Reviews

Synthesis and Application of Carbohydrate-Containing Polymers

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Polysaccharides and glycoproteins participate in a number of biochemical reactions and cell−cell recognition processes in vivo. Synthetic carbohydrate-based polymers are being increasingly explored as biodegradable, biocompatible, and biorenewable materials for use as water absorbents, chromatographic supports, and medical devices. Moreover, synthetic polymers bearing sugar residues can also offer a good surface for cell attachment and, thus, might be applied to cell recognization events in antimicrobial/viral and tissue engineering. This review presents various synthetic approaches for the preparation of sugar-containing polymers.

1. Introduction

The traditional view of carbohydrate polymers as nature's energy source (starch and glycogen) and structural materials (cellulose, collagen, and proteoglycans) has expanded. Today, carbohydrates are known to have a wide variety of biological functions. For example, the sulfated polysaccharide, heparin, plays an essential role in blood coagulation.[1] Another related polysaccharide, hyaluronan, which acts as a lubricant in joints, has been used to protect the corneal endothelium during ophthalmologic surgery.[2] In addition to hyaluronan's lubricating and cushioning properties, its in vitro antiinflammatory activity has prompted its investigation, along with the related chondroitin sulfate, for the treatment of osteoarthritis and rheumatoid arthritis.[3] Cell surface carbohydrates are involved in numerous biological functions, including cellular recognition, adhesion, cell growth regulation, cancer cell metastasis, and inflammation.[4] They also serve as attachment sites for infectious bacteria, viruses, toxins, and hormones that result in pathogenesis.[5] However, the glycopolymers displaying these cell surface carbohydrates are heterogeneous and their chemical structures are illdefined. Synthetic carbohydrate-based polymers are emerging as important well-defined tools for investigating carbohydrate-based interaction processes.[6−9]

Synthetic carbohydrate-based polymers having pendant sugar residues are of great interest, not only as simplified models of biopolymers bearing oligosaccharides but also as artificial glycoconjugates in biochemistry and medicine. They are used as surfactants,[10] texture-enhancing food additives,[11] reverse osmosis membranes, and biologically active polymers.[12,13] Chemically sulfonated dextran and pentosan (1→4 linked xylopyranose) have structural similarity to heparin and show anticoagulant activity.[14] Carbohydrate-based vaccines, such as tumor-associated carbohydrate antigens (e.g., sTn), are an active area of research.[15] Synthetic carbohydrate polymers that are biocompatible and biodegradable are increasingly used in tissue engineering and controlled drug release devices. Specifically, *N*-(2-hydroxypropyl)methacrylamide copolymers modified with galactosamine are known to interact with the asialoglycoprotein receptor on hepatocytes and hepatocarcinomas.[16] Similar copolymers containing galactose, fucosylamine, and mannosamine have been targeted to hepatocytes, mouse leukemia L1210 cells, and macrophages, respectively.[17,18] These specific carbohydrate-based interactions could be applied as drug or gene delivery carriers. A sulfated-glucoside-bearing polymer activates the fibroblast growth factor,[19] suggesting its use as an active component of tissue-engineering matrixes. Modified chitosans are promising polymers as supports for hepatocyte and chondrocyte attachment, which can be applied as carrier materials for transplantation or as a scaffold for tissue engineering.[20−23] The specific interaction of carbohydrates and proteins have also facilitated the application of carbohydrate polymers as specific enzyme inhibitors[24] and in the treatment for infectious diseases.[25] Carbohydrate portions of polymers can mimic natural polysaccharides and bind carbohydrate-specific lectins.[26] This provides high concentration of sugar ligands for specific binding of plant and animal lectins for use in affinity matrixes for biomolecule purification.[27] Chiral sugar-based polymers might provide unique matrixes for gel electrophoresis to separate chiral components.

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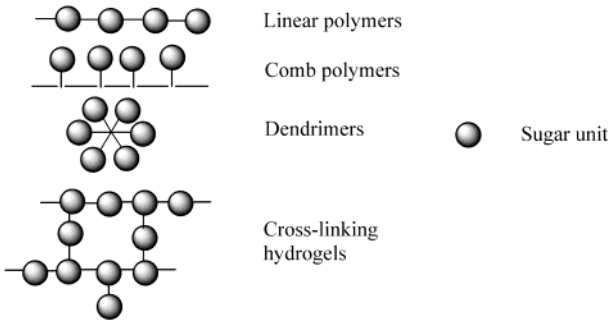


Figure 1. Major classes of synthetic sugar-containing polymers.

Various types of synthetic polymers bearing sugar residues have been described. Linear polymers, comb polymers, dendrimers, and cross-linking hydrogels represent the four major classes (Figure 1). Linear polymers are usually linked through the anomeric position with other hydroxyl groups in the adjacent sugar residue. Comb polymers are synthesized from polymerizable sugar derivatives. Dendritic macromolecules, or dendrimers, are synthetic three-dimensional macromolecules that are prepared in a stepwise fashion from simple branched monomer units. Their unique architecture and monodisperse structure have been shown to result in numerous previously unknown or significantly improved physical and chemical properties when compared to traditional linear polymers. Consequently, dendrimers are now considered to be one of the prime nanometer-scale building blocks for the construction of nanoscale objects, molecular devices, and advanced drug delivery systems.[28]

Hydrogels are lightly cross-linked polymer networks that swell in water. Sugar-based cross-linking hydrogels are hydrophilic and biocompatible, properties that are important in medicine and biomedical engineering as superabsorbents, contact lenses, and matrices for drug delivery systems.[29,30] There are three major synthetic methods for preparing carbohydrate-based polymers: chemical, enzymatic, and chemoenzymatic.

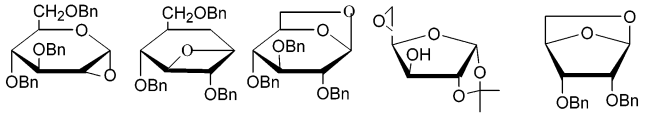
2. Chemical Synthesis

Chemical synthesis of sugar-containing polymers is a challenge to carbohydrate chemists because of the (1) difficulty in achieving selective modification and polymerization due to the presence of multiple hydroxyl groups in sugars with nearly identical reactivities and (2) difficulty in maintaining the stereo regularity found in natural polysaccharides. High-yield glycosylation methods and advanced protection chemistries have largely overcome these difficulties.

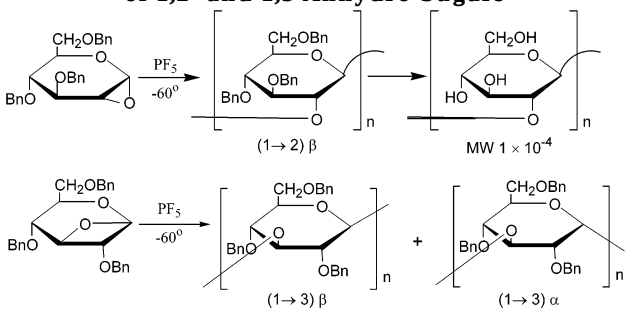
2.1. Ring-Opening Polymerization. Ring-opening polymerization of anhydro sugar derivatives is a popular method for the synthesis of stereo-regular polysaccharides with high molecular weights.[31] Anhydro sugars are usually formed by dehydration of two free hydroxyl groups in the sugar unit (Scheme 1). Polymerization of highly purified monomers can be initiated by Lewis acid, strong electrophiles, or strong bases in dry conditions and under high vacuum.

Schuerch and co-workers[33] synthesized three-membered-ring containing benzylated 1,2-anhydro-α-D-glucopyranoside and β-D-mannopyranoside. Polymerization

Scheme 1. Different Types of Anhydro Sugar Monomers Used in Preparing Stereo-regular Polysaccharides[31,32]



Scheme 2. Lewis Acid-Catalyzed Polymerization of 1,2- and 1,3-Anhydro Sugars



catalyzed with PF_5 afforded a polymer (90%) of molecular weight 10000. Debenzylation gave the fully deprotected polymer as confirmed by ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Scheme 2).

