CHAPTER-1

Introduction

1.1 Carbohydrates

Carbohydrates are the most abundant class of organic compounds present in nature and are the main source of energy for living organisms [1]. Carbohydrates usually exist as glycosides, glycoconjugates, oligosaccharides or polysaccharides and are involved in a wide range of biological processes such as cell-cell recognition, fertilization, embryogenesis, neuronal development, hormonal activities, proliferation, viral and bacterial infections and tumor cell metastasis [2]. Polysaccharides such as cellulose, pectose and xylan are the cell wall structural components of many plants and bacteria that facilitate intercellular communication in different forms [3]. On the other hand, chitin is a major component of the exoskeleton of insects, crabs and lobsters.

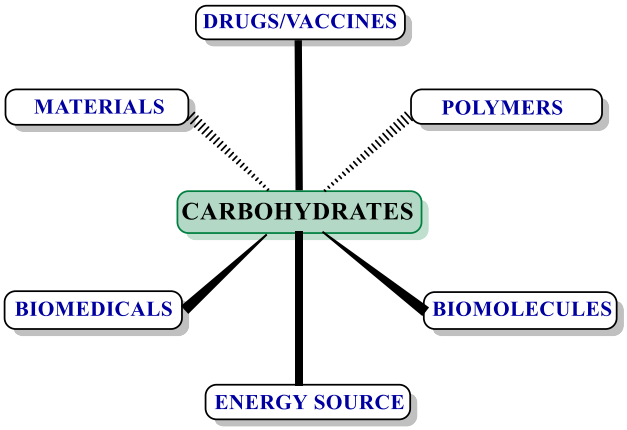


Figure 1.1 Applications of carbohydrates in different fields

Carbohydrates are structurally complex when compared with other linear biopolymers such as proteins, DNA and RNA [4]. Carbohydrates have found various applications in different research fields including chemistry, biology, materials and polymer sciences (**Figure 1.1**) [5, 6].

1.2 Classification of carbohydrates

Carbohydrates are primarily classified as monosaccharides, disaccharides, oligosaccharides and polysaccharides based on the number of carbohydrate units attached through glycosidic bonds. Monosaccharides are single sugar units that act as the building block to assemble disaccharides, oligosaccharides, and polysaccharides (**Figure 1.2**). Further, carbohydrates also exist as peptidoglycans, glycopeptides, glycoproteins, glycolipids and lipopolysaccharides when linked with other biomolecules such as amino acids, proteins, lipids, etc., [7].

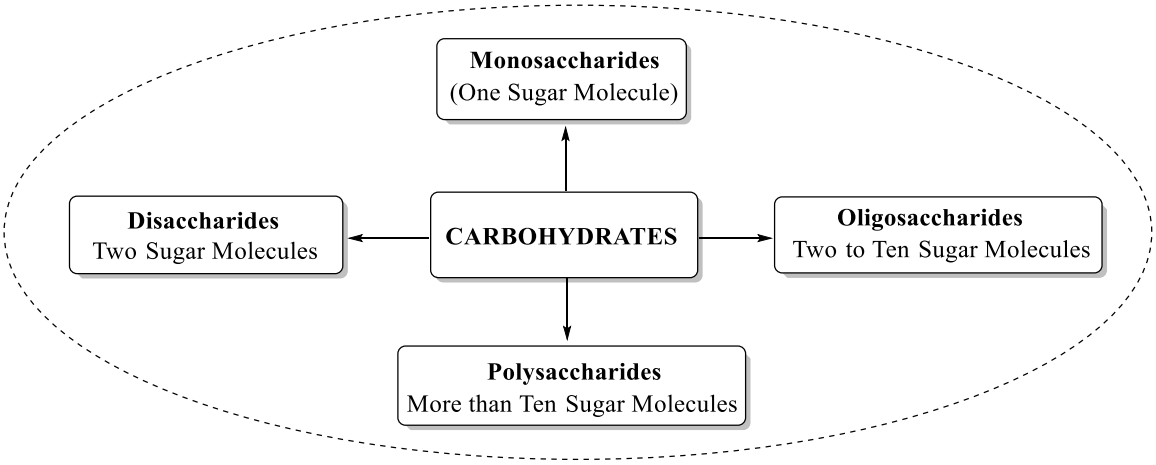


Figure 1.2 Primary classification of carbohydrates

1.3 Sugar carboxylic acids

The importance of organic acids derived from carbohydrates has received great attention in recent years [8]. Aldonic, aldaric and uronic acids are the three main classes of sugar acids known in the literature (Figure 1.3). Aldonic acid is obtained when the aldehyde functional

