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GLYCOCHEMISTRY: OVERVIEW AND PROGRESS

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1.1 INTRODUCTION

Officially, the International Union of Pure and Applied Chemistry (IUPAC) defines glycan as "synonymous with polysaccharides," meaning compounds consisting of a large number of monosaccharides linked to each other through glycosidic bonds [1]. Practically however, the term glycan is all encompassing and often used to describe the carbohydrate portion of glycoconjugates such as glycoproteins and glycolipids. Carbohydrates are the most abundant organic molecules on Earth and are the main products through which the energy of the sun is harnessed and stored. Glucose polysaccharides, such as starch in plants and glycogen in bacteria and animals, serve as a source of energy for essentially all organisms. However, the complex roles of carbohydrates are not limited to simply that of biological fuel stocks or biosynthetic starting materials. DNA and RNA, which transmit and store genetic information, have sugar backbones. Other carbohydrate polymers are essential structural and protective components of the cell walls of plants as cellulose, bacteria as peptidoglycan, and the exoskeletons of arthropods as chitin. They are important constituents of secreted and cell-surface proteins, membrane components in the form of glycolipids and gangliosides, as well as various types of extracellular matrix molecules [2]. The significance of the carbohydrate domains of glycoproteins and glycolipids is further exhibited in their roles as cell-surface recognition elements and as determinants in blood-group typing [3, 4]. Carbohydrates are also appended to various natural products including antibiotics [5]. As such, glycans mediate a wide range of biological processes from

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embryonic development to differentiation, signaling, host–pathogen interactions, metastasis, intracellular trafficking, and localization [6].

The many hydroxy groups that adorn the carbon backbone of glycans give rise to multiple stereoisomers, a fact that has been capitalized on for use as chiral synthons in organic synthesis [7]. The nine common monosaccharides found in mammalian cells can be linked in an astonishing number of ways, resulting in much higher complexity than is possible from amino acid or nucleotide building blocks. Unlike proteins and DNA, glycans encode immense biological information without being template driven or encoded by the genome. The first draft of the human genome revealed a relatively small number of genes associated with the human species—approximately 32,000—as compared to less complex organisms such as fly or worm, which encompasses roughly 13,000 or 18,000 genes, respectively [8–10]. While the origin of biological complexity remains a largely debated topic, one hypothesis accounting for this paradox is the posttranslational modifications of proteins.

Glycosylation is one of the most ubiquitous forms of posttranslational modification and is widely recognized as a modulator of protein structure, localization, and function. Because glycosylation is not under tight genetic control, often complex and unpredictable mixtures of glycoforms with varying properties are produced [11, 12]. Therefore, access to homogeneous glycolipids, glycopeptides, and glycoproteins is an essential step toward furthering our understanding of these important molecules. Over the past century, significant developments have occurred, from the establishment of a carbohydrate nomenclature to discovering the simple building blocks that make up oligosaccharides and how they combine to create unique structures. These advances have enabled studies that reveal the multifaceted roles of glycans.

1.2 NOMENCLATURE, STRUCTURES, AND PROPERTIES OF SUGARS

Most simple sugars have the general formula $C_n(H_2O)_n$, where n is between three and nine. Early nineteenth-century French chemists generically defined carbohydrates as "hydrates de carbone" because they were thought to consist solely of carbon and water in a 1 : 1 ratio. However, the term is used today in a much broader sense. Saccharides can be roughly split into two categories: monosaccharides and complex saccharides such as oligosaccharides and polysaccharides. Depending on their size, oligosaccharides and polysaccharides tend to exhibit different chemical and physical properties as compared to monosaccharides. Polysaccharides can form stable secondary and tertiary structures and are hydrolyzed into smaller subunits upon treatment with aqueous acid, while monosaccharides can be found in a variety of forms including linear and cyclic structures. Monosaccharides are the building blocks from which oligosaccharides and polysaccharides are constructed. They include polyhydroxyaldehydes (aldoses) and polyhydroxyketones (ketoses) as well as the resulting compounds derived thereof by either the reaction of the carbonyl group, via oxidation to form carboxylic acids, or by replacing one or more hydroxy groups with hydrogen, amino, acetamide, thiol, or other functional groups (Fig. 1.1).

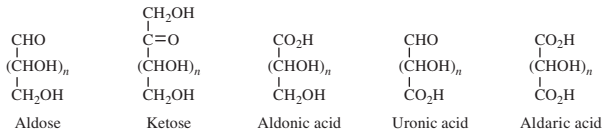


FIGURE 1.1 Common carbohydrate oxidation levels.

Monosaccharides are classified according to the number of carbons in their skeleton per IUPAC recommendations [13]. The suffix -ose is used to indicate an aldose, while -ulose denotes a ketose. Accordingly, the common aldoses in ascending order would be trioses, tetroses, pentoses, hexoses, etc. Prior to their structures being known and the subsequent systematization developed by Emil Fischer, carbohydrates were named based on either their sources (fructose for fruit sugar, glucose for grape sugar, lactose for milk sugar, and sucrose for cane sugar) or physical properties (dextrose for glucose because it rotates plane-polarized light in a clockwise manner (dextrorotation) and levulose for fructose because of its levorotatory nature). Note that each secondary carbon of the sugar alcohols is sp^3 hybridized and represents a stereogenic or chiral center. A uniform method to visualize this tetrahedral geometry in two dimensions came in the form of the Fischer projection. While the Fischer proof is discussed later, this work largely eliminated inconsistencies in the representation and naming of sugars.

1.2.1 Fischer Projection

The Fischer projection is a convenient way of showing the configurations of the linear forms of monosaccharides. This convention depicts the concepts of stereochemistry established by Jacobus Henricus van 't Hoff and Joseph Achille Le Bel in a simplified form. While these abbreviated structural formulas are simple to write and easy to visualize, there are some guidelines that should be taken into account when converting a three-dimensional structure into a Fischer projection and in its manipulation (Fig. 1.2):

1. Orient the molecule in such a way that the chiral center is in the plane of the paper with its vertical bonds toward the back and the horizontal bonds coming out in front.
2. Position the carbon atoms of the chain on the vertical plane with the carbonyl group on top and the primary alcohol at the bottom. The hydrogen and hydroxy moieties should be oriented horizontally. Numbering of the carbon atoms begins with the carbonyl group in the case of aldoses or the terminal carbon closest to the carbonyl group in the case of ketoses.
3. Flatten the resulting model by "pulling" the vertical bonds toward the plane of the paper and "pushing" the horizontal bonds into the plane of the paper. The stereogenic carbon and the attached hydrogen atoms can then be omitted for clarity.

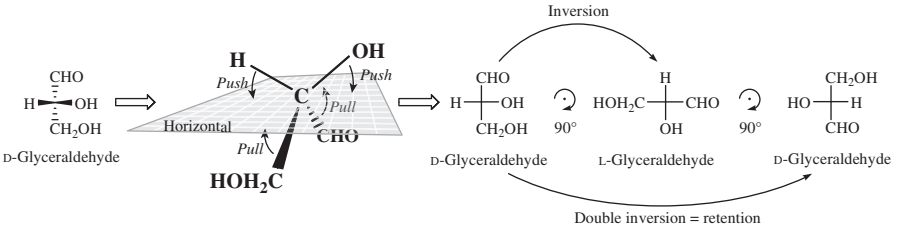


FIGURE 1.2 Fischer projection of glyceraldehyde and its manipulation.

4. Define the most distant stereogenic center from the carbonyl group as the reference atom. The stereoisomer in which the highest priority substituent of the reference atom is pointed to the right is assigned the prefix "D" and to the left is assigned the prefix "L." This prefix designates the absolute configuration at the center of reference. A trivial name, on the other hand, is used to indicate the relative configuration of all other chiral centers in relation to the reference stereogenic center (Fig. 1.3). Consequently, the D- and L-isomers of a given trivial name are mirror images of each other.
5. Fischer projections must only be rotated in increments of 180° as a 90° rotation represents an inversion of configuration at the stereogenic center.

1.2.2 Linear Forms of Monosaccharides

Emil Fischer deduced the stereochemical relationship between monosaccharides using D-glyceraldehyde as the reference molecule. Ultimately, Fischer applied his proof to create the D-aldose family tree (Fig. 1.3), which is still in use to this day. The abbreviated names for aldopentoses and aldohexoses consist of the first three letters of their trivial names except only for "Glc," which is used for glucose ("Glu" had already been assigned to glutamic acid). The "D" (or "L") prefix in the abbreviated names may be omitted when referring to the more abundant isomer. Epimers are carbohydrates that differ only in the configuration at one stereocenter, a relationship that is readily apparent by comparing their Fischer projections. For example, glucose is the C2 epimer of mannose. The trivial names of aldoses may form configurational prefixes, such as *glycero*, *erythro*, *arabino*, *xylo*, *galacto*, *manno*, and *gluco*, in combination with the "D" or "L" notation to describe other sugars. These prefixes point to analogous, but not necessarily contiguous, sequences of chiral centers present in the molecule and may be combined to reflect the stereochemistries embedded in monosaccharides larger than hexoses [13]. Figure 1.4 shows the structures and the trivial and derived names for the D-ketoses as their Fischer projections.

While both the configuration and naming can also be assigned using the Cahn–Ingold–Prelog system, it is typically only used to describe attached chiral substituents as opposed to the stereochemistry of the sugars themselves. The main disadvantages to the application of the Cahn–Ingold–Prelog convention are the lengthy and complicated names that result and the fact that replacement of the terminal carbon may result in prefix changes to unchanged centers of analogues with the same configuration.

