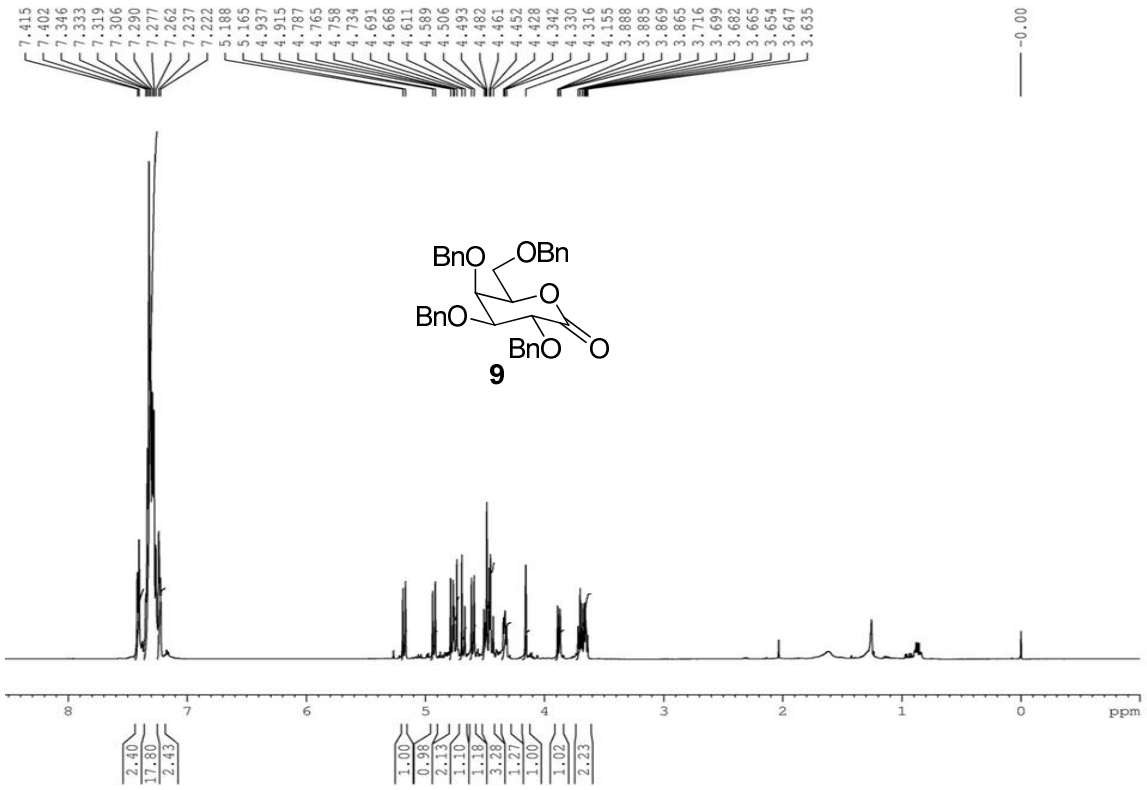
4.50 (m, 3H), 4.58-4.71 (m, 2H), 4.73-4.79 (m, 2H), 4.81-4.85 (m, 2H), 4.92-4.97(m, 2H), 5.29 (d, J = 3.5 Hz), 5.3 (s, 1H), 7.28-7.40 (m, 20H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 68.9, 69.5, 72.9, 73.5, 74.6, 75.0, 78.7, 80.7, 82.2, 91.9, 97.7, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 137.7, 137.8, 138.2, 138.3, 138.4, 138.5; FAB $[M + Na]^+$ m/z 565.06

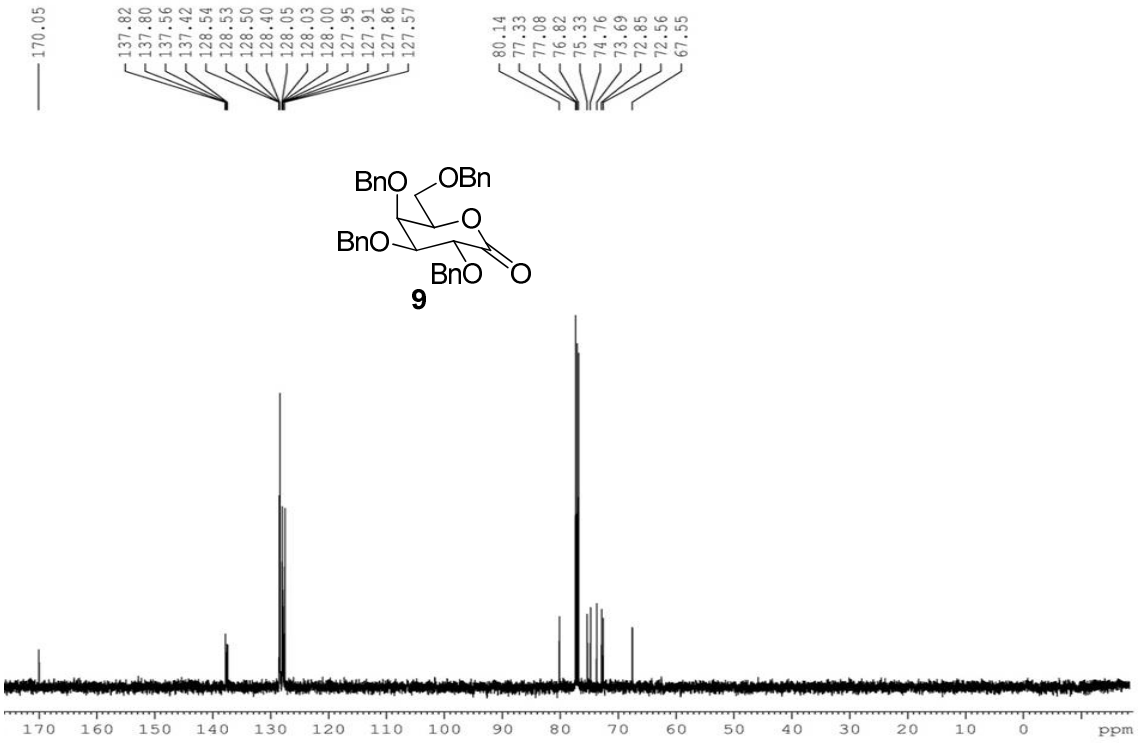
^{1}H and ^{13}C-NMR of compound (**8**)



2,3,4,6-tetra-*O*-benzyl-δ-lactone (9). To a solution of **8** (1.4 g, 2.6 mmol) in CH_2Cl_2 (30 mL) was added Dess-Martin periodinane (1.65 g, 3.9 mmol) and the reaction mixture was stirred at room temperature. After 1.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (40 mL) and saturated aqueous $Na_2S_2O_3$ (40 mL). The product was extracted with CH_2Cl_2 (2 x 30 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (hexane/EtOAc 9:1) afforded lactone **9** (1.28 g, 91%) as pale brown viscous solid. R_f 0.45 (hexane/ EtOAc 2:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.58-3.64 (m, 2H), 3.80 (dd, 1H, J = 2.5, 10 Hz), 4.08 (s, 1H), 4.24-4.27 (m, 1H), 4.24-4.43 (m, 4H), 4.51-4.71 (m, 4H), 4.85 (d, 1H, J = 11.5 Hz), 5.17 (d, 1H J = 11 Hz), 7.15-7.34 (m, 20H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 67.5, 72.5, 72.8, 73.6, 74.7, 75.3, 76.7, 77.3, 80.1, 127.5, 127.8, 127.9, 128.0-128.5, 137.4, 137.5, 137.8, 170.0; FAB [M + Na]$^+$ m/z 562.20.

^{1}H and ^{13}C-NMR of compound (**9**)





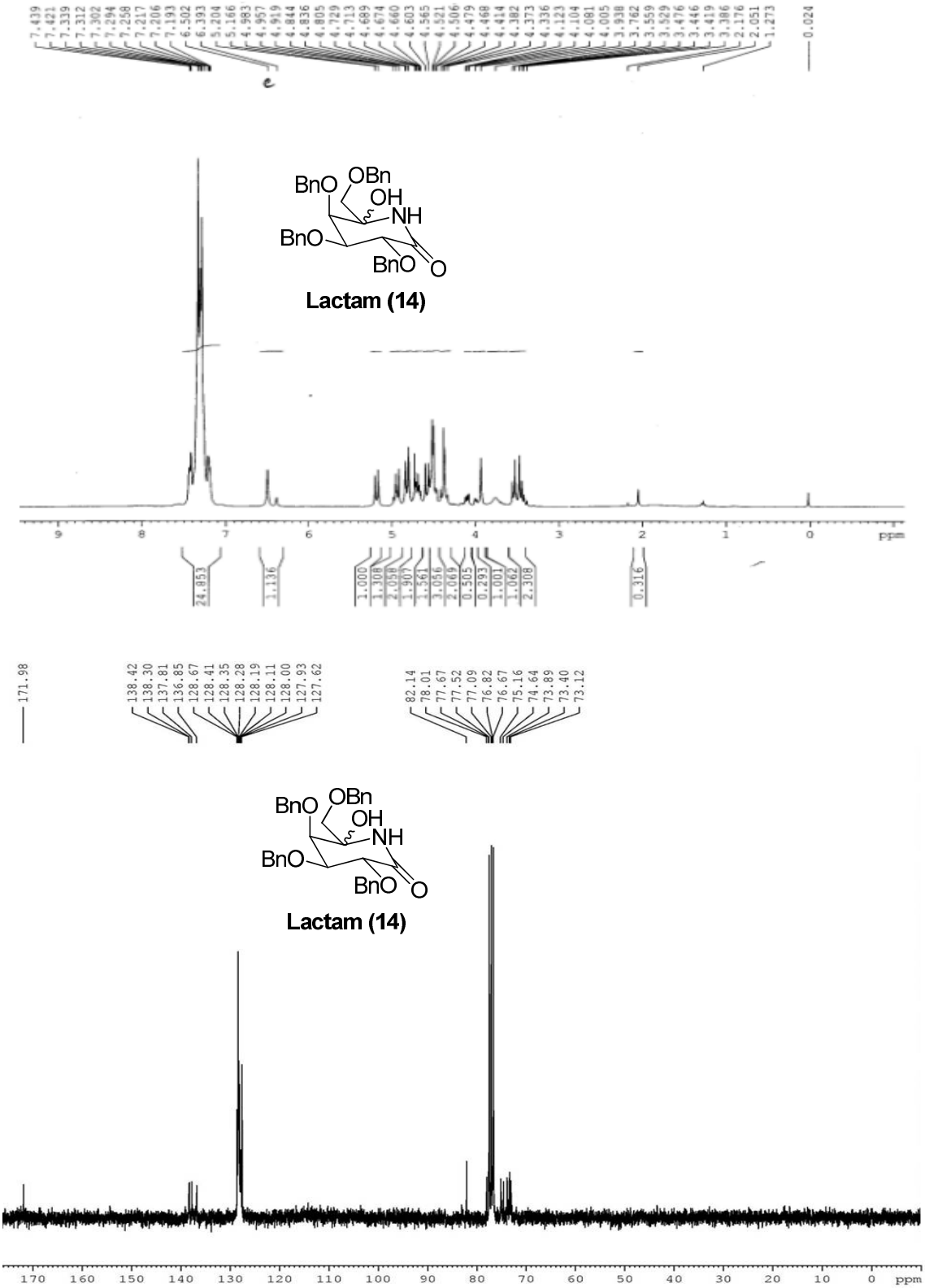
Compound 10. To a solution of lactone **9** (128 mg, 0.23 mmol) in toluene (5 mL), were added benzyl amine (26 μL, 1 eq), catalytic amount of amberlite IR 120 H^+ and then the reaction mixture was stirred under reflux in presence of $4A^0$ molecular sieves. After completion of starting material as indicated by TLC, the reaction mixture was filtered through the celite and quenched with water and extracted with diethyl ether. The organic layer was washed with saturated aqueous sodium bicarbonate followed by brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated; the residue was purified by column chromatography using hexane/EtOAc 80:20 give rise to compound **10** (130 mg) in 84% yield as a colorless viscous solid: R_f 0.28 (hexane/EtOAc 2:1).

Compound 11. To a solution of compound **10** (47 mg, 0.073 mmol, 1 eq) was added (95.8 mg, 0.226 mmol, 3.1 eq) in DCM (5 mL), and then the reaction mixture was stirred for 3h at room temperature. The reaction mixture was treated with saturated aqueous $Na_2S_2O_3$ (10 mL) for 10

minutes and then quenched with sat. aq. $NaHCO_3$, washed with water (2 × 20 mL), extracted with DCM (2 × 50 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography using hexane/EtOAc 80:20 afforded keto-carboxamide **11** (40 mg, 85 %) as a colorless viscous solid: R_f 0.34 (hexane/EtOAc 3:1).

5-Hydroxy-2,3,4,6-tetra-*O*-benzyl-δ-lactam (14). A solution of lactone **9** (310 mg, 0.57 mmol) in 7 N ammonia in MeOH solution (5 mL) were stirred at room temperature for 1 h. The reaction mixture was diluted with MeOH (10 mL) and then concentration afforded carboxamide **13** as a white solid which was recrystallized from hexane/EtOAc (1:1). To a solution of carboxamide (0.57 mmol) in CH_2Cl_2 (10 mL) was added Dess-Martin periodinane (490 mg, 1.15 mmol) and stirred at room temperature for 2.5 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (20 mL) and saturated aqueous $Na_2S_2O_3$ (20 mL) and extracted with CH_2Cl_2 (2 x 25 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (hexane/EtOAc 75:25) afforded **14** (271 mg, 85% two steps). R_f 0.3 (hexane/EtOAc 3:2); ^{1}H NMR ($CDCl_3$, 300MHz): δ 3.37-3.56 (m, 3H), 3.9 (s, 1H), 3.97-4.15 (m, 1H), 4.22-4.39 (m, 2H), 4.45-4.59 (m, 4H), 4.65-4.72 (m, 2H), 4.78-4.84 (m, 2H), 4.91-4.96 (m, 1H), 5.18 (d, 1H, J = 11.4 Hz), 5.94 (m, 1H), 6.92-7.61 (m, 20H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 73.1, 73.4, 73.8, 74.6, 75.2, 77.5, 77.7, 78.0, 82.1, 127.6, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.6, 136.8, 137.8, 138.3, 138.4, 171.9; FAB $[M + H]^+$ m/z 553.15.

^{1}H and ^{13}C-NMR of compound **14**



Compound 15. To a solution of lactam epimers **14** (892 mg, 1.612 mmol) in acetonitrile (20 mL) were added HCOOH (6.6 mL), $NaCNBH_3$ (354 mg, 5.64, 3.5 eq), and then the reaction mixture was stirred under reflux for 2 h under inert atmosphere. The reaction stopped, cooled in ice and then the reaction mixture was quenched with 0.1N HCl (120 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 85:15 to 75:25 afforded lactam **15** (637 mg, 73%) as a colorless sticky solid: R_f 0.30 (hexane/EtOAc 2:1).

Compound 16. To a solution of lactam **15** (342 mg, 0.64 mmol, 1 eq) in dry DCM (10 mL), were added triethyl amine (270 µL, 1.9 mmol, 3 eq), DMAP (23 mg, 0.192, 0.3 eq) and $(Boc)_2O$ (280 mg, 1.28 mmol, 2 eq) and then the resulting reaction mixture was stirred at room temperature for overnight under inert atmosphere. After the non polar spot formed as indicated by TLC, the reaction mixture was quenched with sat. aq. NH_4Cl (50 mL), and extracted with DCM (2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 afforded N-Boc lactam **16** (349 mg, 85%) as a colorless sticky solid: R_f 0.60 (hexane/EtOAc 2:1).

Compound 3. To a solution of compound lactam **16** (430 mg, 0.675 mmol, 1 eq) in dry EtOH (10 mL), was added $NaBH_4$ (205 mg, 5.40 mmol, 8 eq) and the P^H maintained by adding the 1N HCl (2 mL), at -10 ˚C and the resulting reaction mixture was stirred for overnight 40 minutes. After the polar spot formed as indicated by TLC, the reaction mixture was quenched with sat. aq. NH_4Cl (100 mL), and then extracted with EtOAc (2 × 70 mL), washed with sat. aq. $NaHCO_3$ (40 mL), and dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 85:15 afforded N-Boc aza-sugar **3** (290 mg, 67%) as a colorless sticky solid: R_f 0.46 (hexane/EtOAc 4:1).

Compound 17.

a) To the NaN_3 in H_2O: DCM (60 mL, 1:1), was slowly added Tf_2O at 0 ˚C, after stirred for 2 h at the same temperature. The reaction mixture quenched with sat. aq. $NaHCO_3$ (7 mL), and then extracted with DCM (40 mL) resulting TfN_3 stored in separating funnel.

b) To the white solid phytosphingosine in H_2O: DCM (total 80 mL, 1:1), were added K_2CO_3, $CuSO_4.5H_2O$ followed by slow addition of TfN_3 in DCM from the separating funnel (a), and then slowly added MeOH at room temperature and stirred for 5 hours. The reaction stopped and the solvents were evaporated resulting reaction mixture was extracted with EtOAc (2 × 120 mL) and dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 50:50 afforded azido-phytosphingosine **17** (5.40 g) in 96% yield as a white solid: R_f 0.5 (EtOAc).

Compound 18. A solution of azido-alcohol **17** (5.20 g, 15.1 mmol) in DCM were added pyridine (12 mL), trityl chloride (5.46 g, 19.64 mmol, 1.3 eq) at 0 °C and then the reaction mixture stirred for overnight. After the completion of staring material as indicated by TLC, the reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL), extracted with CH_2Cl_2 (2 × 100 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (hexane/EtOAc 100:0 to 85:15) afforded **18** (6.300 g, 71% two steps). R_f 0.3 (hexane/EtOAc 4:1).

Compound 19. To a solution of compound **18** (1.750 g, 3 mmol, 1 eq) in DMF (100 mL) was added at 0 °C NaH 60% suspension in mineral oil (35.7 mg, 8.96 mmol, 3 eq) was added in one portion and the reaction mixture was stirred for 30 min. At the same temperature benzyl bromide (1.53 mL, 8.96 mmol) was added dropwise and let the reaction mixture attain room temperature slowly. After 2.5 h, to the reaction mixture ice water (10 mL) was added at 0 °C slowly and

extracted with ethyl acetate (2 × 100 mL), dried (Na_2SO_4) and concentrated. Purification by flash chromatography (hexane/EtOAc 100:0 to 95:5) afforded compound **19** (1.77 g, 77 %) as brown viscous solid. R_f 0.6 (hexane/EtOAc 4:1).

Compound 4. A solution of compound **19** (1.75 g, 2.3 mmol) in DCM: MeOH (20 mL, 3:2) was added catalytic amount of *p*TSA (40 mg) at room temperature and then the reaction mixture stirred for 3.5 hours. After the completion of staring material as indicated by TLC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (3 × 30 mL), and washed with H_2O, extracted with EtOAc (2 x 70 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (hexane/EtOAc 96:2 to 92:8) give rise to alcohol **4** (833 mg, 70 % two steps). R_f 0.5 (hexane/EtOAc 4.5:0.5).

Compound 5. To a solution of N-Boc protected piperidin-2-ol **3** (90 mg, 0.140 mmol, 1 eq) and phytosphingosine-1-ol **4** (147 mg, 0.281 mmol, 2 eq) in dry THF (5 mL) was added TMSOTf (13 µL, 0.07 mmol, 0.5 eq) at -10 ˚C and the resulting mixture was stirred for 3 h at 0 °C under argon atmosphere. The reaction mixture was quenched with sat. aq. $NaHCO_3$, washed with water (2 × 50 mL), extracted with EtOAc (2 × 50 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 80:20 afforded glycosylated product **5** (93 mg, 58 %) as a colorless viscous solid: R_f 0.5 (hexane/EtOAc 9:1); HR-ESI-MS $[M+Na]^+C_{71}H_{92}N_4O_9Na$ calc'd for m/z 1167.67620, found 1167.67717.

Compound 4b. To a solution of azido-alcohol **4** (580 mg, 1.108 mmol), in benzene (10 mL), were added water (100 µL), PPh_3 (406 mg, 1.55 mmol, 1.4) and then the reaction mixture was stirred for overnight at room temperature. Polar spot formed as indicated by TLC, the reaction stopped and then the solvents were evaporated, resulting crude amine (**4a**) was dried in vacuum.

The crude amine **4a** was dissolved in DCM (15 mL) and then triethylamine (386 μL, 2.77 mmol, 2.5 eq), and $(Boc)_2O$ (483.5 mg, 2.216 mmol, 2 eq) were added at room temperature and then the resulting reaction mixture was stirred for 20 minutes under inert atmosphere. The reaction mixture was quenched with sat. aq. NH_4Cl (50 mL), extracted with CH_2Cl_2 (2 × 70 mL) dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10, 80:20 yielded compound **4b** (633 mg, 95 %, two steps) as a colorless viscous solid: R_f 0.37 (hexane/EtOAc 4:1).

Compound 4c. To a solution of primary alcohol **4b** (205 mg, 0.343 mmol, 1 eq) in DCM (10 mL) was added Dess-Martin periodinane (291 mg, 0.686 mmol, 2 eq), then the reaction mixture was stirred for 1h at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $Na_2S_2O_3$ (30 mL) and sat. aq.$NaHCO_3$ (40 mL), extracted with CH_2Cl_2 (2 × 60 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 give rise to aldehyde **4c** (200 mg, 98 %), as a colorless viscous solid R_f 0.6 (hexane/EtOAc 4:1).

Compound 4d. To a solution of Ohira-Bestmann reagent (771 mg, 4.02 mmol, 2 eq) in CH_3CN (35 mL) was added K_2CO_3 (152 mg, 2.21 mmol, 1.1 eq) and then stirred for 50 minutes at room temperature. A solution of aldehyde **4c** (1.20 g, 2.01 mmol, 1 eq) in MeOH (30 mL) was transferred into the above reaction mixture at room temperature and then the resulting reaction mixture stirred for 1 h. Starting material consumed as indicated by TLC. The reaction mixture was diluted with DCM (20 mL) filtered through celite and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10, 85:15 yielded alkyne **4d** (740 mg, 62 %), as a colorless viscous solid R_f 0.4 (hexane/EtOAc 3:1).

Compound 4e. To a solution of alkyne **4d** (740 mg) in dry EtOAc (30 mL) was added Lindlar catalyst (700 mg), and then H_2 gas was purged for 5 minutes, the reaction mixture was stirred for overnight at room temperature under H_2 atmosphere. The product formation was monitored by ^{1}H-NMR. The reaction mixture filtered through celite, concentrated, resulting in an alkene **4e** in quantitative yield.

Compound 9a. To a solution of lactone **9** (920 mg, 3.42 mmol, 1 eq) in dry THF (10 mL) was cooled at -78 °C and then slowly added vinyl magnesium bromide (3.42 mL, 3.42 mmol) and the resulting reaction mixture was stirred for 1 h at the same temperature. The reaction mixture was quenched with sat. aq. NH_4Cl (50 mL), extracted with EtOAc (2 × 60 mL) dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10, 80:20 yielded compound **9a** (600 mg, 62 %) as a colorless viscous solid: R_f 0.3 (hexane/EtOAc 3:1).

Compound 9b. To a solution of compound **9a** (30 mg, 0.671 mmol, 1 eq) in DCM (10 mL) was added Dess-Martin periodinane (854 mg, 2.01 mmol, 3 eq), then the reaction mixture was stirred for 1h at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $Na_2S_2O_3$ (40 mL) and sat. aq.$NaHCO_3$ (50 mL), extracted with CH_2Cl_2 (2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 give rise to diketone **9b** (250 mg, 66 %), as a colorless viscous solid R_f 0.4 (hexane/EtOAc 4:1).

Compound 9c. To a solution of diketone **9b** (60 mg, 0.106 mmol) in THF: MeOH (4:1, 5 mL) were added $CeCl_3$ (39 mg, 0.106 mmol, 1 eq), $NaBH_4$ (20 mg, 0.52 mmol, 5 eq) at -10 °C and then stirred for 20 minutes under inert atmosphere. The reaction mixture was quenched with sat.

aq. NH_4Cl (20 mL), extracted with EtOAc (2 × 30 mL) dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 75:25 afforded compound **9c** (37 mg, 61 %) as a colorless viscous solid: R_f 0.2 (hexane/EtOAc 4:1).

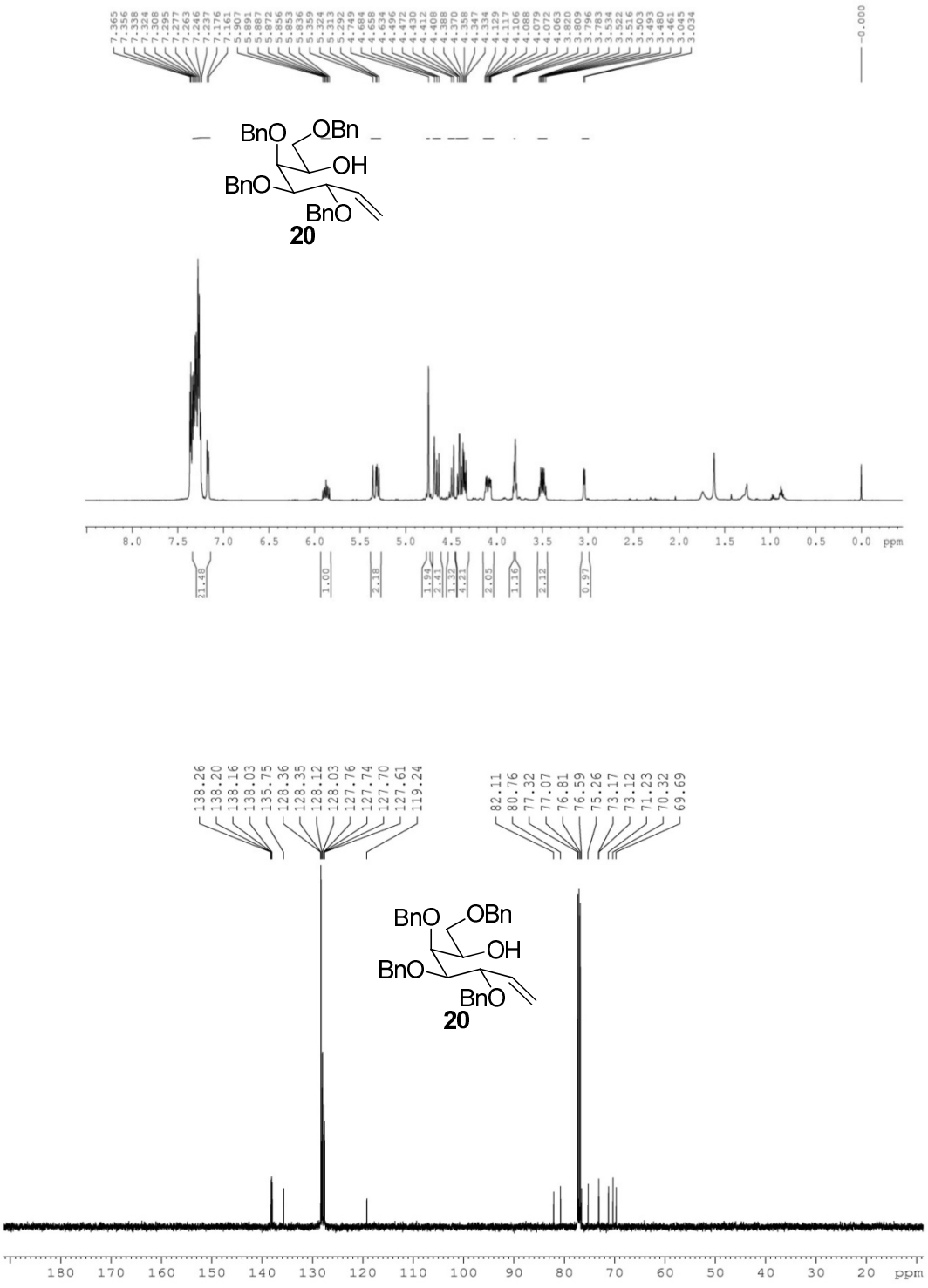
Compound 9d. To a solution of dimethyl methylphosphonate (0.54 mL, 5.53 mmol, 2 eq) in dry THF (10 mL) at -78 °C was slowly added *n*-BuLi (3.16 mL, 5.05 mmol, 2 eq), then the resulting yellow colored solution was stirred for 30 min at the same temperature under argon atmosphere. A solution of lactone **9** (1.36 g, 2.52 mmol, 1 eq) in THF (7 mL) was added, and the resulting mixture was stirred for 1h at -78 °C under argon atmosphere. The reaction mixture was quenched with sat. aq. NH_4Cl (30 mL), extracted with ethyl acetate (2 × 70 mL), washed with water (3 × 100 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography hexane/EtOAc 90:10 to 40:60 afforded β-hydroxy phosphonate **9d** (1.35 g, 81%) as a colorless viscous solid: R_f 0.3 (hexane/EtOAc 1:1).

Compound 9e. To a solution of β-hydroxy phosphonate **9d** (98 mg, 0.148 mmol, 1 eq) in THF (3 mL) $NaBH_4$ (11 mg, 0.29 mmol, 2 eq) was added, and then the reaction mixture was stirred for 1.5 h at room temperature. Reaction stopped and solvents were evaporated resulting in a 1,5-dihydroy phosphonate **9e** (80 mg, 80%) as a colorless viscous solid: R_f 0.2 (hexane/EtOAc 1:3).

Compound 20. A solution of methyltriphenylphosphonium bromide (37.7 g, 105.5 mmol, 3 eq) in dry toluene (200 mL) was stirred for 10 min at room temperature, and then slowly added 1.6M solution of *n*-BuLi in hexane (65.9 mL, 105.5 mmol, 3 eq) at 0 °C under argon atmosphere. The resulting yellow solution was stirred for 2h at 0 °C to room temperature, and then a solution of lactol **8** (19 g, 35.2 mmol, 1 eq) in dry toluene (80 mL)was added at room temperature. After overnight stirring, the reaction mixture was cooled down to 0 °C, quenched with acetone (150

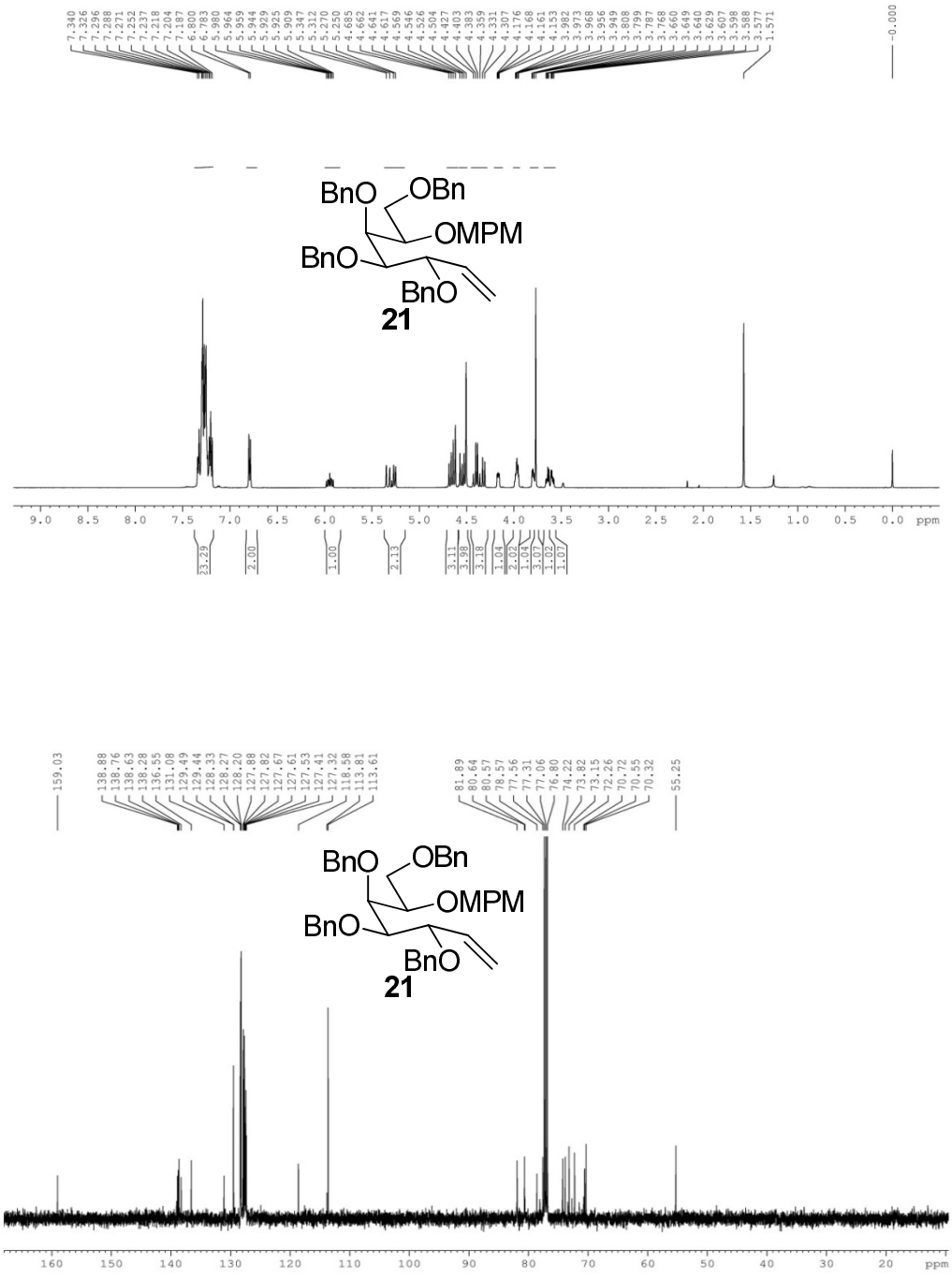
mL), and extracted with Et_2O (2 × 400mL). The organic layer was washed with excess water, dried over anhydrous $MgSO_4$, concentrated and purified by flash chromatography using hexane/EtOAc 95:5 to 90:10 which afforded compound **20** (15.3 g, 81%) as a pale brown viscous solid: R_f0.4 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$,500 MHz) δ 7.36-7.16 (m, 20H), 5.90-5.83 (m, 1H), 5.35-5.29 (m, 2H), 4.74 (s, 2H), 4.68-4.63 (m, 2H), 4.48 (d, *J* = 12 Hz, 1H), 4.49-4.33 (m, 4H), 4.12-4.06 (m, 2H), 3.82-3.78 (m, 1H), 3.53-3.46 (m, 2H), 3.03(d, *J* = 5.5 Hz, 1H);^{13}C NMR ($CDCl_3$,125 MHz) δ 138.3, 138.2, 138.1, 138.0, 135.7, 128.3, 128.1, 128.0, 127.74, 127.70, 127.6, 119.2, 80.7, 76.5, 75.2, 73.1, 71.2, 70.3, 69.6; HR-ESI-MS $[M+Na]^+$ $C_{35}H_{38}O_5Na$ calc'd for m/z 561.26169, found 561.25641.

^{1}H, ^{13}C-NMR spectra of compound **20**



Compound 21. To a solution of compound **20** (9.5 g, 17.6 mmol, 1 eq) in DMF (70mL) at 0 °C was added NaH 60% suspension in mineral oil (1.4 g, 35.3 mmol, 2 eq) and then the reaction mixture was stirred for 30 min. At the same temperature 4-methoxybenzyl chloride (3.6 mL, 26.4 mmol, 1.5 eq) was added dropwise and left the reaction mixture attain room temperature slowly. After 3h, the reaction mixture was quenched with ice water (250 mL) at 0 °C and extracted with ethyl acetate (2 × 300 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 to 90:10 afforded compound **21** (10.9 g, 94%) as a colorless viscous solid: R_f 0.38 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$,500 MHz) δ 7.34-7.18 (m, 22H), 6.79 (d, *J* = 8.5 Hz, 2H), 5.98-5.90 (m, 1H), 5.34-5.25 (m., 2H),4.68-4.61 (m, 3H), 4.56-4.50 (m, 4H), 4.42-4.30 (m, 3H), 4.16 (dd, *J* = 7.5, 4.5 Hz, 1H), 3.98-3.94 (m, 2H), 3.80-3.78 (m, 1H), 3.7 (s, 3H), 3.64 (dd, *J* = 10, 5.5 Hz, 1H), 3.59 (dd, *J* = 9.5, 4.5 Hz, 1H); ^{13}C NMR ($CDCl_3$,125 MHz) δ159.0, 138.8, 138.7, 138.6, 138.2, 136.5, 131.1, 129.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.3, 118.5, 81.8, 78.5, 73.1, 72.2, 70.7, 70.5, 70.3, 55.2; HR-ESI-MS $[M+Na]^+$ $C_{43}H_{46}O_6Na$ calc'd for m/z 681.31921, found 681.31952.

^{1}H, ^{13}C-NMR spectra of compound **21**



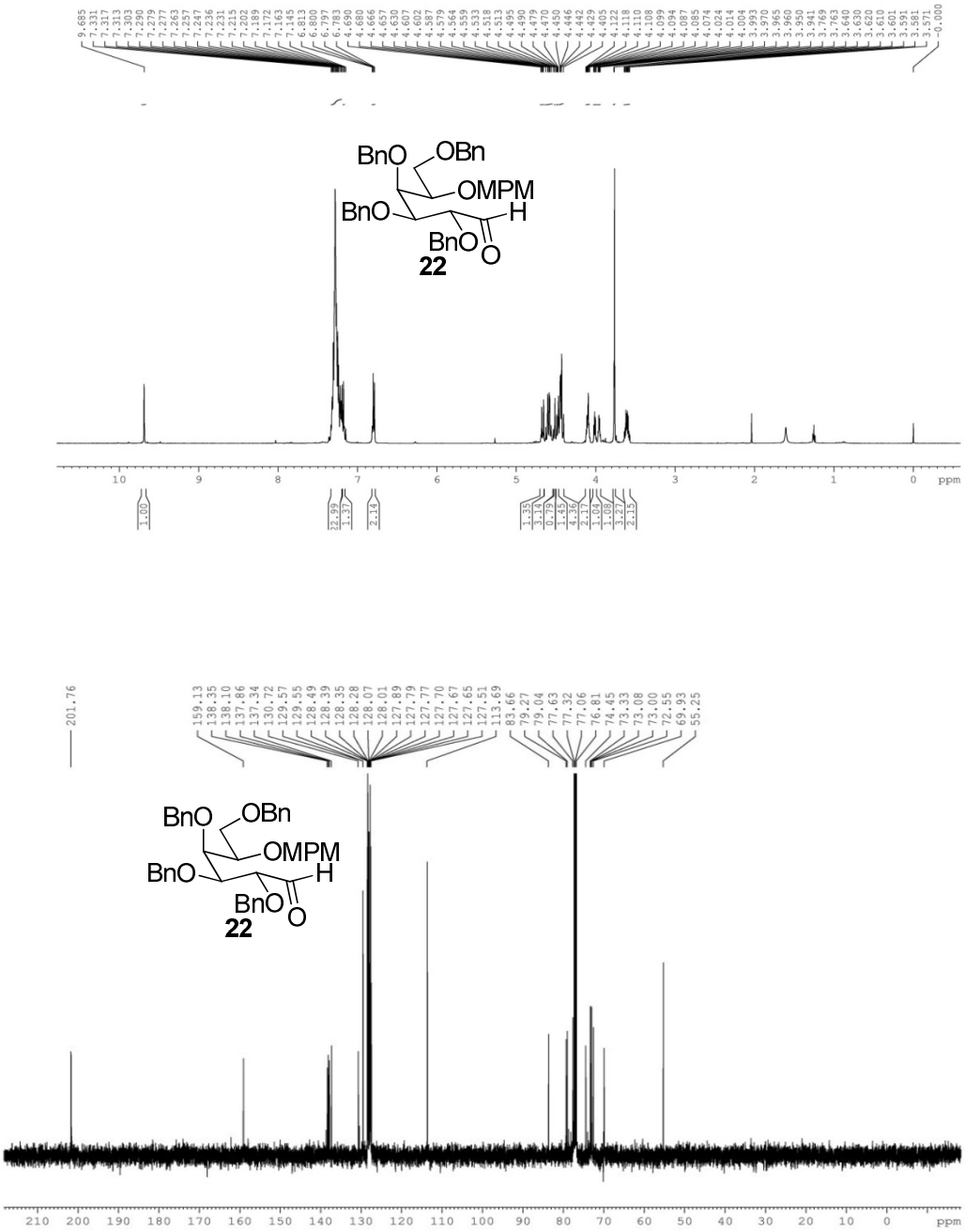
Compound 22. To a solution of compound **21** (10.9 g, 16.5 mmol, 1 eq) in acetone/water (4:1, 150 mL) was added NMO (2.9 g, 24.8 mmol, 1.5 eq), OsO_4 (189.5 mg, 0.74 mmol, 0.045 eq),

and then the reaction mixture was stirred for 8h at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $NaHSO_3$, washed with water (2 × 400mL) and extracted with ethyl acetate (2 × 300mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 70:30 afforded diol as colorless viscous solid: R_f 0.3 (hexane/EtOAc 6:4); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31-7.14 (m, 22H), 6.81 (d, J = 8.5 Hz, 2H), 4.76-4.71 (m, 2H), 4.67-4.60 (m, 2H), 4.58-4.54 (m, 3H), 4.49-4.47 (m, 2H), 4.43-4.38 (m, 2H), 4.09 (app t, J = 5.5 Hz, 1H), 4.00-3.97 (m, 1H), 3.87-3.85 (m, 2H), 3.76 (s, 3H), 3.75 (s, 1H), 3,70-3.66 (m, 1H), 3.60-3.56 (m, 2H), 3.4 (d, J = 4 Hz, 1H), 1.77 (s, 1H); ^{13}C NMR ($CDCl_3$,125 MHz) δ 159.2, 138.2, 138.1, 137.9, 130.6, 129.9, 129.6, 128.4, 128.3, 128.2, 127.9, 127.8, 127.6, 113.7, 79.6, 79.1, 78.2, 73.3, 73.0, 72.9, 71.8, 70.0, 63.8, 55.2; HR-ESI-MS $[M+Na]^+$ $C_{43}H_{48}O_8Na$ calc'd for m/z 715.32469, found 715.32507.

To a solution of diol (4.5 g, 6.5 mmol, 1 eq) in THF (70mL) was added a solution of aqueous $NaIO_4$ (2.79 g, 13.1 mmol, 2 eq) at 0 °Cand stirred overnight at room temperature under argon atmosphere. The reaction mixture was quenched with brine (50 mL), extracted with ethyl acetate (2 × 200 mL), washed with water (2 × 300 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography using hexane/EtOAc 90:5 to 90:10 afforded aldehyde **22** (3.98 g) as a colorless viscous solid (88% yield, 2 steps): R_f 0.4 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$,500 MHz) δ 9.68 (s, 1H), 7.32-7.17 (m, 22H), 6.79 (d, J = 8.5 Hz, 2H), 4.66 (d, J = 11.5 Hz, 1H), 4.63-4.56 (m, 3H), 4.51-4.47 (m, 2H), 4.45-4.40 (m, 4H), 4.11-4.09 (m, 2H), 4.01 (app t, J = 5 Hz, 1H), 3.95-3.94 (m, 1H), 3.77 (s, 3H), 3.61-3.59 (m, 2H); ^{13}C NMR ($CDCl_3$,125 MHz) δ 201.6, 159.1, 138.3, 138.1, 137.8, 130.7, 130.4, 129.5, 128.5, 128.3, 128.0, 127.9, 127.8, 127.6, 127.5, 113.6, 83.6, 79.2, 79.0, 76.8, 74.4, 73.3, 73.08, 73.0, 72.5, 69.9, 55.2;

HR-ESI-MS $[M+Na]^+$ $C_{42}H_{44}O_8Na$ calc'd for m/z 683.29847, found 683.29822 and $[M+MeOH+Na]^+$ 715.32391.

^1H, ^{13}C-NMR spectra of compound **22**

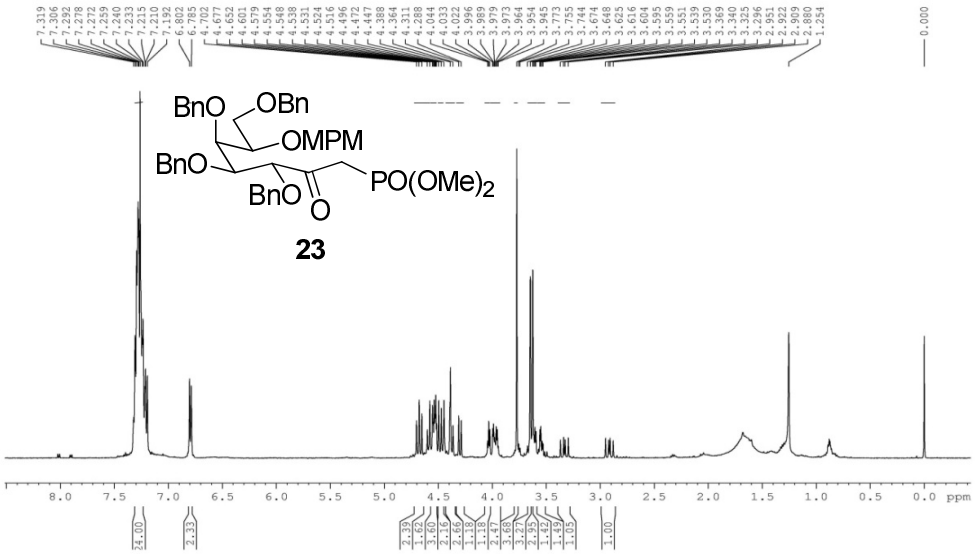


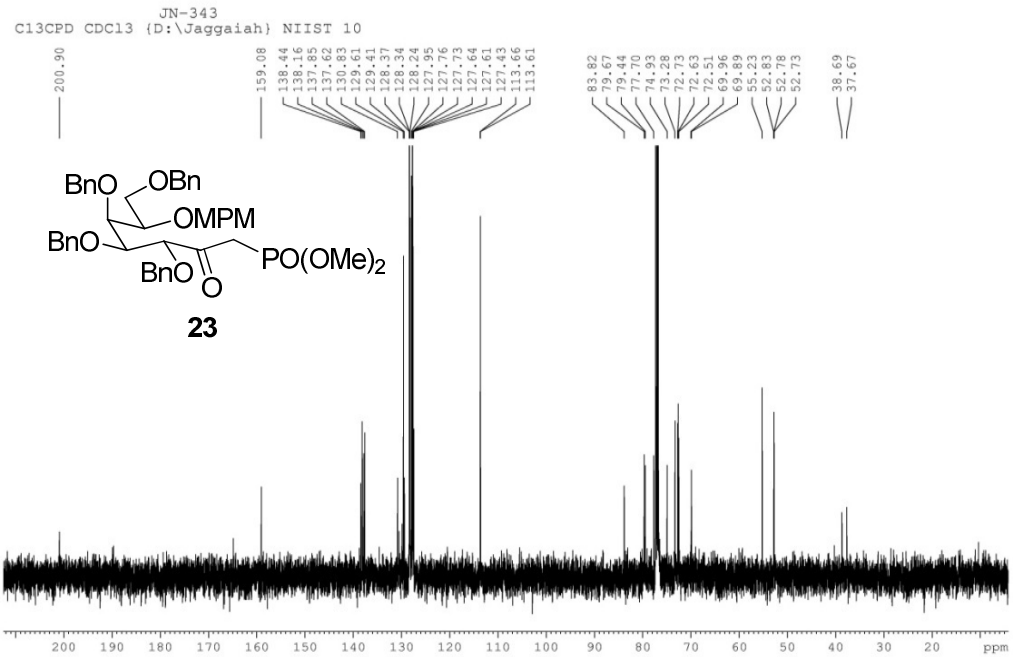
***β*-keto phosphonte 23.**To a solution of dimethyl methylphosphonate (1.28 mL, 11.9 mmol, 2.1 eq) in THF (15 mL) at -78 °C was slowly added *n*-BuLi (7.13 mL, 11.4 mmol, 2 eq), then the resulting yellow colored solution was stirred for 20 min at the same temperature under argon atmosphere. A solution of compound **22** (3.7 g, 5.7 mmol, 1 eq) in THF (30 mL) was added, and the resulting mixture was stirred for 1h at -78 °C under argon atmosphere. The reaction mixture was quenched with sat. aq. NH_4Cl (50 mL), extracted with ethyl acetate (2 × 200 mL), washed with water (3 × 300 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography hexane/EtOAc 90:10 to 40:60 afforded β-hydroxy phosphonate as a mixture: R_f 0.2 (hexane/EtOAc 7:3); HR-ESI-MS $[M+Na]^+C_{45}H_{53}O_{10}PNa$ calc'd for m/z 807.32740, found 807.32819.

To a solution of β-hydroxy phosphonate (3.15 g, 4.02 mmol, 1 eq) in CH_2Cl_2 (30 mL) was added Dess-Martin periodinane (3.4 g, 8.04 mmol, 2 eq), and stirred for 1h at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $Na_2S_2O_3$ (75 mL) and sat. aq.$NaHCO_3$ (100 mL), extracted with CH_2Cl_2 (2 × 150 mL), washed with water (2 × 400 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 to 80:20 to 60:40 afforded compound **23** (2.16 g, 49%, two steps) as a pale yellow viscous solid: R_f 0.3 (hexane/EtOAc 6:4); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31-7.12 (m, 22H), 6.79 (d, J = 8.5 Hz, 2H), 4.68 (app t, J = 12 Hz, 2H), 4.61-4.58 (m, 1H), 4.55-4.51 (m, 3H), 4.49-4.44 (m, 2H), 4.39-4.36 (m, 2H), 4.29 (d, J = 11.5 Hz, 1H), 4.04-4.01 (m, 1H), 3.99-3.94 (m, 2H), 3.77 (s, 3H), 3.64 (s, 3H), 3.62 (s, 3H), 3.60-3.59 (m, 1H), 3.55-3.53 (m, 1H), 3.33 (dd, J = 22, 14.5 Hz, 1H), 2.91 (dd, J = 21, 14.5 Hz, 1H); ^{13}C NMR ($CDCl_3$,125 MHz) δ 200.9, 159.0, 138.4, 138.1, 137.8, 137.6, 130.8, 129.6, 129.4, 128.3, 127.9,

127.4, 113.6, 83.8, 79.6, 79.4, 74.9, 73.2, 72.7, 72.5, 69.8, 55.7, 52.7, 38.7, 37.6; HR-ESI-MS $[M+Na]^+$ $C_{45}H_{51}O_{10}PNa$ calc'd for m/z 805.31175, found 805.31268.

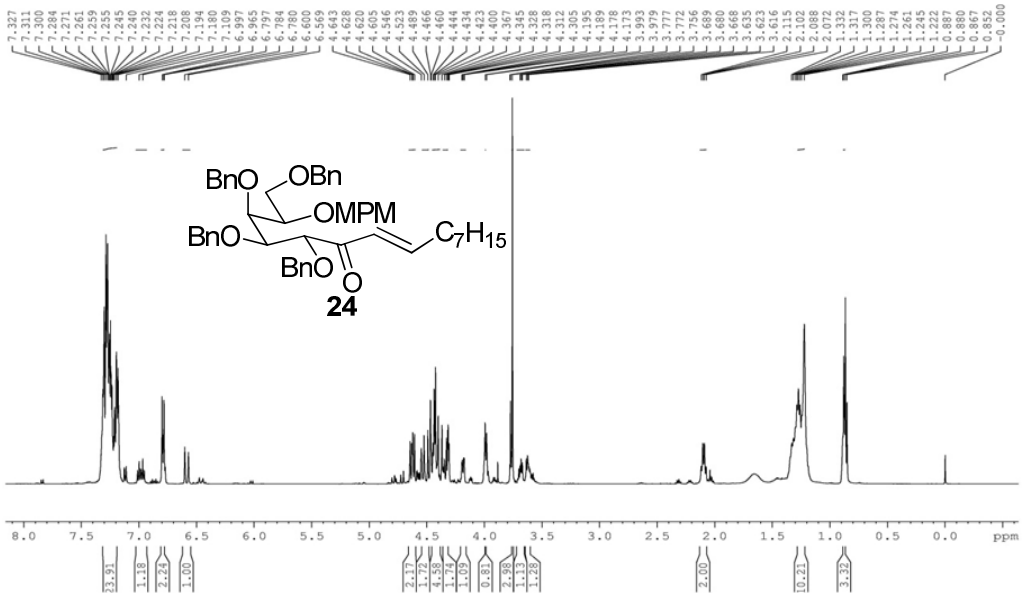
^1H, ^{13}C-NMR spectra of compound **23**

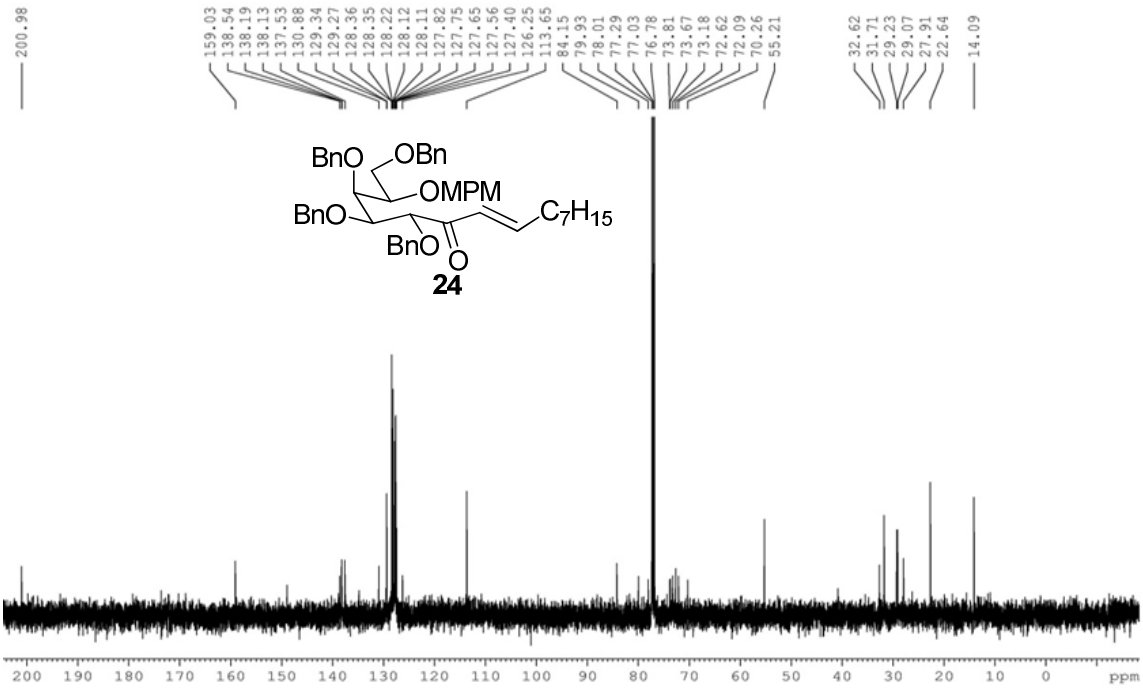




Compound 24. To a solution of compound **23** (295 mg, 0.37 mmol, 1 eq) in THF (6 mL) was added $Ba(OH)_2.8H_2O$ (148.7 mg, 0.47 mmol, 1.25 eq) and then stirred for 30 min at room temperature under argon atmosphere. Octanal (176.7µL, 1.13 mmol, 3 eq) was added directly to the reaction mixture and stirred at room temperature under argon atmosphere. After stirring overnight, the reaction was stopped and solvents were evaporated, the crude mixture was directly subjected to purification by flash chromatography using hexane/EtOAc 95:5 affording compound **24** (190 mg, 64.5%) as a colorless viscous solid: R_f 0.40 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32-7.10 (m, 22H), 7.01-6.95 (m, 1H), 6.78 (d, J = 8.5 Hz, 2H), 6.58 (d, J = 15.5 Hz, 1H), 4.64-4.60 (m, 2H), 4.54-4.46 (m, 2H), 4.44-4.40 (m, 4H), 4.32-4.30 (m, 2H), 4.18 (dd, J = 8.5, 3 Hz, 1H), 3.99-3.97 (m, 1H), 3.75 (s, 3H), 3.68-3.66 (m, 1H), 3.63-3.61 (m, 1H), 2.11-2.07 (m, 2H), 1.33-1.26 (m, 10H), 0.86 (t, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$,125 MHz) δ 200.9, 159.0, 138.5, 138.2, 138.1, 137.5, 130.8, 129.2, 128.3, 128.2, 127.7, 127.5, 126.2, 113.6, 84.1, 79.9, 78.0, 73.8, 73.6, 73.1, 72.6, 72.0, 55.2, 32.6, 31.7, 29.2, 29.0, 27.9, 22.6, 14.0; HR-ESI-MS $[M+Na]^+$ $C_{51}H_{60}O_7Na$ calc'd for m/z 807.42367, found 807.42285.

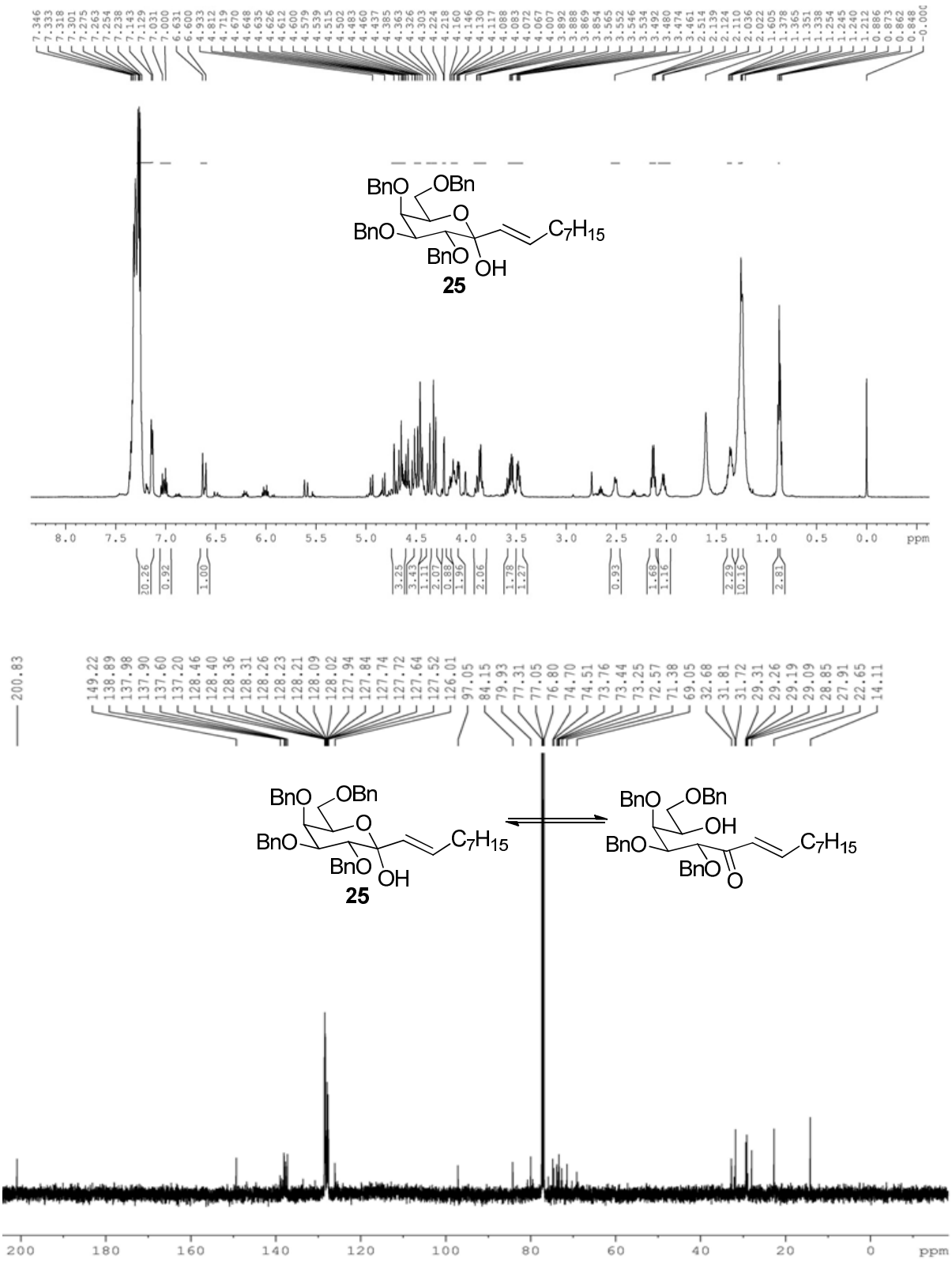
^{1}H, ^{13}C-NMR spectra of compound **24**





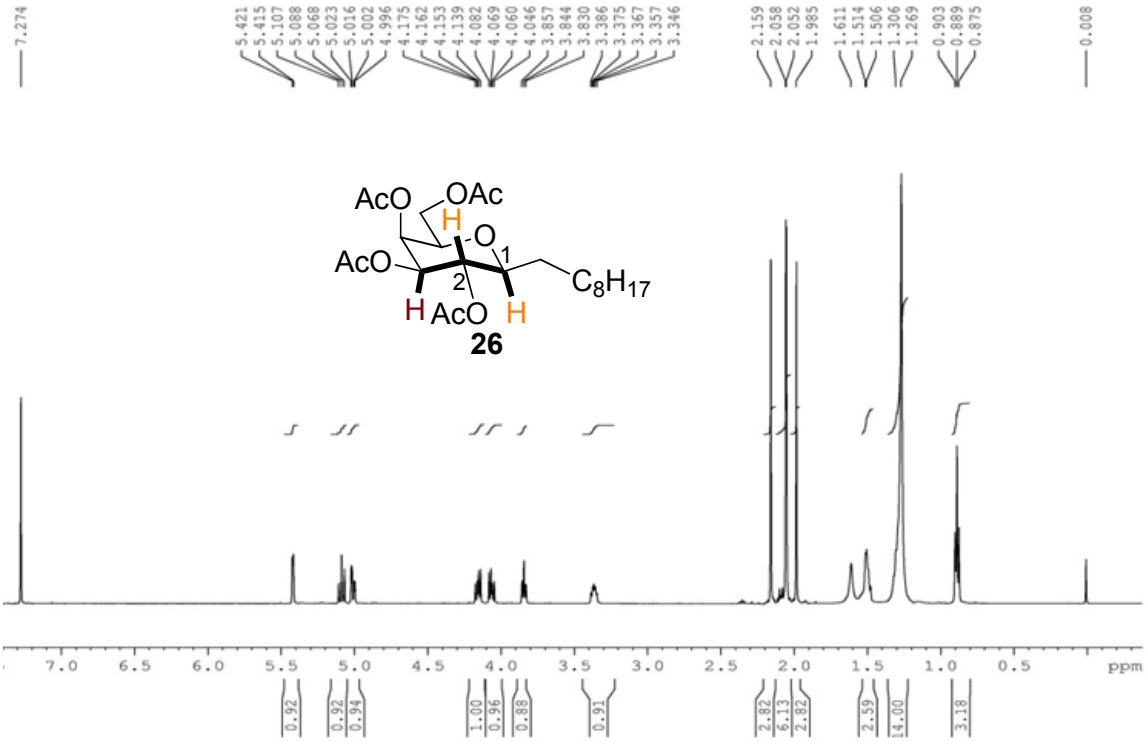
Compound 25. To a solution of compound **24** (170 mg, 0.21 mmol, 1 eq) in CH_2Cl_2(5 mL) was added DDQ (54.1 mg, 0.23 mmol, 1.1 eq) at 0 °Cand the resulting brownish colored reaction mixture was stirred for 2.5 h at room temperature under argon atmosphere. The reaction mixture was then quenched with sat. aq.$NaHCO_3$ (20 mL), extracted with CH_2Cl_2 (2 × 70 mL), washed with water (2 × 250 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 to 90:10 afforded compound **25** (97 mg, 68%) as a pale yellow viscous solid: R_f 0.37 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$,500 MHz) δ 7.34-7.23 (m, 20H), 7.14-7.00 (m, 1H), 6.61 (d, J = 15.5 Hz, 1H), 4.71-4.57 (m, 3H), 4.53-4.43 (m, 3H), 4.38-4.30 (m, 3H), 4.22 (d, J = 3 Hz, 1H), 4.16-4.06 (m, 2H), 3.88-3.85 (m, 2H), 3.59-3.49 (m, 2H), 1.37-01.33 (m, 2H), 1.25-1.21 (m, 10H), 0.87 (t, J = 6.5 Hz, 3H); HR-ESI-MS $[M+Na]^+$ $C_{43}H_{52}O_6Na$ calc'd for m/z 687.36616, found 687.36682.