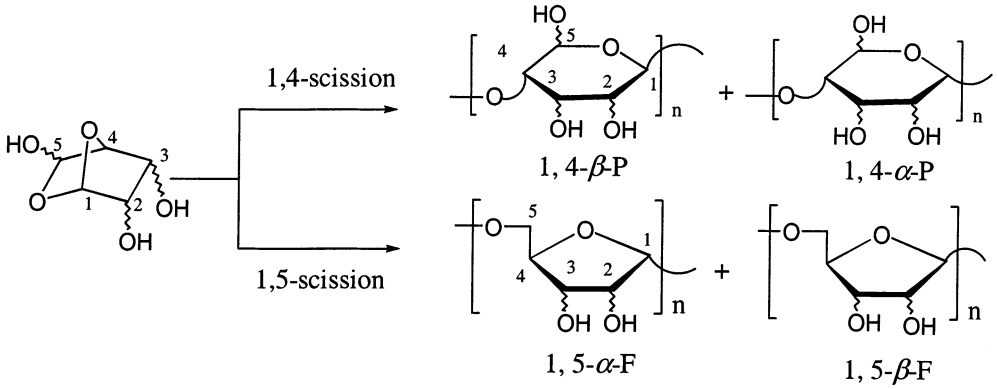
Many (1→3)-β-D-glucopyranan polymers, including lentinan, inhibit tumor cell growth, making them biologically important targets.[34,35] PF_5-catalyzed polymerization of 1,3-anhydro-2,4,6-tri-*O*-benzyl-β-D-glucopyranose gave the α-linked polymer while polymerization with triethylalumimum−water yielded a polymer with the β configuration[36] (Scheme 2).

Ring-opening polymerization of 1,4-anhydrosugar derivatives is complicated by two ring-cleaving pathways. The 1,4-scission pathway affords the (1→4)-pyranoside while the 1,5-scission pathway gives the (1→5)-furanoside.[37] (Scheme 3) Cationic ring-opening polymerization of 1,4-anhydro-D-glucopyranose derivatives yield cellulose and amylose, the most common polysaccharides in nature.[38] Ring-opening polymerization of 1,4-anhydro-2,3,6-tri-*O*-methyl-α-D-galactopyranose and 1,4-anhydro-2,3-di-*O*-methyl-α-L-arabinopyranose gives stereoirregular D-galactan with β-D-furanosidic and pyranosidic structures of L-arabinan.[39] Cationic polymerization of 1,4-anhydro-2,3-di-*O*-methyl-α-D-ribopyranose gave 2,3-di-*O*-methyl-(1→4)-β-D-ribopyranan and 2,3-di-*O*-methyl-(1→5)-β-D-ribofuranan of molecular weight around 30000 with some stereoregularity.[40]

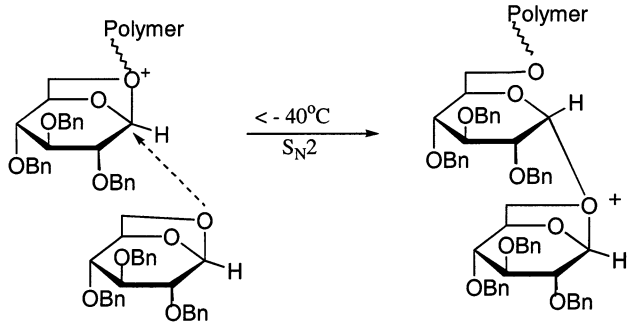
The polymerization of 1,6-anhydrosugar derivatives has been the most successful of all anhydrosugar polymerization, giving both high stereoregularity and high molecular weight. Low-temperature polymerization of 1,6-anhydrosugar derivatives affords (1→6)-α-D-glycopyranans[41−43] (Scheme 4). Because oxonium ions are more stable than oxacarbenium ions at low temperature (less than −40 °C), polymerization reactions are believed to take place through an S_N2-type mechanism to give stereoregular glycopyranans.

Two strategies have been used to synthesize (1→6)-β-D-glycopyranans. The first utilizes neighboring group participation in the nucleophilic reactions and the second controls the reaction outcome through steric hindrance. Kobayashi and co-workers[44,45] synthesized (1→6)-β-D-galactopyranan derivatives by ring-opening

Scheme 3. Two Pathways for Ring-Opening Polymerization of 1,4-Anhydro Sugars



Scheme 4. Mechanism for Low-Temperature Propagation of 1,6-Anhydrosugar Derivatives



polymerization of 1,6-anhydro-2-*O*-benzoyl-3,4-di-*O*-benzyl-*β*-D-galactopyranose (Scheme 5).

The polymerization proceeded at 0−20 °C using 10−50% of PF_5 in dichloromethane to afford a polymer with a molecular weight of 2600−3600. The benzoyl ester protecting C-2 affords a stable five-membered-ring carbocation intermediate, promoting attack from the upper face and affording 1,2-*trans*-(*β*)-glycosides.

Many biologically active polysaccharides are branched, with the degree and position of branching essential for the biological activity. For example, the (1→3)-*β*-D-glucan containing a (1→6)-*β*-D-glucopyranan branch at every third or fourth residue of the main chain shows high antitumor activity, while the linear (1→3)-*β*-D-glucopyranan shows no activity.[46] Moreover, the synthetic α-D-glucopyranose-branched (1→6)-α-D-glucopyranan shows hypoglycemic activity, while the linear polysaccharide is not active.[47] Cationic ring-opening polymerization of anhydrodisaccharide derivatives having a bicyclic acetal skeleton is an excellent method for the chemical synthesis of branched polysaccharides of high molecular weight with well-defined structures[48] (Scheme 6).

2.2. Poly(vinylsaccharide)s. Poly(vinylsaccharide)s belong to a class of water-soluble polymers that are prepared by a vinyl-type free-radical polymerization of well-defined saccharide-based monomers. These monomers are prepared by coupling an unsaturated component to a carbohydrate derivative through an ether, ester, or amido linkage. These types of carbohydrate-based polymers have been the most extensively investigated. The synthesis of pure vinyl sugar monomers is the key step in polymer synthesis. Both protected and unprotected vinyl monomers are polymerized in organic or aqueous media through the use of a radical initiator to afford poly(vinylsaccharide)s. Table 1 lists selected examples of protected vinyl monomers that have been used in preparing poly(vinylsaccharide)s. After reaction of these protected vinyl- and acryloyl-containing sugar monomers with radical initiators (e.g., 2,3-azoisobutyronitrile (AIBN)), the resulting polymers were deprotected.[49]

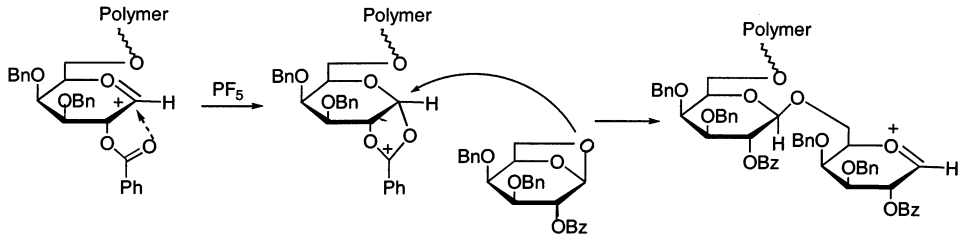
An amino functional group in a carbohydrate can also be used to synthesize vinyl monomers by conversion with reactive (meth)acrylamide at low temperatures in an alkaline aqueous or methanol solvents. Table 2 lists the reagents that have been used to prepare amino sugar monomers. Selective reaction at the amino group in the presence of hydroxyl groups eliminates the need for protection step(s), tremendously simplifying the synthesis. The highest molecular weight poly(vinylsaccharide)s have been prepared using a redox initiator system, such as $(NH_4)_2S_2O_8/Na_2S_2O_5$, at low temperature.

In addition to the sugar polymers described above, a second type of poly(vinylsaccharide) has been synthesized having a polymer backbone connected to the monosaccharide unit through a C−C bond. These polymers can be polymerized either by radical or by anionic polymerization and have a more stable C−C linkage on both the main chain and the side chain.