group in aldose is oxidized into carboxylic acid using mild oxidants. For instance, *gluconic acid* is produced by the selective oxidation of the aldehyde functional group in glucose using hydrogen bromide-water. On the other hand, oxidation of both aldehyde and primary alcohol groups in the monosaccharide (i.e. C-1 and C-6) using a stronger oxidant provides the dicarboxylic acid compound, known as *aldaric acids*. For example, *glucoaldaric acid* is produced when aldehyde and primary alcohol is oxidized to carboxylic acid in glucose using nitric acid.

On the other hand, *uronic acid* has been produced by the selective oxidation of primary alcohols in aldose sugars. Such selective oxidation is usually achieved using anomeric protected sugars with different oxidants (e.g. $PhI(OAc)_2$/TEMPO). The uronic acid produced from glucose via selective oxidation of primary alcohol is called *glucuronic acid*. Unlike other sugar acids, C-1 in uronic acid remains unaffected leading to its occurrence in cyclic form.

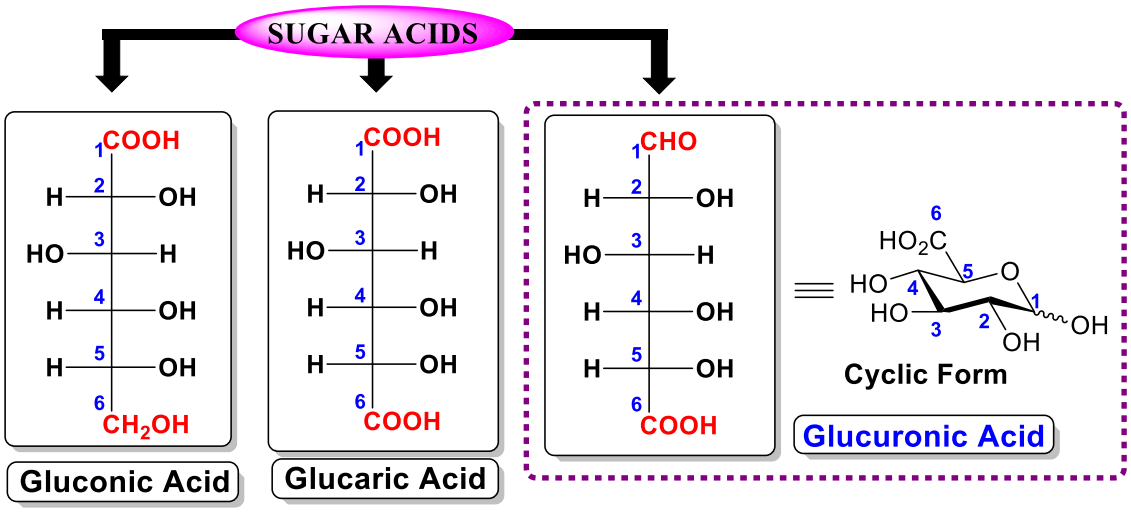


Figure 1.3 Classification of sugar acids

Among the different sugar acids, uronic acids occur naturally in the form of polysaccharides and glycoconjugates and play important roles in various biological processes [9].

1.4 Uronic (aldonic) acids

Uronic acids are an important class of compounds that are present in many biologically relevant polysaccharides. The chemical structures of the most commonly occurring uronic acids such as D-mannuronic acid, D-glucuronic acid, D-galacturonic acid and L-iduronic acid are shown in Figure 1.4.

