Introduction

Polysaccharides are naturally occurring polymers in which the repeat unit consists of monosaccharides linked through glycosidic linkages by a condensation type reaction; the example of cellulose is given in Figure 1. They exist in plants, animals, or microbial worlds where their roles as energy storage or structural materials or as source of biological activity are recognized. Many general books published can be taken into consideration (1–13).

Monosaccharides are most readily obtained from natural sources; in that respect D-glucose plays a central role in the biochemistry of carbohydrates.

In addition, because of the presence of —OH groups, natural polysaccharides may be modified by controlled chemical reaction to give derivatives with new specific properties; industrial derivatives are mainly obtained from cellulose (qv), starch (qv), and chitin and chitosan (qv). The presence of these —OH functional groups is also the origin of interaction with water molecules (hydrophilic character of oligo- and polysaccharides) and intra- and interchain H-bond network formation playing a role in reactivity control, swelling, or dissolution rate.

This article describes the structure of the principal polysaccharides from natural sources, some methods for their characterization, and some physical properties. Few derivatives are described and this article is extended to synthetic polymers having a sugar entity in the basic structure.

Carbohydrate Nomenclature

The international rules of carbohydrate nomenclature adopted by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry have been published (14). A brief summary of the structural basis for naming oligo- and polysaccharides is given in the following.

Structure of Monosaccharides. Monosaccharides (general formula $C_n(H_2O)_n$) are the basic constitutional units of oligo- or polysaccharides. A monosaccharide is a polyhydroxycarbonyl compound classified as tetrose, pentose, hexose, etc, according to the number of carbon atoms in the molecule; a prefix indicates the nature of the carbonyl group which is an aldehyde or a ketone. Thus, aldohexose has six carbons and one end of the molecule (position 1) has an aldehyde group with a reducing character. If the reducing group is a ketone in the second position, a six-carbon chain is called a ketohexose (Table 1) (9,10).
Fig. 1. Cellulosic chain formed from condensed D-glucose.

Fig. 2. The two tetrahedral representations of glyceraldehydes.

**Configuration of Monosaccharides.** Different representations were proposed to show the structure of the monosaccharide. The original Fischer representation allows description of different structures and especially demonstrates the chiral relation between monosaccharides, eg, that D-galactose is the C-4 epimer of D-glucose.

The chirality of monosaccharides is related to the presence of asymmetric carbon in the molecule; aldohexose contains four asymmetric carbons (see Table 1) and consequently 16 stereoisomers can be described.

The first member of the polyhydroxycarbonyl series is the aldotriose glyceraldehyde which contains one asymmetric carbon, and thus two stereoisomers. Figure 2 shows the two different molecules, which are mirror images of each other, in the tetrahedral representation with the asymmetric carbon in the center. The projection on a plane of these molecules corresponds to the Fischer representations (Fig. 3). Initially, one of the forms was found to be dextrorotary (+) and was named

| Table 1. Acyclic Forms of D-Aldoses and D-Ketoses* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Trioses         | Tetroses        | Pentoses        | Hexoses         |
| Aldoses         | CHO             | CHO             | CHO             | CHO             |
|                 | *CHOH           | *CHOH           | *CHOH           | *CHOH           |
|                 | CH₂OH           | CH₂OH           | CH₂OH           | CH₂OH           |
| Ketoses         | CH₂OH           | CH₂OH           | CH₂OH           | CH₂OH           |
|                 | C=O             | C=O             | C=O             | C=O             |
|                 | *CHOH           | *CHOH           | *CHOH           | *CHOH           |
|                 | CH₂OH           | CH₂OH           | CH₂OH           | CH₂OH           |

* Asterisk (*) indicates the presence of asymmetric carbons.
D-glyceraldehyde; the other one was levorotary (−) and called L-glyceraldehyde. From the triose, in the Fischer representation, a series of stereoisomers are generated as indicated in Figure 4. The D-series refers to molecules in which the −OH on the last asymmetric carbon is on the right. The D- or L-identification does not mean that it is dextro (+) or levo (−) (Rosanoff convention). Physical investigation, later after Fischer, concluded that the D- and L-attribution represents the absolute configuration as demonstrated for (+)-D-glyceraldehyde. The majority of natural monosaccharides involved in polymeric systems belongs to the D-series.

**Cyclic Conformation of Monosaccharides.** The crystalline form of glucose was shown as cyclic (six-membered, oxygen-containing ring) from X-ray diffraction (15); infrared spectroscopy detects no carbonyl group, confirming a
cyclic hemiacetal structure. In the hemiacetalic form, the carbonyl carbon changes from sp² to sp³ hybridization and thus becomes chiral with two anomic forms (α and β) on carbon C-1 as shown in Figure 5. Among common hexoses, six-membered (pyranose) and/or five-membered (furanose) rings can be formed. Haworth introduced more realistic pictures of the cyclic forms (Fig. 6). The representation ∼OH means that the two configurations α and β exist in equilibrium. For the D-series, the α-anomer has the hydroxyl at the anomic center (position 1) downwards in the Haworth representation; the β-anomer is upwards.

Considering the six-membered ring, different conformations can be distinguished, taking as reference the cyclohexane. The favored conformations are chairs in the 4C₁ and 1C₄ conformations. The representation of α-D-glucose is
Fig. 8. The different representations for D-glucose in the α conformation for the cyclic form: (a) Fischer, (b) Haworth, (c) 4C1 chair corresponding to the energy minimum.

given in Figure 7; the 4C1 conformation, with all but the OH at C-1 in the equatorial orientation, is preferred compared with the 1C4 conformation. In the α-D geometry, the anomeric hydroxyl (on C-1) is axial but it is equatorial in the β-D conformer.

For D-aldopyranose in general, the conformation 4C1 is usually preferred. A summary of the different representations of D-glucose and the most probable conformation of α-D-glucose are given in Figure 8.

Mutarotation in Solution. In solution, equilibrium exists between the linear conformation and the two anomeric forms of pyranose and furanose cyclic hemiacetal forms (Fig. 9); the percentage of each form as well as the ratio α/β is characteristic of the monosaccharide considered.

A few values of the percentage at equilibrium are given in Table 2. Because of this equilibrium the reducing nature of the sugar is maintained.

1H NMR in D2O allows characterization of the anomeric contents in equilibrium after a short time at 25°C; an example is given in Figure 10: the H-1

Fig. 9. Mutarotation in solution: representation of the different species in equilibrium for D-glucose (see Table 2).
Table 2. Conformation Equilibrium for Some Monosaccharides

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T°C</th>
<th>α-Pyran, %</th>
<th>β-Pyran, %</th>
<th>α-Furan, %</th>
<th>β-Furan, %</th>
<th>Acyclic, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>31</td>
<td>60</td>
<td>35.5</td>
<td>2.5</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>Glucose</td>
<td>31</td>
<td>38</td>
<td>62</td>
<td>—</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Galactose</td>
<td>31</td>
<td>30</td>
<td>64</td>
<td>2.5</td>
<td>3.5</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Reference 10.

The signal corresponding to the α-anomer is located at 5.12 ppm with a narrow doublet ($J = 3.7$ Hz) but the signal for the β-anomer is located 0.6 ppm upfield with a coupling constant $J = 7.9$ Hz. $^{13}$C NMR also allows identification of the equilibrium in solution; in addition, no C=O signal is seen, confirming the cyclic configuration. The NMR characteristics given for D-glucose in water are recalled in Table 3 (16,17).

From this example, it is shown that the coupling constants and the location of the signals allow identification of the nature of the sugar and its configuration (16–19). The NMR technique is one of the most powerful tools for characterizing monosaccharides but also for establishing the structure of polysaccharides. The anomeric equilibrium only affects the reducing end of the chain; for a high molar mass, the spectrum is not perturbed by this effect and only one series of signals appears.

**Uronic Acid and Other Monosaccharides.** Formation of a uronoside requires the oxidation of the primary hydroxyl group of a hexopyranoside. This type of unit is very important in many natural polysaccharides such as alginates, pectins, or some hemicelluloses. Some important monosaccharides are represented in Figure 11.

Some chemical or enzymic methods are recognized to allow the specific oxidation of primary hydroxyls in the C-6 position. The TEMPO method applied to polysaccharides was used to oxidize the N-acetylglucoamine unit in hyaluronan (20); galactose oxidase was used to modify the galactose side groups in galactomannan (21,22).

Table 3. NMR Spectrum Characteristics of D-Glucose in D₂O

<table>
<thead>
<tr>
<th>δ (ppm)</th>
<th>H-1</th>
<th>H-2</th>
<th>H-3</th>
<th>H-4</th>
<th>H-5</th>
<th>H-6</th>
<th>H-6'</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>5.09</td>
<td>3.41</td>
<td>3.61</td>
<td>3.29</td>
<td>3.72</td>
<td>3.72</td>
<td>3.63</td>
</tr>
<tr>
<td>β</td>
<td>4.51</td>
<td>3.13</td>
<td>3.37</td>
<td>3.30</td>
<td>3.35</td>
<td>3.75</td>
<td>3.60</td>
</tr>
<tr>
<td>$J$ (Hz)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α</td>
<td>3.6</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
<td>2.8</td>
<td>5.7</td>
<td>12.8</td>
</tr>
<tr>
<td>β</td>
<td>7.8</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
<td>2.8</td>
<td>5.7</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>C-1</td>
<td>C-2</td>
<td>C-3</td>
<td>C-4</td>
<td>C-5</td>
<td>C-6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>δ (ppm)</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>92.9</td>
<td>72.5</td>
<td>73.8</td>
<td>70.6</td>
<td>72.3</td>
<td>61.6</td>
</tr>
<tr>
<td>β</td>
<td>96.7</td>
<td>75.1</td>
<td>76.7</td>
<td>70.6</td>
<td>76.8</td>
<td>61.7</td>
</tr>
</tbody>
</table>

*Measured at 400 MHz in D₂O at 296 K relative to acetone (2.12 ppm) 16.

*Given in Table 1, Ref. 17.*
Important monosaccharides are the amino sugars which have an amino group at any position other than anomeric carbon: 2-amino-2-deoxy-D-glucose or D-glucosamine also called chitosamine is the monomeric unit constitutive of chitosan, the polymer obtained by deacetylation of chitin which is based on the N-acetyl derivative of D-glucosamine (see Fig. 11). The sugar 2-amino-2-deoxy-D-galactose is found in dermatan and chondroitin sulfate, constituents of mammalian tissues and cartilage.

Oligosaccharides. These oligomers result from partial acid or enzymatic hydrolysis of polysaccharides and they are prepared for structural analysis of polysaccharides. Few disaccharides exist naturally, or are produced by enzymes;
Fig. 11. Representation of some monosaccharides involved in most of the polysaccharides.
the linkage between two monosaccharides is an acetal bond also called an osidic bond. The acetal bond imparts a nonreducing character to the anomeric carbon. For common di- or trisaccharides, trivial names are normally used instead of systematic names. The nonreducing elements of an oligosaccharide are designated by the suffix -yl. In the case of nonreducing disaccharides the monomers are listed in alphabetical order.

Examples of reducing disaccharides are such as lactose and maltose. Lactose occurs in milk of mammals and is named systematically as β-D-galactopyranosyl-(1→4)-D-glucopyranose or 4-O-β-D-galactopyranosyl-D-glucopyranose and abbreviated Galp-β-(1→4)-Glc (the italic p indicates the pyranoid form). Maltose, obtained by hydrolysis of amylose, is named (4-O-α-D-glucopyranosyl-D-glucose or α-D-glucopyranose-(1→4)-D-glucose). The -ose suffix in the systematic name denotes a reducing disaccharide, eg, one with one anomeric carbon in a hemiacetal form. Sucrose or saccharose is a nonreducing disaccharide. It is extracted from sugarbeet or sugar cane, is named β-D-fructofuranosyl-α-D-glucopyranoside, and abbreviated as β-D-Fruf-(2→1)-α-D-Glc. The biosynthesis and structure of disaccharides are developed in Reference 11. The -ide suffix denotes a nonreducing disaccharide wherein both anomeric carbons are in the acetal linkage.

In Figure 13, the 13C NMR spectra of cellobiose (β-D-Glc p-(1→4)-D-Glc) (Fig. 12a) and maltose (α-D-Glc p-(1→4)-D-Glc) (Fig. 12b) are given; the anomeric equilibrium of D-glucose at the reducing end is demonstrated (23); the signals corresponding to the C-1 of the nonreducing D-glucose (indicated as ′) are located at a different chemical shift depending on the anomeric configuration: C-1′β is at 105.3 ppm (cellobiose) but C-1′α is at 102.5 ppm (maltose) (in D2O at 60°C). NMR spectroscopy data are given in the literature for mono- and oligosaccharides (16–19).

