Biotinylated Glycopolymers Synthesized by Atom Transfer Radical Polymerization

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Biotinylated glycopolymers that bind to the protein streptavidin were synthesized by atom transfer radical polymerization (ATRP). Poly(methacrylate)s with pendent *N*-acetyl-D-glucosamines were prepared by polymerizing the protected monomer, followed by deprotection. Alternatively, the unprotected monomer was directly polymerized. Both paths provided well-defined glycopolymers with narrow molecular weight distributions (PDI = 1.07−1.23). The number-average molecular weights determined by gel permeation chromatography increased with increasing initial monomer-to-initiator ratios. The polymers were synthesized using a biotin-functionalized initiator for ATRP. Confirmation of the end group and binding to the protein streptavidin was achieved by ^{1}H NMR and surface plamon resonance.

Introduction

Natural polysaccharides are essential for many processes in the body and can be used exogenously to elicit biological responses.[1] However, differences in macromolecular lengths and structures lead to variations in biological activities.[2] In addition, polysaccharides are challenging to synthesize and modify making some applications, such as attachment to surfaces and protein conjugate formation, difficult. Fortunately, synthetic polymers with pendent saccharides, also known as glycopolymers, exhibit similar bioactivities.[3] For example, it has been demonstrated that sulfated glycopolymers mimic the polysaccharide heparin and similarly act as anticoagulants or modulators of growth factor activity.[4−7] As a result of increasing interest in utilizing the synthetic counterparts of natural polysaccharides, improvements in the syntheses of glycopolymers have been the focus of many groups.

A number of polymerization strategies have been undertaken to prepare well-defined glycopolymers with narrow molecular weight distributions.[3] Kiessling and co-workers have used ring-opening metathesis polymerization (ROMP) to synthesize glycopolymers.[8−16] In many cases, the polymerizations were conducted in aqueous solution or emulsion conditions. These polymers were employed to study carbohydrate−protein interactions and proved to be inhibitors of Concanavalin-A-induced cell agglutination and selectin binding. Davis and co-workers and Lowe et al. have reported the polymerization of methacrylates with pendant glucosides by reversible addition−fragmentation chain transfer (RAFT) polymerization.[17−20] Chaikof and co-workers have prepared *N*-acetyl-D-glucosamine and lactose-based glycopolymers via cyanoxyl-mediated free-radical polymerization.[4,6,7,21−25] The sulfated and nonsulfated variants of these polymers were studied as potential heparin mimics.

A technique that has recently been employed to prepare glycopolymers is atom transfer radical polymerization (ATRP). ATRP is a controlled radical polymerization method that produces polymers with predictable molecular weights and narrow molecular weight distributions.[26,27] This technique is tolerant to a wide range of functional groups[28] and has been successfully used to synthesize glycopolymers.[29−38] For example, Fukuda and co-workers prepared well-defined glucose-substituted homopolymers and block copolymers by ATRP in solution and from a surface-immobilized initiator.[29,30] Armes and co-workers have published the synthesis of sugar side chain poly(methacrylate) homo and block copolymers by ATRP and studied the self-assembly of the block copolymers in solution.[31−33] Hyperbranched glycopolymers and brushes were recently reported by Müller and co-workers.[34,35,38]

One of the many advantages of ATRP is the ability to readily synthesize polymers with functionalized end groups.[28] In particular there has been a lot of interest by our group and others in preparing semitelechelic polymers using functionalized ATRP initiators that react with proteins. We synthesized a pyridyl disulfide initiator that provided poly(2-hydroxyethyl methacrylate) with narrow molecular weight distributions.[39] Since free cysteines are rare in proteins and can be introduced by site-specific mutagenesis,[40] coupling via a disulfide bond represents a way to achieve site-specific conjugation. We showed that the as-synthesized polymers reacted with the free cysteine of bovine serum albumin (BSA) without any postpolymerization modification steps. Haddleton et al. prepared water-soluble poly(methacrylates) with *N*-maleimide end groups by performing a reverse Diels−Alder reaction on the polymer chains; the resulting polymers were conjugated to BSA.[41] In addition, aldehyde and *N*-succiminidyl ester end-functionalized polymers for conjugation to lysine residues of proteins have been synthesized.[42,43]

Polymers that bind to ligand binding sites have also been prepared by ATRP. In particular, biotinylated polymers have been synthesized. Biotin is a ligand for avidin and streptavidin and exhibits a very strong binding interaction with these proteins ($K_a \approx 10^{15}$ M^{-1}).[44] Because four binding sites for biotin are available, this interaction has been exploited to combine different molecules or to engineer surfaces. Wooley and co-workers recently synthesized biotinylated poly(acrylic acid)-*b*-poly(methyl acrylate) (PAA-*b*-PMA) by ATRP and showed that self-assembled structures prepared from the polymers conjugate to

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avidin.[45] We synthesized biotinylated smart polymers, poly(*N*-isopropylacrylamide) (polyNIPAAm), utilizing a biotinylated ATRP initiator and demonstrated binding to the protein streptavidin.[46]

Polymers with pendent saccharides and protein-reactive end groups have many potential applications. Despite this, only a limited number of end-functionalized glycopolymers have been reported.[4,15,23,25,32,37,47] To our knowledge there have been no reported examples of biotinylated glycopolymers prepared by ATRP. Herein, we report the first synthesis by ATRP of biotinylated glycopolymers; specifically polymers with *N*-acetylglucosamine (GlcNAc) attached to the side chains were made. Many proteins are posttranslationally modified at serine and threonine residues with GlcNAc. This dynamic modification is involved in diverse cellular processes and has been detected on transcription factors, protein kinases, and cytoskeletal proteins.[48,49] Additionally, GlcNAc modifications have recently been understood to be involved in diseases such as cancer and diabetes.[50] Therefore, the polymers with GlcNAc side chains and biotin end groups described herein may be employed to modify streptavidin-coated surfaces, for example, commercially available chips for surface plasmon resonance (SPR) and nanoparticles, to investigate biological interactions that involve GlcNAc. The synthesis that we report involves the use of a biotinylated initiator for ATRP and thus is a general approach to prepare a wide range of biotinylated glycopolymers. The synthesis of a suitable initiator, polymerization of protected and unprotected *N*-acetyl-D-glucosamine side chain methacrylates, and end group verification is discussed.

