

Introduction to natural polysaccharides

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1.1 Introduction

Photosynthesis is the most important and fundamental chemical reaction of all living processes. “Inorganic carbon” in the form of carbon dioxide is captured and transformed to carbohydrates next to other important biomolecules in living organisms [1]. Polysaccharides are abundant in nature and regarded as one of the most important biological macromolecules, among others such as proteins, nucleic acids, lipids, etc. Polysaccharides are biopolymers whose monomer units are simple monosaccharides [2, 3]. Monosaccharide units are aldose (six-member pyranose structure) or ketose (five-member furanose structure) sugars present in polysaccharides in numbers of >20 to as high as 60,000. In polysaccharides, monomers are joined by glycosidic bonds with a definite stereochemistry. They are plentiful in nature, produced from various sources, notably, plant origin (cellulose, guar gum, and pectin), animal origin (chitin, hyaluronic acid, and heparin), algae origin (agar and alginate), and microbial origin (gellan and curdlan). Polysaccharides may be linear, branched with a few short branches, long and heavy branches with bush-like structure [4, 5]. Polysaccharides play two major roles in living organisms: (1) key structural machinery of plant cell wall and other [6] (2) used as food reservoir/food storage in the form of starch in plants and glycogen in mammals [7]. Original polysaccharides, or its modified form, have shown a wide range of pharmaceutical applications with a variety of biological properties, such as antioxidant, antiinflammatory agent, anti-HIV agent, antitumor, and anticoagulant agent, etc. [8–13]. Although, it is obvious that not all the natural polysaccharides are bioactive or have showed bioactivities [14] at the highest level owing to their structure and physical properties, including others. Therefore, molecular modification (chemical, physical, and biological) is highly desirable to have the highest level of bioactivities [15–18]. More recently, polysaccharides conjugates can be utilized in different biomedical applications such as drug delivery and imaging, tissue engineering, and other many biomedical processes [19–22]. In this chapter, we present the introductory level of polysaccharides with structure and composition of monosaccharides, biological functions, natural occurrence, classifications, and molecular modifications, particularly by various chemical methods implemented for natural polysaccharides.

1.2 Structure and chemical composition

Because of the immense importance of polymeric carbohydrates (polysaccharides) to the energy production and structural components in cell walls, along with biomedical applications in various sectors, their fundamental structural features are highly advantageous and significant. In addition, the scientific community working in the field of sugar chemistry, primarily applied research, in biomedical applications and industrial biotechnology needs deeper knowledge on molecular structures and the arrangement of repeating units (monosaccharide) and their linkage technique, etc. Especially, the complex structure of the polymeric form of carbohydrates turns them into an interesting structure-activity relationship in supramolecular chemistry (carbohydrate-carbohydrate interaction or carbohydrate-protein interaction). This involves regulating a variety of biochemical processes, such as cell differentiation, proliferation and adhesion, inflammation, and the immune response [23]. In recent trends, polysaccharides alone or polysaccharides conjugates have shown a great importance in pharmaceutical and medicinal fields. In particular, polysaccharide conjugates are widely used in the field of biomedical research and other biomedical applications, such as drug delivery and imaging, tissue engineering, etc. [19–22]. In order to understand the various biomedical and biochemical processes at the molecular level, the short discussion on biosynthesis and degradation of polysaccharides, including the chemical transformation involved in these processes, is necessary to a great extent. Even more, starting from the biosynthesis of monosaccharides and moving to oligosaccharides and polysaccharides is truly significant to all researchers for a better understanding of the fundamental polysaccharide chemistry and their long-standing applications in various fields.

1.2.1 Monosaccharides

In nature, the most frequently encountered carbohydrate units as monosaccharides are: hexoses (six-carbon sugar) and pentoses (five-carbon sugar). Photosynthesis produces a three-carbon-based sugar molecule, which is named 3-phosphoglyceraldehyde [24]. Two molecules of 3-phosphoglyceraldehydes turn into six-carbon sugar, glucose 6-phosphate, effectively via reverse glycolytic pathway. In contrast, 3-phosphoglyceraldehydes can be utilized in the Calvin cycle to access five-carbon pentose sugars, such as ribose 5-phosphate, ribulose 5-phosphate, and xylulose 5-phosphate. The fundamental chemical reactions, such as mutation, epimerization, aldose-ketose interconversions, chain-shortening and chain-elongation modification, and oxidation-reduction including transamination steps, can be used in the biochemical manipulation of monosaccharide structure [25, 26]. Reversible transfer of 2-C “active glycoaldehyde” fragment from a ketose donor (*D*-xylulose-5-phosphate) to an aldose acceptor (*D*-ribose-5-phosphate) catalyzed by transketolase enzyme (Thiamine pyrophosphate is the active catalyst) generates *D*-glyceraldehyde 3-P (chain-shortening) and *D*-sedoheptulose 7-P (Chain-lengthening).

1.2.1.1 Anomeric descriptor (α and β)

For D-glucose in open-chain configuration, the free hydroxyl group (OH-) of C5-carbon intramolecularly attacks the aldehyde group of C1 carbon to form a mixture of two types of isomers: α - and β -anomers with a new stereogenic center at C1-carbon, which is called anomeric carbon.

- i) α -anomer means the hydroxyl group at the new stereogenic center C1-carbon points in the opposite direction of the CH₂OH group at C5-carbon; whereas, the hydroxyl group at the C1-carbon points in the same direction of the CH₂OH group at C5-carbon assigned as β -anomer (Fig. 1.1).

In another way, if the absolute configurations of the two stereogenic centers of sugar molecules (C1-anomeric center and highest chirality center) are the same (*R, R* or *S, S*), they are termed as β -anomer or α anomer if they are alike. A few examples of α and β anomers are depicted as Fig. 1.1.

1.2.1.2 D/L-Notation

The D/L notation can be assigned on the basis of the chirality (as shown in Fischer projection) at the bottommost chiral center (highest numbered chiral center). Note: if hydroxyl group at the highest numbered chiral center is positioned toward the right side, it is named as D or L if positioned to the left side (Fig. 1.2).

1.2.2 Oligosaccharide

Oligosaccharides are formed by typically 2–5 monosaccharide units and as high as 20 monosaccharide units. Each monosaccharide unit is linked with others by a stereoselective α or β -glycosidic bond through a specific carbon center. The biochemical synthesis of natural oligosaccharides or polysaccharides (more than 20 monosaccharides

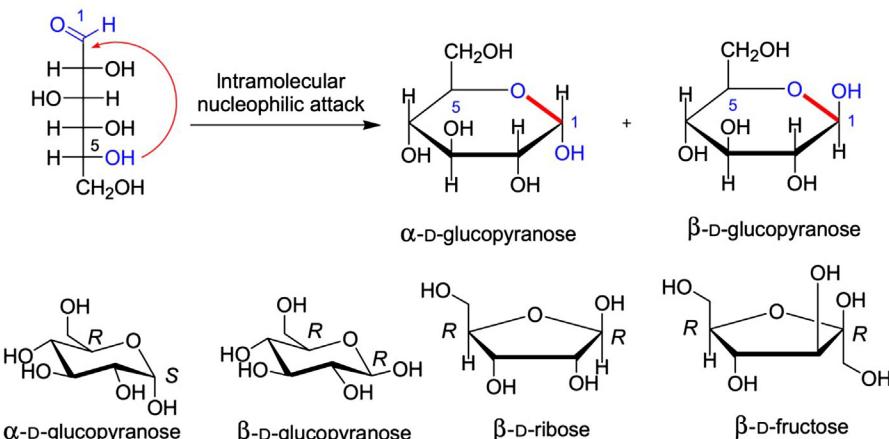


Fig. 1.1 Anomeric descriptor.

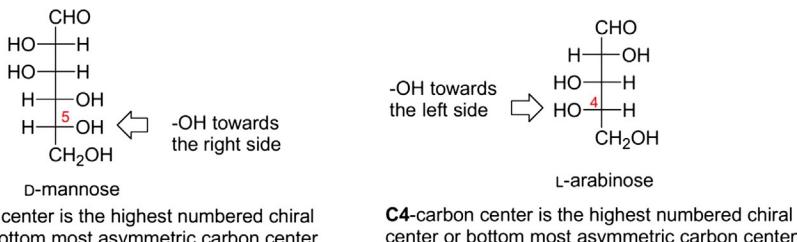


Fig. 1.2 D/L notations in a monosaccharide unit.

