# The Glycopolymer Code: Synthesis of Glycopolymers and Multivalent Carbohydrate–Lectin Interactions



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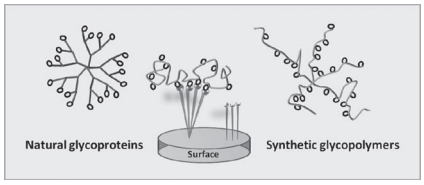
Glycopolymers are becoming more and more important in understanding biological interactions due to their unique recognition properties. Macromolecules with different chain lengths, compositions and architectures provide enormous diversity in the formation of primary and secondary structures that have a major effect on multivalent binding to lectins. It is crucial to control the precise structure of macromolecules to achieve specific and selective carbohydrate-lectin binding. The use of advanced synthesis techniques to prepare well-defined glycopolymers and selected advanced analytical techniques to study multivalent interactions are highlighted in this Feature Article.




## 1. Introduction

In our generation, one of the ultimate goals of a polymer chemist is to achieve the precise synthesis of macromolecules with controlled chain length, architecture, monomer sequence, chain folding, and tertiary structures.[1] When we succeed in this, one will be able to synthesize protein mimics and protein-like complex structures with unique functionalities. The studies on the preparation of artificial cells, which are aimed to have more simplified structures than the natural cells, have already been initiated in the last decade by several research groups.[2] However, our current knowledge on the synthesis of complex macromolecular architectures, on their self-assembly behavior, and more importantly on decoding the communication in between these components is not yet sufficient enough to design and construct an artificial cell that can self-reproduce, self-heal, self-evolve, and self-decide.[2b] In some instances, chemists are able to successfully self-assemble a cell membrane-like structures but understanding the chemical and physical interaction between the cell membrane and other macromolecules has a crucial role in self-functioning systems.[3] In every second, billions of cells in our body interact with nutrients, viruses, fat molecules, and proteins and take an action on how to react against these molecules as a system. An important fraction of these decisions is based on the carbohydrate–lectin interactions. In particular, cell surface oligosaccharides play an essential role in recognition events.

Basically, the sugar code runs the main operations in any living organism.[4] Carbohydrates differ from amino acids and nucleotides by their versatility for isomer formation, which creates the coding. Oligosaccharides have a high-density coding capacity due to the variations in anomeric status, linkage positions, ring size, branching, and introduction of site specific substitutions. These features enable oligosaccharides to be highly specific for protein binding. Oligosaccharides are commonly found on the cell surfaces and are involved in variety of important biological functions.[5] For instance, plant lectins protect plants from fungal attacks and herbivorous animals, involve in establishing symbiosis between plants and bacteria, store proteins and enzymes, modulate the enzymatic activities, and participate in growth regulation. Although animal

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*Scheme 1.* Glycopolymers in different architectures.

lectins play crucial role in cell–cell and cell–matrix interactions, cell migration and routing, intracellular routing of glycoproteins and vesicles, and recognition of foreign glycans.[6] However, the investigation of their functions is rather limited due to the difficulties in synthesizing such structures. Although there have been major developments to chemically synthesize oligosaccharides, such as introduction of good anomeric leaving groups and advances in the solid phase synthesis techniques, it is still demanding to isolate, purify, and analyze the complete structure of oligosaccharides.[7]

Glycopolymers have been considered as alternative structures to oligosaccharides.[8] The current challenge is to synthesize glycopolymers that are able to mimic oligosaccharides in selective binding to lectins.[9] Therefore, it is crucial to have an absolute control over the synthesis of a polymer chain. Development of controlled and living polymerization techniques in combination with click reactions allows the preparation of desired macromolecular architectures, such as linear, star, or graft shaped (Scheme 1). Ionic polymerization techniques provide polymer chains with minimum rate of termination reactions unless any impurities in the reagents react with the reactive chain end. Whereas radical polymerization conditions are more prone to side reactions due to the higher rates of hydrogen abstraction and radical–radical coupling reactions. Nevertheless, it is possible to control the polymer chain length and architecture using controlled/"living" polymerization techniques.

Currently, the major application of glycopolymers is in the drug and gene delivery field due to their biorecognition properties.[10] The research on glycopolymeric drugs mainly focuses on influenza hemagglutinin and neuraminidase inhibitors, human immunodeficiency virus, and Alzheimer's disease, which millions of people are suffering from.[11] However, the majority of these studies are still in preliminary level, and extensive research is required to for clinical trials of glycopolymer-based drugs. Nevertheless, glycopolymers have a great potential to be used in the treatment of cancer and infectious diseases.

The interaction between carbohydrates and lectins is usually very weak and the binding can be enhanced using multivalent ligands. Ligands with multiple carbohydrates on the same molecule, such as glycopolymers, create cluster glycoside effect and simply increases the binding constant. The valency has a large effect in binding but the orientation of the carbohydrates has even larger impact on binding. Several analytical techniques have been developed to measure the carbohydrate-lectin binding. One of the oldest and therefore the most common and easiest technique to measure the carbohydrate-lectin binding is turdimetric measurement.[12] Alternatively, quantitative precipitation and quenching of fluorescence emission techniques have been employed to distinguish effect arising from receptor clustering and receptor proximity, respectively.[13] Relatively more advanced techniques such as ITC, atomic force microscopy (AFM), quartz crystal microbalance (QCM), and surface plasmon resonance (SPR) spectroscopy have also been used widely to investigate carbohydrate–lectin interactions.[14] In this review, selected literature examples have been presented regarding the utilization of QCM and SPR spectroscopy techniques to understand carbohydrate–lectin binding.

