

***Exploration of Silver-Assisted Gold Catalysis for the Synthesis of  
Anticoagulant Heparin Pentasaccharide and a Heptasaccharide  
of Mycobacterium tuberculosis Cell Surface***

*Thesis*

*Submitted in partial fulfilment of the  
Requirements of the degree of*

***Doctor of Philosophy***

*By*

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## *Dedicated to...*

*All those who constantly stood alongside and encouraged me in this journey of life to become what I am today. I do believe with my hard work there are the blessings of my parents and continuous family support.*

---

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## CERTIFICATE

Certified that the work incorporated in the thesis entitled "*Exploration of Silver-Assisted Gold Catalysis for the Synthesis of Anticoagulant Heparin Pentasaccharide and a Heptasaccharide of Mycobacterium Tuberculosis Cell Surface*" submitted by **Mr. Gulab Sudam Walke** was carried out by the 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: May, 2020  
Place: Pune



Prof. Srinivas Hotha  
(Thesis Supervisor)

## DECLARATION

I declare that, this written submission represents my ideas in my own words and where ideas of others have been included; I have adequately cited and referenced the original sources. 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.

Date: May, 2020  
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**Gulab Walke**

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## *General remarks*

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- ❖  $^1\text{H}$  NMR spectra were recorded on AV 200, AV 400, DRX-500 MHz, JEOL ECX 400 or Bruker Avance 500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in ppm units downfield to TMS.
- ❖  $^{13}\text{C}$  NMR spectra were recorded on AV 50, AV 100, DRX-125 MHz, JEOL ECX 100 or Bruker Avance 125 MHz spectrometer.
- ❖ High resolution mass spectroscopy (HRMS) was performed on Waters Synapt G2 or Applied Biosystems MALDI-ToF-ToF spectrometer.
- ❖ IR spectra were recorded on Perkin-Elmer 1310 or Perkin-Elmer 1600 FT-IR spectrometers with sodium chloride optics and are measured in  $\text{cm}^{-1}$ .
- ❖ Specific rotations were measured on a JASCO P-1020 and measured in degrees.
- ❖ All reactions were monitored by Thin-Layer Chromatography carried out on pre-coated Merck silica plates ( $\text{F}_{254}$ , 0.25 mm thickness); compounds were visualized by UV light or by staining with anisaldehyde spray.
- ❖ All reactions were carried out under nitrogen or argon atmosphere with freshly prepared anhydrous solvents under anhydrous conditions and yields refer to chromatographically homogenous materials unless otherwise stated.
- ❖ All evaporations were carried out under reduced pressure on Büchi and Heidolph rotary evaporators below 45 °C unless otherwise specified.
- ❖ Silica gel (100-200) and (230-400) mesh were used for column chromatography.
- ❖ All gold and transition metal salts were purchased from multinational commercial vendors.
- ❖ Materials were obtained from commercial suppliers and were used without further purification.
- ❖ Scheme, Figure and Compound numbers in abstract and individual chapters are different.

## Abbreviations

---

|                                                                  |                                                     |
|------------------------------------------------------------------|-----------------------------------------------------|
| Å – Angstrom                                                     | Lev – Levulinoate                                   |
| Ac – Acetate                                                     | M – Molar                                           |
| AcCl – Acetyl chloride                                           | Manp – mannopyranosyl /<br>mannopyranoside          |
| AcOH – Acetic acid                                               | MeI – Methyl Iodide                                 |
| Ac <sub>2</sub> O – Acetic anhydride                             | MeOD – Methanol-D4                                  |
| AG – Arabinogalactan                                             | mg – milligram                                      |
| Araf – arabinofuranosyl/arabinofuranoside                        | min. – minutes                                      |
| Bn – Benzyl                                                      | MHz – Megahertz                                     |
| BnBr – Benzyl bromide                                            | mL – millilitre                                     |
| Boc – <i>t</i> -butylcarbonyl                                    | mmol – millimolar                                   |
| BSA – Bis(trimethylsilyl)acetamide                               | MS – Molecular sieves                               |
| Bu <sub>2</sub> SnO – Dibutyltin oxide                           | Mtb – Mycobacterium tuberculosis                    |
| Bz – Benzoyl                                                     | NaH – Sodium hydride                                |
| BzCl – Benzoyl chloride                                          | NAP – N-bromomethyl naphthalene                     |
| Calcd – Calculated                                               | NGP – Neighbouring group participation              |
| Cbz – Carbobenzyloxy                                             | NIS – N-Iodosuccinimide                             |
| cat. – catalytic                                                 | NMR – Nuclear Magnetic Resonance                    |
| CDCl <sub>3</sub> – Chloroform-D                                 | NNGP – Non-neighbouring group<br>participation      |
| CHCl <sub>3</sub> – Chloroform                                   | NPG – n-Pentenyl glycoside                          |
| d – days                                                         | PMB – p-Methoxy benzyl                              |
| DBU – 1,8-Diazabicycloundec-7-ene                                | PMBCl – p-Methoxy benzyl chloride                   |
| DDQ – 2,3-Dichloro-5,6-dicyano-1,4-<br>benzoquinone              | Py – Pyridine                                       |
| DCM – Dichloromethane                                            | PTSA, TsOH – <i>p</i> -Toluene sulfonic acid        |
| DCE – Dichloroethane                                             | ppm – parts per million                             |
| DEPT – Distortionless Enhancement by<br>Polarization Transfer    | Ribf – ribofuranoside / ribofuranosyl               |
| DIPEA – <i>N,N'</i> -Diisopropylethylamine                       | rt – room temperature                               |
| DIC – <i>N,N'</i> -Diisopropylcarbodiimide                       | sat. – saturated                                    |
| DMAP – <i>N,N'</i> -Dimethylaminopyridine                        | Tb – tuberculosis                                   |
| DMF – <i>N,N'</i> -Dimethyl formamide                            | TBAI – tetra- <i>n</i> -Butyl ammonium iodide       |
| DMSO- <i>d</i> <sub>6</sub> – Dimethyl sulfoxide<br>(deuterated) | TBDMS – <i>t</i> -Butyldimethylsilyl                |
| D <sub>2</sub> O – Deuterium oxide                               | TBDMSCl – <i>t</i> -Butyldimethylchloride           |
| δ – delta (in ppm)                                               | TBDPS – <i>t</i> -Butyldiphenylsilyl                |
| eq. – equivalents                                                | TBDPSCl – <i>t</i> -Butyldiphenylsilylchloride      |
| Et <sub>3</sub> N – Triethyl amine                               | TCA – Trichloroacetimidate                          |
| EtOAc – Ethyl acetate                                            | TfOH – trifluoromethanesulfonic acid                |
| g – gram                                                         | TFA – Trifluoroacetic acid                          |
| Galf – galactofuranosyl / galactofuranoside                      | THF – Tetrahydrofuran                               |
| h – hour                                                         | TLC – Thin Layer Chromatography                     |
| HOAt – 1-Hydroxy-7-azabenzotriazole                              | TMSOTf –<br>Trimethylsilyltrifluoromethanesulfonate |
| HRMS – High-Resolution Mass<br>Spectrometry                      | µg – microgram                                      |
| Hz – Hertz                                                       | µmol – micromolar                                   |
| Im. – Imidazole                                                  | µL – microliter                                     |
| IR – Infrared                                                    |                                                     |
| <i>J</i> – coupling constant                                     |                                                     |
| Kg – Kilogram                                                    |                                                     |
| LAM – Lipoarabinomannan.                                         |                                                     |

The thesis entitled “*Exploration of Silver-Assisted Gold Catalysis for the Synthesis of Anticoagulant Heparin Pentasaccharide and a Heptasaccharide of Mycobacterium tuberculosis Cell Surface*” is divided into five chapters. Chapter 1 gives the introduction to glycoconjugates, glycosaminoglycans and in brief about Heparin’s antithrombotic activity, chemical structure, synthetic challenges, strategies and limitations. Chapter 2 describes the exploration of proposed strategies for the most challenging iduronic acid building block preparation. In chapter 3, the donor-acceptor study for stereoselective glycosidations on freshly prepared glucosamine (GlcN) and glucuronic acid (GlcA) building blocks was delineated. Chapter 4 communicates the synthetic strategies for anticoagulant heparin pentasaccharide enabling regiodefined sulfate derivatives by silver-assisted gold catalyzed glycosidations. In chapter 5, the branched heptasaccharide subunit of arabinogalactan present in the *Mycobacterium tuberculosis* cell wall was described.

### Chapter 1: Introduction to Heparin and Fondaparinux

As a constituent member of major bio-macromolecules of the living organism, saccharides or carbohydrates play very vital role in cell surface recognition and information transfer. Carbohydrates bind to various biomolecules forming glycoconjugates such as glycoproteins, glycolipids, proteoglycans, etc. Glycosaminoglycans (GAGs) connected to a transmembrane protein core via a serine residue are collectively known as proteoglycans. The chain length of GAG can range from a hundred to several thousand repeating basic disaccharide unit having GlcN or GalN coupled with other sugar unit. Heparin is a well-known linear polysulfated polysaccharide with alternating D-glucosamine (GlcN) and either D-glucuronic acid (GlcA) or L-iduronic acid (IdoA) units of the glycosaminoglycan family.

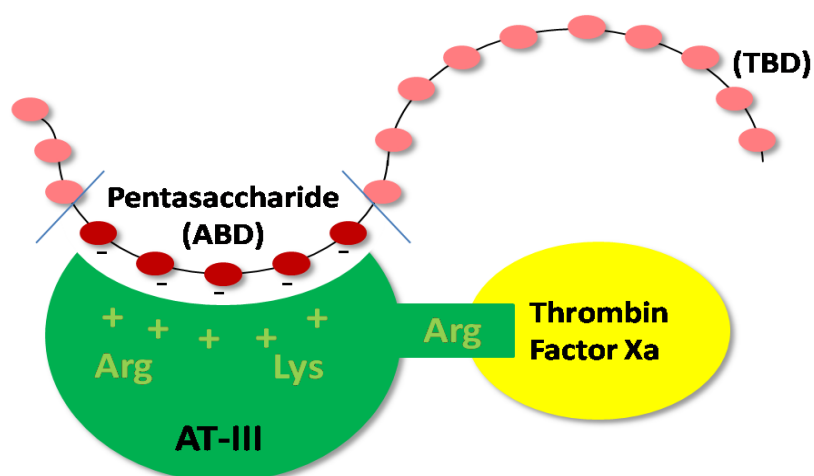


Figure 1: Ternary model for the structure-activity relationship of the anticoagulant activity.

(Ref. Petitou, M.; van Boeckel, C. A. A. *Angew. Chem. Int. Ed.* **2004**, 43, 3118-3133)

Since 1940, it has been used as an antithrombotic agent. In the early 1980s, a unique pentasaccharide domain present in heparin chains capable of activating antithrombin III was discovered. This domain is a serine protease inhibitor that blocks factor Xa in the coagulation cascade. However, isolation of the pentasaccharide from natural heparin by chemical modifications results in a complex mixture of many partially modified products due to incomplete reactions. Thus, the synthesis becomes crucial for pure and homogeneous heparin pentasaccharide. Two pharmaceutical companies, Sanofi and Organon synthesized an analog of this pentasaccharide, which was developed into a novel antithrombotic drug, fondaparinux sodium (Arixtra<sup>®</sup>) in 2002 for the treatment of deep-vein thrombosis. Subsequently, G. A. van der Marel, C-H Wong, S-C Hung, P. Wang, Y. Qin groups reported different routes for the synthesis of anticoagulant heparin pentasaccharide.

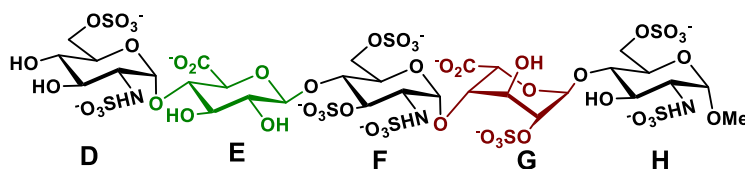


Figure 2: Fondaparinux pentasaccharide or structure of Arixtra<sup>®</sup>

Some of the most important synthetic challenges to be considered while developing a novel strategy for the synthesis of Fondaparinux are: i) preparation of orthogonally protected IduA building block, ii) stereoselectivity in glycosylations, iii) orthogonal protecting groups on pentasaccharide for effective deprotection and sulfation, and iv) number steps and purification issues. The synthesis strategies developed thus far for the heparin oligosaccharides can be categorized as linear synthesis or split react couple synthesis or selective activation or chemoselective glycosidations or latent-active glycosidations, etc. Still, there are exists some challenges in the synthesis of heparin oligosaccharide that need to be addressed; rapid and facile synthesis is in demand particularly for the anticoagulant heparin pentasaccharide (Fondaparinux) in multi kilograms.

## Chapter 2: Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

Iduronic acid (IdoA) (figure 3) is a L-sugar that can also be described as a C5 inverted glucuronic acid (GlcA). GlcA is commercially available; however, the single inversion at C5 disturbs its stability and as a result IdoA exhibits conformers such as <sup>1</sup>C<sub>4</sub>, <sup>4</sup>C<sub>1</sub>, <sup>2</sup>S<sub>0</sub>, etc. in solution. These limitations warrant development of strategies for orthogonally protected iduronic acid derivatives.

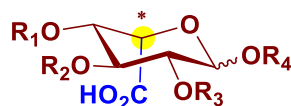


Figure 3: Structure of the iduronic acid (IdoA)

Number of synthetic routes have been developed for iduronic acid building blocks. Most of the strategies utilized glucofuranose having 5,6-diol (**1**) to synthesize L-idopyranose via 5,6-anhydro intermediate **2** by deploying protecting group modifications. Alternately, the oxidation of 5,6-diol **1** was transformed to aldehyde **3** that was used for the nucleophilic addition groups that can act as the carboxylic acid surrogate such as  $-\text{CN}$ ,  $(\text{PhS})_3\text{C}$ , etc to achieve iduronic acid derivatives (Figure 4). Iduronic acid building block remains as one of the major bottlenecks in the heparin pentasaccharide synthesis as it demands 12-16 synthetic steps. Also, the low yield, high-risk reaction conditions, and scale-up shall also be considered while developing a new synthesis route.

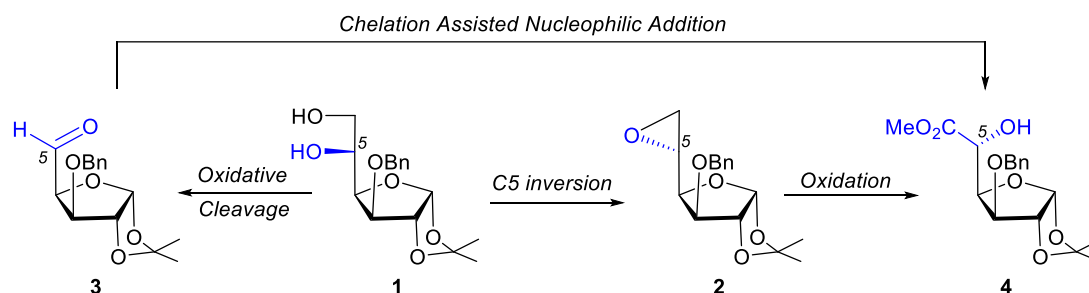


Figure 4: Key reactions that most of the early approaches employed for IdoA synthesis

Accordingly, the synthesis of IdoA was envisioned from easily accessible GlcA **5** by creating 1,3-dicarboxylate at the C-5 position to ensure Krapcho-decarboxylation for the desired Iduronic acid derivative. However, a lot of attempts resulted in the isolation of the  $\beta$ -eliminated product **7** instead of the desired iduronic acid derivative. Later, C5-aldehyde **6** was considered and converted to the hydroxyl-aldehyde **8** under cross Cannizzaro conditions; but to our dismay, several attempts failed to produce 1,3-dicarboxylate from derivative **8**.

In parallel, compound **9** was prepared predominantly as L-Idose derivative from glucofuranoside *via* chelation controlled Grignard reaction on corresponding C5-aldehyde using phenyl magnesium bromide. Further, regioselective oxidation of the phenyl group using *in situ* generated ruthenium tetroxide ( $\text{RuO}_4$ ) from  $\text{RuCl}_3$  and  $\text{NaIO}_4$  followed by the esterification using  $\text{K}_2\text{CO}_3$  and  $\text{CH}_3\text{I}$  revealed that the desired IdoA is a per-*O*-acetylated methyl iduronate (IdoA) **11** rather than the desired 3-*O*-benzyl Iduronate.



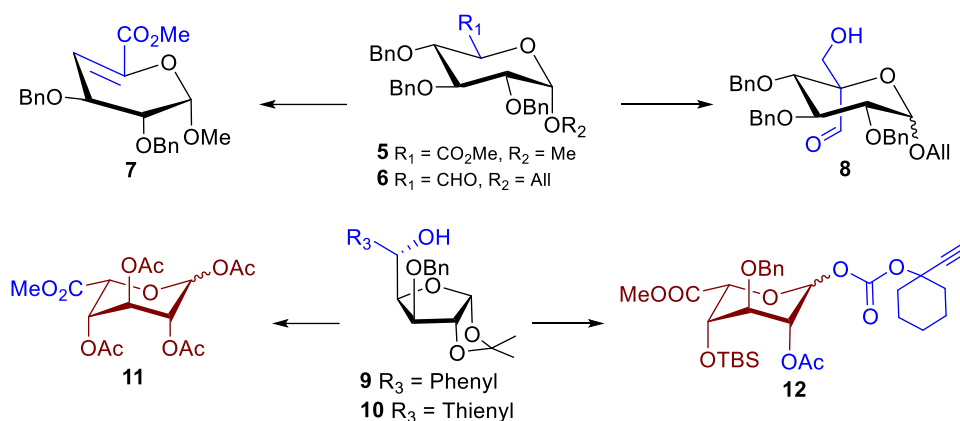


Figure 5: Summarized results from the strategies utilized for IdoA synthesis

However, the synthesis of heparin oligosaccharide warrants an orthogonally protected iduronic acid building block with 3-*O*-benzyl protection. Hence, thienyl moiety was considered as a carboxylate surrogate to afford idofuranoside **10** which underwent regioselective oxidation of the thienyl group under  $\text{RuCl}_3$  and  $\text{NaIO}_4$  in hexane:EtOAc: $\text{H}_2\text{O}$  solvent system to afford corresponding iduronic acid with the 3-*O*-benzyl protection. Finally, careful protecting group conversions enabled us to accomplish iduronic acid donor **12**.

### Chapter 3: Donor-Acceptor Selectivity Studies with GlcA and GlcN Building Blocks

The *cis* or  $\alpha$ -glycosidation is yet another major challenge in the heparin synthesis. We envisioned and synthesized a thoughtfully designed orthogonally protected glucosamine (GlcN) and glucuronic acid (GlcA) derivatives for studying donor-acceptor selectivities keeping in view of the stereoselectivity and the ease of purification in mind. Hence, a diversified set of GlcA acceptors (**14a-14c**) was prepared from easily accessible allyl 4,6-*O*-benzylidene-3-*O*-benzyl glucopyranoside **13** in three simple steps. The lone hydroxyl was protected as either levulinate or acetate or benzoate under standard conditions, benzylidene hydrolysis followed by oxidation of the C6-hydroxyl moiety and base mediated methyl ester preparation afforded acceptors (**14a-14c**). In continuation, orthogonal protections to remaining hydroxyl group at the C4 position, unmasking of the C-1 position using  $\text{PdCl}_2$  and carbonate preparation produced donors (**15a-15c**).

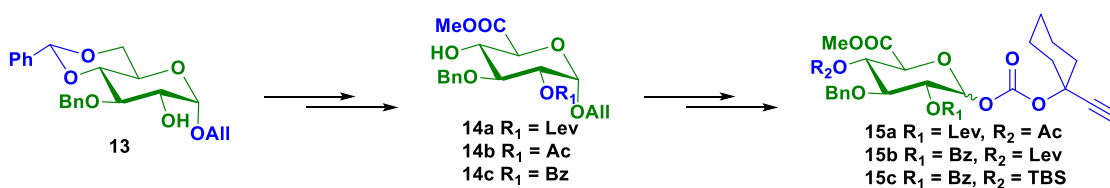


Figure 6: Synthesis of the GlcA building blocks

In parallel, Troc-protected allyl glucosamine **16** was converted to the intermediate **17** in three steps *viz.* benzylidene protection using benzylidenedimethylacetal, Troc-deprotection and azide preparation. Importantly, till intermediate **17** the steps were conducted without any column chromatographic purifications. At this point, a series of protections on the lone hydroxyl group produced **18a-18e** in order to explore orthogonal protections/deprotections at subsequent stages. Further, benzylidene deprotection under acidic conditions, regioselective protection of the C6-OH was performed to get desired aglycons **19a-19g** in excellent yields. Further, protections-deprotections employing either acid or base, allyl deprotection, and carbonate preparation were performed to accomplish desired donors **20a-20e**.

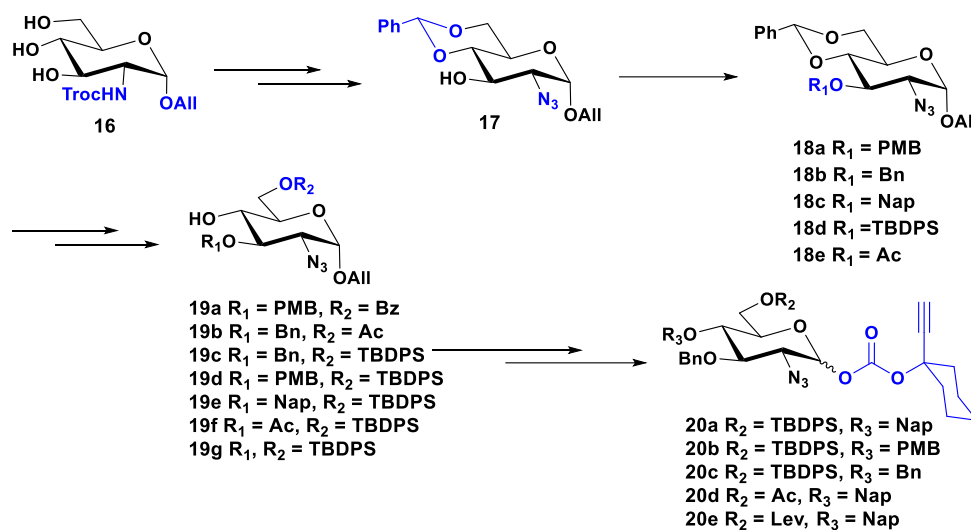


Figure 7: Synthesis of the GlcN building blocks

Donor-acceptor selectivity study was conducted after developing protocols and synthesizing all the identified building blocks in enough quantities. All glycosidations between various orthogonal combinations of the azido-glucosyl donors (**20a-20e**) and acceptors (**14a-14c**) were conducted employing Au-phosphite and AgOTf (8-15 mol% each) in CH<sub>2</sub>Cl<sub>2</sub> containing 4Å MS powder to afford the corresponding disaccharides **21a-21j**. Our explorations revealed that the disaccharide anomers produced with around 5:1 ( $\alpha$ : $\beta$ ) ratio when glycosidation reactions were performed at ambient temperature. Moreover, 1,2-*cis* or  $\alpha$ - linked disaccharide and the unwanted  $\beta$ -isomer are noticed to have similar polarities in most of the cases and hence, demanded multiple column chromatographic separations thereby compromising the overall process efficiency.

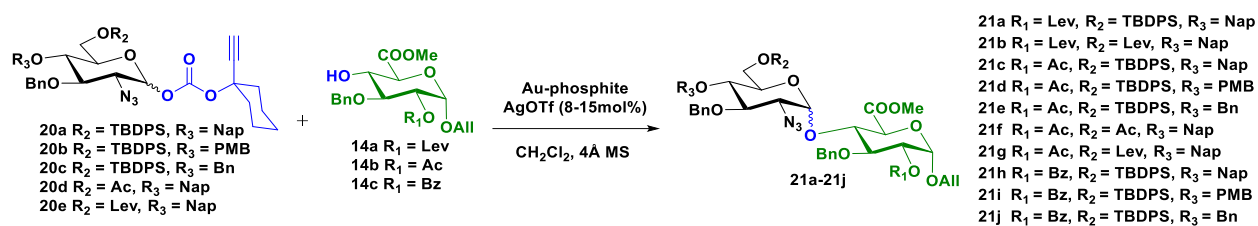


Figure 8: Glycosidations for reciprocal donor-acceptor selectivity study

However, among these, two pairs (**20a+14c** and **20c+14c**) were carefully selected as they gave better anomeric ( $\alpha:\beta$ ) ratio with good  $R_f$  difference. These glycosidations were studied further by lowering the reaction temperature to  $-20\text{ }^\circ\text{C}$  and next to  $-40\text{ }^\circ\text{C}$ . Surprisingly, the  $\alpha$ -stereoselectivity got improved to 20:1 when the reaction between **20c** and **14c** was conducted at  $-40\text{ }^\circ\text{C}$  to produce the disaccharide **21j** that could be easily purified as well under standard silica gel column chromatographic conditions using petroleum ether and ethyl acetate. Further reduction in the reaction temperature was not possible due to the inherent limitation of the gold-catalyzed glycosidation conditions.

#### Chapter 4: Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

The synthesis of Fondaparinux pentasaccharide was targeted by employing our recently discovered silver-assisted gold catalysed glycosidations on strategically synthesized monosaccharide building blocks described in Chapters 2 and 3. The synthesis of Fondaparinux was envisioned from the regioselectively protected pentasaccharide **22**. Trisaccharide **23** was chosen as the key precursor for effecting either 3+2 glycosylation using disaccharide **24** or 3+1+1 elongation by coupling iduronate **25** followed by the glycosidation of the azido-derivative **19b**.

Disaccharide **21j** prepared *vide supra* was conveniently converted to the corresponding carbonate under aforementioned reaction conditions, further subjected to the glycosylation with GlcN acceptors **19d-19g** for synthesizing D-E-F trisaccharide **23**. Several reactions were conducted to obtain the desired DEF trisaccharide; however, all attempts failed and resulted in the isolation of the 1,2-eliminated product (glycal-type derivative).

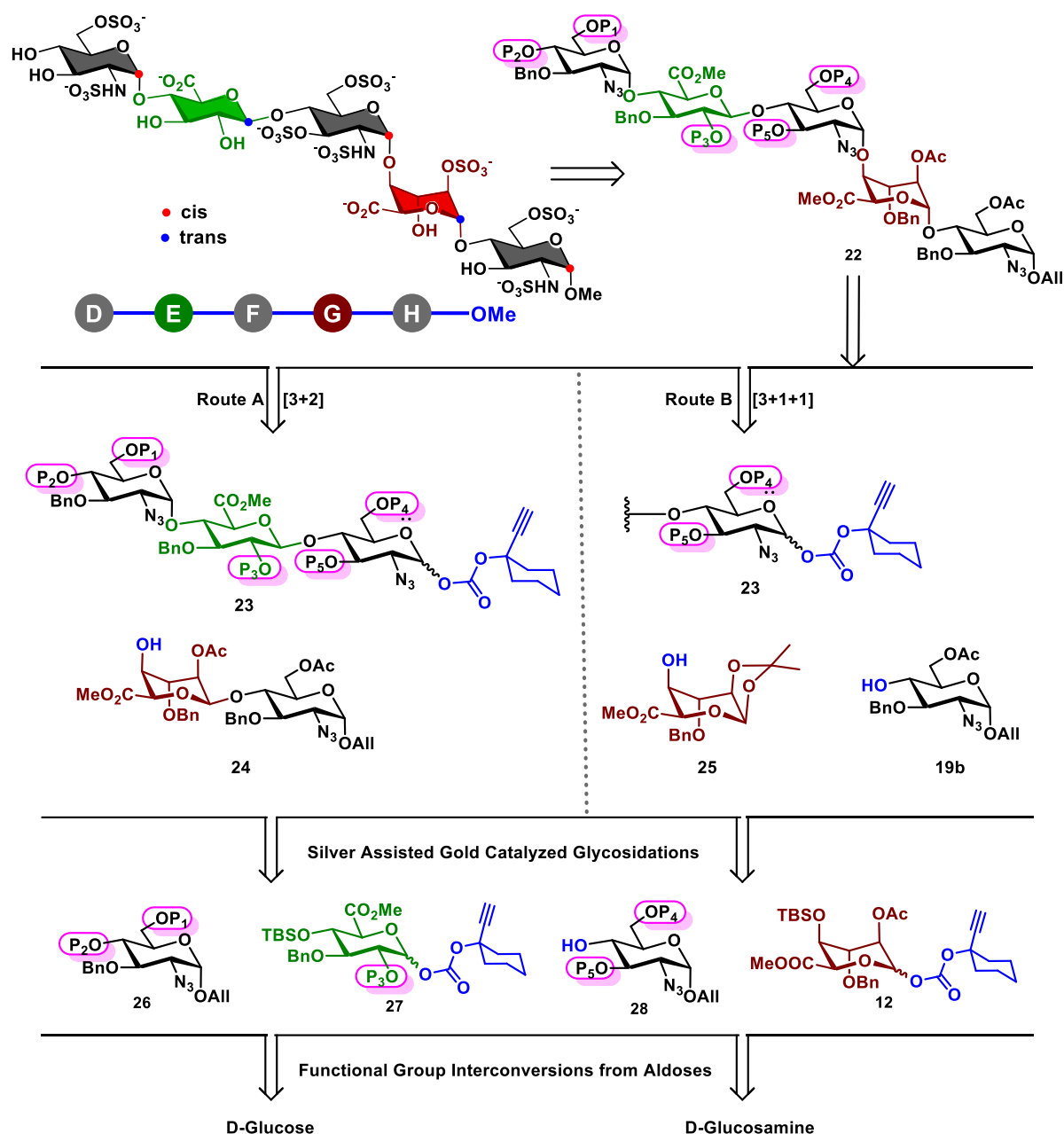


Figure 9: Retrosynthesis of the anticoagulant Fondaparinux pentasaccharide

Hence, the focus shifted to the optimization of EF disaccharide (utilizing various designer monosaccharide donor-acceptor combinations) that demands identification of difficult GlcA glycosidation and discovered that **15c+19a** is the right pair. Subsequently, resulting disaccharide was converted to the silyl deprotected acceptor **29** and glycosidation with donor **20c** at  $-40\text{ }^{\circ}\text{C}$  under standard gold-catalyzed conditions afforded a trisaccharide that was converted to the carbonate donor via hemiacetal intermediate to afford D-E-F trisaccharide **30**. Next in the odyssey is the synthesis of G-H disaccharide acceptor **24** which was easily synthesized by glycosidation between the donor **12** and **19b** followed by the cleavage of the silyl ether. Finally, glycosidation of donor **30** with the acceptor **24** at  $-10\text{ }^{\circ}\text{C}$  afforded the

regiodefined Fondaparinux pentasaccharide **32a** exclusively with desired  $\alpha$ -selectivity in excellent yield.

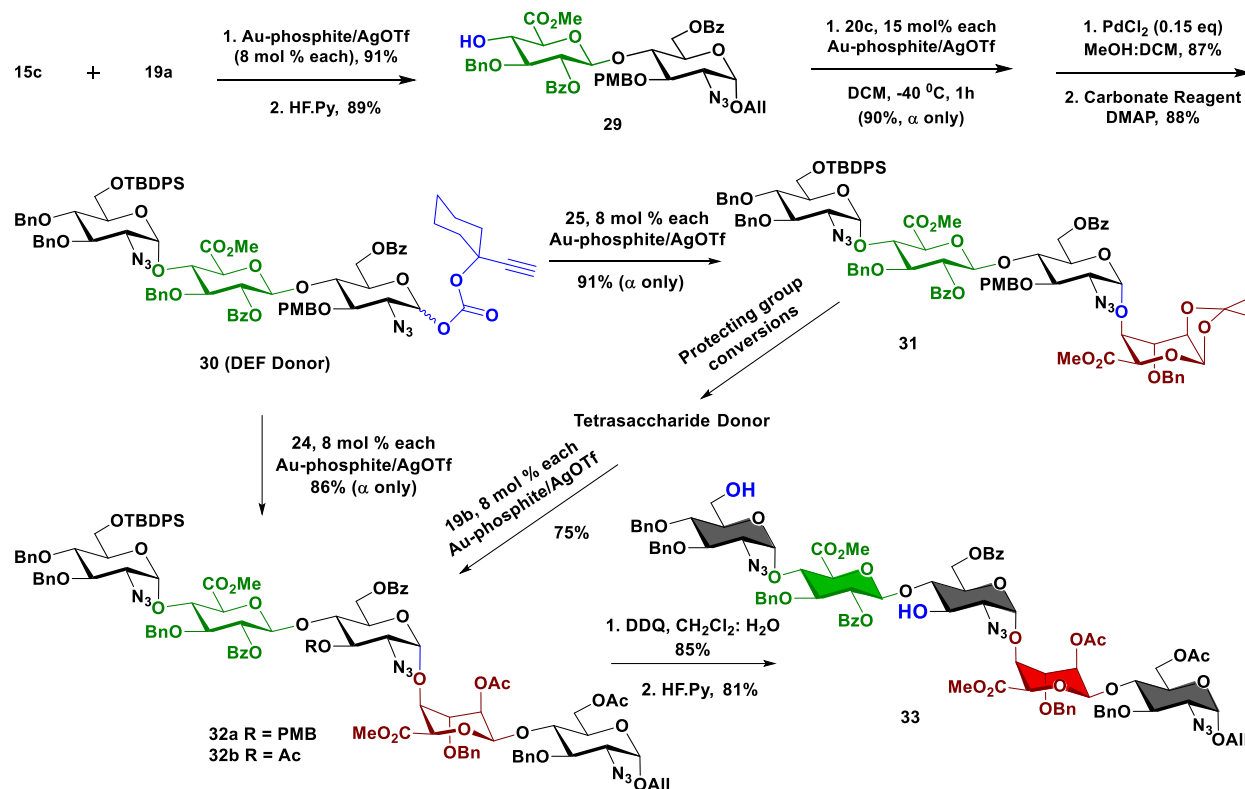
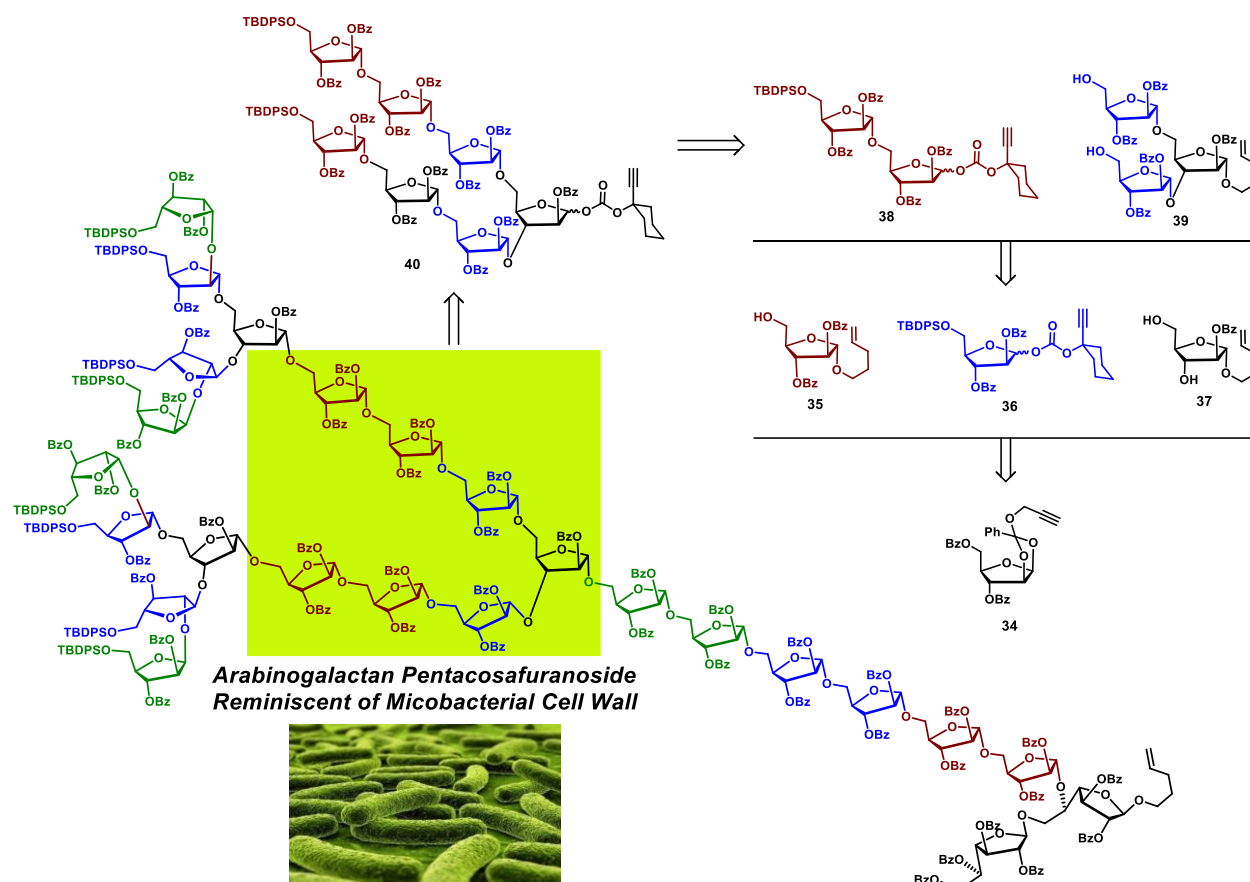


Figure 10: Synthesis of the regiodefined fondaparinux pentasaccharide

In addition, trisaccharide **30** was also glycosidated with IdoA **25** under silver-assisted gold catalysis to obtain exclusive  $\alpha$ -selective tetrasaccharide **31** at 25 °C presumably due to the presence of 1,2-*O*-isopropylidene locking present in the designer aglycon **25**. Further, isopropylidene moiety and PMB group were deprotected using 75% dichloroacetic acid and the regioselective carbonate donor preparation afforded the tetrasaccharide donor that was again coupled with **19b** to accomplish the desired pentasaccharide *via* 3+1+1 strategy. To our satisfaction, all glycosidations were optimized to give desired stereoselectivity and were conducted under silver-assisted gold catalysis to give orthogonally protected Fondaparinux pentasaccharides in excellent yields. Further extrapolation to the therapeutically relevant Fondaparinux is well established by other practitioners of the art and hence, the formal synthesis of Fondaparinux is accomplished in this endeavour. Importantly, the partial deprotection of the pentasaccharide **32a** produced regioisomeric hydroxyls which will enable regioselective sulfation to ensue studies of their biological properties. Cleavage of PMB using DDQ resulted in the pentasaccharide with lone 3-OH on the F-ring which is hitherto very difficult to obtain.

## Chapter 5: Synthesis of Branched Arabinan Heptasaccharide *Mycobacterium tuberculosis* cell surface

As part of the long term studies on the synthesis of giant Arabinogalactan oligosaccharide portion of *Mycobacterium tuberculosis*, a heptasaccharide-donor **40** was required in large quantity for obtaining a pentacosafuranoside moiety. All the interglycosidic linkages are 1,2-*trans* nature and hence invoked the neighboring group assistance by strategically placing a benzoate at the C-2 position. In addition, furanosidations shall be carried out under our group developed silver-assisted gold-catalyzed glycosidations protocol for expanding the scope.



*Figure 11: Retrosynthesis of the branched Arabinan heptasaccharide reminiscent of MTb AG*

Retrosynthesis revealed that propargyl orthoester **34** is suitable for the synthesis of acceptors **35**, **37** and donor **36** via protecting group conversions. Arabinofuranosidation between donor **36** and acceptor **35** resulted in a disaccharide that was converted to the corresponding carbonate donor **38** in two steps via hydrolysis of the pentenyl moiety under NIS/TfOH conditions followed by the treatment with the carbonate reagent. Glycosidation between the donor **36** and diol **37** under silver-assisted gold catalysis followed by the cleavage of silyl ether under

## *Synopsis*

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fluoride ion-mediated reaction afforded trisaccharide **39** in excellent yield. Final coupling of two molar equivalents of the donor **38** with diol **39** was accomplished to obtain the desired heptasaccharide that was further converted to the carbonate donor **40** by aforementioned synthetic sequence. Finally, heptasaccharide donor was utilized in the total synthesis of pentacosafuranoside.

## **Chapter 1**

# ***Introduction to Glycoconjugates, Heparin and Fondaparinux***

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## 1.1 Carbohydrates:

These are optically active polyhydroxy aldehydes or ketones with general formula of  $C_x(H_2O)_y$  which produce units of such type upon hydrolysis. Mostly occur in living tissues and foods in the form of starch, cellulose, and sugars (Greek word saccharides).

Sugars are predominantly found as hexoses and pentoses in natural systems. Hexoses such as glucose contain five chiral centers and five hydroxyl groups (one  $1^\circ$ , four  $2^\circ$ ); whereas pentoses such as ribose contain four chiral centers and four hydroxyl groups (one  $1^\circ$ , three  $2^\circ$ ). Sugars are named depending on the stereochemical configuration of these chiral centers and they often exist in thermodynamically more stable pyranose and furanose hemiacetals (Figure 1.01), although in an aqueous medium it opens up and reshapes into various ring forms;  $\alpha$ - or  $\beta$ - anomers depends on the absolute configuration of the anomeric center and anomeric reference. Anomeric reference is often the farthest chiral carbon center of the sugar (e.g. C5 for pyranose).

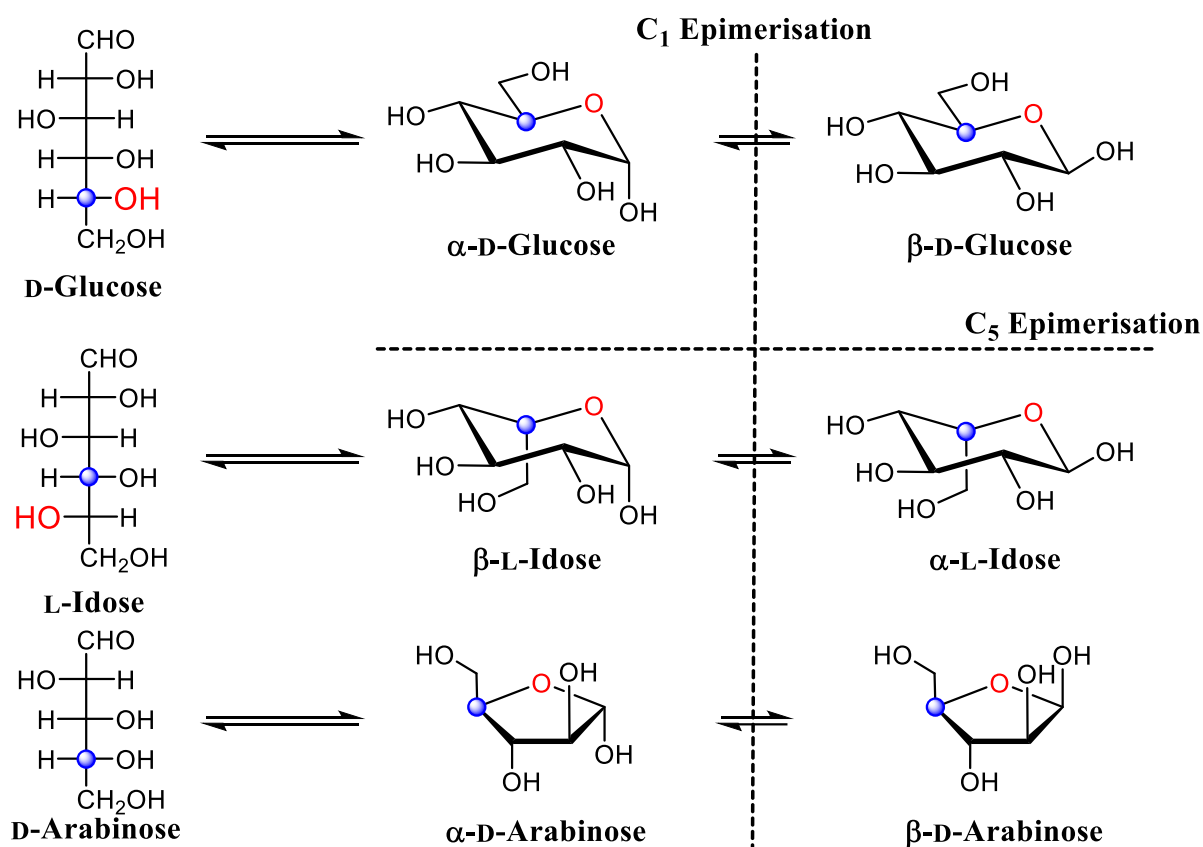


Figure 1.01– Equilibration between cyclic and open-chain forms and the  $\alpha$ - $\beta$  anomeric designation relative to the anomeric reference atom of hexoses and pentoses.

If the absolute configuration of the anomeric center and the reference is identical i.e. R, R or S, S then it is designated as an  $\alpha$ -anomer and if it is different, then it is known as a

$\beta$ -anomer. Anomeric reference also determines the D- or L- configuration. For a sugar drawn in Fischer projection with the most oxidized carbon at the top side then if the  $-\text{OH}$  of the anomeric reference points to the right, then the sugar is D while if the  $-\text{OH}$  is to the left then the sugar is L.

Usually, pyranoses exist in chair configuration closely similar to that of cyclohexane, nevertheless because of the endocyclic oxygen of the sugar ring many other conformers are distinguishable from one another. Apart from well-known two chair conformers ( ${}^1C_4$  and  ${}^4C_1$ ); 12 half chairs, 12 envelopes, 6 boats and 6 skew boats are noticed by IUPAC for pyranosides (Figure 1.02).

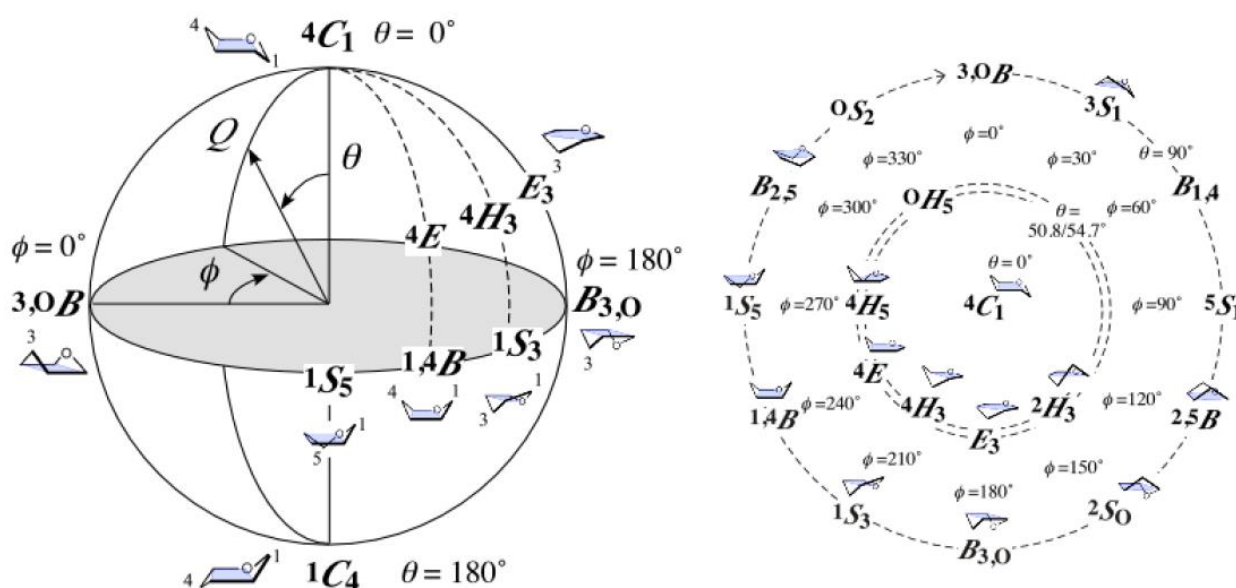


Figure 1.02 – This spherical polar representation of pyranoid ring conformation, meridian angle  $\Phi$ , azimuthal angle  $\theta$ , and radius  $Q$  are used to define each structure, with radius describing the degree of puckering away from a flat 6 membered ring ( $Q=0$ ).<sup>1</sup>

Carbohydrates are further classified as monosaccharide, disaccharide, and polysaccharides. Polysaccharides are routinely found as linear/branched polymeric chains that are conjugated to the other biomolecules such as proteins, peptides, lipids, saccharides and therefore are categorized as glycoproteins, glycopeptides, peptidoglycans, proteoglycans, glycolipids, lipopolysaccharides, and glycosides. These glycoconjugate markers often found in the extracellular matrix, hook up to the phospholipid bilayers of the cell membrane.

### 1.2 Glycoconjugates:

Usually cell surfaces are rich in carbohydrates which are essential because of their crucial role in cellular recognition and information transfer that involves cell-cell interactions, cell-matrix



## Introduction to Glycoconjugates, Heparin and Fondaparinux

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characteristic folding of the protein into its more active ternary structure. Carbohydrates can be simple sugars e.g. glucose, galactose, mannose, xylose; amino sugars e.g. *N*-acetyl glucosamine, *N*-acetyl galactosamine; or acidic sugars e.g. sialic acid, *N*-acetyl neuraminic acid. In *O*-linked glycoproteins, glycans are attached to the proteins either through threonine/serine or sometimes proline within the Golgi complex; whereas, the *N*-linked glycoprotein binds to asparagine, carrying sugars from endoplasmic reticulum membrane to the Golgi complex for modification (Figure 1.04). Glycoproteins are found on the surface of lipid bilayer, function in the physiological environment for cell-cell recognition, binding to other molecules and adds strength and stability to tissue by cross-linking with cells and proteins (e.g. collagen).

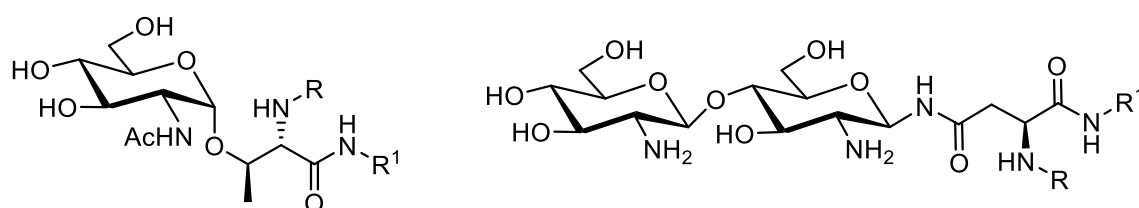


Figure 1.04 – Glycosyl domains can attach through both *O*-linking and *N*-linking.

The surface antibodies (immunoglobulins) of B cells and T cells induce immune response and help in recognizing the right antigen. Lewis group antigens (Le<sup>a</sup> and Le<sup>b</sup>), mucins (Tn, TF, STn), ABO blood compatibility antigens (H-antigen), etc. allow the immune system to set the limits between endogenous and exogenous cellular material. A unimolecular pentavalent KLH-carrier protein conjugate containing Globo-H, GM2, Tn, Tf and STn has brought a new dimension to the anti-cancer treatment (Figure 1.05).<sup>5</sup>

Mucins are yet another class of glycoproteins found in the mucus of the respiratory and digestive tract possessing 5 to 15 monosaccharides<sup>6</sup> giving a considerable water-holding capacity to resist proteolysis by digestive enzymes. As a result, mucins protect sensitive epithelial surfaces including the respiratory, urinary, digestive, and reproductive tracts.<sup>7</sup>

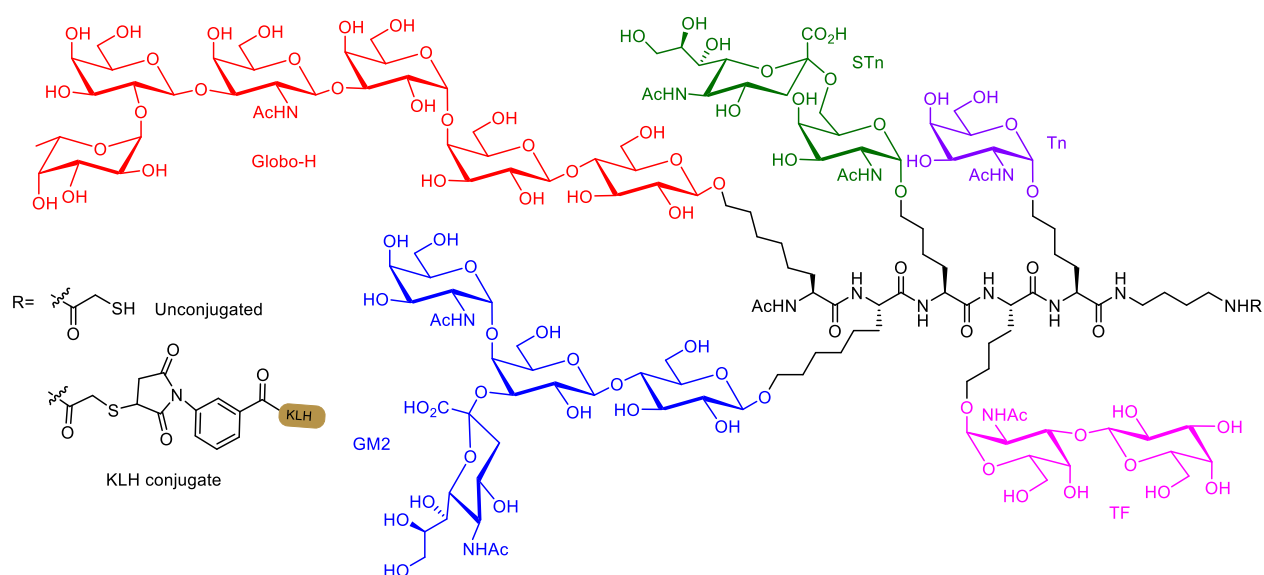


Figure 1.05 – Structure of the unimolecular pentavalent KLH-conjugate containing five TACAs

Unlike DNA, enzymatic addition/removal of carbohydrates to a protein does not have any checking and correction mechanism that interferes in mutations to glycome, results in diverse glycoconjugates known as glycoforms, each has its own characteristic properties. This micro-heterogeneity acts as a fine-tuning mechanism for the biological response.<sup>8</sup>

## 1.2.3 Proteoglycans:

Proteoglycans (PG) are one of the most important backbone units of the glycocalyx that differ from glycoproteins by some of the characteristic post-translationally modified linear polysaccharide chains called glycosaminoglycans (GAGs). GAG chains are negatively charged species such as heparin/heparan sulfate (H/HS), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS). GAGs immerse 50-60% part (95% weight) of PG and they have a common tetrasaccharide composed of glucuronic acid (GlcA), galactose (Gal) and xylose (Xyl) which is first conjugated to a serine of the core protein in the Golgi. The sequence GlcA-Gal-Gal-Xyl-O-Ser-Gly-Asp acts as a handle for further chain elongation (Figure 1.06).<sup>9</sup>

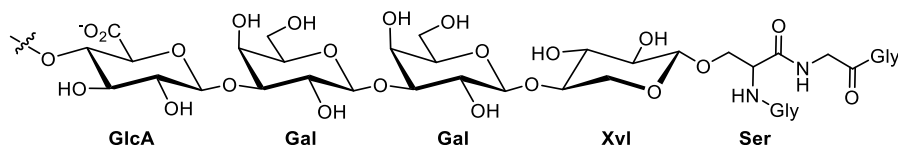


Figure 1.06 – Structure of the Xyl-Gal-Gal-GlcA linked to proteins via a serine residue.<sup>10</sup>

PGs are extremely heterogeneous macromolecules that can be classified by the type of GAG chain decorating the protein core or by their tissue location (intracellular, pericellular, cell

## Introduction to Glycoconjugates, Heparin and Fondaparinux

surface-associated and extracellular).<sup>11</sup> They are further subcategorized according to their gene homology, structural and functional properties, and modular arrangement (figure 1.07).<sup>12</sup>

| Location             | Proteoglycan                                                                                                     | GAG                  | Proteoglycan functions                                                                            |
|----------------------|------------------------------------------------------------------------------------------------------------------|----------------------|---------------------------------------------------------------------------------------------------|
| <b>Intracellular</b> | Serglycin                                                                                                        | CS/DS/<br>HS/Heparin | ✓ Binding of growth factors, cytokines, chemokines, morphogens and protection against proteolysis |
| <b>Cell Surface</b>  | Betaglycan                                                                                                       | CS/HS                |                                                                                                   |
|                      | Syndecans 1-4, Glypicans 1-6                                                                                     | HS                   |                                                                                                   |
|                      | CSPG4 (NG2), Phosphacan                                                                                          | CS                   | ✓ Formation of morphogen gradients                                                                |
| <b>Pericellular</b>  | Perlecan                                                                                                         | HS/CS/KS             |                                                                                                   |
|                      | Collagen XV                                                                                                      | CS/HS                | ✓ Co-receptors for growth factor signaling                                                        |
|                      | Collagen XVIII, Agrin                                                                                            | HS                   |                                                                                                   |
| <b>Extracellular</b> | Aggrecan, Lubricin                                                                                               | CS/KS                | ✓ Extracellular matrix (ECM) assembly                                                             |
|                      | Epiphygan                                                                                                        | DS/CS                | ✓ Basement membrane assembly                                                                      |
|                      | Versican, Neurocan, Brevican, Biglycan                                                                           | CS                   |                                                                                                   |
|                      | Fibromodulin, Lumican, Keratocan, Osteoadherin                                                                   | KS                   | ✓ Direct cell interactions or modulation of cell interactions with other ECM molecules            |
|                      | Decorin                                                                                                          | DS                   | ✓ Biophysical roles in tissue hydration, compression and lubrication                              |
|                      | Testicans 1-3                                                                                                    | HS                   |                                                                                                   |
|                      | ECM2, ECMX, PRELP, Opticin, Osteoglycin, Asporin, Chondroadherin, Nyctalopin, Podocan, Podocan-like 1, Tsukushi, |                      |                                                                                                   |
|                      |                                                                                                                  |                      | ✓ Packaging granular contents in secretory vesicles                                               |

Figure 1.07. Proteoglycan classification based on their location, showing their pendant GAG chains, their major function, and potential biomedical applications.<sup>11</sup>

Initially, it was envisioned that each proteoglycan has a specific type of GAG chain; however, later studies observed that the single protein core can bind to many types of GAG chains.<sup>11</sup> Typically perlecan is known for exclusively HS linked PG derived in the epithelial cells, however, CS and KS decorated perlecan protein core is also observed in chondrocytes, smooth muscle cells, and epithelial cells.<sup>13</sup> Another example is HS, CS, and DS decorated serglycin can be derived in connective tissue-resident mast cells along with other cells,<sup>14</sup> although heparin linked serglycin is only known from the connective tissue-resident mast cells.

## Introduction to Glycoconjugates, Heparin and Fondaparinux

GAG chains are important for extracellular matrix assemblies such as collagen, elastin and laminin polymerization<sup>15</sup> and also responsible for their physical properties including tissue hydration, compression, and lubrication.<sup>16</sup> GAGs with a protein core (PG) plays an additional role in cell adhesion, fibrillogenesis, growth factor (FGF7) signaling, etc.<sup>17</sup>

### 1.3 Glycosaminoglycans:

GAGs are linear polymeric chains of the hundred to several thousand repeating disaccharide units (10–50 kDa) composed of hexosamine and hexose/hexauronic acid sugars (Figure 1.08).

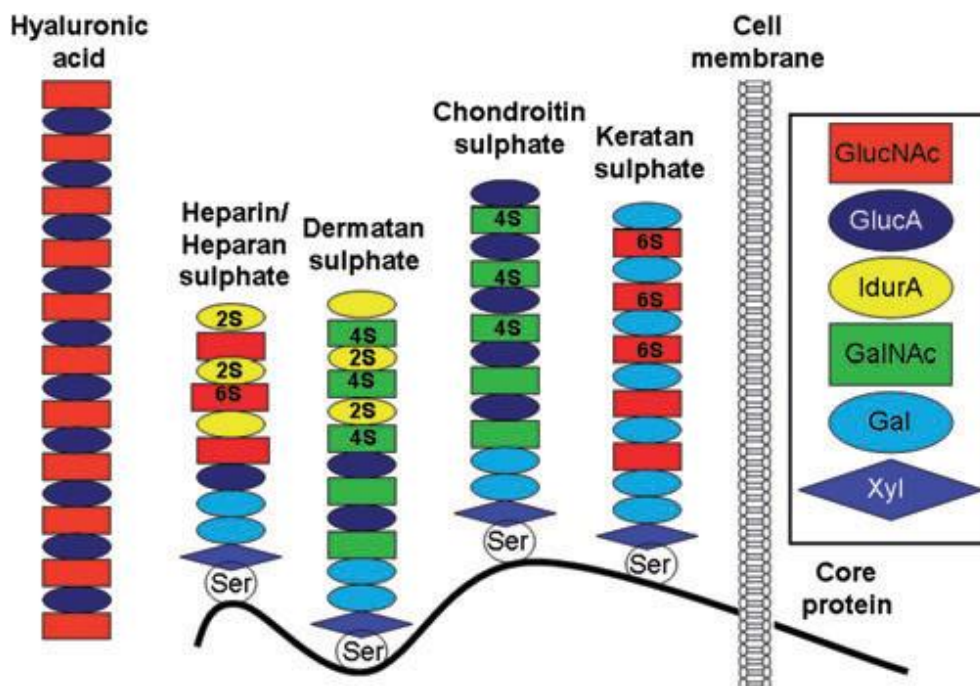


Figure 1.08: Schematic representation of glycosaminoglycans.<sup>22</sup>

It can produce up to  $2 \times 10^{90}$  possible different chain structures<sup>18</sup> because of the high degree of heterogeneity with regards to disaccharide construction, molecular weight, sulfation pattern etc.<sup>19</sup> and often categorized in five different types<sup>20</sup> containing hyaluronic acid (HA), keratan sulfate (KS), chondroitin sulfate (CS), dermatan sulfate (DS), heparin (Hep) and heparan sulfate (HS).<sup>21</sup> With the exception of HA, GAGs are usually covalently attached to a protein core of PG in our body (Figure 1.08).

#### 1.3.1 Types:

##### 1.3.1.1 Hyaluronic Acids (HA):

In 1934, Karl Meyer and John Palmer first isolated HA from the vitreous body of cows' eyes.<sup>23</sup> Later study revealed that it is naturally synthesized on the inner face of the plasma membrane



by three integral membrane protein isoenzymes called HA synthases (HAS1–3)<sup>24</sup> that are localized as complexes within the plasma membrane. HA is the only GAG subgroup that lacks sulfate groups and has the repeating disaccharide linker  $\beta$ -(1 $\rightarrow$ 4)-D-glucuronic acid and  $\beta$ -(1 $\rightarrow$ 3)-D-N-acetyl glucosamine units (Figure 1.09). HA with higher molecular weight ( $2 \times 10^6$  Da) has been obtained as HAS1 and HAS2, whereas HA of lower size ( $2 \times 10^5$  Da) produced by HAS3.<sup>25</sup>

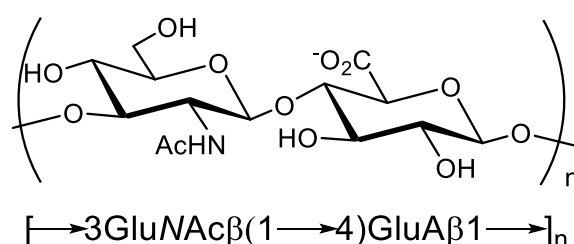


Figure 1.09: Repeating disaccharide unit of Hyaluronic acid

HA with a high concentration often gives rigidity to tissues because of their high viscosity ( $\sim 5000\times$  to that of water as a 10mg/mL solution in water)<sup>26</sup> and it has major implications in many biological activities including extracellular membrane formation, cell adhesion, and cell motility. Essential components of the extracellular matrix and connective tissue systems such as skin, synovial fluid in the joints, the vitreous gel of the eye, heart valves, lungs, and umbilical cord, etc. have this HA in abundance.<sup>27</sup> Further HA pathways are currently investigated as possible therapeutic targets for arresting tumor metastasis.<sup>28</sup>

## 1.3.1.2 Keratins (KS):

KS is a polymer of the disaccharide  $[\rightarrow 4\text{-GlcNAc}\beta(1\rightarrow 3)\text{Gal}\beta 1\rightarrow]$ <sup>29</sup> and the O-6 position of both Gal- and GlcNAc are sulfated by galactose 6-O-sulphotransferase (G6ST) and N-acetyl glucosamine 6-O-sulfotransferase respectively. Further, KS classified as KS-(I, II, III) based on the PG-dependent type of protein linkage (Figure 1.10).

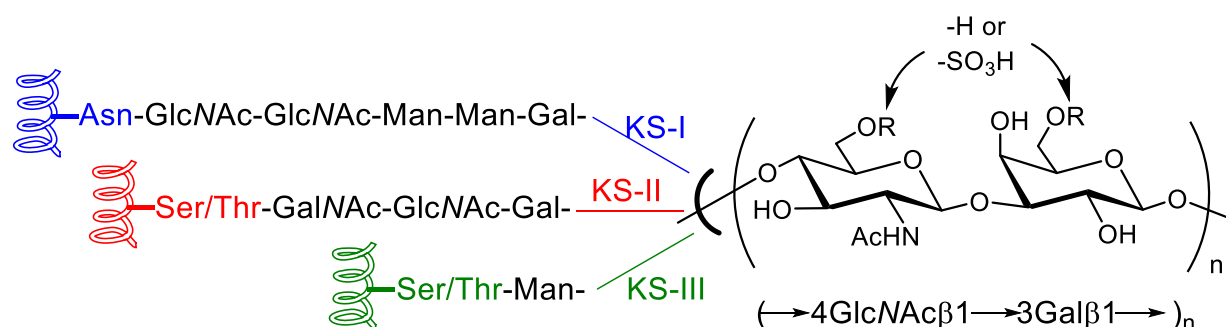


Figure 1.10: Generic chemicals structures of KSI, KSII, KSII.



KS-I is mainly found in the cornea, embryonic lung, and liver. It polymerizes into a complex oligosaccharide that is linked to the L-asparagine residue of the core protein whereas KS-II is located in bones, cartilage, and skin; and is attached to L-serine or L-threonine residue of mucin core-2 structure via GalNAc-O-glycosidic bond. KS-III was recently identified in brain tissues and it is also O-linked to L-serine or L-threonine residue of PG *via* mannose.<sup>25</sup>

### 1.3.1.3 Chondroitin/Dermatan sulfates (CS/DS):

Chondroitin sulfate (CS) is composed of repeating disaccharide units ( $\rightarrow 3\text{-GalNAc}\beta 1\rightarrow 4\text{-D-GlcA}\beta 1\rightarrow$ )<sup>25</sup> *via* sequential addition of GalNAc and GlcA to the non-reducing end of the growing strand. Numerous sulfotransferases are involved in the modification of the elongated chain with various sulfation patterns. The O-6 and O-4 positions of GalNAc and in some cases O-2 position of GlcA is sulfated to produce structurally diverse CS with the combination of eight differently modified disaccharides.<sup>30</sup>

Dermatan sulfate (DS) is the stereoisomeric variant of CS polysaccharide. DS epimerases 1-2 (DSE1-2) epimerize the C-5 position of the chondroitin-GlcA converting D-GlcA to L-IdoA to produce DS with ( $\rightarrow 3\text{-GalNAc}\beta 1\rightarrow 4\text{-IdoA}\alpha 1\rightarrow$ ) as a repeating disaccharide unit (Figure 1.11). Various conformations of the L-IdoA make DS chains more flexible, as a result, it allows specific interactions with many other molecules.<sup>31</sup> Similar to CS, eight different disaccharide units may occur in DS chains providing them with incredible structural diversity. Both CS and DS are highly sulfated in higher animals and act as ligands for proteins to mediate biological functions. Often CS present in human connective tissues and extracellular matrix, including cartilage, cornea, bone, skin, arterial walls and helps to furnish framework, gives elasticity to cartilage, for that CS has been used as a popular dietary supplement.

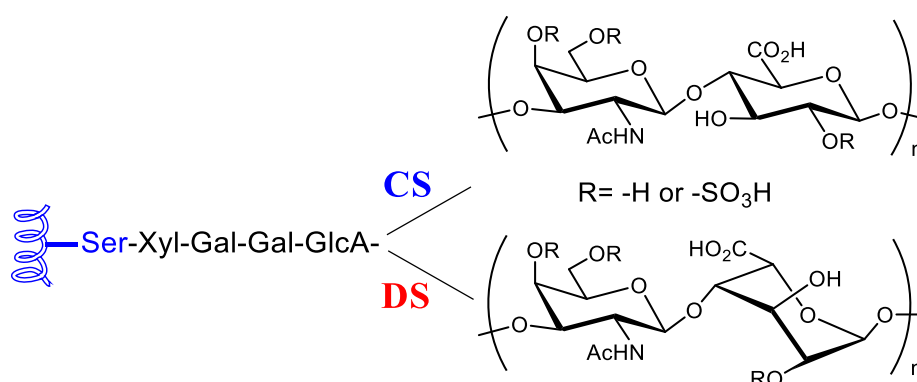


Figure 1.11: CS and DS repeating disaccharides with common tetrasaccharide linker

DS is predominantly present in skin, heart valve, tendons, and blood vessels. CS/DS interacts with growth factors, cytokines, chemokines, adhesion molecules and perform a role in key

cellular events during central nervous system development, inflammation, tumor progression, and wound healing.<sup>32</sup>

### 1.3.1.4 Heparan Sulfate/Heparin (HS/Hep):

HS/Hep chains are composed of the alternate addition of 4GlcA $\beta$ 1 and 4GlcNAc $\alpha$ 1 to the non-reducing end of the growing chain and the process catalyzed by EXTL1 and/or EXTL2 enzymes (glycosyltransferase enzymes). *N*-Deacetylase/*N*-sulfotransferases (NDST1-4) deacetylates GlcNAc and transfers the sulfate group to form GlcNS.<sup>33</sup> D-Glucuronyl C5-epimerase (GLCE) converts GlcA to IdoA, furthermore, HS 2-*O*-sulfotransferase 1 (HS2ST1) transfers sulfate group to *O*-2 of uronic acids to obtain IdoA2S and rarely, GlcA2S. HS 6-*O*-sulfotransferases (HS6ST1-3) catalyzes 6-*O*-sulfation whereas a variety of HS 3-*O*-sulfotransferases (HS3ST1-6) are involved in 3-*O*-sulfation of the GlcNAc to form GlcNAc6S and GlcNS6S, or rarely GlcNAc3S and GlcNS3S or GlcNAc3S6S and GlcNS3S6S.<sup>30</sup> These modifications result in  $\rightarrow 4\text{GlcNAc}1 \rightarrow 4\text{GlcA}\beta 1 \rightarrow$  (major in HS) and  $\rightarrow 4\text{GlcNAc}1 \rightarrow 4\text{IdoA}\beta 1 \rightarrow$  (major in Hep) as a repeating disaccharides with extremely high complexity (Figure 1.12).

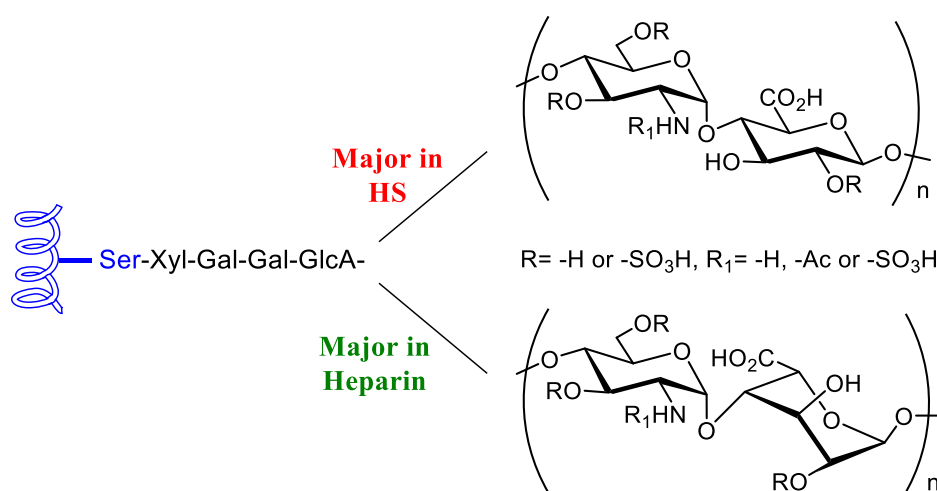


Figure 1.12: HS and Hep repeating disaccharides with common tetrasaccharide linker

Sulfate groups from specific sites can be removed by plasma membrane bound endo-sulfatases altering the overall structure and producing differentially sulfated disaccharides from the intact HS/Hep chains using Heparinases I, II, and III.<sup>34</sup> Each disaccharide unit can give 32(2<sup>6</sup>) possible modifications. HS and Hep both share the same backbone structure and modification process, however, they vary in epimerization and sulfation pattern. The chain modifications usually emerge in clusters, creating highly sulfated clusters with enhanced biological activity.

Consequently, typical HS/Hep shows an extremely complex and information-dense structure (Figure 1.13).

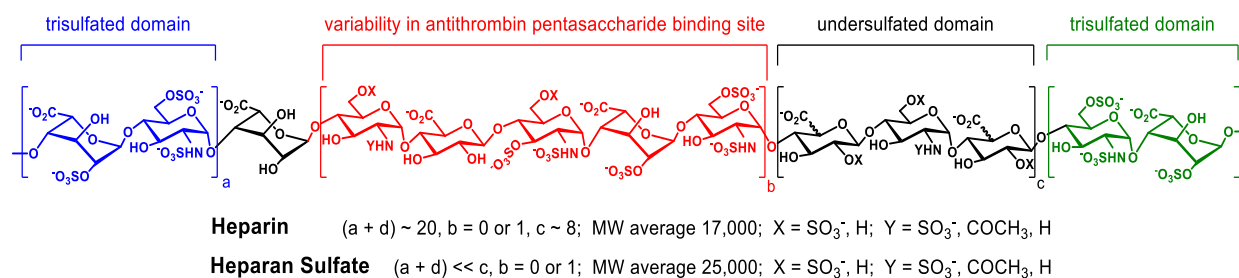


Figure 1.13. Typical HS/Hep chains and their complexity with heterogeneous levels of sulfation.

Heparin exhibits 70-90% uronic acids as GlcA, IdoA and uniformly 2.5 to 3 sulfates per disaccharide unit, depending on the source it is extracted from; whereas, HS has a relatively lower degree of epimerization and sulfation, mostly contains GlcA and exhibits 0.5–1.5 sulfates per disaccharide unit (~ one sulfate/DS).<sup>30</sup> Hep is more negatively charged, structurally complex relative to HS and the domains can be identified by the presence or absence of GlcNS residues. Largely unsulfated NA (*N*-acetylated) domain involves long chains of GlcNAc-containing disaccharides; whereas a highly sulfated NS (*N*-sulfated) domain involves in the shorter sequences of GlcNS-containing disaccharides, which are rich in trisulfated disaccharides of the structure GlcNS6S-IdoA2S disaccharide.<sup>35</sup> Heparin is a widely used anticoagulant drug, usually bound to the serglycin PG in the connective tissue mast cells. The rare pentasaccharide with 3-sulfate group observed in both heparin and HS is an important sequence acting as the antithrombin III (AT) binding site that activates AT which is responsible for the anticoagulant activity.<sup>30</sup>

### 1.3.2 GAG-Protein interaction (binding site):

The negatively charged carboxylate and sulfate groups of the GAGs known as GAG binding sites, often electrostatically interact with a number of different classes of proteins through the positively charged amino acid (AA) binding sites. It may alter the physiological processes like development, cell growth, hemostasis, and lipid transport/absorption. Often in proteins, the binding site includes arginine (Arg) and lysine (Lys) from  $\alpha$ -helices and  $\beta$ -strands interact through its positively charged residues with the negatively charged hexauronic acids and sulfate groups of GAGs. It may be involved in the regulation of enzymatic activity, protein oligomerization, and protection against proteolytic degradation as well as in the ligands-receptor binding in a significant manner. Additionally, it has been found that GAG chains can

interact with more than one ligand, for example, the existence of helical conformation in heparin-FGF interaction assures that GAG residue facing the protein AA interaction from one side whereas the residues from other sides of the helix able to interact with another protein.<sup>36</sup>

The GAG-protein binding has been identified often by affinity-based approaches including affinity chromatography, co-precipitation, fluorescence titration, and surface plasmon resonance. Further, the data regarding structural information has been obtained by X-ray crystallography and solution-state nuclear magnetic resonance (NMR).<sup>37</sup> The majority of GAG-binding proteins interact with Hep or HS (compared to other GAGs), due to its greater heterogeneity and the binding of Arg and Lys residues from antithrombin III.<sup>25</sup>

### **1.4 Heparin:**

#### **1.4.1 History:**

Three decades of intensive research on natural anticoagulants led to the identification of a carbohydrate molecule as the active principle rather than the lipid.<sup>38</sup>

Further, Howell in 1928 correctly identified one of the sugars in heparin to be a uronic acid,<sup>39</sup> and Jorpes and Bergstrom found glucosamine as the second sugar component.<sup>40</sup> Jorpes and later Charles established that heparin contained a high level of covalently linked sulfates making it one of the strongest acids in nature.<sup>41</sup> Subsequent studies also identified the 1→4-linkage between C-1 of glucosamine and C-4 of the uronic acid and the location of the O-sulfate groups.<sup>42</sup> After nearly one and half-decade, in 1950, Jorpes was able to show that the glucosamine in heparin is primarily *N*-sulfated. Wolfrom in 1946, initially identified D-glucuronic acid as heparin's uronic acid whereas Cinonelli and Al Dorfman reported the presence of L-iduronic acid in heparin. Further studies by Arthur Perlin in 1968, confirmed L-iduronic acid as the major uronic acid residue in heparin using NMR spectroscopy.<sup>43</sup> Finally, heparin becomes known as a sulfated, linear polysaccharide with a repeating disaccharide unit containing iduronic acid and glucosamine.

The next critical step obviously was to produce heparin with adequate purity and monitor its anticoagulant activity.<sup>44</sup>

#### **1.4.2 Preparation and Depolymerisation of Heparin**

##### **Preparation of unfractionated heparin (UFH) from animal sources**

The heterogeneous mixture of heparin fragments is known as unfractionated heparin which can be generally extracted from bovine and sheep lungs and porcine intestinal mucosa. The

methods of commercial production of pharmaceutical-grade UFH are well established even on large-scale preparation. The commercial processes typically used to prepare multi-kilogram batches of pharmaceutical-grade heparin are with five basic steps viz. tissue preparation, heparin extraction from tissues, raw heparin recovery, heparin purification, and purified heparin recovery.<sup>45</sup> After the collection of appropriate animal organs from the slaughterhouse, the processing begins for the preparation of tissues. After the protease treatment and heat deactivation, high salt fractions are separated by anion exchange method. Further treatments such as precipitation, bleaching with pH adjustment, cation exchange, filtrations produce heparin as a heterogeneous mixture of various fragments (Figure 1.15).

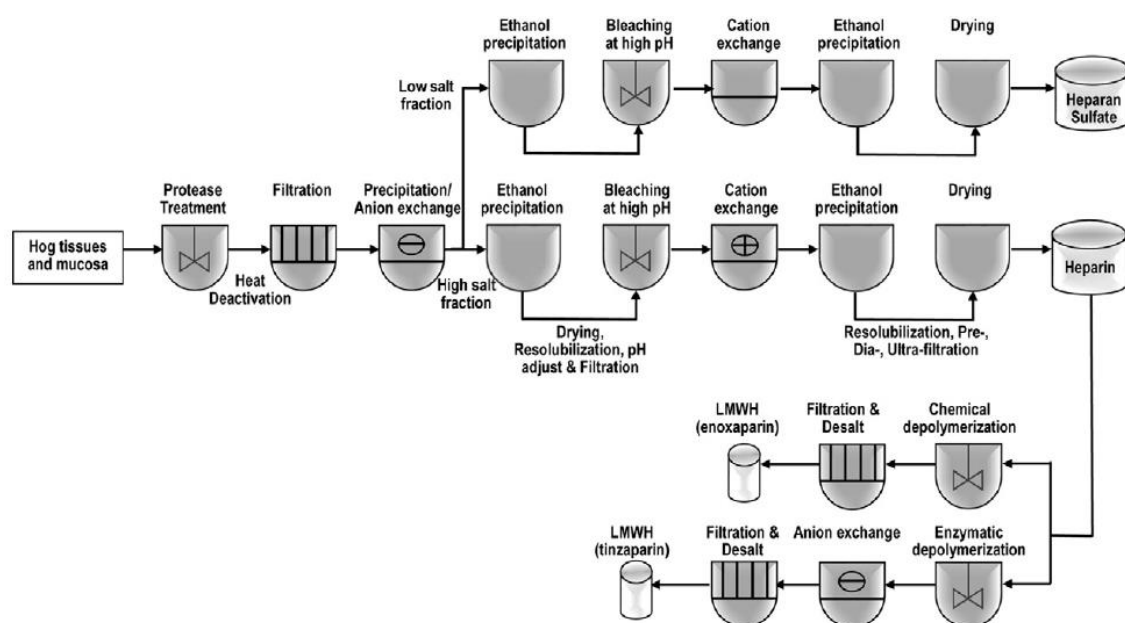


Figure 1.15: Extractive preparation of heparin, HS and LMWHs.

### Chemical and enzymatic depolymerization of UFH to prepare LMWHs

Fractionation of the UFH produces low molecular weight heparins (LMWHs) with a molecular weight ranging from ~3–8 kDa. LMWHs have an important role in a selective anti-FXa anticoagulant/antithrombotic activity.<sup>46</sup> Often the controlled chemical depolymerization method used to prepare LMWHs such as enoxaparin from animal-sourced UFH (Figure 1.16). The reactions such as peroxidative cleavage, nitrous acid cleavage, and chemical  $\beta$ -elimination are used in the chemical depolymerization.<sup>47</sup>

Enzymatic depolymerization is a much milder method to prepare LMWHs. As an example, heparin lyase isolated from *Flavobacterium heparinum* has been used to make LMWHs such as Tinzaparin (Figure 1.16). Most commonly used heparin lyase-I is highly selective for a linkage  $[\rightarrow 4)\text{-}\alpha\text{-D-GlcNS3S6S (1}\rightarrow 4)\text{-}\alpha\text{-L-IdoA2S (1}\rightarrow ]$  that is present in the AT-binding site.

The heparin lyase-II is more selective for  $[\rightarrow 4)\text{-}\alpha\text{-D-GlcNS6S (1}\rightarrow 4)\text{-4)-}\alpha\text{-L-IdoA (1}\rightarrow]$ , hence, it can be act as an alternative enzyme for the LMWH preparation.<sup>48</sup>

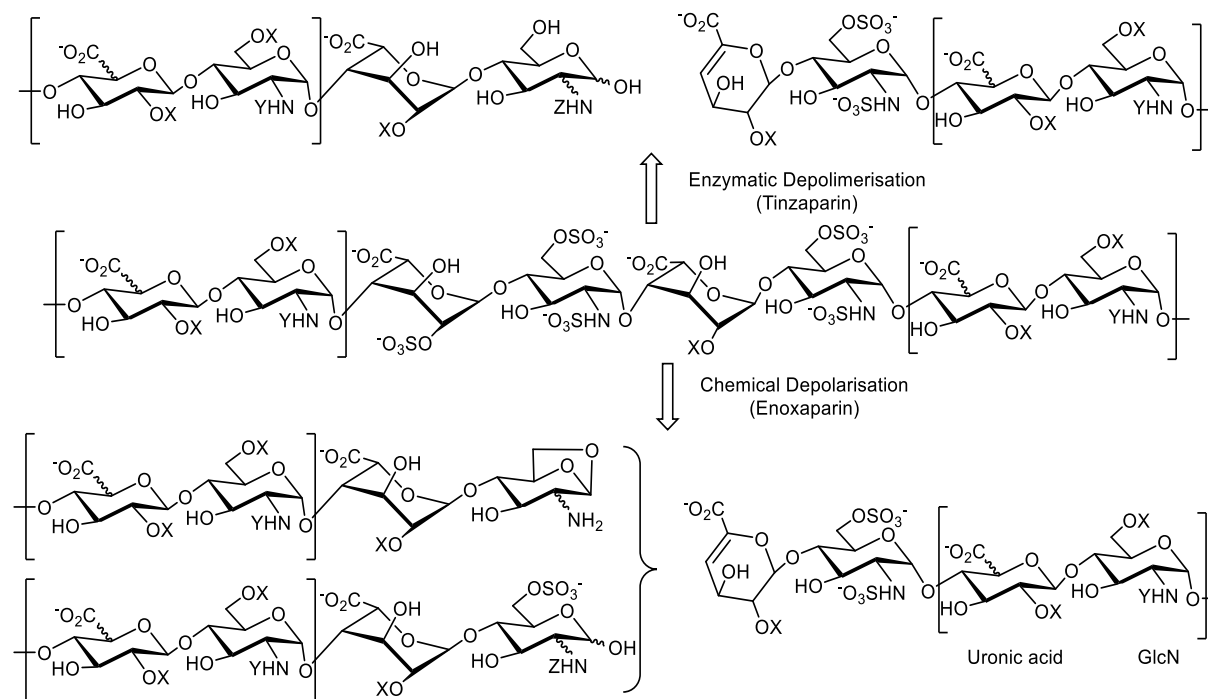


Figure 1.16: Chemical and enzymatic depolymerization methods to prepare LMWHs from UFH.

### Problems and Challenges Associate with Animal-sourced Heparins

Unexpectedly, in 2008, the heparin emergency manifested because of the reduction in the supply of heparin caused due to the contamination of the over sulfated chondroitin sulfate.<sup>49</sup> Supply of the animal is yet another major challenge<sup>50</sup> and sacrificing 1.2 billion pigs is required to produce ~100 tons of heparin per year. Even then the quantity is not sufficient due to the increased worldwide demand; thus production of non-animal sourced synthetic heparin is urgently demanded. On the contrary, LMWHs demand harsh chemical depolymerization conditions thereby causing side reactions;<sup>51</sup> besides, enzymatic depolymerization solves some of these problems, but chain length control, prohibitive cost of enzymes is the major bottleneck.

#### 1.4.3 Structure-activity Relationship Study and the Development of Fondaparinux:

In a major contribution to the antithrombotic field, it was suggested that heparin is an efficient anticoagulant in the presence of a plasma component and also the activity of AT was accelerated by heparin.<sup>52</sup> Abildgaard first time isolated the pure AT<sup>53</sup> and Rosenberg and his collaborators,<sup>54</sup> invented a procedure to prepare AT and performed an experiment by centrifuging a mixture of heparin and AT on a sucrose density gradient to notice that

## Introduction to Glycoconjugates, Heparin and Fondaparinux

approximately 1/3 of the heparin migrated together with AT, whereas 2/3 of the material remained at it is. In agreement with this hypothesis, significant anticoagulant activity was only displayed by the fraction of the polysaccharide that could form a complex and migrate together with AT. In fact, the active anticoagulant fraction exhibited a higher specific activity than the starting material (348–388 vs. 155 U/mg) while the inactive fraction had a very low activity (19–52 U/mg). Next, Lindahl and coworkers prepared standard heparin by affinity chromatographic fractionation on a column of agarose AT.<sup>55</sup> In addition, they tried to evaluate the structural difference between the two fractions, however, a preliminary characterization failed to reveal any structural dissimilarities, apart from a slight difference in charge density. Subsequently, Lindahl showed that heparin fragments as short as hexa- to decasaccharides could bind to AT.<sup>56</sup>

Rosenberg and Lam<sup>57</sup> carried out the experiments in which the heparin glycosidic bonds are cleaved by the treatment of nitrous acid to obtain 2,5-anhydro-mannitol. Though predominantly gives disaccharides the *N*-acetylated glucosamine units do not undergo this transformation, hence they were able to isolate the tetrasaccharide from high-affinity heparin (Figure 1.17). This was found true only in part; the above structures should particularly bear a 3-*O*-sulfonate group to the unit placed at the reducing end of glucuronic acid. This *O*-sulfonate group has become a salient feature of the AT-binding site.<sup>58</sup>

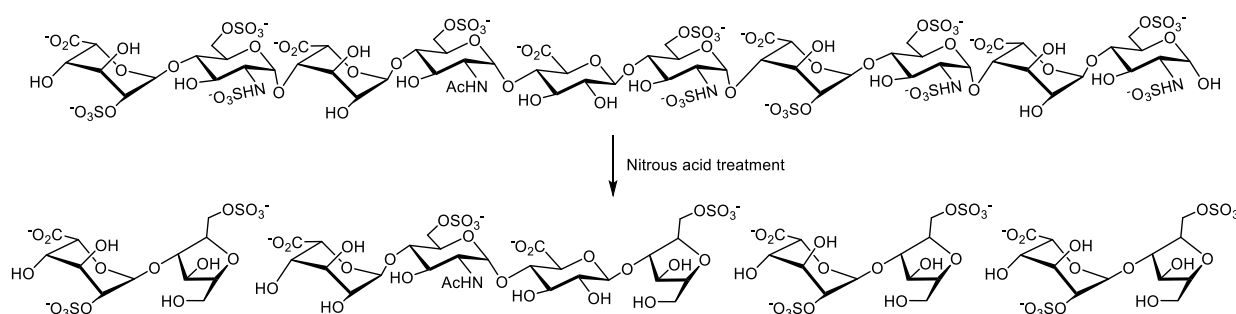


Figure 1.17: The experiments carried out by Rosenberg and Lam.<sup>82</sup>

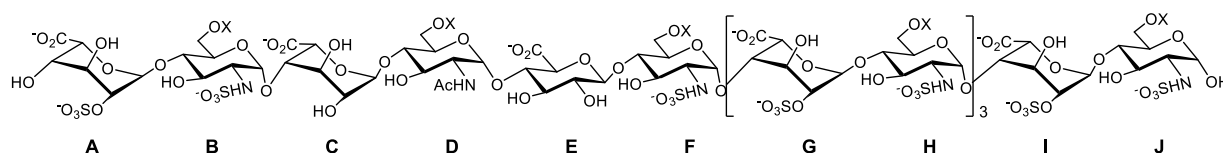


Figure 1.18: The structure of the antithrombin binding site of pig mucosal heparin as viewed by Lindahl et al. in 1979.<sup>58</sup> X stands for either a sulfonate group or a hydrogen atom. The 3-*O*-sulfonate group of unit F is missing.

Lindahl and co-workers<sup>58</sup> employed the very first time the proposed complete heparin oligosaccharide sequence for the AT binding (Figure 1.18) that included the tetrasaccharide previously found by Rosenberg. They emphasized the non-sulfated iduronic acid as the



## Introduction to Glycoconjugates, Heparin and Fondaparinux

functionally important unit and noted that the *N*-acetyl GlcN can be replaced as an *N*-sulfonyl GlcN. Later same group elucidated the structure of a shorter fragment, an octasaccharide, able to bind with high affinity to AT (Figure 1.19, upper structure).<sup>59</sup> The implicated tetrasaccharide sequence constituted the non-reducing terminal half of the AT-binding octasaccharide and the hexasaccharide CDEFGH, common to the two octasaccharides contains the antithrombin binding site (Figure 1.19).

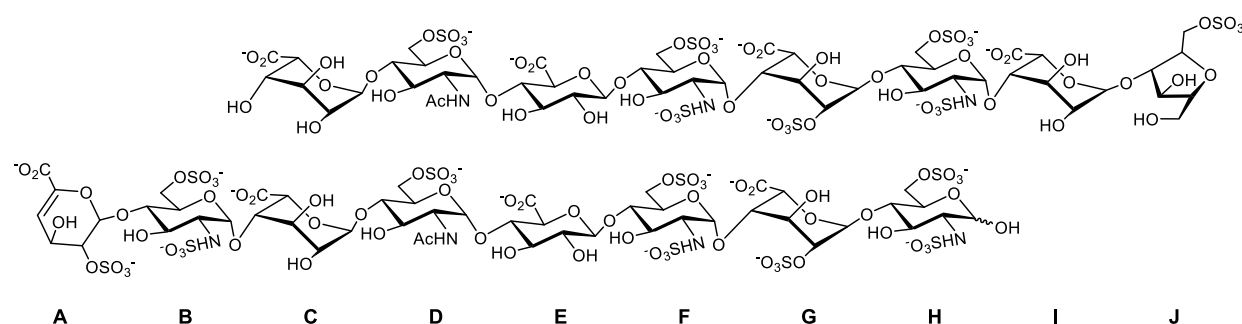


Figure 1.19. Structure of two octasaccharides having a high affinity for antithrombin, obtained from heparin after fragmentation using nitrous acid<sup>59</sup> (upper structure) and heparinase<sup>60b</sup> (lower structure).

Meanwhile, Jean Choay and co-workers launched ambitious research program to identify the shortest sequence able to inhibit factor Xa with several heparin fragments prepared either by direct extraction, enzymatic fragmentation, or else by partial nitrous acid fragmentation.<sup>60</sup> These fragments are subjected to the affinity chromatography on AT-agarose and gel filtration. As a result, the octasaccharide sequence shown in Figure 1.19 (lower structure) was found active. Further, the sequence was analyzed by <sup>13</sup>C NMR, interestingly, it was completely in agreement with the proposed inactive counterpart except the new signal at 58 ppm.<sup>60a, 60c</sup> Finally, Lindahl and his colleagues, indicated the unidentified piece 3,6-di-*O*-sulfonated, *N*-sulfonated glucosamine residue (*F* in Figure 1.21)<sup>58</sup> that was later identified as a typical and decisive 3-*O*-sulfonate group is a marker for the AT-binding site. Further, Choay group identified the hexasaccharide (Figure 1.20, lower sequence), able to bind to antithrombin and to catalyze factor Xa inhibition. Importantly, the typical signal at 58 ppm was explained by the group as a C-2 resonance in a 3-*O*-sulfonated glucosamine unit<sup>60a</sup> that could not have observed from standard heparin as it contains 3-*O*-sulfonated glucosamine in a very low proportion. Moreover, later it was concluded that the C unit appeared unlikely, suggesting that the pentasaccharide DEFGH could be (or could contain) the critical sequence required to bind to antithrombin.<sup>61</sup> Lindahl and his colleagues also reached to the same conclusion<sup>62</sup> and showed that C and I-J disaccharide are not necessary.



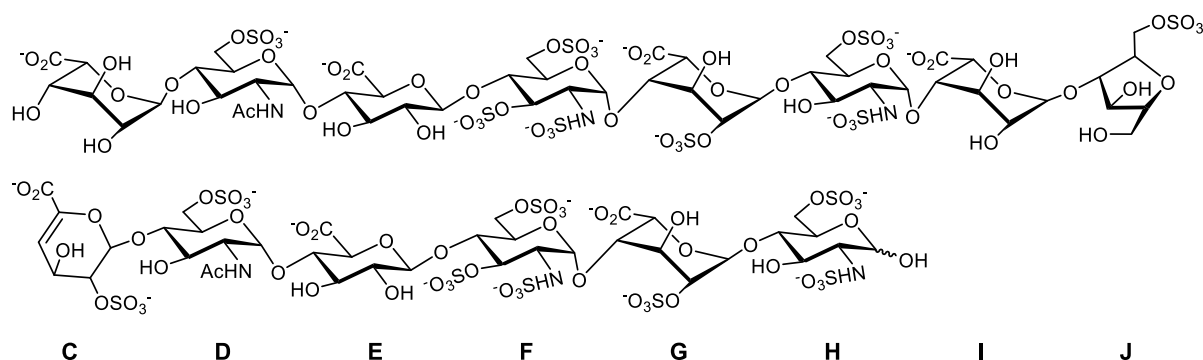


Figure 1.20. Identification of the critical pentasaccharide sequence D–H from the partially degraded high-affinity octasaccharide and hexasaccharide

Sanofi-synth@labo and Organon conducted a myriad of high-risk research and development activities in a joint venture to develop a synthetic route. In collaboration with Sinäy, Choay group synthesized<sup>63</sup> the pentasaccharide sequence DEFGH and the two tetrasaccharides DEFG and EFGH and the structure-property results clearly indicated that the entire pentasaccharide is required for high-affinity binding to AT.<sup>64a</sup> They also elucidated the pentasaccharide lacking the typical 3-*O*-sulfonate group diminished its antithrombin affinity by ~10,000-fold.<sup>64b</sup>

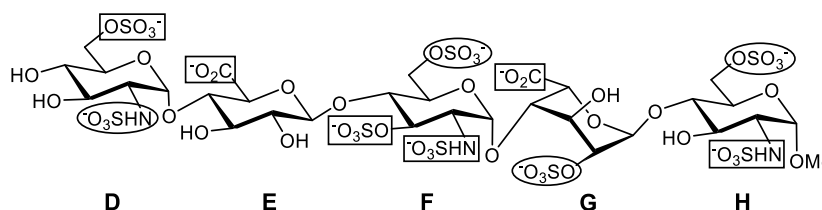


Figure 1.21. Structure of Fondaparinux after the overall structure-activity relationships study

The pentasaccharide with a methyl group at the anomeric center (Figure 1.21) had been prepared by both companies (under the codes SR 90107 and Org 31540, respectively) and subjected to the structure-activity relationship study by the preparation of its corresponding analogs that lacked one particular sulfate or carboxylate group. In this way, it was established that the groups highlighted in the boxes are absolutely essential for the activation of AT-III, whereas the groups in the circles only help to increase the biological activity.

To study the biochemical and pharmacological properties along with toxicology and clinical trials in healthy volunteers, in 1989 chemists provided the highly purified pentasaccharide (SR 90107/Org 31540).<sup>65</sup> Interestingly, in 2001, after the successful clinical development program, it was registered in the USA and Europe as a new antithrombotic drug under the name Arixtra (Fondaparinux).<sup>65c</sup> This drug is currently used for the prevention of venous thromboembolic events following knee- or hip replacement surgery and after hip fractures. Further development

was directed towards other prophylactic indications, as well as the treatment of deep venous thrombosis, pulmonary embolisms, and coronary artery diseases.

### 1.5 Challenges and Strategies in Chemical Synthesis of Heparin and Fondaparinux:

Despite success in the emergence of heparin chemical structure with establishing the AT-III binding site (figure 1.22), heterogeneities of the heparin and HS from natural sources presented major challenges in obtaining sufficient quantities of pure materials. To overcome this limitation, chemical modification of natural heparin had been employed frequently, though, this approach can give a complex mixture of many partially modified products from incomplete reactions.<sup>66</sup>

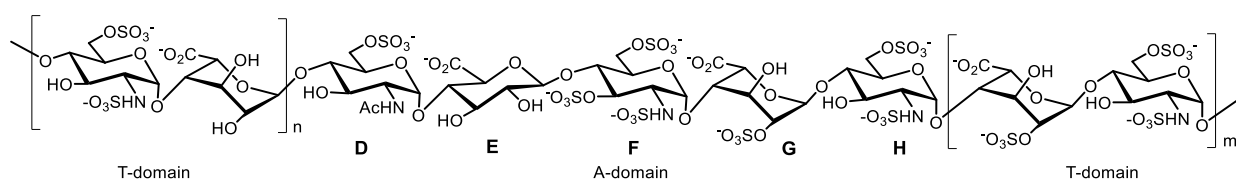


Figure 1.22: Structure of anticoagulant heparin with middle DEFGH pentasaccharide

For instance, more than 60 steps were required in the chemical synthesis of the first pentasaccharide, which was produced in extremely low yield and insufficient purity for use as a synthetic drug. However, multiple factors must be considered for a successful synthetic design. These include (a) synthetic access to L-iduronic acid and/or L-idose; (b) the choice of uronic acid or the corresponding pyranoside as building blocks; (c) formation of the 1,2-*cis* linkage from the GlcN donor; (d) suitable protecting-group strategy to install sulfate groups at desired locations; and (e) methods used for elongation of the backbone sequence. There has been much research in order to overcome these challenges in which some of the recently developed strategies are summarized in this thesis.

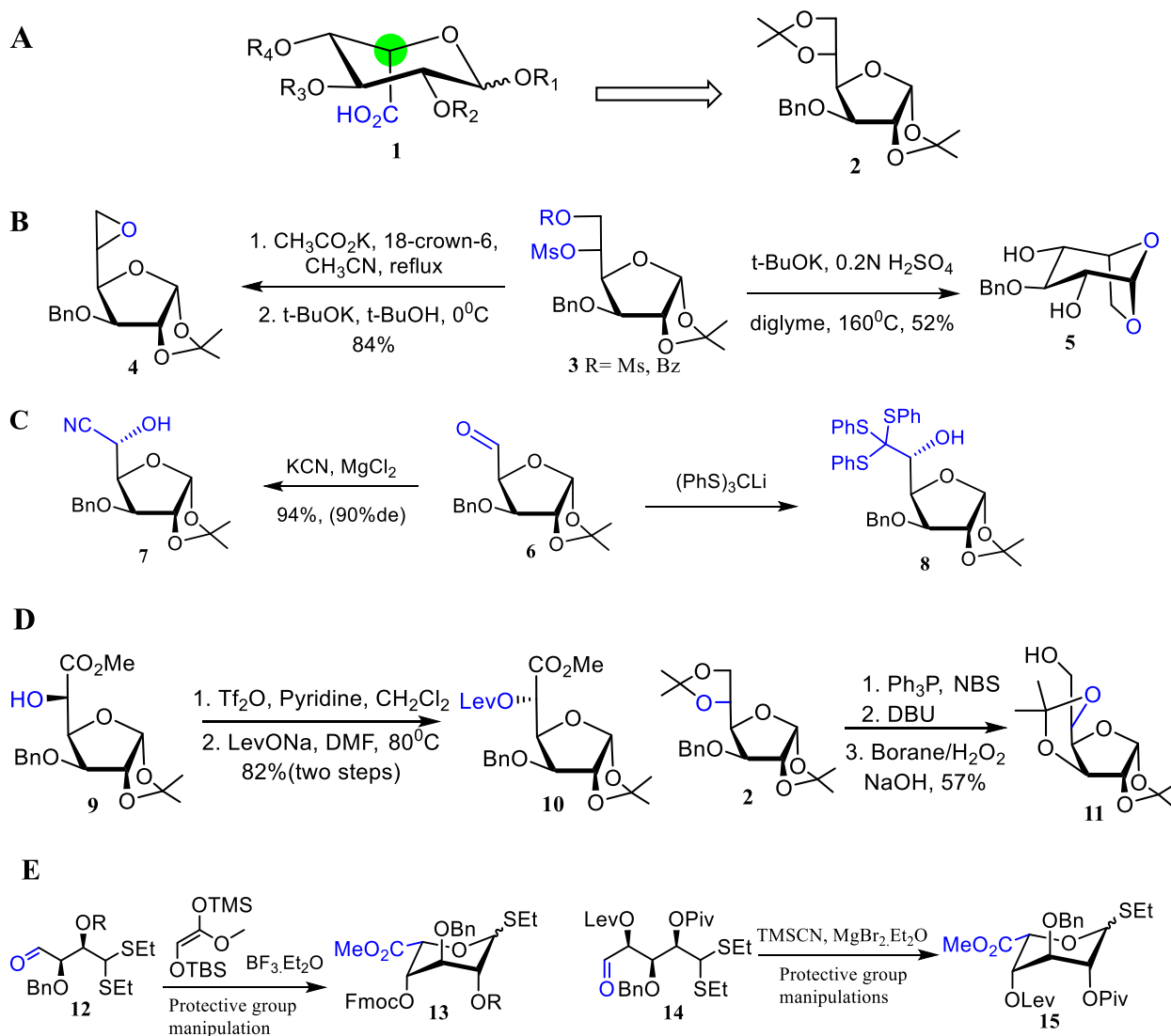
#### 1.5.1 Preparation of L-Iduronic Acid (L-IdoA) and L-Idose:

Unnatural L-Iduronic acid (IduA) or L-Idose is not non-commercial chemical and hence, chemical synthesis is the only alternative. Many routes have been developed to achieve L-idose/L-IdoA (**1**) with orthogonal protections (Scheme 1.01A). Some of the approaches started from 1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose (**2**) followed by the conversion into idopyranose by employing:

- (1) Inversion of configuration at C-5 through formation of an L-ido epoxide<sup>63a, 67a,b</sup> (Scheme 1.01A,B)

- (2) Oxidation of C-5-hydroxyl group to the corresponding aldehyde followed by stereoselective addition of cyano group<sup>67c</sup> or tris(phenylthio)methyl-lithium<sup>67d</sup> (Scheme 1.01C)
- (3) Nucleophilic S<sub>N</sub>2 inversion of the C5-hydroxyl group<sup>67e</sup> (Scheme 1.01D)
- (4) Elimination of the primary hydroxyl group with subsequent hydroboration to invert the stereochemistry at C-5<sup>67f</sup>

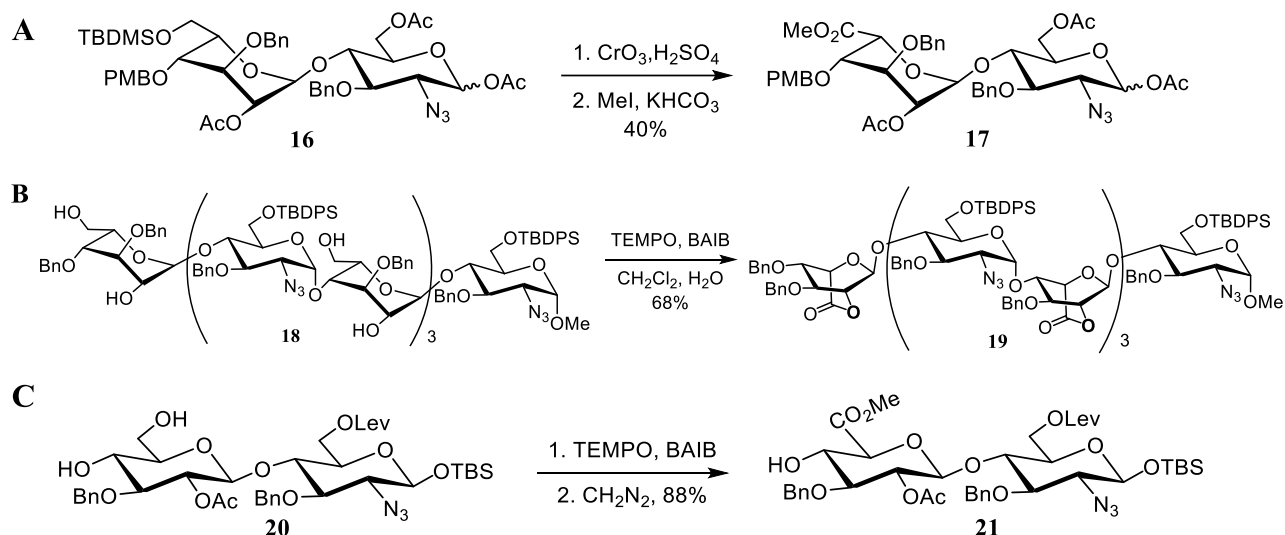
Other alternative routes to L-IdoA have been reported. For example, Seeberger and coworkers reported the *de nova* approaches starting from L-arabinose, but the low selectivity in the Mukaiyama aldol reaction with aldehyde intermediate resulted in a low overall yield (6%) (Scheme 1.01E).<sup>68</sup> Despite many routes for the preparation of L-IdoA or L-idose, synthesis of heparin/HS oligosaccharides remains difficult as 12–16 synthetic steps are required for the preparation of a single monosaccharide building block. Also, low yield and high-risk reaction conditions, particularly for scale-up, is a serious issue in some of the intermediate steps.



*Scheme 1.01: Various strategical key intermediate reactions to achieve an L-idose or L-IdoA efficiently along with orthogonal protection.*

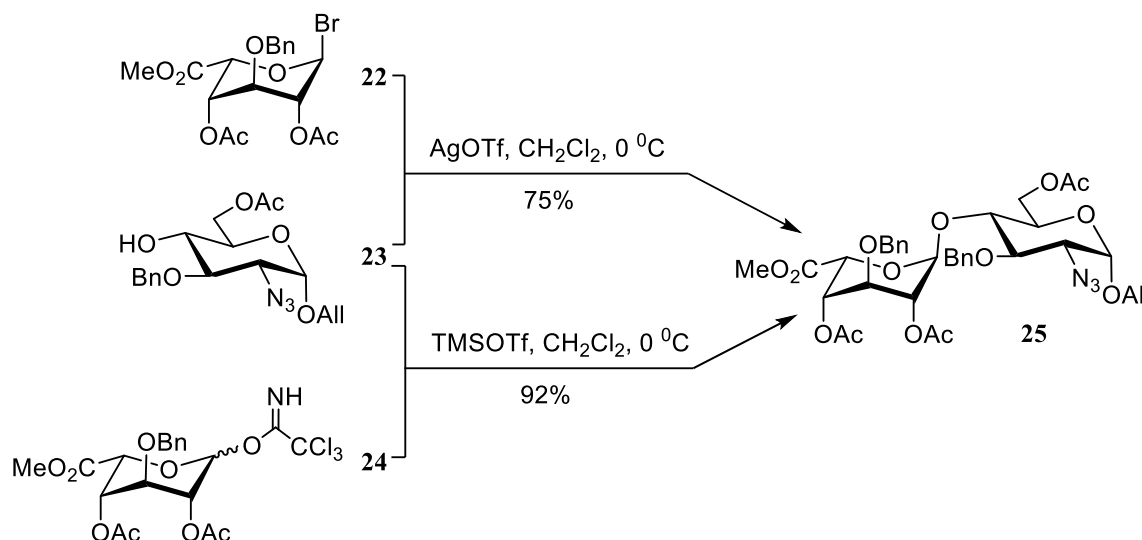
## 1.5.2 The Choice of Uronic Acid Vs Pyranoside as Building Blocks:

To avoid the possibility of epimerization and less yielding problem during the glycosidations from uronic acid donors, late stage oxidation of the corresponding pyranoside donors is preferred for glycosylation.<sup>69</sup> However, Jones oxidation by toxic chromium reagents results in diminished yield of the oxidized product (Scheme 1.02A),<sup>70</sup> later developed TEMPO-mediated mild oxidation condition was used with a co-oxidant such as NaOCl or iodobenzene diacetate (BAIB) enhanced the yield considerably (Scheme 1.02B).<sup>71</sup> Alternatively, oxidation can be invoked at the disaccharides to improve overall synthetic efficiency instead of final-stage oxidation of the larger oligosaccharides (Scheme 1.02C).<sup>72</sup>



*Scheme 1.02. Conversion of glycopyranosides into uronic acids*

However, the suitably designed monosaccharide uronic acids can be used as a glycosyl donor as exemplified by Sinäy *et al.*<sup>63a, 67a</sup> Bonaffée and coworkers synthesized disaccharide **25** in 75% yield by using the IdoA glycosyl bromide **22**, further, the yield was improved to 92% with trichloroacetimidate donor **24** (Scheme 1.03).<sup>73</sup>

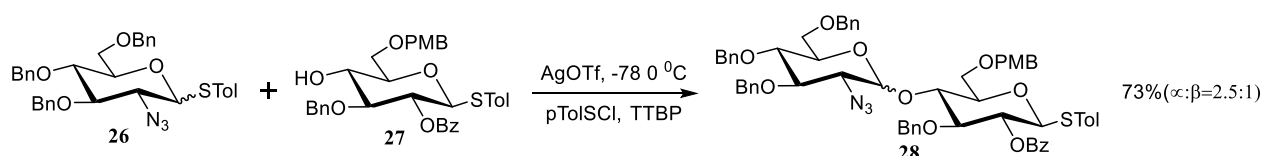


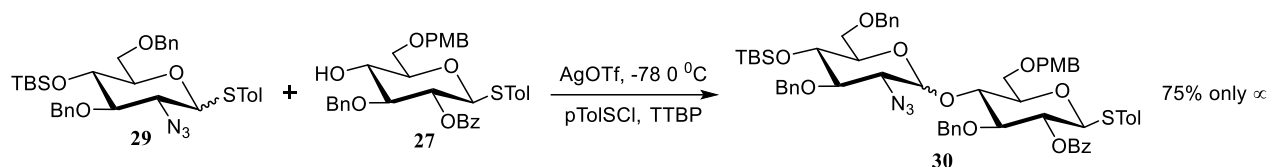
Scheme 1.03. Comparison of glycosyl bromide and trichloroacetimidate donors in glycosylation.

## 1.5.3 Stereochemical Control in the Glycosylation:

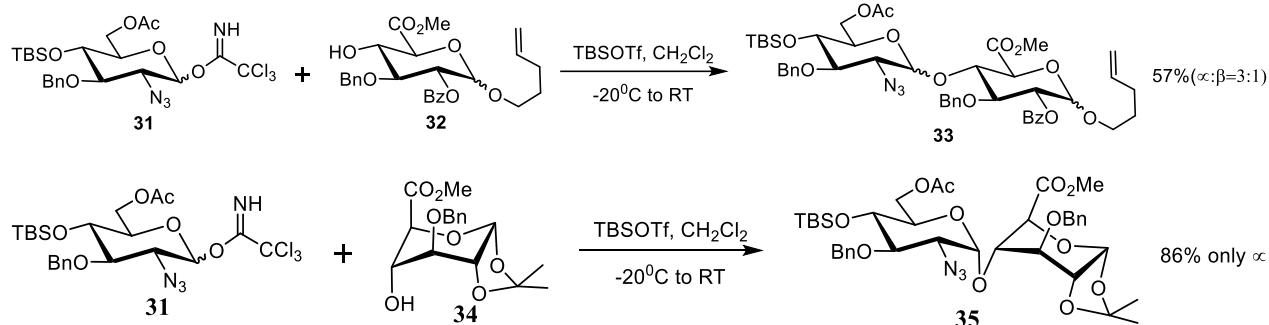
Usually, 1,2-*trans* linkages from uronic acids can be achieved by the C-2 participating groups in the heparin oligosaccharide synthesis; however, installation of the 1,2-*cis* linkage from the GlcN donors is a tough challenge. A widely used linear, non-participating C-2 azide as the surrogate for the C-2-NH<sub>2</sub> that can lead to the thermodynamically more-stable  $\alpha$ -glycoside.<sup>115,116</sup> High  $\alpha$ -selectivity can be achieved with L-idopyranosyl acceptors, though the D-glucopyranosyl based acceptors often leading to the anomeric mixtures and require the fine-tuning of the protecting groups.<sup>75</sup> For example, replacing the 4-*O*-benzyl group from donor **26** to 4-*O*-TBS (**29**), leading to the formation of  $\alpha$ -linked disaccharide **30**. Bulky groups at the *O*-6 position also help in the decrease of  $\beta$ -anomer (Scheme 1.04A).<sup>71</sup> In addition, conformational locking of acceptor (e.g. **34**), exclusively led to the  $\alpha$ -selective disaccharide **35** with an excellent yield that was not noticed with the pentenyl acceptor **32** (Scheme 1.04B).<sup>67e</sup> However, adopting these results in larger oligosaccharides synthesis becomes challenging due to the demand of orthogonal protections. For example, glycosylation of the tetrasaccharide donor **36** with disaccharide acceptor **37**, resulting into the hexasaccharide **38** as an inseparable anomeric mixture (Scheme 1.04C).<sup>76</sup>

### A.

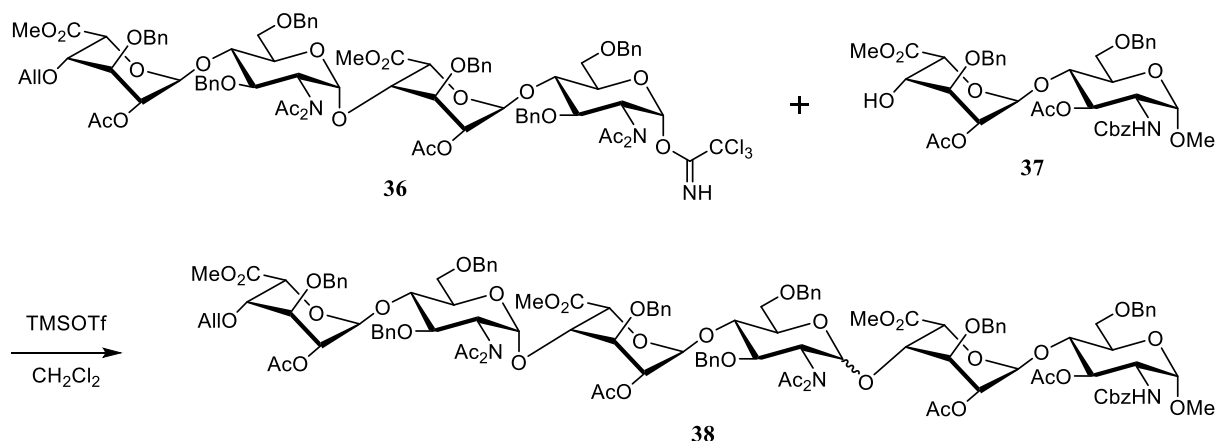




## B.



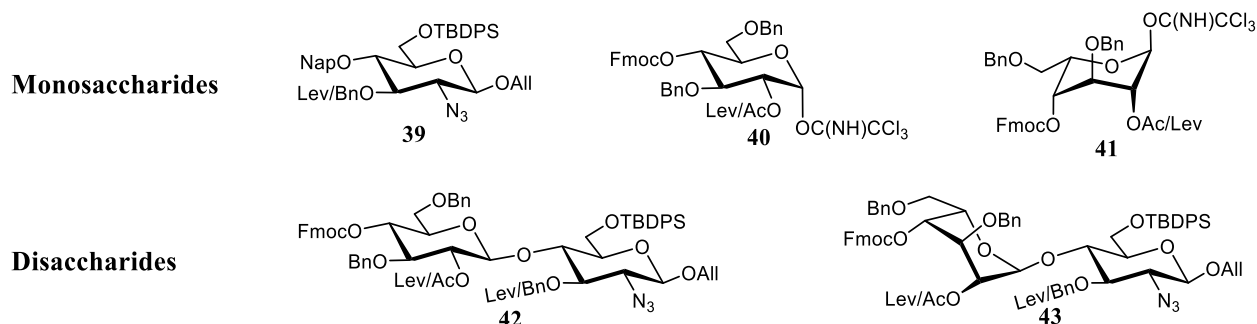
## C.



Scheme 1.04. Strategies for enhancing stereoselectivity in glycosylation A) Protecting group variation B) Conformational locking C) anomeric mixture in the case of oligosaccharides

### 1.5.4 Protecting-Group Strategy:

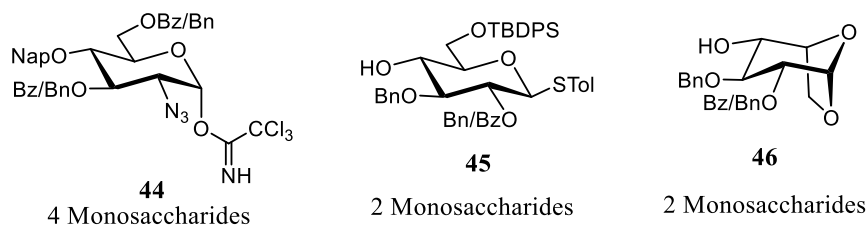
Heparin is well known for its most complex structure, the high level of functionalities and variable sulfation patterns. Thus, the synthesis must be suitably designed to prevent the premature removal of the protecting groups that demand a large number of variable protecting groups. Accordingly, the Boons' group, strategically designed six monosaccharides by a novel set of orthogonal protections that can be employed for synthesizing the disaccharide moieties found in heparin (Scheme 1.05).<sup>72</sup> The systematically protected disaccharides can be transformed into donors and acceptors for further synthesis of well-defined oligosaccharides.



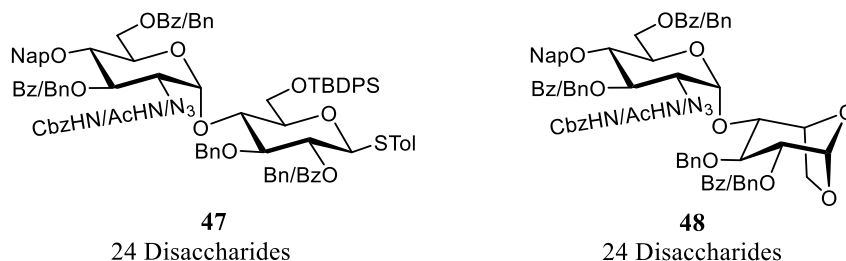
*Scheme 1.05: Orthogonally protected building blocks strategy from the G-J Boon's group that was envisioned towards well-defined oligosaccharides synthesis.*

Further, Hung's group explored the protecting group strategy in the synthesis of all possible heparin disaccharide derivatives (**47** and **48**) which were prepared from 8 monosaccharides (Scheme 1.06).<sup>67b</sup> Importantly, the protecting groups such as benzoyl, benzyl, TBDPS, azide, Cbz, etc. were thoughtfully installed in such a way that they can be regioselectively deprotected and sulfated at the final stage of the targeted oligosaccharides.

## 8 Monosaccharides



## 48 Disaccharides



*Scheme 1.06. Synthesis of all possible heparin/HS disaccharides from eight monosaccharides*

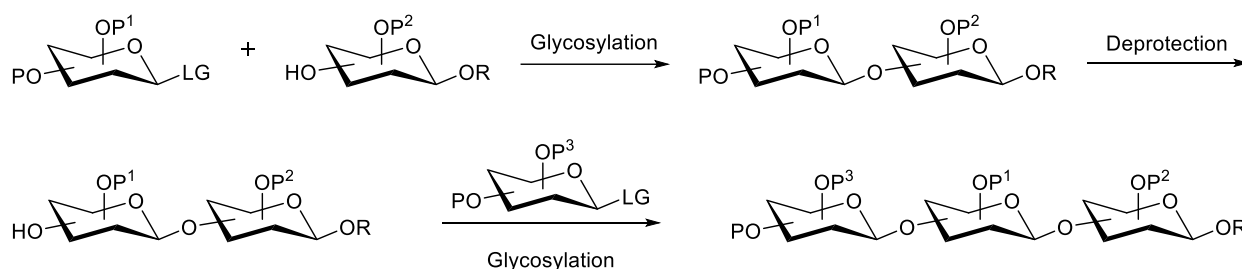
Instead of preparing multiple building units, Wei and coworkers synthesized single GlcA-GlcN disaccharide with orthogonal protection on each hydroxyl group.<sup>77</sup> This strategy proved the divergent synthesis of multiple sulfation patterns from a single unit, however, it demands more synthetic steps.

## 1.5.5. Synthetic Approaches Toward the Heparin Pentasaccharide/Oligosaccharide:

**Linear Synthesis:** The linear approach to produce heparin and other GAGs were explored by early investigators who have targeted the synthesis of heparin.<sup>78</sup> Chemical glycosylation

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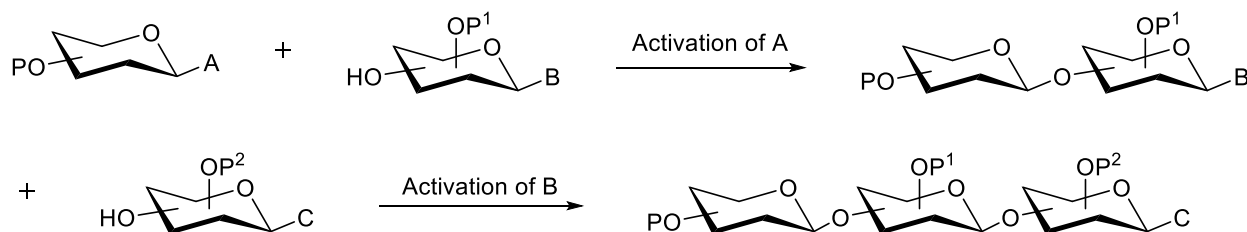
includes activation of a donor to form mostly the oxocarbenium intermediate, followed by a nucleophilic attack of the acceptor to form a new glycosidic linkage. In linear approach for heparin oligosaccharide, C4-hydroxyl protection from the non-reducing end of the newly formed disaccharide is selectively removed, to generate a new acceptor that undergoes further glycosylation. In this way, it leads extension of the chain to form a heparin oligosaccharide backbone from the reducing end to the non-reducing end (Scheme 1.07).



*Scheme 1.07. Linear synthesis of oligosaccharides from the reducing end.*

Boons and coworkers used the linear approach to synthesize a trisaccharide, by using vinyl and trichloroacetimidate donors.<sup>71</sup> Fügedi also used this strategy to synthesize trisaccharides considered to be responsible for interactions of HS with the fibroblast growth factors FGF-1 and FGF-2.<sup>79</sup> Nevertheless, oligosaccharide synthesis prolongs with an overall number of synthetic steps due to the number of intermediates produced and deprotection steps required after each glycosylation. Therefore, linear synthesis is mainly employed for preparing short oligosaccharide sequences.

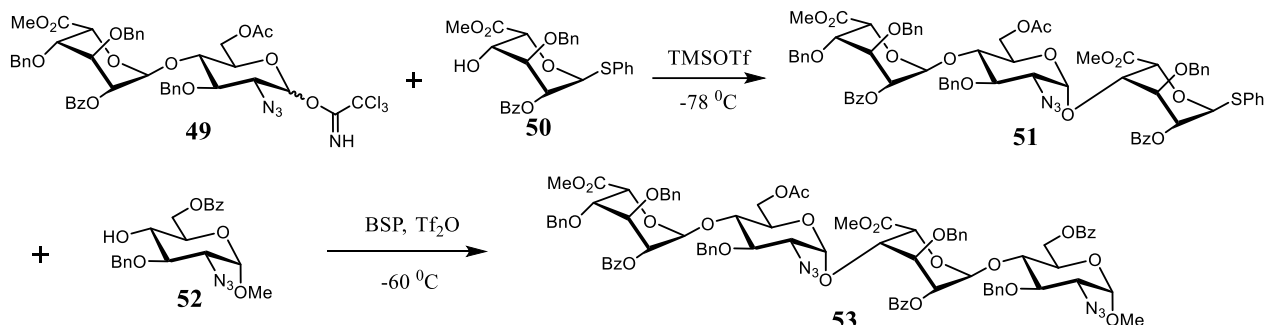
**Selective Activation:** The selective-activation method involves a donor-acceptor system in such a way that chemoselective activation one of the anomeric centers is achieved in the presence of the other. In that way, reduction in the number of steps is possible as the acceptor in one step can become a donor in the subsequent step (Scheme 1.08). However, the challenge is the identification of the right pair of orthogonally activatable glycosyl donors. In this connection, use of trichloroacetimidates and thioglycosides has been investigated to prepare heparin tetrasaccharides (Scheme 1.09).<sup>80</sup>



*Scheme 1.08. Glycosylation strategy employing selective activation.*

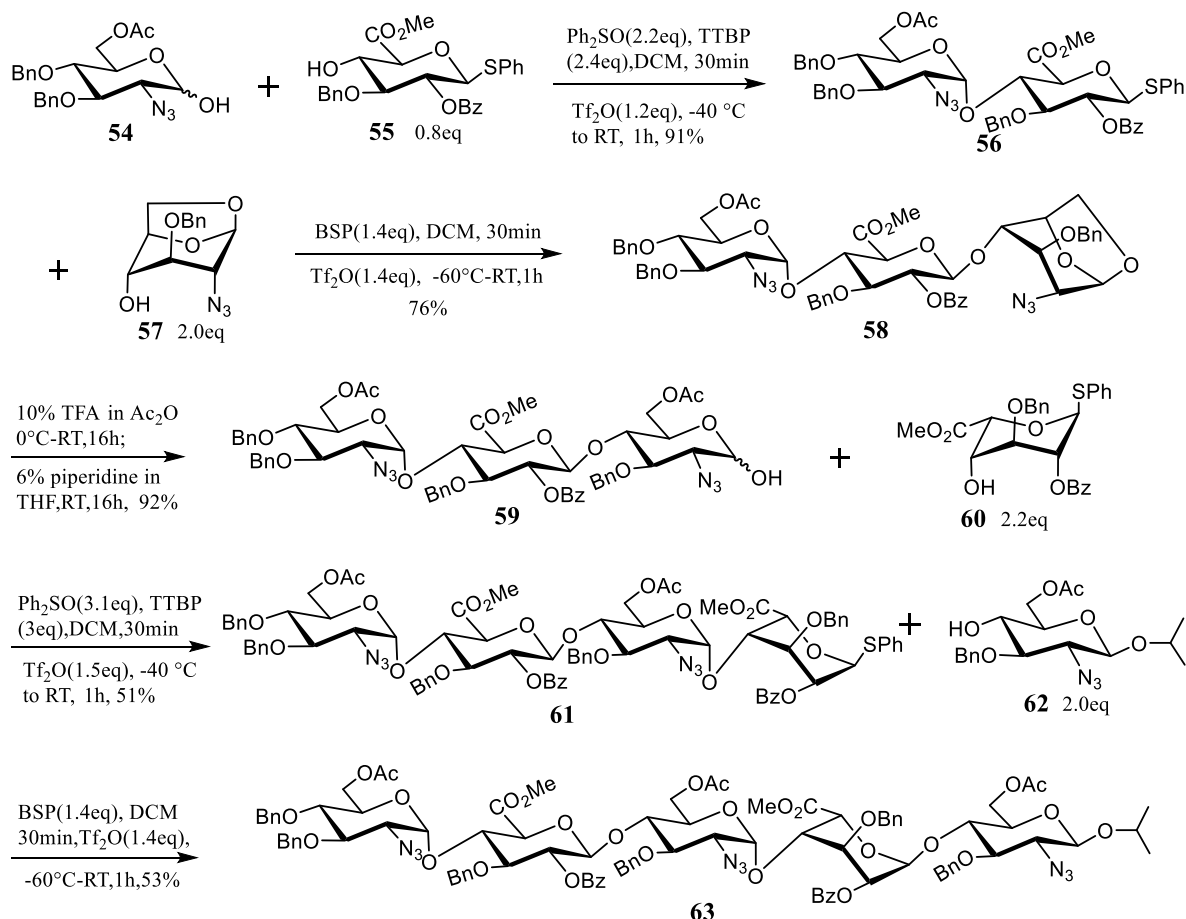


Acetimidate donor **49** was selectively activated by TMSOTf at  $-78\text{ }^{\circ}\text{C}$ , in presence of the thioglycoside acceptor **50**; subsequently, the trisaccharide **51** was directly subjected to further glycosylation with the thiophilic promoter 1-benzene-sulfinyl piperidine (BSP) and  $\text{Tf}_2\text{O}$  to accomplish the synthesis of tetrasaccharide **53**.



*Scheme 1.09. trichloroacetimidate and thioglycoside in a selective-activation strategy.*

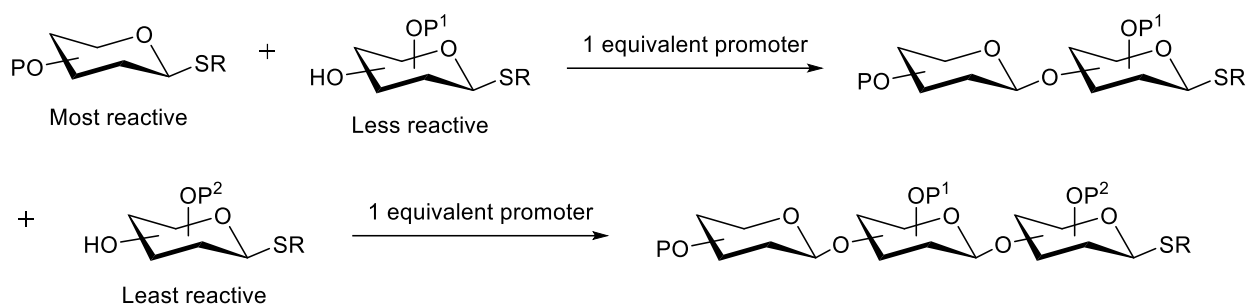
Alternative to trichloroacetimidates and thioglycosides pair, van der Marel and coworkers employed hemiacetals and thioglycosides for selective activation. Importantly, the approach was explored toward the complex pentasaccharide derivative **63**. Diphenyl sulfoxide and  $\text{Tf}_2\text{O}$  promoter system used to activate the hemiacetal **54** selectively that is glycosylated with acceptor **55** (Scheme 1.10). Resulting thiophilic disaccharide donor **56** was subjected to the glycosylation with 1,6-anhydro glucose azide acceptor **57** to obtain compound **58**. Further, trisaccharide hemiacetal donor **59** was prepared under acidic conditions and used for the selective activation during the glycosylation reaction. The resulting thiophilic donor **61** was glycosylated with the acceptor **62** without further modifications.<sup>75b</sup>



*Scheme 1.10. Hemiacetals and thioglycosides in a selective-activation strategy.*

Although selective-activation reduces the intermediate manipulation steps in glycosylation, it needs two different types of glycosyl donors. To simplify the overall synthetic design, it is desirable for a single glycosyl donor to be employed throughout the synthesis.

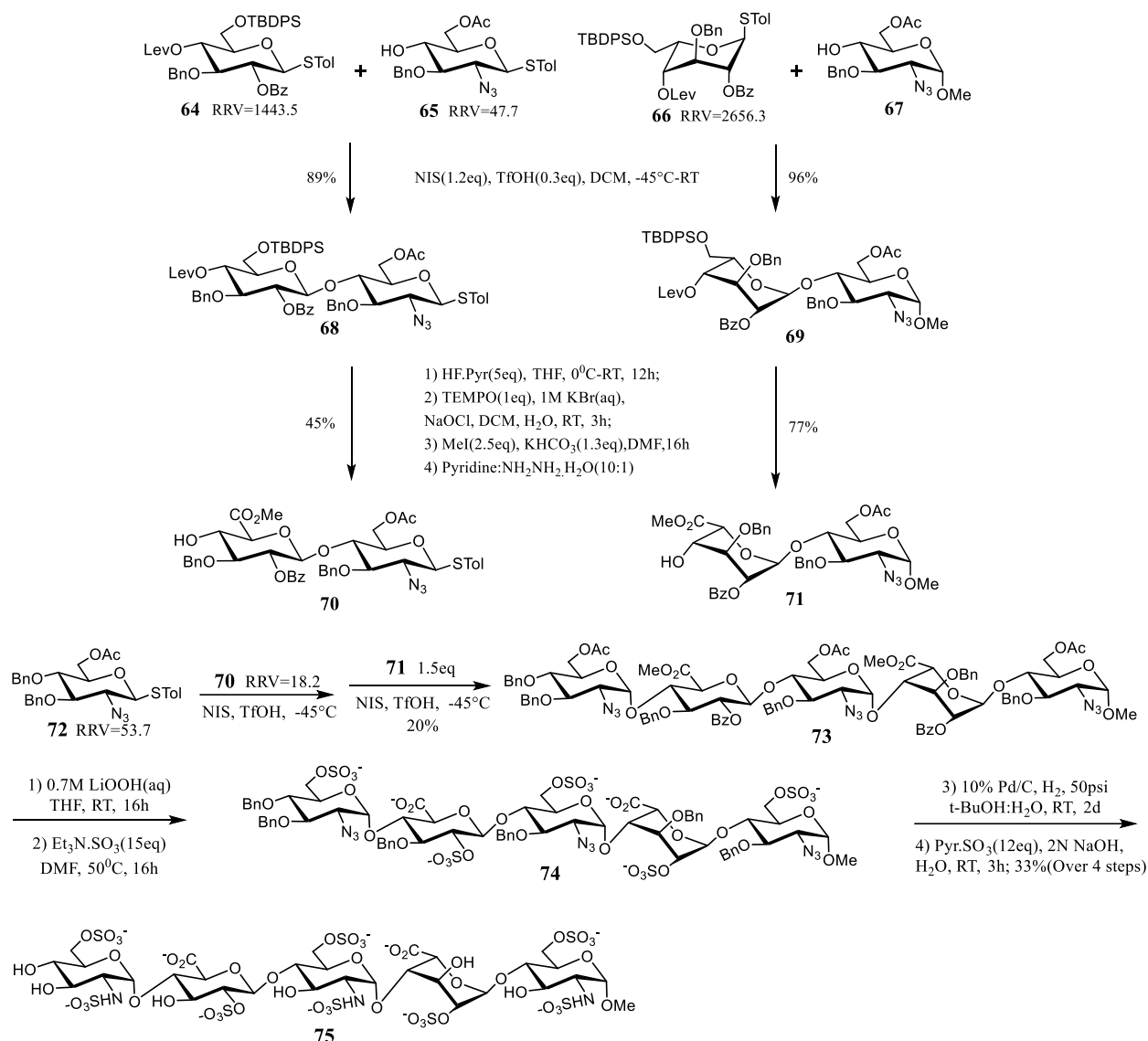
**Reactivity-Based Chemoselective Glycosylation:** This is also known as an armed–disarmed glycosylation method and it was developed on the basis of anomeric reactivity differences, typically thioglycosides used with a limiting amount of promoter. When a mixture of a more-reactive donor (armed) and an acceptor having lower anomeric reactivity (disarmed) is subjected to the glycosylation, the armed donor is activated preferentially by limiting the number of equivalents of the promoter or the temperature (Scheme 1.11). The leading oligosaccharide can be used directly as a donor for further glycosylation under the same condition. The appropriate plan of the building units with sufficient difference in relative anomeric reactivity enables multiple sequential glycosylations in one vessel avoiding the purifications during the intermediate steps.



*Scheme 1.11. Armed–disarmed strategy for chemoselective glycosylation relies on differences in the anomeric reactivities of the building blocks.*

The required anomeric reactivity difference can be achieved by preparing suitable building blocks using proper protecting group strategies<sup>81</sup> or by modification of the aglycon.<sup>82</sup> And the reactivity difference can be calculated by relative reactivity values (RRVs), with the reactivity value of *p*-tolyl 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-mannopyranoside donor toward methanol acceptor being set as 1.0.<sup>81c</sup>

Wong and coworkers employed the relative reactivity-based approach in the synthesis of heparin/HS oligosaccharides. Five monosaccharide building blocks with wide RRVs were designed carefully and applied to the complex heparin pentasaccharide synthesis. Disaccharide **68** was prepared by the chemoselective glycosylation of 30x more reactive armed donor **64** and disarmed acceptor **65** (Scheme 1.12A).<sup>83a</sup> Manipulation of the protecting groups and oxidation of the C-6 of glucopyranoside produced the disaccharide **70** as a new glycosyl donor having RRV of 18.3. In parallel, the disaccharide **71** was prepared from the coupling partners **64** and **65**. Reactivity-based chemoselective glycosylation of gluco-azide donor **72** (RRV= 53.7) with acceptor **70** (RRV= 18.3) was feasible to give the corresponding trisaccharide intermediate in presence of NIS/TfOH as a promoter (Scheme 1.12B). Subsequently, the disaccharide acceptor **71** in the presence of more number of equivalents of promoter completed the one-pot synthesis of fully protected pentasaccharide **73** in 20% overall yield. Orthogonal deprotections and sulfation furnished desired pentasaccharide **75** as a sulfated heparin pentasaccharide. Recently, the same group investigated different combination of the building blocks for improved overall yields.<sup>83b</sup>

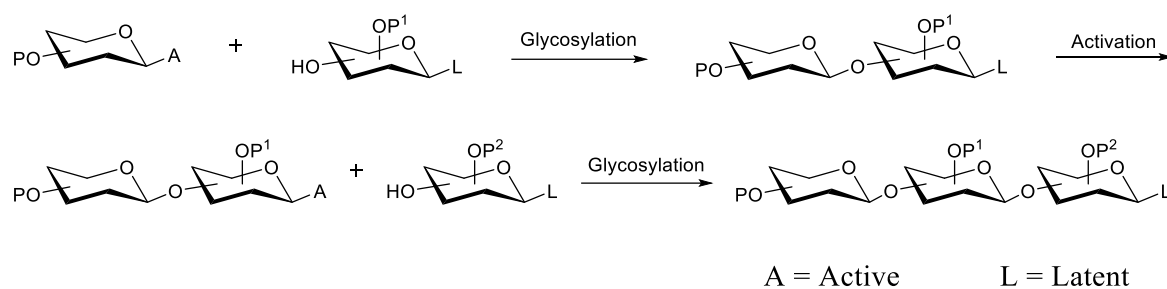


**Scheme 1.12.** (A) preparation of disaccharide building blocks; (B) one-pot synthesis of heparin pentasaccharide by armed-disarmed glycosylation strategy.