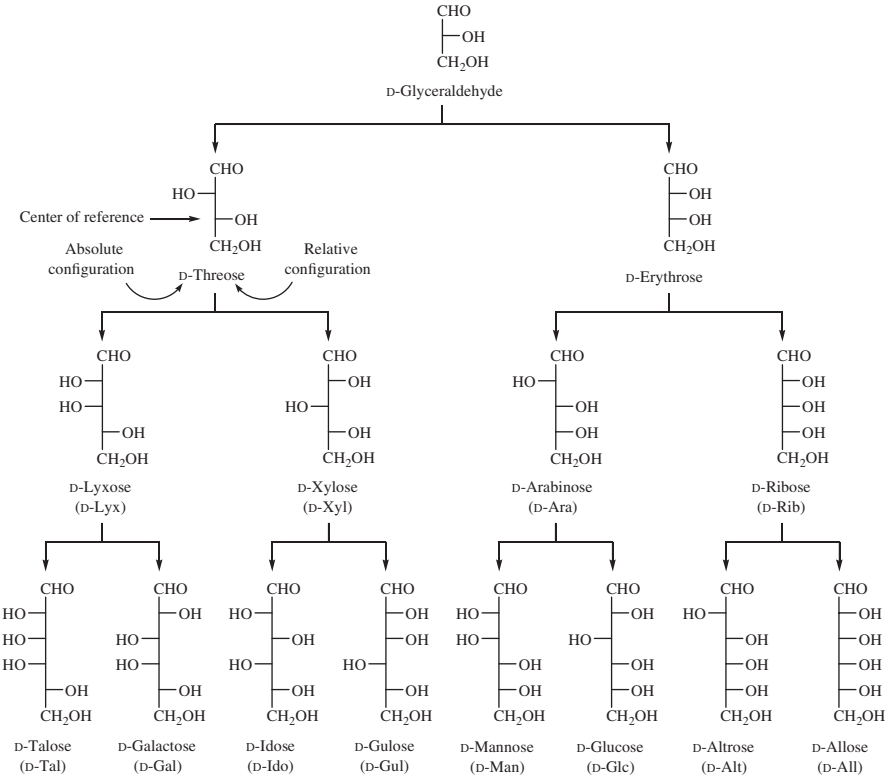


FIGURE 1.3 The family tree of D-aldoses with the trivial and abbreviated names.

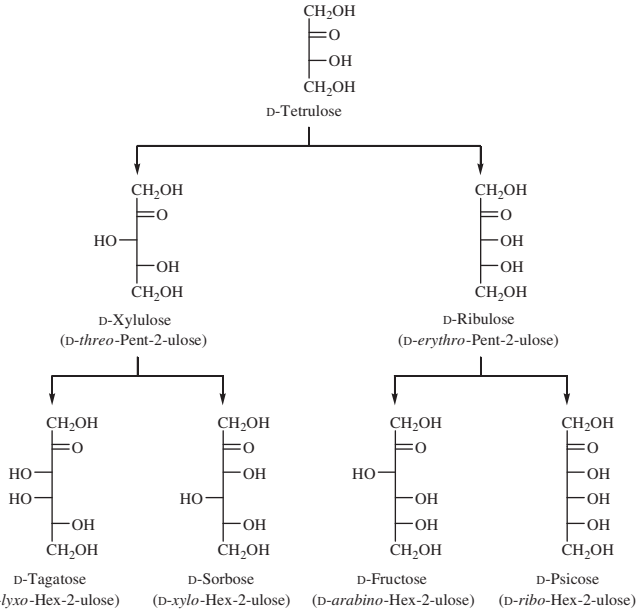


FIGURE 1.4 The family tree of D-ketoses with the trivial and derived names.

1.2.3 Cyclic Forms of Monosaccharides

The electrophilic nature of carbonyl groups is well known. For example, they readily react with nucleophiles including water and alcohols, resulting in the formation of hydrates or hemiacetals in a reversible process. In the case of sugars, this can be an intramolecular process due to the presence of both an electrophilic carbonyl and nucleophilic alcohols. Cyclization typically affords five-membered furanoses or six-membered pyranoses and is entropically favored over intermolecular attack. Further contributing to their stability is the relatively small amount of torsional strain associated with these constructs. As an interesting aside, it was Norman Haworth (1883–1950) who coined the terms "furanose" and "pyranose" in 1927 from tetrahydrofuran and tetrahydropyran, respectively [14]. Cyclic sugars can be depicted using Fischer projections as well. This simply involves drawing a loop between the hydroxyl involved and the former carbonyl carbon. Importantly, as the bond is a continuation of the carbon skeleton, it must enter the carbonyl from the top. This process leads to the formation of an additional chiral center known as the anomeric position and two diastereomers designated as α and β. These newly formed diastereomers known as anomers differ only in the configuration about the anomeric carbon. For simple monosaccharides up to aldohexoses and hept-2-uloses, the α and β designation is based on the relationship between the anomeric exocyclic substituent and the oxygen attached to the center of reference (Fig. 1.5). The anomer is α if these substituents are formally *cis* in a Fischer projection and β if they are formally *trans*. In larger monosaccharides, the reference atom used for the anomeric assignment is the highest-numbered carbon in a configurational prefix formed by the group of chiral centers closest to the anomeric carbon.

1.2.4 Haworth and Mills Projections

A major drawback of cyclic Fischer projections is the unrealistic manner in which the structures are depicted. In 1929, Haworth designed a representation to address this deficiency. Haworth projections provide a simple way to represent cyclic monosaccharides with a three-dimensional perspective. The following process allows the conversion of a Fischer projection into a Haworth representation:

1. Identify the hydroxy group that will be reacting with the carbonyl carbon. In the aldopyranose form, this is 5-OH.

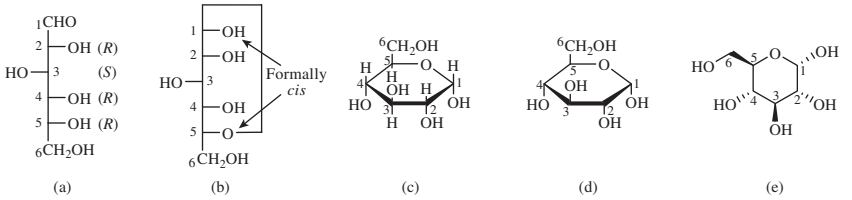


FIGURE 1.5 The (a) linear Fischer projection of D-glucose and the (b) cyclic Fischer, (c) Haworth, (d) simplified Haworth, and (e) Mills projections of α-D-glucopyranose.

2. Manipulate the Fischer projection such that this hydroxyl is at the bottom after exchange with the terminal (C6) functional group.
3. Draw the carbohydrate skeleton such that the ring is drawn on its side. The face closest to the viewer is drawn at the lower side and with a thicker line than the more distant upper side. The ring oxygen is located in the upper right-hand corner for pyranoses and at the top for furanoses.
4. Populate the ring substituents such that those on the right side of the Fischer projection are on the bottom face of the Haworth projection and those on the left side are on the top. Hydrogen atoms are typically omitted for clarity.

William Mills described a similar convention to depict the structures of monosaccharides. While the ring atoms of the Haworth projections are oriented perpendicular to the paper, Mills chose to depict the carbon skeleton in the plane of the paper (Fig. 1.5). Although Fischer, Haworth, and Mills projections are useful tools for depicting the structures of carbohydrates, the planar nature of these representations does not provide an accurate picture of the actual geometry of the molecules. In order to understand carbohydrate function and reactivity, recognition of each distinct conformation and the properties associated with it is required [15].

1.2.5 Reeves Projection

In 1949, Richard Reeves remodeled the Haworth projection by applying the ring conformations of cyclohexane to describe the structures of pyranoses in solution [16]. The Reeves convention is based on the similarity between the geometry of pyranoses to that of the model. Importantly, the assumption that the ring oxygen only introduced a slight conformational perturbation from that of cyclohexane was later confirmed by nuclear magnetic spectroscopy (NMR) spectroscopy. A major advantage of this convention is that it closely resembles the actual shape of the molecule, thereby allowing one to predict the distances and dihedral angles between the substituents. These values can be compared to those determined using the Karplus equation [17, 18] and applied to the interpretation of the NMR spectra. Taken together, it is a relatively trivial task to resolve the predominant averaged conformations of a monosaccharide. Figure 1.6 shows the Reeves projections for all furanose and pyranose structures associated with the α-D-pentoses and α-D-hexoses.

1.2.6 Conformational Analysis

Most molecules tend to favor one conformer over the others based on the stereochemistry of the particular monosaccharide and the steric bulk of the groups that are appended to it. For example, most aldohexoses prefer the chair conformation that places the bulky C5 hydroxymethyl group in the equatorial position. Having said that, the energy barrier between the two possible chair conformations is

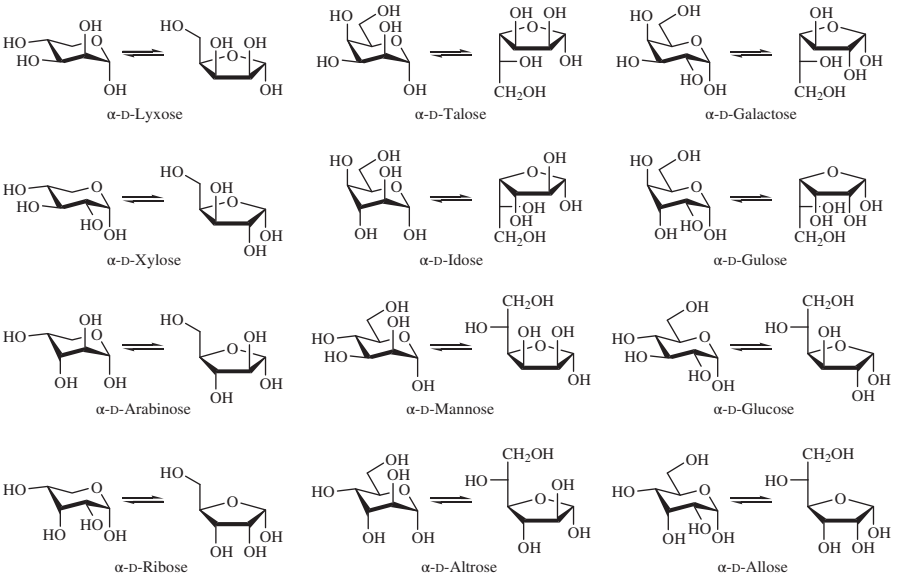


FIGURE 1.6 Reeves projections for α-D-pentoses and α-D-hexoses.

generally low enough to allow conformational flexibility and equilibrium to be established. Interconversion between the two chair conformations involves the rotation of ring atoms and bonds. This process requires the molecule to adopt several distinct conformations with respect to energy and the position of the ring atoms. The current convention for describing a particular conformation begins with assigning reference points. When naming conformations, the reference point above the plane is denoted as a superscript preceding the conformational descriptor and is followed by the one below the plane expressed as a subscript. The names and descriptors of the main conformations of pyranoses are boat (*B*), chair (*C*), envelope (*E*), half-chair (*H*), and skew (*S*). There are 2 discrete chair, 6 boat, 6 skew, 12 half-chair, and 12 envelope conformations (Fig. 1.7) [15]. The reference plane of the boat conformation consists of the two parallel sides of the boat. Of the remaining two out-of-plane atoms, one must be the lowest-numbered ring carbon. The same two parallel sides define the chair conformations as long as the lowest-numbered ring carbon resides above or below the plane. In the half-chair conformation, the reference plane is determined by four adjacent coplanar atoms, leaving the remaining two atoms on opposite sides of the plane. The reference plane of the envelope conformation includes the five adjacent coplanar atoms. The skew conformation contains two exoplanar atoms, one of which must be the lowest-numbered carbon atom. The reference plane is defined as the three adjacent atoms and the remaining coplanar nonadjacent one.