Sugar aldehydes can be treated with Grignard reagents to afford *C*-glycosides (Scheme 7), which can be further polymerized through the use of radical initiators. In another route to synthesize *C*-link polymers, protected sugar monomers are treated with *N*-bromosuccinimide, followed by elimination to obtain exoglycals that can be polymerized or copolymerized with methyl methacrylate, affording high molecular weight polymers.[58−62]

Polyvalency, the ability of a ligand to bind to a target through multiple chemical interactions, is considered an important factor in promoting the binding of pathogens to host cells.[63,64] Influenza A, an orthomyxovirus responsible for the most severe outbreaks of influenza, adheres to the terminal *N*-acetylneuraminic acid (Neu5Ac) of glycolipids and glycoproteins on the surface of epithelial cells. Several groups have developed inhibitors of influenza-virus-induced hemagglutination that are polyvalent in Neu5Ac.[65−68] All the Neu5Ac-based inhibitors are susceptible to glycosidic cleavage by neuraminidase, an exoglycosidase that is present on the surface of influenza virus. Neuraminidases cleave the terminal neuraminic acid (sialic acid) and are thought

Scheme 5. Formation of 1,2-*trans*-Glycosidic Linkage by Neighboring Group Participation



Scheme 6. Chemical Synthesis of Branched Sugar Polymers by Cationic Ring-Opening Polymerization

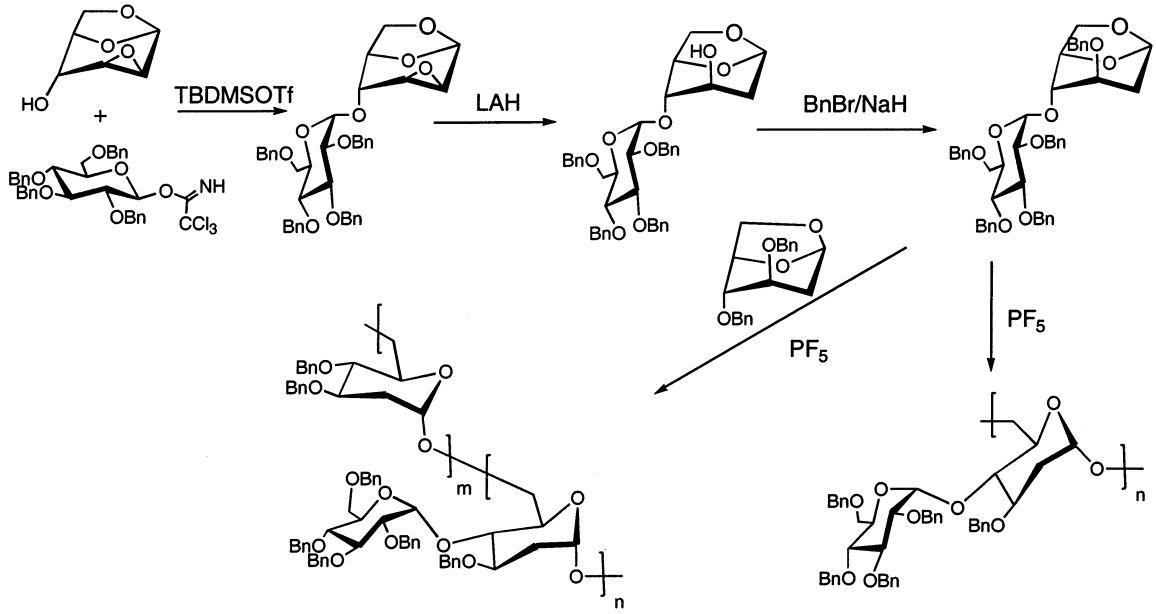
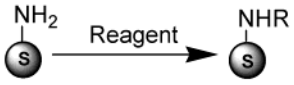
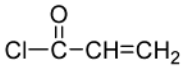
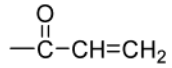
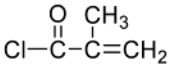
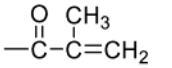
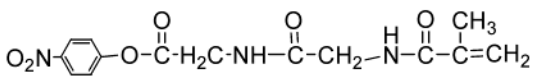
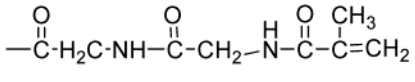


Table 1. Synthesis of Protected Vinyl Monomers

vinylsaccharide monomer	reference
ω-methacryloyloxyalkyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides	49
1-*O*-*p*-vinylbenzyl-β-D-glucoside	50
1,2-dimethyl-5-glucopyranosyl-5-(4-vinylbenzyl) barbiturate	51
1-*O*-(vinyloxy)ethyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimide-β-D-glucopyranoside	26
1,2:3,4-diisopropylidine-6-*O*-vinyl-D-galactopyranose	52
6-*O*-methacryloyl-2,3:4,5-diisopropylidine-D-galactopyranose	53
3-*O*-acryloyl-D-mannitol-1,2,4,5,6-pentanitrate	54

Table 2. Synthesis of Unprotected Vinyl Sugar Monomers

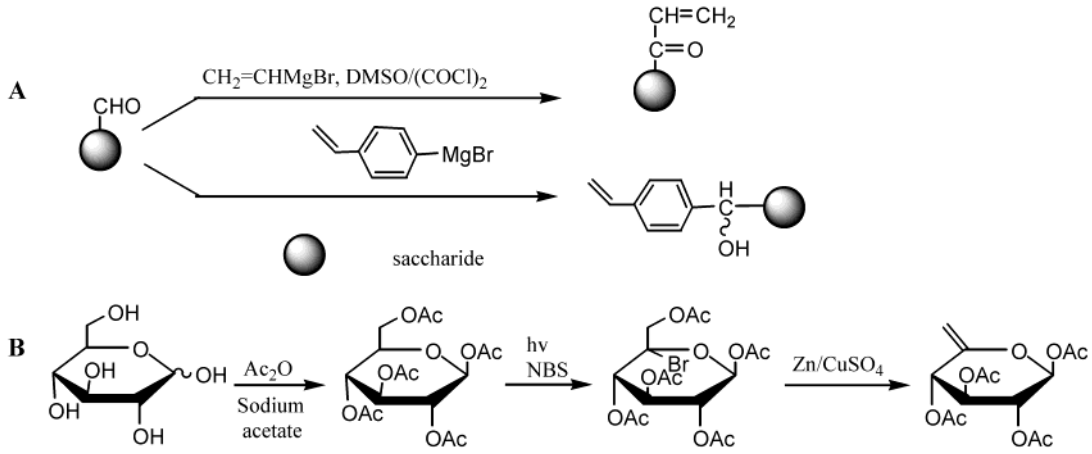


Reagent	R	reference
		55
		55
		56, 57

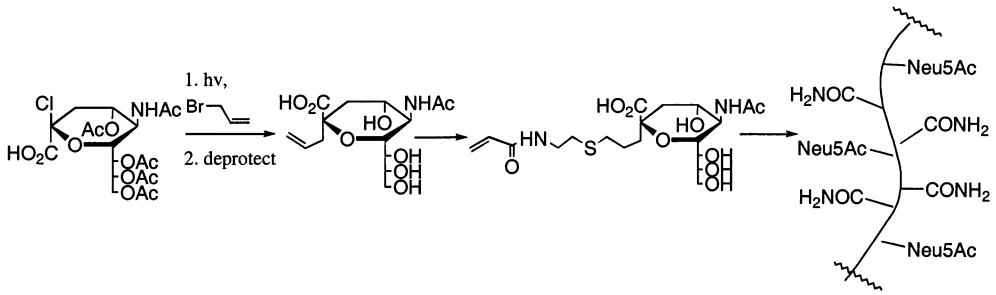
to allow the virus particles to spread in the mammal host. Scheme 8 shows the synthesis of random copolymers incorporating Neu5Ac *C*-glycoside moieties.[69] Polymers are synthesized by photochemical initiation and have been shown to be effective in inhibiting influenza-virus-induced agglutination of erythrocytes in vitro.[70]