RRV is based on the reference to methanol as an acceptor and it gives general guidelines toward the selection of remaining building blocks. However, these values can change according to the structure of acceptors and the reaction conditions and hence, requires a lot of optimizations prior to the onset of the actual synthesis plan.<sup>135</sup>

**Latent Active Glycosylation:** In this approach, acceptor possesses a latent aglycon which is inert to the conditions of glycosyl-donor activation. After glycosylation, the resultant latent aglycon can be converted to active donor for further chain elongation from the nonreducing end to the reducing end (Scheme 1.13).

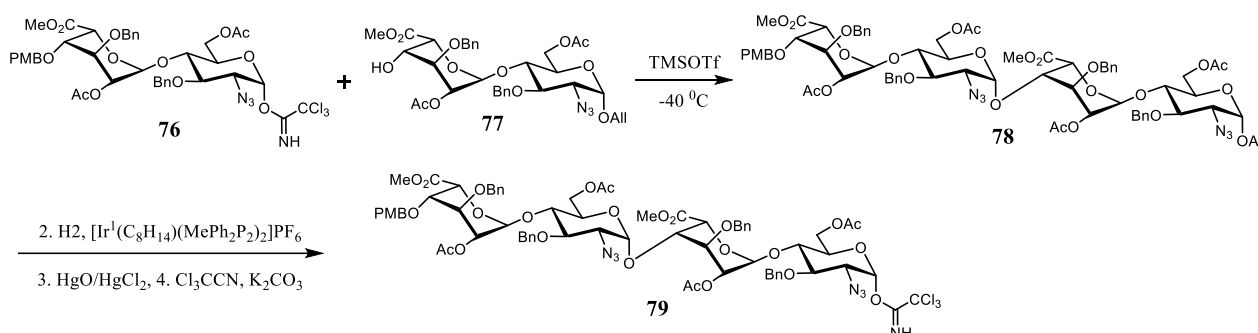
## Introduction to Glycoconjugates, Heparin and Fondaparinux



*Scheme 1.13. Active-latent glycosylation strategy.*

Allyl glycosides are widely employed as latent aglycons because of their inertness towards the general conditions employed for donor activation; resulting allyl glycosides are subsequently transformed into vinyl glycosides as an active glycosyl donors in Lewis acid-catalyzed glycosylations.<sup>72b, 84</sup>

Bonaffée's group glycosylated latent active allyl glycoside **77** and active trichloroacetimidate donor **76** and the resultant allyl glycoside **78** was transformed into active trichloroacetimidate donor **79** (Scheme 1.14). Impressively, the chain was elongated to heparin dodecamer by the this latent-activation strategy.<sup>85</sup>



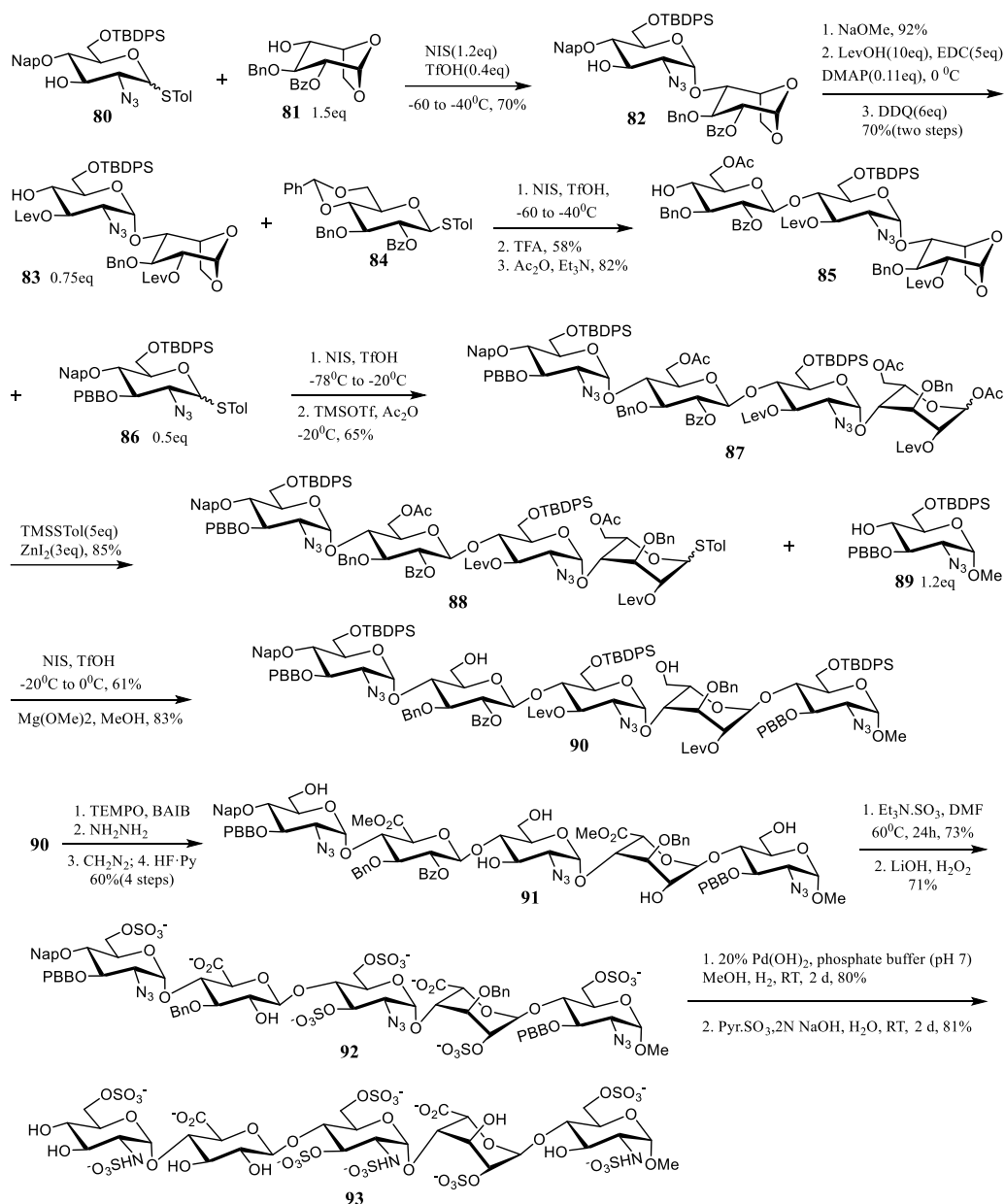
*Scheme 1.14: The Bonnaffe group's active-latent strategy*

However, cleavage of the allyl group requires two synthetic steps additionally and needs to use expensive transition-metal reagents and toxic mercury salts. Alternatively, various silyl protecting groups such as dimethylthexylsilyl (TDS), *t*-butyldimethylsilyl (TBDMS), and trimethylsilyl (TMS) have been utilized for masking anomeric position to make the latent aglycons and employed toward many successful syntheses.<sup>67e, 80b, 86</sup> The combination of silyl and thioglycosides are also explored under variety of activating conditions.<sup>72</sup>

Additionally, isopropylidene acetals<sup>76, 87</sup> and 1,6-anhydro sugars<sup>67f, 75b, 87a</sup> have been used to mask the anomeric position of the latent glycosyl donors. Hung and coworkers successfully employed 1,6-anhydro-L-idose sugar in the preparation of most complex AT-III binding heparin based drug Fondaparinux.<sup>88</sup> Trisaccharide **85** was prepared by the two subsequent linear glycosylation reactions. Acceptor, 1,6-anhydro-L-idose **81** is coupled with thioglycoside donor

# Introduction to Glycoconjugates, Heparin and Fondaparinux

**80** in the presence of NIS/TfOH at  $-60\text{ }^{\circ}\text{C}$  to  $-40\text{ }^{\circ}\text{C}$ . The resultant disaccharide **82** was transformed into acceptor **83** and glycosylated with donor **84**. The latent trisaccharide acceptor **85** and donor **86** were glycosylated followed by the conversion of latent 1,6-anhydro tetrasaccharide intermediate to the active thioglycoside donor **88** via 1,6-diacetyl tetrasaccharide **87**. The remaining building block was coupled to afford desired pentasaccharide, deprotection and sulfation afforded Fondaparinux pentasaccharide **93** (Scheme 1.15).



*Scheme 1.15: Hung and coworkers employed linear and active-latent strategy in the Fondaparinux synthesis.*

In latent activation strategy, the building blocks with a selectively removable protecting group at the non-reducing end shall give corresponding acceptors since the oligosaccharides can be built up from both the non-reducing and reducing end that provides additional versatility in comparison to the linear strategy. Comprehensive elaboration of all the above strategies were employed recently as an optimized modular synthetic strategy for the preparation of Fondaparinux by using a convergent [3 + 2] coupling approach.<sup>89</sup>

A wide range of strategies has been developed for the synthesis of heparin oligosaccharides. Being synthetic, no animal-based contamination issues are perceived and therefore are safer to use clinically. Synthetic heparin oligosaccharides are in a huge demand all over the World because of their activity towards a number of diseases and their use in many surgeries. However, heparin oligosaccharide syntheses remain the major challenge due to its heterogeneity. The prohibitive cost of Fondaparinux due to the multi-step chemical synthesis invoking a variety of protecting groups and glycosylation methods posed as a serious limitation to its extensive use. Performing the reactions and purifications on large scale is yet another the major challenge.

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## **Chapter 2**

### ***Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives***



## 2.1 Introduction:

Number of routes have been developed for the synthesis of iduronic acid building block (Chapter 1, Scheme 1.01) as iduronic acid is unnatural and it is prohibitively expensive. Essentially, majority of those strategies utilized glucofuranose having 5,6-diol to synthesis L-idopyranose *via* 5,6-epoxide intermediate<sup>1</sup> or through the oxidative cleavage to its C5-aldehyde<sup>2</sup> (Figure 2.1). This single building block remains as the major challenge in the synthesis of heparin oligosaccharides as it needs 12-16 synthetic steps.

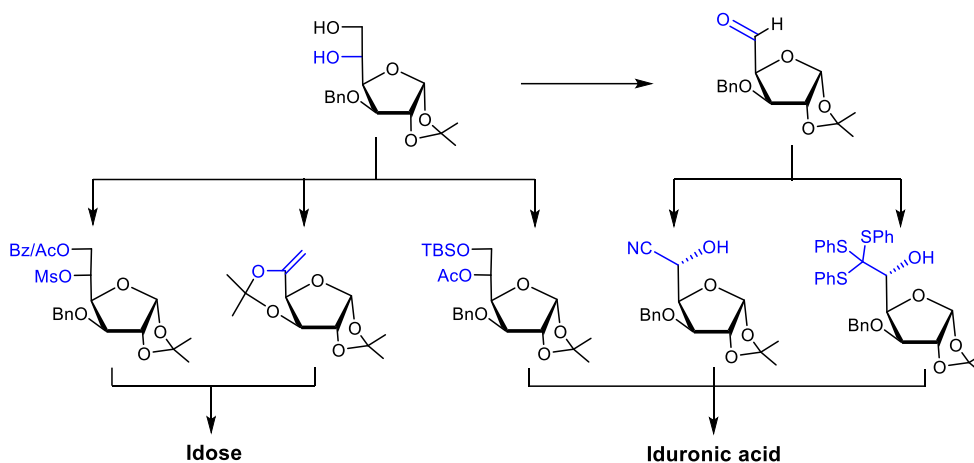


Figure 2.1: Summary of strategies for the synthesis of L-idose or L-iduronic acid (IdoA).

Owing to the significance of L-idose in heparin and in many other biologically active molecules, a novel route of converting glucuronic acid to the iduronic acid was initially planned through epimerization. The enzymatic epimerization is quite cumbersome and is not ideal for large scale preparation of the orthogonally protected iduronic acid synthesis. In this connection, Krapcho decarboxylation from 1,3-diester is envisioned that can be synthesized from the D-glucuronic acid.

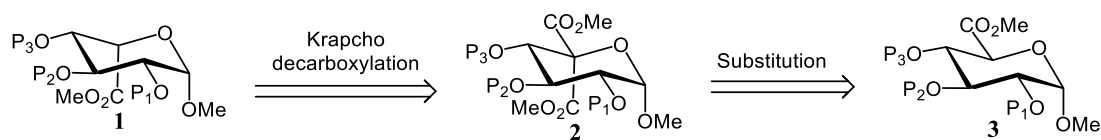
### 2.2-2.4 Present Work

#### 2.2 Krapcho Decarboxylation Approach to Iduronic acid Derivatives:

Krapcho decarboxylation<sup>3</sup> can be affected on  $\beta$ -ketoesters, malonic esters,  $\alpha$ -cyanoesters or  $\alpha$ -sulfonyl esters and it works best with methyl esters since it occurs through an  $S_N2$  reaction at the carbon.<sup>4</sup> Accordingly, methyl iduronate **1** can in principle be realized from the diester **2** which in turn can theoretically be obtained from the methyl glucuronate **3** with a wishful thinking that the less sterically hindered equatorial methylcarboxylate undergoes the decarboxylation to produce the iduronate (Scheme 2.01).



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Scheme 2.01: Krapcho decarboxylation route to the synthesis of iduronate from glucuronate.<sup>3,4</sup>

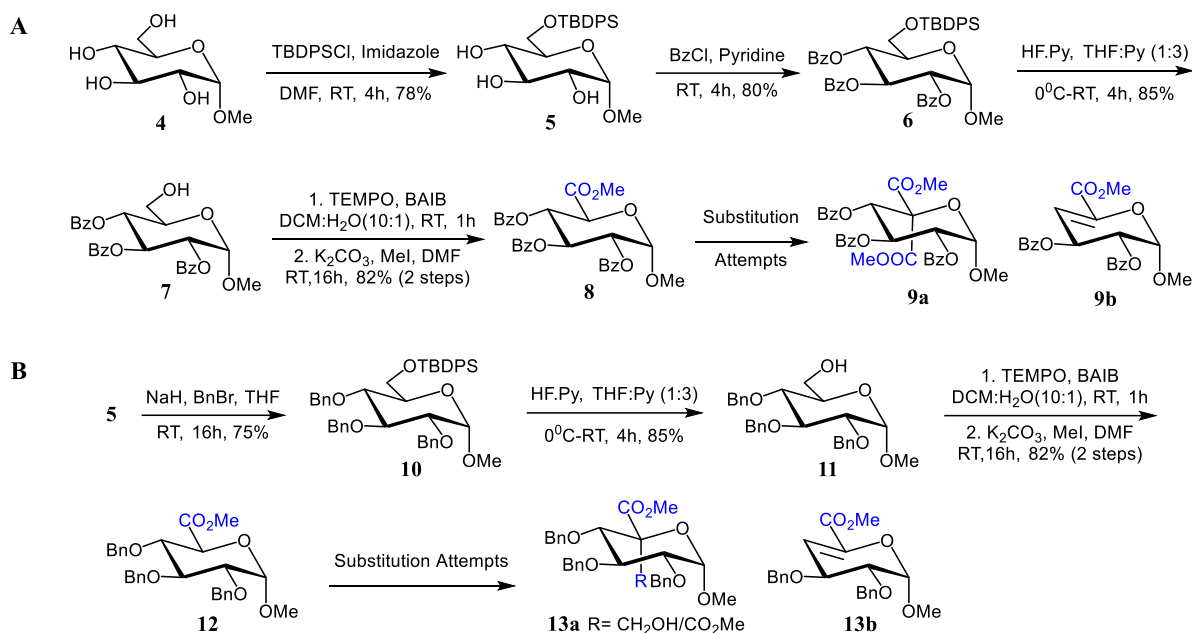
Accordingly, the synthesis of methyl glucuronate **3** from commercially available  $\alpha$ -methyl glucopyranoside **4** was targeted by employing well established reaction protocols (Scheme 2.02A). The synthesis commenced from protection of C-6 hydroxyl as *O*-TBDPS under TBDPSCl/Imidazole conditions to furnish silyl ether **5** which upon esterification using BzCl/py afforded per-*O*-benzoyl derivative **6**. Further, cleavage of the *O*-TBDPS moiety under fluoride ion mediated conditions gave compound **7** which was oxidized using TEMP/BAIB and converted to the methyl ester **8** using K<sub>2</sub>CO<sub>3</sub>/MeI.

The successful synthesis of methyl ester of glucuronic acid encouraged to the extrapolation to the  $\beta$ -ketoester through an alkylation at C-5 position in the presence of a strong base and MeCO<sub>2</sub>Cl. Initial reactions with 1.2 equivalents of LDA as base did not proceed and with excess of LDA, the starting material get decomposed (Table 2.1, Entry 1). Switching the base from LDA to NaH in the presence of dimethyl carbonate at different temperatures was studied. Broadly, the reaction did not proceed until the temperature attained 80 °C; above this temperature, reaction proceeded and produced a new compound that was confirmed to be the  $\beta$ -eliminated (4,5-diene) compound **9b** instead of the desired malonate ester **9a** (Table 2.1, Entry 2). Compound **9b** was confirmed by the thorough NMR and mass spectral analysis. In the aromatic region of <sup>1</sup>H and <sup>13</sup>C NMR, the absence of one of the benzoyl group in the aromatic region was observed, as well as the resonance of C-4 –CH downfield shift at 109 ( $\delta$ ) and C-5 at 141.9. Moreover, the absence of C-5 carbon resonance in DEPT NMR confirmed it as a quaternary carbon. In addition, compound **9b** further confirmed by MALDI-TOF analysis, *m/z* calculated for C<sub>22</sub>H<sub>20</sub>O<sub>8</sub> [M+K]<sup>+</sup>: 451.08, Found: 451.05. This observation showed that the decarboxylation underwent and the presence of electron withdrawing group (-OBz) at the C-4 facilitated elimination over substitution.

Thus, per-*O*-benzyl substitute compound **12** was synthesized quickly from compound **5** in order to avoid the elimination. Compound **12** was prepared from precursor **5** by performing well established benzylation to get **10**, TBDPS deprotection to obtain **11**, oxidation and esterification reactions to obtain ester **12** as shown in Scheme 2.02B. To the surprise, ester **12** also underwent elimination producing the alkene **13b** instead of the desired **13a** while

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performing the Krapcho reaction and the identity of the compound was established based on the spectroscopic data.



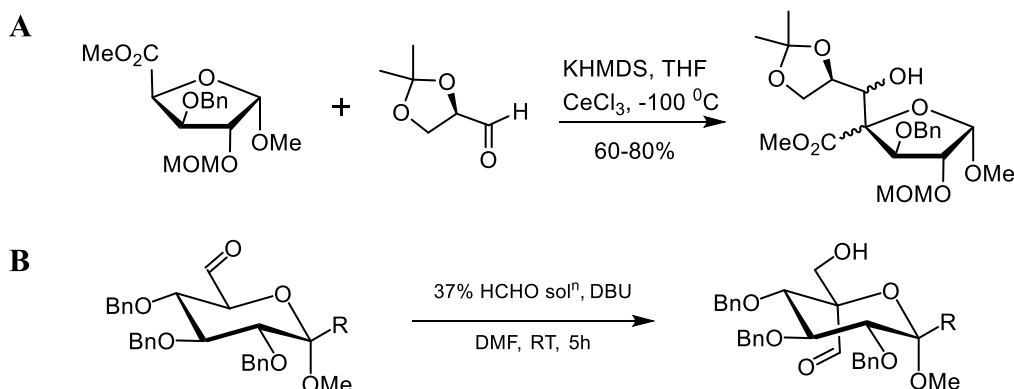
Scheme 2.02: Exploration of Krapcho Reaction for Iduronate Synthesis

Table 2.1: Screening of C<sub>5</sub>-substitution attempts

| Entry | Reactant | Base (1-5 eq)             | Electrophile (1.5-excess eq)                                            | Temp. (°C)     | Solvent               | Result               |
|-------|----------|---------------------------|-------------------------------------------------------------------------|----------------|-----------------------|----------------------|
| 1     | 8        | LDA                       | MeCO <sub>2</sub> Cl                                                    | -78 °C-RT      | THF/Et <sub>2</sub> O | Complex mixture      |
| 2     | 8        | NaH                       | Me <sub>2</sub> CO <sub>3</sub>                                         | RT-100 °C      | THF                   | β-eliminated product |
| 3     | 12       | LDA                       | MeCO <sub>2</sub> Cl                                                    | -78 °C-RT      | THF/Et <sub>2</sub> O | Complex mixture      |
| 4     | 12       | NaH                       | Me <sub>2</sub> CO <sub>3</sub>                                         | RT-100 °C      | THF                   | β-eliminated product |
| 5     | 12       | NaH                       | Me <sub>2</sub> CO <sub>3</sub> /CH <sub>2</sub> I <sub>2</sub> / MEMCl | 80-190 °C (μW) | Neat/THF              | β-eliminated product |
| 6     | 12       | KHMDS, CeCl <sub>3</sub>  | MeCO <sub>2</sub> Cl / Me <sub>2</sub> CO <sub>3</sub>                  | -110 °C        | THF                   | SM remained as such  |
| 7     | 12       | KHMDS, CeCl <sub>3</sub>  | MeCO <sub>2</sub> Cl / Me <sub>2</sub> CO <sub>3</sub>                  | -98 °C         | THF                   | β-eliminated product |
| 8     | 12       | NaHMDS, CeCl <sub>3</sub> | Me <sub>2</sub> CO <sub>3</sub> /CH <sub>2</sub> I <sub>2</sub> / MEMCl | -78 to -98 °C  | THF                   | β-eliminated product |

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Further, all attempts with compound **12** to convert to **13a** by changing the base, reaction temperature and other means of trapping the intermediate produced either the eliminated compound **13b** or there was no reaction at all (Table 2.1). These results nicely corroborated with that of observations made by Fraisse et al in 1999 on ribonoate<sup>5</sup> (Scheme 2.03A).



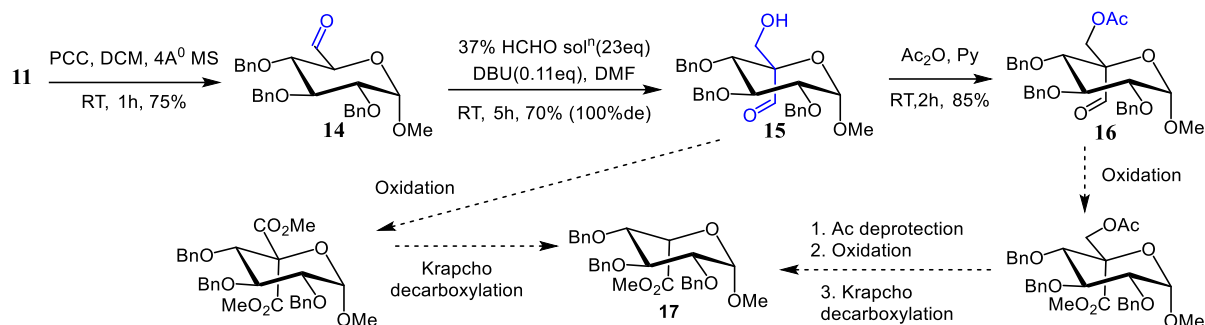
Scheme 2.03: A) P. Fraisse et al reaction of aldol condensation using KHMDS and  $\text{CeCl}_3$  reagents. B) C<sub>5</sub>-substitution reaction on C<sub>6</sub>-aldehyde functionality instead on C<sub>6</sub>-ester

Interestingly, alkylation under cross Cannizzaro conditions was explained in a Patent<sup>6</sup> (WO2015043511A1) utilising 37% HCHO in the presence of DBU (Scheme 2.03B). Accordingly, the ester **12** was treated with 37% formaldehyde and DBU to notice that the starting material remained as such; hence, envisioned that this cross Cannizzaro reaction might work with the C-6 aldehyde. Thus, the aldehyde compound **14** was prepared by the partial oxidation of compound **11** using PCC and surprisingly, aldehyde **14** smoothly got converted to the hydroxymethyl substituted compound **15** in the presence of 37% HCHO/DBU reagents in 70% isolated yield (Scheme 2.04A) with 100% diastereoselectivity.

NMR and mass spectroscopic studies confirmed the presence of both the aldehyde and primary alcohol groups and the structural homogeneity of the compound **15**.  $^1\text{H}$  NMR resonances at  $\delta$  9.9 ppm,  $\delta$  7.3-7.2 ppm,  $\delta$  3.2 ppm,  $\delta$  1.9 ppm were noticed the presence of aldehyde, 15 aromatic protons of benzyl, three protons of OMe, hydroxyl proton of alcohol respectively. Whereas, in the  $^{13}\text{C}$  NMR, anomeric carbon was observed at  $\delta$  99.8 ppm, the characteristic additional  $-\text{CH}_2$  was observed at  $\delta$  62.3 ppm. The absence of C-5 peak in the DEPT MNR spectrum clearly indicated the formation of quaternary C-5 carbon by formaldehyde substitution. Remaining three pyranoside ring  $-\text{CH}$ 's resonates between  $\delta$  76.7-79.9 ppm, benzyl  $-\text{CH}_2$  carbons appeared between  $\delta$  73.7-75.9 ppm and the peak at  $\delta$  56.3 ppm for  $-\text{CH}_2$

## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

OCH<sub>3</sub>carbon confirmed the assigned structure. Compound 15 further confirmed by MALDI-TOF analysis,  $m/z$  calculated for C<sub>29</sub>H<sub>32</sub>O<sub>7</sub> [M+K]<sup>+</sup>: 531.18, Found: 531.06.



*Scheme 2.04A: Aldehyde functionality was successfully utilized for C5-substitution reactions that was envisioned for the preparation of iduronate derivatives.*

Luckily, we could obtain good X-ray diffractable single crystals by the slow evaporation of compound 15 in CH<sub>2</sub>Cl<sub>2</sub> and the relative stereochemistry and overall structural homogeneity was confirmed through the single crystal X-ray diffraction studies (ORTEP diagram in Figure 2.2).

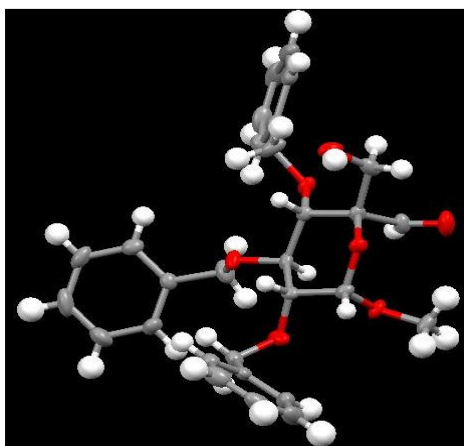
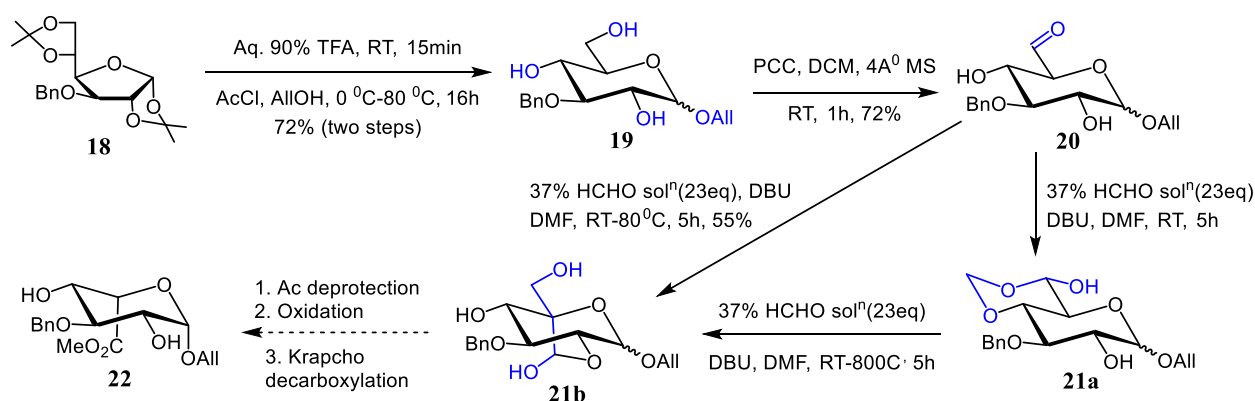


Figure 2.2: ORTEP diagram of Compound 15.

Krapcho decarboxylation is the next target after obtaining and confirming the C5-dialkylated compound for which the  $\beta$ -ketoester is required, that means, we need to oxidize the newly created hydroxymethyl moiety. All our attempts to synthesize compound 17 from compound 15 and the acetate 16 failed (Scheme 2.04A).

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Scheme 2.04B: C<sub>5</sub>-substitution of the aldehyde functionality was envisioned for the preparation of orthogonal iduronate (IdoA) derivatives.

In parallel, Aldehyde **20** was prepared from **18** utilising standard protocols and substituted at C-5 using depicted reaction conditions used *vide supra* imagining in turn the preparation of orthogonal IdoA derivatives. The resultant compound **21b** was observed while performing reactions at 80 °C instead at the room temperature thereby formation of the **21a**. Over again, it was failed to proceed further. However, Compounds **21a** and **21b** confirmed comparing <sup>1</sup>H, <sup>13</sup>C and DEPT NMR spectroscopy. Characteristic comparative signals of the <sup>13</sup>C NMR spectrums of isomeric α/β anomers of the compounds **21a** and **21b** are exposed in **Table 2.2**.

| Entry                       | Compound 21a                                | Compound 21b                              |
|-----------------------------|---------------------------------------------|-------------------------------------------|
| -CH hemiacetal              | 98.2, 97.7, 96.0, 90.0 (four signals)       | 95.6, 94.7, 94.3, 91.3 (four signals)     |
| -CH <sub>2</sub> hemiacetal | 91.3, 85.7 (two signals)                    | --                                        |
| Ring -CH                    | 65.9-79.9 (eight signals)                   | 80.3-69.2 (six signals)                   |
| Quaternary ring carbon      | --                                          | 76.6, 74.7 (two signals)                  |
| C-6 -CH <sub>2</sub> OH     | --                                          | 62.9, 62.3 (two signals)                  |
| Allyl                       | 133.3 (2C), 118.4 (2C), 68.9, 68.7          | 134.1, 133.3, 118.3, 117.7, 69.6, 69.2    |
| Benzyl                      | 138.6, 138.5, 128.4-127.7 (10C), 74.7, 74.8 | 137.5 (2C), 128.6-127.8 (10C), 71.5, 71.7 |

Table 2.2: Characteristic comparative <sup>13</sup>C NMR signals (δ ppm) of the α/β isomeric compounds **21a** and **21b**.

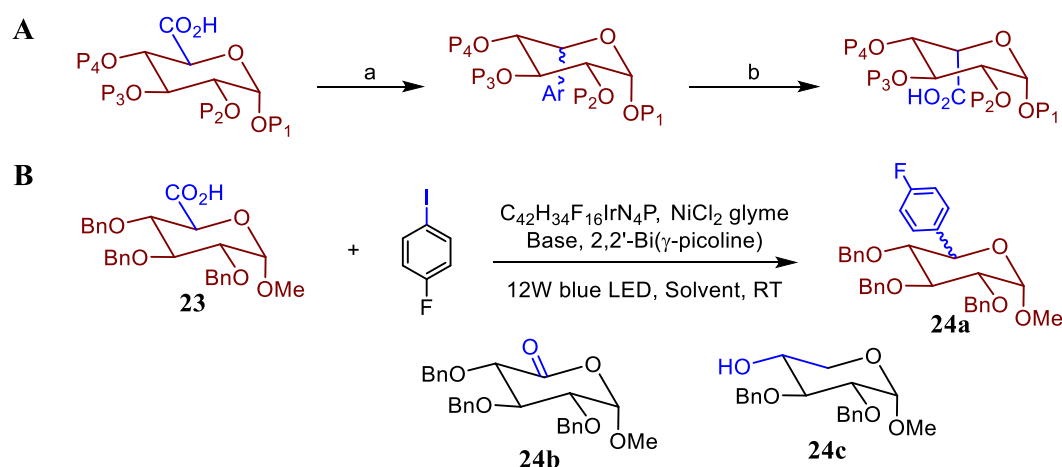
Furthermore, the <sup>1</sup>H NMR spectrums resonances as per the assigned structures indicating the formation of **21a** and **21b**. In addition, MALDI-TOF analysis, m/z calculated for C<sub>17</sub>H<sub>22</sub>O<sub>7</sub>

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$[M+K]^+$ : 377.10, Found: 376.94 and  $m/z$  calculated for  $C_{17}H_{22}O_7$   $[M+Na]^+$ : 361.13, Found: 360.98 confirmed the formation of **21a** and **21b** respectively (Scheme 2.04B).

### 2.3 Photoredox-Metal Catalysis Approach for Iduronate derivatives from Glucuronic Acid:

Recently David W. C. MacMillan and co-workers have been developed a very unique and advantageous photoredox-metal catalysis method for decarboxylative coupling reactions.<sup>7</sup> The photoredox-Ni/Ir-metal catalyzed decarboxylative aryl or vinyl cross-coupling has been exploited in amino acids as well. In yet another effort to obtain the iduronate from glucuronate, MacMillan decarboxylative arylation reaction was thought out so that the aryl group can be oxidized to the carboxylate at a later stage (Scheme 2.05A). Accordingly, glucuronic acid derivative **23** was synthesized from compound **11** prepared *vide supra* by well established TEMPO-BAIB oxidation condition and subjected to the coupling reaction with 4-fluoriodobenzene in the presence of nickel and iridium catalysts using blue LED (Scheme 2.05B).



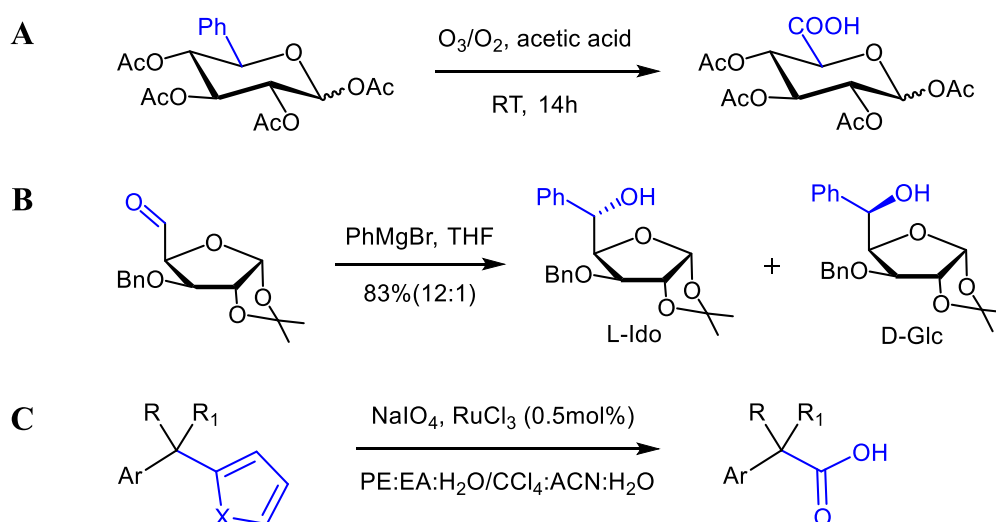
Scheme 2.05: Decarboxylative Arylation for Iduronic Acid Synthesis

Many reactions were conducted by varying base, solvent, time and in none of the cases, the expected product **24a** was not observed though trace quantities of very interesting products **24b-d** were noticed. Compound **24b** was confirmed by the absence of one of the sugar-ring – CH signals in the  $^1H$  and  $^{13}C$  NMR spectrum, the appearance of quaternary carboxyl peak in the  $^{13}C$  NMR spectrum at  $\delta$  169.9 ppm. Furthermore, the assigned compound **24b** was confirmed by MALDI-TOF analysis,  $m/z$  calculated for  $C_{27}H_{28}O_6$   $[M+K]^+$ : 487.15, Found: 487.17.

## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

### 2.4 Chelation Controlled Grignard Reactions and Selective Aryl Oxidations for L-Iduronate Derivatives Preparation:

In parallel, chelation controlled nucleophilic addition of aryl moiety was next envisioned; as such kind of reactions are reported to give high ido- selectivity over the gluco- configuration (Scheme 2.06A)<sup>8</sup> and selective aryl oxidation reactions<sup>9</sup> as well.

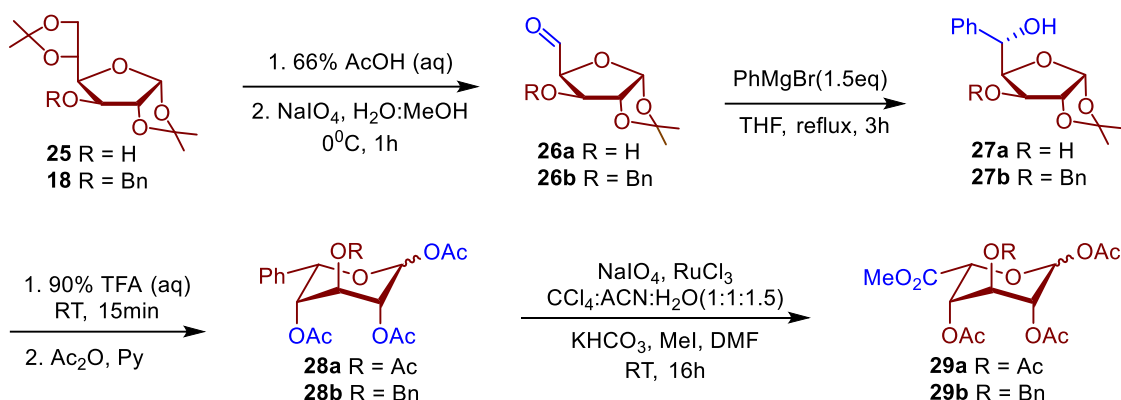


Scheme 2.06: Key reactions found those are imagined for the preparation L-iduronic acid.<sup>8,9</sup>

Accordingly, compound **26a** was prepared from precursor **25** via selective unmasking of the 5,6-acetonide in 66% aqueous acetic acid followed by oxidative cleavage of the diol intermediate employing sodium periodate reagent (Scheme 2.07). Next, **26a** was subjected to the chelation controlled Grignard reaction with phenyl magnesium bromide in THF to notice the formation of L-ido furanoside **27a** predominantly. Then, the acidic treatment of the L-idofuranoside **27a** using trifluoroacetic acid and subsequent acetylation under acetic anhydride/pyridine conditions provided L-idopyranoside **28a**. Further, the oxidation of L-idopyranoside **28a** was smoothly conducted utilizing ruthenium tetraoxide ( $\text{RuO}_4$ )<sup>9b,c</sup> that was generated *in situ* from  $\text{RuCl}_3$  and  $\text{NaIO}_4$ , followed by the treatment of potassium carbonate and methyl iodide successfully afforded much-desired methyl iduronate **29a**. NMR spectral analysis showed sharp singlet at  $\delta$  3.78 ppm for three methyl protons,  $^{13}\text{C}$  resonances for  $-\text{CH}_3$  at  $\delta$  52.9 ppm and the five signals in the carboxyl region, with the absence of phenyl group clearly indicated the formation of **29a** as a methyl iduronate product. Synthesis of heparin oligosaccharide warrants the installation of benzyl ether at the C-3 position of the iduronate building block. Therefore, the aldehyde compound **26b** was prepared from C-3-benzyl ether **18** and utilized for the Grignard reaction with phenyl magnesium bromide to afford **27b** as an

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L-idose isomer predominantly. Subsequently, conversion of compound **27b** to the compound **28b** and further extrapolation to compound **29b** under aforementioned conditions was successfully carried out. At this stage, the regioselective oxidation of the phenyl group in the presence of C-3 benzyl ether posed as a serious challenge. Unfortunately, several oxidation conditions were applied and to our dismay, the regioselectively oxidized product **29b** could not be isolated in satisfactory yield.



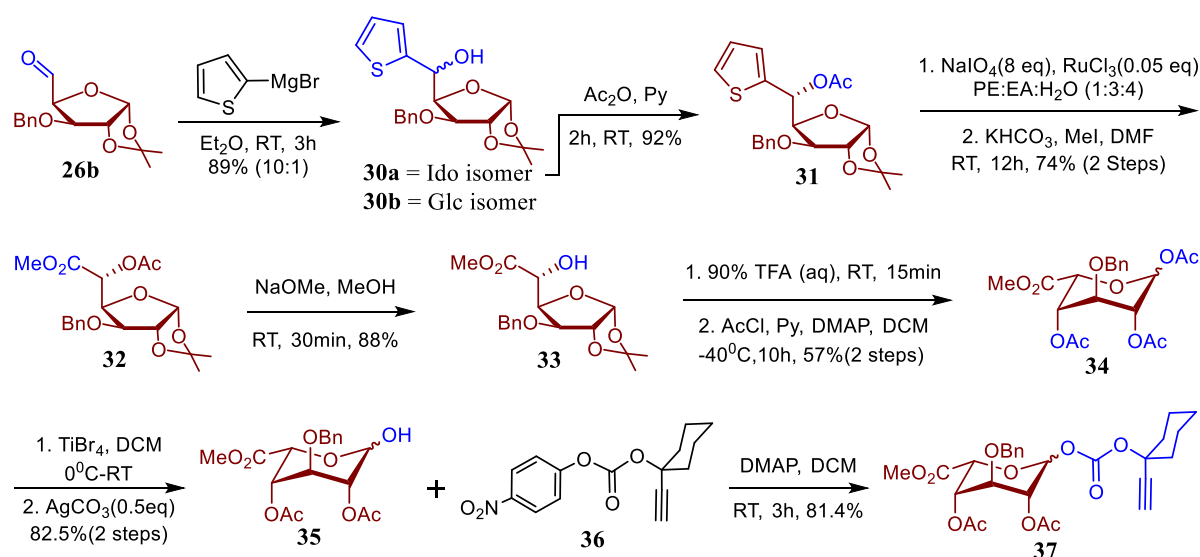
*Scheme 2.07: PhMgBr as a Key Grignard reagent for iduronic acid (IdoA) preparation via chelation controlled Grignard reaction.*

However, the chelation controlled Grignard reaction was noticed as a highly beneficial method for iduronic acid synthesis because of its efficiency towards large scale synthesis and high diastereoselectivity. Hence, the remarkable results from the Ishii<sup>9b</sup> as well as Sato<sup>9c</sup> groups revealed that the addition of hetero-aromatic ring in the presence of C-3 benzyl group followed by selective oxidation under mild oxidizing conditions can resolve the problem. Therefore, aldehyde **26b** was treated with freshly prepared thiophenylmagnesium bromide in diethyl ether as a solvent to notice formation of 10:1 diastereoselective in favour of L-idofuranoside **30a** in 85% yield (Scheme 2.08). In the <sup>1</sup>H NMR spectrum the appearance of aromatic thienyl signals at δ 7.38 – 7.25 (m, 1H along with five benzyl protons), 7.01 (d, *J* = 3.4 Hz, 1H), 6.95 (dd, *J* = 5.0, 3.5 Hz, 1H) and the hydroxyl signal at δ 2.99 (s, 1H). While, in the <sup>13</sup>C NMR spectrum, quaternary carbon resonated at δ 142.6 ppm, aromatic –CH resonances at δ 126.7, 125.3, 125.2 ppm, appearance of the four ring carbon signals and in turn thienyl oxidation confirmed the formation of thienyl compound **30a**. Oxidation of thiophene conditions were optimized and found that *in situ* generated RuO<sub>4</sub> in petroleum ether:ethylacetate:water (1:3:4) can convert compound **30a** to the corresponding carboxylic acid product exclusively. Subsequent, methyl ester was prepared using potassium carbonate and methyl iodide to afford 74% of ester **32** over



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two steps. Zemplén deacetylation afforded alcohol **33** as a L-idofuranoate derivative that was thoroughly confirmed by the analytical data and gratifyingly, the data was in complete agreement with that of the previous reports. Further, the trifluoroacetic acid-mediated 1,2-acetonide deprotection followed by the preparation of 1,2,4-tri-*O*-acetylation using acetyl chloride/pyridine/DMAP/-40 °C afforded idopyranoside **34**. Conversion of the anomeric esters to hemiacetals **35** was accomplished via titanium tetrabromide via corresponding glycosyl bromide intermediate (Scheme 2.08). Finally, the resulting hemiacetals **35** were treated with 1-ethynylcyclohexyl (4-nitrophenyl) carbonate **36** in the presence of a dimethylaminopyridine (DMAP) to obtain the stable IdoA carbonate donor **37** for further silver assisted gold-catalyzed glycosylation reactions.



*Scheme 2.08: Thienyl magnesium bromide as a Key reagent for iduronic acid (IdoA) preparation via chelation controlled Grignard reaction.*

In addition to the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy (Table 2.3, predominantly showing  $\alpha$ -isomer), the successful synthesis of donor **37** was confirmed by mass and IR spectroscopy.  $m/z$  calculated for  $\text{C}_{27}\text{H}_{32}\text{O}_{11}$   $[\text{M}+\text{Na}]^+$ : 555.1842 was found: 555.1840 in the HRMS (ESI-MS) analysis whilst IR analysis noticed the characteristic peaks at  $3272\text{ cm}^{-1}$  (alkynyl C-H) and  $1750\text{ cm}^{-1}$  (carbonyl).

| Entry             | $^1\text{H}$ NMR ( $\delta$ ppm)                                                                                            | $^{13}\text{C}$ NMR ( $\delta$ ppm)                                                                      |
|-------------------|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Anomeric          | 5.97 (d, $J = 1.7$ Hz, 1H)                                                                                                  | 92.7                                                                                                     |
| Carbonate         | --                                                                                                                          | 150.9                                                                                                    |
| Alkynylcyclohexyl | 2.64 (s, 1H) and<br>2.21 – 2.11 (m, 2H), 1.94 – 1.82 (m, 2H), 1.72 – 1.57 (m, 4H), 1.54 – 1.48 (m, 1H), 1.38 – 1.28 (m, 1H) | Quaternary and alkynyl- 82.5, 78.9, 75.4.<br>Cyclohexyl (-CH <sub>2</sub> )- 36.9, 36.6, 25.0, 22.5 (2C) |

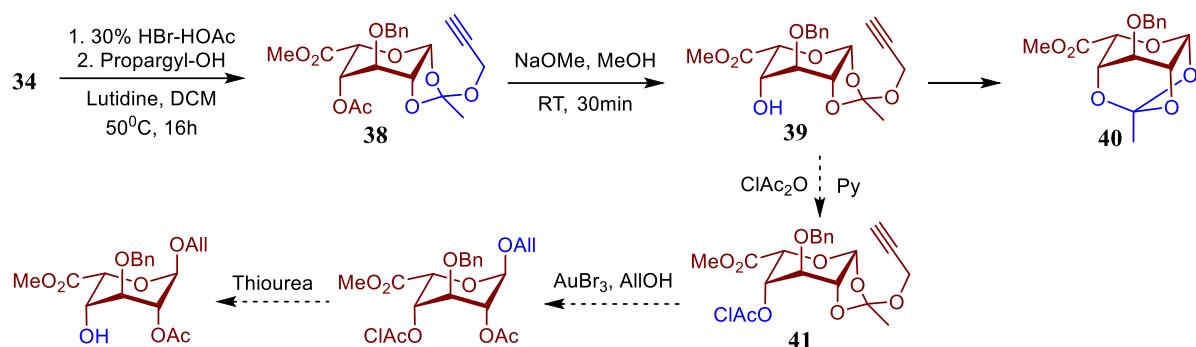
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|        |                            |                                             |
|--------|----------------------------|---------------------------------------------|
|        |                            |                                             |
| -COOMe | 3.77 (s, 3H)               | 52.7 (CH <sub>3</sub> ), 167.4 (carbonyl)   |
| Acetyl | 2.10 (s, 3H), 2.03 (s, 3H) | Carbonyl-170.0, 169.8.<br>Methyl-21.0, 20.7 |

**Table 2.3 Characteristic <sup>1</sup>H and <sup>13</sup>C NMR signals of carbonate donor **37****

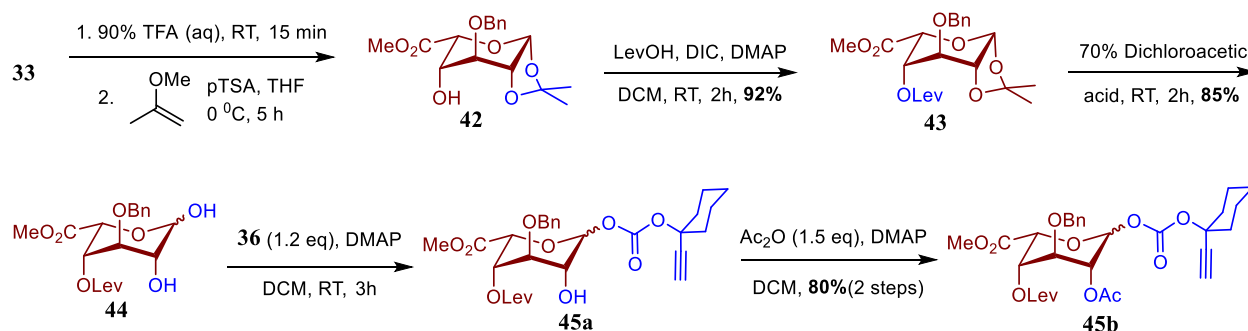
Even though the above IdoA donor **37** was in hand, the difficulties have remained in the identification of C-2 and C-4 positions, as both are protected with acetate groups. As we know for heparin oligosaccharide synthesis, iduronic acid should be orthogonally protected. As the C-1 and C-4 positions participating in the glycosidic bond formation, C-1 acts as a donor while C-4 shall act as an acceptor. Apart from these, C-2 and C-3 should be protected in such a way that the latter can be freed and sulfated. Hence, protecting groups on C-2 and C-4 are required to be orthogonal. Hence, this was imagined from engaging C-2 position as 1,2-orthoester; by the conversion of 1,2,4-tri-*O*-acetylated L-idopyranoate **34** to the propargyl orthoester **38**, via glycosyl bromide intermediate preparation using HBr-HOAc, followed by the propargyl alcohol/2,6-lutidine reaction (Scheme 2.09). The characteristic quaternary carbon resonances at  $\delta$  124.2 ppm with the absence of C1-OAc and C2-OAc resonances and the presence of propargyl moiety in NMR spectra confirmed the formation of orthoester **38**. Further, the Zemplén deacetylation under NaOMe/MeOH underwent smoothly; however, isolation of the product became a nightmare as it led to the formation of the highly stable methyl orthoformate iduronate **40**. Upfield shift of orthoester quaternary carbon from  $\delta$  124.2 to  $\delta$  118.3 ppm with the absence of propargyl moiety in the NMR spectrum confirms the formation of 1,2,4-orthoacetate **40** that also agreed to the previously reported NMR data. However, a very careful work up by slowly pouring over ice-cold water followed by the extraction in cold CH<sub>2</sub>Cl<sub>2</sub> enabled us to isolate crude compound **39** that was immediately subjected to the chloroacetylation reaction to produce compound **41** albeit in poor yield. Overall, increase in the number of steps and work-up sensitive functional groups posed yet another hurdle to proceed further for the large scale preparation of the iduronic acid building block.

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Scheme 2.09: Propargyl orthoester was envisioned for orthogonal protection of L-Iduronate

Alternatively, the locking of 1,2-acetonide in L-idopyranoate **42** was accomplished from precursor **33**. Acid-mediated furanose to pyranose conversion following the reaction with 2-methoxypropene using a catalytic amount of *p*-toluene sulphonic acid in tetrahydrofuran provided isopropylidene **42** in a 57% yield over two steps<sup>10</sup> (Scheme 2.10). Compound **42** can be used as an acceptor for further glycosylation reactions or it can be converted into the glycosyl donor by masking free hydroxyl with a temporary protecting group. Accordingly, 4-*O*-levulinate **43** was prepared by the treatment of levulinic acid, *N,N'*-diisopropyl carbodiimide (DIC) as a coupling reagent and further, unblocking of 1,2-acetonide using 70% dichloroacetic acid was performed to obtain hemiacetals **44**. Reaction between the carbonate reagent **36** and hemiacetals **44** in the presence of DMAP followed acetylation reaction using acetic anhydride afforded the regioselectively key iduronic acid building block **45b** in 80% yield with all the orthogonal protections in place.

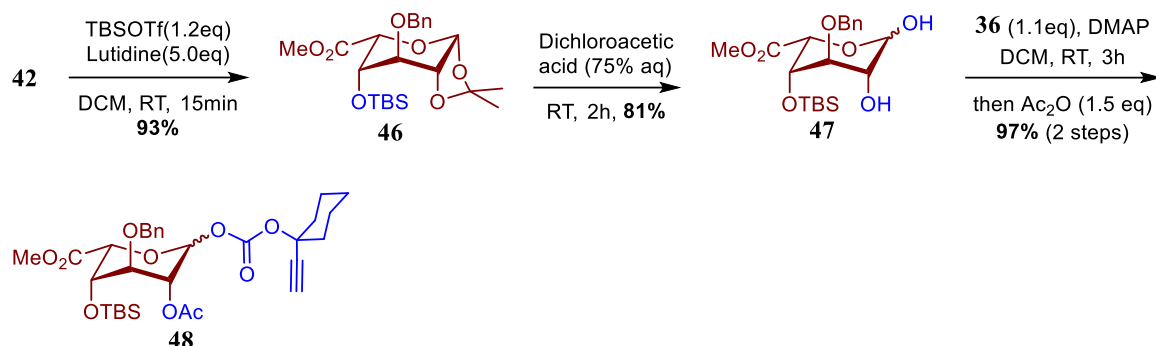


Scheme 2.10: Role of 1,2-acetonide locking in the L-idopyranoate acceptor for orthogonally protected IdoA carbonate donor preparation.

Alternatively, the silyl protection at C-4 position to the iduronate donor may yield better during the glycosylation reaction and thus, compound **42** was also converted to 4-*O*-*t*-butyldimethylsilyl iduronate **46** by using *t*-butyldimethylsilyl trifluoromethane sulfonate (TBSOTf) in the presence of excess amount of 2,6-lutidine. Further, 1,2-acetonide was

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unblocked using 75% dichloroacetic acid, resulting hemiacetals **47** were converted to C4-OTBS idopyranoate donor **48** by using carbonate reagent **36** and DMAP followed by *in situ* 2-O-acetate protection.



Scheme 2.11: Orthogonally protected 4-O-TBS-L-Iduronate **48** synthesized as a carbonate donor that was envisioned for a high yielding glycosylation reactions.

Moreover, with the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy (**Table 2.4**), the successful synthesis of donor **48** was confirmed by mass and IR spectroscopy. HRMS (ESI-MS) analysis,  $m/z$  calculated for  $\text{C}_{31}\text{H}_{44}\text{O}_{10}\text{Si}[\text{M}+\text{Na}]^+$ : 627.2601 was actually found: 627.2605 and the IR analysis noticed the characteristic peaks at  $3289^{-1}$  (alkynyl C-H) and  $1764$ ,  $1742\text{ cm}^{-1}$  (carbonyl).

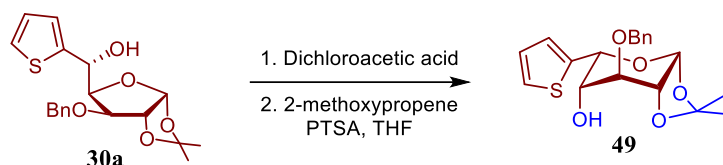
| Entry             | $^1\text{H}$ NMR ( $\delta$ ppm)                                                                    | $^{13}\text{C}$ NMR ( $\delta$ ppm)                                                                      |
|-------------------|-----------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Anomeric          | 5.91 (d, $J = 1.8$ Hz, 1H)                                                                          | 93.1                                                                                                     |
| Carbonate         | --                                                                                                  | 151.1                                                                                                    |
| Alkynylcyclohexyl | 2.62 (s, 1H) and 2.21 – 2.11 (m, 2H), 1.90 – 1.81 (m, 2H), 1.69 – 1.50 (m, 5H), 1.34 – 1.29 (m, 1H) | Quaternary and alkynyl- 82.6, 78.6, 75.4.<br>Cyclohexyl (-CH <sub>2</sub> )- 36.9, 36.7, 25.0, 22.6 (2C) |
| -COOMe            | 3.75 (s, 3H)                                                                                        | 52.3 (CH <sub>3</sub> ), 168.4 (carbonyl)                                                                |
| -OTBS             | 0.78 (s, 9H), -0.12 (s, 3H), -0.23 (s, 3H)                                                          | 25.6 (3C), -4.5, -5.7                                                                                    |
| Acetyl            | 2.08 (s, 3H)                                                                                        | Carbonyl-170.8<br>Methyl-21.2                                                                            |

Table 2.3 Characteristic  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals of orthogonal carbonate donor **48**

Alternatively, thienyl L-idofuranoside derivative **30a** was directly converted to the thienyl L-idopyranoside **49** through an acid-mediated hydrolysis of 1,2-acetonide that converts the furanose to the pyranose and the resulting 1,2-diol was locked as acetonide again (scheme 2.12). Trifluoroacetic acid or dichloroacetic acid used for acetonide unlocking while the 2-methoxypropene/PTSA/THF conditions were employed for locking it as the pyranoside. Formation of compound **49** was confirmed by converting it into **42** prepared *vide supra*

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employing  $\text{RuCl}_3/\text{NaIO}_4$  conditions followed by esterification reaction as delineated above. The thienyl group in this monosaccharide can be explored as a surrogate for the carboxylic for a late stage functional group interconversions.



Scheme 2.12: Conversion of thienyl L-idofuranoside to the thienyl L-idopyranoside **49**.

### 2.5 Conclusions:

In conclusion, a novel route for the synthesis of orthogonally protected iduronic acid monosaccharide building block has been accomplished. Salient features of this endeavour are: (i) chelation controlled Grignard addition of aryl moieties is highly beneficial for the synthesis of iduronate; (ii)  $\text{RuO}_4$  can oxidize the thiophenyl moiety to the carboxylic acid; (iii) a short synthesis of the key building block.

### 2.6 Experimental Section:

**Compound 8: 1°-OH Oxidation and Methyl Ester Preparation:** To a biphasic solution of the 4,6-diol (1 mmol) in 2:1  $\text{CH}_2\text{Cl}_2$ –water (10 mL) was added BAIB (2.5 eq) and TEMPO (0.2 eq) simultaneously, stirred vigorously at 25 °C. After 3 h, the reaction was quenched by the addition of a saturated solution of  $\text{Na}_2\text{SO}_3$  and extracted by  $\text{CH}_2\text{Cl}_2$  (3 x 10 mL), the combined organic phases were washed with brine (10 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude product was redissolved in anhydrous DMF (5 mL) and added 1.5 equivalent of  $\text{K}_2\text{CO}_3$ . After stirring for 15 min, iodomethane (2 eq) was added dropwise under argon atmosphere and stirred for 8h at 25 °C in a dark place. After complete consumption, the reaction was arrested by adding saturated solution of  $\text{Na}_2\text{SO}_3$ , brine (5 mL) and water (10 mL); and extracted with EtOAc (2 x 10 mL). The combined organic phase was washed with brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated under reduced pressure. The crude residue was purified by column chromatography using ethyl acetate and *n*-hexane as mobile phase to obtain the desired product in a 72% yield over two steps.  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  8.02 – 7.94 (m, 4H), 7.91 – 7.86 (m, 2H), 7.57 – 7.50 (m, 2H), 7.45 (t,  $J$  = 7.4 Hz, 1H), 7.42 – 7.37 (m, 4H), 7.31 (t,  $J$  = 7.8 Hz, 2H), 5.96 (t,  $J$  = 9.4 Hz, 1H), 5.73 (t,  $J$  = 9.4 Hz, 1H), 5.58 (dd,  $J$  = 9.4, 7.5 Hz, 1H), 4.83 (d,  $J$  = 7.4 Hz, 1H), 4.41 (d,  $J$  = 9.5 Hz, 1H), 3.73 (s, 3H), 3.60 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  167.5, 165.8, 165.3, 165.1, 133.6,

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133.4, 133.4, 129.9 (6C), 129.2, 128.9, 128.8, 128.5 (4C), 128.4, 128.4, 102.2, 73.1, 72.2, 71.6, 70.3, 57.6, 53.0.

**Compound 9b ( $\beta$ -eliminated):**  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ )  $\delta$  8.06 – 7.97 (m, 4H), 7.58 – 7.52 (m, 2H), 7.44 – 7.38 (m, 4H), 6.25 (d,  $J$  = 2.8 Hz, 1H), 6.09 (dd,  $J$  = 8.4, 2.9 Hz, 1H), 5.58 (dd,  $J$  = 8.3, 2.7 Hz, 1H), 5.38 (d,  $J$  = 2.6 Hz, 1H), 3.85 (s, 3H), 3.57 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ )  $\delta$  165.9, 165.8, 162.2, 141.9, 133.7, 133.6, 130.1, 130.1, 129.9, 129.9, 129.5, 129.1, 128.6 (4C), 109.0, 98.8, 69.7, 67.2, 57.2, 52.8; MALDI-TOF:  $m/z$  calcd for  $\text{C}_{22}\text{H}_{20}\text{O}_8$   $[\text{M}+\text{K}]^+$ : 451.08, Found: 451.05.

**Compound 12:** This compound was prepared from **11**, following the procedure described for the preparation of compound **8**. Yield-74% over two steps.  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ )  $\delta$  7.36 – 7.26 (m, 13H), 7.23 (dd,  $J$  = 7.8, 1.5 Hz, 2H), 4.95 (d,  $J$  = 10.9 Hz, 1H), 4.84 – 4.77 (m, 3H), 4.64 (d,  $J$  = 12.1 Hz, 1H), 4.60 – 4.55 (m, 2H), 4.19 (d,  $J$  = 10.0 Hz, 1H), 3.99 (t,  $J$  = 9.3 Hz, 1H), 3.74 – 3.71 (m, 1H), 3.70 (s, 3H), 3.57 (dd,  $J$  = 9.6, 3.6 Hz, 1H), 3.39 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  170.2, 138.7, 138.0, 138.0, 128.6, 128.6, 128.5 (4C), 128.3, 128.3, 128.2, 128.1 (4C), 127.9, 127.9, 127.8, 127.8, 98.9, 81.5, 79.7, 79.4, 76.0, 75.3, 73.7, 70.2, 55.8, 52.6.

**Compound 13b ( $\beta$ -eliminated):**  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37 – 7.28 (m, 10H), 6.14 (d,  $J$  = 2.8 Hz, 1H), 4.92 (d,  $J$  = 2.5 Hz, 1H), 4.82 (d,  $J$  = 12.2 Hz, 1H), 4.74 – 4.68 (m, 3H), 4.37 (dd,  $J$  = 7.8, 2.8 Hz, 1H), 3.81 – 3.78 (m, 4H), 3.49 (s, 3H); MALDI-TOF:  $m/z$  calcd for  $\text{C}_{22}\text{H}_{24}\text{O}_6$   $[\text{M}+\text{K}]^+$ : 423.12, Found: 423.12.

**Compound 14:** To a solution of alcohol **11** (0.5 g) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added 4Å MS and after 15 min stirring reaction was added 1.5 equivalent of PCC. After 1h stirring at RT, reaction was quenched by the addition of a saturated solution of  $\text{Na}_2\text{SO}_3$  and extracted by  $\text{CH}_2\text{Cl}_2$  (3 x 10 mL), the combined organic phases were washed with brine (10 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. Crude residue was purified by column chromatography using ethyl acetate and *n*-hexane as mobile phase to obtain 75% of aldehyde **14**.  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  9.67 (s, 1H), 7.37 – 7.32 (m, 15H), 5.02 (d,  $J$  = 10.8 Hz, 1H), 4.90 (d, 1H), 4.84 (d,  $J$  = 3.5 Hz, 1H), 4.81 (d,  $J$  = 1.8 Hz, 1H), 4.67 – 4.63 (m, 3H), 4.19 (d,  $J$  = 10.5 Hz, 1H), 4.14 – 4.08 (m, 1H), 3.62 – 3.57 (m, 1H), 3.54 – 3.50 (m, 1H), 3.40 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  197.6, 138.5, 137.9, 137.5, 128.6 (8C), 128.3, 128.2 (3C), 128.1, 128.1, 127.9, 98.5, 81.8, 79.3, 77.9, 76.0, 75.2, 74.3, 73.7, 55.9.

**Compound 15:** To a solution of aldehyde **14** (0.5 g) in DMF (10 mL) was added 37% aqueous formaldehyde solution (23 eq) and DBU (0.1 eq). Reaction mixture was stirred at RT for 5 h. After completion, water was added and extracted with EtOAc, the combined organic phase

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washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Crude residue was purified by column chromatography using ethyl acetate and *n*-hexane afforded **15** in a 70% yield as a single diastereotopic isomer. <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>) δ 9.92 (s, 1H), 7.37 – 7.21 (m, 15H), 5.02 (d, *J* = 10.8 Hz, 1H), 4.93 (d, *J* = 11.3 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 12.0 Hz, 1H), 4.69 (d, *J* = 11.3 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.49 (d, *J* = 3.3 Hz, 1H), 4.30 (t, *J* = 9.6 Hz, 1H), 3.95 (d, *J* = 9.6 Hz, 1H), 3.77 (d, *J* = 12.2 Hz, 1H), 3.54 – 3.43 (m, 2H), 3.18 (s, 3H), 1.86 (s, 1H); <sup>13</sup>C DEPT NMR (100.67 MHz, CDCl<sub>3</sub>) δ 128.6, 128.6, 128.5 (4C), 128.2, 128.1, 128.1, 128.0 (3C), 127.8 (3C), 99.8, 79.9, 79.2, 76.7, 75.9, 75.9, 73.7, 62.3, 56.3; MALDI-TOF: *m/z* calcd for C<sub>29</sub>H<sub>32</sub>O<sub>7</sub> [M+K]<sup>+</sup>: 531.18, Found: 531.06.

**Compound 16:** Compound 15 was dissolved in pyridine followed by the addition of 1.5 eq of acetic anhydride and cat. DMAP. Reaction mixture was stirred at RT for 2 h. After completion, water was added and extracted by EtOAc, the combined organic phase washed with 2NHCl and brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Crude residue was purified by column chromatography using ethyl acetate and *n*-hexane to get **16** in an 85% yield. <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>) δ 9.91 (s, 1H), 7.37 – 7.28 (m, 13H), 7.25 – 7.22 (m, 2H), 5.05 (d, *J* = 10.5 Hz, 1H), 4.91 (d, *J* = 11.1 Hz, 1H), 4.88 – 4.81 (m, 2H), 4.65 (d, *J* = 12.1 Hz, 1H), 4.61 (d, *J* = 11.1 Hz, 1H), 4.56 (d, *J* = 3.5 Hz, 1H), 4.37 – 4.28 (m, 2H), 4.10 (d, *J* = 12.4 Hz, 1H), 3.85 (d, *J* = 9.4 Hz, 1H), 3.53 (dd, *J* = 9.7, 3.5 Hz, 1H), 3.20 (s, 3H), 1.99 (s, 3H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>) δ 197.5, 170.3, 138.3, 137.9, 137.5, 128.7 (4C), 128.6, 128.6, 128.3, 128.2, 128.2, 128.1 (3C), 128.0 (3C), 99.5, 79.9, 79.3, 78.1, 76.7, 76.1, 75.7, 73.8, 62.8, 56.4, 20.9; MALDI-TOF: *m/z* calcd for C<sub>31</sub>H<sub>34</sub>O<sub>8</sub> [M+Na]<sup>+</sup>: 557.22, Found: 557.11.

**Compound 19: Glc-Allyl Glycoside Preparation: α:β (3:1):** To the compound **18** (74 g, 211.18 mmol) in a 1L round bottom flask was added 90% aqueous trifluoroacetic acid (320 mL) and the reaction mixture was stirred for 15 min at 25 °C. The solvent was evaporated under reduced pressure and the residue was co-evaporated twice with water and twice with toluene. The crude was triturated in minimum quantity of the 10% ethyl acetate in hexane, washed with hexane and the trace solvent was removed by applying high vacuum. The crude was redissolved in anhydrous allyl alcohol (50 eq) and the mixture cooled to 0 °C, acetyl chloride (2.5 eq) was added dropwise under argon atmosphere, the reaction mixture was stirred at 0 °C for 1h, refluxed at 80 °C for 12-16 h. After complete conversion, the reaction was quenched by adding excess of Et<sub>3</sub>N and the volatile components were removed under vacuum. The crude residue was purified by silica gel column chromatography using 50% ethyl acetate in *n*-hexane as mobile phase to afford compound **19** (49.5 g, 76%, over two steps) as a thick syrup; [α]<sup>25</sup><sub>D</sub> (CHCl<sub>3</sub>, c1.0): +63.1°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3394, 1923, 1643, 1362, 1216, 1028,



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927, 746, 700;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.38 – 7.25 (m, 10H), 5.97 – 5.85 (m, 2H), 5.30 (ddq,  $J$  = 17.3, 3.0, 1.5 Hz, 2H), 5.24 – 5.19 (m, 2H), 5.0-4.93 (d,  $J$  = 11.5 Hz, 2H), 4.87 (d,  $J$  = 3.5 Hz, 1H), 4.77-4.71 (d,  $J$  = 11.6 Hz, 2H), 4.35 (dt,  $J$  = 5.4, 1.4 Hz, 1H), 4.32 (d,  $J$  = 7.7, 1H), 4.19 (ddt,  $J$  = 12.8, 5.3, 1.4 Hz, 1H), 4.13 – 4.07 (ddt, 1H), 4.04 – 3.98 (m, 1H), 3.85 – 3.81 (m, 1H), 3.76 (d,  $J$  = 3.5 Hz, 2H), 3.72 (m, 1H), 3.66 – 3.54 (m, 5H), 3.53 – 3.47 (m, 1H), 3.39 (t,  $J$  = 9.0 Hz, 1H), 3.32 – 3.27 (m, 1H), 2.91 (s, 1H), 2.71 (s, 1H), 2.47 – 2.28 (m, 2H), 2.26 (m, 2H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  138.6, 138.6, 133.7, 133.6, 128.7(4C), 128.1(5C), 128.0, 118.3, 118.3, 102.0, 97.8, 83.8, 82.9, 75.4, 75.1, 74.9, 74.4, 72.8, 71.4, 70.6, 70.0, 70.0, 68.7, 62.4, 62.2; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{22}\text{O}_6$   $[\text{M}+\text{Na}]^+$ : 333.1314, Found: 333.1316.

**Compound 20:** This compound was prepared from **19**, following the procedure described for the preparation of compound **8**. Yield-72%.

**Compound 21a:** This compound was prepared from **20**, following the procedure described for the preparation of compound **15**.  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.41 – 7.27 (m, 10H), 5.94 (ddt,  $J$  = 16.8, 11.5, 5.9 Hz, 2H), 5.38 (s, 1H), 5.35 (dd,  $J$  = 5.4, 1.5 Hz, 1H), 5.30 (dd,  $J$  = 5.4, 1.5 Hz, 1H), 5.28 – 5.22 (m, 3H), 5.11 (d,  $J$  = 6.6 Hz, 1H), 4.99 (dd,  $J$  = 8.3, 3.8 Hz, 2H), 4.92 (dd,  $J$  = 11.5, 3.5 Hz, 2H), 4.78 (dd,  $J$  = 11.2, 4.2 Hz, 5H), 4.25 (ddd,  $J$  = 18.7, 12.8, 5.3 Hz, 2H), 4.07 (dt,  $J$  = 12.8, 6.4 Hz, 2H), 3.95 – 3.85 (m, 2H), 3.81 – 3.65 (m, 4H), 3.48 – 3.37 (m, 2H), 3.26 (s, 1H), 2.99 (s, 1H), 2.27 (d,  $J$  = 7.6 Hz, 2H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  138.6, 138.5, 133.5, 133.4, 128.5 (4C), 128.0 (4C), 127.9, 127.8, 118.5, 118.5, 98.3, 97.9, 96.2, 91.4, 90.1, 85.9, 79.8, 79.6, 79.2, 74.9, 74.8, 74.8, 72.3, 72.0, 69.2, 69.0, 68.8, 66.1; MALDI-TOF:  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{O}_7$   $[\text{M}+\text{K}]^+$ : 377.10, Found: 376.94.

**Compound 21b:** This compound was prepared from **20**, following the procedure described for the preparation of compound **15** (Reaction temperature- 80 °C). Yield-55%.  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.40 – 7.27 (m,  $J$  = 3.9, 2.7 Hz, 5H), 5.90 (ddq,  $J$  = 16.7, 11.6, 5.9 Hz, 1H), 5.37 – 5.15 (m, 3H), 5.11 – 5.03 (m, 1H), 4.71 (dd,  $J$  = 11.8, 4.3 Hz, 1H), 4.55 (t,  $J$  = 12.7 Hz, 1H), 4.31 (ddd,  $J$  = 29.7, 12.8, 5.3 Hz, 1H), 4.15 – 4.02 (m, 3H), 3.95 – 3.83 (m, 2H), 3.79 – 3.39 (m, 4H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  137.5, 137.5, 134.2, 133.4, 128.6 (4C), 128.1, 128.1, 127.9 (4C), 118.4, 117.8, 95.7, 94.8, 94.4, 91.4, 80.4, 80.2, 76.6, 74.7, 73.7, 72.5, 71.8, 71.6, 69.7, 69.6, 69.4, 69.3, 63.0, 62.4; MALDI-TOF:  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{O}_7$   $[\text{M}+\text{Na}]^+$ : 361.13, Found: 360.98.

**Compound 24b:** To a solution of *p*-fluoro-iodobenzene (90 mg) and GlcA compound **23** (1.5 eq) in anhydrous DMF (20 ml) was added 2,2'-Bi( $\gamma$ -picoline) (0.15 eq), anhydrous cesium carbonate (1.5 eq) and the mixture was purged under nitrogen atmosphere for 15 minute.



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Subsequently, Ir[dF(CF<sub>3</sub>)ppy]<sub>2</sub>(dtbbpy)(PF<sub>6</sub>) (0.01 eq) and NiCl<sub>2</sub>.glyme (0.1 eq) were added and the reaction mixture was stirred vigorously under blue LED lights at 25 °C. After completion (24-36h), ice cold water was added and extracted by EtOAc, the combined organic phase washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Crude residue was purified by column chromatography using ethyl acetate and *n*-hexane. <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>) δ 7.40 (dd, *J* = 7.5, 1.8 Hz, 2H), 7.35 – 7.28 (m, 13H), 5.08 (d, *J* = 11.2 Hz, 1H), 4.97 (d, *J* = 2.5 Hz, 1H), 4.82 (d, *J* = 12.1 Hz, 1H), 4.74 (d, *J* = 6.9 Hz, 3H), 4.70 (d, *J* = 1.9 Hz, 1H), 4.15 (t, *J* = 7.9 Hz, 1H), 4.02 (d, *J* = 7.9 Hz, 1H), 3.74 (dd, *J* = 7.9, 2.5 Hz, 1H), 3.55 (s, 3H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>) δ 169.9, 138.0, 137.6, 137.3, 128.7, 128.7, 128.6 (6C), 128.3, 128.2 (3C), 128.1, 128.1, 128.0, 100.7, 79.3, 78.8, 76.8, 74.9, 74.4, 73.8, 57.5; MALDI-TOF: *m/z* calcd for C<sub>27</sub>H<sub>28</sub>O<sub>6</sub> [M+K]<sup>+</sup>: 487.15, Found: 487.17.

**Compound 24c:** This compound was prepared from **23**, following the procedure described for the preparation of compound **24b** (Note- Solvent changed as DMSO). <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>) δ 7.38 – 7.28 (m, 10H), 4.96 (d, *J* = 11.5 Hz, 1H), 4.75 (d, *J* = 12.1 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 2H), 4.59 (d, *J* = 3.2 Hz, 1H), 3.73 (dq, *J* = 7.5, 3.7 Hz, 1H), 3.69 – 3.60 (m, 2H), 3.58 – 3.48 (m, 2H), 3.41 (s, 3H), 2.39 (s, 1H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>) δ 138.7, 138.1, 128.7, 128.7, 128.6, 128.6, 128.2, 128.2, 128.1 (3C), 128.0, 98.7, 81.2, 79.4, 75.2, 73.4, 69.7, 62.0, 55.6; MALDI-TOF: *m/z* calcd for C<sub>20</sub>H<sub>24</sub>O<sub>5</sub> [M+K]<sup>+</sup>: 383.13, Found: 383.17.

**Compound 26a: Aldehyde Preparation via Diol intermediate:** Diacetonide compound **25** (20 g, 76.84 mmol) was dissolved in 120 mL of the 66.6% aqueous acetic acid and the mixture was stirred at 50 °C for 8 h. After removal of the solvent under reduced pressure, the remaining residue was co-evaporated twice with water and twice with toluene and the crude residue was purified by silica gel column chromatography to afford 14 g (83%) of the diol intermediate as a white solid. Next, diol intermediate (12g, 54.49 mmol) was dissolved in 360 mL of methanol and the mixture was cooled to 0°C. NaIO<sub>4</sub> (1.2 eq) was dissolved in 120 mL of water, slowly added to the reaction mixture and stirred for 45 min. After completion, the reaction was filtered through a bed of Celite®, the filtrate was concentrated *in vacuo* and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 200mL). The combined organic layers were washed with brine solution (200 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude was passed through a silica gel column to obtain the desired aldehyde compound **26a** (6.7g, 65%).

**Compound 26b:** This compound was prepared from **18**, following the procedure invoked for the preparation of compound **26a**. Eluent for purification: 40% ethyl acetate in *n*-hexane; thick syrup; yield 26 g (quantitative) from 29 g; [α]<sub>D</sub><sup>25</sup> (CHCl<sub>3</sub>, c1.0): -45.2°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3485, 2988, 2938, 1737, 1456, 1380, 1260, 1078, 1021, 752; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>): δ 9.67

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(d,  $J = 1.5$  Hz, 1H), 7.37 – 7.29 (m, 4H), 7.25 – 7.23 (m, 1H), 6.12 (d,  $J = 3.5$  Hz, 1H), 4.65 (d,  $J = 3.5$  Hz, 1H), 4.61 (d,  $J = 11.8$  Hz, 1H), 4.57 (dd,  $J = 3.8, 1.5$  Hz, 1H), 4.48 (d,  $J = 11.9$  Hz, 1H), 4.34 (d,  $J = 3.8$  Hz, 1H), 1.47 (s, 3H), 1.33 (s, 3H).;  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  200.0, 136.7, 128.7, 128.7, 128.3, 127.8, 127.8, 112.7, 106.3, 84.7, 83.8, 82.3, 72.4, 27.1, 26.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{18}\text{O}_5$   $[\text{M}+\text{H}]^+$ : 279.1232, Found: 279.1233.

**Compound 27a (Ido:Glc) isomers: Grignard Reaction:** Aldehyde compound **26a** (3.5 g, 18.6 mmol) was dissolved in 50 mL of the anhydrous THF and added slowly to the freshly prepared Grignard reagent (1.5 eq) at 0 °C under argon atmosphere. The reaction mixture was allowed to stir at 25°C for 3h. After completion, the reaction was quenched by the addition of saturated aqueous ammonium chloride solution, water (200 mL) and extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with brine solution (100 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using EtOAc and hexane (30-35%) as a mobile phase to afford **Compound 27a (Ido:Glc) isomers** (4.21 g, 85%, 10:1). **Reagent Preparation:** To a freshly activated Mg-metal (1.5 eq) and 50 mL of the anhydrous THF in two neck round bottom flask equipped with a condenser was added phenyl bromide (1.5 eq) slowly under argon atmosphere and the mixture was stirred at 70 °C for 1h. **Compound 27a (Ido isomer):** white solid; mp 156.8 °C;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +16.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3386, 3020, 1589, 1424, 1218, 772;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.49 – 7.46 (m, 2H), 7.40 – 7.30 (m, 3H), 6.03 (d,  $J = 3.6$  Hz, 1H), 5.05 (t,  $J = 4.9$  Hz, 1H), 4.51 (d,  $J = 3.6$  Hz, 1H), 4.34 (dd,  $J = 4.7, 2.8$  Hz, 1H), 4.13 – 4.09 (m, 1H), 3.18 (d,  $J = 4.8$  Hz, 1H), 2.85 (d,  $J = 5.4$  Hz, 1H), 1.46 (s, 3H), 1.31 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  140.2, 128.8, 128.8, 128.5, 126.9, 126.9, 112.2, 105.2, 85.6, 82.8, 76.3, 72.8, 27.0, 26.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{14}\text{H}_{18}\text{O}_5$   $[\text{M}+\text{Na}]^+$ : 289.1052, Found: 289.1053. **Compound 27a (Glc isomer):** thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  0.8): -23.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3778, 3022, 1595, 1426, 1216, 1021, 929, 761, 670;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.46 – 7.30 (m, 5H), 6.03 (d,  $J = 3.6$  Hz, 1H), 5.27 (d,  $J = 4.0$  Hz, 1H), 4.49 (d,  $J = 3.6$  Hz, 1H), 4.29 (s, 1H), 4.20 (dd,  $J = 4.0, 2.6$  Hz, 1H), 4.15 (d,  $J = 2.1$  Hz, 1H), 3.24 (s, 1H), 1.46 (s, 3H), 1.30 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  139.1, 128.9, 128.4, 126.1, 111.9, 105.1, 85.3, 82.2, 75.5, 73.8, 26.9, 26.2; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{14}\text{H}_{18}\text{O}_5$   $[\text{M}+\text{Na}]^+$ : 289.1052, Found: 289.1054.

**Compound 27b (Ido:Glc) isomers:** These compounds were prepared from **26b**, following the procedure identified for the compounds **27a (Ido:Glc) isomers**. Eluent for purification: 18-20% ethyl acetate in *n*-hexane; yield 530 mg (83%, 12:1) from 500 mg; **27b (Ido isomer):** thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): -44.6°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3779, 3022, 1593, 1427, 1216, 930,

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766, 670;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.31 – 7.28 (m, 2H), 7.27 – 7.19 (m, 8H), 5.92 (d,  $J = 3.8$  Hz, 1H), 4.96 (d,  $J = 7.6$  Hz, 1H), 4.49 (d,  $J = 3.8$  Hz, 1H), 4.43 (d,  $J = 11.5$  Hz, 1H), 4.23 (dd,  $J = 7.6, 3.2$  Hz, 1H), 4.18 (d,  $J = 11.5$  Hz, 1H), 3.51 (d,  $J = 3.2$  Hz, 1H), 2.93 (s, 1H), 1.39 (s, 3H), 1.20 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  139.8, 137.0, 128.6, 128.6, 128.4, 128.4, 128.1, 128.0, 127.7, 127.7, 127.1, 127.1, 111.9, 105.2, 84.6, 82.2, 82.1, 72.4, 71.8, 26.8, 26.3; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{24}\text{O}_5$   $[\text{M}+\text{Na}]^+$ : 379.1521, Found: 379.1523. **27b (Glc isomer)**: thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0):  $-77.2^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3688, 3022, 2929, 1595, 1427, 1216, 1079, 1026, 928, 767, 671;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.39 – 7.28 (m, 10H), 6.03 (d,  $J = 3.9$  Hz, 1H), 5.10 (t,  $J = 6.4$  Hz, 1H), 4.69 (d,  $J = 11.5$  Hz, 1H), 4.63 (d,  $J = 3.9$  Hz, 1H), 4.46 (d,  $J = 11.5$  Hz, 1H), 4.33 (dd,  $J = 6.2, 3.2$  Hz, 1H), 4.00 (d,  $J = 3.2$  Hz, 1H), 3.26 (d,  $J = 6.9$  Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  141.4, 136.8, 128.9, 128.9, 128.5 (3C), 128.2, 128.2, 127.8, 126.2, 126.2, 111.8, 105.3, 82.9, 82.6, 81.8, 72.4, 72.2, 26.9, 26.3; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{24}\text{O}_5$   $[\text{M}+\text{Na}]^+$ : 379.1521, Found: 379.1515.

**Compound 28a: Idopyranose Preparation:** Compound **27a (Ido isomer)** (440 mg, 1.30 mmol) was dissolved in 90% aqueous trifluoroacetic acid (3 mL) and stirred for 15 min at room temperature. The solvent was evaporated and the residue was co-evaporated twice with water and twice with toluene to afford the crude product which was dissolved in anhydrous pyridine (5 mL), acetic anhydride (1.2 eq) was added dropwise at  $0^\circ\text{C}$  under an argon atmosphere, followed by the addition of a catalytic amount of DMAP. The reaction mixture was gradually warmed up to  $25^\circ\text{C}$  and stirred for 3 h. After completion, ice-cooled water (50 mL) was added and extracted with EtOAc (2 x 25 mL). The organic layer was washed with aqueous 1N HCl (2 x 50 mL) and brine solution (1x50 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using 25% ethyl acetate in *n*-hexane as a mobile phase to obtain Compound **28a** (410 mg, 74%, over two steps) as a thick syrup.  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 2.0):  $+23.0^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3022, 1756, 1594, 1427, 1216, 1044, 929, 761, 670;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.40 – 7.37 (m, 2H), 7.35 – 7.32 (m, 2H), 7.31 – 7.29 (m, 1H), 6.18 (d,  $J = 1.8$  Hz, 1H), 5.24 (t,  $J = 3.1$  Hz, 1H), 5.17 (d,  $J = 1.8$  Hz, 1H), 5.08 – 5.06 (m, 1H), 5.05 – 5.03 (m, 1H), 2.20 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 1.88 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.7, 169.3, 168.9, 168.5, 135.7, 128.3 (3C), 126.5, 126.5, 90.6, 76.1, 68.4, 67.7, 65.7, 21.0 (3C), 20.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{19}\text{H}_{22}\text{O}_9$   $[\text{M}+\text{Na}]^+$ : 417.1162, Found: 417.1165.

**Compound 28b:** This compound was prepared from Compound **27b (Ido isomer)**, following the procedure developed for compound **28a**. Eluent for purification: 20% ethyl acetate in *n*-

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hexane; thick syrup; yield 190 mg (69%) from 120 mg;  $[\alpha]^{25}_D$  ( $\text{CHCl}_3$ ,  $c$ 2.0): +10.9°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3024, 1755, 1599, 1431, 1214, 1110, 1050, 931, 758, 669;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.40 (s, 1H), 7.39 (s, 2H), 7.36 – 7.32 (m, 5H), 7.31 – 7.30 (m, 2H), 6.25 (d,  $J$  = 1.7 Hz, 1H), 5.22 (d,  $J$  = 1.3 Hz, 1H), 5.11 (ddd,  $J$  = 2.7, 1.7, 0.9 Hz, 1H), 5.06 – 5.04 (m, 1H), 4.82 (d,  $J$  = 11.8 Hz, 1H), 4.76 (d,  $J$  = 11.8 Hz, 1H), 3.95 (t,  $J$  = 2.8 Hz, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 1.86 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.2, 169.9, 168.9, 137.2, 136.3, 128.8, 128.8, 128.4, 128.2, 128.2, 128.0(3C), 126.5, 126.5, 90.9, 75.8, 74.3, 73.1, 68.5, 66.7, 21.1, 21.1, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{24}\text{H}_{26}\text{O}_8[\text{M}+\text{Na}]^+$ : 465.1525, Found: 465.1531.

**Compound 29a: Phenyl Oxidation and Methyl Ester Preparation:** To a solution of Compound 28a (200 mg, 0.507 mmol) in  $\text{CCl}_4\text{:CH}_3\text{CN}$  (20 mL, 1:1) was added solution of  $\text{NaIO}_4$  (8 eq) in  $\text{H}_2\text{O}$  (15 mL) at 25 °C and stirred for 10 min.  $\text{RuCl}_3\cdot 3\text{H}_2\text{O}$  (5mol%) was added and the mixture was stirred vigorously for 24 h.  $\text{NaCl}$  was added to saturate the aqueous layer and extracted with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  50 mL). Combined organic phases were washed with brine solution (50 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. To the solution of crude product in anhydrous DMF (5 mL) was added 1.5 equivalent of  $\text{K}_2\text{CO}_3$ . After stirring for 15 min, iodomethane (2 eq) was added under argon atmosphere and the mixture was stirred at 25 °C for 8h in a dark place. After complete consumption, the reaction was quenched by the addition of excess amount of saturated solution of  $\text{Na}_2\text{SO}_3$ , followed by addition of brine (5 mL), water (10 mL) and extracted with EtOAc (2  $\times$  20 mL). The combined organic phases were washed with water, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The crude residue was purified by column chromatography using 35% ethyl acetate in *n*-hexane as a mobile phase to obtain the compound 29a (138 mg, 72%). thick syrup;  $[\alpha]^{25}_D$  ( $\text{CHCl}_3$ ,  $c$ 0.8): -2.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3022, 2926, 1761, 1594, 1427, 1216, 1056, 929, 766, 670;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.05 (d,  $J$  = 1.8 Hz, 1H), 5.28 (t,  $J$  = 3.5 Hz, 1H), 5.10 (t,  $J$  = 2.5 Hz, 1H), 5.03 – 4.99 (m, 1H), 4.71 (d,  $J$  = 2.3 Hz, 1H), 3.78 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.6, 169.4, 168.7, 168.3, 166.9, 89.9, 73.5, 67.3, 66.4, 65.4, 52.9, 20.9 (3C), 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{20}\text{O}_{11}[\text{M}+\text{Na}]^+$ : 399.0903, Found: 399.0909.

**Compound 30a:30b Grignard Reaction:** Aldehyde compound 26b (10 mmol) was dissolved in 30 mL of the anhydrous  $\text{Et}_2\text{O}$  or THF and added dropwise to the freshly prepared Grignard reagent (1.5 eq) at 0°C under argon atmosphere. Reaction mixture was allowed to stir at 25 °C for 3 h. After completion, the reaction was quenched by the addition of excess amount of saturated aqueous ammonium chloride solution, water (150 mL) and extracted with EtOAc (3

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x 100 mL). The combined organic phases were washed with brine solution (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using 10-15% ethyl acetate in *n*-hexane as mobile phase to afford the compound **30a:30b** [89% (10:1) when Et<sub>2</sub>O as a solvent whereas 82% (5:1) when THF as a solvent] as Ido:Glc isomers. **Reagent Preparation:** To a freshly activated Mg-metal (1.5 eq) suspended in 30 mL of the anhydrous Et<sub>2</sub>O or THF in a two neck flask equipped with reflux condenser was added 2-thienyl bromide (1.5 eq) slowly under argon atmosphere and mixture was stirred at 45 °C for 1h). **Compound 30a:** thick syrup; [ $\alpha$ ]<sup>25</sup><sub>D</sub>(CHCl<sub>3</sub>, c1.0): -46.3°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3496, 3010, 2923, 1456, 1379, 122, 1215, 1075, 1018, 746, 703; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>):  $\delta$  7.38 – 7.25 (m, 6H), 7.01 (d, *J* = 3.4 Hz, 1H), 6.95 (dd, *J* = 5.0, 3.5 Hz, 1H), 6.02 (d, *J* = 3.8 Hz, 1H), 5.35 (d, *J* = 7.6 Hz, 1H), 4.63 (d, *J* = 3.8 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.41 – 4.35 (m, 2H), 3.80 (d, *J* = 3.3 Hz, 1H), 2.99 (s, 1H), 1.50 (s, 3H), 1.32 (s, 3H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>):  $\delta$  142.6, 137.1, 128.7, 128.7, 128.2, 127.7, 127.7, 126.7, 125.3, 125.2, 112.2, 105.4, 84.6, 82.3, 82.3, 72.0, 68.7, 27.0, 26.4; HRMS (ESI-MS): *m/z* calcd for C<sub>19</sub>H<sub>22</sub>O<sub>5</sub>S[M+Na]<sup>+</sup>: 385.1086, Found: 385.1086. **Compound 30b:** thick syrup; [ $\alpha$ ]<sup>25</sup><sub>D</sub>(CHCl<sub>3</sub>, c0.45): -61.4°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3489, 3010, 2929, 1498, 1379, 1261, 1217, 1077, 1025, 753, 703; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>):  $\delta$  7.39 – 7.30 (m, 5H), 7.26 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.02 (dt, *J* = 2.5, 1.0 Hz, 1H), 6.98 (dd, *J* = 5.0, 3.5 Hz, 1H), 6.03 (d, *J* = 3.8 Hz, 1H), 5.31 (t, *J* = 6.4 Hz, 1H), 4.68 (d, *J* = 11.4 Hz, 1H), 4.64 (d, *J* = 3.8 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.38 (dd, *J* = 6.2, 3.3 Hz, 1H), 4.16 (d, *J* = 3.3 Hz, 1H), 3.38 (d, *J* = 7.1 Hz, 1H), 1.49 (s, 3H), 1.32 (s, 3H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>):  $\delta$  145.4, 136.8, 128.9, 128.9, 128.5, 128.1, 128.1, 127.0, 125.0, 124.5, 112.0, 105.4, 83.1, 82.6, 81.9, 72.5, 69.0, 27.0, 26.4; HRMS (ESI-MS): *m/z* calcd for C<sub>19</sub>H<sub>22</sub>O<sub>5</sub>S[M+Na]<sup>+</sup>: 385.1086, Found: 385.1083.

**Compound 31: Acetyl Protection:** To a solution of 13.0 g (35.87mmol) of the compound **30a** in anhydrous CH<sub>2</sub>Cl<sub>2</sub>(65 mL) was added DMAP (1.2eq) under argon atmosphere, followed by dropwise addition of the acetic anhydride (1.5 eq). The reaction mixture was stirred at 25 °C for 1 h. After completion, the mixture was purified by silica gel column chromatography using 18% ethyl acetate in *n*-hexane as a mobile phase to obtain compound **31** (13.8 g, 95%) as a thick syrup. [ $\alpha$ ]<sup>25</sup><sub>D</sub>(CHCl<sub>3</sub>, c1.0): +20.8°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 2988, 2933, 1742, 1453, 1375, 1226, 1076, 1018, 752, 705; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>):  $\delta$  7.35 – 7.21 (m, 6H), 7.10 – 7.05 (m, 1H), 6.94 (dd, *J* = 5.1, 3.5 Hz, 1H), 6.40 (d, *J* = 9.3 Hz, 1H), 6.00 (d, *J* = 3.8 Hz, 1H), 4.62 (dd, *J* = 9.3, 3.3 Hz, 1H), 4.56 (d, *J* = 3.8 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.21 (d, *J* = 11.4 Hz, 1H), 3.67 (d, *J* = 3.3 Hz, 1H), 2.05 (s, 3H), 1.54 (s, 3H), 1.31 (s, 3H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>):  $\delta$  170.0, 139.1, 137.1, 128.5, 128.5, 128.0, 127.7, 127.7, 127.2, 126.7, 125.9,

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112.0, 105.7, 82.2, 82.2, 81.8, 72.1, 69.7, 27.0, 26.4, 21.4; HRMS (ESI-MS):  $m/z$  calcd for  $C_{21}H_{24}O_6S[M+Na]^+$ : 427.1191, Found: 427.1187.

**Compound 32: Thienyl Oxidation and Methyl Ester Preparation:** To a solution of compound **31** (13.2 g, 32.63 mmol) in hexane–EtOAc (240 mL, 1:3) was added a solution of  $NaIO_4$  (8 eq) in  $H_2O$  (240 mL) at 25 °C and stirred for 10 min. Catalyst  $RuCl_3 \cdot 3H_2O$  (5mol%) was added and the mixture was stirred vigorously for 24 h.  $NaCl$  was added to saturate the aqueous layer, two phases were separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine solution (100 mL), dried over  $Na_2SO_4$  and concentrated *in vacuo*. The crude product was dissolved in anhydrous DMF (100 mL) and added 1.5 equivalent of  $K_2CO_3$ . After stirring for 15 min, iodomethane (2 eq) was added under argon atmosphere and the mixture was stirred at 25 °C for 8 h in a dark place. After complete consumption, the reaction was quenched by adding excess of saturated solution of  $Na_2SO_3$ , brine (100 mL) and water (200 mL) and extracted by EtOAc (2 x 200 mL). The combined organic phases washed with brine, dried over anhydrous  $Na_2SO_4$  and concentrated under reduced pressure. The crude was purified by column chromatography using 18% ethyl acetate in *n*-hexane as a mobile phase to obtain the desired compound **32** (9.2 g, 74%) over two steps. Thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.8):  $-3.4^\circ$ ; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 2956, 1747, 1446, 1376, 1216, 1076, 1024, 745, 700;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ ):  $\delta$  7.38 – 7.27 (m, 5H), 5.97 (d,  $J$  = 3.6 Hz, 1H), 5.52 (d,  $J$  = 7.1 Hz, 1H), 4.66 – 4.58 (m, 3H), 4.51 (d,  $J$  = 11.4 Hz, 1H), 4.16 (d,  $J$  = 3.8 Hz, 1H), 3.69 (s, 3H), 2.11 (s, 3H), 1.50 (s, 3H), 1.34 (s, 3H);  $^{13}C$  NMR (100.67 MHz,  $CDCl_3$ ):  $\delta$  170.0, 168.5, 137.2, 128.6, 128.6, 128.1, 127.9, 127.9, 112.6, 105.3, 83.0, 82.7, 78.8, 72.5, 70.8, 52.7, 27.2, 26.7, 20.9; HRMS (ESI-MS):  $m/z$  calcd for  $C_{19}H_{24}O_8[M+Na]^+$ : 403.1369, Found: 403.1369.

**Compound 33: Acetate Deprotection:** To a solution of the compound **32** (9.18 g, 24.13 mmol) in anhydrous  $CH_3OH$  (30 mL, 1:1), freshly prepared 1M  $NaOMe$  in  $MeOH$  (0.3 eq) was added and the reaction mixture was stirred at 25 °C for 30 minute. After complete consumption of the starting material, Amberlite IR 120 ( $H^+$ ) (1.0 g per mol) was added to the reaction mixture to quench the  $NaOMe$ . After complete neutralization, the solid resin was filtered off and the filtrate was concentrated *in vacuo*, the crude product was purified by silica gel column chromatography to obtain the compound **33** (7.7 g, 94%). Eluent for purification: 30% ethyl acetate in *n*-hexane; thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0):  $-39.8^\circ$ ; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3448, 2988, 2954, 1740, 1451, 1378, 1255, 1214, 1075, 1025, 861, 743, 701;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ):  $\delta$  7.36 – 7.27 (m, 5H), 5.99 (d,  $J$  = 3.9 Hz, 1H), 4.70 (d,  $J$  = 11.6 Hz, 1H), 4.66 (d,  $J$  = 3.8 Hz, 1H), 4.54 – 4.48 (m, 3H), 4.17 (d,  $J$  = 3.4 Hz, 1H), 3.71 (s, 3H), 3.40 (d, 1H), 1.46 (s,



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3H), 1.32 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  172.0, 136.8, 128.6, 128.6, 128.2, 128.0, 128.0, 112.5, 105.2, 83.0, 83.0, 80.3, 72.4, 69.9, 52.7, 27.1, 26.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{O}_7$   $[\text{M}+\text{Na}]^+$ : 361.1263, Found: 361.1266.

**Compound 34:** Compound **33** was dissolved in 90% aqueous trifluoroacetic acid and stirred for 15 min at room temperature. The solvent was evaporated and the residue was co-evaporated twice both with water and toluene to afford the crude product which was dissolved in  $\text{CH}_2\text{Cl}_2$  and pyridine (10 eq), acetyl chloride (6 eq) and cat. DMAP was added dropwise at  $-40^\circ\text{C}$  under argon atmosphere. The reaction mixture was stirred for 10 h. After completion, ice-cooled water was added and extracted with EtOAc. The organic layer was washed with aqueous 1N HCl (2 x 50 mL) and brine solution (1x50 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography. Eluent for purification: 38% ethyl acetate in n-hexane; white solid; yield 5.1 g (58%) from 7 g.  **$\beta$  isomer:**  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0):  $+9.3^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2954, 1747, 1443, 1374, 1218, 1060, 949, 753;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38 – 7.28 (m, 5H), 6.07 (d,  $J$  = 1.7 Hz, 1H), 5.14 (ddd,  $J$  = 2.9, 2.1, 1.0 Hz, 1H), 5.02 (ddd,  $J$  = 2.9, 1.8, 1.0 Hz, 1H), 4.78 (d,  $J$  = 2.1 Hz, 1H), 4.76 – 4.69 (m, 2H), 3.94 (t,  $J$  = 3.0 Hz, 1H), 3.75 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  169.9, 169.8, 168.6, 167.4, 136.6, 128.6, 128.6, 128.3, 127.9, 127.9, 90.0, 73.3, 73.0, 72.9, 67.1, 66.1, 52.6, 20.9, 20.9, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{20}\text{H}_{24}\text{O}_{10}$   $[\text{M}+\text{Na}]^+$ : 447.1267, Found: 447.1265.  **$\alpha$ -isomer:**  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0):  $-49.7^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2955, 1746, 1441, 1373, 1218, 1099, 1054, 1014, 971, 753;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38 – 7.28 (m, 5H), 6.23 (s, 1H), 5.24 – 5.21 (m, 1H), 4.96 (d,  $J$  = 1.9 Hz, 1H), 4.93 – 4.90 (m, 1H), 4.73 (s, 2H), 3.87 – 3.84 (m, 1H), 3.77 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ )  $\delta$  169.9, 169.5, 168.6, 168.3, 137.3, 128.6, 128.6, 128.1, 127.6, 127.6, 91.4, 72.5, 71.4, 67.9, 67.6, 65.7, 52.8, 21.0, 20.9, 20.8; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{20}\text{H}_{24}\text{O}_{10}$   $[\text{M}+\text{Na}]^+$ : 447.1267, Found: 447.1262.

**Compound 35:** To a solution of compound **34** (200 mg) in  $\text{CH}_2\text{Cl}_2$  (4 ml) was added  $\text{TiBr}_4$  (1.0 eq) at  $0^\circ\text{C}$  and the reaction mixture was stirred for 1.5 h. After completion, ice cold sat. solution of  $\text{NaHCO}_3$  was added and extracted by  $\text{CH}_2\text{Cl}_2$ . The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated *in vacuo*. Resulting crude was dissolved in a acetone:water (10:1, 4 ml) and added 0.5 equivalent of  $\text{AgCO}_3$ . After stirring for 2h, solvent was evaporated and extracted by  $\text{CH}_2\text{Cl}_2$  by adding brine. Crude residue was purified by silica gel column chromatography using 30% ethyl acetate in n-hexane to afford hemiacetals **35** in 82.5% yield.

**Compound 37:** Eluent for purification: 20% ethyl acetate in n-hexane; thick syrup; yield 160 mg (92%) from 125 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0):  $+2.8^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3272, 2941, 2864,

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1750, 1447, 1373, 1222, 1074, 909, 754;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.41 – 7.30 (m, 5H), 5.97 (d,  $J$  = 1.7 Hz, 1H), 5.15 (t,  $J$  = 2.3 Hz, 1H), 5.08 – 5.05 (m, 1H), 4.81 – 4.69 (m, 3H), 4.01 (t,  $J$  = 3.3 Hz, 1H), 3.77 (s, 3H), 2.64 (s, 1H), 2.21 – 2.11 (m, 2H), 2.10 (s, 3H), 2.03 (s, 3H), 1.94 – 1.82 (m, 2H), 1.72 – 1.57 (m, 4H), 1.54 – 1.48 (m, 1H), 1.38 – 1.28 (m, 1H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  170.0, 169.8, 169.8, 169.4, 168.2, 167.4, 150.9, 150.5, 137.3, 136.8, 128.7, 128.7, 128.5, 128.5, 128.4, 128.0, 127.9, 127.9, 127.8, 127.8, 94.3, 92.7, 82.6, 82.5, 78.7, 78.6, 75.5, 75.4, 73.3, 73.2, 73.0, 72.4, 71.1, 67.9, 67.6, 67.4, 66.5, 65.4, 52.7, 52.7, 36.9, 36.9, 36.7, 36.6, 25.0, 25.0, 22.6, 22.6, 22.5, 22.5, 21.0, 20.9, 20.8, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{27}\text{H}_{32}\text{O}_{11}$   $[\text{M}+\text{Na}]^+$ : 555.1842, Found: 555.1840.

**Compound 38:** IdoA **34** (150 mg) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  and cooled to 0 °C. 30% HBr in acetic acid (700  $\mu\text{L}$ ) was added dropwise and the reaction mixture stirred at 0 °C for 2 h. After completion, ice cold water was added and extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with ice-cold saturated  $\text{NaHCO}_3$  (1x25 mL), brine solution (1x25 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo* to afford corresponding bromoside which was immediately dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$ . Propargyl alcohol (2 eq), 2,6-lutidine (2.2 eq) and tetra *n*-butyl ammonium iodide (0.1 eq) were added and the reaction mixture was refluxed at 65 °C for 16 h. After completion, water, sat. aq. solution of oxalic acid was added and extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with saturated  $\text{NaHCO}_3$ , brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. Crude residue was purified by silica gel column chromatography using EtOAc and Hexane as mobile phase to obtain propargyl orthoester of the IdoA.  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.40 – 7.32 (m, 5H), 5.56 (d,  $J$  = 2.2 Hz, 1H), 5.18 (dt,  $J$  = 2.7, 1.5 Hz, 1H), 4.80 (d,  $J$  = 11.8 Hz, 1H), 4.68 (d,  $J$  = 11.8 Hz, 1H), 4.55 (d,  $J$  = 1.3 Hz, 1H), 4.17 (dd,  $J$  = 2.5, 0.6 Hz, 2H), 4.12 (q,  $J$  = 1.8 Hz, 2H), 3.78 (s, 3H), 2.35 (t,  $J$  = 2.5 Hz, 1H), 2.03 (s, 3H), 1.77 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  170.3, 168.1, 136.9, 128.8, 128.8, 128.5, 128.2, 128.2, 124.2, 96.8, 79.7, 76.0, 73.6, 73.1, 71.3, 69.8, 66.9, 52.8, 50.3, 25.3, 20.9; MALDI-TOF:  $m/z$  calcd for  $\text{C}_{21}\text{H}_{24}\text{O}_9$   $[\text{M}+\text{K}]^+$ : 459.11, Found: 459.12.

**Compound 42: 1,2-Acetonide Idopyranose Preparation:** Compound **33** (1.54 g, 4.55 mmol) was dissolved in 90% aqueous trifluoroacetic acid (10 mL) and stirred for 15 min at room temperature. The solvent was evaporated and the residue was co-evaporated twice with water and twice with toluene. The crude product in anhydrous THF (6 mL) was added 2-methoxypropene (8.7 mL, 20 eq). The reaction mixture was cooled to 0 °C and PTSA (0.1 eq) in THF (0.5 mL) was added dropwise with stirring under argon atmosphere. The mixture was allowed to warm to room temperature and stirred for 4 h. The reaction was quenched by the



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addition of Et<sub>3</sub>N, water (50 mL) was added and extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with saturated aqueous NaHCO<sub>3</sub> and brine solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude residue was purified by column chromatography using 20% ethyl acetate in *n*-hexane as a mobile phase to obtain 850 mg (55%, over two steps) of the desired compound **42** as a thick syrup;  $[\alpha]^{25}_D$  (CHCl<sub>3</sub>, *c*1.0): -15.8°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3535, 2924, 2857, 1763, 1737, 1458, 1379, 1229, 1119, 1061, 846, 703; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 7.40 – 7.30 (m, 5H), 5.36 (d, *J* = 1.9 Hz, 1H), 4.71 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.49 (s, 1H), 4.12 – 4.06 (m, 2H), 3.97 (q, *J* = 2.0 Hz, 1H), 3.81 (s, 3H), 3.12 (d, *J* = 11.3 Hz, 1H), 1.63 (s, 3H), 1.38 (s, 3H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>): δ 169.4, 137.1, 128.8, 128.8, 128.4, 127.9, 127.9, 112.0, 96.5, 75.5, 73.5, 72.9, 72.2, 67.4, 52.5, 28.4, 25.7; HRMS (ESI-MS): *m/z* calcd for C<sub>17</sub>H<sub>22</sub>O<sub>7</sub> [M+Na]<sup>+</sup>: 361.1263, Found: 361.1261.

**Compound 43: Protection of Levulinoate:** To a solution of **42** (200 mg) and levulinic acid (1.5 eq) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was added 0.2 equivalent of DMAP and reaction was cooled to 0 °C, *N, N'*-diisopropyl carbodiimide (1.2 eq) was added drop-wise under argon atmosphere and the mixture was stirred at 25 °C for 2 h. After complete consumption, the reaction mixture was concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using ethyl acetate in *n*-hexane as mobile phase to afford the levulinate ester **43** in a 92% yield. <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>) δ 7.39 – 7.29 (m, 5H), 5.36 (d, *J* = 2.4 Hz, 1H), 5.19 (s, 1H), 4.81 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.52 (d, *J* = 1.3 Hz, 1H), 4.07 (t, *J* = 2.2 Hz, 1H), 3.91 (s, 1H), 3.78 (s, 3H), 2.74 (q, *J* = 6.4 Hz, 2H), 2.55 (t, *J* = 6.6 Hz, 2H), 2.17 (s, 3H), 1.61 (s, 3H), 1.36 (s, 3H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>) δ 206.4, 171.9, 168.3, 137.2, 128.7, 128.7, 128.3, 128.0, 128.0, 112.1, 96.6, 75.2, 73.0, 72.2, 70.1, 67.3, 52.7, 37.9, 29.8, 28.2, 28.0, 26.4; MALDI-TOF: *m/z* calcd for C<sub>22</sub>H<sub>28</sub>O<sub>9</sub> [M+K]<sup>+</sup>: 475.14, Found: 475.24.

**Compound 44: Acetonide Unlocking:** To the compound **43** (150 mg) in a 50 mL flask was added 75% aq. dichloroacetic acid (4 mL) at 0 °C and the solution was stirred at 0-25 °C for 1-2 h. After completion, the reaction was diluted with ice-cold water (10 mL), neutralized with NaHCO<sub>3</sub> (1 g) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The combined organic phases were washed with saturated aqueous NaHCO<sub>3</sub> and brine solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude residue was further purified by silica gel column chromatography using 45% ethyl acetate in *n*-hexane as mobile phase to afford 85% of the compound **44** as a thick syrup.

## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

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**Compound 45a:** To a solution of **44** (100 mg, 375.71  $\mu\text{mol}$ ) in anhydrous  $\text{CH}_2\text{Cl}_2$  (2 mL), DMAP (1.5 eq) and ethynyl cyclohexyl (4-nitrophenyl) carbonate **36** (1.2 eq) were added and the reaction mixture was stirred at 25  $^\circ\text{C}$  for 3 h. After complete consumption of the starting hemiacetals, solvent was concentrated *in vacuo* at bath temperature 30  $^\circ\text{C}$ . Crude residue was purified by silica gel column chromatography using ethyl acetate in *n*-hexane as mobile phase to afford compound **45a** as a thick syrup.  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37 – 7.31 (m, 5H), 5.89 (d,  $J$  = 1.3 Hz, 1H), 5.22 (dd,  $J$  = 3.4, 1.5 Hz, 1H), 4.78 – 4.74 (m, 2H), 4.66 (d,  $J$  = 11.7 Hz, 1H), 3.95 (t,  $J$  = 3.1 Hz, 1H), 3.85 – 3.81 (m, 1H), 3.80 (d,  $J$  = 3.5 Hz, 1H), 3.79 (s, 3H), 2.74 (td,  $J$  = 6.3, 2.5 Hz, 2H), 2.65 (s, 1H), 2.54 (dt,  $J$  = 8.0, 5.9 Hz, 2H), 2.24 – 2.19 (m, 1H), 2.18 (s, 3H), 2.16 – 2.11 (m, 1H), 1.93 – 1.82 (m, 2H), 1.73 – 1.61 (m, 5H), 1.54 – 1.50 (m, 1H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ )  $\delta$  206.3, 171.4, 167.4, 151.1, 137.0, 128.7, 128.7, 128.4, 128.0, 128.0, 94.5, 75.5, 75.0, 73.7, 73.0, 67.7, 67.3, 52.8, 38.0, 37.1, 36.7, 29.8, 28.1, 25.0, 22.6, 22.6; MALDI-TOF:  $m/z$  calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_{11} [\text{M}+\text{K}]^+$ : 585.17, Found: 585.18.

**Compound 46: TBDMS protection:** To a solution of alcohol **42** (280 mg, 0.828 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (5 mL) was added 5.0 equivalent of 2,6-lutidine. TBDMSOTf (1.2 eq) was added dropwise at 0  $^\circ\text{C}$  under argon atmosphere and the resulting reaction mixture was stirred at 25  $^\circ\text{C}$ . After 15 min, ice-cold water (10 mL) was added and extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 10 mL). The combined organic phases were washed with brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*, crude residue was purified by silica gel column chromatography using 10% ethyl acetate in *n*-hexane as a mobile phase to obtain 350 mg (93%) of the compound **46** as a thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  0.8):  $-61.4^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2926, 2858, 1770, 1461, 1376, 1238, 1144, 1099, 1068, 835, 776;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.40 – 7.30 (m, 5H), 5.32 (d,  $J$  = 2.5 Hz, 1H), 4.65 (q,  $J$  = 12.0 Hz, 2H), 4.38 (d,  $J$  = 1.3 Hz, 1H), 4.07 (dt,  $J$  = 2.5, 1.2 Hz, 1H), 3.95 (t,  $J$  = 2.7 Hz, 1H), 3.83 (t, 1H), 3.76 (s, 3H), 1.59 (s, 3H), 1.38 (s, 3H), 0.82 (s, 9H),  $-0.05$  (s, 3H),  $-0.07$  (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.8, 137.4, 128.8, 128.3, 128.0, 112.3, 96.9, 75.3, 75.1, 72.8, 72.6, 68.0, 52.2, 28.3, 26.6, 25.6, 18.0,  $-4.5$ ,  $-5.3$ ; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{36}\text{O}_7\text{Si} [\text{M}+\text{Na}]^+$ : 475.2128, Found: 475.2130.

**Compound 47:** This compound was prepared from **46**, following the procedure developed for compound **44**. Eluent for purification: 30% ethyl acetate in *n*-hexane; thick syrup; yield 230 mg (81%) from 310 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  0.7):  $-7.7^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3504, 2956, 2925, 2858, 1740, 1461, 1373, 1214, 1140, 1082, 916, 839, 777;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.43 – 7.30 (m, 5H), 4.98 (d,  $J$  = 12.4 Hz, 1H), 4.68 (d,  $J$  = 12.1 Hz, 1H), 4.66 – 4.55 (m, 2H), 4.09 – 4.05 (m, 1H), 3.81 – 3.71 (m, 4H), 3.70 – 3.62 (m, 1H), 3.47 (d,  $J$  = 11.7 Hz, 1H), 0.83 (s, 9H),  $-0.03$  (d,  $J$  = 2.7 Hz, 6H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.4, 137.2, 128.9,

## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

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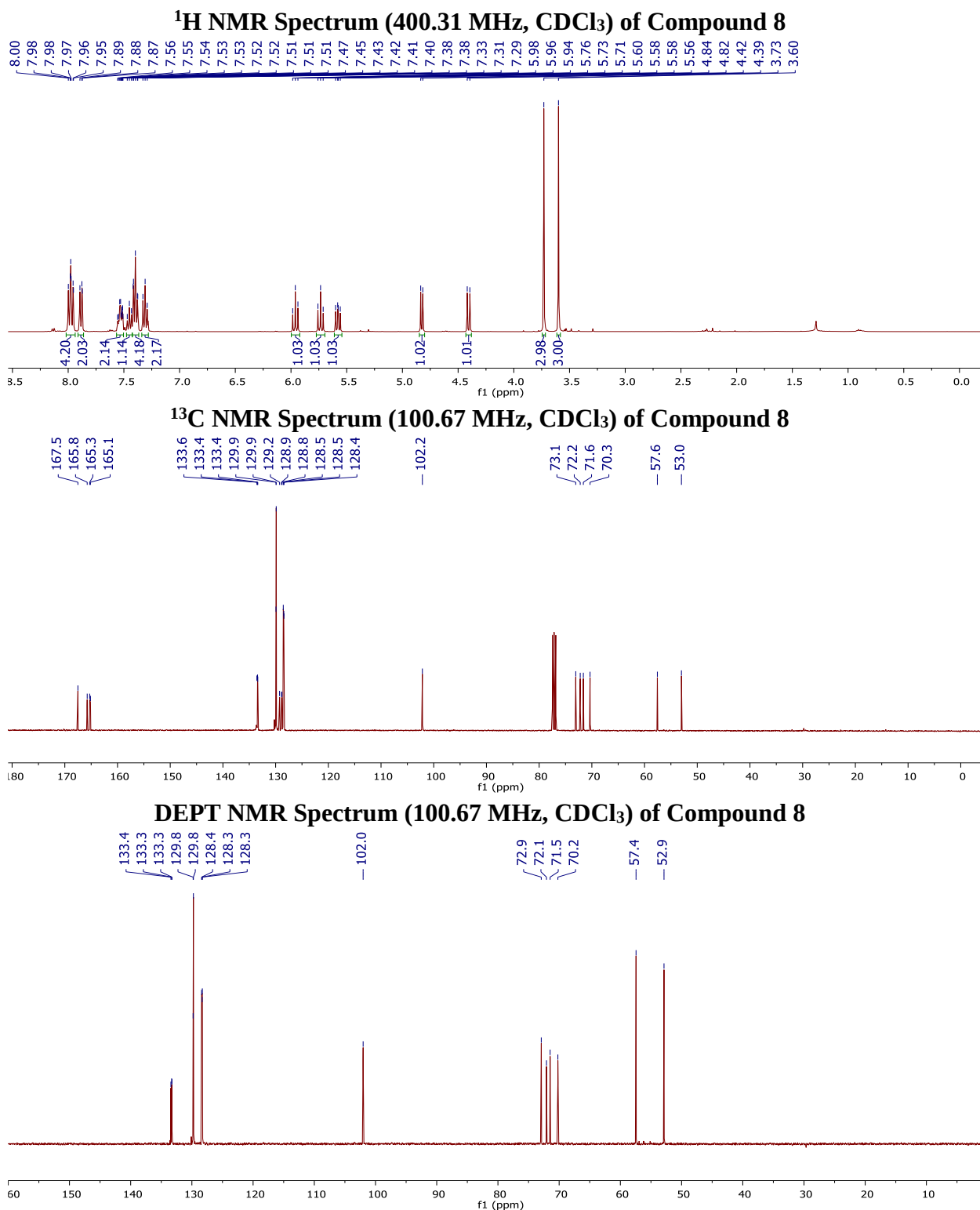
128.9, 128.5, 128.1, 128.1, 93.1, 75.4, 74.6, 72.6, 69.2, 68.5, 52.5, 25.6 (3C), 18.0, -4.8, -5.4; HRMS (ESI-MS):  $m/z$  calcd for  $C_{20}H_{32}O_7Si[M+Na]^+$ : 435.1815, Found: 435.1818.

**Compound 48: Selective Carbonate and Acetate Protection:** To a solution of **47** (155 mg, 375.71  $\mu$ mol) in anhydrous  $CH_2Cl_2$  (2 mL), DMAP (138 mg, 3.0 eq) and ethynyl cyclohexyl (4-nitrophenyl) carbonate **36** (130 mg, 1.2 eq) were added and the reaction mixture was stirred at 25 °C for 3 h. After complete consumption of the starting hemiacetal, acetic anhydride (71  $\mu$ L, 2.0 eq) was added and the stirring continued for another 2 h. The reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography using 15% ethyl acetate in *n*-hexane as mobile phase to obtain Compound **48** (220 mg, 97%) of the desired carbonate donor as a thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 0.8): -24.7°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3289, 2929, 2860, 1764, 1742, 1455, 1370, 1223, 1147, 1075, 1012, 915, 835, 755;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ):  $\delta$  7.37 – 7.30 (m, 5H), 5.91 (d,  $J$  = 1.8 Hz, 1H), 5.06 – 5.03 (m, 1H), 4.76 (d,  $J$  = 12.1 Hz, 1H), 4.60 (d,  $J$  = 7.0 Hz, 1H), 4.59 (d,  $J$  = 2.8 Hz, 1H), 3.94 (t,  $J$  = 2.3 Hz, 1H), 3.75 – 3.73 (m, 4H), 2.62 (s, 1H), 2.21 – 2.11 (m, 2H), 2.08 (s, 3H), 1.90 – 1.81 (m, 2H), 1.69 – 1.50 (m, 5H), 1.34 – 1.29 (m, 1H), 0.78 (s, 9H), -0.12 (s, 3H), -0.23 (s, 3H);  $^{13}C$  NMR (100.53 MHz,  $CDCl_3$ ):  $\delta$  170.8, 168.4, 151.1, 137.0, 128.7, 128.7, 128.4, 128.3, 128.3, 93.1, 82.6, 78.6, 76.4, 75.4, 75.0, 72.6, 68.2, 66.3, 52.3, 36.9, 36.7, 25.6 (3C), 25.0, 22.6, 22.6, 21.2, 17.9, -4.5, -5.7; HRMS (ESI-MS):  $m/z$  calcd for  $C_{31}H_{44}O_{10}Si[M+Na]^+$ : 627.2601, Found: 627.2605.

**Compound 49:** This compound was prepared from **30a**, following the procedure described for the preparation of compound **44**. And then the acetone locking procedure described for the preparation of compound **42**. Eluent for purification: 20% ethyl acetate in *n*-hexane; thick syrup;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ )  $\delta$  7.39 – 7.35 (m, 4H), 7.33 – 7.30 (m, 2H), 7.14 (dt,  $J$  = 3.5, 1.0 Hz, 1H), 7.00 (dd,  $J$  = 5.1, 3.5 Hz, 1H), 5.66 (d,  $J$  = 4.7 Hz, 1H), 4.98 (d,  $J$  = 8.5 Hz, 1H), 4.81 (d,  $J$  = 11.8 Hz, 1H), 4.74 (d,  $J$  = 7.6 Hz, 1H), 4.29 (t,  $J$  = 4.7 Hz, 1H), 3.88 – 3.81 (m, 2H), 2.23 (d,  $J$  = 4.2 Hz, 1H), 1.60 (s, 3H), 1.39 (s, 3H);  $^{13}C$  NMR (100.67 MHz,  $CDCl_3$ )  $\delta$  141.9, 137.8, 128.7, 128.7, 128.1 (3C), 126.9, 126.0, 125.9, 109.5, 97.6, 80.5, 76.8, 73.6, 72.5, 71.5, 27.5, 26.4.