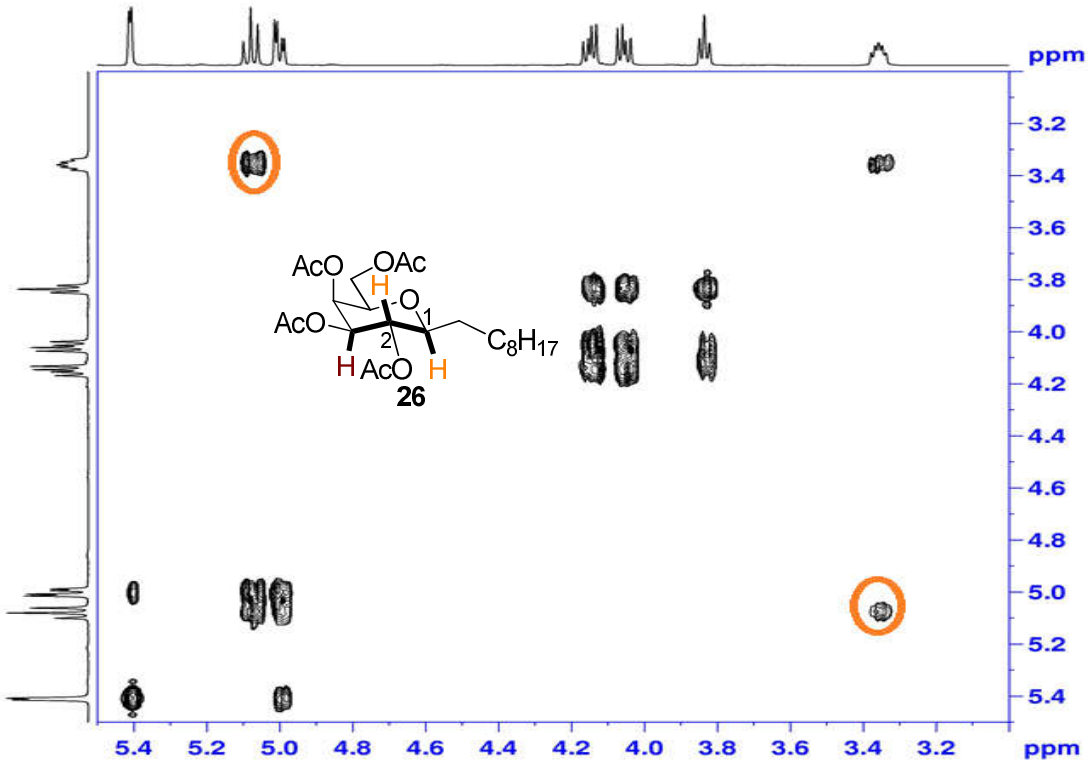
^{1}H, ^{13}C-NMR spectra of compound **25**



Compound 26. To solution of compound **25** (39 mg, 0.06 mmol, 1 eq) in CH_3CN (3 mL) was added Et_3SiH (94 µL, 0.58 mmol, 10 eq), and TMSOTf (32 µL, 0.17 mmol, 3 eq), at 0 °C and stirred for 20 min. The reaction mixture was quenched with sat. aq.$NaHCO_3$ (10 mL), extracted with EtOAc (2 × 30 mL), washed with brine (25 mL), dried over anhydrous $MgSO_4$ and concentrated. The crude mixture was dissolved in 3 mL of EtOH/THF (6:1) and then added 10 % Pd/C (30 mg) and TFA (18 µL, 0.23 mmol, 4 eq). The resulting mixture was purged with H_2 gas for 5 min at room temperature, and then the reaction mixture was stirred overnight under H_2 atmosphere (balloon). The reaction mixture was then diluted with EtOH (3 mL), filtered through celite pad, washed with MeOH, and the filtrate was concentrated in vacuum resulting in a white solid in a quantitative yield. The resulting product was dissolved in pyridine (2 mL) and Ac_2O (0.5 mL) and stirred the reaction mixture overnight at room temperature under argon atmosphere. The reaction mixture was diluted with H_2O (5 mL), extracted with EtOAc (2 × 25 mL), and the organic layer washed with sat. aq. $NaHCO_3$ (2 × 10 mL), dried over anhydrous $MgSO_4$ and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 85:15 afforded compound **26** (13 mg, 48%, three steps) as a white solid: R_f 0.46 (hexane/EtOAc 6:4); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.41 (d, J = 3 Hz, 1H), 5.08 (app t, J = 9.5 Hz, 1H), 5.02 (dd, J = 10.5, 3.5 Hz, 1H), 4.14 (dd, J = 9.5, 5 Hz, 1H), 4.06 (dd, J = 11, 6.5 Hz, 1H), 3.84 (app t, J = 6.5 Hz, 1H), 3.38-3.34 (m, 1H), 2.15 (s, 3H), 2.05 (s, 6H), 1.98 (s, 3H), 1.51-1.50 (m, 2H), 1.30-1.26 (m, 14H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$,125 MHz) 170.5, 170.4, 170.3, 169.9, 78.3, 74.0, 72.3, 69.5, 67.7, 31.9, 31.8, 31.4, 29.7, 29.5, 29.3, 25.1, 22.6, 20.8, 20.74, 20.6, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{23}H_{38}O_9Na$ calc'd for m/z 481.24135, found 481.23959.

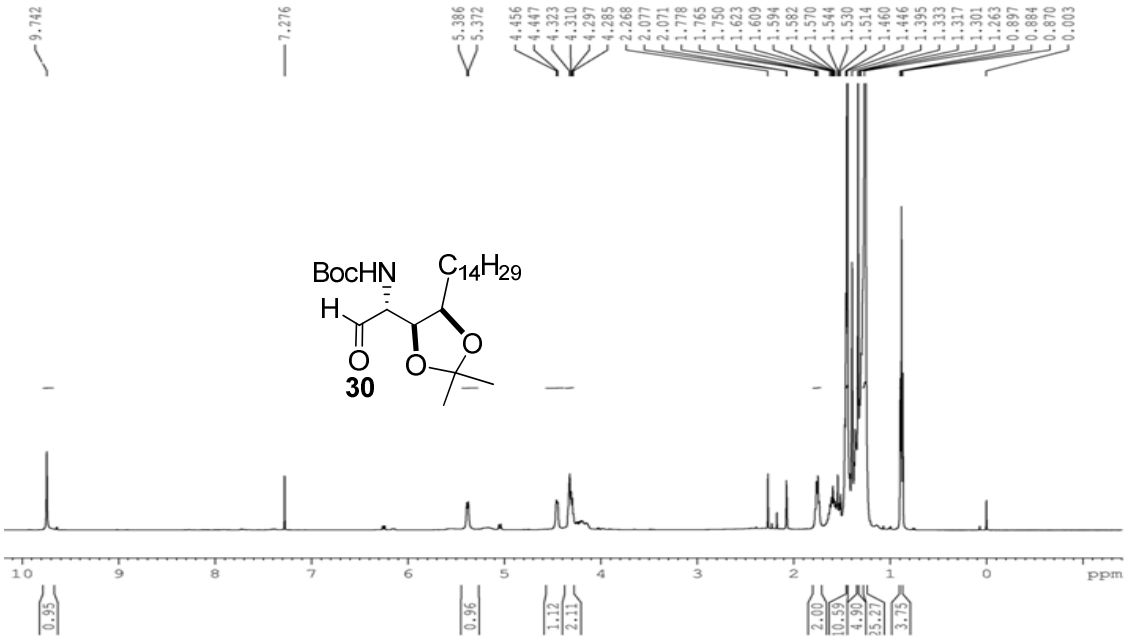
^{1}H and COSY-NMR spectra of compound **26**

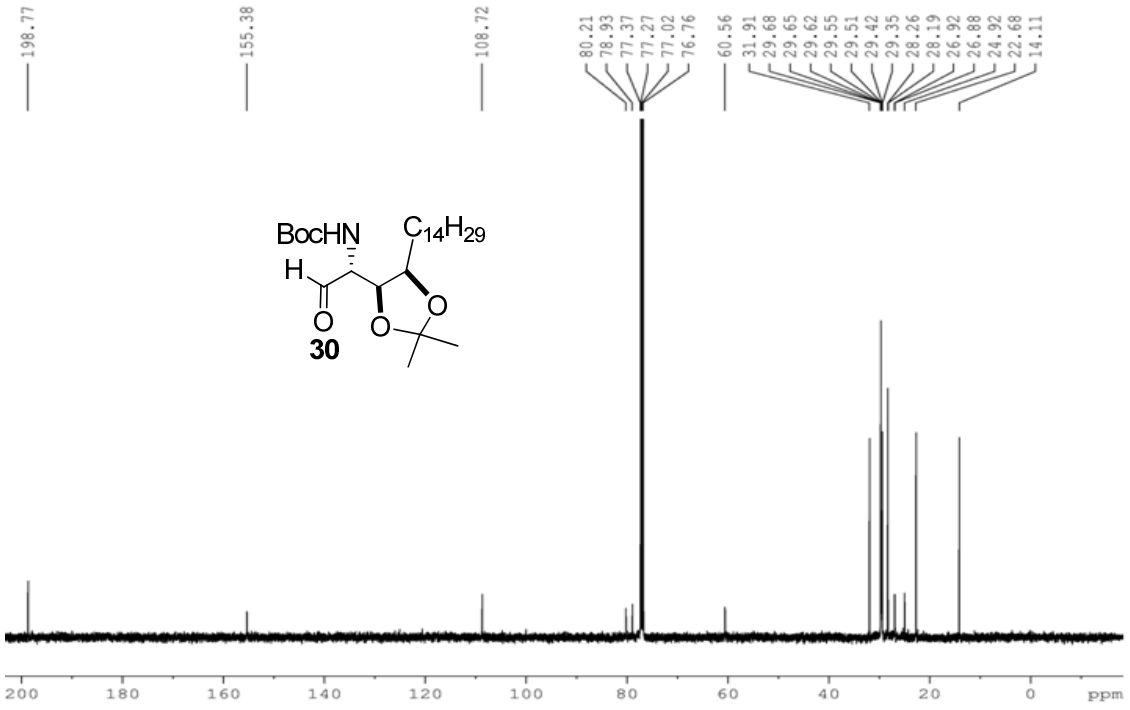




Compound 30. To a solution of primary alcohol **29a** (100 mg, 0.22 mmol, 1 eq) in CH_2Cl_2 (5 mL) was added $NaHCO_3$ (45.9 mg, 0.54 mmol, 2.5 eq) and Dess-Martin periodinane (185.5 mg, 0.43 mmol, 2 eq), and the reaction mixture was stirred for 1 h at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $Na_2S_2O_3$ (20 mL) and sat. aq. $NaHCO_3$ (50 mL), extracted with CH_2Cl_2(2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated and passed through short silica pad by washing with ethylacetate, the resulting filtrate was dried over anhydrous Na_2SO_4 and concentrated to afford phytosphingosine-1-al **30** as a white solid which was directly used for the next step without further purification R_f 0.48 (hexane/EtOAc 7:3); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.74 (s, 1H), 5.37 (d, J = 7 Hz), 4.45 (d, J = 5 Hz), 4.32-4.28 (m, 2H), 1.77-1.73 (m, 2H), 1.46 (s, 9H), 1.39 (s, 6H), 1.26 (m, 24H), 0.88 (t, J = 6.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 198.7, 155.3, 108.7, 80.2, 78.9, 60.5, 31.9, 29.68, 29.65, 29.62, 29.55, 29.51, 29.42, 29.35, 28.2, 28.1, 26.9, 24.9, 22.6, 14.1; HR-ESI-MS $[M+MeOH+Na]^+C_{27}H_{53}NO_6Na$ calc'd for m/z 510.37706, found 510.37799.

^{1}H, ^{13}C-NMR spectra of compound **30**

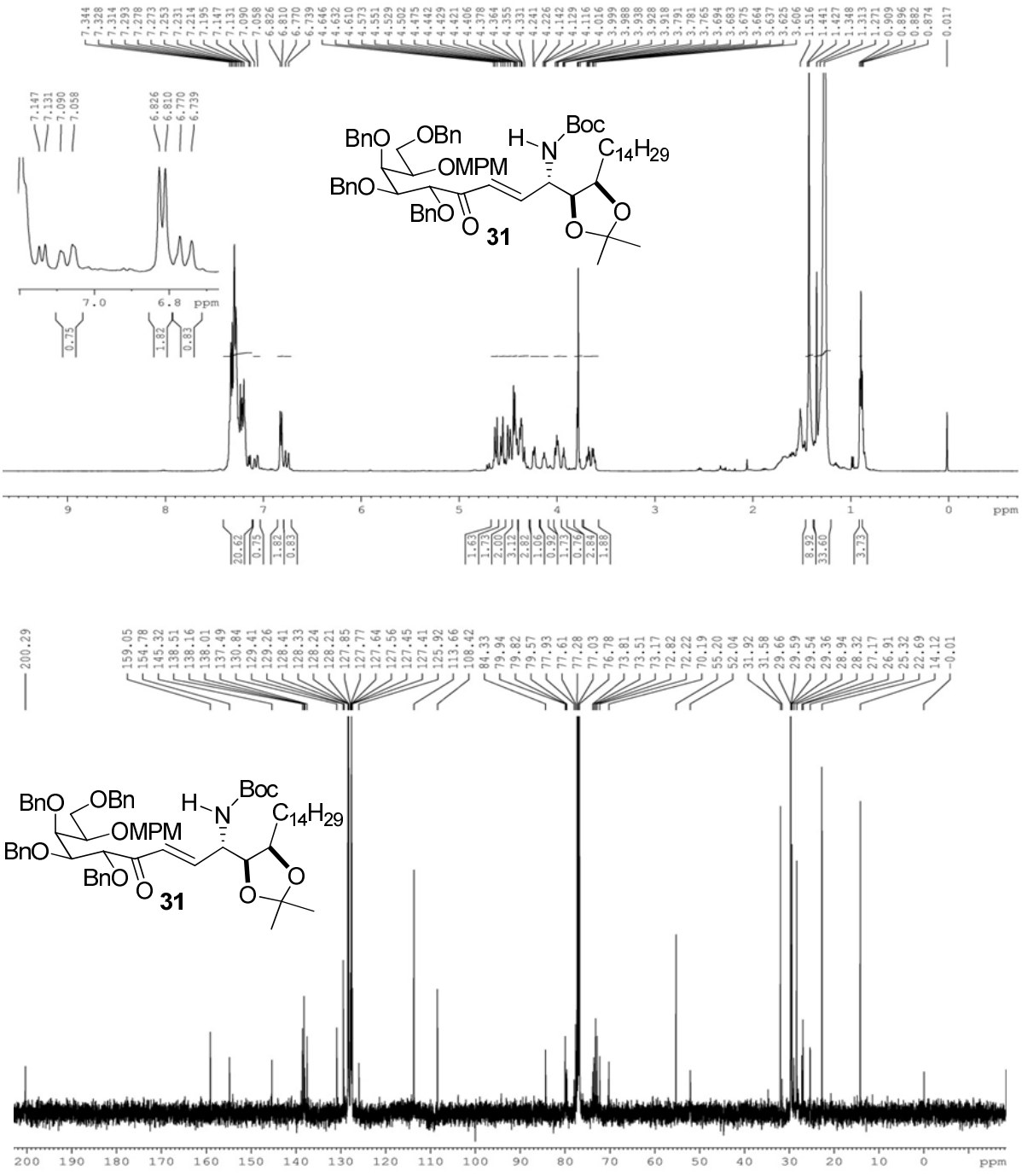




Compound 31. To a solution of compound **23** (341 mg, 0.44 mmol, 2 eq) in EtOH (4 mL) was added a solution of phytosphingosine-1-al **30** (0.22 mmol) in EtOH (2.5 mL), K_2CO_3(90.6 mg, 0.65 mmol, 3 eq) and stirred at room temperature for 1h under argon atmosphere. The reaction mixture was quenched with aqueous citric acid (1 gm in 5 mL of H_2O), washed with H_2O (30 mL), extracted with EtOAc (2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 to 90:10 afforded compound **31** (95 mg, 39%, two steps) as a colorless viscous solid: R_f 0.45 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34-7.19 (m, 22H), 7.10 (dd, J = 16, 8 Hz, 1H), 6.81 (d, J = 8 Hz, 2H), 6.75 (d, J = 15.5 Hz, 1H), 4.64-4.63 (m, 2H), 4.61-4.57 (m, 2H), 4.50-4.47 (m, 2H), 4.44-4.40 (m, 3H), 4.37-4.33 (m, 3H), 4.23 (d, J = 7.5 Hz, 1H), 4.14-4.11 (m, 1H), 4.01-3.98 (m, 2H), 3.93-3.91 (m, 1H), 3.78 (s, 3H), 3.69-3.60 (m, 2H), 1.44 (s, 9H), 1.34 (s, 6H), 1.31-1.27 (m, 26H),0.89 (t, J = 6.5 Hz, 3H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 200.3, 159.0, 154.7, 145.3, 138.5, 138.1, 138.0, 137.5, 130.8, 129.2, 128.4, 128.2, 127.7, 127.5, 127.4, 125.9, 113.6, 108.4, 84.3,

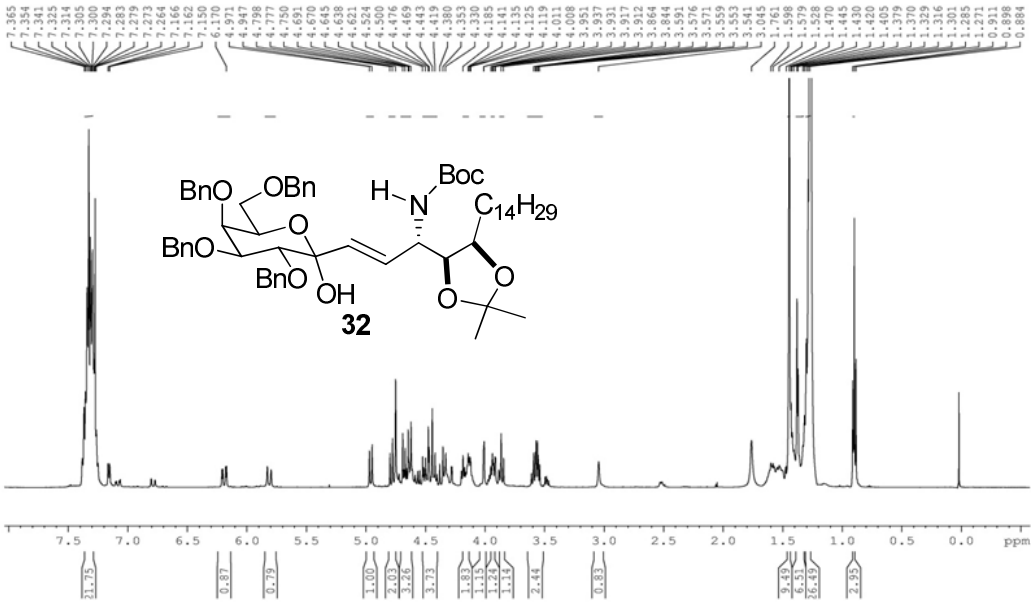
79.9, 79.5, 77.9, 73.5, 73.1, 72.8, 72.2, 70.2, 55.2, 52.0, 31.9, 31.5, 29.6, 29.3, 28.9, 28.3, 27.1, 26.9, 25.3, 22.7, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{69}H_{93}NO_{11}Na$ calc'd for m/z 1134.66463 , found 1134.66481.

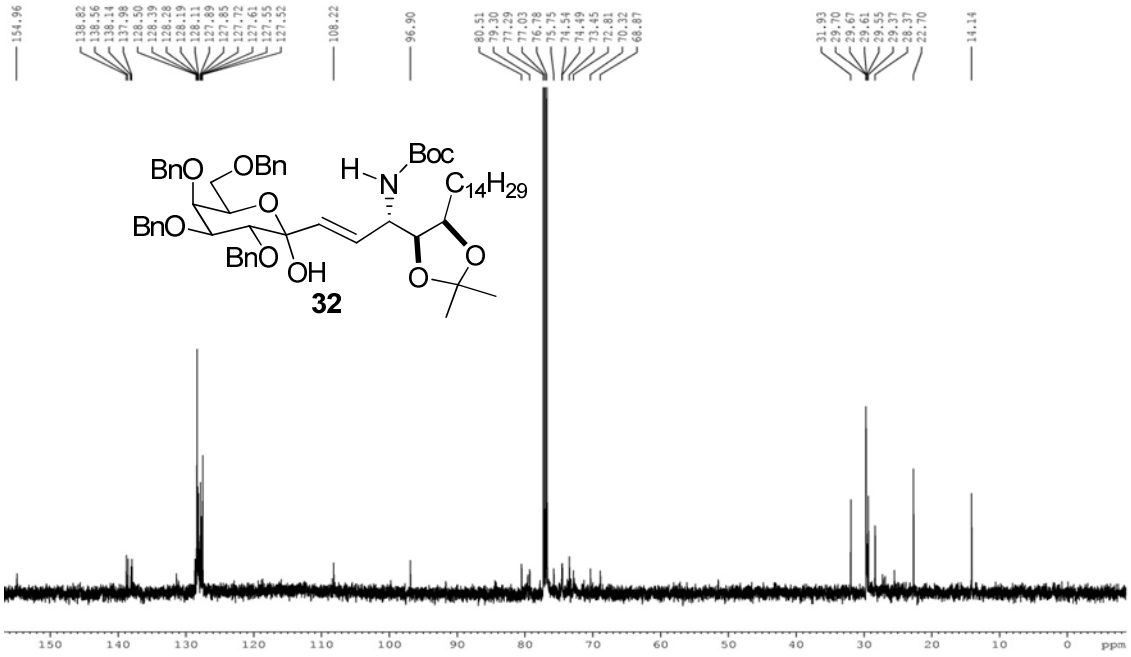
^1H, ^{13}C-NMR spectra of compound **31**



Compound 32. To a solution of compound **31** (200 mg, 0.18 mmol, 1 eq) in CH_2Cl_2/H_2O (10:1, 11 mL) was added DDQ (69 mg, 0.3 mmol, 1.7 eq) at 0 °C, the reaction mixture was allowed to warm to room temperature under argon atmosphere. After 5h, the reaction mixture was quenched with sat. aq. $NaHCO_3$(2 x 50 mL), extracted with CH_2Cl_2(2 × 75 mL), washed with water (2× 100 mL) and brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 80:20 afforded compound **32** (123 mg, 69%) as a colorless viscous solid. R_f 0.23 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36-7.26 (m, 20H), 7.15 (dd, *J* = 8, 2 Hz, 1H),6.19 (dd, *J* = 16, 4 Hz, 1H), 5.81 (d, *J* = 16 Hz, 1H),4.95 (d, *J* = 12 Hz, 1H), 4.79-4.75 (m, 2H), 4.63-4.61 (m, 3H), 4.52-4.33 (m, 3H), 4.19-4.11 (m, 2H), 4.0 (d, *J* = 1.5 Hz, 1H), 3.95-3.91 (m, 1H), 3.85 (d, *J* = 10 Hz, 1H), 3.59-3.54 (m, 2H), 3.04 (s, 1H), 1.43 (s, 9H), 1.37 (s, 6H), 1.32-1.27 (m, 26H), 0.89 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.9, 138.8, 138.5, 138.1, 137.9, 128.5, 128.3, 128.1, 127.9, 127.7, 127.5, 108.2, 96.9, 80.5, 79.3, 75.7, 74.4, 73.4, 72.8, 70.3, 68.8, 31.9, 29.7, 29.6, 29.5, 29.3, 28.3, 22.7, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{61}H_{85}NO_{10}Na$ calcd for m/z 1014.60712, found 1014.60669.

^{1}H, ^{13}C-NMR spectra of compound **32**

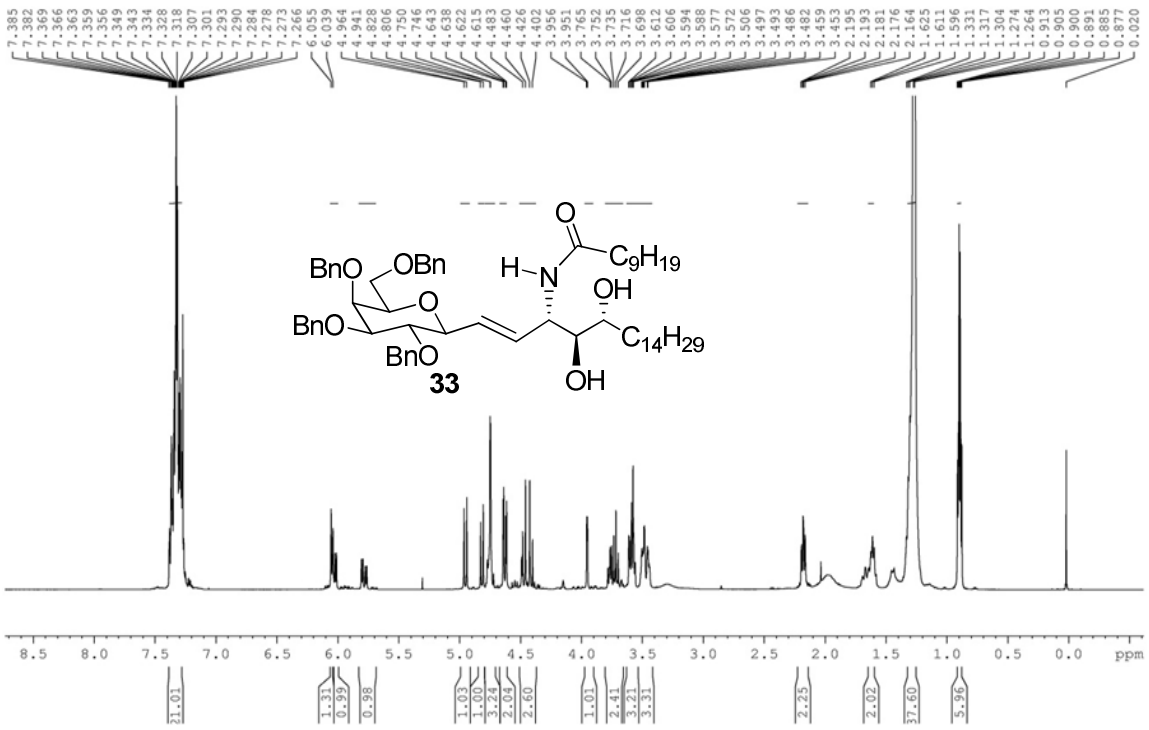


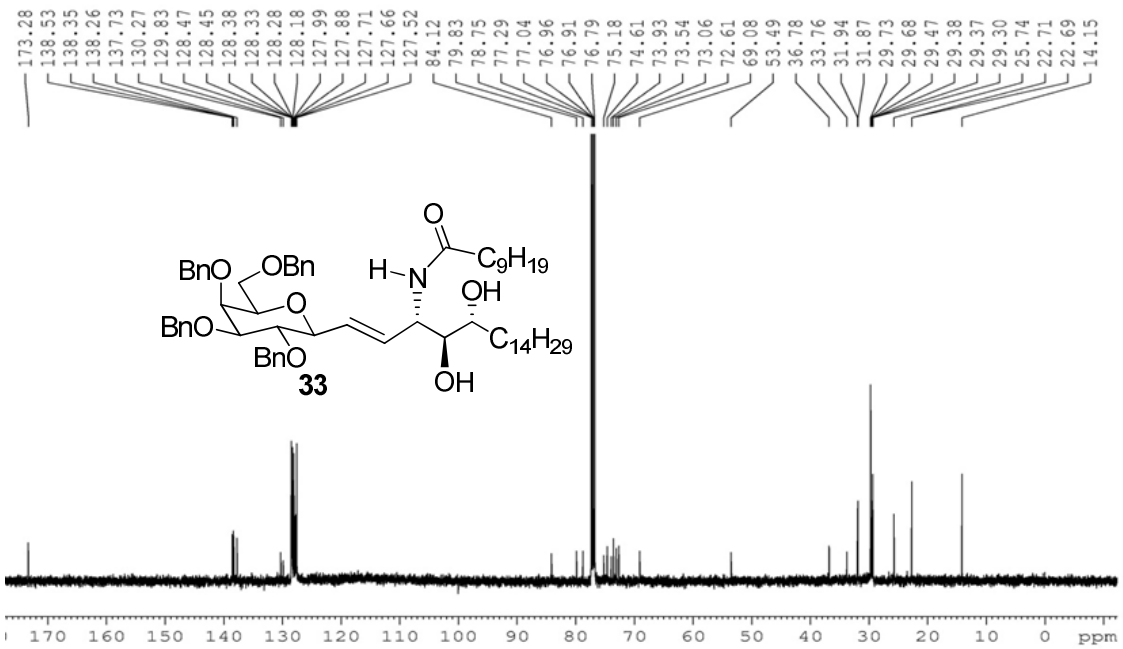


Compound 33. To a solution of compound **32** (40 mg, 0.04 mmol, 1 eq) in CH_3CN (5 mL) was added Et_3SiH (64 µL, 0.40 mmol, 10 eq) and TMSOTf (22 µL, 0.12 mmol, 3 eq) at 0 °C and stirred for 10 min. The reaction mixture was quenched with sat. aq.$NaHCO_3$ (30 mL), extracted with EtOAc (2 × 50 mL), washed with H_2O (30 mL), the organic layer was dried over anhydrous Na_2SO_4 and concentrated. Without further purification the resulting crude mixture was treated with 4 mL of EtOH/2M HCl/THF (4:1:1) at 70 °C overnight. The reaction mixture was cooled down to room temperature and then diluted with 20 mL of H_2O, extracted with $CHCl_3$:MeOH (7:1, 3 × 30 mL), dried over anhydrous Na_2SO_4 and concentrated. The resulting crude free 1° amine was dried in vacuum and then dissolved in 2 mL of DCM/DMF (5:2). 4-Nitrophenyl decanoate (17.1 mg, 0.06 mmol, 1.5 eq) and K_2CO_3 (17 mg, 0.12 mmol, 3 eq) were added and stirred vigorously overnight at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $NaHCO_3$(15 mL), extracted with EtOAc (2 × 30 mL), the organic layer was thoroughly washed with water (3 × 30 mL), brine(10 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10, to

60:40 afforded compound **33** (21mg, 53%, three steps)as a white solid: R_f 0.37 (hexane/EtOAc 7:3); [α] = -3.03° (0.33, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.26 (m, 20H), 6.04 (d, *J* = 8 Hz, 1H), 6.01 (d, *J* = 6 Hz, 1H),5.78 (dd, *J* = 15.5, 6 Hz, 1H), 4.95 (d, *J* = 11.5 Hz, 1H), 4.81 (d, *J* = 12 Hz, 1H), 4.75-4.74 (m, 3H), 4.62 (dd, *J* = 10.5, 2.5 Hz, 2H), 4.48-4.40 (m, 2H), 3.95 (d, *J* = 2.5 Hz, 1H), 3.78-3.69 (m, 2H), 3.61-3.55 (m, 3H), 3.50-3.48 (m, 3H), 2.18-2.16 (m, 2H), 1.65-1.60 (m, 2H), 1.33-1.26 (m, 38H), 0.87-0.91 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 138.5, 138.3, 138.2, 137.7, 130.2, 129.8, 128.4, 128.3, 128.1, 127.9, 127.7, 127.6, 127.5, 84.1, 79.8, 78.7, 75.1, 74.6, 73.9, 73.5, 73.0, 72.6, 69.0, 53.5, 36.7, 33.7, 31.9, 31.8, 29.7, 29.6, 29.4, 29.3, 25.7, 22.7, 22.6, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{63}H_{91}NO_8Na$ calc'd for m/z 1012.66424, found 1012.66579.

^{1}H, ^{13}C-NMR spectra of compound **33**

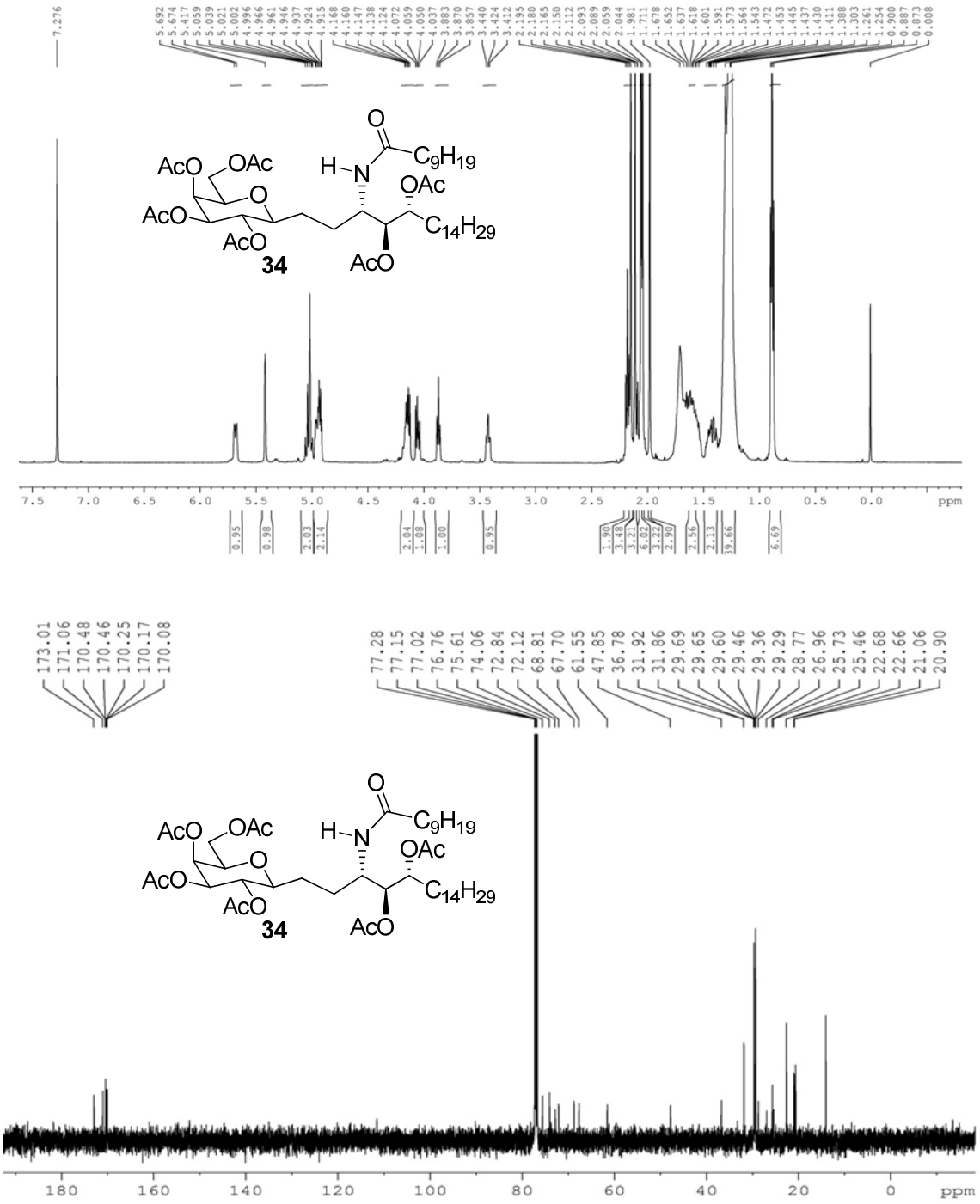




Compound 34. A solution of compound **33** (45 mg, 0.04 mmol, 1 eq) in EtOH (4 mL) was added 35 mg of 10% Pd/C and TFA (14µL, 0.18 mmol, 4 eq). H_2 gas was purged into the reaction mixture for 2 min, and stirred overnight under H_2atmosphere (balloon).The reaction mixture was diluted with MeOH (60 mL) filtered through celite pad and concentrated to afford compound **1** in a quantitative yield. To 18 mg (0.03 mmol) of compound **1** in pyridine (2 mL) was added acetic anhydride (0.3 mL), and then the reaction mixture was stirred at room temperature overnight under argon atmosphere. The reaction mixture was diluted with H_2O (10 mL), extracted with EtOAc (2 × 25 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 80:20 to 50:50 afforded compound **34** (11mg)as a white solid R_f 0.60 (EtOAc 100%); [α] = +9.09° (0.11, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ5.71 (d, *J* = 10 Hz, 1H), 5.41 (s, 1H), 5.05-4.99 (m, 2H), 4.96-4.91 (m, 2H), 4.15-4.12 (m, 2H), 4.05 (dd, *J* = 11, 7.5 Hz, 1H), 3.86 (t, *J* = 6.5 Hz, 1H),3.43-3.41 (m, 1H),2.19-2.16 (m, 2H), 2.14 (s, 3H), 2.11 (s, 3H), 2.05 (s, 6H), 2.04 (s, 3H), 1.98 (s, 3H),1.61-1.54 (m, 2H), 1.45-1.38 (m, 2H), 1.30-1.25 (m, 40H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz)

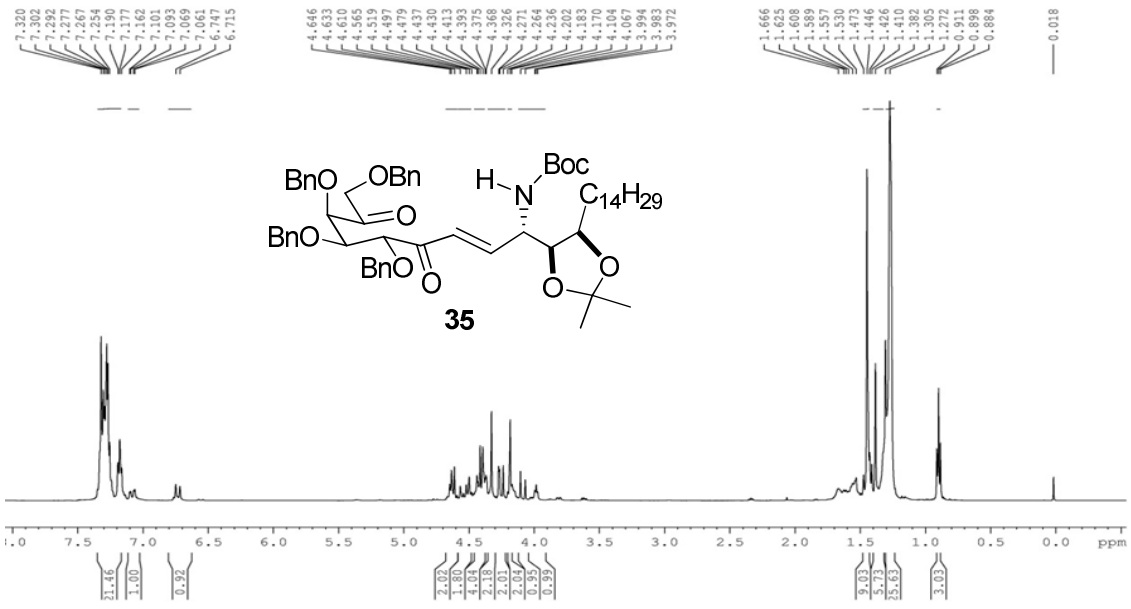
δ173.0, 171.0, 170.48, 170.46, 170.2, 170.1, 170.0, 75.6, 74.0, 72.8, 72.1, 67.8, 67.7, 61.5, 47.8, 36.7, 31.9, 31.8, 29.7, 29.6, 29.4, 29.3, 29.2, 28.7, 26.9, 25.7, 25.6, 22.6, 21.0, 20.9, 20.8, 20.7, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{47}H_{81}NO_{14}Na$ calc'd for m/z 906.55548, found 906.55286.

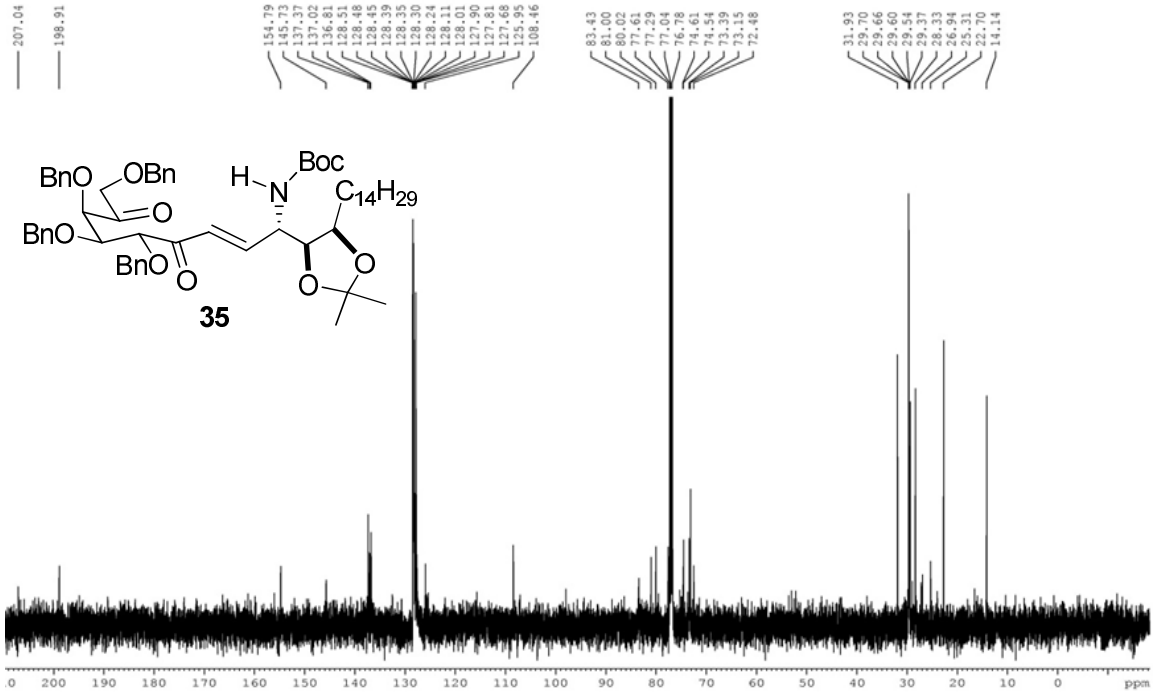
^1H, ^{13}C-NMR spectra of compound **34**



Compound 35. To a solution of hemiketal **32** (160 mg, 0.16 mmol, 1 eq) in DCM (15 mL) was added $NaHCO_3$ (67.7 mg, 0.8 mmol, 5 eq) and Dess-Martin periodinane (205.4 mg, 0.48 mmol, 3eq), then the reaction mixture was stirred for 1h at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq. $Na_2S_2O_3$ (30 mL) and sat. aq.$NaHCO_3$ (50 mL), extracted with CH_2Cl_2 (2×80 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 85:15 afforded diketone **35** (145 mg, 91%), as a colorless viscous solid R_f 0.47 (hexane/EtOAc 7:3), [α] = -5.00° (0.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32-7.16(m, 20H), 7.08 (dd, *J* = 16, 4 Hz, 1H), 6.73 (d, *J* = 16 Hz, 1H), 4.64-4.61 (m, 2H), 4.56-4.47 (m, 2H), 4.43-4.36 (m, 4H), 4.32 (s, 2H), 4.26-4.23 (m, 2H),4.20-4.17 (m, 2H),4.10-4.06 (m, 1H), 3.98 (app t, *J* = 5.5 Hz, 1H),1.44 (s, 9H), 1.38 (s, 6H),1.30-1.27 (m, 26H),0.89 (t, *J* = 6.5 Hz, 3H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 207.0, 198.9, 154.7, 145.7, 137.3, 137.0, 136.8, 128.5, 128.39, 128.30, 128.1, 128.0, 127.9, 127.8, 127.6, 125.9, 108.4, 83.4, 81.0, 80.0, 74.6, 74.5, 73.3, 73.1, 72.4, 31.9, 29.7, 29.6, 29.5, 28.3, 26.9, 25.3, 22.7, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{61}H_{83}NO_{10}Na$ calc'd for m/z 1012.59147, found 1012.59229.

^{1}H, ^{13}C-NMR spectra of compound **35**

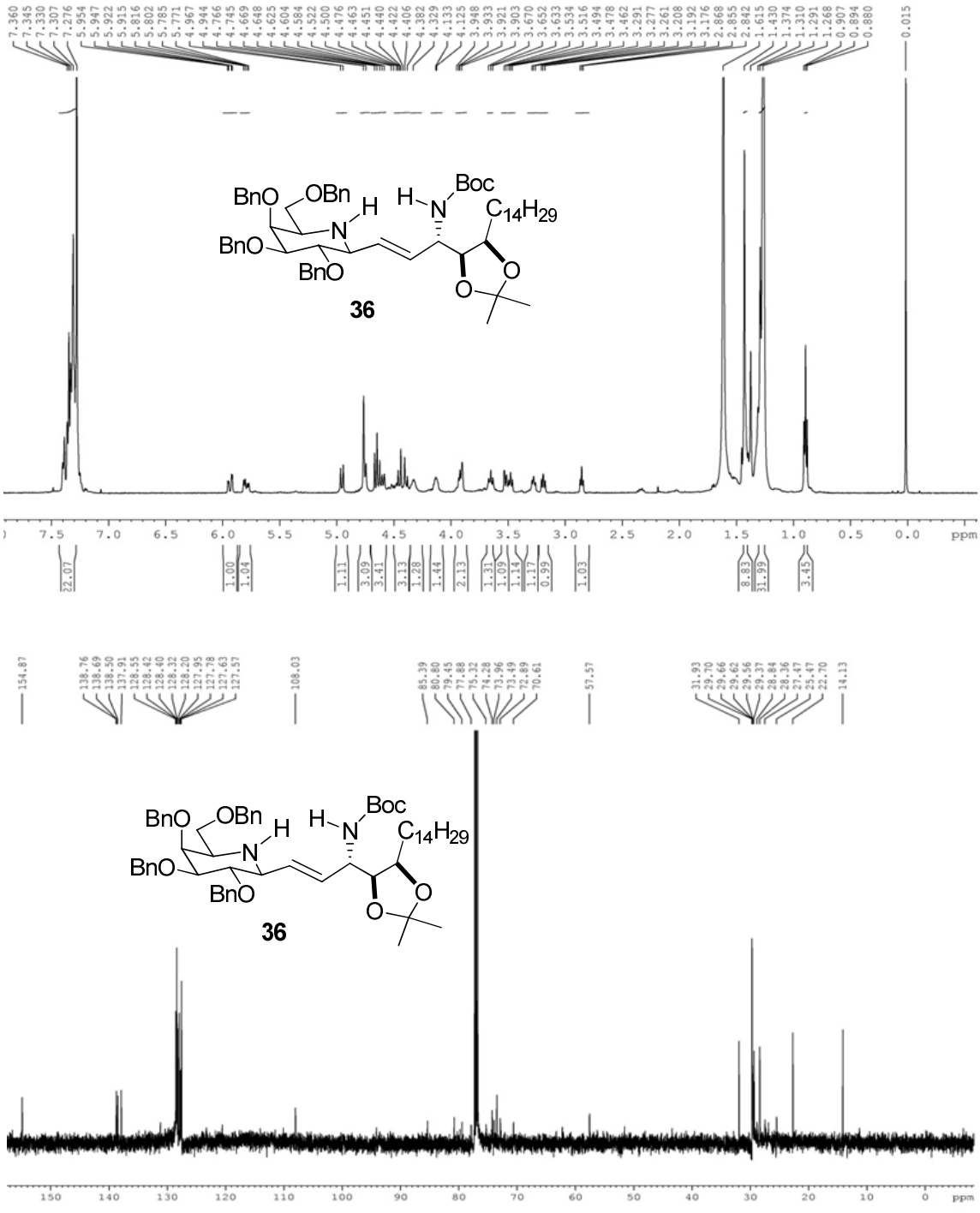




Compound 36. To a solution of diketone **35** (140 mg, 0.14 mmol, 1 eq) in MeOH (8 mL) was added ammonium formate (35.5 mg, 0.56 mmol, 4 eq) and $NaCNBH_3$ (35.6 mg, 0.56 mmol, 4 eq), and then the reaction mixture was stirred overnight at room temperature under argon atmosphere. The reaction mixture was quenched with sat. aq.$NaHCO_3$ (30 mL), extracted with EtOAc (2×80 mL), the organic layer was washed with H_2O (2× 70 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 70:30 afforded compound **36** (90mg, 65.5%) as a pale yellow viscous solid: R_f0.40 (hexane/EtOAc 5:3); [α] = -7.89° (0.38, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36-7.27 (m, 20H), 5.93 (dd, *J* = 16, 3.5 Hz, 1H),5.79 (dd, *J* = 15.5, 7 Hz, 1H), 4.95 (d, *J* = 11.5 Hz, 1H), 4.96-4.74 (m, 3H), 4.66-4.58 (m, 3H), 4.50-4.38 (m, 3H), 4.32 (m, 1H), 4.13-4.12 (m, 1H), 3.94-3.90 (m, 2H), 3.65 (app t, *J* = 9 Hz, 1H), 3.53-3.46 (m, 2H), 3.27 (app t, *J* = 7 Hz, 1H), 3.19 (app t, *J* = 8 Hz, 1H),2.85 (app t, *J* = 6.5 Hz, 1H), 1.43 (s, 9H), 1.29-1.31(m, 32H),0.89 (t, *J* = 6.5 Hz, 3H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.8, 138.7, 138.6, 138.5, 137.9, 128.5, 128.4, 127.7,

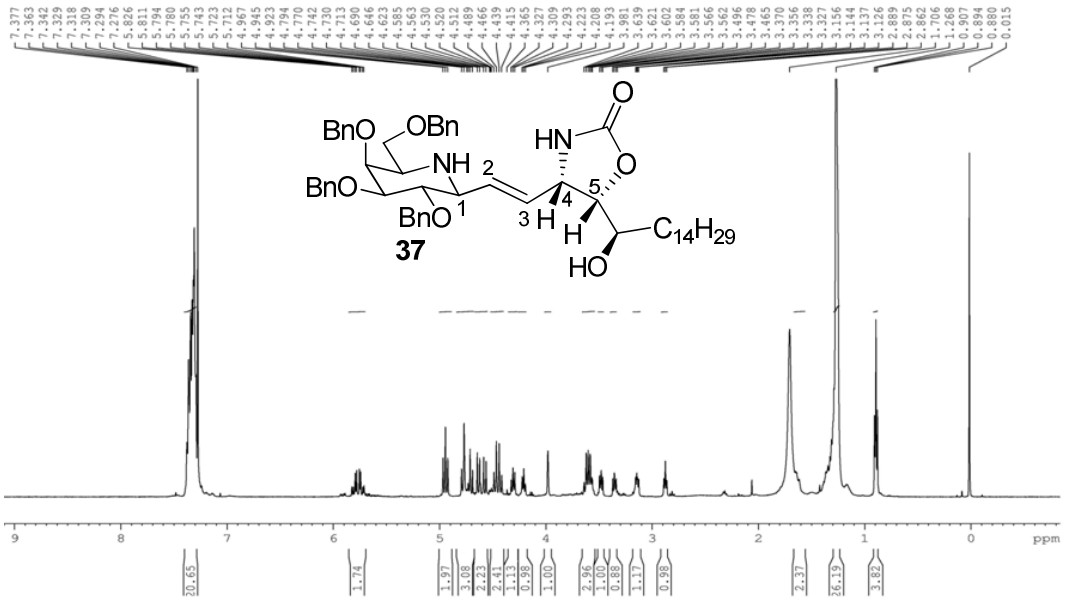
127.5, 80.0, 80.8, 79.4, 75.3, 74.2, 73.9, 73.4, 72.8, 70.6, 57.5, 31.9, 29.7, 29.6, 29.5, 29.3, 28.8, 28.3, 25.4, 22.7, 14.1; HR-ESI-MS $[M+H]^+$ $C_{61}H_{87}N_2O_8$ calc'd for m/z 975.64624, found 975.64839.

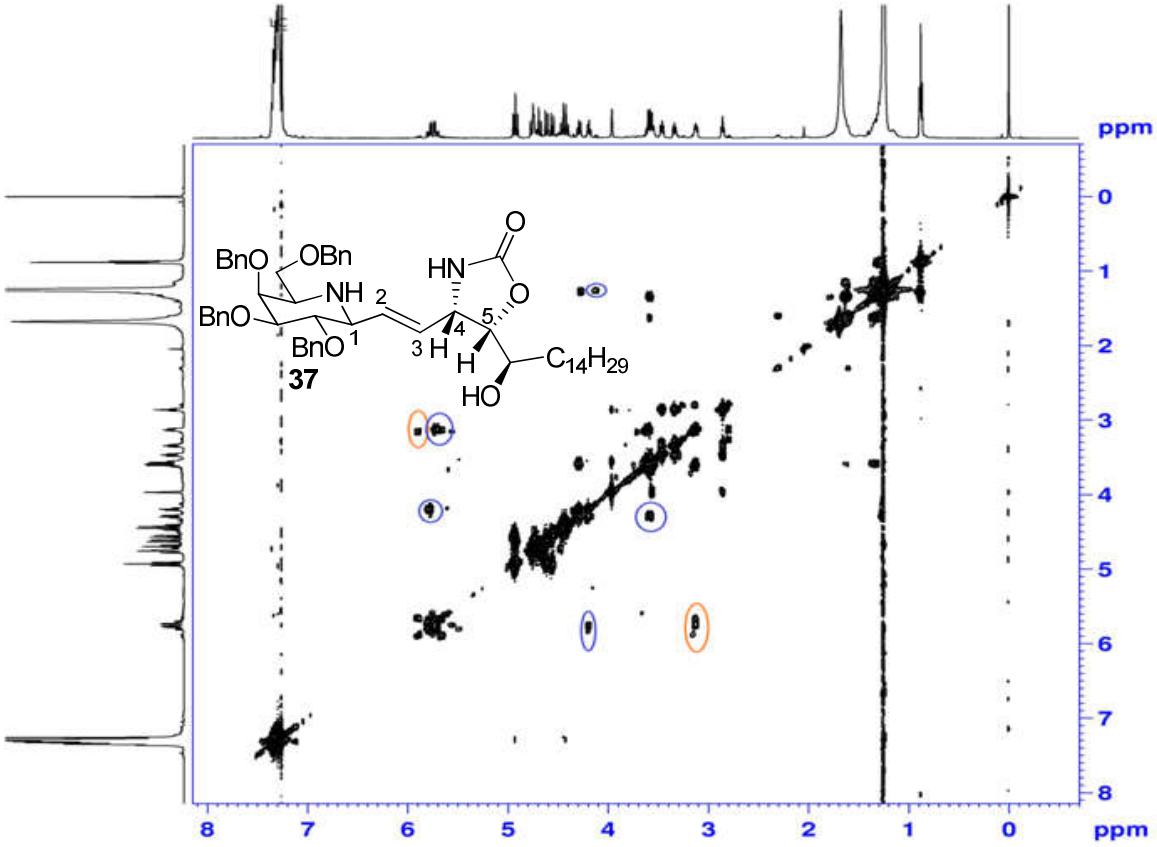
^1H, ^{13}C-NMR spectra of compound **36**



Compound 37. A solution of compound **36** (28 mg, 0.03 mmol) in 3 mL of EtOH/2M HCl (4:1) was refluxed for 3 h at 70 °C. After completion of starting material as indicated by TLC, the reaction mixture was diluted with MeOH (10 mL) and evaporated. The resulting crude mixture was lyophilized over benzene (3 mL). To 12 mg (0.014 mmol) of free amine in dry THF (1 mL) was added N,N-carbonyldiimidazole (21 mg, 0.126 mmol, 9 eq) at 0 °C, and then the reaction mixture was stirred for 6h at room temperature under argon atmosphere. Evaporation of solvent and purification by flash chromatography using hexane/EtOAc 80:20 to 40:60 afforded compound **37** (6 mg, 49% two steps) as a viscous solid: R_f0.44 (hexane/EtOAc 2:8); ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.37-7.27 (m, 20H), 5.80 (H-3, dd, *J* = 15.5, 7.5 Hz, 1H), 5.73 (H-2, dd, *J* = 15.5, 6 Hz, 1H), 4.96-4.92 (m, 2H), 4.79-4.69 (m, 3H), 4.64-4.56 (m, 2H), 4.48-4.36 (m, 2H), 4.30 (H-5, app t, *J* = 8.0 Hz, 1H), 4.20 (H-4, app t, *J* = 7.5 Hz,1H), 3.98 (s, 1H), 3.63-3.56 (m, 3 H), 3.47 (app t, *J* = 9 Hz, 1H), 3.35 (app t, *J* = 8 Hz, 1H), 3.14 (H-1, dd, *J* = 9.5, 6 Hz, 1H), 2.87 (t, *J* = 7 Hz, 1H), 1.26 (m, 26H), 0.89 (t, *J* = 6.5 Hz, 3H); HR-ESI-MS $[M+H]^+$ $C_{54}H_{73}N_2O_7$ calc'd for m/z 861.54178, found 861.54407.

^{1}H, COSY-NMR spectra of compound **37**

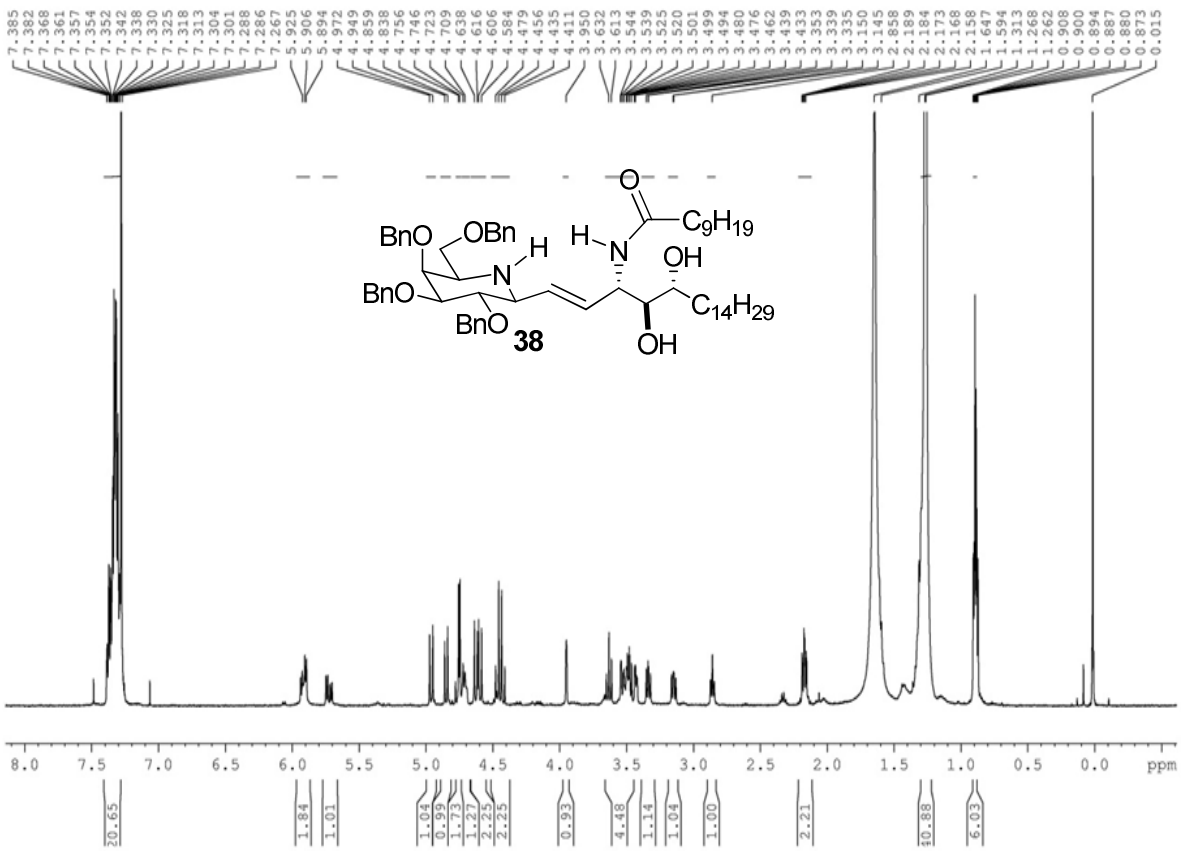


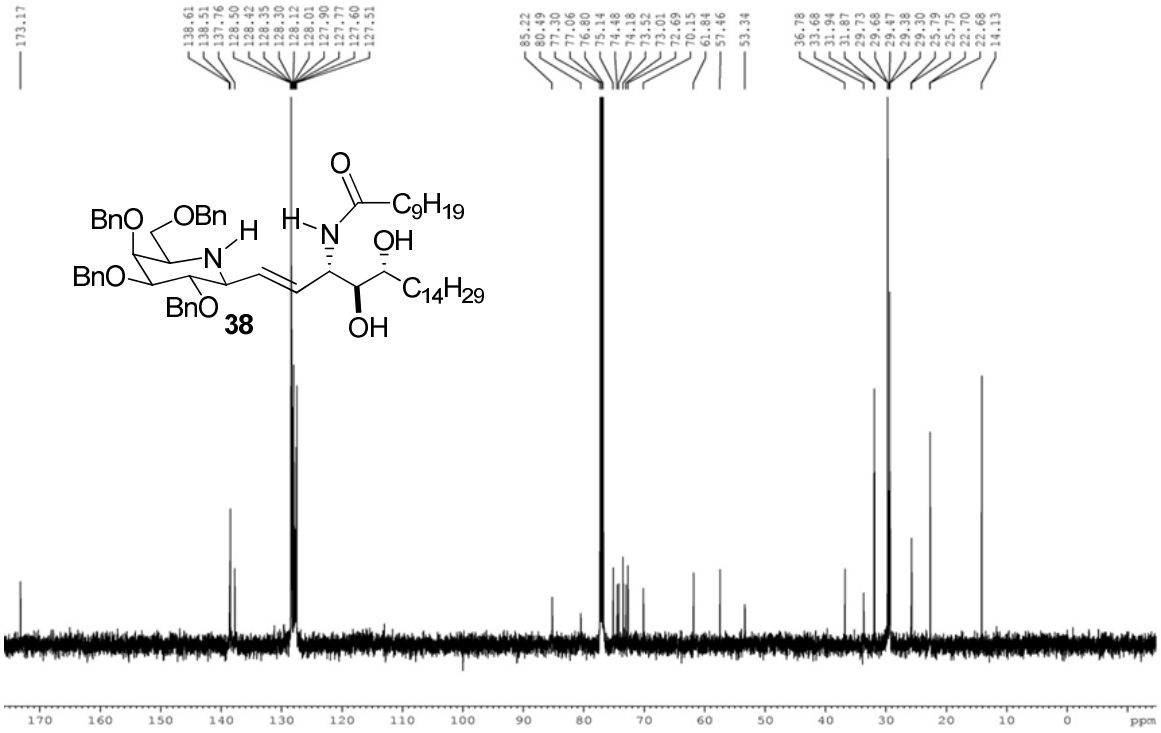


Compound 38. Compound **36** (80 mg, 0.08 mmol) was dissolved in 8 mL of EtOH/2M HCl (4:1), and then refluxed for 3 h at 70°C. After completion of starting material as indicated by TLC, the reaction mixture was diluted with H_2O (10 mL), extracted with $CHCl_3$/MeOH (7:1, 4 × 50 mL), the combined organic layers were dried over anhydrous $Na_2SO_4$and concentrated. The resulting crude free amine was dried in vacuum and dissolved in 8 mL of DMF/DCM (2:5), and then added 4-nitrophenyl decanoate (36.1 mg, 0.12 mmol, 1.5 eq), K_2CO_3(34 mg, 0.24 mmol, 3 eq), and stirred vigorously overnight at room temperature under argon atmosphere. After completion of the starting material as indicated by TLC, the reaction mixture was quenched with sat. aq. $NaHCO_3$ (2 × 10 mL), extracted with EtOAc (2 × 75 mL), the organic layer was thoroughly washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography (hexane/EtOAc 80:20 to 60:40 to 40:60)afforded

compound **38** (60 mg, 74%, two steps)as a white solid: R_f0.55 (EtOAc 100%), [α] = 10.7° (0.28, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.26 (m, 20H), 5.93 (dd, *J* = 15.5, 6 Hz, 1H), 5.91 (m, 1H),5.72 (dd, *J* = 16, 7 Hz, 1H), 4.96 (d, *J* = 11.5 Hz, 1H), 4.84 (d, *J* = 10.5 Hz, 1H),4.77-4.69 (m, 3H), 4.63-4.58 (m, 2H), 4.47-4.41 (m, 2H), 3.95 (s, 1H), 3.63 (app t, *J* = 9 Hz, 1H), 3.54-3.46 (m, 4H), 3.42 (dd, *J* = 6.5, 3 Hz, 1H), 3.33 (dd, *J* = 9, 7 Hz, 1H), 3.14 (dd, *J* = 9, 7 Hz, 1H),2.85 (t, *J* = 7 Hz, 1H), 2.18-2.16 (m, 2H), 1.31-1.26 (m, 40H), 0.87-0.90 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 138.6, 138.5, 137.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 85.2, 80.4, 76.8, 75.1, 74.4, 74.1, 73.0, 72.6, 70.1, 61.8, 57.6, 53.3, 36.7, 36.6, 31.9, 31.8, 29.7, 29.6, 29.4, 29.3, 25.7, 22.6, 14.1; HR-ESI-MS $[M+H]^+$ $C_{63}H_{93}N_2O_7$ calc'd for m/z 989.69828, found 989.69965.