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## 2. Discussion

### 2.1. Preparation of Glycopolymers via Various Synthesis Techniques

Synthesis of glycopolymers became more and more popular in the last two decades due to better understanding of their biorecognition properties. Most of the initial reports on this field are based on the polymerization of glycomonomers. However, there are several drawbacks in the synthesis and characterization of glycopolymers that are prepared directly from glycomonomers. For instance, the selection of the reaction medium is crucial to sufficiently solubilize of glycomonomer and glycopolymer and

the common reaction solvent has been selected as water in case the glycomonomer is not protected. Indeed, it is not possible to conduct ionic polymerization in an aqueous medium and the glycomonomer should be protected, that is, by acetylation of the hydroxyl groups, prior to the ionic polymerization. Following to the polymerization of the protected glycomonomer, deprotection reaction has to be performed. Ionic polymerization techniques provide glycopolymers with a well-defined chain length though, these reactions are quite sensitive to impurities and require additional protection and deprotection steps.[15]

Controlled radical polymerization (CRP) techniques have been developed in 1990s and versatile chain transfer agents, stable radical mediators, and catalyst systems have been investigated. The most commonly employed CRP techniques have been reversible addition–fragmentation chain transfer polymerization (RAFT), nitroxide-mediated polymerization (NMP), and Cu-catalyzed living radical polymerizations (TMM-LRP, ATRP, and SET-LRP).[16] These techniques are relatively more tolerant to different reaction solvents and functional monomers and almost as successful as ionic polymerizations in controlling the kinetic chain length and macromolecular.[17]

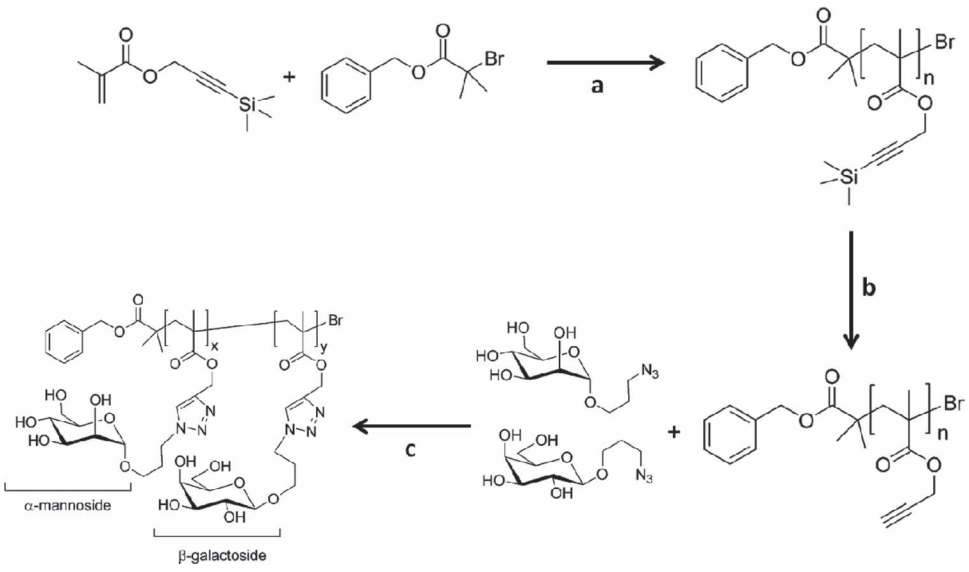
Another major turning point in polymer science has happened in 2001 when Sharpless and co-workers[18] highlighted the importance of highly efficient organic reactions. These reactions have been called as click reactions and nowadays there are around 20 organic reactions that are claimed to be potential click reactions. Most of these click reactions have cons and pros but more importantly they allow polymer chemists to prepare tailor-made macromolecular structures in a well-defined manner.[19] In the last decade, selected click reactions in combination with ionic or radical polymerization techniques have been used to prepare glycopolymers in controlled chain length, composition, and architecture.[9]

### 2.1.1. Cu-Mediated Controlled Radical Polymerization

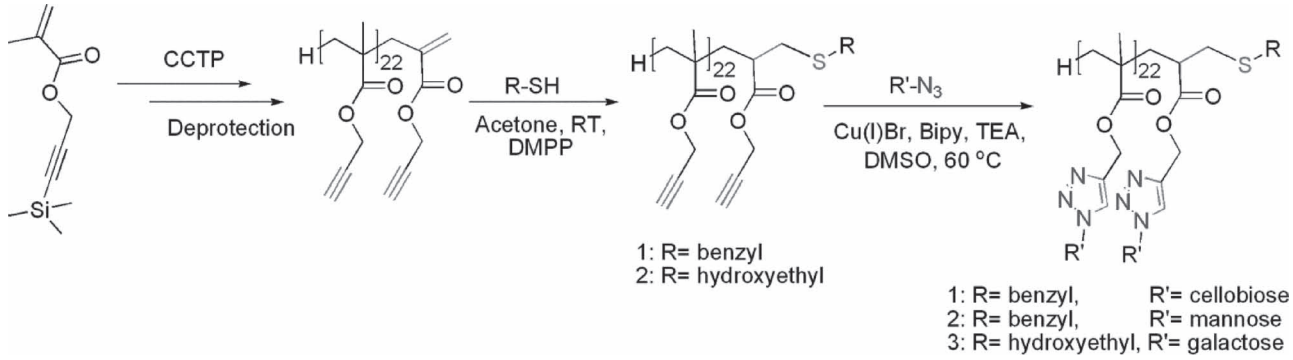
Haddleton et al.[20] reported the synthesis of neoglycopolymers by combination of click reactions and living radical polymerization. The first step of the synthesis involves the Cu(I)-mediated living radical polymerization of (trimethylsilyl)propargyl methacrylate initiated by a benzylic α–bromo ester) (Scheme 2). The aromatic hydrogens of the initiator appear around 7 ppm in the $^1$H NMR spectrum and provide a more accurate characterization of the polymer. Trimethylsilyl (TMS) group was used for protecting the alkyne group from radical attacks during the radical polymerization. The removal of this group is straightforward and can be achieved by using TBAF in the presence of acetic acid. After complete deprotection, evidenced by the disappearance of the TMS peak at around 0.2 ppm in $^1$H NMR spectrum, Cu(I)-catalyzed sugar –azide and poly(alkyne) reaction was performed in the presence of DIPEA. The click reaction can be followed by the disappearance of the alkyne peak (2.5 ppm in $^1$H NMR) and the azide peak (≈2100 cm$^{-1}$ in FTIR) and appearance of the triazole ring (≈8.0 ppm in $^1$H NMR). These polymers are typically purified by precipitation, dialysis, and freeze-drying.

