Synthetic Approaches and Biological Functions of *N*-sulfated Heparan Sulfate Oligosaccharides and Proteoglycan Mimetics

विद्या वाचस्पति की
उपाधि की अपेक्षाओं की आंशिक पूर्ति में प्रस्तुत शोध प्रबंधक

A Thesis Submitted in partial fulfillment of the requirements of the degree Doctor of Philosophy

द्वारा / By

रायगवळी राकेश भास्कर / Raigawali Rakesh Bhaskar

ID- 20183593

शोध प्रबंधक पर्यवेक्षक / Thesis Supervisor

Prof. Raghavendra Kikkeri

प्रोफेसर राघवेंद्र किक्केरी



Indian Institute of Science Education and Research, Pune

2024

I would like to dedicate my thesis to my beloved family

CERTIFICATE

Certified that the work incorporated in this thesis entitled **"*Synthetic Approaches and Biological Functions of N-sulfated Heparan Sulfate Oligosaccharides and Proteoglycan Mimetics*"** submitted by **Raigawali Rakesh Bhaskar** was carried out by candidate under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.



Date: 06/01/2025

Supervisor
Prof. Raghavendra Kikkeri
Professor
IISER, Pune



Date: 06/01/2025

Co-Supervisor
Prof. H. N. Gopi
Professor
IISER, Pune

DECLARATION

Name of Student: **Raigawali Rakesh Bhaskar**

Reg. No.: **20183593**

Thesis Supervisor(s): **Prof. Raghavendra Kikkeri**

Department: **Chemistry**

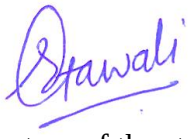
Date of joining program: **1st August 2018**

Date of Pre-Synopsis Seminar: **9th August 2024**

Title of Thesis: **Synthetic Approaches and Biological Functions of N-sulfated Heparan Sulfate Oligosaccharides and Proteoglycan Mimetics.**

I declare that this written submission represents my idea in my own words and where others' ideas have been included; I have adequately cited and referenced the original sources. I declare that I have acknowledged collaborative work and discussions wherever such work has been included. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

The work reported in this thesis is the original work done by me under the guidance of **Prof. Raghavendra Kikkeri**



Date: 06/01/2025 Signature of the student

Acknowledgements

I would like to extend my heartfelt gratitude to my supervisor, Dr. Raghavendra Kikkeri. This thesis is as much his as it is mine. His unwavering dedication, relentless efforts, and boundless patience have been the cornerstone of my journey throughout my Ph.D. tenure. I could not have asked for a more supportive and inspiring mentor. His "never give up" attitude kept me moving forward, even during the most challenging and stressful times. His passion for research and creative thinking has been a constant source of motivation, encouraging me to work not only harder but also smarter. His enduring faith in me, even in moments of failure, has helped me grow into a more confident and resilient individual.

I would like to thank my Co-Supervisor Prof. H.N. Gopi for his valuable support during my PhD.

I am deeply thankful to my collaborators, Dr. Vered, from Tel Aviv University, Israel, for microarray experiments and Dr. Boopathy Gnanaprakasam for the Continuous flow Project. I also extend my gratitude to my RAC members, Dr. Boopathy Gnanaprakasam from IISER and Dr. Ravindar Kontham from NCL Pune, for their insightful guidance and suggestions.

I am immensely grateful to IISER Pune for providing financial support and an excellent research environment. My sincere thanks to Prof. Sunil S. Bhagwat, Director of IISER Pune; Prof. Jayant Udgaonkar, former Director of IISER Pune; and Prof. Nirmalya S. Ballav, Chair of Chemistry and Prof. H.N. Gopi, former Chair of Chemistry, for their leadership and for fostering a world-class research infrastructure. A special thanks to the chemistry technical team and instrument operators, particularly Dr. Sandeep Kanade (mass spectrometry), Dr. Sandeep Mishra (NMR), Mr. Nitin Dalavi (NMR), Mr. Karan (NMR), and Mr. Mahesh Jadhav whose expertise made the PhD process much smoother.

To my lab mates, whom I now consider family, thank you for your unwavering support throughout this journey. I owe a special thanks to my senior, Chethan, for teaching me the foundations of chemistry the right way and for his hard work that paved the path for the rest of us. My gratitude also goes to Preeti Ma'am (Lunchmate) for mentoring me in and being a guiding light. I am also grateful to Bala anna for his helpful nature in any need. I am thankful to Sandhya for her incredible support. My deepest thanks to my lab mates Saurabh, Virendra (Chaimate), Ankita, Deepak and Haritha for making the lab a vibrant and joyful place. I am equally grateful to the lab alumni—Prashant, Suraj, Vijendra, and Trimbak dada for their encouragement during my early days.

I am thankful to Kamesh and all my batchmates (Dec. 2018), for unforgettable memories and Ganesh Dada for guidance.

I wish to acknowledge my family especially my Parents for their patience and constant support.

Last but not least, I am heartily grateful to my wife Manali for her immense patience and her constant support and encouragement throughout my PhD journey.

Above all, I am deeply grateful to God Almighty for the blessings and strength that have guided me every step of the way.

Rakesh Raigawali

Contents

Abbreviations ..... ***ix***

Abstract ..... ***xiv***

Publications ..... ***xvi***

Chapter – 1 ..... ***18***

Introduction ***18***

1.1 Abstract 19

1.2 Introduction 20

1.3 Synthesis of Heparan Sulfate oligosaccharides 21

1.4 Universal building blocks 25

1.5 Solid-phase synthesis 28

1.6 Heparan Sulfate Binding Proteins (HSBPs) 30

1.7 Conclusions 32

1.8 References 33

Chapter – 2 ..... ***41***

Synthesis of Heparan Sulfate Disaccharides and its Microarray Evaluation with Growth Factors and Chemokines ***41***

2.1. Abstract 42

2.2. Introduction 43

2.3. Results and Discussion 44

2.3.1. Synthesis of L-iduronic acid based HS disaccharides. 44

2.3.2 Synthesis of *N*-sulfated D-glucuronic acid based HS disaccharides. 44

2.3.4. Synthesis of *N*-acetate D-glucuronic acid based HS disaccharides. 46

2.3.5. Microarray analysis. 47

2.4. Conclusion 48

2.5 Experimental Section 49

2.5.1 General Information 49

2.6. Referances 66

2.7. NMR Spectras 70

Chapter – 3 ..... ***98***

Probing Fondaparinux Activity in the Blood Microenvironment Using ^{19}F-NMR Tag ***98***

3.1. Abstract: 99

3.2. Introduction 100

3.3. Results and discussion. 101

3.3.1 Synthesis of monosaccharide building blocks. 101

3.3.2. Synthesis of GluN-Glu disaccharide. 105

3.3.3. Synthesis of Trisaccharide acceptor. 105
3.3.4. Synthesis of fondaparinux Pentasaccharide. 107
3.3.5 ^{19}F-NMR studies. 109
3.3.6. Blood coagulation assay. 109
3.3.7. ^{19}F-NMR study of blood anticoagulation. 111
3.4. Conclusion. 112
3.5. Experimental Section 112
3.5.1 General Information 112
3.6. References 129
3.7. NMR Spectras 132
Chapter – 4 ..... ***157***
Continuous-Flow Accelerated Sulfation of Heparan Sulfate Intermediates ***157***
4.1 Abstract 158
4.2 Introduction 159
4.3 Results and discussion: 161
4.4 Conclusion 168
4.5 Experimental section: 169
4.5.1 Synthesis of glucosamine substrates and its sulfation 169
4.5.2 General procedure for *O*-Sulfation in batch process. 172
4.5.3 *O*-Sulfation of HS Disaccharide Intermediates 175
4.5.4 *O*-Sulfation of HS Tetrasaccharides Intermediates 177
4.5.5 Synthesis of Fully Deprotected HS Oligosaccharides 186
4.6 References 189
4.7 NMR Spectras 193
Chapter- 5 ..... ***217***
Sulfated L-Idose instead of L-Iduronic acid in Heparinoid Structures Influences Amphiphilic Glycopeptide Cell Membrane Dynamics ***217***
5.1 Abstract 218
5.2 Introduction 219
5.3 Results and Discussion 219
5.4 Conclusion 224
5.5 Experimental section 224
5.5.1 General Information 224
5.5.2 General Procedure for Conjugation of Hexasaccharide to Peptide Template 235
5.6 References 237
5.5.3 NMR Spectras and other characterisations 238

Abbreviations

A

AT	Antithrombin

B

BAIB	Bis(acetoxy)iodobenzene
BMP-2	Bone Morphogenetic Protein 2
BM	Basement Membrane

C

CAMs	Cell Adhesion Molecules
CS	Chondroitin Sulfate
CNS	Central Nervous System
CSPG	Chondroitin Sulfate Proteoglycans
CP (cp)	Cis-platin
CSA	Camphore Sulfonic Acid
Cy-5	Cyanine-5
CTCF	Corrected Total Cell Fluorescence

D

DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's Modified Eagle's Medium
DMF	Dimethylformamide
DS	Dermatan Sulfate
DIPEA	N,N-Diisopropylethylamine
DBCO	Dibenzocyclooctyne
DCC	Dicyclohexyl Carbodiimide
DiO	3,3′-Dioctadecyloxacarbocyanine Perchlorate

DOSY	Diffusion Ordered Spectroscopy
E	
ECM	Extracellular Matrix
Erk-1/2	Extracellular Signal-Regulated Protein Kinases 1 and 2
Ext1	Exostosin-1
EVs	Extracellular Vesicles
F	
FBS	Fetal Bovine Serum
FGF-1	Fibroblast Growth Factor - 1
FGF-2	Fibroblast Growth Factor - 2
FGF	Fibroblast Growth Factor
FITC	Fluorescein Isothiocyanate
Fmoc-Cl	Fluorenylmethyloxycarbonyl Chloride
G	
GAGs	Glycosaminoglycans
Gal	Galactose
GalN	Galactose Amine
GlcA	Glucuronic Acid
GFs	Growth Factors
GPI	Glycosylphosphatidylinositol
GDFs	Growth Differentiation Factor
H	
Hep	Heparin
HR-ESI-MS	High resolution Electrospray Ionisation Mass Spectrometry
HS	Heparan Sulfate
HSBPs	Heparan Sulfate Binding Proteins
HSPGs	Heparan Sulfate Proteoglycans

HIV	Human Immunodeficiency Viruses
HSV	Herpes Simplex Virus
HOBT	Hydroxybenzotriazole
HBTU	Hexafluorophosphate Benzotriazole Tetramethyl Uronium
HPLC	High-Performance Liquid Chromatography
HCMV	Human Cytomegalovirus
HPV	Human Papillomavirus
DENV	Dengue Virus

I

IdoA	Iduronic Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IR	Infra-Red

K

KS	Keratan Sulfate

M

MAPK	Mitogen-activated Protein Kinases
MS	Molecular Sieves
mESC	Mouse Embryonic Stem Cell
MsCl	Methanesulfonyl Chloride
MALDI-TOF-MS	Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

N

NAP-Br	2-(Bromomethyl)naphthalene
NHS	N-Hydroxysuccinimide
NIS	N-Iodosuccinimide
NMR	Nuclear Magnetic Resonance

NMP	N-Methylpyrrolidone
N-CAM	Neural Cell Adhesion Molecule
nM	Nano Molar
NRP1	Neuropilin-1

P

PBS	Phosphate Buffered Saline
PTSA	p-Toluenesulfonic Acid
py	Pyridine
PG	Proteoglycan
PM	Plasma Membrane
PEG	Polyethylene glycol

R

RT	Room Temperature
RMSD	Root Mean Square Deviation

T

TBDPS-Cl	tert-Butyldimethylsilyl Chloride
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy
THF	Tetrahydrofuran
TLC	Thin-layer Chromatography
TMSSPh	Phenyl Trimethylsilyl Sulphide
TGF-β	Transforming Growth Factor Beta
TFA	Trifluoroacetic acid
TMSOtf	Trimethylsilyl Trifluoromethanesulfonate
TIPS	Triisopropyl Silane

U

UV	Ultra-violet

µM	micromolar

V

VEGF	Vascular Endothelial Growth Factor
VGIC	Voltage Gated Ion Channel
V3	Third Ventricle
VZV	Varicella-Zoster Virus

Abstract

Heparan Sulfate (HS), a member of the glycosaminoglycan family, is composed of repeating units of α (1-4) linked D-glucosamine and uronic acid residues (L-iduronic acid and D-glucuronic acid) with diverse sulfation patterns. For instance, HS disaccharides can arrange themselves into 48 distinct possibilities, and this structural diversity enables binding to numerous proteins, thereby modulating a wide array of biological processes. Despite significant progress in the synthesis of structurally defined HS oligosaccharides, the importance of *N*-sulfated HS ligands remains underexplored. In my thesis, I synthesized a wide range of *N*-sulfated HS ligands and examined their structure-activity relationships.

Chapter 1 outlines the divergent approach employed for synthesizing HS oligosaccharides. It includes a comprehensive review of the synthesis of disaccharide building blocks with various orthogonal protecting groups, promoters, and optimized conditions to improve glycosylation yields. It also discusses the synthesis of universal disaccharide building blocks, one-pot synthesis, and automated solid-phase synthesis of HS oligosaccharides. Finally, the chapter highlights the current understanding of the structure-activity relationships of heparan sulfate-binding proteins (HSBPs).

Chapter 2 focuses on the synthesis of *N*-sulfated HS disaccharides using a divergent strategy and their subsequent characterization. Employing a panel of 14 disaccharides, we conducted high-throughput microarray analyses involving 9 growth factors and 14 chemokines. Our findings revealed that highly sulfated *N*-sulfated L-iduronic acid ligands exhibit a strong tendency to bind a broad range of growth factors compared to their D-glucuronic acid counterparts. Furthermore, we observed that 3-*O*-sulfation of HS disaccharides plays a pivotal role in fine-tuning the binding interactions with several growth factors and chemokines.

Chapter 3 describes the synthesis of ^{19}F-NMR-sensitive fondaparinux to monitor blood-fondaparinux interactions. To achieve this, trifluoromethyl phenyl glycosylated fondaparinux was synthesized using a [2+3] glycosylation strategy. The ^{19}F-NMR analysis of the conjugate exhibited a strong T_1 value and detectable ^{19}F-NMR signals at micromoles concentrations. When treated with blood, a 0.2 ppm shift in the NMR signal of fondaparinux was observed. Further *in vivo* activity studies and ^{19}F-NMR analysis of the blood microenvironment behavior of the complex are currently underway.

Chapter 4 outlines a continuous flow strategy for the insertion of sulfation groups onto HS disaccharides and tetrasaccharides. Using flow chemistry, conditions critical for 6-*O*-sulphation and 3-*O*-sulphation with SO_3.salts complex at varying temperatures were optimized. These optimied conditions were then employed to synthesie a broad range of sulfation patterns on HS disaccharides and tetrasaccharides. The results demonstrated that flow chemistry is an efficient approach to sulfate HS in a short timeframe with high yields.

Chapter 5 details the synthesis of HS hexasaccharides containing L-iduronic acid and L-idose. When these two hexasaccharides were functionalized on a proteoglycan backbone and expressed on cancer cell surfaces, the L-idose-based hexasaccharide underwent endocytosis significantly faster than the L-iduronic acid-based variant. This highlights the importance of L-iduronic acid in maintaining HS oligosaccharides on the cell membrane. Furthermore, L-idose-based proteoglycan mimetics were observed to localize in the nuclear region after 1–2 hours, suggesting their potential as markers for targeting the nucleus of cells using proteoglycan mimetics.

Publications

1. **Rakesh Raigawali**, Saurabh Anand, Ankita Chandra, Preeti Ravindra Bhoge, Jancy Nixon Abraham, and Raghavendra Kikkeri "Comparative Analysis of Sulfated L-Idose and L-Iduronic Acid in Neoproteoglycan Cell Surface Engineering" (*Manuscript submitted*)
2. **Rakesh Raigawali**, Sharath S. Vishweshwara, Saurabh Anand and Raghavendra Kikkeri "Synthesis of sulfated carbohydrates – glycosaminoglycans" (Book Chapter)
3. Saurabh Anand, Sandhya Mardhekar, **Rakesh Raigawali***, Nirmala Mohanta, Prashant Jain, Chethan D. Shanthamurthy, Boopathy Gnanaprakasam, and Raghavendra Kikkeri "Continuous-Flow Accelerated Sulfation of Heparan Sulfate Intermediates" *Org. Lett*, **2020**, 22, 9, 3402-3406. (* *equal contribution*)
4. Preeti Ravindra Bhoge, **Rakesh Raigawali**, Sandhya Mardhekar, Saurabh Anand**,** and Raghavendra Kikkeri "Synergestic interplay of uronic acid and sulfation composition of heparan sulfate on molecular recognition to activity" *Carbohydr. Res*, **2023,** 532, 108919.
5. Saurabh Anand**,** Preeti Ravindra Bhoge, **Rakesh Raigawali**, Srinivas Vinod Saladi and Raghavendra Kikkeri "NeoMProbe: a new class of fluorescent cellular and tissue membrane probe" *Chem. Sci.*, **2024**, 15, 19962-19969.
6. Sharath S. Vishweshwara, Preeti Ravindra Bhoge, Saurabh Anand, **Rakesh Raigawali**, Ankita Chandra Srinivas Vinod Saladi and Raghavendra Kikkeri "Immunogenic Sulfated L-Idose Homo Oligosaccharides Elicit Neutralizing Antibody Against Native Heparan Sulfate with Biomarker and Therapeutic Application" *J. Med. Chem*, **2024**, 67, 20, 18465-18477.
7. Ariel Shitrit, Sandhya Mardhekar, Israel Alshanski, Prashant Jain, **Rakesh Raigawali**, Chethan D. Shanthamurthy, Raghavendra Kikkeri, Shlomo Yitzchaik, and Mattan Hurevich "Profiling Heparan Sulfate-Heavy Metal Ions Interaction Using Electrochemical Techniques". *Chem. Eur. J.*, **2022**, 28, e202202193.
8. Pragati Kishore Prasad, Suraj Toraskar, Suman Khan, Tom Granot, Yael Fridmann Sirkis, Eliane Hadas Yardeni, Shira Albeck, Tamar Unger, Ekaterina Petrovich-Kopitman, Yoseph Addadi, **Rakesh Raigawali**, Saurabh Anand, Sharath, S. Vishweshwara, Chethan D. Shanthamurthy, Raghavendra Kikkeri, Ori Avinoam, Leila Motiei, and David Margulies "Turn-On Fluorescence Sensing of the ACE2–

SARS-CoV-2 RBD Interaction Using a Low-Background, His-Tag Binding Probe". (*Manuscript submitted*)

Chapter – 1

Introduction

1.1 Abstract

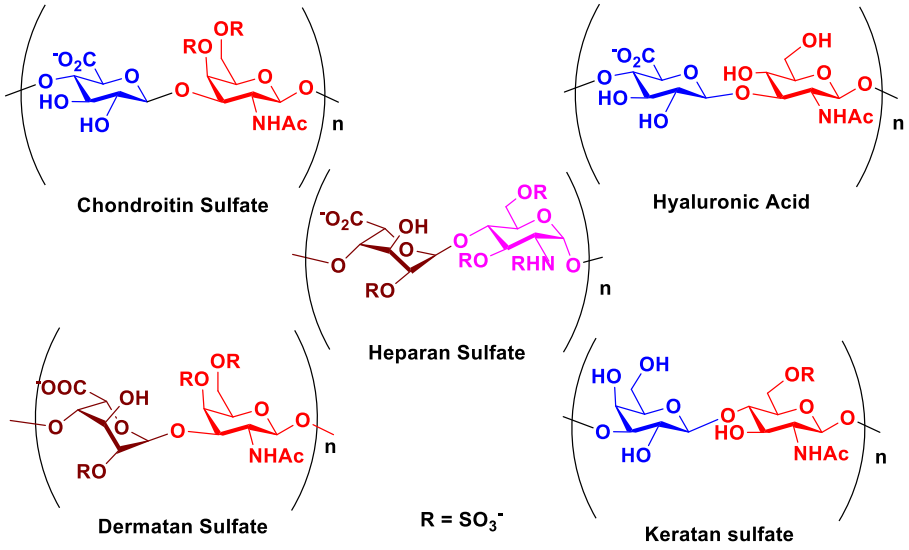
Glycosaminoglycans (GAGs) are polysaccharides that are universally present on the surface of all mammalian cells, where they engage with numerous proteins and play critical roles in both physiological and pathological processes. Among these, heparan sulfate (HS) is particularly notable for its complex structure, making it a key cell-surface molecule involved in regulating a broad spectrum of cellular functions. As a result, exploring the structure-activity relationships (SARs) using well-defined HS ligands has become a promising direction for drug discovery and biosensor development. This chapter presents a modular, divergent approach for synthesizing HS oligosaccharides to facilitate SAR studies. It includes a comprehensive review of existing literature on the synthesis of disaccharide building blocks, focusing on the use of orthogonal protecting groups, promoters, and optimized conditions to enhance their applicability for subsequent oligosaccharide assembly. Additionally, it discusses the creation of universal disaccharide building blocks derived from natural polysaccharides and provides insights into methods such as one-pot synthesis and automated solid-phase synthesis of HS oligosaccharides. Finally, the chapter examines the current understanding of SARs for key heparan sulfate-binding proteins (HSBPs).

1.2 Introduction

Mammalian cell surfaces are adorned with a diverse array of carbohydrate structures, ranging from simple monosaccharides to complex polysaccharides. Among these, glycosaminoglycans (GAGs) stand out as intricate polyanionic polysaccharides, characterized by repeating hexosamine-uronic acid disaccharide units.[1] GAGs are found abundantly on cell surfaces and within extracellular matrices, with each tissue exhibiting a distinct GAG composition that mediates interactions with various proteins.[2] These molecules play pivotal roles in physiological and pathological processes by serving as reservoirs for proteins, protecting them from proteolytic degradation, enhancing local protein concentrations, promoting interactions with receptors and other proteins, and occasionally acting as receptors themselves.[3-4] Understanding the structure-activity relationships (SARs) of GAGs holds great potential for driving innovations in biosensor technologies and the development of next-generation therapeutics.

Glycosaminoglycans (GAGs) are classified into four main groups based on their structural characteristics: hyaluronan or hyaluronic acid (HA), chondroitin sulfate (CS) and dermatan sulfate (DS), heparin and heparan sulfate (HS), and keratan sulfate (KS) (Scheme 1).[5-6] These polysaccharides can be further divided into sulfated and non-sulfated forms. HA, a non-sulfated GAG, consists of N-acetyl β-D-glucosamine (β-GlcNAc) and β-D-glucuronic acid (β-GlcA). In contrast, sulfated GAGs, such as CS and DS (composed of N-acetyl β-D-galactosamine, β-GalNAc), as well as heparin and HS (containing α-GlcNAc as the hexosamine unit), undergo extensive tissue-specific modifications that contribute to their structural diversity. For instance, DS, heparin, and HS chains are modified by converting β-GlcA to α-L-iduronic acid (α-IdoA) through the action of C5-epimerases.[7] Sulfation patterns vary among GAGs: in CS and DS, sulfation can occur at the O2 position of the uronic acid and the O4 and O6 positions of β-GalNAc, while in heparin and HS, sulfation may occur at the O2 position of the uronic acid and the O3 and O6 positions of the glucosamine. Additionally, glucosamine units in heparin and HS can be *N*-acetylated or *N*-sulfated, although the presence of free amine groups (*N*-unsubstituted) is uncommon.[6,8] Unlike other GAGs, KS lacks a uronic acid and is composed of β-GlcNAc and β-Gal units, with sulfation and fucosylation occurring at specific sites. The inherent complexity and heterogeneity of GAG structures make the chemical synthesis of pure GAGs with defined chain lengths particularly challenging. Advances in chemoenzymatic and chemical approaches, including automated synthesis techniques, are providing new opportunities to obtain specific GAG

structures for research.[9] This chapter focuses on summarizing the strategies used for chemical and automated synthesis of GAGs, with a special emphasis on the synthesis of heparan sulfate oligosaccharides.



Scheme 1. Structures of GAG disaccharides.

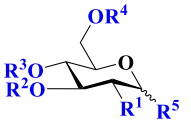
1.3 Synthesis of Heparan Sulfate oligosaccharides

A. Solution-phase synthesis

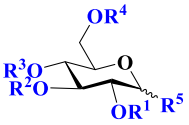
Over the last two decades, significant progress has been made in the synthesis of heparan sulfate (HS) oligosaccharides.[9,10] However, the chemical synthesis of structurally precise HS ligands remains a considerable challenge due to their extensive structural complexity. Compounding this difficulty is the lack of a readily available commercial source for L-iduronic acid and the intricate stereocontrol required for glycosylation between glucosamine and uronic acid residues.[11] These challenges have spurred chemists to explore various synthetic methodologies for generating a diverse array of HS oligosaccharides. Among these strategies, the modular convergent synthetic approach has emerged as particularly promising.[12] This method involves the synthesis of versatile disaccharide building blocks that can be easily modified into either glycosyl donors or acceptors, streamlining the construction of HS oligosaccharides.[13,23] For instance, the anomeric hydroxyl group of the glucosamine building block is protected using either a stable thiotoluene donor or a t-hexyldimethylsilyl (TDS) ether, which can be efficiently removed and converted into a donor for glycosylation.

Temporary protective groups, such as acetate (Ac), levulinoyl esters (Lev), 9-fluorenylmethyl carbonate (Fmoc), or 2-naphthylmethyl (NAP), are applied to the C4 hydroxyls of glucosamine and uronic acid to facilitate selective cleavage and subsequent glycosyl acceptor generation.

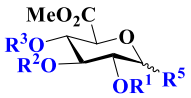
In contrast, permanent protective groups, including benzyl (Bn), tert-butyldimethylsilyl (TBS), and 2-NAP, are introduced to the C4 hydroxyl of the non-reducing end glucosamine and the C3 hydroxyl of uronic acid to prevent sulfation, with their removal reserved for the final global deprotection step. Strategic combinations of temporary protective groups, such as tert-butyldiphenylsilyl (TBDPS), 2-NAP, Lev, p-bromobenzyl (PBB), and allyl ether (Alloc), at the C6 and C3 hydroxyl positions of glucosamine enable remote and steric participation in α-glycosylations. These are followed by selective deprotection and sulfation, producing the desired sulfated HS sequences (Scheme 2). The glucosamine's amine group is masked with azide or imide groups to enhance α-stereoselective glycosylation through the anomeric effect. Similarly, the C2 hydroxyl group of uronic acid building blocks is temporarily protected with benzoyl (Bz), Lev, or 4-acetoxy-2,2-dimethylbutanoyl (PivOAc) ester groups to leverage neighboring group participation for 1,2-trans glycosylation, enabling subsequent deprotection and sulfation. A notable advancement includes the synthesis of the L-iduronic acid building block from 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose via six straightforward steps, yielding anhydrous L-Idose. This intermediate undergoes glycosylation with glucosamine, followed by anhydrous ring opening and oxidation, ultimately yielding the GlcN-IdoA disaccharide building block. The synthesis of HS oligosaccharides was then achieved through [2+2] glycosylation, demonstrating the viability of this approach for constructing defined HS structures.



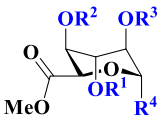
$R^1 = N_3$/ NHTCA/ NHTFA/*2*-CF_3-Ph CH=N
R^2 = 2-NAP/Lev/Bn/PBB
R^4 = TBDPS/Lev/Ac/Bn/PMB/CA
R^3 = Bn/ 2-NAP/TBS/Fmoc/CA/Lev/Alloc
R^5 = STol /OTDS/SEt/OC=NH(CCl_3)/OC=N(Ph)CF_3



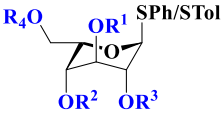
R^1 =Bz/Lev/Piv
R^2 = Bn
R^3 = Bn/ 2-NAP/TBS
R^4 = TBDPS/Lev/CA/Ac/Fmoc
R^5 = STol /OTDS//OC=NH(CCl_3)/OC=N(Ph)CF_3



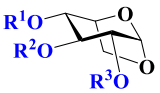
R^1 =Bz/Lev/Ac
R^2 = Bn
R^3 = Fmoc
R^5 = STol /OTDS



R^1 =Bn
R^2 = Fmoc/Lec
R^3 = Ac/Lev
R^4= STol/SPh/OC=NH(CCl_3)



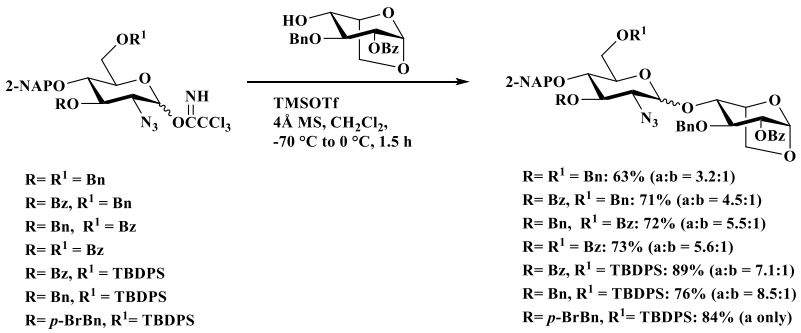
R^1 =Bn
R^2 = TBS/PMB
R^3 = Bz/Lev
R^4 = PMB/Lev/TBDPS



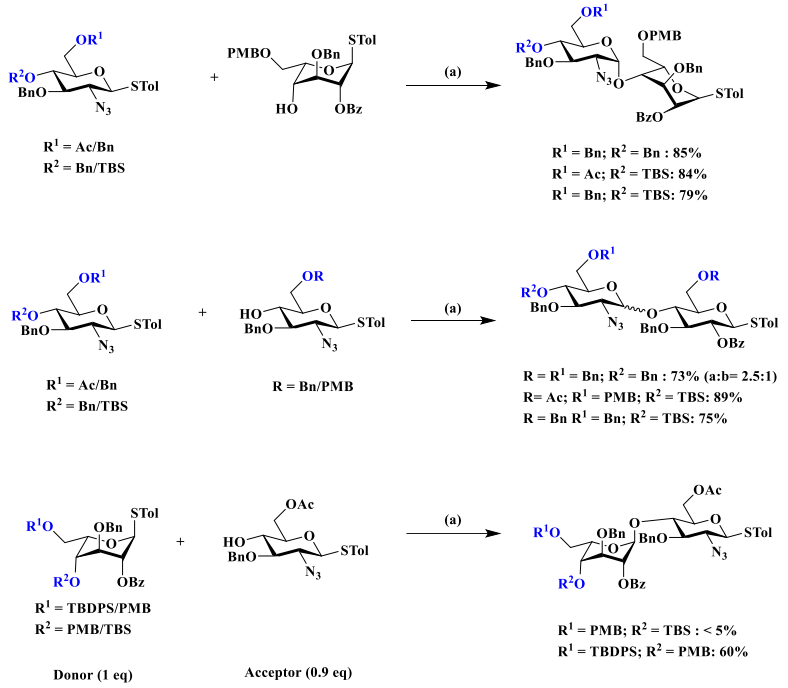
R^1 =PMB/H
R^2 = Bn
R^3 = Bz

Scheme 2. Monosaccharide building blocks used in the assemble HS oligosaccharides.

Trihaloacetimidate and thioglycosyl donors have been widely employed to improve stereoselectivity in forming the 1,2-cis α-D-glucosamine glycosidic bond, utilizing carefully selected orthogonal protecting groups and glycosylation promoters (Scheme 3).[15,24] In an alternative approach, HS disaccharides were synthesized by activating a glucosamine thiosyl donor in the presence of p-toluene sulfenyl chloride (p-TolSCl) and silver triflate (AgOTf). This method facilitated glycosylation with thiosyl idose or glucose thioglycosyl acceptors, yielding exclusively the α-anomer (Scheme 4).[25-31] Subsequent steps involved adjusting protecting groups, modifying disaccharide donor/acceptors, performing post-glycosylation oxidation, and implementing sulfation and global deprotection to achieve the desired HS oligosaccharides. While these techniques are primarily designed to produce HS oligosaccharides with uniform sulfation patterns, synthesizing oligosaccharides with heterogeneous sulfation patterns requires a different approach. To address this, two distinct disaccharide building blocks were developed. In one, the C2 position of glucosamine was protected using either a trichloroacetyl group (TCA) or ortho-trichloro benzylimine. In the other, a 2-azido-2-deoxy-D-glucose building block was used to enable selective *N*-sulfation and *N*-acetylation, allowing for the controlled introduction of varied sulfation patterns within HS oligosaccharides.[32]



Scheme 3. Synthesis of HS disaccharides in the presence of different orthogonal protections.

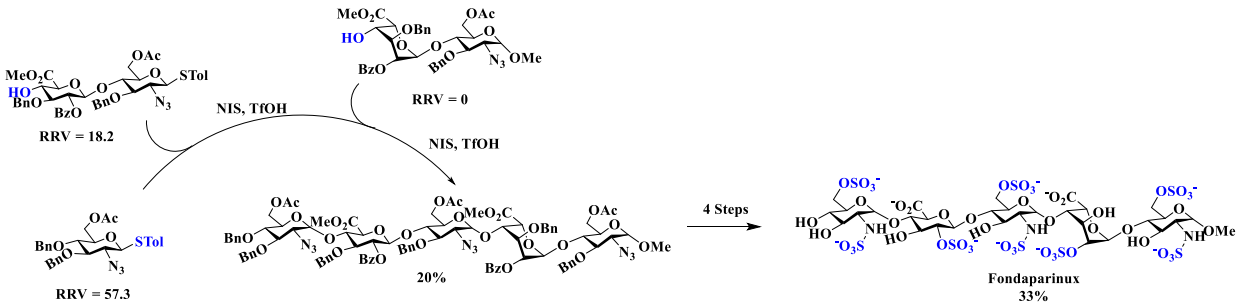


Scheme 4. Synthesis of HS disaccharides: (a) AgOTf, *p*-TolSCl, -78°C, acceptor, TTBP.

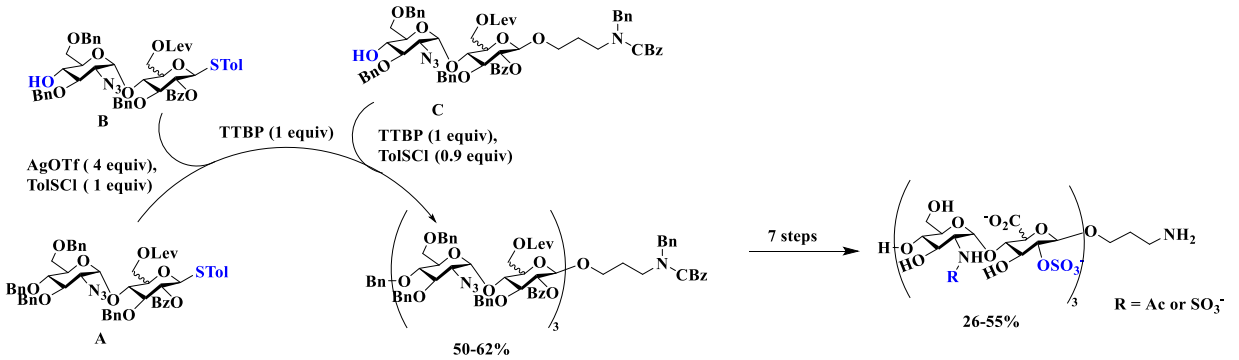
A. One-pot synthesis

The one-pot assembly of HS oligosaccharides significantly accelerates synthesis by enabling the formation of oligosaccharides within a few hours, a stark contrast to the step-by-step method, which can take several days or even weeks. This approach relies on building blocks with carefully tuned relative reactivity differences, ensuring that each glycosylation step achieves 100% efficiency to prevent the formation of side products that could complicate the synthesis. Using this method, fondaparinux was synthesized by first activating a more reactive donor in the presence of a glucuronic acid disaccharide acceptor, followed by the activation of a second disaccharide acceptor with different relative reactivity values (RRVs) (Scheme 5). The process was finalized with selective deprotection, *O*- and *N*-sulfation, and global deprotection to yield the desired pentasaccharides.[33] Although the one-pot approach is

rapid, its application becomes challenging when identical repeating units or similar RRVs are involved. To address this limitation, the preactivation strategy offers a more effective solution. In this method, a HS hexasaccharide was assembled by preactivating the non-reducing end disaccharide with TolSOTf, followed by the addition of an acceptor and 2,4,6-Tri-tert-butylpyrimidine (TTBP) to facilitate glycosylation (Scheme 6).[18] After completing the initial glycosylation, the resulting tetrasaccharide was activated and further glycosylated with the reducing end HS disaccharide unit, successfully assembling the full oligosaccharide. This preactivation strategy demonstrates its efficiency in overcoming challenges associated with similar RRVs while maintaining the rapid synthesis benefits of the one-pot approach.



Scheme 5. One-pot synthesis of fondaparinux



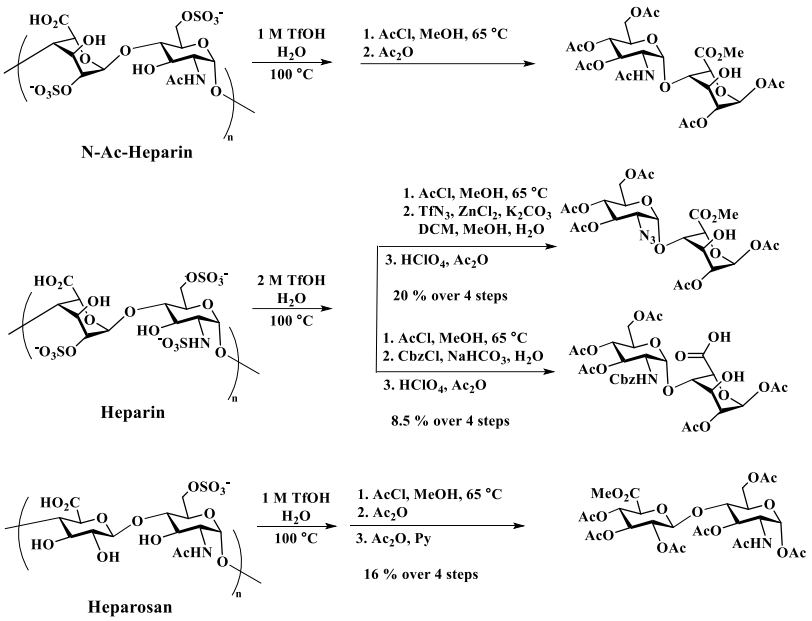
Scheme 6. One-pot synthesis of HS oligosaccharides.

1.4 Universal building blocks

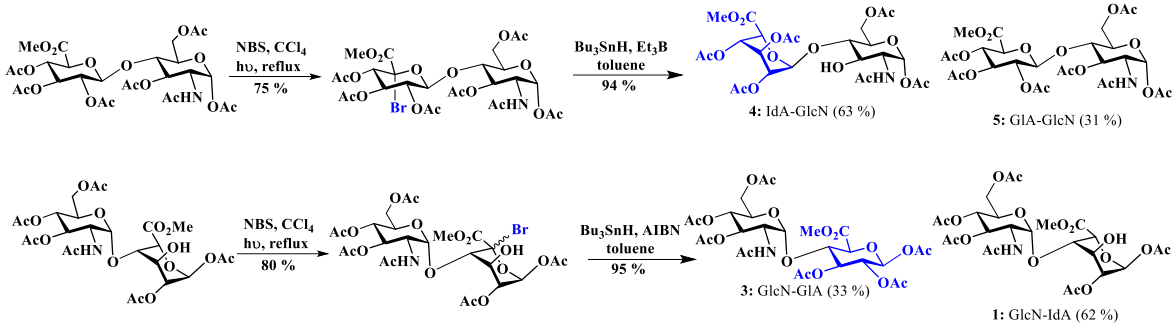
To date, all 48 combinations of HS disaccharides have been synthesised and characterised.[34-35] However, for biological investigations, a tetrasaccharide is the minimum required length of HS oligosaccharide.[36] Hence, synthesising all 2,304 combinations of HS tetrasaccharides

necessitated a novel and robust approach to synthesising disaccharide building blocks. To enhance the prospect of synthesising disaccharide building blocks on gram scales, heparin/heparan sulfate polysaccharides were hydrolysed and chemically modified to synthesise disaccharide intermediates in a minimal number of steps for the production of universal HS disaccharide ligands to construct an oligosaccharide library.[36-37] Controlled hydrolysis of *N*-acetyl heparin was carried out in the presence of 1 M TfOH at 100 °C, followed by esterification and acetylation, resulting in the GlcN–IdoA disaccharide. Similarly, the hydrolysis of sodium heparinate with triflic acid (TfOH), followed by esterification and azidation in the presence of triflic azide (TfN_3), potassium carbonate (K_2CO_3), and zinc chloride ($ZnCl_2$), and subsequent anomeric demethylation and peracetylation, yielded the $GlcN_3$–IdoA disaccharide in a 20% yield over four steps. Alternatively, the use of benzyl chloroformate introduced an *N*-Cbz protecting group in place of the *N*-acetyl group in HS disaccharide. Through the hydrolysis of heparosan and subsequent esterification and peracetylation, the GlcA–GlcN disaccharide was obtained with an overall yield of 16% over four steps (Scheme 7). Utilising these uronic acid HS disaccharide building blocks, other uronic acid-based HS disaccharides were synthesized through epimerization. Here, the GlcA-containing building block was subjected to bromination using NBS in the presence of UV light, resulting in the C-5 bromo derivatives with a yield of 75%. Subsequently, treatment with Et_3B and Bu_3SnH at 20 °C yielded the highest amount of epimerized IdoA–GlcN product (63% yield), alongside the recovery of the valuable starting material with a yield of 31%. On the other hand, the NBS-mediated bromination of GlcN-IdoA resulted in an epimeric mixture of the C-5 bromo HS disaccharides. Subsequent α-dehalogenation using AIBN and Bu_3SnH at 110°C yielded only 33% epimerization (Scheme 8). In summary, these streamlined routes offer a novel approach to synthesise core HS disaccharide modules for oligosaccharide assembly. Subsequently, utilising these disaccharide building blocks, 64 potential HS tetrasaccharides were synthesised and characterized. To date, all 48 combinations of HS disaccharides have been successfully synthesized and characterized.[34-35] However, biological studies require a minimum oligosaccharide length of a tetrasaccharide,[36] leading to the need for an efficient approach to produce all 2,304 possible combinations of HS tetrasaccharides. This ambitious goal necessitated the development of novel strategies for synthesizing disaccharide building blocks on a gram scale.

To achieve this, heparin and heparan sulfate polysaccharides were hydrolyzed and chemically modified to generate disaccharide intermediates through a minimal number of steps, enabling the production of universal HS disaccharide ligands for constructing a comprehensive oligosaccharide library.[36-37] Controlled hydrolysis of *N*-acetyl heparin in the presence of 1 M triflic acid (TfOH) at 100 °C, followed by esterification and acetylation, yielded the GlcN–IdoA disaccharide. Similarly, hydrolysis of sodium heparinate with TfOH, followed by esterification and azidation using triflic azide (TfN_3), potassium carbonate ($K2CO_3$), and zinc chloride ($ZnCl_2$), and subsequent anomeric demethylation and peracetylation, produced the $GlcN_3$–IdoA disaccharide with an overall yield of 20% across four steps. Alternatively, introducing an N-Cbz protecting group using benzyl chloroformate in place of the *N*-acetyl group in the HS disaccharide provided an additional synthetic route. Hydrolysis of heparosan, followed by esterification and peracetylation, generated the GlcA–GlcN disaccharide with an overall yield of 16% across four steps (Scheme 7). Using these uronic acid-based HS disaccharide building blocks, additional HS disaccharides were synthesized via epimerization. For example, the GlcA-containing disaccharide was subjected to bromination with NBS under UV light, yielding C-5 bromo derivatives with a 75% yield. Subsequent treatment with Et3B and Bu_3SnH at 20 °C produced the IdoA–GlcN epimer in a 63% yield, along with 31% recovery of the starting material. In contrast, NBS-mediated bromination of GlcN–IdoA resulted in an epimeric mixture of C-5 bromo HS disaccharides. Dehalogenation using AIBN and Bu_3SnH at 110 °C yielded a modest 33% epimerization (Scheme 8). In summary, these streamlined methods provide an innovative approach for synthesizing core HS disaccharide building blocks. These modules were subsequently utilized to synthesize and characterize 64 potential HS tetrasaccharides, showcasing the efficiency and scalability of these synthetic strategies.



Scheme 7. Synthesis of HS disaccharide building blocks from natural polysaccharides.

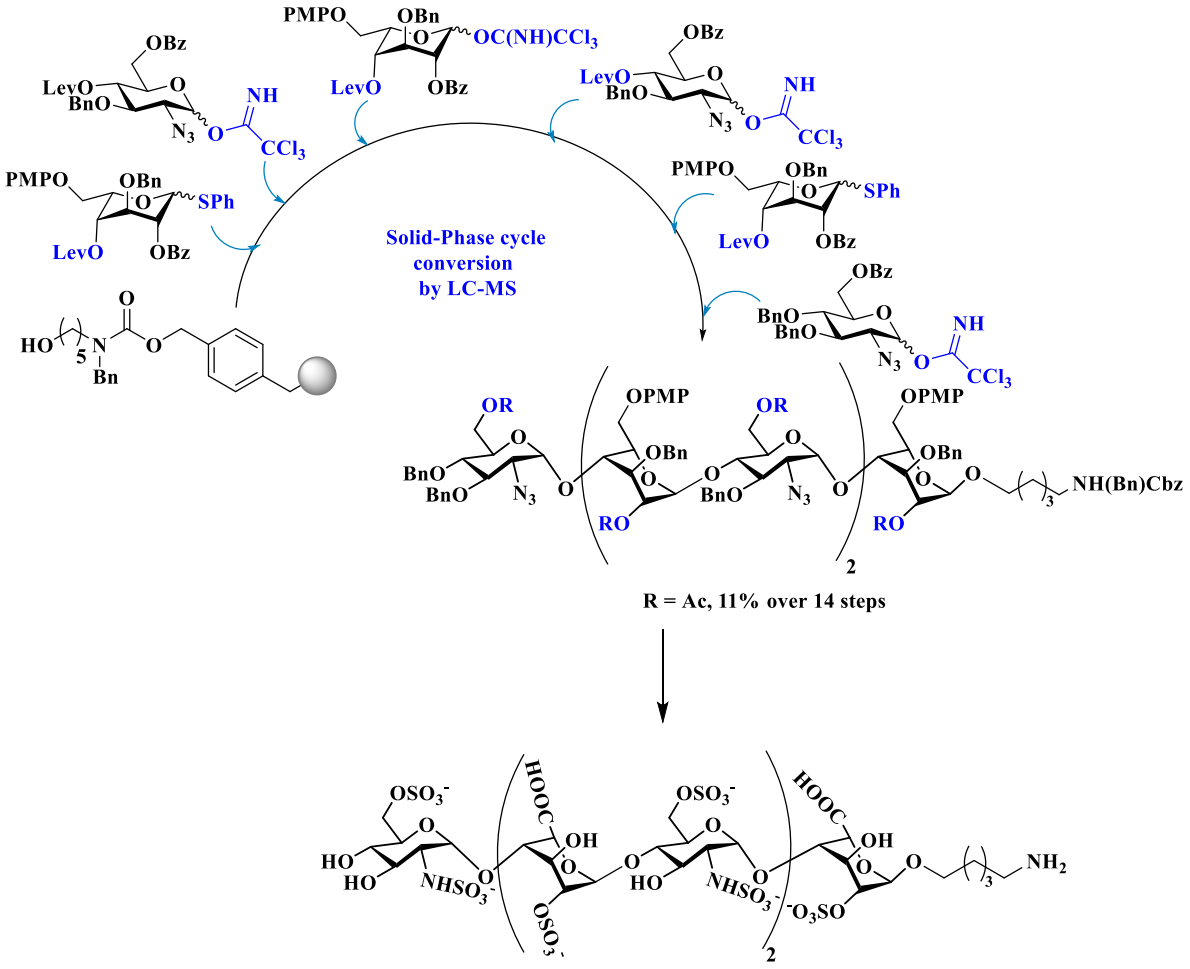


Scheme 8. Synthesis of epimerized HS disaccharide building blocks.

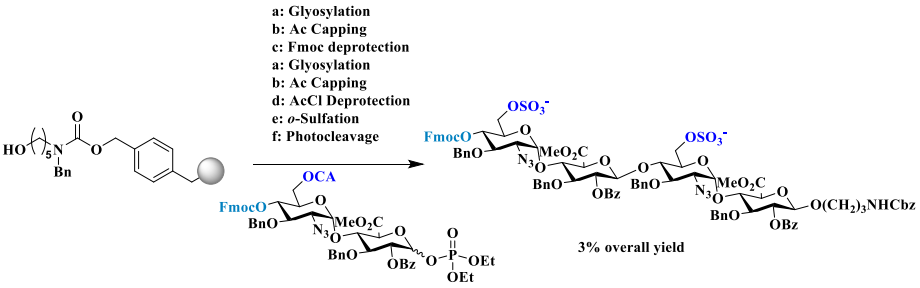
1.5 Solid-phase synthesis

To streamline and accelerate the assembly of disaccharides into oligosaccharides, automated synthesis using a solid-phase oligosaccharide synthesizer has proven invaluable. This approach enables the efficient production of well-defined oligosaccharides within days, rather than the years required by traditional methods. Solid-phase synthesis has been successfully applied to the assembly of HS oligosaccharides. In one instance, a semi-automated glycan assay was carried out using Merrifield resin functionalized with a photolabile nitrobenzyl ether-based linker. This system was glycosylated with IdoA and glucosamine building blocks to produce hexasaccharide analogs with an overall yield of 11%.[38] Another significant development involved immobilizing an HS disaccharide intermediate onto synphase lanterns. This setup employed a divergent deprotection and sulfation strategy, yielding 16 distinct HS disaccharides with high efficiency.[39] The first fully automated assembly of an HS

tetrasaccharide was accomplished using a D-GlcN-α-D-GlcA disaccharide building block. In this method, the chloroacetate group of the tetrasaccharide precursor was selectively cleaved while still on resin, followed by sulfation and photocleavage from the resin, resulting in a final product yield of 3%.[40] In summary, automated solid-phase synthesis represents a promising strategy for the rapid assembly of HS oligosaccharides. However, further optimization of glycosylation, sulfation, and final deprotection steps is essential to improve yields and streamline the production process, making it more suitable for broader applications.



Scheme 8. Merrifield resin based solid-phase HS oligosaccharide synthesis.



Scheme 9. Automated glycan assembly of HS tetrasaccharide.

1.6 Heparan Sulfate Binding Proteins (HSBPs)

Nearly 500 proteins have been recognized as potential heparan sulfate (HS) binding proteins.[7,41] The interactions between these proteins and HS are largely influenced by the negatively charged groups on HS, such as sulfate and carboxylic acid residues. Proteins interact with HS through ionic bonds formed by lysine, arginine, and histidine residues, while hydrogen bonding is facilitated by polar residues like asparagine, glutamine, and histidine. Based on their functions, HS-binding proteins (HSBPs) are grouped into five main categories: blood coagulation factors; growth factors, chemokines, and cytokines; extracellular proteins; transmembrane signaling receptors; and cell adhesion proteins. These proteins exhibit binding affinities for HS in the micromolar to nanomolar range.[42-43] Understanding the structure-activity relationships of these proteins using synthetic HS could lead to the discovery of new biomarkers and therapeutic agents.

Blood Coagulation Factors

Heparin is a widely used anticoagulant that works by binding to antithrombin (AT), a serine protease inhibitor. This interaction forms an AT-heparin complex that inhibits thrombin and Factor Xa, thereby preventing blood coagulation.[44] Unfractionated Heparin (UFH) and Low Molecular Weight Heparin (LMWH), both derived from porcine mucosa, have been staples of anticoagulant therapy for decades.[45,46] In the early 1980s, researchers discovered a unique pentasaccharide sequence within heparin that is crucial for binding to AT.[47-49] The 3-*O*-sulfation of the glucosamine residue at the reducing end and the $^{2}S_{0}$ conformation of L-iduronic acid in this sequence were found to be essential for AT activity.[50] This discovery led to the development of Fondaparinux (Arixtra), the only FDA-approved heparin-based drug. Heparan sulfate (HS) also regulates serine protease inhibitors such as antithrombin III (ATIII) and heparin cofactor II (HCII), both of which contribute to thrombin inhibition. Recently, Desai and colleagues employed computational tools and a synthetic HS hexasaccharide library to identify the rare involvement of 2-*O*-sulfated glucuronic acid in activating HCII.[51]

Serine proteases, including thrombin, HCII, Factor Xa, and Factor IXa, play essential roles in the blood coagulation cascade. While structure-activity relationship (SAR) studies of HS-serine protease interactions have advanced significantly, challenges persist, particularly in selectively targeting specific serine proteases to minimize unintended side effects.

Growth factors

Growth factors (GFs) are a diverse group of proteins critical for regulating cellular processes such as proliferation, migration, differentiation, and survival.[52-56] Dysregulated GF signaling is often associated with various diseases, including cancer. Many GFs interact with heparan sulfates (HS) found on heparan sulfate proteoglycans (HSPGs), influencing cellular signaling. For instance, fibroblast growth factors (FGF-1 and FGF-2) bind to HS to form a ternary complex with FGF receptors (FGFRs), a key step in their activation and signaling pathways. HS also protects FGFs from proteolytic degradation and inactivation.[57-59]

Hung and colleagues synthesized 48 HS-based disaccharide analogues to study their binding interactions with FGF-1 and FGF-2.[34,60] Using ITC binding studies and X-ray crystallography, they identified 3-*O*-sulfated *N*-sulfated glucosamine and L-iduronic acid as critical residues for FGF binding. These results were further validated with limited synthetic HS ligands at the tetrasaccharide and hexasaccharide levels.[13,61] Beyond FGFs, HS oligosaccharides regulate other growth factors, including bone morphogenetic protein-2 (BMP-2), heparin-binding epidermal growth factor (HB-EGF), and vascular endothelial growth factors (VEGFs). Linhardt and collaborators demonstrated that VEGF activity requires highly sulfated *N*-sulfated HS ligands with a minimum hexasaccharide length.[62] Similarly, BMP-2 and HB-EGF specifically bind to 6-*O*-sulfated HS oligosaccharides.[63] However, the limited availability of comprehensive HS tetrasaccharide and hexasaccharide libraries continues to pose challenges in identifying optimal ligands for these growth factors, making it a focal point of ongoing research.

Chemokines

Heparan sulfate (HS) plays a key role in modulating the activity of cytokines and chemokines, acting as a co-receptor for nearly 50 distinct chemokines. This interaction regulates processes such as leukocyte migration, angiogenesis, breast cancer metastasis, and leukocyte degranulation.[64-66] Upon stimuli like bacterial or viral infections, cells release chemokines to recruit leukocytes to the site of damage, facilitating immune responses to

eliminate pathogens.[67-70] Under normal conditions, chemokines exist in monomeric form, but during inflammation, HS binds to chemokines, promoting their polymerization and enhancing their activity. Understanding the structure-activity relationships (SARs) between HS and chemokines could lead to the development of anti-inflammatory agents with fewer side effects. Using synthetic HS libraries, researchers have investigated these SARs. Lindahl and colleagues found that interleukin-8 (CXCL8/IL-8) binds preferentially to HS structures containing 2-*O*-sulfated L-iduronic acid and 6-*O*-sulfated *N*-sulfated glucosamine units, enhancing neutrophil trafficking.[71] Gallagher and coworkers identified 2-*O*-sulfated iduronic acid as critical for CXCL4 chemokine tetramerization and cell surface receptor binding.[72] Hsieh-Wilson's team revealed that *N*-sulfated HS oligosaccharides strongly bind to the CCL5 chemokine, reducing cell migration.[73] Seeberger's group demonstrated that a simple 2,4-*O*-disulfated iduronic acid binds strongly to CCL20, helping to mitigate asthmatic responses.[74] Meanwhile, Boons and coworkers discovered that CCL2 interacts with highly sulfated HS compounds, showing no specific preference for the uronic acid component.[13]Despite these advances, challenges remain in designing HS ligands with high selectivity and specificity for chemokine targeting, limiting their potential as therapeutic candidates. Continued refinement of HS libraries and a deeper understanding of chemokine-HS interactions are essential for overcoming these limitations.

1.7 Conclusions

Developing efficient strategies for building block synthesis and precise stereoselective glycosylation techniques is essential for the chemical synthesis of HS oligosaccharides. Optimized α-glycosylation between glucosamine and uronic acid residues has been achieved through the use of diverse orthogonal protecting groups and promoters. Advances such as solution-based one-pot synthesis and the activation of glucosamine thiosyl donors using p-toluene sulfenyl chloride (p-TolSCl) and silver triflate (AgOTf) have further enhanced the potential to synthesize more complex HS oligosaccharides. Despite these improvements, synthesizing HS disaccharide building blocks, which typically requires 18–24 steps, remains a formidable challenge. A recent approach, which simplifies the process to 4–5 steps by extracting universal disaccharide building blocks from natural heparin polysaccharides, has significantly improved the outlook for HS synthesis. Additionally, automated synthesis methods are expected to provide rapid access to a wide range of diverse and complex sequences tailored to biological needs. Although HS synthetic libraries have shown exceptional binding capabilities with blood coagulants, growth factors, and chemokines,

further systematic exploration and expansion of these libraries could lead to the discovery of novel therapeutic compounds for various diseases and infections.

1.8 References

1. Taylor, K. R. Gallo, R. L. Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation. *FASEB J*. 2006, 20, 9–22. DOI: 10.1096/fj.05-4682rev
2. Bulow, H. E. Hobert, O. The molecular diversity of glycosaminoglycans shapes animal development. *Annu. Rev. Cell Dev. Biol*. 2006, 22, 375–407. DOI: 10.1146/annurev.cellbio.22.010605.093433
3. Sasisekharan, R. Raman, R. Prabhakar, V. Glycomics approach to structure-function relationships of glycosaminoglycans. *Annu. Rev. Biomed. Eng*. 2006, 8, 181–231 DOI: 10.1146/annurev.bioeng.8.061505.095745
4. Kamhi, E. Joo, E. J. Dordick, J. S. Linhardt, R. Glycosaminoglycans in infectious disease. *J. Biol. Rev*. 2013, 88, 928–943 DOI: 10.1111/brv.12034
5. Kjellén, L. Lindahl, U. Proteoglycans: structures and interactions. *Annu. Rev. Biochem*. 1991, 60, 443–475 DOI: 10.1146/annurev.bi.60.070191.002303
6. Couchman, J. R. Pataki, C. A. An introduction to proteoglycans and their localization. *J. Histochem. Cytochem*. 2012, 60, 885–897 DOI: 10.1369/0022155412464638
7. Xu, D. Esko, J. D. Demystifying heparan sulfate-protein interactions. *Annu. Rev. Biochem*. 2014, 83, 129–157 DOI: 10.1146/annurev-biochem-060713-035314
8. Bhoge. P. R. Raigawali, R. S. Mardhekar, Anand, S. Kikkeri. R. Synergestic interplay of uronic acid and sulfation composition of heparan sulfate on molecular recognition to activity. *Carbohydr Res*. 2023, 532. 108919 DOI: 10.1016/j.carres.2023.108919
9. Panza, M. Pistorio, S. G. Stine, K. J. Demchenko, A. V. Automated chemical oligosaccharide synthesis: novel approach to traditional challenges. *Chem. Rev*. 2018, 118, 8105–8150 DOI: 10.1021/acs.chemrev.8b00051
10. Mende, M. Bednarek, C. Wawryszyn, M. Sauter, P. Biskup, M. B. Schepers, U. Bräse, S. Chemical synthesis of glycosaminoglycans. *Chem Rev*. 2016, 116, 14, 8193-9255 DOI: 10.1021/acs.chemrev.6b00010
11. Hansen, S. U. Dalton, C. E. Barath, M. Kwan, G. Raftery, J. Jayson, G. C. Miller, G. J. Gardiner, J. M. Synthesis of l-iduronic acid derivatives via [3.2.1] and [2.2.2] l-

iduronic lactones from bulk glucose-derived cyanohydrin hydrolysis: a reversible conformationally switched superdisarmed/rearmed lactone route to heparin disaccharides. *J. Org. Chem*. 2015, 80, 3777–3789 DOI: 10.1021/jo502776f

12. Codee, J. D. Stubba, B. Schiattarella, M. Overkleeft, H. S. van Boeckel, C. A. A. van Boom, J. H. van der Marel, G. A. (2005) A modular strategy toward the synthesis of heparin-like oligosaccharides using monomeric building blocks in a sequential glycosylation strategy. *J. Am. Chem. Soc*. 2005, 127, 3767–3773 DOI: 10.1021/ja045613g
13. Zong C. Venot, A. Li, X. Lu, W. Xiao, W. Wilkes, J-S. L. Salanga, C. L. Handel, T. M. Wang, L. Wolfert, M. A. Boons, G.-J. Heparan sulfate microarray reveals that heparan sulfate–protein binding exhibits different ligand requirements *J. Am. Chem. Soc*. 2017, 139, 9534 – 9543 DOI:10.1021/jacs.7b01399
14. Zong, C. Huang, R. Condac, E. Chiu, Y. Xiao, W. Li, Xiuru, Lu, W. Ishihara, M. Wang, S. Ramiah, A. Stickney, M. Azadi, P. Amster, I. J. Moremen, K. W. Wang, L. Sharp, J. S. Boons, G. J. Integrated approach to identify heparan sulfate ligand requirements of Robo1. *J. Am. Chem. Soc*. 2016, 138, 13059–13067 DOI**:** 10.1007/978-1-0716-1398-6_14
15. Zulueta, M. M. Liu, S. Y. Lin, Y. T. Huang, C. J. Wang, C. C. Ku, C. C. Shi, Z. Chyan, C. L. Irene, D. Lim, L. H. Tsai, T. I. Hu, Y. P. Arco, S. D. Wong, C. H. Hung, S. C. α-Glycosylation by D-glucosamine-derived donors: synthesis of heparosan and heparin analogues that interact with mycobacterial heparin-binding hemagglutinin. *J. Am. Chem. Soc.* 2012, 134, 8988–8995 DOI: 10.31635/ccschem.023.202303086
16. Noti, C. de Paz, J. L. Polito, L. & Seeberger, P. H. Preparation and use of microarrays containing synthetic heparin oligosaccharides for the rapid analysis of heparin-protein interactions. *Chem. Eur. J*. 2006, 12, 8664–8686 DOI: 10.1002/chem.200601103
17. Hu, Y. P. Lin. S. Y. Huang, C. Y. Zulueta, M. M. L. Liu, J. Y. Chang, W. Hung, S. C. Synthesis of 3-O-sulfonated heparan sulfate octasaccharides that inhibit the herpes simplex virus type 1 host–cell interaction. *Nat. Chem*. 2011, 3, 557–563 DOI: 10.1038/NCHEM.1073
18. Wang, Z. Xu, Y. Yong, B. Tiruchinapally, G. Sun, B. Liu, R. Dulaney, S. Liu, J. Huang, X. Preactivation-based, one-pot combinatorial synthesis of heparin-like hexasaccharides for the analysis of heparin-protein interactions. *Chem. Eur. J.* 2010, 16, 8365–8375 DOI: 10.1002/chem.201000987

19. Jain. P. Shanthamurthy, C. D. Leviatan Ben-Arye, S. Woods, R. J. Kikkeri, R. Padler-Karavani, V. Discovery of rare sulfated *N*-unsubstituted glucosamine based heparan sulfate analogs selectively activating chemokines. *Chem. Sci.* 2021, 12, 3674-3681 DOI: 10.1039/d0sc05862a
20. Anand, S. Mardhekar, S. Raigawali, R. Mohanta, N. Jain, P. Shanthurthy, C. D. Gnanaprakasam, B. Kikkeri, R. Continuous-flow accelerate sulfation of heparan sulfate intermediates. *Org. Lett*. 2020, 22, 3402-3406 DOI: 10.1021/acs.orglett.0c00878
21. Jain, P. Shanthamurthy, C. D. Leviatan Ben-Arye, S. Yeshuda, S. Nandikol, S. S. Thulasiram, H. V. Palder-Karavani, V. Kikkeri, R. Synthetic heparan sulfate ligands for vascular endothelial growth factor to modulate angiogenesis. *Chem. Commun.* 2021, 57, 3516-3519 DOI: 10.1039/d1cc00964h
22. Dey, S. & Wong, C. H. Programmable one-pot synthesis of heparin pentasaccharides enabling access to regiodefined sulfate derivatives. *Chem. Sci*. 2018 9, 6685–6691 DOI: 10.1039/c8sc01743c
23. Zong, C. Venot, A. Li, X. Lu, W. Xiao, W. Wilkes, J. S. L. Salanga, C. L. Handel, T. M. Wang, L. Wolfert, M. A. Boons, G. J. (2017) Heparan sulfate microarray reveals that heparan sulfate-protein binding exhibits different ligand requirements. *J. Am. Chem. Soc.* 2017, 139, 9534–9543 DOI: 10.1021/jacs.7b01399
24. Hung, T. Y. Irena, D. Zulueta, M. M. L. Tai, T. J. Lain, S. H. Cheng, C. P. Tsai, P. X. Lin, S. Y. Chen, Z. G. Ku, C. C. Hsiao, C. D. Chyan, C. L. Hung, S. C. Structure of the complex between a heparan sulfate octasaccharide and mycobacterial heparin binding hemoaglutinin. *Angew. Chem. Int. Ed*. 2017, 56, 4192-4196 DOI: 10.1002/anie.201612518
25. Yang, W. Yoshida, K. Yang, B. Huang, X. Obstacles and solutions for chemical synthesis of syndecan-3 (53-62) glycopeptides with two heparan sulfate chains. *Carbohydr Res*. 2016, 435 180-194 DOI: 10.1016/j.carres.2016.10.005
26. Dulaney, S. B. Xu, Y. Wang, P. Tiruchinapally, G. Wang, Z. Kathawa, J. El-dakdouki, M. H. Yang, B. Liu, J. Huang, X. Divergent synthesis of heparan sulfate oligosaccharides. *J. Org. Chem.* 2015, 80, 12265-12279 DOI: 10.1021/acs.joc.5b02172
27. Wang, P. Zhao, J. Nasr, S. H. Otieno, S. A. Zhang, F. Qiang, W. Linhardt, R. J. Huang, X. Probing amyloid β interactions with synthetic heparan sulfate

oligosaccharides. *ACS Chem. Biol*. 2021, 16, 1894-1899 DOI: 10.1021/acschembio.0c00904

28. Yang, B. Yoshida, K. Yin, Z. Dai, H. Kavunja, H. El-Dakdouki, M. H. Sungsuwan, S. Dulaney, S. B. Huang, X. Chemical synthesis of a heparan sulfate glycopeptide: syndecan-1. *Angew. Chem. Int. Ed.* 2012, 51, 10185-10189 DOI: 10.1002/anie.201205601
29. Yoshida, K. Yang, B. Yang, W. Zhang, Z. Zhang, J. Huang, X. Chemical synthesis of syndecan-3 glycopeptides bearing two heparan sulfate glycan chains. *Angew. Chem. Int. Ed*. 2014, 53, 9051-9058 DOI: 10.1002/anie.201404625
30. Dhamale, O. P. Zong, C. Al-Mafraji, K. Boons, G. J., New glucuronic acid donors for the modular synthesis of heparan sulfate oligosaccharides. *Org. Biomol. Chem*. 2014, 12, 2087-2096 DOI: 10.1039/c3ob42312c
31. Baryal, K. N. Ramadan, S. Su, G. Huo, C. Zhao, Y. Liu, J. Hsieh-Wilson, L. C. Huang, X., synthesis of a systematic 64-membered heparan sulfate tetrasaccharide library. *Angew. Chem. Int. Ed.* 2023, 63. E202211985 DOI: 10.1002/anie.202211985
32. Chopra, P. Joshi, A. Wu, J. Lu, W. Yadavalli, T. Wolfert, M. A. Shukla, D. Zaia, J. Boons, G. J. The 3-O-sulftion of heparan sulfate modulates protein binding and lyase degradation. *PNAS*, 2021, 118, e2012935118 DOI: 10.1073/pnas.2012935118
33. Polat, T. Wong, C.-H. Anomeric reactivity based one-pot synthesis of heparin-like oligosaccharides. *J. Am. Chem. Soc*. 2007, 129, 12795–12800 DOI: 10.1021/ja073098r
34. Hu, Y. P. Zhong, Y. Q. Chen, Z. G. Chen, C. Y. Shi, Z. Zulueta, M. M. L. Ku, C. C. Lee, P. Y. Wang, C. C. Hung, S. C. Divergent synthesis of 48 heparan sulfate-based disaccharides and probing the specific sugar-fibroblast growth factor-1 interaction. *J. Am. Chem. Soc.* 2012, 134, 20722–20727 DOI: 10.1021/ja3090065
35. Lu, L. D. Shie, C. R. Kulkarni, S. S. Pan, G. R. Lu, X. A. Hung, S. C. Synthesis of 48 disaccharide building blocks for the assembly of a heparin and heparna sulfate oligosaccharide library. *Org. Lett.* 2006, 8, 5995-5998 DOI: 10.1021/ol062464t
36. Pawar, N. J. Wang, L. Higo, T. Bhattacharya, C. Kancharla, P. K. Zhang, F. Baryal, K. Huo, C. X. Liu, J. Linhardt, R. J. Huang, X. Hsieh-Wilson, L. C. Expedient synthesis of core disaccharide building blocks from natural polysaccharides for heparan sulfate oligosaccharide assembly. *Angew. Chem. Int. Ed.* 2019, 58, 18577–18583 DOI:10.1021/acs.joc.3c01108

37. Wang, L. Sorum, A. W. Huang, B. S. Kern, M. K. Su. Guowei, Pawr, N. Huang, X. Liu, J. Pohl, N. L. B. Hsieh-Wilson, L. C. Efficient platform for synthesizing comprehensive heparan sulfate oligosaccharide libraries for decoding glycosaminoglycan-protein interactions. *Nat. Chem.* 2023, 15, 1108-1117 DOI: 10.1038/s41557-023-01248-4
38. Guedes, N. Kopitzki, S. Echeverria, B. Pazos, R. Elosegui, E. Calvo, Reichardt, N. C. Solid-phase assembly of glycosaminoglycan oligosaccharide precursors. *RSC Adv.* 2015, 5, 9325-9327 DOI: 10.1039/d3ob01909h
39. Ramadan, S. Su, G. Baryal, K. Hsieh-Wilson, L. C. Liu, J. Huang, X. Automated solid phase assisted synthesis of heparan sulfate disaccharide library. *Org. chem. Front.* 2022, 9, 2910-2920 DOI: 10.1039/d2qo00439a
40. Pongener, I. Sletten, E. T. Danglad-Flores, J. Seeberger, P. H. Miller, G. J. Synthesis of a heparan sulfate tetrasaccharide using automated glycan assembly. *Org. Biomol. Chem*. 2023, 22, 1395-1399 DOI: 10.1039/D3OB01909H
41. Whitelock, J. M. Iozzo, R. V. Heparan sulfate: A complex polymer charged with biological activity. Chem. Rev. 2005, 105, 2745–2764 DOI: 10.1021/cr010213m
42. Raman, R. Sasisekharan, V. Sasisekharan, R. Structural Insights into biological roles of protein-glycosaminoglycan interactions. *Chem. Biol*. 2005, 12, 267 277 DOI: 10.1016/j.chembiol.2004.11.020
43. Bishop, J. R. Schuksz, M. Esko, J. D. Heparan sulphate proteoglycans fine tune mammalian physiology. *Nature,* 2007, 446, 1030–1037 DOI: 10.1038/nature05817
44. Rosenberg, D. R. Hichs, M. Damus S. P. Anticoagulant Action of Heparin. *Nature,* 1973, 246, 355-357 DOI: 10.1038/246355a0
45. Bray, B. Lane, D. A. Freyssinet, J. M. Pejler, G. Lindahl, U. Anti-thrombin activities of heparin. Effect of saccharide chain length on thrombin inhibition by heparin cofactor II and by antithrombin. *Biochem. J*. 1989, 262, 225–232 DOI: 10.1042/bj2620225
46. Herbert, J. M. Petitou, M. Lormeau, J. C. Cariou, R. Necciari, J. Magnani, H. N. Zandberg, P. van Amstredam, R. G. M. van Boeckel, C. A. A. Meuleman, D. G. SR 90107A/Org 31540, a novel anti-factor Xa antithrombotic agent. *Cardiovasc. Drug Rev.* 1997, 15, 1–26 DOI: **10.1111/j.1527**-3466.1997.tb00321.x
47. Petitou, M. Van Boeckel, C. A. A. A synthetic antithrombin III binding pentasaccharide is now a drug! What comes next? *Angew. Chemie Int. Ed*. 2004, 43, 3118–3133 DOI: 10.1002/anie.200300640

48. Visser, A. Grootenhius P. D. J. Van Boeckel, C. A. A. A mechanism of haparin indused potentiation of antithrombin III. *Nat. Struct. Biol*. 1994, 1, 423-425 DOI: 10.1038/nsb0794-423
49. Dementiev, A. Petitou, M. Herbert, J. M. Gettins, P. G. W. The ternary complex of antithrombin-anhydrothrombin-heparin reveals the basis of inhibitor specificity. *Nat. Struct. Mol. Biol*. 2004, 11, 863–867 DOI: 10.1038/nsmb810
50. Demeter, F. Gyöngyösi, T. Bereczky, Z. Kövér, K. E. Herczeg, M. Borbás, A. Replacement of the L-iduronic acid unit of the anticoagulant pentasaccharide idraparinux by a 6-deoxy-L-talopyranose – Synthesis and conformational analysis. *Sci. Rep.* 2018, 8, 2–11 DOI:10.1038/s41598-018-31854-z
51. Sankarayanarayanan, N. V. Strebel, T. R. Boothello, R. S. Sheerin, K. Raghuraman, A. Sallas, F. Mosier, P. D. Watermeyer, N. D. Oscarson, S. Desai, U. R. A hexasaccharide containing rare 2-O-sulfate-glucuronic acid residues selectively activates heparin cofactor II. *Angew. Chem. Int .Ed.* 2017, 56, 2312-2317 DOI: 10.1002/anie.201609541
52. Beenken, A. Mohammadi, M. The FGF family: Biology, pathophysiology and therapy. *Nat. Rev. Drug Discov*. 2009, 8, 235–253 DOI: 10.1038/nrd2792
53. Turner, N. Grose, R. Fibroblast growth factor signalling: From development to cancer. *Nat. Rev. Cancer*, 2010, 10, 116–129 DOI: 10.1038/nrc2780
54. Yun, Y. R. Won, J. E. Jeon, E. Lee, S. Kang, W. Jo, H. Jang, J. H. Shin, U. S. Kim, H. W. Fibroblast growth factors: Biology, function, and application for tissue regeneration. *J. Tissue Eng.*2010, 1, 1–18 DOI: 10.4061/2010/218142
55. Pellegrini, L. Role of heparan sulfate in fibroblast growth factor signalling: a structural view. *Curr. Opin. Struct. Biol.* 2001, 11, 629-634 DOI: 10.1016/s0959-440x(00)00258-x
56. Maccarana, M. Casu, B. Lindahl, U. Minimal sequence in heparin/heparan sulfate required for binding of basic fibroblast growth factor. *J. Biol. Chem.* 1993, 268 (32), 23898-23905
57. Allen, B. L. Filla, M. S. Rapraeger, A. C. Role of heparan sulfate as a tissue specific regulator of FGF-4 and FGF receptor recognition. *J. Cell. Biol.* 2001, 155, 845-857 DOI: 10.1083/jcb.200106075
58. Ashikari-Hada, S. Habuchi, H. Kariya, Y. Itoh, N. Reddi, A. H. Kimata, K. Characterization of Growth Factor-binding Structures in Heparin/Heparan Sulfate

Using an Octasaccharide Library. *J. Biol. Chem*. 2004, 279, 12346–12354 DOI: 10.1074/jbc.M313523200

59. Turnbull, J. E. Fernig, D. G. Ke, Y. Wilkinson, M. C. Gallagher, J. T. Identification of the basic fibroblast growth factor binding sequence in fibroblast heparan sulfate. *J. Biol. Chem*. 1992, 267, 10337–10341
60. Li, Y. C. Ho, I. H. Ku, C. C. Zhong, Y. Q. Hu, Y. P. Chen, Z. G. Chen, C. Y. Lin, W. C. Zulueta, M. M. Hung, S. C. Lin, M. G. Wang, C. C. Hsiao, C. D Interactions that influence the binding of synthetic heparan sulfate based disaccahrides to fibroblast growth factor-2. *ACS Chem. Biol*. 2014, 8, 1712-1717
61. Horton, M. Su, G. Yi, L. Wang, Z. Xu, Y. Pagadala, V. Zhang, F. Zaharoff, D. A. Pearce, K. Linhardt, R. J. Liu, J. Construction of heparan sulfate microarray for investigating the binding of specific saccharide sequences to proteins. *Glycobiology*, 2021, 31, 188-199 DOI: 10.1093/glycob/cwaa068
62. Zhao, W. McCallum, S. A. Xiao, Z. Zhang, F. Linhardt, R. J. Binding affinities of vascular endothelial growth factor (VEGF) for heparin-derived oligosaccharides. *Biosci. Rep*. 2012, 32, 71-81 DOI: 10.1042/BSR20110077
63. Jain, P. Shanthamurthy, C. D. Leviatan Ben-Arye, S. Yehuda, S. Nandikol, S. S. Thulasiram, H. V. Padler-Karavani, V. Kikkeri, R. Synthetic heparan sulfate ligands for vascular endothelial growth factor to modulate angiogenesis. *Chem. Commun.* 2021, 57, 3516-3519 DOI: 10.1039/d1cc00964h
64. Hughes, C. E. Nibbs, R. J. A guide to chemokines and their receptors. *FEBS J.* 2018, 285 (16), 2944-2971 DOI: 10.1111/febs.14466
65. Farrugia, B. L. Lord, M. S. Melrose, J. Whitelock, J. M. The role of heparan sulfate in inflammation, and the development of biomimetics as anti-inflammatory strategies. *J. Histochem. Cytochem*. 2018, 66 (4), 321-336 DOI: 10.1369/0022155417740881
66. Proudfoot, A. E. Johnson, Z. Bonvin, P. Handel, T. M. Glycosaminoglycan interactions with chemokines add complexity to a complex system. *Pharmaceuticals* 2017, 10 (3), 70 DOI: 10.3390/ph10030070
67. Raman, D. Sobolik-Delmaire, T. Richmond, A. Chemokines in health and disease. *Exp. Cell Res.* 2011, 317 (5), 575-589.
68. Parish, C. R. Heparan sulfate and inflammation. *Nat. Immunol.* 2005, 6, 861–862 DOI: 10.1038/ni0905-861

69. Belperio, J. A. Keane, M. P. Arenberg, D. A. Addison, C. L. Ehlert, J. E. Burdick, M. D. Strieter, R. M. CXC chemokines in angiogenesis. *J. Leukoc. Biol.* 2000, 68, 1–8 DOI: 10.1189/jlb.68.1.1
70. Handel, T. M. Johnson, Z. Crown, S. E. Lau, E. K. Proudfoot, A. E. Regulation of protein function by glycosaminoglycans - As exemplified by chemokines. *Annu. Rev. Biochem.* 2005, 74, 385–410 DOI: 10.1146/annurev.biochem.72.121801.161747
71. Spillmann, D. Witt, D. Lindahl, U. Defining the interleukin-8-binding domain of heparan sulfate. *J. Biol. Chem.* 1998, 273 (25), 15487-15493 DOI: 10.1074/jbc.273.25.15487
72. Stringer, S. E. Gallagher, J. T. Specific binding of the chemokine platelet factor 4 to heparan sulfate. *J. Biol. Chem.* 1997, 272 (33), 20508-20514 DOI: 10.1074/jbc.272.33.20508
73. Sheng, G. J. Oh, Y. I. Chang, S.-K. Hsieh-Wilson, L. C. Tunable heparan sulfate mimetics for modulating chemokine activity. *J. Am. Chem. Soc.* 2013, 135 (30), 10898-10901 DOI: 10.1021/ja4027727
74. Nonaka, M. Bao, X. Matsumura, F. Götze, S. Kandasamy, J. Kononov, A. Broide, D. H. Nakayama, J. Seeberger, P. H. Fukuda, M. Synthetic di-sulfated iduronic acid attenuates asthmatic response by blocking T-cell recruitment to inflammatory sites. *Proc. Natl. Acad. Sci. USA.* 2014, 111, 8173-8178 DOI: 10.1073/pnas.1319870111

Chapter – 2

Synthesis of Heparan Sulfate Disaccharides and its Microarray Evaluation with Growth Factors and Chemokines

2.1. Abstract

Heparan sulfate (HS) binds to various growth factors and chemokines, thereby playing a crucial role in regulating cell signalling. The structural heterogeneity of HS significantly contributes to the specific binding and modulation of morphogens. As such, there is considerable interest in synthesizing structurally well-defined HS ligands, conducting high-throughput analyses with various growth factors and chemokines on a single platform, and decoding their structure-activity relationships. Herein, we report the synthesis of 14 HS disaccharides, comprising both L-iduronic acid and D-glucuronic acid in *N*-sulphated domain ligands, and analyze their binding affinities with a wide range of growth factors and chemokines. Our studies revealed that 6SNSGlc-IdoA(2S) is a potential ligand for FGF1, HER, and HB-EGF growth factors, while 63SGlc-IdoA(2S) shows strong potential as a ligand for FGF2, VEGF, and BMP2 growth factors, clearly highlighting the importance of 3-O-sulfation in fine-tuning binding specificity. A similar trend was observed in chemokines. Overall, the high-throughput analysis demonstrated that highly sulfated L-iduronic acid based ligands are promising ligands for targeting the majority of growth factors and chemokines compared to D-glucuronic acid counterparts.

2.2. Introduction

Heparan sulfate (HS) is a complex and highly sulfated glycosaminoglycan that plays a pivotal role in numerous biological processes, including cell signalling, development, and tissue homeostasis. Found ubiquitously on the surface of mammalian cell surface and extracellular matrix, HS binds to a broad spectrum of proteins, such as growth factors, chemokines, and morphogens. These interactions are crucial for modulating key physiological processes, including angiogenesis, wound healing, and immune responses. The specificity of these interactions, however, is dictated by the structural heterogeneity of HS, which is a direct result of its highly variable patterns of sulfation, acetylation, and epimerization.[1-6]

The structural diversity of HS enables it to act as a molecular code, fine-tuning the activity of growth factors and chemokines by influencing their binding affinities and downstream signalling pathways. For instance, growth factors such as fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), and bone morphogenetic proteins (BMPs) depend on HS as a co-receptor for stabilization and activation.[7-13] Similarly, chemokines utilize HS to establish concentration gradients critical for immune cell recruitment and tissue repair. Despite its importance, the precise mechanisms by which HS structure governs these interactions remain poorly understood due to the inherent complexity and variability of its biosynthesis.[14-18]

Deciphering the structure-activity relationship of HS is a key challenge in glycobiology. Synthetic approaches to generate structurally defined HS oligosaccharides provide an unparalleled opportunity to unravel these interactions at a molecular level. By systematically varying the sulfation patterns and uronic acid composition of HS, it becomes possible to study their impact on specific protein binding and functional outcomes[19-23]. Such insights are vital for designing HS-based therapeutics targeting growth factor and chemokine signalling in diseases such as cancer, inflammation, and fibrosis. This study aims to bridge this gap by synthesising a library of HS oligosaccharides and evaluating their binding specificities across a panel of growth factors and chemokines, shedding light on the molecular basis of HS-protein interactions.[24-26]

2.3. Results and Discussion

2.3.1. Synthesis of L-iduronic acid based HS disaccharides.

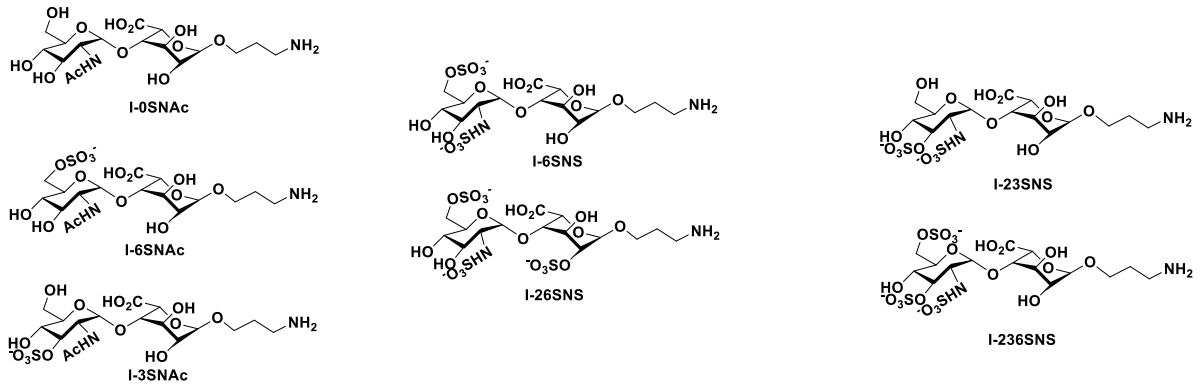
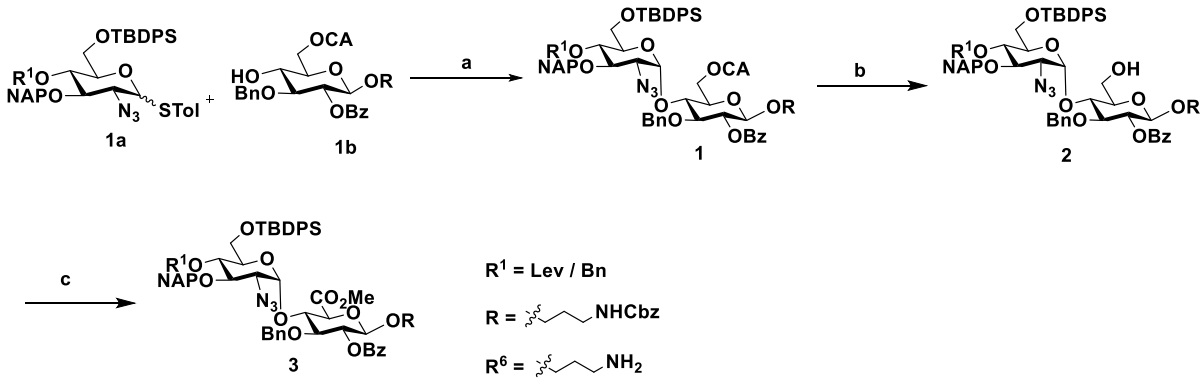
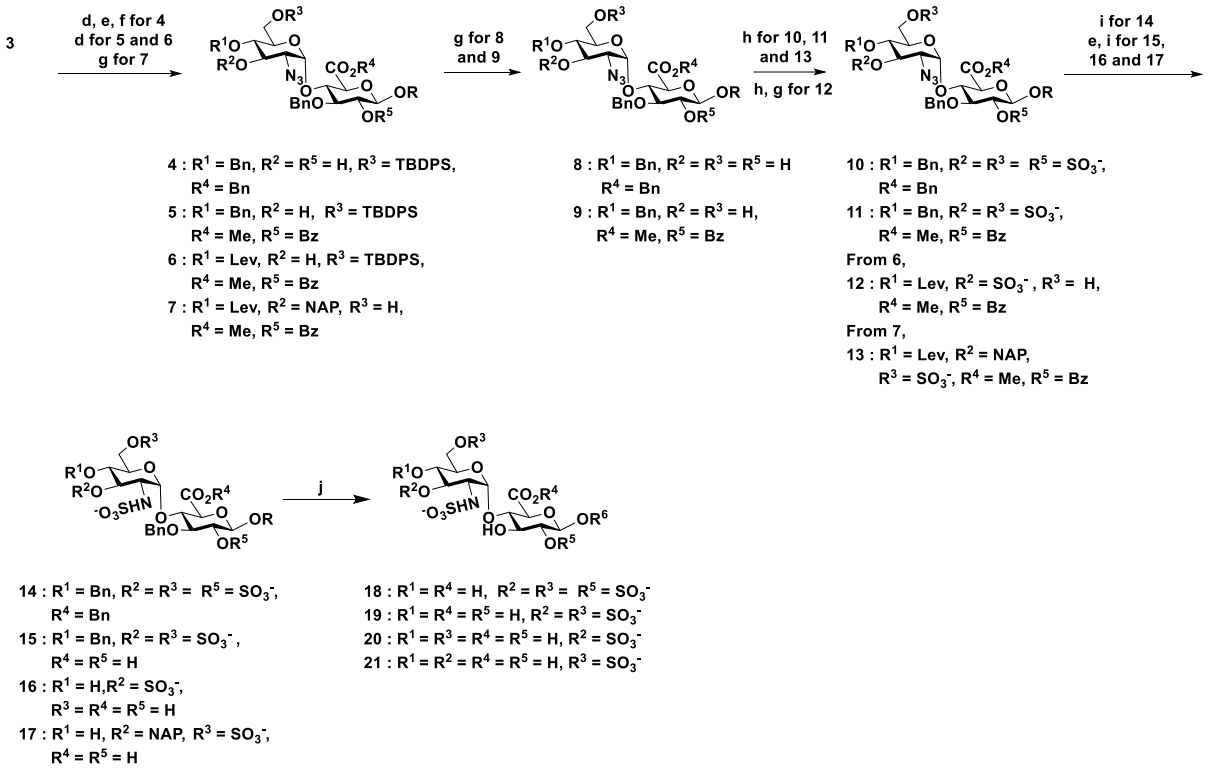


Fig 1. Chemdraw structures of L-iduronic acid based HS disaccharides.

We employed a divergent synthetic strategy to obtain seven disaccharide HS ligands composed of L-iduronic acid. Both *N*-acetylated and *N*-sulfated derivatives, as well as highly sulfated and non-sulfated variants, were synthesized (fig.1). All compounds were glycosylated with an amino-propanol linker to enable their conjugation onto microarray slides. The final compounds were thoroughly characterized using NMR and mass spectrometry, and the details have been reported in my colleague Saurabh's thesis.

2.3.2 Synthesis of *N*-sulfated D-glucuronic acid based HS disaccharides.



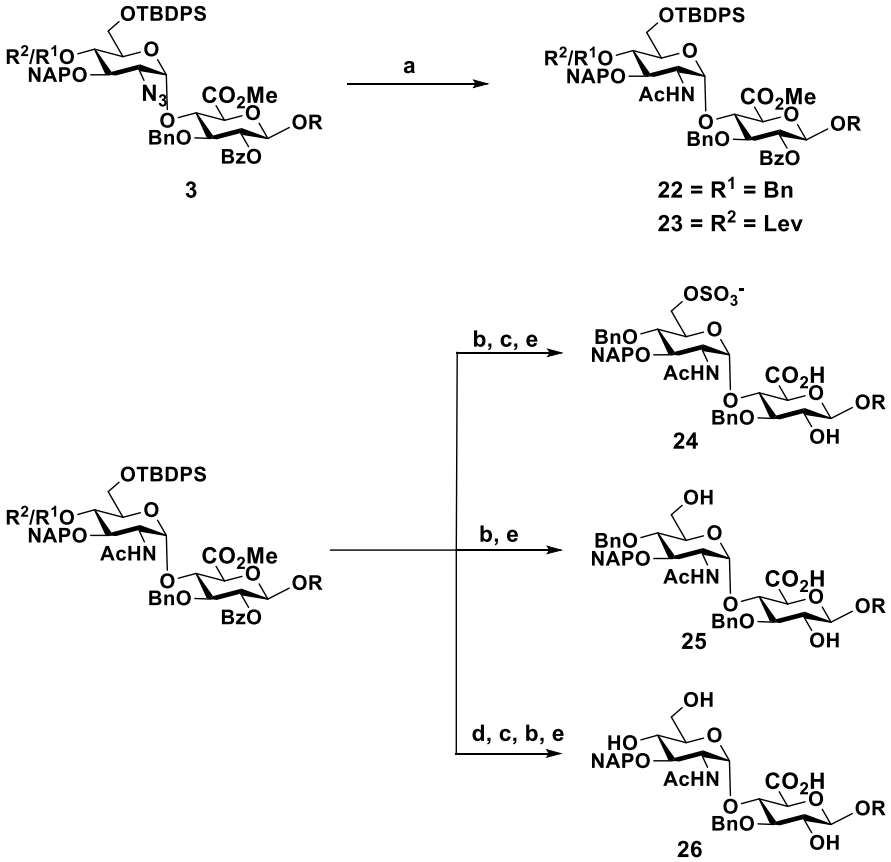


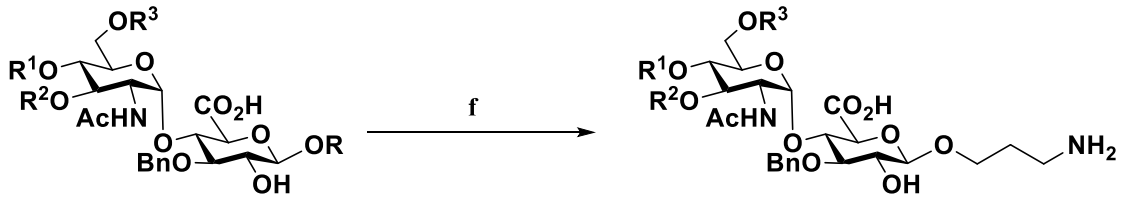
Scheme S1. Synthesis of Glucuronic acid based HS disaccharides : (a) NIS, TMSOTf, DCM, -78 °C, 15 min, 77.57 %; (b) Thiourea, Py : MeOH (1 : 1), 6 h, 89.34%; (c) (i) TEMPO, BAIB, DCM:H2O (1:1), RT, 10 h; (ii) MeI, K_2CO_3, DMF, 6 h, 72.85%; (d) DDQ, DCM:H_2O (18:1), RT, 1 h, 85-90%; (e) LiOH, H_2O : THF(1 : 1), 12 h, 91%; (f) BnBr, $NaHCO_3$, DMF, 60 °C, 2 h, 82%; (g) HF.Py, Py, 0 °C, 12 h, 63-66% ; (h) SO_3.NMe_3, DMF, 60 °C, 24 h, 59-69% ; (i) (i) PMe_3.THF, 12 h; (ii) SO_3.Py. MeOH, 1N NaOH, 48 h, 41-58%; (j) H_2, $Pd(OH)_2$, H_2O, 48 h, 40-69% .

The synthesis of HS disaccharides began with the glycosylation of compound **1a** with compound **1b** using N-iodosuccinimide (NIS) as the activator and trimethylsilyl triflate (TMSOTf) as the promoter in dichloromethane (DCM) at -78 °C for 15 minutes. This reaction yielded the disaccharide **1**. Compound **1** was then subjected to selective chloroacetate deprotection using thiourea in a pyridine:methanol mixture (1:1) for 6 hours. Subsequently, the primary hydroxyl group of compound **2** was oxidised using TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and BAIB (bis(acetoxy)iodobenzene) in a DCM:water mixture (1:1) at room temperature for 10 hours. This was followed by methylation using methyl iodide (MeI) in the presence of potassium carbonate in dimethylformamide (DMF) for 6 hours, producing compound **3**. Compound **3** underwent selective protection for specific modifications. For 3-O-sulfation, NAP deprotection was performed using DDQ

(dichlorodicyanoquinone) in a DCM:water mixture (18:1) at room temperature for 1 hour. For 6-O-sulfation, compound **3** was treated with HF:pyridine in pyridine to deprotect the TBDPS group, followed by sulphation using the SO_3·TMA complex in DMF at 60 °C for 24 hours, resulting in the O-sulphated intermediates **11**, **12**, and **13**. The methyl ester groups were then hydrolysed, and the azide group was reduced to an amine using trimethylphosphine in THF for 12 hours. N-sulphation was subsequently achieved using the SO_3·pyridine complex, yielding the desired disaccharides **14-17**. These disaccharides then underwent global deprotection via hydrogenolysis using $Pd(OH)_2$ as a catalyst in water under hydrogen gas for 48 hours. This step removed the benzyl protecting groups, yielding the fully deprotected HS derivatives **18**, **19**, **20**, and **21**, each with specific sulphation patterns (scheme S1).[27-30]

2.3.4. Synthesis of *N*-acetate D-glucuronic acid based HS disaccharides.





24 : R^1 = H, R^2 = NAP, R^3 = SO_3^-
25 : R^1 = Bn, R^2 = NAP, R^3 = H
26 : R^1 = Bn, R^2 = SO_3^-, R^3 = H

27 : R^1 = R^2 = H, R^3 = SO_3^-
28 : R^1 = R^2 = R^3 = H
29 : R^1 = R^3 = H, R^2 = SO_3^-

Scheme S2. Synthesis of Glucuronic acid based NHAc disaccharides : (a) Zn dust, THF:AcOH:Ac_2O (3:2:1), RT, 12h, 82%; (b) HF.Py, Py, 0 °C, 12 h, 65-68%; (c) SO_3.NMe_3, DMF, 60 °C, 24 h, 61-72% ; (d) DDQ, DCM:H_2O (18:1), RT, 1 h, 86-92%; (e) LiOH, H_2O : THF (1 : 1), 12 h, 92%; (**f**) H_2, $Pd(OH)_2$, H_2O, 24 h, 55-69%.

The synthesis of N-acetate HS disaccharides is outlined in Scheme S2. Briefly, compound **2** was reduced using zinc dust in a THF:AcOH:Ac_2O mixture (3:2:1) at room temperature for 12 hours, yielding compounds **22** and **23**. Subsequently, as described earlier, selective deprotection of TBDPS or NAP was performed, followed by sulfation and hydrolysis of the ester bond on the glucuronic acid unit. Finally, global deprotection was carried out through hydrogenolysis using Pd(OH)2 as the catalyst in water under hydrogen gas for 24 hours. This reaction removed all benzyl protecting groups, resulting in the fully deprotected glucuronic acid-based NHAc disaccharides **27**, **28**, and **29**.[30]

2.3.5. Microarray analysis.

				FGF1	FGF2	HER	VEGF	EGF	HB-EGF	mphiregu	BMP2
Skeleton	9OAc	ID	Name	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank
GlcNAc-IdoA	0	831	GlcNAcα(1-4)IdoA-αProNH2	0	26	17	22	45	10	29	20
	3S	834	GlcNAc(3S)α(1-4)IdoA-αProNH2	0	45	21	31	15	32	25	35
	6S	833	GlcNAc(6S)α(1-4)IdoA-αProNH2	0	45	35	38	9	41	29	29
GlcNAc-GlcA	0	832	GlcNAcα(1-4)GlcA-βProNH2	0	2	40	0	16	18	5	2
	3S	836	GlcNAc(3S)α(1-4)GlcA-βProNH2	0	38	18	25	18	24	34	22
	6S	835	GlcNAc(6S)α(1-4)GlcA-βProNH2	0	53	25	29	9	55	24	35
GlcNS-IdoA	3S	840	GlcNS(3S)α(1-4)IdoA-αProNH2	1	67	15	33	8	33	48	50
	6S	839	GlcNS(6S)α(1-4)IdoA-αProNH2	1	77	18	38	21	54	41	63
	6S-2S	846	GlcNS(6S)α(1-4)IdoA(2S)-αProNH2	100	90	100	98	59	100	72	77
	6S,3S-6S	847	GlcNS(6S,3S)α(1-4)IdoA(2S)-αProNH2	34	93	87	94	57	78	62	90
GlcNS-GlcA	3S	842	GlcNS(3S)α(1-4)GlcA-βProNH2	8	87	35	64	13	67	38	66
	6S	841	GlcNS(6S)α(1-4)GlcA-βProNH2	3	78	42	53	16	59	49	60
	6S,3S	868	GlcNS(6S,3S)α(1-4)GlcA-βProNH2	14	85	42	62	20	51	56	58
	6S,3S-2S	869	GlcNS(6S,3S)α(1-4)GlcA(2S)-βProNH2	21	77	55	57	37	56	53	52
Natural Heparin	0	1547	Heparin	53	38	79	49	79	23	63	28

Fig 2. Microarray analysis of HS-disaccharides with various growth factors.

				CCL2	CCL5	CCL7	CCL13	CCL21	CCL28	CXCL10	CXCL12	CXCL13
Skeleton	9OAc	ID	Name	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank	Mean Rank
GlcNAc-IdoA	0	831	GlcNAcα(1-4)IdoA-αProNH2	13	18	16	30	11	39	28	21	3
	3S	834	GlcNAc(3S)α(1-4)IdoA-αProNH2	25	22	54	53	41	40	65	52	4
	6S	833	GlcNAc(6S)α(1-4)IdoA-αProNH2	15	26	35	50	42	13	33	52	3
GlcNAc-GlcA	0	832	GlcNAcα(1-4)GlcA-βProNH2	0	0	0	1	0	0	2	1	3
	3S	836	GlcNAc(3S)α(1-4)GlcA-βProNH2	26	41	36	50	59	21	42	48	1
	6S	835	GlcNAc(6S)α(1-4)GlcA-βProNH2	21	31	42	55	65	28	49	58	6
GlcNS-IdoA	3S	840	GlcNS(3S)α(1-4)IdoA-αProNH2	21	48	42	46	42	11	52	52	4
	6S	839	GlcNS(6S)α(1-4)IdoA-αProNH2	24	55	58	60	59	24	48	65	3
	6S-2S	846	GlcNS(6S)α(1-4)IdoA(2S)-αProNH2	90	91	87	100	45	96	58	98	100
	6S,3S-6S	847	GlcNS(6S,3S)α(1-4)IdoA(2S)-αProNH2	95	84	89	88	41	84	92	80	53
GlcNS-GlcA	3S	842	GlcNS(3S)α(1-4)GlcA-βProNH2	55	66	51	72	54	26	55	61	9
	6S	841	GlcNS(6S)α(1-4)GlcA-βProNH2	55	50	58	61	53	40	63	61	8
	6S,3S	868	GlcNS(6S,3S)α(1-4)GlcA-βProNH2	72	75	74	72	39	63	65	64	18
	6S,3S-2S	869	GlcNS(6S,3S)α(1-4)GlcA(2S)-βProNH2	73	85	65	76	31	53	57	62	30
Natural Heparin	0	1547	Heparin	42	91	81	29	73	20	61	34	69

Fig 3. Microarray analysis of HS-disaccharides with different chemokines.

To identify hidden HS disaccharide-binding active sites on growth factors, we developed a microarray platform comprising both D-glucuronic acid and L-iduronic acid-based ligands. The synthetic HS disaccharides were printed onto epoxide-functionalized microarray slides in quadruplicate, as described in the Experimental Section, and tested against HS-binding growth factors (FGF1, FGF2, VEGF, HB-EGF, amphiregulin, and BMP2) as well as three homeostatic chemokines (CCL28, CXCL12, and CCL12) and six inflammatory chemokines [CXCL8 (IL-8), CXCL10 (IP-10), CCL2 (MCP-1), CCL7 (MCP-3), CCL13 (MCP-4), and CCL5 (RANTES)].[31] To validate the binding order of HS disaccharides to growth factors, each glycan was ranked based on the percentage of its relative fluorescent intensity compared to the optimal fluorescent signal(fig.2). Our results revealed that sulfated ligands bind strongly to most growth factors and chemokines, with a preference for highly sulfated oligosaccharides. Among these, 6SNSGlc-IdoA(2S) emerged as a potential ligand for FGF1, HER, and HB-EGF growth factors, while 63SGlc-IdoA(2S) demonstrated strong potential as a ligand for FGF2, VEGF, and BMP2 growth factors, underscoring the critical role of 3-O-sulphation in fine-tuning binding specificity(fig.3). Similarly, highly sulfated L-iduronic acid ligands exhibited stronger binding to various chemokines compared to their D-glucuronic acid counterparts. Overall, our findings indicate that highly sulfated HS ligands are promising substrates for further biological investigations. Currently, we are evaluating their binding affinities using surface plasmon resonance (SPR) and exploring their growth factor-mediated biological activities.[32]

2.4. Conclusion

We report divergent strategy to synthesize 14 HS disaccharides featuring L-iduronic acid and D-glucuronic acid ligands with various sulfation patterns. Our preliminary microarray

analysis indicated that highly sulfated oligosaccharides demonstrated preferential binding to the majority of growth factors and chemokines. Further analyses, including surface plasmon resonance (SPR) binding studies and biological activity assessments of these molecules, are currently in progress.

2.5 Experimental Section

2.5.1 General Information

All chemicals were reagent grade and used without further purification unless otherwise noted. Reactions were carried out in anhydrous solvents under a nitrogen atmosphere. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F_{254}. Spots on TLC plate were visualized under UV light or by dipping the TLC plate in CAM/ninhydrin solution followed by heating. Column chromatography was carried out using Flukakieselgel 60 (230-400 mesh). ^{1}H and ^{13}C NMR spectra of compounds were measured with Bruker 400 MHz, Bruker 600 MHz and Jeol 400 MHz using residual solvents as an internal reference ($CDCl_3$ δH 7.26 ppm, δC 77.3 ppm, CD_3OD δH 3.31 ppm, δC 49.0 ppm, and D_2O δH 4.79 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-(2-azido-2-deoxy-3-_O_-naphthylmethyl-4-_O_-levulinoyl-6-_O_-_tert_-butyldiphenylsilyl-α-D-glucopyranosyl)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-6-_O_-chloroacetyl-β-D glucopyranoside (1)

To a solution of compound **1a** donor (2g, 2.53 mmol) and **1b** acceptor (1.3 g, 2.03 mmol) in CH_2Cl_2 (30 mL) was stirred under N_2 atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. The mixture solution was cooled down to -78 ℃ followed by the addition of NIS (0.685 g, 3.04 mmol) and TMSOTf (91 µL, 0.50 mmol). The temperature was gradually increased to -20 ℃ and reaction mixture was left for stirring for another 15 minutes. After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et_3N. Molecular sieves were filtered using celite and organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/12, v/v) to obtain compound **1** (2.56 g, 77.57 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 – 8.01 (m, 2H), 7.87 – 7.79 (m, 4H), 7.64 (tt, *J* = 4.9, 1.4 Hz, 4H), 7.56 (d, *J* = 7.4 Hz, 1H), 7.50 – 7.29 (m, 18H), 7.20 (tdd, *J* = 7.8, 4.9, 1.6

Hz, 5H), 5.61 (t, *J* = 3.4 Hz, 1H), 5.32 (dt, *J* = 8.6, 4.3 Hz, 1H), 5.25 (t, *J* = 9.4 Hz, 1H), 5.06 (s, 3H), 4.96 (dd, *J* = 11.3, 3.8 Hz, 1H), 4.85 (d, *J* = 11.4 Hz, 1H), 4.81 – 4.72 (m, 2H), 4.67 – 4.56 (m, 2H), 4.19 (dd, *J* = 11.8, 5.1 Hz, 1H), 4.01 (q, *J* = 10.1, 9.3 Hz, 2H), 3.89 (dtd, *J* = 23.6, 12.1, 10.7, 4.8 Hz, 3H), 3.81 – 3.63 (m, 5H), 3.62 – 3.52 (m, 2H), 3.45 – 3.34 (m, 2H), 3.17 (dq, *J* = 20.1, 6.7 Hz, 2H), 2.50 (tdd, *J* = 8.6, 5.8, 3.0 Hz, 2H), 2.38 – 2.31 (m, 2H), 2.06 (d, *J* = 1.7 Hz, 3H), 1.75 – 1.68 (m, 2H), 1.03 (s, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 206.00, 171.19, 165.21, 156.47, 136.80, 135.81, 135.77, 135.73, 135.13, 133.50, 133.30, 133.16, 133.13, 133.06, 129.76, 129.73, 128.61, 128.50, 128.45, 128.21, 128.09, 128.04, 127.87, 127.77, 127.69, 127.65, 126.82, 126.11, 126.01, 125.96, 100.73, 97.47 (d, *J* = 5.2 Hz), 82.94, 77.87, 77.75, 74.76, 74.24, 73.75, 73.34, 72.53, 71.75, 70.18, 67.29, 66.47, 64.62, 62.83, 62.10, 40.57, 38.06, 37.66, 29.72, 29.69, 29.50, 27.91, 26.81, 19.30, 14.15. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{71}H_{77}ClN_4O_{16}Si$, 1327.4692; found, 1327.4612.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-(2-azido-2-deoxy-3-_O_-naphthylmethyl-4-_O_-levulinoyl-6-_O_-_tert_-butyldiphenylsilyl-α-D-glucopyranosyl)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-β-D glucopyranoside (2)

To a solution of **1** (1.30 g, 0.99 mmol) in pyridine/MeOH (1:1, 15 ml) at 80 °C was added thiourea (0.150 g, 1.9 mmol) and stirred under nitrogen atmosphere for 6 h. After completion of the reaction, the solvent was evaporated, then dissolved in EtOAc and washed with 1 N HCl and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 1/6, v/v), to obtain compound compound **2** (1.09 g 89.34 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06 (d, *J* = 7.7 Hz, 2H), 7.88 – 7.79 (m, 4H), 7.66 (ddd, *J* = 7.8, 2.7, 1.4 Hz, 4H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.51 – 7.31 (m, 16H), 7.24 – 7.15 (m, 5H), 5.65 (d, *J* = 3.8 Hz, 1H), 5.33 (t, *J* = 8.4 Hz, 1H), 5.24 – 5.09 (m, 2H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.88 – 4.71 (m, 3H), 4.61 (d, *J* = 7.9 Hz, 1H), 4.09 – 3.95 (m, 3H), 3.86 (q, *J* = 9.4, 8.0 Hz, 3H), 3.80 – 3.55 (m, 5H), 3.35 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.21 (q, *J* = 6.4 Hz, 2H), 2.46 (p, *J* = 6.1 Hz, 2H), 2.32 (t, *J* = 6.4 Hz, 2H), 2.05 (s, 3H), 1.72 (dt, *J* = 12.2, 6.1 Hz, 2H), 1.04 (s, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 205.96, 171.21, 165.23, 156.46, 137.40, 135.76, 135.75, 135.21, 133.43, 129.76, 129.71, 129.65, 128.59, 128.51, 128.42, 128.19, 128.10, 128.07, 128.05, 127.80, 127.68, 127.65, 126.75, 126.10, 125.99, 125.93, 101.06, 97.22, 83.34, 77.88, 75.12, 74.75, 74.08, 73.92, 72.56, 71.66, 70.38, 67.34, 66.54, 62.86,

62.42, 62.22, 38.02, 37.65, 29.70, 29.55, 27.85, 26.80, 19.26, 0.03.HR-ESI-MS *(m/z)*: $[M+Na]_+$ calcd for $C_{69}H_{76}N_4O_{15}Si$, 1251.4976; found, 1251.4956.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Methyl(2-azido-2-deoxy-3-_O_-naphthylmethyl-4-_O_-levulinoyl-6-_O_-_tert_-butyldiphenylsilyl-α-D-glucopyranosyl)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-β-D-gluco pyranosyluronate] (3)

To a solution of **2** (1.10 g, 0.89 mmol) in DCM/H_2O (2:1, 12 mL) were added 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical (TEMPO, 0.027 g, 0.17 mmol) and [Bis(acetoxy)iodo] benzene (BAIB) (1.44 g, 4.47 mmol), and stirred at RT for 10 h. The organic layer was washed with saturated NH_4Cl solution and concentrated. Then, the crude product was dissolved in DMF (10 mL), mixed with K_2CO_3 (0.37 g, 2.66 mmol), MeI (0.50 mL, 8.04 mmol), and stirred overnight at RT. After completion of the reaction, the reaction mixture was extracted with EtOAc and brine. The organic layer was concentrated and purified through silica gel column chromatography (EtOAc/Hexane = 1/8, v/v) to obtain compound compound **3** (816 mg 72.85 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 – 8.02 (m, 2H), 7.86 – 7.78 (m, 4H), 7.64 (ddt, *J* = 9.8, 6.6, 1.5 Hz, 4H), 7.57 (s, 1H), 7.46 (ddt, *J* = 7.9, 6.2, 3.1 Hz, 5H), 7.42 – 7.27 (m, 13H), 7.21 (d, *J* = 1.6 Hz, 4H), 5.48 (d, *J* = 3.6 Hz, 1H), 5.39 – 5.31 (m, 2H), 5.06 (d, *J* = 17.6 Hz, 2H), 4.95 (d, *J* = 11.4 Hz, 1H), 4.83 – 4.70 (m, 3H), 4.65 (d, *J* = 7.1 Hz, 1H), 4.27 (dd, *J* = 9.2, 8.2 Hz, 1H), 4.12 (d, *J* = 9.2 Hz, 1H), 4.05 – 3.85 (m, 3H), 3.66 (qd, *J* = 11.7, 2.7 Hz, 2H), 3.59 – 3.49 (m, 5H), 3.35 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.16 (dp, *J* = 26.9, 6.8 Hz, 2H), 2.49 (dt, *J* = 9.1, 6.6 Hz, 2H), 2.34 – 2.26 (m, 2H), 2.05 (s, 3H), 1.70 (dd, *J* = 12.9, 6.8 Hz, 2H), 1.03 (s, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 206.08, 156.47, 137.24, 135.82, 135.76, 133.52, 133.30, 129.79, 129.64, 128.62, 128.47, 128.43, 128.17, 128.02, 127.98, 127.88, 127.69, 127.62, 126.87, 126.12, 126.04, 126.01, 100.76, 97.38, 81.93, 77.74, 74.64, 74.52, 74.08, 73.80, 73.21, 71.11, 69.89, 67.22, 66.41, 63.01, 61.64, 52.57, 37.94, 37.68, 29.73, 29.35, 27.84, 26.78, 19.31. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{70}H_{76}N_4O_{16}Si$, 1279.4926; found, 1279.4924.

General Experimental Procedures

General procedure for TBDPS deprotection

To a solution of TBDPS protected HS oligosaccharides (1 mmol) in pyridine (5 ml) was added 70% HF.Py (5 mmol) at 0 °C and stirred at RT overnight. After completion, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine. The organic

layer was dried using anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica column chromatography or sephadex LH-20 column depending on the sulfate group in the starting material.

General procedure for NAP deprotection

To a solution of NAP-protected HS oligosaccharides in DCM/H2O (18:1, 10 mL for 0.620 mg) was added DDQ (3 equiv) and stirred under nitrogen atmosphere at 0 °C for 3-4 h. After completion of the reaction, the mixture was diluted with DCM and washed with brine. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/Hexane).

General procedure for *O*-Sulfation

To a solution of 6-*O* / 3-*O* / 2-*O*-hydroxyl group HS oligosaccharides (1 mmol) in DMF (5 mL) was added $SO_3{\cdot}NMe_3$/$SO_3{\cdot}NEt_3$ (10 mmol per -OH) and stirred at 60 °C for 72 h. DMF was evaporated and the crude product was purified by column chromatography (MeOH/DCM).

General procedure for Saponification

To a solution of HS oligosaccharides (1 mmol) in a mixture of THF/H_2O (1:1, 4 ml) was added 1 M $LiOH{\cdot}H_2O$ (3 mmol) and stirred at RT for 15 h. After completion of the reaction, the reaction mixture was diluted with MeOH and neutralized using Dowex 50WX8 H+ resin. The reaction was filtered and concentrated under reduced pressure and purified by bond elute C-18 column (H_2O/ACN).

General procedure for Global deprotection

To a solution of HS oligosaccharides (1 mmol) in H_2O (4 ml) was added $Pd(OH)_2$ (10% by wt.) and stirred for 24 h under hydrogen atmosphere. After completion of the reaction, the reaction mixture was diluted with H_2O, filtered and concentrated, and finally purified by bond elute C-18 column (H_2O).

General procedure for Azide reduction

Trimethyl phosphine (PMe_3, 1.0 M sol in THF, 8 equiv. per azide group) and aq. NaOH (1 M, 2.0 equiv.) solution were added to the solution of starting material in THF (1.0 mL for 0.013 mmol) and the reaction mixture was stirred for 12 h. The progress of reaction was

monitored by TLC (EtOAc/MeOH/H2O, 7/2/1, v/v/v). Upon completion, the pH was adjusted to 8.0–8.5 by careful addition of glacial AcOH, and the mixture was concentrated in vacuo. The residue obtained was purified by reverse phase chromatography (C-18 silica gel column). The appropriate fractions were concentrated in vacuo, and the residue was passed through a column DowexR 50 x 8 Na+ resin using MeOH/H_2O as eluent. The appropriate fractions were combined and lyophilized to obtain desired product as sodium salt.

General procedure for *N*-sulfation

To the stirring solution of starting material in a mixture of MeOH (1.0 mL for 0.006 mmol), Et_3N (0.3 mL) and aq. NaOH solution (1 M, 2.0 equiv.) at 0 ºC was added SO_3.Py complex (5.0 equiv. per amine). The progress of reaction was monitored by TLC (EtOAc/pyridine/H_2O/AcOH 8/5/3/1, v/v/v/v). Two additional portions of SO_3.Py complex were added at 0 ºC after 1h and 2h. After stirring for an additional 8h, the reaction mixture was concentrated in vacuo and the residue was passed through a column of DowexR 50 x 8 Na+ resin using H_2O/MeOH as eluent to obtain desired product. The appropriate fractions were concentrated in vacuo and further was purified by reverse phase C-18 silica gel column using H_2O/MeOH as eluent. Appropriate fractions were lyophilized to obtain desired product as sodium salt.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Methyl(2-azido-2-deoxy-4-*O*-levulinoyl-6-*O*-*tert*-butyl diphenyl silyl-α-D-glucopyranosyl)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-gluco pyranosyluronate] (6)**

Disaccharide **3** (490 mg, 0.38 mmol) was subjected to the NAP deprotection according to the general procedures to give disaccharide **6** (405 mg, 85.49 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 – 8.00 (m, 2H), 7.64 (tt, *J* = 7.0, 1.5 Hz, 4H), 7.59 – 7.53 (m, 1H), 7.46 – 7.28 (m, 14H), 7.22 (d, *J* = 1.2 Hz, 4H), 5.46 (d, *J* = 3.7 Hz, 1H), 5.37 – 5.29 (m, 1H), 5.20 (t, *J* = 9.6 Hz, 1H), 5.05 (d, *J* = 12.4 Hz, 3H), 4.81 (d, *J* = 10.6 Hz, 1H), 4.73 (d, *J* = 10.6 Hz, 1H), 4.64 (d, *J* = 7.1 Hz, 1H), 4.26 (t, 1H), 4.08 (d, *J* = 9.3 Hz, 2H), 3.99 (t, 1H), 3.90 (ddd, 1H), 3.72 (m, 2H), 3.56 (d, *J* = 7.2 Hz, 4H), 3.40 (d, *J* = 3.9 Hz, 1H), 3.28 – 3.07 (m, 3H), 2.89 (ddd, *J* = 18.6, 8.9, 5.3 Hz, 1H), 2.77 – 2.66 (m, 1H), 2.51 (dd, *J* = 8.9, 5.0 Hz, 1H), 2.38 (s, 1H), 2.19 (s, 3H), 1.72 (d, *J* = 5.9 Hz, 2H), 1.03 (s, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 207.70, 156.46, 135.76, 135.70, 133.50, 129.77, 129.69, 128.60, 128.46, 128.40, 127.99, 127.97, 127.92, 127.85, 127.66, 127.64, 100.78, 97.40, 82.07, 77.26,

74.58, 74.28, 74.00, 73.34, 71.58, 70.72, 70.29, 67.23, 66.40, 62.98, 61.70, 52.56, 38.49, 37.93, 29.78, 29.33, 28.12, 26.79, 19.28. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{59}H_{68}N_4O_{16}Si$, 1139.4300; found, 1139.4312.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Methyl(2-azido-2-deoxy-3-_O_-naphthylmethyl-4-_O_-levulinoyl-α-D-glucopyranosyl)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-β-D-gluco pyranosyluronate] (7)

Disaccharide **3** (380 mg, 0.38 mmol) was subjected to the TBDPS deprotection as described in general procedures to give disaccharide **7** (208 mg, 67 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06 – 8.00 (m, 2H), 7.86 – 7.77 (m, 4H), 7.56 (d, *J* = 7.4 Hz, 1H), 7.51 – 7.40 (m, 6H), 7.39 – 7.29 (m, 6H), 7.23 – 7.15 (m, 5H), 5.53 (d, *J* = 3.8 Hz, 1H), 5.37 – 5.29 (m, 1H), 5.15 (d, *J* = 7.3 Hz, 1H), 5.06 (s, 2H), 4.99 (dd, *J* = 10.8, 7.8 Hz, 2H), 4.86 (d, *J* = 11.8 Hz, 1H), 4.80 – 4.70 (m, 2H), 4.68 (d, *J* = 6.8 Hz, 1H), 4.34 (dd, *J* = 9.4, 8.3 Hz, 1H), 4.14 (d, *J* = 9.5 Hz, 1H), 4.03 – 3.90 (m, 3H), 3.76 (s, 3H), 3.68 – 3.59 (m, 1H), 3.58 – 3.46 (m, 3H), 3.36 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.20 (dp, *J* = 25.7, 6.9 Hz, 2H), 2.70 – 2.54 (m, 2H), 2.51 – 2.46 (m, 1H), 2.45 – 2.41 (m, 1H), 2.25 – 2.15 (m, 1H), 2.09 (s, 3H), 1.76 – 1.70 (m, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 206.45, 172.91, 168.94, 165.19, 156.64, 137.24, 135.34, 133.65, 133.35, 133.09, 129.87, 128.71, 128.58, 128.33, 128.14, 128.11, 127.97, 127.92, 127.78, 126.57, 126.32, 126.16, 125.74, 100.79, 97.58, 82.13, 77.64, 77.37, 75.08, 74.84, 74.52, 74.39, 73.62, 71.11, 70.92, 67.42, 66.54, 62.99, 60.70, 53.00, 38.14, 37.70, 29.39, 27.84. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{54}H_{58}N_4O_{16}$, 1041.3748; found, 1041.3726.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Methyl(2-azido-2-deoxy-4-_O_-levulinoyl-3-_O_-sulfonate-α-D-glucopyranoside)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-β-D-gluco pyranosyluronate] (12)

Disaccharide **6** (360 mg, 0.32 mmol) was subjected to the sequence of *O*-sulfation and TBDPS deprotection as described in general procedures to give disaccharide **12**(142 mg, 46.01 % over 2 steps).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.12 – 8.04 (m, 2H), 7.67 – 7.58 (m, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 7.38 – 7.28 (m, 5H), 7.17 (s, 5H), 5.59 (d, *J* = 3.6 Hz, 1H), 5.23 (t, *J* = 8.1 Hz, 1H), 5.11 (t, *J* = 9.6 Hz, 1H), 5.04 (s, 2H), 4.92 (d, *J* = 10.7 Hz, 1H), 4.84 – 4.80 (m, 1H), 4.70 (d, *J* = 10.6 Hz, 1H), 4.59 (s, 1H), 4.28 – 4.12 (m, 3H), 3.85 (s, 4H), 3.71 – 3.65 (m, 1H), 3.61 – 3.54 (m, 3H), 3.43 – 3.37 (m, 1H), 3.07 (td, *J* = 6.7, 4.6 Hz, 2H), 2.86 (dt, *J* = 7.8, 6.5 Hz,

2H), 2.64 (dd, *J* = 6.9, 5.2 Hz, 2H), 2.19 (s, 3H), 1.67 (dq, *J* = 13.0, 6.7, 5.5 Hz, 2H). ^{13}C NMR (400 MHz, Methanol-d_4) δ 208.97, 172.49, 169.28, 165.54, 137.51, 133.37, 129.43, 128.42, 128.10, 127.91, 127.59, 127.40, 127.37, 100.79, 97.82, 82.12, 75.85, 75.07, 74.64, 74.07, 73.70, 70.70, 68.63, 67.19, 65.94, 61.91, 59.50, 52.22, 48.29, 48.08, 47.87, 47.65, 47.44, 47.23, 47.01, 37.39, 29.37, 28.34, 27.88. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{43}H_{49}N_4O_{19}S^-$, 957.2717; found, 957.2716.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Methyl(2-azido-2-deoxy-3-_O_-naphthylmethyl-4-_O_-levulinoyl-6-O-sulfonate-α-D-glucopyranosyl)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-β-D-gluco pyranosyluronate] (13)

Disaccharide **7** (208 mg, 0.20 mmol) was subjected to *O*-sulfation and as described in general procedures to give disaccharide **13** (156 mg, 69.64 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 – 7.96 (m, 2H), 7.82 – 7.73 (m, 4H), 7.57 – 7.51 (m, 1H), 7.46 – 7.37 (m, 5H), 7.30 (q, *J* = 7.0, 6.5 Hz, 4H), 7.11 (q, *J* = 3.2, 2.1 Hz, 4H), 5.38 (d, *J* = 3.2 Hz, 1H), 5.27 (t, *J* = 7.5 Hz, 1H), 5.18 – 5.01 (m, 3H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.80 – 4.72 (m, 2H), 4.62 (dd, *J* = 16.2, 9.0 Hz, 2H), 4.24 (t, *J* = 8.9 Hz, 1H), 4.04 (dt, *J* = 18.7, 10.3 Hz, 3H), 3.91 (dt, *J* = 13.6, 9.1 Hz, 3H), 3.77 (d, *J* = 9.1 Hz, 3H), 3.64 (d, *J* = 8.6 Hz, 1H), 3.49 – 3.41 (m, 1H), 3.37 – 3.28 (m, 1H), 3.07 (p, *J* = 6.3 Hz, 2H), 2.64 (s, 2H), 2.58 – 2.46 (m, 3H), 2.34 – 2.23 (m, 1H), 1.99 (s, 3H), 1.69 – 1.57 (m, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 209.02, 172.66, 169.17, 165.16, 156.58, 137.24, 136.87, 135.19, 133.47, 133.23, 132.99, 129.76, 129.36, 128.57, 128.45, 128.36, 128.14, 128.01, 127.92, 127.81, 127.67, 126.85, 126.15, 126.02, 100.79, 97.52, 81.96, 74.48, 73.40, 67.67, 66.41, 62.50, 53.50, 38.01, 37.46, 29.66, 29.41, 27.94. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{54}H_{57}N_4O_{19}S^-$, 1120.3243; found, 1120.3259.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-(2-sulfamido2-deoxy-3-_O_-naphthylmethyl-6-O-sulfonate-α-D-glucopyranosyl)-(1→4)-_O_-3-_O_-benzyl-β-D-gluco pyranosyluronate (17)

Disaccharide **13** (156 mg, 0.14 mmol) was subjected to the sequence of saponification, azide reduction and *N*-sulfation according to the general procedures to give sulfated disaccharide **17** (60.20 mg, 45.33 % over 3 steps).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.90 (d, *J* = 1.6 Hz, 1H), 7.86 – 7.78 (m, 3H), 7.57 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.35 (d, *J* = 1.8 Hz, 1H), 7.33 (d, *J* = 1.5 Hz, 1H), 7.31 – 7.22 (m, 8H), 5.59 (d, *J* = 3.5 Hz, 1H), 4.97 (d, *J* = 11.3 Hz, 3H), 4.88 – 4.82 (m, 1H), 4.78 – 4.71 (m, 2H), 4.35 – 4.30 (m, 1H), 4.20 (dd, *J* = 11.0, 3.1 Hz, 1H), 4.07 (dd, *J* =

10.9, 2.1 Hz, 1H), 3.84 – 3.76 (m, 2H), 3.71 (td, *J* = 8.0, 6.5, 3.7 Hz, 3H), 3.59 – 3.51 (m, 3H), 3.39 (t, *J* = 8.3 Hz, 1H), 3.32 – 3.26 (m, 1H), 3.11 (q, *J* = 6.4 Hz, 2H), 1.67 (p, *J* = 6.4 Hz, 2H).

