Adv Polym Sci (2013) 262: 39–60
DOI: 10.1007/12_2013_254

Published online: 6 November 2013

Synthetic Glycopolymers: Some Recent Developments

Qiang Zhang and David M. Haddleton

Abstract Glycopolymers are synthetic macromolecules containing sugar moieties. They have shown promise in biorelated applications and the number of synthetic approaches for making these molecules is expanding rapidly. This field benefits from the rapid development of synthetic polymer chemistry, which has seen dramatic progress in the synthesis of functional glycopolymers. Strategies employed in glycopolymer synthesis have been generally carried out as either direct polymerization of glycomonomers or post-glycosylation of pre-formed polymers. This contribution is a short overview of some of the recent developments and will hopefully direct the reader to many papers of interest.

Keywords Glycopolymer · Living radical polymerization · Molecular recognition · Polymerization

Contents

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Glycopolymers are generally considered as synthetic macromolecules featuring sugar moieties and have showed promise in some biorelated applications [1]. This field has benefited from the development of elegant synthetic polymer chemistry, and the past two decades have evidenced dramatic progress in the synthesis of functional glycopolymers. Glycopolymer synthesis has been generally carried out by either direct polymerization of glycomonomers or post-glycosylation of pre-formed polymers [2]. As a special case, glycopolymers can also be synthesised via simultaneous copper-catalyzed azide-alkyne cycloaddition (CuAAC) and living radical polymerization (LRP), which is a hybrid of the previous two strategies [3].

By the combination of living polymerization and click chemistry, different strategies have been developed for the efficient synthesis of glycopolymers with defined structure and function. These strategies have already been discussed in detailed reviews separately by Haddleton, Stenzel, Cameron, Maynard and co-authors [1, 2, 4–6]. The applications of glycopolymers such as therapeutic drug delivery, multivalent recognitions with lectins and signal transduction have been summarized in recent reviews by Cameron, Stenzel, Remzi, Kiessling and co-authors [2, 7–9]. Thus, there has been very intensive research on glycopolymer synthesis and application, and most of the research until 2011 has been summarized in previous reviews. However, new strategies have been constantly emerging during 2011–2013 and are described below.

1 Novel Strategies in the Direct Polymerization of Glycomonomers

1.1 Ring-Opening Polymerization

Ring-opening polymerization includes cationic, anionic and enzymatic ring-opening polymerization, which depend on whether the catalyst type or the reactive centre of the propagating chain is a carbocation or carbanion. It has had a long history since the 1950s and has been widely used for polymerization of different functional cyclic monomers [10]. However, its application in the direct polymerization of carbohydrate-containing cyclic monomers has been limited [11, 12]. Recently, the Schubert group synthesized a glucose-substituted 2-oxazoline monomer (Fig. 1) via

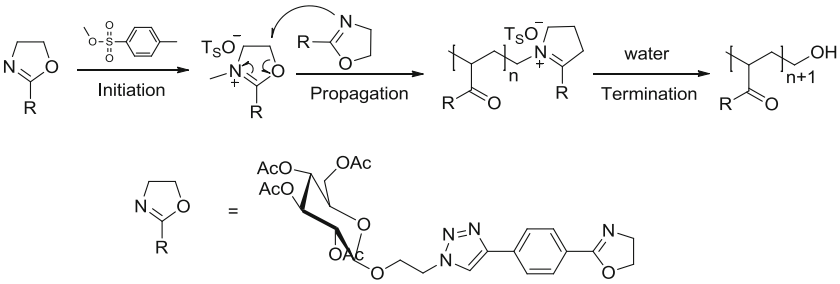


Fig. 1 Synthesis of glyco-poly(2-oxazoline)s by ring-opening polymerization

CuAAC and used this for cationic ring-opening copolymerization (CROP) with 2-oxazoline-based monomers, yielding well-defined glycopolymers bearing functional groups for thiol-ene reactions to tune the properties [13].

Although the polymerization of protected glycomonomers requires high reaction temperatures (~120°C) and long reaction times (overnight) for this CROP, the final glycopolymers show relatively narrow molecular weight distribution (~1.3) and the poly(2-oxazoline) backbones are biocompatible and considered as analogues of poly(amino acids), which may have potential application in drug delivery.

1.2 Copper-Mediated Living Radical Polymerization

Radical species usually have poor chemo- and regioselectivity in organic reactions and tend to undergo bimolecular termination and disproportionation in polymerizations. Thus, in order to have precise control in radical polymerization, a reversible and dynamic equilibrium between active radical growing species and dormant species (Fig. 2) is necessary so that the concentration of active radicals can be kept at a low level. The relatively stable dormant species could avoid side reactions or propagation yet is still able to generate intermediates capable of propagation by dissociation of the leaving groups via chemical catalysis or physical stimuli [14]. Different strategies have been developed to perturb this equilibrium with different leaving groups, including halides, stable radicals and thiolcarbonylthio compounds, via varying dissociation methods such as metal catalysis and addition-fragmentation chain transfer etc. Most of the current methods in living radical polymerizations are based on this concept [15].