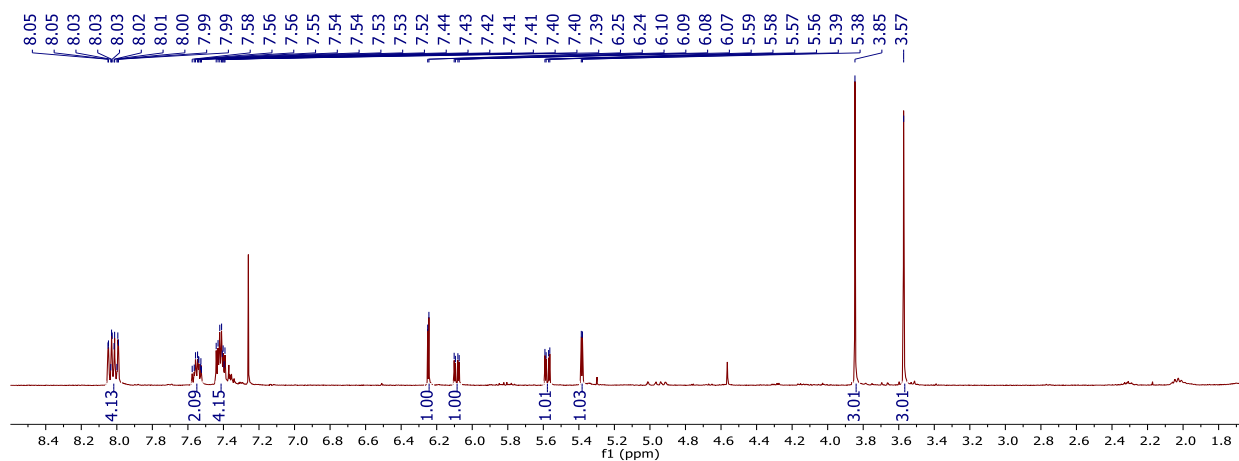
# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

## 2.7 Spectral Charts of Representative Compounds:

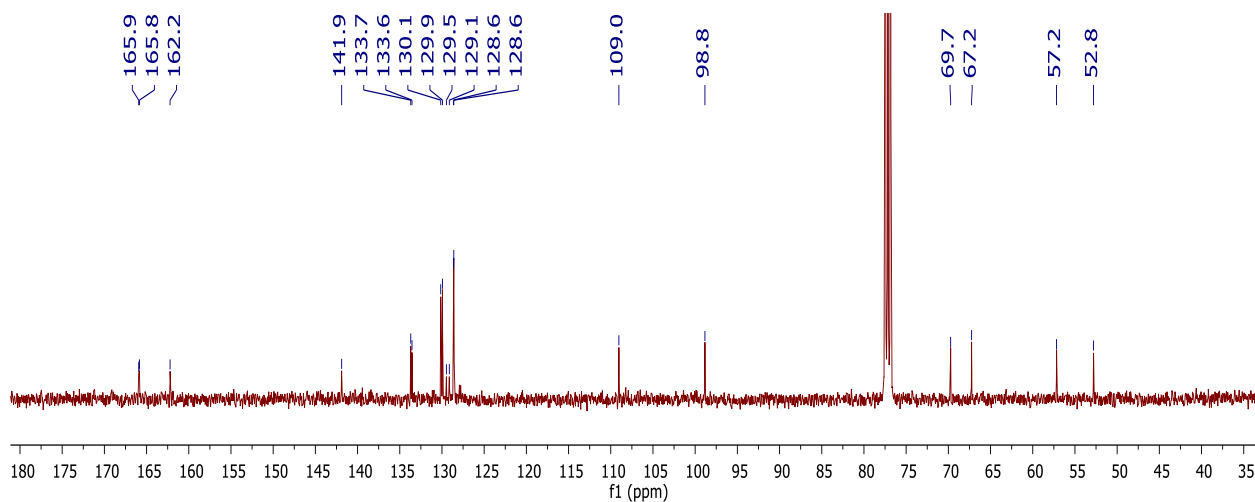


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

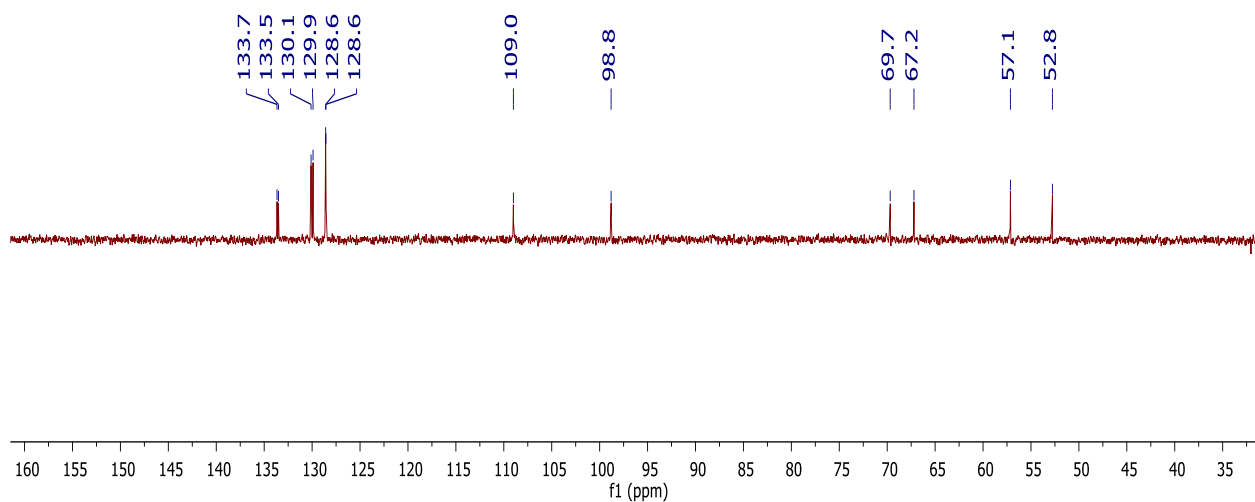
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 9b (β-eliminated)**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 9b (β-eliminated)**

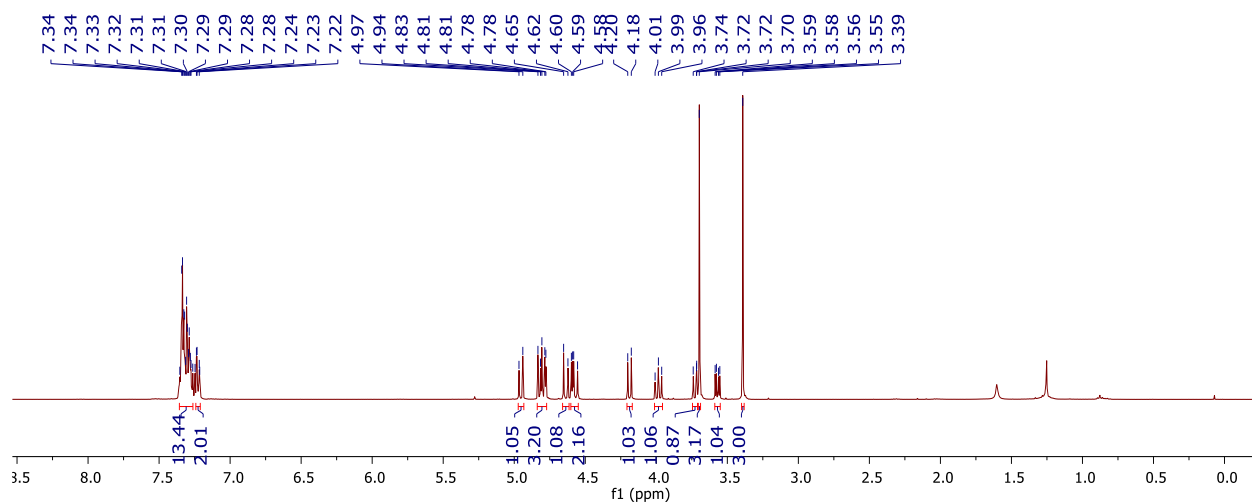


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 9b (β-eliminated)**

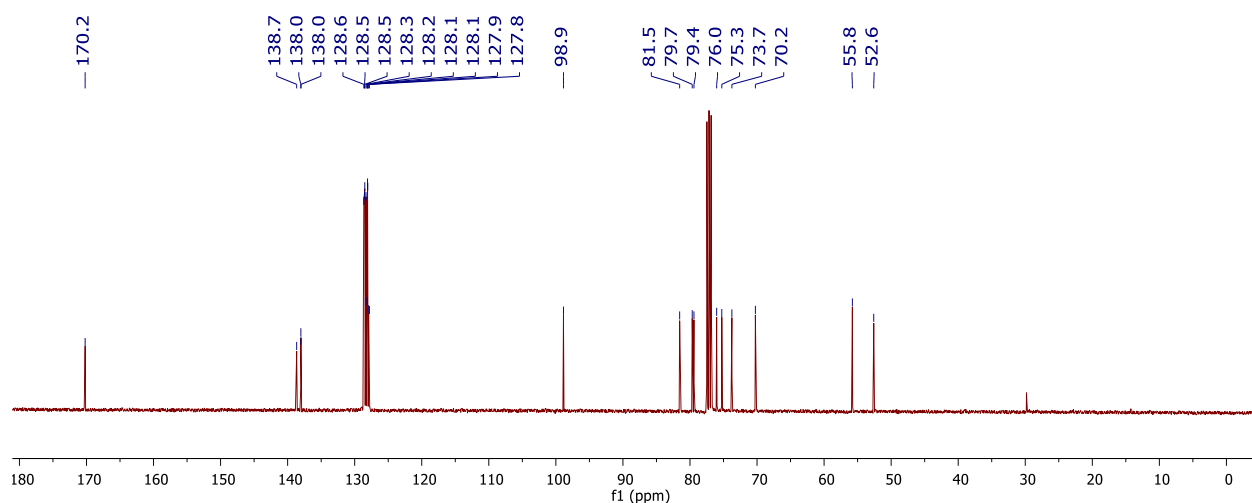


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

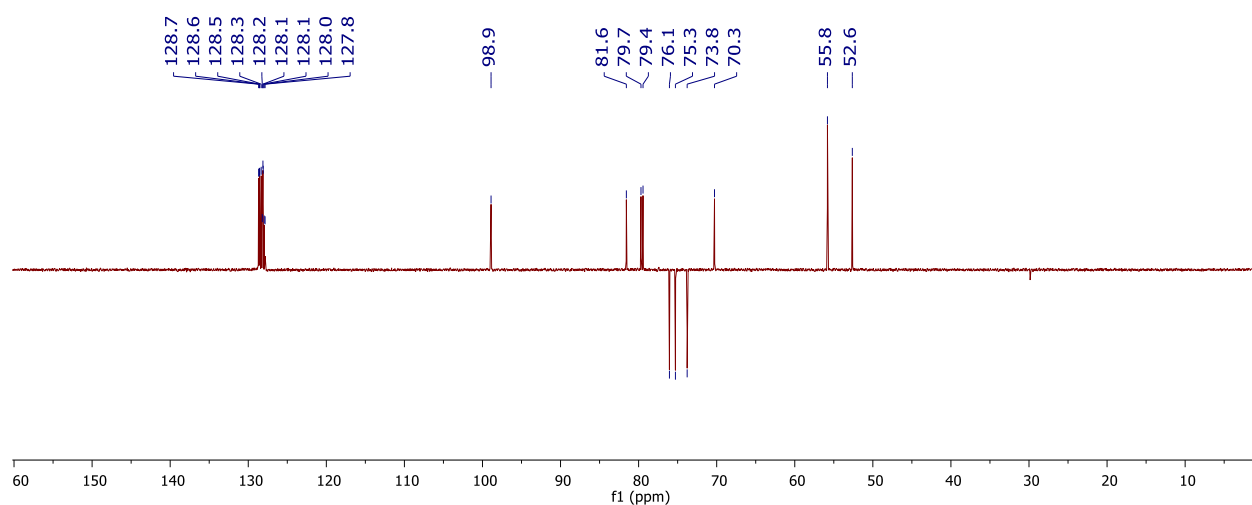
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 12**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 12**

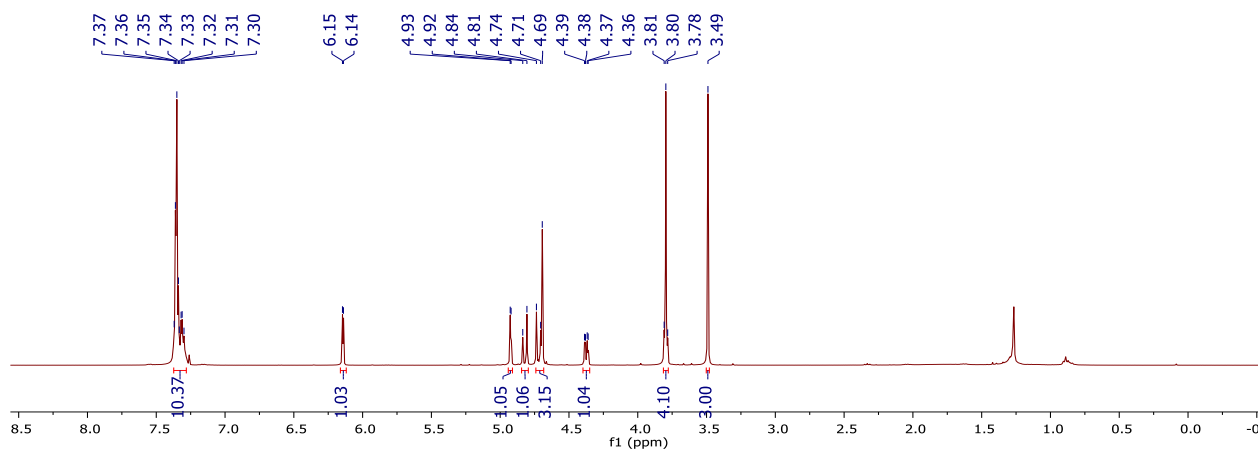


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 12**

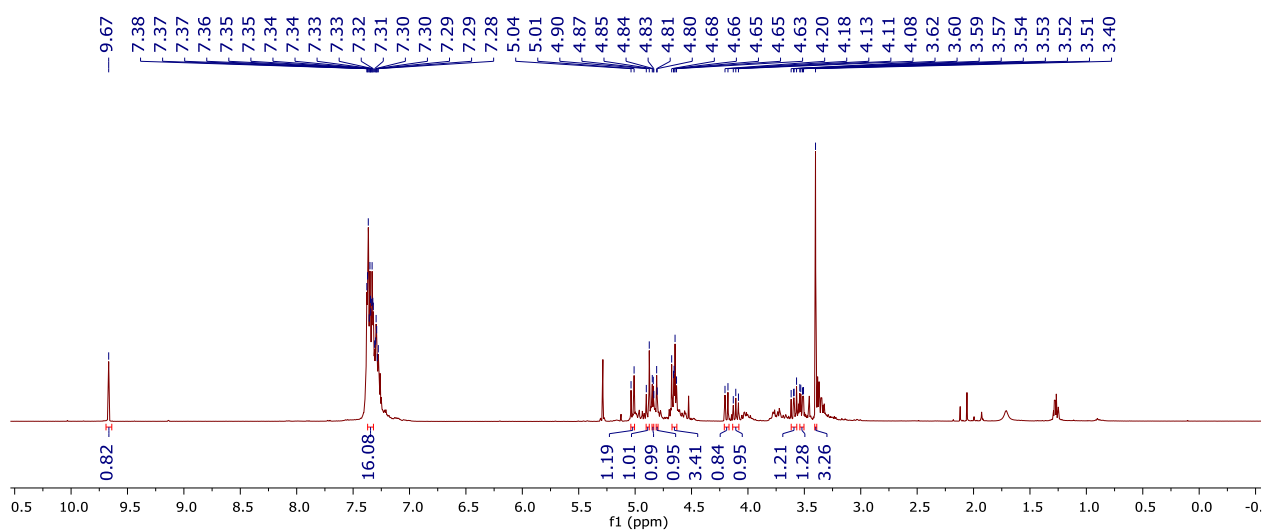


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

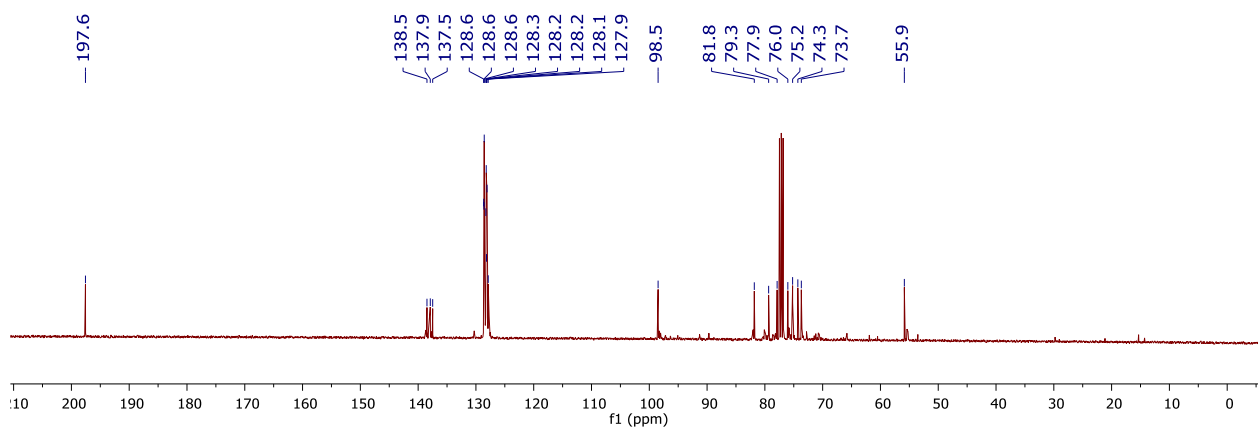
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 13b ( $\beta$ -eliminated)**



**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 14**

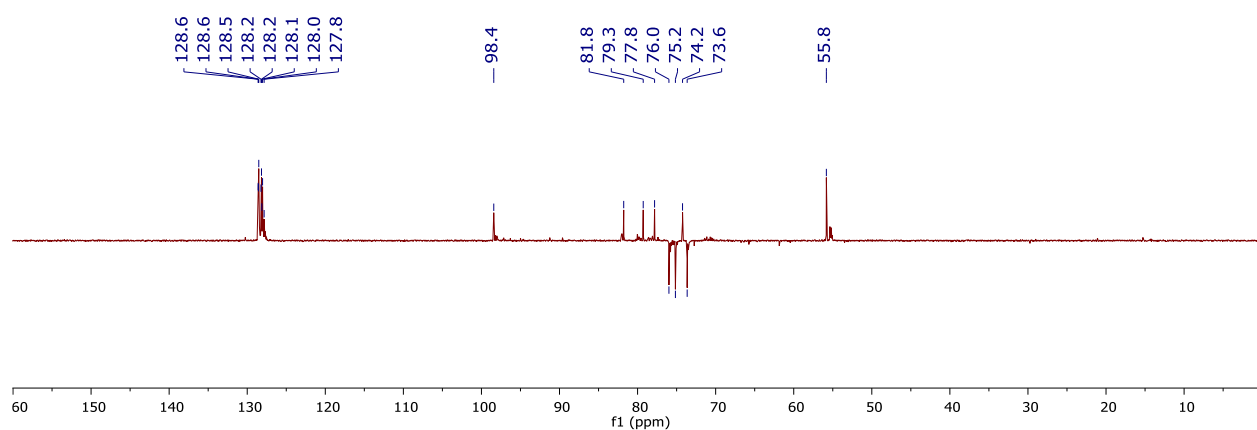


**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 14**

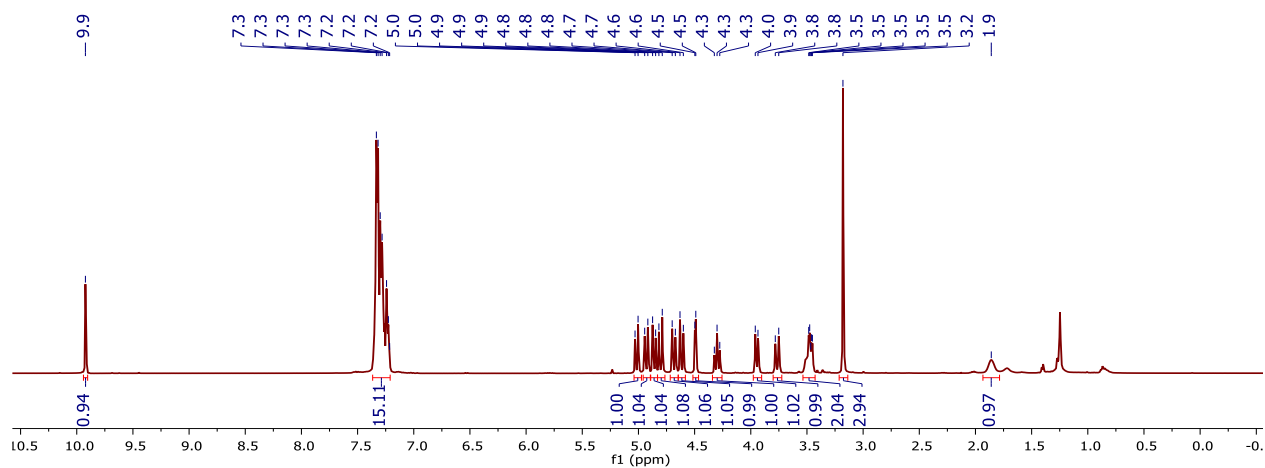


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

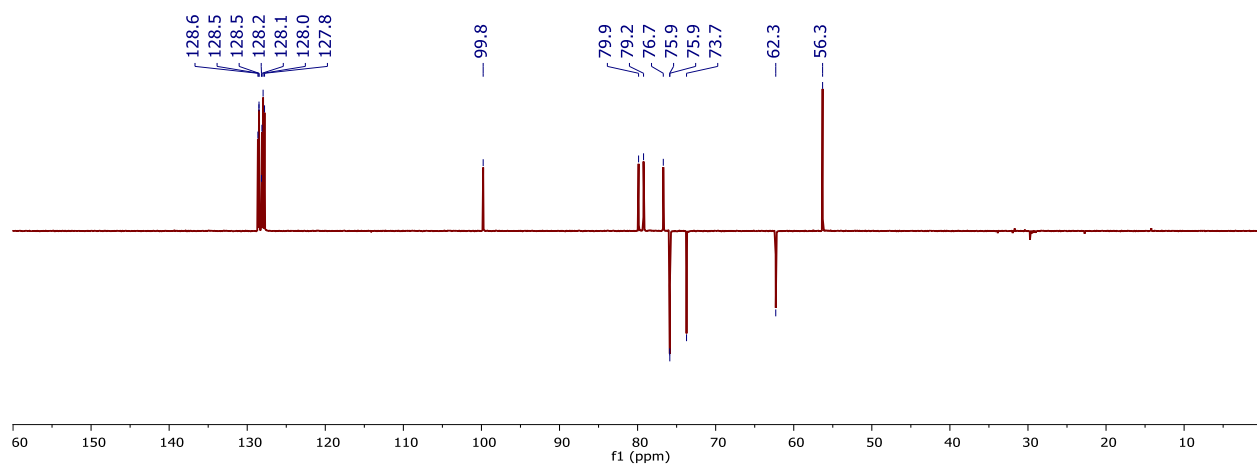
DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 14



<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 15



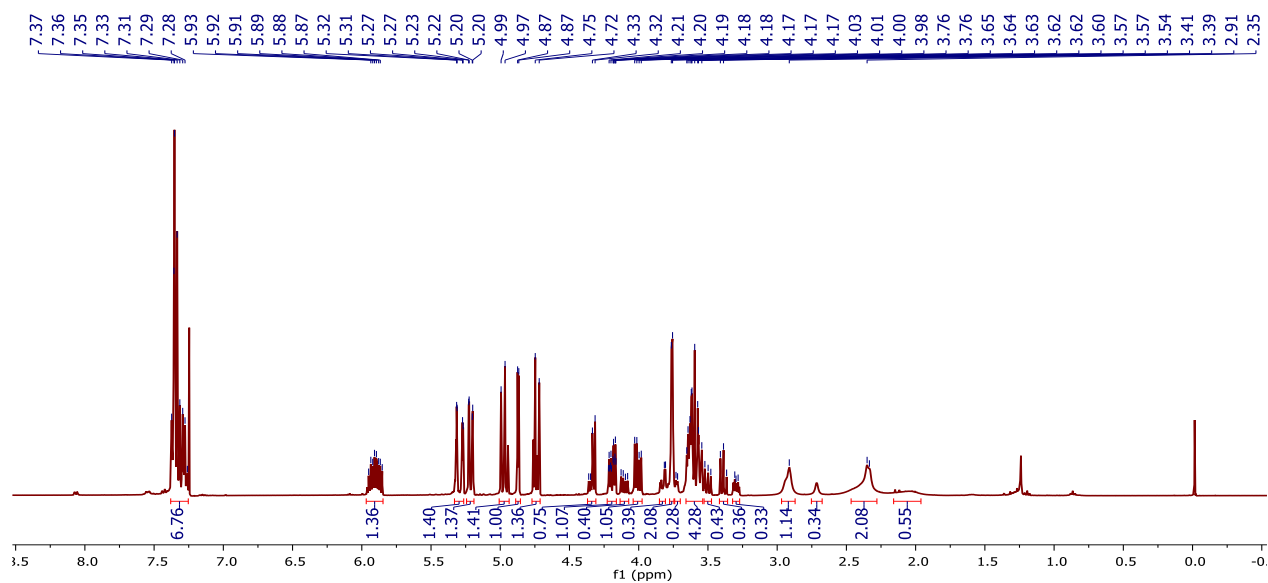
DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 15



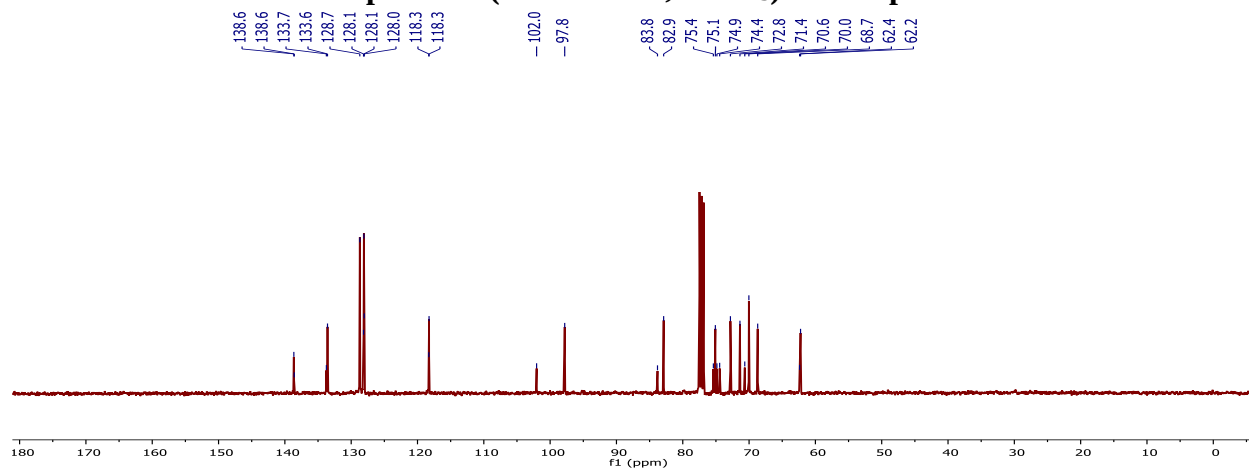


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

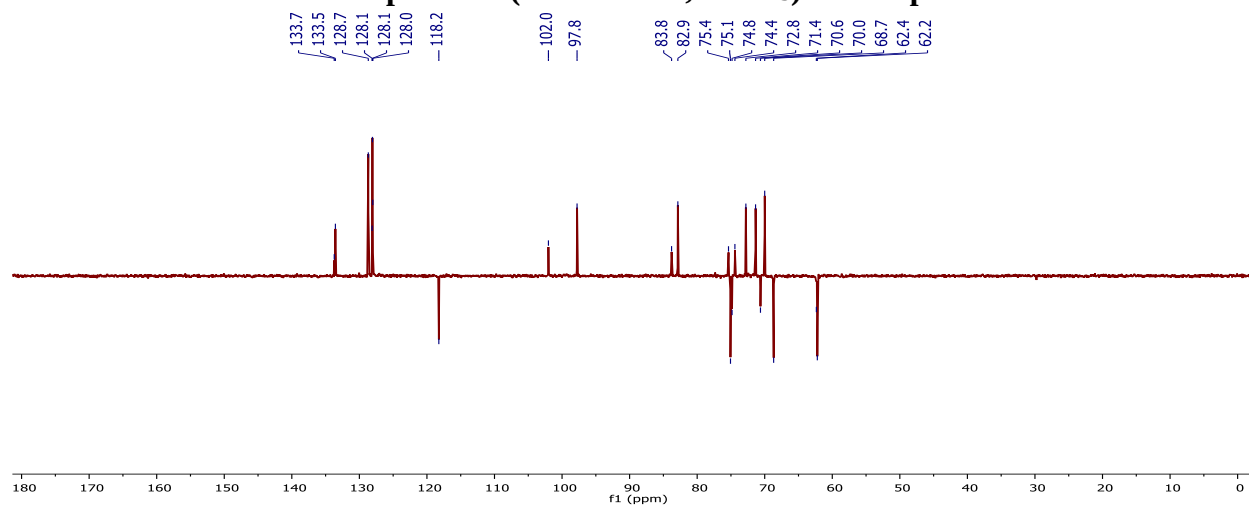
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 19**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 19**

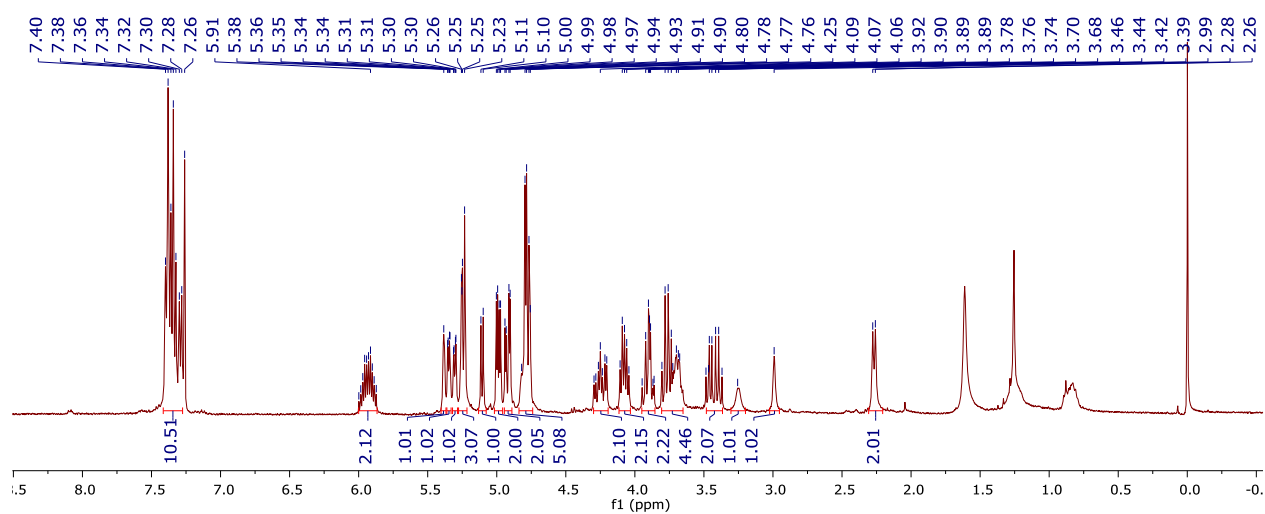


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 19**

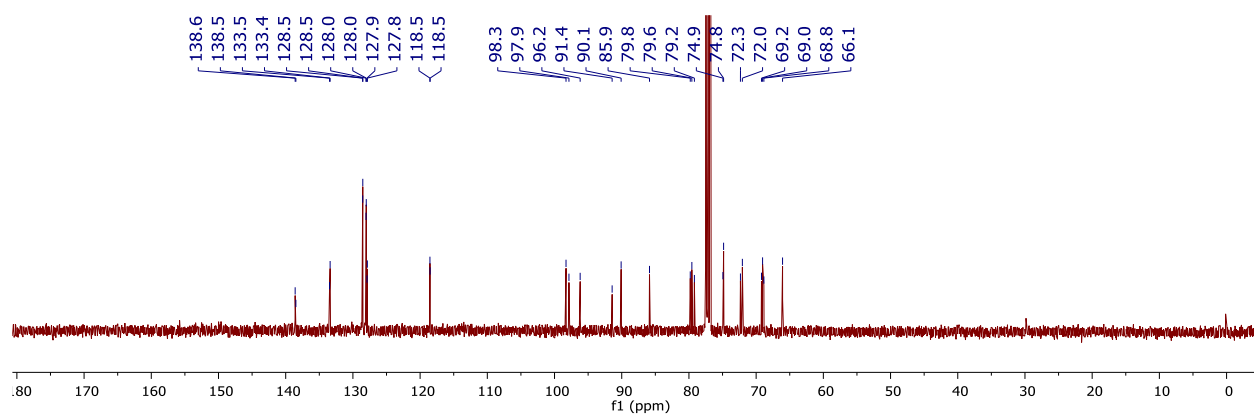


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

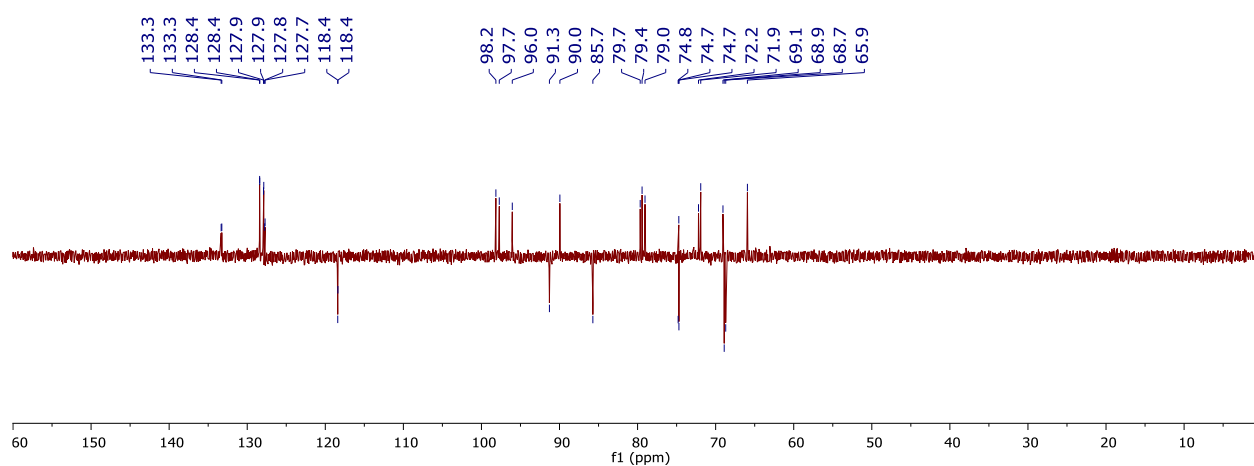
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 21a**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 21a**

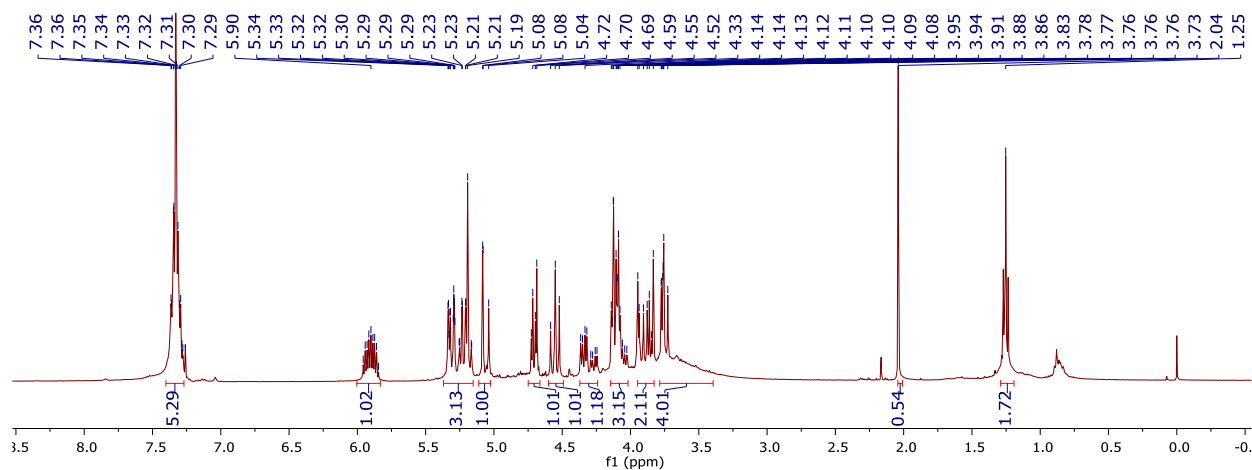


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 21a**

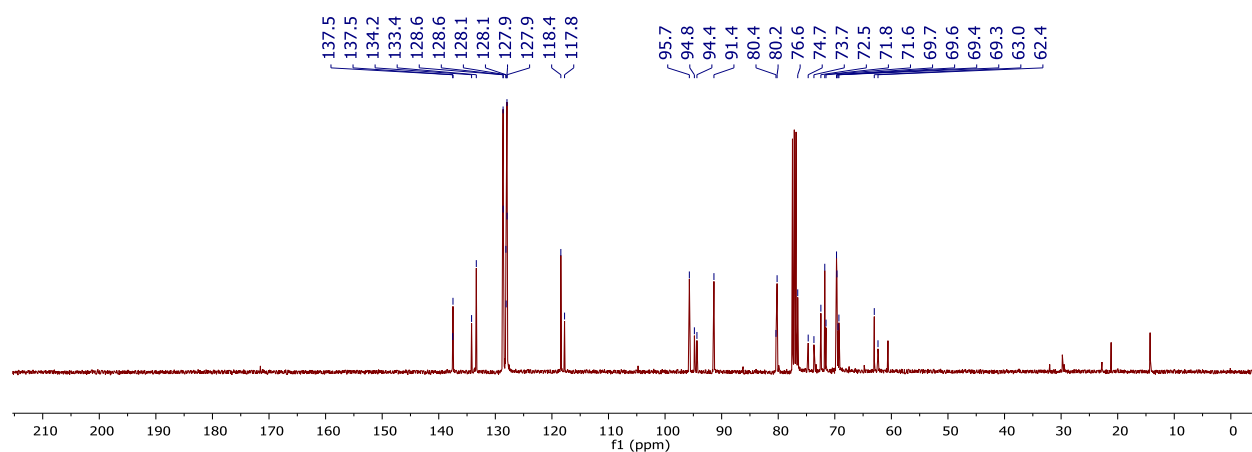


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

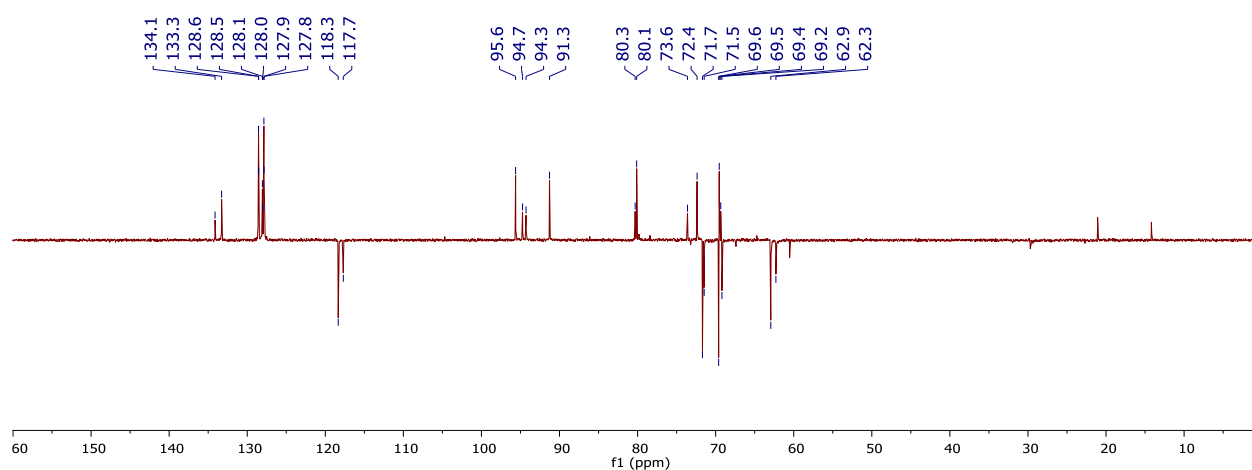
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 21b**



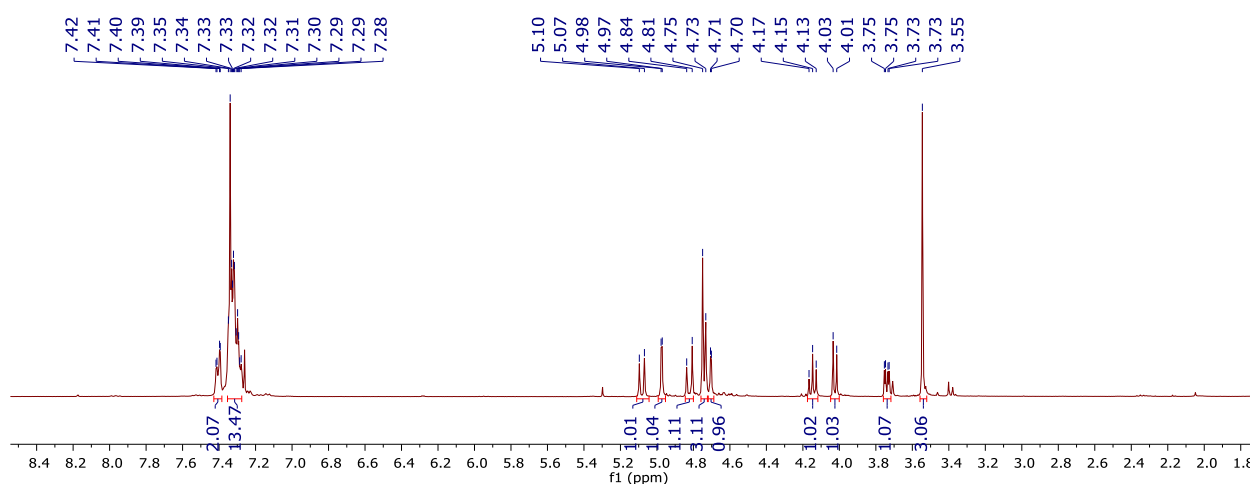
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 21b**



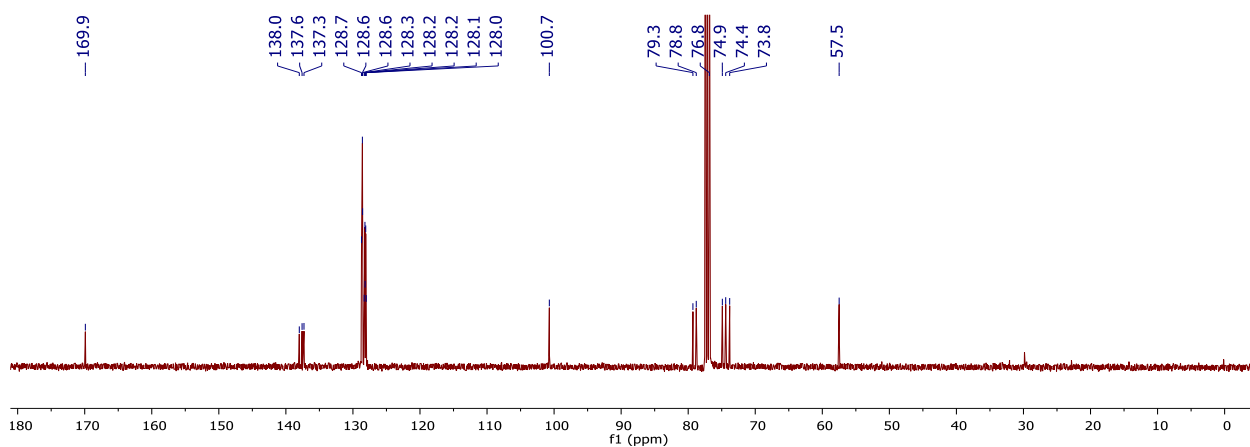
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 21b**



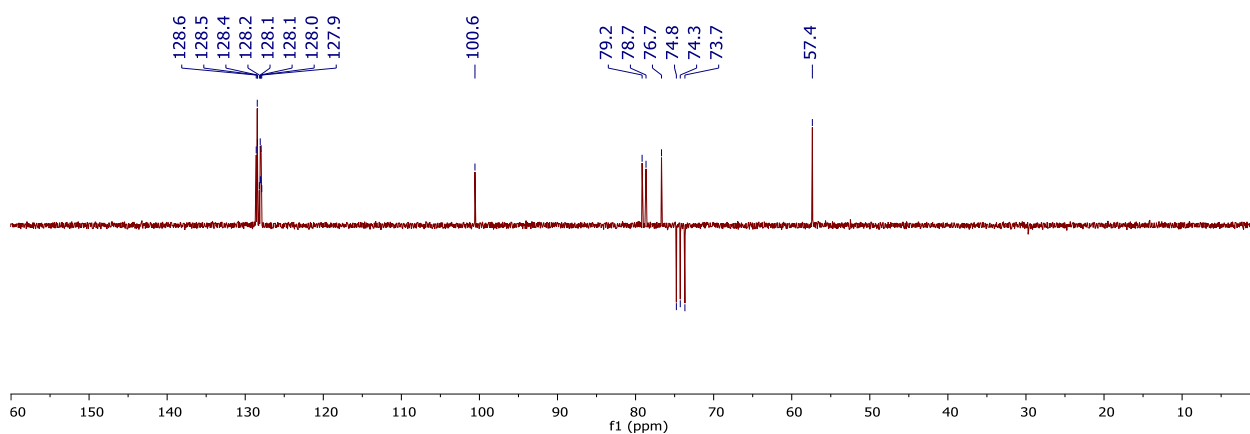
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 24b**



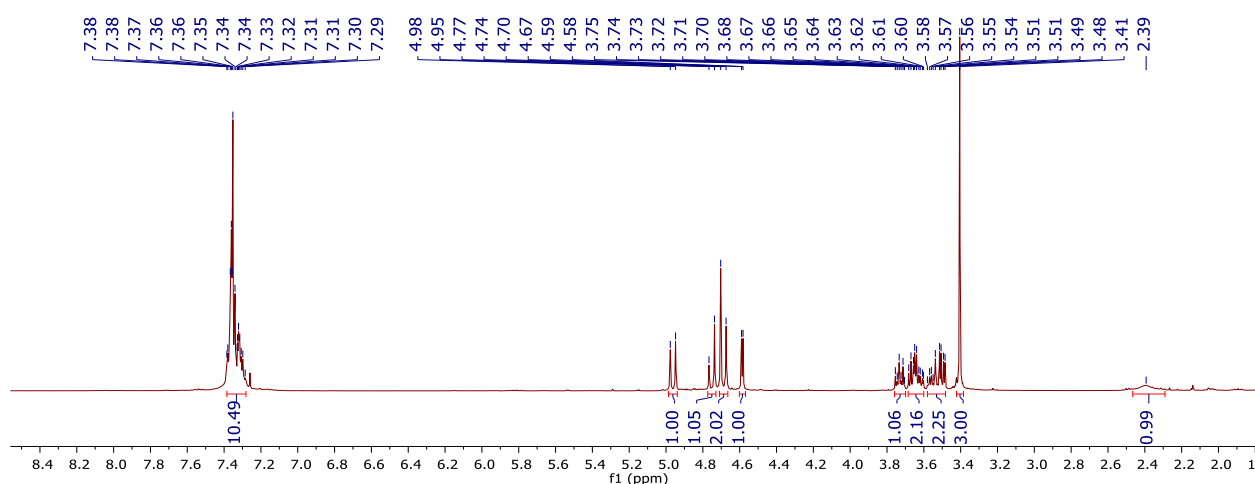
**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 24b**



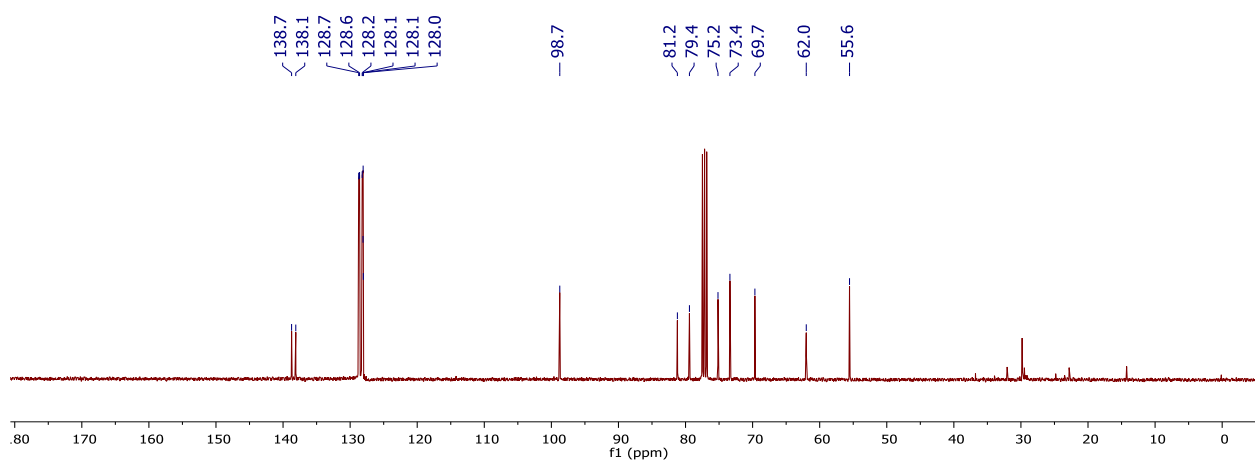
**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 24b**



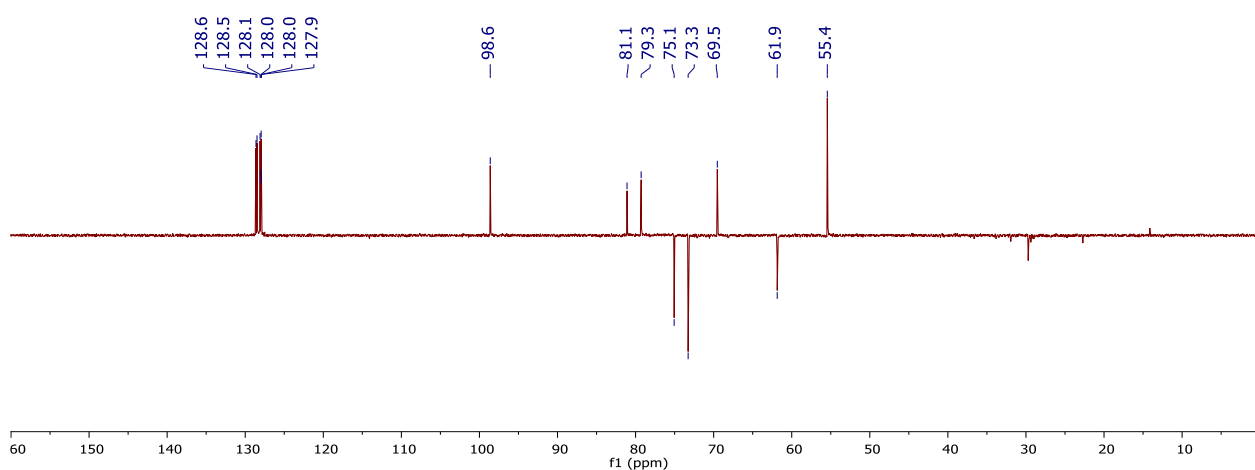
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 24c**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 24c**

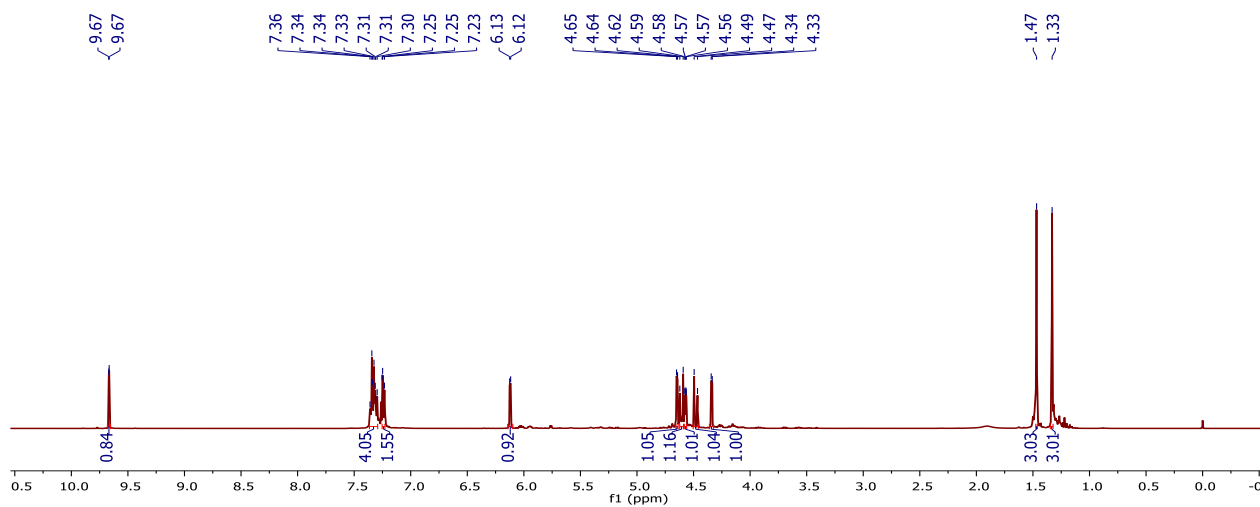


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 24c**

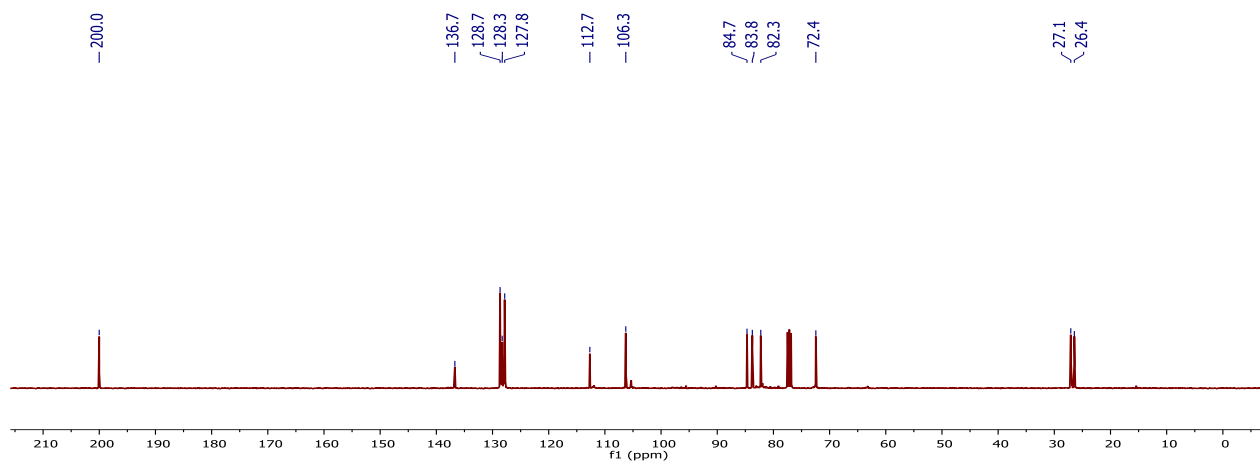


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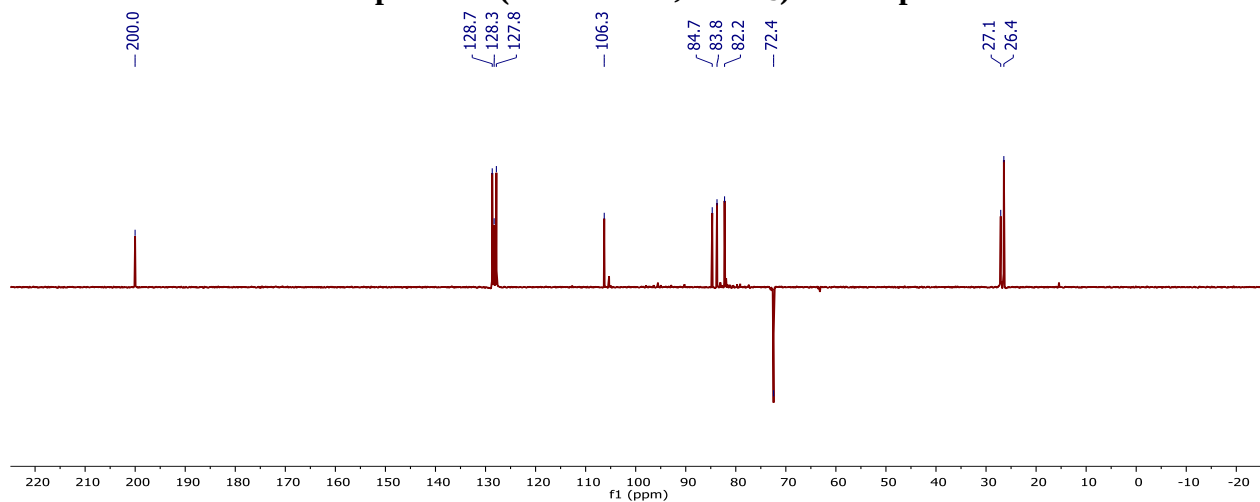
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 26b**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 26b**

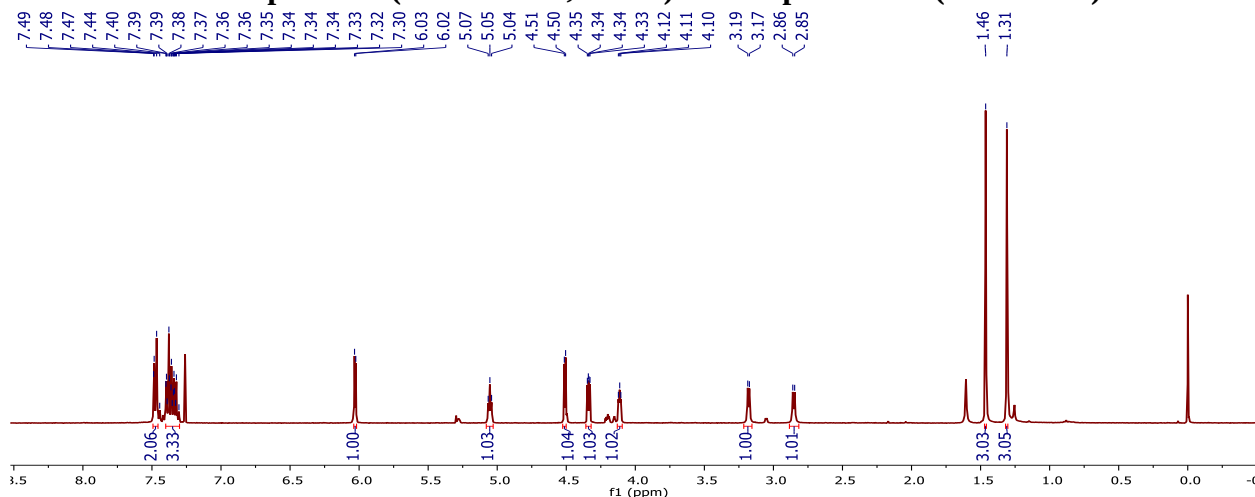


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 26b**

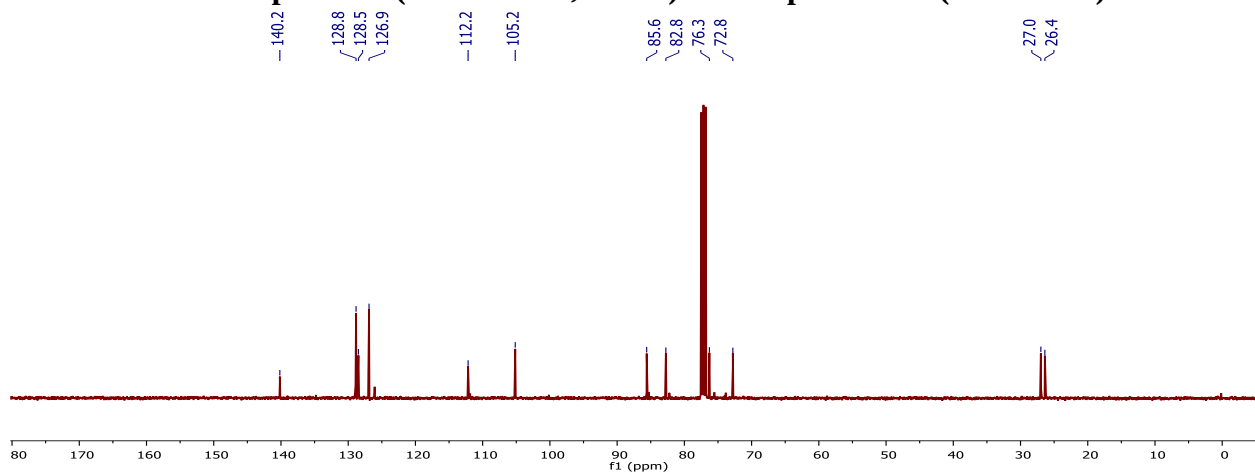


## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

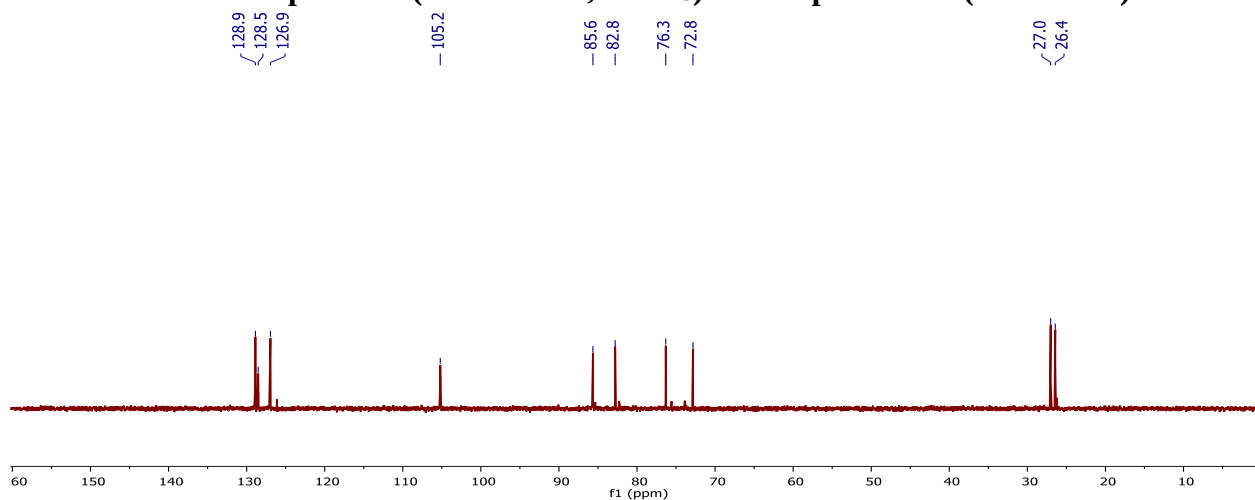
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 27a (Ido isomer)**



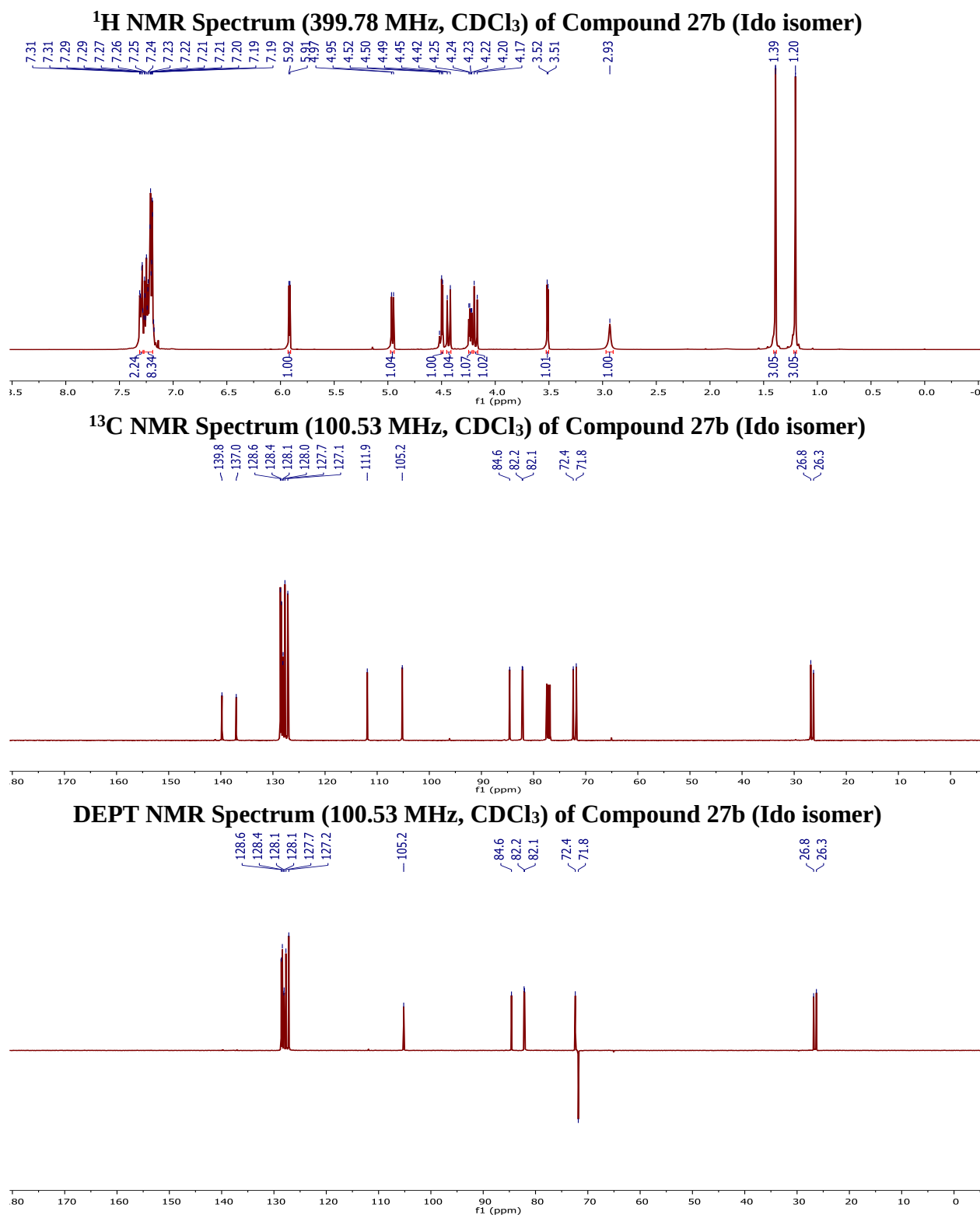
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 27a (Ido isomer)**



**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 27a (Ido isomer)**



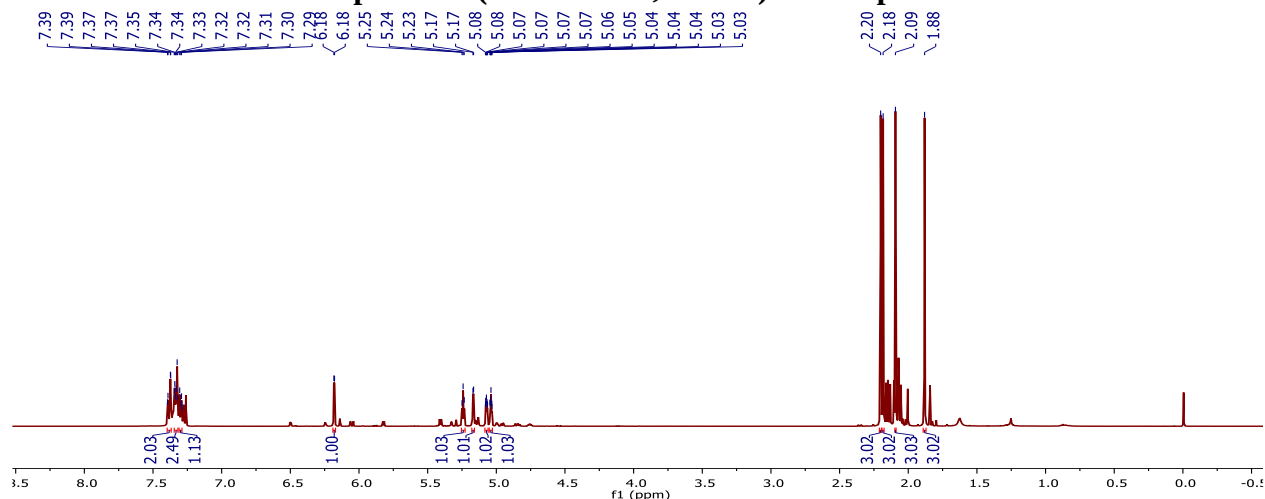
## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives



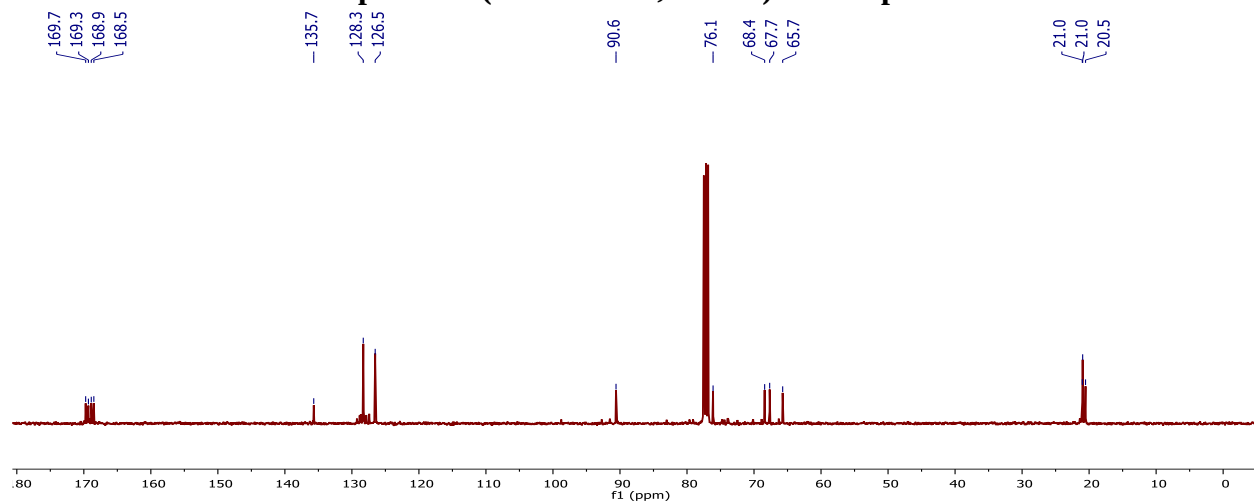


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

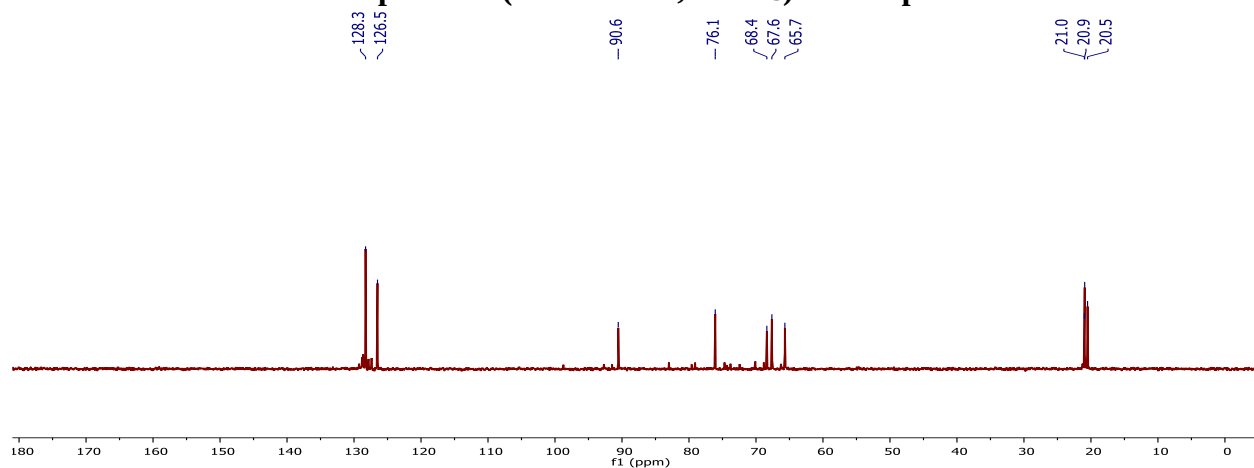
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 28a**



**<sup>13</sup>C NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 28a**

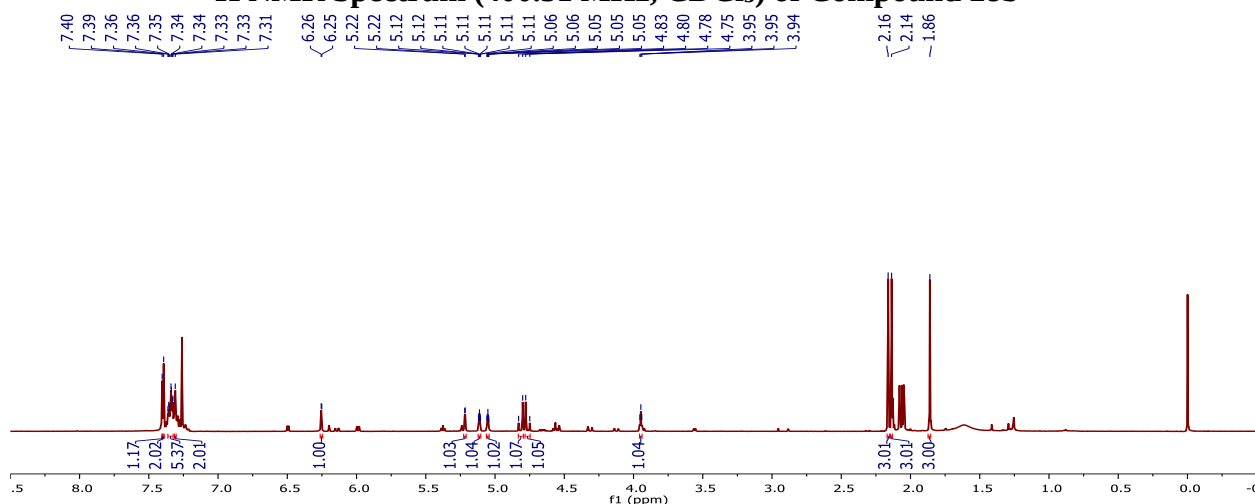


**DEPT NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 28a**

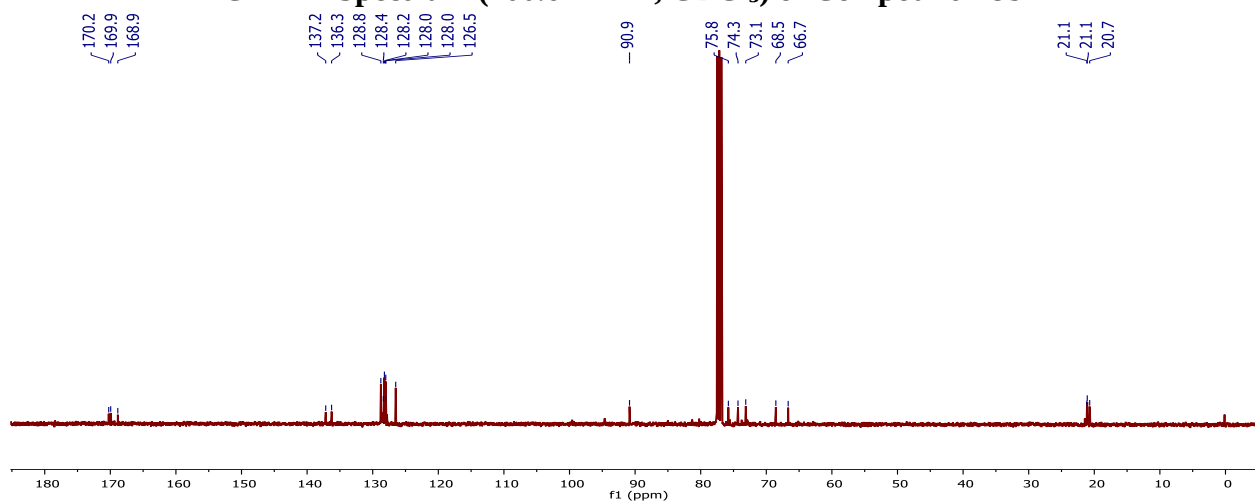


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

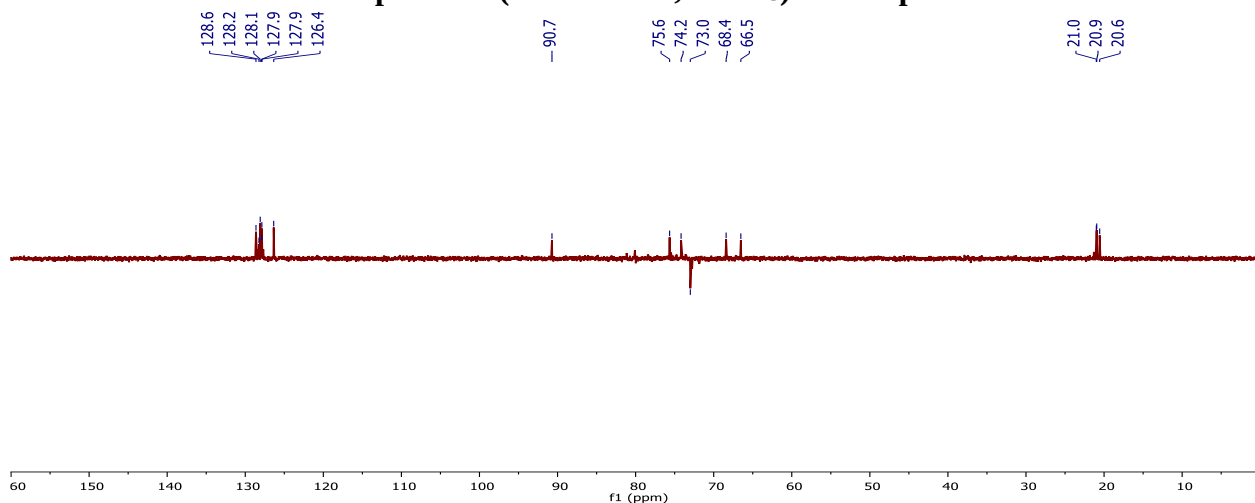
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 28b**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 28b**

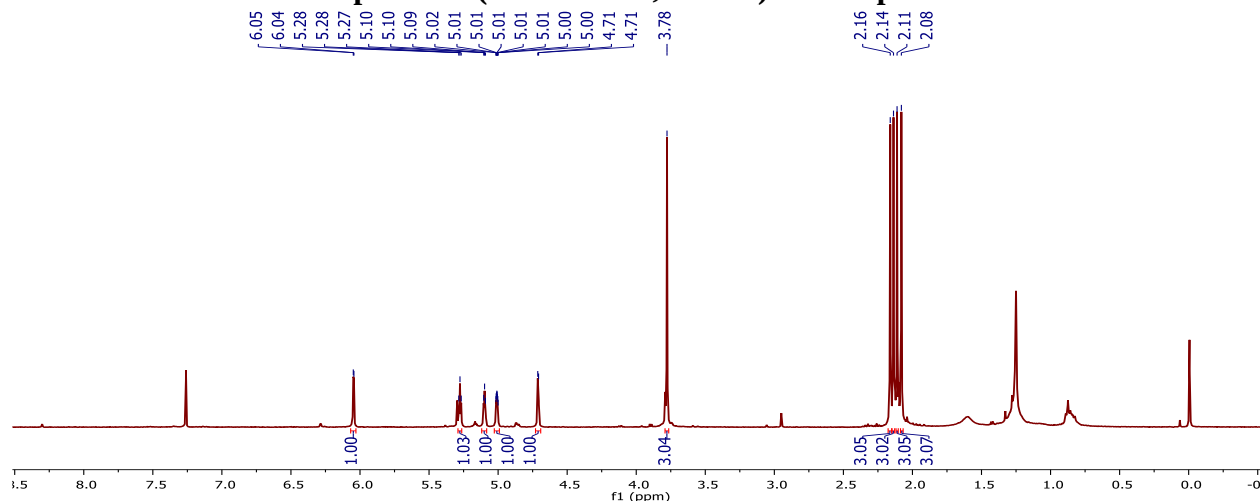


**DEPT NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 28b**

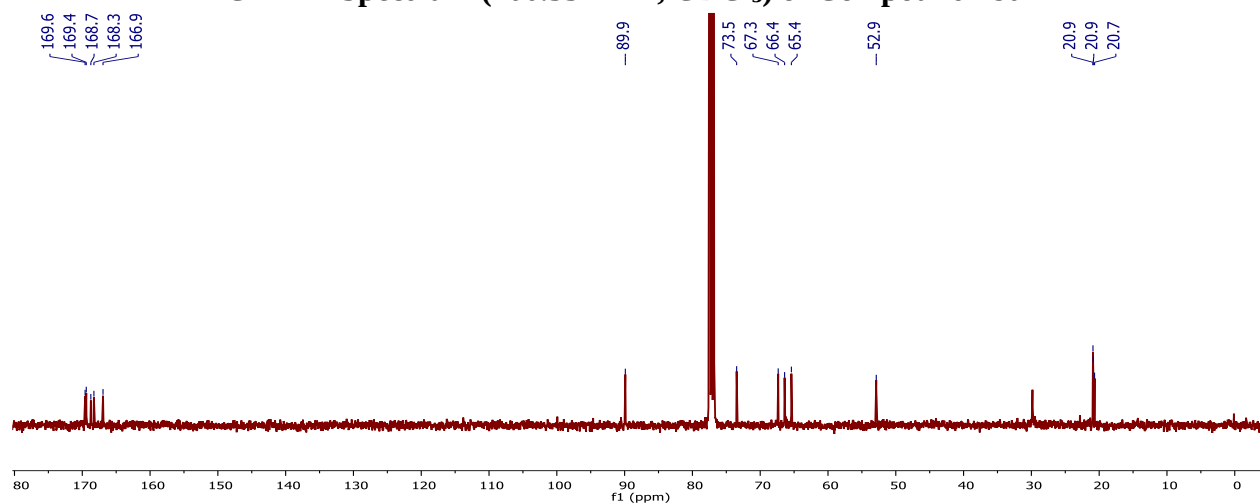


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

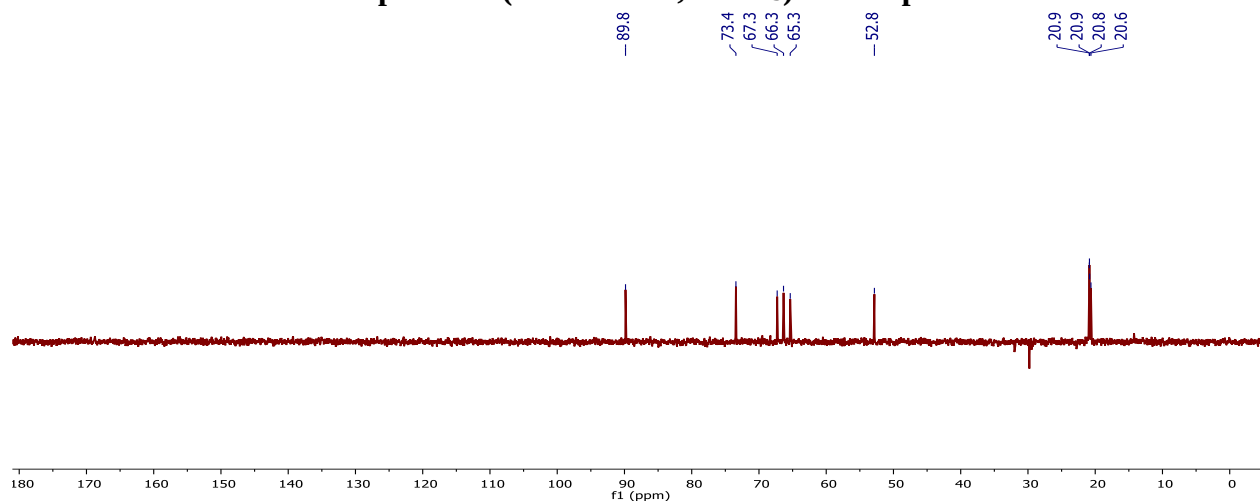
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 29a**

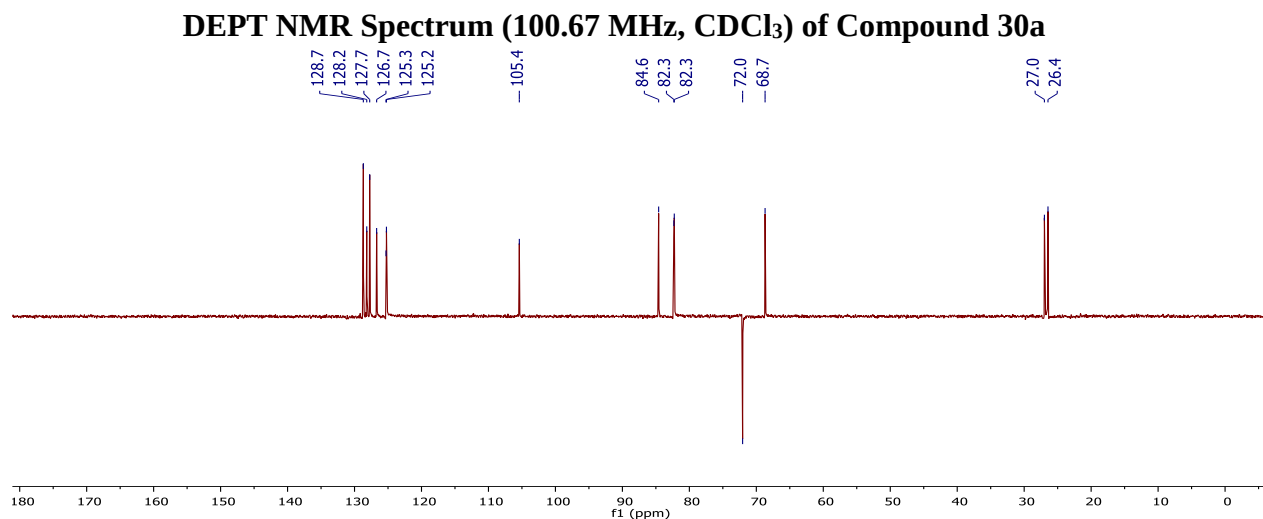
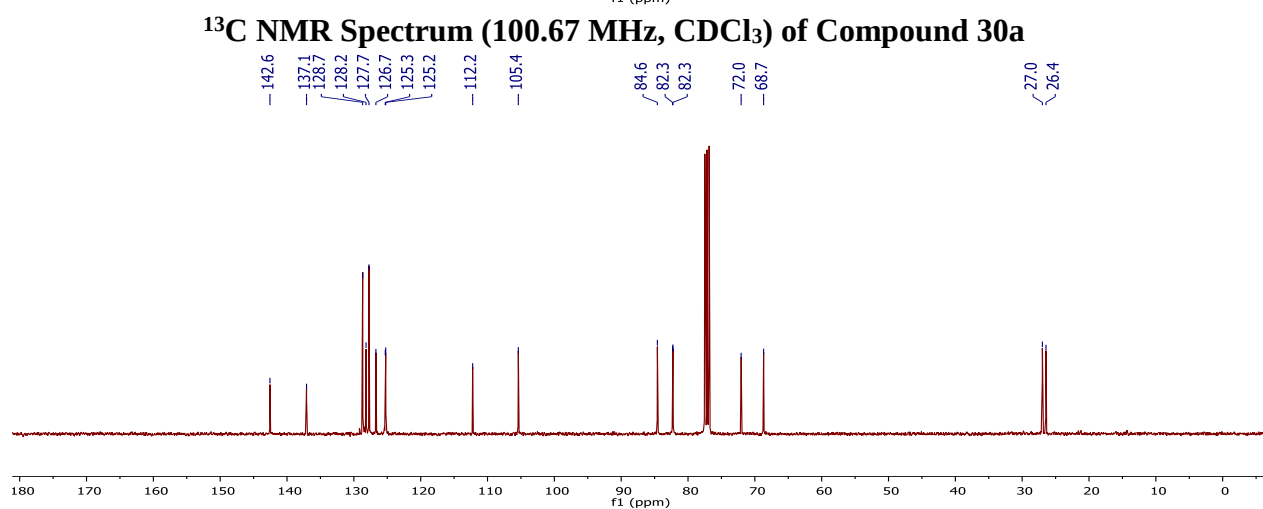
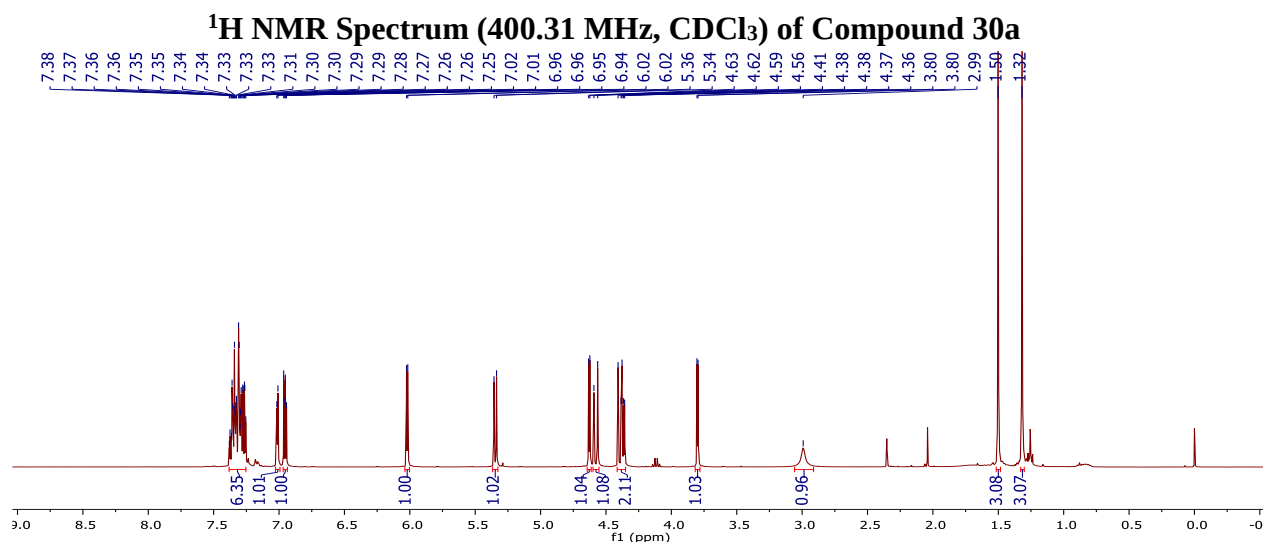


**<sup>13</sup>C NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 29a**

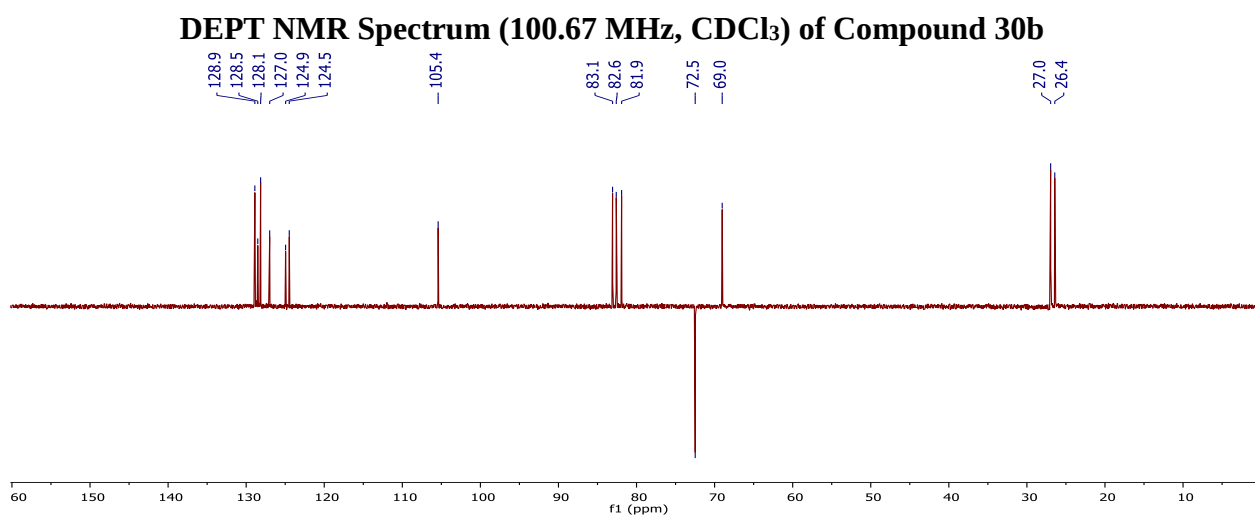
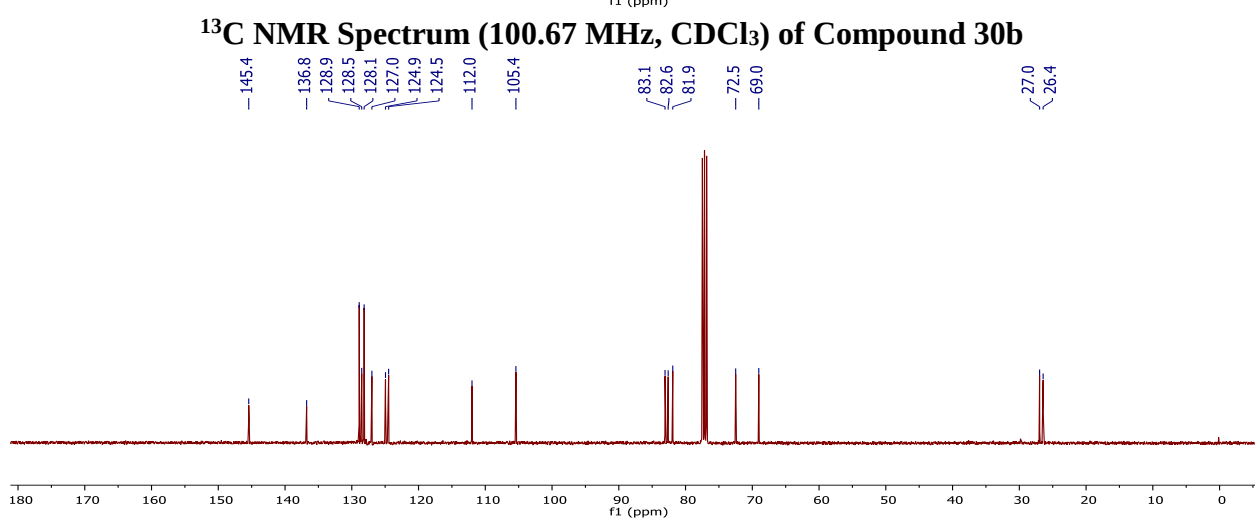
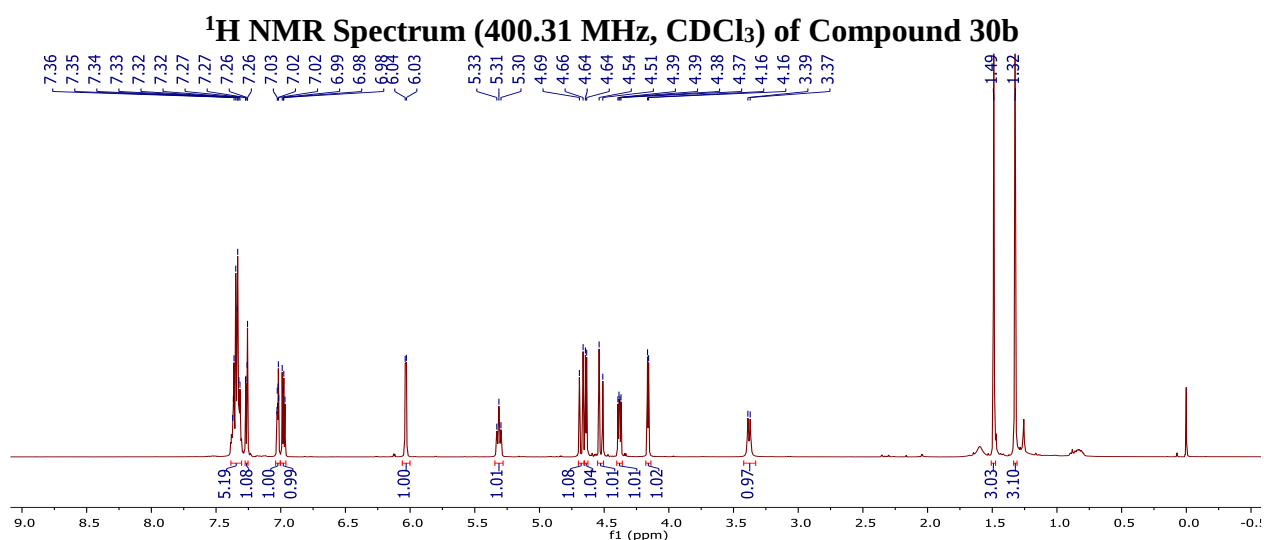


**DEPT NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 29a**



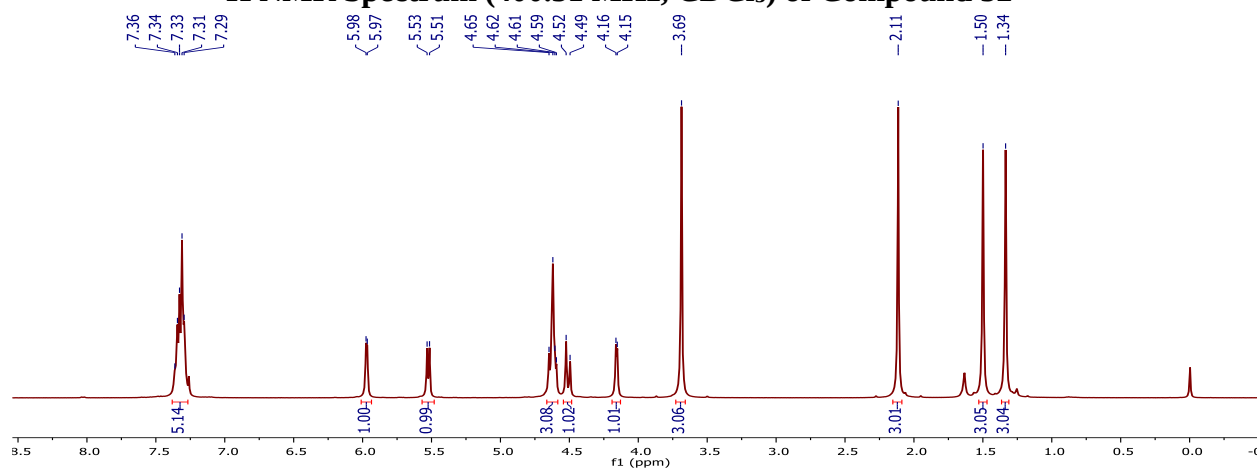


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

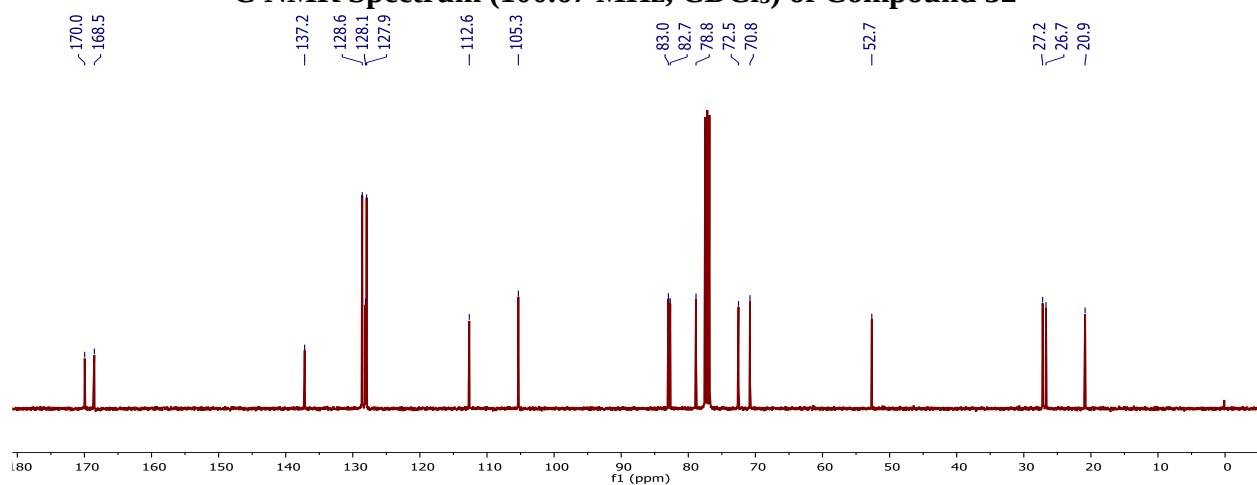


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

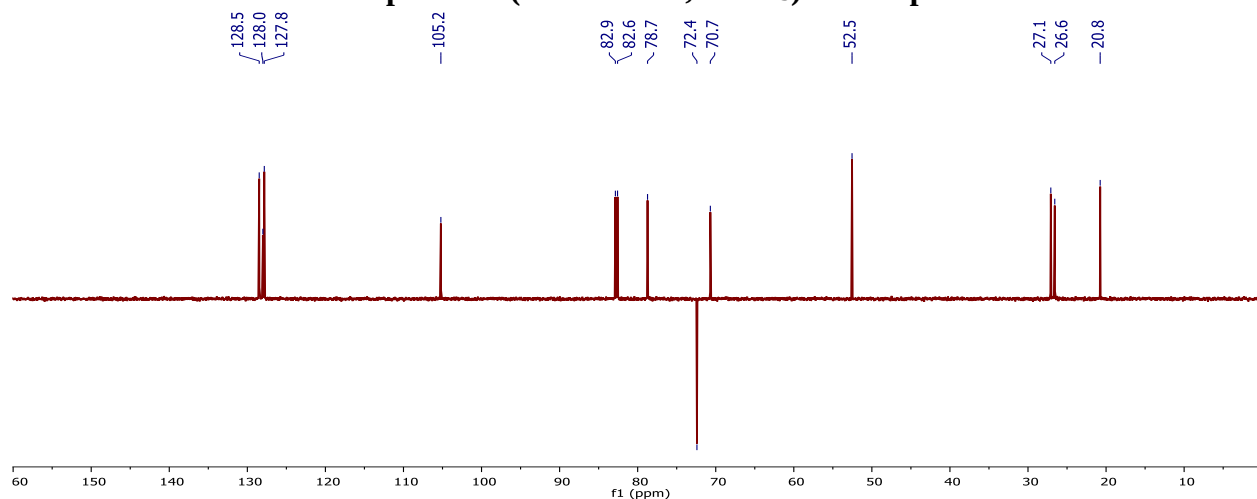
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 32**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 32**

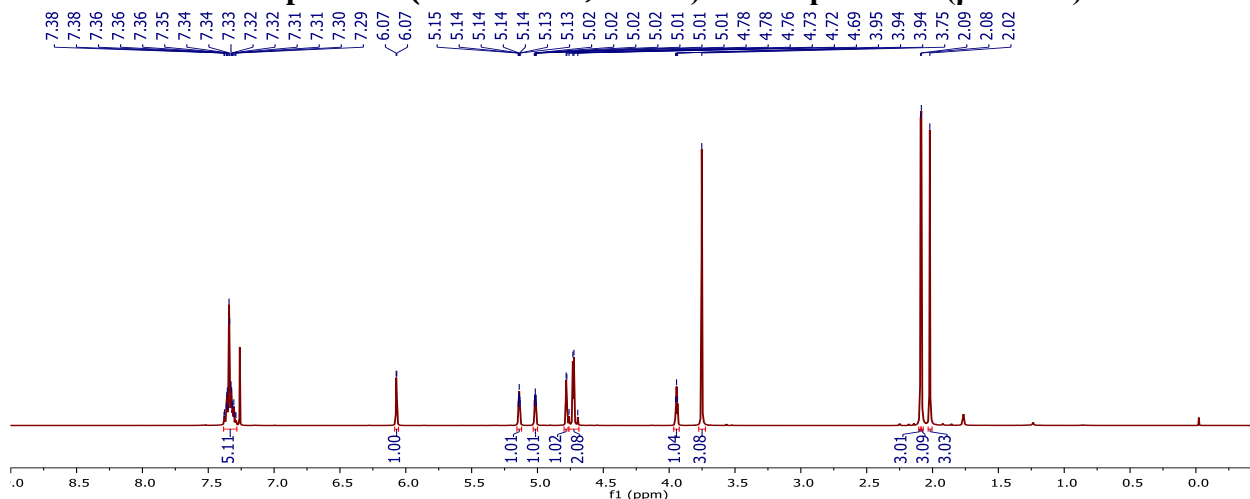


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 32**

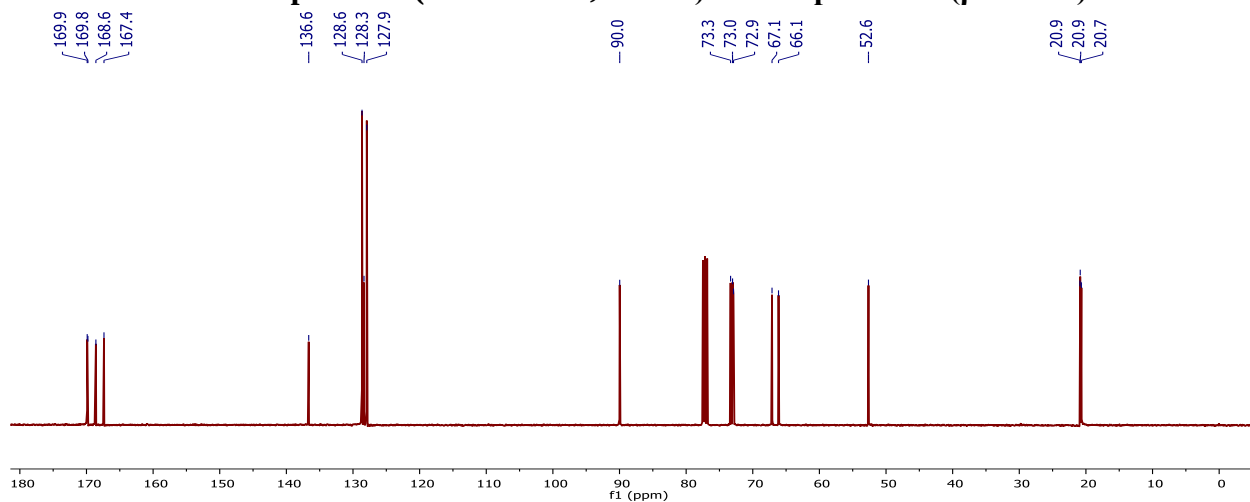


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

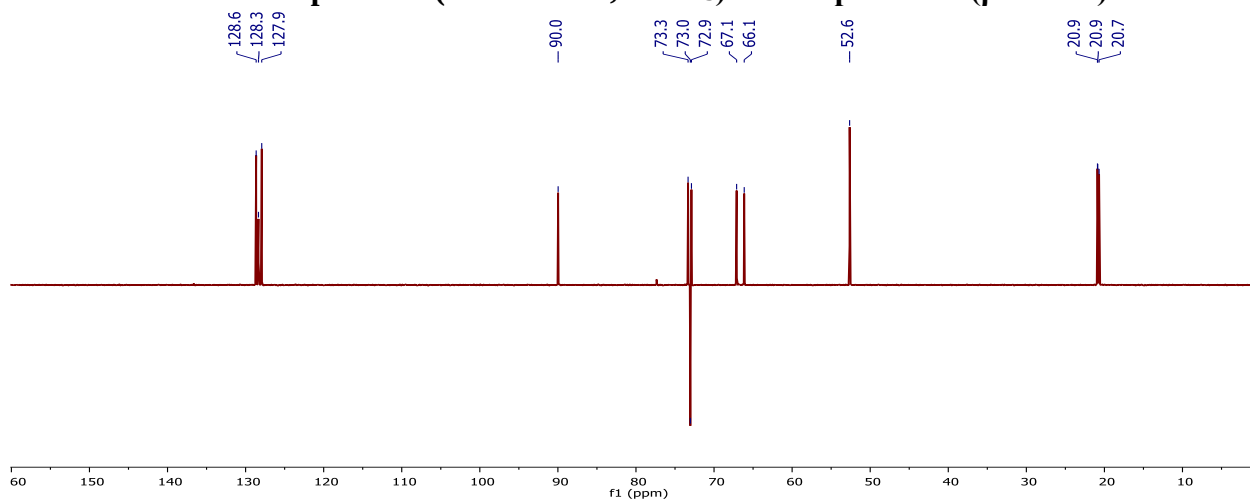
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 34 (β isomer)**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 34 (β isomer)**

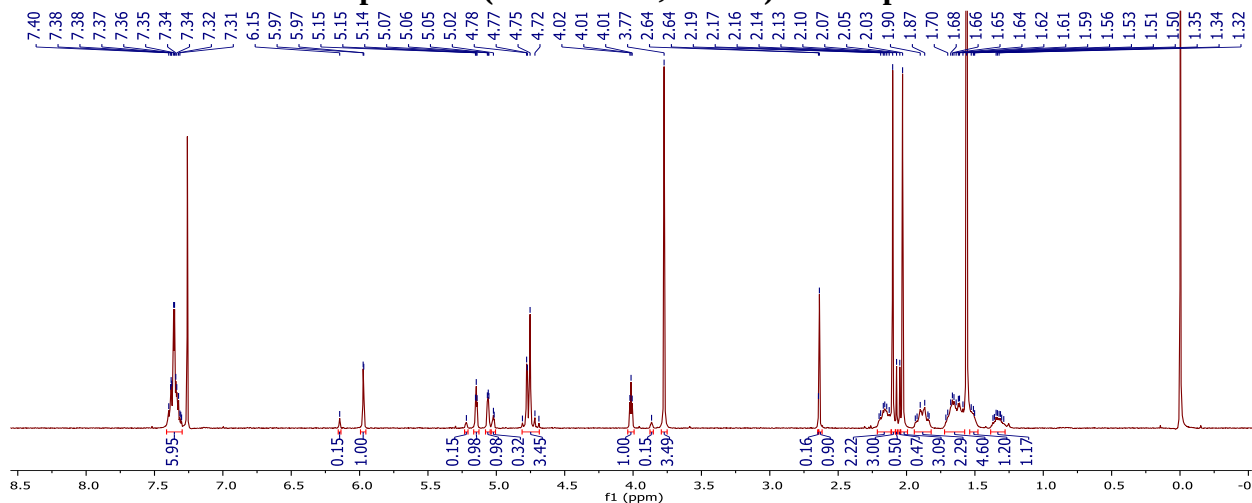


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 34 (β isomer)**

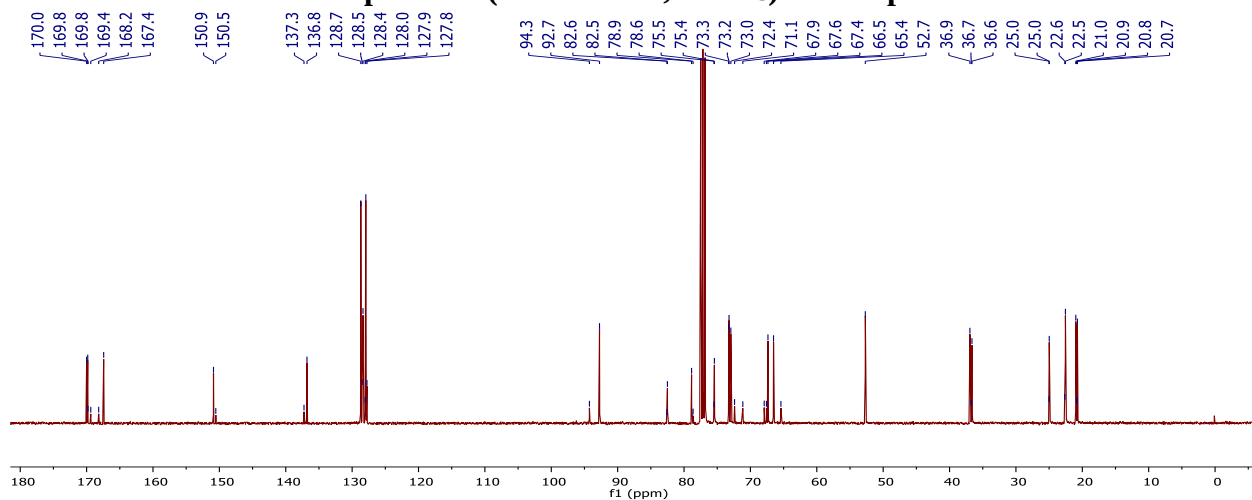


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

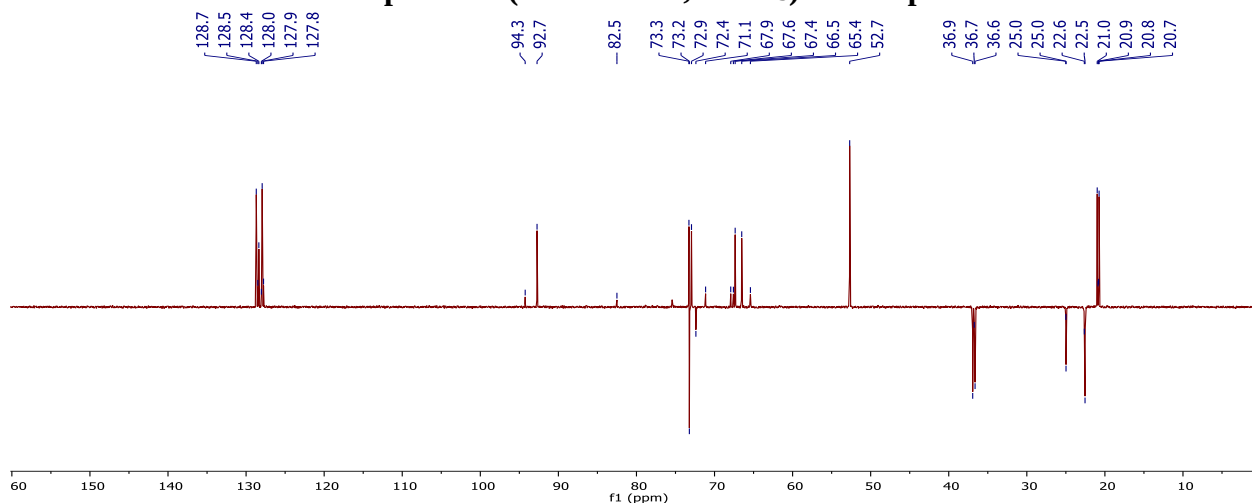
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 37**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 37**



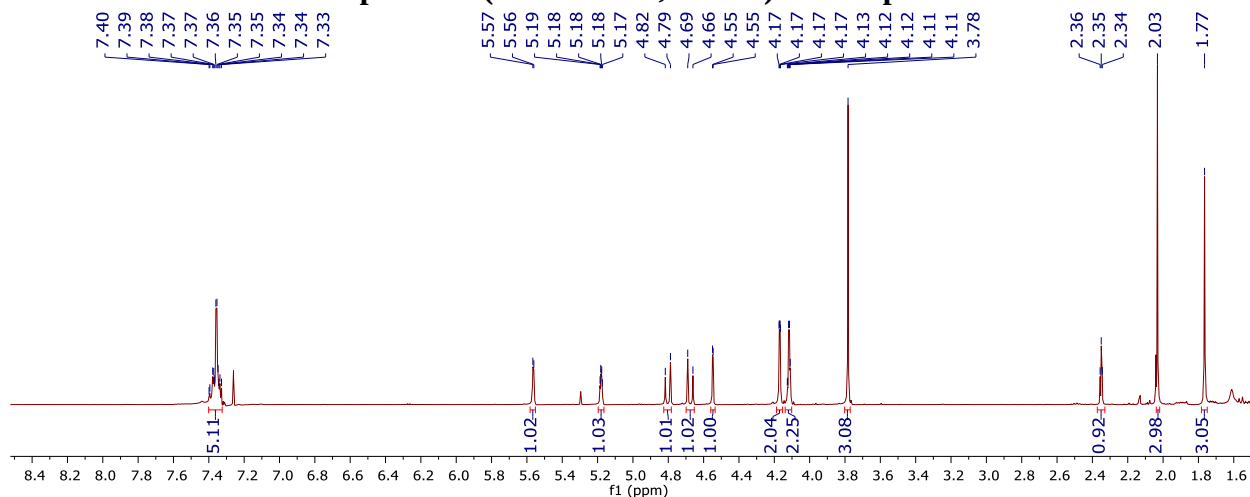
**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 37**



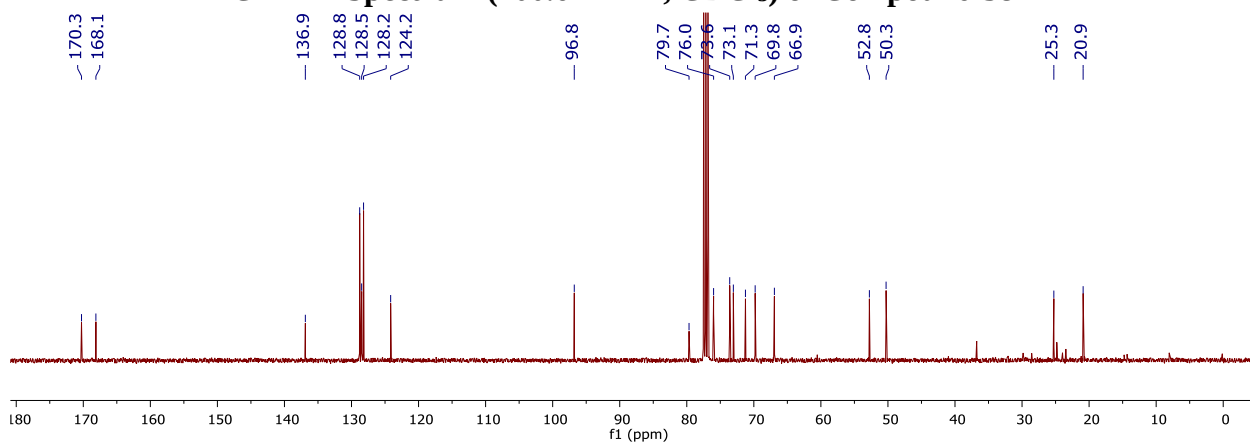


## Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

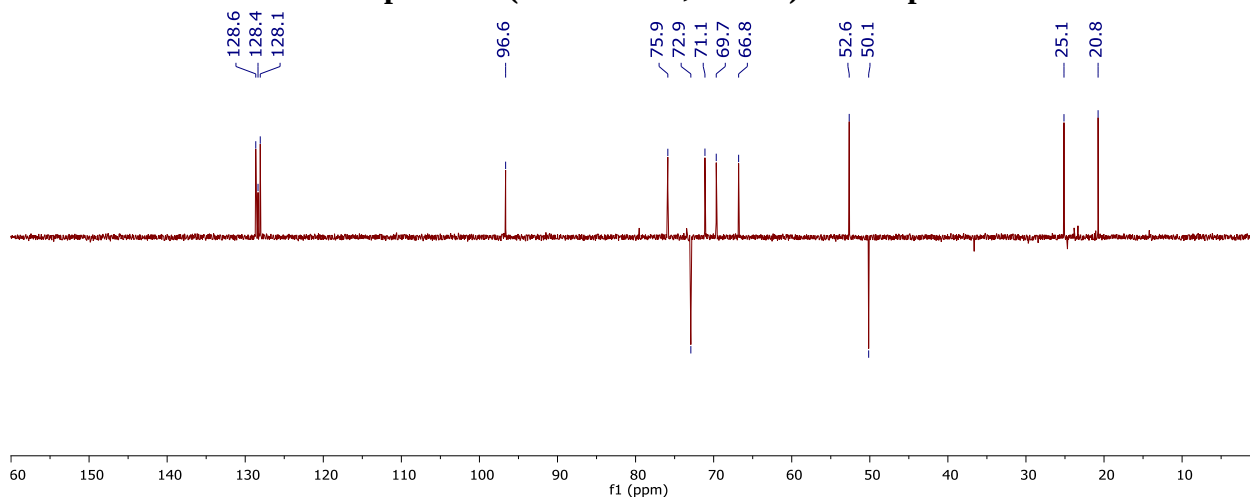
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 38**



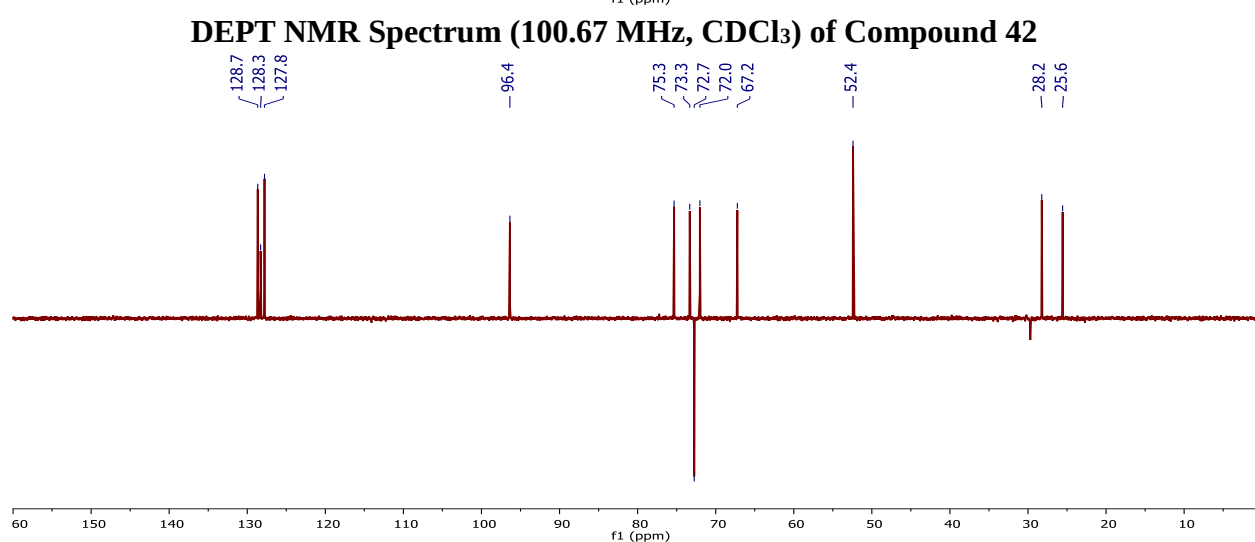
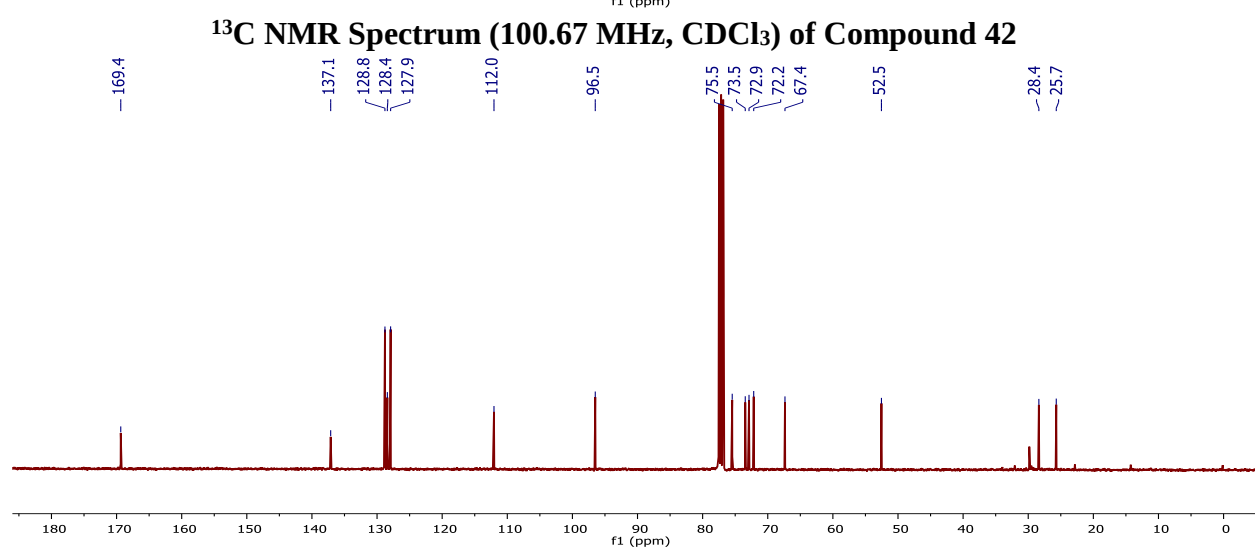
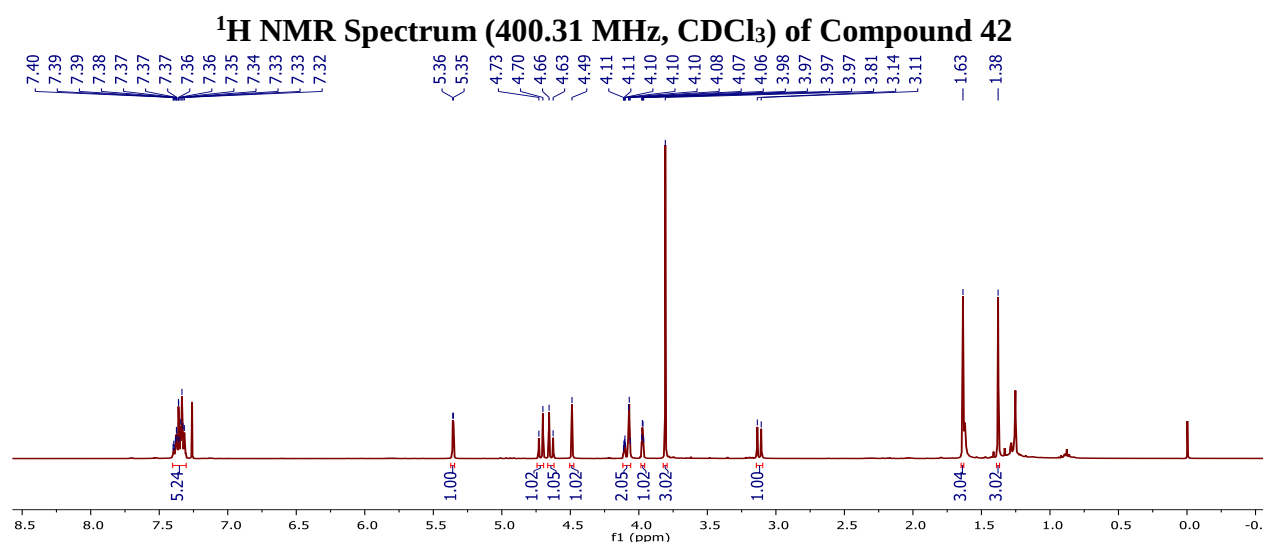
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 38**



**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 38**

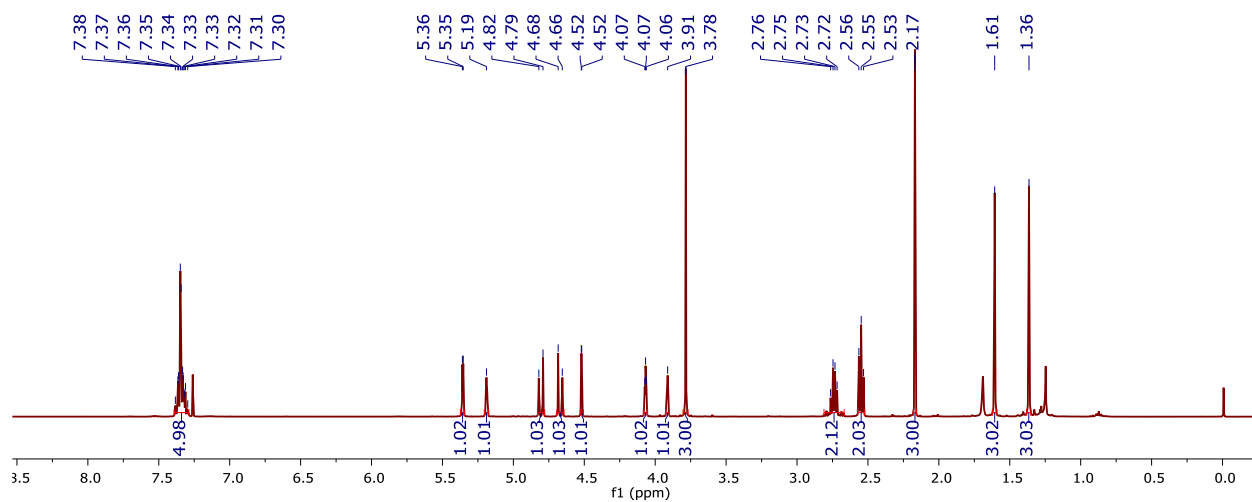


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

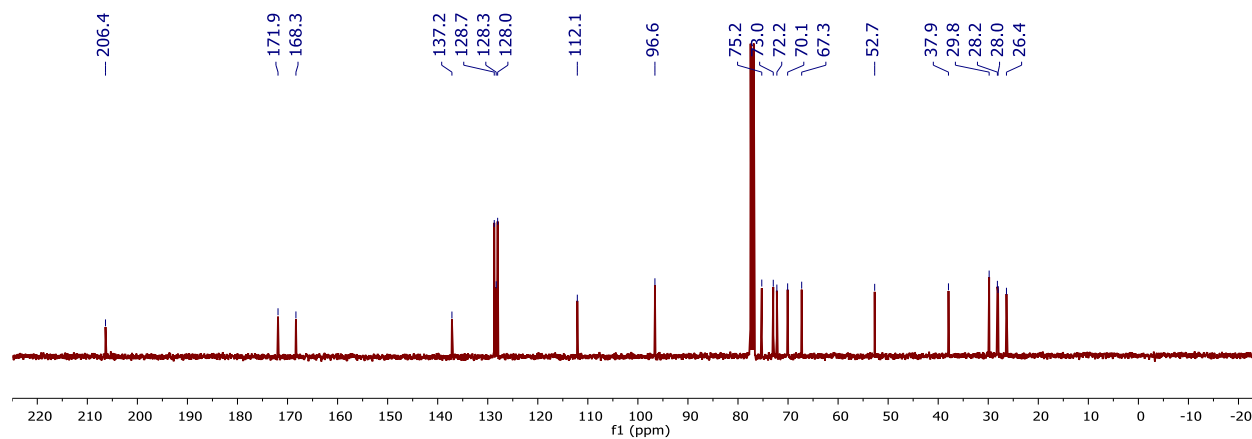


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

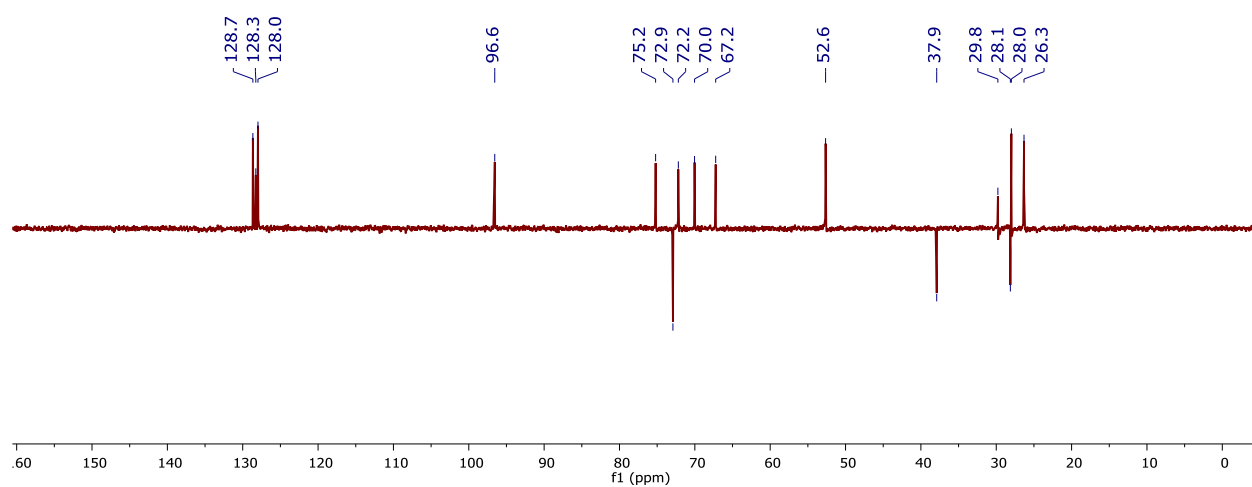
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 43**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 43**

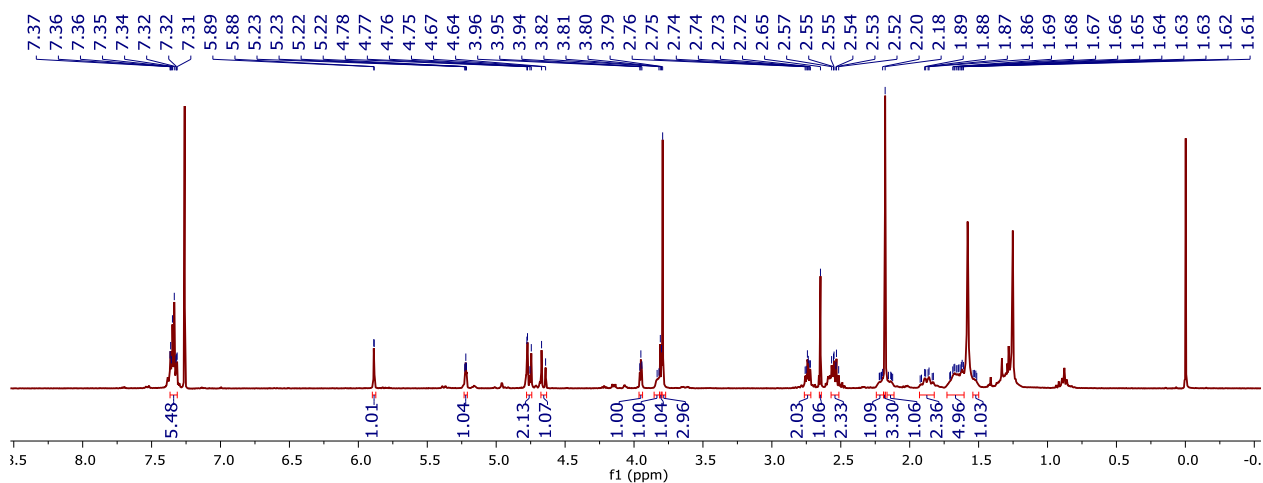


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 43**

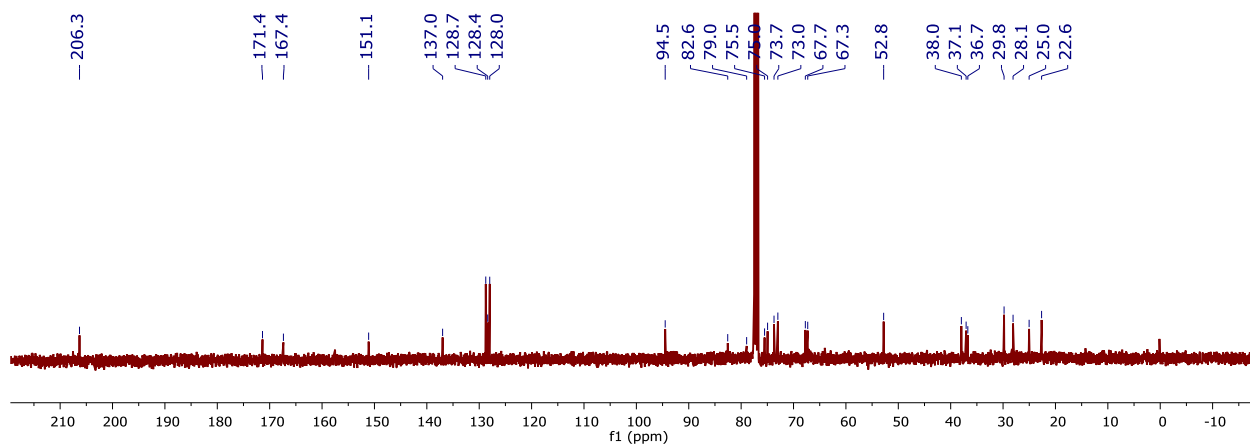


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

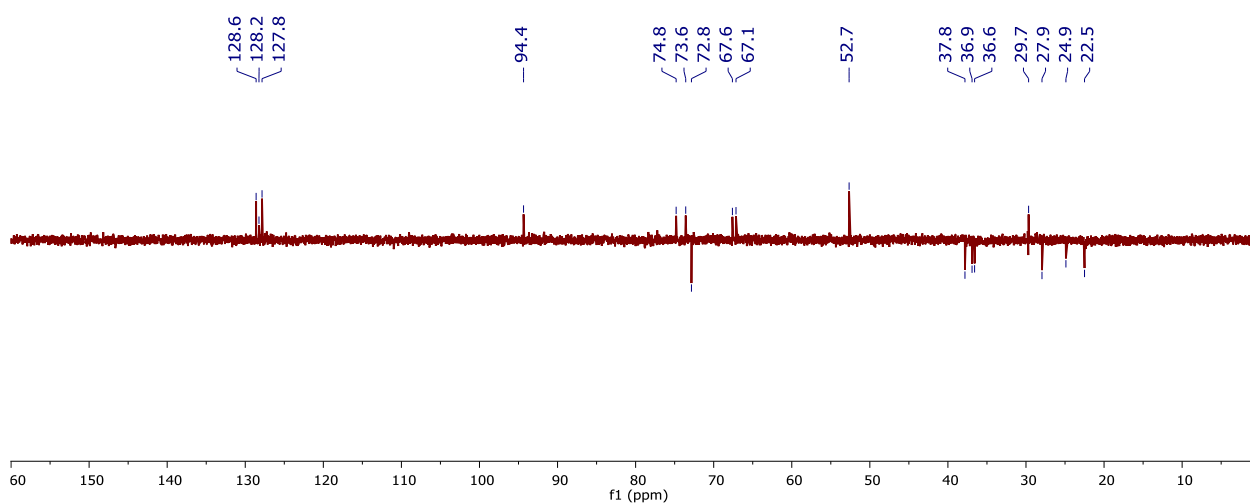
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 45a**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 45a**

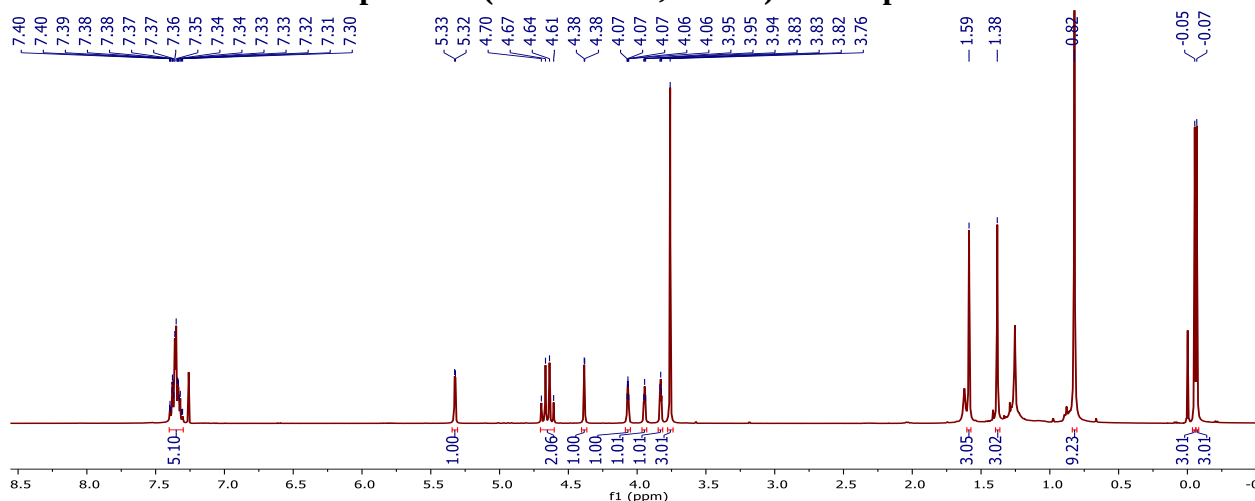


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 45a**

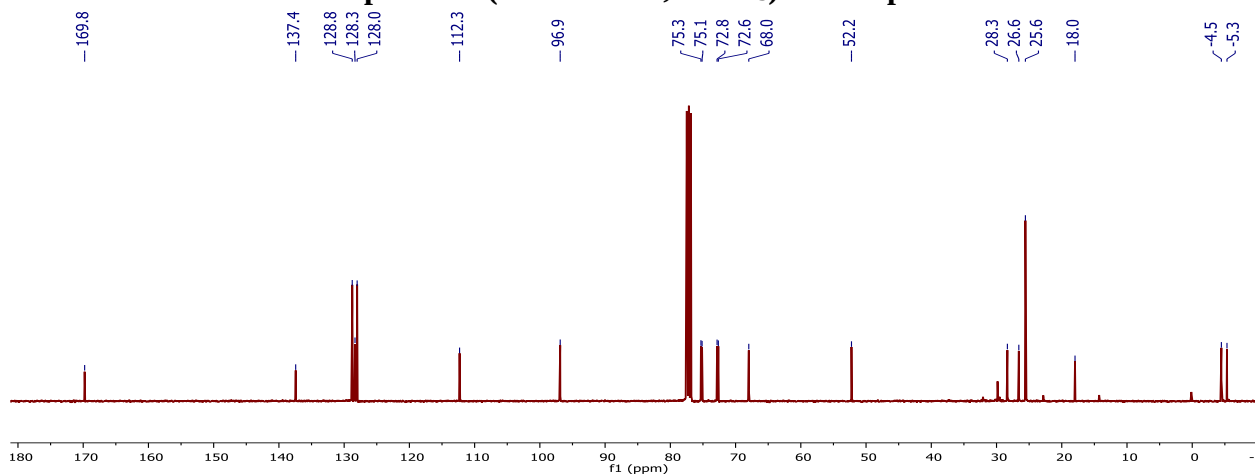


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

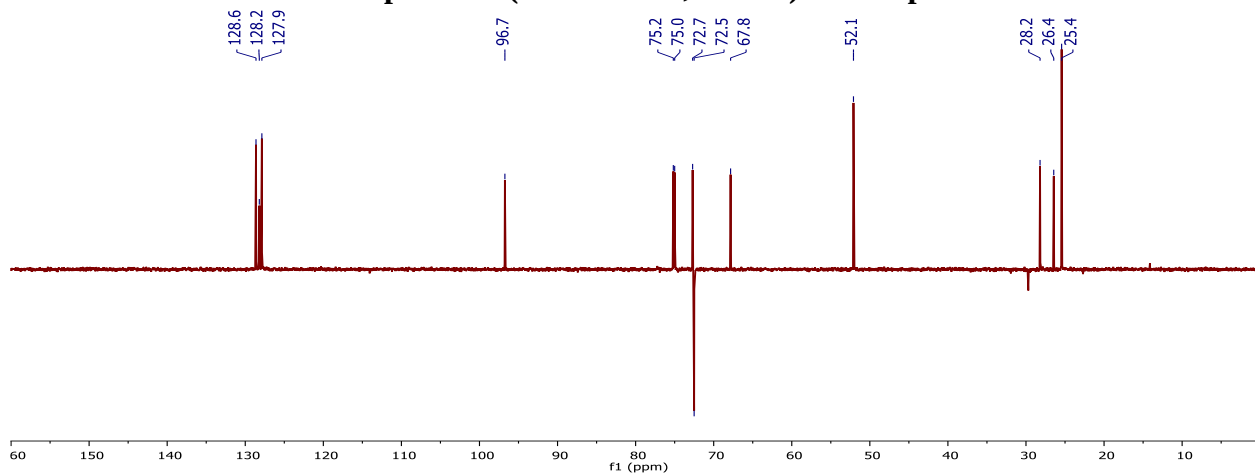
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 46**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 46**

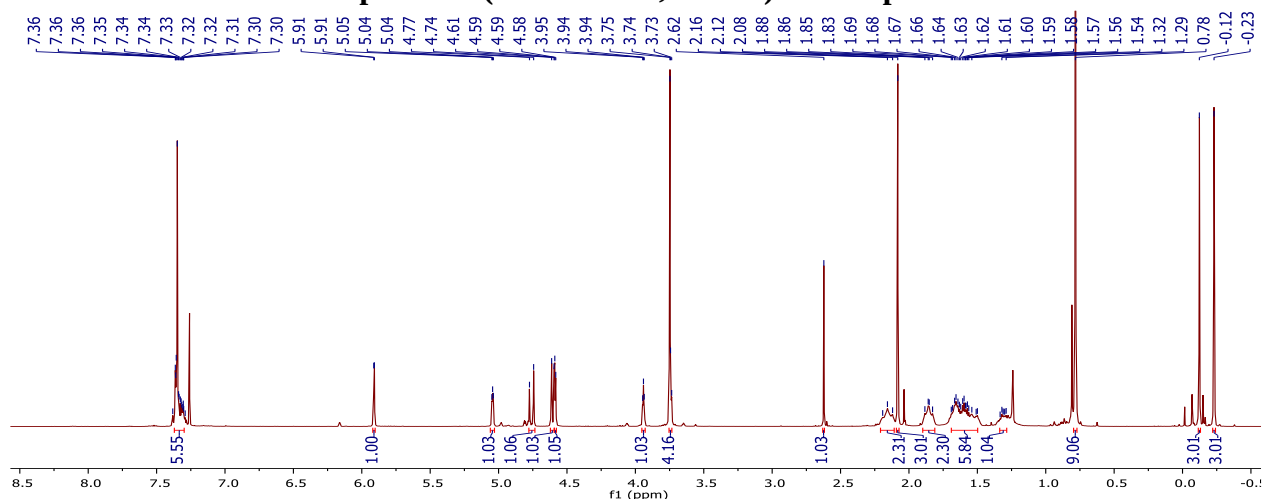


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 46**

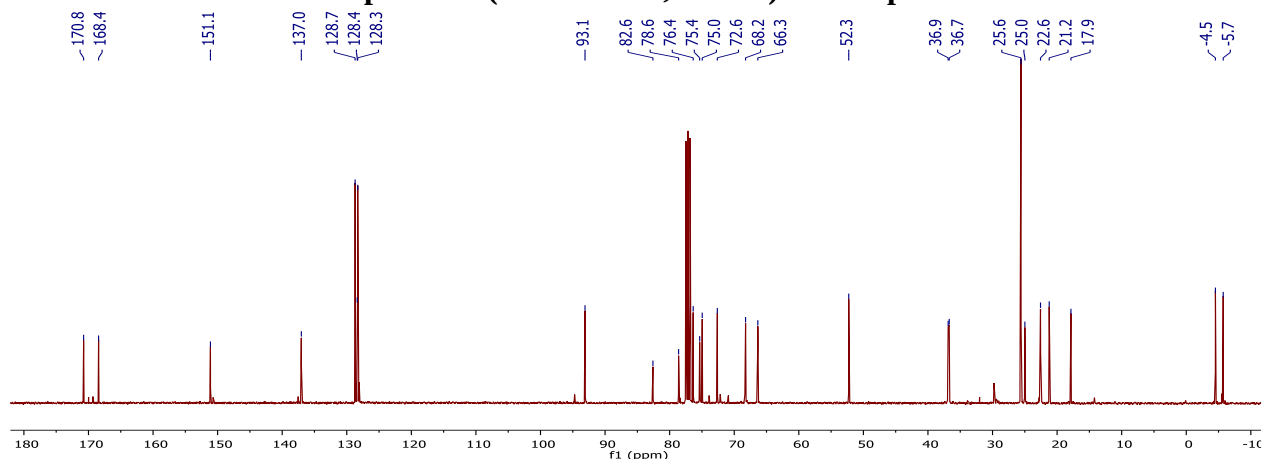


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

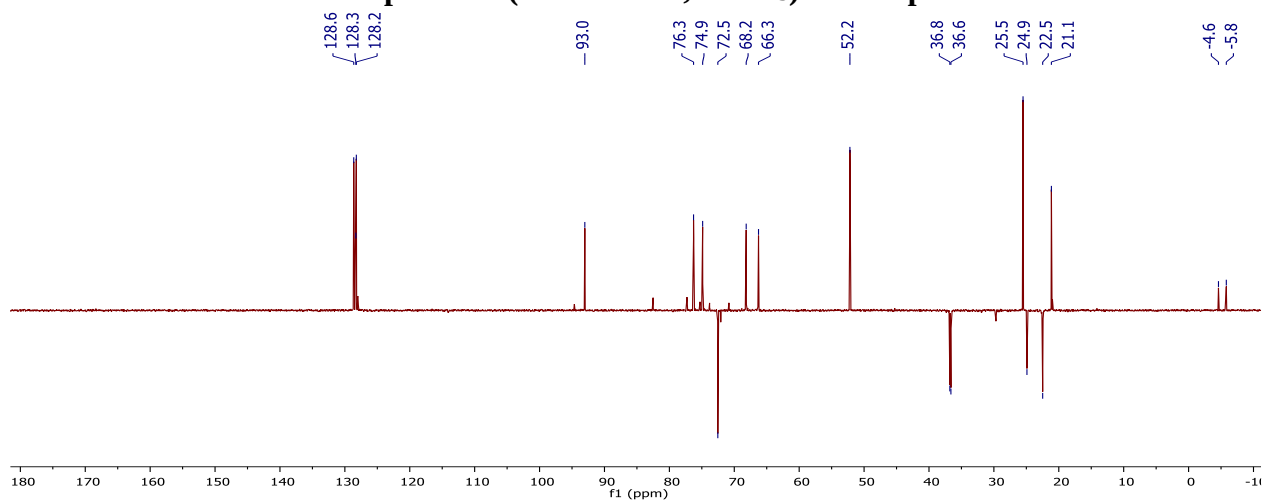
**$^1\text{H}$  NMR Spectrum (399.78 MHz,  $\text{CDCl}_3$ ) of Compound 48**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 48**

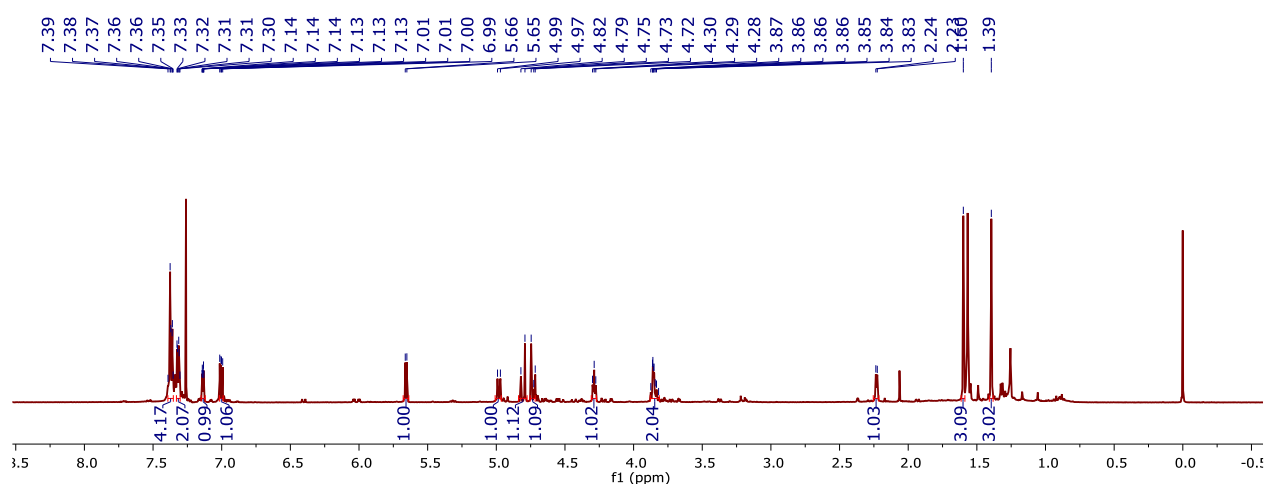


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 48**

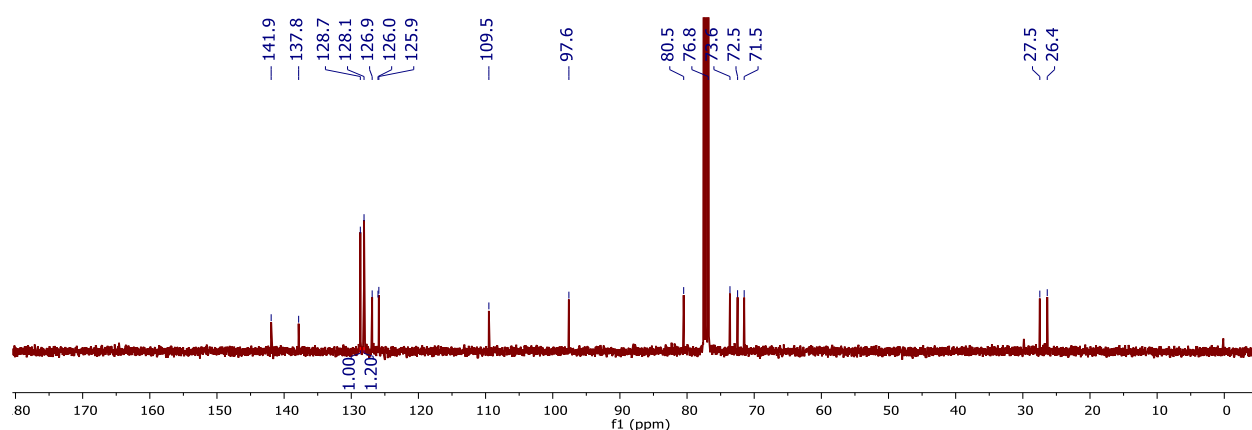


# Strategies for the Synthesis of Iduronic Acid (IdoA) Derivatives

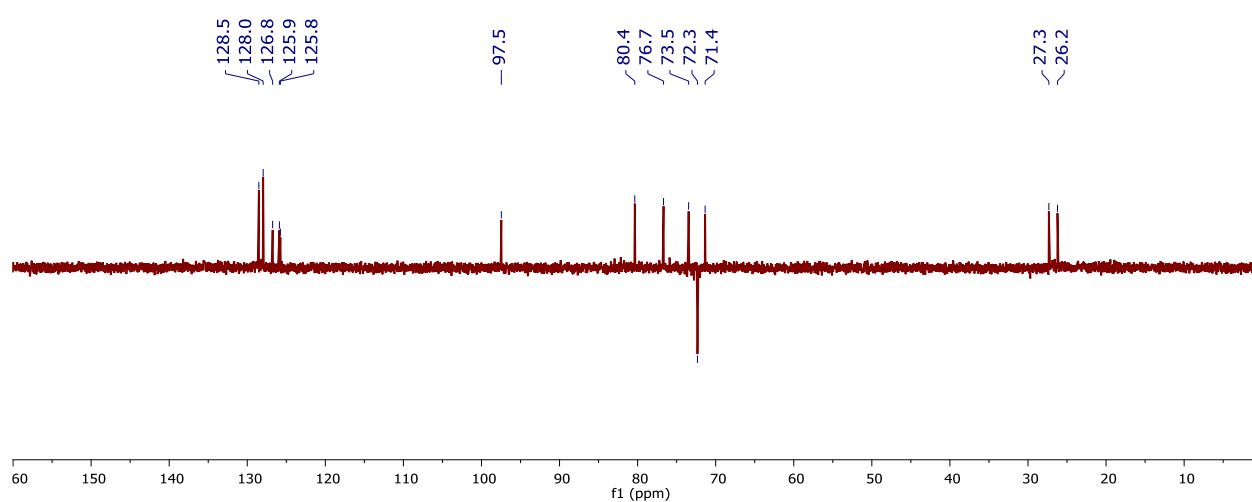
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 49**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 49**



**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 49**



### 2.8 References:

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- 2) (a) Hansen, S. U.; Baráth, M.; Salameh, B. A. B.; Pritchard, R. G.; Stimpson, W. T.; Gardiner, J. M.; Jayson, G. C. *Org. Lett.* **2009**, 11, 4528–4531. (b) Lubineau, A.; Gabvard, O.; Alais, J.; Bonnaffé, D. *Tetrahedron Lett.* **2000**, 41, 307–311.
- 3) (a) Banks, H. D.; *J. Org. Chem.* **1981**, 46, 1743–1745; Sulake, R. S.; Jiang, Y.-F.; Lin, H.-H.; Chen, C. *J. Org. Chem.* **2014**, 79, 10820–10828. (b) Takeda, K.; Toyota, M. *Tetrahedron* 2011, 67, 9909–9921; Roy, A.; Paul, T.; Michael, G. B. Drew, M. G. B.; Mukherjee; D. *Tet. Lett.* **2003**, 44, 4835–4837.
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- 6) Patent-PCT/CN2014/087587 (WO2015043511A1).
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## **Chapter 3**

### ***Donor Acceptor Selectivity Studies with GlcA and GlcN Building Blocks***

### 3.1 Introduction:

Chemical synthesis of heparin oligosaccharide demands orthogonally protected iduronic acid, glucuronic acid and glucosamine units. A novel route to the synthesis of orthogonally protected iduronic acid donor/acceptor are described in chapter 2 wherein chelation assisted Grignard addition of aryl group, furanose to pyranose conversion and Ru-mediated oxidation as key steps. Synthesis of orthogonally protected glucuronic and glucosamine building is the next logical extension in our journey. It is well known that reactivity, polarity and thus purification largely depend on the nature of the protecting groups, stereoelectronic factors imparted by them. Needless to mention that the neighbouring group participation in carbohydrate chemistry for accomplishing the 1,2-*trans* diastereoselective glycosidations. Also, the use of N<sub>3</sub> at the C-2 position of glucose serves as surrogate for the NH<sub>2</sub> and also the stereoelectronic nature of N<sub>3</sub> facilitates the 1,2-*cis* selectivity while undergoing the glycosidation.<sup>1</sup> Accordingly, before venturing out, a large set of thoughtfully designed building block synthesis keeping orthogonality issues and predictive polarities was initiated. Our careful analysis revealed that the preparation of allyl glycopyranosides, followed by the benzylidene locking at O-4,6-positions at the early stage<sup>2</sup>, can make orthogonal installation feasible with various essential protecting groups. In this endeavor, we have prepared diversified sets of glucuronic acid and glucosamine acceptors, as well as donors, and utilized them for donor-acceptor studies.