2.3. Dendritic Glycosides. Dendrimer synthesis is a relatively new field of synthetic polymer chemistry focused on the preparation of regular, highly branched monomers that upon polymerization lead to a monodis-

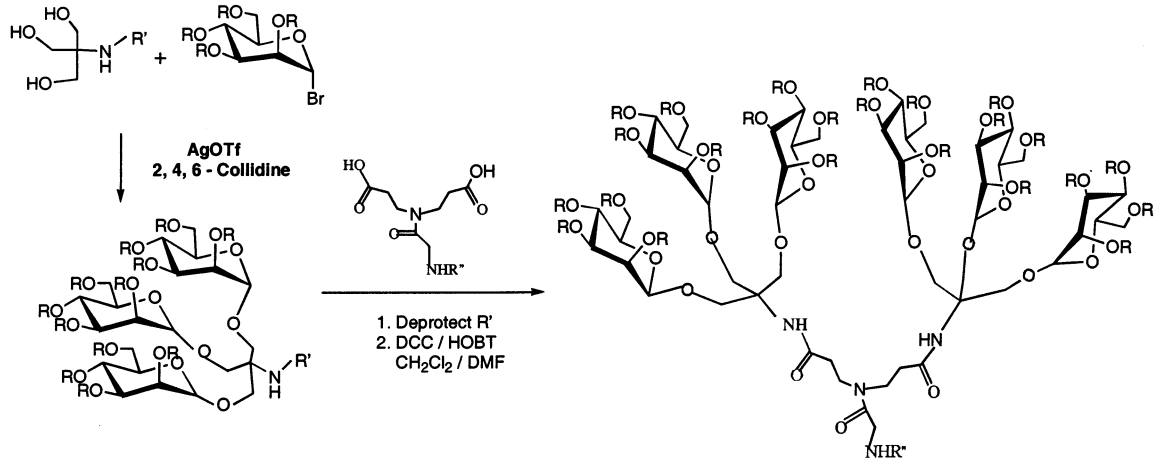
Scheme 7. Polymerable Monosaccharide Gives C−C Connection



Scheme 8. Synthesis of Neu5Ac (Sialic Acid)-Based C-Glycoside Polymer



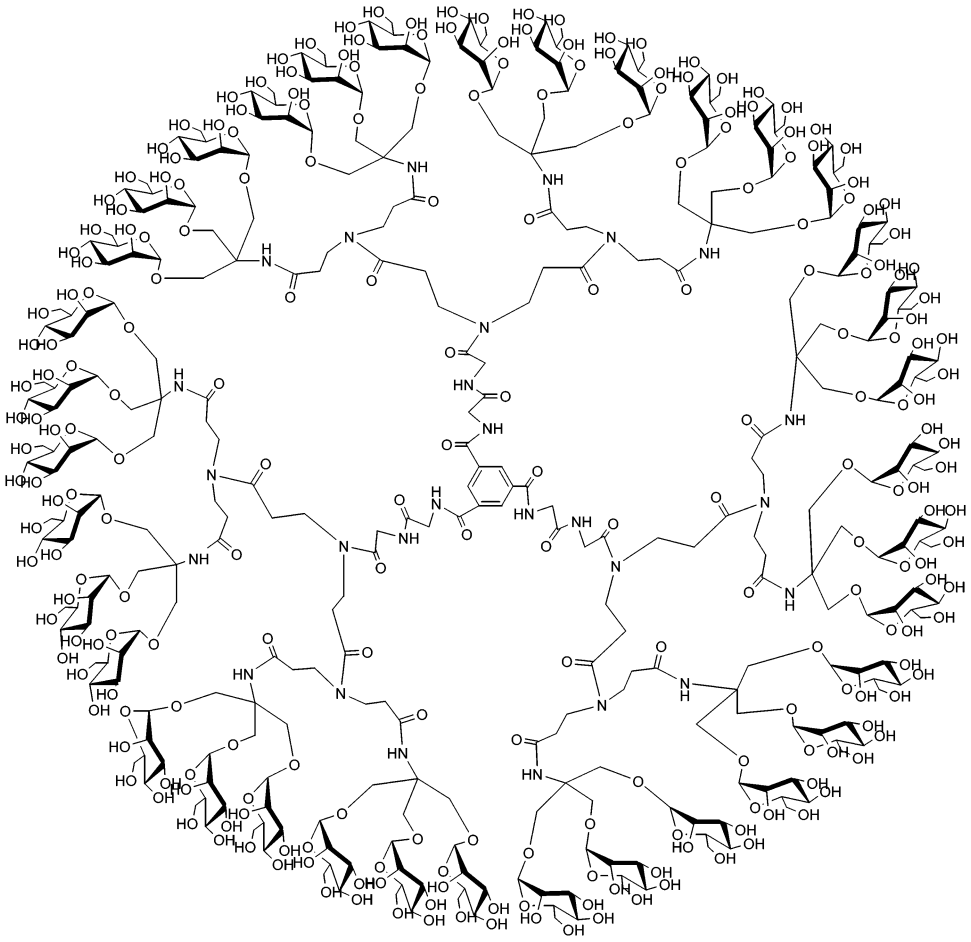
Scheme 9. Synthesis of a Mannopyranoside-Containing Dendritic Wedge



perse, treelike (or generational) structure. The synthesis of monodisperse polymers demands a high level of synthetic control, which can be achieved through stepwise reactions, in which a dendrimer is built up one monomer layer, or "generation," at a time. Interactions between monovalent carbohydrate ligands and a single binding site of a complementary receptor protein are weak with millimolar dissociation constants. The multivalent nature of cell surface carbohydrates acts cooperatively to increase the avidity of carbohydrate−protein interactions that induce various cellular functions.[71,72] Analogues that mimic naturally occurring di- and tribranched oligosaccharides with mannose at the reducing terminus display binding affinities approximately 1000-fold and 1000000-fold greater than that of the singly branched oligosaccharide. Dendrimers with covalently attached glycoside residues in the outer layer are well-defined glycopolymer models of cell surface multiantennary glycoproteins.[73]

Amoebiasis, a parasitic infection caused by *Entamoeba histolytica*, is initiated by adherence to the colonic wall. Adherence involves recognition of colonic mucin and epithelial glycoconjugates by an amoebic cell surface lectin, which recognizes nonreducing terminal Gal and GalNAc residues.[74] Previous studies demonstrated that 5−10 mM galactose or *N*-acetylgalactosamine inhibited *E. histolytica* adherence to target cells. The glycoproteins asialoorosomucoid or asialofetuin, with polyvalent nonreducing terminal Gal and GalNAc residues, are more potent micromolar inhibitors of amoebic adherence. Partially purified rat and human colonic mucins (MW ∼ 900000) are highly polyvalent molecules and very potent inhibitors ($IC_{50} < 10^{-10}$ M). These data are characteristic of other carbohydrate recognition systems,

Scheme 10. Structure of α-D-Mannopyranoside-Containing 36-mer Dendrimer

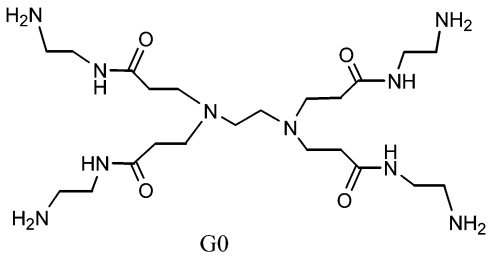


where multiple appropriately spaced nonreducing terminal carbohydrates afford high affinity binding to cellular lectins.[75,76]

Stoddart and co-workers[74] designed a convergent synthetic methodology for α-D-mannopyranoside-containing dendrimers. The tris-branched mannopyranoside derivatives were prepared (Scheme 9), and after group manipulation was protected, the "3-mer" was further reacted with branched carboxylic acid derivatives to form higher generation dendritic wedges. Following the same convergent synthesis strategy, the α-D-mannopyranoside-containing 9-mer, 18-mer, and 36-mer were prepared in 96%, 88%, and 30% yields, respectively. The structure of the 36-mer is shown in Scheme 10. Structural characterizations of dendritic wedges and dendrimers were carried out by ^{1}H NMR, ^{13}C NMR, and matrix-assisted laser-desorption time-of-flight (MALDI-TOF) mass spectrometry. Biological evaluation showed those dendrimers exhibited much greater inhibitory effects than monomeric analogues.[77,78]

PAMAM dendrimers[79] are prepared by the Michael addition of methyl acrylate to ethylenediamine (EDA), followed by amidation of the tetraester product with an excess of EDA to afford the generation zero (G0) product (Scheme 11). Continuing similar alternating Michael addition/amidation reactions on G0 leads to PAMAM dendrimers of the desired generation, having a defined number of primary amine end groups. PAMAM dendrimers are currently available in commercial quantities.