Since its discovery in 1994, transition metal-catalyzed LRP has been one of the most popular, versatile and robust polymerization methods for synthesis of various functional polymers with controlled chain length, architecture and molecular weight distribution [16, 17]. The initiators are generally organic halides with potentially active carbon–halogen bonds for radical generation or conventional radical initiators, both of which are either commercially available or can be easily synthesized. The transition metal catalysts generally contain transition metals of groups 8–11, typically including iron, nickel, ruthenium and copper. Copper

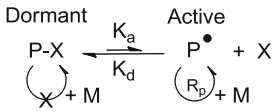


Fig. 2 Reversible and dynamic equilibrium between active radical growing species and dormant species (K_a means rate constant of activation; K_d means rate constant of deactivation; R_p means rate constant of propargation; M means monomer; P-X represents dormant polymer species;P* represents reactive polymer radical species)

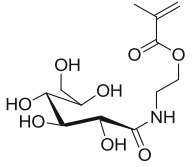
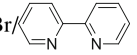
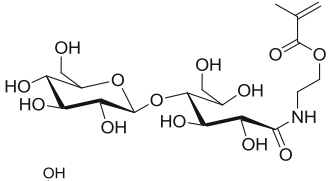
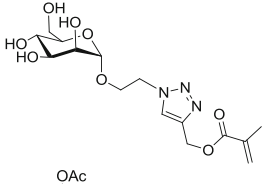
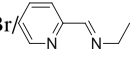
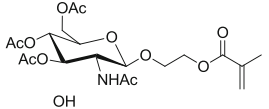
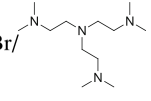
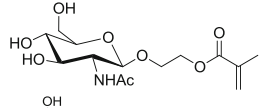
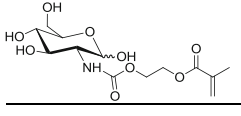
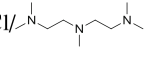
catalysts have been the most popular of the transition metal catalysts and are easily handled and highly efficient [15].

Of the copper(I) systems, probably the most well-known is the so-called atom transfer radical polymerization (ATRP), which utilizes the lower oxidation state copper(I) halide and (usually) nitrogen-based ligand complexes as the catalyst. Further research resulted in development of systems such as simultaneous reverse and normal initiation (SR&NI) ATRP, activators generated by electron transfer (AGET) ATRP, activators regenerated by electron transfer (ARGET) ATRP, initiators for continuous activator regeneration (ICAR) ATRP and electrochemically mediated ATRP (eATRP). In these systems, copper (I) generated by reduction of higher oxidation state copper(II) was believed to be always present and act as the predominant activator [18].

For the copper(0) systems, copper(I) is used as a catalyst precursor to generate copper(0), which reacts with organic halides for radical generation. Previous research has suggested that in polar solvents copper(I) halides and nitrogen-based ligand complexes are often unstable to sometimes rapid disproportionation into copper (0) and copper (II) halide and this disproportionation facilitates an fast LRP, in which the radicals are generated from the nascent copper(0) atomic species and the deactivation is mediated by copper(II) halide. Both steps are proposed to proceed via a low activation energy outer-sphere single-electron-transfer mechanism and thus the polymerization was named single electron transfer living radical polymerization (SET-LRP) [19, 20].

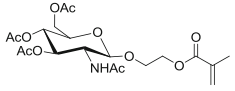
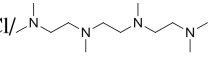
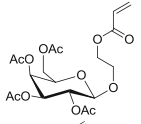
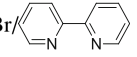
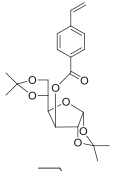
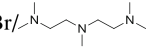
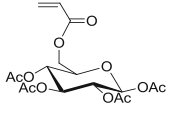
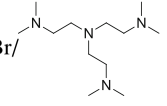
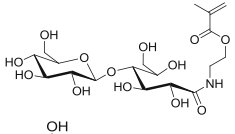
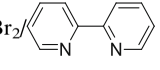
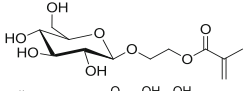
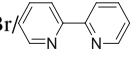
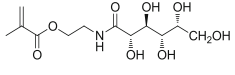
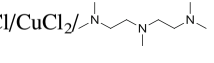
The direct polymerization of a protected glycomonomer via ATRP was first reported in 1998 using CuBr/4,4′-di-*n*-heptyl-2, 2′-bipyridine catalyst in veratrole at 80°C [21] (see Table 1). Direct copper-mediated polymerization of unprotected glycomonomers was generally performed in highly polar solvents such as alcohols, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), *N*-methyl-2-pyrrolidone (NMP) or mixtures with water [22]. The main reason for choosing such highly polar solvents is to solubilize the glycomonomer and the obtained glycopolymer, yet in some cases it resulted in low initiation efficiency or polymerization that was out of control [22, 23]. Previous research also revealed that direct aqueous ATRP of unprotected glycomonomers showed poor living character and that high ratios of alcohol as the co-solvent had to be used [24, 25]. The main reason is due to the fast propagation yet inefficient deactivation and the presence of side reactions under aqueous condition, such as hydrolysis of initiator and propagating polymer chain and, more importantly, disproportionation of copper catalyst [26]. Pure water has only been used as the solvent for surface-initiated polymerization, in which cases