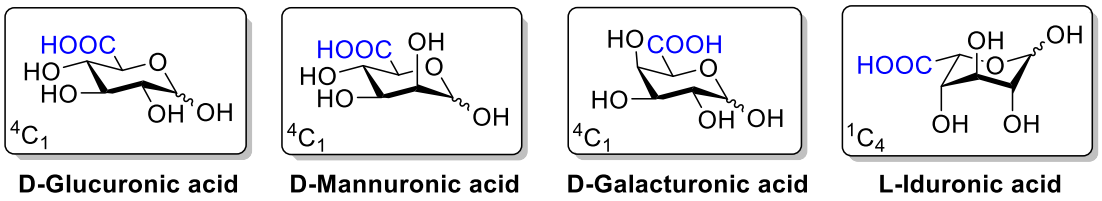


Figure 1.4 Some common uronic acids

D-glucuronic acid and L-Iduronic acid (or IdoA) are the major uronic acid components of glycosaminoglycans (GAGs) such as hyaluronan, chondroitin sulfate, dermatan sulfate, heparin and heparin sulfate. In general, pyranose sugars are stable in one of two chair conformations $^{1}C_4$ or $^{4}C_1$. In particular, D-sugars are more stable in $^{4}C_1$ conformation while L-sugars are stable in $^{1}C_4$ conformation. In some cases, sugars can adopt more than one conformation. Homoglycuronans, the structurally and functionally distinct class of polysaccharides, are made-up of only uronic acid building blocks. Examples of homoglycuronans are i) alginate, which is composed of D-mannuronic acid and L-guluronic acid, and ii) pectin, which is mainly composed of D-galacturonic acid. These

homoglycuronans are often used in the food and pharma industries as gelling and emulsifying agents.

1.5 Polysaccharides with uronic acid units:

There are several polysaccharides present in nature with uronic acid units [9b, 10]. Some of the biologically important polysaccharides containing uronic acids are enlisted below with a brief description:

1.5.1 Heparin

Heparin is a highly sulfated polysaccharide from the glycosaminoglycan family and it has been used as an anticoagulant drug since 1935 (Figure 1.5). Heparin binds to toserine protease inhibitor antithrombin III (AT) and activates. The activated AT blocks thrombin and deactivates Xa and IIa factors which are responsible for blood coagulation [9b]. Structurally, heparin is a linear polysaccharide that is made up of disaccharide repeating units composed of L-iduronic acid and D-glucosamine. Heparin is usually found in animal tissues in the form of heparin sulphate.

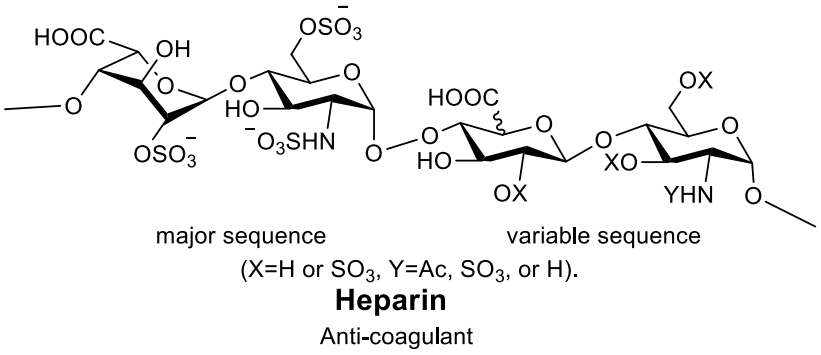


Figure 1.5 Structure of Heparin.

However, the structural heterogeneity, contamination, and side effects of natural heparin create limitations to their widespread clinical applications. To overcome these issues, synthetic heparin mimetics such as fondaparinux and idraparinux have been rationally developed and clinically used [9c, d].