Cyclodextrins (qv) represents a series of cyclic α-(1→4)-linked D-glucopyranose oligomers obtained by enzymic degradation of starch using a cyclodextrin glucotransferase (11). The most common cyclodextrins are called α, β, γ cyclodextrins with 6, 7, 8 sugars respectively resulting in intrasaccharide α-1,4-glycosidic bonds.

They have a toroidal structure with hydrophilic exteriors and a hydrophobic cavity in which hydrophobic organic compounds can be trapped.

They are used to stabilize vitamins against temperature or increase solubility of pharmaceutical products. They also show an interesting selectivity in relation with the dimensions of the cavities (Fig. 13).

Fructosans are oligomers or polymers of β-D-fructofuranose and belong to the inulin-type ((2→1)-β-linked) or to the phlein-type (2→6 linked); inulin oligomers are found in Jerusalem artichoke or dahlia tubers where they are carbohydrate reserves. They are usually low molecular weight polymers (degree of polymerization, DP ≤ 50). The high yield in fructosans in Jerusalem artichoke may be considered as a source of fructose for the food industry. The juice extracted from grounded tubers was isolated and studied by liquid chromatography (HPLC). The oligosaccharide distribution was obtained by size exclusion chromatography (SEC) and by normal-phase HPLC (24–29). After separation of the different constituents and NMR analysis, it is concluded that the juice consists of an oligomer series formed of oligo-β-fructofuranosyl units (2→1) ended by one α-D-glucopyranosyl unit (Fig. 14).
Fig. 12. $^{13}$C NMR of (a) cellobiose and (b) maltose at 60°C in D$_2$O at 62.86 MHz. The anomeric equilibrium is clearly observed on the C-1 position. Reproduced from Ref. 23, with the permission of John Wiley & Sons (Copyright 2003).
**Fig. 13.** Representation of the α-cyclodextrin (six glucose units) in the cyclic and toroidal forms showing the hydrophobic cavity and the hydrophilic faces.

**Oligosaccharins.** It has been demonstrated that specific oligosaccharides are used to control biological processes in plant. Albersheim who was a pioneer in this work recognized that specific oligosaccharide components of plant cell walls (named oligosaccharins) can have biochemical activity which results in regulatory response (30). α-(1→4)-linked D-galacturonic acid oligosaccharides obtained from pectins have regulatory effects on plant growth and development.

In connection with the biological activity of mono- and oligosaccharides, it is important to mention plant lectins: lectins are proteins possessing at least one noncatalytic domain which binds reversibly to specific mono- and oligosaccharides (31,32); lectins are carbohydrate-binding (glyco)proteins of nonimmune origin capable of specific recognition of carbohydrates and reversible binding to them, without altering their covalent structure. Many of plant-based food ingredients contain lectins, some with striking biological activities.
**Glycans.** The generic name for polysaccharides is glycan using the suffix “an.” The names of polysaccharides should be D-glucan, or D-mannan for homopolymers constituted of D-glucose or D-mannose respectively. Certain historic names have been retained such as cellulose ($\beta$-(1$\rightarrow$4)-D-glucan) or amylose ($\alpha$-(1$\rightarrow$4)-D-glucan). The homopolymers are also named homoglycans, whereas polysaccharides composed of two or more monosaccharides are named heteroglycans. An example is given with the bacterial polysaccharide called Fucogel® based on a trisaccharide repeating unit:

\[
3\alpha-L-Fucp-(1\rightarrow3)\alpha-D-Galp-(1\rightarrow3)\alpha-D-GalpA-(1\rightarrow)
\]

in which the sequence, the name of the constitutive sugars, the type of bonds, and the configuration are all given (33). A labile acetyl substituent was located on the GalpA unit.

Single words such as xylans or mannans may also be used to refer to related groups of polysaccharides without implying that they are homopolymers. The name xylan is used for arabininoxylan and glucuronoxylan, heterogeneous polysaccharides found in plants. The term mannan is used for glucomannan, galactomannan, and galactoglucomannan.

The extract from a natural source is often a mixture of different polymers, especially from plant sources; the extract must be fractionated but each fraction still contains molecules with minor structural features (partial acetylation or length of side chains). The fraction is polydisperse in molecular structure; the difference in local structure is also termed the microstructure.

Bacterial polysaccharides produced by a specific strain are usually perfectly homogeneous; the only difference between molecules being in the DP (or the
molecular weight); the sample is characterized by the polydispersity index, which is defined as the ratio between the mass-average molar mass \(M_w\) and the number-average molar mass \(M_n\) (34) (see Molecular Weight Determination).

**Molecular Modeling**

The physical and biological properties of macromolecules depend on their three-dimensional structures. Knowledge of the different stable conformations is required to better understand and to predict their behavior in different environments. This knowledge is also important to control and modify the role played by these macromolecules in recognition and interaction processes. Conformational data can be obtained by both experimental and computational methods. Experimental studies give data that are often incomplete, dependant on conformational equilibra or specific for a frozen state. An alternative method is molecular modeling where results depend unfortunately on human decisions. However, conjunction, these two approaches lead to a good understanding of the three-dimensional arrangements of the studied molecule.

**Monosaccharides.** Most of the monosaccharides exist as pyranose rings. The most stable conformation of such six-membered ring systems is usually one of the chair forms (C). In principle the pyranoid ring can also adopt energetically less favorable conformations. Six different skew conformers (S) separated by six different boat conformers can be identified on the pseudorotational itinerary (35). Three puckering parameters define unambiguously the position of the individual forms of the pyranoid ring on the conformational sphere (Fig. 15), \(Q\) is the maximum puckering amplitude, the parameters \(\theta\) and \(\phi\) are angles in the range \(0^\circ < \theta < 180^\circ\) and \(0^\circ < \phi < 360^\circ\), and can be thought as polar and azimuthal angles for a sphere of radius \(Q\). The two poles \(\phi = 0^\circ\) and \(180^\circ\) represent the energy wheels of the chair conformations; \(1C_4\) and \(4C_1\).

All 12 flexible forms are located at the equator. In unsubstituted cyclohexane, the two chair forms are the prominent species. Substitution of heteroatom in the ring and addition of hydroxyls or other exocyclic substituents further stabilize or destabilize ring conformers in reference to cyclohexane. As a general rule, the equatorial position of bulky substituents would be preferred because of 1, 3 syn–di axial interaction that causes steric clashes. The \(4C_1\) of glucopyranose having all ring substituents in the equatorial position is preferred to the \(1C_4\) conformer in which all the substituents are in axial orientation. However, at high temperature conformational transition to this form can arise spontaneously as demonstrated by the formation of levoglucosan (1,6-anhydro-\(\beta\)-D-glucopyranose). Besides, the \(\alpha\)-L-iduronate ring, which is a constituent of the glycosaminoglycans (heparin, heparan sulfate, and dermatan sulfate), shows conformational mobility. Three forms, namely \(1C_4\), \(2S_0\), and \(4C_1\), of this ring have been suggested to be responsible for the biological activities of these compounds.

Very important furanose molecules (five-membered rings) are commonly found in nature. For example, D-ribose and D-deoxyribose are found as the building units of nucleic acids, and fructose is a constituent of sucrose. These rings are not planar, either one (envelope form) or two (twist form) atoms are out of the plane containing the others. The different envelope and twist conformations
are of similar energy, and the barrier to their interconversion is small, therefore mixtures of different conformations are expected in solution. The different conformations of the furanoid ring are described by a pseudorotation circle (Fig. 16). Two puckering parameters are needed to define a conformation for those rings, the puckering amplitude $\nu$ and the phase angle $P$ (35).

Several low energy conformations are accessible for the primary hydroxyl exocyclic groups. The energies of the different ring conformations are affected by the orientations of the hydroxymethyl group. This group usually exists in three staggered positions called gauche–gauche, gauche–trans, and trans–gauche (Fig. 17).

In this terminology, the torsion angle $\omega(O-5-C-5-C-6-O-6)$ is stated first, followed by the torsion angle $\omega'(C-4-C-5-C-6-O-6)$. It is known from crystallographic studies, NMR measurements, and theoretical calculations that the conformational equilibrium around the C-5–C-6 bond in aldopyranoses depends significantly upon the configuration at C-4. For the “gluco” configuration (O-4 equatorial) the trans–gauche is high in energy and the remaining two conformations are almost equally populated, while the trans–gauche and gauche–trans positions are preferred for those having a “galacto” configuration (36).

The secondary hydroxyl groups undergo almost free rotational transitions. All these hydroxyl groups can participate in the creation of hydrogen bonds. As a result of the many possible orientations of such groups, prediction of the hydrogen bonding network is a difficult task. Most carbohydrates offer an exceptionally
high ratio of hydroxyl groups per saccharide residue. Such a hydrogen-bonding potential is satisfied by association with neighboring carbohydrate molecules, glycoproteins, or surrounding water molecules.

**Anomeric Effect.** The anomeric effect describes the axial preference for an electronegative substituent of the pyranose ring adjacent to the ring oxygen, whereas the exo-anomeric effect describes the rotational preference of the glycosidic C-1–O bonds (37). These stereoelectronic effects are of general importance for all molecules having two heteroatoms linked to a tetrahedral center. Survey of X-ray crystallographic data reveals that these effects have geometrical consequences. The most obvious feature of the experimental data on both the α and β configuration is a marked difference in the molecular geometry around the acetal group. By way of example, in the axial configuration one observes a general shortening of the C-1–O-5 bond, a lengthening of the C-O-X bond (for a 1→X linkage), and an increase in the O-5–C-1–O-X bond angle value. Molecular orbital
theory accounts for these observations. The magnitude of the anomeric effect varies with the nature of the electronegative group, the polarity of the solvent, and the location of the other substituents in the molecule.

The exo-anomeric effect influences the rotations around the glycosidic C-1–O bond and is therefore important in determining the relative orientations of saccharide units in carbohydrate chains. The exo-anomeric effect is a balance between electronic and steric effects. The three staggered orientations for rotation about the glycosidic bond are not equivalent; the exo-anomeric effect causes preference for the \(+\text{synclinal}\) orientation of the aglycone group in the \(\alpha\) series and \(\text{synclinal}\) for the \(\beta\) series (see Fig. 18 for the definition of the torsion angle domains).

**Disaccharides.** Monosaccharides can be condensed to produce oligomeric structures through glycosidic bond formation. Water is eliminated between the anomeric hydroxyl and any one of the hydroxyls of a second monosaccharide or oligosaccharide. The glycosidic linkage is constituted by two bonds, the glycosidic C-1–O-\(X\) and the aglycone O-\(X\)–C-\(X\). In the case of (1\(\rightarrow\)6) linkages between two pyranosic units, three bonds connect the successive sugar rings.

The low energy conformers of a disaccharide can be estimated using molecular mechanics. The conformational parameters that occur for a simple disaccharide are as follows:

- OH rotation: 7 torsion angles
- CH\(_2\) OH rotation: 2 torsions
- Aglycone rotation: 1 torsion
- Glycosidic bond: 2 torsions
- Puckering: 6 variables

If we assume three minima that should be explored for each variable, there are \(3^{18}\) combinations to consider, far beyond the available computer resources.
It is therefore important to recognize that the global shape of a disaccharide depends mainly on rotations around the glycosidic linkages, because the flexibility of the pyranose ring is rather limited and the different orientations of the pendant groups do not participate in the description of the backbone trajectories. The relative orientations of saccharide units are therefore expressed in terms of the glycosidic linkage torsional angles $\Phi_1$ and $\Psi_1$ which have the definition $\Phi_1 = \angle O-5-C-1-O-X-C-X'$ and $\Psi_1 = \angle C-1-O-X-C'-X-C'-(X+1)$ for a $(1\rightarrow X)$ linkage. The $\Phi_1, \Psi_1$ space is then explored in a systematic fashion. Both torsions are sequentially rotated in small increments over the full $360^\circ$ range. At each point on the grid, energy contributions calculated from the force field in use are evaluated. It is then possible to represent the energies of all the conformations available as a contour map in the $\Phi_1, \Psi_1$ space. These contour maps enable graphical description of energy change as related to the relative orientation of the monosaccharides; this is a three-dimensional cross section of the complex conformational space of disaccharides. They indicate the shape and position of minima, the routes for interconversion between conformers, and the heights of the transitional barriers. Among the many different methodologies for calculating contour maps, adiabatic procedures are now widely accepted. In such procedures, the strain produced by steric interactions inherent from rotation of monosaccharide residues is relieved by the inclusion of bond length and angle adjustment in the form of minimization, with respect to all degrees of freedom of the system (except $\Phi, \Psi$), at each point of the space. However, as minimization will only lead to conformations “downhill” from the starting structure, the torsional dimension where most conformational variation occurs is limited to only one orientational well. It is possible that rotation of pendant groups over torsional barriers could produce lower energy conformations at that point in $\Phi, \Psi$ space. Ideally at each point in $\Phi, \Psi$ space investigation of all possible combinations of pendant group orientations is required. Adiabatic
maps attempt to represent the lowest energy of all possible pendant group orientations at each point in the $\Phi,\Psi$ space. An example of an adiabatic map is given in Figure 19.