^{13}C NMR (400 MHz, Deuterium Oxide) δ 175.00, 137.50, 135.66, 133.00, 132.85, 128.84, 128.81, 128.68, 128.39, 128.22, 128.08, 127.72, 127.56, 127.05, 126.44, 102.18, 96.32, 83.70, 79.32, 76.89, 74.79, 73.50, 73.07, 70.25, 68.92, 67.84, 66.86, 66.50, 57.38, 37.42, 28.75. HR-ESI-MS *(m/z)*: $[M-2H]^{2-}$ calcd for $C_{41}H_{46}N_2O_{19}S_2^{2-}$, 467.1073; found, 467.1072.

3-aminopropyl-*O*-(2-deoxy-2-sulfamido-6-O-sulfonate-α-D-glucopyranosyl)-(1→4)-β-D-gluco pyranosyluronate (21)

Compound **17** (48.35 mg, 0.05 mmol) was subjected to Global deprotection as described in General procedure to obtain **21** (16.4 mg, 62.50%).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.56 (d, *J* = 3.9 Hz, 1H), 4.45 (dt, *J* = 8.0, 1.7 Hz, 1H), 4.25 (dt, *J* = 10.7, 3.0 Hz, 1H), 4.09 (dt, *J* = 11.2, 2.5 Hz, 1H), 3.95 – 3.89 (m, 1H), 3.77 (dddd, *J* = 24.4, 16.1, 9.2, 3.0 Hz, 5H), 3.54 – 3.46 (m, 2H), 3.34 – 3.27 (m, 1H), 3.22 – 3.17 (m, 1H), 3.08 (q, *J* = 6.0, 5.3 Hz, 2H), 1.92 (t, *J* = 6.7 Hz, 2H).^{13}C NMR (400 MHz, Deuterium Oxide) δ 174.32, 102.19, 97.37, 76.12, 75.93, 72.64, 71.03, 69.86, 68.98, 67.98, 66.36, 57.82, 37.64, 26.66. HR-ESI-MS *(m/z)*: $[M-2H]^{2-}$ calcd for $C_{15}H_{26}N_2O_{17}S_2^{2-}$, 285.0342; found, $^{-}$ 285.0345.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Methyl(2-azido-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate] (9)**

Disaccharide **6** (364 mg, 0.32 mmol) was subjected to the TBDPS deprotection as described in general procedures to give disaccharide **9** (182 mg, 63.63 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.05 – 7.98 (m, 2H), 7.60 – 7.53 (m, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.37 – 7.28 (m, 5H), 7.23 – 7.15 (m, 5H), 5.49 (d, *J* = 3.8 Hz, 1H), 5.35 – 5.28 (m, 1H), 5.15 (t, *J* = 6.0 Hz, 1H), 5.04 (s, 2H), 4.85 (t, *J* = 9.5 Hz, 1H), 4.80 – 4.64 (m, 3H), 4.33 (dd, *J* = 9.5, 8.3 Hz, 1H), 4.10 (dd, *J* = 9.6, 6.8 Hz, 2H), 4.00 – 3.88 (m, 2H), 3.77 (s, 3H), 3.71 – 3.63 (m, 1H), 3.60 – 3.49 (m, 3H), 3.29 – 3.10 (m, 3H), 2.88 – 2.78 (m, 2H), 2.63 – 2.48 (m, 2H), 2.19 (s, 3H), 1.72 (q, *J* = 6.1 Hz, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 207.83, 173.32, 168.95, 165.14, 156.57, 137.16, 133.54, 129.77, 129.31, 128.60, 128.48, 128.37, 128.04, 128.01, 127.90, 127.86, 100.68, 97.45, 82.13, 74.68, 74.44, 74.33, 73.56, 71.89, 70.33, 69.99, 67.34, 66.46, 62.81, 60.88, 52.90, 38.44, 38.08, 29.71, 29.67, 29.29, 28.11. HR-ESI-MS *(m/z)*: $[M+Na]_+$ calcd for $C_{43}H_{50}N_4O_{16}$, 901.3122; found, 901.3156.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[(2-deoxy-2-sulfamino-3-*O*-sulfonate-α-D-glucopyranoside]-(1→4)-*O*-3-*O*-benzyl-β-D-glucopyranosyluronate (16)**

Disaccharide **12** (142 mg, 0.14 mmol) was subjected to the sequence of saponification, azide reduction and *N*-sulfation according to the general procedures to give sulfated disaccharide **16** (62 mg, 41.38 % over 3 steps).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.55 – 7.35 (m, 10H), 5.74 (d, *J* = 3.3 Hz, 1H), 5.12 (s, 2H), 5.01 (d, *J* = 10.2 Hz, 1H), 4.90 (d, *J* = 10.3 Hz, 1H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.40 (dd, *J* = 10.6, 8.2 Hz, 1H), 3.93 (s, 3H), 3.84 – 3.77 (m, 2H), 3.76 (d, *J* = 0.9 Hz, 1H), 3.72 – 3.61 (m, 3H), 3.48 (t, *J* = 8.2 Hz, 1H), 3.40 (dd, *J* = 10.6, 3.3 Hz, 1H), 3.25 (d, *J* = 5.8 Hz, 2H), 1.82 (q, *J* = 6.5 Hz, 2H). ^{13}C NMR (400 MHz, Deuterium Oxide) δ 174.43, 158.41, 137.42, 136.59, 128.95, 128.78, 128.64, 128.33, 128.27, 127.60, 102.09, 96.37, 83.66, 78.45, 74.33, 73.68, 72.22, 68.70, 67.84, 66.79, 60.29, 56.60, 55.43, 37.35, 28.67, 27.50, 19.12. HR-ESI-MS *(m/z)*: $[M-2H]^{2-}$, calcd for $C_{30}H_{38}N_2O_{19}S_2^{2-}$, 397.0760; found, 397.0726.

3-aminopropyl-*O*-(2-deoxy-2-sulfamino-3-*O*-sulfonate-α-D-glucopyranoside)-(1→4)-β-D-glucopyranosyluronate (20)

Compound **16** (62 mg, 0.06 mmol) was subjected to Global deprotection as described in General procedure to obtain **20** (16 mg, 45.84%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.61 (d, *J* = 3.5 Hz, 1H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.38 (dd, *J* = 10.7, 8.9 Hz, 1H), 4.02 (dt, *J* = 11.5, 5.9 Hz, 1H), 3.92 – 3.77 (m, 7H), 3.69 (t, *J* = 9.3 Hz, 1H), 3.43 – 3.36 (m, 2H), 3.18 (t, *J* = 6.9 Hz, 2H), 2.02 (p, *J* = 6.6 Hz, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 104.73, 100.75, 81.17, 80.52, 79.19, 75.06, 74.27, 70.88, 70.53, 62.46, 59.34, 40.17, 29.19. HR-ESI-MS *(m/z)*: $[M-2H]^{2-}$, calcd for $C_{15}H_{26}N_2O_{17}S_2^{2-}$ is 285.0342; found, 285.0341.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Methyl(2-azido-2-deoxy-4-*O*-Benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-glucopyranosyl)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl uronate] (5)**

Disaccharide **3 (R^1= Bn)** (1g, 0.80 mmol) was subjected to the NAP deprotection according to the general procedures to give disaccharide **5** (800 mg, 90.19 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.05 – 8.01 (m, 2H), 7.67 (tt, *J* = 6.5, 1.5 Hz, 4H), 7.59 – 7.54 (m, 1H), 7.45 – 7.38 (m, 4H), 7.36 – 7.29 (m, 12H), 7.27 (d, *J* = 2.2 Hz, 1H), 7.25 (d, *J* = 1.7 Hz, 1H), 7.22 (d, *J* = 3.4 Hz, 5H), 5.44 (d, *J* = 3.7 Hz, 1H), 5.34 (dd, *J* = 8.1, 7.0 Hz, 1H), 5.08 (t, *J* = 5.6 Hz, 1H), 5.04 (s, 2H), 4.83 – 4.77 (m, 3H), 4.74 (d, *J* = 10.6 Hz, 1H),

4.63 (d, *J* = 7.0 Hz, 1H), 4.24 (dd, *J* = 9.2, 8.2 Hz, 1H), 4.08 – 4.00 (m, 2H), 4.00 – 3.94 (m, 2H), 3.92 – 3.85 (m, 2H), 3.75 (t, *J* = 9.4 Hz, 1H), 3.52 (s, 3H), 3.41 (dt, *J* = 9.8, 2.0 Hz, 1H), 3.24 – 3.04 (m, 3H), 1.70 (dd, *J* = 13.2, 7.1 Hz, 2H), 1.08 (s, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 168.71, 165.19, 156.47, 138.32, 137.31, 136.83, 135.90, 135.63, 133.58, 133.50, 132.90, 129.78, 129.75, 129.40, 128.69, 128.60, 128.46, 128.40, 128.02, 127.99, 127.96, 127.95, 127.86, 127.76, 127.69, 127.66, 100.73, 97.53, 82.15, 77.98, 77.27, 74.96, 74.50, 74.01, 73.40, 72.01, 71.59, 67.19, 66.40, 62.93, 61.98, 52.63, 37.91, 29.31, 26.97, 19.43. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{61}H_{68}N_4O_{14}Si$, 1131.4401; found 1131.4426.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Benzyl(2-azido-2-deoxy-4-_O_-Benzyl-6-_O_-_tert_-butyldiphenylsilyl-α-D-glucopyranosyl)-(1→4)-_O_-3-_O_-benzyl-β-D-glucopyranosyl uronate] (4)

Disaccharide **3** (800 mg, 0.64 mmol) was subjected to the sequence of NAP deprotection and saponification according to the general procedures to give crude disaccharide. Then, the crude product was dissolved in DMF (10 mL), mixed with $NaHCO_3$ (77 mg, 0.90 mmol), BnBr (0.087 mL, 0.7 mmol), TBAI (94 mg, 0.25 mmol) and stirred 2 h at 60 ℃. After completion of the reaction, the reaction mixture was extracted with EtOAc and brine. The organic layer was concentrated and purified through silica gel column chromatography (EtOAc/Hexane = 1/8, v/v), and yielded compound **4** (314 mg, 45.35 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (ddd, *J* = 9.8, 8.0, 1.5 Hz, 4H), 7.45 – 7.38 (m, 3H), 7.38 – 7.26 (m, 17H), 7.22 (dt, *J* = 7.0, 2.5 Hz, 1H), 7.19 (s, 3H), 7.18 – 7.14 (m, 1H), 5.55 (d, *J* = 3.6 Hz, 1H), 5.08 (d, *J* = 7.2 Hz, 2H), 5.04 – 4.94 (m, 2H), 4.90 (d, *J* = 12.4 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.75 (s, 2H), 4.25 (d, *J* = 7.5 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.98 – 3.93 (m, 1H), 3.93 – 3.85 (m, 3H), 3.72 (dt, *J* = 16.6, 8.9 Hz, 2H), 3.62 (t, *J* = 8.2 Hz, 1H), 3.56 – 3.37 (m, 3H), 3.27 (s, 1H), 3.20 (dq, *J* = 14.0, 5.7 Hz, 1H), 3.10 (dd, *J* = 10.4, 3.6 Hz, 1H), 1.68 (d, *J* = 7.6 Hz, 2H), 1.07 (s, 9H).^{13}C NMR (400 MHz, Chloroform-*d*) δ 168.32, 156.80, 138.41, 138.35, 136.53, 135.91, 135.65, 134.68, 133.65, 132.98, 129.71, 128.62, 128.55, 128.51, 128.48, 128.37, 128.25, 128.17, 127.97, 127.88, 127.81, 127.74, 127.73, 127.64, 102.98, 97.27, 83.97, 78.05, 75.05, 74.54, 74.38, 73.61, 71.95, 71.56, 67.46, 67.03, 66.82, 62.94, 61.99, 37.66, 29.66, 26.98, 19.43. HR-ESI-MS *(m/z)*: $[M+Na]^+$ calcd for $C_{60}H_{68}N_4O_{13}Si$, 1103.4452; found 1103.4426.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Benzyl(2-azido-2-deoxy-4-*O*-Benzyl-α-D-glucopyranosyl)-(1→4)-*O*-3-*O*-benzyl-β-D-glucopyranosyl uronate] (8)**

Disaccharide **4** (314 mg, 0.29 mmol) was subjected to the TBDPS deprotection as described in general procedures to give disaccharide **8** (163 mg, 66.61 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.32 (m, 2H), 7.32 – 7.21 (m, 18H), 5.50 (d, *J* = 3.8 Hz, 1H), 5.15 (d, *J* = 12.3 Hz, 1H), 5.07 – 4.99 (m, 4H), 4.77 (d, *J* = 10.8 Hz, 1H), 4.69 (d, *J* = 1.2 Hz, 2H), 4.22 (d, *J* = 7.5 Hz, 1H), 4.08 (dd, *J* = 9.6, 8.4 Hz, 1H), 3.95 – 3.84 (m, 3H), 3.76 (ddd, *J* = 12.2, 4.2, 2.1 Hz, 1H), 3.70 – 3.54 (m, 3H), 3.50 (ddd, *J* = 9.6, 7.9, 4.2 Hz, 1H), 3.46 – 3.38 (m, 2H), 3.35 (dd, *J* = 8.5, 5.0 Hz, 1H), 3.23 – 3.12 (m, 1H), 3.03 (dd, *J* = 10.5, 3.8 Hz, 1H), 1.72 – 1.59 (m, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 168.49, 156.83, 138.31, 137.98, 136.50, 134.64, 128.70, 128.65, 128.59, 128.56, 128.49, 128.44, 128.19, 128.08, 127.98, 127.74, 103.06, 97.45, 83.97, 77.85, 75.04, 74.80, 74.47, 71.62, 67.79, 67.14, 66.85, 62.91, 61.27, 37.69, 29.66. HR-ESI-MS *(m/z)*: $[M+Na]^{+}$ calcd for $C_{44}H_{50}N_4O_{13}$, 865.3274; found 865.3264.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Benzyl(2-azido-2-deoxy-4-*O*-Benzyl-3-*O*-sulfonate-6-*O*-sulfonate-α-D-glucopyranoside)-(1→4)-*O*-2-*O*-sulfonate-3-*O*-benzyl-β-D-gluco pyranosyluronate] (10)**

Disaccharide **8** (163 mg, 0.19 mmol) was subjected to *O*-sulfation as described in general procedures to give disaccharide **10** (123 mg, 58.90 %).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.48 – 7.44 (m, 2H), 7.40 – 7.34 (m, 2H), 7.25 – 7.06 (m, 17H), 5.40 (d, *J* = 3.6 Hz, 1H), 5.09 – 4.99 (m, 3H), 4.96 – 4.90 (m, 3H), 4.78 (d, *J* = 4.2 Hz, 1H), 4.67 – 4.63 (m, 1H), 4.54 (dd, *J* = 12.3, 9.9 Hz, 2H), 4.39 – 4.34 (m, 1H), 4.32 – 4.19 (m, 2H), 4.09 – 4.02 (m, 2H), 3.86 (dd, *J* = 8.5, 5.3 Hz, 1H), 3.67 (dt, *J* = 9.7, 6.1 Hz, 1H), 3.58 (dd, *J* = 10.0, 8.6 Hz, 1H), 3.52 – 3.46 (m, 1H), 3.43 – 3.35 (m, 1H), 3.12 (t, *J* = 6.4 Hz, 2H), 2.97 (dd, *J* = 10.4, 3.6 Hz, 1H), 1.60 (dt, *J* = 13.8, 6.8 Hz, 2H). ^{13}C NMR (400 MHz, Methanol-d_4) δ 169.91, 157.52, 138.46, 137.95, 137.04, 135.07, 129.23, 128.41, 128.33, 128.24, 128.09, 128.06, 127.90, 127.65, 127.52, 127.40, 127.22, 100.71, 97.82, 81.68, 79.20, 78.38, 75.95, 74.70, 73.62, 72.86, 69.98, 67.45, 66.59, 66.00, 65.31, 62.05, 47.71, 47.50, 47.29, 47.08, 37.40, 29.08. HR-ESI-MS *(m/z)*: $[M-3H]^{3-}$, calcd for $C_{44}H_{47}N_4O_{22}S_3^{3-}$, 359.7287; found 359.7269.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Methyl(2-azido-2-deoxy-4-*O*-Benzyl-α-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl 3-*O*-benzyl-β-D-gluco pyranosyluronate] (9)**

Disaccharide **5** (421 mg, 0.37 mmol) was subjected to the TBDPS deprotection as described in general procedures to give disaccharide **9** (232 mg, 70.23 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 – 7.96 (m, 2H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.26 (m, 10H), 7.16 – 7.10 (m, 5H), 5.46 (d, *J* = 3.8 Hz, 1H), 5.31 (dd, *J* = 8.0, 6.8 Hz, 1H), 5.25 (d, *J* = 6.0 Hz, 1H), 5.03 (s, 2H), 4.74 (d, *J* = 6.5 Hz, 2H), 4.70 (d, *J* = 9.1 Hz, 1H), 4.67 – 4.62 (m, 1H), 4.34 (t, *J* = 9.0 Hz, 1H), 4.07 (d, *J* = 9.4 Hz, 1H), 3.98 (d, *J* = 13.9 Hz, 1H), 3.95 – 3.82 (m, 3H), 3.73 (s, 3H), 3.48 (dd, *J* = 12.0, 6.9 Hz, 2H), 3.40 (d, *J* = 2.8 Hz, 1H), 3.25 – 3.10 (m, 3H), 1.70 (d, *J* = 6.8 Hz, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 169.08, 165.22, 156.62, 138.06, 137.20, 136.81, 133.51, 129.77, 129.30, 128.71, 128.57, 128.48, 128.34, 128.17, 128.03, 128.01, 127.99, 127.95, 127.82, 100.66, 97.57, 82.26, 77.72, 74.92, 74.45, 74.25, 73.51, 71.77, 67.22, 66.44, 62.89, 61.06, 52.90, 38.00, 29.22. HR-ESI-MS *(m/z)*: $[M+Na]^{+}$ calcd for $C_{445}H_{50}N_4O_{14}$ is 893.3224; found 893.3245.

***N*-benzyloxycarbonyl-3-aminopropyl-*O*-[Methyl(2-azido-2-deoxy-4-*O*-Benzyl-3-*O*-sulfonate-6-*O*-sulfonate-α-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate] (11)**

Disaccharide **9** (232 mg, 0.26 mmol) was subjected to *O*-sulfation as described in general procedures to obtain disaccharide **11**(184 mg, 67.13 %)

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.07 (d, *J* = 7.8 Hz, 1H), 7.67 – 7.56 (m, 2H), 7.50 (t, *J* = 7.8 Hz, 1H), 7.39 – 7.23 (m, 4H), 7.17 (tq, *J* = 6.8, 2.5, 1.8 Hz, 2H), 5.53 – 5.44 (m, 1H), 5.21 (dd, *J* = 9.1, 5.6 Hz, 2H), 5.03 (s, 2H), 4.97 (dd, *J* = 10.7, 6.5 Hz, 1H), 4.85 – 4.80 (m, 5H), 4.68 (dd, *J* = 15.3, 10.0 Hz, 2H), 4.35 (dd, *J* = 10.8, 2.7 Hz, 1H), 4.25 – 4.08 (m, 4H), 3.87 (dq, *J* = 10.9, 6.0 Hz, 1H), 3.80 – 3.75 (m, 3H), 3.68 – 3.50 (m, 2H), 3.06 (t, *J* = 6.7 Hz, 2H), 1.66 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (400 MHz, Methanol-d_4) δ 169.10, 165.47, 157.35, 138.49, 137.73, 137.03, 133.22, 129.55, 129.41, 129.09, 128.33, 128.05, 127.80, 127.66, 127.56 (d, *J* = 6.1 Hz), 127.35, 127.17, 127.11, 100.82, 98.38, 81.67, 78.54, 76.18, 75.96, 74.70, 74.64, 74.26, 73.53, 70.39, 67.09, 65.88, 65.17, 62.64, 52.21, 37.16, 29.35.

HR-ESI-MS *(m/z)*: $[M-2H]^{2-}$, calcd for $C_{45}H_{48}N_4O_{20}S_2^{2-}$, 514.1157; found, 514.1123.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Benzyl(2-deoxy-2-sulfamino-3-_O_-sulfonate-4-_O_-benzyl-6-_O_-sulfonate-α-D-glucopyranoside-(1→4)-_O_-2-_O_-sulfonate-3-_O_-benzyl-β-D-glucopyranosyluronate] (14)

Disaccharide **10** (123 mg, 0.11 mmol) was subjected to the sequence of azide reduction and *N*-sulfation according to the general procedures to give disaccharide **14** (75 mg, 58.13 % over 2 steps).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.56 – 7.42 (m, 6H), 7.40 – 7.25 (m, 14H), 5.62 (s, 1H), 5.02 (s, 2H), 4.95 (d, *J* = 9.5 Hz, 1H), 4.85 (s, 2H), 4.59 (d, *J* = 9.6 Hz, 2H), 4.46 (t, *J* = 9.5 Hz, 1H), 4.28 (d, *J* = 10.6 Hz, 1H), 4.22 (s, 1H), 4.09 (d, *J* = 10.8 Hz, 1H), 3.96 (s, 2H), 3.77 (ddd, *J* = 26.0, 18.6, 8.6 Hz, 6H), 3.56 (s, 1H), 3.38 (dd, *J* = 10.7, 3.2 Hz, 1H), 3.14 (t, *J* = 6.5 Hz, 2H), 1.70 (d, *J* = 7.1 Hz, 2H). ^{13}C NMR (400 MHz, Deuterium Oxide) δ 166.65, 137.28, 137.05, 129.46, 129.06, 128.78, 128.62, 128.48, 128.29, 128.09, 127.57, 100.44, 96.65, 81.63, 79.80, 77.87, 75.73, 75.12, 73.91, 69.60, 66.71, 66.01, 56.85, 37.39.

HR-ESI-MS *(m/z)*: $[M-4H]^{4-}$, calcd for $C_{44}H_{48}N_2O_{25}S_4^{4-}$ 283.0362; found, 283.0346.

3-aminopropyl-_O_-2-deoxy-2-sulfamino-3-_O_-sulfonate-6-_O_-sulfonate-α-D-gluco pyranoside-(1→4)-_O_-2-_O_-sulfonate-β-D-glucopyranosyluronate (18)

Disaccharide **14** (56 mg, 0.04 mmol) was subjected to Global deprotection as described in General procedure to give disaccharide **18** (23 mg, 63.88 %).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.68 (d, *J* = 3.5 Hz, 1H), 4.71 (d, *J* = 7.6 Hz, 1H), 4.39 – 4.31 (m, 2H), 4.24 – 4.14 (m, 3H), 4.11 – 4.02 (m, 3H), 3.97 – 3.93 (m, 1H), 3.92 – 3.86 (m, 1H), 3.82 (dt, *J* = 11.1, 5.6 Hz, 1H), 3.75 (dd, *J* = 10.1, 9.0 Hz, 1H), 3.41 (dd, *J* = 10.7, 3.5 Hz, 1H), 3.20 (t, *J* = 6.3 Hz, 3H), 2.01 (q, *J* = 6.1 Hz, 2H). ^{3}C NMR (600 MHz, Deuterium Oxide) δ 173.34, 100.54, 98.15, 79.76, 78.32, 77.03, 75.13, 74.30, 70.23, 68.58, 67.91, 66.28, 56.51, 38.23, 26.34. HR-ESI-MS *(m/z)*: $[M-4H]^{4-}$, calcd for $C_{15}H_{24}N_2O_{23}S_4^{4-}$ 181.9918; found, 181.9975.

3-aminopropyl-_O_-2-deoxy-2-sulfamino-3-_O_-sulfonate-6-_O_-sulfonate-α-D-glucopyrano side-(1→4)-β-D-glucopyranosyluronate (19)

Disaccharide **15** (63 mg, 0.06 mmol) was subjected to Global deprotection as described in General procedure to give disaccharide **19** (17 mg, 40.09 %).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.54 (d, *J* = 3.6 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.32 – 4.24 (m, 2H), 4.11 (dd, *J* = 11.1, 2.1 Hz, 1H), 3.97 – 3.87 (m, 2H), 3.86 – 3.72 (m, 4H), 3.67 (t, *J* = 9.6 Hz, 1H), 3.37 – 3.34 (m, 1H), 3.34 – 3.30 (m, 1H), 3.09 (t, *J* = 6.8 Hz,

2H), 1.93 (p, *J* = 6.6 Hz, 2H). ^{13}C NMR (400 MHz, Deuterium Oxide) δ 173.80, 102.22, 98.26, 78.28, 77.76, 75.93, 75.53, 72.47, 70.15, 68.03, 67.83, 66.26, 56.67, 37.64, 26.67. HR-ESI-MS *(m/z)*: $[M-3H]^{3-}$, calcd for $C_{15}H_{25}N_2O_{20}S_3^{3-}$ is 216.3393; found, 216.3348.

_N_-benzyloxycarbonyl-3-aminopropyl-_O_-[Methyl(2-acetamido-2-deoxy-3-_O_-naphthyl methyl-4-_O_-levulinoyl-6-_O_-_tert_-butyldiphenylsilyl-α-D-glucopyranosyl)-(1→4)-_O_-2-_O_-benzoyl-3-_O_-benzyl-β-D-gluco pyranosyluronate] (23)

To a solution of compound **3** (1 g, 0.79 mmol) in THF (6 mL), AcOH (4 mL) and Ac_2O (2 mL) was added Zn dust (1.04 g, 15.9 mmol) and stirred for 12 h at room temperature. Upon completion, the reaction mixture was filtered through celite and volatiles were evaporated. The remaining residue was extracted with ethyl acetate, saturated $NaHCO_3$ and washed with brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/2.5, v/v) to obtain compound **23** (708 mg, 70.09 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 – 7.98 (m, 2H), 7.82 – 7.77 (m, 3H), 7.71 – 7.63 (m, 6H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.46 – 7.32 (m, 18H), 7.13 – 7.09 (m, 4H), 5.88 (d, *J* = 9.4 Hz, 1H), 5.45 (t, *J* = 9.5 Hz, 1H), 5.33 (dd, *J* = 8.4, 6.8 Hz, 1H), 5.14 (d, *J* = 3.4 Hz, 1H), 5.03 (d, *J* = 11.2 Hz, 3H), 4.77 – 4.59 (m, 5H), 4.53 (d, *J* = 10.5 Hz, 1H), 4.46 – 4.36 (m, 1H), 4.18 (t, *J* = 9.0 Hz, 1H), 3.98 (d, *J* = 9.0 Hz, 1H), 3.95 – 3.83 (m, 3H), 3.83 – 3.76 (m, 2H), 3.72 – 3.68 (m, 2H), 3.51 (s, 3H), 3.20 (dd, *J* = 13.4, 6.7 Hz, 1H), 3.12 (p, *J* = 6.4 Hz, 1H), 2.58 (t, *J* = 7.1 Hz, 2H), 2.52 – 2.29 (m, 3H), 2.08 (s, 3H), 1.06 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 206.22, 170.99, 170.08, 168.11, 165.10, 156.45, 136.36, 135.82, 135.79, 133.63, 133.37, 133.30, 133.20, 132.90, 129.73, 129.66, 129.22, 128.66, 128.48, 128.38, 128.35, 128.01, 127.98, 127.94, 127.64, 126.47, 126.05, 125.96, 125.84, 101.10, 99.29, 80.73, 77.74, 77.35, 77.03, 76.72, 75.25, 74.45, 73.60, 72.19, 71.44, 69.10, 67.26, 66.45, 62.14, 52.70, 51.57, 37.86, 29.77, 29.37, 27.90, 26.80, 22.63, 19.28. HR-ESI-MS *(m/z)*: $[M]^-$ calcd for $C_{58}H_{61}N_2O_{18}S^-$, 1295.5126; found, 1295.5136.

N-benzyloxycarbonyl-3-aminopropyl-_O_-[(2-acetamido-2-deoxy-3-_O_-naphthylmethyl-4-_O_-benzyl-6-_O_-sulfonate-_α_-D-glucopyranosyl)]-(1→4)-_O_-3-_O_-benzyl-1-β-D-glucopyranosiduronate (24)

Compound **22** (600 mg, 0.48 mmol) was subjected to the sequence of TBDPS deprotection, *O*-sulfation and Saponification as described in general procedures to give disaccharide **24** (173 mg, 32.64 % over 3 steps).

^{1}H NMR (400 MHz, Methanol-d4) δ 7.18 – 6.88 (m, 22H), 5.26 (d, J = 3.5 Hz, 1H), 4.81 (s, 1H), 4.77 (s, 2H), 4.72 (d, J = 11.5 Hz, 1H), 4.66 – 4.58 (m, 2H), 4.49 (d, J = 10.2 Hz, 1H), 4.37 (d, J = 10.5 Hz, 1H), 4.24 (d, J = 10.1 Hz, 1H), 4.13 – 3.94 (m, 4H), 3.74 (q, J = 10.6, 10.0 Hz, 2H), 3.67 – 3.52 (m, 3H), 3.52 – 3.42 (m, 1H), 3.37 – 3.17 (m, 2H), 3.01 – 2.89 (m, 2H), 1.62 (s, 3H), 1.53 – 1.42 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 175.1, 171.8, 157.8, 138.4, 138.4, 137.2, 136.9, 136.3, 133.4, 133.0, 130.3, 129.1, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 126.0, 125.9, 125.7, 125.6, 102.9, 97.5, 84.9, 80.7, 78.1, 76.9, 75.3, 74.7, 74.3, 74.0, 69.9, 67.0, 66.3, 66.0, 53.1, 48.8, 48.6, 48.4, 48.2, 48.0, 47.8, 47.6, 47.3, 37.4, 29.7, 22.0. HR-ESI-MS *(m/z)*: $[M]^-$ calcd for $C_{58}H_{61}N_2O_{18}S^-$, 1128.3546; found, 1128.3595.

N-benzyloxycarbonyl-3-aminopropyl-*O*-[(2-acetamido-2-deoxy-6-*O*-*tert*-butyldiphenyl silyl-3-*O*-sulfonate-*α*-D-glucopyranosyl)]-(1→4)-*O*-3-*O*-benzyl-1-β-D-glucopyranosidu ronate (26)

Compound **23** (452 mg, 0.35 mmol) was subjected to the sequence of NAP deprotection, *O*-sulfation and TBDPS deprotection as described in general procedures to give disaccharide **26** (158 mg, 51.97 % over 3 steps).^{1}H NMR (400 MHz, Methanol-d4) δ 7.38 – 7.18 (m, 10H), 5.44 (d, J = 3.9 Hz, 1H), 5.04 (d, J = 15.7 Hz, 3H), 4.65 (d, J = 10.6 Hz, 1H), 4.50 (dd, J = 10.7, 8.9 Hz, 1H), 4.31 (d, J = 7.7 Hz, 1H), 4.06 (ddd, J = 10.6, 6.5, 3.8 Hz, 1H), 4.00 – 3.87 (m, 2H), 3.80 (dd, J = 21.6, 10.5 Hz, 3H), 3.68 (d, J = 8.8 Hz, 2H), 3.61 – 3.52 (m, 2H), 3.48 (dd, J = 8.9, 7.8 Hz, 1H), 3.24 (dt, J = 8.9, 6.6 Hz, 2H), 1.83 (s, 3H), 1.77 (q, J = 6.2 Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 172.0, 157.6, 138.5, 128.1, 127.9, 127.6, 127.6, 127.4, 127.1, 103.0, 97.4, 84.5, 78.7, 75.5, 74.6, 74.0, 72.2, 69.7, 66.7, 66.0, 60.9, 52.1, 48.3, 48.0, 47.8, 47.6, 47.4, 47.2, 47.0, 37.3, 29.4, 21.5. HR-ESI-MS *(m/z)*: $[M]^-$ calcd for $C_{39}H_{47}N_2O_{17}S^-$, 870.2501; found, 870.2532.

3-aminopropyl-*O*-[(2-acetamido-2-deoxy-6-*O*-sulfonate-*α*-D-glucopyranosyl)]-(1→4)-*O*-β-D-glucopyranosiduronate (27)

Disaccharide **24** (46 mg, 0.05 mmol) was subjected to Global deprotection as described in General procedure to give disaccharide **27** (15 mg, 55.55 %).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.43 (d, *J* = 3.8 Hz, 1H), 4.48 (dd, *J* = 8.0, 1.3 Hz, 1H), 4.37 – 4.30 (m, 1H), 4.18 (dt, *J* = 11.1, 1.8 Hz, 1H), 4.01 – 3.95 (m, 1H), 3.94 – 3.82 (m, 3H), 3.81 – 3.66 (m, 4H), 3.58 (dd, *J* = 10.4, 9.1, 1.3 Hz, 1H), 3.37 – 3.32 (m, 1H), 3.16 (q, *J* = 6.7 Hz, 2H), 2.05 (s, 3H), 1.99 (p, *J* = 6.4 Hz, 2H). ^{13}C NMR (151 MHz, D_2O) δ 175.0,

174.3, 102.2, 96.8, 76.6, 76.5, 75.5, 73.4, 70.6, 70.0, 69.0, 67.9, 66.3, 53.5, 37.6, 26.7, 21.9. HR-ESI-MS *(m/z)*: $[M]^-$ calcd for $C_{17}H_{29}N_2O_{15}S^-$, 556.1181; found, 556..1136.

3-aminopropyl-*O*-[(2-acetamido-2-deoxy-*α*-D-glucopyranosyl)]-(1→4)-*O*-β-D-gluco pyranosiduronate (28)

Disaccharide **25** (49 mg, 0.05 mmol) was subjected to Global deprotection as described in General procedure to give disaccharide **28** (17 mg, 69.41 %).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.32 (d, J = 3.7 Hz, 1H), 4.38 (d, J = 8.0 Hz, 1H), 3.93 – 3.84 (m, 1H), 3.82 – 3.74 (m, 2H), 3.73 – 3.67 (m, 4H), 3.66 – 3.59 (m, 3H), 3.39 (dd, J = 10.1, 9.1 Hz, 1H), 3.28 – 3.19 (m, 1H), 2.01 – 1.84 (m, 5H). ^{13}C NMR (101 MHz, D_2O) δ 175.2, 174.4, 100.4, 94.4, 73.1, 71.9, 71.1, 69.6, 68.7, 68.4, 67.3, 66.4, 60.1, 53.5, 38.2, 26.4, 21.9. HR-ESI-MS *(m/z)*: $[M]^-$ calcd for $C_{17}H_{30}N_2O_{12}$, 477.1699; found, 477.1632.

3-aminopropyl-*O*-[(2-acetamido-2-deoxy-3-*O*-sulfonate-*α*-D-glucopyranosyl)]-(1→4)-*O*-β-D-glucopyranosiduronate (29)

Disaccharide **26** (38 mg, 0.04 mmol) was subjected to Global deprotection as described in General procedure to give disaccharide **29** (14 mg, 58.3 %).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.36 (d, J = 3.6 Hz, 1H), 4.52 – 4.36 (m, 2H), 4.04 (dd, J = 10.6, 3.6 Hz, 1H), 3.94 (dt, J = 11.2, 5.8 Hz, 1H), 3.88 (d, J = 8.4 Hz, 1H), 3.84 – 3.77 (m, 2H), 3.74 (dd, J = 8.0, 5.6 Hz, 3H), 3.64 (t, J = 9.3 Hz, 1H), 3.43 – 3.25 (m, 2H), 3.15 (t, J = 7.2 Hz, 2H), 2.05 – 1.91 (m, 5H). ^{13}C NMR (151 MHz, D_2O) δ 174.8, 174.3, 102.2, 102.2, 97.5, 79.6, 76.9, 76.8, 76.7, 76.2, 76.2, 73.3, 71.8, 68.2, 67.9, 67.7, 59.9, 52.3, 50.8, 42.5, 37.5, 26.7, 26.0, 22.1, 18.1, 18.1. HR-ESI-MS *(m/z)*: $[M]^-$ calcd for $C_{17}H_{29}N_2O_{15}S^-$, 556.1194; found, 556.1146.

Glycan microarray Materials.

PBSx10 was purchased from Hy-labs, ethanolamine from Fisher, ovalbumin (Grade V), sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, Tween-20 and Tris/HCl were purchased from Sigma-Aldrich. Antibodies were purchased from Peprotech: Human SD1α CXCL12, human IL8 CXCL8 72 aa, human exodus-2 CCL21, human MCP-3 CCL7, human MCP-4 CCL13, human IP-10 CXCL10, human MEC CCL28, human RANTES CCL5, human MCP-1/MCAF CCL2, biotinylated antigen affinity-purified goat-antimurine SDF-1α, biotinylated-rabbit-anti-human IL8 CXCL8, biotinylated-rabbit-anti-human Exodus-2 CCL21, biotinylated goat-anti-human MCP-3, biotinylated anti-H-

MCP-4, biotinylated rabbit-anti-human IP-10, biotinylated anti-human MEC, biotinylated-rabbit-anti-human MCAF/MCP-1. Biotinylated anti-Rantes was purchased from R&D. Cy3-sterptavidin (Cy3-SA) was purchased from Jackson ImmunoResearch. IVIG GammaGard 10% (Baxter, USA) were a kind gift from Adriana Tremolot, Childrens hospital UCSD.[32-34]

Heparin disaccharide microarray fabrication.

Arrays were fabricated with NanoPrint LM60 Microarray Printer (Arrayit) on epoxide-derivatized slides (PolyAn 2D) with 16 sub-array blocks on each slide. Glycoconjugates were distributed into 384-well source plates using 4 replicate wells per sample and 7 µl per well. Each glycoconjugate diluted into 50 and 100 µM in an optimized printing buffer (300 mM phosphate buffer, pH 8.4 Version VrHI.01. To monitor printing Alexaflour-555-hydraside (Invitrogen, at 1 ng/µl in 178 mM phosphate buffer, pH 5.5) was used for each printing run. The arrays were printed with four 946MP3 pins (5 µm tip, 0.25 µl sample channel, ~100 µm spot diameter; Arrayit). The humidity level in the arraying chamber was maintained at about 70% during printing. Printed slides were left on arrayer deck overnight, allowing humidity to drop to ambient levels (40-45%). Next, slides were packed, vacuumsealed and stored at room temperature (RT) until used.[35,36]

HS tetrasaccharide microarray binding assay.

Slides were developed and analyzed as previously described1 with some modifications. Slides were rehydrated with dH2O and incubated for 30 min in a staining dish with 50°C pre-warmed ethanolamine (0.05 M) in Tris-HCl (0.1 M, pH 9.0) to block the remaining reactive epoxy groups on the slide surface, then washed with 50 °C pre-warmed dH2O. Slides were centrifuged at 200×g for three min then fitted with ProPlate™ Multi-Array 16-well slide module (Grace Bio-lab P37001) to divide into the sub-arrays (blocks). Slides were washed with PBST (0.1% Tween 20), aspirated and blocked with 200 µl/sub-array of blocking buffer (PBS pH 7.3 + 1% w/v ovalbumin) for 1 hour at RT with gentle shaking. Next, the blocking solution was aspirated and 100 µl/block of primary detection proteins (for each detection, 3 serially decreasing concentrations were used, see Table S1) diluted in PBS pH 7.3 + 1% w/v ovalbumin, were incubated with gentle shaking for 2 hours at RT. Slides were washed 4 times with PBST, then with PBS (without Tween-20) for 2 min. Bound antibodies were detected by incubating with biotinylated secondary detections (1 ng/µl, see Table S1) diluted in PBS, 200 µl/block at RT for 1 hour. Slides were washed 4 times with PBST, then with PBS (without Tween-20) for 2 min and biotinylated antibodies were detected with Cy3-SA

(1.2µg/ml). Slides were washed 4 times with PBST, then with PBS for 10 min followed by removal from ProPlate™ Multi-Array slide module and immediately dipping in a staining dish with dH2O for 10 min with shaking. Slide then were centrifuged at 200×g for 3 min. and the dry slides immediately scanned.[32,34]

Array slide processing.

Processed slides were scanned and analyzed as described at 10 µm resolution with a Genepix 4000B microarray scanner (Molecular Devices) using 350 gain. Image analysis was carried out with Genepix Pro 4.0 analysis software (Molecular Devices). Spots were defined as circular features with a variable radius as determined by the Genepix scanning software. Local background subtraction was performed. RFU from each spot was calculated and Ranking (per printed glycan per concentration, per each chemokine dilution, per printed block) was used to compare the data between detections; since each glycans was printed at 2 concentration, 100% binding was set separately for each concentration. Then, binding to all the other glycans at the same concentration was ranked in comparison to the maximal binding, and the average rank binding and SEM for each glycan across the two glycan concentrations and three examined dilutions of each chemokine was calculated (n = 6, 2 glycan concentrations across 3 chemokine dilutions).[34]

2.6. Referances

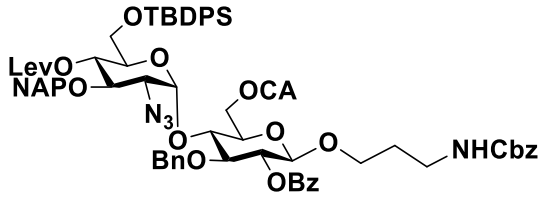
1. Mantovani, A.; Bonecchi, R.; Locati, M., Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat. Rev. Immunol.* **2006,** *6* (12), 907-918.
2. Mackay, C. R., Chemokines: immunology's high impact factors. *Nat. Immunol.* **2001,** *2* (2), 95-101.
3. Nibbs, R. J.; Graham, G. J., Immune regulation by atypical chemokine receptors. *Nature Rev. Immunol.* **2013,** *13* (11), 815-829.
4. Moser, B.; Loetscher, P., Lymphocyte traffic control by chemokines. *Nat. Immunol.* **2001,** *2* (2), 123-128.
5. Mortier, A.; Van Damme, J.; Proost, P., Overview of the mechanisms regulating chemokine activity and availability. *Immunol. Lett.* **2012,** *145* (1-2), 2-9.

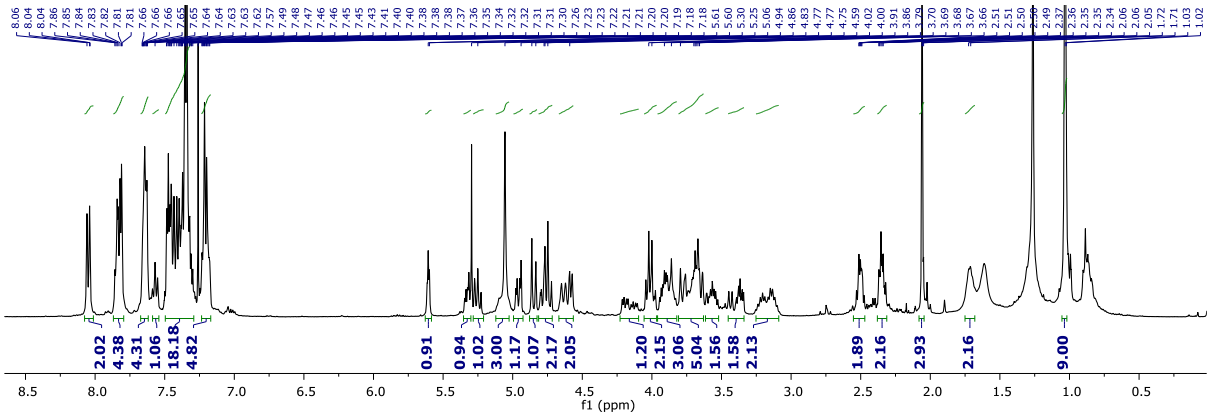
6. Jöhrer, K.; Pleyer, L.; Olivier, A.; Maizner, E.; Zelle-Rieser, C.; Greil, R., Tumour-immune cell interactions modulated by chemokines. *Expert Opin. Biol. Th.* **2008,** *8* (3), 269-290.
7. Liang, W. G.; Triandafillou, C. G.; Huang, T.-Y.; Zulueta, M. M. L.; Banerjee, S.; Dinner, A. R.; Hung, S.-C.; Tang, W.-J., Structural basis for oligomerization and glycosaminoglycan binding of CCL5 and CCL3. *Proc. Natl. Acad. Sci.* **2016,** *113* (18), 5000-5005.
8. Dyer, D. P.; Salanga, C. L.; Volkman, B. F.; Kawamura, T.; Handel, T. M., The dependence of chemokine–glycosaminoglycan interactions on chemokine oligomerization. *Glycobiology* **2016,** *26* (3), 312-326.
9. Wang, L.; Fuster, M.; Sriramarao, P.; Esko, J. D., Endothelial heparan sulfate deficiency impairs L-selectin-and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nat. Immunol.* **2005,** *6* (9), 902-910.
10. Parish, C. R., The role of heparan sulphate in inflammation. *Nature Rev. Immunol.* **2006,** *6* (9), 633-643.
11. Proudfoot, A. E.; Handel, T. M.; Johnson, Z.; Lau, E. K.; LiWang, P.; Clark-Lewis, I.; Borlat, F.; Wells, T. N.; Kosco-Vilbois, M. H., Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. *Proc. Natl. Acad. Sci. USA.* **2003,** *100* (4), 1885-1890.
12. Johnson, Z.; Proudfoot, A.; Handel, T., Interaction of chemokines and glycosaminoglycans: a new twist in the regulation of chemokine function with opportunities for therapeutic intervention. *Cytokine Growth Factor Rev.* **2005,** *16* (6), 625-636.
13. Sepuru, K. M.; Rajarathnam, K., Structural basis of chemokine interactions with heparan sulfate, chondroitin sulfate, and dermatan sulfate. *J. Biol. Chem.* **2019,** *294* (43), 15650-15661.
14. Stringer, S. E.; Gallagher, J. T., Specific binding of the chemokine platelet factor 4 to heparan sulfate. *J. Biol. Chem.* **1997,** *272* (33), 20508-20514.
15. Spillmann, D.; Witt, D.; Lindahl, U., Defining the interleukin-8-binding domain of heparan sulfate. *J. Biol. Chem.* **1998,** *273* (25), 15487-15493.
16. Jayson, G. C.; Hansen, S. U.; Miller, G. J.; Cole, C. L.; Rushton, G.; Avizienyte, E.; Gardiner, J. M., Synthetic heparan sulfate dodecasaccharides reveal single sulfation site interconverts CXCL8 and CXCL12 chemokine biology. *Chem. Comm.* **2015,** *51* (72), 13846-13849.

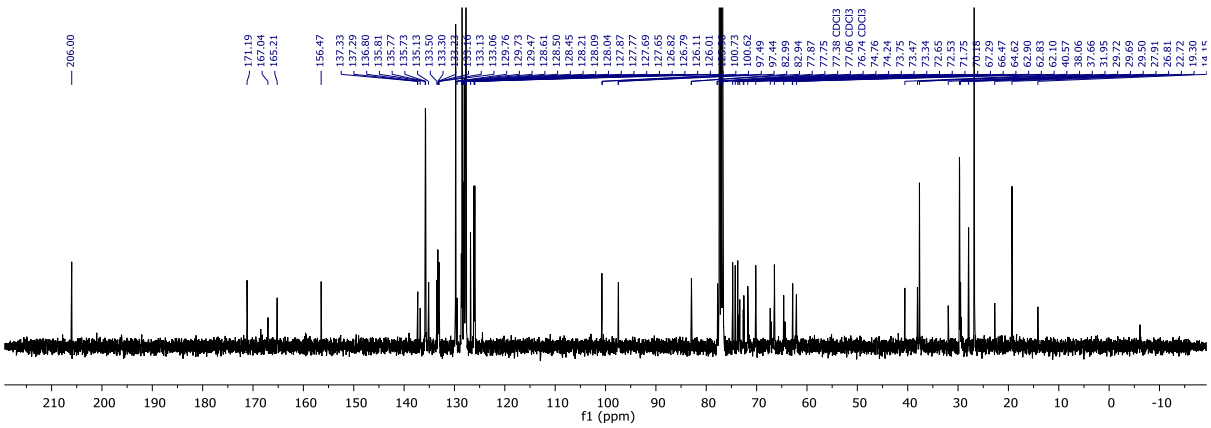
17. Sheng, G. J.; Oh, Y. I.; Chang, S.-K.; Hsieh-Wilson, L. C., Tunable heparan sulfate mimetics for modulating chemokine activity. *J. Am. Chem. Soc.* **2013,** *135* (30), 10898-10901.
18. de Paz, J. L.; Moseman, E. A.; Noti, C.; Polito, L.; von Andrian, U. H.; Seeberger, P. H., Profiling heparin–chemokine interactions using synthetic tools. *ACS Chem. Biol.* **2007,** *2* (11), 735-744.
19. Zong, C.; Venot, A.; Li, X.; Lu, W.; Xiao, W.; Wilkes, J.-S. L.; Salanga, C. L.; Handel, T. M.; Wang, L.; Wolfert, M. A., Boons, G. J., Heparan sulfate microarray reveals that heparan sulfate–protein binding exhibits different ligand requirements. *J. Am. Chem.Soc.* **2017,** *139* (28), 9534-9543.
20. Toida, T.; Yoshida, H.; Toyoda, H.; Koshiishi, I.; Imanari, T.; Hileman, R. E.; Fromm, J. R.; Linhardt, R. J., Structural differences and the presence of unsubstituted amino groups in heparan sulphates from different tissues and species. *Biochem. J.* **1997,** *322* (2), 499-506.
21. Westling, C.; Lindahl, U., Location of N-unsubstituted glucosamine residues in heparan sulfate. *J. Biol. Chem.* **2002,** *277* (51), 49247-49255.
22. Vanpouille, C.; Deligny, A.; Delehedde, M.; Denys, A.; Melchior, A.; Liénard, X.; Lyon, M.; Mazurier, J.; Fernig, D. G.; Allain, F., The heparin/heparan sulfate sequence that interacts with cyclophilin B contains a 3-O-sulfated N-unsubstituted glucosamine residue. *J. Biol. Chem.* **2007,** *282* (33), 24416-24429.
23. Wei, Z.; Lyon, M.; Gallagher, J. T., Distinct substrate specificities of bacterial heparinases against N-unsubstituted glucosamine residues in heparan sulfate. *J. Biol. Chem.* **2005,** *280* (16), 15742-15748.
24. Wei, Z.; Deakin, J. A.; Blaum, B. S.; Uhrín, D.; Gallagher, J. T.; Lyon, M., Preparation of heparin/heparan sulfate oligosaccharides with internal N-unsubstituted glucosamine residues for functional studies. *Glycoconj. J.* **2011,** *28* (8-9), 525-535.
25. Koenig, A.; Norgard-Sumnicht, K.; Linhardt, R.; Varki, A., Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J. Clin. Invest.* **1998,** *101* (4), 877-889.
26. Norgard-Sumnicht, K.; Varki, A., Endothelial heparan sulfate proteoglycans that bind to L-selectin have glucosamine residues with unsubstituted amino groups. *J. Biol. Chem.* **1995,** *270* (20), 12012-12024.

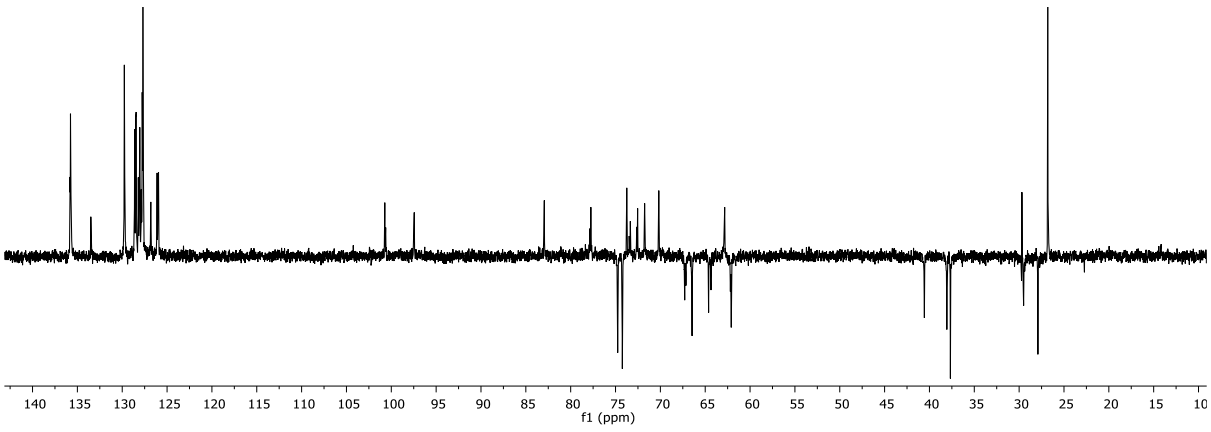
27. Mende, M.; Bednarek, C.; Wawryszyn, M.; Sauter, P.; Biskup, M. B.; Schepers, U.; Bräse, S., Chemical synthesis of glycosaminoglycans. *Chem. Rev.* **2016,** *116* (14), 8193-8255.
28. Hu, Y.-P.; Zhong, Y.-Q.; Chen, Z.-G.; Chen, C.-Y.; Shi, Z.; Zulueta, M. M. L.; Ku, C.-C.; Lee, P.-Y.; Wang, C.-C.; Hung, S.-C., Divergent synthesis of 48 heparan sulfate-based disaccharides and probing the specific sugar–fibroblast growth factor-1 interaction. *J. Am. Chem. Soc.* **2012,** *134* (51), 20722-20727.
29. Hansen, S. U.; Miller, G. J.; Jayson, G. C.; Gardiner, J. M., First gram-scale synthesis of a heparin-related dodecasaccharide. *Org. Lett.* **2013,** *15* (1), 88-91.
30. Arungundram, S.; Al-Mafraji, K.; Asong, J.; Leach III, F. E.; Amster, I. J.; Venot, A.; Turnbull, J. E.; Boons, G.-J., Modular synthesis of heparan sulfate oligosaccharides for structure-activity relationship studies. *J. Am. Chem. Soc.* **2009,** *131* (47), 17394-17405.
31. Noti, C.; de Paz, J. L.; Polito, L.; Seeberger, P. H., Preparation and use of microarrays containing synthetic heparin oligosaccharides for the rapid analysis of heparin–protein interactions. *Chem. Eur. J.* **2006,** *12* (34), 8664-8686.
32. Padler-Karavani, V.; Song, X.; Yu, H.; Hurtado-Ziola, N.; Huang, S.; Muthana, S.; Chokhawala, H. A.; Cheng, J.; Verhagen, A.; Langereis, M. A., Cross-comparison of protein recognition of sialic acid diversity on two novel sialoglycan microarrays. *J. Biol. Chem.* **2012,** *287* (27), 22593-22608.
33. Ben-Arye, S. L.; Yu, H.; Chen, X.; Padler-Karavani, V., Profiling anti-Neu5Gc IgG in human sera with a sialoglycan microarray assay. *J. Vis. Exp.* **2017,** (125), e56094.
34. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E., UCSF Chimera-a visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004,** *25* (13), 1605-1612.
35. Jain, P.; Shanthamurthy, C. D.; Ben-Arye, S. L.; Woods, R. J.; Kikkeri, R.; Padler-Karavani, V. Discovery of Rare Sulfated N-Unsubstituted Glucosamine Based Heparan Sulfate Analogs Selectivly Activating Chemokines. Chem Sci. ,**12**, 3674-3681 (2021).
36. Chethan D. Shanthamurthy, Prashant Jain, S. Yehuda, J. T. Monteiro, S. L. Ben-Arye, Vered Padler-karavani, Bernd Lepenies, Raghavendra Kikkeri. - "ABO Antigens Active Tri and Disaccharides Microarray to Evaluate C-type Lectin Receptor Binding Preference". *Scientific reports*. **26**, 6603, (2018).

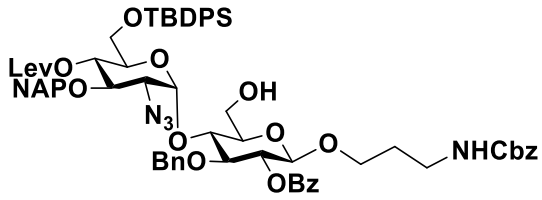
2.7. NMR Spectras

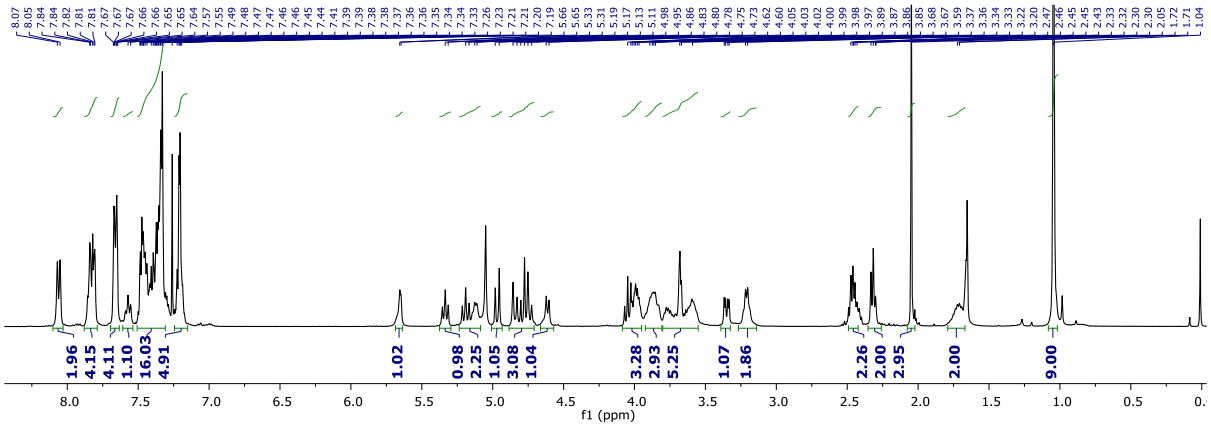


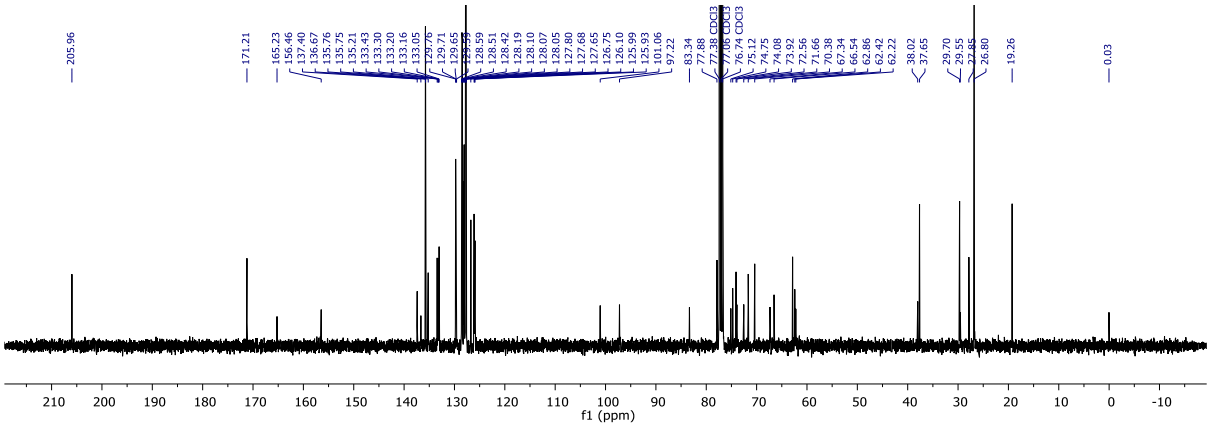


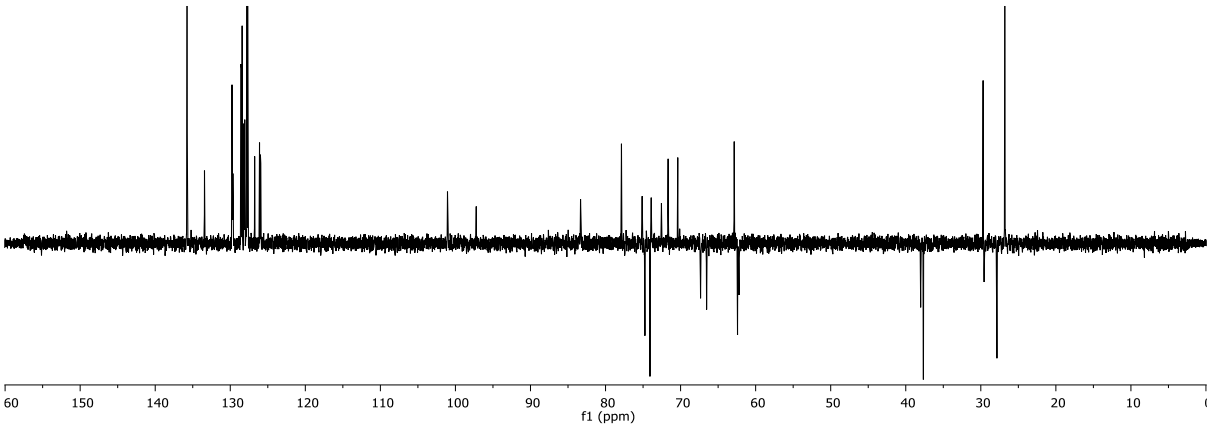


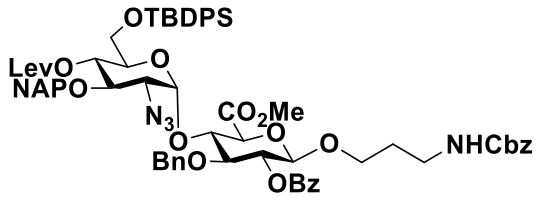


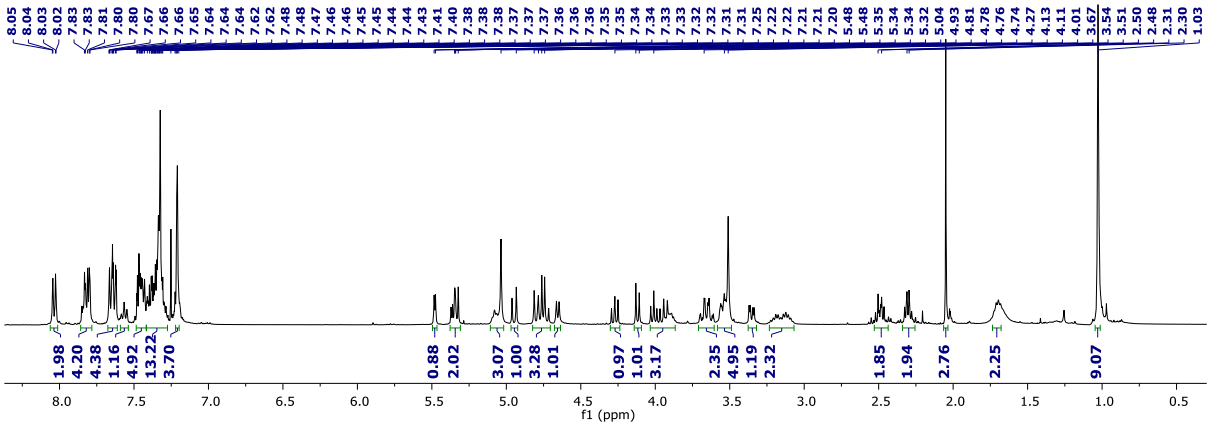

OTBDPS
LevO
NAPO
O
N3
OH
O
O
BnO
O
NHCbz
OBz

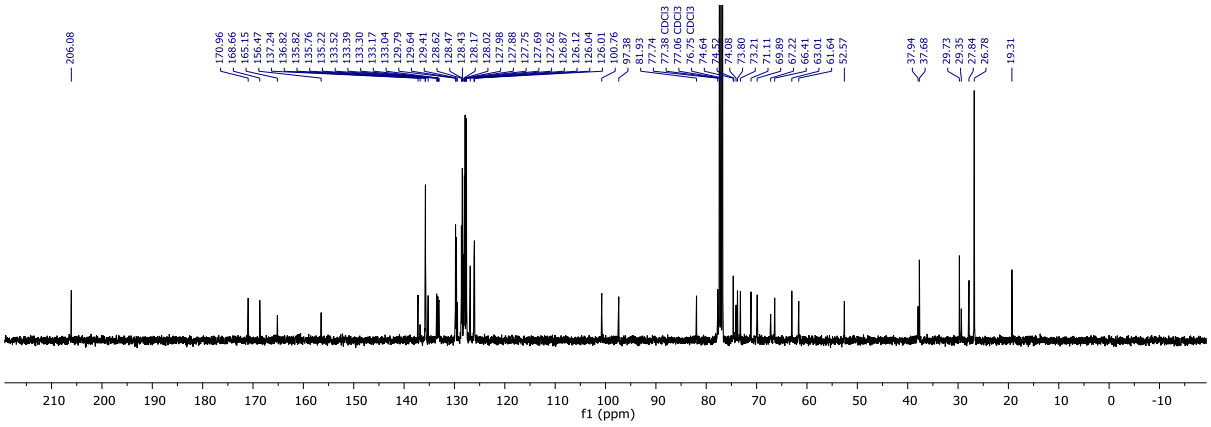

1.96
4.15
4.11
1.10
16.03
4.91
1.02
0.98
2.25
1.05
3.08
1.04
3.28
2.93
5.25
1.07
1.86
2.26
2.00
2.95
2.00
9.00
f1 (ppm)

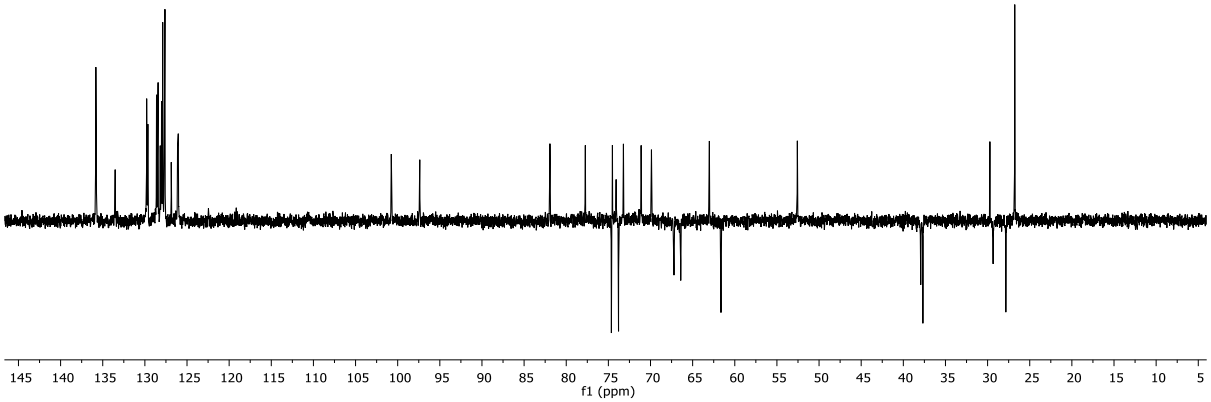

f1 (ppm)

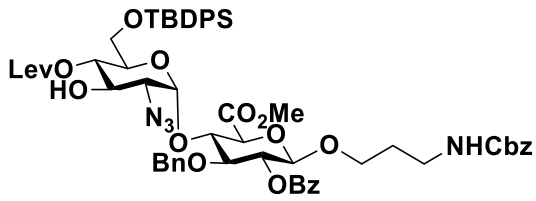

f1 (ppm)

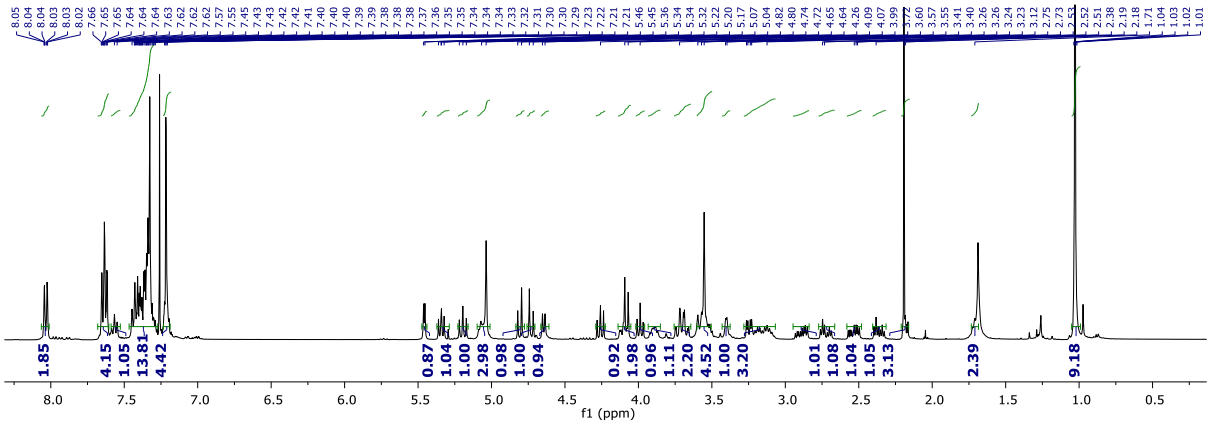

OTBDPS
LevO
NAPO
O
N3
CO2Me
O
O
BnO
O
NHCbz
OBz

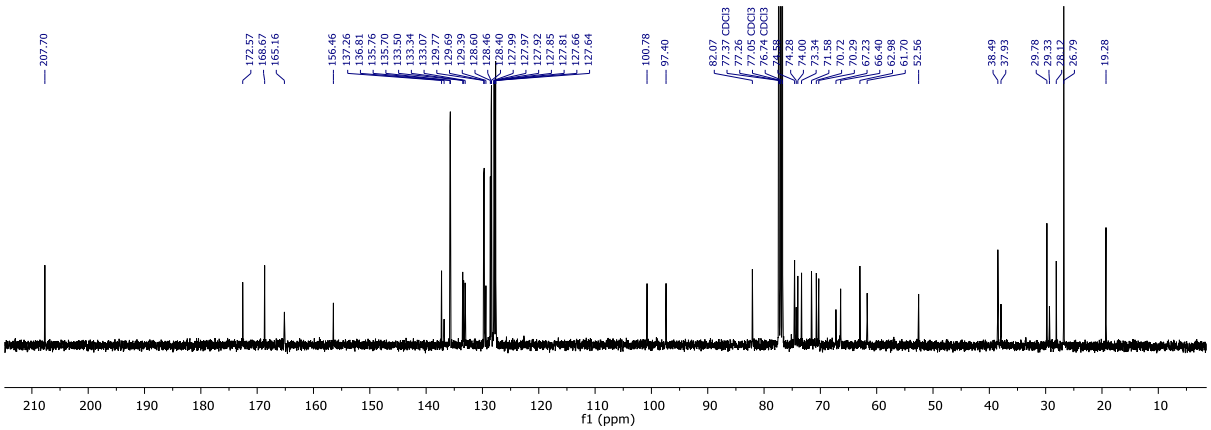

1.98
4.20
4.38
1.16
4.92
13.22
3.70
0.88
2.02
3.07
1.00
3.28
1.01
0.97
1.01
3.17
2.35
4.95
1.19
2.32
1.85
1.94
2.76
2.25
9.07
f1 (ppm)

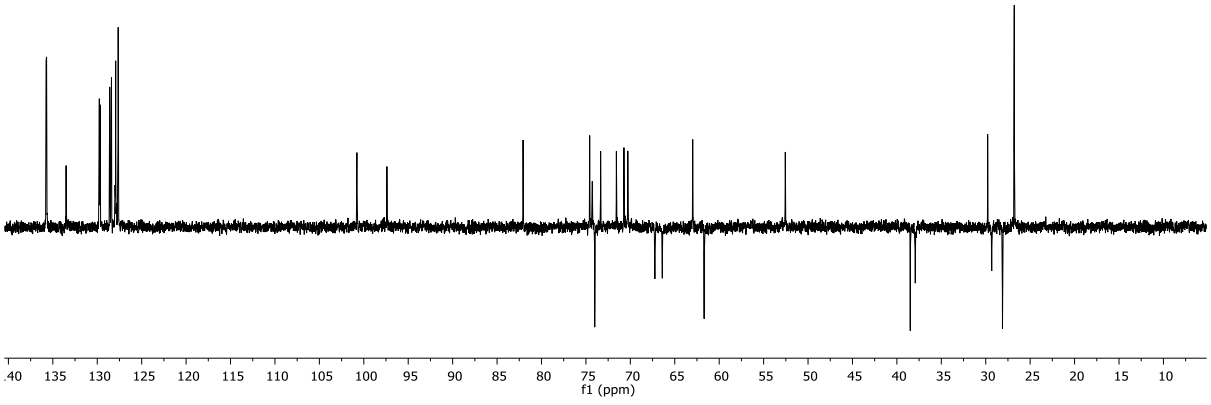

f1 (ppm)

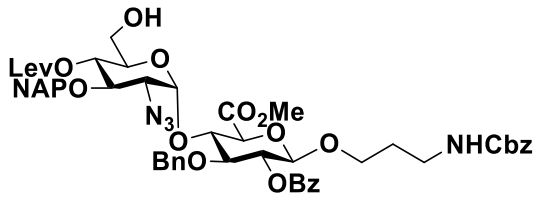

f1 (ppm)

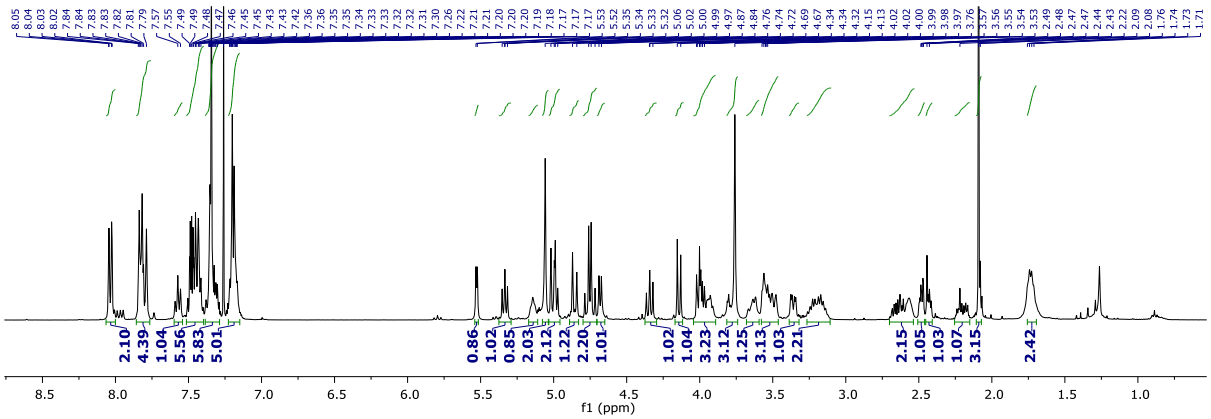

OTBDPS
LevO
HO
O
N3
CO2Me
O
O
NHCbz
BnO
OBz

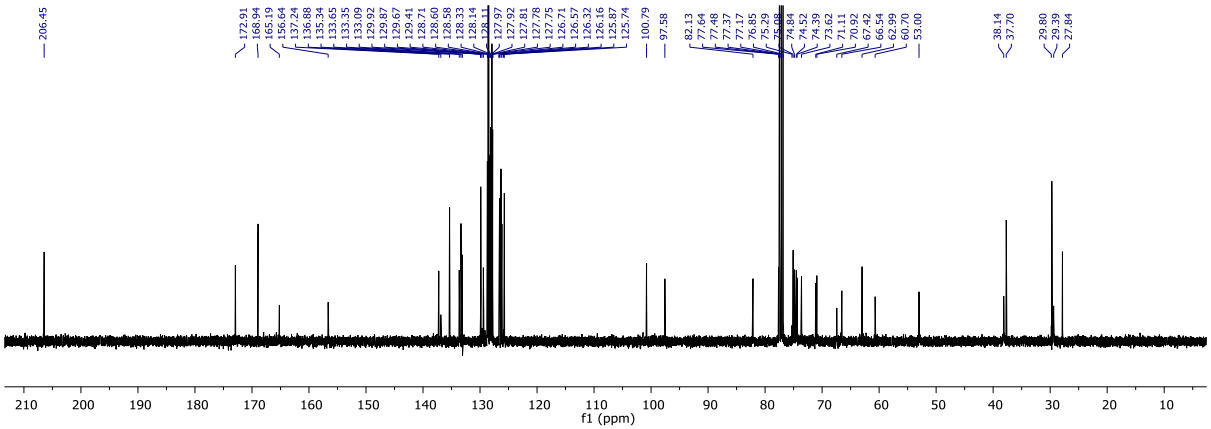

1.85
4.15
1.05
13.81
4.42
0.87
1.04
1.00
2.98
0.98
1.00
0.94
0.92
1.98
0.96
1.11
2.20
4.52
1.00
3.20
1.01
1.08
1.04
1.05
3.13
2.39
9.18
f1 (ppm)

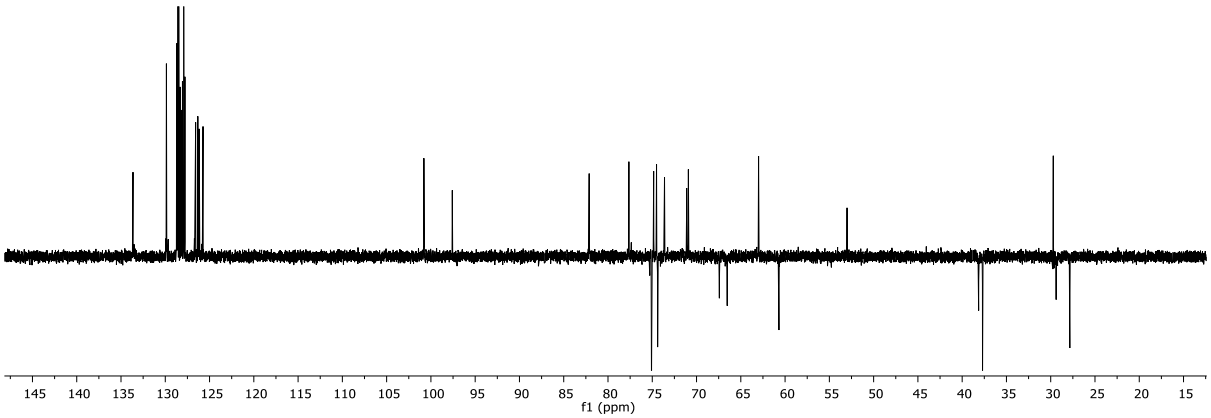

f1 (ppm)

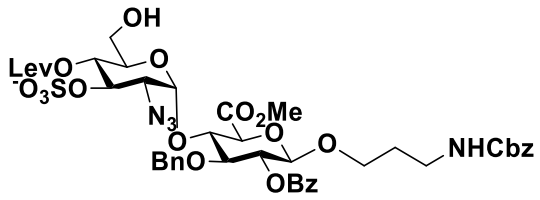

f1 (ppm)

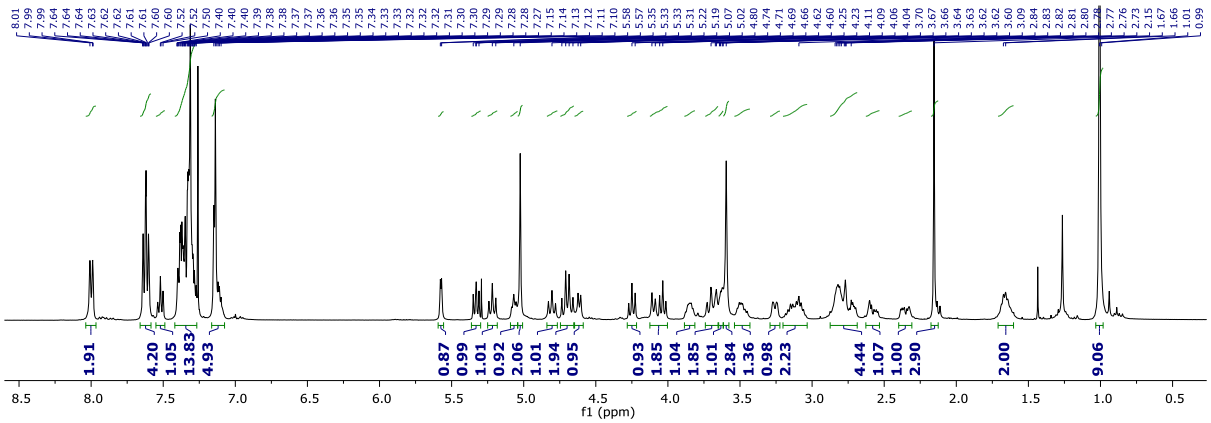

OH
LevO
NAPO
O
N3
CO2Me
O
O
BnO
O
NHCbz
OBz

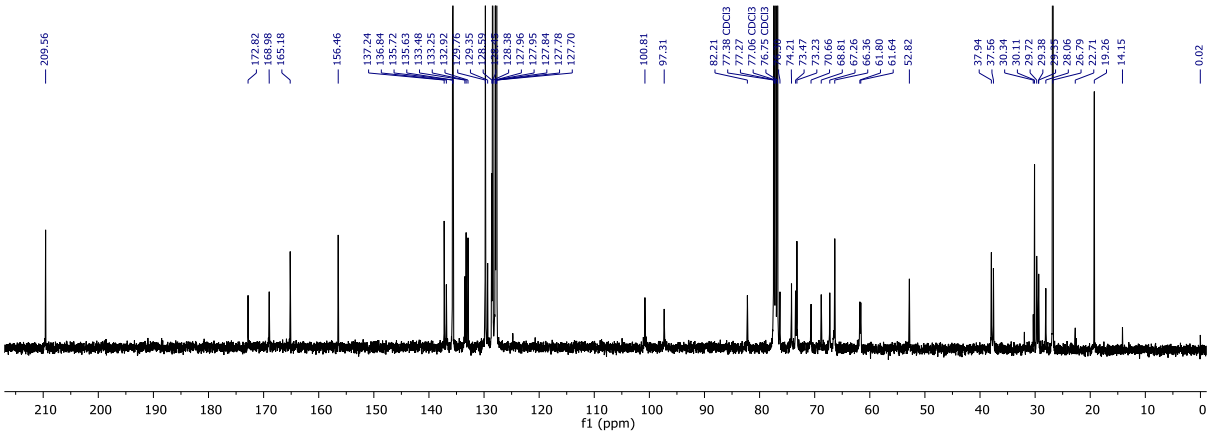

2.10
4.39
1.04
5.56
5.83
5.01
0.86
1.02
0.85
2.03
2.12
1.22
2.20
1.01
1.02
1.04
3.23
3.12
1.25
3.13
1.03
2.21
2.15
1.05
1.03
1.07
3.15
2.42
f1 (ppm)

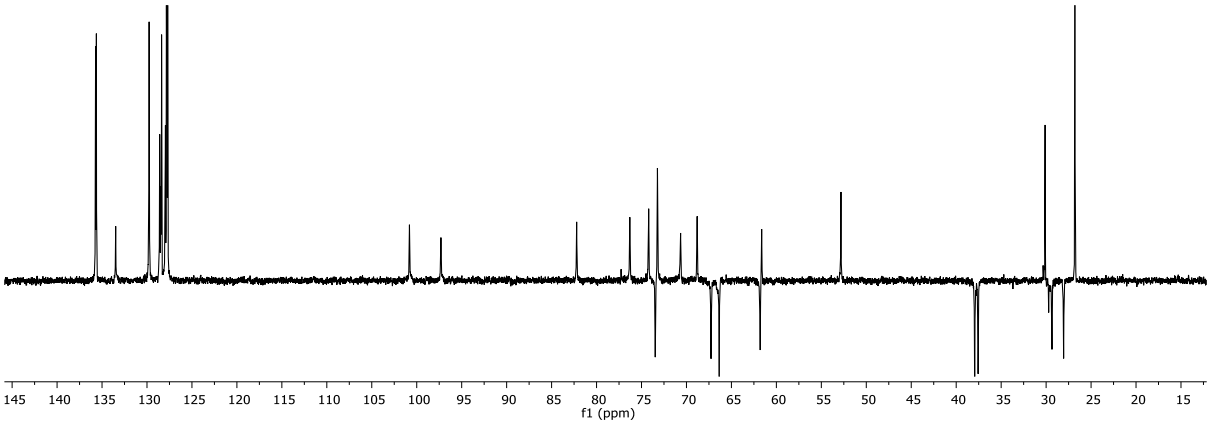

206.45
172.91
168.94
165.19
156.64
137.24
136.88
135.34
133.65
133.35
129.99
129.92
129.87
129.67
129.41
128.71
128.60
128.58
128.33
128.14
127.97
127.92
127.81
127.78
127.75
126.71
126.57
126.32
126.16
125.87
125.74
100.79
97.58
82.13
77.64
77.48
77.37
77.17
76.85
75.29
75.08
74.84
74.52
74.39
73.62
71.11
70.92
67.42
66.54
62.99
60.70
53.00
38.14
37.70
29.80
29.39
27.84
f1 (ppm)

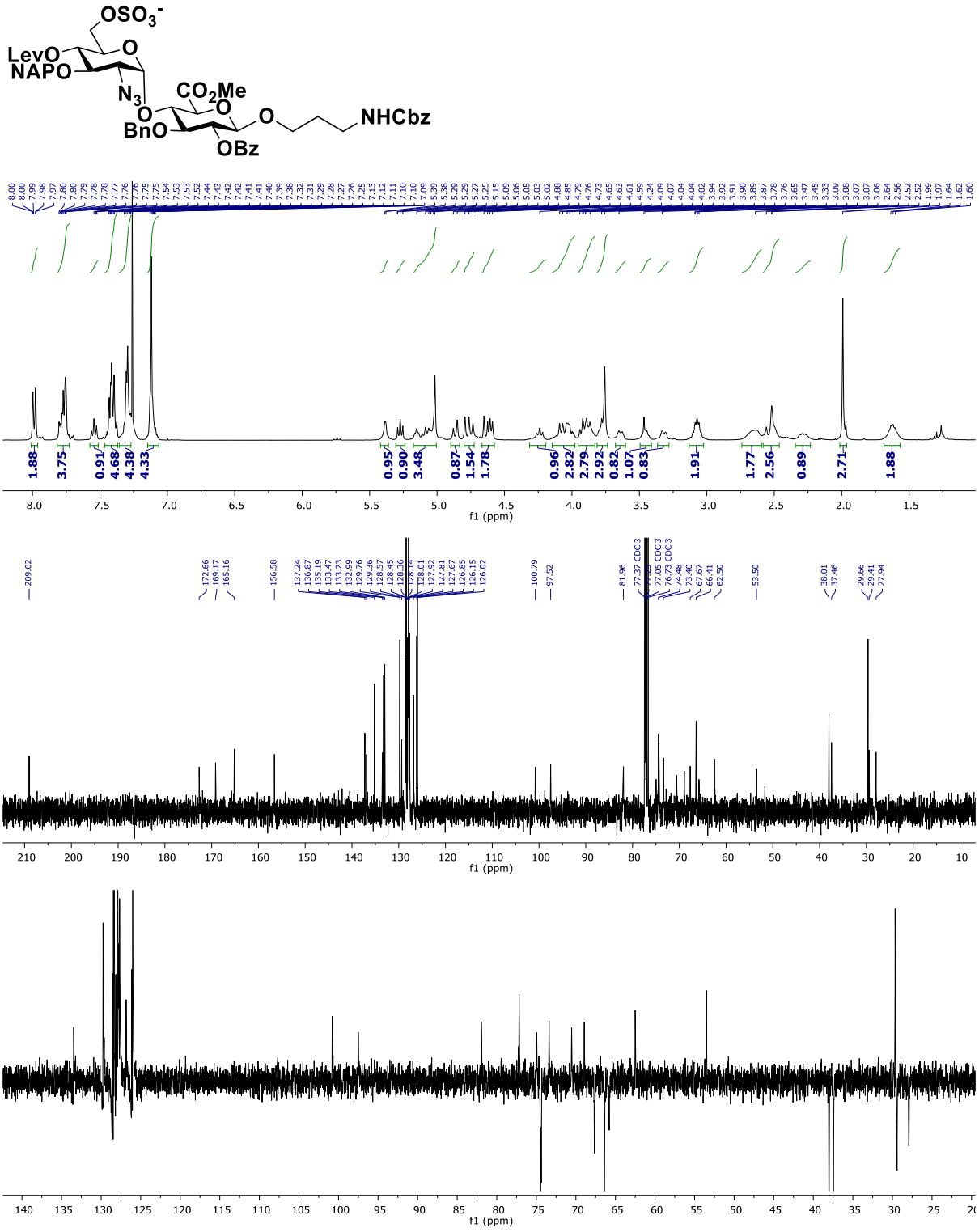

f1 (ppm)

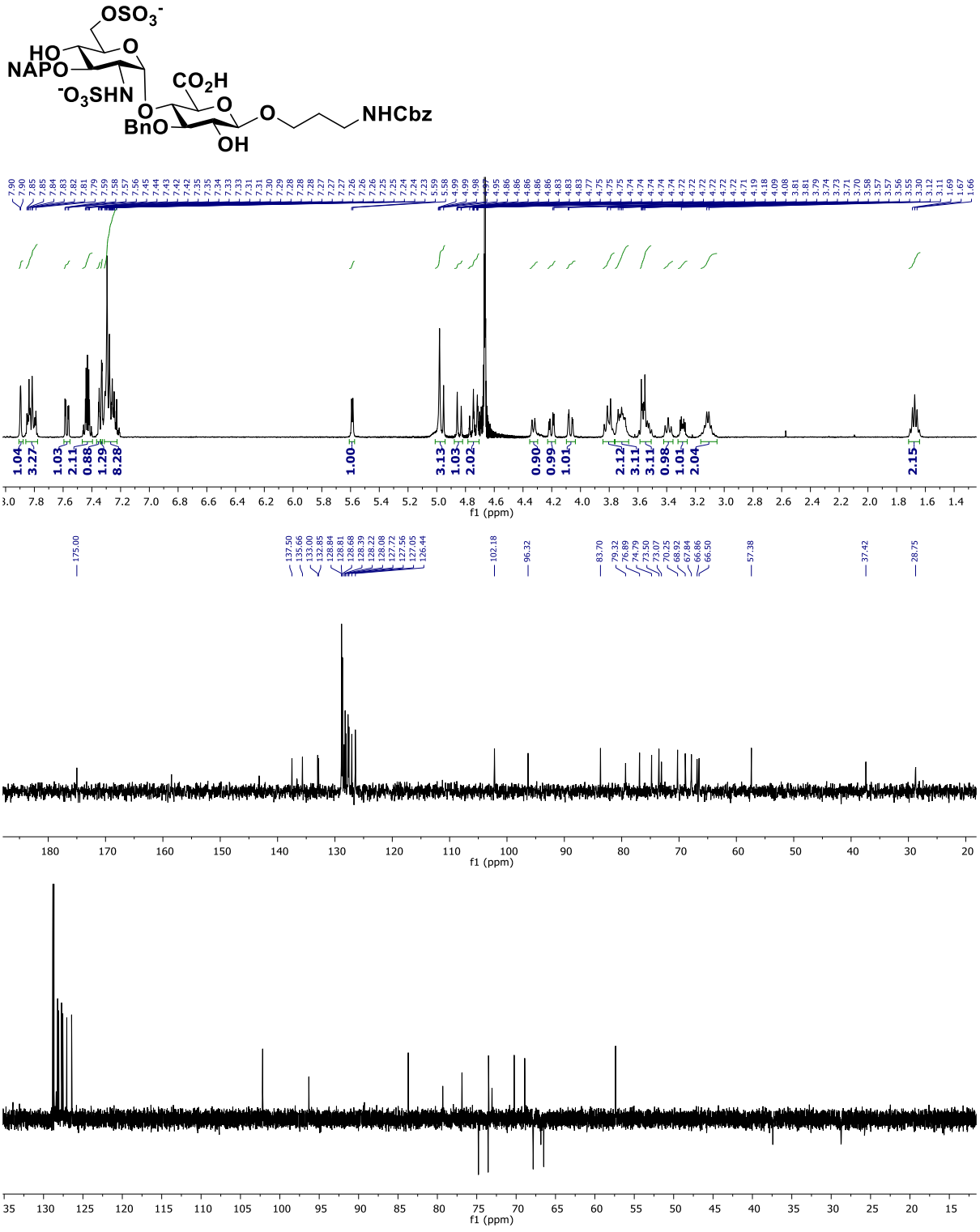

OH
LevO
O
$^{-}O_3SO$
N_3
CO_2Me
O
O
BnO
O
NHCbz
OBz

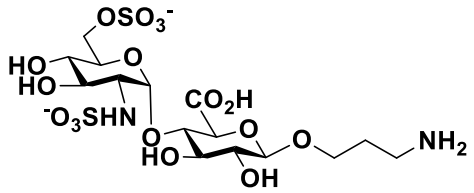

1.91
4.20
1.05
13.83
4.93
0.87
0.99
1.01
0.92
2.06
1.01
1.94
0.95
0.93
1.85
1.04
1.85
1.01
2.84
1.36
0.98
2.23
4.44
1.07
1.00
2.90
2.00
9.06
f1 (ppm)

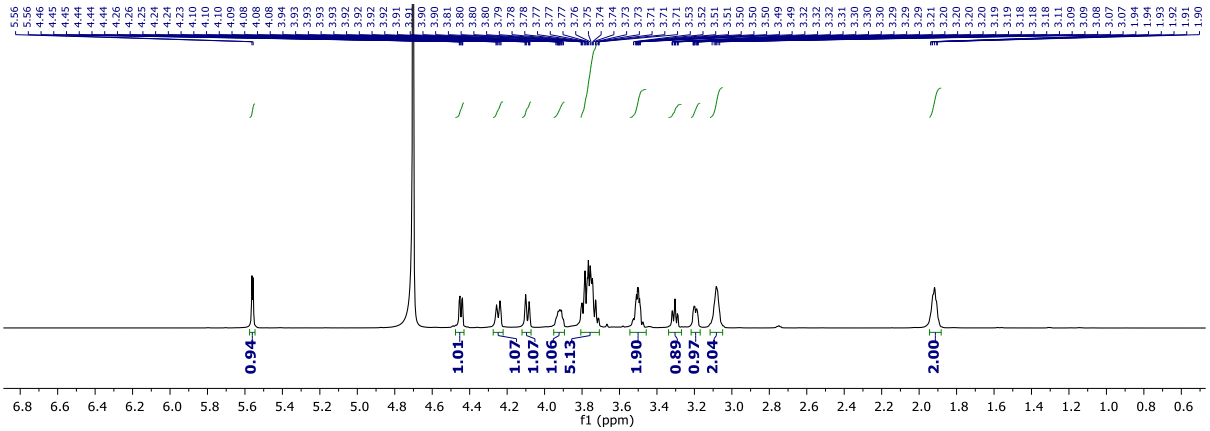

209.56
172.82
168.98
165.18
156.46
137.24
136.84
135.72
135.63
133.48
133.25
132.92
129.76
129.35
128.53
128.38
127.96
127.95
127.84
127.78
127.70
100.81
97.31
82.21
77.38 CDCl3
77.27
77.06 CDCl3
76.75 CDCl3
74.21
73.47
73.23
70.66
68.81
67.26
66.36
61.80
61.64
52.82
37.94
37.56
30.34
30.11
29.72
29.38
29.23
28.06
26.79
22.71
19.26
14.15
0.02
f1 (ppm)

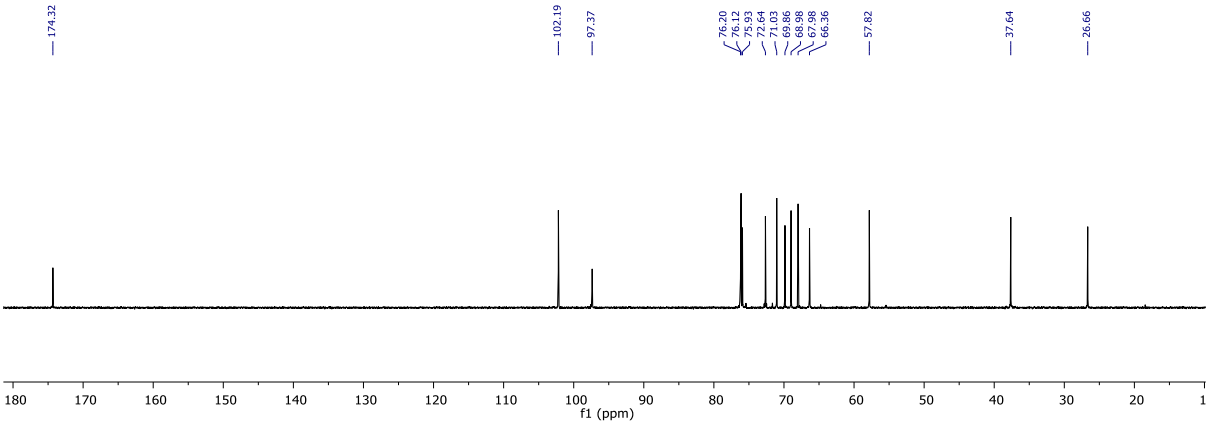

f1 (ppm)

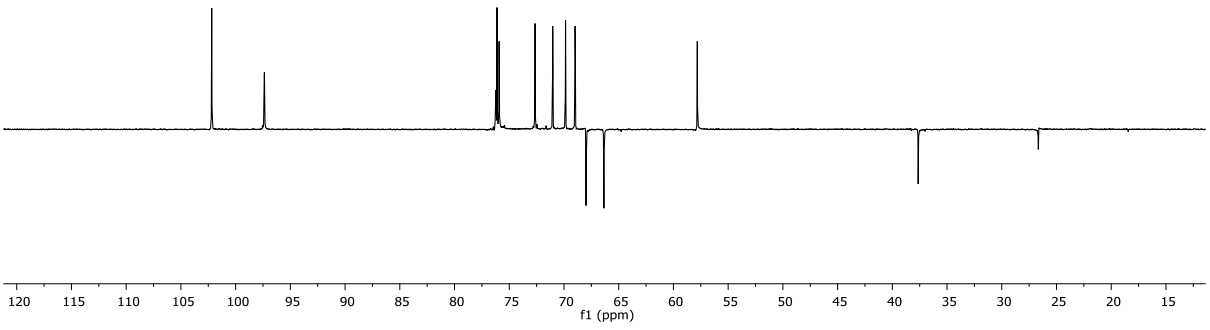

OSO3-
LevO
NAPO
O
N3
CO2Me
O
O
NHCbz
BnO
OBz
f1 (ppm)

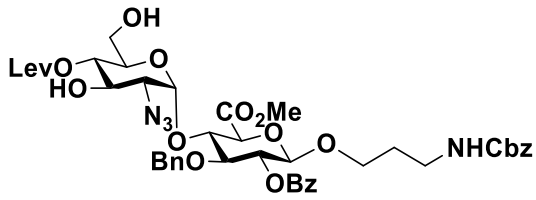

OSO3-
HO
NAPO
-O3SHN
CO2H
O
BnO
OH
NHCbz
f1 (ppm)

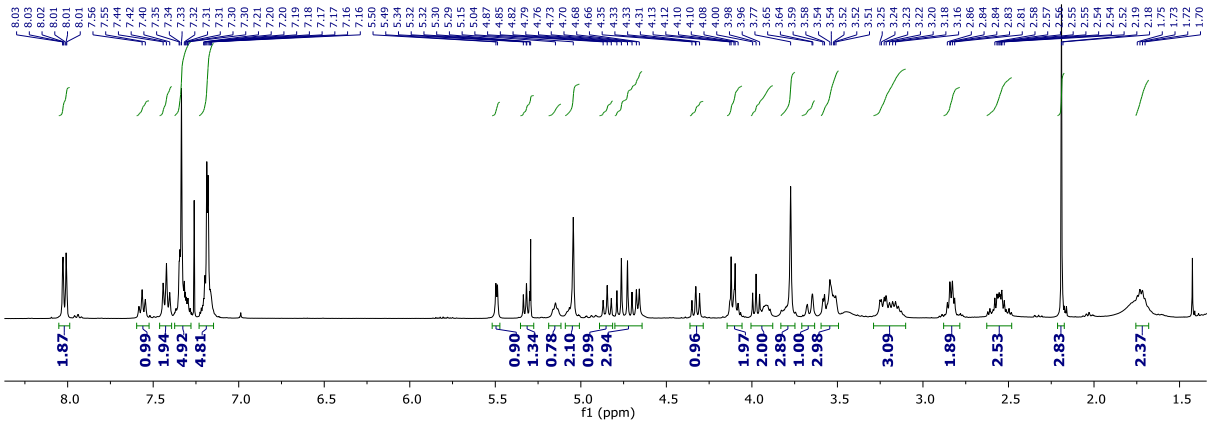

OSO3-
HO
HO
-O3SHN
CO2H
O
HO
OH
O
O
NH2

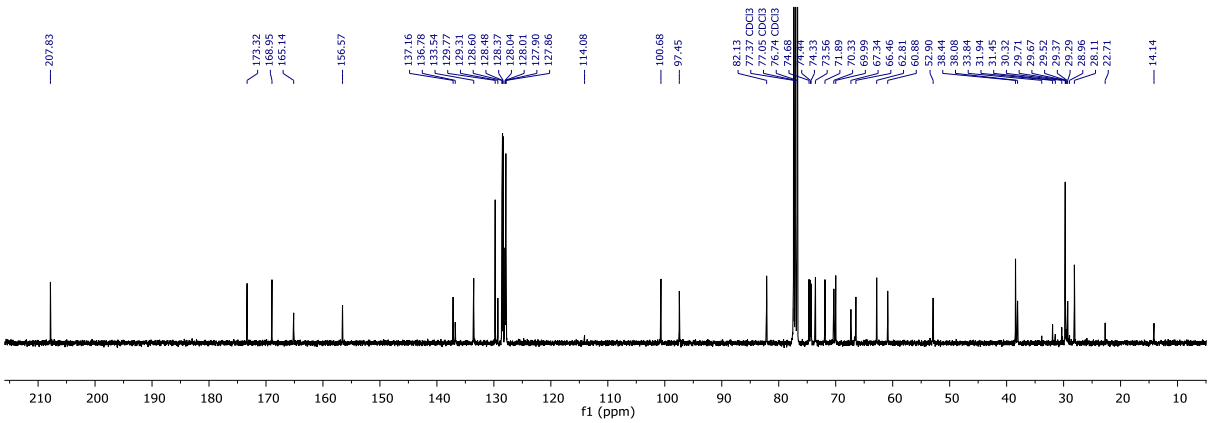

0.94
1.01
1.07
1.07
1.06
5.13
1.90
0.89
0.97
2.04
2.00
f1 (ppm)

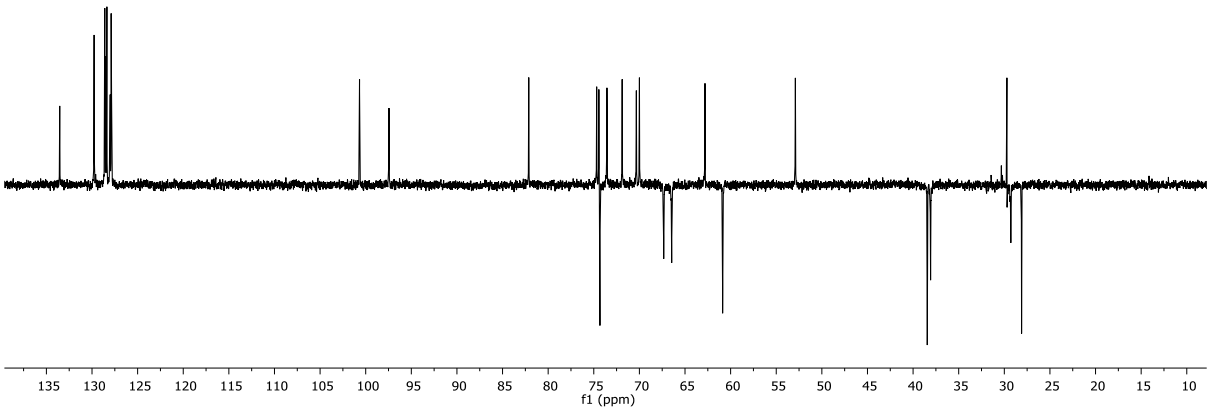

174.32
102.19
97.37
76.20
76.12
75.93
72.64
71.03
69.86
68.98
67.98
66.36
57.82
37.64
26.66
f1 (ppm)

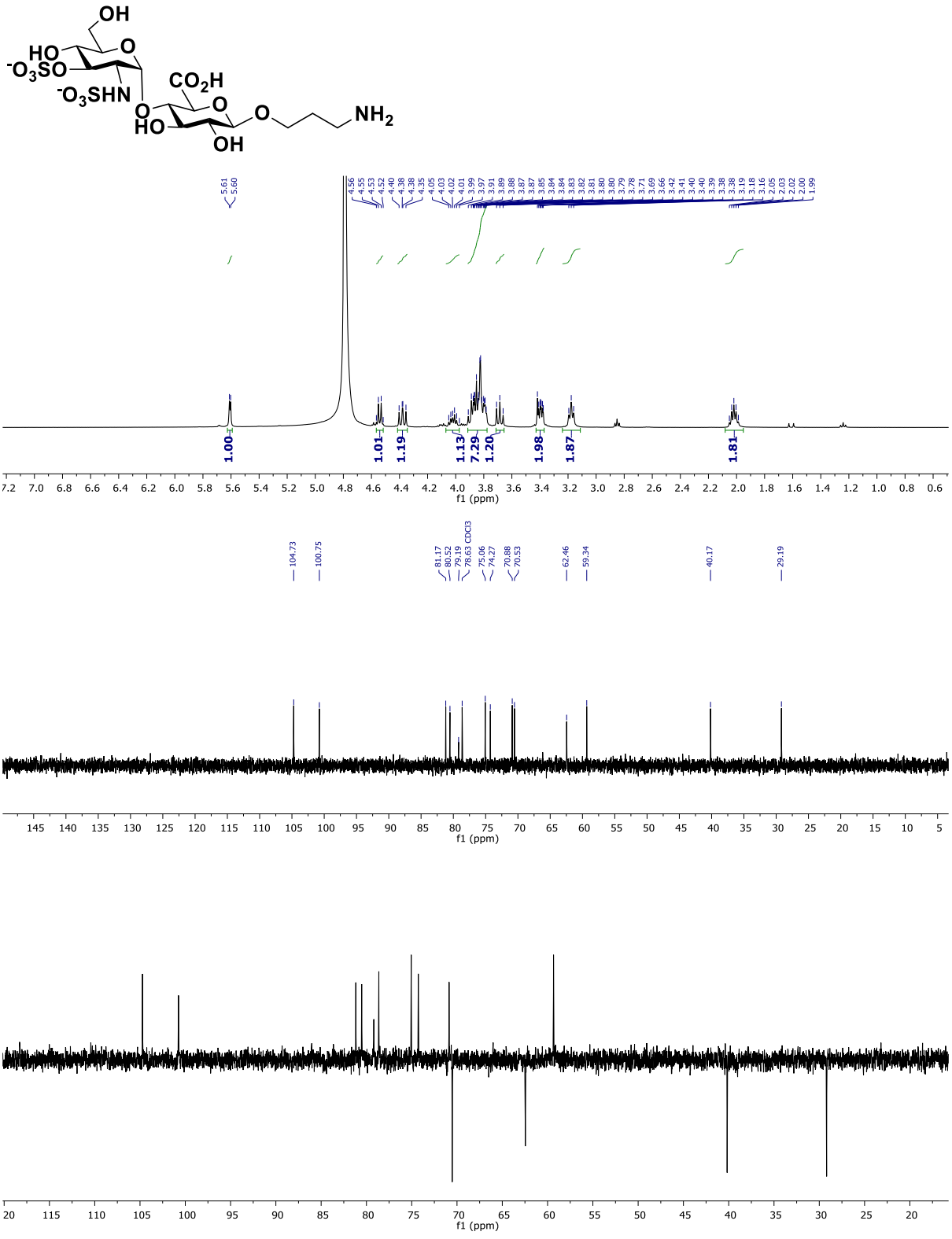

f1 (ppm)

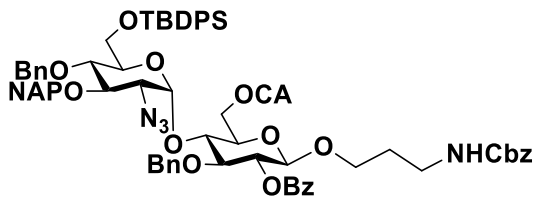

OH
LevO
HO
O
N3
CO2Me
O
O
BnO
O
NHCbz
OBz

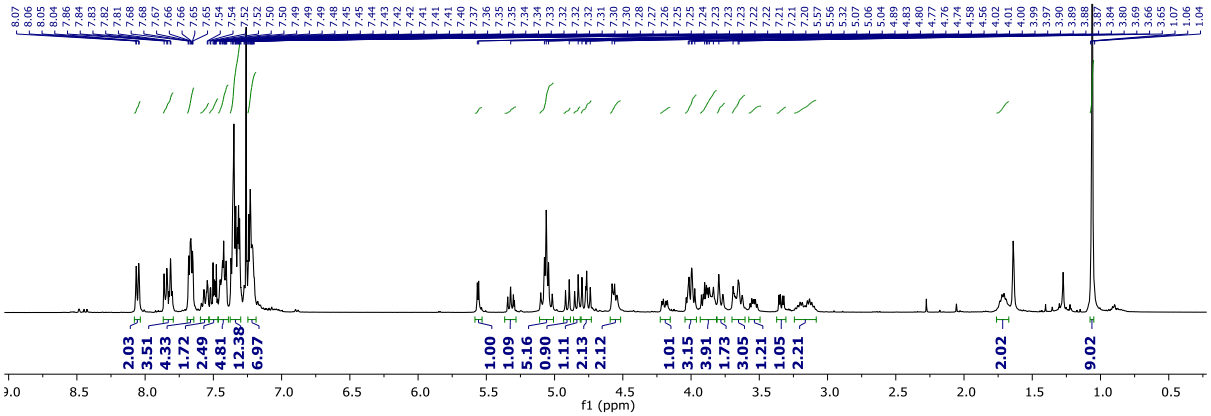

1.87
0.99
1.94
4.92
4.81
0.90
1.34
0.78
2.10
0.99
2.94
0.96
1.97
2.00
2.89
1.00
2.98
3.09
1.89
2.53
2.83
2.37
f1 (ppm)

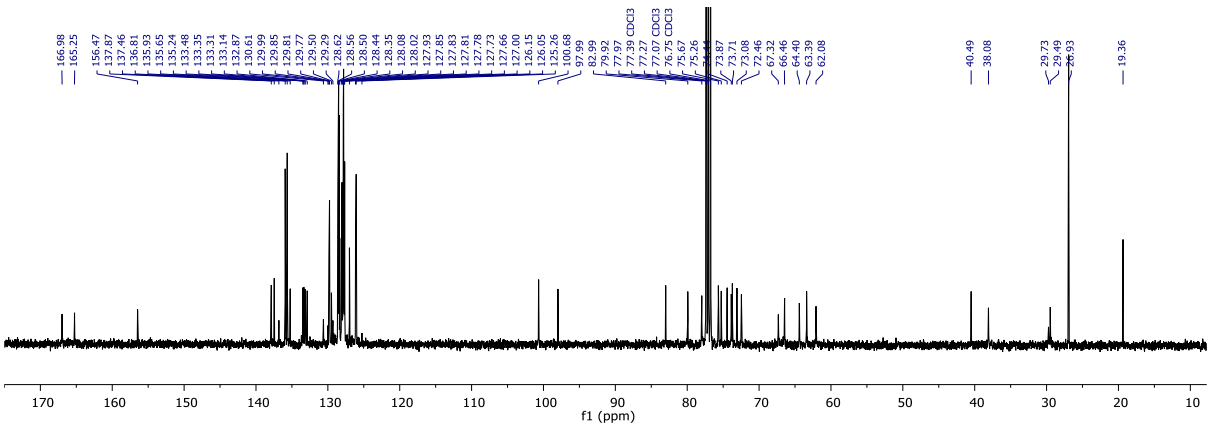

207.83
173.32
168.95
165.14
156.57
137.16
136.78
133.54
129.77
129.31
128.60
128.48
128.37
128.04
128.01
127.90
127.86
114.08
100.68
97.45
82.13
77.32 CDCl3
77.05 CDCl3
76.74 CDCl3
74.68
74.44
74.33
73.56
71.89
70.33
69.99
67.34
66.46
62.81
60.88
52.90
38.44
38.08
33.84
31.94
31.45
30.32
29.71
29.67
29.52
29.37
29.29
28.96
28.11
22.71
14.14
f1 (ppm)

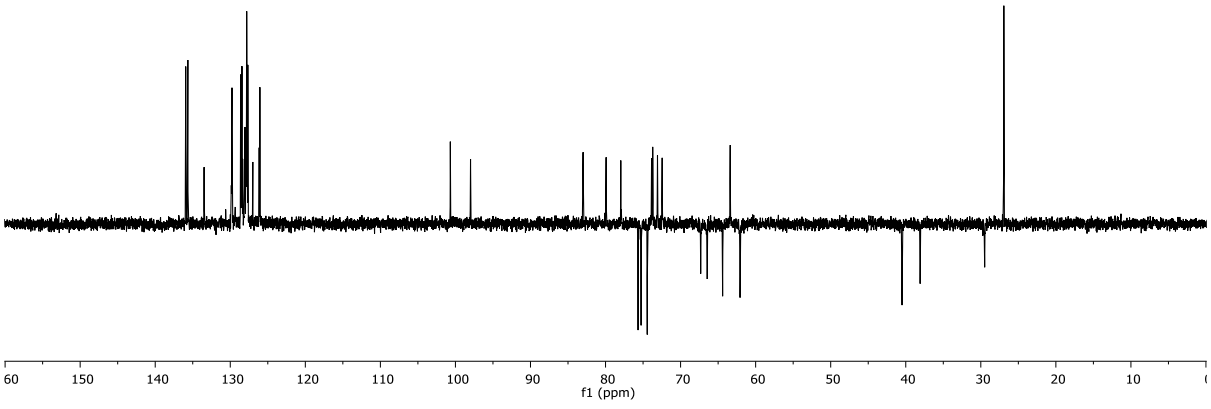

f1 (ppm)

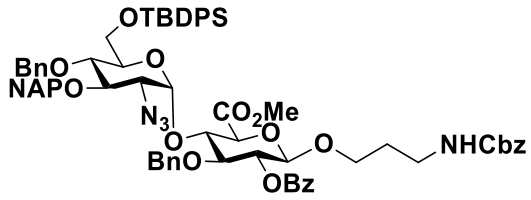

OH
HO
O
-O3SO
-O3SHN
CO2H
O
O
NH2
HO
OH
1.00
1.01
1.19
1.13
7.29
1.20
1.98
1.87
1.81
f1 (ppm)
104.73
100.75
81.17
80.52
79.19
78.63
CDCl3
75.06
74.27
70.88
70.53
62.46
59.34
40.17
29.19
f1 (ppm)
f1 (ppm)

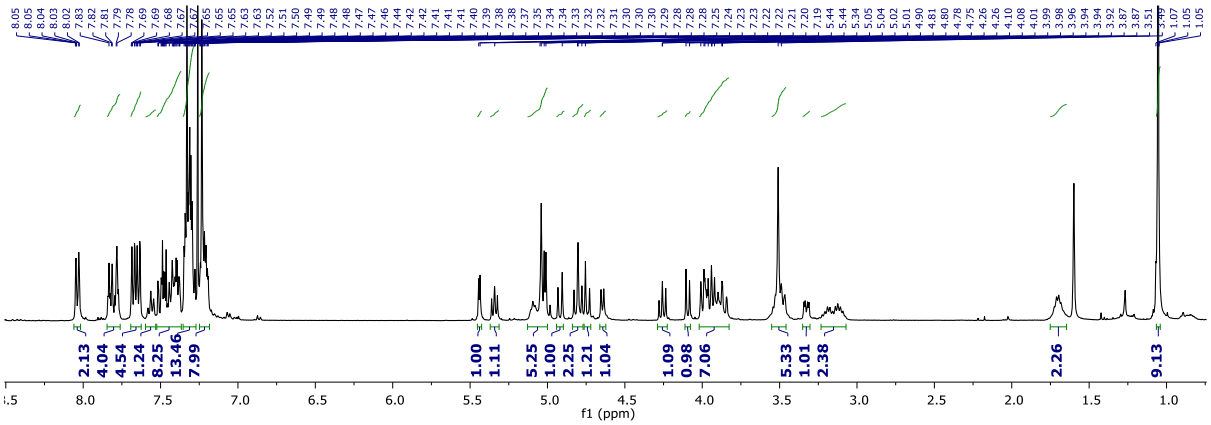

OTBDPS
BnO
NAPO
O
N3
OCA
O
O
BnO
O
NHCbz
OBz

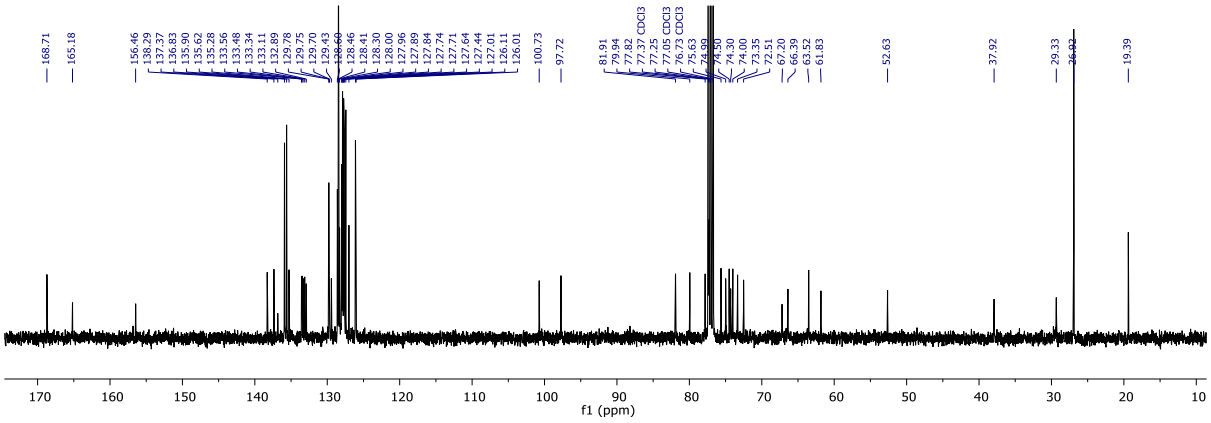

2.03
3.51
4.33
1.72
2.49
4.81
12.38
6.97
1.00
1.09
5.16
0.90
1.11
2.13
2.12
1.01
3.15
3.91
1.73
3.05
1.21
1.05
2.21
2.02
9.02
f1 (ppm)

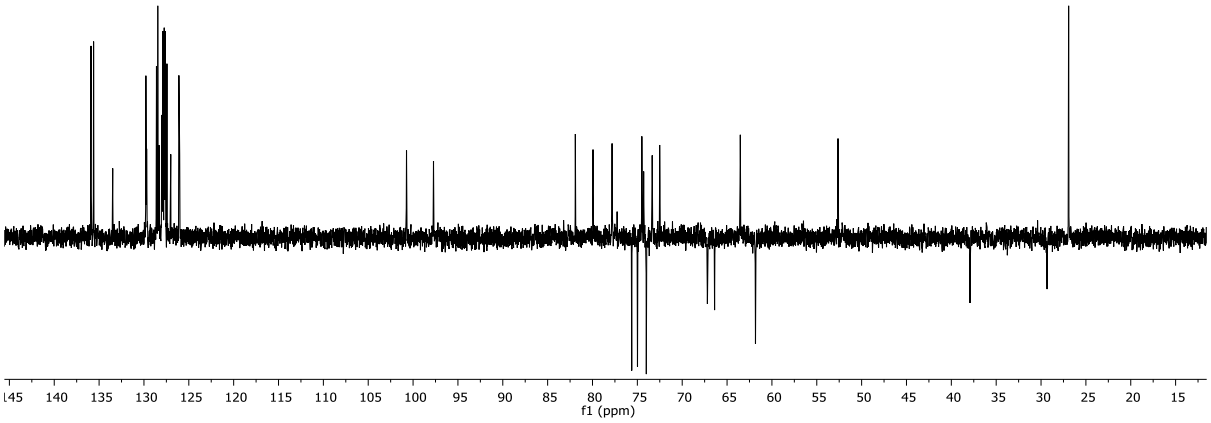

f1 (ppm)

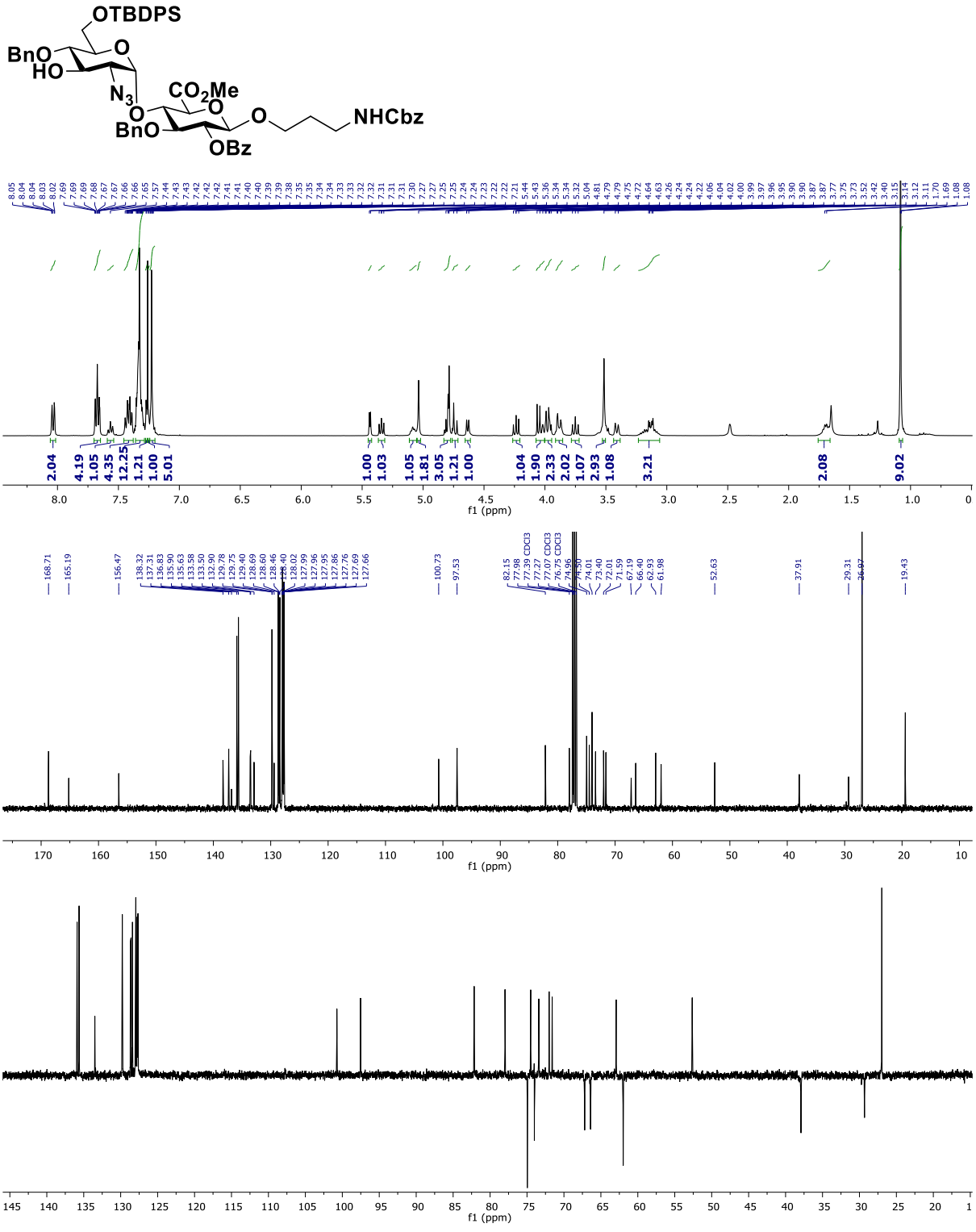

f1 (ppm)