Materials and Methods

Materials. All chemicals were purchased from Aldrich or Acros and used as received, unless otherwise specified. Copper bromide (CuBr) was purified by stirring in glacial acetic acid overnight and rinsing with ethanol and diethyl ether prior to drying under vacuum. The oxazoline **2**,[51] the ligand tris(2-(dimethylamino)ethyl)amine (Me_6-TREN),[52] and the biotin-conjugated ATRP initiator **5**[45,46] were synthesized according to published procedures.

Analytical Techniques. ^{1}H NMR spectra were recorded on a Bruker Avance 500 spectrometer; all polymer spectra were taken with a minimum delay time of 10 s. Gel permeation chromatography (GPC) was conducted on a Shimadzu high-performance liquid chromatography system equipped with a refractive index detector RID-10A and two Polymer Laboratories PLgel 5 μm mixed D columns (with guard column). LiBr (0.1 M) in *N*,*N*-dimethylformamide (DMF) at 40 °C was used as a solvent (flow rate 0.8 mL/min). Near-monodisperse poly(methyl methacrylate) standards (Polymer Laboratories) were employed for calibration. Chromatograms were processed with the EZStart 7.2 chromatography software. SPR measurements were performed with a Biacore X instrument equipped with a streptavidin-coated sensor chip (Biacore). A buffer composed of 20 mM Tris, 150 mM sodium chloride, and 0.005% surfactant P20 was used with a flow rate of 5 μL/min. Infrared spectra were obtained with a Perkin-Elmer Spectrum One instrument equipped with a universal attenuated total reflectance (ATR) accessory.

Synthesis of Protected Glycomonomer 3. This reaction was performed as described by Tokura and co-workers[53] with some modification. In a 50 mL round-bottom flask, **2** (1.0 g, 3.04 mmol) and 10-camphorsulfonic acid (CSA, 70 mg, 0.30 mmol) were dissolved in dry 1,2-dicholoroethane (15 mL). To the mixture, 2-hydroxyethyl methacrylate (HEMA, 0.55 mL, 4.56 mmol) and 4-methoxyphenol (~200 ppm) were added, and the reaction mixture was heated to reflux for 6 h under argon. The reaction was quenched by the addition of Et_3N and cooled to room temperature. The crude mixture was concentrated and purified by column chromatography (ethyl acetate/toluene, 9:1). The overall yield for the reaction was 72%. ^{1}H NMR (DMSO-d_6): δ 7.94 (d, 1H, NH, J = 2.0 Hz), 6.05 (s, 1H, C=CH_2), 5.68 (s, 1H, C=CH_2), 5.08 (t, 1H, H3, J = 10.2 Hz), 4.82 (t, 1H, H4, J = 9.9 Hz), 4.68 (d, 1H, H1, J = 8.5 Hz), 4.20−4.15 (m, 3H, H6b, CH_2OCO), 3.99 (dd, 1H, H6a, J = 2.1 and J = 12.2 Hz), 3.92−3.80 (m, 2H, H5, OCH_2), 3.74−3.68 (m, 2H, H2, OCH_2), 2.0 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$), 1.87 (s, 3H, CCH_3), 1.71 (s, 3H, $COCH_3$). IR (cm^{-1}): 3336 w, 2960 w, 1743 s, 1672 m, 1547 m, 1369 m, 1227 s, 1037 s.

Synthesis of Glycomonomer 4. In a 25 mL round-bottom flask **3** (0.50 g, 1.10 mmol) was dissolved in methanol (5 mL) and stirred for 10 min under argon. Sodium methoxide in a 30% methanol solution (21 μL, 0.11 mmol) was added, and the reaction was followed by thin-layer chromatography (TLC; acetonitrile/water, 9:1). After 7 min the reaction was terminated by addition of DOWEX 50W, and the mixture was stirred for another 20 min. The solution was filtered to remove the resin, concentrated, and purified by column chromatography (chloroform/methanol, 9:1). The yield was 66%. ^{1}H NMR (D_2O): δ 5.99 (s, 1H, C=CH_2), 5.58 (s, 1H, C=CH_2), 4.39 (d, 1H, H1, J = 8.5 Hz), 4.14 (t, 2H, CH_2OCO, J = 4.3 Hz), 3.96−3.91 (m, 1H, OCH_2), 3.77−3.72 (m, 2H, OCH_2, H6b), 3.60−3.49 (m, 2H, H6a, H2), 3.38−3.33 (m, 1H, H5), 3.29−2.25 (m, 2H, H3, H4), 1.80 (s, 3H, $HCOCH_3$), 1.76 (s, 3H, CCH_3). IR (cm^{-1}): 3264 s, 2962 w, 1717 m, 1651 m, 1553 m, 1320 m, 1297 m, 1070 s, 1030 s.