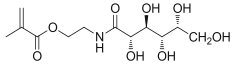
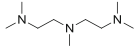
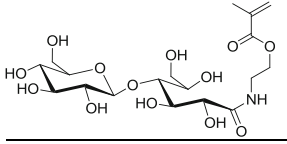
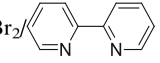
Table 1 Specific polymerization conditions for the synthesis of glycopolymers via copper-mediated living radical polymerization

Glycomonomers	Catalysts	Solvents	Reaction temperature (°C)	References
	CuBr/ 	H_2O ×, MeOH, MeOH/H_2O, NMP	20	[24]
				
	CuBr/ 	MeOH/H_2O (5:2)	25	[25]
	CuBr/ 	DMSO, MeOH	30	[22]
				
	CuBr or CuCl/ 	DMSO ×, DMF	40 (CuBr), 90 (CuCl)	[23, 30–32]

(continued)

Table 1 (continued)

Glycomonomers	Catalysts	Solvents	Reaction temperature (°C)	References
	CuCl/ 	Anisole, DMSO	25 (DMSO), 60 (anisole)	[33, 34]
	CuBr/ 	Chlorobenzene	80	[35, 36]
	CuBr/ 	THF	60	[37, 38]
	CuBr/ 	EtOAc	100	[39]
	CuBr/CuBr$_2$/ 	Pyridine/H_2O	25	[40]
	CuBr/ 	MeOH/H_2O	25	[41]
	CuCl/CuCl$_2$/ 	H_2O	30	Surface-initiated polymerization [29]

	Ascorbic acid/$CuCl_2$ / 	H_2O	30	Surface-initiated polymerization [28]
	CuBr/$CuBr_2$/ 	H_2O, NMP	25	Surface-initiated polymerization [27]

the chain end fidelity and molecular weight distribution tend to be difficult to elucidate [27–29]. Thus, more efforts are necessary to develop a proper catalyst system that could efficiently catalyse the polymerization of glycomonomers under different conditions, especially in aqueous media.[1]

1.3 Reversible Addition-Fragmentation Chain Transfer Polymerization

Since the discovery of reversible addition-fragmentation chain transfer (RAFT) in 1998 it has become one of the most popular living polymerization processes because it is tolerant of a wide variety of functional monomers and reaction conditions and also is promising in bio-applications [42, 43]. For the synthesis of glycopolymers, RAFT is probably the most popular LRP route at present (with about twice as many published papers than ATRP/transition metal-mediated strategies for the synthesis of glycopolymers) and different strategies have been developed for polymerization of both protected and unprotected glycomonomers [2, 43]. As an interesting case, direct RAFT polymerization of unprotected glycomonomers in pure water was reported in 2003, at which time direct aqueous ATRP of glycomonomers was still a challenge [24, 44]. Now, most RAFT polymerizations of glycomonomers are conducted in aqueous systems with some ratio of organic solvents (DMF, alcohol, DMSO etc.) with the aim of solubilizing the RAFT agents and radical sources. Most of these polymerizations are carried out at 60–80°C, although use of aqueous RAFT at ambient temperature has already been reported (Table 2) [45].

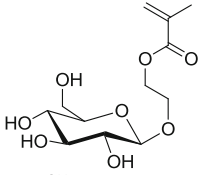
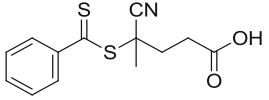
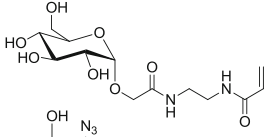
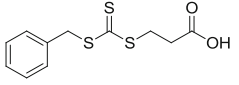
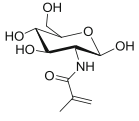
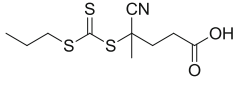
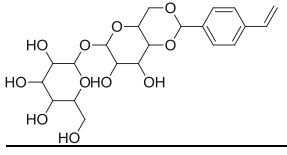
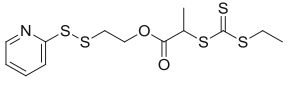
2 Novel Strategies in the Post-glycosylation of Pre-formed Polymers

2.1 Copper-Catalyzed Azide–Alkyne Cycloaddition Reaction

Copper-catalyzed azide–alkyne cycloaddition (CuAAC) has been widely used in the post-glycosylation of pre-formed polymers, for which the protected alkyne monomers can be first polymerized by various LRP strategies followed by removal of trimethylsilyl (TMS) protection groups using tetrabutylammonium fluoride (TBAF)/acetic acid for click reaction with azido functional sugars (Fig. 3) [59, 60]. This approach avoids the use of hazardous azide-functionalized monomers and utilizes the diversity of well-documented azido functional sugars [59].