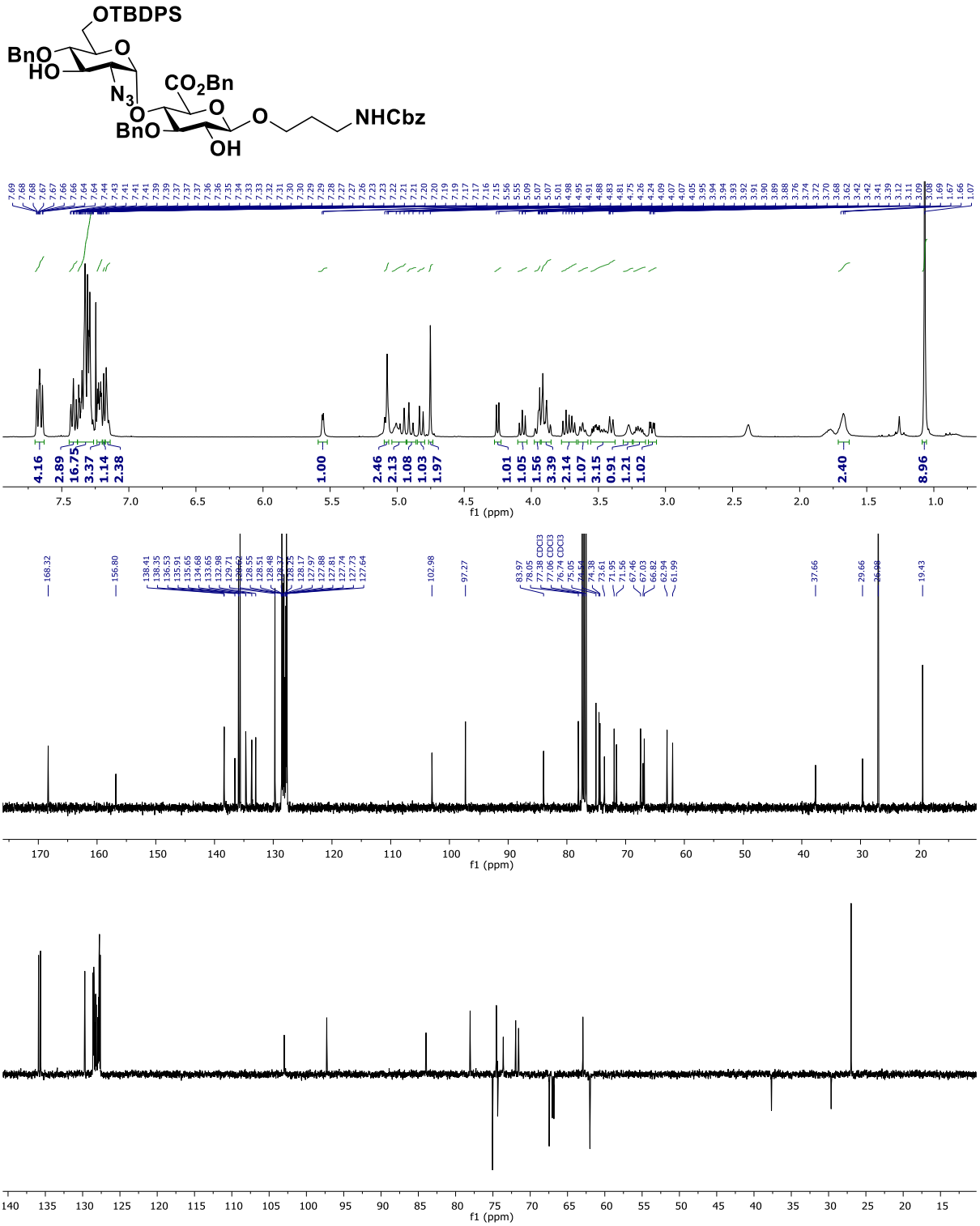

OTBDPS
BnO
NAPO
O
N3
CO2Me
O
O
O
BnO
NHCbz
OBz

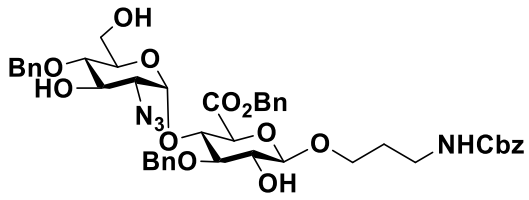

2.13
4.04
4.54
1.24
8.25
13.46
7.99
1.00
1.11
5.25
1.00
2.25
1.21
1.04
1.09
0.98
7.06
5.33
1.01
2.38
2.26
9.13
f1 (ppm)

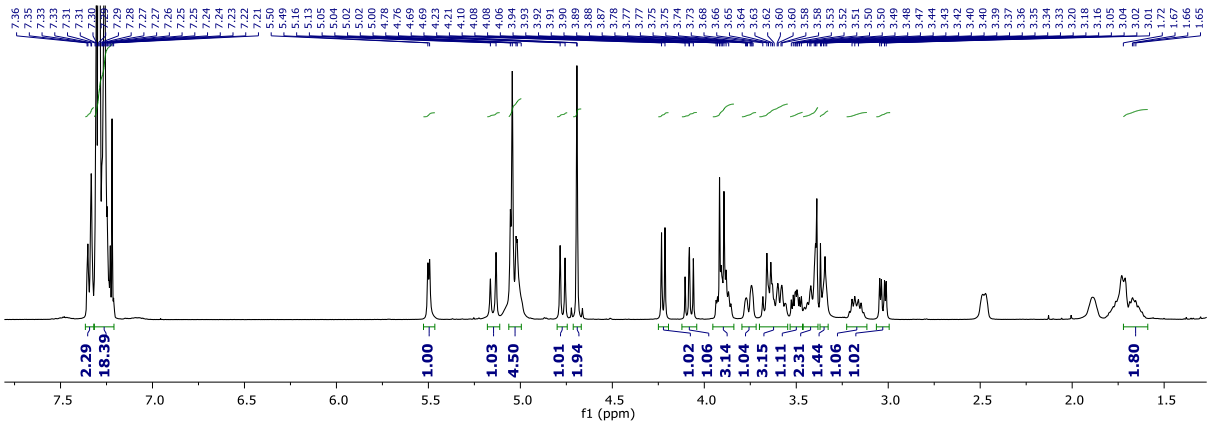

f1 (ppm)

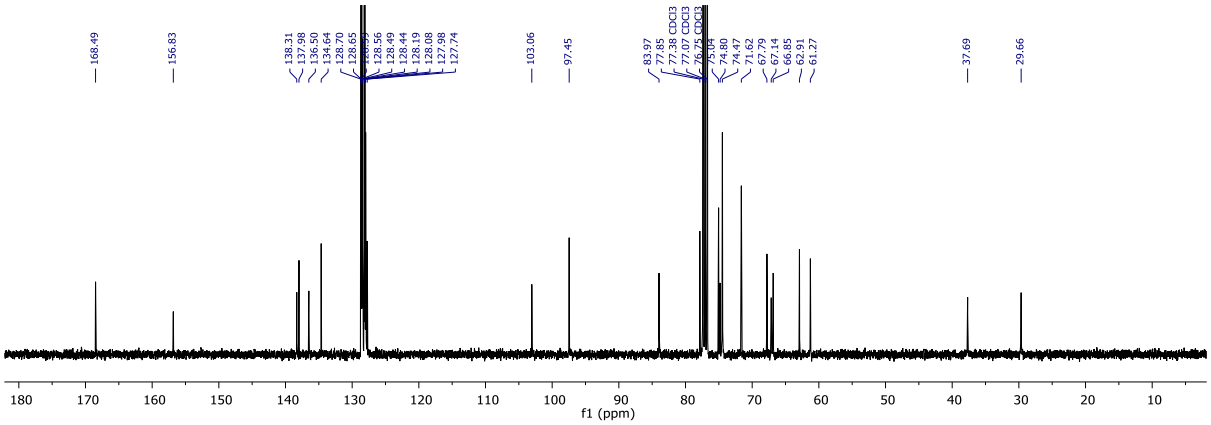

f1 (ppm)

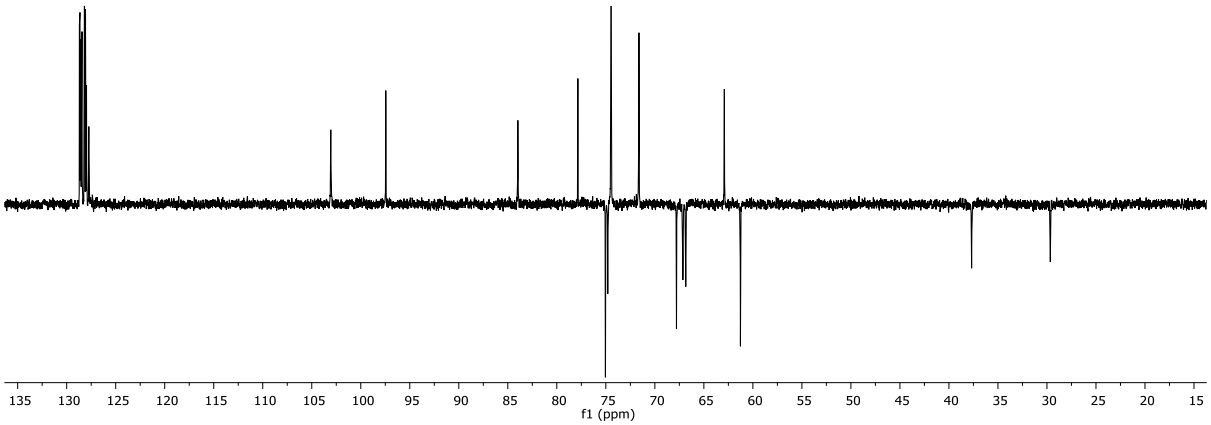

OTBDPS
BnO
HO
O
N3
CO2Me
O
O
O
NHCbz
BnO
OBz
f1 (ppm)

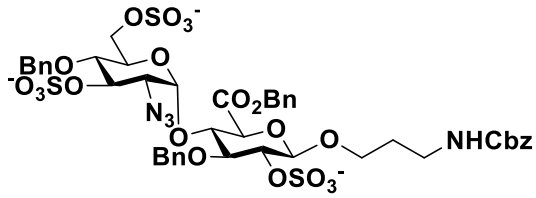

OTBDPS
BnO
HO
O
N3
CO2Bn
O
O
O
NHCbz
BnO
OH
4.16
2.89
16.75
3.37
1.14
2.38
1.00
2.46
2.13
1.08
1.03
1.97
1.01
1.05
1.56
3.39
2.14
1.07
3.15
0.91
1.21
1.02
2.40
8.96
f1 (ppm)
f1 (ppm)
f1 (ppm)

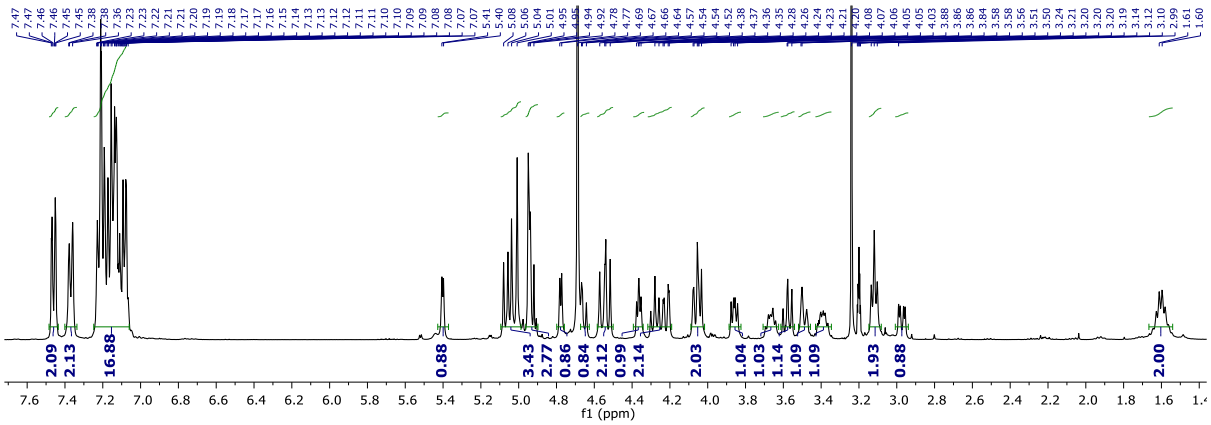

OH
BnO
HO
O
N3
CO2Bn
O
O
O
NHCbz
BnO
OH

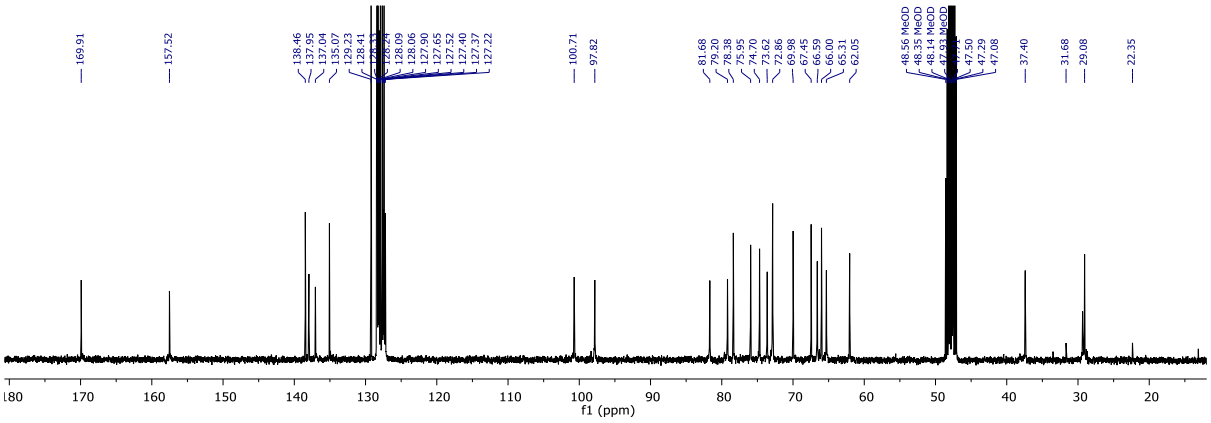

2.29
18.39
1.00
1.03
4.50
1.01
1.94
1.02
1.06
3.14
1.04
3.15
1.11
2.31
1.44
1.06
1.02
1.80
f1 (ppm)

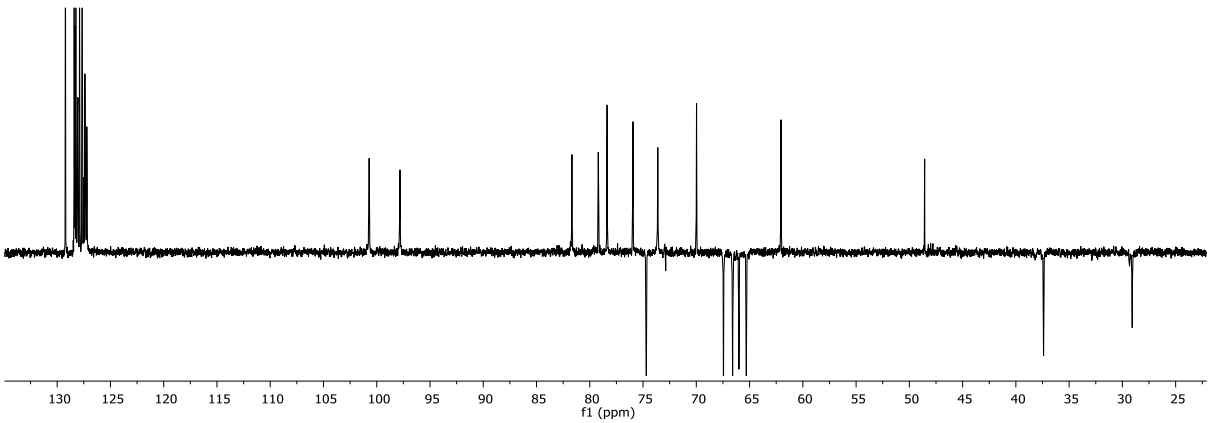

168.49
156.83
138.31
137.98
136.50
134.64
128.70
128.65
128.56
128.49
128.44
128.19
128.08
127.98
127.74
103.06
97.45
83.97
77.85
77.38 CDCl3
77.07 CDCl3
76.75 CDCl3
75.04
74.80
74.47
71.62
67.79
67.14
66.85
62.91
61.27
37.69
29.66
f1 (ppm)

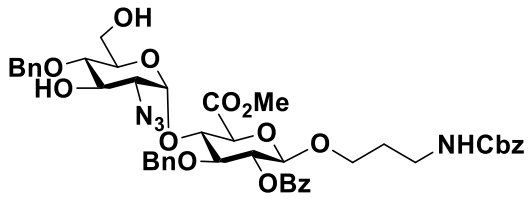

f1 (ppm)

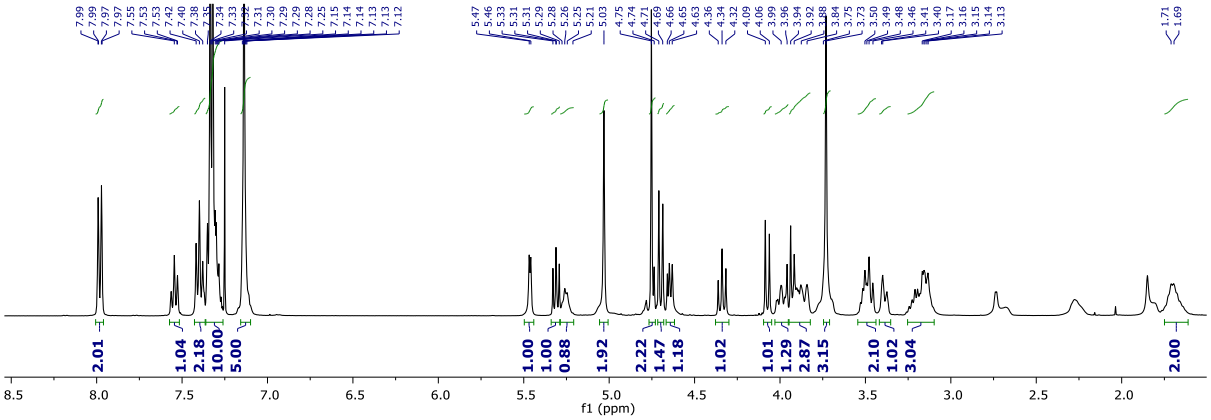

OSO3-
BnO
-O3SO
O
N3
CO2Bn
O
O
NHCbz
BnO
OSO3-

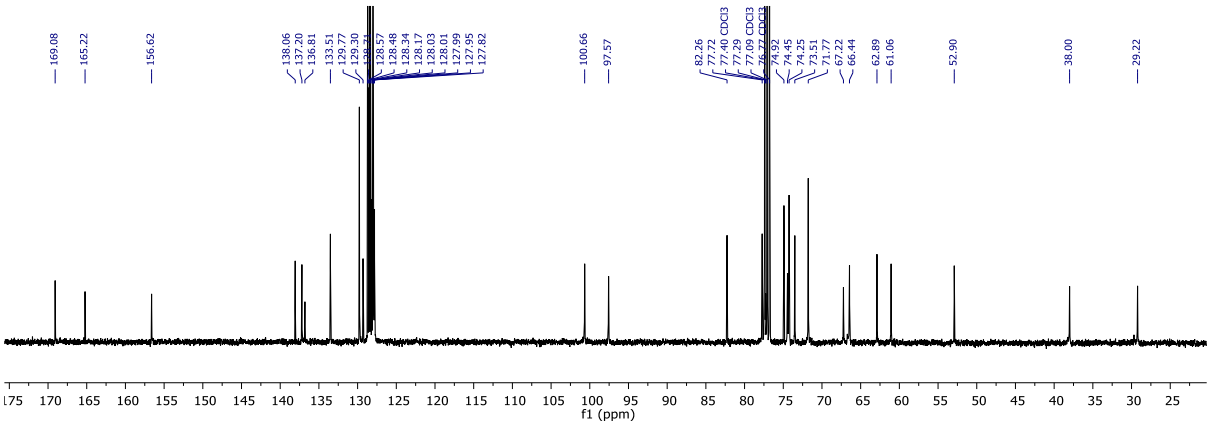

2.09
2.13
16.88
0.88
3.43
2.77
0.86
0.84
2.12
0.99
2.14
2.03
1.04
1.03
1.14
1.09
1.09
1.93
0.88
2.00
f1 (ppm)

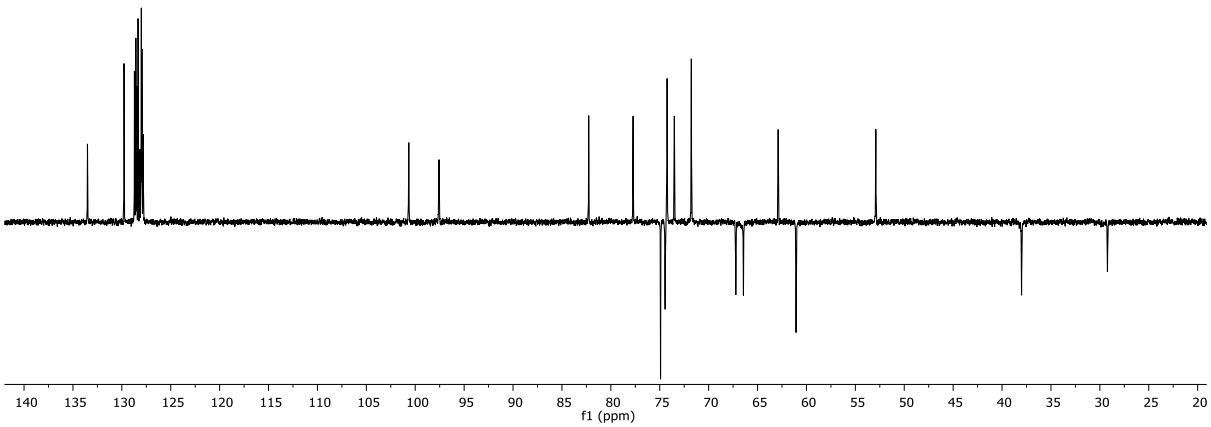

169.91
157.52
138.46
137.95
137.04
135.07
129.23
128.41
128.09
128.06
127.90
127.65
127.52
127.40
127.37
127.22
100.71
97.82
81.68
79.20
78.38
75.95
74.70
73.62
72.86
69.98
67.45
66.59
66.00
65.31
62.05
48.56 MeOD
48.35 MeOD
48.14 MeOD
47.93 MeOD
47.50
47.29
47.08
37.40
31.68
29.08
22.35
f1 (ppm)

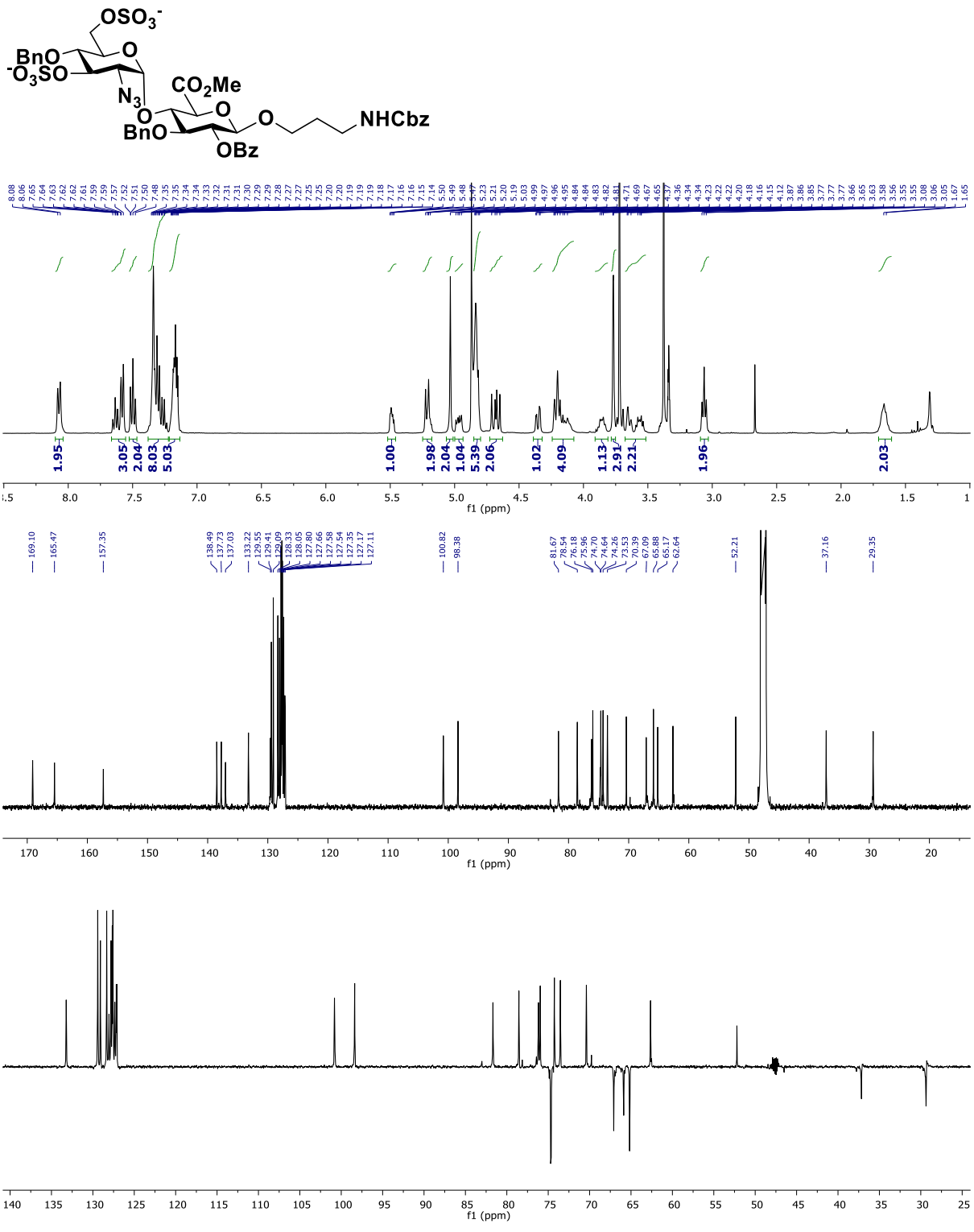

f1 (ppm)

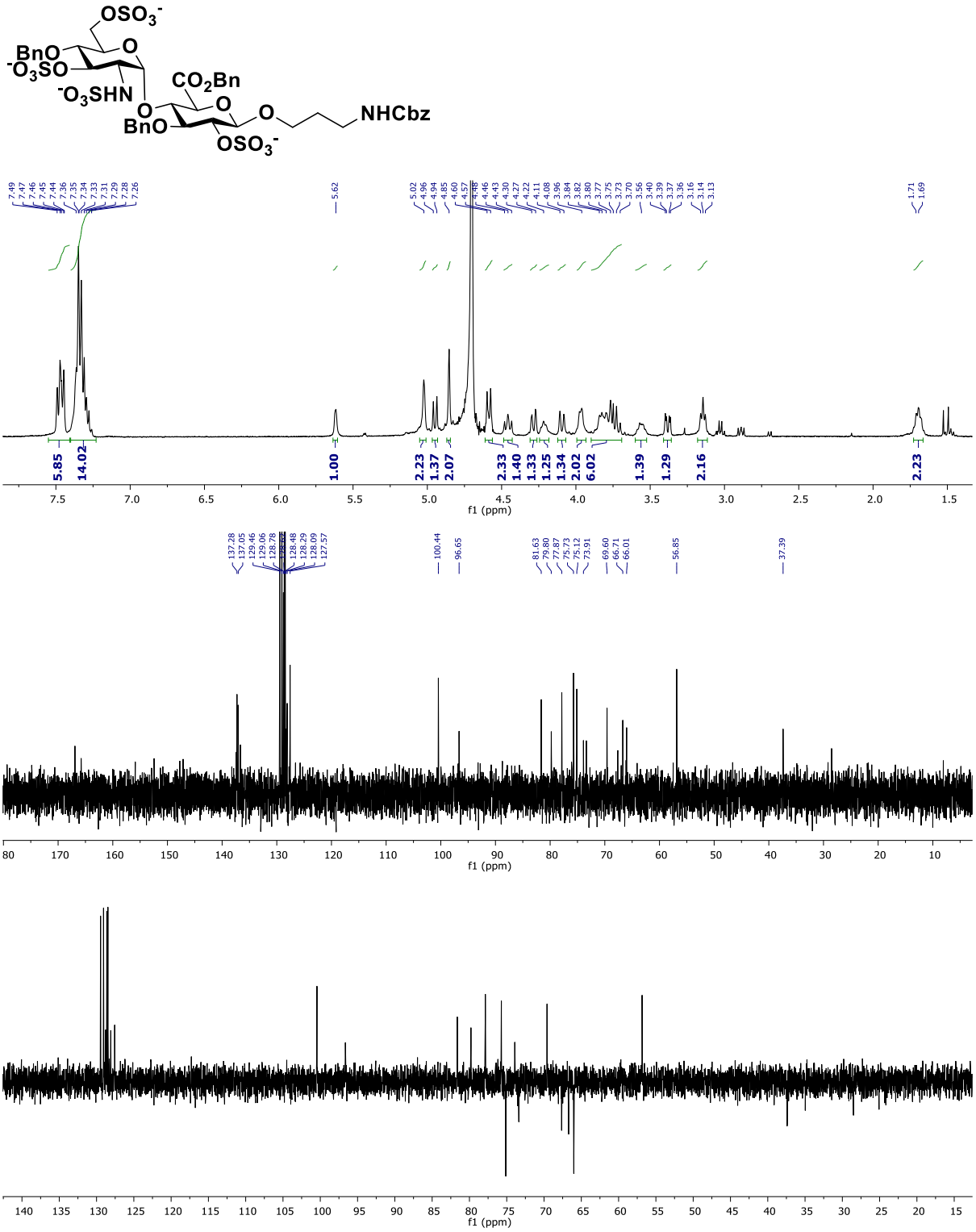

OH
BnO
HO
O
N3
CO2Me
O
O
O
NHCbz
BnO
OBz

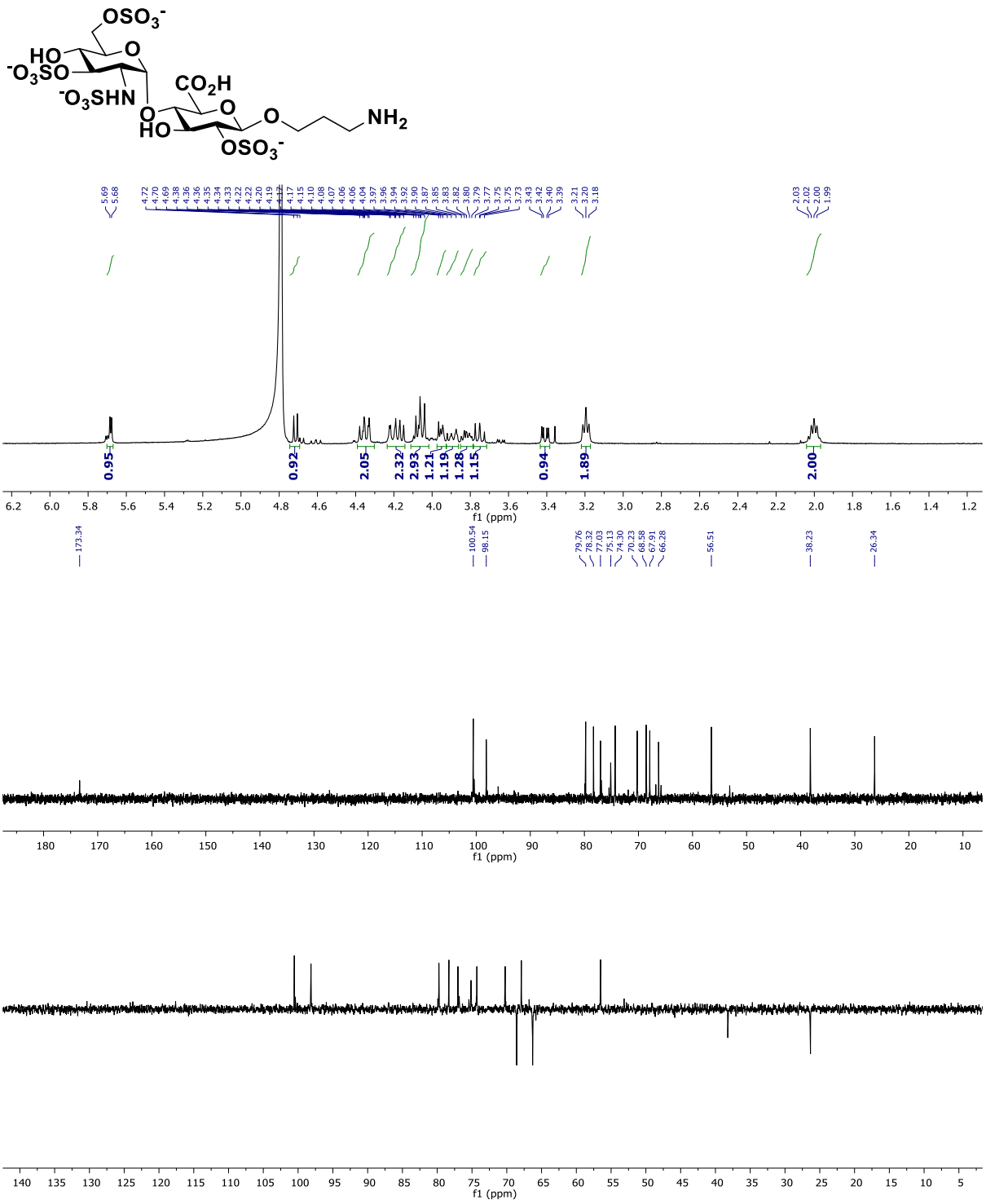

2.01
1.04
2.18
10.00
5.00
1.00
1.00
0.88
1.92
2.22
1.47
1.18
1.02
1.01
1.29
2.87
3.15
2.10
1.02
3.04
2.00
f1 (ppm)

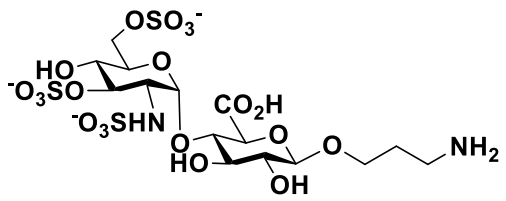

f1 (ppm)

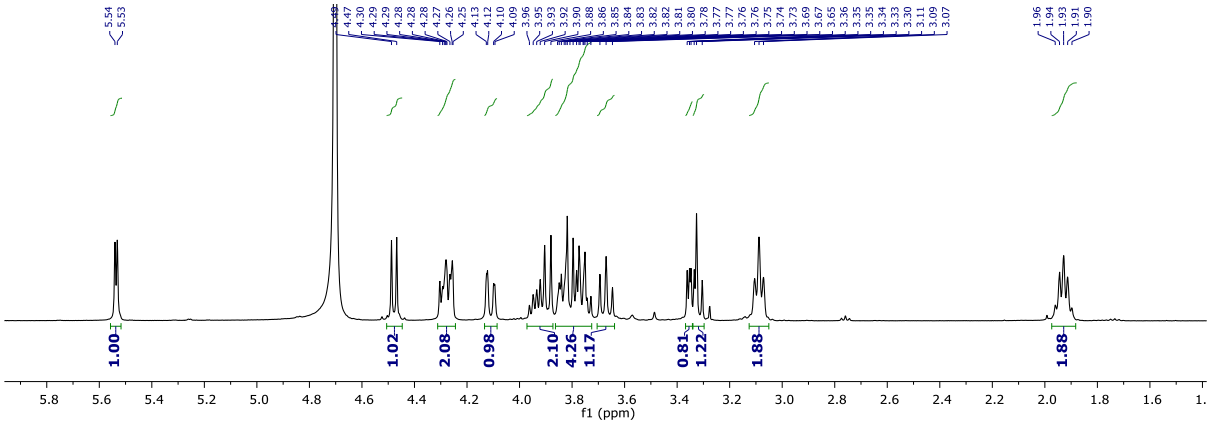

f1 (ppm)

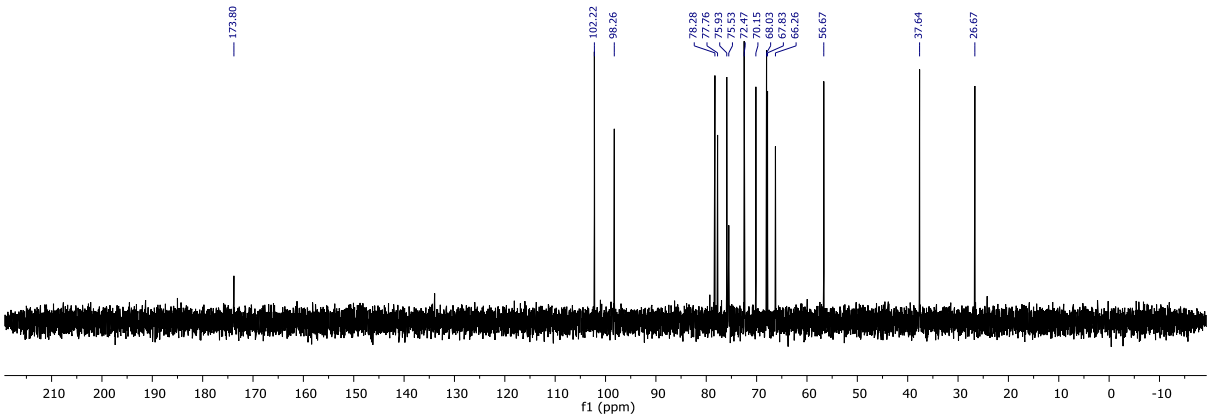

OSO3-
BnO
-O3SO
O
N3
CO2Me
O
O
BnO
O
NHCbz
OBz
f1 (ppm)

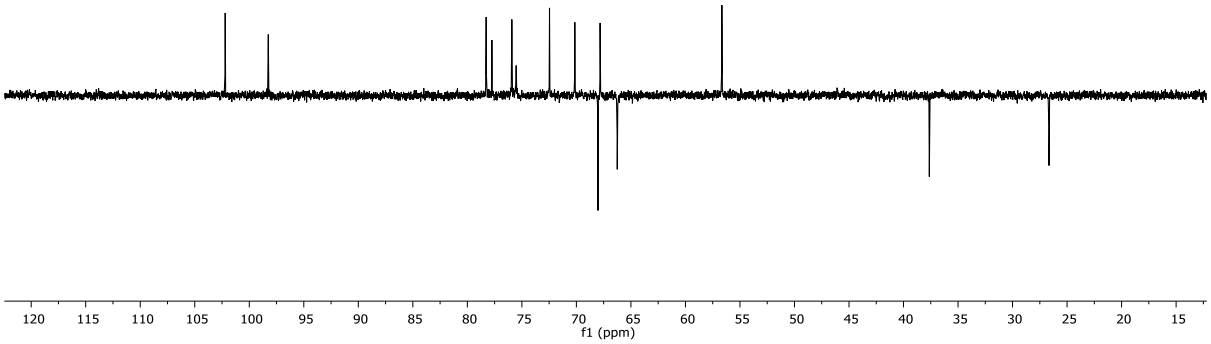

OSO3-
BnO
-O3SO
-O3SHN
CO2Bn
BnO
OSO3-
NHCbz
f1 (ppm)
f1 (ppm)
f1 (ppm)

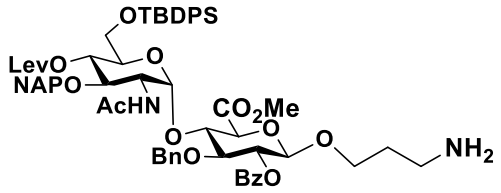

OSO3-
HO
-O3SO
-O3SHN
CO2H
O
HO
OSO3-
NH2
f1 (ppm)

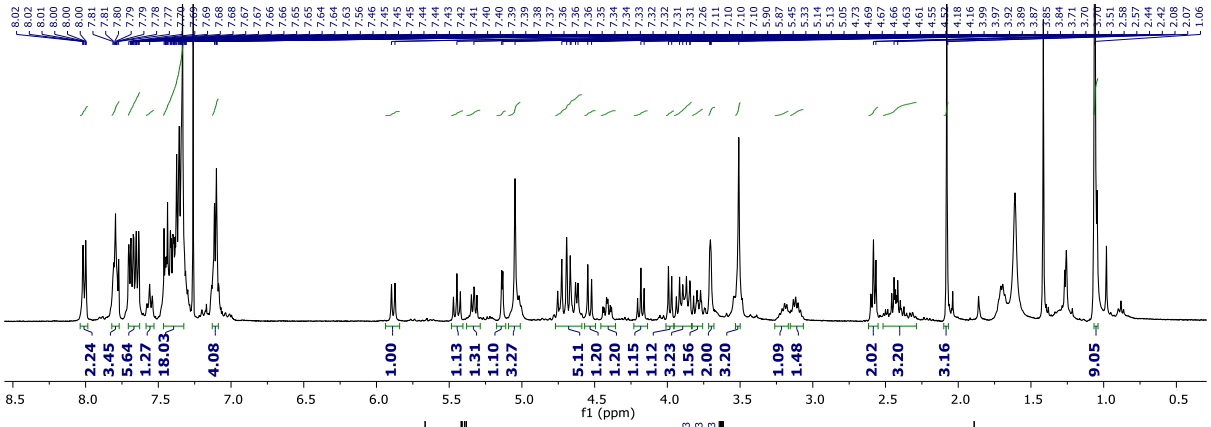

OSO3-
HO
-O3SO
-O3SHN
CO2H
O
HO
OH
NH2

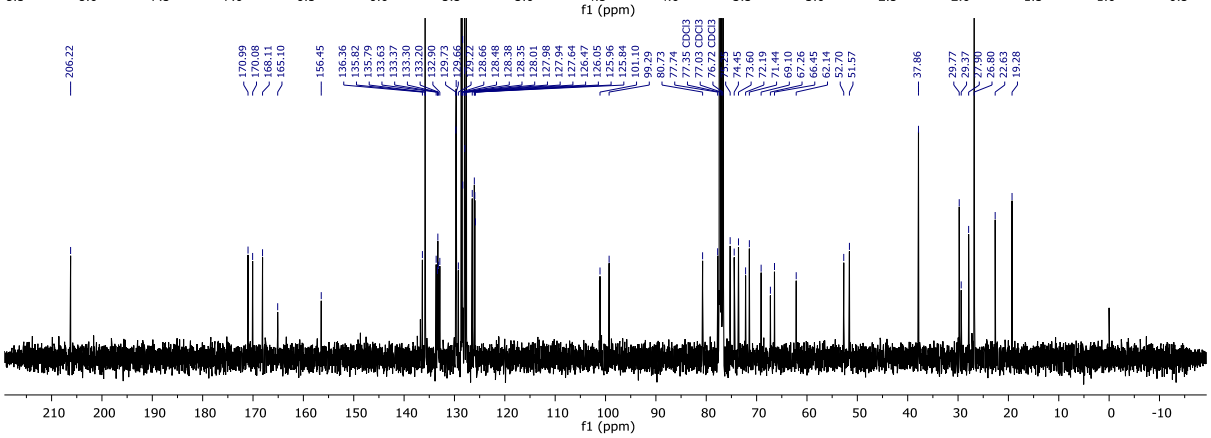

1.00
1.02
2.08
0.98
2.10
4.26
1.17
0.81
1.22
1.88
1.88
f1 (ppm)

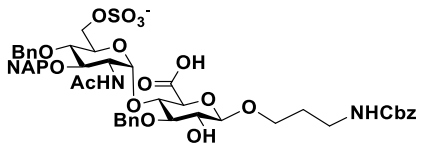

173.80
102.22
98.26
78.28
77.76
75.93
75.53
72.47
70.15
68.03
67.83
66.26
56.67
37.64
26.67
f1 (ppm)

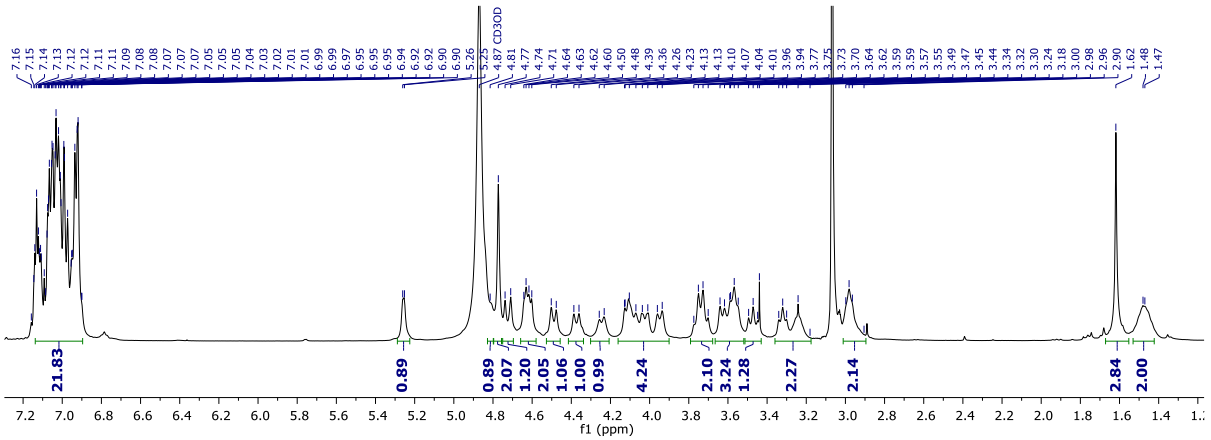

f1 (ppm)

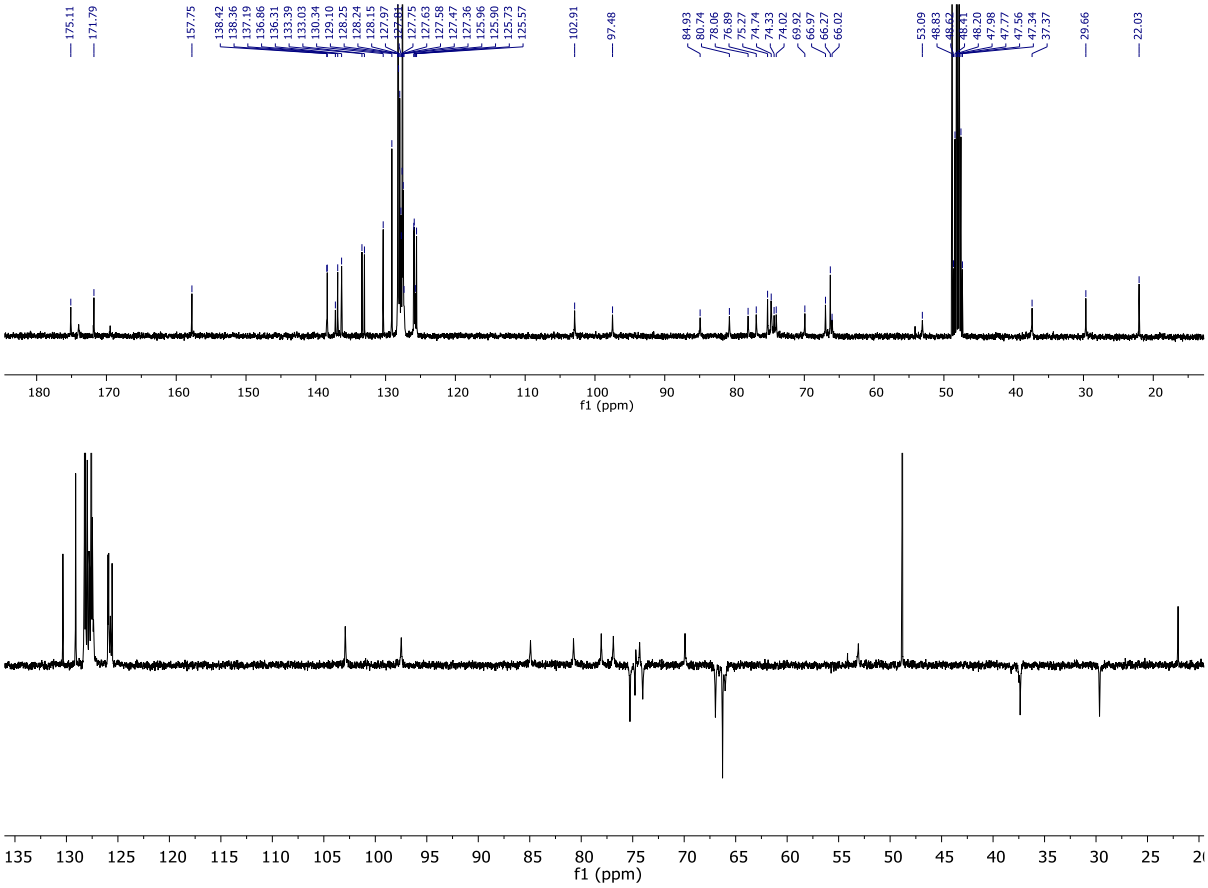

OTBDPS
LevO
NAPO
AcHN
O
CO2Me
BnO
BzO
NH2

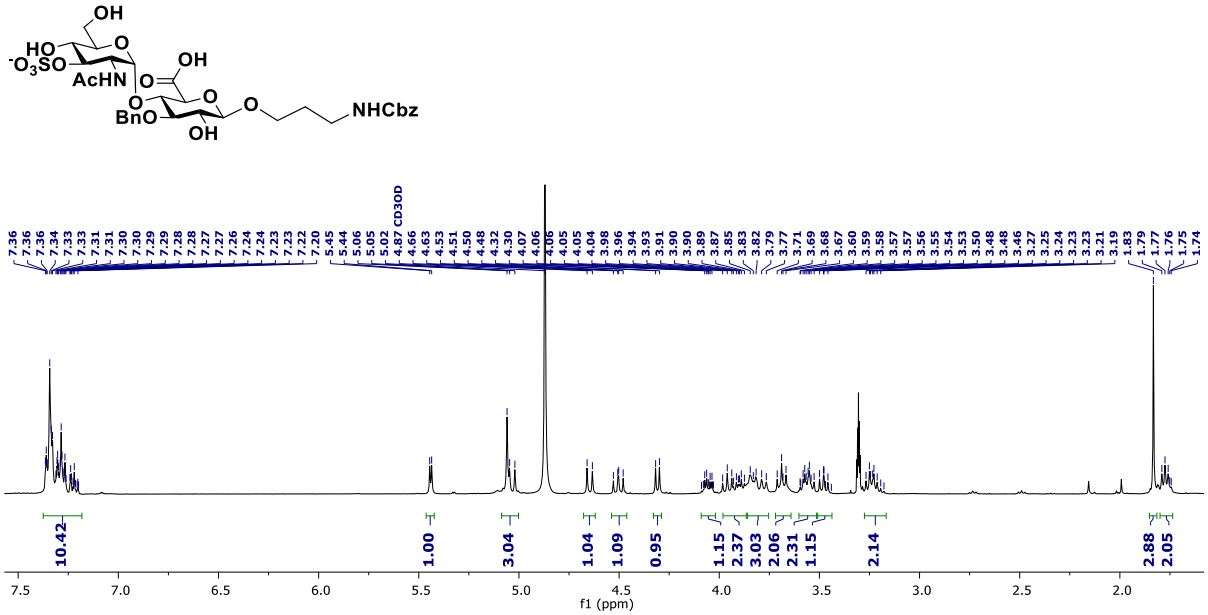

2.24
3.45
5.64
1.27
18.03
4.08
1.00
1.13
1.31
1.10
3.27
5.11
1.20
1.20
1.15
1.12
3.23
1.56
2.00
3.20
1.09
1.48
2.02
3.20
3.16
9.05
f1 (ppm)

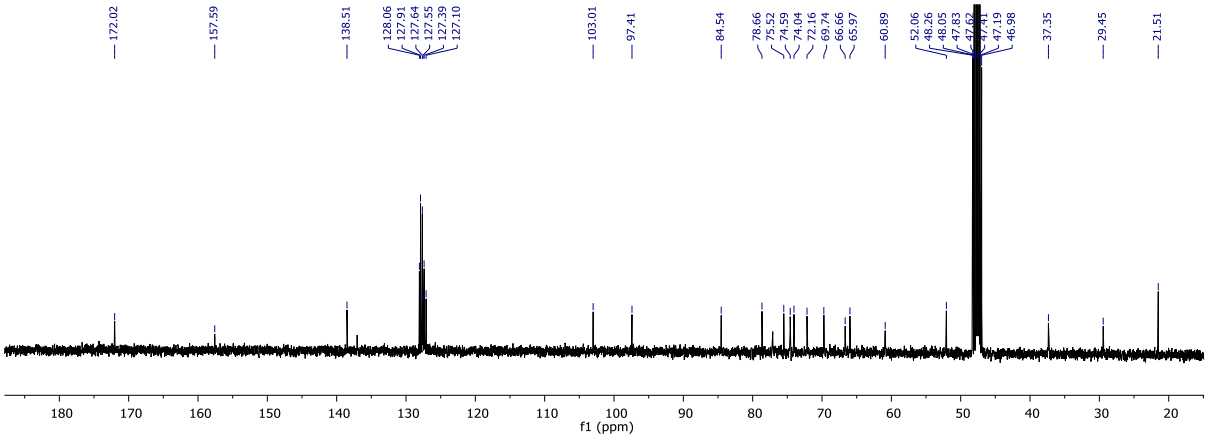

206.22
170.99
170.08
168.11
165.10
156.45
136.36
135.82
135.79
133.63
133.37
133.30
133.20
132.90
129.73
129.66
128.66
128.48
128.38
128.35
128.01
127.98
127.94
127.64
126.47
126.05
125.96
125.84
101.10
99.29
80.73
77.74
77.35 CDCl3
77.03 CDCl3
76.72 CDCl3
74.45
74.19
72.39
71.44
69.10
67.26
66.45
62.14
52.70
51.57
37.86
29.77
29.37
27.90
26.80
22.63
19.28
f1 (ppm)

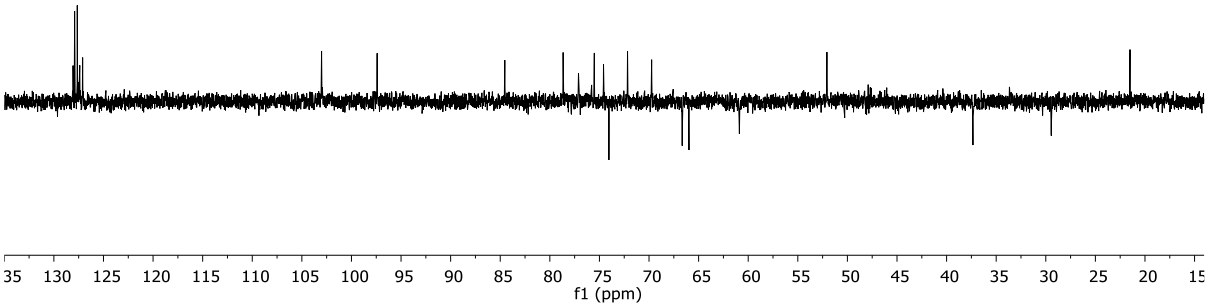

OSO3-
BnO
NAPO
AcHN
O
OH
BnO
OH
NHCbz

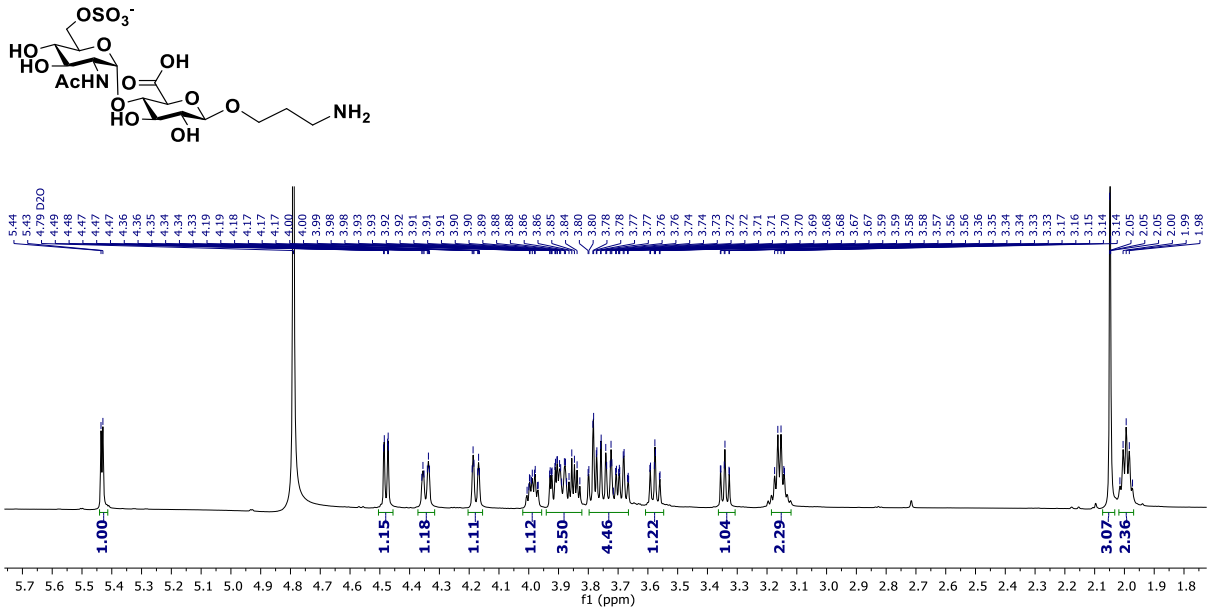

CD3OD
21.83
0.89
0.89
2.07
1.20
2.05
1.06
1.00
0.99
4.24
2.10
3.24
1.28
2.27
2.14
2.84
2.00
f1 (ppm)

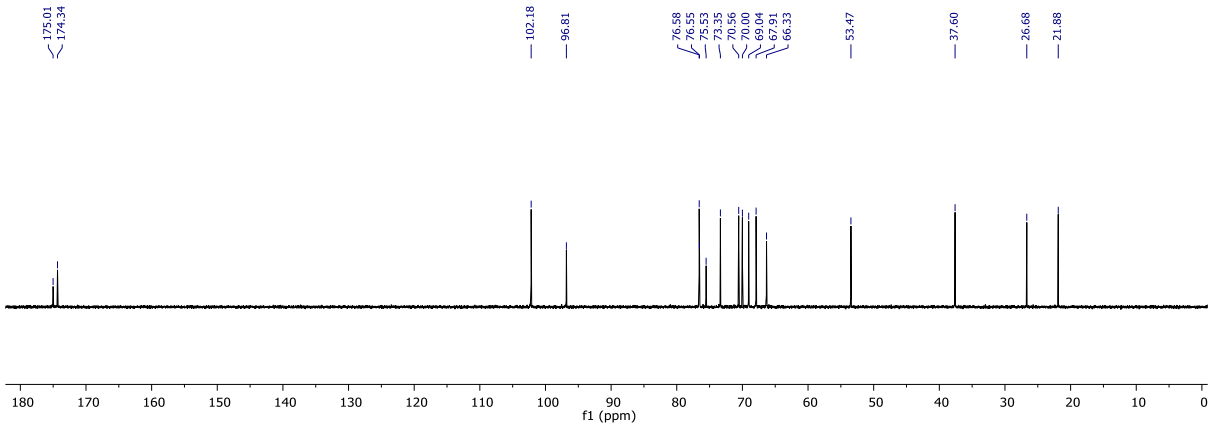

175.11
171.79
157.75
138.42
138.36
137.19
136.86
136.31
133.39
133.03
130.34
129.10
128.25
128.24
128.15
127.97
127.88
127.75
127.63
127.58
127.47
127.36
125.96
125.90
125.73
125.57
102.91
97.48
84.93
80.74
78.06
76.89
75.27
74.74
74.33
74.02
69.92
66.97
66.27
66.02
53.09
48.83
48.62
48.41
48.20
47.98
47.77
47.56
47.34
37.37
29.66
22.03
f1 (ppm)
f1 (ppm)

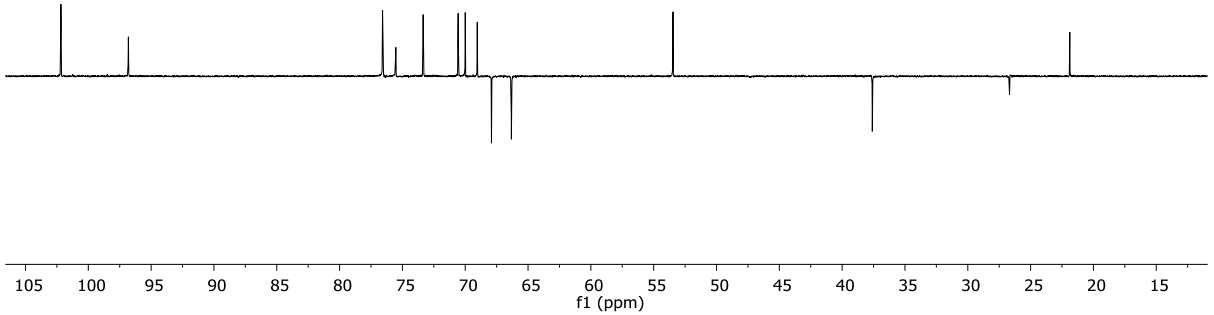

OH
HO
O
-O3SO
AcHN
O
OH
O
O
BnO
O
NHCbz
OH
CD3OD
10.42
1.00
3.04
1.04
1.09
0.95
1.15
2.37
3.03
2.06
2.31
1.15
2.14
2.88
2.05
f1 (ppm)

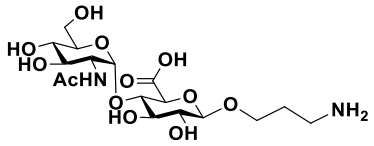

172.02
157.59
138.51
128.06
127.91
127.64
127.55
127.39
127.10
103.01
97.41
84.54
78.66
75.52
74.59
74.04
72.16
69.74
66.66
65.97
60.89
52.06
48.26
48.05
47.83
47.62
47.41
47.19
46.98
37.35
29.45
21.51
f1 (ppm)

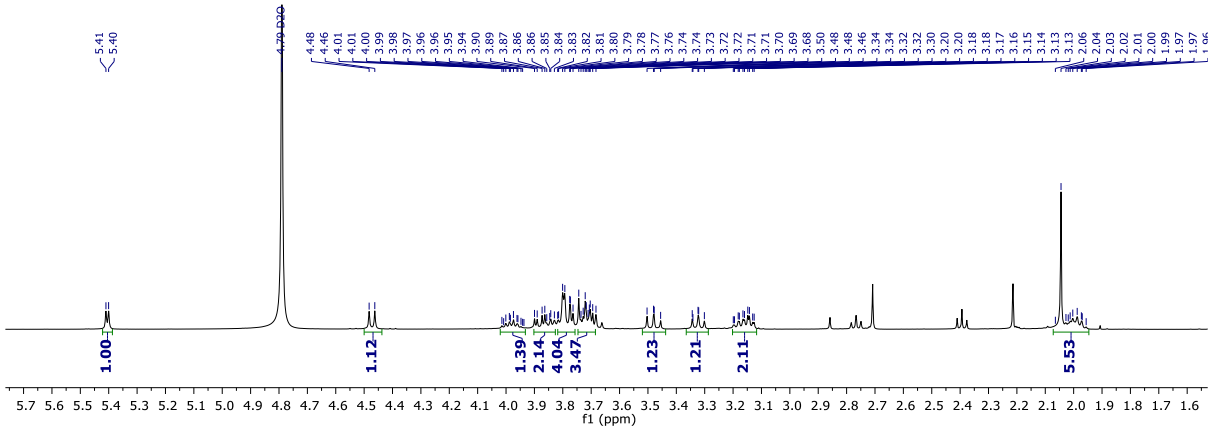

f1 (ppm)

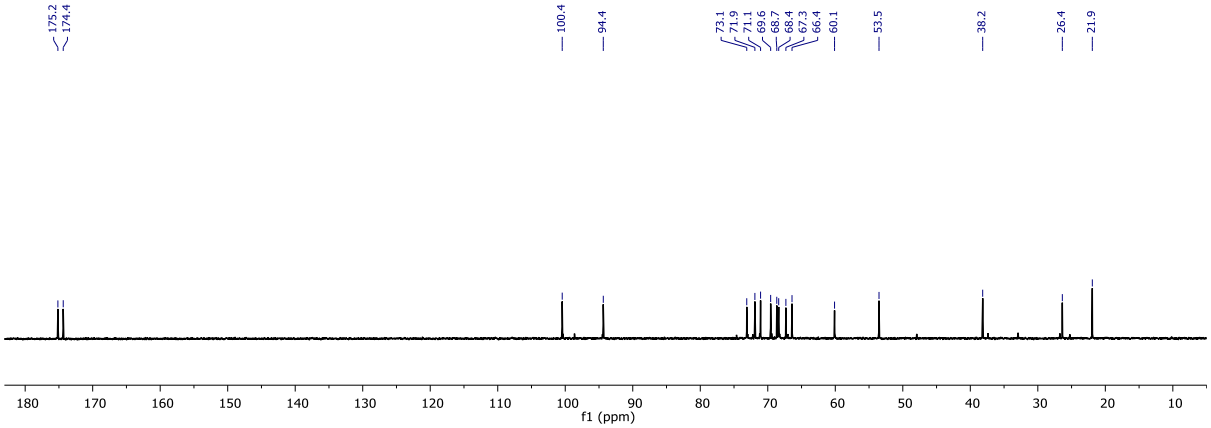

OSO3-
HO
HO
AcHN
O
OH
O
O
HO
OH
O
NH2
1.00
1.15
1.18
1.11
1.12
3.50
4.46
1.22
1.04
2.29
3.07
2.36
f1 (ppm)

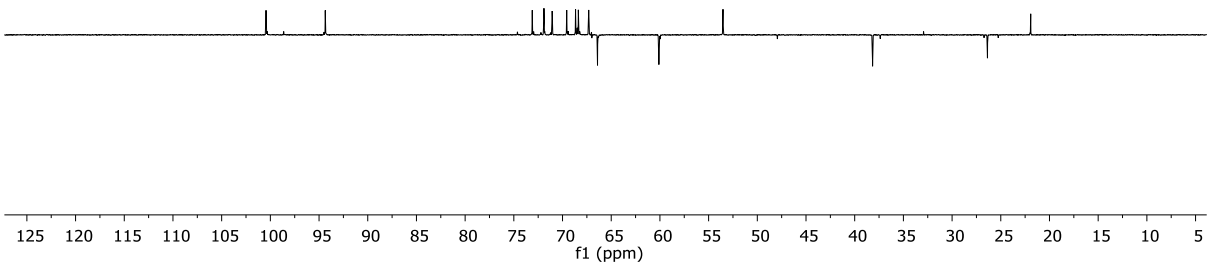

175.01
174.34
102.18
96.81
76.58
76.55
75.53
73.35
70.56
70.00
69.04
67.91
66.33
53.47
37.60
26.68
21.88
f1 (ppm)

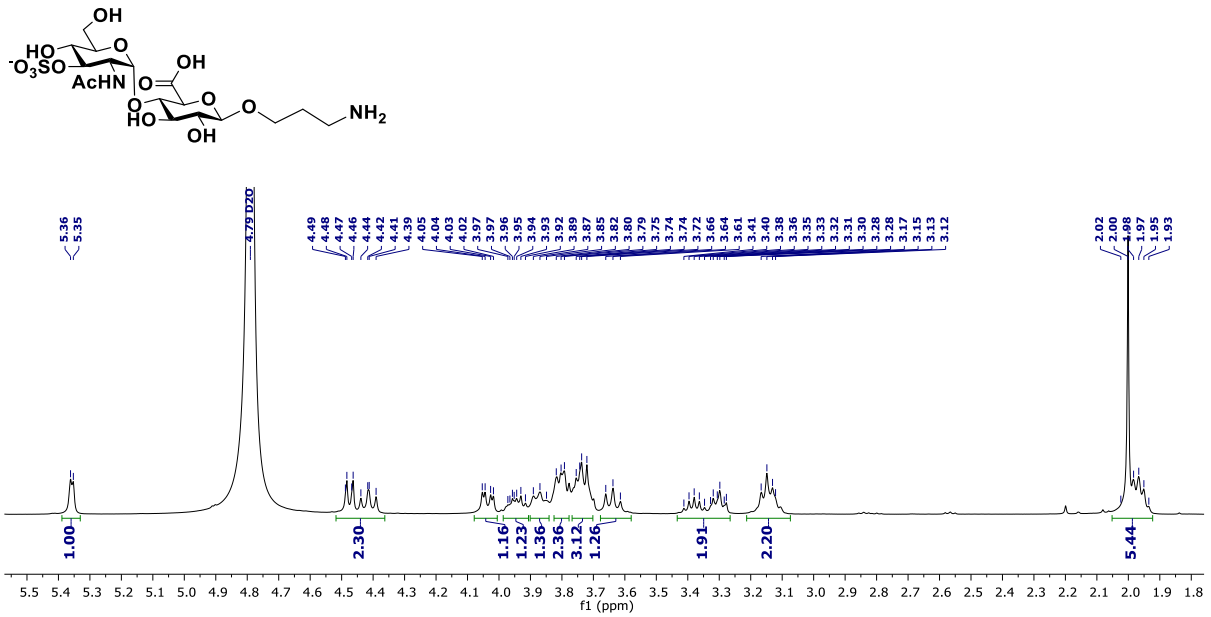

f1 (ppm)

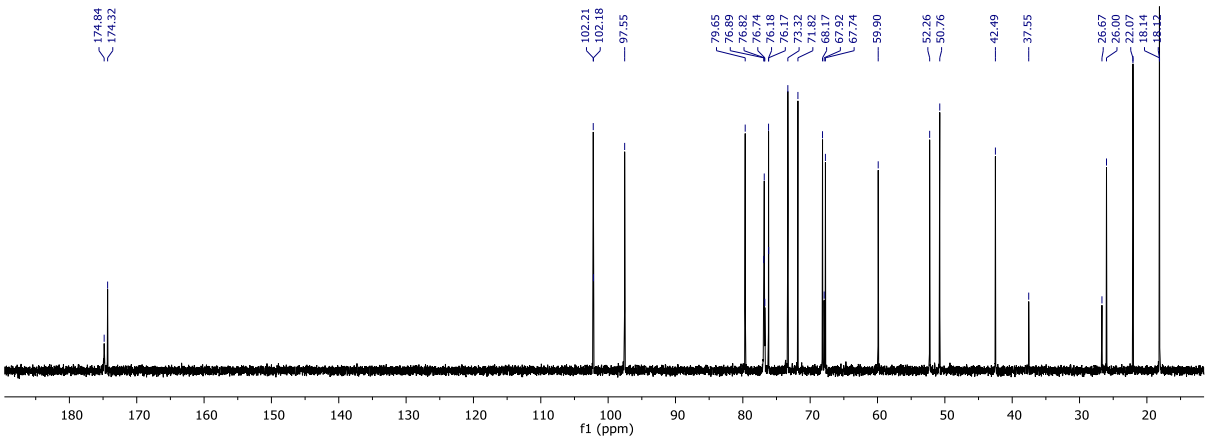

OH
HO
HO
O
AcHN
O
OH
O
O
HO
O
NH2
OH

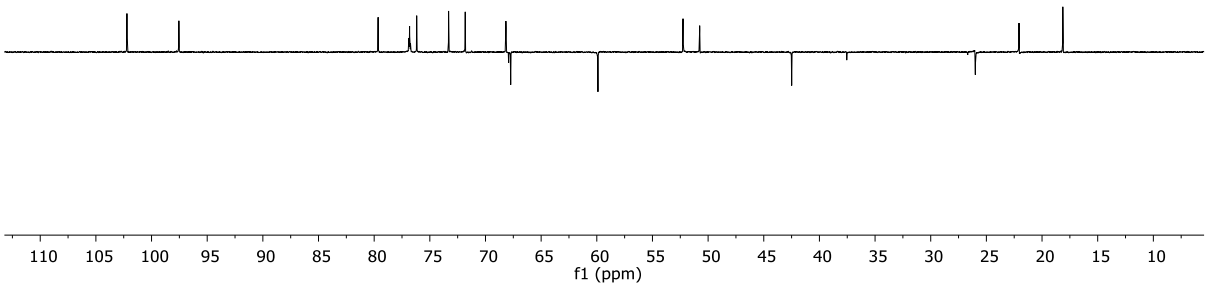

5.41
5.40
4.79 D2O
1.00
1.12
1.39
2.14
4.04
3.47
1.23
1.21
2.11
5.53
f1 (ppm)


175.2
174.4
100.4
94.4
73.1
71.9
71.1
69.6
68.7
68.4
67.3
66.4
60.1
53.5
38.2
26.4
21.9
f1 (ppm)


f1 (ppm)


5.36
5.35
4.79 D2O
4.49
4.48
4.47
4.46
4.44
4.42
4.41
4.39
4.05
4.04
4.03
4.02
3.97
3.97
3.96
3.95
3.94
3.93
3.92
3.89
3.87
3.85
3.82
3.80
3.79
3.75
3.74
3.74
3.72
3.66
3.64
3.61
3.41
3.40
3.38
3.36
3.35
3.33
3.32
3.31
3.30
3.28
3.17
3.15
3.13
3.12
2.02
2.00
1.98
1.97
1.95
1.93
1.00
2.30
1.16
1.23
1.36
2.36
3.12
1.26
1.91
2.20
5.44
f1 (ppm)


174.84
174.32
102.21
102.18
97.55
79.65
76.89
76.82
76.74
76.18
76.17
73.32
71.82
68.17
67.92
67.74
59.90
52.26
50.76
42.49
37.55
26.67
26.00
22.07
18.14
18.12
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)

Chapter – 3

Probing Fondaparinux Activity in the Blood Microenvironment Using ^{19}F-NMR Tag

3.1. Abstract:

Fluorinated drugs dominate modern medicinal chemistry due to their ability to modulate molecular properties and biological efficacy. Here, we report the synthesis of trifluoromethyl phenyl tagged fondaparinux (CF_3-Fond) as a novel ^{19}F-NMR probe for tracking anticoagulant activity. Docking studies revealed that the anomeric substitution of CF_3-phenyl group minimally affects interactions with Factor Xa and platelet growth factors, however ensure anticoagulant activity equivalent to native fondaparinux. CF_3-Fond displayed a robust T_1 relaxation signal of 1.2 ms and a distinct 0.2 ppm shift in blood coagulation assays. Following intravenous administration of CF_3-Fond in mice, ^{19}F-NMR of the blood sample collected at different time interval demonstrated the stability of CF_3-Fond for 24 hours and slow degradation after 48 hours. These results validate the application of CF_3-Fond in ^{19}F-NMR in dynamic, real-time anticoagulant monitoring and related enzymatic processes.

3.2. Introduction

Carbohydrates are unique biological molecules that form a crucial part of the communication systems used by living organisms across the animal kingdom. Understanding this "communication language of carbohydrates" and finding ways to use it to enable specific functions is a key goal in the fields of glycobiology.[1,2] Compared to proteins and nucleic acids, carbohydrates have complex and diverse structures. This complexity allows them to form precise hydrogen bond networks, ensuring they can accurately interact with specific proteins. However, even though carbohydrate-protein interactions play a crucial role in cell behaviour, their binding strength is usually quite weak (in the millimolar range), which limits their use in developing effective drug and vaccine development. A noticeable exception is the heparin-protein interaction, which is in micromolar to picomolar range. Heparin and heparan sulfate (HS) are commonly used as anticoagulant drugs during major surgeries, including cardiopulmonary bypass, knee replacement, and hip replacement, to prevent venous thrombosis. However, challenges in ensuring the purity of these biomacromolecules have led to the use of low molecular weight heparin (LMWH), particularly pentasaccharide heparin, commonly called fondaparinux, which has proven to be clinically effective as a specific FXa inhibitor. Fondaparinux is FDA approved drug, whose pharmacokinetics and pharmacodynamics properties and specificity to Factor Xa has been well established. The structure-activity relationship of fondaparinux highlights the critical roles of uronic acid composition, the conformational flexibility of L-iduronic acid, and the sulfation pattern, particularly the 3-O-sulfation of the glucosamine unit, in binding to Factor Xa.[17-18] Despite extensive biological studies, the behaviour of fondaparinux within the blood microenvironment remains poorly understood, posing challenges for developing orally active drug modifications.[3-16] To enable real-time imaging of the anticoagulant activity of fondaparinux, fluorination at the anomeric residue—an area not directly involved in biological activity—was proposed. Moreover, bioisosteric modification with a trifluoromethylphenyl group would not only introduce a ^{19}F-NMR reporter nucleus for solution-phase binding studies, but also serve as a bioisosteric replacement for the carboxylic acid group, potentially enhancing binding affinity with heparin binding proteins. Additionally, trifluoromethylphenyl modified fondaparinux (**CF_3-Fond**) offers the potential for qualitative analyses of HS protein interactions and heparinase activity, paving the way for its development as a novel biomarker for heparin and HS activities.

3.3. Results and discussion.

The synthesis of **CF3-Fond** is not straightforward, because, fondparinux is a synthetic drug. Hence, it is necessary to follow the fondaparinux synthetic strategy to incorporate the CF3-phenyl group. Pioneering work by Petitou, Hung, and others optimized strategies for synthesizing individual monosaccharide building blocks in high yield, identifying orthogonal protecting group strategies to improve stereospecific glycosylation and site-specific deprotection to incorporate sulfation patterns. Ultimately, a one-pot synthetic approach, either employing a 3+2 or 3+1+1 glycosylation strategy was used to produce the desired pentasaccharide.[19-23] More recently, gold-catalyzed glycosylation has been applied to achieve stereoselective glycosylation and the synthesis of fondaparinux. Herein, we employed the 3+2 synthetic strategy to obtain **CF3-Fond**. We used CF3-phenyl glycosylated glucosamine building block as the starting material to synthesize the fondaparinux derivative. ^{19}F-NMR analysis at each step revealed significant changes in the fluorine peak positions after each sulfation and global deprotection step, indicating that the electron-withdrawing nature of the glycosylation influences the peak shifts.

3.3.1 Synthesis of monosaccharide building blocks.

Synthesis of L-idose building block.

The synthesis of the L-iduronic acid building block is challenging due to the unavailability of commercially sourced L-idose and the difficulty in achieving stereoselective α-glycosylation with L-iduronic acid. Previously, we reported a linear approach for synthesising oligo-iduronic acid building blocks and utilised a divergent strategy to create a library of sulphated iduronic acid oligosaccharides.[24-30] Here, we intend to employ the same process to obtain the desired building block. Briefly, 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose was 3-O-benzylated using benzyl bromide and sodium hydride in DMF as the solvent. Selective 2,3-diol deprotection, followed by benzoylation, mesylation, epoxidation, and ring expansion, yielded anhydro-idose in an overall good yield. A regioselective ring-opening reaction of compound **7** with acetic anhydride in the presence of $Cu(OTf)_2$ at 0 °C afforded compound 8 with an 83% yield. Subsequent reaction of compound **8** with p-thiocresol in the presence of boron trifluoride:diethyl ether complex in DCM produced the L-idose thiophenol donor (compound 10).[31-34] Compound **10** was subjected to deacetylation, followed by selective protection of the C6- and C4-OH groups with benzylidene and protection of the C2-OH

group with benzoyl protection resulting in the desired L-idose donor suitable for the synthesis of fondaparinux (fig.1).[35-37]

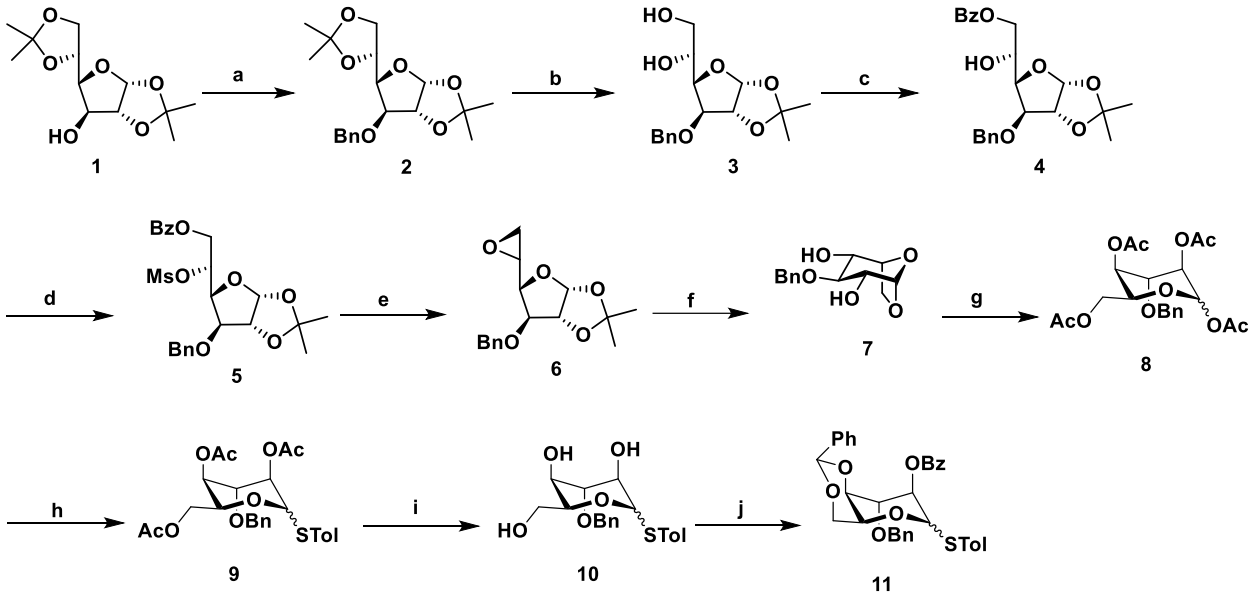


Fig. 1: Synthesis of Iduronic acid building blocks and reagents. a. BnBr, NaH, DMF, 0°C, 12 h, 86% ; **b.** 64% AcOH (in H2O), 40 °C, 3 h, 76%; **c.** BzCl, DCM:Py (3:1), 0 °C, 30 min, 71%; **d.** MsCl, Py, 0 °C, 12 h, 87%; **e.** ^{t}BuOK, ^{t}BuOH:DCM (1:1), 0 °C, 12 h, 73%; **f.** 2N H2SO4, 1,4 Dioxane, 12 h, 62%; **g.** Cu(OTf)2, Ac_2O, 0°C, 2 h, 83%; **h.** *P*-Thiocresol, $BF_3.O(C_2H_5)_2$, DCM, 0 °C, 12 h, 88%; **i.** NaOMe, MeOH:DCM (2:1), 5 h, 79%; **J.** (i) $PhCH(OMe)_2$, CSA, ACN, 4 h; (ii) BzCl, DCM:Py (4:1), 0 °C, 1 h, 68%.

Synthesis of glucosamine building block

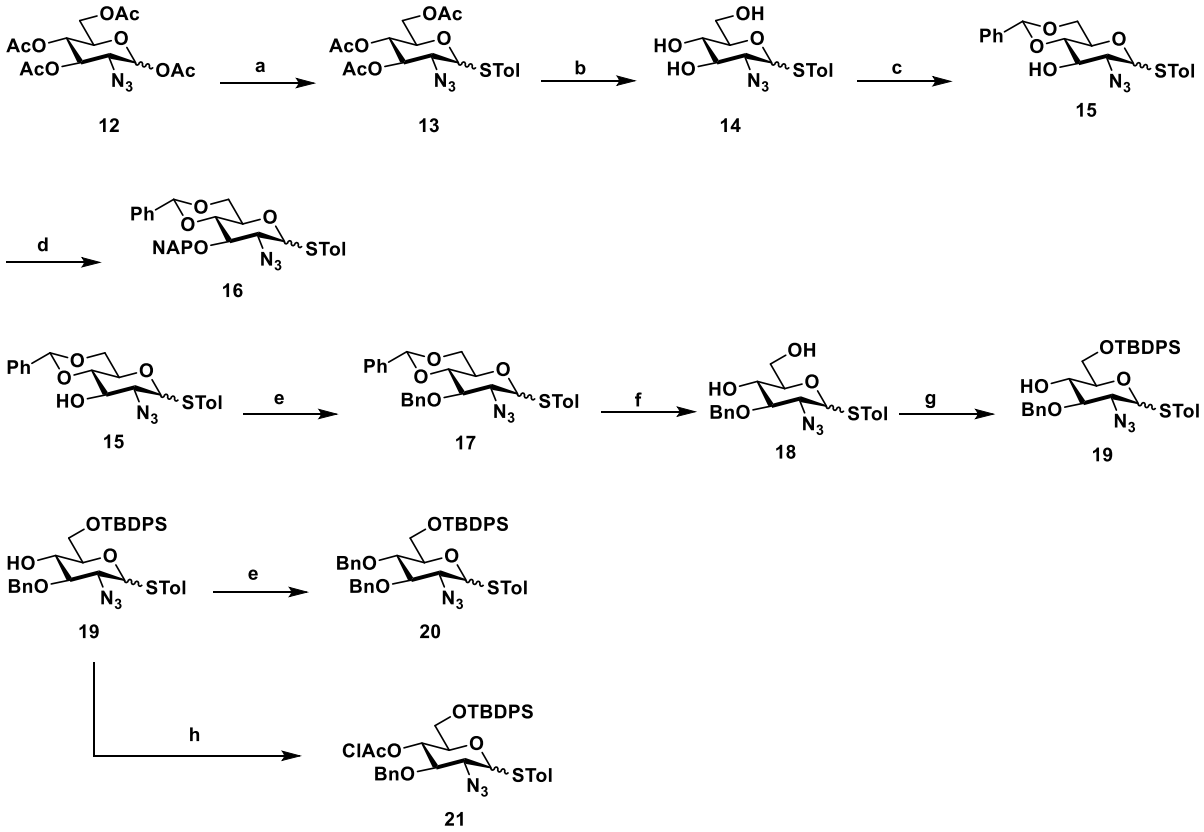


Fig. 2: Synthesis of Glucosamine building blocks, Reagents. a. *P*-Thiocresol, $BF_3.O(C_2H_5)_2$, DCM, 0 °C, 12 h, 85%; **b.** NaOMe, MeOH:DCM (2:1), 5 h, 84%; **c.** $PhCH(OMe)_2$, CSA, ACN, 4 h, 68%; **d.** NapBr, NaH, DMF, 0 °C, 15 min, 73%; **e.** BnBr, NaH, DMF, 0 °C, 15 min, 82%; **f.** PTSA, DCM:MeOH (2:1), 2 h, 75%; **g.** TBDPSCl, Et3N, DMAP, DCM, 0 °C, 12 h, 81%; **h. (**$ClAc)_2O$, Py, 0 °C, 6 h, 90%.

Commercially available D-glucosamine was converted into 2-azido-2-deoxy-glucosamine, as the azide group at the C-2 position strongly influences stereoselective 1,2-cis (α-anomeric) glycosylation.[42] The amine group was converted into an azide using freshly synthesized azidosulfonylimidazole, followed by per-acetylation with acetic anhydride and pyridine. The resulting compound was purified by flash column chromatography, yielding 85% of compound **13**, which was then subjected to thiotoluene glycosylation using boron trifluoride:diethyl ether complex over 72 hours to produce the thiotoluene glycosylated product, compound **14**, with an 84% yield. Compound **14** was deacetylated using sodium methoxide and underwent a series of protection and deprotection steps to obtain the desired three different glucosamine building block. Initially, benzylidene acetal was used to protect

the C6-OH and C4-OH groups of glucosamine, followed by protection of the C3-OH group with either naphthyl or benzyl groups. The benzylidene group was then removed, and the C6-OH group was protected with a silyl group using TBDPS-chloride and triethylamine. Finally, the C4-OH group was protected with either chloroacetate or benzyl groups, yielding the desired glucosamine building blocks. The C3-naphthyl, C4-chloroacetate-protected glucosamine building block **16** was employed for selective incorporation of C3-O-sulfation. Conversely, the C3- and C4-dibenzyl-protected glucosamine building block **20** was used for the non-reducing end glucosamine unit, while the C3-benzyl, C4-chloroacetate-protected glucosamine building block **21** was used for the reducing end sugar (fig. 2).

Synthesis of D-glucose building block

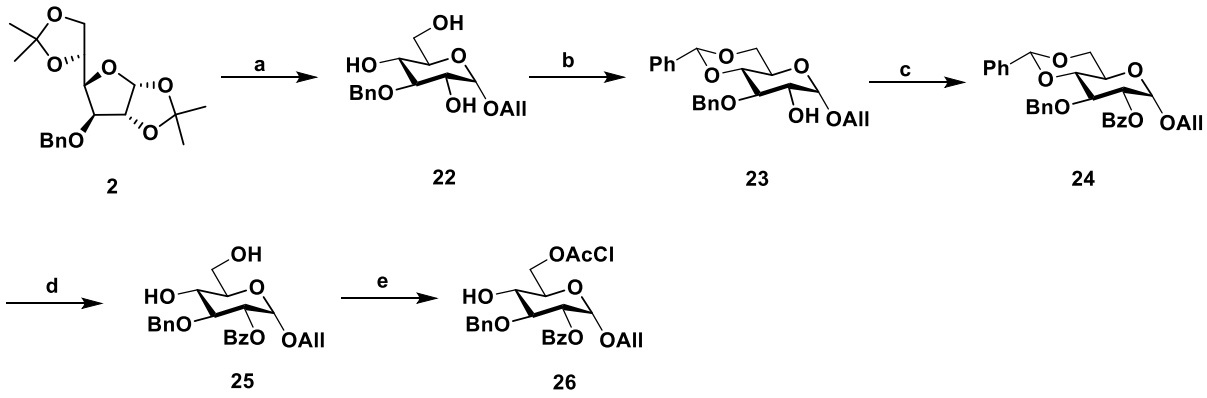


Fig. 3: Synthesis of Glucuronic acid building blocks, Reagents. a. (i) TFA-H2O (9:1), 25 °C, 15 min, (ii) AcCl, AllOH, 0-80 °C, 16 h, 52%; **b.** $PhCH(OMe)_2$, CSA, ACN, 4 h, 62%; **c.** BzCl, Pyridine, DMAP (cat), 0 °C, 2 h, 75%; **d.** PTSA, DCM:MeOH (2:1), 2 h, 71%; **e.** **(**$ClAc)_2O$, Py, 0 °C, 6 h, 86%.

The synthesis of the D-glucose building block was carried out using 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose, which underwent anomeric allylation in a 9:1 mixture of trifluoroacetic acid and water. After 15 minutes, the reaction mixture was evaporated and co-evaporated with toluene to remove excess trifluoroacetic acid and allyl alcohol. The resulting compound was purified by flash column chromatography. Compound **22** was then subjected to a series of protection and deprotection steps to obtain the desired building block, compound **26**. Briefly, compound **22** was initially protected with a benzylidene group, followed by C2-benzoylation. The benzylidene group was then deprotected, and selective C6-chloroacetate protection was performed at low temperature, yielding compound **26** (fig. 3).

3.3.2. Synthesis of GluN-Glu disaccharide.

Once the required monosaccharide building blocks, compounds **20** and **26**, were synthesised, glycosylation was performed using N-iodosuccinimide and TMSOTf at -20 °C, yielding 81% of the desired disaccharide precursor, compound **27**. Compound **27** then underwent allyl deprotection using palladium chloride in acetic acid, followed by acetylation with copper triflate in acetic anhydride, resulting in compound **29** with an 85% yield. Finally, compound **29** was treated with TMSSPh and ZnI_2 in dry DCM to produce the thio-phenyl disaccharide donor **30**, achieving a 80% yield (fig. 4).[38,40]

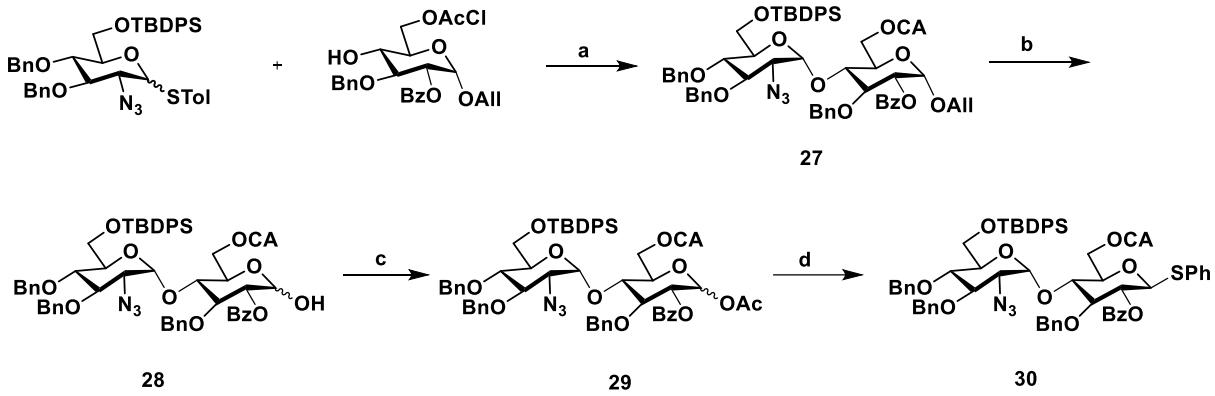


Fig. 4: Synthesis of D-E disaccharide donor, Reagents. a. NIS, TMSOTf, DCM, -78 °C, 15 min, 81%; **b.** $PdCl_2$, DCM:MeOH (1:4), 6 h, 86%; **c.** $Cu(OTf)_2$, Ac_2O, 0°C, 2 h, 85%; **d.** TMSSPh, ZnI_2, DCM, 1 h, 80%.

3.3.3. Synthesis of Trisaccharide acceptor.

Efforts to incorporate trifluoromethylphenol into glucosamine building blocks (**16–19**) encountered challenges due to low yields and difficulties in separating the alpha and beta anomers. However, glycosylation of glucosamine building block **21** under optimised conditions produced a 9:1 ratio of alpha to beta anomers, with a significant difference in R_f values, allowing effective separation of the mixture by column chromatography. Subsequently, the C4-chloroacetate group was deprotected using hydrazine, yielding building block **32**, suitable for further glycosylation reactions. Glycosylation of the L-idose donor 11 and glucosamine acceptor **32** in the presence of NIS and TMSOTf in DCM at -40 °C yielded the desired disaccharide **33**. This was then subjected to benzylidene deprotection and oxidation of the primary hydroxyl group of L-idose using a TEMPO and BAIB mixture in a DCM-water system, followed by esterification, to produce the desired L-iduronic acid-based

disaccharide acceptor **35**. Finally, glycosylation of the glucosamine donor **16** with disaccharide acceptor **35** in the presence of NIS and TMSOTf in DCM at -78 °C yielded the desired trisaccharide acceptor. The product was purified and characterised by ^{1}H, ^{13}C, ^{19}F-NMR, and mass spectrometry (fig. 5).

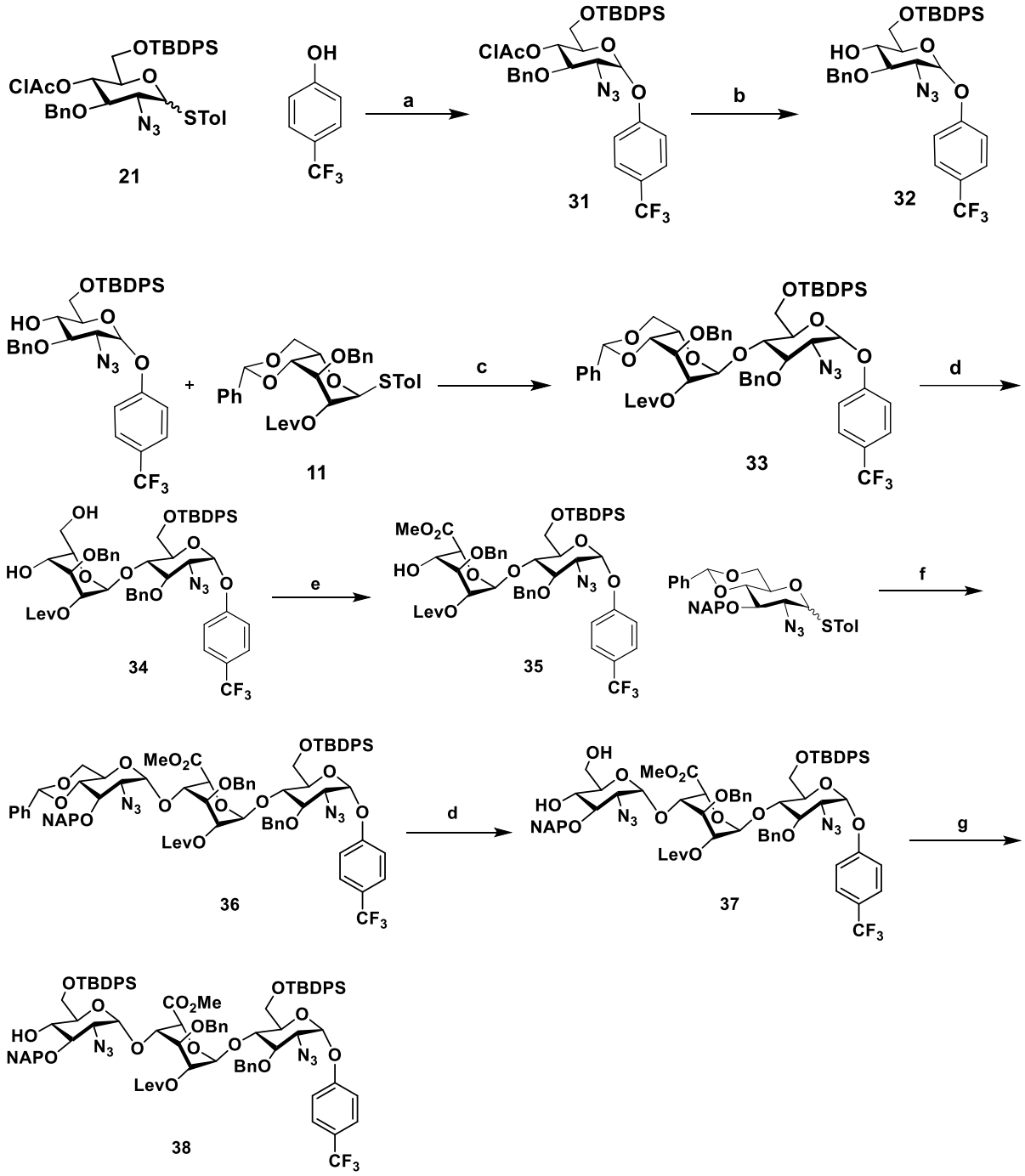


Fig. 5: Synthesis of trisaccharide acceptor, Reagents. a. NIS, TMSOTf, DCM, -78 °C, 15 min, 93%; **b.** Thiourea, Py : MeOH (1 : 1), 6 h, 83%; **c.** NIS, TMSOTf, DCM, -40 °C, 20 min, 76%; **d.** AcOH/H_2O (8:2), 80 °C, 2h, 75%; **e.** (i)TEMPO, BAIB, DCM:H2O (1:1), 10 h,

(ii) MeI, K_2CO_3, DMF, 6 h, 63%; **f.** NIS, TMSOTf, DCM, -78 °C, 1 h, 84%; **g.** TBDPSCl, Et_3N, Imidazole, DMAP, DCM, 0 °C, 12 h, 91%.

3.3.4. Synthesis of fondaparinux Pentasaccharide.

Next, we employed a 2+3 strategy to synthesise the fondaparinux precursor. Glycosylation of compounds **30** and **38** in the presence of NIS and TMSOTf in DCM at -40 °C yielded the desired pentasaccharide **39**, which was then subjected to chloroacetate deprotection using thiourea in a pyridine/methanol mixture to produce compound **40**. Compound **40** was further subjected to TEMPO/BAIB-mediated oxidation, followed by methyl esterification, to yield the desired fully protected fondaparinux pentasaccharide **41** (fig. 6).

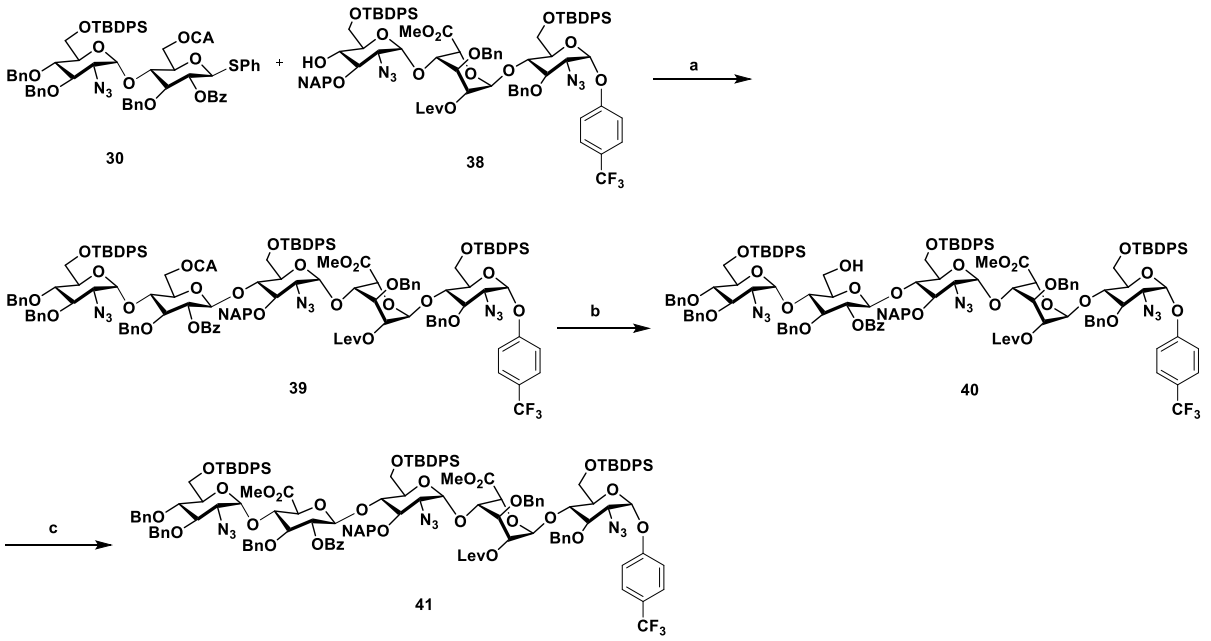


Fig. 6: Synthesis of protected fondparinux pentasaccharide, Reagents. a. NIS, TMSOTf, DCM, -40 °C, 20 min, 43%; **b.** Thiourea, Py:MeOH (1:1), 6 h, 62%; **c.** (i)TEMPO, BAIB, DCM:H2O (1:1), 10 h; (ii) MeI, K_2CO_3, DMF, 6 h, 66%.

Compound **41** was subjected to step-by-step deprotection and sulphation to achieve the desired sulphation pattern on fondaparinux. Initially, levulinic acid and naphthyl groups were deprotected using hydrazine and DDQ, respectively. Naphthyl deprotection proved to be highly challenging, leading to significant degradation of benzyl groups. To address this, reactions were performed in small batches of 50 mg to improve yields. After successfully deprotecting the naphthyl and levulinic groups, the TBDPS group was removed, and compound **44** was sulphated using an SO_3·TEA complex in a microwave reactor. The desired

sulphated product was purified using ion exchange resin to remove excess SO_3 salts, followed by purification with regular silica chromatography to eliminate unreacted products and impurities. The compound was characterised by NMR and mass spectrometry. Subsequently, the ester was hydrolysed using lithium hydroxide, and the azide group was converted to an amine using trimethyl phosphine, followed by sulphation with an SO_3·pyridine complex. Finally, global deprotection was performed through hydrogenolysis, yielding the desired final fondaparinux analogue (fig. 7).

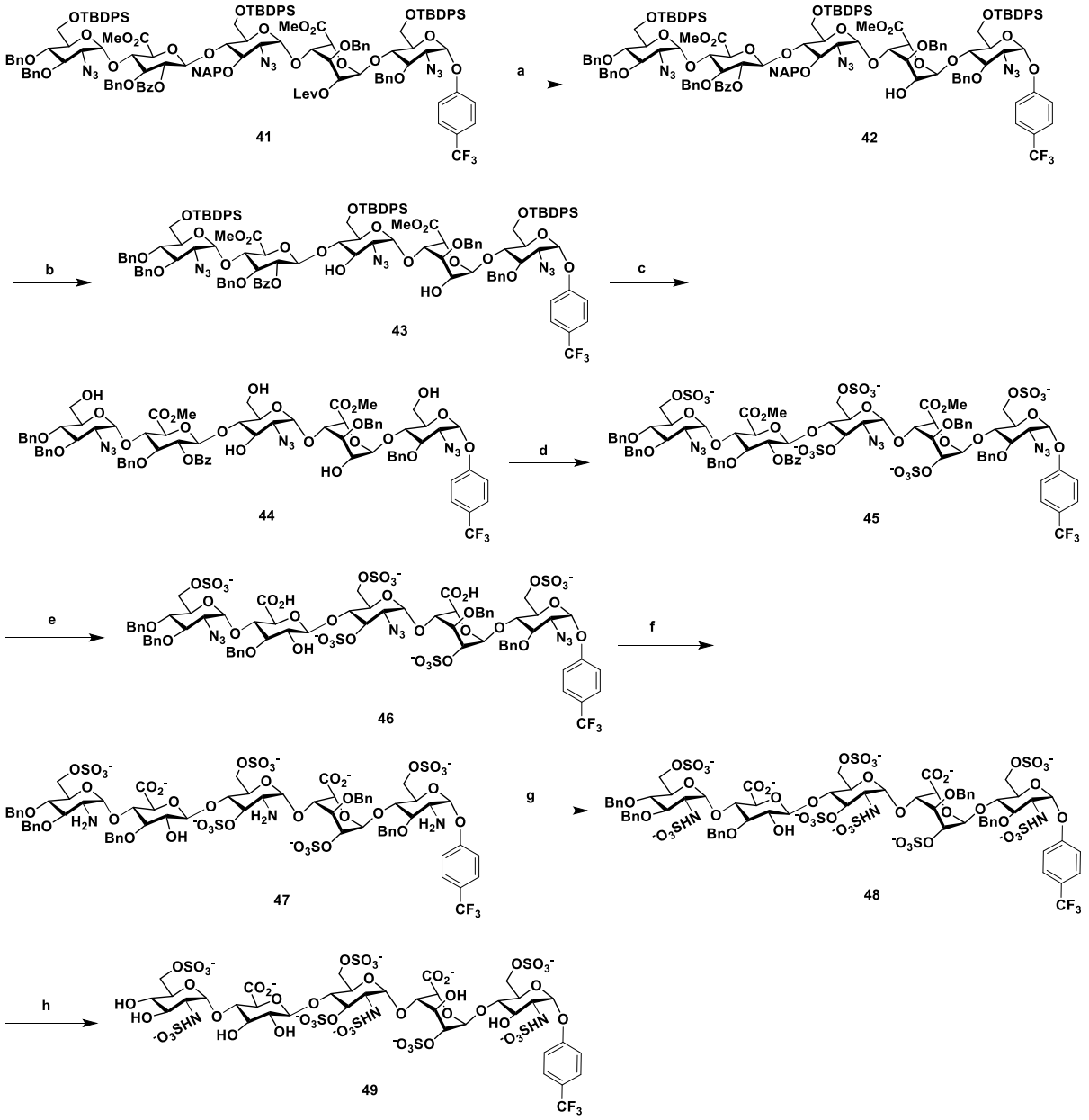


Fig. 7: Synthesis of deprotected fondparinux pentasaccharide, Reagents. a. $N_2H_4.H_2O$, AcOH, 6h, 89%; **b.** DDQ, DCM : H_2O (18:1), 1 h, 78%; **c.** HF.Py, Py, 0 °C, 12 h, 70%; **d.** $SO_3.NMe_3$, DMF, MW, 100 °C, 1h, 47%; **e.** LiOH, H2O : THF(1:1), 12 h, 95%; **f.**

PMe_3.THF, 1 M NaOH, THF, 12 h, 80%; **g.** SO_3.Py, MeOH, 1M NaOH, 0 °C, 48 h,58% ; **h**. H_2, $Pd(OH)_2$, H_2O, 48 h, 57%.

3.3.5 ^{19}F-NMR studies.

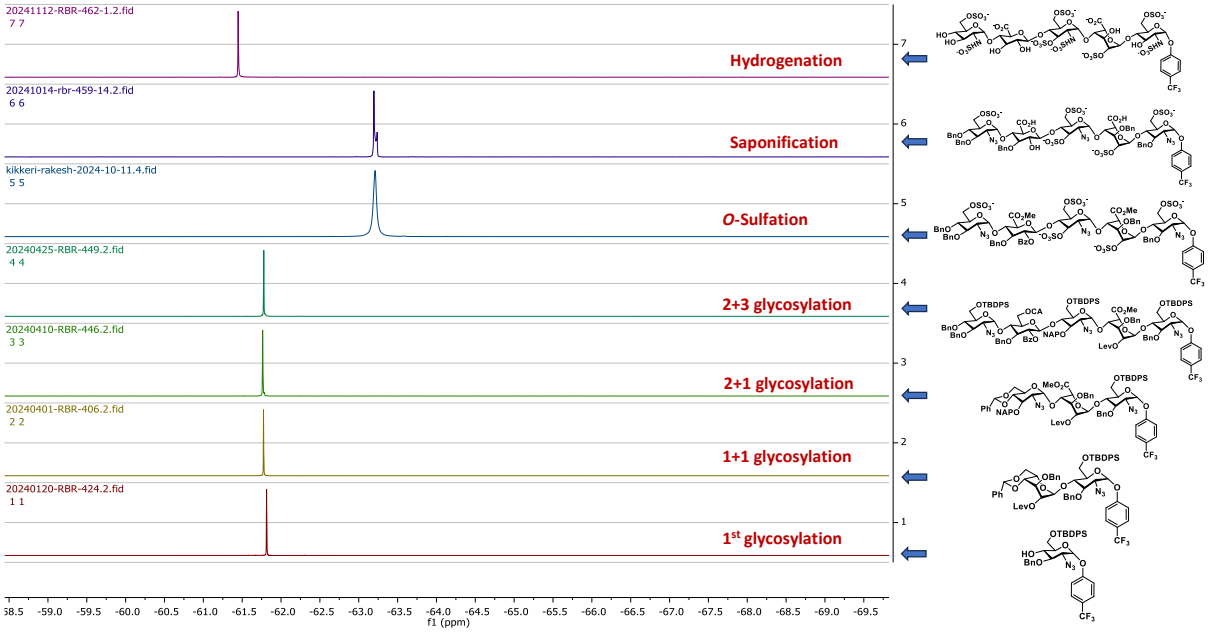


Fig 8. ^{19}F-NMR of monosaccharide to fondaparinux.

The ^{19}F-NMR spectrum collected at different synthetic stages of **CF3-Fond** revealed that the fluorine peak for the protected mono- to pentasaccharide remained consistent at -61.7 ppm. However, the deprotection and sulphation of the compound caused a significant shift in the fluorine peak to -63.4 ppm. While some solvent effects were observed, the ~2 ppm shift in the NMR spectra indicates that sulphation pulls electron density, resulting in the shift of the fluorine peak. Interestingly, the final **CF3-Fond** compound exhibited a fluorine peak at -61.4 ppm, highlighting that the hydroxyl groups and sulphation significantly contribute to pulling electron density from the anomeric position, inducing a pronounced shift in the 19F-NMR signal(fig. 8). These findings demonstrate that sulphation has a substantial impact on the fluorine chemical shift in the final compound. Furthermore, T_1 value measurements confirmed that CF3-Fond holds promise as a potential biomarker for ^{19}F-MRI studies.

3.3.6. Blood coagulation assay.

The effect of this **CF_3-Fond** on the biological activity was examined by blood anti-coagulation assay using human blood sample. To this end, we incubated human blood (100

µl) with heparin or fondparinux or **CF_3-Fond** respectively at different concentration. We observed blood anti-coagulation of all three compounds. However, to our surprise, **CF_3-Fond** displayed blood anti-coagualtion even at 40 µM concentration. Whereas, fondaparinux showed anticoagulation activity at 100 µM concentration. These results showed the anomeric modification of fondaparinux didn't interfere the anticoagulation activity, infact it was enhanced in the presence of CF3-phenyl group. Motivated by these results. We performed ^{19}F-NMR studies of blood anticoagulation.

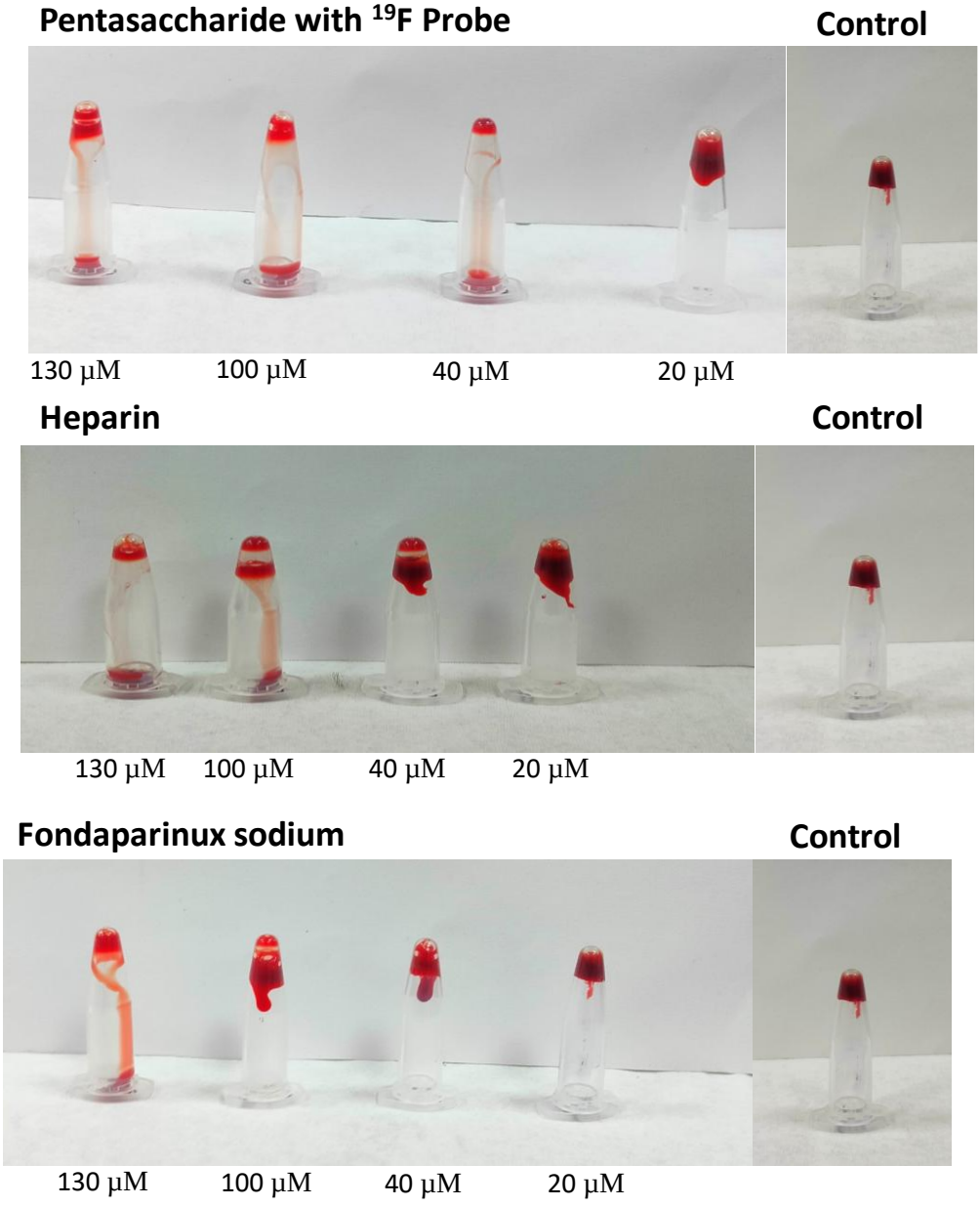


Fig 9. Blood coagulation studies by incubating 100 µl of blood with CF3-Fond, heparin and fondaparinux respectively.

Ex Vivo Blood Coagulation Assay: Freshly collected blood was utilized for the assay without additional processing. Aliquots (100 μL) were prepared, to which **CF_3-Fond**, heparin and fondaparinux at different concentrations (fig.9) were added. These samples under different conditions were placed in Eppendorf tubes, and a picture was captured after 15 min. Clot formation was verified by gently rotating the Eppendorf vials.

3.3.7. ^{19}F-NMR study of blood anticoagulation.

Motivated by the anticoagulation assay data, we next conducted a live ^{19}F-NMR study to monitor blood anticoagulation over different time intervals using varying concentrations of blood and CF_3-Fond molecules. As shown in Figure 10, the addition of a drop of blood to CF_3-Fond resulted in a shift in the ^{19}F-NMR signal, accompanied by peak broadening. Increasing the concentration of blood caused a pronounced shift in the ^{19}F-NMR peak of the CF_3 group. After the addition of 12 drops of blood, no further changes in the peak were observed. Consequently, we ceased adding blood and maintained the sample for 24 and 48 hours, recording spectra at these time points. While a slight shift in the peak was observed, no significant further shifts occurred. Next, we diluted the mixture in D_2O to optimize the minimum detectable signal. We found that at a concentration of 0.312 mM, a significant shift in the ^{19}F-NMR signal was still detectable. Currently, we are investigating the potential reasons for the observed shifts and are also measuring the spectra at 600 MHz to achieve better resolution.

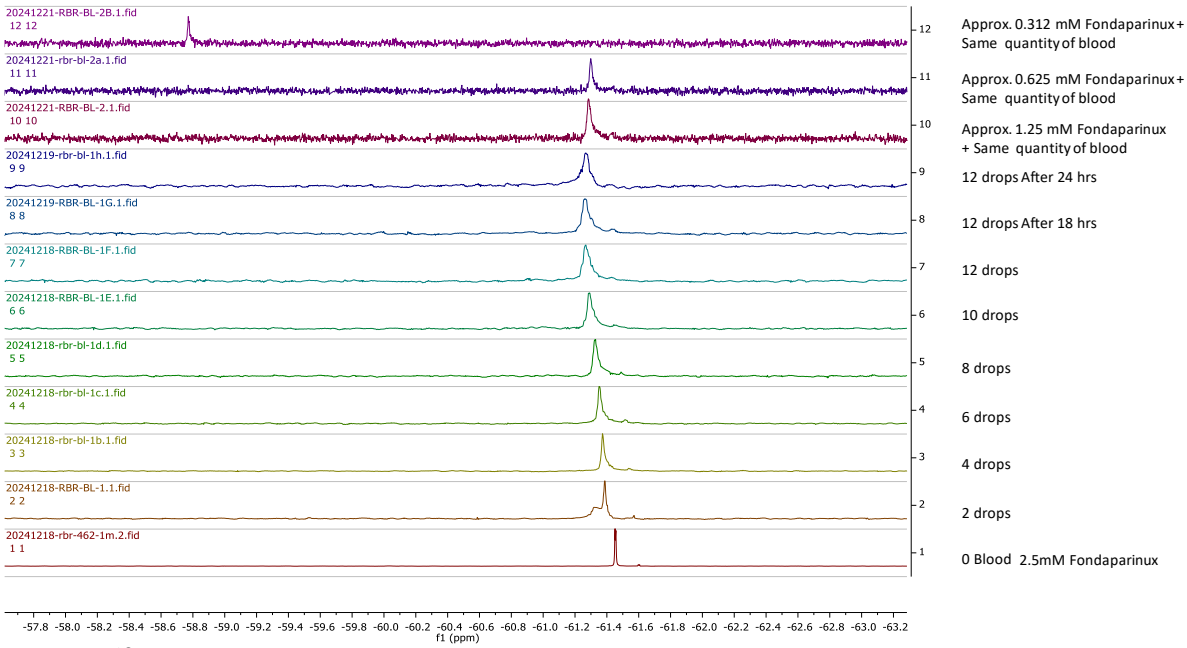


Fig. 10. ^{19}F-NMR study of blood anticoagulation.

3.4. Conclusion.

In conclusion, we have optimized the monosaccharide building blocks for the synthesis of **CF_3-Fond**. Using the [2+3] strategy, we successfully assembled the pentasaccharide and removed the protective groups to introduce the desired sulfation patterns. Preliminary docking studies indicated that the CF3-phenyl group has minimal influence on antithrombin and platelet factor 4 binding. Furthermore, blood anticoagulation assays confirmed that **CF_3-Fond** exhibits enhanced anticoagulant activity compared to native fondaparinux. ^{19}F-NMR studies revealed that **CF_3-Fond** binds to more than one protein in the blood and inhibits anticoagulant activity. *In vivo* studies are currently in progress.

3.5. Experimental Section

3.5.1 General Information

All chemicals were reagent grade and used without further purification unless otherwise noted. Reactions were carried out in anhydrous solvents under a nitrogen atmosphere. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F_{254}. Spots on TLC plate were visualized under UV light or by dipping the TLC plate in CAM/ninhydrin solution followed by heating. Column chromatography was carried out using Fluka kiesel gel 60 (230-400 mesh). ^{1}H and ^{13}C NMR spectra of compounds were measured with Bruker 400 MHz, Bruker 600 MHz and Jeol 400 MHz using residual solvents as an internal reference ($CDCl_3$ δH 7.26 ppm, δC 77.3 ppm, CD_3OD δH 3.31 ppm, δC 49.0 ppm, and D_2O δH 4.79 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. UV-visible measurements were performed with Evolution 300 UV-visible spectrophotometer (Thermo Fisher Scientific, USA). Fluorescence spectra were measured with FluoroMax-4 spectrofluorometer (Horiba Scientific, U.S.A.). All microscopy images were captured using Leica SP8 confocal microscope and processed using Image J software.

Allyl-*O*-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-*α*-D-glucopyranoside (27)

The solution of compound **20** (3.00 g, 4.10 mmol) and **26** (1.61 g, 3.28 mmol) in CH_2Cl_2 (40 mL) was stirred under N_2 atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. The mixture solution was cooled down to -78 °C followed by the addition of NIS (1.10 g, 4.93 mmol) and TMSOTf (150 μL, 0.82 mmol). The temperature

was gradually increased to -20 °C and the reaction mixture was left for stirring for another 15 minutes. After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et_3N. Molecular sieves were filtered using celite and the organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/12, v/v) to obtain compound **27** (3.66 g, 81.33 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 – 8.06 (m, 2H), 7.83 (dt, *J* = 8.9, 7.2 Hz, 4H), 7.67 (ddt, *J* = 8.6, 7.2, 1.5 Hz, 4H), 7.62 – 7.57 (m, 1H), 7.54 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.51 – 7.45 (m, 4H), 7.45 – 7.39 (m, 3H), 7.38 – 7.28 (m, 8H), 7.25 – 7.19 (m, 7H), 5.89 – 5.76 (m, 1H), 5.62 (d, *J* = 4.0 Hz, 1H), 5.28 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.19 – 5.10 (m, 3H), 5.10 – 5.03 (m, 2H), 4.98 – 4.87 (m, 3H), 4.79 (d, *J* = 10.9 Hz, 1H), 4.50 (dd, *J* = 11.9, 2.3 Hz, 1H), 4.35 (dd, *J* = 9.5, 8.5 Hz, 1H), 4.27 – 4.14 (m, 2H), 4.07 – 3.96 (m, 4H), 3.94 – 3.80 (m, 3H), 3.76 (dd, *J* = 11.7, 1.7 Hz, 1H), 3.72 – 3.62 (m, 2H), 3.40 – 3.34 (m, 1H), 1.06 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.93, 165.81, 137.96, 137.94, 135.96, 135.66, 135.29, 133.45, 133.35, 133.13, 132.92, 129.80, 129.78, 129.49, 128.58, 128.54, 128.41, 128.33, 128.04, 127.88, 127.79, 127.72, 127.67, 127.64, 127.48, 126.99, 126.12, 126.07, 126.02, 117.82, 98.16, 95.11, 80.24, 80.10, 78.00, 77.37, 77.06, 76.74, 75.71, 75.21, 74.94, 74.26, 74.20, 73.03, 68.73, 68.11, 64.55, 63.52, 62.11, 40.49, 29.73, 26.92, 19.36. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{61}H_{66}N_3ClO_{12}Si$, 1118.4004; found, 1118.4014.

2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-*α*-D-glucopyranoside (28)

To a solution of the disaccharide **27** (3.5 g, 3.19 mmol) in 4:1 MeOH: DCM (40 mL) was added $PdCl_2$ (85 mg, 0.47 mmol) and the reaction mixture was stirred for 4-8 h at 25 °C, the reaction was quenched by adding an excess of Et_3N and filtered through Celite. The filtrate was concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography (ethyl acetate/ hexane= 1/10, v/v) to obtain **28** (2.86 g, 86.66 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.09 (m, 2H), 7.90 – 7.81 (m, 5H), 7.70 (ddt, *J* = 8.1, 6.3, 1.4 Hz, 5H), 7.62 – 7.55 (m, 2H), 7.53 – 7.47 (m, 3H), 7.47 – 7.42 (m, 4H), 7.41 – 7.37 (m, 3H), 7.37 – 7.32 (m, 6H), 7.28 – 7.22 (m, *J* = 2.7, 2.3 Hz, 9H), 5.65 (d, *J* = 3.9 Hz, 1H), 5.50 (t, *J* = 3.6 Hz, 1H), 5.19 – 5.12 (m, 1H), 5.11 – 5.04 (m, 2H), 5.04 – 4.86 (m, 4H), 4.82 (dd, *J* = 10.9, 6.6 Hz, 1H), 4.59 – 4.51 (m, 1H), 4.41 (dd, *J* = 9.8, 8.5 Hz, 1H), 4.28 – 4.19 (m, 2H), 4.11 – 4.00 (m, 3H), 3.99 – 3.86 (m, 3H), 3.84 – 3.77 (m, 2H), 3.71 – 3.66 (m, 2H), 3.42 – 3.33 (m, 2H), 1.09 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.10, 165.89,

137.95, 137.92, 137.88, 135.99, 135.96, 135.68, 135.31, 133.55, 133.38, 133.15, 132.95, 129.91, 129.85, 129.82, 129.40, 128.66, 128.63, 128.58, 128.50, 128.45, 128.36, 128.06, 127.94, 127.92, 127.89, 127.83, 127.75, 127.66, 127.59, 127.55, 127.52, 127.05, 127.02, 126.17, 126.15, 126.09, 126.05, 98.19, 98.06, 95.57, 90.22, 80.04, 79.72, 78.02, 77.98, 77.42, 77.30, 77.10, 76.78, 75.71, 75.26, 74.96, 74.44, 74.10, 74.05, 73.02, 68.13, 64.47, 63.52, 62.13, 40.56, 40.54, 26.95, 19.39. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{58}H_{62}N_3ClO_{12}Si$, 1078.3691; found, 1078.3684.

Phenyl-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-1-thio-*α*-D-glucopyranoside (30)

A solution of compound **28** (3.4 g, 3.22 mmol) in Ac_2O (30 mL) was stirred at ice-cold temperature for 15 minutes. $Cu(OTf)_2$ (116 mg, 0.32 mmol) was added to the reaction mixture. After 12 h, the reaction mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate, $NaHCO_3$ and washed with brine. The combined organic layer was dried over Na_2SO_4, filtered, concentrated and preceded further without any purification. Next, the as obtained acetylated solution of compound (3.42 g, 3.11 mmol), ZnI_2 (2.08 g, 6.54 mmol) and phenyl trimethylsilyl sulphide (1.82 ml, 9.66 mmol) in DCM (20 mL) was stirred under N2 atmosphere for 2 h. Upon completion, the reaction mixture was filtered through celite, evaporated and purified through silica gel column chromatography (ethyl acetate/ hexane= 2/8, v/v) to obtain compound **30** (2.96 g, 80.21 % over two steps).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.08 (m, 2H), 7.66 – 7.63 (m, 4H), 7.62 – 7.58 (m, 1H), 7.51 – 7.37 (m, 10H), 7.32 (dddd, *J* = 13.3, 11.8, 6.2, 3.5 Hz, 14H), 7.26 – 7.18 (m, 8H), 5.52 (d, *J* = 4.0 Hz, 1H), 5.32 (t, *J* = 9.3 Hz, 1H), 4.93 (d, *J* = 10.6 Hz, 1H), 4.89 – 4.78 (m, 4H), 4.73 (dd, *J* = 12.9, 10.5 Hz, 2H), 4.58 (dd, *J* = 11.9, 2.4 Hz, 1H), 4.18 (dd, *J* = 11.9, 5.2 Hz, 1H), 4.06 – 3.91 (m, 3H), 3.91 – 3.80 (m, 3H), 3.78 – 3.72 (m, 2H), 3.72 – 3.66 (m, 2H), 3.61 – 3.57 (m, 1H), 3.29 (dd, *J* = 10.2, 3.9 Hz, 1H), 1.05 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.76, 165.20, 137.82, 137.71, 137.33, 135.92, 135.65, 133.52, 133.31, 133.21, 132.84, 131.94, 129.91, 129.83, 129.80, 129.60, 128.85, 128.62, 128.58, 128.55, 128.44, 128.25, 128.19, 128.05, 127.93, 127.87, 127.84, 127.80, 127.66, 98.04, 85.76, 84.56, 79.91, 77.89, 77.37, 77.26, 77.06, 76.74, 76.26, 75.65, 75.24, 74.75, 73.73, 73.09, 72.61, 64.55, 63.31, 62.04, 40.50, 26.92, 19.34. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{64}H_{66}N_3ClO_{11}Si$, 1170.3776; found, 1170.3786.