**Solvation.** It is important to recognize that most procedures are designed to treat molecules in the isolated state. Omission of the effect of the environment from the calculation results in a neglect of the fraction of the energy contribution that arises from these interactions.

Several different approaches have been proposed to treat solvation effects (38). In the simplest one, the effect of the solvent is achieved by increasing the dielectric constant for calculations of electrostatic interactions or by the use of a distance-dependent dielectric constant. Another possible way is to explicitly
include a limited number of solvent molecules in the calculation. By way of example, in aqueous solution the potential importance of hydrogen bonding and the great strength and directionality of hydrogen bonds requires explicit consideration of the water molecules surrounding the solute. An example obtained on maltose–water interaction is given in Reference 39. To account for such a solvent effect, molecular dynamics with the inclusion of explicit water molecules around the carbohydrate can be used. An alternative approach is to treat the solvent as a dielectric continuum. The conformational free energy of a given conformer in a particular solvent may be described as arising from the contribution of the energy of the isolated state and the solvation free energy. The latter is evaluated from the continuum model and is partitioned into cavity, electrostatic, and dispersion contributions.

**Probing Potential Energy Surfaces.** The accuracy of the predicted potential energy surfaces has to be evaluated. Indeed, many observable properties of oligosaccharides can be calculated and have been shown to be sensitive to the details of the conformational energy surface. Many crystal structure determinations of carbohydrates, nucleosides, and nucleotides are available in the Cambridge Crystallographic Database. X-ray analysis gives the best data for the conformation of an oligosaccharide. Precise atomic coordinates are provided, along with an explicitly defined environment. Comparisons with crystal structures are among the most precise test of modeling available for carbohydrate molecules, provided that packing forces are taken into account. These surveys of crystalline conformations are indicative of the structural variations and the conformational flexibility that the glycosidic linkages exhibit in the solid state.

In solution, the method of choice to study the three-dimensional structure of saccharides is NMR, through the parameters represented by chemical shifts, coupling constants, nuclear Overhauser effects (nOe), and also relaxation time measurements. Although the conformational dependence of the carbon chemical shifts is far from understood, coupling constants can be used to evaluate the magnitude of the torsion angles, and nOe measurements can provide estimations of distances between protons located in rather close proximity. In addition, relaxation time measurements give information on the mobility and the behavior of molecules in solution.

Three bond coupling constants provide a source of information with regards to torsional angles about the bonds. They can be interpreted by trigonometric formulas with suitable parameters derived from empirical studies on model compounds. $^3J_{H-H}$ coupling constants allow the extraction of information about the sugar ring pucker. For the exocyclic group of pyranose, the vicinal coupling constants between H-5 and H-6 define the torsional angle about C-5–C-6, provided that the stereospecific assignment of the two H-6 protons is possible. The relative magnitudes of the coupling constants, $^3J_{H5-H6}$ and $^3J_{H5'-H6'}$ can be used to determine the distribution of conformers about the $\omega$ angle in the case of (1→6)-linked pyranoses. As for the torsional angles $\Phi$ and $\Psi$ about the glycosidic linkage, only heteronuclear coupling constants $^3J_{C-H}$ and $^1J_{C-H}$ can be used, and empirical trigonometric formulas relating $^3J_{C-H}$ to torsional angles have been proposed. The angular dependence that incorporates the solvent effects of $^1J_{C-H}$ have been established in $\alpha$- and $\beta$-oligosaccharides (40,41).
The nOe data may be obtained by either one- or two-dimensional methods. These through-space effects may be used to estimate internuclear distances. However, nOe cross-peak volumes are inversely proportional to the sixth power of the distance between the correlated protons only in the case of rigid spherical molecules whose tumbling is isotropic. The simplest method that has been proposed for the interpretation of nOe-derived distance data is the “isolated spin pair approximation.” The matrix treatment is more rigorous, although relying also on several approximations. A set of equations describing the cross-relaxation pathways of all the protons in the molecule is cast into a matrix form and solved.

All these NMR parameters represent average values, because of the acknowledged flexibility of carbohydrates and of all the rapidly interconverting conformations accessible to the molecule on the NMR time scale. This means that a straightforward interpretation of experimental data leads only to the definition of a “virtual conformation” without real physical meaning. Conformational averaging based on the abundances of the predicted structures gives averaged values which can be compared safely to the experimental ones.

**The Disordered State of Polysaccharides.** The polysaccharide chains in solution tend to adopt a more or less coiled structure. Such a dissolved coil would fluctuate between local and overall conformations. Polysaccharides are able to assume an enormous variety of spatial arrangements around the glycosidic linkages because these molecules have extensive conformational freedom; they may sample a large part of the space around the glycosidic and aglycone bonds. Theoretical polysaccharide models are based on studies of the relative abundance of the various conformations, of a given polysaccharide, in conjunction with the statistical theory of polymer chain conformation (42). Possible interactions between residues of the polysaccharide chain that are not nearest neighbors in the primary sequence of the polymer are ignored.

The Monte Carlo sample then reflects the number of conformations of polymer molecules. This means that observable parameters describing the solution behavior of polysaccharides are averages of the properties of individual conformations. This approach yields properties corresponding to the equilibrium state of the chain. Results refer to a model for an unperturbed chain that ignores the consequences of the long range excluded volume effect, because only nearest-neighbor interactions are accounted for in the computation of the $\Phi, \Psi$ surfaces.

Given a sufficient Monte Carlo sample of unperturbed polysaccharide chains, it is possible to assess many mean properties of the polymer in question simply by computing unweighted arithmetic averages over the chains of the sample. For example, the mean-square end-to-end distance, the average square radius of gyration, the mean persistence length, and dipole moment, are all average geometric properties readily computed from a knowledge of the coordinates of the atoms or atomic groups making up the Monte Carlo sample.

Models of polymer chain extension were first used to compare the effect of the glycosidic linkage geometry of simple polysaccharide chains, e.g., cellulose and amylose (43). Both polymers are 1,4-linked glucans; the only difference is in the anomeric configuration on the C-1 atom of the monomeric unit, $\alpha$ for amylose and $\beta$ for cellulose. The calculated data show a remarkable pseudohelical chain
trajectory of the amylosic chains, the characteristic ratio of 5 denotes a moderately compact chain configuration. This behavior seems to be the consequence of the glycosidic bond geometry because changing this geometry from the $\alpha$ to the $\beta$ configuration has a dramatic effect on the character of the chain trajectory. Relative to amylose, the cellulosic characteristic ratio of 100 is an increase of a factor of 20. This reflects the extended character of the cellulosic chains. Investigation of the solvent effects on those two representative polysaccharides has also been carried out (44). In good concordance with the experimentally observed solvent dependence, significant changes in the unperturbed chain dimensions were found; the characteristic ratio is larger in water than in vacuum for amylose, whereas for cellulose it is smaller.

The answer to the question of how reliable is the predicted conformational behavior of the polysaccharide chains lies again in the comparison of theoretically and experimentally measured properties. A few examples were analyzed (chitin, chitosan, hyaluronan, galactomannan) and the local stiffness of the chain reflected by the persistence length $L_p$ has been compared with the value obtained experimentally using multidetection SEC (45–47).

**Molecular Structure**

The polymer may be linear (such as hyaluronan) (Fig. 20a) or branched with a long side chain (as in xanthan) (Fig. 20b) or with many short chains or monosaccharide side groups (as in galactomannan) (Fig. 20c) or branched with side chains themselves branched giving rise to a tree-like structure (as in amylopectin) (Fig. 20d).

The determination of the structure is a very difficult task especially in heteroglycans. The structure must be established on a pure sample isolated from its natural source with a minimum degradation.

![Fig. 20. Structural patterns of polymer chains.](image)
Considering the repeat unit of a polysaccharide represented by the scheme,

\[
\begin{array}{c}
\text{A} \\
\text{B} \\
\text{C} \\
\text{D}
\end{array}
\]

the answers to the different following questions are required:

1. What is the nature and relative proportion of the A, B, C, D monosaccharides involved in this oligomer, including ring size and their configuration (D- or L-)?
2. At which positions \((u, v, x)\) are A, B, D engaged in the osidic linkage and what is the anomeric geometry \((\alpha\) or \(\beta)\) of each glycosidic linkage?
3. Unit C is substituted at \(w\) and \(y\) positions but which position corresponds to the attachment of the side chain and which to the main chain linkage?
4. Which is the exact sequence of the four sugars in the repeat unit?

The general methods are reviewed in many books (3) and in this article only general overview will be given.

If there are any substituents on the rings as is frequent in plant or bacterial polysaccharides (acetyl, pyruvyl, succinyl groups), they must be located on a specific position, but often they are labile in acidic or mainly basic conditions and the structure determination becomes even more difficult.

**Extraction–Purification.** Purification of polymeric molecules is often easier than for small molecules. It is relatively easy to separate small (impurities) from large molecules on solubility criteria, gel filtration, or by dialysis.

If the polymer is water soluble as in exocellular bacterial polysaccharides, it is often a polyelectrolyte (containing uronic acid); it can be precipitated using a nonsolvent such as ethanol or 2-propanol (in a ratio around 1:1 water/alcohol) after clarification of the extract by filtration and addition of monovalent salt excess to exchange the multivalent counterions. The presence of bivalent counterions left in the sample will prevent resolubilization after drying. For a strongly interacting polymer with calcium (such as pectin) the use of oxalate as chelating agent is useful.

Before extraction of polysaccharides from plants or woods, it is recommended

1. to extract these materials with organic solvents (toluene, chloroform, methanol, etc) to remove pigments, waxes, or oils;
2. then, to eliminate Lignin (qv), a highly cross-linked polyaromatic compound, by chemical treatment (chlorite, ozone, sulfite, etc).

For neutral polymers (cellulose being excluded), extraction can be performed using DMSO (48), \(N\)-methylmorpholine \(N\)-oxide (49), NaOH, or KOH at different concentrations. Over pH 12, alcololate is formed on the \(-\text{OH}\) groups, increasing the solubility of polysaccharides. When hemicellulos have to be extracted from a plant source, KOH 24% or NaOH 18% are often used; these hydroxides produce
swelling of the cellulosic matrix by rupturing hydrogen-bonding associations, allowing better diffusion of other polymers (50).

The extract may contain different polysaccharides and it is useful to resolubilize this material after first purification and to perform SEC in addition to ion-exchange chromatography before establishment of the structure. This allows separation of fractions with different average molecular weights and different average charge densities.

**NMR Spectroscopy.** The structure can be established as mentioned before using $^1$H and $^{13}$C NMR; many examples are given in the literature. NMR is very useful especially for analyzing polysaccharides with a regular structure. Figure 21 shows the NMR analysis of a bacterial polysaccharide (hyaluronan) based on a 2 sugars repeat unit. On the $^1$H NMR spectrum, the two anomic C-1 are identified as well as the acetyl of the $N$-acetyl-$d$-glucosamine unit. On the $^{13}$C NMR spectrum, two different C=O appear in the range of 175 ppm due to the carboxylic group and to the acetyl group. The attribution of all the signals was given by Haxaire (51). NMR is also useful for estimating the population of substituents, the number of sugars in the repeat units, and to determining the anomery as well as the position of linkage and the sequence.

**Composition.** The most usual method to determine the composition of a polysaccharide (nature of the sugar units) is the complete hydrolysis by heating in acidic conditions ($1\ M\ H_2SO_4$ followed by neutralization with barium carbonate, or with $2\ M$ trifluoroacetic acid, which is volatile and easily eliminated after reaction) (52,53). Nevertheless, no recommendation can be proposed a priori; especially, for heteropolysaccharides, the rate of hydrolysis of the osidic linkage depends on the sugars involved: glycosidic bonds of deoxysugars and furanose sugars (like arabinose and fructose) are more labile whereas the glycosidic bond of the uronic moiety is more stable. In the latter case, it is better to reduce the polymer before hydrolysis (54,55). Methanolyis is sometimes preferred to simple acid hydrolysis, leading to methyl-glycoside derivatives (56).