Synthesis of Glycopolymers 6 and 7 from Biotin Initiator 5. The glycomonomer and biotin initiator were evacuated−refilled in a 25 mL air-free reaction tube with argon three times, and then degassed dimethyl sulfoxide (DMSO) was added. From an oxygen-free stock solution, an aliquot containing CuBr and Me_6-TREN in DMSO was added to the reaction tube to start the polymerization. The polymerization was terminated after 15 min by exposure to oxygen. The protected glycopolymer **6** was diluted in methanol and precipitated from ether three times. Polymer **6** IR (cm^{-1}): 3321, 2952, 1741, 1667, 1536, 1433, 1367, 1223, 1157, 1034. Glycopolymer **7** was isolated by evaporation of the DMSO, diluted in water, and extensively dialyzed for 3 days against water (10 × 5 L). The polymer was collected as a white solid after lyophilization. Polymer **7** IR (cm^{-1}): 3331, 2926, 2461, 1722, 1634, 1423, 1272, 1155, 1117, 1074, 1015.

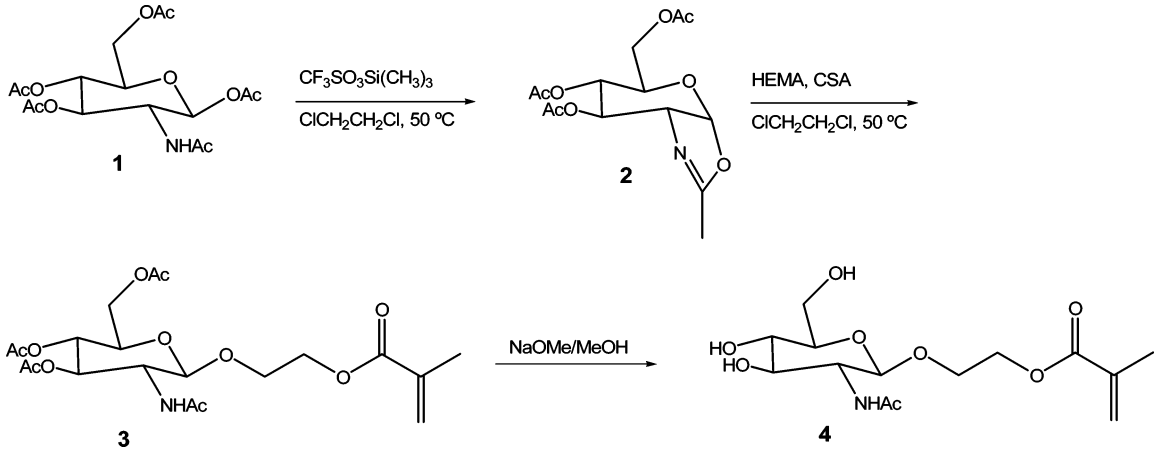
Deprotection of Glycopolymer 6. In a 15 mL round-bottom flask **6** (25 mg, 0.054 mmol) was dissolved in 1 mL of chloroform/methanol (1:1) and stirred under argon for 15 min. A catalytic amount of sodium methoxide in a 30% methanol solution (10 μL, 0.005 mmol) was added to the solution, which was left stirring under argon gas. After 1 h the mixture was centrifuged, and the precipitate was redissolved in deionized water and neutralized with 1 M HCl. The polymer was isolated by lyophilization. The solids were diluted in water and extensively dialyzed for 3 days in water (10 × 5 L). The purified polymer was collected as a white solid after lyophilization. IR (cm^{-1}): 3355, 2938, 2465, 1714, 1634, 1471, 1425, 1271, 1155, 1114, 1070, 1028

Kinetic Studies of the Polymerization of 3 in Methanol. The glycomonomer (**3**) and biotin initiator (**5**) were added to a 25 mL air-free reaction tube and evacuated−refilled with argon gas three times to create an oxygen-free atmosphere. Degassed methanol was then added. From a stock solution, an aliquot of CuBr and 2,2′-bipyridine (bipy) in degassed methanol was added to the reaction tube, and the mixture was immediately placed in a 30 °C oil bath to start the polymerization. Samples were taken from the mixture at defined time points. The methanol was removed, and the samples were diluted into DMSO-d_6 for ^{1}H NMR analysis and DMF for GPC analysis. After 90 min, the protected glycopolymer **6d** was diluted in methanol and precipitated from ether three times