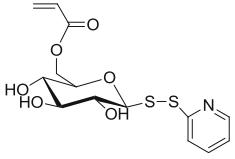
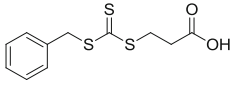
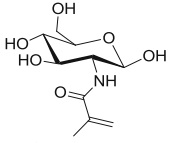
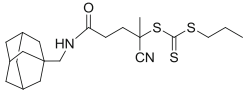
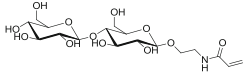
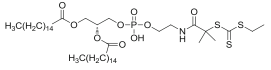
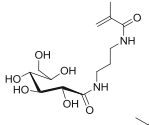
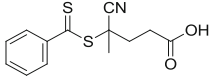
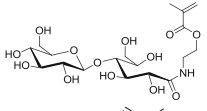
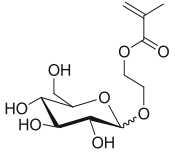
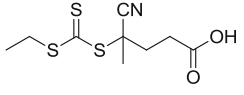
[1] X means that in the corresponding literatures H_2O or DMSO were used as the solvent for polymerization, but the polymerization is not successful or out of control under relevant conditions.

Table 2 Specific polymerization conditions for the synthesis of glycopolymers via RAFT

Glycomonomers	RAFT agent	Solvents	Reaction temperature (°C)	References
		H_2O	70	[44]
		H_2O/DMF (5:1)	70	[46]
		Acetate buffer/ethanol (4:1)	70	[47]
		DMF	80	[48]

(continued)

Table 2 (continued)

Glycomonomers	RAFT agent	Solvents	Reaction temperature (°C)	References
		Chlorobenzene	60	[49]
		D_2O/DMSO	70	[50]
		H_2O/DMF (4:1)	70	[51]
		H_2O/DMF (5:1)	70	[52–54]
				
		H_2O/EtOH (3:1)	70	[55]

		D_2O	60	[56]
		Toluene	75	[57]
		H_2O/DMF (3:7)	75	[58]

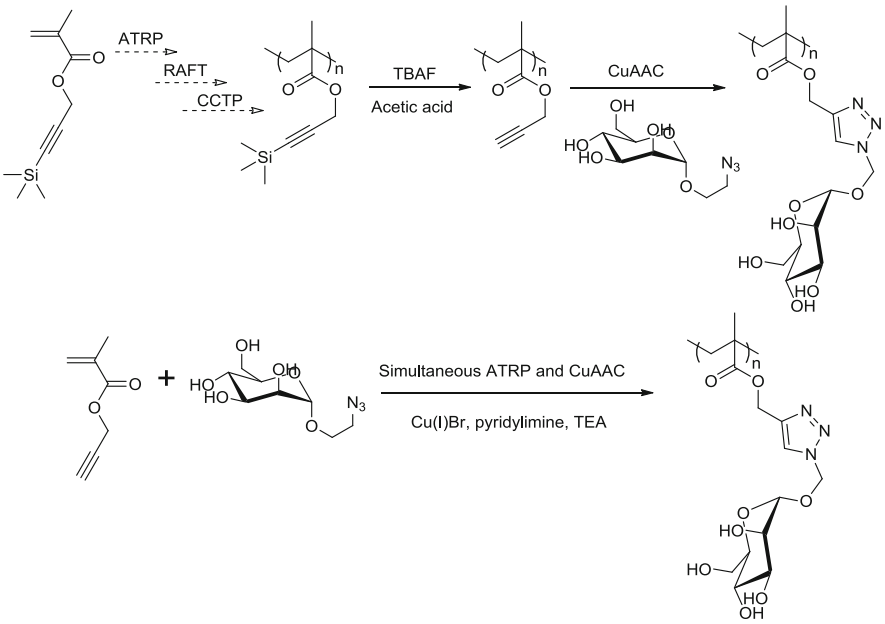


Fig. 3 Synthesis of glycopolymers via CuAAC of azide sugar with alkyne functional polymer or monomer

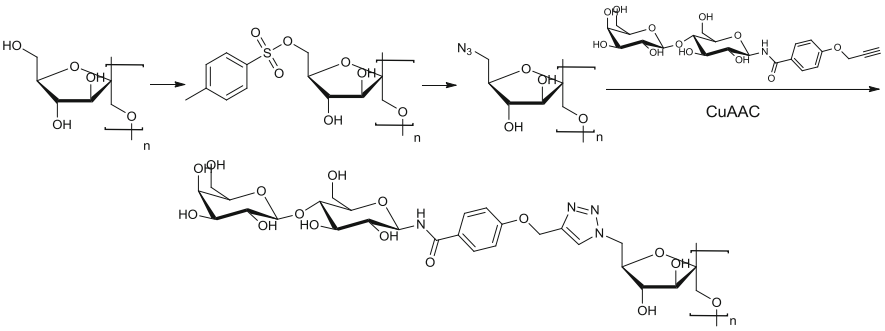


Fig. 4 Synthesis of glycopolymers via CuAAC of alkyne sugar with azido functional insulin

As an inverse approach, an insulin-based glycopolymer was synthesized by sequential chemical modification using tosylation, azidation and subsequent click reaction with alkyne sugars [61]. Due to the low ratio of tosylation, the azido functional insulin tends to be safe and the obtained insulin-based glycopolymers showed enhanced lectin affinity and gelation properties (Fig. 4).