^{1}H, ^{13}C and COSY-NMR spectra of compound **38**

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74.48
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73.01
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57.46
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36.78
33.68
31.94
31.87
29.73
29.68
29.47
29.38
29.30
25.79
25.75
22.70
22.68
14.13
BnO
OBn
H
N
BnO
BnO
38
O
H
N
C9H19
OH
C14H29
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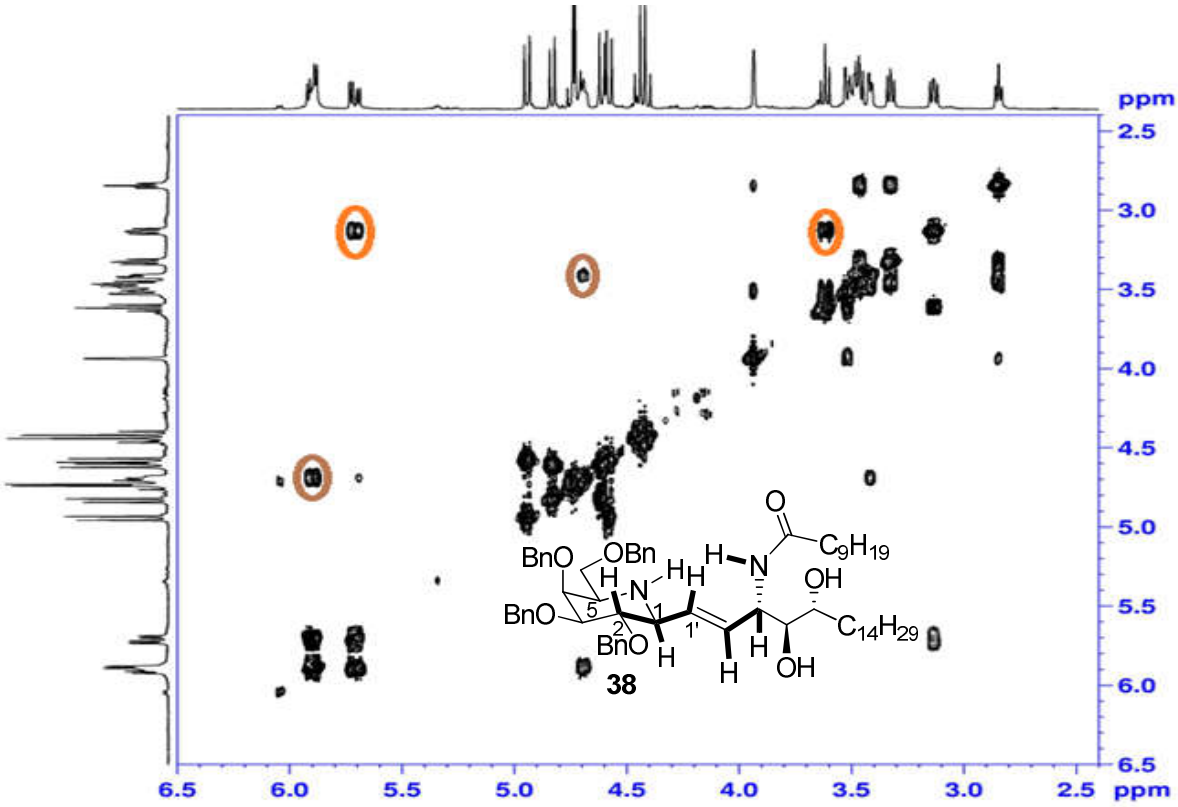
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BnO
5
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H
OH
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O
C9H19
OH
C14H29
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6.0
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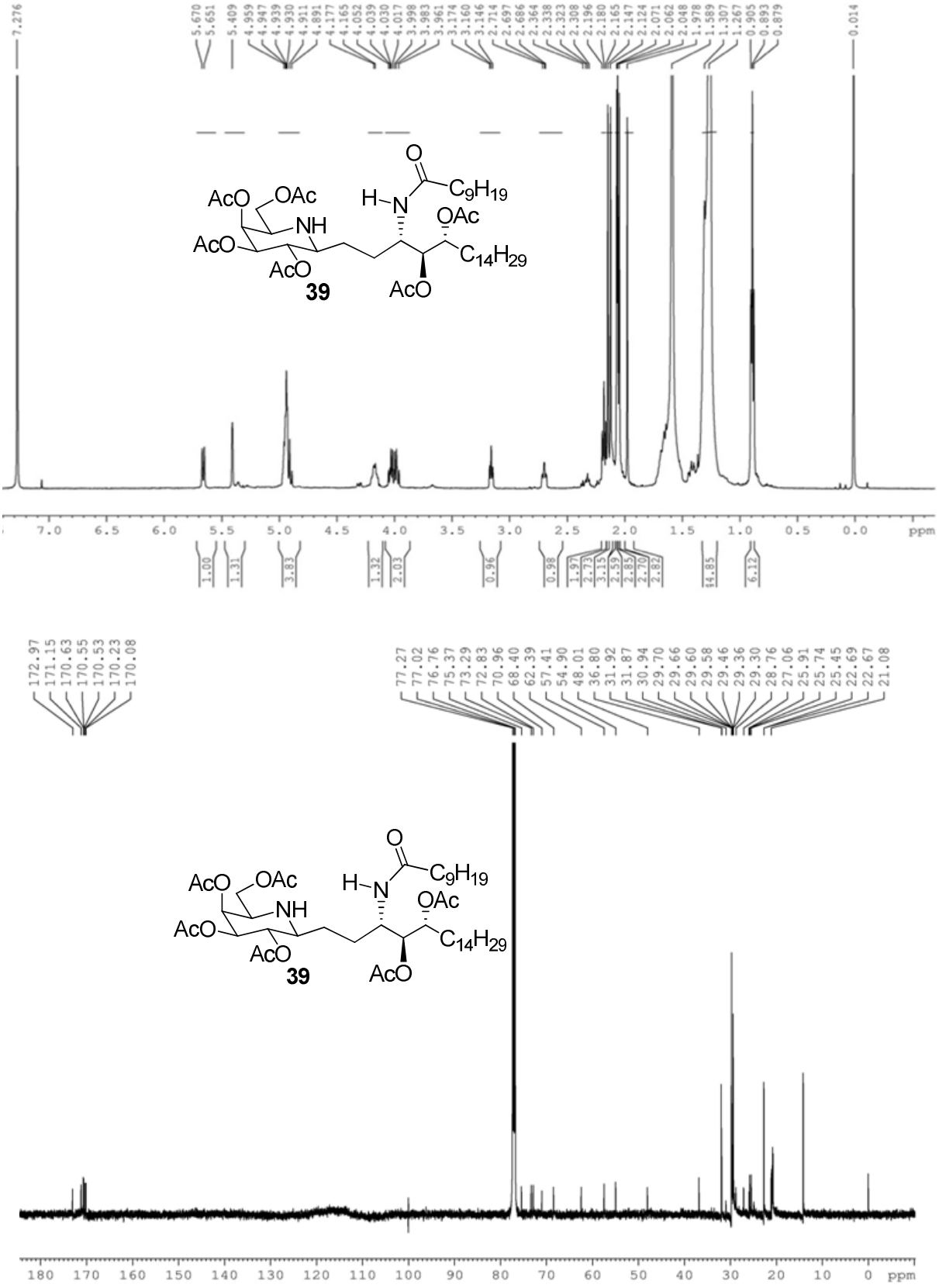
5.0
4.5
4.0
3.5
3.0
2.5
ppm

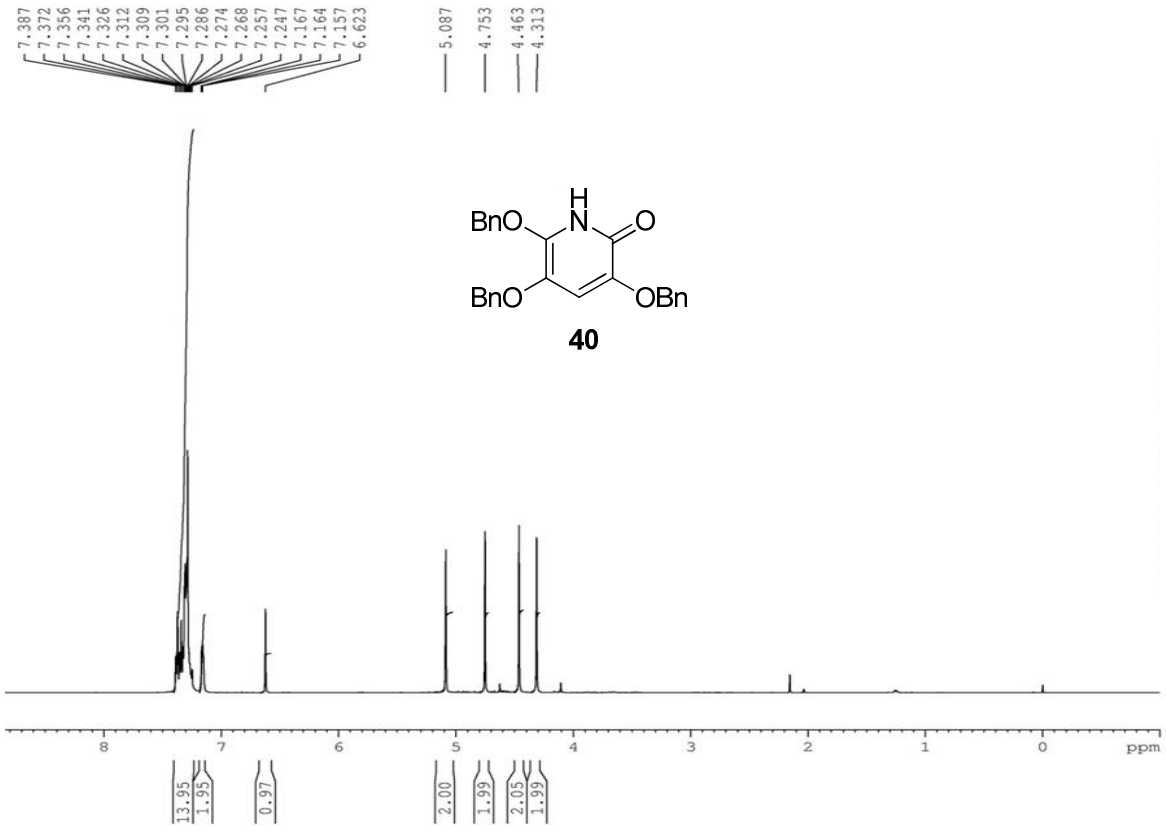
Compound 39. To a solution of compound **38** (55 mg, 0.05mmol, 1 eq) in EtOH (5 mL) was added 40 mg of 10% Pd/C and 2 drops of conc. HCl. H_2 gas was purged into the reaction mixture for 2 min, and stirred overnight under H_2 atmosphere (balloon), the reaction mixture was diluted with MeOH (50 mL), filtered through celite pad and concentrated to afford the desired compound **2** in a quantitative yield. To 20 mg (0.03 mmol) of compound **2** was added pyridine (2 mL) and acetic anhydride (0.5mL). The resulting mixture was stirred overnight under argon atmosphere at room temperature. The reaction mixture was diluted with H_2O (10 mL), extracted with EtOAc (2 × 25 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 80:20 to 40:60 afforded compound **39** (8 mg)as a white solid: R_f 0.45 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ5.66 (d, J = 9.5 Hz, 1H), 5.40 (s, 1H), 4.95-4.89 (m, 4H), 4.17-4.16 (m, 1H), 4.05-3.95 (m, 2H), 3.16 (app t, J = 7 Hz, 1H), 2.71-2.68 (m, 1H), 2.18 (t, J = 7.5 Hz, 2H),2.14 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H),2.06 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H), 1.30-1.26 (m, 44H), 0.89 (t, J = 6Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9, 171.1, 170.6, 170.55, 170.53, 170.2, 170.0, 75.3, 73.2, 72.8, 70.9, 68.4, 62.3, 57.4, 54.9, 48.0, 36.8, 31.9, 31.8, 30.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.0, 25.9, 25.7, 25.4, 21.0, 20.9, 20.8, 20.7, 14.1; HR-ESI-MS $[M+H]^+$ $C_{47}H_{83}N_2O_{13}$ calc'd for m/z 883.58952, found 883.58728.

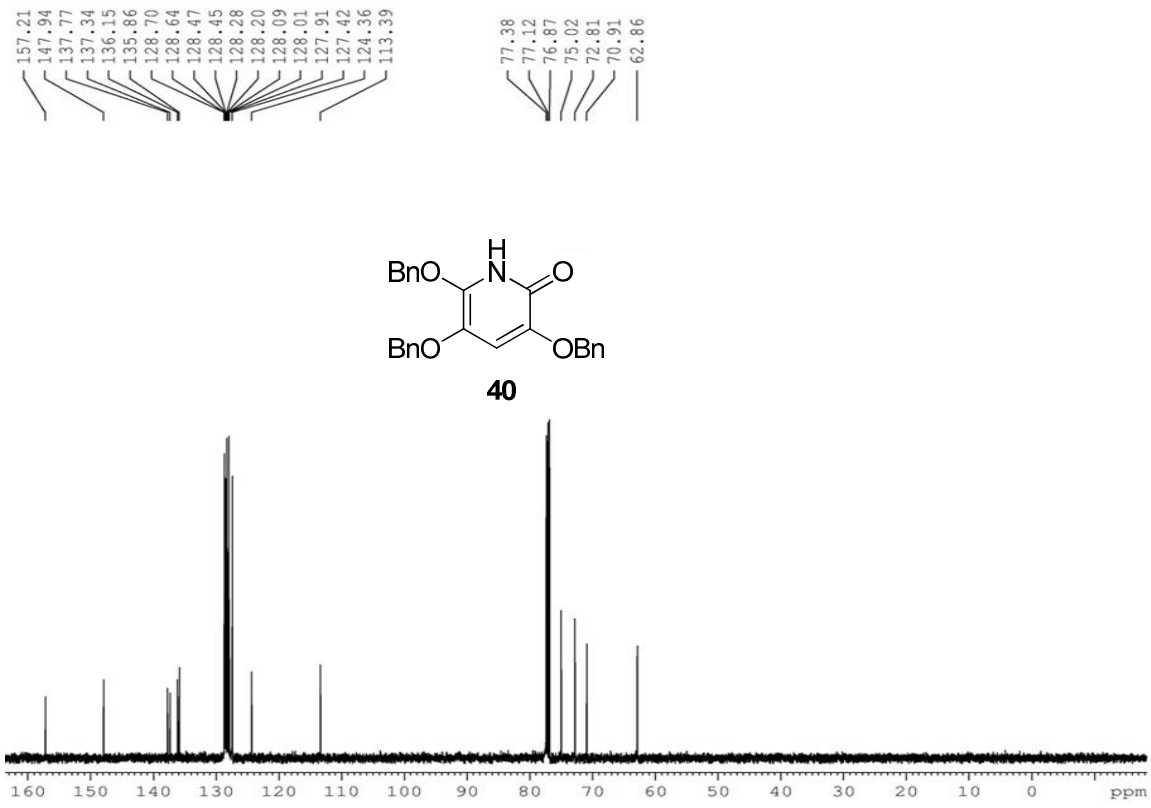
^{1}H, ^{13}C-NMR spectra of compound **39**



3,5-dibenzyloxy-6-benzyloxymethyl-2-pyridone (40). To solution of lactam **14** (240 mg, 0.434 mmol) in EtOH:H_2O (1:1, 9 mL) was added KOH (120 mg, 2.13 mmol), and the resulting mixture was stirred at reflux for 5 h. The reaction mixture was quenched with 1 N HCl (10 mL), extracted with ethyl acetate (2 x 15 mL), dried (Na_2SO_4), concentrated and purified by flash chromatography (hexane/ EtOAc 1:4) affording 2-pyridone **40** (130 mg, 70%). R_f 0.5 (EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.31 (s, 2H), 4.46 (s, 2H), 4.75 (s, 2H), 5.08 (s, 2H), 6.62 (s, 1H), 7.15-7.38 (m, 15H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 62.8, 71.0, 72.8, 75.0, 113.4, 124.3, 127.4, 127.9, 128.0, 128.2, 128.27, 128.4, 128.6, 128.7, 135.9, 136.2, 137.3, 137.7, 147.9, 157.2; HR-ESI-MS $[M + H]^+$ $C_{27}H_{26}NO_4$ calcd for m/z 428.18618, found 428.18638.

^{1}H, ^{13}C-NMR spectra of compound **40**

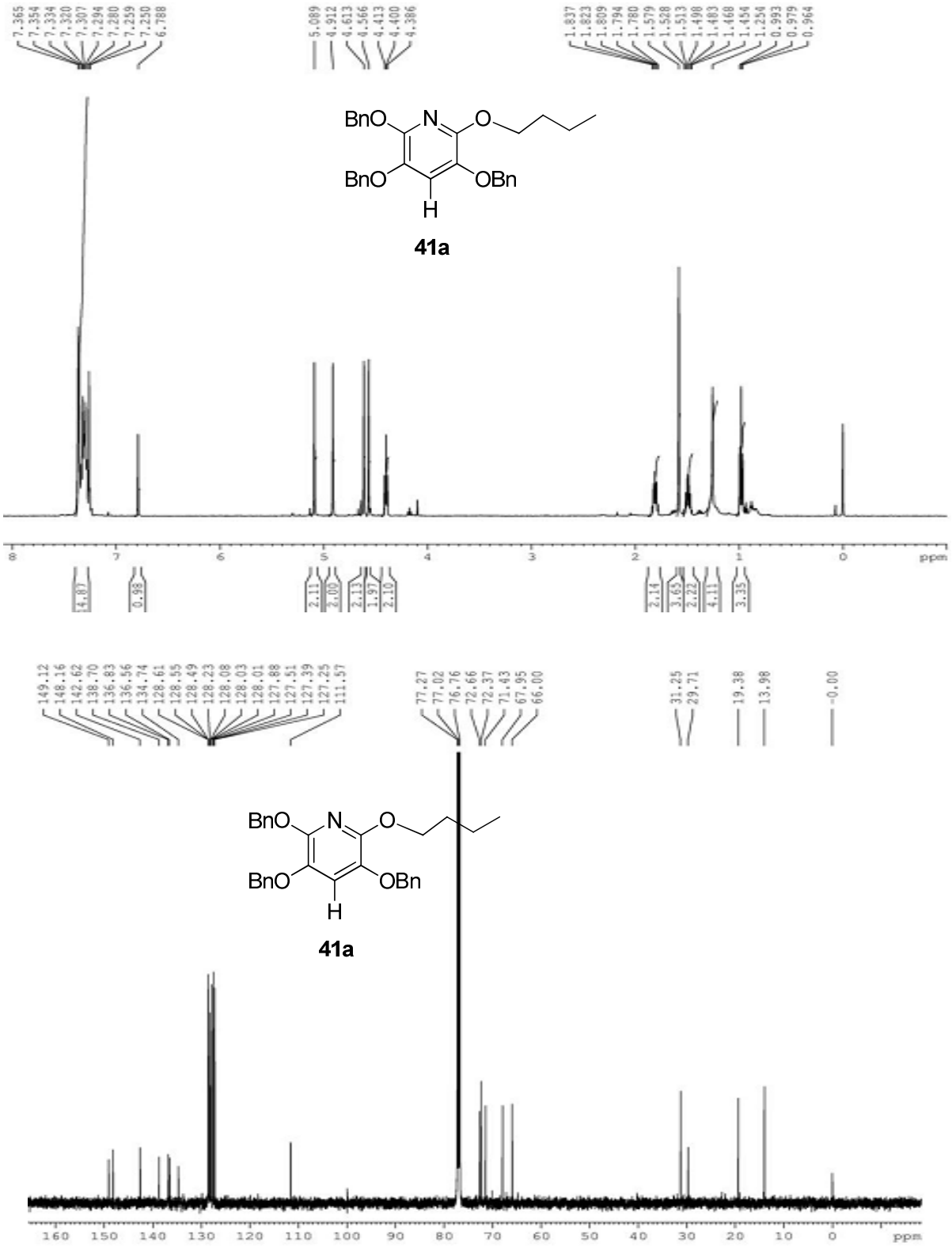




40

2-*O*-Butyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41a). To a solution of 2-pyridone **40** (23 mg, 0.053 mmol) in DMF (1.5 mL) was added Cs_2CO_3 (43 mg, 2.5 mmol), *n*-butyl bromide (12 μL, 0.107 mmol), and the reaction mixture was stirred overnight at room temperature. The reaction was quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (20 mL), dried (Na_2SO_4), concentrated and purified by flash chromatography (hexane/EtOAc 15:1) affording **41a** (24 mg, 94%). R_f 0.7 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.97 (t, 3H, J = 7 Hz), 1.49 (m, 2H), 1.83 (m, 2H), 4.40 (t, 2H, J = 6.5 Hz), 4.56 (s, 2H), 4.61 (s, 2H), 4.91 (s, 2H), 5.08 (s, 2H), 6.78 (s, 1H), 7.3 (m, 15H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.9, 19.3, 29.7, 31.2, 66.0, 67.9, 71.4, 72.3, 72.6, 111.5, 127.2, 127.3, 127.5, 127.8, 128.0-128.6, 134.7, 136.5, 136.8, 138.7, 142.6, 148.1, 149.1; HR-ESI-MS $[M + H]^+$ $C_{31}H_{34}NO_4$ calcd for m/z 484.24878, found 484.24770.

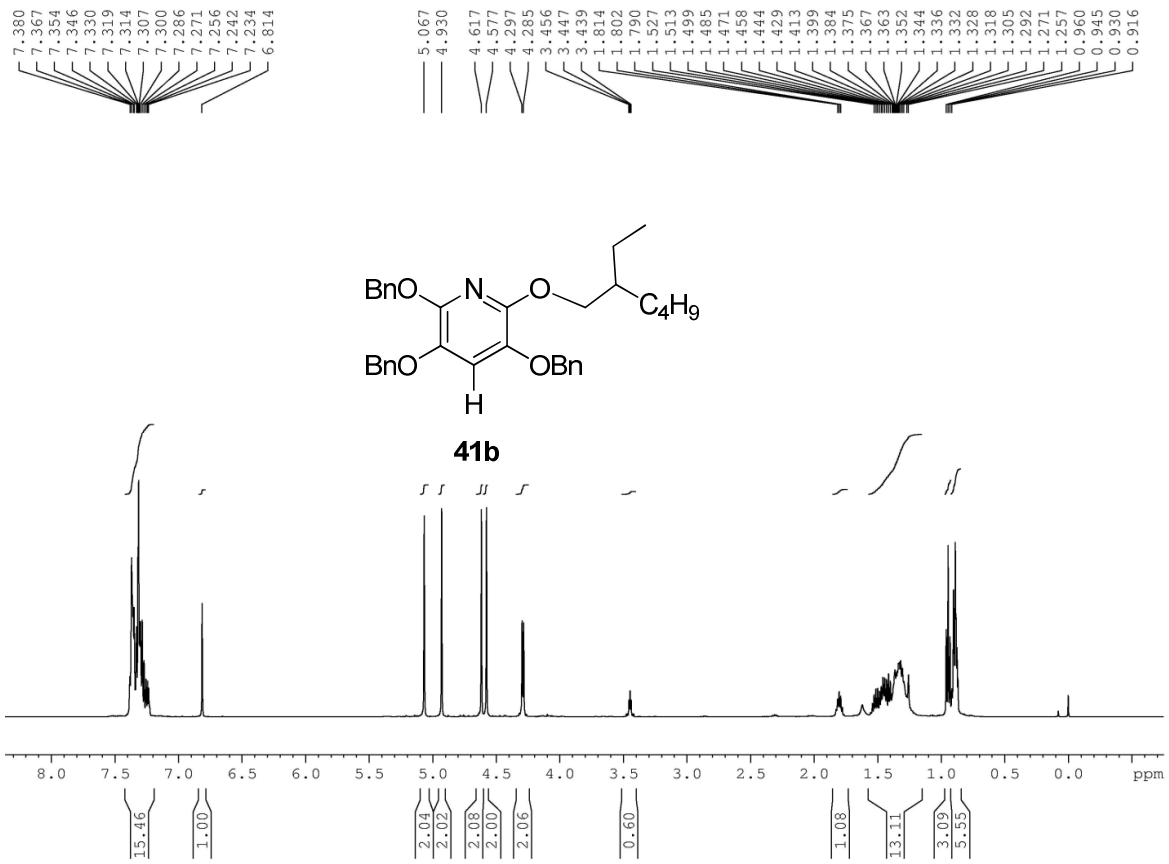
^{1}H and ^{13}C-NMR of compound **41a**

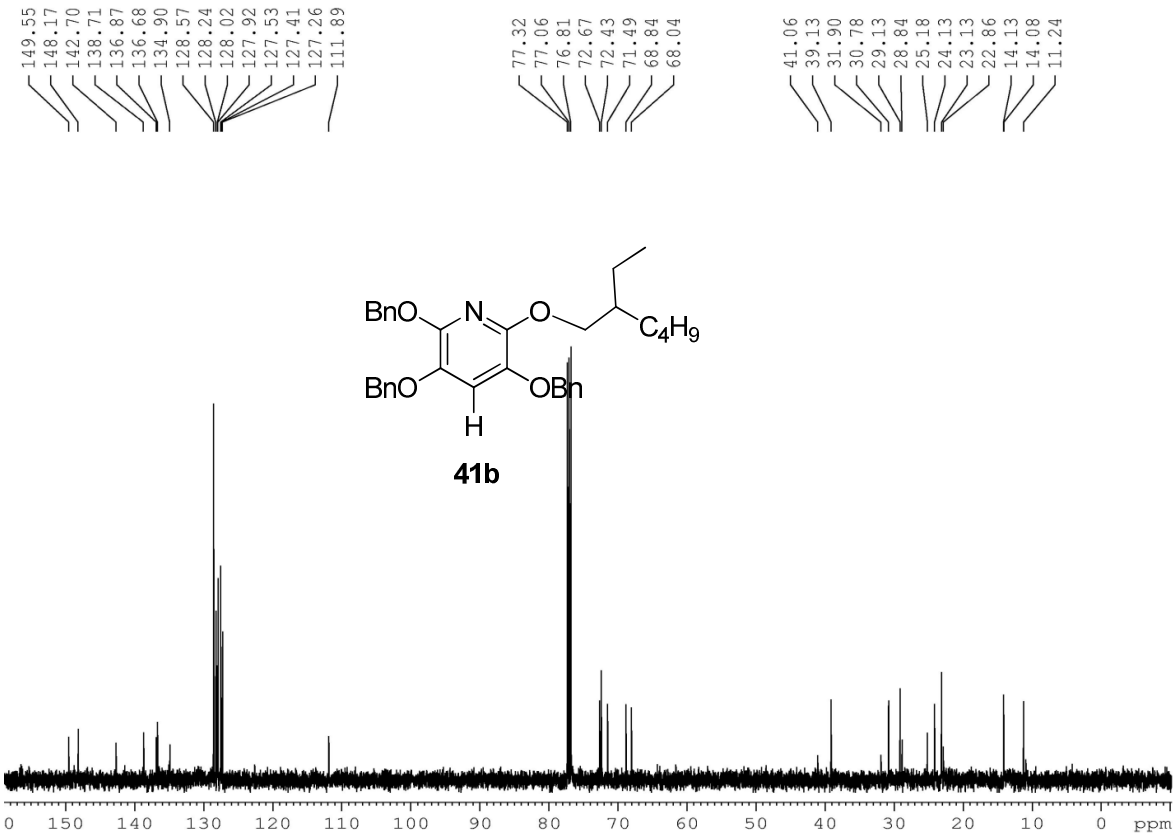


Procedure similar to synthesis of compound 41a was followed for synthesis of compounds 41b-g. For preparation of 41f *n*-pentyl chloride was used.

2-*O*-2'-ethyl-hexyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41b) ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.88 (t, 3H, *J* = 7 Hz), 0.94 (t, 3H, *J* = 7.5 Hz), 1.27-1.52 (m, 8H), 1.79-1.81 (m, 1H), 4.28 (t, 2H, *J* = 6 Hz), 4.57 (s, 2H), 4.61 (s, 2H), 4.93 (s, 2H), 5.06 (s, 2H), 6.81 (s, 1H), 7.23-7.38 (m, 15H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 11.2, 14.0, 23.1, 24.1, 29.1, 30.7, 39.1, 68.0, 68.8, 71.4, 72.4, 72.6, 111.8, 127.2, 127.4, 127.5, 127.9, 128.02, 128.2, 128.5, 134.9, 136.6, 136.8, 138.7, 142.7, 148.1, 149.5; HR-ESI-MS $[M + H]^+$ $C_{35}H_{42}NO_4$ calcd for m/z 540.30691, found 540.31079.

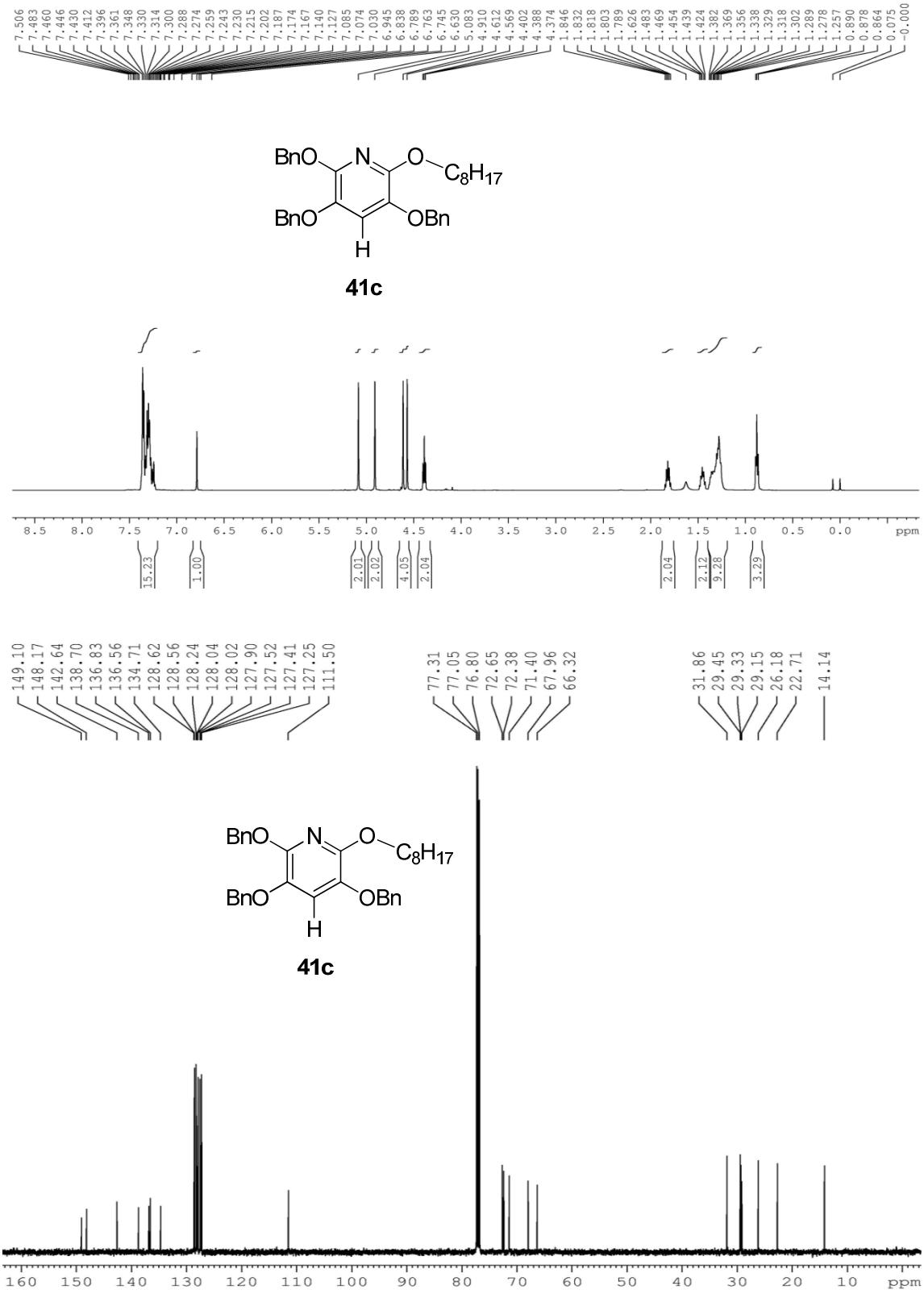
^{1}H and ^{13}C-NMR of compound **41b**





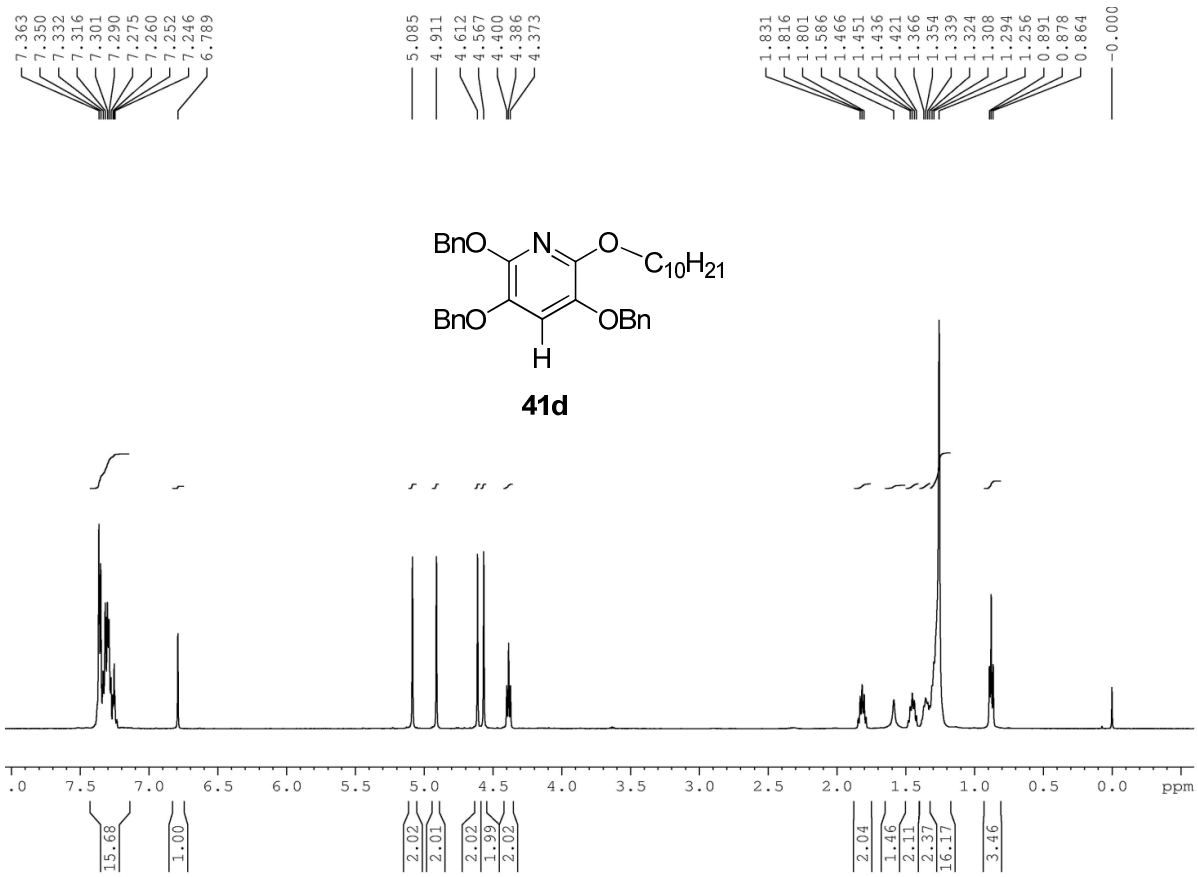
2-*O*-Octyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41c). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87 (t, 3H, J = 6.5 Hz), 1.25-1.38 (m, 8H), 1.42-48 (m, 2H), 1.78-1.84 (m, 2H), 4.38 (t, 2H, J = 7 Hz), 4.56 (s, 2H), 4.61 (s, 2H), 4.91 (s, 2H), 5.08 (s, 2H), 6.63 (s, 1H), 6.76-7.50 (m, 15H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.1, 22.7, 26.1, 29.1, 29.3,29.4, 31.8, 66.3, 67.9, 71.4, 72.3, 72.6, 111.5, 127.2, 127.4, 127.5, 127.9, 128.02, 128.04, 128.2, 128.5, 128.6, 134.7, 136.5, 136.8, 138.7, 142.6, 148.1, 149.1; HR-ESI-MS $[M + H]^+$ $C_{35}H_{42}NO_4$ calcd for m/z 540.31138, found 540.31044.

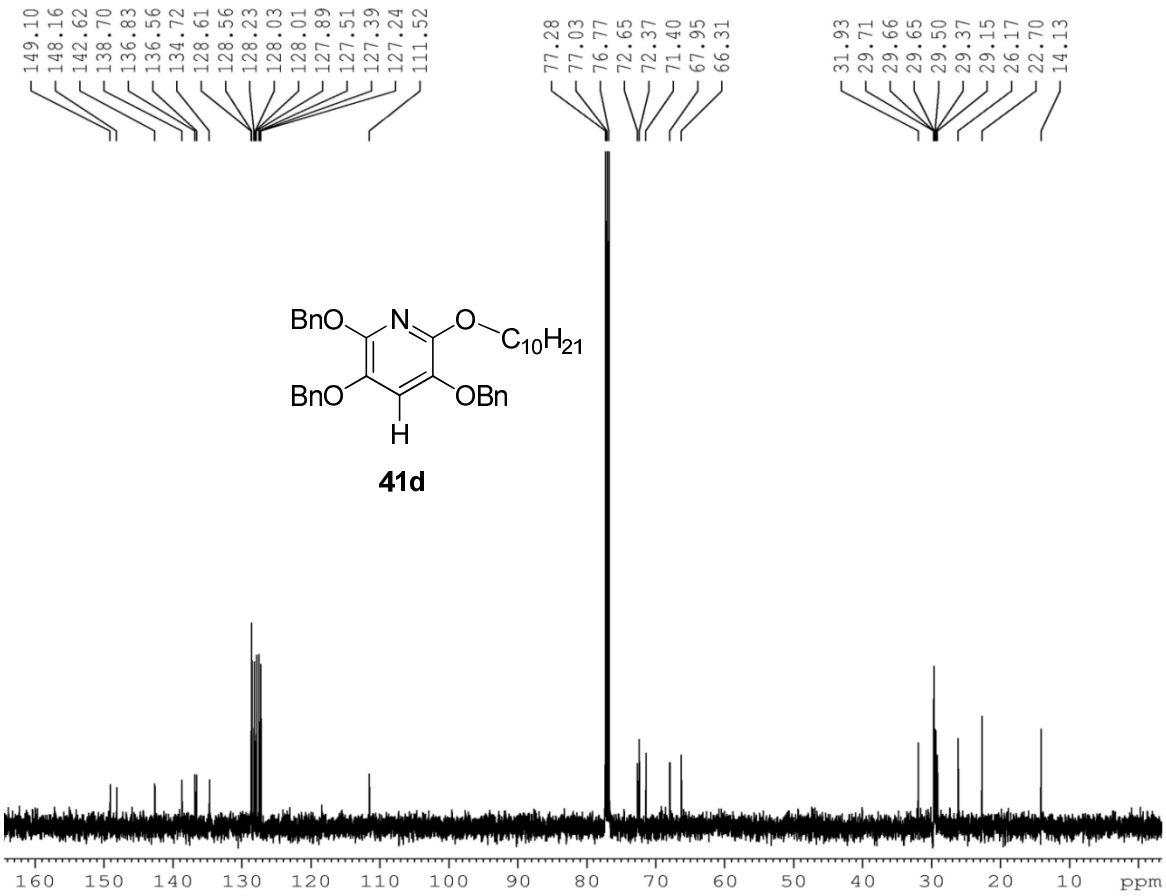
^{1}H and ^{13}C-NMR of compound **41c**



2-*O*-Decyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41d). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87 (t, 3H, *J* = 6.5 Hz), 1.25-1.36 (m, 12H), 1.42-1.46 (m, 2H), 1.80-1.83 (m, 2H), 4.38 (t, 2H, *J* = 6.5 Hz), 4.56 (s, 2H), 4.61 (s, 2H), 4.91 (s, 2H), 5.08 (s, 2H), 6.78 (s, 1H), 7.24-7.36 (m, 15H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.1, 22.7, 26.1, 29.1, 29.3, 29.5, 29.65, 29.66, 31.9, 66.3, 67.9, 71.4, 72.3, 72.6, 111.5, 127.2, 127.3, 127.5, 127.8, 128.01, 128.03, 128.2, 128.5, 128.6, 134.7, 136.5, 136.8, 138.7, 142.6, 148.1, 149.1; HR-ESI-MS $[M + H]^+$ $C_{37}H_{46}NO_4$ calcd for m/z 568.34268, found 568.34241.

^{1}H and ^{13}C-NMR of compound **41d**

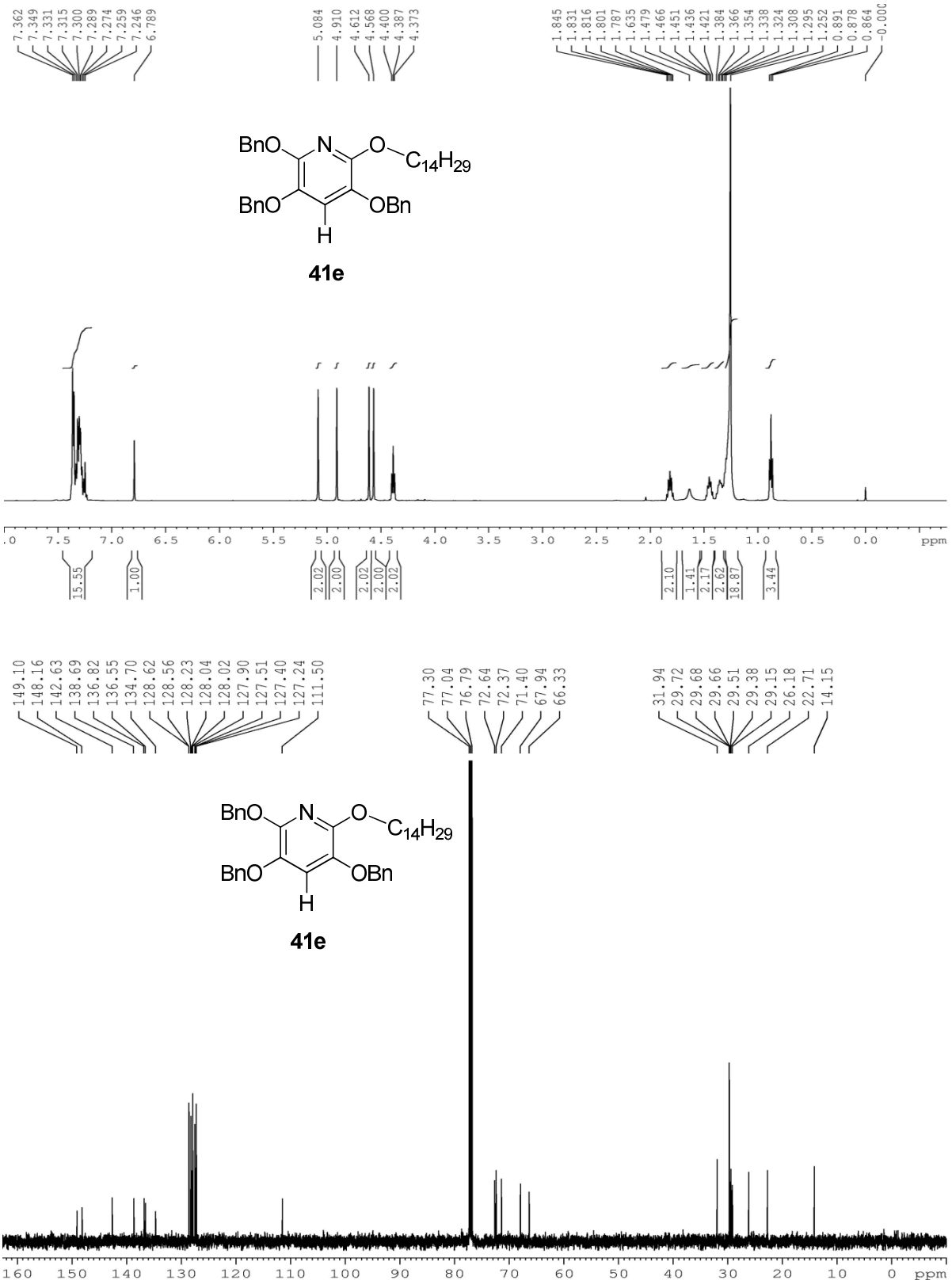




41d

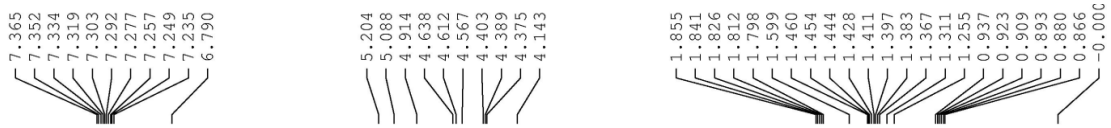
2-*O*-tetradecyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41e). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87 (t, 3H, J = 6.5 Hz), 1.25-1.38 (m, 20H), 1.42-1.47 (m, 2H), 1.78-1.84 (m, 2H), 4.38 (t, 2H, J = 6.5 Hz), 4.56 (s, 2H), 4.61 (s, 2H), 4.91 (s, 2H), 5.08 (s, 2H), 6.78 (s, 1H), 7.24-7.36 (m, 15H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.1, 22.7, 26.1, 29.1, 29.3, 29.5, 29.66, 29.68, 29.72, 31.9, 66.3, 67.9, 71.4, 72.3, 72.6, 111.5, 127.2, 127.4, 127.5, 127.9, 128.02, 128.04, 128.2, 128.5, 128.6, 134.7, 136.5, 136.8, 138.6, 142.6, 148.1, 149.1; HR-ESI-MS $[M + H]^+$ $C_{41}H_{54}NO_4$ calcd for m/z 624.40528 found 624.40509.

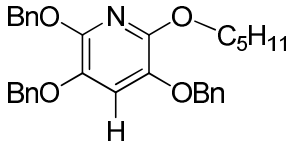
^{1}H and ^{13}C-NMR of compound **41e**



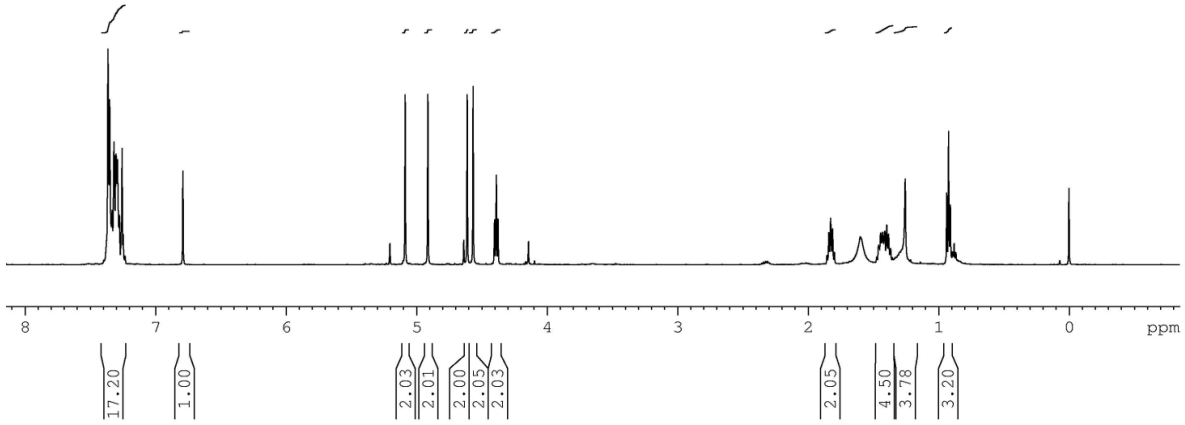
2-*O*-Pentyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41f). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.84 (t, 3H, *J* = 7.5 Hz), 1.29-1.38 (m, 4H), 1.73-1.76 (m, 2H), 4.31 (t, 2H, *J* = 7 Hz), 4.49 (s, 2H), 4.53 (s, 2H), 4.83 (s, 2H), 5.01 (s, 2H), 6.71 (s, 1H), 7.28-7.17 (m, 15H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.1, 22.5, 28.3, 28.8, 66.3, 67.9, 71.3, 72.3, 72.6, 111.4, 127.2, 127.4, 127.5, 127.9, 128.0, 128.1, 128.2, 128.5, 128.6, 134.6, 136.5, 136.8, 138.6, 142.6, 148.1, 149.1; HR-ESI-MS $[M + H]^+$ $C_{32}H_{36}NO_4$ calcd for m/z 498.26443, found 498.29413.

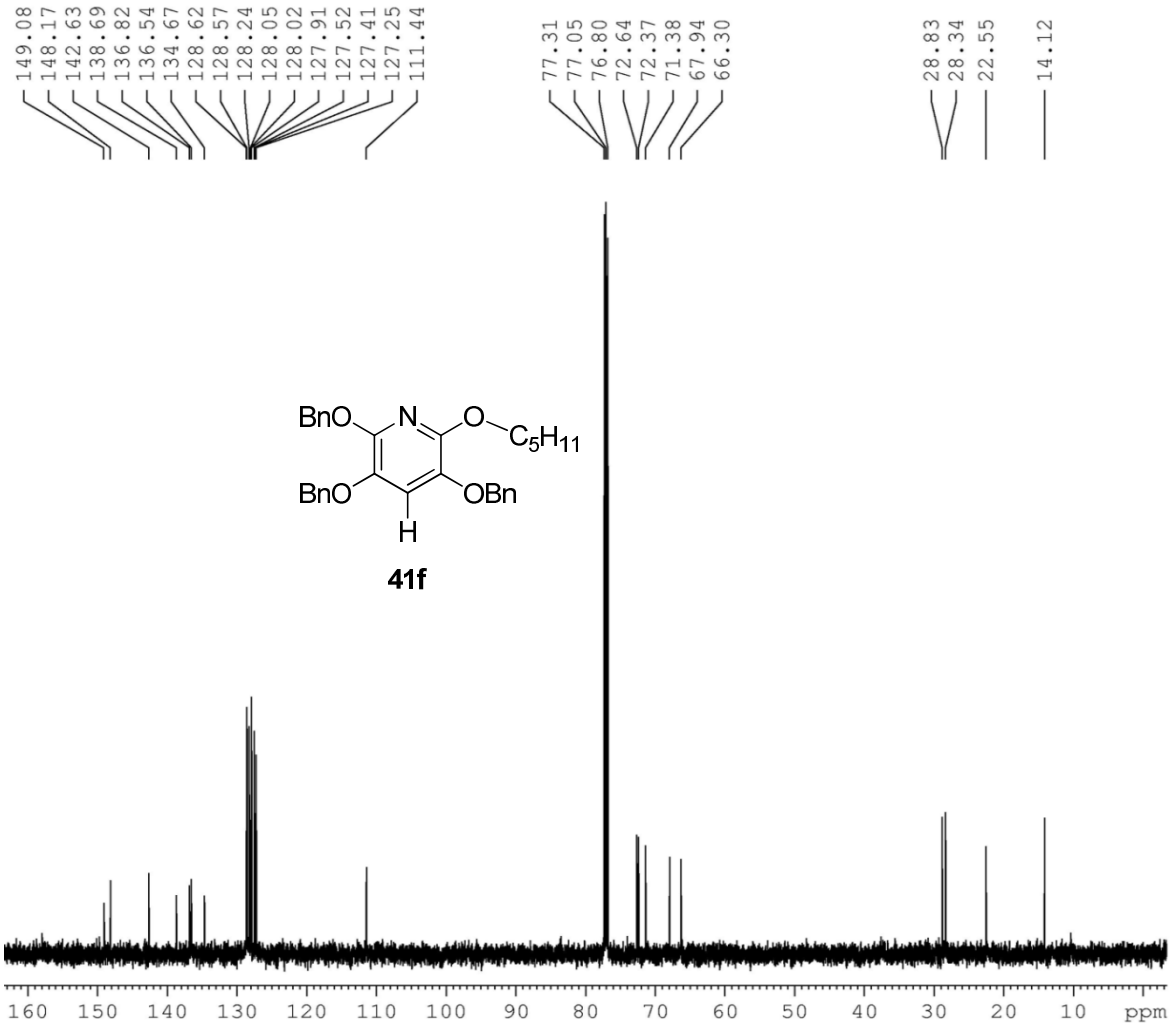
^{1}H and ^{13}C-NMR of compound **41f**





41f

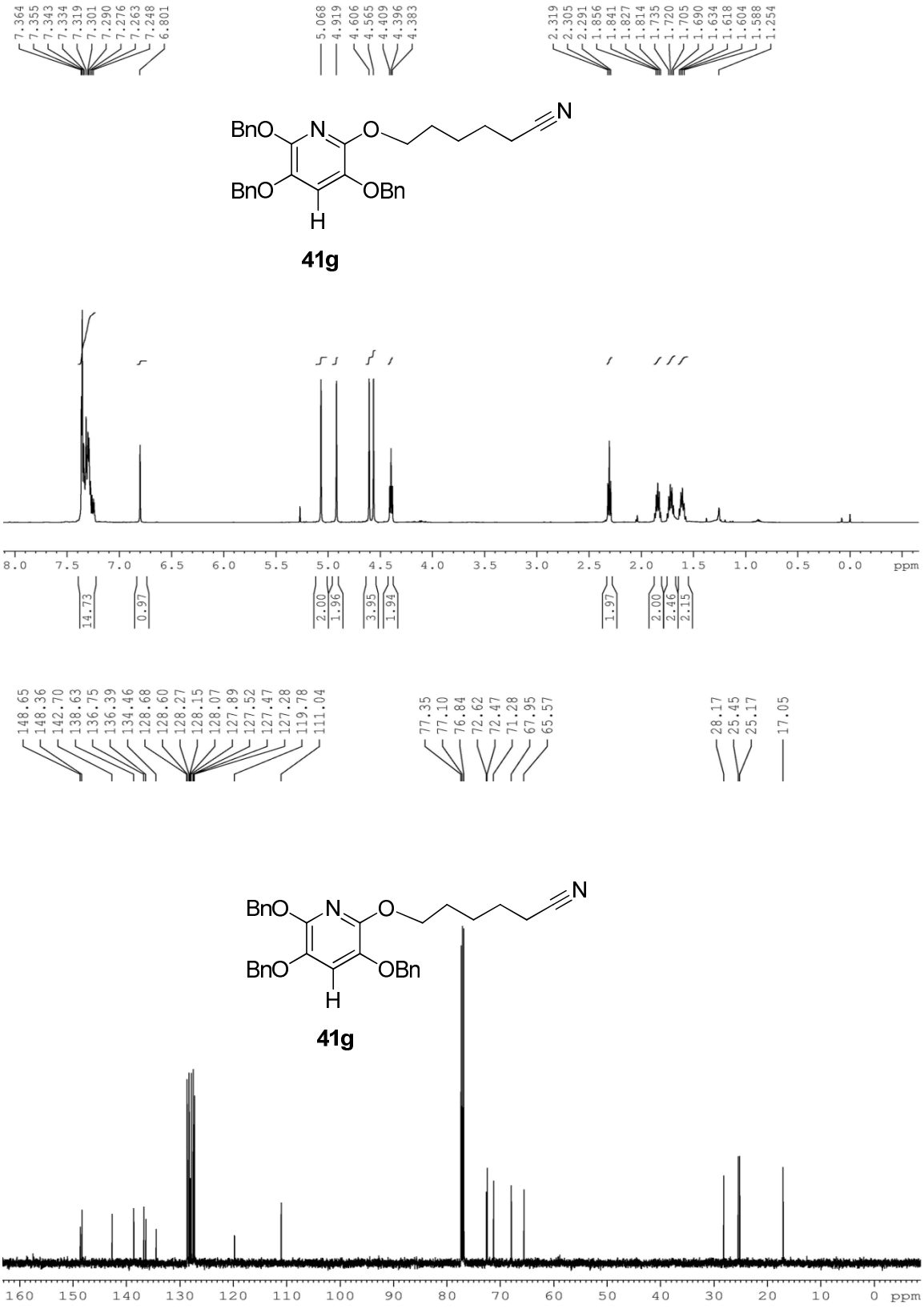




41f

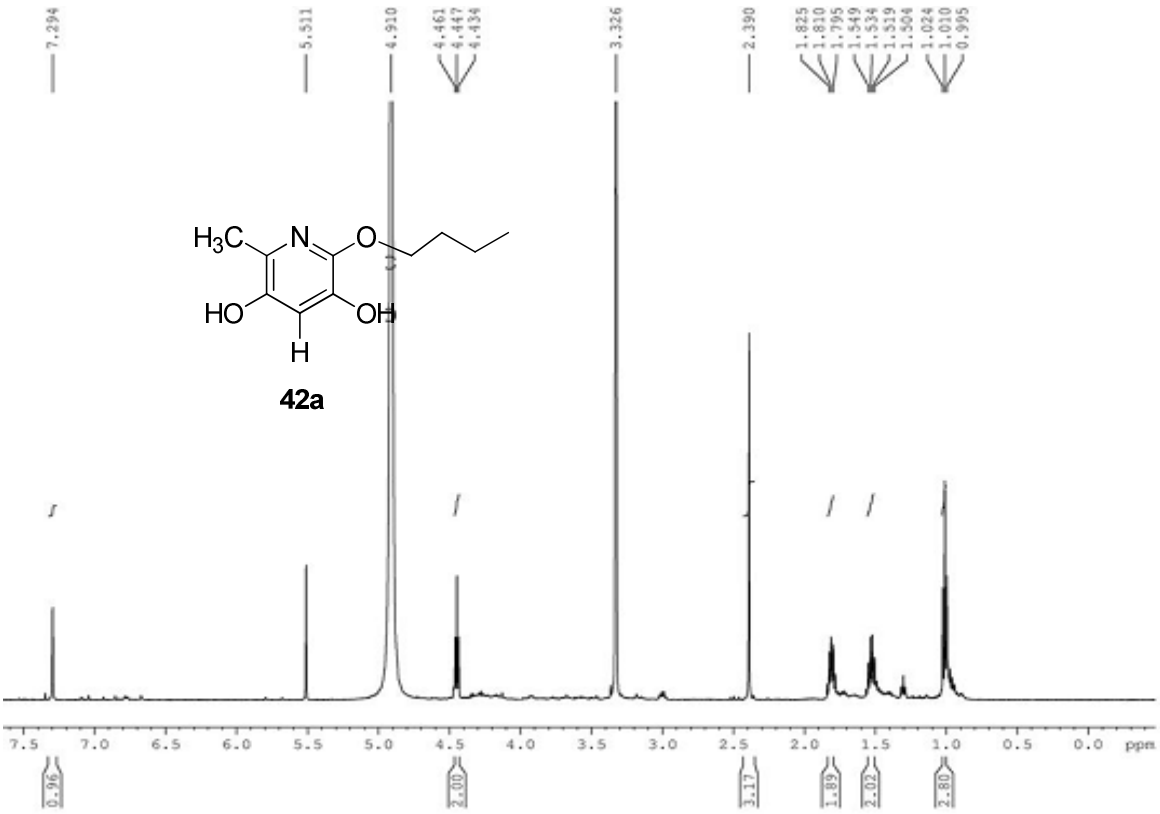
2-*O*-5'-cyano-pentyl-3,5-dibenzyloxy-6-benzyloxymethylpyridine (41g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.58-1.63 (m, 2H), 1.69-1.73 (m, 2H), 1.81-1.85 (m, 2H), 2.30 (t, 2H, *J* = 7 Hz), 4.39 (t, 2H, *J* = 6.5 Hz), 4.56 (s, 2H), 4.60 (s, 2H), 4.91 (s, 2H), 5.06 (s, 2H), 6.80 (s, 1H), 7.24-7.36 (m, 15H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 17.0, 25.1, 25.4, 28.1, 65.5, 67.9, 71.2, 72.4, 72.6, 111.04, 119.04, 127.2, 127.4, 127.5, 127.8, 128.07, 128.1, 128.2, 128.6, 128.68, 134.4, 136.3, 136.7, 138.6, 142.7, 148.3, 148.6; HR-ESI-MS $[M + H]^+$ $C_{33}H_{35}N_2O_4$ calcd for m/z 523.25968, found 523.25916.