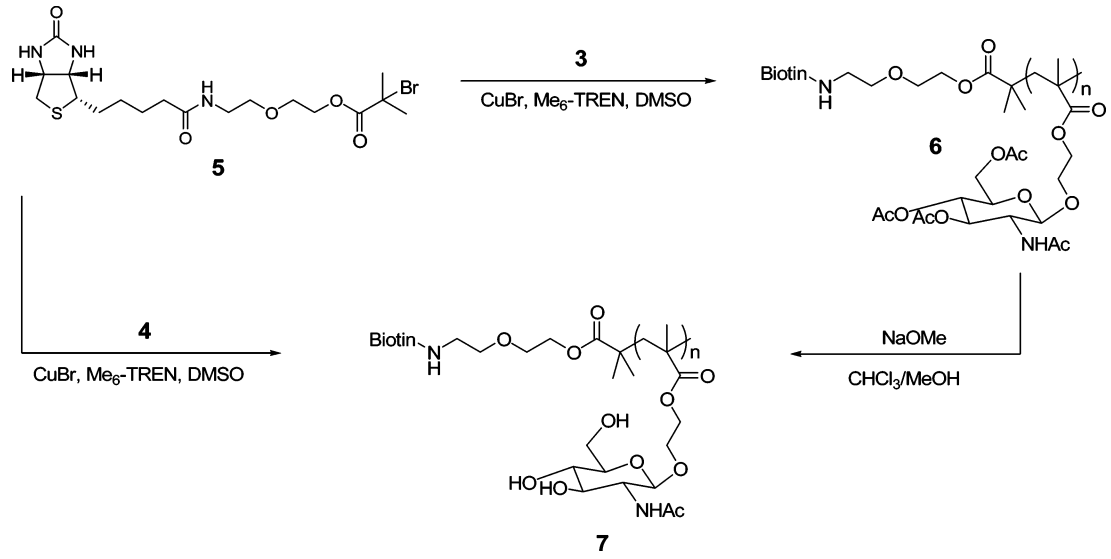
Results and Discussion

Glycomonomer Synthesis. The protected glycomonomer was synthesized in two steps (Scheme 1) starting with the commercially available 2-acetamido-2-deoxy-β-D-glucopyranose

Scheme 1. Synthesis of the Glycomonomers



Scheme 2. Polymerization of the Glycomonomers from the Biotinylated Initiator



1,3,4,6-tetraacetate (**1**). Intermediate **2** was synthesized following a procedure described by Hirabayashi and co-workers using trimethylsilyl trifluoromethanesulfonate (TMS-triflate).[51] Oxazoline **2** was then treated with HEMA in the presence of CSA to provide the glycomonomer **3** in a 72% yield. We initially attempted the deprotection of the monomer with potassium carbonate, which caused hydrolysis of the methacrylate ester. Instead, we obtained **4** in 66% yield using a catalytic amount of sodium methoxide and quenching after 7 min. Longer times resulted in cleavage of the methacrylate group. Both the protected and the unprotected monomers were subjected to polymerization.

Polymerization of Protected Glycomonomer 3. ATRP of the protected glycomonomer **3** (Scheme 2) was explored. A biotin-conjugated initiator (**5**) was synthesized as previously described[45,46] and utilized in the polymerization ($[\text{monomer}]_0/[\text{initiator}]_0 = 50{:}1$). ATRP of **3** was conducted in DMSO with CuBr and Me_6-TREN. The polymerization was fast and reached a high conversion of 94% to polymer **6b** in 15 min. It was possible to change the initial monomer-to-initiator ratios and maintain good conversions. When ATRP with 10:1 and 100:1 ratios were employed, 88% and 73% conversions to **6a** and **6c**, respectively, were achieved (Table 1).

Gel permeation chromatography was performed to analyze the molecular weights of the polymers. The GPC traces (Figure 1) did indicate slight low molecular weight tailing at higher monomer-to-initiator ratios, which suggested some early termination of the polymer chains. However, the molecular weight distributions were all narrow (PDIs = 1.17−1.23), which demonstrated that well-defined polymers were synthesized. The number-average molecular weights (M_n's) were between 16 100 and 52 600 (Table 1). These values were significantly higher than the targeted molecular weights (4000−33 500). The discrepancy could be partially attributed to the GPC calibration using poly(methyl methacrylate) standards. To determine the molecular weights more accurately, the ^{1}H NMR spectra were inspected. For polymers **6b** and **6c** it was not possible to observe the end group. For polymer **6a**, several peaks at 4.5, 3.2, and 2.8 ppm originating from the biotin were identified (Figure 2). The protected polymers were purified by precipitation, which may not have removed residual unreacted initiator. Therefore, polymer **6a** was deprotected to render it soluble in water and extensively dialyzed prior to molecular weight analysis by NMR.

Deprotection of Glycopolymer 6. Polymer **6a** was subjected to a catalytic amount of sodium methoxide in a 1:1 mixture of chloroform and methanol (Scheme 2). Fortunately, as the product formed, it precipitated out of solution. This effectively protected the ester group of the poly(methacrylate) from cleavage. The polymer became soluble in water as expected and was extensively dialyzed against water for 3 days to remove any unbound biotinylated initiator and impurities prior to analysis by ^{1}H NMR. Inspection of the peaks centered at 2 ppm in the ^{1}H NMR spectrum of the resulting polymer (Figure 3) indicated that the acetate groups were removed. Only the peak

Table 1. Molecular Weights of Glycopolymers **6** and **7**

polymer	$[M]_0/[I]_0$	solvent	conversion[a] (%)	M_n (GPC)	M_n (^{1}H NMR)	M_n (theory)	PDI (GPC)
6a[b]	10:1	DMSO	88	16 100	14 300	4000	1.17
6b[b]	50:1	DMSO	94	40 700		21 600	1.17
6c[b]	100:1	DMSO	73	52 600		33 500	1.23
6d[c]	10:1	MeOH	83	13 000		3800	1.13
7a[b]	10:1	DMSO	78	14 300	11 400	2600	1.14
7b[b]	50:1	DMSO	86	43 100		14 300	1.07
7c[b]	100:1	DMSO	94	68 400		31 300	1.16
7d[c]	10:1	MeOH	98	11 800	9100	3300	1.08

[a] Determined by ^{1}H NMR. [b] Polymerization conditions $[M]_0$ = 50% w/v, DMSO, 23 °C, $[\mathbf{5}]_0/[CuBr]_0/[Me_6\text{-TREN}]_0$ = 1:1:1, reaction time = 15 min. [c] Polymerization conditions $[M]_0$ = 25% w/v, MeOH, 30 °C, $[\mathbf{5}]_0/[CuBr]_0/[bipy]_0$ = 1:1:2, reaction time = 1.5 h.