Based on this combination of CuAAC and LRP, one-pot simultaneous ATRP and CuAAC was developed as a new tool for glycopolymer synthesis that utilized unprotected alkyne monomer and azido sugar (Fig. 3) [3]. As an inverse approach, a

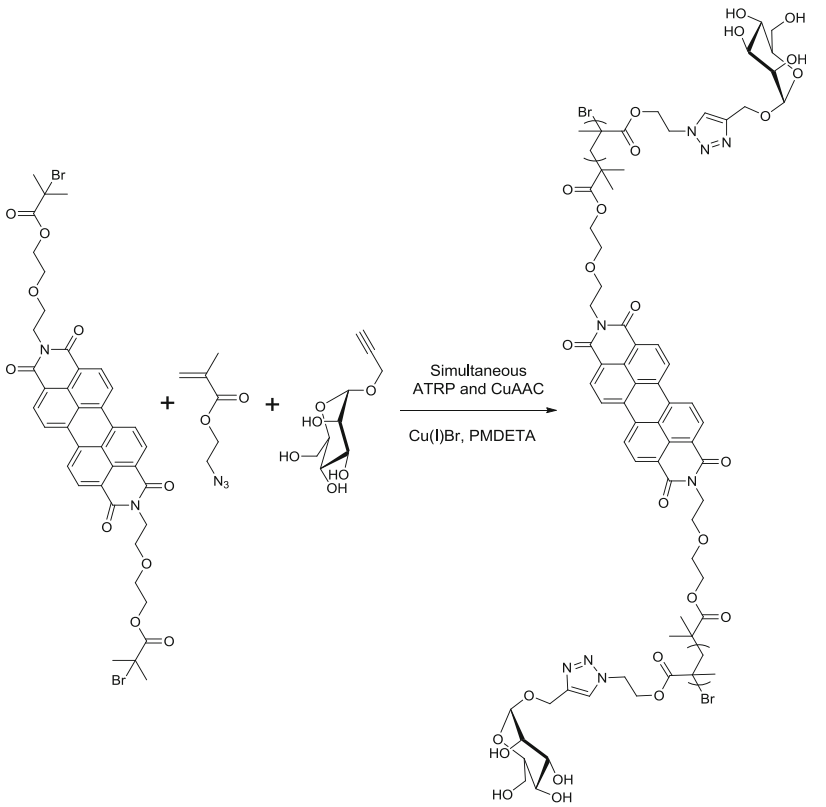


Fig. 5 Synthesis of glycopolymers via simultaneous ATRP and CuAAC using azido monomer and alkyne sugars

fluorescent glycopolymer could be synthesized via similar one-pot ATRP and CuAAC strategy using 2-azidoethyl methacrylate and alkyne mannose (Fig. 5) [62].

2.2 Thiol Click Chemistry

Thiol groups can react with many chemical species with high yields under benign conditions and thus many thiol-related reactions, such as thiol-ene, thiol-yne, thiol-epoxy, thiol-isocyanate and thiol-halogen reactions, are considered to be click-type reactions [63].

The thiol-yne coupling reaction is versatile, robust and can tolerate different functional groups due to its radical nature. It allows facile addition of two thiols to one alkyne group, which is suitable for construction of complex polymer structures such as networks, dendrimers and hyperbranched polymers [63, 64]. Successful glycosylation of linear polymers and dendrimers can be performed via radical-

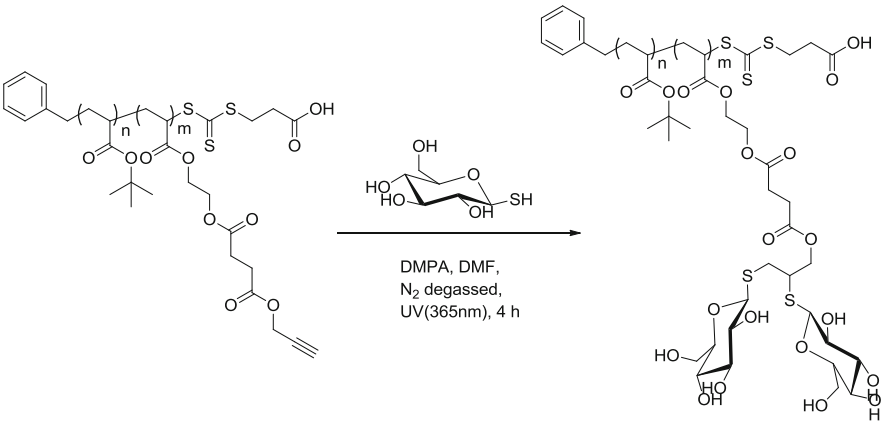


Fig. 6 Synthesis of glycopolymers via thiol-alkyne click reaction

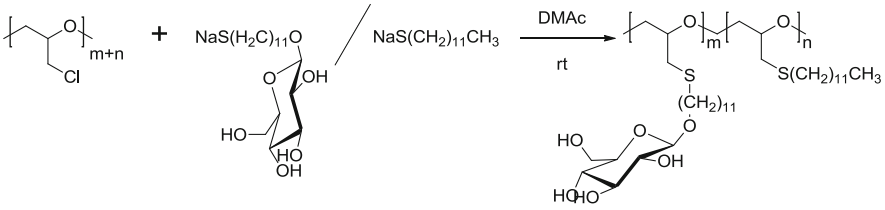


Fig. 7 Synthesis of glycopolymers via thiol-halogen click reaction

mediated thiol-alkyne click reaction, in which the 1-thiol-β-D-glucose reacts with the alkyne group in the presence of photo-initiator and UV light (Fig. 6) [65].

