Chemical Glycobiology
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Chemical tools have proven indispensable for studies in glycobiology. Synthetic oligosaccharides and glycoconjugates provide materials for correlating structure with function. Synthetic mimics of the complex assemblies found on cell surfaces can modulate cellular interactions and are under development as therapeutic agents. Small molecule inhibitors of carbohydrate biosynthetic and processing enzymes can block the assembly of specific oligosaccharide structures. Inhibitors of carbohydrate recognition and biosynthesis can reveal the biological functions of the carbohydrate epitope and its cognate receptors. Carbohydrate biosynthetic pathways are often amenable to interception with synthetic unnatural substrates. Such metabolic interference can block the expression of oligosaccharides or alter the structures of the sugars presented on cells. Collectively, these chemical approaches are contributing great insight into the myriad biological functions of oligosaccharides.

Oligosaccharides and glycoconjugates (glycoproteins and glycolipids) have intrigued biologists for decades as mediators of complex cellular events. With respect to structural diversity, they have the capacity to far exceed proteins and nucleic acids. This structural variance allows them to encode information for specific molecular recognition and to serve as determinants of protein folding, stability, and pharmacokinetics. Given that glycosylation is one of the most ubiquitous posttranslational modifications, the unexpectedly small number of genes identified in the initial analyses of the human genome sequence provides even more impetus for understanding the biological roles of oligosaccharides.

Oligosaccharide functions are now being elucidated in molecular detail, but advances in glycobiology have been slow to arrive compared with the pace of revelations in protein or nucleic acid biochemistry. The same structural diversity that has captivated biologists has also frustrated efforts to define oligosaccharide expression patterns on proteins and cells and to correlate structure with function. Some technical challenges are analytical in nature; determination of the oligosaccharide sequence on a specific glycoprotein is still far from routine. Others originate from glycoconjugate biosynthesis, which is neither template-driven nor under direct transcriptional control. Oligosaccharides are assembled in step-wise fashion primarily in the endoplasmic reticulum and Golgi apparatus (Fig. 1), a process that affords significant product microheterogeneity (1). As a result, it is difficult to obtain homogeneous and chemically defined glycoconjugates from biological sources. Without such materials in hand, biological functions are difficult to unravel.

Genetic approaches have contributed considerably to our appreciation of oligosaccharide function. The availability of entire genome sequences has revealed the multiplicity of enzymes that contribute to glycoconjugate assembly. Their deletion in model organisms has provided substantial insight. For example, mice deficient in α-mannosidase II expressed an altered portfolio of N-linked glycans on their cell surface glycoproteins (2). The mice were prone to a systemic autoimmune response, suggesting that abnormalities in N-glycosylation in humans may be a factor in the pathogenesis of autoimmunity. Still, cell surface presentation of simple as well as complex glycans requires many genes to be expressed in concert, which complicates the analysis of single gene “knockouts” or “knockins.”

As outlined in this review, chemical approaches are powerful allies to genetics and biochemistry in the study of glycobiology. Chemical tools have been used to probe glycosylation at many levels. For example, cell surface carbohydrate-receptor binding events (Fig. 1) can be interrogated with synthetic oligosaccharides, glycoconjugates, and their analogs. The biosynthesis of oligosaccharide structures can be disrupted or modulated by synthetic enzyme inhibitors. Unnatural sugars that substitute for native monosaccharides or their downstream metabolic intermediates can be used to inhibit or control any stage of this process.

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(Fig. 1) can intercept biosynthetic pathways, leading to changes in cell surface glycosylation. These chemical strategies allow one to perturb glycosylation and oligosaccharide-receptor interactions in a cellular context. Furthermore, chemically synthesized molecules that disrupt pathological carbohydrate-dependent processes are emerging as important therapeutic agents.

**Synthesis of Oligosaccharides and Glycoproteins**

Access to structurally defined oligosaccharides and glycoconjugates is a prerequisite for unraveling their function. Chemical routes to the production of oligosaccharides are, therefore, essential. Advances on this front are providing materials for the assessment of glycancan function, the establishment of the structural features important for function, the elucidation of biosynthetic pathways, the creation of carbohydrate-based vaccines, the production of non-natural glycosylated antibiotics, and the generation of inhibitors of glycoconjugate function.