^{1}H and ^{13}C-NMR of compound **41g**

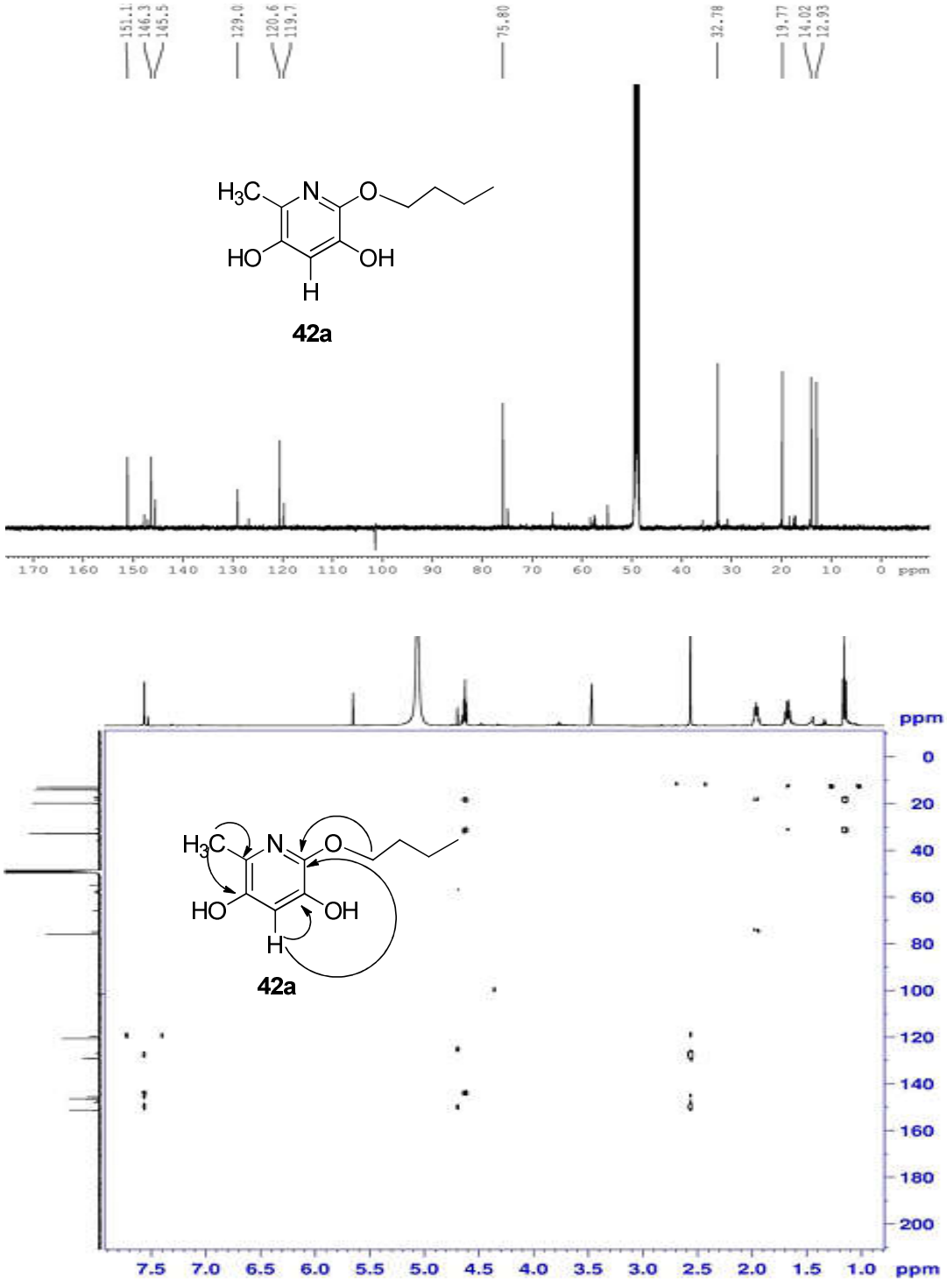


2-*O*-Butyl-3,5-dihydroxy-6-methylpyridine (42a). To a solution of pyridine **41a** (26.5 mg, 0.055 mmol) in EtOH (2.5 mL) was added one drop of conc. HCl and Pd/C 10% (22 mg). The reaction mixture was stirred at room temperature under H_2. After 1 h, the reaction was filtered on a celite pad and the resulting filtrate was concentrated. To the resulting residue CH_2Cl_2 was added to precipitate the product as a red colored solid **42a** (10.5 mg, 98%). R_f 0.61 (hexane/EtOAc 3:7); ^{1}H NMR (CD_3OD, 500 MHz): δ 1.01 (t, 3H, J = 7 Hz), 1.53 (m, 2H), 1.81 (m, 2H), 2.39 (s, 3H), 4.44 (t, 2H, J = 7 Hz), 7.29 (s, 1H). ^{13}C-NMR (CD_3OD, 125 MHz): δ 12.9, 14.0, 19.7, 32.7, 75.8, 120.6, 129.0, 145.5, 146.3, 151.1; HR-ESI-MS $[M + H]^+$ $C_{10}H_{16}NO_3$ calcd for m/z 198.11302, found 198.11305.

^{1}H-NMR of compound **42a**



^{13}C-NMR and HMBC spectra of compound **42a**



Procedure similar to the preparation of compound 42a was followed for synthesis of compounds 42b-g.

2-*O*-2'-ethyl-hexyl-3,5-dihydroxy-6-methylpyridine (42b). ^{1}H NMR (CD_3OD, 500 MHz): δ 0.95 (t, 3H, *J* = 7 Hz), 1.98 (t, 3H *J* = 7 Hz), 1.31-1.58 (m, 8H), 1.76-1.78 (m, 1H) 2.40 (s, 3H), 4.37 (t, 2H, *J* = 5.5 Hz), 7.36 (s, 1H); HR-ESI-MS $[M + H]^+$ $C_{14}H_{24}NO_3$ calcd for m/z 254.17561, found 254.17474.

2-*O*-Octyl-3,5-dihydroxy-6-methylpyridine (42c). ^{1}H NMR (CD_3OD, 500 MHz): δ 0.80 (t, 3H, *J* = 6.5 Hz), 1.19-1.41 (m, 10H), 1.70-1.73 (m, 2H), 2.30 (s, 3H), 4.35 (t, 2H, *J* = 6.5 Hz), 7.28 (s, 1H); HR-ESI-MS $[M + H]^+$ $C_{14}H_{24}NO_3$ calcd for m/z 254.17561, found 254.17493.

2-*O*-decyl-3,5-dihydroxy-6-methylpyridine (42d). ^{1}H NMR (CD_3OD, 500 MHz): δ 0.80 (t, 3H, *J* = 6.5 Hz), 1.20-1.41 (m, 14H), 1.68-1.73 (m, 2H), 2.30 (s, 3H), 4.35 (t, 2H, *J* = 6.5 Hz), 7.28 (s, 1H); HR-ESI-MS $[M + H]^+$ $C_{16}H_{28}NO_3$ calcd for m/z 282.20691, found 282.20609.

2-*O*-tetradecyl-3,5-dihydroxy-6-methylpyridine (42e). ^{1}H NMR (CD_3OD, 500 MHz): δ 0.80 (t, 3H, *J* = 7 Hz), 1.19-1.63 (m, 22H), 1.68-1.73 (m, 2H), 2.29 (s, 3H), 4.34 (t, 2H, *J* = 6.5 Hz), 7.25 (s, 1H); HR-ESI-MS $[M + H]^+$ $C_{20}H_{36}NO_3$ calcd for m/z 338.26951, found 338.26874.

2-*O*-Pentyl-3,5-dihydroxy-6-methylpyridine (42f). ^{1}H NMR (CD_3OD, 500 MHz): δ 0.96 (t, 3H, *J* = 7 Hz), 1.20-1.54 (m, 4H), 1.75-1.87 (m, 2H), 2.4 (s, 3H), 4.8 (t, 2H, *J* = 7 Hz), 7.4 (s, 1H); HR-ESI-MS $[M + H]^+$ $C_{11}H_{18}NO_3$ calcd for m/z 212.12866, found 212.12780.

2-*O*-5'-cyano-pentyl-3,5-dihydroxy-6-methylpyridine (42g). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.66-1.76 (m, 4H), 1.87-1.90 (m, 2H), 2.42 (s, 3H), 2.51 (t, 2H, *J* = 6.5 Hz), 4.50 (t, 2H, *J* = 6.5 Hz), 7.44 (s, 1H); HR-ESI-MS $[M + H]^+$ $C_{12}H_{17}N_2O_3$ calcd for m/z 237.12391, found 237.12331; $[M - H]^-$ $C_{12}H_{15}N_2O_3$ calcd for m/z 235.10826, found 235.10828.

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Chapter-3

Synthesis of N-alkyl and 1,2,3-triazole linked 2-pyrrolidinone derivatives from D-galactose

3.1. Abstract:

Synthetic analogs of glycosphingolipids (GSLs) have been demonstrated as potential therapeutic interventions in certain patho-physiological conditions. KRN7000, a synthetic GSL which is a *α*-galactosylceramide (*α*-GalCer) is a potent immunomodulatory agent. Bittman et al. reported several modifications of *C*-glycosides of KRN7000 with an eye towards achieving selective cytokine response during iNKT cell activation. However, GSLs with azasugar variants were not explored which inspired us to derive a polyhydroxy 2-pyrrolidinone azasugar from D-galactose and then the lactam was appended with the simple short and long chain alkyl groups giving rise to polyhydroxy-N-alkyl-2-pyrrolidinone derivatives **9a-e** (Scheme 3.3.1A) and extended to phytoceramide via a 1,2,3-triazole linker to afford GSL analogs **21a-d** (Scheme 3.4A).

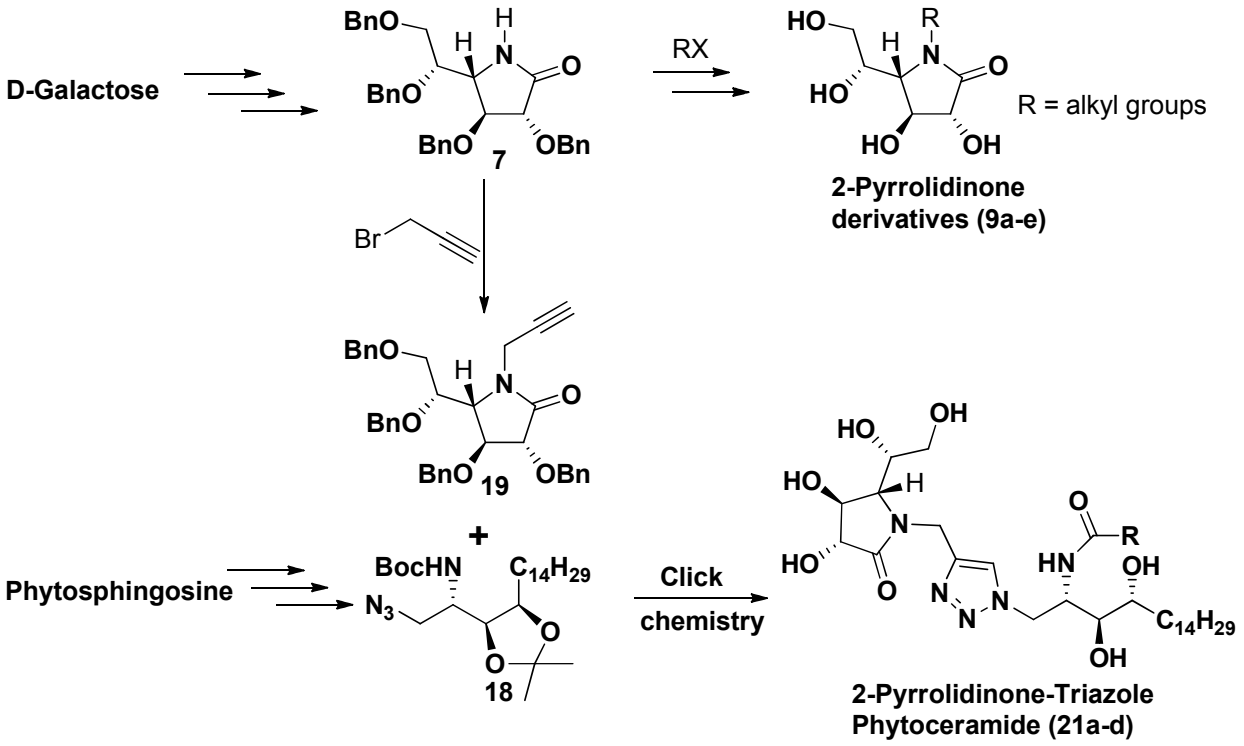


Figure 3.1: Synthesis of N-alkyl and 1,2,3-triazole-2-pyrrolidinone phytoceramide derivatives

3.2. Introduction:

Glycosphingolipids (GSLs) present in the outer leaflet of the plasma membrane are the primary attachment sites for various pathogens.[1] Bittman et al. designed and synthesized various GSL analogs for therapeutic intervention in certain patho-physiological conditions.[2] A fluorescent tagged BODIPY^{TM}-lactosylceramide (LacCer) GSL analog was used to demonstrate that caveolar endocytosis, a mechanism for uptake of certain pathogens, is stimulated by exogenous GSLs.[3] To study the effect of stereochemistry of the sphingosine backbone, BODIPY^{TM}-tagged natural (D-erythro) and non-natural (L-threo) LacCer analogs were examined in the endocytosis and the results indicated that the natural analog is internalized via caveolae and the non-natural analog is excluded from uptake via caveolae.[4] Furthermore, *β*-D-lactosyl-*N*-octanoyl-L-threo-sphingosine with non-natural stereochemistry was shown to selectively inhibit caveolar

endocytosis and SV40 virus infection, block lipid rafts in the plasma membrane, and inhibit β1-integrin activation and downstream signaling.[5] In a related family of GSLs, a structure-activity derived synthetic GSL analog of a naturally occurring agelasphin with phytoceramide known as KRN7000 (α-GalCer) activate iNKT cells which in turn results in release of cytokines that are potent immunomodulatory agents.[6] The *C*-glycoside of KRN7000 (α-*C*-GalCer) was found to be 100 times more potent in a melanoma challenge assay on C57BL/6 mice than *O*-glycoside.[7] Ever since it was discovered that *α*-*C*-GalCer and related *C*-glycoside ligands promote biased cytokine response that is essential for therapeutic potential,[8] a series of *C*-glycosides were designed and synthesized by Bittman et al. The design of these *C*-glycosides involved structural variations essential in understanding activation of iNKT cells and its subsequent selective cytokine response. The first analog of *α*-*C*-GalCer reported was a truncated nonisosteric *α*-*C*-glycoside where the anomeric carbon is directly appended to the C1 of phytoceramide that promoted higher IFN-γ cytokine production, displaying its potential as an adjuvant for immunotherapy.[9] The nonisosteric *α*-*C*-glycoside analog was further synthesized in an alternative approach to confirm the stereochemistry in the previous synthesis.[10] *α*-*C*-glycoside analogs of KRN-7000 with variations in the linker area viz. *E*-alkene, acetylene, and exo-methylene were prepared in a stereocontrolled synthesis to promote biased cytokine responses.[11] Finally, KRN7000 bearing different alkyl chains at meta or para positions of phenyl group that is embedded within the amide chain were synthesized. The biological results of the latter compounds indicated that meta- and para- substituted compounds displayed increased Th2 response and IFN-γ secretion, respectively, compared to the analog bearing phenyl group at the amide chain terminus.[12] Inspired by synthetic GSL analogs reported by Bittman et al. (vide supra), we report herein a N-alkyl-2-pyrrolidinone derivatives **9a-e** (Scheme 3.3.1A) and novel GSL analog **21a-d** (Scheme 3.

4A) with a polyhydroxy 2-pyrrolidinone and phytoceramide linked via a 1,2,3-triazole. Among the *C*-glycoside analogs with linkers reported by Bittman et al. the exo-methylene analog served as an agonist for both human and mouse iNKT cells, perhaps due to flexibility in the latter compound allowing the sugar to rotate along the methylene unit in the linker (Figure. 3.2). For the same reason, our design involves the attachment of the sugar lactam via methylene to the triazole linker produced by "click chemistry" which is widely employed in appending biomolecules. Structural variants and mimics of KRN7000 with a presence of triazole moiety at different positions (Figure 3.2) afforded selective cytokine responses.[13] Recently, we have reported for the first time synthesis of a novel GSL analog which is an aza-variant of *β*-*C*-GalCer where the ring oxygen is replaced with nitrogen that can be further functionalized for introducing diversity.[14] The rationale for introducing an azasugar was due to their ability to exhibit a broad range of biological activities.[15] To further prepare more GSL analogs with different azasugars, we propose herein the azasugar moiety as a polyhydroxy 2-pyrrolidinone that was derived from D-galactose. Finally, the present design offers a new entry to the repertoire of GSL analogs of therapeutic significance with aza-sugar.

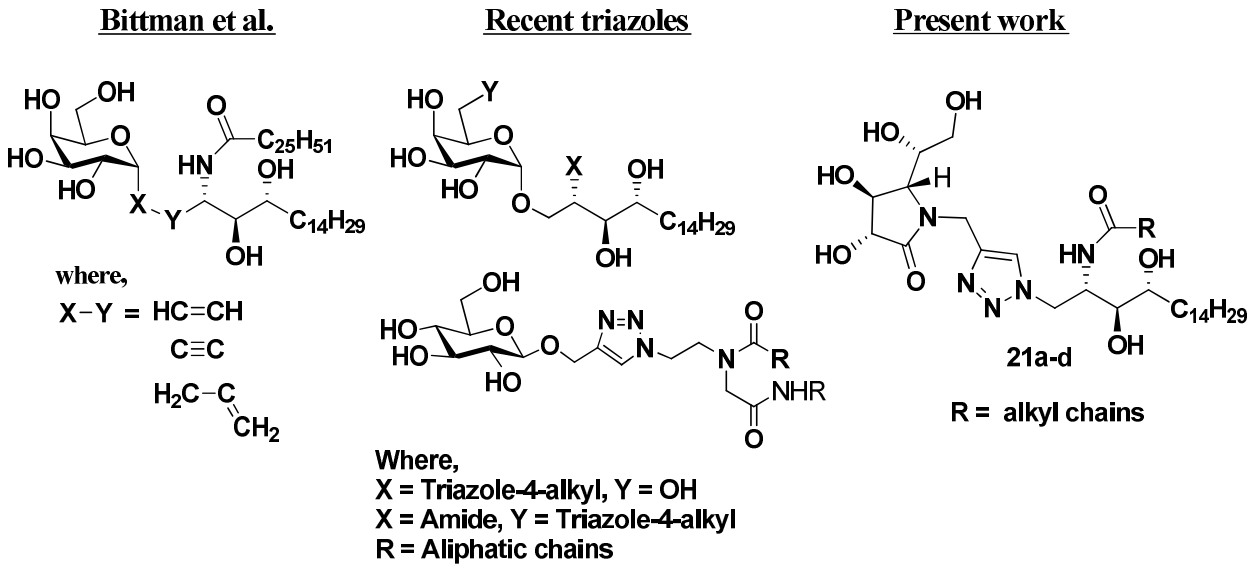


Figure 3.2: Design of the novel GSL analog:: 2-Pyrrolidinone-Triazole-Phytoceramide **21a-d**

3.2.1. Previous methods for the synthesis of 2-pyrrolidinone derivatives:

Conjugate addition of nitromethane to unsaturated ester in the presence of a bulky bicyclic guanidine base led to the corresponding 3-nitromethyl adduct. Reduction of the nitro group with Raney-Ni in the presence of H_2PtCl_6 afforded the lactam (Figure 3.2.1A).[16]

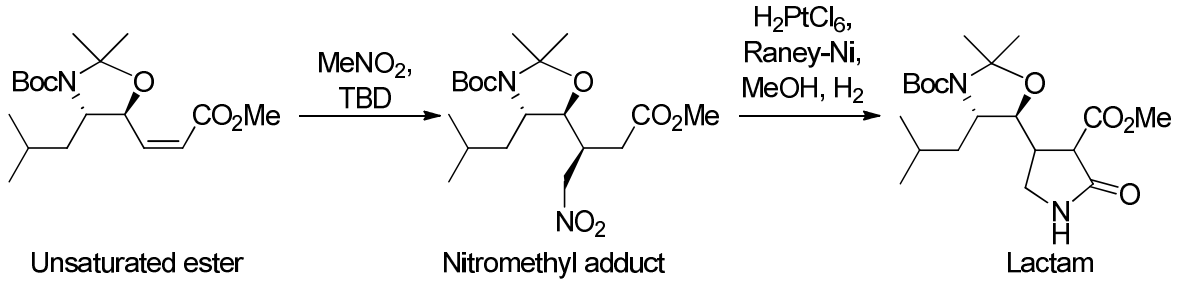


Figure 3.2.1A: Synthesis of 2-pyrrolidinone from unsaturated ester via nitromethane addition reaction

Synthesis of pyrrolidinone derivative has been reported using an efficient three-component nitro-Mannich/lactamization cascade of methyl 3-nitropropanoate with *in situ* formed acyclic imine

(Figure 3.2.1B).[17] Copper-catalyzed conjugate addition of diorgano zinc reagents to nitroacrylate followed by a subsequent nitro-Mannich reaction and *in situ* lactamization led to an efficient one-pot synthesis of highly substituted 4-nitropyrrolidin-2-ones.[18]

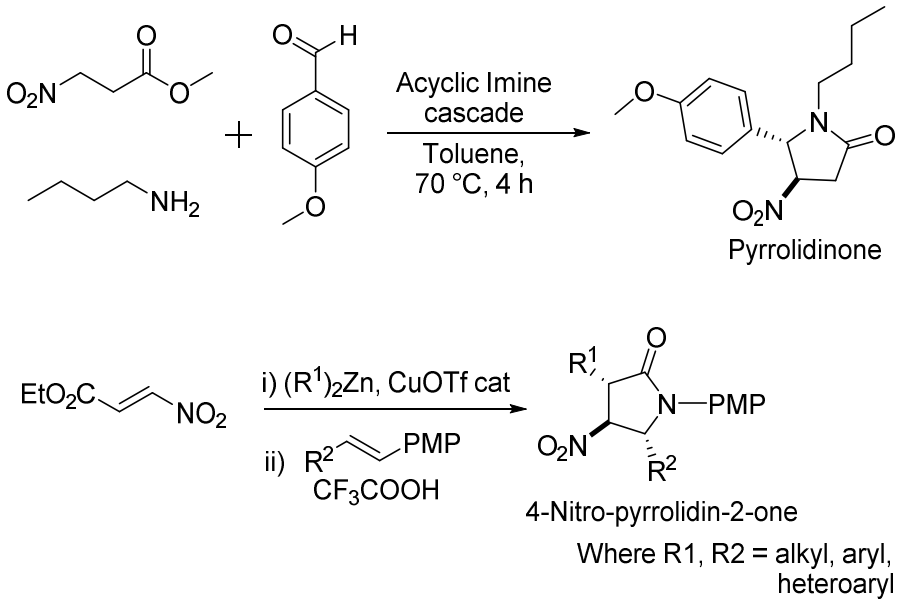


Figure 3.2.1B: Synthesis of highly substituted 2-pyrrolidinones via nitro-Mannich/lactamization reaction

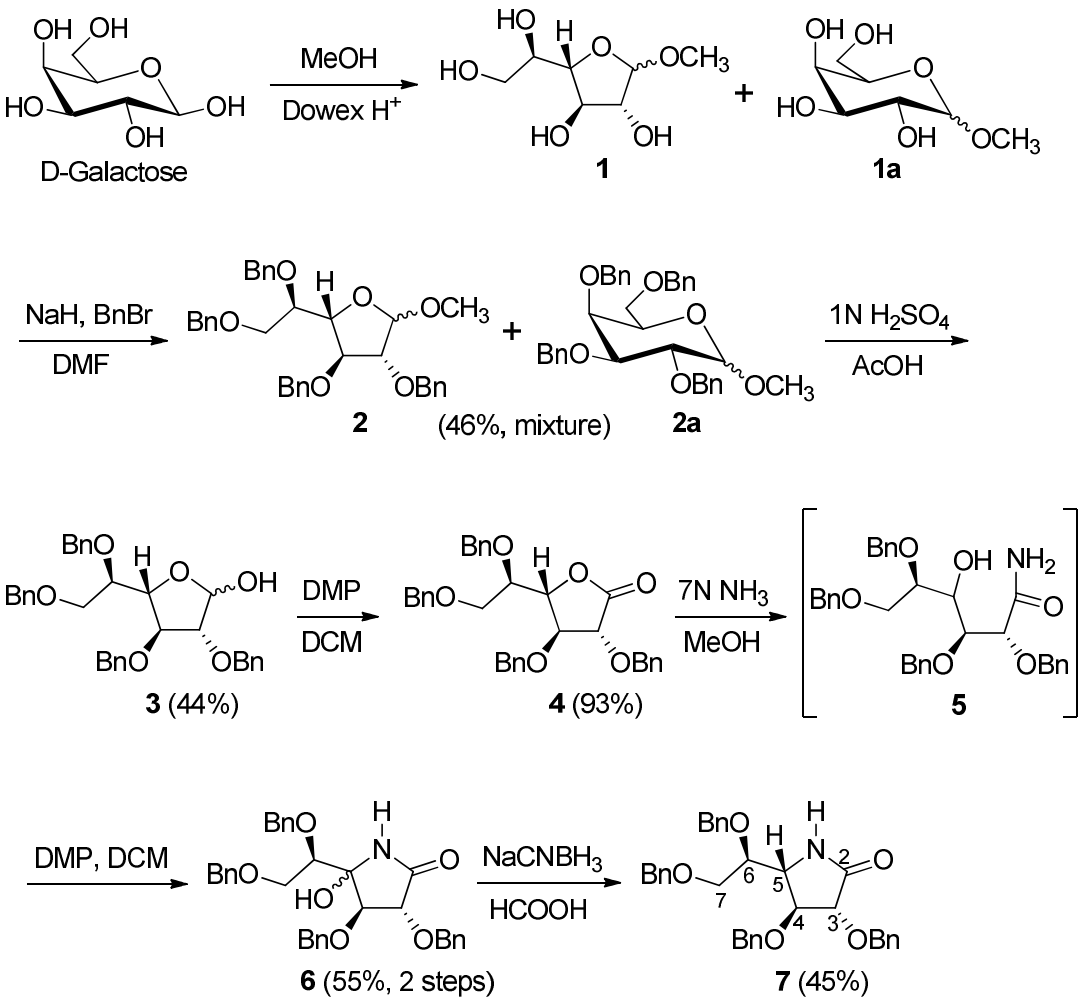
3.3. Results and Discussion:

In our earlier work, we have reported the synthesis of *β-C*-GalCer with an aza-variant, where the ring oxygen of D-galactose was replaced with nitrogen.[14] This is the first report of introducing azasugar as the carbohydrate moiety in GSLs, using the Horner-Wadsworth-Emmons reaction as the key step in combining the sugar and aglycone portions. In order to prepare more GSL analogs with different azasugars of therapeutic significance, we report in the present chapter polyhydroxy 2-pyrrolidinone as the azasugar which is appended to aliphatic chains as N-alkyls

9a-e (Scheme 3.3.1) and phytoceramide via a 1,2,3-triazole linker to afford GSL analogs **21a-d** (Scheme 3.4A).

3.3.1 Synthesis of polyhydroxy 2-pyrrolidinone 7 from D-Galactose and its N-alkyl derivatives (9a-e):

Scheme 3.3.1 depicts the synthesis of polyhydroxy 2-pyrrolidinone **7** from D-Galactose. Glycosidation of D-Galactose with methanol in the presence of Dowex H^+ resin afforded the mixture of glycosides **1** and **1a**.[19] Perbenzylation of the mixture of methyl galactosides **1** and **1a** using benzyl bromide in the presence of NaH afforded the mixture of tetra-*O*-benzyl methyl galactosides **2** and **2a** in 46% yield over two steps. Demethylation of the mixture of galactosides **2** and **2a** with 1 N H_2SO_4 in acetic acid followed by purification provided the desired 1-hydroxy-galactofuranoside **3** in 44% yield. Dess-Martin periodinane oxidation of lactol **3** afforded D-galactono-1,4-lactone **4** in 93% yield. A clear distinction in the ^1H and ^{13}C NMR of δ-lactone derived from D-galactose,[20a] and γ-lactone **4** was observed (see experimental section),[20b] thus, confirming the 1,4-lactone required for the present work to synthesize 2-pyrrolidinone. Treatment of γ-lactone **4** with 7 N NH_3 in MeOH afforded the ring-opening product **5** which was oxidized without purification under Dess-Martin periodinane conditions to provide the ketone and further cyclization generated lactam **6** in 55% yield over two steps. Reductive amination of hemiaminal **6** with $NaCNBH_3$ and HCOOH under reflux conditions,[21] provided the desired polyhydroxy 2-pyrrolidinone **7** in 45% yield (Scheme 3.3.1).



Scheme 3.3.1: Synthesis of polyhydroxy 2-pyrrolidinone (**7**) from D-Galactose

A single proton H-2 at δ_H 4.22 (d, J = 6.5 Hz) and presence of COSY (Figure 3.3.1) correlation with H-3 at δ_H 4.05 (app t, J = 6.5 Hz, 1H) indicates that the two protons H-2 and H-3 are neighbors. Additionally the H-3 is having correlation with H-4 at δ_H 3.51 (app t, J = 5.5 Hz, 1H). Furthermore the H-5 at δ_H3.70 (dd, J = 11.5, 4.5 Hz, 1H) is having correlation with H-6 at δ_H 3.46 (m, 2H) which helped us to determine the partial structure of lactam **7**.

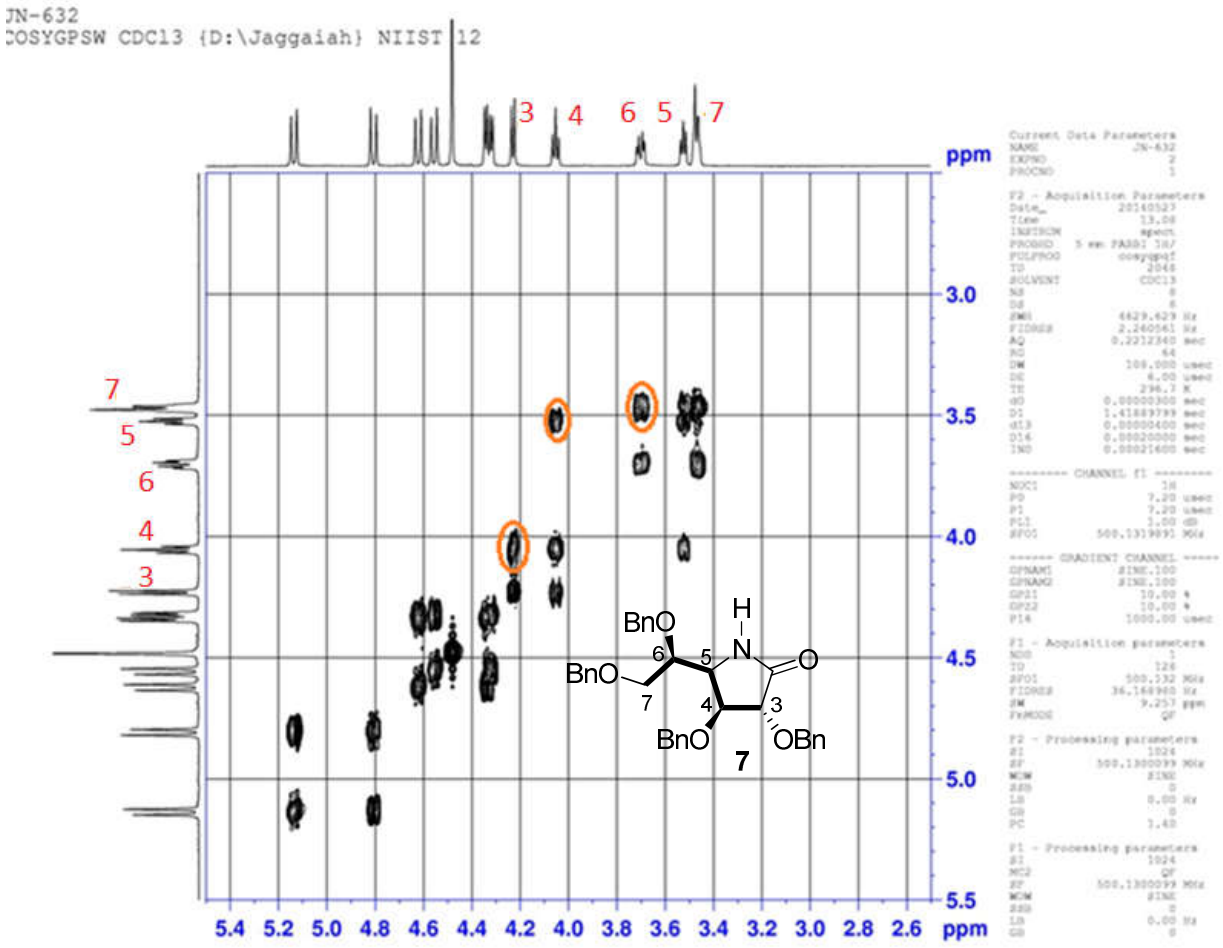
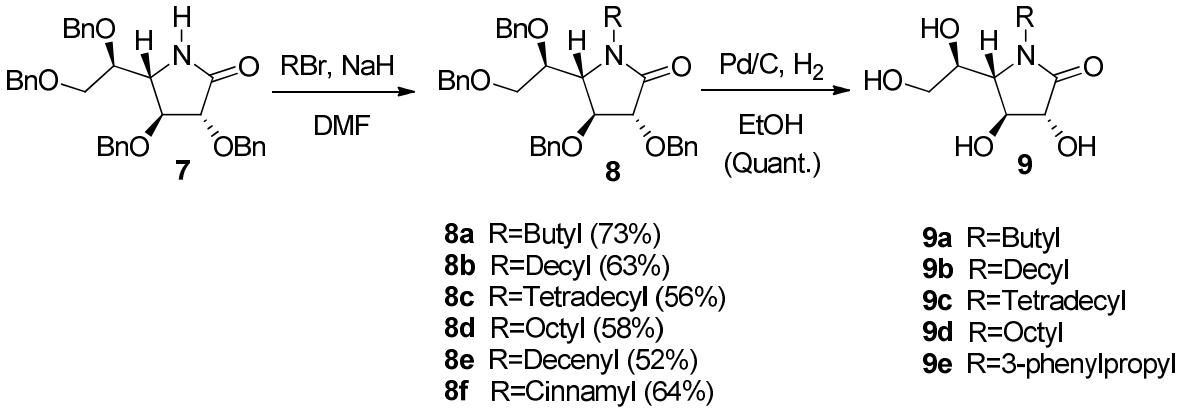


Figure 3.3.1: COSY correlations of 2-pyrrolidinone (**7**)

The lactam **7** was then treated with different alkyl bromides in presence of NaH in DMF to produce the N-alkyl γ-lactams. In our initial attempt lactam **7** was treated with 3 equivalents of NaH at room temperature for overnight under inert atmosphere surprisingly C-2 and C-5 benzyloxy groups have undergone elimination resulting in undesired products. However, we succeeded in synthesizing γ-lactam derivatives (**8a-f**) in good yields (Scheme 3.3.1A) by reacting **7** with different alkyl bromides using 1.5 equivalents of NaH in DMF at 0 °C to room temperature for 3-5 h under inert atmosphere. Finally the N-alkylated derivatives (**8a-f**) were subjected to global debenzylation with 10% Pd/C with few drops of conc. HCl under H_2

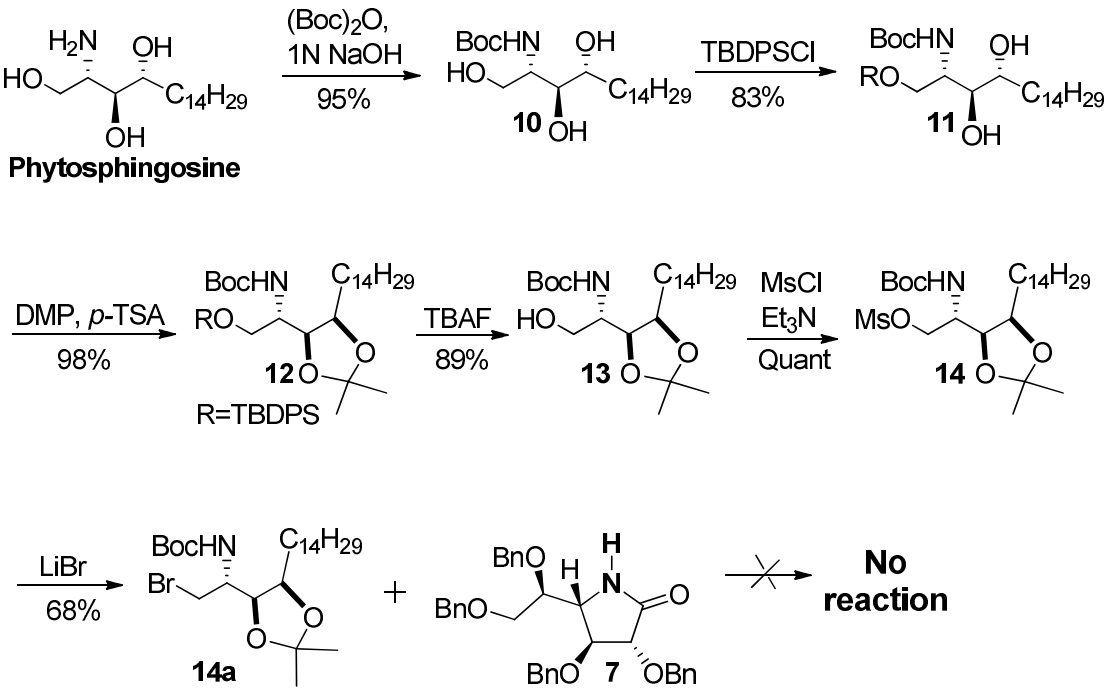
atmosphere for overnight which resulted in polyhydroxy-N-alkyl γ-lactam derivatives (**9a-e**) in quantitative yields.



Scheme 3.3.1A: Synthesis of *N*-alkylated polyhydroxy γ-lactams **9a-e**

3.3.2 Attempts towards N-alkylation reaction of 1-bromophytosphingosine (14a) with 2-pyrrolidinone (7):

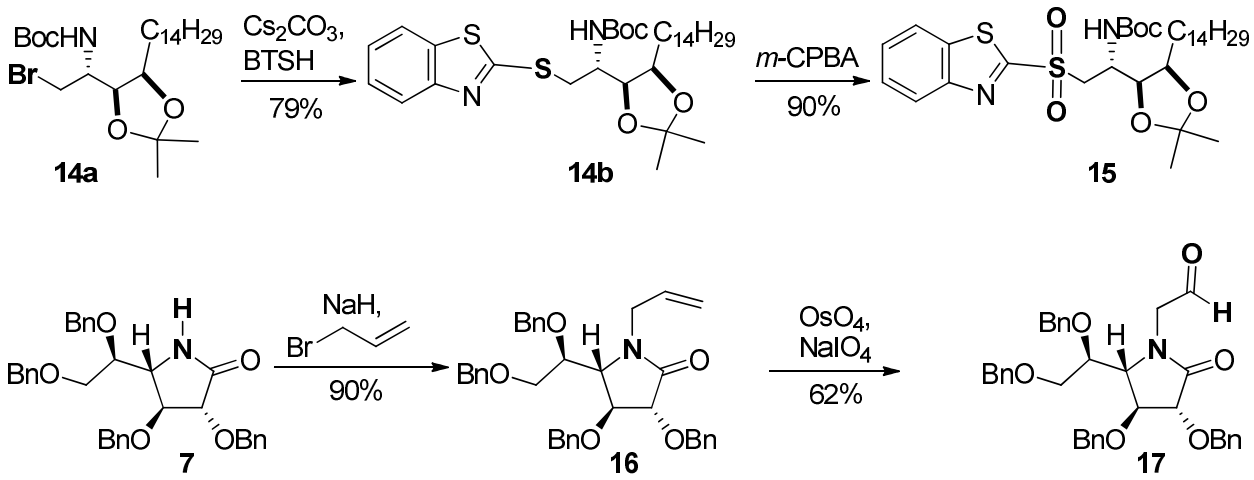
1-bromophytosphingosine (**14a**) synthesis commenced from commercially available phytosphingosine which was initially treated with $(Boc)_2O$ giving rise to N-Boc protected phytosphingosine (**10**, Scheme 3.3.2). The primary alcohol was protected with TBDPS group which resulted in compound **11** with 83% yield. The diol was treated with 2,2-dimethoxypropane (DMP) in the presence of a catalytic amount of *p*-TSA affording acetonide **12** in 98% yield, and then the silyl group was removed with a fluoride source affording primary alcohol **13** in 89% yield as a colorless solid.[22] The primary alcohol was converted into mesylate **14** in quantitative yield which was substituted with bromide using lithium bromide resulting in 1-bromo-phytosphingosine **14a** in 68% yield. Unfortunately, treatment of lactam **7** with **14a** using NaH resulted in no reaction.



Scheme 3.3.2: Attempts towards N-alkylation reaction on 2-pyrrolidinone **7**

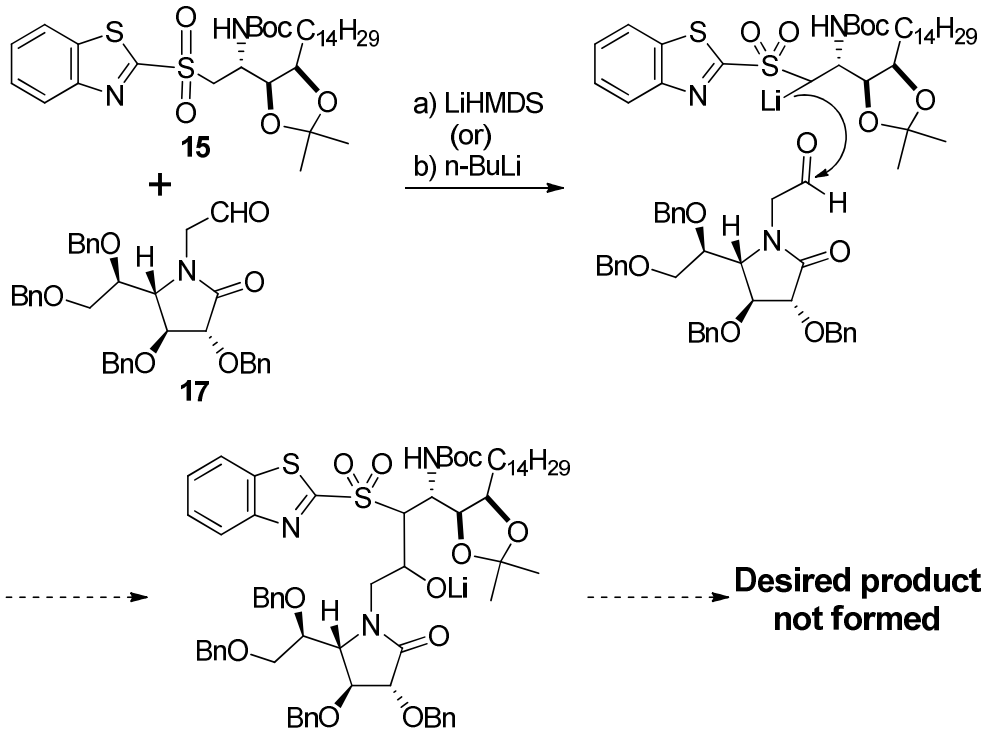
3.3.3. Attempts for connecting sugar and lipid moieties via Julia reaction:

Julia precursor BT-sulfone **15** synthesis commenced from 1-bromo-phytosphingosine **14a** which was prepared in six steps (Scheme 3.3.2). SN2 reaction with in situ generated thiolate anion, from the 2-mercaptobenzothiazole, afforded thioether **14b** which on further oxidation using *m*-CPBA produced BT-sulfone **15** in good yield (Scheme 3.3.3).[22] The aldehyde **17** was synthesized from lactam **7** which was initially treated with allyl bromide under basic conditions to afford N-ally-2-pyrrolidinone (**16**) in 90% yield. Compound **16** was subjected to dihydroxylation reaction followed by oxidative cleavage of diol using OsO_4 and $NaIO_4$, respectively, which produced the desired aldehyde **17** in 62% yield (Scheme 3.3.3).



Scheme 3.3.3: Synthesis of BT-sulfone (**15**) and aldehyde (**17**)

Reaction of BT-sulfone **15** and aldehyde **17** under Julia reaction conditions failed to afford the desired coupled product either while using *n*-BuLi or LiHMDS (Scheme 3.3.3A).



Scheme 3.3.3A: Attempted synthetic route for the C-C bond formation via Julia reaction

3.4. Successful synthesis of 1,2,3-triazol-phytoceramide-2-pyrrolidinone derivatives (21a-d):

Figure 3.4 outlines the retrosynthetic analysis for synthesis of 1*H*-1,2,3-triazole, 1-[phytoceramide]-4-methyl-2'-pyrrolidinone **21a-d** by "click chemistry". The required azasugar moiety can be obtained from *N*-propargyl-2-pyrrolidinone 1**8**, which in turn was derived from D-galactose. The 1-azidophytosphingosine **19** moiety can be derived from commercially available phytosphingosine in six steps.

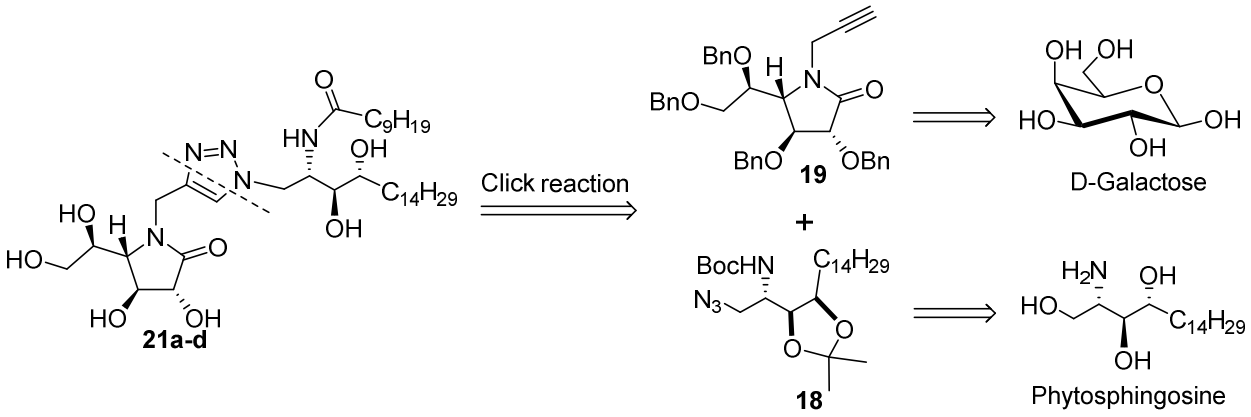
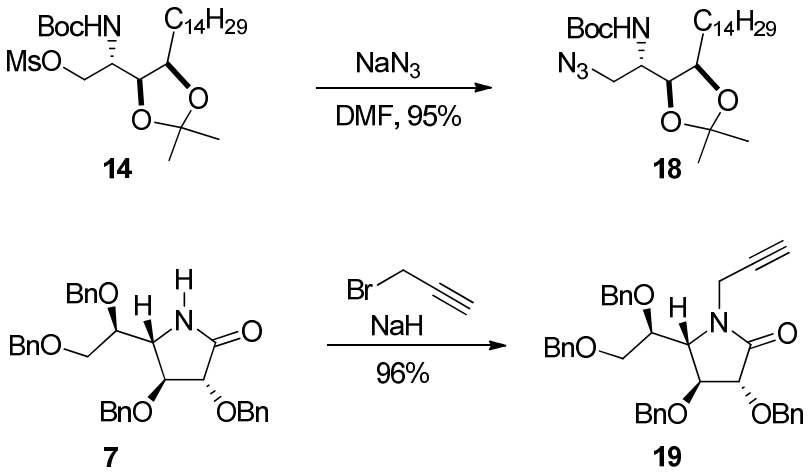


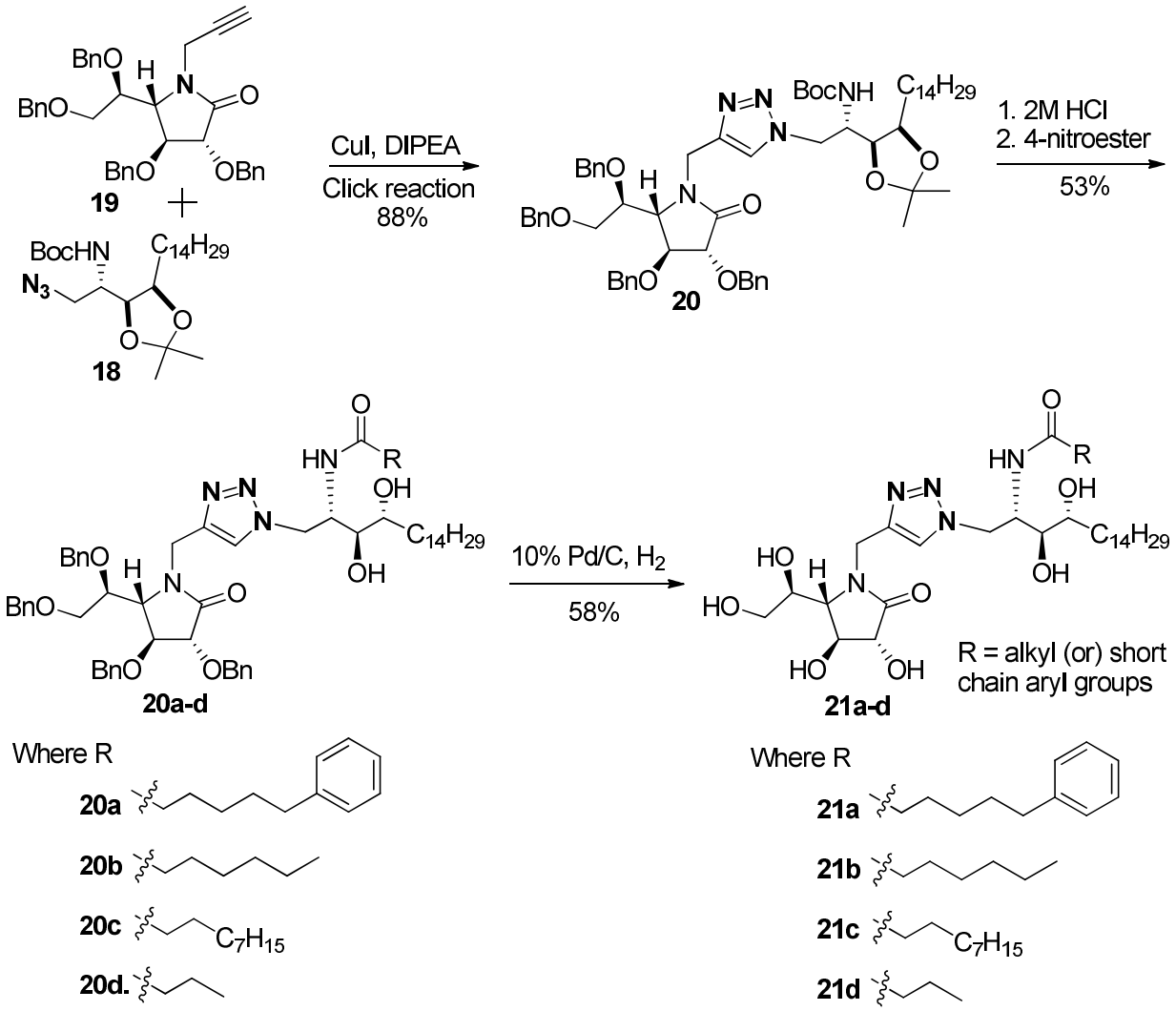
Figure 3.4: Retrosynthetic route to 2-pyrrolidinone phytoceramides (**21a-d**)

N-Propargylation of 2-pyrrolidinone (**7**) using propargyl bromide in the presence of NaH afforded *N*-propargyl-2-pyrrolidinone **19** in 96% yield (Scheme 3.4). The azido-phytosphingosine **18** was synthesized from mesylate **14** under standard SN2 conditions using NaN_3 in DMF. Mesylate **14** was derived from commercially available phytosphingosine by reported conditions.



Scheme 3.4: Synthesis of click-chemistry precursors azide **18** and alkyne **19**

N-Propargyl-2-pyrrolidinone **19** and azido-phytosphingosine **18** were coupled by employing Cu-catalyzed azide-alkyne cycloadditon conditions in the presence of CuI and DIPEA,[23] resulting in a single 1,4-triazole isomer **20** in 88% yield(Scheme 3.4A). Deprotection of the Boc and acetonide groups under acidic conditions, and subsequent *N*-acylation of the resulting amine using 4-nitrophenyl esters afforded the ceramide products **20a-d**. Global debenzylation of compounds **20a-d** using 10% Pd/C under H_2 afforded the desired 1*H*-1,2,3-triazole, 1-[phytoceramide]-4-methyl-2'-pyrrolidinone derivatives **21a-d**.



Scheme 3.4A: Synthesis of 1*H*-1,2,3-triazole, 1-[phytoceramide]-4-methyl-2'-pyrrolidinone derivatives **21a-d**

3.5. Structural confirmation of 2-pyrrolidinones 7 & 19:

The trans coupling constants observed for 2-pyrrolidinone **7** ($J_{3\text{-}4,\ 4\text{-}5}$ = 6 Hz) was in agreement with the literature.[21,24] Key NOESY cross peaks were observed between H-3 at δ_H 4.22 (d, *J* = 6 Hz) and H-5 at δ_H 3.51 (app t, *J* = 6 Hz) (Figure 3.5A); indicating the presence of these two protons on the same side. The Key HMBC correlations of 2-pyrrolidinone **19** can be observed at δ_C 171 ppm C-2 with H-3, H-4, H-5 and H-8, confirming the five-membered ring (Figure 3.5B).

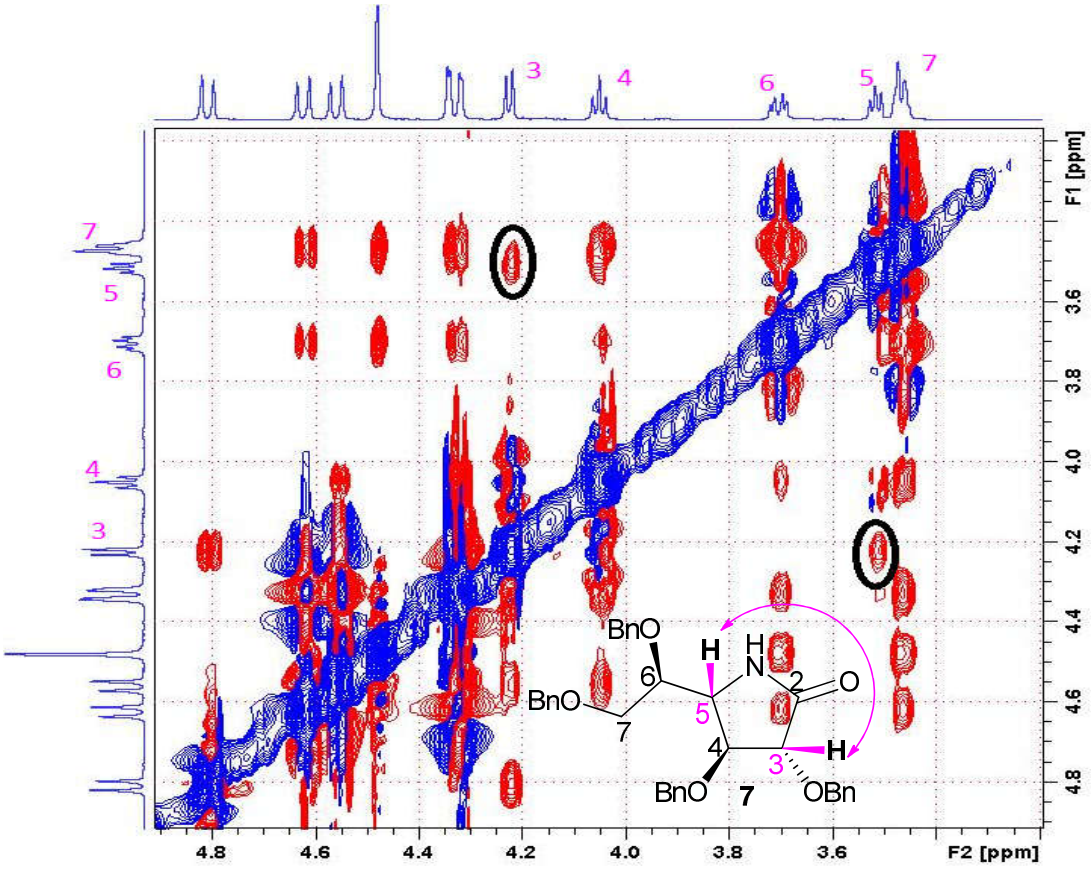


Figure 3.5A: Key NOESY correlations for C-5 stereochemistry of 2-pyrrolidinone **7**

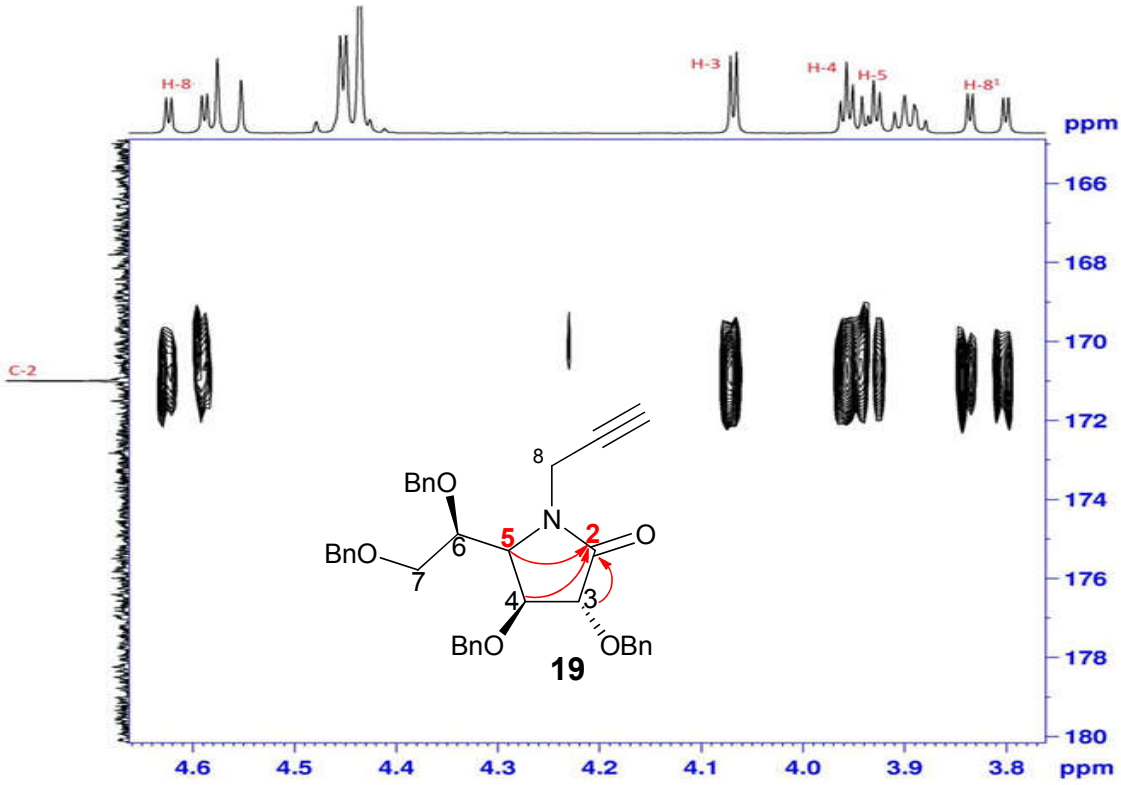


Figure 3.5B: Key HMBC correlations for the ring determination of compound **19**

3.6. Conclusion:

In summary, N-alkyl 2-pyrrolidinone derivatives (**9a-e**) and novel GSL analogs **21a-d** with a polyhydroxy 2-pyrrolidinone appended to the phytoceramide via a 1,2,3-triazole linker has been synthesized in a convergent manner from a single intermediate lactam **7** derived from D-galactose. *N*-Propargyl-2-pyrrolidinone **19** and azido-phytosphingosine **18** derived from D-galactose and commercially available phytosphingosine, respectively, were coupled by employing standard "click chemistry" conditions. The immunological cytokine response of compounds **9a-d** and novel GSL analogs **21a-d** upon iNKT cell activation is currently under screening and the results will be presented elsewhere.

3.7. Experimental section:

The solvents were dried as follows: CH_2Cl_2 and CH_3CN were distilled over calcium hydride, and MeOH was dried over magnesium turnings. All reactions were carried out under nitrogen atmosphere using oven-dried glassware. Silica gel 60 F_{254} aluminum TLC plates were used to monitor the reactions with short-wavelength ultraviolet light, iodine staining, and by charring the TLC plate after spraying with 15% sulfuric acid to visualize the spots. Column chromatography was performed on silica gel 120-200 and 230-400 mesh. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively. Chemical shifts are given in parts per million and coupling constants in hertz. HR-ESI-MS analysis was performed on a Thermo Scientific Exactive mass spectrometer with ions given in m/z.