Thiol-halogen reactions, such as nucleophilic substitution reaction of thiocarbohydrate sodium salt with halogen-containing polymers, have been used for direct synthesis of glycopolymers [66]. This is a relatively slow reaction; however, no catalyst is needed and hazardous side products are also avoided. Thus, further research was reported utilizing similar methods (Fig. 7) [67].

2.3 Amine Chemistry

Condensation reactions between ketone groups and aminooxy sugars have become a tool for synthesis of glycopolymers and glycopeptides (Fig. 8) [68–70]. Generally, the reactions can be performed in acetate buffer or organic solvent/water mixtures at ambient temperature or higher temperatures (up to 95°C). The reaction conversion is only partial at ambient temperature but close to full conversion at higher temperature; however, reaction times can be as long as 4–7 days.

In order to eliminate the multistep reactions required for glycopolymer synthesis, free reducing sugars were used directly for the reaction with hydrazide functional polymer (Fig. 9) under acidic conditions in the presence of aniline catalyst [71].

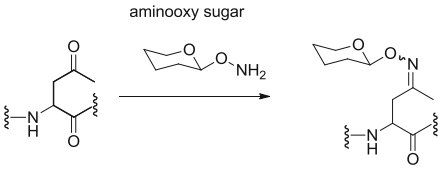


Fig. 8 Synthesis of glycopolymers by the reaction of ketones with aminooxy sugars

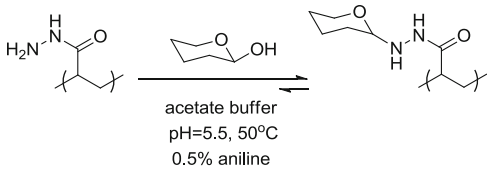


Fig. 9 Synthesis of glycopolymers by reaction of free reducing sugar with hydrazide functional polymer

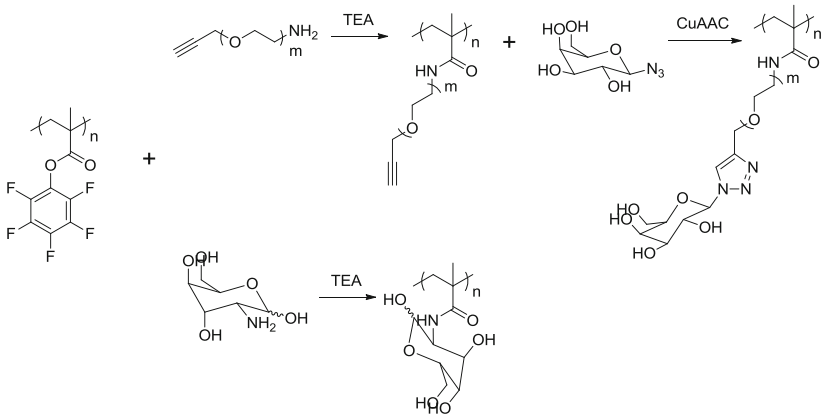


Fig. 10 Synthesis of glycopolymers by reaction of poly(pentafluorophenyl methacrylate) with functional amines

Different sugars, including mannose, fucose, lactose, xylose and panose, were used with this reaction, giving conversions ranging from 34% up to 95%.

Poly(pentafluorophenyl methacrylate) (PPFMA) bearing active ester groups could react with a wide variety of functional amines (Fig. 10). Glycopolymers have been synthesized by direct reaction of PPFMA with glucose amine or first with propargyl amine then with azido sugar via CuAAC, in which case the linker length and density of the glycopolymer could be adjusted by the length of propargyl amines [72, 73].

Other polymers bearing active ester groups, such as highly reactive *p*-nitrophenyl carbonate groups, can also react with amine functional sugars for the synthesis of glycopolymers (Fig. 11) [74, 75]. Utilizing the nucleophilic ring

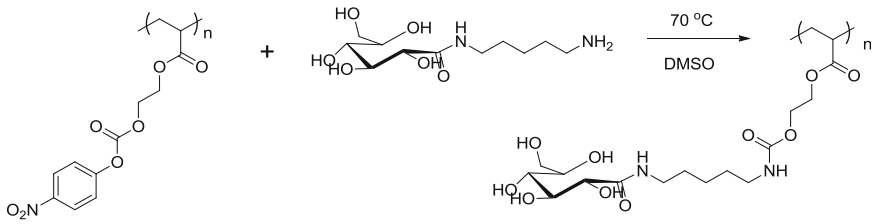


Fig. 11 Synthesis of glycopolymers by reaction of polymers bearing reactive *p*-nitrophenyl carbonate with amine functional sugar