Furanose rings also exhibit a degree of conformational mobility, albeit to a lesser extent. The two predominant conformations adopted by these five-membered rings are envelope (*E*) and twist (*T*). There are 10 individual envelope

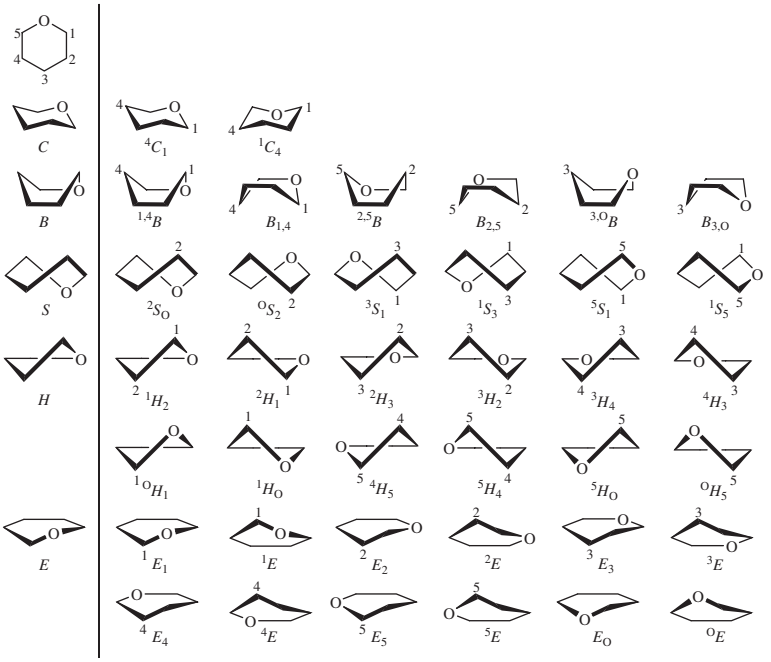


FIGURE 1.7 Pyranose ring nomenclature and conformations.

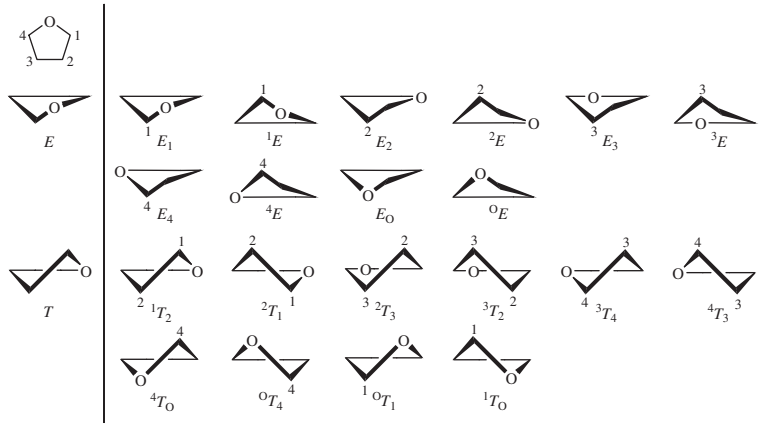


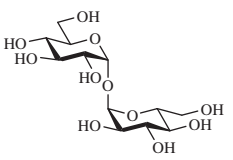
FIGURE 1.8 Furanose ring nomenclature and conformations.

and 10 twist conformations (Fig. 1.8) [15]. The reference plane of the envelope conformation is defined by the four adjacent coplanar atoms, with the remaining one either above or below this plane. For the twist, the reference plane is defined by three contiguous coplanar atoms, with the remaining two atoms placed on opposite sides of the plane.

1.2.7 Disaccharides, Oligosaccharides, and Polysaccharides

While the aforementioned monosaccharides are important in their own right, they also serve as building blocks for the assembly of more complex carbohydrates or glycans. The simplest of these is disaccharides, which are formed by the condensation of two monosaccharides. The two monomers are joined through at least one of the anomeric carbons via an acetal bridge. This newly formed bond is known as a glycosidic linkage. Typically, this process involves linking the anomeric carbon of one sugar to a nonanomeric hydroxyl of another, forming a reducing disaccharide. However, both of the constituent monosaccharides can be coupled through their anomeric centers to produce a nonreducing disaccharide. Either sugar can be present in its pyranose or furanose form and in a combination of both α and β anomers. While many disaccharides have long-standing trivial names, all of these variables can make their systematic naming rather tedious. The nomenclature of these compounds includes the abbreviated names of the constituent monosaccharides, the ring size (pyranose (*p*) or furanose (*f*)), the configuration of the anomeric centers, and the location of the glycosidic bond. The position of the anomeric linkage is reported in parenthesis. For nonreducing disaccharides, the positions should be separated by a double-headed arrow, while for reducing disaccharides a single-headed arrow pointing in the direction of the nonanomeric position is used (Fig. 1.9). A condensed form of this nomenclature is also allowed wherein the descriptors referring to the more abundant form of the sugar residue are omitted.

Even though the term "oligosaccharide" is not rigorously defined, it is generally used to describe complex carbohydrates composed of between 2 and 10 monosaccharide units. Oligosaccharides can be linear or branched in nature but are of distinct structure. If there are branches present, the longer arm is designated as the parent, and all connections are explicitly stated within square brackets. If two or more of the branches are of equal length, they are prioritized from the branching point. Thus, the parent arm is the one with the lowest point of attachment. Polysaccharides are biopolymers consisting of more than 10 monosaccharides. These complex structures are found as homopolysaccharides or heteropolysaccharides. Polysaccharides can assume highly ordered secondary and tertiary structures or exhibit random behavior. Their physical properties are determined largely by chain conformation, intra- and intermolecular interactions, and the solvent they are dissolved in.

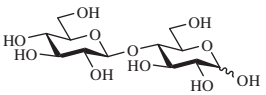


Trehalose

Extended form: α-D-Glc*p*-(1↔1)-α-D-Glc*p*

Condensed form: Glc(α1↔α1)Glc

Nonreducing disaccharide



Cellobiose

Extended form: β-D-Glc*p*-(1→4)-D-Glc

Condensed form: Glc(β1→4)Glc

Reducing disaccharide

FIGURE 1.9 Nomenclatures and structures of selected disaccharides.

1.2.8 Anomeric Effect

It is widely known that equatorial substituents of six-membered cyclic hydrocarbons are less sterically demanding and energetically preferred as compared to their axial counterparts. This is largely due to unfavorable 1,3-diaxial interactions associated with axial substituents. However, in contrast to cyclohexane, pyranose rings contain an endocyclic oxygen atom (O5 in aldohexoses) adjacent to the anomeric center (C1). In 1955, John Edward identified an axial bias for the anomeric substituents of sugars [19]. Raymond Lemieux later defined this phenomenon as the "anomeric effect" [20]. Although there are a number of theories, this effect is largely attributed to a combination of hyperconjugative and electrostatic effects [21]. In both cases, the nonbonding electron pairs of O5 play a major role. This is especially apparent when there is an electronegative moiety at the anomeric position. The combination of the adjacent ring oxygen and an electronegative substituent X having nonbonding electrons (where X is defined as O, S, N, F, Cl, Br, or I) at the anomeric position renders C1 particularly electron deficient. When X is in the axial position, a lone pair of electrons from O5 is positioned antiperiplanar to the C1—X antibonding orbital. Delocalization of these electrons can stabilize the electron-deficient anomeric center through hyperconjugation. This stabilizing n–σ* interaction is not possible when X is in an equatorial position (Fig. 1.10a) and also explains why the anomeric effect becomes more dominant as the electronegativity of X increases. Electrostatics also plays a role in the observed axial preference of electronegative anomeric substituents. While hyperconjugation is a stabilizing interaction, some electronic effects can be described in terms of alleviating unfavorable dipole–dipole interactions. When X is in an equatorial orientation, its exocyclic lone pairs exhibit a strong repulsive

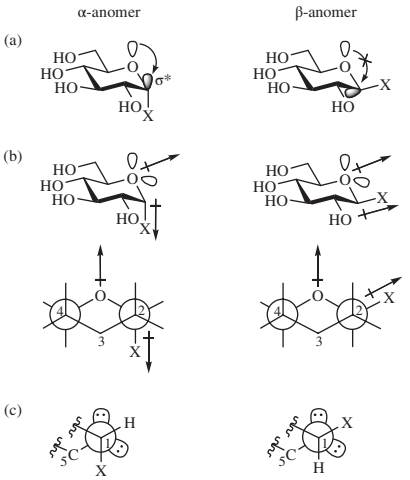


FIGURE 1.10 The anomeric effect. (a) The n–σ* interaction stabilizes the α anomer. (b) The β anomer experiences unfavorable dipole–dipole interaction that is reduced in the α anomer. (c) Greater electrostatic repulsion between the lone-pair electrons of the endocyclic oxygen and the electronegative anomeric substituent in the β anomer.