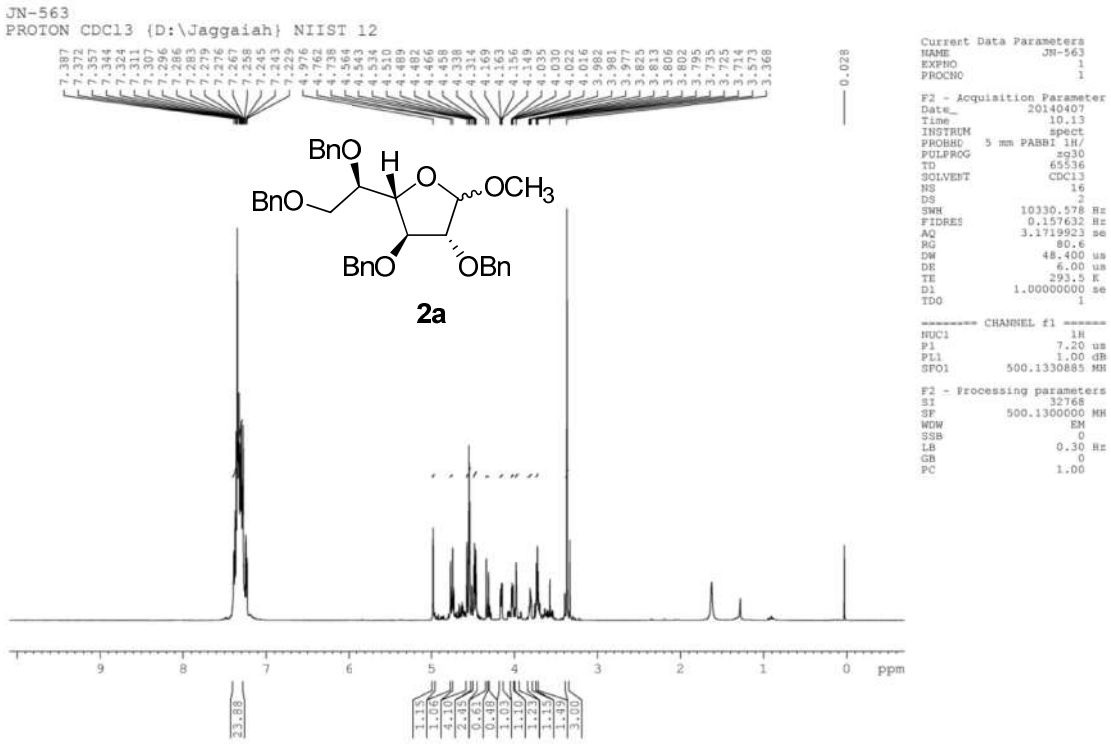
Methyl-D-galactofuranoside (1) and Methyl-D-galactopyranoside (1a):

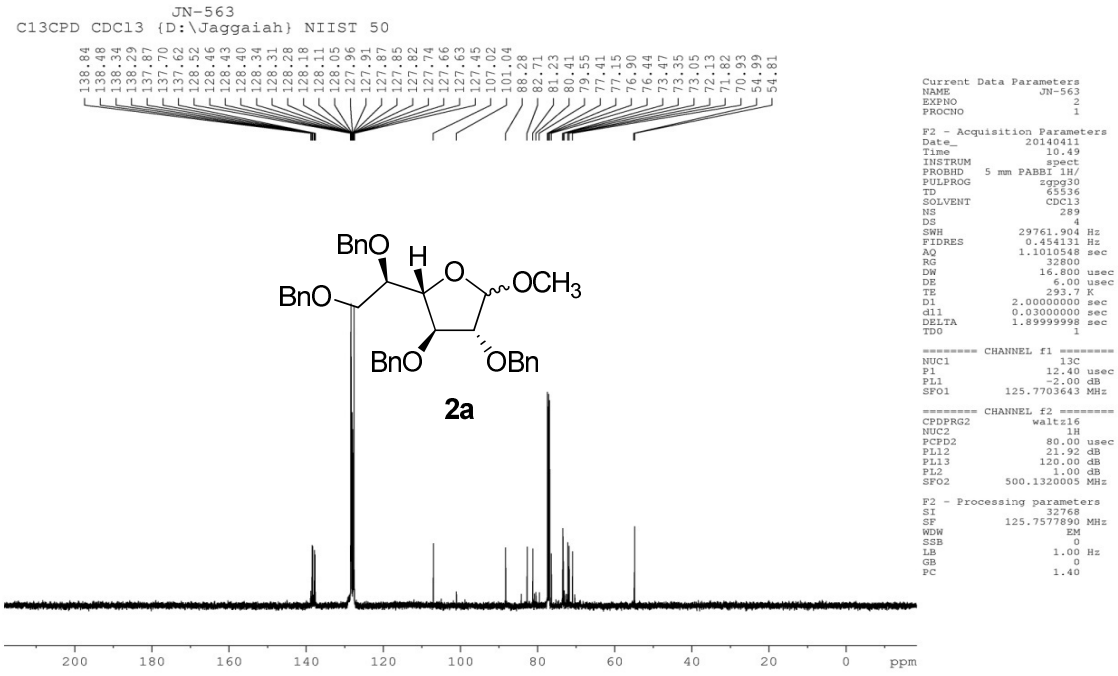
To a solution of D-galactose (5 g, 27.7 mmol, 1 equiv) in MeOH (80 mL) was added Dowex H^+ resin (2 g) and the resulting mixture was heated at reflux. After 3 h, the reaction mixture was allowed to attain room temperature, filtered through cotton plug and washed with methanol (50 mL), concentrated, and dried under vacuum for 4 h which resulted in a colorless sticky mixture of compounds **1** and **1a** (5.37 g). The crude mixture was directly used for the next step without further purification.

2,3,5,6-Tetra-O-benzyl-methyl-D-galactofuranoside (2) and 2,3,4,6-tetra-O-benzyl-methyl-D-galactopyranoside (2a): To a solution of the mixture of isomers **1** and **1a** (5.37 g) in DMF (150 mL) was added 60% NaH (8.84 g, 222.1 mmol, 8 equiv) at 0 °C and stirred for 30 minutes under nitrogen atmosphere. BnBr (26.4 mL, 222.1 mmol, 8 equiv) was added slowly at 0 °C and the resulting mixture was continued to stir at room temperature overnight. The reaction mixture was quenched by adding cold water (100 mL) drop wise at 0 °C and extracted with EtOAc (2 ×

250 mL), the extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc (95:5 to 90:10), afforded perbenzylated isomers **2** and **2a** (7.10 g) as a mixture (46%, over two steps). Compound **2**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.36 (s, 3H), 3.71−3.73 (m, 2H), 3.79−3.82 (m, 1H), 3.97−3.98 (m, 1H), 4.02 (dd, *J* = 2.5, 6.5 Hz, 1H), 4.15 (dd, *J* = 3.5, 6.5 Hz, 1H), 4.32 (d, *J* = 12 Hz, 1H), 4.47 (dd, *J* = 3.5, 11.5 Hz, 2H), 4.53−4.56 (m, 4H), 4.75 (d, *J* = 12 Hz, 1H), 4.97 (s, 1H), 7.22−7.38 (m, 20H); ^{13}C NMR ($CDCl_3$, 125 MHz), δ 54.9, 70.9, 71.8, 72.1, 73.0, 73.4, 76.4, 81.2, 82.7, 88.2, 107.0, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 137.6, 137.8, 138.3, 138.8; HR-ESI-MS $[M+Na]^+$ cacld for m/z $C_{35}H_{38}NaO_6^+$ 577.2566, found 577.2575.

^{1}H, ^{13}C-NMR spectra of compound **2a**





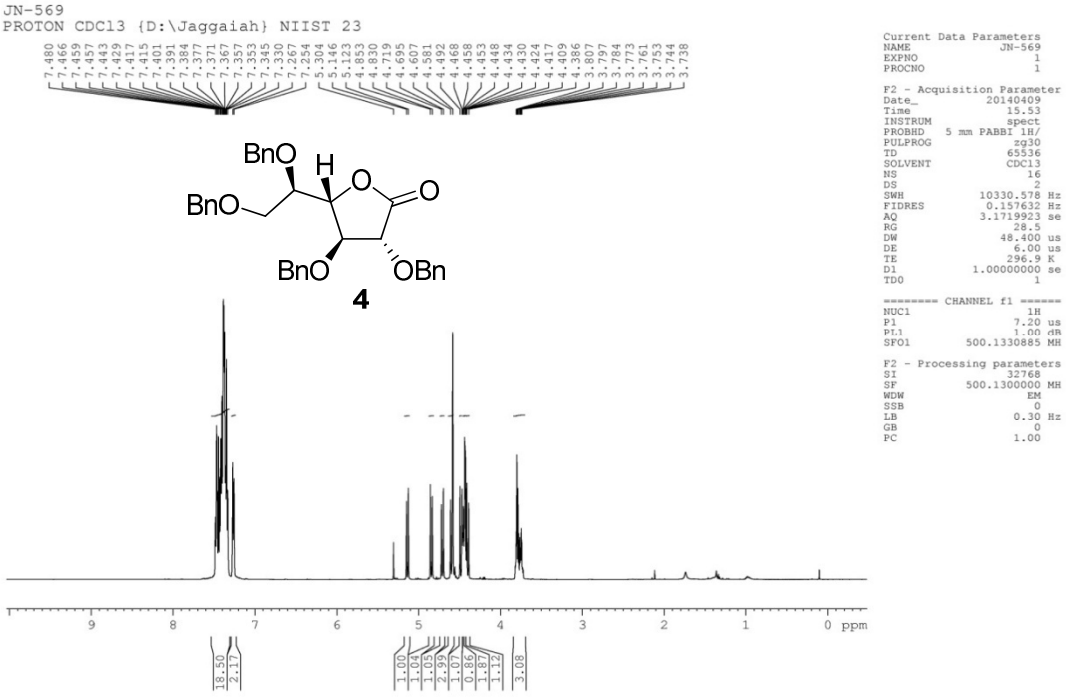
1-Hydroxy-2,3,5,6-tetra-O-benzyl-D-galactofuranoside (3):

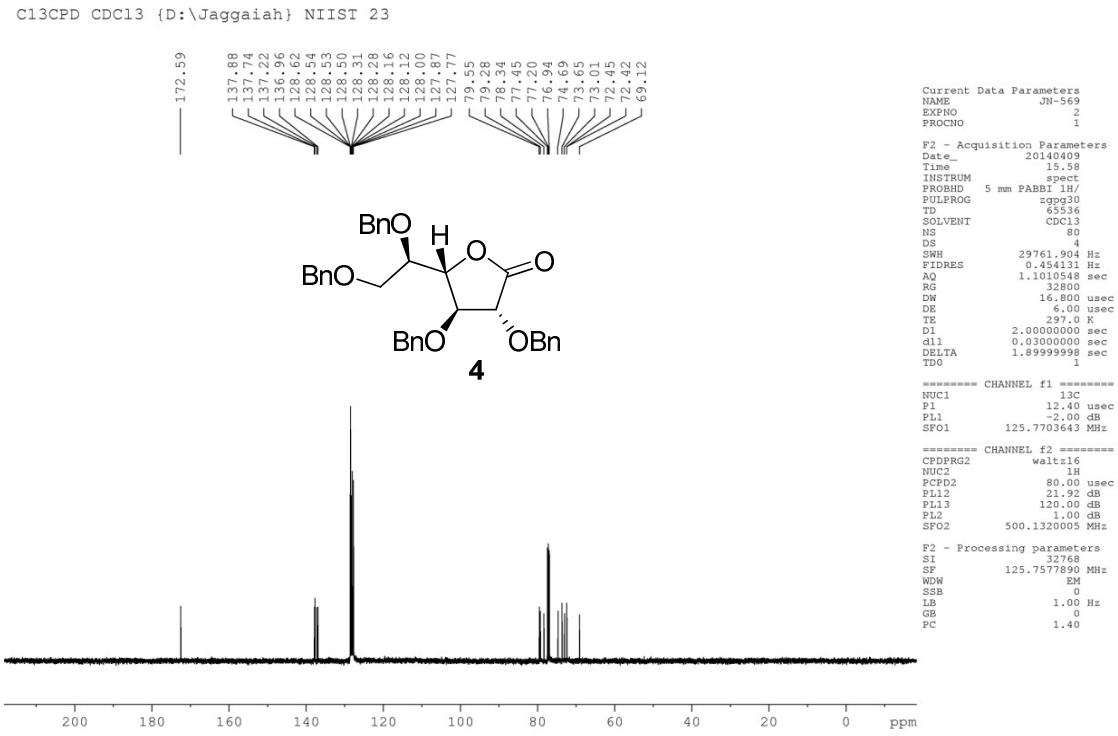
To a solution of the mixture of compounds **2** and **2a** (12.1 g, 21.7 mmol, 1 equiv) in acetic acid (100 mL) was added 1N H_2SO_4 (30 mL) and heated at reflux for 2 h. The reaction mixture was allowed to attain room temperature, diluted with water (50 mL) and extracted with DCM (4 × 150 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (4 × 100 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 80:20 afforded *α,β*-anomeric mixture of lactol **3** (5.22 g, 44%) as a colorless sticky solid: R_f 0.27 (hexane/EtOAc 7.5:2.5); HR-ESI-MS $[M+Na]^+$ cacld for m/z $C_{34}H_{36}NaO_6^+$ 563.2410, found 563.2412.

2,3,5,6-Tetra-O-benzyl-D-galactono-1,4-lactone (4): To a solution of lactol **3** (5.22 g, 9.6 mmol, 1 equiv) in DCM (100 mL) was added Dess−Martin periodinane (8.20 g, 19.3 mmol, 2 equiv), and the reaction mixture was stirred for 2 h at room temperature under nitrogen

atmosphere. The reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ (100 mL) and saturated aqueous $NaHCO_3$ (100 mL), extracted with DCM (3 × 150 mL), and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2 × 100 ml), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 afforded lactone **4** (4.86 g, 93%) as a colorless sticky solid: R_f 0.53 (hexane/EtOAc 6:4); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.73−3.80 (m, 3H), 4.39 (d, *J* = 11.5 Hz, 1H), 4.41−4.43 (m, 2H), 4.44−4.45 (m, 1H), 4.48 (d, *J* = 12 Hz, 1H), 4.58−4.60 (m, 3H), 4.70 (d, *J* = 12 Hz, 1H), 4.84 (d, *J* = 11.5 Hz, 1H), 5.13 (d, *J* = 11.5 Hz, 1H), 7.25−7.46 (m, 20H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 69.1, 72.4, 73.0, 73.6, 74.6, 78.3, 79.2, 79.5, 127.7, 127.8, 128.1, 128.3, 128.5, 128.6, 136.9, 137.2, 137.7, 137.8, 172.5; HR-ESI-MS $[M+Na]^+$ cacld for m/z $C_{34}H_{34}NaO_6^+$ 561.2253, found 561.2262.

^{1}H, ^{13}C-NMR spectra of compound **4**



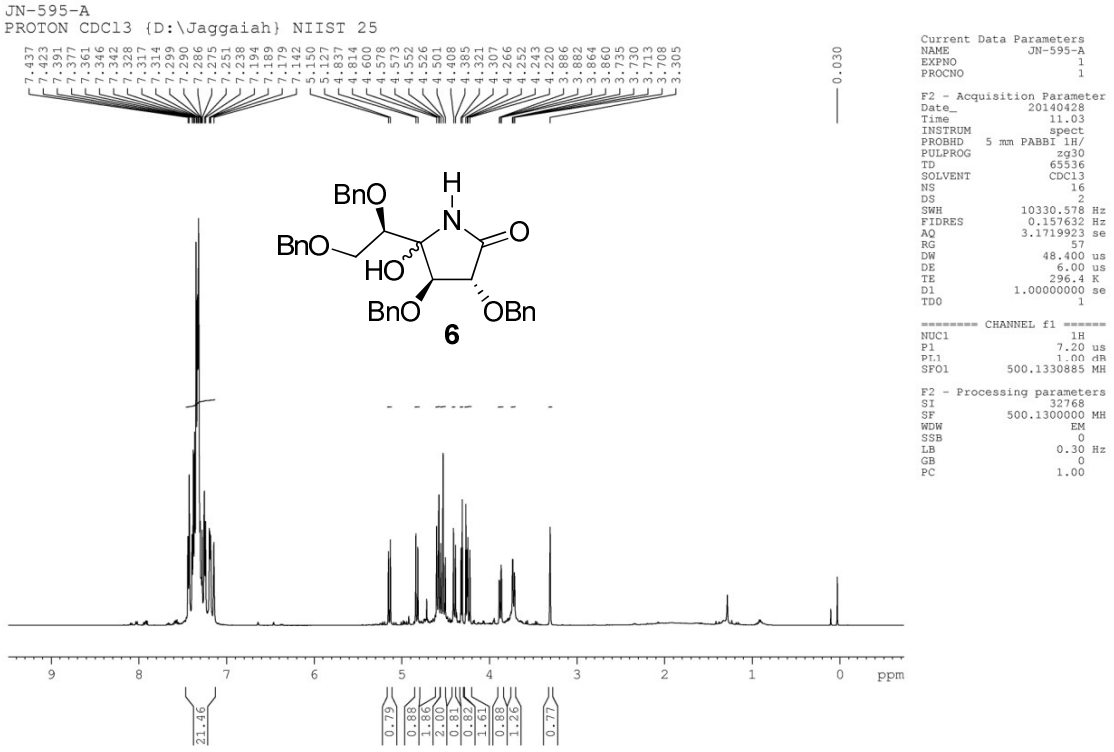


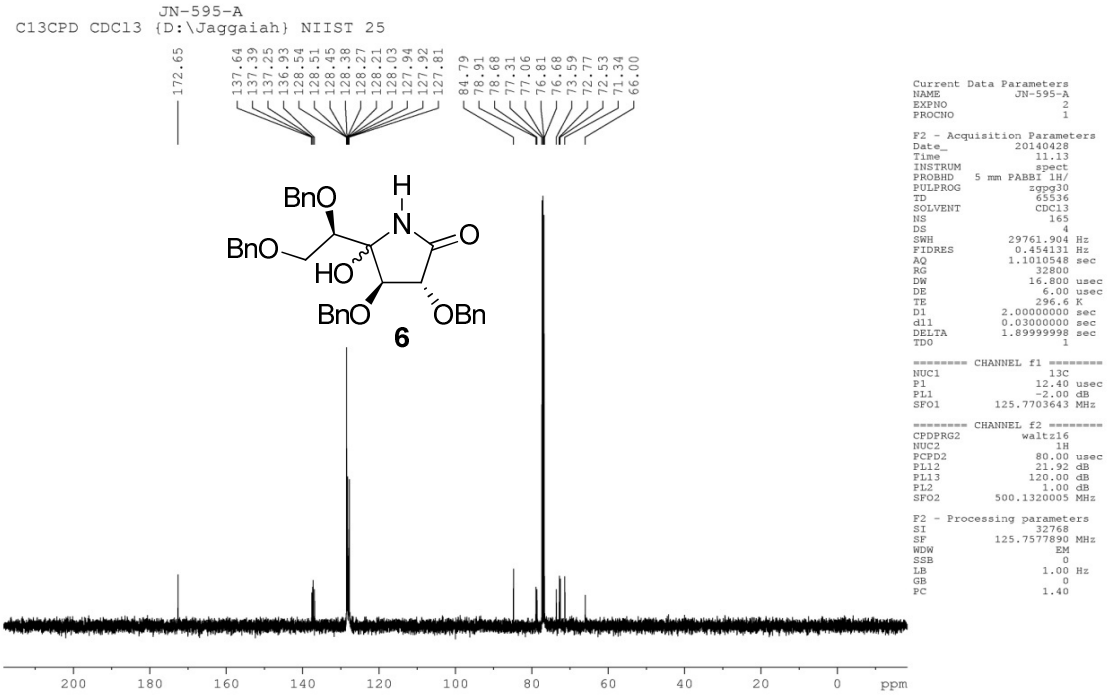
4-Hydroxy-2,3,5,6-tetra-O-benzyl-D-galactono-1,4-lactam (6):

Lactone **4** (1.40 g, 2.6 mmol, 1 equiv) was dissolved in 7 N ammonia in MeOH solution (10 mL) and stirred for 1 h at room temperature under nitrogen atmosphere. After consumption of the starting material as indicated by TLC (hexane/EtOAc 3:2), the reaction mixture was diluted with MeOH (20 mL) and evaporated to afford carboxamide **5** as a pale yellow solid which was recrystallized using hexane/ethyl acetate (1:1). To a solution of carboxamide **5** (2.6 mmol, 1 equiv) in DCM (15 mL) was added Dess−Martin periodinane (1.65 g, 3.9 mmol, 1.5 equiv) and the reaction mixture was stirred for 2 h at room temperature under nitrogen atmosphere. The reaction mixture was then quenched with saturated aqueous $Na_2S_2O_3$ (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) and extracted with DCM (2 × 150 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (50 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 to 80:20

afforded compound **6** (793 mg, 55% over two steps) as a white solid: R_f 0.60 (hexane/EtOAc 6:4); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.30 (s, 1H), 3.72 (dd, *J* = 2.5, 11 Hz, 1H), 3.87 (dd, *J* = 2, 11 Hz, 1H), 4.22−4.26 (m, 2H), 4.31 (d, *J* = 7 Hz, 1H), 4.39 (d, *J* = 11.5 Hz, 1H), 4.50−4.55 (m, 2H), 4.57−4.60 (m, 2H), 4.82 (d, *J* = 11.5 Hz, 1H), 5.13 (d, *J* = 11.5 Hz, 1H), 7.14−7.43 (m, 20H), ^{13}C NMR ($CDCl_3$, 125 MHz) δ 66.0, 71.3, 72.5, 72.7, 73.6, 78.7, 78.9, 84.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 136.9, 137.2, 137.3, 137.6, 172.6; HR-ESI-MS $[M+H]^+$ cacld for m/z $C_{34}H_{36}NO_6^+$ 554.2542, found 554.2558.

^{1}H, ^{13}C-NMR spectra of compound **6**

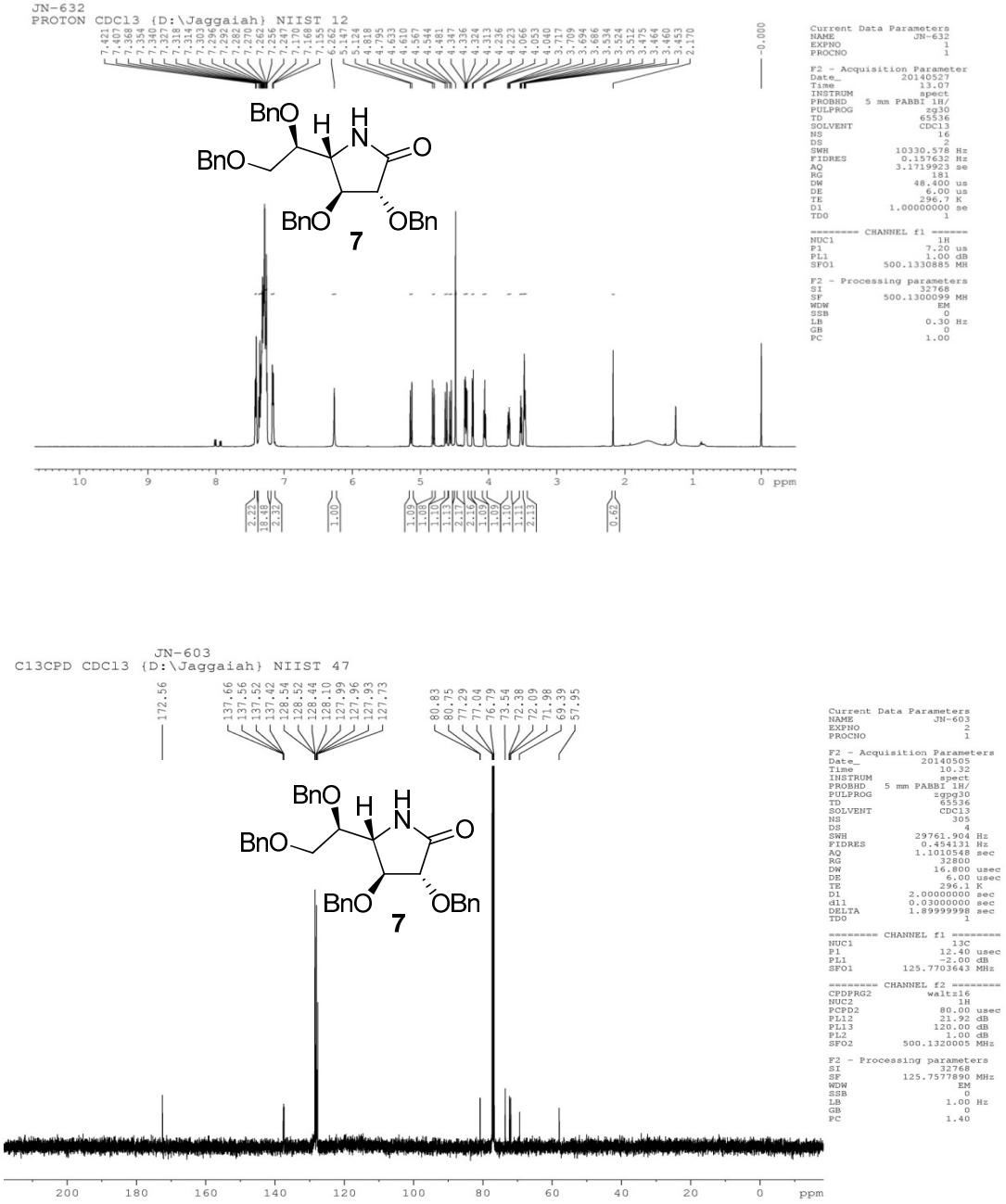




2-Pyrrolidinone (7): To a solution of compound **6** (870 mg, 1.5 mmol, 1 equiv) in distilled CH_3CN (35 mL) was added $NaCNBH_3$ (1.97 g, 31.4 mmol, 20 equiv) and HCOOH (8 mL), the reaction mixture was then heated at reflux for 3 h under nitrogen atmosphere. After formation of the non-polar product as indicated by TLC (hexane/EtOAc 7:3), the reaction mixture was quenched with 0.1N HCl (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 to 90:10 to 85:15 afforded 2-pyrrolidinone **7** (380 mg, 45%) as a colorless sticky solid: R_f 0.40 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.44−3.47 (m, 2H, H-7), 3.51 (app t, *J* = 6 Hz, 1H, H-5), 3.70 (dd, *J* = 4.0, 11.5 Hz, 1H, H-6), 4.05 (app t, *J* = 6 Hz, 1H, H-4), 4.22 (d, *J* = 6 Hz, 1H, H-3), 4.32 (dd, *J* = 2, 11.5, Hz, 2H), 4.48 (s, 2H), 4.55 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 12 Hz, 1H), 4.80 (d, *J* = 11.5 Hz, 1H), 5.13 (d, *J* = 11.5 Hz, 1H), 6.26 (s, 1H), 7.15−7.42 (m, 20H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 57.9, 69.3, 71.9,

72.1, 72.3, 73.5, 80.7, 80.8, 127.7, 127.9, 128.1, 128.4, 128.5, 137.4, 137.52, 137.56, 137.6, 172.5; HR-ESI-MS $[M+H]^+$ calcd for m/z $C_{34}H_{36}NO_5^+$ 538.2593, found 538.2598.

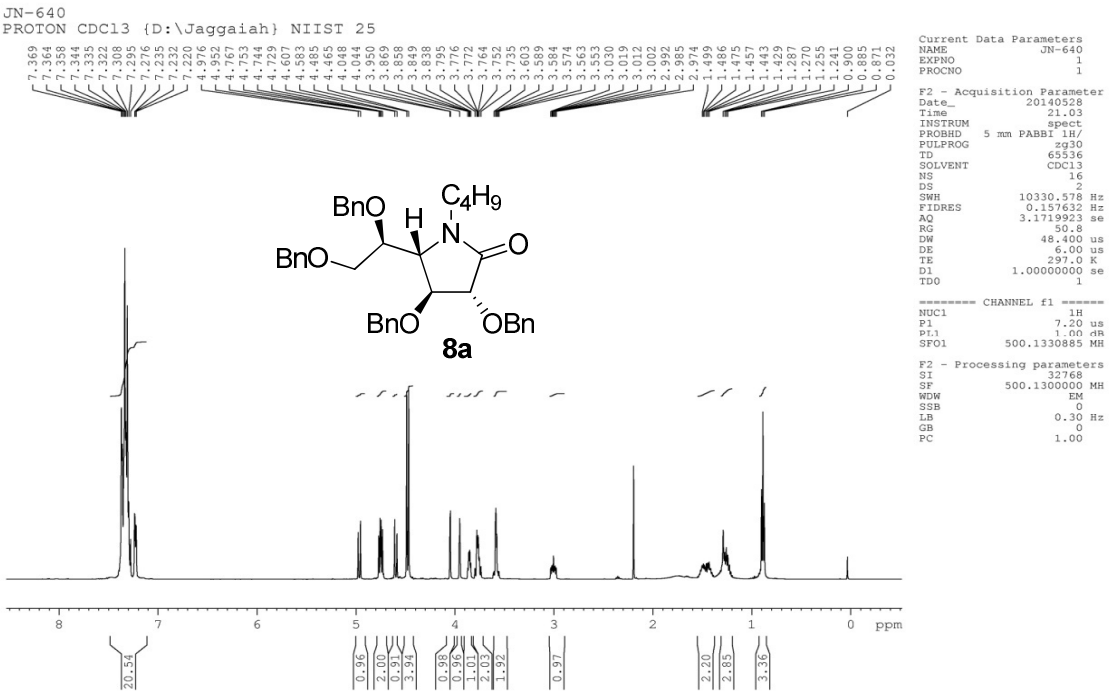
^1H, ^{13}C-NMR spectra of compound **7**

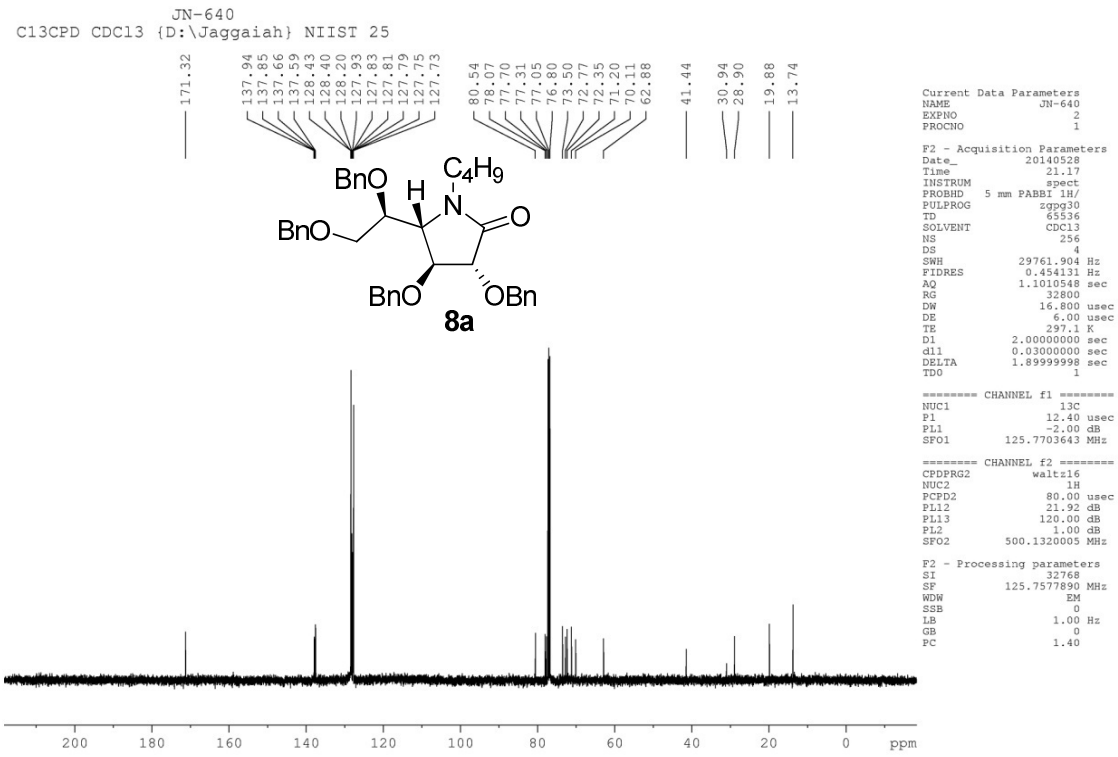


N-butylpyrrolidin-2-one (8a): To a solution of compound **7** (50 mg, 0.093 mmol, 1 equiv) in DMF (1.5 mL) was added 60% NaH (5 mg, 0.139 mmol, 1.5 equiv) at 0°C and stirred for 15

minutes under inert atmosphere. Then Butyl bromide (30 µL, 0.279 mmol, 3 equiv) was added at 0°C and stirred for 5 hours. After the completion of the starting material as indicated by TLC, the reaction mixture was quenched with H_2O (5 mL), extracted with EtOAc (2 x 30 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash column chromatography using hexane/EtOAc 95/5 afforded the compound **8a** (40 mg, 73%). R_f 0.39 (hexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37-7.22 (m, 20H), 4.96 (d, *J* = 12 Hz, 1H), 4.74 (dd, *J* = 11.5, 7 Hz, 2H), 4.59 (d, *J* = 12 Hz, 1H), 4.48 (s, 2H), 4.46 (s, 2H), 4.04 (d, *J* = 2 Hz, 1H), 3.95 (bs, 1H), 3.85 (app q, *J* = 5.5 Hz, 1H), 3.79-3.73 (m, 2H), 3.60-3.55 (m, 2H), 3.00 (ddd, *J* = 14, 9, 5.5 Hz, 1H), 1.49-1.42 (m, 2H), 1.28-1.24 (m, 2H), 0.88 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 137.9, 137.8, 137.6, 137.5, 128.4, 127.9, 127.8, 127.7, 80.5, 78.1, 77.7, 73.5, 72.7, 72.3, 71.2, 70.1, 62.8, 41.4, 28.9, 19.8, 13.7; HR-ESI-MS $[M+H]^+$ $C_{38}H_{44}NO_5$ calcd for m/z 594.3219 , found 594.3219.

^{1}H, ^{13}C-NMR spectra of compound **8a**

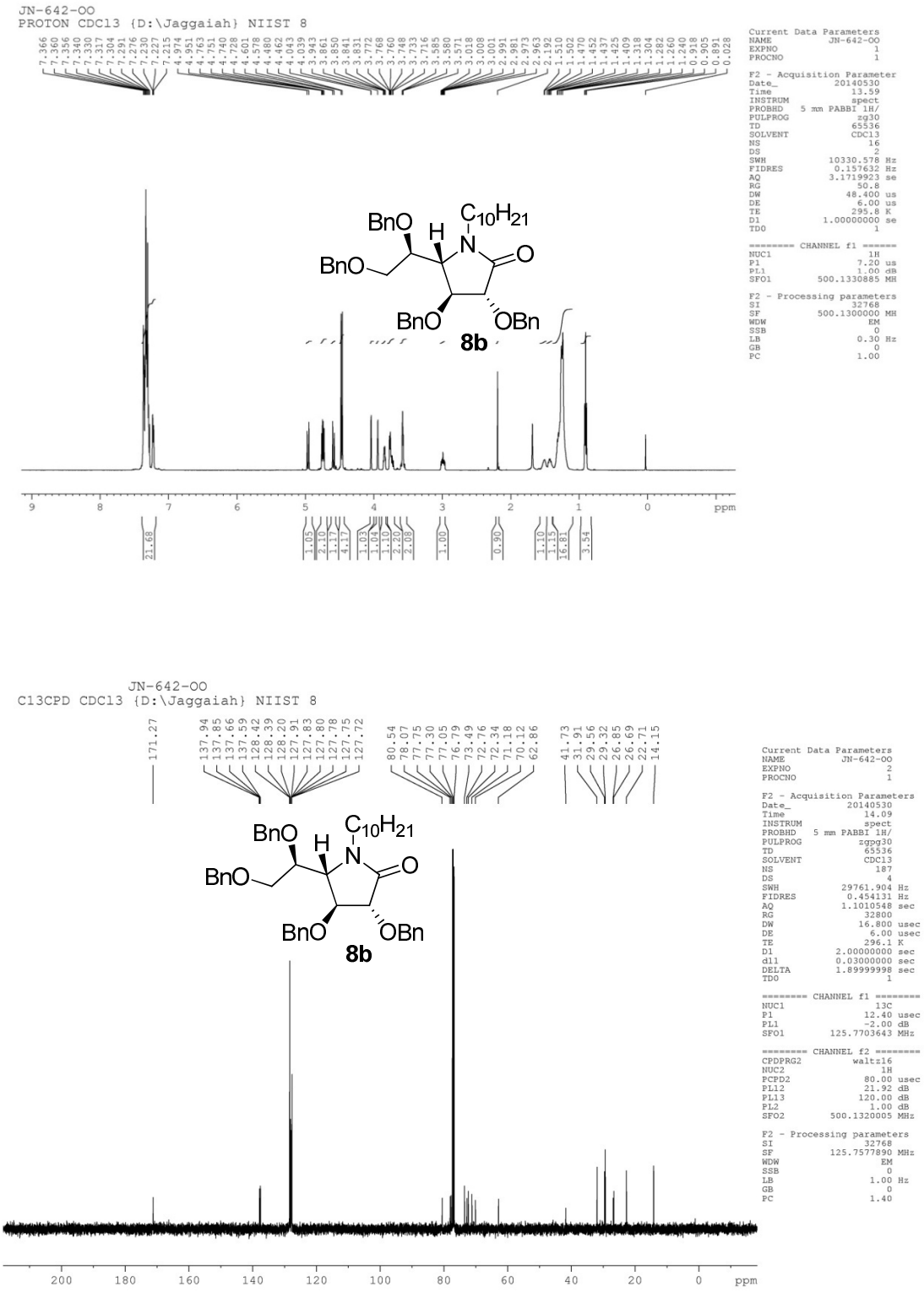




Procedure similar to synthesis of compound 8a was followed for the synthesis of compounds 8b-f:

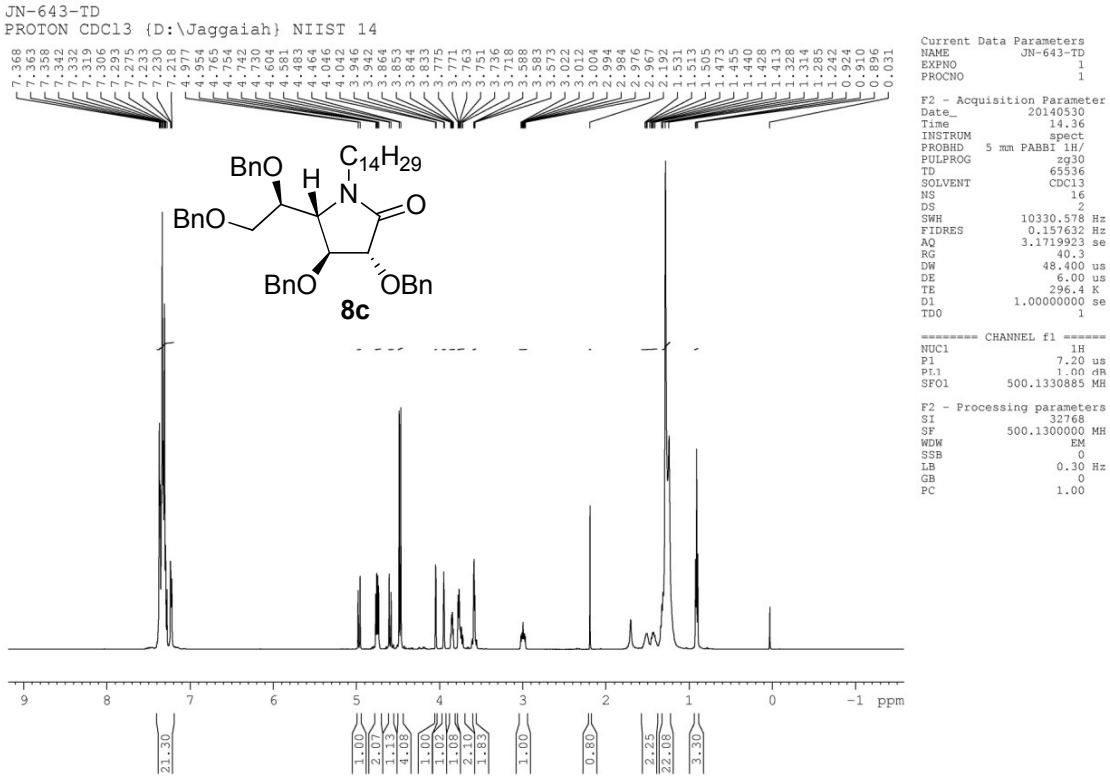
Compound 8b: R_f 0.46 (hexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36-7.21 (m, 20H), 4.96 (d, *J* = 11.5 Hz, 1H), 4.74 (dd, *J* = 11.5, 6 Hz, 2H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.48 (s, 2H), 4.46 (s, 2H), 4.04 (d, *J* = 2 Hz, 1H), 3.94 (bs, 1H), 3.84 (app q, *J* = 5.5 Hz, 1H), 3.77-3.71 (m, 2H), 3.58-3.57 (m, 2H), 2.99 (ddd, *J* = 13.5, 8.5, 5 Hz, 1H), 1.47-1.40 (m, 2H), 1.31-1.24 (m, 16H), 0.90 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (CDCl3, 125 MHz) δ 171.2, 137.9, 137.8, 137.6, 137.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 80.5, 78.1, 77.7, 73.4, 72.7, 72.3, 71.2, 70.1, 62.8, 41.7, 31.9, 29.5, 29.3, 26.8, 26.6, 22.7, 14.1; HR-ESI-MS $[M+H]^+$ $C_{44}H_{56}NO_5$ cacld for m/z 678.4158 , found 678.4160.

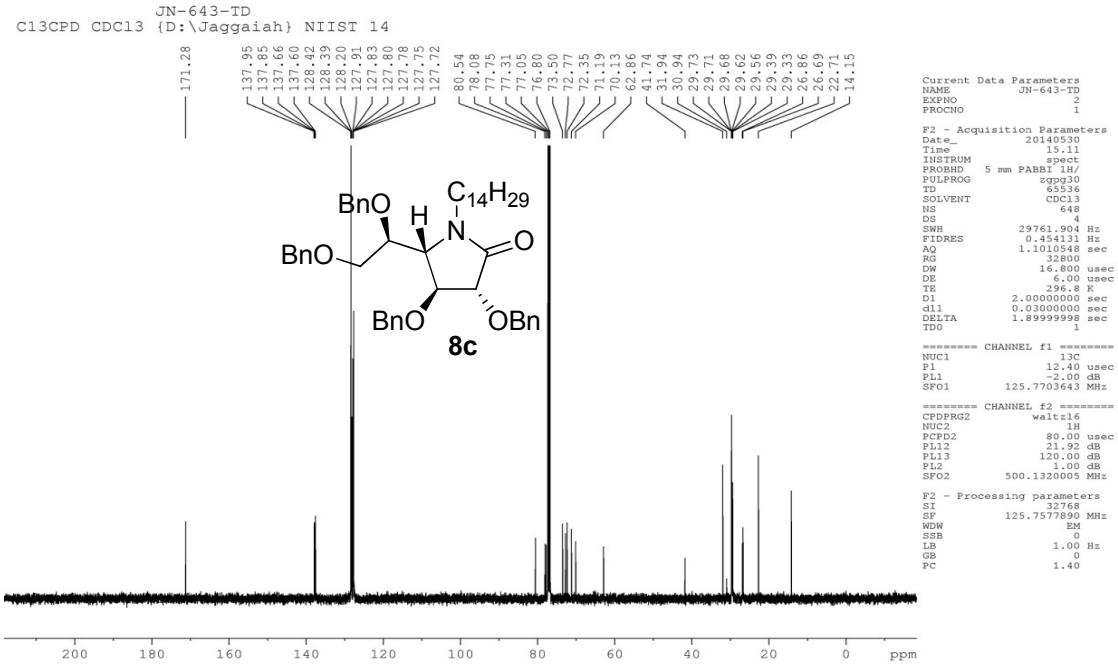
^{1}H, ^{13}C-NMR spectra of compound **8b**



Compound 8c: R_f 0.40 (hexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36-7.21 (m, 20H), 4.96 (d, *J* = 11.5 Hz, 1H), 4.74 (dd, *J* = 11.5, 5.5 Hz, 2H), 4.59 (d, *J* = 11.5 Hz, 1H), 4.48 (s, 2H), 4.46 (s, 2H), 4.04 (d, *J* = 2 Hz, 1H), 3.94 (bs, 1H), 3.84 (app q, *J* = 5.5 Hz, 1H), 3.77-3.71 (m, 2H), 3.58-3.57 (m, 2H), 2.99 (ddd, *J* = 14, 9, 5. Hz, 1H), 1.51-1.41 (m, 2H), 1.32-1.24 (m, 22H), 0.91 (t, *J* = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.2, 137.9, 137.8, 137.6, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 80.5, 78.0, 77.7, 73.5, 72.7, 72.3, 71.1, 70.1, 62.8, 41.7, 31.9, 29.7, 29.6, 29.5, 29.3, 26.8, 26.6, 22.7, 14.1; HR-ESI-MS $[M+H]^+$ $C_{48}H_{64}NO_5$ calcd for m/z 734.4784 , found 734.4779.

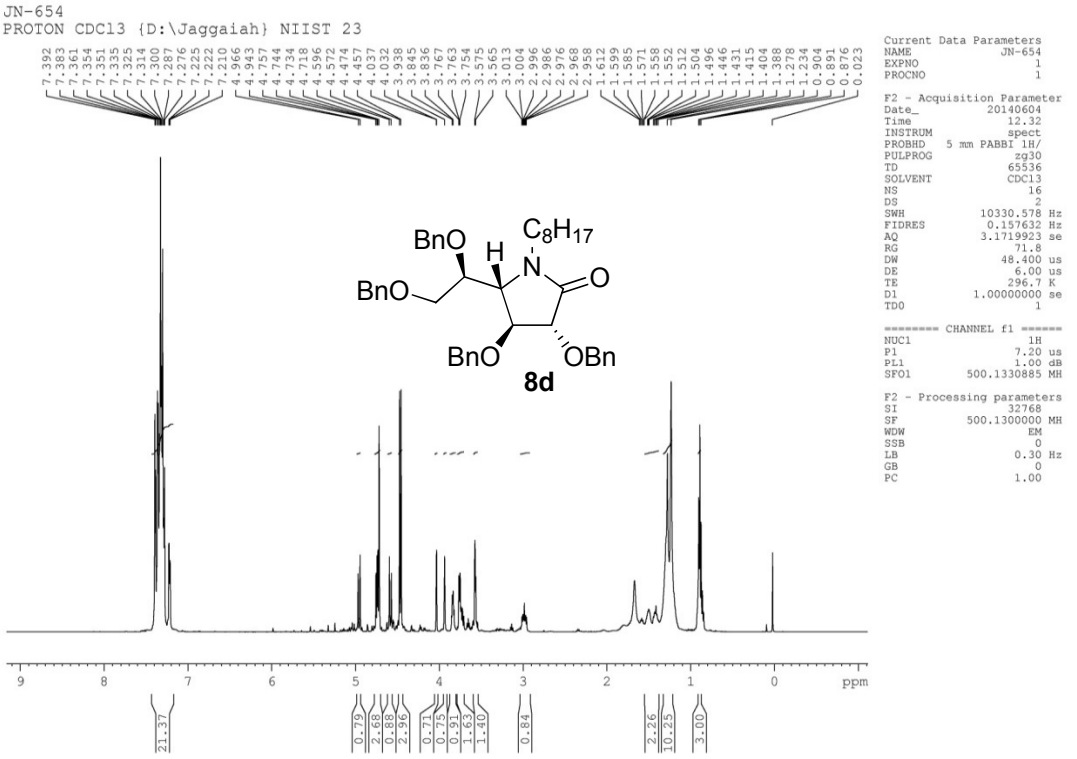
^{1}H, ^{13}C-NMR spectra of compound **8c**

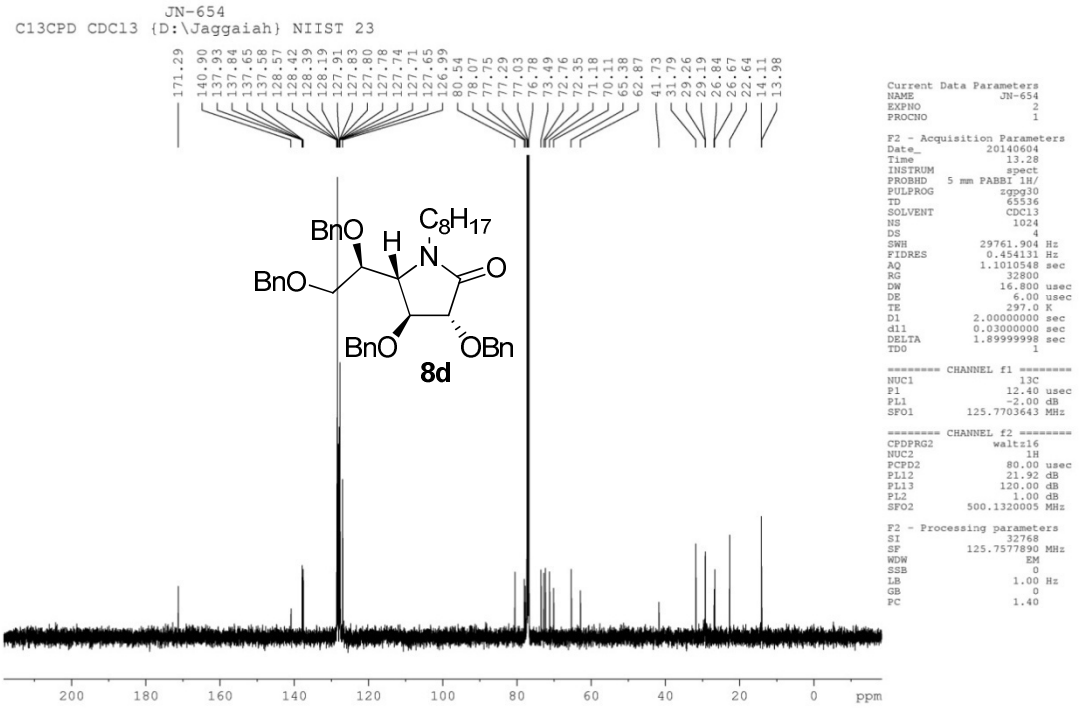




Compound 8d: R_f 0.50 (hexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.3-7.21 (m, 20H), 4.95 (d, *J* = 11.5 Hz, 1H), 4.75-4.71 (m, 4H), 4.58 (d, *J* = 12 Hz, 1H), 4.47 (s, 2H), 4.45 (s, 2H), 4.03 (d, *J* = 2.5 Hz, 1H), 3.93 (bs, 1H), 3.84 (app q, *J* = 5.5 Hz, 1H), 3.76-3.75 (m, 2H), 3.57-3.56 (m, 2H), 2.98 (ddd, *J* = 13.5, 8.5, 4.5. Hz, 1H), 1.58-1.44 (m, 2H), 1.41-1.23 (m, 10H), 0.91 (t, *J* = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.2, 140.9, 137.9, 137.8, 137.6, 137.5, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 126.9, 80.5, 78.0, 77.7, 73.4, 72.7, 72.3, 71.1, 70.1, 65.3, 62.8, 41.7, 31.7, 29.2, 29.1, 26.8, 26.6, 22.6, 14.1, 13.9.

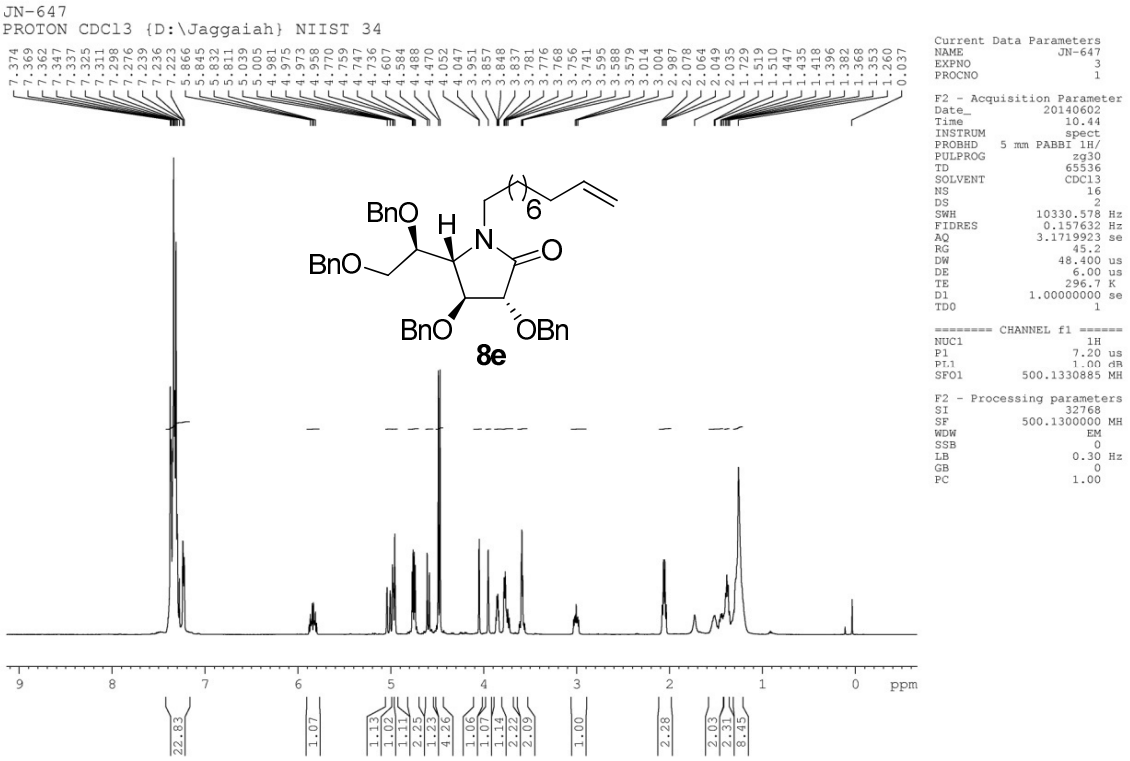
^{1}H, ^{13}C-NMR spectra of compound **8d**

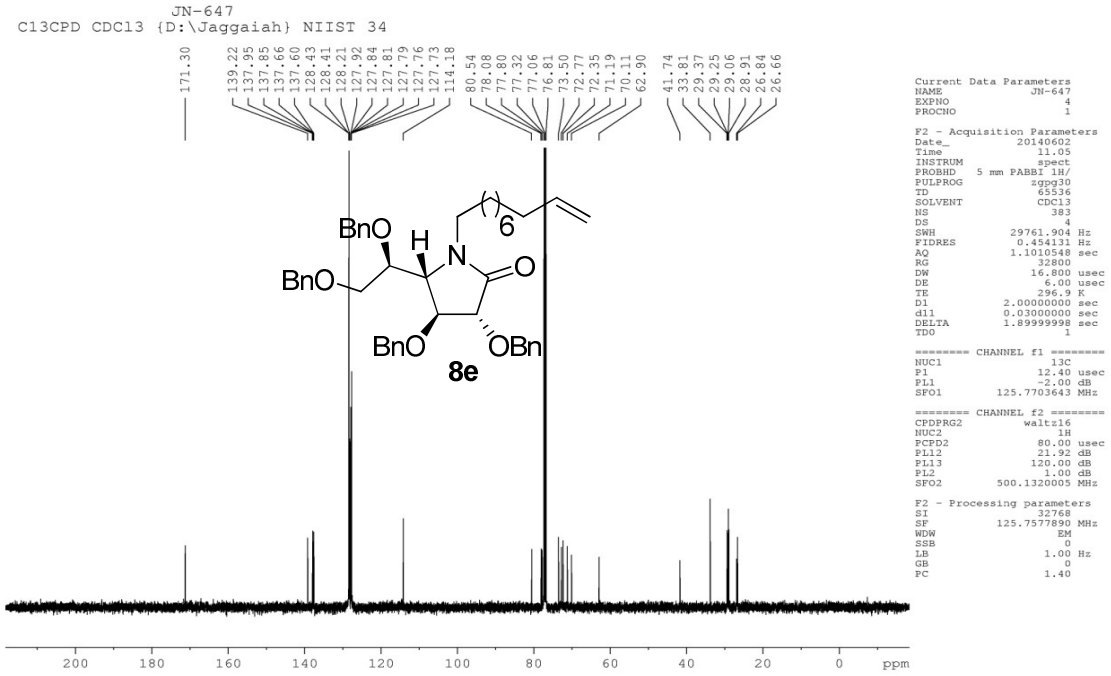




Compound 8e: R_f 0.45 (hexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37-7.22 (m, 22H), 5.86-5.81 (m, 1H), 5.03-4.95 (m, 3H), 4.77-4.73 (m, 2H), 4.59 (d, *J* = 11.5 Hz, 1H), 4.48-4.47 (m, 4H), 4.04 (d, *J* = 2.5 Hz,1H), 3.95 (s, 1H), 3.84 (app q, *J* = 5.5 Hz, 1H), 3.78-3.72 (m, 2H), 3.60-3.56 (m, 2H), 3.00 (ddd, *J* = 14, 9, 5 Hz, 1H), 2.07 (q, *J* = 7 Hz, 2H), 1.51-1.41 (m, 4H), 1.26 (bs, 8H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 139.2, 137.9, 137.8, 137.7, 137.6, 128.4, 128.2, 127.9, 127.8, 127.7, 114.1, 80.5, 78.1, 73.5, 72.7, 72.4, 71.2, 70.1, 62.9, 41.7, 33.8, 29.3, 29.2, 29.1, 28.9, 26.8, 26.6; HR-ESI-MS $[M+H]^+$ $C_{44}H_{54}NO_5$ calcd for m/z 676.4002 , found 676.4005.

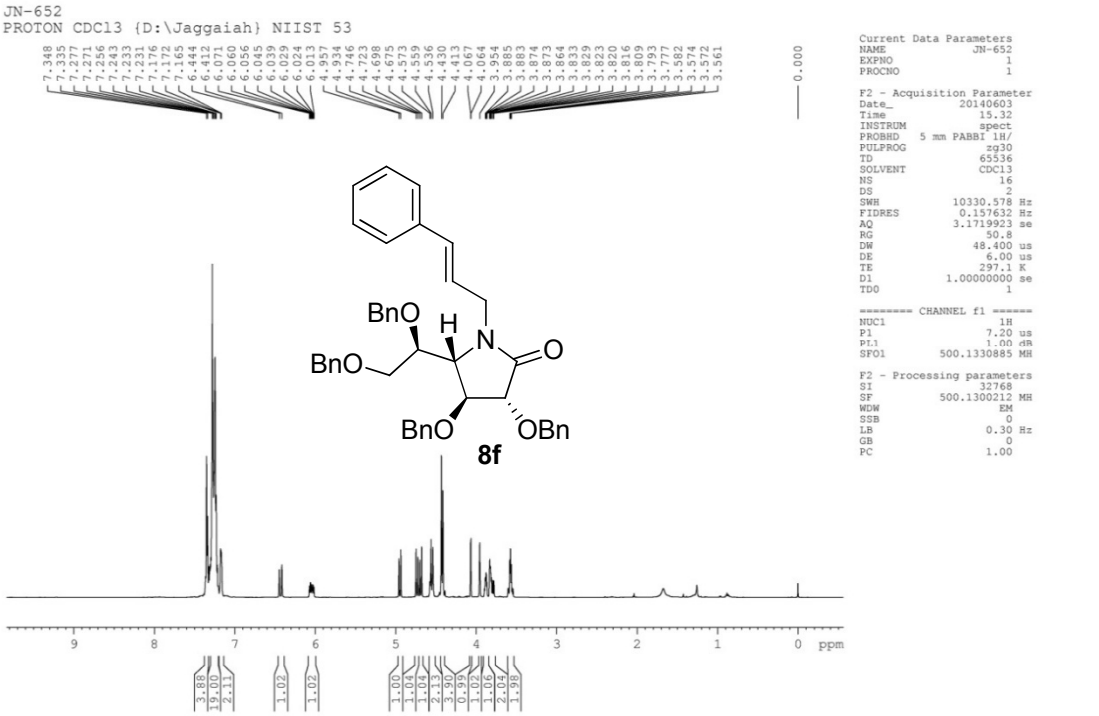
^{1}H, ^{13}C-NMR spectra of compound **8e**

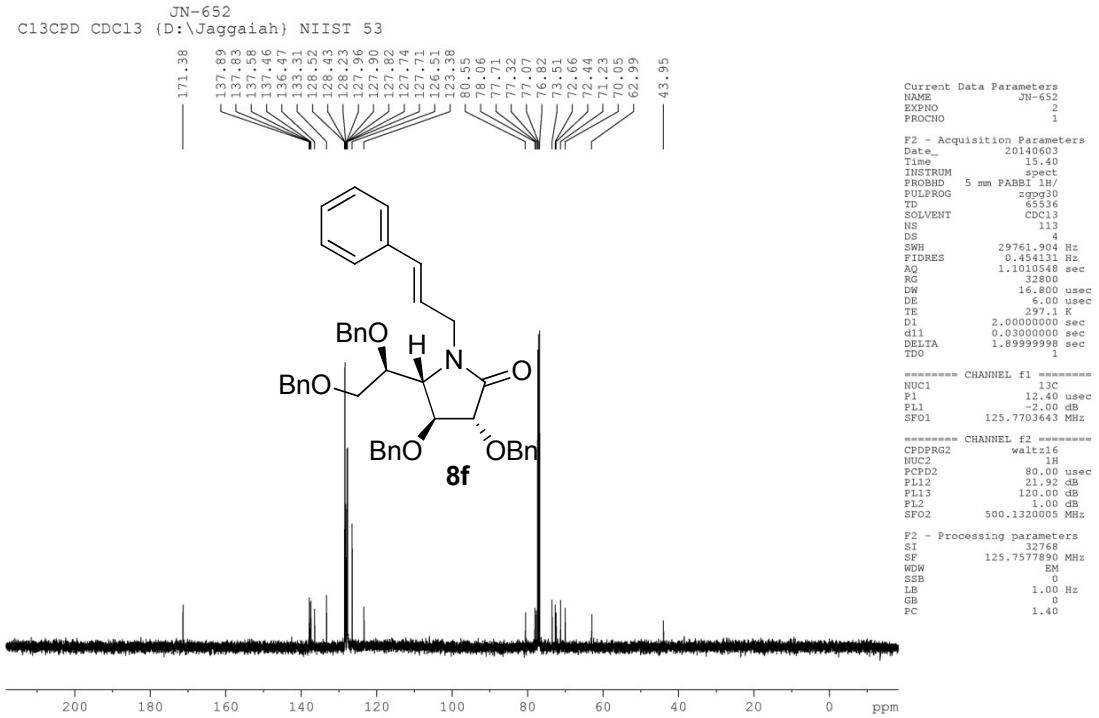




Compound 8f: R_f 0.35 (hexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 500 MHz) 7.34-7.16 (m, 25H), 6.42 (d, *J* = 16 Hz, 1H), 6.04 (ddd, *J* = 5.5, 7.5, 16, Hz, 1H), 4.94 (d, *J* = 11.5 Hz, 1H), 4.73 (d, *J* = 11.5 Hz, 1H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.57-4.53 (m, 2H), 4.43 (s, 2H), 4.41 (s, 2H), 4.06 (d, *J* = 1.5 Hz , 1H), 3.95 (bs, 1H), 3.88-3.86 (m, 1H), 3.83-3.77 (m, 2H), 3.58-3.56 (m, 2H); ^{13}C NMR (CDCl3, 125 MHz) δ 171.3, 137.8, 137.8, 137.5, 137.4, 136.4, 133.3, 128.5, 128.4, 128.2, 127.9, 127.8, 127.7, 126.5, 123.3, 80.5, 78.1, 73.5, 72.6, 72.4, 71.2, 70.1, 62.9, 43.9.