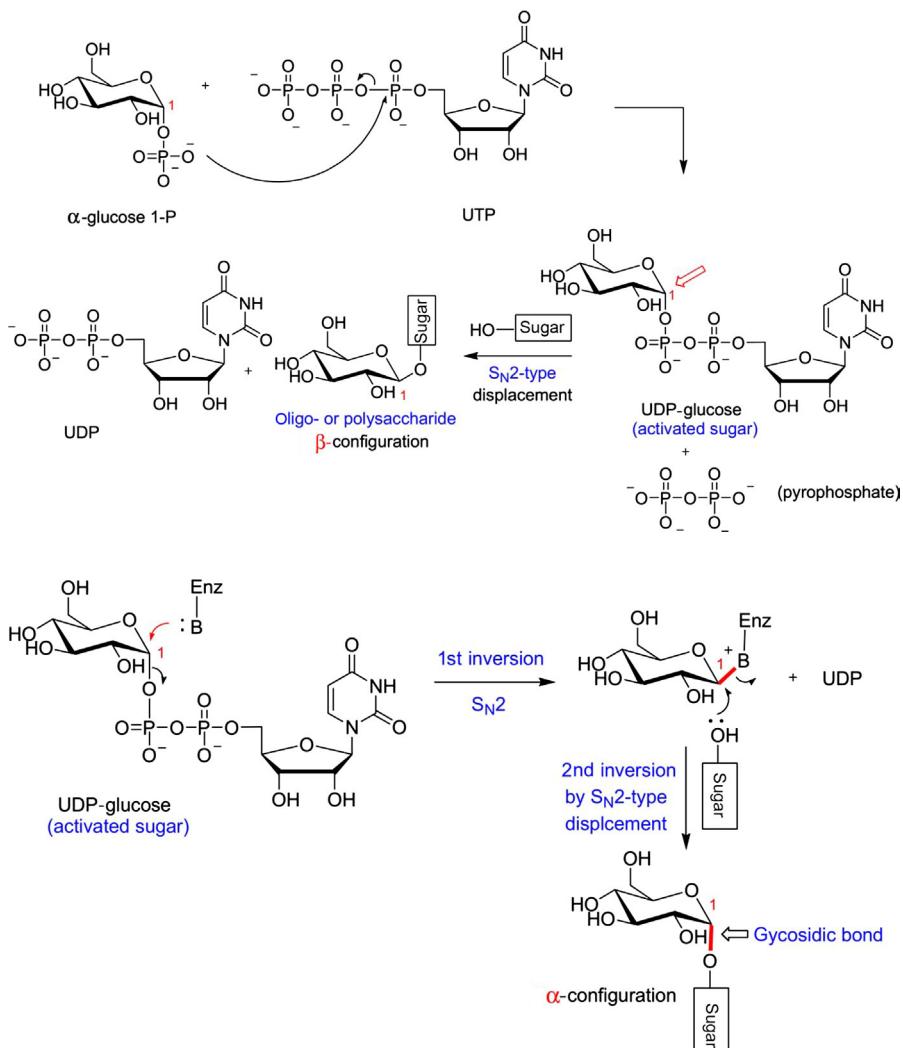
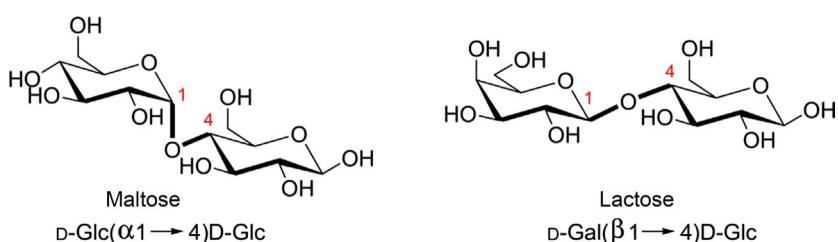
joined together in the linear or branched form) depends on an activated sugar bound to a nucleoside diphosphate. The UDP is typically encountered as a nucleoside diphosphate, although sometimes ADP and GDP are also involved. As depicted in Fig. 1.3, an activated sugar UDP-glucose is formed by the nucleophilic displacement reaction of α -D-glucose 1-P with UTP. Next, S_N2 -type of displacement reaction with a suitable nucleophile [-OH groups of sugar molecule] generates a new oligosaccharide or polysaccharide along with free UDP. Mechanistically, this reaction is S_N2 -type, so the absolute configuration at anomeric center (C1) would be inversion of configuration, i.e., β , if the starting D-glucose is with α -configuration [26]. Many times the linkages between glucose monomers are shown to have α -linkages (maltose or maltotriose). This is possible if double inversions occur at the reactive centers; first S_N2 inversion takes place involving enzyme and followed by second S_N2 -displacement by an appropriate sugar molecule (Fig. 1.3) [26].

Simple oligosaccharide molecules can be exemplified as maltose, a hydrolysis product of starch that consists of two glucose units linked through α 1 \rightarrow 4 glycosidic bond, and lactose, which is found in cow's milk and has galactose linked with glucose through β 1 \rightarrow 4 glycosidic bond (Fig. 1.4).

1.2.3 Polysaccharide

Polysaccharide means a polymer of >20 to 60,000 monosaccharide units linked by O-glycosidic bonds in linear or branched fashion. Mostly, the monosaccharides are joined in a linear fashion in the structural polysaccharides, and several linear polymeric chains are closely packed together one after another. The presence of weak secondary interaction, predominantly hydrogen bonding between the hydroxyl groups of the chain layers, makes them more rigid with high tensile strength and high viscous material. Cellulose, a natural polysaccharide developed by both plants and animals, although almost exclusively by plants, is structural polysaccharides of glucose molecules joined by β 1 \rightarrow 4 glycosidic bonds and growth observed in a linear approach [27]. The glucose chains (approximately 60–70 glucose chains) lay side by side to make a thicker, stronger, and fibrous bond in nature. Large numbers of hydrogen bonding among the free hydroxyl groups of the glucose chains makes a structure called microfibril, and it provides enormous physical strength (Fig. 1.5).

On the other hand, the polysaccharides, which serve mainly as energy storage in plants or animals in the form of starch or glycogen, respectively, are commonly

**Fig. 1.3** Oligosaccharide biosynthesis.**Fig. 1.4** Representative examples of oligosaccharide: Maltose and lactose with structure.

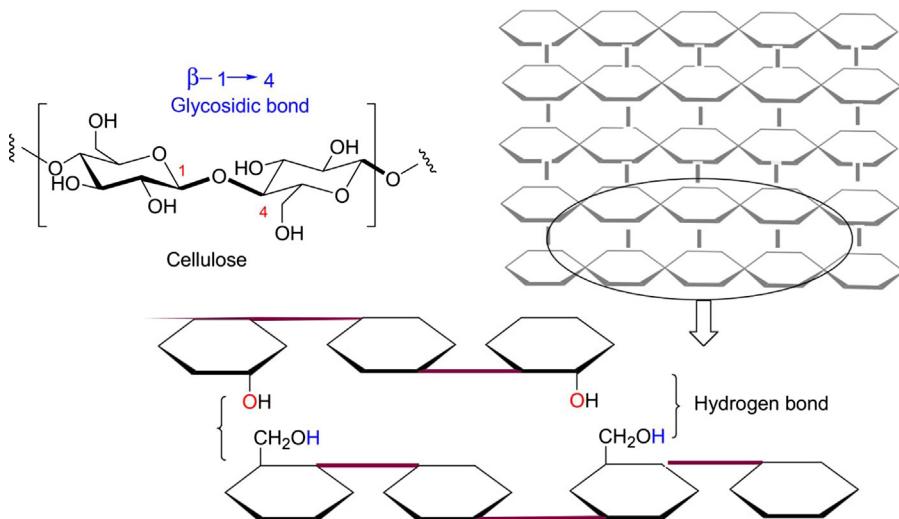


Fig. 1.5 Schematic diagram of glucose polysaccharide layers, which are held together by hydrogen bond.

branched or in some cases (starch) a mixture of linear or branched. Obviously, the branched polysaccharides are less packed thus making them easily soluble in water. Very often this type of polysaccharides has a helical shape, rather than a linear shape. Plants produce starch as their main food reserve, and it is composed of a glucopyranoses unit with two different polysaccharides named amylose and amylopectin. Amylose is a linear shaped polysaccharide composed of glucopyranoses linked by α 1→4 glycosidic bond. Whereas, amylopectin is a branched polysaccharide assembled with glucose residues linked by both the 1→4 linearly and α 1→6 branched glycosidic bonds (Fig. 1.6). Amylose structure contains fewer glucose residues (about 1000–2000) than amylopectin. Amylopectin is a much larger polymer with glucose units as high as 10^6 , and it forms a branched polymer. At every 20 glucopyranoses linearly linked with α 1→4, a branch starts with α 1→6 glycosidic bond, and the branch propagates up to 20 residues linked with α 1→4. The next subsequent second branch starts

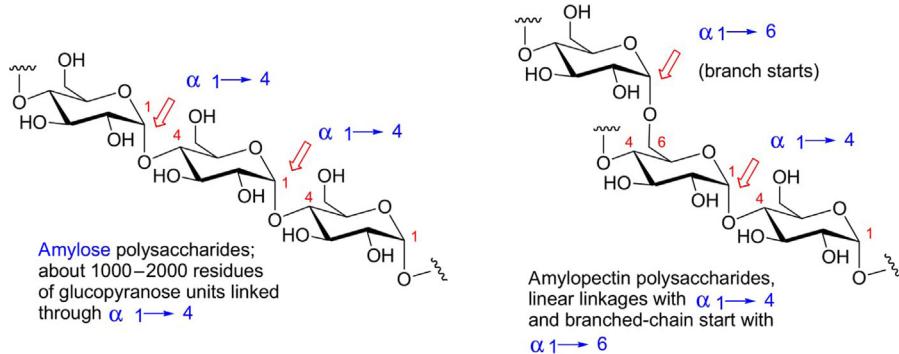


Fig. 1.6 Structure of amylose and amylopectin polysaccharides.

with α 1→6 glycosidic bond, and the process continues. Finally, the overall structure of starch looks like a tree structure. In mammalian carbohydrates, storage polymer is a glycogen, which is analogous of amylopectin molecule in structure, although more branched-chains at every 10 residues of glucopyranoses are observed [4, 28–30].

1.3 Natural occurrence and classifications

The isolation, synthesis, and structural modification of natural polysaccharides and their applications in the various areas, particularly in the biomedical field, recently have progressed rapidly and can now be considered as the most important biopolymers existing at a higher percentage in nature along with others such as proteins and nucleic acids, etc. Their systematic structural chemistry and classification is highly important; however, it is not an easy task to classify the polysaccharides in one dimension. Because of their great variety in polymolecularity and structure with different functionality [3, 31], the classification of polysaccharides always remained a great challenge to the scientific community. In this section, we attempt to categorize the natural polysaccharides based on origins or sources, shape, charge, chemical and structural features of monosaccharide units, and physiochemical properties.