There are several advantages of using combination of atom transfer radical polymerization (ATRP) and Cu-catalyzed azide–alkyne cycloaddition (CuAAC) reactions to prepare glycopolymers. ATRP conditions have been widely studied and the controlled polymerization of TMS-MA could easily be achieved. Besides, ATRP technique is tolerant to most functional initiators. For instance, it is possible to use initiators with azide, disulphide, and protected alkyne or maleimide functionalities that would provide successful postpolymerization modification of polymer chains.[21]

The preparation of sugar azides had required several steps until an elegant approach has been reported by Tanaka et al. in 2009.[22] They have demonstrated the one-pot synthesis of β-glycosyl azides using unprotected sugars, sodium azide, and 2-chloro-1,3-dimethylimidazolinium




*Scheme 2.* Synthesis of glycopolymers by combination of Cu(I)-catalyzed living radical polymerization and Cu(I)-catalyzed azide-alkyne click reaction. (a) *N*-(*n*-ethyl)-2-pyridylmethanimine, CuBr, Toluene, 70 °C; (b) tetra-*N*-butylammonium fluoride (TBAF), acetic acid, THF, –20 to +25 °C; (c) [$(PPh_3)_3$]CuBr, DIPEA.




*Scheme 3.* Synthesis of glycopolymers by combination of cobalt-catalyzed chain transfer polymerization and Cu(I)-catalyzedazide–alkyne click reaction.

chloride (DMC) in appropriate solvent. This approach was extended to synthesize glycopolymers with mono, di-, and tri- saccharides by Gibson et al.[23] Last but not least, it is possible to conduct one-pot simultaneous CuAAc click and polymerization reactions to prepare glycopolymers since both reactions use the same catalyst system where click reaction proceeds much faster than the polymerization.[24]

### 2.1.2. Cobalt-Catalyzed Chain Transfer Polymerization

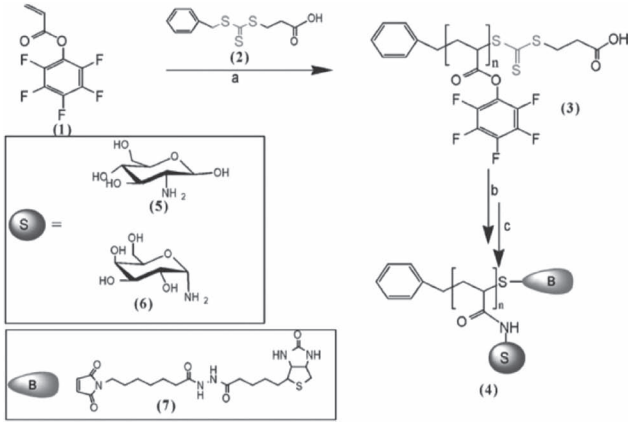
Alternatively, TMS-methacrylate can be polymerized by cobalt-catalyzed chain transfer polymerization (CCTP) that yields polymers with terminal vinyl groups. The main advantages of this polymerization technique are very high chain-end fidelity and ultra-low amount of catalyst required for the reaction.[25] However, this radical polymerization technique is more powerful for synthesizing relatively shorter polymer chains (DP < 50). Haddleton et al.[26] demonstrated the synthesis of glycopolymers combining CCTP and two different types of click reactions.[26] In this case, CCTP of TMSMA provided relatively shorter polymers (DP = 22) with a high ratio of vinyl end groups (Scheme 3). Subsequent to the deprotection of the alkyne groups, phosphine-catalyzed Michael addition reaction was performed to functionalize the chain ends with either benzyl or hydroxyethyl groups. It should be noted that thiols do not react with alkyne groups under base catalyzed thiol-ene click reaction conditions but it would react under radical mediated thiol-ene click reaction conditions. Finally, three different sugar–azides were clicked on the poly(alkyne) backbone under CuAAc conditions.

### 2.1.3. RAFT polymerization

There are three main advantages of using RAFT polymerization; (i) conducting homogeneous polymerization in aqueous medium is possible, (ii) higher molecular weights are easily achievable, and (iii) preparation of thiol end functional polymers are relatively easy.[27] Besides, the syntheses of chain transfer agents are straightforward and recently selected RAFT agents have become commercially available. Therefore, RAFT polymerization has become the most employed technique for polymerization of glycomonomers in aqueous medium.[28]

Stenzel and co-workers[29] reported the synthesis of various glycopolymers architectures via RAFT polymerization. They have demonstrated the preparation of homo, block, and star shaped polymers of poly(acryloyl glucosamine) typically in $H_2O$:EtOH (5:1) mixtures. The resulting polymers have a molar mass in the range of 3000 to 120 000 g mol$^{-1}$ with relatively low polydispersity indices. Moreover, Stenzel and co-workers[30] reported the preparation of mannose containing vinyl acetate block copolymers combining RAFT polymerization and CuAAc click reaction.[30] They performed RAFT polymerization of 6-*O*-methacryloyl mannose in *N,N*-dimethyl acetamide (DMAc) at 70 °C initiated by TMS protected alkyne-functionalized dithiobenzoate chain transfer agent. Similarly, they prepared azide end-functionalized poly(vinyl acetate) using azide-functionalized xanthate chain transfer agent. Finally, they obtained poly(vinyl acetate)-*b*-(6-*O*-methacryloyl mannose) ($\bar{M}_{n,GPC}$ = 15 400 g mol$^{-1}$, PDI = 1.48) by reacting two functional homopolymers under CuAAc click reaction conditions.