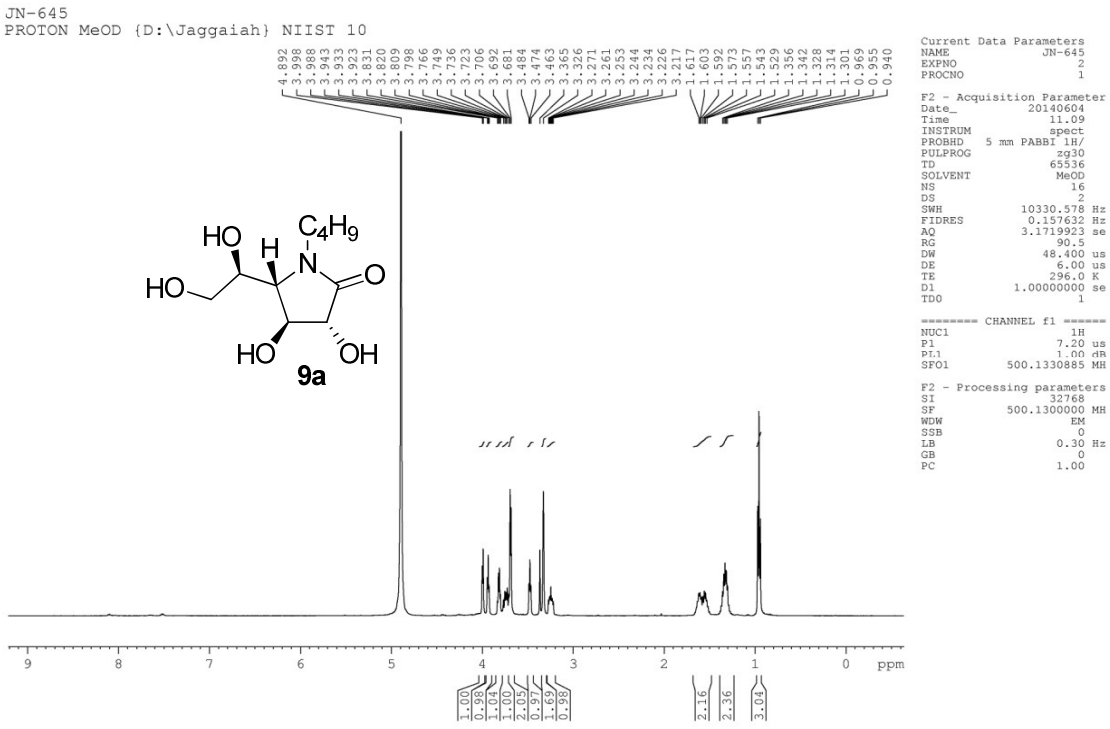
^{1}H, ^{13}C-NMR spectra of compound **8f**

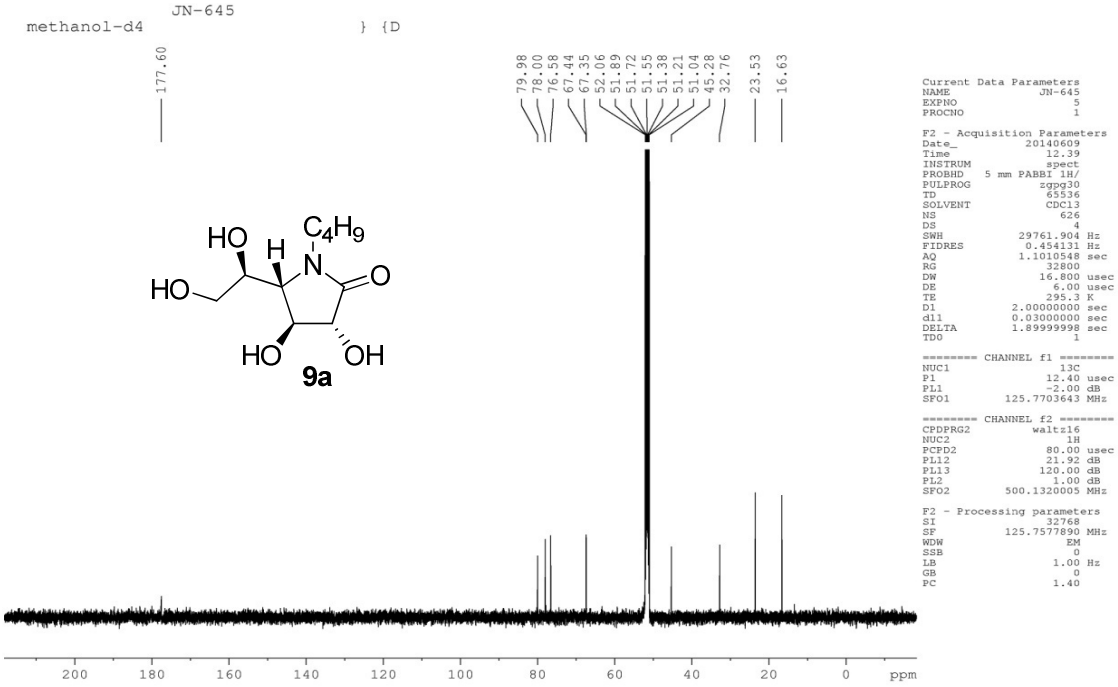




N-Butyl poly hydroxyl pyrrolidin-2-one (9a): To a solution of compound **8a** in EtOH (4 mL), was added Pd/C (30 mg) and few drops of conc. HCl and then H_2 gas was purged for 2 minutes, reaction mixture was stirred for overnight under H_2 atmosphere The reaction mixture was diluted with MeOH and filtered through a Celite pad by washing with MeOH and the filtrate was concentrated and then the resulted residue was precipitated with DCM afforded **9a** (quantitative): ^{1}H NMR (CD_3OD, 500 MHz) δ 3.99 (d, J = 5 Hz, 1H), 3.93 (app t, J = 5 Hz, 1H), 3.81 (app q, J = 5.5 Hz, 1H), 3.76-3.70 (m, 1H), 3.68 (d, J = 5.5 Hz, 2H), 3.47 (app t, J = 5 Hz, 1H), 3.36 (bs, 1H), 3.24 (ddd, J = 13.5, 9, 5 Hz, 1H), 1.61-1.52 (m, 2H), 1.35-1.30 (m, 2H), 0.95 (t, J = 7 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.6, 79.9, 78.0, 76.5, 67.4, 52.0, 51.8, 45.2, 32.7, 23.5, 16.6; ^{13}C DEPT 135^0 NMR (CD_3OD, 125 MHz) δ 79.9, 78.0, 76.5, 67.4, 67.3, 45.2, 32.7, 23.5, 16.6; HR-ESI-MS $[M+H]^+$ $C_{10}H_{20}NO_5$ calcd for m/z 234.1341 , found 234.1338.

^{1}H, ^{13}C-NMR spectra of compound **9a**

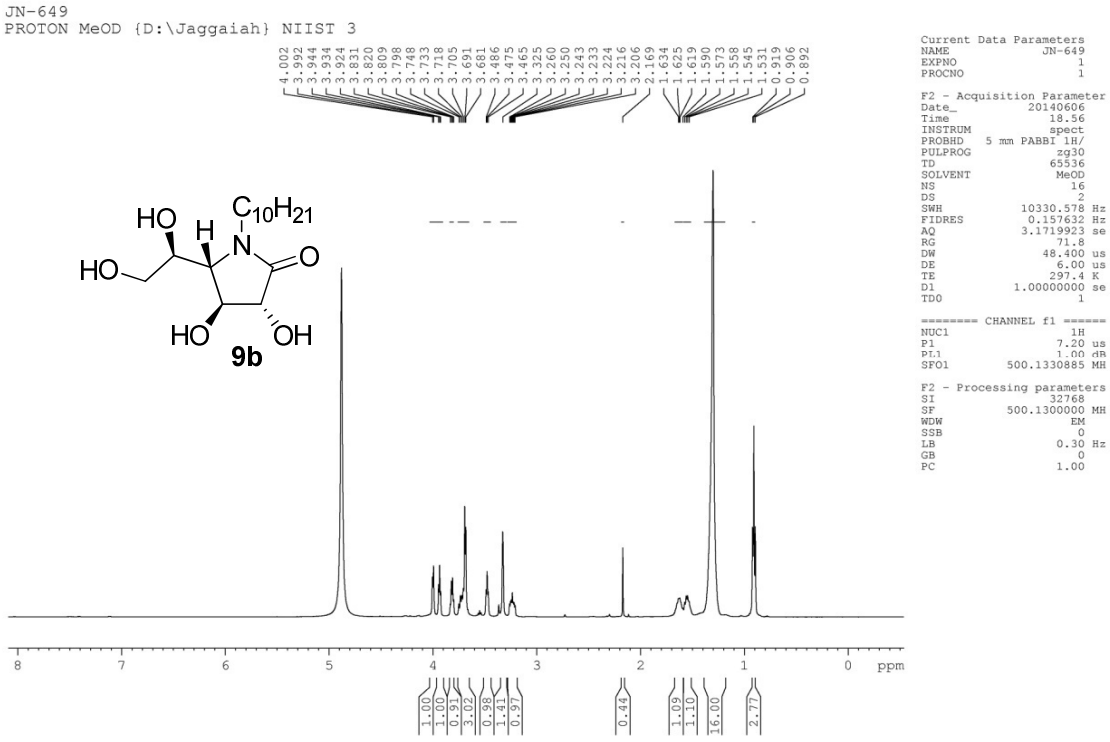




Procedure similar to synthesis of compound 9a was followed for the synthesis of compounds 9b-e:

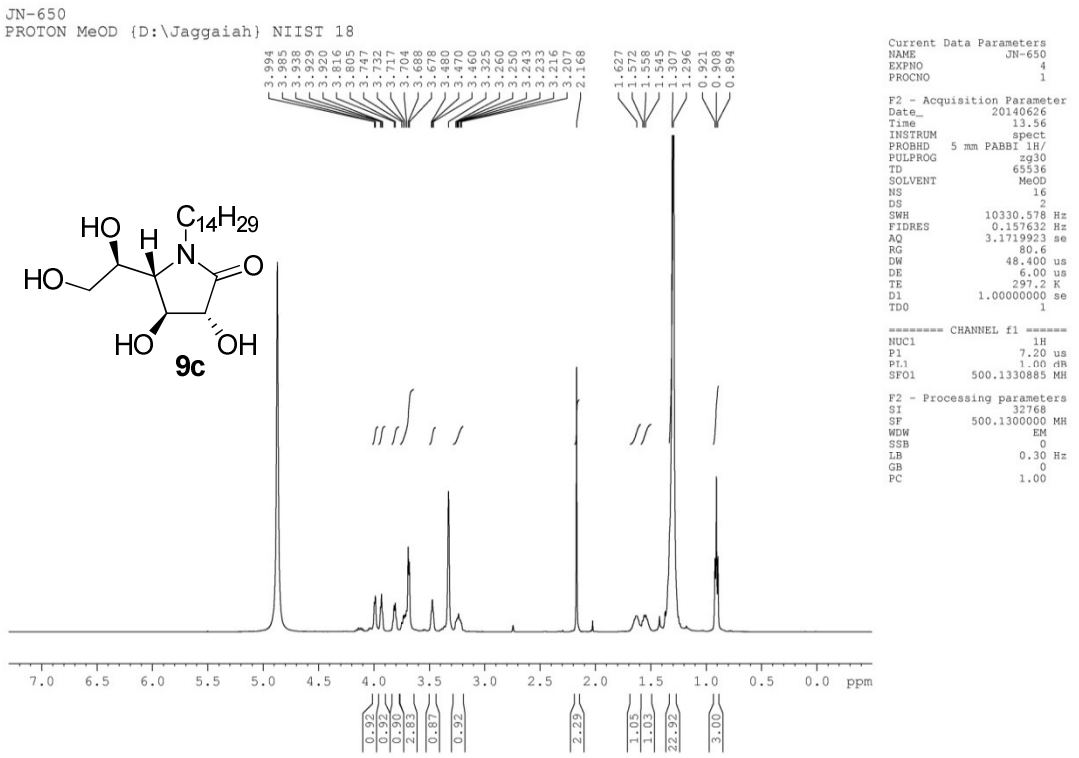
Compound 9b: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.99 (d, J = 5 Hz, 1H), 3.93 (app t, J = 5Hz, 1H), 3.81 (app q, J = 5.5 Hz, 1H), 3.74-3.71 (m, 1H), 3.68 (d, J = 5 Hz, 2H), 3.47 (app t, J = 5.5 Hz, 1H), 3.23 (ddd, J = 13.5, 8.5, 5. Hz, 1H), 1.63-1.53 (bs, 16H), 0.90 (t, J = 6.5 Hz, 3H); ^{13}C DEPT 135^{0} NMR (CD_3OD, 125 MHz) δ 76.0, 74.0, 72.6, 63.5, 63.4, 42.3, 31.6, 20.2, 29.0, 26.7, 26.4, 22.3, 13.0; HR-ESI-MS $[M + H]^+$ $C_{16}H_{32}NO_5$ calcd for m/z 318.2280, found 318.2280.

^{1}H-NMR spectra of compound **9b**



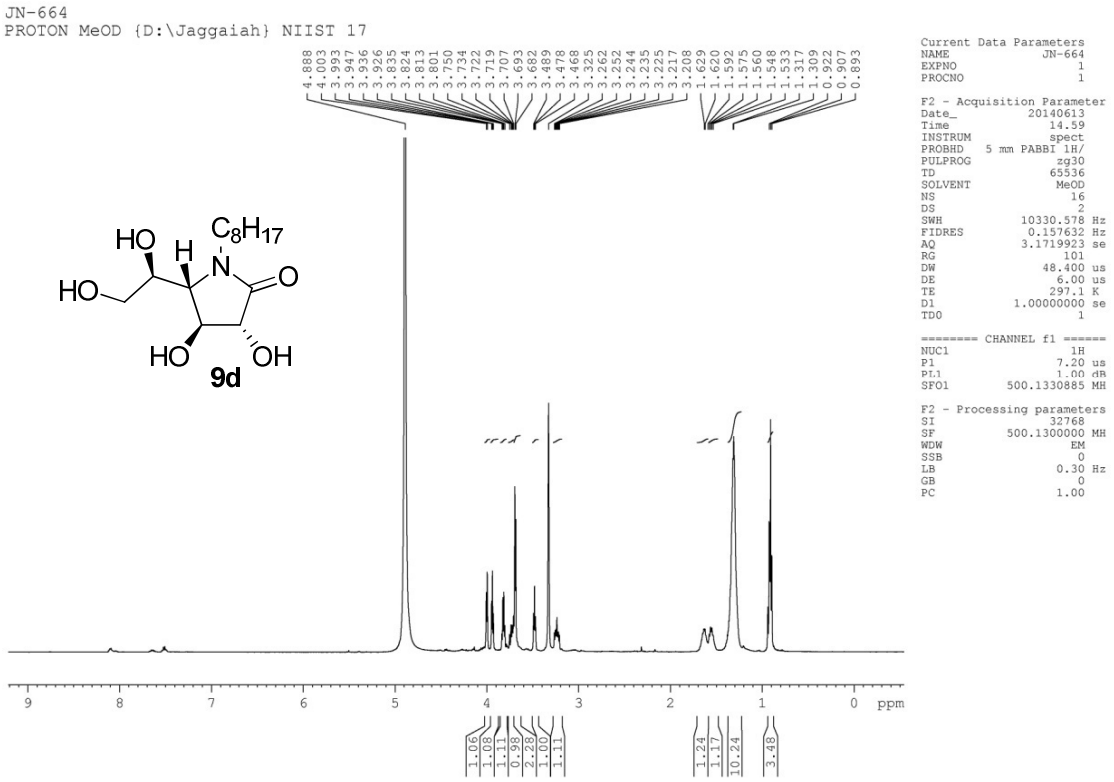
Compound 9c: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.98 (d, J = 4.5 Hz, 1H), 3.92 (t, J = 4.5 Hz, 1H), 3.81 (d ,J = 5.5 Hz, 1H), 3.74-3.70 (m, 1H), 3.68 (d, J = 5 Hz, 2H), 3.47 (t, J = 5 Hz, 1H), 3.23 (ddd, J = 13.5, 8.5, 5 Hz, 1H), 1.62-1.53 (m, 2H), 1.30 (bs, 22H), 0.90 (t, J = 6.5 Hz, 3H) ; ^{13}C DEPT 135^{0} (CD_3OD, 125 MHz) δ 79.9, 78.0, 76.5, 67.4, 67.3, 45.5, 35.6, 33.3, 33.2, 33.0, 32.9, 30.6, 30.4, 26.2, 26.7, 16.9; HR-ESI-MS $[M + H]^{+}C_{20}H_{40}NO_5$ calcd for m/z 374.2906, found 374.2907.

^{1}H-NMR spectra of compound **9c**



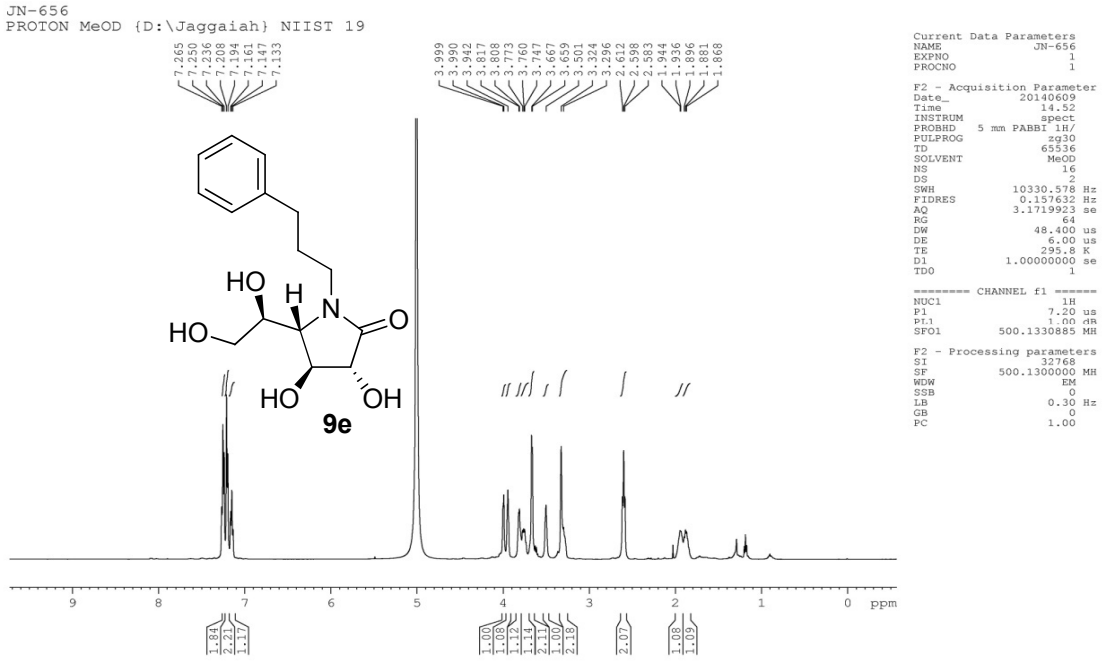
Compound 9d: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.99 (d, *J* = 5 Hz, 1H), 3.93 (app t, *J* = 5.5 Hz, 1H), 3.81 (app q, *J* = 5.5 Hz, 1H), 3.75-3.70 (m, 1H), 3.68 (d, *J* = 5.5 Hz, 2H), 3.47 (app t, *J* = 5.5 Hz, 1H), 3.23 (ddd, *J* = 13.5, 9, 5 Hz, 1H), 1.62-1.53 (m, 2H), 1.31-1.30 (bs, 10H), 0.9 (t, *J* = 7.5 Hz, 3H); ^{13}C DEPT 135^{0} (CD_3OD, 125 MHz) δ 76.0, 74.0, 72.6, 63.5, 63.4, 41.6, 31.5, 28.9, 26.7, 26.4, 22.3, 22.0, 13.0; HR-ESI-MS $[M + H]^+$ $C_{14}H_{27}NO_5$ calcd for m/z 290.1967, found 290.1974.

^{1}H-NMR spectra of compound **9d**



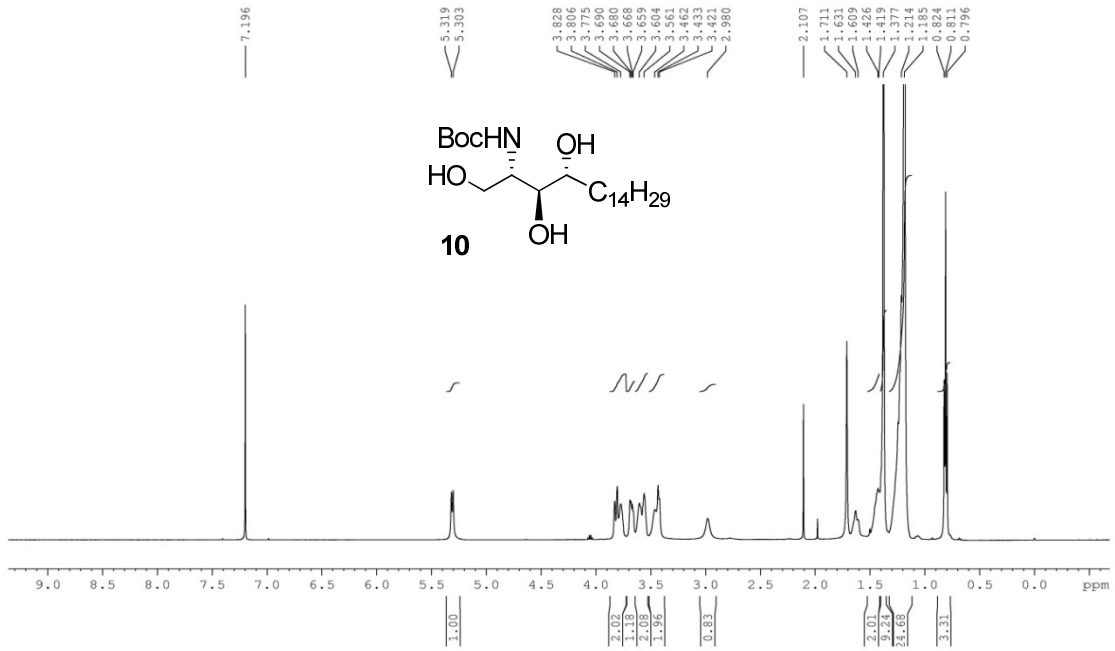
Compound 9e: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (t, *J* = 7.5 Hz, 2H), 7.20 (d, *J* = 7 Hz, 2H), 7.14 (t, *J* = 7 Hz, 1H), 3.99 (d, *J* = 4.5 Hz, 1H), 3.94 (bs, 1H), 3.81 (d, *J* = 4.5 Hz, 1H), 3.77-3.74 (m, 1H), 3.66 (d, *J* = 4 Hz, 2H), 3.50 (bs, 1H), 3.29 (bs, 1H), 2.59 (t, *J* = 7 Hz, 2H), 1.94-1.86 (m, 2H);^{13}C DEPT 135^{0} (CD_3OD, 125 MHz) δ 128.0, 125.5, 76.0, 74.1, 72.6, 63.6, 63.4, 41.6, 32.7, 28,6; HR-ESI-MS $[M + H]^{+}$ $C_{15}H_{21}NNaO_5$ calcd for m/z 318.1317, found 318.1321.

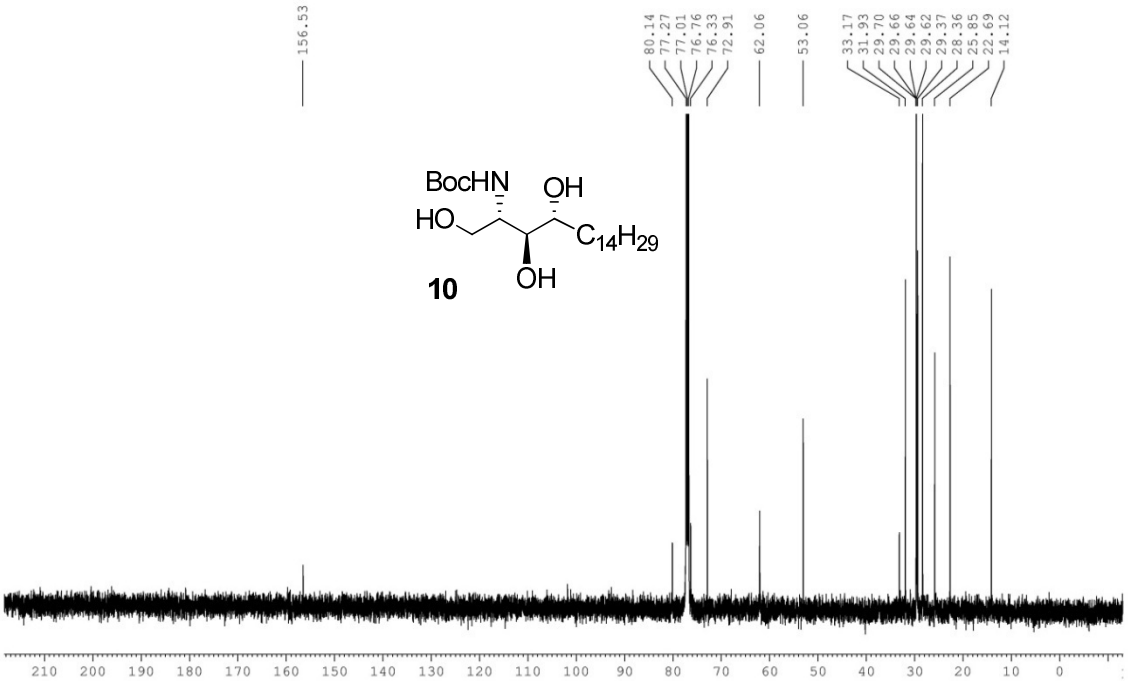
^{1}H-NMR spectra of compound **9e**



Compound 10: To a stirred suspension of phytosphingosine (2 g, 6.303 mmol, 1 eq), in 30 ml of EtOH: H2O (2:1), was added 1N NaOH (9.45 ml, 9.45 mmol, 1.5 eq), reaction mixture was in heterogeneous, and then added $(Boc)_2O$ (2.06 g, 9.45 mmol, 1.5 eq), reaction mixture became homogeneous, again reaction mixture became heterogeneous and stirred for 2 h at room temperature under argon atmosphere. Reaction mixture quenched with sat. aq. NH_4Cl (50 ml), and extracted with EtOAc (2×300 ml), dried over $NaSO_4$ and concentrated. Purification on flash chromatography (hexane/EtOAc 30:70), afforded compound **10** (2.49 g, 95%) as white solid. R_f 0.40 (hexane/EtOAc 3:7); ^{1}H NMR ($CDCl_3$, 500 MHz)δ 5.31 (d, J = 8 Hz, 1H),3.82-3.77 (m, 2H), 3.67 (dd, J = 11, 5 Hz, 1H), 3.60-3.56 (m, 2H), 3.46-3.42 (m, 2H), 2.98 (bs, 1H), 1.42-1.41 (m, 2H), 1.37 (s, 9H), 1.21-1.18 (m, 24H), 0.81 (t, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.5, 80.1, 72.9, 62.0, 53.0, 33.1, 31.9, 29.7, 29.6, 29.3, 28.3, 25.8, 22.7, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{23}H_{47}NNaO_5$ calc'd for m/z 440.33519, found 440.33459.

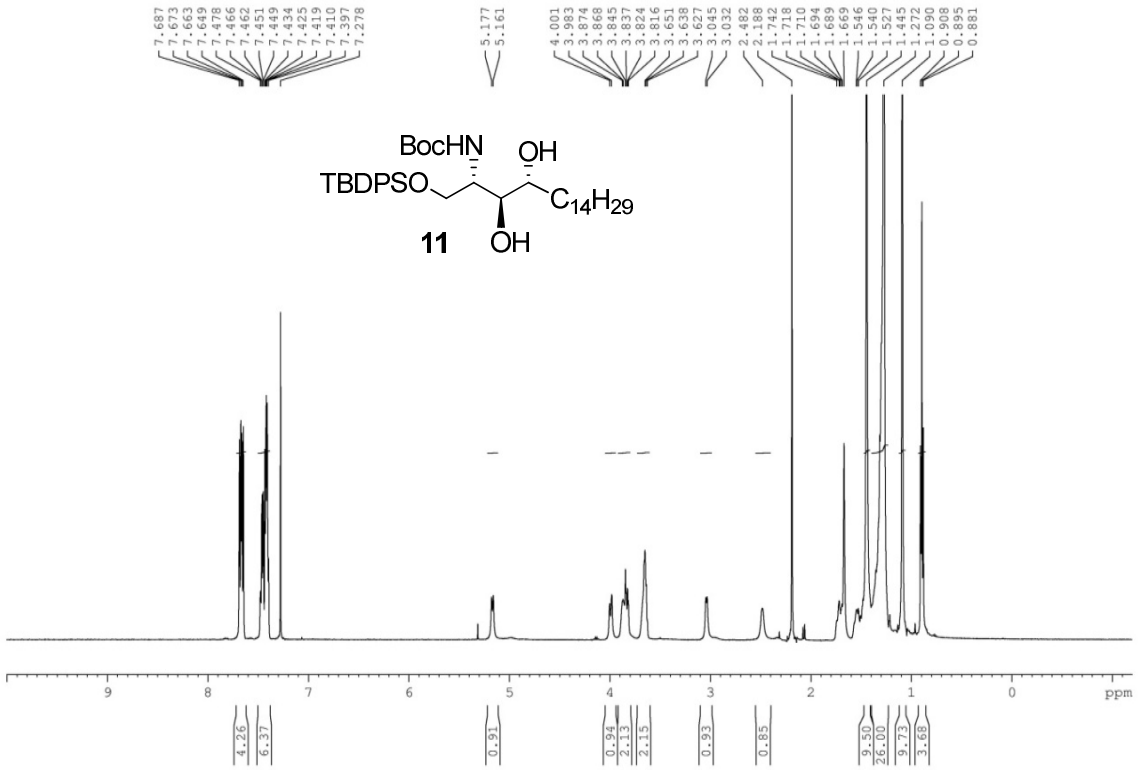
^{1}H, ^{13}C-NMR of compound **10**

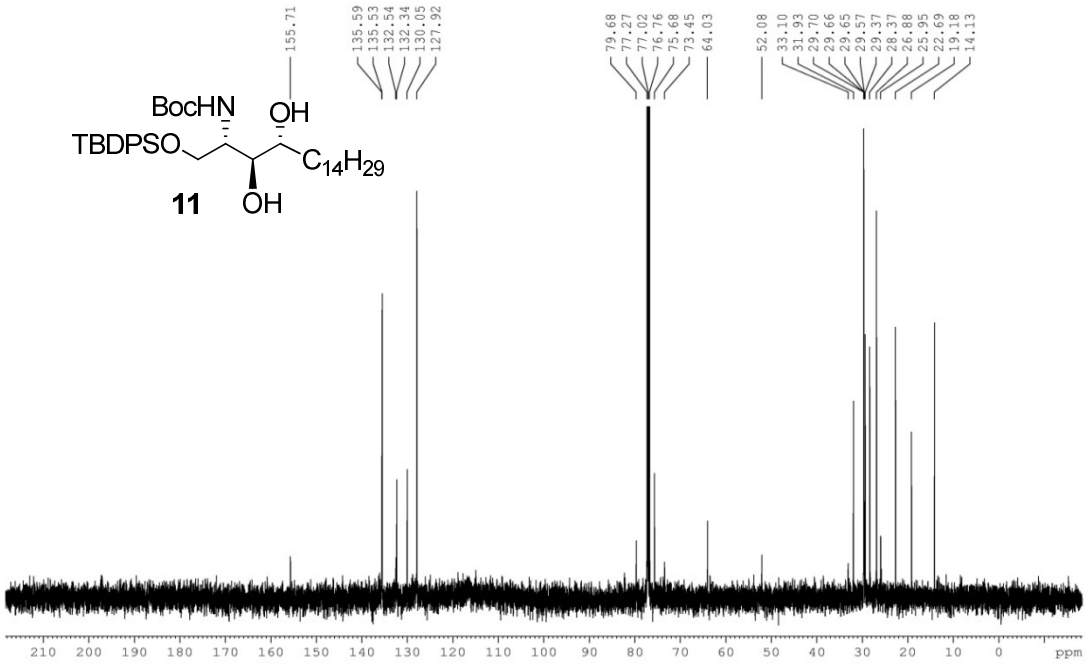




Compound 11: To a solution of compound **10** (500 mg, 1.198 mmol, 1 eq), in CH_2Cl_2 (15 ml), was added imidazole (210 mg, 3.0 mmol, 2.5 eq), and then slowly added TBDPSCl (460 µl, 1.790 mmol, 1.5 eq), and then the reaction mixture became cloudy, and stirred for 20 minutes under argon atmosphere, solvents were evaporated. Purification on flash chromatography (hexane/EtOAc 95:5 to 50:50), afforded compound **11** (750 mg, 95.5%) as colorless viscous solid. R_f 0.66 (hexane/EtOAc 6:4); ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.68-7.64 (m, 4H), 7.46-7.39 (m, 6H), 5.16 (d, J = 8 Hz, 1H),3.98 (d, J = 9 Hz, 1H),3.87-3.81(m, 2H), 3.65-3.62 (m, 2H), 3.03(d, J = 6.5 Hz, 1H), 2.48 (s, 1H), 1.44 (s, 9H), 1.27 (m, 26H), 1.08 (s, 9H), 0.89 (t, J = 6.5 Hz, 3H);^{13}C NMR ($CDCl_3$, 125 MHz) δ155.7, 135.5, 132.3, 130.0, 127.9, 79.7, 75.6, 73.4, 64.0, 52.0, 33.1, 31.9, 29.7, 29.6, 29.3, 26.8, 25.9, 22.7, 19.1, 14.1;HR-ESI-MS $[M+Na]^+$ $C_{39}H_{65}NNaO_5Si$ calc'd for m/z 678.45297, found 678.45258.

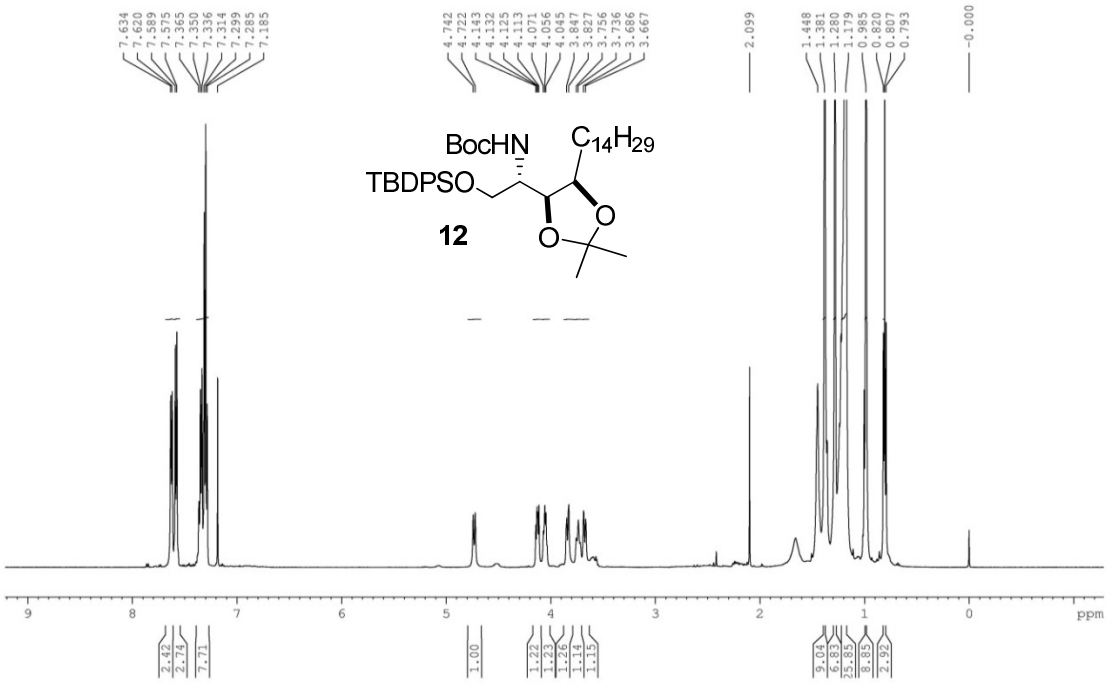
^{1}H, ^{13}C-NMR of compound **11**

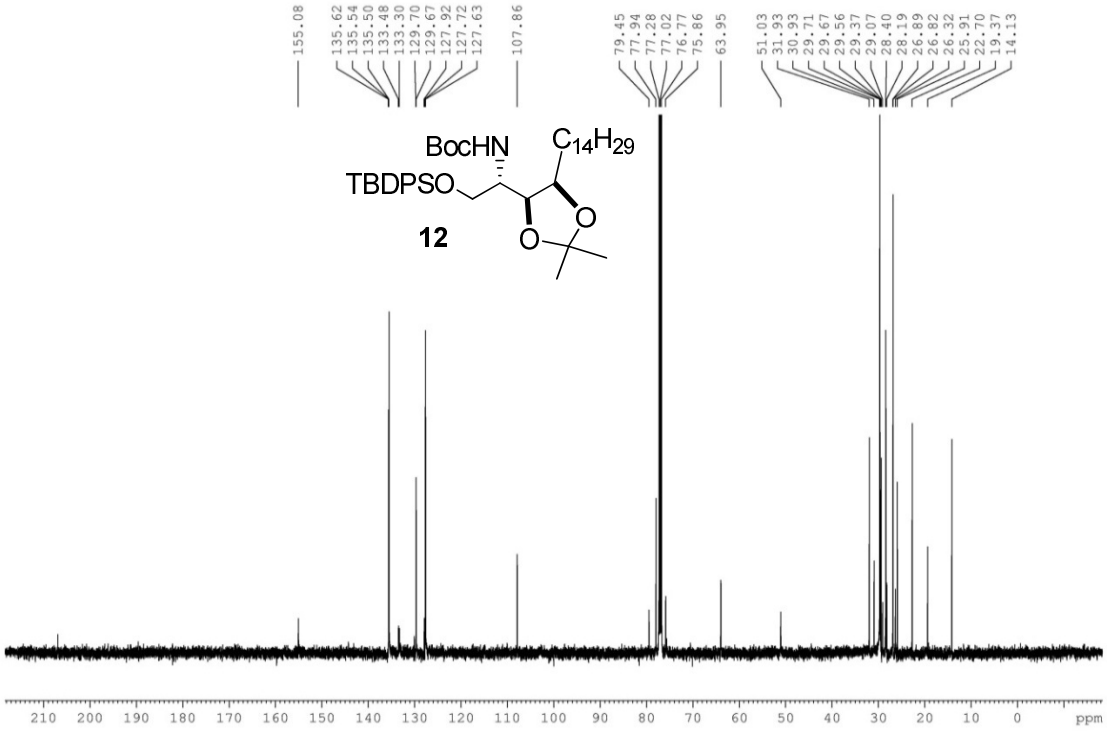




Compound 12: To a solution of compound **11** (725 mg, 1.105 mmol, 1 eq), in acetone (10 ml), was added 2,2-dimethoxypropane (204 µl, 1.65 mmol, 1.5 eq), and catalytic amount of *p*-TSOH (10 mg), then stirred for 1 h at room temperature under argon atmosphere. Reaction mixture quenched with sat. aq. $NaHCO_3$ and extracted with EtOAc (2×150 ml), organic layer washed with brine (50 ml) and separated organic layer dried over Na_2SO_4 and concentrated.Purification on flash chromatography (hexane/EtOAc 50:50), afforded compound **12** (740 mg, 97%) as colorless viscous solid. R_f 0.70 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.62 (d, J = 7 Hz, 2H), 7.58 (d, J = 7 Hz, 2H), 7.36-7.28 (m, 6H), 4.73 (d, J = 10 Hz, 1H),4.12 (dd, J = 9, 5.5 Hz, 1H),4.07-4.04 (m, 1H), 3.84-3.82 (m, 1H), 3.75-3.73 (m, 1H), 3.67 (d, J = 9.5 Hz, 1H),1.38 (s, 9H), 1.28 (s, 6H), 1.17-1.18 (m, 26H), 0.98 (s, 9H), 0.80 (t, J = 6.5 Hz, 3H);^{13}C NMR ($CDCl_3$, 125 MHz) δ155.0, 135.5, 133.5, 133.3, 129.7, 129.6, 127.9, 127.7, 127.6, 107.8, 79.4, 77.9, 75.8, 63.9, 51.0, 31.9, 30.9, 29.7, 29.5, 29.3, 29.0, 28.4, 28.2, 26.8, 26.3, 25.9, 22.7, 19.3, 14.1; HR-ESI-MS $[M+Na]^+$ $C_{42}H_{69}NNaO_5Si$ calc'd for m/z 718.48427, found 718.48370.

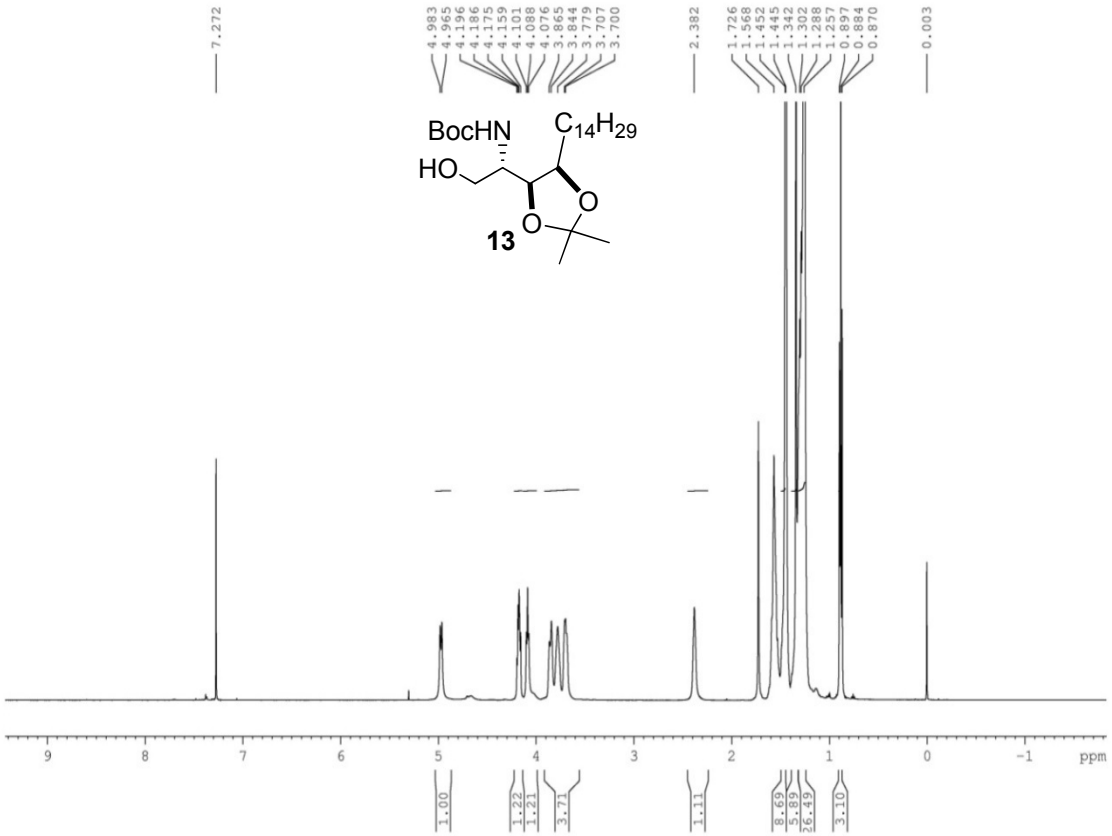
^{1}H, ^{13}C-NMR of compound **12**

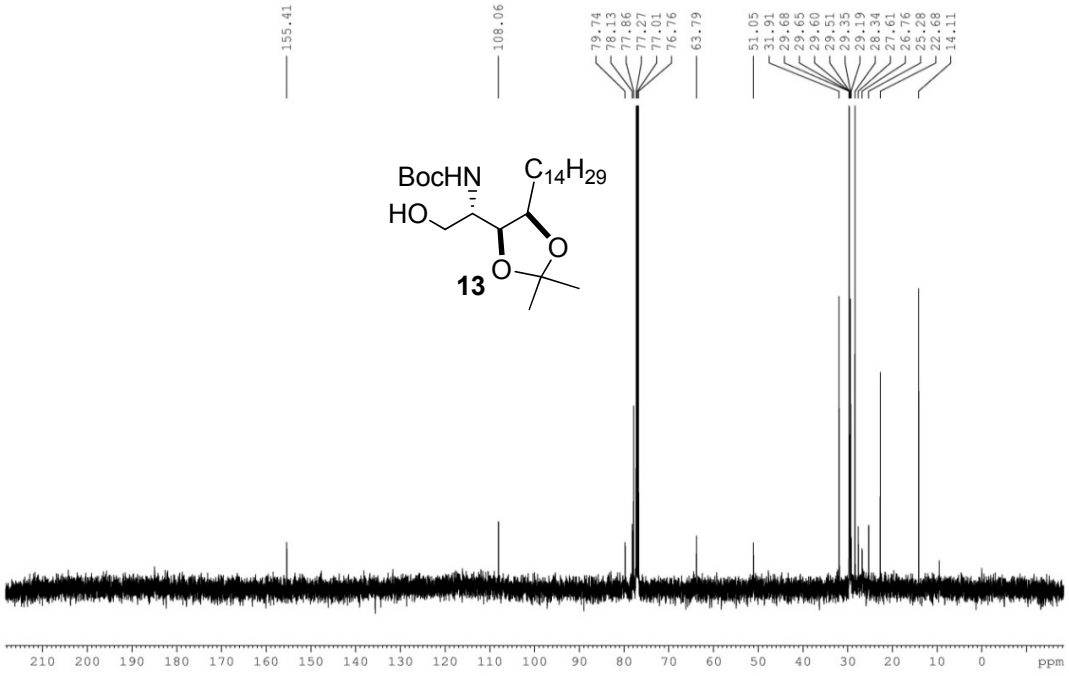




Compound 13: To a solution of compound **12** (710 mg, 1.02 mmol, 1 eq), in THF (8 ml), was added TBAF (2.04 ml, 2.04 mmol, 2 eq), and stirred for 0.5 h at room temperature under argon atmosphere. Reaction mixture diluted with H_2O (10 ml), extracted with EtOAc (2×100 ml), separated organic layer dried over Na_2SO_4 and concentrated. Purification on flash chromatography (hexane/EtOAc 50:50), afforded compound **13** (415mg, 91%) as white solid. R_f 0.27 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ4.97 (d, J = 10 Hz, 1H), 4.17 (dd, J = 10.5, 5 Hz, 1H), 4.10-4.07 (m, 1H), 3.86-3.77 (m, 2H), 3.70 (d, J = 3.5 Hz, 1H), 2.38 (s, 1H), 1.44 (s, 9H), 1.34 (s, 6H), 1.28-1.25 (m, 26H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ155.4, 108.0, 79.7, 78.1, 63.8, 51.0, 31.9, 29.6, 29.5, 29.3, 29.2, 28.3, 27.6, 26.7, 25.2, 22.6, 14.1;HR-ESI-MS $[M+Na]^+$ $C_{26}H_{51}NNaO_5$ calc'd for m/z 480.36649, found 480.36639.

^{1}H, ^{13}C-NMR of compound **13**





Compound 14: To a solution of compound **13** (530 mg, 1.158 mmol, 1 equiv), in dry DCM (10 mL), were added triethylamine (356 μL, 2.54 mmol, 2.2 equiv) and mesyl chloride (135 μL, 1.73 mmol, 1.5 equiv) at 0°C and stirred for 20 minutes under inert atmosphere. After the completion of the starting material as indicated by TLC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with DCM (2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated resulting in a mesylated product **14** in quantitative yield as a white solid: R_f 0.30 (hexane/EtOAc 7/3).

Compound 14a: To a solution of mesylated product **14** (1.158 mmol, 1 equiv) in double dried acetone (15 mL), was added Lithium bromide (1.5 g, 17.37 mmol, 15 equiv) and stirred for overnight at room temperature under inert atmosphere. Reaction stopped and solvents were evaporated and the crude reaction mixture was extracted with EtOAc (2 × 70 mL), washed with H_2O (100 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification

by flash chromatography using hexane/EtOAc 97:3 to 95:5 afforded 1-bromo-phytosphingosine **14a** (410 mg, 68%) as a white solid: R_f 0.40 (hexane/EtOAc 7:3).

Compound 14b: A solid 2-mercaptobenzothiazole (193.3 mg, 1.15 mmol, 1.5 equiv) in DMF (6 mL) was treated with Cs_2CO_3 for 15 minutes at 0°C under inert atmosphere and then a solution of 1-bromo-phytosphingosine **14a** (400 mg, 0.770 mmol, 1 equiv) in DMF (3 mL) was added into the above reaction mixture at 0°C and the resulting reaction mixture was stirred at room temperature for 6 hours under inert atmosphere. The reaction mixture was quenched with water (60 mL), extracted with EtOAc (2 × 60 mL), washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 97:3 to 95:5 resulting in a thioether **14b** (370 mg, 79%) yield: R_f 0.40 (hexane/EtOAc 8:2).

Compound 15: To a of thioether **14b** (200 mg, 0.33 mmol, 1 equiv) in dry DCM (10 mL), was added *m*CPBA (262 mg, 0.99 mmol, 4.5 equiv) at 0°C and the resulting reaction mixture was stirred at room temperature for overnight under inert atmosphere. After the formation of polar spot as indicated by TLC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with DCM (2 × 100 mL), dried over anhydrous Na_2SO_4 and concentrated Purification by flash chromatography using hexane/EtOAc 70:30 gave rise to BT-sulfone **15** (190 mg, 90%) yield as a white solid: R_f 0.20 (hexane/EtOAc 7:3).

Compound 16: To a solution of 2-pyrrolidinone **7** (98 mg, 0.18 mmol, 1 equiv) in DMF (2 mL) was added 60% NaH (14.5 mg, 0.36 mmol, 2 equiv) at 0 °C and stirred for 15 minutes under nitrogen atmosphere. Allyl bromide (62 µL, 0.72 mmol, 4 equiv) was then added at 0 °C to the reaction mixture. The resulting mixture was then allowed to attain room temperature and continued stirring for 1.5 h. After formation of the non-polar product as indicated by TLC (hexane/EtOAc 7:3), the reaction mixture was quenched with H_2O (25 mL) and extracted with

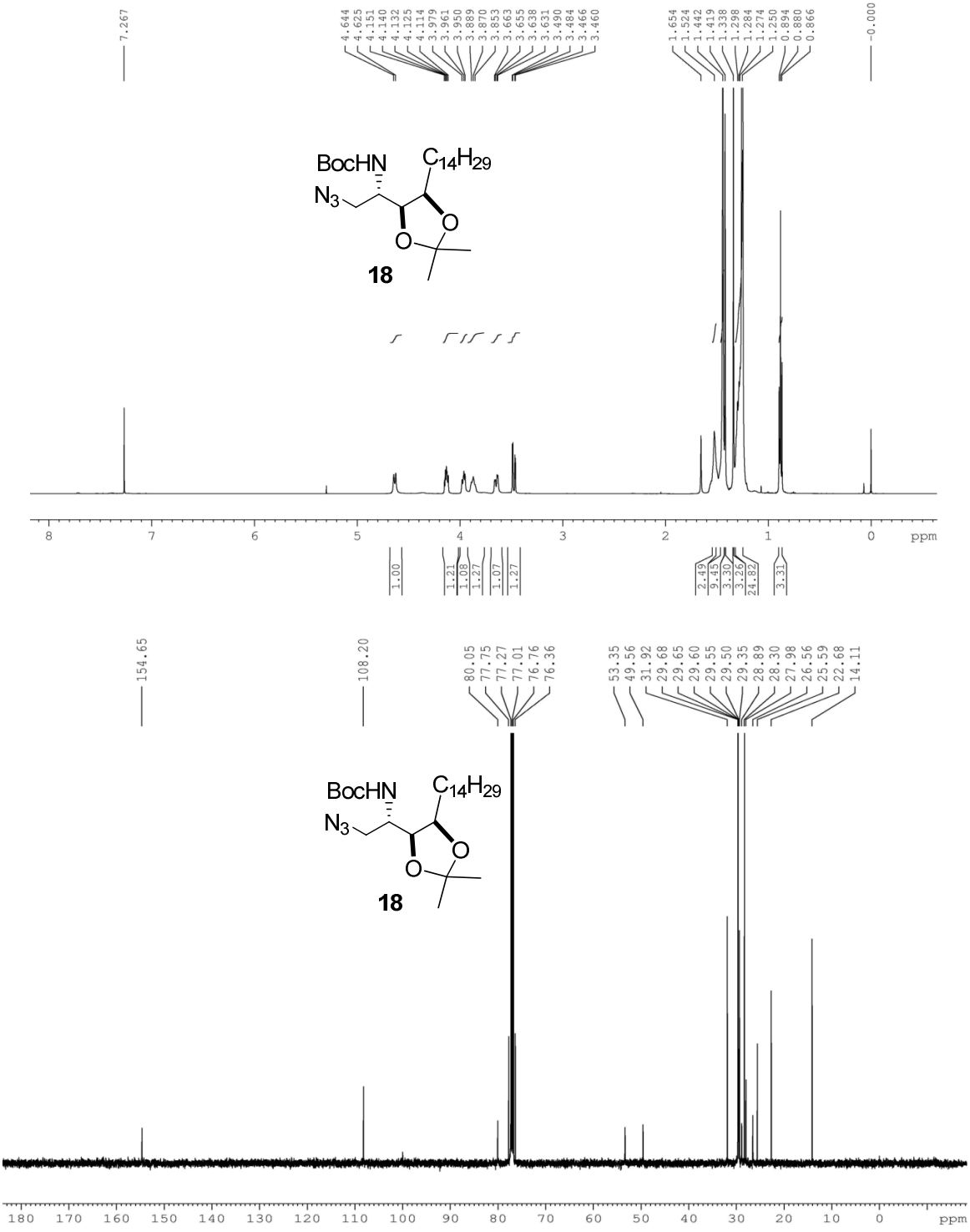
EtOAc (2 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 85:15 afforded the *N*-allyl-2-pyrrolidinone **16** (95 mg, 90%) as a colorless sticky solid: R_f 0.50 (hexane/EtOAc 7:3).

Compound 17: To a solution of allyl-2-pyrrolidinone **16** (80 mg, 0.138 mmol, 1 equiv) in THF: H_2O 2:1 (6 mL) were added catalytic amount 2 pinches of OsO_4 and $NaIO_4$ (88.7 mg, 0.41 mmol, 5 equiv). The reaction mixture was stirred at room temperature for overnight under inert atmosphere. Reaction stopped and quenched with saturated aqueous NH_4Cl (60 mL) and extracted with EtOAc (2 × 40 mL), dried over anhydrous Na_2SO_4 and concentrated Purification by flash chromatography using hexane/EtOAc 85:15 afforded to aldehyde **17** (50 mg, 62%) yield as sticky solid: R_f 0.40 (hexane/EtOAc 6:4).

(2S,3S,4R)-1-Azido-2-[(tert-butoxycarbonyl)amino]-3,4-O-isopropylidene-octadecanol (18): A solution of mesylate **14** (362 mg, 0.7 mmol, 1 equiv) in DMF (5 mL) was transferred into a pressure tube and then added NaN_3 (110 mg, 1.7 mmol, 2.5 equiv). The resulting mixture was stirred at 60 °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 (50 mL) and extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 afforded azide **18** (310 mg, 95%) as a white solid: R_f 0.58 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, *J* = 7 Hz, 3H), 1.25−1.29 (m, 24H), 1.33 (s, 3H), 1.41 (s, 3H), 1.44 (s, 9H), 1.52 (m, 2H), 3.47 (dd, *J* = 3, 12 Hz, 1H), 3.64 (dd, *J* = 3.5, 12.5 Hz, 1H), 3.85−3.88 (m,1H), 3.95−3.97 (m, 1H), 4.11−4.15 (m, 1H), 4.63 (d, *J* = 9.5 Hz, 1H), ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1, 22.6, 25.5, 26.5, 27.9, 28.3, 28.8, 29.3, 29.50, 29.55, 29.60, 29.65, 29.7, 31.9, 49.5, 53.3,

80.0, 108.2, 154.6; HR-ESI-MS $[M+Na]^+$ calcd for m/z $C_{26}H_{50}N_4NaO_4^+$ 505.3729, found 505.3720.

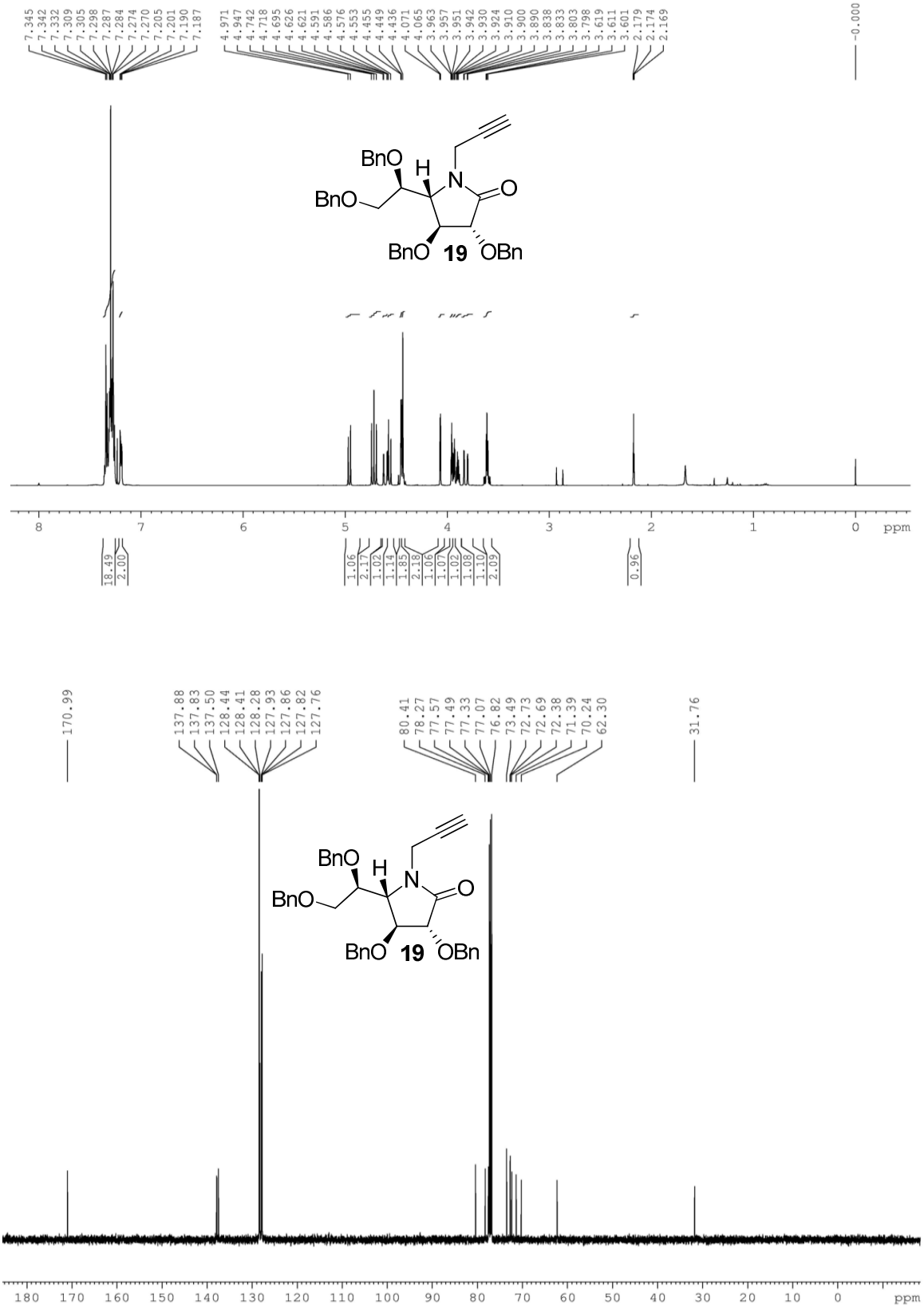
^1H, ^{13}C-NMR spectra of compound **18**



N-Propargyl-2-pyrrolidinone (19):

To a solution of 2-pyrrolidinone **7** (110 mg, 0.2 mmol, 1 equiv) in DMF (4 mL) was added 60% NaH (20.4 mg, 0.5 mmol, 2.5 equiv) at 0 °C and stirred for 15 minutes under nitrogen atmosphere. Propargyl bromide (46.5 μL, 0.6 mmol, 3 equiv) was then added at 0 °C to the reaction mixture. The resulting mixture was then allowed to attain room temperature and continued stirring for 1 h. After formation of the non-polar product as indicated by TLC (hexane/EtOAc 7:3), the reaction mixture was quenched with H_2O (25 mL) and extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 90:10 afforded the *N*-propargyl-2-pyrrolidinone **19** (113 mg, 96%) as a colorless sticky solid: R_f 0.50 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.17 (s, 1H, H-10), 3.60−3.61 (m, 2H, H-7), 3.81 (dd, *J* = 2.5, 16.5 Hz, 1H, H-8'), 3.90 (dd, *J* = 5, 10 Hz, 1H, H-6), 3.93 (dd, *J* = 3, 6 Hz, 1H, H-5), 3.95 (t, *J* = 3 Hz, 1H, H-4), 4.06 (d, *J* = 3 Hz, 1H, H-3), 4.43−4.45 (m, 4H, CHPh), 4.56 (d, *J* = 11.5 Hz, 1H, CHPh), 4.60 (dd, *J* = 2.5, 16.5 Hz, 1H, H-8), 4.71 (app t, *J* = 12 Hz, 2H, CHPh), 4.95 (d, *J* = 12 Hz, 1H, CHPh), 7.18−7.34 (m, 20H, Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.7, 62.3, 70.2, 71.3, 72.38, 72.6, 72.7, 73.4, 77.5, 78.3, 80.4, 127.7, 127.82, 127.86, 127.9, 128.2, 128.41, 128.44, 137.5, 137.83, 137.88, 170.9; HR-ESI-MS $[M+H]^+$ calcd for m/z $C_{37}H_{38}NO_5^+$ 576.2750, found 576.2745.