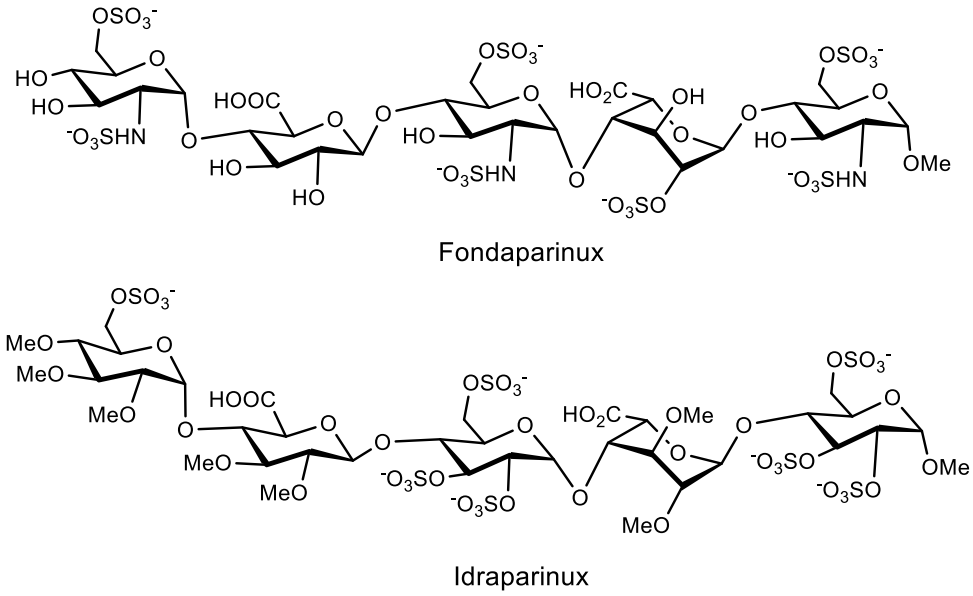


Figure 1.6 Structures of heparin memetics Fondaparinux and Idraparinux

1.5.2 Chondroitin sulfate

Chondroitin sulfate (CS) or Chondroitin sulfate A (CS-A) is another uronic acid containing polysaccharide that comes from glycosaminoglycan family, usually found in the cartilage and extracellular matrix [9e]. Chondroitin sulfate is a linear polysaccharide composed of disaccharide repeating units containing *N*-acetyl galactosamine (GalNAc) and D-glucuronic acid joined by β-1,4 or β-1,3 linkages respectively (Figure 1.7).

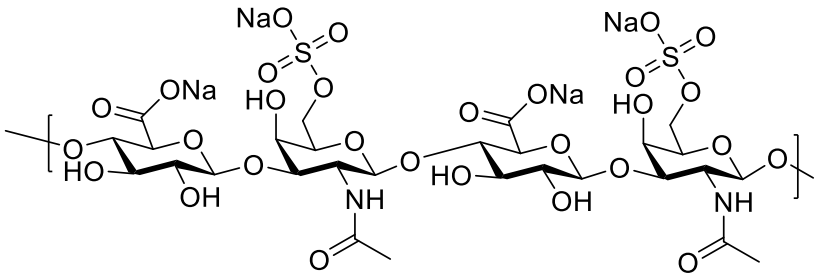


Figure 1.7 Structure of Chondroitin Sulfate

Chondroitin sulfate has been used as a supplement in the treatment of osteoarthritis and cataracts due to its anti-inflammatory activity.

1.5.3 Dermatan Sulphate

Dermatan sulphate (DS) also known as chondroitin sulfate B (CS-B), is also a linear polysaccharide from glycosaminoglycan family. Dermatan sulphate is composed of disaccharide repeating units containing *N*-acetyl galactosamine (GalNAc) and L-iduronic acid joined by β 1,4 or 1,3 linkages respectively (Figure 1.8). Dermatan sulphate is found in the skin, blood vessels, heart valves, tendons, lungs, and intestinal mucosa. Dermatan sulphate is the constituent of tissues and organs and is involved in wound repair, regulation of blood coagulation and the response to infection [9f].