Another elegant approach on one-pot synthesis and biofunctionalization of glycopolymers via RAFT polymerization and thiol-ene reactions was reported by Boyer and Davis.[31] They modified an activated ester-functionalized polymer with sugar-amines and performed a simultaneous aminolysis-thiol-ene reaction involving the RAFT end-group yield biotin-functionalized glycopolymer in one pot. As shown in Scheme 4, pentafluorophenyl acrylate was polymerized in the presence of 3-(benzylsulfanylthiocarbonylsulfanyl)-propionic acid as a chain transfer agent in benzene at 70 °C. The purified polymer was subsequently modified by reaction with D-glucose amine or D-galactose amine in DMF. Near quantitative conversion was achieved in 1 h. As the final step, in situ aminolysis of the RAFT end-group and the addition of thiol onto biotin



*Scheme 4.* Synthesis of glycopolymers by RAFT polymerization. Conditions: (a) RAFT polymerization in the presence of AIBN in benzene at 70 °C, (b) nucleophilic addition of amine, in DMF–water (50/50 vol%) at room temperature, (c) in situ aminolysis of the RAFT end-group and the addition of thiol onto biotin-modified maleimide.

modified maleimide was performed. Eventually, biotin-functionalized glucose or galactose glycopolymers could be obtained in one-pot synthesis.

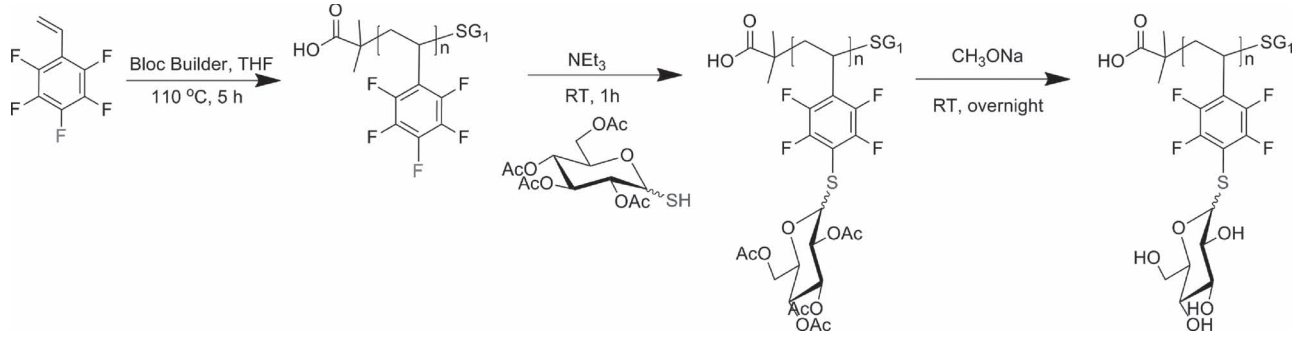
### 2.1.4. Nitroxide-Mediated Polymerization

As discussed earlier, there are two main routes to prepare glycopolymers, which are the direct polymerization of glycomonomers and postpolymerization functionalization of reactive polymer chains. There are several earlier reports on the synthesis of glycomonomers and their polymerization via ionic or radical polymerization techniques.[32] The first report on radical polymerization of a glycomonomer was published by Fukuda and co-workers[33] where they have performed NMP of *N*-(*p*-vinylbenzyl)-[O-β-D-galactopyranosyl-(1 → 4)]-D-gluconamide (VLA), a styrene derivative with an oligosaccharide moiety, in DMF at 90 °C. However, they found it crucial to acetylate the glycomonomer, to use di-*tert*-butyl nitroxide instead of TEMPO, and to initiate the polymerization with di-cumyl-peroxide radical initiator. Nevertheless, they could obtain glycopolymers with molar mass around 20 000 to 40 000 g mol$^{-1}$ and relatively low polydispersity values.

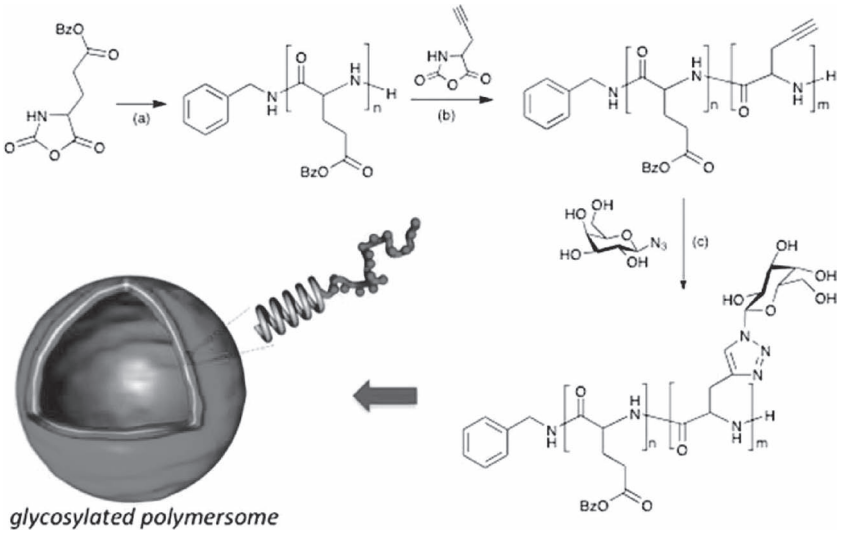
NMP technique has attracted a great attention from material chemists who preferred performing metal-free or sulfur-free radical polymerizations.[34] However, there was a need of developing free nitroxide compounds with improved stability and activation/deactivation rates.[35] The first and most commonly used nitroxide compound is TEMPO and required relatively high polymerization temperatures and provided limited control for most of the monomer classes. Following to this, several nitroxide compounds have been developed and two of those, namely TIPNO and SG1 showed relatively good control over polymerization under various reaction conditions.[34a,36] The majority of the research on NMP has shifted to unimolecular systems where the initiator and the free nitroxide groups present on the same molecule whereas previously bimolecular systems were preferred. The main drawback of NMP on preparation of glycopolymers could be the relatively high polymerization temperature required for polymerizing glycomonomers. The usual reactions temperatures exceed 100 °C and this may cause decomposition of glycomonomers at extended reaction times.