The generic term “glycose” stands for a monosaccharide, and “glycan” stands for a polysaccharide. On the basis of type of monosaccharides, a polysaccharide can be divided in two parts: homopolysaccharides (homoglycans), which consist of only one kind of monosaccharide such as cellulose or glycogen, and heteropolysaccharides (heteroglycans), which may have more than two or more different kinds of monosaccharides linked with a variety of glycosidic bonds. Most importantly, in heteroglycans, the different kinds of monosaccharide units are arranged with uniform and definite repeating structures, rather than a random fashion. Heparin, a heteroglycan [32], consists of α -L-iodopyranosyluronic acid 2-sulfate and 2-deoxy-2-sulfoamino- α -D-glucopyranose 6-sulfate. The linking pattern of these monosaccharides are either α - or β at the anomeric center. Thus on the basis of linkages, a polysaccharide may be classified as homolinkages if all the glycosidic bonds are α - or β -configuration and as heterolinkages if the bonds are mixing of both α - and β -configurations. Polysaccharides can be linear or have a branched structure. In branched types, there is a variety: a few long branches, branch-on-branch structures formed in clusters, or bush-like structures. In a polysaccharide chain, only one reducing end is present. Thus, a polysaccharide chain can have different sequences of monosaccharide units with a uniform repeating pattern [33], different sequences of glycosidic linkages, and different kinds of branching.

In respect to mass, linear polysaccharides are the most abundant in nature and are found mostly in higher plants, marine algae, and weeds; however, branched polysaccharides have also been found in nature. The degree of polymerization (DP) or degree of polydispersity, i.e., the number of monosaccharides linked in a polymeric carbohydrate, average molecular weight, and range of molecular weights, can vary from source to source because polysaccharides are polydisperse, which means the biosynthetic route of polysaccharides is not a template-basis [34].

In addition to simple monosaccharide units, polysaccharides may contain other functionality such as ester (acetate, phosphate, glycolate, succinate, sulfate, etc.), ether

(methyl and ethyl ether mostly), acetals including amido groups, in which the acid moiety is acetic, glycolic, and sulfuric acids (amino sugars). Polysaccharides may be attached to other important biopolymer protein (protein-polysaccharides if originated from plants and proteoglycans [35] if originated from animals) and lipids as well. Polysaccharides attached to lipids along with O-antigen are called lipopolysaccharides [36] and are found in outer cell membranes in Gram-negative bacteria. Glycans are attached to amino acids in the form of peptides called peptidoglycans, which are mostly present in all bacterial cell walls as structural components [37]. On the basis of charge, polysaccharides can be classified in three categories: neutral, cationic, and anionic forms [38]. The plant kingdom produces a variety of polysaccharides in nature. The animal kingdom, including microbial (fungal and bacterial) and marine algae or seaweeds, are also equally effective to produce polysaccharides with different physiochemical properties [39]. These natural occurring polysaccharides containing a wide variety of structural components that show tremendous applications in biomedicines and tissue engineering owing to their biocompatibility, nontoxicity, biodegradability, and some specific wound healing and drug delivery properties. In industrial applications, generally, natural polysaccharides can be categorized on the basis of sources or origins (Table 1.1), or on the basis of structure and function (Table 1.2), which is illustrated as follows with some selective examples [5].

Table 1.1 Natural polysaccharides originated from various sources

Classification of polysaccharides by source/origin	
Origin	Selective examples with composition
Plant sources	<p><i>Dietary fiber and wood</i></p> <ul style="list-style-type: none"> • Cellulose [β-(1→4) linked D-glucopyranose, linear and homopolysaccharides] • Hemicelluloses [Four classes of structurally different cell-wall polysaccharides including xylans, mannans, β-glucans with mixed linkages, and xyloglucans] • Pectins [α-(1→4)-D-galacturonic acid and rhamnose backbone, arabinose, galactose, xylose side chains, partially O-methyl/ acetylated] • β-Glucans [β-(1→4)-D-glucose and β-(1→3)-D-glucose] • Gums [Galactan, xylan, xyloglucan, glucuronic mannan, galacturonic rhamnosan type] • Inulin • Xylan • Glucomannans • Arabinans • Galactan <p><i>Herbs</i></p> <ul style="list-style-type: none"> • Ginseng polysaccharides [(1→4)-Linked homogalacturonan backbone. (1→2 or 3)-Linked rhamnose on position 4 as a part of backbone or ramified regions. (1→5 or 2)-Linked arabinose with branch points at position 3. (1→3 or 4)-Linked terminal galactose] • Astragalus polysaccharides [α-(1→4)-D-glucan with α-(1→6)-branches]

Table 1.1 Continued

Classification of polysaccharides by source/origin	
Origin	Selective examples with composition
Algae and lichens	<ul style="list-style-type: none"> Green algae sulfated polysaccharides [(1→3 or 6)-linked galactose, (1→3 or 4)-linked arabinose, (1→4)-linked glucose and terminal, (1→4)-linked xylose residues. Sulfations occur on O₆ of galactose and O₃ of arabinose. Sulfate ester content: 9% Brown algae sulfated polysaccharides Fucan: [(1→3)-linked α-fucopyranosyl backbone, mostly sulfated at C4, and branched at C₂ with nonsulfated fucofuranosyl and fucopyranosyl units, and 2-sulfated fucopyranosyl units. Sulfate ester content: 30%–34% Galactan: D-galactopyranose units linked on C₃ and C₆ and sulfation mostly occur on C4. Sulfate ester content: 21%–24% Red algae sulfated polysaccharides Porphyran: Backbone of alternating β-(1→3)-linked D-galactosyl units and α-(1→4)-linked L-galactosyl, (1→6)-sulfate or 3, 6-anhydro-α-L-galactosyl units. Sulfate ester content: 17%
Bacterial and fungal	<ul style="list-style-type: none"> Xanthan Dextran Curdlan Pullan Gellan Baker's yeast glycan
Animal origin	<ul style="list-style-type: none"> Chitin Chitosan Heparin [(1→4)-linked glucuronic/iduronic acid and N-acetylglucosamine disaccharide units with variable 2/3 or 6-O-sulfonation] Chondroitin sulfate [(1→3)-linked glucuronic/iduronic acid and (1→4)-linked N-acetylglucosamine disaccharide units with variable 2/3 or 6-O-sulfonation] Hyaluronic acid

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1.4 IUPAC nomenclature

In sugar chemistry, particularly for polysaccharides, a separate and conventional nomenclature is described in detail at the website: <http://www.chem.qmw.ac.uk/iupac>. In this introductory section, we highlight a simple basis of IUPAC nomenclature for polysaccharides for better understanding, which is discussed below [40]:

- I. In a polysaccharide, each glucose (monosaccharide) ring size is indicated by an italic *f* or *p* for five-membered furanose or six-membered pyranose ring structures, respectively. The term furanose is derived from furan, and pyranose is derived from pyran ring structure,

Table 1.2 Classification of unmodified polysaccharides by structure and functions with examples

Classification by structure (chemical composition)	
Linear molecules	<p><i>Unbranched</i></p> <ul style="list-style-type: none"> • Neutral homoglycans <ol style="list-style-type: none"> 1. Cellulose 2. Laminarans 3. Yeast glucans 4. Cereal β-glucans 5. Amylose 6. Inulins 7. Yeast mannans • Neutral diheteroglycans <ol style="list-style-type: none"> 1. Konjac mannans 2. Agarose component of agar • Anionic/acidic homoglycans <ol style="list-style-type: none"> 1. Lambda-carrageenan 2. Pectins, pectic acids • Anionic/acidic diheteroglycans <ol style="list-style-type: none"> 1. Algins/alginate 2. Kappa-carrageenan 3. Iota-carrageenan • Anionic/acidic diheteroglycans <ol style="list-style-type: none"> 1. Gellan gum • Cationic/basic homoglycans <ol style="list-style-type: none"> 1. Chitosan <p><i>Linear with short branches/side chain units</i></p> <ul style="list-style-type: none"> • Branches irregularly spaced <ol style="list-style-type: none"> 1. Neutral homoglycans <ol style="list-style-type: none"> (a) Fungal (mushroom)-β-glucans 2. Neutral diheteroglycans <ol style="list-style-type: none"> (a) Galactomannans (guar gum, locust bean gum, tara gum) (b) Flour arabinoxylans (c) Larch arabinogalactan 3. Neutral tetraheteroglycans <ol style="list-style-type: none"> (a) Xyloglucans • Branches regularly spaced <ol style="list-style-type: none"> 1. Neutral homoglycans <ol style="list-style-type: none"> (a) Yeast mannan 2. Anionic/acidic triheteroglycan <ol style="list-style-type: none"> (a) Xanthan gum
Nonlinear molecules	<ul style="list-style-type: none"> • Branches in clusters, homoglycans <ol style="list-style-type: none"> 1. Amylopectins • Highly branched/branch-on-branch structures, anionic/acidic <ol style="list-style-type: none"> 1. Tetraheteroglycans <ol style="list-style-type: none"> (a) Gum karayas (b) Okra gum 2. Pentaheteroglycans <ol style="list-style-type: none"> (a) B-type hemicelluloses of cereal brans, etc. (arabinoxylans) (b) Gum arabic (c) Psyllium seed gum