Scheme 11. PAMAM Dendrimer G0

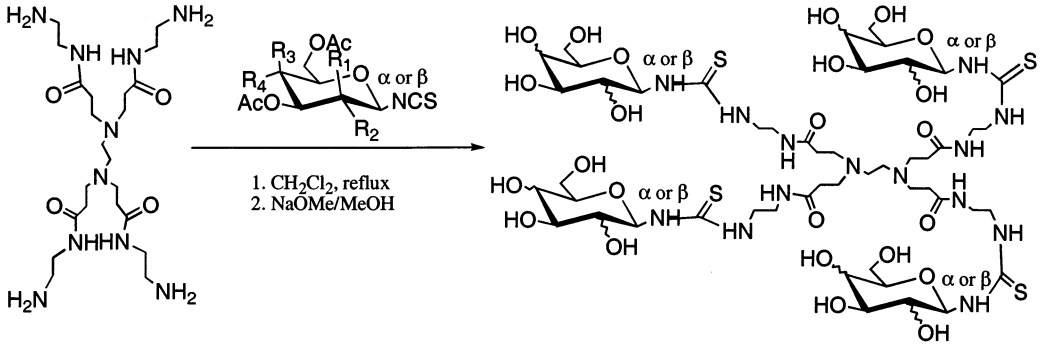


G0

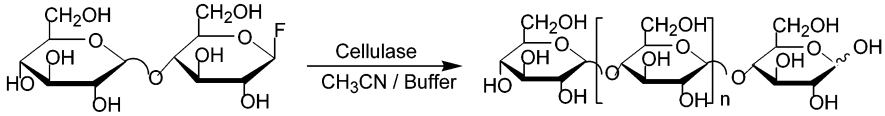
Disaccharide lactones of lactose, maltose, and other sugars have been conjugated to PAMAM to obtain highly branched dendritic glycosides of well-defined structure.[80−83] Lactose and maltose persubstituted PAMAM dendrimers ($G = 2.0-4.0$) are globular carbohydrate macromolecules with specific binding to ConA and peanut agglutinin (PNA, *Arachis hypogaea*), respectively. Acetylated glycosyl isothiocyanates have also been linked with dendritic polyamines to give throurea-bridged cluster glycosides with defined structures (Scheme 12).[84]

Baker and co-workers[65] synthesized sialic acid conjugated dendritic polymers by using PAMAM as a core structure. With different types of PAMAM structures, various sialic acid fictionalized macromolecular architectures were formed. Hemagglutination inhibition assays (HAI) performed on each polymer showed that variations in dendritic architecture have a profound effect on the potency of sialic acid based polymeric viral inhibitors. On the basis of the HAI screening data,

Scheme 12. Glycosyl Isothiocyanates in Dendrimer Synthesis



Scheme 13. Enzymatic Synthesis of Cellulose



linear and spheroidal polymers were 32−256-fold more effective than monomeric neuraminic acid at inhibiting hemagglutination toward H2N2 influenza. Linear-dendron copolymers were 1025−8200-fold more effective against H2N2 influenza, X31 influenza, and Sendai viruses. The most effective were the comb-branched and dendrigraft inhibitors, which shows up to 50000-fold increased activities against these viruses. The effectiveness of the inhibitors increases with the degree of sialic acid substitution on the surface of these polymers.

3. Enzymatic Synthesis

Enzymes have been increasingly used as catalysts in organic synthesis. The advantage of enzymatic catalysis over synthetic techniques is the high degree of reaction selectivity (stereo-, regio-, and chemoselectivity) and the use of mild conditions (ambient temperature and pressure) that enable exquisite control of polymer properties. In addition, genetic engineering can afford a large quantity of enzymes with tunable catalytic properties and selectivities. Enzymes that catalyze the synthesis of polysaccharides are usually restricted to glycosidases, glycosyltransferases, and phosphorylases. The mechanism of glycosylation generally involves the formation of an activated enzyme glycosyl donor intermediate, followed by nucleophilic attack of a glycosyl acceptor to form a glycosidic bond.

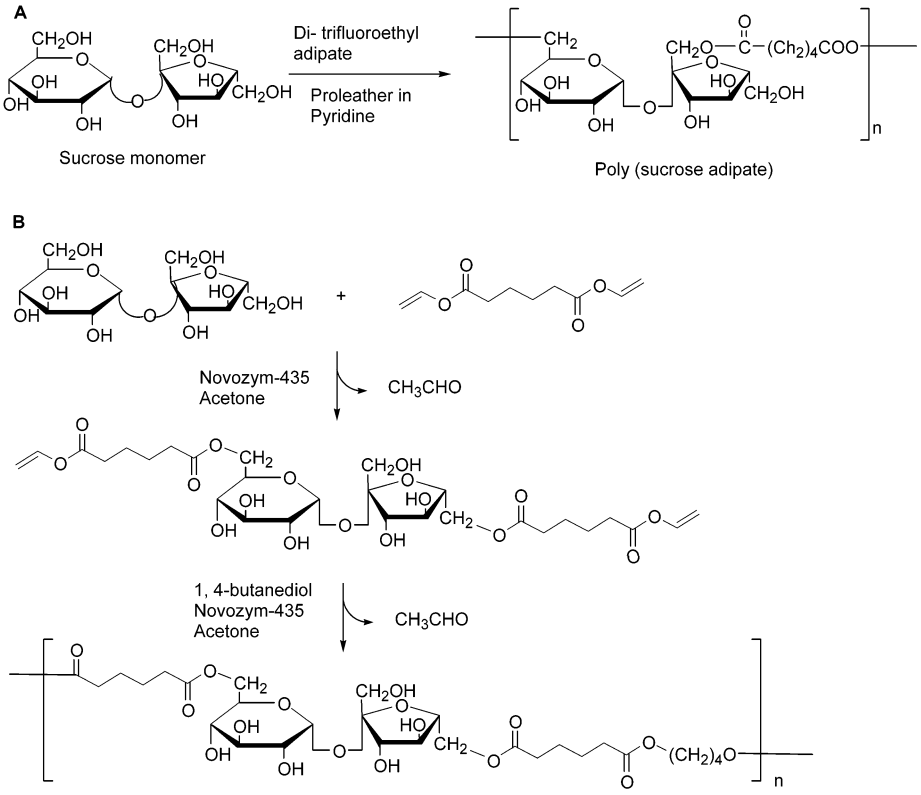
Glycoside formation occurs via a condensation reaction (e.g., reverse hydrolysis), and this is possible even in the presence of water, although the equilibrium is not highly favorable. The equilibrium toward synthesis can be improved by (1) increasing the substrate concentration, (2) decreasing the water content, and/or (3) removing the glycosidic product from the reaction mixture. Condensation reactions of 10% (w/v) galactose and 70% sucrose catalyzed by α-galactosidase and incubated for 48 h at 37 °C yielded two trisaccharides, planteose and raffinose, in a 2:3 ratio at ≈10% yield.[85] Isolation of products is generally complicated and can be simplified by continuous reaction methods using immobilized enzyme. Nonetheless, glycosidase-catalyzed polycondensation reactions invariably result in small polymers and low yields.