Quantitative determinations of sugars are achieved by liquid chromatography (HPLC) or gas chromatography (GC). HPLC has the advantage of utilizing sugars directly but each sugar can give two or more peaks corresponding to the anomic equilibrium; an example of HPLC chromatogram obtained on C18-µ Bondapak on maltodextrins is given in Figure 22. The peaks corresponding to DP $= 4–6$ show the anomic equilibrium (26).

Reduction of the saccharide gives alditols and only one peak per sugar. The same occurs in GC; but, in addition, as sugars are not volatile, they must be transformed into alditol acetates by reduction and peracetylation to get a single peak per sugar in GC (57).

**Methylation.** Methylation involves the complete conversion of all free hydroxyl groups in a polysaccharide into methoxyl groups (−OH → −OCH$_3$). The most used method is the Hakomori procedure using DMSO, sodium hydride, and methyl iodide (58). To give significant results, methylation must be perfectly completed; then, total hydrolysis yields monomeric units with different degrees of methylation. The position of nonetherified −OH indicates the position of the glycosidic bond. Thus, a tetra-O-methylhexose is representative of a terminal unit (nonreducing end). In a linear oligo- or polysaccharide, the fraction of these units can be used to estimate the number-average degree of polymerization.
Fig. 21. (a) $^1$H and (b) $^{13}$C NMR spectra for hyaluronan in D$_2$O at 85 °C in the presence of DMSO as standard at 300 and 75 MHz respectively (51).
A tri-O-methylhexose is an in-chain unit; di-O-methylhexose indicates the existence of a branched point. The partially methylated sugars can be fractionated by HPLC. This technique allowed determination of the distribution of methyl substituents in methylcellulose (59); Figure 23 shows the chromatogram obtained for partially methylated glucose after total hydrolysis of the methylcellulose. Eight modified D-glucose derivatives are obtained including unsubstituted glucose.

Usually, the methylated sugars are derivatized to alditol acetates and separated by GC combined with mass spectroscopy (60–63).

**Partial Acid and Enzymic Hydrolysis.** Partial hydrolysis with acid under mild conditions gives a mixture of oligosaccharides. Homoglycan gives a series of homologous oligomers, whereas heteroglycan gives a series of products depending on the relative acid labilities of the different glycosidic bonds. The fragments permit the sequencing of the monosaccharide residues in the polysaccharides.

Establishment of the polysaccharide structure can be obtained by the use of specific enzymes. The most developed studies concern the amylopectin molecular structure using α- and β-amylase and debranching enzymes (isoamylase, pullulanase, glucoamylase). These enzymes hydrolyze specifically the α(1→6) and/or α(1→4) linkages, allowing separation of the side chains. The different sizes of side chains in amylopectins were separated by SEC (64,65). Endo- and exoglucanases specific for the cellulose (β(1→4) linkage) were also investigated; a cocktail of these types of enzymes is also used to disrupt the plant cell walls. Bacteriophages possess a highly specific endoglycanase for a single glycosidic bond in each repeat unit of the corresponding bacterial polysaccharide, thus giving a single oligosaccharide motive in good yield, identified as the repeat unit, easier to characterize (66).
**Molecular Weight Determination.** Whenever the polysaccharide is perfectly water soluble it is easy to perform SEC; but the polyhydroxyl nature of polysaccharides causes specific problems: owing to the large number of –OH groups in the molecules, cooperative interaction may form aggregates; the associated molecules are connected by a network of H-bonds, often created by the conditions of isolation and drying of the pure polysaccharide. The presence of aggregates disturbs the chromatogram which becomes difficult to interpret.

In addition, when uronic acid or pyruvic acid substituents are present, the polysaccharide becomes a polyelectrolyte (see POLYELECTROLYTES). It is necessary to shield the polymer from electrostatic interactions; this implies that the polymer has to be solubilized in the presence of an external 1–1 electrolyte (67–70). A multidetection experimental device in which a complete molecular weight distribution can be obtained without any calibration has been developed (71).

Another important parameter is the intrinsic viscosity $[\eta]$ (expressed in mL/g). It is obtained by extrapolation to zero polymer concentration of the reduced viscosity ($\eta_{\text{red}}$) of the polymeric solution plotted as a function of the polymer concentration $C$ (g/mL) in accordance with the Huggins relation:

$$\eta_{\text{red}} = (\eta - \eta_0)/\eta_0 C = [\eta] + k'[\eta]^2 C$$  

in which $\eta$ is the viscosity of the polymer solution, $\eta_0$ is the viscosity of the solvent, and $k'$ is the Huggins constant. There is a relative relation between the intrinsic viscosity and the molecular weight of the polymer following the Mark–Houwink relationship:

$$[\eta] = K M_v^a$$
Table 4. Mark–Houwink Parameters for Cellulose and Some Cellulosic Derivatives and for Starch and Starch Derivatives

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent</th>
<th>T, °C</th>
<th>(K, 10^{-3})</th>
<th>(\alpha)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Cupriethylenediamine</td>
<td>25</td>
<td>10.1</td>
<td>0.9</td>
<td>92</td>
</tr>
<tr>
<td>Cellulose nitrate</td>
<td>Tetrahydrofuran</td>
<td>25</td>
<td>1.32</td>
<td>1.01</td>
<td>73</td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td>Acetone</td>
<td>30</td>
<td>16.0</td>
<td>0.82</td>
<td>74</td>
</tr>
<tr>
<td>Cellulose acetate butyrate</td>
<td>Acetone</td>
<td>25</td>
<td>13.7</td>
<td>0.85</td>
<td>75</td>
</tr>
<tr>
<td>Cellulose triacetate</td>
<td>Acetone</td>
<td>25</td>
<td>14.9</td>
<td>0.82</td>
<td>76</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>Aqueous NaCl (0.1 M)</td>
<td>25</td>
<td>12.3</td>
<td>0.91</td>
<td>77</td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>Water</td>
<td>25</td>
<td>316</td>
<td>0.55</td>
<td>78</td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>Water</td>
<td>20</td>
<td>280</td>
<td>0.55</td>
<td>79</td>
</tr>
<tr>
<td>Amylose</td>
<td>Dimethyl sulfoxide</td>
<td>25</td>
<td>15.1</td>
<td>0.70</td>
<td>80</td>
</tr>
<tr>
<td>Amylose</td>
<td>Dimethyl sulfoxide</td>
<td>25</td>
<td>30.6</td>
<td>0.64</td>
<td>81</td>
</tr>
<tr>
<td>Amylose</td>
<td>Water</td>
<td>20</td>
<td>13.2</td>
<td>0.68</td>
<td>82</td>
</tr>
<tr>
<td>Amylose triacetate</td>
<td>Aqueous KOH (0.15 M)</td>
<td>25</td>
<td>8.36</td>
<td>0.77</td>
<td>80</td>
</tr>
<tr>
<td>Amylose triacetate</td>
<td>Chloroform</td>
<td>30</td>
<td>1.06</td>
<td>0.92</td>
<td>83</td>
</tr>
</tbody>
</table>

in which \(M_v\) is the viscometric average molecular weight; \(K\) and \(\alpha\) are two parameters depending on the polymer, the solvent, and the temperature. Some values are given in Table 4.

**Plant Polysaccharides**

Polysaccharides from the biomass consist largely of renewable polymers which have been used for a long time in many industrial applications. The main polysaccharides concerned are cellulose (qv) and starch (qv) (84).

Cellulose is the main structural polymer in wood (representing around 50% of the dried matter) or agricultural resources (normally between 35 and 75% of the dried matter depending on the source; in the case of cotton (qv), values are between 90 and 99%) (85–88). Cellulose is found in a very organized fibrous structure. In contrast, starch constitutes the reserve polysaccharide organized in a granular structure. Both of these polymers are semicrystalline polymers (qv) based respectively on \(\beta-(1\rightarrow4)\)-d-glucose and \(\alpha-(1\rightarrow4), \alpha-(1\rightarrow6)\)-d-glucose (89,90).

Derivatization may be used to extend the domains of application for these natural polymers; nevertheless, their relative lack of solubility and their high degree of organization lead usually to heterogeneous chemical modifications. Heterogeneity of the substitution gives bad reproducibility of the samples, depending also on the sources of polymers. Because of this problem, a large variety of parameters relating the degree of substitution and molecular weight to the physical properties can be found in the literature.

The supramolecular structure of the initial materials is a very important point. The swelling and/or dissolution in different conditions depend on the morphology of the native substrate. Cellulose in cellulosic fibers and
amylose/amylopectin in starch granules are tightly packed in a more or less regular organization, depending on their source but also on the presence of other additional components. Thus, the accessibility of the reactive groups (—OH) depends on this supramolecular structure in addition to the degree of crystallinity (diffusion control of the reactants) and on the intra- or interchain H-bonds modulating the —OH reactivity. This is why cellulose and starch are not thermoplastic materials but in presence of water playing the role of plasticizer; especially starch becomes thermoplastic. Only a complete destructure passing through a solution state avoids a memory effect of the original state of organization and attainment of regular chemical modification.

The two main characteristics of cellulose and starch derivatives are the distribution in molecular weight (the weight average DP$_w$ or $M_w$) in addition to the degree of substitution represented by DS (DS is the average number of substituents per glucose unit; $0 < \text{DS} < 3$).

### Cellulose and Cellulose Derivatives

The solubilization of cellulose to determine its molecular weight is delicate; one of the most powerful solvents is cupriethylenediamine for which the Mark–Houwink parameters relating the intrinsic viscosity to the molecular weight ($\eta = K M^\alpha$) have been determined (see Table 4). Some authors previously prepared cellulose derivatives (nitrocellulose or cellulose tricarbanilate) to determine the molecular weight and molecular weight distribution, assuming no polymer degradation (72–83,91,92).

Cellulose derivatives are the most important derivatives obtained from natural sources. Chemical modifications of cellulose are very useful to enhance solubility in organic or aqueous solvents or to obtain new thermoplastic materials. The main derivatives are described in Table 5.

In designing derivatives for various properties and applications the degree of substitution is a very important parameter, as well as the chemical structure of the substituent. Recent studies have been done in selective cellulose functionalization obtaining regioselectively substituted esters and ethers (93–97).

### Table 5. Main Substituents in Cellulose Derivatives

<table>
<thead>
<tr>
<th>Product</th>
<th>Functional group (R=)^a</th>
<th>DS^b</th>
<th>MS^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose nitrate</td>
<td>−NO$_2$</td>
<td>1.8–2.8</td>
<td></td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td>−C(=O)CH$_3$</td>
<td>0.6–3.0</td>
<td></td>
</tr>
<tr>
<td>Carboxymethylcellulose</td>
<td>−CH$_2$COONa</td>
<td>0.5–2.9</td>
<td></td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>−CH$_3$</td>
<td>0.4–3.0</td>
<td></td>
</tr>
<tr>
<td>Hydroxyethylcellulose</td>
<td>−[CH$_2$CH$_2$O]$_x$ H (x = 1, 2, 3, …)</td>
<td>0.8–1.2</td>
<td>1.7–3.0</td>
</tr>
<tr>
<td>Hydroxypropylcellulose</td>
<td>−[CH$_2$CH(CH$_3$)O]$_y$H (x = 1, 2, …)</td>
<td>&lt;4.6</td>
<td></td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>−CH$_2$CH$_3$</td>
<td>0.5–4.6</td>
<td></td>
</tr>
</tbody>
</table>

*a $0 < \text{DS} < 3$ is the degree of substitution.

*b MS is the molar substitution (gives the average number of monomoles of reactant added to one anhydroglucose unit).
Among the large variety of cellulose derivatives, two main groups of products are prepared: cellulose esters and cellulose ethers. Worldwide, around $1.4 \times 10^6$ t/year of cellulosic dissolving pulp is used in the production of cellulose esters, whereas $5.4 \times 10^5$ t/year is used in the production of cellulose ethers (98).