4-Trifluoromethylphenyl-*O*-(2-azido-2-deoxy-3-*O*-benzyl-4-*O*-chloroacetyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (31)

The solution of compound **21** (3.14 g, 4.38 mmol) and **4-(trifluoromethyl) phenol** (0.78 g, 4.82 mmol) in CH_2Cl_2 (30 mL) was stirred under N_2 atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. The mixture solution was cooled down to -78 ℃ followed by the addition of NIS (1.18 g, 5.25 mmol) and TMSOTf (159 µL, 0.87 mmol). The temperature was gradually increased to -20 ℃ and reaction mixture was left for stirring for another 15 minutes. After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et_3N. Molecular sieves were filtered using celite and organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/12, v/v) to obtain compound **31** (3.07 g, 93.03 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 (ddd, *J* = 6.7, 3.3, 1.6 Hz, 4H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.47 – 7.41 (m, 2H), 7.40 – 7.31 (m, 9H), 7.22 (d, *J* = 8.5 Hz, 2H), 5.64 (d, *J* = 3.4 Hz, 1H), 5.29 (dd, *J* = 10.3, 9.2 Hz, 1H), 4.95 (d, *J* = 11.3 Hz, 1H), 4.69 (d, *J* = 11.3 Hz, 1H), 4.20 (dd, *J* = 10.2, 9.2 Hz, 1H), 3.88 (ddd, *J* = 10.3, 4.9, 2.3 Hz, 1H), 3.72 – 3.64 (m, 2H), 3.63 – 3.59 (m, 3H), 1.00 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.78, 158.56, 137.44, 135.72, 135.58, 132.94, 132.92, 129.84, 129.81, 128.67, 128.20, 128.19, 127.74, 127.66, 127.21, 127.17, 127.13, 127.09, 125.74, 125.49, 125.42, 125.09, 124.76, 122.79, 116.80, 96.49, 77.73, 77.37, 77.26, 77.05, 76.74, 75.09, 71.57, 71.37, 62.99, 62.20, 40.29, 26.64, 19.16. ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.81. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{38}H_{39}N_3ClF_3O_6Si$, 776.2146; found, 776.2135.

4-Trifluoromethylphenyl-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (32)

To a solution of **31** (3.07 g, 4.07 mmol) in pyridine/MeOH (1:1, 30 ml) at 80 ℃ was added thiourea (1.20 g, 15.89 mmol) and stirred under nitrogen atmosphere for 2 h. After completion of the reaction, the solvent was evaporated, then dissolved in EtOAc and washed with 1 N HCl and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 1/9, v/v), to obtain compound compound **32** (2.31 g, 83.69 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.64 (ddd, *J* = 7.0, 3.6, 1.7 Hz, 4H), 7.55 – 7.51 (m, 2H), 7.48 – 7.39 (m, 7H), 7.39 – 7.32 (m, 5H), 7.14 (d, *J* = 8.6 Hz, 2H), 5.56 (d, *J* = 3.4 Hz, 1H), 5.01 – 4.90 (m, 2H), 4.05 (dd, *J* = 10.2, 8.6 Hz, 1H), 3.90 – 3.81 (m, 3H), 3.72 (dt, *J* = 9.7, 4.1 Hz, 1H), 3.46 (dd, *J* = 10.2, 3.4 Hz, 1H), 2.53 (d, *J* = 3.0 Hz, 1H), 1.04 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 158.65, 137.98, 135.68, 135.55, 132.95, 132.87, 129.93, 129.92, 128.71, 128.21, 128.19, 127.80, 127.76, 127.10, 127.06, 127.03, 126.99, 116.72, 96.71, 79.66, 77.36, 77.24, 77.04, 76.73, 75.35, 72.16, 72.05, 63.74, 62.55, 26.83, 26.76, 19.24. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.79. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{36}H_{38}N_3F_3O_5Si$, 700.2333; found, 700.2345.

4-Trifluoromethylphenyl-*O*-(2-*O*-levulinyl-3-*O*-benzyl-4,6-*O*-benzilidine-α-D idopyranoside)-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (33)

To a solution of compound **11 (donor)** (2.3 g, 4.09 mmol) and **32 (acceptor)** (2.22 g, 3.27 mmol) in CH_2Cl_2 (50 mL) was stirred under N_2 atmosphere in a round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. The mixture solution was cooled down to -40 ℃ followed by the addition of NIS (1.10 g, 4.91 mmol) and TMSOTf (148 µL, 0.81 mmol). The temperature was gradually increased to -20 ℃ and reaction mixture was left for stirring for another 15 minutes. After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et_3N. Molecular sieves were filtered using celite and organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/12, v/v) to obtain compound **33** (3.20 g, 76.29 %).

H NMR (400 MHz, Chloroform-*d*) δ 7.73 – 7.69 (m, 2H), 7.68 – 7.64 (m, 2H), 7.53 – 7.43 (m, 6H), 7.42 – 7.28 (m, 20H), 7.08 (d, *J* = 8.5 Hz, 2H), 5.54 (d, *J* = 3.5 Hz, 1H), 5.31 (s, 1H), 5.27 (d, *J* = 2.2 Hz, 1H), 5.00 (dd, *J* = 4.0, 2.3 Hz, 1H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.77 – 4.69 (m, 2H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.21 (t, *J* = 9.5 Hz, 1H), 4.04 – 3.94 (m, 2H), 3.89 (s, 1H), 3.87 – 3.79 (m, 3H), 3.77 – 3.72 (m, 1H), 3.68 (q, *J* = 3.3, 2.7 Hz, 1H), 3.56 (dd, *J* = 10.2, 3.5 Hz, 1H), 3.12 (dd, *J* = 12.8, 1.9 Hz, 1H), 2.49 (dd, *J* = 7.0, 5.3 Hz, 2H), 2.37 (dt, *J* = 11.7, 6.8 Hz, 2H), 1.91 (s, 3H), 1.08 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.16, 171.78, 158.71, 138.35, 138.08, 137.72, 135.98, 135.73, 133.56, 133.34, 129.78, 129.71, 129.02, 128.42, 128.35, 128.23, 128.15, 128.04, 127.72, 127.69, 127.57, 127.35, 127.06, 127.02, 126.99, 126.37, 125.53, 125.02, 124.69, 122.84, 116.55, 100.63, 97.42, 96.44, 78.61, 77.38,

77.27, 77.06, 76.74, 74.87, 74.03, 73.14, 72.57, 72.12, 69.01, 66.97, 63.61, 62.35, 60.04, 37.71, 29.55, 28.07, 26.87, 19.50. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.77. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{61}H_{64}N_3F_3O_{12}Si$, 1138.4111; found, 1138.4154

4-Trifluoromethylphenyl-*O*-(2-*O*-levulinyl-3-*O*-benzyl-α-D-idopyranoside)-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (34)

The compound **33** (3.2 g, 3.11mmol) was dissolved in AcOH/H_2O (8:2, 60 ml) and stirred the solution at 80 °C under nitrogen atmosphere for 2 h. After completion of the reaction, the solvent was evaporated, then dissolved in EtOAc and washed with saturated aqueous solution of $NaHCO_3$ and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography to obtain compound **34** (2.41 g, 75.31 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.73 – 7.67 (m, 2H), 7.67 – 7.61 (m, 2H), 7.51 – 7.27 (m, 18H), 7.01 (d, *J* = 8.5 Hz, 2H), 5.47 (d, *J* = 3.6 Hz, 1H), 5.23 (s, 1H), 4.99 – 4.95 (m, 1H), 4.78 (dd, *J* = 10.9, 4.2 Hz, 2H), 4.65 (t, *J* = 11.4 Hz, 2H), 4.25 – 4.18 (m, 2H), 4.02 – 3.93 (m, 2H), 3.80 (dd, *J* = 12.2, 1.8 Hz, 1H), 3.69 (dt, *J* = 15.4, 2.7 Hz, 2H), 3.63 (d, *J* = 8.6 Hz, 1H), 3.50 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.35 (dt, *J* = 11.8, 5.9 Hz, 1H), 3.24 (dt, *J* = 11.3, 4.8 Hz, 1H), 2.71 – 2.47 (m, 4H), 2.36 (dt, *J* = 17.2, 6.2 Hz, 1H), 2.12 (s, 3H), 1.06 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 206.09, 171.42, 158.61, 137.62, 137.60, 135.99, 135.76, 133.59, 133.34, 129.81, 129.77, 128.49, 128.42, 128.40, 128.20, 128.05, 127.91, 127.62, 127.54, 127.03, 126.99, 125.50, 125.01, 124.69, 122.81, 116.44, 97.13, 96.28, 78.73, 77.36, 77.25, 77.05, 76.73, 75.37, 75.31, 72.94, 72.32, 72.27, 68.00, 66.87, 63.74, 62.63, 62.19, 37.80, 29.71, 27.90, 26.88, 19.50. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.79. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{54}H_{60}N_3F_3O_{12}Si$, 1050.3798; found, 1050.3725.

4-Trifluoromethylphenyl-*O*-[Methyl (2-*O*-levulinyl-3-*O*-benzyl-4,6-*O*-benzilidine-α-D idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (35)

To a solution of **34** (2.4 g, 2.33 mmol) in DCM/H_2O (2:1, 25 mL) were added 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical (TEMPO, 0.072 g, 0.46 mmol) and [Bis(acetoxy)iodo] benzene (BAIB) (1.87 g, 5.8 mmol), and stirred at RT for 10 h. The organic layer was washed with saturated NH_4Cl solution and concentrated. Then, the crude product was dissolved in DMF (10 mL), mixed with K_2CO_3 (0.954 g, 2.30 mmol), MeI (1.29 mL, 20.72 mmol), and stirred overnight at RT. After completion of the reaction, the reaction

mixture was extracted with EtOAc and brine. The organic layer was concentrated and purified through silica gel column chromatography (EtOAc/Hexane = 1/8, v/v) to obtain compound compound **35** (1.52 g 63.33 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.72 – 7.68 (m, 2H), 7.66 (dd, *J* = 7.9, 1.7 Hz, 2H), 7.50 – 7.43 (m, 4H), 7.43 – 7.35 (m, 9H), 7.34 – 7.26 (m, 7H), 7.01 (d, *J* = 8.5 Hz, 2H), 5.45 (d, *J* = 3.6 Hz, 1H), 5.33 (s, 1H), 4.97 – 4.92 (m, 2H), 4.75 (dd, *J* = 21.0, 11.1 Hz, 2H), 4.65 (dd, *J* = 16.1, 11.1 Hz, 2H), 4.18 (t, *J* = 9.5 Hz, 1H), 4.01 – 3.92 (m, 3H), 3.83 – 3.75 (m, 2H), 3.69 (dt, *J* = 9.9, 2.6 Hz, 1H), 3.47 (s, 3H), 2.70 – 2.62 (m, 2H), 2.59 (t, *J* = 6.0 Hz, 1H), 2.55 – 2.46 (m, 1H), 2.38 – 2.30 (m, 1H), 2.12 (s, 3H), 1.07 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.79. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{55}H_{60}N_3F_3O_{13}Si$, 1078.3748; found, 1078.3723.

4-Trifluoromethylphenyl-*O*-[Methyl-(2-azido-2-deoxy-3-*O*-napthylmethyl-4,6-*O*-benzilidine-*α*-D-glucopyranoside-(1→4)-2-*O*-levulinyl-3-*O*-benzyl-4,6-*O*-benzilidine-*α*-D idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (36)

Compound **35** (3 g, 1.98 mmol) was subjected to glycosylation reaction with glucosamine monosaccharide donor as per the synthetic procedure for the preparation of compound **2** to obtain disaccharide **36** (3.31 g 84.87 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.82 – 7.77 (m, 3H), 7.75 – 7.69 (m, 5H), 7.51 – 7.43 (m, 9H), 7.39 (ddd, *J* = 9.4, 8.2, 6.0 Hz, 13H), 7.34 – 7.27 (m, 6H), 7.03 (d, *J* = 8.6 Hz, 2H), 5.56 (s, 1H), 5.48 (dd, *J* = 5.3, 3.4 Hz, 2H), 5.12 – 5.02 (m, 2H), 4.97 – 4.91 (m, 2H), 4.85 (d, *J* = 10.7 Hz, 1H), 4.77 – 4.69 (m, 4H), 4.26 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.19 (t, *J* = 9.5 Hz, 1H), 4.02 (ddd, *J* = 16.4, 7.5, 4.0 Hz, 4H), 3.95 (d, *J* = 9.5 Hz, 1H), 3.86 (dt, *J* = 9.9, 4.9 Hz, 1H), 3.79 (dd, *J* = 12.1, 2.0 Hz, 1H), 3.69 (td, *J* = 10.2, 9.8, 3.8 Hz, 3H), 3.50 (dd, *J* = 10.3, 3.5 Hz, 1H), 3.42 (s, 3H), 3.34 (dd, *J* = 9.9, 3.7 Hz, 1H), 2.57 – 2.47 (m, 1H), 2.47 – 2.37 (m, 2H), 2.34 (d, *J* = 9.3 Hz, 1H), 2.27 – 2.18 (m, 1H), 1.99 (s, 3H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.79, 171.97, 168.79, 158.70, 137.85, 137.46, 137.34, 136.08, 135.87, 135.32, 133.86, 133.52, 133.41, 133.28, 133.07, 130.11, 129.70, 129.04, 128.49, 128.27, 128.23, 128.18, 128.11, 127.99, 127.78, 127.67, 127.64, 127.56, 127.50, 126.98, 126.94, 126.90, 126.13, 126.04, 125.93, 124.92, 124.59, 122.85, 116.55, 101.50, 98.44, 97.83, 96.36, 82.45, 78.39, 77.38, 77.06, 76.74, 75.83, 75.05, 74.84, 74.23, 74.00, 73.70, 73.43, 72.78, 69.14, 68.99, 68.53, 63.22, 63.02, 61.78, 51.77, 37.57, 36.66, 31.96, 29.73, 29.59, 28.59, 27.53, 26.89, 24.71, 22.72, 21.38, 19.35, 14.16. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.76. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{79}H_{81}N_6F_3O_{17}Si$, 1493.5280; found, 1493.5205.

4-Trifluoromethylphenyl-*O*-[Methyl-(2-azido-2-deoxy-3-*O*-napthylmethyl-*α*-D-gluco pyranoside-(1→4)-2-*O*-levulinyl-3-*O*-benzyl-4,6-*O*-benzilidine-α-D-idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (37)

Compound **36** (3.3 g, 2.24 mmol) was subjected to benziridine deprotection reaction as per the synthetic procedure for the preparation of compound **34** to obtain trisaccharide **37** (2.16 g 69.67 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 – 7.80 (m, 4H), 7.71 (ddd, *J* = 7.5, 5.7, 1.6 Hz, 4H), 7.48 (ddt, *J* = 13.3, 10.1, 3.2 Hz, 7H), 7.43 – 7.36 (m, 9H), 7.35 – 7.27 (m, 6H), 7.01 (d, *J* = 8.6 Hz, 2H), 5.46 (dd, *J* = 7.2, 3.3 Hz, 2H), 5.08 – 5.03 (m, 2H), 4.96 – 4.89 (m, 2H), 4.85 – 4.77 (m, 2H), 4.76 – 4.66 (m, 3H), 4.18 (t, *J* = 9.5 Hz, 1H), 4.06 – 3.95 (m, 4H), 3.82 – 3.66 (m, 6H), 3.61 (td, *J* = 9.0, 3.4 Hz, 1H), 3.48 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.38 (s, 3H), 3.19 (dd, *J* = 10.2, 3.5 Hz, 1H), 2.51 (tdd, *J* = 20.9, 8.7, 5.0 Hz, 4H), 2.34 – 2.24 (m, 1H), 2.02 (s, 3H), 1.06 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.14, 172.01, 169.09, 158.67, 137.75, 137.36, 136.08, 135.85, 135.37, 133.57, 133.43, 133.31, 133.09, 129.72, 128.58, 128.50, 128.29, 128.23, 128.16, 128.01, 127.78, 127.73, 127.65, 127.54, 127.50, 126.94, 126.28, 126.15, 125.76, 116.53, 97.75, 97.65, 96.33, 79.09, 78.38, 77.35, 77.04, 76.72, 75.04, 74.70, 74.06, 73.65, 73.25, 72.81, 72.09, 71.01, 68.88, 68.61, 63.26, 62.86, 62.14, 61.86, 51.81, 37.69, 29.65, 27.59, 26.95, 19.39. ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.78. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{72}H_{77}N_6F_3O_{17}Si$, 1405.4967; found, 1405.4932.

4-Trifluoromethylphenyl-*O*-[Methyl(2-azido-2-deoxy-3-*O*-napthylmethyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside-(1→4)-2-*O*-levulinyl-3-*O*-benzyl-4,6-*O*-benzilidine-α-D-idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-*α*-D-glucopyranoside (38)

To a solution of **37** (2 g, 1.44 mmol) in DCM (30 ml) at 0 °C was added Triethylamine (1 ml, 7.22 mmol), DMAP (88 mg, 0.72 mmol) and Imidazole (345 mg, 5.05 mmol) stirred under nitrogen atmosphere for 5 min. TBDPSCl (1.87 ml, 7.22 mmol) was added dropwise to the reaction mixture at the same temperature and left the reaction under continuous stirring overnight. After the reaction was completed, the solvent was evaporated, then dissolved in EtOAc and washed with $NaHCO_3$ and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 4/6, v/v), to obtain compound **38** (2.1 g, 91.30 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 – 7.81 (m, 4H),

7.73 – 7.65 (m, 8H), 7.54 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.49 – 7.35 (m, 17H), 7.34 – 7.27 (m, 6H), 7.22 – 7.15 (m, 2H), 7.14 – 7.07 (m, 1H), 7.03 (d, *J* = 8.6 Hz, 2H), 5.47 (dd, *J* = 8.7, 3.7 Hz, 2H), 5.09 (d, *J* = 3.5 Hz, 1H), 5.00 (s, 2H), 4.94 (t, *J* = 4.4 Hz, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.72 (d, *J* = 1.2 Hz, 2H), 4.70 – 4.65 (m, 2H), 4.15 (t, *J* = 9.5 Hz, 1H), 4.06 (t, *J* = 5.2 Hz, 1H), 4.00 (ddd, *J* = 8.9, 6.8, 3.4 Hz, 3H), 3.87 (td, *J* = 11.1, 3.2 Hz, 1H), 3.83 – 3.63 (m, 6H), 3.46 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.37 (s, 3H), 3.22 (dd, *J* = 10.1, 3.4 Hz, 1H), 2.59 – 2.40 (m, 4H), 2.30 – 2.19 (m, 1H), 2.02 (s, 3H), 1.05 (d, *J* = 5.0 Hz, 18H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 205.86, 171.96, 168.97, 158.70, 137.81, 137.53, 136.05, 135.86, 135.66, 135.60, 135.55, 133.55, 133.36, 133.10, 133.02, 132.97, 129.95, 129.92, 129.69, 128.43, 128.14, 128.08, 128.03, 127.99, 127.88, 127.86, 127.82, 127.78, 127.71, 127.64, 127.50, 127.46, 126.97, 126.95, 126.91, 126.18, 126.04, 125.95, 116.57, 97.82, 97.50, 96.37, 79.03, 78.24, 77.36, 77.24, 77.04, 76.72, 75.01, 74.94, 74.67, 73.97, 73.50, 72.75, 72.44, 71.79, 71.74, 69.60, 69.52, 63.49, 63.12, 62.70, 61.75, 51.70, 37.66, 29.72, 29.62, 27.53, 26.94, 26.90, 19.36, 19.30. ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.78. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{88}H_{95}N_6F_3O_{17}Si_2$, 1643.6144; found, 1643.6147.

4-Trifluoromethylphenyl-*O*-[Methyl(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-*α*-D-glucopyranoside-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-β-D-glucopyranoside-(1→4)-2-azido-2-deoxy-3-*O*-napthylmethyl-6-*O*-*tert*-butyldiphenyl silyl-*α*-D-glucopyranoside-(1→4)-2-*O*-levulinyl-3-*O*-benzyl-α-D idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (39)

Glycosyl donor **30** (3.15 g, 2.74 mmol) and glycosyl acceptor **38** (2 g, 1.37 mmol) were subjected to a glycosylation reaction as per the synthetic procedure followed for the synthesis of compound **33** to obtain pentasaccharide **39** (3.13 g, 43.47 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 – 7.75 (m, 2H), 7.73 (s, 1H), 7.71 – 7.68 (m, 1H), 7.67 (d, *J* = 1.3 Hz, 1H), 7.66 – 7.61 (m, 8H), 7.55 (dq, *J* = 6.6, 1.6 Hz, 4H), 7.52 – 7.48 (m, 3H), 7.43 – 7.38 (m, 3H), 7.38 – 7.32 (m, 8H), 7.32 – 7.27 (m, 10H), 7.26 (d, *J* = 1.9 Hz, 2H), 7.25 – 7.19 (m, 15H), 7.17 – 7.14 (m, 3H), 7.12 (dd, *J* = 6.8, 2.8 Hz, 3H), 7.08 (dd, *J* = 7.4, 1.9 Hz, 4H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.81 – 6.71 (m, 3H), 5.47 (d, *J* = 3.9 Hz, 1H), 5.38 (dd, *J* = 8.7, 4.4 Hz, 2H), 5.33 – 5.26 (m, 1H), 5.16 (d, *J* = 11.8 Hz, 1H), 4.95 (d, *J* = 3.4 Hz, 1H), 4.91 – 4.87 (m, 1H), 4.85 – 4.74 (m, 6H), 4.73 – 4.61 (m, 4H), 4.60 – 4.50 (m, 3H), 4.21 (dd, *J* = 11.9, 2.8 Hz, 1H), 4.14 – 3.97 (m, 4H), 3.96 – 3.80 (m, 8H), 3.75 – 3.66 (m, 5H), 3.66 – 3.55 (m, 5H), 3.51 – 3.40 (m, 2H), 3.33 (dd, *J* = 9.9, 3.6 Hz, 1H), 3.30 (s, 2H), 3.29 – 3.24 (m, 1H), 3.19 (ddd, *J* = 10.0, 6.1, 3.7 Hz, 2H), 3.00 (s, 3H), 2.41 (td, *J* = 6.3, 3.7 Hz,

2H), 2.26 – 2.18 (m, 2H), 2.06 (ddd, *J* = 17.6, 7.1, 5.8 Hz, 1H), 2.01 – 1.94 (m, 1H), 1.90 (s, 3H), 1.05 (s, 9H), 0.95 (d, *J* = 7.4 Hz, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.63, 171.82, 168.91, 166.66, 164.67, 158.72, 139.29, 137.88, 137.80, 137.73, 137.31, 136.07, 135.94, 135.88, 135.71, 135.63, 133.76, 133.48, 133.33, 133.29, 133.25, 132.90, 132.25, 130.30, 130.10, 129.77, 129.66, 129.45, 128.96, 128.71, 128.57, 128.52, 128.43, 128.42, 128.19, 128.17, 128.04, 127.93, 127.90, 127.87, 127.82, 127.78, 127.75, 127.69, 127.63, 127.60, 127.53, 127.46, 127.17, 126.91, 126.53, 126.00, 125.91, 125.73, 116.62, 114.08, 99.87, 98.13, 97.71, 96.39, 83.54, 79.41, 78.10, 77.86, 77.35, 77.23, 77.03, 76.89, 76.71, 76.17, 75.55, 75.36, 75.20, 75.02, 74.74, 74.19, 74.06, 73.08, 72.63, 72.09, 71.75, 70.87, 70.53, 64.55, 63.23, 62.93, 62.86, 62.13, 61.96, 61.44, 60.80, 51.31, 40.16, 37.51, 33.83, 31.94, 31.46, 30.23, 29.71, 29.68, 29.63, 29.56, 29.52, 29.37, 29.17, 28.97, 27.48, 27.02, 26.99, 26.90, 24.89, 22.70, 19.43, 19.31, 14.12. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.78. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{146}H_{155}ClN_9F_3O_{28}Si_3$, 2680.9830; found, 2680.9886.

4-Trifluoromethylphenyl-*O*-[Methyl(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-*α*-D-glucopyranoside-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyrano side-(1→4)-2-azido-2-deoxy-3-*O*-napthylmethyl-6-*O*- *tert*-butyldiphenyl silyl-*α*-D-gluco pyranoside-(1→4)-2-*O*-levulinyl-3-*O*-benzyl-α-D-idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (40)

Compound **39** (3 g, 1.12 mmol) was subjected to Chloroacetate deprotection reaction as per the synthetic procedure followed for the preparation of compound **32** to obtain pentasaccharide **40** (1.82 g 62.54 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 (ddd, *J* = 15.1, 7.9, 2.6 Hz, 5H), 7.71 (ddt, *J* = 11.6, 6.8, 1.7 Hz, 11H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.57 – 7.48 (m, 7H), 7.48 – 7.27 (m, 37H), 7.24 – 7.20 (m, 4H), 7.19 – 7.10 (m, 8H), 7.03 (d, *J* = 8.6 Hz, 2H), 6.90 – 6.76 (m, 3H), 5.57 (d, *J* = 3.9 Hz, 1H), 5.46 (dd, *J* = 9.0, 4.3 Hz, 2H), 5.38 (t, *J* = 8.6 Hz, 1H), 5.17 (d, *J* = 10.8 Hz, 1H), 5.04 (d, *J* = 3.6 Hz, 1H), 4.94 – 4.84 (m, 7H), 4.80 – 4.72 (m, 3H), 4.69 – 4.59 (m, 3H), 4.24 – 4.14 (m, 2H), 4.08 – 3.92 (m, 5H), 3.89 (d, *J* = 3.0 Hz, 1H), 3.88 – 3.83 (m, 1H), 3.82 – 3.64 (m, 10H), 3.50 (d, *J* = 9.9 Hz, 2H), 3.43 (dd, *J* = 10.0, 3.9 Hz, 2H), 3.34 – 3.24 (m, 3H), 3.16 (s, 3H), 3.13 – 3.05 (m, 1H), 2.54 (t, *J* = 6.5 Hz, 2H), 2.40 – 2.31 (m, 1H), 2.17 (dt, *J* = 17.5, 6.2 Hz, 1H), 2.01 (s, 3H), 1.10 (s, 9H), 1.04 (d, *J* = 1.7 Hz, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.72, 171.89, 168.91, 164.76, 138.03, 137.85, 137.73, 137.37, 136.08, 135.97, 135.88, 135.66, 135.50, 133.69, 133.55, 133.49, 133.31, 133.22, 132.91, 132.44, 130.26, 130.05, 129.88, 129.78, 129.68, 129.49, 129.06, 128.75, 128.56, 128.46,

128.44, 128.24, 128.18, 128.16, 128.00, 127.95, 127.92, 127.86, 127.77, 127.73, 127.66, 127.48, 127.18, 126.93, 126.34, 126.09, 125.78, 125.65, 116.60, 99.94, 97.84, 97.75, 97.59, 96.34, 83.89, 79.68, 78.14, 77.98, 77.61, 77.37, 77.05, 76.73, 75.87, 75.58, 75.30, 75.11, 74.78, 74.31, 74.19, 73.13, 72.63, 71.87, 70.64, 70.41, 63.29, 63.06, 62.93, 61.94, 61.73, 51.39, 37.56, 31.95, 31.47, 30.22, 29.73, 29.62, 29.39, 27.50, 27.03, 26.99, 26.85, 22.72, 19.47, 19.33, 19.30, 14.15. ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.78. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{144}H_{154}N_9F_3O_{27}Si_3$, 2633.0063; found, 2633.0014.

4-Trifluoromethylphenyl-*O*-[(methyl-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-*α*-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-gluco pyranate-(1→4)-(methyl-(2-azido-2-deoxy-3-*O*-napthylmethyl-6-*O*- *tert*-butyl diphenyl silyl-*α*-D-glucopyranoside)-(1→4)-2-*O*-levulinyl-3-*O*-benzyl-α-L idopyrano nate)]-(1→4) -2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyrano side (41)

Compound **40** (1.82 g, 0.70 mmol) was subjected to Oxidation and esterification reaction as per the synthetic procedure followed for the preparation of compound **35** to obtain pentasaccharide **41** (1.20 g 66.66 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 (dt, *J* = 5.9, 2.1 Hz, 3H), 7.83 – 7.62 (m, 16H), 7.62 – 7.56 (m, 4H), 7.52 – 7.27 (m, 37H), 7.25 – 7.15 (m, 9H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.92 – 6.81 (m, 3H), 5.51 (dd, *J* = 5.9, 4.6 Hz, 2H), 5.48 – 5.42 (m, 2H), 5.33 (d, *J* = 10.7 Hz, 1H), 5.05 (d, *J* = 3.6 Hz, 1H), 4.99 – 4.85 (m, 6H), 4.84 – 4.73 (m, 4H), 4.71 – 4.57 (m, 3H), 4.27 – 4.20 (m, 2H), 4.15 (d, *J* = 5.3 Hz, 1H), 4.08 – 3.87 (m, 9H), 3.85 – 3.65 (m, 8H), 3.47 – 3.38 (m, 3H), 3.30 (dt, *J* = 10.4, 3.7 Hz, 2H), 3.23 (s, 3H), 3.17 (s, 3H), 2.53 (td, *J* = 6.3, 2.8 Hz, 2H), 2.33 (dt, *J* = 17.2, 6.3 Hz, 1H), 2.22 – 2.14 (m, 1H), 2.00 (s, 3H), 1.15 (s, 9H), 1.06 (s, 18H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 205.67, 171.86, 168.97, 167.69, 164.58, 158.72, 138.33, 137.90, 137.86, 137.74, 137.29, 136.10, 135.91, 135.73, 135.64, 135.52, 133.73, 133.60, 133.48, 133.38, 133.32, 133.04, 132.90, 132.29, 130.36, 130.09, 129.76, 129.70, 129.47, 128.91, 128.70, 128.57, 128.45, 128.42, 128.28, 128.23, 128.04, 127.95, 127.88, 127.80, 127.75, 127.73, 127.68, 127.65, 127.53, 127.50, 127.48, 127.41, 127.20, 126.93, 126.78, 125.67, 125.62, 124.58, 116.61, 100.18, 98.04, 97.71, 96.36, 82.94, 79.62, 78.13, 77.83, 77.37, 77.06, 76.74, 76.19, 75.85, 75.55, 75.45, 75.35, 75.09, 74.93, 74.72, 74.30, 73.54, 73.07, 72.62, 72.37, 71.77, 70.99, 70.61, 63.42, 62.92, 62.68, 61.77, 61.40, 60.72, 52.37, 51.43, 37.55, 36.66, 31.96, 29.73, 29.60, 29.40, 27.50, 27.01, 26.94, 24.71, 22.73, 19.39, 19.33, 14.16. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.76. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{145}H_{154}N_9F_3O_{28}Si_3$, 2633.0063; found, 2633.0015.

4-Trifluoromethylphenyl-*O*-[(methyl-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-*α*-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyra nate-(1→4)-(methyl-(2-azido-2-deoxy-3-*O*-napthylmethyl-6-*O*- *tert*-butyldiphenylsilyl-*α*-D-glucopyranoside)-(1→4)-3-*O*-benzyl-α-L-idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (42)

To a solution of **41** (1.20 g, 0.45 mmol) in DCM (15 ml) at was added N2H4.H2O (0.105 ml, 3.21 mmol), AcOH (0.184 ml, 3.21 mmol) and stirred under nitrogen atmosphere for 10 h. After the reaction was completed, the solvent was evaporated, then dissolved in EtOAc and washed with $NaHCO_3$ and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 4/6, v/v), to obtain compound **42** (1.03 g, 89.56 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 – 7.82 (m, 3H), 7.80 – 7.64 (m, 16H), 7.60 – 7.52 (m, 4H), 7.52 – 7.36 (m, 18H), 7.36 – 7.27 (m, 18H), 7.25 – 7.15 (m, 10H), 7.07 (dt, *J* = 8.9, 3.5 Hz, 5H), 5.51 (d, *J* = 3.5 Hz, 1H), 5.48 (t, *J* = 2.6 Hz, 1H), 5.45 – 5.38 (m, 1H), 5.31 (d, *J* = 10.9 Hz, 2H), 5.00 – 4.96 (m, 1H), 4.96 – 4.87 (m, 4H), 4.83 (dd, *J* = 15.1, 10.8 Hz, 2H), 4.77 – 4.69 (m, 2H), 4.68 – 4.55 (m, 5H), 4.20 (q, *J* = 10.0 Hz, 2H), 4.07 – 3.86 (m, 9H), 3.85 – 3.67 (m, 7H), 3.61 (d, *J* = 10.7 Hz, 1H), 3.55 – 3.50 (m, 1H), 3.48 – 3.39 (m, 2H), 3.31 (ddd, *J* = 18.5, 10.3, 2.7 Hz, 2H), 3.19 (d, *J* = 1.5 Hz, 3H), 3.17 (s, 1H), 2.68 (d, *J* = 1.5 Hz, 3H), 1.15 (d, *J* = 1.5 Hz, 9H), 1.06 (q, *J* = 1.9, 1.1 Hz, 18H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.08, 167.64, 164.37, 158.68, 138.34, 137.86, 137.76, 137.47, 137.27, 135.99, 135.97, 135.91, 135.78, 135.71, 135.64, 135.39, 133.72, 133.62, 133.44, 133.37, 133.18, 133.16, 133.01, 132.87, 132.50, 130.34, 130.00, 129.82, 129.79, 129.71, 129.60, 129.02, 128.61, 128.57, 128.55, 128.45, 128.41, 128.25, 128.23, 128.19, 128.08, 128.03, 128.00, 127.83, 127.79, 127.76, 127.74, 127.68, 127.66, 127.60, 127.52, 127.45, 127.42, 127.26, 127.05, 127.01, 126.85, 125.62, 125.58, 122.83, 116.60, 101.16, 100.13, 98.02, 96.51, 95.11, 82.96, 79.62, 78.56, 78.25, 77.83, 77.38, 77.27, 77.06, 76.75, 76.08, 75.85, 75.54, 75.12, 74.91, 74.76, 74.15, 73.39, 73.03, 72.90, 72.61, 72.36, 71.62, 71.45, 68.23, 67.65, 63.41, 62.98, 62.91, 62.33, 61.75, 60.56, 52.34, 51.21, 31.96, 29.73, 29.40, 27.00, 26.95, 22.73, 19.44, 19.39, 19.36, 14.17. ^{19}F NMR (400 MHz, $CDCl_3$) δ -61.78. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{140}H_{148}N_9F_3O_{26}Si_3$, 2534.9695; found, 2534.9676.

4-Trifluoromethylphenyl-*O*-[(methyl-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-*α*-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-gluco pyranate-(1→4)-(methyl-(2-azido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyra noside)-(1→4)-3-*O*-benzyl-α-L idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranoside (43)

To a solution of **42** (1.03 g, 0.40 mmol) in DCM/H2O (18:1, 15 ml) was added DDQ (0.372 g, 1.63 mmol) and stirred under nitrogen atmosphere at 0 °C for 3-4 h. After completion of the reaction, the mixture was diluted with DCM and washed with $NaHCO_3$ and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 4/6, v/v), to obtain compound **43** (765 mg, 78.06 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.85 – 7.79 (m, 2H), 7.72 – 7.62 (m, 12H), 7.59 – 7.27 (m, 37H), 7.24 – 7.16 (m, 9H), 7.13 – 7.05 (m, 4H), 7.04 – 6.98 (m, 1H), 5.51 (d, *J* = 3.5 Hz, 1H), 5.46 (d, *J* = 3.6 Hz, 1H), 5.40 – 5.29 (m, 2H), 4.91 (dd, *J* = 10.4, 3.1 Hz, 3H), 4.87 – 4.73 (m, 8H), 4.66 (d, *J* = 11.1 Hz, 2H), 4.61 (d, *J* = 2.4 Hz, 3H), 4.19 (t, *J* = 8.8 Hz, 1H), 4.05 (dd, *J* = 9.5, 3.9 Hz, 2H), 4.01 – 3.94 (m, 3H), 3.93 – 3.82 (m, 8H), 3.78 (dd, *J* = 11.9, 2.2 Hz, 1H), 3.67 (dd, *J* = 17.8, 5.8 Hz, 4H), 3.59 (d, *J* = 2.2 Hz, 4H), 3.47 (dd, *J* = 10.3, 3.2 Hz, 2H), 3.37 (q, *J* = 3.9 Hz, 1H), 3.30 (dt, *J* = 17.8, 6.1 Hz, 2H), 3.16 (d, *J* = 10.3 Hz, 1H), 3.04 (d, *J* = 2.3 Hz, 3H), 1.10 (s, 9H), 1.06 (d, *J* = 2.4 Hz, 18H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.21, 167.97, 164.47, 158.67, 138.22, 137.78, 137.75, 137.54, 137.08, 135.98, 135.88, 135.79, 135.66, 135.63, 133.66, 133.55, 133.49, 133.20, 133.16, 132.89, 132.85, 130.14, 129.95, 129.81, 129.74, 129.61, 128.86, 128.69, 128.56, 128.51, 128.45, 128.21, 128.12, 128.08, 128.03, 127.97, 127.95, 127.85, 127.76, 127.72, 127.66, 127.57, 127.49, 127.47, 127.29, 125.02, 124.70, 116.57, 101.12, 100.32, 97.85, 96.47, 95.27, 82.33, 79.76, 78.32, 78.18, 77.79, 77.37, 77.26, 77.06, 76.74, 75.60, 75.31, 75.00, 74.38, 74.27, 73.25, 73.00, 72.76, 72.58, 71.73, 70.63, 70.49, 68.66, 68.14, 63.38, 63.01, 62.84, 62.20, 61.84, 61.09, 52.98, 51.62, 31.96, 31.47, 30.22, 29.73, 29.39, 27.00, 26.94, 26.92, 22.72, 19.53, 19.39, 19.34, 14.15. ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.77. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{129}H_{140}N_9F_3O_{26}Si_3$, 2394.9069; found, 2394.9046.

4-Trifluoromethylphenyl-*O*-[(methyl-(2-azido-2-deoxy-3,4-*O*-benzyl-*α*-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranate-(1→4)-(methyl-(2-azido-2-deoxy-*α*-D-glucopyranoside)-(1→4)-3-*O*-benzyl-α-L idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-*α*-D-glucopyranoside (44)

To a solution of **43** (765 mg, 0.31 mmol) in pyridine (5 ml) was added 70% HF.Py (5 mmol) at 0 °C and stirred at RT overnight. After completion, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 8/2, v/v), to obtain compound **44** (361 mg, 70.23 %).^{1}H NMR (401 MHz, Chloroform-*d*) δ 8.05 – 8.01 (m, 2H), 7.64 – 7.56 (m, 3H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.37 – 7.27 (m, 15H), 7.25 – 7.15 (m, 12H), 5.61 (d, *J* = 3.5 Hz, 1H), 5.44 (d, *J* = 3.7 Hz, 1H), 5.34 – 5.27 (m, 2H), 4.93 – 4.82 (m, 5H), 4.81 – 4.72 (m, 4H), 4.72 – 4.65 (m, 3H), 4.62 (d, *J* = 11.7 Hz, 1H), 4.38 (dd, *J* = 9.2, 7.9 Hz, 1H), 4.25 (d, *J* = 9.3 Hz, 1H), 4.21 – 4.17 (m, 1H), 4.09 (t, *J* = 9.3 Hz, 1H), 4.03 (d, *J* = 9.7 Hz, 1H), 4.00 – 3.96 (m, 2H), 3.96 – 3.91 (m, 1H), 3.90 – 3.86 (m, 1H), 3.85 – 3.83 (m, 1H), 3.82 – 3.76 (m, 6H), 3.68 (d, *J* = 16.9 Hz, 2H), 3.64 – 3.50 (m, 6H), 3.48 – 3.39 (m, 2H), 3.32 (dt, *J* = 10.4, 3.3 Hz, 2H), 3.20 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.59, 168.95, 164.81, 158.56, 137.83, 137.75, 137.70, 137.29, 136.93, 133.85, 129.73, 128.86, 128.78, 128.58, 128.53, 128.51, 128.48, 128.27, 128.19, 128.17, 128.04, 127.99, 127.97, 127.80, 127.47, 127.41, 127.18, 127.14, 125.49, 125.28, 124.95, 122.79, 116.59, 100.75, 100.64, 97.40, 96.59, 95.48, 82.08, 79.85, 79.78, 78.25, 77.73, 77.37, 77.25, 77.05, 76.73, 75.42, 75.05, 74.55, 74.27, 74.19, 73.90, 73.82, 73.55, 73.16, 72.74, 72.70, 72.29, 71.08, 70.46, 68.58, 68.08, 63.24, 63.09, 62.73, 61.06, 60.24, 53.32, 52.05, 31.94, 29.71. ^{19}F NMR (377 MHz, $CDCl_3$) δ -61.78. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{81}H_{86}N_9F_3O_{26}Si_3$, 1680.5536; found, 1680.5541.

4-Trifluoromethylphenyl-*O*-[(methyl-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranoside)-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranate-(1→4)-(methyl-(2-azido-2-deoxy-3,6-*O*-sulfonate-*α*-D-glucopyranoside)-(1→4)-2-*O*-sulfonate-3-*O*-benzyl-α-L-idopyranonate)]-(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranoside (45)

To a solution of **44** (361 mg, 0.21 mmol) in DMF (8 mL) was added $SO_3{\cdot}NMe_3$ (1.64 g, 11.97 mmol) and stirred at 100 °C for 1 h. in the microwave reactor. DMF was evaporated and the

crude product was purified by by bond elute C-18 column MeOH/H2O (9/1), to obtain compound **45** (212 mg, 47.42 %).

^{1}H NMR (600 MHz, Methanol-d_4) δ 8.32 – 8.26 (m, 2H), 7.80 – 7.74 (m, 1H), 7.67 (td, *J* = 8.3, 7.5, 2.3 Hz, 4H), 7.51 – 7.48 (m, 2H), 7.43 – 7.38 (m, 2H), 7.38 – 7.32 (m, 7H), 7.31 (d, *J* = 1.8 Hz, 1H), 7.31 – 7.22 (m, 4H), 7.15 – 7.13 (m, 2H), 7.12 – 7.06 (m, 4H), 7.04 (t, *J* = 7.7 Hz, 2H), 6.83 (tt, *J* = 7.3, 1.4 Hz, 1H), 5.77 (d, *J* = 3.5 Hz, 1H), 5.42 – 5.38 (m, 2H), 5.25 (t, *J* = 8.5 Hz, 1H), 5.17 – 5.12 (m, 2H), 4.93 – 4.88 (m, 3H), 4.85 – 4.82 (m, 2H), 4.78 (d, *J* = 10.6 Hz, 1H), 4.68 (d, *J* = 10.9 Hz, 1H), 4.59 (d, *J* = 10.5 Hz, 1H), 4.57 – 4.55 (m, 1H), 4.50 (d, *J* = 10.7 Hz, 1H), 4.44 (d, *J* = 10.7 Hz, 1H), 4.36 – 4.32 (m, 2H), 4.30 – 4.27 (m, 2H), 4.25 (t, *J* = 2.7 Hz, 1H), 4.22 (dd, *J* = 10.8, 2.0 Hz, 1H), 4.21 – 4.18 (m, 1H), 4.17 (d, *J* = 2.7 Hz, 1H), 4.15 (d, *J* = 1.9 Hz, 1H), 4.14 – 4.11 (m, 1H), 4.10 (d, *J* = 9.6 Hz, 1H), 3.98 (ddd, *J* = 9.8, 3.2, 1.9 Hz, 1H), 3.92 – 3.86 (m, 3H), 3.83 (s, 3H), 3.75 – 3.69 (m, 3H), 3.68 – 3.58 (m, 3H), 3.48 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.24 (dd, *J* = 10.3, 3.6 Hz, 1H), 3.15 (dt, *J* = 10.0, 2.1 Hz, 1H), 2.78 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.24, 169.28, 165.21, 159.11, 138.28, 137.99, 137.62, 137.41, 133.66, 129.97, 129.20, 128.82, 128.21, 128.04, 127.95, 127.92, 127.80, 127.70, 127.68, 127.57, 127.46, 127.39, 127.36, 127.28, 127.23, 127.11, 126.81, 126.77, 125.73, 124.68, 124.36, 123.05, 116.84, 100.00, 97.76, 97.34, 96.47, 93.59, 82.04, 79.44, 78.32, 77.60, 77.33, 75.95, 75.00, 74.71, 74.56, 74.39, 74.06, 73.09, 72.12, 71.40, 71.09, 70.55, 69.61, 68.97, 68.51, 66.48, 65.71, 65.20, 63.97, 63.53, 63.34, 62.89, 52.35, 51.15, 48.49, 48.28, 48.06, 47.85, 47.64, 47.43, 47.21, 47.00. ^{19}F NMR (400 MHz, MeOD) δ -63.21. HR-ESI-MS *(m/z)*: $[M-5H]^{-5}$ calcd for $C_{72}H_{73}N_9F_3O_{40}Si_3^{5-}$, 410.4623; found, 410.4624.

4-Trifluoromethylphenyl-*O*-(2-azido-2-deoxy-3,4-*O*-benzyl-6-*O*-sulfonate-*α*-D-gluco pyranoside)-(1→4)-*O*-3-*O*-benzyl-β-D-glucopyranate-(1→4)-(2-azido-2-deoxy-3,6-*O*-sulfonate-*α*-D-glucopyranoside)-(1→4)-2-*O*-sulfonate-3-*O*-benzyl-α-L idopyranonate) -(1→4)-2-azido-2-deoxy-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranoside (46)

To a solution of **45** (212 mg, 0.10 mmol) in a mixture of THF/H_2O (1:1, 4 ml) was added 1 M LiOH·H_2O (130 mg, 3.09 mmol) and stirred at RT for 15 h. After completion of the reaction, the reaction mixture was diluted with MeOH and neutralized using Dowex 50WX8 H+ resin. The reaction was filtered and concentrated under reduced pressure and purified by bond elute C-18 column MeOH/H2O (8/2), to obtain compound **46** (183 mg, 95.31 %).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62 (d, *J* = 8.6 Hz, 2H), 7.41 (dd, *J* = 7.5, 2.5 Hz, 4H), 7.36 – 7.20 (m, 23H), 5.69 (d, *J* = 3.4 Hz, 1H), 5.56 (d, *J* = 3.8 Hz, 1H), 5.44 (s, 1H), 5.17 (d,

J = 10.6 Hz, 1H), 5.07 (d, *J* = 3.7 Hz, 1H), 4.99 – 4.89 (m, 4H), 4.84 – 4.80 (m, 3H), 4.76 – 4.67 (m, 2H), 4.67 – 4.62 (m, 1H), 4.57 (s, 1H), 4.52 – 4.47 (m, 1H), 4.46 – 4.35 (m, 3H), 4.26 – 4.11 (m, 7H), 4.06 (dd, *J* = 17.4, 9.6 Hz, 2H), 4.01 – 3.95 (m, 2H), 3.94 – 3.88 (m, 2H), 3.86 – 3.81 (m, 1H), 3.77 – 3.71 (m, 1H), 3.66 (td, *J* = 9.4, 2.9 Hz, 2H), 3.53 – 3.44 (m, 2H), 3.38 (d, *J* = 3.6 Hz, 1H), 3.24 (dd, *J* = 10.5, 3.8 Hz, 1H). ^{13}C NMR (400 MHz, MeOD) δ 174.35, 174.17, 159.12, 139.03, 138.00, 137.86, 128.24, 128.11, 128.04, 127.99, 127.93, 127.86, 127.77, 127.73, 127.43, 127.39, 127.09, 126.69, 116.79, 102.44, 97.95, 97.70, 96.52, 93.62, 85.13, 79.72, 78.11, 77.90, 76.75, 75.63, 75.13, 75.05, 74.67, 72.81, 72.08, 71.18, 71.05, 70.78, 69.83, 69.71, 69.17, 68.91, 66.03, 65.70, 65.34, 63.96, 63.59, 62.90, 48.50, 48.29, 48.26, 48.08, 48.05, 47.87, 47.83, 47.65, 47.62, 47.44, 47.41, 47.23, 47.20, 47.01, 46.98. ^{19}F NMR (400 MHz, MeOD) δ -63.20. HR-ESI-MS *(m/z)*: $[M-5H]^{-5}$ calcd for $C_{72}H_{73}N_9F_3O_{40}Si_3^{5-}$, 384.0507; found, 384.0504.

4-Trifluoromethylphenyl-*O*-(2-sulfamido-2-deoxy-3,4-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranoside)-(1→4)-*O*-3-*O*-benzyl-β-D-glucopyranate-(1→4)-(2-sulfamido-2-deoxy-3,6-*O*-sulfonate-*α*-D-glucopyranoside)-(1→4)-2-*O*-sulfonate-3-*O*-benzyl-α-L idopyranonate)-(1→4)-2-sulfamido-2-deoxy-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranoside (48)

To the stirring solution of **47** (106 mg, 0.05 mmol) in a mixture of MeOH (3 ml), Et_3N (2 ml) and 1 M NaOH solution (2 ml) at 0 ºC was added SO_3.Py complex (274 mg, 1.72 mmol). The progress of reaction was monitored by TLC (EtOAc/pyridine/H_2O/AcOH 8/5/3/1, v/v/v/v). Two additional portions of SO_3.Py complex were added at 0 ºC after 1h and 2h. After stirring for an additional 8h, the reaction mixture was concentrated in vacuo and the residue was passed through a column of DowexR 50 x 8 Na+ resin using H_2O/MeOH as eluent. The appropriate fractions were concentrated in vacuo and further was purified by reverse phase C-18 silica gel column using H_2O/MeOH as eluent to obtain compound **48** (61 mg, 58.65 %) as sodium salt.

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.80 – 7.71 (m, 2H), 7.66 – 7.28 (m, 27H), 6.02 – 5.95 (m, 1H), 5.68 (t, *J* = 3.5 Hz, 1H), 5.40 – 5.28 (m, 1H), 5.12 (d, *J* = 3.8 Hz, 1H), 5.03 – 4.94 (m, 3H), 4.93 – 4.82 (m, 3H), 4.76 – 4.66 (m, 5H), 4.65 – 4.57 (m, 1H), 4.57 – 4.35 (m, 3H), 4.35 – 4.11 (m, 6H), 4.06 – 3.91 (m, 6H), 3.86 (t, *J* = 9.6 Hz, 2H), 3.80 – 3.60 (m, 5H), 3.44 (dt, *J* = 10.3, 3.1 Hz, 1H), 3.38 – 3.32 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.41, 172.91, 158.65, 137.65, 137.50, 137.30, 137.10, 137.06, 136.96, 136.86, 129.10, 129.03, 128.97, 128.83, 128.79, 128.74, 128.69, 128.63, 128.59, 128.56, 128.41, 128.32, 128.27,

128.22, 127.20, 127.17, 127.15, 127.05, 125.26, 124.56, 124.34, 124.13, 123.91, 123.47, 121.67, 117.17, 102.10, 101.90, 98.10, 97.96, 97.82, 96.68, 95.80, 95.77, 93.67, 83.25, 83.21, 79.68, 77.39, 77.27, 77.21, 77.14, 76.81, 76.08, 75.67, 75.29, 75.11, 75.05, 74.26, 74.02, 73.85, 73.80, 73.75, 73.72, 73.70, 72.82, 72.56, 71.58, 70.59, 69.94, 69.37, 69.28, 69.20, 69.06, 68.90, 68.58, 67.44, 67.03, 66.49, 66.41, 66.17, 65.81, 62.23, 57.91, 57.58, 57.43, 57.31, 48.88. ^{19}F NMR (400 MHz, D_2O) δ -61.40. HR-ESI-MS *(m/z)*: $[M-8H]^{-8}$ calcd for $C_{65}H_{70}N_9F_3O_{49}S_8^{8-}$, 248.6355; found, 248.6354.

4-Trifluoromethylphenyl-*O*-(2-sulfamido-2-deoxy-6-*O*-sulfonate-*α*-D-gluco pyranoside)-(1→4)-*O*-(β-D-glucopyranate)-(1→4)-(2-sulfamido-2-deoxy-3,6-*O*-sulfonate-*α*-D-gluco pyranoside)-(1→4)-2-*O*-sulfonate-(α-L idopyranonate) -(1→4)-2-sulfamido-2-deoxy-6-*O*-sulfonate-*α*-D-glucopyranoside (49)

To a solution of **48** (60 mg, 0.03 mmol) in H_2O (3 ml) was added $Pd(OH)_2$ (10% by wt.) and stirred for 48 h under hydrogen atmosphere. After completion of the reaction, the reaction mixture was diluted with H_2O, filtered and concentrated, and finally purified by bond elute C-18 column (H_2O) to obtain compound **49** (28 mg, 57.14 %)

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.72 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 5.98 (d, *J* = 3.5 Hz, 1H), 5.63 (d, *J* = 3.8 Hz, 1H), 5.44 (d, *J* = 6.7 Hz, 1H), 5.28 (s, 1H), 5.03 (d, *J* = 20.0 Hz, 1H), 4.66 – 4.58 (m, 1H), 4.46 (t, *J* = 9.7 Hz, 1H), 4.38 (s, 1H), 4.36 – 4.23 (m, 5H), 4.20 (d, *J* = 7.8 Hz, 1H), 4.16 – 4.01 (m, 3H), 3.97 (t, *J* = 9.3 Hz, 1H), 3.91 – 3.73 (m, 6H), 3.71 – 3.63 (m, 1H), 3.60 (d, *J* = 8.8 Hz, 1H), 3.52 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.42 (t, *J* = 8.7 Hz, 2H), 3.29 (t, *J* = 10.9 Hz, 2H). ^{13}C NMR (151 MHz, D_2O) δ 173.47, 158.60, 127.09, 127.06, 125.21, 124.30, 124.09, 123.42, 117.19, 116.92, 102.04, 101.86, 99.09, 98.71, 98.19, 98.12, 97.98, 95.59, 91.26, 77.31, 77.24, 77.05, 76.75, 76.60, 76.42, 75.54, 75.49, 75.16, 74.88, 74.74, 72.70, 72.64, 72.61, 71.01, 70.46, 70.10, 70.02, 69.75, 69.66, 69.54, 69.30, 69.09, 68.92, 67.73, 66.66, 66.53, 66.48, 66.29, 65.75, 65.50, 62.70, 57.84, 57.70, 57.63, 57.55, 53.61, -3.02. ^{19}F NMR (400 MHz, D_2O) δ -61.45. HR-ESI-MS *(m/z)*: $[M-8H]^{-8}$ calcd for $C_{37}H_{46}N_9F_3O_{49}S_8^{8-}$, 203.6120; found, 203.6126.

3.6. References

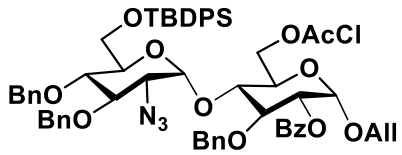
1. Linhardt, R. J. Heparin: structure and activity. *J. Med. Chem.* **2003**, 46, 2551–2564.
2. Linhardt, R. J., Witczak, Z., Nieforth, K. *Carbohydrates as Drugs. eds.* (Dekker, New York) **1998**, 277–341.
3. Walenga, J. M. et al. Development of a synthetic heparin pentasaccharide: fondaparinux. *Turk. J. Haematol*. **2002**, 19, 137–150.
4. Mueller, R. L. & Scheidt, S. History of drugs for thrombotic disease. Discovery, development, and directions for the future. *Circulation* **1994**, 89, 432–449.
5. Petitou, M., Casu, B. & Lindahl, U. 1976-1983, a critical period in the history of heparin: the discovery of the antithrombin binding site. *Biochemie* **2003**, 85, 83–89.
6. Mackman, N. Triggers, targets and treatments for thrombosis. *Nature* **2008**, 451, 914–918.
7. Xu, Y. et al. Homogeneous low-molecular-weight heparins with reversible anticoagulant activity. *Nat. Chem. Biol.* **2014**, 10, 248–250.
8. Brill-Edwards, P., Ginsberg, J. S., Johnston, M. & Hirsh, J. Establishing a therapeutic range for heparin therapy. *Ann. Intern. Med*. **1993**, 119, 104–109.
9. Gerotziafas, G. T., Petropoulou, A. D., Verdy, E., Samama, M. M. & Elalamy, I. Effect of the anti-factor Xa and anti-factor IIa activities of low-molecularweight heparins upon the phases of thrombin generation. *J. Thromb. Haemost.* **2007**, 5, 955–962.
10. Hemker, H. C., Al Dieri, R. & Béguin, S. Heparins: a shift of paradigm. *Front. Med.* **2019**, 6, 254.
11. Dou, H., Song, A., Jia, S. & Zhang, L. Heparinoids Danaparoid and Sulodexide as clinically used drugs. *Prog. Mol. Biol. Transl. Sci.* **2019**, 163, 55–74.
12. Hetzel, G. R. & Sucker, C. The heparins: all a nephrologist should know *Nephrol. Dial. Transplant.* **2005**, 20, 2036–2042
13. Cohen, M. et al. A comparison of low-molecular-weight heparin with unfractionated heparin for unstable coronary artery disease. *N. Engl. J. Med.* **1997**, 337, 447–452 .
14. Guerrini, M. et al. Oversulfated chondroitin sulfate is a contaminant in heparin associated with adverse clinical events. *Nat. Biotechnol.* **2008**, 26, 669–675.
15. Blossom, D. B. et al. Outbreak of adverse reactions associated with contaminated heparin. *N. Engl. J. Med.* **2008** 359, 2674–2684.

16. Lindahl, U., Bäckström, G., Thunberg, L. & Leder, I. G. Evidence for a 3-Osulfated D-glucosamine residue in the antithrombin-binding sequence of heparin. *Proc. Natl Acad. Sci. USA* **1980**, 77, 6551–6555.
17. Choay, J. et al. Anti-Xa active heparin oligosaccharides. *Thromb. Res.* **1980**, 18, 573–578.
18. Thunberg, L., Bäckström, G. & Lindahl, U. Further characterization of the antithrombin-binding sequence in heparin. *Carbohydr. Res.* **1982**, 100, 393–410.
19. Petitou, M. & van Boeckel, C. A. A. A synthetic antithrombin III binding pentasaccharide is now a drug! What comes next? *Angew. Chem. Int. Ed.* **2004**, 43, 3118–3133.
20. Chang, C. H. et al. Synthesis of the heparin-based anticoagulant drug fondaparinux. *Angew. Chem. Int. Ed.* **2014**, 53, 9876–9879.
21. Petitou, M. et al. Synthesis of heparin fragments: a methyl alpha-pentaoside with high affinity for antithrombin III. *Carbohydr. Res.***1987**, 167, 67–75.
22. Sinaÿ, P. et al. Total synthesis of a heparin pentasaccharide fragment having high affinity for antithrombin III. *Carbohydr. Res.* **1984**, 132, C5–C9.
23. Codée, J. D. C. et al. A modular strategy toward the synthesis of heparin-like oligosaccharides using monomeric building blocks in a sequential glycosylation strategy. *J. Am. Chem. Soc.* **2005**, 127, 3767–3773.
24. Arungundram, S. et al. Modular synthesis of heparan sulfate oligosaccharides for structure−activity relationship studies. *J. Am. Chem. Soc.* **2009**, 131, 17394–17406.
25. Orgueira, H. A. et al. Modular synthesis of heparin oligosaccharides. *Chem. Eur. J.* **2003**, 9, 140–169.
26. Dey, S. & Wong, C.-H. Programmable one-pot synthesis of heparin pentasaccharides enabling access to regiodefined sulfate derivatives. *Chem. Sci.* **2018**, 9, 6685–6691.
27. Li, T. et al. Total synthesis of anticoagulant pentasaccharide fondaparinux. *ChemMedChem.* **2014**, 9, 1071–1080.
28. Dey, S., Lo, H.-J. & Wong, C.-H. An Efficient Modular One-Pot Synthesis of Heparin-Based Anticoagulant Idraparinux. *J. Am. Chem. Soc.* **2019**, 141, 10309–10314.
29. Jin, H. et al. Preactivation-based, iterative one-pot synthesis of anticoagulant pentasaccharide fondaparinux sodium. *Org. Chem. Front.* **2019**, 6, 3116–3120).
30. Mishra, B., Neralkar, M. & Hotha, S. Stable alkynyl glycosyl carbonates: catalytic anomeric activation and synthesis of a tridecasaccharide reminiscent of

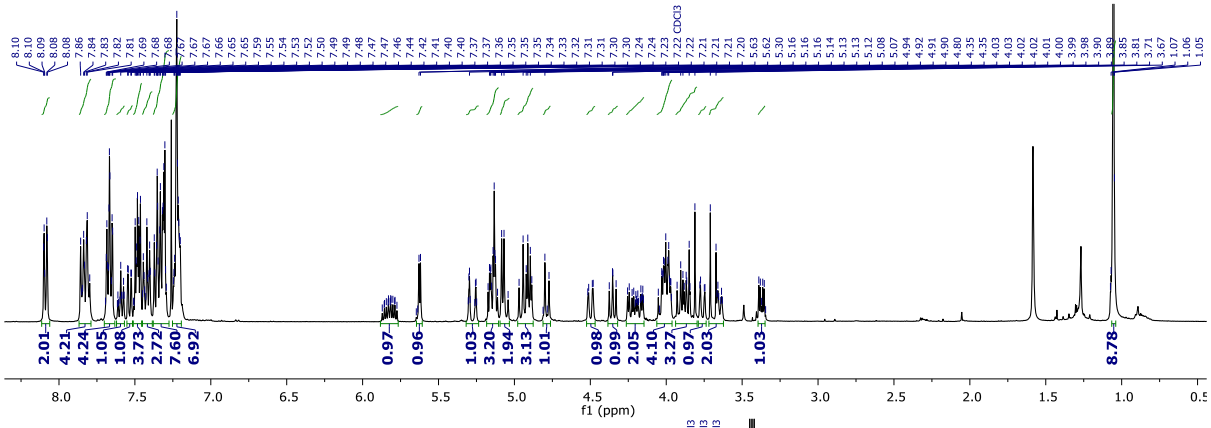
Mycobacterium tuberculosis cell wall lipoarabinomannan. *Angew. Chem. Int. Ed.* **2016**, 55, 7786–7791.

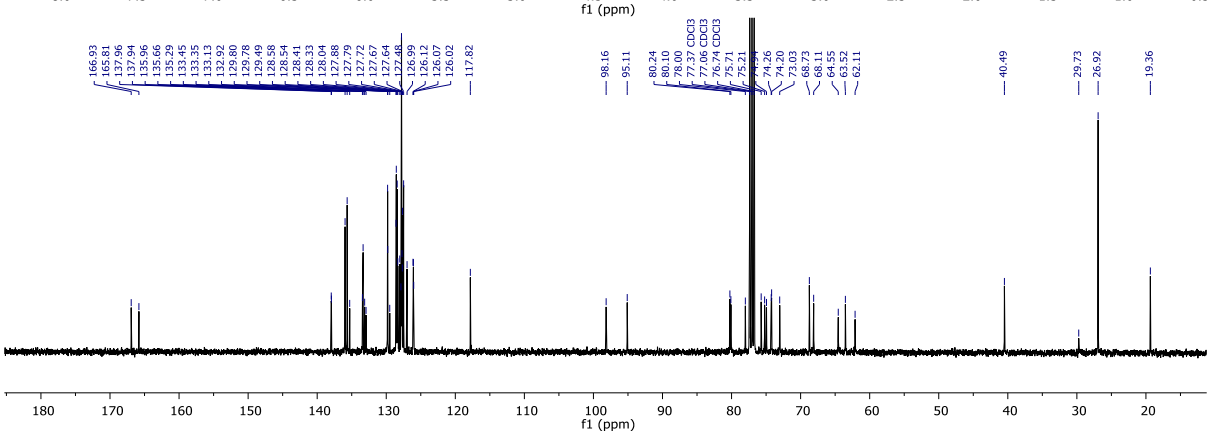
31. Prakash, K. R. C., Rao, S. P. Ethoxycarbonylmethylenetriphenylphosphorane in carbohydrate chemistry, Part III: a short and highly efficient synthesis of (-)-epigoniofufurone. *Synlett* **1993**, 123–124.
32. Lohman, G. J. S., Hunt, D. K., Högermeier, J. A. & Seeberger, P. H. Synthesis of iduronic acid building blocks for the modular assembly of glycosaminoglycans. *J. Org. Chem.* **2003**, 68, 7559–7561.
33. Noji, M., et al. A novel synthetic route to 2-arylalkanoic acids by a rutheniumcatalyzed
34. chemoselective oxidation of furan rings. *Synthesis* **2008**, 3835–3845.
35. Nunez, M. T. & Martin, V. S. Efficient oxidation of phenyl groups to carboxylic acids with ruthenium tetraoxide. A simple synthesis of (R)-.γ.-caprolactone, the pheromone of Trogoderma granarium. *J. Org. Chem.* **1990**, 55, 1928–1932.
36. Lubineau, A., Gabvard, O., Alais, J. & Bonnaffé, D. New accesses to l-iduronyl synthons. *Tetrahedron Lett.* **2000**, 41, 307–311.
37. Hansen, S. U. et al. Scalable synthesis of l-iduronic acid derivatives via stereocontrolled cyanohydrin reaction for synthesis of heparin-related disaccharides. *Org. Lett.* **2009**, 11, 4528–4531.
38. Tatai, J., Osztrovszky, G., Kajtár-Peredy, M. & Fügedi, P. An efficient synthesis of l-idose and l-iduronic acid thioglycosides and their use for the synthesis of heparin oligosaccharides. *Carbohydr. Res.* **2008**, 343, 596–606.
39. Lu, L.-D. et al. Synthesis of 48 disaccharide building blocks for the assembly of a heparin and heparan sulfate oligosaccharide library. *Org. Lett.* **2006**, 26, 5995–5998.
40. Nakamura, T., Shiozaki, M. & Kurakata, S.-I. Synthesis of ceramidated GLA-60 derivatives. *Carbohydr. Res.* **2002**, 337, 1343–1349.
41. Goddard-Borger, E. D. & Stick, R. V. An efficient, inexpensive, and shelfstable diazotransfer reagent: imidazole-1-sulfonyl azide hydrochloride. *Org. Lett.* **2007**, 9, 3797–3800.

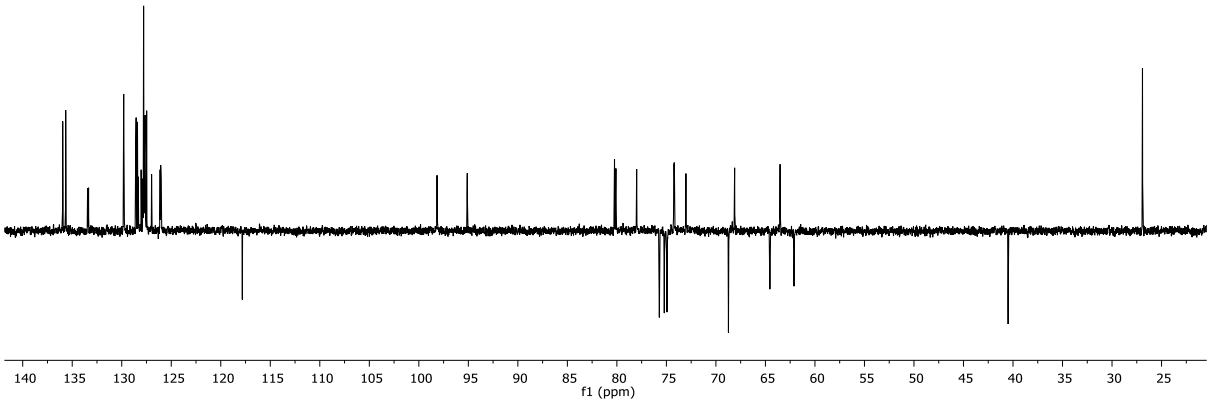
3.7. NMR Spectras

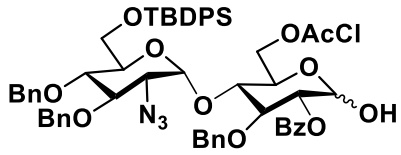


27

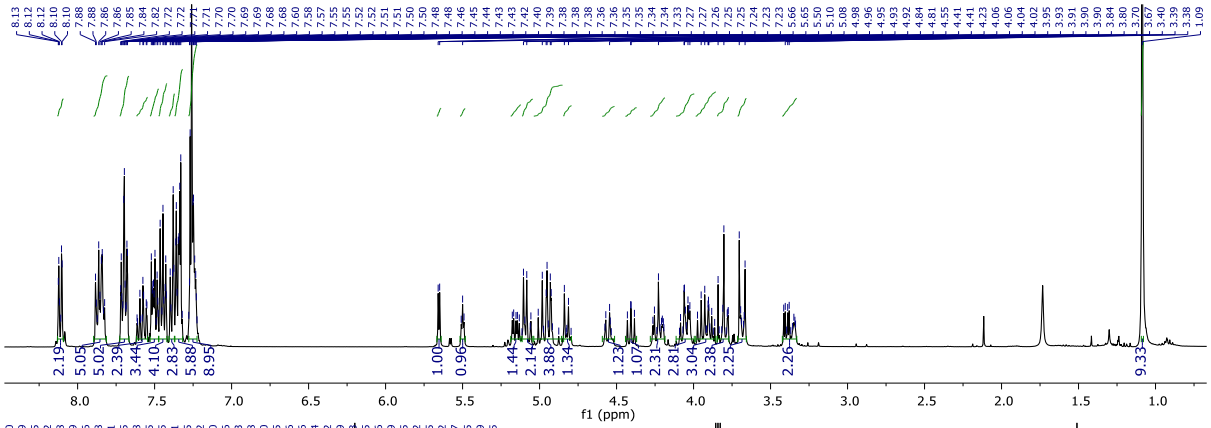


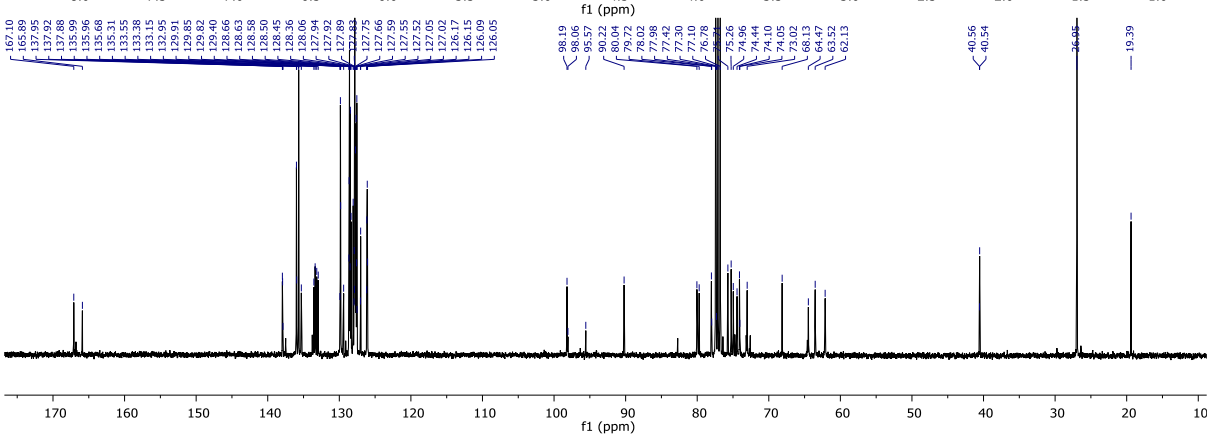


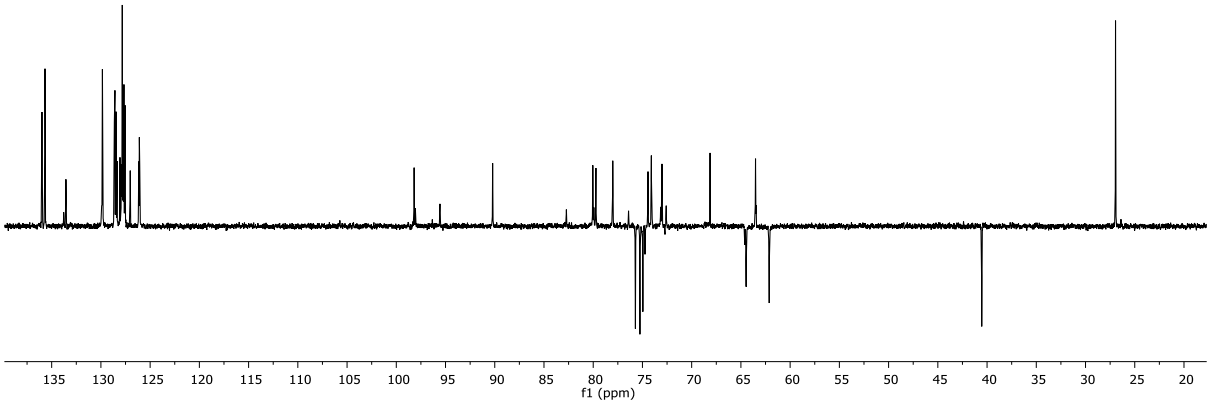


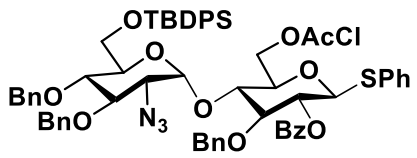


28

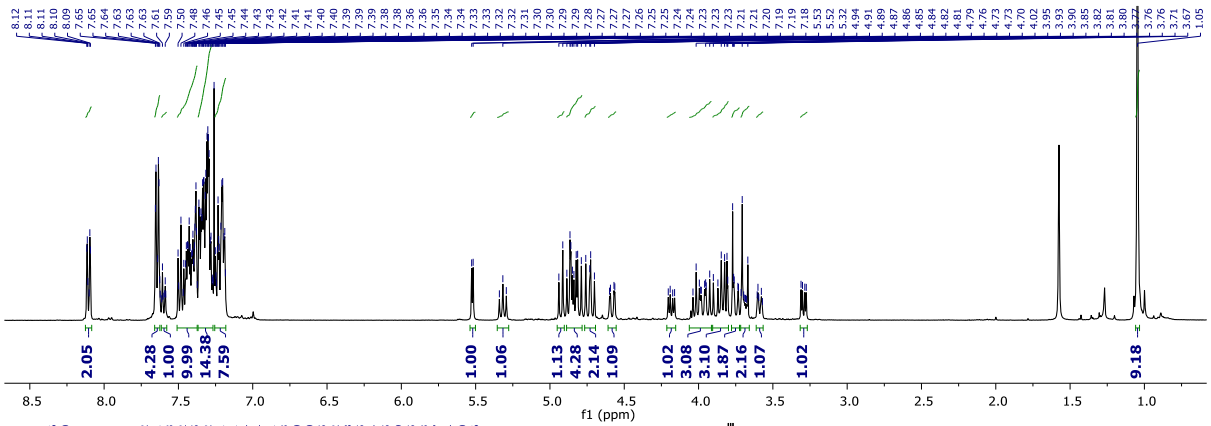


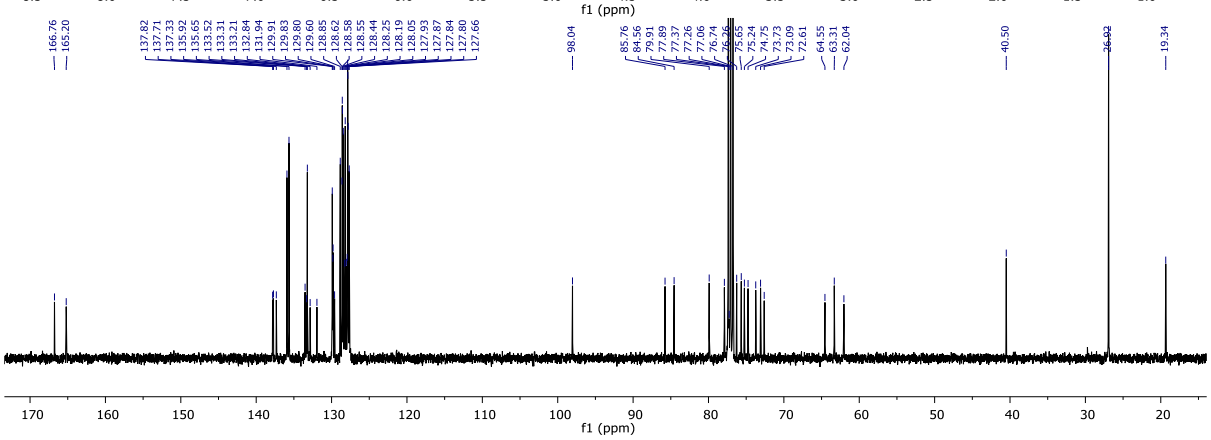


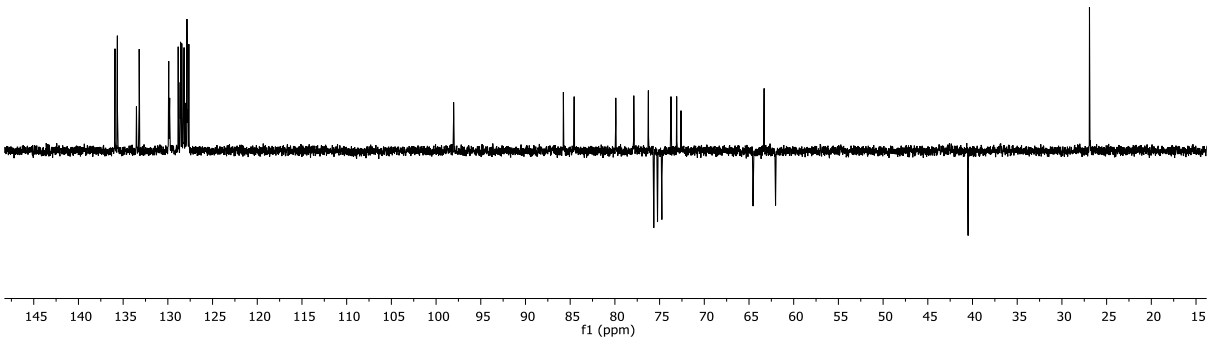


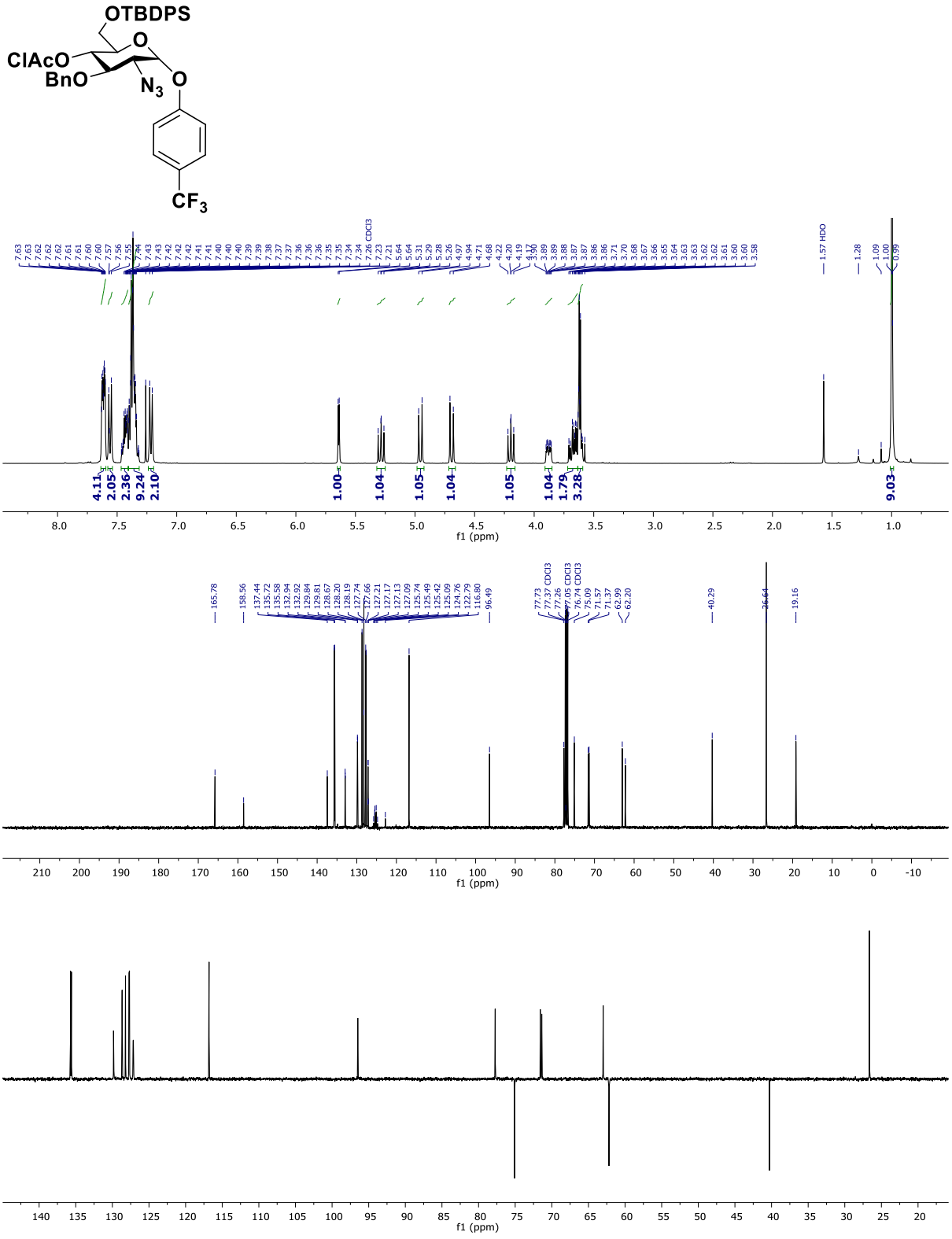

OTBDPS
O
OAcCl
O
SPh
BnO
BnO
N3
O
BnO
BzO

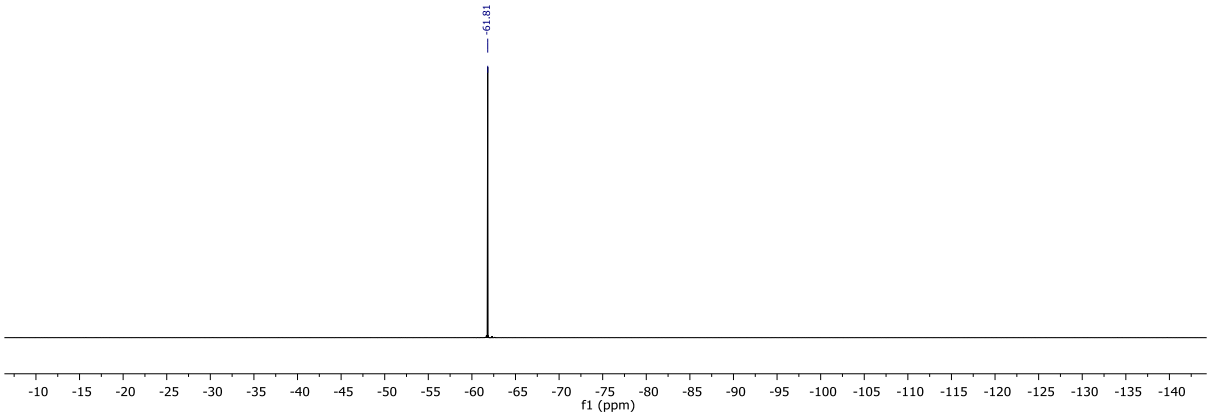
30

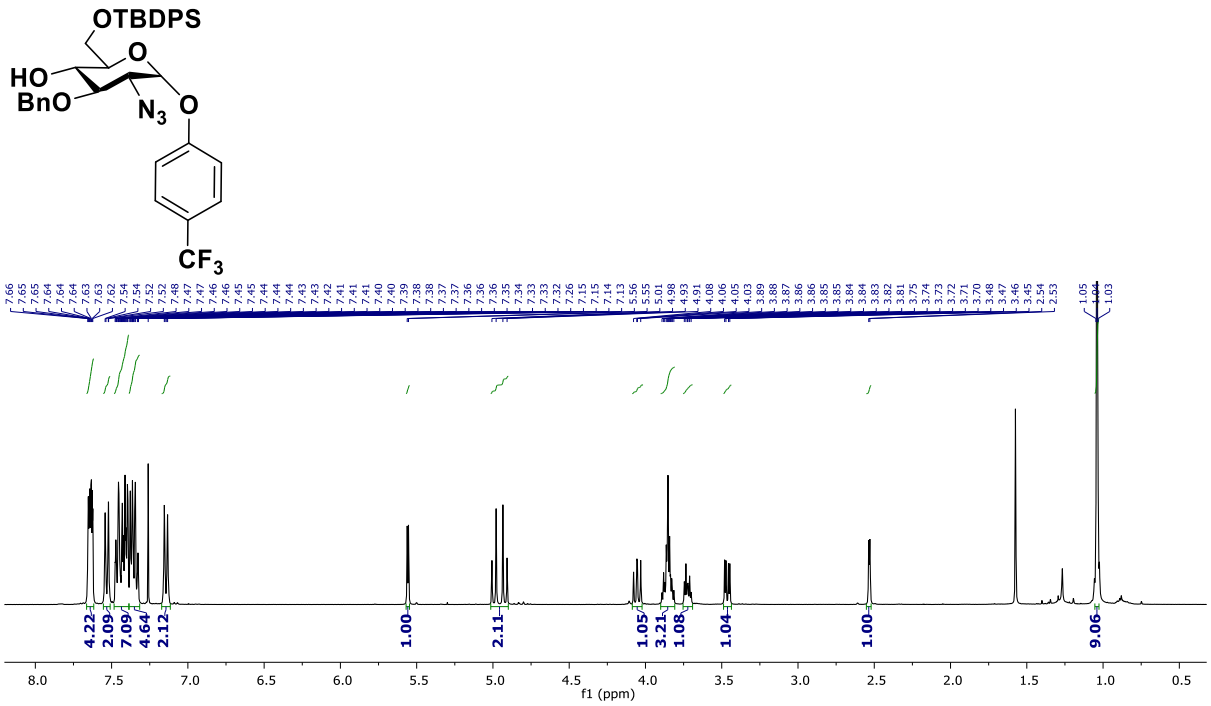

2.05
4.28
1.00
9.99
14.38
7.59
1.00
1.06
1.13
4.28
2.14
1.09
1.02
3.08
3.10
1.87
2.16
1.07
1.02
9.18
f1 (ppm)

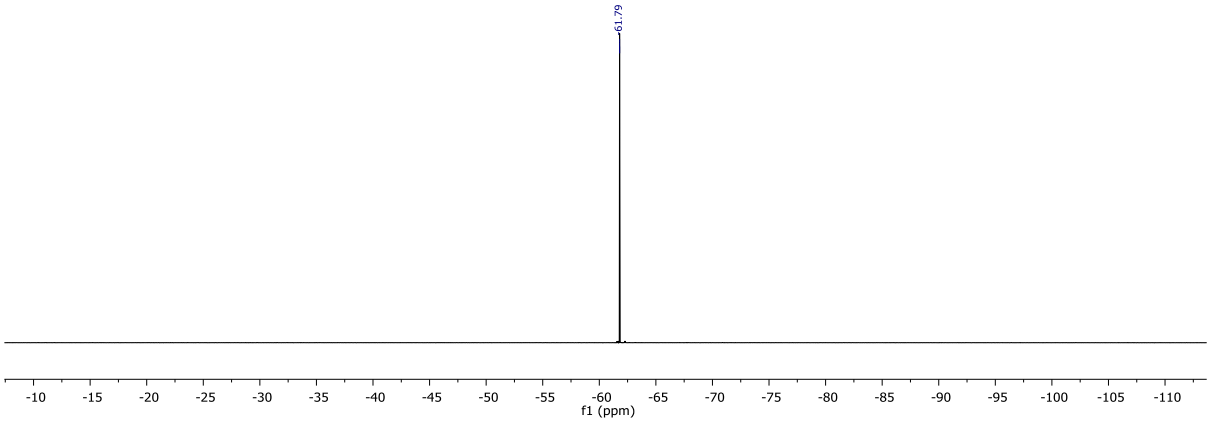

f1 (ppm)

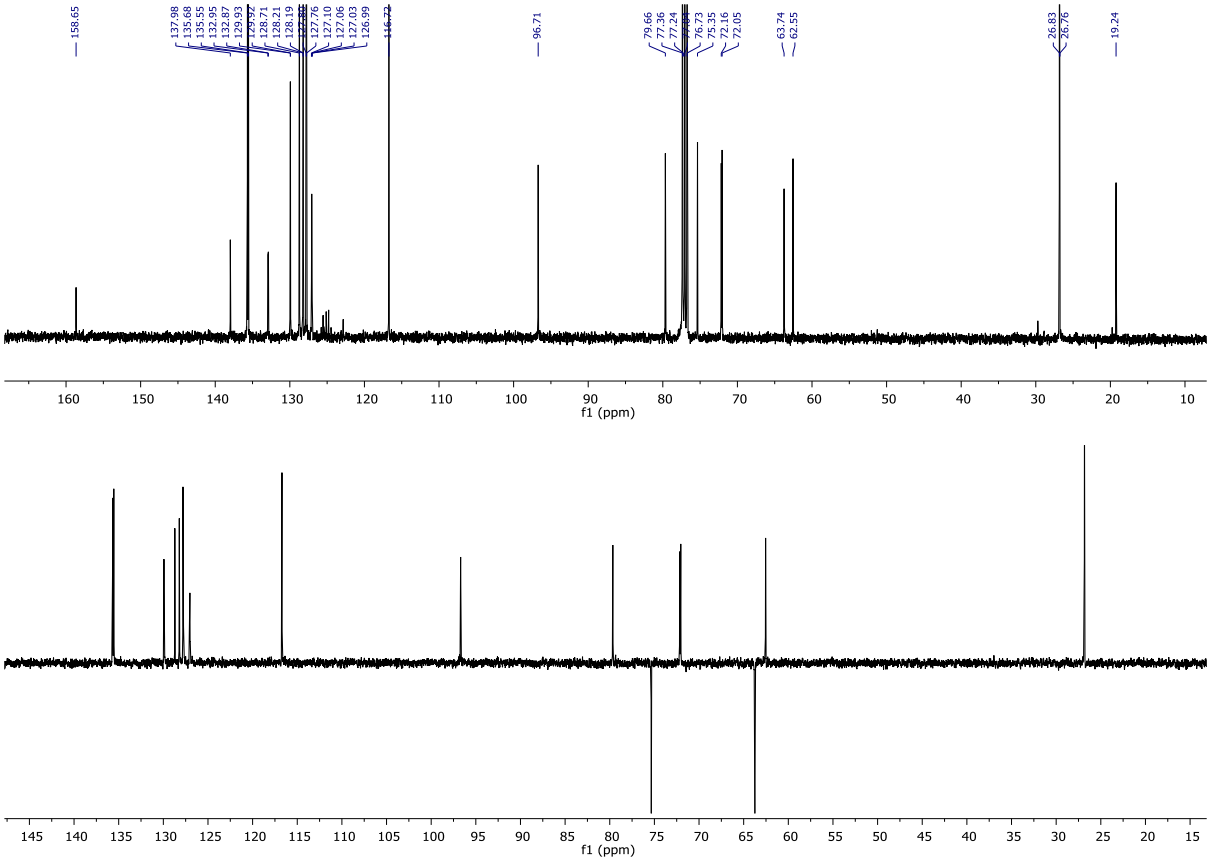

f1 (ppm)


OTBDPS
O
ClAcO
BnO
N3
O
CF3
CDCl3
1.57 HDO
1.28
4.11
2.05
2.36
9.24
2.10
1.00
1.04
1.05
1.04
1.05
1.04
1.79
3.28
9.03
f1 (ppm)
77.37 CDCl3
77.05 CDCl3
77.74 CDCl3
f1 (ppm)
f1 (ppm)

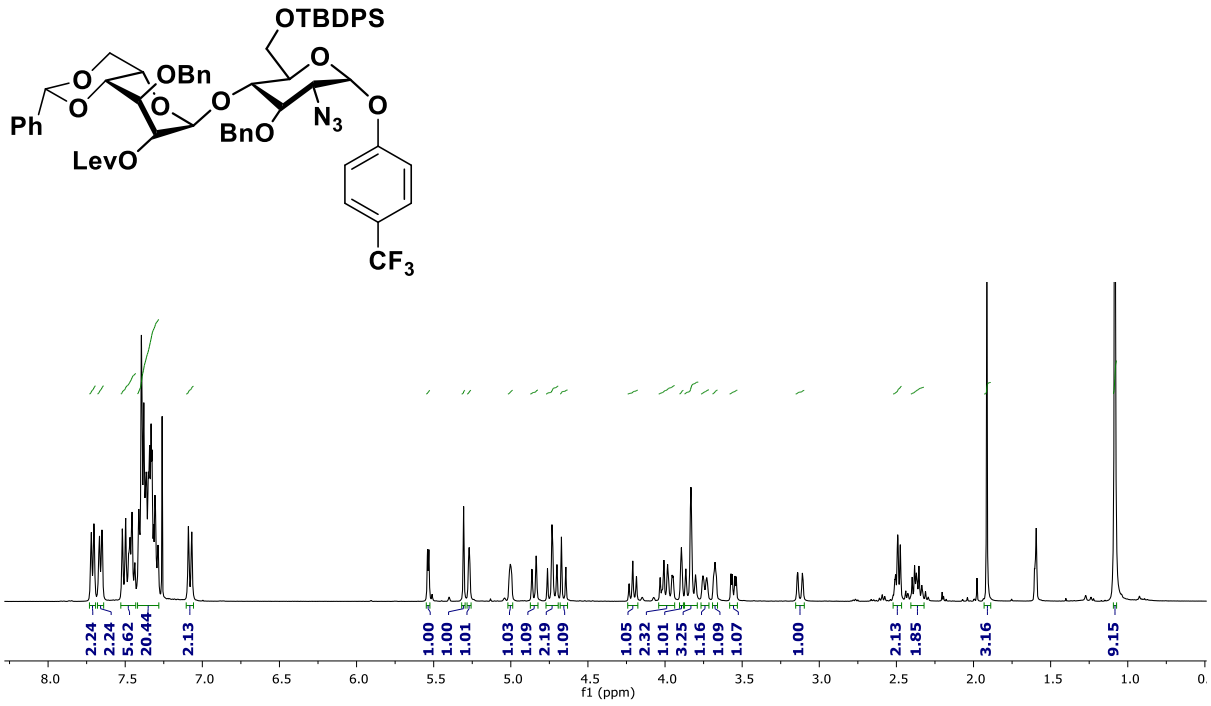

-61.81
-10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140
f1 (ppm)

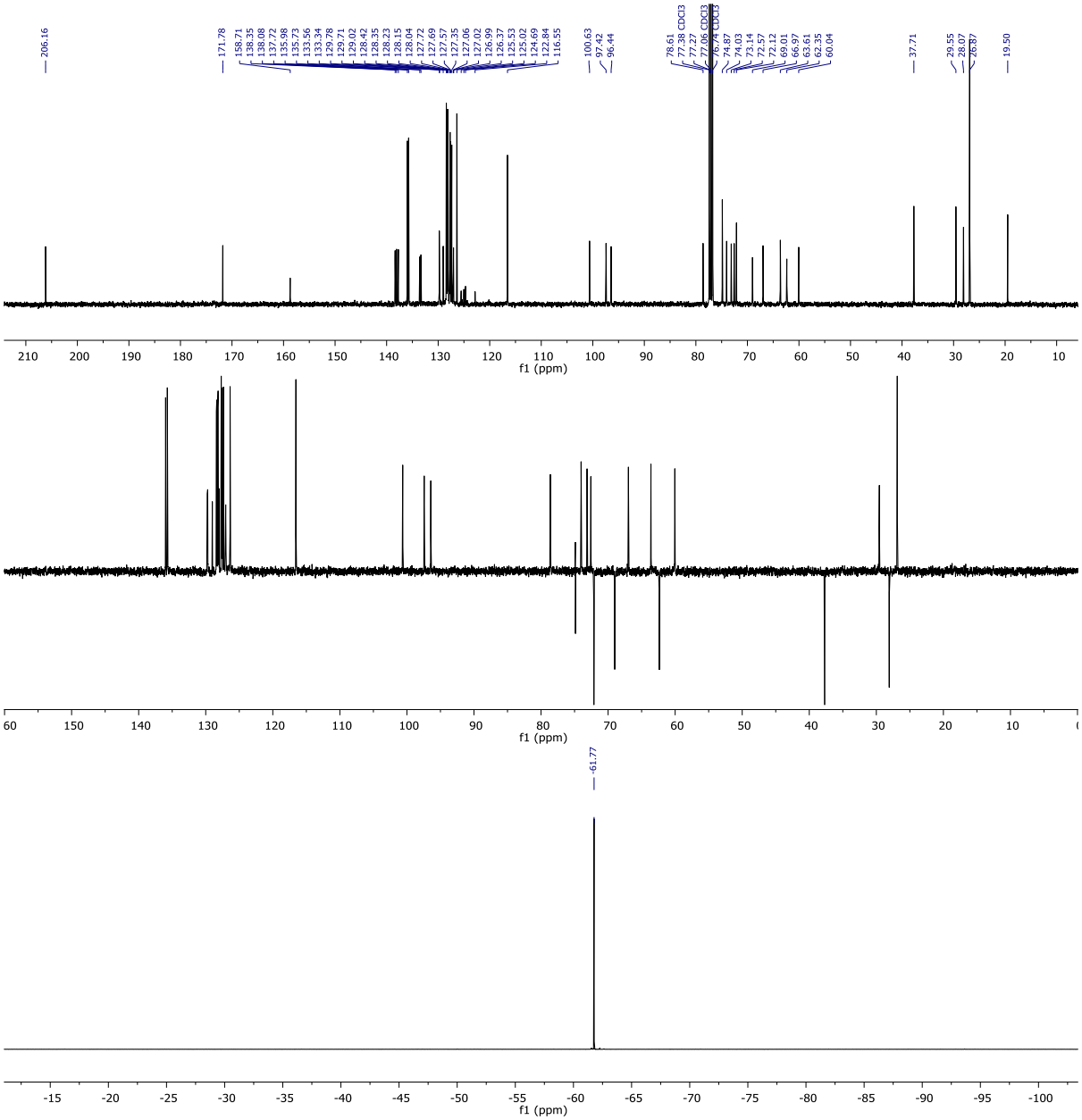

OTBDPS
O
HO
BnO
N3
O
CF3
4.22
2.09
7.09
4.64
2.12
1.00
2.11
1.05
3.21
1.08
1.04
1.00
9.06
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

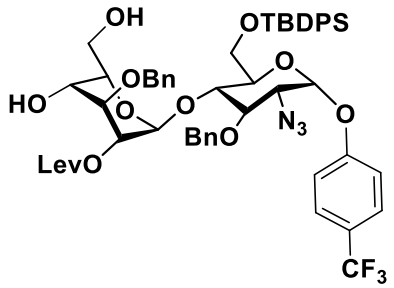

-61.79
-10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110
f1 (ppm)

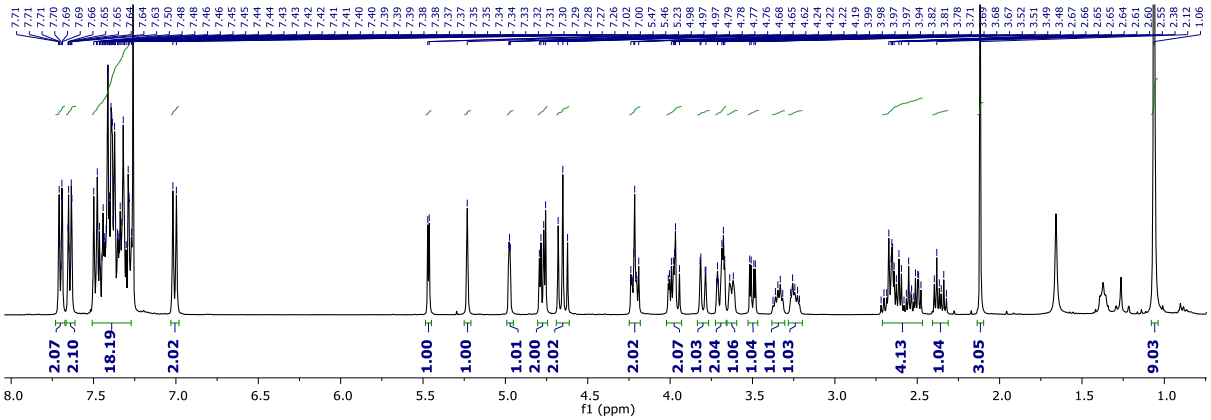

158.65
137.98
135.68
135.55
132.95
132.87
129.93
129.92
128.71
128.21
128.19
128.18
127.76
127.10
127.06
127.03
126.99
116.72
96.71
79.66
77.36
77.24
76.73
75.35
72.16
72.05
63.74
62.55
26.83
26.76
19.24
f1 (ppm)

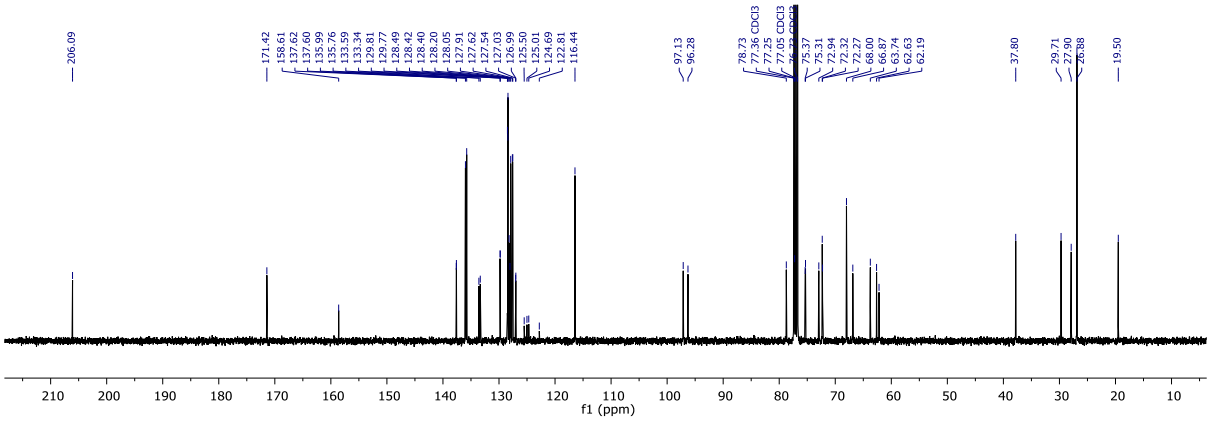
f1 (ppm)

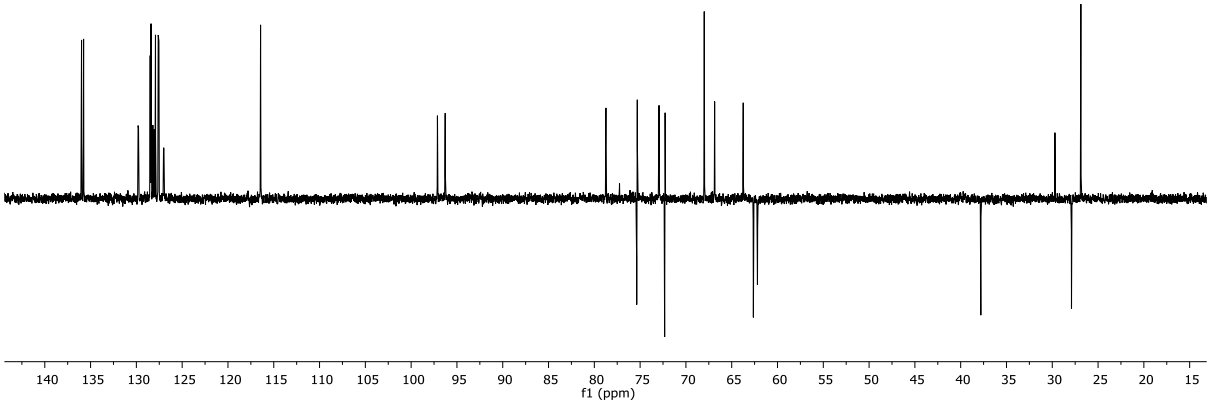

OTBDPS
OBn
Ph
LevO
BnO
N3
CF3
2.24
2.24
5.62
20.44
2.13
1.00
1.00
1.01
1.03
1.09
2.19
1.09
1.05
2.32
1.01
3.25
1.16
1.09
1.07
1.00
2.13
1.85
3.16
9.15
f1 (ppm)

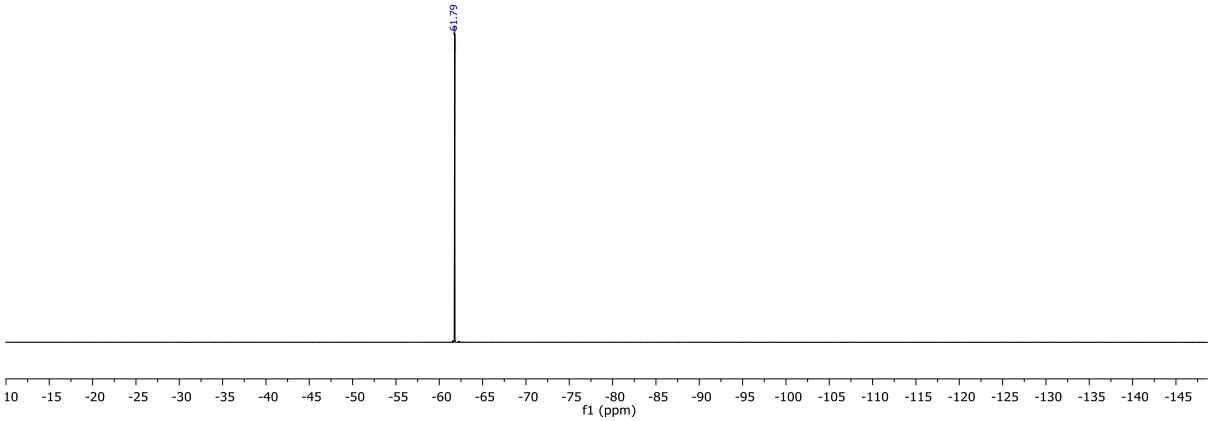

206.16
171.78
158.71
138.35
138.08
137.72
135.98
133.73
133.56
133.34
129.78
129.71
129.02
128.42
128.35
128.23
128.15
128.04
127.72
127.69
127.57
127.35
127.06
127.02
126.99
126.37
125.53
125.02
124.69
122.84
116.55
100.63
97.42
96.44
78.61
77.38 CDCl3
77.27
77.06 CDCl3
76.74 CDCl3
74.87
74.03
73.14
72.57
72.12
69.01
66.97
63.61
62.35
60.04
37.71
29.55
28.07
26.87
19.50
f1 (ppm)
f1 (ppm)
-61.77
f1 (ppm)

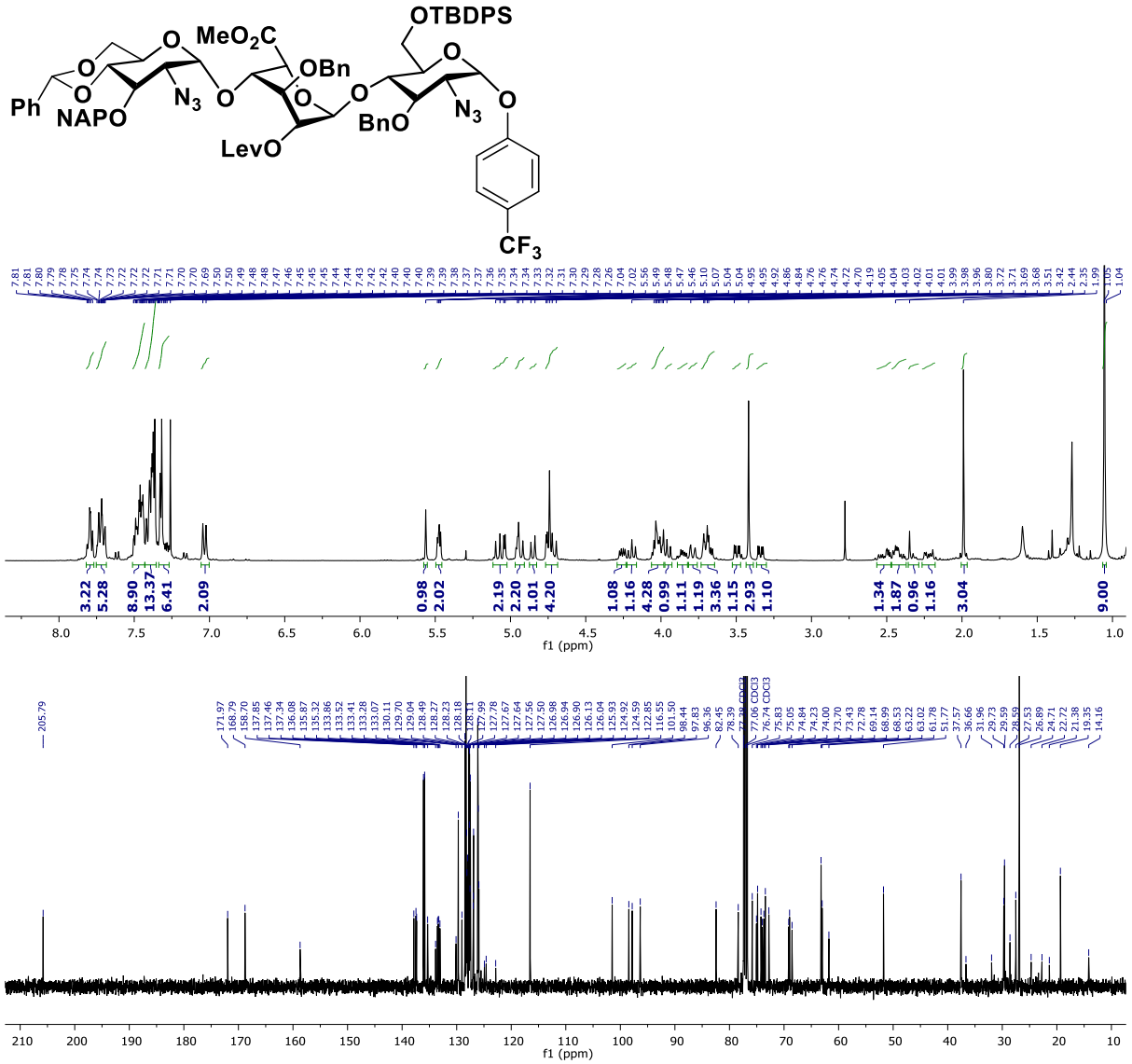

OH
OTBDPS
OBn
HO
O
O
O
O
BnO
N3
LevO
CF3

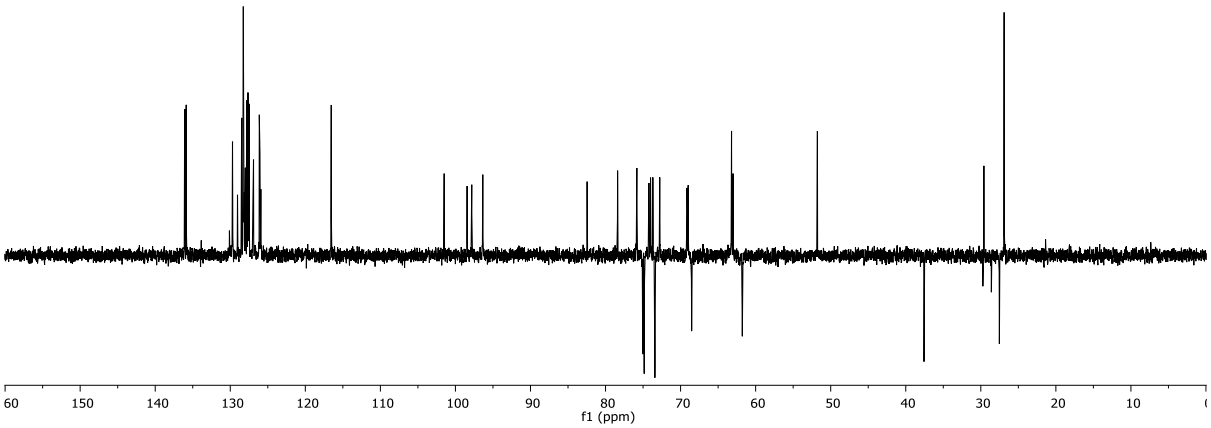

2.07
2.10
18.19
2.02
1.00
1.00
1.01
2.00
2.02
2.02
2.07
1.03
2.04
1.06
1.04
1.01
1.03
4.13
1.04
3.05
9.03
f1 (ppm)

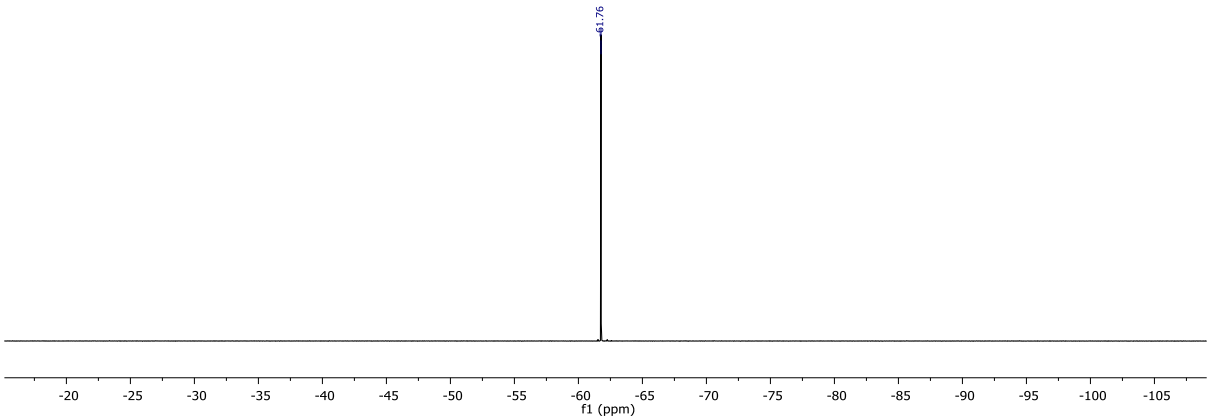

f1 (ppm)

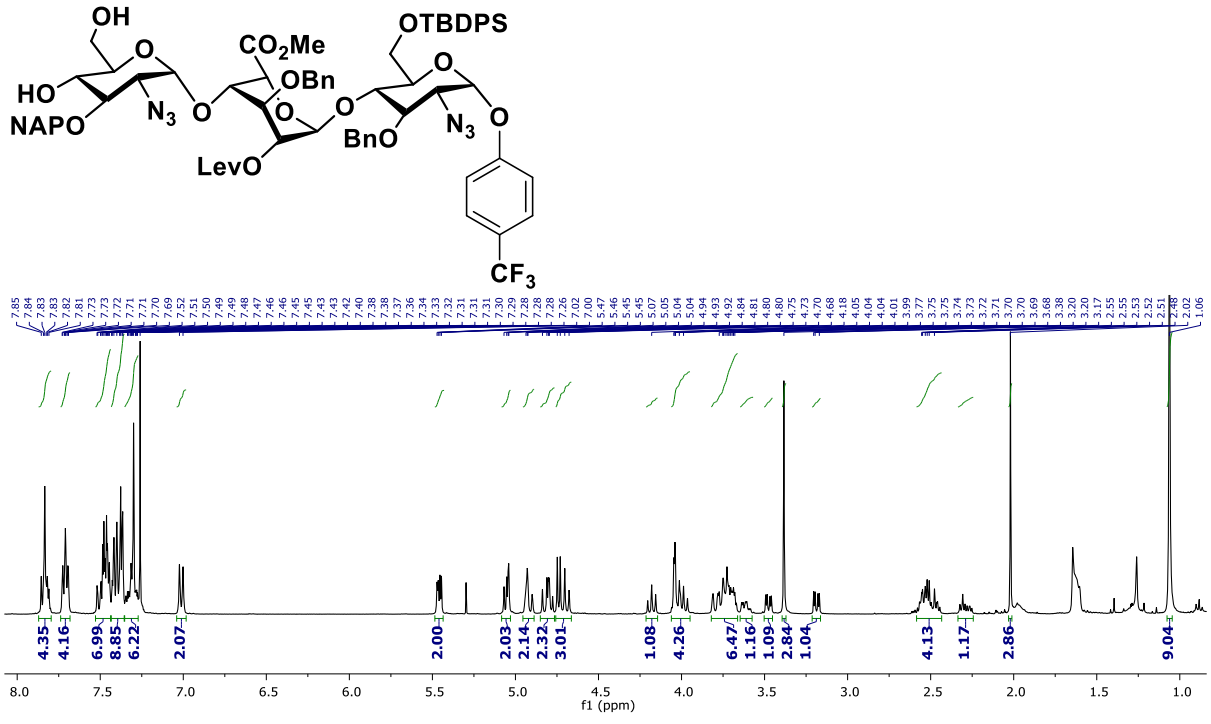

f1 (ppm)

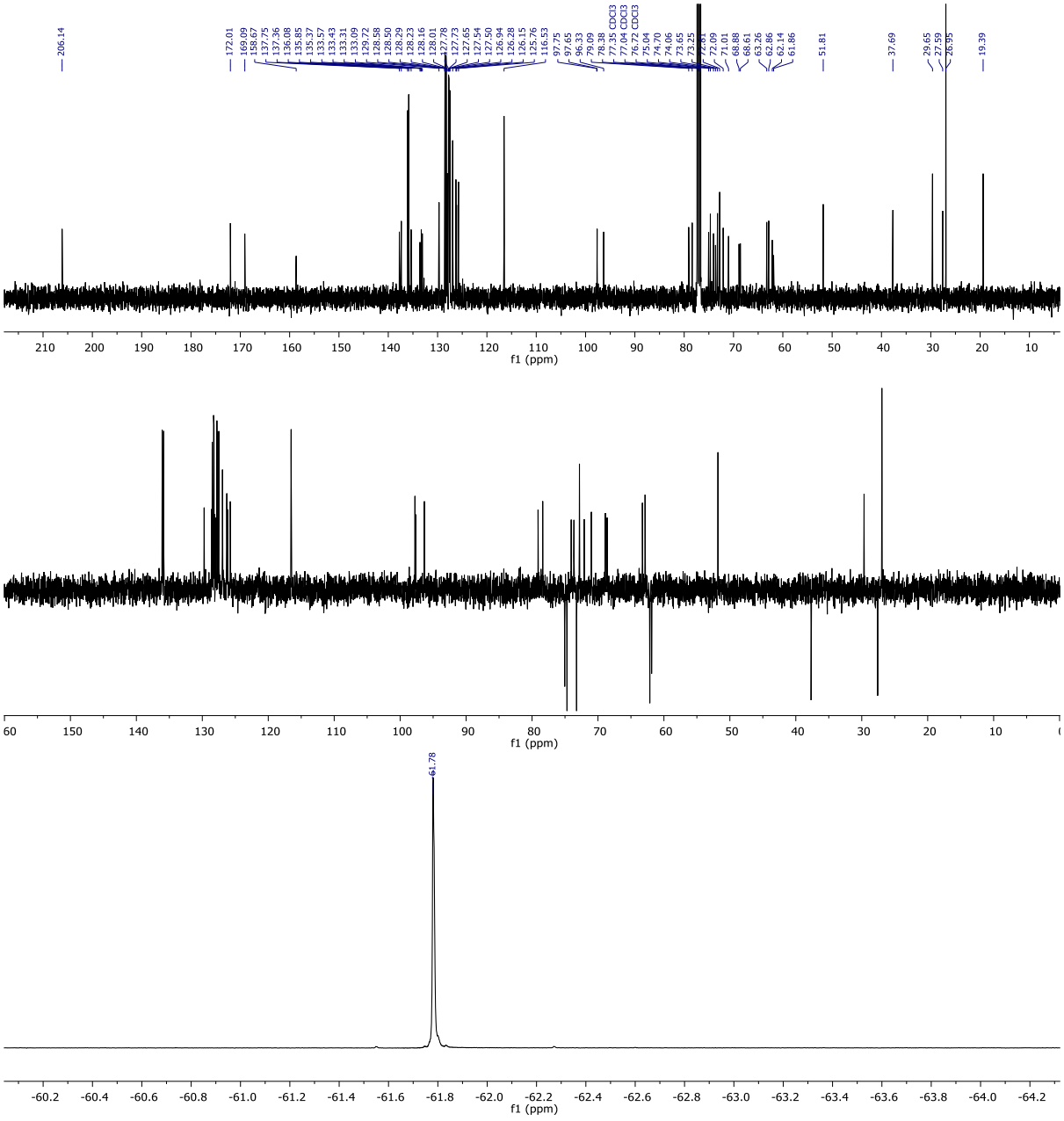

-61.79
f1 (ppm)

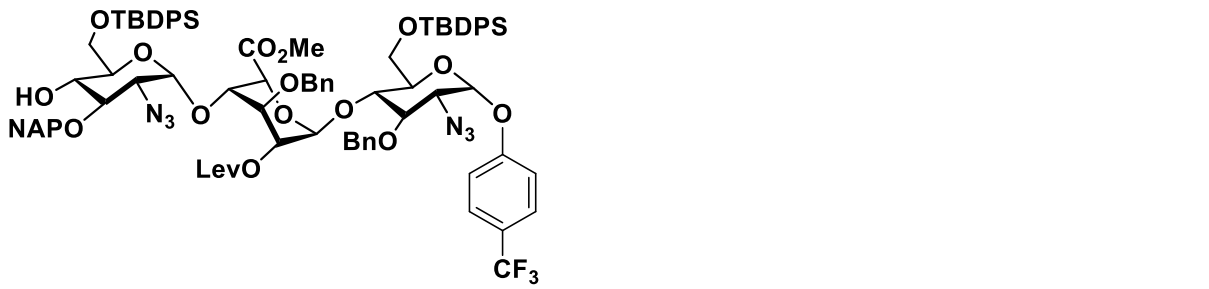

OTBDPS
MeO2C
OBn
Ph
NAPO
N3
LevO
BnO
N3
CF3
3.22
5.28
8.90
13.37
6.41
2.09
0.98
2.02
2.19
2.20
1.01
4.20
1.08
1.16
4.28
0.99
1.11
1.19
3.36
1.15
2.93
1.10
1.34
1.87
0.96
1.16
3.04
9.00
f1 (ppm)

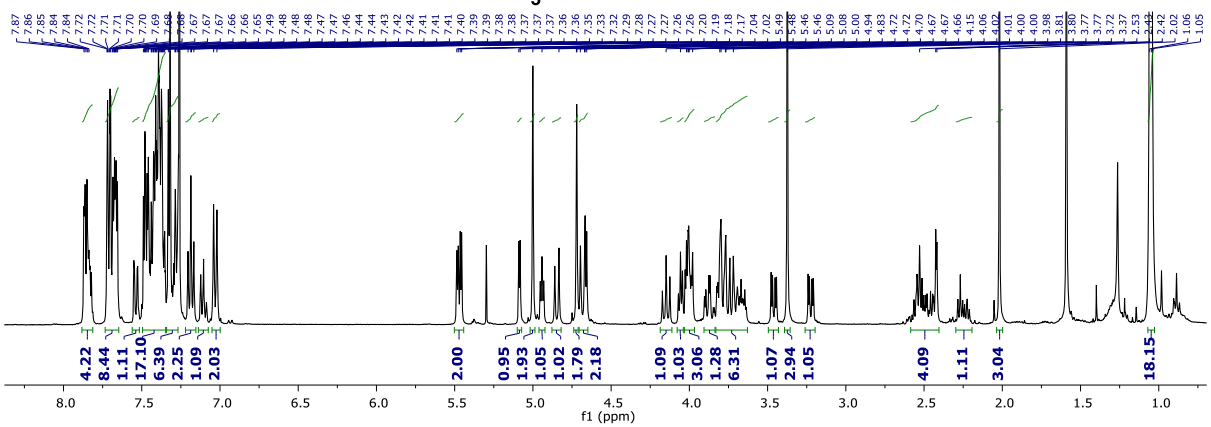

f1 (ppm)

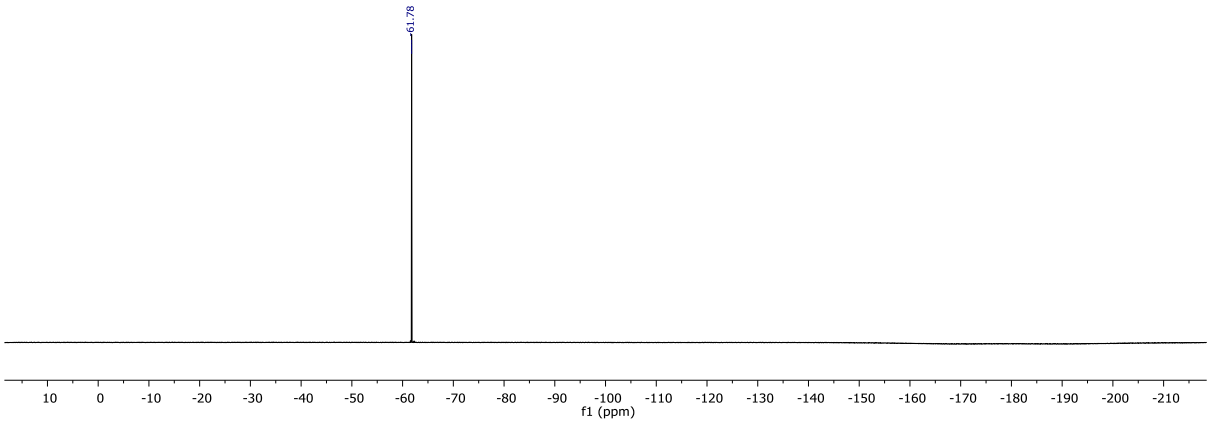

61.76
f1 (ppm)