Two general strategies are used for in vitro oligosaccharide production: enzymatic (including chemoenzymatic) synthesis and chemical synthesis. In enzymatic and chemoenzymatic routes, saccharide intermediates are elaborated with enzymes, typically glycosyltransferases or glycosidases, to generate oligosaccharides (Fig. 2A) (3–6). Chemoenzymatic synthesis is distinguished from enzymatic synthesis by its reliance on both chemical synthetic and enzymatic transformations. In chemical synthesis, the appropriate building blocks are produced and assembled into oligosaccharides (Fig. 2B). In both approaches, the focus is on forming the critical connection that links saccharide building blocks: the glycosidic bond.

Chemical synthesis and enzyme-based routes are complementary. Enzymes can be used to effect glycosylation with absolute regio- and stereo-control. If the necessary enzyme is available, the desired bond can be formed (Fig. 2A), often with high efficiency. In comparison, chemical synthesis offers exceptional flexibility. Natural and non-natural saccharide building blocks can be assembled with natural or non-natural linkages. Although some enzymes will act on alternative substrates, chemical synthesis provides the means to generate any oligosaccharide, oligosaccharide analog, or glycoconjugate.

The chemical synthesis of oligosaccharides is formidable. It requires stereochemical and regiochemical control in glycosidic linkage formation. The first viable method for controlled glycosidic bond formation, the Koenigs-Knorr glycosylation, was reported in 1901 (7). Although the search for alternative glycosylation reactions is ongoing (8), chemists have made remarkable strides in carbohydrate synthesis. The problem of obtaining the proper regiochemistry of glycosylation has been largely solved by the development of orthogonal hydroxyl group protection strategies. Thus, groups can be installed to block reaction at one site and later removed to unmask specific hydroxyl groups for glycosylation. To devise safer and less toxic procedures, reactions that require heavy metal activators are being replaced with milder, more environmentally sound methods (9, 10). Our understanding of how to obtain the desired configuration of a glycosidic bond is also more sophisticated. Stereochemical control can be achieved by employing stereospecific activation methods, using protecting groups that direct the orientation of the glycosidic bond through intermolecular (neighboring group participation) or intramolecular (tethered aglycone delivery) participation, altering the steric environment around the anomic position to bias the desired outcome, or exploiting the intrinsic stereoelectronic preferences for reaction at the anomic position.

Two major advances are being applied to streamline the chemical synthesis of oligosaccharides: one-pot reactions (11, 12) and polymer-supported synthesis (13, 14). In one-pot processes, glycosylation reactions occur sequentially in a single reaction vessel; the most reactive glycosyl donor is triggered first and the least is coerced to engage in the final reaction. A key concept underlying progress on this front is that there are “armed” (reactive) and “disarmed” (less reactive) glycosyl donors (10, 15). A number of research groups have exploited this knowledge to efficiently assemble oligosaccharides using one-pot reactions (12, 16–20). Solid-phase synthesis of oligosaccharides similarly offers powerful advantages for oligosaccharide synthesis in comparison to conventional methods because it circumvents multiple purification steps needed in traditional solution syntheses. Pioneering studies in solid-phase oligosaccharide synthesis were initiated in the early 1970s (21) after Merrifield’s successful demonstration of solid-phase peptide synthesis (22). Unfortunately, the harsh conditions needed for some of the early glycosylation reactions and the difficulty of monitoring the reaction on the solid support hindered progress for 20 years. Newly developed glycosylation methods and advances in solid-phase organic chemistry rejuvenated interest in solid-phase oligosaccharide synthesis in the 1990s (13, 14). The productivity from these renewed efforts is evident from the diversity of and complexity of oligosaccharides that have been synthesized, including sequences containing up to 12 residues (23). Progress on this front also has facilitated the generation of oligosaccharide libraries containing up to 1300 compounds (23–25). Thus, complex oligosaccharides and oligosaccharide libraries are becoming accessible to a large community of scientists.