Cellulose ethers (qv) are obtained by substitution of hydroxyl groups by ether groups, and depending on the substituent they can be soluble in water or organic solvents. Carboxymethylcellulose (CMC) is the ether of cellulose of largest production. Approximately 300,000 t/year is produced each year. It is a water-soluble polymer in the sodium salt form and when DS is larger than 0.5. It is produced by reaction of the alkalicellulose with sodium chloroacetate or reaction with sodium in liquid nitrogen in the presence of chloroacetic acid (99–101). On a laboratory scale, soluble CMC with DS varying from 0.5 up to 3 has been produced. Typical raw materials for cellulose-ether production are wood pulp or cotton linters. Cotton linters are used for the production of high viscosity ethers because of their higher DP. CMC sodium salt is a white, odorless, and nontoxic solid. Viscosities range in aqueous solution from 10 to over 50,000 mPa⋅s (=cP) for 2% aqueous solutions. The DS of commercial samples may be between 0.3 and 1.2 although clear and fiber-free CMC solutions require a minimum DS value of about 0.5. The majority of commercial products have a DS between 0.65 and 0.85. Most CMC solutions are highly pseudoplastic (102,103).

Methylcellulose (MC) is produced, as in the case of CMC, by the Williamson synthesis, ie, by reaction of alkali cellulose with methyl chloride. World annual production is around 80,000 t/year (98). Other hydroxyalkyl derivatives or mixed ethers such as hydroxyethylmethylcellulose, hydroxypropylmethylcellulose, hydroxybutylmethylcellulose, ethylmethylcellulose, and carboxymethylmethylcellulose have similar properties to MC.

MC is a white to slightly off-white, essentially odorless and tasteless powder. The solubility of MC varies as a function of DS. In fact, commercial MC is divided into two types of products according to DS. MC with a degree of substitution between 1.4 and 2.0 are soluble in cold water, whereas lower substituted materials are soluble in dilute alkali. MC is also soluble in ethanol, acetone, ethyl acetate, benzene, and toluene for higher DS. Just as in the case of CMC, viscosity values of MC vary as a function of (average) molecular weight. Apparent viscosity at 2% and 20°C can vary between 10 and 19,000 mPa⋅s (=cP) in a range of average molar mass between 13,000 and 140,000. MC and its derivatives form gels at specific temperatures when heated (104).

The mechanism of gelation is related to the distribution of substituents along the chain; two steps in the gelation mechanism have been demonstrated. The first step of gelation is the formation of a clear gel related to the existence of blocks of highly substituted glucose units (105–108). The second step is the phase separation giving a turbid gel when temperature is larger than 60°C. Below their gel point, solutions of MCs exhibit pseudoplastic rheology, but appear to be Newtonian at low shear rates. MCs have excellent water retention properties.

The two main types of commercial hydroxyalkylcelluloses are hydroxyethylcellulose (HEC) and hydroxypropylcellulose (HPC). HEC is produced in larger amount with around 60,000 t/year, but investments are planned in order to increase the capacities of HPC, in future years. HEC and HPC are synthesized by reaction of alkali cellulose with ethylene oxide or propylene oxide respectively in a
slurry process in an organic medium. In the case of HPC it is possible to use liquid propylene oxide as a medium of reaction; after etherification the crude product is purified by washing with hot water (109–111).

HEC is available in a wide range of viscosities (from 10 to 100,000 mPa·s (=cP) in 2% aqueous solution at ambient temperature). It is soluble in cold and hot water or some mixtures of water and water-miscible organic solvents. The nonionic chemical structure and its solubility in both cold and hot water are the main advantages for the utilization of HEC. HPC has a thermal gel point like MC and it is thermoplastic. Because of its high level of substitution (molar substitution about 4) and its remarkable hydrophobic character it is soluble in a number of organic solvents as well as water.

Many general books develop the structure and reactivity of cellulosic materials and the preparation of their derivatives (112–115).

**Starch and Starch Derivatives.** The starch granule dimensions depend on the sources as well as the composition and degree of crystallinity. The molecular structure of the principal component in the starch granules consists of a linear (1→4)-D-glucose chain, amylose. Amylose is a flexible polymer having a relatively low thickening power; its molecular weight seems to remain relatively low (less than 1 million). The second major component is a highly grafted polymer, amylopectin. It is a very compact molecule with a very high molecular weight (10–100 millions have been cited in the literature). Also, in some materials a third component is a slightly grafted amylose called the intermediate material (116,117).

The main techniques to characterize starch molecules consist of iodine or butanol complex formation to estimate the fraction of linear amylose (118). A colored complex is formed with iodine; its wavelength of absorption is characteristic of the length of the helicoidal segments involved in the complex formed with iodine. This technique has been examined by Salemis (119). The second important characteristic is the molecular weight or better, the molecular weight distribution. It is often difficult to solubilize starch in aqueous solution. In one SEC investigation of starch in aqueous systems containing KOH (120), it was shown that amylopectin has a low solubility. SEC studies were also done in DMSO (121,122). The determination of the molecular weights can be performed using the empirical relation relating the intrinsic viscosity to the viscometric average molecular weight. Some parameters are given in Table 4.

Nowadays, starch is used in industry as native starch (world production: \((10–12) \times 10^6 \text{ t/year}\)), gelatinized starch, modified starch \((7 \times 10^6 \text{ t/year})\), or as partially or totally degraded starch \(((16,17) \times 10^6 \text{ t/year})\), (98). First, the characteristics of the products depend on the parent starch sample.

The hydroxyl groups of starch can be derivatized as esters or ethers, as in the case of cellulose. Table 6 gives the main substituents for starch.

Starch esters are synthesized by reaction of a carboxylic acid or an acyl chloride or an acid anhydride with the hydroxyl groups of amylose or amylopectin chains. As in the case of cellulose derivatives, there are organic and inorganic esters depending on the origin of the substituent.

Acetate of starch is the most important ester of starch industrially produced. Commercial products are traditionally low substituted derivatives \((\text{DS} < 0.3)\) (123–125). In this range of DS it is possible to derivatize preserving the granule
structure, to purify by washing with water, and to recover by centrifugation or filtration. The acetylating agent is commonly acetic anhydride in aqueous medium in the presence of dilute sodium hydroxide. Derivatization eases the formation and stability of colloidal dispersions of starch and the adjustment of the colloidal properties to the requirements of the application. Products obtained have high purity and low ash content. The principal uses are in food (paste clarity and viscosity stability) and the pharmaceutical and textile industry (warp sizing, finishing operations). Other higher acetylated starches are those obtained with a DS of 2–3 (126,127). Their characteristics of solubility and thermoplasticity permit their utilization as materials. Nevertheless, this type of material has not been developed commercially because they could not compete with similar cellulose derivatives in terms of strength and cost. The glass transition ($T_g$) stabilizes around 150°C. These polymers have been proposed to prepare hydrophobic thermoplastic materials or to coat hydrophilic films (128–130). Even up to DS ~ 3, the polymers remain biodegradable (129).

Syntheses of longer chain organic esters (C$_3$–C$_{18}$) have been carried out on a laboratory scale, but have not yet been industrially produced because at present they are not profitable (125,130–133).

Starch can be etherified by reaction of an alkyl halide or an epoxy in alkaline medium. Depending on the substituent it is possible to obtain anionic, hydroxylalkyl, and cationic products.

The two main types of hydroxylalkyl ethers industrially produced are hydroxyethyl starch and hydroxypropyl starch. These compounds are synthesized by reaction of ethylene oxide or propylene oxide respectively with starch under alkaline conditions (134).

Low MS hydroxylalkyl starch products (MS < 0.1) are prepared by reaction of the epoxy in an aqueous and alkaline starch slurry. A salt such as sodium sulfate is often added to protect the starch from swelling so that relatively high levels of caustic can be added to increase the reaction efficiency. In this case usual temperatures are between 25 and 50°C. If the temperature is increased and the salt eliminated, it is possible to do the reaction in a starch solution and obtain higher molar substitutions.

High MS hydroxylalkyl products are prepared by reaction of the epoxy in alcohol slurry and alkaline conditions. The alcohols typically used are methanol, ethanol, and 2-propanol. Another possibility is to combine starch with a low level
of moisture (5–15%) with ethylene or propylene oxide under pressure in presence of an alkaline catalyst. In this process the starch retains its granular form.

Low MS hydroxyalkyl starches are very similar in appearance to unmodified starch. Nevertheless, there are two main differences in their properties owing to the presence of hydroxyalkyl groups. On one hand, these groups weaken the hydrogen bonding between starch molecules and facilitate the reduction of pasting temperature. On the other hand, when the product is solubilized, retrogradation of the starch chains is inhibited, resulting in a more fluid paste with improved clarity, viscosity stability, freeze–thaw stability, and cohesiveness. Although the substituted starch paste thickens when it cools, reheating will return it to its original hot viscosity and clarity.

When the MS increases the pasting temperature decreases, until the product is soluble in cold water. These products have excellent viscosity stability over a wide range of conditions. The viscosity of high MS hydroethyl starch solution is quite stable to shear changes, changes in pH, and enzymatic attack. Salts have little effect on these products.

Hydroxyethyl starch is used in papermaking, textile manufacturing, medical and pharmaceutical industries. Hydroxypropyl starch is used in the food industry, textile and paper products, building materials, cosmetics, and pharmaceuticals (134).

Cationic starches are obtained by reaction of starch with reagents containing amino, imino, ammonium, sulfonium groups. The two main types of commercial products are the tertiary amino and quaternary ammonium starch ethers (135).

Quaternary Ammonium Starch Ethers. Among the reagents that can add quaternary ammonium groups to starch probably the most popular is 2,3-epoxypropyltrimethylammonium chloride. Ethers of this type with different DS have been prepared and tested for their role in adsorption on calcium carbonate in relation with the mechanisms of dispersion and flocculation of small particles (136).

Cross-linking. Starch polymer chains can be cross-linked with difunctional reagents to form diethers or diesters. These derivatives are distinguished from starch ethers and starch esters because the properties obtained by the cross-linking of starch are specific. In general terms cross-linking reinforces granule integrity, modifies the water retention capacity, and provides higher mechanical resistance and improves film properties.

Cross-linking is employed when a stable, high viscosity starch paste is needed, and particularly when the dispersion is to be subjected to high temperature, high shear, and/or low pH (137).

Hemicelluloses. Hemicelluloses constitute a series of polysaccharides associated with cellulose in plants. In wood, in addition to lignin and cellulose, hemicelluloses represent around 25% by weight (138). Some structures are given in Figure 24. The composition of hemicelluloses depends on the source: deciduous trees (hardwoods) have mainly glucuronoxylan; coniferous trees (softwoods) yield more glucomannans, O-acetylgalactoglucomannans, and xylans having both arabinofuranosyl and 4-0-CH3-galacturonic side chains. Some softwoods also have significant amounts of arabinogalactan (eg in larch wood) (138).

Hemicelluloses from annual plants (cereals crops) are mainly of the xylan type; mixed-linked β-1,3-1,4 glucans are also found. The structure has been
Fig. 24. Partial chemical structure of some hemicelluloses: A = O-acetyl-(4-O-methylglucurono)xylan from hardwood; B = arabino-(4-O-methylglucurono)xylan from softwood; C = O-acetyl-galactoglucomannan; D = arabinogalactan from larch wood (138).
Cooperative interactions may be stabilized between xanthan and galactomannan depending on the conformation of xanthan (helical or coiled). Two types of gels can be identified: one around 20°C involving disordered xanthan and galactomannan whatever its content in galactose; the second one, around 60°C, engages helical xanthan and it is the usual gel identified when these two polymers are mixed (145–152). Molecular modeling was applied for the determination of the persistence length of a galactomannan model for which M/G ≈ 1 (47); the value predicted as a function of the DP is given in Figure 26. The role of the microstructure of the polymer (random or blockwise distribution of galactose, content of galactose) was also analyzed by molecular modeling (153).

![Fig. 25. Representation of the galactomannan structure. * represents the C-6 position where –OH can be specifically modified.](image)

![Fig. 26. Persistence length of galactomannan (for G/M = 1) as a function of the degree of polymerization x. L_∞ is equal to 9.6 nm. Reproduced from Ref. 47, with permission from Elsevier (Copyright 2003).](image)
Glucomannans are also obtained from seeds or roots; the structure is a random arrangement of $\rightarrow 4)-\beta$-Glc-(1 and $\rightarrow 4)-\beta$-Man-(1. The ratio Glc/Man depends on the source and varies from 1:1 in Iris bulbs to 1:5 in certain gymnosperms. Konjac glucomannan has been developed mainly in Japan for food applications (154,155).