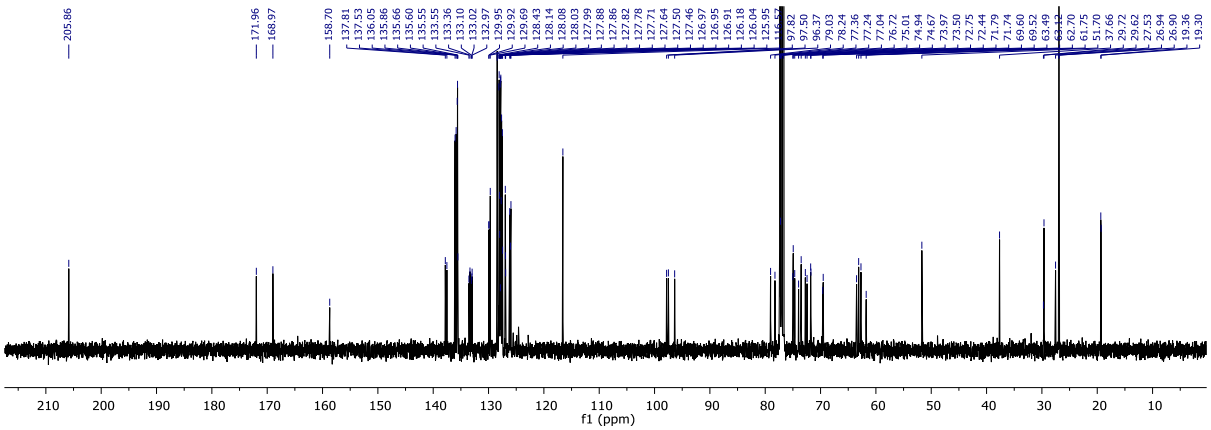

OH
HO
NAPO
O
N3
CO2Me
OBn
LevO
OTBDPS
BnO
CF3
4.35
4.16
6.99
8.85
6.22
2.07
2.00
2.03
2.14
2.32
3.01
1.08
4.26
6.47
1.16
1.09
2.84
1.04
4.13
1.17
2.86
9.04
f1 (ppm)

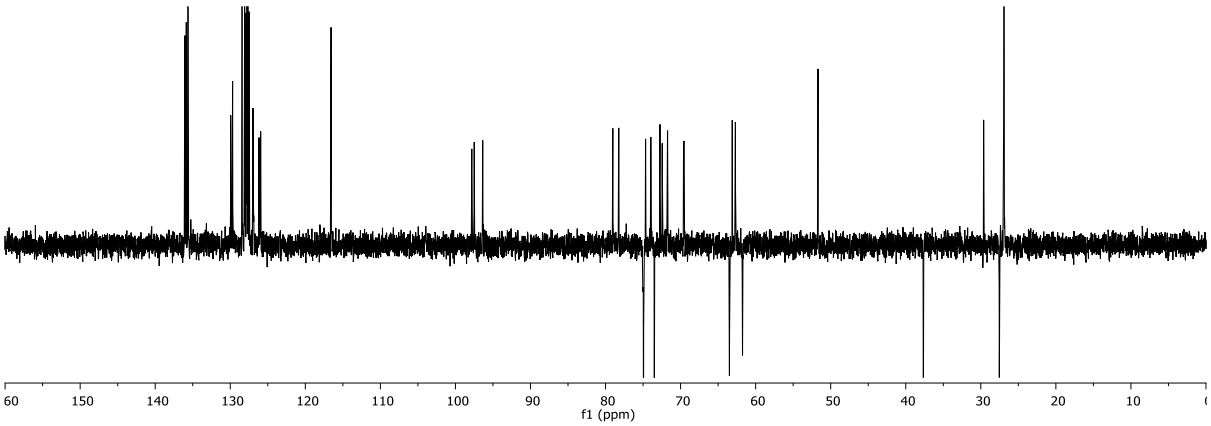

206.14
172.01
169.09
159.67
137.75
137.36
136.08
135.85
135.37
133.57
133.43
133.31
133.09
129.72
128.58
128.50
128.29
128.23
128.16
128.01
127.78
127.73
127.65
127.54
127.50
126.94
126.28
126.15
125.76
116.53
97.75
97.65
96.33
79.09
78.38
77.35 CDCl3
77.04 CDCl3
76.72 CDCl3
75.94
74.70
74.06
73.65
73.25
72.81
72.09
71.01
68.88
68.61
63.26
62.86
62.14
61.86
51.81
37.69
29.65
27.59
26.95
19.39
f1 (ppm)
f1 (ppm)
61.78
f1 (ppm)

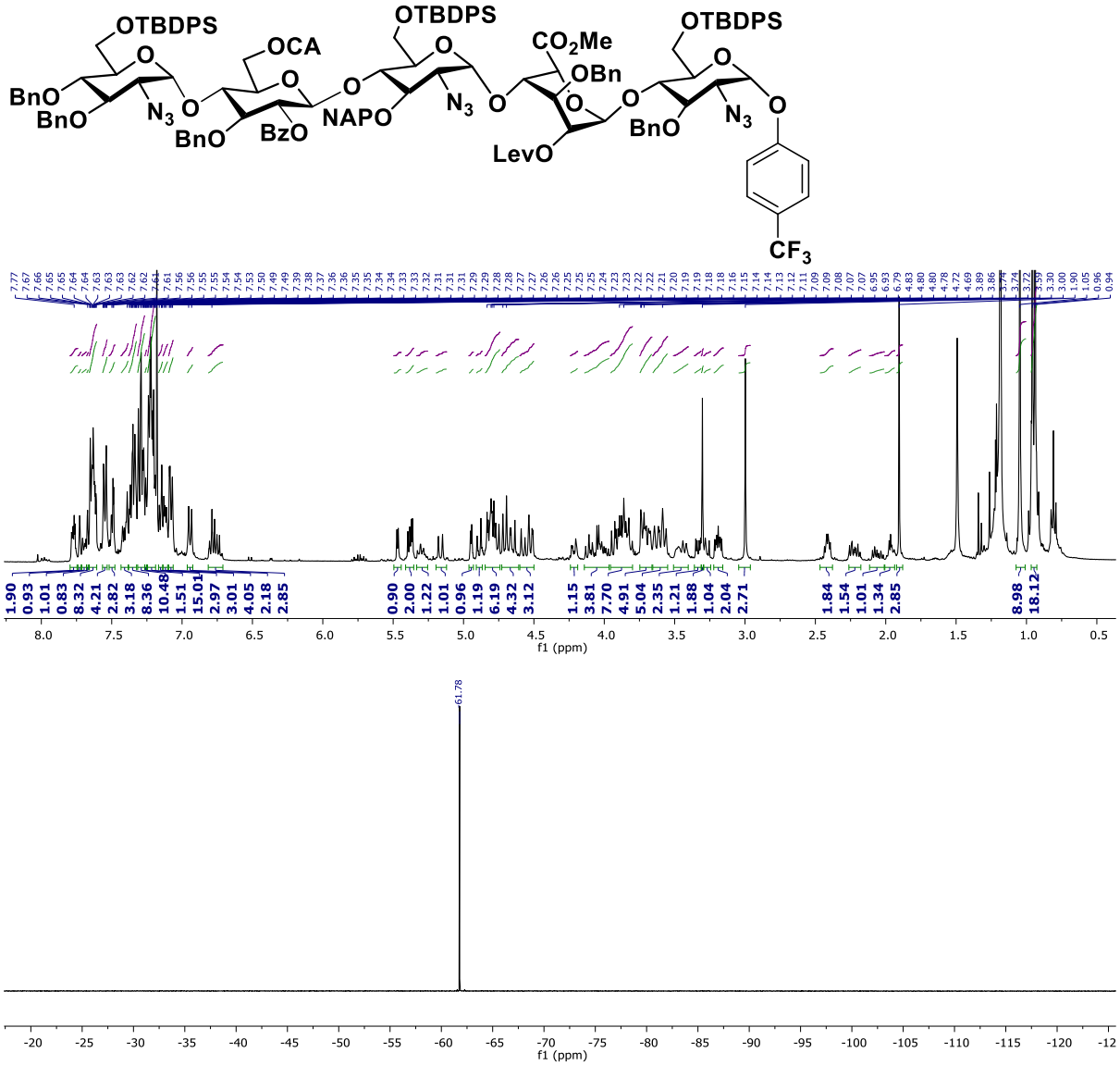

OTBDPS
HO
NAPO
N3
O
CO2Me
OBn
LevO
OTBDPS
BnO
N3
CF3


4.22
8.44
1.11
17.10
6.39
2.25
1.09
2.03
2.00
0.95
1.93
1.05
1.02
1.79
2.18
1.09
1.03
3.06
1.28
6.31
1.07
2.94
1.05
4.09
1.11
3.04
18.15
f1 (ppm)

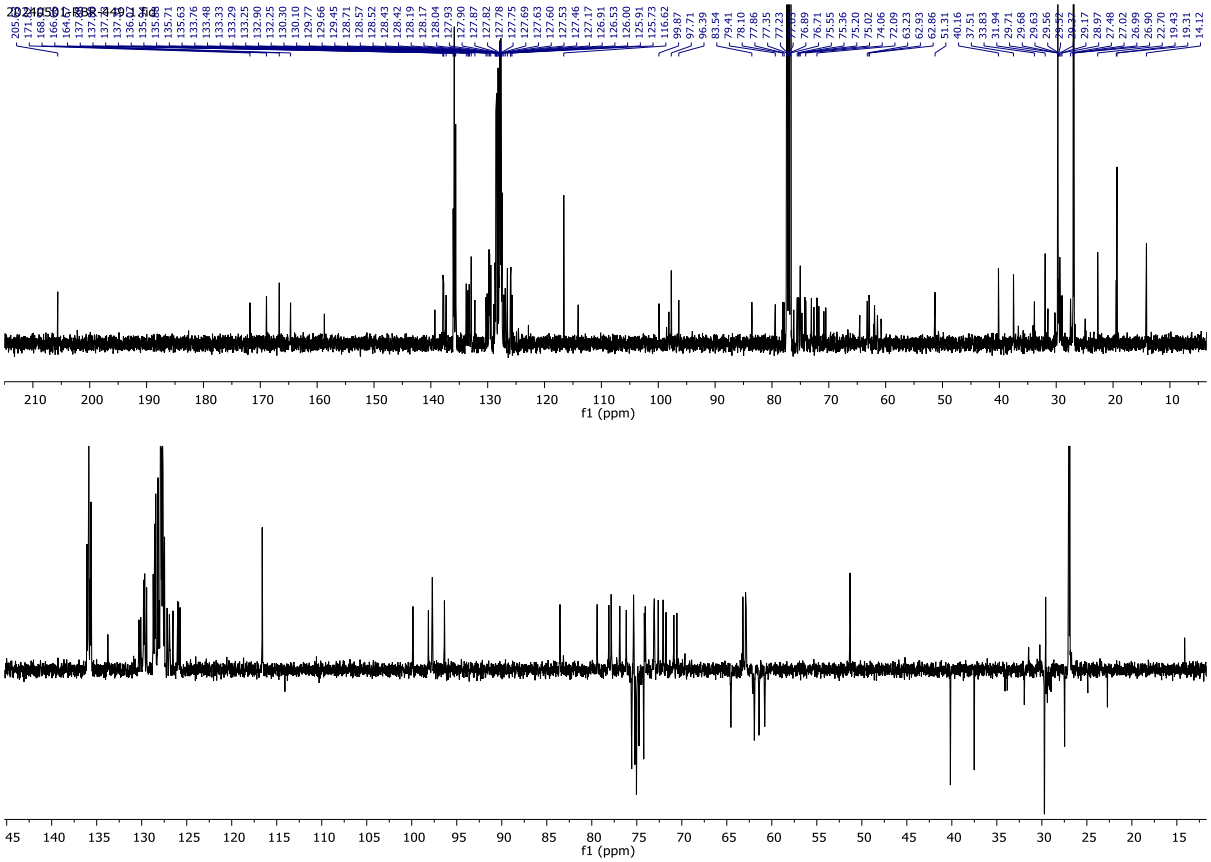

61.78
f1 (ppm)

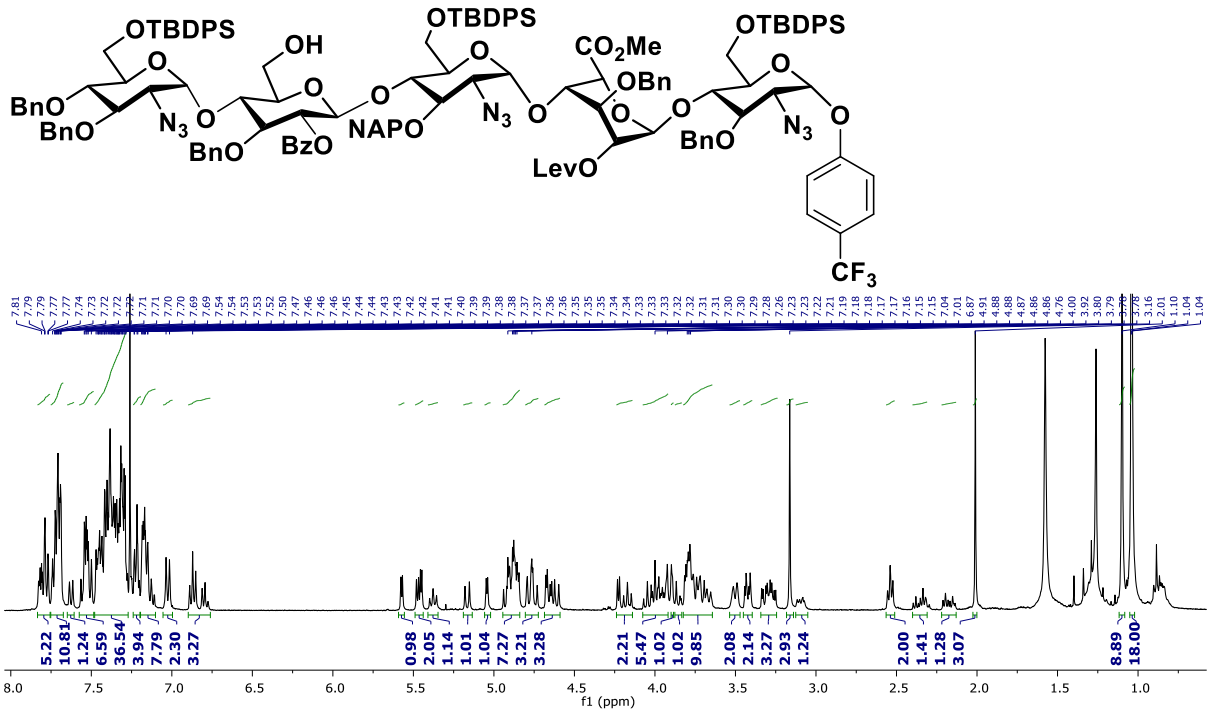

205.86
171.96
168.97
158.70
f1 (ppm)

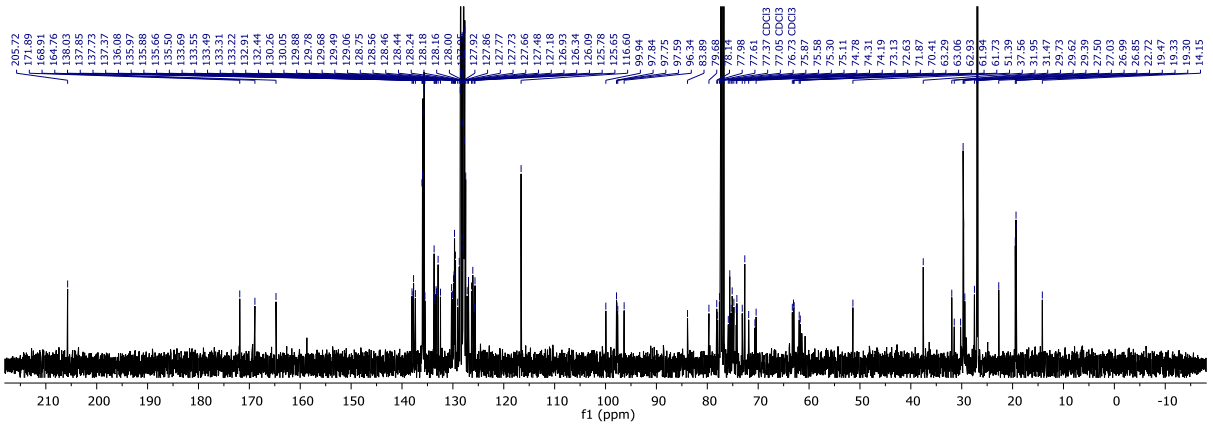

60 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

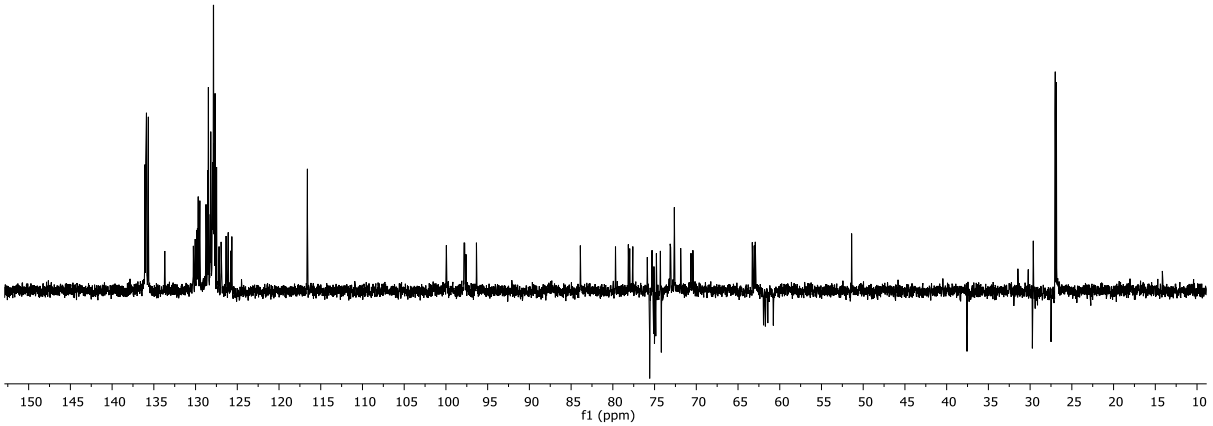

OTBDPS
OCA
OTBDPS
CO2Me
OTBDPS
BnO
BnO
N3
BnO
BzO
NAPO
N3
OBn
LevO
BnO
N3
CF3
1.90 0.93 1.01 0.83 8.32 4.21 2.82 3.18 8.36 10.48 1.51 15.01 2.97 3.01 4.05 2.18 2.85
0.90 2.00 1.22 1.01 0.96 1.19 6.19 4.32 3.12
1.15 3.81 7.70 4.91 5.04 2.35 1.21 1.88 1.04 2.04 2.71
1.84 1.54 1.01 1.34 2.85
8.98 18.12
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

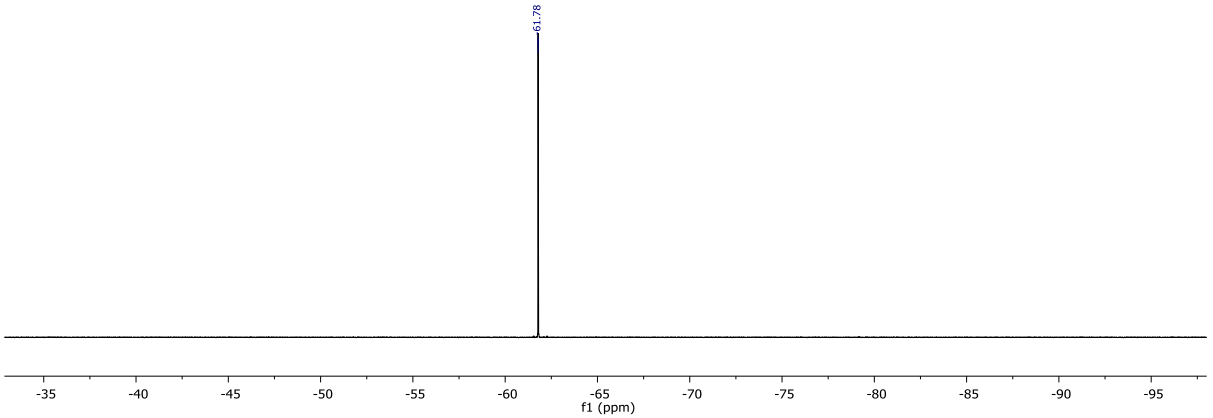
61.78
-20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -12
f1 (ppm)

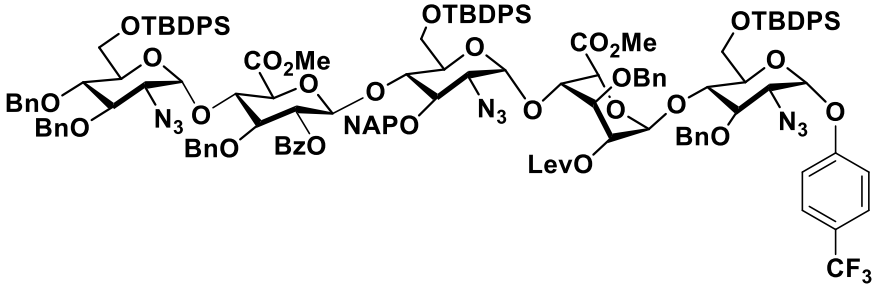

f1 (ppm)
f1 (ppm)

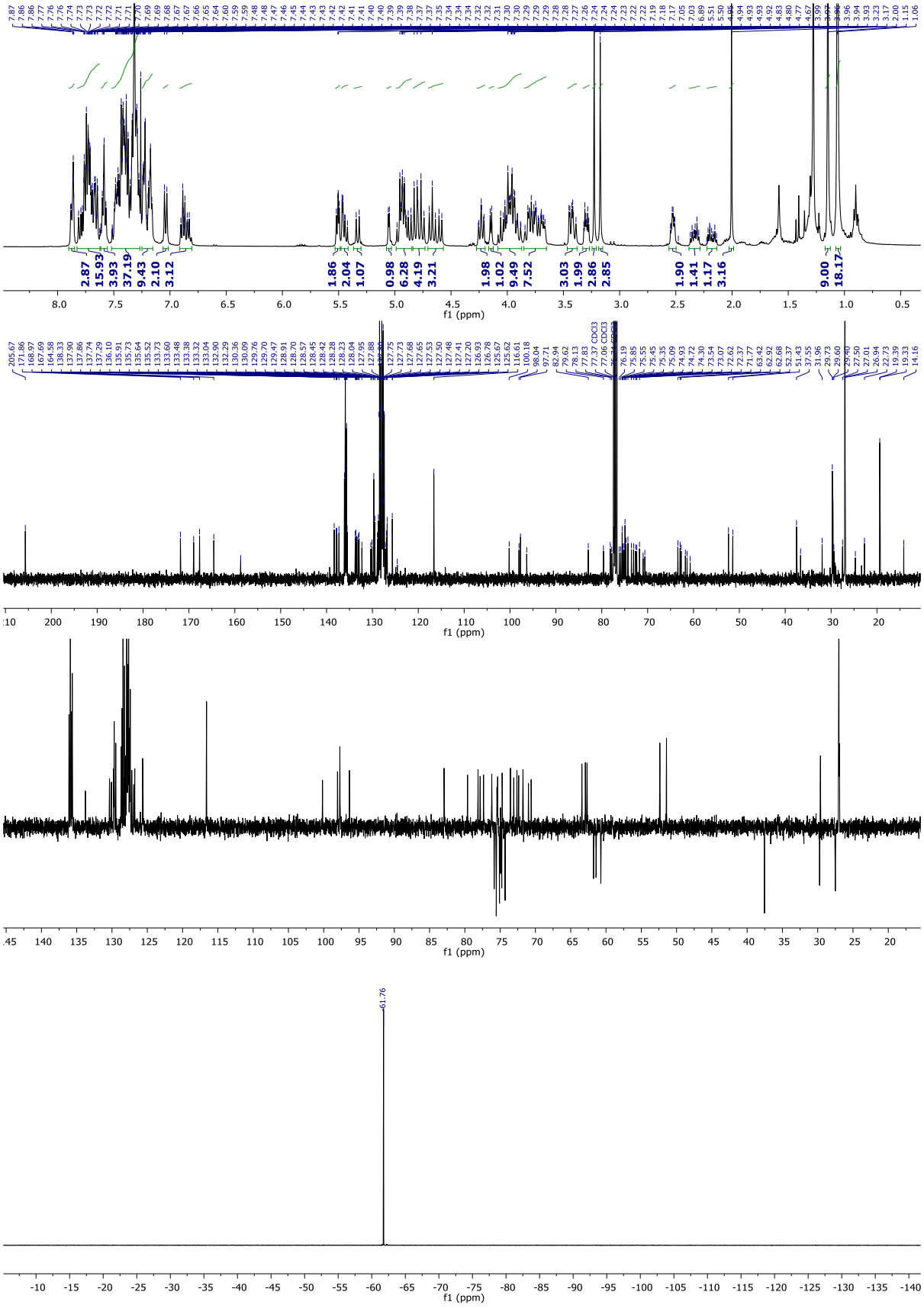

OTBDPS
OH
OTBDPS
CO2Me
OTBDPS
BnO
BnO
N3
BnO
BzO
NAPO
N3
OBn
LevO
BnO
N3
CF3
5.22
10.81
1.24
6.59
36.54
3.94
7.79
2.30
3.27
0.98
2.05
1.14
1.01
1.04
7.27
3.21
3.28
2.21
5.47
1.02
1.02
9.85
2.08
2.14
3.27
2.93
1.24
2.00
1.41
1.28
3.07
8.89
18.00
f1 (ppm)

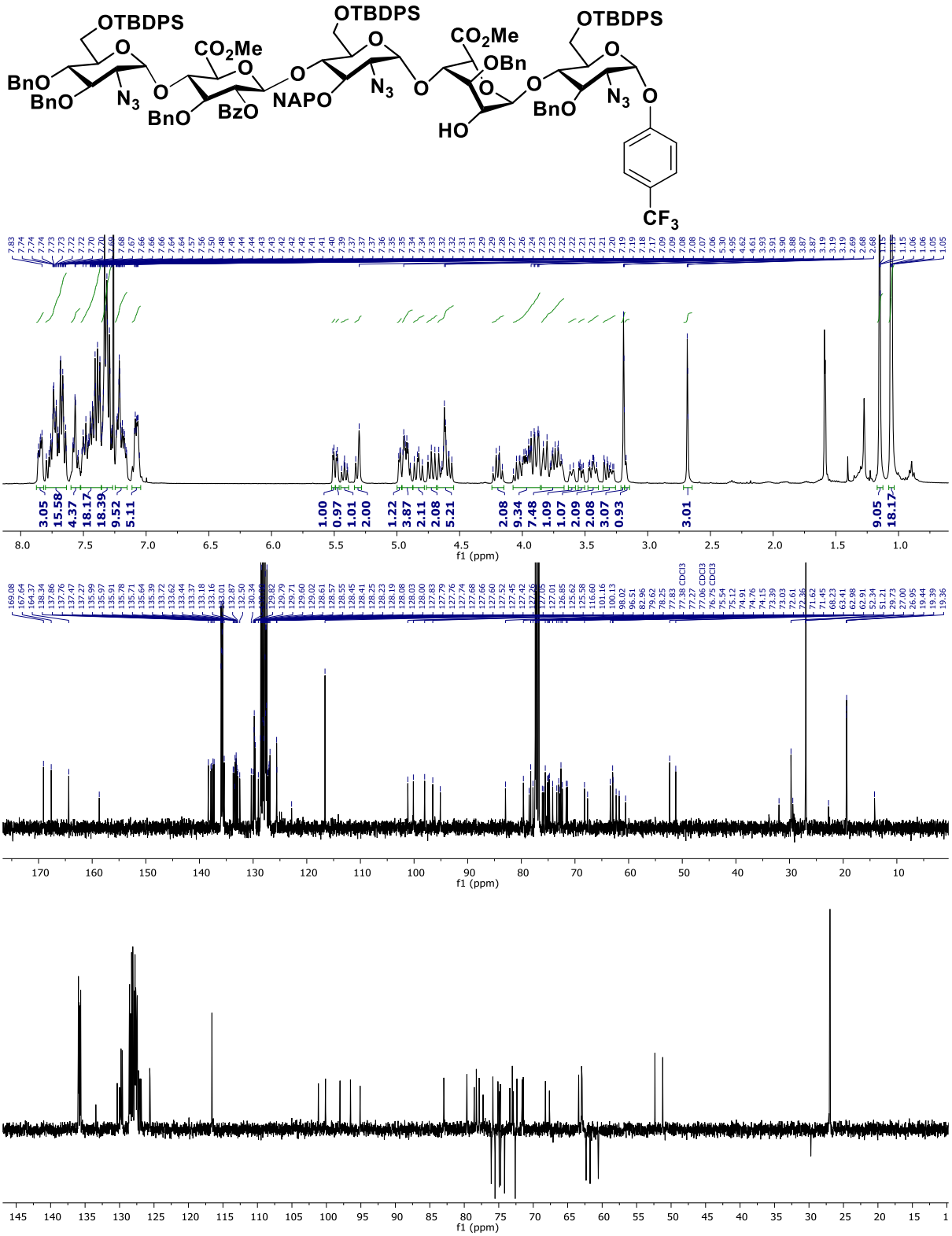

205.72
171.89
168.91
164.76
138.03
137.85
137.73
137.37
136.08
135.97
135.88
135.66
135.50
133.69
133.55
133.49
133.31
133.22
132.91
132.44
130.26
130.05
129.88
129.78
129.68
129.49
129.06
128.75
128.56
128.46
128.44
128.24
128.18
128.16
128.00
127.92
127.86
127.77
127.73
127.66
127.48
127.18
126.93
126.34
126.09
125.78
125.65
116.60
99.94
97.84
97.75
97.59
96.34
83.89
79.68
78.14
77.98
77.61
77.37 CDCl3
77.05 CDCl3
76.73 CDCl3
75.87
75.58
75.30
75.11
74.78
74.31
74.19
73.13
72.63
71.87
71.44
63.29
63.06
62.93
61.94
61.73
51.39
37.56
31.95
31.47
29.73
29.62
29.39
27.50
27.03
26.99
26.85
22.72
19.47
19.33
19.30
14.15
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

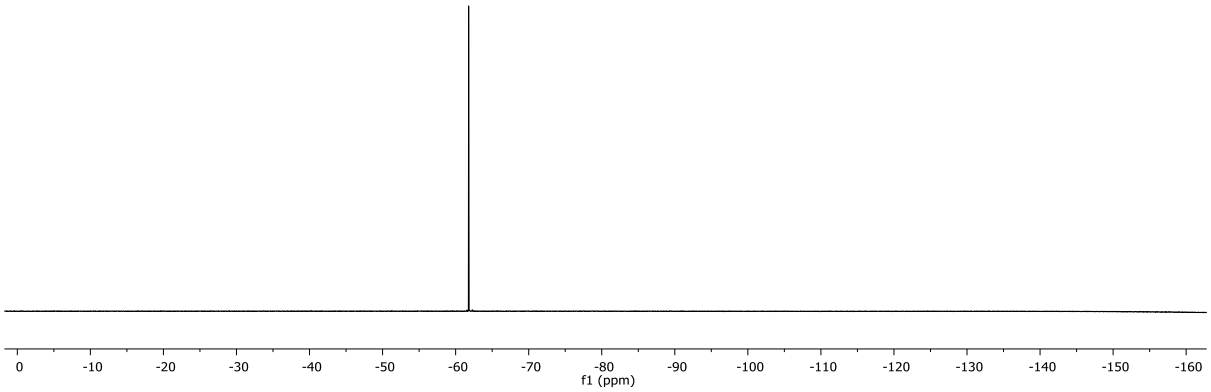

150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)

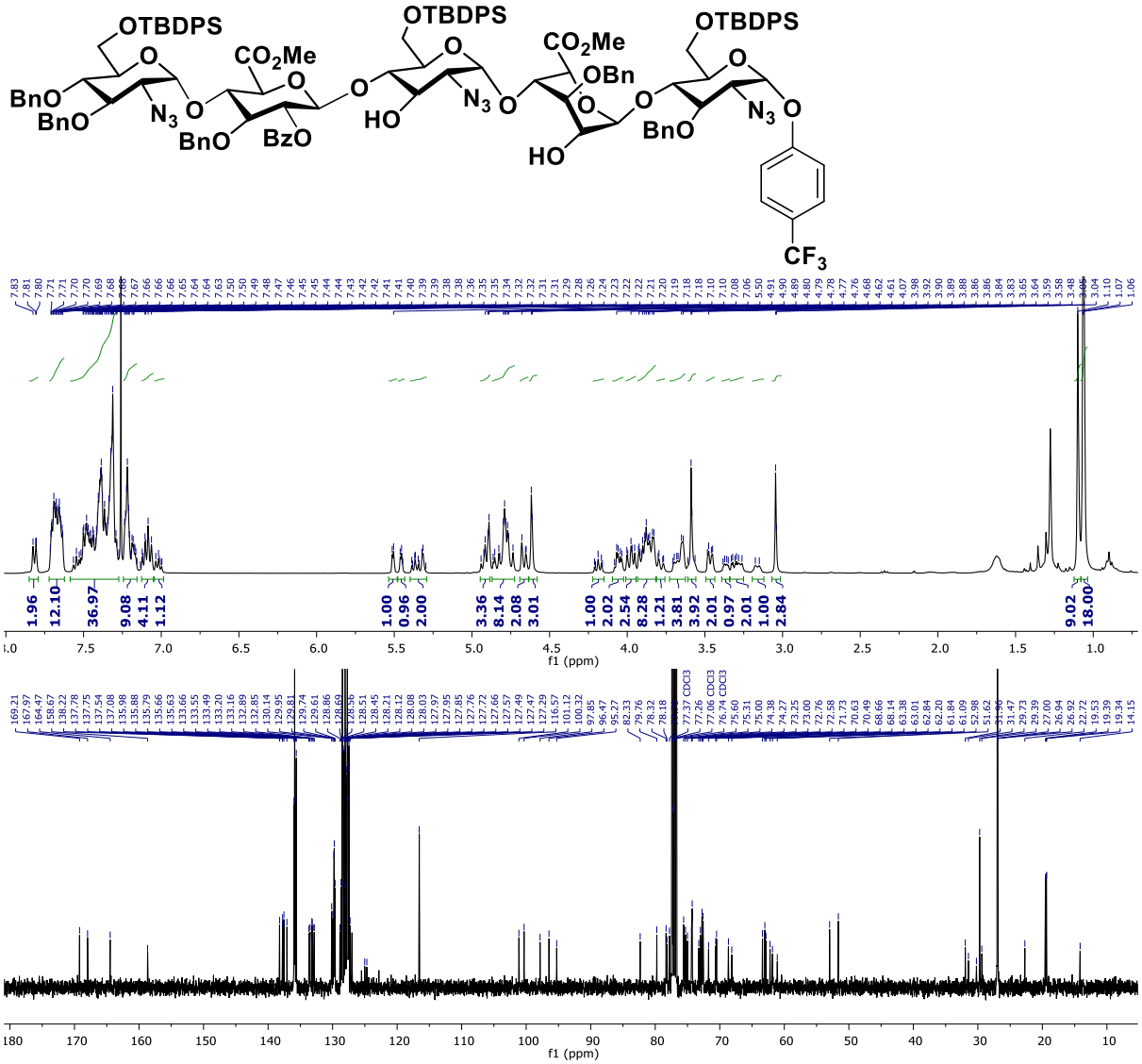

61.78
-35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95
f1 (ppm)

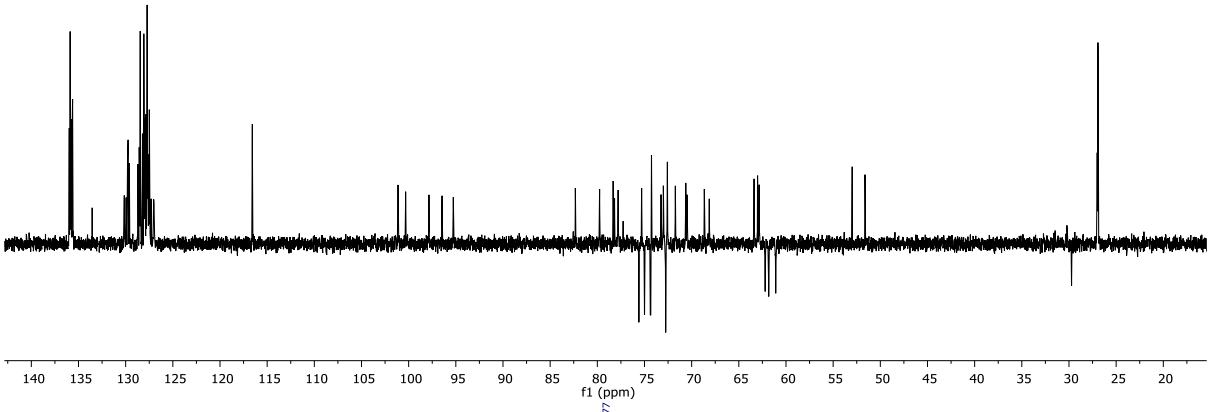

OTBDPS
BnO
BnO
N3
CO2Me
BnO
BzO
NAPO
OTBDPS
N3
CO2Me
OBn
LevO
OTBDPS
BnO
N3
CF3

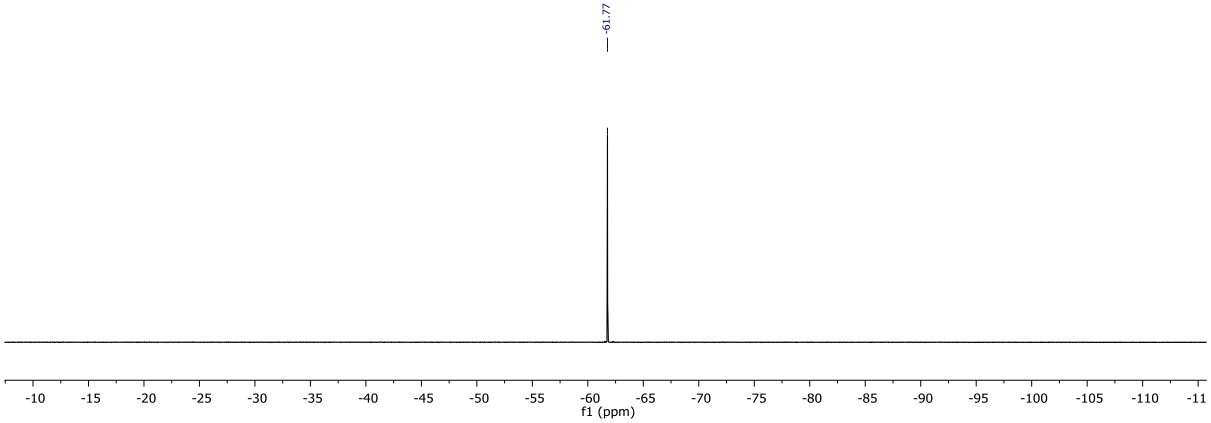


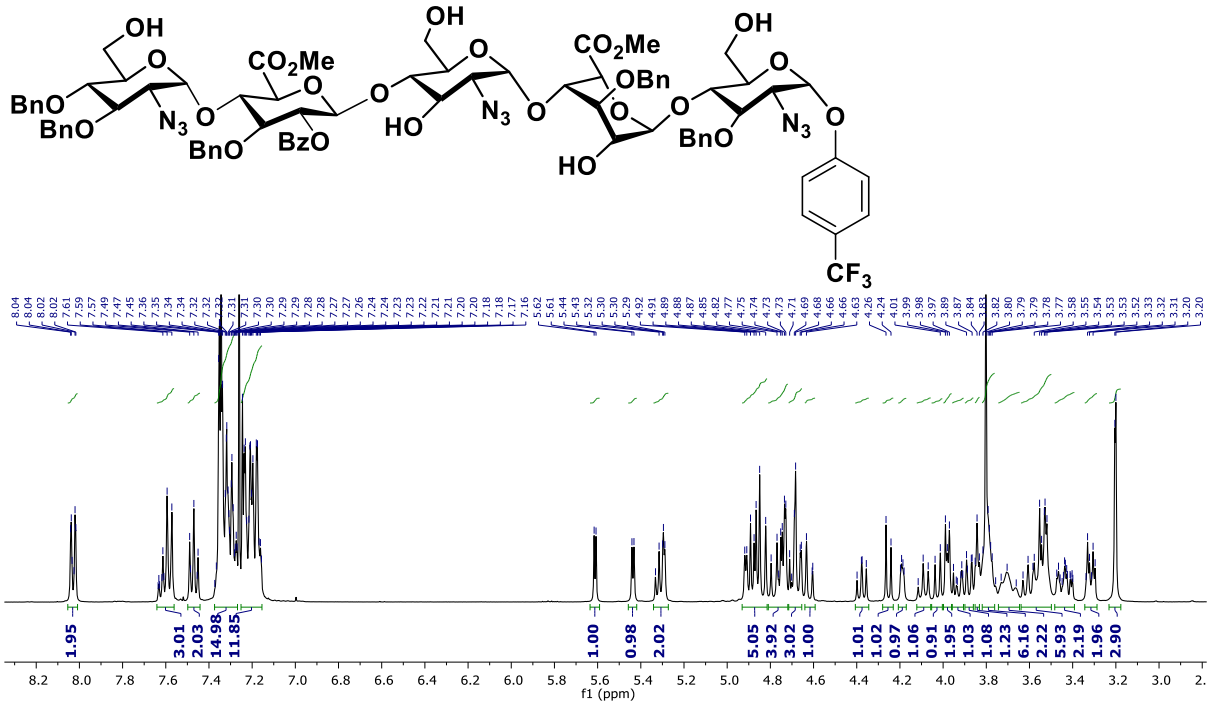

OTBDPS
BnO
BnO
N3
O
CO2Me
O
BnO
BzO
O
NAPO
OTBDPS
O
N3
O
CO2Me
OBn
O
HO
O
OTBDPS
O
BnO
N3
O
CF3
f1 (ppm)
f1 (ppm)
f1 (ppm)

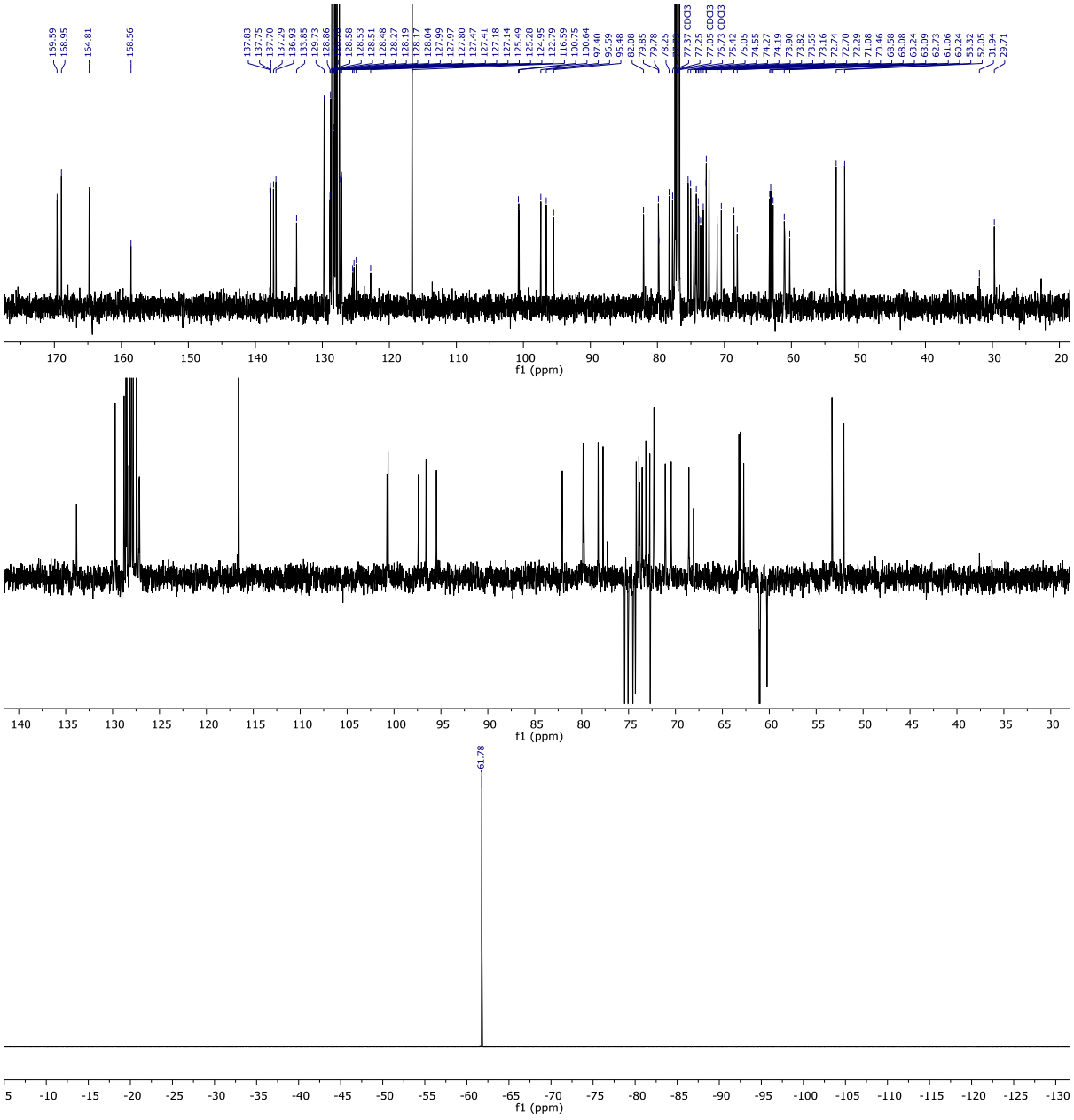

0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
f1 (ppm)

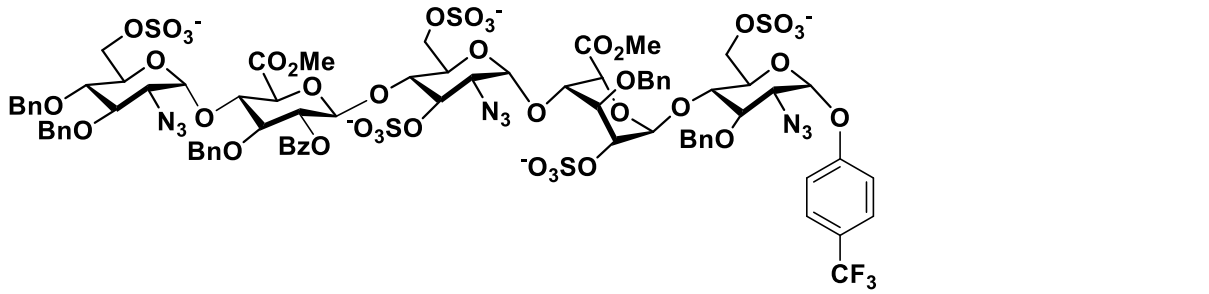

OTBDPS
CO2Me
OBn
BnO
BnO
BzO
HO
N3
CF3
1.96
12.10
36.97
9.08
4.11
1.12
1.00
0.96
2.00
3.36
8.14
2.08
3.01
1.00
2.02
2.54
8.28
1.21
3.81
3.92
2.01
0.97
2.01
1.00
2.84
9.02
18.00
f1 (ppm)

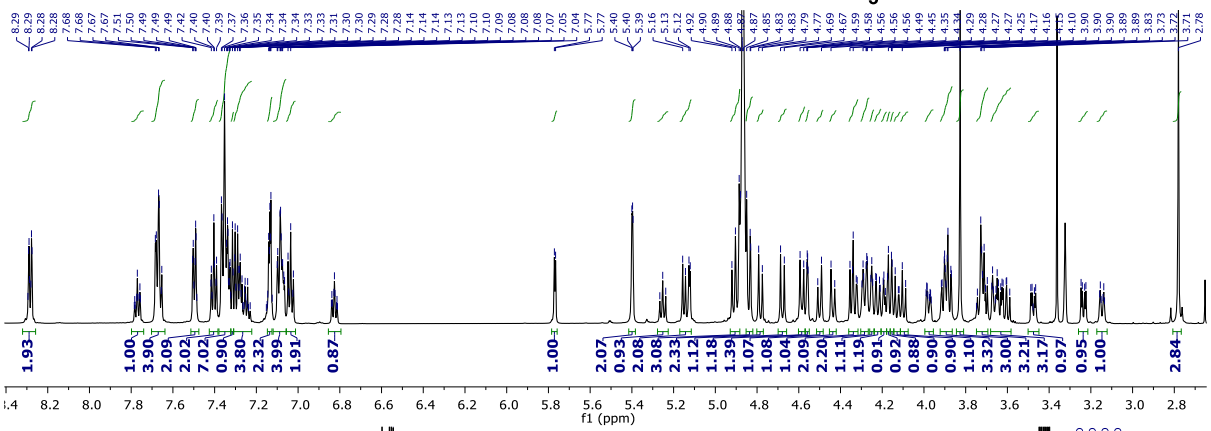

f1 (ppm)

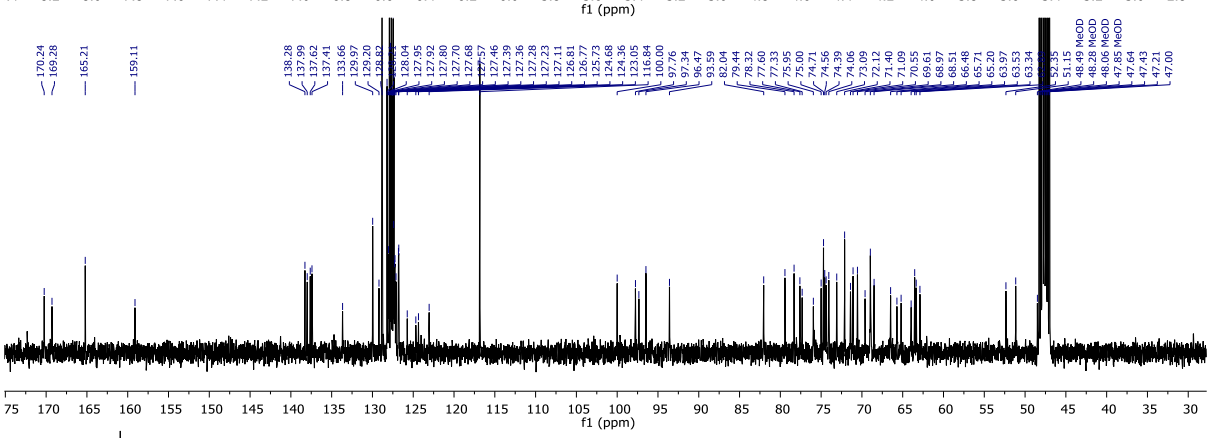

-61.77
f1 (ppm)

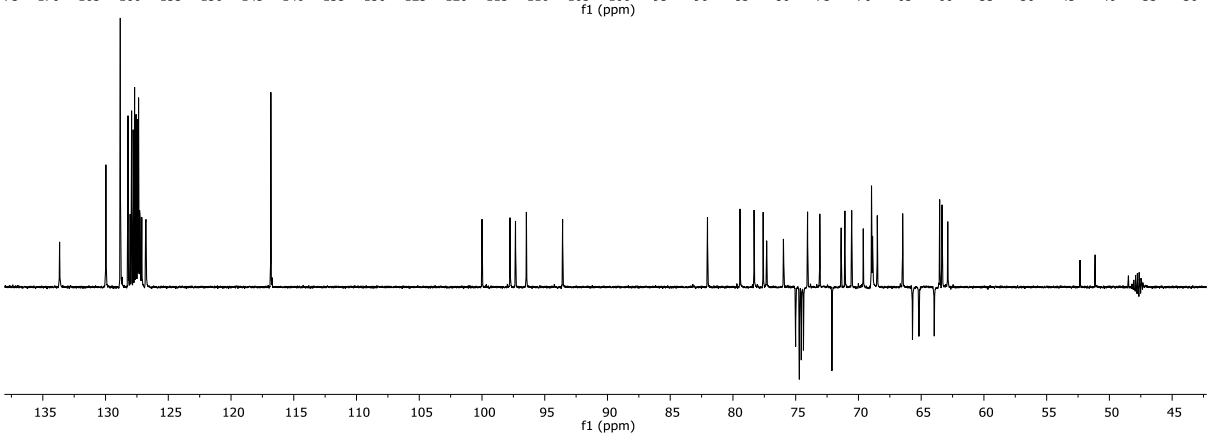

OH
BnO
BnO
N3
CO2Me
BzO
HO
OBn
CF3
1.95
3.01
2.03
14.98
11.85
1.00
0.98
2.02
5.05
3.92
3.02
1.00
1.01
1.02
0.97
1.06
0.91
1.95
1.03
1.08
1.23
6.16
2.22
5.93
2.19
1.96
2.90
f1 (ppm)

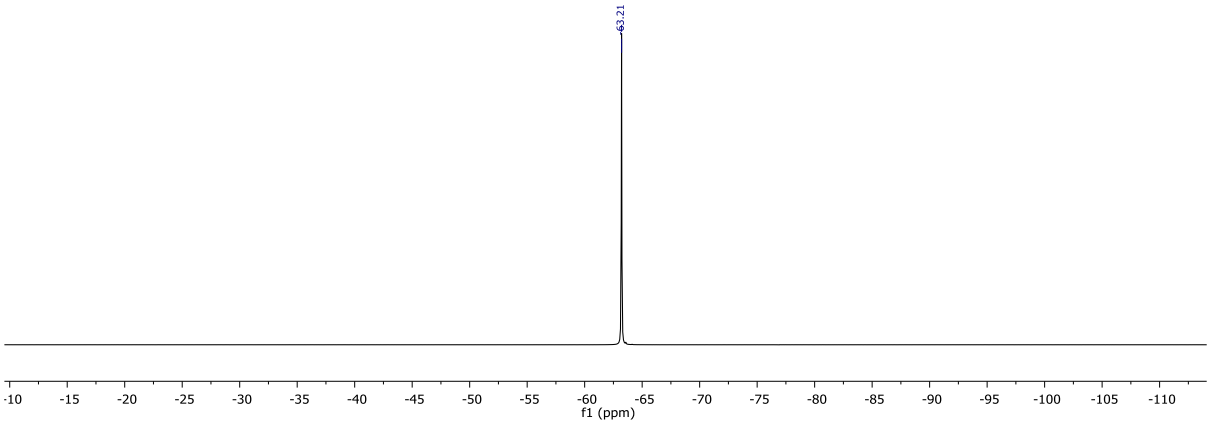

169.59
168.95
164.81
158.56
137.83
137.75
137.70
137.29
136.93
133.85
129.73
128.86
128.58
128.53
128.51
128.48
128.27
128.19
128.17
128.04
127.99
127.97
127.80
127.47
127.41
127.18
127.14
125.49
125.28
124.95
122.79
116.59
100.75
100.64
97.40
96.59
95.48
82.08
79.85
79.78
79.75
78.45
77.37 CDCl3
77.25
77.05 CDCl3
76.73 CDCl3
75.42
75.05
74.55
74.27
74.19
73.90
73.82
73.55
73.16
72.74
72.70
72.29
71.08
70.46
68.58
68.08
63.24
63.09
62.73
61.06
60.24
53.32
52.05
31.94
29.71
f1 (ppm)
61.78
f1 (ppm)

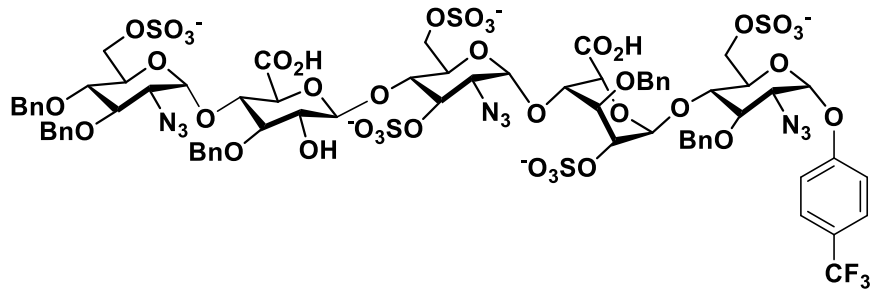

OSO3-
CO2Me
OBn
BnO
BzO
N3
O
-O3SO
CF3

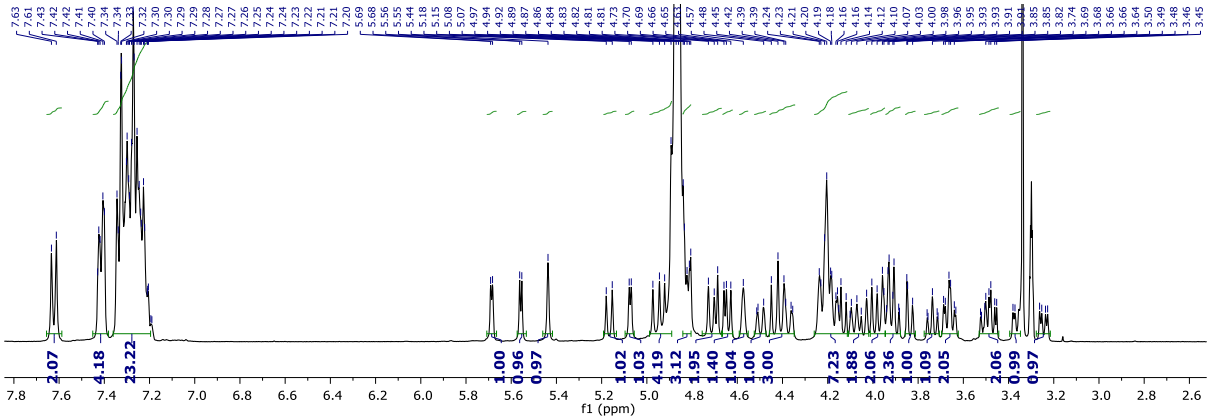


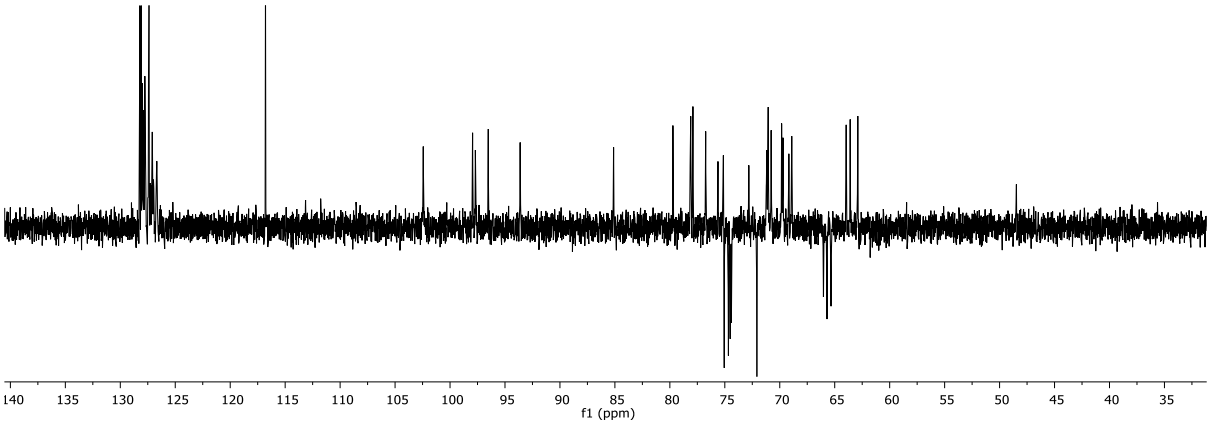


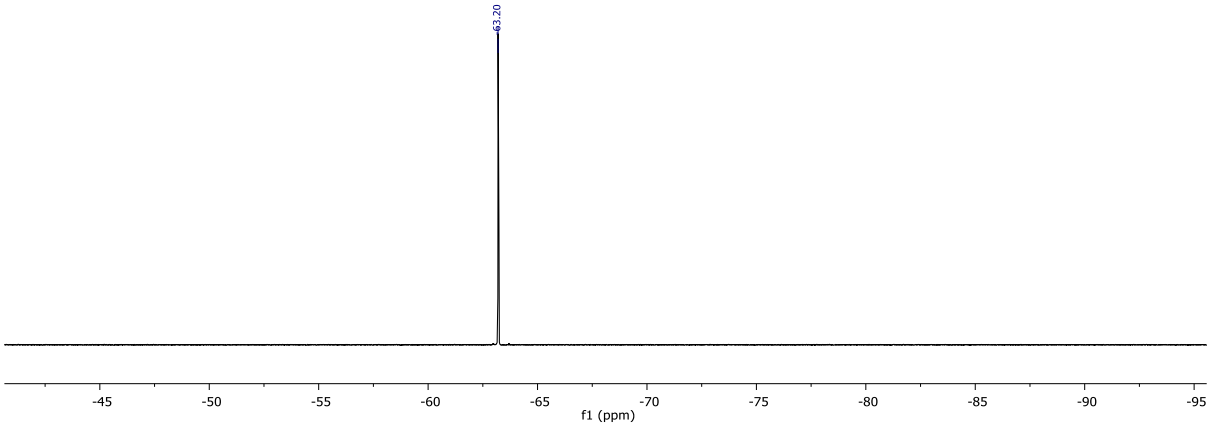


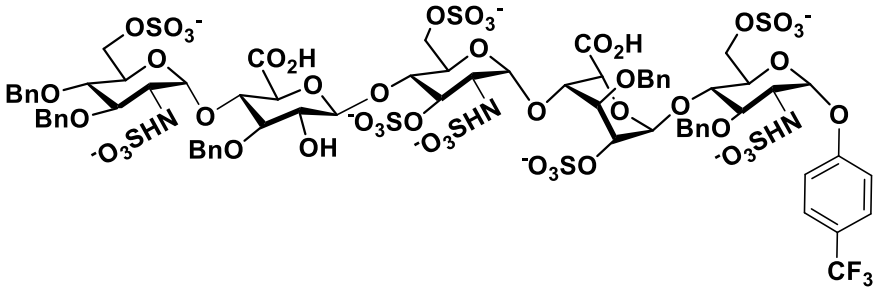

-63.21
f1 (ppm)

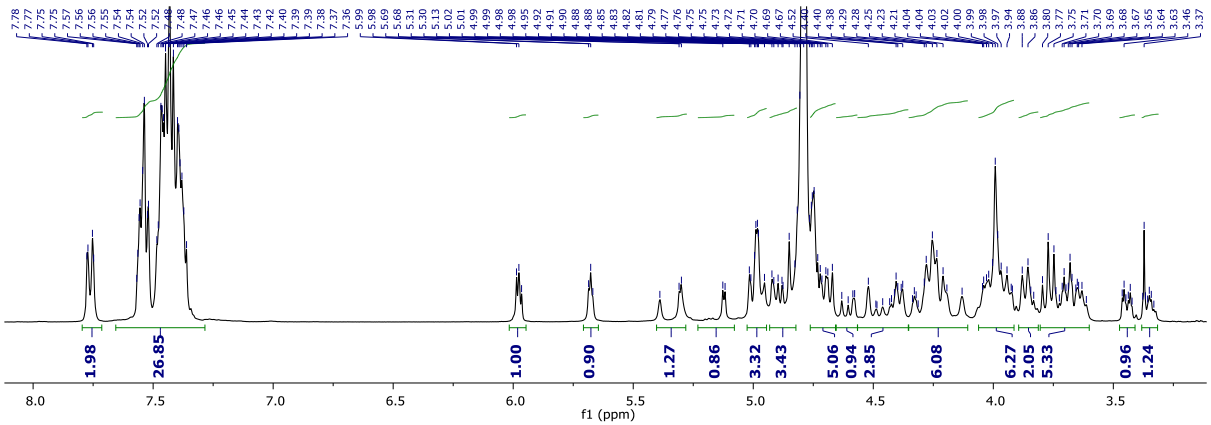

OSO3-
CO2H
OBn
BnO
N3
O
-O3SO
OH
CF3

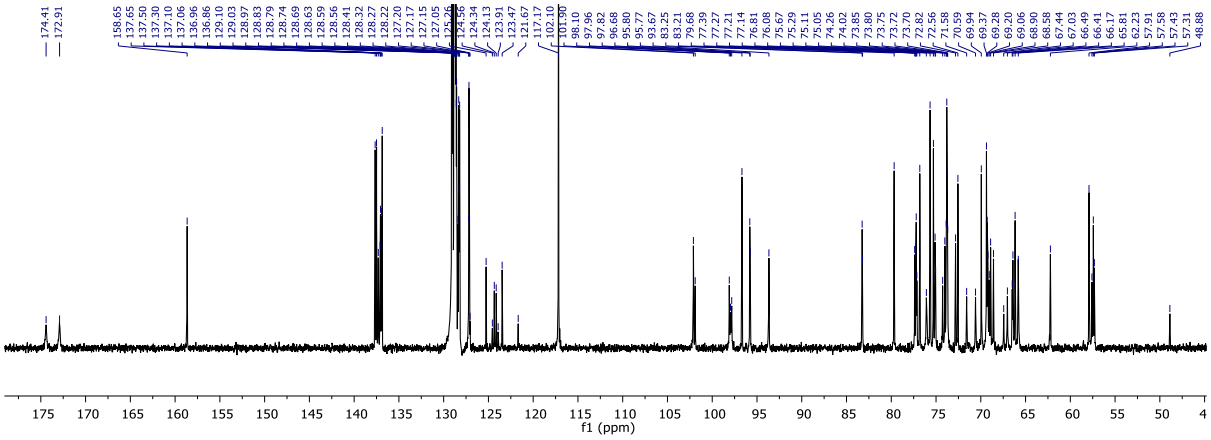

2.07
4.18
23.22
1.00
0.96
0.97
1.02
1.03
4.19
3.12
1.95
1.40
1.04
1.00
3.00
7.23
1.88
2.06
2.36
1.00
1.09
2.05
2.06
0.99
6.97
f1 (ppm)

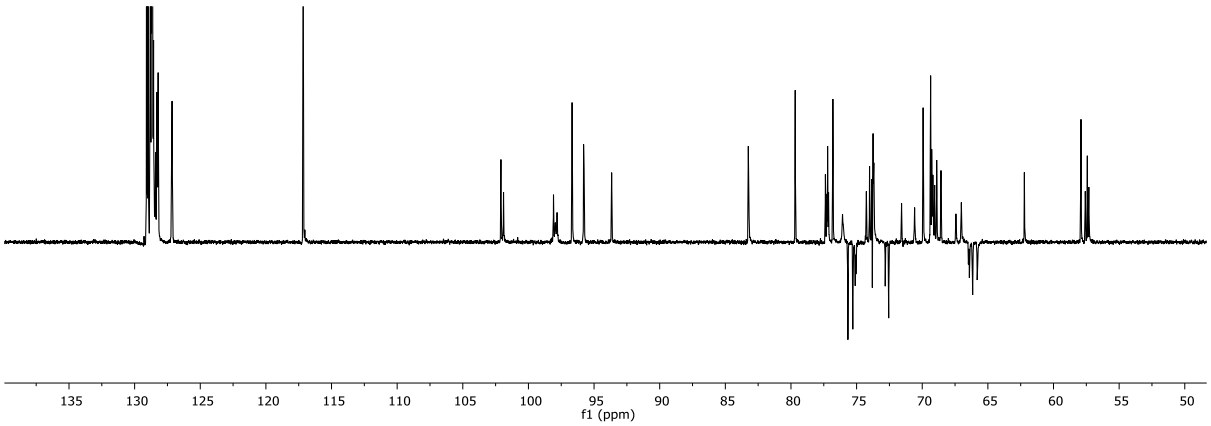

f1 (ppm)

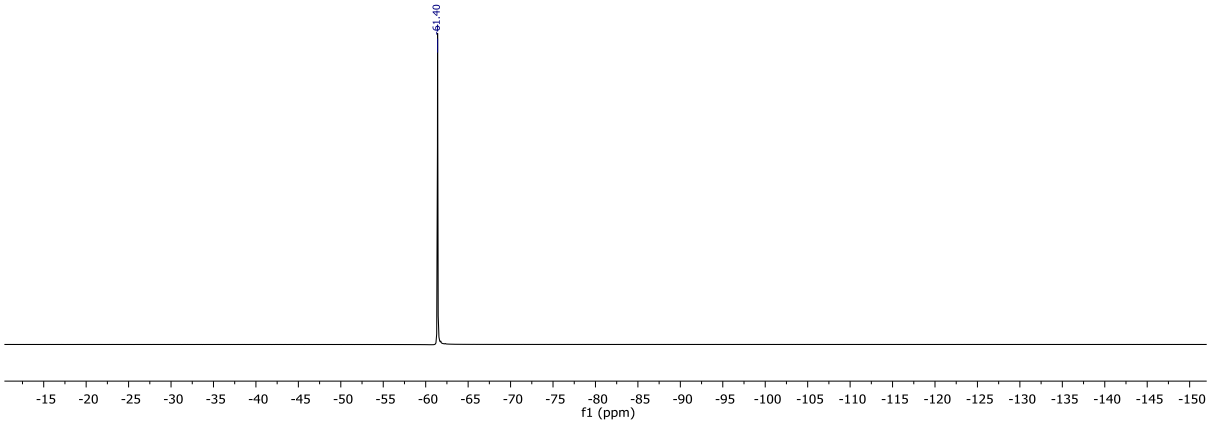

-63.20
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
f1 (ppm)

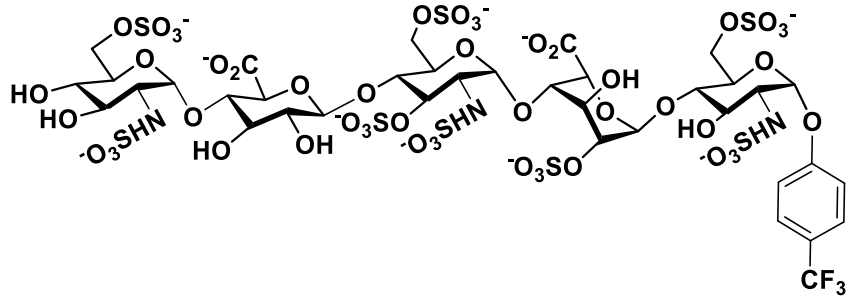

OSO3-
CO2H
OBn
BnO
-O3SHN
-O3SO
OH
CF3

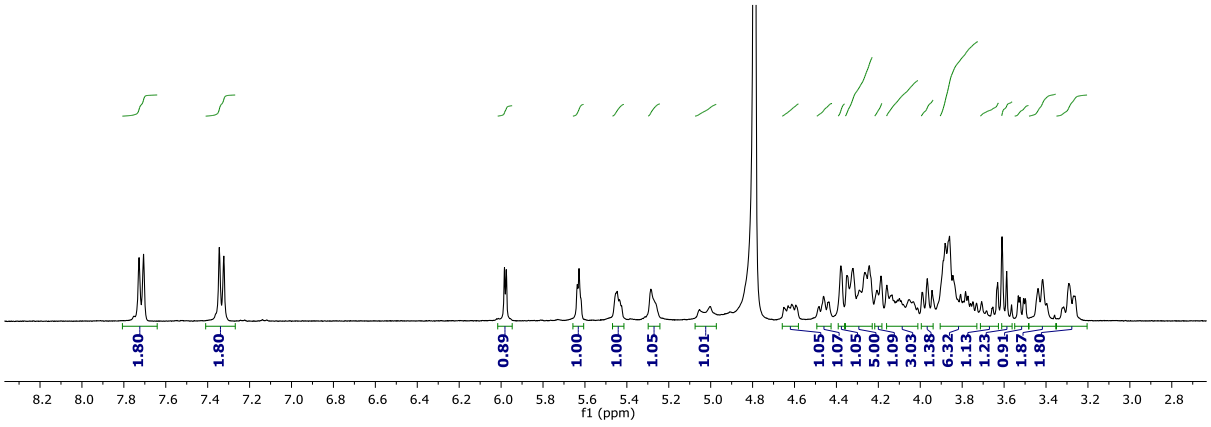

1.98
26.85
1.00
0.90
1.27
0.86
3.32
3.43
5.06
0.94
2.85
6.08
6.27
2.05
5.33
0.96
1.24
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
f1 (ppm)

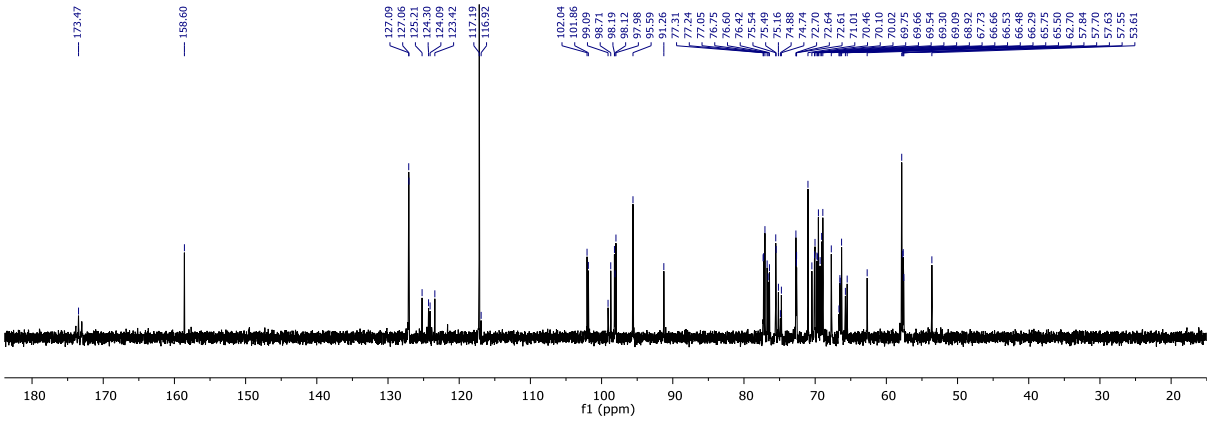

174.41
172.91
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
4
f1 (ppm)

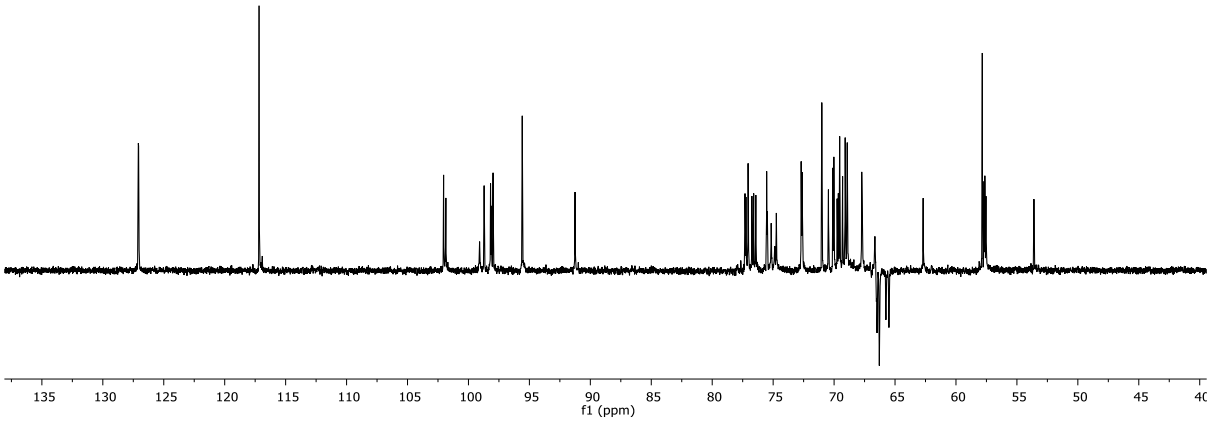

135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50
f1 (ppm)

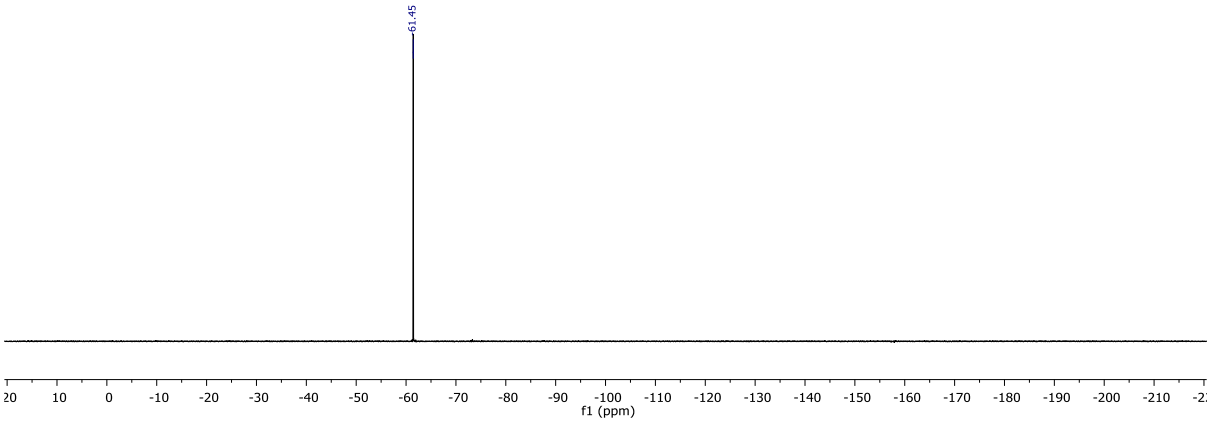

-61.40
-15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150
f1 (ppm)


OSO3-
O
HO
HO
-O3SHN
-O2C
OH
-O3SO
CF3


1.80 1.80 0.89 1.00 1.00 1.05 1.01 1.05 1.07 1.05 5.00 1.09 3.03 1.38 6.32 1.13 1.23 0.91 1.87 1.80
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8
f1 (ppm)


173.47
158.60
127.09
127.06
124.21
124.20
124.09
123.42
117.19
116.92
102.04
101.86
99.09
98.71
98.19
98.12
97.98
95.59
91.26
77.31
77.24
77.05
76.75
76.60
76.42
75.54
75.49
75.16
74.88
74.74
72.70
72.64
72.61
71.01
70.46
70.10
70.02
69.75
69.66
69.54
69.30
69.09
68.92
67.73
66.66
66.53
66.48
66.29
65.75
65.59
62.70
57.84
57.70
57.63
57.55
53.61
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

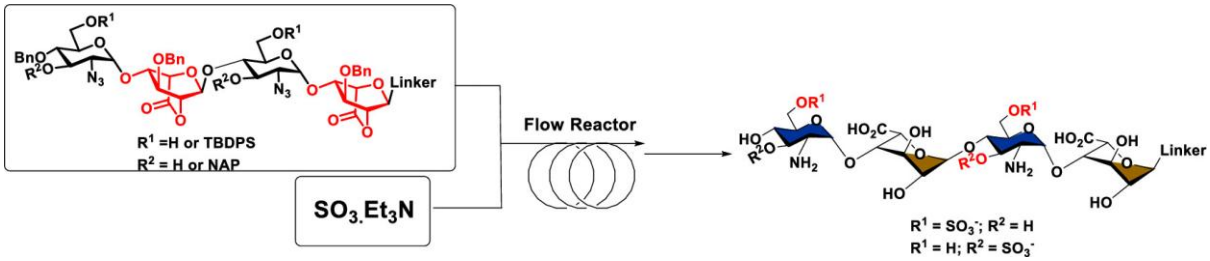

135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40
f1 (ppm)


61.45
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

Chapter – 4
Continuous-Flow Accelerated Sulfation of Heparan Sulfate Intermediates

4.1 Abstract

We present a novel continuous-flow approach for performing *O*-sulfation modifications on heparan sulfate (HS) oligosaccharides. By systematically analyzing the impact of flow parameters on the sulfation of glucosamine monosaccharides, this method offers a fast, reliable, and efficient strategy for *O*-sulfation of HS oligosaccharide precursors. Subsequent deprotection of the sulfated intermediates enabled the creation of an extensive, biologically inspired oligosaccharide library. This library serves as a valuable resource for investigating the critical structure-function relationships of HS.



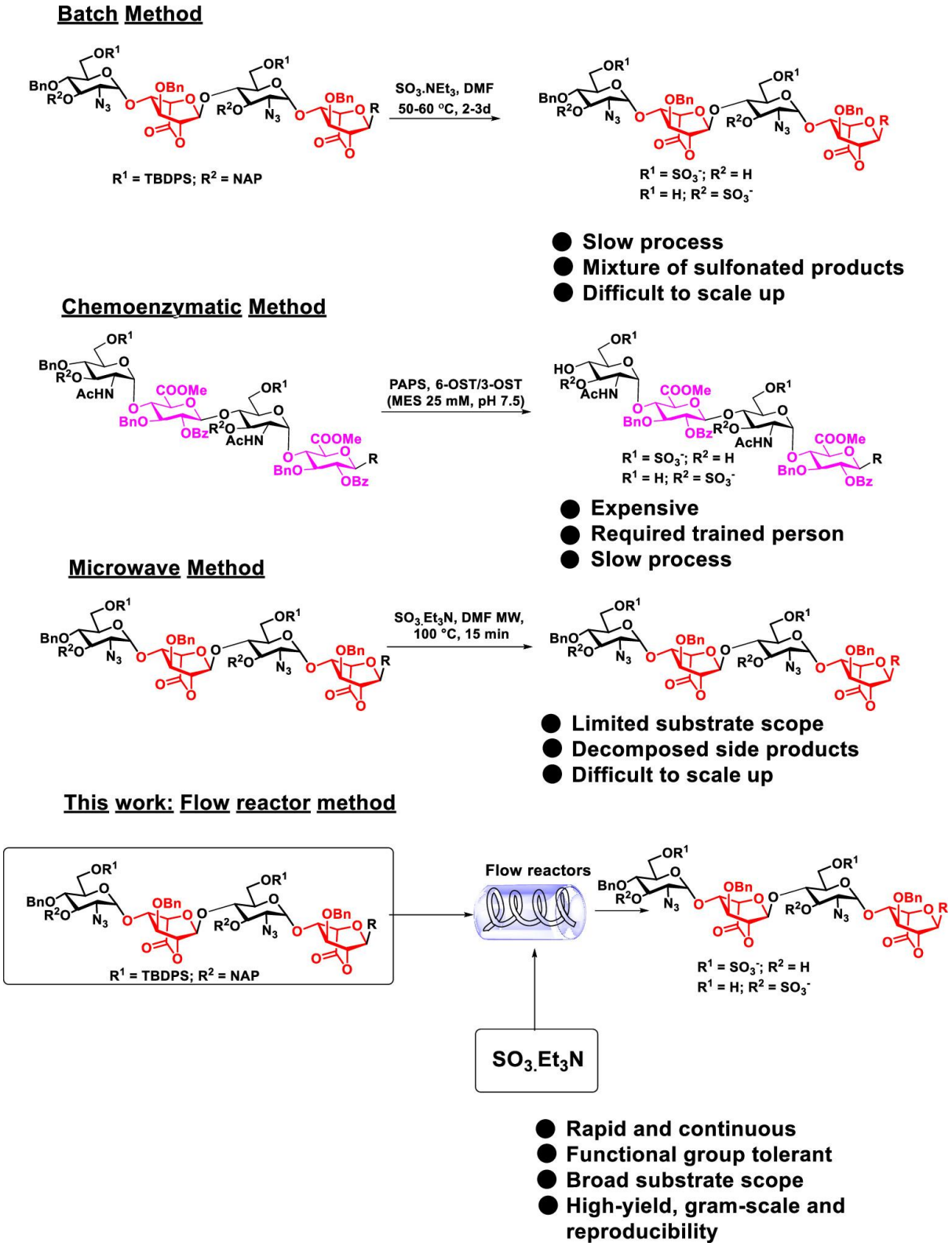
4.2 Introduction

Heparan sulfate (HS) and heparin (HP) are part of the glycosaminoglycan (GAG) family, which consists of linear polysaccharides widely distributed on mammalian cell surfaces, playing a crucial role in various biological processes.[1] One of the most notable characteristics of HS and HP is their structural microheterogeneity, arising from repeating disaccharide units (glucosamine and uronic acid), epimers of uronic acid (D-glucuronic and L-iduronic acid), and sulfate groups.[2] The sulfate groups in particular are vital to the interactions mediated by HS and HP, a subject that has been thoroughly studied over the last two decades.[3] For instance, fibroblast growth factor (FGF) signaling relies on the 6-*O*-sulfated and *N*-sulfated glucosamine residues in HS,4 while the 3-*O*-sulfated and *N*-sulfated glucosamine groups in fondaparinux and enoxaparin are crucial for regulating antithrombin III activity.[5] Further research has highlighted the importance of 6-*O*-sulfated HS in binding to heparin-binding epidermal growth factor (HB-EGF) and vascular endothelial growth factors (VEGF),[6] and the role of 3-*O*-sulfation in binding neuropilin-1, tau protein, and facilitating herpes simplex virus 1 entry.[7] Given the broad biological and pharmaceutical applications of the "sulfation code" in HS oligosaccharides, significant efforts have been made to develop efficient methods for large-scale synthesis of well-defined HS fragments.

Several research groups have made strides in stereoselective glycosylation, carefully selecting protecting groups to synthesize the HS backbone on a commercial scale.[8] However, challenges remain, as the yields of poststructural modifications such as oxidation, sulfation, and global deprotection are often poor, limiting large-scale production of HS oligosaccharides.[9] Traditionally, *O*-sulfation of GAG intermediates has been carried out using sulfur trioxide complexes ($SO_3 \cdot NMe_3$ or $SO_3 \cdot NEt_3$) in batch processes at 40–60°C for 2–3 days.[9] This method suffers from low yields and the formation of partially sulfated side products, due to the weak nucleophilicity of hydroxyl groups and anionic repulsion between sulfate groups in GAG oligosaccharides. As a result, gram-scale sulfation and selective multiple sulfation remain challenging. Alternatively, chemoenzymatic methods have been used for sulfating HS oligosaccharides, but these approaches, while milder, require expensive enzymes, skilled operators, and extended reaction times (Scheme 1).[10] Microwave-mediated sulfation has also been explored to synthesize sulfated HS oligosaccharides at 100 °C within 15–20 minutes.[11] However, this method is limited to specific substrates and produces decomposition side products, hindering its scalability.[12]

In contrast, flow chemistry has made significant advancements in chemical processes, offering solutions to many challenges encountered in batch reactions. Flow chemistry allows for better control over reaction conditions, such as temperature, pressure, and reactant mixing, leading to improved reaction kinetics.[13] Building on these advantages, we present a study in which we applied flow chemistry to perform mono- and multiple sulfation of biologically inspired oligosaccharides, successfully synthesizing sulfated HS oligosaccharide intermediates for the first time. Additionally, we demonstrated the concomitant deprotection of the final sulfated oligosaccharides to obtain the desired HS oligosaccharides. This novel sulfation approach is versatile and could potentially be applied to other GAG oligosaccharides, including dermatan, chondroitin, and keratan sulfates.

4.3 Results and discussion:



In the quest to develop a mild, selective, and high-yielding *O*-sulfation reaction in a continuous flow system, we first standardized the flow optimization on 6-*O*- and 3-*O*-

sulfation of a glucosamine precursor. Glucosamine substrate 1 was converted to sulfate precursor 2 by deprotection of the TBDPS group using HF·pyridine. A 0.05 M solution of 2 in DMF and a 0.5 M solution of SO_3·NEt_3 in DMF were pumped at 0.05 and 0.1 mL/min, respectively, into a 10 mL stainless steel coiled reactor in a Vaportec R-series system at room temperature and 3.5 bar pressure, giving a retention time of 66.6 min (graph (i) in Figure 1). Evaporation of the volatile solvent from the collected fractions followed by column chromatography purification afforded the product 3 in 68% yield (Table 1). Increasing the flow rate (0.1 mL/min for 2 and 0.2 mL/min for SO_3·NEt_3, retention time = 33.3 min) resulted in a slight decrease in yield to 62% (graph (ii) in Figure 1). Byproducts formed by deprotection of Cbz/NAP/Troc groups were not observed under continuous flow. Using higher temperatures (60−120 °C) with the same retention time (66.6 min) and pressure (3.5 bar) resulted in moderate to good yields of 3 (71−86%). Under the same conditions, SO_3·NMe_3 as the precursor gave similar yields (Table 2), indicating that both precursors were ideal for the O-sulfation reaction. However, the use of SO_3·DMF resulted in a 6% yield. A control experiment was performed under batch conditions and gave a 73% yield of 3 after 24 h. Overall, we optimized the retention time, temperature, and pressure essential for effective 6-*O*-sulfation of glucosamine 1 and showed that the sulfation of the glucosamine precursor is more efficient in the flow reactor compared with the batch process Having established the optimum flow conditions for 6-*O*sulfated glucosamine 3, we next sought to investigate 3-*O* sulfation of glucosamine 4 under optimized continuous-flow reaction conditions. Because of the steric hindrance and poor nucleophilicity of the 3-hydroxyl group of glucosamine 4, we observed the sulfation reaction at C-3 to be slow (graphs (i) and (ii) in Figure 2).

S.No.	Flow Rate (R_1) $mlmin^{-1}$	Flow Rate (R_2) $mlmin^{-1}$	Retention Time (min)	Temperature (°C)	Pressure (bar)	Yield (%)
1	0.05	0.1	66.6	25	3.5	68
2	0.1	0.2	33.3	25	3.5	62
3	0.05	0.1	66.6	60	3.5	79
4	0.1	0.2	33.3	60	3.5	71
5	0.05	0.1	66.6	100	3.5	84
6	0.1	0.2	33.3	100	3.5	76
7	0.05	0.1	66.6	120	3.5	86
8	0.1	0.2	33.3	120	3.5	78

Table 1: Effect of flow rates and temperature at constant pressure and reagent $SO_3.NEt_3$

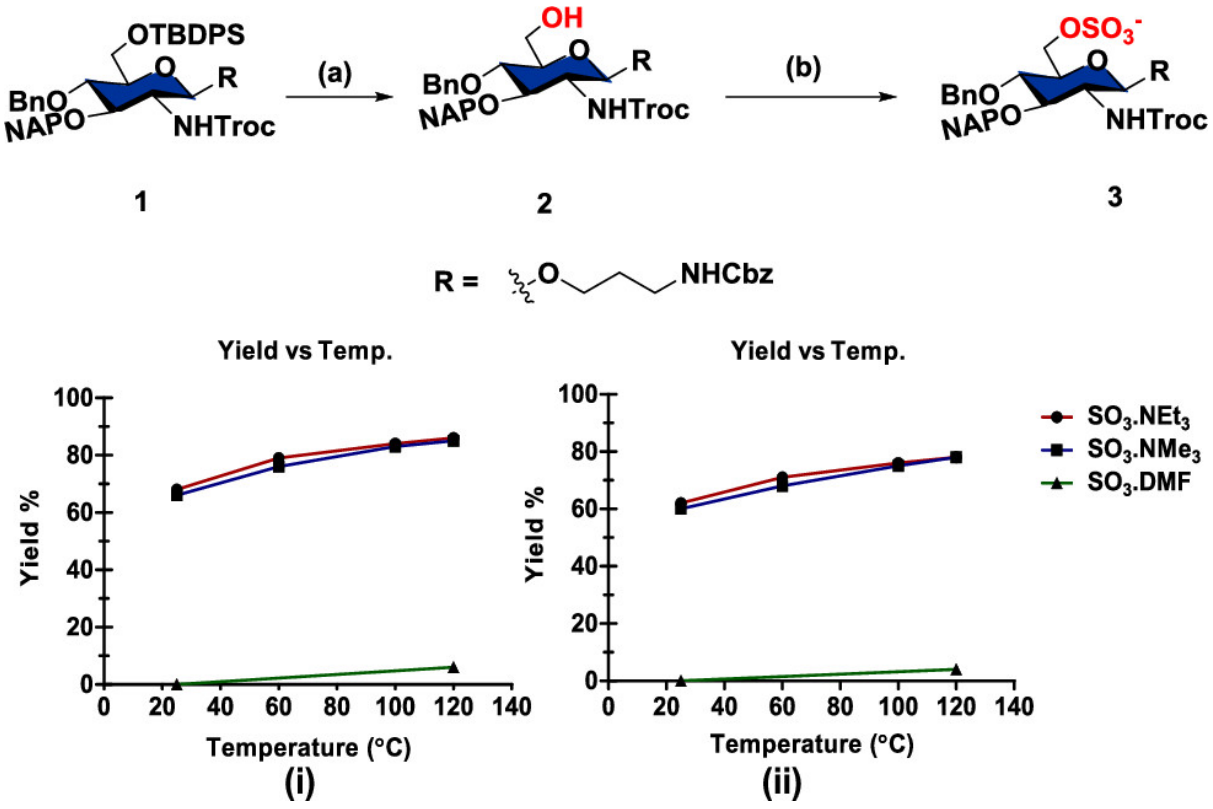


Figure 1. (top) Continuous 6-*O*-sulfation of glucosamine precursor 2. Reagents and conditions: (a) 70% HF·py, Py, 0 °C, 12 h; (b) SO_3·NEt_3 or SO_3·NMe_3 or SO_3·DMF in DMF and 2 in DMF were flowed into the 10 mL stainless steel coil flow reactor (Vaportec R-

series). (bottom) Comparisons of 6-*O*-sulfation yields vs temperature for different sulfur trioxide precursors at 3.5 bar pressure: (i) retention time = 66.6 min (2 in DMF at 0.05 mL/min and $SO_3 \cdot NEt_3$ in DMF at 0.1 mL/min); (ii) retention time = 33.3 min (2 in DMF at 0.1 mL/ min and $SO_3 \cdot NEt_3$ in DMF at 0.2 mL/min).

At room temperature, no conversion was observed at flow rates of 0.05 mL/min for glucosamine precursor 4 and 0.1 mL/min for the $SO_3 \cdot NEt_3$ precursor. As the temperature increased, the conversion rate showed a nearly linear correlation with the yield. Maximum conversion of 4 was observed at 120 °C with the $SO_3 \cdot NEt_3$ complex. With an increase in the precursor flow rate from 0.1 to 0.2 mL/min, the yield of the sulfation is decreased (Tables 3 and 4). This result suggests that the flow rate of the precursor exerts a strong influence on 3-*O*-sulfation. A similar reaction in a batch process afforded a 65% yield of 5 after 2 days at 60 °C, and at a high temperature (120 °C) we observed fragmentations.

S.No.	Flow Rate (R_1) $mlmin^{-1}$	Flow Rate (R_2) $mlmin^{-1}$	Retention Time (min)	Temperature (^oC)	Pressure (bar)	Yield (%)
1	0.05	0.1	66.6	25	3.5	66
2	0.1	0.2	33.3	25	3.5	60
3	0.05	0.1	66.6	60	3.5	76
4	0.1	0.2	33.3	60	3.5	68
5	0.05	0.1	66.6	100	3.5	83
6	0.1	0.2	33.3	100	3.5	75
7	0.05	0.1	66.6	120	3.5	85
8	0.1	0.2	33.3	120	3.5	78

Table 2: Effect of flow rates and temperature at constant pressure and reagent $SO_3.NMe_3$

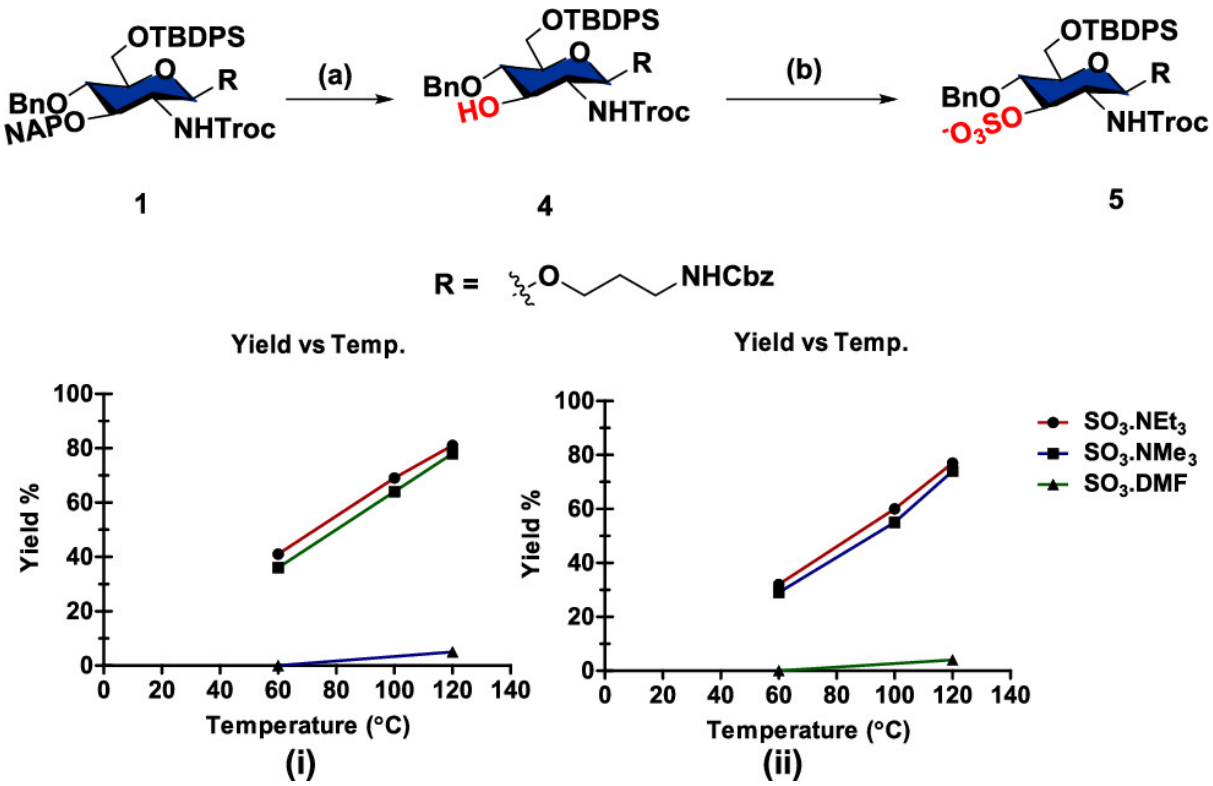


Figure 2. (top) Continuous 3-*O*-sulfation of glucosamine precursor 4. Reagents and conditions: (a) DDQ, DCM/H_2O (18:1), RT, 1 h; (b) SO_3·NEt_3 or SO_3·NMe_3 or SO_3·DMF. (bottom) Comparisons of 3-*O*sulfation yields vs temperature for different sulfur trioxide precursors at 3.5 bar pressure: (i) retention time = 66.6 min (4 in DMF at 0.05 mL/min and SO_3·NEt_3 in DMF at 0.1 mL/min), pressure; (ii) retention time = 33.3 min (4 in DMF at 0.1 mL/min and SO_3·Et_3N in DMF at 0.2 mL/min).

Overall, these results revealed that a fine optimization between the retention time of the precursor and a high reaction temperature was essential for 3-*O*-sulfation of glucosamine in the flow reactor. After having optimized the flow conditions for *O*-sulfation using glucosamine residues, we were more interested in studying the continuous-flow *O*-sulfation chemistry with more fascinating biomolecules, heparinoids. Surprisingly, the flow chemistry provided excellent sulfation with di- and tetrasaccharide intermediates with different sulfation patterns in better yields than for the batch process (Figure 3).

S.No.	Flow Rate (R_1) $mlmin^{-1}$	Flow Rate (R_2) $mlmin^{-1}$	Retention Time (min)	Temperature (^oC)	Pressure (bar)	Yield (%)
1	0.05	0.1	66.6	60	3.5	41
2	0.1	0.2	33.3	60	3.5	32
3	0.05	0.1	66.6	100	3.5	69
4	0.1	0.2	33.3	100	3.5	60
5	0.05	0.1	66.6	120	3.5	81
6	0.1	0.2	33.3	120	3.5	77

Table 3: Effect of flow rates and temperature at constant pressure and reagent $SO_3.NEt_3$

S.No.	Flow Rate (R_1) $mlmin^{-1}$	Flow Rate (R_2) $mlmin^{-1}$	Retention Time (min)	Temperature (^oC)	Pressure (bar)	Yield (%)
1	0.05	0.1	66.6	60	3.5	36
2	0.1	0.2	33.3	60	3.5	29
3	0.05	0.1	66.6	100	3.5	65
4	0.1	0.2	33.3	100	3.5	54
5	0.05	0.1	66.6	120	3.5	78
6	0.1	0.2	33.3	120	3.5	74

Table 4: Effect of flow rates and temperature at constant pressure and reagent $SO_3.NMe_3$

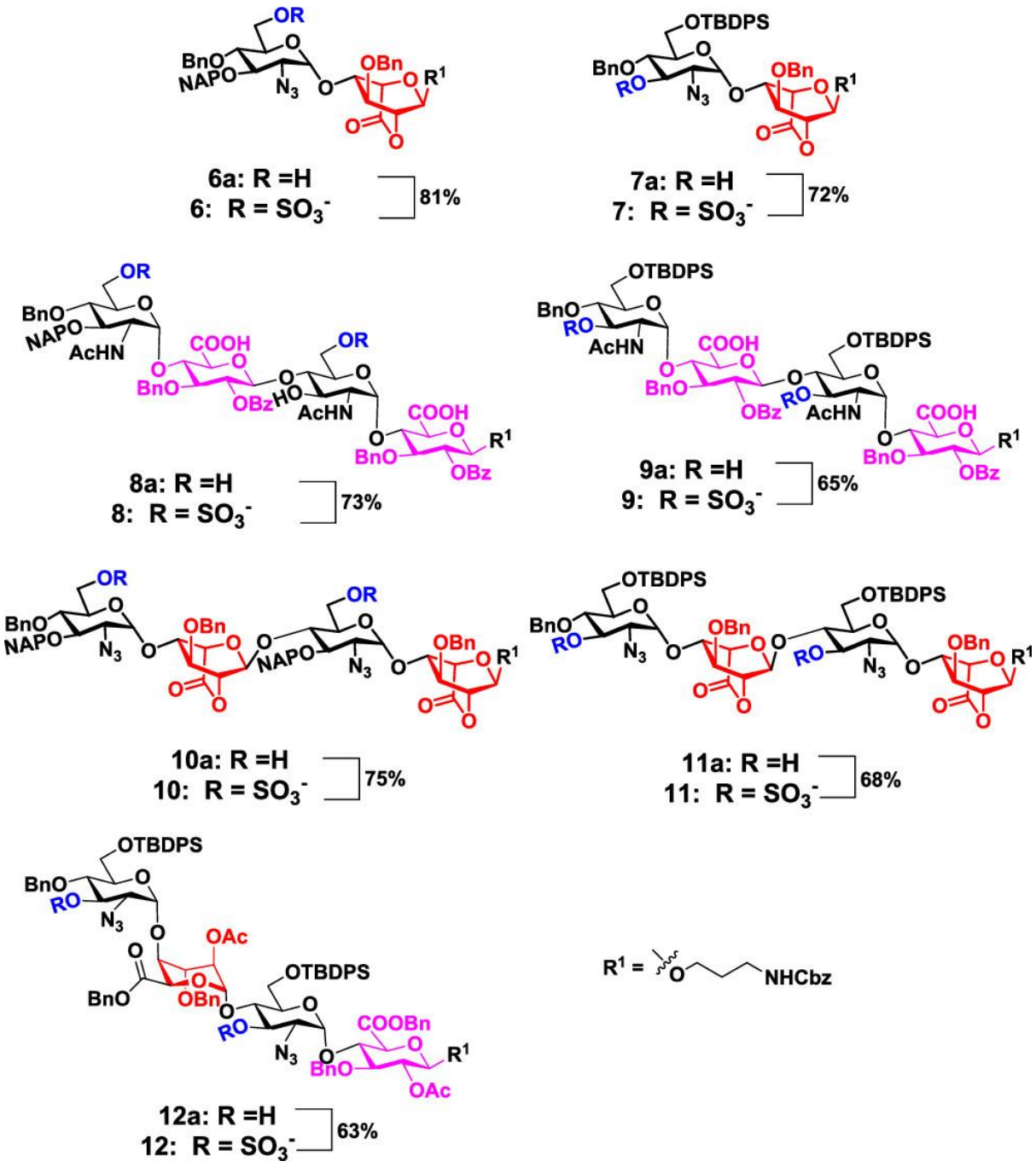


Figure 3. Substrate scope of mono- and disulfation of disaccharides and tetrasaccharides.

Disaccharide 6a was persulfated at the 6-position using an optimized flow rate in an excellent yield of 81%. Similarly, 3-*O*-sulfation of disaccharide 7 furnished 72% conversion. In all cases, a minor quantity of unreacted starting material was observed in the reaction mixture and recovered by column purification for further reaction. Tetrasaccharides 8a−12a with different uronic acid compositions (L-iduronic and D-glucuronic acid precursors) and glucosamine derivatives showed excellent yields of 6-*O*- and 3-*O*-sulfation products (63−75%). Moreover, the gram-scale conversion of 6a demonstrated the potential of the flow

chemistry. Finally, persulfated 6 and 8−11 were then fully deprotected using HF·pyridine (in the case of 9 and 11) followed by LiOH·H_2O and hydrogenolysis to afford the desired HS tetrasaccharides 13−17 (Figure 4).

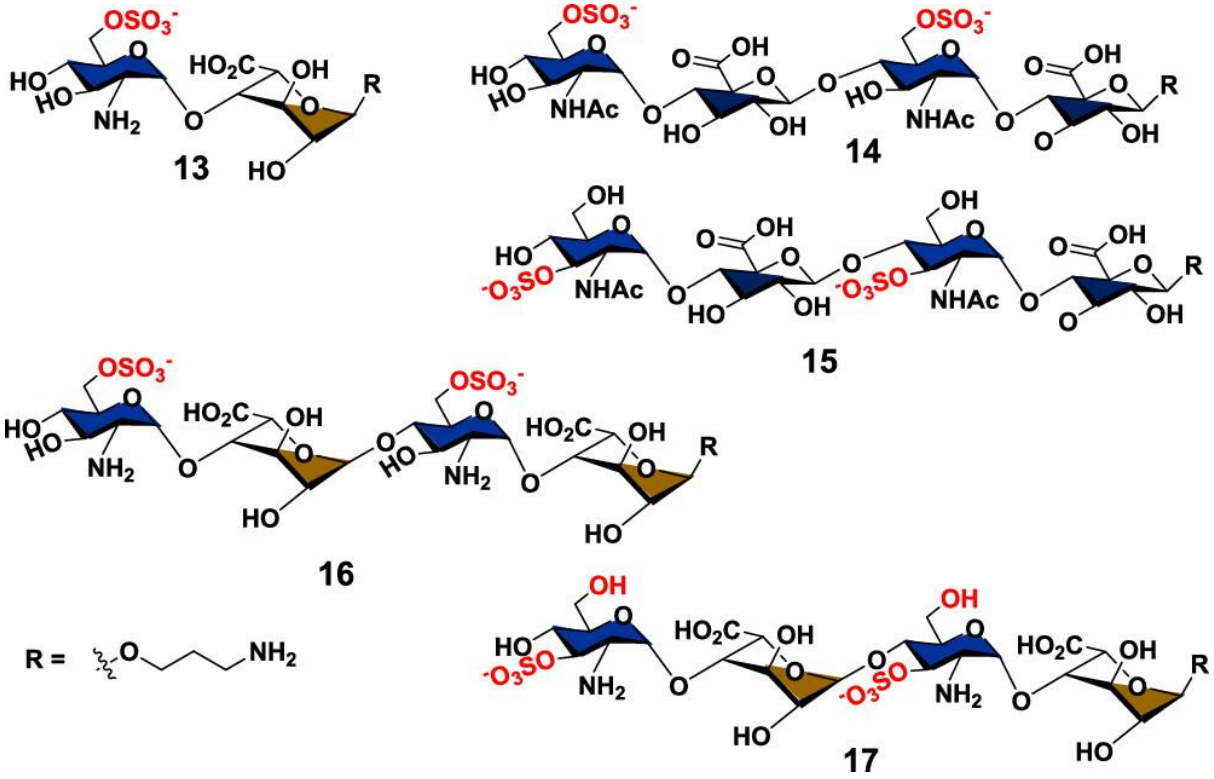


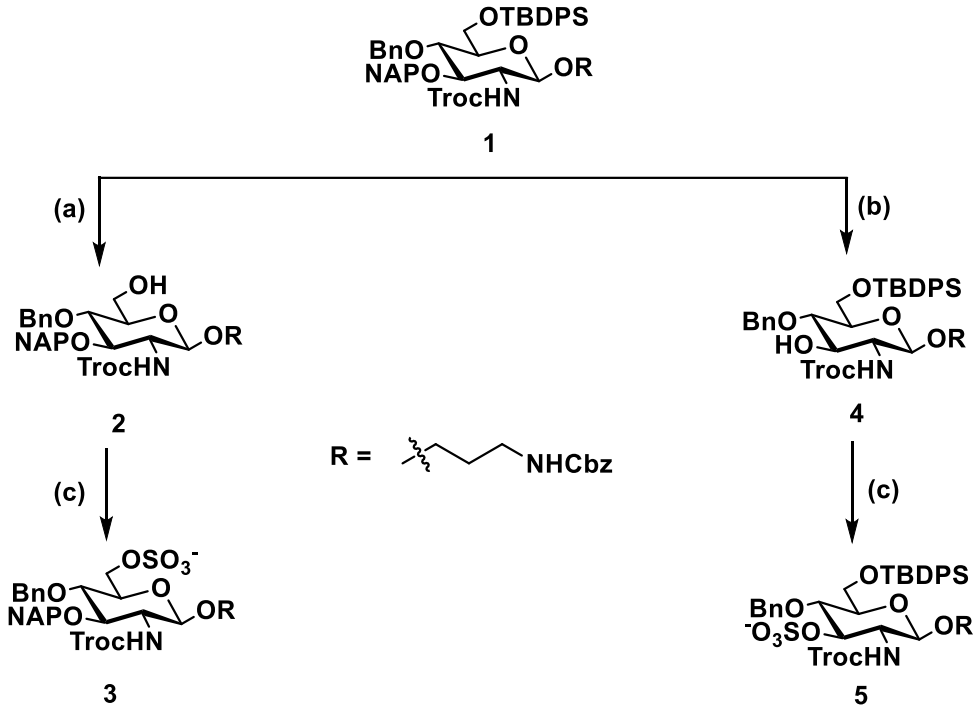
Figure 4. Heparan sulfate oligosaccharides 13−17 after complete deprotection.

4.4 Conclusion

In conclusion, we have successfully developed a machine-accelerated *O*-sulfation method for the first time for biomolecules, such as heparan sulfate (HS) oligosaccharides, in a continuous flow system. By optimizing the flow rate, temperature, and pressure, we efficiently sulfated the 6-*O* and 3-*O* hydroxyl groups of glucosamine, HS disaccharides, and HS tetrasaccharide intermediates with excellent yields (65–81%). The resulting tetrasaccharides were fully deprotected under mild conditions to produce the desired HS oligosaccharides with >88% yield. This approach enabled the synthesis of tetrasaccharides with different uronic acids. The general applicability of the reaction conditions and the scalability of the process are expected to significantly accelerate the production of HS oligosaccharides with various sulfation patterns. We are currently exploring the simultaneous 6-*O*/3-*O*-sulfation and *N*-sulfation of these oligosaccharides.

4.5 Experimental section:

4.5.1 Synthesis of glucosamine substrates and its sulfation



Scheme 1. Synthesis of glucosamine precursors: Reaction condition and reagents: (a) HF.Py., pyridine, 0 °C, 12 h, 83%; (b) DDQ, DCM:H_2O (18:1), RT, 1 h 74%; (c) Flow reactor, SO_3 precursors, DMF.

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-2-(2,2,2-trichloroethoxycarbonylamino)-4-Obenzyl-3-O-(2- naphthylmethyl)-2-deoxy-α-D-glucopyranosyl (2)**

To a solution of compound **1** (1.5 g, 1.48 mmoles) in pyridine (3 mL) was added 70% HF.py. (4 equivalents per TBDPS) dropwise manner under N_2 atmosphere at 0 °C and kept the reaction mixture stirring for 12 h at RT. After completion of reaction the residue was diluted with ethyl acetate (E.A) and it was washed by 1 N HCl and brine solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Finally, the residue was purified by column chromatography (50% E.A/P.E) and 954 mg, 83% **2** was obtained. 1H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.77 (m, 3H), 7.73 (s, 1H), 7.49 – 7.43 (m, 3H), 7.35 – 7.29 (m, 10H), 5.45 (s, 1H), 5.18 – 5.07 (m, 3H), 5.01 (d, *J* = 11.5 Hz, 1H), 4.90 – 4.86 (m, 2H), 4.70 (d, *J* = 11.0 Hz, 1H), 4.63 – 4.52 (m, 2H), 3.91 – 3.81 (m, 3H), 3.75 – 3.71 (m, 1H), 3.64 (t, *J* = 9.1 Hz, 1H), 3.55 – 3.35 (m, 3H), 3.25 – 3.17 (m, 1H), 1.76 – 1.68 (m, 2H). 13C NMR (100 MHz, $CDCl_3$)

δ 156.7, 154.4, 138.0, 136.7, 135.5, 133.3, 133.2, 128.7, 128.4, 128.3, 128.2, 128.1, 128.1, 127.8, 127.1, 126.3, 126.3, 126.2, 101.1, 95.7, 81.0, 78.5, 77.2, 74.4, 67.5, 66.8, 61.8, 57.9, 38.0, 29.8. HRMS (ESI - TOF) m/z: [M+Na]+ calcd. for $C_{38}H_{41}Cl_3N_2O_9Na$ 799.1774; Found 799.1774.

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-2-(2,2,2-trichloroethoxycarbonylamino)-4-Obenzyl-6-*O*- *tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl (4)**

To a solution of compound **1** (1.6 g, 1.58 mmoles) in 18:1 ratio of DCM:H2O (19 mL) was stirred at RT. After that DDQ (743 mg, 3.28 mmoles) was added in four portions and kept the reaction mixture for 15 minute stirring. After completion of the reaction 10 mL saturated solution of $NaHCO_3$ was added to quench the reaction and the residue was washed with DCM/$NaHCO_3$ and brine solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Finally, the residue was purified by column chromatography (25%E.A/P.E) and 1022 mg, 74% **4** was obtained. 1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.9 Hz, 2H), 7.70 (d, *J* = 6.7 Hz, 2H), 7.43 – 7.41 (m, 2H), 7.38 – 7.35 (m, 8H), 7.33 – 7.29 (m, 6H), 6.30 (s, 1H), 5.14 – 5.03 (m, 3H), 4.92 (d, *J* = 11.2 Hz, 1H), 4.82 – 4.69 (m, 3H), 4.28 (d, *J* = 8.2 Hz, 1H), 3.98 – 3.91 (m, 3H), 3.79 (t, *J* = 8.9 Hz, 1H), 3.66 (t, *J* = 9.0 Hz, 1H), 3.54 (q, *J* = 8.1 Hz, 2H), 3.46 – 3.32 (m, 3H), 3.18 – 3.13 (dd, *J* = 13.9, 4.9 Hz, 1H), 1.98 – 1.75 (m, 2H), 1.08 (s, 9H). 13C NMR (100 MHz, $CDCl_3$) δ 156.9, 138.5, 136.7, 136.0, 135.7, 133.7, 133.3, 129.8, 128.7, 127.9, 100.9, 95.7, 78.4, 77.2, 75.9, 75.0, 74.7, 66.9, 63.0, 58.6, 37.7, 30.1, 29.8, 27.0, 19.4. HRMS (ESI - TOF) m/z: [M+H]+ calcd. For C43H51Cl3N2O9SiH 873.2508; Found 873.2498.

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-2-(2,2,2-trichloroethoxycarbonylamino)-4-Obenzyl-6-*O*-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl (3)**

The compound **2** (600 mg, 0.77 mmoles) and $SO_3.NEt_3$ (2.8 g, 15.5 mmoles) was dissolved separately in DMF(15 mL) and passed through the SS coil reactor (10 mL) with 0.05 mLmin-1 flow rate for **2** and 0.1 mLmin-1 for $SO_3.NEt_3$ at 120 oC, 3.5 bar and the retention time was 66.6 minute as shown in figure **1**. The conversion was monitored by TLC. After that solvent was evaporated under reduce pressure and finally reaction mixture was purified by column chromatography (5% MeOH/DCM) and again solvent was evaporated under reduce pressure, and the crude product was

purified by size exclusion Sephadex LH-20 column followed by amberlite IR 120 resin and 566 mg, 86% **3** was obtained.

1H NMR (400 MHz, CD_3OD) δ 7.84 – 7.77 (m, 3H), 7.74 (s, 1H), 7.48 – 7.41 (m, 3H), 7.36 –7.23 (m, 10H), 5.07 (s, 2H), 5.00 – 4.90 (m, 3H), 4.86 – 4.79 (m, 3H), 4.71 – 4.61 (m, 3H), 4.43 – 4.34 (m, 3H), 4.29 – 4.18 (m, 2H), 3.92 (dt, *J* = 10.3, 5.7 Hz, 1H), 3.73 – 3.51 (m, 3H), 1.75 (dq, *J* = 11.9, 6.1 Hz, 2H). 13C NMR (100 MHz, CD_3OD) δ 156.8, 139.6, 138.5, 137.3, 134.7, 134.4, 129.5, 129.3, 128.9, 128.7, 128.7, 127.4, 127.1, 127.0, 126.9, 102.8, 97.1, 84.0, 79.3, 76.3, 75.9, 75.4, 74.9, 68.4, 67.5, 67.3, 58.8, 49.0, 45.5, 38.8, 30.9. HRMS (ESI - TOF) m/z: [M]- calcd. for $C_{38}H_{40}Cl_3N_2O_{12}S$- 853.1373; Found 853.1364.

_N_-Benzyloxycarbonyl-3-aminopropyl-_O_-2-(2,2,2-trichloroethoxycarbonylamino)-4-Obenzyl-6-_O_-_tert_-butyldiphenylsilyl-3-O-sulfonato-2-deoxy-α-D-glucopy ranosyl (5)

The compound **4** (550 mg, 0.94 mmoles) and $SO_3.NEt_3$ (3.45 g, 18.92 mmoles) was dissolved separately in DMF (15 mL) and passed through the SS coil reactor (10 mL) with 0.05 mLmin-1 flow rate for **4** and 0.1 mLmin-1 for $SO_3.NEt_3$ at 120 oC, 3.5 bar and the retention time is 66.6 minute. The conversion was monitored by TLC. After that solvent was evaporated under reduce pressure and finally purified by column chromatography (5% MeOH/DCM) and again solvent was evaporated under reduce pressure, and the crude product was purified by size exclusion Sephadex LH-20 column followed by amberlite IR 120 resin and 726 mg, 81% **5** was obtained. 1H NMR (400 MHz, CD3OD) δ 7.71 – 7.0 (m, 2H), 7.66 – 7.64 (m, 2H), 7.43 – 7.39 (m, 2H), 7.37 – 7.24 (m, 11H), 7.19 – 7.18 (m, 3H), 5.10 (d, *J* = 10.5 Hz, 1H), 5.04 (s, 1H), 5.01 – 4.97 (m, 1H), 4.58 – 4.45 (m, 4H), 3.93 – 3.88 (m, 1H), 3.82 (s, 2H) 3.72 (t, *J* = 9.2 Hz, 1H), 3.62 – 3.50 (m, 2H), 3.40 – 3.34 (m, 1H), 3.23 – 3.18 (m, 2H), 1.80 – 1.73 (m, 2H), 1.02 (s, 9H). 13C NMR (100 MHz, CD3OD) δ 158.8, 157.1, 139.9, 138.4, 137.0, 136.7, 134.8, 134.3, 130.8, 128.5, 102.7, 97.2, 82.1, 78.2, 76.7, 76.0, 75.6, 68.2, 67.3, 64.0, 58.4, 49.0, 39.1, 30.9, 27.3, 20.1. HRMS (ESI - TOF) m/z: [M]- calcd. for C43H50Cl3N2O12SiS- 951.1925; Found 951.1917.

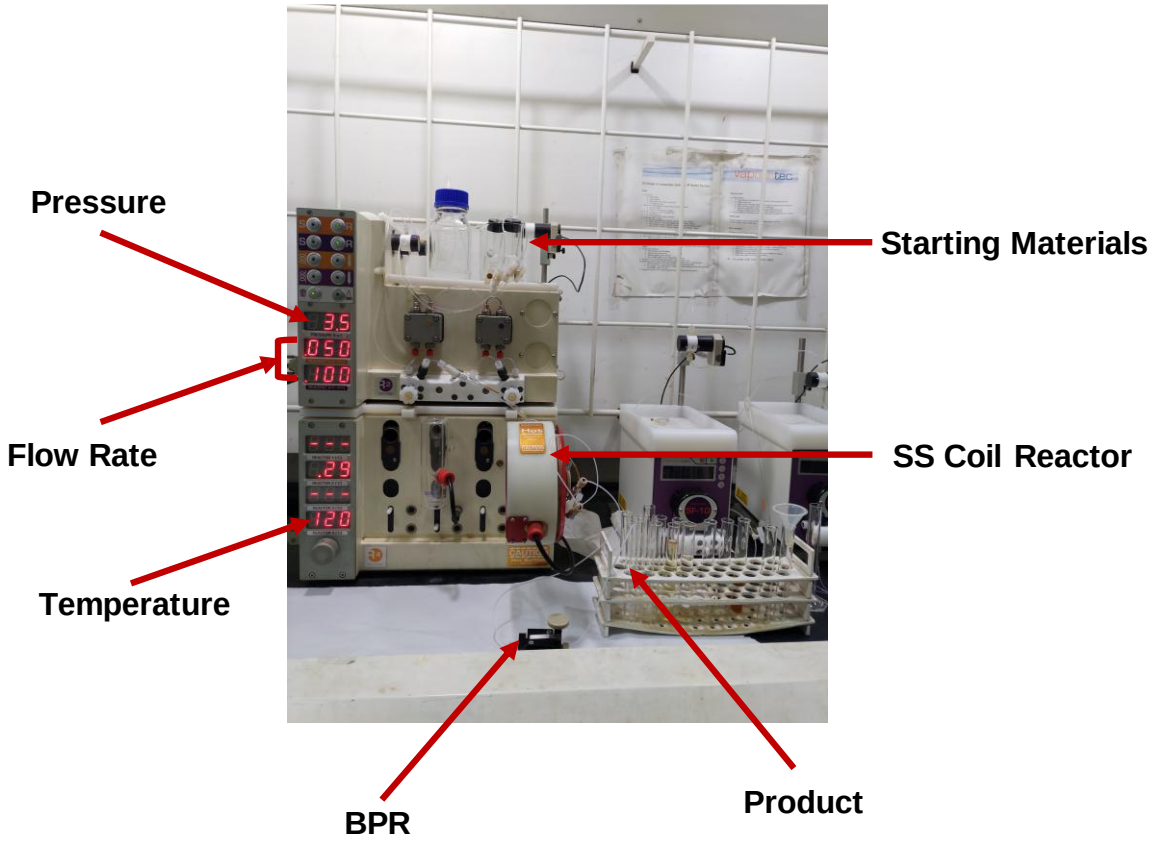


Figure 1: Full setup of continuous flow reactor

4.5.2 General procedure for *O*-Sulfation in batch process.

To a solution of starting material (1 equivalent) in DMF (2 mL) was added $SO_3.NEt_3$ complex (10 equivalents) under N_2 atmosphere and stirred at 60 oC for 1-2 days on oil bath. Upon completion of reaction, aqueous $NaHCO_3$ (20 mmol) was added to reaction mixture and stirred for another 16 h. Next, the reaction mixture was concentrated under reduced pressure and the resulting residue was filtered with MeOH through wattman filter paper. The filtrate was concentrated and purified using silica column chromatography.

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-2-(2,2,2-trichloroethoxycarbonylamino)-4-Obenzyl-6-*O*-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl (3)**

Compound **3** was synthesized from compound **2** (50 mg, 0.064 mmoles) using above mentioned general procedure after 24 h to obtain 40 mg, 73% of **3**. 1H NMR (400 MHz, CD_3OD) δ 7.84 – 7.77 (m, 3H), 7.74 (s, 1H), 7.48 – 7.41 (m, 3H), 7.36 – 7.23 (m, 10H), 5.07 (s, 2H), 5.00 – 4.90 (m, 3H), 4.86 – 4.79 (m, 3H), 4.71 – 4.61 (m, 3H), 4.43 – 4.34 (m, 3H), 4.29 – 4.18 (m, 2H), 3.92 (dt, *J* = 10.3, 5.7 Hz, 1H), 3.73 – 3.51 (m, 3H), 1.75 (dq, *J*

= 11.9, 6.1 Hz, 2H). 13C NMR (100 MHz, CD_3OD) δ 156.8, 139.6, 138.5, 137.3, 134.7, 134.4, 129.5, 129.3, 128.9, 128.7, 128.7, 127.4, 127.1, 127.0, 126.9, 102.8, 97.1, 84.0, 79.3, 76.3, 75.9, 75.4, 74.9, 68.4, 67.5, 67.3, 58.8, 49.0, 45.5, 38.8, 30.9. HRMS (ESI - TOF) m/z: [M]- calcd. for $C_{38}H_{40}Cl_3N_2O_{12}S$- 853.1373; Found 853.1369.

N-benzyloxycarbonyl-3-aminopropyl-*O*-2-(2,2,2-trichloroethoxycarbonylamino)-4-Obenzyl-6-*O*-*tert*-butyldiphenylsilyl-3-O-sulfonato-2-deoxy-α-D-glucopyrano syl (5)

Compound **5** was synthesized from compound **4** (50 mg, 0.089 mmoles) using above mentioned general procedure after 2 days to obtain 55 mg, 65% of **5**.

1H NMR (400 MHz, CD_3OD) δ 7.71 – 7.0 (m, 2H), 7.66 – 7.64 (m, 2H), 7.43 – 7.39 (m, 2H), 7.37 – 7.24 (m, 11H), 7.19 – 7.18 (m, 3H), 5.10 (d, *J* = 10.5 Hz, 1H), 5.04 (s, 1H), 5.01 – 4.97 (m, 1H), 4.58 – 4.45 (m, 4H), 3.93 – 3.88 (m, 1H), 3.82 (s, 2H) 3.72 (t, *J* = 9.2 Hz, 1H), 3.62 – 3.50 (m, 2H), 3.40 – 3.34 (m, 1H), 3.23 – 3.18 (m, 2H), 1.80 – 1.73 (m, 2H), 1.02 (s, 9H). 13C NMR(100 MHz, CD_3OD) δ 158.8, 157.1, 139.9, 138.4, 137.0, 136.7, 134.8, 134.3, 130.8, 128.5, 102.7, 97.2, 82.1, 78.2, 76.7, 76.0, 75.6, 68.2, 67.3, 64.0, 58.4, 49.0, 39.1, 30.9, 27.3, 20.1. HRMS (ESI - TOF) m/z: [M]- calcd. for $C_{43}H_{50}Cl_3N_2O_{12}SiS$- 951.1925; Found 951.1919.