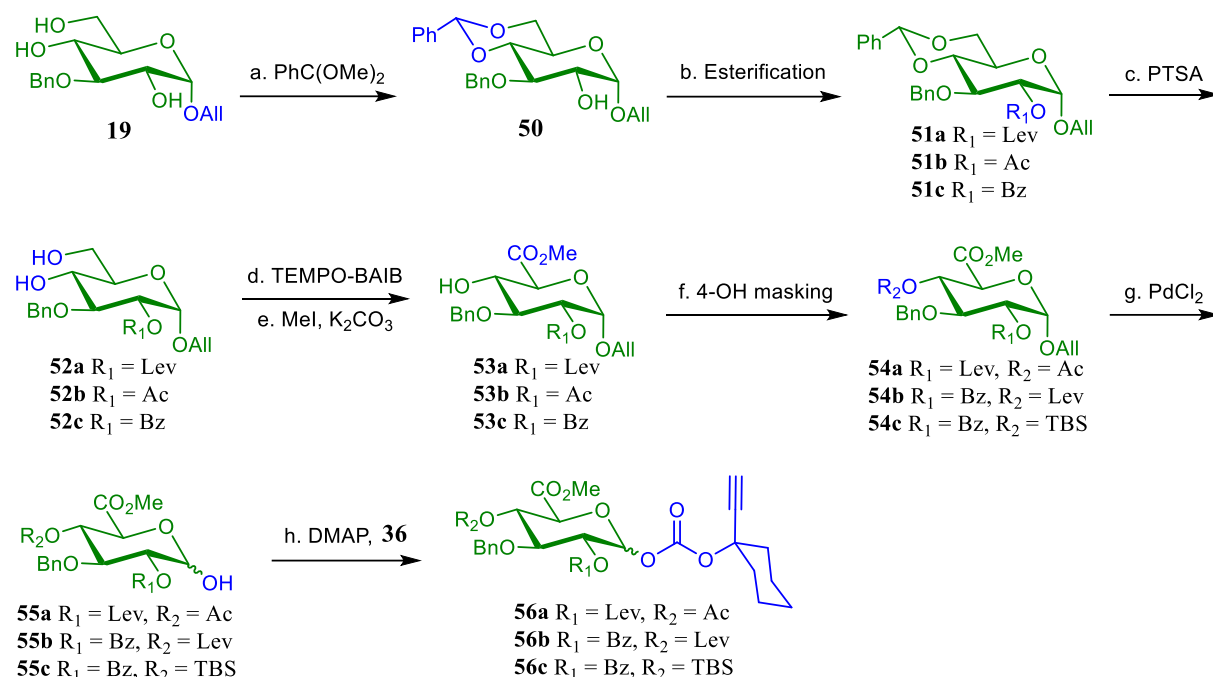
### 3.2-3.4 Present Work

#### 3.2 Synthesis of Glucuronic Acid (GlcA) Building Blocks:

Synthesis of a diversified set of glucuronic acid acceptors and donors commenced with the allyl glucopyranoside preparation. The commercially available benzyl protected diacetone glucofuranose **18** (Chapter 2, Scheme 2.04B) was converted into pyranoside under acidic conditions. The resultant intermediate was refluxed in allyl alcohol in the presence of catalytic amount of acetyl chloride to furnish the allyl glycoside **19**. Further, 4,6-*O*-benzylidene locking reaction was accomplished with benzylidene dimethylacetal and a catalytic amount of a camphor sulfonic acid to afford compound **50** in a 75% yield (Scheme 3.01). Compound **50** was divided into three parts to protect the remaining C-2 hydroxyl group with either levulinate (**51a**) or acetate (**51b**) or benzoate (**51c**) under well-established protocols. The presence of C2-*O*-ester group would facilitate the required 1,2-*trans* glycosylation via neighboring group participation.<sup>3</sup> Levulinate was protected by using levulinic acid and *N,N*-diisopropyl

## Donor-Acceptor Selectivity Studies with GlcA and GlcN Building Blocks

carbodiimide (DIC), acetate was prepared by using acetic anhydride while benzoate by using benzoyl chloride in pyridine.



*Scheme 3.01: Preparation of the diverse set of glucuronic acid (GlcA) acceptors and donors.*

Reagents.(a) PhCH(OMe)<sub>2</sub>, CSA, DMF, 25 °C, 4 h, 75%, (b) LevOH, DIC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0-25 °C, 2 h, **51a**: 92% or Ac<sub>2</sub>O/ BzCl, Pyridine, DMAP (cat), 0-25 °C, 2 h, **51b**: 95%, **51c**: 92%; (c) PTSA, MeOH-CH<sub>2</sub>Cl<sub>2</sub> (1:1), 25 °C; (d) TEMPO, BAIB, CH<sub>2</sub>Cl<sub>2</sub>-H<sub>2</sub>O (2:1), 25 °C, 3 h; (e) K<sub>2</sub>CO<sub>3</sub>, MeI, DMF, 25 °C, 8 h, **53a**: 66%, **53b**: 66%, **53c**: 67% over three steps (c,d,e); (f) (i) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 30 min, **54a**: 87%; (ii) LevOH, DIC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0-25 °C, 2 h, **54b**: 86%; (iii) TBDMSOTf, 2,6-lutidine, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 20 min, **54c**: 93%; (g) PdCl<sub>2</sub>, MeOH-CH<sub>2</sub>Cl<sub>2</sub> (3:1), 25 °C, 4-8 h; (h) **36**, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 3 h, **56a**: 72%, **56b**: 72%, **56c**: 71% over two steps (g,h).

Subsequently, unlocking of the 4,6-benzylidene protection of compounds **51a-51c** was performed using a catalytic amount of *p*-toluenesulfonic acid in a 1:1 mixture of CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub>. C-6 Hydroxyl group of the resulting diols **52a-52c** were oxidized under TEMPO/BAIB<sup>4</sup> conditions to obtain corresponding glucuronic acids which were extrapolated to corresponding methyl esters **53a-53c** respectively under potassium carbonate and methyl iodide conditions. Characteristic resonances from the <sup>1</sup>H and <sup>13</sup>C NMR spectrums (**Table 3.1a**) and the mass spectroscopic analysis (**Table 3.1b**) confirmed the synthesis of assigned acceptors **53a-53c**.

| Entry    | <sup>1</sup> H NMR (δ ppm)               | <sup>13</sup> C NMR (δ ppm) | Emerging Compounds |
|----------|------------------------------------------|-----------------------------|--------------------|
| Anomeric | merged with allyl and sugar ring protons | 95.9-95.5                   | <b>53a-53c</b>     |

|            |                                                                           |                                                                                            |                |
|------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|----------------|
| Allyl      | 5.96 – 5.84 (dddd, $J = 16.9$ , 10.9, 6.0, 5.1 Hz, 1H)                    | 133.3, 118.2, 69.0                                                                         | <b>53a-53c</b> |
| -COOMe     | 3.87-3.80 (s, 3H)                                                         | 170.7 (carbonyl), 52.9 (-CH <sub>3</sub> )                                                 | <b>53a-53c</b> |
| Levulinate | 2.73 – 2.44 (m, 4H), 2.14 (s, 3H)                                         | 206.1, 171.5, 37.8 (-CH <sub>2</sub> ), 29.9 (-CH <sub>3</sub> ), 27.9 (-CH <sub>2</sub> ) | <b>53a</b>     |
| Acetate    | 2.05 (s, 3H)                                                              | 170.3 (carbonyl), 21.0 (-CH <sub>3</sub> )                                                 | <b>53b</b>     |
| Benzoate   | 8.11-8.05 (m, 2H), 7.61 (t, $J = 7.4$ Hz, 1H), 7.48 (t, $J = 7.7$ Hz, 2H) | Carbonyl- 165.9<br>Aromatic- 133.2(p-CH), 129.9 (2C), 129.7 (quat), 128.5 (2C)             | <b>53c</b>     |

**Table 3.1a: Characteristic <sup>1</sup>H and <sup>13</sup>C NMR signals resonating region of the distinctive acceptors 53a-53c.**

| <b>m/z</b> | <b>53a {C<sub>22</sub>H<sub>28</sub>O<sub>9</sub> [M+Na]<sup>+</sup>}</b> | <b>53b {C<sub>19</sub>H<sub>24</sub>O<sub>8</sub> [M+Na]<sup>+</sup>}</b> | <b>53c {C<sub>24</sub>H<sub>26</sub>O<sub>8</sub> [M+Na]<sup>+</sup>}</b> |
|------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Calculated | 459.1631                                                                  | 403.1369                                                                  | 465.1525                                                                  |
| Found      | 459.1648                                                                  | 403.1362                                                                  | 465.1519                                                                  |

**Table 3.1b: HRMS analysis for compounds 53a-53c.**

Compounds **53a-53c** can be used for glycosylation reactions as acceptors or these can be converted into orthogonally protected donors by protecting lone hydroxyl with chosen protecting group. In continuation, C4-OH of the precursor **53a** was protected as an acetate using acetic anhydride to afford **54a** in 87% yield. While compound **53c** was utilized to protect C4-O-levulinate as well as to protect C4-O-silyl ether. C4-O-Levulinate protection was accomplished using levulinic acid, DIC, DMAP as a reaction conditions to afford fully protected **54b** whereas *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) was used in the presence of excess 2,6-lutidine to obtain C4-O-TBS protected glucuronic acid derivative **54c** within 20 min in 93% yield. Important to note that TBS protection under TBSCl/imidazole/DMAP took 36 h for 59% conversion.

Furthermore, compounds **54a-54c** were subjected to the allyl deprotection in the presence of a catalytic amount of palladium(II) chloride in a 3:1 mixture of methanol: dichloromethane.<sup>5</sup> Thus resulting hemiacetals **55a-55c** were treated with carbonate reagent **36** (Chapter 2, Scheme 2.08) in the presence of a stoichiometric amount of 4-(dimethylamino)pyridine to accomplish the synthesis of donors **56a-56c** in excellent yields.<sup>6</sup> Characteristic resonances from the <sup>1</sup>H and

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$^{13}\text{C}$  NMR spectrums (**Table 3.2a**) and the mass spectroscopic analysis (**Table 3.2b**) confirmed the synthesis of assigned donors **56a-56c** as a  $\alpha/\beta$  isomeric mixtures.

| Entry                       | $^1\text{H}$ NMR ( $\delta$ ppm)          | $^{13}\text{C}$ NMR ( $\delta$ ppm)                                                       | Emerging Compounds   |
|-----------------------------|-------------------------------------------|-------------------------------------------------------------------------------------------|----------------------|
| Anomeric                    | 6.4-5.6 (d, 1H)                           | 95.0-92.2                                                                                 | <b>56a-56c</b>       |
| Alkynylcyclohexyl carbonate | 2.45 – 2.35 (s, 1H),<br>2.21-1.2 (m, 10H) | 151.2-150.5, 82.5-75.4 (3C), 37.0-22.5 (5C)                                               | <b>56a-56c</b>       |
| -COOMe                      | 3.78-3.69 (s, 3H)                         | 168.8-167.4 (carbonyl), 52.9 (-CH <sub>3</sub> )                                          | <b>56a-56c</b>       |
| Levulinate                  | 2.75-2.41 (m, 4H),<br>2.15 (s, 3H)        | 206.1, 171.5, 37.8, 29.8, 27.8                                                            | <b>56a &amp; 56b</b> |
| Acetate                     | 1.96 (s, 3H)                              | 169.4 (carbonyl), 20.6 (-CH <sub>3</sub> )                                                | <b>56a</b>           |
| Benzoate                    | 8.0-7.3 (5H)                              | 165.2 (C=O), 133.4 (p-CH), 130.0 (2C), 129.9-129.2 (quat), 128.4 (2C)                     | <b>56b &amp; 56c</b> |
| -OTBS                       | 0.86 (s, 9H), -0.01 (d, 6H)               | 25.8 (3CH <sub>3</sub> ), 18.0 (quat), -3.9 (-CH <sub>3</sub> ), -5.2 (-CH <sub>3</sub> ) | <b>56c</b>           |

**Table 3.2a: Characteristic  $^1\text{H}$  and  $^{13}\text{C}$  NMR resonances of compounds 56a-56c**

| m/z        | <b>56a</b> {C <sub>30</sub> H <sub>36</sub> O <sub>12</sub> [M+Na] <sup>+</sup> } | <b>56b</b> {C <sub>35</sub> H <sub>38</sub> O <sub>12</sub> [M+Na] <sup>+</sup> } | <b>56c</b> {C <sub>36</sub> H <sub>46</sub> O <sub>10</sub> Si[Na] <sup>+</sup> } |
|------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Calculated | 611.2104                                                                          | 673.2260                                                                          | 689.2758                                                                          |
| Found      | 611.2097                                                                          | 673.2263                                                                          | 689.2758                                                                          |

**Table 3.2b: HRMS analysis for compounds 56a-56c**

### 3.3 Diverse Route for the Synthesis of Glucosamine (GlcN) Building Blocks:

In parallel, the synthesis of regioselectively protected glucosamine building blocks commenced with the preparation of Troc-protected  $\alpha$ -O-allyl glucosamine derivative **58** adopting known procedures.<sup>7</sup> Glucosamine **57** reaction with 2,2,2-trichloro-ethoxycarbonyl chloride in the presence of sodium bicarbonate provided the Troc protection to the amine functionality. The resultant intermediate was converted to its allyl  $\alpha$ -D-glycoside **58** in 94% yield under AcCl/allyl alcohol/0-80 °C (Scheme 3.02). The benzylidene locking on the triol **58** was performed using benzylidene dimethyl acetal and a catalytic amount of CSA to afford benzylidene **59** as a white solid after precipitation. Further, lone hydroxyl moieties of compound **59** was attempted to protect with benzyl ether under the sodium hydride-THF reaction conditions that were unsuccessful in the presence of Troc protecting group. Thus, Troc protection was removed by Zinc mediated reaction<sup>8</sup> by using acetic acid-methanol-dichloromethane solvent mixture to obtain 77% of glucosamine **60**. Azide at the C-2 position

strongly influences the stereochemical outcome of glycosidation in favor of desired 1,2-*cis* or  $\alpha$ -glucoside. Hence, the conversion of amine to azide was easily accomplished by the use of freshly prepared azido-sulfonyl imidazole<sup>9</sup> in the presence of potassium carbonate and catalytic copper(II) sulfate pentahydrate in CH<sub>3</sub>OH-THF to afford compound **61** in 82% yield. Gratifyingly, scaffold **61** was scaled up (performing reactions on >100 g scale) avoiding the column chromatographic purifications in the intermediate stages. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic study (**Table 3.3**) along with HRMS (ESI-MS) analysis, m/z calculated for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub> [M+Na]<sup>+</sup>: 356.1222, Found: 356.1209 and the appearance of sharp IR peak at 2109 cm<sup>-1</sup> for the azide group confirmed the formation of assigned scaffold **61**.

| Entry           | <sup>1</sup> H NMR ( $\delta$ ppm)                                                | <sup>13</sup> C NMR ( $\delta$ ppm)                     |
|-----------------|-----------------------------------------------------------------------------------|---------------------------------------------------------|
| Anomeric        | 4.92 (d, <i>J</i> = 3.6 Hz, 1H)                                                   | 97.6                                                    |
| Allyl           | 5.98-5.86 (dddd, 1H), 5.35 (dq, <i>J</i> = 17.3, 1.5 Hz, 1H), 5.28 – 5.22 (m, 1H) | 133.2, 118.2, 68.8                                      |
| Benzylidene     | 7.51 – 7.46 (m, 2H), 7.40 – 7.35 (m, 3H), 5.51 (s, 1H)                            | 137(quat), 129.5 (p-CH), 128.5 (2C), 126.4 (2C), 102.1, |
| Ring resonances | Ranges from 4.3 to 3.2                                                            | 81.9, 68.9, 68.8, 63.1, 62.6                            |

**Table 3.3 Characteristic <sup>1</sup>H and <sup>13</sup>C NMR signals of scaffold 61.**

At this point, a series of protections on the lone 3-*O*-hydroxyl group were considered in order to explore orthogonal protections/deprotections at a subsequent stage. Accordingly, the lone hydroxyl group was protected as *p*-methoxybenzyl ether (**62a**) or benzyl ether (**62b**) or naphthyl ether (**62c**) or silyl ether (**62d**) or acetate (**62e**) using appropriate reaction conditions in an excellent reaction yields. The 3-*O*-ethers **62a-62c** were accomplished in the presence of sodium hydride in THF using corresponding *p*-methoxybenzyl chloride or benzyl bromide or naphthyl bromide. Silyl ether **62d** was synthesized by using TBDPSCl/Et<sub>3</sub>N/cat. DMAP whereas 3-*O*-acetate **62e** was accomplished using Ac<sub>2</sub>O/DMAP conditions in 84% yield. Addition of these distinctive protections was identified by the <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy (**Table 3.4**). In addition, formation of the compounds **62a-62e** was confirmed by mass spectroscopic analysis.

| Entry  | <sup>1</sup> H NMR ( $\delta$ ppm)                     | <sup>13</sup> C NMR ( $\delta$ ppm)        |
|--------|--------------------------------------------------------|--------------------------------------------|
| PMB    | 7.35 – 7.30 (m, 2H), 6.90 – 6.85 (m, 2H), 3.80 (s, 3H) | 159.5, 130.0 (3C), 113.9 (2C), 74.8, 55.3  |
| Benzyl | 7.46 – 7.40 (m, 5H)                                    | 137.9, 128.4 (2C), 128.3 (2C), 127.9, 75.1 |

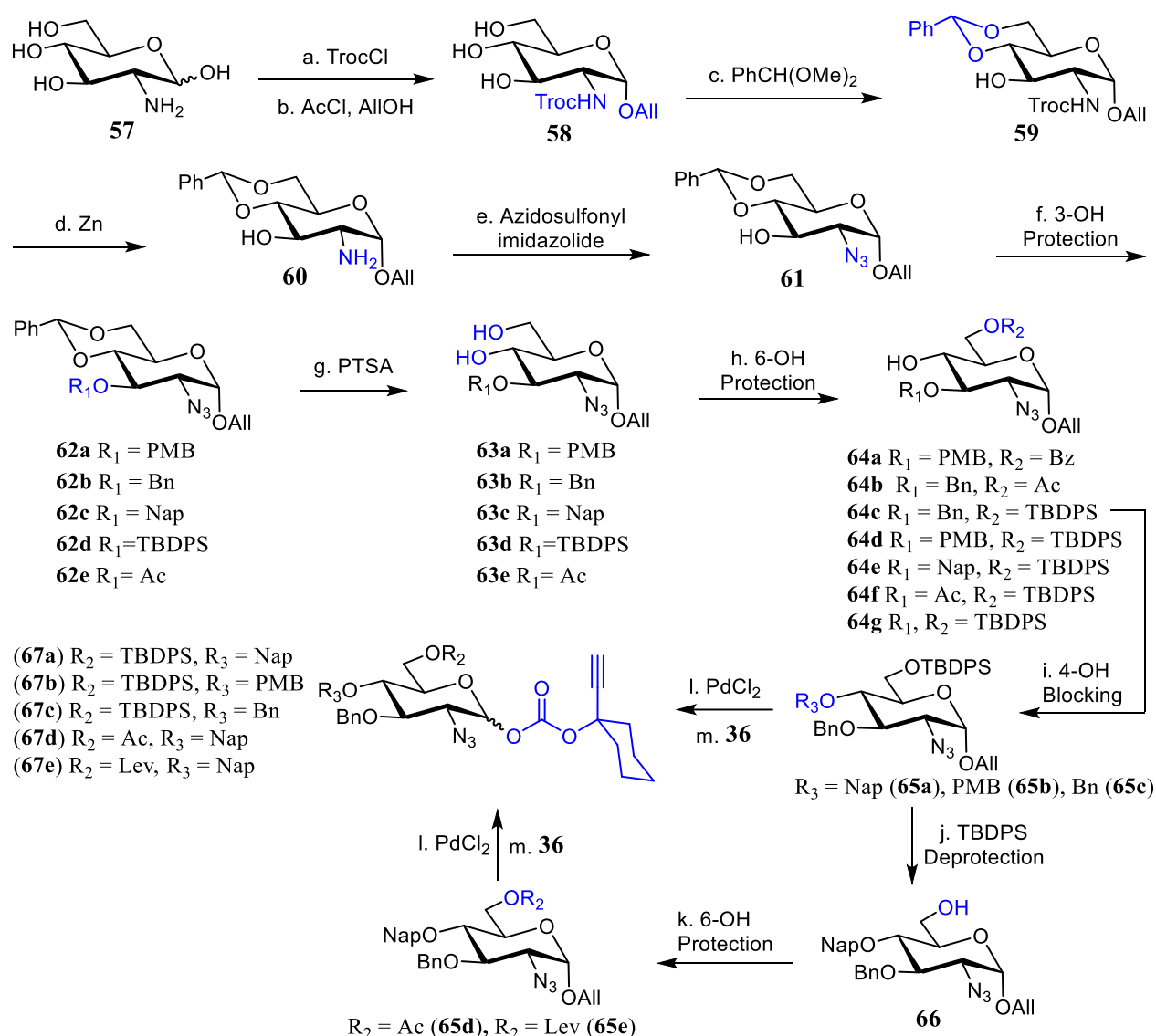
|         |                                                                                                          |                                                                                             |
|---------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| TBDPS   | 7.59 – 7.53 (m, 4H), 7.17 (dt, $J = 15.2$ , 7.6 Hz, 4H), 6.76 (dd, $J = 8.1$ , 1.0 Hz, 2H), 0.99 (s, 9H) | 136.5 (2C), 135.6 (2C), 134.8, 132.2, 129.7, 129.4, 127.4 (2C), 127.3 (2C), 26.8 (3C), 19.7 |
| Acetate | 2.13 (s, 3H)                                                                                             | 169.9, 21.0                                                                                 |

**Table 3.4 Distinctive  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals of 62a-62e**

In continuation, benzylidene acetal of compounds **62a-62e** was hydrolyzed under acidic conditions using catalytic *p*-toluenesulfonic acid in methanol-dichloromethane solvent mixture. Subsequently, the resulting diols **63a-63e** were regioselectively protected at the C6 position as a benzoate (**64a**) or acetate (**64b**) or silyl ether (**64c-64g**). The ester protections in diols **64a,64b** were accomplished using corresponding anhydrides in the presence of excess triethylamine in dichloromethane at ambient temperature; whereas, the C6-*O*-TBDPS protections of compounds **64c-64g** were carried out using stoichiometric amount of TBDPSCl with excess triethylamine and a catalytic amount of DMAP. The NMR, mass and IR analysis confirmed the synthesis of the orthogonally protected acceptor compounds **64a-64g**.<sup>6</sup> These can be utilized directly for glycosylation reactions or can be converted to the corresponding donors via blocking of the lone hydroxyl with an appropriate protecting group.

Optimization of donor-acceptor combination towards the  $\alpha$ -selective glycosylations with glucosamine donors requires orthogonally protected glycosyl donors **67a-67e**. Accordingly, the lone hydroxyl of the compound **64c** was protected as either naphthyl ether (**65a**), PMB ether (**65b**) or benzyl ether (**65c**) using sodium hydride in THF under inert conditions. Subsequently, cleavage of the C6-*O*-TBDPS of the naphthyl ether **65a** was accomplished under hydrogen fluoride-pyridine conditions. The C6-OH of the resulting compound **66** was protected either with acetate (**65d**) or levulinate (**65e**) by aforementioned procedures. Compounds **65a-65e** are quite interesting as they possess orthogonal protecting groups that can be unmasked on demand without affecting the other. In the next sequence, the  $\alpha$ -*O*-allyl glycosides of compound **65a-65e** were unlocked by using  $\text{PdCl}_2$  in methanol-dichloromethane. Finally, the resulting hemiacetal intermediates were conveniently transformed to the much-desired carbonate donors **67a-67e** using carbonate reagent **36** and DMAP as a base in excellent yields (Scheme 3.02). Characteristic resonances of alkynylcyclohexyl carbonates with the absence of allyl signals in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra and the good agreement of mass and IR analysis confirmed the synthesis of the  $\alpha/\beta$  anomeric mixtures of assigned **67a-67e** donors.<sup>6</sup>

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*Scheme 3.02: Synthesis of orothogonally protected glucosamine (GlcN) acceptors and donors.*

Reagents. (a) TrocCl, NaHCO<sub>3</sub>, H<sub>2</sub>O; (b) AcCl, AlIOH, 0-80 °C, 16 h, 94%; (c) PhCH(OMe)<sub>2</sub>, CSA, DMF, 25 °C, 4 h, 74%; (d) Zn, AcOH:CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub> (1:2:1), 0-25 °C, 1 h, 77%; (e) Azidosulfonylimidazolidine, K<sub>2</sub>CO<sub>3</sub>, CuSO<sub>4</sub>·5H<sub>2</sub>O, MeOH-THF (4:1), 25 °C, 2 h, 82%; (f) (i) NaH, PMB-Cl/BnBr/NapBr, TBAI (cat.), THF, 25 °C, 3 h, **62a**: 81%, **62b**: 87 %, **62c**: 84 %; or (ii) TBDPSCl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 15 h, **62d**: 78%; or (iii) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 30 min, RT; **62e**: 84%; (g) PTSA, MeOH-CH<sub>2</sub>Cl<sub>2</sub> (1:1), 25 °C, 1 h; then (h) (i) Bz<sub>2</sub>O/Ac<sub>2</sub>O, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 12 h; **64a**: 74%, **64b**: 75%; over two steps; or (ii) TBDPSCl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 15 h; **64c**: 87%, **64d**: 71%, **64e**: 79%, **64f**: 76%, **64g**: 74%; over two steps (i) NaH, NapBr/PMB-Cl/BnBr, TBAI (cat.), THF, 25 °C, 3 h **65a**: 92%, **65b**: 87%, **65c**: 93%; (j) HF·py, pyridine-THF (8:2), 5 h, 0-25 °C, 89%. (k) (i) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 30 min; **65d**: 86%; (ii) LevOH, DIC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0-25 °C, 2h **65e**: 93%; (l) PdCl<sub>2</sub>, MeOH-CH<sub>2</sub>Cl<sub>2</sub> (3:1), 25 °C, 4-8 h; then (m) **36**, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 3 h **67a**: 79%; **67b**: 68%; **67c**: 78%; **67d**: 69%; **67e**: 71%; over two steps.

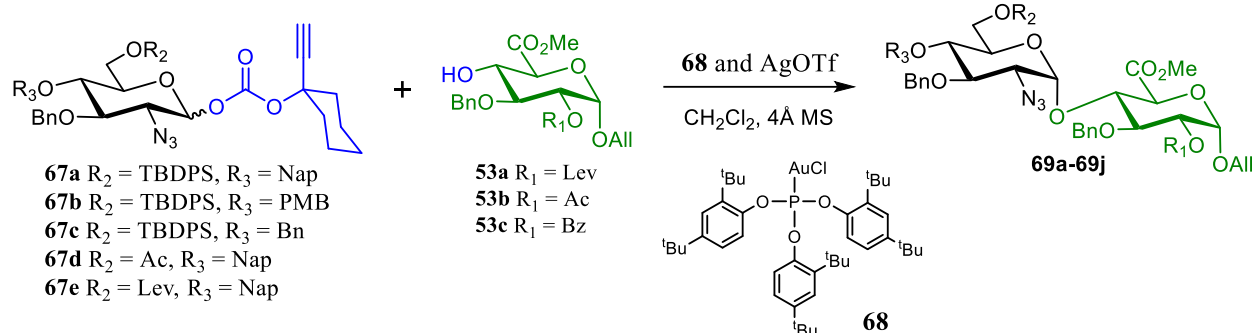
### 3.4 Optimization of reaction conditions for Stereoselective Glycosylations:

Synthesis of the heparin oligosaccharides is contingent after developing protocols and synthesizing all the identified building blocks in enough quantities. The chain elongation was



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commenced with the optimization of reaction conditions with silver-assisted gold catalysis<sup>10</sup> for the stereoselective  $\alpha$ -glycosylation of the D-E disaccharide. The various combinations of the donors **67a-67e** and aglycons **53a-53c** prepared *vide supra* were utilized in the stereoselectivity method development. All glycosylation reactions between azidoglucosyl donors and acceptors were conducted by employing Au-phosphite (**68**) and silver triflate (AgOTf) in dichloromethane containing freshly activated 4Å powdered molecular sieves (MS) to afford the disaccharides **69a-69j** (Scheme 3.03).<sup>10</sup>



*Scheme 3.03: The donor-acceptor combinations employed to achieve D-E disaccharide selectivity.* Reagents: Au phosphate **68** and AgOTf [8mol% each for reactions at 25 °C, 12mol% each for reactions at -20 °C, 15mol% each for reactions -40 °C], CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, 15 min to 1 h.

Our explorations on donor-acceptor selectivity studies commenced with the silver assisted gold-catalyzed glycosidation with the donor **67a** and acceptor **53a** that have orthogonal protecting groups TBDPS and Nap on glycosyl donor; lev- and benzyl- moieties to afford D-E disaccharide **69a** in 77% yield with 4:1 ( $\alpha$ : $\beta$ ) ratio (Table 3.1, Entry 1). Changing the C6-*O*-TBDPS protecting group of the donor to the levulinate as in **67e** resulted into multiple spots on TLC which could not purified even after multiple flash chromatographic separations to obtain **69b** (Entry 2).

Table 3.1: Reciprocal donor-acceptor selectivity studies

| Entry | Donor      | Acceptor   | T (°C) | Product    | % Yield | $\alpha$ : $\beta$ ratio |
|-------|------------|------------|--------|------------|---------|--------------------------|
| 1     | <b>67a</b> | <b>53a</b> | 25     | <b>69a</b> | 77      | 4:1                      |
| 2     | <b>67e</b> | <b>53a</b> | 25     | <b>69b</b> | 55      | -                        |
| 3     | <b>67a</b> | <b>53b</b> | 25     | <b>69c</b> | 68      | 4:1                      |
| 4     | <b>67b</b> | <b>53b</b> | 25     | <b>69d</b> | 83      | 5:1                      |

|    |            |            |     |            |    |      |
|----|------------|------------|-----|------------|----|------|
| 5  | <b>67c</b> | <b>53b</b> | 25  | <b>69e</b> | 87 | 4:1  |
| 6  | <b>67d</b> | <b>53b</b> | 25  | <b>69f</b> | 76 | 6:1  |
| 7  | <b>67e</b> | <b>53b</b> | 25  | <b>69g</b> | 68 | 5:1  |
| 8  | <b>67a</b> | <b>53b</b> | -20 | <b>69c</b> | 79 | 8:1  |
|    |            |            |     |            |    |      |
| 9  | <b>67a</b> | <b>53c</b> | 25  | <b>69h</b> | 82 | 5:1  |
| 10 | <b>67b</b> | <b>53c</b> | 25  | <b>69i</b> | 75 | 4:1  |
| 11 | <b>67c</b> | <b>53c</b> | 25  | <b>69j</b> | 80 | 4:1  |
| 12 | <b>67a</b> | <b>53c</b> | -20 | <b>69h</b> | 78 | 10:1 |
| 13 | <b>67c</b> | <b>53c</b> | -20 | <b>69j</b> | 77 | 10:1 |
| 14 | <b>67a</b> | <b>53c</b> | -40 | <b>69h</b> | 81 | 17:1 |
| 15 | <b>67c</b> | <b>53c</b> | -40 | <b>69j</b> | 85 | 20:1 |

Hence, instead of changing the protecting group of the donor, the acceptor with C2-*O*-acetate was preferred as compound **53b** did not alter the  $\alpha$ : $\beta$  selectivity ratio of disaccharide **69c**; isomers could not be separated even after multiple purification attempts (Entry 3). Next in sequence, the change in C4-*O*-naphthyl protection to the *p*-methoxybenzyl ether (**67b**) or benzyl ether (**67c**), glycosylation with donor **53b** did not show that much improvement in  $\alpha$ : $\beta$  selectivity, however, the slight different difference in *R<sub>f</sub>* values for the  $\alpha$ : $\beta$  isomers was observed on TLC with the enhancement in the reaction yield (Entries 4 and 5). Next, C6-*O*-TBDPS was replaced with the acetate (**67d**) or levulinate (**67e**) and glycosylated with donor **53b** to observe marginal improvement in the  $\alpha$ : $\beta$  selectivity without any separation of the isomers on TLC (Entries 6 and 7). However, a quantum jump in the  $\alpha$ : $\beta$  ratio to 8:1 was noticed when the glycosylation was carried out between the glycosyl donor **67a** and donor **53b** at -20 °C with very high yield (Entry 8). Though the selectivity got improved, the 1,2-*cis* or  $\alpha$ -linked disaccharide and the unwanted  $\beta$ -isomer are noticed to have similar polarities and hence, demanded multiple column chromatographic separations thereby compromising the overall process efficiency.

Moreover, the glycosylation between the donors **67a**-**67c** and C2-*O*-benzoyl acceptor **53c** were performed at 25 °C to obtain the disaccharide **69h**-**69j** with almost similar  $\alpha$ : $\beta$  ratio of D-E disaccharides (Entries 9, 10, 11). To our satisfaction, good *R<sub>f</sub>* difference was noticed on TLC for compounds **69h** and **69j** thereby facilitating easy purifications. Further, the temperature

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dependence of the glycosylation between 4-*O*-naphthyl donor **67a** and acceptor **53c** was performed at -20 °C with the addition of 12mol% each Au-phosphite (**68**) and AgOTf and observed a significant enhancement of  $\alpha$ : $\beta$  ratio to 10:1 (Entry 12). A similar selectivity ratio was observed even after altering the 4-*O*-naphthyl ether to the 4-*O*-benzyl ether (Entry 13). Importantly, glycosylations at -40 °C in the presence of 15mol% [Au]/[Ag]-catalysts with the donor-acceptor combinations of entry 12 and entry 13 were successfully achieved with an impressive  $\alpha$ : $\beta$  selectivity ratio as 17:1 and 20:1 respectively with very high reaction yields (Entries 14, 15). Further decrease in the reaction temperature to less than -40°C was not possible since gold-catalyzed glycosylations do not proceed below this temperature. Thus, the donor-acceptor studies showed that glycosylation shall be carried out at -40 °C using glycosyl donor **67c** and acceptor **53c** to accomplish up to 20:1  $\alpha$ -selectivity (Entry 15, Table 3.1). Hence,  $\alpha$ -selective disaccharide **69j** was produced in sufficient quantity that can be extrapolated to the heparin pentasaccharide. Both the  $\alpha$  and  $\beta$  anomers of **69j** were isolated and identified by thorough NMR analysis. Allyl was  $\alpha$ -glycosidic in both of these isomers and hence, the corresponding anomeric carbon resonated at around  $\delta$  95.6 ppm. However, another anomeric carbon resonates at  $\delta$  98.2 ppm indicated the synthesis of  $\alpha$ -anomeric **69j** disaccharide whereas resonance at  $\delta$  101.3 ppm indicates the formation of the  $\beta$ -anomer. All remaining  $^{13}\text{C}$  as well as  $^1\text{H}$  NMR resonances<sup>6</sup> matches perfectly with the assigned structure. Furthermore, the **69j** disaccharide formation was confirmed by HRMS (ESI-MS) analysis,  $m/z$  calculated for  $\text{C}_{60}\text{H}_{65}\text{N}_3\text{O}_{12}\text{Si}[\text{M}+\text{Na}]^+$ : 1070.4235, Found: 1070.4225.

### 3.5 Conclusions:

Subsequent to the iduronic acid donor-acceptor development from chapter 2, protocols for the synthesis of remaining building blocks of glucuronic acid and glucosamine were developed. Various orthogonally protected glucuronic acid donors and acceptors were prepared by diverse methods from commercially available 3-*O*-benzyl-1,2,5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose. Further, the glucosamine building blocks were synthesized by adopting known procedures. The intermediate scaffold 4,6-*O*-benzylidene-2-*deoxy*-2-azido- $\alpha$ -*O*-allyl glucosamine **61** was prepared from Troc-glucosamine by a modification of the standard procedures and prepared in 100g scale without column chromatographic purification. The donor-acceptor  $\alpha$ , $\beta$  selectivity study with the glucosamine donors **67a-67e** and glucuronic acid acceptors **53a-53c** showed that the glycosylation between donor **67c** and acceptor **53c** at -40 °C

provides up to 20:1  $\alpha$ -selectivity with excellent reaction yield as well as good Rf difference for better separation.

### 3.6 Experimental Section:

**Compound 50: Glc-Benzylidene Protection:** To a solution of the compound **19** (30 g, 96.67 mmol) in anhydrous DMF (150 mL) was added benzaldehyde dimethyl acetal (17.41 mL, 116 mmol), followed by the camphor sulfonic acid (4.49 g, 19.33 mmol) was added portion-wise under argon atmosphere and the reaction mixture stirred at 25 °C for 4 h. Ice-cold saturated aq NaHCO<sub>3</sub> solution (150 mL) and water (150 mL) were added to neutralize the reaction and extracted with EtOAc (3 x 100 mL), organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The product was precipitated by stirring crude residue in the combination of CH<sub>2</sub>Cl<sub>2</sub>-hexane solvent mixture and the residue was washed with hexane, dried over high vacuum to afford 25.5 g of white solid. The filtrate was evaporated and purified by column chromatography resulting in the overall 28.9 g (75%) of the compound **50**. Eluent for purification: 25% ethyl acetate in *n*-hexane; white solid; mp 143.1 °C;  $[\alpha]^{25}_{\text{D}}$  (CHCl<sub>3</sub>, c1.0): +90.4°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3432, 3025, 2925, 2869, 1449, 1367, 1217, 1076, 1032, 748, 694; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>):  $\delta$  7.54 – 7.48 (m, 2H), 7.44 – 7.36 (m, 5H), 7.36 – 7.27 (m, 3H), 5.94 (dddd, *J* = 16.8, 10.4, 6.3, 5.4 Hz, 1H), 5.58 (s, 1H), 5.33 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.25 (dd, *J* = 10.4, 1.3 Hz, 1H), 5.00 – 4.95 (m, 2H), 4.82 (d, *J* = 11.6 Hz, 1H), 4.30 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.24 (ddt, *J* = 12.8, 5.4, 1.4 Hz, 1H), 4.08 (ddt, *J* = 12.8, 6.4, 1.2 Hz, 1H), 3.94 – 3.88 (m, 1H), 3.86 (d, *J* = 9.2 Hz, 1H), 3.76 (t, *J* = 10.3 Hz, 2H), 3.66 (t, *J* = 9.3 Hz, 1H), 2.38 (s, 1H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>):  $\delta$  138.6, 137.4, 133.5, 129.1, 128.5, 128.5, 128.4, 128.4, 128.1, 128.1, 127.8, 126.1, 126.1, 118.4, 101.4, 98.1, 82.0, 79.1, 75.0, 72.5, 69.1, 68.9, 62.9; HRMS (ESI-MS): *m/z* calcd for C<sub>23</sub>H<sub>26</sub>O<sub>6</sub> [M+Na]<sup>+</sup>: 421.1627, Found: 421.1628.

**Compound 51a: Protection of Levulinoate:** To a solution of **50** (4.28 g) and levulinic acid (1.5 eq) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL) was added 0.2 equivalent of DMAP and reaction was cooled to 0 °C, *N, N'*-diisopropyl carbodiimide (1.2 eq) was added drop-wise under argon atmosphere and the mixture was stirred at 25 °C for 2 h. After complete consumption of the starting alcohol, the reaction mixture was concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using 25% ethyl acetate in *n*-hexane as mobile phase to afford the levulinate ester compound **51a** (3.95 g, 92%) as a thick syrup;  $[\alpha]^{25}_{\text{D}}$  (CHCl<sub>3</sub>, c1.0): +67.3°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3020, 2923, 2869, 1738, 1453, 1368, 1214, 1089,

1038, 751, 699;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.51 – 7.47 (m, 2H), 7.40 – 7.34 (m, 3H), 7.34 – 7.22 (m, 5H), 5.96 – 5.84 (m, 1H), 5.58 (s, 1H), 5.30 (dq,  $J$  = 17.3, 1.5 Hz, 1H), 5.21 (dd,  $J$  = 10.4, 1.3 Hz, 1H), 5.05 (d,  $J$  = 3.8 Hz, 1H), 4.89 (dd,  $J$  = 10.5, 4.2 Hz, 2H), 4.72 (d,  $J$  = 11.8 Hz, 1H), 4.28 (dd,  $J$  = 10.2, 4.8 Hz, 1H), 4.18 (ddt,  $J$  = 12.9, 5.2, 1.3 Hz, 1H), 4.10 – 3.98 (m, 3H), 3.92 (td,  $J$  = 10.0, 4.8 Hz, 1H), 3.81 – 3.67 (m, 2H), 2.76 – 2.49 (m, 4H), 2.14 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.2, 172.2, 138.7, 137.4, 133.6, 129.1, 128.4, 128.4, 128.3, 128.3, 127.8, 127.8, 127.7, 126.1, 126.1, 118.1, 101.4, 96.0, 82.2, 76.3, 74.9, 73.2, 69.0, 68.9, 62.6, 37.9, 29.9, 28.1; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{28}\text{H}_{32}\text{O}_8$   $[\text{M}+\text{Na}]^+$ : 519.1995, Found: 519.1995.

**Compound 51b:** This compound was prepared from **50**, following procedure described for the compound **31**. Eluent for purification: 10% ethyl acetate in *n*-hexane; Thick syrup; yield 3.35 g (95%) from 3.2 g, forwarded as such for the next step.

**Compound 51c: Benzoyl protection:** A solution of the compound **50** (20 g, 50.19 mmol) in anhydrous pyridine (100 mL) was cooled to 0 °C and the benzoyl chloride (1.2 eq) was added drop-wise under argon atmosphere, catalytic amount of DMAP was added. The reaction mixture was gradually warmed up to 25 °C and stirred for 2 h. After completion, ice cooled water (500 mL) was added and extracted with EtOAc (2 x 250 mL). The organic layer was washed with aqueous 1N HCl (3 x 300 mL) and brine solution (500 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using 10% ethyl acetate in *n*-hexane as mobile phase to afford 23.2 g (92%) of the compound **51c** as a white solid. mp 93.6 °C;  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +146.6°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3024, 2930, 2867, 1727, 1601, 1453, 1370, 1270, 1217, 1096, 997, 752, 702;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.16 – 8.09 (m, 2H), 7.66 (tt,  $J$  = 7.0, 1.3 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.55 – 7.43 (m, 5H), 7.34 – 7.29 (m, 2H), 7.29 – 7.20 (m, 3H), 5.88 (dddd,  $J$  = 17.1, 10.5, 5.9, 5.2 Hz, 1H), 5.69 (s, 1H), 5.36 – 5.27 (m, 2H), 5.26 – 5.17 (m, 2H), 4.97 (d,  $J$  = 11.8 Hz, 1H), 4.85 (d,  $J$  = 11.8 Hz, 1H), 4.39 (dd,  $J$  = 10.2, 4.8 Hz, 1H), 4.32 (t,  $J$  = 9.4 Hz, 1H), 4.25 (ddt,  $J$  = 13.2, 5.1, 1.5 Hz, 1H), 4.11 – 4.03 (m, 2H), 3.88 (td,  $J$  = 9.9, 9.4, 2.4 Hz, 2H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.0, 138.4, 137.5, 133.6, 133.3, 130.0, 130.0, 129.8, 129.1, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 127.6, 126.2, 126.2, 117.7, 101.5, 96.3, 82.4, 76.1, 74.9, 73.6, 69.1, 68.8, 62.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{30}\text{H}_{30}\text{O}_7$   $[\text{M}+\text{Na}]^+$ : 525.1889, Found: 525.1879.

**Compound 53a-c: 1°OH Oxidation and Methyl Ester Preparation:** To a biphasic solution of the 4,6-diol (1 mmol) in 2:1  $\text{CH}_2\text{Cl}_2$ -water (10 mL) was added BAIB (2.5 eq) and TEMPO (0.2 eq) simultaneously, stirred vigorously at 25 °C. After 3 h, the reaction was quenched by

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the addition of a saturated solution of Na<sub>2</sub>SO<sub>3</sub> and extracted by CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL), the combined organic phases were washed with brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude product was redissolved in anhydrous DMF (5 mL) and added 1.5 equivalent of K<sub>2</sub>CO<sub>3</sub>. After stirring for 15 min, iodomethane (2 eq) was added dropwise under argon atmosphere and stirred for 8h at 25 °C in a dark place. After complete consumption, the reaction was arrested by adding saturated solution of Na<sub>2</sub>SO<sub>3</sub>, brine (5 mL) and water (10 mL); and extracted with EtOAc (2 x 10 mL). The combined organic phase was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure. The crude residue was purified by column chromatography using ethyl acetate and *n*-hexane as mobile phase to obtain the desired product.

**Compound 53a:** Eluent for purification: 42% ethyl acetate in *n*-hexane; Thick syrup; yield 1.3 g (72%, over two steps) from 1.7 g; [ $\alpha$ ]<sup>25</sup><sub>D</sub> (CHCl<sub>3</sub>, c1.0): +107.2°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3467, 3023, 2958, 1741, 1442, 1366, 1213, 1050, 750; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>):  $\delta$  7.36 – 7.24 (m, 5H), 5.96 – 5.84 (m, 1H), 5.31 (dq, *J* = 17.4, 1.5 Hz, 1H), 5.22 (dd, *J* = 10.5, 1.2 Hz, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 4.86 – 4.81 (m, 2H), 4.77 (d, *J* = 11.7 Hz, 1H), 4.26 – 4.18 (m, 2H), 4.04 (ddt, *J* = 12.6, 5.8, 1.2 Hz, 1H), 3.94 – 3.85 (m, 2H), 3.80 (s, 3H), 3.35 (s, 1H), 2.73 – 2.44 (m, 4H), 2.14 (s, 3H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>):  $\delta$  206.4, 172.1, 170.6, 138.6, 133.3, 128.5, 128.5, 127.8, 127.7, 127.7, 118.2, 95.5, 78.7, 75.2, 72.5, 72.1, 70.6, 69.0, 52.8, 37.8, 29.9, 27.9; HRMS (ESI-MS): *m/z* calcd for C<sub>22</sub>H<sub>28</sub>O<sub>9</sub> [M+Na]<sup>+</sup>: 459.1631, Found: 459.1648.

**Compound 53b:** Eluent for purification: 30% ethyl acetate in *n*-hexane; Thick syrup; yield 320 mg (74%, over two steps) from 400 mg; [ $\alpha$ ]<sup>25</sup><sub>D</sub> (CHCl<sub>3</sub>, c1.0): +110.0°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3452, 2954, 2920, 1740, 1448, 1369, 1236, 1042, 747, 699; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>):  $\delta$  7.39 – 7.25 (m, 5H), 5.88 (dddd, *J* = 16.9, 10.9, 6.0, 5.1 Hz, 1H), 5.36 – 5.28 (m, 1H), 5.23 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.14 (d, *J* = 3.6 Hz, 1H), 4.88 – 4.82 (m, 2H), 4.78 (d, *J* = 11.7 Hz, 1H), 4.28 – 4.19 (m, 2H), 4.04 (dd, *J* = 13.1, 6.2 Hz, 1H), 3.95 – 3.88 (m, 2H), 3.83 (s, 3H), 3.06 (d, *J* = 2.1 Hz, 1H), 2.05 (s, 3H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>):  $\delta$  170.7, 170.3, 138.6, 133.2, 128.6, 128.6, 127.9, 127.7, 127.7, 118.3, 95.6, 78.8, 75.4, 72.5, 72.2, 70.5, 69.0, 52.9, 21.0; HRMS (ESI-MS): *m/z* calcd for C<sub>19</sub>H<sub>24</sub>O<sub>8</sub> [M+Na]<sup>+</sup>: 403.1369, Found: 403.1362.

**Compound 53c:** Eluent for purification: 20% ethyl acetate in *n*-hexane; thick syrup; yield 6.35 g (74%, over two steps) from 8.0 g; [ $\alpha$ ]<sup>25</sup><sub>D</sub> (CHCl<sub>3</sub>, c1.0): +143.6°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3467, 3017, 2956, 1731, 1602, 1448, 1364, 1269, 1213, 1101, 1050, 925, 748, 709; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>):  $\delta$  8.11 – 8.05 (m, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.31 – 7.22 (m, 5H), 5.91 – 5.78 (m, 1H), 5.34 – 5.27 (m, 2H), 5.17 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.13 (dd, *J* = 9.9, 3.7 Hz, 1H), 4.86 (s, 2H), 4.33 (d, *J* = 9.8 Hz, 1H), 4.26 (ddt, *J* = 13.2, 5.0, 1.4

Hz, 1H), 4.15 – 4.09 (m, 1H), 4.09 – 3.98 (m, 2H), 3.87 (s, 3H), 3.16 (s, 1H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.7, 165.9, 138.3, 133.4, 133.2, 129.9, 129.9, 129.7, 128.6, 128.6, 128.5, 128.5, 128.0, 128.0, 127.8, 118.0, 95.9, 78.6, 75.4, 72.9, 72.4, 70.6, 69.1, 52.9; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{24}\text{H}_{26}\text{O}_8$   $[\text{M}+\text{Na}]^+$ : 465.1525, Found: 465.1519.

**Compound 54a:** This compound was prepared from **53a** following procedure described for the preparation of compound **31**. Eluent for purification: 30% ethyl acetate in *n*-hexane; Thick syrup; yield 335 mg (87%) from 350 mg;  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +79.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3020, 1747, 1718, 1521, 1214, 1052, 744, 668;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.36 – 7.31 (m, 2H), 7.30 – 7.25 (m, 3H), 5.90 (dddd,  $J$  = 16.7, 10.4, 6.2, 5.3 Hz, 1H), 5.32 (dq,  $J$  = 17.2, 1.5 Hz, 1H), 5.27 – 5.22 (m, 1H), 5.19 – 5.12 (m, 2H), 4.92 (dd,  $J$  = 9.8, 3.7 Hz, 1H), 4.74 (d,  $J$  = 11.9 Hz, 1H), 4.66 (d,  $J$  = 11.9 Hz, 1H), 4.29 (d,  $J$  = 10.0 Hz, 1H), 4.22 (ddt,  $J$  = 13.0, 5.2, 1.4 Hz, 1H), 4.09 – 4.02 (m, 2H), 3.74 (s, 3H), 2.79 – 2.47 (m, 4H), 2.17 (s, 3H), 1.97 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.2, 171.9, 169.6, 168.6, 138.2, 133.2, 128.4, 128.4, 127.8, 127.6, 127.6, 118.4, 95.3, 76.7, 75.0, 72.6, 71.1, 69.3, 69.0, 52.9, 37.8, 29.9, 27.9, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{24}\text{H}_{30}\text{O}_{10}$   $[\text{M}+\text{Na}]^+$ : 501.1737, Found: 501.1741.

**Compound 54b:** This compound was prepared from compound **53c** following procedure described for the preparation of compound **51a**. Eluent for purification: 20% ethyl acetate in *n*-hexane; Thick syrup; yield 527 mg (86%) from 500 mg;  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +95.8°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3020, 2922, 2853, 1748, 1719, 1540, 1360, 1267, 1213, 1154, 1106, 1067, 928, 749, 668;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.07 – 8.00 (m, 2H), 7.59 (tt,  $J$  = 7.0, 1.3 Hz, 1H), 7.49 – 7.42 (m, 2H), 7.25 – 7.15 (m, 5H), 5.80 (dddd,  $J$  = 16.5, 10.5, 6.0, 5.2 Hz, 1H), 5.31 (d,  $J$  = 3.6 Hz, 1H), 5.30 – 5.20 (m, 2H), 5.19 – 5.12 (m, 2H), 4.77 – 4.68 (m, 2H), 4.35 (d,  $J$  = 10.0 Hz, 1H), 4.26 – 4.19 (m, 2H), 4.02 (ddt,  $J$  = 13.2, 6.1, 1.3 Hz, 1H), 3.75 (s, 3H), 2.75 – 2.38 (m, 4H), 2.16 (s, 3H);  $^{13}\text{C}$  NMR (100.37 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.2, 171.6, 168.7, 165.7, 138.0, 133.5, 133.1, 129.9, 129.9, 129.6, 128.6, 128.6, 128.4, 128.4, 127.9, 127.9, 127.8, 118.2, 95.6, 76.8, 75.1, 72.9, 71.6, 69.3, 69.0, 53.1, 37.8, 30.0, 27.8; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{29}\text{H}_{32}\text{O}_{10}$   $[\text{M}+\text{Na}]^+$ : 563.1893, Found: 563.1884.

**Compound 54c:** This compound was prepared from compound **53c** following procedure described for the compound **46**. Eluent for purification: 5% ethyl acetate in *n*-hexane; Thick syrup; yield 4.1 g (93%) from 3.5 g;  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +145.7°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2938, 2860, 1753, 1725, 1601, 1453, 1328, 1264, 1102, 1051, 842, 777, 744, 710;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.91 – 7.86 (m, 2H), 7.45 – 7.40 (m, 1H), 7.32 – 7.26 (m, 2H), 7.09 – 7.01 (m, 5H), 5.77 – 5.65 (m, 1H), 5.17 (dq,  $J$  = 17.3, 1.6 Hz, 1H), 5.14 – 5.08 (m, 2H), 5.03 (dq,  $J$  = 10.6, 1.3 Hz, 1H), 4.74 (d,  $J$  = 11.2 Hz, 1H), 4.66 (d,  $J$  = 11.3 Hz, 1H), 4.24 – 4.18 (m, 1H),



4.13 (ddt,  $J = 13.2, 5.1, 1.5$  Hz, 1H), 3.99 – 3.87 (m, 3H), 3.69 (s, 3H), 0.78 (s, 9H), -0.09 (s, 3H), -0.10 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.8, 165.8, 138.2, 133.4, 133.3, 129.8, 129.8, 129.6, 128.5, 128.5, 128.2, 128.2, 127.4, 127.3, 127.3, 118.0, 99.0, 78.0, 75.3, 73.9, 72.6, 72.6, 69.0, 52.5, 25.9(3C), 18.0, -3.9, -5.1; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{30}\text{H}_{40}\text{O}_8\text{Si}[\text{M}+\text{Na}]^+$ : 579.2390, Found: 579.2394.

### **Compounds 56a-e: Allyl Deprotection and Synthesis of Ethynyl Cyclohexyl Glycosyl**

**Carbonate Donor:** To a biphasic solution of the alkyl glycoside (1.0 mmol) in 3:1  $\text{CH}_3\text{OH}:\text{CH}_2\text{Cl}_2$  (20 mL) was added 0.15 equivalent of  $\text{PdCl}_2$  and the reaction mixture was stirred for 4-8 h at 25 °C, the reaction was quenched by adding excess of  $\text{Et}_3\text{N}$  and filtered through a bed of Celite®. The filtrate concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using ethyl acetate and *n*-hexane as a mobile phase to obtain desired hemiacetals.

To a solution of glycosyl hemiacetal (1.0 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (5 mL) was added DMAP (1.5 eq) and ethynyl cyclohexyl (4-nitrophenyl) carbonate **36** (1.2 eq), the reaction mixture was stirred at 25 °C for 3 h. After complete consumption of the starting hemiacetal, the reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography using EtOAc and hexane as a mobile phase to obtain carbonate donor.

**Compound 56a:** Prepared from compound **54a**. Eluent for purification: 25% ethyl acetate in *n*-hexane; white solid; yield 370 mg (72%) from 420 mg (over two steps); mp 92.1 °C;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +31.1°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3277, 3018, 2942, 2863, 2115, 1751, 1444, 1367, 1224, 1152, 1040, 903, 752, 700;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.35 – 7.22 (m, 10H), 6.23 (d,  $J = 3.6$  Hz, 1H), 5.61 (d,  $J = 7.2$  Hz, 1H), 5.26 (t,  $J = 8.8$  Hz, 1H), 5.20 – 5.13 (m, 2H), 5.08 (dd,  $J = 9.8, 3.6$  Hz, 1H), 4.74 (d,  $J = 11.9$  Hz, 1H), 4.69 – 4.60 (m, 3H), 4.38 (d,  $J = 10.1$  Hz, 1H), 4.09 (d,  $J = 8.9$  Hz, 1H), 4.05 (t,  $J = 9.5$  Hz, 1H), 3.82 – 3.77 (m, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 2.79 – 2.59 (m, 6H), 2.54 – 2.48 (m, 4H), 2.24 – 2.08 (m, 10H), 1.97 (s, 3H), 1.95 (s, 3H), 1.93 – 1.73 (m, 4H), 1.72 – 1.54 (m, 10H), 1.35 – 1.28 (m, 2H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.1, 205.9, 171.5, 171.0, 169.4, 169.4, 167.6, 167.3, 151.0, 150.5, 137.9, 137.6, 128.4(4C), 127.8(4C), 127.6(2C), 94.4, 92.3, 82.4(2C), 78.9, 78.8, 78.2, 76.1, 75.6, 75.4, 75.0, 74.0, 73.3, 71.1, 71.1, 70.9, 70.4, 70.1, 52.9, 52.9, 37.8, 37.7, 37.0, 36.8, 36.5, 36.4, 29.8, 29.8, 27.8, 27.7, 24.9, 24.9, 22.5(4C), 20.6, 20.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{30}\text{H}_{36}\text{O}_{12}[\text{M}+\text{Na}]^+$ : 611.2104, Found: 611.2097.

**Compound 56a:β ratio 6:1:** Prepared from compound **54b**. Eluent for purification: 35% ethyl acetate in *n*-hexane; white solid; yield 705 mg (72%) from 810 mg (over two steps); mp 86.1 °C;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +74.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3284, 3012, 2940, 2862, 2117, 1747,



1447, 1366, 1228, 1152, 1101, 1015, 900, 753, 709;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.02 – 7.95 (m, 2H), 7.60 – 7.53 (m, 1H), 7.46 – 7.38 (m, 2H), 7.24 – 7.14 (m, 5H), 6.41 (d,  $J$  = 3.6 Hz, 1H), 5.40 – 5.34 (m, 1H), 5.27 (dd,  $J$  = 10.1, 9.2 Hz, 1H), 4.73 (s, 2H), 4.48 (d,  $J$  = 10.1 Hz, 1H), 4.25 (t,  $J$  = 9.5 Hz, 1H), 3.73 (s, 3H), 2.75 – 2.62 (m, 2H), 2.61 – 2.52 (m, 1H), 2.50 – 2.41 (m, 1H), 2.39 (s, 1H), 2.16 (s, 3H), 2.15 – 2.05 (m, 1H), 2.05 – 1.96 (m, 1H), 1.88 – 1.74 (m, 2H), 1.67 – 1.43 (m, 5H), 1.29 (dq,  $J$  = 9.6, 4.8 Hz, 1H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.1, 171.5, 167.8, 165.2, 150.4, 137.8, 133.5, 130.0, 130.0, 129.20, 128.5, 128.5, 128.4, 128.4, 127.9, 127.8, 127.8, 92.6, 82.2, 79.0, 76.4, 75.4, 75.2, 71.4, 71.0, 71.0, 53.1, 37.7, 36.7, 36.6, 29.9, 27.7, 24.9, 22.6, 22.6; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{35}\text{H}_{38}\text{O}_{12} [\text{M}+\text{Na}]^+$ : 673.2260, Found: 673.2263.

**Compound 56c  $\alpha:\beta$  ratio 4:1:** Prepared from compound **54c**. Eluent for purification: 5% ethyl acetate in *n*-hexane; Thick syrup; yield 2.1 g (86%) from 1.9 g;  $[\alpha]_{\text{D}}^{25}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +72.9°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3279, 2941, 2861, 2117, 1760, 1451, 1271, 1243, 1149, 1100, 1043, 1010, 899, 844, 754, 708;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.97 – 7.90 (m, 4H), 7.57 – 7.49 (m, 2H), 7.42 – 7.34 (m, 4H), 7.18 – 7.10 (m, 10H), 6.35 – 5.82 (m, 2H), 5.45 – 5.33 (m, 2H), 4.82 – 4.64 (m, 4H), 4.45 – 4.02 (m, 6H), 3.78 (s, 6H), 2.42 (s, 2H), 2.21 – 1.96 (m, 4H), 1.95 – 1.73 (m, 4H), 1.66 – 1.38 (m, 10H), 1.35 – 1.23 (m, 2H), 0.86 (s, 18H), -0.01 (d, 12H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.8, 168.4, 165.3, 165.0, 151.1, 150.8, 137.9, 137.5, 133.4, 133.4, 130.0, 130.0, 129.9, 129.9, 129.4, 129.2, 128.4(4C), 128.2(4C), 127.8, 127.8, 127.6, 127.5, 127.4, 127.4, 95.0, 93.1, 82.5, 82.3, 81.7, 79.5, 78.8, 78.5, 77.5, 75.5, 75.3, 75.1, 74.7, 74.6, 72.5, 72.3, 72.0, 71.7, 52.6, 52.6, 36.7, 36.7, 36.6, 36.5, 25.8(6C), 25.0, 24.9, 22.6, 22.6, 22.4, 22.3, 18.0, 18.0, -3.9, -4.1, -5.1, -5.2; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{36}\text{H}_{46}\text{O}_{10}\text{Si} [\text{M}+\text{Na}]^+$ : 689.2758, Found: 689.2758.

**Compound 58: Allyl Glycoside Preparation of the Troc-GlcN:** A solution of the Troc-GlcN compound (115 g, 324.34 mmol) in anhydrous allyl alcohol (1.1 L, 16.22 mol) was cooled to 0°C and acetyl chloride (57.86 mL, 810.86 mmol) was added dropwise under argon atmosphere. After stirring at 0 °C for 1 h, the reaction mixture was refluxed at 80 °C for 15 h, the reaction was quenched with  $\text{Et}_3\text{N}$  and the volatile components were removed under reduced pressure. The product was precipitated by stirring crude compound in methanol- $\text{CH}_2\text{Cl}_2$ -hexane (1:2:4) solvent mixture. The residue was filtered through Whatman filter, washed with  $\text{CH}_2\text{Cl}_2$ -Hexane (1:4) and dried over high vacuum; successfully afforded 100 g of the pure white solid. The filtrate was evaporated and purified by column chromatography to get overall 120 g (94%) of compound **58**.

**Compound 59: Troc-GlcN Benzylidene Protection:** To a solution of the compound **58** (120 g, 304.09 mmol) and benzaldehyde dimethyl acetal (68.46 mL, 456.13 mmol) in anhydrous DMF (500 mL) was added camphor sulfonic acid (14.13 g, 60.82 mmol) portion-wise under argon atmosphere and the mixture stirred at 25 °C for 4 h. After completion, the reaction was quenched by dropwise addition of the ice-cold saturated aq NaHCO<sub>3</sub> solution (500 mL), then 500 mL of water was added and extracted with EtOAc (3 x 300 mL). The organic layers were washed with brine (3 x 300 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The product was precipitated by stirring crude residue in the combination of methanol-water (1:1) solvent mixture and after filtration, residue was washed with methanol-water (1:1) followed by CH<sub>2</sub>Cl<sub>2</sub>-Hexane (1:4), dried under high vacuum to afford 98 g of the pure white. Further the filtrate was evaporated and purified by column chromatography to get an overall 108 g (74%) of the compound **59**. mp 172.5 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> (CHCl<sub>3</sub>, c1.0): +66.9°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3336, 2955, 2870, 1710, 1541, 1375, 1269, 1086, 754; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>):  $\delta$  7.54 – 7.47 (m, 2H), 7.42 – 7.35 (m, 3H), 5.88 (ddt, *J* = 16.6, 10.8, 5.8 Hz, 1H), 5.52 (s, 1H), 5.39 (d, *J* = 9.2 Hz, 1H), 5.30 (d, *J* = 17.1 Hz, 1H), 5.23 (d, *J* = 10.3 Hz, 1H), 4.90 (d, *J* = 3.5 Hz, 1H), 4.83 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 1H), 4.25 (dd, *J* = 10.0, 4.6 Hz, 1H), 4.18 (dd, *J* = 12.8, 5.3 Hz, 1H), 4.02 – 3.89 (m, 2H), 3.88 – 3.79 (m, 2H), 3.72 (t, *J* = 10.2 Hz, 1H), 3.54 (t, *J* = 9.1 Hz, 1H), 3.11 (d, *J* = 3.3 Hz, 1H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>):  $\delta$  155.0, 137.1, 133.3, 129.4, 128.4, 128.4, 126.4, 126.4, 118.4, 102.0, 97.1, 95.5, 81.9, 74.8, 69.8, 68.8, 68.7, 62.7, 55.8; HRMS (ESI-MS): *m/z* calcd for C<sub>19</sub>H<sub>22</sub>Cl<sub>3</sub>NO<sub>7</sub> [M+Na]<sup>+</sup>: 504.0360, Found: 504.0360 & 506.0321.

**Compound 60: Troc Deprotection:** To a solution of the compound **59** (98 g, 203.01 mmol) in 1:2 CH<sub>2</sub>Cl<sub>2</sub>-MeOH (60 mL) was added 20 mL of glacial acetic acid. Zinc (239 g, 3.65 mol) was added portion-wise at 0 °C and the mixture was stirred at 25 °C for 1 h filtered through a bed of Celite®, the residue was washed with excess CH<sub>2</sub>Cl<sub>2</sub> and filtrate was concentrated under reduced pressure. The resulting crude residue was treated with saturated aq NaHCO<sub>3</sub> solution (300 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 200 mL). The combined organic phases were washed with brine (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude residue was passed through a small bed of silica gel using 4% methanol in CH<sub>2</sub>Cl<sub>2</sub> as the mobile phase to afford 48 g (77%) of the compound **60** as a thick syrup.

**Compound 61 GlcN Azide Preparation:** To a solution of the compound **60** (50g, 162.68 mmol) in MeOH:THF (750 mL, 4:1) was added K<sub>2</sub>CO<sub>3</sub> (53.96 g, 390.44 mmol) and CuSO<sub>4</sub>•5H<sub>2</sub>O (406 mg, 1.63 mmol). Imidazole-1-sulfonyl azide hydrochloride (47.74 g, 227.76 mmol) was added portion-wise and the mixture stirred at 25 °C for 2 h. The mixture was

concentrated, diluted with ice-cold water (1 L) and extracted with EtOAc (2 × 500 mL). The combined organic layers were washed with brine solution (300 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude was purified by silica gel column chromatography to afford 44.5 g (82 %) of the compound **61**. Eluent for purification: 15% ethyl acetate in *n*-hexane; white solid; mp 164.0 °C;  $[\alpha]^{25}_{\text{D}}$  (CHCl<sub>3</sub>, *c*1.0): +89.6°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3390, 2920, 2868, 2109, 1648, 1459, 1381, 1267, 1092, 1032, 756; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>): δ 7.51 – 7.46 (m, 2H), 7.40 – 7.35 (m, 3H), 5.98 – 5.86 (m, 1H), 5.51 (s, 1H), 5.35 (dq, *J* = 17.3, 1.5 Hz, 1H), 5.28 – 5.22 (m, 1H), 4.92 (d, *J* = 3.6 Hz, 1H), 4.28 – 4.14 (m, 3H), 4.03 (ddt, *J* = 12.9, 6.0, 1.3 Hz, 1H), 3.86 (td, *J* = 10.0, 4.8 Hz, 1H), 3.70 (t, *J* = 10.3 Hz, 1H), 3.47 (t, *J* = 9.3 Hz, 1H), 3.25 (dd, *J* = 10.1, 3.7 Hz, 1H), 3.08 (s, 1H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>): δ 137.0, 133.2, 129.5, 128.5, 128.5, 126.4, 126.4, 118.2, 102.1, 97.6, 81.9, 68.9, 68.8, 68.8, 63.1, 62.6; HRMS (ESI-MS): *m/z* calcd for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub> [M+Na]<sup>+</sup>: 356.1222, Found: 356.1209.

**Compound 62a-c: Benzyl/PMB/Nap protection:** To a solution of alcohol (1 mmol) in anhydrous THF (2.5 mL) was added NaH (60% in mineral oil, 1.2 eq per -OH) in portions under argon atmosphere. After the evolution of hydrogen ceased, BnBr/PMB-Cl or Nap-Br (1.1 eq per OH) was added dropwise, followed by the addition of catalytic amount of tetrabutylammonium iodide and the mixture was stirred at 25 °C for 3 h. After completion, ice cold water (10 mL) was added slowly to the reaction mixture and extracted with EtOAc (2 x 10 mL), combined organic phases were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* to a crude residue that was purified by silica gel column chromatography using EtOAc and hexane as mobile phase to afford the corresponding ether product. **62a:** Eluent for purification: 12% ethyl acetate in *n*-hexane as a thick syrup; yield 7.85 g (81%) from 7.12 g;  $[\alpha]^{25}_{\text{D}}$  (CHCl<sub>3</sub>, *c*1.0): +47.8°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 2922, 2866, 2107, 1612, 1513, 1460, 1374, 1248, 1089, 1036, 999, 823, 755, 700; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>): δ 7.55 – 7.49 (m, 2H), 7.45 – 7.37 (m, 3H), 7.35 – 7.30 (m, 2H), 6.90 – 6.85 (m, 2H), 6.00 – 5.89 (m, 1H), 5.60 (s, 1H), 5.37 (dq, *J* = 17.3, 1.5 Hz, 1H), 5.27 (dq, *J* = 10.6, 1.3 Hz, 1H), 4.95 (d, *J* = 3.7 Hz, 1H), 4.90 (d, *J* = 10.6 Hz, 1H), 4.76 (d, *J* = 10.6 Hz, 1H), 4.30 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.24 (ddt, *J* = 12.9, 5.1, 1.4 Hz, 1H), 4.14 – 4.04 (m, 2H), 3.94 (td, *J* = 10.0, 4.8 Hz, 1H), 3.80 (s, 3H), 3.79 – 3.74 (m, 1H), 3.71 (t, *J* = 9.3 Hz, 1H), 3.42 (dd, *J* = 10.0, 3.7 Hz, 1H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>): δ 159.5, 137.3, 133.2, 130.0 (3C), 129.2, 128.4, 128.4, 126.1, 126.1, 118.3, 113.9, 113.9, 101.5, 97.5, 82.9, 76.0, 74.8, 69.0, 68.8, 63.1, 62.9, 55.3; HRMS (ESI-MS): *m/z* calcd for C<sub>24</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub> [M+Na]<sup>+</sup>: 476.1798, Found: 476.1791. **Compound 62b:** Eluent for purification: 10% ethyl acetate in *n*-hexane; white solid; yield 9.9 g (87%) from 9.0 g as a solid; mp = 174.5 °C;  $[\alpha]^{25}_{\text{D}}$  (CHCl<sub>3</sub>, *c*1.0): +53.3°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 2919, 2868, 2107, 1457, 1375, 1268, 1088, 997, 752,

698;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.57 – 7.52 (m, 2H), 7.46 – 7.40 (m, 5H), 7.39 – 7.30 (m, 3H), 5.97 (ddt,  $J$  = 16.3, 10.7, 5.7 Hz, 1H), 5.62 (s, 1H), 5.43 – 5.36 (m, 1H), 5.29 (d,  $J$  = 10.4 Hz, 1H), 5.00 (d,  $J$  = 11.0 Hz, 1H), 4.98 (d,  $J$  = 3.7 Hz, 1H), 4.85 (d,  $J$  = 11.0 Hz, 1H), 4.32 (dd,  $J$  = 10.2, 4.8 Hz, 1H), 4.26 (dd,  $J$  = 12.9, 5.2 Hz, 1H), 4.16 (t,  $J$  = 9.5 Hz, 1H), 4.09 (dd,  $J$  = 12.9, 6.1 Hz, 1H), 3.97 (td,  $J$  = 10.0, 4.8 Hz, 1H), 3.82 – 3.77 (m, 1H), 3.77 – 3.72 (m, 1H), 3.45 (dd,  $J$  = 9.9, 3.6 Hz, 1H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  137.9, 137.3, 133.2, 129.1, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 127.9, 126.1, 126.1, 118.3, 101.5, 97.5, 82.9, 76.3, 75.1, 68.9, 68.8, 63.1, 62.9; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_5$   $[\text{M}+\text{Na}]^+$ : 446.1692, Found: 446.1688.

**Compound 62d: TBDPS Protection:** To a solution of the compound **61** (1.5 g, 4.5 mmol) and  $\text{Et}_3\text{N}$  (5 eq) in anhydrous  $\text{CH}_2\text{Cl}_2$  (20 mL) was added 0.5 equivalents of DMAP. TBDPSCl (1.2 eq) was added drop-wise with stirring under argon atmosphere and the reaction mixture was stirred at 25 °C for 15 h. Ice-cold water (150 mL) was added and extracted with  $\text{CH}_2\text{Cl}_2$  (3x100 mL), organic layer was washed with brine solution (2 x 200 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo* and the crude product was purified by flash silica gel column chromatography using EtOAc and hexane as a mobile phase to obtain the compound **62d** (2.0 g, 78%) as a thick syrup;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.59 – 7.53 (m, 4H), 7.39 – 7.33 (m, 2H), 7.30 – 7.24 (m, 3H), 7.17 (dt,  $J$  = 15.2, 7.6 Hz, 4H), 6.76 (dd,  $J$  = 8.1, 1.0 Hz, 2H), 5.87 (dddd, 1H), 5.27 (dq,  $J$  = 17.3, 1.6 Hz, 1H), 5.20 (dq,  $J$  = 10.5, 1.3 Hz, 1H), 4.99 – 4.93 (m, 2H), 4.19 (d,  $J$  = 9.8 Hz, 1H), 4.17 – 4.10 (m, 2H), 4.00 (ddt,  $J$  = 13.1, 6.1, 1.4 Hz, 1H), 3.66 (dd,  $J$  = 9.6, 4.5 Hz, 1H), 3.58 (t,  $J$  = 10.1 Hz, 1H), 3.50 (t,  $J$  = 9.1 Hz, 1H), 3.29 (dd,  $J$  = 9.8, 3.7 Hz, 1H), 0.99 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  136.7, 136.5, 136.5, 135.6, 135.6, 134.8, 133.3, 132.2, 129.7, 129.4, 128.8, 127.8, 127.8, 127.4, 127.4, 127.3, 127.3, 126.5, 126.5, 118.0, 101.8, 98.3, 82.2, 70.3, 68.8, 68.8, 64.7, 62.7, 26.8 (3C), 19.7.

**Compound 62e:** This compound was prepared from compound **61**, following the procedure adopted for the preparation of compound **31**. Eluent for purification: 13% ethyl acetate in *n*-hexane; thick syrup; yield 9.5 g (84%) from 10 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +146.7°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2959, 2912, 2105, 1732, 1460, 1377, 1267, 1230, 1090, 1040, 990, 757, 701;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.48 – 7.43 (m, 2H), 7.39 – 7.34 (m, 3H), 6.02 – 5.89 (m, 1H), 5.68 – 5.61 (m, 1H), 5.51 (s, 1H), 5.37 (dq,  $J$  = 17.3, 1.5 Hz, 1H), 5.27 (dq,  $J$  = 10.3, 1.1 Hz, 1H), 5.04 (d,  $J$  = 3.6 Hz, 1H), 4.31 – 4.24 (m, 2H), 4.09 (ddt,  $J$  = 12.8, 6.1, 1.2 Hz, 1H), 4.00 (td,  $J$  = 10.0, 4.9 Hz, 1H), 3.75 (t,  $J$  = 10.3 Hz, 1H), 3.63 (t,  $J$  = 9.6 Hz, 1H), 3.24 (dd,  $J$  = 10.3, 3.6 Hz, 1H), 2.13 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.9, 137.0, 133.0, 129.2, 128.3,

128.3, 126.3, 126.3, 118.5, 101.8, 98.0, 79.6, 69.1, 69.1, 68.8, 63.0, 61.7, 21.0; HRMS (ESI-MS):  $m/z$  calcd for  $C_{18}H_{21}N_3O_6 [M+Na]^+$ : 398.1328, Found: 398.1334.

**Compound 63a-e: Benzylidene Deprotection:** To a solution of benzylidene protected compound (1.0 mmol) in 1:1  $CH_3OH:CH_2Cl_2$  (2 mL) was added 0.3 equivalent of the *p*-toluenesulfonic acid monohydrate and the mixture was stirred at 25 °C for 1 h. After completion, the reaction was quenched with  $Et_3N$  and the volatiles were evaporated under reduced pressure, the crude residue was purified by silica gel column chromatography using EtOAc and hexane as mobile phase to obtain the desired product.

**Compound 64a: Selective Benzoyl Protection:** A solution of the 4,6-diol **63a** (5.45 g, 31.15 mmol) in anhydrous  $CH_2Cl_2$  (50 mL) was added 9.0 eq of  $Et_3N$  and the benzoic anhydride (1.2 eq) was added slowly at 0 °C under argon atmosphere. The reaction mixture was stirred at 25 °C for 12 h, quenched by adding methanol (5 mL), volatiles were evaporated under vacuum and the residue was purified by silica gel column chromatography using 12% ethyl acetate in *n*-hexane as a mobile phase to afford compound **64a** (5.86 g, 86%) as a thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): +74.0°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3465, 2919, 2106, 1717, 1611, 1514, 1455, 1271, 1109, 1033, 930, 822, 756, 713;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ):  $\delta$  8.04 (dt,  $J$  = 8.4, 1.6 Hz, 2H), 7.60 – 7.55 (m, 1H), 7.46 – 7.41 (m, 2H), 7.37 – 7.31 (m, 2H), 6.92 – 6.87 (m, 2H), 5.94 (dddd,  $J$  = 16.8, 10.3, 6.1, 5.4 Hz, 1H), 5.34 (dq,  $J$  = 17.1, 1.5 Hz, 1H), 5.24 (dq,  $J$  = 10.5, 1.4 Hz, 1H), 4.97 (d,  $J$  = 3.6 Hz, 1H), 4.87 (d,  $J$  = 10.7 Hz, 1H), 4.76 (d,  $J$  = 10.7 Hz, 1H), 4.71 (dd,  $J$  = 12.2, 4.4 Hz, 1H), 4.47 (dd,  $J$  = 12.2, 2.1 Hz, 1H), 4.23 (ddt,  $J$  = 12.9, 5.2, 1.4 Hz, 1H), 4.08 (ddt,  $J$  = 13.1, 6.3, 1.3 Hz, 1H), 3.94 (ddd,  $J$  = 10.0, 4.3, 2.1 Hz, 1H), 3.88 (dd,  $J$  = 10.2, 8.7 Hz, 1H), 3.79 (s, 3H), 3.57 (td,  $J$  = 9.9, 3.6 Hz, 1H), 3.34 (dd,  $J$  = 10.2, 3.5 Hz, 1H), 2.91 (d,  $J$  = 3.4 Hz, 1H);  $^{13}C$  NMR (100.53 MHz,  $CDCl_3$ ):  $\delta$  167.2, 159.7, 133.5, 133.3, 130.1, 130.0, 130.0, 129.9, 129.9, 129.6, 128.6, 128.6, 118.4, 114.2, 114.2, 97.0, 79.5, 75.1, 70.8, 70.4, 68.8, 63.5, 63.1, 55.4; HRMS (ESI-MS):  $m/z$  calcd for  $C_{24}H_{27}N_3O_7 [M+Na]^+$ : 492.1746, Found: 492.1743.

**Compound 64b: Selective Acetyl Protection:** A solution of the 4,6-diol **63b** (2.32 g, 6.92 mmol) in anhydrous  $CH_2Cl_2$  (40 mL) was added 9.0 eq of  $Et_3N$  and the acetic anhydride (1.2 eq) dropwise at 0 °C under argon atmosphere. The reaction mixture was stirred at 25 °C for 12 h, quenched by adding methanol (4 mL), volatiles were evaporated and the residue was purified by silica gel column chromatography using 10% ethyl acetate in *n*-hexane as mobile phase to afford compound **64b** (2.2 g, 84%) as a thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): +60.8°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3452, 2920, 2108, 1737, 1455, 1367, 1245, 1097, 1037, 921, 747, 701;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ ):  $\delta$  7.42 – 7.29 (m, 5H), 5.94 (dddd,  $J$  = 17.1, 10.4, 6.2, 5.3 Hz, 1H), 5.35

(dq,  $J = 17.2, 1.6$  Hz, 1H), 5.25 (dq,  $J = 10.4, 1.3$  Hz, 1H), 4.96 (d,  $J = 3.6$  Hz, 1H), 4.93 (d,  $J = 11.0$  Hz, 1H), 4.82 (d,  $J = 11.0$  Hz, 1H), 4.51 (dd,  $J = 12.3, 4.1$  Hz, 1H), 4.23 – 4.16 (m, 2H), 4.06 (ddt,  $J = 12.9, 6.2, 1.3$  Hz, 1H), 3.87 (dd,  $J = 10.2, 8.7$  Hz, 1H), 3.81 (ddd,  $J = 10.0, 4.0, 2.3$  Hz, 1H), 3.49 (td,  $J = 9.9, 3.4$  Hz, 1H), 3.33 (dd,  $J = 10.2, 3.6$  Hz, 1H), 2.75 (d,  $J = 3.5$  Hz, 1H), 2.11 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.9, 138.0, 133.2, 128.8, 128.8, 128.3 (3C), 118.4, 97.0, 79.7, 75.4, 70.7, 70.2, 68.8, 63.0, 63.0, 21.0; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_6$   $[\text{M}+\text{Na}]^+$ : 400.1485, Found: 400.1489.

**Compound 64c-g: Selective TBDPS Protection:** To a solution of the 4,6-diol compound (1.0 mmol) and  $\text{Et}_3\text{N}$  (5 eq) in anhydrous  $\text{CH}_2\text{Cl}_2$  (5 mL) was added 0.5 equivalent of DMAP. The TBDPSCl (1.2 eq) was added drop-wise with stirring under argon atmosphere and the reaction mixture was stirred at 25 °C for 15 h. Ice-cold water (30 mL) was added to the reaction mixture and extracted with  $\text{CH}_2\text{Cl}_2$  (3x20 mL), organic layer was washed with brine solution (2 x 50 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude product was purified by flash silica gel column chromatography using EtOAc and hexane as a mobile phase to obtain the desired product.

**Compound 64c:** This compound was prepared from compound **63b**, following the above described procedure for **64c-g**. Eluent for purification: 8% ethyl acetate in *n*-hexane; Thick syrup; yield 10.03 g (98%) from 6.0 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +40.5°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3382, 3069, 2932, 2861, 2106, 1465, 1355, 1268, 1103, 1049, 752, 701;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 – 7.69 (m, 4H), 7.47 – 7.31 (m, 11H), 6.01 – 5.85 (m, 1H), 5.34 (dq,  $J = 17.3, 1.5$  Hz, 1H), 5.29 – 5.18 (m, 1H), 4.96 – 4.91 (m, 2H), 4.87 (d,  $J = 11.1$  Hz, 1H), 4.19 (ddt,  $J = 12.9, 5.1, 1.4$  Hz, 1H), 4.07 – 4.00 (m, 1H), 3.92 – 3.87 (m, 3H), 3.76 – 3.71 (m, 2H), 3.34 (dd,  $J = 10.2, 3.6$  Hz, 1H), 2.55 (d,  $J = 1.9$  Hz, 1H), 1.09 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  138.3, 135.8, 135.8, 135.7, 135.7, 133.4, 133.2, 133.0, 130.0, 130.0, 128.7, 128.7, 128.3, 128.3, 128.1, 127.9(4C), 118.1, 96.7, 80.1, 75.3, 72.7, 71.2, 68.4, 64.4, 63.0, 27.0(3C), 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{32}\text{H}_{39}\text{N}_3\text{O}_5\text{Si}$   $[\text{M}+\text{H}]^+$ : 574.2737, Found: 574.2726.

**Compound 64d:** This compound was prepared from compound **63a**, following the above delineated procedure for compounds **64c-g**. Eluent for purification: 7% ethyl acetate in *n*-hexane; Thick syrup; yield 1.18 g (83%) from 0.865 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +30.3°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3488, 2930, 2869, 2107, 1613, 1514, 1465, 1257, 1109, 1043, 754, 704;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.76 – 7.70 (m, 4H), 7.50 – 7.37 (m, 8H), 6.96 – 6.92 (m, 2H), 6.01 – 5.90 (m, 1H), 5.36 (dq,  $J = 17.1, 1.5$  Hz, 1H), 5.28 – 5.23 (m, 1H), 4.96 (d,  $J = 3.5$  Hz, 1H), 4.88 (d,  $J = 10.7$  Hz, 1H), 4.80 (d,  $J = 10.7$  Hz, 1H), 4.21 (ddt,  $J = 13.0, 5.3, 1.4$  Hz, 1H), 4.05 (ddt,  $J = 13.2, 6.3, 1.3$  Hz, 1H), 3.92 – 3.87 (m, 3H), 3.82 (s, 3H), 3.78 – 3.69 (m, 2H), 3.35



(dd,  $J = 10.2, 3.5$  Hz, 1H), 2.57 (d,  $J = 2.2$  Hz, 1H), 1.11 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.5, 135.7(4C), 133.4, 133.2, 133.0, 130.4, 129.9(4C), 127.9, 127.9, 127.8, 127.8, 118.1, 114.1, 114.1, 96.7, 79.8, 74.9, 72.5, 71.3, 68.3, 64.2, 63.0, 55.3, 26.9(3C), 19.3; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{33}\text{H}_{41}\text{N}_3\text{O}_6\text{Si}[\text{M}+\text{H}]^+$ : 604.2843, Found: 604.2844.

**Compound 64e:** This compound was prepared from compound **63c**, following the above described procedure of compounds **64c-g**. Eluent for purification: 7% ethyl acetate in *n*-hexane; thick syrup; yield 660 mg (91%) from 450 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +36.5°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3492, 3055, 2928, 2862, 2106, 1692, 1466, 1339, 1267, 1106, 1048, 817, 753, 702;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.92 – 7.85 (m, 4H), 7.76 – 7.72 (m, 4H), 7.59 (dd,  $J = 8.4, 1.7$  Hz, 1H), 7.52 – 7.40 (m, 8H), 5.96 (dddd,  $J = 16.6, 10.4, 6.1, 5.2$  Hz, 1H), 5.37 (dq,  $J = 17.2, 1.6$  Hz, 1H), 5.26 (dq,  $J = 10.4, 1.3$  Hz, 1H), 5.12 (d,  $J = 11.3$  Hz, 1H), 5.06 (d,  $J = 11.4$  Hz, 1H), 4.98 (d,  $J = 3.6$  Hz, 1H), 4.21 (ddt,  $J = 13.0, 5.2, 1.5$  Hz, 1H), 4.06 (ddt,  $J = 13.0, 6.2, 1.3$  Hz, 1H), 4.01 – 3.96 (m, 1H), 3.92 (d,  $J = 3.7$  Hz, 2H), 3.80 – 3.75 (m, 2H), 3.39 (dd,  $J = 10.2, 3.6$  Hz, 1H), 2.65 (s, 1H), 1.12 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  135.8, 135.8, 135.7(3C), 133.5, 133.5, 133.2, 133.1, 133.0, 130.0, 130.0, 128.5, 128.1, 127.9(4C), 127.8, 127.0, 126.2, 126.1, 126.1, 118.1, 96.8, 80.1, 75.3, 72.8, 71.3, 68.4, 64.4, 63.0, 27.0(3C), 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{36}\text{H}_{41}\text{N}_3\text{O}_5\text{Si}[\text{M}+\text{Na}]^+$ : 646.2712, Found: 646.2718.

**Compound 64f:** This compound was prepared from compound **63e**, following the above delineated procedure for **64c-g**. Eluent for purification: 12% ethyl acetate in *n*-hexane; Thick syrup; yield 10.1 g (89%) from 6.2 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +10.9°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3384, 2931, 2862, 2109, 1740, 1467, 1371, 1235, 1096, 1047, 754, 703;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 – 7.68 (m, 4H), 7.47 – 7.38 (m, 6H), 5.98 – 5.86 (m, 1H), 5.41 – 5.30 (m, 2H), 5.23 (dq,  $J = 10.4, 1.3$  Hz, 1H), 4.98 (d,  $J = 3.5$  Hz, 1H), 4.20 (ddt,  $J = 12.9, 5.3, 1.4$  Hz, 1H), 4.03 (ddt,  $J = 12.9, 6.2, 1.3$  Hz, 1H), 3.91 (d,  $J = 4.2$  Hz, 2H), 3.82 – 3.69 (m, 2H), 3.24 (dd,  $J = 10.6, 3.5$  Hz, 1H), 2.84 (d,  $J = 4.1$  Hz, 1H), 2.19 (s, 3H), 1.08 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.5, 135.7(4C), 133.2, 133.1, 133.0, 130.0, 130.0, 127.9(4C), 118.2, 96.7, 73.6, 71.7, 71.0, 68.6, 64.1, 61.0, 26.9(3C), 21.1, 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{27}\text{H}_{35}\text{N}_3\text{O}_6\text{Si}[\text{M}+\text{Na}]^+$ : 548.2193, Found: 548.2192.

**Compound 64g:** This compound was prepared from compound **63d**, following the above mentioned procedure for compounds **64c-g**. Eluent for purification: 7% ethyl acetate in *n*-hexane; Thick syrup; yield 680 mg (91%) from 500 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +76.6°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3110, 2930, 2861, 2107, 1457, 1268, 1102, 1036, 752, 702;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.88 – 7.83 (m, 2H), 7.77 – 7.73 (m, 2H), 7.70 – 7.66 (m, 4H), 7.51 – 7.42 (m, 8H), 7.41 – 7.35 (m, 4H), 5.92 – 5.81 (m, 1H), 5.23 (dq,  $J = 17.3, 1.6$  Hz, 1H), 5.17 (dq,  $J = 10.4,$

1.4 Hz, 1H), 5.01 (d,  $J = 3.6$  Hz, 1H), 4.16 (ddt,  $J = 13.3, 4.9, 1.6$  Hz, 1H), 4.07 (dd,  $J = 10.1, 8.4$  Hz, 1H), 3.99 (ddt,  $J = 13.3, 5.9, 1.3$  Hz, 1H), 3.86 – 3.71 (m, 3H), 3.55 (ddd,  $J = 9.8, 4.2, 2.8$  Hz, 1H), 3.23 (dd,  $J = 10.1, 3.5$  Hz, 1H), 1.67 (d,  $J = 4.5$  Hz, 1H), 1.13 (s, 9H), 1.09 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  136.3, 136.3, 135.8, 135.8, 135.7, 135.7, 135.4, 135.4, 134.9, 133.5, 133.5, 133.4, 132.3, 130.1, 129.9, 129.8, 129.8, 128.1, 128.1, 127.9, 127.9, 127.8(4C), 117.4, 97.2, 74.1, 71.9, 71.7, 68.0, 64.0, 63.4, 26.9(6C), 19.7, 19.4.

**Compound 65a:** This compound was prepared from compound **64c**, following the above mentioned procedure for compounds **62a-c**. Eluent for purification: 5% ethyl acetate in *n*-hexane; Thick syrup; yield 6.2 g (92%) from 5.4 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +22.7°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3040, 2932, 2860, 2107, 1596, 1464, 1356, 1268, 1153, 1089, 1059, 815, 751, 700;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.90 – 7.86 (m, 1H), 7.82 – 7.77 (m, 4H), 7.74 (dd,  $J = 8.1, 1.3$  Hz, 2H), 7.69 (s, 1H), 7.55 – 7.51 (m, 2H), 7.48 – 7.34 (m, 12H), 5.98 (dddd,  $J = 16.8, 10.5, 6.2, 5.4$  Hz, 1H), 5.38 (dq,  $J = 17.5, 1.5$  Hz, 1H), 5.30 – 5.25 (m, 1H), 5.10 (d,  $J = 11.1$  Hz, 1H), 5.06 (d,  $J = 3.6$  Hz, 1H), 4.99 (s, 2H), 4.91 (d,  $J = 11.1$  Hz, 1H), 4.25 (ddt,  $J = 12.7, 4.9, 1.2$  Hz, 1H), 4.19 – 4.13 (m, 1H), 4.09 (ddt,  $J = 12.7, 6.3, 1.2$  Hz, 1H), 4.05 – 3.95 (m, 2H), 3.93 – 3.85 (m, 2H), 3.52 (dd,  $J = 10.2, 3.6$  Hz, 1H), 1.13 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  138.0, 136.0, 136.0, 135.7, 135.7, 135.6, 133.6, 133.5, 133.4, 133.2, 133.1, 129.8, 129.8, 128.6, 128.6, 128.3(3C), 128.0, 128.0, 127.8(3C), 127.7, 127.7, 126.5, 126.2, 126.1, 125.8, 118.1, 96.6, 80.7, 78.5, 75.8, 75.3, 72.2, 68.3, 63.8, 62.7, 26.9(3C), 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{43}\text{H}_{47}\text{N}_3\text{O}_5\text{Si}[\text{M}+\text{Na}]^+$ : 714.3363, Found: 714.3353.

**Compound 65b:** This compound was prepared from compound **64b**, following the above described procedure for compounds **62a-c**. Eluent for purification: 5% ethyl acetate in *n*-hexane; Thick syrup; yield 525 mg (87%) from 500 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +71.6°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2954, 2924, 2859, 2106, 1612, 1513, 1462, 1363, 1249, 1111, 1045, 822, 743, 703, 611;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 – 7.68 (m, 4H), 7.46 – 7.41 (m, 4H), 7.40 – 7.35 (m, 6H), 7.34 – 7.29 (m, 1H), 7.09 – 7.05 (m, 2H), 6.81 – 6.77 (m, 2H), 5.91 (dddd,  $J = 16.8, 10.4, 6.2, 5.2$  Hz, 1H), 5.30 (dq,  $J = 17.2, 1.6$  Hz, 1H), 5.21 (dq,  $J = 10.4, 1.3$  Hz, 1H), 4.98 (d,  $J = 3.6$  Hz, 1H), 4.91 (s, 2H), 4.79 (d,  $J = 10.4$  Hz, 1H), 4.58 (d,  $J = 10.4$  Hz, 1H), 4.17 (ddt,  $J = 12.9, 5.3, 1.5$  Hz, 1H), 4.06 – 3.99 (m, 2H), 3.92 – 3.85 (m, 2H), 3.79 (s, 3H), 3.75 – 3.69 (m, 2H), 3.41 (dd,  $J = 10.3, 3.6$  Hz, 1H), 1.07 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.4, 138.1, 136.0, 136.0, 135.8, 135.8, 133.7, 133.5, 133.3, 130.3, 129.8, 129.8, 129.6, 129.6, 128.7, 128.7, 128.3, 128.3, 128.1, 127.9, 127.9, 127.7, 127.7, 118.1, 114.0, 114.0, 96.6, 80.7, 78.3, 75.7, 75.0, 72.2, 68.2, 63.8, 62.7, 55.4, 26.9 (3C), 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{40}\text{H}_{47}\text{N}_3\text{O}_6\text{Si}[\text{M}+\text{Na}]^+$ : 716.3132, Found: 716.3127.



**Compound 65c:** This compound was prepared from compound **64c**, following the above described procedure for compounds **62a-c**. Eluent for purification: 6% ethyl acetate in *n*-hexane; Thick syrup; yield 8.6 g (93%) from 8.0 g;  $[\alpha]^{25}_D$  (CHCl<sub>3</sub>, *c*1.0): +55.9°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3029, 2932, 2861, 2107, 1461, 1268, 1151, 1093, 1060, 752, 700; <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>): δ 7.76 – 7.70 (m, 4H), 7.48 – 7.41 (m, 5H), 7.41 – 7.39 (m, 2H), 7.39 – 7.37 (m, 2H), 7.37 – 7.32 (m, 3H), 7.32 – 7.30 (m, 2H), 7.21 (dd, *J* = 6.7, 2.9 Hz, 2H), 5.94 (dddd, *J* = 16.7, 10.3, 6.2, 5.3 Hz, 1H), 5.34 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.24 (dq, *J* = 10.5, 1.1 Hz, 1H), 5.02 (d, *J* = 3.6 Hz, 1H), 4.94 – 4.89 (m, 3H), 4.71 (d, *J* = 10.8 Hz, 1H), 4.21 (ddt, *J* = 12.9, 5.1, 1.4 Hz, 1H), 4.11 – 4.03 (m, 2H), 3.96 (dd, *J* = 11.2, 2.9 Hz, 1H), 3.91 (dd, *J* = 11.2, 0.8 Hz, 1H), 3.82 – 3.78 (m, 2H), 3.46 (dd, *J* = 10.2, 3.6 Hz, 1H), 1.10 (s, 9H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>): δ 138.1, 138.0(3C), 135.7, 135.7, 133.6, 133.5, 133.2, 129.8, 129.8, 128.6(4C), 128.4, 128.4, 128.1, 127.9(3C), 127.8, 127.8, 127.7, 127.7, 118.1, 96.6, 80.6, 78.5, 75.8, 75.3, 72.2, 68.3, 63.8, 62.6, 26.9(3C), 19.4; HRMS (ESI-MS): *m/z* calcd for C<sub>39</sub>H<sub>45</sub>N<sub>3</sub>O<sub>5</sub>Si[M+Na]<sup>+</sup>: 664.3206, Found: 664.3215.

**Compound 66: TBDPS Deprotection:** To a solution of the silyl ether **65a** (500 mg, 0.7 mmol) in 4:1 anhydrous pyridine-THF (5 mL) was added dropwise 1.0 mL of the 70% HF•py solution at 0 °C and the reaction mixture was stirred at 25 °C for 5 h. Reaction mixture was diluted with ice-cold water (20 mL) and extracted with EtOAc (2 x 20 mL), the organic layer was washed with aqueous 1N HCl (25 mL), saturated aq. NaHCO<sub>3</sub> (25 mL), brine solution (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using 20% ethyl acetate in *n*-hexane as mobile phase to furnish 295 mg (89%) of the compound **66** as a thick syrup.

**Compound 65d:** This compound was prepared from compound **66**, following the above described procedure for the compound **31**. Eluent for purification: 10% ethyl acetate in *n*-hexane; Thick syrup; yield 112 mg (86%) from 120 mg;  $[\alpha]^{25}_D$  (CHCl<sub>3</sub>, *c*1.0): +95.0°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3026, 2919, 2107, 1741, 1456, 1366, 1238, 1087, 1037, 820, 754, 700; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 7.86 – 7.80 (m, 3H), 7.71 (s, 1H), 7.52 – 7.47 (m, 2H), 7.44 – 7.40 (m, 3H), 7.39 – 7.30 (m, 3H), 5.95 (dddd, *J* = 17.2, 10.4, 6.1, 5.3 Hz, 1H), 5.36 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.26 (dq, *J* = 10.4, 1.3 Hz, 1H), 5.02 (d, *J* = 11.1 Hz, 1H), 4.98 (dd, *J* = 7.1, 3.6 Hz, 2H), 4.91 (d, *J* = 10.6 Hz, 1H), 4.78 (d, *J* = 11.1 Hz, 1H), 4.35 – 4.26 (m, 2H), 4.22 (ddt, *J* = 12.9, 5.3, 1.5 Hz, 1H), 4.13 – 4.04 (m, 2H), 3.98 – 3.93 (m, 1H), 3.64 (dd, *J* = 10.1, 8.7 Hz, 1H), 3.43 (dd, *J* = 10.3, 3.5 Hz, 1H), 1.92 (s, 3H); <sup>13</sup>C NMR (100.66 MHz, CDCl<sub>3</sub>): δ 170.7, 137.9, 135.0, 133.3, 133.2, 133.2, 128.7, 128.7, 128.5, 128.2, 128.2, 128.1, 128.1, 127.8, 127.0,

126.3, 126.2, 126.0, 118.4, 96.8, 80.7, 77.7, 75.7, 75.2, 69.3, 68.8, 63.6, 62.8, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $C_{29}H_{31}N_3O_6 [M+Na]^+$ : 540.2111, Found: 540.2128.

**Compound 65e:** This compound was prepared from compound **66**, following procedure described for the compound **51a**. Eluent for purification: 20% ethyl acetate in *n*-hexane; Thick syrup; yield 280 mg (93%) from 250 mg;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): +73.8°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3028, 2919, 2107, 1734, 1360, 1156, 1087, 1062, 920, 820, 751, 700;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ ):  $\delta$  7.85 – 7.79 (m, 3H), 7.72 (s, 1H), 7.51 – 7.46 (m, 2H), 7.44 – 7.39 (m, 3H), 7.38 – 7.31 (m, 3H), 5.95 (dddd,  $J$  = 17.2, 10.4, 6.1, 5.3 Hz, 1H), 5.37 (dq,  $J$  = 17.2, 1.6 Hz, 1H), 5.26 (dq,  $J$  = 10.4, 1.4 Hz, 1H), 5.03 (d,  $J$  = 11.1 Hz, 1H), 4.99 – 4.95 (m, 2H), 4.91 (d,  $J$  = 10.7 Hz, 1H), 4.79 (d,  $J$  = 11.1 Hz, 1H), 4.36 – 4.30 (m, 2H), 4.22 (ddt,  $J$  = 12.9, 5.3, 1.5 Hz, 1H), 4.12 – 4.04 (m, 2H), 3.95 (dt,  $J$  = 10.0, 3.2 Hz, 1H), 3.64 (dd,  $J$  = 10.1, 8.8 Hz, 1H), 3.43 (dd,  $J$  = 10.2, 3.6 Hz, 1H), 2.65 (t,  $J$  = 6.2 Hz, 2H), 2.56 – 2.40 (m, 2H), 2.15 (s, 3H);  $^{13}C$  NMR (100.66 MHz,  $CDCl_3$ ):  $\delta$  206.3, 172.5, 137.9, 135.2, 133.3, 133.2, 133.1, 128.6, 128.6, 128.4, 128.2, 128.2, 128.1, 128.1, 127.8, 127.0, 126.3, 126.1, 126.0, 118.3, 96.8, 80.6, 77.9, 75.6, 75.2, 69.4, 68.7, 63.5, 62.9, 37.8, 29.9, 27.8; HRMS (ESI-MS):  $m/z$  calcd for  $C_{32}H_{35}N_3O_7 [M+Na]^+$ : 596.2373, Found: 596.2354.

**Compounds 67a-e:** These compounds were prepared from compounds **65a-65e** following the procedures described for compounds **56a-56e**.

**Compound 67a:** Eluent for purification: 8% ethyl acetate in *n*-hexane; yield 530 mg (96%) from 450 mg; thick syrup;  **$\alpha$ -isomer:**  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): +24.3°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3035, 2932, 2860, 2110, 1763, 1458, 1361, 1268, 1112, 1030, 753, 700;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ )  $\delta$  7.87 – 7.82 (m, 1H), 7.80 – 7.75 (m, 2H), 7.71 – 7.63 (m, 5H), 7.50 (dt,  $J$  = 6.3, 3.6 Hz, 2H), 7.44 – 7.33 (m, 9H), 7.32 – 7.27 (m, 3H), 6.17 (d,  $J$  = 3.6 Hz, 1H), 5.07 (d,  $J$  = 11.1 Hz, 1H), 4.95 (s, 2H), 4.90 (d,  $J$  = 11.1 Hz, 1H), 4.10 – 4.05 (m, 1H), 4.02 – 3.97 (m, 2H), 3.92 (dd,  $J$  = 10.0, 7.2 Hz, 2H), 3.67 (dd,  $J$  = 9.9, 3.6 Hz, 1H), 2.64 (s, 1H), 2.23 – 2.14 (m, 2H), 1.90 (q,  $J$  = 9.2 Hz, 2H), 1.74 – 1.60 (m, 4H), 1.57 – 1.50 (m, 1H), 1.37 – 1.31 (m, 1H), 1.08 (s, 9H);  $^{13}C$  NMR (100.53 MHz,  $CDCl_3$ ) [ $\alpha$ -isomer]:  $\delta$  151.0, 137.8, 136.0, 136.0, 135.7, 135.7, 135.5, 133.5, 133.4, 133.1, 133.0, 129.8, 129.8, 128.7, 128.7, 128.4(3C), 128.2, 128.1, 127.9, 127.9 127.8(3C), 126.4, 126.3, 126.1, 125.6, 94.3, 82.7, 80.7, 78.4, 77.6, 76.0, 75.3, 75.3, 74.4, 63.2, 62.2, 36.8, 36.8, 27.0(3C), 25.0, 22.7, 22.6, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $C_{49}H_{53}N_3O_7Si [M+Na]^+$ : 846.3550, Found: 846.3548.  **$\beta$ -isomer:** thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): -35.5°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3277, 3035, 2934, 2861, 2111, 1765, 1458, 1270, 1084, 1017, 909, 754, 701;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ) [ $\beta$ -isomer]:  $\delta$  7.87 – 7.82 (m, 1H), 7.81 – 7.76 (m, 2H), 7.72 (dd,  $J$  = 7.8, 1.6 Hz, 2H), 7.68 (dd,  $J$  = 8.0, 1.2 Hz, 3H), 7.51 (dt,  $J$  = 6.2, 3.3 Hz,

2H), 7.43 – 7.33 (m, 11H), 7.31 (d,  $J = 7.0$  Hz, 1H), 5.40 – 5.36 (m, 1H), 5.07 (d,  $J = 11.0$  Hz, 1H), 4.97 – 4.90 (m, 3H), 4.03 – 3.97 (m, 3H), 3.68 – 3.60 (m, 2H), 3.49 (dt,  $J = 9.7$ , 2.2 Hz, 1H), 2.67 (s, 1H), 2.28 – 2.20 (m, 2H), 2.05 – 1.95 (m, 2H), 1.78 – 1.64 (m, 4H), 1.57 – 1.53 (m, 1H), 1.46 – 1.36 (m, 1H), 1.08 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ) [ $\beta$ -isomer]:  $\delta$  151.1, 137.7, 136.1, 136.1, 135.7, 135.7, 135.5, 133.6, 133.4, 133.1, 132.8, 129.8, 129.7, 128.7, 128.7, 128.5, 128.5, 128.4, 128.2, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 126.6, 126.3, 126.2, 125.8, 96.0, 83.4, 82.6, 78.8, 77.3, 76.6, 76.1, 75.5, 75.4, 65.1, 62.1, 37.0, 36.7, 26.9(3C), 25.1, 22.6, 22.6, 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{49}\text{H}_{53}\text{N}_3\text{O}_7\text{Si}[\text{M}+\text{Na}]^+$ : 846.3550, Found: 846.3545.

**Compound 67b  $\alpha$ : $\beta$  ratio 5:1:** Eluent for purification: 5% ethyl acetate in *n*-hexane; Thick syrup; yield 155 mg (84%) from 150 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0):  $-30.6^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3289, 2925, 2860, 2111, 1765, 1613, 1513, 1459, 1361, 1241, 1081, 1019, 911, 822, 745, 702, 613;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.69 (td,  $J = 8.1$ , 1.4 Hz, 4H), 7.45 – 7.33 (m, 11H), 7.10 (d,  $J = 8.7$  Hz, 2H), 6.82 (d,  $J = 8.6$  Hz, 2H), 5.60 (d, 1H), 4.90 (s, 2H), 4.82 (d,  $J = 10.3$  Hz, 1H), 4.67 (d,  $J = 10.4$  Hz, 1H), 3.94 – 3.84 (m, 3H), 3.80 (s, 3H), 3.64 – 3.52 (m, 2H), 3.41 (dt,  $J = 9.6$ , 2.2 Hz, 1H), 2.64 (d,  $J = 11.9$  Hz, 1H), 2.26 – 2.17 (m, 2H), 2.01 – 1.86 (m, 2H), 1.76 – 1.62 (m, 4H), 1.56 – 1.51 (m, 1H), 1.42 – 1.36 (m, 1H), 1.06 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.5, 151.1, 137.7, 136.1, 136.1, 135.7, 135.7, 133.7, 132.9, 130.1, 129.8, 129.7 (3C), 128.7, 128.7, 128.4, 128.4, 128.2, 127.9, 127.9, 127.7, 127.7, 114.1, 114.1, 96.0, 83.4, 82.6, 78.8, 77.0, 76.7, 76.1, 75.5, 75.0, 65.1, 62.0, 55.4, 37.0, 36.7, 27.0 (3C), 25.1, 22.6, 22.6, 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{46}\text{H}_{53}\text{N}_3\text{O}_8\text{Si}[\text{M}+\text{Na}]^+$ : 826.3500, Found: 826.3499.

**Compound 67c  $\alpha$ : $\beta$  ratio 2.3:1:** Eluent for purification: 8% ethyl acetate in *n*-hexane; Thick syrup; yield 6.2 g (92%) from 6.7 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0):  $+5.3^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3280, 3032, 2936, 2862, 2111, 1765, 1765, 1457, 1357, 1269, 1096, 1018, 909, 754, 700;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.70 (dd,  $J = 16.0$ , 6.8 Hz, 8H), 7.45 – 7.30 (m, 28H), 7.26 – 7.18 (m, 4H), 6.16 (d,  $J = 3.4$  Hz, 1H), 5.36 (d,  $J = 7.6$  Hz, 1H), 4.92 (d,  $J = 9.6$  Hz, 6H), 4.77 (t,  $J = 11.1$  Hz, 2H), 4.05 (t,  $J = 9.4$  Hz, 1H), 4.00 – 3.84 (m, 7H), 3.67 – 3.56 (m, 3H), 3.45 (d,  $J = 9.6$  Hz, 1H), 2.67 (s, 1H), 2.64 (s, 1H), 2.28 – 2.14 (m, 4H), 2.04 – 1.84 (m, 4H), 1.78 – 1.62 (m, 8H), 1.58 – 1.48 (m, 2H), 1.45 – 1.34 (m, 2H), 1.07 (s, 18H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  151.1, 151.0, 138.0, 138.0, 137.8, 137.7, 136.0(4C), 135.7(4C), 133.6, 133.5, 133.0, 132.9, 129.8(3C), 129.7, 128.7(4C), 128.6(4C), 128.5(2C), 128.4(2C), 128.2(2C), 128.0, 127.9(9C), 127.8, 127.7(3C), 96.0, 94.3, 83.3, 82.7, 82.6, 80.7, 78.8, 78.4, 77.6, 77.3, 76.6, 76.1, 76.0, 75.5, 75.3(3C), 74.3, 65.1, 63.2, 62.2, 62.0, 37.0, 36.8(2C), 36.7, 27.0(3C), 26.9(3C), 25.1(2C),

22.6(4C), 19.5, 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $C_{45}H_{51}N_3O_7Si[M+Na]^+$ : 796.3394, Found: 796.3386.

**Compound 67d  $\alpha:\beta$  ratio 5:1:** Eluent for purification: 12% ethyl acetate in *n*-hexane; thick syrup; yield 90 mg (85%) from 81 mg;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): +22.0°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3294, 2927, 2863, 2111, 1764, 1455, 1367, 1271, 1237, 1088, 1016, 906, 755;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ):  $\delta$  7.84 – 7.78 (m, 3H), 7.70 – 7.68 (m, 1H), 7.51 – 7.46 (m, 2H), 7.41 – 7.33 (m, 6H), 5.60 (d, 1H), 5.03 – 4.94 (m, 2H), 4.90 (d, 1H), 4.76 (d,  $J$  = 11.1 Hz, 1H), 4.36 – 4.30 (m, 1H), 4.27 – 4.22 (m, 1H), 3.71 – 3.56 (m, 4H), 2.67 (s, 1H), 2.23 – 2.15 (m, 2H), 1.96 – 1.89 (m, 2H), 1.87 (s, 3H), 1.74 – 1.61 (m, 4H), 1.56 – 1.50 (m, 1H), 1.39 – 1.30 (m, 1H);  $^{13}C$  NMR (100.53 MHz,  $CDCl_3$ ):  $\delta$  170.7, 150.9, 137.5, 134.7, 133.3, 133.2, 128.7, 128.7, 128.6, 128.3, 128.3, 128.3, 128.1, 127.9, 127.2, 126.4, 126.3, 126.0, 95.8, 83.5, 82.4, 79.1, 76.6, 76.0, 75.6, 75.2, 73.9, 65.0, 62.4, 36.9, 36.7, 25.0, 22.6, 22.6, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $C_{35}H_{37}N_3O_8[M+Na]^+$ : 650.2478, Found: 650.2472.

**Compound 67e:** Eluent for purification: 30% ethyl acetate in *n*-hexane; Thick syrup; yield 130 mg (83%) from 123 mg;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): -5.6°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3291, 2938, 2865, 2113, 1765, 1740, 1452, 1362, 1272, 1239, 1091, 1017, 910, 759;  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ):  $\delta$  7.85 – 7.79 (m, 3H), 7.72 – 7.69 (m, 1H), 7.50 – 7.46 (m, 2H), 7.41 – 7.33 (m, 6H), 5.39 – 5.32 (m, 1H), 5.02 – 4.94 (m, 2H), 4.89 (d,  $J$  = 10.7 Hz, 1H), 4.78 (d,  $J$  = 11.1 Hz, 1H), 4.32 (d,  $J$  = 2.6 Hz, 2H), 3.71 – 3.59 (m, 4H), 2.68 (s, 1H), 2.63 (td,  $J$  = 6.7, 6.1, 3.4 Hz, 2H), 2.53 – 2.38 (m, 2H), 2.23 – 2.16 (m, 2H), 2.15 (s, 3H), 1.97 – 1.87 (m, 2H), 1.75 – 1.67 (m, 2H), 1.66 – 1.51 (m, 3H), 1.40 – 1.29 (m, 1H);  $^{13}C$  NMR (100.53 MHz,  $CDCl_3$ ):  $\delta$  206.5, 172.4, 150.9, 137.5, 134.9, 133.3, 133.2, 128.7, 128.7, 128.5, 128.3, 128.3, 128.2, 128.1, 127.8, 127.1, 126.3, 126.2, 126.0, 95.8, 83.4, 82.4, 79.1, 76.7, 76.0, 75.6, 75.2, 73.9, 64.9, 62.4, 37.8, 36.9, 36.7, 29.9, 27.8, 25.0, 22.6, 22.6; HRMS (ESI-MS):  $m/z$  calcd for  $C_{38}H_{41}N_3O_9[M+Na]^+$ : 706.2740, Found: 706.2751.

**Compounds 69a-j: Glycosidation:** To a solution of glycosyl donor (1.0 eq) and acceptor (0.8 eq) in anhydrous  $CH_2Cl_2$  (5 mL/mmol) was added freshly activated 4Å MS powder (0.400 g/mmol) at 25 °C under argon atmosphere. After 15 min of vigorous stirring at 25 °C, chloro[tris(2,4-di-*tert*butylphenyl) phosphite]gold(I) **68** and AgOTf [8mol% each for reactions at 25 °C, 12mol% each for reactions at -20 °C and 15mol% each for reactions -40 °C] were added simultaneously to the reaction mixture at given temperature (Ref. Table 1) and stirred for 15 min to 1h. After completion, the reaction was quenched with  $Et_3N$  and filtered through a bed of Celite®, the filtrate was concentrated *in vacuo* and crude residue was purified by silica

gel column chromatography using ethyl acetate and hexane as mobile phase to afford the desired glycoside product.

**Compound 69a:**  $\alpha:\beta$  ratio (4:1): Eluent for purification: 20% ethyl acetate in *n*-hexane; Thick syrup; yield 58 mg (77%) from 30 mg;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.90 – 7.85 (m, 1H), 7.83 – 7.66 (m, 7H), 7.55 – 7.51 (m, 2H), 7.46 – 7.33 (m, 14H), 7.32 – 7.28 (m, 2H), 7.26 – 7.16 (m, 1H), 5.94 (dddd,  $J$  = 16.8, 10.4, 6.3, 5.2 Hz, 1H), 5.52 (d,  $J$  = 3.6 Hz, 1H), 5.40 – 5.32 (m, 1H), 5.31 – 5.25 (m, 1H), 5.14 – 5.04 (m, 2H), 5.04 – 4.80 (m, 6H), 4.46 – 3.97 (m, 8H), 3.94 – 3.89 (m, 1H), 3.88 (s, 1H), 3.57 (s, 2H), 3.54 – 3.36 (m, 2H), 2.79 – 2.32 (m, 4H), 2.18 (s, 3H), 1.08 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.2, 206.1, 172.1, 172.0, 169.9, 169.4, 139.1, 138.5, 137.9, 137.9, 136.0, 136.0, 135.9, 135.9, 135.8, 135.8, 135.7, 135.7, 135.6, 135.6, 133.7, 133.4, 133.4, 133.3(3C), 133.1, 133.0, 133.0, 132.9, 129.9, 129.8(3C), 128.6(4C), 128.3(4C), 128.2, 128.2, 128.1(3C), 128.0(3C), 127.9(4C), 127.8(4C), 127.7(6C), 127.4, 127.3, 127.3, 127.2, 126.4, 126.2, 126.2, 126.1, 126.0, 126.0, 125.7, 125.6, 118.4, 118.2, 101.3, 98.1, 95.5, 95.3, 83.5, 80.1, 79.8, 78.1, 77.9, 77.4, 76.2, 75.8, 75.8, 75.2, 75.1, 75.1, 74.9, 74.6, 73.4, 72.5, 72.4, 70.6, 70.2, 69.2, 69.2, 69.1, 67.2, 63.7, 62.3, 62.0, 52.9, 52.7, 37.9, 37.9, 29.9, 29.9, 27.9, 27.9, 27.0(6C), 19.5, 19.3;

**Compound 69c:** Eluent for purification: 12% ethyl acetate in *n*-hexane; thick syrup; yield 75 mg (79%) from 35 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ , c1.0): +32.3°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3002, 2929, 2860, 2108, 1750, 1460, 1367, 1268, 1039, 754, 703;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.86 – 7.82 (m, 1H), 7.79 – 7.74 (m, 2H), 7.69 – 7.62 (m, 5H), 7.52 – 7.47 (m, 2H), 7.35 (dddd,  $J$  = 17.4, 6.9, 5.3, 3.3 Hz, 15H), 7.28 – 7.24 (m, 2H), 5.91 – 5.81 (m, 1H), 5.47 (d,  $J$  = 3.6 Hz, 1H), 5.30 (dq,  $J$  = 17.2, 1.5 Hz, 1H), 5.23 (dd,  $J$  = 10.3, 1.3 Hz, 1H), 5.10 (d,  $J$  = 3.6 Hz, 1H), 5.05 (d,  $J$  = 11.3 Hz, 1H), 4.96 (d,  $J$  = 11.2 Hz, 1H), 4.95 – 4.88 (m, 4H), 4.84 (d,  $J$  = 11.2 Hz, 1H), 4.29 (d,  $J$  = 9.5 Hz, 1H), 4.21 (ddt,  $J$  = 13.2, 5.1, 1.4 Hz, 1H), 4.18 – 4.12 (m, 1H), 4.09 – 4.04 (m, 1H), 4.04 – 3.98 (m, 2H), 3.96 (dd,  $J$  = 7.4, 2.4 Hz, 2H), 3.85 (dd,  $J$  = 11.2, 1.5 Hz, 1H), 3.52 (s, 3H), 3.51 – 3.44 (m, 2H), 3.38 – 3.32 (m, 1H), 2.01 (s, 3H), 1.06 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.3, 169.4, 138.4, 137.9, 136.0, 136.0, 135.9, 135.7, 135.7, 133.7, 133.4, 133.2, 133.0, 133.0, 129.8, 129.8, 128.7(4C), 128.3, 128.3, 128.2, 128.1, 128.0, 127.8(4C), 127.7, 127.7, 127.3, 127.3, 126.3, 126.2, 126.0, 125.6, 118.4, 98.2, 95.3, 80.2, 79.8, 77.9, 75.8, 75.3, 75.1, 75.0, 73.2, 72.5, 70.7, 69.0, 63.7, 62.0, 52.7, 27.0(3C), 20.9, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{59}\text{H}_{65}\text{N}_3\text{O}_{12}\text{Si}[\text{M}+\text{Na}]^+$ : 1058.4235, Found: 1058.4208.

**Compound 69d:** Eluent for purification: 20% ethyl acetate in *n*-hexane; Thick syrup; yield 60 mg (83%) from 27 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ , c1.0): +42.5°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3036, 2926, 2859, 2108, 1748, 1613, 1513, 1460, 1362, 1241, 1147, 1108, 1034, 820, 744, 700;  $^1\text{H}$  NMR (399.78 MHz,

$\text{CDCl}_3$ ):  $\delta$  7.69 – 7.64 (m, 4H), 7.42 – 7.30 (m, 16H), 7.13 – 7.09 (m, 2H), 6.84 – 6.80 (m, 2H), 5.91 – 5.81 (m, 1H), 5.44 (d,  $J$  = 3.7 Hz, 1H), 5.30 (dq,  $J$  = 17.0, 1.4 Hz, 1H), 5.23 (dd,  $J$  = 10.3, 1.3 Hz, 1H), 5.09 (d,  $J$  = 3.6 Hz, 1H), 4.97 – 4.89 (m, 3H), 4.87 (d,  $J$  = 10.5 Hz, 1H), 4.82 (dd,  $J$  = 10.9, 3.7 Hz, 2H), 4.68 (d,  $J$  = 10.5 Hz, 1H), 4.26 (d,  $J$  = 9.4 Hz, 1H), 4.20 (ddt,  $J$  = 13.3, 5.0, 1.4 Hz, 1H), 4.16 – 4.10 (m, 1H), 4.07 – 4.03 (m, 1H), 4.02 – 3.94 (m, 2H), 3.93 – 3.85 (m, 2H), 3.84 – 3.79 (m, 4H), 3.49 (s, 3H), 3.41 (d,  $J$  = 9.2 Hz, 1H), 3.31 (dd,  $J$  = 9.9, 3.7 Hz, 1H), 2.00 (s, 3H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.4, 169.3, 159.3, 138.4, 138.0, 136.0, 136.0, 135.8, 135.8, 133.7, 133.2, 133.1, 130.6, 129.8, 129.8, 129.3, 129.3, 128.7, 128.7, 128.6, 128.6, 128.3, 128.3, 128.1, 127.8(3C), 127.7, 127.7, 127.3, 127.3, 118.4, 113.9, 113.9, 98.1, 95.3, 80.1, 79.7, 77.7, 75.7, 75.2, 75.0, 74.8, 73.2, 72.5, 70.7, 68.9, 63.7, 61.9, 55.4, 52.7, 27.0(3C), 20.9, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{56}\text{H}_{65}\text{N}_3\text{O}_{13}\text{Si}[\text{M}+\text{Na}]^+$ : 1038.4184, Found: 1038.4170.

**Compound 69e:** Eluent for purification: 15% ethyl acetate in *n*-hexane; Thick syrup; yield 63 mg (87%) from 28 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +48.1°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3034, 2926, 2859, 2108, 1748, 1459, 1363, 1235, 1147, 1107, 1034, 742, 699;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.67 (dd,  $J$  = 8.1, 1.4 Hz, 2H), 7.63 (dd,  $J$  = 8.1, 1.3 Hz, 2H), 7.40 – 7.29 (m, 19H), 7.22 – 7.19 (m, 2H), 5.86 (dddd,  $J$  = 16.9, 10.6, 6.3, 5.1 Hz, 1H), 5.45 (d,  $J$  = 3.7 Hz, 1H), 5.30 (dq,  $J$  = 17.1, 1.5 Hz, 1H), 5.23 (dd,  $J$  = 10.3, 1.3 Hz, 1H), 5.09 (d,  $J$  = 3.6 Hz, 1H), 4.95 (d,  $J$  = 11.2 Hz, 1H), 4.92 (d,  $J$  = 3.6 Hz, 1H), 4.91 – 4.85 (m, 3H), 4.83 (d,  $J$  = 11.2 Hz, 1H), 4.77 (d,  $J$  = 11.1 Hz, 1H), 4.27 (d,  $J$  = 9.5 Hz, 1H), 4.20 (ddt,  $J$  = 13.3, 5.0, 1.3 Hz, 1H), 4.16 – 4.11 (m, 1H), 4.08 – 4.03 (m, 1H), 4.02 – 3.95 (m, 2H), 3.94 – 3.87 (m, 2H), 3.82 (dd,  $J$  = 11.5, 1.2 Hz, 1H), 3.50 (s, 3H), 3.43 (d,  $J$  = 8.9 Hz, 1H), 3.32 (dd,  $J$  = 10.0, 3.6 Hz, 1H), 2.00 (s, 3H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.3, 169.4, 138.4, 138.4, 137.9, 136.0, 136.0, 135.7, 135.7, 133.7, 133.2, 133.0, 129.8, 129.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.1, 127.8(4C), 127.7, 127.7, 127.5, 127.5, 127.3, 127.3, 118.5, 98.1, 95.3, 80.1, 79.8, 77.9, 75.8, 75.2, 75.0, 75.0, 73.2, 72.5, 70.7, 69.0, 63.7, 61.9, 52.7, 27.0(3C), 20.9, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{55}\text{H}_{63}\text{N}_3\text{O}_{12}\text{Si}[\text{M}+\text{Na}]^+$ : 1008.4079, Found: 1008.4063.

**Compound 69f:** Eluent for purification: 25% ethyl acetate in *n*-hexane; thick syrup; yield 45 mg (76%) from 27 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +71.8°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3037, 2924, 2858, 2107, 1744, 1687, 1454, 1367, 1235, 1128, 1030, 746, 699;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.84 – 7.77 (m, 3H), 7.68 – 7.65 (m, 1H), 7.51 – 7.46 (m, 2H), 7.41 – 7.27 (m, 11H), 5.88 (dddd,  $J$  = 16.8, 10.3, 6.2, 5.1 Hz, 1H), 5.51 (d,  $J$  = 3.7 Hz, 1H), 5.32 (dq,  $J$  = 17.1, 1.4 Hz, 1H), 5.25 (dq,  $J$  = 10.4, 1.3 Hz, 1H), 5.11 (d,  $J$  = 3.6 Hz, 1H), 4.97 (d,  $J$  = 11.0 Hz, 1H), 4.94 – 4.87 (m, 4H), 4.82 (d,  $J$  = 11.1 Hz, 1H), 4.74 (d,  $J$  = 11.2 Hz, 1H), 4.31 – 4.28 (m, 1H), 4.27 – 4.19 (m,



3H), 4.18 – 4.10 (m, 2H), 4.03 (ddt,  $J = 12.9, 5.9, 1.2$  Hz, 1H), 3.95 (dd,  $J = 10.4, 8.4$  Hz, 1H), 3.78 (s, 3H), 3.63 (dt,  $J = 10.1, 2.6$  Hz, 1H), 3.57 (dd,  $J = 10.0, 8.4$  Hz, 1H), 3.34 (dd,  $J = 10.3, 3.7$  Hz, 1H), 1.99 (s, 3H), 1.87 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.8, 170.3, 169.4, 138.3, 137.8, 135.0, 133.3, 133.2, 133.2, 128.7, 128.7, 128.6, 128.6, 128.5, 128.2(3C), 128.1, 127.9, 127.8, 127.2, 127.2, 127.0, 126.4, 126.3, 126.0, 118.5, 98.0, 95.4, 80.4, 79.7, 77.2, 75.7, 75.6, 75.2, 75.1, 73.3, 70.4, 69.8, 69.0, 63.5, 62.3, 52.9, 20.9, 20.7; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{45}\text{H}_{49}\text{N}_3\text{O}_{13}$   $[\text{M}+\text{Na}]^+$ : 862.3163, Found: 862.3164.

**Compound 69g:** Eluent for purification: 30% ethyl acetate in *n*-hexane; thick syrup; yield 35 mg (68%) from 22 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +53.8°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2922, 2860, 2108, 1743, 1645, 1457, 1363, 1237, 1154, 1047, 747, 699;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.84 – 7.76 (m, 3H), 7.69 – 7.66 (m, 1H), 7.50 – 7.45 (m, 2H), 7.40 – 7.27 (m, 11H), 5.88 (dddd,  $J = 17.0, 10.6, 6.2, 5.3$  Hz, 1H), 5.50 (d,  $J = 3.7$  Hz, 1H), 5.32 (dq,  $J = 17.3, 1.6$  Hz, 1H), 5.24 (dd,  $J = 10.3, 1.3$  Hz, 1H), 5.11 (d,  $J = 3.5$  Hz, 1H), 4.99 – 4.93 (m, 2H), 4.92 – 4.87 (m, 3H), 4.81 (d,  $J = 11.1$  Hz, 1H), 4.76 (d,  $J = 11.2$  Hz, 1H), 4.30 – 4.20 (m, 4H), 4.16 – 4.10 (m, 2H), 4.05 – 3.99 (m, 1H), 3.94 (dd,  $J = 10.3, 8.1$  Hz, 1H), 3.77 (s, 3H), 3.66 – 3.56 (m, 2H), 3.35 (dd,  $J = 10.3, 3.7$  Hz, 1H), 2.68 – 2.59 (m, 2H), 2.52 – 2.38 (m, 2H), 2.13 (s, 3H), 1.99 (s, 3H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.5, 172.5, 170.3, 169.5, 138.3, 137.8, 135.2, 133.3, 133.2, 133.1, 128.7, 128.7, 128.6, 128.6, 128.4, 128.2, 128.2, 128.1, 128.1, 127.8, 127.8, 127.3, 127.3, 126.9, 126.3, 126.2, 126.0, 118.5, 98.0, 95.4, 80.3, 79.7, 77.4, 75.7, 75.6, 75.2, 75.1, 73.3, 70.4, 69.9, 69.0, 63.5, 62.4, 53.0, 37.9, 29.9, 27.8, 20.9; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{48}\text{H}_{53}\text{N}_3\text{O}_{14}$   $[\text{M}+\text{Na}]^+$ : 918.3425, Found: 918.3400.

**Compound 69h:** Eluent for purification: 8% ethyl acetate in *n*-hexane; Thick syrup; yield 70 mg (81%) from 35 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +29.5°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3036, 2926, 2859, 2108, 1731, 1459, 1267, 1103, 1038, 816, 745, 702;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.09 (dd,  $J = 8.3, 1.2$  Hz, 2H), 7.88 – 7.84 (m, 1H), 7.81 – 7.77 (m, 2H), 7.72 – 7.64 (m, 6H), 7.60 – 7.56 (m, 1H), 7.53 – 7.50 (m, 2H), 7.48 – 7.41 (m, 3H), 7.41 – 7.32 (m, 11H), 7.31 – 7.29 (m, 1H), 7.28 – 7.27 (m, 2H), 7.25 – 7.23 (m, 1H), 5.83 (dddd,  $J = 16.7, 10.3, 6.1, 5.0$  Hz, 1H), 5.51 (d,  $J = 3.6$  Hz, 1H), 5.29 (dq,  $J = 17.3, 1.6$  Hz, 1H), 5.25 (d,  $J = 3.6$  Hz, 1H), 5.22 (dd,  $J = 9.3, 3.6$  Hz, 1H), 5.16 (dd,  $J = 10.4, 1.4$  Hz, 1H), 5.06 (d,  $J = 11.2$  Hz, 1H), 4.96 (d,  $J = 10.4$  Hz, 2H), 4.90 (dd,  $J = 10.4, 3.7$  Hz, 3H), 4.41 (d,  $J = 9.4$  Hz, 1H), 4.37 – 4.32 (m, 1H), 4.24 (ddt,  $J = 13.1, 5.0, 1.5$  Hz, 1H), 4.16 (dd,  $J = 9.3, 8.5$  Hz, 1H), 4.06 – 4.00 (m, 2H), 3.99 – 3.95 (m, 2H), 3.89 (dd,  $J = 11.5, 1.4$  Hz, 1H), 3.56 (s, 3H), 3.54 (d,  $J = 7.9$  Hz, 1H), 3.39 – 3.33 (m, 1H), 1.08 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.5, 165.9, 138.0, 137.9, 136.0, 136.0, 135.9, 135.7, 135.7, 133.7, 133.5, 133.4, 133.3, 133.1, 133.0, 129.9, 129.9, 129.8, 129.8, 129.6, 128.7,

128.7, 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8(4C), 127.7(4C), 126.3, 126.2, 126.0, 125.7, 118.1, 98.2, 95.5, 80.2, 79.6, 78.0, 75.7, 75.3, 75.1, 75.0, 73.4, 72.5, 70.9, 69.1, 63.7, 62.1, 52.7, 27.1(3C), 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $C_{64}H_{67}N_3O_{12}Si[M+Na]^+$ : 1120.4392, Found: 1120.4384.

**Compound 69i:** ( $\alpha$ : $\beta$  ratio 4:1): Eluent for purification: 15% ethyl acetate in *n*-hexane; Thick syrup; yield 55 mg (75%) from 30 mg;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.5): +43.4; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3034, 2928, 2860, 2106, 1728, 1611, 1513, 1457, 1259, 1102, 1035, 819, 742, 701;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ ):  $\delta$  8.08 – 8.03 (m, 2H), 7.69 – 7.60 (m, 4H), 7.59 – 7.54 (m, 1H), 7.46 – 7.30 (m, 14H), 7.22 (d,  $J$  = 9.2 Hz, 4H), 7.17 – 7.09 (m, 2H), 6.87 – 6.79 (m, 2H), 5.80 (dddd,  $J$  = 17.0, 10.8, 6.1, 5.0 Hz, 1H), 5.46 (d,  $J$  = 3.7 Hz, 1H), 5.30 – 5.21 (m, 2H), 5.20 – 5.12 (m, 2H), 4.92 (d,  $J$  = 10.7 Hz, 1H), 4.89 – 4.84 (m, 2H), 4.84 – 4.78 (m, 2H), 4.68 (d,  $J$  = 10.4 Hz, 1H), 4.36 (d,  $J$  = 9.4 Hz, 1H), 4.31 (dd,  $J$  = 9.3, 8.4 Hz, 1H), 4.24 – 4.18 (m, 1H), 4.12 (dd,  $J$  = 9.4, 8.3 Hz, 1H), 4.04 – 3.93 (m, 2H), 3.89 (dd,  $J$  = 16.7, 8.5 Hz, 2H), 3.84 – 3.77 (m, 4H), 3.58 (s, 3H), 3.45 (d,  $J$  = 9.4 Hz, 1H), 3.30 (dd,  $J$  = 9.9, 3.6 Hz, 1H), 1.05 (s, 9H);  $^{13}C$  NMR (100.67 MHz,  $CDCl_3$ ):  $\delta$  169.4, 165.9, 159.3, 138.1, 137.9, 136.1, 136.1, 135.8, 135.8, 133.8, 133.5, 133.3, 133.1, 130.6, 129.9, 129.9, 129.8, 129.8, 129.6, 129.3, 129.3, 128.7(3C), 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 127.8(3C), 127.7(4C), 118.1, 113.9, 113.9, 98.2, 95.5, 80.1, 79.6, 77.8, 75.7, 75.3, 75.0, 74.8, 73.4, 72.6, 70.9, 69.1, 63.7, 62.0, 55.4, 52.7, 27.1(3C), 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $C_{61}H_{67}N_3O_{13}Si[M+Na]^+$ : 1100.4341, Found: 1100.4325.

**Compound 69j:** Eluent for purification: 7% ethyl acetate in *n*-hexane; yield 686 mg (85%) from 340 mg;  **$\alpha$  isomer:** thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c$ 1.0): +6.9°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3036, 2933, 2862, 2108, 1741, 1462, 1356, 1265, 1107, 1051, 871, 744, 699;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ ):  $\delta$  8.10 (dd,  $J$  = 8.3, 1.3 Hz, 2H), 7.72 – 7.65 (m, 4H), 7.59 (tt,  $J$  = 7.0, 1.3 Hz, 1H), 7.48 – 7.30 (m, 17H), 7.28 – 7.26 (m, 2H), 7.26 – 7.20 (m, 4H), 5.83 (dddd,  $J$  = 16.6, 10.5, 6.1, 5.1 Hz, 1H), 5.50 (d,  $J$  = 3.7 Hz, 1H), 5.29 (dq,  $J$  = 17.3, 1.6 Hz, 1H), 5.25 (d,  $J$  = 3.5 Hz, 1H), 5.22 (dd,  $J$  = 9.3, 3.6 Hz, 1H), 5.16 (dq,  $J$  = 10.4, 1.3 Hz, 1H), 4.98 – 4.84 (m, 5H), 4.80 (d,  $J$  = 11.1 Hz, 1H), 4.41 (d,  $J$  = 9.4 Hz, 1H), 4.38 – 4.32 (m, 1H), 4.25 (ddt,  $J$  = 13.2, 5.0, 1.5 Hz, 1H), 4.16 (dd,  $J$  = 9.3, 8.5 Hz, 1H), 4.06 – 3.99 (m, 2H), 3.98 – 3.89 (m, 2H), 3.87 (dd,  $J$  = 11.5, 1.4 Hz, 1H), 3.56 (s, 3H), 3.51 (d,  $J$  = 9.1 Hz, 1H), 3.34 (dd,  $J$  = 10.0, 3.7 Hz, 1H), 1.08 (s, 9H);  $^{13}C$  NMR (100.67 MHz,  $CDCl_3$ ):  $\delta$  169.4, 165.8, 138.4, 138.0, 137.9, 136.0, 136.0, 135.7, 135.7, 133.7, 133.5, 133.3, 133.0, 129.9, 129.9, 129.8, 129.8, 129.6, 128.7, 128.7, 128.6, 128.6, 128.5(4C), 128.3, 128.3, 128.1, 127.8(4C), 127.7(4C), 127.5, 127.5, 118.1, 98.2, 95.5, 80.1, 79.6, 77.9, 75.7, 75.3, 75.0, 75.0, 73.4, 72.5, 70.9, 69.1, 63.7, 62.0, 52.7, 27.0(3C), 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $C_{60}H_{65}N_3O_{12}Si[M+Na]^+$ : 1070.4235, Found: 1070.4225.  **$\beta$**



**isomer:** thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +48.9°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3033, 2926, 2860, 2111, 1731, 1456, 1268, 1105, 1057, 748, 701;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.93 (dd,  $J$  = 8.3, 1.3 Hz, 2H), 7.74 – 7.70 (m, 2H), 7.64 (dd,  $J$  = 8.0, 1.3 Hz, 2H), 7.57 (tt,  $J$  = 7.1, 1.3 Hz, 2H), 7.44 – 7.27 (m, 16H), 7.15 – 7.02 (m, 7H), 5.87 – 5.76 (m, 1H), 5.27 (dq,  $J$  = 17.2, 1.6 Hz, 1H), 5.23 (d,  $J$  = 3.7 Hz, 1H), 5.18 (dd,  $J$  = 9.5, 3.8 Hz, 1H), 5.14 (dq,  $J$  = 10.4, 1.3 Hz, 1H), 4.90 – 4.81 (m, 3H), 4.77 – 4.66 (m, 3H), 4.46 – 4.39 (m, 2H), 4.33 (dd,  $J$  = 9.6, 8.5 Hz, 1H), 4.26 (ddt,  $J$  = 13.2, 5.0, 1.5 Hz, 1H), 4.16 (dd,  $J$  = 9.4, 8.5 Hz, 1H), 4.03 (ddt,  $J$  = 13.3, 6.1, 1.3 Hz, 1H), 3.89 – 3.77 (m, 6H), 3.47 – 3.35 (m, 2H), 3.17 (dq,  $J$  = 9.4, 1.7 Hz, 1H), 1.01 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.0, 165.8, 138.6, 138.1, 138.0, 136.0, 136.0, 135.8, 135.8, 133.3(3C), 132.9, 130.0, 130.0, 129.9, 129.8, 129.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.1(3C), 128.0, 128.0, 127.9(3C), 127.8, 127.8, 127.5, 127.5, 127.2, 118.0, 101.3, 95.7, 83.5, 78.2, 77.4, 77.0, 76.2, 75.9, 75.1, 74.3, 72.8, 70.3, 69.0, 67.2, 62.2, 52.9, 27.0(3C), 19.3; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{60}\text{H}_{65}\text{N}_3\text{O}_{12}\text{Si}[\text{M}+\text{Na}]^+$ : 1070.4235, Found: 1070.4233.