Most oligosaccharides are linked covalently to proteins. As oligosaccharides become more accessible, the next challenge is to integrate them into glycoproteins. Several laboratories have used protected glycosylated amino acids as building blocks for automated solid-phase peptide synthesis (SPPS) to gen-

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**Fig. 2. Generic examples of glycosylation reactions.** (A) Enzyme-catalyzed glycosylation. Glycosyltransferases catalyze the formation of glycosyl bonds from sugar nucleotide donors. The specific regiosomer and stereoisomer produced depends on the enzyme employed. (B) Chemical glycosylation. Most such glycosylations involve the reaction of a glycosyl donor, equipped with a leaving group (X) at the anomeric position, with a glycosyl acceptor. To produce the desired regiosomer, protecting groups (P) are used to block other reactive sites. The desired stereoisomer can be generated by altering the nature of X or the protecting group.
erate glycosylated peptide fragments reminiscent of natural glycoproteins (26). Modern FMOC-based peptide synthesis methods are sufficiently mild that the oligosaccharide remains intact throughout the synthesis. As an illustration, Danishefsky and co-workers chemically synthesized glycopeptide 2, derived from the mucin-like leukocyte antigen leukosialin (CD43), using trisaccharide–amine acid 1 as a building block (Fig. 3A) (27). The oligosaccharide structures and their arrangement on the polypeptide backbone are characteristic of tumor-associated glycoproteins and, hence, such synthetic assemblies may serve as tumor vaccine components.

The extension of these methods to full-length glycoproteins has proved more troublesome, largely due to limitations inherent to linear, step-wise SPPS. Peptides larger than 50 to 60 residues are difficult to obtain using conventional methods due to poor yields and accumulating byproducts. Much larger polypeptides can be produced using recombinant DNA technology; thus, several groups have exploited recombinant proteins as starting materials for the synthesis (i.e., “semi-synthesis”) of homogeneous glycoproteins. Whereas the glycosidic linkage is too difficult a bond to be made between an oligosaccharide and a large protein, analogs of glycoproteins bearing unnatural linkages are readily prepared (28). These “neoglycoproteins” lend themselves to a facile convergent assembly from proteins and synthetic oligosaccharides. But, their non-native linkages may affect their overall structures and perturb their biological activities.

The total chemical synthesis of native glycoproteins has recently been facilitated by breakthroughs in protein chemistry. In particular, the “native chemical ligation” method (29, 30) has enabled the convergent condensation of unprotected glycopeptide fragments, each generated by automated SPPS, to form full-length, fully functional glycoproteins (31) (Fig. 3B). The related “expressed protein ligation” (32) permits the chemical union of recombinant protein fragments with synthetic glycopeptides, further relieving the burden of large protein synthesis. O-linked (33) and N-linked (34) glycoproteins bearing homogeneous and chemically defined glycans have been prepared in this fashion, suggesting that any glycoprotein may now be obtained in the quantities required for structure determination and functional analysis. These synthetic advances are very important with respect to development of glycoprotein therapeutics. Glycosylated biotechnology products such as monoclonal antibodies, erythropoietin, and tissue plasminogen activator may benefit from methods for their semi-synthesis.

What future breakthroughs can we expect in the synthesis of oligosaccharides and glycoconjugates? Success in the automated solid-phase synthesis of complex oligosaccharides appears imminent (35) and efforts to automate one-pot assembly methods are advancing. These developments offer nonspecialists access to oligosaccharides that can be used to address problems in biology. Although a standard method for the synthesis of oligosaccharides has yet to emerge, the diversity of glycosidic linkages may demand multiple synthetic approaches. Applications of these methods will afford diverse combinatorial libraries of oligosaccharides and more complex glycoconjugates. This increased repertoire of compounds will facilitate the identification of specific oligosaccharide and glycoprotein ligands for proteins and provide new leads for inhibitor design.

Inhibitors of Glycan Biosynthesis and Processing

The discovery of diverse biological roles for oligosaccharides and glycoconjugates is fueling interest in the development of chemical tools that block their formation and/or function. Two general types of inhibitors are being sought: those that block glycoconjugate biosynthesis and those that interfere with glycoconjugate recognition. Effective inhibitors of various biosynthetic steps in glycoconjugate assembly have the potential to transform our understanding of carbohydrate function. By blocking the production of specific glycoconjugates, their biological roles can be ascertained (36). Similarly, antagonists that prevent glycoconjugate recognition can illuminate the function of the natural interactions (37). Progress on all of these fronts is accelerating.