Table 1.2 Continued

Classification by functions	
Cell wall polysaccharides ^a	<ul style="list-style-type: none"> • Higher land plants <ol style="list-style-type: none"> 1. Cellulose 2. Hemicellulose <ol style="list-style-type: none"> (a) Arabinoxylans (b) Galactoglucomannans (c) β-Glucans (d) Glucomannans (e) Mannans (f) Xylans (g) Xyloglucans 3. Pectic polysaccharides <ol style="list-style-type: none"> (a) Arabinans (b) Arabinogalactans (c) Galatans (d) Galacturonans (e) Rhamnogalacturonans • Marine algae <ol style="list-style-type: none"> 1. Algins 2. Cellulose 3. L-fucans 4. Galactans <ol style="list-style-type: none"> (a) Agars (b) Carrageenans (c) Furcellarans 5. β-Glucans 6. Mannans 7. Xylans • Fungi and yeasts <ol style="list-style-type: none"> 1. Cellulose 2. Chitin 3. β-Glucans
Energy storage polysaccharides	<ul style="list-style-type: none"> • Higher land plants <ol style="list-style-type: none"> 1. Fructans 2. Galactans 3. Galactomannans 4. Glucomannans 5. Starches 6. Xyloglucans • Marine algae <ol style="list-style-type: none"> 1. Fructans 2. α-Glucans 3. β-Glucans 4. Xylans

Table 1.2 Continued

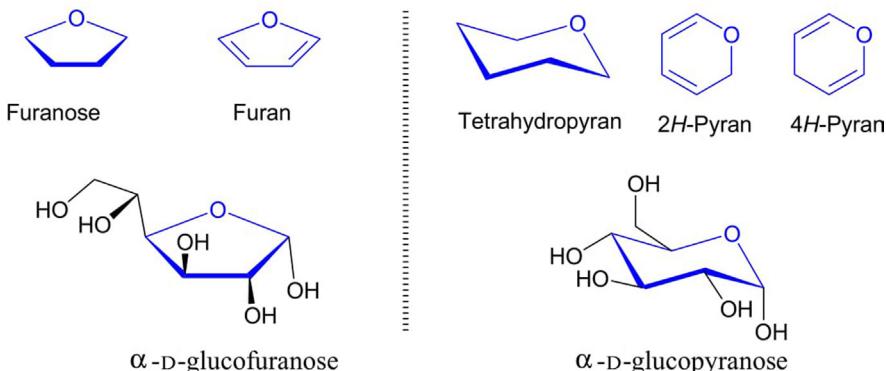
Classification by functions	
	<ul style="list-style-type: none"> • Freshwater algae <ol style="list-style-type: none"> 1. α-Glucans 2. β-Glucans • Fungi and yeasts <ol style="list-style-type: none"> 1. α-Glucans 2. β-Glucans

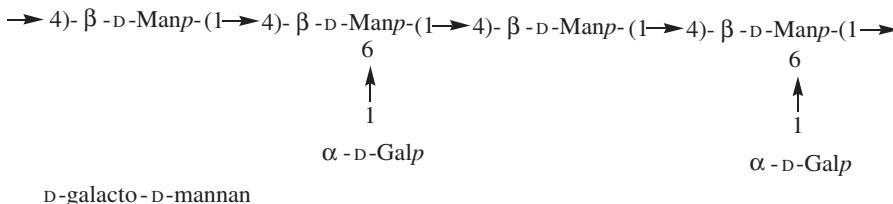
^a Many of those polysaccharides may contain monosaccharide units other than those indicated in the name. For example, xylans often contain uronic acid unit, and L-fucan contains, in addition to main monosaccharide unit L-fucose, D-galactose, D-glucuronic acid, D-mannose, and D-xylose units.

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although both the furanose and pyranose structures have no double bond. The nomenclature for any sugar molecule initiated by anomeric descriptor α - or β - for α/β -anomer and next with the configuration symbols D- and L-configuration with a hyphen is followed by the symbol for a monosaccharide unit (Fig. 1.7).

- II.** A homopolysaccharide (homoglycans) consists of one kind of glycosidic residue and can be depicted by replacing the ending -ose of the glycosidic residue by the suffix -an. For example: xylan for polymers of xylose, mannan for polymers of mannose, and glucan for glucose monomer present both in cellulose and starch.
- III.** A heteropolysaccharide (heteroglycans) consists of two or more kinds of glycosidic residues: a long chain repeating and similar kind of glycosidic units (parent or principal chain). It can be cited at the end by replacing -ose with the suffix -an, with other glycosidic residues written in alphabetical order as glyco- in prefix, if different kinds of monosaccharides present. A heteropolysaccharide, guran composed of D-mannose and D-galactose monosaccharides, where mannose present in a principal chain named as D-mannan at the last and with D-galactose present in side chain named as D-galacto- in prefix.

**Fig. 1.7** Furanose and pyranose cores in a sugar molecule.



- IV.** For an oligosaccharide, glycosidic units are linked with a glycosidic bond and the location of linkages is presented in parenthesis between the symbols by an arrow ($\rightarrow/\leftrightarrow$) indicating the glycosidic linkage in the direction from the anomeric carbon center to the carbon center of the next monosaccharide unit. Single-headed arrow indicates glycosidic linkage from anomeric carbon center (C_1) to the next monosaccharide's other carbon center except anomeric position. Whereas the double-headed arrow indicates the glycosidic linkage of two subsequent monosaccharide units linked by two anomeric centers. Oligosaccharide without a free hemiacetal group i.e., no reducing end is named as glycosyl glycoside. For example, raffinose is a trisaccharide composed of galactose (Gal), glucose (Glc), and fructose (Fru) monosaccharide units. It can be represented as α -D-galactopyranosyl-(1 \rightarrow 6)- α -D-glucopyranosyl- β -D-fructofuranoside, or α -D-Galp-(1 \rightarrow 6)- α -D-Glcp-(1 \leftrightarrow 2)- β -D-Fruf (Fig. 1.8).

V. Linear oligosaccharide consisting of a free hemiacetal group or with reducing end is illustrated with a nonreducing glycosyl unit on the left side and reducing glycosyl unit on the right side in a linear polymeric chain. As a trisaccharide, cellotriose can be represented as β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 4)-glucopyranose or β -D-Glcp-(1 \rightarrow 4)- β -D-Glcp-(1 \rightarrow 4)-D-Glcp (Fig. 1.9).

1.5 Structure determination (ring size and linkage type)

Most polysaccharides except bacterial polysaccharides and some plant-generated polysaccharides are heterogeneous in terms of chemical composition of glycosidic linkages and/or presence of variety of α - or β -linkages at the different positions and other noncarbohydrate

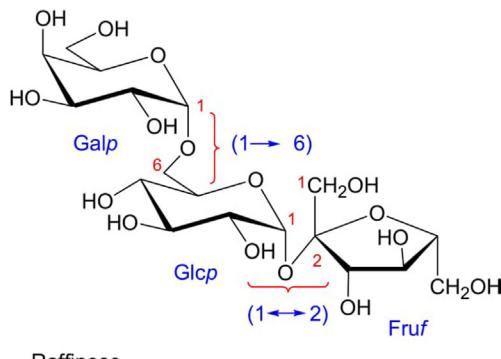


Fig. 1.8 Representation of raffinose polysaccharide

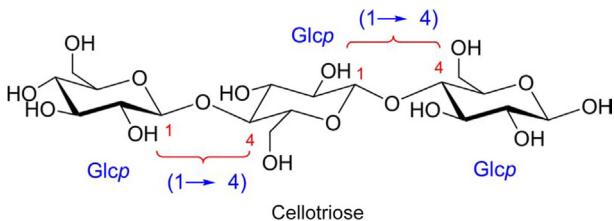


Fig. 1.9 Representation of cellotriose polysaccharide.

building blocks such as protein, polynucleotide, lipids, etc. In addition, the presence of more substitution patterns and linkages in the sugar molecules make it complicated to solve the structure with ease [41]. Therefore, the absolute structure determination of glycans from extracts of plants or a fermentation culture medium of bacteria, obviously, is not an easy task. Both the chemical methods and instrumental methods are used to determine the most probable statistical structure of polysaccharides. At the primary level, various fractional distillations [42], chemical [43–45], or more specific enzymatic methods [46, 47] are adopted in order to achieve information about monosaccharides, linkages' types, ring structures, etc. Finally, various spectroscopic techniques [48, 49] are utilized only after subject to appreciable degree of purity of the sample checked by chromatography. In this section, we discuss the techniques, particularly, various chemical/enzymatic methods to determine the primary structure of the polysaccharides, which are as follows:

- Chemical composition of monosaccharides
- Linkage types

1.5.1 Extraction

In order to get a certain degree of purity of the polysaccharides for the next level structure determination, extraction from the sources, whether from plant polysaccharides or from the fermentation of the culture medium in case of bacterial polysaccharides, is very significant. In the laboratory scale, the extraction from plant polysaccharides begins with replacing the noncarbohydrate portions from the sugar residues, and it can be done by adding simple water or, sometimes, alkaline aq. solution to lipids, proteins, or lignins, etc. Subsequently, new polysaccharides are formed, which again can be subjected to precipitation techniques to achieve the highest degree of purity.

1.5.2 Fractional analysis

In a polysaccharide, the presence of the monosaccharide residues (homoglycan or heteroglycan) can be analyzed by the fractional analysis, which is often carried out in the presence of acid-catalyzed hydrolysis. The monosaccharides, or in some cases in the form of oligopolysaccharides, released upon partial hydrolysis are next evaluated both qualitatively and quantitatively by high-performance liquid chromatography or LC/MS spectroscopic techniques. Sometimes, the linkage-types/anomeric centers (C_1) bonded to what extent of other carbon centers ($C_2/C_3/C_4$ or C_6) of next monosaccharide unit also can be determined by the stability of the glycosidic bonds [42]. For example, in

hexopyranoses, the $1\rightarrow 6$ linkages are comparatively more stable than other linkages ($1\rightarrow 2$, $1\rightarrow 3$ or $1\rightarrow 4$), and α -linkages are less stable than its corresponding β -anomers.