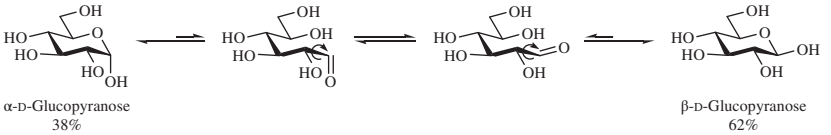


FIGURE 1.11 Mutarotation of D-glucose in water at pH 7.

electrostatic interaction with the O5 lone-pair electrons. These destabilizing interactions are drastically reduced when X is in the axial position (Fig. 1.10b). Another feature attributed to the anomeric effect is the preference for synclinal (*gauche*) over antiperiplanar (*anti*)conformations such as in the system C5—O5—C1—X (Fig. 1.10c). Looking along the C1—O5 bond, one can see that, in the *anti*-conformer, the electronegative heteroatom is placed between two lone pairs, resulting in greater electrostatic repulsion.

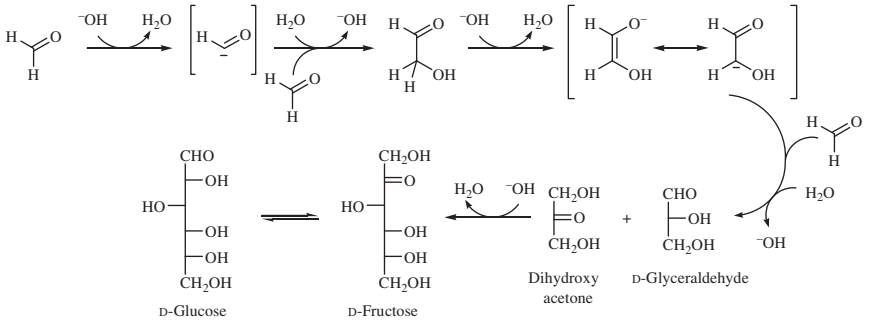
There is a balance between the stabilizing anomeric effect (which favors the α anomer) and other factors that contribute to the anomeric preference of a particular sugar, such as solvent effects and sterics (which can favor the β anomer). A good example to illustrate this duality is the mutarotation process.

1.2.9 Mutarotation

Mutarotation [22] is defined as a change in optical rotation due to epimerization. When a crystalline sample of pure α-D-glucose is dissolved in water at neutral pH, its initial optical rotation value is +112°; but after approximately 3 h at 20°C, this value decreases until an equilibrium value of +52.7° is reached. The same equilibrium value is observed if one starts with a sample of pure β-D-glucose. In the crystalline form, the ring size and anomeric stereochemistry are fixed. However, in solution, ring opening and hydrolysis contribute to an equilibrium, resulting in a combination of both anomers (Fig. 1.11) and to a lesser degree a mixture of pyranose and furanose forms. This process results in a change in the optical rotation of the solution as the equilibrium is established. If anomeric or steric effects were solely responsible for the configuration at the anomeric position, the equilibrium would lie heavily to one side, which is not the case.

1.3 HISTORICAL OVERVIEW OF CARBOHYDRATE RESEARCH

Sugars, such as fructose and glucose from honey, have been harvested and processed by humans since the Stone Age [23]. The use of sucrose as a sweetener dates back to the eighth century BC and could only be afforded by royalty and the very wealthy [24]. More recently, these natural products become critical in a variety of industries focused on the production of paper, pulp, textiles, and pharmaceuticals. As often is the case, industrial applications ultimately provided the economic impetus for investigations into carbohydrate synthesis, purification, and characterization in the late nineteenth century.



SCHEME 1.1 The formose reaction.

Any discussion of the beginning of carbohydrate chemistry should include Alexander Butlerov's discovery of the formose reaction in 1861 [25]. Subsequent experiments identified that out of all the possible formose products, glucose was present in the highest concentration [26]. Indeed, glucose is the most abundant sugar found in nature. This finding is of historical significance as the formose reaction provides a plausible route to ribose and other sugars from simple formaldehyde building blocks (Scheme 1.1).

1.3.1 Emil Fischer (1852–1919): The Father of Carbohydrate Chemistry

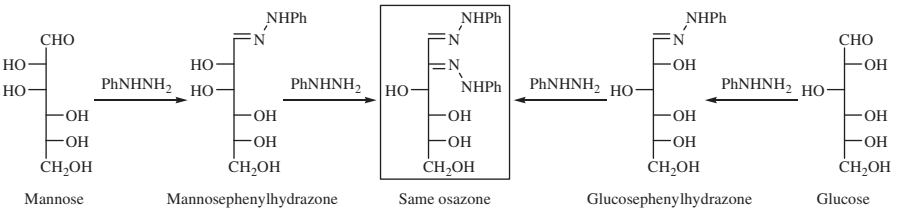
The structures and properties described in the previous sections would not be possible without the knowledge and insight provided by the "father of carbohydrate chemistry," Emil Fischer. Until the work of Fischer [27, 28], progress was made largely by the empirical observations of alchemists. In 1891, his pioneering work and that of his students provided the structural characterization and relative configurations of monosaccharides via a combination of analytical (polarimetry) and chemical techniques [29, 30]. It is a combination of the complexity and limited chemical and analytical tools available that made the Fischer proof so profound. While this skillfully planned and beautifully executed work has been reported in detail elsewhere [27, 28, 31–34], its significance warrants highlighting.

In 1870, only two aldohexoses (glucose and galactose) and one ketose (fructose) were known. Three disaccharides (lactose, maltose, and sucrose) had also been identified. Perhaps the first milestone on a path that ultimately led Fisher to the Nobel Prize in Chemistry (1902) was the serendipitous discovery of the reagent phenylhydrazine in 1875 [35]. Although he found it reacted rapidly with aldehydes, resulting in the formation of the corresponding phenylhydrazones, it would be 9 years until he applied this tool to the characterization of carbonyl compounds and an additional 7 years before his structural assignment of the sugars was achieved. At that time, it was known that glucose was composed of 6 carbons, 6 oxygens, and 12 hydrogens and that it reduced Tollens' reagent. Heinrich Kiliani also described the conversion of glucose and galactose to *n*-heptanoic acid, thereby confirming that they are aldohexoses. Taken together, these experiments supported the hypothesis

that glucose is a pentahydroxy aldehyde. Moreover, Kiliani identified fructose as a 2-ketohexose via the isolation of 2-methylhexanoic acid. The process he used involved the formation and subsequent hydrolysis of the corresponding cyanohydrins followed by reduction with hydrogen iodide and red phosphorus [36]. In order for a compound to be considered pure at the time, it needed to be isolated in crystalline form and possess a constant melting point and optical rotation. Fortunately, osazones (1,2-bishydrazones) formed by the reaction of sugars and phenylhydrazine were often crystallizable and readily characterized. Fischer utilized this property to demonstrate that the osazones of glucose and fructose were identical, providing evidence that they share the same configuration at C3, C4, and C5. In a subsequent paper, Fischer reported that the isolation and identification of phenylhydrazone intermediates were possible when the reaction was conducted at a reduced temperature. It was this discovery that ultimately led to the conclusion that glucose and mannose are C2 epimers, as they yielded the same osazone but different hydrazones (Scheme 1.2).

Although the postulate of Le Bel and van 't Hoff was based solely on theoretical considerations, it provided an explanation for the occurrence of the numerous isomers that were inexplicable on the basis of the structural formulas of the time [37, 38]. Fischer applied this theory as the foundation of his stereochemical deductions, ultimately resulting in the assignment of a tetrahedral geometry for carbon atoms. The next major breakthrough came in 1889 when Fischer discovered that sodium amalgam could be used to reduce the lactones of sugar acids to their corresponding aldoses. For example, mannonic acid lactone was reduced to mannose in this manner [39]. The combination of this reaction with the known cyanohydrin procedure led to the conclusion that the D-enantiomers of arabinose, glucose, mannose, and fructose all share the same configuration at the three highest-numbered chiral centers. Polarimetry was critical for establishing the final piece of evidence needed to determine the configuration of the aldopentoses. Of the 1,5-dicarboxylic acids derived from the nitric acid oxidation of pentoses, D-arabinose was optically active, while those of D-ribose and D-xylose were not. Therefore, they were identified as *meso*-compounds, allowing correlation between the configuration and optical activity of aldopentoses.

Finally, oxidation experiments on D-gluconic and D-gulonic acid produced the head-to-tail enantiomers D/L-glucaric acid. Thus, it was apparent that these acids could only be derived from D-glucose and D-gulose. In a seminal report published in 1891, Fischer

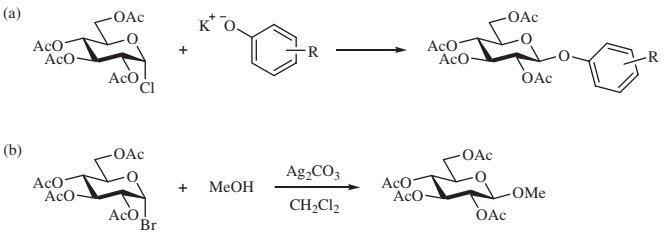


SCHEME 1.2 Convergence of glucose and mannose to the same osazone.

described the configuration of glucose, mannose, and fructose in accordance with the van 't Hoff convention [29]. Just over 2 months later, Fischer replaced the van 't Hoff notation with his own projections to eliminate the confusion associated with the depictions [30]. Fischer projections have since become the universally accepted way to represent the linear form of sugars in two dimensions [40]. As an interesting aside, Fischer arbitrarily decided to place the hydroxy substituent of the lowest chiral center of D-glucose (C5) to the right, a choice that was later confirmed by X-ray crystallography some 60 years later [41]. It should be emphasized that Fischer set out to accomplish this monumental task with limited knowledge of carbohydrate chemistry, without an understanding of the concept of stereochemistry, having crystallization as the primary method of purification, and limited access to reference compounds. In the end, it was a mixture of brilliance, determination, and luck that resulted in the Fischer solution. He not only provided a strong foundation upon which the disciplines of organic chemistry and biochemistry were built, but his inspiration was transferred to over 300 doctoral students and postdoctoral researchers, stimulating the next generation of scientists.