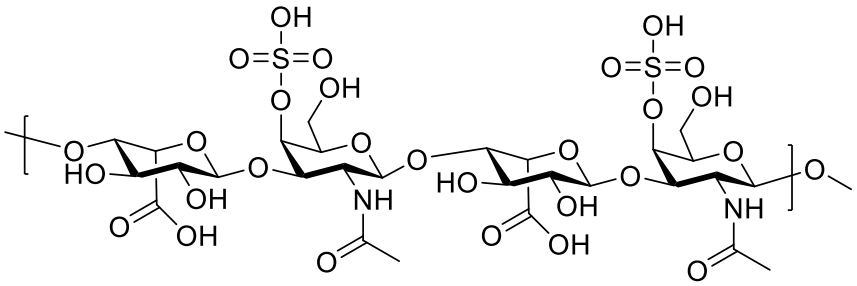


Figure 1.8 Structure of Dermatan Sulphate

1.5.4 Hyaluronic acid (Hyaluronan)

Hyaluronic acid (HA) also comes from glycosaminoglycan family, however without any sulphate group. It is also a linear polysaccharide made up of disaccharide repeating units bearing *N*-acetylglucosamine and D-glucuronic acid with alternating β-1,4 and β-1,3 glycosidic linkages (Figure 1.9). Hyaluronic acid is found primarily in the extracellular and pericellular matrix, but also occurs intracellularly. The biological functions of HA include migration, regulation of cell division, differentiation, maintenance of the elastoviscosity of liquid in connective tissues, tissue hydration and water transport, etc. Hyaluronic acid is also used as a supplement in the treatment of joint pain, wound repair, ophthalmologic and cosmetic treatment, etc., [9g].

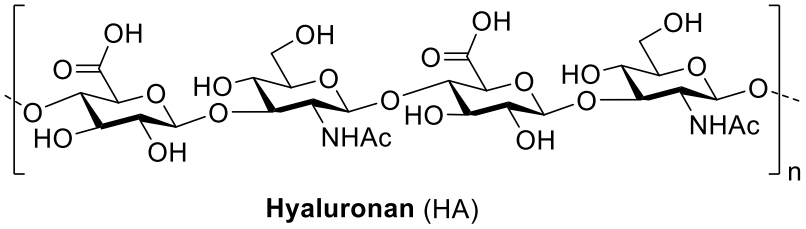


Figure 1.9 Chemical structure of HA

1.5.5 Xanthan

Xanthan, also known commercially as xanthan gums, is a polysaccharide with many industrial uses, including food additives, thickening agents, emulsifiers and stabilizers which prevent ingredients from separating [9h]. Xanthan is composed of a branched pentasaccharide repeating unit in which one unit of D-glucuronic acid is present.

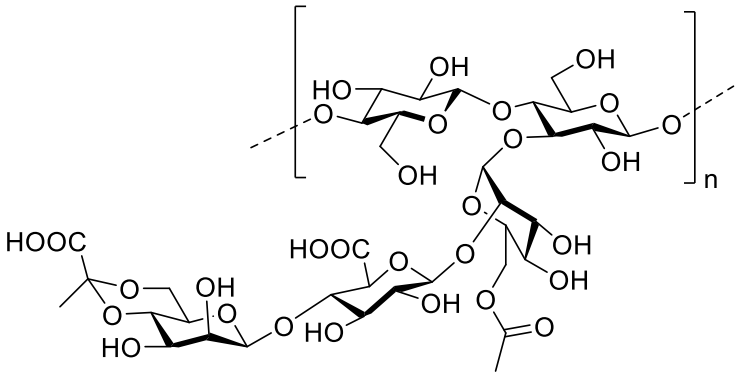


Fig 1.10 Chemical structure of Xanthan

It can be produced from simple sugars using a fermentation process using the bacteria Xanthomonas campestris.

1.5.6 Glucuronan

Glucuronan is one of the less abundant polysaccharides which is produced by some bacteria, fungi, and algae [9h]. Glucuronan is composed of β-D-glucuronic acids with 1, 4-glycosidic linkages. The C-3 and/or C-2 positions of glucuronan are partially acetylated. Glucuronan is used in the food and pharmacological industries due to its rheological and biological properties.

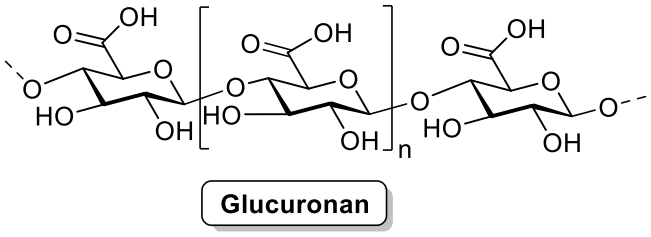


Fig 1.11 Chemical structure of Glucuronan

1.5.7 Alginate (Mannuronate)

Alginate is a linear polysaccharide mainly composed of β-D-mannuronic acid building blocks with 1, 4- glycosidic linkages. Alginate is produced by various genera of brown seaweed and some bacteria that belongs to the Pseudomonas and Azotobacter [9h, i]. Alginate polysaccharides obtained from brown seaweed are widely used in the food and pharmaceutical industries due to their chelation and gelling properties.