Fig. 3. Chemical synthesis of glycopeptides and glycoproteins. (A) Glycopeptide synthesis using glycosylated amino acid building blocks. Synthesis of a mucin-like fragment of leukosialin (CD43) (2) by incorporation of glycosylated FMOC amino acids (1) into solid-phase peptide synthesis (SPPS). (B) Glycoprotein synthesis by convergent coupling of glycopeptide fragments. Assembly from synthetic glycopeptide fragments using the technique of native chemical ligation. One fragment is functionalized as a COOH-terminal thioester (“thioester), and the other bears an NH2-terminal cysteine residue. A transthioesterification reaction between the two components produces an intermediate thioester that rearranges to the peptide bond shown in the product.
Though active against the enzyme, polar and charged compounds of this type are unlikely to be effective in cells or organisms due to their membrane impermeability. Thus, strategies for the design and discovery of glycosyltransferase inhibitors that can access their targets inside cells are needed. The oligosaccharide transferase inhibitor 4 (Fig. 4) is capable of transport across the endoplasmic reticulum membrane where it can act on its target and abrogate N-linked glycosylation (45), and other glycosyltransferase inhibitors demonstrate efficacy in whole cells (38). Library screening approaches that are now routine in pharmaceutical industry might be applied to the glycosyltransferases (46). The availability of combinatorial methods for synthesizing libraries of drug-like (and, hence, bioavailable) compounds has revolutionized the search for pharmacological tools in academic laboratories. Indeed, library screens have produced cell-permeable, small molecule inhibitors of a class of carbohydrate modifying enzymes called sulfotransferases (5, Fig. 4), and these may be effective tools for delineating biological function (47). The prospects for advances in glycosyltransferase inhibitor identification are, therefore, excellent. In addition, the rapid increase in structural information available for this class of proteins will aid in inhibitor design and discovery (48, 49).

Inhibitors of Glycan Recognition

The generation of compounds that block glycan recognition remains a major challenge. Many oligosaccharide binding sites are relatively shallow and solvent exposed, and binding interactions can take place over large surface areas (50). Those confronted with the problem of inhibiting protein–saccharide complexation encounter many of the same challenges as those seeking to block protein–protein interactions. Moreover, in solution many oligosaccharides bind their protein targets with relatively low affinities (e.g., with a dissociation constant $K_d \approx 10^{-3}$ to $10^{-7}$ M); thus, initial lead compounds tend to require much optimization. Lastly, structural data is often lacking, so information on the important binding contacts can be obtained only through the synthesis and evaluation of analogs. Given these barriers, the progress that has been made in identifying inhibitors of one family of carbohydrate-binding proteins, the selectins, is impressive.

The selectins are a family of three carbohydrate-binding proteins that have been the object of numerous investigations because of their role in leukocyte recruitment to sites of inflammation (51). The discovery of the selectins led to the initial identification of a ligand, the tetrasaccharide sialyl Lewis x (sLe$^\alpha$). The findings raised questions illustrative of those generally relevant for protein–carbohydrate interactions: What functional groups are needed for recognition by the selectins? Can the sLe$^\alpha$ tetrasaccharide be modified such that it binds specific selectin family members? Can compounds that bind more tightly be discovered? Access to sLe$^\alpha$ and a wide array of analogs, conjugates, and mimics has been instrumental in addressing these questions.