1.3.2 Koenigs–Knorr Reaction

With limited information about the structure and function of carbohydrates, the work of a few brilliant scientists during this early period provided the foundation for the advances that were just around the corner. For example, Arthur Michael reported the first chemical glycosylation in 1879. The reaction involved the nucleophilic displacement of an anomeric halide by the potassium salts of various phenols (Scheme 1.3a) [42, 43]. The beginning of the twentieth century was marked by the discovery of perhaps the most commonly used glycosylation method, the Koenigs–Knorr reaction (Scheme 1.3b) [44, 45]. Initial reaction conditions involved the displacement of an anomeric halide with excess Ag_2CO_3 in methanol to afford the corresponding methyl glycoside. Since then, the reaction has been successfully applied to the synthesis of a wide range of alkyl and aryl O-glycosides as well as O-linked oligosaccharides. In fact, the procedure was utilized for the first stereoselective formation of an α-linked glycoside [46]. However, low reaction efficiencies with unreactive acceptors, stoichiometric amounts of toxic heavy metals, halophilic promoters, and facile donor 1,2-elimination provided motivation for a significant number of modifications and refinements [47–51].



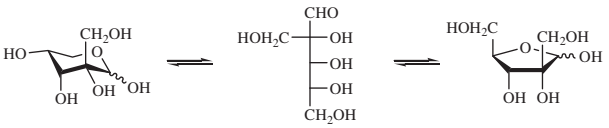
SCHEME 1.3 Early examples of chemical glycosylations. (a) The first reported chemical glycosylation. (b) A Koenigs–Knorr reaction. Ac, acetyl.

1.3.3 Karl Freudenberg (1886–1983)

Karl Freudenberg [52] conducted his doctoral and postdoctoral studies under the guidance of Emil Fischer. As such, it is not surprising that a major portion of his research was dedicated to investigations on the absolute configuration of "sterically related compounds." His observations led to the "optical shift rule," which has been frequently invoked to assign the absolute configuration of molecules with one or two chiral centers [53–56]. Freudenberg is perhaps better known for his contributions to the field of carbohydrate chemistry. His investigations of sugar cyclic acetals provided the structures of di-*O*-isopropylidene derivatives of glucose, galactose, mannose, and xylose [56]. Some of these compounds served as regioselectively functionalized substrates for pioneering studies with tosyl esters. Freudenburg was the first to report the synthesis of 3-*O*-tosyl-D-glucose and 6-*O*-tosyl-D-galactose, which have since become valuable intermediates for the construction of complex glycosides as well as modified sugars such as deoxy, deoxyamino, and deoxyhalogeno [57–62]. The work of Freudenberg et al. also contributed to our understanding of the structure and functions of glycans such as amylose, cellulose, and cyclodextrins. In 1939, he postulated that hydrogen bonding would cause macromolecules such as starch and proteins to assume a helical structure [63]. His work on the natural product witch hazel tannin resulted in the identification of the first naturally occurring branched-chain sugar, hamamelose (Fig. 1.12).

1.3.4 Burckhardt Helferich (1887–1982)

Concurrent with Freudenberg, another Fischer alumnus was at the center of a highly contested debate over the most stable conformations assumed by carbohydrates. Burckhardt Helferich [64] began his studies into the cyclization of hydroxyaldehydes in 1919. He used these simple substrates as model systems to analyze their cyclic and linear characteristics. Until that time, it was largely accepted that cyclic sugars assumed a 1,4-furanosyl structure. Helferich's investigations pointed toward a 1,5-pyranoid structure, which was ultimately shown to be correct [65]. Arguably, the central theme of his research was the discovery and development of methods to assemble complex carbohydrates. In order to facilitate the chemical construction of these molecules, he sought to develop methods to obtain regioselectively and orthogonally protected carbohydrate building blocks and to efficiently couple them. In one such study, Helferich determined that the yield of the Koenigs–Knorr reaction could be improved by replacing Ag_2CO_3 or Ag_2O with mercury(II) salts and by conducting the reaction in more polar solvents such as acetonitrile or nitromethane [66].



D-Hamamelose

FIGURE 1.12 The first identified branched-chain sugar, hamamelose.

An attractive feature of these conditions was that no water was formed as the reaction progressed, alleviating the need to add drying agents as required under more classical conditions. These optimized conditions were later termed the Helferich modification [67, 68]. In 1923, his discovery of the trityl group revolutionized the way in which chemists approached the regioselective protection of organic molecules [69]. Helferich later employed the trityl moiety for the chemical synthesis of several di-, tri-, and tetrasaccharides. He also explored the synthetic utility of glycals in glycosylation reactions [70]. Like Freudenberg, he saw the usefulness of sulfonyl esters for organic synthesis. Indeed, it was his laboratory that introduced the methanesulfonyl (mesyl) group.

1.3.5 Hermann Fischer (1888–1960)

Hermann Fischer [56] conducted his postdoctoral research in the laboratory of his father, Emil Fischer, before leaving for World War I in 1914. Shortly after returning from 4 years of service, his father passed away in July of 1919. Hermann started his independent career focusing primarily on the difficult chemistry of triose phosphates. His synthesis of D-glyceraldehyde and D-glyceraldehyde-3-phosphate from 1,2,5,6-di-*O*-isopropylidene-D-mannitol is worthy of note as these molecules are chiral synthons for a wide range of biological and industrial processes. His other main research interests involved the natural products quinic acid and shikimic acid. These unique natural products are formed biosynthetically from the phosphate precursors described earlier. Hermann Fisher's studies of the structure and absolute configuration of quinic acid ultimately resulted in its correct assignment [71].

In 1948, Hermann Fischer joined the faculty of the University of California at Berkeley, where he continued his work on the synthesis of amino sugars and phospho sugars among other compounds of biological importance. During the beginning of the twentieth century, there was a significant power shift taking place in the field of carbohydrate chemistry. The discipline, which had largely been dominated by German scientists, was transitioning to American leadership, and Hermann Fischer was one of several exceptional scientists that contributed to this change.

1.3.6 Claude Hudson (1881–1952)

Claude Hudson [72] was awarded a Ph.D. (*magna cum laude*) from Princeton in 1907. He spent the better part of the next decade moving between institutions, serving in a variety of capacities, before settling into a long-term relationship with US government laboratories. One such position was as a visiting researcher in the laboratory of van 't Hoff in Berlin. Hudson's research interests were primarily concerned with the stereochemistry of the reducing or anomeric carbon of sugars. Indeed, he conducted extensive kinetic studies of the mutarotation and oxidation of lactose and glucose with the assistance of his colleague Horace Isbell [73–76]. He also extended van 't Hoff's ideas on optical superposition to a wide range of optically active substrates (sugars), which laid the groundwork for Hudson's *isorotation rule* [77].

As with any rule, there are exceptions. In particular, some inconsistencies were noted between his studies and those of Haworth's methylation analysis [78]. However, they were both indispensable methods to assign the anomeric configuration and structure of carbohydrates for several decades until they were replaced with physical methods such as NMR and X-ray diffraction. In addition to the large amount of information gained from the analysis of these compounds, Hudson published several papers concerned with the isolation, preparation, and purification of the numerous sugars that were required in high purity for the aforementioned studies. In search of a more fitting substitute for Fischer's phenylosazones, Hudson found phenylosotriazole derivatives, which he readily obtained through oxidation of the corresponding phenylosazone with $CuSO_4$ [79]. Another interest of Hudson was the way in which enzymes act on carbohydrates. For example, his studies on the hydrolysis of sucrose with invertase revealed that the reaction was irreversible and that α-D-glucose was liberated as a product of the hydrolysis [80–83].

1.3.7 Horace Isbell (1898–1992)

In 1926, Horace Isbell [84] earned his Ph.D. degree at the University of Maryland where his studies focused on the research of organogold compounds. In 1927, he obtained a position at the National Bureau of Standards (NBS) in Washington, DC, where he met Claude Hudson and remained there for more than 40 years. During his tenure at the NBS and later at American University, he allocated the bulk of his research to carbohydrate chemistry, resulting in several notable discoveries. Indeed, in a seminal report, Isbell identified the important roles neighboring groups play during the course of reactions [85]. He also developed the current system for describing the conformation of pyranoid sugars [86], which built on the pioneering work of Haworth [87, 88], and investigated the effects these conformations have on reactivity. Perhaps the most significant contribution of Isbell was his development of the first practical methods to synthesize ^{14}C- and ^{3}H-radiolabeled sugars and their derivatives [89]. This work revolutionized the way in which complex biological processes were probed and visualized.

1.3.8 Melville Wolfrom (1900–1969)

Melville Wolfrom [90] earned a Ph.D. in 1927 from Northwestern University. He then began postdoctoral studies under the mentorship of Claude Hudson at NBS. From there, Wolfrom moved to the Rockefeller Institute for Medical Research, where he worked in the laboratory of Phoebus Levene. His research focused on the structural elucidation of biologically relevant carbohydrates. In 1929, Wolfrom accepted a position at Ohio State University, where he remained for the rest of his career. His research interests can be described by the broad heading of carbohydrate structure and reactivity. He developed methods to obtain acetylated straight-chain sugars, including their dithioacetals, and demonstrated their use as reactive sugar intermediates [90]. Some of these acyclic sugars (*keto*-acetates) were utilized for the synthesis of branched carbohydrates. Extending his dithioacetal work, Wolfrom developed a

method for their reductive desulfurization [91]. One of his exceptional postdoctoral researchers, Raymond Lemieux, later employed this reaction to correlate the stereochemistry of amino acids and sugars through the transformation of 2-amino-2-deoxy-D-glucose to an L-alanine derivative.