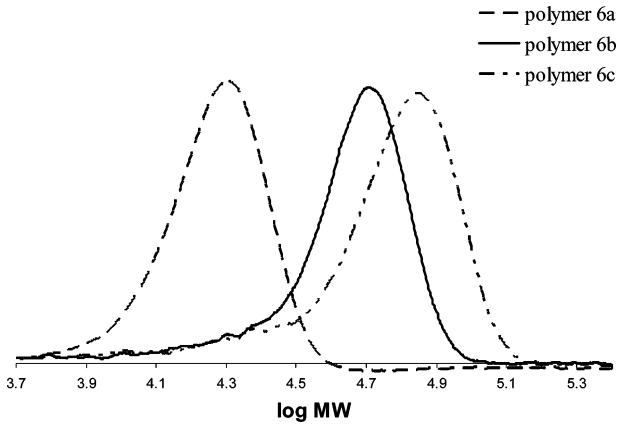


Figure 1. GPC chromatograms of protected biotinylated glycopolymers synthesized with CuBr/Me_6-TREN as the catalyst ($[\mathbf{3}]_0$ = 50% w/v, DMSO, 23 °C, $[\mathbf{5}]_0/[CuBr]_0/[Me_6\text{-TREN}]_0$ = 1:1:1, 15 min; conversions 88%, 94%, 73% for $[M]_0/[I]_0$ = 10 (**6a**), 50 (**6b**), 100 (**6c**), respectively).

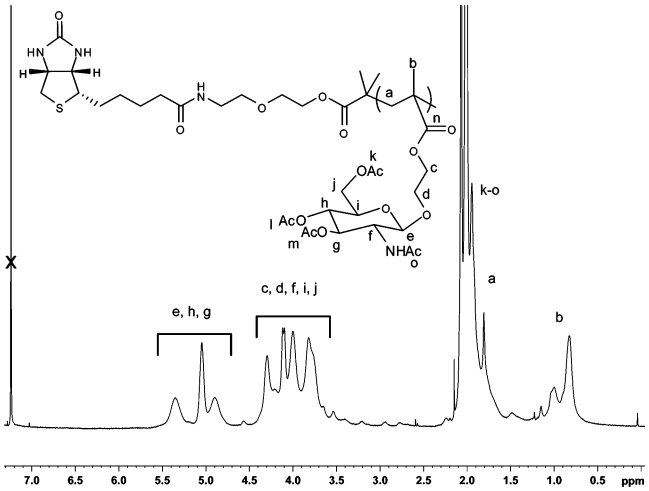


Figure 2. ^{1}H NMR spectrum (500 MHz) in $CDCl_3$ of glycopolymer **6a** ($[M]_0/[I]_0/[CuBr]_0/[Me_6\text{-TREN}]_0$ = 10:1:1:1).

corresponding to the *N*-acetyl group remained. Integration of the peaks corresponding to the polymer confirmed that hydrolysis of the sugar side chains had not occurred (Supporting Information). Importantly, peaks corresponding to biotin were still detectable, demonstrating that the deprotection did not affect the end group. Infrared characterization also confirmed that the deprotection was successful (see Supporting Information for the spectra). The band corresponding to the carbonyl stretch of the esters became less intense relative to the amide carbonyl stretch in the unprotected polymer (1714 and 1634 cm^{-1}, respectively) compared to that of the protected polymer (1741 and 1667 cm^{-1},

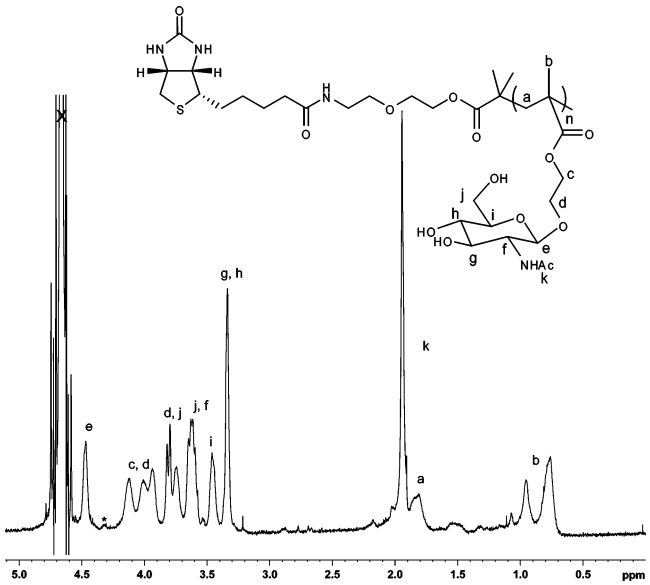


Figure 3. ^{1}H NMR spectrum (500 MHz) in D_2O of the product of the deprotection of glycopolymer **6a** with NaOMe in a 30% methanol solution. The biotin peak used for molecular weight analysis is marked by an asterisk.

respectively). In addition, the C−O stretch of the acetates in **6a** at 1223 cm^{-1} was no longer visible, and the O−H stretch at 3355 cm^{-1} was obvious after deprotection.

The molecular weight of the deprotected polymer was calculated by comparison of the biotin peaks (*, Figure 3) to the proton peaks of the polymer backbone methyl groups (b, Figure 3). The resulting M_n of the deprotected polymer was calculated to be 10 400 and corresponded to a M_n of 14 300 (Table 1) of the original protected glycopolymer **6a**. These results showed that the molecular weight discrepancy was not solely due to calibration with PMMA standards.