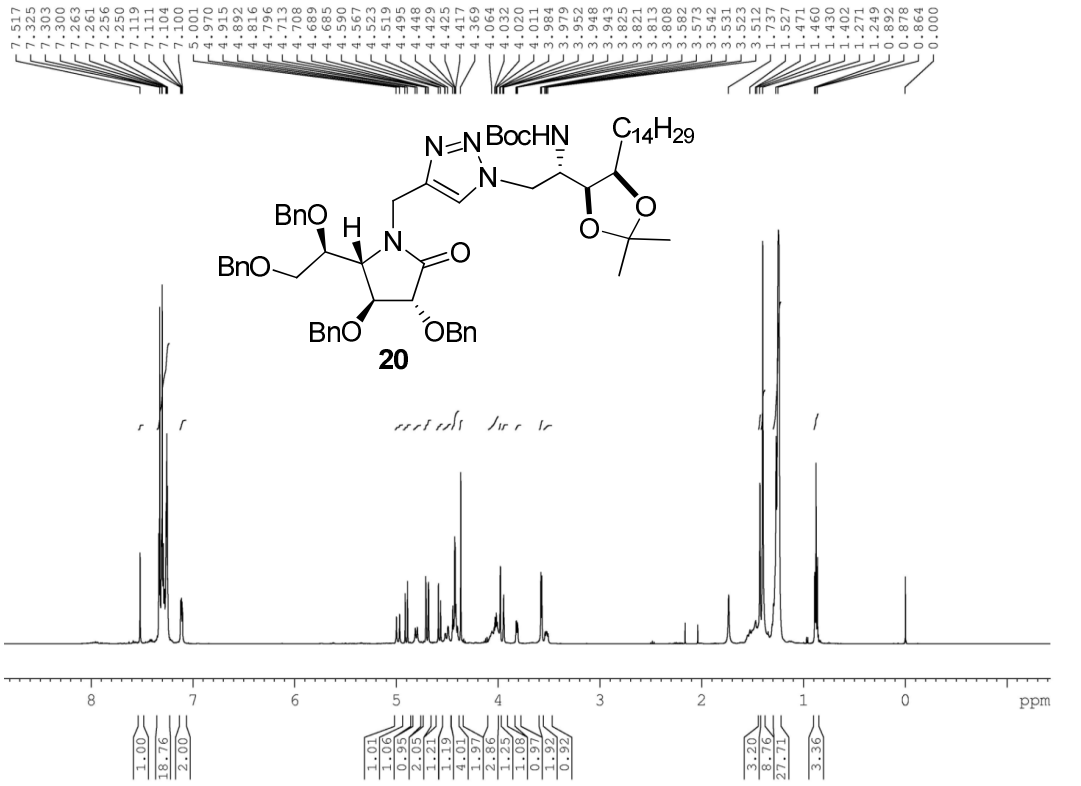
^{1}H, ^{13}C-NMR spectra of compound **19**

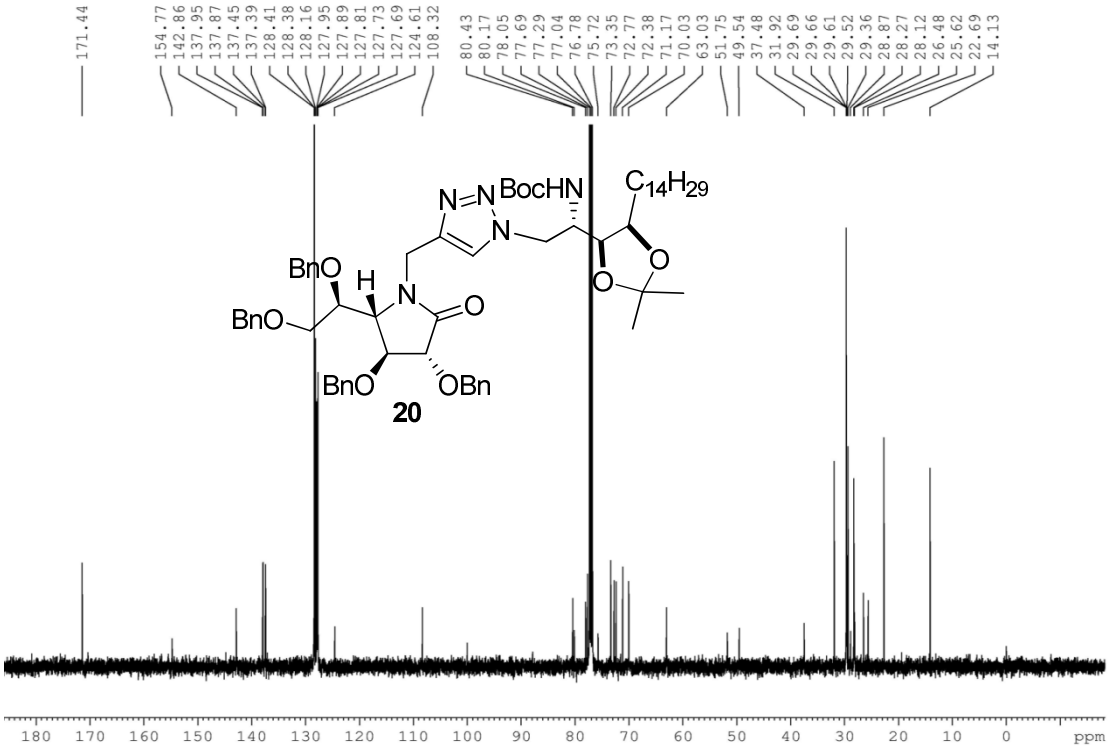


1***H***-1,2,3-Triazole,1-[**(2S,3S,4R)-1-methyl-2-[(tert-butoxycarbonyl)amino]-3,4-O isopropylidene-octadecanol**]-4-yl-methyl-N-[**2,3,5,6-tetra-O-benzyl-D-galactono-1,4-lactam] (20):**

To a solution of 2-pyrrolidinone **19** (105 mg, 0.18 mmol, 1 equiv) and azide **18** (87.9 mg, 0.18 mmol, 1 equiv) in acetonitrile (10 mL) at 0 °C were added CuI (69.4 mg, 0.36 mmol, 2 equiv) and DIPEA (95 μL, 0.54 mmol, 3 equiv). The resulting mixture was stirred for 1 h under nitrogen atmosphere and after complete consumption of both the starting materials was indicated by TLC (hexane/EtOAc 7:3), the reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by column chromatography using hexane/EtOAc 80:10 to 60:40 yielded compound **20** (170 mg, 88%) as a colorless sticky solid: R_f 0.29 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, *J* = 7 Hz, 3H), 1.24−1.27 (m, 27H), 1.40 (s, 9H), 1.43 (s, 3H), 1.46−1.52 (m, 2H), 3.52 (dd, *J* = 5.5, 9.5 Hz, 1H), 3.57 (d, *J* = 4.5 Hz, 2H), 3.81 (dd, *J* = 2, 6 Hz, 1H), 3.94 (app t , *J* = 2.5 Hz, 1H), 3.98 (d, *J* = 2.5 Hz, 1H), 4.01−4.10 (m, 3H), 4.36 (s, 2H), 4.41−4.44 (m, 4H), 4.49−4.52 (m, 1H), 4.57 (d, *J* = 11.5 Hz, 1H), 4.69 (dd, *J* = 2.5, 12 Hz, 2H), 4.80 (d, *J* = 10 Hz, 1H), 4.90 (d, *J* = 11.5 Hz, 1H), 4.98 (d, *J* = 15.5 Hz, 1H), 7.10−7.32 (m, 20H), 7.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1, 22.6, 25.6, 26.4, 28.1, 28.3, 28.9, 29.3, 29.5, 29.61, 29.66, 29.7, 31.9, 37.0, 49.5, 51.7, 63.0, 70.0, 71.1, 72.3, 72.7, 73.3, 75.7, 77.6, 78.0, 80.1, 108.3, 124.6, 127.6, 127.7, 127.81, 127.89, 127.9, 128.1, 128.4, 137.3, 137.4, 137.8, 137.9, 142.8, 154.7, 171.4; HR-ESI-MS $[M+H]^+$ calcd for m/z $C_{63}H_{88}N_5O_9^+$ 1058.6582, found 1058.6577.

^{1}H, ^{13}C-NMR spectra of compound **20**

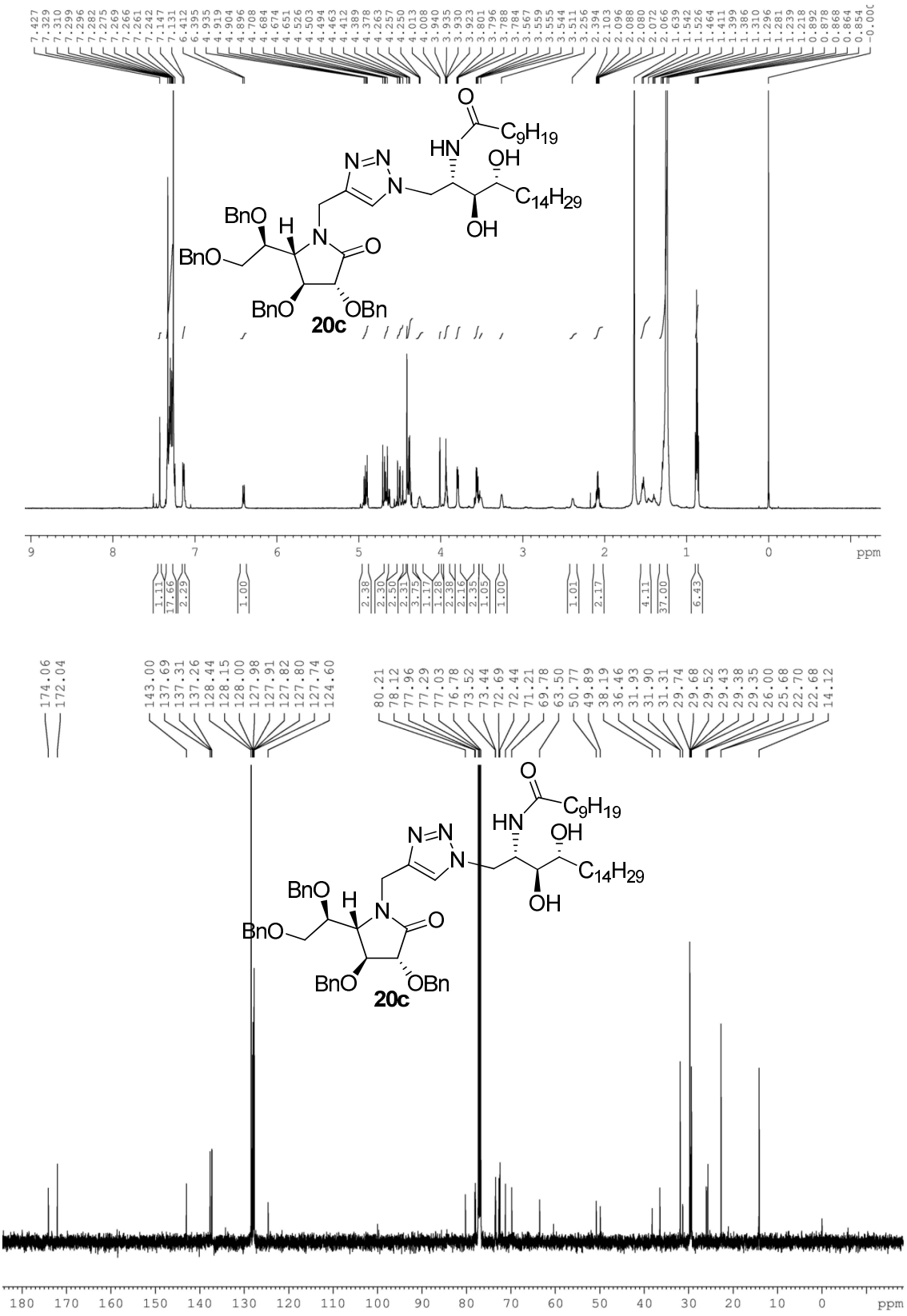




1***H***-1,2,3-Triazole,1-[**(2S,3S,4R)-1-methyl-2-[(decanoyl)amino]-3,4-O-isopropylidene-octadecanol**]-4-yl-methyl-N-[**2,3,5,6-tetra-O-benzyl-D-galactono-1,4-lactam] (20c):**

Compound **20** (130 mg, 0.12 mmol) in 20 mL of EtOH/2 M HCl (4:1) was heated at reflux for 4 h under nitrogen atmosphere. After formation of the polar product as indicated by TLC, the reaction mixture was extracted with $CHCl_3$/MeOH (7:1, 3 × 10 mL) and the resulting intermediate was dried under vacuum for 2 h. The crude amine hydrochloride was dissolved in 12 mL of DMF/DCM (2:5), and then added 4-nitrophenyl decanoate (144 mg, 0.5 mmol, 4 equiv), K_2CO_3 (153 mg, 1.1 mmol, 9 equiv), and the resulting mixture was stirred vigorously for 48 hours at room temperature under nitrogen atmosphere. After completion of the starting material as indicated by TLC (EtOAc 100%), the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (4 × 50 mL), and extracted with EtOAc (3 × 75 mL). The combined organic layers were thoroughly washed with water (3 × 60 mL), dried over anhydrous Na_2SO_4 and concentrated. Purification by chromatography (hexane/EtOAc 80:20 to 60:40 to 40:60) afforded acylated product **20c** (70.0 mg, 53% over two steps) as a colorless sticky solid : R_f 0.55 (EtOAc 100%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.85−0.89 (m, 6H), 1.21−1.28 (m, 36H), 1.29−1.52 (m, 4H), 2.06−2.10 (m, 2H), 2.39 (bs, 1H), 3.25 (bs, 1H), 3.52 (bs, 1H), 3.55 (ddd, *J* = 4.5, 11, 15 Hz, 2H), 3.79 (dd, *J* = 2.5, 6 Hz, 1H), 3.91−3.95 (m, 2H), 4.01 (d, *J* = 2.5 Hz, 1H), 4.25−4.26 (m, 1H), 4.35−4.41 (m, 4H), 4.46−4.52 (m, 2H), 4.62−4.70 (m, 4H), 4.89−4.93 (m, 2H), 6.41 (d, *J* = 9 Hz, 1H), 7.13−7.33 (m, 20H), 7.42 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1, 22.6, 22.7, 25.6, 26.0, 29.35, 29.38, 29.4, 29.5, 29.6, 29.7, 31.3, 31.90, 31.93, 36.4, 38.1, 49.8, 50.7, 63.5, 69.7, 71.2, 72.4, 72.6, 73.4, 73.5, 80.2, 124.6, 127.7, 127.80, 127.82, 127.91, 127.98, 128.0, 128.1, 128.4, 137.2, 137.3, 137.6, 143.0, 172.0, 174.0; HR-ESI-MS $[M+H]^+$ calcd for m/z $C_{65}H_{94}N_5O_8^+$ 1072.7102, found 1072.7078.

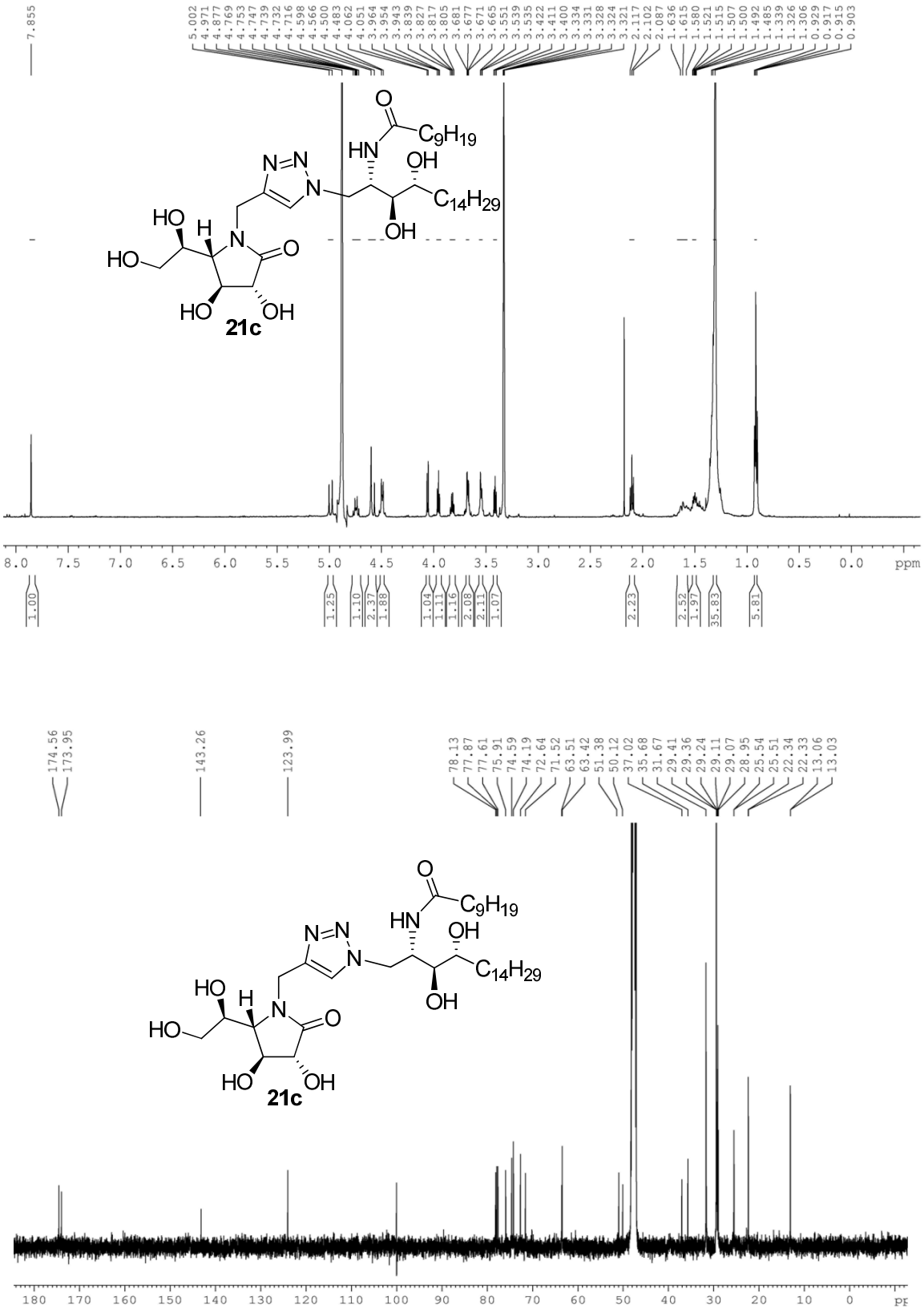
^{1}H, ^{13}C-NMR spectra of compound **20c**



1***H***-1,2,3-Triazole,1-[**(2S, 3S, 4R)-1-methyl-2-[(decanoyl)amino]-3,4-dihydroxy-octadecanol**] -4-yl-methyl-N-[**2,3,5,6-tetrahydroxy-D-galactono-1,4-lactam] (21c):**

To a solution of compound **20c** (26.0 mg, 0.02 mmol, 1 equiv) in EtOH (3 mL) was added 10% Pd/C (15.0 mg) and one drop of concentrated HCl and the resulting mixture was purged with H_2 gas for 1 minute. The reaction mixture was stirred at room temperature under H_2 atmosphere (balloon) for 1 h. The reaction mixture was diluted with MeOH (10 mL) and filtered through a celite pad. The celite pad was further washed with MeOH (15 ml) and the combined filtrates were concentrated. The resulting residue was purified by column chromatography using $CHCl_3$/MeOH (95:5) which afforded the final product **21c** (10.0 mg, 58%) as a white solid: R_f 0.4 (MeOH/$CHCl_3$, 2:8); ^{1}H NMR (CD_3OD, 500 MHz) δ 0.90−0.92 (m, 6H), 1.30−1.34 (m 36H), 1.48−1.63 (m, 4H), 2.10 (t, *J* = 7.5 Hz, 1H), 3.41 (app t, *J* = 5.5 Hz, 1H), 3.53−3.55 (m, 2H), 3.66−3.68 (m, 2H), 3.82 (dd, *J* = 6, 11 Hz, 1H), 3.95 (t, *J* = 5 Hz, 1H), 4.05 (d, *J* = 5 Hz, 1H), 4.48−4.50 (m, 2H), 4.56−4.59 (m, 2H), 4.71−4.77 (m, 1H), 4.98 (d, *J* = 15.5 Hz, 1H), 7.85 (s, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 13.03, 13.06, 22.33, 22.34, 25.51, 25.54, 28.9, 29.0, 29.1, 29.2, 29.3, 29.4, 31.6, 35.6, 37.0, 50.1, 51.3, 63.4, 63.5, 71.5, 72.6, 74.1, 74.5, 75.9, 77.6, 77.8, 78.1, 123.9, 143.0, 173.9, 174.5; HR-ESI-MS $[M+H]^+$ calcd for m/z $C_{37}H_{70}N_5O_8^+$ 712.5224, found 712.5208.

^{1}H, ^{13}C-NMR spectra of compound **21c**



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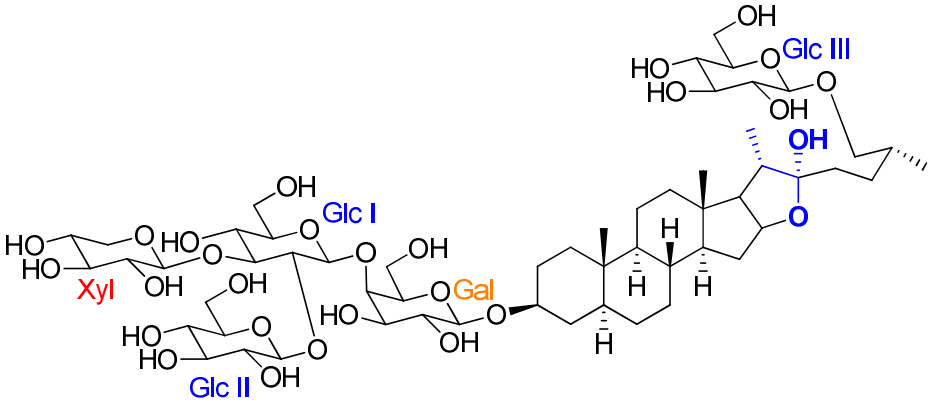
Chapter 4

Structure elucidation of steroidal saponin uttroside B.

4.1. Abstract:

A complete structural elucidation of steroidal saponin uttroside B by 1D- and 2D- NMR techniques are explained in this chapter. The steroidal saponin was isolated from the methanolic extract of leaves of *Solanum nigrum*. Complex ^{1}H-NMR of this saponin in CD_3OD and pyridine-d5 led us to derivatize with an acetyl group to afford a peracetylated saponin which afforded a well resolved ^{1}H-NMR spectra in 700 MHz NMR. Using a series of NMR experiments such as ^{1}H-NMR, ^{13}C-NMR, DEPT-135, HSQC, COSY, HMBC, TOCSY, ROESY, and HRESIMS along with MS-MS experiments helped in unambiguous identification of the isolated saponin as uttroside B (Figure 4.1A). The detailed structural analysis of uttroside B from *S. nigrum* is reported by us for the first time in Scientific Reports [DOI: 10.1038/srep36318].



Uttroside B

Figure 4.1A: Structure of Uttroside B (**1**)

4.2. Introduction:

Steroidal saponin isolation and their anticancer activity against different cancer cell lines have been widely reported from the *Solanum* genera.[1,2] More than 40 *Solanum* species examined were found to contain a large number of steroidal glycosides possessing cytotoxicity, antifeedant, and anti HSV-13 activities.[3] Bioactives isolated from *Solanum* species display a broad range of biological activities *viz* anti-cancer, anti-cholesterol, anti-microbial, anti-inflammatory, anti-nociceptive, and anti-pyretic effects.[4-5] Saponins with spirostanol aglycone appended to mono, di, tri, tetra and penta saccharides with a *β*-glycosidic linkage have shown good anti-cancer activity against human lung carcinoma (A549), human adenocarcinoma (SK-OV-3), human malignant melanoma, metastasis to skin of thigh (SK-MEL-2), human central nervous system tumor (XF498), human colon adenocarcinoma (HCT15), and breast adenocarcinoma (MCF-7) cell lines.[6-7] Uttroside B was first reported by Sharma et al. in 1983 from the *Solanum nigrum* Linn.[8] Uttroside B is characterized by presence of *β*-D-glucopyranosyl unit at C-26 of the furostanol and *β*-lycotetraosyl unit at C-3 (Figure 4.1A). Among the other genera where uttroside B was isolated includes *Tribulus terrestris*[9] and *Polianthes tuberosa*.[10] Uttroside B isolated from ethanolic extract of *Polianthes tuberosa* exhibited moderate cytotoxic activity (IC_{50} 18.83 µg/mL) against HeLa cells as determined by MTT assay.[10] However, uttroside B was found to be more effective against PC-12 (IC_{50} 1.20 µM) and HCT-116 (IC_{50} 2.33 µM) cells.[11] In the present study, we have isolated and characterized the steroidal saponin uttroside B from the methanolic extract of the *Solanum nigrum* Linn. Uttroside B was evaluated both *in vitro* and *in vivo* against hepatocellular carcinoma (HepG2 cells).[12] Minor structural variations in the saponins have a dramatic effect on the antitumor activity against hepatocellular carcinoma (HepG2).[13] Therefore, structure

elucidation of the isolated saponin in the present study carries high relevance for unambiguous determination.

4.3. Results and Discussion:

4.3.1. General experimental conditions for the structural elucidation of saponin-SN (1, saponin from *S.nigrum*):

^{1}H- and ^{13}C- NMR of compound **1** in methanol-d4 were recorded at 500 MHz and 125 MHz, respectively. ^{1}H- and ^{13}C- NMR of compound **2** in $CDCl_3$ were recorded at 700 MHz and 176 MHz, respectively. Chemical shifts are given in parts per million and coupling constants in Hz. HR-ESI-MS analysis was performed on a Thermo Scientific Exactive mass spectrometer with ions given in *m*/*z*. Other than ^{1}H- and ^{13}C- NMR, DEPT-135 and a series of 2D NMR experiments *viz.* HSQC, COSY, HMBC, TOCSY, ROESY of compound **2** were used for structure determination.

4.3.2. ^{1}H and ^{13}C NMR of compound 1 in pyridine-d5 and CD_3OD:

Compound **1** was isolated by conventional column chromatography using silica gel followed by reverse phase HPLC purification. The isolated compound **1** was initially dissolved in pyridine-d5 and ^{1}H-NMR was recorded (Figure 4.3.2A) which resulted in a complex pattern of signals in the carbohydrate region of δ_H 3 to 4 ppm. Changing the NMR solvent to methanol-d4 for compound **1** also couldn't afford a ^{1}H-NMR with dispersion of peaks (Figure 4.3.2B). However, ^{13}C-NMR assisted in finding the anomeric hemikeal carbon that appeared at δ_C 112 ppm (Figure 4.3.2C).

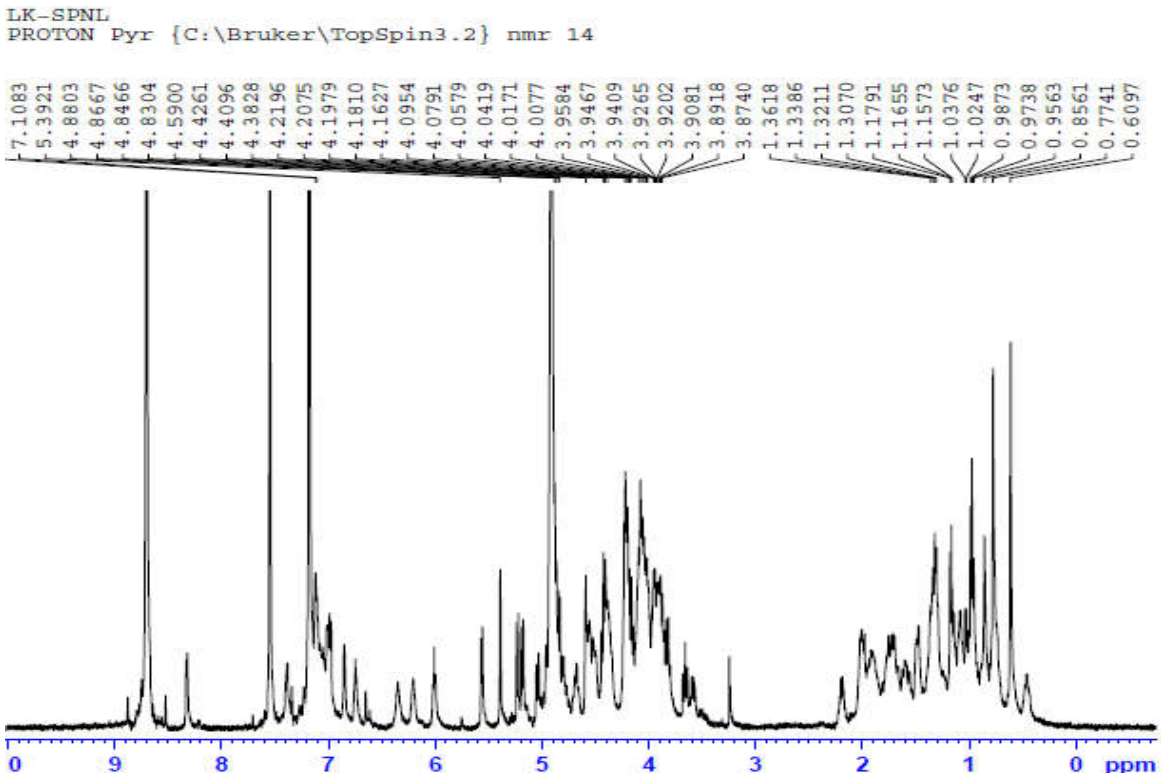


Figure.4.3.2A: ^{1}H-NMR spectra of compound (**1**) in pyridine-d5

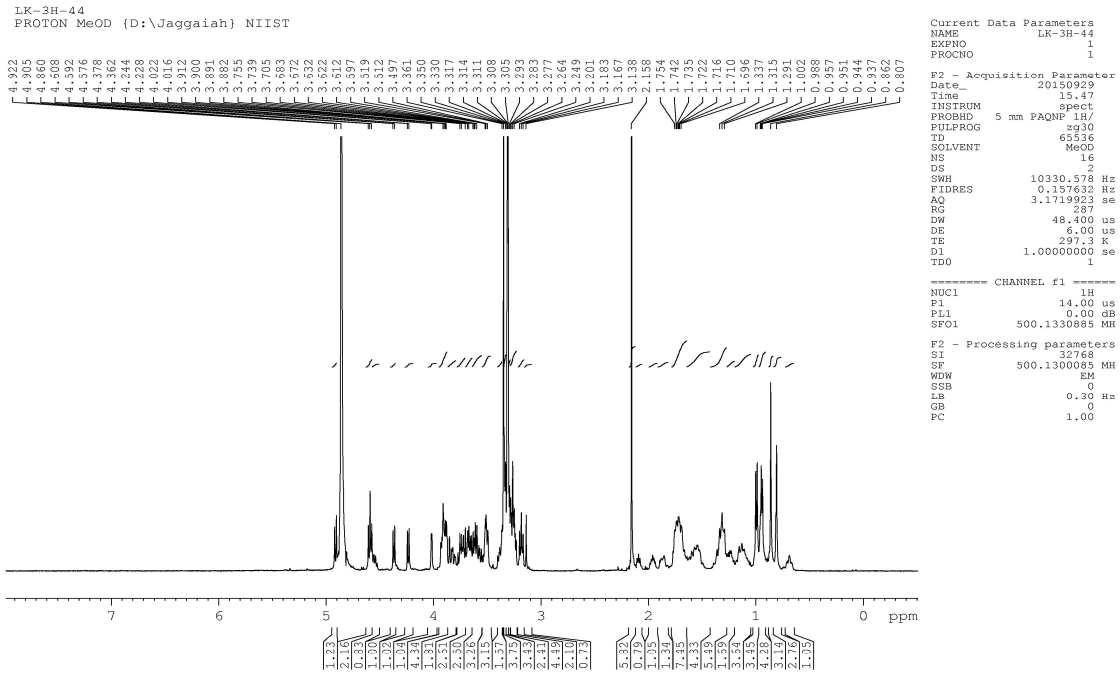


Figure.4.3.2B: ^{1}H-NMR spectra of compound (**1**) in CD_3OD

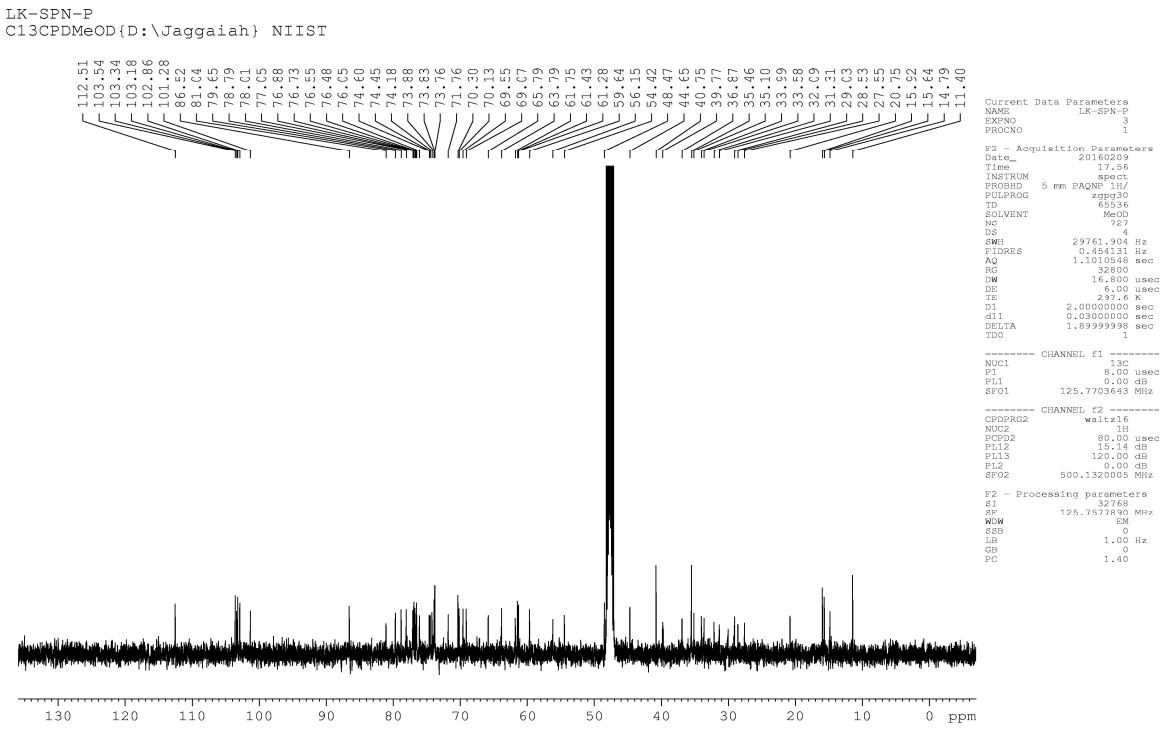


Figure.4.3.2C: ^{13}C-NMR spectra of compound (**1)** in CD_3OD

4.3.3. Peracetylation of compound 1: Compound **1** was per acetylated to peracetylated-SN (**2**, peracetylated saponin from *S.nigrum*) (Figure 4.3.3A) in order to make it less polar. Initially, compound **2** ^{1}H-NMR (Figure 4.3.3B) was recorded in $CDCl_3$ which resulted in a well dispersed peak pattern. Remaining NMR experiments: ^{13}C-NMR (Figure 4.3.3C), HSQC, DEPT-135°, COSY, TOCSY, HMBC, and ROESY experiments were recorded in the same solvent. Excluding the methyl peaks from the acetyl group, a total of 72 carbon signals, 4 methyls, 6 methylenes, 32 methines and four quarternary carbons were observed with the help of DEPT-135° (Figure 4.3.3D) and 16 carbonyl carbons from acetyl groups.

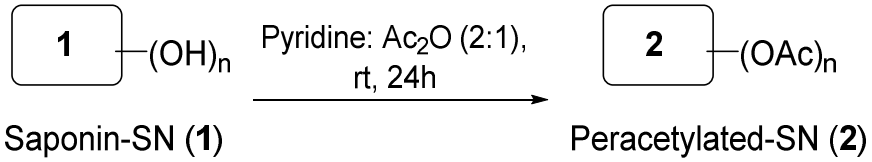


Figure 4.3.3A: Peracetylated-SN (**2**) from saponin-SN (**1**)

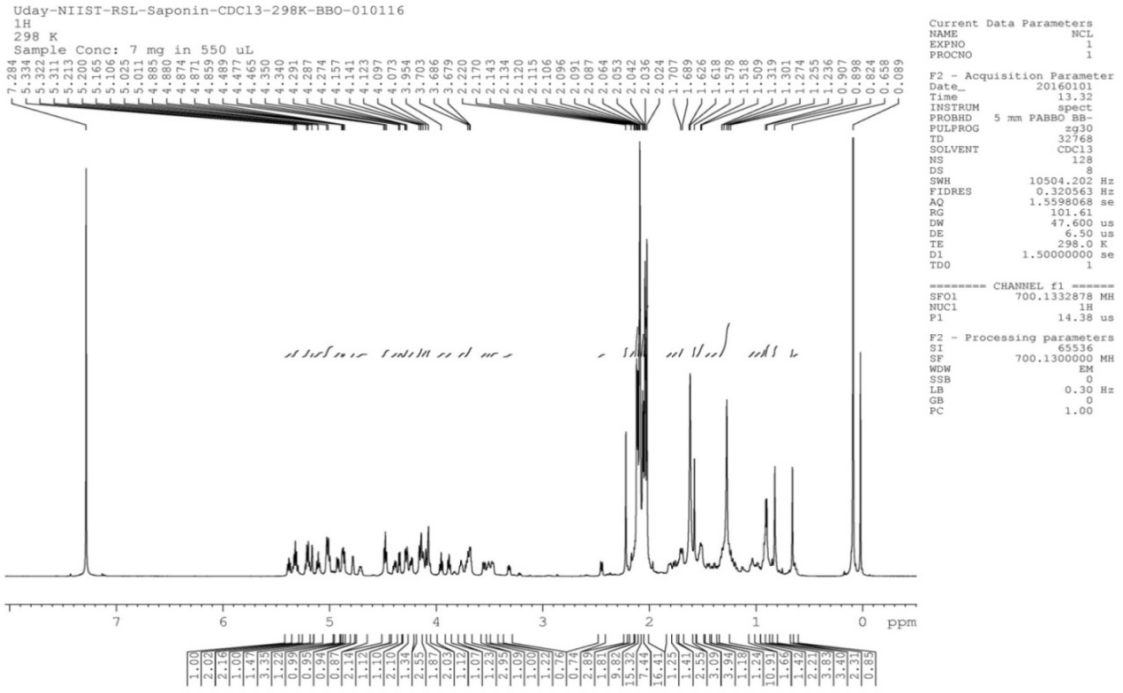


Figure 4.3.3B: ^{1}H-NMR spectra of compound **2**

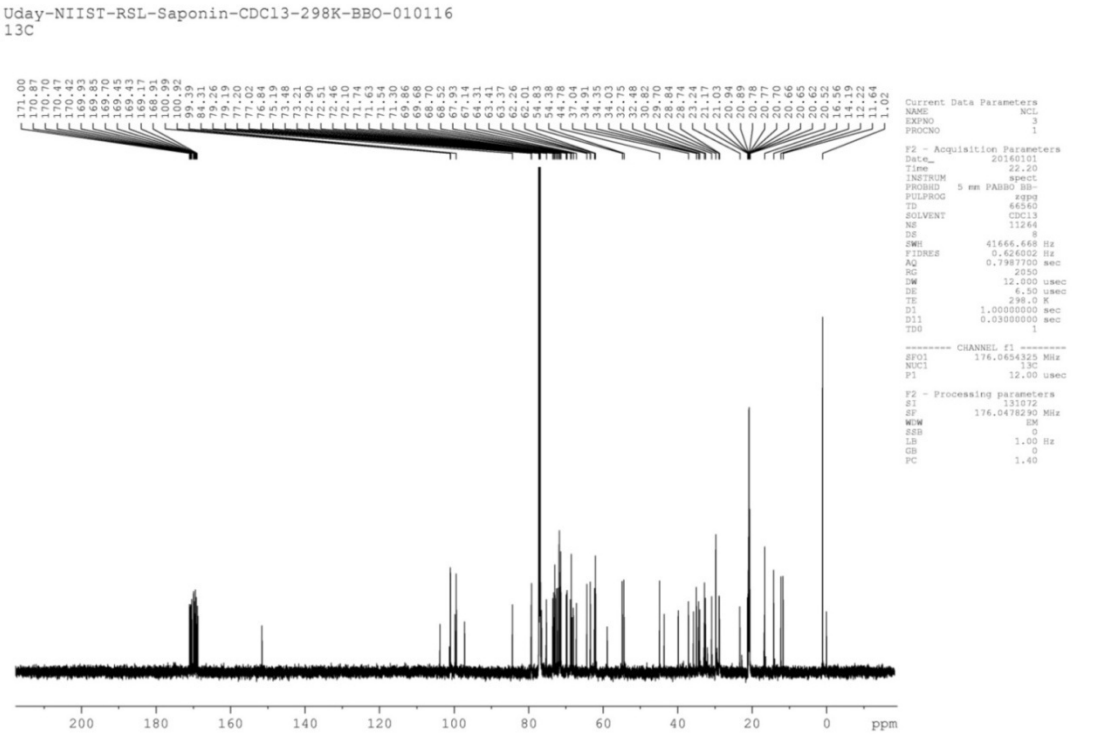


Figure 4.3.3C: ^{13}C-NMR spectra of compound **2**

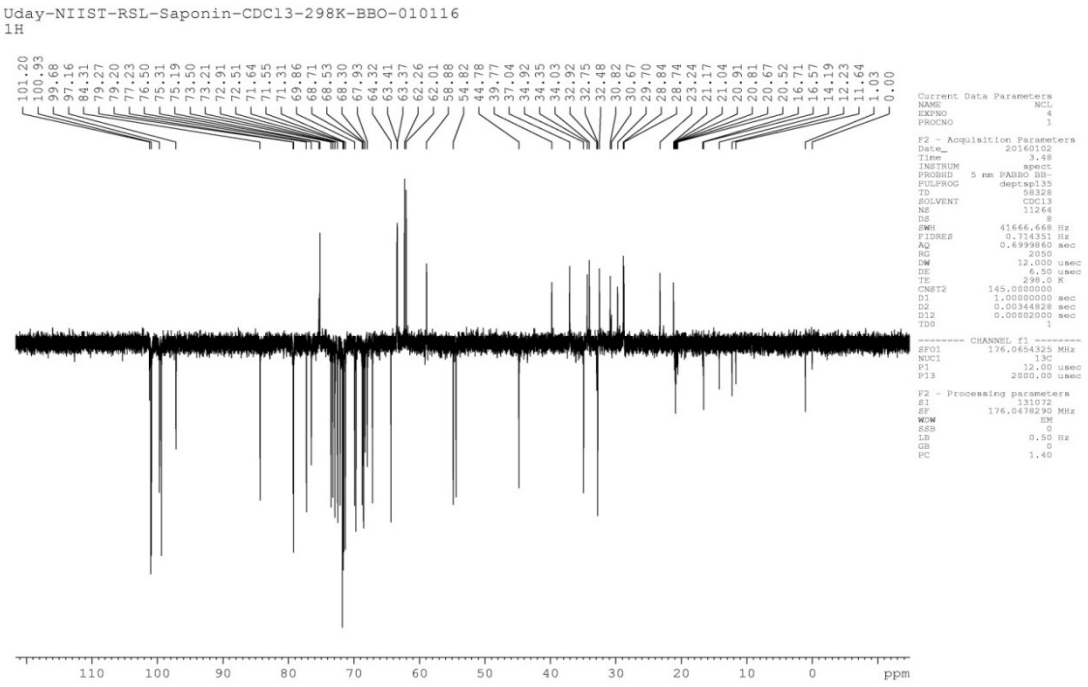


Figure 4.3.3D: DEPT-135° spectra of compound **2**

4.3.4. Identification of anomeric carbons using HSQC spectra:

The ^{1}H and ^{13}C-NMR spectral values of compound **2** are shown in Table 4.1. The loss of C-22 hydroxyl group resulted in presence of two olefin carbons at δ_C 103.7, 151.7, thus, confirming the loss of the hydroxyl group at hemiketal carbon at δ_C 113.0 in CD_3OD in compound **1**. Presence of five anomeric protons from HSQC spectra was clearly located from the five anomeric carbons (Figure 4.3.4A). The anomeric peaks appeared at [δ_H 4.34 (d, J = 7.0 Hz)/ δ_C 100.92, δ_H 4.45 (d, J = 8.4 Hz)/ δ_C 99.64, δ_H 4.48 (d, J = 8.4 Hz)/ δ_C 100.99, δ_H 4.85 (d, J = 8.4 Hz)/ δ_C 99.3, δ_H 5.16 (m)/ δ_C 97.1] (Figure 4.3.5A & Table 4.1). The identified five anomeric protons exhibited a coupling constant of J = 7-9 Hz which determines the β-configuration (axial-H-1 and axial-H-2, trans) of all the glycosidic linkages (Figure 4.3.4B).

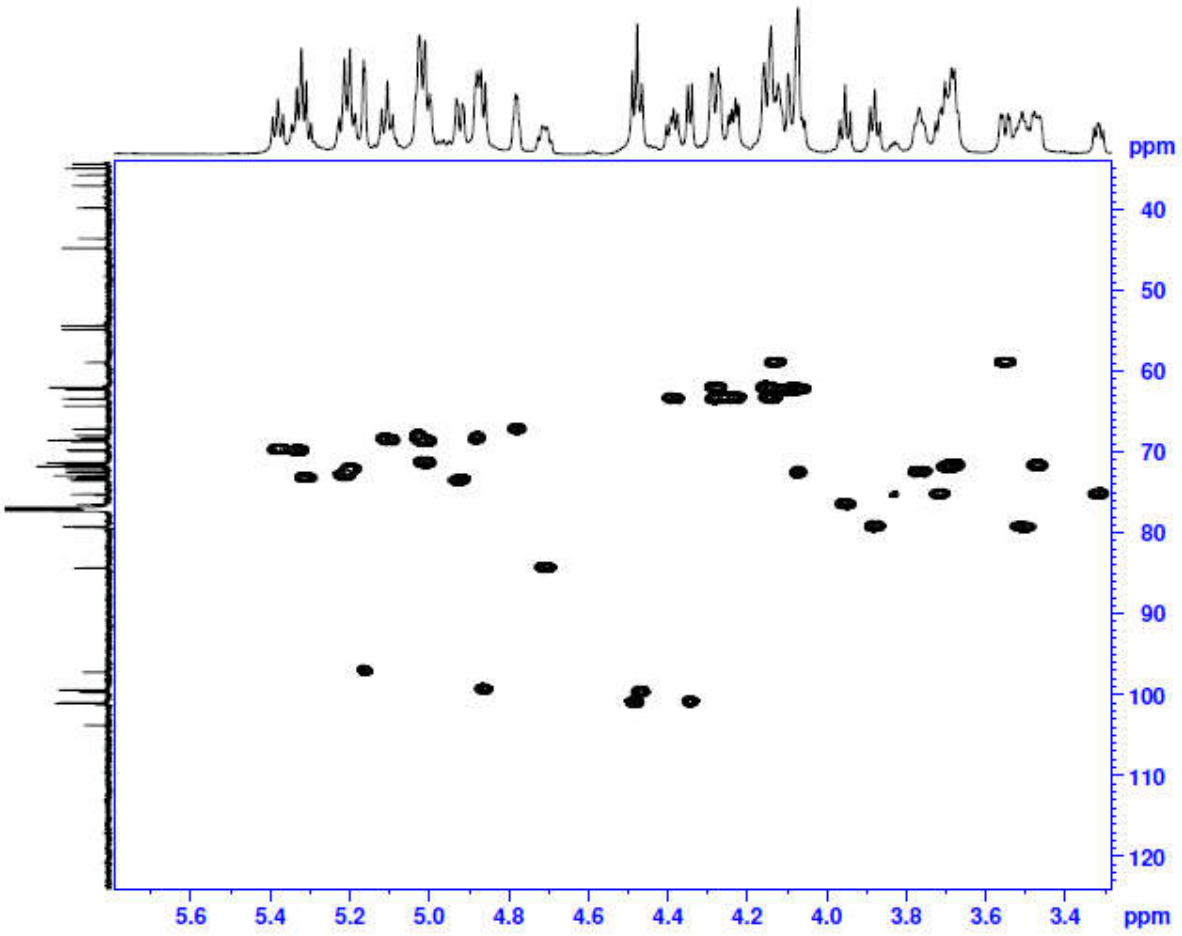


Figure 4.3.4A: HSQC spectra of compound **2**

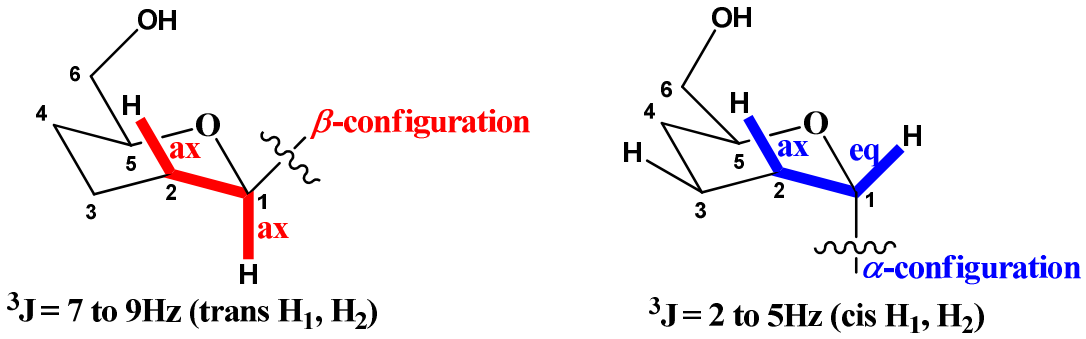


Figure 4.3.4B: Representation of configuration of α, β-glycosidic linkage

4.3.5. Construction of sugar rings using COSY, TOCSY, HMBC and ROESY spectral analysis: Sequential correlations beginning with five anomeric protons using COSY

and TOCSY helped in identification of the monosaccharide units. Among the five sugars, one monosaccharide was found as xylose using COSY and HMBC. Anomeric proton at δ_H 4.45 (d, *J* = 8.4 Hz, H-1) exhibited COSY correlation with H-2 at δ_H 5.38 (app t, *J* = 9.1 Hz) (Figure 4.3.5A). COSY correlations that were observed include H-2 with H-1 and H-3 at δ_H 4.92 (dd, *J* = 2.8, 9.1 Hz); H-3 with H-2 and H-4 at δ_H 4.05-4.06 (m); and H-4 with H-5 (δ_H 4.14, 1H, m) and H-6 (δ_H 4.23, 1H, dd, *J* = 5.6, 11.9). The key axial H-3 correlation with equatorial H-4 showed lower coupling constant (*J* = 2.8 Hz), and axial H-3 correlation with axial H-2 showed higher coupling constant (*J* = 9.1 Hz) which indicates the presence of galactose as a monosaccharide unit.

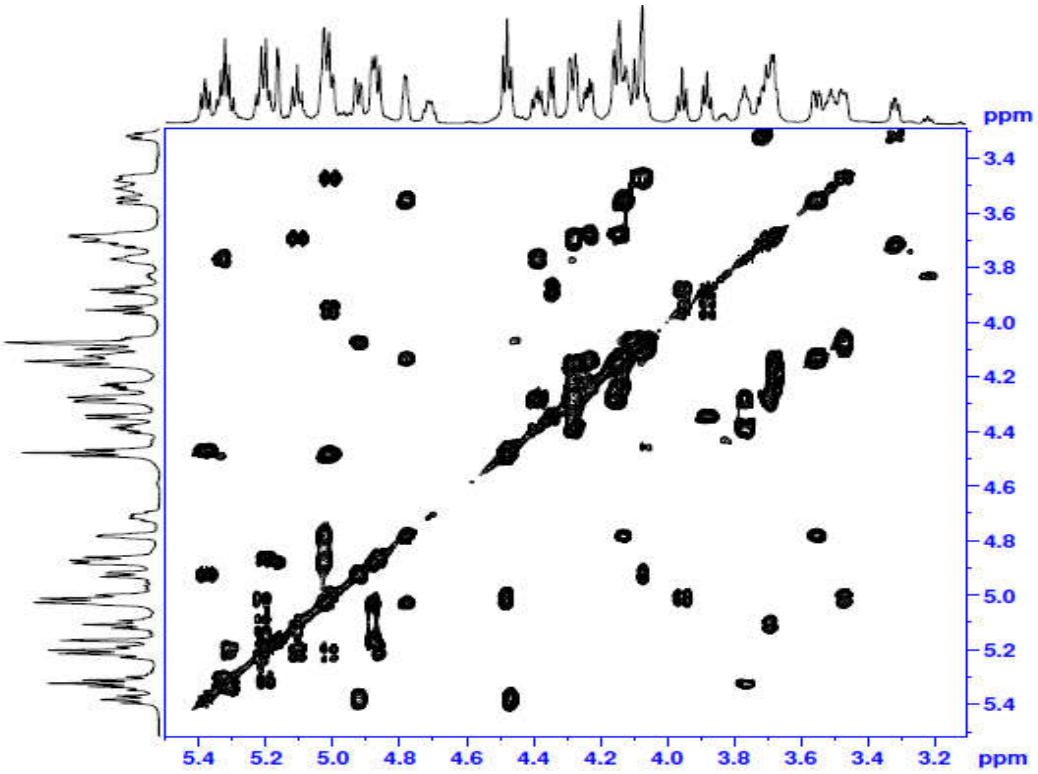


Figure 4.3.5A: COSY spectra of sugar portion of compound **2**

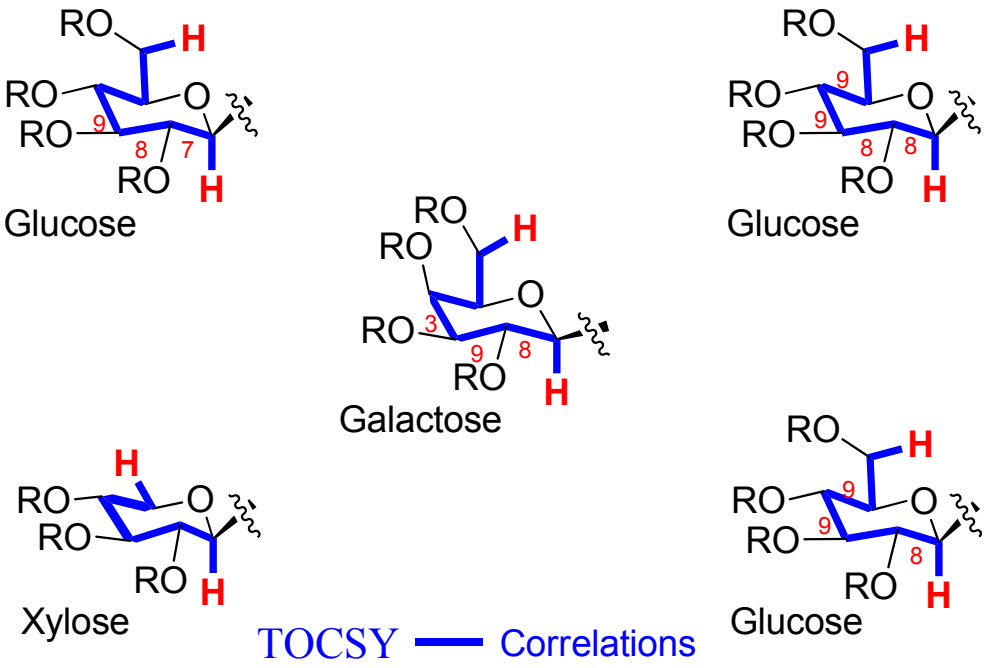


Figure 4.3.5B: TOCSY correlations for the determination of sugar rings

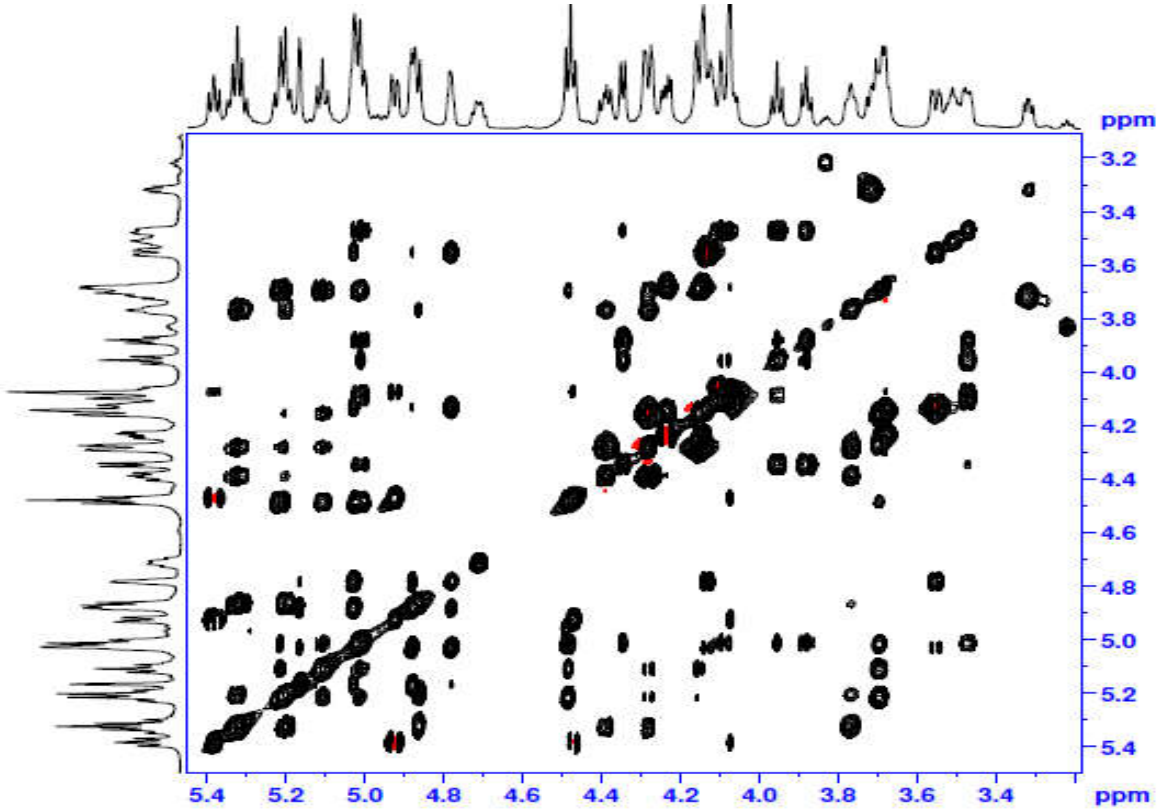


Figure 4.3.5C: TOCSY spectra of compound **2**

The anomeric carbon at δ_H 4.45 (d, J = 8.4 Hz)/δ_C 99.64 exhibited key HMBC correlations with H-3 at δ_H 3.50-3.52 (m) of steroid and H-2 of galactose (Figure 4.3.5D) with higher coupling constant indicating that the galactose is attached to furostanol via β-glycoside linkage. Furthermore, β-glycoside linkage was established with the help of ROESY correlations (Figure 4.3.5E) exhibiting spatial interaction of gal H-1 with H-3 at δ_H 3.50-3.52 (m)/δ_C 79.2 of steroid (Table 4.1).

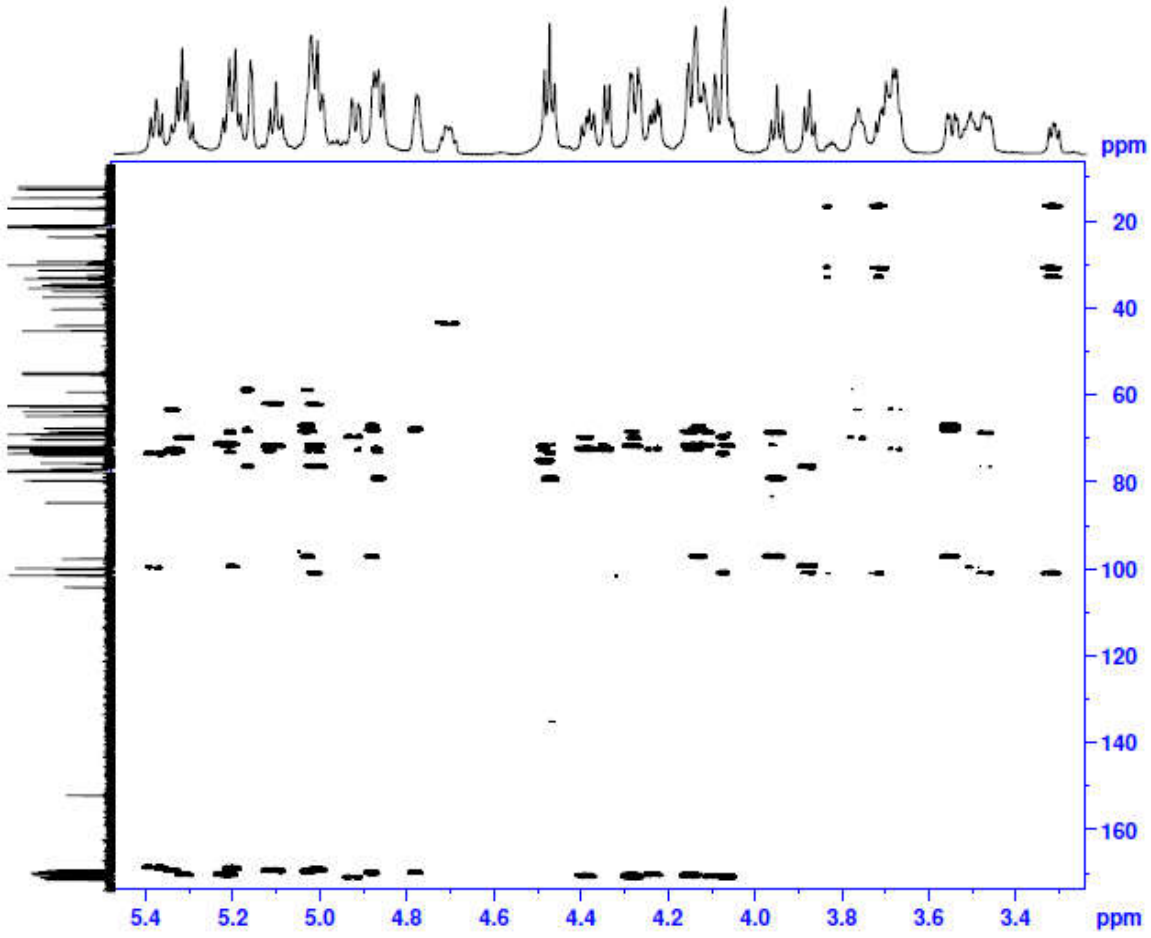


Figure 4.3.5D: HMBC spectra of compound (**2**)

The second monosaccharide *β*-O-glycoside linkage was found between gal C-4 at δ_C 72.51 which showed strong HMBC interaction (^{3}J) with H-1′ at δ_H 4.45 (1H, d, *J* = 8.4 Hz) indicating that the second monosaccharide unit was connected to C-4 of gal via a *β*-O-glycoside linkage as shown in (Figure 4.3.5D). Furthermore, the second sugar ring was determined with the help of COSY, TOCSY and HMBC spectra. The axial H-1′ at δ_H 4.45 (1H, d, *J* = 8.4 Hz) exhibited COSY correlation with axial H-2′ at δ_H 3.87 (app t, *J* = 8.4 Hz). COSY correlations were seen for H-2′ with axial H-3′ at δ_H 3.95 (app t, *J* = 9.1 Hz), a higher coupling constant indicates that the H-3′ and H-4′ are axial to each other depicting the presence glucose I as the second sugar unit. COSY correlations of H-4′ at δ_H 4.99 (m) with H-5′ at δ_H 3.46-3.48 (m) and H-5′ with H-6′ at δ_H 4.07-4.09 (2H, m) along TOCSY correlations were observed (Figure 4.3.5C).