1.3.9 "Sugar" Raymond Lemieux (1920–2000)

One of the most prolific scientists of the second half of the twentieth century was Raymond Lemieux. His discoveries spanned a wide range of scientific disciplines including carbohydrate chemistry, organic synthesis, NMR, stereochemistry, and their resulting biological implications. While a postdoctoral researcher under Melville Wolfrom, his research primarily dealt with the structural elucidation of streptomycin [92]. In 1947, Lemieux started his independent research career at the University of Saskatchewan. It was during this time that he began investigating the chemical and physical properties of carbohydrates. The results of these studies provided the foundation for the first chemical synthesis of sucrose [93]. In 1954, Lemieux accepted the position of professor and chair of the Department of Chemistry at the University of Ottawa. It was there that he collaborated with Harold Bernstein and William Schneider of the National Research Council to study sugars using NMR. This formative work not only showed a correlation between chemical shift and the local environment of the protons, but also it demonstrated the utility of $^{1}H{-}^{1}H$ couplings for the determination of the preferred conformation of per-O-acetylated sugars in solution for the first time. These studies were perhaps the most significant development in the field of carbohydrate chemistry since the Fischer proof as they experimentally validated the Karplus equation before it was even published [94]. About this time, Lemieux also published a seminal report detailing the anomeric effect. Moreover, Lemieux's studies of the conformation of glycosides led to his identification of the reverse anomeric [95] and *exo*-anomeric effects [96–98]. He believed the *exo*-anomeric effect was the reason for the orientation of the sugars in higher-order structures such as polysaccharides and oligosaccharides in solution, a theory that was ultimately confirmed by NMR. It was the combination of theoretical results and extensive mechanistic studies that paved the way for the development of novel glycosylation methods, which was a central theme of his research interests.

A long-standing problem facing carbohydrate chemists is the formation of 1,2-*cis*-glycosides. Lemieux's answer to this challenge was the halide ion-catalyzed glycosylation reaction. This extraordinary work permitted, for the first time, the efficient and reproducible synthesis of 1,2-*cis*-glycosides in a completely stereoselective manner. At the heart of this approach was a rapid equilibrium between the relatively stable α-halide and its far more reactive β anomer [99]. This process is known as Lemieux-type *in situ* anomerization and is still one of only a few methods to obtain α-linked glycosides stereoselectively. Some recent methods that have been particularly effective in achieving stereochemical control during the formation of 1,2-*cis*-glycosides include the use of stereospecific activators, novel participating groups, and intramolecular aglycone delivery systems [12].

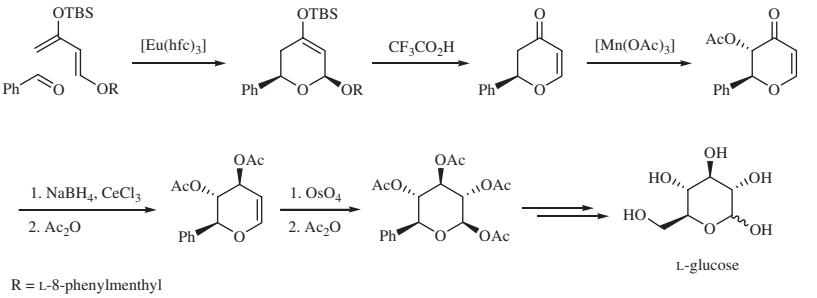
Lemieux sought to answer biological questions through chemistry. The discovery of the multifaceted roles of glycans in key biological processes not only inspired him but also marked the dawn of glycobiology and chemical biology. Indeed, his group synthesized numerous biologically relevant natural products including six human blood-group antigens, which he then utilized for immunization and animal studies. The resulting monoclonal antibodies against these synthetic sugars were harvested and purified, and their binding affinities were quantified [100]. For the first time, a picture of the complex interactions between glycans and their protein receptors (lectins) emerged on a molecular level. Lemieux attracted top-tier students and postdoctoral fellows from around the world. Together they helped cement the way in which chemistry was applied to answer biological questions.

1.3.10 Ascent of De Novo Sugar Synthesis

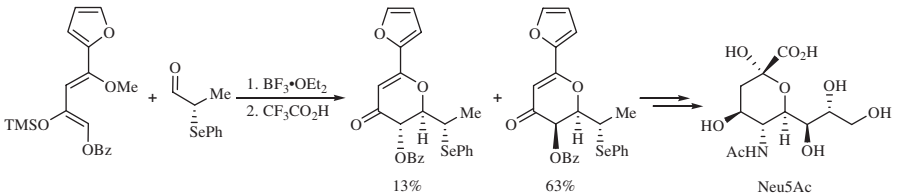
During the time of Lemieux, the field of organic synthesis was rapidly expanding. In laboratories across the globe, complex natural products were submitting to total synthesis. Occasionally, total synthesis endeavors would include carbohydrates, but for the most part, this was left to those skilled in the art. The high density of largely equivalent functional groups, poor solubility in organic solvents, and nontrivial purifications associated with carbohydrate synthesis posed considerable challenge. However, as the biological relevance of glycans became increasingly evident, carbohydrate chemistry began to garner the attention of scientists from a wide range of backgrounds. The synthesis of carbohydrate-based molecules became a target for noncarbohydrate chemists who expanded the chemical toolbox to include carbenes [101], carbanions [102], carbocations [43], organometallics [103], and radicals [104–106] for the synthesis of sugars and sugar derivatives. Although these reactions often afforded product mixtures, novel purification and characterization methodologies were also introduced. As a result, a wide range of elongated, branched, carbocyclic, and C-linked glycosides and nucleosides were created.

A particularly elegant example is the application of Danishefsky's diene [107] to the total synthesis of carbohydrates and carbohydrate derivatives. While it was known that activated aldehydes undergo cycloaddition with electron-rich dienes, the process was not efficient with typical aldehydes under thermal conditions. A major breakthrough was realized [108] with the development of the Lewis acid-catalyzed diene–aldehyde cyclocondensation (LACDAC) reaction, which provided a new strategy for the synthesis of carbohydrates and other polyoxygenated natural products (Scheme 1.4) [109].

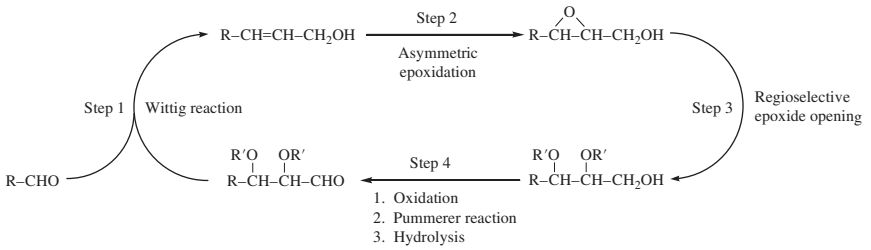
Initially, the de novo synthesis of enantiomerically pure carbohydrates [110] and glycolipids [111] using transition metal complexes and chiral auxiliaries afforded only modest success. Ultimately, it was the use of enantiomerically pure aldehydes, such as the *R* and *S* enantiomers of 2-(phenylseleno)propionaldehyde, to convey facial selectivity upon the LACDAC reaction that enabled the synthesis of optically pure glycals. Syntheses of several complex monosaccharides such as the main sialic acid-type *N*-acetylneuraminic acid (Neu5Ac) and *rac*-3-deoxy-*manno*-2-octulosonic acid (KDO) were accomplished with this technology [112, 113]. The LACDAC



SCHEME 1.4 Enantioselective synthesis of L-glucose via the LACDAC reaction. hfc, 3-(heptafluoropropylhydroxymethylene)-D-camphorato; TBS, *tert*-butyldimethylsilyl.



SCHEME 1.5 LACDAC reaction in the total synthesis of Neu5Ac. Bz, benzoyl; TMS, trimethylsilyl.



SCHEME 1.6 General scheme for a reagent-controlled approach to the total synthesis of all eight L-hexoses.

reaction that eventually led to the total synthesis of Neu5Ac is shown in Scheme 1.5. A noteworthy critical element of the synthesis is that a furan ring was employed as the carboxylic acid surrogate.

While Samuel Danishefsky and coworkers were optimizing the LACDAC reaction, many other groups were also developing methods for the total synthesis of natural and nonnatural sugars. For example, William Roush et al. reported the stereoselective synthesis of several dideoxyhexoses from allylic alcohol precursors [114–116]. Moreover, in a seminal report by Saturo Masamune, K. Barry Sharpless, and coworkers, a reagent-controlled approach to the total synthesis of all eight L-hexoses was achieved via a reiterative two-carbon extension cycle consisting of four key transformations (Scheme 1.6) [117]. This cycle began with the conversion

of an aldehyde to a two-carbon extended allylic alcohol via Wittig reaction. The starting material can also be readily prepared from commercially available (*Z*)-2-butene-1,4-diol via successive monoprotection, oxidation/isomerization, and reduction. Thus, step one of the first cycle is not required but is shown here for completeness. The second step involved asymmetric epoxidation, followed by the regioselective (and stereospecific) opening of the resultant epoxide. Finally, oxidation afforded a bis-homologated aldehyde, which was primed for another cycle.

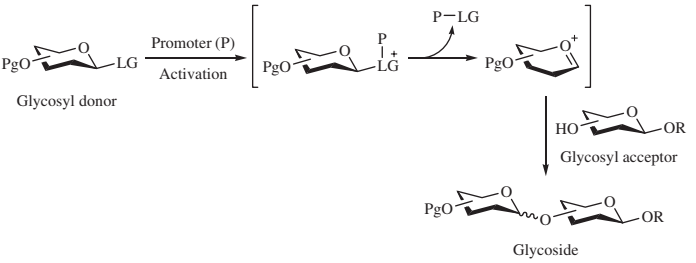
These synthetic achievements shifted the paradigm to de novo synthesis of pyranoses [118], rather than begin with naturally occurring sugar building blocks, and provided the necessary tools to assemble glycans with unprecedented structural complexity. In fact, the origin of glycomics can be traced back to this period. Numerous novel methods for the manipulation of sugars were discovered at a rapid pace. Application of the methodologies afforded reasonable quantities of both natural and unnatural analogues that were available for biological evaluation for the first time.