Time required for 1 g 6-O-sulfation of monosaccharide by flow reactor

Molecular weight = 776.101

Number of moles (n) = 1.288

Concentration (C) = 0.05M

Volume of HS precursor = (n/C) = 25.8mL

Volume of $SO_3.NEt_3$ = 51.6mL

Flow rate of HS precursor = 0.05mLmin^{-1}

Flow rate of $SO_3.NEt_3$ = 0.1mLmin^{-1}

$$\text{Time required} = \frac{\left(\text{Volume of HS} + \text{Volume of } SO_3.NEt_3\right)}{\left(\text{Flow rate of HS} + \text{Flow rate of } SO_3.NEt_3\right)}$$

$$= \frac{25.8 + 51.6}{0.05 + 0.1}$$

Time required = 516 min = 8h 36 min

Time required for 1 g 3-O-sulfation of monosaccharide by flow reactor

Molecular weight = 874.321

Number of moles (n) = 1.143

Concentration (C) = 0.05 M

Volume of HS precursor = (n/C) = 22.9 mL

Volume of $SO_3.NEt_3$ = 45.8 mL

Flow rate of HS precursor = 0.05 $mLmin^{-1}$

Flow rate of $SO_3.NEt_3$ = 0.1 $mLmin^{-1}$

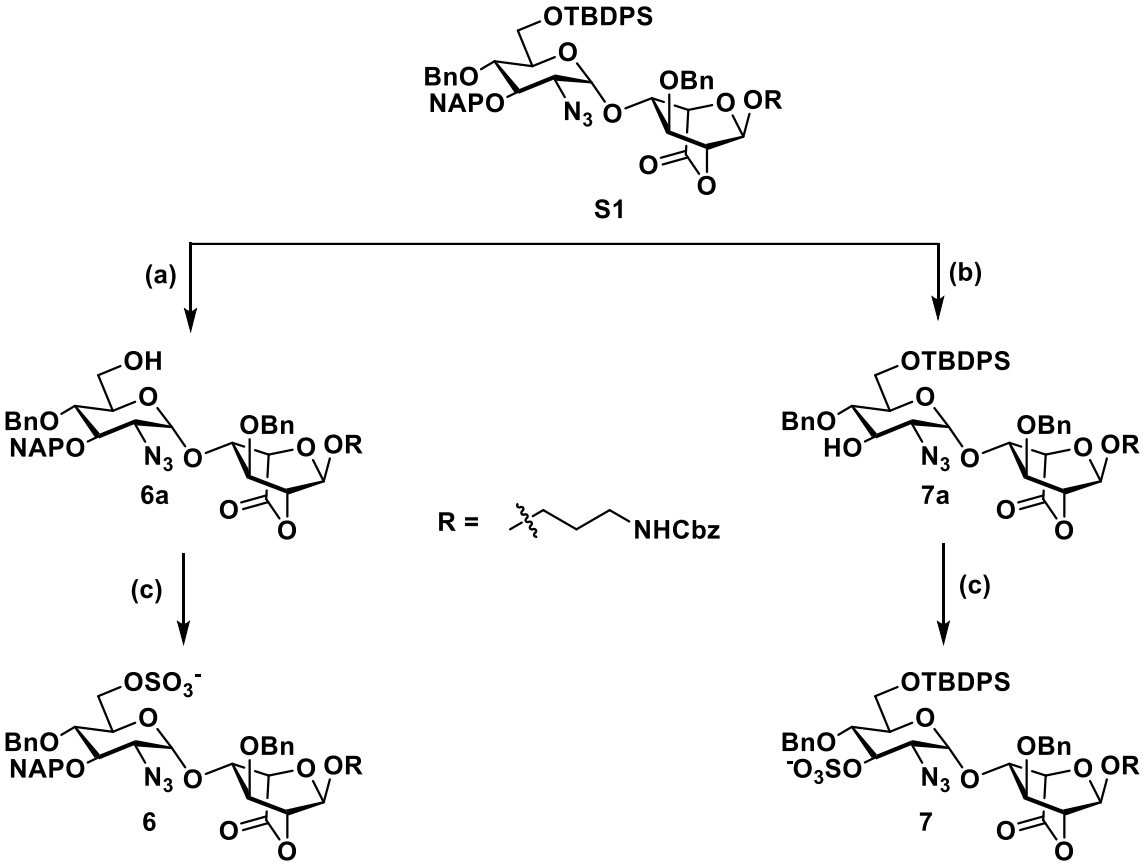
Temperature is 120 ^oC

$$\text{Time required} = \frac{\left(\text{Volume of HS} + \text{Volume of } SO_3.NEt_3\right.}{\left(\text{Flow rate of HS} + \text{Flow rate of } SO_3.NEt_3\right)}$$

$$= \frac{22.9 + 45.8}{0.05 + 0.1}$$

Time required = 458 min = 7 h 38 min

4.5.3 *O*-Sulfation of HS Disaccharide Intermediates



Scheme 2. Synthesis of glucosamine precursors: Reaction condition and reagents: (a) HF.py., pyridine, 0°C, 12 h, 83%; (b) DDQ, DCM:H_2O (18:1), RT, 1 h 74%; (c) Flow reactor, SO_3.NEt_3, DMF

N-Benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-4-*O*-benzyl-3-*O*-(2-naphthylmethyl)-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-3-*O*-benzyl-*α*-L-idopyranosidurono-6,2-lactone (6a)

Compound **6a** was synthesized from compound **S1**[1,2] (1.5 g, 1.34 mmoles) using same procedure as described above for compound **2** and 973 mg, 83% **6a** was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.78 (m, 4H), 7.51 – 7.47 (m, 3H), 7.37 – 7.26 (m, 14H), 5.78 (s, 1H), 5.14 – 4.97 (m, 6H), 4.85 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 12.4 Hz, 1H), 4.66 (dd, *J* = 14.8, 11.9 Hz, 2H), 4.57 (d, *J* = 3.1 Hz, 1H), 4.48 (s, 1H), 4.26 (s, 1H), 3.93 (q, *J* = 8.7, 7.1 Hz, 3H), 3.82 – 3.76 (m, 2H), 3.73 – 3.69 (m, 1H), 3.62 – 3.54 (m, 2H), 3.50 – 3.37 (m, 2H), 3.32 – 3.25 (m, 1H), 2.06 (s, 2H), 1.85 – 1.83 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 156.7, 137.8, 136.9, 136.8, 135.3, 128.6, 128.4, 128.1, 127.9, 127.0, 126.2, 126.1, 126.0, 99.8, 96.8, 80.6, 80.1, 78.5, 78.1, 77.2, 75.7, 75.2, 72.7, 72.6, 72.3, 69.5, 69.2, 67.2, 66.7,

63.6, 61.6, 50.9, 39.9, 31.0, 29.8, 29.5. HRMS (ESI - TOF) m/z: $[M+Na]^+$ calcd. for $C_{48}H_{50}N_4O_{12}Na$ 897.3323; Found 897.3334.

_N_-Benzyloxycarbonyl-3-aminopropyl-_O_-[(2-azido-4-_O_-benzyl-6-_O_-_tert_-butyldiphenylsilyl-2-deoxy-_α_-D-glucopyranosyl]-(1→4)-_O_-3-_O_-benzyl-_α_-L-idopyranosidurono-6,2-lactone (7a)

Compound **7a** was synthesized from compound **S1**[1,2] (1.5 g, 1.34 mmoles) using same procedure as described above for compound **4** and 965 mg, 74% **7a** was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.68 (m, 4H), 7.45 – 7.28 (m, 19H), 7.25 – 7.22 (m, 2H), 5.82 (s, 1H), 5.12 – 4.99 (m, 3H), 4.90 (d, *J* = 3.4 Hz, 1H), 4.77 – 4.71 (m, 3H), 4.61 (d, *J* = 12.3 Hz, 1H), 4.40 (t, *J* = 2.7 Hz, 1H), 4.36 (d, *J* = 3.2 Hz, 1H), 4.19 – 4.17 (m, 1H), 4.01 – 3.91 (m, 4H), 3.81 – 3.73 (m, 2H), 3.66 – 3.55 (m, 2H), 3.48 – 3.40 (m, 1H), 3.34 – 3.27 (m, 1H), 3.20 (dd, *J* = 10.4, 3.6 Hz, 1H), 2.62 (s, 1H), 1.86 – 1.84 (m, 2H), 1.11 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 156.7, 138.1, 137.0, 136.8, 136.0, 135.7, 133.5, 133.2, 129.8, 128.8, 128.6, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 100.0, 96.8, 80.8, 78.9, 78.2, 77.2, 75.0, 72.6, 72.5, 72.3, 71.6, 69.5, 69.3, 66.7, 63.0, 62.5, 40.0, 29.8, 29.5, 27.0. HRMS (ESI - TOF) m/z: $[M+Na]^+$ calcd. for $C_{53}H_{60}N_4O_{12}SiNa$ 995.3875; Found 995.3868.

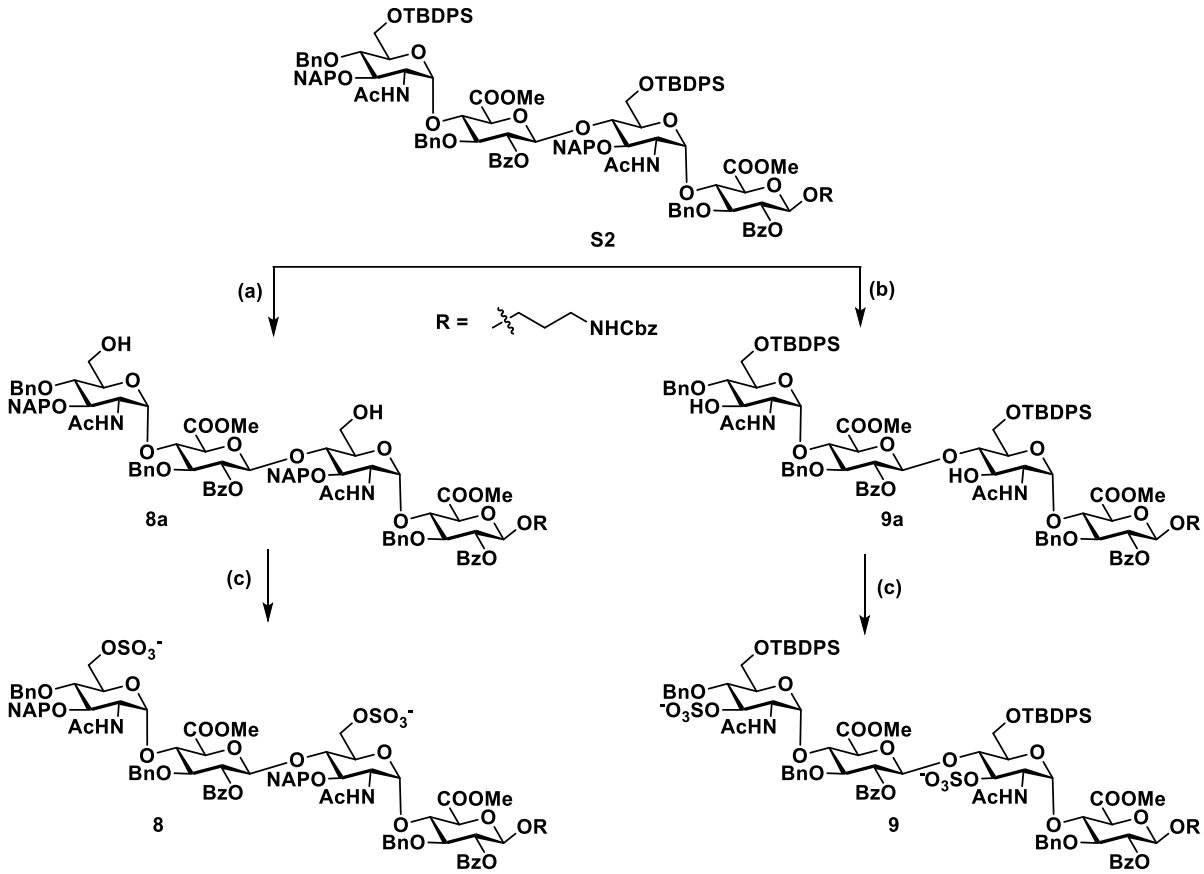
_N_-Benzyloxycarbonyl-3-aminopropyl-_O_-[(2-azido-4-_O_-benzyl-6-_O_-sulfonato-3-_O_-(2-naphthylmethyl)-2-deoxy-_α_-D-glucopyranosyl]-(1→4)-_O_-3-_O_-benzyl-_α_-L-idopyranosidurono-6,2-lactone (6)

Compound **6** was synthesized from **6a** (800 mg, 0.91 mmoles) using same procedure as described above for compound **3** and 703 mg, 81% **6** was obtained. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.82 – 7.72 (m, 4H), 7.46 – 7.42 (m, 3H), 7.35 – 7.21 (m, 15H), 5.12 (d, *J* = 3.5 Hz, 1H), 5.08 (s, 1H), 5.04 (s, 2H), 4.96 – 4.90 (m, 2H), 4.78 – 4.74 (m, 2H), 4.66 (s, 2H), 4.53 (d, *J* = 3.2 Hz, 1H), 4.41 – 4.30 (m, 3H), 3.92 – 3.71 (m, 5H), 3.57 – 3.52 (m, 1H), 3.41 (dd, *J* = 10.2, 3.5 Hz, 0H), 3.28 – 3.16 (m, 2H), 1.82 – 1.73 (m, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 169.9, 158.7, 139.5, 138.7, 138.3, 136.9, 134.7, 134.4, 129.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.9, 128.7, 127.6, 127.1, 127.0, 127.0, 100.7, 98.2, 81.2, 81.0, 80.4, 78.9, 76.2, 76.0, 73.5, 72.8, 71.9, 70.8, 68.9, 67.4, 66.9, 64.5, 49.0, 39.6, 30.6. HRMS (ESI - TOF) m/z: $[M]^-$ calcd. for $C_{48}H_{49}N_4O_{15}S^-$ 953.2921; Found 953.2922.

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-4-*O*-benzyl-6-*O*-*tert*-butyldiphenyl silyl-3-*O*-sulfonate-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-3-*O*-benzyl-*α*-L-idopyran osidurono-6,2-lactone (7)**

Compound **7** was synthesized from **7a** (750 mg, 0.71 mmoles) using same procedure as described above for compound **5** and 538 mg, 72% **7** was obtained. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.69 – 7.62 (m, 4H), 7.42 – 7.37 (m, 5H), 7.35 – 7.31 (m, 5H), 7.29 – 7.26 (m, 4H), 7.25 – 7.19 (m, 4H), 7.17 – 7.12 (m, 3H), 5.16 – 5.11 (m, 2H), 5.07 – 4.98 (m, 3H), 4.79 – 4.67 (m, 4H), 4.53 – 4.48 (m, 2H), 4.34 – 4.32 (m, 1H), 3.93 – 3.82 (m, 4H), 3.71 – 3.67 (m, 1H), 3.60 – 3.53 (m, 2H), 3.26 – 3.19 (m, 2H), 1.79 – 1.70 (m, 2H), 1.01 (s, 9H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 169.3, 158.4, 139.3, 138.5, 138.1, 136.7, 136.5, 134.3, 134.0, 130.5, 130.1, 129.4, 129.2, 129.1, 128.8, 128.7, 128.5, 128.4, 128.2, 101.3, 98.1, 81.1, 80.6, 79.5, 77.5, 75.7, 73.2, 73.1, 72.9, 70.5, 68.7, 67.2, 63.9, 63.4, 49.0, 39.4, 30.3, 27.1. HRMS (ESI - TOF) m/z: $[M]^-$ calcd. for $C_{53}H_{59}N_4O_{15}SiS^-$ 1051.3472; Found 1051.3477.

4.5.4 *O*-Sulfation of HS Tetrasaccharides Intermediates



Scheme 3. Synthesis of glucosamine precursors: Reaction condition and reagents: (a) HF.py., pyridine, 0°C, 12 h, 80%; (b) DDQ, DCM:H_2O (18:1), RT, 1 h 71%; (c) Flow reactor, SO_3.NEt_3, DMF

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-[2-azido-4-O-benzyl-3-O-(2- naphthylmethyl)-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-(2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside)-(1→4)-*O*-[2-azido-4-O-benzyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside (8a)**

Compound **8a** was synthesized from **S2**[1,2] (1.2 g, 0.53 mmoles) using same procedure as described above for compound **2** and 744 mg, 80% **8a** was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.5 Hz, 2H), 7.96 (d, *J* = 7.5 Hz, 2H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.79 (s, 2H), 7.75 (t, *J* = 5.2 Hz, 3H), 7.70 (s, 1H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.53 (d, *J* = 7.3 Hz, 1H), 7.49 – 7.45 (m, 5H), 7.45 – 7.40 (m, 5H), 7.37 (s, 1H), 7.33 (d, *J* = 3.2 Hz, 9H), 7.05 (ddd, *J* = 11.9, 8.3, 3.6 Hz, 10H), 5.67 (d, *J* = 9.5 Hz, 1H), 5.50 – 5.44 (m, 2H), 5.24 (t, *J* = 7.2 Hz, 1H), 5.12 – 5.08 (m, 2H), 5.05 – 4.92 (m, 6H), 4.86 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 11.8 Hz, 1H), 4.70 – 4.66 (m, 2H), 4.57 (dd, *J* = 14.7, 10.5 Hz, 3H), 4.42 (t, *J* = 10.7 Hz, 2H), 4.27 (td, *J* = 10.0, 3.5 Hz, 1H), 4.19 – 4.13 (m, 2H), 4.09 (t, *J* = 9.3 Hz, 1H), 4.04 (d, *J* = 8.8 Hz, 1H), 3.92 (t, *J* = 9.3 Hz, 1H), 3.87 – 3.82 (m, 3H), 3.79 (d, *J* = 6.5 Hz, 1H), 3.76 (s, 1H), 3.69 (dd, *J* = 9.4, 4.3 Hz, 3H), 3.65 – 3.61 (m, 2H), 3.55 (d, *J* = 9.7 Hz, 2H), 3.46 (d, *J* = 2.8 Hz, 6H), 3.29 (d, *J* = 10.0 Hz, 1H), 3.16 – 3.10 (m, 2H), 2.12 (t, *J* = 6.2 Hz, 2H), 2.04 (s, 1H), 1.68 (d, *J* = 5.7 Hz, 2H), 1.42 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.1, 168.5, 168.0, 165.1, 165.0, 156.6, 138.0, 136.8, 136.5, 136.5, 136.4, 135.8, 133.9, 133.7, 133.4, 133.3, 133.0, 132.9, 129.8, 129.7, 129.2, 129.0, 128.9, 128.7, 128.7, 128.6, 128.6, 128.4, 128.3, 128.1, 128.1, 128.0, 127.7, 127.7, 126.6, 126.4, 126.3, 126.2, 126.1, 126.1, 126.0, 125.9, 100.8, 100.8, 99.1, 98.4, 81.3, 81.2, 80.2, 77.9, 77.4, 77.3, 77.2, 75.8, 75.6, 75.3, 75.1, 74.9, 74.7, 74.7, 74.3, 73.8, 73.5, 73.4, 72.3, 67.5, 66.6, 61.7, 60.1, 52.9, 52.7, 52.7, 52.2, 38.3, 29.3, 22.9, 22.8. HRMS (ESI - TOF) m/z: $[M+H]^+$ calcd. for $C_{98}H_{103}N_3O_{27}H$ 1754.6812; Found 1754.6812.

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-[2-azido-4-O-benzyl-6-O-tert-butyldiphenyl silyl-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-(2-O-benzoyl-3-O-benzyl-β-D-gluco pyranoside)-(1→4)-O-[2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy -α-D-glucopyranosyl]-(1→4)-O-2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside (9a)**

Compound **9a** was synthesized from **S2**[1,2] (1.2 g, 0.53 mmoles) using same procedure as described above for compound **4** and 734 mg, 71% **9a** was obtained. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.02 (d, *J* = 7.6 Hz, 2H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.71 – 7.65 (m, 7H), 7.61 (d, *J* = 6.7 Hz, 2H), 7.55 (t, *J* = 7.2 Hz, 2H), 7.41 (dt, *J* = 13.6, 7.0 Hz, 11H), 7.32 (dd, *J* = 7.1, 2.7 Hz, 14H), 7.22 (dt, *J* = 5.3, 2.4 Hz, 6H), 7.17 (d, *J* = 2.8 Hz, 1H), 7.14 (dd, *J* = 5.9, 3.2 Hz, 3H), 6.39 (d, *J* = 8.8 Hz, 1H), 5.92 (d, *J* = 9.2 Hz, 1H), 5.45 – 5.41 (m, 1H), 5.29 (t, *J* = 7.8 Hz, 1H), 5.05 (d, *J* = 8.2 Hz, 4H), 4.97 (d, *J* = 3.2 Hz, 2H), 4.81 (d, *J* = 7.9 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.68 (d, *J* = 10.4 Hz, 1H), 4.61 (dd, *J* = 15.4, 9.2 Hz, 3H), 4.54 (d, *J* = 7.0 Hz, 1H), 4.08 (t, *J* = 8.6 Hz, 4H), 4.00 (d, *J* = 9.3 Hz, 1H), 3.95 (d, *J* = 9.4 Hz, 1H), 3.87 (dd, *J* = 10.7, 5.1 Hz, 3H), 3.80 (dd, *J* = 9.2, 5.7 Hz, 4H), 3.74 – 3.61 (m, 6H), 3.44 (s, 3H), 3.32 (d, *J* = 9.8 Hz, 1H), 3.28 (s, 3H), 3.16 – 3.09 (m, 2H), 3.06 (s, 1H), 2.95 (d, *J* = 3.6 Hz, 1H), 1.66 (d, *J* = 5.8 Hz, 2H), 1.50 (s, 3H), 1.36 (s, 3H), 1.09 (d, *J* = 3.8 Hz, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 170.6, 168.0, 167.4, 165.2, 164.9, 156.5, 138.7, 136.5, 136.0, 136.0, 135.8, 135.8, 134.0, 133.9, 133.7, 133.5, 133.2, 132.7, 130.1, 130.1, 129.9, 129.9, 129.8, 129.7, 129.3, 129.0, 128.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.6, 128.1, 128.0, 127.9, 127.8, 101.2, 100.5, 99.9, 99.0, 81.6, 81.3, 78.3, 78.2, 77.2, 76.4, 75.5, 75.2, 75.0, 75.0, 74.6, 73.7, 73.2, 72.8, 71.6, 69.9, 67.5, 66.5, 61.4, 54.9, 53.4, 53.2, 52.6, 38.0, 29.8, 29.5, 27.2, 27.1, 22.8, 22.8, 22.3, 19.7, 19.5. HRMS (ESI - TOF) m/z: $[M]^+$ calcd. for $C_{108}H_{123}N_3O_{27}Si_2$ 1950.7916; Found 1950.7912.

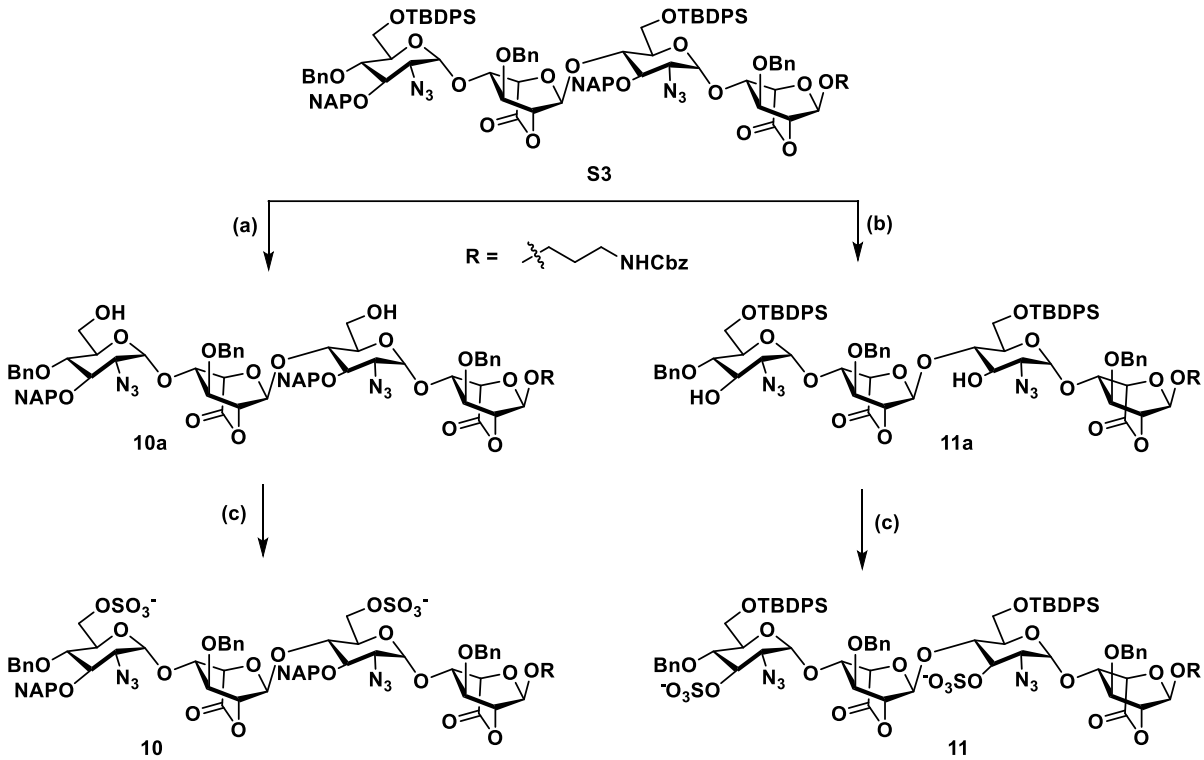
_N_-Benzyloxycarbonyl-3-aminopropyl-_O_-[2-azido-4-O-benzyl-6-_O_-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-(2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside)-(1→4)-_O_-[2-azido-4-O-benzyl-6-_O_-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside (8)

Compound **8** was synthesized from **8a** (500 mg, 0.28 mmoles) and $SO_3.NEt_3$ (20 equivalents, 10 equivalents per OH) using same procedure as described above for compound **3** and 264 mg, 73% **8** was obtained. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.27 (d, *J* = 7.5 Hz, 2H), 7.99 (d, *J* = 7.7 Hz, 2H), 7.90 (d, *J* = 6.0 Hz, 1H), 7.83 – 7.79 (m, 4H), 7.77 (d, *J* = 6.6 Hz, 2H), 7.71 (s, 1H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.56 (dt, *J* = 15.2, 7.6 Hz, 3H), 7.48 – 7.37 (m, 8H), 7.31 (s, 7H), 7.26 (d, *J* = 7.5 Hz, 3H), 7.10 – 7.08 (m, 3H), 7.07 – 7.04 (m, 3H), 6.99 – 6.94 (m, 4H), 5.39 – 5.30 (m, 4H), 5.25 (d, *J* = 11.2 Hz, 1H), 5.12 (t, *J* = 8.2 Hz, 1H), 5.00 (s, 2H), 4.97 (s, 1H), 4.79 (d, *J* = 10.7 Hz, 1H), 4.70 (d, *J* = 10.7 Hz, 2H), 4.60 (d, *J* = 3.8 Hz, 2H), 4.50 (d, *J* = 16.2 Hz, 3H), 4.39 (d, *J* = 9.0 Hz, 1H), 4.34 – 4.21 (m, 5H), 4.15 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.12 – 3.97 (m, 5H), 3.78 (q, *J* = 11.7, 10.3 Hz, 2H), 3.70 (d, *J* = 8.8 Hz, 1H), 3.67 – 3.59 (m, 5H), 3.47 (s, 4H), 3.37 (d, *J* = 7.8 Hz, 1H), 3.17 (s, 3H), 3.01 (dd, *J* = 13.2, 6.8 Hz, 2H), 1.83 (s, 3H), 1.80 (s, 3H), 1.63 – 1.56 (m, 2H). ^{13}C NMR (100 MHz, Methanol-

d$_4$) δ 173.3, 173.1, 170.2, 166.5, 166.4, 158.6, 139.6, 138.2, 137.6, 137.3, 134.8, 134.6, 134.3, 131.1, 130.6, 130.6, 130.4, 130.0, 129.6, 129.3, 129.2, 128.8, 128.7, 128.5, 127.7, 127.5, 127.0, 126.9, 126.6, 102.0, 101.6, 98.9, 98.6, 83.6, 83.3, 81.5, 79.2, 78.9, 78.3, 76.2, 75.7, 75.4, 74.9, 74.8, 71.6, 71.3, 71.1, 68.3, 67.1, 66.8, 54.0, 53.3, 53.1, 52.8, 49.0, 38.4, 30.6, 22.8. HRMS (ESI - TOF) m/z: $[M]^-$ calcd. for $C_{98}H_{101}N_3O_{33}S_2^{2-}$ 956.2901; Found 956.2859.

_N_-Benzyloxycarbonyl-3-aminopropyl-_O_-[2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-_O_-sulfonato-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-(2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside)-(1→4)-O-[2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-2-O-benzoyl-3-O-benzyl-β-D-glucopyranoside (9)

Compound **9** was synthesized from **9a** (500 mg, 0.25 mmoles) using same procedure as described above for compound **5** and 343 mg, 65% **9** was obtained. ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 8.07 (d, *J* = 7.8 Hz, 2H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.81 (d, *J* = 3.8 Hz, 2H), 7.74 (d, *J* = 6.9 Hz, 2H), 7.72 (s, 1H), 7.70 (s, 2H), 7.69 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 4.1 Hz, 3H), 7.50 (s, 2H), 7.49 – 7.46 (m, 3H), 7.44 (d, *J* = 6.5 Hz, 3H), 7.40 (d, *J* = 7.6 Hz, 4H), 7.36 (d, *J* = 5.7 Hz, 5H), 7.36 – 7.27 (m, 7H), 7.23 – 7.20 (m, 2H), 7.15 (dd, *J* = 5.9, 2.4 Hz, 7H), 5.69 – 5.66 (m, 1H), 5.55 (d, *J* = 3.3 Hz, 1H), 5.43 (d, *J* = 9.9 Hz, 2H), 5.18 (d, *J* = 16.7 Hz, 1H), 5.04 (s, 2H), 4.98 – 4.92 (m, 1H), 4.83 (d, *J* = 10.8 Hz, 1H), 4.78 (d, *J* = 7.4 Hz, 1H), 4.71 (d, *J* = 10.7 Hz, 1H), 4.64 (dd, *J* = 10.5, 5.1 Hz, 2H), 4.57 (d, *J* = 10.6 Hz, 1H), 4.54 – 4.48 (m, 1H), 4.18 (t, *J* = 9.1 Hz, 2H), 4.12 (dd, *J* = 9.1, 5.5 Hz, 4H), 4.08 (s, 1H), 4.01 (dd, *J* = 15.8, 5.3 Hz, 3H), 3.94 (d, *J* = 9.5 Hz, 1H), 3.92 – 3.87 (m, 2H), 3.81 (d, *J* = 10.5 Hz, 2H), 3.68 (s, 3H), 3.63 (s, 1H), 3.52 (d, *J* = 9.7 Hz, 2H), 3.27 (d, *J* = 9.7 Hz, 1H), 3.19 (s, 3H), 3.05 (q, *J* = 6.2 Hz, 2H), 1.94 (d, *J* = 3.7 Hz, 6H), 1.67 – 1.62 (m, 2H), 1.13 (s, 9H), 1.11 (s, 9H). ^{13}C NMR (100 MHz, Methanol-*d*$_4$) δ 174.6, 174.4, 172.0, 172.0, 171.1, 167.5, 166.9, 159.3, 140.9, 139.4, 139.3, 139.0, 137.7, 137.6, 137.3, 135.5, 135.5, 135.3, 135.1, 134.7, 134.3, 132.2, 131.9, 131.6, 131.4, 131.4, 131.3, 131.0, 130.9, 130.5, 130.3, 130.0, 130.0, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.5, 129.4, 129.3, 129.0, 102.6, 101.9, 98.8, 98.7, 84.3, 83.8, 79.2, 78.0, 76.7, 76.5, 76.4, 76.3, 75.9, 75.7, 75.7, 75.5, 75.0, 74.7, 74.2, 71.5, 71.3, 69.0, 67.8, 67.0, 63.9, 62.8, 56.3, 55.2, 54.7, 53.7, 49.0, 39.1, 34.6, 32.4, 31.3, 28.2, 28.0, 23.7, 23.6, 21.0, 20.8. HRMS (ESI - TOF) m/z: $[M+Na]^+$ calcd. for $C_{108}H_{121}N_3O_{33}Si_2NaS_2^{2-}$ 2130.6771; Found 2130.6771.



Scheme 4. Synthesis of glucosamine precursors: Reaction condition and reagents: (a) HF.py., pyridine, 0 ^{o}C, 12 h, 80%; (b) DDQ, DCM:H_2O (18:1), RT, 1 h 70%; (c) Flow reactor, SO_3.NEt_3, DMF

***N*-Benzyloxycarbonyl-3-aminopropyl-O-[(2-azido-4-*O*-benzyl-3-*O*-(2-naphthylmethyl)-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-O-(3-*O*-benzyl-*α*-L-idopyranosidurono-6,2-lactone)-(1→4)-O-[(2-azido-3-*O*-(2-naphthylmethyl)-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-O-3-O-benzyl-*α*-L-idopyranosidurono-6,2-lactone (10a)**

Compound **10a** was synthesized from **S3**[1,2] (1.2 g, 0.62 mmoles) using same procedure as described above for compound **2** and 719 mg, 80% **10a** was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.89 – 7.76 (m, 6H), 7.72 (s, 1H), 7.53 – 7.50 (m, 2H), 7.49 – 7.45 (m, 3H), 7.44 – 7.40 (m, 2H), 7.38 (s, 2H), 7.37 – 7.31 (m 8H), 7.30 – 7.29 (m, 7H), 7.24 (dd, J = 7.5, 1.9 Hz, 2H), 5.75 (t, J = 5.1 Hz, 1H), 5.35 (s, 1H), 5.16 (d, J = 12.1 Hz, 1H), 5.09 – 5.07 (m, 3H), 5.01 (s, 1H), 4.98 – 4.93 (m, 3H), 4.79 – 4.70 (m, 3H), 4.67 – 4.64 (m, 1H), 4.62 – 4.56 (m, 2H), 4.52 (t, J = 3.4 Hz, 2H), 4.46 (t, J = 2.6 Hz, 1H), 4.30 – 4.28 (m, 2H), 3.95 – 3.85 (m, 5H), 3.82 – 3.76 (m, 2H), 3.73 (td, J = 3.1, 1.5 Hz, 1H), 3.71 – 3.68 (m, 3H), 3.66 – 3.61 (m, 2H), 3.57 (d, J = 3.8 Hz, 1H), 3.51 – 3.27 (m, 6H), 2.83 (dd, J = 10.4, 3.7 Hz, 1H), 2.05 (s, 1H), 1.85 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 168.0, 156.7, 137.9, 137.1, 136.9, 136.8, 136.1, 135.3, 133.6, 133.4, 133.2, 133.0, 129.8, 128.7, 128.7, 128.6, 128.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8,

127.7, 127.0, 126.3, 126.2, 126.1, 126.1, 126.0, 125.2, 124.8, 99.8, 99.6, 97.1, 96.8, 80.9, 80.8, 79.7, 78.6, 78.3, 78.1, 77.8, 77.4, 77.2, 76.6, 75.7, 75.1, 74.1, 72.8, 72.8, 72.4, 72.4, 72.2, 72.2, 69.6, 69.4, 69.3, 66.8, 63.4, 63.3, 61.2, 39.9, 29.6. HRMS (ESI - TOF) m/z: $[M+Na]^+$ calcd. for $C_{78}H_{79}N_7O_{21}Na$ 1472.5227; Found 1472.5231.

***N*-Benzyloxycarbonyl-3-aminopropyl-O-[(2-azido-4-*O*-benzyl-6-O-*tert*-butyldiphenylsilyl-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-O-(3-O-benzyl-*α*-L-idopyranosidurono-6,2-lactone)-(1→4)-O-[(2-azido-6-O-*tert*-butyldiphenylsilyl-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-O-3-*O*-benzyl-*α*-L-idopyranosidurono-6,2-lactone (11a)**

Compound **11a** was synthesized from **S3**[1,2] (1.2 g, 0.62 mmoles) using same procedure as described above for compound **4** and 715 mg, 70% **11a** was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (t, J = 1.3 Hz, 1H), 7.70 – 7.67 (m, 3H), 7.63 – 7.61 (m, 4H), 7.46 – 7.42 (m, 3H), 7.41 – 7.34 (m, 12H), 7.33 – 7.29 (m, 13H), 7.26 – 7.23 (m, 4H), 5.82 (t, J = 5.4 Hz, 1H), 5.12 – 5.08 (m, 3H), 5.04 (dd, J = 2.3, 1.0 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.91 (d, J = 3.6 Hz, 1H), 4.81 – 4.69 (m, 3H), 4.62 (d, J = 12.4 Hz, 1H), 4.54 (d, J = 11.6 Hz, 1H), 4.45 – 4.42 (m, 2H), 4.36 (dd, J = 5.9, 3.3 Hz, 2H), 4.22 – 4.19 (m, 1H), 4.13 – 4.11 (m, 1H), 4.03 (t, J = 2.6 Hz, 1H), 4.00 – 3.92 (m, 5H), 3.90 (dd, J = 12.0, 1.8 Hz, 1H), 3.81 – 3.79 (m, 2H), 3.77 – 3.75 (m, 2H), 3.72 (s, 1H), 3.70 – 3.68 (m, 2H), 3.65 – 3.59 (m, 2H), 3.49 – 3.41 (m, 1H), 3.35 – 3.28 (m, 1H), 3.24 (dd, J = 10.4, 3.7 Hz, 1H), 3.18 (dd, J = 10.5, 3.7 Hz, 1H), 2.46 (s, 1H), 1.86 (s, 1H), 1.09 (s, 9H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 166.5, 156.7, 138.1, 137.0, 136.8, 136.7, 136.1, 135.9, 135.8, 135.7, 133.5, 133.2, 132.9, 130.1, 130.0, 130.0, 129.9, 128.8, 128.8, 128.7, 128.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 100.3, 100.0, 98.0, 96.9, 81.2, 80.6, 79.9, 79.2, 78.8, 78.2, 77.4, 77.2, 75.0, 72.8, 72.7, 72.4, 71.8, 71.3, 71.2, 70.4, 69.7, 69.6, 69.4, 66.8, 62.8, 62.6, 62.4, 61.8, 40.0, 29.6, 27.1. HRMS (ESI - TOF) m/z: $[M+Na]^+$ calcd. for $C_{88}H_{99}N_7O_{21}Si_2Na$ 1668.6330; Found 1668.6327.

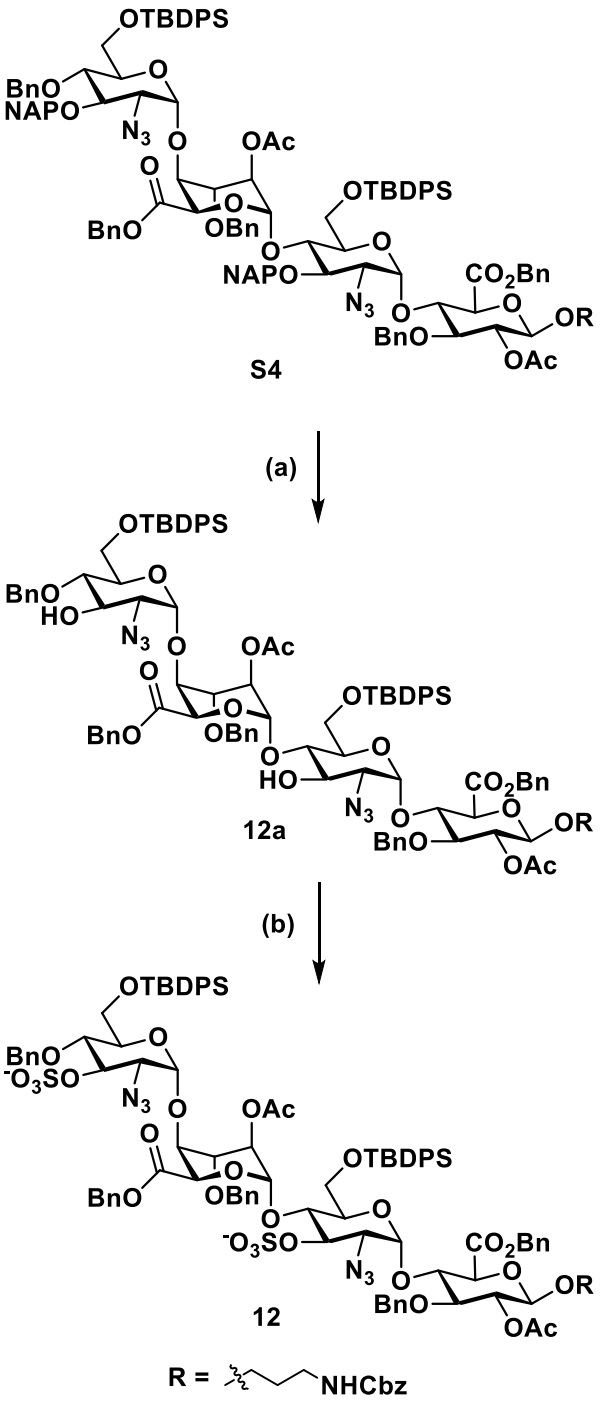
***N*-Benzyloxycarbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-O-(3-*O*-benzyl-*α*-L-idopyranosidurono-6,2-lactone)-(1→4)-O-[(2-azido-6-*O*-sulfonato-3-*O*-(2-naphthylmethyl)-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-O-3-*O*-benzyl-*α*-L-idopyranosidurono-6,2-lactone (10)**

Compound **10** was synthesized from **10a** (450 mg, 0.31 mmoles) using same procedure as described above for compound **3** and 374 mg, 75% **10** was obtained. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.85 – 7.80 (m, 4H), 7.79 (d, *J* = 2.2 Hz, 1H), 7.76 – 7.74 (m, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.58- 7.54 (m, 2H), 7.52 – 7.50 (m, 1H), 7.49 (d, *J* = 2.1 Hz, 1H), 7.48 – 7.39 (m, 8H), 7.38 – 7.26 (m, 13H), 5.29 (s, 1H), 5.18 (d, *J* = 3.2 Hz, 1H), 5.15 (d, *J* = 3.5 Hz, 1H), 5.07 (s, 2H), 4.99 (d, *J* = 2.9 Hz, 1H), 4.93 (s, 2H), 4.82 (d, *J* = 3.1 Hz, 2H), 4.80 – 4.79 (m, 2H), 4.76- 4.75 (m, 5H), 4.34 – 4.26 (m, 2H), 4.23 (dt, *J* = 4.9, 2.4 Hz, 1H), 4.20 – 4.18 (m, 1H), 4.12 (d, *J* = 9.4 Hz, 1H), 4.04 (t, *J* = 3.8 Hz, 1H), 3.98- 3.93 (m, 3H), 3.91 – 3.84 (m, 3H), 3.78 – 3.68 (m, 4H), 3.67 – 3.58 (m, 3H), 3.55 (dd, *J* = 9.9, 3.5 Hz, 1H), 3.45 (d, *J* = 9.5 Hz, 1H), 3.26 (tt, *J* = 13.0, 6.5 Hz, 2H), 1.86- 1.79 (m, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 171.6, 171.4, 158.8, 139.7, 139.4, 139.3, 138.3, 136.9, 136.5, 134.8, 134.7, 134.4, 134.4, 132.4, 129.9, 129.7, 129.5, 129.4, 129.4, 129.2, 129.2, 129.1, 129.0, 129.0, 128.9, 128.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 127.7, 127.6, 127.3, 127.0, 127.0, 126.9, 102.9, 101.9, 97.6, 97.0, 81.4, 80.3, 78.6, 76.2, 76.1, 75.8, 75.4, 74.2, 74.0, 73.7, 73.1, 72.1, 71.8, 70.0, 69.9, 69.7, 69.4, 69.1, 67.8, 67.4, 67.0, 66.7, 65.5, 64.9, 53.1, 52.2, 49.0, 40.2, 39.4, 34.9, 33.1, 32.1, 30.5, 30.1, 24.9, 24.0, 23.7. HRMS (ESI - TOF) m/z: $[M]^{2-}$ calcd. for $C_{78}H_{77}N_7O_{27}S_2^{2-}$ 803.7160; Found 803.7170.

_N_-Benzyloxycarbonyl-3-aminopropyl-_O_-[(2-azido-4-_O_-benzyl-6-_O_-_tert_-butyldiphenylsilyl-3-_O_-sulfonato-2-deoxy-_α_-D-glucopyranosyl]-(1→4)-_O_-(3-_O_-benzyl-_α_-L-idopyranosidurono-6,2-lactone)-(1→4)-_O_-[(2-azido-6-_O_-_tert_-butyldiphenylsilyl-3-O-sulfonato-2-deoxy-_α_-D-glucopyranosyl]-(1→4)-_O_-3-_O_-benzyl-_α_-L-idopyranosidurono-6,2-lactone (11)

Compound **11** was synthesized from **11a** (450 mg, 0.31 mmoles) using same procedure as described above for compound **5** and 380 mg, 68% **11** was obtained. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.68 – 7.67 (m, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.65 – 7.61 (m, 4H), 7.60 (s, 1H), 7.58 (d, J = 1.3 Hz, 1H), 7.41 (d, J = 7.4 Hz, 2H), 7.39 – 7.34 (m, 4H), 7.32 – 7.29 (m, 9H), 7.29 – 7.22 (m, 13H), 7.20 – 7.13 (m, 4H), 5.49 (s, 1H), 5.18 (d, J = 10.6 Hz, 1H), 5.13 (d, J = 3.4 Hz, 1H), 5.06 (s, 1H), 5.02- 5.00 (m, 2H), 4.98 – 4.87 (m, 2H), 4.76 – 4.70 (m, 5H), 4.56 (dd, J = 18.2, 11.0 Hz, 2H), 4.43 – 4.42 (m, 1H), 4.41- 4.39 (m, 2H), 4.35 – 4.32 (m, 1H), 4.07 – 3.94 (m, 3H), 3.87- 3.83 (m, 3H), 3.80- 3.65 (m, 5H), 3.55 (dt, J = 9.3, 5.5 Hz, 1H), 3.26 – 3.16 (m, 3H), 1.78 – 1.74 (m, 2H), 0.99 (s, 9H), 0.96 (s, 9H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 170.0, 169.2, 158.7, 139.8, 139.0, 138.7, 138.3, 137.0, 136.9, 136.8,

136.8, 134.7, 134.4, 134.3, 134.1, 130.9, 130.9, 130.8, 129.7, 129.6, 129.5, 129.5, 129.4, 129.4, 129.1, 129.0, 129.0, 129.0, 128.8, 128.8, 128.7, 128.7, 128.4, 101.5, 101.1, 98.4, 95.9, 81.2, 80.9, 80.8, 80.5, 79.9, 79.4, 77.9, 76.9, 76.1, 75.4, 73.3, 73.2, 72.9, 72.7, 70.9, 70.8, 69.0, 67.4, 64.2, 63.9, 63.8, 63.5, 49.0, 39.7, 30.6, 27.4. HRMS (ESI - TOF) m/z: $[M-2H]^{2-}$ calcd. for $C_{88}H_{97}N_7O_{27}S_2Si_2^{2-}$ 901.7711; Found 901.7706.



Scheme 5. Synthesis of glucosamine precursors: Reaction condition and reagents: (a) DDQ, DCM:H_2O (18:1), RT, 1 h 70%; (b) Flow reactor, $SO_3.NEt_3$, DMF

***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-4-*O*-benzyl-6-*O*-*tert*-butyldiphenyl silyl-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-(2-O-acetyl-3-*O*-benzyl-6-benzylcarbonate-α-L-idopyranosidurono)-(1→4)-*O*-[(2-azido-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-2-*O*-acety-3-*O*-benzyl-6-benzylcarbonate-β-D-glucopyranoside (12a)**

Compound **12a** was synthesized from **S4**[1,2] (1.3 g, 0.58 mmoles) using same procedure as described above for compound **4** and 847 mg, 75% **12** was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 6.4 Hz, 2H), 7.70 (dt, *J* = 7.5, 4.8 Hz, 6H), 7.46 – 7.29 (m, 38H), 7.24 – 7.21 (m, 4H), 5.47 (d, *J* = 6.2 Hz, 1H), 5.37 (d, *J* = 3.4 Hz, 1H), 5.15 (d, *J* = 9.4 Hz, 1H), 5.11 (d, *J* = 3.0 Hz, 4H), 5.08 – 5.07 (d, *J* = 6.0 Hz, 2H), 5.02 (d, *J* = 12.3 Hz, 1H), 4.95 (t, *J* = 6.4 Hz, 1H), 4.86 – 4.79 (m, 3H), 4.75 – 4.65 (m, 6H), 4.50 (d, *J* = 6.8 Hz, 1H), 4.23 – 4.17 (m, 1H), 4.12 – 4.08 (m, 2H), 4.03 (d, *J* = 9.2 Hz, 1H), 3.96 – 3.91 (m, 3H), 3.88 – 3.85 (m, 4H), 3.82 – 3.78 (m, 3H), 3.78 – 3.74 (m, 1H) 3.67 (t, *J* = 9.7 Hz, 1H), 3.57 – 3.52 (m, 2H), 3.43 (d, *J* = 9.5 Hz, 1H), 3.17 – 3.11 (m, 2H), 2.28 – 2.26 (m, 1H), 2.02 (s, 3H), 1.78 – 1.72 (m, 5H), 1.10 (s, 9H), 1.09 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.8, 168.9, 168.7, 156.9, 138.6, 138.0, 137.9, 137.2, 136.4, 136.2, 136.1, 135.2, 135.1, 134.0, 133.7, 133.4, 133.3, 130.2, 130.2, 129.5, 129.1, 129.0, 129.0, 128.9, 128.9, 128.8, 128.8, 128.7, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 100.9, 99.9, 98.9, 97.3, 82.5, 80.4, 78.7, 77.2, 76.8, 75.5, 74.8, 74.6, 74.2, 74.2, 74.0, 73.0, 72.9, 72.4, 72.0, 71.8, 71.4, 70.3, 67.9, 67.8, 67.5, 66.9, 63.2, 63.0, 62.9, 61.5, 38.6, 29.8, 27.4, 27.3, 21.3, 20.9, 19.8, 19.7. HRMS (ESI - TOF) m/z: [M+H] calcd. for $C_{106}H_{119}N_7O_{25}Si_2H$ 1947.7906; Found 1947.7892.

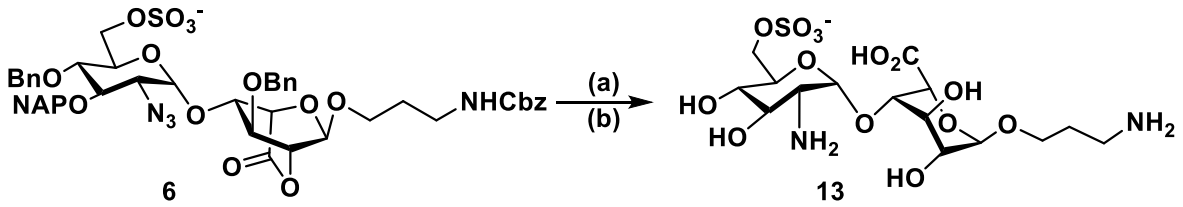
***N*-Benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-4-*O*-benzyl-6-*O*-*tert*-butyldiphenyl silyl-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-(2-O-acetyl-3-*O*-benzyl-6-benzylcarbonate-α-L-idopyranosidurono)-(1→4)-*O*-[(2-azido-4-*O*-benzyl-6-*O*-*tert*-butyl diphenylsilyl-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-2-*O*-acety-3-*O*-benzyl-6-benzylcarbonate-β-D-glucopyranoside (12)**

Compound **12** was synthesized from **12a** (500 mg, 0.25 mmoles) using same procedure as described above for compound **5** and 331 mg, 63% **12** was obtained. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.65 (t, *J* = 7.5 Hz, 6H), 7.58 (d, *J* = 7.0 Hz, 2H), 7.42 – 7.35 (m, 8H), 7.33 (m, 13H), 7.27 – 7.22 (m, 6H), 7.19 – 7.01 (m, 15H), 5.39 – 5.31 (m, 4H), 5.11 – 5.02 (m, 4H), 4.94 (dd, *J* = 12.1, 5.3 Hz, 3H), 4.85 – 4.80 (m, 2H), 4.75 – 4.70 (m, 3H), 4.60 – 4.56

(m, 1H), 4.50 – 4.45 (m, 2H), 4.34 (d, *J* = 12.2 Hz, 1H), 4.07 – 4.00 (m, 5H), 3.94 – 3.85 (m, 3H), 3.79 – 3.70 (m, 4H), 3.61 (t, *J* = 11.9 Hz, 2H), 3.51 – 3.47 (m, 1H), 3.42 (d, *J* = 9.7 Hz, 1H), 3.15 – 3.06 (m, 4H), 2.07 (s, 3H), 2.02 (s, 3H), 1.71 – 1.65 (m, 2H), 1.01 (s, 9H), 0.99 (s, 9H). ^{13}C NMR (400 MHz, Methanol-d_4) δ 171.9, 171.5, 170.1, 158.8, 140.1, 139.3, 138.7, 138.4, 137.3, 137.0, 137.0, 136.8, 136.2, 136.2, 134.9, 134.6, 134.6, 134.5, 130.9, 130.8, 130.8, 130.8, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.4, 129.3, 129.1, 129.0, 128.9, 128.9, 128.8, 128.7, 128.5, 128.5, 101.8, 101.5, 98.7, 97.6, 83.4, 80.1, 77.9, 77.8, 77.7, 76.3, 75.3, 75.1, 74.9, 74.3, 73.8, 73.5, 73.2, 72.4, 71.5, 69.6, 69.4, 68.9, 68.5, 68.1, 67.3, 63.8, 63.5, 63.1, 49.0, 38.7, 30.7, 27.6, 27.5, 21.1, 21.0, 20.2, 20.1. HRMS (ESI - TOF) m/z: [M] calcd. for $C_{106}H_{117}N_7Si_2S_2^{2-}$ 1052.3409; Found 1052.3449.

4.5.5 Synthesis of Fully Deprotected HS Oligosaccharides

A. Deprotection of Disaccharide Substrate



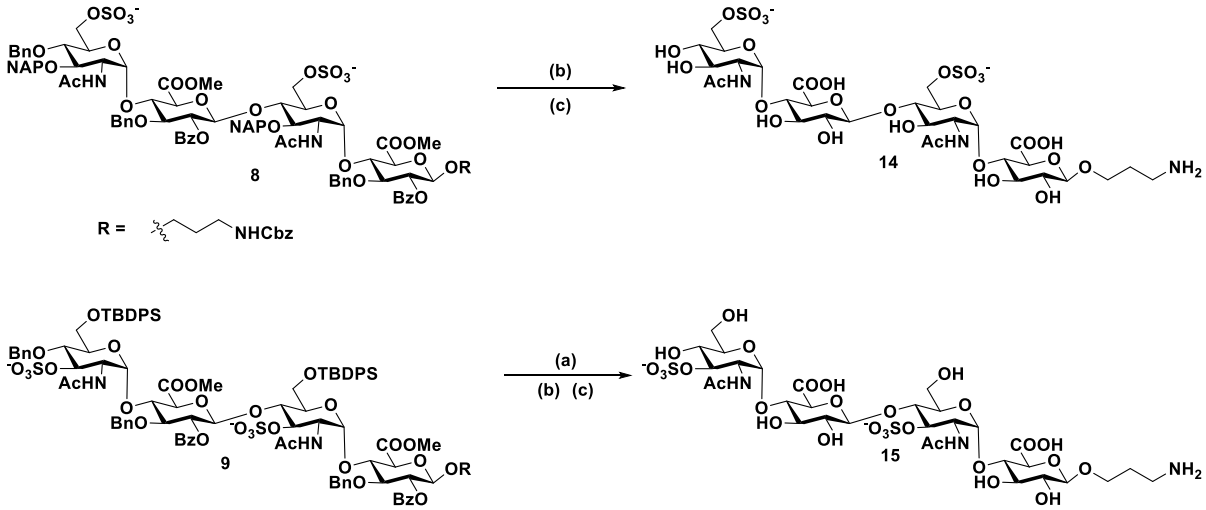
Scheme 6. Reaction condition and reagents: (a) LiOH.H_2O, MeOH, RT, 1h, 95%; (b) H_2, $Pd(OH)_2$, MeOH, RT, 40 h

3-Aminopropyl-*O*-(2-amino-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-*α*-L-idopyranosiduronate (13)

To a solution of **6** (100 mg, 0.104mmoles) in 1:1 ratio of water (1 ml) and methanol (1 mL) LiOH.H_2O was added and kept it for stirring for 1 h at RT. Upon completion, the reaction was quenched by using Dowex 50WX8 H^+ resin. The mixture was filtered, concentrated and passed through Bond Elute C-18 column (H_2O/ MeOH = 1/5, v/v). Then the combined fraction was concentrated under reduce pressure and dissolve in methanol for hydrogenolysis. The reaction was stirred with $Pd(OH_2)$ (catalytic amount) for 36 h under H_2 atmosphere. Upon completion, the reaction mixture was filtered, concentrated and passed through Bond Elute C-18 column using H_2O as eluent. The combined H_2O fraction was lyophilized to yield

fully deprotected 6-*O*- sulfated disaccharide **13** in 51 mg, 70% yield. ^{1}H NMR (400 MHz, D_2O) δ 5.45 (d, *J* = 3.6 Hz, 1H), 4.84 (d, *J* = 2.9 Hz, 2H), 4.50 – 4.47 (m, 1H), 4.31 (dd, *J* = 11.2, 3.4 Hz, 1H), 4.22 (dd, *J* = 11.2, 2.0 Hz, 1H), 4.13 – 4.12 (m, 1H), 4.06 – 4.02 (m, 1H), 3.97 (dt, *J* = 10.0, 2.5 Hz, 1H), 3.90 – 3.78 (m, 3H), 3.71 – 3.65 (m, 2H), 3.54 (t, *J* = 9.7 Hz, 1H), 3.36 – 3.31 (m, 1H), 1.99 – 1.94 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 100.6, 92.1, 73.0, 70.4, 69.2, 68.7, 68.7, 68.6, 67.4, 66.4, 66.2, 53.9, 38.1, 26.4. HRMS (ESI - TOF) m/z: [M] calcd. for $C_{15}H_{27}N_2O_{14}S^-$ 491.1188; Found 491.1182.

B. Deprotection of Tetrasaccharide Substrates



Scheme 7. Reaction condition and reagents: (a) HF.py, pyridine, 0°C, 12 h, 83%; (b) LiOH.H_2O, MeOH, RT, 1h, 95%; (c) H_2, Pd(OH_2), MeOH, RT, 40 h

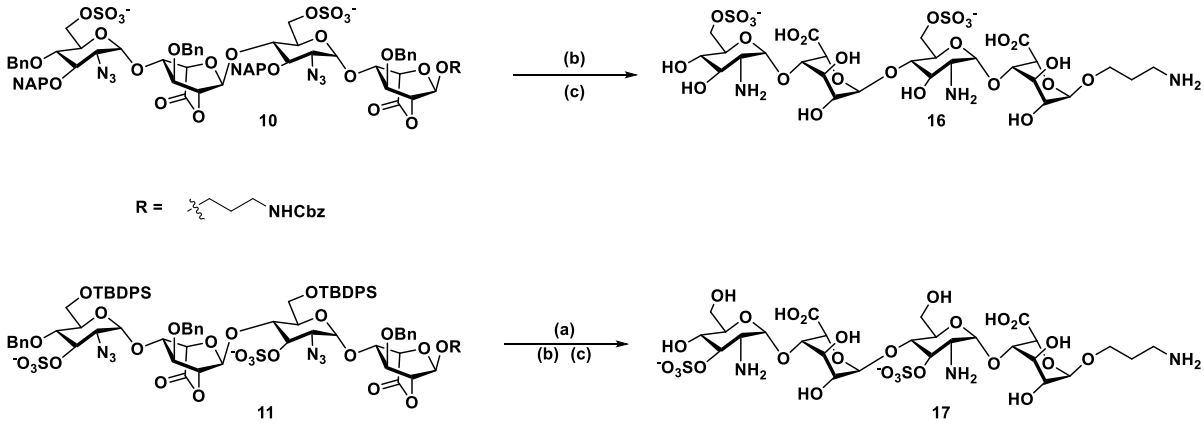
3-Aminopropyl-*O*-(2-acetamido-6-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosiduronate)-(1→4)-*O*-(2-acetamido-6-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosiduronate (14)

Compound **14** was synthesized from **8** (100 mg, 0.052 mmoles) using same procedure as described above for compound **13** and 47 mg, 91% **11** was obtained. ^{1}H NMR (400 MHz, D_2O) δ 5.28 (t, *J* = 3.2 Hz, 2H), 4.55 (d, *J* = 7.9 Hz, 1H), 4.44 (d, *J* = 8.0 Hz, 1H), 4.35 (s, 1H), 4.24 (dd, *J* = 11.0, 2.8 Hz, 1H), 4.11 (t, *J* = 11.4 Hz, 2H), 3.98 (d, *J* = 9.5 Hz, 1H), 3.91 (d, *J* = 9.3 Hz, 1H), 3.87 (d, *J* = 5.5 Hz, 1H), 3.85 (d, *J* = 5.4 Hz, 2H), 3.76 (d, *J* = 9.7 Hz, 3H), 3.71 (m, 3H), 3.66 (d, *J* = 5.2 Hz, 2H), 3.62 (d, *J* = 9.6 Hz, 2H), 3.49 (t, *J* = 9.6 Hz, 1H), 3.28 (q, *J* = 8.4 Hz, 2H), 3.07 (t, *J* = 6.8 Hz, 2H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 – 1.90 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 174.3, 173.0, 102.2, 101.9, 97.7, 97.3, 77.4, 76.7, 76.7,

76.0, 75.8, 75.0, 74.6, 73.3, 73.2, 70.4, 70.4, 69.1, 69.0, 68.8, 68.0, 66.3, 65.7, 53.4, 53.1, 37.6, 26.7, 21.9. HRMS (ESI - TOF) m/z: $[M]^{2-}$ calcd. for $C_{31}H_{49}N_3O_{29}S_2^{2-}$ 495.5952; Found 495.6048.

3-Aminopropyl-*O*-(2-acetamido-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosiduronate)-(1→4)-*O*-(2-acetamido-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosiduronate (15)

Compound **15** was synthesized from **9** (100 mg, 0.061 mmoles) using same procedure as described above for compound **13** and 53 mg, 88% **15** was obtained. ^{1}H NMR (400 MHz, D_2O) δ 5.39 (t, J = 4.1 Hz, 2H), 4.62 (d, J = 7.8 Hz, 1H), 4.56 (d, J = 9.7 Hz, 1H), 4.51 (d, J = 8.2 Hz, 1H), 4.48 – 4.42 (m, 1H), 4.09 (ddd, J = 14.1, 10.7, 3.6 Hz, 2H), 4.03 – 3.95 (m, 3H), 3.93 (d, J = 2.6 Hz, 1H), 3.89 – 3.80 (m, 7H), 3.75 (q, J = 7.6, 6.4 Hz, 4H), 3.69 (d, J = 8.9 Hz, 1H), 3.42 (t, J = 8.5 Hz, 1H), 3.33 (s, 1H), 3.16 (t, J = 6.6 Hz, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 1.99 (d, J = 6.2 Hz, 2H). ^{13}C NMR (100 MHz, D_2O) δ 176.9, 104.7, 103.7, 100.2, 99.9, 82.1, 79.5, 79.3, 79.1, 78.6, 78.4, 75.9, 75.8, 75.7, 74.5, 74.0, 70.6, 70.5, 62.3, 61.9, 55.2, 54.7, 40.1, 29.2, 24.7, 24.6. HRMS (ESI - TOF) m/z: $[M]^{2-}$ calcd. for $C_{31}H_{49}N_3O_{29}S_2^{2-}$ 495.5952; Found 495.6048.



Scheme 8. Reaction condition and reagents: (a) HF.py, pyridine, 0^{o}C, 12 h, 83%; (b) LiOH.H_2O, MeOH, RT, 1h, 95%; (c) H_2, Pd(OH_2), MeOH, RT, 40 h

3-Aminopropyl-*O*-(2-amino-6-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-(*α*-L-idopyranosiduronate)-(1→4)-*O*-[(2-amino-6-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-*α*-L-idopyranosiduronate (16)

Compound **16** was synthesized from **8** (100 mg, 0.052 mmoles) using same procedure as described above for compound **13** and 47 mg, 92% **16** was obtained. ^{1}H NMR (600 MHz, D_2O) δ 5.41 (d, J = 3.7 Hz, 1H), 5.39 (d, J = 3.6 Hz, 1H), 5.04 – 5.03 (m, 3H), 4.15 – 4.11 (m, 1H), 4.07 – 3.97 (m, 1H), 3.85 – 3.81 (m, 2H), 3.80- 3.72 (m, 5H), 3.70 – 3.59 (m, 5H), 3.50 – 3.39 (m, 2H), 3.30 (td, J = 10.7, 10.0, 3.6 Hz, 1H), 3.08 - 3.05 (m, 2H), 2.76 (d, J = 5.2 Hz, 1H), 1.96 - 1.88 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 171.3, 102.0, 100.9, 93.2, 92.7, 76.7, 73.4, 73.0, 72.5, 71.9, 69.4, 69.0, 68.5, 68.0, 67.8, 67.4, 66.8, 66.0, 60.0, 59.3, 54.0, 53.8, 38.0, 26.3. HRMS (ESI - TOF) m/z: $[M]^{2-}$ calcd. for $C_{27}H_{45}N_3O_{27}S_2^{2-}$ 453.5846 Found 453.5833.

3-Aminopropyl-*O*-(2-amino-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-(*α*-L-idopyranosiduronate)-(1→4)-*O*-[(2-amino-3-*O*-sulfonato-2-deoxy-*α*-D-glucopyranosyl]-(1→4)-*O*-*α*-L-idopyranosiduronate (17)

Compound **17** was synthesized from **9** (100 mg, 0.061 mmoles) using same procedure as described above for compound **13** and 54 mg, 89% **17** was obtained. ^{1}H NMR (600 MHz, D_2O) δ 5.51 (d, J = 3.5 Hz, 1H), 5.41 – 5.39 (m, 1H), 4.95 (d, J = 2.8 Hz, 1H), 4.87 (d, J = 4.0 Hz, 1H), 4.56 – 4.50 (m, 1H), 4.48 – 4.42 (m, 2H), 4.19 (t, J = 3.6 Hz, 1H), 4.08 – 4.05 (m, 1H), 3.99 (t, J = 3.8 Hz, 1H), 3.93 (t, J = 9.6 Hz, 1H), 3.89 (s, 1H), 3.88 – 3.84 (m, 2H), 3.82 – 3.77 (m, 6H), 3.69- 3.67 (m, 1H), 3.64 – 3.59 (m, 4H), 3.55 (dd, J = 10.4, 3.6 Hz, 1H), 3.06 – 3.04 (m, 2H), 1.96 – 1.87 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 174.6, 100.9, 100.6, 92.6, 92.4, 77.3, 74.9, 74.8, 73.7, 72.1, 71.5, 71.4, 70.0, 69.8, 69.5, 68.6, 68.1, 67.4, 67.3, 66.4, 59.8, 59.2, 53.6, 53.1, 38.1. HRMS (ESI - TOF) m/z: $[M]^{2-}$ calcd. for $C_{27}H_{45}N_3O_{27}S_2^{2-}$ 453.5846 Found 453.5826.

4.6 References

1. (a) Zong, C.; Venot, A.; Li, X.; Lu, W.; Xiao, W.; Wilkes, J-S. L.; Salanga, C. L.; Handel, T. M.; Wang, L.; Wolfert, M. A.; Boons, G.-J. *J. Am. Chem. Soc.* **2017**, 139, 9534−9543. (b) Vlodavsky, I.; Gross-Cohen, M.; Weissmann, M.; Ilan, N.; Sanderson, R. D. *Trends Biochem. Sci.* **2018**, 43, 18−31. (c) Kaltenbach, D. D.; Jaishankar, D.; Hao, M.; Beer, J. C.; Volin, M. V.; Desai, U. R.; Tiwari, V. *Front. Pharmacol*. **2018**, 9, 1315. (d) Xu, D.; Esko, J. D. *Annu. Rev. Biochem.* **2014**, 83, 129−157. (e) Gama, C. I.; Hsieh-Wilson, L. C. Curr. Opin. Chem. Biol. **2005**, 9, 609−619. (f) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002**, 41, 390−412. (g)

Zhang, P.; Lu, H.; Peixoto, R. T.; Pines, M. K.; Ge, Y.; Oku, S.; Siddiqui, T. J.; Xie, Y.; Wu, W.; Archer-Hartmann, S.; Yoshida, K.; Tanaka, K. F.; Aricescu, A. R.; Azadi, P.; Gordon, M. D.; Sabatini, B. L.; Wong, R. O. L.; Craig, A. M. *Cell* **2018**, 174, 1450−1464. (h) Iozzo, R. V. *Nat. Rev. Mol. Cell Biol*. **2005**, 6, 646−656.

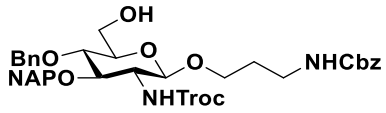
2. (a) Brickman, Y. G.; Ford, M. D.; Gallagher, J. T.; Nurcombe, V.; Bartlett, P. F.; Turnbull, J. E. *J. Biol. Chem.* **1998**, 273, 4350−4359. (b) Feyzi, E.; Saldeen, T.; Larsson, E.; Lindahl, U.; Salmivirta, M. *J. Biol. Chem.* **1998**, 273, 13395−13398. (c) Nadanaka, S.; Kitagawa, H. J. Biochem. 2008, 144, 7−14. (d) Shi, X.; Zaia, J. *J. Biol. Chem.* **2009**, 284, 11806−11814.
3. (a) Sakamoto, K.; Ozaki, T.; Ko, Y. C.; Tsai, C. F.; Gong, Y.; Morozumi, M.; Ishikawa, Y.; Uchimura, K.; Nadanaka, S.; Kitagawa, H.; Zulueta, M. M. L.; Bandaru, A.; Tamura, J. I.; Hung, S. C.; Kadomatsu, K. *Nat. Chem. Biol*. **2019**, 15, 699−709. (b) de Paz, J. L.; Seeberger, P. H. *Mol. BioSyst.* **2008**, 4, 707−711. (c) Yamada, M.; Hamaguchi, T. *J. Biol. Chem.* **2018**, 293, 10841−10842. (d) Grand, E.; Kovensky, J.; Pourceau, G.; Toumieux, S.; Wadouachi, A. *Carbohydr. Chem*. **2014**, 40, 195−235.
4. (a) Li, Y. C.; Ho, I. H.; Ku, C. C.; Zhong, Y. Q.; Hu, Y. P.; Chen, Z. G.; Chen, C. Y.; Lin, W. C.; Zulueta, M. M.; Hung, S. C.; Lin, M. G.; Wang, C. C.; Hsiao, C. D. *ACS Chem. Biol*. **2014**, 9, 1712−1717. (b) Zulueta, M. M. L.; Chyan, C. L.; Hung, S. C. *Curr. Opin. Struct. Biol.* **2018**, 50, 126−133. (c) Hu, Y. P.; Zhong, Y. Q.; Chen, Z. G.; Chen, C. Y.; Shi, Z.; Zulueta, M. M.; Ku, C. C.; Lee, P. Y.; Wang, C. C.; Hung, S. C. *J. Am. Chem. Soc.* **2012**, 134, 20722−20727. (d) Noti, C.; de Paz, J. L.; Polito, L.; Seeberger, P. H. *Chem. - Eur. J.* **2006**, 12, 8664−8686. (e) Huang, M. L.; Smith, R. A.; Trieger, G. W.; Godula, K. J. *Am. Chem. Soc.* **2014**, 136, 10565−10568. (f) Naticchia, M. R.; Laubach, L. K.; Tota, E. M.; Lucas, T. M.; Huang, M. L.; Godula, K. *ACS Chem. Biol.* **2018**, 13, 2880−2887.
5. (a) Thacker, B. E.; Xu, D.; Lawrence, R.; Esko, J. D. *Matrix Biol.* **2014**, 35, 60−72. (b) Langeslay, D. J.; Young, R. P.; Beni, S.; Beecher, C. N.; Mueller, L. J.; Larive, C. K. *Glycobiology* **2012**, 22, 1173−1182. (c) Guerrini, M.; Elli, S.; Mourier, P.; Rudd, T. R.; Gaudesi, D.; Casu, B.; Boudier, C.; Torri, G.; Viskov, C. *Biochem. J.* **2013**, 449 (2), 343− 351. (d) Sankaranarayanan, N. V.; Strebel, T. R.; Boothello, R. S.; Sheerin, K.; Raghuraman, A.; Sallas, F.; Mosier, P. D.; Watermeyer, N. D.; Oscarson, S.; Desai, U. R. *Angew. Chem., Int. Ed*. **2017**, 56, 2312− 2317.

6. (a) Cole, C. L.; Rushton, G.; Jayson, G. C.; Avizienyte, E. *J. Biol. Chem.* **2014**, 289, 10488−10501. (b) Corti, F.; Wang, Y.; Rhodes, J. M.; Atri, D.; Archer-Hartmann, S.; Zhang, J.; Zhuang, Z. W.; Chen, D.; Wang, T.; Wang, Z.; Azadi, P.; Simons, M. *Nat. Commun.* **2019**, 10, 1562. (c) Ferreras, C.; Rushton, G.; Cole, C. L.; Babur, M.; Telfer, B. A.; van Kuppevelt, T. H.; Gardiner, J. M.; Williams, K. J.; Jayson, G. C.; Avizienyte, E. *J. Biol. Chem.* **2012**, 287, 36132−36146.
7. (a) Thacker, B. E.; Seamen, E.; Lawrence, R.; Parker, M. W.; Xu, Y.; Liu, J.; Vander Kooi, C. W.; Esko, J. D. *ACS Chem. Biol.* **2016**, 11, 971−980. (b) Wang, Z.; Hsieh, P. H.; Xu, Y.; Thieker, D.; Chai, E. J. E.; Xie, S.; Cooley, B.; Woods, R. J.; Chi, L.; Liu, J. *J. Am. Chem. Soc.* **2017**, 139, 5249−5256. (c) Alavi Naini, S. M.; Soussi-Yanicostas, N. *Front. Cell Dev. Biol.* **2018**, 6, 163. (d) Zhao, J.; Zhu, Y.; Song, X.; Xiao, Y.; Su, G.; Liu, X.; Wang, Z.; Xu, Y.; Liu, J.; Eliezer, D.; Ramlall, T. F.; Lippens, G.; Gibson, J.; Zhang, F.; Linhardt, R. J.; Wang, L.; Wang, C. *Angew. Chem., Int. Ed.* **2020**, 59, 1818−1827.
8. (a) Hansen, S. U.; Miller, G. J.; Cliff, M. J.; Jayson, G. C.; Gardiner, J. M. *Chem. Sci.* **2015**, 6, 6158−6164. (b) Hu, Y. P.; Lin, S. Y.; Huang, C. Y.; Zulueta, M. M. L.; Liu, J. Y.; Chang, W.; Hung, S. C. *Nat. Chem.* **2011**, 3, 557−563. (c) Zhang, X.; Pagadala, V.; Jester, H. M.; Lim, A. M.; Pham, T. Q.; Goulas, A. M. P.; Liu, J.; Linhardt, R. J. *Chem. Sci.* **2017**, 8, 7932−7940. (d) de Paz, J. L.; Noti, C.; Seeberger, P. H. *J. Am. Chem. Soc.* **2006**, 128, 2766−2767. (e) Pawar, N. J.; Wang, L.; Higo, T.; Bhattacharya, C.; Kancharla, P. K.; Zhang, F.; Baryal, K.; Huo, C.-X.; Liu, J.; Linhardt, R. J.; Huang, X.; Hsieh-Wilson, L. C. *Angew. Chem., Int. Ed*. **2019**, 58, 18577−18583. (f) Boothello, R. S.; Sarkar, A.; Tran, V. M.; Nguyen, T. K.; Sankaranarayanan, N. V.; Mehta, A. Y.; Alabbas, A.; Brown, S.; Rossi, A.; Joice, A. C.; Mencio, C. P.; Quintero, M. V.; Kuberan, B.; Desai, U. R. *ACS Chem. Biol.* **2015**, 10, 1485−1494. (g) Shanthamurthy, C. D.; Kikkeri, R. *Eur. J. Org. Chem.* **2019**, 2019, 2950−2953.
9. Mende, M.; Bednarek, C.; Wawryszyn, M.; Sauter, P.; Biskup, M. B.; Schepers, U.; Brase, S. *Chem. Rev.* **2016**, 116, 8193−8255.
10. (a) Lu, W.; Zong, C.; Chopra, P.; Pepi, L. E.; Xu, Y.; Amster, I. J.; Liu, J.; Boons, G. J. *Angew. Chem., Int. Ed*. **2018**, 57, 5340−5344. (b) Zhang, X.; Pagadala, V.; Jester, H. M.; Lim, A. M.; Pham, T. Q.; Goulas, A. M. P.; Liu, J.; Linhardt, R. J. *Chem. Sci.* **2017**, 8, 7932− 7940. (c) Wang, Z.; Hsieh, P. H.; Xu, Y.; Thieker, D.; Chai, E. J. E.;

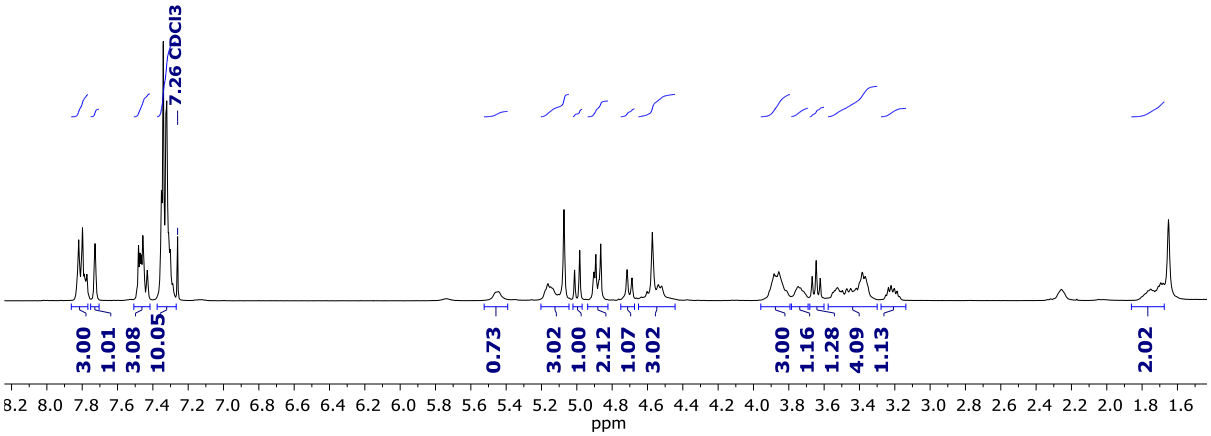
Xie, S.; Cooley, B.; Woods, R. J.; Chi, L.; Liu, J. *J. Am. Chem. Soc.* **2017**, 139, 5249−5256.

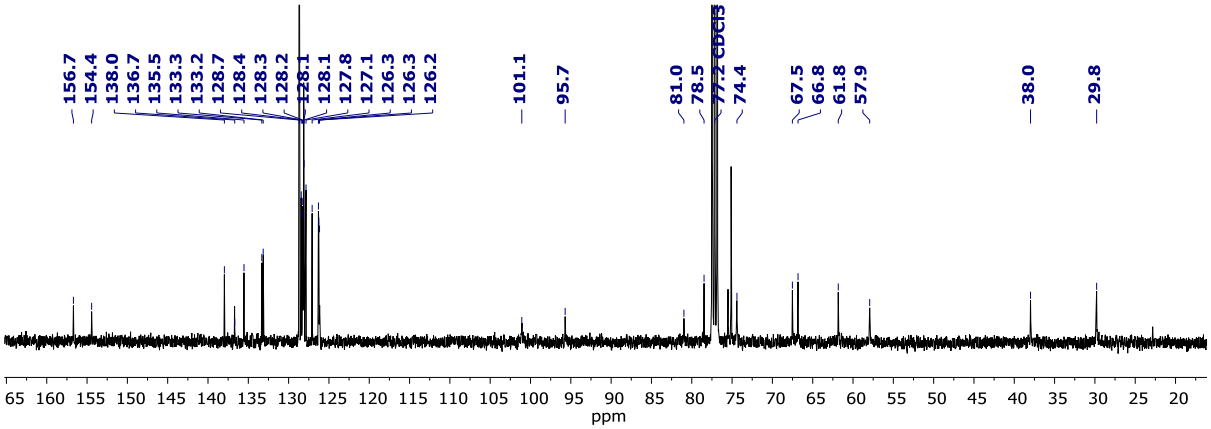
11. (a) Xu, P.; Laval, S.; Guo, Z.; Yu, B. *Org. Chem. Front.* **2016**, 3, 103−109. (b) Maza, S.; de Paz, J. L.; Nieto, P. M. *Tetrahedron Lett.* **2011**, 52, 441−443.
12. Díaz-Ortiz, Á.; Prieto, P.; de la Hoz, A. *Chem. Rec.* **2019**, 19, 85−97.
13. (a) Chatterjee, S.; Moon, S.; Hentschel, F.; Gilmore, K.; Seeberger, P. H. *J. Am. Chem. Soc.* **2018**, 140, 11942−11953. (b) Plutschack, M. B.; Pieber, B.; Gilmore, K.; Seeberger, P. H. *Chem. Rev*. **2017**, 117, 11796−11893. (c) Hurevich, M.; Kandasamy, J.; Ponnappa, B. M.; Collot, M.; Kopetzki, D.; McQuade, D. T.; Seeberger, P. H. *Org. Lett.* **2014**, 16, 1794−1797. (d) Laurino, P.; Kikkeri, R.; Seeberger, P. H. *Nat. Protoc.* **2011**, 6, 1209−1220. (e) Chaudhari, M. B.; Moorthy, S.; Patil, S.; Bisht, G. S.; Mohamed, H.; Basu, S.; Gnanaprakasam, B. *J. Org. Chem.* **2018**, 83, 1358−1368. (f) Chaudhari, M. B.; Jayan, K.; Gnanaprakasam, B. *J. Org. Chem.* **2020**, 85, 3374−3382. (g) Ledru, H.; D'Attoma, J.; Bostyn, S.; Routier, S.; Buron, F.; Lopin-Bon, C. *Chemistry Select,* **2017**, 2, 9637− 9641.

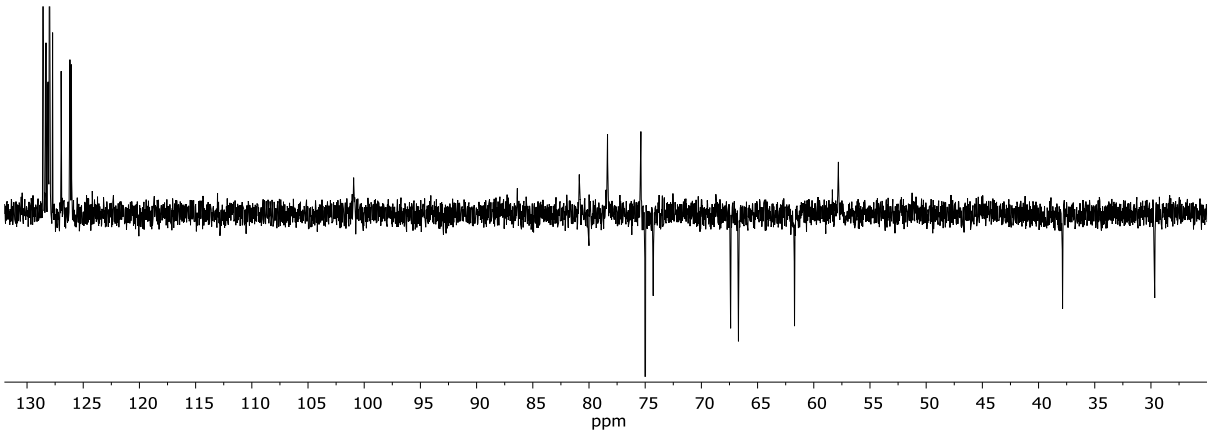
4.7 NMR Spectras

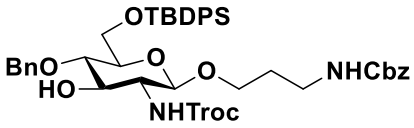


2

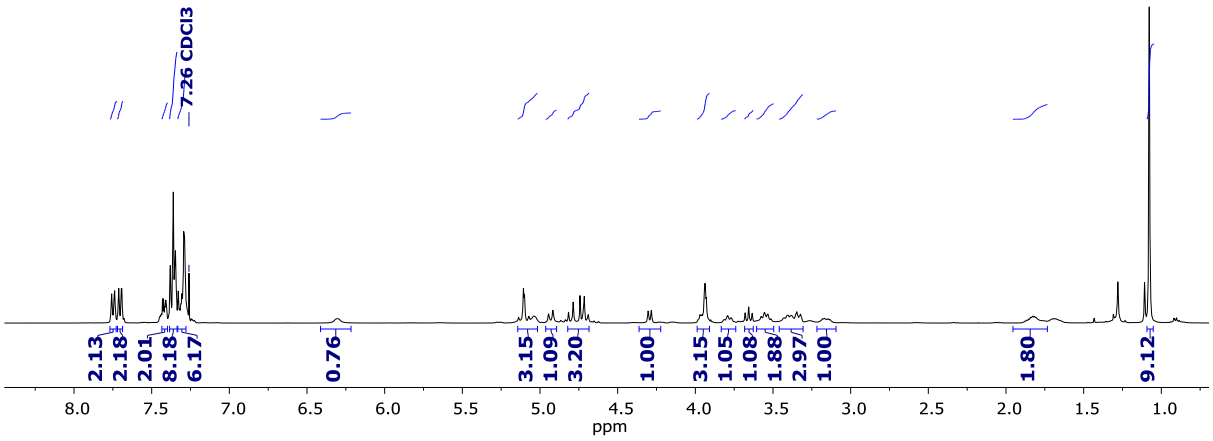


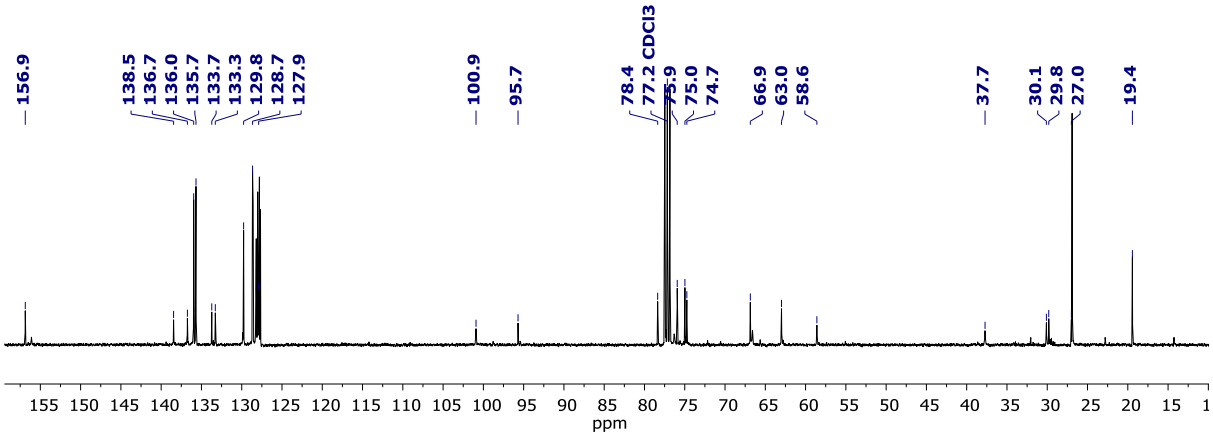


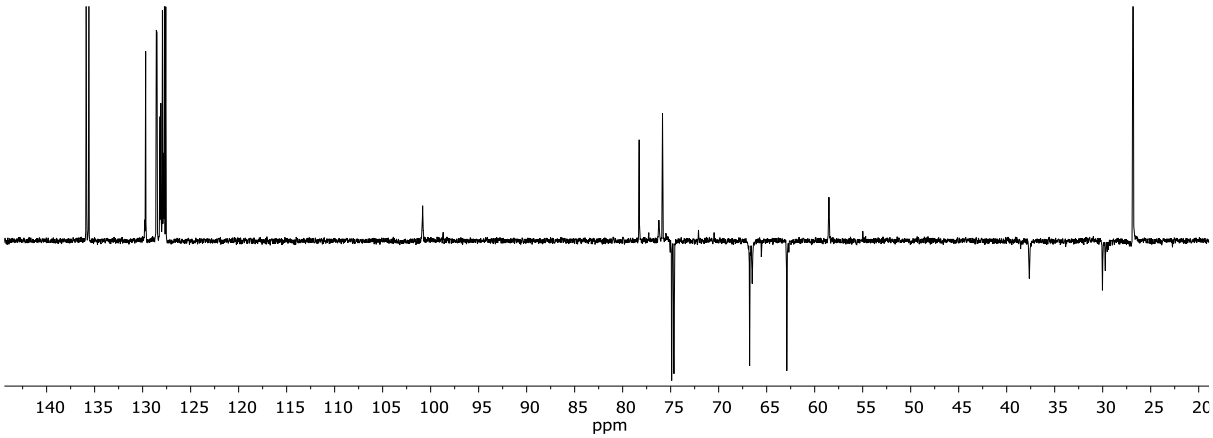

130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
ppm

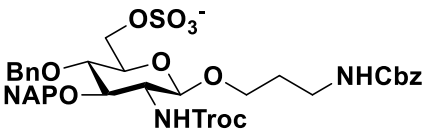

OTBDPS
BnO
HO
O
O
NHCbz
NHTroc

4

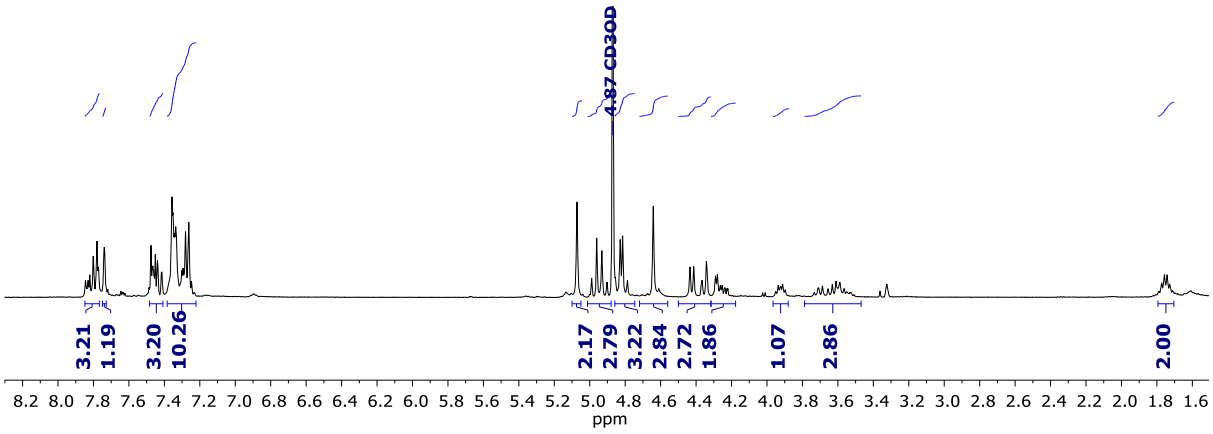

7.26 CDCl3
2.13
2.18
2.01
8.18
6.17
0.76
3.15
1.09
3.20
1.00
3.15
1.05
1.08
1.88
2.97
1.00
1.80
9.12
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm

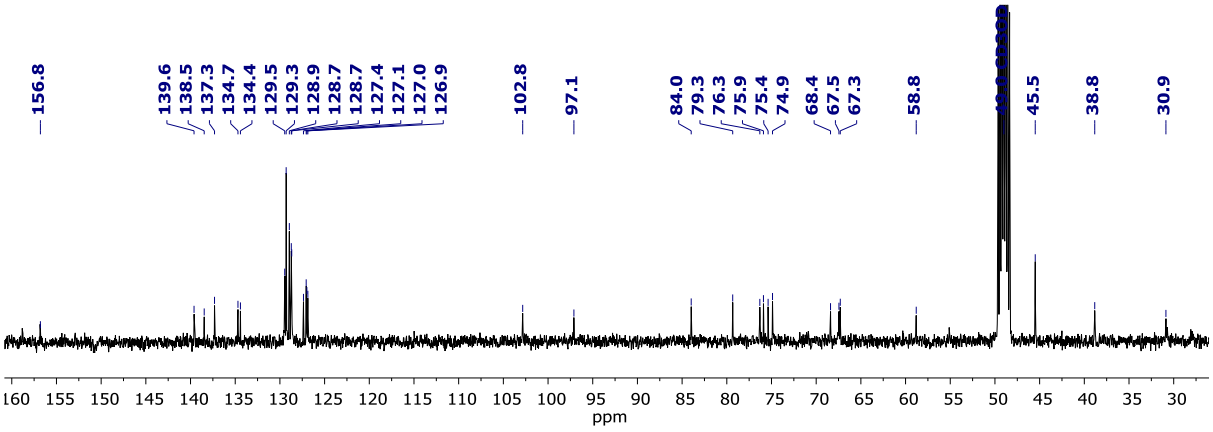

156.9
138.5
136.7
136.0
135.7
133.7
133.3
129.8
128.7
127.9
100.9
95.7
78.4
77.2 CDCl3
75.9
75.0
74.7
66.9
63.0
58.6
37.7
30.1
29.8
27.0
19.4
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 1
ppm

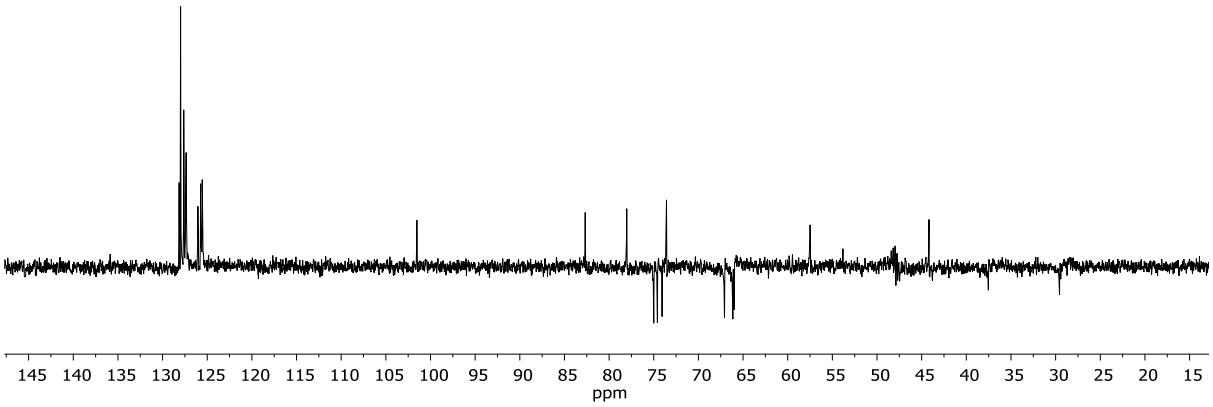

140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
ppm

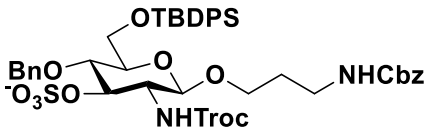

OSO3-
O
BnO
NAPO
O
NHCbz
NHTroc

3

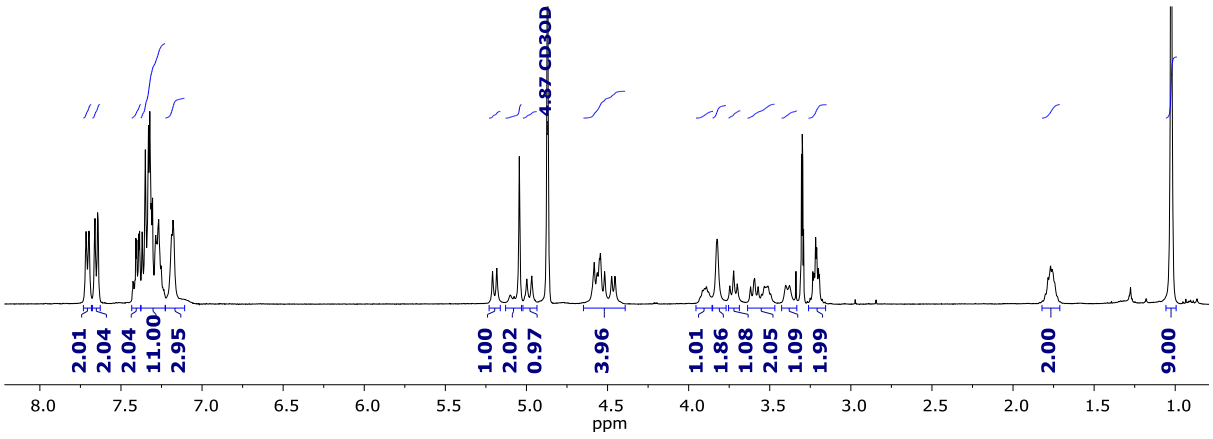

4.87 CD3OD
3.21
1.19
3.20
10.26
2.17
2.79
3.22
2.84
2.72
1.86
1.07
2.86
2.00
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
ppm

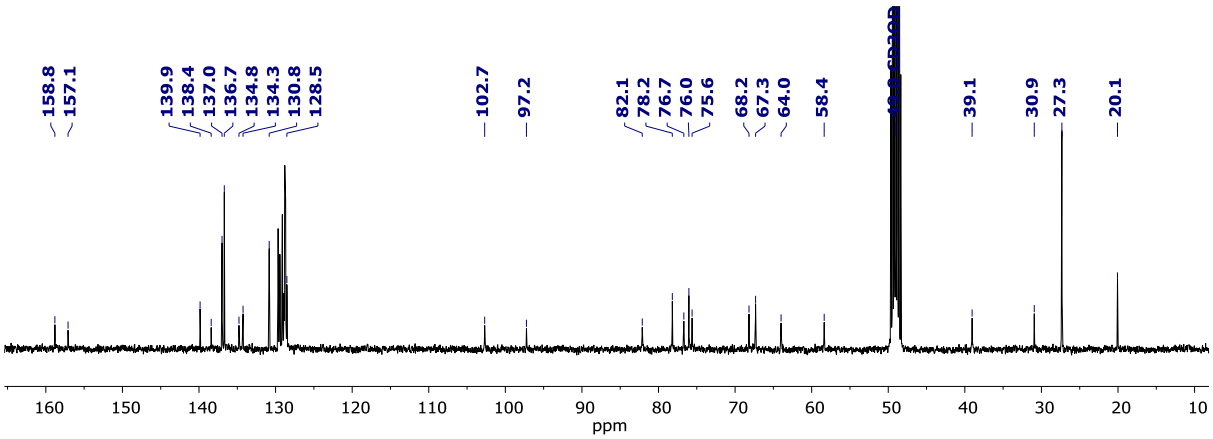

156.8
139.6
138.5
137.3
134.7
134.4
129.5
129.3
128.9
128.7
128.7
127.4
127.1
127.0
126.9
102.8
97.1
84.0
79.3
76.3
75.9
75.4
74.9
68.4
67.5
67.3
58.8
45.5
38.8
30.9
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
ppm

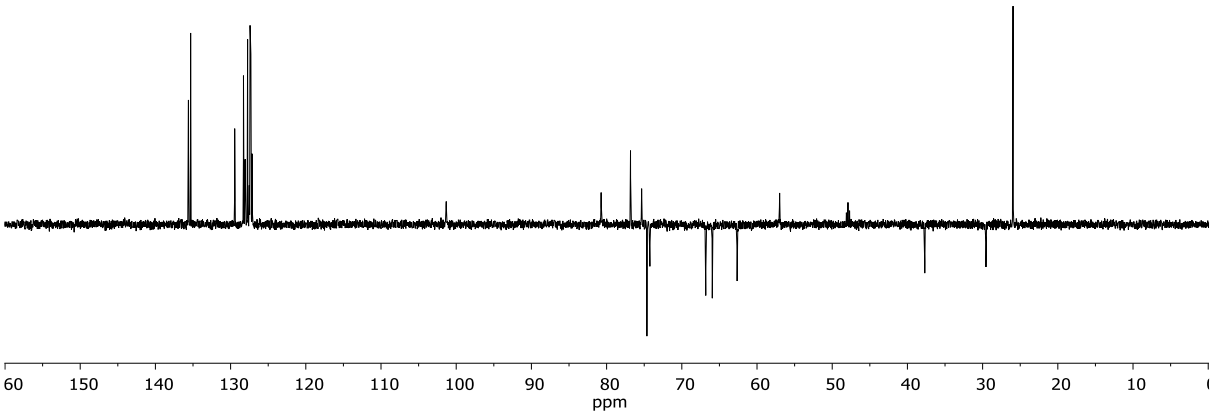

145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15
ppm

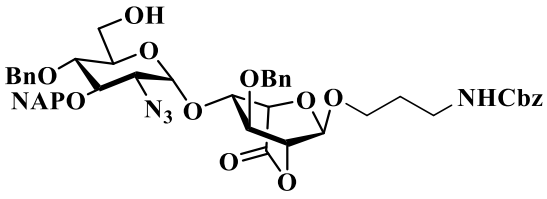

OTBDPS
BnO
$^{-}O_3SO$
O
O
NHCbz
NHTroc

5

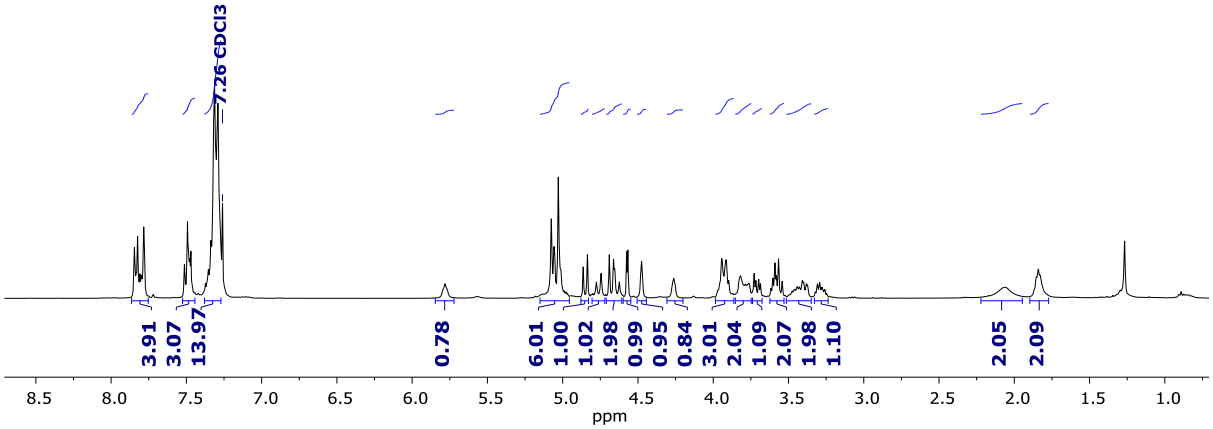

4.87 CD3OD
2.01 2.04 2.04 11.00 2.95 1.00 2.02 0.97 3.96 1.01 1.86 1.08 2.05 1.09 1.99 2.00 9.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm

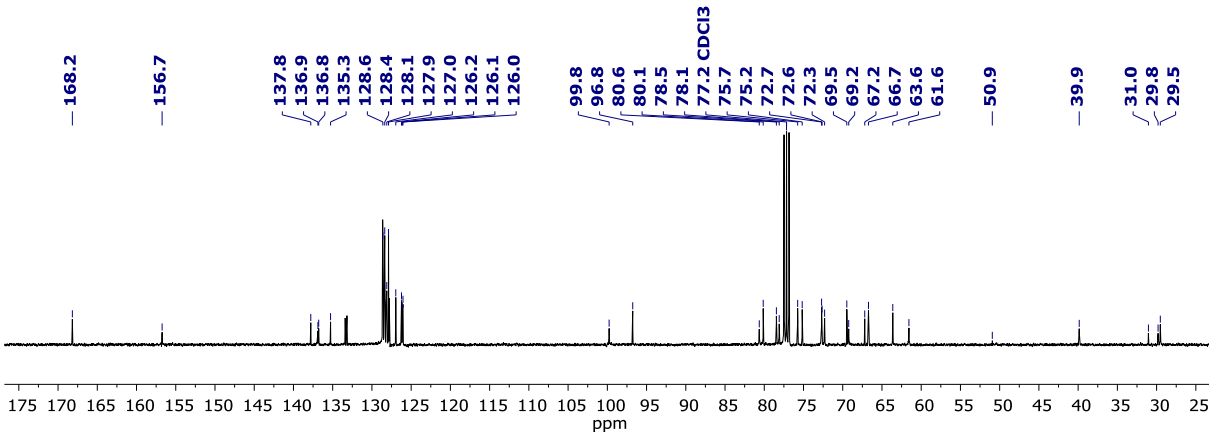

158.8 157.1 139.9 138.4 137.0 136.7 134.8 134.3 130.8 128.5 102.7 97.2 82.1 78.2 76.7 76.0 75.6 68.2 67.3 64.0 58.4 49.0 CD3OD 39.1 30.9 27.3 20.1
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

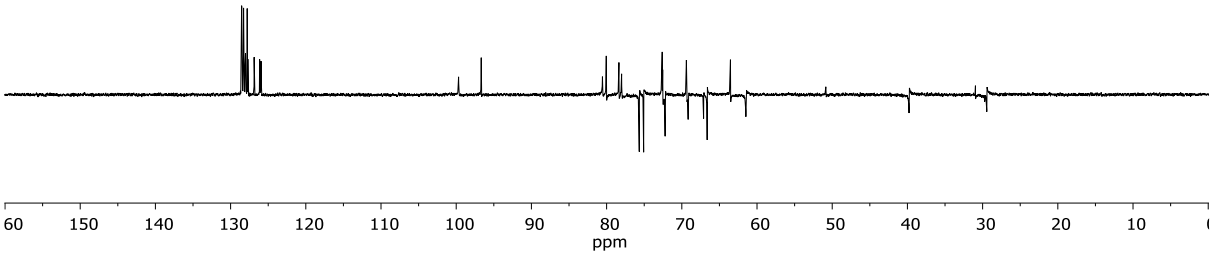

60 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

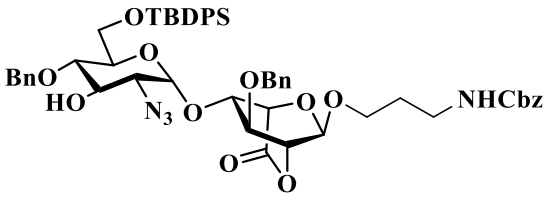

OH
O
BnO
NAPO
N3 O
OBn
O O
NHCbz
O
O

6a

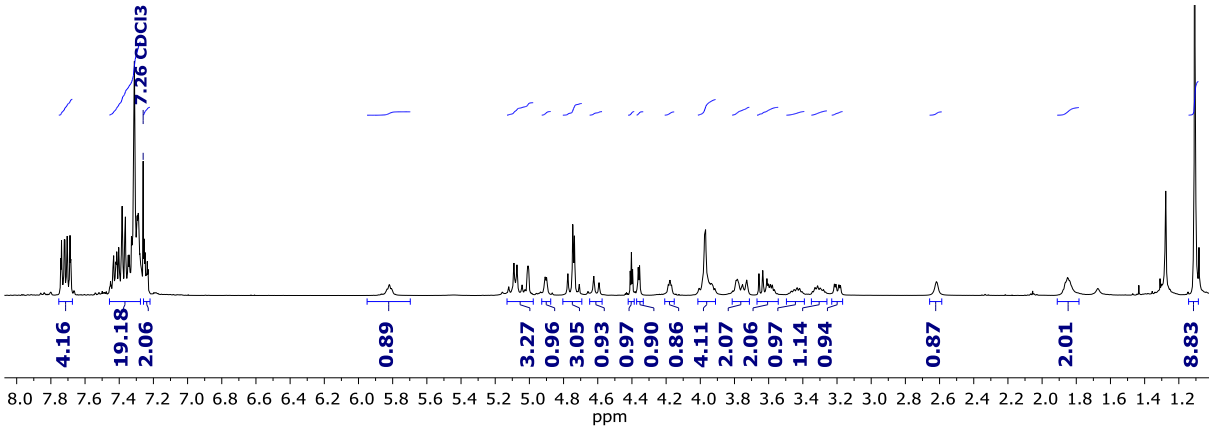

7.26 CDCl3
3.91 3.07 13.97 0.78 6.01 1.00 1.02 1.98 0.99 0.95 0.84 3.01 2.04 1.09 2.07 1.98 1.10 2.05 2.09
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm

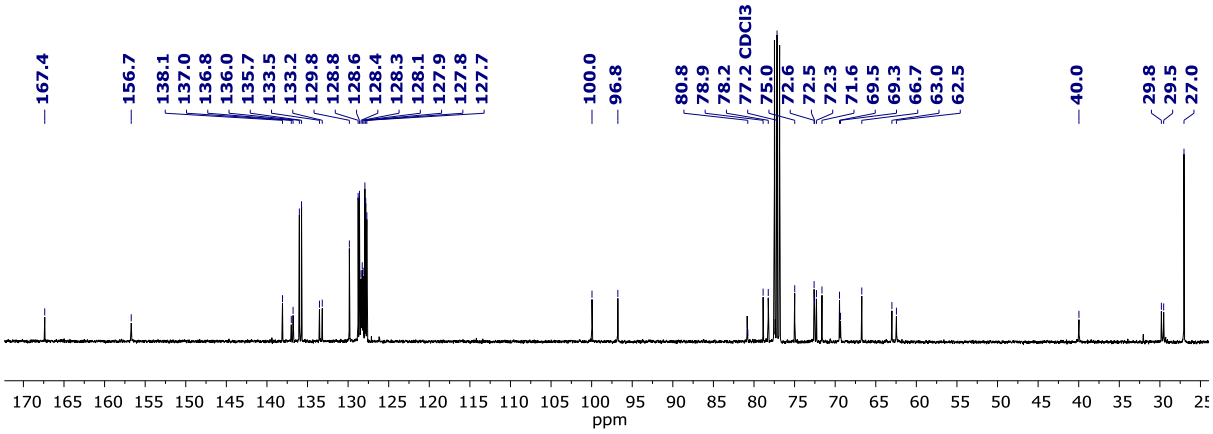

168.2 156.7 137.8 136.9 136.8 135.3 128.6 128.4 128.1 127.9 127.0 126.2 126.1 126.0 99.8 96.8 80.6 80.1 78.5 78.1 77.2 CDCl3 75.7 75.2 72.7 72.6 72.3 69.5 69.2 67.2 66.7 63.6 61.6 50.9 39.9 31.0 29.8 29.5
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
ppm

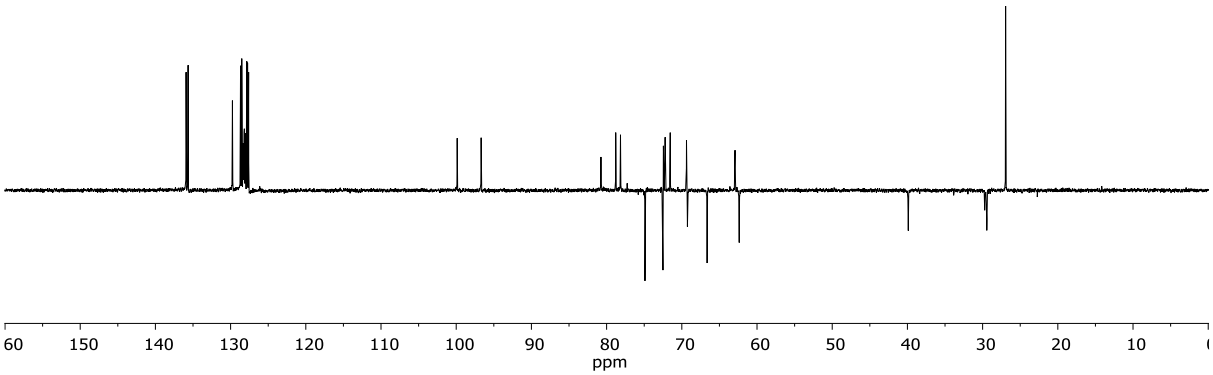

60 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

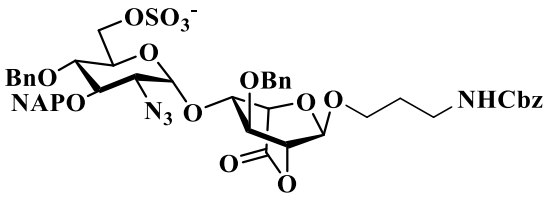

OTBDPS
O
BnO
HO
N3
O
OBn
O
O
NHCbz
O
O

7a

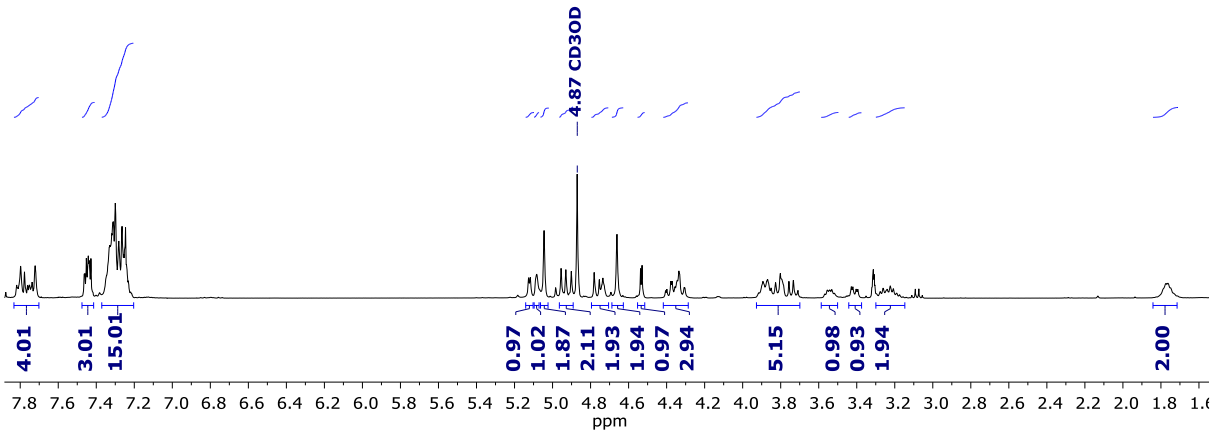

7.26 CDCl3
4.16
19.18
2.06
0.89
3.27
0.96
3.05
0.93
0.97
0.90
0.86
4.11
2.07
2.06
0.97
1.14
0.94
0.87
2.01
8.83
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
ppm

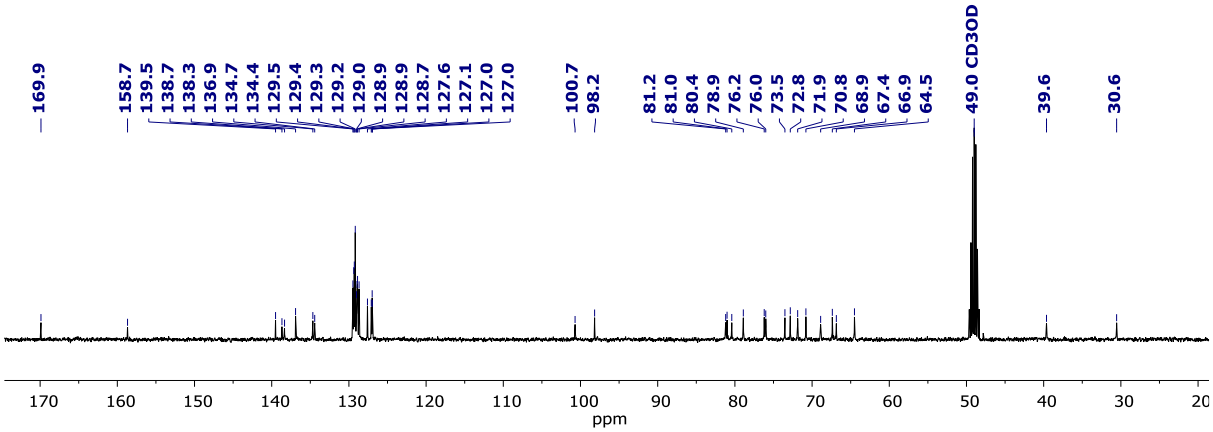

167.4
156.7
138.1
137.0
136.8
136.0
135.7
133.5
133.2
129.8
128.8
128.6
128.4
128.3
128.1
127.9
127.8
127.7
100.0
96.8
80.8
78.9
78.2
77.2 CDCl3
75.0
72.6
72.5
72.3
71.6
69.5
69.3
66.7
63.0
62.5
40.0
29.8
29.5
27.0
ppm

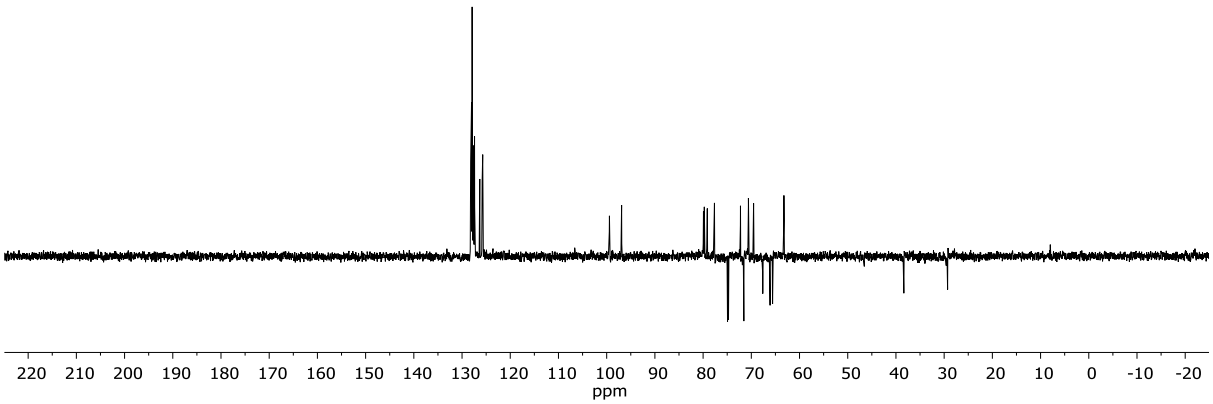

ppm

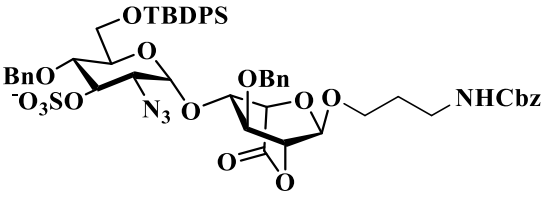

OSO3-
O
BnO
NAPO
N3
O
OBn
O
O
NHCbz
O
O

6

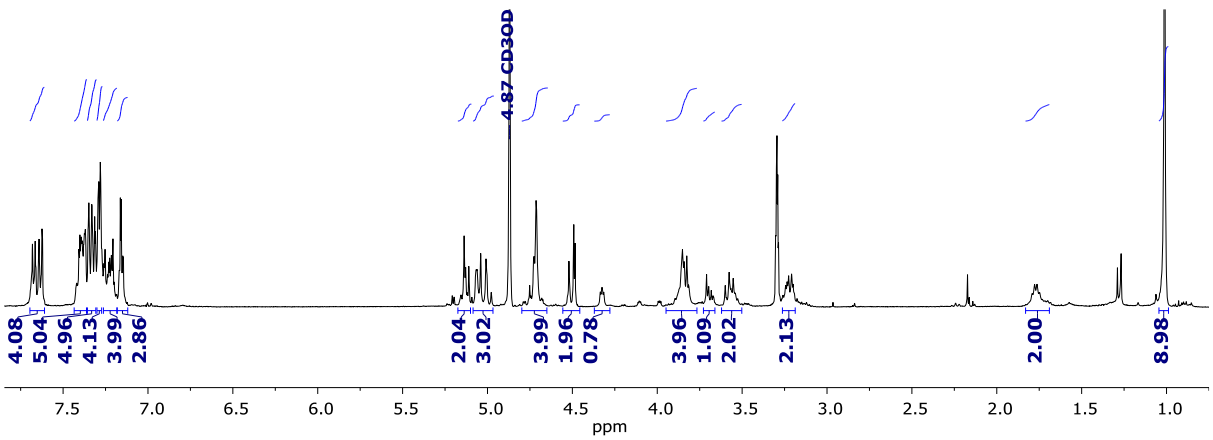

4.87 CD3OD
4.01
3.01
15.01
0.97
1.02
1.87
2.11
1.93
1.94
0.97
2.94
5.15
0.98
0.93
1.94
2.00
ppm

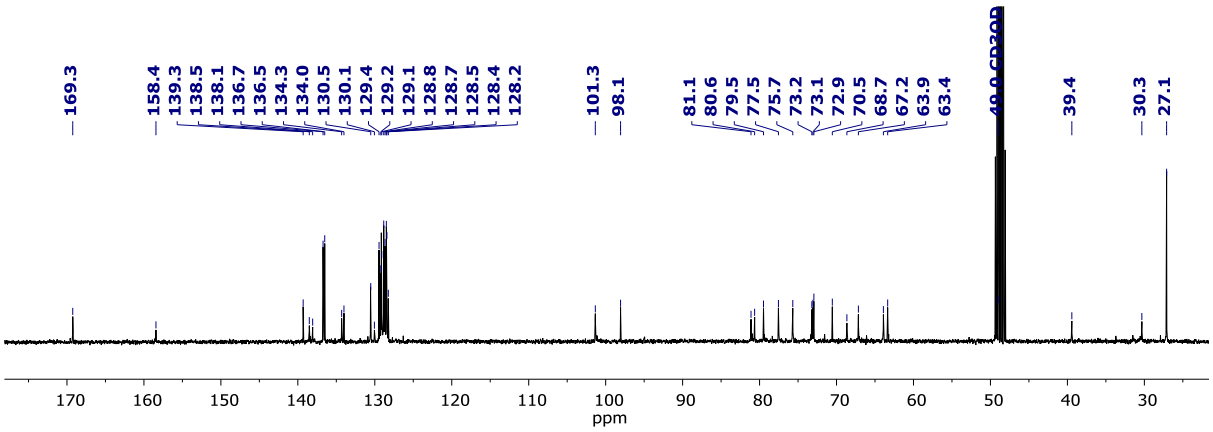

169.9
158.7
139.5
138.7
138.3
136.9
134.7
134.4
129.5
129.4
129.3
129.2
129.0
128.9
128.9
128.7
127.6
127.1
127.0
127.0
100.7
98.2
81.2
81.0
80.4
78.9
76.2
76.0
73.5
72.8
71.9
70.8
68.9
67.4
66.9
64.5
49.0 CD3OD
39.6
30.6
ppm