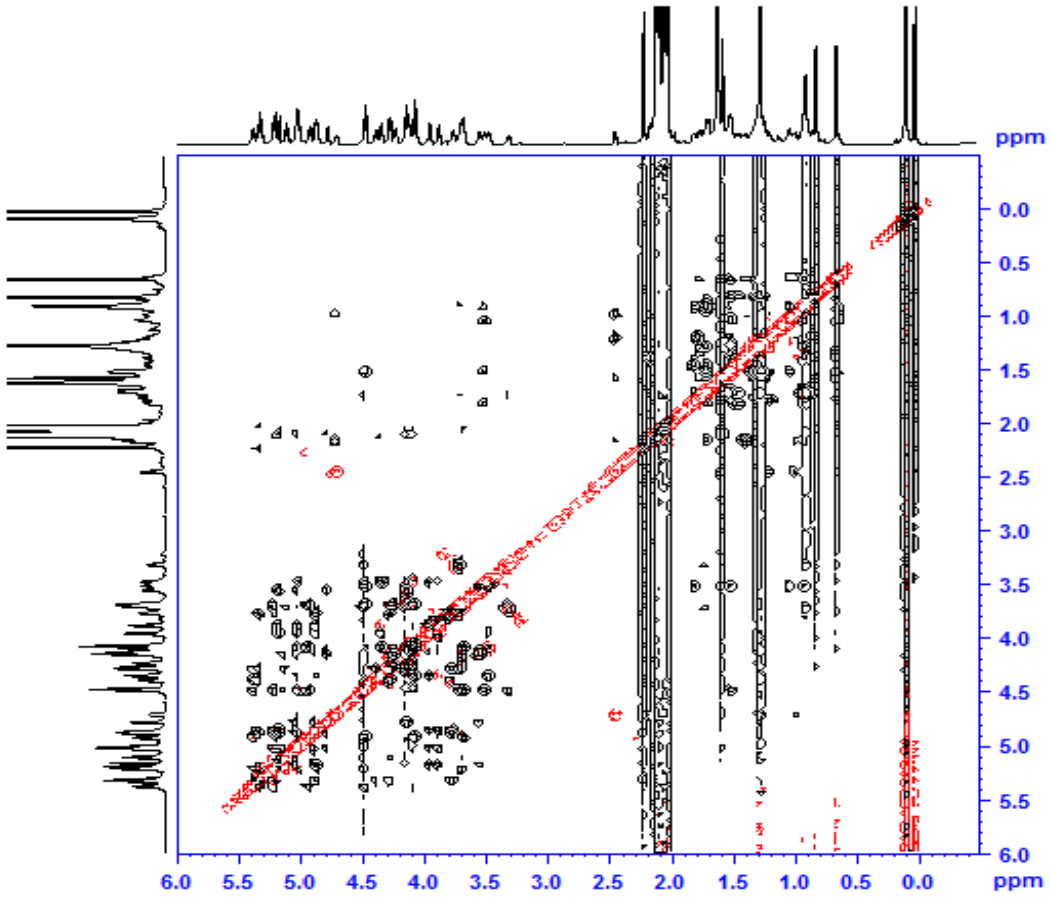


Figure 4.3.5E: ROESY spectra of compound (**2**)

The third glycosidic linkage between C-2′ at δ_C 79.19 with H-1″ at δ_H 4.85 (1H, d, *J* = 8.4 Hz) was determined with the help of HMBC. The third anomeric proton H-1″ at δ_H 4.85 (1H, d, *J* = 8.4 Hz) showed COSY correlation with H-2″ at δ_H 5.20 (app t, *J* = 8.4 Hz); H-2″ in turn showed COSY correlation with H-3″ at δ_H 5.31 app t (*J* = 9.1 Hz). Further COSY correlation include H-3″ with H-4″ at δ_H 5.1 (app t, *J* = 9.1 Hz), H-4″ with H-5″, and H-5″ (δ_H 4.27, m) with H-6″ at δ_H 4.39 (dd, *J* = 7.7, 11.9 Hz). The axial H-3″ and axial H-4″ evident from their coupling constants indicates that the third monosaccharide unit is glucose II. The fourth glycoside connection was established with help of strong HMBC correlation between the fourth monosaccharide unit anomeric carbon at δ_C 97.16 with H-3′ at δ_H 5.31 (app t, *J* = 9.1 Hz). The anomeric proton H-1 at δ_H 5.16 (m) of xylose showed COSY correlation with H-2 at δ_H 4.88 (m). Further COSY correlations observed include H-2 with H-3 at δ_H 5.02-5.04 (m); H-3 with H-4 at δ_H 4.77-4.78 (m); H-4 with methylene H-5 protons at δ_H 3.55 (dd, 1H, *J* = 4.2, 12 Hz), 4.12 (1H, m). ROESY correlations (Figure 4.3.5E) were observed for the anomeric proton at δ_H 5.16 (m) with H-3′ at δ_H 5.31 (app t, *J* = 9.1 Hz) of glc I. These aforementioned results indicated that the fourth monosaccharide was xylose.

4.3.6. Determination of glycosidic linkages using HMBC spectral analysis:

The anomeric carbon C-1′ at δ_C 100.92 showed HMBC corrleation with H-4 of galactose at δ_H 4.05-4.06 (m); C-2′ at δ_C 79.1 was having correlation with H-1″ at δ_H 4.85 (1H, d, *J* = 8.4 Hz); C-3′ at δ_C 76.4 showed ^{3}J interaction with H-1 at δ_H 5.16 (m) of xylose indicating that the glucopyranosyl-I was connected to *β*-O-galactopyranosyl, *β*-O-glucopyranosyl-II and *β*-O-xylopyranosyl units. On the basis of the aforementioned evidence the four sugar units were linked as 3-O-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-

(1→4)-*β*-D-galactopyranoside (Figure 4.3.6A), thus, indicating the presence of tetrasaccharide lycoterosyl unit in saponin-SN (**1**).

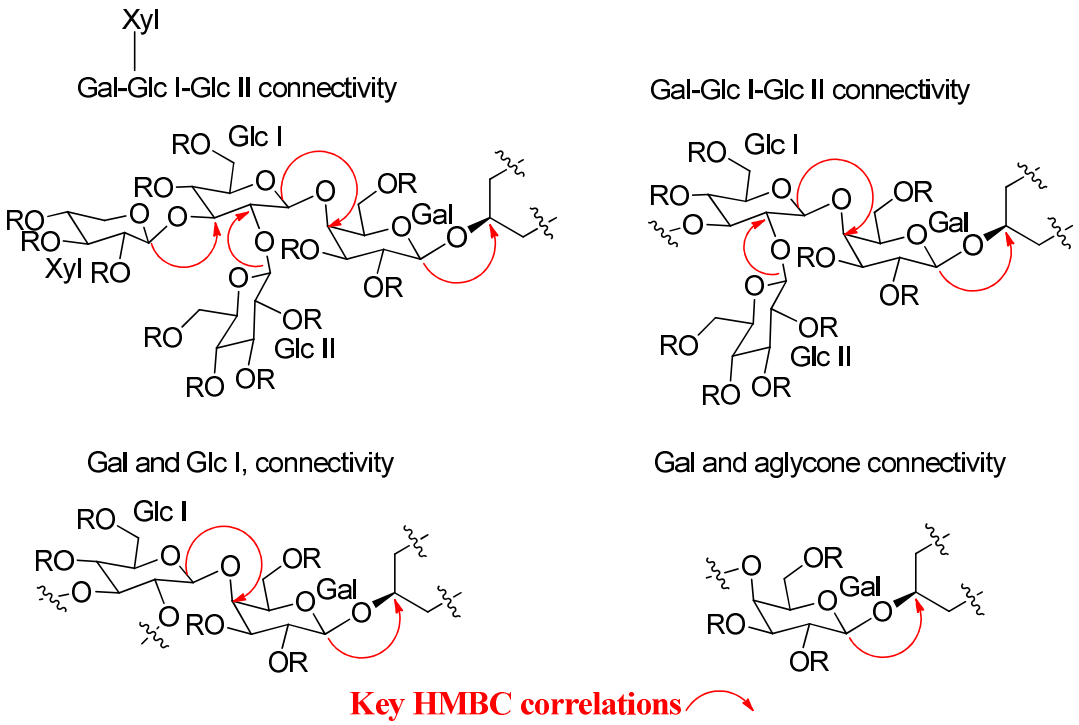


Figure 4.3.6A: Determination of glycosidic linkages using HMBC correlations

The fifth monosaccharide unit was attached to the other end of furostanol ring via a *β*-O-glycoside linkage which was proven with the help of HMBC correlation. The fifth anomeric carbon C-1‴ at δ_C 100.99 showed strong HMBC (Figure 4.3.5D) correlation with H-26 at δ_H 3.31 (1H, dd, J = 6.3, 9.1 Hz), 3.71-3.72 (1H, m). Futhermore, C-26 at δ_C 75.1 exhibited ^{3}J interaction with H-27 at δ_H 0.90 (d, J = 6.3 Hz). The anomeric axial proton H-1‴ at δ_H 4.48 (d, J = 8.4 Hz) was having COSY correlation with axial H-2‴ at δ_H 5.01 (m). The higher coupling constant indicates the presence of *β*-O-glycoside linkage between the steroid and the fifth monosaccharide unit (Figure 4.3.6B). COSY correlations include H-2‴ with axial H-3‴ at δ_H

5.22 (app t, J = 9.1 Hz); H-3‴ with axial H-4‴ at δ_H 5.1 (app t, J = 9.1 Hz); H-4‴ with H-5‴ at δ_H 3.70-3.71 (m); and H-5‴ with H-6‴ at δ_H 4.15 (1H, m), 4.28 (1H, m). The axial H-3‴ and axial H-4‴ (^2J = 9.1 Hz) with higher coupling constant indicates that the fifth monosaccharide unit was glucose (Glc III).

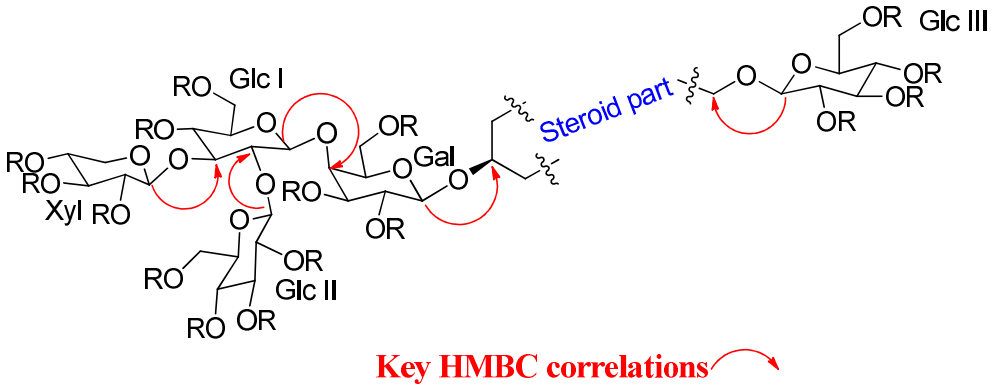


Figure 4.3.6B: Determination of fifth glycosidic linkage using HMBC correlation

The key ROESY (Figure 4.3.6E) correlation of axial H-1‴ with axial H-5‴ at δ_H 3.70-3.71 (m) was observed. Thus, the five monosaccharide units (gal-glc-I-glc-II-xyl and glc-III), and their β-O-glycoside linkages were established with the help of key COSY, TOCSY HMBC and ROESY correlations.

4.3.7. Identification of steroidal portion:

The steroidal portion was established with the help of 1D and 2D NMR experiments. The furostanol C-3 carbon at δ_C 79.2 showed HMBC (Figure 4.3.5D) correlation with H-1 at δ_H 4.45 (d, J = 8.4 Hz) of galactose, and the other end carbon C-26 at δ_C 75.1 was having ^{3}J interaction with H-1‴ at δ_H 4.48 (d, J = 8.4 Hz) of glc-III. This result indicates the presence of β-O-galactopyranosyl and β-O-glucopyranosyl units at both ends of furostanol. The H-3 at δ_H 3.50-

3.52 (1H, m) was having COSY correlations with H-2 at δ_H 1.43-1.45 (1H, m), 1.80 (1H, m) and H-4 at δ_H 1.21-1.23 (1H, m), 1.51 (1H, m), H-2 with H-1 at δ_H 0.92 (1H, m), 1.70 (1H, m), however, H-1 did not exhibit any correlations. The HMBC correlations between C-10 at δ_C 35.72 with H-1 at δ_H 0.92 (1H, m), 1.70 (1H, m) indicates the presence of quaternary carbon adjacent to the H-1. Similarly, H-4 with H-5 at δ_H 1.03 (1H, m) indicates the presence of tertiary carbon adjacent to the H-4. H-5 and H-1 showed HMBC ^2J interactions with quaternary carbon C-10 at δ_C 35.72 which depicts ring A. Quaternary carbon C-10 at δ_C 35.72 showed HMBC correlation with H-19 at δ_H 0.82 (3H, s) indicating that the methyl group is attached to C-10 (Figure 4.3.7A).

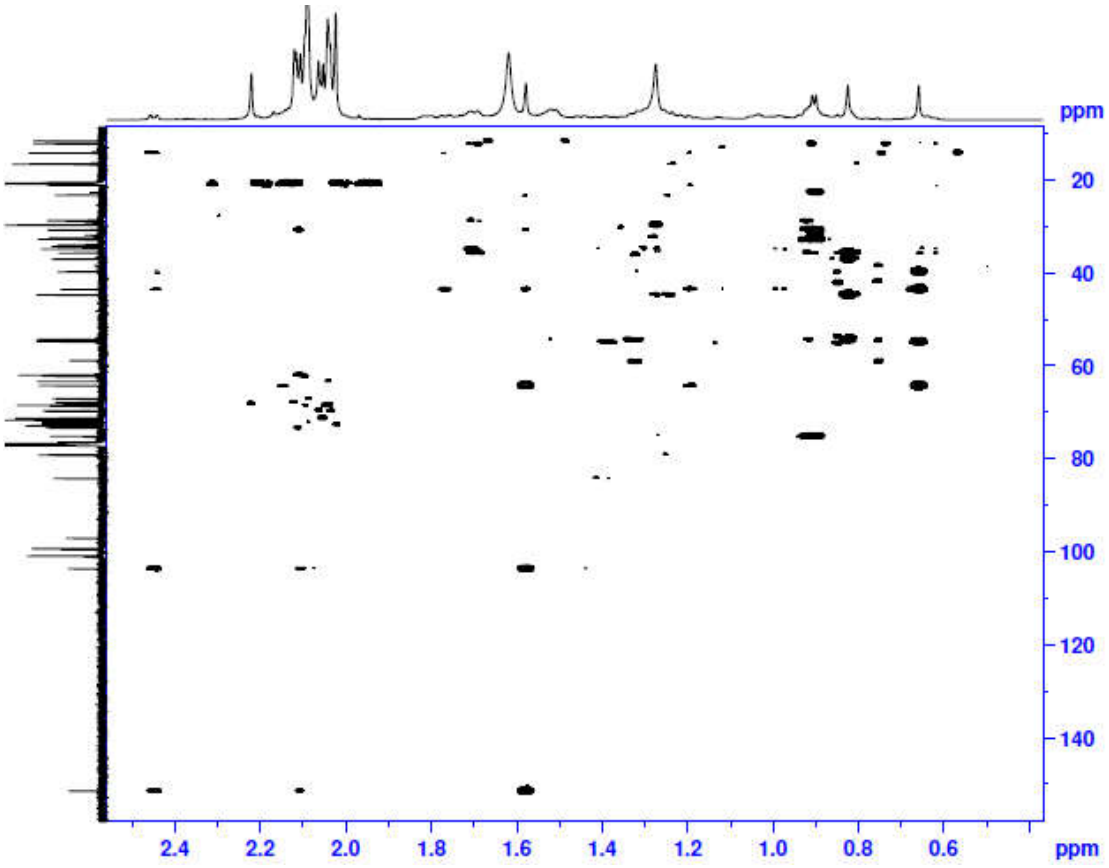


Figure 4.3.7A: Key HMBC correlations for the construction of steroidal portion

H-5 at δ_H 1.03 (1H, m) showed vicinal interaction with H-6 at δ_H 1.27 (2H, m) and H-4 at δ_H 1.21-1.23 (1H, m), 1.51 (1H, m) which indicates that the two methylene groups were attached to C-5 tertiary carbon. Furthermore, H-6 showed correlation with H-7 at δ_H 0.87 (1H, m), 1.68 (1H, m), and H-7 with H-8 at δ_H 1.49-1.51 (1H, m) which provided information that C-8 is attached to a single proton. Similarly, H-8 was having COSY correlation with H-9 at δ_H 0.61-0.63 (1H, m), and H-14 at δ_H 0.96-0.98 (1H, m) and C-8 at δ_C 34.91 showed HMBC correlation with H-7, H-9, H-13, H-14, H-15 which indicates the presence of two tertiary carbons were attached to C-8. Key HMBC correlation between C-10 at δ_C 35.72 with a single proton H-9 at δ_H 0.61-0.63 (1H, m), and methyl group H-19 at δ_H 0.82 (3H, s) indicates the methyl group attachment with C-10. C-10 showed HMBC correlation with A ring of H-1 at 0.92 (1H, m), 1.70 (1H, m), and H-9 of B ring which indicates the presence of fused ring carbon C-10 that is connecting ring A and B. The construction of ring C began from H-8 at δ_H 1.49-1.51 (1H, m) which showed COSY correlation with H-9 and an important correlation with a single proton H-14 at δ_H 0.96-0.98 (1H, m) which shows that the H-8 was having two neighboring protons with a single proton integration. Furthermore, H-9 showed correlation with H-11 at δ_H 1.30-1.33 (2H, m), and H-11 with H-12 at δ_H 1.17-1.19 (1H, m), 1.75-1.77 (1H, m). H-12 did not show any correlation which indicates the presence of quaternary carbon C-13. The quaternary carbon C-13 at δ_C 43.56 showed a strong HMBC interaction with H-12 and methyl group H-18 at δ_H 0.65 (3H, s). Considering the H-8 vicinal interaction with H-14 which showed COSY correlation with H-15 at δ_H 1.36-1.41 (1H, m), 2.14-2.17 (1H, m), and H-15 having vicinal interaction with a single proton H-16 at higher δ_H 4.69-4.72 (1H, m) indicates that H-16 would be alpha to the oxygen atom. A single proton H-16 showed a strong ^3J interaction with a single allylic proton at H-17 δ_H 2.45 (1H, d, J = 9.8 Hz), the higher coupling constants indicates that H-16 and H-17 are trans to each other which further

indicates that H-16 was located between H-15 and H-17. Importantly, H-17 showed only one COSY correlation with H-16. Two quaternary carbons C-13 at δ_C 43.56 and C-20 at δ_C 103.74 showed strong two bond HMBC correlation with H-17 which indicates that H-17 was located between two quaternary carbons. Similarly ring D was established using COSY and HMBC connections. A single proton H-16 at higher δ_H 4.69-4.72 (1H, m) showed a strong COSY correlation with a single allylic proton at H-17 at δ_H 2.45 (1H, d, J = 9.8 Hz), further H-17 was having allylic interaction with H-21 at δ_H 1.57 (3H, s). HMBC data revealed the presence of two and three bond correlations of quaternary carbons C-20 at δ_C 103.74, C-22 at higher δ_C 151.7 with H-17, H-21 and H-23 (Figure 4.3.7B). These higher chemical shifts for C-20 at δ_C 103.74 and C-22 at δ_C 151.7 confirmed the presence of double bond between C-20 and C-22.

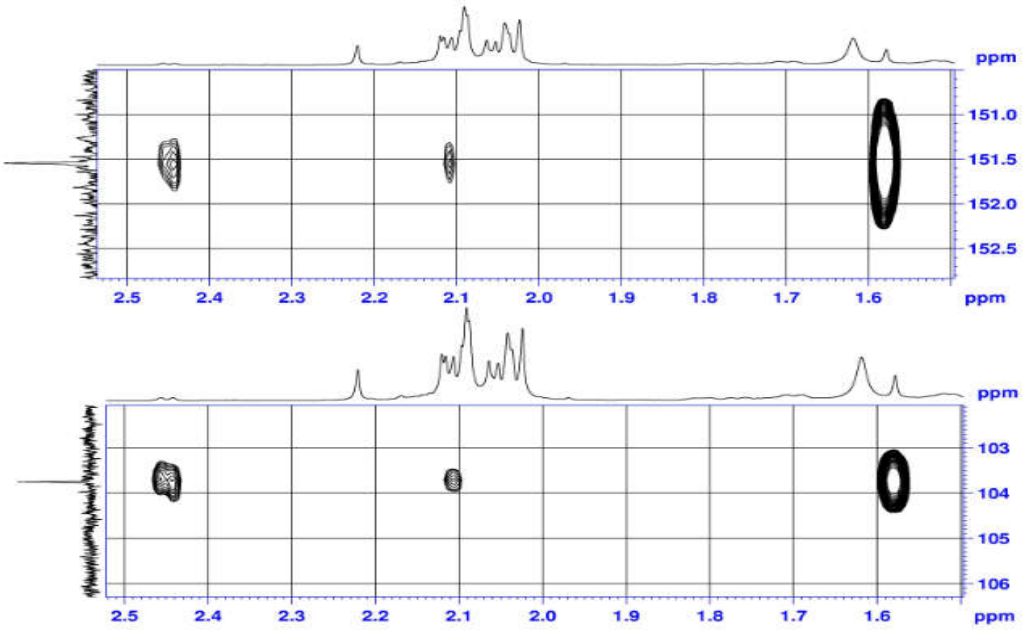


Figure 4.3.7B: Identification of new olefin bond formation in steroidal portion using HMBC correlations

Similarly, ring E was established using COSY and HMBC experimental data. The key information pertaining to steroidal furanose ring include H-21 methyl group at δ_H 0.99 (3H, d, *J* = 7 Hz), and hemiketal carbon C-22 at δ_C 112.5 (Figure.4.3.2C). Surprisingly, after acetylation the H-21 methyl group exhibited a downfield shift at δ_H 1.57 (3H, s), and H-17 at δ_H 2.45 [1H, d, *J* = 9.8 Hz (Table 4.1 and Figure 4.3.3B)]. In ^{13}C-NMR, the hemiketal carbon peak disappeared and two additional peaks appeared at δ_C 103.7 and 151.7 indicating carbons C-20 and C-22, respectively (Table 4.1 and Figure 4.3.3C). The aforementioned observations by NMR led us to believe the appearance of a new olefinic bond in the furanose ring due to loss of a water molecule during acetylation. Both the carbons C-20 and C-22 showed strong HMBC correlations with H-21, H-17 and H-23. The key COSY correlations observed include: H-23 at δ_H 2.08 (2H, m) and H-24 at δ_H 1.25 (1H, m), 1.52 (1H, m), and H-24 with H-25 at δ_H 1.73-1.75 (1H, m). Similarly, H-25 showed correlations with methyl group H-27 at δ_H 0.9 (3H, d, *J* = 6.3 Hz) and H-26 at higher δ_H 3.31 (1H, dd, 1H, *J* = 6.3, 9.1 Hz), 3.71-3.72 (1H, m) which confirmed the presence of two methylene and one methyl groups as vicinal to H-25.

4.3.8. Acid hydrolysis of compound 1:

The saponin (20 mg) in 3 mL of ethanol: 2M HCl (4:1) was heated under reflux at 100 °C for 3 hours. The reaction was allowed to attain room temperature and the reaction mixture was quenched with 5 mL of saturated aq. $NaHCO_3$ and extracted with 10 mL twice with $CHCl_3$/MeOH (7/1), dried over Na_2SO_4 and concentrated. The resulting crude mixture was subjected to mass spectral analysis. The HR-ESI-MS (Figure 4.3.8B) showed the molecular ion peak at m/z 615.3522 (M+Cl+H), which indicates that the tetrasaccharide glycosidic linkage underwent hydrolysis and the monosaccharide gluc-III was still intact, showing a

monosaccharide attached gluc-chloro-furostane (**3**). The loss of water molecule followed by the addition of chlorine on the double bond led to the formation of gluc-chloro-furostane product **3** (Figure 4.3.8A).

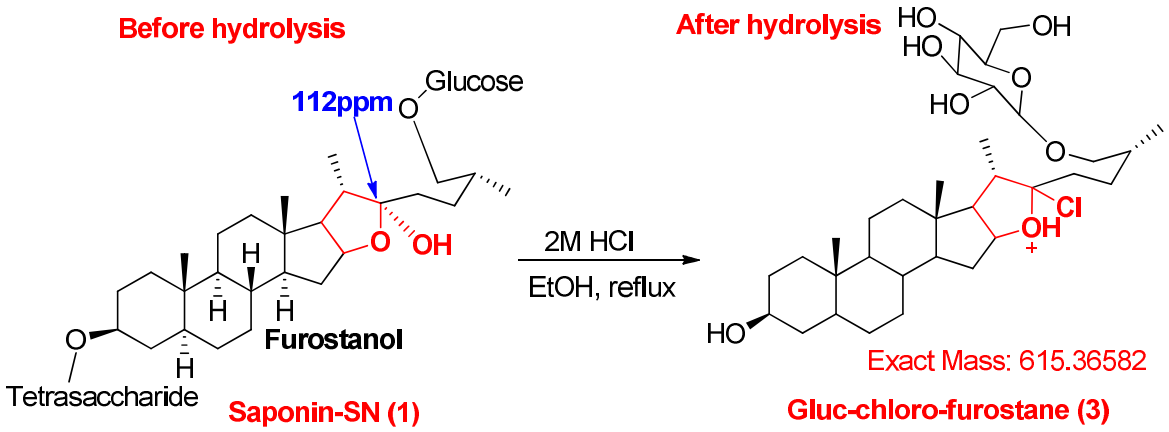


Figure 4.3.8A: Formation of Gluc-chloro-furostane (**3**) during acid hydrolysis of saponin-SN (**1**)

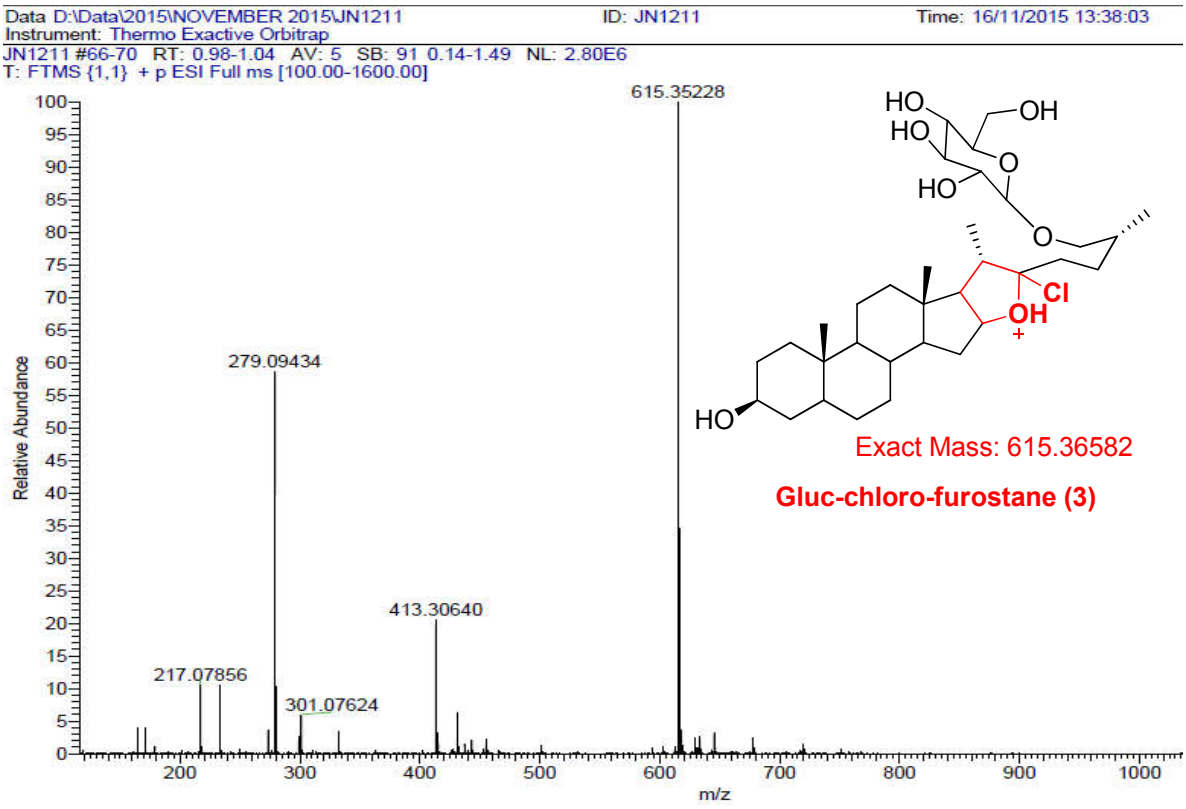


Figure 4.3.8B: Mass spectra of Gluc-chloro-furostane (**3**)

4.3.9. Key COSY, HMBC and ROESY correlations of compound 2:

The structure was finally elucidated as 26-O-β-D-glucopyranosyl-(25R)-5R-furost-3β,22R,26-triol-3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. A thorough literature survey provided further structural confirmation of the isolated compound **1** as uttroside B (Figure 4.1A).[8]

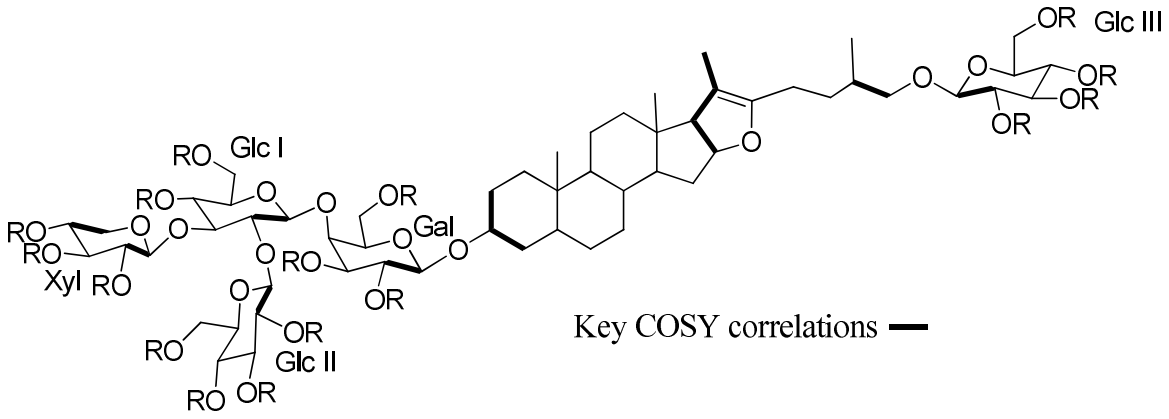


Figure 4.3.9A: Key COSY correlations of compound **2**

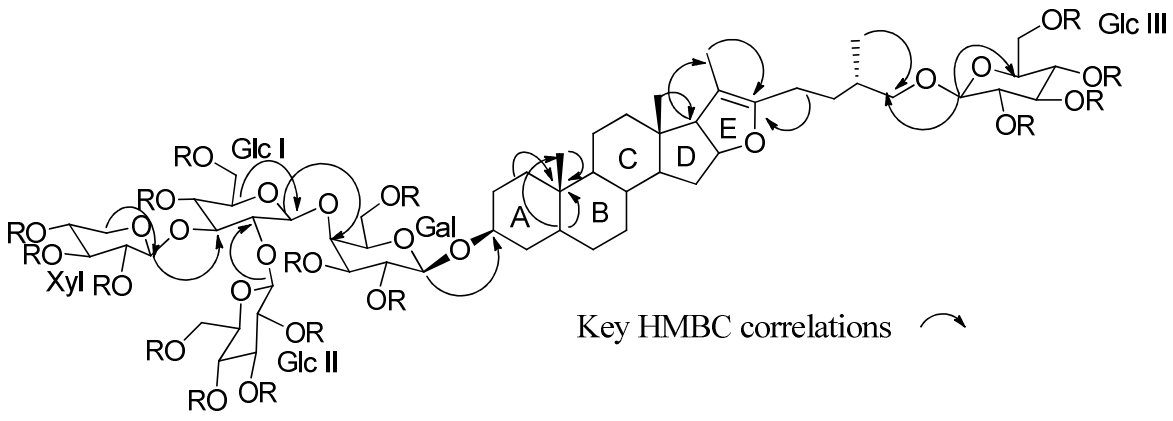


Figure 4.3.9B: Key HMBC correlations of compound **2**

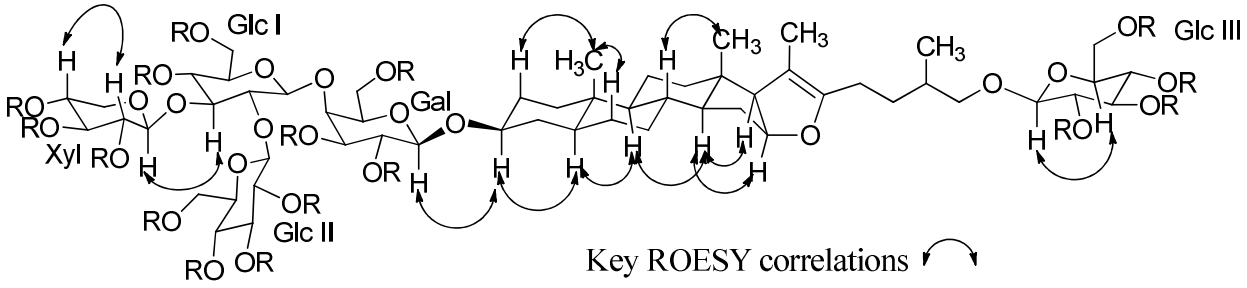


Figure 4.3.9C: Key ROESY correlations of compound **2**

4.3.10. HR-ESI-MS analysis of compound 2:

The molecular ion mass was expected at 1909.75 (M+Na) for compound **2**. Surprisingly, the molecular ion mass was observed at 1891.74 (M-H_2O+Na) which indicates that the loss of water molecule from compound **1** during acetylation reaction conditions (Figure 4.3.10A). The mass of the compound **2** provided information of the presence of 16 hydroxyl groups in compound **1**.

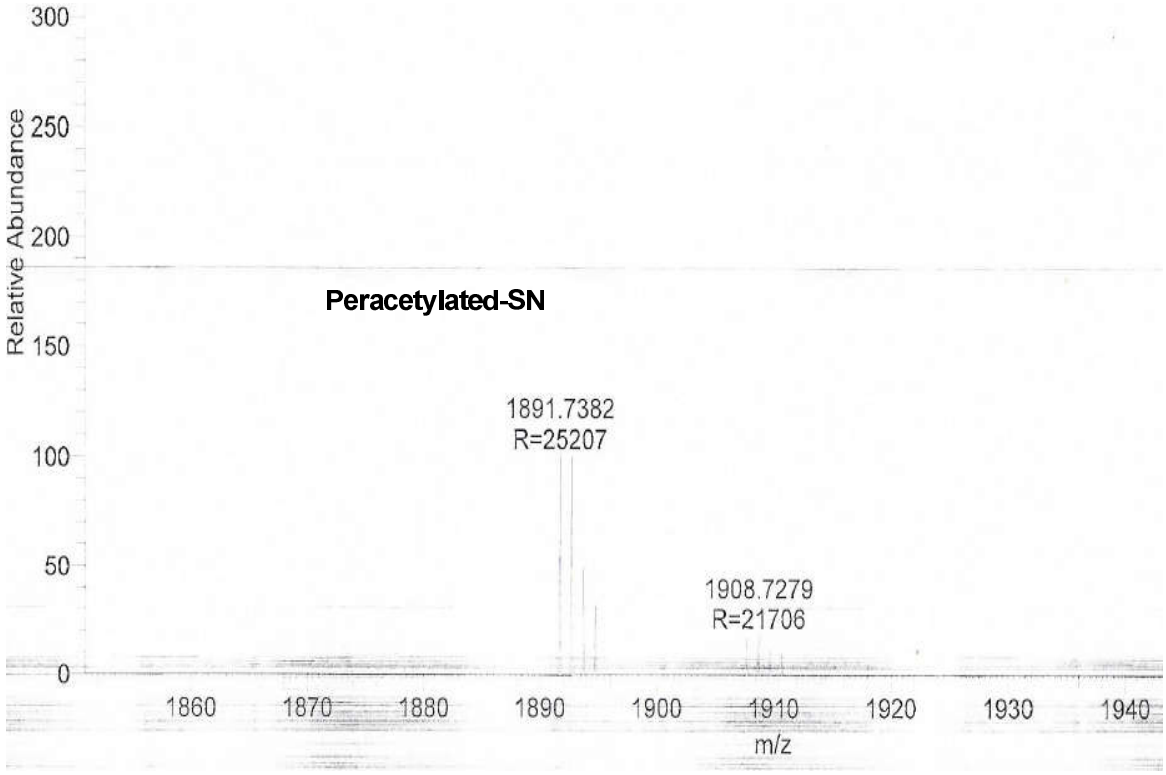


Figure 4.3.10A: HR-ESI-MS analysis data of compound (**2**)

4.4. ^{1}H and ^{13}C NMR data of compound 2:

Table 4.1: ^{1}H NMR (700 MHz in $CDCl_3$), ^{13}C NMR (176 MHz in $CDCl_3$) data of compound **2**

Position	δ ^{13}C	δ ^{1}H mult [*J* (Hz)]	Position	δ ^{13}C	δ ^{1}H mult [*J* (Hz)]
1	37.0	0.92 (m)	Gal		
		1.70 (m)	1	99.6	4.45 d (8.4)
2	28.7	1.43-1.45 (m), 1.80 (m)	2	69.6	5.38 app t (9.1)
3	79.2	3.50-3.52 (m)	3	73.4	4.92 dd (2.8, 9.1)
4	34.2	1.21-1.23 (m)	4	72.5	4.05-4.06 (m)
		1.51 (m)	5	71.6	3.67-3.68 (m)
5	44.7	1.03 (m)	6	63.4	4.14 (m)
					4.23 dd (5.6, 11.9)
6	29.7	1.27 (m)	Glc I		
7	32.4	0.87 (m), 1.68 (m)	1′	100.92	4.34 d (7.0)
8	34.9	1.49-1.51 (m)	2′	79.1	3.87 app t (8.4)
9	54.3	0.61-0.63 (m)	3′	76.4	3.95 app t (9.1)
10	35.7		4′	68.7	4.99 (m)
11	28.8	1.30-1.33 (m)	5′	71.5	3.46-3.48 (m)
12	39.7	1.17-1.19 (m)	6′	62.2	4.07-4.09 (m)
		1.75-1.77 (m)	Glc II		
13	43.5		1″	99.3	4.85 d (8.4)
14	54.8	0.96-0.98 (m)	2″	72.1	5.20 app t (8.4)
15	34.0	1.36-1.41 (m)	3″	73.2	5.31 app t (9.1)
		2.14-2.17 (m)	4″	69.8	5.33 app t (9.1)

16	84.3	4.69-4.72 (m)	5″	72.4	3.75-3.77 (m)
17	64.3	2.45 d (9.8)	6″	63.3	4.27 (m)
					4.39 dd (7.7, 11.9)
18	14.1	0.65 (s)	Xyl		
19	12.2	0.82 (s)	1	97.1	5.16 (m)
20	103.7		2	68.2	4.88 (m)
21	11.6	1.57 (s)	3	67.9	5.02-5.04 (m)
22	151.7		4	67.1	4.77-4.78 (m)
23	23.2	2.08 (m)	5	58.8	3.55 dd
					4.12 (m), (4.2, 12)
24	30.8	1.25 (m)	Glc III		
		1.52 (m)	1‴	100.99	4.48 d (8.4)
25	32.7	1.73-1.75 (m)	2‴	71.3	5.01 (m)
26	75.1	3.31 dd (6.3, 9.1)	3‴	72.9	5.22 app t (9.1)
		3.71-3.72 (m)	4‴	68.5	5.1 app t (9.1)
27	16.5	0.90 d (6.3)	5‴	71.7	3.70-3.71 (m)
			6‴	62.0	4.15 (m), 4.28 (m)

4.5. Mass spectral analysis of uttroside B (compound 1):

The HR-ESI-MS of uttroside B in (CH_3CN:H_2O) mobile phase using Q-TOF negative mode analyzer showed $(M-H)^-$ ion at *m/z* 1213.61450 (Figure 4.5A) which indicates a molecular formula of $C_{56}H_{93}O_{28}$. Surprisingly, uttroside B in ($MeOH$:H_2O) mobile phase while using orbitrap analyzer showed $(M+Na+OMe)^+$ ion at *m/z* 1251.59829 (Figure 4.5B) which indicates a molecular formula $C_{57}H_{96}O_{28}Na$; negative mode $(M+MeOH-H)^-$ ion at *m/z* 1227.60154 (Figure 4.5C) indicates a molecular formula of $C_{57}H_{95}O_{28}$. The loss of C-22 attached hydroxyl group resulting in psedomolecular ion at *m/z* 1197.5490 $(M-H_2O+H)^+$ was observed in Orbitrap and Q-TOF analyzers (Figure 4.5D).

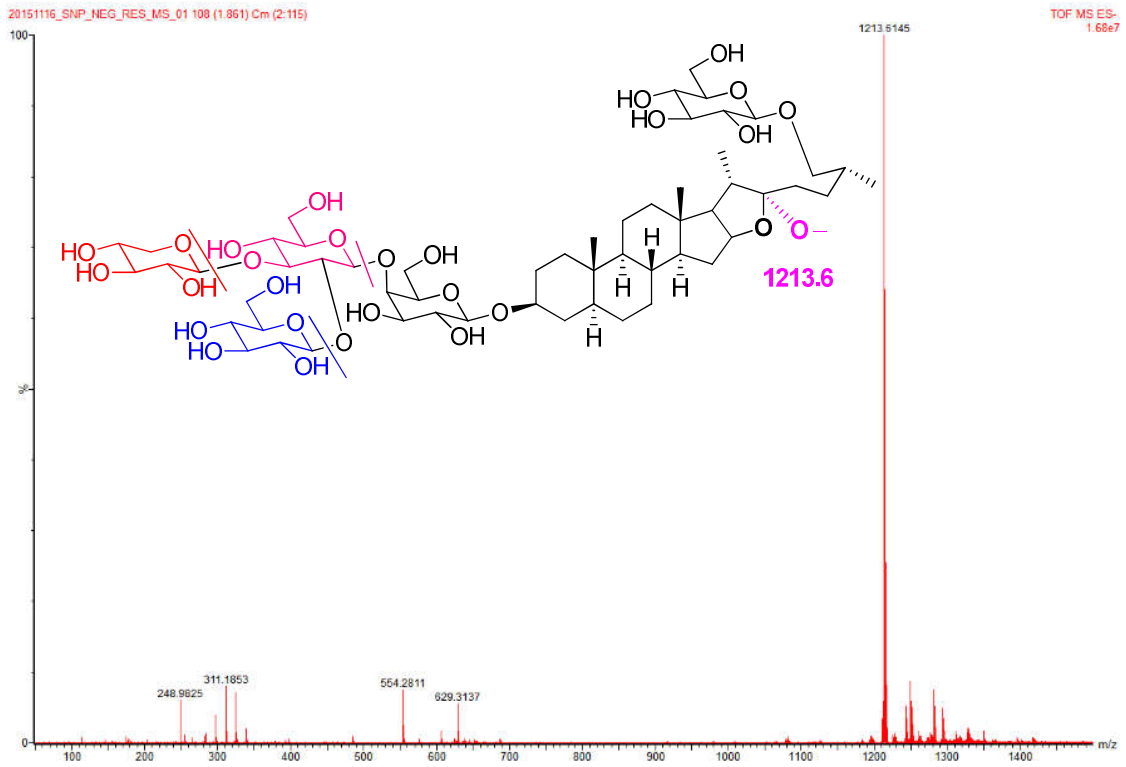


Figure 4.5A: HR-ESI-MS analysis [Q-TOF (CH_3CN/H_2O)] of uttroside B in negative mode

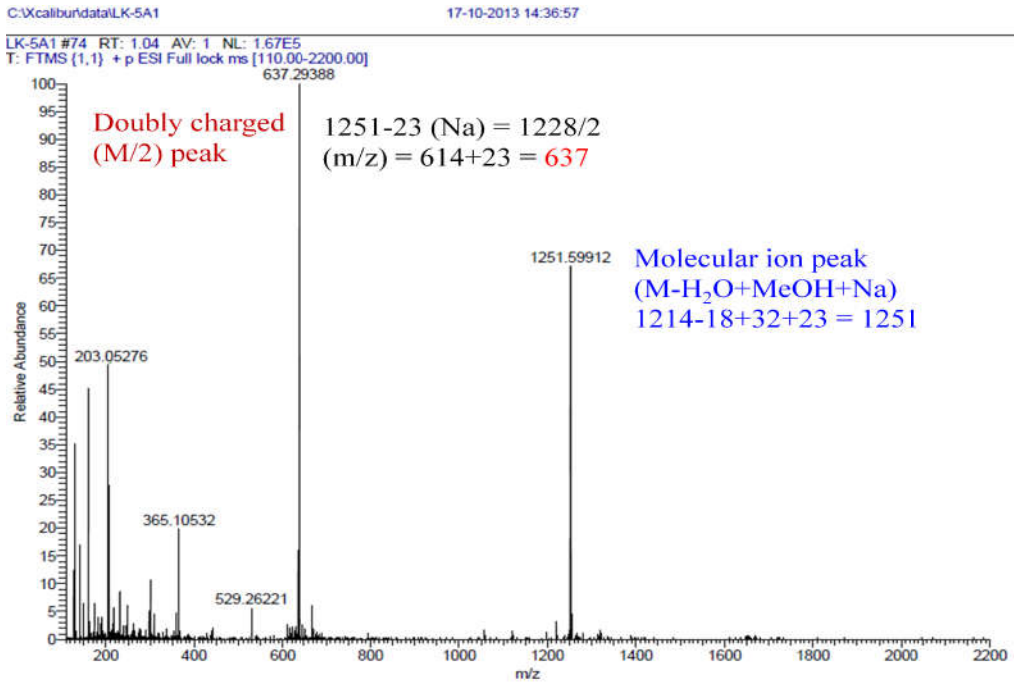


Figure 4.5B: HR-ESI-MS analysis [Orbitrap (MeOH/H_2O)] of uttroside B in positive mode

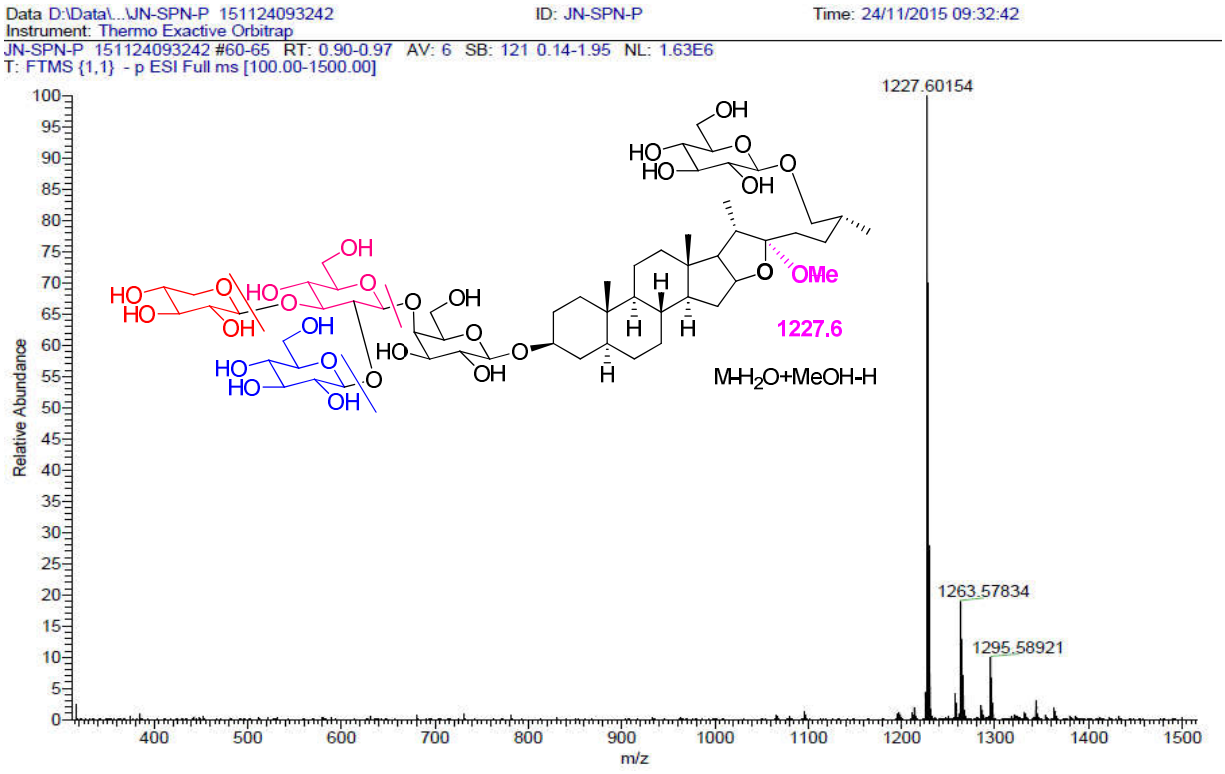


Figure 4.5C: HR-ESI-MS analysis [Orbitrap (MeOH/H_2O)] of uttroside B in negative mode

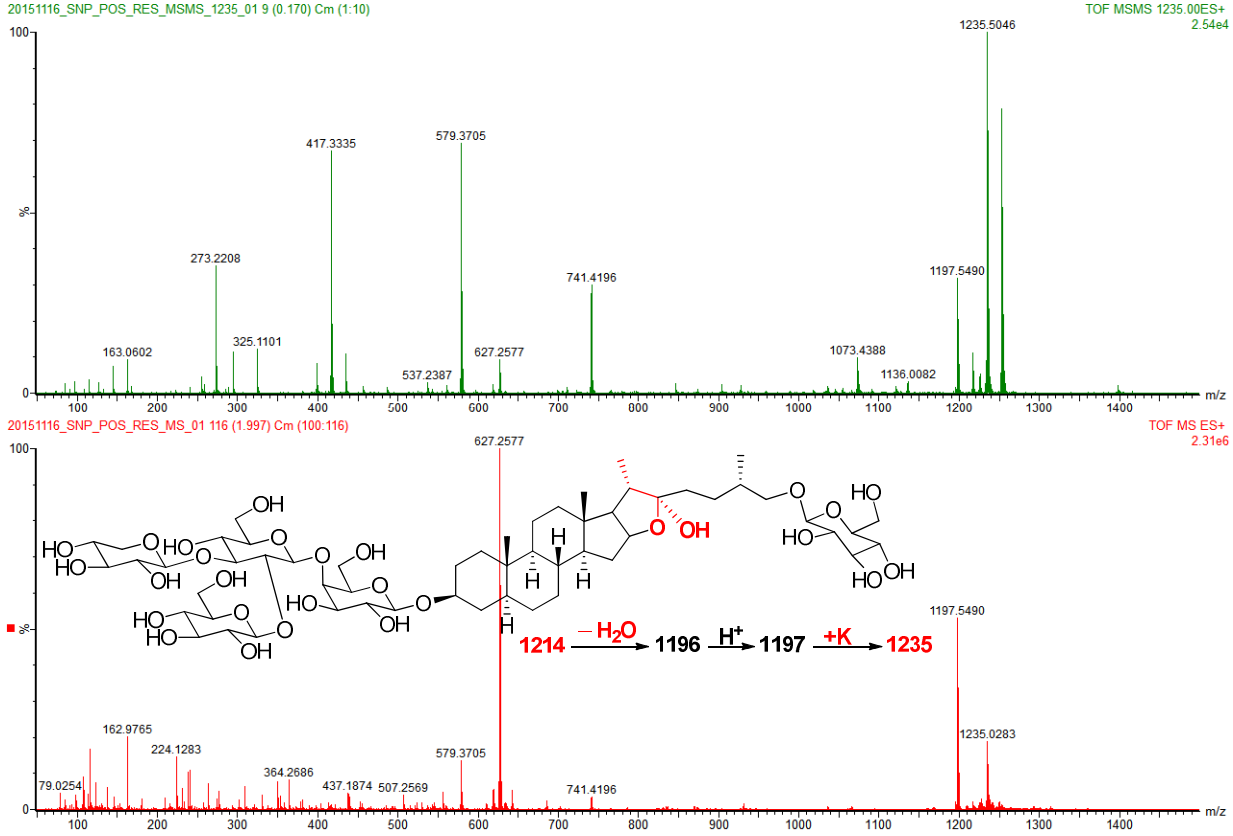


Figure 4.5D: HR-ESI-MS analysis [Q-TOF (CH_3CN/H_2O)] of uttroside B in positive mode

4.5.1. MS-MS analysis of uttroside B:

One of the most common practice in structural determination of oligosaccharides is using MS-MS analysis.[15] MS-MS negative mode (Figure 4.5.1A) analysis showed fragmented ions at *m/z* 1213.6145 (M-H), 1081.5756 (M-xyl-H), 919.5200 (M-glc I-H), 757.4646 (M-glc II-H). Positive mode (Figure 4.5.1B) analysis showed fragmented ions at *m/z* 1235.5046 (M-H_2O+K), 1197.5490 (M-H_2O+H), 1073.4675 (M-H_2O-glcIII+K), 741.4196 (M-H_2O-glcI-xyl+H), 579.3705 (M-H_2O-glc II+H), 417.3335 (M-H_2O-gal+H), 163.0658 (M-furostanol+H).

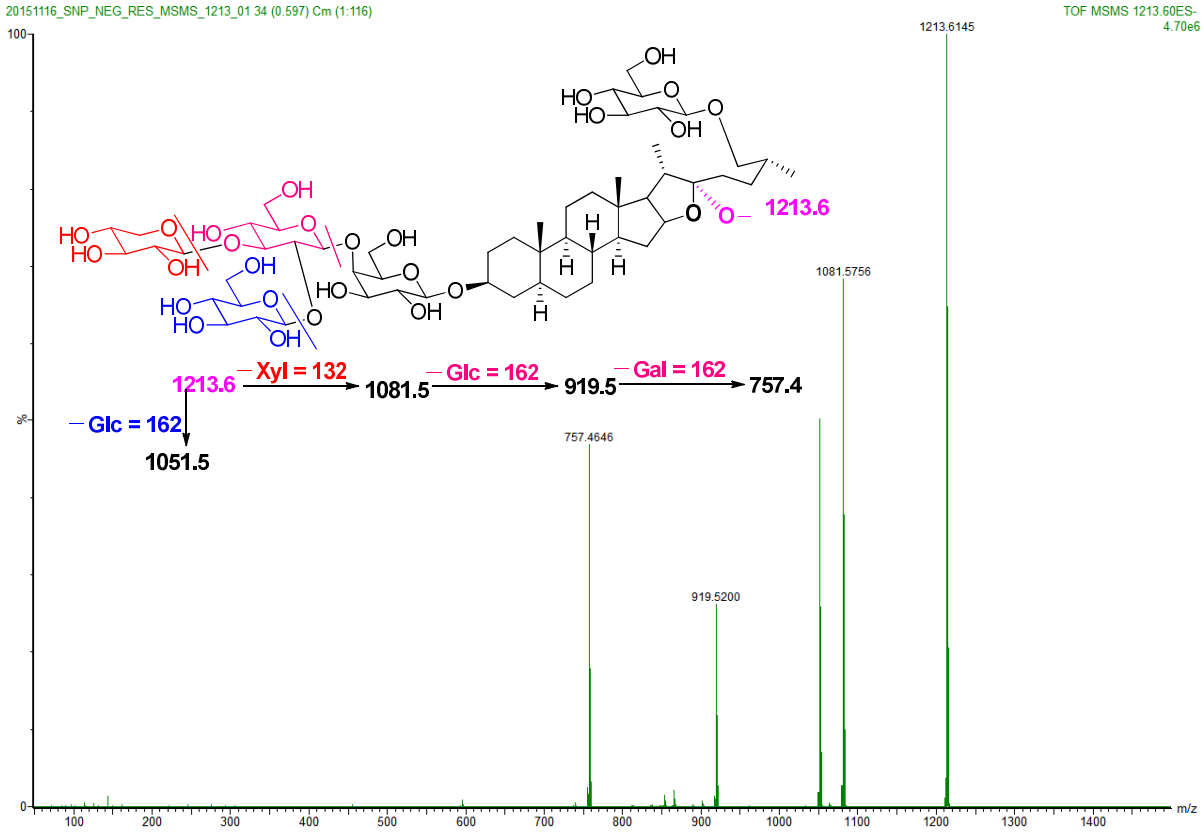


Figure 4.5.1A: MS-MS analysis of uttroside B in negative mode

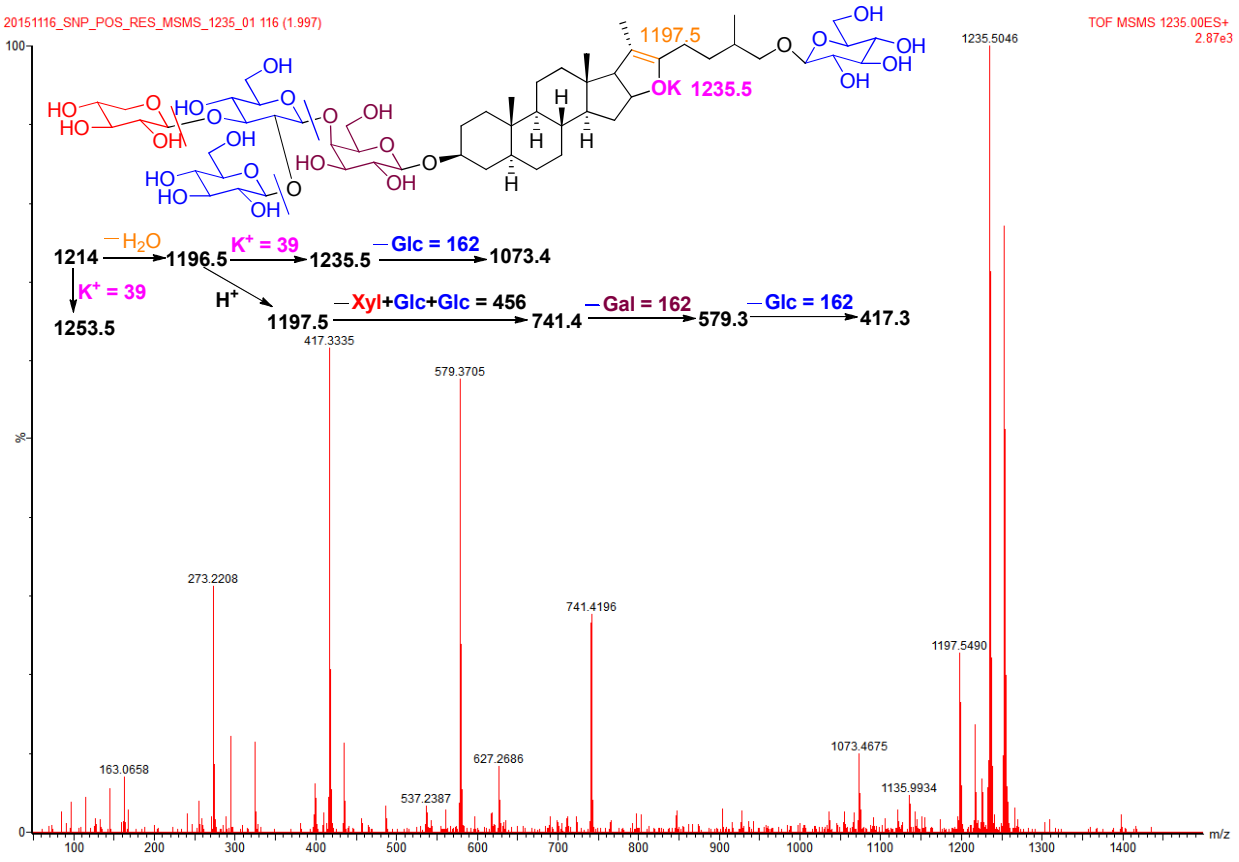


Figure 4.5.1B: MS-MS analysis of uttroside B in positive mode

4.6. Conclusion:

We have successfully isolated a steroidal saponin from the methanolic extract of the leaves of *Solanum nigrum*. The steroidal saponin exhibited excellent anti-cancer activity against liver cancer (HepG2 cell lines) both *in vivo* and *in vitro*. Derivatization of the saponin by acetylation was employed for complete characterization of uttroside B. The ^{1}H-NMR of this saponin presented a complex pattern of signals in CD_3OD and pyridine-d5. To reduce the complexity in the spectra, the saponin was derivatized to peracetylated saponin which resulted in a well resolved ^{1}H-NMR spectra in 700 MHz NMR which followed by sequential 1D, 2D NMR, HRESIMS and MS-MS analysis helped in an unambiguous structure elucidation of uttroside B for the first time from *S. nigrum*.

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List of Publications

1. An unusual synthesis of 2-pyridone and 3,5-dihydroxypyridine from a carbohydrate **Jaggaiah N. Gorantla**, Divya Kovval, Ravi S. Lankalapalli*. *Tetrahedron Lett*. **2013**, *54*, 3230–3232.
2. Isolation and antifungal properties of cyclo (D-Tyr- L-Leu) diketopiperazine isolated from Bacillus sp. associated with rhabditid entomopathogenic nematode. Nishanth Kumar, **Jaggaiah N. Gorantla**, C. Mohandas, Bala Nambisan, Ravi S. Lankalapalli. *Nat. Prod. Res* .**2013***, 27*, 2168–2172.
3. Purification and characterization of antifungal phenazines from a fluorescent *Pseudomonas* strain FPO4 against medically important fungi. **Jaggaiah N. Gorantla**, Nishanth Kumar S, Nisha, G.V., Sudaresan A., Sree Kumar M.M., Ravi S. Lankalapalli, Sumandu A.S., Dileep C., Dileep Kumar B.S. *J. Mycol. Med.* **2014**, *24*, 185–192.
4. Total synthesis of β-*C*-galactosyl ceramide and its new aza variant via Horner-Wadsworth-Emmons reaction. **Jaggaiah N. Gorantla**, Ravi S. Lankalapalli*. *J. Org. Chem.* **2014**, *79*, 5193–5200.
5. Cytotoxicity studies of semi-synthetic derivatives of theveside derived from the aqueous extract of leaves of 'suicide tree' Cerbera odollam. **Jaggaiah N. Gorantla**, Jamsheena Vellekkatt, Lekshmi R. Nath, Ruby John Anto, Ravi S. Lankalapalli*. *Nat. Prod. Res.* **2014**, *28*, 1507–1512.
6. A novel 2-alkoxy-3,5-dihydroxypyridine mediated regulation of gelatinases. Nambiar, Jyotsna, Kumar, Geetah B, Sanjana, S. R, **Jaggaiah N. Gorantla,** Ravi S. Lankalapalli, Nair, bipin G. *Intn. J. Pharm. Bio Sci.* **2015**, *6*, 1435–1444.

7. Isolation and characterization of flavonoids from the DCM extract of *Chromolaena odorata* and evaluation of the anticancer potential of the most active compound, Kaempferide, in HeLa cells. Lekshmi R. Nath,[δ] **Jaggaiah N. Gorantla**,[δ] Sophia Margaret Joseph, Jayesh Antony, Ravi S. Lankalapalli*, Ruby John Anto*. *RSC. Adv*. **2015**, *5*, 100912–100922. **δ – Contributed equally**

8. Design and synthesis of a novel glycosphingolipid derived from polyhydroxy 2-pyrrolidinone and phytoceramide appended by a 1,2,3-triazole linker. **Jaggaiah N. Gorantla**, Akkarammal Faseela, Ravi S. Lankalapalli*. *Chem. Phys. Lipids*. **2016**, *194*, 158–164.

9. Evaluation of uttroside B, a saponin from *Solanum nigrum* Linn, as a promising chemotherapeutic agent against hepatocellular carcinoma. Lekshmi R. Nath[δ], **Jaggaiah N. Gorantla[δ],** Arunkumar T. Thulasidasan, Vinod Vijayakurup, Shabna Shah, Shabna Anwer, Sophia M. Joseph, Jayesh Antony, Kollery Suresh Veena, Sankar Sundaram, Udaya K. Marelli, Ravi S. Lankalapalli,* Ruby John Anto.* *Sci. Rep.* **2016,** *6,* 36318.

δ – Contributed equally

Conference/Posters/Oral Presentations

1. Unusual synthesis of 2-pyridone and 3,5-dihydroxypyridine from a carbohydrate. **Best paper award -- Oral presentation** in 26th Kerala Science Congress (KSC), Wayanad, Kerala, January, 28 to 31, **2013**. **Jaggaiah N. Gorantla,** Ravi S. Lankalapalli.
2. A general strategy towards synthesis of *β-C*-glycosides via Horner-Wadsworth-Emmons (HWE) reaction and its application towards total synthesis of *β-C*-Galactosylceramide. **Poster presentation** in 27th International Carbohydrate Symposium (ICS), organised by IISC-Banglore, January 12 to 17, **2014**. **Jaggaiah N. Gorantla,** Ravi S. Lankalapalli.
3. Total Synthesis of an unprecedented aza-variant of C-glycoside of KRN-7000. **Oral Presentation** in 1st National symposium on Transcending Frontiers in Organic Chemistry (TFOC), organized by CSIR-NIIST, Trivandrum, Kerala, October 09-11, **2014**. **Jaggaiah N. Gorantla,** Ravi S. Lankalapalli.
4. Design and synthesis of a novel polyhydroxy 2-pyrrolidinone phytoceramides appended via a 1, 2, 3-triazole linker. **Poster presentation** in 10th Mid-CRSI, organised by NIT-Trichy, July 23-25, **2015**. **Jaggaiah N. Gorantla,** Ravi S. Lankalapalli.