Polymerization of Monomer 3 in Methanol. The ATRP of **3** in methanol was also explored. The initial monomer-to-initiator ratio was 10:1, the catalyst was CuBr and bipy, and the polymerization was conducted at 30 °C. The reaction was slower than that in DMSO, reaching a high conversion in 90 min. The polymerization was monitored, and the linear first-order kinetic plot (Figure 4a) indicated that the concentration of radicals remained constant to 83% conversion. The M_n by GPC of 13 000 after 90 min of polymerization was higher than expected (Table 1) indicating initiator inefficiency. However, the molecular weight versus percent conversion (Figure 4b) plot was linear throughout the reaction, and the molecular weight distributions were narrow. Taken together these data indicate a degree of control for the ATRP of **3** using biotin initiator **5**.

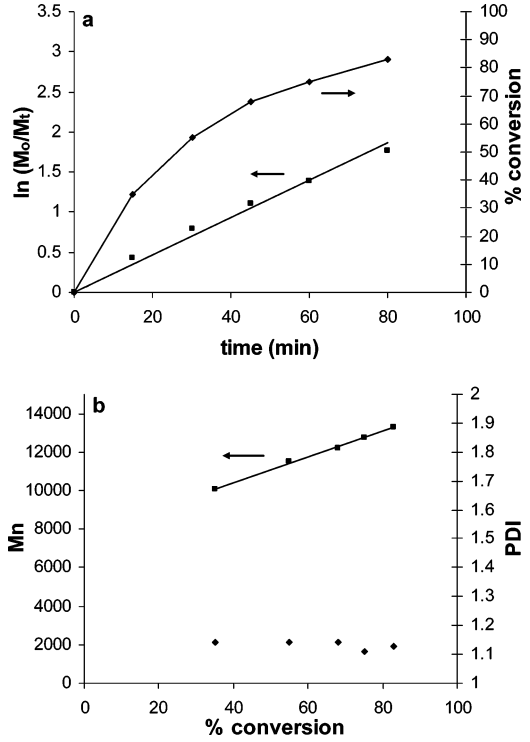


Figure 4. Polymerization of monomer **3** in methanol from initiator **5** at 30 °C [**3**]/[**5**]/[CuBr]$_0$/[bipy]$_0$ = 10:1:1:2): (a) kinetic plot; (b) experimental M_n (from GPC) and PDI vs conversion.

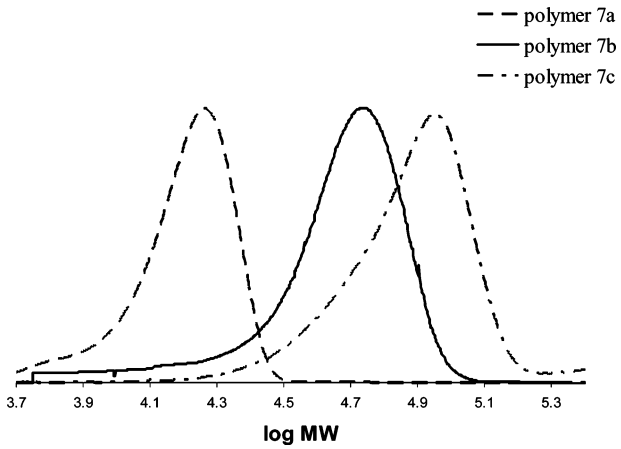


Figure 5. GPC chromatograms of deprotected biotinylated glycopolymers synthesized with CuBr/Me$_6$-TREN as the catalyst ([**4**]$_0$ = 50% w/v, DMSO, 23 °C, [**5**]$_0$/[CuBr]$_0$/[Me$_6$-TREN]$_0$ = 1:1:1, 15 min; conversions 78%, 86%, 94% for [M]$_0$/[I]$_0$ = 10 (**7a**), 50 (**7b**), 100 (**7c**), respectively).

Polymerization of Monomer 4. Instead of synthesizing the polymer and then deprotecting the monosaccharide pendant group, it was also possible to polymerize the unprotected monomer directly. Monomer **4** was polymerized with **5** as the initiator in DMSO with CuBr and Me$_6$-TREN or methanol with CuBr and bipy.[54] ATRP was conducted using different initial monomer-to-initiator ratios. The resulting polymers (**7a**−**d**) were obtained with high conversions (78−98%) and narrow molecular weight distributions (PDIs = 1.07−1.16, Table 1). The GPC traces (Figure 5 for **7a**−**c**) exhibited low molecular weight tailing, particularly when higher monomer-to-initiator ratios were employed. For polymers **7a** ([M]$_0$/[I]$_0$ = 10:1, DMSO) and **7d** ([M]$_0$/[I]$_0$ = 10:1, MeOH) it was possible to detect the peaks arising from the biotin moiety in the ^{1}H NMR spectra. The peak at 4.3 ppm (*, Figure 6 for **7a**) was compared to the backbone