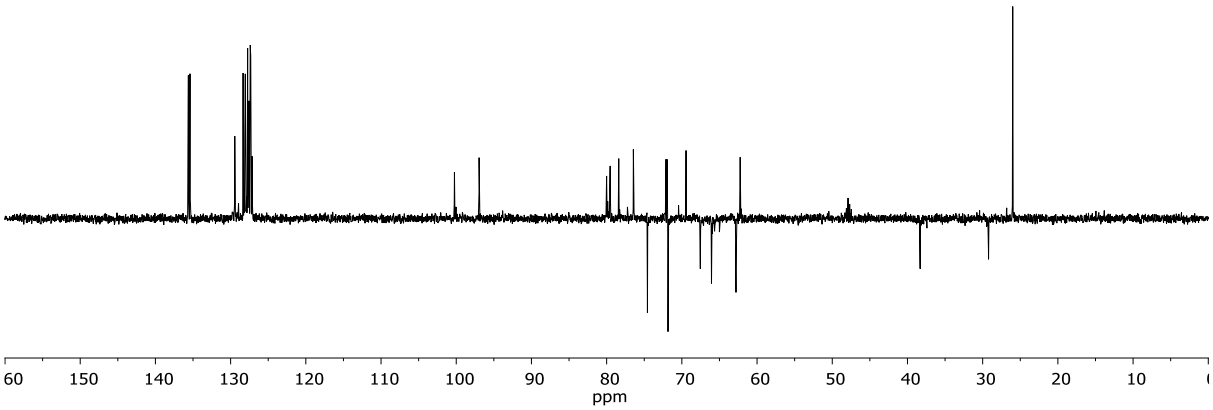

ppm

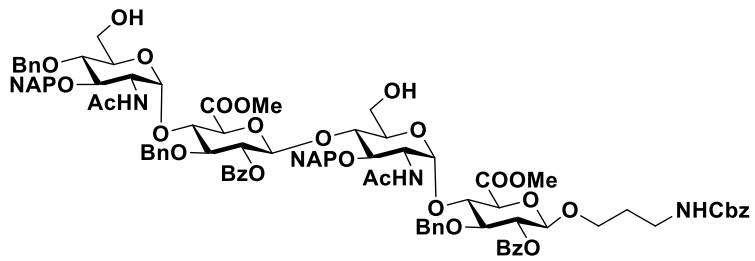

OTBDPS
O
BnO
OBn
$^{-}O_3SO$
N_3
O
O
O
NHCbz
O
O

7

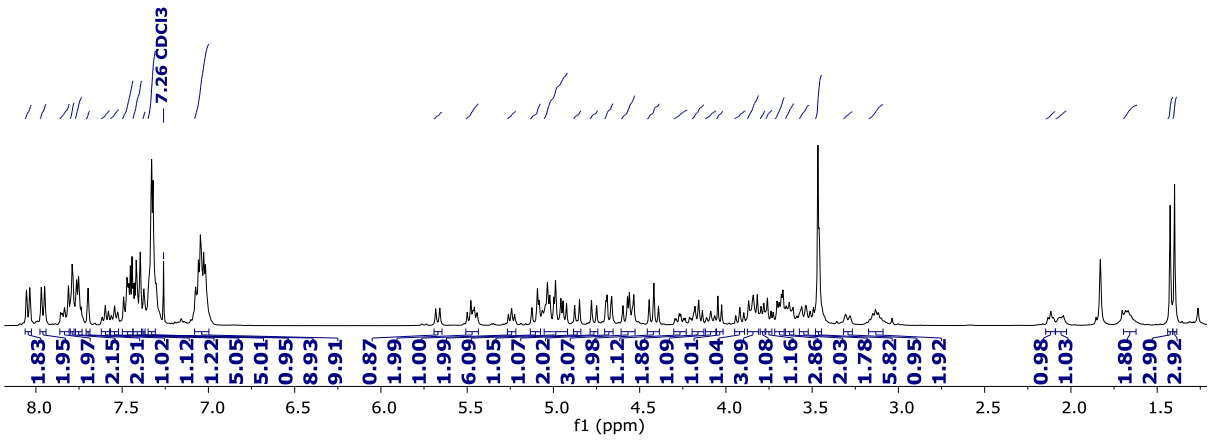

4.87 CD3OD
4.08
5.04
4.96
4.13
3.99
2.86
2.04
3.02
3.99
1.96
0.78
3.96
1.09
2.02
2.13
2.00
8.98
ppm

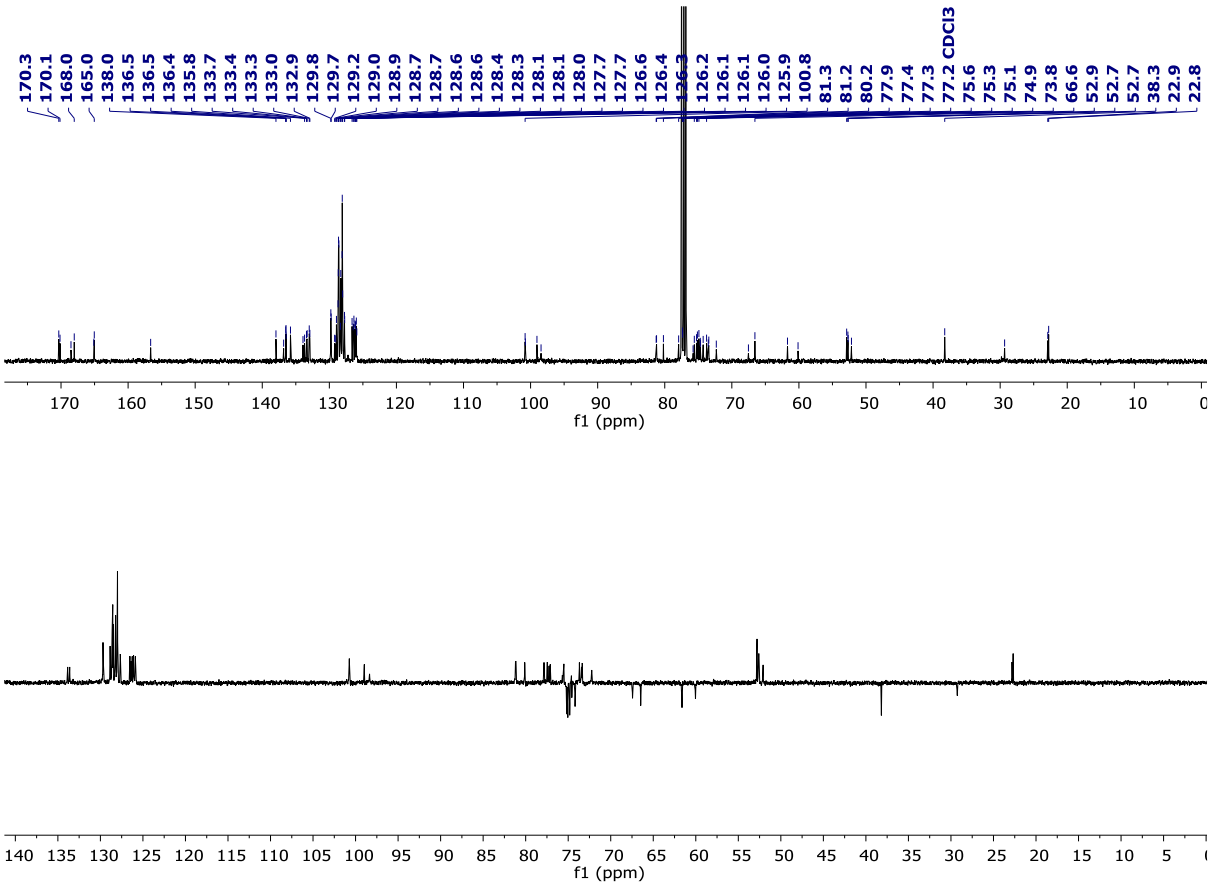

169.3
158.4
139.3
138.5
138.1
136.7
136.5
134.3
134.0
130.5
130.1
129.4
129.2
129.1
128.8
128.7
128.5
128.4
128.2
101.3
98.1
81.1
80.6
79.5
77.5
75.7
73.2
73.1
72.9
70.5
68.7
67.2
63.9
63.4
49.0 CD3OD
39.4
30.3
27.1
ppm

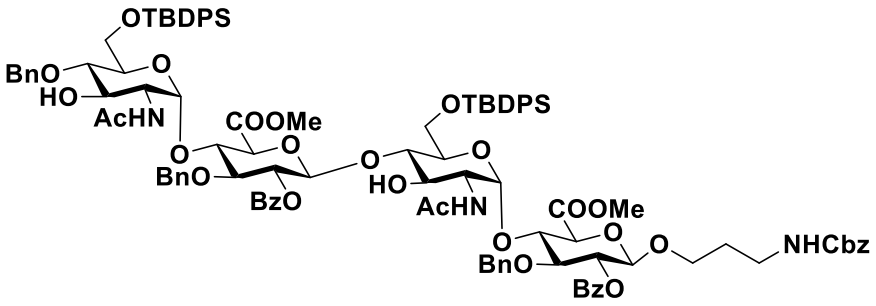

ppm


OH
BnO
O
NAPO
AcHN
COOMe
O
O
BnO
BzO
O
NAPO
OH
O
AcHN
COOMe
O
O
O
NHCbz
BnO
BzO

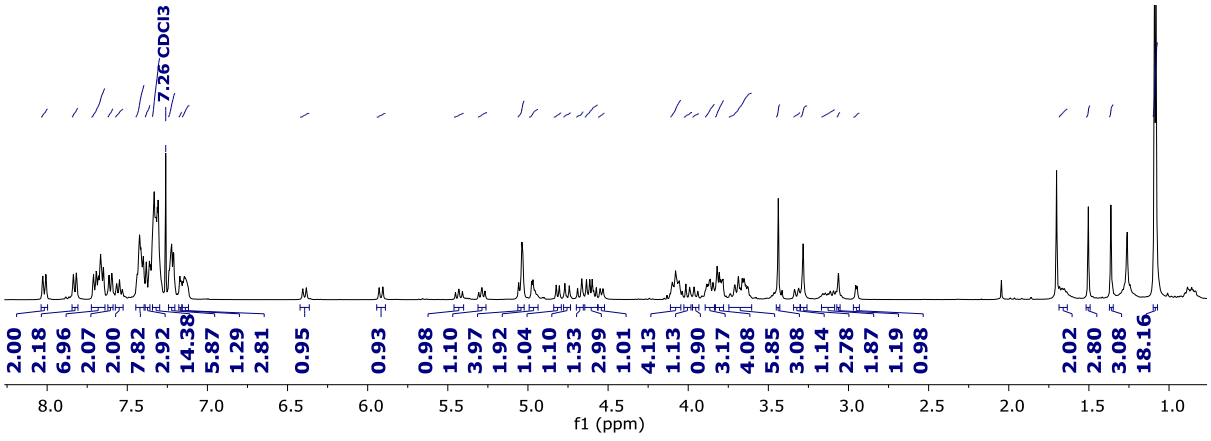
8a

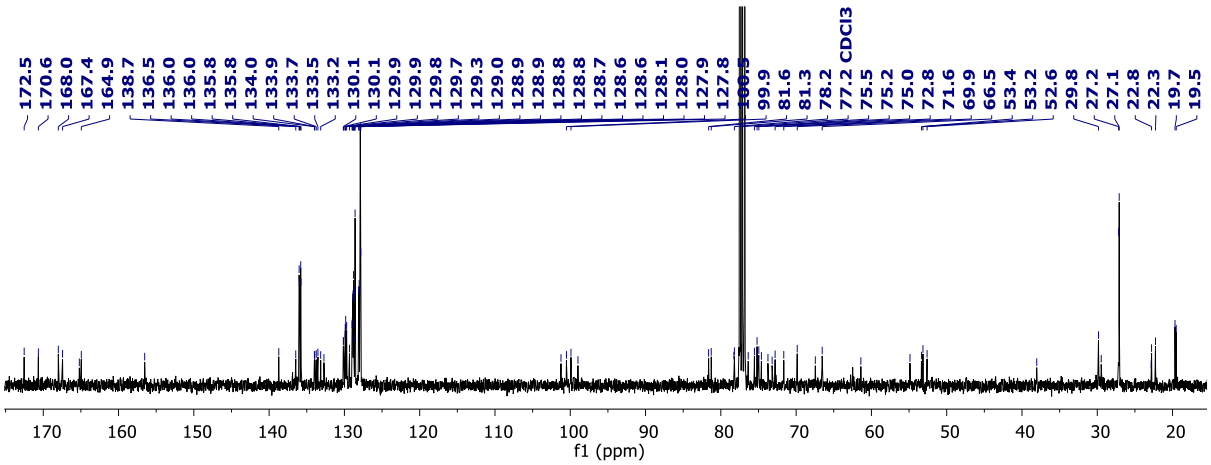

7.26 CDCl3
1.83
1.95
1.97
2.15
2.91
1.02
1.12
1.22
5.05
5.01
0.95
8.93
9.91
0.87
1.99
1.00
1.99
6.09
1.05
1.07
2.02
3.07
1.98
1.12
1.86
1.09
1.01
1.04
3.09
1.08
1.16
2.86
2.03
1.78
5.82
0.95
1.92
0.98
1.03
1.80
2.90
2.92
f1 (ppm)

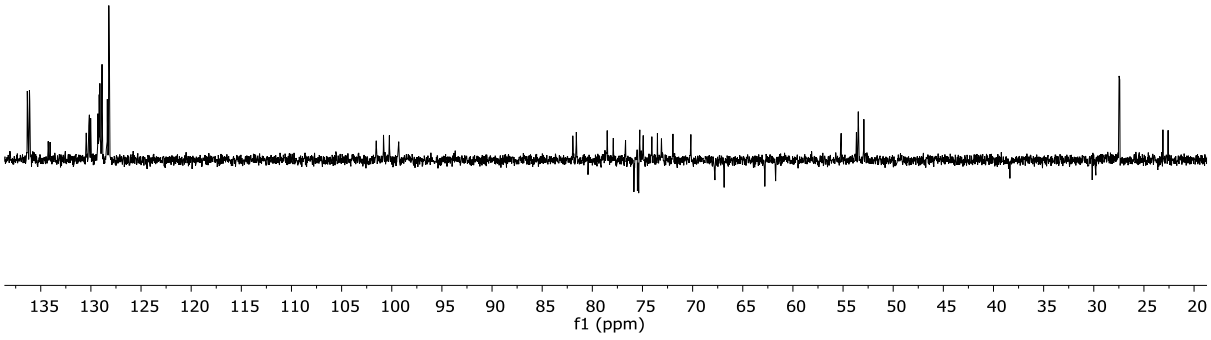

170.3
170.1
168.0
165.0
138.0
136.5
136.5
136.4
135.8
133.7
133.4
133.3
133.0
132.9
129.8
129.7
129.2
129.0
128.9
128.7
128.7
128.6
128.6
128.4
128.3
128.1
128.1
128.0
127.7
127.7
126.6
126.4
126.3
126.2
126.1
126.1
126.0
125.9
100.8
81.3
81.2
80.2
77.9
77.4
77.3
77.2 CDCl3
75.6
75.3
75.1
74.9
73.8
66.6
52.9
52.7
52.7
38.3
22.9
22.8
f1 (ppm)
f1 (ppm)

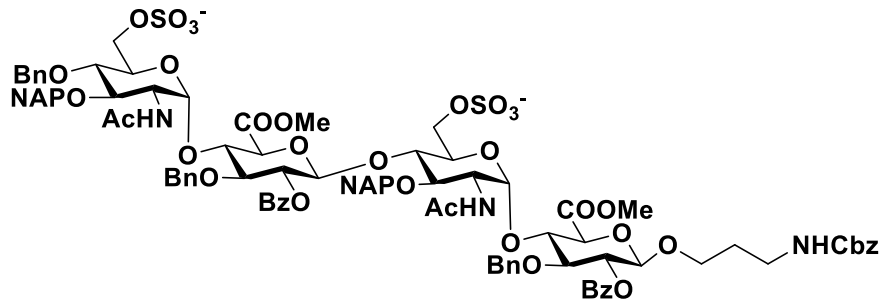

OTBDPS
BnO
HO
AcHN
O
COOMe
BnO
BzO
OTBDPS
HO
AcHN
COOMe
BnO
BzO
NHCbz

9a

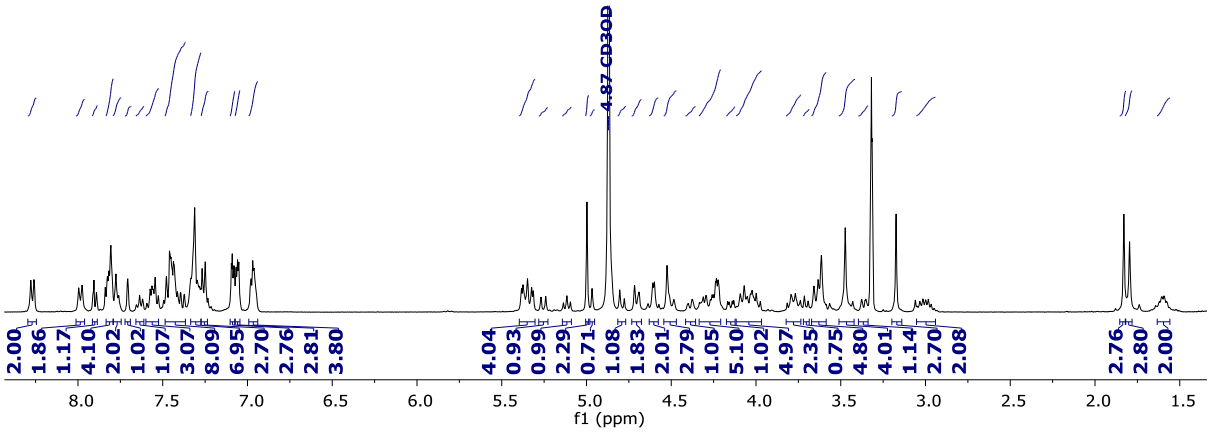

7.26 CDCl3
2.00
2.18
6.96
2.07
2.00
7.82
2.92
14.38
5.87
1.29
2.81
0.95
0.93
0.98
1.10
3.97
1.92
1.04
1.10
1.33
2.99
1.01
4.13
1.13
0.90
3.17
4.08
5.85
3.08
1.14
2.78
1.87
1.19
0.98
2.02
2.80
3.08
18.16
f1 (ppm)

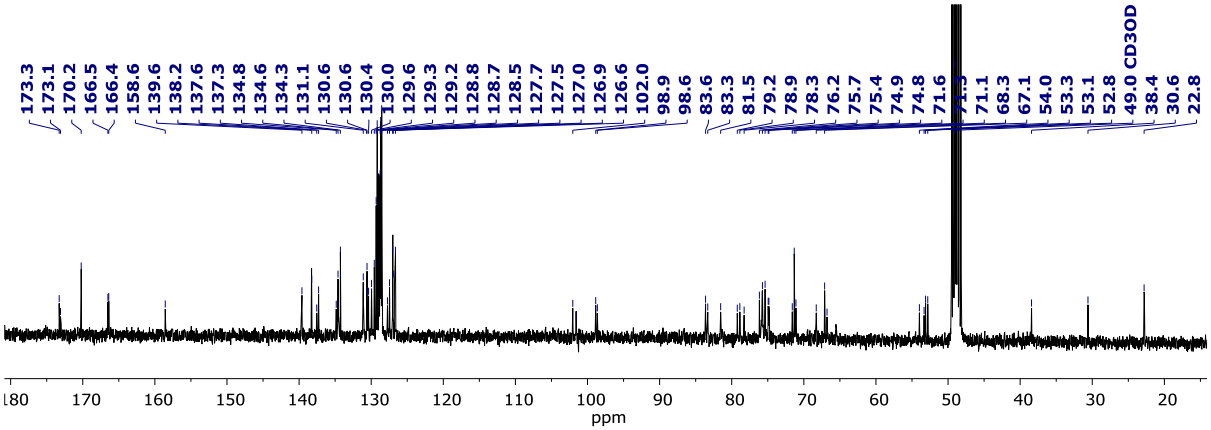

172.5
170.6
168.0
167.4
164.9
138.7
136.5
136.0
136.0
135.8
135.8
134.0
133.9
133.7
133.5
133.2
130.1
130.1
129.9
129.9
129.8
129.7
129.3
129.0
128.9
128.9
128.8
128.8
128.7
128.6
128.6
128.1
128.0
127.9
127.8
100.3
99.9
81.6
81.3
78.2
77.2 CDCl3
75.5
75.2
75.0
72.8
71.6
69.9
66.5
53.4
53.2
52.6
29.8
27.2
27.1
22.8
22.3
19.7
19.5
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

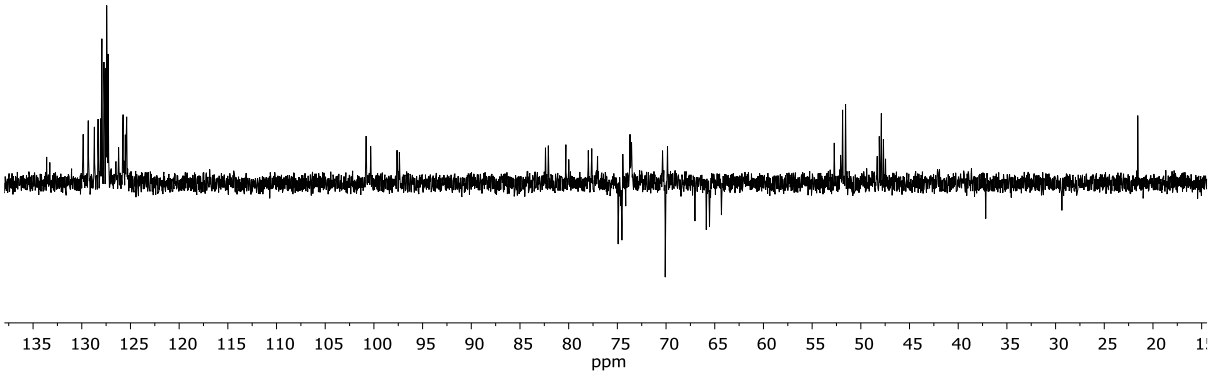

135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

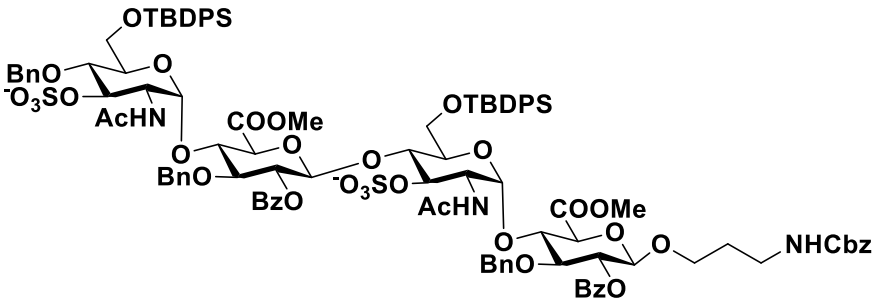

OSO3-
BnO
NAPO
AcHN
O
COOMe
BnO
BzO
NAPO
OSO3-
AcHN
COOMe
BnO
BzO
NHCbz

8

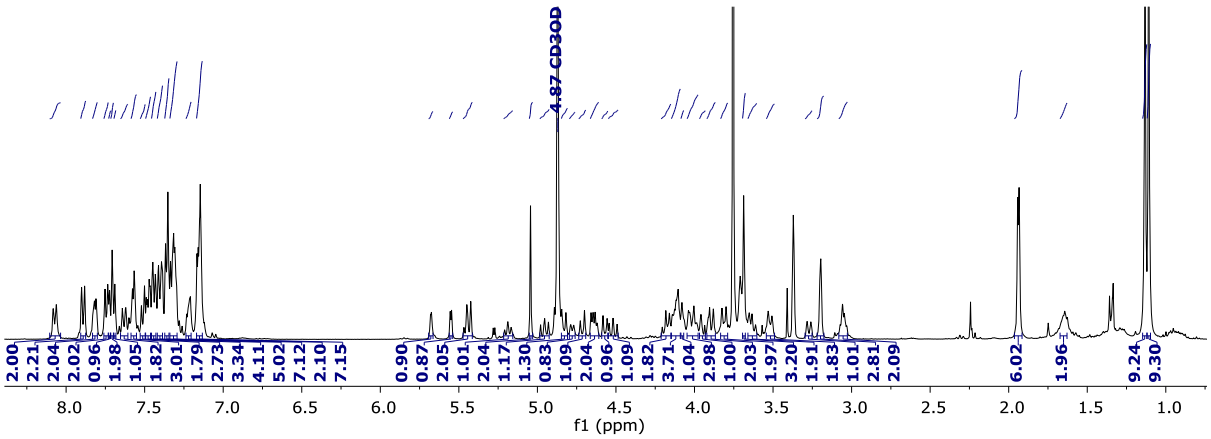

4.87 CD3OD
2.00
1.86
1.17
4.10
2.02
1.02
1.07
3.07
8.09
6.95
2.70
2.76
2.81
3.80
4.04
0.93
0.99
2.29
0.71
1.08
1.83
2.01
2.79
1.05
5.10
1.02
4.97
2.35
0.75
4.80
4.01
1.14
2.70
2.08
2.76
2.80
2.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

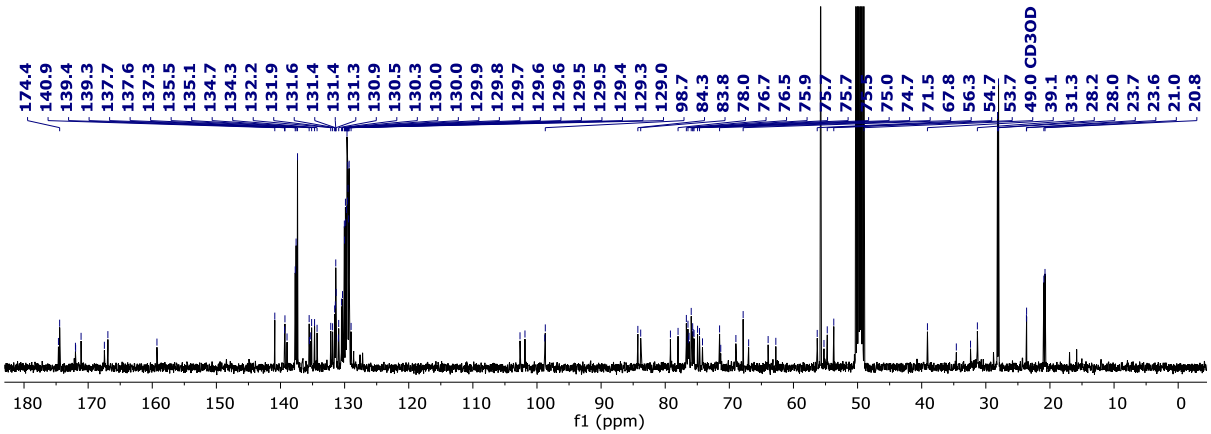

173.3
173.1
170.2
166.5
166.4
158.6
139.6
138.2
137.6
137.3
134.8
134.6
134.3
131.1
130.6
130.6
130.4
130.0
129.6
129.3
129.2
128.8
128.7
128.5
127.7
127.5
127.0
126.9
126.6
102.0
98.9
98.6
83.6
83.3
81.5
79.2
78.9
78.3
76.2
75.7
75.4
74.9
74.8
71.6
71.1
68.3
67.1
54.0
53.3
53.1
52.8
49.0 CD3OD
38.4
30.6
22.8
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm

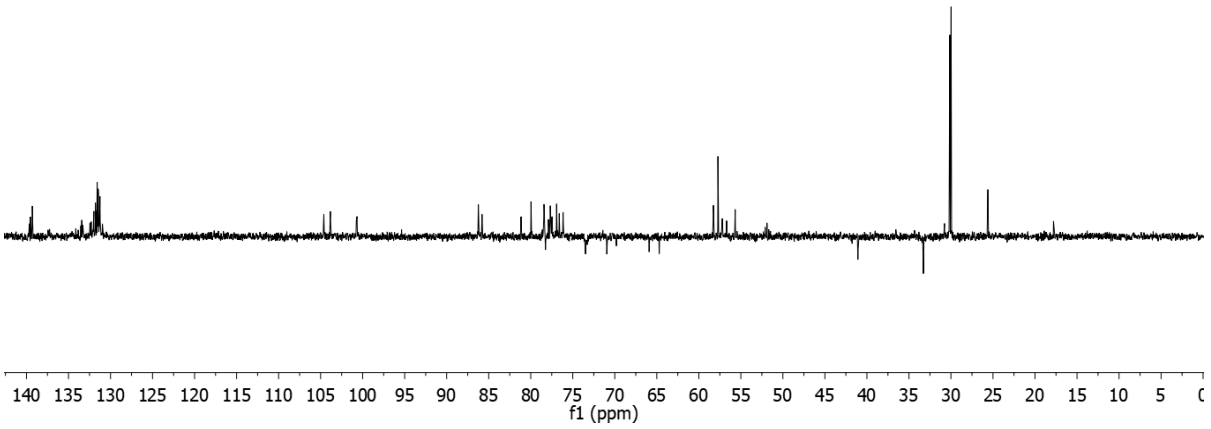

135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
ppm

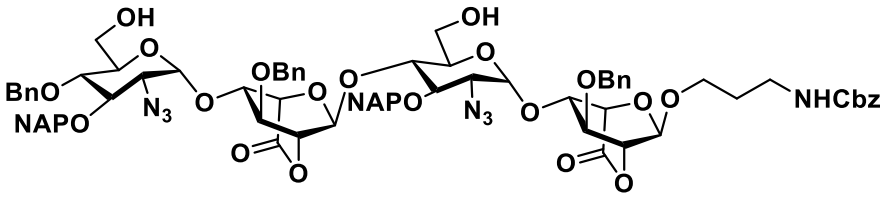

OTBDPS
BnO
O
$^{-}O_3SO$
AcHN
COOMe
O
O
BnO
BzO
O
$^{-}O_3SO$
OTBDPS
O
AcHN
COOMe
O
O
BnO
BzO
O
NHCbz

9

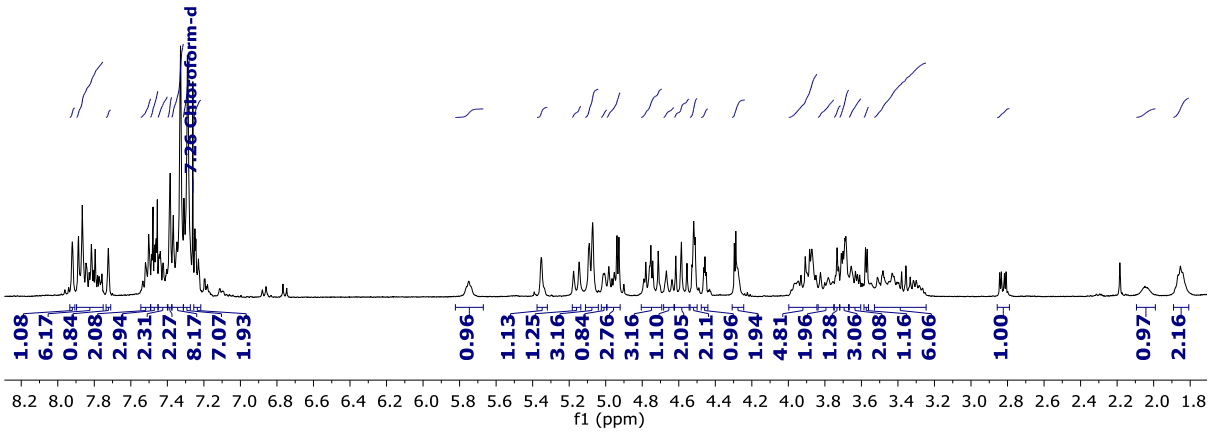

4.87 CD3OD
2.00
2.21
2.04
2.02
0.96
1.98
1.05
1.82
3.01
1.79
2.73
3.34
4.11
5.02
7.12
2.10
7.15
0.90
0.87
2.05
1.01
2.04
1.17
1.30
0.83
1.09
2.04
0.96
1.09
1.82
3.71
1.04
2.98
1.00
2.03
1.97
3.20
1.91
1.83
1.01
2.81
2.09
6.02
1.96
9.24
9.30
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

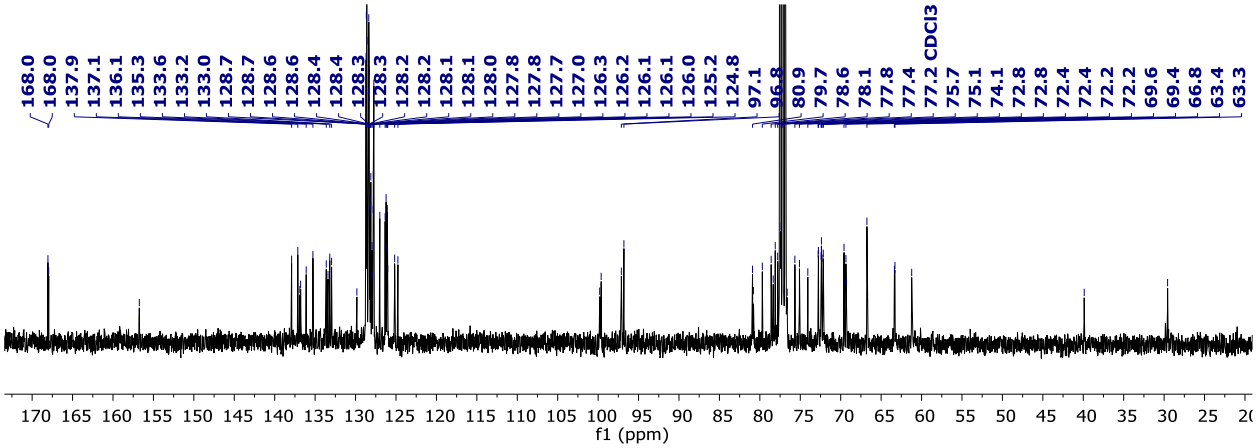

174.4
140.9
139.4
139.3
137.7
137.6
137.3
135.5
135.1
134.7
134.3
132.2
131.9
131.6
131.4
131.4
131.3
130.9
130.5
130.3
130.0
130.0
129.9
129.8
129.7
129.6
129.6
129.5
129.5
129.4
129.3
129.0
98.7
84.3
83.8
78.0
76.7
76.5
75.9
75.7
75.7
75.5
75.0
74.7
71.5
67.8
56.3
54.7
53.7
49.0 CD3OD
39.1
31.3
28.2
28.0
23.7
23.6
21.0
20.8
f1 (ppm)

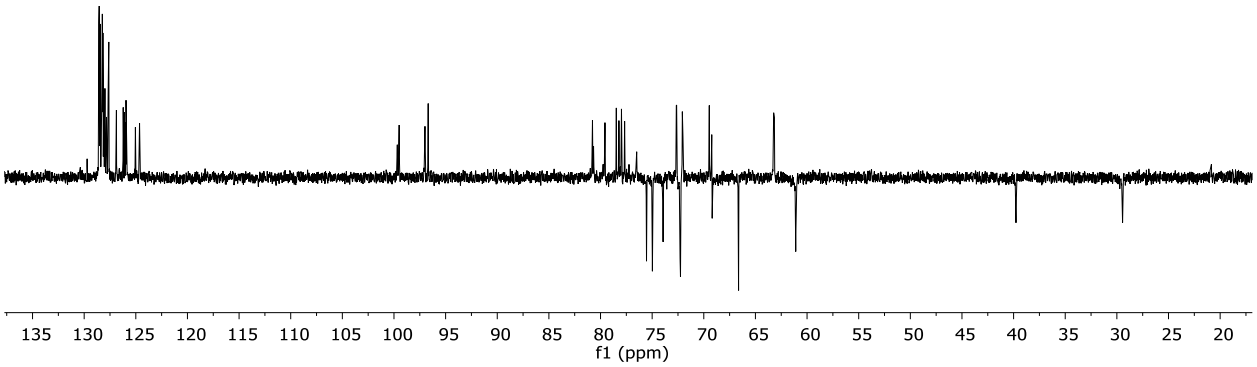

f1 (ppm)

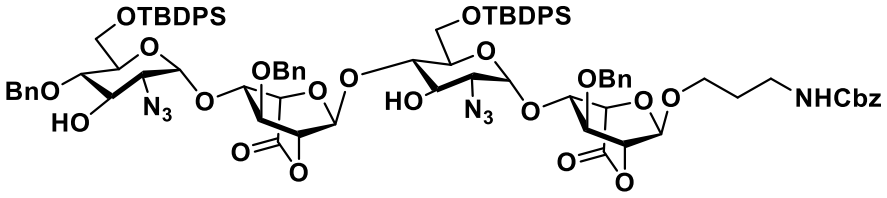

OH
O
BnO
NAPO
N3
O
OBn
O
O
O
O
OH
O
O
NAPO
N3
O
OBn
O
O
O
O
NHCbz

10a

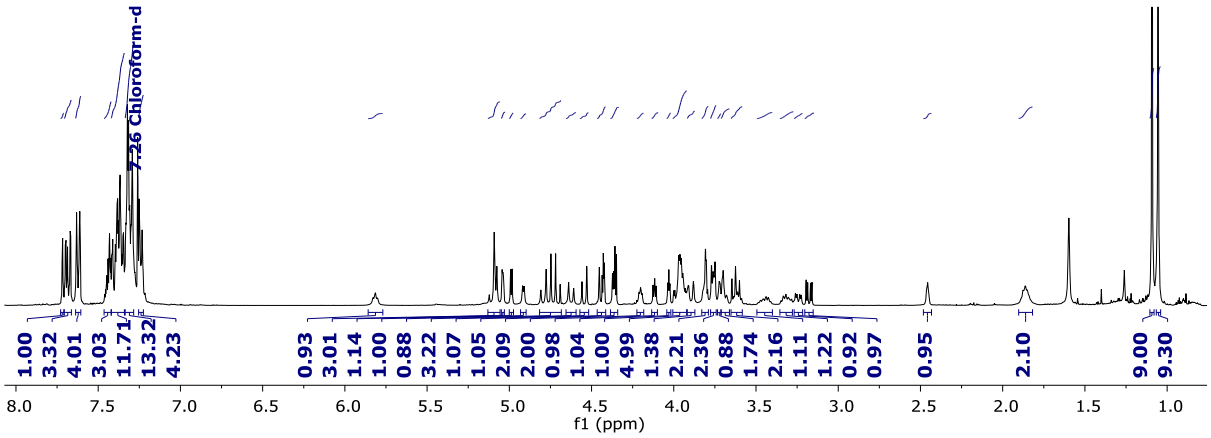

7.26 Chloroform-d
1.08
6.17
0.84
2.08
2.94
2.31
2.27
8.17
7.07
1.93
0.96
1.13
1.25
3.16
0.84
2.76
3.16
1.10
2.05
2.11
0.96
1.94
4.81
1.96
1.28
3.06
2.08
1.16
6.06
1.00
0.97
2.16
f1 (ppm)

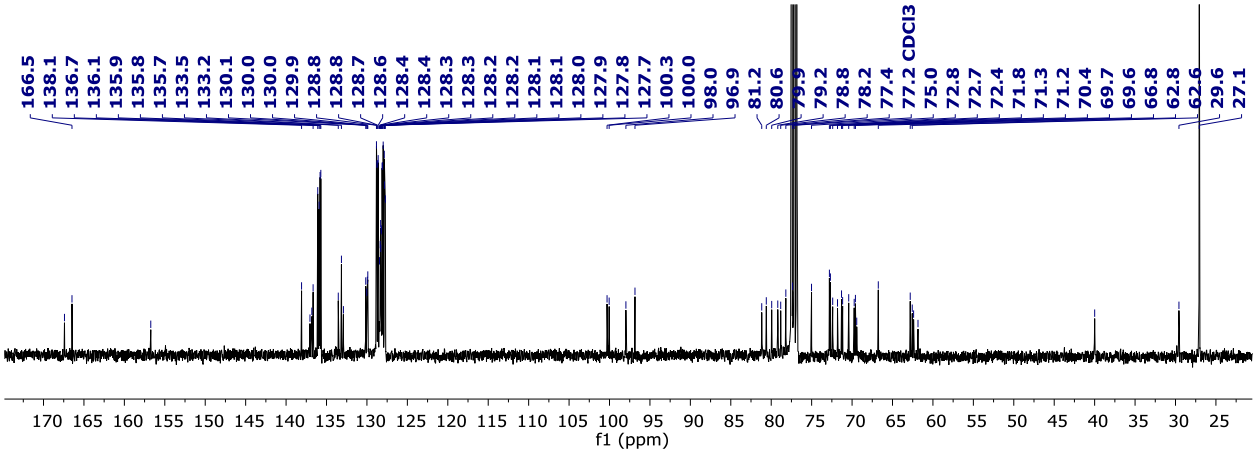

168.0
168.0
137.9
137.1
136.1
135.3
133.6
133.2
133.0
128.7
128.7
128.6
128.6
128.4
128.4
128.3
128.3
128.2
128.2
128.1
128.1
128.0
127.8
127.8
127.7
127.0
126.3
126.2
126.1
126.1
126.0
125.2
124.8
97.1
96.8
80.9
79.7
78.6
78.1
77.8
77.4
77.2 CDCl3
75.7
75.1
74.1
72.8
72.8
72.4
72.4
72.2
72.2
69.6
69.4
66.8
63.4
63.3
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

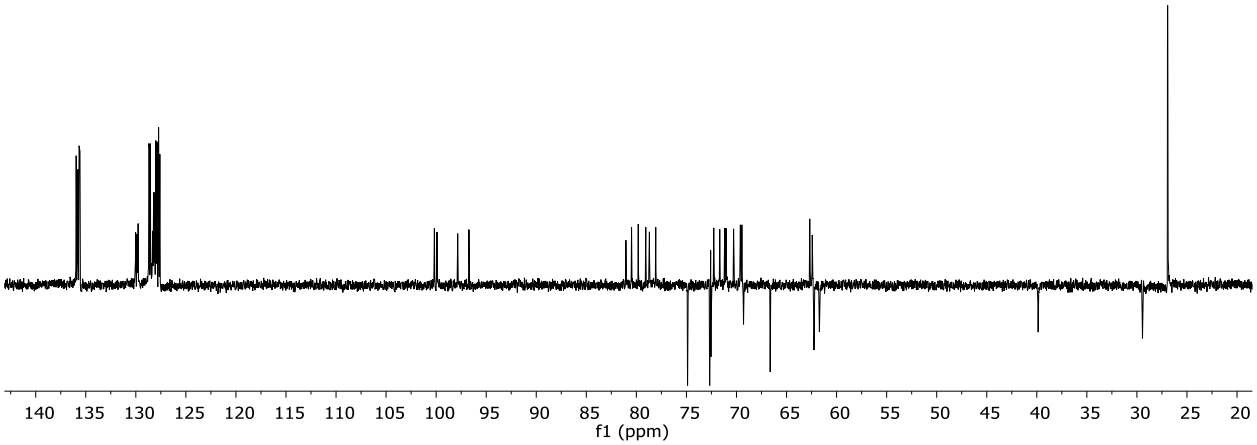

135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

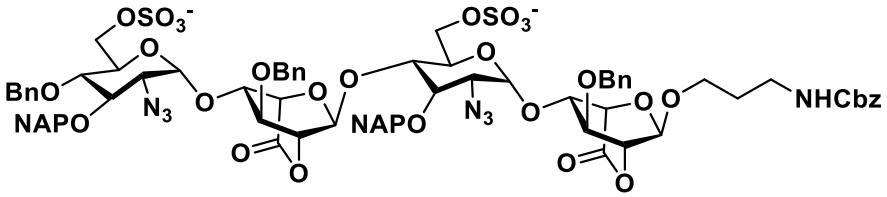

OTBDPS
BnO
HO
N3
OBn
OTBDPS
HO
N3
OBn
NHCbz

11a

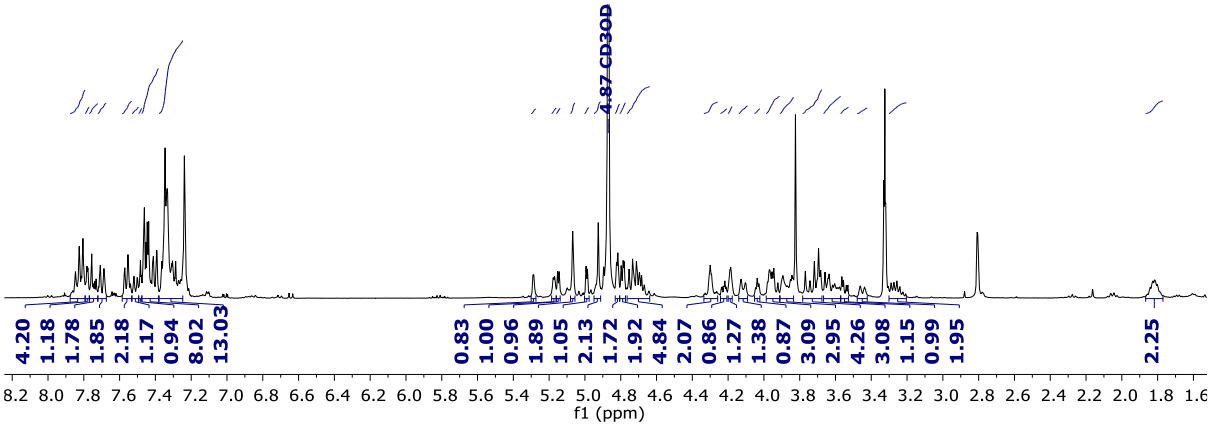

7.26 Chloroform-d
1.00 3.32 4.01 3.03 11.71 13.32 4.23 0.93 3.01 1.14 1.00 0.88 3.22 1.07 1.05 2.09 2.00 0.98 1.04 1.00 4.99 1.38 2.21 2.36 0.88 1.74 2.16 1.11 1.22 0.92 0.97 0.95 2.10 9.00 9.30
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

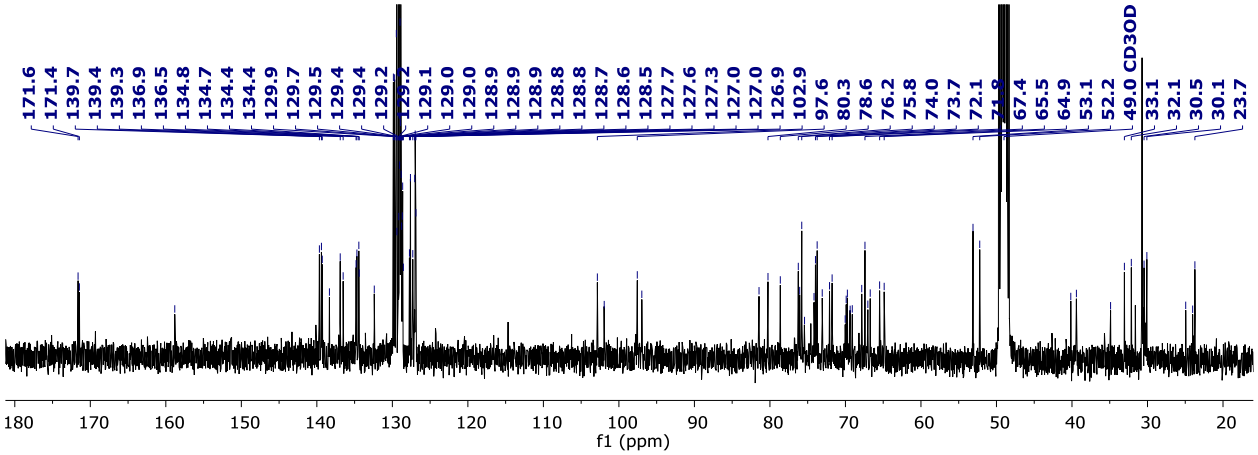

166.5 138.1 136.7 136.1 135.9 135.8 135.7 133.5 133.2 130.1 130.0 130.0 129.9 128.8 128.8 128.7 128.6 128.4 128.4 128.3 128.3 128.2 128.2 128.1 128.1 128.0 127.9 127.8 127.7 100.3 100.0 98.0 96.9 81.2 80.6 79.9 79.2 78.8 78.2 77.4 77.2 CDCl3 75.0 72.8 72.7 72.4 71.8 71.3 71.2 70.4 69.7 69.6 66.8 62.8 62.6 29.6 27.1
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)

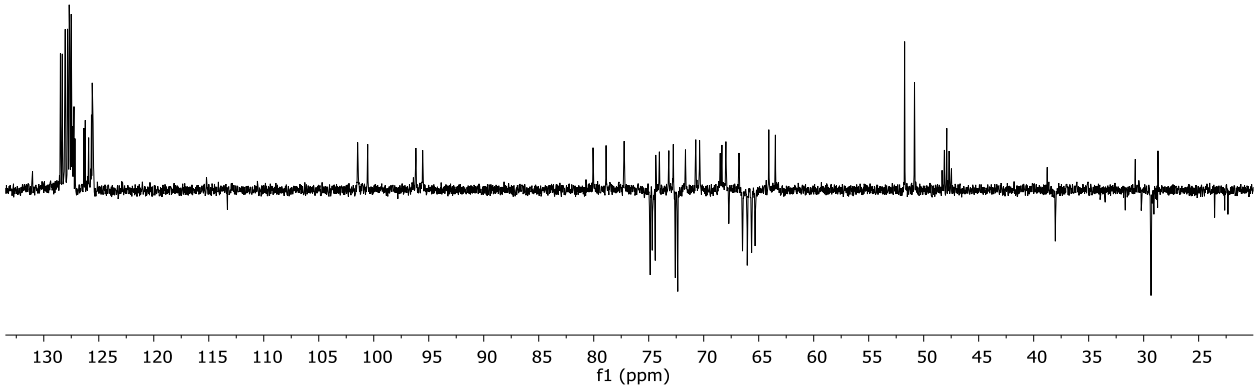

140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

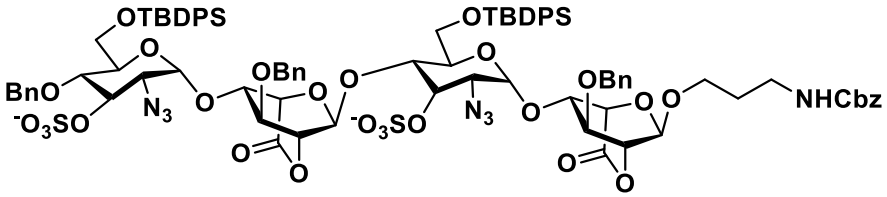

OSO3-
O
BnO
NAPO
N3
O
OBn
O
O
O
O
OSO3-
O
NAPO
N3
O
OBn
O
O
O
O
NHCbz

10

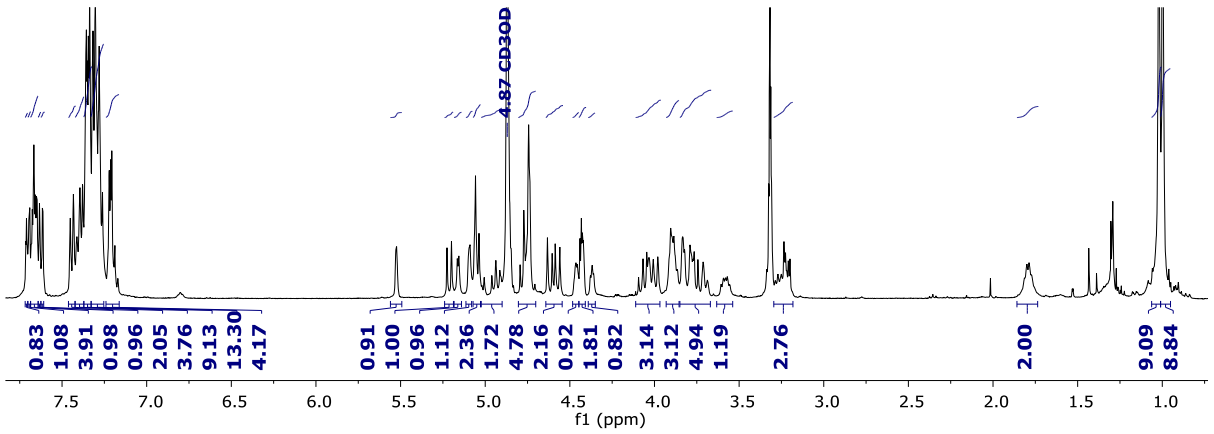

4.87 CD3OD
4.20 1.18 1.78 1.85 2.18 1.17 0.94 8.02 13.03
0.83 1.00 0.96 1.89 1.05 2.13 1.72 1.92 4.84 2.07 0.86 1.27 1.38 0.87 3.09 2.95 4.26 3.08 1.15 0.99 1.95
2.25
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
f1 (ppm)

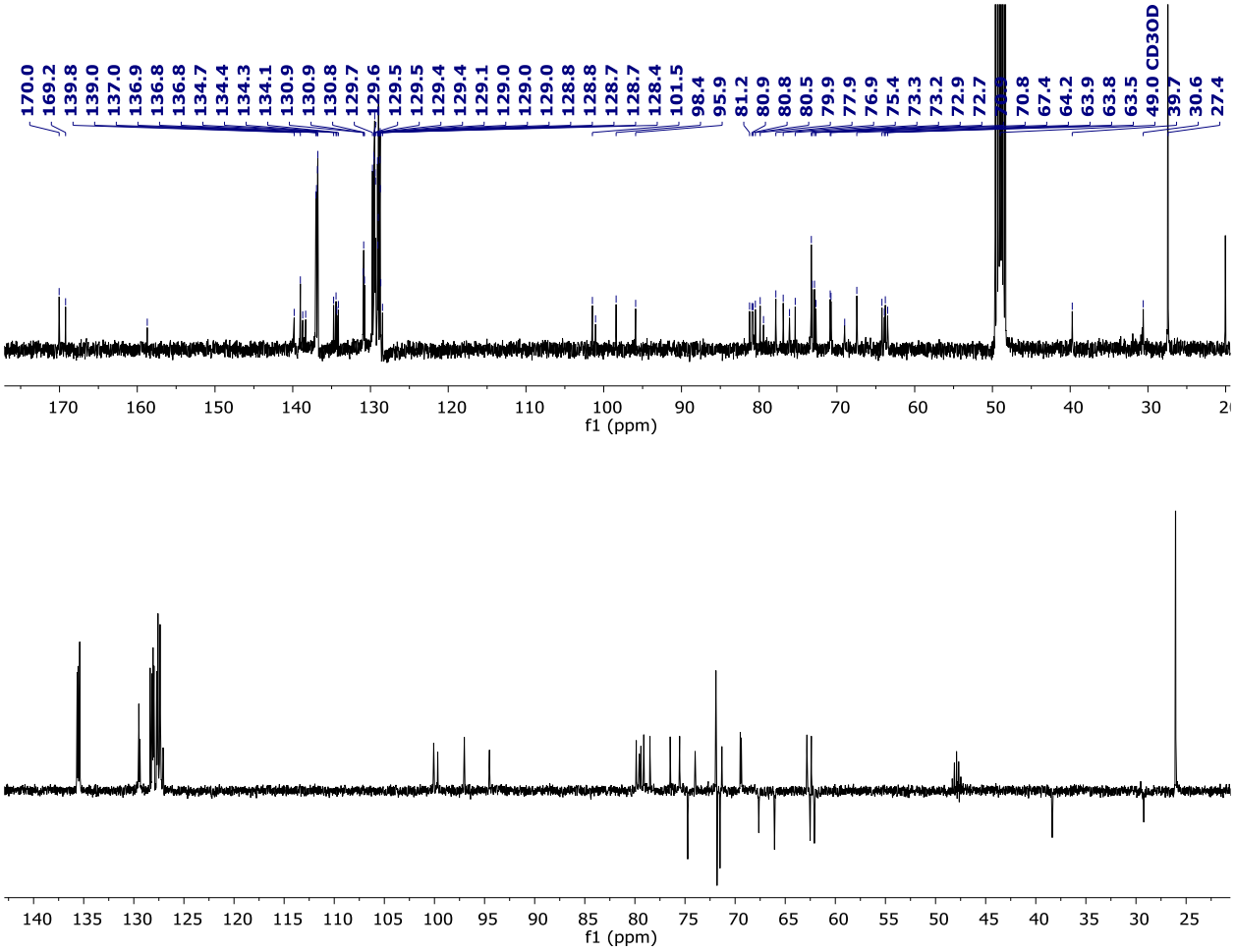

171.6
171.4
139.7
139.4
139.3
136.9
136.5
134.8
134.7
134.4
134.4
129.9
129.7
129.5
129.4
129.4
129.2
129.2
129.1
129.0
129.0
128.9
128.9
128.8
128.8
128.7
128.6
128.5
127.7
127.6
127.3
127.0
127.0
126.9
102.9
97.6
80.3
78.6
76.2
75.8
74.0
73.7
72.1
71.8
67.4
65.5
64.9
53.1
52.2
49.0 CD3OD
33.1
32.1
30.5
30.1
23.7
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

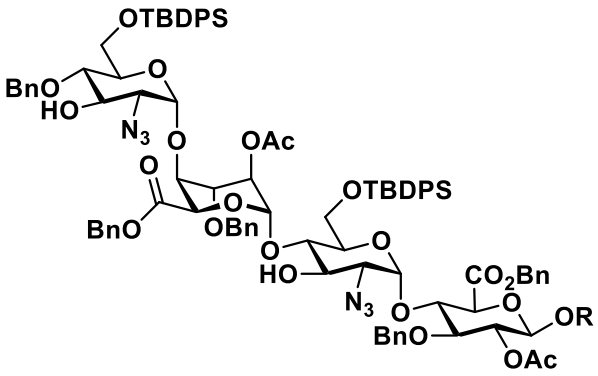

130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)


OTBDPS
BnO
$^{-}O_3SO$
N_3
OBn
OTBDPS
$^{-}O_3SO$
N_3
OBn
NHCbz

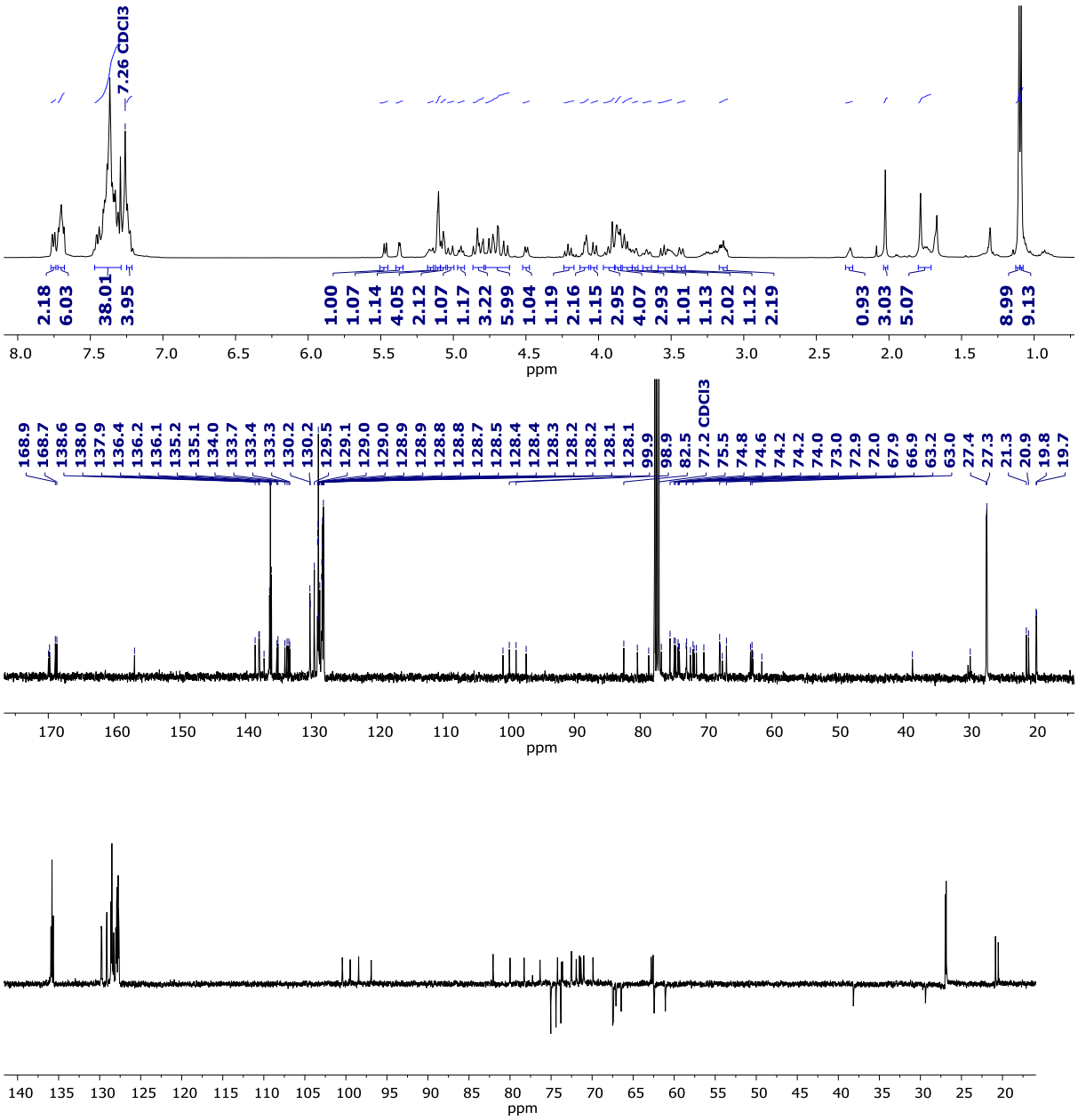
11

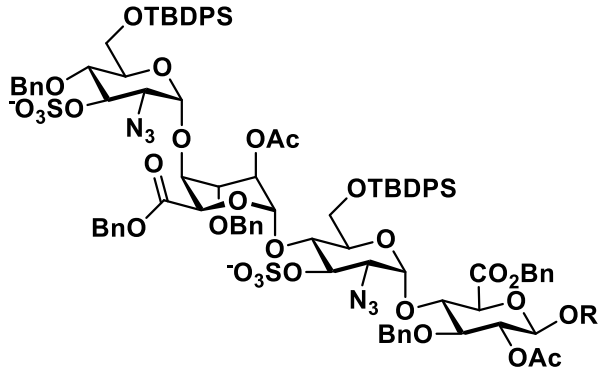

4.87 CD3OD
0.83 1.08 3.91 0.98 0.96 2.05 3.76 9.13 13.30 4.17
0.91 1.00 0.96 1.12 2.36 1.72 4.78 2.16 0.92 1.81 0.82 3.14 3.12 4.94 1.19 2.76 2.00 9.09 8.84
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)


170.0
169.2
139.8
139.0
137.0
136.9
136.8
136.8
134.7
134.4
134.3
134.1
130.9
130.9
130.8
129.7
129.6
129.5
129.5
129.4
129.4
129.1
129.0
129.0
129.0
128.8
128.8
128.7
128.7
128.4
101.5
98.4
95.9
81.2
80.9
80.8
80.5
79.9
77.9
76.9
75.4
73.3
73.2
72.9
72.7
70.8
67.4
64.2
63.9
63.8
63.5
49.0 CD3OD
39.7
30.6
27.4
f1 (ppm)
f1 (ppm)

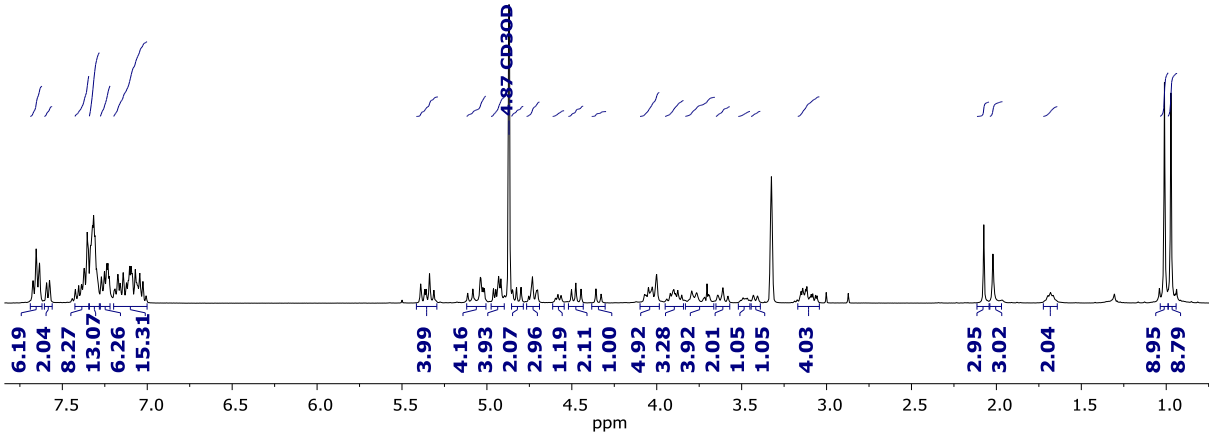

OTBDPS
BnO
HO
O
N3
O
OAc
O
BnO
O
OBn
OTBDPS
O
HO
O
CO2Bn
N3
O
O
OR
BnO
OAc

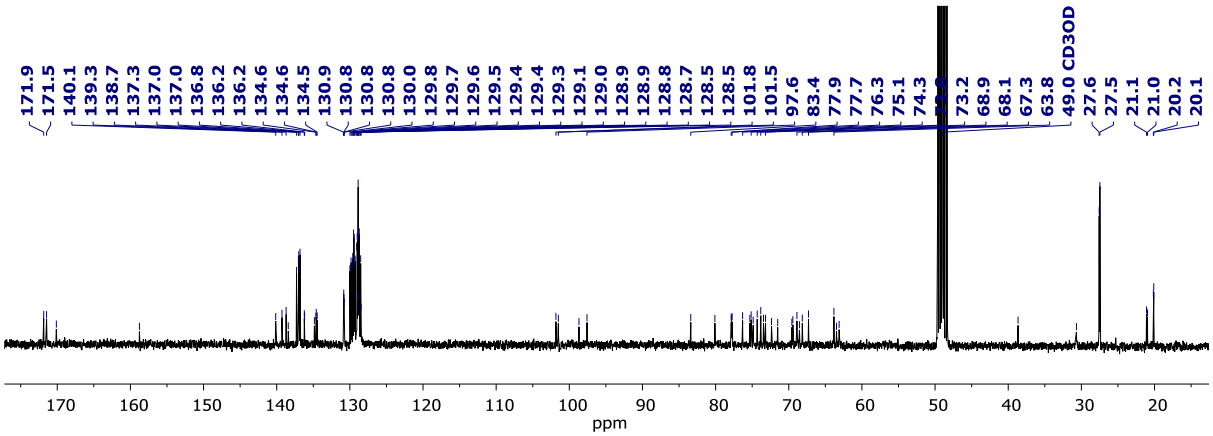
12a

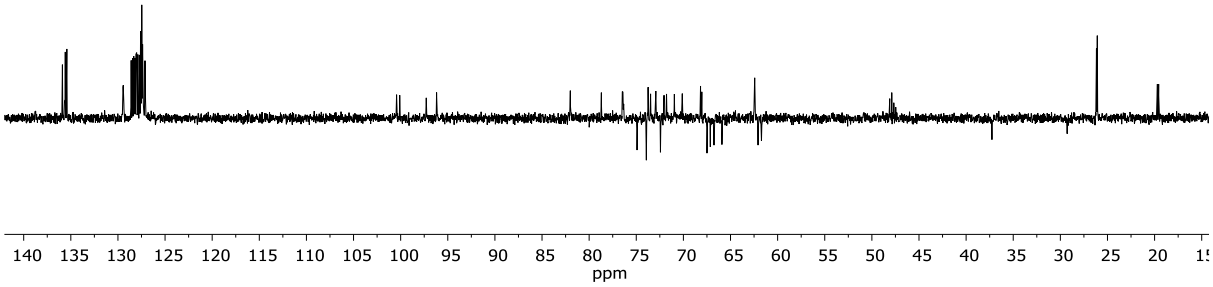

7.26 CDCl3
2.18 6.03 38.01 3.95 1.00 1.07 1.14 4.05 2.12 1.07 1.17 3.22 5.99 1.04 1.19 2.16 1.15 2.95 4.07 2.93 1.01 1.13 2.02 1.12 2.19 0.93 3.03 5.07 8.99 9.13
ppm
168.9 168.7 138.6 138.0 137.9 136.4 136.2 136.1 135.2 135.1 134.0 133.7 133.4 133.3 130.2 130.2 129.5 129.1 129.0 129.0 128.9 128.9 128.8 128.8 128.7 128.5 128.4 128.4 128.3 128.2 128.2 128.1 128.1 99.9 98.9 82.5 77.2 CDCl3 75.5 74.8 74.6 74.2 74.2 74.0 73.0 72.9 72.0 67.9 66.9 63.2 63.0 27.4 27.3 21.3 20.9 19.8 19.7
ppm
ppm

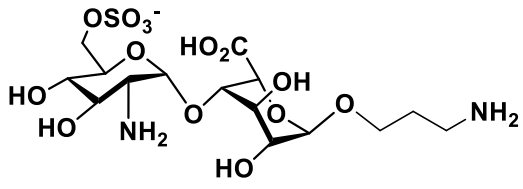

OTBDPS
BnO
-O3SO
N3
O
OAc
BnO
OBn
OTBDPS
-O3SO
CO2Bn
N3
BnO
OR
OAc

12

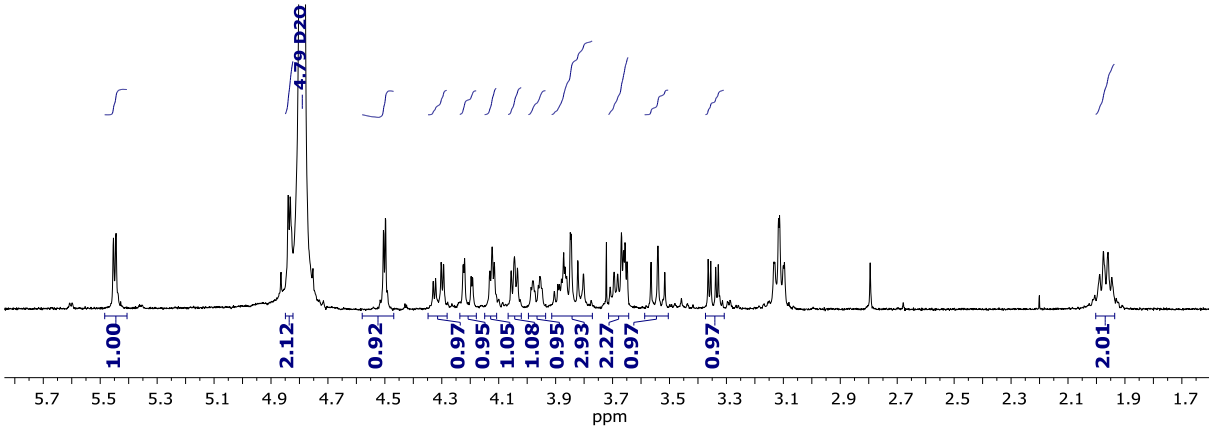

4.87 CD3OD
6.19 2.04 8.27 13.07 6.26 15.31 3.99 4.16 3.93 2.07 2.96 1.19 2.11 1.00 4.92 3.28 3.92 2.01 1.05 1.05 4.03 2.95 3.02 2.04 8.95 8.79
ppm

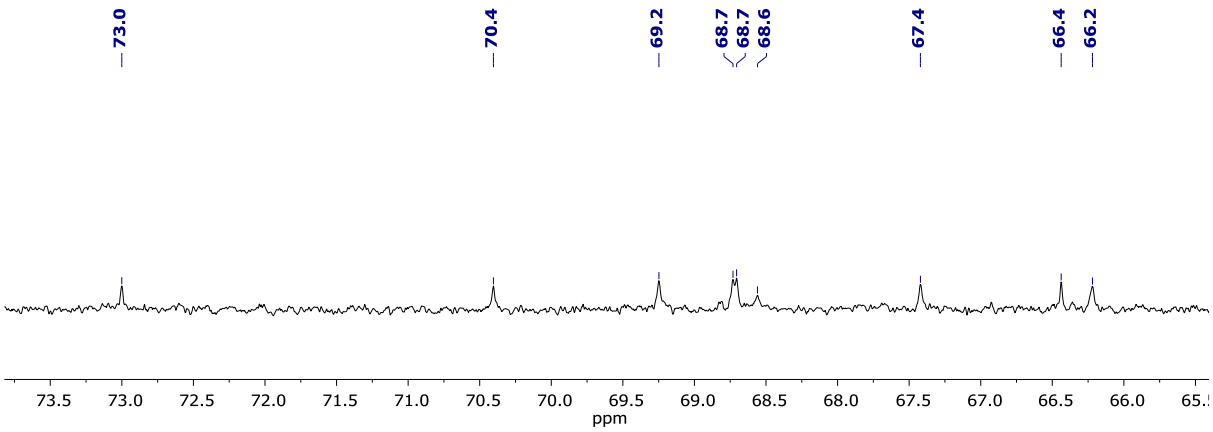

171.9 171.5 140.1 139.3 138.7 137.3 137.0 137.0 136.8 136.2 136.2 134.6 134.6 134.5 130.9 130.8 130.8 130.8 130.0 129.8 129.7 129.6 129.5 129.4 129.4 129.3 129.1 129.0 128.9 128.9 128.8 128.7 128.5 128.5 101.8 101.5 97.6 83.4 77.9 77.7 76.3 75.1 74.3 73.2 68.9 68.1 67.3 63.8 49.0 CD3OD 27.6 27.5 21.1 21.0 20.2 20.1
ppm

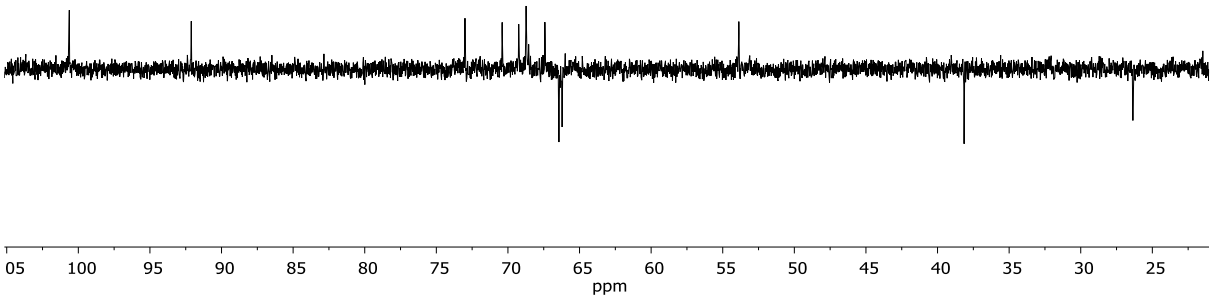

ppm

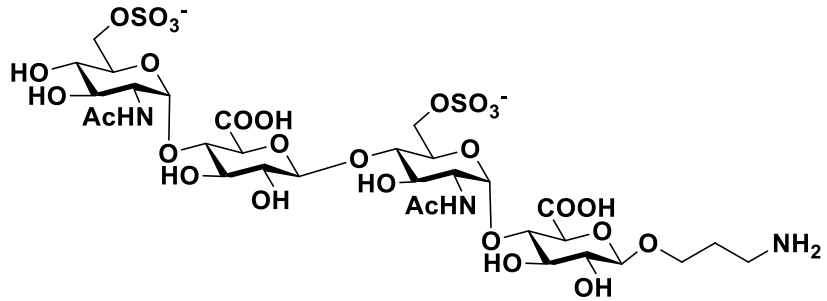

OSO3-
O
HO2C
OH
HO
HO
NH2
O
O
O
NH2
HO

13

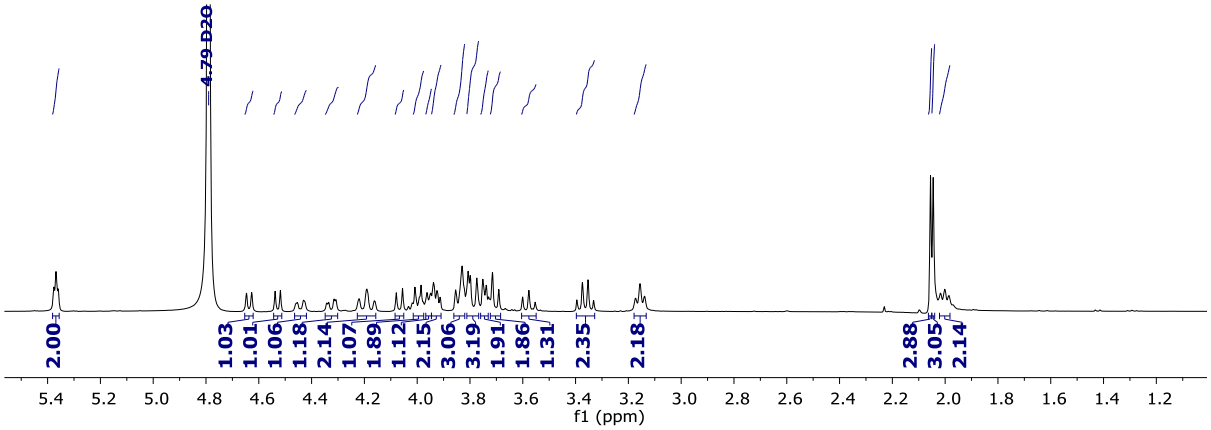

4.79 D2O
1.00
2.12
0.92
0.97
0.95
1.05
1.08
0.95
2.93
2.27
0.97
0.97
2.01
ppm

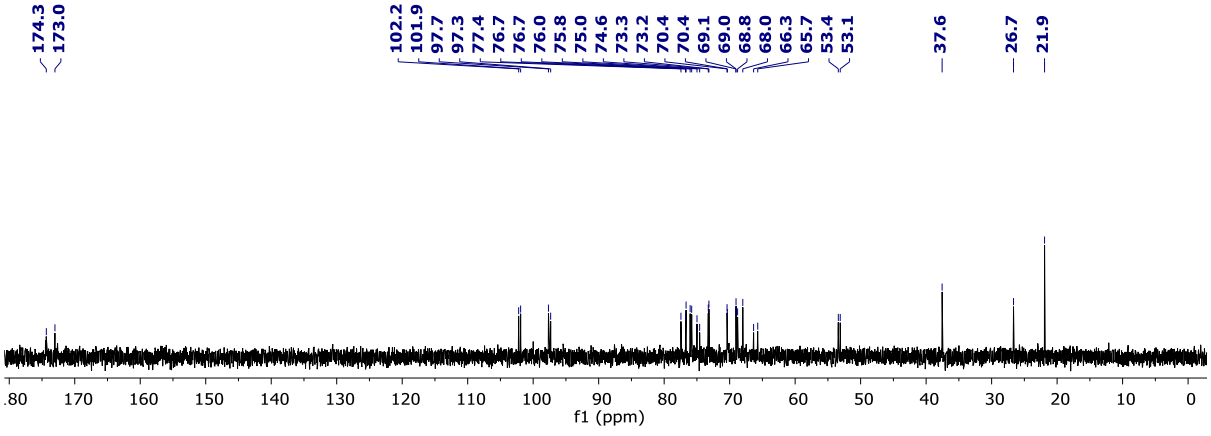

73.0
70.4
69.2
68.7
68.7
68.6
67.4
66.4
66.2
ppm

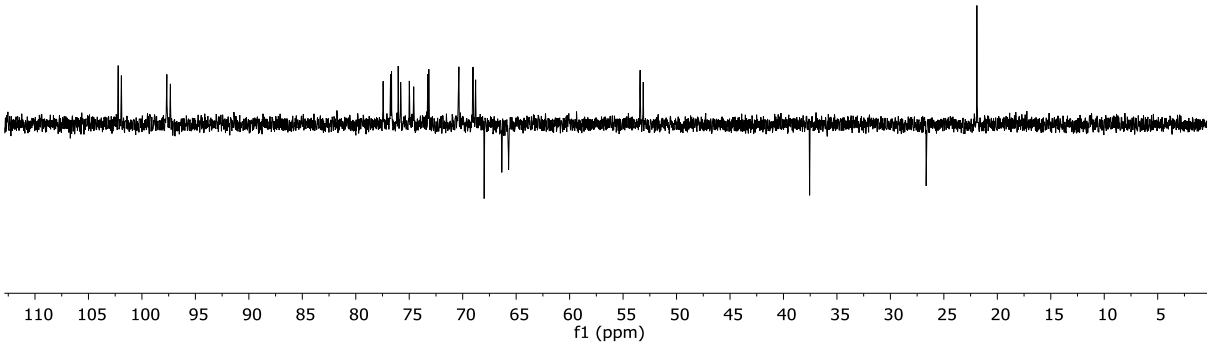

ppm

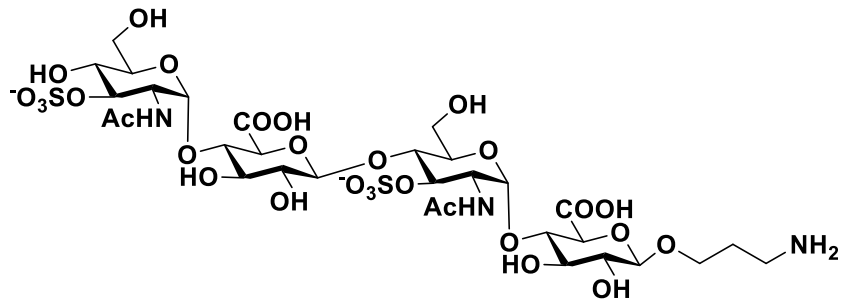

OSO3-
HO
HO
AcHN
O
COOH
O
HO
OH
O
OSO3-
O
HO
AcHN
O
COOH
O
HO
OH
O
NH2

14

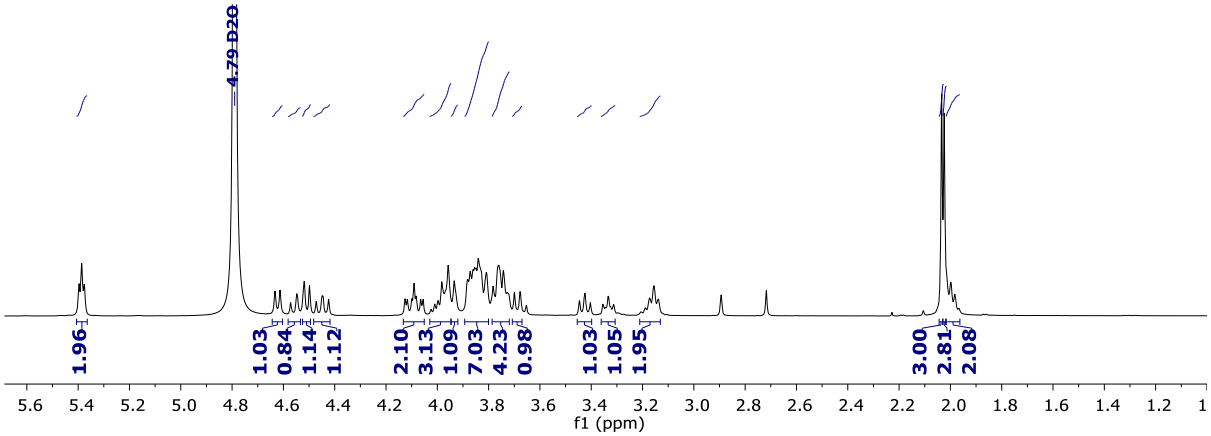

4.79 D20
2.00
1.03
1.01
1.06
1.18
2.14
1.07
1.89
1.12
2.15
3.06
3.19
1.91
1.86
1.31
2.35
2.18
2.88
3.05
2.14
f1 (ppm)

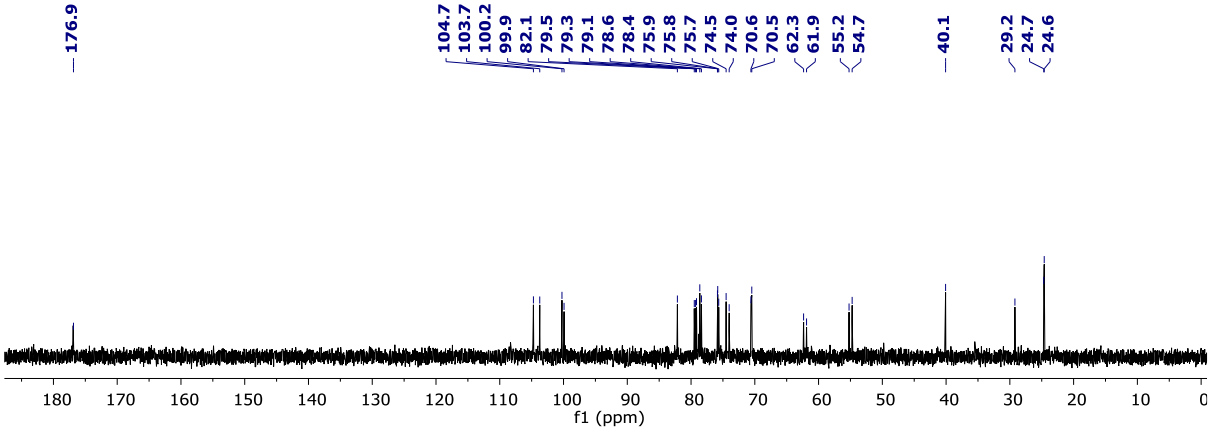

174.3
173.0
102.2
101.9
97.7
97.3
77.4
76.7
76.7
76.0
75.8
75.0
74.6
73.3
73.2
70.4
70.4
69.1
69.0
68.8
68.0
66.3
65.7
53.4
53.1
37.6
26.7
21.9
f1 (ppm)

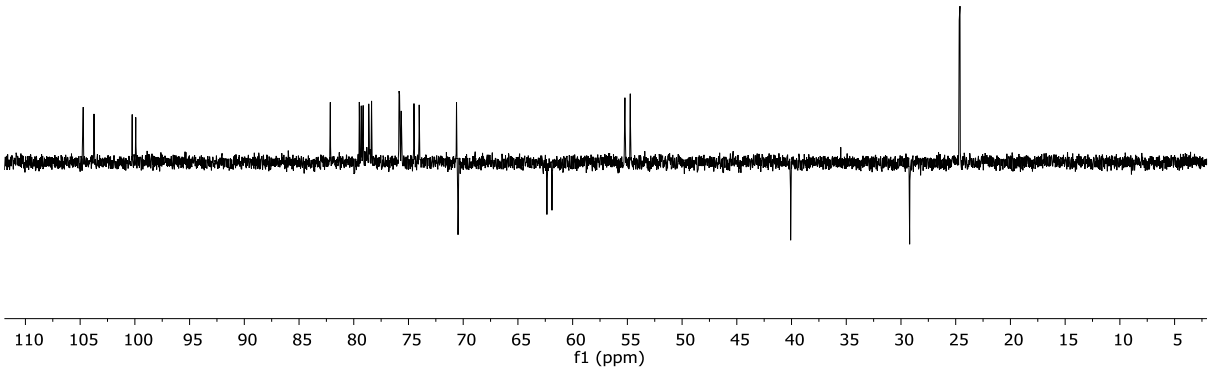

f1 (ppm)

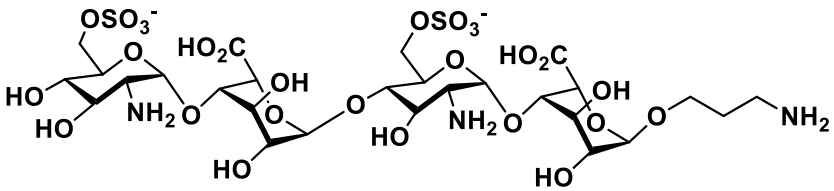

OH
HO
O
-O3SO
AcHN
COOH
O
HO
OH
O
-O3SO
OH
AcHN
COOH
O
HO
OH
O
NH2

15

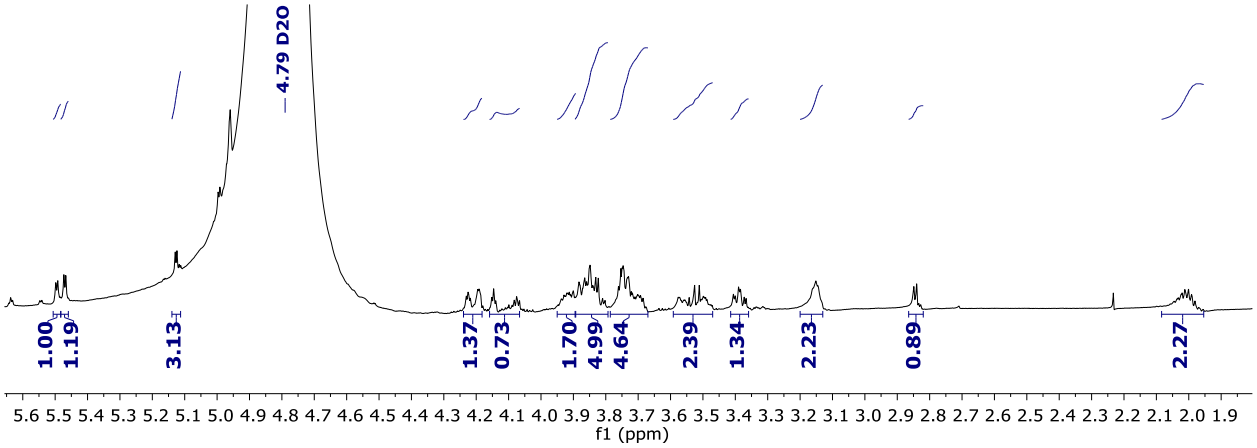

4.79 D2O
1.96
1.03
0.84
1.14
1.12
2.10
3.13
1.09
7.03
4.23
0.98
1.03
1.05
1.95
3.00
2.81
2.08
f1 (ppm)

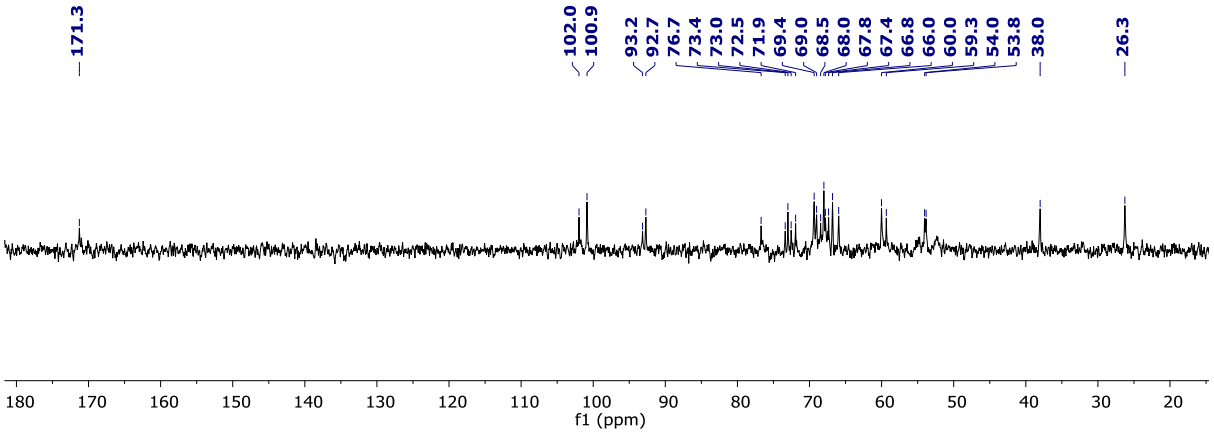

176.9
104.7
103.7
100.2
99.9
82.1
79.5
79.3
79.1
78.6
78.4
75.9
75.8
75.7
74.5
74.0
70.6
70.5
62.3
61.9
55.2
54.7
40.1
29.2
24.7
24.6
f1 (ppm)

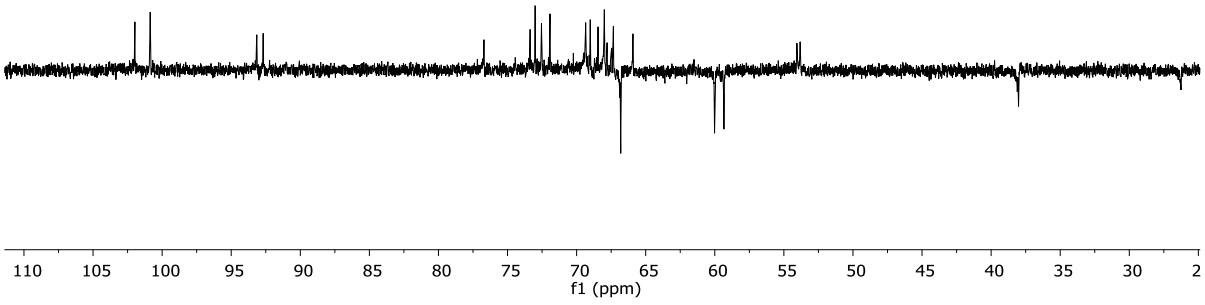

f1 (ppm)

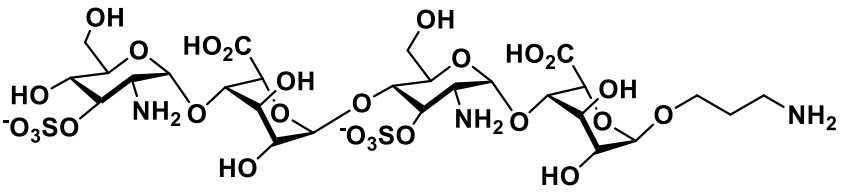

OSO3-
HO2C
OH
HO
NH2
O
HO
OSO3-
HO2C
OH
NH2
NH2

16

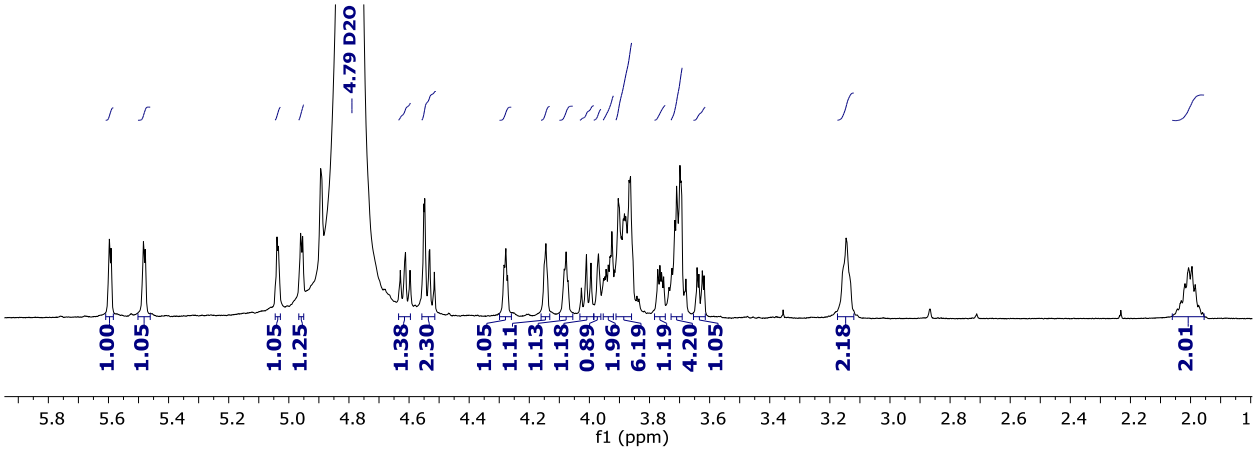

4.79 D2O
1.00
1.19
3.13
1.37
0.73
1.70
4.99
4.64
2.39
1.34
2.23
0.89
2.27
5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9
f1 (ppm)

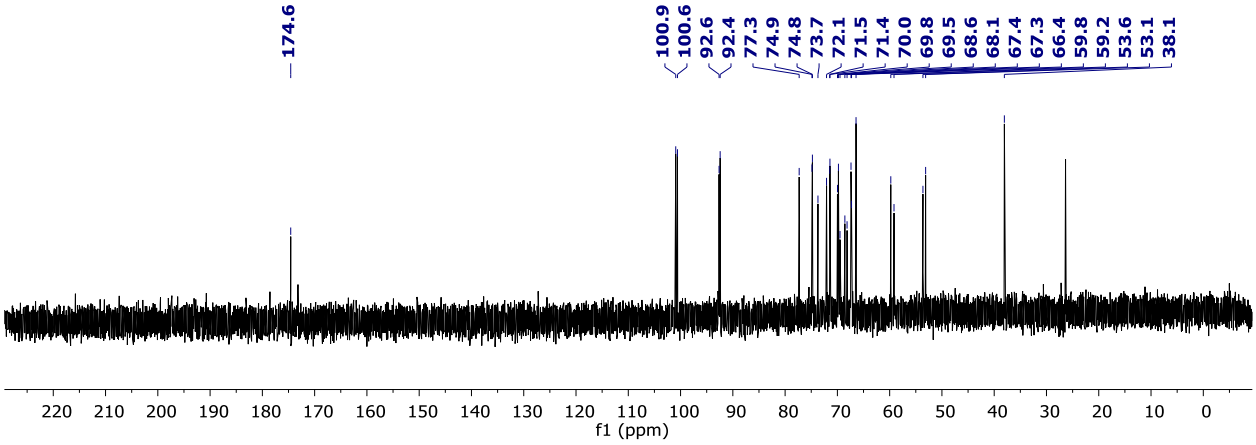

171.3
102.0
100.9
93.2
92.7
76.7
73.4
73.0
72.5
71.9
69.4
69.0
68.5
68.0
67.8
67.4
66.8
66.0
60.0
59.3
54.0
53.8
38.0
26.3
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

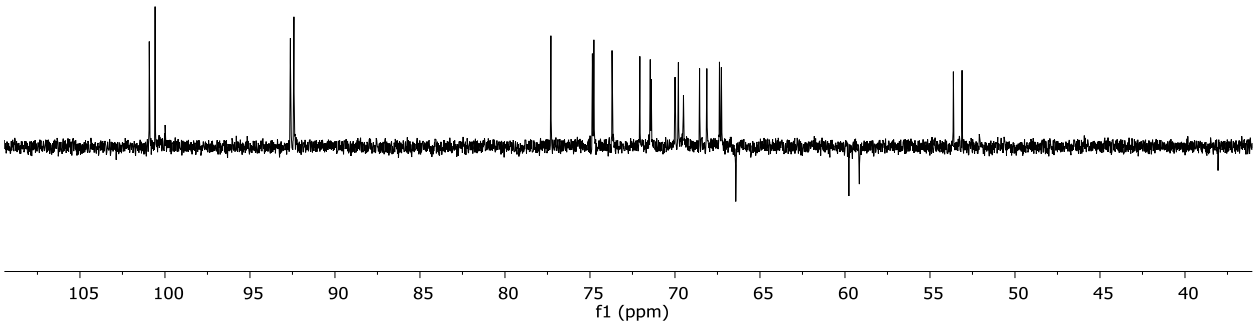

110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 2
f1 (ppm)


OH
HO
HO2C
NH2
O
-O3SO
NH2
NH2

17


4.79 D2O
1.00
1.05
1.05
1.25
1.38
2.30
1.05
1.11
1.13
1.18
0.89
1.96
6.19
1.19
4.20
1.05
2.18
2.01
f1 (ppm)


174.6
100.9
100.6
92.6
92.4
77.3
74.9
74.8
73.7
72.1
71.5
71.4
70.0
69.8
69.5
68.6
68.1
67.4
67.3
66.4
59.8
59.2
53.6
53.1
38.1
f1 (ppm)


f1 (ppm)

Chapter- 5

Sulfated L-Idose instead of L-Iduronic acid in Heparinoid Structures Influences Amphiphilic Glycopeptide Cell Membrane Dynamics

5.1 Abstract

Molecular editing is a potent strategy for elucidating the significance of specific functionalities within a given molecule. Herein, we edited heparan sulphate hexasaccharide by replacing L-iduronic acid with sulfated L-idose and investigated their role in glycocalyx remodeling. Employing a [2+2+2] disaccharide glycosylation strategy, we synthesized *N*-sulfated HS hexasaccharides containing either L-iduronic acid or sulfated L-idose. These hexasaccharides were functionalized onto a fluorescent amphiphilic peptide backbone and expressed on cancer cells. The sulfated L-idose in proteoglycan mimetics (**PG@HH662S**) exhibited rapid endocytosis compared to the native L-iduronic acid-based mimetics (**PG@HH62S**). The internalization of **PG@HH662S** was found to be independent of clathrin- and caveolae-mediated pathways. Notably, after 2 hours, a fraction of the molecules was sequestered in the nuclear region. We are currently investigating the pathway underlying the nuclear localization of **PG@HH662S** and its potential role in modulating histone activity.

5.2 Introduction

Heparan sulphate (HS) is a polysulfated glycosaminoglycan (GAG) ubiquitously expressed on cell surfaces and within the extracellular matrix. HS interacts with numerous proteins, including growth factors, chemokines, and enzymes, to regulate signaling pathways critical for cell growth, differentiation, immune responses, and angiogenesis. Structurally, HS comprises repeating disaccharide units of D-glucuronic acid (GlcA) or its epimer L-iduronic acid (IdoA), linked to glucosamine (GlcN) residues, with varying patterns of sulfation and acetylation. Moreover, the flexibility of IdoA, particularly its ability to adopt multiple conformations such as the chair ($^1C_4$), skew-boat ($^2S_0$), and boat forms, enables specific interactions with proteins.[1-4] These structural variations confer HS with remarkable functional diversity and specificity in biological recognition events. Consequently, there is significant research interest in understanding the role of different HS variants in biological processes.[5-8] Previous studies have demonstrated that 3-O-sulfation is crucial for binding many viral proteins, while the $^2S_0$ conformation of L-iduronic acid is essential for anticoagulant activity. Similarly, D-glucuronic acid plays a vital role in HCII anticoagulation activity. However, the rationale behind nature's preference for the sulfate-carboxylic acid combination in the ionic interactions of HS during biological recognition remains unclear. To investigate this, herein, we synthesized two hexasaccharides incorporating L-iduronic acid or sulphated L-idose as the uronic acid residue and functionalized them on a neoproteoglycan backbone to study their role in cell surface engineering. Despite both molecules carrying 12 anionic residues, they exhibited significant differences in their cell surface arrangement and internalization processes.

5.3 Results and Discussion

We synthesized sulfated L-idose HS oligosaccharide using [2+2+2] glycosylation strategy comprising donor **5** and acceptor **8** disaccharide respectively. The disaccharide assemblies **5** and **8** were obtained from orthogonally protected D-glucosamine donors **1** and **6** and acceptor L-idopyranosyl **2** under standard glycosylation conditions. The thiodonor **6** carries C-4 chloroacetyl as a facile protecting group which can be removed easily at the later stage for further modification and chain elongation. The disaccharide donor **5** was obtained by acetolysis of **3** using acetic anhydride and copper (II) trifluoromethanesulfonate as a catalyst followed by phenyl trimethylsilyl sulphide and ZnI_2 treatment to generate

corresponding thioglycoside in excellent yield. Finally, glycosylation of donor **5** and acceptor **8** by utilizing *N*-iodosuccinimide (NIS) and the trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoter resulted in 90% of tetrasaccharide **9** (Fig. 1 & 2).[9,10]

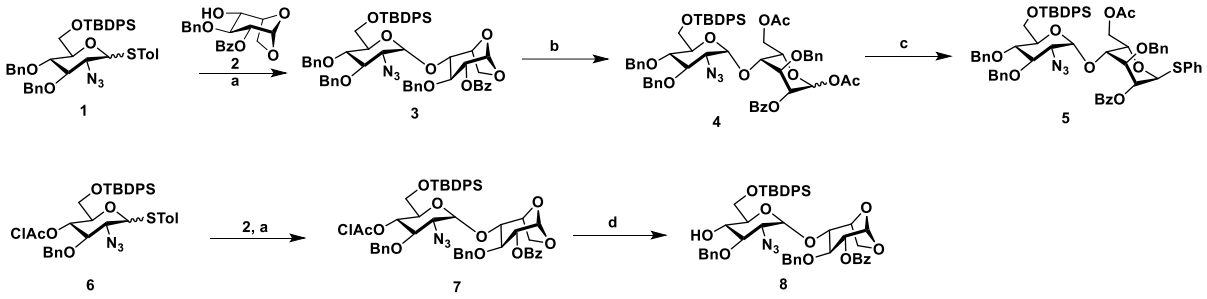


Fig. 1 Synthesis of disaccharide building block. Reagents. Scheme 1: a. NIS, TMSOTf, 4 Å MS, -78 ℃ to -20 ℃, DCM, 30 min, 78-82%. **b.** Ac_2O, $Cu(OTf)_2$, rt, 12 h, 87%. **c.** TMSSPh, ZnI_2, DCM, rt, 2 h, 76%. **d.** Thiourea, MeOH/ Py (1:1), 80 ℃, 1 h, 84%.

Sequential acetolysis, thiophenol glycosylation and acceptor glycosylation **8** at the reducing end of IdoA resulted hexasaccharide **12** (Fig. 3). Selective deprotection of TBDPS using hydrogen fluoride: pyridine complex in pyridine followed by deacetylation and debezoylation using lithium hydroxide solution and O-sulfation in the presence of $SO_3 \cdot Et_3N$ in DMF to yield sulfated L-idose hexasaccharide precursor **18**. The C-2 azide of **18** was converted to amine in the presence of trimethyl phosphine yielding 72% of **19**[11-13]. Using the SO_3.pyridine complex, amine was *N*-sulfated. Finally, global deprotection by hydrogenolysis yielded desired HS hexasaccharide **HH662S** (fig. 4). Synthesis of **HH62S** was reported in Saurabh's thesis.

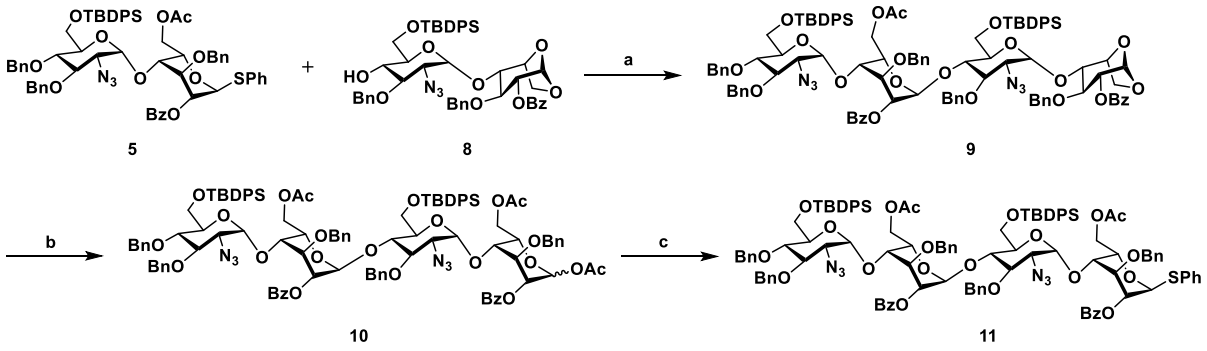


Fig. 2 Synthesis of tetrasaccharide building block. Reagents. a. NIS, TMSOTf, 4 Å MS -10 °C, DCM, 30 min., 78%; **b.** Ac_2O, $Cu(OTf)_2$, rt, 12 h, 80% **c.** TMSSPh, ZnI_2, DCM, rt, 2 h, 70%.

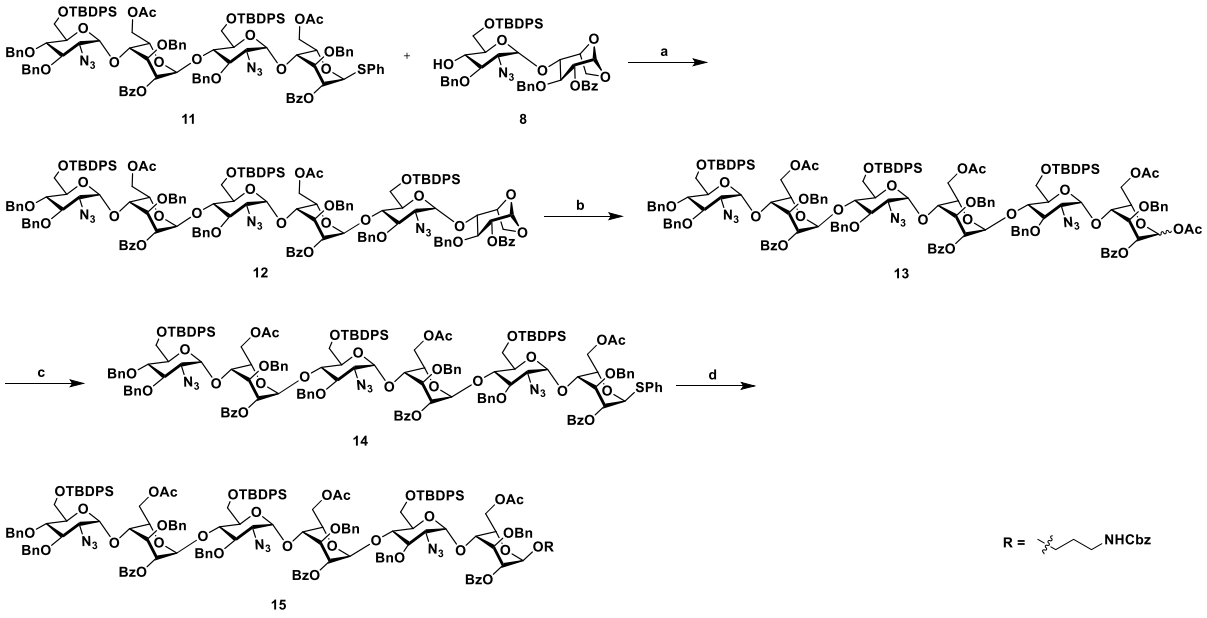


Fig. 3 Synthesis of protected Hexasaccharide building block. Reagents. a. NIS, TMSOTf, 4 Å MS -10 °C, DCM, 30 min., 59% **b.** Ac_2O, $Cu(OTf)_2$, rt, 12 h, 80% **c.** TMSSPh, ZnI_2, DCM, rt, 2 h, 83%. **d.** Benzyl (3-hydroxypropyl)carbamate, NIS, TMSOTf, 4 Å MS, 0 °C, DCM, 10 min., 80%.

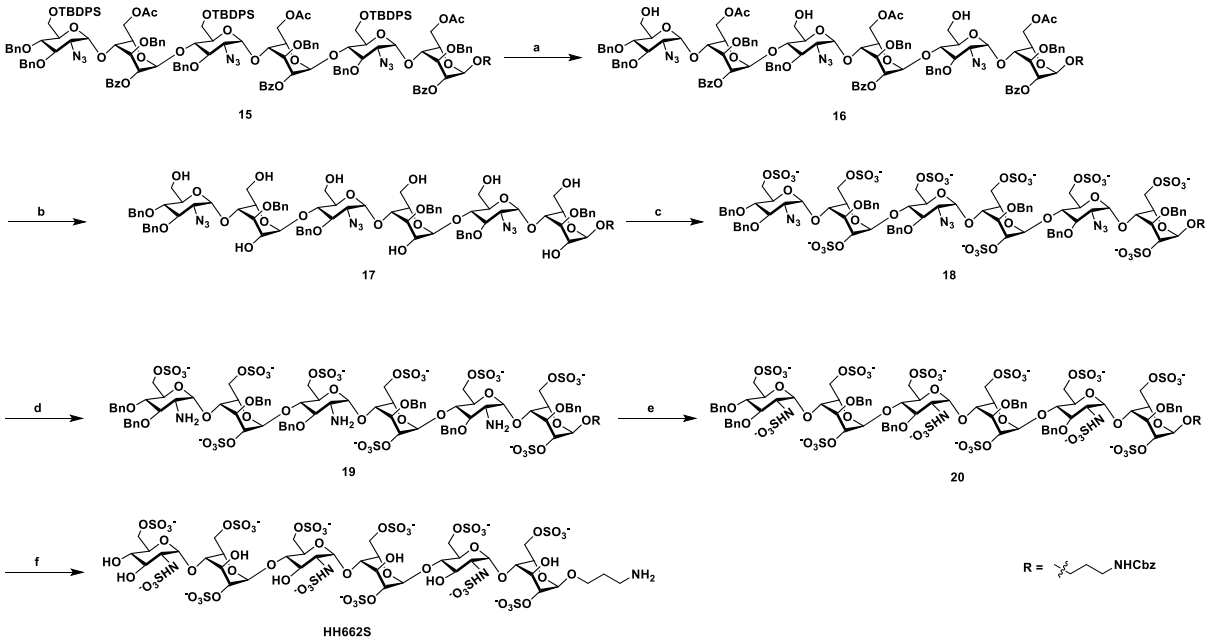


Fig. 4 Synthesis of final deprotected Hexasaccharide. Reagents. a. HF.Py, Py, 0 °C, 12 h, 84%; **b.** LiOH, H2O : THF(1 : 1), rt, 12 h, 90%; **c.** $SO_3.NMe_3$, DMF, MW, 100 °C, 30 min,

54%; **d.** PMe_3.THF, THF, 1N NaOH, rt, 12 h, 76%; **e.** SO_3.Py. MeOH, 1N NaOH, 48 h, 74%; **f.** H_2, $Pd(OH)_2$, H_2O, rt, 48 h, 63%.

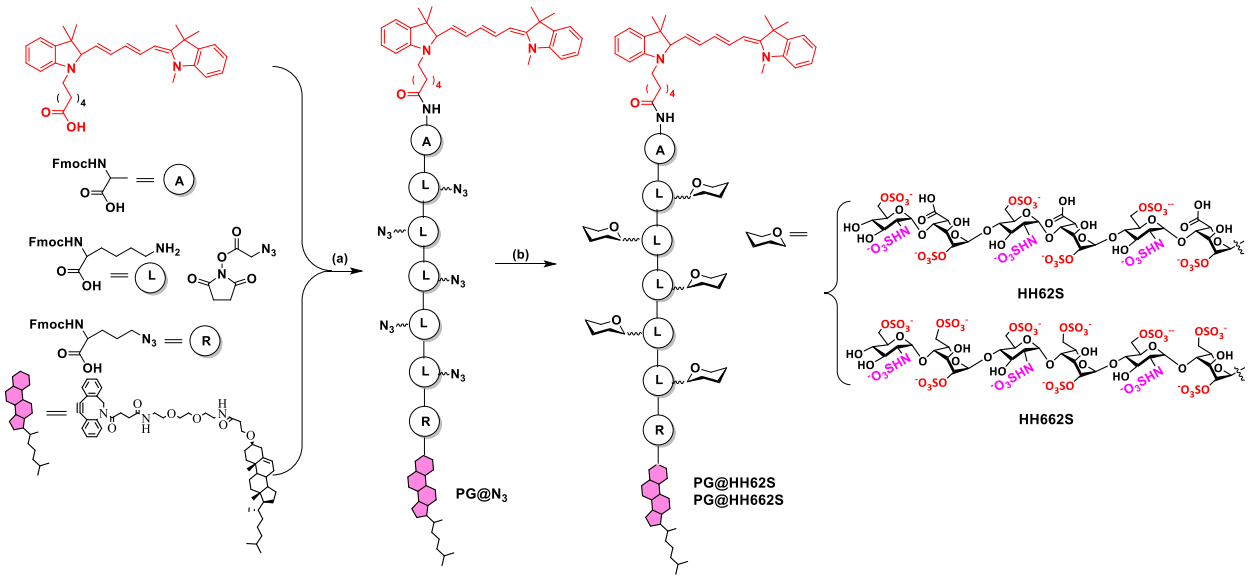


Fig. 5. Synthesis of neoproteoglycan carrying **HH62S** and **HH662S** ligands. (a) Rink amide resin peptide synthesis; (b) **HH62S-DBCO** or **HH662S-DBCO** in DMF at RT for 7 days.

We next constructed neoproteoglycans (neoPG: **PG@HH62S** and **PG@HH662S**) using **PG@N3**, which was synthesized as reported previously using solid state peptide synthesizer and Fmoc amino acid precursors (**A**, **L** & **R**), Cy5 dye (**Cy5**) and cholesterol linker (**Chol-DBCO**). Cy5 fluorescent was attached at C-terminal of the 7-mer peptide for real-time imaging studies and a cholesterol moiety was incorporated at the N-terminal to immobilize the neoPG on the cell membrane. Next, **HH62S** and **HH662S** ligand was treated with DBCO active ester linker, followed by copper free click reaction using stoichiometric amount of **PG@N3** and HS ligands for seven days (Fig. 5). Compound was purified by reverse phase HPLC employing MeOH/H_2O gradient system. UV-visible and fluorescent spectra of the neoPGs showed characteristic peaks of Cy5 with 5-6 nm bathochromic shift compared to the Cy5 as such, indicating the glycoconjugation. Quantum yield of the both compounds was 22.7 and 22.8, compared to atto 655 quantum yield of 30. illustrating the brightness of the probe.[14-16]

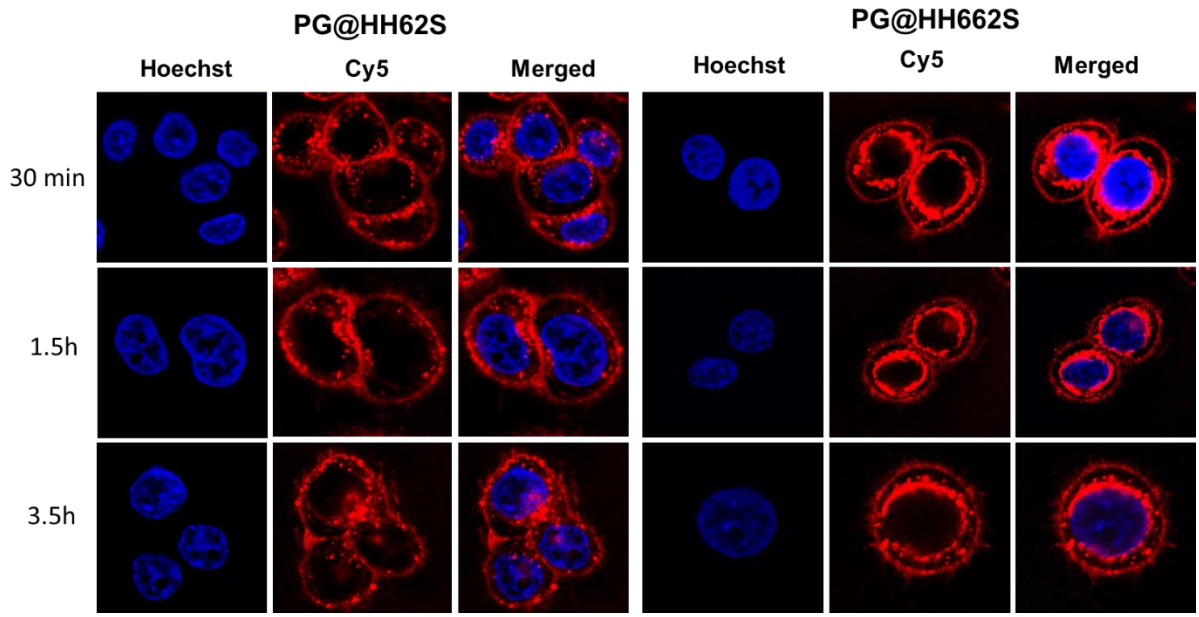


Fig. 6. Time dependent confocal imaging of PG@HH62S and PG@HH662S in MDA-MB-468 cells.

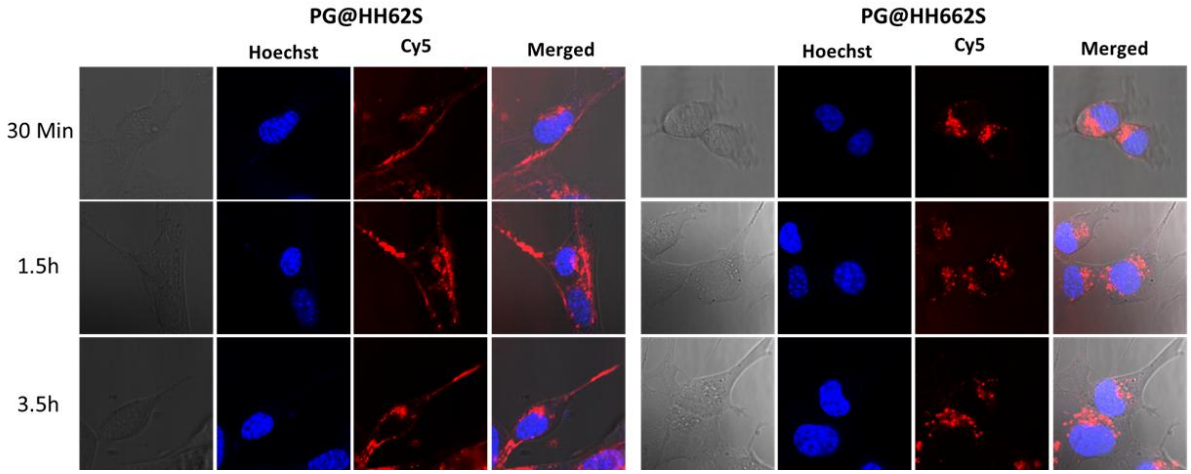


Fig. 7. Time dependent confocal imaging of PG@HH62S and PG@HH662S in NIH3T3 cells.

we examined the potential of **PG@HH62S** and **PG@HH662S** in cell surface engineering utilizing MDA-MB-468 breast cancer. A solution of neoPG (1 µM) was added to the cells and washed after 30 min incubation and confocal images displayed significant difference in the plasma membrane (PM) expression and internalization of the neoPGs. To our surprise **PG@HH662S** showed internalization within 0.5 h (Figure 6), whereas **PG@HH62S** displayed intense fluorescence on PM and continue to express even after 3.5 h in MDA-MB-468 cell line, indicating that **PG@HH62S** well support cell membrane decoration over

PG@HH662S. We have performed a similar confocal study of both proteoglycans with healthy cells i.e. NIH3T3 keeping the same parameters and found the same results (Figure 7). Currently, we are investigating the endocytosis process of **PG@HH62S** and its potential application.

5.4 Conclusion

We synthesized HS hexasaccharides incorporating L-iduronic acid (**HH62S**) and 6-O-sulphated L-idose residue (**HH662S**) using a [2+2+2] glycosylation strategy. HS ligands were functionalized on amphiphilic glycopeptides *via* a copper-free click reaction. The cell surface engineering of these two molecules revealed significant differences. **PG@HH62S** remained on the cell membrane for an extended period, whereas **PG@HH662S** was internalized within a few seconds. Additionally, a portion of these molecules persisted in the nuclear region. Overall, these findings demonstrate that the sulfated-carboxylic acid combination is critical for the cell surface decoration of proteoglycans, whereas complete sulfation of HS ligands leads to endocytosis. Currently, we are investigating the mechanism and potential cargo delivery applications of **HH662S**.

5.5 Experimental section

5.5.1 General Information

All chemicals were reagent grade and used without further purification unless otherwise noted. Reactions were carried out in anhydrous solvents under a nitrogen atmosphere. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F_{254}. Spots on TLC plate were visualized under UV light or by dipping the TLC plate in CAM/ninhydrin solution followed by heating. Column chromatography was carried out using Fluka kiesel gel 60 (230-400 mesh). ^{1}H and ^{13}C NMR spectra of compounds were measured with Bruker 400 MHz, Bruker 600 MHz and Jeol 400 MHz using residual solvents as an internal reference ($CDCl_3$ δH 7.26 ppm, δC 77.3 ppm, CD_3OD δH 3.31 ppm, δC 49.0 ppm, and D_2O δH 4.79 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. UV-visible measurements were performed with Evolution 300 UV-visible spectrophotometer (Thermo Fisher Scientific, USA). Fluorescence spectra were measured with FluoroMax-4 spectrofluorometer (Horiba Scientific, U.S.A.). All microscopy images were captured using Leica SP8 confocal microscope and processed using Image J software.

1,6-Anhydro-[2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-*β*-L- idopyranose (3)

To a solution of compound **1** glycosyl donor (2.09 g, 2.86 mmol) and **2** glycosyl acceptor (0.818 g, 2.29 mmol) in CH_2Cl_2 (40 mL) was stirred under N_2 atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. The mixture solution was cooled down to -78 ℃ followed by the addition of NIS (0.773 g, 3.43 mmol) and TMSOTf (104 µL, 0.57 mmol). The temperature was gradually increased to -20 ℃ and reaction mixture was left for stirring for another 15 minutes. After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et_3N. Molecular sieves were filtered using celite and organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/10, v/v) to obtain compound **3** (2.25 g, 81.81 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 – 8.04 (m, 2H), 7.69 (dt, *J* = 7.9, 1.4 Hz, 4H), 7.63 – 7.56 (m, 1H), 7.49 – 7.43 (m, 6H), 7. 42 – 7.33 (m, 8H), 7.33 – 7.27 (m, 6H), 7.25 – 7.19 (m, 2H), 7.17 (dd, *J* = 6.6, 2.9 Hz, 2H), 5.53 (d, *J* = 1.8 Hz, 1H), 5.29 (d, *J* = 3.8 Hz, 1H), 5.09 (dd, *J* = 7.9, 1.8 Hz, 1H), 4.97 – 4.93 (m, 2H), 4.92 (d, *J* = 4.2 Hz, 1H), 4.87 (d, *J* = 10.5 Hz, 1H), 4.78 (d, *J* = 10.8 Hz, 1H), 4.65 – 4.58 (m, 2H), 4.14 (d, *J* = 7.7 Hz, 1H), 4.08 (t, *J* = 8.0 Hz, 1H), 4.05 – 3.97 (m, 2H), 3.88 (t, *J* = 2.2 Hz, 2H), 3.73 – 3.66 (m, 3H), 3.42 (dd, *J* = 10.4, 3.7 Hz, 1H), 1.08 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.77, 137.97, 137.69, 137.49, 135.80, 135.67, 133.42, 133.18, 132.87, 129.91, 129.88, 129.84, 129.46, 128.62, 128.49, 128.41, 128.20, 128.13, 128.12, 127.94, 127.83, 127.77, 127.75, 99.69, 99.28, 79.98, 79.37, 78.91, 78.30, 77.38, 77.06, 77.03, 76.74, 75.65, 75.51, 75.02, 74.25, 72.85, 65.72, 63.64, 62.57, 26.84, 19.24, 0.04. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{56}H_{59}N_3O_{10}Si$ is 984.3870; found, 984.3842.

Phenyl-[2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-1-thio-*α*-L- idopyranoside (5)

A solution of compound **3** (2.25 g, 2.33 mmol) in Ac_2O (20 mL) was stirred at ice cold temperature for 15 minutes. $Cu(OTf)_2$ (0.084 g, 0.23 mmol) was added to the reaction mixture. After 12 h, the reaction mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate, NaHCO3 and washed with brine. The combined organic layer was dried over Na2SO4, filtered, concentrated and preceded further without any purification. Next, the as obtained acetylated solution of compound (1.98 g, 1.86 mmol),

ZnI2 (1.24 g, 3.90 mmol) and phenyl trimethylsilyl sulphide (1.09 ml, 5.76 mmol) in CH2Cl2 (20 mL) was stirred under N2 atmosphere for 2 h. Upon completion, the reaction mixture was filtered through celite, evaporated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/5, v/v) to obtain compound **5** (1.97 g, 76.06 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 – 8.12 (m, 2H), 7.67 (ddt, *J* = 9.4, 6.8, 1.5 Hz, 4H), 7.58 – 7.54 (m, 2H), 7.51 – 7.47 (m, 2H), 7.41 (qd, *J* = 5.6, 3.2 Hz, 4H), 7.37 – 7.29 (m, 15H), 7.27 (d, *J* = 1.7 Hz, 2H), 7.19 – 7.15 (m, 4H), 5.60 (d, *J* = 1.7 Hz, 1H), 5.39 (t, *J* = 2.4 Hz, 1H), 4.99 (d, *J* = 11.7 Hz, 1H), 4.88 (ddd, *J* = 7.5, 4.5, 2.6 Hz, 1H), 4.78 (dd, *J* = 11.2, 8.2 Hz, 2H), 4.67 (d, *J* = 10.8 Hz, 1H), 4.61 (d, *J* = 3.7 Hz, 1H), 4.39 (dt, *J* = 8.2, 4.7 Hz, 2H), 4.15 (dt, *J* = 7.0, 4.5 Hz, 2H), 4.08 (d, *J* = 10.3 Hz, 1H), 3.98 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.86 (d, *J* = 12.1 Hz, 1H), 3.79 – 3.70 (m, 2H), 3.67 – 3.60 (m, 2H), 3.32 (dd, *J* = 10.1, 3.6 Hz, 1H), 1.90 (s, 3H), 1.05 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.36, 165.75, 138.03, 137.59, 137.35, 135.89, 135.62, 135.54, 133.50, 133.25, 133.05, 131.73, 129.88, 129.71, 129.69, 128.90, 128.62, 128.49, 128.44, 128.24, 128.08, 127.91, 127.81, 127.80, 127.74, 127.60, 127.50, 98.89, 85.86, 80.67, 77.75, 77.38, 77.26, 77.06, 76.74, 75.27, 75.11, 74.97, 73.12, 72.86, 71.96, 69.74, 66.45, 64.05, 63.09, 62.16, 26.87, 20.71, 19.35, 0.04. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{64}H_{67}N_3O_{11}SSi$ is 1136.4166; found, 1136.4146.

1,6-Anhydro-[2-azido-2-deoxy-3-O-benzyl-4-*O*-chloroacetyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-*β*-L- idopyranose (7)

Compound **6** (3.30 g, 4.60 mmol) was subjected to glycosylation reaction as per the synthetic procedure for the preparation of compound **3** to obtain disaccharide **7** (3.44 g 78.89 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 – 8.05 (m, 2H), 7.66 – 7.58 (m, 6H), 7.49 – 7.35 (m, 11H), 7.34 – 7.31 (m, 3H), 7.27 (q, *J* = 1.4, 0.9 Hz, 3H), 7.26 (s, 1H), 7.24 – 7.22 (m, 1H), 5.55 (d, *J* = 1.8 Hz, 1H), 5.34 (d, *J* = 3.8 Hz, 1H), 5.14 – 5.06 (m, 2H), 4.90 (dd, *J* = 11.1, 8.0 Hz, 2H), 4.79 (d, *J* = 10.8 Hz, 1H), 4.68 – 4.62 (m, 2H), 4.19 (d, *J* = 7.6 Hz, 1H), 4.14 – 4.03 (m, 2H), 3.95 (t, *J* = 9.7 Hz, 1H), 3.74 (ddd, *J* = 11.9, 6.7, 3.8 Hz, 2H), 3.70 – 3.58 (m, 4H), 3.48 (dd, *J* = 10.2, 3.8 Hz, 1H), 1.06 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.77, 165.73, 137.86, 137.40, 135.66, 135.61, 133.47, 132.81, 132.70, 129.92, 129.89, 129.41, 128.64, 128.51, 128.44, 128.17, 128.12, 127.85, 127.82, 127.79, 99.38, 99.30, 79.43, 78.92, 77.54, 77.37, 77.25, 77.05, 76.73, 74.97, 74.88, 74.17, 71.71, 71.34, 65.68, 63.24, 62.59, 40.30, 29.72, 26.75, 19.14, 0.02. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{51}H_{54}ClN_3O_{11}Si$ is 970.3116; found, 970.3125.