1.5.3 Methylation analysis

In methyl analysis, particularly, the positions of glycosidic linkages and nature of the ring sizes are determined. In this chemical analysis, polysaccharides are fully methylated in the presence of excess methylating agents [50] and then hydrolyzed in the presence of acidic conditions. In hydrolysis reaction, only the hydroxyl groups involved in glycosidic bond formation now become free, except all other hydroxy groups remained methylated. Next, the individual monosaccharide units and/or oligosaccharides are characterized further by spectroscopic techniques to get the information about the position and nature of the linkages. To illustrate this method, a simple lactose disaccharide can be considered. Lactose, a disaccharide is composed of two monosaccharide subunits; D-galactose in acetal form linked with $1\rightarrow 4$ glycosidic bond of D-glucose, which is in hemiacetal form. On exhaustive methylation reaction (Ag_2O , MeI , or Hakomori reagents [50], NaH , MeI in dry DMSO), all the hydroxyl groups in lactose are methylated. Fully methylated lactose is hydrolyzed in acidic condition. On hydrolysis, two acetal linkages hydrolyzed and provided two different products, 2,3,4,6-tetra-O-methylgalactose and 2,3,6-tri-O-methylglucose, which can be characterized by spectroscopic techniques. This provides information about the position of the linkage and nature of the subunits whether in acetal or hemiacetal form (Fig. 1.10).

1.5.4 Periodate oxidation

The stoichiometric HIO_4 -oxidation of vicinal diol (1,2-diol) provided oxidized products aldehyde by cleaving C-C single bond. This technique is employed mostly to determine the ring sizes of monosaccharides subunits present in a polysaccharide. Oxidation of vicinal triol by HIO_4 afforded 1 mole of formic acid and 2 moles of aldehydes (Fig. 1.11). To evaluate the furanose or pyranose ring structure and the

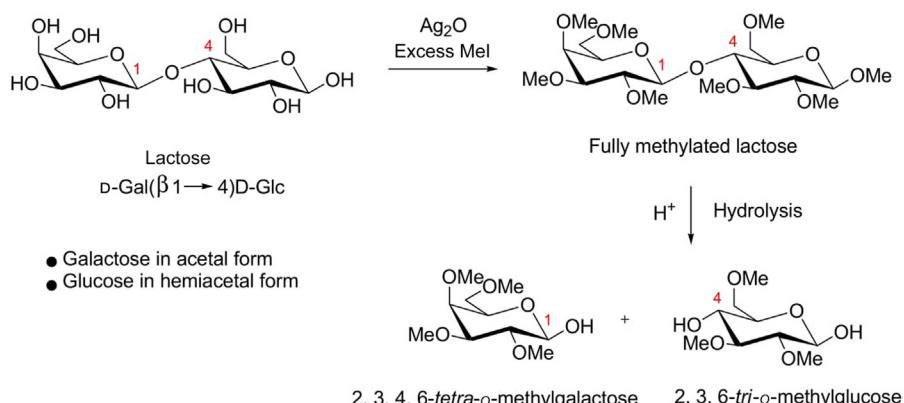


Fig. 1.10 Representative example of methylation analysis of lactose sugar.

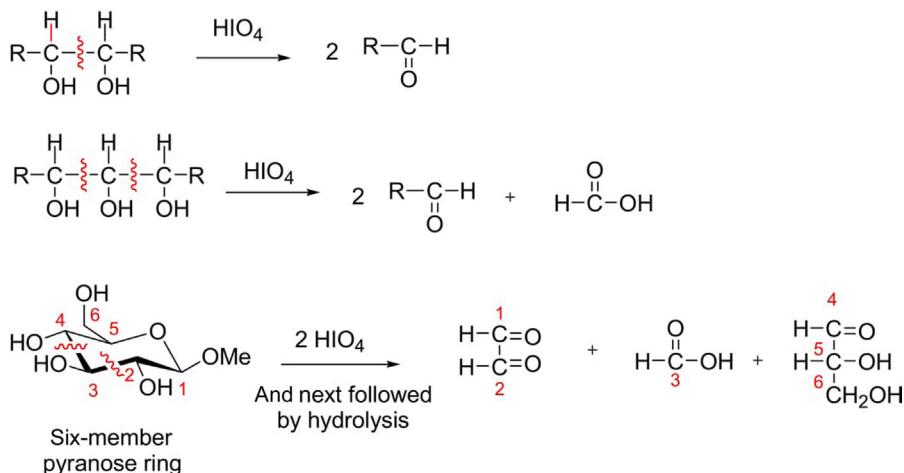


Fig. 1.11 HIO_4 -mediated oxidation analyses in structure determination of polysaccharide.

differentiation among the linkages in a sugar molecule with qualitative and quantitative manner, this technique is very significant ([Table 1.3](#)) [[51](#)].

1.5.5 Enzymatic method

Because of the specificity of the enzymatic reaction in a polysaccharide (glycosidic bond hydrolyzes at a specific position), the enzymatic method is supported to determine the sequence of monosaccharide units [[46](#), [47](#)]. In general, two types of

Table 1.3 Quantitative periodate oxidation of various linked hexopyranoses units

Hexose linkage	Observation per residue
1,2-linkage:	Inner residues Terminal reducing residue
1,3-linkage:	Inner residues Terminal reducing residue
1,4-linkage:	Inner residues Terminal reducing residue
1,6-linkage:	Inner residues Terminal reducing residue

Note: For glycans of hexopyranoses, the terminal nonreducing unit consumes 2-moles of periodate with the formation of one mole of formic acid (C_3).

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glycosidase enzymes (exoglycosidase and endoglycosidase) are utilized for the partial or complete hydrolysis at a specific position ([Table 1.4](#)).

Finally, after completion of chemical/enzymatic methods, the individual monosaccharides, or in some cases, oligosaccharide (partial hydrolysis) are further characterized by the various spectroscopic techniques, such as polarimetric method, mass spectrometric analysis (FAB=fast atom bombardment, MALDI=matrix assisted laser desorption/ionization, ESI=electrospray ionization), nuclear magnetic resonance (^1H -, ^{13}C -NMR; 1D and 2D) analysis, etc., to deduce the most appropriate primary structure of a polysaccharide [48, 49, 52, 53].

1.6 Molecular modification

The natural polysaccharides obtained from different sources have shown a wide range of bioactivities applied to clinical practices or under clinical trials. Since 1943 [54, 55], many research groups have been devoted to finding out biological activities of natural polysaccharides or its molecular level alteration, which includes a variety of chemical, physical, and biological methods. In recent times, the application of these polysaccharides in modern medicinal fields, particularly in the area of tissue engineering, controlled drug delivery and release and wound healing, much more

Table 1.4 Selected enzymes mostly used in glycan structure analysis

Enzymes	Source	Specificity
Exoglycosidases		
α -D-Glucosidase	<i>Saccharomyces cerevisiae</i>	Glc α 1 \rightarrow 4
β -D-Glucosidase	Almond emulsin	Glc β 1 \rightarrow 4
α -D-Galactosidase	Green coffee bean	Gal α 1 \rightarrow 3,4,6
β -D-Galactosidase	<i>Escherichia coli</i>	Gal β 1 \rightarrow 4Glc
α -D-Mannosidase	<i>Aspergillus phoenicis</i>	Man α 1 \rightarrow 2
	Jack bean	Man α 1 \rightarrow 2,3,6
β -D-Mannosidase	<i>Helix pomatia</i>	Man β 1 \rightarrow 4
α -N-acetyl-D-galactosaminidase	Chicken/porcine liver	GalNAc α 1 \rightarrow
α -L-Fucosidase	Bovine epididymis	Fuc α 1 \rightarrow 6,2,3,4
α -D-Sialidase	<i>Archrobacter ureafaciens</i>	NeuNAc α 2 \rightarrow 6,3
	<i>Clostridium perfringens</i>	NeuNAc α 2 \rightarrow 3,6
Endoglycosidases		
α -Amylase	Pig pancreas	↓
	<i>Bacillus amyloliquefaciens</i>	Glc α 1 \rightarrow 4Glc
Endoglycosidase H	<i>Streptomyces plicatus/griseus</i>	↓ (Man)n-GlcNAc-GlcNAc

significantly. In view of the research results, it is evident that the polysaccharides have shown enormous bioactivities in specific manner owing to their own structural features, i.e., the types of glycosidic linkages, solubility [56], degree of polymerization [57], and stereochemistry [58]. The inbuilt properties of natural polysaccharides, such as bonding type (glycosidic bond), anomeric nature (α - or β), and chemical composition of monosaccharides, are not changeable for augmenting the bioactivities. Although, in the laboratory, some polysaccharides can be synthesized to change these natural properties with the enhancement of bioactivities [15–17]. On the other hand, the rest of the area, particularly, the substituent groups attached at various positions, can be easily functionalized by modification, which mainly includes chemical, physical, and biological modification [18].

Perhaps, the chemical modification is the most acceptable and widely implemented method for the modification or functionalization of polysaccharides besides physical and biological modification techniques, in order to increase the highest level of biological activities. In this molecular modification, a variety of well-studied chemical methods is reviewed. Specific reagents and reaction conditions with general mechanism of chemical methods which include etherification (alkylation, hydroxyalkylation, carboxymethylation, sulfoalkylation etc.), esterification (acetylation, sulfation, phosphorylation etc), selenylation, amine alkylation and others, are discussed in this section (Fig. 1.12) [18].