Data from many synthetic sLe$^\alpha$ derivatives have revealed the critical contacts for selectin binding, information that has guided the generation of more potent inhibitors. The functional groups important for binding to each of the selectin family members (E-, P- and L-selectin) have been identified. For instance, the hypothesis that sulfation of sLe$^\alpha$ would increase its affinity for L-selectin (52) was confirmed with derivatives produced by chemical synthesis (53–55). Similarly, a chemoenzymatic route was used to generate sLe$^\alpha$-substituted glycopeptides to elucidate the critical binding epitope of PSGL-1 (56), the physiological ligand for P-selectin. With knowledge of the sLe$^\alpha$ functional groups important for complexation, mimics of the tetrasaccharide have been synthesized that are potent selectin antagonists (57). For example, tetrasaccharide mimic 6 (Fig. 5) is >50-fold more active at blocking E-selectin than sLe$^\alpha$ (58). In a P-selectin inhibition assay, macrocyclic sLe$^\alpha$ analog 7 exhibits a dramatic enhancement (about $10^3$) relative to sLe$^\alpha$ (59). These data underscore the tremendous progress made in generating efficacious glycomimetics.

Synthesis and Biological Activity of Glycoassembles

The function of many carbohydrates is contingent on their multidentate presentation. The binding of proteins to monovalent carbohydrate determinants is often weak, yet the strength and specificity required for recognition in physiological settings is high. The simultaneous formation of multiple protein–carbohydrate interactions is one binding mode that can be exploited to achieve the necessary avidity. In physiological settings, saccharide epitopes and their protein receptors are arranged such that multiple binding events can occur simultaneously. Naturally occurring carbohydrate displays are widespread: examples include highly glycosylated proteins (e.g., mucins), the carbohydrate surfaces of bacteria and other pathogens, and the outer membranes of mammalian cells. Carbohydrate-binding proteins also tend to be oligomeric or present in multiple copies at the surface of a cell. The interaction of multivalent presentations can result in the formation of numerous simultaneous complexation events that proceed to afford a high observed affinity and a high functional affinity (60).
What are the physiological advantages conferred by multivalent binding? Synthetic arrays have provided key answers to this question. First, these interactions have been shown to be highly specific and versatile (61–63). For example, binding can be modulated by changing the individual saccharide residues or by altering their spacing. Second, the kinetics exhibited by such binding events are likely critical for biological systems. For example, relative to monovalent binding, multivalent interactions exhibit greater reversibility in the presence of competing ligands (64). Thus, low affinity, multivalent interactions are less likely to entrap cells in unproductive binding events. In addition, binding events mediated by multiple weak interactions are expected to be more resistant to shear stress, such as that encountered when cells interact in the bloodstream (65). To understand the roles of multivalency in carbohydrate recognition, platforms that display multiple copies of recognition elements have been generated (66–68). A number of diverse scaffolds have been used for multivalent presentation; these include low–molecular weight displays (e.g., dimers and trimers), dendrimers, polymers, and liposomes. The structure of the display determines what features of selected naturally occurring multivalent ligands it mimics. For example, a low–molecular weight ligand or dendrimer can resemble a branched oligosaccharide chain, such as those displayed by glycoproteins. Alternatively, larger displays such as polymers or liposomes can more effectively mimic a glycoprotein or a glycolipid array.

There are several distinct mechanisms that contribute to the high activities often observed for multivalent ligands. An understanding of these is critical for optimizing ligand performance and for understanding how natural systems function. Relevant mechanisms include the chelate effect, occupation of adjacent subsites, and ligand-induced protein clustering. When the chelate effect operates (Fig. 6A), multiple interactions occur after formation of the first contact; these are facilitated by the high effective concentration of the binding groups (69). This mode of binding can give rise to large enhancements in activity if the orientation and display of binding groups are favorably disposed. Because multipoint binding typically involves ligand organization, it exhibits a conformational entropy penalty that may offset the advantages of chelation. Alternatively, multivalent displays may be effective ligands because they occupy subsites as well as the primary binding site on the target protein. Many lectins possess secondary sites adjacent the key binding cleft (Fig. 6B) (50). Lastly, the activities of many small and large multivalent carbohydrate derivatives are due to their abilities to cluster their receptors (Fig. 6C) (70, 71). Interestingly, ligands with this property may serve not only as antagonists but also as agonists. Because cell surface receptor clustering can facilitate signaling transduction, carbohydrate displays that cluster receptors or lectins that cluster glycoproteins can elicit cellular responses. Thus, multivalent ligands can exhibit a wide range of different activities, depending on their binding modes.