138.6  
137.4  
133.5  
129.1  
128.5  
128.4  
128.1  
127.8  
126.1  
118.4  
  
101.4  
98.1  
  
82.0  
79.1  
75.0  
72.5  
69.1  
68.9  
62.9

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

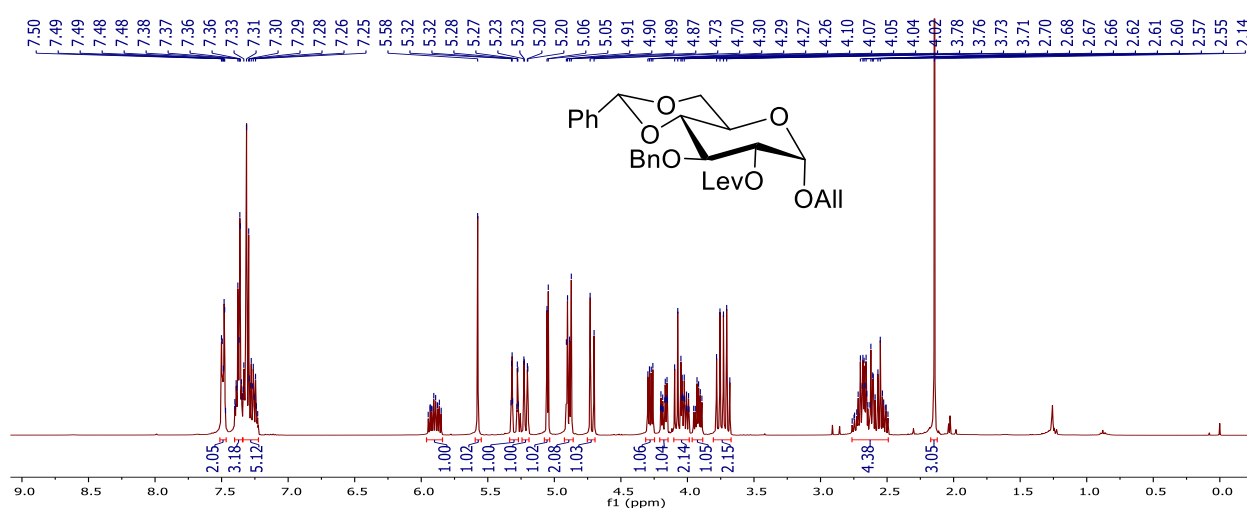
f1 (ppm)

133.5  
129.1  
128.5  
128.4  
128.1  
127.8  
126.1  
118.4

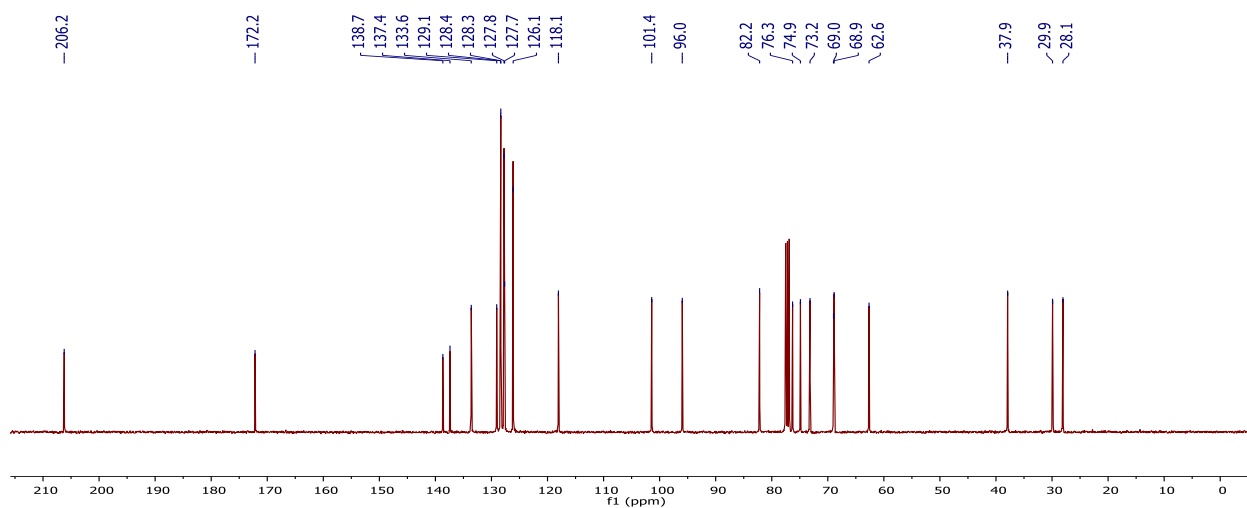
101.4  
98.1  
82.0  
79.1  
75.0  
72.5  
69.1  
68.9  
62.9

f1 (ppm)

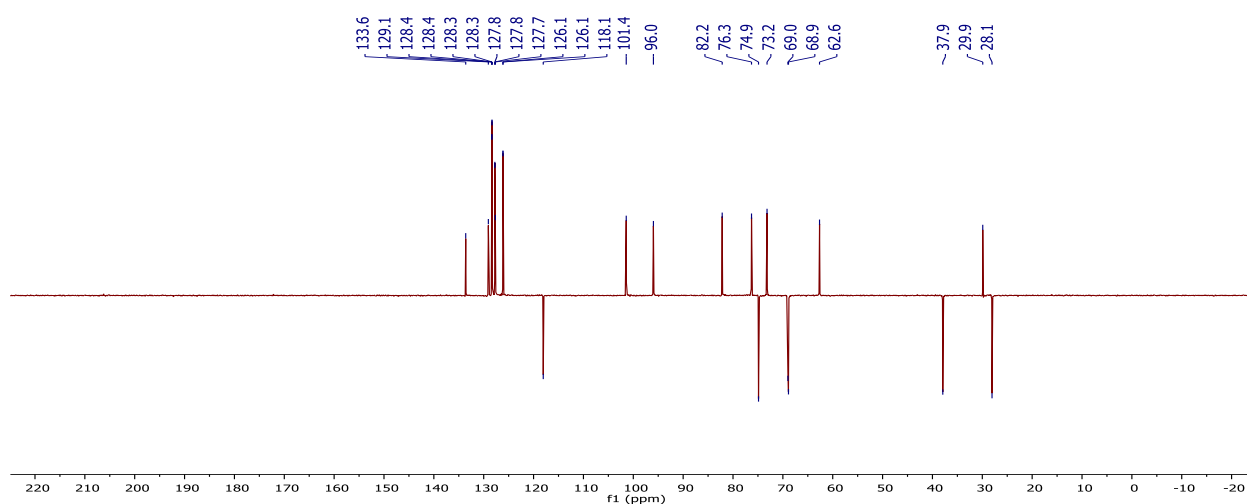
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 51a**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 51a**

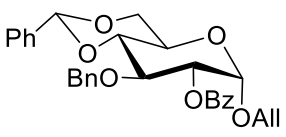


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 51a**



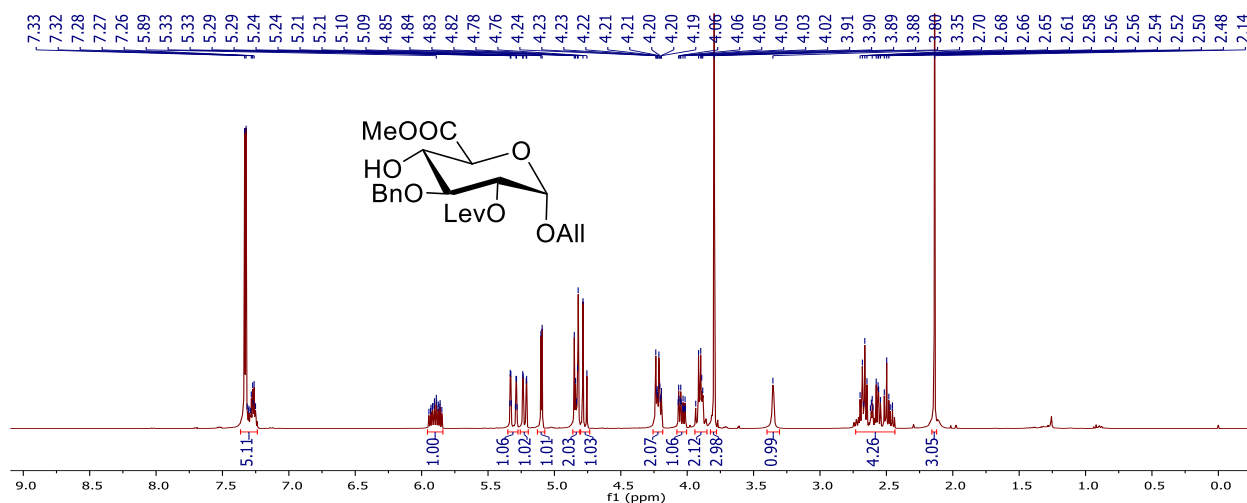
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— — — — —

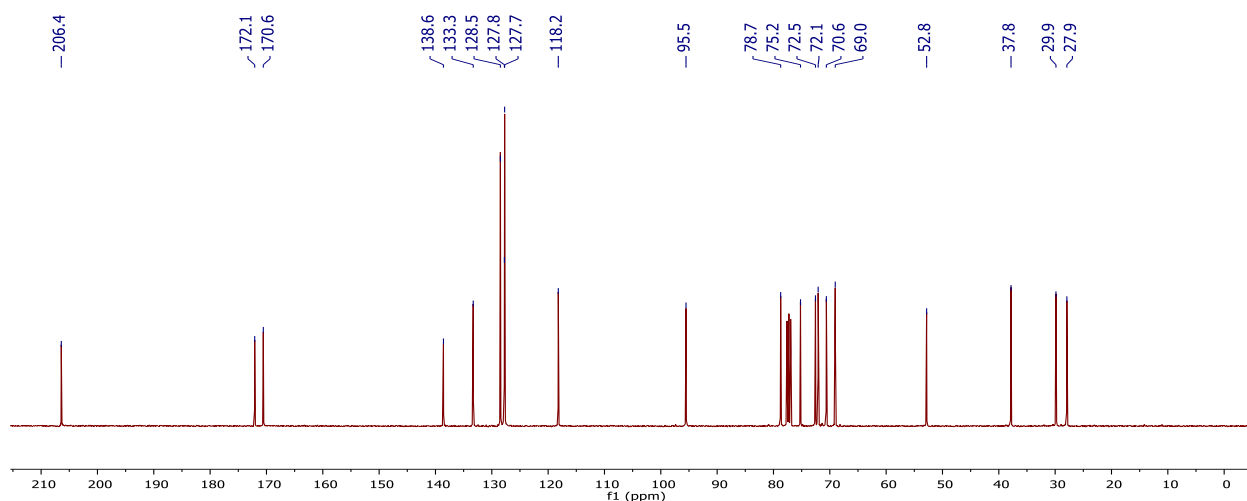


— — — — —

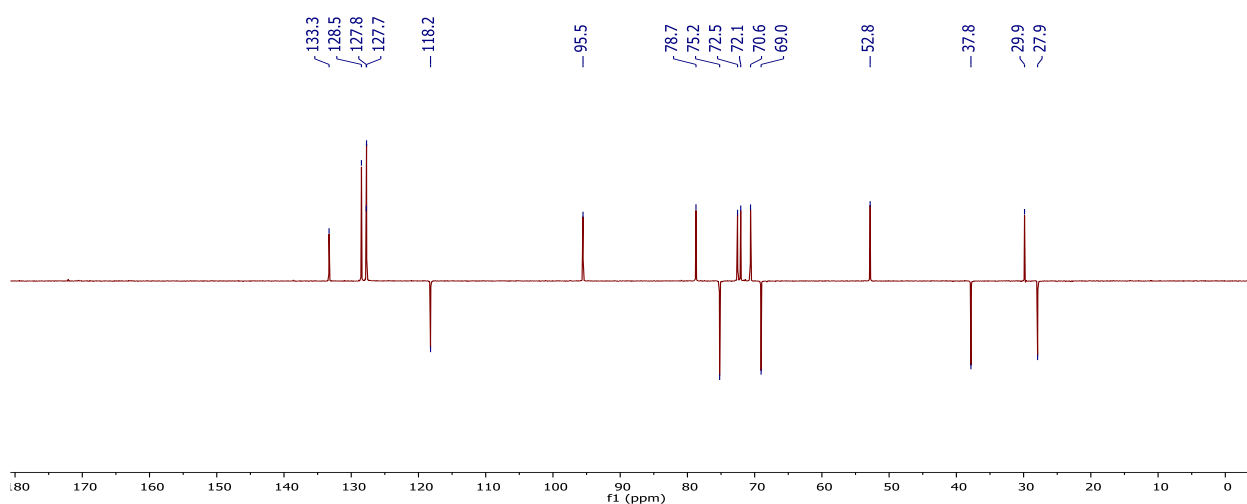
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 53a**



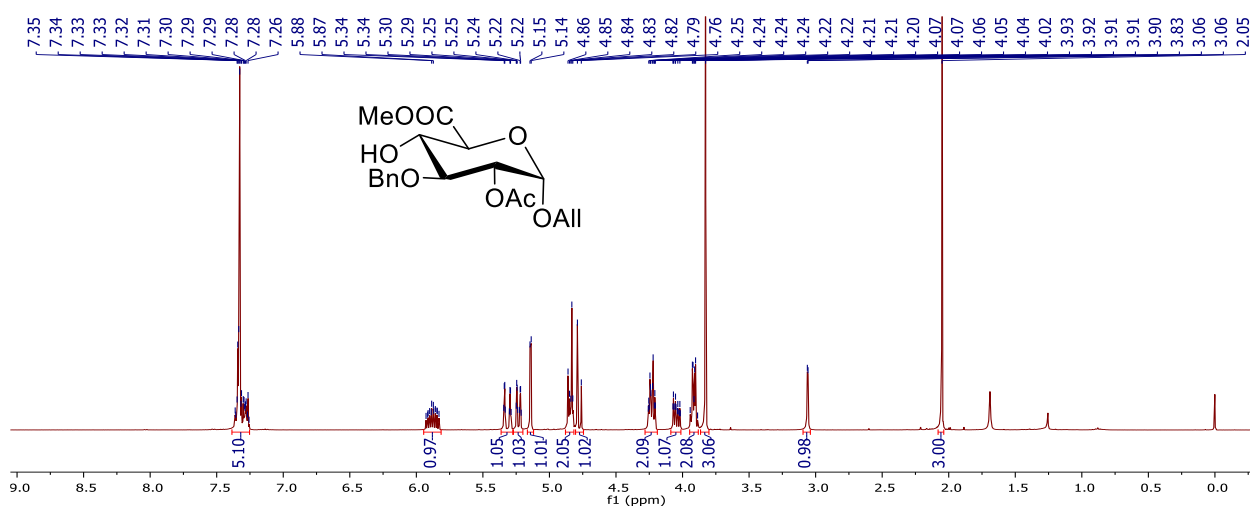
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 53a**



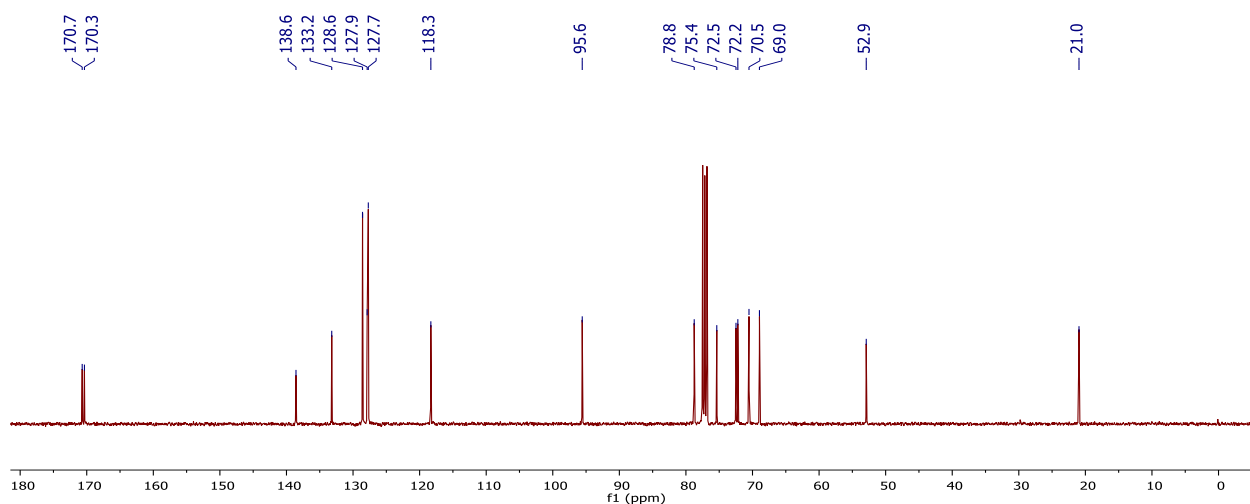
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 53a**



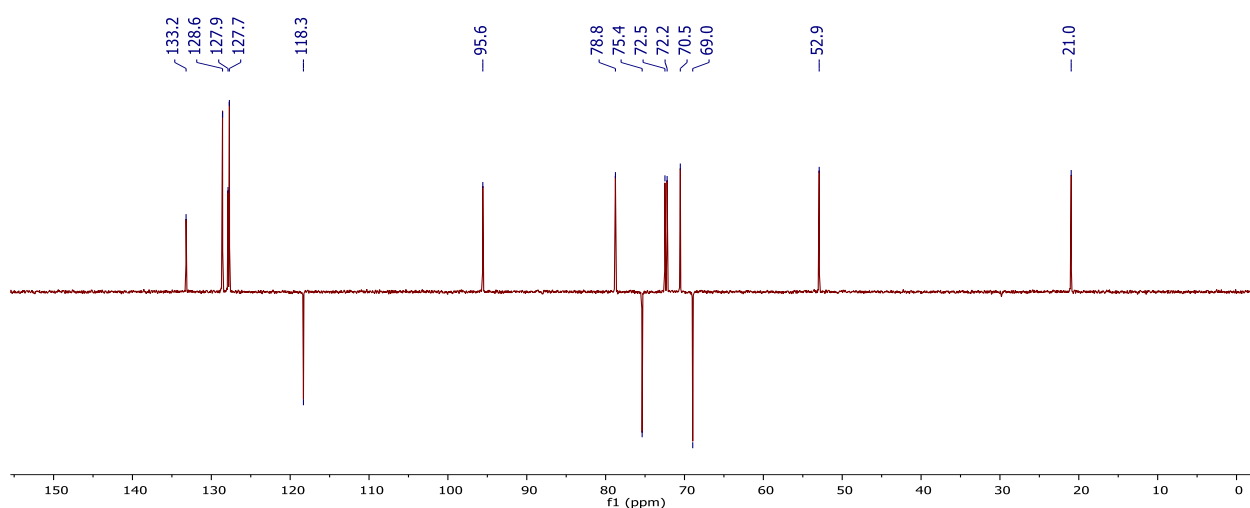
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 53b**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 53b**

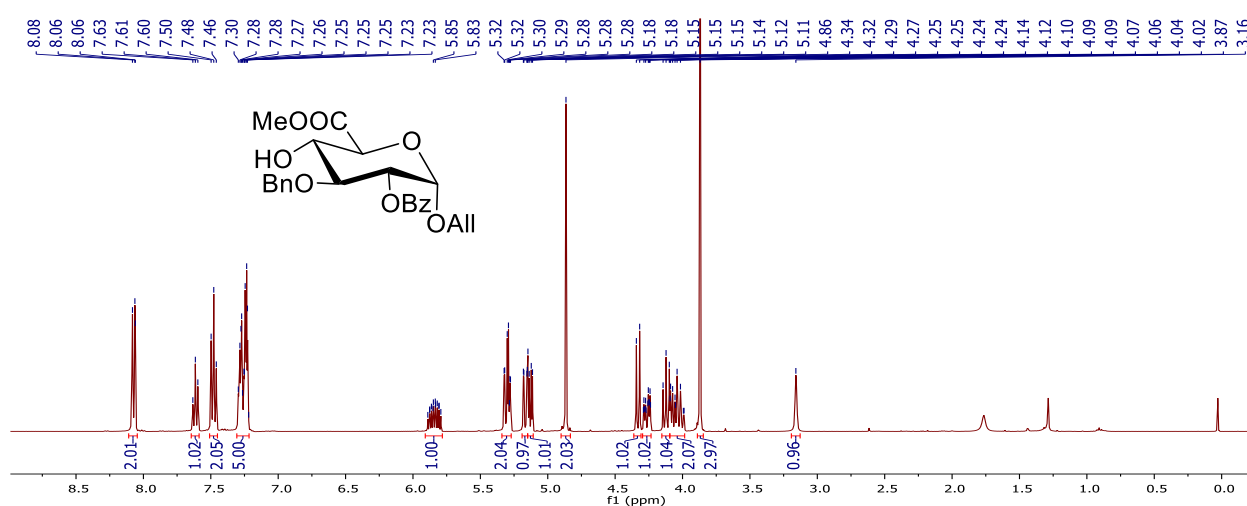


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 53b**

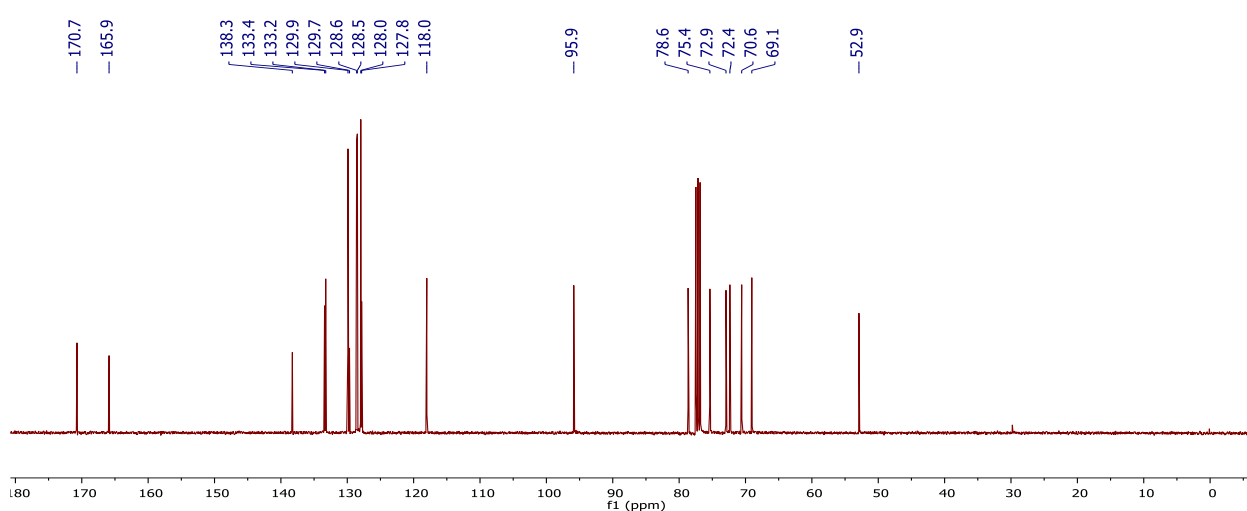


## Donor-Acceptor Selectivity Studies with GlcA and GlcN Building Blocks

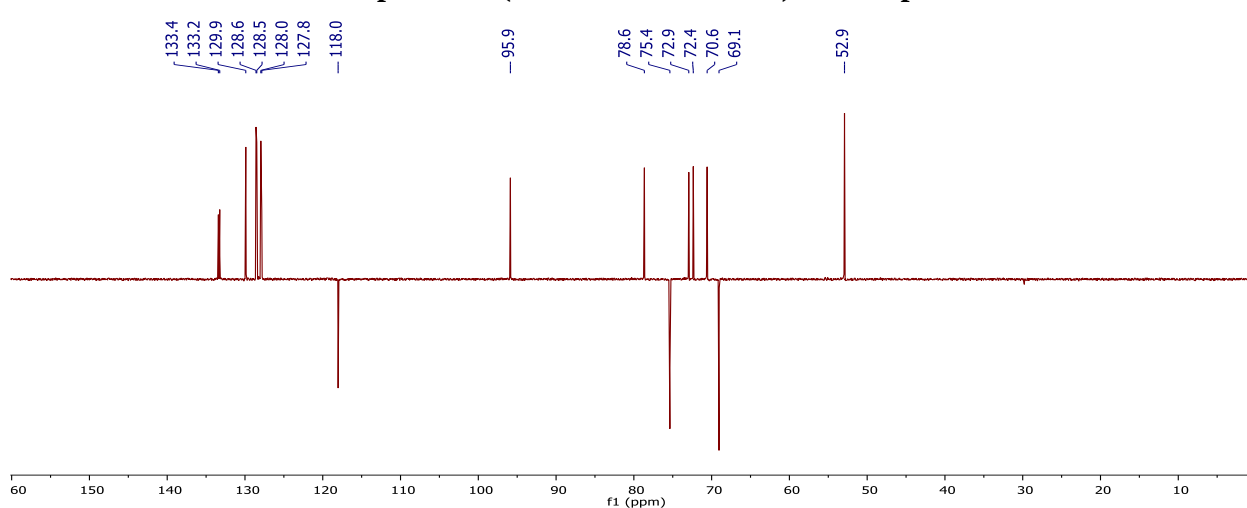
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 53c**



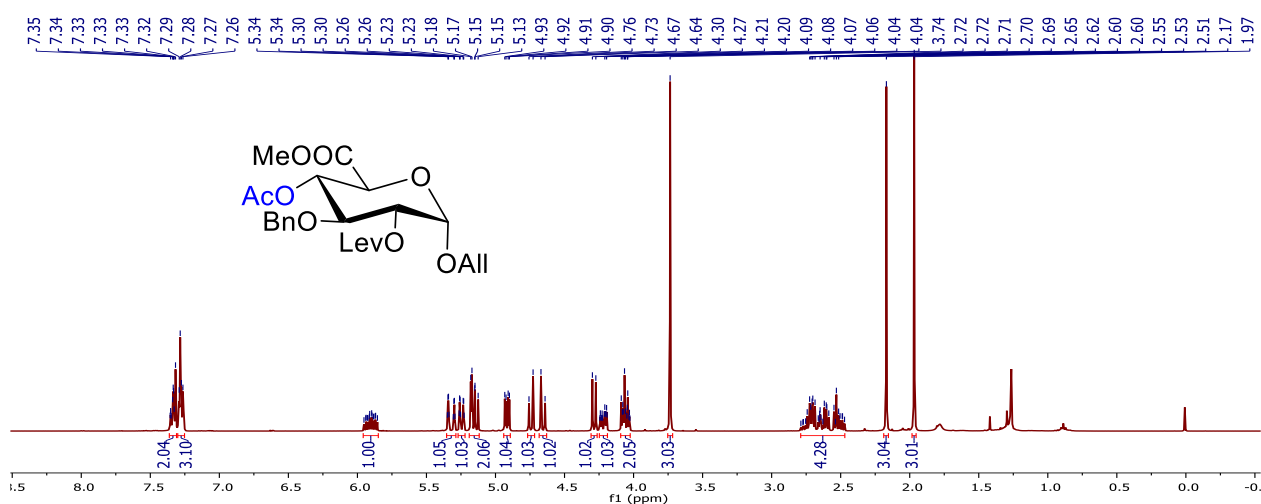
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 53c**



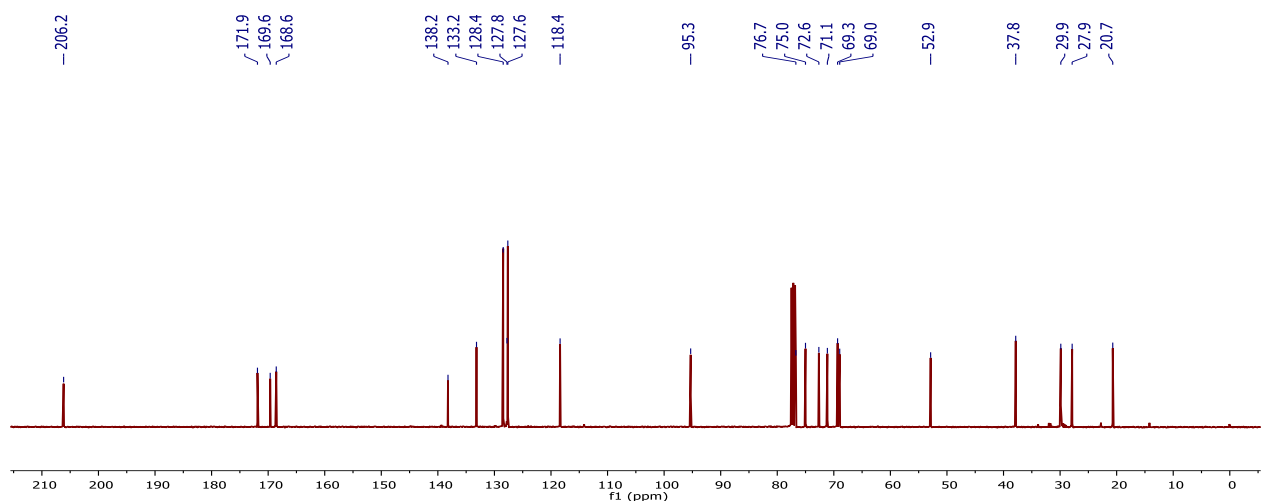
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 53c**



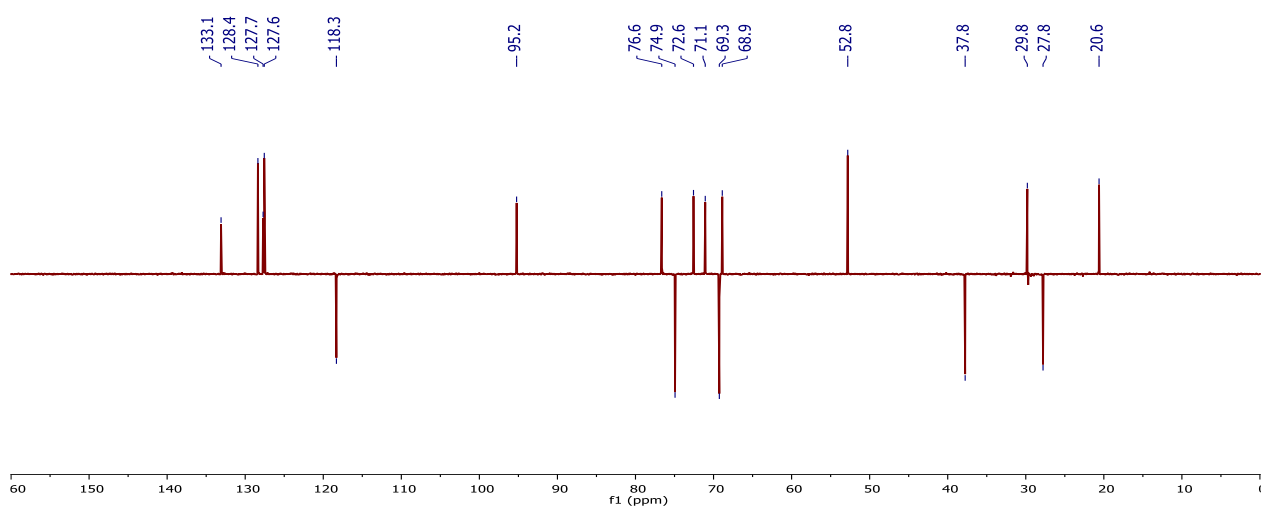
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 54a**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 54a**

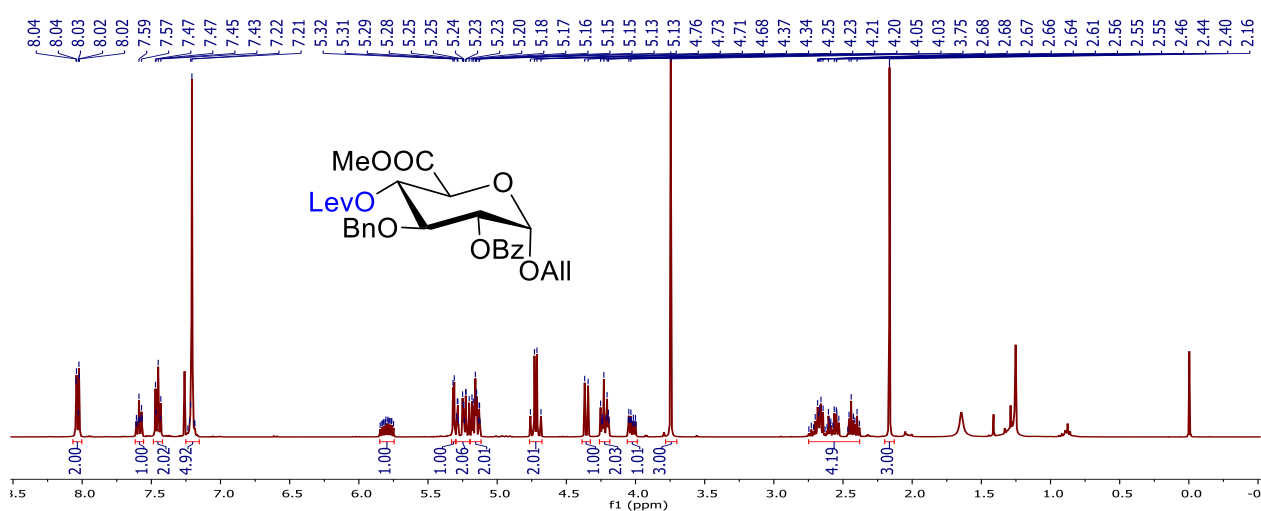


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 54a**

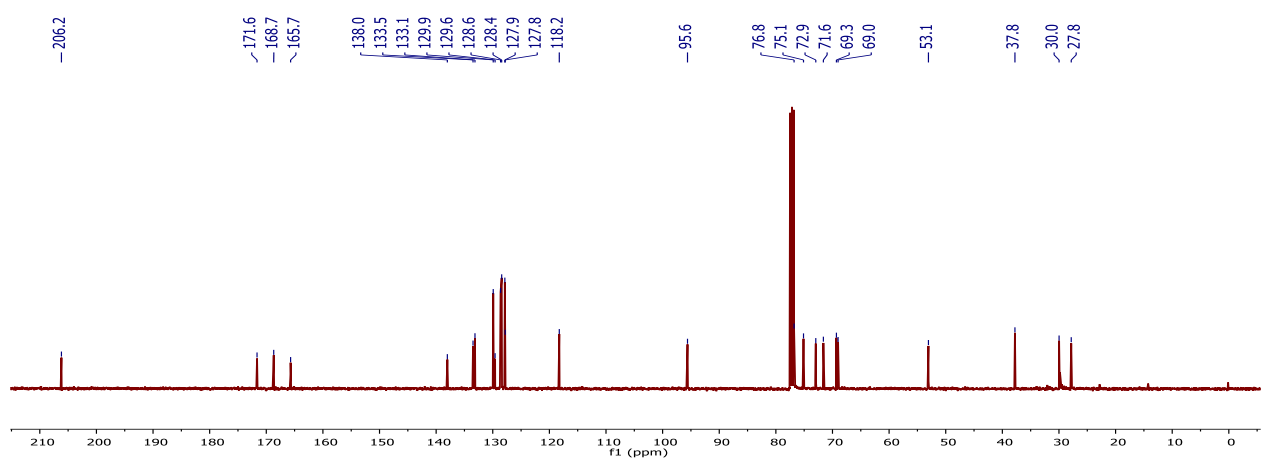




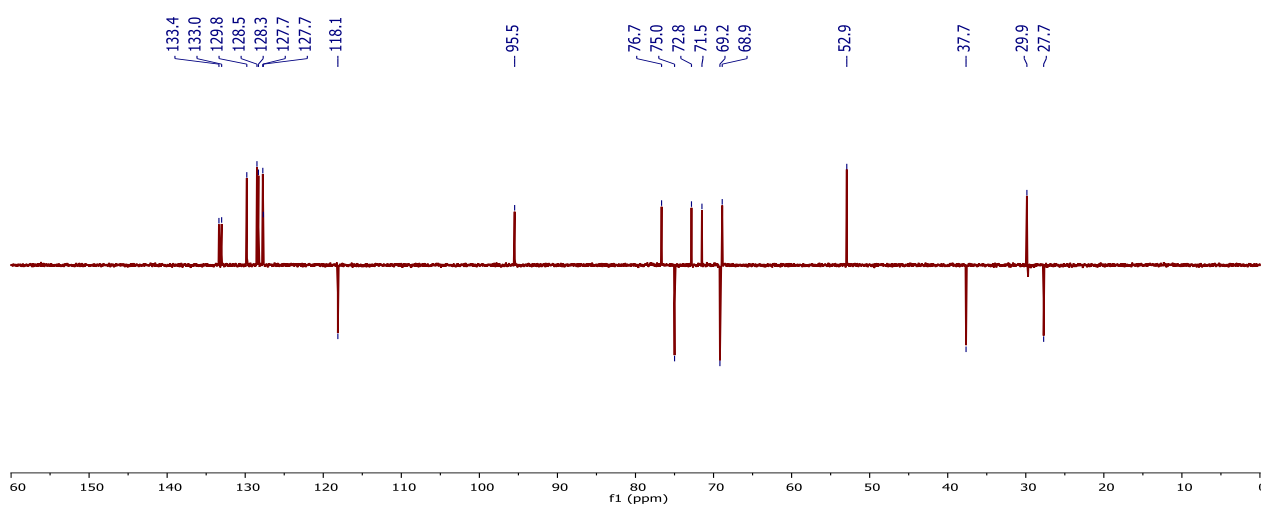
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 54b**



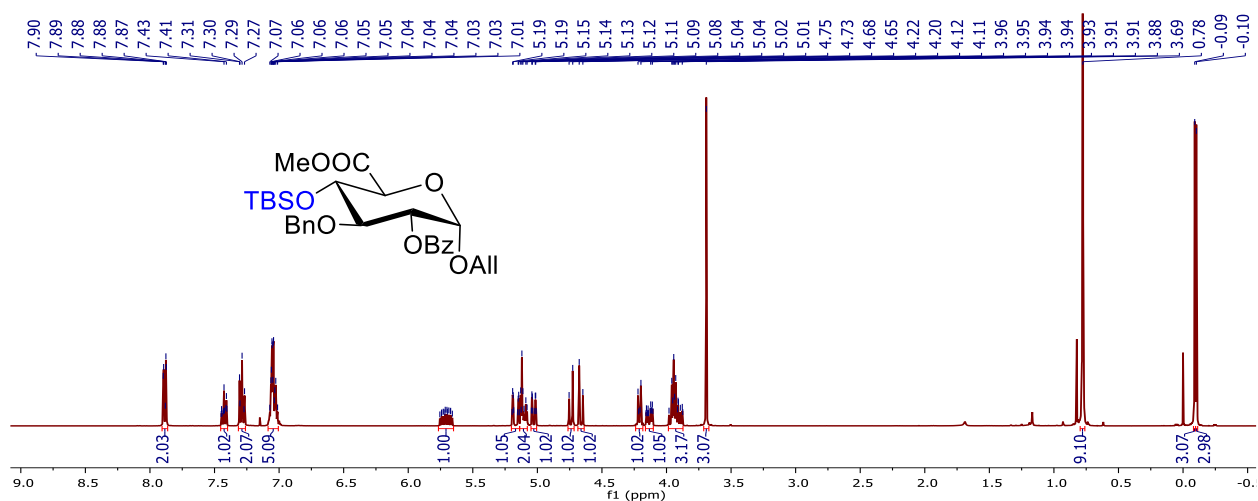
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 54b**



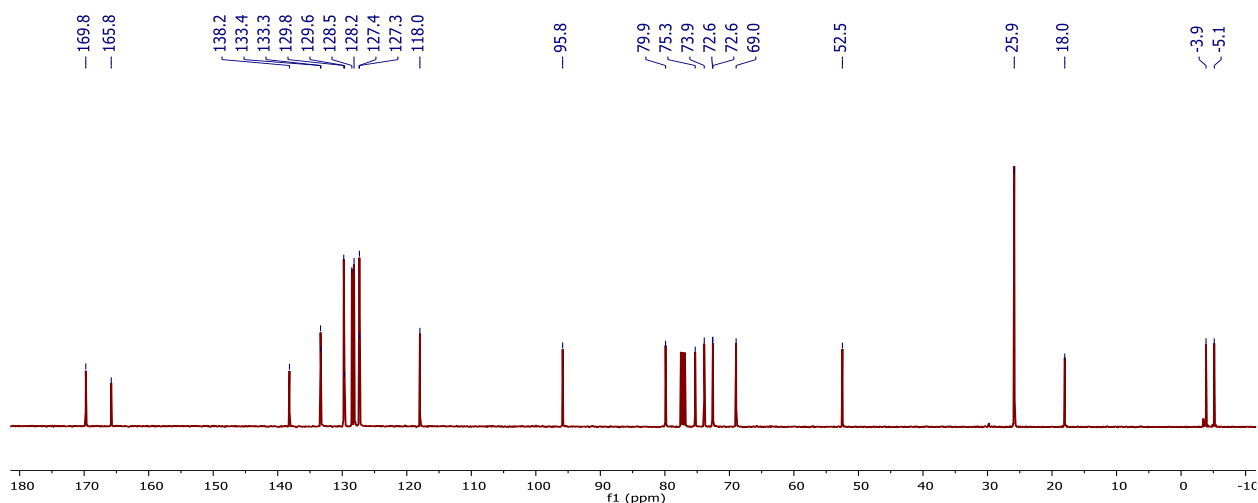
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 54b**



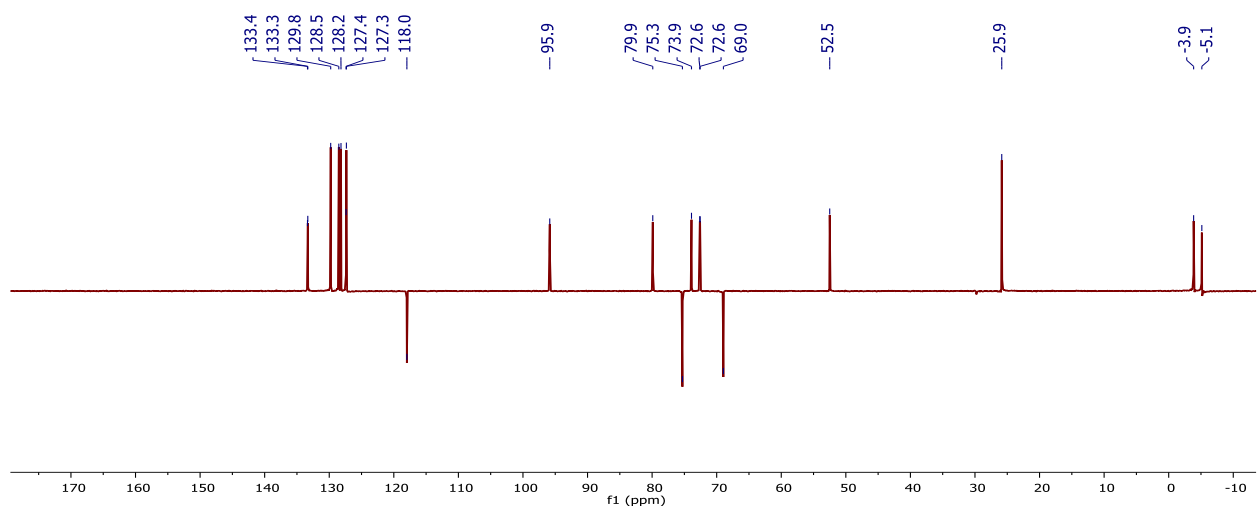
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 54c**



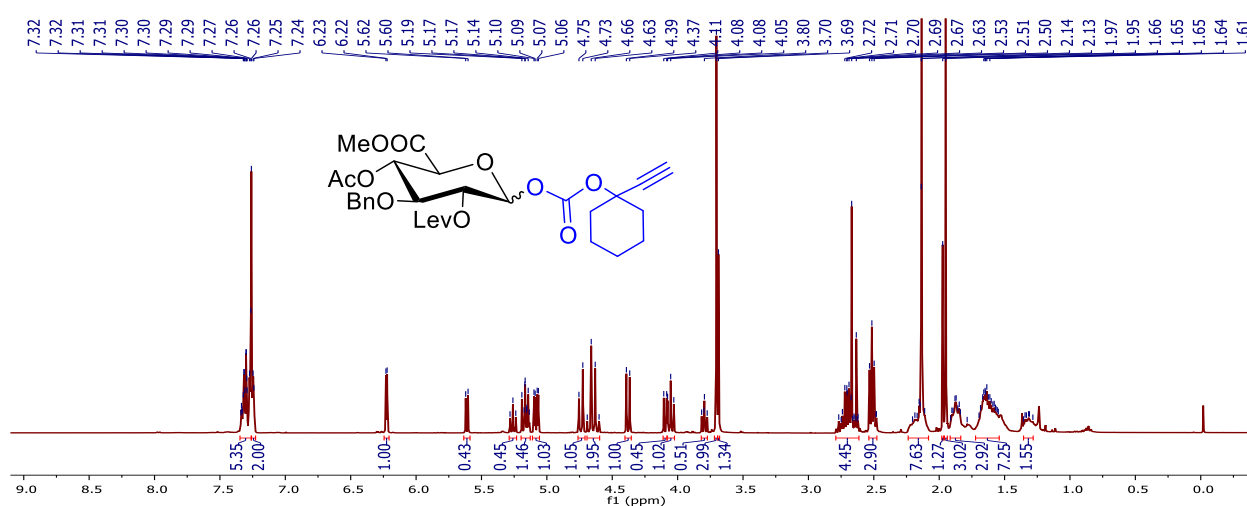
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 54c**



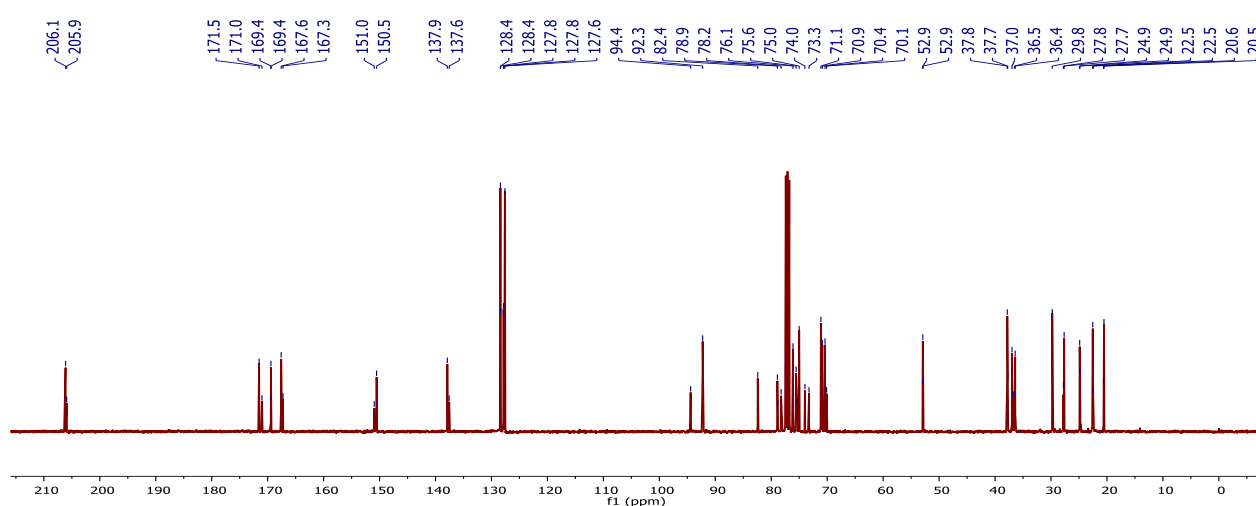
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 54c**



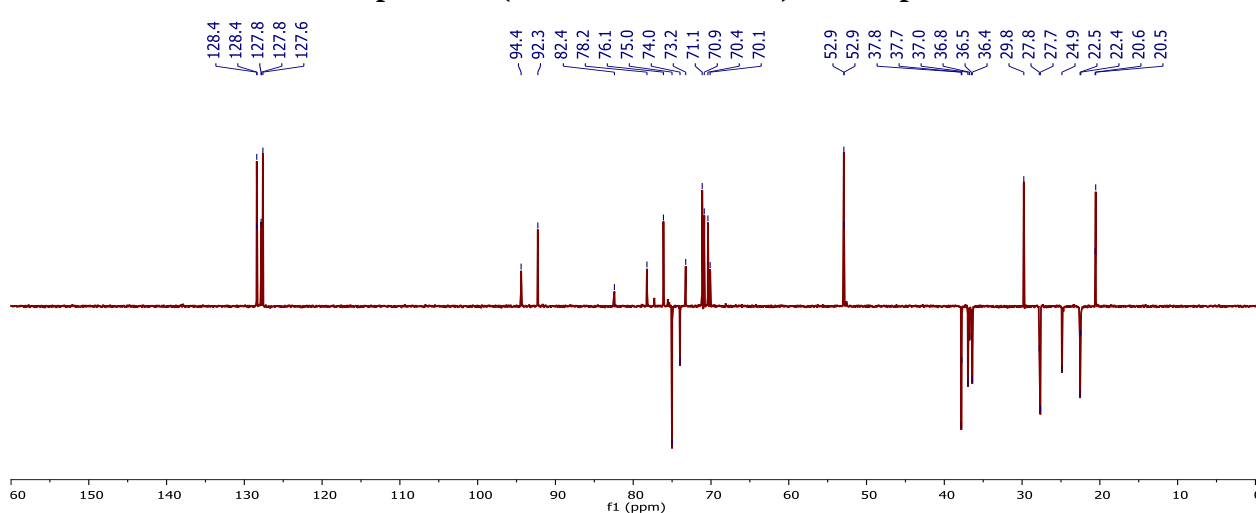
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 56a**



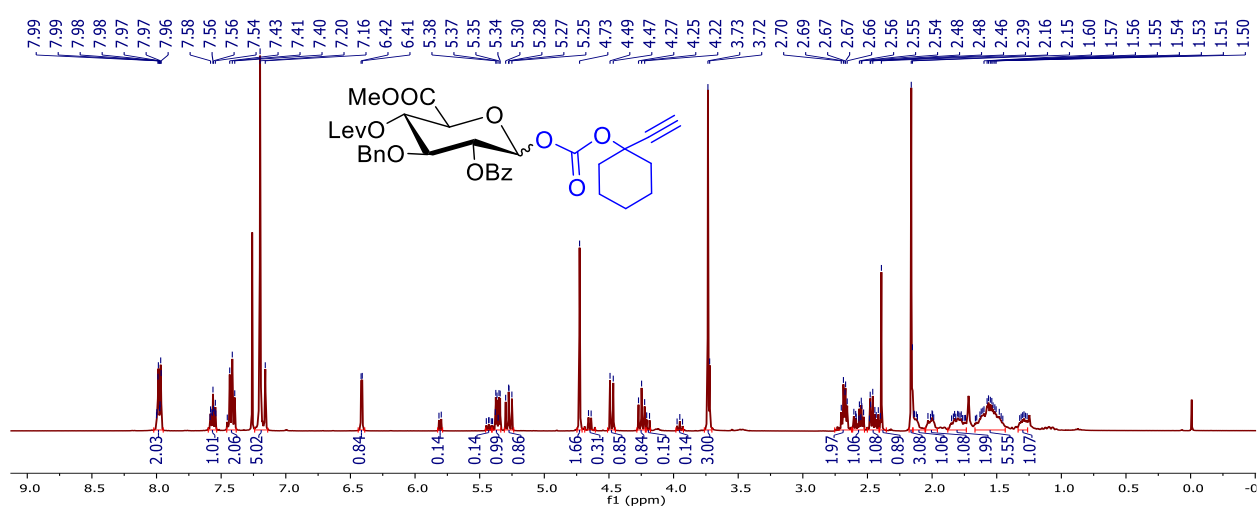
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 56a**



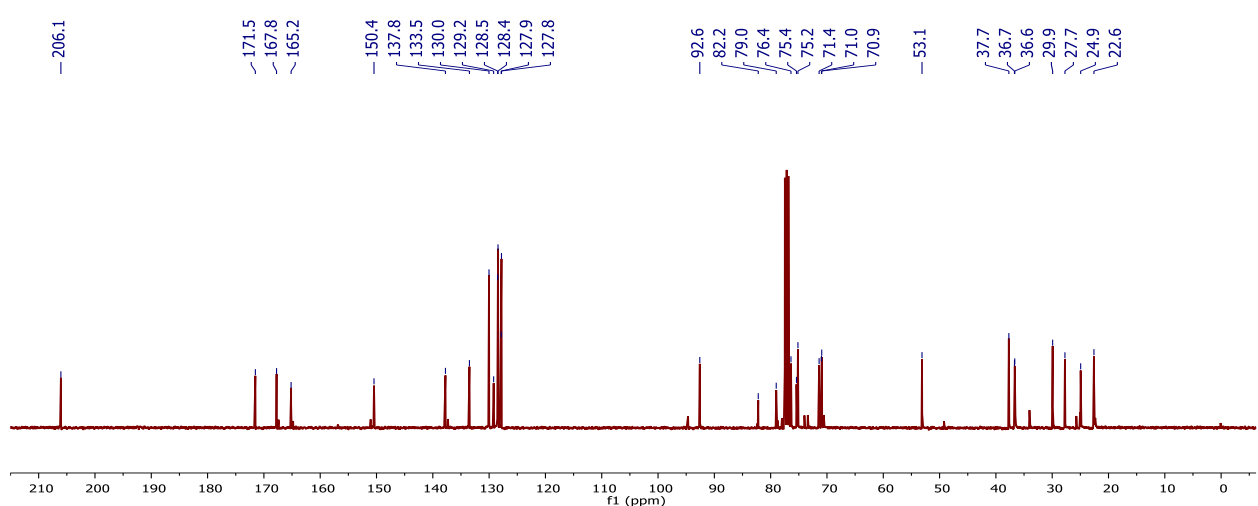
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 56a**



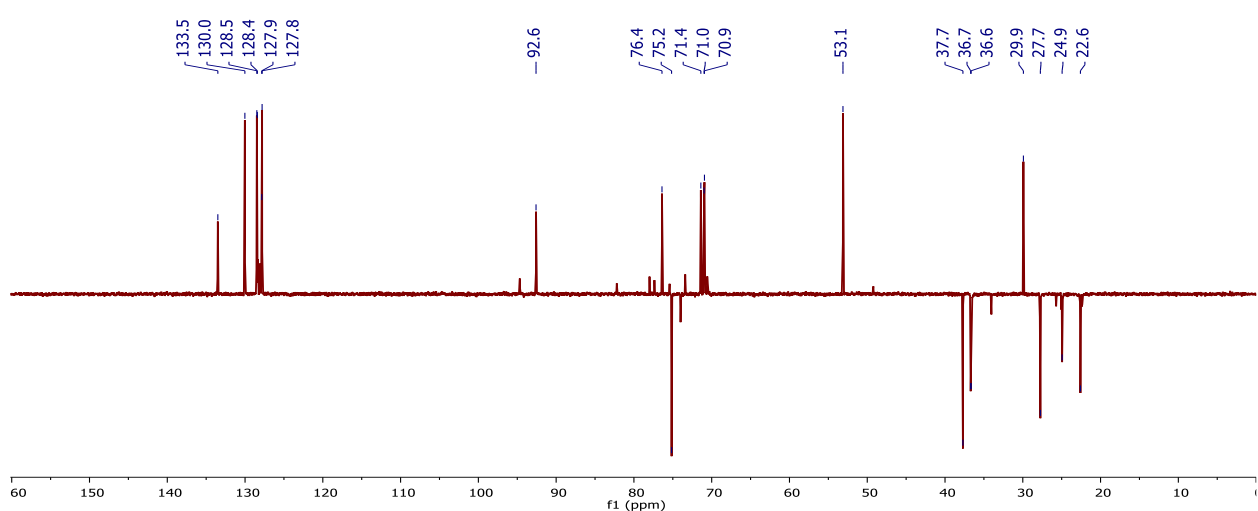
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 56b**



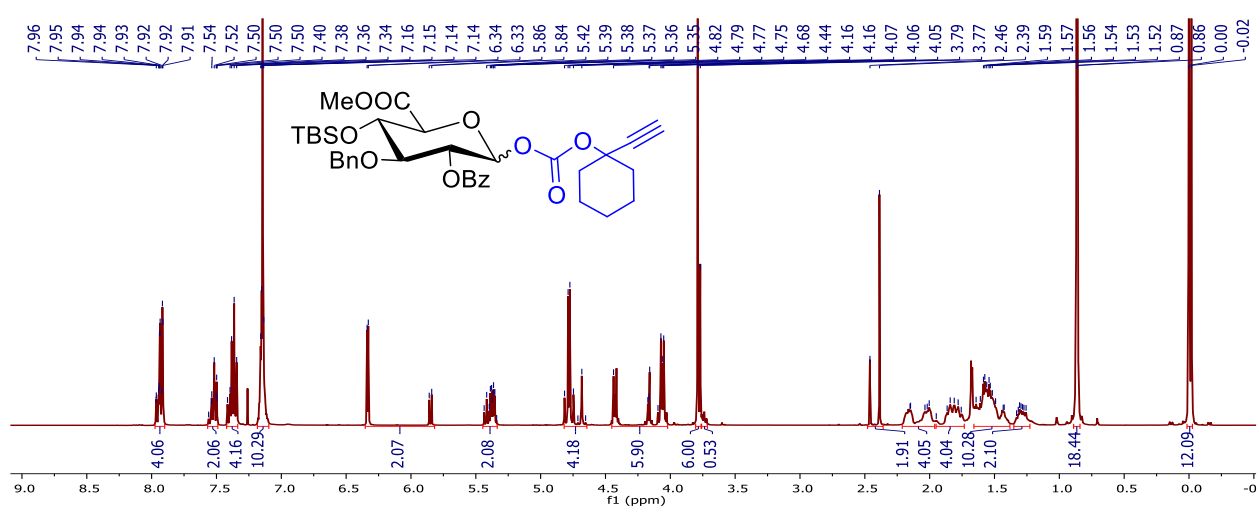
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 56b**



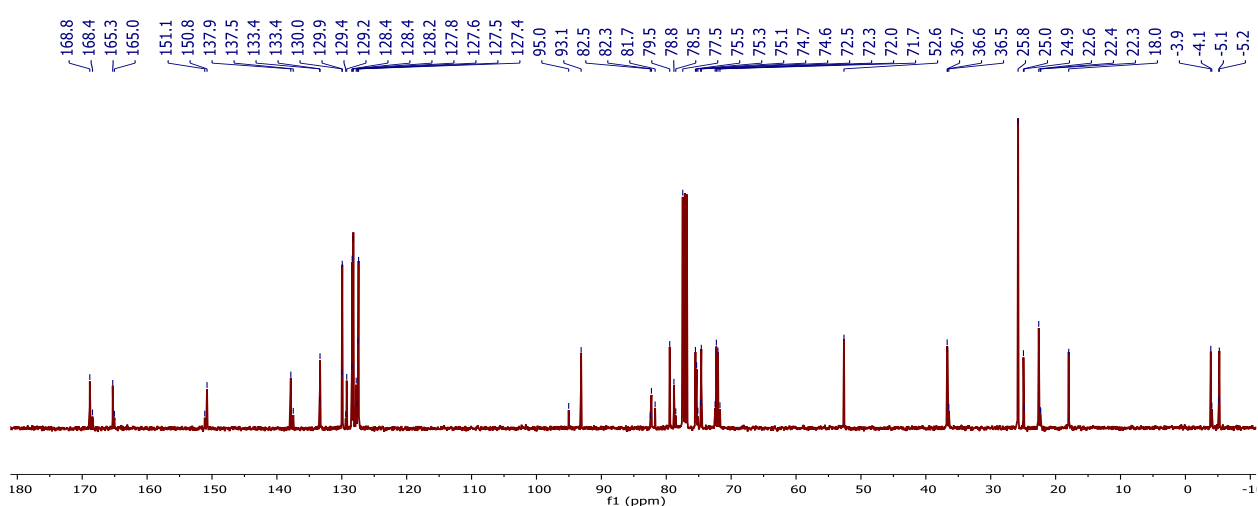
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 56b**



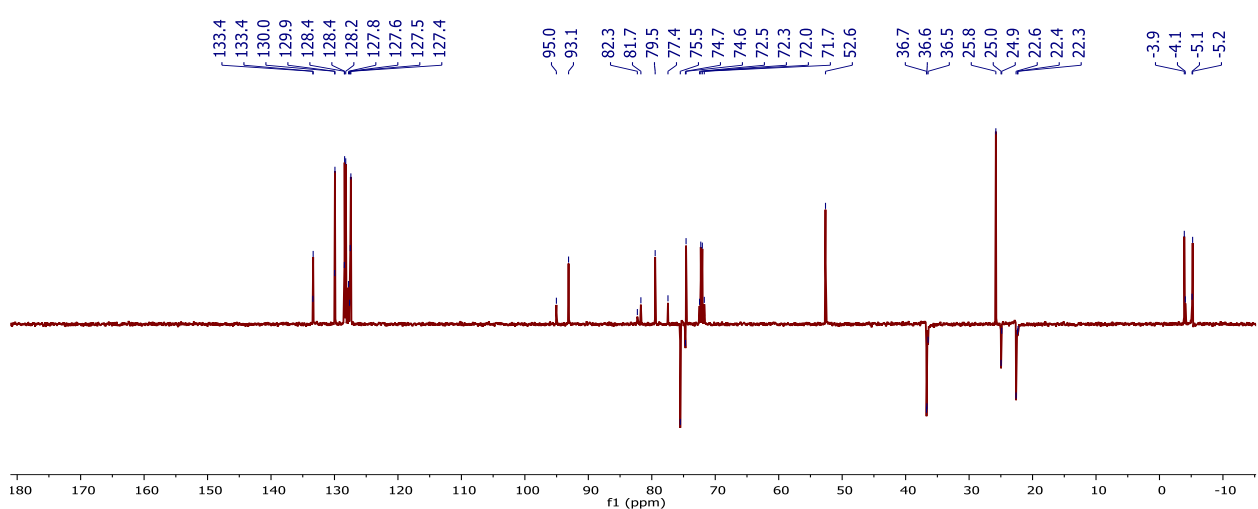
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 56c**



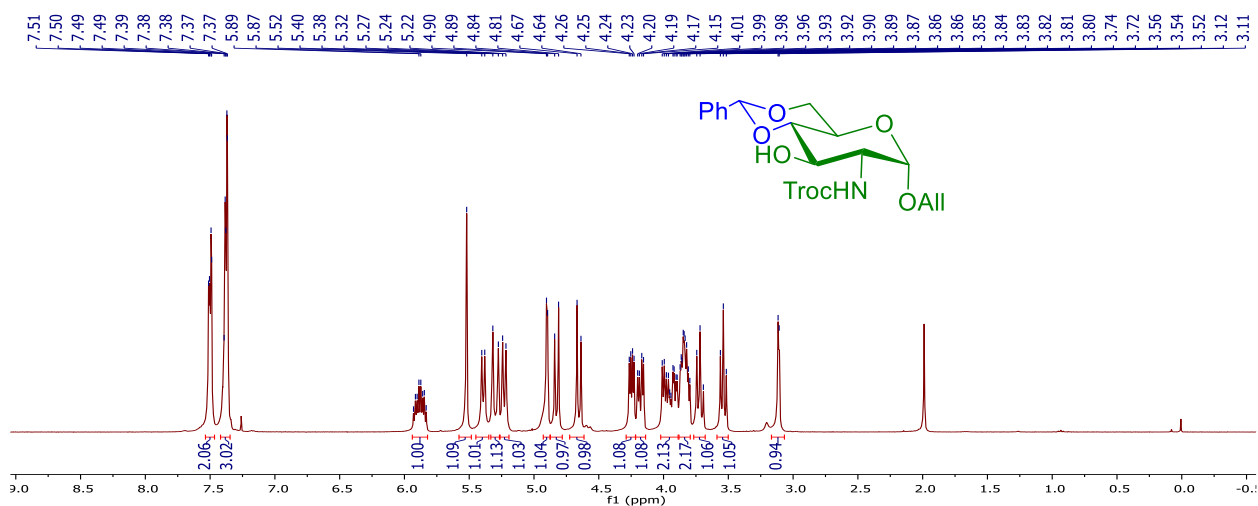
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 56c**



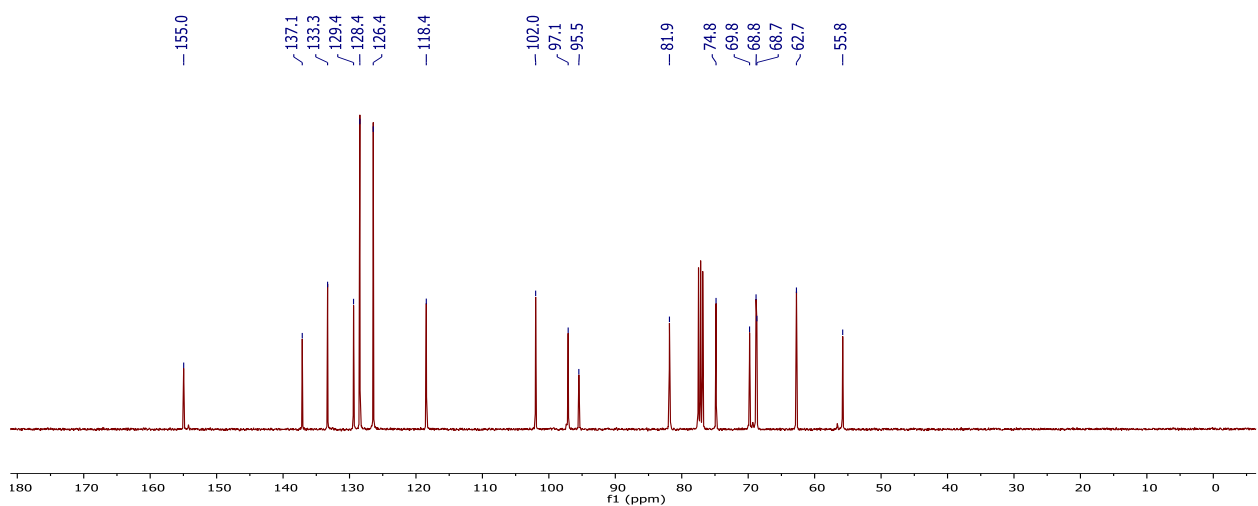
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 56c**



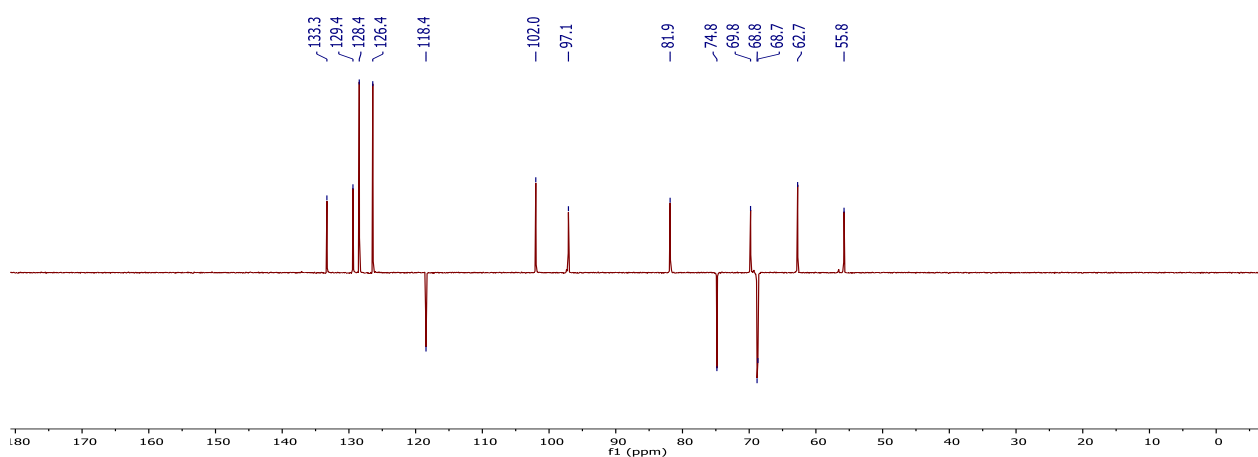
## <sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 59

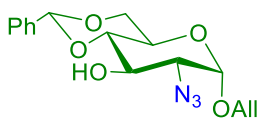


## <sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 59



## DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 59

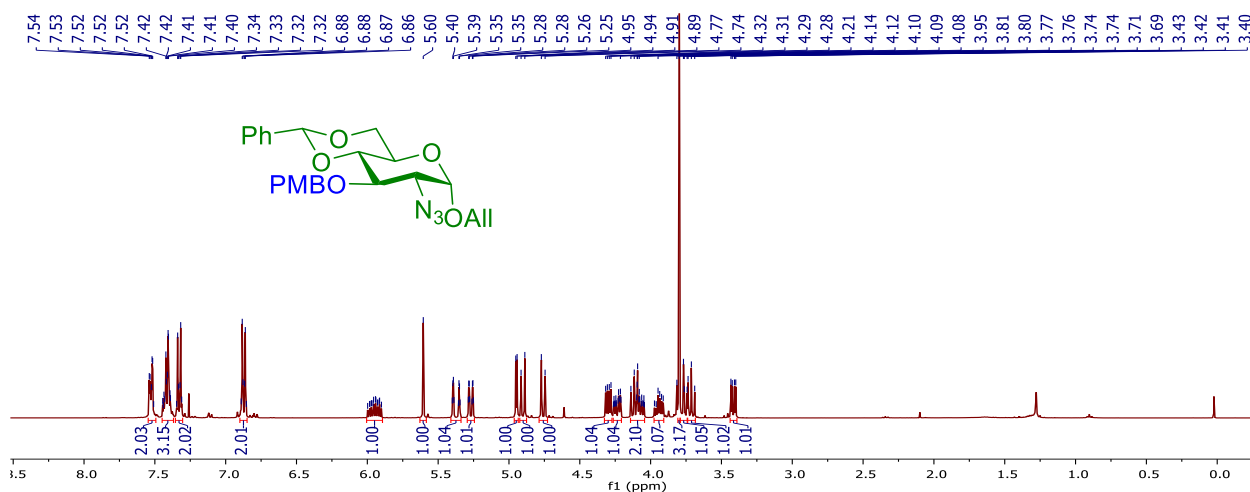




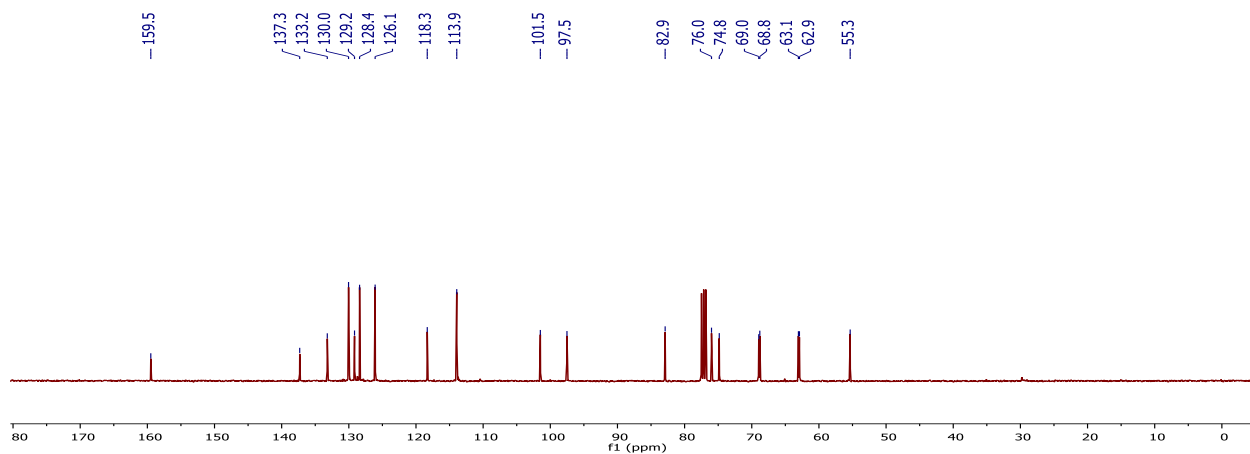
*Journal of Management Inquiry* 20(6) 798–814



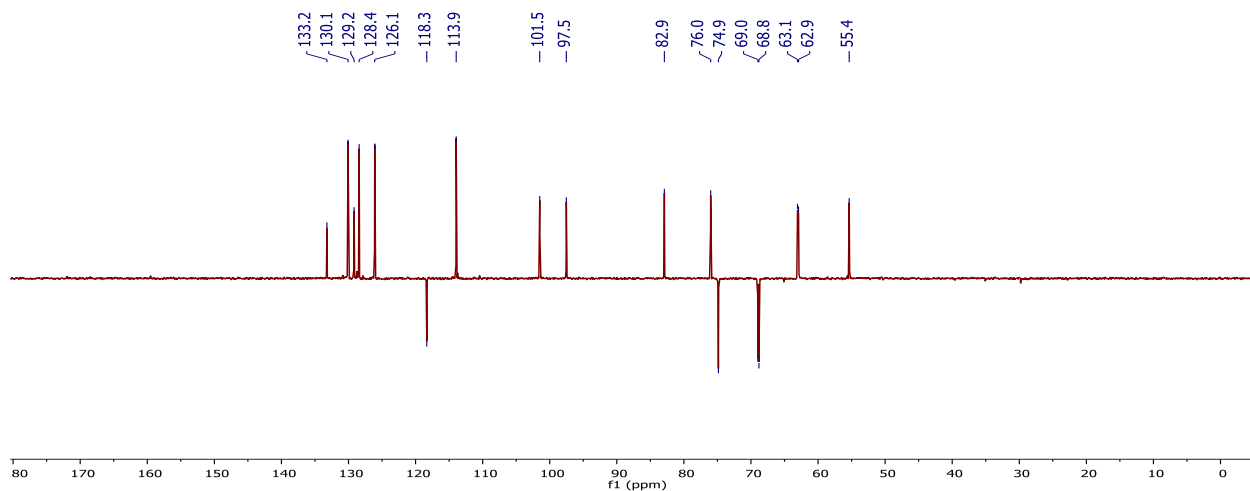
**<sup>1</sup>H NMR Spectrum (399.78 MHz, CDCl<sub>3</sub>) of Compound 62a**



**<sup>13</sup>C NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 62a**

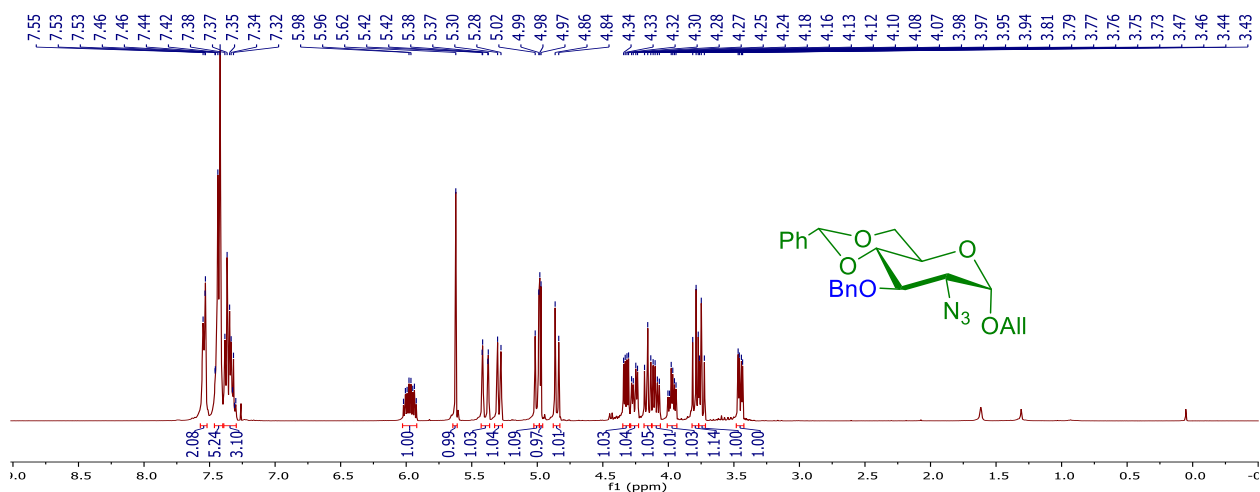


**DEPT NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 62a**

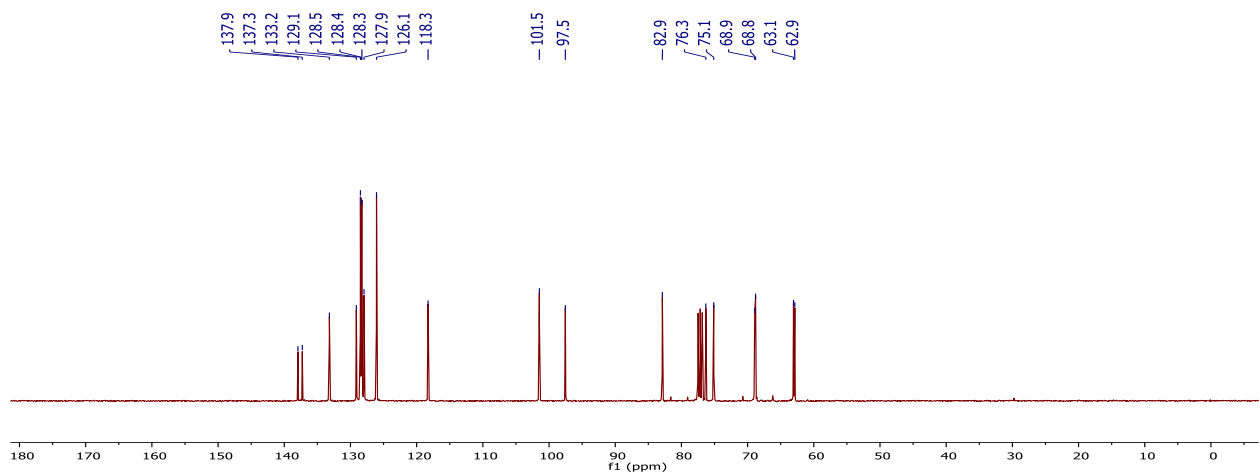




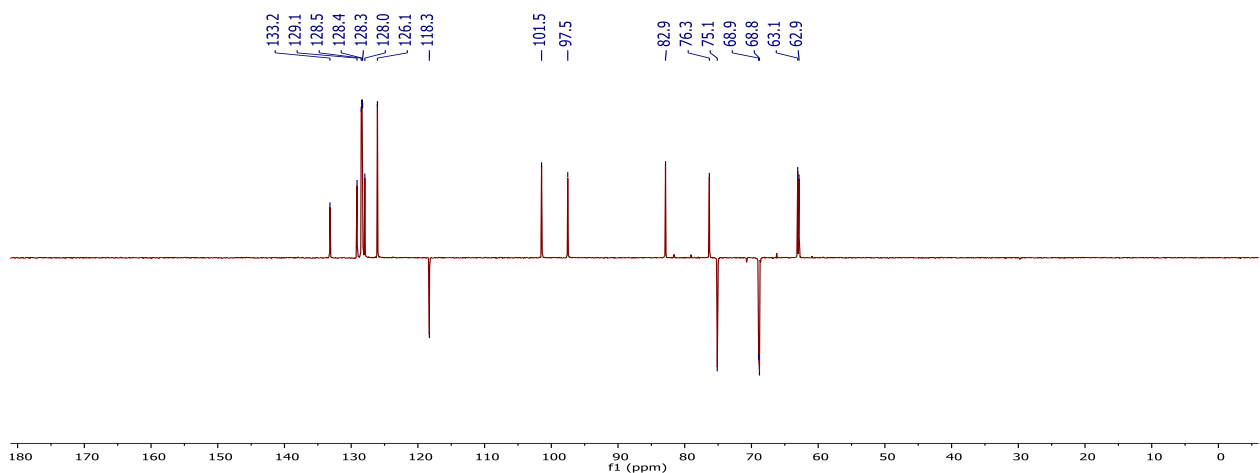
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 62b**

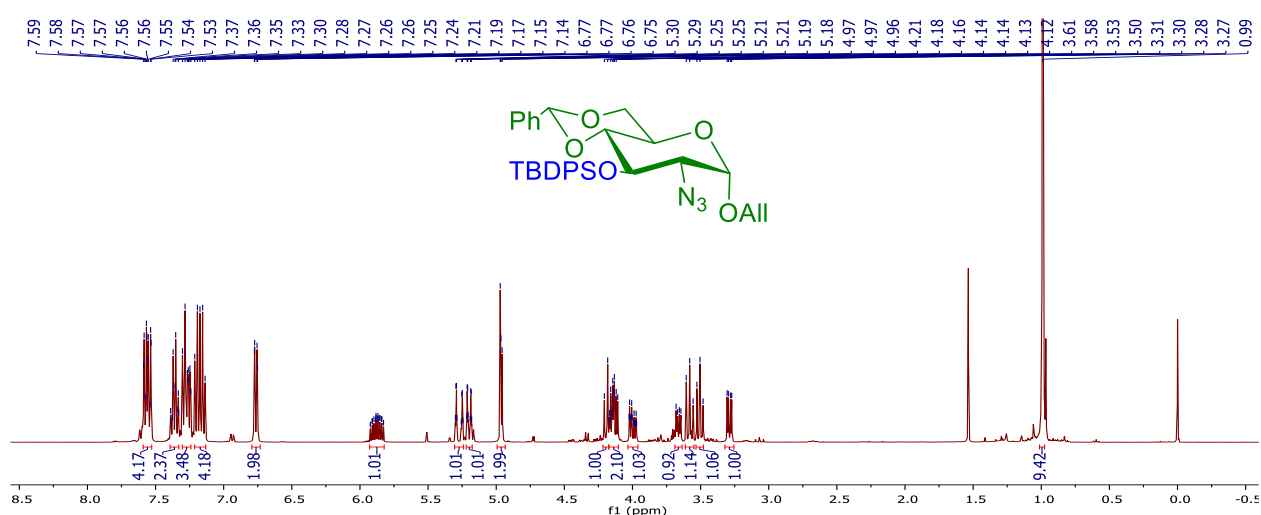


**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 62b**

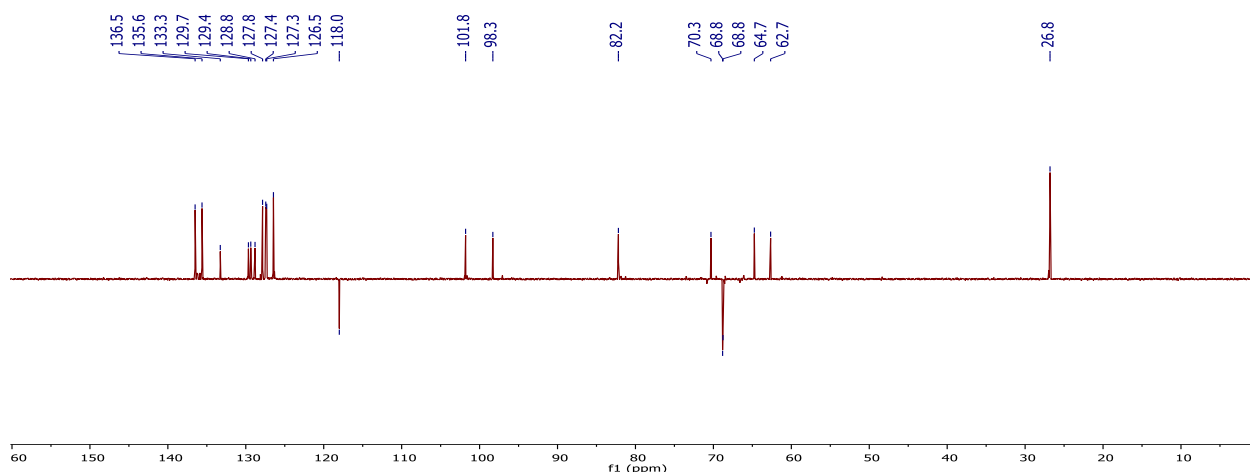
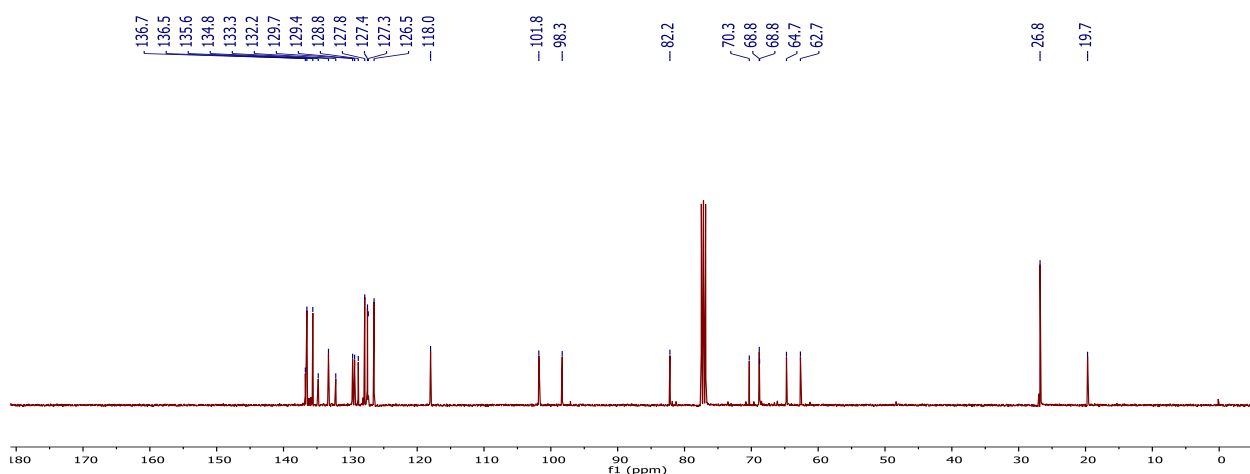


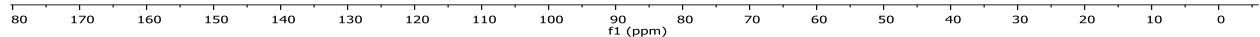
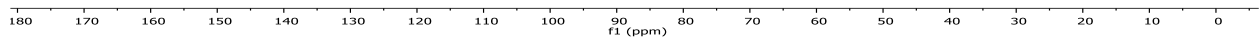
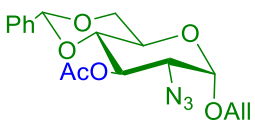
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 62b**



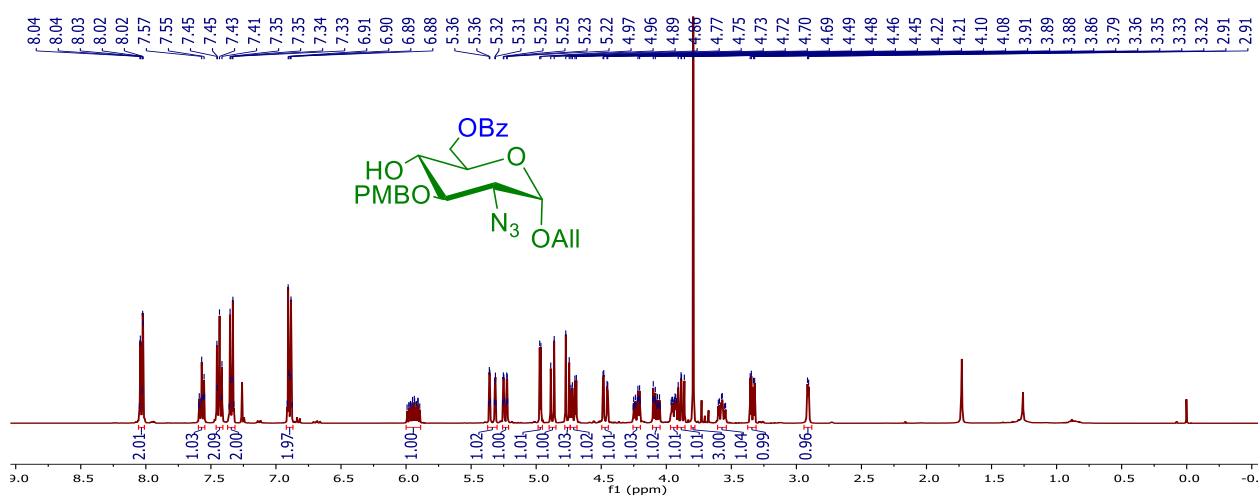


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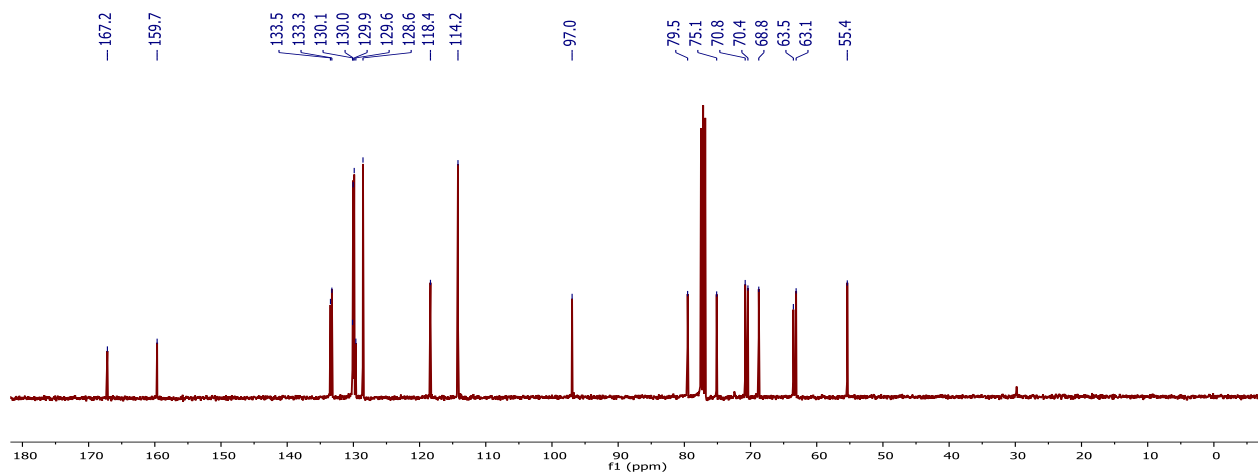




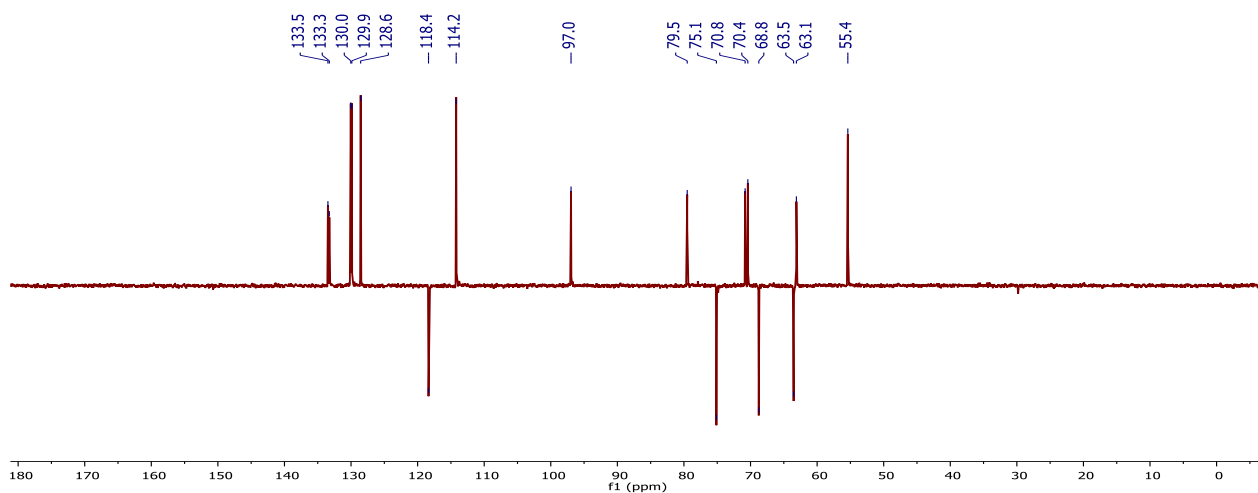
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 64a**



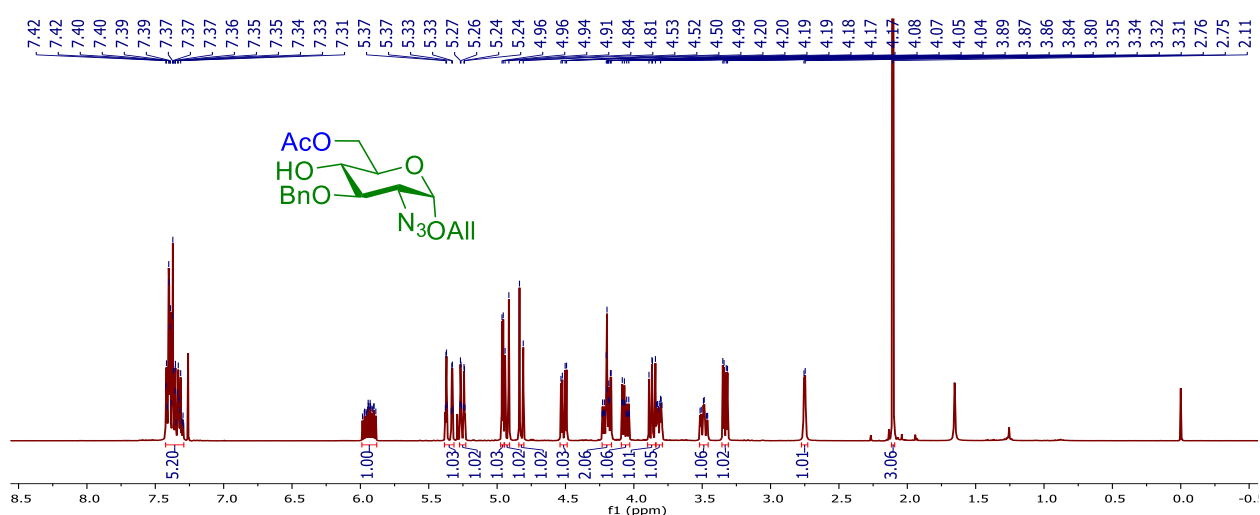
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64a**



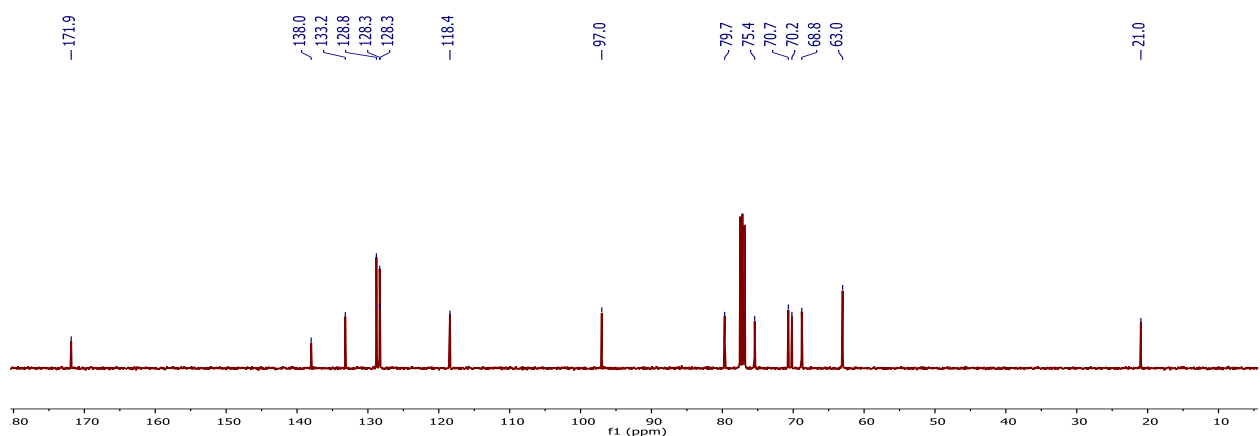
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64a**



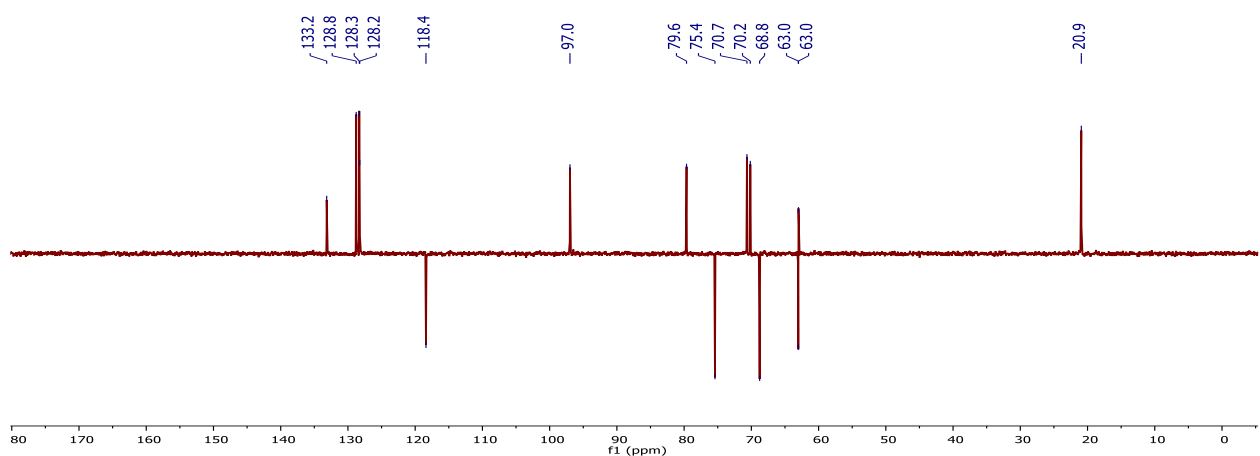
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 64b**



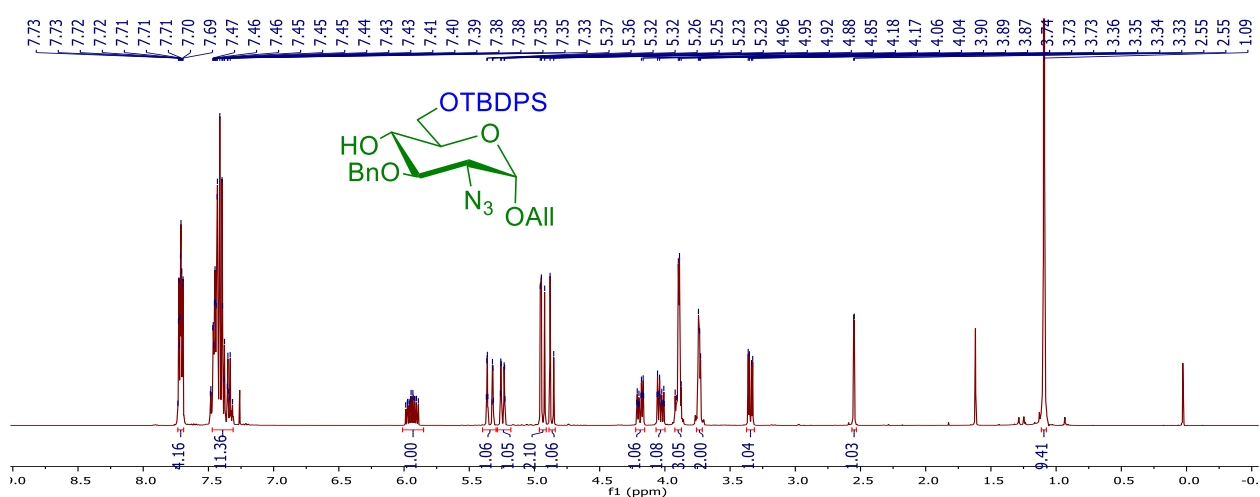
**<sup>13</sup>C NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 64b**



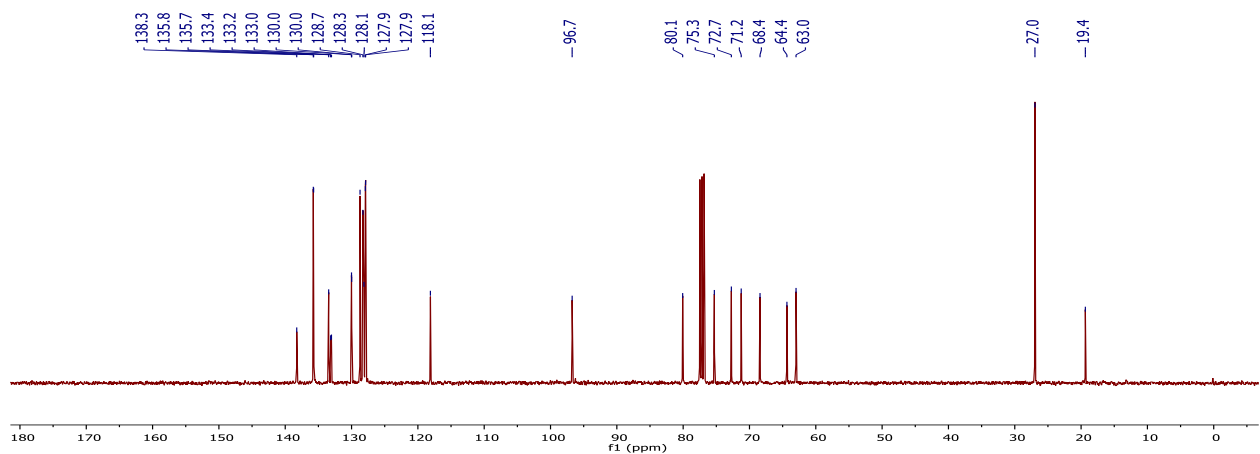
**DEPT NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 64b**



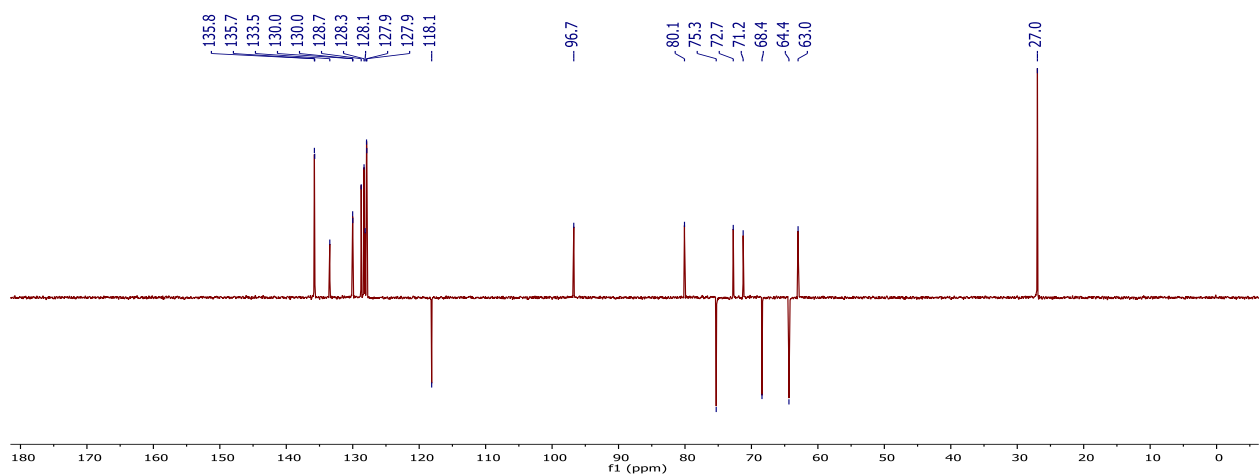
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 64c**



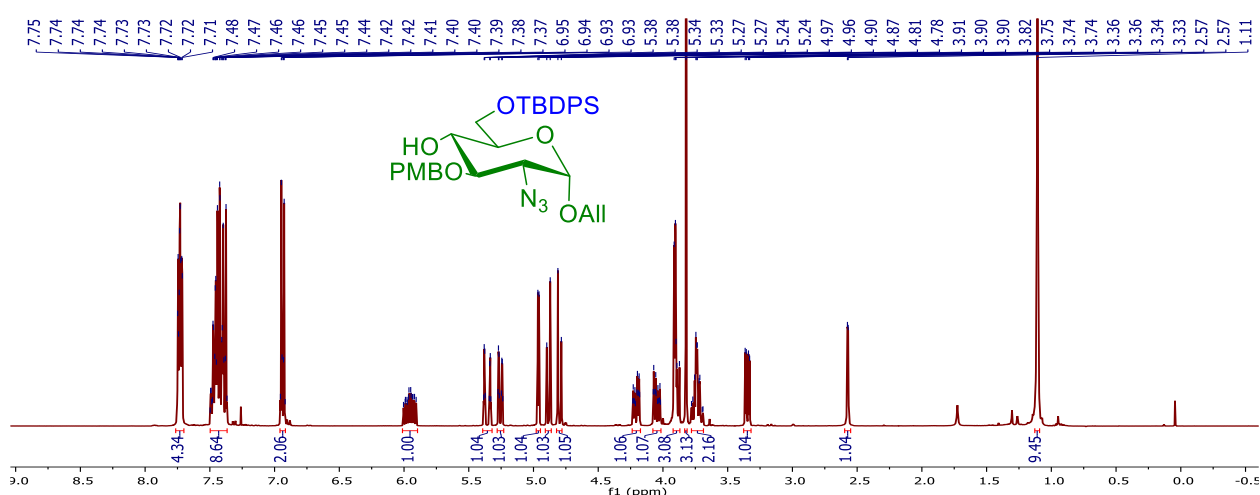
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64c**



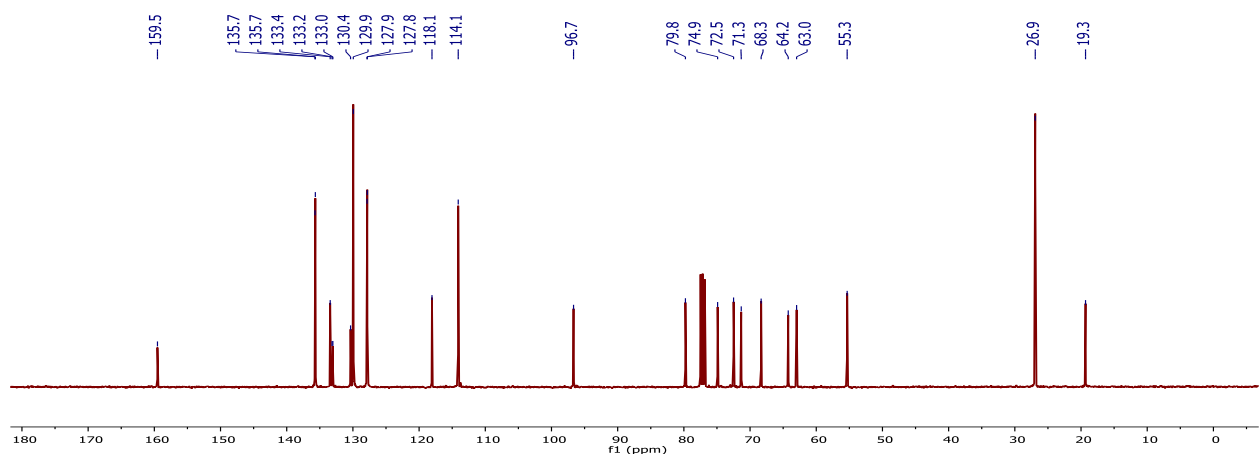
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64c**



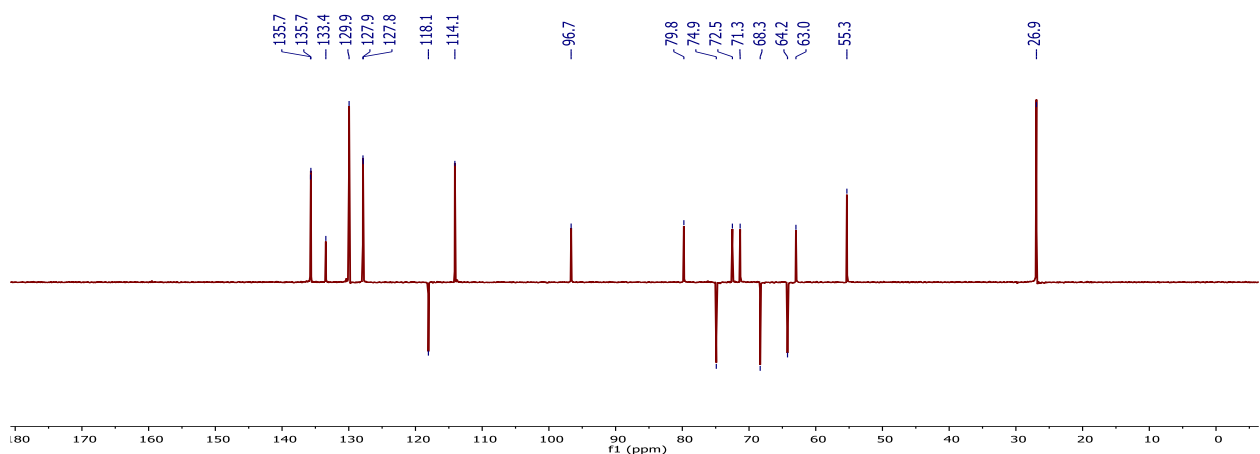
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 64d**

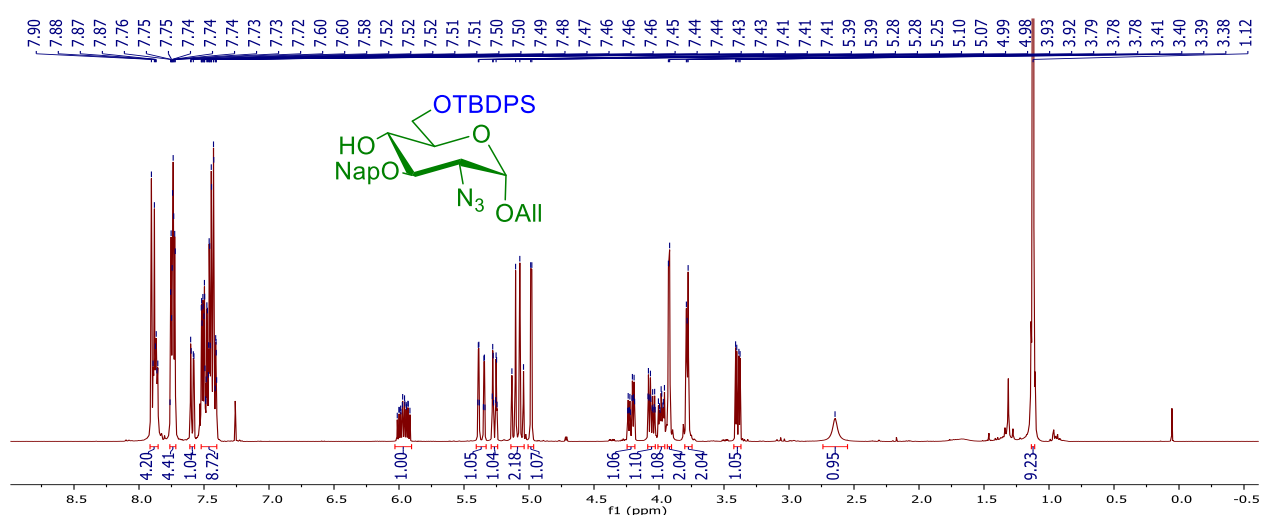


**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64d**

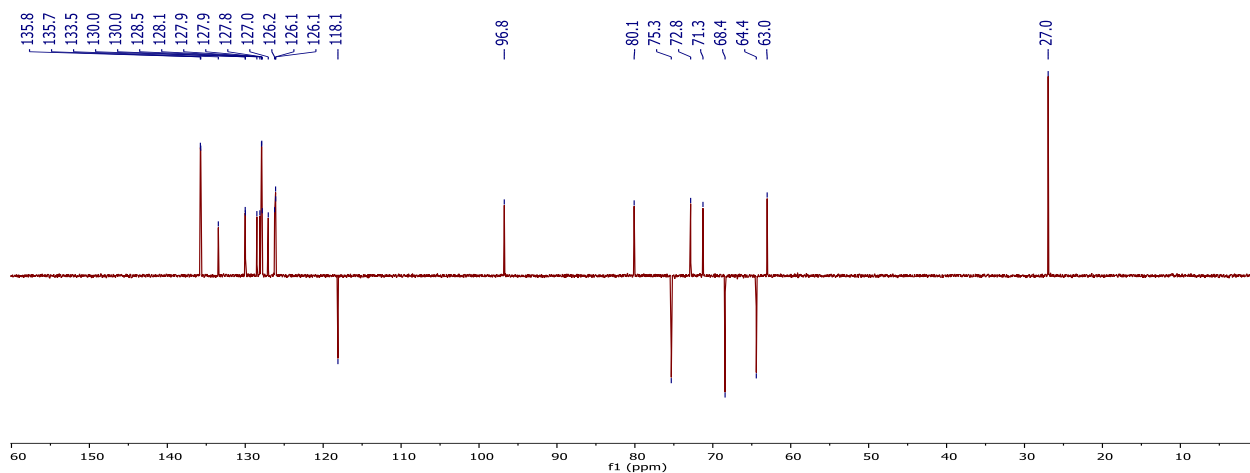
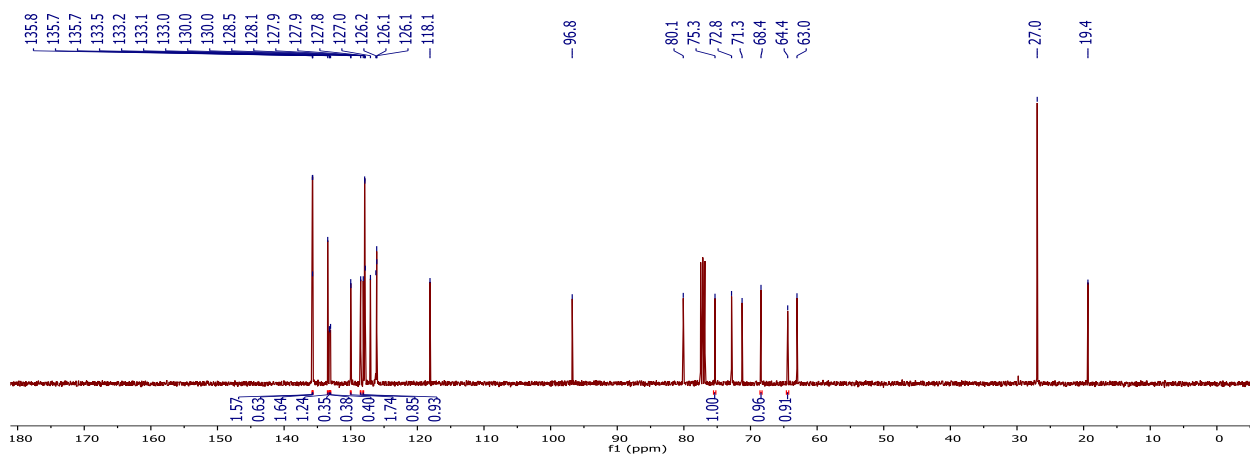


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64d**



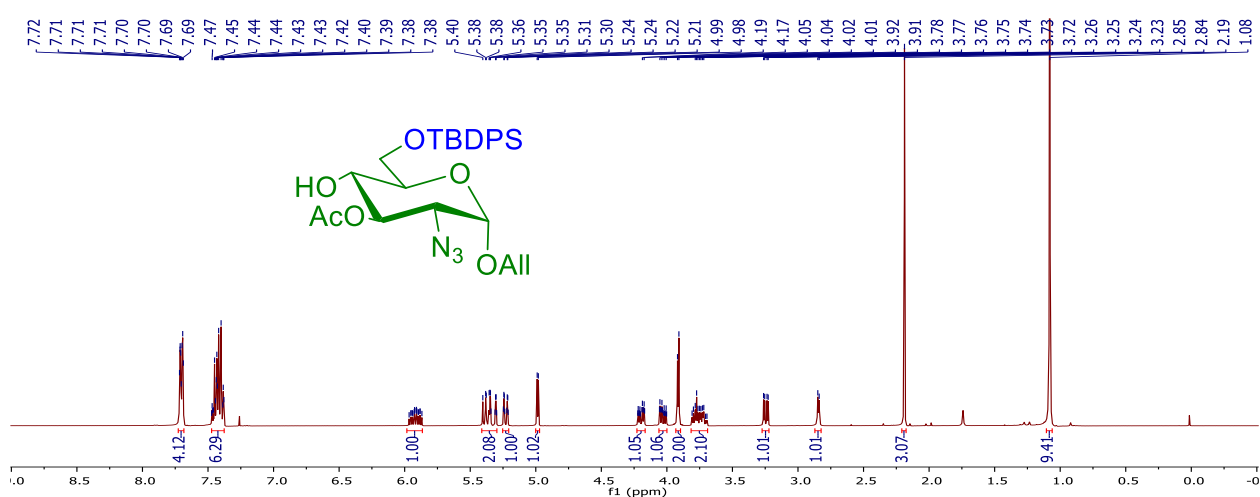


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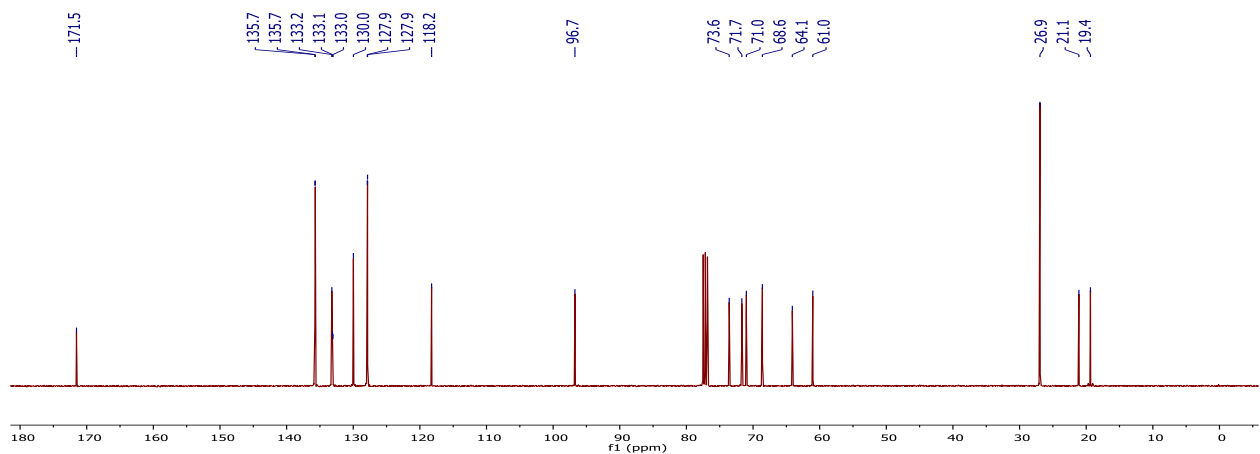




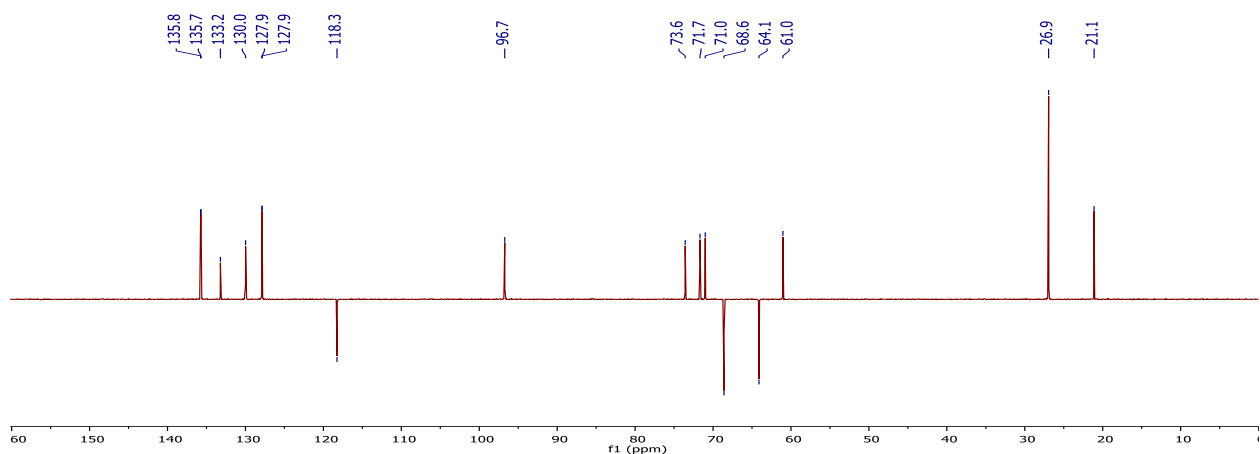
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 64f**



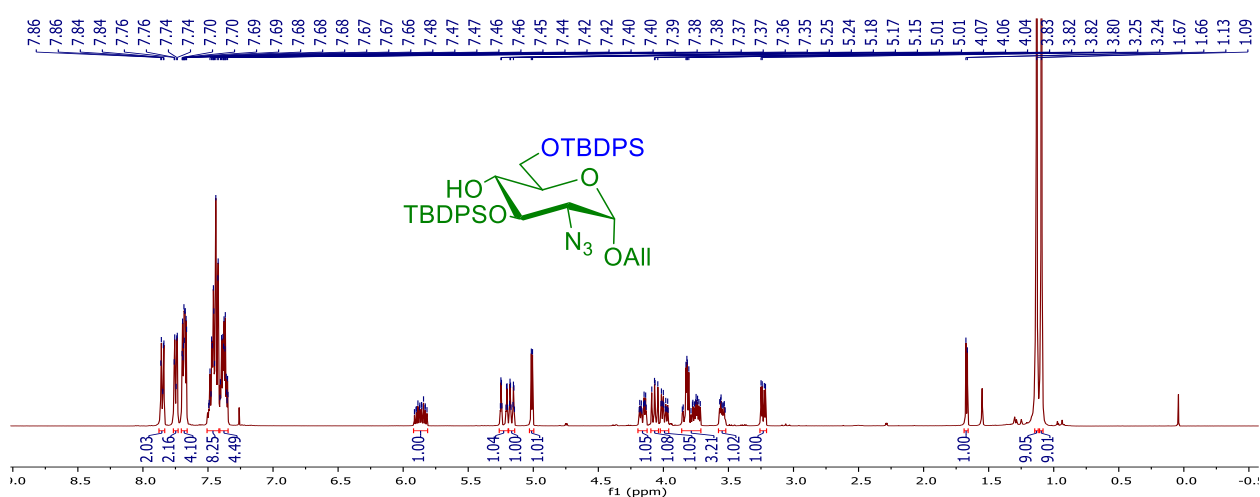
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64f**



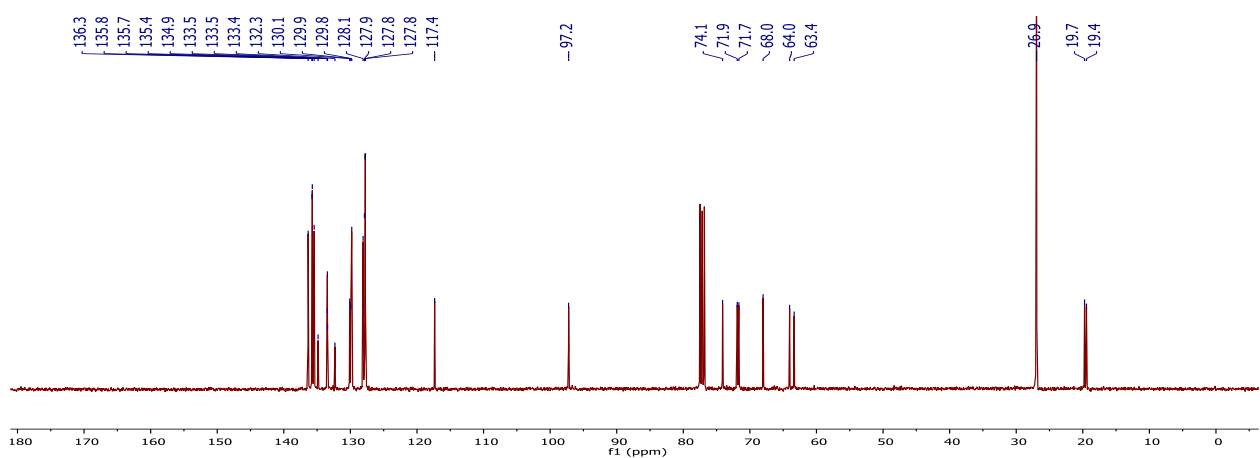
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64f**



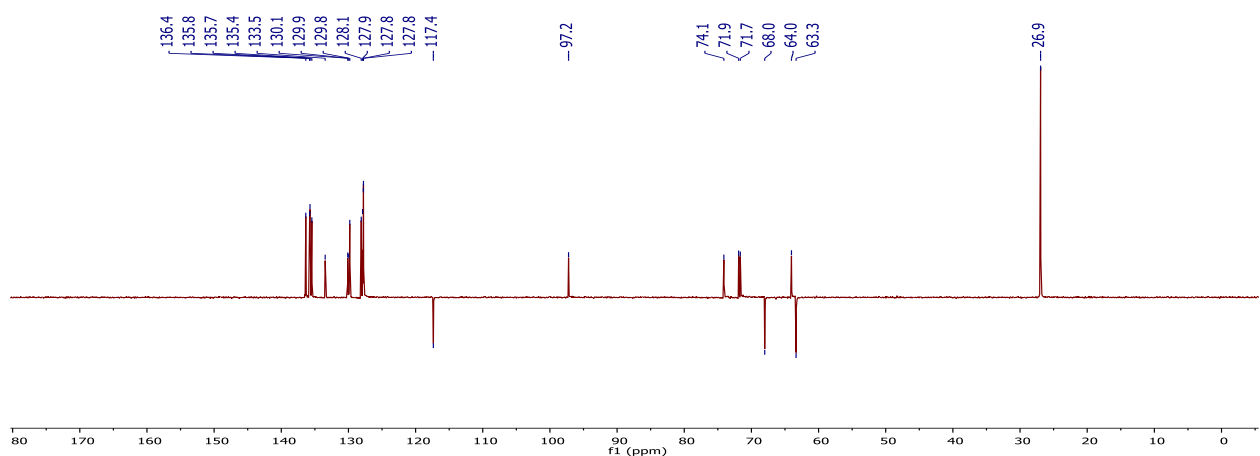
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 64g**



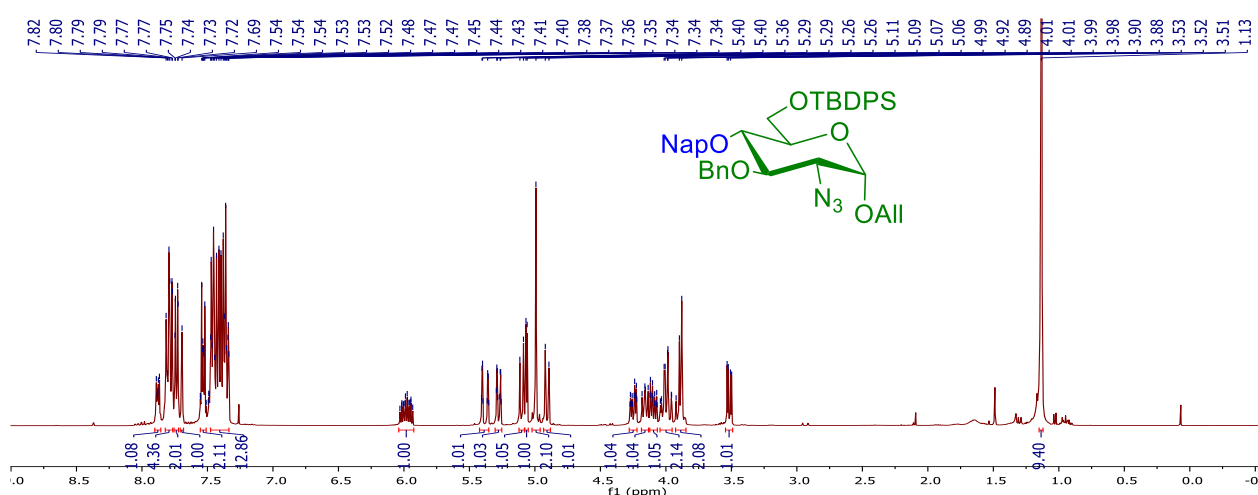
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64g**



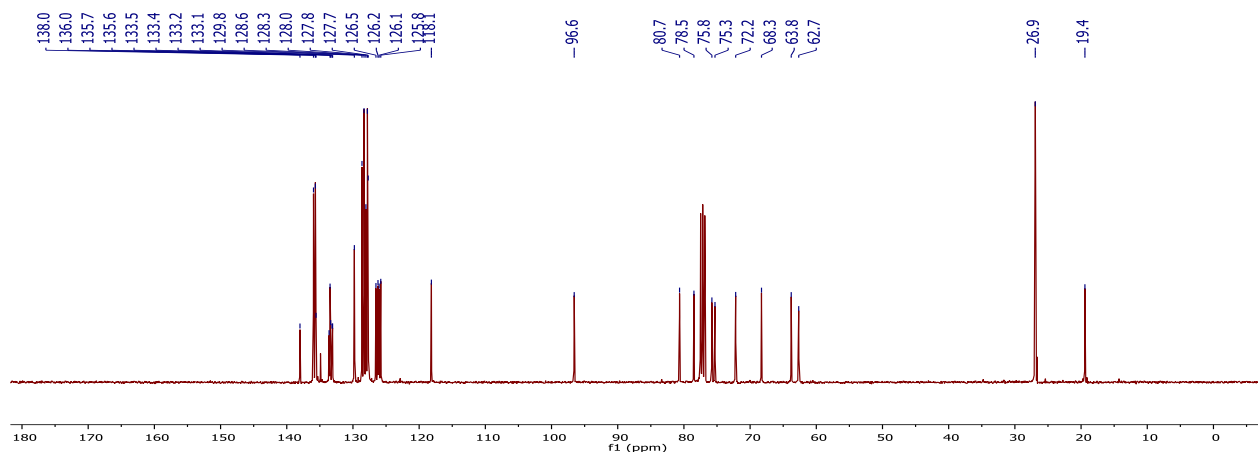
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 64g**



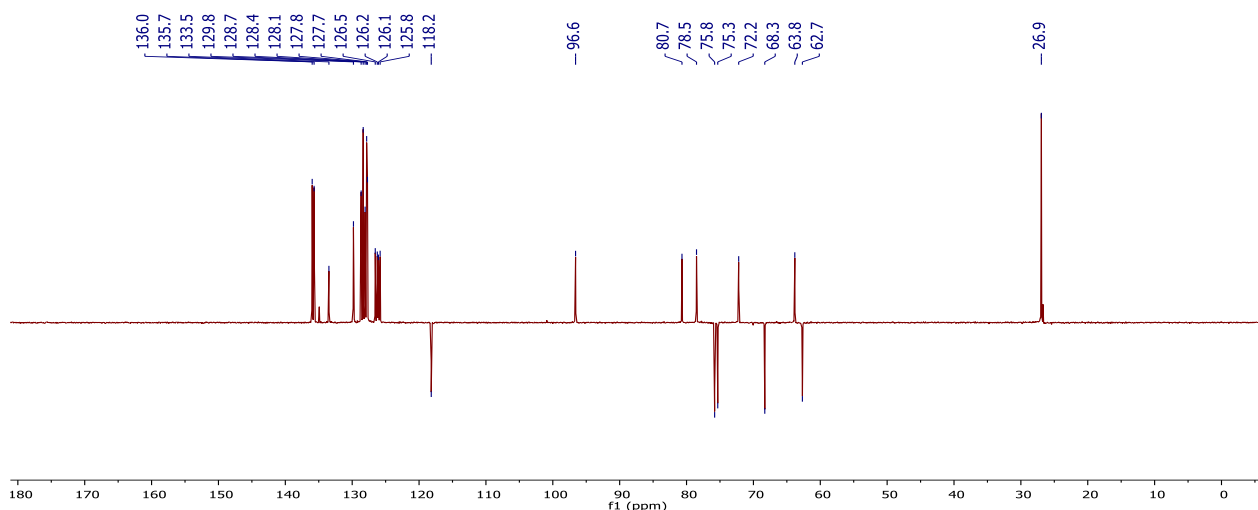
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 65a**



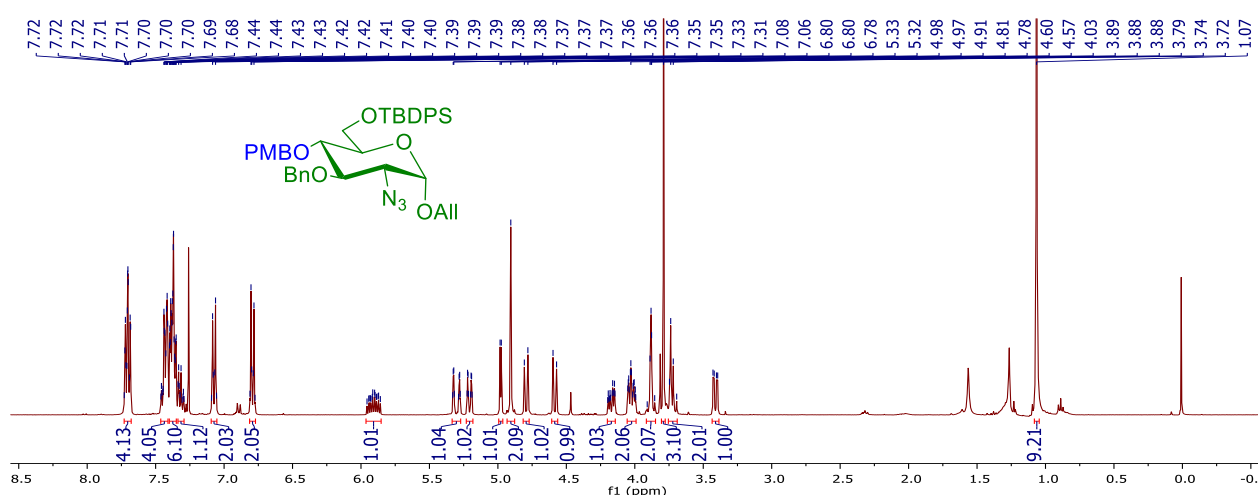
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 65a**



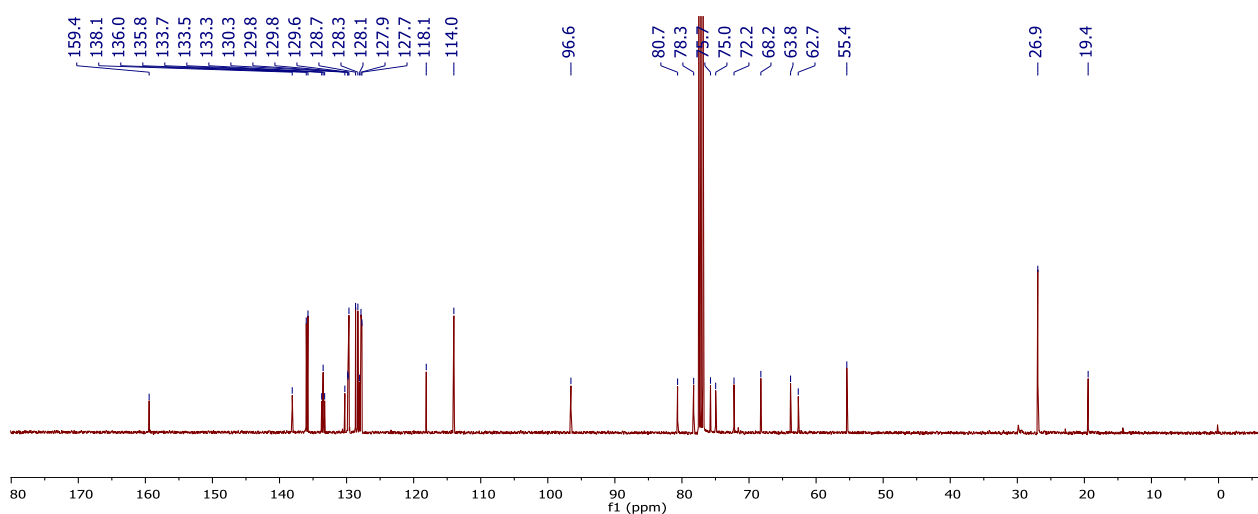
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 65a**



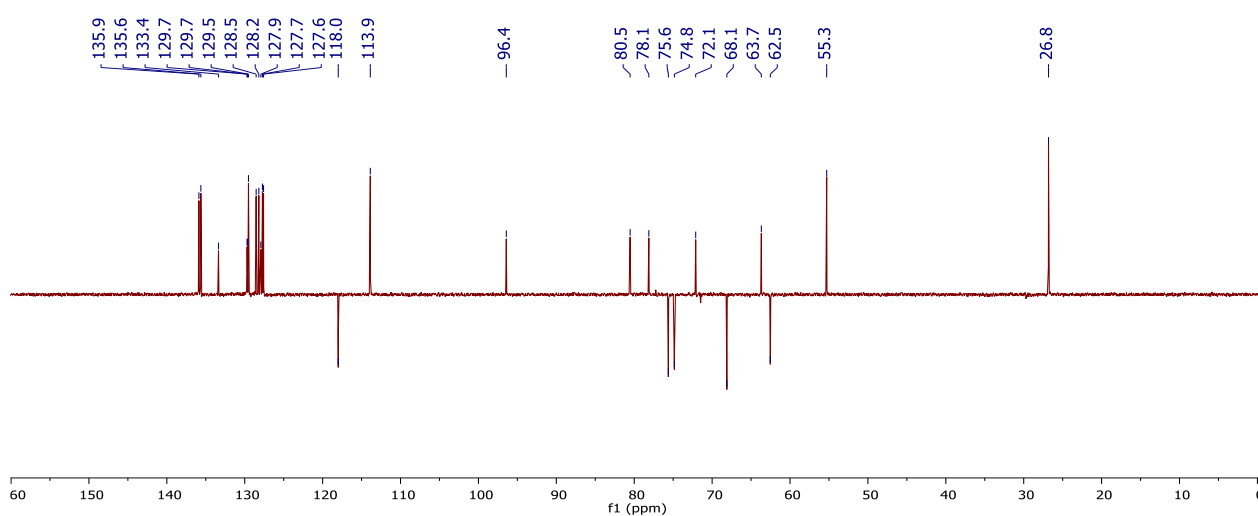
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 65b**