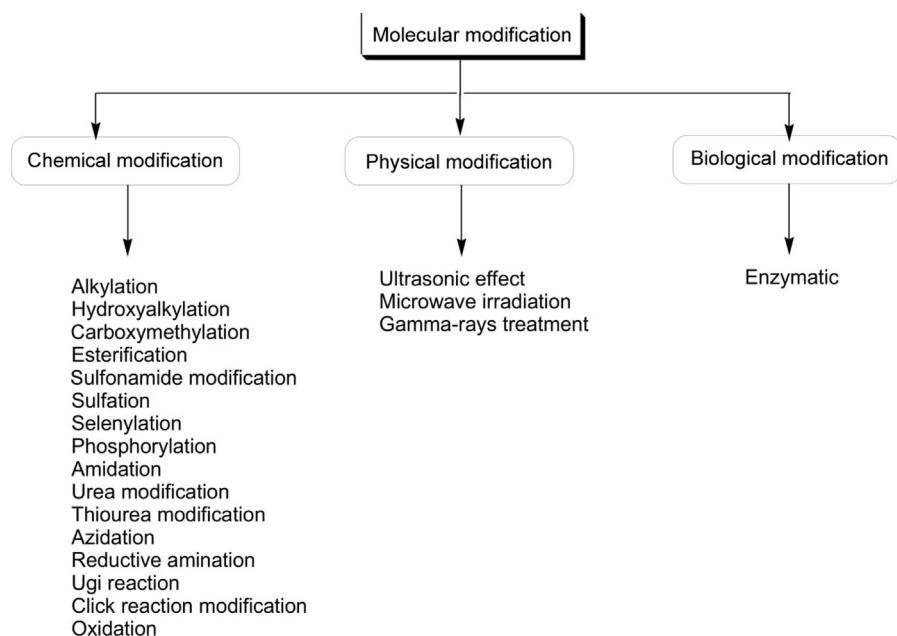


Fig. 1.12 Schematic representations of different types of modification techniques.

1.6.1 Chemical modification

1.6.1.1 Hydroxyl group modification

Etherification

Natural polysaccharides used as alcohol in chemical modification plays an important role, and the resultant modified polysaccharides can be utilized in various biomedical applications. Mostly, the study of the polysaccharides like cellulose, starch, arabinogalactan, and pullan consisting only hydroxyl groups is extensively investigated. Halogenated alkane (alkyl halide) under basic conditions is mostly investigated for simple alkylative modification [59, 60]. Hydroxyalkylation reaction is generally carried out using oxirane as an electrophile under basic condition [60]. In this etherified modification, the basic condition is employed in most of the cases for the alkylation via ring opening of oxirane from less steric hindrance side. Carboxymethylation reaction is also carried out by mono chloroacetic acid, which yielded anionic materials used for ion exchange applications. This reaction is generally carried out in organic solvent or aqueous medium. Some reports on successful carboxymethylation of cellulose [61, 62] and chitin [63] polysaccharides have shown better solubility in water and enhanced bioactivities. In addition, the etherification modification with sulfoalkylating agents [64] has also been investigated for the same purposes. The cationic ion exchange resins of cellulose can be prepared by etherification modification employing 2-chloroethyl diethylamine [65]. Silylation of hydroxyl groups in a polysaccharide utilizing trimethylsilyl chloride (TMSCl) or hexamethyldisilazane reagents afforded trimethylsilyl ether, which can be used to increase hydrophobicity of the polysaccharides [66]. Most importantly, in the etherification modification, the electrophile, such as epichlorohydrin consisting bis-electrophilic centers, can be used in order to functionalize in second times. Initially, the hydroxyl group of the polysaccharide reacts with epoxide to form ring-opened product (halohydrin) that subsequently removes HCl to form new oxirane product [67]. Further, various nucleophiles (N-, S-, O-, and carbon nucleophiles) react with epoxide-derived new modified polysaccharide, which gave additional functionalization to the polysaccharides for better bioactivities (Fig. 1.13).

Esterification

In 1988, Mizumoto et al. first installed sulfate group to a monosaccharide structure and observed that the sulfated modified structure inhibits the T lymphocyte virus [68]. After that, many research groups are engaged to modify sugar molecules with sulfating agents for the improvement of bioactivities. The most commonly used methods for sulfated esterification are chlorosulfonic acid-pyridine (CSA-pyridine) [69] and amino sulfonic acid-pyridine method (ASA-pyridine) [70]. Besides that, the other methods such as SO_3 -pyridine [71] and oleum-DMF [72] etc. are also employed to sulfate the hydroxyl groups of the polysaccharides.

Judging by the recent trends, phosphorylation of sugar molecules and its bioactivities investigation is very rare, most probably owing to the very limited number of

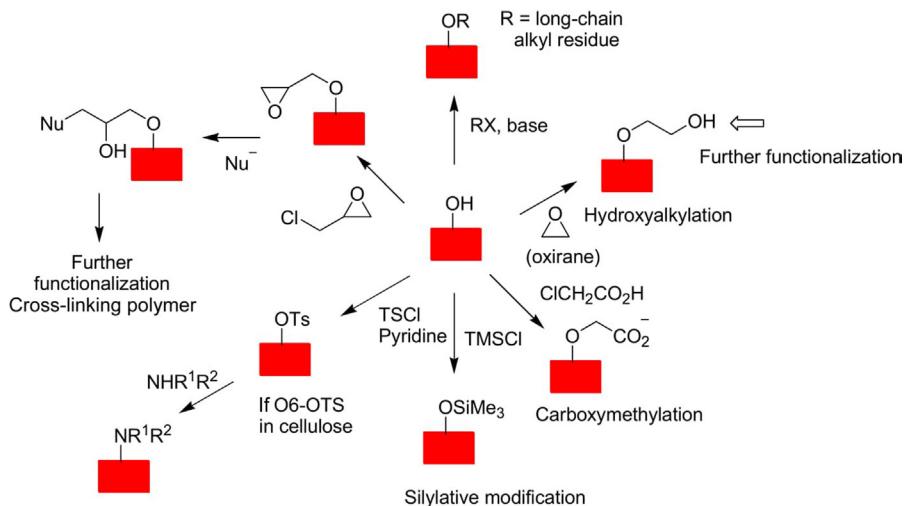


Fig. 1.13 Various chemical modifications with hydroxyl group in a polysaccharide.

natural phosphated glycosidic units present in nature [73]. In synthetic point of view, only a few reagent systems, such as simple phosphoric acid or phosphoric anhydride under acid condition in DMSO solvent, POCl_3 and salts of phosphoric acids, are considered to introduce the phosphate functionality to a polysaccharide. By introducing the phosphate group, it has been proven that modified phosphorylated polysaccharides showed specific bioactivities owing to the presence of charged phosphate groups [74, 75]. In addition, this modified polysaccharide can improve the properties related with solubility, molecular weight and change, and sometimes the structural parameters.

Selenylated modification to polysaccharides is quite important as selenium (Se) plays a significant role in the reduction of hydrogen peroxide catalyzed by glutathione peroxidase and also acting as a radical scavenger in the living system [76]. Although inorganic selenium compounds are toxic, selenylated polysaccharides, an organic selenium compound, can be treated in a safe mode, and both Se and polysaccharide synergistically works together [77, 78]. This modification can be successfully completed by using selenious acid or selenite in the presence of strong acid conditions.

Acetyl modified polysaccharides, mainly, the side chain residue or in some cases ring hydroxyl groups to a polysaccharide are acetylated in the presence of acetic anhydride as reagent in water or DMSO solvents. The acetylated polysaccharide showed a greater level of the solubility in water medium, and so it has shown better biological activities [79].

1.6.1.2 Amine group modification

Judging by the recent trends, amine functionalization of mainly chitosan and chitin polysaccharides and, after that, resultant modified biopolymer's usage, mainly in pharmaceutical, biomedical, and biotechnological fields, has been gradually increased [80, 81]. Various methods such as oligomerization, *N*-alkylation, and followed by

quaternization, reductive amination (condensation reaction with aldehydes and next reduction), *N*-carboxymethylation, *N*-acylation including azidation, urea or thiourea formation by reaction with -NCO or -NCS, and many more are well-studied in the literature [19, 82, 83]. Some methods of amine functionalization are presented in Fig. 1.14.

1.6.1.3 Carboxylic group modification

Polysaccharides, such as hyaluronic acid (HA), and alginic acids can be modified by a variety of reactions strategy based on free carboxylic acid group or prefunctionalized activated ester group [83–86]. Among them, Ugi condensation reaction (Figs. 1.15 and 1.16), amidation, and esterification (Fig. 1.17) including ring opening reaction with substituted oxiranes, hydrazination, etc. are important. The carboxylic acid is activated by mostly EDC or DCC reagent for esterification; whereas, NHS or HOBT reagent is for amidation modification (Fig. 1.18).