1.4 ONWARD TO THE TWENTY-FIRST CENTURY

The structural complexity and numerous isoforms found in naturally occurring glycans pose significant challenges in isolating pure and homogeneous samples of glycolipids (variations in carbohydrates, linkages, and lipids), glycoconjugates (differences in sugars and connectivity), and glycoproteins (existing as a diverse collection of posttranslational modifications). Because even slight impurities can intensely affect bioactivity, access to structurally and compositionally defined samples is essential for biological evaluation. Much of the responsibility for providing these samples rests on the shoulders of the synthetic chemists. The synthesis of glycans is an arduous task, requiring specialized knowledge, considerable resources, and, most importantly, creativity. At the heart of the matter is the regio- and stereoselective assembly of oligosaccharides and glycoconjugates. While the diversity of orthogonal protecting groups currently available has largely solved the issue of regioselectivity, achieving stereoselective formation of glycosidic linkages in a controlled manner remains one of the central challenges of modern synthetic chemistry. Fortunately, the chemists of today have many more tools at their disposal than those of only a few decades ago.

1.4.1 Glycosyl Donors and Glycosylation Systems

Perhaps the one area that has yielded the highest dividends during the latter part of the twentieth century is the design and development of new glycosylation methods. These processes traditionally involve two components: the glycosyl donor and the glycosyl acceptor. A glycosyl donor is the species that contributes the anomeric center to the resulting glycoside and is typically electrophilic in nature. The acceptor, in majority of glycosylation reactions, provides the nucleophile. For O-glycosides, the glycosylation pathway generally begins with donor activation upon addition of a



SCHEME 1.7 General glycosylation pathway.

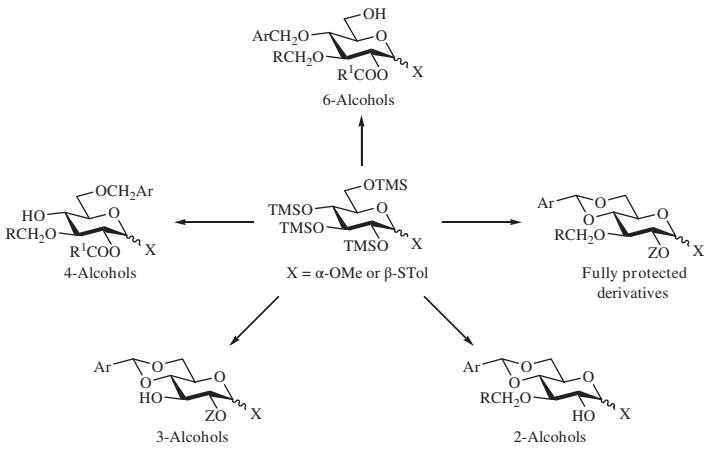
promoter. Once activated, the donor reacts with a nucleophilic hydroxy group of the glycosyl acceptor or aglycone (Scheme 1.7). There are many factors that can have a profound effect on the reactivity and selectivity of a glycosylation event and a wide range of mechanisms by which the reaction can proceed [119]. This section provides a few examples of key developments in glycosylation techniques and applications from a historical perspective. More detailed analyses can be found in subsequent chapters of this book.

Some donors such as glycosyl bromides and chlorides were employed in the early chemical glycosylations and are still in use today, albeit under optimized conditions. Within the last two decades, glycosyl iodides have been increasingly employed due to our ability to tame their reactivity through careful choice of protecting groups. Although stable glycosyl iodides protected with acetates had been discovered by Emil Fischer in the first part of the twentieth century [120], ether-protected analogues were typically found to be too reactive to be useful glycosyl donors [121–123]. Thanks, in part, to the work of Conrad Schuerch [124], Joachim Thiem and Bernd Meyer [125], and others [126], these valuable donors enjoyed increasing popularity since the 1970s. This is especially true for the synthesis of 1,2-*cis*-glycosides [127–132]. While glycosyl fluorides had been known since 1923, it was not until Teruaki Mukaiyama introduced them as glycosyl donors in 1981 that their popularity increased [133]. Another glycosyl donor introduced at about this time is the novel O-imidate leaving group by Pierre Sinaÿ in 1977 [134]. Further refinements by Richard Schmidt led to the trichloroacetimidate donors 3 years later [135]. Trichloroacetimidates enjoyed widespread application due to their stability, efficiency, and the relatively mild conditions required for activation. Possibly the most versatile family of glycosyl donors to date is the thioglycosides. First reported in 1909 by Emil Fischer [136], the anomeric thiol moiety is stable toward a wide range of reaction conditions including those typically required for the manipulation of protecting groups. Thioglycosides can be activated under relatively mild conditions that are often orthogonal to those required for other donors [137–139]. This property made them particularly useful for the synthesis of oligosaccharides in one-pot and iterative couplings [140]. As workhorses of carbohydrate chemistry, thioglycosides are readily converted into other glycosyl donors such as hemiacetals, imidates, halides, and sulfoxides. When used in this manner, one can consider the thioacetal moiety as a transient anomeric protecting group. The nucleophilicity of the

anomeric thiol has been employed for the synthesis of S-linked oligosaccharides and glycoproteins via S_N2 [141], conjugate addition [142], and radical mechanisms [143, 144]. Many of the novel glycosyl donors described herein are sufficiently stable to be purified, manipulated, and stored for extended periods of time.

1.4.2 Automated and One-Pot Methods for Oligosaccharide Synthesis

Recently, significant resources have been dedicated to the development of one-pot strategies for the synthesis of oligosaccharides. While many variations were reported, they generally utilize chemoselective, orthogonal, or preactivation strategies [145]. Two examples of particular significance are Chi-Huey Wong's automated one-pot synthesis of oligosaccharides and Shang Cheng Hung's regioselective one-pot protection method. In 1999, Wong and coworkers developed a custom computer program for the automated one-pot synthesis of oligosaccharides that they named OptiMer. To accomplish this, they derived and tabulated the relative reactivity values (RRVs) of a library of thioglycoside building blocks. Their studies showed that selective activation of the anomeric leaving group could be achieved through careful choice of the protecting groups. A database containing the reactivity profiles and the target oligosaccharide sequence was loaded into Optimer. The software predicted the optimal set of these building blocks and the order in which they should be added to accomplish the synthesis [146]. A drawback of this technology is the need to synthesize large libraries of orthogonally protected donors offering a wide range of RRVs. In 2007, Hung et al. addressed this shortcoming with a trimethylsilyl triflate-catalyzed one-pot approach for the direct and efficient preparation of hundreds of thioglucoside building blocks (Scheme 1.8) [147]. This technology represents a paradigm shift for the way in which regioselectively protected monosaccharides are obtained.



SCHEME 1.8 Regioselective one-pot protection of carbohydrates by Hung et al.

1.4.3 Solid-Phase Oligosaccharide Synthesis

Inspired by the success of Robert Bruce Merrifield's solid-phase peptide synthesis (SPPS) [148], Jean Frechet and Conrad Schuerch disclosed the first synthesis of oligosaccharides on solid support in 1971 [149]. Although the utility of the process was readily apparent, the technology went largely unused for over 20 years due to limitations associated with reaction monitoring, the large excesses required of costly reagents, and the lack of automation. Interest in solid-phase oligosaccharide synthesis was rejuvenated in 1993 with the advent of new glycosyl donors and linkers [150]. In 2001, Peter Seeberger modified a peptide synthesizer to produce the first automated solid-phase carbohydrate synthesizer [150–153]. This technology resulted in the generation of large oligosaccharide libraries, which could prove to be particularly useful for high-throughput screening assays [154]. In general, most solid-phase oligosaccharide strategies may be categorized as either donor-bound, acceptor-bound or bidirectional, referring to the reactant component that is attached to the solid support. In a seminal report in which the donor-bound strategy was utilized, Danishefsky employed silicon to tether glycal donors to the resin. The glycosylations were performed with an excess of the solution-based acceptor. Following iterative coupling reactions, the oligosaccharide was released upon addition of tetrabutylammonium fluoride and acetic acid. One striking advantage of this process over solution-based chemistries is that the excess acceptor and promoter can be removed by rinsing after each coupling reaction [150]. The results for acceptor-bound [149, 155] and bidirectional [153] strategies are equally promising. While progress has been made, solid-phase oligosaccharide synthesis remains an area of intense investigation with the goal of simplifying the process to the extent that nonchemists would be able to perform the synthesis of complex glycosides in an automated and programmable fashion.

1.4.4 Natural Product Synthesis

The aforementioned synthetic tools and other discoveries enabled the assembly of carbohydrates with unprecedented structural complexity, such as that of the potent adjuvant QS-21A (Fig. 1.13). Investigations into the dynamic functions of these

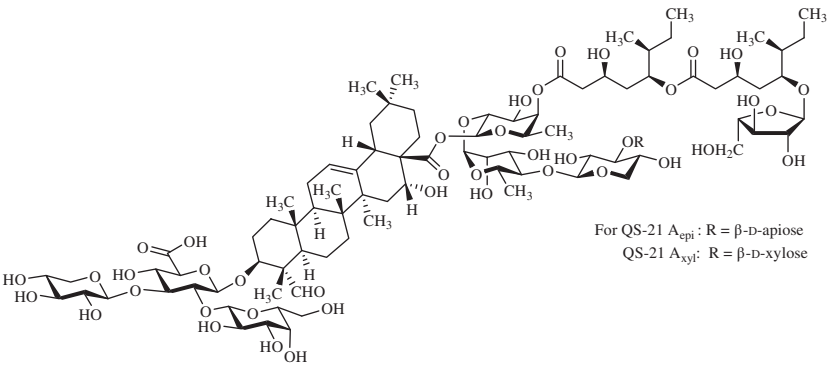


FIGURE 1.13 The immunological adjuvant QS-21A.