**Pectins.** Pectins are located in the middle lamella and primary cell walls of plant tissues. They are extracted from apple skins and citrus peels. Pectins consist primarily of 1,4-linked $\alpha$-D-galacturonic units or their methyl esters with some interruption by a rhamnogalacturonan region kinking the linear polygalacturonic backbone (156). They also contain branched chains composed of neutral sugars such as galactose or arabinose. These neutral sugars amount to 10–15% of the pectic dried weight and are concentrated in blocks (called the hairy region; Fig. 27) (157).

In nature, they occur often with different degrees of esterification (DE) around 70%. Because of enzymic degradation, they can have a lower DE; in that case (if DE < 50%), they are insolubilized by calcium cross-linkage. Extraction requires the use of chelating agent.

The interaction between calcium ions and carboxyl groups of the pectins forms gels; the interaction with calcium is very cooperative owing to the stereochemistry of the 1,4-linked monomeric units, leading to the formation of a polar cavity that can be occupied by calcium or related cations. The mechanism of interaction is described by the egg-box model. A series of galacturonic acid oligomers was investigated to test the minimum carboxylic group necessary to get a stable junction (158–160). A two-step process leads to gelation: first dimer formation followed by aggregation forming junction zones (161–163).

The ability to gel can be determined from the dependence of viscosity as a function of counterions added (164). At a given concentration, the critical amount of cations at the gel point is directly related to the distribution of carboxyl groups along the chain (Fig. 28).

It is shown that the divalent counterions form gels with a sequence of affinity $\text{Ba} > \text{Sr} > \text{Ca}$, but Mg does not form gels nor dimers. The interaction with counterions as well as change in conformation have been identified by X rays (165) and by circular dichroism (162).
Fig. 28. Change in reduced viscosity and scattered light during addition of CaCl$_2$ to pectins (polymer concentration: 0.2 g/L; 5 × 10$^{-3}$ M CaCl$_2$). Role of the carboxyl groups distribution: (a) DE = 30% blockwise distribution obtained by pectinesterase hydrolysis; (b) DE = 30% random distribution obtained by alkaline hydrolysis. Reproduced from Ref. 164, with permission from John Wiley & Sons, Inc., on behalf of the Society of Chemical Industry (Copyright SCI 2003).

There are different categories of pectins: HM pectins (high methoxyl; DE > 50%) form gels in acid conditions and in the presence of sucrose to decrease the water activity. The gelation is based on H-bond association and is thermoreversible. Only, LM pectins (low methoxyl; DE < 50%) form gels in presence of calcium.

HM pectins can be extracted by water, mineral acids, or bases. LM pectins immobilized in situ via metallic ions need a sequestering agent to displace the counterions. Alkaline treatment also has a role in the deesterification. Different treatments were compared on sugar beet and potato pulps. HM pectins are naturally occurring in sugar beet pulp; they were extracted in alkaline conditions (50 mM NaOH, pH 12) and precipitated with ethanol after neutralization to pH 6.5–7. A pretreatment of the plant material is necessary to inactivate enzymes (water at 85°C, 20 min). On the contrary, for potato pulp containing an LM pectin the extraction is performed in acid conditions (pH 3.5 in presence of 0.75% hexametaphosphate, 75°C, 1 h). The pectins are precipitated at pH 2 with HCl. The precipitate is redispersed in water with NaOH up to pH 6.5–7 and reprecipitated by ethanol. Potato pulp was also extracted in alkaline conditions with hexametaphosphate, precipitated at pH 2, redispersed in water to pH 6.5–7, and precipitated by ethanol as previously. The LM pectins obtained in alkaline conditions have a higher gelling ability due to deesterification and partial deacetylation (166,167).

Seaweed Polysaccharides

Algae are an important sources of polysaccharides with different structures. Among the different groups of algae, green algae (Chlorophycea), red algae
Polysaccharides (Rhodophyceae), and brown algae (Phaeophyceae), only the last two series are used on an industrial scale (168). Many different polysaccharides may be extracted but alginates and carrageenans are the more developed especially as physical gel formers (169). Fucoidans also are now under investigation because of the importance of sulfated polysaccharides for biological applications (170, 171).

**Alginates.** Alginates are linear copolymers of α-L-guluronate (G) and α-D-mannuronate (M) (Fig. 29). They are also called polyuronides and are extracted from brown algae. Their gelling properties derive from the cooperative binding of divalent cations on the G blocks in the egg-box model (similar to that proposed in LM pectins).

Alginates behave as polyelectrolytes in the presence of monovalent counterions, and in 0.1 M NaCl, the intrinsic viscosity allows determination of the average molar mass (172).

Mechanical properties of calcium alginate gels are related to the ratio M/G and also to the distribution of the G units along the chain and to the molar mass of the polymer (173). Alginate is often used for encapsulation of pharmaceuticals, bacteria, or yeasts in biotechnological processes. The beads of gel are formed by a dripping technique; drops of alginate solution falling in 1 N CaCl₂ solution form beads which are washed in water after a few hours maturation. Smaller and more regular particles can be obtained by emulsification (174, 175); the porosity of these gels is smaller than for that obtained by the dripping method.

**Agarose and Carrageenans.** These polysaccharides represented a family of alternating (AB) type copolymers with different degrees of sulfation; agarose is the neutral polymer form. They are extracted from red algae. Their structures are given in Figure 30. They consist of alternating (1→3)-β-D-galactose and (1→4)-α-(3,6)anhydro-D-galactose or (1→4)-α-(3,6)anhydro-L-galactose (in agarose).
Agarose, κ-carrageenans, and ι-carrageenans are recognized as gelling polymers; the conditions of gelation as well as the mechanism of gelation have been abundantly discussed in the literature. The gels are thermoreversible based on H-bond stabilized conformations. Gelation is based on the formation of double helices which associate forming an aggregate or a junction zone which is the basis of the network formation; a two-step gelation process has been demonstrated clearly. Gelation and melting of the gel present a hysteresis in temperature directly related with the degree of aggregation of double helices and to the charge density of the polymers (176–178). The rigidity of the gels follows the same trend. A sequence of solubility has been established: Agarose < κ-carrageenan < ι-carrageenan < λ-carrageenan following the order of sulfate density: No sulfate < 1 sulfate < 2 sulfates < 3 sulfates The stiffness of the gels formed in presence of K⁺ counterions varies in the following order: Agarose > κ-carrageenan > ι-carrageenan The polymer λ-carrageenan never forms gels or helical conformation.

For charged polysaccharides, the formation of double helices and gelation depends on the ionic concentration, nature of electrolyte, and temperature. A phase diagram relating the total ionic concentration and the inverse of the temperature for conformational change \((T_m^{-1})\) has been established for each of them. For κ-carrageenan, the ionic selectivity among monovalent cations is very important (179,180); the sequence is as follows:

\[
\text{Rb}^+ > \text{K}^+, \text{Cs}^+ > \text{Na}^+ > \text{Li}^+, \text{NH}_4^+ > \text{R}_4\text{N}^+
\]
The mechanical properties of the gels formed in the presence of different counterions follow the same order: Rb-carrageenan gel has a higher modulus and a higher melting temperature than those of Na-carrageenan gel. The molecular weight also plays a role on the elastic modulus which increases up to a limit around $M \sim 250,000$ (181). The mechanism of gelation in two steps is described in Reference 182.

In the series of anions, a characteristic behavior was observed with $I^-$; it stabilizes the double helix but prevents gelation (180).

**Microbial Polysaccharides**

Fungi and bacteria are sources of polysaccharides and especially of exopolysaccharides which can be produced in culture media on an industrial scale. They are a source of new additives for cosmetic or food applications but also for biological activity. Many of them are now in development; a review will be published in the second edition of Reference 12. Many of these polysaccharides are water soluble and able to compete with natural polysaccharides as described before (alginites, carrageenans, galacto- and glucomannans, chitosans, pectins, etc) especially in the domain of food additives. Many books discuss their applications (183–187).

The best examples of fungal polysaccharides are the $\beta(1\rightarrow3)$ glucans with a few $\beta(1\rightarrow6)$ glucose units as side groups. They often form a triple helical conformation with a tendency to give physical gel. There have been studies on scleroglucan, lentinan, schizophillan, etc (139,188,189). Because they are neutral, solubility depends on the fraction of side glucose units and their distribution.

Bacterial polysaccharides represent a large variety of polymers biosynthesized by bacteria. Their chemical structures and also their physical properties in solution or in the solid state may vary widely. They often contain uronic acid and then become polyelectrolytes (190). Many new polysaccharides have been developed from bacteria for industrial purposes. Exocellular polysaccharides are produced on a large scale by the usual techniques of microbiology and fermentation. This procedure allows good control of the characteristics of the polymers and allows purification of the polysaccharides more easily than from other natural sources (191–194). Extension of such production also allows reducing the price and extends the range of applications. A good example remains the hyaluronan previously produced by extraction from animal sources but in which some fraction of proteins remained. Bacterial hyaluronan can be prepared in a very pure form (195).

In general, polysaccharides are especially important in the domain of water-soluble polymers; they play an important role as thickening, gelling, emulsifying, hydrating, film forming, and suspending polymers. Especially important is the fact that some polysaccharides give physical gels in well-defined thermodynamic conditions. They constitute a very important class of materials in food, cosmetics, or pharmaceutical applications.

Specific methods for the purification of the polysaccharides have been developed. They are usually isolated in their sodium salt forms by precipitation from aqueous solution with ethanol or 2-propanol. This step is the most important one to get reproducible experimental characteristics. In fact, because of the presence
of many —OH groups in the molecule, the polysaccharides have a tendency to form cooperative intra- and interchain H-bonds, causing some insolubility or at least the presence of aggregates when solutions are prepared. Because of their stereoregularity, they are often able to form helical conformations in solution. Their ordered conformation has a semirigid character and its stability depends on temperature and ionic concentration if the polysaccharide structure contains uronic acid unit or ionic substituents; an example is given for succinoglycan (196,197).

Details of the chemical structure of bacterial polysaccharides have been given previously (198,199). In the following, the main industrial bacterial polysaccharides (xanthan, succinoglycan, gellan) are described briefly.

Succinoglycans are produced by many soil bacteria of the species *Pseudomonas, Rhizobium, Agrobacterium*, and *Alcaligenes*. These polysaccharides have a general chemical structure based on an height sugar repeat unit (D-glucuronic acid/D-galactose ratio equals 7:1); this structure is given in Figure 31.

Different substituents are also present in the molecule such as acetyl, succinyl, and pyruvyl groups, depending on the strain and on the conditions of fermentation and/or isolation (200–203). The content in substituents is easily determined by $^1$H NMR in the presence of an internal standard to calibrate the signals and get a quantitative determination.

Succinoglycan, whatever its origin, is water soluble and, being stereoregular, it adopts a single-chain helical conformation at least in dilute solution. As shown by different techniques, the conformational transition is reversible and very cooperative at least in the presence of some salt excess. The stability of the ordered conformation depends on the salt concentration in solution as is usual for charged polysaccharides. On the two sides of the transition, the stiffness is completely different. In the helical conformation, the chain behaves as a semirigid chain having an intrinsic persistence length ($L_p$) estimated to be 35 nm and becoming more flexible at higher temperature in the coiled conformation with $L_p \sim 5$ nm. The persistence length in the helical conformation has been confirmed by AFM from the analysis of chain curvature (204).

The rheological behavior in dilute and semidilute solution is quite usual and typical of viscoelastic systems (197).

Xanthan was the first bacterial polysaccharide produced by the strain *Xanthomonas campestris* and developed on a large scale. The chemical structure of xanthan is recalled in Figure 31; it was confirmed by NMR. The $^{13}$C spectrum allows identification of the different sugar units as well as the substituents (205). $^1$H allows quantification of the yield in substituents. Xanthan is a semirigid polymer for which, in the native conformation, a persistence length around 40 nm was found (206–208). It has been demonstrated that when the native form is heated to coil conformation (with a much lower stiffness) and renatured by cooling, the conformation is changed. Xanthan has many industrial applications due to its suspending character (stabilizer of solid suspensions or foams), its pseudoplastic and thickening properties, good stability of the rheological properties as a function of pH, temperature, and salt concentration, and also its ability to be cross-linked and gelled. It is used in paints and coatings, cosmetics, ceramics, etc. In Figure 32 the rheological behavior characteristic for these semirigid polysaccharides is shown. The dependence of the viscosity as a function of shear rate and polymer concentration demonstrates the existence of a Newtonian plateau for low shear
rate followed by a decrease of the viscosity due to the viscoelastic character of the solution (209).