1.6.1.4 Click chemistry

In addition, the most significant modification of polysaccharides investigated, so far, is “click chemistry”. This reaction involved Huisgen 1,3-dipolar cycloaddition between azide and terminal alkyne in the presence of Cu-salt [87]. Both the preinstalled azide group, mostly at the C₆-position of sugar molecule, and external terminal alkyne,

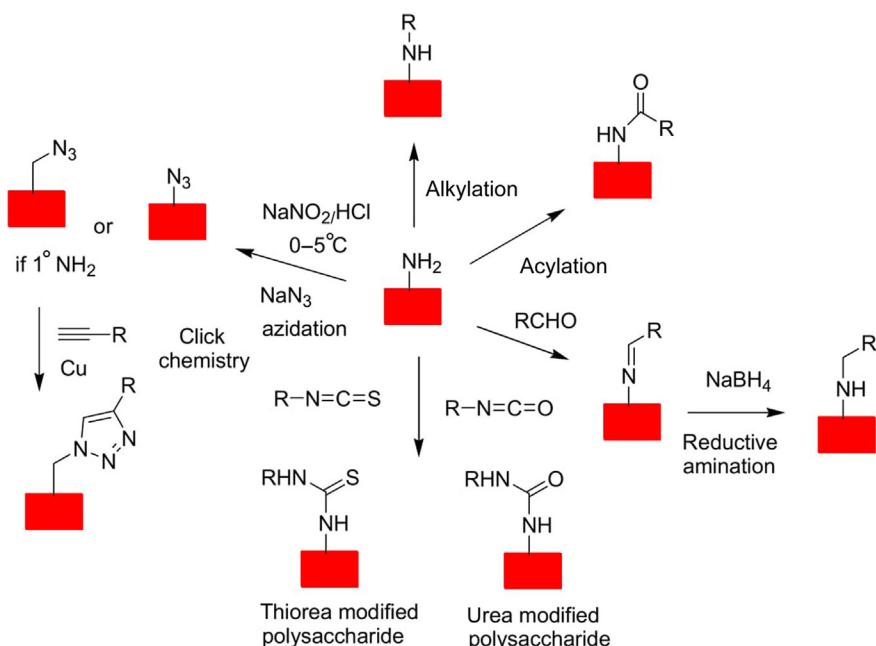


Fig. 1.14 A variety of techniques adopted for the amine group modifications. ART: Delete the unwanted arrow

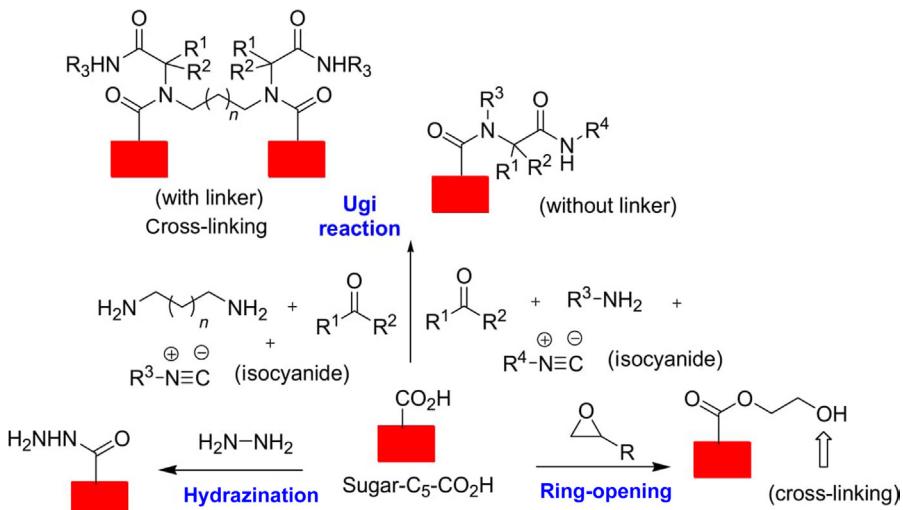


Fig. 1.15 Ugi reaction, hydrazination, and epoxide ring-opening reactions.

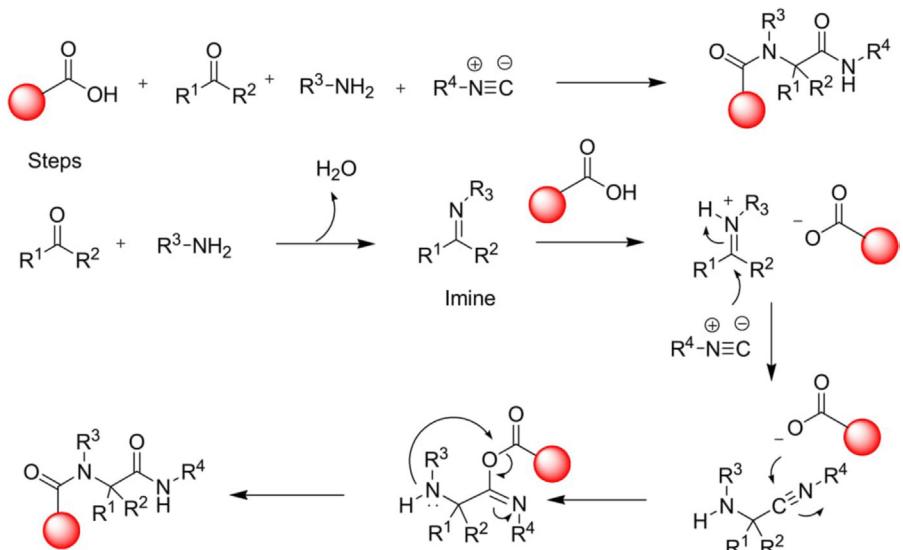


Fig. 1.16 Mechanism of Ugi reaction.

or vice-versa, are involved in this reaction. To introduce the azide functionality, in general, the hydroxyl group is tosylated, a good leaving group, and next using sodium azide, a polysaccharide is azidated via S_N2 displacement reaction [88]. Finally, azidated polysaccharide reacts with externally added terminal alkyne in presence of Cu-slat as Lewis acid gives the triazole-modified polysaccharide at C₆-carbon center. Alternately, the free hydroxyl group can be propargylated, first under basic conditions

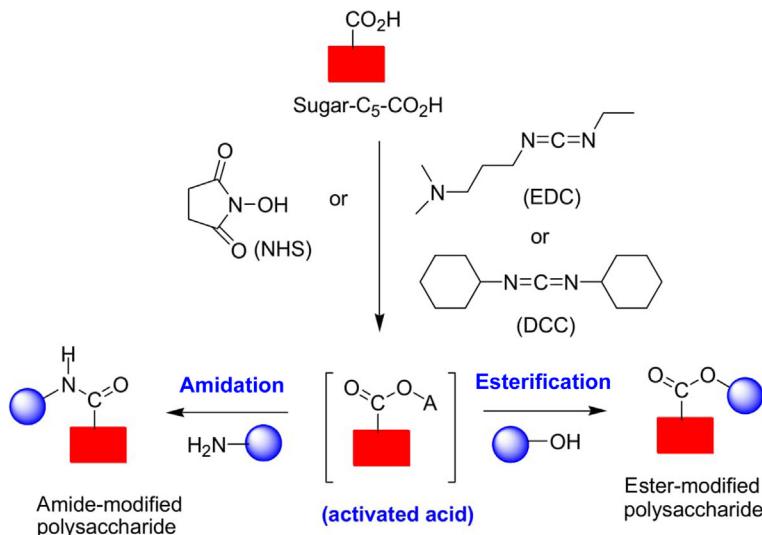


Fig. 1.17 Esterification and amidation of polysaccharides consisting of acid functionality.

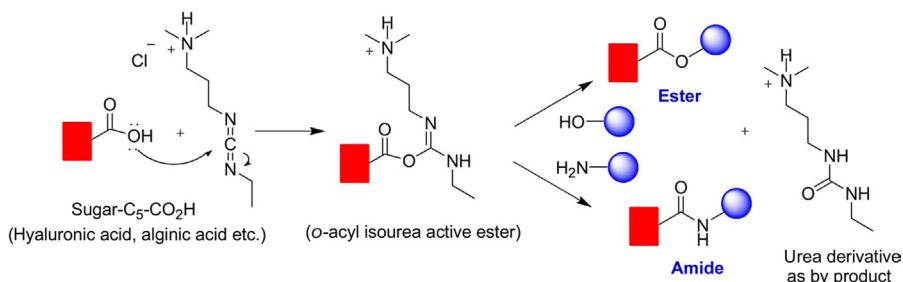


Fig. 1.18 Mechanism of EDC/DCC or NHS-coupling reaction.

and followed by click reaction with externally added azide; this could provide the same triazole-based polysaccharide. For chitosan, azide functionality introduced by replacing amino functionality at the C₂-position by diazotization step and followed by reacted with sodium azide [83]. Similarly, azidated chitosan reacts with terminal alkyne to afford triazole-modified chitosan. Here the mechanism of click chemistry [89] and “click-chemistry”-induced modification of polysaccharides is illustrated in Fig. 1.19.

As an example, both the two differently functionalized hyaluronic acid derivatives prepared by an esterification reaction, react in aqueous medium to afford a new cross-linked hyaluronic acid via a “click” reaction, even in the absence of Cu-catalyst (Figure 1.20). The new cross-linked polymer has lost the hydrophilic properties and forms a very rigid hydrogel due to incorporation of triazole-ring substituted cyclooctane ring as a linker between the hyaluronic acids chain [90].