1,6-Anhydro-[2-azido-2-deoxy-3-O-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-*β*-L- idopyranose (8)

To a solution of **7** (3 g, 3.16 mmol) in pyridine/MeOH (1:1, 30 ml) at 80 °C was added thiourea (0.938 g, 12.33 mmol) and stirred under nitrogen atmosphere for 2 h. After completion of the reaction, the solvent was evaporated, then dissolved in EtOAc and washed with 1 N HCl and brine solution. The organic layer was dried using anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, crude mixture was purified through silica gel column chromatography (EtOAc/Hexane = 1/9, v/v), to obtain compound compound **8** (2.32 g, 84.36 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 – 8.04 (m, 2H), 7.89 – 7.82 (m, 4H), 7.67 (td, *J* = 6.8, 1.4 Hz, 4H), 7.62 – 7.54 (m, 2H), 7.52 – 7.37 (m, 11H), 7.28 – 7.17 (m, 6H), 5.53 (d, *J* = 1.8 Hz, 1H), 5.25 (d, *J* = 3.8 Hz, 1H), 5.13 – 5.02 (m, 3H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.56 (t, *J* = 4.6 Hz, 1H), 4.16 (d, *J* = 7.7 Hz, 1H), 4.07 (t, *J* = 8.2 Hz, 1H), 3.99 (dd, *J* = 8.2, 4.3 Hz, 1H), 3.90 – 3.81 (m, 3H), 3.72 (ddd, *J* = 11.1, 6.3, 2.3 Hz, 2H), 3.60 (dt, *J* = 9.5, 4.6 Hz, 1H), 3.37 (dd, *J* = 10.5, 3.7 Hz, 1H), 1.07 (s, 9H). ^{13}C NMR (400 MHz, CHLOROFORM-*D*) δ 165.82, 137.97, 135.68, 135.66, 135.44, 133.50, 133.21, 132.82, 132.71, 130.12, 130.09, 129.96, 129.48, 128.60, 128.56, 128.45, 128.08, 127.97, 127.94, 127.83, 127.06, 126.34, 126.21, 125.97, 99.75, 99.32, 79.50, 79.43, 78.89, 77.43, 77.30, 77.11, 76.79, 75.32, 75.03, 74.31, 72.58, 71.90, 65.73, 64.34, 62.95, 26.91, 19.26. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{49}H_{53}N_3O_{10}Si$ is 894.3398; found, 894.3304.

1,6-Anhydro-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-*β*-L-idopyranose (9)

To a solution of compound **5** glycosyl donor (2 g, 1.79 mmol) and **8** glycosyl acceptor (1.25 g, 1.43 mmol) in CH_2Cl_2 (40 mL) was stirred under N_2 atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. The mixture solution was cooled down to -10 ℃ followed by the addition of NIS (0.646 g, 2.87 mmol) and TMSOH (98 µL, 0.53 mmol) and reaction mixture was left for stirring for another 10 minutes. After 10 minutes reaction completion was monitored by TLC and quenched using few drops of Et_3N. Molecular sieves were filtered using celite and organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated

and purified through silica gel column chromatography (ethyl acetate/ hexane= 2/10, v/v) to obtain tetrasaccharide **9** (2.62 g, 78.20 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 – 8.00 (m, 4H), 7.69 – 7.57 (m, 10H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.44 – 7.27 (m, 30H), 7.25 – 7.15 (m, 14H), 7.15 – 7.10 (m, 1H), 5.50 (d, *J* = 1.8 Hz, 1H), 5.32 (d, *J* = 2.2 Hz, 1H), 5.18 (dd, *J* = 9.3, 3.3 Hz, 2H), 5.04 (dd, *J* = 8.2, 1.8 Hz, 1H), 4.97 (d, *J* = 11.0 Hz, 1H), 4.88 (s, 1H), 4.85 (d, *J* = 4.0 Hz, 1H), 4.83 – 4.78 (m, 2H), 4.75 (d, *J* = 2.2 Hz, 1H), 4.73 – 4.65 (m, 4H), 4.51 (d, *J* = 10.5 Hz, 1H), 4.43 (tt, *J* = 7.0, 3.5 Hz, 1H), 4.35 (t, *J* = 4.6 Hz, 1H), 4.24 (d, *J* = 10.4 Hz, 1H), 4.17 – 4.06 (m, 4H), 4.05 – 3.96 (m, 3H), 3.88 (dt, *J* = 11.5, 3.5 Hz, 2H), 3.81 (dq, *J* = 13.4, 5.5, 4.7 Hz, 3H), 3.73 – 3.67 (m, 3H), 3.63 – 3.57 (m, 2H), 3.53 – 3.48 (m, 1H), 3.31 (ddd, *J* = 11.9, 10.1, 3.7 Hz, 2H), 1.76 (s, 3H), 1.05 (s, 9H), 1.00 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.13, 165.77, 165.69, 138.09, 137.93, 137.70, 137.43, 135.90, 135.83, 135.67, 135.61, 133.45, 133.43, 133.34, 133.18, 133.02, 132.96, 129.90, 129.86, 129.83, 129.79, 129.73, 129.60, 129.44, 128.64, 128.52, 128.50, 128.47, 128.43, 128.41, 128.39, 128.28, 128.21, 128.16, 128.11, 127.91, 127.89, 127.86, 127.81, 127.78, 127.75, 127.63, 127.54, 127.52, 99.33, 99.21, 98.51, 97.45, 80.34, 79.22, 79.04, 78.40, 77.71, 77.38, 77.26, 77.06, 76.95, 76.74, 75.30, 75.21, 75.15, 74.99, 74.12, 74.03, 73.59, 73.24, 72.99, 72.89, 72.76, 69.15, 65.73, 65.51, 64.03, 63.97, 62.69, 62.29, 62.03, 26.89, 26.85, 26.81, 20.77, 19.36, 19.34. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{107}H_{114}N_6O_{21}Si_2$ is 1897.7473; found, 1897.7413.

Phenyl-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-1-thio-*α*-L-idopyranoside (11)

Compound **9** (2.56 g, 1.36 mmol) was subjected to ring opening reaction and thioglycosylation reaction respectively as per the synthetic procedure given for the preparation of compound **5** to obtain tetrasaccharide **11** (1.90 g 70.37 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.08 (m, 2H), 8.02 – 7.97 (m, 2H), 7.68 – 7.53 (m, 11H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.41 – 7.26 (m, 31H), 7.22 (d, *J* = 5.8 Hz, 3H), 7.20 – 7.08 (m, 9H), 7.07 – 7.01 (m, 3H), 5.59 (s, 1H), 5.36 – 5.31 (m, 2H), 5.15 – 5.11 (m, 1H), 4.93 (d, *J* = 11.7 Hz, 1H), 4.85 – 4.76 (m, 3H), 4.74 – 4.67 (m, 3H), 4.59 (t, *J* = 2.9 Hz, 1H), 4.54 (d, *J* = 10.9 Hz, 1H), 4.49 – 4.42 (m, 2H), 4.31 (q, *J* = 8.2, 5.7 Hz, 2H), 4.17 (d, *J* = 10.2 Hz, 1H), 4.08 – 3.93 (m, 6H), 3.92 – 3.80 (m, 4H), 3.76 (td, *J* = 11.9, 10.2, 4.2 Hz, 3H), 3.63 (td, *J* = 14.7, 12.7, 7.2 Hz, 3H), 3.53 – 3.45 (m, 3H), 3.27 – 3.17 (m, 2H), 1.84 – 1.82 (m,

3H), 1.68 – 1.64 (m, 3H), 1.05 – 1.02 (m, 9H), 0.98 – 0.95 (m, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 135.89, 135.65, 135.60, 133.30, 133.16, 131.75, 129.86, 129.80, 129.70, 129.64, 128.91, 128.63, 128.56, 128.47, 128.43, 128.41, 128.39, 128.22, 128.20, 128.11, 128.08, 127.87, 127.82, 127.76, 127.62, 127.54, 127.49, 127.41, 127.18, 98.43, 97.99, 97.09, 85.81, 80.32, 79.02, 77.65, 77.25, 75.24, 75.09, 74.81, 74.14, 74.05, 73.18, 73.02, 72.89, 72.86, 72.78, 72.74, 71.64, 69.80, 68.92, 66.34, 65.15, 64.46, 64.00, 62.83, 62.65, 62.20, 61.97, 26.89, 26.81, 20.70, 20.62, 19.42, 19.36. HR-ESI-MS *(m/z)*: [M+Na]+ calcd for $C_{115}H_{122}N_6O_{22}SSi_2$ is 2049.7769; found, 2049.7721.

1,6-Anhydro-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-*β*-L-idopyranose (12)

Compound **11** glycosyl donor (1.15 g, 0.56 mmol) was subjected to glycosylation reaction with glcosyl acceptor **8** (0.397 g, 0.45 mmol) as per the synthetic procedure given for the preparation of compound **5** to obtain hexasaccharide **12** (0.94 g 59.49 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 – 7.99 (m, 6H), 7.72 – 7.56 (m, 15H), 7.49 – 7.28 (m, 39H), 7.25 – 7.16 (m, 17H), 7.15 – 7.07 (m, 7H), 7.05 (dd, *J* = 7.5, 1.7 Hz, 1H), 5.51 (t, *J* = 1.9 Hz, 1H), 5.41 (s, 1H), 5.29 (d, 1H), 5.18 (h, *J* = 2.2 Hz, 3H), 5.05 (dt, *J* = 8.2, 1.9 Hz, 1H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.87 (ddd, *J* = 8.9, 7.1, 1.8 Hz, 2H), 4.84 – 4.78 (m, 1H), 4.75 (d, *J* = 2.0 Hz, 2H), 4.74 – 4.67 (m, 5H), 4.61 (q, *J* = 3.5, 2.1 Hz, 2H), 4.46 (dd, *J* = 10.4, 1.9 Hz, 1H), 4.36 (dq, *J* = 9.9, 4.5, 2.8 Hz, 3H), 4.22 – 4.17 (m, 2H), 4.16 – 4.10 (m, 2H), 4.10 – 4.02 (m, 5H), 4.02 – 3.94 (m, 3H), 3.89 (dd, *J* = 10.0, 5.1 Hz, 5H), 3.84 (d, *J* = 1.9 Hz, 1H), 3.79 (dd, *J* = 12.1, 3.3 Hz, 2H), 3.72 (s, 1H), 3.68 (d, *J* = 8.0 Hz, 3H), 3.62 – 3.53 (m, 3H), 3.52 – 3.46 (m, 3H), 3.33 (ddd, *J* = 10.4, 3.8, 2.1 Hz, 1H), 3.30 – 3.25 (m, 1H), 3.23 – 3.17 (m, 1H), 1.75 (d, *J* = 2.0 Hz, 3H), 1.69 (d, *J* = 2.0 Hz, 3H), 1.06 (d, *J* = 1.8 Hz, 9H), 1.03 (d, *J* = 1.9 Hz, 9H), 1.00 (d, *J* = 1.8 Hz, H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 170.06, 169.99, 165.77, 165.71, 165.64, 138.17, 138.01, 137.95, 137.90, 137.73, 137.46, 137.38, 135.91, 135.89, 135.64, 135.62, 133.50, 133.43, 133.29, 133.24, 133.04, 132.90, 129.91, 129.88, 129.81, 129.72, 129.67, 129.48, 129.44, 128.76, 128.59, 128.51, 128.45, 128.43, 128.41, 128.21, 128.15, 128.08, 127.89, 127.85, 127.77, 127.76, 127.68, 127.64, 127.59, 127.57, 127.50, 127.45, 127.21, 99.30, 99.21, 98.47, 97.47, 97.39, 97.02, 80.34, 79.24, 79.02,

78.72, 78.37, 77.67, 77.27, 76.96, 75.25, 75.11, 75.00, 74.11, 73.36, 73.18, 73.02, 72.85, 72.71, 72.57, 69.61, 68.84, 66.18, 65.51, 65.02, 64.33, 64.04, 63.94, 62.71, 62.32, 62.15, 61.99, 26.90, 26.84, 26.81, 20.73, 20.71, 19.46, 19.37, 19.34. HR-ESI-MS (m/z): [M+Na]+ calcd for $C_{158}H_{169}N_9O_{32}Si_3$ is 2811.1079; found, 2811.1023.

Phenyl-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-gluco pyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-1-thio-*α*-L-idopyranoside (14)

Compound **12** (0.94 g, 0.32 mmol) was subjected to ring opening reaction and thioglycosylation reaction respectively as per the synthetic procedure given for the preparation of compound **5** to obtain hexasaccharide **14** (0.80 g 83.89 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 (dt, *J* = 8.4, 1.7 Hz, 2H), 7.99 (dd, *J* = 7.8, 2.8 Hz, 4H), 7.68 – 7.53 (m, 15H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.41 – 7.25 (m, 41H), 7.23 – 7.12 (m, 12H), 7.12 – 7.03 (m, 4H), 7.00 (d, *J* = 4.1 Hz, 4H), 6.92 (h, *J* = 4.1 Hz, 1H), 5.59 (s, 1H), 5.39 (s, 1H), 5.35 (d, *J* = 2.5 Hz, 1H), 5.29 (d, *J* = 2.7 Hz, 1H), 5.13 (t, *J* = 3.7 Hz, 2H), 4.94 (d, *J* = 11.7 Hz, 1H), 4.82 (t, *J* = 10.3 Hz, 2H), 4.78 – 4.63 (m, 7H), 4.55 (dd, *J* = 7.0, 3.6 Hz, 2H), 4.52 – 4.39 (m, 3H), 4.38 – 4.27 (m, 2H), 4.23 (t, *J* = 4.9 Hz, 1H), 4.17 – 4.08 (m, 3H), 4.08 – 3.72 (m, 17H), 3.69 – 3.54 (m, 5H), 3.54 – 3.44 (m, 4H), 3.38 (t, *J* = 3.5 Hz, 1H), 3.27 – 3.12 (m, 3H), 1.82 (d, *J* = 2.0 Hz, 3H), 1.66 (d, *J* = 2.3 Hz, 6H), 1.03 (s, 9H), 0.98 (d, *J* = 5.8 Hz, 18H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 170.21, 170.06, 169.90, 165.82, 165.65, 165.63, 138.18, 138.04, 137.86, 137.74, 137.47, 137.32, 135.98, 135.94, 135.91, 135.64, 135.62, 135.44, 133.57, 133.51, 133.47, 133.32, 133.27, 133.17, 133.04, 131.77, 129.88, 129.83, 129.78, 129.72, 129.68, 129.63, 128.93, 128.78, 128.58, 128.55, 128.51, 128.49, 128.45, 128.43, 128.40, 128.25, 128.22, 128.15, 128.11, 127.87, 127.85, 127.80, 127.78, 127.64, 127.62, 127.59, 127.52, 127.50, 127.46, 127.19, 98.47, 98.10, 97.23, 97.10, 96.96, 85.84, 80.35, 79.03, 78.70, 77.67, 77.28, 75.24, 75.10, 74.91, 74.79, 74.31, 74.14, 73.21, 73.14, 72.93, 72.86, 72.47, 71.64, 69.78, 69.38, 68.78, 66.32, 65.65, 64.91, 64.40, 64.05, 62.82, 62.74, 62.37, 62.27, 62.06, 61.99, 26.91, 26.85, 26.81, 20.72, 20.67, 20.63, 19.48, 19.44, 19.38.HR-ESI-MS (m/z): [M+Na]+ calcd for $C_{166}H_{177}N_9O_{33}SSi_3$ is 2963.1375; found, 2963.1346.

N-benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-*α*-D-glucopyranosyl)]-(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranoside (15)

A solution of glycosyl donor **14** (0.80 g, 0.27 mmol) and linker benzyl (3-hydroxypropyl) carbamate (0.062 g, 0.29 mmol) in CH_2Cl_2 (15 mL) was stirred under N_2 atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 1 h. NIS (0.098 g, 0.43 mmol) and TfOH (2.5 μL, 0.27 mmol) was added to the reaction mixture at room temperature and reaction completion was monitored by TLC, quenched using few drops of Et_3N. Molecular sieves were filtered using celite and organic layer was washed with aqueous $Na_2S_2O_3$ and brine. The collected organic layer was dried over Na_2SO_4, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/3, v/v) to obtain compound **15** (657 mg 80.12 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.12 – 8.08 (m, 2H), 8.00 (td, *J* = 7.5, 6.9, 1.4 Hz, 4H), 7.69 – 7.65 (m, 2H), 7.65 – 7.57 (m, 10H), 7.42 – 7.36 (m, 9H), 7.36 – 7.27 (m, 35H), 7.23 (d, *J* = 3.1 Hz, 3H), 7.22 – 7.14 (m, 10H), 7.13 – 7.05 (m, 5H), 7.02 (d, *J* = 4.5 Hz, 4H), 6.95 (h, *J* = 4.0 Hz, 1H), 5.63 (t, *J* = 5.9 Hz, 1H), 5.40 (s, 1H), 5.30 (d, *J* = 2.6 Hz, 1H), 5.14 (q, *J* = 3.2 Hz, 2H), 5.08 (s, 2H), 5.05 (t, *J* = 2.4 Hz, 1H), 4.95 (d, *J* = 1.8 Hz, 1H), 4.87 – 4.80 (m, 2H), 4.80 – 4.76 (m, 2H), 4.74 – 4.61 (m, 5H), 4.59 – 4.51 (m, 3H), 4.44 (d, *J* = 10.4 Hz, 1H), 4.39 – 4.22 (m, 5H), 4.18 – 4.10 (m, 3H), 4.05 (d, *J* = 3.5 Hz, 1H), 4.04 – 3.90 (m, 8H), 3.89 – 3.78 (m, 6H), 3.78 – 3.70 (m, 2H), 3.66 (dt, *J* = 12.0, 7.0 Hz, 3H), 3.60 – 3.44 (m, 7H), 3.39 (d, *J* = 3.5 Hz, 2H), 3.28 – 3.21 (m, 2H), 3.15 (ddd, *J* = 10.8, 7.1, 3.6 Hz, 2H), 1.83 (s, 5H), 1.67 (d, *J* = 2.4 Hz, 6H), 1.05 (s, 9H), 1.00 (d, *J* = 5.8 Hz, 18H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 170.35, 170.05, 169.89, 165.78, 165.64, 165.62, 156.57, 138.17, 138.03, 137.86, 137.73, 137.47, 137.45, 136.72, 135.96, 135.92, 135.90, 135.63, 135.61, 135.58, 133.57, 133.49, 133.46, 133.25, 133.14, 133.03, 129.87, 129.81, 129.76, 129.70, 129.66, 128.77, 128.56, 128.52, 128.47, 128.43, 128.41, 128.39, 128.33, 128.28, 128.20, 128.14, 128.11, 128.03, 127.86, 127.83, 127.79, 127.76, 127.62, 127.58, 127.51, 127.49, 127.45, 127.19, 98.45, 98.10, 97.85, 97.21, 97.06, 96.95, 80.34, 78.95, 78.69, 77.65, 77.26, 75.22, 75.09, 74.91, 74.11, 73.81, 73.10, 72.91, 72.80, 72.48, 71.64, 69.35, 68.76, 67.03, 66.59, 65.60, 65.07, 64.90, 64.35, 64.03, 62.96, 62.72, 62.31, 61.97, 39.66, 29.44, 26.89, 26.82,

26.80, 20.71, 20.65, 20.61, 19.46, 19.44, 19.36. HR-ESI-MS (m/z): [M+Na]+ calcd for $C_{171}H_{186}N_{10}O_{36}Si_3$ is 3062.2237; found, 3062.2214.

N-benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-*α*-D-gluco pyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-*α*-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-*α*-D-glucopyranosyl)] -(1→4)-*O*-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-acetyl-*α*-L-idopyranoside (16)

To a solution of **15** (650 mg, 0.21 mmol) in pyridine (5 ml) was added 70% HF.Py (482 µl 5.34 mmol) at 0 °C and stirred at RT overnight under N_2 atmosphere. After completion, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine. The organic layer was dried using anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica column chromatography (ethyl acetate/ hexane= 6/4, v/v) to obtain compound **16** (412 mg 84.42 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.05 (m, 6H), 7.53 – 7.47 (m, 1H), 7.46 – 7.39 (m, 5H), 7.39 – 7.27 (m, 29H), 7.24 – 7.16 (m, 10H), 7.13 – 7.09 (m, 2H), 5.67 (s, 1H), 5.17 (dq, *J* = 16.7, 3.4 Hz, 4H), 5.08 (s, 1H), 5.04 (s, 1H), 4.96 (s, 1H), 4.82 (dd, *J* = 11.3, 4.8 Hz, 3H), 4.78 – 4.70 (m, 4H), 4.70 – 4.63 (m, 3H), 4.63 – 4.57 (m, 1H), 4.52 (d, *J* = 10.4 Hz, 1H), 4.45 (d, *J* = 10.6 Hz, 1H), 4.38 – 4.24 (m, 6H), 4.23 – 4.10 (m, 5H), 4.09 – 3.94 (m, 5H), 3.91 – 3.82 (m, 2H), 3.75 (ddd, *J* = 23.2, 12.6, 6.7 Hz, 9H), 3.63 (qd, *J* = 9.8, 5.3 Hz, 8H), 3.44 (dt, *J* = 18.9, 9.1 Hz, 4H), 3.22 (ddd, *J* = 12.6, 8.8, 3.6 Hz, 3H), 3.14 (dd, *J* = 10.1, 3.7 Hz, 1H), 2.02 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H), 1.89 – 1.83 (m, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 170.90, 170.77, 170.60, 165.69, 165.57, 165.54, 156.58, 137.80, 137.64, 137.61, 137.29, 136.67, 133.37, 129.82, 129.79, 129.75, 129.62, 129.56, 128.71, 128.64, 128.57, 128.55, 128.51, 128.47, 128.45, 128.42, 128.32, 128.17, 128.14, 128.11, 128.08, 128.06, 127.95, 127.89, 127.84, 127.52, 127.43, 99.27, 98.66, 98.21, 97.62, 97.45, 80.32, 78.94, 78.74, 77.84, 77.26, 75.17, 75.10, 74.81, 74.68, 74.45, 74.17, 73.31, 73.24, 72.83, 72.56, 72.38, 71.21, 70.36, 70.00, 68.36, 67.12, 66.91, 66.63, 64.91, 64.11, 64.01, 63.88, 62.83, 62.54, 61.57, 61.06, 39.90, 29.41, 20.90, 20.86, 20.81. HR-ESI-MS (m/z): [M+Na]+ calcd for $C_{123}H_{132}N_{10}O_{36}$ is 2347.8703; found, 2347.8746.

N-benzyloxycarbonyl-3-aminopropyl-*O*-[(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-O-sulfonate-*α*-D-glucopyranosyl)-(1→4)-*O*-(2,6-di-*O*-sulfonate-3-*O*-benzyl-*α*-L-ido pyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranosyl)-

(1→4)-*O*-(2,6-di-*O*- sulfonate-3-*O*-benzyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-azido-2-deoxy-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranosyl)]-(1→4)-*O*-2,6-di-*O*-sulfonate-3-*O*-benzyl-*α*-L-idopyranoside (18)

To a solution of **16** (360 mg, 0.15 mmol) in a mixture of THF/H_2O (1:1, 4 ml) was added 1 M $LiOH{\cdot}H_2O$ (195 mg, 4.64 mmol) and stirred at RT for 15 h. After completion of the reaction, the reaction mixture was diluted with MeOH and neutralized using Dowex 50WX8 H+ resin. The reaction was filtered and concentrated under reduced pressure and purified by bond elute C-18 column MeOH/H_2O (8/2). Next, to the obtained solution of compound (286 mg, 0.15 mmol) in DMF (8 mL), $SO_3{\cdot}NMe_3$ (1.89 g, 13.63 mmol) was added and stirred at 100 °C for 1 h. in the microwave reactor. DMF was evaporated and the crude product was purified by bond elute (C-18 column) MeOH/H_2O (9/1), to obtain compound **18** (214 mg, 54.88 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 – 8.05 (m, 1H), 7.58 (td, *J* = 7.3, 1.5 Hz, 1H), 7.48 – 7.42 (m, 5H), 7.40 (d, *J* = 5.2 Hz, 4H), 7.36 – 7.27 (m, 22H), 7.24 (d, *J* = 8.5 Hz, 3H), 7.18 – 7.09 (m, 4H), 5.65 (s, 1H), 5.02 (ddd, *J* = 19.6, 10.6, 6.8 Hz, 5H), 4.92 (d, *J* = 2.1 Hz, 1H), 4.82 (ddt, *J* = 22.2, 16.5, 5.9 Hz, 6H), 4.73 (d, *J* = 5.4 Hz, 1H), 4.67 (d, *J* = 23.5 Hz, 2H), 4.61 – 4.46 (m, 8H), 4.32 (d, *J* = 7.2 Hz, 1H), 4.13 (t, *J* = 7.5 Hz, 1H), 4.08 – 4.01 (m, 1H), 3.92 – 3.71 (m, 22H), 3.65 (dt, *J* = 16.1, 5.4 Hz, 8H), 3.54 (ddd, *J* = 27.8, 10.8, 4.9 Hz, 5H), 3.44 – 3.33 (m, 2H), 3.32 – 3.15 (m, 4H), 1.88 – 1.78 (m, 2H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 169.50, 156.81, 138.10, 138.05, 137.49, 137.26, 137.23, 136.51, 133.41, 130.09, 128.72, 128.68, 128.65, 128.63, 128.57, 128.52, 128.49, 128.46, 128.42, 128.26, 128.13, 128.10, 128.03, 128.00, 127.89, 127.79, 127.12, 127.01, 101.10, 100.30, 94.85, 94.64, 94.52, 81.33, 79.69, 78.13, 77.28, 75.89, 75.09, 74.93, 74.84, 73.40, 73.27, 72.52, 72.40, 71.97, 70.37, 69.53, 66.93, 66.73, 66.44, 66.06, 64.54, 63.89, 61.54, 61.48, 61.35, 60.48, 60.18, 39.68, 31.94, 30.33, 29.71, 22.71, 14.15. HR-ESI-MS *(m/z)*: $[M\text{-}9H]^{-9}$ calcd for $C_{96}H_{105}N_{10}O_{57}S_9^{9-}$, 288.7022; found, 288.7021.

N-benzyloxycarbonyl-3-aminopropyl-*O*-[(2-deoxy-2-sulfamido-3,4-di-*O*-benzyl-6-O-sulfonate -*α*-D-gluco pyranosyl)-(1→4)-*O*-(2,6-di-*O*- sulfonate-3-*O*-benzyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-deoxy-2-sulfamido-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-gluco pyranosyl)-(1→4)-*O*-(2,6-di-*O*-sulfonate-3-*O*-benzyl-*α*-L-idopyranosyl)-(1→4)-*O*-(2-deoxy-2-sulfamido-3-*O*-benzyl-6-*O*-sulfonate-*α*-D-glucopyranosyl)]-(1→4)-*O*-2,6-di-*O*-sulfonate-3-*O*-benzyl-*α*-L-idopyranoside (20)

To the solution of **18** (150 mg, 0.05 mmol) Trimethyl phosphine (PMe3, 1.0 M sol in THF) (287 µl, 1.38 mmol) and 1 M NaOH solution (2 ml) were added in THF and the reaction

mixture was stirred for 5 h. The progress of reaction was monitored by TLC (EtOAc/MeOH/H2O, 7/2/1, v/v/v). Upon completion, the P^H was adjusted to 8.0–8.5 by careful addition of glacial AcOH, and the mixture was concentrated in vacuo. The residue obtained was purified by C- 18 column, H2O/MeOH, 9/1→1/1, v/v). The appropriate fractions were concentrated in vacuo, and the residue was passed through a column DowexR 50 x 8 Na+ resin (0.6 x 5 cm) using MeOH/H2O (1/9, v/v) as eluent. The appropriate fractions were combined and lyophilized to afford desired product as sodium salt. Next to the stirring solution of obtained product (108 mg, 0.04 mmol) in a mixture of MeOH (3 ml), Et_3N (2 ml) and 1 M NaOH solution (2 ml) at 0 ºC was added SO_3.Py complex (273 mg, 1.71 mmol). The progress of reaction was monitored by TLC (EtOAc/pyridine/H_2O/AcOH 8/5/3/1, v/v/v/v). Two additional portions of SO_3.Py complex were added at 0 ºC after 1h and 2h. After stirring for an additional 8h, the reaction mixture was concentrated in vacuo and the residue was passed through a column of DowexR 50 x 8 Na+ resin using H_2O/MeOH as eluent. The appropriate fractions were concentrated in vacuo and further was purified by reverse phase C-18 silica gel column using H_2O/MeOH as eluent to obtain compound **20** (82 mg, 74.54 %) as sodium salt

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.64 – 7.26 (m, 40H), 5.27 (q, *J* = 15.1, 9.8 Hz, 2H), 5.19 – 5.03 (m, 5H), 4.96 – 4.80 (m, 3H), 4.64 (dtt, *J* = 48.0, 21.7, 8.7 Hz, 5H), 4.44 – 4.15 (m, 11H), 4.08 – 3.76 (m, 14H), 3.75 – 3.62 (m, 3H), 3.53 – 3.42 (m, 2H), 3.34 – 3.23 (m, 2H), 1.87 (dq, *J* = 13.3, 6.7, 6.3 Hz, 2H). ^{13}C NMR (400 MHz, Deuterium Oxide) δ 158.34, 136.98, 129.22, 129.13, 128.97, 128.83, 128.79, 128.70, 128.65, 128.56, 128.52, 128.39, 127.82, 98.10, 97.87, 97.49, 94.14, 79.24, 78.41, 78.18, 76.94, 75.60, 75.48, 75.35, 74.97, 73.23, 72.56, 72.40, 72.15, 71.87, 71.28, 70.78, 70.28, 70.15, 69.76, 69.00, 67.43, 66.84, 66.36, 66.21, 65.86, 65.24, 65.08, 62.86, 38.73, 28.39. HR-ESI-MS *(m/z)*: $[M-12H]^{-12}$ calcd for $C_{96}H_{108}N_4O_{66}S_{12}^{12-}$, 229.7664; found 229.7664.

3-aminopropyl-*O*-[(2-deoxy-2-sulfamido-6-O-sulfonate -*α*-D-glucopyranosyl)-(1→4)-*O*-(2,6-di-*O*- sulfonate-*α*-L-idopyranosyl)-(1→4)-*O*-(2-deoxy-2-sulfamido-6-*O*- sulfonate-*α*-D-glucopyranosyl)-(1→4)-*O*-(2,6-di-*O*-sulfonate-3-*α*-L-idopyranosyl)-(1→4)-*O*-(2-deoxy -2-sulfamido-3-6-*O*-sulfonate-*α*-D-glucopyranosyl)]-(1→4)-*O*-2,6-di-*O*-sulfonate-*α*-L-idopyranoside (21)

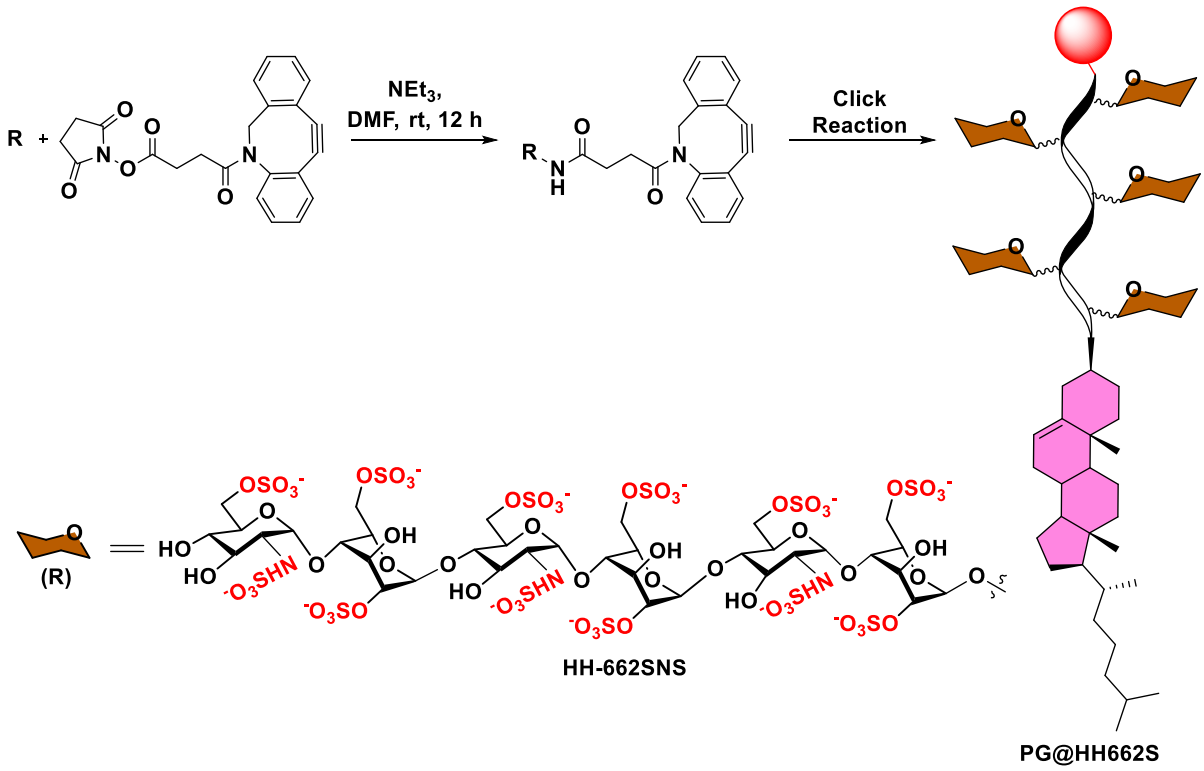
To a solution of **20** (50 mg, 0.01 mmol) in H_2O (2 ml) was added $Pd(OH)_2$ (10% by wt.) and stirred for 48 h under hydrogen atmosphere. After completion of the reaction, the reaction

mixture was diluted with H_2O, filtered and concentrated, and finally purified by bond elute C-18 column (H_2O) to obtain compound **21** (23 mg, 63.88 %)

^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.27 (dd, *J* = 6.2, 3.7 Hz, 2H), 5.20 (d, *J* = 3.7 Hz, 1H), 4.77 (dd, *J* = 5.4, 2.7 Hz, 2H), 4.74 – 4.72 (m, 2H), 4.25 (dq, *J* = 8.8, 4.4 Hz, 2H), 4.15 – 4.11 (m, 1H), 3.90 – 3.84 (m, 4H), 3.83 – 3.66 (m, 21H), 3.65 – 3.59 (m, 2H), 3.56 (dt, *J* = 9.6, 4.7 Hz, 3H), 3.54 – 3.49 (m, 3H), 3.36 (t, *J* = 9.6 Hz, 1H), 3.12 – 2.99 (m, 5H), 1.91 (pd, *J* = 6.9, 4.6 Hz, 2H). ^{13}C NMR (600 MHz, Deuterium Oxide) δ 101.19, 101.18, 99.85, 96.01, 95.83, 78.27, 78.23, 75.13, 75.00, 74.07, 73.09, 71.86, 71.81, 71.71, 71.66, 71.51, 71.11, 70.70, 70.68, 70.08, 69.89, 69.41, 68.54, 66.61, 60.34, 60.18, 59.76, 59.37, 59.32, 54.30, 38.01, 26.35. HR-ESI-MS *(m/z)*: $[M-12H]^{-12}$ calcd for $C_{96}H_{108}N_4O_{66}S_{12}^{12-}$, 229.7664; found 229.7664.

5.5.2 General Procedure for Conjugation of Hexasaccharide to Peptide Template

The hexasaccharide was dissolved in 400 µL of dry DMF and stirred for 5 minutes. NEt_3 (2 equivalents) was then added, followed by the dropwise addition of a DBCO-NHS solution in 400 µL of DMF, all under continuous stirring at room temperature. After 12 hours, the DMF was evaporated, and the crude product was purified via LH-20 size exclusion chromatography. Separately, a compound with an azide linker (1 equivalent) was dissolved in a mixture of 500 µL of $CHCl_3$ and 500 µL of MeOH. A sugar molecule solution (1.2 equivalents per azide of peptide) in 1 mL of H_2O was then added, followed by an additional 500 µL of MeOH. The mixture was stirred at room temperature for 7–8 days, with reaction progress monitored using infrared spectroscopy (IR) (Fig. 5) Once the reaction was complete, the solvents were removed under reduced pressure.



The resulting crude product was purified using a 10 kDa centrifuge filter and a reversed-phase C-18 column with high-performance liquid chromatography (HPLC) employing a gradient of MeOH and water. The final product was confirmed using IR, NMR, and HRMS-ESI.

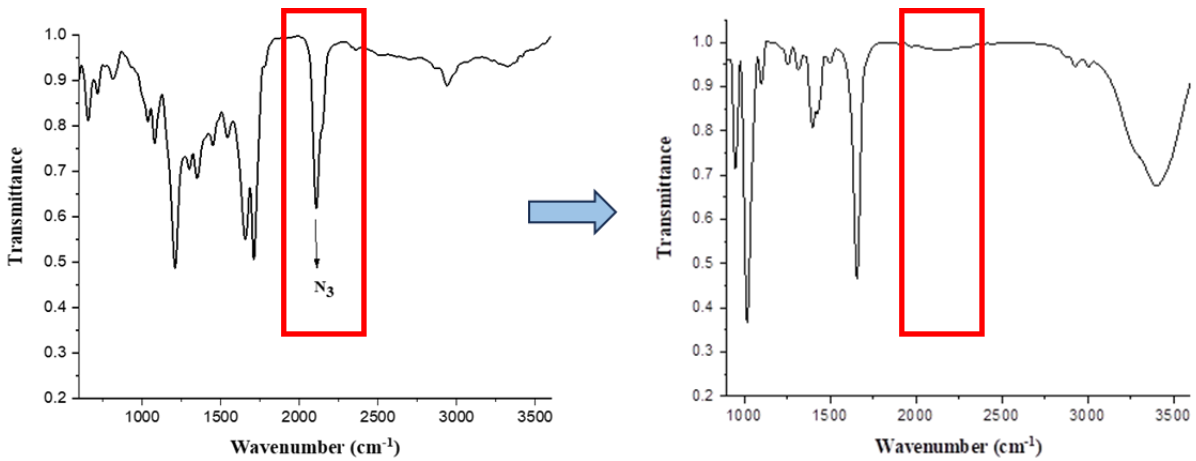


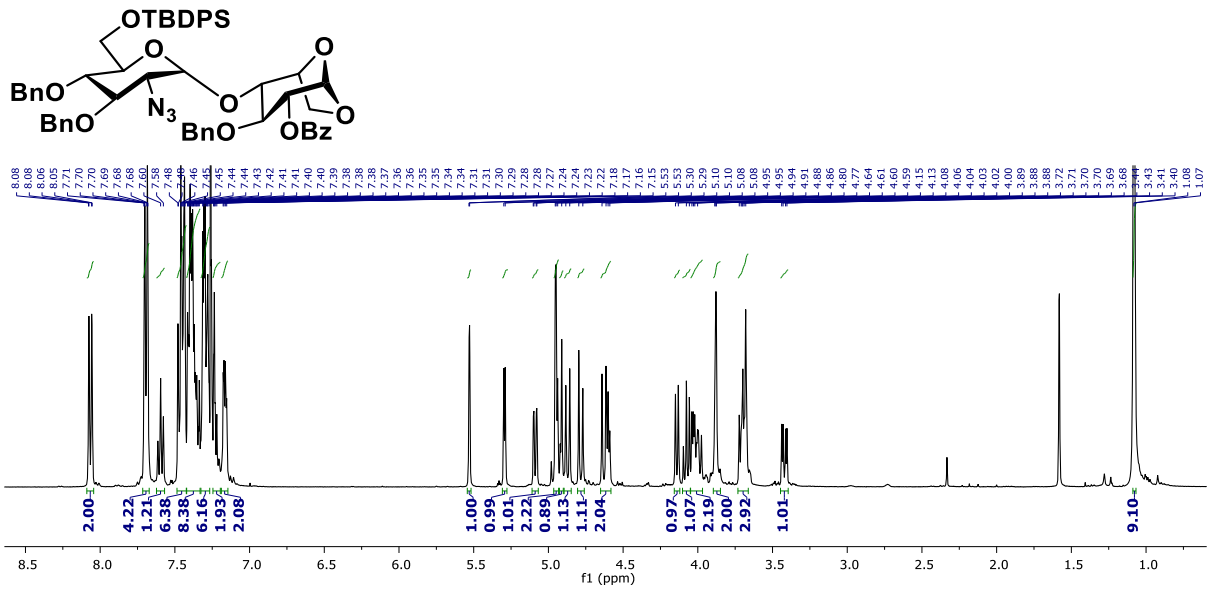
Fig. 5. Disappearance of azide peak in IR spectrum **b** indicates the completion of click reaction between Hexasaccharide and peptide template

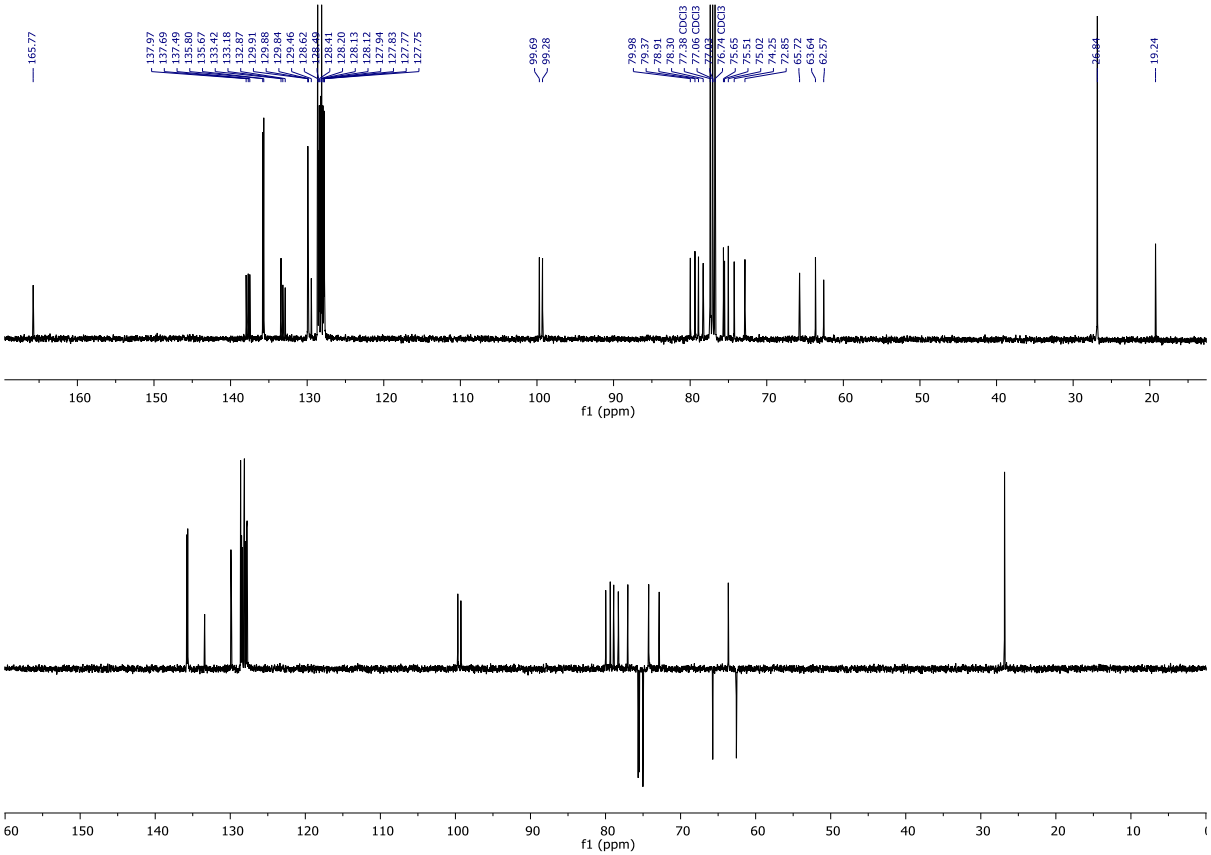
5.6 References

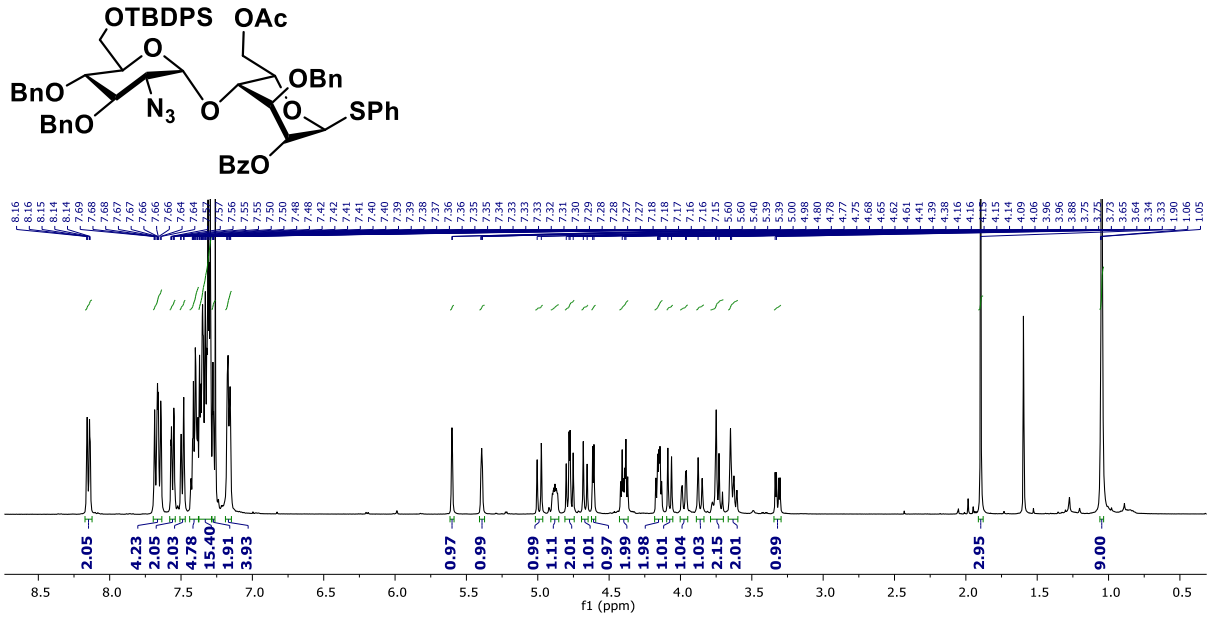
1. Muńoz-Garcìa, J. C., Corzana, F., de Paz, J. L., Angulo, J.& Nieto, P. M. Conformations of the iduronate ring in short heparin fragments described by time-averaged distance restrained molecular dynamics. *Glycobiology*. **2013**, 23, 1220-1229.
2. Muńoz-Garcìa, J. C. et al. Effect of the substituents of the neighboring ring in the conformational equilibrium of iduronate in heparin-like trisaccharides. *Chem. Eur. J.* **2013**, 18, 16319-16331.
3. Hsieh, P. H., Thieker, D. F., Guerrini, M., Woods, R. J.& Liu, J. Uncovering the relationship between sulphation patterns and conformation of iduronic acid in heparan sulfate. *Sci. Rep*.**2016**, 6, 29602.
4. Canales, A. et al. Conformational flexibility of a synthetic glycosaminoglycan bound to a fibroblast growth factor. FGF-1 recognized both the 1C4 and 2S0-conformations of a bioactive heparin-like hexasaccharide. *J. Am. Chem. Soc*. **2005**, 127, 16, 5778-5779.
5. Sarrazin, S.; Lamanna, W. C.; Esko, J. D., Heparan sulfate proteoglycans. *Cold Spring Harbor perspectives in biology* **2011,** *3* (7), a004952.
6. Shriver, Z.; Capila, I.; Venkataraman, G.; Sasisekharan, R., Heparin and heparan sulfate: analyzing structure and microheterogeneity. In *Heparin-A Century of Progress*, Springer: 2012; pp 159-176.
7. Rabenstein, D. L., Heparin and heparan sulfate: structure and function. *Nat. Prod. Rep.* **2002,** *19* (3), 312-331.
8. Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Darvill AG, Kinoshita T, Packer NH, Prestegard JH, Schnaar RL, Seeberger PH, editors. Essentials of Glycobiology [Internet]. 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015–2017.
9. Anand S, Mardhekar S, Raigawali R, Mohanta N, Jain P, D Shanthamurthy C, Gnanaprakasam B, Kikkeri R. Continuous-Flow Accelerated Sulfation of Heparan Sulfate Intermediates. Org Lett. **2020**, *22(9)*, 3402-3406.
10. Jain, P.; Shanthamurthy, C. D.; Leviatan Ben-Arye, S.; Woods, R. J.; Kikkeri, R.; Padler-Karavani, V. Discovery of Rare Sulfated: N -Unsubstituted Glucosamine Based Heparan Sulfate Analogs Selectively Activating Chemokines. Chem. Sci. 2021, 12 (10), 3674–3681.

11. Jain, P.; Shanthamurthy, C. D.; Chaudhary, P. M.; Kikkeri, R. Rational Designing of Glyco-Nanovehicles to Target Cellular Heterogeneity. Chem. Sci. 2021, 12 (11), 4021–4027.
12. Shitrit A, Mardhekar S, Alshanski I, Jain P, Raigawali R, Shanthamurthy CD, Kikkeri R, Yitzchaik S, Hurevich M. Profiling Heparan Sulfate-Heavy Metal Ions Interaction Using Electrochemical Techniques. Chemistry. **2022** Oct 4;28(55):e202202193.
13. Anand, S.; Mardhekar, S.; Bhoge, P. R.; Mishra, S. K.; Kikkeri, R. Molecular Recognition and Proteoglycan Mimic Arrangement: Modulating Cisplatin Toxicity. Chem. Commun. **2024**, 60 (33), 4495–4498.
14. Bernfield, M.; Götte, M.; Park, P. W.; Reizes, O.; Fitzgerald, M. L.; Lincecum, J.; Zako, M., Functions of cell surface heparan sulfate proteoglycans. *Ann. Rev. Biochem.* **1999,** *68* (1), 729-777.
15. Xu, D.; Esko, J. D., Demystifying heparan sulfate–protein interactions. *Ann. Rev. Biochem.* **2014,** *83*, 129-157.
16. Linhardt RJ. Analysis of glycosaminoglycans with polysaccharide lyases. Curr Protoc Mol Biol. **2001,** *Chapter 17*,13B.

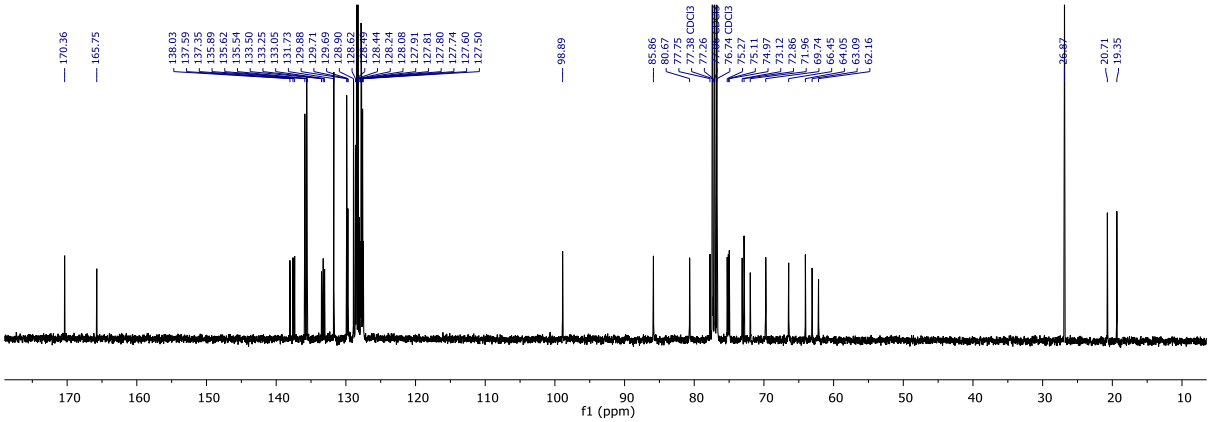
5.5.3 NMR Spectras and other characterisations

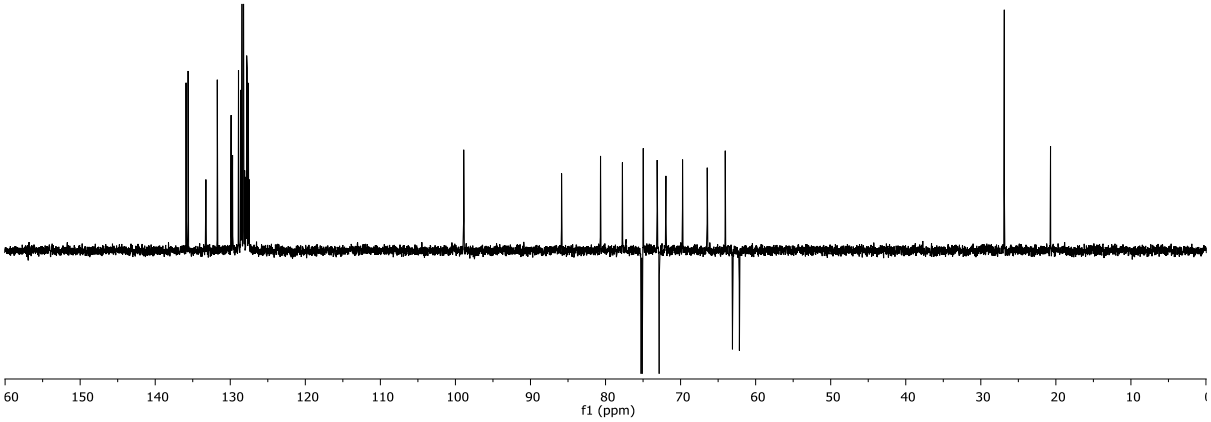


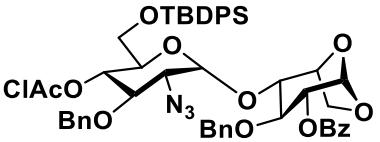

165.77
137.97
137.69
137.49
135.80
135.67
133.42
133.18
132.87
129.91
129.88
129.84
129.46
128.62
128.41
128.20
128.13
127.94
127.83
127.77
127.75
99.69
99.28
79.98
79.37
78.91
78.30
77.38 CDCl3
77.06 CDCl3
76.74 CDCl3
75.65
75.51
75.02
74.25
72.85
65.72
63.64
62.57
26.84
19.24
f1 (ppm)

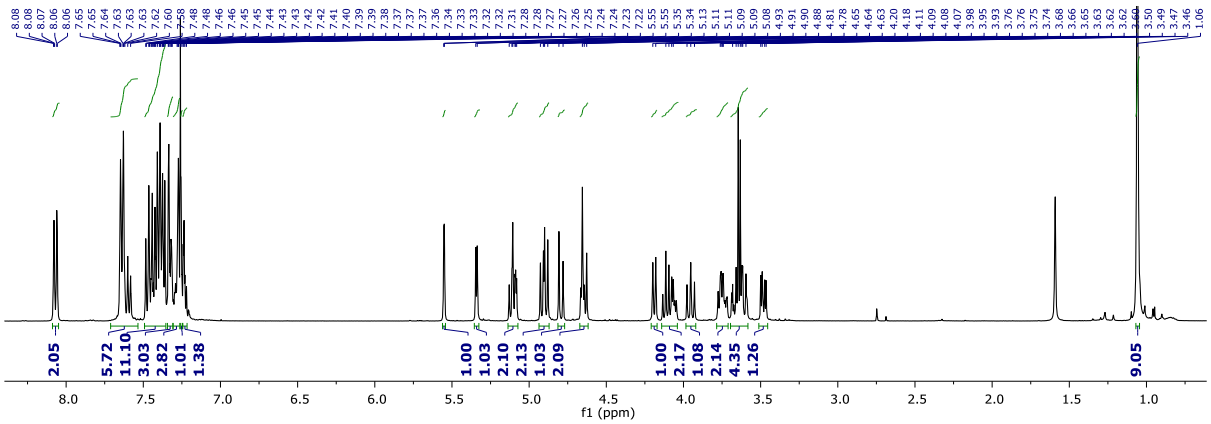

OTBDPS
OAc
O
BnO
BnO
N3
O
OBn
O
SPh
BzO

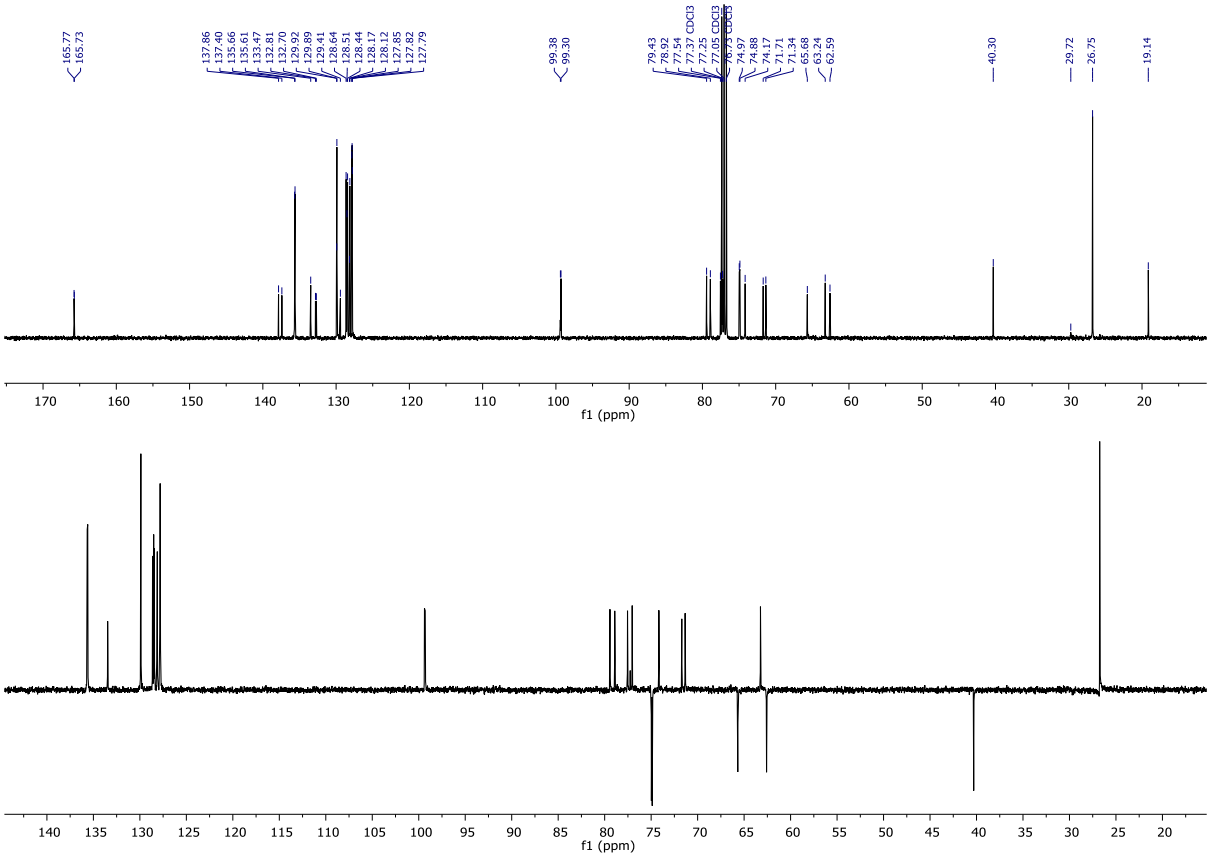
2.05
4.23
2.05
2.03
4.78
15.40
1.91
3.93
0.97
0.99
0.99
1.11
2.01
1.01
0.97
1.99
1.01
1.04
1.03
2.15
2.01
0.99
2.95
9.00
f1 (ppm)


170.36
165.75
138.03
137.59
137.35
135.89
135.62
135.54
133.50
133.25
131.05
131.03
129.88
129.71
129.69
128.90
128.62
128.44
128.24
128.08
127.91
127.81
127.80
127.74
127.60
127.50
98.89
85.86
80.67
77.75
77.38 CDCl3
77.26
76.74 CDCl3
75.27
75.11
74.97
73.12
72.86
71.96
69.74
66.45
64.05
63.09
62.16
26.87
20.71
19.35
f1 (ppm)

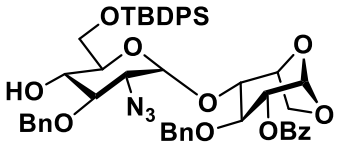

f1 (ppm)

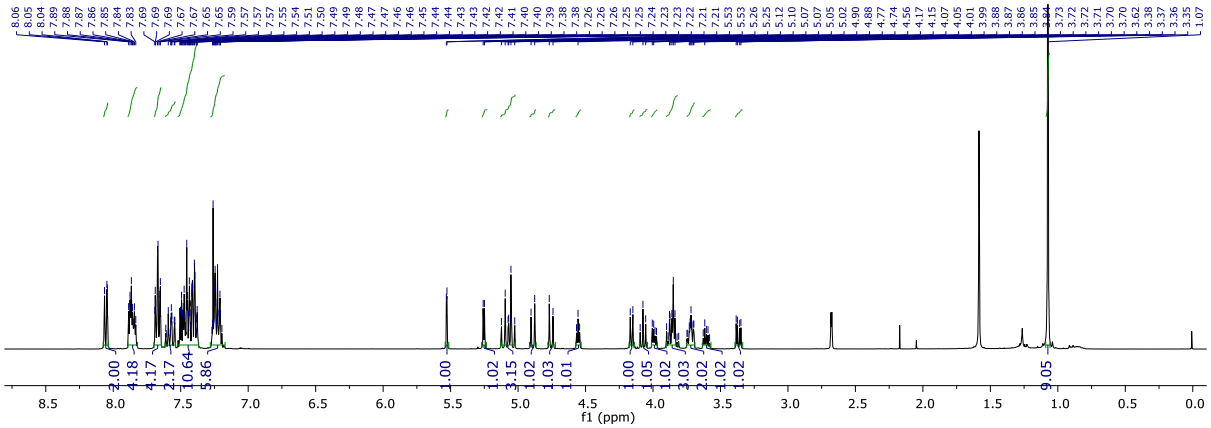

OTBDPS
O
ClAcO
N3
O
O
BnO
BnO
OBz
O

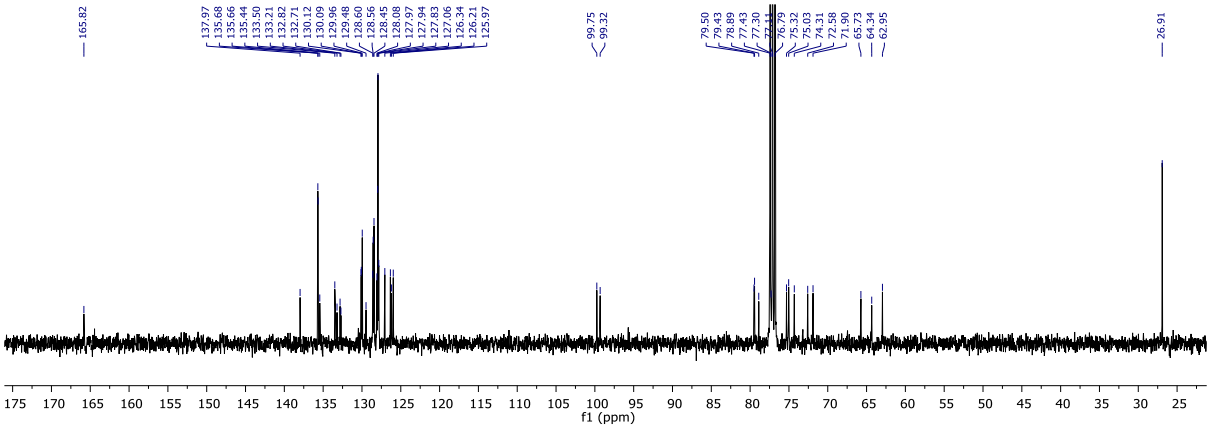

2.05
5.72
11.10
3.03
2.82
1.01
1.38
1.00
1.03
2.10
2.13
1.03
2.09
1.00
2.17
1.08
2.14
4.35
1.26
9.05
f1 (ppm)

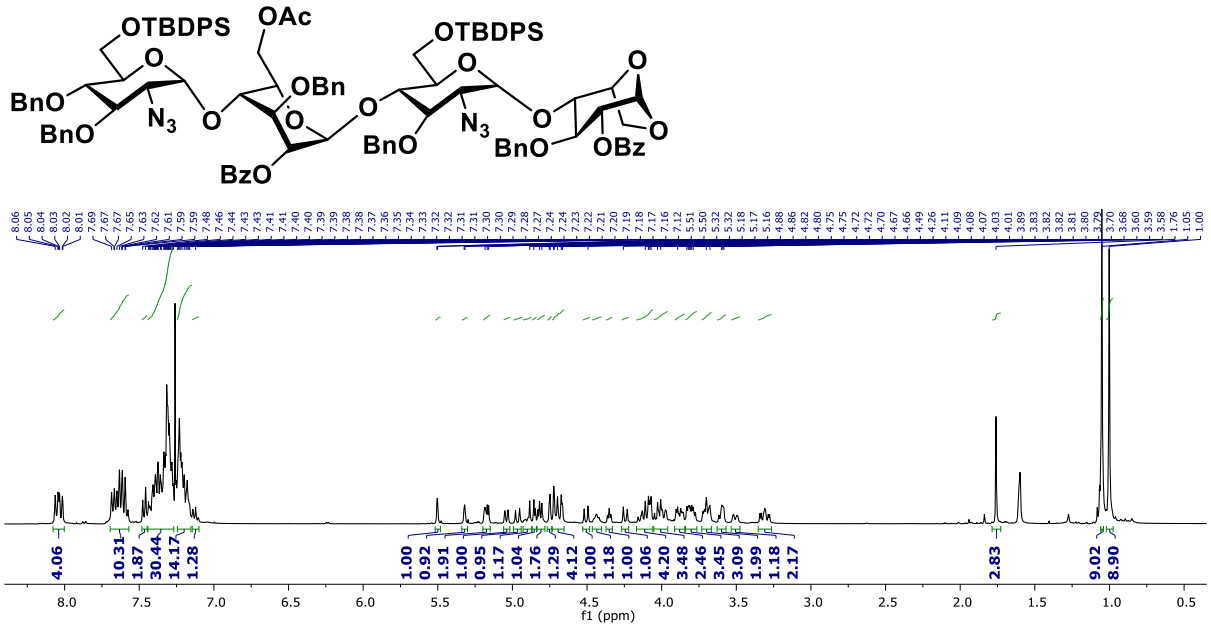

165.77
165.73
137.86
137.40
135.66
135.61
133.47
132.81
132.70
129.92
129.89
129.41
128.64
128.51
128.44
128.17
128.12
127.85
127.82
127.79
99.38
99.30
79.43
78.92
77.54
77.37 CDCl3
77.25
77.05 CDCl3
76.73 CDCl3
74.97
74.88
74.17
71.71
71.34
65.68
63.24
62.59
40.30
29.72
26.75
19.14
f1 (ppm)

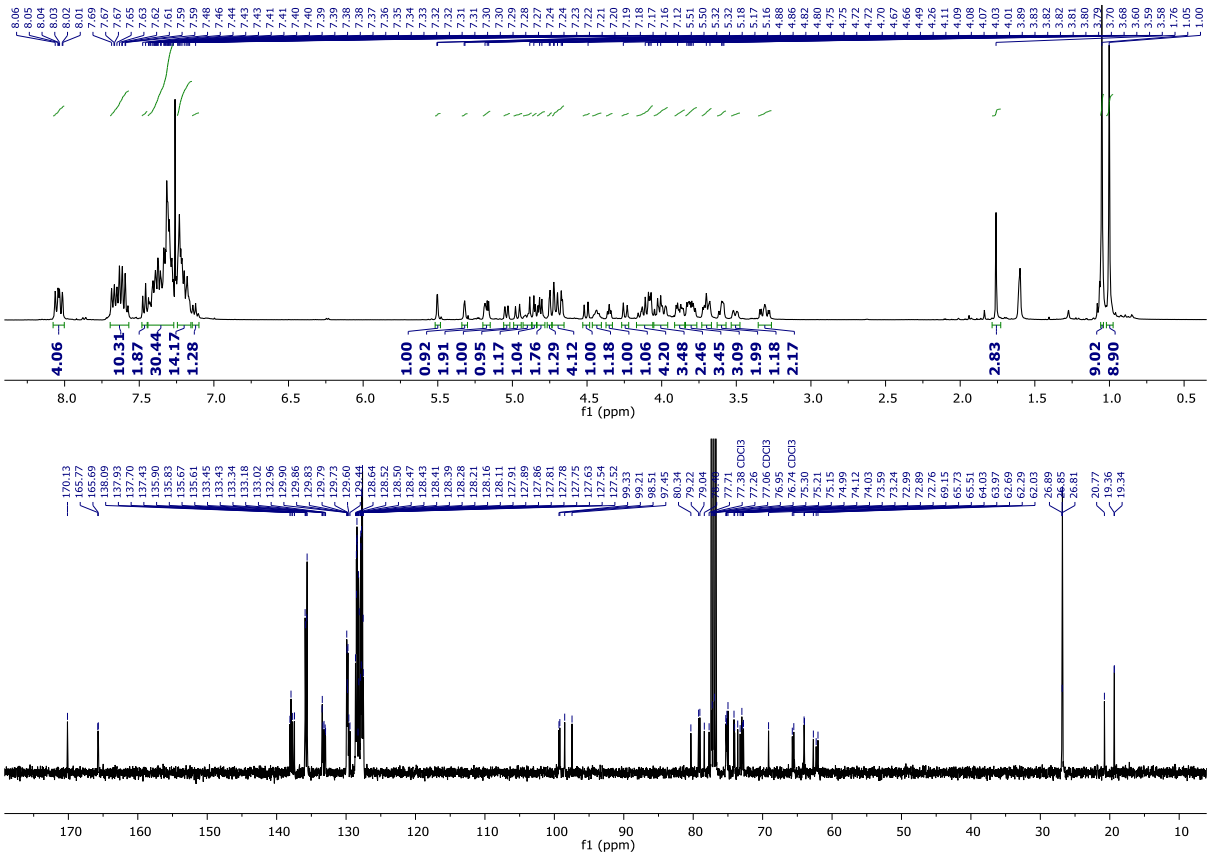
f1 (ppm)

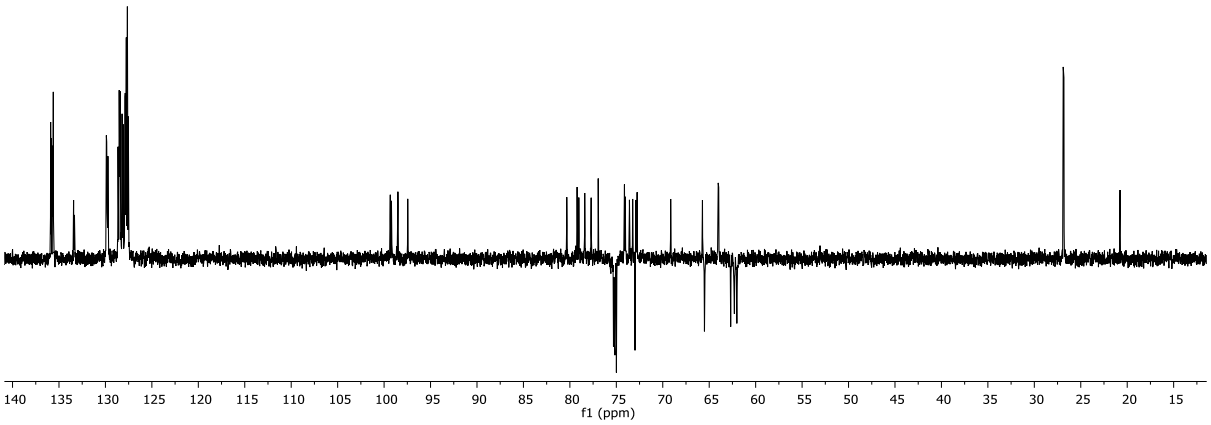

OTBDPS
O
HO
BnO
N3
O
BnO
O
OBz
O

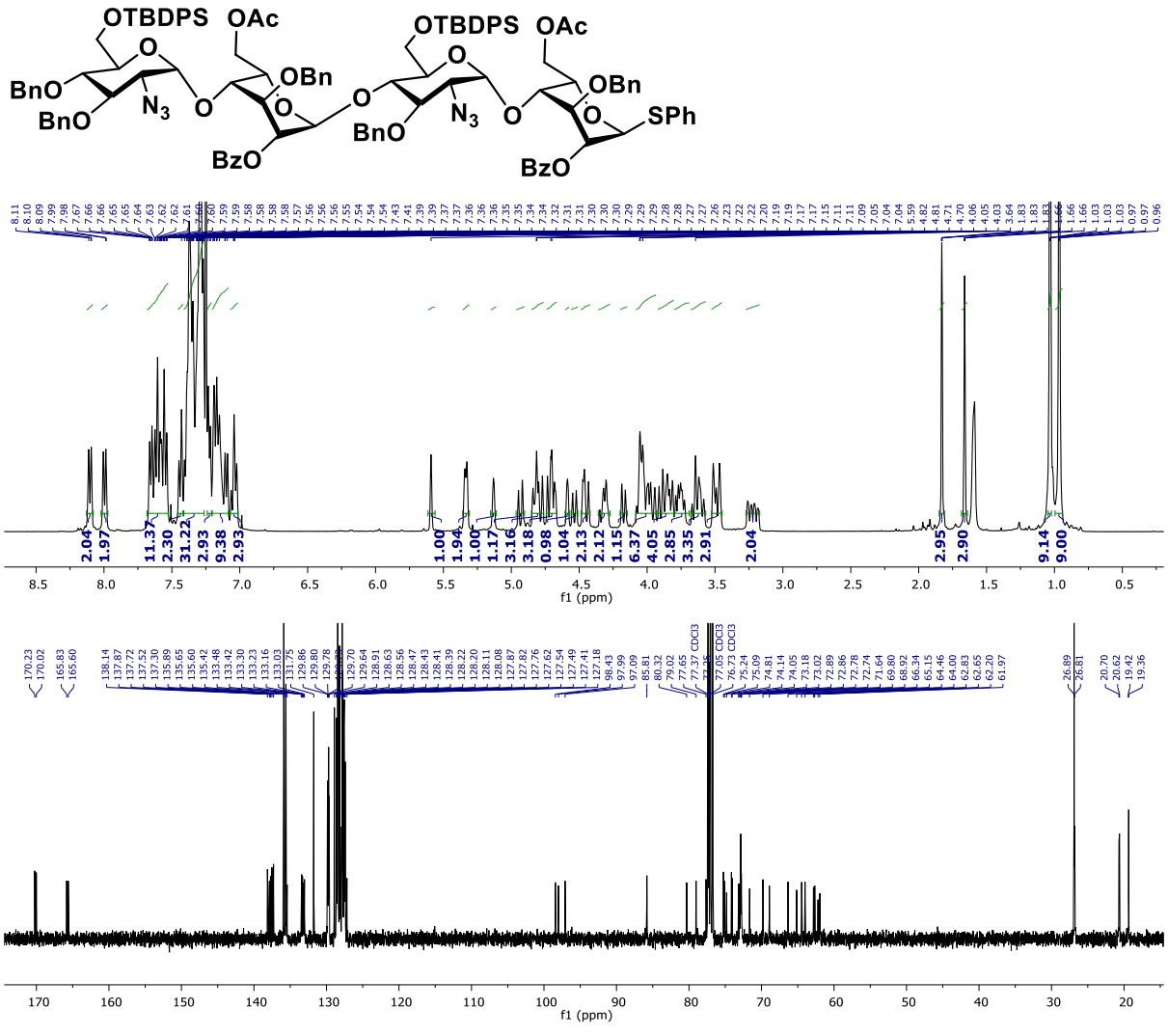

2.00
4.18
4.17
2.17
10.64
5.86
1.00
1.02
3.15
1.02
1.03
1.01
1.00
1.05
1.02
3.03
2.02
1.02
1.02
9.05
f1 (ppm)

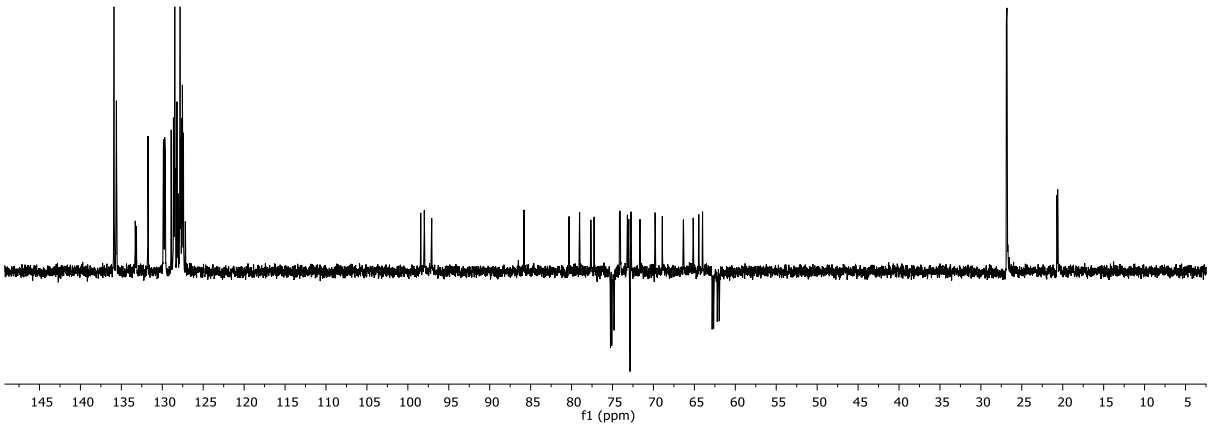


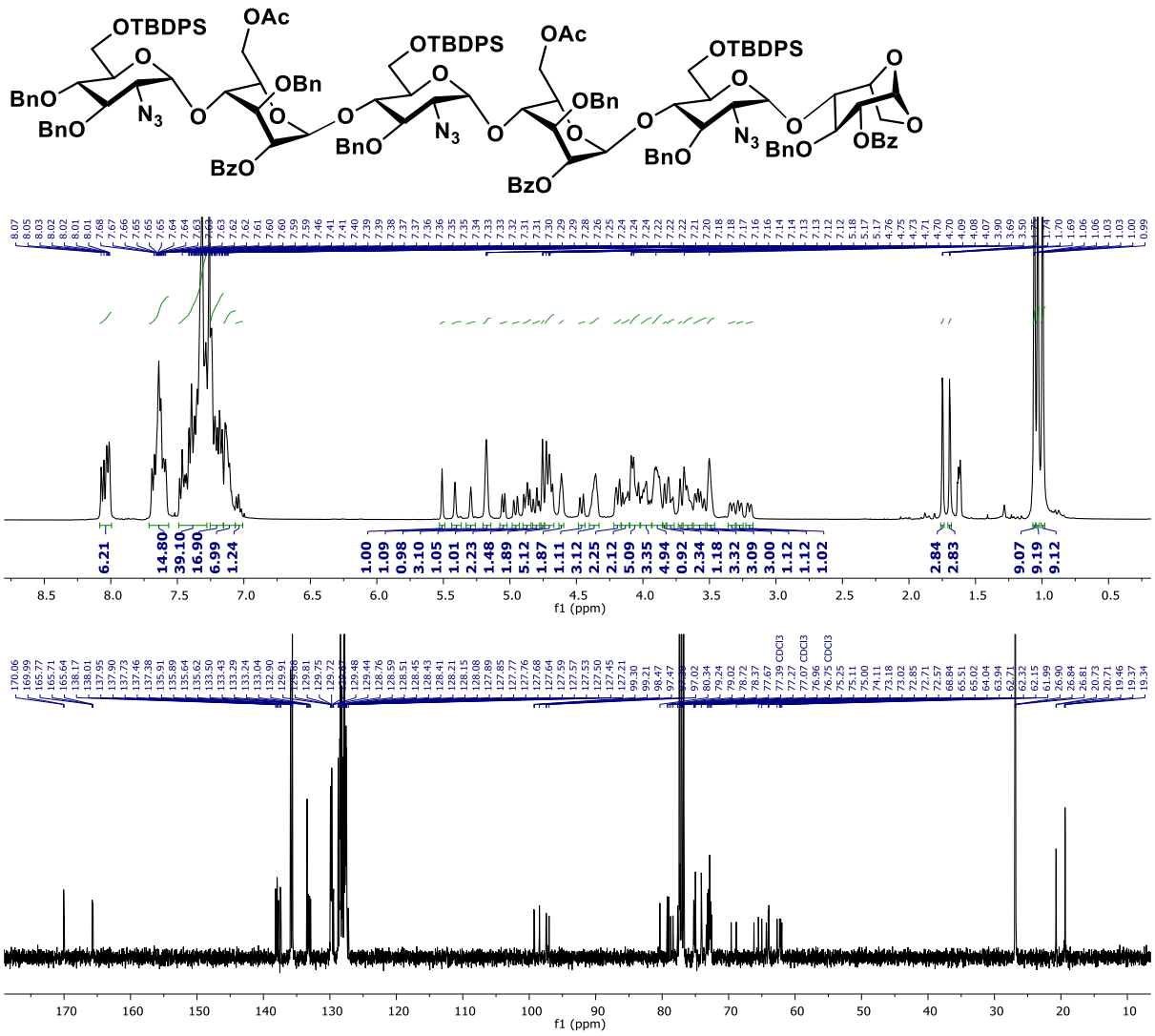

OTBDPS
OAc
OBn
BnO
BnO
N3
O
BzO
OTBDPS
BnO
N3
BnO
OBz

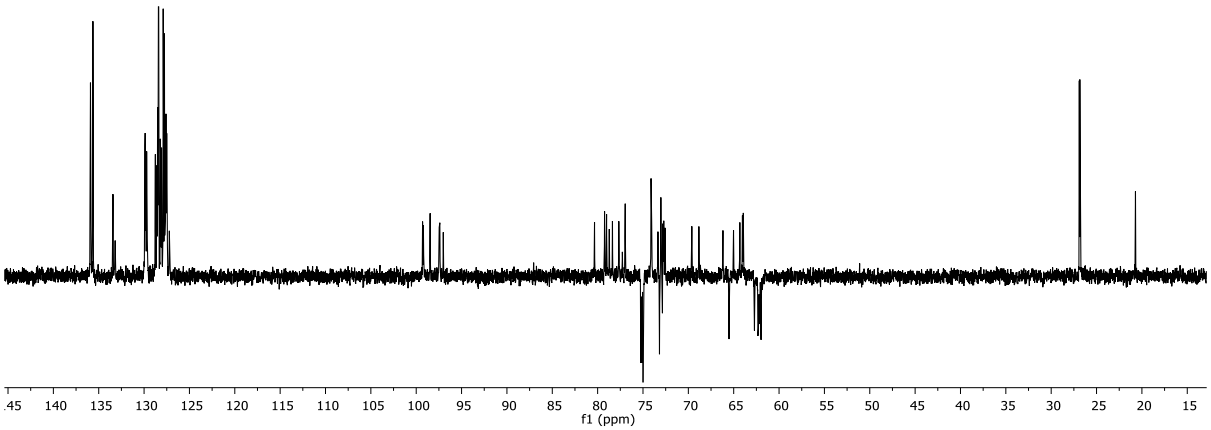


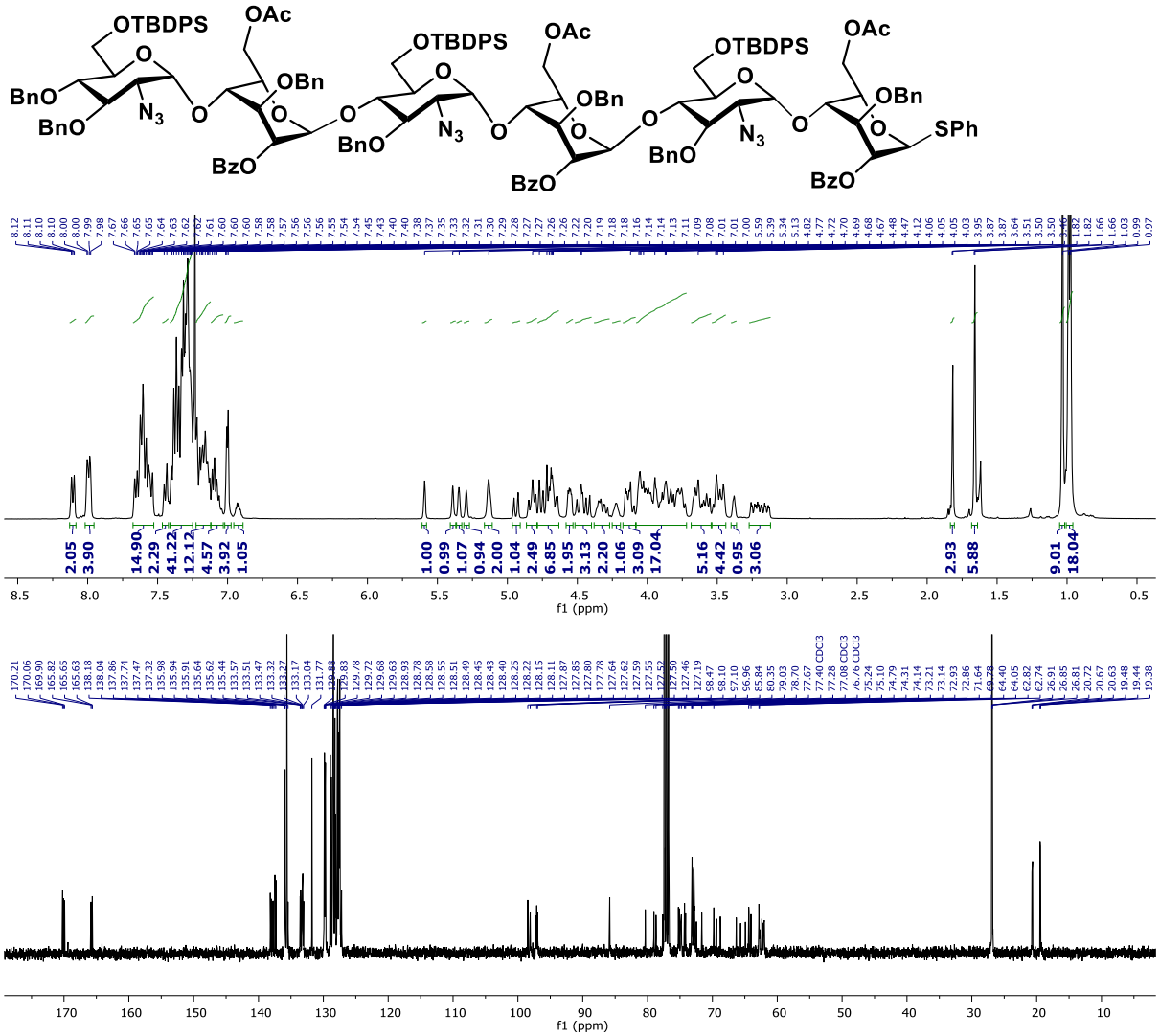

f1 (ppm)

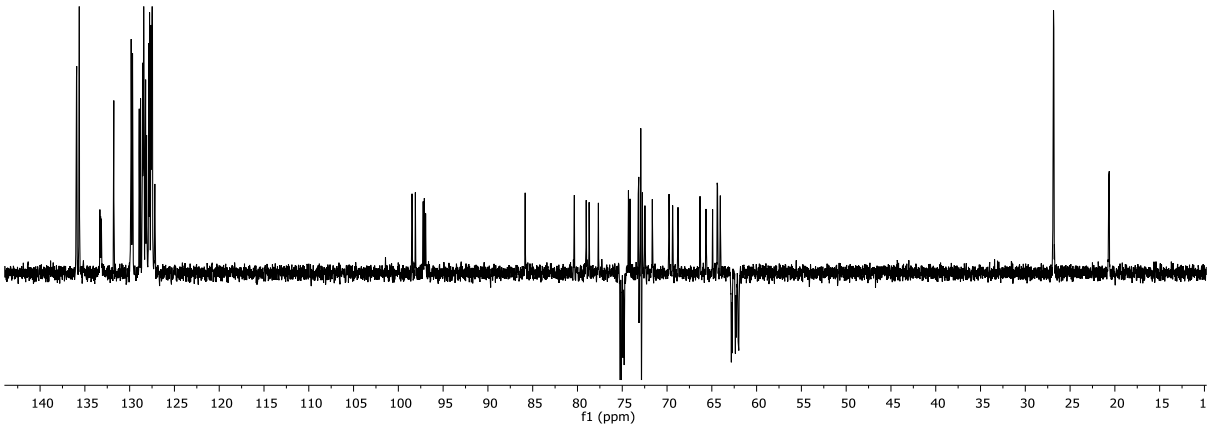

OTBDPS
OAc
OBn
BnO
BnO
N3
O
BzO
SPh
f1 (ppm)

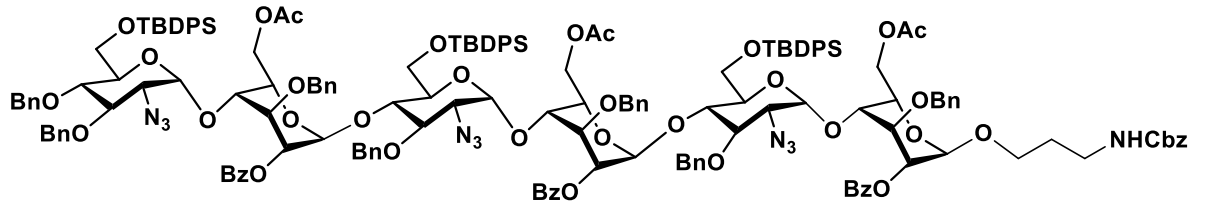

145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

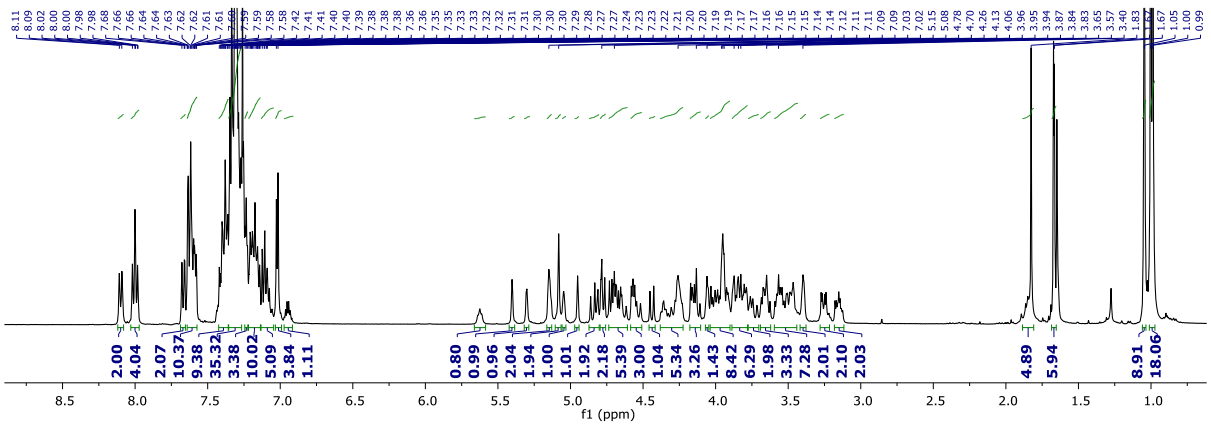

OTBDPS
OAc
OBn
BnO
N3
BzO
OBz
6.21 14.80 39.10 16.90 6.99 1.24 1.00 1.09 0.98 3.10 1.05 1.01 2.23 1.48 1.89 5.12 1.87 1.11 3.12 2.25 2.12 5.09 3.35 4.94 0.92 2.34 1.18 3.32 3.09 3.00 1.12 1.12 1.02 2.84 2.83 9.07 9.19 9.12
f1 (ppm)

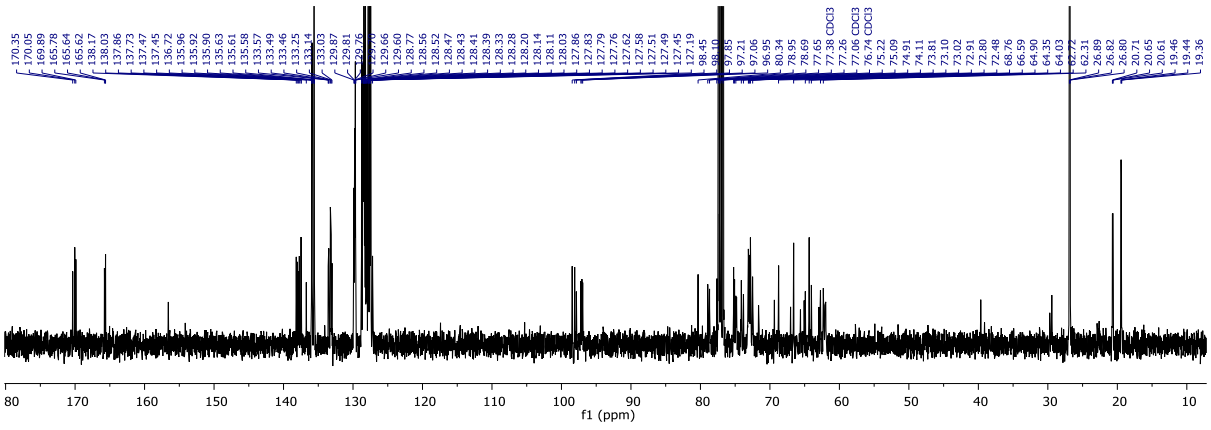

f1 (ppm)

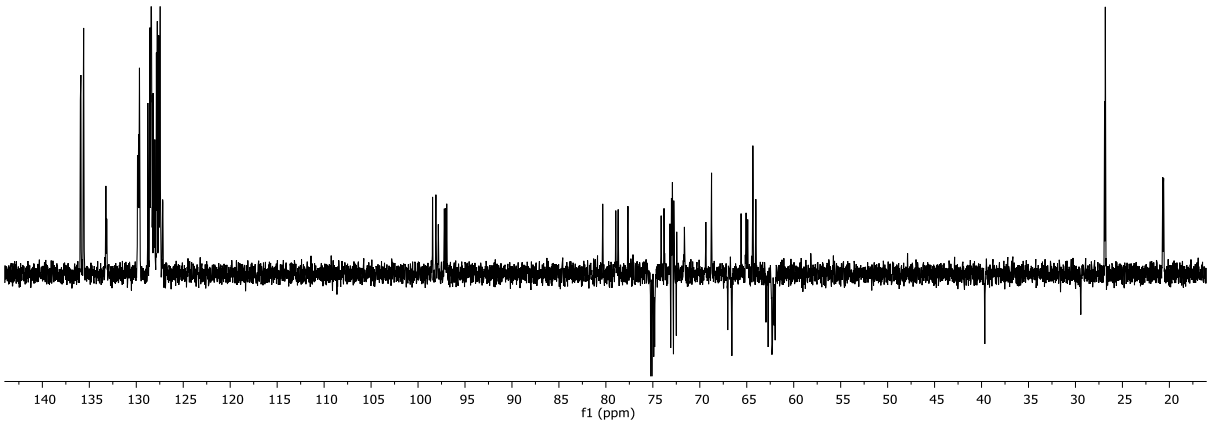

OTBDPS
OAc
OBn
BnO
BnO
N3
O
BzO
SPh
2.05
3.90
14.90
2.29
41.22
12.12
4.57
3.92
1.05
1.00
0.99
1.07
0.94
2.00
1.04
2.49
6.85
1.95
3.13
2.20
1.06
3.09
17.04
5.16
4.42
0.95
3.06
2.93
5.88
9.01
18.04
f1 (ppm)

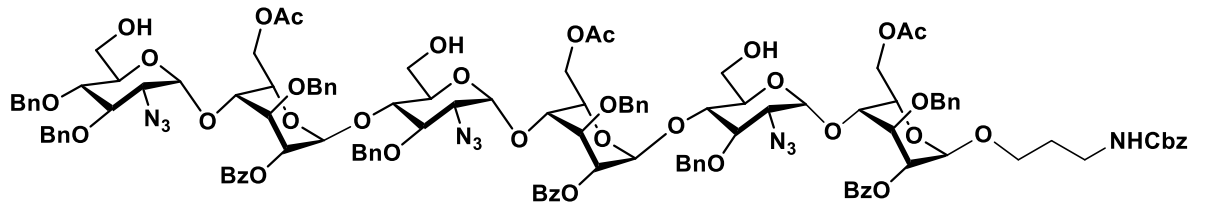

140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 1
f1 (ppm)

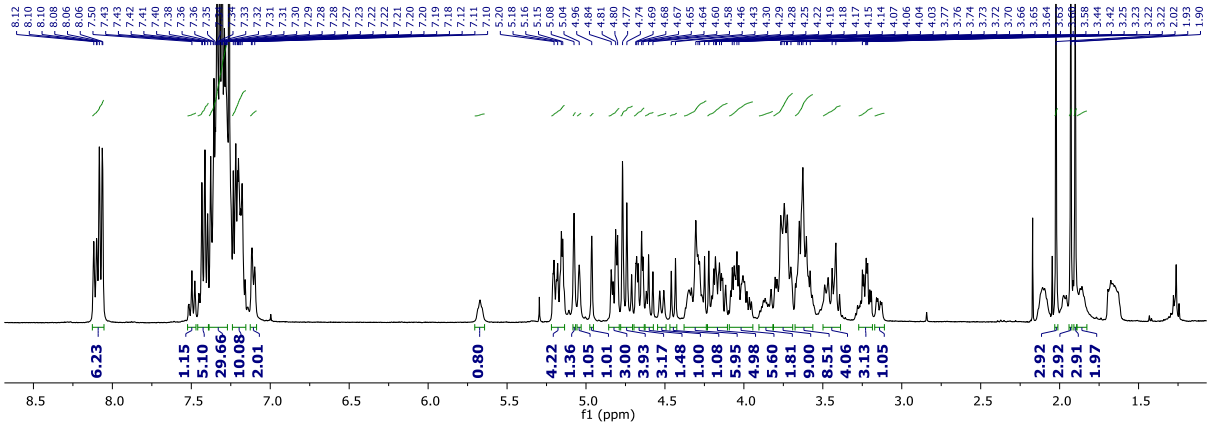

OTBDPS
OAc
OBn
BnO
BnO
N3
O
BzO
NHCbz

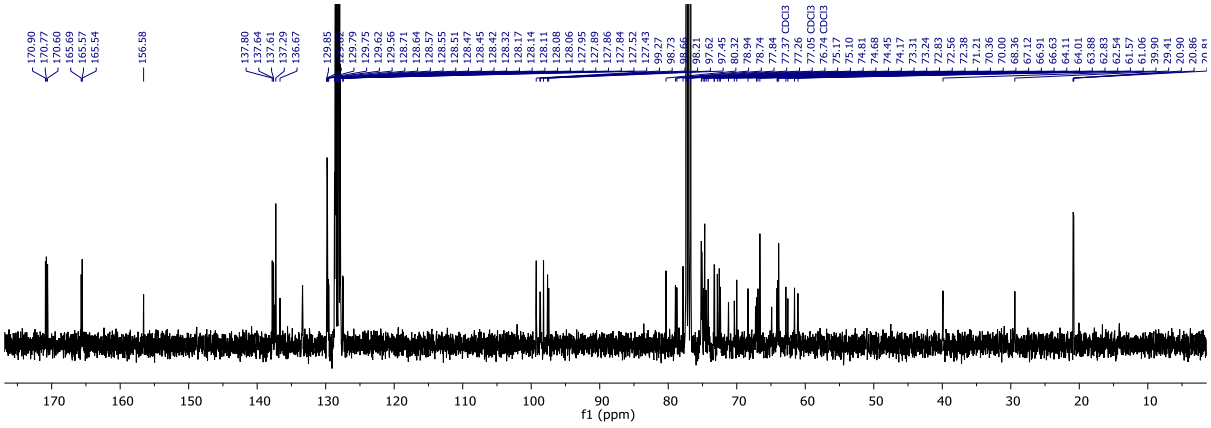

2.00 4.04 2.07 10.37 9.38 35.32 3.38 10.02 5.09 3.84 1.11 0.80 0.99 0.96 2.04 1.94 1.00 1.01 1.92 2.18 5.39 3.00 1.04 5.34 3.26 1.43 8.42 6.29 1.98 3.33 7.28 2.01 2.10 2.03 4.89 5.94 8.91 18.06
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

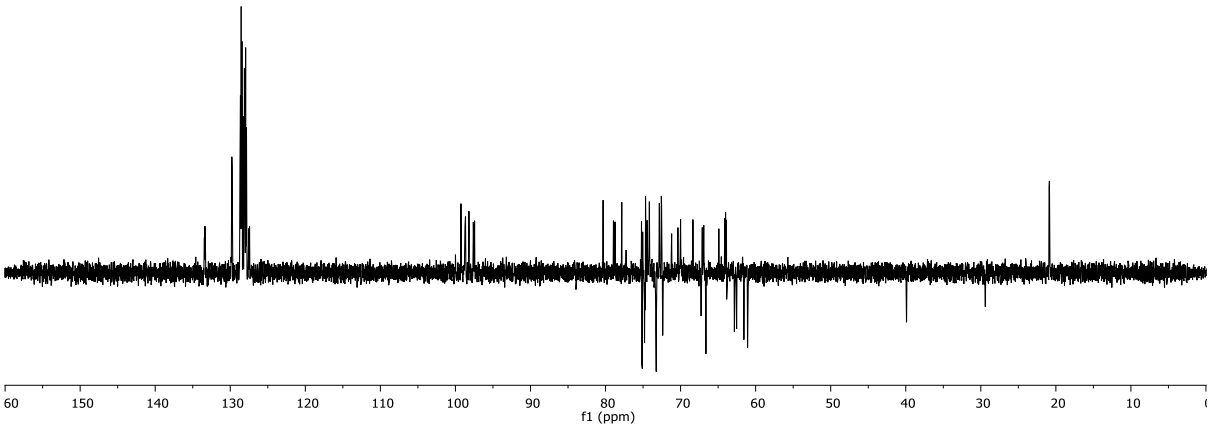

80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

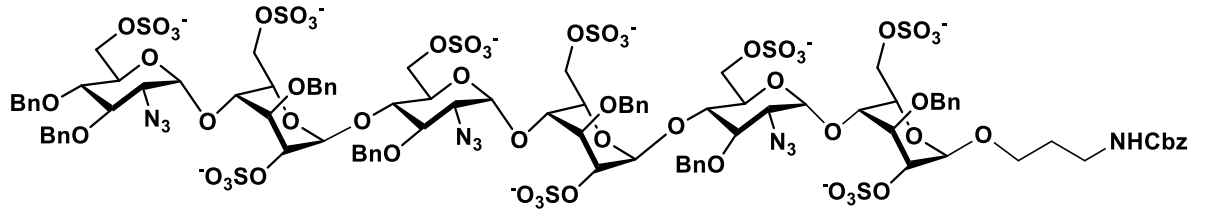

140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

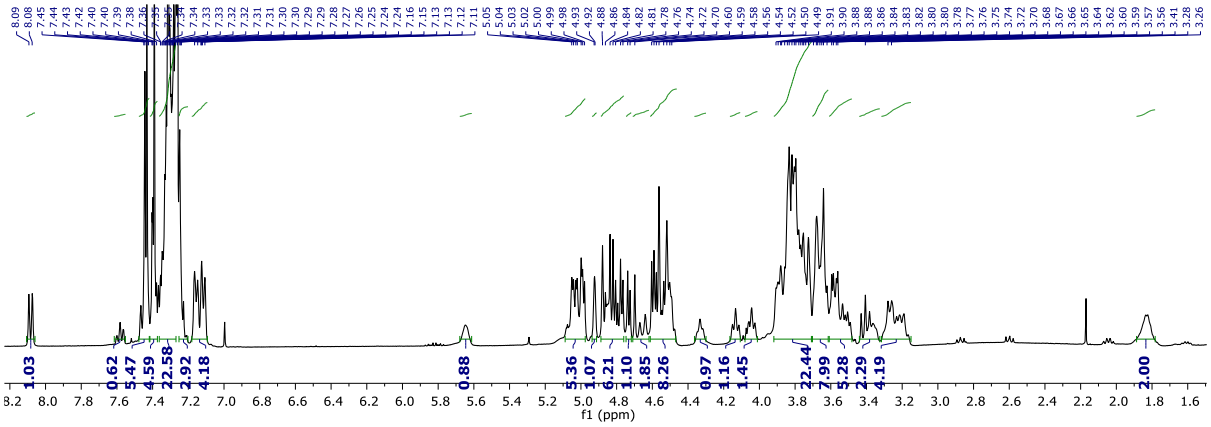

OH
OAc
OBn
BnO
BnO
N3
O
BzO
NHCbz

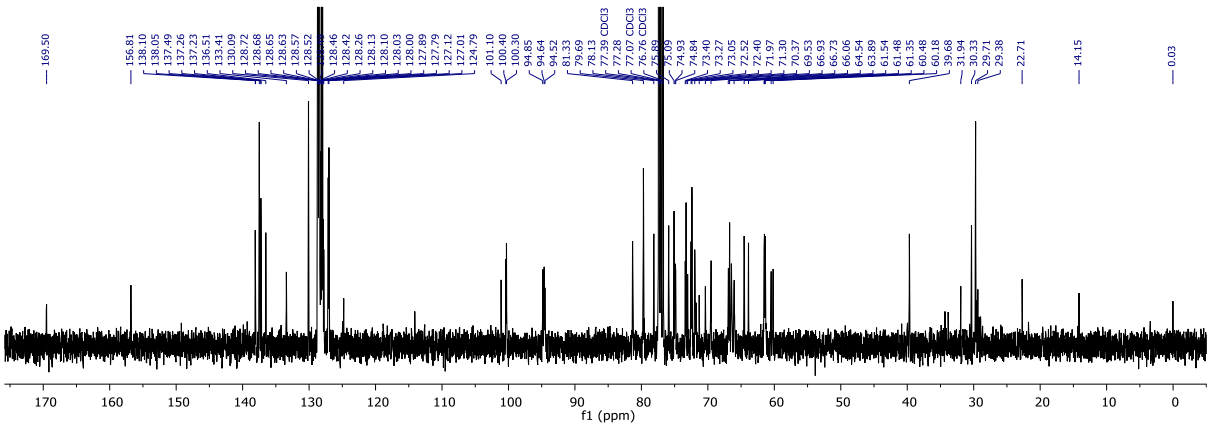

6.23
1.15
5.10
29.66
10.08
2.01
0.80
4.22
1.36
1.05
1.01
3.00
3.93
3.17
1.48
1.00
1.08
5.60
4.98
1.81
9.00
8.51
4.06
3.13
1.05
2.92
2.92
2.91
1.97
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

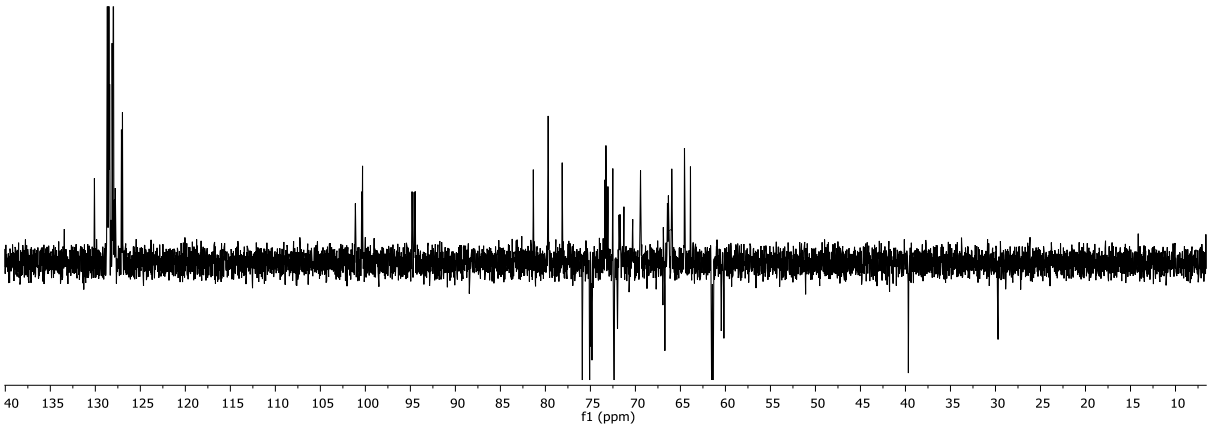

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

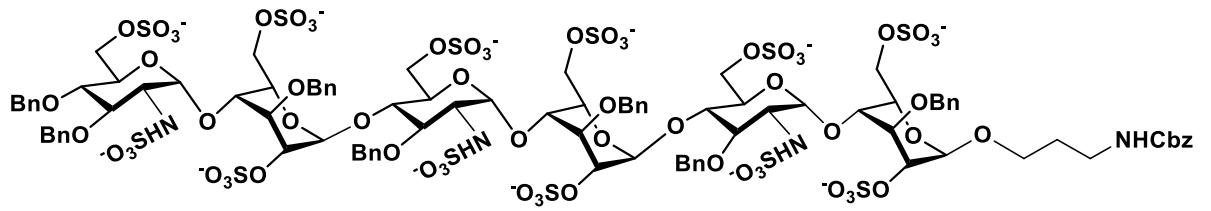

60 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

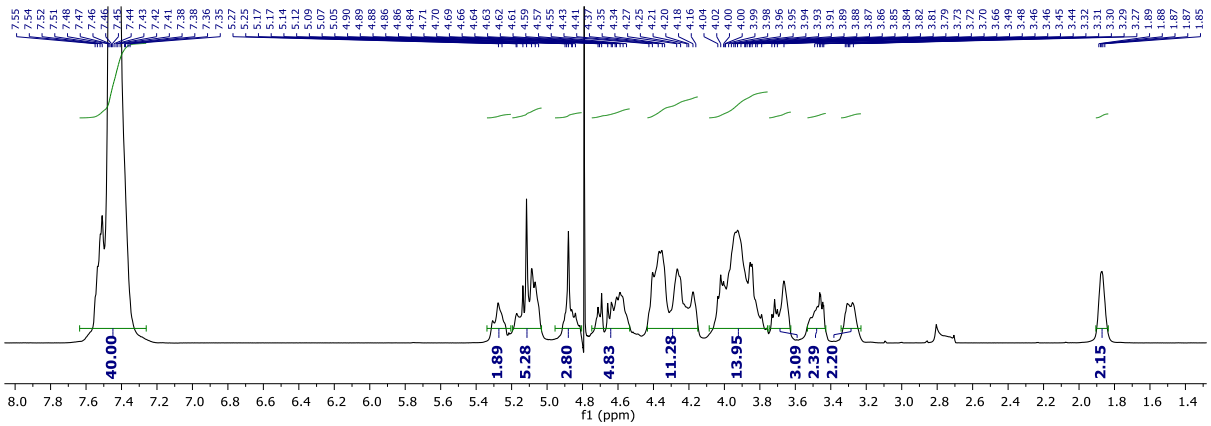

OSO3-
BnO
N3
OBn
-O3SO
NHCbz

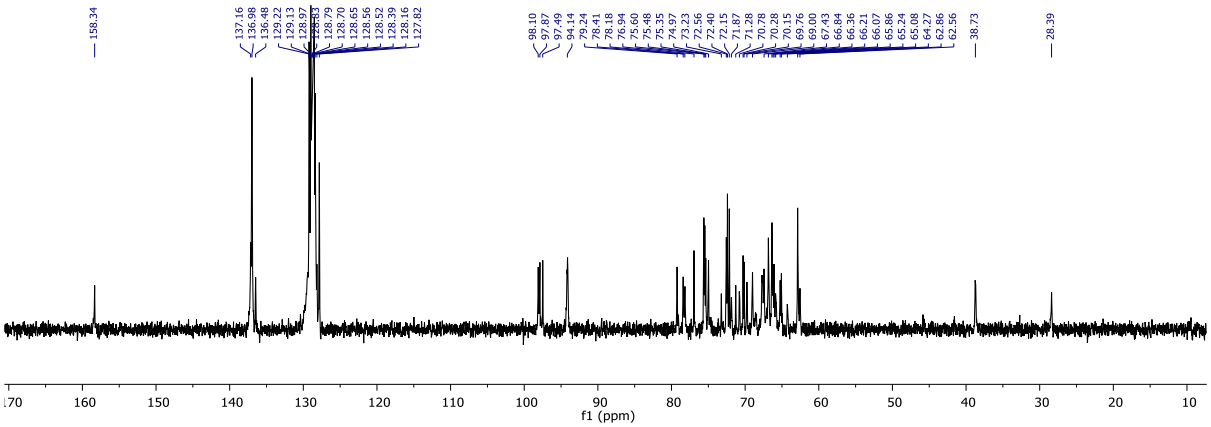

1.03 0.62 5.47 4.59 22.58 2.92 4.18 0.88 5.36 1.07 6.21 1.10 1.85 8.26 0.97 3.16 1.45 22.44 7.99 5.28 2.29 4.19 2.00
f1 (ppm)

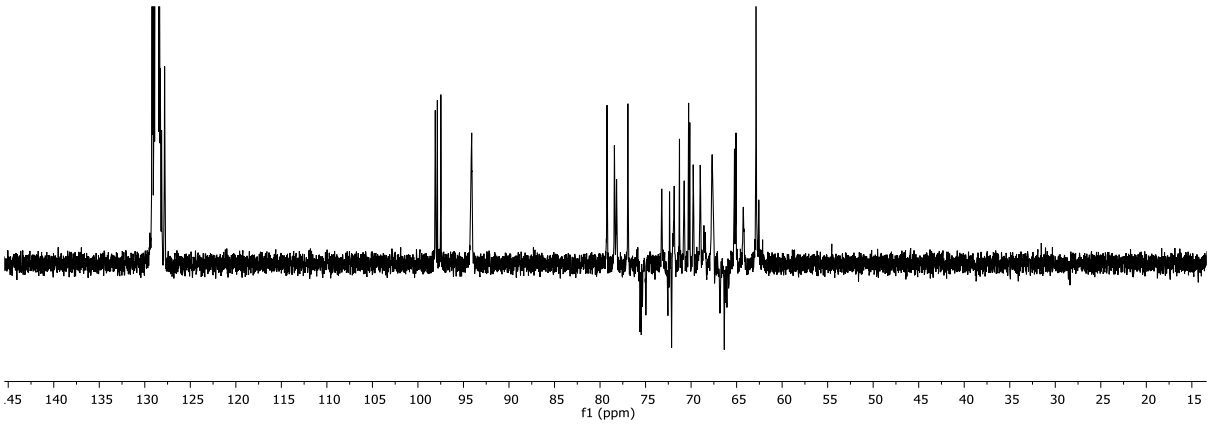

f1 (ppm)

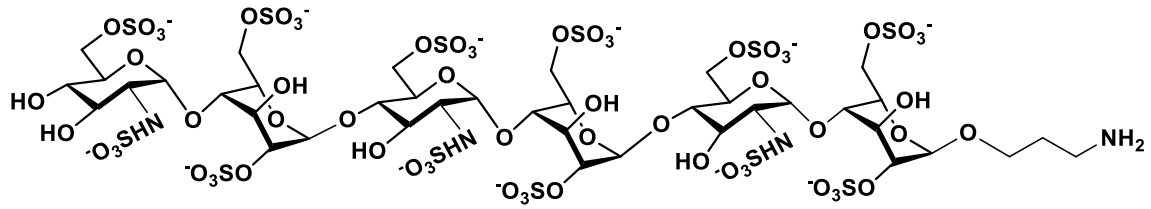

f1 (ppm)

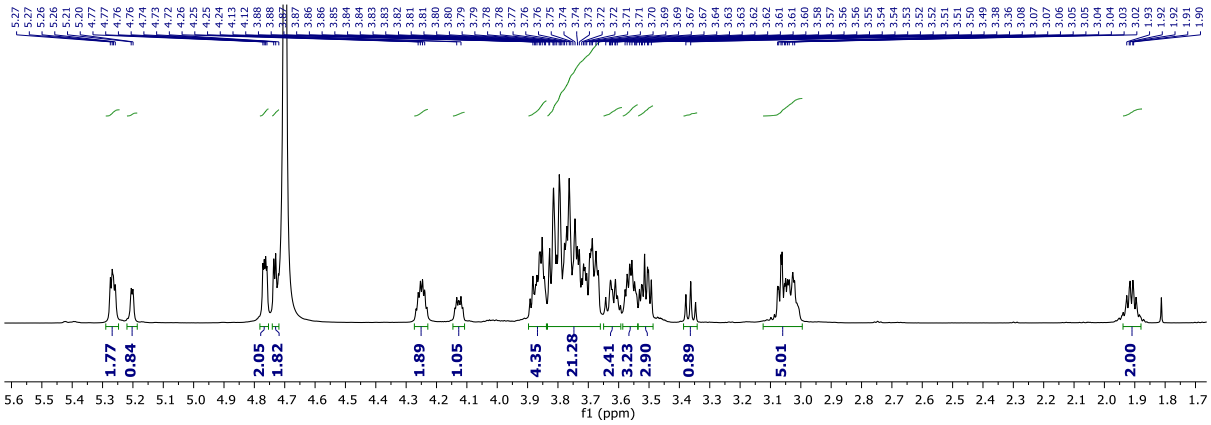

OSO3-
BnO
OBn
-O3SHN
-O3SO
NHCbz

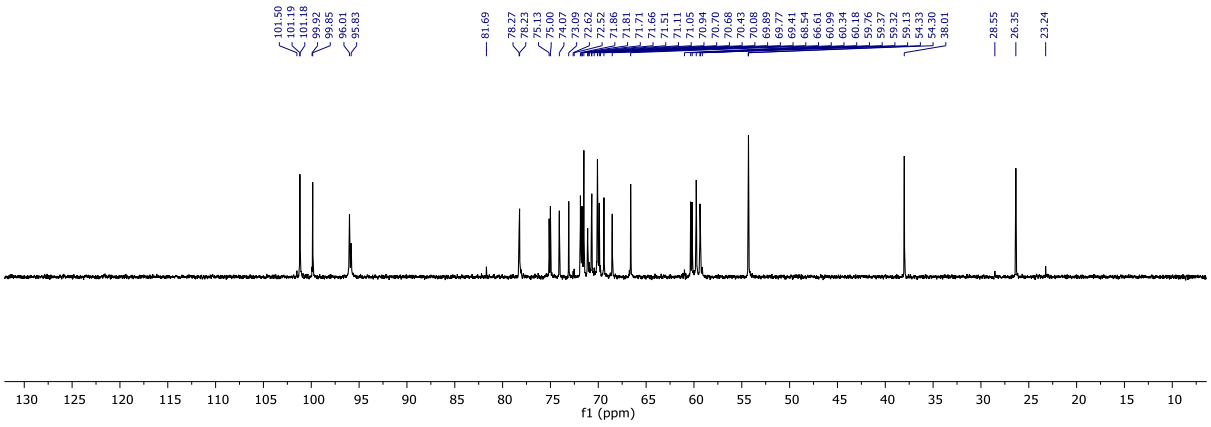

40.00
1.89
5.28
2.80
4.83
11.28
13.95
3.09
2.39
2.20
2.15
f1 (ppm)

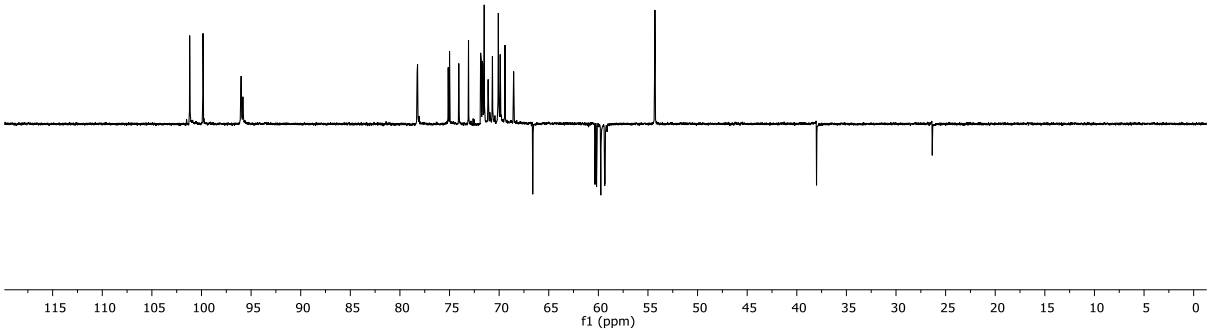

158.34
137.16
136.98
136.48
129.22
129.13
128.97
128.79
128.70
128.65
128.56
128.52
128.39
128.16
127.82
98.10
97.87
97.49
94.14
79.24
78.41
78.18
76.94
75.60
75.48
75.35
74.97
73.23
72.56
72.40
72.15
71.87
71.28
70.78
70.28
70.15
69.76
69.00
67.43
66.84
66.36
66.21
66.07
65.86
65.24
65.08
64.27
62.86
62.56
38.73
28.39
f1 (ppm)


f1 (ppm)

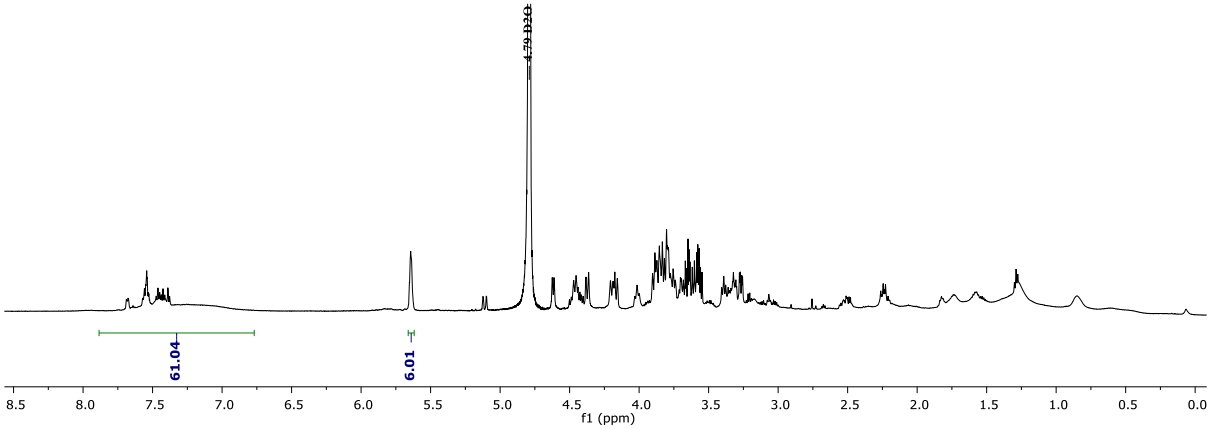

OSO3-
HO
-O3SHN
OH
-O3SO
NH2

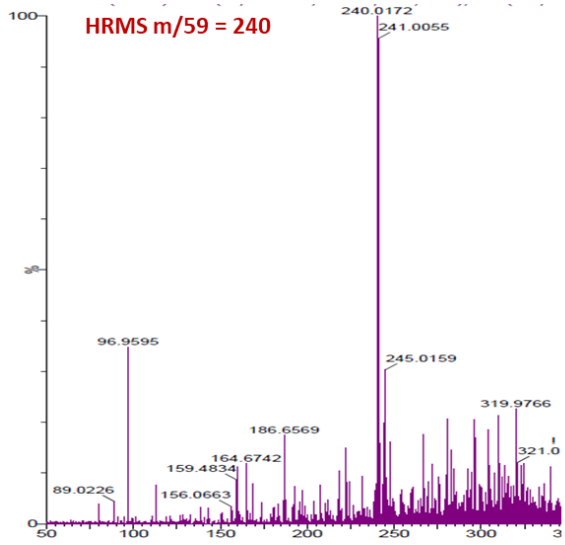

1.77
0.84
2.05
1.82
1.89
1.05
4.35
21.28
2.41
3.23
2.90
0.89
5.01
2.00
f1 (ppm)


f1 (ppm)

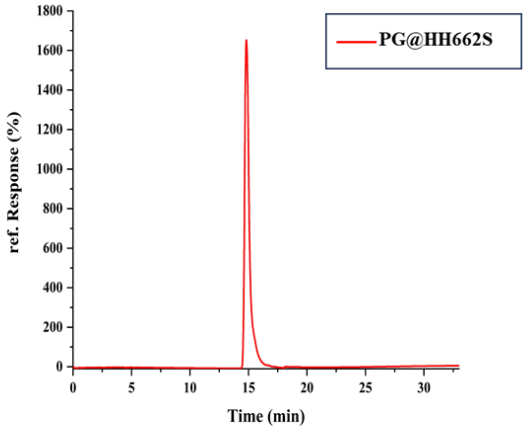


PG@HH662S

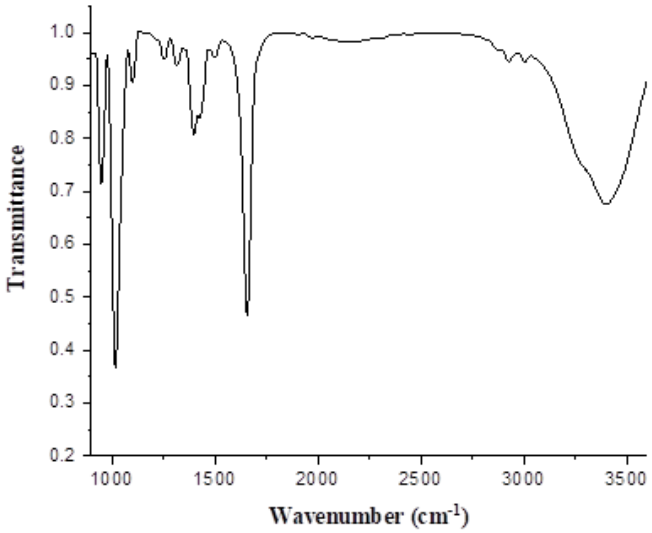




HRMS data of PG@HH62S



HPLC Profile of PG@HH662S



IR data of PG@HH662S