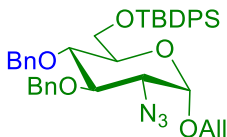


**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 65b**

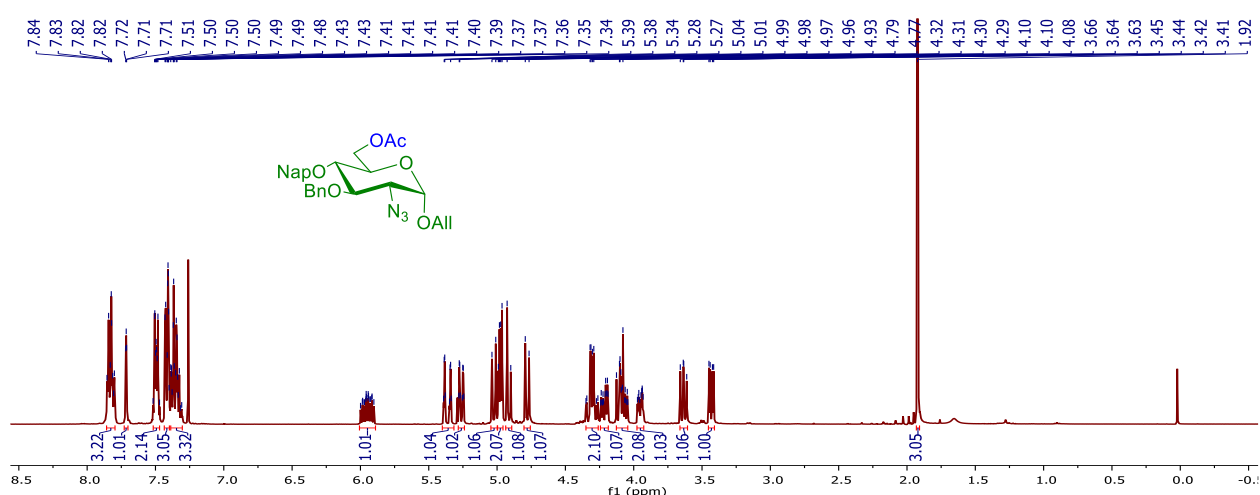


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 65b**

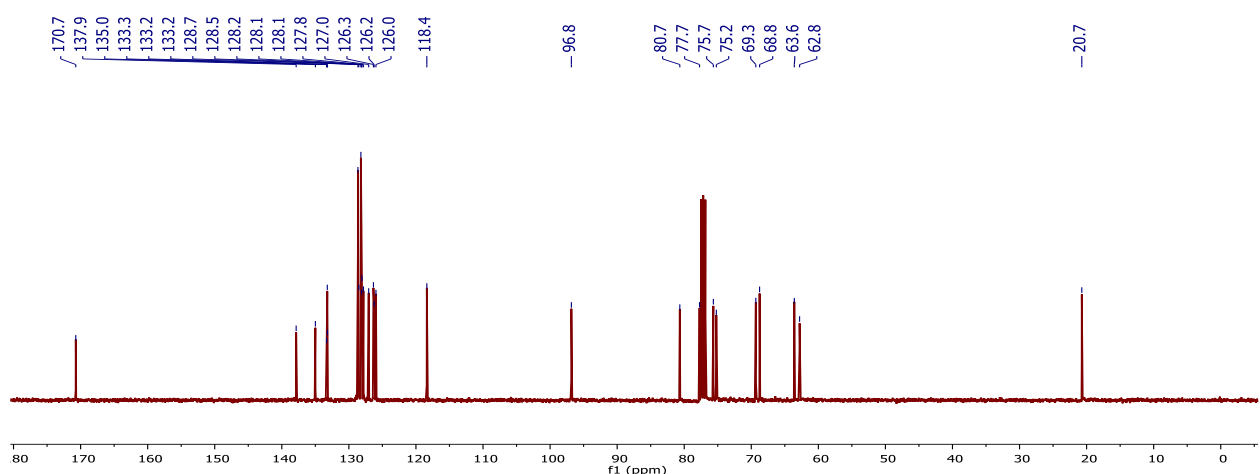


[illegible]

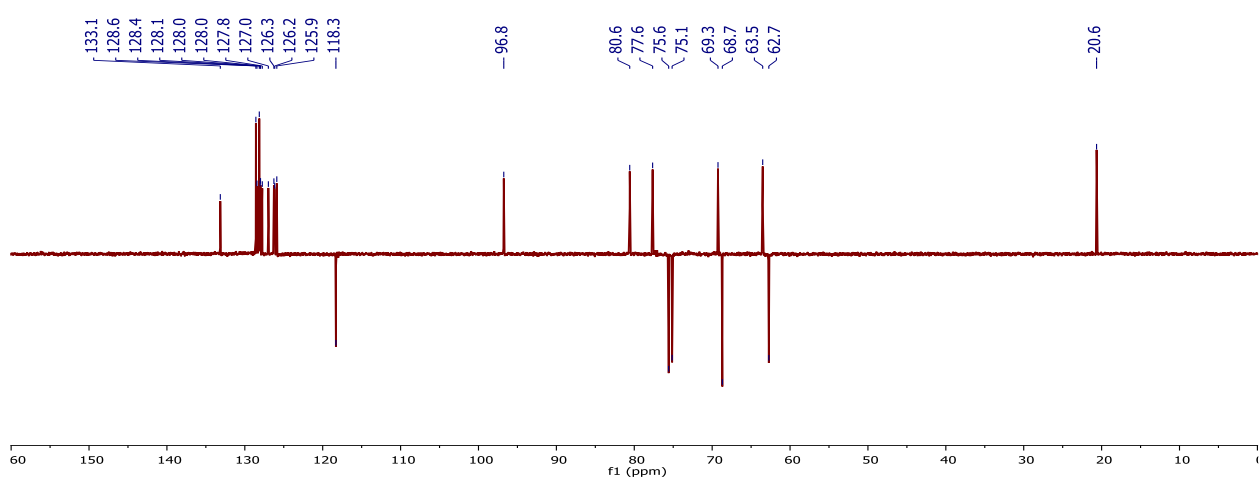
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 65d**



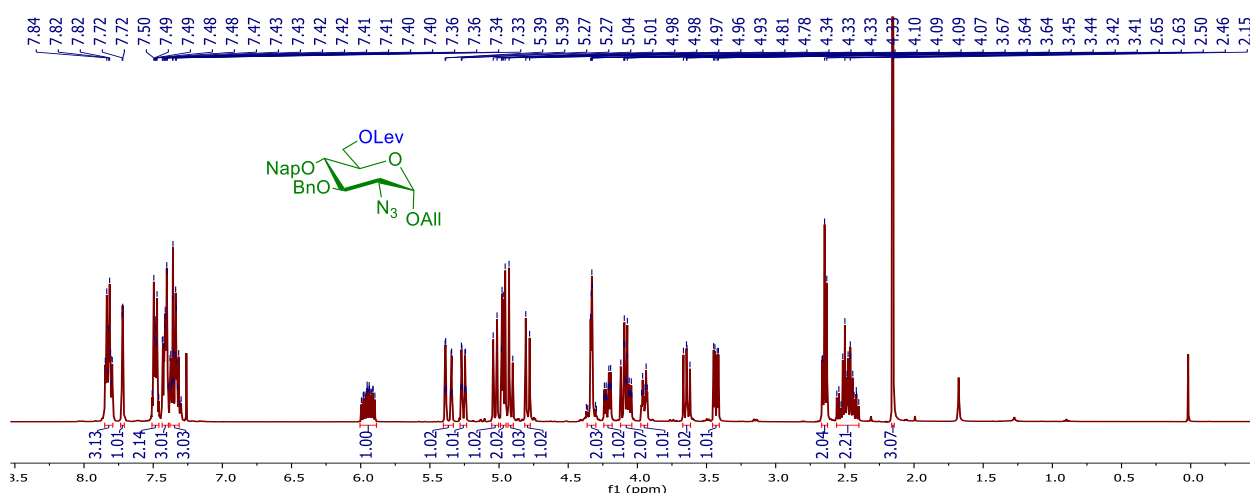
**<sup>13</sup>C NMR Spectrum (100.66 MHz, CDCl<sub>3</sub>) of Compound 65d**



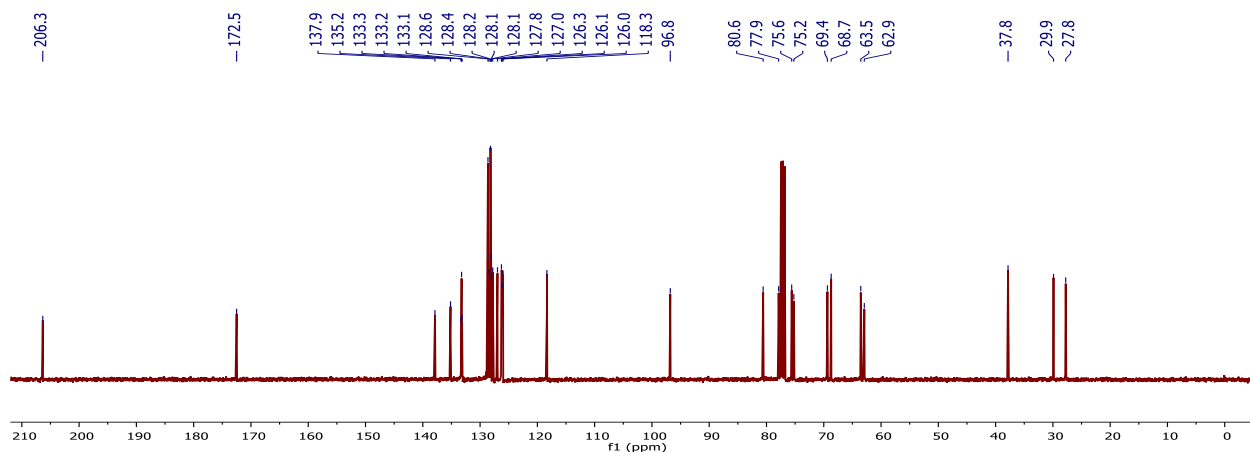
**DEPT NMR Spectrum (100.66 MHz, CDCl<sub>3</sub>) of Compound 65d**



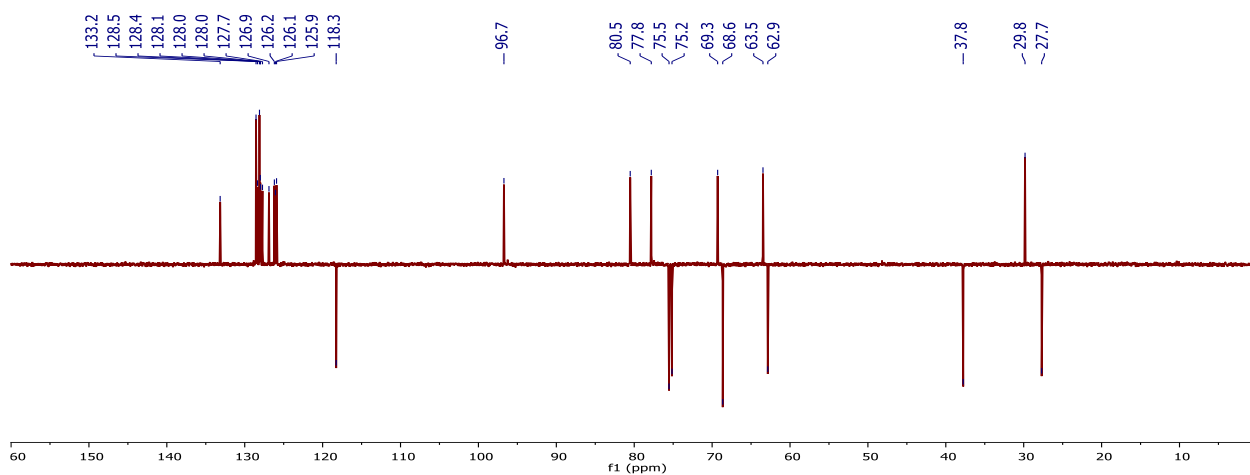
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 65e**



**<sup>13</sup>C NMR Spectrum (100.66 MHz, CDCl<sub>3</sub>) of Compound 65e**

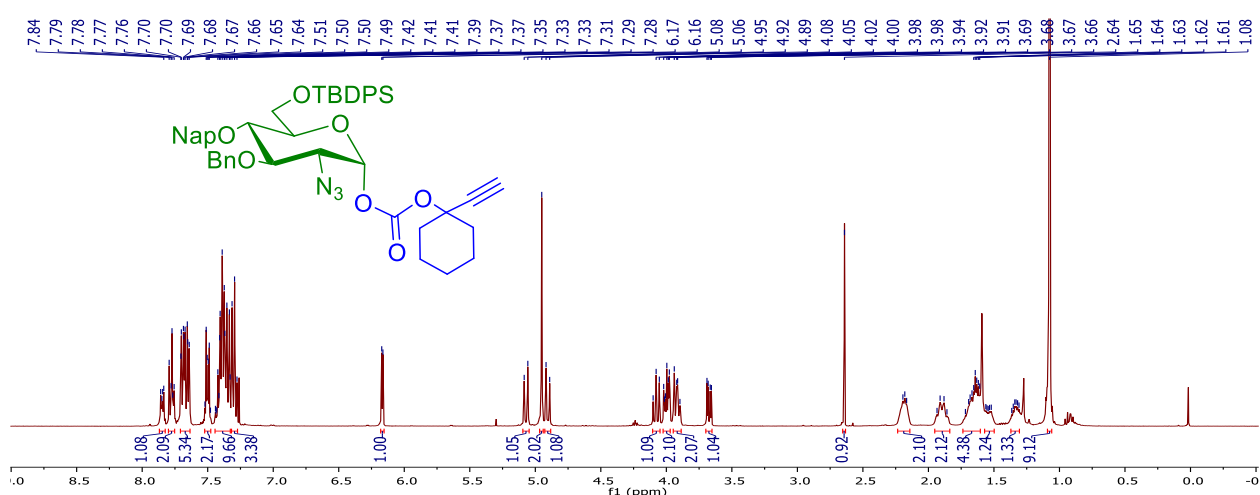


**DEPT NMR Spectrum (100.66 MHz, CDCl<sub>3</sub>) of Compound 65e**

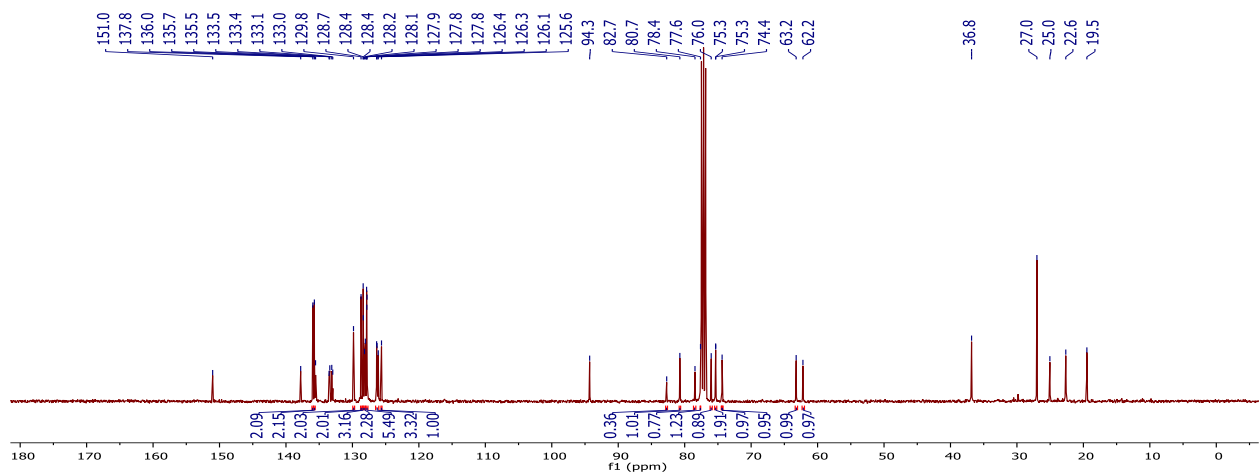


## Donor-Acceptor Selectivity Studies with GlcA and GlcN Building Blocks

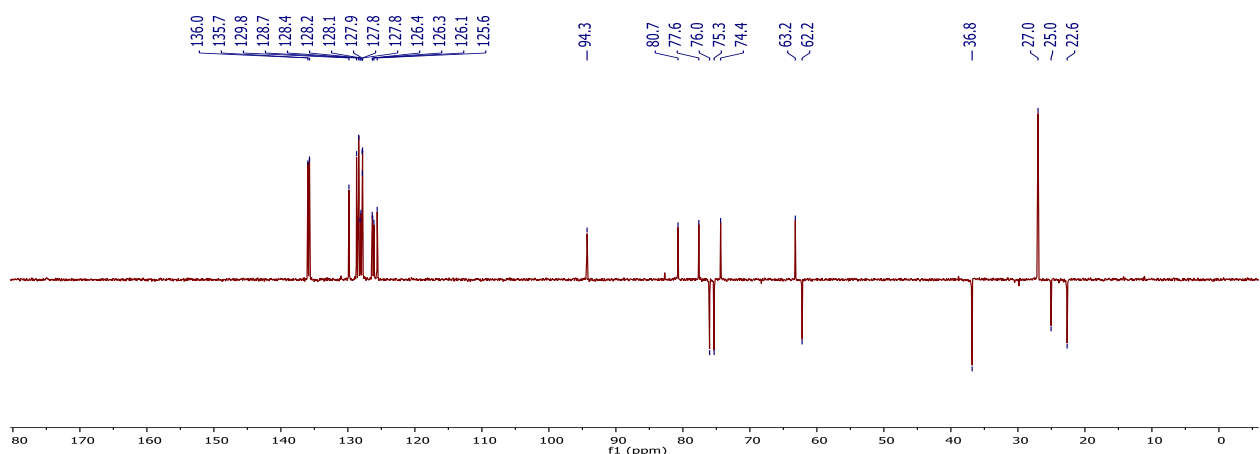
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 67a ( $\alpha$  isomer)**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 67a ( $\alpha$  isomer)**

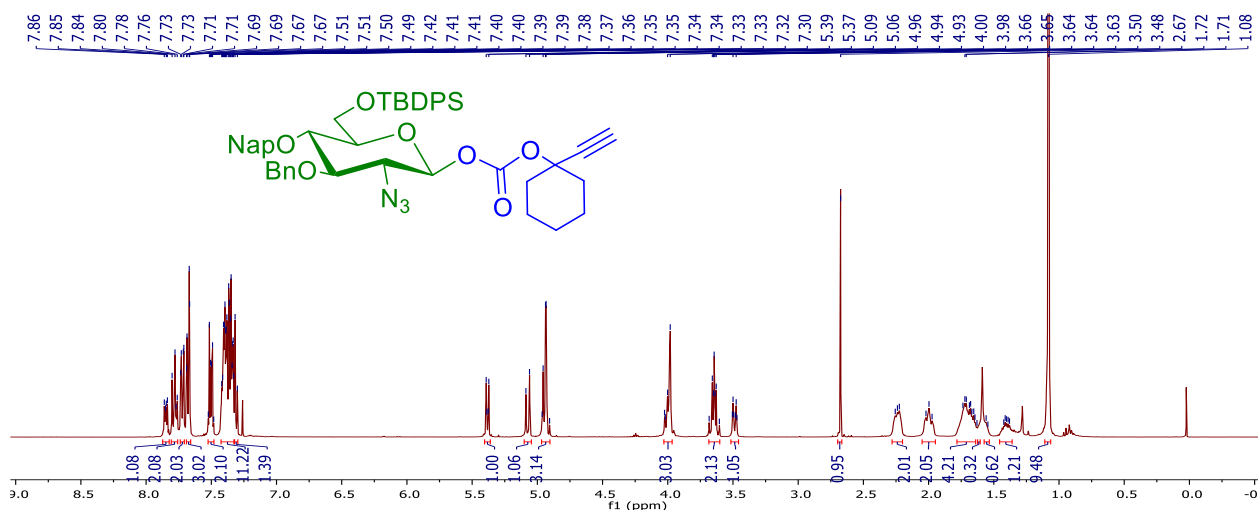


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 67a ( $\alpha$  isomer)**

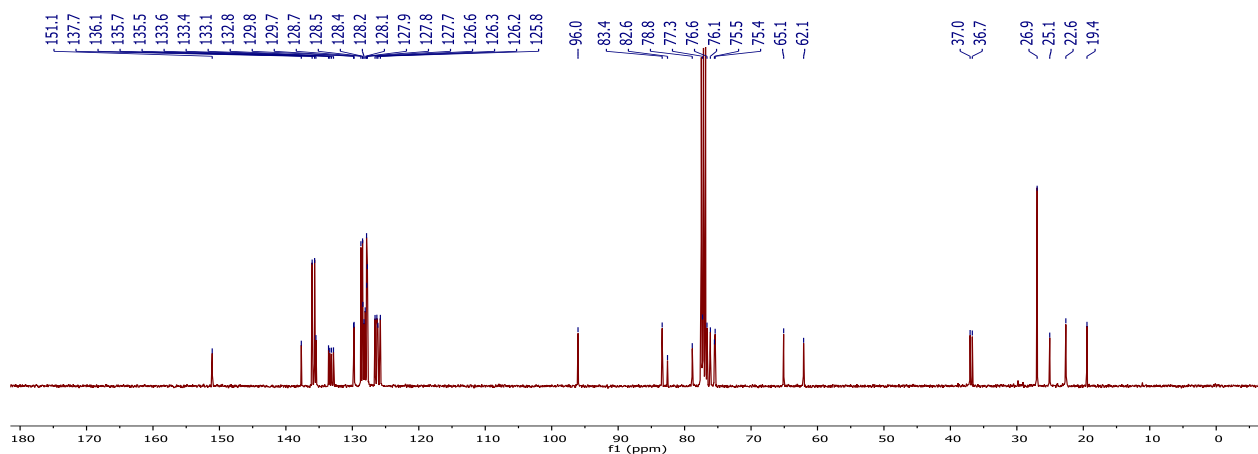




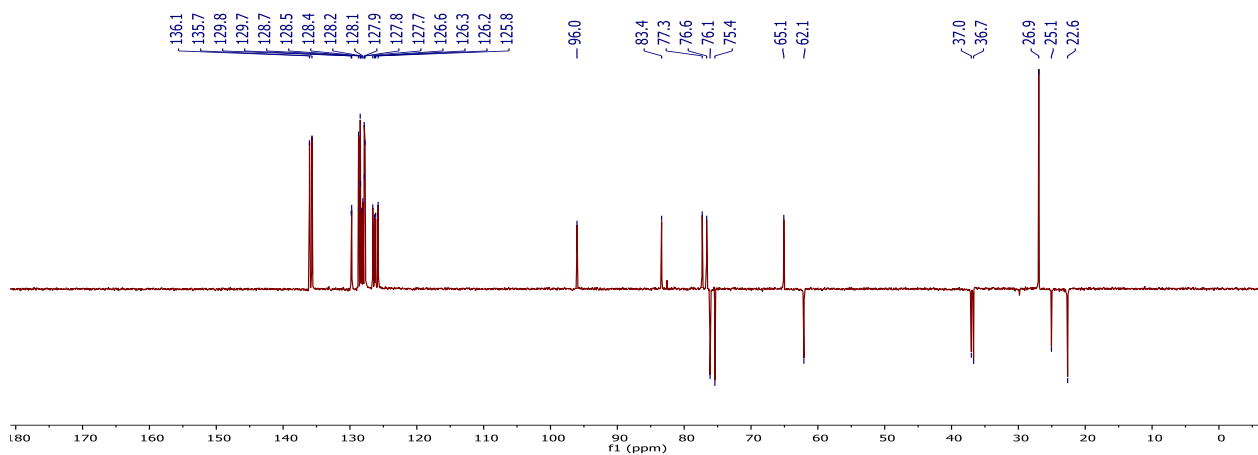
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 67a ( $\beta$  isomer)**



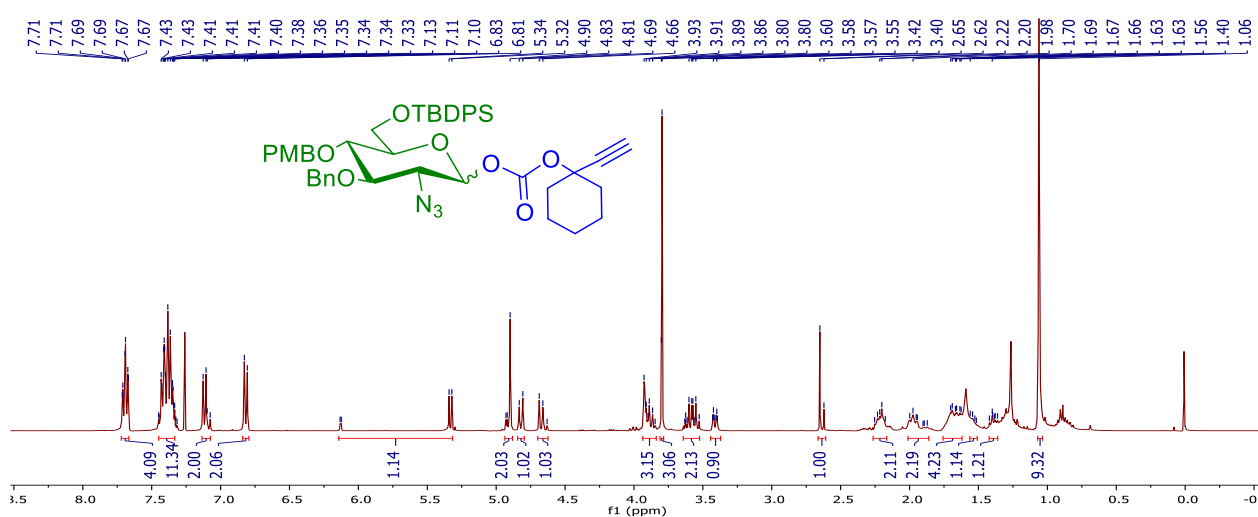
**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 67a ( $\beta$  isomer)**



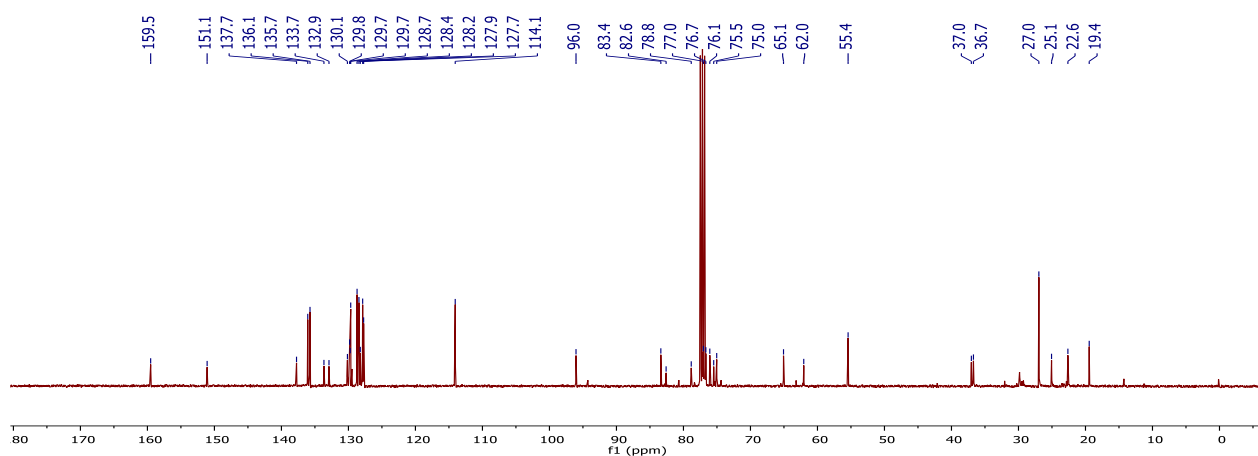
**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 67a ( $\beta$  isomer)**



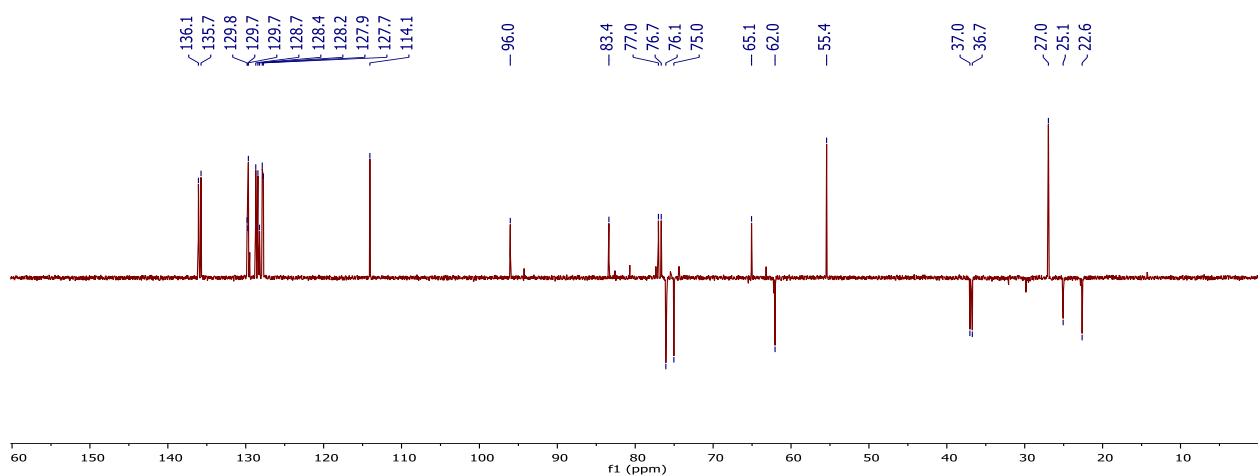
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 67b**



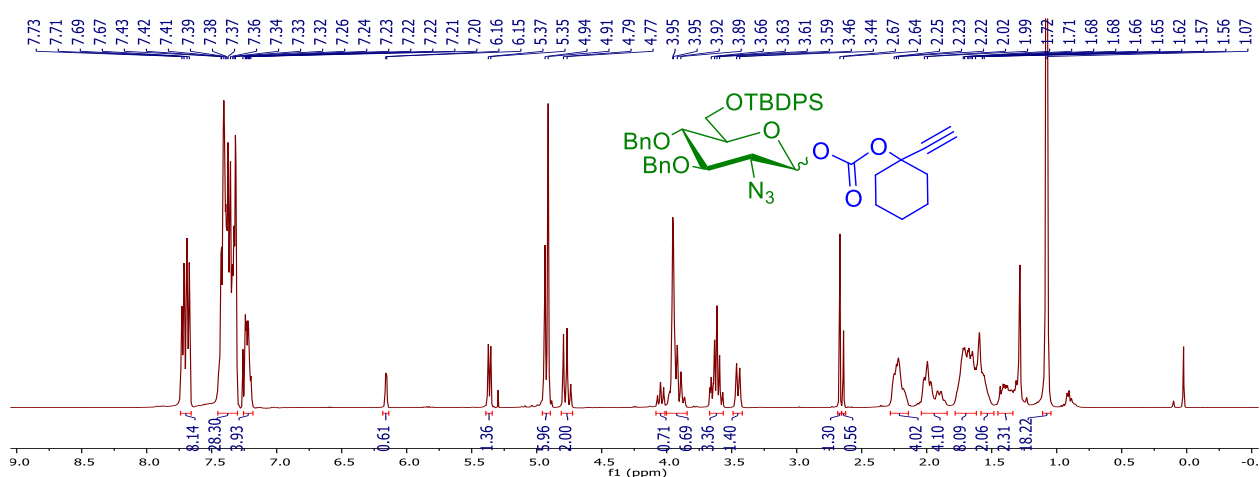
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 67b**



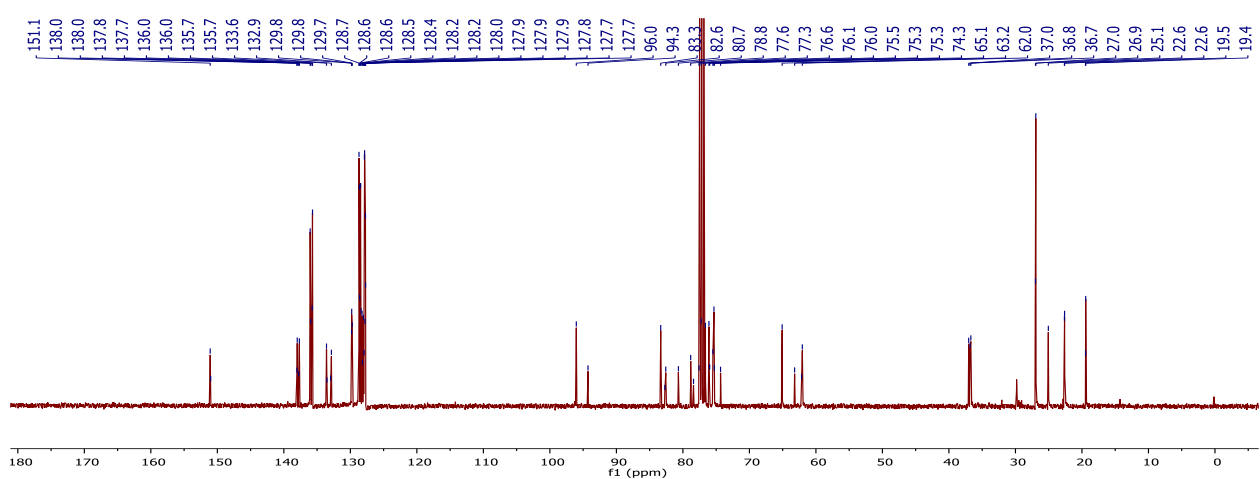
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 67b**



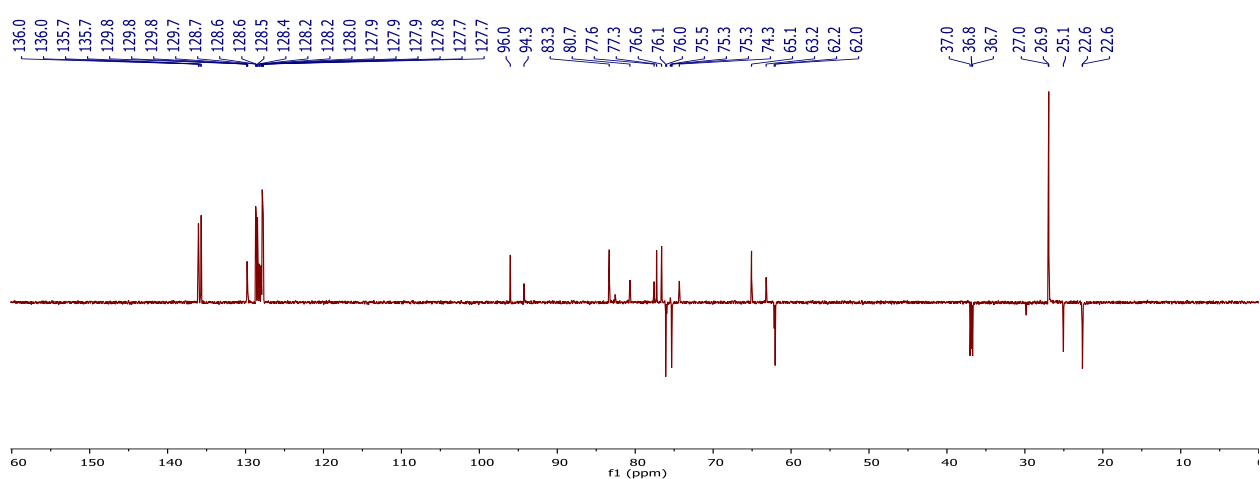
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 67c**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 67c**

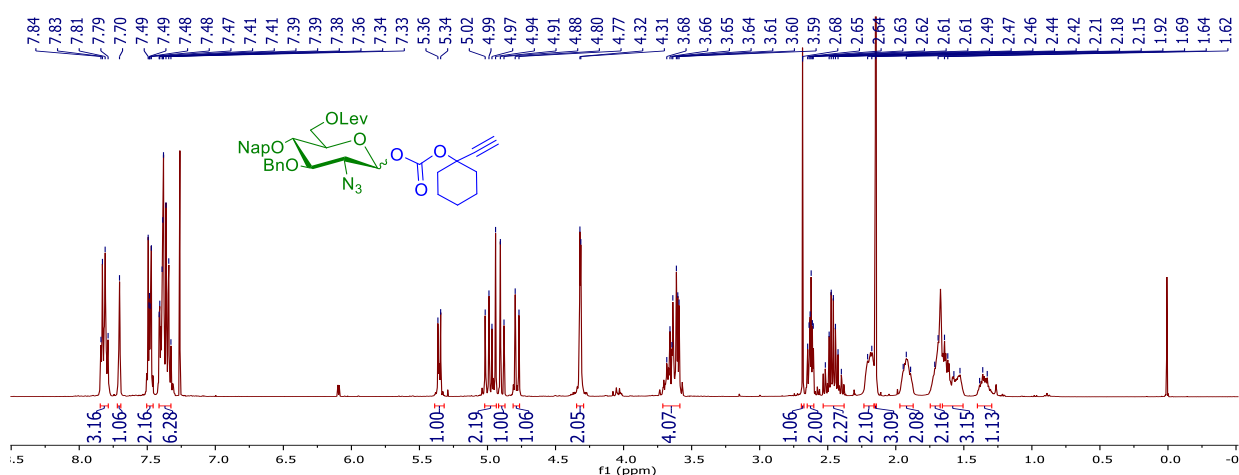


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 67c**

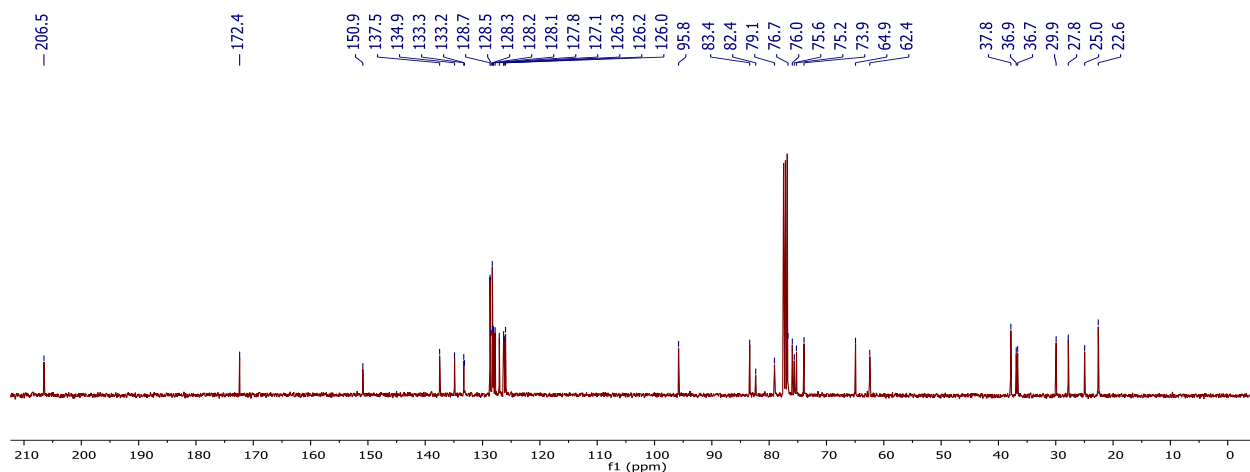




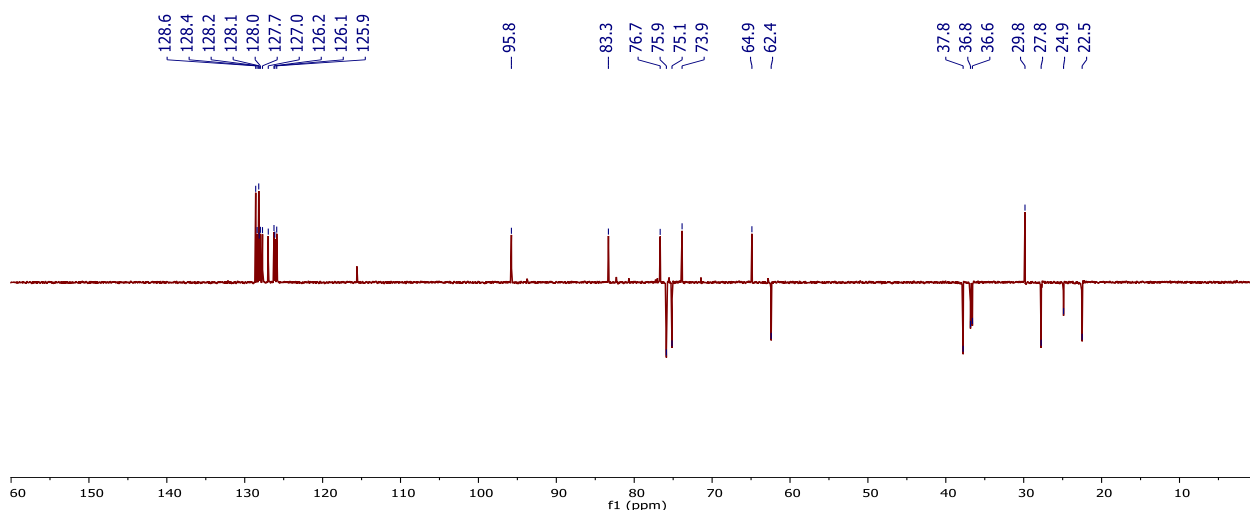
**<sup>1</sup>H NMR Spectrum (399.78 MHz, CDCl<sub>3</sub>) of Compound 67e**



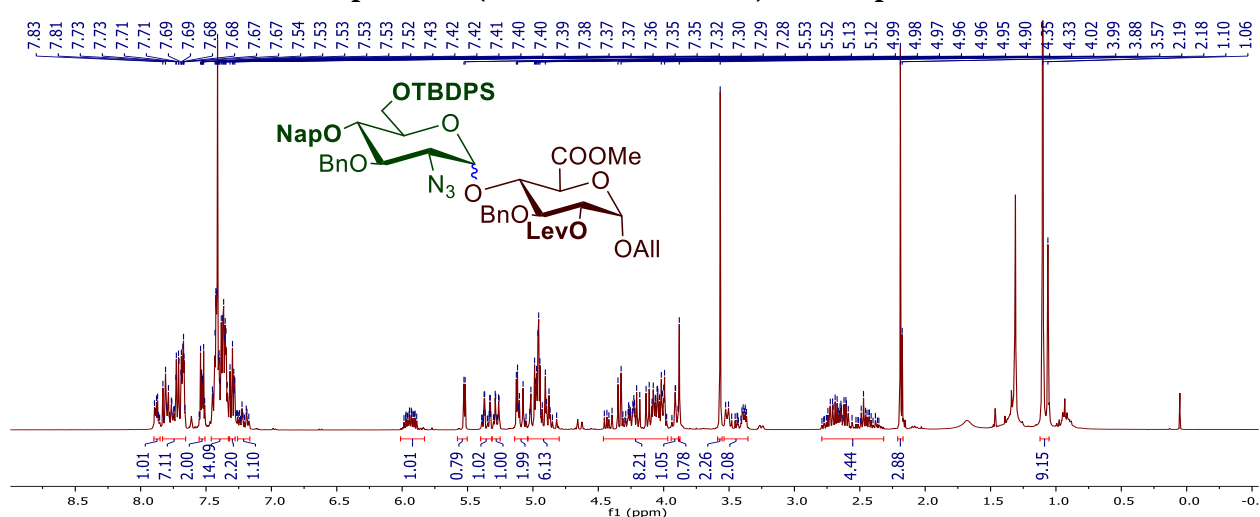
**<sup>13</sup>C NMR Spectrum (100.53 MHz, CDCl<sub>3</sub>) of Compound 67e**



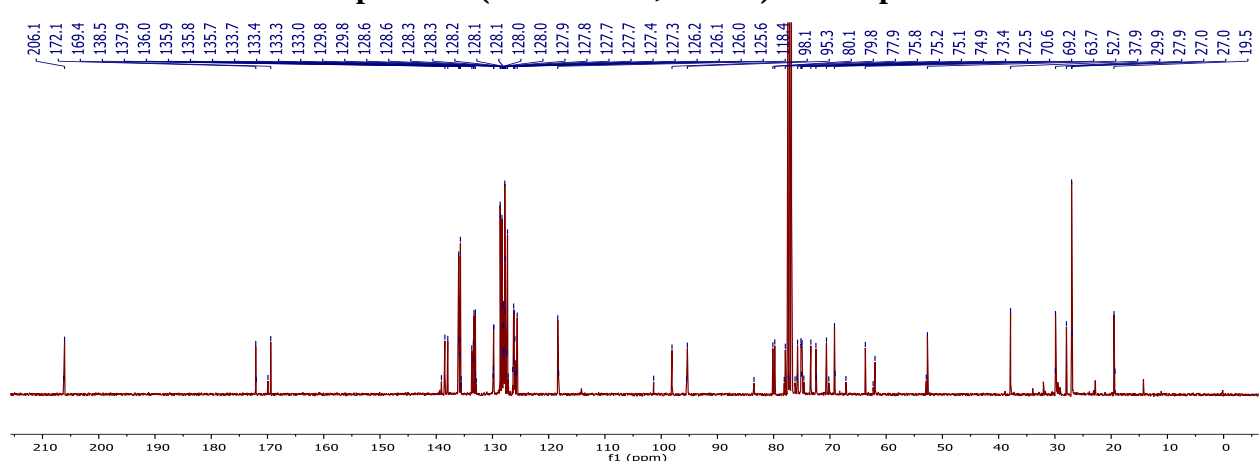
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 67e**



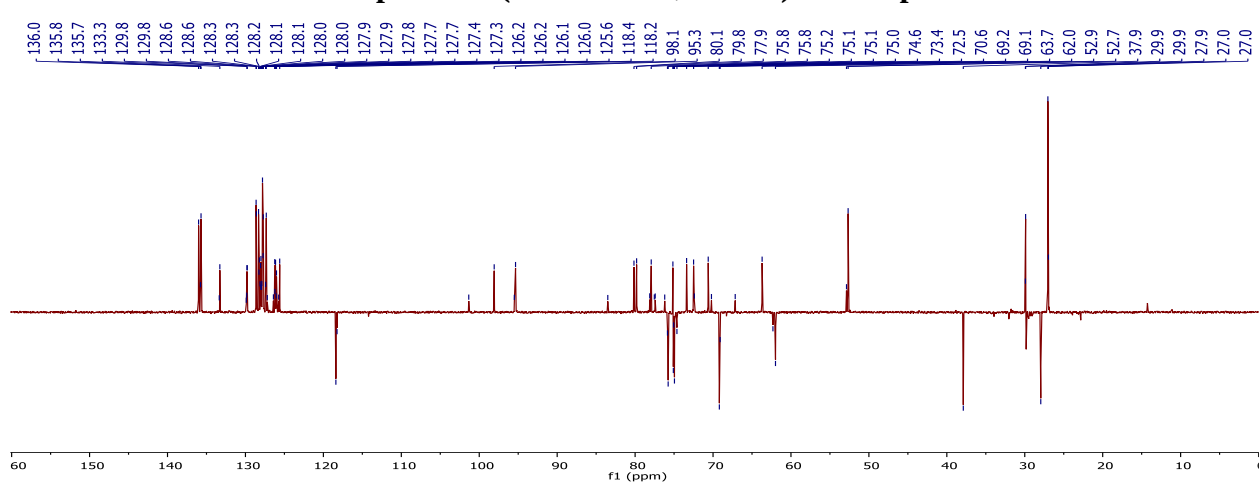
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69a**



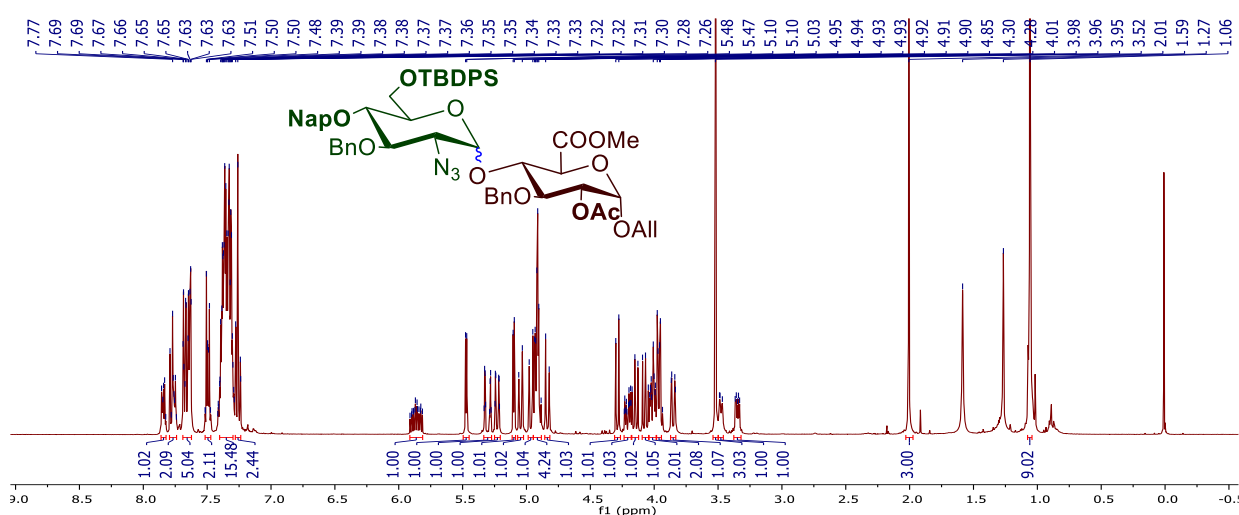
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69a**



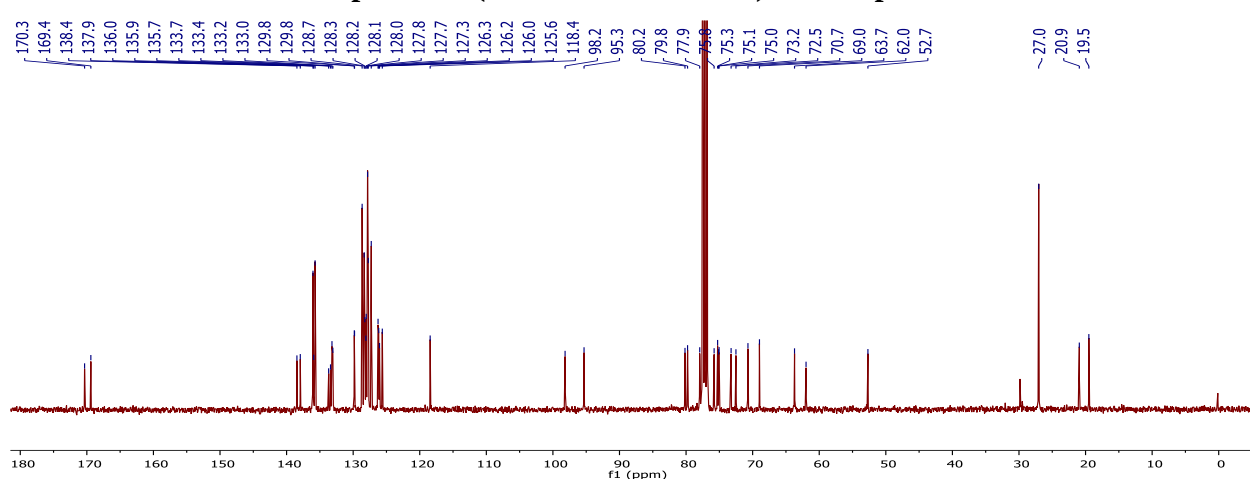
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69a**



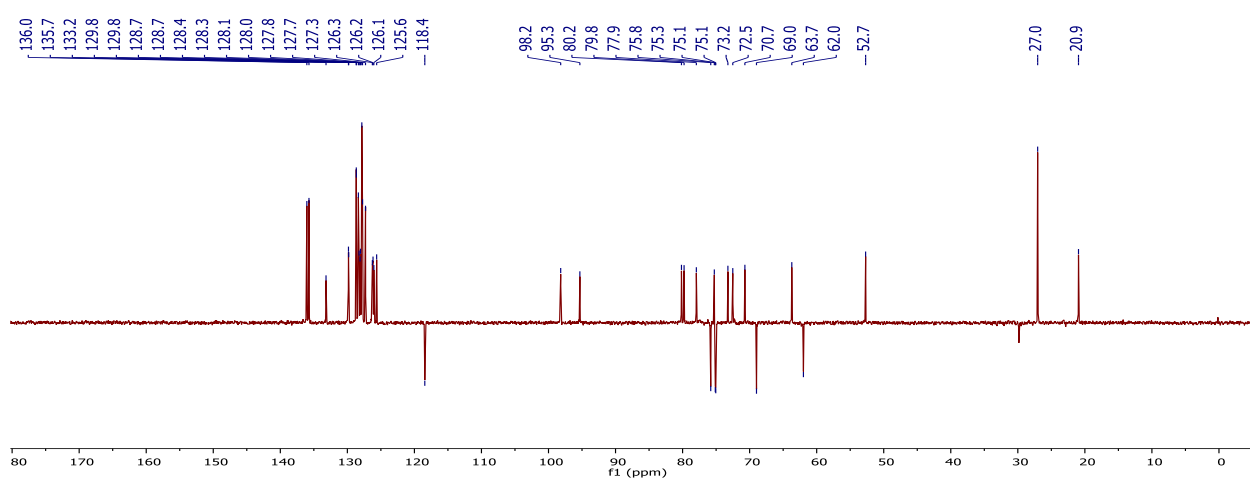
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69c**



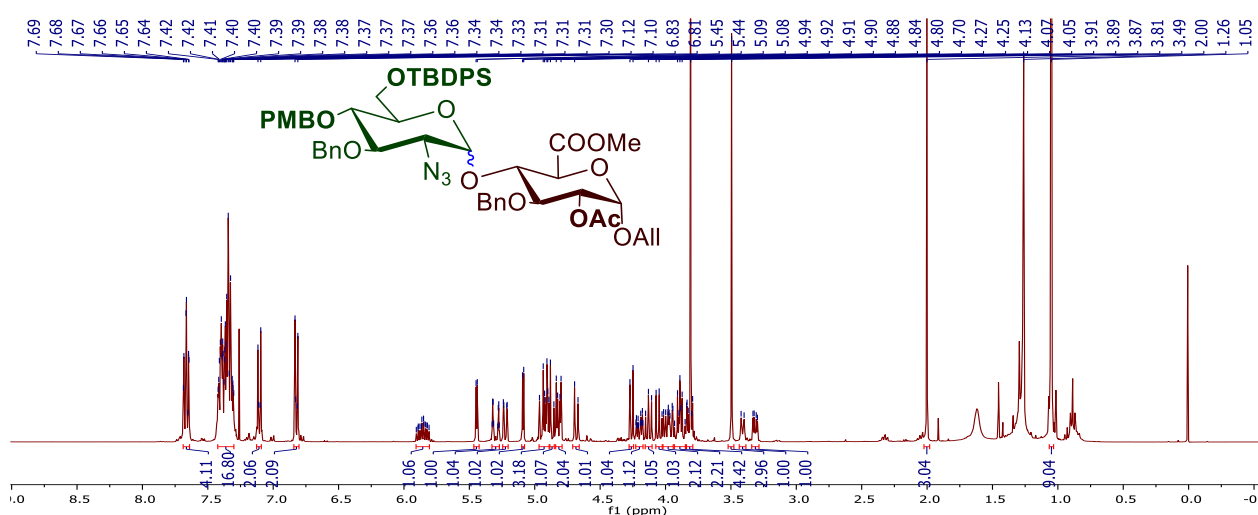
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69c**



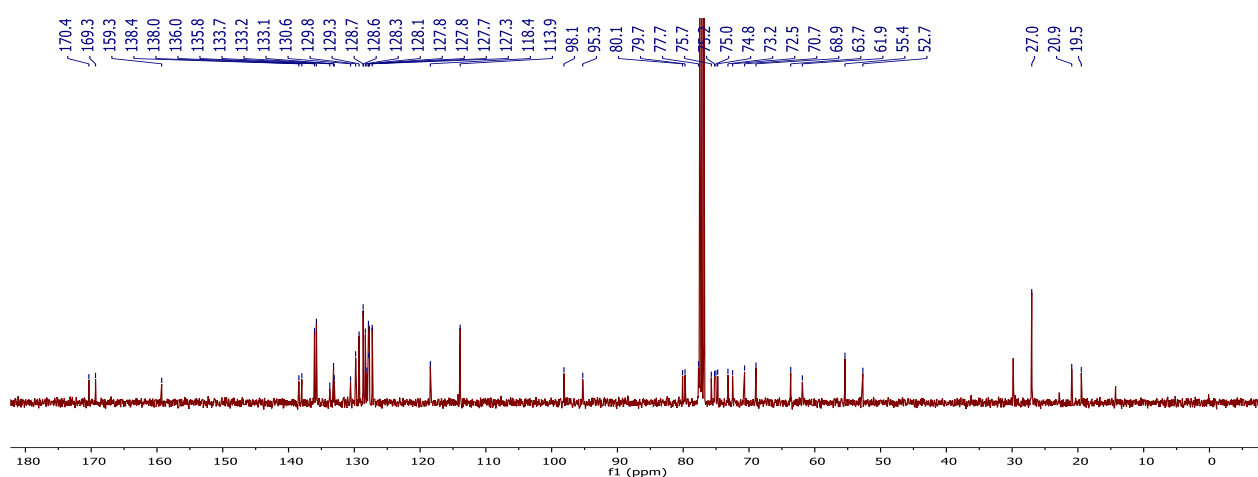
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69c**



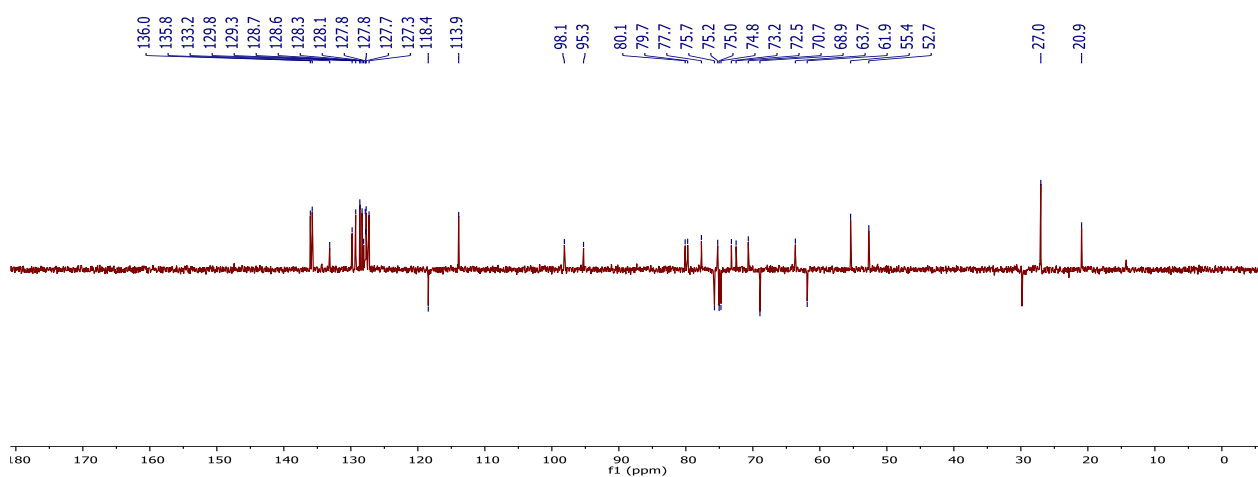
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69d**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69d**

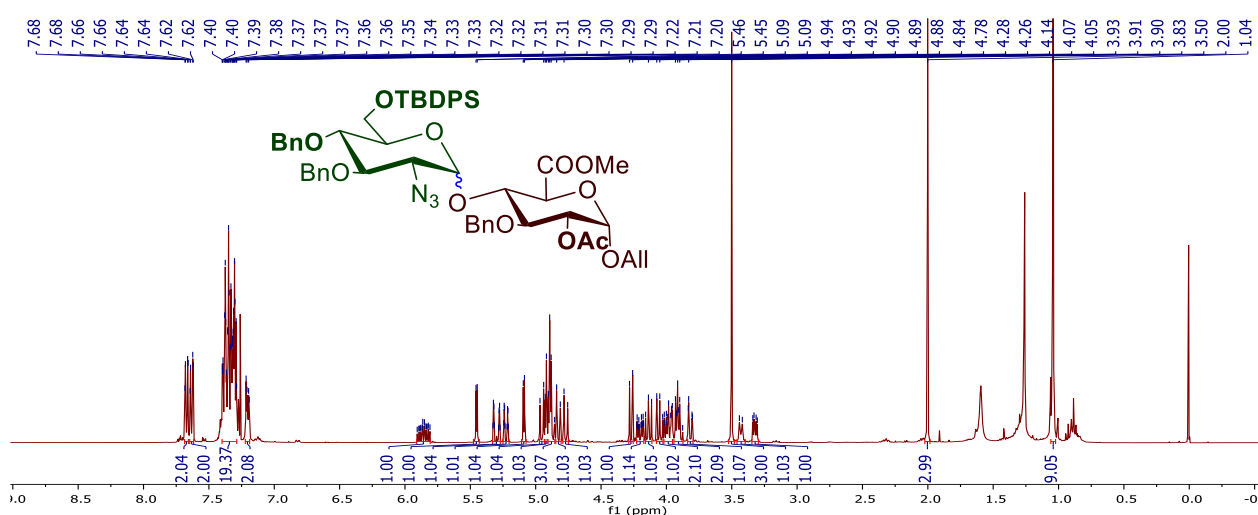


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69d**

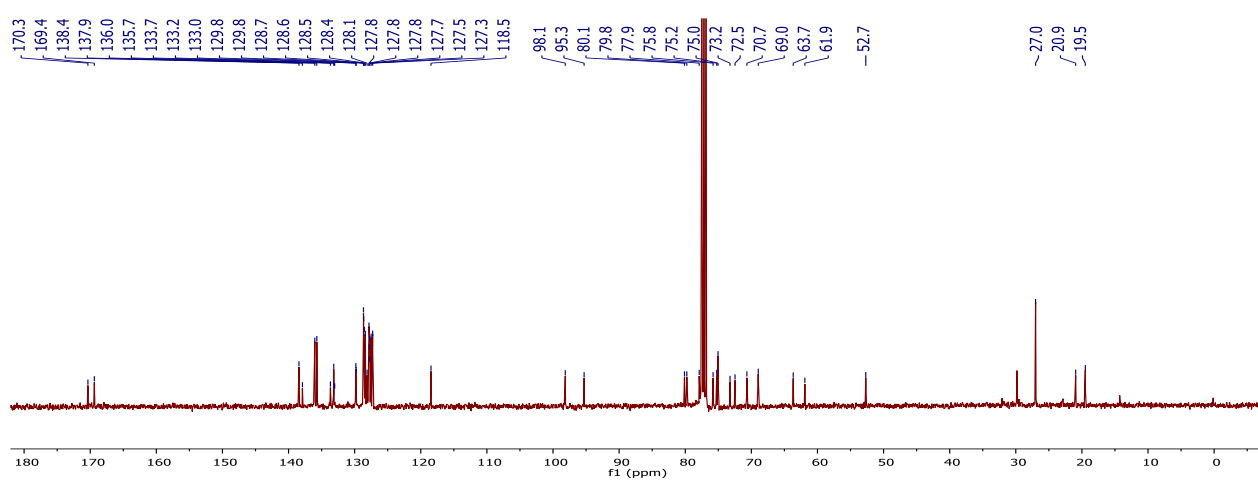




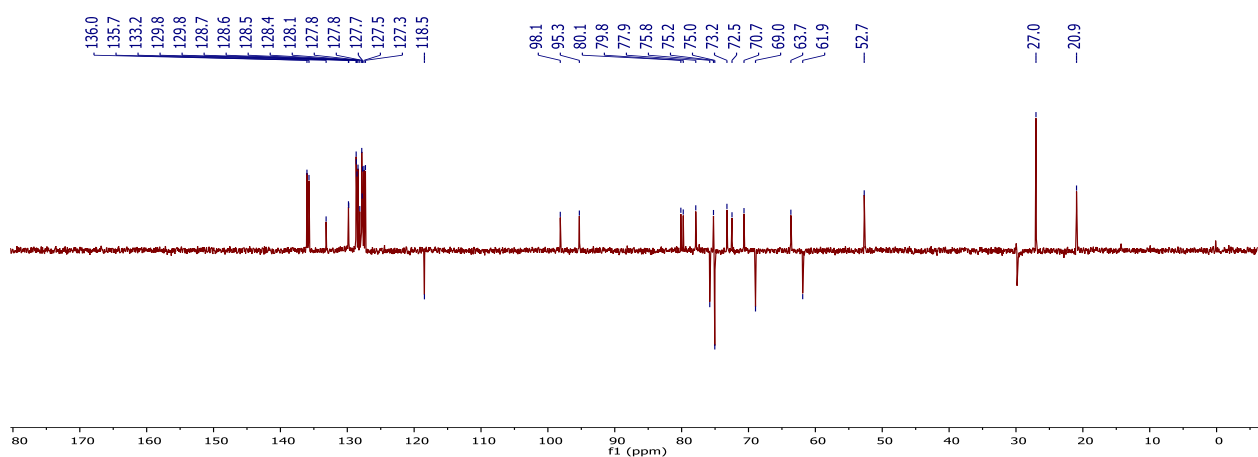
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69e**



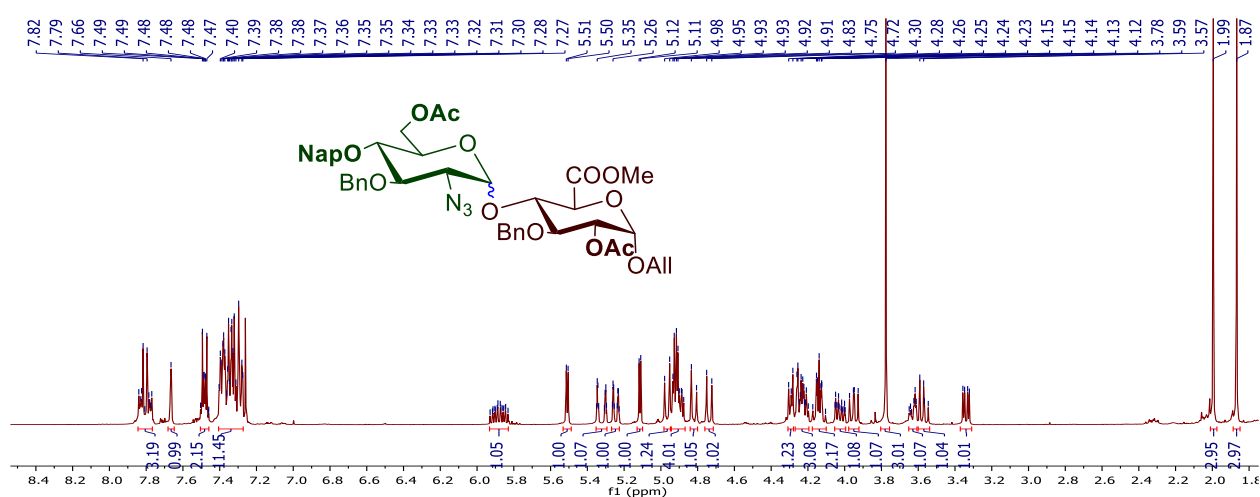
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69e**



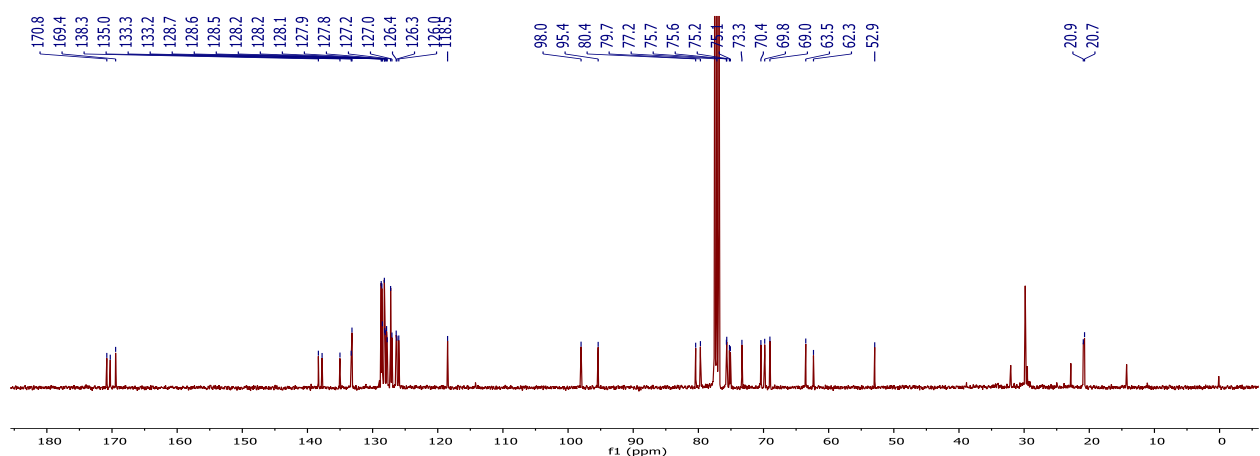
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69e**



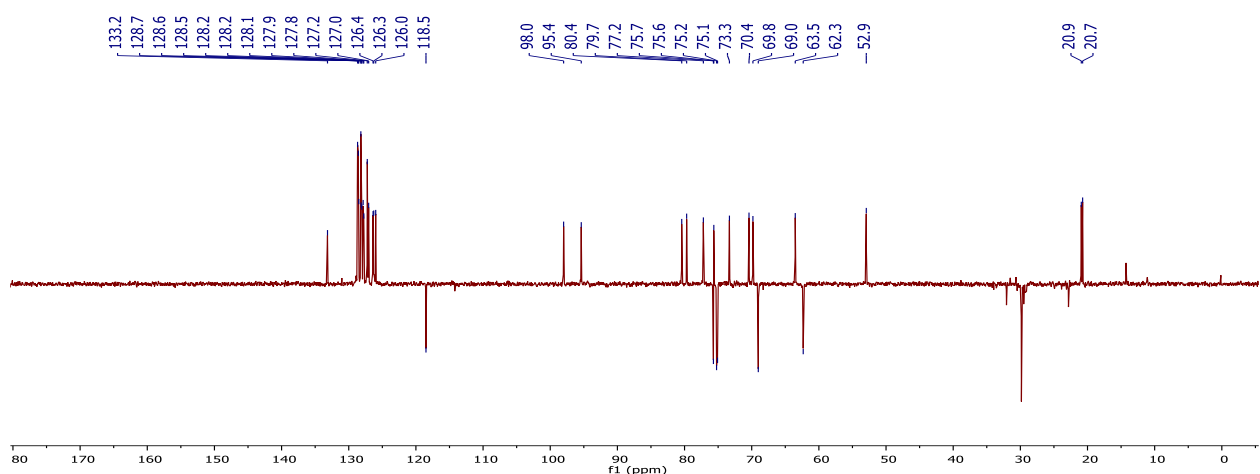
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69f**



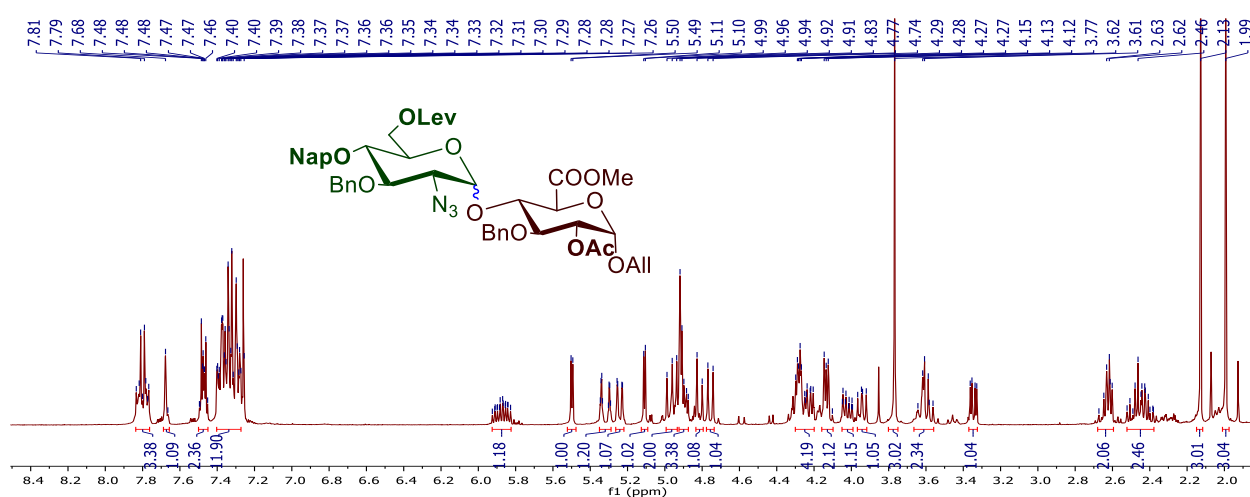
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69f**



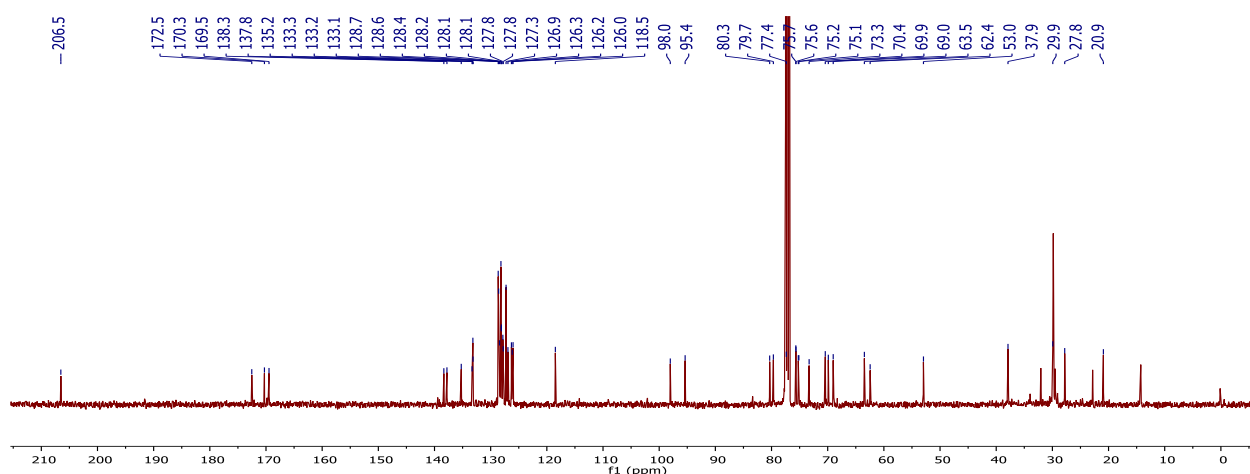
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69f**



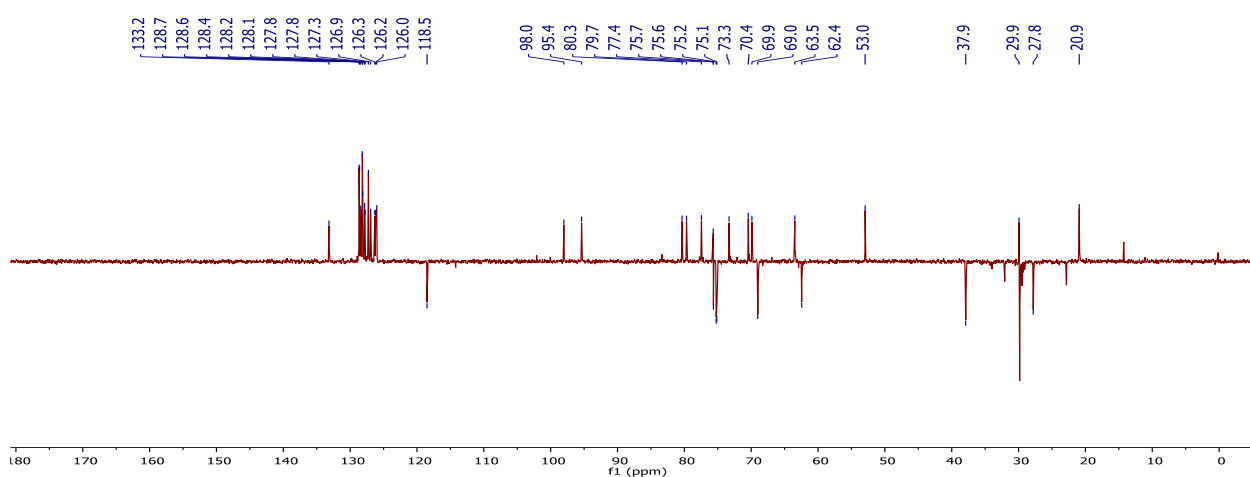
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69g**



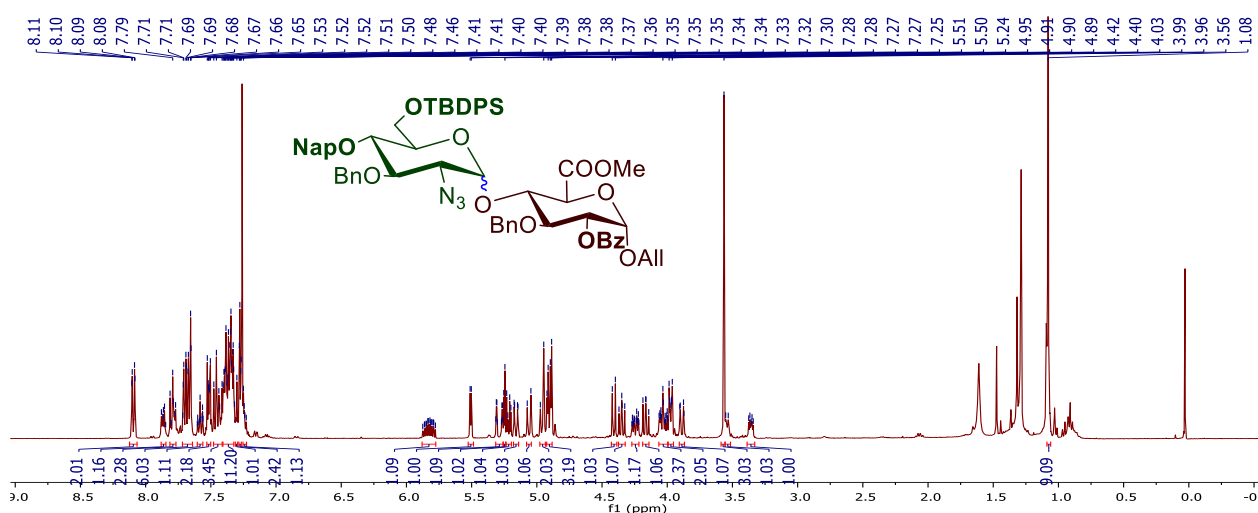
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69g**



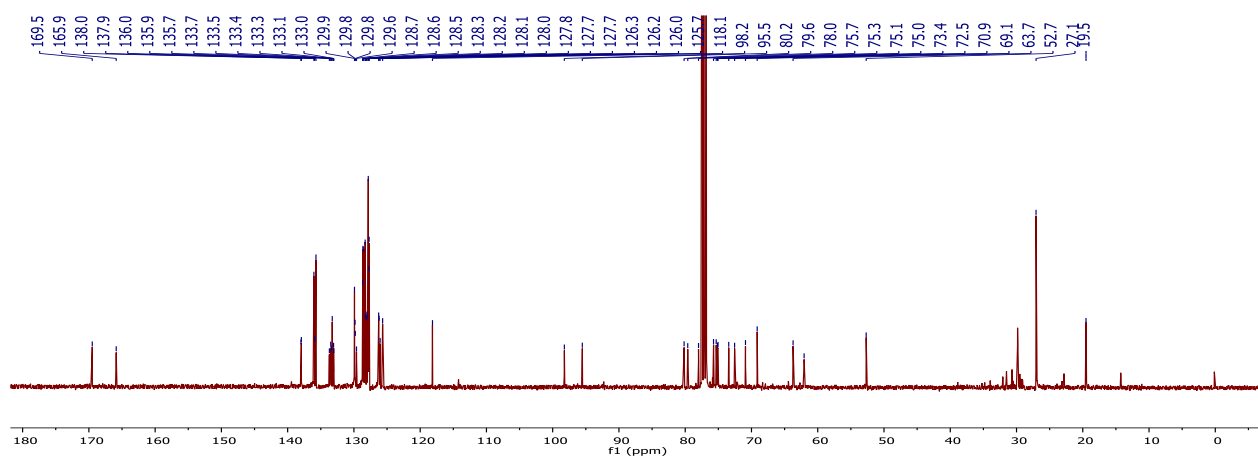
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69g**



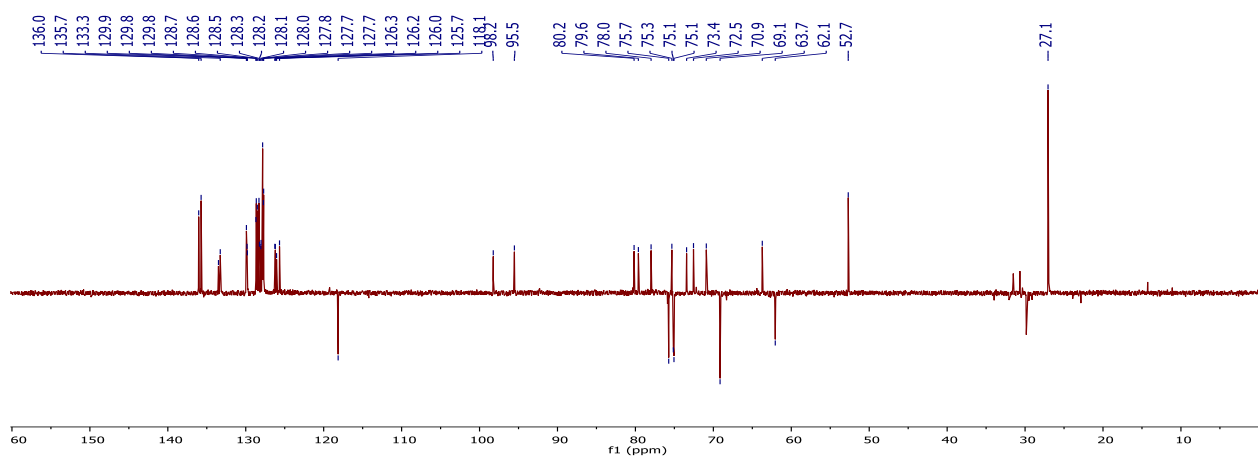
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69h**



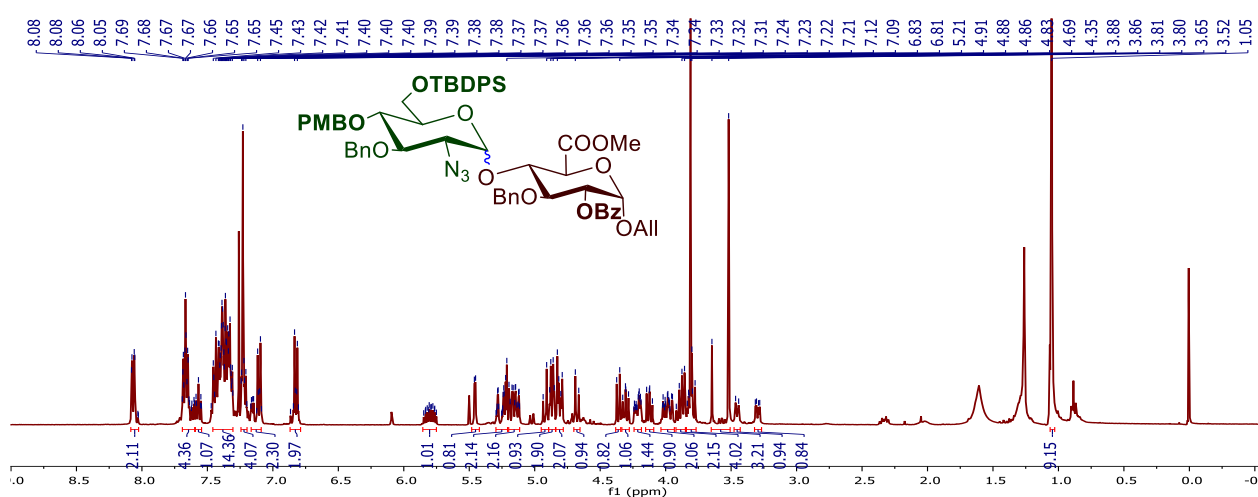
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69h**



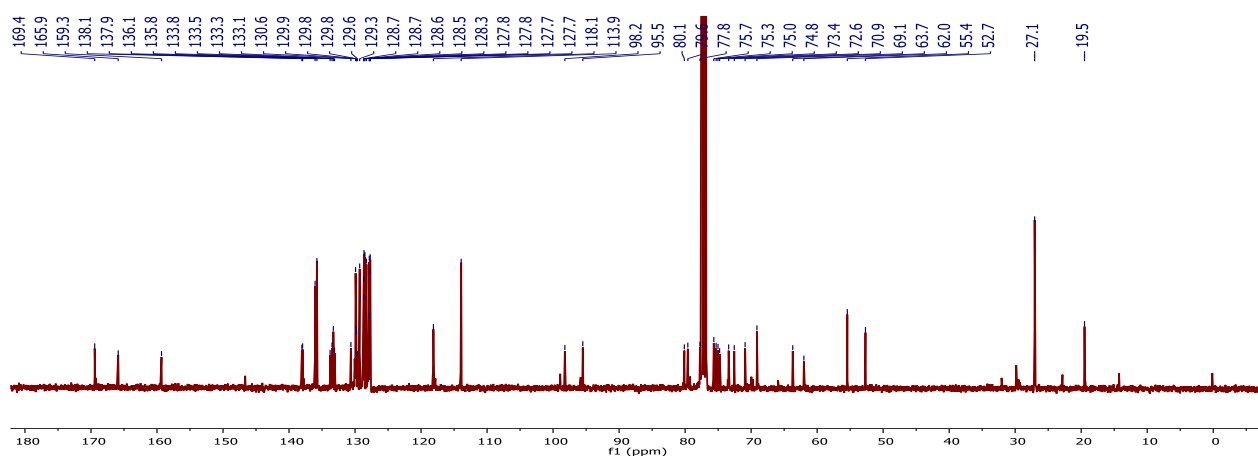
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69h**



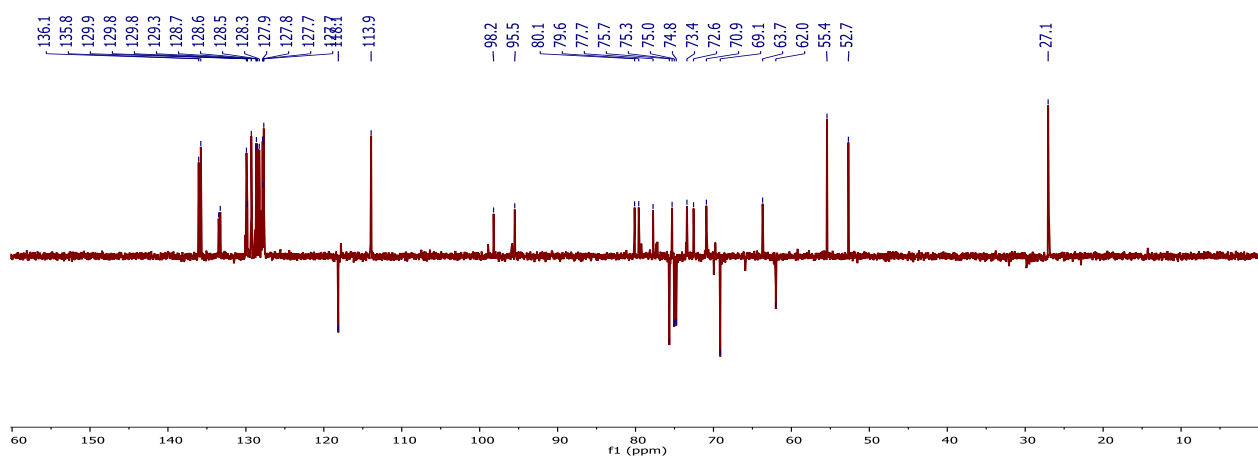
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69i**



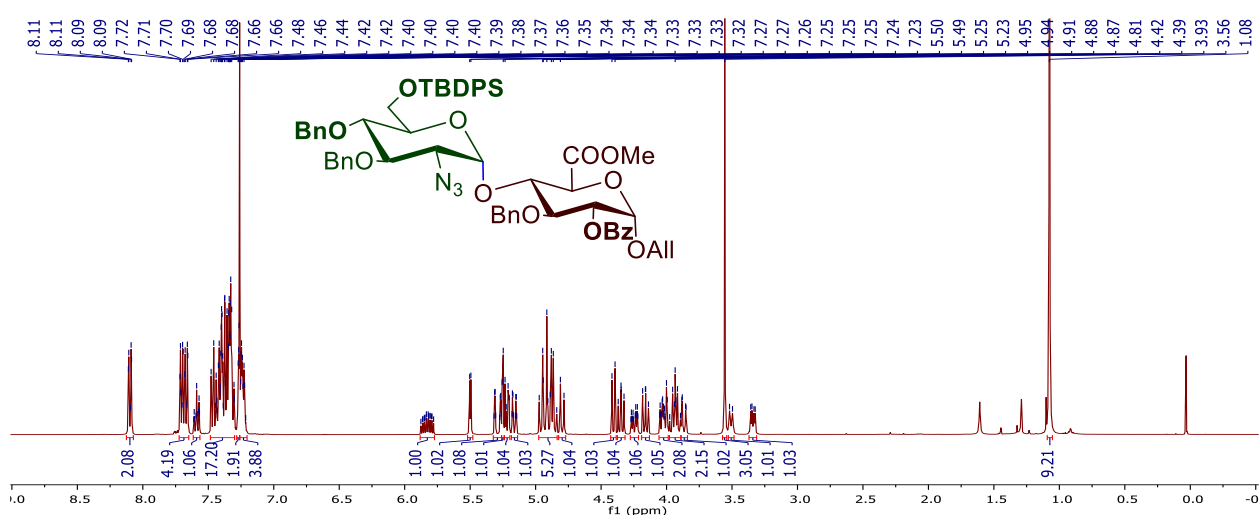
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69i**



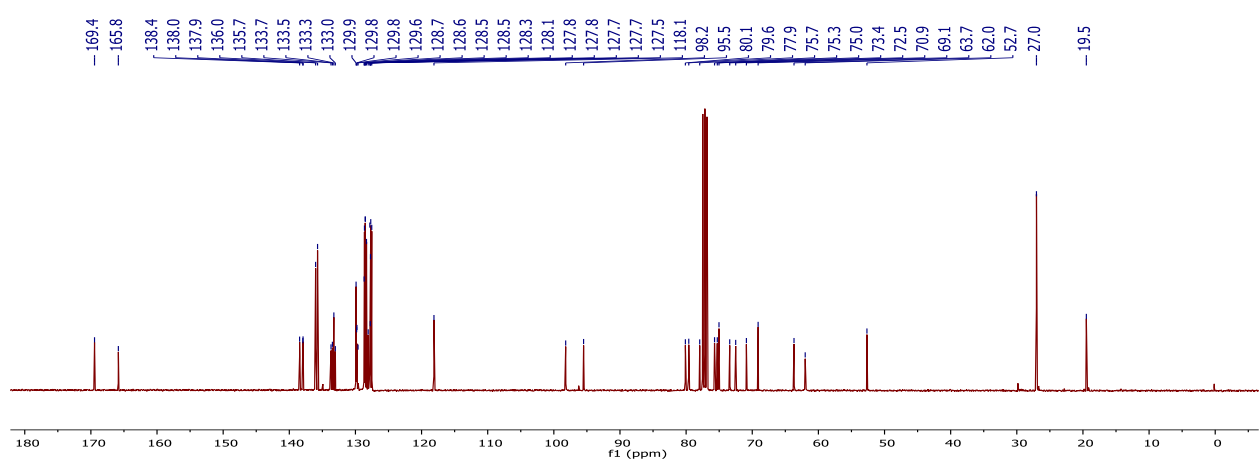
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69i**



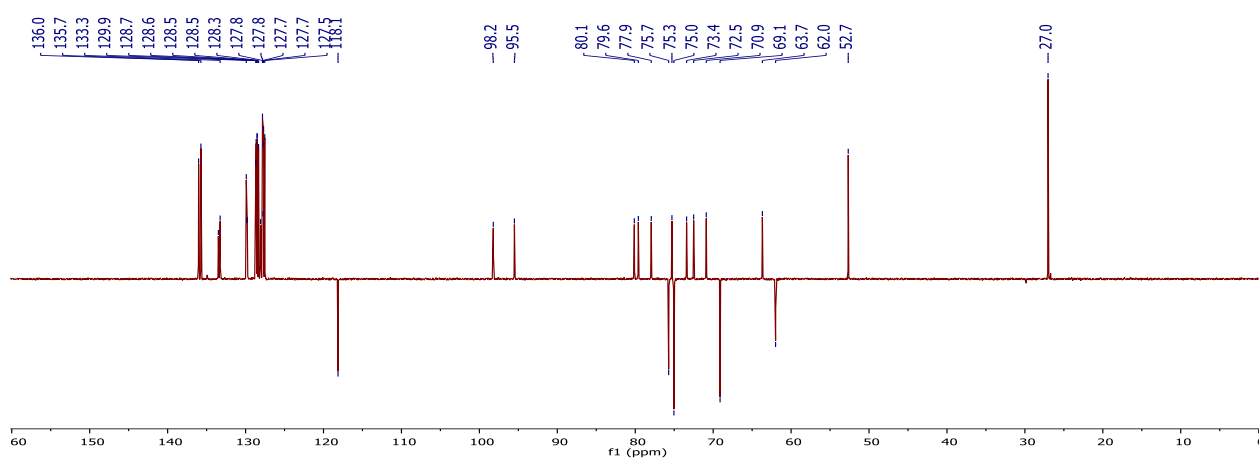
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69j (α isomer)**



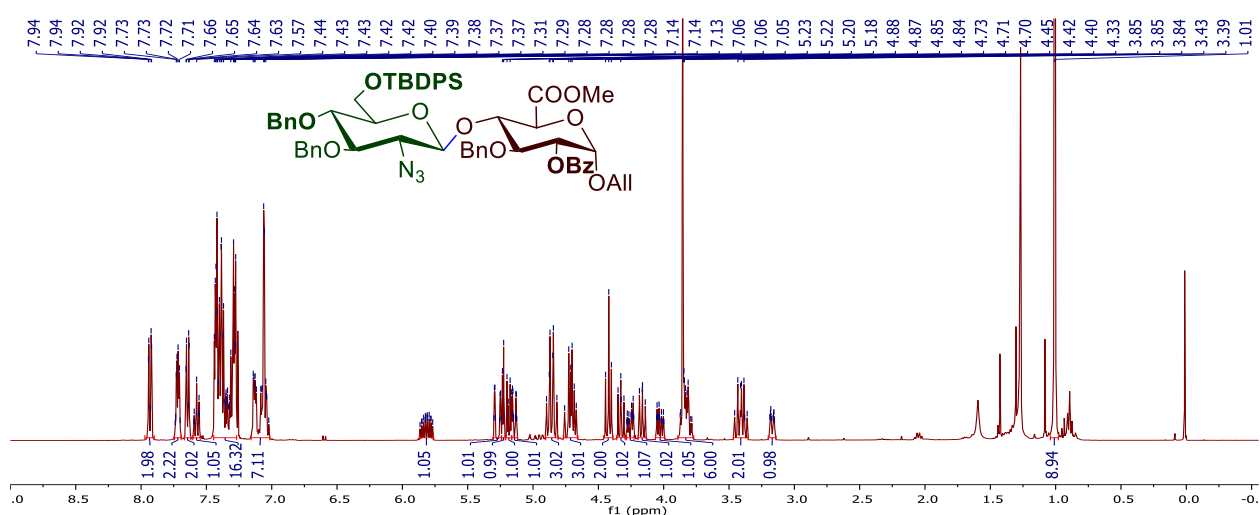
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69j (α isomer)**



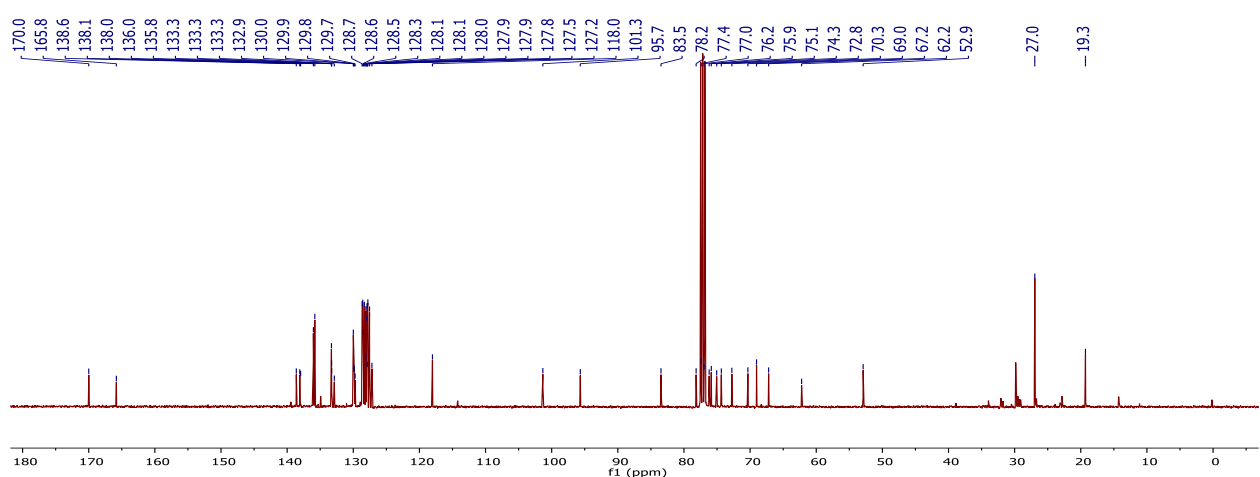
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69j (α isomer)**



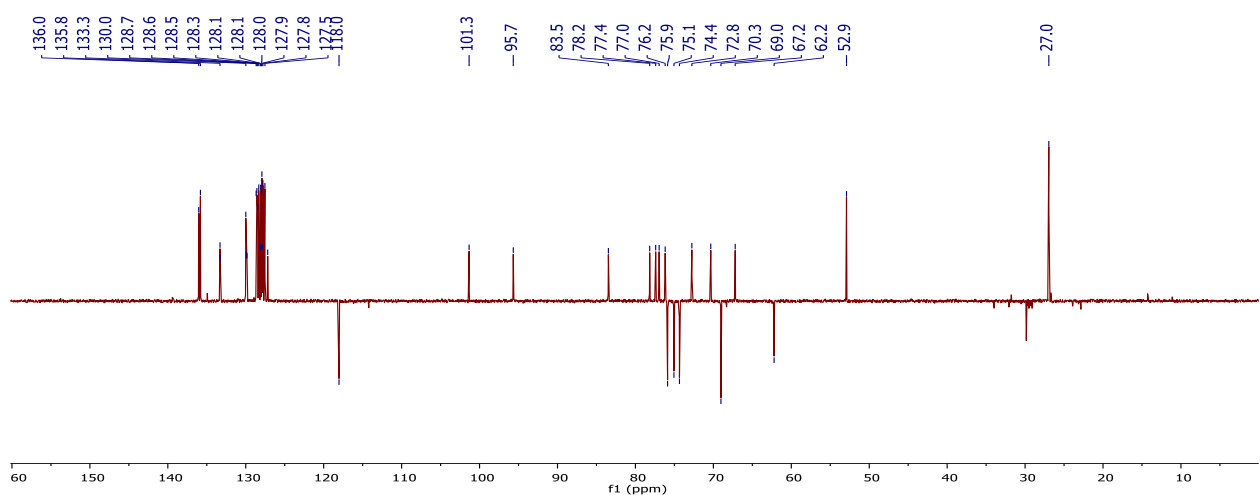
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 69j (β isomer)**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69j (β isomer)**



**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 69j (β isomer)**



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## Chapter 4

### *Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide*

### 4.1 Introduction:

Sulfated linear polysaccharides consisting of alternating disaccharide units of  $\alpha$ -1,4-linked glucosamine and either glucuronic acid or iduronic acid such as heparin (H) and heparin sulfate (HS) are present on the surface of most animal cells, membranes, and extracellular matrices.<sup>1</sup> They play pivotal role in diverse biological pathways including tumor metastasis, cell growth, cell adhesion, wound healing, inflammation, diseases of central nervous system etc.<sup>2</sup> Both H and HS are heavily *O*- and *N*-sulfated, they belong to glycosaminoglycan polysaccharides and are extracted and isolated from natural animal sources (porcine intestine or bovine lung or sometimes from turkeys, mice, camel, whales, lobsters, etc.).<sup>3</sup> H and HS are routinely used as anticoagulant drugs during major surgeries such as cardiopulmonary bypass, knee replacement, hip replacement in order to prevent occurrence of venous thrombosis.<sup>4</sup>

Porcine or bovine-derived Heparin has been used in clinics for many decades as an anticoagulant drug due to its strong binding affinity with anti-thrombin III thereby preventing the venous thrombosis.<sup>4</sup> Unfractionated heparin (UFH) ( $MW_{avg} \sim 15000$ ,  $\sim 45$  monosaccharide chains) and low molecular weight heparin (LMWH) ( $MW_{avg} \sim 6000$ ) are marketed for a long time.<sup>5</sup> Several LMWHs are marketed under different trade names such as Enoxaparin, Nadroparin, Reviparin, Dalteparin, Tinzaparin, Certoparin, Danaparoid depending on the type of depolymerization.<sup>6</sup> For example, Enoxaparin is isolated from UFH after  $\beta$ -eliminative cleavage employing alkali through peeling off reaction whereas Nadroparin is obtained by deaminative-cleavage employing nitrous acid.<sup>7</sup>

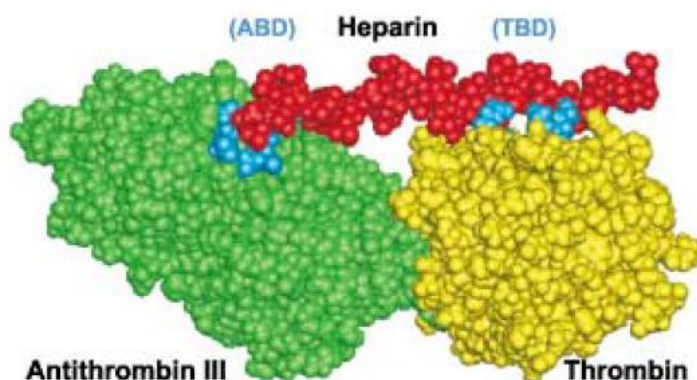


Figure 4.01: Molecular model of the ternary AT-III/heparin/thrombin complex.<sup>8</sup>

To recognize the heparin-binding site, molecular model of the ternary AT-III, heparin, and thrombin complex were designed by using the crystal structure of thrombin (Figure4.01).<sup>8</sup> It shows that heparin forms bridge between the positively charged regions of antithrombin and thrombin proteins with 6 to 8 sulfated monosaccharides, does not take part in the interaction

## Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

with positively charged protein residues. Mechanistic investigations revealed that the heparin binds to antithrombin with high affinity, brings in a conformational change thereby converting it to a rapid (1000x) inhibitor of thrombin (FIIa).<sup>9</sup>

Apart from thrombin, antithrombin interacts with coagulation factor Xa (FXa). LMWHs derived by chemical and/or enzymatic depolymerization procedures from UFH vary in their relative abilities to enhance the inhibition of FXa and FIIa (anti-FIIa) and also in their physicochemical properties. It has been noticed that specific FXa inhibitory activity increases as the mean molecular weight decreases. For example, UFH ( $MW_{avg} \sim 15000$ ) has Anti-FXa/Anti-FIIa activity ratio of 1.0 whereas the same ratio for Enoxaparin ( $MW_{avg} \sim 4200$ ) is 3.9 and Bemiparin ( $MW_{avg} \sim 3600$ ) was 8.0.<sup>7</sup> After the comprehensive structure-activity relationship studies, a unique pentasaccharide domain of heparin was found to be clinically effective as an antithrombin-binding domain (ABD), activates AT-III to produce those conformational changes which exclusively accelerate the inhibition of Factor Xa whereas longer heparin fragments comprising both the ABD and a thrombin-binding domain (TBD) are required for stimulating antithrombin activity (Figure 4.02).<sup>8</sup>

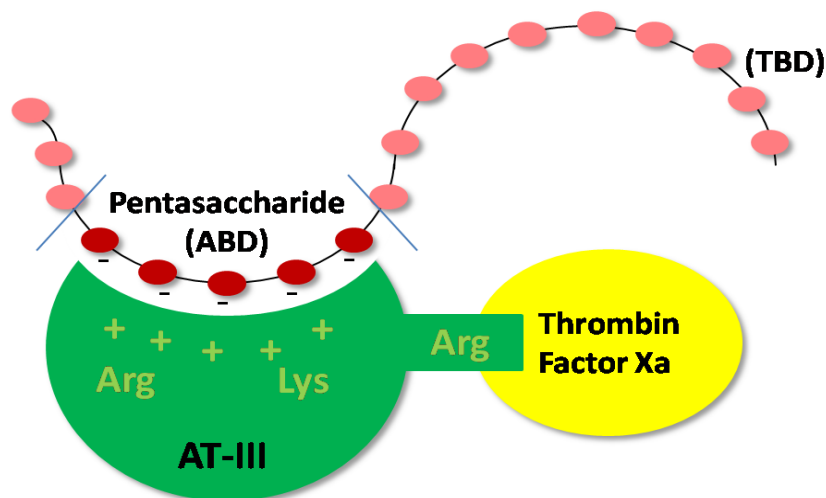


Figure 4.02: Cartoon representation of how heparin interacts with antithrombin III (AT-III).<sup>8</sup>

However, chemically and enzymatically extracted H and HS from animal sources suffer from microheterogeneity, presence of viral or prion contaminants; and hence, strongly influence their purity and quality from batch to batch.<sup>10</sup> The problem manifested into a pinnacle due to the worldwide distribution of contaminated animal-sourced heparin about a decade ago. Thus, the investigation of chemical synthesis to the heparin pentasaccharide that later led to the launch of the first synthetic anticoagulant antithrombotic Fondaparinux (Arixtra®) **70** in 2002 (Figure 4.03).<sup>8</sup> Fondaparinux ( $MW = 1725$ ) has well-controlled pharmacokinetic and pharmacodynamic properties, is free from any viral or prion impurities, and importantly, is a

specific FXa inhibitor. Extensive structure-property relationship studies proved that essential sulfate and carboxylic acid groups shall be located at opposite sides of the pentasaccharide. Despite its predictable anticoagulant dose and long half-life, Fondaparinux (**70**) is very expensive compared to animal-derived H and HS since its synthesis demands a long and tedious procedure diminishing the overall efficiency.<sup>11</sup> Indeed, synthesis of fondaparinux pentasaccharide and other heparin oligosaccharides is a herculean task due to intricacies involved in the installation and/or unblocking of multiple orthogonal protecting groups.

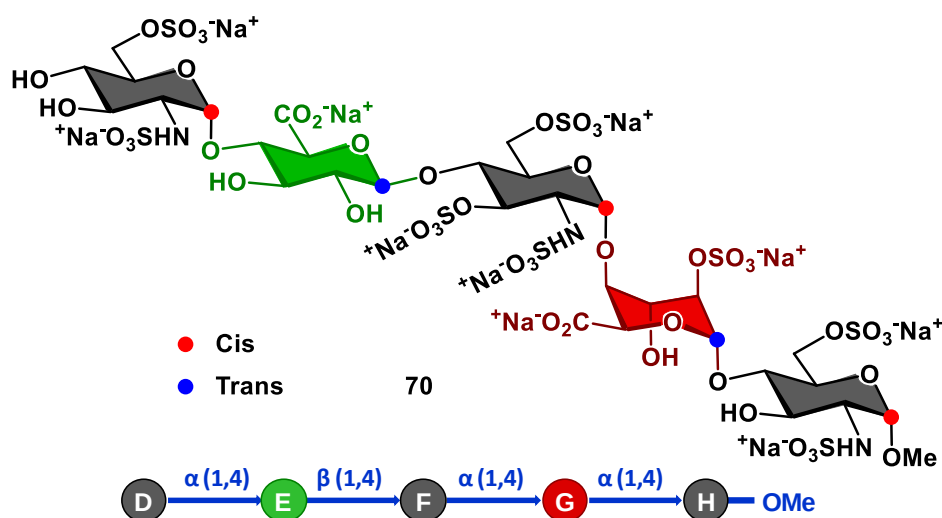
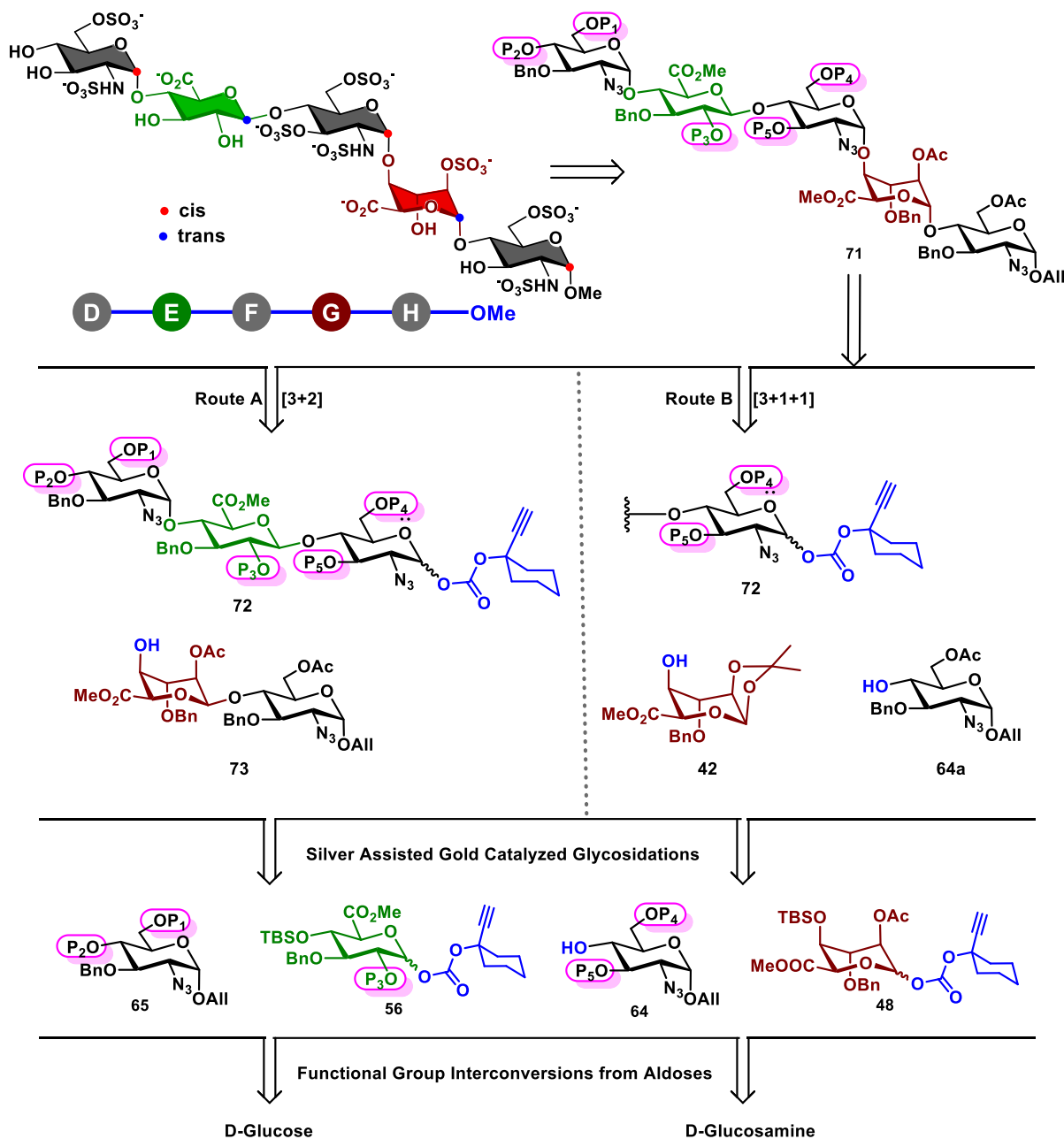


Figure 4.03. Structure of Fondaparinux

Since the first heroic synthesis of the pentasaccharide by Petitou in 1987,<sup>12a</sup> several synthetic strategies have been reported for the synthesis of heparin fragments involving stepwise glycosylation invoking many protecting groups and glycosylation protocols. Given the complexity of Fondaparinux, very few total syntheses<sup>12</sup> are reported to date and they mainly focused on the following aspects in order to improve the overall yield: (i) optimizing chemistry of individual monosaccharides; (ii) identification of right pairs of orthogonal protecting groups; (iii) stereoselective glycosylation chemistry (chapter 1, section 1.5). On the contrary, HS polymerase, sulfotransferases, and epimerases were employed for the enzymatic synthesis of the Fondaparinux. In spite of these, access to differentially substituted derivatives is very significant for the structure-property relationships of the Fondaparinux. Reported syntheses of the pentasaccharide **70** till date have modeled their convergent or linear or multiple one-pot strategies either by a 3+2 or 3+1+1 combination of modular saccharide building blocks (chapter 1, section 1.5.5). A rapid and facile synthetic strategy for compound **70** that also enables the creation of diverse molecular entities that differ in sulfation pattern is still in demand.

## 4.2 Retrosynthesis of the Pentasaccharide:



Scheme 4.01: Retrosynthesis of the Fondaparinux Pentasaccharide

The synthesis of Fondaparinux pentasaccharide by employing our recently discovered silver-assisted gold-catalyzed glycosidations {Chapter 3, Ref. 10} on strategically designed monosaccharide building blocks was targeted. The synthesis of Fondaparinux pentasaccharide **70** was envisioned from the regioselectively protected pentasaccharide **71**. Trisaccharides **72** was chosen as the key precursor for effecting either 3+2 glycosylation using disaccharide **73** or 3+1+1 elongation by coupling iduronate **42** followed by the azido-derivative **64a**. Trisaccharides **72** can be synthesized by stepwise glycosylation using alkynyl glycosyl

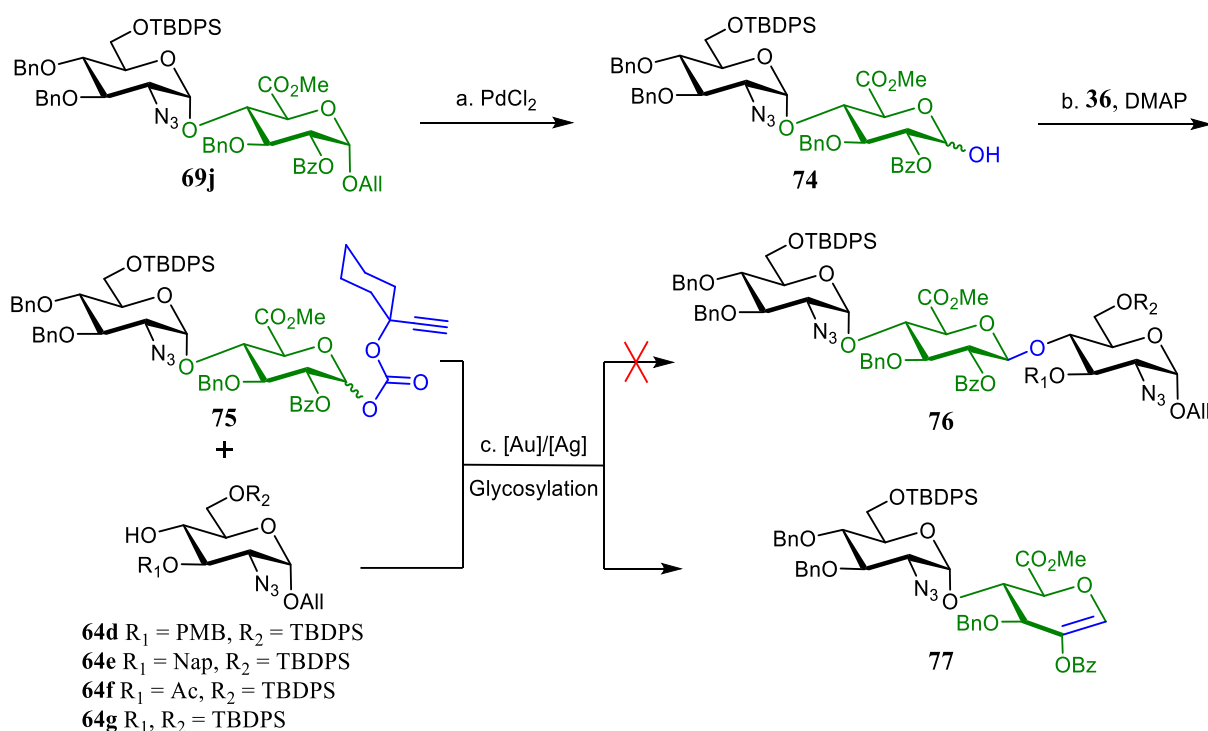
carbonates and Au- and Ag- salts as catalysts. Iduronates (**42**, **48**) and glucuronate **56** were envisaged from D-Glucose whereas building blocks **64** and **65** were imagined from D-Glucosamine (Scheme 4.01). Importantly, the building blocks were designed and synthesized by a diverse route that efficiently produced variety of different orthogonal combinations of the donors as well as acceptors (see Chapter 2 and 3). The donor-acceptor  $\alpha,\beta$  selectivity study from Chapter 3 optimized the glycosidation conditions and confirmed that the D-E disaccharide **69j** is having higher  $\alpha$ -selectivity with the better R<sub>f</sub> difference between anomeric diastereomers.

### 4.3-4.6 Present Work

#### 4.3 Optimisation of GlcA glycosidation conditions for DEF-Trisaccharide Synthesis:

The next milestone is the synthesis of D-E-F Trisaccharide and it was envisioned through the glycosidation between D-E and F by [2+1] strategy. In this direction, allyl moiety of the disaccharide **69j** was smoothly unblocked by Pd-catalyzed reaction conditions in 1:1 mixture of CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub> to afford the hemiacetals **74** in 95% yield. Subsequently, compound **74** was treated with the carbonate reagent **36** in the presence of DMAP in CH<sub>2</sub>Cl<sub>2</sub> to afford D-E disaccharide donor **75** in 85% yield that can be used for the synthesis of D-E-F trisaccharide (Scheme 4.02).

Donor **75** was subjected to the silver-assisted gold catalysed glycosidation reactions with glucosamine acceptors. These acceptors are carefully selected for the orthogonal protections of the expected D-E-F trisaccharide. C6-OH was protected as a silyl ethers using TBDPSCl whereas C3-OH was protected with *p*-methoxybenzyl ether (**64d**) or naphthalyl ether (**64e**) or acetate (**64f**) or silyl ether (**64g**) that can give rare C3-*O*-sulfate during the final stages. However, glycosidation of disaccharide donor **75** with aglycons **64d-64g** failed to produce desired trisaccharide **76**. Instead, it resulted in the isolation of 1,2-eliminated disaccharide product **77** in yields ranging from 45-70% (Scheme 4.02). The <sup>1</sup>H, <sup>13</sup>C and DEPT NMR spectroscopic analysis<sup>13</sup> and the HRMS (ESI-MS) analysis, m/z calculated for C<sub>57</sub>H<sub>59</sub>N<sub>3</sub>O<sub>11</sub>Si[M+Na]<sup>+</sup>: 1012.3817, Found: 1012.3810 was confirmed formation of the illustrated structure of compound **77**. Downfield shifting of one of the anomeric carbon (-CH) and formation of the quaternary carbon centre of the C-2 position was clearly indicated from the resonances at  $\delta$  138.6 ppm and  $\delta$  129.9 ppm respectively. All the remaining residual carbons and the <sup>1</sup>H NMR spectrum matching perfectly with the depicted structure.



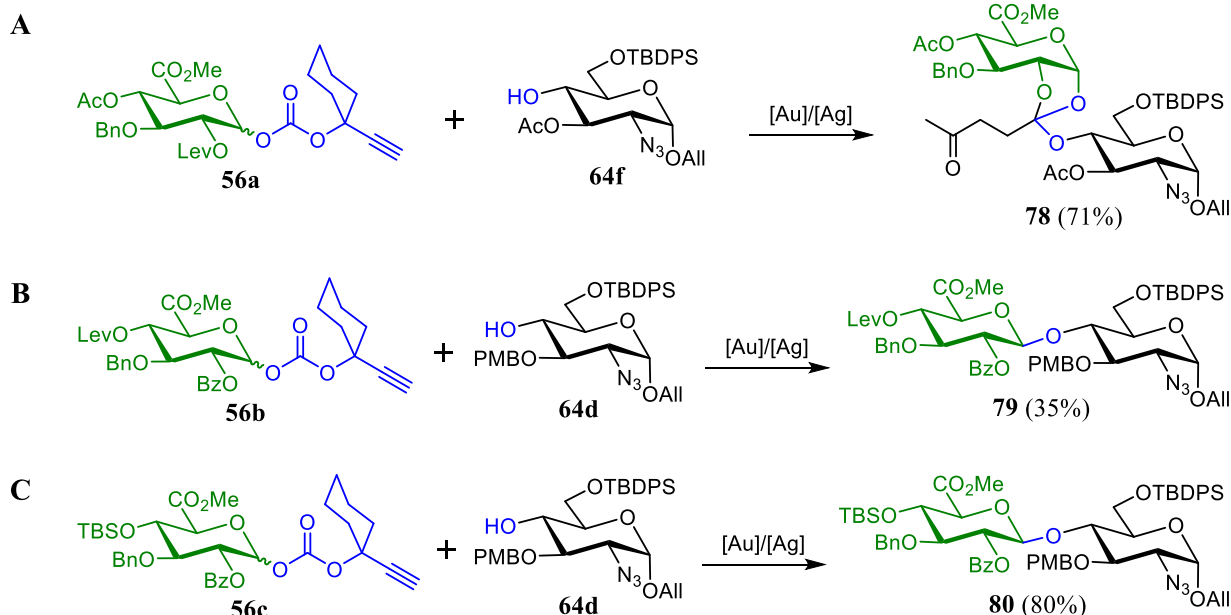
*Scheme 4.02: Trisaccharide was envisioned via [2+1] strategy by D-E and F glycosidation.*

Reagents. (a)  $\text{PdCl}_2$ ,  $\text{MeOH-CH}_2\text{Cl}_2$  (3:1), 25  $^\circ\text{C}$ , 4 h, 95%, (b) **36**, DMAP,  $\text{CH}_2\text{Cl}_2$ , 25  $^\circ\text{C}$ , 3 h, 85%; (c) 8mol% each of **68** and  $\text{AgOTf}$ ,  $\text{CH}_2\text{Cl}_2$ , 4Å MS, 25  $^\circ\text{C}$ , 15 min, **77**: 45-70%.

Discouraged by the above results while executing the DE+F [2+1] strategy for D-E-F trisaccharide, exploration of the D+EF [1+2] glycosidation strategy was investigated. In this direction, some of the selected orthogonal combinations of the E and F building blocks were envisioned for the E-F disaccharide. Accordingly, the glycosylation of donor **56a** with aglycon **64f** was performed and noticed that there is a formation of 1,2-orthoester **78** in the presence of C2-*O*-levulinate, instead of the formation of desired E-F disaccharide (scheme 4.03A). The  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT NMR  $^{13}\text{C}$  spectral signals, quaternary carbon signal at  $\delta$  124.3 ppm and the absence of levulinoxyl carbon signal, along with perfectly-matching of all other resonances was clearly indicated the formation of levulinyl orthoester **78**. In addition, it was confirmed by HRMS (ESI-MS) analysis,  $m/z$  calculated for  $\text{C}_{48}\text{H}_{59}\text{N}_3\text{O}_{15}\text{Si}[\text{M}+\text{Na}]^+$ : 968.3613, Found: 968.3600. However, the change in donor-acceptor combination to **56b** and **64d** produced the desired E-F disaccharide **79** albeit in poor yield due to the loss while separating the desired E-F disaccharide from myriad of uncharacterized products (Scheme 4.03B). Interestingly, alteration of the 4-*O*-levulinate of donor **56b** to the 4-*O*-silyl ether as a donor **56c** afforded the desired E-F disaccharide **80** in 80% yield. Still the polarity of the acceptor **64d** and the product **80** were almost similar thereby warranting multiple flash purifications (Scheme 4.03C). In addition,

## Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

hydrolysis of the TBS-ether in the presence of TBDPS-ether also diminished the net yield in the subsequent step.



*Scheme 4.03: Optimisation of the glucuronic acid glycosidation for EF disaccharide synthesis.*

Reagents. 8mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4 Å MS, 25 °C, 15 min.

Thus, the C6-*O*-silyl ether of the acceptor **64d** was altered to C6-*O*-benzoate as in acceptor **64a** and performed the glycosylation reaction with donor **56c** to obtain the desired E-F disaccharide **81** with 1,2-*trans* interglycosidic-linkage in an excellent 91% yield (Scheme 4.04). Continuing our synthesis, cleavage of the 4-*O*-silyl ether of the E-F disaccharide **81** was smoothly achieved under HF•pyridine reaction conditions to obtain E-F disaccharide acceptor **82**. Compound **82** was subjected to the silver-assisted gold catalyzed glycosidation with the glycosyl donor **67c** under the optimised reaction conditions (Chapter 3, Table 3.1) to accomplish the desired D-E-F trisaccharide **83** in 90% yield.

Gratifyingly, D-E-F trisaccharide **83** was noticed that the glycosidation reaction was happened in a completely stereoselective fashion and resulted in a single 1,2-*cis* or  $\alpha$ -anomer at -40 °C. Anomeric mixture of D-E-F trisaccharides was noticed above -20 °C. Symbolized structure of the trisaccharide **83** was identified by careful NMR spectroscopic analysis<sup>13</sup> (**Table 4.1**). In addition, it was confirmed by HRMS (ESI-MS) analysis, *m/z* calculated for C<sub>81</sub>H<sub>86</sub>N<sub>6</sub>O<sub>18</sub>Si[M+Na]<sup>+</sup>: 1481.5666, Found: 1481.5657 and from the IR spectra which having sharp signal at 2107 cm<sup>-1</sup> specifying the presence of azide moiety.

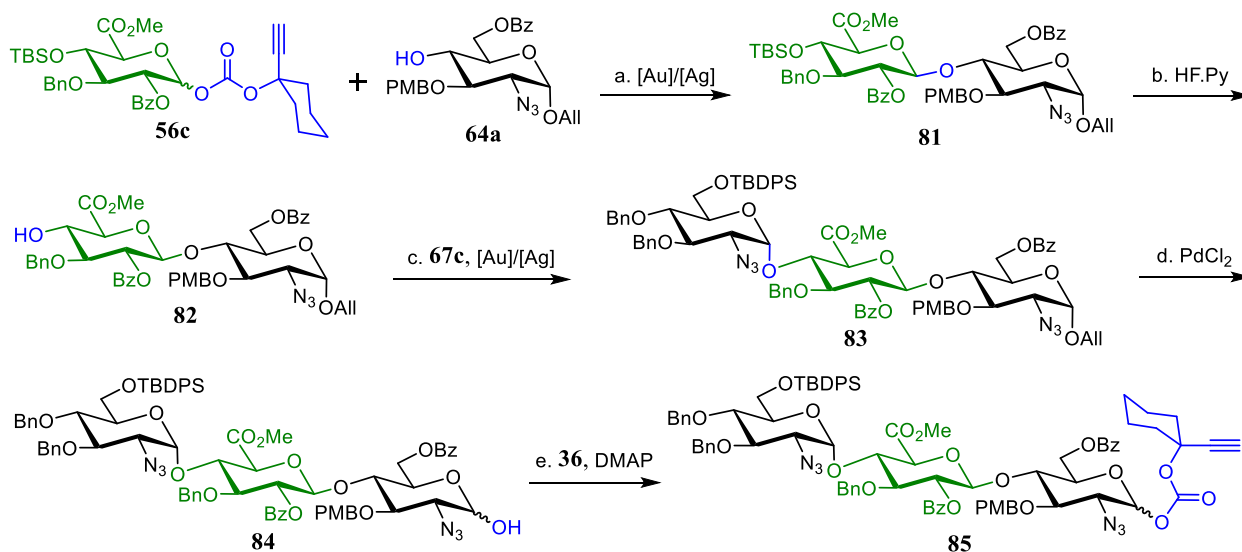
| Entry | <sup>1</sup> H NMR ( $\delta$ ppm) | <sup>13</sup> C NMR ( $\delta$ ppm) |
|-------|------------------------------------|-------------------------------------|
|-------|------------------------------------|-------------------------------------|



|                                   |                                                                                                                                   |                                                                  |
|-----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| Anomeric                          | 5.40 (d, $J = 3.7$ Hz, 1H), 5.04 (d, $J = 10.3$ Hz, 1H), 4.91 – 4.80 (merged)                                                     | 101.2, 97.7, 96.6                                                |
| Allyl                             | 5.83 (dddd, $J = 16.6, 10.4, 6.2, 5.3$ Hz, 1H), 5.23 (dq, $J = 17.2, 1.5$ Hz, 1H), 5.15 (dq, $J = 10.4, 1.2$ Hz, 1H)              | 133.1, 118.4, 68.8                                               |
| TBDPS                             | 7.15-7.45 (10H, merged), 1.05 (s, 9H)                                                                                             | 136.0 (2C), 135.7 (2C), 133.7, 133.0, 27.0(3C), 19.5             |
| -COOMe                            | 3.73 (s, 3H)                                                                                                                      | 167.9, 52.6                                                      |
| PMB                               | 7.22 – 7.17 (m, 2H, merged), 6.85 – 6.80 (m, 2H), 3.40 (s, 3H)                                                                    | 159.3, 130.4, 129.9, 129.8, 113.9 (2C), 74.6, 55.3               |
| Benzoate                          | 8.09 (dd, $J = 8.3, 1.3$ Hz, 2H), 7.95 (dd, $J = 8.4, 1.3$ Hz, 2H), 7.69 – 7.63 (m, 4H), 7.58 – 7.53 (m, 1H), 7.51 – 7.46 (m, 1H) | 166.1, 165.0, 133.7, 133.5, 130.2 (2C), 129.9 (2C), 129.6, 129.1 |
| Benzyl                            | 7.45-7.30 (m, 15H, merged), 4.9-3.3 (6H, merged),                                                                                 | 138.4, 137.9, 137.4, 128.5-127.5 (15C), 75.7, 75.4, 75.1         |
| CH <sub>2</sub> (C <sub>6</sub> ) | 4.9-3.3 (4H, merged)                                                                                                              | 62.5, 61.9                                                       |

**Table 4.1: Characteristic <sup>1</sup>H and <sup>13</sup>C NMR signals of trisaccharide **83****

Further, trisaccharide **83** was extrapolated to the carbonate donor **85** in two aforementioned steps. Hydrolysis of the allyl glycoside was accomplished by Pd-catalysis using CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub> as a solvent mixture to obtain trisaccharide hemiacetals **84**. Subsequently, compound **84** was treated with the carbonate reagent **36** in the presence of DMAP in CH<sub>2</sub>Cl<sub>2</sub> to furnish the much desired D-E-F trisaccharide donor **85** (Scheme 4.04). Donor **85** was confirmed by the NMR, IR and mass spectral analysis<sup>13</sup> and utilized further for the synthesis of Fondaparinux pentasaccharide.

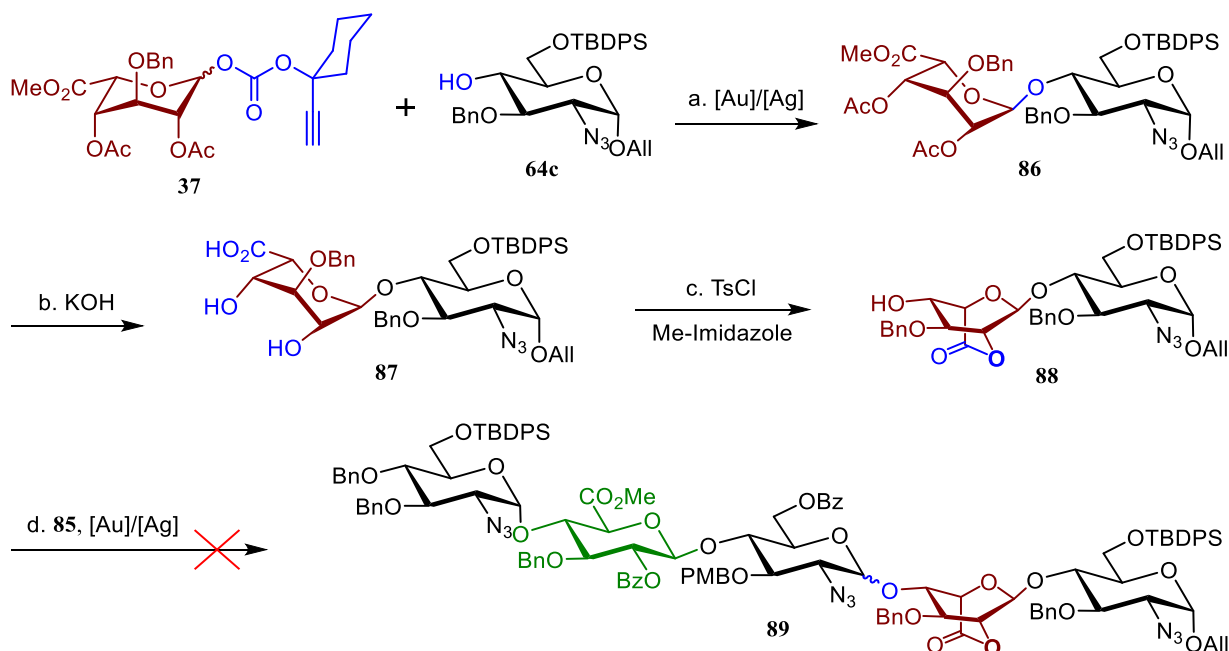


*Scheme 4.04: Synthesis of D-E-F trisaccharide donor via [1+2] strategy by D and E-F glycosidation.*

Reagents. (a) 8mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4 Å MS, 25 °C, 15 min, 91%; (b) HF•py, pyridine, 0-25 °C, 5 h, 89%; (c) 15mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, -40 °C, 1 h, 90% (α only); (d) PdCl<sub>2</sub>, MeOH-CH<sub>2</sub>Cl<sub>2</sub> (3:1), 25 °C, 4 h, 87%; (e) **36**, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 3 h, 88%.

## 4.4 [3+2] Glycosidation Strategy for the Synthesis of Fondaparinux Pentasaccharide:

After the trisaccharide donor synthesis, we moved on to accomplish the synthesis of Fondaparinux pentasaccharide. The orthogonally protected pentasaccharide was planned by the glycosidation of D-E-F trisaccharide and G-H disaccharide *via* [3+2] coupling strategy. Accordingly, the synthesis of G-H disaccharide **86** was accomplished by the silver-assisted gold catalysed glycosylation of the 2,4-O-acetate donor **37** and acceptor **64c** (Scheme 4.05). However, regioselective deprotection of 4-O-acetate in the presence of 2-O-acetate is cumbersome. Thus, disaccharide **86** was converted to L-iduronate [2,2,2]-bicyclic lactone **88**. The complete ester hydrolysis was carried out in the presence of potassium hydroxide in the THF/CH<sub>3</sub>OH/H<sub>2</sub>O as a solvent mixture to obtain carboxylic acid **87**. Subsequently, lactonization through tosylate of the resulting diol-acid **87** afforded disaccharide acceptor **88**. However, at this stage, many attempts for glycosylation between D-E-F trisaccharide donor **85** and GH disaccharide aglycon **88** were unsuccessful to produce desired pentasaccharide **89** (Scheme 4.05).



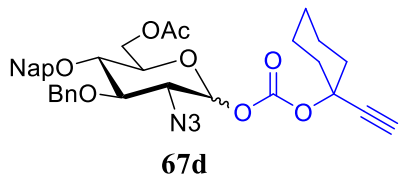
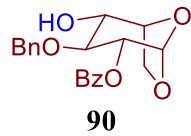
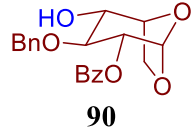
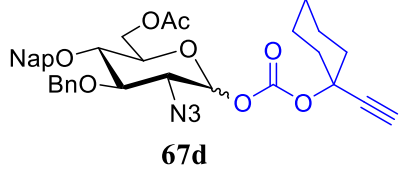
*Scheme 4.05: Preparation of the [2,2,2] bicyclic iduronate-acceptor as a GH disaccharide that was envisioned for pentasaccharide preparation.*

## Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

Reagents. (a) 8mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4 Å MS, 25 °C, 15 min, 91%; (b) KOH (3.0 eq), THF:CH<sub>3</sub>OH:H<sub>2</sub>O; then (c) TsCl (1.1 eq), methyl imidazole (2.2 eq), CH<sub>2</sub>Cl<sub>2</sub>, 3h, 72% over two steps.

Earlier studies by Hung group showed that bicyclic 1,6-anhydro-L-idose **90** undergoes the glycosidation. The difference between the compound **88** and compound **90** is that the former has a lactone ring and it is a [2.2.2]-bicyclic system whereas the latter has [3.2.1]-bicyclic system with an ether linkage and this is the nearest congener that has been reported in the literature. The model glycosidation between the carbonate donor **67d** and the Hung's derivative **90** underwent without any problems {monitored by TLC-MS} (**Table 4.2**); similarly, the glycosidation performed between the trisaccharide donor **85** and the aglycon **90** also was successful based on the TLC-MS. However, the glycosidation between donor **67d** and the aglycon **88** failed to show any progress thereby signifying the inherent problem with the substrate.