Gellan is produced by the bacteria *Sphingomonas elodea* (210). The native gellan as produced by the bacteria contains two additional substituents (acetyl and L-glyceryl) compared to the commercial polymer named Gelrite® (Fig. 31).
The native gellan gives highly viscous solutions with a loose gel-like behavior. After deacylation, gellan gives a strong and rigid gel; a comparison between the native and deacylated gellan has been done (211,212). The chemical structures of the repeat units of the native and deacylated gellans are easily controlled by $^1$H NMR.

The mechanism of gelation of deacylated gellan was examined and shown as a two-step process similar to the mechanism proposed for $\kappa$-carrageenan (182,213). Deacylated gellan adopts an ordered double-helix conformation in salt excess and the temperature for conformational change $T_m$ increases when the salt concentration increases as is usual for stereoregular Polyelectrolytes (qv). The helical conformation is more stable in the presence of divalent counterions but no ionic selectivity appears among monovalent counterions (Li, Na, K, TMA) on one side and among divalent counterions (Ca, Mg) on the other side (212). When the salt and polysaccharide concentrations increase, the double helices interact to give a physical gel with strong ionic selectivity. The sequence of selectivity is

$$K^+ > Na^+ > Li^+$$

$K^+$ promotes gel formation and gives the higher elastic modulus.

Many new bacterial polysaccharides are now under development when they have specific physical behavior.
Mammalian Polysaccharides

Two homopolysaccharides are found in animals: glycogen and chitin. The others are heteroglycans based on disaccharide repeat units (they are also named glycosaminoglycans).

**Glycogen.** It is a storage polysaccharide able to produce D-glucose depending on the nutritional level. It is a highly branched neutral polysaccharide, analogue to amylopectin but more highly branched; thus, its solubility in water is larger.

**Chitin.** It is found in bacteria, fungi (such as *Aspergillus niger*), and crustaceans but only crustaceans are important commercial sources (see CHITIN AND CHITOSAN). Especially crab or shrimp shells are the main sources of chitin; *in situ* chitin is embedded in calcium carbonate and proteins. Chitin is a linear polymer made of $\rightarrow 4(\beta-D-N\text{-acetyl-D-glucosamine})-1$. Treatment of shells with acid and alkali allows isolation of chitin partially deacetylated. When chitin in decetylated to about 50% of the free amine form it is referred to as chitosan. Chitin is the renewable polysaccharide of most importance in quantity produced per year after cellulose. It has found many applications, at least on laboratory scale in food, medical, cosmetic applications in which their original structure is appreciated. After partial deacetylation, the polymer becomes soluble in acidic conditions by protonation of the amino group in C-2 position; the intrinsic $pK$ of the $\text{NH}_2$ groups is around 6 (214,215). Methods for characterizing chitosans with different degrees of acetylation (DA) have been re-examined using infrared spectroscopy and NMR (216–218). The molar mass distribution was examined by SEC (219). This polymer is the only natural water soluble polycation; it may be used for flocculation in water purification. It is recognized for its mucoadhesive properties. Unlike cellulose or starch, chitosan is able to be chemically modified on the specific C-2 position of the glucosamine unit. Several derivatives have been prepared. Alkylation gives alkylchitosans (220,221), amphiphilic polymers which are good thickeners. Carboxymethylation gives an anionic polymer (222) and quaternization of $-\text{NH}_2$ gives a cationic polymer (223,224). Grafted cyclodextrin–chitosan has also been prepared (225,226). Another original property of chitosan is its chelating properties; a specific complex is formed with $\text{Cu}^{2+}$ and its structure has been proposed (227,228). The applications and biochemical properties of chitosan have been reviewed in two books (229,230).

**Proteoglycans.** They are carbohydrate–protein polymers in which many long polysaccharide chains are covalently linked to the protein core. The percentage of protein is low. They are also classified as glycoproteins, but the name proteoglycan is now preferred to the historical term mucopolysaccharide. These polymers are widely distributed in connective tissue, cartilage, tendon, or synovial fluid. Carbohydrate components (with an alternating structure (AB)$_n$ divided into six groups) based on disaccharide repeat units (Table 7) are joined to the protein core in different ways by a N- or O-glycosidic linkage. Depending on their composition, glycoaminoglycans are known as hyaluronan, chondroitin sulfate (in 4- or 6-position), dermatan sulfate, heparin, heparan sulfate, and keratan sulfate. Much research is in progress on these biopolymers; a review on the structure, properties, medical and biological applications of hyaluronan has been published (231).
Table 7. Disaccharide Units Constitutive of Proteoglycans

<table>
<thead>
<tr>
<th>Basic disaccharide unit</th>
<th>Common names</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlcNAcβ1,4-GlcAβ1,3</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td>GalNAc(6 or 4-SO₃⁻)β1,4-GlcAβ1,3</td>
<td>Chondroitin sulfate</td>
</tr>
<tr>
<td>GalNAc(4-SO₃⁻)β1,4-L-IduAα1,3</td>
<td>Dermatan sulfate</td>
</tr>
<tr>
<td>GlcNSO₃(6-SO₃⁻)α1,4-L-IduAα1,4</td>
<td>Heparin</td>
</tr>
<tr>
<td>GlcNSO₃β1,4-L-IduAα1,4</td>
<td>Heparan sulfate</td>
</tr>
<tr>
<td>GlcNAc(6-SO₃⁻)β1,3Gal(6-SO₃⁻)β1,4</td>
<td>Keratan sulfate</td>
</tr>
</tbody>
</table>

Glycoconjugates

Glycoconjugates, eg glycoproteins and glycolipids, are biopolymers; the name indicates that the carbohydrate is the minor component. In the glycoconjugates, oligosaccharide moieties consist of up to 20 monomers. They are basic components of all the cell membranes. Thus, certain oligosaccharides can be associated with degenerative cell growth and can be used in cancer diagnostics as so-called tumor-associated antigens.

Glycoproteins. They are proteins containing one or several oligosaccharide side chains. The linkages are N- or O-glycosidic linkages or by ethanolamine phosphate. The O-glycoproteins seem to be more involved in the stabilization and protection of protein structures rather than as signaling molecules in cell communication.

Glycolipids. They are amphiphilic molecules of low molecular weight. The lipophilic part consists of 1,2-di-O-diacylglycerol or N-acylsphingosin; the hydrophilic part is a phosphate group and/or a carbohydrate moiety. There are many glycolipids with different covalent structures with specific biological roles.

Another series of glycoconjugates (but usually not joined in this classification) is represented by natural plant gums such as Arabic gum which is recognized as very specific to stabilize emulsion. In these polymers, the glycosidic part is the most important in weight fraction compared to the protein fraction.

Glycopolymers

Under this term we refer to synthetic polymers in which at least one of the monomers engaged contains a saccharidic unit.

The extensive participation of saccharides in recognition processes has sparked the synthesis of new polymeric materials expected to have sophisticated functions similar to or even superior to natural glycoconjugates. Numerous synthetic polymers carrying various kinds of saccharide residues as information elements have therefore been developed. It has been shown that such glycoconjugates may have enhanced binding capacity with lectins based on a polymer sugar-cluster effect whereas the monomeric carbohydrate derivatives exhibit only weak affinity to the same lectins (232–235). These “glycopolymers” have thus diverse potential uses, such as multivalent inhibitors of cell or virus binding, cell-specific culture substrata, artificial antigens, targeted drug delivery systems. The
term “glycopolymers” was first introduced by Roy and co-workers (236) as a replacement for “pseudo-polysaccharide” previously used, to designate water-soluble as well as insoluble polymers bearing covalently bound carbohydrates. In the following, the term glycopolymer refers to synthetic (or natural) linear polymers possessing sugar moieties as pendant or terminal groups irrespective of whether they are water soluble or not. Glycopolymers can be obtained by polymerization of sugar-carrying monomers. An alternative synthetic method consists of the chemical modification of preformed polymers using carbohydrate-containing agents. Nevertheless, both strategies generally rely on the same carbohydrate precursors.

In accordance with the great attention that these glycoconjugates have attracted for the last 10 years, several reviews on the synthesis and biological functions of these macromolecules have been published (237–243). In fact, the selection and design of a glycopolymer for a given application is a challenging task because of the inherent diversity of structures and to the impact of the latter and solution conformation of the sugar-containing polymers on their biorecognition. Herein is considered glycopolymers with an emphasis on their structures, their physicochemical and biological properties, and their applications.

**Synthesis Strategy.** Polymer chemistry offers the possibility to prepare glycopolymers with custom-designed properties since several groups with specific properties can be easily introduced along the polymer chain, as shown in the scheme:

![Synthesis Scheme](image)

Indeed, besides the saccharide moieties which are expected to act, in most cases, as recognition signals, various molecules such as hydrophobic chains, fluorescent probes, and drugs can be used to modulate the solution properties of the polymer, label the cells, or deliver a given drug on site. In addition, the polymer backbone can be adjusted to confer desired physical or biological properties.

The most commonly used strategies for the syntheses of such glycopolymers involve direct homopolymerization of sugar-bearing monomers or copolymerization of the latter with one or two other monomers. The next most used strategy involves chemical modification of preformed polymers with suitably functionalized carbohydrate residues. Generally, both methodologies require the same glycosyntons and several methods leading to activated carbohydrate derivatives suitable to be covalently linked to either a preformed polymer or a polymerizable anchor have been described. These methods belong to five main categories, discussed below and depicted in Figures 33–37.

The first one relies on standard glycosylation chemistry, providing O-, C-, or S-glycosides having, for example, alkene, styrene or amine groups in the aglycon portion (Fig. 33) (236,244–255) The intermediate amine-containing glycoside can be used in graft copolymerizations or acryloylated to give a monomer precursor.
The second approach is based on the reductive amination of the saccharide (lactose, maltose, N-acetylchitooligosaccharides, etc) by ammonium acetate, hydrazine, N-methylamine, or 4-acrylamidobenzylamine, followed by N-acryloylation (256,257), addition of 4-vinylbenzyl isocyanate (258) or N-acetylation (259,260) (Fig. 34).

This method is quite easy and rapid; however, it leads to an altered saccharide, the reducing sugar being transformed into a linear polyol amine derivative. Therefore, it is rather applied to oligosaccharides since at least, the terminal sugar is preserved.
In the third approach, the reducing end of the carbohydrate (maltose, lactose, maltotriose, etc) is oxidized by hypoiodite and then, the resulting lactone can be condensed with p-vinylbenzylamine (261) or 1,4-diaminobutane (262) (Fig. 35). For the same reasons as those described above, this reaction is rather applied to oligosaccharides.

In the fourth approach, the carbohydrate can be reversibly converted into a glycosylamine by treatment with an ammonium salt (263,264) or an aliphatic amine (265,266). However, these derivatives are quite unstable in slightly acidic medium or neutral medium. They are further stabilized by acylation with, for example, anhydride acetic (265), p-vinylbenzoyl chloride (267), acryloyl chloride (263), or chloroacetic anhydride, followed by ammonolysis (268) (Fig. 36). The N-linked structure of these glycosynthons is distinct from the open chain structure of the carbohydrate derivatives described above. This method may be useful to introduce complex oligosaccharides.

These different methodologies generally provide unprotected activated glycosynthons. Carbohydrate hydroxyl or amine residues at positions other than the anomeric center can also be used to introduce functional groups for direct or graft polymerization. These carbohydrate precursors can be fully protected or unprotected. However, in the former case, the resulting glycopolymers must undergo a final deprotection step of the carbohydrate moieties under the usual conditions. Some examples of these glycosynthons such as N-methacryloylglycglycylgalactosamine (MA-GG-GalN) (269,270), diacetone glucofuranose acrylate (271), p-vinylbenzylether (272) are given in Figure 37.

![Fig. 36. Synthetic routes to polymerizable glycosylamides.](image1)

![Fig. 37. Examples of carbohydrates monomers bearing a polymerizable group at a position other than the anomeric center.](image2)
In this last approach, although it has been shown in some cases that the carbohydrate–protein interaction remains efficient (269,270), the carbohydrate modification at a position other than the anomeric center may decrease its affinity toward the protein.

**Glycopolymer Syntheses by Homo- or Copolymerization.** The radical polymerization of N-acryloylated carbohydrate precursors with acrylamide and/or acrylamide derivatives with specific properties is likely one of the most commonly used strategies for the syntheses of glycopolymers. Indeed, this is a versatile method which can be easily and efficiently performed in aqueous solution from unprotected sugar-carrying monomers. Moreover, it generally leads to water-soluble derivatives. This method has been extensively used for the preparation of sialic acid bearing polyacrylamides as inhibitors of influenza virus adhesion (251–253,273). Several custom-designed glycopolymers made of two or more components were also synthesized according to this methodology. Various applications of such copolymers have been envisaged as discussed in the following section.