In 2009, we have reported a route to synthesize glycopolymers using metal-free polymerization and click reaction techniques.[37] In many cases, it is necessary to protect the clickable groups of the monomer to avoid any side reactions during radical polymerization. However, pentafluorostyrene has a *para*-fluoro group, which is reactive toward thiols or amines but not reactive with any radicals present in the reaction medium. There have been reports on ATRP, RAFT, and NMP of PFS and in all cases well-defined polymers could be obtained.[38] Additionally, PFS is a commercially available monomer and has a very similar solubility and reactivity behavior to styrene, and there are wide range of thiol or amine compounds that can be reacted with *para*-fluoro functional group.

As the synthesis route shown in Scheme 5, PFS was polymerized in THF using Bloc Builder and purified by pre-




*Scheme 5.* Preparation of glycopolymers using metal-free polymerization and click reaction techniques.




*Scheme 6.* Synthesis of glycopeptide block copolymer via NCA polymerization and CuAAc click reaction. (a) DMF, benzylamine, 0 °C; (b) DMSO, room temperature; (c) $Cu(PPh_3)_3Br$, $Et_3N$, DMSO, 30 °C.

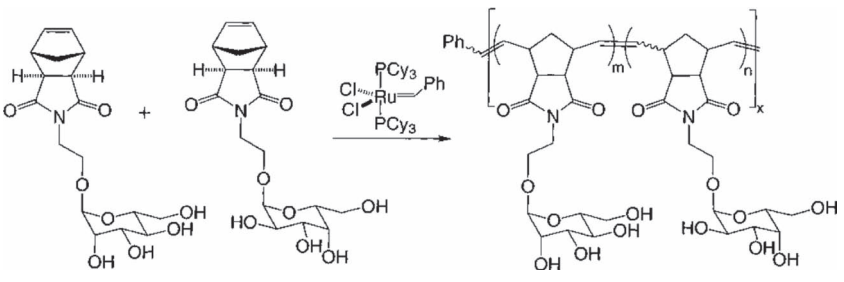
cipitation into methanol. Following to that, *p*-fluoro-thiol glucose click reaction was performed in the presence of triethylamine (TEA) under ambient conditions.[37] Deacetylation of sugar units was performed as the final step of the synthesis. Poly(pentafluorostyrene) (PPFS) could be obtained in desired chain length with low polydispersity (3 500 g mol, 1.03) using Bloc Builder alkoxyamine. Thiols are known as soft nucleophiles in comparison to amines or alcohols, hence displaying higher reactivity in nucleophilic substitution reactions.[39] Therefore, thio-glucose (1.2 eq.) reacted quantitatively with PPFS (1.0 eq. per PFS unit) in the presence of TEA (3.0 eq.) at room temperature. White solid could be obtained by precipitation of the acetylated glycopolymer into methanol (yield = 93%). Deacetylation reaction was performed in DMF and reacted with sodium methanolate at room temperature for 1 h. Complete deacetylation reaction was proved by $^{13}C$ NMR and $^{1}H$ NMR measurements. The GPC analysis showed relatively high molar mass of glycopolymers according to polystyrene calibration standards. The initial polymer had a molar mass of 3 500 g $mol^{-1}$, the acetylated glycopolymer had a molar mass of 9 200 g $mol^{-1}$ and the final glycopolymer had a molar mass of 19 200 g $mol^{-1}$. This is due to the dramatic change in the hydrodynamic volume of polymer in the GPC eluent, *N,N*-dimethylacetamide.

### 2.1.5. Ring-Opening Polymerization

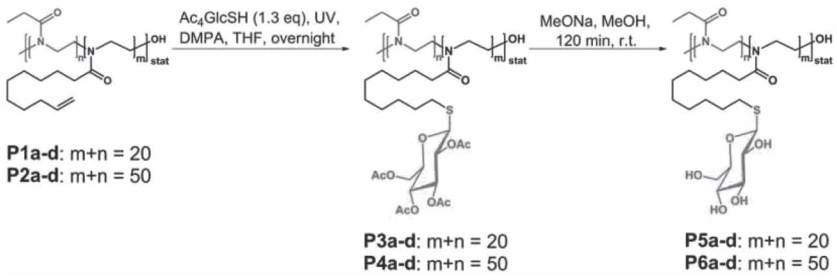
Ring opening polymerization of glycomonomers has been studied for many years.[40] Different catalyst systems and reaction conditions have been employed for successful synthesis of glycopolymers. For instance, N-carboxyanhydrides (NCA) can be polymerized via amine initiation, functionalized cyclic carbonates can be polymerized via organo-catalyzed ROP, oxazolines can be polymerized via cationic mechanism, and norbornene functional glycomonomers can be polymerized via ring-opening metathesis polymerization.[41] Some selected examples are discussed below.

Heise and co-workers[42] reported the synthesis of polypeptide block copolymers with different block length ratios using sequential ring-opening polymerization of benzyl-L-glutamate and propargylglycine (PG) N-carboxyanhydrides (NCA), Scheme 6.[42] Moreover, poly(PG) block copolymer was functionalized with galactose-azide by CuAAc click reaction. All copolymers were self-assembled using the nanoprecipitation method to obtain spherical and worm-like micelles as well as polymersomes depending on the block length ratio and the nanoprecipitation conditions. These structures display bioactive galactose units in the polymersome shell, as proven by selective $RCA_{120}$ lectin binding experiments.