An alternative to condensation is transglycosylation. A wide range of glycosyl donors have been used with the key feature being the presence of an activated leaving group. *p*-Nitrophenyl (PNP) and *o*-nitrophenyl glycoside donors are useful for transglycosylation. They are efficiently recognized by glycosidases and their high reactivity enables rapid glycosyl transfer to acceptors, reducing competing hydrolysis of glycosylated products.[86,87] Their water solubility is a problem as a result of the hydrophobicity of the nitrophenyl group. This limits their utility since reactions require high concentrations of substrates to favor glycosylation over donor hydrolysis. New glycosyl donors, 3-nitro-2-pyridyl and 5-nitro-2-pyridyl glycosides, have proven to be effective for transglycosylation reactions catalyzed by *β*-glucosidase, *β*-galactosidase, and *N*-acetyl-*β*-hexosaminidase. Their high water solubility and reactivity have enabled reactions to be run at high donor concentrations, resulting in rapid glycosyl transfer to acceptors.[88]

Glycosyl fluorides, in which the anomeric hydroxyl group is replaced by fluorine, are powerful candidates for glycosidase-catalyzed polysaccharide synthesis. Glycosyl fluorides are prepared chemically by using fluorinated reagents such as (dimethylamino) sulfur trifluoride (DAST) or tris(dimethylamino) sulfonium difluorotrimethyl silicate (TASF).[89] The glycosyl fluorides are relatively stable, and the resulting covalent glycosyl-enzyme intermediate is easily formed by cleavage of the carbon−fluorine bond of the glycosyl fluoride. One of the more interesting reactions performed using glycosyl fluorides is the cellulase-catalyzed synthesis of cellulose with a molecular weight of 6300 after peracetylation (Scheme 13).[90]

In enzymatic transglycosylation reactions, a lack of regioselectivity is usually encountered when glycosyl fluorides are used as activated donors. With monosaccharide donors and hexopyranosides as acceptors, the formation of (1→6)-glycosidic linkages generally predominates. Transglycosylation with enzymes such as cellulase and 1,3- and 1,4-glucanase afford strict regioselective transfer of disaccharide fluorides. Disaccharide donors are usually transferred to equatorially oriented *O*-4 of mono-, di-, tri-, and oligosaccharide acceptors.[91] Cellulase catalyzes the transglycosylation of the cellobiosyl moiety onto the 4′-hydroxyl group of another cellobiosyl fluoride, resulting in the elimination of HF. The polymerization is performed in aqueous−

Scheme 14. Enzymatic Synthesis of Sucrose-Based Polyesters



organic cosolvents such as acetonitrile−water to promote the formation of the desired transglycosylation reaction product and to minimize the competitive formation of the hydrolytic breakdown product. The structure of the water-insoluble "synthetic cellulose" product was confirmed by X-ray and ^{13}C NMR analyses.[90]

The enzymatic polycondensation of glycosyl fluorides in aqueous−organic cosolvents has been extended to amylase-catalyzed synthesis of maltooligosaccharides.[92] These oligosaccharides are useful components as food additives, pharmaceuticals, and enzyme substrates for clinical reactions. In addition to their utility in the synthesis of natural products, glycosidases such as cellulase and xylanase are able to recognize unnatural substrates, leading to the synthesis of unnatural polysaccharides. For example, cellulase catalyzes the regio- and enantioselective polymerization of 6-*O*-methyl-β-cellobiosyl fluoride to afford 6-*O*-methylated cellulose.[93]

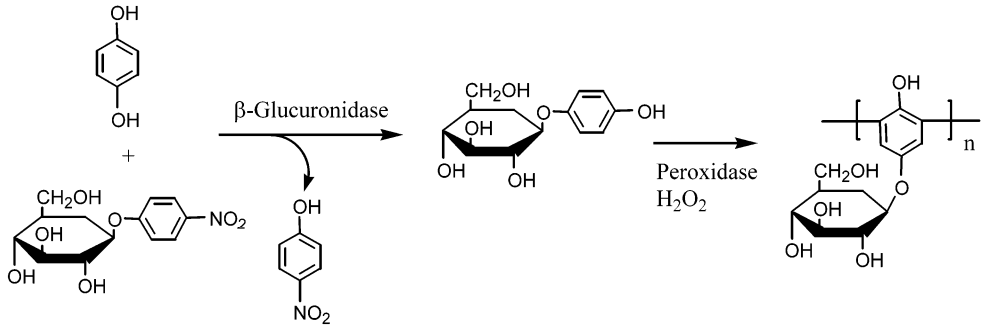
Chitin, β -(1→4)-linked poly(*N*-acetylglucosamine), and its deacetylated derivative chitosan have a number of potentially useful properties, including antibacterial activity, wound-healing activity, and biodegradability.[23,94] In vitro synthesis of chitin has been achieved in nearly quantitative yield through the ring-opening polyaddition of an oxazoline derivative of chitobiose catalyzed by Chitinase.[95] In addition to enzymatic polymerizations catalyzed by glycosidases, phosphorylases have been used to catalyze polysaccharide synthesis. Specifically, potato phosphorylase catalyzes amylose synthesis from α-D-glucosyl-1-phosphate and has been used to synthesize linear-, star-, and comb-shaped primers.[96,97]

Enzymes have been used to synthesize sugar-containing polyesters in nearly anhydrous organic solvents. Once again, the high selectivity of enzymes allows them to distinguish specific hydroxyl moieties in the polyol. Specifically, subtilisin has been used to catalyze the regioselective acylation of maltose, sucrose, and other sugars as well as nucleosides and related compounds in *N*,*N*-dimethylformamide.[98−100] Such acylation can be used to generate polymers, as evidenced by the Proleather (a *Bacillus* protease), which catalyzed the synthesis of sucrose-based linear polyesters (Scheme 14A) using di(2,2,2-trifluoroethyl)adipate as the acyl donor. The trifluoroethylester acyl donor is an activated functionality for the protease-catalyzed polyester synthesis reaction, while ensuring that the alcohol-leaving group acts as a poor nucleophile. This shifts the reaction equilibrium toward polymer formation. In addition, enzymes are kinetically activated by the trifluorethyl ester. Proleather is an extremely selective enzyme; only the 6 and 1′ hydroxyls of sucrose are acylated. Water-soluble polymers were prepared with over 100 sucrose units. In addition to sucrose, polymers of raffinose, lactose, and fructose were prepared using this method.[101]

Lipases have also been used to catalyze polycondensation reactions on disaccharides. For example, Novozym-435 (lipase B from *Candida antarctica*) catalyzes the synthesis of sucrose 6,6′-*O*-divinyladipate in acetone (Scheme 14B).[102] The diester was then employed as the monomer in polycondensation reactions with diols (aliphatic and aromatic) catalyzed by the same enzyme to yield linear polyesters with molecular weights up to 22000.