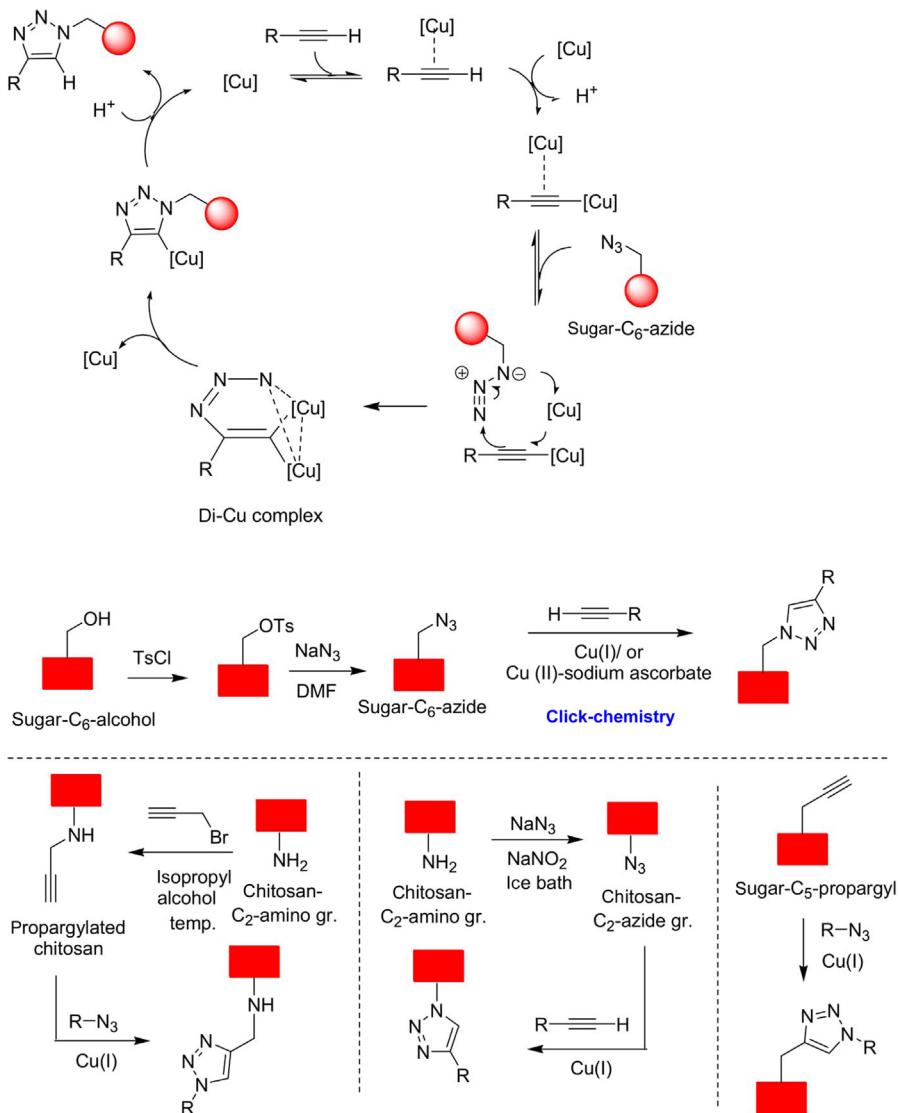


Fig. 1.19 General reaction mechanism of click-chemistry and a variety of Cu(I)-mediated polysaccharide modifications.

1.6.1.5 Oxidation in modification

Dextran and arabinogalactan are frequently encountered polysaccharides for conjugation via oxidation reaction employing external oxidants NaIO₄ [91] and peroxide-mediated if vicinal diol (C2-C3-diol). For the C6-OH position, DMP-oxidation and TEMPO [92] are utilized. Next, oxidized products of polysaccharides (aldehydes) react with a variety of polyamines or amine-group containing polysaccharide and

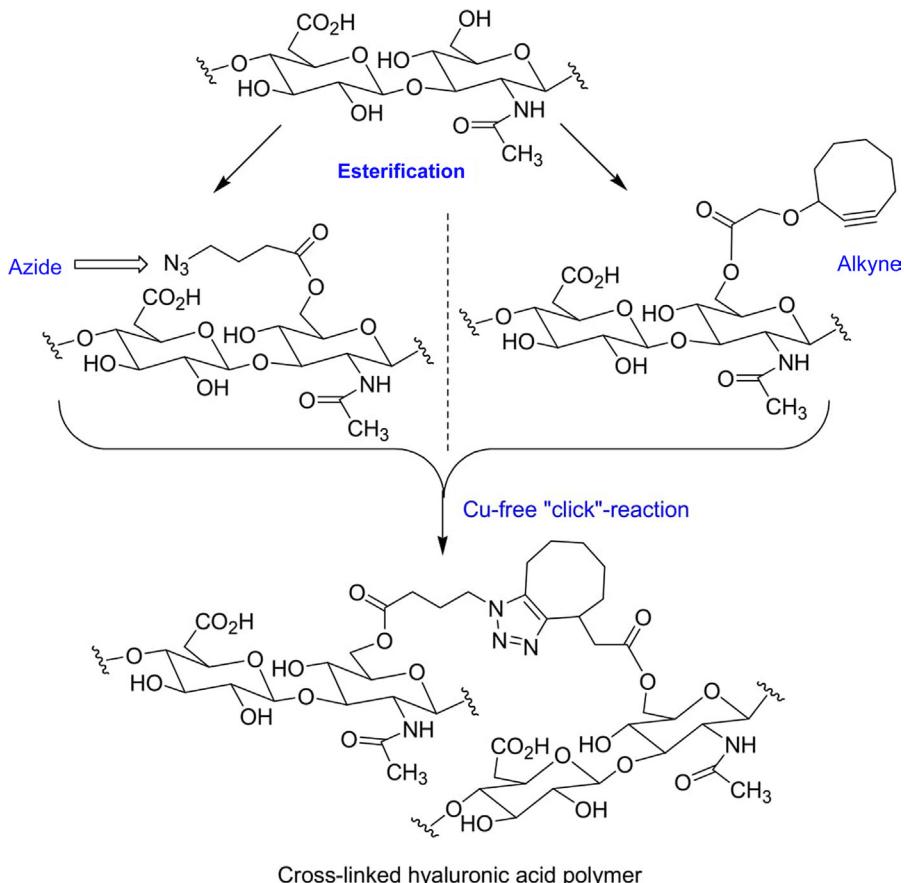
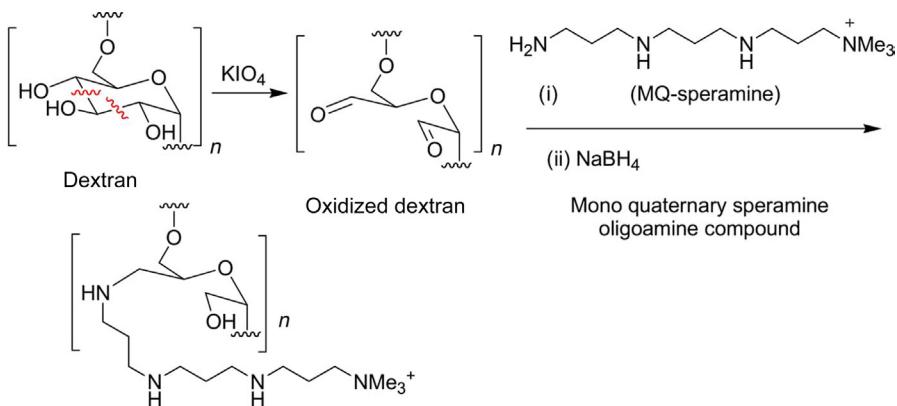


Fig. 1.20 “Click”-reaction in hyaluronic acid for making rigid hydrogel. ART: Delete (XXC)

followed by reductive amination generates a self assemble cationic core in aqueous solution. This kind of polysaccharide, which conjugates with cationic core such as dextran-spermine, is useful in gene delivery (Fig. 1.21) [93].

1.6.2 Physical modification

The high molecular weight containing polysaccharides have difficulties exerting a certain level of pharmacological effects for better bioactivities as it would be unfavorable for bioactive polysaccharides to penetrate multiple cell membrane barriers exhaustively. Disconnect the chain length of the natural bioactive polymers without alteration of the composition of monosaccharides or decomposition of main chain by applying physical methods. The physical methods, which mainly include ultrasonic disruption [94], microwave exposure [95], or very powerful radiation-induced treatment [96] to polysaccharide long chain with high molecular weight, may be fragmented. The



Oxidized dextran with cationic polysaccharide

Fig. 1.21 Oxidized dextran conjugate with oligoamine derivatives (MS-Q-Speramine).

lowering of chain length obviously increases the solubility, and a variety of pharmacological properties helps to increase the bioactivities of shorter chain length containing modified polysaccharides. Many natural polysaccharides, such as cellulose, starch, and hyaluronic acid including chitosan, can be modified by applying γ -radiation and, consequently, their solubility level increased satisfactorily [97, 98].

1.6.3 Biological modification

The enzymatic degradation of polysaccharides is known as biological modification, which can be applied more specifically at the target area to degrade the polysaccharide backbone compared to other physical and chemical methods owing to high specificity of enzyme actions. The enzymatic modification of polysaccharides is only applicable to a certain type of polysaccharides only, and there is a huge scope to develop this particular area and, consequently, to increase the bioactivities [17, 99].

1.7 Conclusion

We highlighted a few basics of natural polysaccharide chemistry, such as natural occurrence, classification, structural components, etc. A simple chemistry, starting from monosaccharide to oligosaccharides and finally ending with polysaccharides, is described. The biochemical reactions in monosaccharides are presented with figures for the better understanding. Next, various important chemical and enzymatic methods are described to analyze the primary structure of polysaccharides. Natural polysaccharides obtained from microorganisms, plants, and animals show a broad spectrum of bioactivities, such as antioxidative, immune regulatory, antiinflammatory, anti-HIV, antitumor, and anticancer activities. Sometimes, modified natural polysaccharides also have shown greater bioactivities compared to natural ones. With this

view, various molecular modifications, which include chemical, physical, and biological modifications of natural polysaccharides, are illustrated in detail. Some of the chemical modifications are discussed with reaction mechanism in detail for deeper knowledge. Overall, fundamental knowledge about polysaccharide chemistry, which includes origin, classification, and structural parameters, is attempted in this chapter.

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