Kiessling and co-workers[41c,43] have demonstrated the control of multivalent interactions by altering the binding epitope density. They utilized ring opening metathesis polymerization (ROMP) to prepare mannose- and galactose-containing glycopolymers. Even though they have used the glycomonomer approach to prepare glycopolymers they succeeded to prepare polymers that are of similar length and vary only in proportion of mannose and galactose residues (Scheme 7). Moreover, they have discussed several mechanisms of multivalent ligand binding, that is, chelate effect, subsite binding, steric stabilization, statistical effect, and receptor clustering.[44] The latter is mainly influenced by stoichiometry, rate of clustering, and the receptor proximity. Therefore, it is crucial to achieve absolute control over the composition and architecture of glycopolymers.



*Scheme 7.* Preparation of glycopolymers by ring-opening metathesis polymerization.




*Scheme 8.* Synthesis of glycopolymers with long spacer using cationic ring-opening polymerization and thiol-ene click reaction.

Schubert and co-workers[41d] reported the preparation of thermoresponsive glycopolymers by combining cationic ring opening copolymerization and thiol-ene click reaction. A series of 2-(dec-9-enyl)-2-oxazoline and 2-ethyl-2-oxazoline copolymers with different chain lengths was prepared in acetonitrile using a microwave reactor (Scheme 8). Moreover, the glycopolymers were obtained by the photoaddition of 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glycopyranose onto the double bonds followed by deacetylation of the saccharide residues. Turbidimetric measurements of the respective glycopolymers revealed a decreasing cloud-point temperature with increasing amount of glucose moieties, proposed to be caused by hydrogen bonding between the sugars and the polymer amide groups, which is enabled by the flexibility of the long decyl spacer.

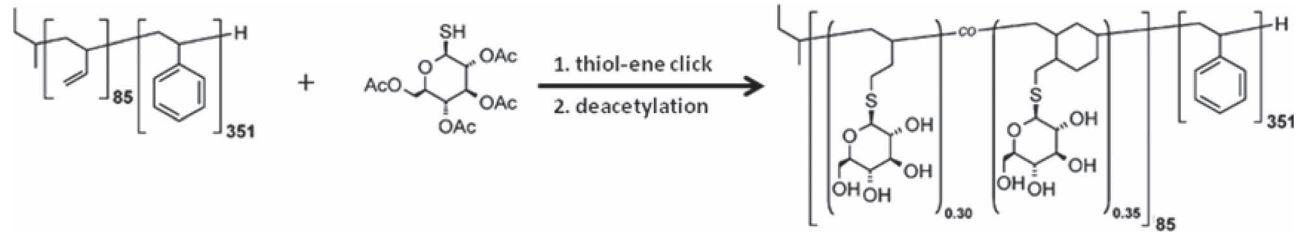
### 2.1.6. Anionic Polymerization

Anionic polymerization is also highly selective and is limited to vinyl monomers possessing electron-withdrawing groups such as nitrile, carbonyl, and phenyl. Anionic polymerizations are very sensitive to oxygen, require aprotic solvents and low reaction temperatures. All reactants should be of highest purity and monomers should not have any acidic protons to avoid side reactions. Several reports have been published on the synthesis of glycopolymers via anionic polymerization.[15a–15c,45]

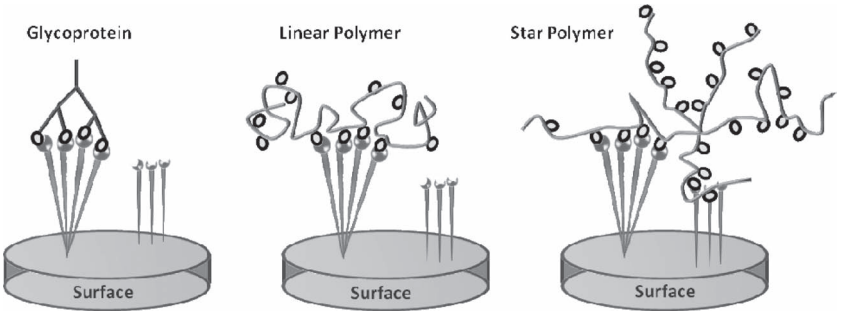
You and Schlaad[46] have demonstrated a simple route for the preparation of glycosomes by combining sequential anionic polymerization and radical-mediated thiol-ene click reaction, Scheme 9. Poly(1,2-butadiene)$_{85}$-*b*-(styrene)$_{351}$ was prepared by anionic polymerization with narrow molar mass distribution, 1.15. Then, a degassed mixture of 2,3,4,6-tetra-O-acetyl-β-D-1-thioglucopyranose, and azoisobutyronitrile (AIBN) ([C = C]/[SH]/[AIBN] = 1:6:0.33) in dry THF was stirred for 5 h under irradiation with a hand held mercury lamp. The resulting polymer was purified by precipitation into methanol and it contained 55 thioglucose units. This suggests the random formation of cyclic units along the poly(1,2-butadiene) segment of the block copolymer. Nevertheless, this is one of the initial reports performing thiol-ene click reactions on well-defined polymers and presents and easy methodology to prepare amphiphilic glycopolymer.

## 3. Typical Lectins Utilized in Glycopolymer Interactions

Plant lectins play important roles in external and internal activities of plants.[47] For instance, they protect the plant from fungal attack and herbivorous animals. Whereas, they also participate in growth regulation, storage of proteins, modulation of enzymatic activities, and adjustment to altered environmental conditions. The most popular and the cheapest plant lectin is *Canavalia ensiformis* (Concanavalin A, ConA) and it binds specifically to mannose and glucose. ConA is the first lectin that is isolated by crystallization in 1919.[48] Usually, ConA is used as a model lectin to investigate the multivalent binding of glycopolymers.[49] ConA is a tetramer at neutral pH with four subunits in a tetrahedral orientation where the binding sites are 72 Å apart from each other. Similar to ConA, *Arachis hypogaea* (peanut agglutinin, PNA) is also from the Leguminosae family. However, PNA binds to galactose and has wide applications in histochemistry.[50] *Triticum vulgare* (wheat germ agglutinin, WGA) is in Gramineae family and a dimeric lectin with eight binding sites for GlcNAc that are separated by distances of 14 Å. WGA has a potential function in plant defence




*Scheme 9.* Synthesis of amphiphilic glycopolymer using anionic polymerization and radical-mediated thiol-ene click reaction.