Two recent studies dramatically illustrate the power of multivalent presentation in inhibitor design and the interplay of different binding mechanisms. Highly potent pentavalent inhibitors of bacterial toxin binding to host cells were synthesized. The toxins targeted, heat-labile enterotoxin (LT-1) and shiga-like toxin I (SLT-I), are members of the class of AB, toxins. The AB, class, which can be subdivided into the cholera, pertussis, and shiga toxin families, possess a pentagonal arrangement of five B subunits and a single A subunit (72). These toxins, which are responsible for millions of human fatalities each year (73), invade cells by multivalent binding of the B subunit to the carbohydrate residues of gangliosides. Thus, a logical strategy is to generate multivalent ligands that can occupy all five binding sites simultaneously. Fan et al. applied this strategy to produce candidate inhibitors of LT-1, which were generated by appending galactose residues to a pentacyclic core (Fig. 7) (74). A potent antagonist of LT-1 was found, a compound 105 times more active than the corresponding monovalent galactose derivative. This excellent potency appears to arise from its ability to interact simultaneously with the five toxin B subunits. Kitov et al. pursued an alternative strategy for inhibiting SLT-I (75). Structural studies of SLT-I complexed with the glycolipid Gb3 revealed that each B subunit possessed subsites adjacent to the primary carbohydrate-binding site. They synthesized dimers of the appropriate trisaccharide binding element that could, in principle, occupy both the primary and secondary site. Although their divalent ligands exhibited only modest increases in potency (40-fold), attachment of these units to a pentavalent scaffold afforded one of the most potent (107 times more active than the trisaccharide alone) multivalent ligands (8, Fig. 7) yet described. Structural analysis of compound 8 bound to SLT-I did not reveal the 1:1 complex envisioned but rather a complex containing two equivalents of the pentameric protein. This ligand appears to act both through dimerizing the target receptor as well as through the chelate effect. The excellent potencies of the toxin inhibitors highlight the advantages of multivalent presentation, and the wide range of unique binding modes multivalent ligands can employ.

Multivalent presentation is also beneficial for eliciting rather than inhibiting biological responses. This role has long been appreciated in the context of vaccine development. As early as 1929 (76), it was recognized that displays of oligosaccharides conjugated to proteins elicit the production of specific antibodies to carbohydrate. In 1975, Lemieux and coworkers showed that defined synthetic glycoconjugates could be synthesized and used to elicit an immune response (77). These investigations provided the foundation for understanding how natural systems function.
for current approaches to generating synthetic carbohydrate-based vaccines. One such effort is directed at exploring the features required to generate the first vaccine against <i>Shigella dysenteriae</i> (78). Synthetic anti-cancer vaccines are also being pursued. Danishefsky and co-workers have used state-of-the-art synthetic methods to generate complex glycoconjugates (79) with promising anti-cancer activities. To optimize the potency of carbohydrate-based vaccines, more data addressing how the structure of the conjugate (epitope spacing, valency, backbone composition) affects its immunogenicity is needed.

Multivalent ligands can also act as effectors of one response and inhibitors of another. In one study, a multivalent saccharide derivative was designed to effect a process that disables the receptor, L-selectin (80, 81). L-Selectin is a transmembrane protein found on neutrophils and lymphocytes that facilitates the transient attachment and rolling of leukocytes through its interaction with highly O-glycosylated proteins on the endothelium (82). A soluble form of L-selectin can be released into circulation by a membrane-associated protease (83). Given the role of cell surface L-selectin, ligands that induce its downregulation could serve as anti-inflammatory agents. To test this hypothesis, human neutrophils were treated with monovalent and multivalent oligosaccharides that mimic features of physiologic L-selectin ligands. The levels of L-selectin after addition of monovalent oligosaccharides were unchanged, but upon exposure to multivalent display 9 (Fig. 7) L-selectin was lost from the surface (80, 81). Multivalent ligand 9 also inhibits L-selectin-mediated cell rolling, suggesting that its ability to downregulate L-selectin makes it a highly effective inhibitor (84). These results suggest a new approach to manipulating the cell surface interactions. The design of multivalent ligands that selectively disarm receptors by activating endogenous processes is an uncharted territory with high potential.