Peroxidases (from horseradish (HRP) and soybean (SBP)) have been used to catalyze sugar-containing polymer formation. Peroxidases catalyze the oxidative dehydrogenative polymerization of phenols, using H_2O_2 as the terminal oxidant, and this reaction can be performed on phenolic-derivatized sugars. Scheme 15

Scheme 15. Multienzymatic Synthesis of Polyphenols



shows the preparation of phenolic polymer by multienzymatic synthesis.[103]

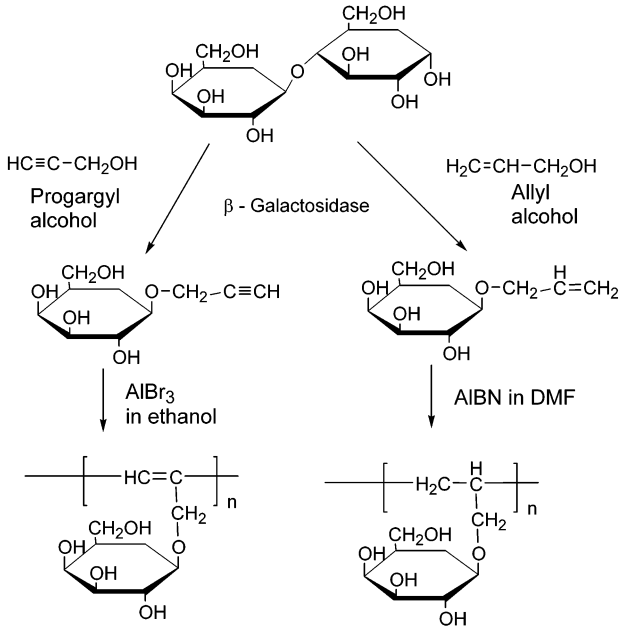
A combinatorial strategy for biocatalytic polymer synthesis was also achieved by Dordick and co-workers.[104] A library of polymers was synthesized in 96 deep-well plates using AA−BB polycondensations of acyl donors and acceptors. The library included a variety of aliphatic/aromatic diols including carbohydrates and nucleic acids. The lipase from *C. antarctica* was active in acetonitrile and was capable of catalyzing the polycondensation reactions, affording products of molecular weight up to 20000. Combinatorial approaches can facilitate the rapid enzymatic production of novel polymeric materials. Enzymatic synthesis of sugar-based polymers usually affords polymers with a high degree of regioselectivity and anomeric selectivity. However, the slow rate of these reactions and difficulties in obtaining high molecular weight polymers limits enzymatic methods. In many cases, only oligosaccharides are formed in enzyme-catalyzed syntheses.

4. Chemoenzymatic Methods

The relatively low rate of polymer synthesis and the low molecular weights of the polymers obtained can be overcome using chemoenzymatic synthesis. In these cases, the high selectivity of the biocatalyst is coupled with the high reactivity of chemical routes to yield high molecular weight polymers. Strategies for chemoenzymatic synthesis include the following: (1) lipase-, protease-, or glycosidase-catalyzed transesterification or glycosylation of saccharides to obtain sugar monomers that contain acetylenic and ethylenic functional groups for chemical polymerization; and (2) chemical synthesis of core structures that are further modified by glycosyltransferases.

The formation of propargyl- and allyl-*β*-D-galactopyranoside has been achieved by *β*-galactosidase-catalyzed transglycosylation of lactose with propargyl alcohol and allyl alcohol, respectively, in aqueous buffer (Scheme 16). While the polymerization of a propargyl sugar derivative under free radical conditions (AIBN and benzoyl peroxide) failed, cationic polymerization using $AlBr_3$ formed the desired carbohydrate-based polymer. When the triple bond was replaced by double bonds, a higher molecular weight polymer (M_w 37400) was obtained using a nickel-based catalyst. Free radical initiation was effective in polymerizing allyl sugar derivatives, giving carbohydrate-based polymers. Both AIBN and 2,2′-azobis-(2-amidinopropane) (ABAP) catalyzed the formation of higher molecular weight, water-soluble, and highly hygroscopic products.[105]

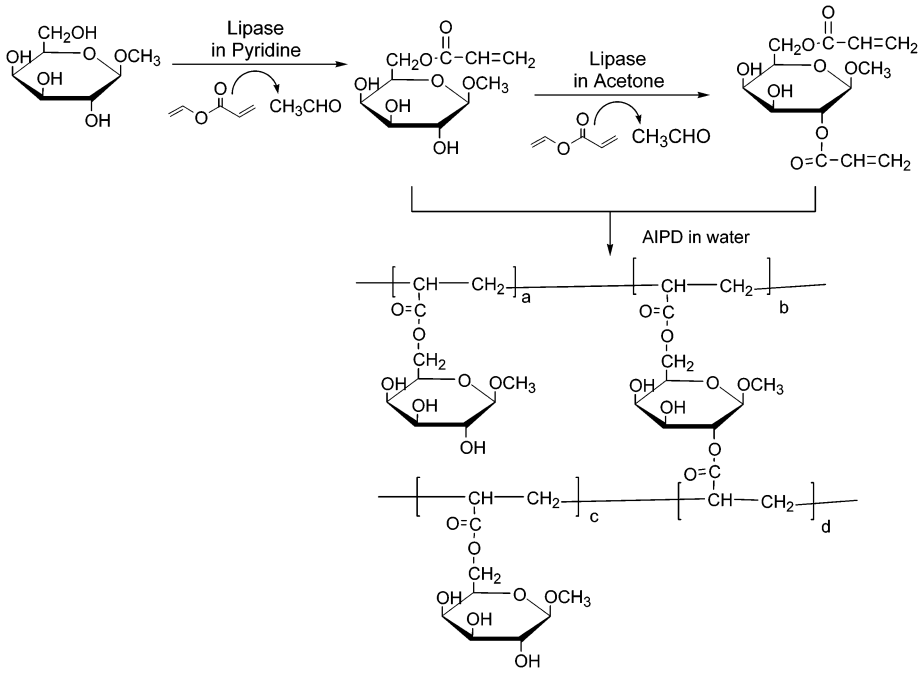
Scheme 16. Enzymatic Derivatization of Saccharides and Their Chemical Polymerization



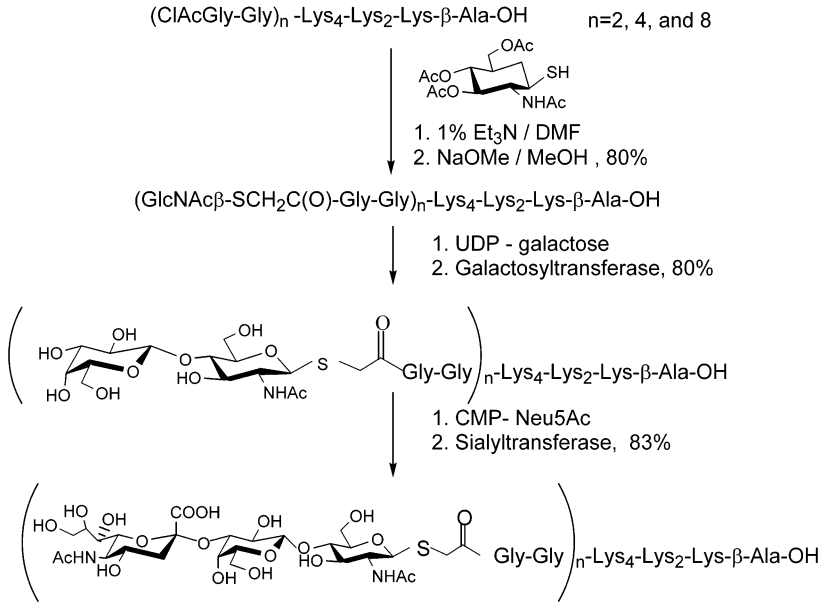
The aforementioned polymers are not cross-linked. Nonetheless, highly hygroscopic sugar-based materials can be prepared to contain cross-links. These materials, which can be considered as hydrogels, have applications ranging from water absorbents and biomedical devices to chromatographic supports and functional components of permselective membranes.[106,107] The high water content, homogeneity, stability, and expected nontoxicity make sugars ideal building blocks for hydrogels. Dordick and co-workers[108,109] used a chemoenzymatic method to synthesize sucrose- and galactose-based hydrogels (Scheme 17).