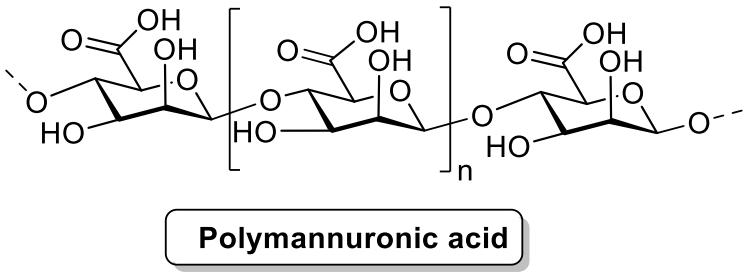


Fig 1.12 Chemical structure of Polymannuronic acid

1.5.8 Ethyl glucuronide

Ethyl glucuronide is a direct metabolite of ethanol that can be detected in various body fluids, tissues, urine, serum and hair. Ethyl glucuronide is primarily used to detect alcohol consumption in human beings [9j].

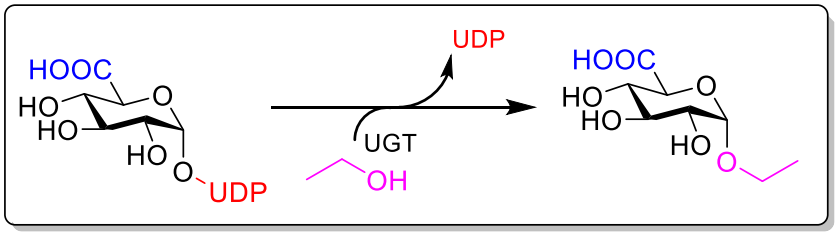
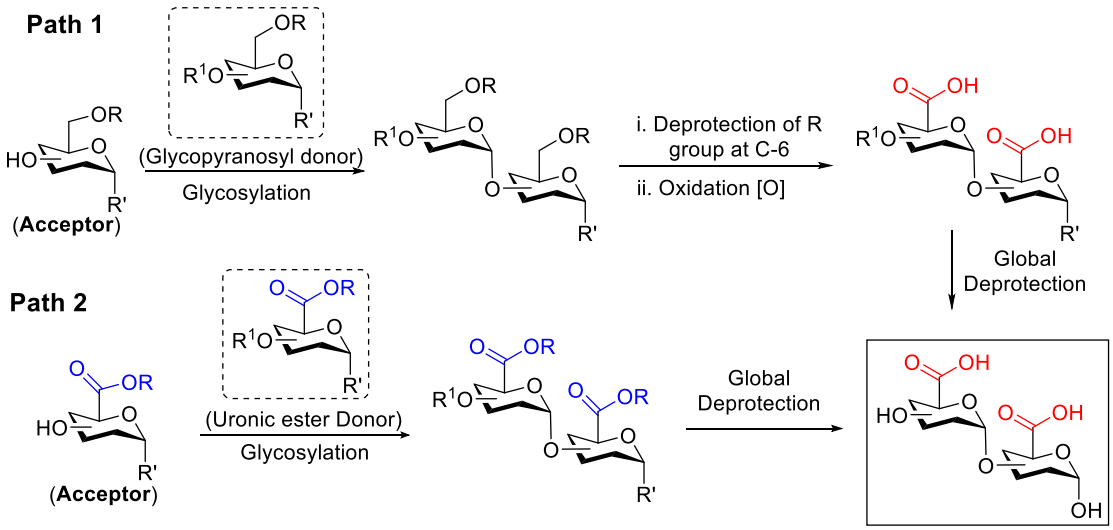


Fig 1.13 Enzymatic formation of Ethyl Glucuronide

1.6 Synthesis of uronic acids containing oligosaccharides/ polysaccharides

Structurally well-defined oligosaccharides are highly important for understanding their exact biological functions. In this context, the synthesis of uronic acid containing polysaccharides received great attention owing to their structural complexity and diverse biological properties.