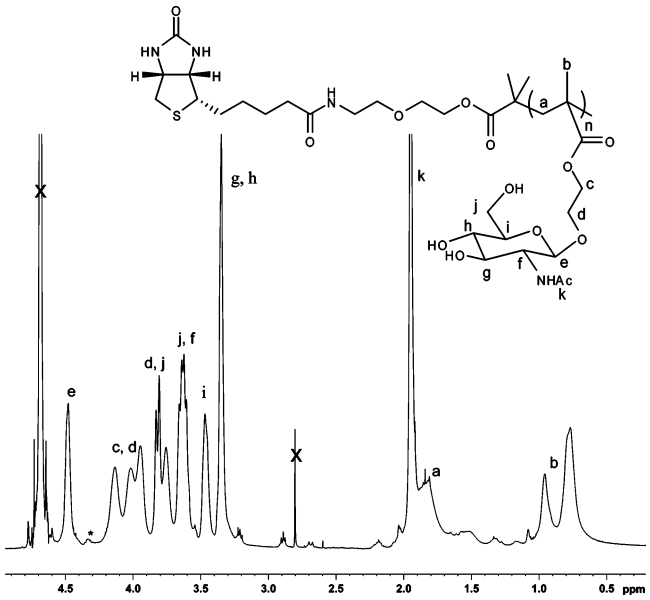


Figure 6. ^{1}H NMR spectrum (500 MHz) in D_2O of glycopolymer **7a** ([M]$_0$/[I]$_0$/[CuBr]$_0$/[Me$_6$-TREN]$_0$ = 10:1:1:1). The biotin peak used for molecular weight analysis is marked by an asterisk.

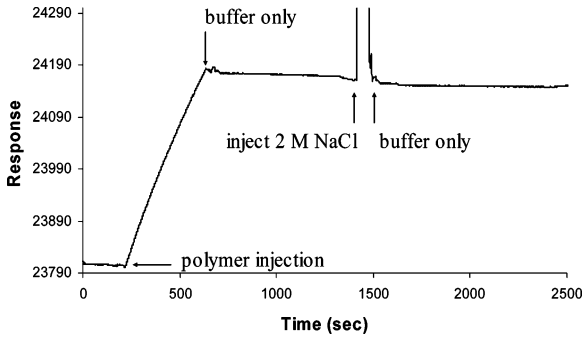


Figure 7. SPR sensorgram of glycopolymer **7b** (data taken on a Biacore X equipped with a streptavidin-coated sensor chip; running buffer = 20 mM Tris, 150 mM sodium chloride, 0.005% surfactant P20; flow rate = 5 μL/min).

methyl proton peaks (b, Figure 6 for **7a**) to calculate the molecular weights. For **7a** it was determined that the degree of polymerization was approximately 34, corresponding to a M_n of 11 400. For **7d** the M_n was 9100. Similar to polymer **6a**, the molecular weight values thus obtained by ^{1}H NMR were slightly lower than that determined by GPC and significantly higher than the targeted M_n (Table 1). Nonetheless, different molecular weights (between 11 800 and 68 400, Table 1) were readily accessible by altering the initial monomer-to-initiator ratios. As expected IR analysis of the polymers revealed spectra identical to that of the deprotected **6a** (Supporting Information).

Polymer Interaction with Streptavidin. We tested the ability of the biotinylated polymers to bind to the protein streptavidin using SPR. SPR is a powerful tool to directly observe binding events. The unprotected glycopolymers were injected into the SPR chamber containing a streptavidin-coated chip. Immediately a change in response units was observed (sensorgram provided for **7b** in Figure 7). The affinity of biotin for streptavidin is strong ($K_a \approx 10^{15}$ M^{-1}), and as expected, polymer binding to the streptavidin surface was not affected by the injection of buffer containing 2 M NaCl, which would disrupt electrostatic interactions. These results clearly demonstrate that the polymers bind to the protein streptavidin. Importantly, this binding was observed for polymers where the biotin end group was not visible in the ^{1}H NMR spectrum, further confirming that the

end group was present. These results also indicated that it is feasible to utilize these glycopolymers for surface engineering with the protein streptavidin as a linker to the substrate.

Conclusions

Well-defined biotinylated glycopolymers were synthesized by ATRP. The glycopolymers were prepared from biotinylated initiators either by direct polymerization of the unprotected monosaccharide side chain monomers or by polymerization of the protected monomer. The protected polymer was readily deprotected without cleaving the end group. Both routes provided glycopolymers with narrow molecular weight distributions. Polymerization of the deprotected monomer provided a facile way to prepare glycopolymers without postpolymerization modifications. The polymers readily bound to surfaces coated with streptavidin. Since synthetic glycopolymers are mimics of natural polysaccharides, these polymers could be employed to prepare biologically active substrates by using the biotin groups to anchor the polymers to streptavidin-coated surfaces.