*Scheme 10.* Schematic representation for multivalent binding of a glycoprotein, synthetic linear glycopolymer, and star-shaped glycopolymer.

mechanisms.[51] *Ricinus communis* (ricin) is isolated from castor bean and binds selectively to galactose. Ricin is a ribosome-inactivating protein and also used for generating immunotoxins.[14d,52]

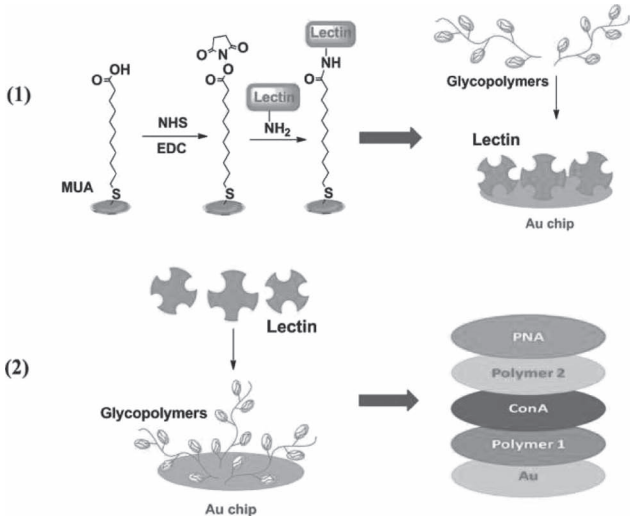
Animal lectins can be categorized in five families, which are C-, I-, P- type lectins, pentraxins, and galectins. These lectins are relatively more complex in structure and activities in comparison to plant lectins. Therefore, the number of studies conducted on understanding their interactions with glycopolymers is very limited. However, animal lectins have crucial roles in animal organisms such as intracellular routing of glycoconjugates, cellular growth regulation, cell–cell interactions, recognition of foreign molecules, and cells and cell type specific endocytosis.

## 4. Multivalent Carbohydrate–Lectin Interactions

Half of all proteins in our body are known to be glycosylated and many critical biochemical reaction pathways are dependent on the sugar code. Glycans, which are compounds that include monosaccharides, oligosaccharides, polysaccharides and their conjugates such as peptidoglycan, glycolipids, and glycoproteins are ubiquitous components of all organisms. The increased appreciation for the importance of glycans to human health motivated researchers to pursue interdisciplinary approaches. Different approaches have been followed to elucidate the molecular mechanisms in particular in fertilization and development, hormone function, cell profileration and organization, host–pathogen interactions, and inflammatory, and immune responses.[53] However, the synthesis of glycans are still challenging and requires multi-step organic reactions to obtain the complimentary glycoprotein of a lectin.[54] There are also several techniques developed for the synthesis of glycodendrimers and glycodendrons but these structures also require extensive synthesis and purification steps.[55] Alternatively, synthetic polymers can be used for multivalent binding to lectins (Scheme 10). The selectivity of glycopolymers would not be as good as glycoproteins but the binding rates might be manipulated by the density of sugar, type of sugar, and the architecture of the polymer.[56]

Monovalent carbohydrate ligands have relatively low binding constants ($10^3 \approx 10^6$ $M^{-1}$) to lectins. Usually, stronger and selective binding is required for any useful biological activity. Natural and synthetic multivalent ligands present multiple copies of a receptor-binding element that they can bind to receptors with high avidity and specificity. Thus, they can serve as powerful inhibitors. Moreover, they can be potent effectors that promote a specific biological response via signal transduction.

Various analytical methods can be employed to study multivalent carbohydrate –lectin interactions. The most widely applied technique is turbidimetry and based on the determination of the turbidity of the solution up on aggregation of lectin and polymer chains.[23,57] UV–vis spectrometer can be utilized to determine the turbidity of the solution at varying ratio of lectin to glycopolymers.[58] More advanced technique such as analytical ultracentrifugation can be utilized to measure the sedimentation velocity, which is related to the binding rate of lectins and carbohydrates. Alternatively, isothermal titration calorimetry (ITC) technique can be applied to investigate the interaction between lectins and multivalent ligands.[59] The thermodynamics of binding (K, $\Delta G$, $\Delta H$, $\Delta S$ and n) can be determined from ITC data by using and appropriate binding model.[60] Single molecule force spectroscopy has developed into a highly sensitive tool for the investigation of single biomolecule interactios in the last decade.[61] AFM or optical




*Scheme 11.* Formation of layer-by-layer assembly using quartz crystal microbalance technique.

tweezers have been used to measure dissociation forces of single ligand-receptor complexes in the picoNewton range.

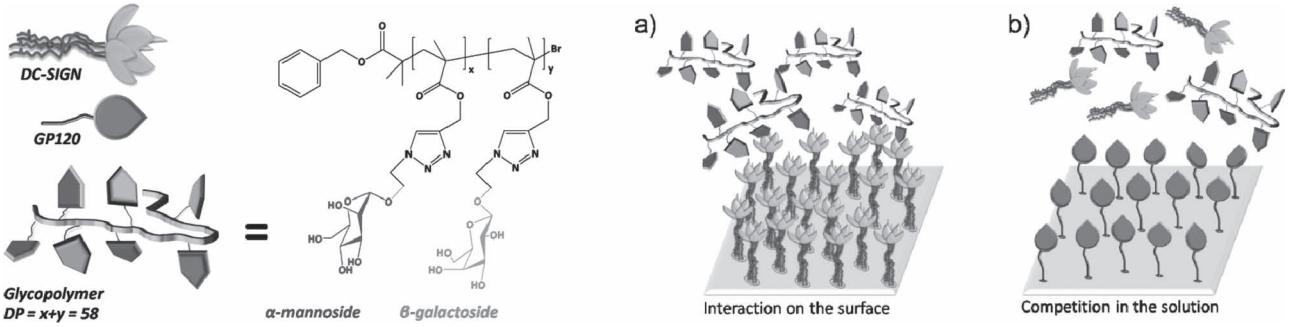
Quartz crystal microbalance (QCM) is a simple, cost effective, high-resolution mass sensing technique, which has been employed to study a wide range of molecular systems at the solution–surface interface.[62] Quartz crystal microbalance with dissipation monitoring (QCM-D) technique relies upon the piezoelectric effect in quartz crystals, whose frequency of oscillation changes in proportion to the amount of mass adsorbed onto their surface.[63] The detection range of QCM-D is relatively wide, so that it is capable of detecting monolayer surface coverage by small molecules on a polymer film. Another important feature of this system is the ability to measure simultaneously the mass and energy dissipation properties of films forming. According to Sauerbrey equation,[64] there is a linear relationship between the mass adsorbed on the piezoelectric crystal surface and the resonance frequency of the crystal.