There are two distinct strategies which have been followed for the preparation of uronic acids containing oligosaccharides [11]. One of the strategies relies on the post-assembly oxidation in which initially oligosaccharide is assembled by keeping unique protecting groups on the appropriate primary hydroxyl groups. After completion of the oligosaccharide assembly, the protecting groups present on the primary alcohols are removed selectively and oxidized to uronic acids before or after global deprotection (Scheme 1.1, Path 1). The other strategy entails the direct use of pre-synthesized uronic acid building blocks as shown in scheme 1.1 (Path 2).

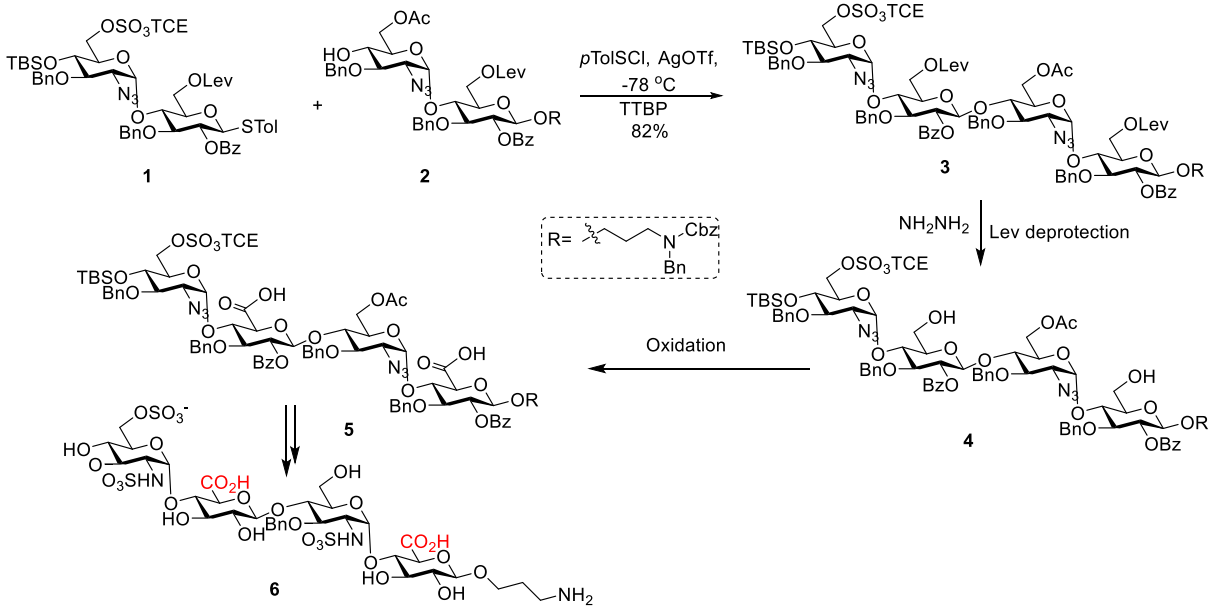


Scheme 1.1 Strategies of synthesis of uronic acids containing oligosaccharides.

For example, the synthesis of uronic acids containing oligosaccharides via different strategies has been shown below [12].