Lipase from *Pseudomonas cepacia* catalyzed regioselective acryloylation at the 6-hydroxyl moiety of *β*-*O*-methyl galactopyranoside in anhydrous pyridine to give the monoacrylate. Conversion of the monoacrylate to the 2,6-diacrylate cross-linker can be performed in anhydrous acetone by the same enzyme. Polymer synthesis in the presence of 0.5% (mol/mol) diacrylate yields the desired hydrogel network. The water absorbency of the resulting hydrogels is correlated to synthetic conditions. Important variables include initial monomer concentration, cross-link density, reaction temperature, and the absence or presence of ionic groups in the polymers. A sugar-based cross-linker does not dramatically affect the hydrating capacity of the hydrogels. At 20% (w/w) diacrylate cross-linker, a poly(α-methylgalactoside acry-

Scheme 17. Preparation of Sugar Hydrogels



Scheme 18. Chemoenzymatic Synthesis of Dendritic Sialyl Lewis^x

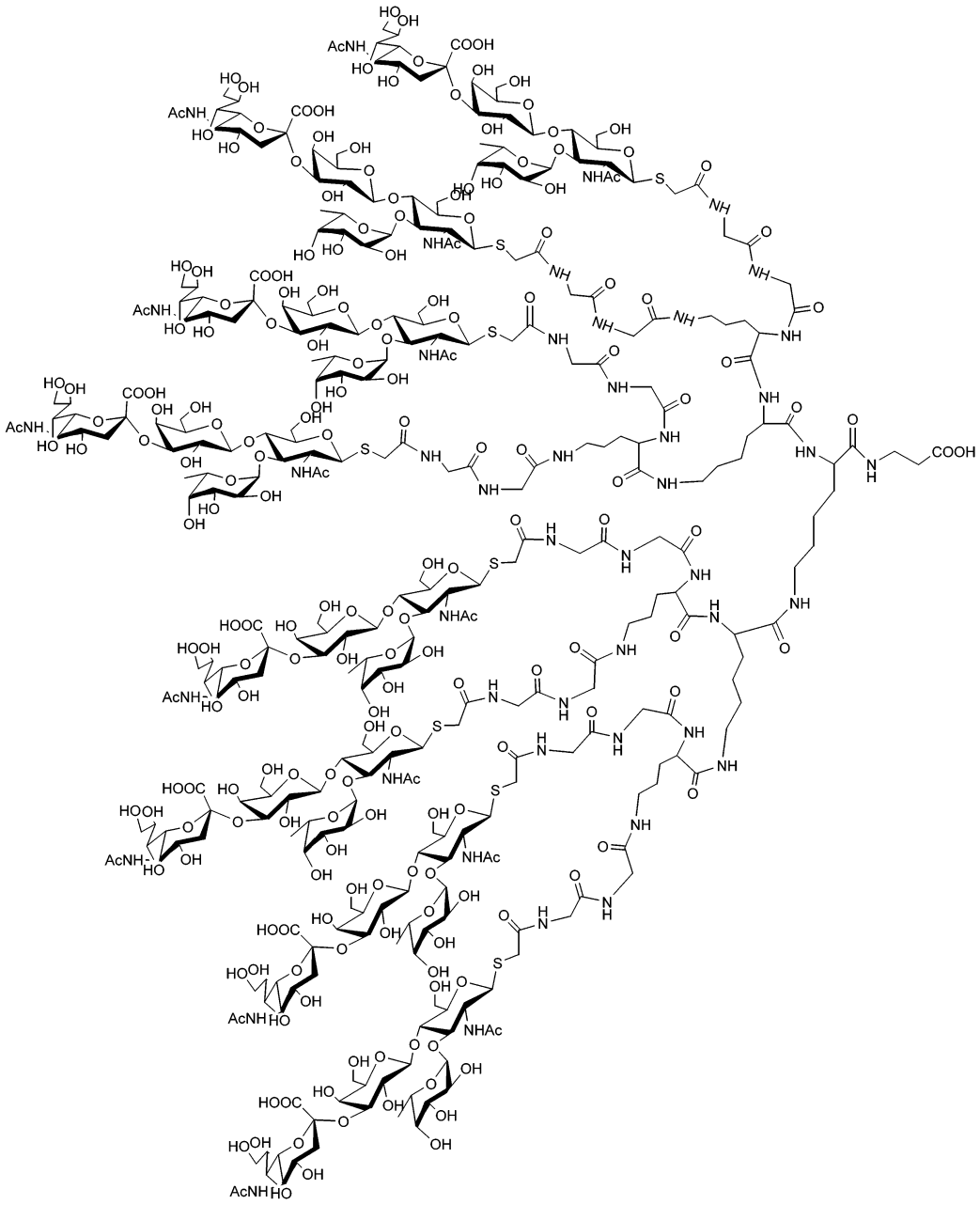


late) hydrogel still swells to 72 times its weight in water. However, at fixed cross-linker concentration, an increase in the initial concentration of monomer from 5 to 25% (w/w) decreases the swelling ratio in water from 23 to 4. Polymers with swelling ratios of 20−1000 have been prepared by adjusting the cross-linker ratio and the ionic content of the polymers. When the dicrylate cross-linker was increased to 20% (w/w), sugar-based hydrogels were obtained with comparable mechanical strength to more conventional hydrogels such as poly(hema) and poly(acrylamide).[110] A wide range of pore sizes can be obtained by this method, suggesting that they may be especially useful as matrixes for enzyme immobilization and controlled delivery of biological macromolecules.[111]

L, P, and E selectins are a well-known family of transmembrane glycoproteins responsible for the adhesion of leukocytes to the vascular endothelium in the early cascade of events leading to inflammation.[112] Sialylated Lewis^x (sLe^x) tetrasaccharide (α-Neu5Ac-(2→3)-*β*-Gal-(1→4)-[α-L-Fuc(1→3)]-*β*-D-GlcNAc-OR are key ligands expressed on leukocytes and on endothelial cell surfaces that are initially recognized by L-selectin on leukocytes. sLe^x and related oligosaccharides represent lead compounds in the development of drugs for inflammatory diseases. Chemoenzymatic synthesis of dendritic sLe^x has been the focus of several research groups[113−115] (Scheme 18).

N-Chloroacetylated-glycine-ending clusters were prepared on the solid phase using Wang resin and Fmoc protecting groups.[116,117] Chloroacetyl groups at the end of each branch were substituted with peracetylated 1-thio-GlcNAc derivatives. Hydrolysis from the resin, followed by *O*-acetyl protecting group removal, afforded the starting series of glycodendrimers. The GlcNAc-

Scheme 19. Structure of Octavalent sLe^x Dendrimer



based dendrimers were then enzymatically galactosylated using UDP-galactose and bovine milk β-(1→4)-galactosyltransferase. The reactions were monitored by matrix-assisted laser desorption ionization (MALDI)-MS. The resulting *N*-acetyl-lactosamine (LacNAc)-based dendrimers were further elongated by the addition of *N*-acetylneuraminc acid using CMP-Neu5Ac and a cloned α-(2→3)-sialyltransferase from rat liver. In the third and final enzymatic transformation, sialyl LacNAc dendrimers were fucosylated using GDP-fucose and a mixture of human milk α-(1→3/4)-fucosyltransferase. The resulting dendrimers were purified by gel-permeation chromatography and analyzed by MALDI-MS. The structure of the octavalent sLe^x (Scheme 19) was elucidated by NMR spectroscopy.

5. Future Significance

Modern organic and carbohydrate chemistries allow the preparation of homogeneous and structurally defined polysaccharides and their analogues. Polymers containing carbohydrate residues have a wide range of applications as coating agents, molecular recognition biomedical materials for use in biotechnology. The polyvalency inherent in carbohydrate-based polymers is an important feature, which allow these materials to serve as cell surface mimicks to understand and manipulate carbohydrate−protein interaction. Carbohydrates are also important raw materials for the development of biodegradable, biocompatible, and biorenewable materials in the chemical industry and in medicine. Here, chemoenzymatic routes are particularly promising. Resulting hydrogel networks may find application as biomaterials because of their high water content, homogeneity, stability, and expected nontoxicity. In addition to the aforementioned synthetic strategies, modification of natural polymers, such as cyclodextrins as neoglycoconjugates, should provide new gene therapy vectors and controlled release drug delivery devices.[118,119]

Synthesis of polymers bearing specific lectin-binding carbohydrate units, with tight multivalent interaction, might be useful during drug candidates or in the preparation of biolayers against pathogen infection. Finally, the synthesis of dendrimers and related carbohydrate-based materials may find application in the emerging nanotechnology arena. Structures that can be tailored with respect to size and shape and that can be functionalized with chemical and biological moieties and catalysts will likely find applications in a new generation of smart materials in structures as diverse as medical implants and antifouling materials.

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CM0200137