We have demonstrated the layer-by-layer formation of glycopolymer and lectin using QCM-D (Scheme 11). Gold chip was chemically modified with 11-mercaptoundecanoic acid (MUA), followed by 1-[3-(dimethylamino)-propyl]-3-ethyl carbodiimide (EDC) hydrochloride and N-hydroxysuccinimide (NHS). Subsequently, ConA was bound to the surface via nucleophilic substitution of lysine and ethanolamine hydrochloride was used to block unreacted NHS groups to prevent their interaction with glycopolymers. Mannose glycopolymer was flown over the surface to determine the rate of binding.

In the second approach, disulfide-containing mannose glycopolymer was flown over naked gold surface. Because of a good binding between disulfide and gold, we were able to immobilize mannose glycopolymer on the surface. Then, ConA solution was passed through the chip to form the first glycopolymer-lectin layer. In the next step, glycopolymers with varying ratios of mannose and galactose content were flown over gold surfaces in parallel. It is well known that ConA and PNA selectively bind to mannose and galactose, respectively. By using mannose-galactose copolymers, we assured that we can use these polymers to link two different lectins together. After forming the second glycopolymer layer, PNA was flown over the QCM chip and sufficient binding was achieved because of the existing galactose units in the glycopolymer. This model study demonstrates that glycocopolymers with at least two different sugar types can be used to bring different biological entities selectively to a close proximity to function together.

Surface plasmon resonance (SPR) spectrometer monitors the interaction of two or more molecules or molecular assemblies in real time.[65] Basically, SPR utilizes the flow of analyte solution over a functionalized gold surface resulting in a change in the refractive index. This technique is very sensitive and can be used to detect association of glycoproteins or glycopolymers in picomolar concentrations.[66] Very recently, we reported a study on investigation of the potential of mannose-containing glycopolymers to interact with human DC-SIGN and the ability of these glycopolymers to inhibit the interactions between DC-SIGN and the HIV envelope glycoprotein gp120.[67] We used a library of glycopolymers that are prepared via combination of Cu(I)-mediated living radical polymerization and azide–alkyne [3 + 2] Huisgen cycloaddition reaction. We demonstrated that a relatively simple glycopolymer can effectively prevent the interactions between a human dendritic cell associated lectin (DC-SIGN) and the viral envelope glycoprotein gp120. Multichannel surface plasmon resonance (MC-SPR) was used to investigate the binding affinity of a library of glycopolymers with bacterially expressed soluble recombinant human DC-SIGN tetramers. DC-SIGN was immobilized onto an SPR sensor chip and the interactions between DC-SIGN and the glycopolymers were probed as a function of glycopolymer concentration (Scheme 12). The homopolymer of mannose exhibited the highest binding in the set of mannose–galactose glycocopolymers.

A therapeutic role for mannose-containing glycopolymers would lie in their potential to interrupt viral adhesion to host receptors, a process referred to as anti-adhesion therapy. We therefore established a competition assay within the MC-SPR system to examine




*Scheme 12.* Investigation on glycopolymer–lectin interactions on the surface and competition reactions in the solution.

whether the glycopolymers could inhibit DC-SIGN interactions with HIV gp120. An increase in the mannose content of the polymers from 25% to 100% reduces the IC50 value from 1453 to 37 $\times$ $10^{-9}$ M, highlighting the enhanced avidity due to multivalent presentation of the binding epitopes, such as the cluster glycoside effect.

## 5. Conclusions and Outlook

The synthesis and characterization of synthetic macromolecules have been developed significantly in the last couple of decades. In particular, macromolecules with pendant sugar units can easily be prepared by employing CRP techniques and selected click reactions. These techniques not only provide glycopolymers with controlled degree of polymerization but they also allow varying the architecture of glycopolymers. Moreover, glycopolymers can further be functionalized with other compounds such as proteins or tags by postpolymerization modification reactions. In this min-ireview, we have highlighted selected recent and successful examples of different combination of CRP and click reaction techniques to prepare glycopolymers in different architectures. Currently, we are able to prepare glycopolymers with one or more different types of sugar attached but it is still relatively difficult to control the sequence of sugars along the polymer chain. The current challenge in the polymer synthesis field is to gain absolute control over the repeating sequence of synthetic polymers, which will then be more similar to perfectly sequenced natural macromolecules. This will open new avenues for synthetic glycopolymers and their applications in the near future.

Glycans are key compounds for several biochemical reactions and synthetic multivalent ligands might be designed for investigating the low-affinity interactions. Many methods have been developed to study multivalent carbohydrate –lectin interactions. In particular, QCM and SPR techniques are highly sensitive in detection and provide more realistic measurements because the ligands or analytes can be immobilized on a surface. Indeed, there is a need for combination of different techniques and development of precisely sequence controlled synthesis techniques to have a better understanding in glycopolymer–lectin binding activities and to create the *synthetic glycopolymer code*.

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