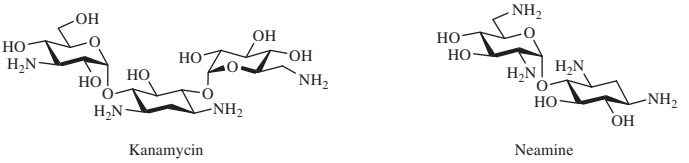


FIGURE 1.14 Structures of the aminoglycoside antibiotics kanamycin and neamine.

compounds often begin with their isolation from natural sources. This complex triterpene–oligosaccharide–normonoterpene conjugate is a heavily glycosylated saponin originally isolated from *Quillaja saponaria* Molina as both apiose and xylose forms [156].

Many of the same chromatographic and spectroscopic techniques were applied to the isolation and identification of natural products such as carbohydrates and glycolipids. The discovery, isolation, computational analysis, synthesis, and structure–activity relationship studies of antibiotics such as the aminoglycosides kanamycin and neamine are of particular note (Fig. 1.14) [157, 158].

1.4.5 Carbohydrate-Based Therapeutics

Carbohydrates in the form of glycoproteins, GPI anchors, or glycoconjugates such as glycolipids and gangliosides (Fig. 1.15) are ubiquitous cell-surface components of animal as well as some plant cells [159, 160]. They are also found on the surface of virions and bacterial cells. Gangliosides, such as GM1, are found on the cell surface in lipid rafts. They are believed to modulate signal transduction and are considered as possible therapeutics for neurodegenerative disorders. Members of this class of biologically relevant molecules facilitate a myriad of biological and pathological processes including cell–cell communication, growth, fertility, recognition, adhesion, fusion, replication, metastasis, and immune system evasion [161, 162].

In 1993, a Kirin Pharmaceuticals research team isolated the first reported α-linked galactosylceramides (α-GalCer) from an extract of the marine sponge *Agelas mauritianus* off the coast of Okinawa, Japan [163]. These glycolipids, also known as agelasphins, were found to possess antitumor activity. The carbohydrate moiety of these compounds varied from mono- to tetrasaccharides, and related compounds have also been isolated from the pathogenic microbe *Borrelia burgdorferi* [164, 165]. Subsequent studies into the mode of action of these unique compounds revealed that their therapeutic effects stemmed from activation of invariant natural killer T cells in a CD1d-dependent manner. The formation of the T-cell receptor/α-GalCer/CD1d triplex results in the initiation of a cascade of immunological events. The progression primarily involves the secretion of the proinflammatory T helper 1 cytokine interferon-γ as well as the immunoregulatory T helper 2 cytokine interleukin-4. Research has shown that the immunological response can be tuned by altering the structure of the glycolipid. As such, research into the identification of analogues that elicit biased cytokine production is of high interest for the treatment of a wide range of maladies ranging from viral and bacterial infections and tumor

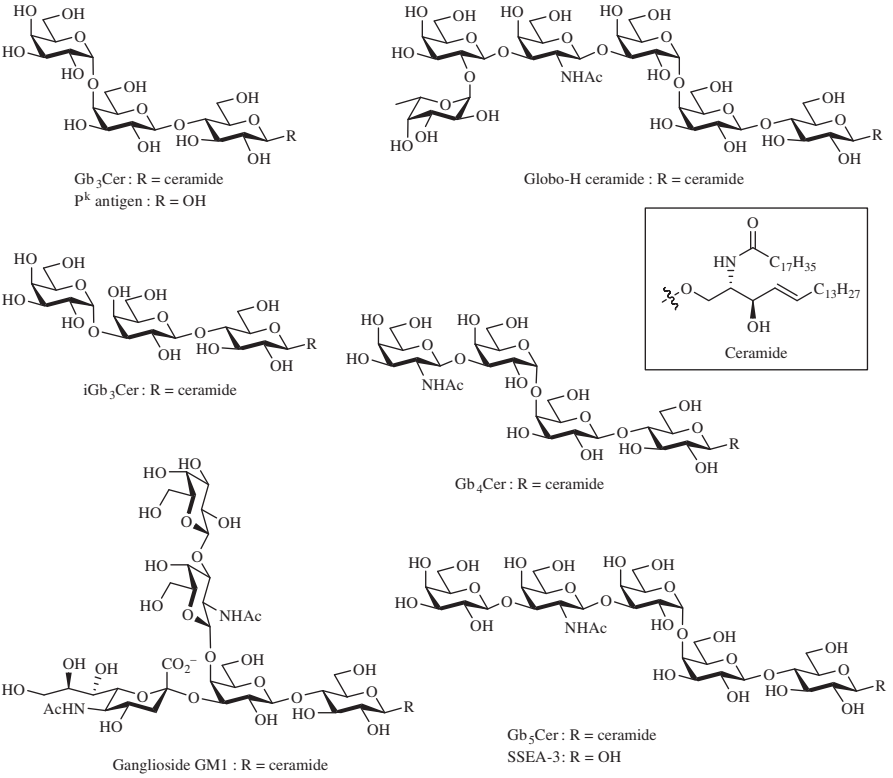


FIGURE 1.15 Structures of immunological glycolipids and gangliosides.

growth inhibition (tumor immunotherapy) to certain autoimmune diseases such as type 1 diabetes and multiple sclerosis. The dense surface distribution and characteristic glycan composition presented by a wide range of pathogens and malignant cells render them attractive targets for vaccines [166]. Indeed, glycoconjugates such as trehalose glycolipids and glycosylceramides are being investigated for use as adjuvants and key vaccine components [167–169]. The structure and properties of glycolipids, including their interactions with proteins, are not fully understood due to several reasons including the lack of synthetic methods for their efficient preparation; their properties that are closely correlated to their local environment such as microdomains or lipid rafts, which are hard to mimic; and their existence as heterogeneous mixtures with a high degree of structural flexibility. The combination of spectroscopic techniques, molecular dynamic simulations, biomimetic membrane chemistry, and carbohydrate chemistry has recently begun to shed some light on the multifaceted roles of these multifunctional compounds. As a result, interest in the synthesis and application of glycolipids and gangliosides has been increasing over the last two decades. A wide range of industries are interested in capitalizing on their highly amphiphilic character for use as environmentally friendly detergents, surfactants, and emulsifiers.

With the rapid spread of antimicrobial-resistant microorganisms, the prevention of parasitic, bacterial, and viral infections is an urgent global necessity. The idea of using glycans to provide protection has been known for the better part of a century. Indeed, in 1923, a seminal report by Michael Heidelberger indicated that capsular polysaccharides could be used to induce immunity [170]. Unfortunately, the combination of short-lived antibody response to carbohydrate-based vaccines and the discovery of antibiotics and chemotherapeutics dampened research and development. Recently however, rational vaccine design, modern synthetic and semisynthetic vaccine conjugates, and the advent of glycomics brought attention back to carbohydrate-vaccine development. Advances in glycan analysis, synthesis, purification, screening, and structural determination have provided astonishing results. These techniques have also been applied to the field of nanotechnology, resulting in a veritable tool chest for glycomics including affinity-labeled species, neoglycoproteins, fluorescent tags, multivalent quantum dots, and targeted magnetic nanoparticles [171]. Nanoparticles bearing carbohydrates revolutionized the diagnosis, imaging, and treatment of a wide range of biological phenomenon. The fruits of this translational research allowed the modern scientist to rationally design carbohydrate-based therapeutics with higher efficacies and in a more efficient manner than ever before.

1.5 CONCLUSION AND OUTLOOK

The topics presented in this chapter are meant to provide an overview of the evolution of glycan chemistry from the time of Emil Fischer to the burgeoning field of glycomics. Worthy of note is the way in which pioneering chemists contributed to the understanding of a broad range of sciences such as organic stereochemistry, carbohydrate chemistry, computational science, biology, and immunology. Although we are gaining a better understanding of the biological roles played by these polyols, glycobiology and chemical glycobiology are scientific disciplines still in their infancy. While carbohydrate chemistry has enjoyed remarkable progress, the search for alternative glycosylation strategies for the synthesis of biologically important compounds is an ongoing endeavor. Oligosaccharide synthesis is in no way routine. Some of the challenges that remain include the stereoselective formation of 1,2-*cis*-glycosides and the incompatibility associated with matched–mismatched donor–acceptor pairs. Advances in solid-supported and one-pot protocols are being successfully applied to the synthesis of oligosaccharides, which should help to streamline the synthesis and purification processes, thereby increasing the overall efficiency and will undoubtedly play a major role in the expansion of the field to nonchemists.

In nature, most oligosaccharides are covalently linked to peptides, proteins, or lipids. The advent of protein and peptide conjugation methods including SPPS, site-selective protein modification [172], native chemical ligation [173], and expressed protein ligation [174] has provided extraordinary access to the corresponding glycopeptides and glycoproteins. The efforts of chemists and biologists combined with recent advances in computational and spectroscopic techniques will undoubtedly

yield answers to many of the questions surrounding the structure and function of glycolipids, gangliosides, and the microdomains in which they reside.

The diverse repertoire of glycoconjugates available today also expedited the identification of glycolipid- and glycoprotein-based ligands and inhibitors. This could not have happened at a better time as the emergence of antibiotic resistance has become a worldwide crisis. One solution may be the rational design of potent inhibitors to block entry, propagation, or other enzymatic processes such as glycosylation or hydrolysis. Recent developments in the multivalent presentation of carbohydrate-based high-affinity ligands resulted in some of the most potent inhibitors to date [175]. Another weapon in our arsenal is the development of synthetic and semisynthetic glycan-based vaccines and adjuvants. In combination with high-throughput screening methods such as carbohydrate microarrays, the future of carbohydrate-based vaccines appears bright. Indeed, the intrinsic diversity and complex relationship between nucleic acids, proteins, glycolipids, and carbohydrates will certainly put the technologies described herein to the test. However, if the past is any indication of the future, these challenges will continue to inspire researchers to invent even more ingenious solutions.

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