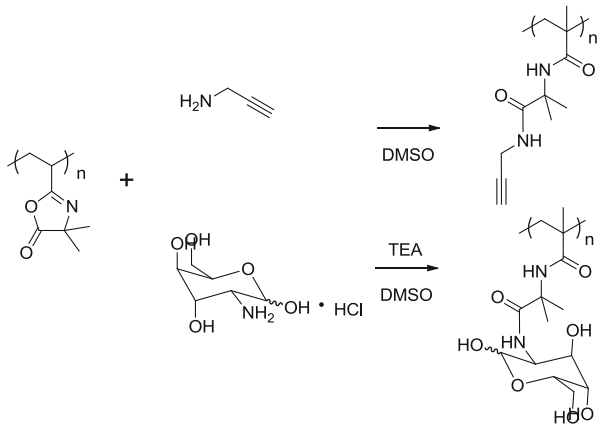


Fig. 12 Synthesis of glycopolymers by reaction of poly(azlactone) with amine functional sugar

opening reaction of azlactone with amine, poly(galactose) glycopolymers with long linker length between carbohydrate and backbone were synthesized by direct post-polymerization modification of poly(azlactone) scaffold and were shown to be very active against cholera toxin (Fig. 12) [76].

3 Novel Applications of Glycopolymers

3.1 Therapeutic Application: Anticancer and Anti-HIV

Carbohydrate-based anticancer agents have been explored with the aim of increasing the efficacy and decreasing the side effects of traditional anticancer Pt-based drugs [77, 78]. Recently, glycopolymer-based dithiocarbamates conjugates modified with gold(I) phosphine (Fig. 13) were synthesized and their cytotoxicity profiles examined. The results suggested that the gold conjugates showed higher accumulation and cytotoxicity to cancer cells due to the existence of glycopolymers and that their effect on normal breast cells was not significant [79].

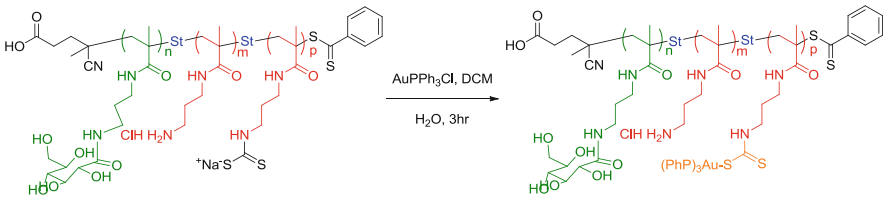


Fig. 13 Synthesis of glycopolymer–dithiocarbamate gold conjugates

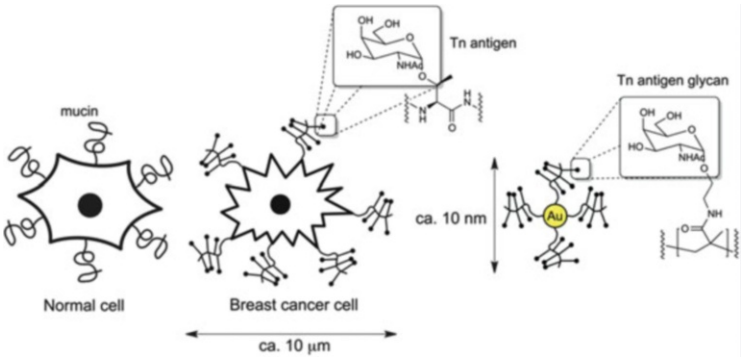


Fig. 14 Multicopy multivalent glycopolymer-stabilized gold nanoparticles as potential synthetic cancer vaccines [58]

Alison et al. synthesized a series of glycopolymers based on *N*-acetyl-D-glucosamine using RAFT and subsequently conjugated these glycopolymers to gold nanoparticles, yielding a type of multicopy multivalent nanoscale glycoconjugate (Fig. 14) [58]. These glycopolymer-stabilized gold nanoparticles could generate strong and long-lasting production of antibodies for selective recognition with Tn-antigen and thus have the potential to be used as a novel anticancer vaccine.

Relatively simple mannose-containing glycopolymers can effectively bind to human dendritic cell-associated lectin (DC-SIGN) and disrupted the interaction of DC-SIGN interactions with HIV envelope glycoprotein gp120, which could be seen as a new therapeutic approach (Fig. 15) [80].

3.2 Biocompatible Materials

Hyperbranched glycopolymers have been synthesized via RAFT (Fig. 16) and tested for blood biocompatibility. The results revealed that glycopolymers are highly haemocompatible and do not induce clot formation, red blood cell aggregation and immune response, suggesting a fine biocompatible material [53].