**Table 4.2:** Cross-checking experiments

| S. No. | Donor                                                                                             | Acceptor                                                                                         | Inference based on TLC-MS |
|--------|---------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------|
| 1      | <br><b>67d</b>  | <br><b>90</b> | Product observed          |
| 2      | <b>85</b>                                                                                         | <br><b>90</b> | Product observed          |
| 3      | <br><b>67d</b> | <b>88</b>                                                                                        | No reaction               |

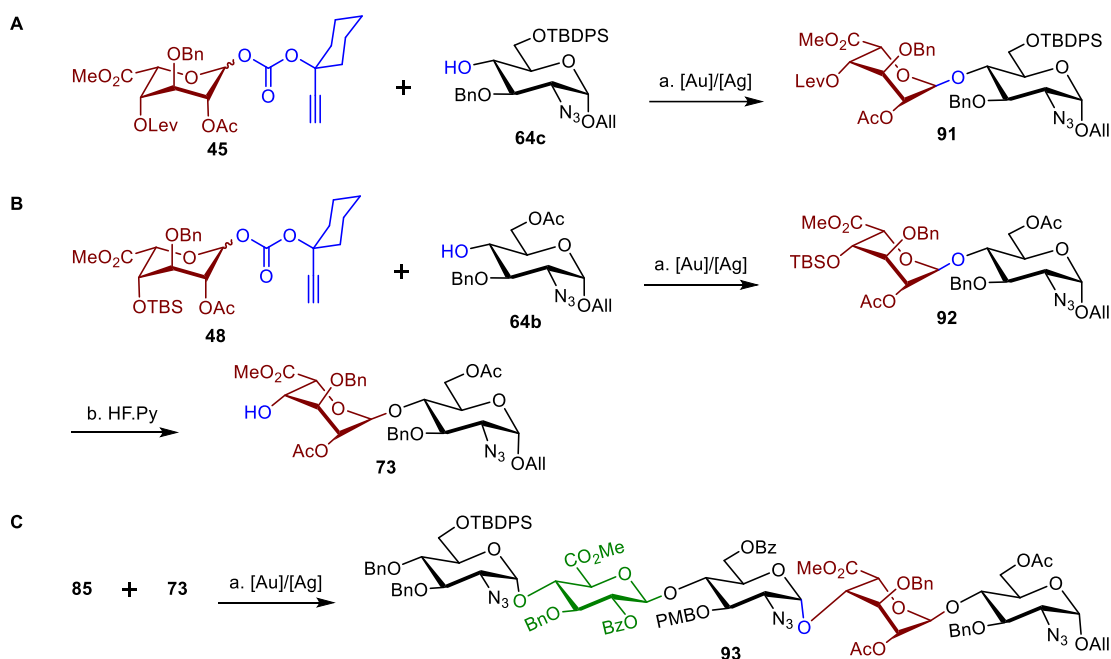
Further, in continuation to the [3+2] strategy for pentasaccharide synthesis, the G-H disaccharide was envisaged from the 4-O-L-iduronate donor **45**, thereby differing the C-2 and C-4 functionality. Accordingly, donor **45** and acceptor **64c** were subjected to the glycosylation to notice the formation of disaccharide **91** albeit in poor yield due to the formation of many products which could not be isolated and characterized (Scheme 4.06A). However, reactivity of the glucuronate donors increased by the replacement of the 4-O-levulinate of **56b** to the 4-O-silyl ether as in **56c** (see above scheme 4.03). Accordingly, donor **45** was replaced with 4-O-

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TBS protected donor **48** and performed the glycosylation with 6-*O*-acetate **64b** to afford the GH disaccharide **92** in excellent yield. Further, the silyl ether deprotection was carried out under HF•py conditions to afford the C4-OH acceptor **73** (Scheme 4.06B). Finally, acceptor **73** was subjected to the glycosylation with D-E-F trisaccharide donor **85** that was prepared *vide supra* (Scheme 4.05) to furnish much desired pentasaccharide **93** reminiscent of the Fondaparinux. Gratifyingly, the glycosylation reaction was accomplished the synthesis of pentasaccharide **93** with completely 1,2-*cis* or  $\alpha$ -selective manner in an excellent yield at ambient temperature (Scheme 4.06C). Assigned structure of the pentasaccharide **93** was verified from comprehensive NMR spectroscopic study<sup>13</sup> (Table 4.3). Furthermore, HRMS (ESI-MS) analysis, *m/z* calculated for C<sub>81</sub>H<sub>86</sub>N<sub>6</sub>O<sub>18</sub>Si[M+Na]<sup>+</sup>: 1481.5666, Found: 1481.5657 and the IR spectral data<sup>13</sup> was confirmed formation of the pentasaccharide.

| Entry                             | <sup>1</sup> H NMR (δ ppm)                                                                                                                         | <sup>13</sup> C NMR (δ ppm)                                                         |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Anomeric                          | 5.37 – 5.33 (m, 1H), 5.27 (d, <i>J</i> = 10.3 Hz, 1H), 5.03 (d, <i>J</i> = 10.3 Hz, 1H), 4.93 (d, <i>J</i> = 3.7 Hz, 1H), 4.90 – 4.85 (1H, merged) | 100.8, 97.9, 97.8, 97.1, 96.6                                                       |
| Allyl                             | 5.93 (ddt, <i>J</i> = 16.6, 11.0, 5.7 Hz, 1H), 5.39 (d, <i>J</i> = 3.7 Hz, 1H), 5.19 (d, <i>J</i> = 4.0 Hz, 1H)                                    | 133.1, 118.5, 68.8                                                                  |
| TBDPS                             | 7.45-7.10 (m, 10H, merged), 1.03 (s, 9H)                                                                                                           | 136.0 (2C), 135.7 (2C), 133.6, 132.9, 27.0(3C), 19.5                                |
| -COOMe                            | 3.84 (s, 3H, merged), 3.71 (s, 3H)                                                                                                                 | 169.3, 167.7, 52.7, 51.8                                                            |
| PMB                               | 7.15 (dd, <i>J</i> = 9.3, 7.2 Hz, 2H), 6.81 (d, <i>J</i> = 8.6 Hz, 2H), 3.36 (s, 3H, merged)                                                       | 159.3, 130.1, 129.9, 129.8, 113.8 (2C), 73.6, 55.3                                  |
| Acetate                           | 2.12 (s, 3H), 2.04 (s, 3H)                                                                                                                         | 170.9, 170.2, 21.0, 20.9                                                            |
| Benzoate                          | 8.08 (d, <i>J</i> = 7.7 Hz, 2H), 7.99 (d, <i>J</i> = 7.7 Hz, 2H), 7.65 (dd, <i>J</i> = 19.8, 7.0 Hz, 4H), 7.53 (t, <i>J</i> = 7.4 Hz, 2H)          | 166.0, 164.8, 133.9, 133.6, 130.2 (2C), 129.9 (2C), 129.5, 128.9                    |
| Benzyl                            | 7.45-7.10 (m, 25H, merged), 4.9-3.2 (10H, merged)                                                                                                  | 138.3, 137.9, 137.7, 137.4, 137.3, 129.0- 127.5 (25C), 75.8, 75.5, 75.0, 74.9, 74.8 |
| CH <sub>2</sub> (C <sub>6</sub> ) | 5.45-3.2 (6H, merged)                                                                                                                              | 62.2, 62.0, 61.8                                                                    |

**Table 4.3: Characteristic <sup>1</sup>H and <sup>13</sup>C NMR signals of the pentasaccharide 93**



*Scheme 4.06: Preparation of the GH disaccharide acceptor and its final glycosylation by [3+2] strategy to achieve the pentasaccharide reminiscent of the Fondaparinux.*

Reagents. (A) 8mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, 25 °C, 15 min; (B) (a) 8mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, 25 °C, 15 min, 89%; (b) HF•py, pyridine, 5 h, 0-25 °C, 86%; (C) 10mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, -10 °C to 25 °C, 30 min, 86% (α only).

#### 4.5 [3+1+1] Glycosidation Strategy for the Synthesis of Fondaparinux Pentasaccharide:

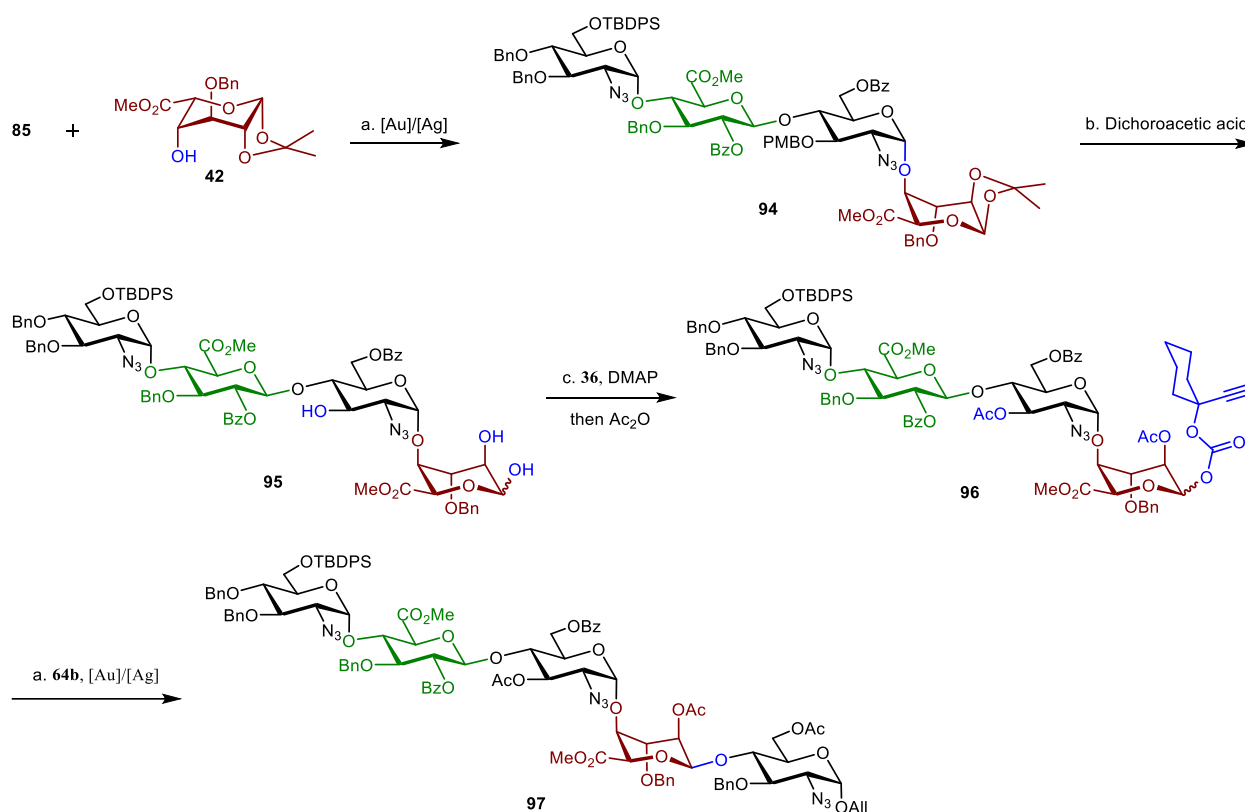
In addition, pentasaccharide reminiscent of the Fondaparinux was also envisioned from the [3+1+1] strategy by [D-E-F]+G+H glycosidation. In this direction, trisaccharide donor **85** was treated with the iduronate aglycon **42** under the [Au]/[Ag]-catalyzed glycosylation conditions to afford the tetrasaccharide **94** in 91% yield within 15 min at 25 °C (Scheme 4.07). Gratifyingly, the glycosidation between donor **85** and acceptor **42** resulted in the completely 1,2-*cis* or α-selectivity<sup>13</sup> at 25 °C presumably due to the presence of isopropylidene locking of the aglycon as noticed by Seeberger group.<sup>12g</sup> Symbolized structure of the tetrasaccharide **94** was identified by thorough NMR spectroscopic analysis<sup>13</sup> (Table 4.4). In addition, it was confirmed by HRMS (ESI-MS) analysis, *m/z* calculated for C<sub>95</sub>H<sub>102</sub>N<sub>6</sub>O<sub>24</sub>Si[M+Na]<sup>+</sup>: 1761.6612, Found: 1761.6615 and the IR spectra having sharp signal at 2109 cm<sup>-1</sup> specified the presence of azide moiety.

| Entry    | <sup>1</sup> H NMR (δ ppm)                                                                                                           | <sup>13</sup> C NMR (δ ppm) |
|----------|--------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| Anomeric | 5.40 (d, <i>J</i> = 3.6 Hz, 1H), 5.26 (d, <i>J</i> = 2.3 Hz, 1H), 5.02 (d, <i>J</i> = 10.3 Hz, 1H), 4.90 (d, <i>J</i> = 10.9 Hz, 1H) | 100.8, 97.7, 97.6, 96.9     |

|                                   |                                                                                    |                                                                        |
|-----------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Acetonide                         | 1.43 (s, 3H), 1.36 (s, 3H)                                                         | 112.3, 27.9, 26.3                                                      |
| TBDPS                             | 7.45-7.10 (m, 10H, merged), 1.05 (s, 9H)                                           | 136.0 (2C), 135.7 (2C), 133.7, 133.0, 27.0(3C), 19.5                   |
| -COOMe                            | 3.72 (s, 3H), 3.54 (s, 3H),                                                        | 169.0, 167.7, 52.6, 52.4                                               |
| PMB                               | 7.23 – 7.15 (m, 2H, merged), 6.82 (d, $J = 8.6$ Hz, 2H), 3.39 (s, 3H)              | 159.3, 130.3, 129.9, 129.8, 113.8 (2C), 72.8, 55.3                     |
| Benzoate                          | 8.11 – 8.06 (m, 2H), 8.03 – 7.99 (m, 2H), 7.69 – 7.63 (m, 4H), 7.57 – 7.50 (m, 2H) | 166.0, 164.8, 133.7, 133.5, 130.4 (2C), 129.9 (2C), 129.7, 129.1       |
| Benzyl                            | 7.45-7.10 (m, 20H, merged), 4.9-3.3 (8H, merged)                                   | 138.4, 137.8, 137.4, 137.1, 128.9- 127.5 (20C), 75.7, 75.4, 75.0, 74.7 |
| CH <sub>2</sub> (C <sub>6</sub> ) | 4.9-3.3 (4H, merged)                                                               | 62.2, 61.8                                                             |

**Table 4.4: Characteristic <sup>1</sup>H and <sup>13</sup>C NMR signals of the tetrasaccharide **94****

Further, hydrolysis of the isopropylidene and PMB-ether of the tetrasaccharide **94** was effected under acidic conditions using 75% dichloroacetic acid to afford the triol hemiacetals **95** in 68% yield. Anomeric position of the tetrasaccharide **95** was transformed regioselectively into the alkynyl carbonate donor using carbonate reagent **36** in the presence of DMAP in CH<sub>2</sub>Cl<sub>2</sub> and the per acetylated using acetic anhydride and pyridine to afford {D-E-F-G} tetrasaccharide donor **96**. Finally, the donor **96** was coupled with the acceptor **64b** affording the desired pentasaccharide **97** in 75% yield. Comparative NMR spectroscopic study<sup>13</sup> of the pentasaccharide **97** with the compounds **83**, **93** and **94** prepared *vide supra*, and the HRMS (ESI-MS) analysis,  $m/z$  calculated for C<sub>106</sub>H<sub>115</sub>N<sub>9</sub>O<sub>30</sub>Si[M+Na]<sup>+</sup>: 2044.7417, Found: 2044.7214 was confirmed the synthesis of depicted compound **97**.



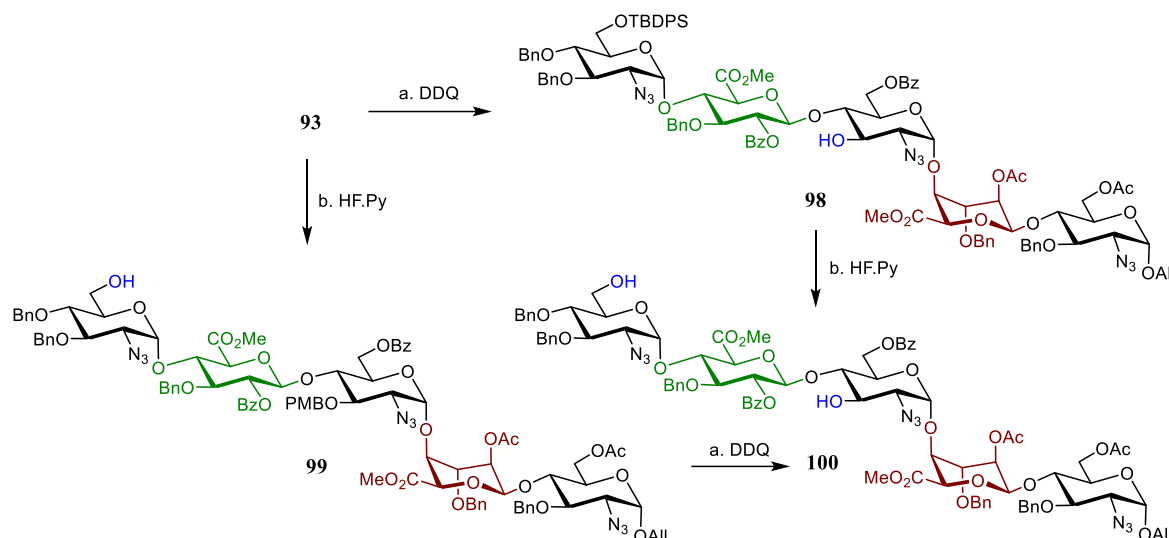
*Scheme 4.07: Preparation of the pentasaccharide reminiscent of the Fondaparinux by [3+1+1] strategy.*

Reagents. (a) 8mol% each of **68** and AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, 25 °C, 15 min; **94**: 91% ( $\alpha$  only), **97**: 75%; (b) 75% Dichloroacetic acid (aq), 0 °C, 1-2 h, 68%, (c) **36**, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 2-3 h, then Ac<sub>2</sub>O, 25 °C, 2 h, 71% over two steps.

## 4.6 Partial Deprotection of Pentasaccharide Fondaparinux

Pentasaccharide **93** was split into two portions and subjected to selective deprotections. Unmasking of the 3-*O*-*p*-methoxybenzyl ether of the F-unit was successfully accomplished by the action of DDQ in CH<sub>2</sub>Cl<sub>2</sub>-H<sub>2</sub>O solvent mixture at 25 °C within 30 min to afford compound **98** in an 85% yield (Scheme 4.08). The monosulfated derivative of the compound **98**, which is otherwise very difficult to synthesize,<sup>14</sup> shows very significant improvement in the anti-coagulant activity. Further, the fluoride ion mediated cleavage of the 6-*O*-silyl ether of the D-unit of **98** afforded the diol **100** in 81% yield. In parallel, the second portion of the pentasaccharide **93** was subjected to the deprotection of 6-*O*-silyl ether of the D-unit first to afford pentasaccharide **99**, followed by the cleavage of 3-*O*-PMB-ether of the F-unit afforded diol compound **100**. Disappearance of the signals of PMB and TBDPS resonances in the <sup>1</sup>H, <sup>13</sup>C and DEPT NMR<sup>13</sup> spectral analysis was clearly indicated the successful selective deprotections. Moreover, formation of the compounds **98**, **99** and **100** was confirmed by mass spectroscopic analysis; **98**: *m/z* calculated for C<sub>104</sub>H<sub>113</sub>N<sub>9</sub>O<sub>29</sub>Si[M+Na]<sup>+</sup>: 2002.7311, Found:

2002.7004, **99**:  $m/z$  calculated for  $C_{96}H_{103}N_9O_{30} [M+Na]^+$ : 1884.6709, Found: 1884.6536. **100**:  $m/z$  calculated for  $C_{88}H_{95}N_9O_{29} [M+Na]^+$ : 1764.6133, Found: 1764.6050.



*Scheme 4.08: Partial regioselective deprotection of pentasaccharide Fondaparinux.*

Reagents. (a) DDQ,  $CH_2Cl_2$ :  $H_2O$  (10:1), 25 °C, 30 min, **98**: 85%, **100**: 76%; (b)  $HF \cdot py$ , pyridine, 0 °C to 25 °C, 5 h, **99**: 83%, **100**: 81%.

Further extrapolation through regioselective deprotections and sulfation to the therapeutically relevant Fondaparinux is well established by other practitioners of the art<sup>12</sup> and hence, the formal synthesis of Fondaparinux is accomplished in this endeavour. However, the partially deprotected regioisomeric hydroxyls will enable regioselective sulfation to ensue studies of their biological properties.

## 4.7 Conclusions:

A novel, flexible, modular and highly efficient synthetic strategy has been developed for the synthesis of Fondaparinux pentasaccharide. This unique route is constructed upon coupling of thoughtfully identified monosaccharide building blocks which can be synthesized from shared precursors. All glycosidations are conducted under silver-assisted gold-catalyzed conditions utilizing recently discovered alkynyl cyclohexyl carbonate donor chemistry. All glycosidations were optimized to give exclusive stereoselectivity thereby minimizing the tedious purifications. This strategy offers a new route to the synthesis of mono 3-*O*-sulfation at the F-ring of fondaparinux pentasaccharide which is hitherto very difficult to obtain. This significantly improved route for fondaparinux pentasaccharide illustrates a new set of building blocks, utility of gold-catalyzed glycosidations and promised to yield orthogonally protected hydroxyl groups that facilitate installation of sulfates in a regiodefined fashion. Further



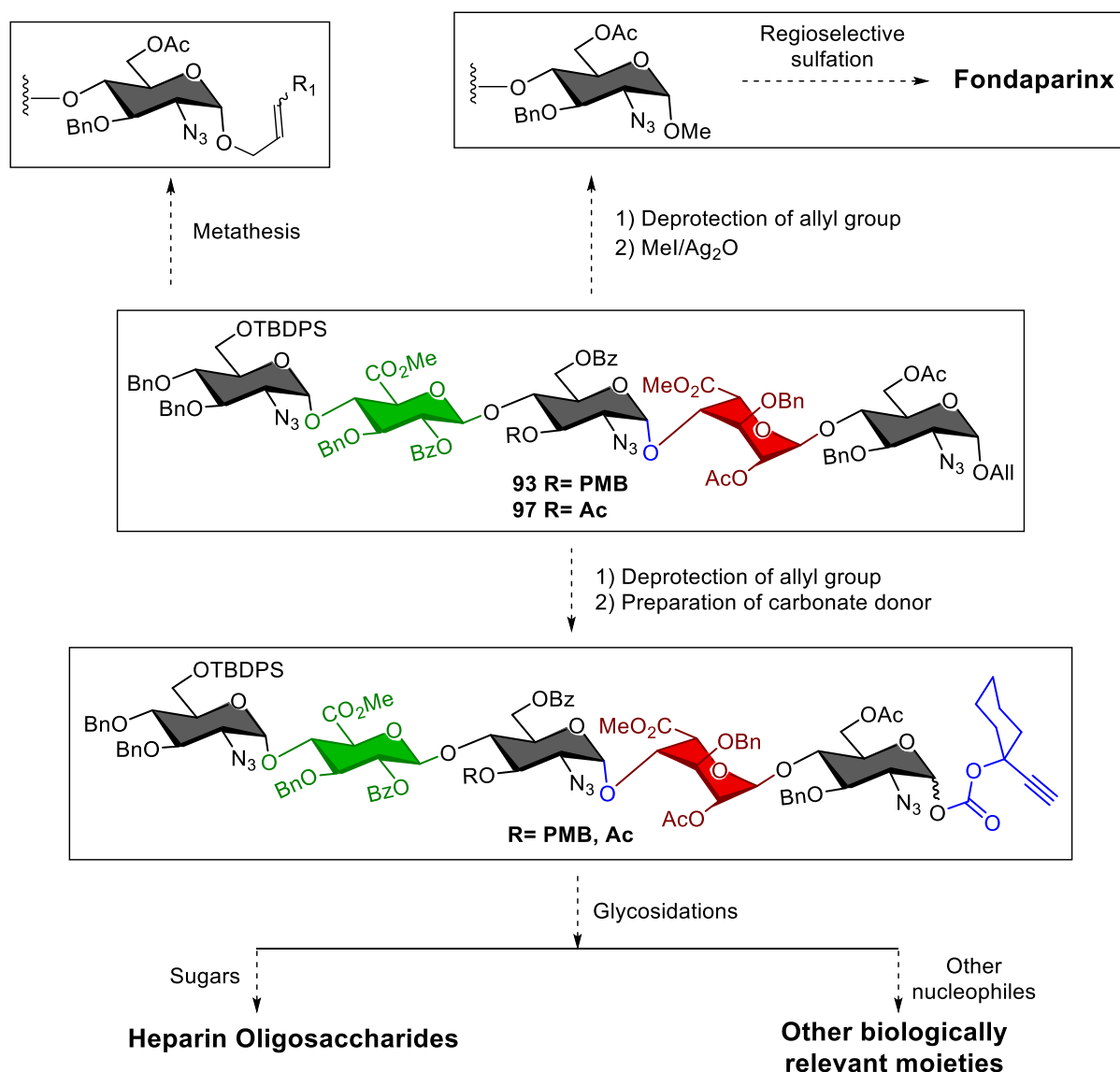
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research on exploitation of this strategy for the synthesis of other glycosaminoglycan derivatives of therapeutic significance is currently underway.

### 4.8 Comparative Summary Chart for the Synthesis of Regiodefined Anticoagulant Pentasaccharide (Fondaparinux):

| Group/Lit           | Synthetic steps of | Current Work<br>Walke <i>et al</i> | Codee <i>et. al</i><br>JACS, 2005,<br>127, 3767                | Wong JACS,<br>2007 | Hung (Ang Chem,<br>2014) |                | Shen, Zhao<br>&Wang(Chem<br>Med Chem,<br>2014)                         | Tang & Qin<br>JOC, 2016  |
|---------------------|--------------------|------------------------------------|----------------------------------------------------------------|--------------------|--------------------------|----------------|------------------------------------------------------------------------|--------------------------|
|                     |                    |                                    |                                                                |                    | Insitu more<br>steps     | Total<br>Steps |                                                                        |                          |
| D                   |                    |                                    |                                                                |                    |                          |                |                                                                        |                          |
| F                   |                    |                                    |                                                                |                    |                          |                |                                                                        |                          |
| H                   |                    |                                    |                                                                |                    |                          |                |                                                                        |                          |
| D, F, H Total steps |                    | 13                                 | 15                                                             | 16                 | 8                        | 12             | 18                                                                     | >16                      |
| E                   | Structure          |                                    |                                                                |                    |                          |                |                                                                        |                          |
|                     | Steps              | 11                                 | 11                                                             | 11                 | 5                        | 7              | 10                                                                     | 8                        |
| G                   | Structure          |                                    |                                                                |                    |                          |                |                                                                        |                          |
|                     | Steps              | 9                                  | 13                                                             | 13                 | 6                        | 9              | 14                                                                     | 13                       |
| Coupling            |                    | 8                                  | 6                                                              | 11                 | 12                       | 14             | 9                                                                      | 14                       |
| Total Steps         |                    | 41                                 | 45                                                             | 51                 | 31                       | 42             | 51                                                                     | 51                       |
| Coupling Strategy   |                    | 3+2 & 3+1+1                        | Sequential                                                     | 1 Pot (1+2+2)      | 3+1+1                    |                | 3+2                                                                    | 3+2                      |
| Glycosylation       | Donor              | Carbonate                          | Lactol, SPh                                                    | STol               | STol                     |                | NCCl <sub>3</sub> , Br, SPh                                            | NCCl <sub>3</sub> , STol |
|                     | Catalyst           | Au Phosphite-<br>AgOTf             | Ph <sub>2</sub> SO-Tf <sub>2</sub> O,<br>BSP-Tf <sub>2</sub> O | NIS-TfOH           | NIS-TfOH                 |                | TBS/TMSOTf,<br>Ag <sub>2</sub> CO <sub>3</sub> , BSP-Tf <sub>2</sub> O | TfOH, NIS-TfOH           |

## 4.9 Future Plan:



## 4.10 Experimental Section:

**Compound 74:** This compound was prepared from compound **69j** following the procedure described for the preparation of compounds **67 (a-e)** intermediate hemiacetals. Eluent for purification: 25% ethyl acetate in *n*-hexane; Thick syrup; yield 210 mg (95%) from 230 mg;

**Compound 75:** This compound was prepared from compound **74** by following the procedure described for compounds **67a-e**. Eluent for purification: 10% ethyl acetate in *n*-hexane; Thick syrup; yield 205 mg (85%) from 210 mg;  $[\alpha]^{25}_D$  (CHCl<sub>3</sub>, *c*1.0): +34.4°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3286, 3031, 2934, 2861, 2107, 1759, 1454, 1270, 1240, 1143, 1099, 1032, 906, 747, 701; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 8.04 (td, *J* = 8.5, 1.4 Hz, 4H), 7.69 – 7.62 (m, 8H), 7.58 – 7.51 (m, 2H), 7.44 – 7.36 (m, 12H), 7.36 – 7.27 (m, 24H), 7.25 – 7.19 (m, 10H), 6.34 (d, *J* = 3.5 Hz,

1H), 5.87 (d,  $J = 6.2$  Hz, 1H), 5.47 – 5.41 (m, 2H), 5.38 (dd,  $J = 9.4, 3.6$  Hz, 1H), 5.34 (d,  $J = 3.7$  Hz, 1H), 4.94 (d,  $J = 10.6$  Hz, 1H), 4.90 (d,  $J = 6.7$  Hz, 1H), 4.88 – 4.74 (m, 9H), 4.69 (d,  $J = 10.5$  Hz, 1H), 4.50 (d,  $J = 9.4$  Hz, 1H), 4.36 – 4.30 (m, 3H), 4.17 (dd,  $J = 9.5, 8.5$  Hz, 1H), 4.06 (ddd,  $J = 7.3, 4.9, 2.4$  Hz, 1H), 3.98 (ddd,  $J = 11.6, 4.3, 2.5$  Hz, 2H), 3.91 – 3.80 (m, 6H), 3.58 – 3.55 (m, 1H), 3.54 (s, 3H), 3.53 (s, 3H), 3.45 (d,  $J = 7.8$  Hz, 1H), 3.36 – 3.28 (m, 2H), 2.50 (s, 1H), 2.42 (s, 1H), 2.20 – 1.97 (m, 4H), 1.90 – 1.70 (m, 5H), 1.69 – 1.50 (m, 14H), 1.35 – 1.30 (m, 2H), 1.05 (s, 18H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.6, 168.3, 165.4, 165.1, 151.1, 150.7, 138.4, 138.4, 137.9, 137.9, 137.6, 137.4, 136.0(4C), 135.7(4C), 133.7, 133.7, 133.6, 133.5, 133.1, 133.0, 130.1(4C), 129.9, 129.8(3C), 129.3, 129.3, 128.7(4C), 128.6(6C), 128.5(6C), 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0(4C), 127.8(6C), 127.7(8C), 127.5, 127.5, 98.5, 98.2, 94.7, 92.6, 82.5, 82.4, 80.2, 80.0, 80.0, 79.3, 78.9, 78.6, 77.9, 77.9, 75.7, 75.6, 75.4, 75.3, 75.2, 75.1, 75.1, 74.9, 74.6, 74.5, 74.0, 72.8, 72.7, 72.6, 71.9, 71.3, 63.7, 63.6, 62.0, 61.9, 52.8, 52.8, 36.8, 36.8, 36.7, 36.6, 27.0(6C), 25.0, 25.0, 22.6, 22.6, 22.5, 22.4, 19.5, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{66}\text{H}_{71}\text{N}_3\text{O}_{14}\text{Si}[\text{M}+\text{Na}]^+$ : 1180.4603, Found: 1180.4590.

**Compound 77: Glycosylation:** To a solution of glycosyl donor (1.0 eq) and acceptor (0.8 eq) in anhydrous  $\text{CH}_2\text{Cl}_2$  (5 ml/mmol) was added freshly activated 4Å MS powder (0.400 g/mmol) at 25 °C under argon atmosphere. After 15 min of vigorous stirring at 25 °C, chloro[tris(2,4-di-*t*-butylphenyl) phosphite]gold(I) (8mol%) **68** and AgOTf (8mol%) were added simultaneously to the reaction mixture and stirred for 15 min. After completion, the reaction mixture was quenched by adding excess of  $\text{Et}_3\text{N}$  and filtered through a bed of Celite, the filtrate was concentrated *in vacuo* and the crude residue by silica gel column chromatography using 10% ethyl acetate and hexane as a mobile phase. Thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CH}_2\text{Cl}_2$ ,  $c$  1.1): +31.2°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3032, 2925, 2858, 2106, 1738, 1688, 1457, 1261, 1447, 1038, 816, 742, 700;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.07 – 8.03 (m, 2H), 7.69 (ddt,  $J = 11.6, 6.8, 1.5$  Hz, 4H), 7.59 (tt,  $J = 7.0, 1.3$  Hz, 1H), 7.47 – 7.39 (m, 6H), 7.39 – 7.30 (m, 8H), 7.29 – 7.26 (m, 4H), 7.23 – 7.15 (m, 5H), 6.95 (s, 1H), 5.20 (d,  $J = 3.6$  Hz, 1H), 4.93 – 4.91 (m, 3H), 4.87 (d,  $J = 10.7$  Hz, 1H), 4.66 (d,  $J = 10.7$  Hz, 1H), 4.59 – 4.52 (m, 3H), 4.29 (dd,  $J = 2.4, 1.2$  Hz, 1H), 4.10 (dd,  $J = 10.3, 9.0$  Hz, 1H), 3.96 – 3.86 (m, 3H), 3.74 (t,  $J = 9.3$  Hz, 1H), 3.55 (s, 3H), 3.33 (dd,  $J = 10.3, 3.6$  Hz, 1H), 1.06 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  167.7, 164.8, 138.6, 138.1, 137.9, 137.5, 136.0, 136.0, 135.8, 135.8, 133.5, 133.5, 133.2, 130.2, 130.2, 129.9, 129.8, 129.8, 129.6, 128.7, 128.7, 128.6(4C), 128.5, 128.5, 128.4, 128.4, 128.1(5C), 128.0, 128.0, 127.9, 127.9, 127.7, 127.7, 99.0, 79.8, 78.4, 75.7, 75.5, 74.0, 73.4, 73.1, 71.2, 69.7, 63.5, 62.6, 52.3, 27.0(3C), 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{57}\text{H}_{59}\text{N}_3\text{O}_{11}\text{Si}[\text{M}+\text{Na}]^+$ : 1012.3817, Found: 1012.3810.

**Compound 78:** This compound was observed in the glycosylation reaction performed between the donor **56a** and acceptor **64f** by following the above described procedure for the compound **77**. Eluent for purification: 20% ethyl acetate in *n*-hexane; Thick syrup; yield 45 mg (71%) from 35 mg;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.71 (td,  $J = 6.8, 6.3, 1.7$  Hz, 4H), 7.43 – 7.38 (m, 5H), 7.37 – 7.31 (m, 4H), 7.30 – 7.28 (m, 2H), 6.03 (d,  $J = 3.3$  Hz, 1H), 5.97 – 5.86 (m, 1H), 5.43 – 5.35 (m, 1H), 5.34 – 5.27 (m, 2H), 5.22 (dd,  $J = 10.3, 1.3$  Hz, 1H), 4.98 (d,  $J = 3.6$  Hz, 1H), 4.67 (d,  $J = 11.8$  Hz, 1H), 4.55 – 4.50 (m, 2H), 4.24 (ddt,  $J = 12.8, 5.0, 1.2$  Hz, 1H), 4.15 (t,  $J = 2.6$  Hz, 1H), 4.06 – 4.00 (m, 1H), 3.92 (dd,  $J = 11.2, 1.9$  Hz, 1H), 3.88 (t,  $J = 2.7$  Hz, 1H), 3.80 (dd,  $J = 11.3, 5.8$  Hz, 1H), 3.75 – 3.71 (m, 1H), 3.64 – 3.58 (m, 1H), 3.53 (s, 3H), 3.21 (dd,  $J = 10.8, 3.5$  Hz, 1H), 2.56 – 2.36 (m, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 1.77 (s, 3H), 1.06 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  207.8, 170.2, 169.8, 169.1, 136.5, 135.9, 135.9, 135.7, 135.7, 133.8, 133.4, 133.2, 129.8, 129.8, 128.7, 128.7, 128.5, 128.2, 128.2, 127.8, 127.8, 127.7, 127.7, 124.3, 118.3, 96.1, 94.1, 76.1, 72.7, 72.4, 71.6, 71.1, 70.6, 70.1, 68.4, 66.2, 62.8, 61.1, 52.4, 38.6, 33.7, 30.0, 27.0, 20.9(3C), 20.9, 20.9, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{48}\text{H}_{59}\text{N}_3\text{O}_{15}\text{Si}[\text{M}+\text{Na}]^+$ : 968.3613, Found: 968.3600.

**Compound 79:** This compound was prepared by the glycosylation reaction of the donor **56b** and acceptor **64d** following the above described procedure for the preparation of compound **77**. Eluent for purification: 25% ethyl acetate in *n*-hexane; Thick syrup; yield 22 mg (35%) from 35 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$  1.0): +68.8°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3032, 2928, 2859, 2109, 1739, 1644, 1455, 1365, 1256, 1150, 1079, 1034, 752, 704;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.78 (t,  $J = 7.9$  Hz, 4H), 7.70 – 7.67 (m, 2H), 7.52 – 7.43 (m, 7H), 7.38 (t,  $J = 7.2$  Hz, 2H), 7.31 (t,  $J = 7.7$  Hz, 2H), 7.16 – 7.12 (m, 5H), 6.91 (d,  $J = 8.6$  Hz, 2H), 5.76 (ddt,  $J = 16.8, 11.3, 5.7$  Hz, 1H), 5.39 (dd,  $J = 9.4, 8.3$  Hz, 1H), 5.34 (t,  $J = 9.6$  Hz, 1H), 5.21 – 5.18 (m, 1H), 5.16 (s, 1H), 5.12 – 5.08 (m, 1H), 4.98 (d,  $J = 8.1$  Hz, 1H), 4.87 (d,  $J = 3.7$  Hz, 1H), 4.65 (d,  $J = 11.2$  Hz, 1H), 4.60 (d,  $J = 7.1$  Hz, 1H), 4.55 (d,  $J = 11.6$  Hz, 1H), 4.23 – 4.18 (m, 1H), 3.96 (dd,  $J = 13.0, 5.2$  Hz, 1H), 3.93 – 3.84 (m, 4H), 3.81 (s, 3H), 3.71 – 3.66 (m, 5H), 3.33 (dd,  $J = 10.3, 3.7$  Hz, 2H), 2.72 (t,  $J = 6.3$  Hz, 2H), 2.63 – 2.48 (m, 2H), 2.18 (s, 3H), 1.10 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.1, 171.4, 167.3, 164.6, 159.3, 137.6, 136.0, 136.0, 135.6, 135.6, 134.0, 133.3, 133.3, 132.5, 130.7, 130.5, 130.5, 130.2, 130.0, 129.7, 129.2, 128.6, 128.5, 128.3, 128.3, 128.1, 128.1, 128.0, 127.8(4C), 118.1, 113.8, 113.8, 100.3, 96.5, 79.8, 77.7, 76.7, 75.6, 74.2, 73.2, 73.1, 71.9, 71.2, 68.5, 62.9, 61.1, 55.4, 52.9, 37.8, 29.9, 27.9, 26.9(3C), 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{59}\text{H}_{67}\text{N}_3\text{O}_{15}\text{Si}[\text{M}+\text{Na}]^+$ : 1108.4239, Found: 1108.4225.

**Compound 81:** This compound was prepared by the glycosylation reaction performed between the donor **56c** and acceptor **64a** following the above described procedure for the preparation of

## Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

compound **77**. Eluent for purification: 8% ethyl acetate in *n*-hexane; white solid; yield 2.8 g (91%) from 1.5 g;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +88.2°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3028, 2948, 2862, 2109, 1733, 1610, 1514, 1456, 1261, 1141, 1100, 1068, 1036, 844, 754, 708;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.00 – 7.94 (m, 4H), 7.63 – 7.58 (m, 1H), 7.48 – 7.39 (m, 5H), 7.37 – 7.32 (m, 2H), 7.16 – 7.10 (m, 5H), 6.92 – 6.88 (m, 2H), 5.82 (dddd,  $J$  = 16.7, 10.4, 6.2, 5.3 Hz, 1H), 5.40 (t,  $J$  = 8.2 Hz, 1H), 5.21 (dq,  $J$  = 17.3, 1.6 Hz, 1H), 5.16 – 5.08 (m, 2H), 4.85 – 4.80 (m, 2H), 4.67 – 4.62 (m, 3H), 4.45 (dd,  $J$  = 12.2, 2.1 Hz, 1H), 4.39 (dd,  $J$  = 12.1, 4.1 Hz, 1H), 4.12 (t,  $J$  = 8.3 Hz, 1H), 4.07 (ddt,  $J$  = 12.9, 5.3, 1.5 Hz, 1H), 3.96 – 3.90 (m, 3H), 3.86 (d,  $J$  = 8.6 Hz, 1H), 3.81 (s, 3H), 3.76 – 3.71 (m, 1H), 3.67 – 3.62 (m, 4H), 3.34 – 3.27 (m, 1H), 0.82 (s, 9H), -0.04 (s, 6H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.4, 166.1, 165.0, 159.3, 137.7, 133.5, 133.5, 133.1, 130.6, 130.3, 130.3, 129.8, 129.8, 129.7, 129.7, 129.2, 128.7(4C), 128.2, 128.2, 127.5(3C), 118.4, 113.9, 113.9, 101.2, 96.6, 82.6, 78.2, 77.8, 77.3, 75.5, 75.0, 74.2, 72.3, 69.1, 68.8, 63.0, 62.6, 55.4, 52.5, 25.8(3C), 18.0, -3.9, -5.1; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{51}\text{H}_{61}\text{N}_3\text{O}_{14}\text{Si}[\text{M}+\text{Na}]^+$ : 990.3820, Found: 990.3820.

**Compound 82: TBDMS Deprotection:** To a solution of the compound **81** (2.8 g) in anhydrous pyridine (15 mL) was added dropwise 8 mL of the 70%  $\text{HF}\cdot\text{py}$  solution at 0 °C and the reaction mixture was allowed to stir at 25 °C for 5 h. After completion, ice-cold water (50 mL) was added and extracted with EtOAc (2 x 30 mL). The organic layer was washed with aqueous 1N HCl (50 mL), saturated aq.  $\text{NaHCO}_3$  (50 mL) and brine solution (100 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography using 35% ethyl acetate in *n*-hexane as mobile phase to furnish the corresponding disaccharide acceptor **82** (2.2 g, 89%) as a white solid; mp 133.9 °C;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0): +97.4°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3515, 3025, 2921, 2854, 2108, 1727, 1608, 1513, 1452, 1259, 1032, 826, 751, 705;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05 – 8.01 (m, 2H), 7.95 – 7.91 (m, 2H), 7.58 (ddt,  $J$  = 8.7, 7.1, 1.3 Hz, 1H), 7.48 – 7.41 (m, 3H), 7.40 – 7.33 (m, 4H), 7.16 – 7.12 (m, 5H), 6.90 – 6.86 (m, 2H), 5.83 (dddd,  $J$  = 17.2, 10.4, 6.2, 5.3 Hz, 1H), 5.33 (dd,  $J$  = 9.5, 8.0 Hz, 1H), 5.23 (dq,  $J$  = 17.2, 1.6 Hz, 1H), 5.15 (dq,  $J$  = 10.4, 1.3 Hz, 1H), 5.06 (d,  $J$  = 10.5 Hz, 1H), 4.85 (d,  $J$  = 3.6 Hz, 1H), 4.78 – 4.66 (m, 4H), 4.46 – 4.38 (m, 2H), 4.12 – 4.01 (m, 2H), 3.99 – 3.91 (m, 3H), 3.81 – 3.77 (m, 4H), 3.73 (d,  $J$  = 9.8 Hz, 1H), 3.68 – 3.63 (m, 4H), 3.33 (ddd,  $J$  = 8.8, 3.7, 1.2 Hz, 1H), 2.99 (d,  $J$  = 2.8 Hz, 1H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.2, 166.1, 165.0, 159.3, 137.8, 133.5, 133.5, 133.1, 130.7, 129.9, 129.9, 129.7(4C), 129.2, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.1, 128.1, 127.9, 118.4, 113.8, 113.8, 101.4, 96.6, 81.1, 78.2, 77.9, 75.1, 74.8, 74.5, 73.3, 72.2, 69.0, 68.8, 63.0, 62.6, 55.4, 52.9; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{45}\text{H}_{47}\text{N}_3\text{O}_{14}[\text{M}+\text{Na}]^+$ : 876.2956, Found: 876.2934.

**Compound 83: Glycosylation:** To a solution of glycosyl donor **67c** (1.7 g, 2.2 mmol) and acceptor **82** (1.5 g, 0.8 eq) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added freshly activated 4Å MS powder (1.2 g/mmol) at 25 °C under argon atmosphere, the reaction mixture was stirred vigorously at -45 °C for 30 min. Then chloro[tris(2,4-di-tertbutylphenyl)phosphite]gold(I) and AgOTf (15mol% each) were added simultaneously and the mixture stirred at -40 °C for 1h. After completion, the reaction was quenched by adding Et<sub>3</sub>N and filtered through a bed of Celite, the filtrate was concentrated *in vacuo* and crude residue was purified by silica gel column chromatography using 15% ethyl acetate in hexane as a mobile phase to afford the glycosylated trisaccharide **83** (2.3 g, 90%) selectively as  $\alpha$ -isomer. Thick syrup;  $[\alpha]^{25}_D$  (CHCl<sub>3</sub>, *c*1.0): +64.9°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3031, 2931, 2863, 2107, 1731, 1609, 1512, 1456, 1261, 1069, 1033, 749, 703; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>):  $\delta$  8.09 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.95 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.69 – 7.63 (m, 4H), 7.58 – 7.53 (m, 1H), 7.51 – 7.46 (m, 1H), 7.45 – 7.30 (m, 21H), 7.22 – 7.17 (m, 6H), 6.85 – 6.80 (m, 2H), 5.83 (dddd, *J* = 16.6, 10.4, 6.2, 5.3 Hz, 1H), 5.46 (dd, *J* = 8.8, 7.9 Hz, 1H), 5.40 (d, *J* = 3.7 Hz, 1H), 5.23 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.15 (dq, *J* = 10.4, 1.2 Hz, 1H), 5.04 (d, *J* = 10.3 Hz, 1H), 4.91 – 4.80 (m, 6H), 4.77 (d, *J* = 11.1 Hz, 1H), 4.70 (d, *J* = 10.4 Hz, 1H), 4.62 (d, *J* = 10.3 Hz, 1H), 4.45 (qd, *J* = 12.2, 3.0 Hz, 2H), 4.26 – 4.21 (m, 1H), 4.08 (ddt, *J* = 12.9, 5.3, 1.4 Hz, 1H), 3.97 (dd, *J* = 3.4, 2.0 Hz, 1H), 3.96 – 3.89 (m, 5H), 3.89 – 3.83 (m, 3H), 3.78 – 3.74 (m, 1H), 3.73 (s, 3H), 3.42 – 3.36 (m, 4H), 3.30 (ddd, *J* = 10.0, 8.0, 3.7 Hz, 2H), 1.05 (s, 9H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>):  $\delta$  167.9, 166.1, 165.0, 159.3, 138.4, 137.9, 137.4, 136.0, 136.0, 135.7, 135.7, 133.7, 133.7, 133.5, 133.1, 133.0, 130.4, 130.2, 130.2, 129.9(3C), 129.8, 129.7, 129.7, 129.6, 129.1, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5(4C), 128.3, 128.3, 128.1, 127.9, 127.8(5C), 127.7, 127.7, 127.5, 127.5, 118.4, 113.9, 113.9, 101.2, 97.7, 96.6, 82.4, 80.1, 78.3, 77.8, 77.8, 75.7, 75.4, 75.1, 75.1, 74.8, 74.6, 73.8, 72.6, 69.1, 68.8, 63.6, 63.0, 62.5, 61.9, 55.3, 52.6, 27.0(3C), 19.5; HRMS (ESI-MS): *m/z* calcd for C<sub>81</sub>H<sub>86</sub>N<sub>6</sub>O<sub>18</sub>Si[M+Na]<sup>+</sup>: 1481.5666, Found: 1481.5657.

**Compound 84:** This compound was prepared from compound **83** following the procedure for the preparation of **67(a-e)** intermediate hemiacetals. Eluent for purification: 25% ethyl acetate in *n*-hexane; Thick syrup; yield 1.7 g (87%) from 2.0 g.

**Compound 85:** This compound was prepared from compound **84** following the procedure for the preparation of compounds **67a-e**. Eluent for purification: 18% ethyl acetate in *n*-hexane; White solid; yield 1.65 g (88%) from 1.7 g; mp 78.7 °C;  $[\alpha]^{25}_D$  (CH<sub>2</sub>Cl<sub>2</sub>, *c*1.0): +30.9°; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3284, 2934, 2862, 2110, 1742, 1730, 1608, 1513, 1454, 1261, 1076, 1030, 911, 744, 702; <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>):  $\delta$  8.10 – 8.05 (m, 2H), 7.99 – 7.95 (m, 2H), 7.65 (ddt, *J* = 12.9, 6.7, 1.5 Hz, 4H), 7.57 – 7.53 (m, 1H), 7.52 – 7.48 (m, 1H), 7.45 – 7.36 (m, 7H), 7.36 –

7.27 (m, 14H), 7.21 – 7.16 (m, 6H), 6.86 – 6.81 (m, 2H), 5.43 (dd,  $J = 9.1, 7.9$  Hz, 1H), 5.39 (d,  $J = 3.7$  Hz, 1H), 5.21 – 5.17 (m, 1H), 4.98 (d,  $J = 10.3$  Hz, 1H), 4.90 – 4.81 (m, 4H), 4.75 (d,  $J = 11.1$  Hz, 1H), 4.72 (d,  $J = 7.8$  Hz, 1H), 4.69 (d,  $J = 10.5$  Hz, 1H), 4.65 (d,  $J = 10.4$  Hz, 1H), 4.48 – 4.38 (m, 2H), 4.20 (dd,  $J = 9.3, 8.5$  Hz, 1H), 3.98 – 3.89 (m, 3H), 3.88 – 3.80 (m, 4H), 3.74 (s, 3H), 3.54 – 3.48 (m, 2H), 3.46 – 3.42 (m, 1H), 3.40 (s, 3H), 3.37 – 3.33 (m, 1H), 3.29 (dd,  $J = 9.9, 3.7$  Hz, 1H), 2.57 (s, 1H), 2.16 – 2.06 (m, 2H), 1.89 – 1.77 (m, 2H), 1.67 – 1.63 (m, 1H), 1.58 – 1.46 (m, 4H), 1.33 – 1.27 (m, 1H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  167.8, 166.0, 165.0, 159.5, 150.8, 138.4, 137.9, 137.4, 136.0, 136.0, 135.7, 135.7, 133.8, 133.7, 133.5, 133.0, 130.5, 130.5, 129.9(7C), 129.8, 129.5, 128.9, 128.9, 128.7(4C), 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.1, 127.9, 127.8(7C), 127.5, 127.5, 113.9, 113.9, 101.0, 97.7, 95.5, 82.4, 82.3, 80.7, 80.1, 79.1, 77.8, 77.1, 75.7, 75.7, 75.6, 75.1, 75.0, 74.7, 74.7, 73.8, 73.7, 72.6, 64.3, 63.6, 62.2, 61.9, 55.3, 52.7, 36.8, 36.7, 27.0(3C), 25.0, 22.6, 22.6, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{87}\text{H}_{92}\text{N}_6\text{O}_{20}\text{Si}[\text{M}+\text{Na}]^+$ : 1591.6033, Found: 1591.6047.

**Compound 86:** This compound was prepared from compounds **37** and **64c** following the procedure delineated for the preparation of **77**. Eluent for purification: 15% ethyl acetate in n-hexane; thick syrup; yield 590 mg (86%) from 420 mg;  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ,  $c$  1.0):  $+10.5^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2927, 2859, 2107, 1745, 1460, 1373, 1262, 1226, 1157, 1106, 1040, 754, 702;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70 (ddd,  $J = 7.8, 4.4, 1.5$  Hz, 4H), 7.41 – 7.34 (m, 10H), 7.33 – 7.18 (m, 6H), 5.91 (dddd,  $J = 16.9, 10.4, 6.2, 5.4$  Hz, 1H), 5.30 (dq,  $J = 17.3, 1.5$  Hz, 1H), 5.26 – 5.21 (m, 2H), 5.03 (t,  $J = 3.0$  Hz, 1H), 4.94 – 4.90 (m, 2H), 4.90 – 4.87 (m, 1H), 4.75 – 4.66 (m, 2H), 4.62 (s, 2H), 4.15 (ddt,  $J = 13.1, 5.3, 1.3$  Hz, 1H), 4.04 – 3.86 (m, 4H), 3.83 – 3.77 (m, 2H), 3.72 (ddd,  $J = 9.9, 3.8, 2.2$  Hz, 1H), 3.41 – 3.35 (m, 4H), 1.98 (s, 3H), 1.90 (s, 3H), 1.07 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ )  $\delta$  170.0, 169.6, 168.6, 137.9, 137.3, 136.0, 136.0, 135.9, 135.9, 133.7, 133.4, 133.4, 129.7, 129.7, 128.6, 128.6, 128.3(3C), 128.2, 128.2, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 118.3, 97.3, 96.1, 78.7, 74.7, 74.3, 72.7, 72.3, 72.2, 68.2, 68.2, 67.1, 66.5, 63.9, 62.7, 52.0, 27.0(3C), 20.8, 20.8, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{50}\text{H}_{59}\text{N}_3\text{O}_{13}\text{Si}[\text{M}+\text{Na}]^+$ : 960.3715, Found: 960.3973.

**Compound 88:** Eluent for purification: 10% ethyl acetate in n-hexane; thick syrup; yield 120 mg (72%) from 190 mg;  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ,  $c$  1.0):  $+5.2^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3386, 2929, 2861, 2108, 1790, 1461, 1268, 1150, 1104, 1034, 753, 700;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ )  $\delta$  7.68 – 7.64 (m, 4H), 7.46 – 7.43 (m, 1H), 7.41 (d,  $J = 7.8$  Hz, 5H), 7.38 – 7.33 (m, 4H), 7.32 – 7.25 (m, 6H), 5.91 (ddt,  $J = 16.7, 10.7, 5.7$  Hz, 1H), 5.44 – 5.40 (m, 1H), 5.32 (dd,  $J = 17.2, 1.5$  Hz, 1H), 5.26 – 5.21 (m, 1H), 4.98 (d,  $J = 3.6$  Hz, 1H), 4.95 (d,  $J = 10.8$  Hz, 1H), 4.81 (d,  $J = 10.7$  Hz, 1H), 4.51 – 4.44 (m, 2H), 4.27 (t,  $J = 2.7$  Hz, 1H), 4.14 (dd,  $J = 12.9, 5.3$  Hz, 1H), 4.09 –



4.06 (m, 1H), 4.06 – 4.00 (m, 2H), 3.99 (d,  $J = 3.3$  Hz, 1H), 3.97 – 3.92 (m, 1H), 3.79 – 3.74 (m, 1H), 3.69 (dd,  $J = 11.7, 2.8$  Hz, 1H), 3.66 – 3.61 (m, 2H), 3.39 (dd,  $J = 10.0, 3.6$  Hz, 1H), 1.86 (s, 1H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ )  $\delta$  167.9, 138.4, 137.4, 136.0, 136.0, 135.8, 135.8, 133.4, 133.2, 132.8, 130.2, 129.9, 128.7, 128.7, 128.5, 128.5, 128.3, 128.1, 128.1, 127.9, 127.9, 127.8(3C), 118.3, 98.0, 96.8, 81.1, 78.7, 78.0, 74.8, 72.5, 72.3, 71.4, 71.4, 71.2, 68.6, 63.5, 62.1, 27.0(3C), 19.3; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{45}\text{H}_{51}\text{N}_3\text{O}_{10}\text{Si}[\text{M}+\text{Na}]^+$ : 844.3241, Found: 844.3237.

**Compound 92:** This compound was prepared from compounds **48** and **64b** following the procedure for the preparation of compound **77**. Eluent for purification: 20% ethyl acetate in *n*-hexane; thick syrup; yield 230 mg (89%) from 120 mg of the acceptor **64b**. thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.0):  $+17.0^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2955, 2860, 2109, 1742, 1460, 1371, 1237, 1091, 1040, 837, 701;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.39 – 7.36 (m, 2H), 7.35 – 7.27 (m, 7H), 7.27 – 7.24 (m, 1H), 5.94 (dddd,  $J = 16.6, 10.4, 6.1, 5.3$  Hz, 1H), 5.35 (dq,  $J = 17.2, 1.5$  Hz, 1H), 5.30 (d,  $J = 5.0$  Hz, 1H), 5.25 (dq,  $J = 10.4, 1.2$  Hz, 1H), 4.94 (d,  $J = 10.4$  Hz, 1H), 4.92 (d,  $J = 3.7$  Hz, 1H), 4.89 (t,  $J = 4.7$  Hz, 1H), 4.76 – 4.70 (m, 2H), 4.67 (d,  $J = 11.8$  Hz, 1H), 4.59 (d,  $J = 4.5$  Hz, 1H), 4.43 (dd,  $J = 12.1, 2.0$  Hz, 1H), 4.22 – 4.16 (m, 2H), 4.04 (ddt, 1H), 4.01 – 3.98 (m, 1H), 3.97 – 3.85 (m, 3H), 3.67 – 3.63 (m, 1H), 3.56 (s, 3H), 3.40 (dd,  $J = 9.8, 3.7$  Hz, 1H), 2.12 (s, 3H), 2.00 (s, 3H), 0.83 (s, 9H), -0.04 (s, 3H), -0.09 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.8, 170.2, 170.1, 138.2, 137.9, 133.3, 128.6, 128.6, 128.3, 128.3, 128.2, 128.2, 128.0 (3C), 127.6, 118.4, 98.0, 96.7, 78.3, 77.2, 76.3, 75.0, 73.3, 72.4, 70.9, 69.4, 69.3, 68.8, 63.2, 62.2, 51.8, 25.7 (3C), 21.1, 21.0, 17.9, -4.6, -5.3; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{40}\text{H}_{55}\text{N}_3\text{O}_{13}\text{Si}[\text{M}+\text{Na}]^+$ : 836.3402, Found: 836.3409.

**Compound 73:** This compound was prepared from compound **92** following procedure described for the compound **82**. Eluent for purification: 60% ethyl acetate in *n*-hexane; white solid; yield 170 mg (86%) from 230 mg; thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 1.2):  $+13.0^\circ$ ; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3446, 3024, 2956, 2925, 2109, 1739, 1451, 1371, 1225, 1093, 1035, 752, 700;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.43 – 7.38 (m, 4H), 7.38 – 7.34 (m, 1H), 7.33 – 7.30 (m, 4H), 7.28 – 7.23 (m, 1H), 5.98 (dddd,  $J = 16.6, 10.4, 6.1, 5.4$  Hz, 1H), 5.39 (dq,  $J = 17.2, 1.5$  Hz, 1H), 5.30 (dq,  $J = 10.4, 1.2$  Hz, 1H), 5.09 (s, 1H), 4.98 (d,  $J = 3.7$  Hz, 1H), 4.96 (dt,  $J = 2.5, 1.2$  Hz, 1H), 4.93 (d,  $J = 2.0$  Hz, 1H), 4.77 (d,  $J = 11.3$  Hz, 2H), 4.68 (t,  $J = 11.0$  Hz, 2H), 4.44 (dd,  $J = 12.3, 1.1$  Hz, 1H), 4.28 – 4.21 (m, 2H), 4.09 (ddt,  $J = 12.9, 6.2, 1.3$  Hz, 1H), 4.01 – 3.96 (m, 1H), 3.93 – 3.84 (m, 3H), 3.76 – 3.73 (m, 1H), 3.50 (s, 3H), 3.42 (dd,  $J = 9.7, 3.6$  Hz, 1H), 2.64 (d,  $J = 11.3$  Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.7, 169.6, 169.3, 137.9, 137.3, 133.2, 128.7, 128.7, 128.3 (3C), 128.2, 128.2, 127.6, 127.6, 127.5,



118.5, 98.2, 96.7, 78.7, 75.2, 74.7, 74.5, 72.5, 69.4, 68.9, 68.6, 67.8, 67.2, 63.7, 62.3, 52.2, 21.1, 21.0; HRMS (ESI-MS):  $m/z$  calcd for  $C_{34}H_{41}N_3O_{13}$   $[M+Na]^+$ : 722.2537, Found: 722.2545.

**Compound 93:** This compound was prepared from compounds **85** and **73** following the procedure for the preparation of **77**. Eluent for purification: 20% ethyl acetate in n-hexane; thick syrup; yield 18 mg (86%) from 7 mg of the acceptor **73**. Thick syrup;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c0.9$ ): +102.9°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 2956, 2926, 2862, 2108, 1737, 1456, 1369, 1256, 1146, 1105, 1071, 1035, 745, 706;  $^1H$  NMR (600.40 MHz,  $CDCl_3$ ):  $\delta$  8.08 (d,  $J$  = 7.7 Hz, 2H), 7.99 (d,  $J$  = 7.7 Hz, 2H), 7.65 (dd,  $J$  = 19.8, 7.0 Hz, 5H), 7.53 (t,  $J$  = 7.4 Hz, 2H), 7.46 – 7.39 (m, 6H), 7.38 – 7.35 (m, 4H), 7.34 – 7.30 (m, 11H), 7.29 – 7.27 (m, 4H), 7.25 – 7.24 (m, 1H), 7.22 – 7.17 (m, 8H), 7.15 (dd,  $J$  = 9.3, 7.2 Hz, 2H), 6.81 (d,  $J$  = 8.6 Hz, 2H), 5.93 (ddt,  $J$  = 16.6, 11.0, 5.7 Hz, 1H), 5.43 (t,  $J$  = 8.5 Hz, 1H), 5.39 (d,  $J$  = 3.7 Hz, 1H), 5.37 – 5.33 (m, 1H), 5.27 (d,  $J$  = 10.3 Hz, 1H), 5.19 (d,  $J$  = 4.0 Hz, 1H), 5.03 (d,  $J$  = 10.3 Hz, 1H), 4.93 (d,  $J$  = 3.7 Hz, 1H), 4.90 – 4.85 (m, 4H), 4.84 – 4.80 (m, 2H), 4.78 – 4.73 (m, 3H), 4.69 – 4.64 (m, 4H), 4.53 (d,  $J$  = 10.4 Hz, 1H), 4.49 (d,  $J$  = 4.2 Hz, 1H), 4.46 (d,  $J$  = 12.2 Hz, 1H), 4.40 – 4.34 (m, 2H), 4.24 – 4.15 (m, 3H), 4.04 (dd,  $J$  = 12.9, 6.3 Hz, 1H), 3.96 – 3.86 (m, 6H), 3.86 – 3.81 (m, 6H), 3.73 – 3.69 (m, 4H), 3.66 (dd,  $J$  = 10.3, 8.6 Hz, 1H), 3.41 – 3.34 (m, 5H), 3.32 (dd,  $J$  = 10.0, 3.7 Hz, 1H), 3.25 (s, 3H), 3.21 (dd,  $J$  = 10.3, 3.6 Hz, 1H), 2.12 (s, 3H), 2.04 (s, 3H), 1.03 (s, 9H);  $^{13}C$  NMR (150.99 MHz,  $CDCl_3$ ):  $\delta$  170.9, 170.2, 169.3, 167.7, 166.0, 164.8, 159.3, 138.3, 137.9, 137.7, 137.4, 137.3, 136.0, 136.0, 135.7, 135.7, 133.9, 133.6, 133.6, 133.1, 132.9, 130.2, 130.2, 130.1, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.5, 129.0, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6 (4C), 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9 (3C), 127.8 (7C), 127.7, 127.7, 127.6, 127.5, 127.5, 118.5, 113.8, 113.8, 100.8, 97.9, 97.8, 97.1, 96.6, 82.5, 80.2, 78.4, 77.7, 77.4, 77.2, 75.9, 75.8, 75.5, 75.0, 75.0, 74.9, 74.9, 74.8, 73.8, 73.6, 73.6, 72.6, 72.5, 69.6, 69.5, 69.3, 69.2, 68.8, 63.6, 63.3, 62.6, 62.2, 62.0, 61.8, 55.3, 52.7, 51.8, 27.0 (3C), 21.0, 20.9, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $C_{112}H_{121}N_9O_{30}Si[M+Na]^+$ : 2122.7886, Found: 2122.7530.

**Compound 94:** This compound was prepared from substrates **85** and **42** following the procedure for the preparation of **77**. Eluent for purification: 18% ethyl acetate in n-hexane; thick syrup; yield 286 mg (91%) from 61 mg of the acceptor **73**. White solid; mp 88.1 °C;  $[\alpha]^{25}_D$  ( $CHCl_3$ ,  $c1.0$ ): +49.0°; IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3025, 2932, 2109, 1732, 1513, 1456, 1369, 1265, 1216, 1138, 1071, 1036, 750, 707;  $^1H$  NMR (400.31 MHz,  $CDCl_3$ ):  $\delta$  8.11 – 8.06 (m, 2H), 8.03 – 7.99 (m, 2H), 7.69 – 7.63 (m, 4H), 7.57 – 7.50 (m, 2H), 7.46 – 7.41 (m, 4H), 7.39 – 7.36 (m, 5H), 7.35 – 7.30 (m, 13H), 7.29 – 7.28 (m, 2H), 7.23 – 7.15 (m, 8H), 6.82 (d,  $J$  = 8.6 Hz, 2H),

5.47 – 5.41 (m, 1H), 5.40 (d,  $J = 3.6$  Hz, 1H), 5.26 (d,  $J = 2.3$  Hz, 1H), 5.02 (d,  $J = 10.3$  Hz, 1H), 4.90 (d,  $J = 10.9$  Hz, 1H), 4.86 – 4.81 (m, 3H), 4.80 – 4.75 (m, 3H), 4.67 (d,  $J = 10.4$  Hz, 1H), 4.62 – 4.55 (m, 4H), 4.39 (dd,  $J = 12.4, 3.1$  Hz, 1H), 4.34 (d,  $J = 1.4$  Hz, 1H), 4.22 (t,  $J = 8.9$  Hz, 1H), 4.13 – 4.09 (m, 1H), 3.99 – 3.94 (m, 3H), 3.94 – 3.89 (m, 3H), 3.88 – 3.83 (m, 3H), 3.82 – 3.76 (m, 2H), 3.72 (s, 3H), 3.54 (s, 3H), 3.39 (s, 3H), 3.38 – 3.29 (m, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.0, 167.7, 166.0, 164.8, 159.3, 138.4, 137.8, 137.4, 137.1, 136.0, 136.0, 135.7, 135.7, 133.7, 133.7, 133.5, 133.0, 130.4, 130.4, 130.3, 129.9 (3C), 129.8, 129.7 (3C), 129.1, 128.9, 128.9, 128.7 (4C), 128.6, 128.6, 128.5 (4C), 128.5, 128.3 (3C), 128.1, 127.9, 127.9, 127.8 (6C), 127.7, 127.7, 127.5, 127.5, 113.8, 113.8, 112.3, 100.8, 97.7, 97.6, 96.9, 82.6, 80.2, 77.8, 77.3, 77.2, 75.7, 75.4, 75.4, 75.1, 75.0, 74.9, 74.7, 73.6, 73.1, 72.8, 72.6, 72.5, 71.3, 69.9, 63.6, 63.3, 62.2, 61.8, 55.3, 52.6, 52.4, 27.9, 27.0 (3C), 26.3, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{95}\text{H}_{102}\text{N}_6\text{O}_{24}\text{Si}[\text{M}+\text{Na}]^+$ : 1761.6612, Found: 1761.6615.

**Compound 95: Acetonide and PMB Deprotection:** To the compound **94** (275 mg, 0.158 mmol) in a 25mL flask was added 75% aq. dichloroacetic acid (5 mL) at 0 °C and the solution was stirred for 1 h. The reaction mixture was diluted with ice-cold water (25 mL), neutralized by portion-wise addition of the solid  $\text{NaHCO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 15 mL). The combined organic phases were washed with brine solution, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*, the crude residue was further purified by column chromatography using ethyl acetate and *n*-hexane as mobile phase to afford 175 mg (70%) of the compound **95**.

**Compound 96: Selective Anomeric Carbonate Donor and Diacetate Protection:** To a solution of **95** (148 mg, 0.094 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (2 mL) was added 3.0 eq of DMAP followed by the portion-wise addition of the ethynyl cyclohexyl (4-nitrophenyl) carbonate **36** (1.2 eq) and the reaction mixture was stirred at 25 °C for 3 h. After complete consumption of the starting hemiacetal, acetic anhydride (2.0 eq) was added and the stirring continued for another 2 h. The reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography using 20% ethyl acetate in *n*-hexane as a mobile phase to obtain compound **96** (120 mg, 71%) of the desired carbonate donor. Thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 0.8): +77.1°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2954, 2924, 2829, 2109, 1747, 1457, 1371, 1269, 1222, 1142, 1074, 1037, 910, 750, 705;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.10 – 8.02 (m, 2H), 8.00 – 7.94 (m, 2H), 7.68 – 7.61 (m, 4H), 7.57 (t,  $J = 7.4$  Hz, 1H), 7.48 – 7.27 (m, 24H), 7.22 – 7.14 (m, 7H), 5.91 (d,  $J = 1.7$  Hz, 1H), 5.38 (d,  $J = 3.6$  Hz, 1H), 5.35 – 5.26 (m, 2H), 5.07 (dd,  $J = 3.4, 1.7$  Hz, 1H), 4.90 – 4.80 (m, 3H), 4.79 – 4.68 (m, 4H), 4.65 (d,  $J = 10.8$  Hz, 2H), 4.60 (dd,  $J = 12.6, 2.6$  Hz, 2H), 4.42 – 4.30 (m, 2H), 4.16 – 4.06 (m, 2H), 3.94 (dq,  $J = 6.1, 3.7$  Hz, 2H), 3.90 –

3.82 (m, 5H), 3.77 (dd,  $J = 19.0, 9.5$  Hz, 2H), 3.59 (s, 3H), 3.51 (s, 3H), 3.36 – 3.24 (m, 3H), 2.65 (s, 1H), 2.23 – 2.09 (m, 5H), 2.01 (s, 3H), 1.93 – 1.83 (m, 2H), 1.75 – 1.63 (m, 3H), 1.58 – 1.49 (m, 2H), 1.39 – 1.31 (m, 1H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.1, 169.6, 168.1, 168.0, 165.9, 164.6, 151.0, 138.4, 137.8, 137.4, 136.9, 136.0, 136.0, 135.7, 135.7, 133.7, 133.6, 133.5, 132.9, 129.9 (3C), 129.8, 129.7 (3C), 129.0, 128.8, 128.8, 128.7, 128.7, 128.6 (4C), 128.5 (4C), 128.3 (3C), 128.1 (3C), 127.9, 127.8 (7C), 127.5, 127.5, 101.2, 98.0, 97.7, 93.3, 82.6, 82.5, 80.1, 78.8, 77.8, 75.8, 75.7, 75.4, 75.1, 75.0, 74.6, 74.4, 74.3, 74.0, 73.2, 73.2, 73.1, 72.6, 70.1, 69.6, 66.4, 63.6, 62.0, 61.9, 61.7, 52.6, 52.6, 36.8, 36.8, 27.0 (3C), 25.0, 22.7, 22.7, 20.9, 20.7, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{97}\text{H}_{104}\text{N}_6\text{O}_{27}\text{Si}[\text{M}+\text{Na}]^+$ : 1835.6616, Found: 1835.6498.

**Compound 97:** This compound was prepared from substrates **96** and **64b** following the procedure for the preparation of **77**. Eluent for purification: 20% ethyl acetate in *n*-hexane; thick syrup; yield 20 mg (75%) from 5 mg of the acceptor **64b**. White solid;  $[\alpha]_{\text{D}}^{25}$  ( $\text{CHCl}_3$ ,  $c$ 0.3): +206.7°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2957, 2925, 2863, 2109, 1742, 1457, 1371, 1264, 1225, 1145, 1073, 1036, 744, 705;  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ )  $\delta$  8.05 (d,  $J = 7.3$  Hz, 2H), 7.95 (d,  $J = 7.3$  Hz, 2H), 7.67 – 7.61 (m, 4H), 7.55 (t,  $J = 7.4$  Hz, 1H), 7.49 – 7.42 (m, 3H), 7.41 – 7.37 (m, 4H), 7.37 – 7.26 (m, 18H), 7.25 – 7.24 (m, 1H), 7.21 – 7.12 (m, 10H), 5.93 (ddt,  $J = 16.5, 10.8, 5.7$  Hz, 1H), 5.39 – 5.24 (m, 6H), 4.95 (dd,  $J = 18.9, 3.4$  Hz, 2H), 4.90 – 4.82 (m, 4H), 4.81 – 4.74 (m, 3H), 4.71 (d,  $J = 16.0$  Hz, 1H), 4.68 – 4.61 (m, 4H), 4.44 (d,  $J = 4.4$  Hz, 1H), 4.41 – 4.30 (m, 3H), 4.23 – 4.16 (m, 2H), 4.11 (t,  $J = 9.0$  Hz, 1H), 4.04 (dd,  $J = 12.9, 6.1$  Hz, 1H), 3.97 – 3.82 (m, 12H), 3.78 (d,  $J = 9.4$  Hz, 1H), 3.54 (s, 3H), 3.50 (s, 3H), 3.39 – 3.31 (m, 2H), 3.28 (dd,  $J = 9.8, 3.6$  Hz, 1H), 3.13 (dd,  $J = 10.8, 3.2$  Hz, 1H), 2.12 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (150.97 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.0, 170.3, 169.8, 169.6, 168.0, 165.9, 164.6, 138.3, 138.0, 137.8, 137.5, 137.3, 136.0, 136.0, 135.7, 135.7, 133.8, 133.6, 133.6, 133.2, 132.9, 129.9, 129.8 (3C), 129.7, 129.7, 129.6, 128.9 (3C), 128.7 (4C), 128.6 (4C), 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9 (5C), 127.8 (7C), 127.6 (3C), 118.5, 101.2, 98.0, 97.8, 97.7, 96.6, 82.5, 80.1, 78.4, 77.7, 76.2, 75.8, 75.8, 75.2, 75.1, 75.0, 74.9, 74.5, 74.3, 73.8, 73.3, 73.2, 72.6, 70.0, 69.8, 69.5, 69.5, 69.3, 68.8, 63.5, 63.3, 62.1, 61.8, 61.8, 61.1, 52.7, 52.4, 27.0 (3C), 21.0, 20.9, 20.8, 19.5; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{106}\text{H}_{115}\text{N}_9\text{O}_{30}\text{Si}[\text{M}+\text{Na}]^+$ : 2044.7417, Found: 2044.7214.

**Compound 98: PMB deprotection:** To a biphasic solution of the pentasaccharide **93** (15 mg, 0.007 mmol) in 10:1  $\text{CH}_2\text{Cl}_2$ : $\text{H}_2\text{O}$  (1 mL) was added DDQ (2.5 eq) at 25 °C and stirred for 30 min. After complete consumption of the starting material, the reaction mixture was filtered through a bed of Celite®, washed with  $\text{CH}_2\text{Cl}_2$ . The filtrate was sequentially washed with

## Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

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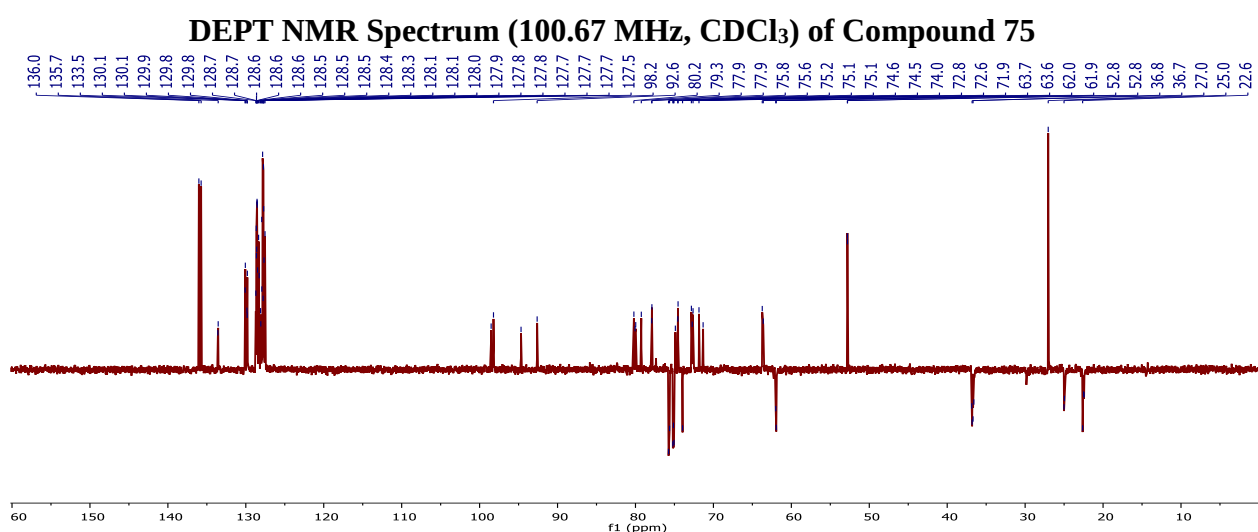
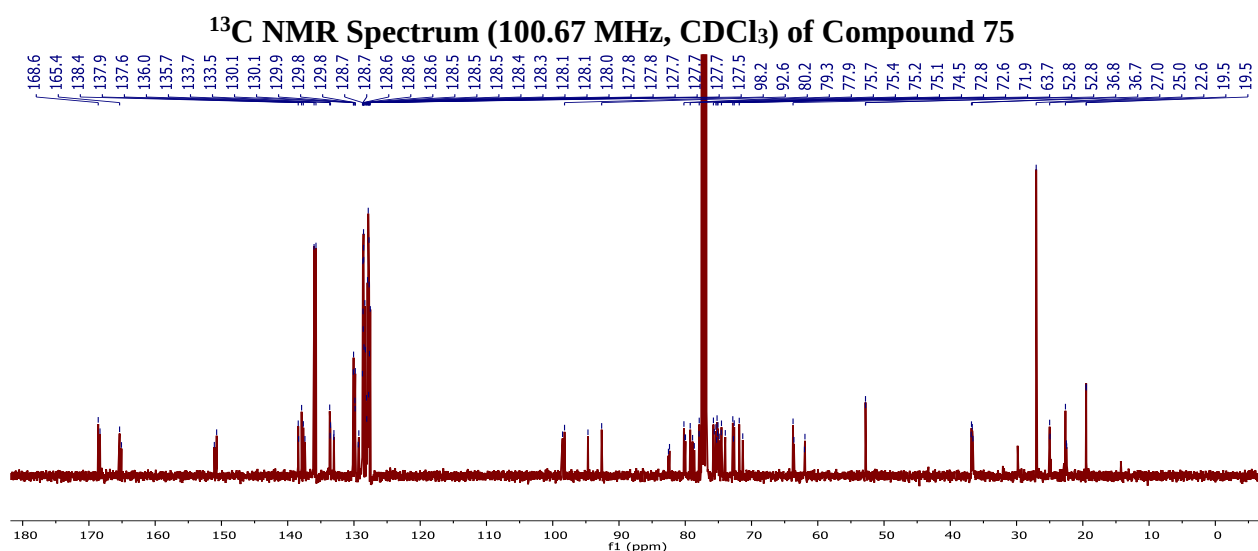
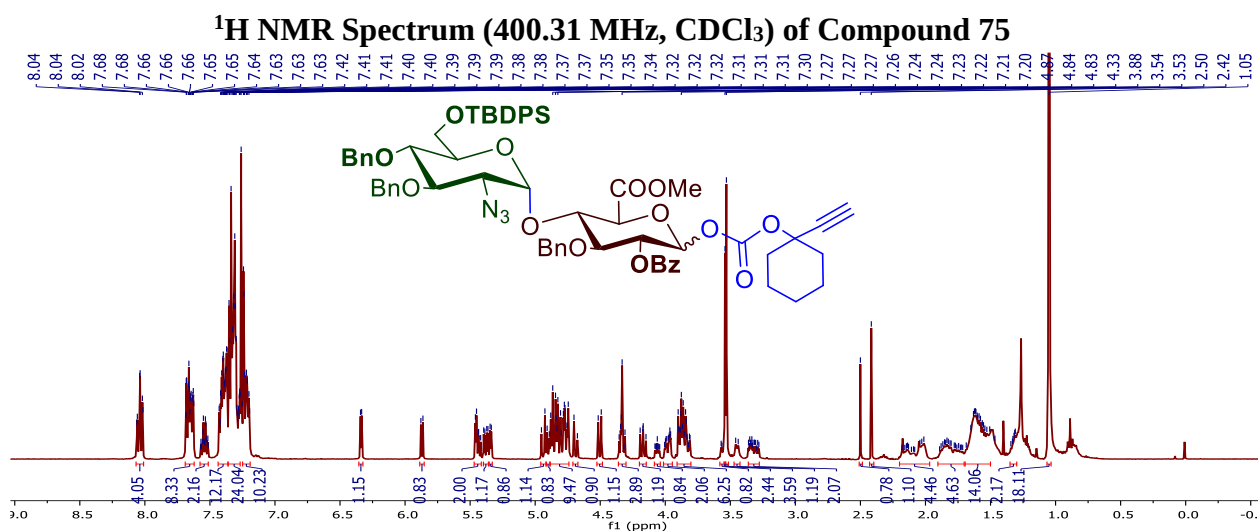
saturated aq.  $\text{Na}_2\text{S}_2\text{O}_3$  and brine solution. Organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using 25% ethyl acetate in *n*-hexane as mobile phase to obtain 12 mg (85%) of the compound **98**. Thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 0.3): +69.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2919, 2851, 2110, 1736, 1458, 1376, 1216, 1109, 1031, 757, 707;  $^1\text{H}$  NMR (600.40 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.03 (d,  $J$  = 7.3 Hz, 2H), 7.95 (d,  $J$  = 7.2 Hz, 2H), 7.63 (dd,  $J$  = 15.0, 6.8 Hz, 4H), 7.56 (t,  $J$  = 7.4 Hz, 1H), 7.52 (t,  $J$  = 7.4 Hz, 1H), 7.43 (d,  $J$  = 7.8 Hz, 2H), 7.42 – 7.38 (m, 5H), 7.36 – 7.28 (m, 17H), 7.24 (s, 1H), 7.22 – 7.15 (m, 9H), 7.09 (t,  $J$  = 6.8 Hz, 1H), 5.93 (ddt,  $J$  = 16.6, 11.3, 5.9 Hz, 1H), 5.39 – 5.33 (m, 3H), 5.28 – 5.25 (m, 1H), 5.20 (d,  $J$  = 3.6 Hz, 1H), 4.93 (d,  $J$  = 3.6 Hz, 1H), 4.88 – 4.84 (m, 4H), 4.79 (dd,  $J$  = 10.4, 4.1 Hz, 2H), 4.75 – 4.72 (m, 3H), 4.67 (d,  $J$  = 10.4 Hz, 4H), 4.55 (d,  $J$  = 3.7 Hz, 1H), 4.38 (d,  $J$  = 11.7 Hz, 1H), 4.24 (dd,  $J$  = 12.3, 2.9 Hz, 1H), 4.22 – 4.17 (m, 4H), 4.10 (d,  $J$  = 8.9 Hz, 1H), 4.05 – 4.02 (m, 2H), 3.97 (t,  $J$  = 8.4 Hz, 1H), 3.95 – 3.92 (m, 1H), 3.92 – 3.89 (m, 2H), 3.88 – 3.85 (m, 5H), 3.83 (d,  $J$  = 7.9 Hz, 1H), 3.80 – 3.76 (m, 2H), 3.55 (s, 3H), 3.54 – 3.50 (m, 1H), 3.43 – 3.38 (m, 5H), 3.32 (dt,  $J$  = 6.4, 3.5 Hz, 1H), 3.10 (dd,  $J$  = 10.4, 3.4 Hz, 1H), 2.12 (s, 3H), 2.06 (s, 3H), 1.03 (s, 9H);  $^{13}\text{C}$  NMR (150.97 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.0, 170.3, 169.3, 167.8, 165.9, 164.9, 138.1, 137.8, 137.6, 137.4, 137.1, 136.0, 136.0, 135.7, 135.7, 134.0, 133.5, 133.4, 133.1, 132.8, 129.9(4C), 129.7, 129.7, 129.6, 129.5, 128.9, 128.9, 128.7(4C), 128.6(6C), 128.4(2C), 128.2(3C), 128.1, 128.0(5C), 127.9(3C), 127.8(4C), 127.6(2C), 127.5, 118.6, 101.2, 98.0, 97.6, 97.3, 96.5, 81.8, 81.1, 80.0, 78.4, 77.7, 75.8, 75.8, 75.1, 74.8, 74.6, 74.4, 73.8, 73.5, 73.5, 72.9, 72.8, 72.7, 69.6, 69.4, 69.2, 68.9, 68.8, 68.5, 63.4, 63.3, 62.2, 62.0, 61.8, 61.8, 53.2, 52.2, 27.0(3C), 21.0, 20.8, 19.4; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{104}\text{H}_{113}\text{N}_9\text{O}_{29}\text{Si}[\text{M}+\text{Na}]^+$ : 2002.7311, Found: 2002.7004.

**Compound 99:** This compound was prepared from **93** following the procedure described for the preparation of compound **66**. Eluent for purification: 40% ethyl acetate in *n*-hexane; Thick syrup; yield 11 mg (85%) from 15 mg;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c$ 0.3): +63.3°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3020, 2922, 2109, 1735, 1470, 1412, 1380, 1214, 1100, 1028, 747, 668;  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.08 – 8.04 (m, 2H), 8.03 – 7.98 (m, 2H), 7.58 (t,  $J$  = 7.4 Hz, 1H), 7.52 (t,  $J$  = 7.4 Hz, 1H), 7.47 – 7.40 (m, 4H), 7.38 – 7.31 (m, 9H), 7.31 – 7.26 (m, 8H), 7.25 (s, 2H), 7.22 – 7.13 (m, 6H), 7.10 (dd,  $J$  = 7.1, 2.4 Hz, 2H), 6.88 (d,  $J$  = 8.7 Hz, 2H), 5.98 – 5.88 (m, 1H), 5.43 – 5.38 (m, 2H), 5.35 (dd,  $J$  = 17.2, 1.5 Hz, 1H), 5.28 – 5.24 (m, 1H), 5.18 (d,  $J$  = 3.9 Hz, 1H), 5.03 (d,  $J$  = 10.2 Hz, 1H), 4.92 (d,  $J$  = 3.6 Hz, 1H), 4.88 – 4.74 (m, 8H), 4.69 – 4.62 (m, 5H), 4.56 (d,  $J$  = 10.3 Hz, 1H), 4.50 (d,  $J$  = 4.1 Hz, 1H), 4.48 – 4.43 (m, 1H), 4.41 – 4.34 (m, 2H), 4.29 – 4.24 (m, 1H), 4.22 – 4.16 (m, 2H), 4.04 (dd,  $J$  = 12.9, 6.2 Hz, 1H), 3.98 – 3.93 (m, 1H), 3.93 – 3.89 (m, 2H), 3.87 (d,  $J$  = 9.5 Hz, 1H), 3.85 – 3.81 (m, 4H), 3.79 (s, 3H), 3.77 – 3.70

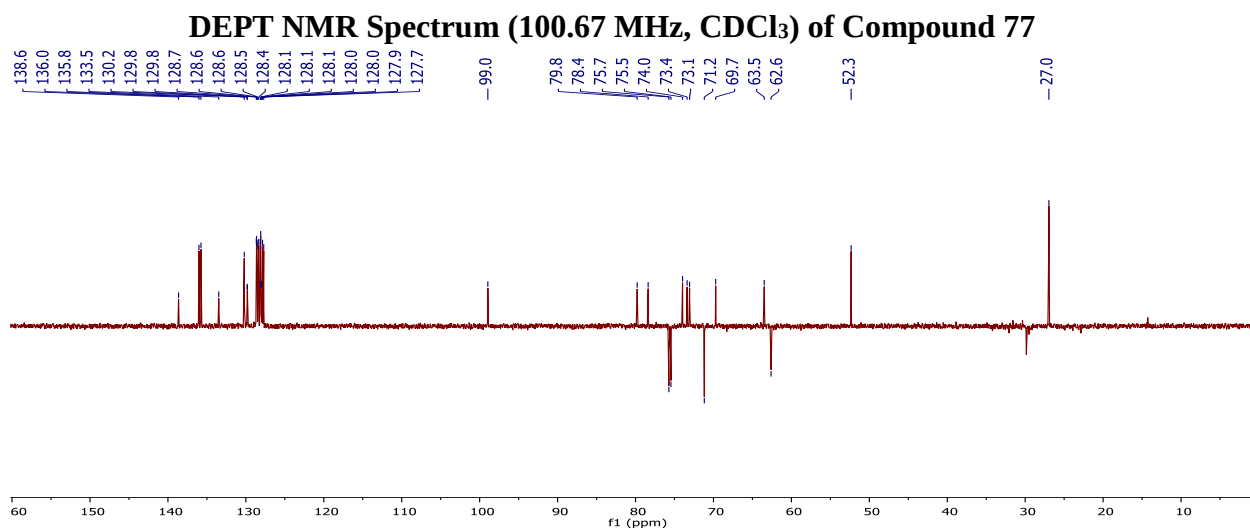
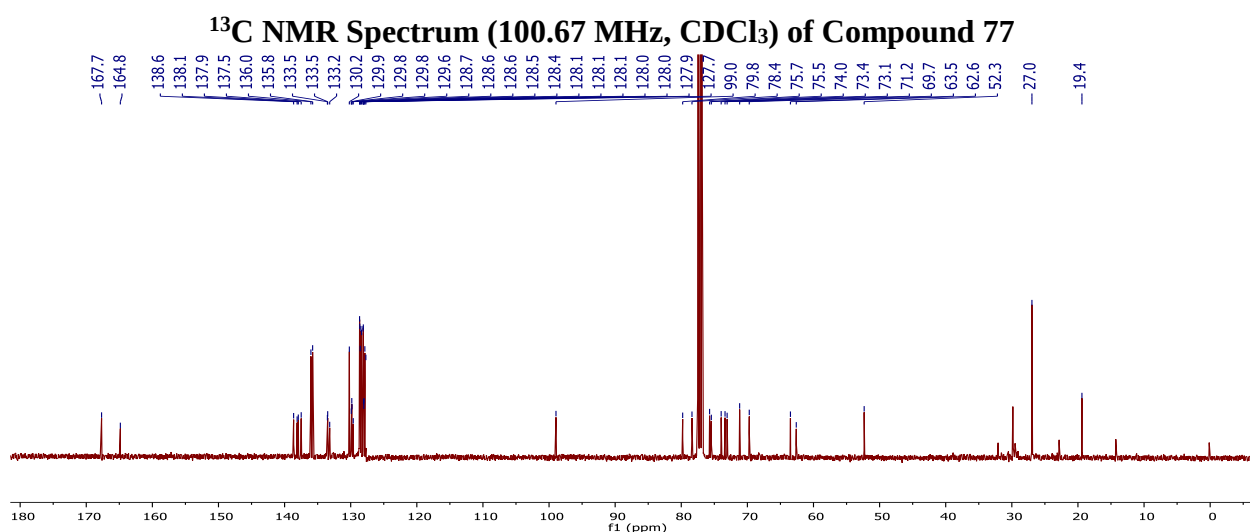
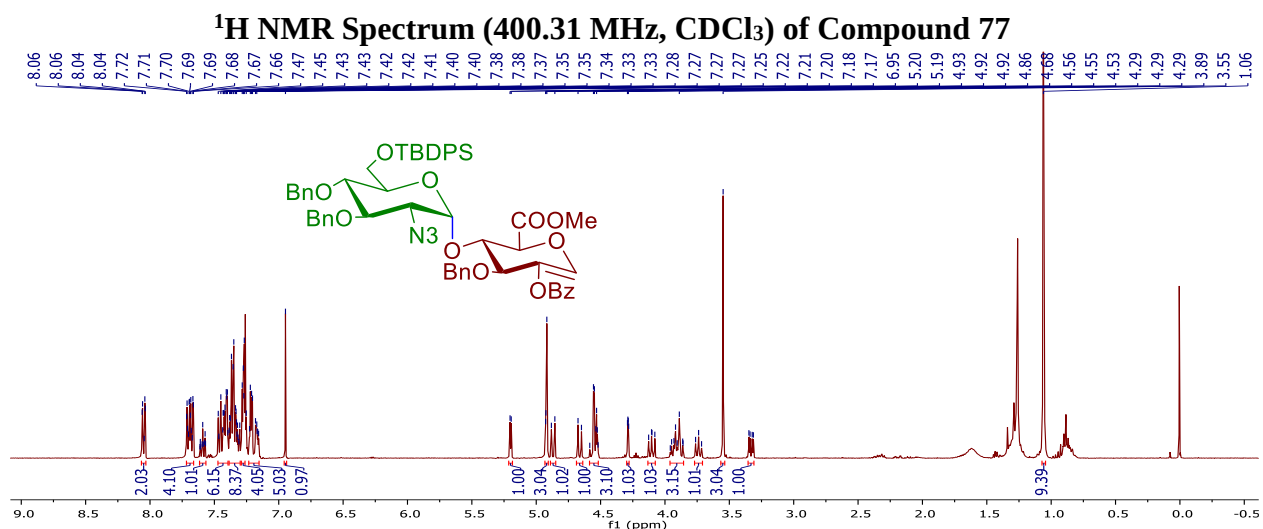
(m, 2H), 3.70 – 3.64 (m, 2H), 3.63 (s, 3H), 3.52 (t,  $J = 9.4$  Hz, 1H), 3.41 – 3.34 (m, 2H), 3.31 – 3.24 (m, 4H), 3.22 (dd,  $J = 10.3$ , 3.6 Hz, 1H), 2.11 (s, 3H), 2.04 (s, 3H);  $^{13}\text{C}$  NMR (150.99 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.8, 170.1, 169.4, 168.1, 166.1, 164.8, 159.4, 138.0, 137.9, 137.8, 137.5, 137.3, 133.9, 133.6, 133.2, 130.2(3C), 129.8, 129.8, 129.7(3C), 129.6, 129.0, 129.0, 128.8, 128.8, 128.7, 128.7, 128.6(4C), 128.5, 128.5, 128.3, 128.3, 128.2, 128.2, 128.1(3C), 128.0(4C), 127.9, 127.8(4C), 127.6, 118.5, 113.9, 113.9, 100.9, 98.0, 97.7, 97.2, 96.7, 82.5, 80.1, 78.4, 77.8, 77.3, 77.3, 76.0, 75.6, 75.5, 75.3, 75.2, 75.1, 74.9, 74.9, 73.9, 73.8, 73.6, 72.7, 72.3, 69.6, 69.6, 69.3, 69.3, 68.9, 63.5, 63.4, 62.8, 62.3, 62.1, 61.4, 55.4, 53.0, 51.9, 21.0, 20.9; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{96}\text{H}_{103}\text{N}_9\text{O}_{30} [\text{M}+\text{Na}]^+$ : 1884.6709, Found: 1884.6536.

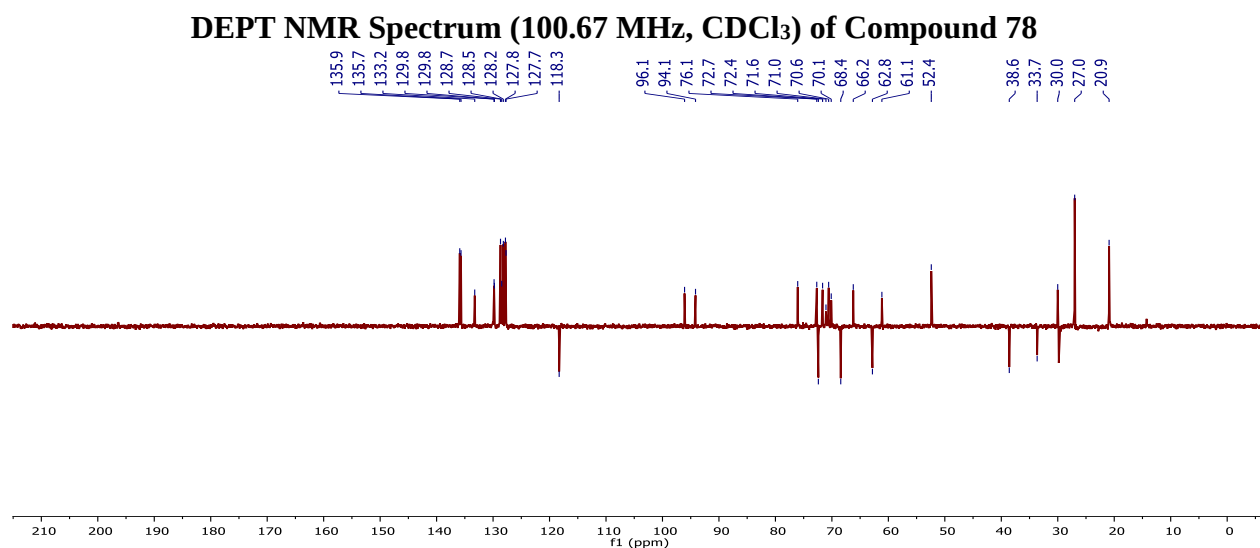
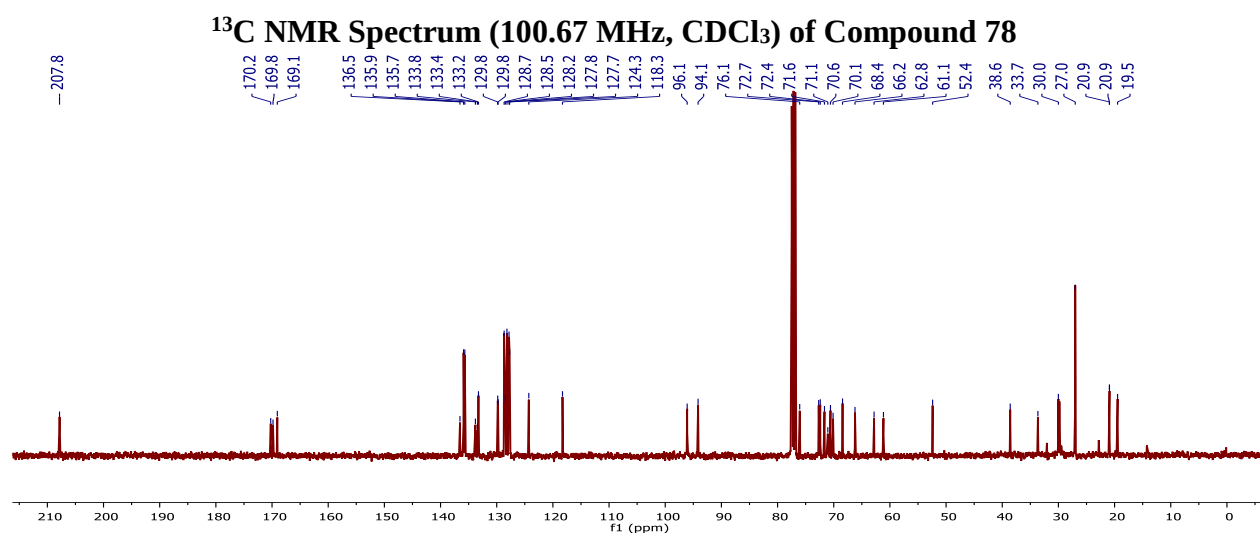
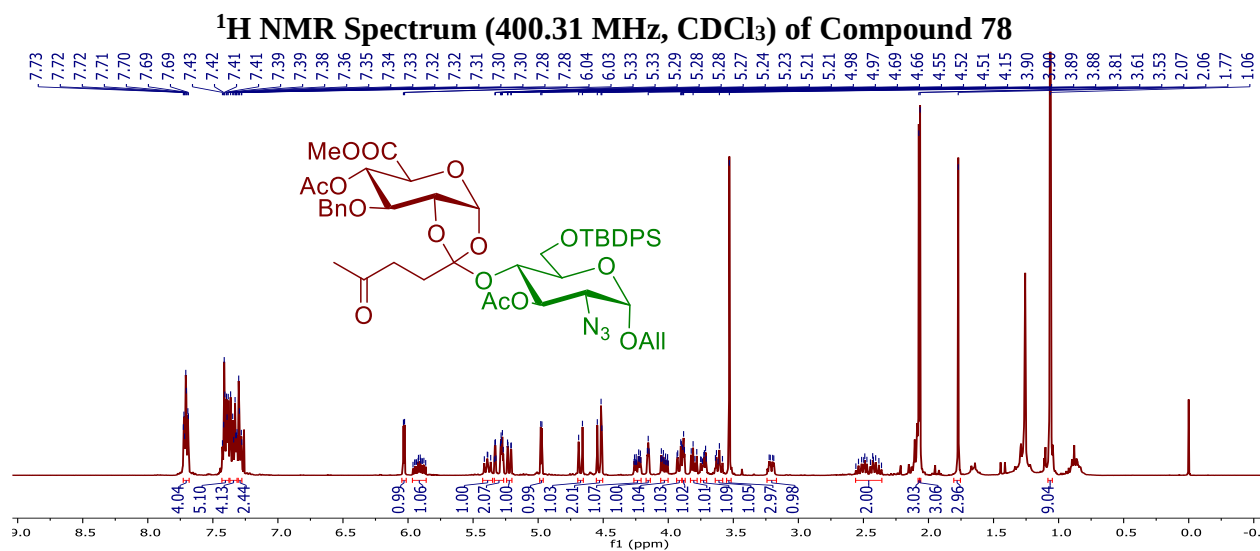
**Compound 100:** This compound was prepared from compound **99** by following the above described procedure for the preparation of compound **98** [yield 5 mg (76%) from 7 mg], In addition, this compound was prepared from compound **98**, following the above procedure for compound **66** [yield 5 mg (81%) from 7 mg]. Eluent for purification: 45% ethyl acetate in *n*-hexane; Thick syrup;  $[\alpha]^{25}_{\text{D}}$  ( $\text{CHCl}_3$ ,  $c_{0.2}$ ): +48.0°; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 2922, 2109, 1733, 1456, 1376, 1216, 1027, 756;  $^1\text{H}$  NMR (600.40 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.04 (d,  $J = 7.8$  Hz, 2H), 7.99 (d,  $J = 7.5$  Hz, 2H), 7.59 (t,  $J = 7.3$  Hz, 1H), 7.55 – 7.53 (m, 1H), 7.45 (t,  $J = 7.6$  Hz, 2H), 7.41 (t,  $J = 7.5$  Hz, 3H), 7.37 – 7.32 (m, 12H), 7.24 – 7.13 (m, 10H), 5.96 (ddt,  $J = 16.0$ , 10.6, 5.4 Hz, 1H), 5.45 (d,  $J = 3.2$  Hz, 1H), 5.41 – 5.36 (m, 2H), 5.28 (d,  $J = 10.1$  Hz, 1H), 5.24 – 5.19 (m, 1H), 4.96 – 4.94 (m, 1H), 4.92 – 4.88 (m, 2H), 4.87 – 4.82 (m, 4H), 4.77 (d,  $J = 9.4$  Hz, 2H), 4.72 (d,  $J = 12.3$  Hz, 2H), 4.71 – 4.69 (m, 2H), 4.67 (d,  $J = 5.8$  Hz, 1H), 4.63 (d,  $J = 3.1$  Hz, 1H), 4.40 (d,  $J = 12.1$  Hz, 1H), 4.35 – 4.29 (m, 2H), 4.28 – 4.19 (m, 5H), 4.16 (s, 1H), 4.07 (dd,  $J = 12.6$ , 5.9 Hz, 1H), 4.01 – 3.96 (m, 3H), 3.92 – 3.90 (m, 1H), 3.89 – 3.86 (m, 4H), 3.83 (s, 3H), 3.82 – 3.80 (m, 1H), 3.79 (d,  $J = 9.2$  Hz, 1H), 3.69 – 3.66 (m, 1H), 3.57 (t,  $J = 9.2$  Hz, 2H), 3.47 (d,  $J = 10.3$  Hz, 1H), 3.43 (s, 3H), 3.41 – 3.39 (m, 1H), 3.32 (dd,  $J = 10.1$ , 3.4 Hz, 1H), 3.15 (dd,  $J = 10.3$ , 2.9 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H);  $^{13}\text{C}$  NMR (150.99 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.9, 170.3, 169.3, 168.5, 166.0, 164.9, 138.0, 137.9, 137.8, 137.4, 137.0, 133.9, 133.5, 133.2, 129.9(3C), 129.7, 129.7, 128.9, 128.9, 128.7(4C), 128.6(6C), 128.5, 128.2(6C), 128.1(6C), 128.0, 128.0, 127.8, 127.8, 127.5, 118.5, 101.3, 98.1, 97.5, 97.2, 96.7, 82.1, 81.5, 80.0, 78.5, 77.7, 75.8, 75.6, 75.2, 74.8, 74.7, 74.2, 74.0, 73.4, 73.4, 73.1, 72.7, 72.4, 69.8, 69.4, 69.4, 68.9, 68.9, 68.6, 63.5, 63.4, 62.3, 62.1, 62.0, 61.3, 53.5, 52.2, 21.0, 20.8; HRMS (ESI-MS):  $m/z$  calcd for  $\text{C}_{88}\text{H}_{95}\text{N}_9\text{O}_{29} [\text{M}+\text{Na}]^+$ : 1764.6133, Found: 1764.6050.

## 4.11 Spectral Charts of Representative Compounds:



# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

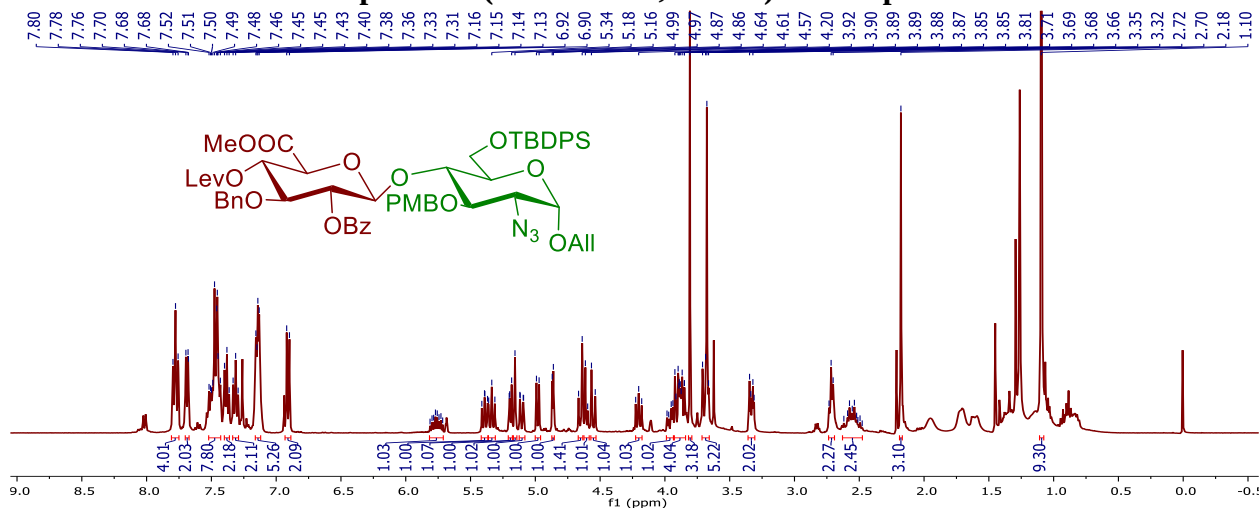




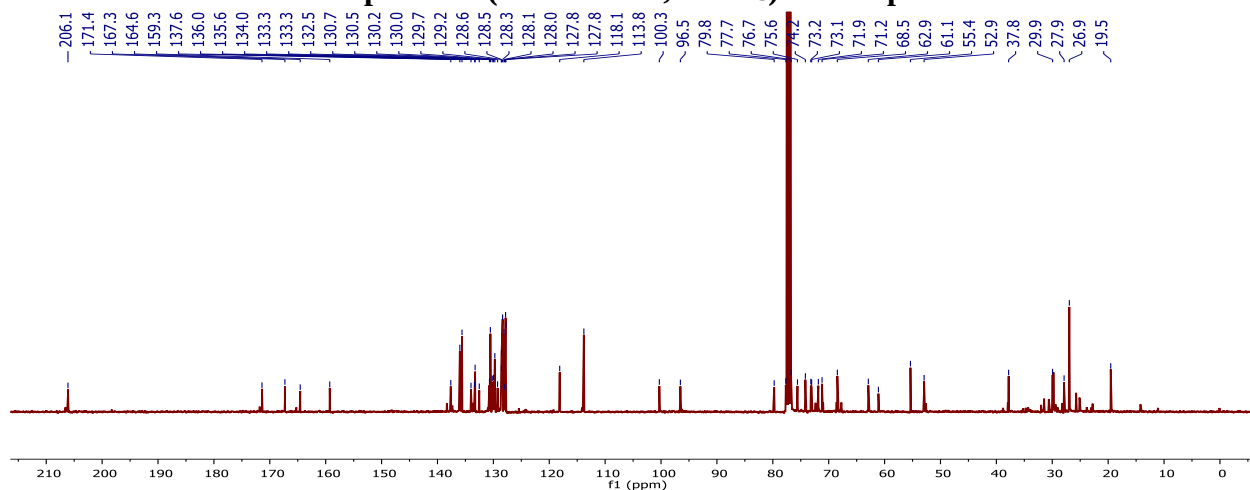


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

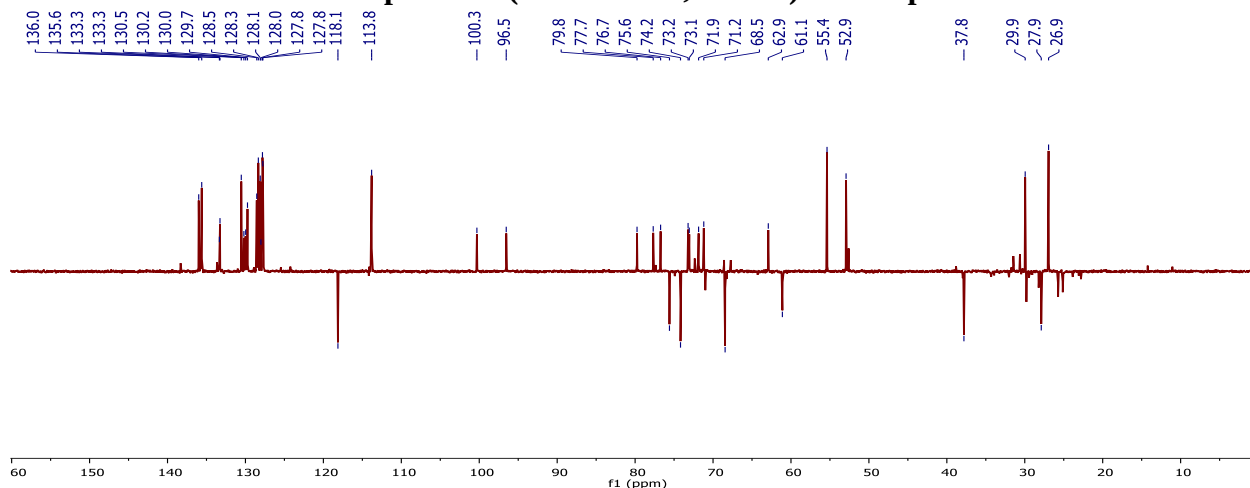
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 79**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 79**

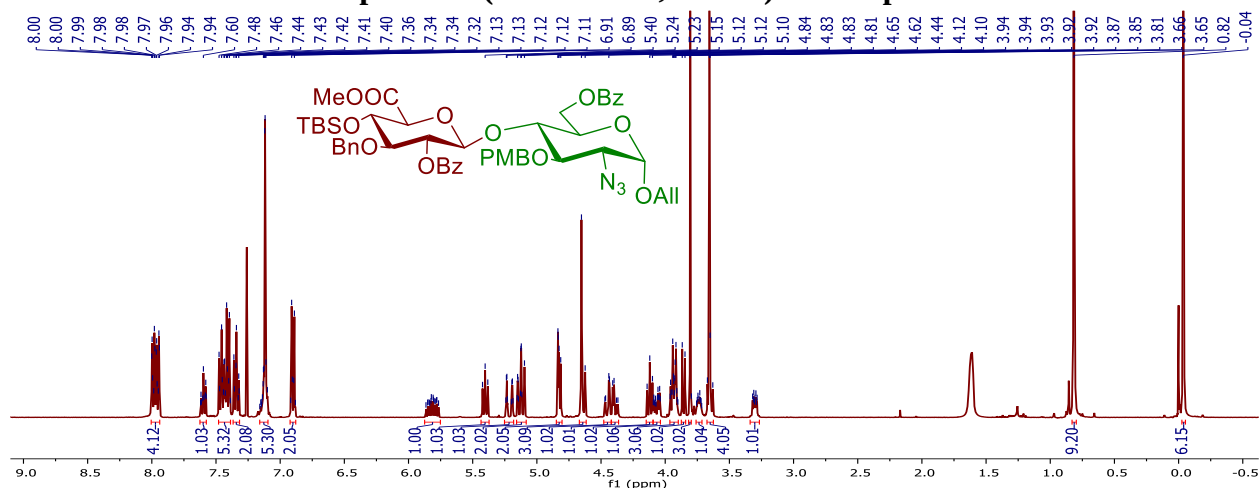


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 79**

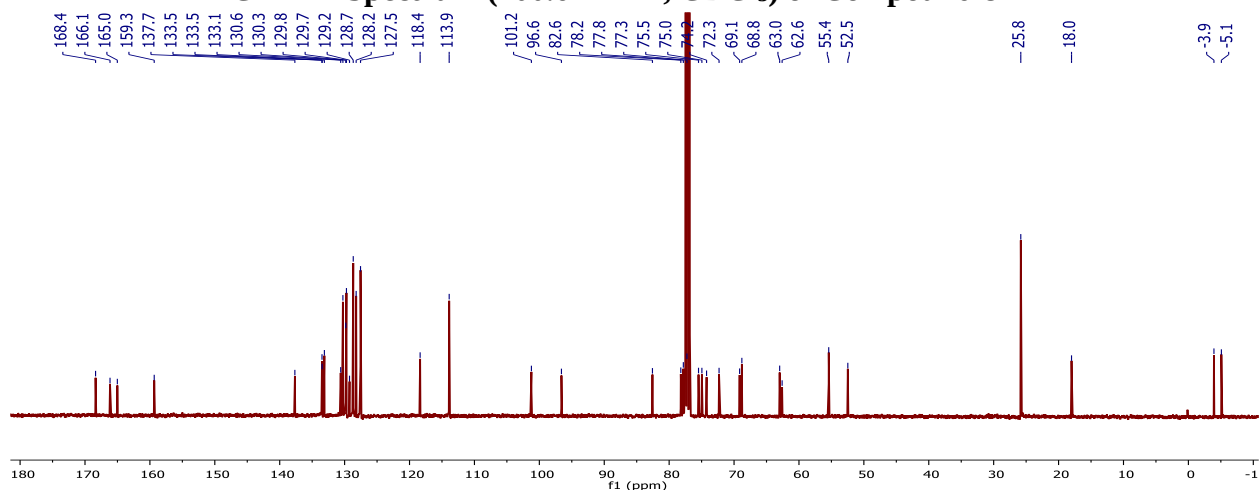


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

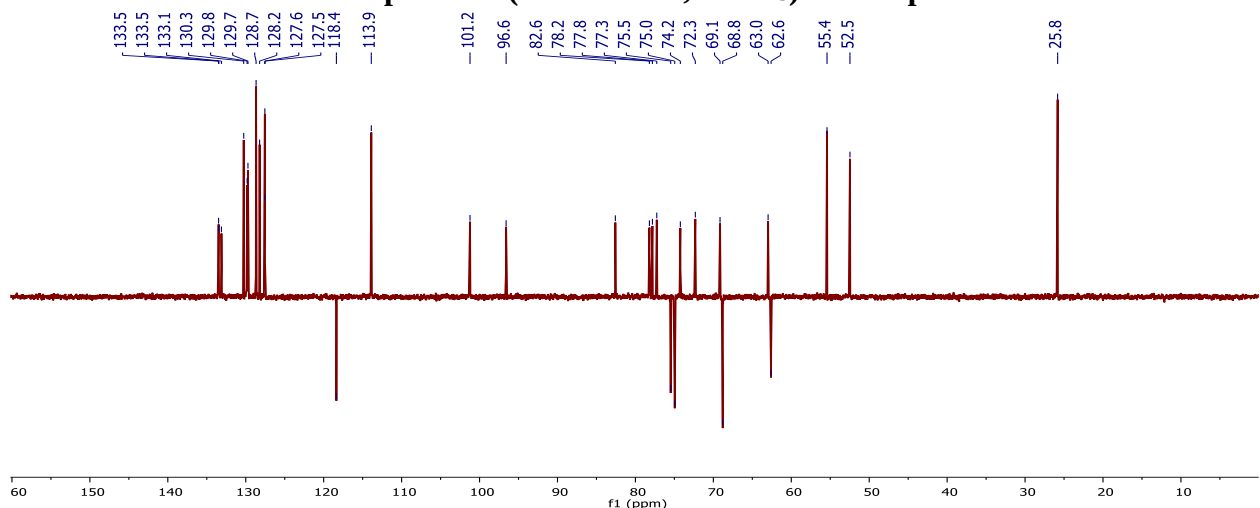
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 81**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 81**

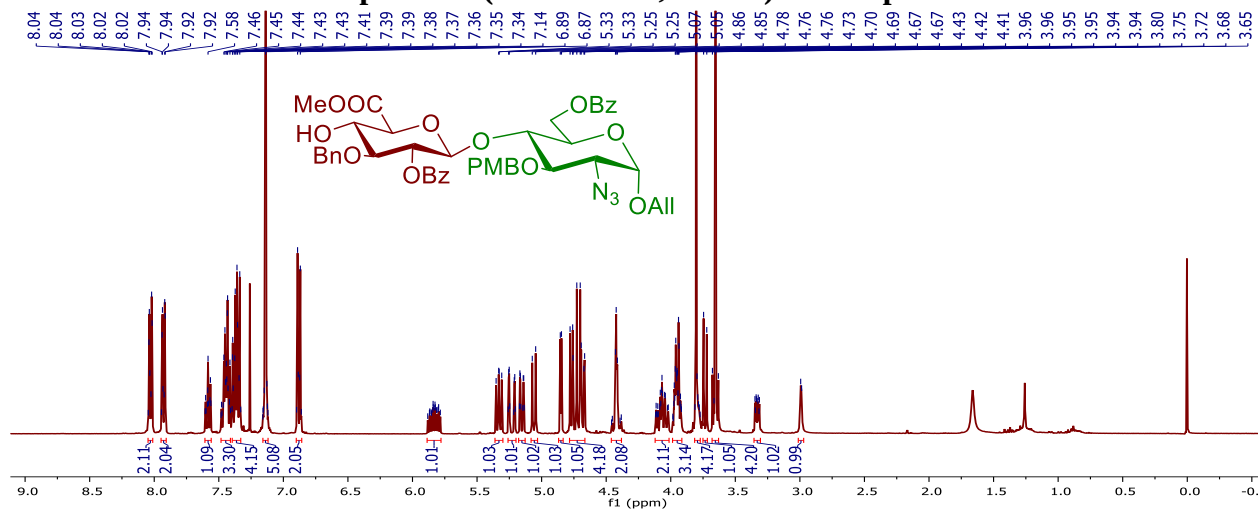


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 81**

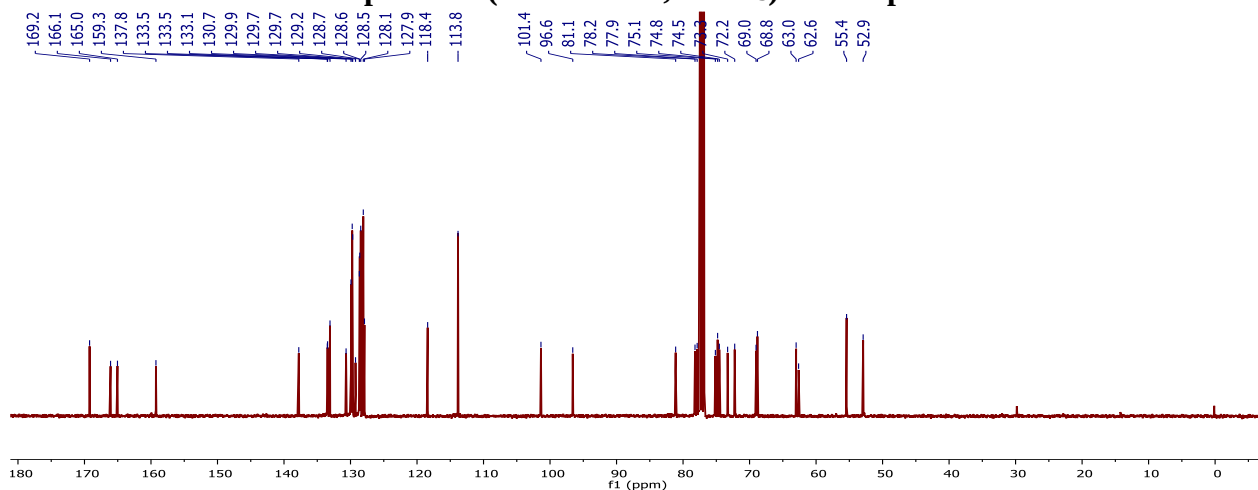


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

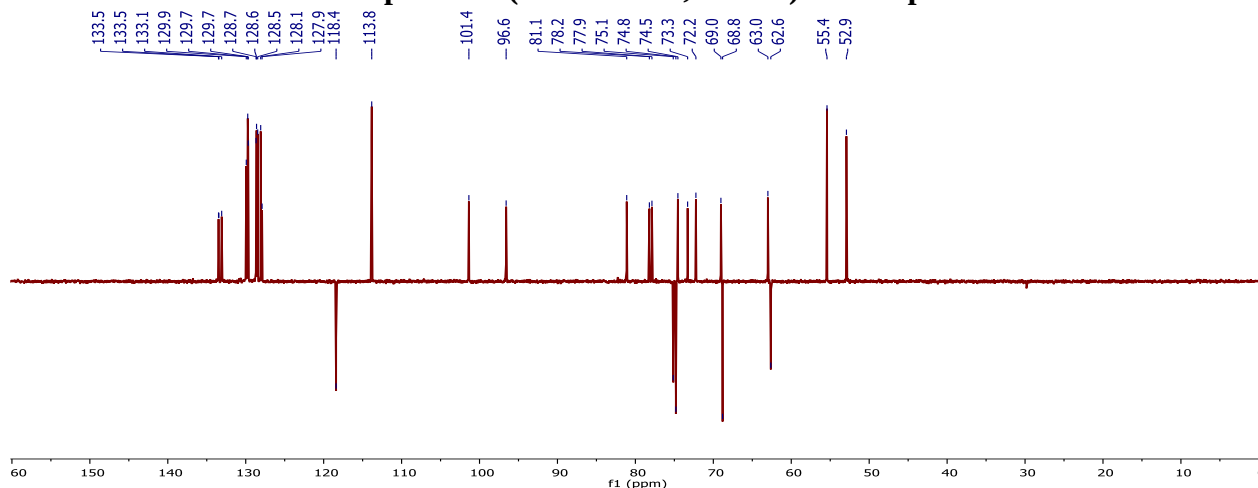
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 82**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 82**

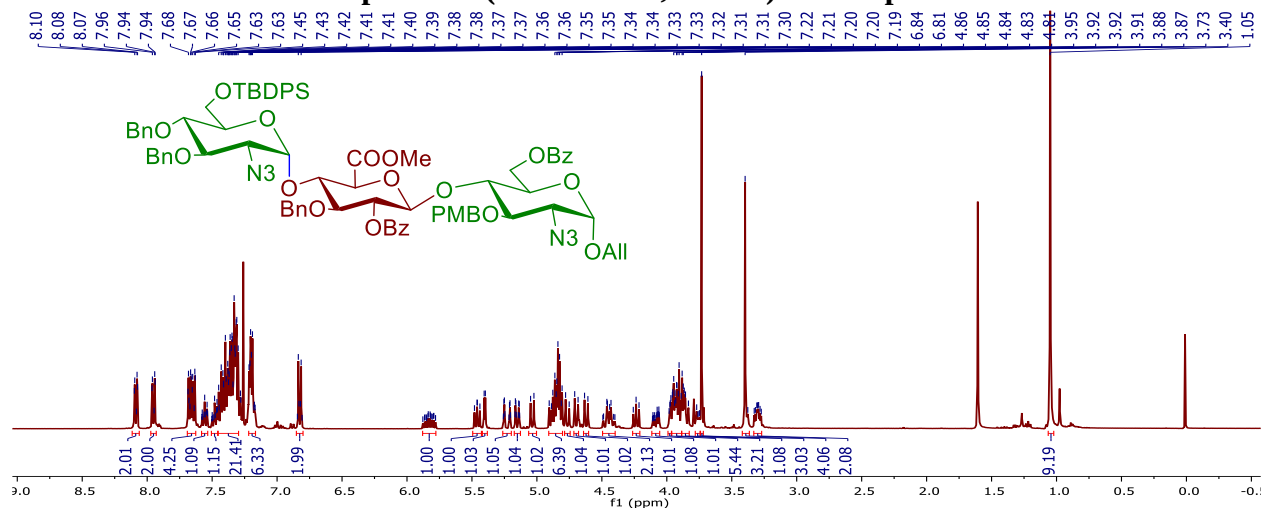


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 82**

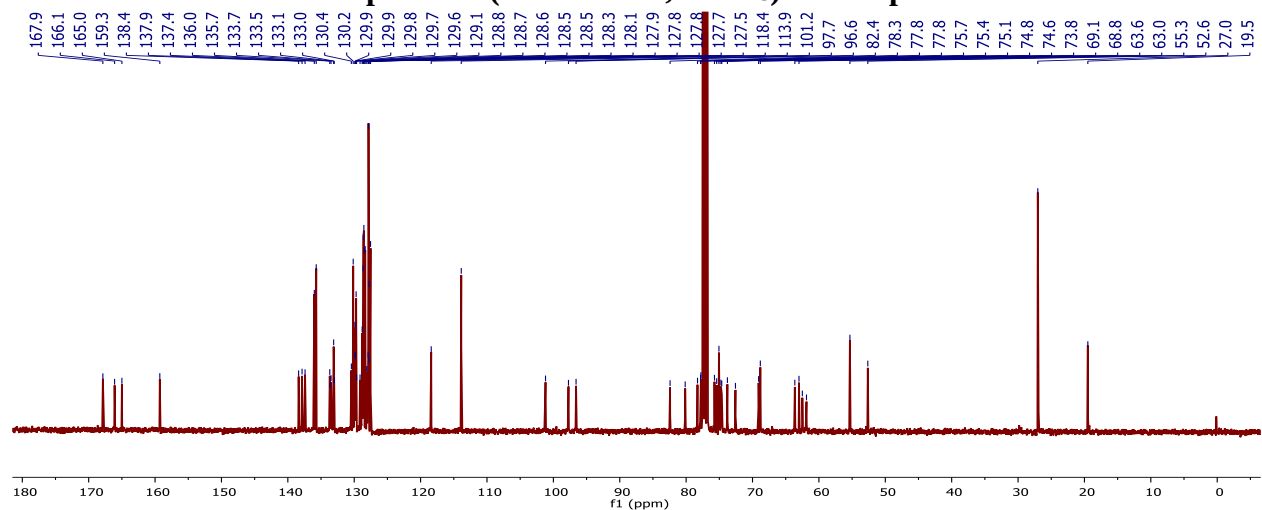


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

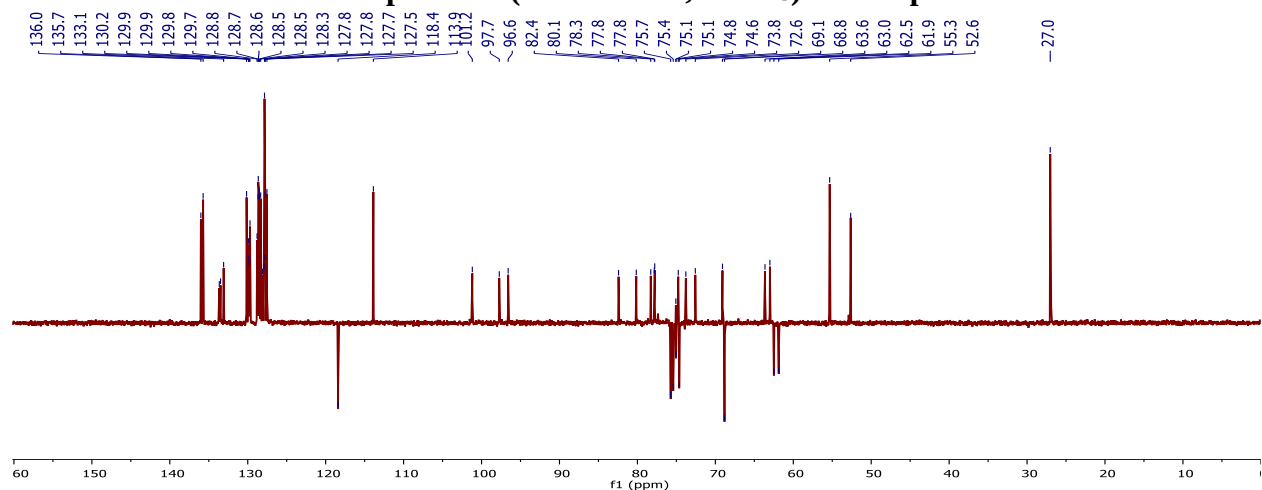
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 83**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 83**



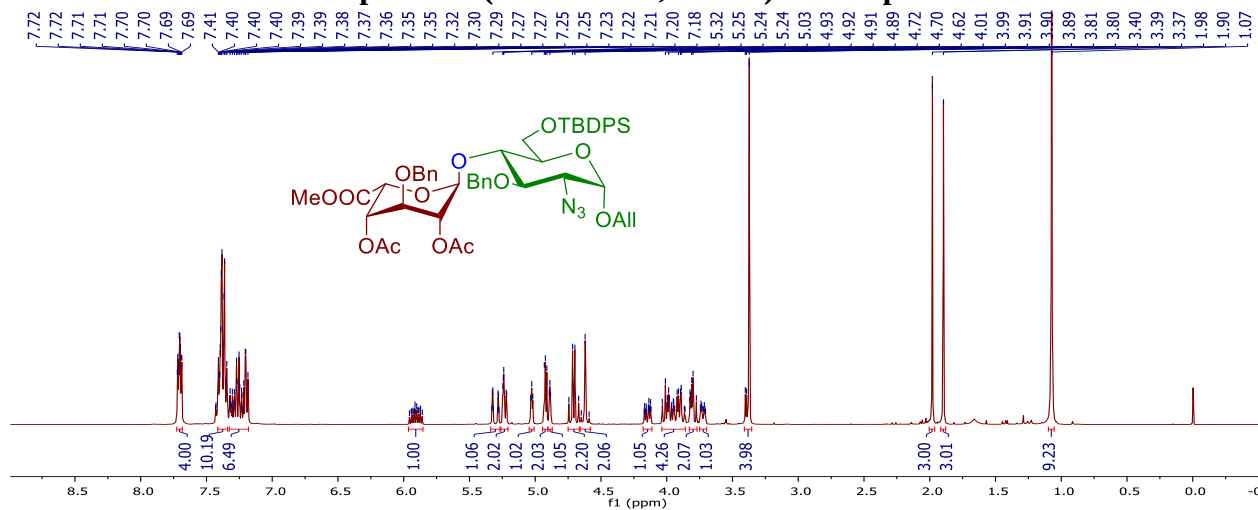
**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 83**



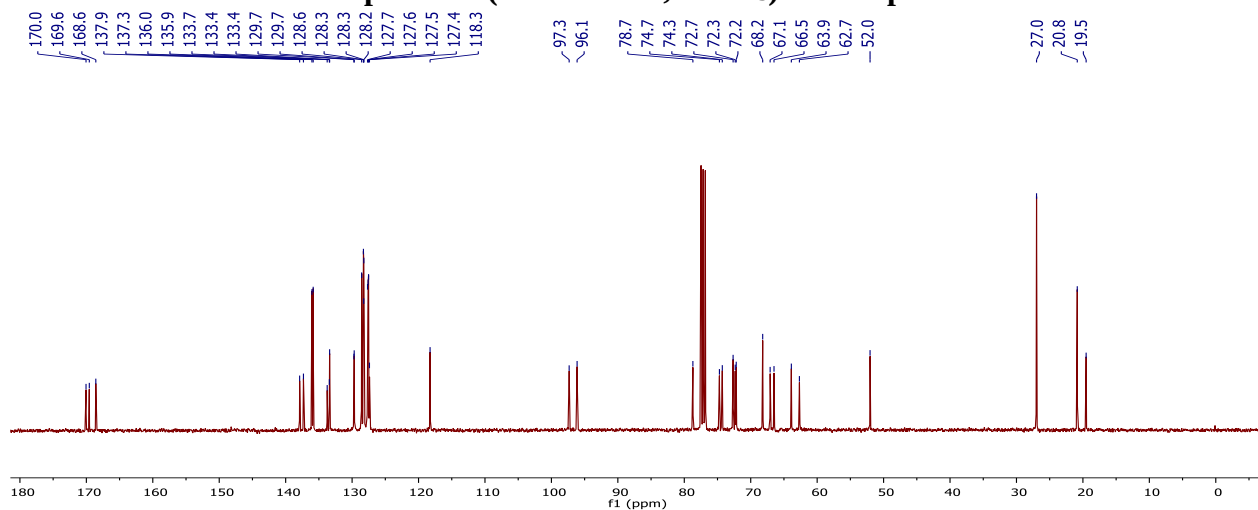
|   | 1    | 2    | 3    | 4    |
|---|------|------|------|------|
| 1 | 1    | 0.99 | 0.99 | 0.99 |
| 2 | 0.99 | 1    | 0.99 | 0.99 |
| 3 | 0.99 | 0.99 | 1    | 0.99 |
| 4 | 0.99 | 0.99 | 0.99 | 1    |

# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

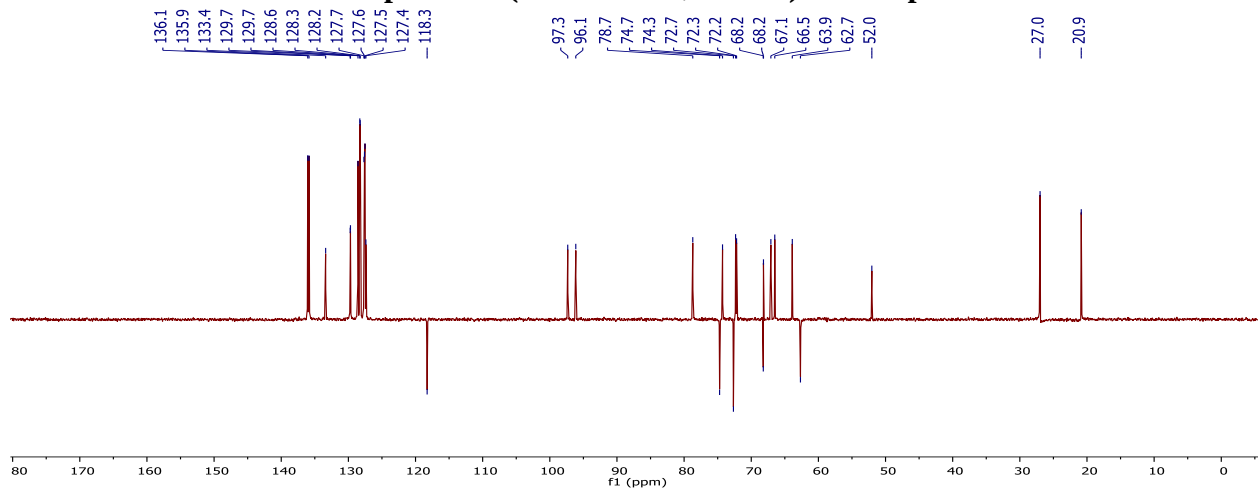
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 86**



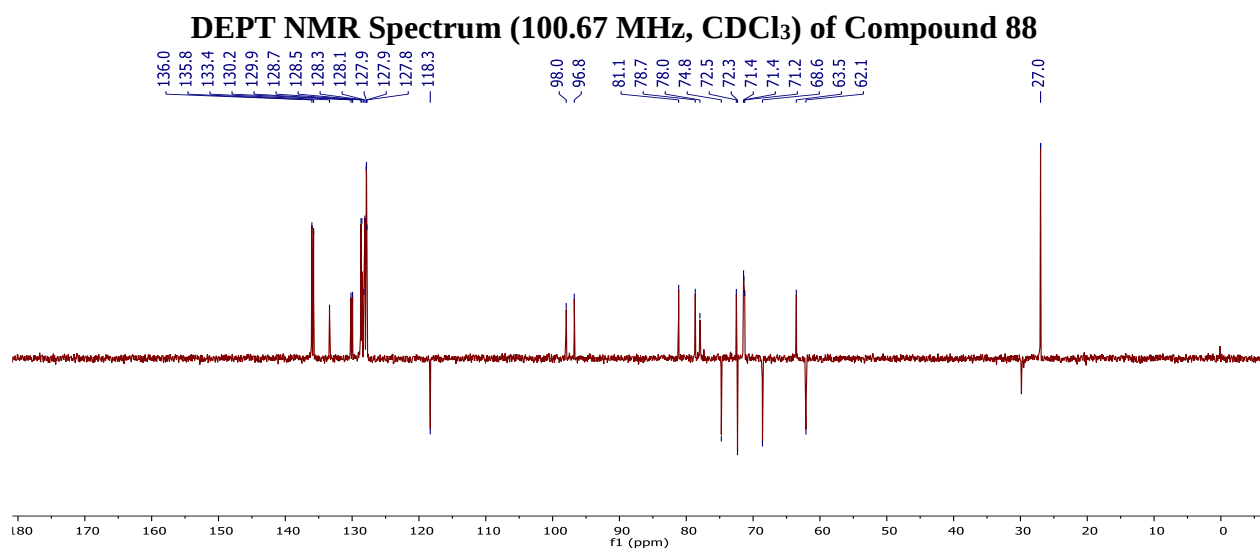
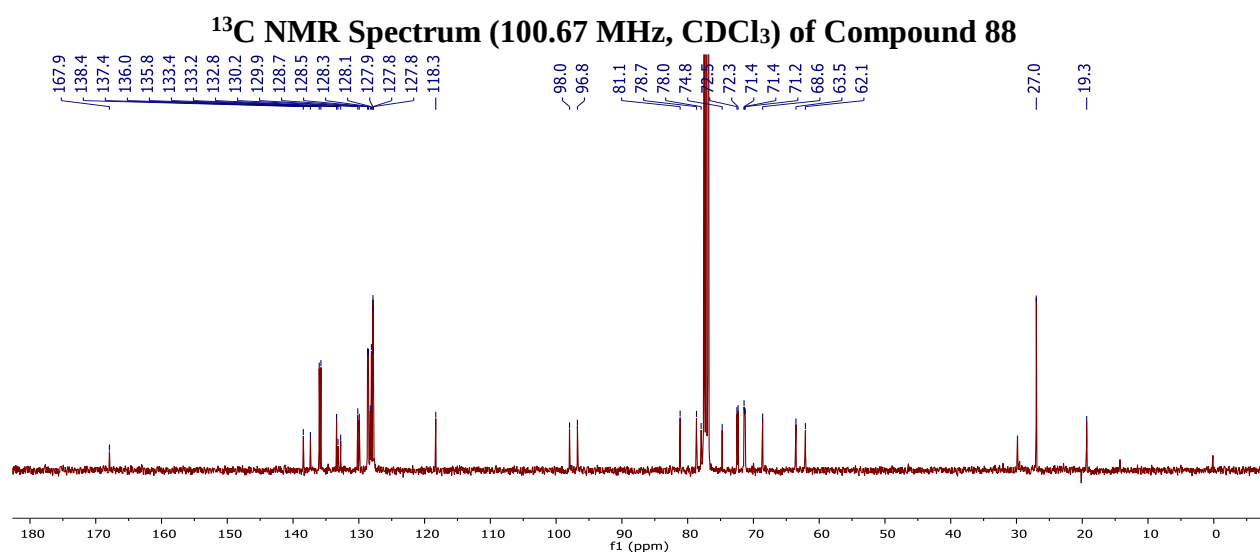
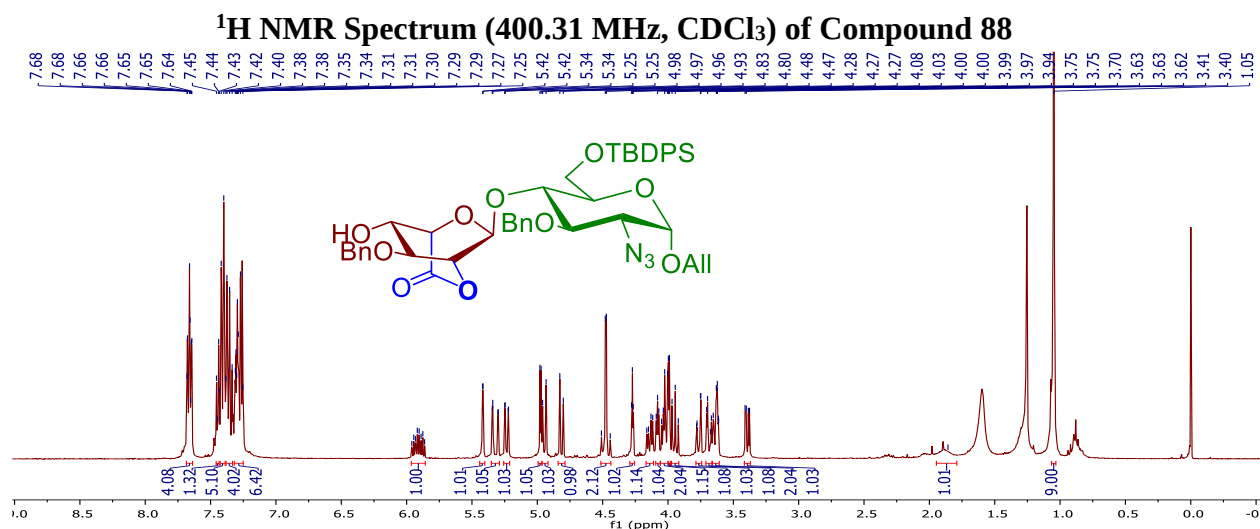
**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 86**