1.6.1 Strategy 1

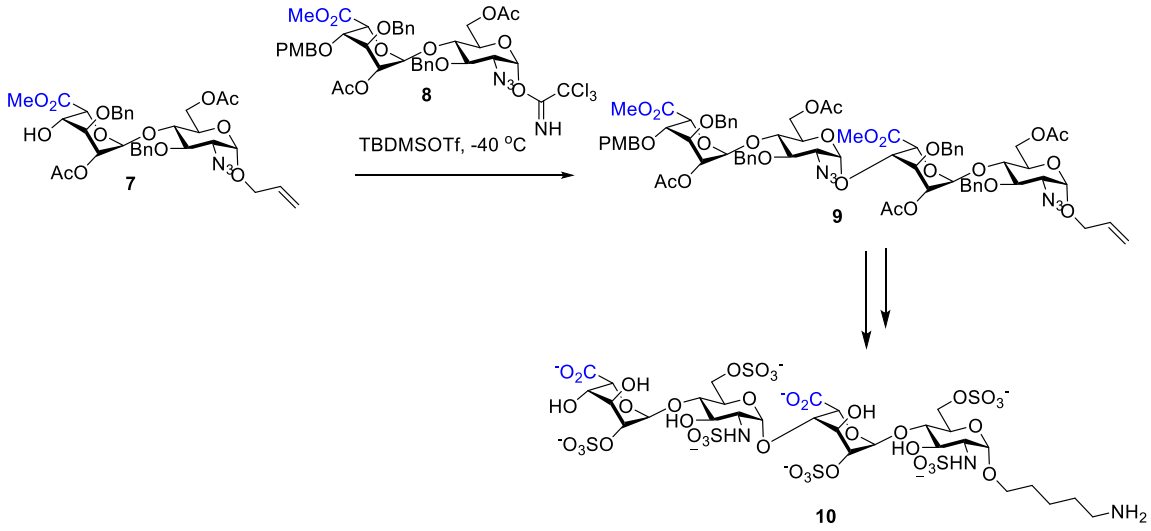
The assembly of sulphated uronic acid containing oligosaccharide **6** has been achieved from the disaccharide building blocks **1** and **2** via a post-assembly oxidation strategy (Scheme 1.2). The glycosylation of acceptor **2** with donor **1** provides the tetrasaccharide **3**. Compound **3** possesses Lev protection at the specific primary alcohols which is deprotected using hydrazine hydrate. Resulting primary alcohols in compound **4** were oxidized to corresponding carboxylic acids using TEMPO/ $PhI(OAc)_2$ to generate uronic acid containing oligosaccharide **5**. The compound **5** was further converted into sulphated uronic acid containing oligosaccharide **6** using additional steps [13, 14].



Scheme 1.2 Post-assembly oxidation strategy for the synthesis of uronic acids containing oligosaccharides

1.6.2 Strategy 2

As an alternative path, the uronic acid containing oligosaccharide **10** was prepared using pre-synthesized uronic acid building blocks (Scheme 1.3). Glycosylation of the acceptor **7** with uronic acid methyl ester donor **8** provided the tetrasaccharide **9.** The compound **9** was further converted into sulphated uronic acid containing oligosaccharide **10** with additional steps including deacetylation, sulphation, hydrolysis of the methyl esters and hydrogenolysis [15, 16].



Scheme 1.3 Uronic acid glycosyl donors in oligosaccharide synthesis

1.7 Conclusions and Objective of the thesis

Carbohydrates are an integral part of living systems and have immense biological importance. The recent advancements in this field have led to the development of several carbohydrates-based therapeutic tools which include drugs, vaccines, bio-materials, biomedical devices and so on. Polysaccharides with uronic acid residues are widespread in

nature and display a wide range of physical and biological properties. Hence, the synthesis of uronic acids and related oligo- and polysaccharides are highly important to understand their biological roles.

In this context, the current thesis aimed to explore the synthesis of uronic acid building blocks through different routes and their applications. With this goal, following projects have been explored,

- A highly efficient TEMPO mediated oxidation of sugar primary alcohols into uronic acids using 1-chloro-1,2-benziodoxol-3-one at room temperature
- An efficient and direct esterification of uronic acids using recyclable H_2SO_4-SiO_2 at room temperature under solvent free conditions
- Synthesis of photolabile protecting group (PPG) protected uronic acid building blocks: applications in carbohydrate synthesis with the assistance of a continuous flow photoreactor
- Synthesis of photolabile group protected anomeric acetals and their application in carbohydrate synthesis with the assistance of continuous flow photoreactor (*As an Appendix*)

The aforementioned projects have been well-planned and executed systematically in the last five years (2016-2022) at IIT (BHU). The outcomes of the project have been placed in four chapters in addition to "Introduction" and "Summary and Conclusion".

1.8 References

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