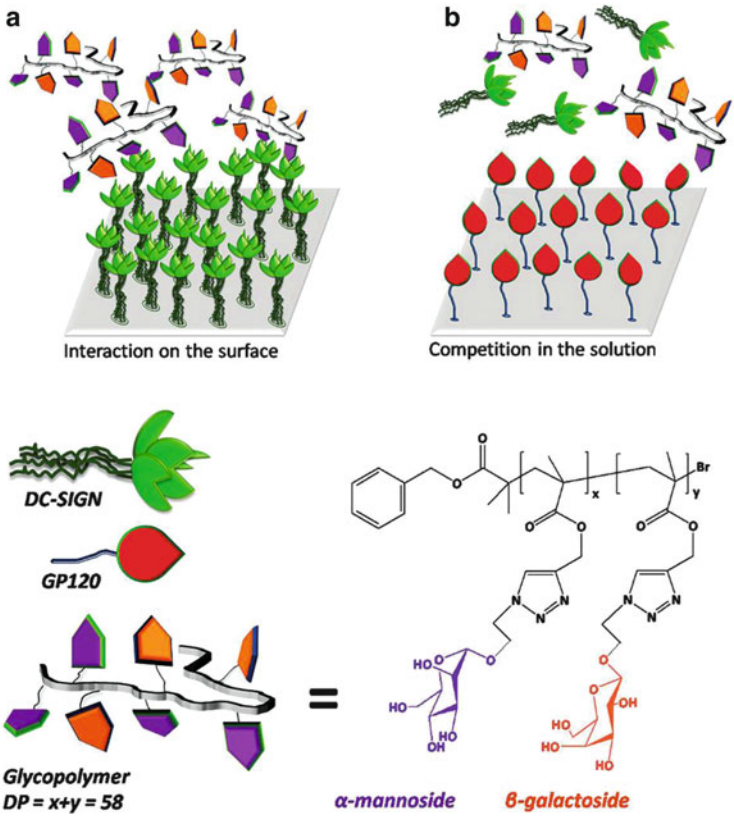


Fig. 15 High-affinity glycopolymer binding to human DC-SIGN and disruption of DC-SIGN interactions with gp120 [80]

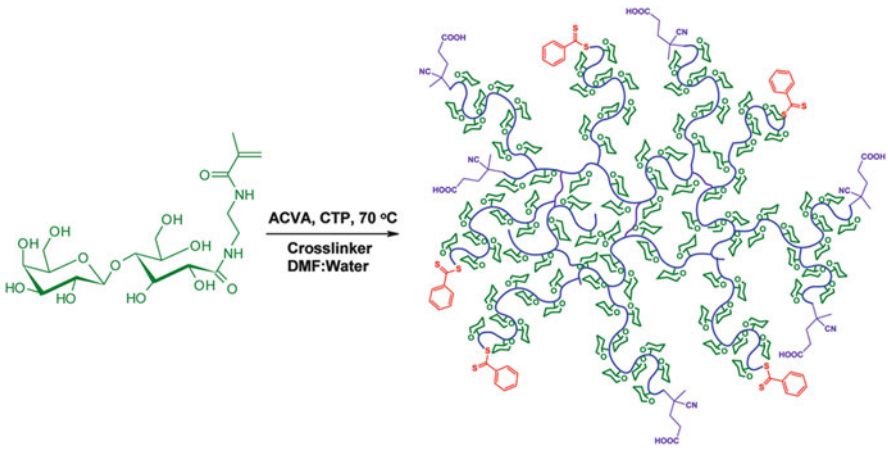


Fig. 16 Synthesis of hyperbranched glycopolymers via RAFT [53]

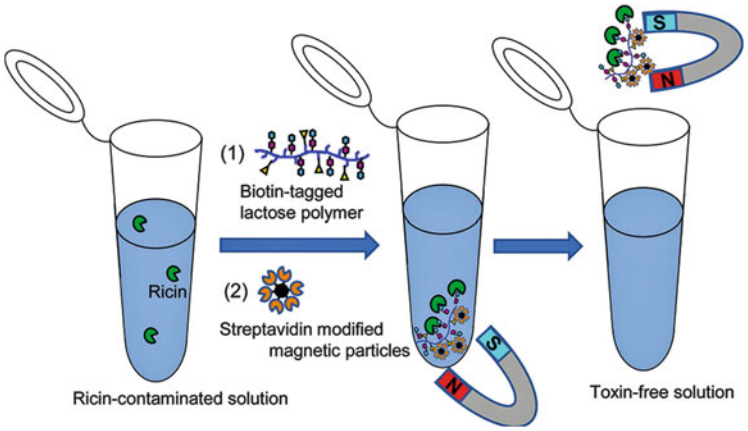


Fig. 17 Ricin decontamination using biotin-tagged lactose polymer [81]

Lactose and biotin-tagged glycopolymer could effectively absorb ricin and the obtained toxin–glycopolymer conjugate could be transferred onto streptavidin-modified magnetic particles for decontamination (Fig. 17) [81].

4 Summary

Glycopolymers represent a challenging and useful target for the synthetic polymer chemist. New polymerization strategies have resulted in a wide range of polymers that show really excellent recognition properties towards lectins. The polymer approach relying on multiple sugar epitopes and a flexible backbone is very different to the traditional organic chemistry approach where complex and elegant synthetic routes are used to put certain sugars in the right spatial orientation for lectin binding. We will see over the next few years if these glycopolymers will find a breakthrough application and, hopefully, this will occur in the near future.

Acknowledgment We appreciate financial support from the University of Warwick and China Scholarship Council (Q.Z.). Equipment used in this research was supported by the Innovative Uses for Advanced Materials in the Modern World (AM2), with support from Advantage West Midlands (AWM), and partially funded by the European Regional Development Fund (ERDF). D.M.H. is a Royal Society/Wolfson Fellow.

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http://www.springer.com/978-3-319-03718-9

Hierarchical Macromolecular Structures: 60 Years after the Staudinger Nobel Prize II

Percec, V. (Ed.)
2013, XVI, 452 p. 300 illus., 122 illus. in color.,
Hardcover
ISBN: 978-3-319-03718-9