**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 86**

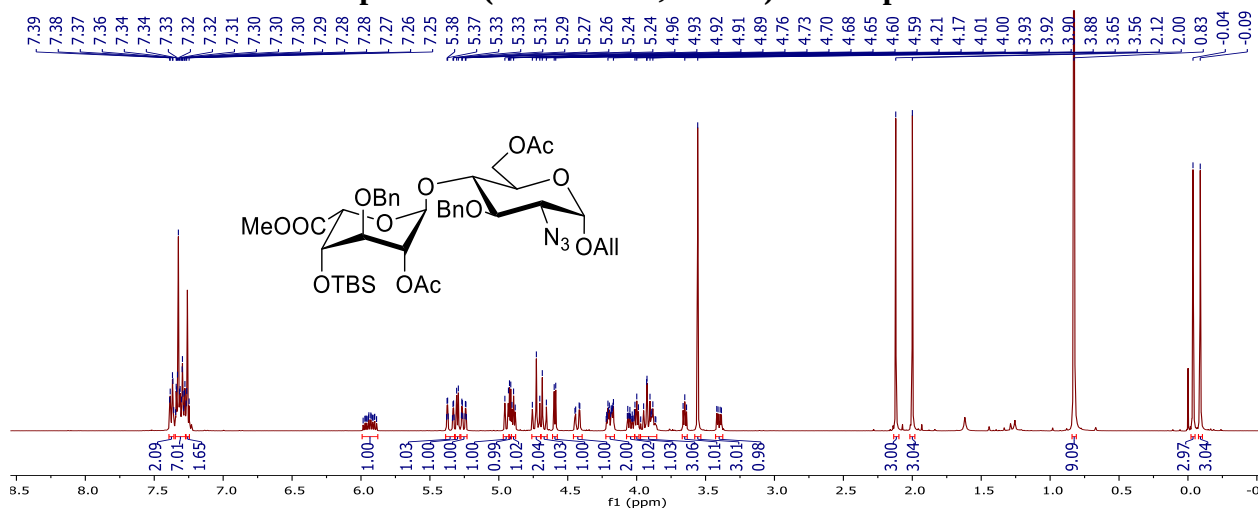


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

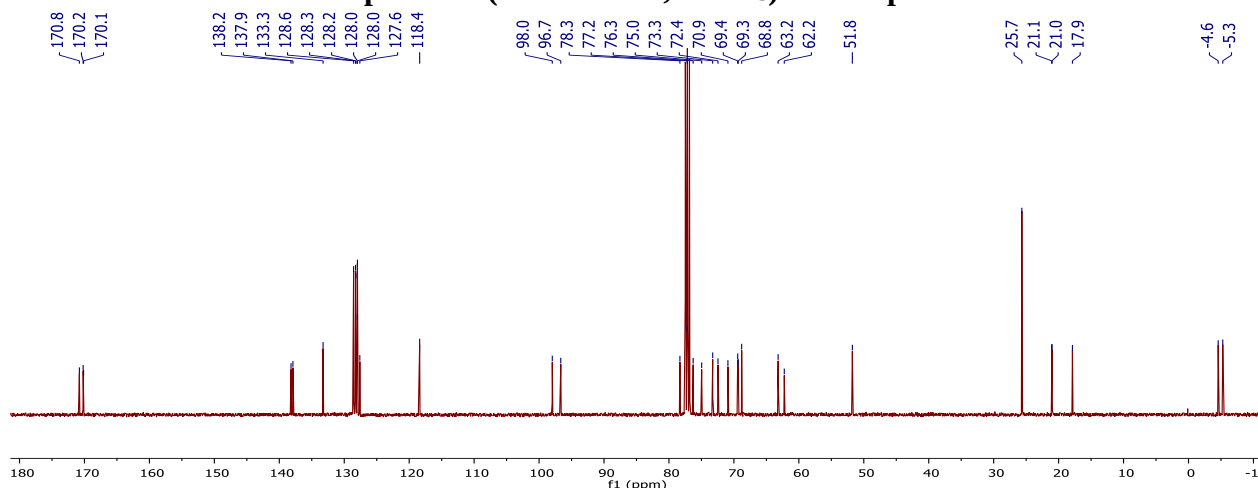


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

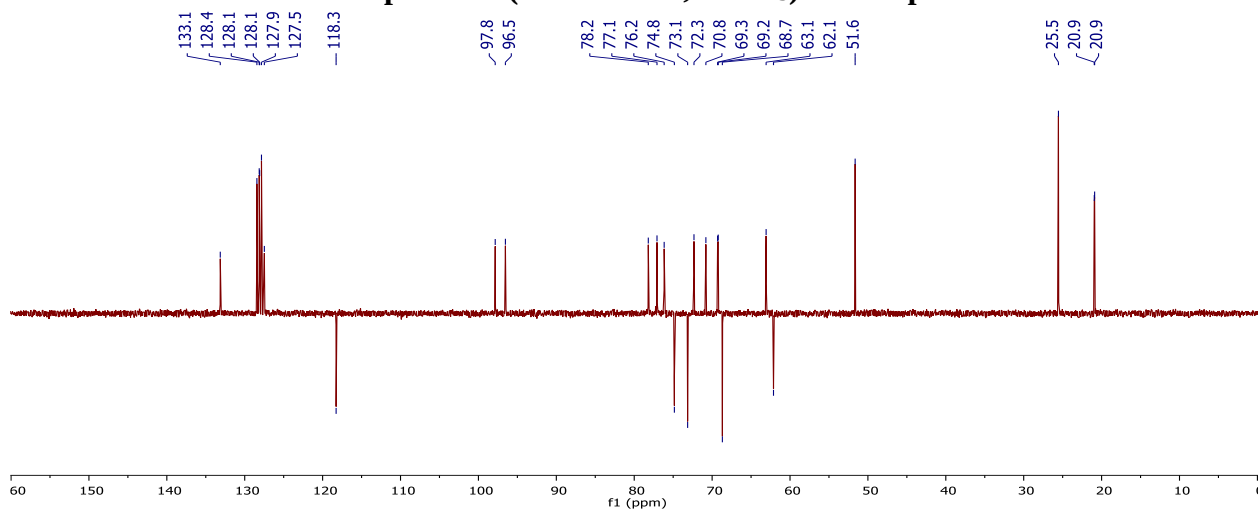
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 92**



**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 92**

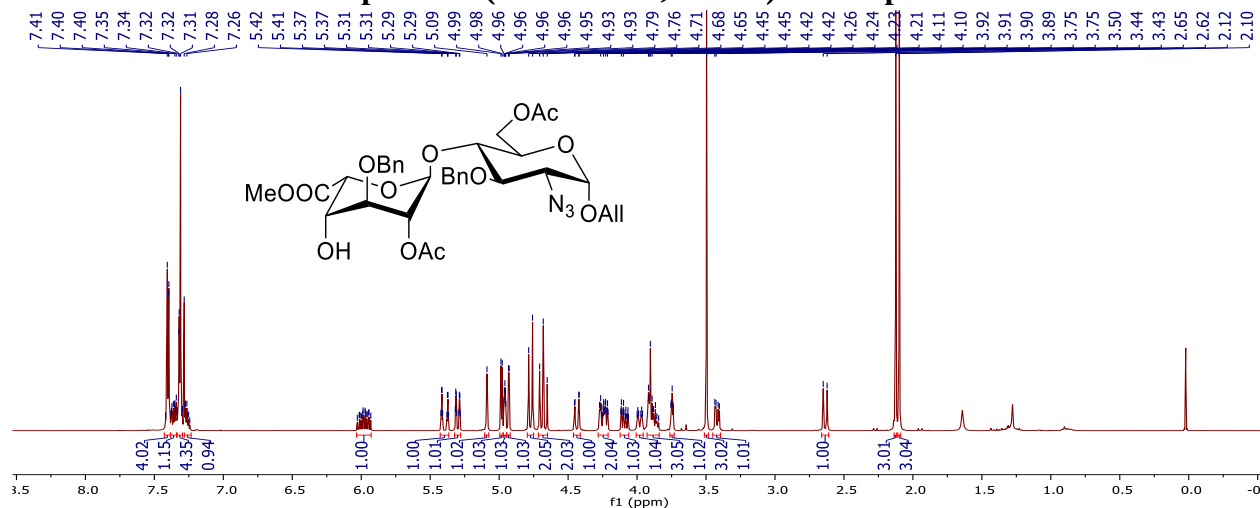


**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 92**

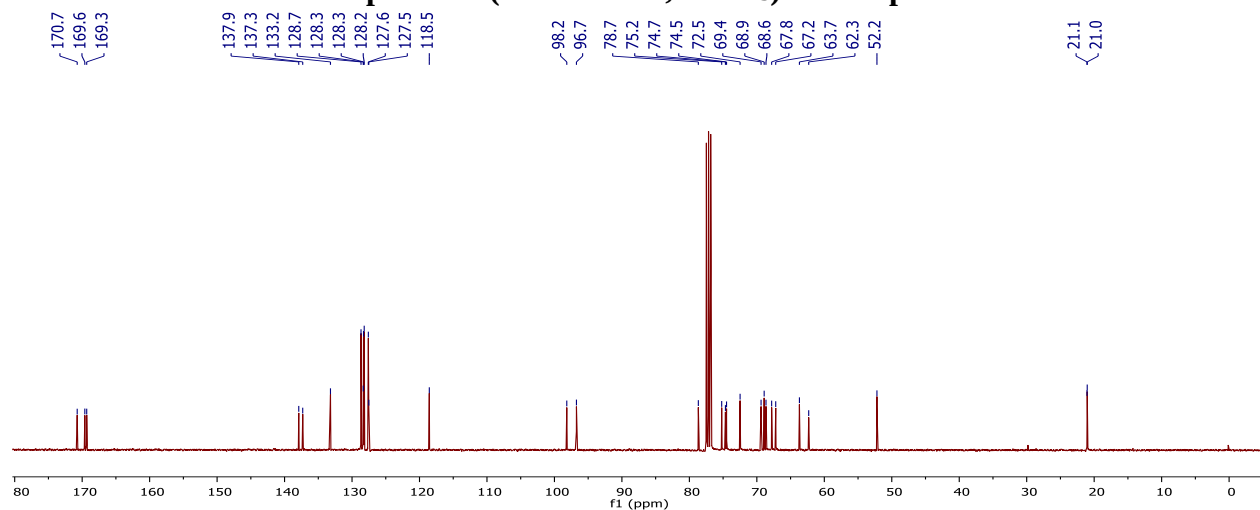




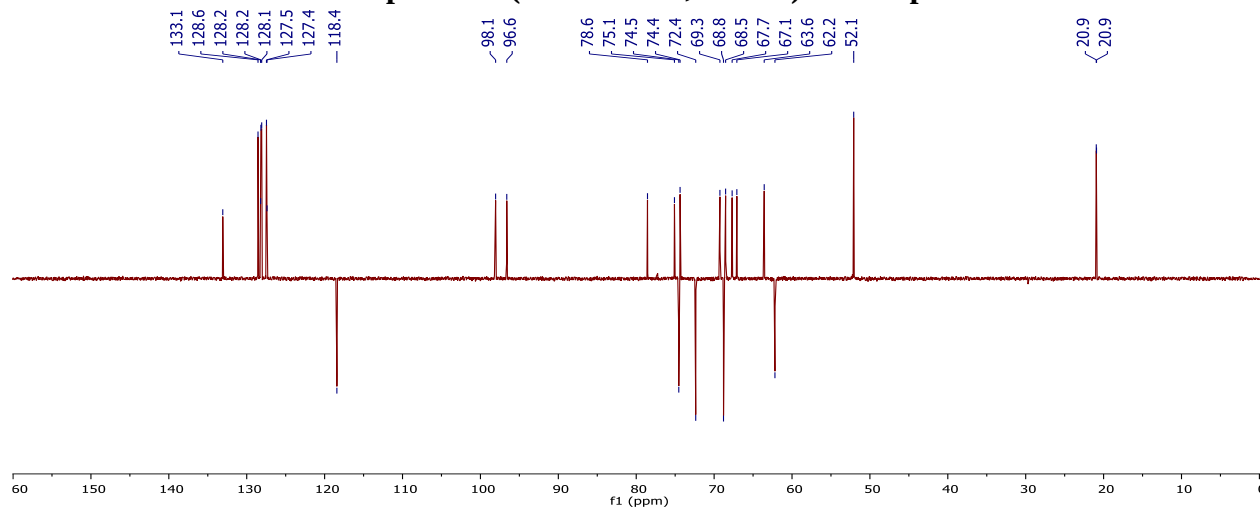
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 73**



**<sup>13</sup>C NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 73**

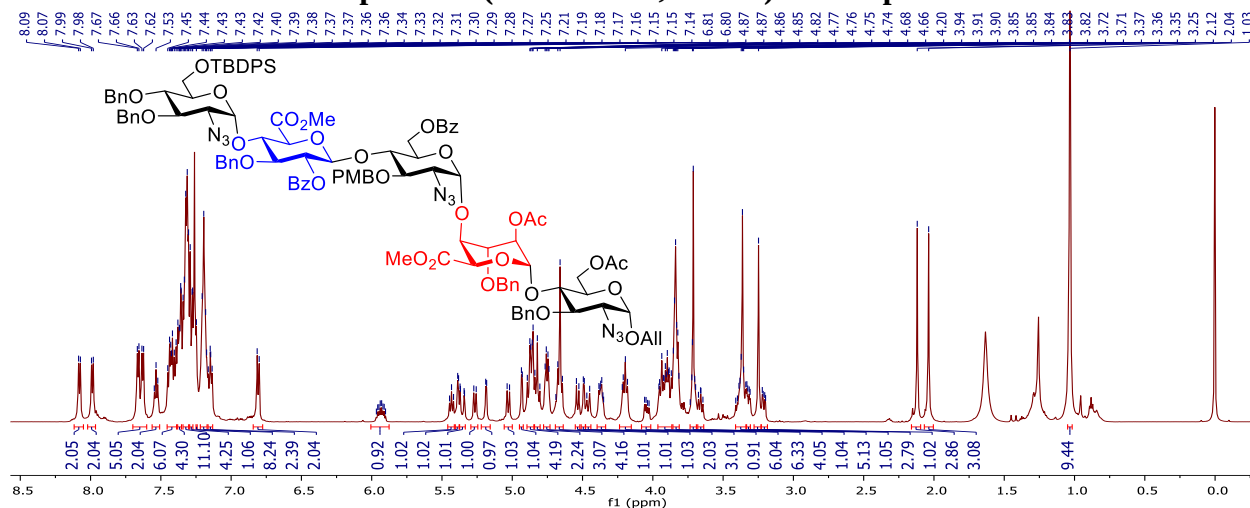


**DEPT NMR Spectrum (100.67 MHz, CDCl<sub>3</sub>) of Compound 73**

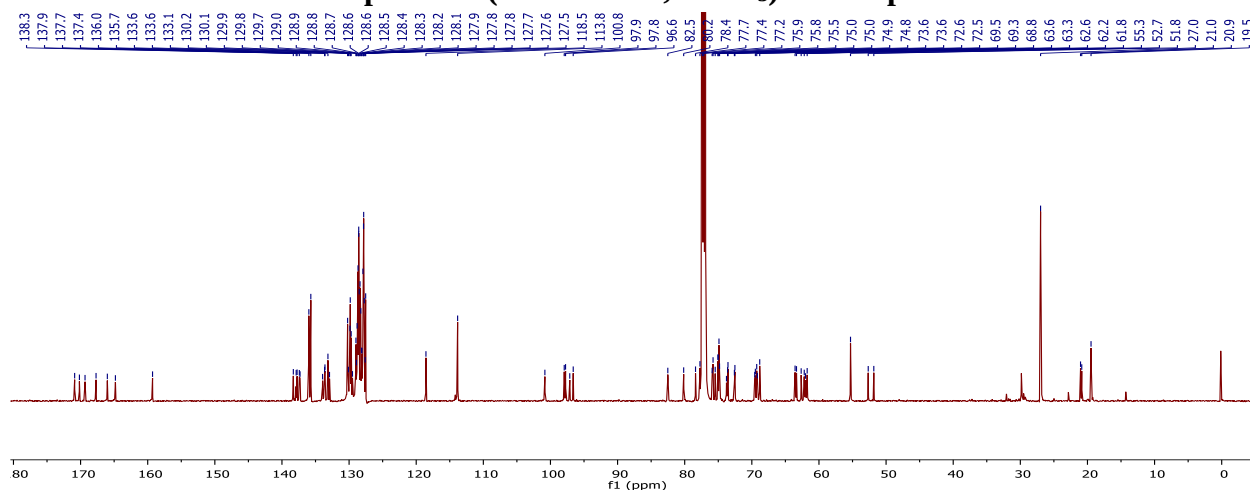


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

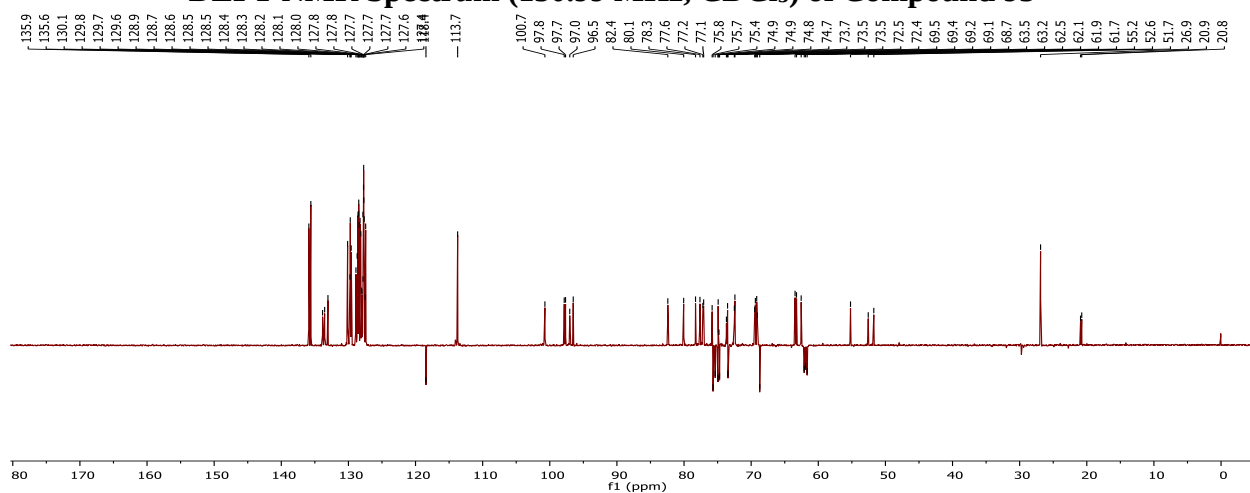
**<sup>1</sup>H NMR Spectrum (600.40 MHz, CDCl<sub>3</sub>) of Compound 93**

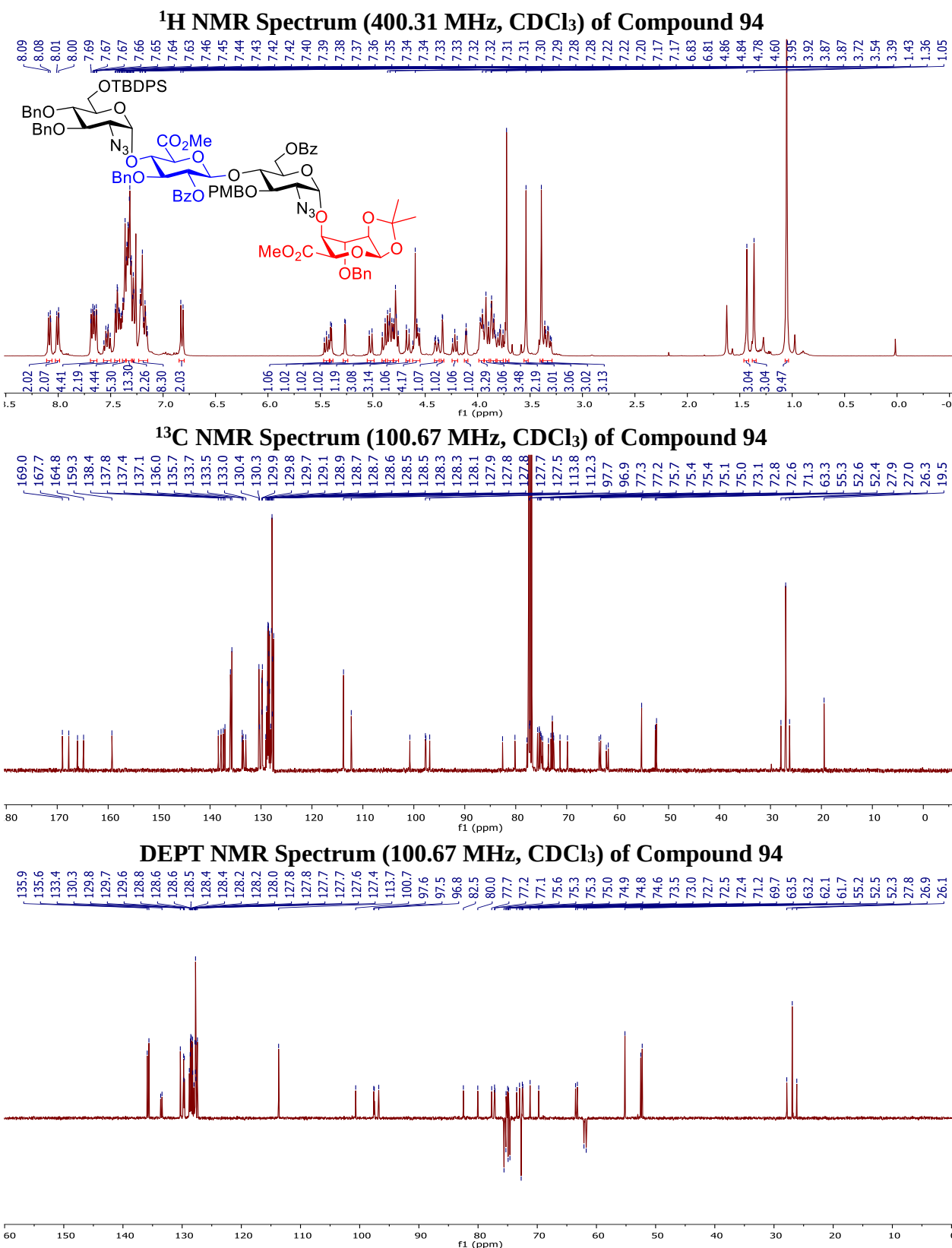


**<sup>13</sup>C NMR Spectrum (150.99 MHz, CDCl<sub>3</sub>) of Compound 93**



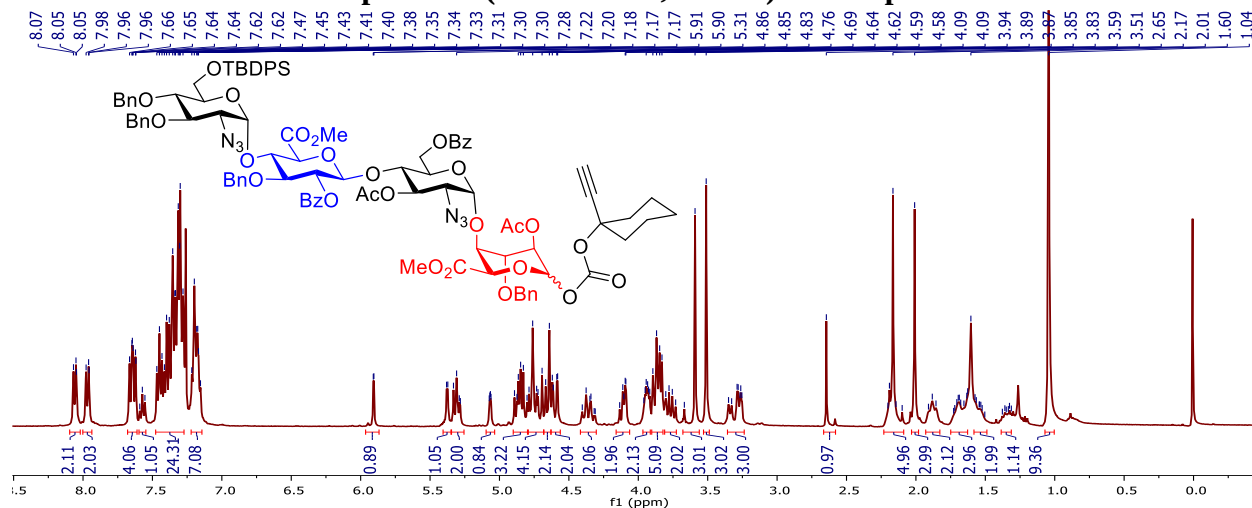
**DEPT NMR Spectrum (150.99 MHz, CDCl<sub>3</sub>) of Compound 93**



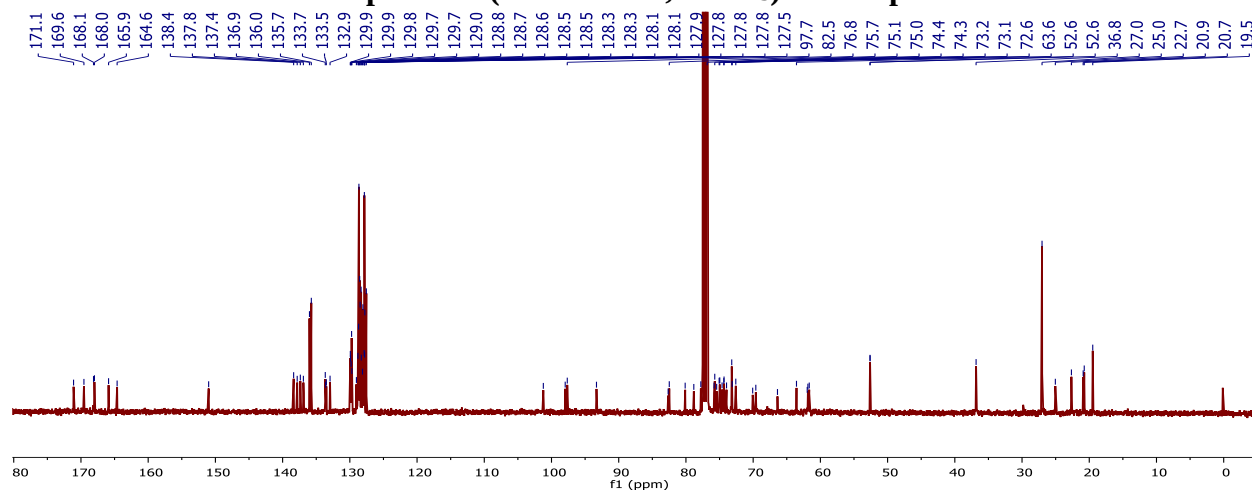


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

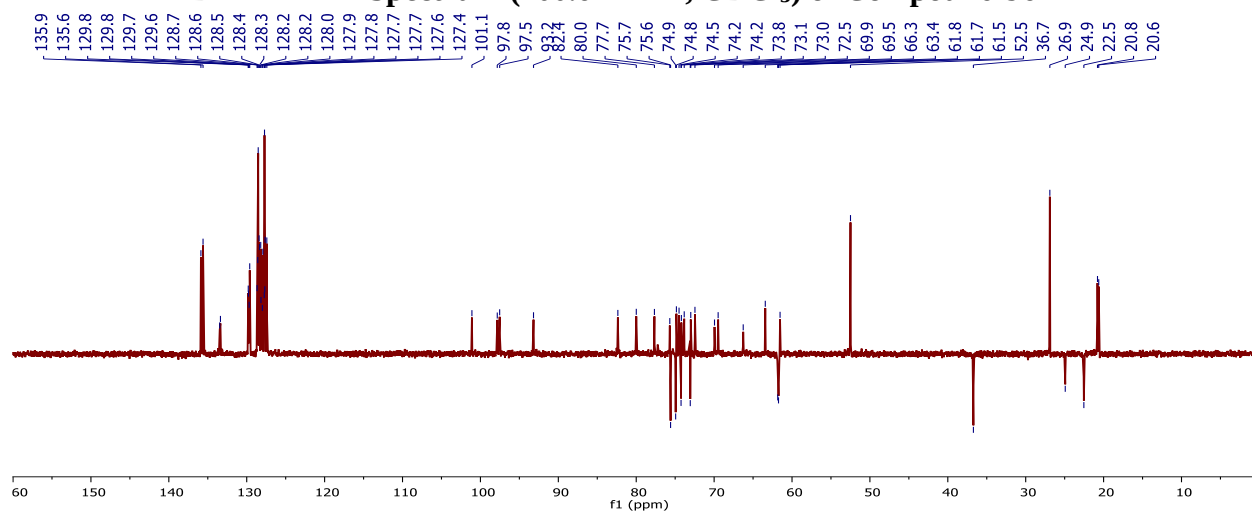
**$^1\text{H}$  NMR Spectrum (400.31 MHz,  $\text{CDCl}_3$ ) of Compound 96**



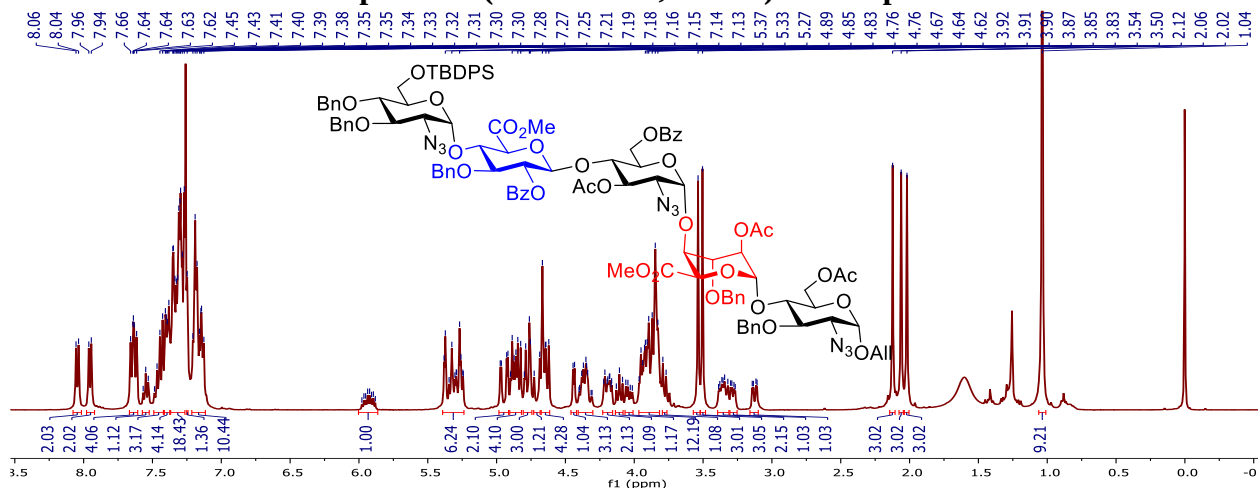
**$^{13}\text{C}$  NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 96**



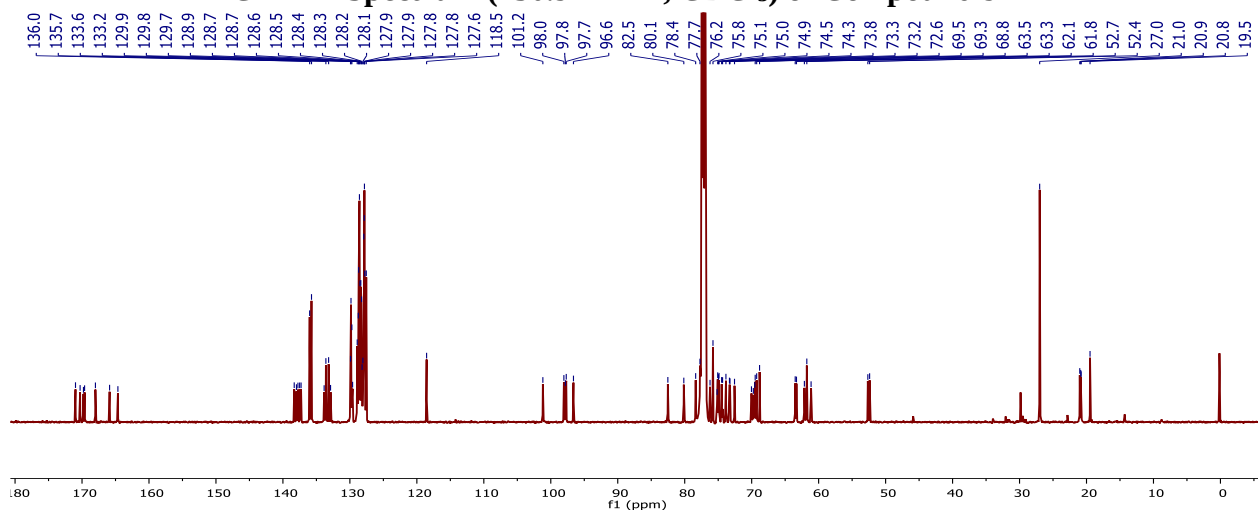
**DEPT NMR Spectrum (100.67 MHz,  $\text{CDCl}_3$ ) of Compound 96**



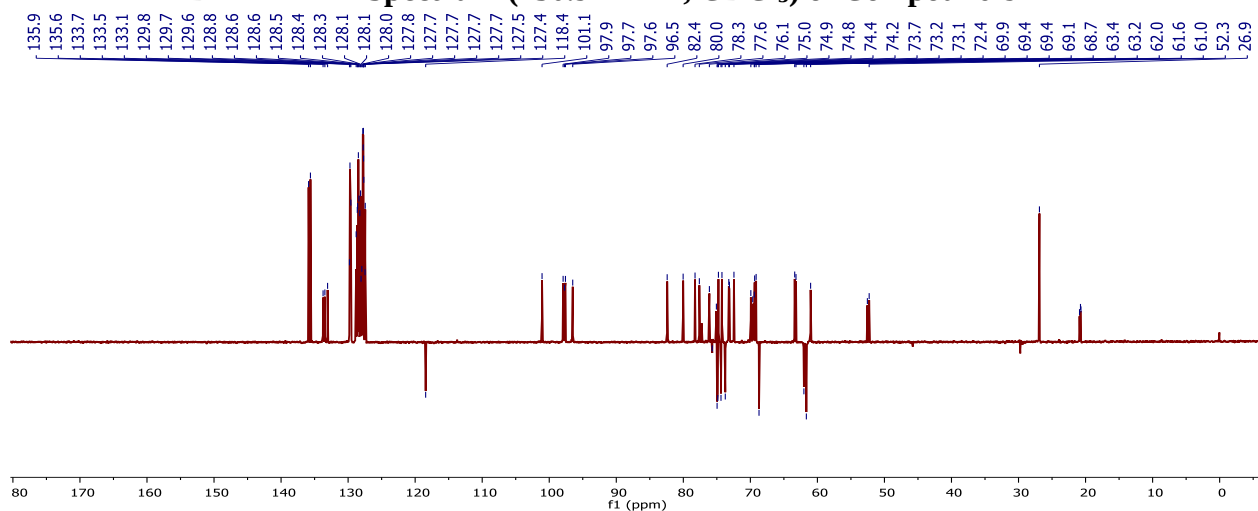
**<sup>1</sup>H NMR Spectrum (399.78 MHz, CDCl<sub>3</sub>) of Compound 97**



**<sup>13</sup>C NMR Spectrum (150.97 MHz, CDCl<sub>3</sub>) of Compound 97**

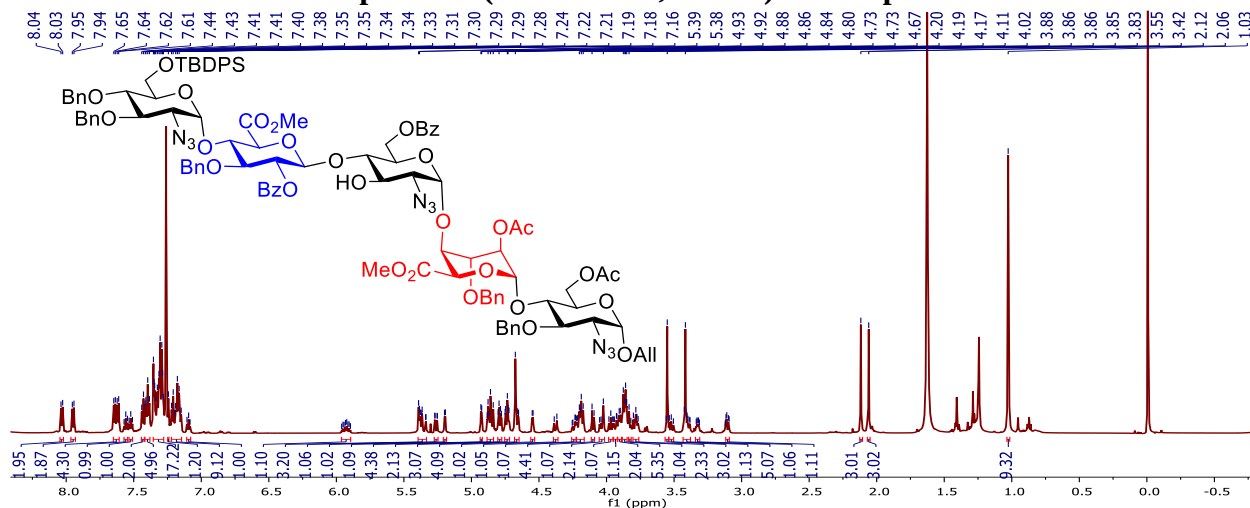


**DEPT NMR Spectrum (150.97 MHz, CDCl<sub>3</sub>) of Compound 97**

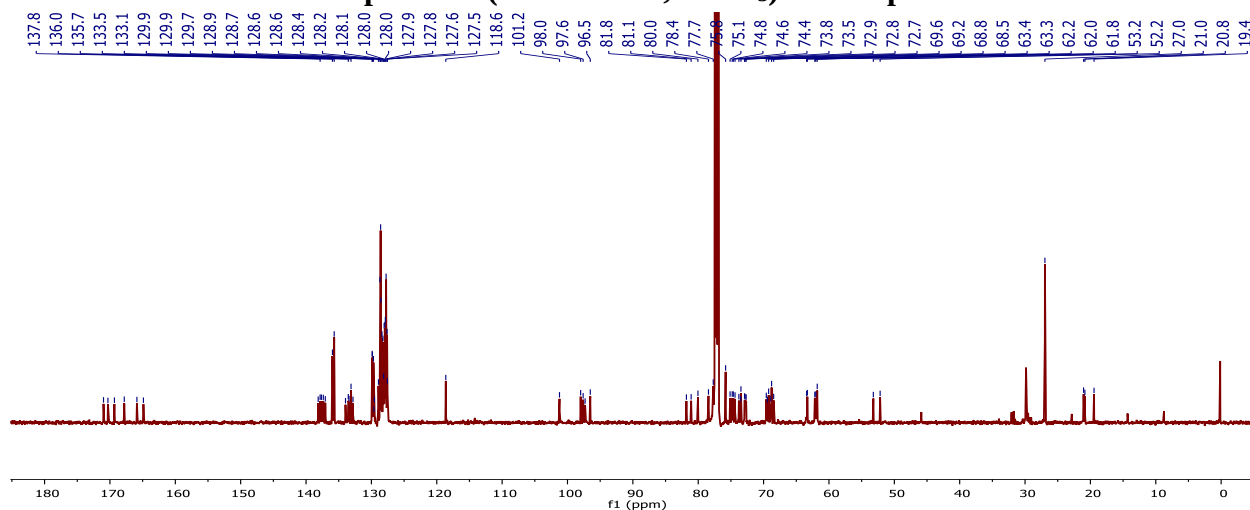


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

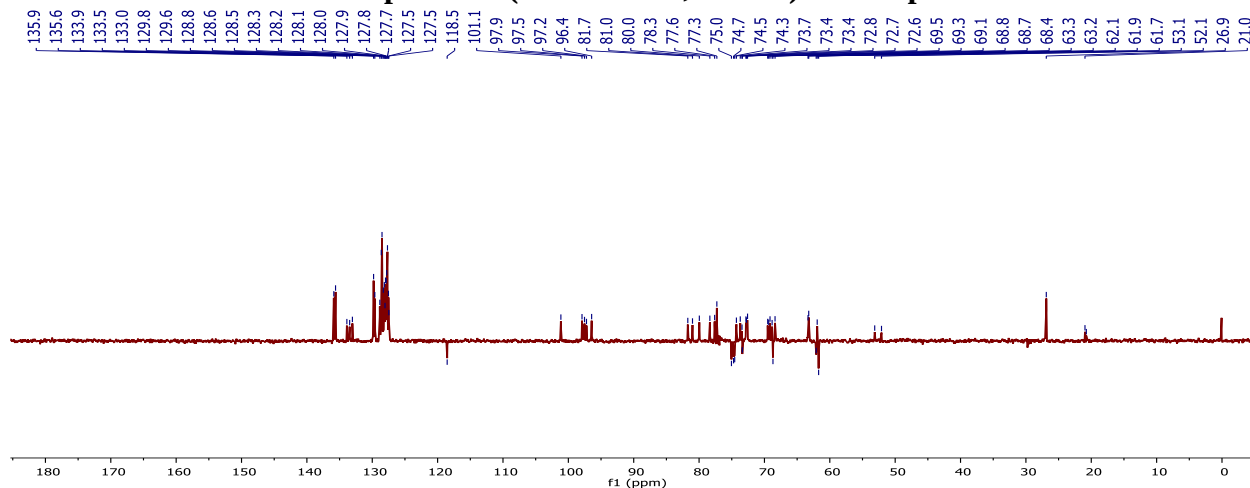
**<sup>1</sup>H NMR Spectrum (600.40 MHz, CDCl<sub>3</sub>) of Compound 98**



**<sup>13</sup>C NMR Spectrum (150.97 MHz, CDCl<sub>3</sub>) of Compound 98**

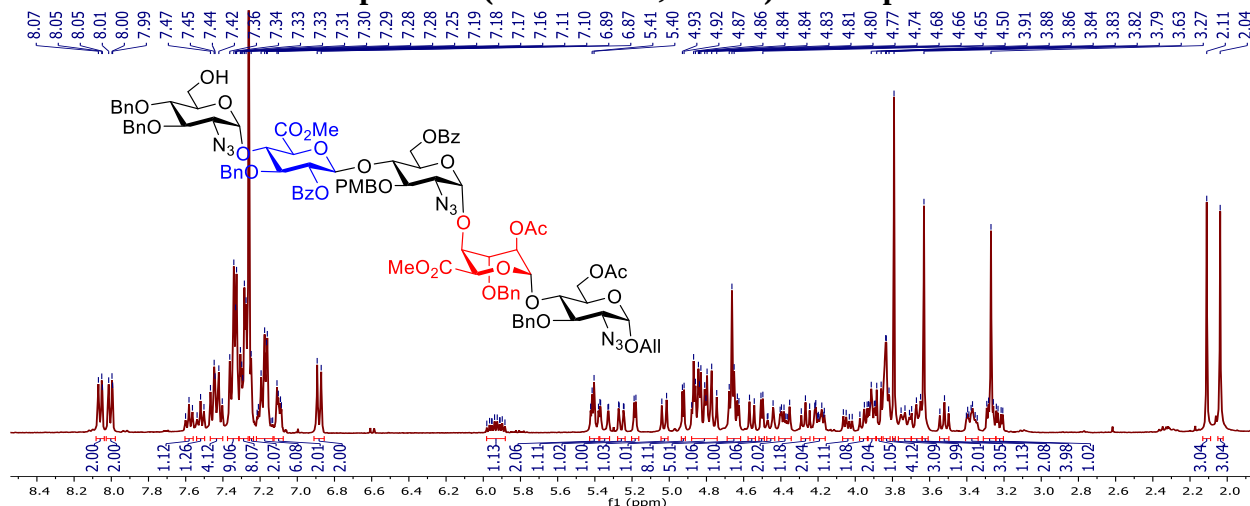


**DEPT NMR Spectrum (150.97 MHz, CDCl<sub>3</sub>) of Compound 98**

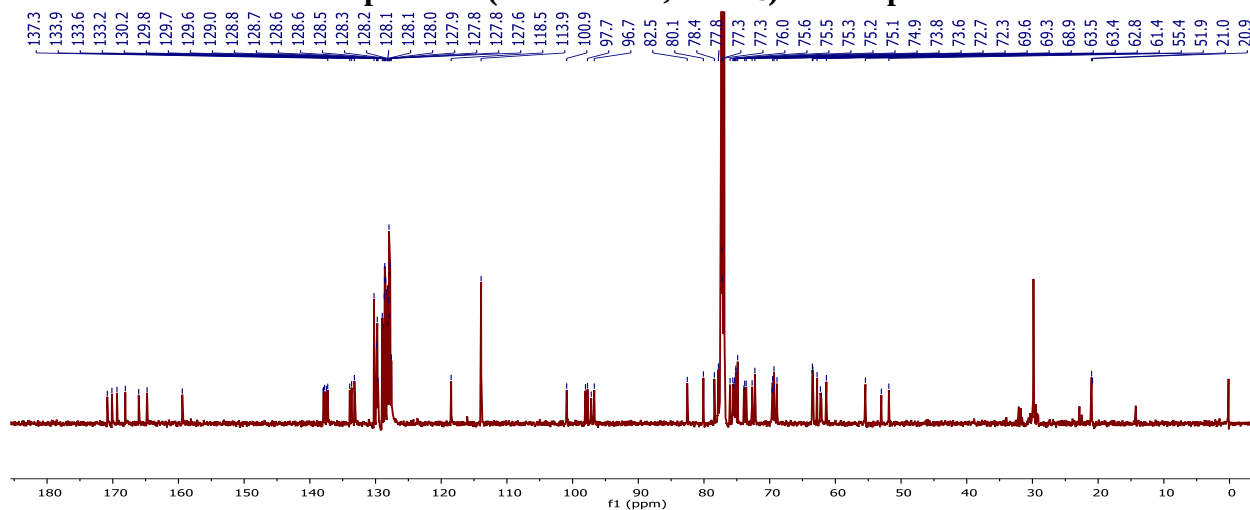


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

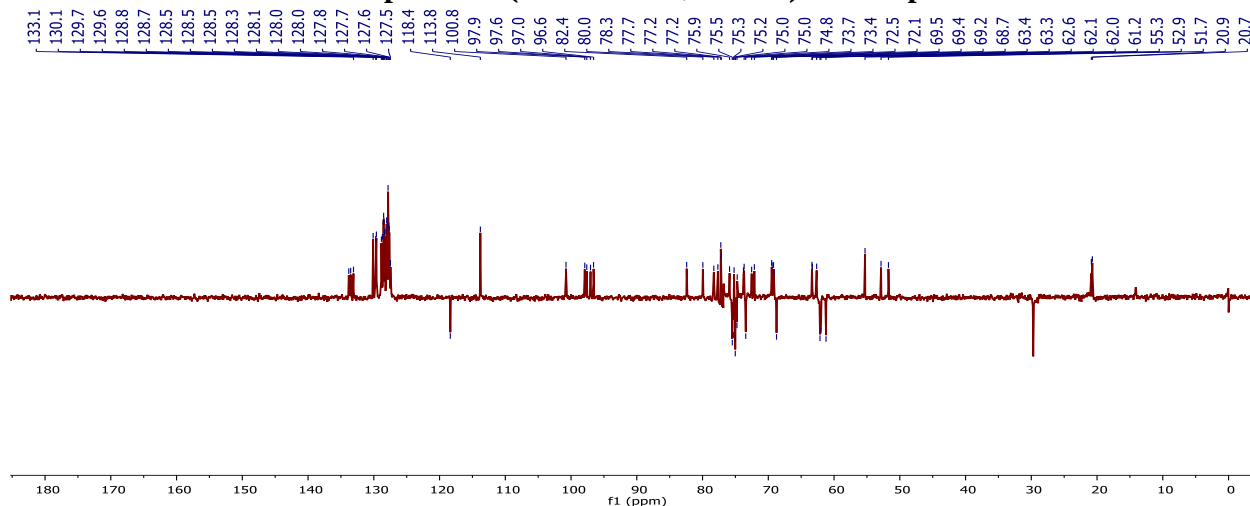
**<sup>1</sup>H NMR Spectrum (400.31 MHz, CDCl<sub>3</sub>) of Compound 99**



**<sup>13</sup>C NMR Spectrum (150.99 MHz, CDCl<sub>3</sub>) of Compound 99**

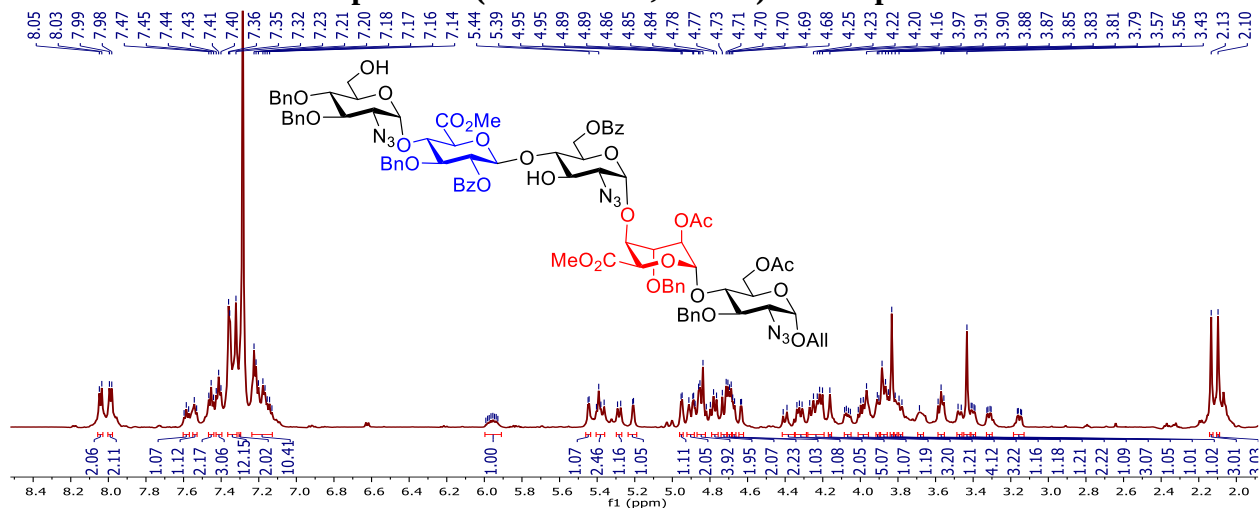


**DEPT NMR Spectrum (150.97 MHz, CDCl<sub>3</sub>) of Compound 99**

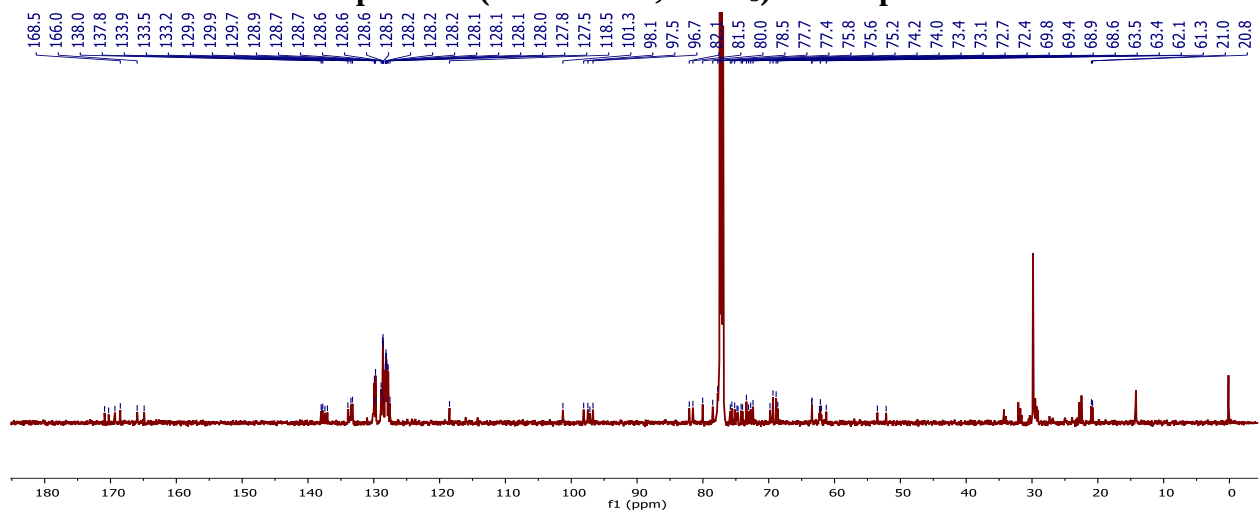


# Formal Synthesis of Regiodefined Anticoagulant Heparin Pentasaccharide

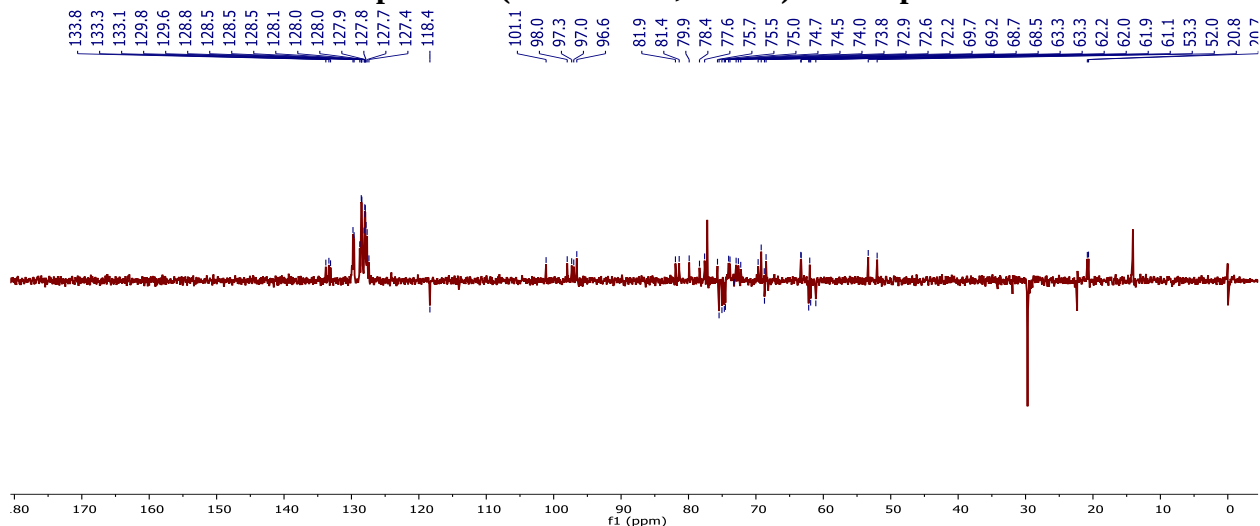
**<sup>1</sup>H NMR Spectrum (600.40 MHz, CDCl<sub>3</sub>) of Compound 100**



**<sup>13</sup>C NMR Spectrum (150.99 MHz, CDCl<sub>3</sub>) of Compound 100**



**DEPT NMR Spectrum (150.97 MHz, CDCl<sub>3</sub>) of Compound 100**





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## Chapter 5

### *Synthesis of Branched Arabinan Heptasaccharide of Mycobacterium tuberculosis cell surface*

# Synthesis of Branched Arabinan Heptasaccharide of *Mycobacterium tuberculosis* cell surface

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## 5.1 Introduction:

Tuberculosis (TB) is one of the lethal diseases known for more than 134 years, 124 years since the first vaccine trial and still it persists as a global health hazard. As per the 2018 World Health Organisations (WHO) Global Tuberculosis Report,<sup>1</sup> about 10 million people effected by TB disease along with a staggering 1.6 million deaths, from which 1.3 million-deaths are having HIV-negative people and the remaining are HIV-positive.<sup>1</sup> Complex situations of the increased incidence of TB infections, co-infection with HIV and emerging multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains causing major public health crisis. Therefore, in turn to restrict TB infected deaths and further transmission to the healthy ones, rapid and accurate diagnosis of tuberculosis (TB), HIV-associated TB and drug-resistant TB, followed by appropriate treatment in line with international standards is highly recommended. X-ray, sputum smear microscopy, skin tuberculin test or acid-fast stain and culture-polymerase based methods are some of the most frequently used TB diagnostic tests available. Currently, WHO recommended Xpert® MTB/RIF assay (Cepheid, USA) as a rapid diagnostic test which can display the results within 2 hours. Nearly 100 years ago, Albert Calmette and Camille Guérin discovered first vaccine to prevent TB known as Bacilli Calmette- Guérin (BCG).<sup>2</sup>

### 5.1.1 *Mycobacterium tuberculosis* (Mtb):

An event 'World TB Day' has been celebrated every year since March 24, 1882. On this day, Robert Koch declared the discovery of tubercle bacillus, *Mycobacterium tuberculosis* (Mtb), as the causative agent of tuberculosis (TB) in humans. Mtb is a species of pathogenic bacteria belonging to the family Mycobacteriaceae, primarily affects on the immune(respiratory) system by the deposition onto lung alveolar surfaces. It can attack on other parts of the body such as central nervous system, lymph nodes, bones, skin and many other organ systems. Among the species of complex mycobacteria such as *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. Canettii* and *M. Microti*, first three cause human TB infection. These three are slow-growing, with a doubling time of 12-20h at 34-80 °C in a laboratory experiment and generally taking more than 4-6 weeks to appear as visible colonies of Mtb on Lowenstein-Jensen (L-J) or 7H10/7H11 Middlebrooks agar.<sup>3</sup> Mtb is an aerobic, rod like shaped having lipid-rich unique cell wall in *Actinomycetes*, including the genera, *Mycobacterium*, *Rhodococcus*, *Corynebacterium*, and *Nocardia*. Rich and flexible metabolic range permits Mtb to stay alive for longer time, hence it can persist in human for a decade and activates to cause post-primary

## Synthesis of Branched Arabinan Heptasaccharide of *Mycobacterium tuberculosis* cell surface

TB.<sup>4</sup>The Mtb infection usually spreads *via* sneezing, coughing or talking to a person suffering from pulmonary tuberculosis.

**Structure of Mtb Cell Wall:** A number of researchers have contributed to the identification of compositional and architectural complexity of the Mtb cell wall. Mainly, it has become crucial after the continuous increase in the incidence of as multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. Usually, treatment of drug-susceptible tuberculosis requires chemotherapy with multiple antibiotics administered over prolonged periods. Mtb cell wall is significantly different from Gram-negative and Gram-positive bacteria as it has high content of lipid, ~40% of the dry mass of the cell. It makes Mtb cell wall unusually dense, waxy, and unique glycocalyx acts as a robust permeability barrier to any foreign components, as well antibiotics and thus, it requires prolonged chemotherapy.<sup>5</sup>

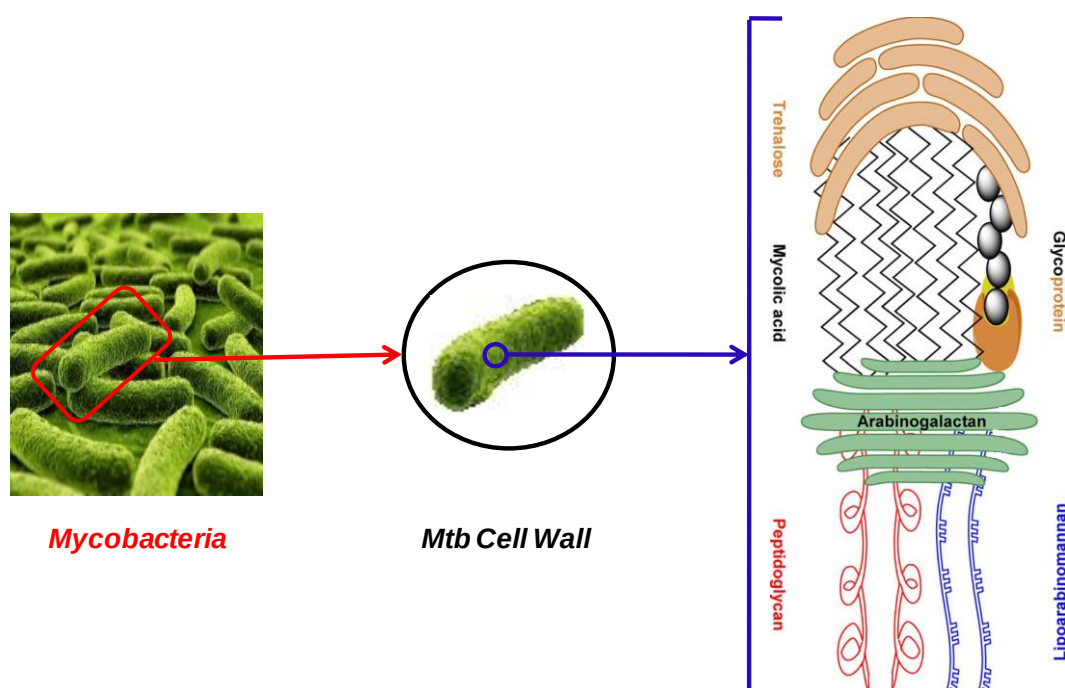


Figure 5.1. Schematic representation of the mycobacterial cell wall.<sup>6</sup>

Late 19<sup>th</sup> century, numerous efforts directed towards identifying the mechanism of the new anti-tubercular drugs led to the identification of the fine structure of the Mtb cell wall *via* deep analytical investigations mostly with mass spectroscopic characterization. Brennan *et al.* in 1980, deduced the cellular envelope of the Mtb cell wall comprises with three major interconnected oligosaccharides and the non-oligosaccharide part.<sup>7</sup>Arabinogalactan (AG), Lipomannan (LM) and Lipoarabinomannan (LAM) are the interconnected oligosaccharides

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whereas non-oligosaccharide long chains ( $C_{70}$ - $C_{90}$ ) of mycolic acid is attached to the non-reducing part of AG.<sup>8</sup> Apart from these, Mtb envelope contains trehalose and peptidoglycan.

Mycolic acids are long chain fatty acids containing  $\beta$ -hydroxy carboxylic acid with a long  $\alpha$ -alkyl side chain (Figure 5.2). Mtb is mainly characterized by very hydrophobic ( $C_{54}$  to  $C_{63}$ ) fatty acids with linear saturated  $\alpha$ -alkyl ( $C_{22}$  or  $C_{24}$ ) chain. These chains are functionalized with alpha ( $\alpha$ ), methoxy or keto mycolic acid which is also known for major classification of Mtb. Typically,  $\alpha$ -mycolic acid presents in major form (>70%), whereas methoxy- and keto-mycolic acid are the minor components (10 to 15%). Mycolic acid contents are rich at outer membrane of the cell wall which is important for the initial contact with receptors.<sup>9</sup>

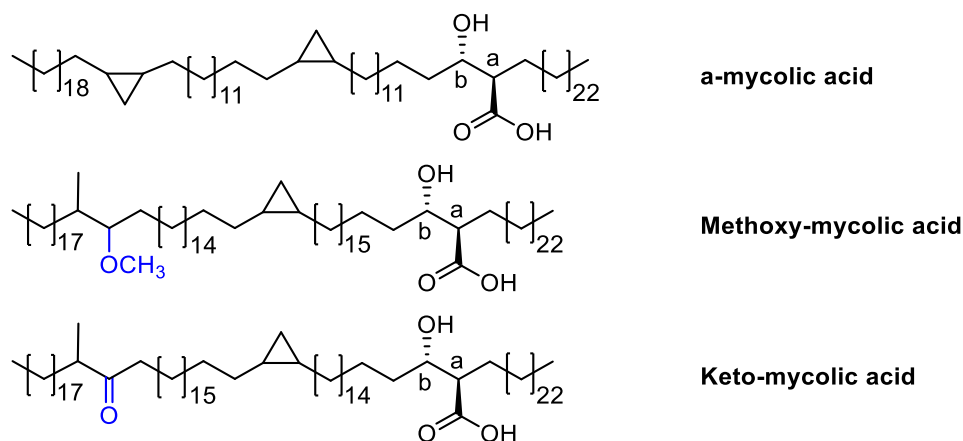


Figure 5.2: Structures of mycolic acids found in *Mtb*

Lipoarabinomannan (LAM) and lipomannan (LM) are collectively known as lipoglycans, present in Mtb plasma membrane mainly composed from mannosyl-phosphate inositol anchor, mannan backbone and capping moieties. LAM consists of 120 sugar residues having arabinose linkages in furanosyl form at non reducing side whereas mannose linkages in pyranosyl form attached to LM at the reducing end. Common backbone of LAM and LM compose linear mannopyranosides  $\alpha$ -Man(1 $\rightarrow$ 6)Man linkages of 21-34 monosaccharide units. Mycolic acid is absent in LAM whereas it presents with arabinogalactan (AG) to form mycolyl arabinogalactan polysaccharide.<sup>10</sup>

### 5.1.2 Arabinogalactan (AG):

Mycolyl arabinogalactan (AG) polysaccharide is one of the major structural components of the unique glycocalyx of Mtb cell wall wherein, mycolic acid typically as a cyclopropanated fatty acid is esterified at the non-reducing end of the arabinan whereas the reducing end is attached

## Synthesis of Branched Arabinan Heptasaccharide of *Mycobacterium tuberculosis* cell surface

as a pendant to the galactan (Figure 5.3). The fine structure of the mycolyl glycolyx revealed that the arabinose and galactose are in the furanosyl form. Most of the glycosidic linkages are in 1,2-*trans*or  $\alpha$ -fashion between the Araf-Araf or Galf-Galf or Araf-Gal except the terminal four Araf residues. Most of the Arabinan segment is composed of  $\alpha$ -D-Araf-(1 $\rightarrow$ 5)- $\alpha$ -D-Araf while there are three  $\alpha$ -D-Araf-(1 $\rightarrow$ 3)- $\alpha$ -D-Araf linkages at the branching arrangement. Mycolic acids are attached at the non-reducing side of the terminal four arabinans while the reducing end linkages are 1,2-*cis*or  $\beta$ -Araf residues that are, in turn, linked to the C2-position of the penultimate 1,2-*trans*or  $\alpha$ -Araf residue. The galactan portion is composed of  $\beta$ -D-Galf-(1 $\rightarrow$ 5)- $\beta$ -D-Galf-(1 $\rightarrow$ 6)- $\beta$ -D-Gal repeating unit containing pendant arabinan polysaccharide at three sites through the C5-D-Galf. Admitting the chemical synthesis of homogeneous oligosaccharide fragments could considerably boost the discovery of carbohydrate-based vaccines and diagnostics.<sup>7,11</sup>

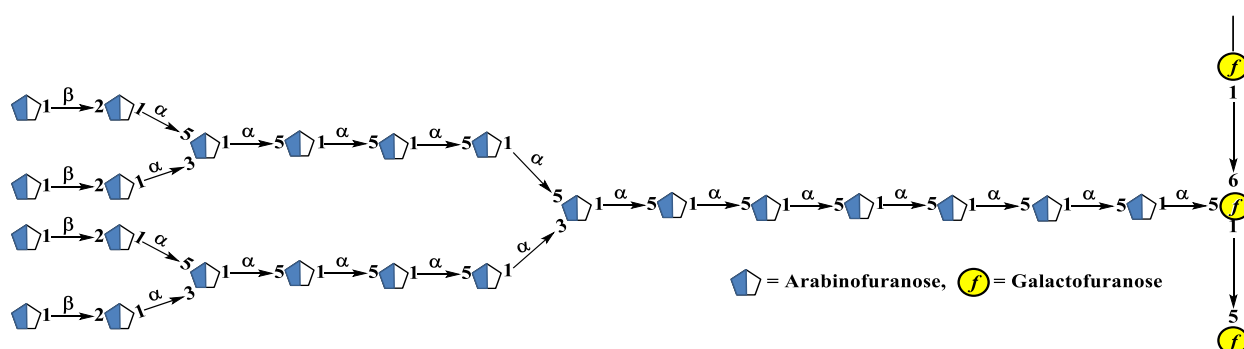


Figure 5.3. Cartoon representation of the arabinogalactan

Unlike peptides and nucleotides, the syntheses of large oligosaccharides remain as a significant challenge, although many well designed methodologies for chemical glycosidation have been developed. Fine tuning of the suitable strategically designed glycosyl donor chemistry with appropriate reagents is essential in order to achieve multi-step oligosaccharide synthesis. However, synthesis of the glycolyx fragments typically AG and LAM fragments from research groups led mainly by Ogawa (1993), Pozsgay (2000), Fraser-Reid (2005), Lowary (2007), Ito (2011), heneicosasaccharide (21 residues) of LAM (2017) and AG (2017), Seeberger (2017), Ye (2018) and syntheses by our group are some of the notable efforts in the literature that describe synthesis of challenging oligosaccharides.<sup>12</sup> Lowary explored trichloroacetimidates and thiotolyl donor chemistry, whereas Ito utilized thioglycosides for assembling the docosasaccharide.

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## 5.2 Present Work:

Arabinogalactan (AG) is one of the major constituents of Mtb cell wall. However, preparation of homogeneous AG fragment is a long standing challenge in chemical synthesis. The major aim of this investigation is to synthesize a major oligosaccharide portion of the arabinogalactan. Drawing upon the early strands of the synthetic research, this study was conducted to synthesize various linear and branched oligosaccharides employing one strategic silver assisted gold catalysis glycosidations. Strategically placed *n*-pentenyl moiety at the reducing end enables ligation of biomolecular probes through celebrated cross metathesis or thiol-ene click reactions. These oligosaccharides are envisioned to be excellent probes for understanding disease biology thereby facilitating discovery of novel antitubercular agents, vaccines and/or diagnostics.

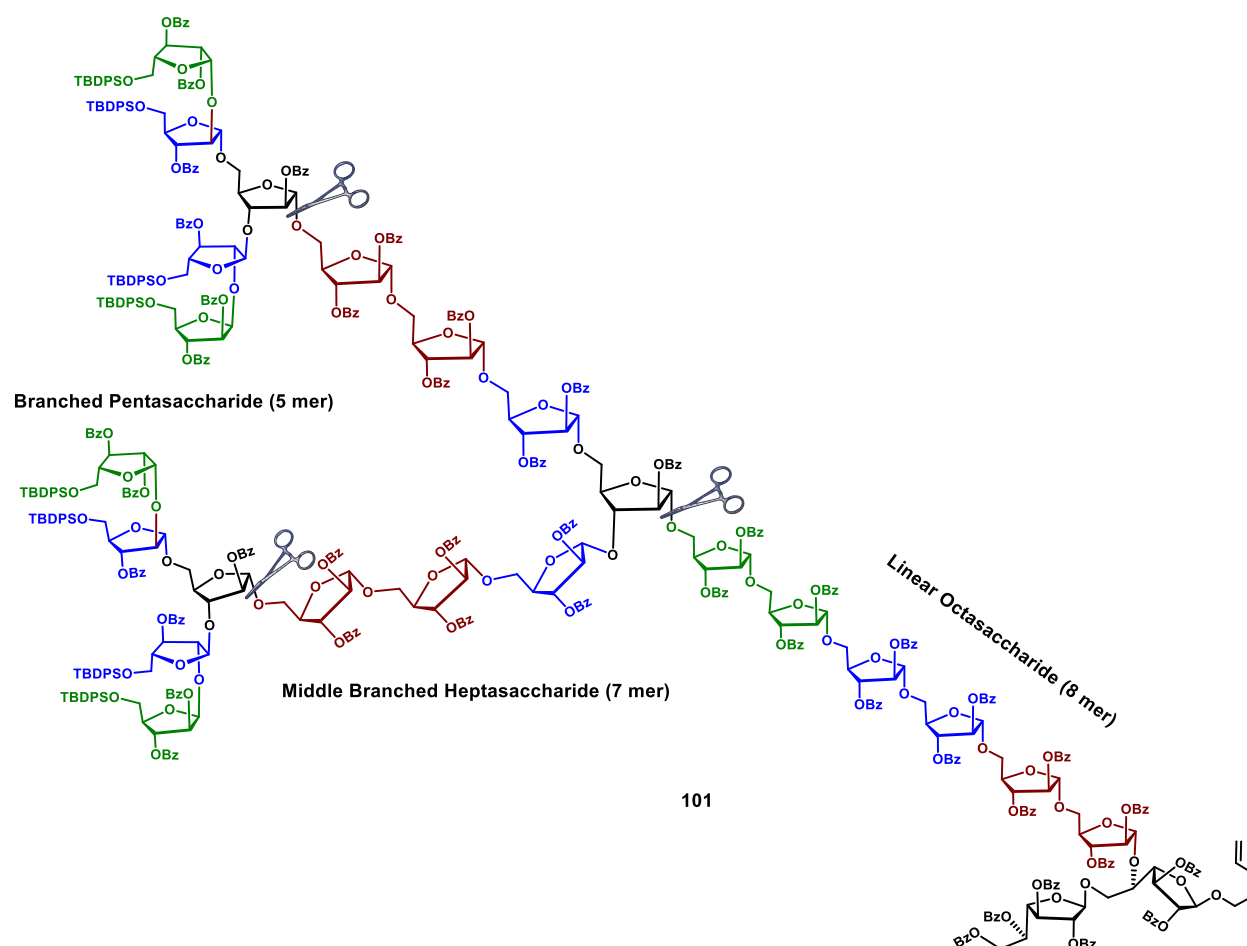


Figure 5.4. Strategic disconnection of the pentacosafuranoside (25 mer)



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The target molecule called as pentacosafuranoside (25 mer) **101** contains 23-Araf and 2-Galfresidues. It has been planned to install 1,2-*trans* linkages through neighbouring group participation and oxidation-reduction strategy for the 1,2-*cis*Araf linkage. Careful analysis revealed that **101** can be synthesized from three partners as branched pentasaccharide (5-mer), middle branched heptasaccharide (7-mer) and linear octasaccharide (8-mer) (Figure 5.4).

### 5.2.1 Retrosynthesis of Middle Branched Heptasaccharide Subunit:

As part of the long term studies on the synthesis of giant Arabinogalactan oligosaccharide portion of *Mycobacterium tuberculosis*, middle branched heptasaccharide was required in large quantity. The heptasaccharide donor **102** was designed to place carbonate moiety at the reducing end in turn for coupling with linear octasaccharide while two non-reducing ends masking with silyl group for glycosidation of 2 molar equivalents of pentasaccharide (Figure 5.5). All the interglycosidic linkages in the heptasaccharide **102** are 1,2-*trans* in nature and hence invoked the neighbouring group assistance by strategically placing a benzoate at the C-2 position. Considering minimal number of complex reactions and protecting groups as a key aspect, furanosidations developed in our group using silver-assisted gold-catalyzed glycosidations protocol were considered for glycosidations.

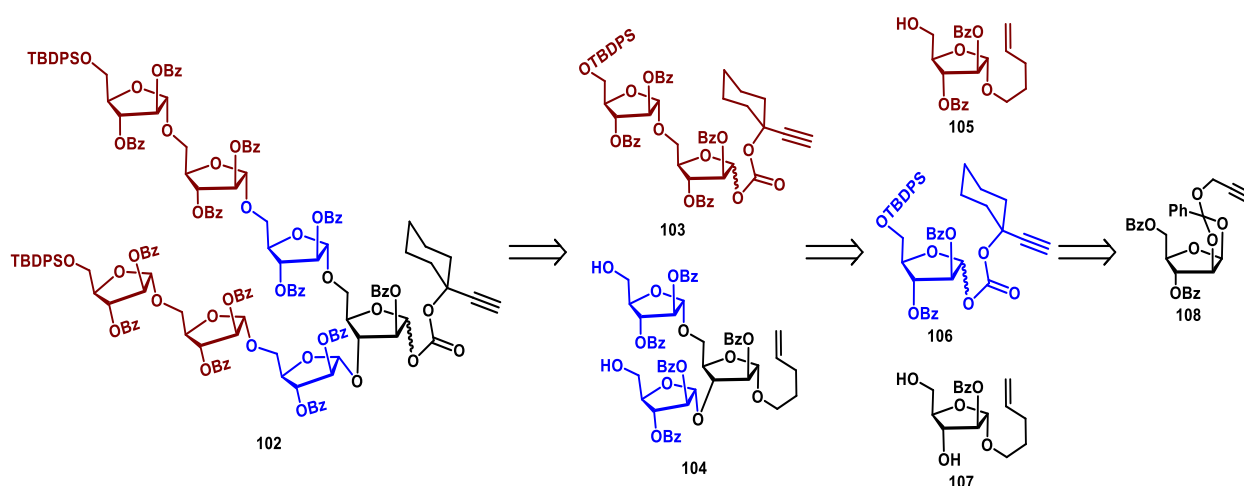


Figure 5.5. Retrosynthesis of the middle branched heptasaccharide (7 mer).

In the retrosynthetic analysis, we envisioned that the heptasaccharide can be assembled from the disaccharide donor **103** and a trisaccharide diol **104** by [2x2+3] glycosylation strategy. Monosaccharide carbonate donor **106** was envisaged to enable synthesis of both disaccharide donor **103** as well as trisaccharide diol **104**. Finally, the monosaccharide acceptors **105**, **107** and

## Synthesis of Branched Arabinan Heptasaccharide of *Mycobacterium tuberculosis* cell surface

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donor **106** were synthesized from single propargyl orthoester building block **108** which is well explored in our group.

### 5.2.2 Split-React-Couple Strategy for Disaccharide Synthesis:

Fitting arabinofuranose (Araf) monosaccharide construction for a glycosidic chain elongation requires to play with the C-3 and C-5 protecting group conversions without affecting the C-2 ester protections. Therefore, propargyl orthoester **108** was synthesized by invoking a protocol developed in our group. Subsequently, the saponification under Zemplén conditions easily transformed **108** to the intermediate diol **109** without affecting C-2 position (Figure 5.6). Further, regioselective silyl protection of the C-5 position using TBDPS chloride in the presence of imidazole and catalytic amount of DMAP produced orthoester **110**. Next, blocking of the lone hydroxyl moiety of orthoester **110** by benzoate protections using standard benzoylation protocol. Orthoester opening of the resulting compound **111** by 4-pentene-1-ol utilizing gold(III) chloride catalyzed glycosidations was afforded orthogonally protected *n*-pentenyl glycoside **112** in 75% yield. In the  $^1\text{H}$  NMR spectrum<sup>13</sup> of compound **112**, the resonances at  $\delta$  5.89 (ddt,  $J = 16.9, 10.2, 6.7$  Hz, 1H); 5.09 (ddd,  $J = 17.1, 3.5, 1.6$  Hz, 1H), 5.02 (ddt,  $J = 10.2, 2.2, 1.2$  Hz, 1H) ppm corresponds to methine and methylene protons were noticed. Benzoyl and TBDPS protons appeared at  $\delta$  8.15 – 8.09 (m, 2H), 8.07– 8.02 (m, 2H), 7.81 – 7.75 (m, 4H), 7.65 – 7.56 (m, 2H) ppm and  $\delta$  7.52 – 7.46 (m, 2H), 7.46 – 7.35 (m, 8H), 1.12 (s, 9H) ppm respectively. In the  $^{13}\text{C}$  NMR spectrum, presence of the anomeric, distinctive methylene, two methoxy, methylene and *t*-butyl carbons at  $\delta$  105.8 ppm,  $\delta$  115.0 ppm,  $\delta$  66.8, 63.9 ppm,  $\delta$  30.4, 28.9 ppm and  $\delta$  26.9 (3C), 19.4 ppm was clearly indicated the successful synthesis of *n*-pentenyl glycoside **112**. In addition, it was corroborated by the HRMS (ESI-MS) analysis,  $m/z$  calculated for  $\text{C}_{40}\text{H}_{44}\text{NaO}_7\text{Si}[\text{M}+\text{Na}]^+$ , 687.2753; found 687.2744.

## Synthesis of Branched Arabinan Heptasaccharide of *Mycobacterium tuberculosis* cell surface

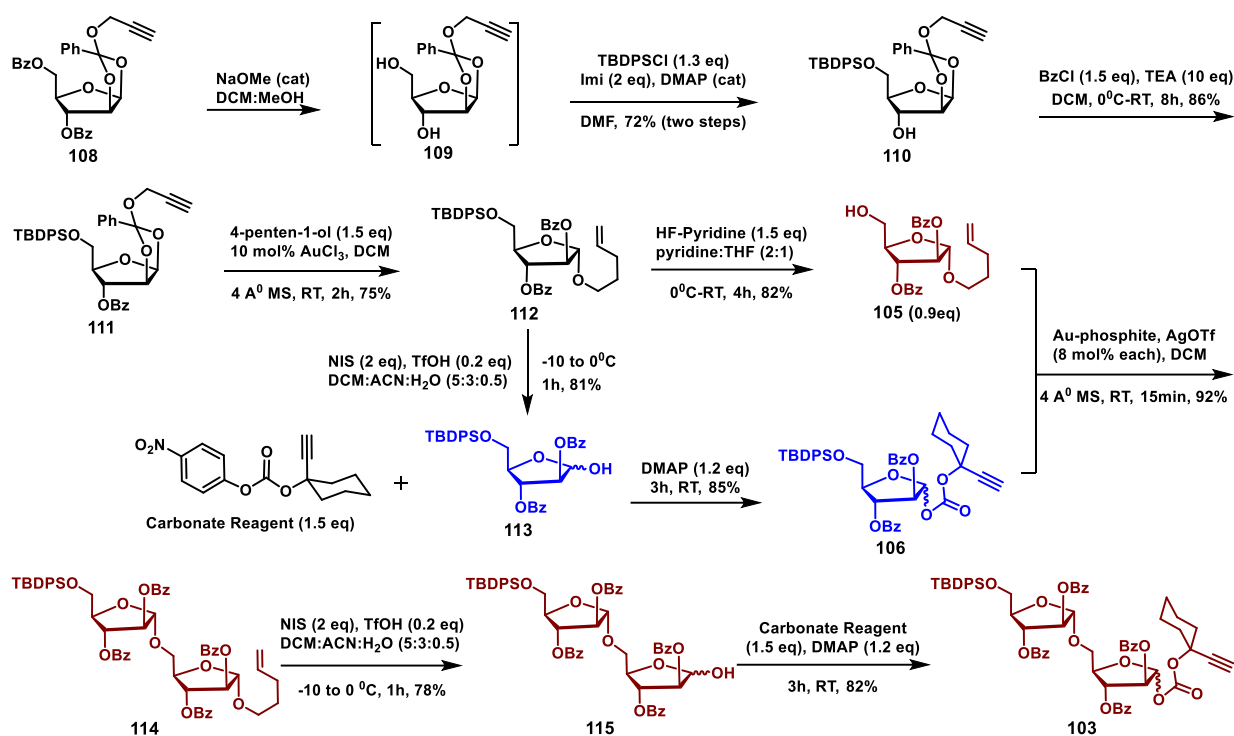


Figure 5.6. Synthesis of the disaccharide donor via split react couple strategy.

At this point, exploration of the split react couple strategy invited by splitting compound **112** into two portions. One portion was utilized to prepare the desired acceptor **105** by fluoride ion-mediated cleavage of the silyl group, while, another portion was carefully hydrolysed using NIS and catalytic amount of TfOH obtaining hemiacetals **113** in very high yield. Subsequently, the desired carbonate donor **106** was prepared by the nucleophilic attack of the hemiacetal anion on carbonate reagent utilising DMAP as a base. Subsequently, glycosyl donor **106** and acceptor **105** were glycosylated employing 8mol% each of the gold(I) phosphite and silver triflate (AgOTf) in the presence of 4Å molecular sieves powder in anhydrous dichloromethane resulting in the isolation of pentenyl disaccharide **114** in excellent yield. Anomeric resonances in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectrum<sup>13</sup> at  $\delta$  5.40 (s, 1H), 5.24 (s, 1H) ppm and  $\delta$  106.1, 105.8 ppm respectively along with the appearance of other residual signals as per the assigned structure confirmed disaccharide **114**. Further hydrolysis of the pentenyl moiety under aforementioned conditions afforded hemiacetals **115** that was treated with carbonate reagent and DMAP to obtain desired disaccharide donor **103** in 82% yield.

### 5.2.3 [2D+A] Glycosidations for Branched Trisaccharide and Heptasaccharide Synthesis:

Coupling of two molar equivalents of the glycosyl donor with one molar equivalent of the acceptor diol was designed for the branched oligosaccharide synthesis by [2x1+1] strategy for

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which the preparation of acceptor-diol **107** from orthoester**108** was initiated. Saponification under Zemplén conditions followed by protection of diol using TBDPSCl (2 eq.)/Imidazole/DMAP reagents afforded orthoester**116** in a 74% yield over two steps. Subsequently, pentenyl glycoside **117** was prepared by orthoester opening in the presence of catalytic amount of gold(III) chloride. Two silyl ethers were cleaved off by fluoride ion-mediated reaction awarding the desired acceptor-diol **107**. At this point, glycosidation reaction of the acceptor diol **107** with two units of donor **106** prepared *vide supra* using silver assisted gold catalysis in the presence of 4Å molecular sieves afforded the branched trisaccharide **118** in excellent yield. In addition to the  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT and NMR spectrum<sup>13</sup> (**Table 5.1**), the HRMS (ESI-MS) analysis,  $m/z$  calculated for  $\text{C}_{87}\text{H}_{90}\text{NaO}_{18}\text{Si}_2[\text{M}+\text{Na}]^+$ , 1501.5563; Found 1501.5552 was confirmed the synthesis of trisaccharide **118**.

| Entry                  | $^1\text{H}$ NMR ( $\delta$ ppm)                                                                                                                                | $^{13}\text{C}$ NMR ( $\delta$ ppm)                                                                                                                            |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Anomeric               | 5.50 (s, 1H), 5.40 (s, 1H), 5.27 (s, 1H)                                                                                                                        | 106.2, 106.0, 105.4                                                                                                                                            |
| TBDPS                  | 7.44–7.28 (m, 20H), 1.08 (s, 9H), 1.04 (s, 9H)                                                                                                                  | 135.7 (6C), 135.6 (2C), 133.2 (3C), 133.1, 129.7 (4C), 127.7 (8C), 26.9(3C), 26.8 (3C), 19.4, 19.3                                                             |
| Pentenyl               | 5.84 (ddt, $J = 16.9, 10.4, 6.7$ Hz, 1H), 5.07 (d, $J = 17.1$ Hz, 1H), 4.99 (d, $J = 10.2$ Hz, 1H), 2.19 (q, 2H), 1.76 (p, $J = 6.9$ Hz, 2H)                    | 138.1, 115.0, 66.7, 30.3, 28.7                                                                                                                                 |
| Benzoyl                | 8.13 (d, $J = 7.7$ Hz, 2H), 8.05 (d, $J = 7.5$ Hz, 3H), 8.01 (d, $J = 8.3$ Hz, 5H), 7.76 (d, $J = 6.6$ Hz, 4H), 7.71 (t, $J = 6.3$ Hz, 4H), 7.62 – 7.44 (m, 7H) | 165.6, 165.5 (2C), 165.2 (2C), 133.4, 133.3 (3C), 133.2, 130.1 (2C), 130.0 (2C), 129.9 (4C), 129.8 (2C), 129.5, 129.4 (2C), 129.3 (2C), 128.5 (4C), 128.4 (6C) |
| C-5(-CH <sub>2</sub> ) | 5.75-3.5 (6H)                                                                                                                                                   | 66.3, 63.6, 63.4                                                                                                                                               |
| Ring -CH               | 5.75-3.5 (9H)                                                                                                                                                   | 84.0, 83.5, 82.7, 82.2, 82.1, 81.7, 80.9, 77.3 (2C)                                                                                                            |

**Table 5.1 Characteristic  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR signals of trisaccharide **118****

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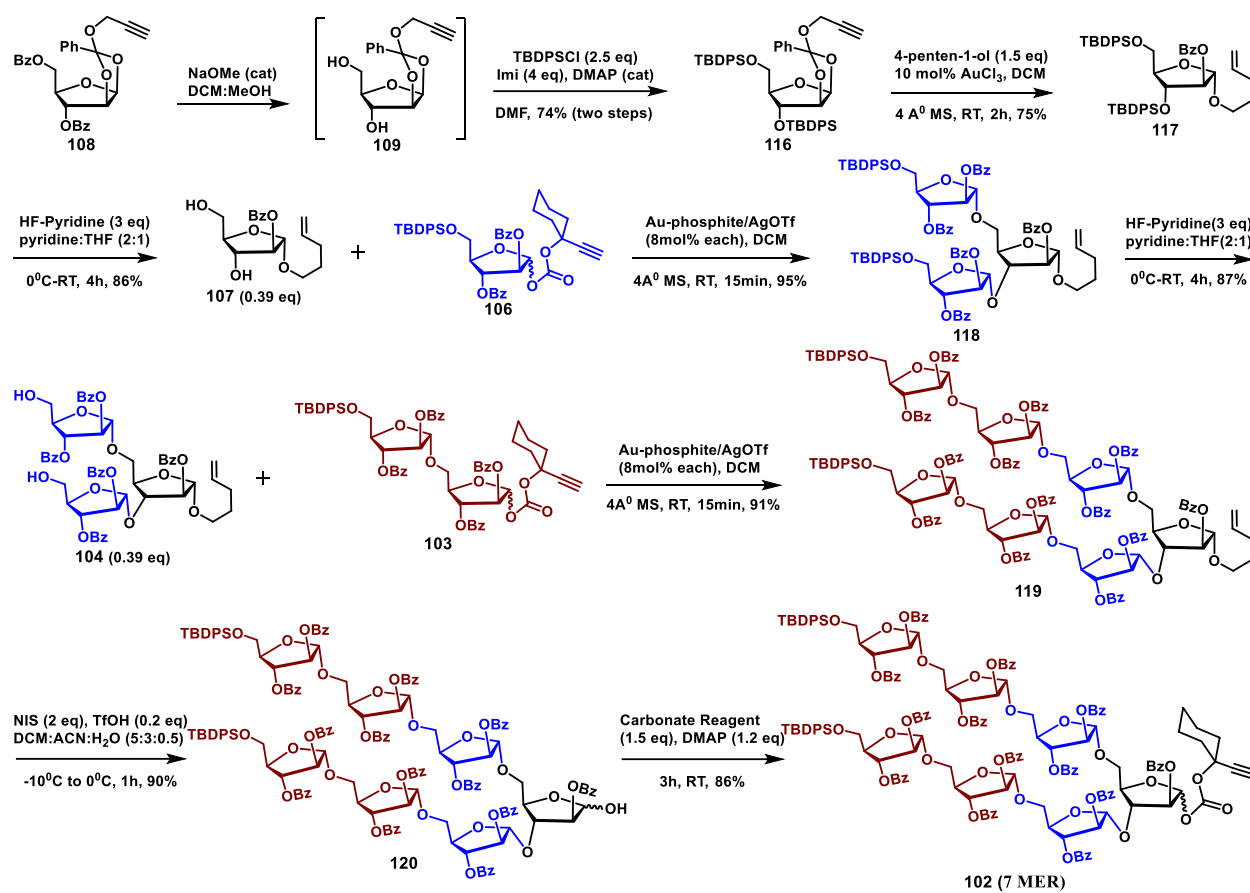


Figure 5.7. Synthesis of the branched trisaccharide and heptasaccharide donor.

Subsequently, cleavage of the silyl ether of trisaccharide **118** using HF•pyridine produced acceptor **104** for the heptasaccharide preparation. Here again, gold-silver catalysed glycosidation of the trisaccharide acceptor-diol **104** with two molar equivalents of disaccharide donor **103** under aforementioned silver-assisted gold-catalysed glycosidation conditions afforded the branched heptasaccharide **119** in 91% isolated yield. The thorough NMR spectroscopic analysis<sup>13</sup> (Table 5.2) corroborated the portrayed structure of heptasaccharide **119**. Furthermore, it was confirmed by the mass spectroscopic analysis (MALDI-TOF), *m/z* calculated for C<sub>163</sub>H<sub>154</sub>NaO<sub>42</sub>Si<sub>2</sub>[M+Na]<sup>+</sup>, 2862.9384; Found 2862.9297.

| Entry    | <sup>1</sup> H NMR (δ ppm)                                                              | <sup>13</sup> C NMR (δ ppm)                                                                  |
|----------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Anomeric | 5.50 (d, <i>J</i> = 1.1 Hz, 1H), 5.46 (s, 1H), 5.41 (s, 1H), 5.38 (s, 1H), 5.26 (s, 1H) | 106.1 (2C), 106.0 (4C), 105.3                                                                |
| TBDPS    | 7.48–7.29 (m, 20H, merged), 1.10 (d, <i>J</i> = 1.6 Hz, 18H)                            | 135.8 (4C), 135.7 (4C), 133.4 (2C), 133.3 (2C), 129.7 (4C), 127.7 (8C), 26.9 (6C), 19.4 (2C) |
| Pentenyl | 5.87 (ddt, <i>J</i> = 16.9, 10.2, 6.7 Hz, 1H), 5.09 (dd, <i>J</i> = 17.1, 1.7 Hz,       | 138.2, 115.0, 66.7, 30.3, 28.8                                                               |

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|                        |                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                        | 1H), 5.02 (d, $J = 10.2$ Hz, 1H),<br>2.21 (q, $J = 7.2$ Hz, 2H), 1.77 (dtd,<br>$J = 13.6, 6.9, 3.2$ Hz, 2H)                                                                                                                 |                                                                                                                                                                                                                                                |
| Benzoyl                | 8.11 – 8.09 (m, 2H), 8.08 – 8.01<br>(m, 15H), 7.96 (dt, $J = 12.2, 6.2$ Hz,<br>8H), 7.80 – 7.76 (m, 8H), 7.64 –<br>7.59 (m, 2H), 7.56 (dd, $J = 13.4,$<br>6.8 Hz, 4H), 7.53 – 7.48 (m, 5H),<br>7.48 – 7.33 (m, 21H, merged) | 165.7 (5C), 165.6 (2C), 165.3,<br>165.2(2C), 165.2 (2C), 165.1, 133.5,<br>133.4 (4C), 133.3 (4C), 133.2 (2C),<br>133.1 (2C), 130.0 (6C), 129.9 (18C),<br>129.8 (2C), 129.4 (3C), 129.3 (3C),<br>129.2 (5C), 129.1 (5C), 128.6 – 128.2<br>(26C) |
| C-5(-CH <sub>2</sub> ) | 5.75-3.5 (14H)                                                                                                                                                                                                              | 66.2, 65.9, 65.8 (2C), 65.7, 63.5 (2C)                                                                                                                                                                                                         |
| Ring -CH               | 5.75-3.5 (21H)                                                                                                                                                                                                              | 83.3 (2C), 82.9, 82.8, 82.6, 82.2 (5C),<br>81.6(5C), 81.5, 80.6, 77.3 (2C), 77.2,<br>77.0                                                                                                                                                      |

**Table 5.2 Characteristic <sup>1</sup>H NMR and <sup>13</sup>C NMR signals of heptasaccharide **119****

At this point, hydrolysis of the pentenyl glycoside of compound **119** was carried out using NIS and catalytic amount of triflic acid in dichloromethane:acetonitrile:water (5:3:0.5) solvent mixture at 0 °C to obtain 90% of heptasaccharide hemiacetals **120**. Above 0 °C, hydrolysis of the silyl groups was noticed. Finally, the conversion of hemiacetals **120** to the heptasaccharide donor **102** was accomplished using alkynyl cyclohexyl carbonate in the presence of DMAP at room temperature. Heptasaccharide donor **102** was utilized further in the total synthesis of pentacosafuranoside (25 mer) in our group.<sup>6</sup>

### 5.3 Conclusions:

Silver-assisted gold-catalysed glycosidation protocol was explored for the synthesis of arabinan oligosaccharides including di-, tri- and hepta- saccharides which in turn were utilized in the total synthesis of pentacosafuranoside invoking salient features of alkynyl carbonate glycosyl donor strategy. Monosaccharide building blocks identified from single orthoester and split react couple strategy are the salient features of this endeavour. Modular and convergent [2D+A] strategy was investigated for the synthesis of branched oligosaccharide utilizing one strategic glycosylation protocol. Neighbouring group assistance of C-2 benzoyl group enabled installation of all 1,2-*trans* linkages. The presence of an *n*-pentenyl moiety can be exploited for conjugating immunogenic proteins, biomarkers, and chemical probes for further biological explorations.

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## 5.4 Experimental Section:

### 5.4.1 Experimental Procedures:

**Procedure A: Deprotection of Benzoates (Saponification) Followed by TBDPS-Protection:** To a solution of the benzoate (1.0 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (4 mL, 1:1), a freshly prepared 1M NaOMe in MeOH (0.3 mmol per benzoate) was added and the reaction mixture was stirred at 25 °C for 3 h. After complete consumption of the starting material, Amberlite IR 120 (H+) [1.0 g per mol) was added to the reaction mixture to quench the NaOMe or until neutralization. The solid resin was filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography to obtain the alcohol. Subsequently, solution of the alcohol compound (1.0 mmol) and imidazole (1.3 mmol per -OH) in anhydrous DMF (5 mL) was cooled to 0 °C and *tert*-butyldiphenylchlorosilane (1.2 mmol per -OH) was added drop-wise under argon atmosphere. The reaction mixture was gradually warmed up to 25 °C and stirred for 2 h. After completion, the reaction mixture was quenched by adding ice-cold water (10 mL). The reaction mixture was extracted with ethyl acetate (3x25 mL). The organic layer was washed with water (2x100 mL) and brine solution (1x50 mL). The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (EtOAc/hexane) to obtain the desired product.

**Procedure B: Benzoate Ester Protection:** A solution of the compound (1.0 mmol) in anhydrous pyridine (5 mL) was cooled to 0 °C and benzoyl chloride (1.2 mmol per alcohol) was added drop-wise under argon atmosphere. The reaction mixture was gradually warmed up to 25 °C and stirred for 4 h. After completion, the reaction mixture was quenched by adding ice cooled water and extracted with ethyl acetate and the EtOAc layer was washed with 1N HCl (2x100 mL) and brine solution (1x50 mL). The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using EtOAc and hexane as mobile phase to obtain the desired product in 86% yield.

**Procedure C: Pent-4-ene-1-ol Glycosides from Propargyl-1,2-Orthoester:** To a solution of propargyl-1,2-orthoester (1.0 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (4 mL), pent-4-ene-1-ol (6 mmol) and freshly activated 4Å MS powder (0.200 g) were added under argon atmosphere and stirred for 15 min at 25 °C.  $\text{AuCl}_3$  (10mol%) was added and the reaction mixture was allowed to stir for 3-4 h and was passed through a bed of Celite®. The filtrate was concentrated *in vacuo* and



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purified by silica gel column chromatography using ethyl acetate and hexane as mobile phase to obtain corresponding glycoside with C-2 benzoate.

**Procedure D: Deprotection of the TBDPS-ethers:** To a solution of the silylated ether (1.0 mmol) in anhydrous pyridine and THF (4 mL, 3:1), HF.py (3 mmol per TBDPS) was added at 0 °C and the reaction mixture was allowed to stir at 25 °C for 6-8 h. After completion, the reaction mixture was quenched by the drop-wise addition of 2NHCl at 0 °C and diluted with ethyl acetate. The organic layer was successively washed with 1NHCl (25 mL), saturated aqueous solution of NaHCO<sub>3</sub> (25 mL) and brine solution (50mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude residue was purified by silica gel column chromatography using ethyl acetate and hexane to furnish corresponding alcohol in very high yields.

**Procedure E: Deprotection of Pent-4-ene-1-ol Glycosides:** The pent-4-enylglycoside (1.0 mmol) was dissolved in a mixture of solvents CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN/H<sub>2</sub>O (5.0:3.0:0.5) and the reaction mixture was cooled to -10 °C. After 15 min, NIS (2.2mmol) and TfOH (0.2 mmol) were added to the reaction mixture simultaneously and the reaction was allowed to stir at 0 °C for another 1 h. After completion, the reaction mixture was quenched with NaHCO<sub>3</sub> solution and diluted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was successively washed with water (25 mL), brine solution (25 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in *vacuo* to obtain crude that was purified by silica gel column chromatography using ethyl acetate and hexane to furnish corresponding hemiacetal alcohol in 78-90% yields.

**Procedure F: Synthesis of Ethynyl Cyclohexyl Glycosyl Carbonate Donors:** To a solution of arabinofuranosyl hemiacetal (1.0 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL), ethynyl cyclohexyl (4-nitrophenyl) carbonate 15 (1.2 mmol) and DMAP (1.1 mmol) were added and the reaction mixture was stirred at 25 °C for 5 h. After complete consumption of the starting hemiacetal, the reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography (EtOAc/Hexane as mobile phase) to obtain ethynyl cyclohexyl glycosyl carbonate donors in 80-90% yield.

**Procedure G: Glycosidation using Ethynyl Cyclohexyl Glycosyl Carbonate Donors:** To a solution of acceptor (1.0 mmol) and donor (1.1 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added freshly activated 4Å MS powder (0.200 g) under argon atmosphere. After 15 min of vigorous stirring at 25 °C, chloro[tris(2,4-di-*tert*butylphenyl)phosphite]gold(I) and AgOTf (8mol% each) were added simultaneously to the reaction mixture and stirred for 15 min to 1 h. After consumption of the glycosyl acceptor, the reaction mixture was filtered through a bed of



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Celite® and the filtrate was concentrated *in vacuo*, purified by silica gel column chromatography using EtOAc and hexane as mobile phase to afford desired glycosides in a very high (>90%) yield.

### 5.4.2 Analytical Data:

**Compound 111:** This compound is prepared from compound **108** using the above delineated procedures A and B; Yield = 61% (in three steps);  $[\alpha]_{\text{D}}^{25} = -31.1$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 8.13 (d, *J* = 1.2 Hz, 1H), 7.81 – 7.76 (m, 4H), 7.60 – 7.55 (m, 5H), 7.44 – 7.35 (m, 10H), 6.38 (d, *J* = 4.3 Hz, 1H), 5.72 (s, 1H), 5.14 (d, *J* = 4.0 Hz, 1H), 4.51 (t, *J* = 7.4 Hz, 1H), 3.99 (d, *J* = 1.7 Hz, 2H), 3.75 – 3.63 (m, 2H), 2.41 (t, *J* = 2.4 Hz, 1H), 1.14 (s, 9H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>): δ 165.3, 135.6 (2C), 135.5 (2C), 134.5, 133.6, 133.4, 133.3, 129.9 (2C), 129.7 (3C), 129.5, 128.6 (2C), 128.4 (2C), 127.8 (2C), 127.7 (2C), 126.5 (2C), 122.6, 106.7, 87.4, 85.2, 79.5, 77.8, 73.8, 63.7, 52.0, 26.9 (3C), 19.2; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3471.92, 3065.15, 2938.78, 2859.86, 1725.56, 1597.85, 1458.43, 1430.22, 1363.73, 1265.47, 1106.63, 1029.56, 822.43, 745.98, 703.45; HRMS (ESI-MS) [M+Na]<sup>+</sup> *m/z* calcd for C<sub>38</sub>H<sub>38</sub>NaO<sub>7</sub>Si, 657.2284; found 657.2274.

**Compound 112:** This compound was prepared from compound **111** using the above delineated procedure C; Yield = 75%;  $[\alpha]_{\text{D}}^{25} = -15.1$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 8.15 – 8.09 (m, 2H), 8.07 – 8.02 (m, 2H), 7.81 – 7.75 (m, 4H), 7.65 – 7.56 (m, 2H), 7.52 – 7.46 (m, 2H), 7.46 – 7.35 (m, 8H), 5.89 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.68 (dd, *J* = 5.0, 1.0 Hz, 1H), 5.52 (d, *J* = 1.5 Hz, 1H), 5.28 (s, 1H), 5.09 (ddd, *J* = 17.1, 3.5, 1.6 Hz, 1H), 5.02 (ddt, *J* = 10.2, 2.2, 1.2 Hz, 1H), 4.44 (q, *J* = 4.8 Hz, 1H), 4.14 – 4.02 (m, 2H), 3.85 (dt, *J* = 9.5, 6.6 Hz, 1H), 3.60 (dt, *J* = 9.6, 6.2 Hz, 1H), 2.29 – 2.18 (m, 2H), 1.88 – 1.75 (m, 2H), 1.12 (s, 9H). <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>): δ 165.7, 165.6, 138.2, 135.8 (4C), 133.5, 133.4 (3C), 130.0 (4C), 129.8 (2C), 129.7, 129.4, 128.5 (4C), 127.8 (4C), 115.0, 105.9, 83.1, 82.5, 77.6, 66.8, 63.9, 30.4, 28.9, 26.9 (3C), 19.4; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3066.46, 2935.74, 2863.24, 1726.04, 1598.37, 1454.46, 1361.86, 1262.58, 1105.43, 1066.34, 969.39, 913.84, 792.15, 744.84, 705.06; HRMS (ESI-MS) [M+Na]<sup>+</sup> *m/z* calcd for C<sub>40</sub>H<sub>44</sub>NaO<sub>7</sub>Si, 687.2753; found 687.2744.

**Compound 105:** This compound is prepared from compound **112** using the above delineated procedure D; Yield = 82%;  $[\alpha]_{\text{D}}^{25} = -45.1$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 8.11 – 8.01 (m, 4H), 7.58 (td, *J* = 7.3, 5.3 Hz, 2H), 7.49 – 7.40 (m, 4H), 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.53 (d, *J* = 1.3 Hz, 1H), 5.44 (dd, *J* = 4.7, 0.8 Hz, 1H), 5.23 (s, 1H), 5.00 (ddd, *J* = 17.1, 3.5, 1.6 Hz, 1H), 4.95 (ddt, *J* = 10.2, 2.1, 1.2 Hz, 1H), 4.32 (dd, *J* = 8.4, 4.1 Hz,

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1H), 4.06 – 3.93 (m, 2H), 3.78 (dt,  $J = 9.5, 6.6$  Hz, 1H), 3.53 (dt,  $J = 9.5, 6.2$  Hz, 1H), 2.52 (s, 1H), 2.23 – 2.11 (m, 2H), 1.80 – 1.68 (m, 2H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.2, 165.4, 138.1, 133.6 (2C), 130.0(2C), 129.9 (2C), 129.3, 129.2, 128.6 (4C), 115.0, 105.6, 83.8, 81.9, 77.9, 66.8, 62.5, 30.3, 28.8; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3498.64, 2932.82, 1720.87, 1599.82, 1450.68, 1358.74, 1260.19, 1175.99, 1107.31, 1062.87, 969.88, 913.63, 865.89, 711.19; HRMS (MALDITOF)  $[\text{M}+\text{Na}]^+$   $m/z$  calcd for  $\text{C}_{24}\text{H}_{26}\text{NaO}_7$ , 449.1576; found 449.1582.

**Compound 113:** This compound was prepared from compound **112** using the above delineated procedure E; Yield = 81%;  $[\alpha]_{\text{D}}^{25} = -72.8$  (c 0.7,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (399.78 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.02 – 7.92 (m, 6H), 7.91 – 7.85 (m, 2H), 7.76 – 7.68 (m, 2H), 7.62 (d,  $J = 6.5$  Hz, 6H), 7.51 – 7.42 (m, 4H), 7.40 – 7.20 (m, 20H), 5.95 (dd,  $J = 5.6, 4.2$  Hz, 1H), 5.64 (dd,  $J = 10.4, 4.7$  Hz, 1H), 5.59 – 5.55 (m, 1H), 5.53 (d,  $J = 2.6$  Hz, 1H), 5.48 (t,  $J = 5.2$  Hz, 1H), 5.43 – 5.36 (m, 1H), 4.48 (q,  $J = 4.6$  Hz, 1H), 4.32 (d,  $J = 10.5$  Hz, 1H), 4.20 – 4.14 (m, 1H), 3.99 (dd,  $J = 11.2, 2.9$  Hz, 1H), 3.94 – 3.87 (m, 2H), 3.84 (dd,  $J = 11.1, 2.3$  Hz, 1H), 3.40 (d,  $J = 3.6$  Hz, 1H), 1.03 (s, 9H), 0.95 (s, 9H);  $^{13}\text{C}$  NMR (100.53 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.2, 165.9, 165.7(2C), 136.0 (2C), 135.8 (2C), 135.7 (2C), 135.6 (2C), 133.6, 133.5 (3C), 133.3, 133.2, 132.1, 131.8, 130.3, 130.2, 130.1 (2C), 130.0 (4C), 129.9 (2C), 129.82 (2C), 129.4, 129.3, 129.2 (2C), 128.6 – 128.5 (8C), 128.1 (4C), 127.8 (4C), 101.2, 95.5, 83.4, 83.1, 82.7, 79.4, 77.6, 76.5, 65.1, 63.7, 26.0 (3C), 26.9 (3C), 19.4 (2C); IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3467.05, 3065.36, 3940.81, 2861.99, 1725.61, 1598.46, 1453.95, 1362.60, 1269.03, 1109.24, 1027.61, 751.03, 707.20; HRMS (ESI-MS)  $[\text{M}+\text{Na}]^+$   $m/z$  calcd for  $\text{C}_{35}\text{H}_{36}\text{NaO}_7\text{Si}$ , 619.2127; found 619.2123.

**Compound 106:** This compound is prepared from **113** using the above delineated procedure F; Yield = 85%;  $[\alpha]_{\text{D}}^{25} = -32.3$  (c 0.7,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.15 – 7.95 (m, 8H), 7.75 – 7.69 (m, 7H), 7.65 – 7.54 (m, 5H), 7.52 – 7.45 (m, 4H), 7.44 – 7.41 (m, 2H), 7.41 – 7.33 (m, 14H), 6.54 – 6.47 (m, 1H), 6.34 (s, 1H), 6.14 (t,  $J = 7.4$  Hz, 1H), 5.80 – 5.74 (m, 2H), 5.69 (s, 1H), 4.61 (d,  $J = 4.6$  Hz, 1H), 4.36 (q,  $J = 6.4, 5.9$  Hz, 1H), 4.06 – 3.94 (m, 4H), 2.67 (s, 1H), 2.39 (s, 1H), 2.23 – 2.13 (m, 3H), 2.07 (m, 1H), 1.96 (m, 3H), 1.75 – 1.63 (m, 6H), 1.59 – 1.44 (m, 5H), 1.35 (m, 2H), 1.06 (s, 9H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  165.6, 165.5 (2C), 165.2, 151.1, 151.1, 135.8 (4C), 135.7 (4C), 133.6, 133.5 (3C), 133.3, 133.2 (2C), 133.1, 130.3, 130.1(8C), 130.0, 129.8 (2C), 129.5, 129.4, 129.1, 128.9, 128.6 – 128.5 (8C), 127.8 (8C), 102.6, 97.1, 85.5 (2C), 82.7 (2C), 82.3, 81.6 (2C), 78.4, 78.0, 76.9 (2C), 76.7, 75.4, 75.0, 74.9, 64.8, 63.2, 36.9 (2C), 36.8, 36.6, 26.9 (6C), 25.1, 25.0, 22.7, 22.5, 19.4, 19.3; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3298.19, 2937.72, 2860.85, 1728.59, 1598.12, 1452.58, 1363.51,

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1239.14, 1105.12, 1011.56, 905.41, 822.11, 754.08, 705.87; HRMS (ESI-MS)[M+Na]<sup>+</sup> m/z calcd for C<sub>44</sub>H<sub>46</sub>NaO<sub>9</sub>Si, 769.2808; found 769.2797.

**Compound 114:** This compound was prepared from **105** and **106** using the above delineated procedure G; Yield = 92%; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -33.2 (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR(400.31 MHz, CDCl<sub>3</sub>)  $\delta$  8.12 – 8.05 (m, 2H), 8.03 – 7.98 (m, 4H), 7.96 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.72 (ddd, *J* = 7.8, 3.6, 1.6 Hz, 4H), 7.61 – 7.54 (m, 2H), 7.50 – 7.31 (m, 16H), 5.84 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.65 (d, *J* = 4.9 Hz, 2H), 5.60 (d, *J* = 1.3 Hz, 1H), 5.53 (d, *J* = 1.2 Hz, 1H), 5.40 (s, 1H), 5.24 (s, 1H), 5.03 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 4.97 (ddt, *J* = 10.2, 2.1, 1.2 Hz, 1H), 4.55 (q, *J* = 4.6 Hz, 1H), 4.50 (dd, *J* = 7.8, 4.6 Hz, 1H), 4.22 (dd, *J* = 11.2, 4.6 Hz, 1H), 4.01 (dd, *J* = 4.5, 1.2 Hz, 2H), 3.96 (dd, *J* = 11.1, 3.0 Hz, 1H), 3.81 (dt, *J* = 9.5, 6.6 Hz, 1H), 3.54 (dt, *J* = 9.5, 6.2 Hz, 1H), 2.24 – 2.13 (m, 2H), 1.79 – 1.72 (m, 2H), 1.04 (s, 9H); <sup>13</sup>C NMR (100.67 MHz, CDCl<sub>3</sub>)  $\delta$  165.8, 165.6, 165.5, 165.4, 138.2, 135.8 (4C), 133.5 (2C), 133.40 (2C), 133.3, 133.2, 130.1 (2C), 130.0 (4C), 129.9 (2C), 129.8 (2C), 129.5, 129.4 (2C), 129.2, 128.6 (4C), 128.5 (2C), 128.3 (2C), 127.8 (4C), 115.0, 106.1, 105.8, 83.3, 82.3, 82.0, 81.9, 77.5 (2C), 66.8, 66.3, 63.5, 30.4, 28.9, 26.9 (3C), 19.4; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3066.16, 2938.60, 2863.83, 1725.40, 1598.60, 1452.96, 1362.55, 1261.11, 1174.81, 1103.97, 1028.06, 965.24, 824.18, 752.60, 706.11; HRMS (ESI-MS) [M+Na]<sup>+</sup> m/z calcd for C<sub>59</sub>H<sub>60</sub>NaO<sub>13</sub>Si, 1027.3701; found 1027.3698.

**Compound 115:** This compound was prepared from compound **114** using the above delineated procedure E; Yield = 78%; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -30.2 (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR(399.78 MHz, CDCl<sub>3</sub>):  $\delta$  8.07 – 8.02 (m, 5H), 8.01 – 7.93 (m, 10H), 7.74 – 7.68 (m, 8H), 7.58 – 7.52 (m, 4H), 7.49 – 7.27 (m, 33H), 5.99 (t, 1H), 5.74 – 5.72 (m, 2H), 5.65 – 5.62 (m, 3H), 5.57 – 5.54 (m, 2H), 5.53 (d, 1H), 5.50 (d, *J* = 1.5 Hz, 1H), 5.41 (s, 1H), 5.37 (s, 1H), 4.69 – 4.64 (m, 1H), 4.59 (q, *J* = 5.1 Hz, 1H), 4.51 (q, *J* = 4.8 Hz, 1H), 4.33 – 4.29 (m, 1H), 4.24 – 4.15 (m, 3H), 4.02 (d, *J* = 4.4 Hz, 2H), 3.98 (d, *J* = 4.7 Hz, 3H), 3.96 – 3.94 (m, 1H), 3.92 (d, *J* = 3.1 Hz, 1H), 1.03 (d, *J* = 10.4 Hz, 18H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>):  $\delta$  166.0 (3C), 165.8 (2C), 165.6 (2C), 165.4, 135.8 (8C), 133.6 (3C), 133.4 (5C), 133.3 (4C), 130.1 – 129.8 (16C), 129.4 (2C), 129.3 (5C), 129.2 (3C), 129.0 (2C), 128.6 (6C), 128.5 (8C), 128.4 (2C), 127.8 (8C), 106.6, 106.1, 101.0, 95.6, 83.3 (3C), 82.9, 82.5, 82.3 (2C), 80.3, 78.3, 77.6, 77.4, 77.0, 76.1, 67.4, 66.5, 63.5 (2C), 26.9 (6C), 19.4 (2C); IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3469.78, 3014.93, 2941.89, 2865.61, 1724.25, 1599.37, 1452.63, 1362.43, 1265.08, 1107.20, 1065.96, 967.25, 754.66, 707.16; HRMS(ESI-MS) [M+Na]<sup>+</sup> m/z calcd for C<sub>54</sub>H<sub>52</sub>NaO<sub>13</sub>Si, 959.3074; found 959.3073.

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**Compound 103:** This compound was prepared from compound **115** using the above delineated procedure F; Yield = 82%;  $[\alpha]_D^{25} = -19.1$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>): δ 8.02 – 7.97 (m, 4H), 7.94 – 7.82 (m, 12H), 7.62 (tdd, *J* = 7.9, 3.2, 1.6 Hz, 8H), 7.51 – 7.40 (m, 6H), 7.40 – 7.31 (m, 6H), 7.31 – 7.20 (m, 24H), 6.41 (d, *J* = 4.5 Hz, 1H), 6.26 (s, 1H), 5.98 – 5.92 (m, 1H), 5.70 (dd, *J* = 7.3, 4.5 Hz, 1H), 5.63 (d, *J* = 3.2 Hz, 2H), 5.60 (d, *J* = 4.9 Hz, 1H), 5.54 (d, *J* = 4.9 Hz, 1H), 5.45 – 5.42 (m, 2H), 5.27 (s, 2H), 4.59 (q, *J* = 4.0 Hz, 1H), 4.43 – 4.39 (m, 3H), 4.08 (ddd, *J* = 22.4, 11.1, 5.1 Hz, 2H), 3.95 – 3.82 (m, 6H), 2.56 (s, 1H), 2.25 (s, 1H), 2.18 – 1.96 (m, 4H), 1.88 – 1.78 (m, 3H), 1.64 – 1.51 (m, 6H), 1.49 – 1.30 (m, 7H), 0.93 (s, 18H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>): δ 165.7, 165.6 (4C), 165.4, 165.3, 165.1, 151.1, 151.0, 135.8 – 135.7 (8C), 133.7, 133.6 (2C), 133.5, 133.4 – 133.2 (8C), 130.2 (4C), 130.1 – 129.9 (12C), 129.7 (4C), 129.6, 129.3 (3C), 129.2, 129.0, 128.9, 128.7, 128.6 – 128.4 (16C), 127.8 (8C), 106.3, 106.0, 102.4, 96.8, 84.4, 83.6, 83.4, 82.6 (2C), 82.5, 82.2, 81.0, 80.8, 78.4, 78.3, 77.3, 77.1, 76.9, 76.3, 75.5, 75.1, 74.9, 67.7, 65.9, 63.4 (2C), 36.9, 36.8, 36.6 (2C), 26.8 (6C), 25.0, 24.8, 22.6 (4C), 19.4 (2C); IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3297.05, 2939.04, 2863.24, 1726.14, 1599.13, 1452.10, 1363.48, 1260.88, 1106.35, 1020.09, 965.38, 851.64, 755.16, 707.51; HRMS (ESI-MS) [M+Na]<sup>+</sup> *m/z* calcd for C<sub>63</sub>H<sub>62</sub>NaO<sub>15</sub>Si, 1109.3755; found 1109.3757.

**Compound 117:** This compound was prepared from compound **108** using the above delineated procedures A and C; Yield = 55% (in three steps);  $[\alpha]_D^{25} = +42.7$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (399.78 MHz, CDCl<sub>3</sub>): δ 7.74 – 7.71 (m, 2H), 7.63 – 7.59 (m, 6H), 7.58 (d, *J* = 1.7 Hz, 1H), 7.52 – 7.47 (m, 1H), 7.42 – 7.34 (m, 3H), 7.33 – 7.26 (m, 7H), 7.23 – 7.15 (m, 5H), 5.87 (ddt, *J* = 16.9, 10.1, 6.6 Hz, 1H), 5.20 (d, *J* = 2.0 Hz, 1H), 5.06 (m, 1H), 4.97 (ddt, *J* = 10.3, 2.2, 1.0 Hz, 1H), 4.95 (s, 1H), 4.43 (dd, *J* = 5.8, 2.0 Hz, 1H), 4.31 (q, *J* = 2.6 Hz, 1H), 3.80 – 3.72 (m, 2H), 3.65 (dd, *J* = 11.3, 5.2 Hz, 1H), 3.50 (dt, *J* = 9.6, 6.2 Hz, 1H), 2.22 (m, 2H), 1.78 (m, 2H), 1.05 (s, 9H), 0.98 (s, 9H); <sup>13</sup>C NMR (100.53 MHz, CDCl<sub>3</sub>): δ 165.4, 138.5, 136.0, 136.0, 135.9, 135.9, 135.8, 135.8, 135.7, 135.7, 133.6, 133.5, 133.3, 133.1, 130.0, 129.9 (4C), 129.7, 129.7, 129.6, 128.2, 128.2, 127.8 (4C), 127.7 (4C), 114.9, 106.0, 85.1, 85.0, 77.3, 66.7, 63.4, 30.5, 29.1, 27.0, 27.0, 27.0, 26.9, 26.9, 26.9, 19.4, 19.3; IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 3066.57, 2935.42, 2860.78, 1727.17, 1465.12, 1428.92, 1363.36, 1304.09, 1264.90, 1107.15, 822.39, 743.13, 701.60; HRMS (ESI-MS) [M+Na]<sup>+</sup> *m/z* calcd for C<sub>49</sub>H<sub>58</sub>NaO<sub>6</sub>Si<sub>2</sub>, 821.3669; found 821.3673.

**Compound 107:** This compound was prepared from compound **117** using the above delineated procedure D; Yield = 86%;  $[\alpha]_D^{25} = +138.6$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400.31 MHz, CDCl<sub>3</sub>): δ 7.92 (d, *J* = 8.3 Hz, 2H), 7.46 (td, *J* = 7.3, 1.5 Hz, 1H), 7.32 (m, 2H), 5.72 (m, 1H), 5.13 (s, 1H), 5.08 (s, 1H), 4.95 (dt, *J* = 17.1, 1.7 Hz, 1H), 4.89 (d, *J* = 10.1 Hz, 1H), 4.12 (m, 3H), 3.83 (dd,

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$J = 12.2, 3.3$  Hz, 1H), 3.76 – 3.66 (m, 2H), 3.40(m, 1H), 3.34 (br, 1H), 2.05 (q,  $J = 7.0$  Hz, 2H), 1.63 (p,  $J = 6.8$  Hz, 2H);  $^{13}\text{C}$  NMR(100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.3, 137.8, 133.4, 129.6, 129.6, 129.0, 128.3, 128.3, 114.8, 105.3, 85.7, 83.9, 76.2, 67.0, 61.6, 30.0, 28.4; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3458.07, 3072.39, 2933.61, 1719.36, 1641.99, 1601.36, 1449.50, 1369.13, 1309.13, 1268.62, 1109.69, 1032.10, 985.18, 913.81, 860.62, 713.53; HRMS (ESI-MS)  $[\text{M}+\text{Na}]^+$   $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{NaO}_6$ , 345.1313; found 345.1317.

**Compound 118:** This compound was prepared from compounds **106** and **107** using the above delineated procedure G; Yield = 95%;  $[\alpha]_{\text{D}}^{25} = +13.8$  (c 0.7,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR(400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.13 (d,  $J = 7.7$  Hz, 2H), 8.05 (d,  $J = 7.5$  Hz, 3H), 8.01 (d,  $J = 8.3$  Hz, 5H), 7.76 (d,  $J = 6.6$  Hz, 4H), 7.71 (t,  $J = 6.3$  Hz, 4H), 7.62 – 7.44 (m, 8H), 7.44 – 7.28 (m, 19H), 5.84 (ddt,  $J = 16.9, 10.4, 6.7$  Hz, 1H), 5.71 (dd,  $J = 9.4, 4.6$  Hz, 2H), 5.66 (s, 2H), 5.61 (s, 1H), 5.50 (s, 1H), 5.40 (s, 1H), 5.27 (s, 1H), 5.07 (d,  $J = 17.1$  Hz, 1H), 4.99 (d,  $J = 10.2$  Hz, 1H), 4.55 (d,  $J = 5.8$  Hz, 1H), 4.51 – 4.44 (m, 3H), 4.15 (dd,  $J = 11.2, 4.9$  Hz, 1H), 4.07 – 3.92 (m, 5H), 3.83 (q,  $J = 6.8$  Hz, 1H), 3.56 (m, 1H), 2.19 (q, 2H), 1.76 (p,  $J = 6.9$  Hz, 2H), 1.08 (s, 9H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  165.6, 165.5 (2C), 165.2 (2C), 138.1, 135.7 (6C), 135.6 (2C), 133.4, 133.3 (3C), 133.2 (4C), 133.1, 130.1 (2C), 130.0 (2C), 129.9 (4C), 129.8 (2C), 129.7 (4C), 129.5, 129.4 (2C), 129.3 (2C), 128.5 (4C), 128.4 (6C), 127.7 (8C), 115.0, 106.2, 106.0, 105.4, 84.0, 83.5, 82.7, 82.2, 82.1, 81.7, 80.9, 77.3 (2C), 66.7, 66.3, 63.6, 63.4, 30.3, 28.7, 26.9 (3C), 26.8 (3C), 19.4, 19.3; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3065.56, 2937.03, 2864.26, 1725.51, 1598.56, 1453.58, 1363.34, 1261.27, 1104.49, 969.75, 753.49, 704.74; HRMS (ESIMS)  $[\text{M}+\text{Na}]^+$   $m/z$  calcd for  $\text{C}_{87}\text{H}_{90}\text{NaO}_{18}\text{Si}_2$ , 1501.5563; found 1501.5552.

**Compound 104:** This compound was prepared from compound **118** using the above delineated procedure D; Yield = 87%;  $[\alpha]_{\text{D}}^{25} = +1.4$  (c 0.7,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR(400.31 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.99 – 7.89 (m, 8H), 7.85 (d,  $J = 7.7$  Hz, 2H), 7.51 (t,  $J = 7.4$  Hz, 2H), 7.44 (q,  $J = 7.1$  Hz, 2H), 7.40 – 7.25 (m, 9H), 7.19 – 7.09 (m, 2H), 5.72 (ddt,  $J = 16.9, 10.2, 6.6$  Hz, 1H), 5.54 (s, 1H), 5.49 (s, 1H), 5.31 (s, 1H), 5.24 (s, 1H), 5.21 (s, 2H), 5.18 (d, 1H), 5.10 (s, 1H), 4.94 (d,  $J = 17.2$  Hz, 1H), 4.87 (d,  $J = 10.2$  Hz, 1H), 4.45 (d,  $J = 6.0$  Hz, 1H), 4.31 (q,  $J = 4.4$  Hz, 1H), 4.23 (q,  $J = 4.3$  Hz, 2H), 3.94 (dd,  $J = 11.6, 3.6$  Hz, 2H), 3.89 – 3.75 (m, 4H), 3.72 – 3.62 (m, 1H), 3.43 (dt,  $J = 9.6, 6.4$  Hz, 1H), 2.63 (s, 2H), 2.07 (q,  $J = 7.1$  Hz, 2H), 1.64 (p,  $J = 7.1$  Hz, 2H);  $^{13}\text{C}$  NMR (100.67 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.4, 166.1, 165.7, 165.2, 165.1, 138.2, 133.7 (2C), 133.6 (2C), 133.5, 130.0 (3C), 130.0 (4C), 129.9 (4C), 129.5, 129.2 (3C), 129.1, 128.7 (2C), 128.6 (6C), 128.5 (2C), 115.1, 106.0, 105.4, 105.3, 84.6, 84.2, 82.9, 81.6, 81.4, 81.1, 80.5, 78.3, 77.9, 66.9, 65.2, 62.8 (2C), 30.3, 28.8; IR ( $\text{cm}^{-1}$ ,  $\text{CHCl}_3$ ): 3503.58, 2933.36, 1720.59, 1599.80, 1451.41,

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1360.34, 1260.24, 1176.08, 1105.81, 1065.10, 1029.55, 970.51, 914.82, 864.18, 756.76, 711.16; HRMS (ESI-MS)  $[M+Na]^+$   $m/z$  calcd for  $C_{55}H_{54}NaO_{18}$ , 1025.3207; found 1025.3214.

**Compound 119:** This compound was prepared from compounds **103** and **104** using the above delineated procedure G; Yield = 91%;  $[\alpha]_D^{25} = +43.4$  (c 0.7,  $CHCl_3$ );  $^1H$  NMR (500.20 MHz,  $CDCl_3$ ):  $\delta$  8.11 – 8.09 (m, 2H), 8.08 – 8.01 (m, 15H), 7.96 (dt,  $J = 12.2, 6.2$  Hz, 8H), 7.80 – 7.76 (m, 8H), 7.64 – 7.59 (m, 2H), 7.56 (dd,  $J = 13.4, 6.8$  Hz, 4H), 7.53 – 7.48 (m, 5H), 7.48 – 7.33 (m, 38H), 7.32 (s, 1H), 7.31 (s, 1H), 7.29 (s, 1H), 5.87 (ddt,  $J = 16.9, 10.2, 6.7$  Hz, 1H), 5.79 (d,  $J = 4.6$  Hz, 1H), 5.74 (d,  $J = 4.3$  Hz, 2H), 5.71 (d,  $J = 4.8$  Hz, 5H), 5.69 (s, 1H), 5.67 (s, 1H), 5.65 (d,  $J = 0.8$  Hz, 1H), 5.64 (d,  $J = 0.8$  Hz, 1H), 5.62 (s, 1H), 5.50 (d,  $J = 1.1$  Hz, 1H), 5.46 (s, 1H), 5.45 (s, 2H), 5.41 (s, 1H), 5.38 (s, 1H), 5.26 (s, 1H), 5.09 (dd,  $J = 17.1, 1.7$  Hz, 1H), 5.02 (d,  $J = 10.2$  Hz, 1H), 4.69 (d,  $J = 3.6$  Hz, 2H), 4.60 – 4.55 (m, 4H), 4.52 (dd,  $J = 7.2, 3.5$  Hz, 1H), 4.48 – 4.44 (m, 1H), 4.31 – 4.22 (m, 4H), 4.12 (dd,  $J = 11.3, 5.0$  Hz, 1H), 4.08 – 4.03 (m, 4H), 4.00 – 3.93 (m, 4H), 3.91 (dd,  $J = 11.1, 2.5$  Hz, 1H), 3.82 (dt,  $J = 9.6, 6.6$  Hz, 1H), 3.56 (dt,  $J = 9.7, 6.3$  Hz, 1H), 2.21 (q,  $J = 7.2$  Hz, 2H), 1.77 (dtd,  $J = 13.6, 6.9, 3.2$  Hz, 2H), 1.10 (d,  $J = 1.6$  Hz, 18H);  $^{13}C$  NMR (125.78 MHz,  $CDCl_3$ ):  $\delta$  165.7 (5C), 165.6 (2C), 165.3, 165.2 (2C), 165.2 (2C), 165.1, 138.2, 135.8 (4C), 135.7 (4C), 133.5, 133.4 (6C), 133.3 (6C), 133.2 (2C), 133.1 (2C), 130.0 (6C), 129.9 (16C), 129.8 (2C), 129.7 (4C), 129.4 (5C), 129.3 (2C), 129.2 (5C), 129.1, 128.6 – 128.5 (12C), 128.4 (6C), 128.3 (8C), 127.7 (10C), 115.0, 106.1 (2C), 106.0 (4C), 105.3, 83.3 (2C), 82.9, 82.8, 82.6, 82.2 (5C), 81.6 (5C), 81.5, 80.6, 77.3 (2C), 77.2, 77.0, 66.7, 66.2, 65.9, 65.8 (2C), 65.7, 63.5 (2C), 30.3, 28.8, 26.9 (6C), 19.4 (2C); IR ( $cm^{-1}$ ,  $CHCl_3$ ): 3018.53, 2936.24, 1722.23, 1599.20, 1452.70, 1360.87, 1258.92, 1174.95, 1103.72, 1028.24, 966.15, 863.71, 753.30, 705.17; HRMS (MALDI-TOF)  $[M+Na]^+$   $m/z$  calcd for  $C_{163}H_{154}NaO_{42}Si_2$ , 2862.9384; found 2862.9297.

**Compound 102:** This compound was prepared from compound **119** using the above delineated procedure E and F; Yield = 77% (in two steps);  $[\alpha]_D^{25} = +12.7$  (c 0.7,  $CHCl_3$ );  $^1H$  NMR (399.78 MHz,  $CDCl_3$ ):  $\delta$  7.99 – 7.92 (m, 4H), 7.89 – 7.82 (m, 28H), 7.80 – 7.71 (m, 18H), 7.62 – 7.56 (m, 16H), 7.44 – 7.40 (m, 4H), 7.39 – 7.36 (m, 4H), 7.35 – 7.10 (m, 96H), 6.36 (d,  $J = 4.4$  Hz, 1H), 6.24 (s, 1H), 5.66 – 5.61 (m, 2H), 5.59 – 5.51 (m, 14H), 5.50 (d,  $J = 7.5$  Hz, 4H), 5.47 (d,  $J = 1.0$  Hz, 2H), 5.46 – 5.44 (m, 2H), 5.44 – 5.42 (m, 2H), 5.33 – 5.18 (m, 11H), 4.53 – 4.24 (m, 16H), 4.14 – 4.03 (m, 8H), 4.00 – 3.83 (m, 11H), 3.82 – 3.66 (m, 10H), 2.54 (s, 1H), 2.26 (s, 1H), 2.16 – 1.86 (m, 4H), 1.80 – 1.71 (m, 4H), 1.64 – 1.36 (m, 10H), 1.34 – 1.24 (m, 2H), 0.91 (s, 36H);  $^{13}C$  NMR (100.53 MHz,  $CDCl_3$ ):  $\delta$  165.8, 165.6 (7C), 165.5 (4C), 165.3 (2C), 165.2 (8C), 165.1 (4C), 151.2, 151.0, 135.7 (16C), 133.1 – 133.6 (34C), 130.1 – 129.6 (60C), 129.4 –



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128.7 (26C), 128.6 - 128.2 (52C), 127.7 (16C), 106.2, 106.1, 106.0 (4C), 105.9 (3C), 105.8, 105.5, 105.3, 102.8, 96.5, 83.8, 83.2 (4C), 82.9, 82.7, 82.6, 82.5, 82.2 (9C), 81.9, 81.7, 81.6, 81.5 (4C), 80.3, 80.0, 78.5, 78.1, 77.7, 77.6, 77.4 (5C), 77.2 (5C), 77.0 (2C), 76.8 (2C), 76.6, 75.5, 75.1, 67.0, 65.8 (3C), 65.7 (3C), 65.6 (2C), 65.4, 63.4 (4C), 36.9, 36.7, 36.6, 36.5, 26.8 (12C), 25.0, 24.8, 22.7 (3C), 22.5, 19.4 (4C); IR (cm<sup>-1</sup>, CHCl<sub>3</sub>): 2940.15, 1725.00, 1599.48, 1452.23, 1363.71, 1262.90, 1175.23, 1106.64, 966.60, 910.93, 859.31, 754.87, 707.83; HRMS (MALDI-TOF)[M+Na]<sup>+</sup> m/z calcd for C<sub>167</sub>H<sub>156</sub>NaO<sub>44</sub>Si<sub>2</sub> 2944.9439; found: 2944.9578.

### 5.5 Spectral Data<sup>6</sup>

For spectral data (NMR and Mass), kindly refer the supporting information of the publication: S. Pasari, S. Manmode, G. Walke, S. Hotha *Chem. Eur. J.* **2018**, *24*, 1128 – 1139.

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- 13) See experimental section



## List of publications

1. [Au]/[Ag]-Catalyzed Expedient Synthesis of Branched Henicosafuranosyl Arabinogalactan Motif of Mycobacterium Tuberculosis Cell Wall; Shivaji Thadke, Bijoyananda Mishra, Maidul Islam, Sandip Pasari, Sujit Manmode, Boddu Venkateswara Rao, Mahesh Neralkar, Ganesh P. Shinde, **Gulab Walke** & Srinivas Hotha; *Nature Communications*, **2017**, 8, 14019.
2. A Versatile Synthesis of Pentacosafuranoside Subunit Reminiscent of Mycobacterial Arabinogalactan Employing One Strategic Glycosidation Protocol; Sandi Pasari, Sujit Manmode, **Gulab Walke** and Srinivas Hotha; *Chem. Eur. J.* **2018**, 24, 1128 –1139.
3. Synthesis of Anticoagulant Fondaparinux Pentasaccharide Enabling Regiodefined Sulfate Derivatives by Silver-assisted Gold Catalysis; **Gulab Walke**, Nitesh Kasdekar, Yogesh Sutar, Srinivas Hotha (*Manuscript under revision*).
4. An Efficient Glycosylation Protocol by Benchtop Stable Propiolate Glycosyl Donors; **Gulab Walke**, Nitesh Kasdekar, Srinivas Hotha (*Manuscript under preparation*).
5. Active Crotonate Donors in a One-Pot Glycosidation Reactions using Catalytic Triflates; Nitesh Kasdekar, **Gulab Walke**, Kshitij Deshpande, Srinivas Hotha (*Manuscript under preparation*).
6. Convergent Synthesis of Lipoarabinomannan (LAM) Triacontasaccharide (30 Mer) Subunit Reminiscent of Mycobacterial Arabinogalactan Employing One Strategic Glycosidation Protocol; Maidul Islam, Ganesh Shinde, **Gulab Walke**, Srinivas Hotha (*Manuscript under preparation*).
7. Total Synthesis of Highly Branched N-linked Core Hexasaccharide from Chloroviruses; Bijoyananda Mishra, Sujit Manmode, **Gulab Walke**, Mahesh Neralkar, Saptashwa Chakraborty and Srinivas Hotha (*Manuscript under preparation*).

## Current Projects

1. Synthesis of Heparin Oligosaccharides by Employing Chelation Assisted Grignard Reaction and Late Stage Oxidation Protocol; **Gulab Walke**, Kamesh Bhat, Srinivas Hotha (*Work is under progress*).
2. Synthesis of Hyaluronic Acid Oligomers by Split React Coupled Protocols Employing Silver-Assisted Gold Catalysed Glycosidations; **Gulab Walke**, Yogesh Sutar, Nitesh Kasdekar, Srinivas Hotha (*Work is under progress*).