Numerous carbohydrate styrene monomers such as (p-vinylbenzamido)-β-lactose and N-(p-vinylbenzyl)-4-O-β-D-galactopyranosyl-D-gluconamide have been homopolymerized (or copolymerized) in an organic solvent (dimethyl sulfoxide) using 2,2′-azobisisobutyronitrile (261,267). The resulting polystyrenes, having amphiphilic structures, were shown to have strong affinity toward specific lectins. Moreover, the hydrophobic effect of the styrene moiety allowed preparation of polymeric nanospheres with a polystyrene core and a glycopolymer corona. These nanospheres were synthesized by free radical copolymerization in a polar solvent (ethanol/water mixture) of styrene with a hydrophilic carbohydrate macromonomer such as the glucosyloxyethyl methacrylate macromonomer (Fig. 38) (274). The concanavalin A lectin recognized the glucose on the nanospheres with a better binding activity than with the monomeric glucose.

Living polymerization techniques have been successfully used for the synthesis of glycopolymers with controlled molecular weights and molecular weight...
distributions. They have also served for the preparation of well-defined block copolymers. These methods are based on “living” radical polymerizations such as nitroxide-controlled polymerization (271,275) and atom transfer radical polymerization (276), living cationic (277–279) or anionic (280) polymerization (see LIVING POLYMERIZATION, CATIONIC; ANIONIC POLYMERIZATION; CARBOCATIONIC POLYMERIZATION). Thus these polymerization strategies allowed preparation of new amphiphilic block copolymers, the hydrophilic segment having pendant sugar moieties (see Fig. 39). However, one of the drawbacks of the approach is that it involves protected carbohydrate monomers and thus, a final deprotection step of the glycopolymer is necessary.

An alternative method developed by Kiessling and co-workers is ring-opening metathesis polymerization (ROMP) (281–285). A significant advantage of this strategy is that living polymerization can be performed from unprotected carbohydrate monomers including, for example, sulfated sugar-carrying monomers. Moreover, besides the possibility of preparing block copolymers, specific endlabels can be introduced. However, although the molecular weight distribution of glycopolymers obtained by the ROMP strategy is narrower than polymers prepared through classical radical polymerization approaches, the control of molecular weights remains difficult.

Glycopolymer Syntheses by Reactions on Preformed Polymers.

Compared to the methodologies based on polymerizations of sugar-carrying monomers, the chemical modification of preformed polymers using carbohydrate-containing reagents is generally advantageous as it requires fewer reaction steps and it allows easy control of the number of sugars along the polymeric chain. Moreover, large quantities of preformed polymers can be synthesized with the desired molecular weight. Therefore, the same polymer backbone can be used to prepare glycopolymers with different carbohydrate contents and/or carbohydrate residues. Of course, the preformed polymers must have reactive functionalities such as amine, carboxylic acid, or alcohol groups. These polymers may be activated or not. For instance, polyacrylates with \( p \)-nitrophenyl (249,268) or succinimidyl esters (252,255) react at room temperature with carbohydrate amines in aprotic polar solvents (DMSO or DMF) to give glycopolymers after the quenching of excess active esters with sodium hydroxide, ammonia, or ethanolamine.
Carbohydrate amines also react with polymers possessing pendant hydroxyl groups partially functionalized with \( p \)-nitrophenyl carbonate groups (286). Maleic anhydride copolymers have the advantage of being directly modified by carbohydrate amines under mild conditions. This method allowed efficient preparation of various glycopolymers useful for solid-phase assays or new drug delivery systems (262, 287–289). Various carbohydrate derivatives have also been grafted to natural polymers such as chitosan. The free amine group at the C-2 position of the glucosamine unit can easily react with aldehydes which allows preparation of a wide variety of compounds such as L-fucose-, D-fucose-, D-mannose-branched chitosan (290), cyclodextrin-grafted chitosans (225, 291, 292), and a sialic acid dendron-grafted chitosan (293).

Moreover, the pendant carbohydrate chains of synthetic glycopolymers have been shown to be further elongated by enzymatic reactions. Thus, by this approach, Nishimura and co-workers have been able to prepare a glycopolymer having a sialylloligosaccharide using glycosyl transferases and transglycosidases (294). More recently, this strategy allowed the selective modification of a glycopolymer possessing both lactose and mannose residues (254). Indeed, using a galactosyl transferase, a galactose unit could be introduced specifically on the pendant lactose moieties.

**Role of Glycopolymer Structure.** The properties of glycopolymers of course depend on the nature of the pendant saccharide chain as a biorecognizable element, but also on the whole chemical structure. Indeed, the latter influences conformation, charge, biodegradability, etc, which are important parameters for the glycopolymer functions. For example, glycosylated polystyrenes have been shown to exhibit strong binding affinities to lectins, viruses, and cells (267, 295, 296). The strong binding affinity has been attributed to the characteristic conformations based on the amphiphilic structure of glycopolystyrenes. Indeed, the hydrophobic main chain tends to form a hydrophobic core that is sheltered from water, and hence the saccharide chains gather on the outside of the polymer in water. In addition, as mentioned above, the hydrophobic effect of the styrene moiety allowed preparation of original polymeric nanospheres having a polystyrene core and a glycopolymer corona. Furthermore, the significant enhancement of protein–carbohydrate interactions due to hydrophobic associations in water has been well evidenced by Kopecek and co-workers (269) who have synthesized glycopolymers with photoresponsive benzospiropyran side chains. Upon irradiation by UV light, benzospiropyrans change to a red colored polar (zwitterionic) merocyanine form. On exposure to visible light, merocyanine converts back to the nonpolar (hydrophobic) spiro form. Thus, under visible light, the photoresponsive glycopolymers show a high lectin binding due to contraction of the polymer chains, which tends to increase the probability of formation of clustered saccharide chains. After irradiation, the binding decreases as a result of the expansion of polymer chains.

The flexibility of glycopolymers may also have a large influence on their biorecognition. In the case of flexible glycopolymers, the increased content of carbohydrate side chains generally increases biorecognition. This is not confirmed at all in the case of rigid ones. Indeed, rigid cylindrical phenyl isocyanide (PPI) glycopolymers have been shown to exhibit little specific interactions with lectins (297). In these systems, the saccharide arrays are crowded too thickly to be accessible or to be induced-fit to the binding sites. The decrease of saccharide density
by insertion of comonomer units into the rigid PPI glycopolymer backbone was found to increase the binding affinity to the lectin.

**Applications of Glycopolymers.** Most applications of glycopolymers arise from the specific molecular and cell recognition of saccharides. Therefore, many bio- and immunochemical properties have been demonstrated for a large number of glycopolymers. The synthesis of sugar-based hydrogels from carbohydrate monomers and cross-linking agents or by covalent attachment of carbohydrates to commercially available affinity supports made of agarose, cellulose, or polyacrylamide allowed preparation of new bioaffinity supports (298–300). The latter have been used for the purification of antibodies, enzymes, lectins, and myeloma proteins. Sugar-based hydrogels also show potential as substrates for cell growth. Indeed, the replacement of agar, used in culture plates, by hydrogels containing specific sugars may enhance the adhesion of cells. Such properties have also been evidenced by a lactose-carrying styrene homopolymer (301,302). The latter has been shown to be adsorbed on the surface of glass culture dishes and the resulting polymer-coated dishes have been found to be useful for hepatic cell cultures.

Moreover, glycopolymers have been shown to constitute very sensitive coating antigens in solid-phase enzyme-linked immunosorbent assays (ELISA). In comparison to protein glycoconjugates, glycopolymers offer several advantages such as lower cost productions and improved thermal and biological stabilities. Therefore, many examples of applications of glycopolymers in the detection of carbohydrate-binding proteins including serodiagnosis of bacterial antigens have been reported. These have been summarized in several reviews (240–242).

Glycopolymers can also be advantageously used to inhibit cell adhesion. Typical examples are polymers bearing pendant α-sialoside groups which show potent anti-influenza activity (249,251–253,268,273,303). Indeed, it is now well established that human infections by influenza viruses are mediated by the binding of the viral membrane protein hemagglutinin (hyaluronan) to sialosides on cell surface glycolipids and glycoproteins. The inhibitory properties of such glycopolymers illustrate the value of cooperative polyvalent interaction in the design of potent inhibitors of viral adherence to the host cell.

Glycopolymers also show promising properties as drug-targeting delivery agents. One of the most successful polymer–drug–saccharide conjugate systems reported are the N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–adriamycin (ADR)–sugar conjugates, in which both ADR and sugar were attached to a polymer backbone via lysosomally cleavable oligopeptide spacers (304). The conjugates containing galactosamine moieties were reported to accumulate in the liver selectively (305). Other glycopolymers such as lactose-carrying styrene polymers were also found to be very efficient liver-specific targeting materials (306).

Besides these fields of applications, Wulff and co-workers (307,308) investigated the potential ability of glycopolymers to modify surface properties. Thus, they copolymerized several isopropylidene-protected vinyl sugars with styrene, methyl methacrylate, and acrylonitrile. After eliminating the isopropylidene protecting groups from the copolymer surfaces by acid hydrolysis, these surfaces were shown to become hydrophilic with improved dyeability and surface conductivity.
Synthesis of Oligo- and Polysaccharides

The chemical synthesis of oligosaccharides or their analogues is well developed now; nevertheless, it is a difficult task. Protection of labile groups of the –OH position which are not engaged in an osidic linkage is needed. Then, reaction with specific groups of the anomeric and nonanomeric position must be done with a control of the stereochemistry of the anomeric position. Oligosaccharides in which glycosidic oxygen atoms are replaced by sulfur atoms can be routinely synthesized by iterative or convergent approaches (309,310) and these nonnatural compounds are not hydrolyzed by various glycosidases (311,312).

Polysaccharides having a regular structure were first obtained by polymerization of anhydrosugars or step-by-step elongation. For synthesis of irregular structures, a step-by-step elongation by polycondensation is the usual method at least to produce the repeating unit which is then polymerized. These routes have been discussed previously in the review of Kochetkov (313).

A different but interesting approach comes from the enzymatic polymerization of the corresponding mono- or disaccharide precursors with controlled structure.

Synthesis of Regular Polysaccharides. The first attempt at such synthesis appears with the work of Haq and Whelan in 1956 (314); they proposed, without success, the polycondensation of 2,3,4,6-ti-O-acetyl-α-D-glucosyl bromide in the presence of Ag₂O and CaSO₄ to get a (1–6)-β-D-glucan. Other negative attempts have been published (313).

The most fruitful results came with the synthesis of polysaccharides by polymerization of anhydrosugars. Schuerch (315–317) investigated the mechanism of cationic polymerization in solution of 1,6-anhydro-hexopyranoses wherein the 2,3,4 positions were protected by benzyl groups. He got high molecular weight (1,6)-α-D-glucan and the benzyl groups were easily removed at the end of polymerization. The 1,4; 1,3; 1,2 anhydrosugar polymerizations were also performed with less success. However, (1–3)-α-D-glucopyranans and (1–3)-α-D-mannopyranans were obtained with high stereoregularity. These results are discussed in the review by Kochetkov (313).

Chemoenzymatic Synthesis. Original oligosaccharides bifunctionalized with fluorescent entities were synthetized in the presence of a mutant of Humicola insolens endoglucanase (318). Hemithiocellodextrins with degree of polymerization from 4 to 14 were synthetized in the presence of a cellulase in buffer/organic solvent (319).

Homopolymers were also obtained with mutant of Barley (1,3)-β-D-glucan endohydrolase producing (1,3)-β-D-glucan and with mutant of cellulase producing β-(1→4)-oligo- and polysaccharides (320,321).

A very active research is lead by the Kobayashi group in Japan; in a recent review, cellulose, chitin, and xylan syntheses were described using Enzymatic Polymerization (qv) (322).

Hydrolases were most often used as catalyst forming the glycosidic bonds in vitro. Recently, synthetic polysaccharides were synthetized using other enzymes such as glycosyltransferase, phosphorylases, oxidoreductases, and lipases (323).
Copolymers may also be produced using combinations of chemically modified mono- or disaccharides and enzymatic condensation. Such a route has been proposed to prepare an artificial hyaluronan, GlucA-β-(1→3) GlcNAc oxazoline monomer reacting with hyaluronidase (324).

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