**Modulating Cell Surface Glycosylation by Metabolic Interference**

The ability to alter glycoconjugate structures expressed on cell surfaces is important for understanding their biological functions. An alternative to enzyme inhibitors is the use of unnatural metabolic substrates that can intercept carbohydrate biosynthetic pathways (85). Metabolic interference can
produce several outcomes on the cell surface (Fig. 8). An unnatural substrate might divert oligosaccharide elaboration away from endogenous scaffolds destined for the cell surface (Fig. 8A). The result is a reduction in the amount of mature structures expressed by the cell. Alternatively, unnatural substrates might be designed to engage a biosynthetic pathway, resulting in their incorporation into cell surface glycoconjugates (Fig. 8B). The result is the presentation of an unnatural epitope that might display different receptor binding properties than its native counterpart. Subtle modifications to the fine structure of a monosaccharide can be engendered in this fashion. Incorporation of unnatural sugars with reactive functional groups into cell surface glycoconjugates (Fig. 8C) provides a scenario in which the glycan structure can be further altered by chemical reactions at the cell surface (Fig. 8D).

An example of cell surface glycan suppression using metabolic decays is provided by the “oligosaccharide primers” described by Esko and co-workers. When fed a scenario in which the glycan structure can be further altered by chemical reactions at the cell surface (Fig. 8D).

In summary, advances in oligosaccharide and glycoprotein synthesis have provided the critical material for biological investigations. Genome sequencing efforts have unveiled the various enzymes that participate in glycoconjugate biosynthesis and processing, and a detailed understanding of these pathways will allow the judicious choice of targets for inhibitor design and metabolic interference. The convergence of chemical tools with frontier genetic and biochemical technologies has created an exciting platform from which to tackle problems in glycoscience.
Intracellular Functions of N-Linked Glycans
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N-linked oligosaccharides arise when blocks of 14 sugars are added cotranslationally to newly synthesized polypeptides in the endoplasmic reticulum (ER). These glycans are then subjected to extensive modification as the glycoproteins mature and move through the ER via the Golgi complex to their final destinations inside and outside the cell. In the ER and in the early secretory pathway, where the repertoire of oligosaccharide structures is still rather small, the glycans play a pivotal role in protein folding, oligomerization, quality control, sorting, and transport. They are used as universal “tags” that allow specific lectins and modifying enzymes to establish order among the diversity of maturing glycoproteins. In the Golgi complex, the glycans acquire more complex structures and a new set of functions. The division of synthesis and processing between the ER and the Golgi complex represents an evolutionary adaptation that allows efficient exploitation of the potential of oligosaccharides.

In mature glycoproteins, N-linked glycan moieties are structurally diverse. The sugar composition and the number and size of branches in the sugar tree varies among glycans bound to a protein, among glycoproteins, and among cell types, tissues, and species (1). However, when initially added in the ER to growing nascent polypeptides, the glycans do not display such heterogeneity. The “core glycans” are homogeneous and relatively simple (Fig. 1).

The trimming and processing that the glycans undergo when the glycoprotein is still in the ER introduce only limited additional diversity, because the alterations are shared by all glycoproteins. Thus, the spectrum of glycoforms remains rather uniform until the glycoproteins reach the medial stacks of the Golgi apparatus, where structural diversification is introduced through a series of nonuniform modifications. Particularly in vertebrate and plant cells, it is the terminal glycosylation in the Golgi complex that gives rise to the tremendous diversity seen in glycoconjugates that reach the cell surface.

The switch from structural uniformity in the ER to diversification in the Golgi complex coincides with a marked change in glycan function. In the early secretory pathway, the glycans have a common role in promoting protein folding, quality control, and certain sorting events. Later, Golgi enzymes prepare them for the spectrum of novel functions that the sugars display in the mature proteins (3). Here, we mainly address events in the early secretory pathway. We focus on observations that are starting to unmask the logic of the various early trimming and modification events. We also discuss glycan structure and function in light of fundamental differences between the two biosynthetic organelles, the ER and the Golgi complex.

N-Linked Glycan Synthesis and Modification
During the synthesis of N-linked glycans in mammalian cells (Fig. 2), a 14-saccharide