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Supporting Information Available. ^{1}H NMR spectrum of deprotected glycopolymer with integrations and IR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Dwek, R. A. *Chem. Rev.* **1996,** *96*, 683−720.
(2) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002,** *41*, 391−412.
(3) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004,** *40*, 431−449.
(4) Sun, X. L.; Grande, D.; Baskaran, S.; Hanson, S. R.; Chaikof, E. L. *Biomacromolecules* **2002,** *3*, 1065−1070.
(5) Akashi, M.; Sakamoto, N.; Suzuki, K.; Kishida, A. *Bioconjugate Chem.* **1996,** *7*, 393−395.
(6) Baskaran, S.; Grande, D.; Sun, X. L.; Yayon, A.; Chaikof, E. L. *Bioconjugate Chem.* **2002,** *13*, 1309−1313.
(7) Guan, R.; Sun, X. L.; Hou, S. J.; Wu, P. Y.; Chaikof, E. L. *Bioconjugate Chem.* **2004,** *15*, 145−151.
(8) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994,** *116*, 12053−12054.
(9) Mortell, K. H.; Weatherman, R. V.; Kiessling, L. L. *J. Am. Chem. Soc.* **1996,** *118*, 2297−2298.
(10) Schuster, M. C.; Mortell, K. H.; Hegeman, A. D.; Kiessling, E. L. *J. Mol. Catal. A: Chem.* **1997,** *116*, 209−216.
(11) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997,** *119*, 9931−9932.
(12) Manning, D. D.; Hu, X.; Beck, P.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997,** *119*, 3161−3162.
(13) Manning, D. D.; Strong, L. E.; Hu, X.; Beck, P. J.; Kiessling, L. L. *Tetrahedron* **1997,** *53*, 11937−11952.
(14) Sanders, W. J.; Gordon, E. J.; Dwir, O.; Beck, P. J.; Alon, R.; Kiessling, L. L. *J. Biol. Chem.* **1999,** *274*, 5271−5278.
(15) Gordon, E. J.; Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L. *Chem. Biol.* **2000,** *7*, 9−16.
(16) Mowery, P.; Yang, Z. Q.; Gordon, E. J.; Dwir, O.; Spencer, A. G.; Alon, R.; Kiessling, L. L. *Chem. Biol.* **2004,** *11*, 725−732.
(17) Albertin, L.; Kohlert, C.; Stenzel, M.; Foster, L. J. R.; Davis, T. P. *Biomacromolecules* **2004,** *5*, 255−260.
(18) Albertin, L.; Stenzel, M.; Barner-Kowollik, C.; Foster, L. J. R.; Davis, T. P. *Macromolecules* **2004,** *37*, 7530−7537.
(19) Albertin, L.; Stenzel, M. H.; Barner-Kowollik, C.; Foster, L. J. R.; Davis, T. P. *Macromolecules* **2005,** *38*, 9075−9084.
(20) Lowe, A. B.; Sumerlin, B. S.; McCormick, C. L. *Polymer* **2003,** *44*, 6761−6765.
(21) Grande, D.; Baskaran, S.; Baskaran, C.; Gnanou, Y.; Chaikof, E. L. *Macromolecules* **2000**, *33*, 1123−1125.
(22) Grande, D.; Baskaran, S.; Chaikof, E. L. *Macromolecules* **2001,** *34*, 1640−1646.
(23) Sun, X. L.; Faucher, K. M.; Houston, M.; Grande, D.; Chaikof, E. L. *J. Am. Chem. Soc.* **2002,** *124*, 7258−7259.
(24) Faucher, K. M.; Sun, X. L.; Chaikof, E. L. *Langmuir* **2003,** *19*, 1664−1670.
(25) Hou, S. J.; Sun, X. L.; Dong, C. M.; Chaikof, E. L. *Bioconjugate Chem.* **2004,** *15*, 954−959.
(26) Matyjaszewski, K.; Xia, J. H. *Chem. Rev.* **2001,** *101*, 2921−2990.
(27) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001,** *101*, 3689−3745.
(28) Coessens, V.; Pintauer, T.; Matyjaszewski, K. *Prog. Polym. Sci.* **2001,** *26*, 337−377.
(29) Ohno, K.; Tsujii, Y.; Fukuda, T. *J. Polym. Sci., Part A: Polym. Chem.* **1998,** *36*, 2473−2481.
(30) Ejaz, M.; Ohno, K.; Tsujii, Y.; Fukuda, T. *Macromolecules* **2000,** *33*, 2870−2874.
(31) Narain, R.; Armes, S. P. *Chem. Commun.* **2002,** 2776−2777.
(32) Narain, R.; Armes, S. P. *Biomacromolecules* **2003,** *4*, 1746−1758.
(33) Narain, R.; Armes, S. P. *Macromolecules* **2003,** *36*, 4675−4678.
(34) Muthukrishnan, S.; Mori, H.; Muller, A. H. E. *Macromolecules* **2005,** *38*, 3108−3119.
(35) Muthukrishnan, S.; Jutz, G.; Andre, X.; Mori, H.; Muller, A. H. E. *Macromolecules* **2005,** *38*, 9−18.
(36) Meng, J. Q.; Du, F. S.; Liu, Y. S.; Li, Z. C. *J. Polym. Sci., Part A: Polym. Chem.* **2005,** *43*, 752−762.
(37) Sen Gupta, S.; Raja, K. S.; Kaltgrad, E.; Strable, E.; Finn, M. G. *Chem. Commun.* **2005,** 4315−4317.
(38) Muthukrishnan, S.; Zhang, M. F.; Burkhardt, M.; Drechsler, M.; Mori, H.; Muller, A. H. E. *Macromolecules* **2005,** *38*, 7926−7934.
(39) Bontempo, D.; Heredia, K. L.; Fish, B. A.; Maynard, H. D. *J. Am. Chem. Soc.* **2004,** *126*, 15372−15373.
(40) Duncan, R. *Nat. Rev. Drug Discovery* **2003,** *2*, 347−360.
(41) Mantovani, G.; Lecolley, F.; Tao, L.; Haddleton, D. M.; Clerx, J.; Cornelissen, J. J. L. M.; Velonia, K. *J. Am. Chem. Soc.* **2005,** *127*, 2966−2973.
(42) Tao, L.; Mantovani, G.; Lecolley, F.; Haddleton, D. M. *J. Am. Chem. Soc.* **2004,** *126*, 13220−13221.
(43) Lecolley, F.; Tao, L.; Mantovani, G.; Durkin, I.; Lautru, S.; Haddleton, D. M. *Chem. Commun.* **2004,** 2026−2027.
(44) Weber, P. C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. *Science* **1989,** *243*, 85−88.
(45) Qi, K.; Ma, Q. G.; Remsen, E. E.; Clark, C. G.; Wooley, K. L. *J. Am. Chem. Soc.* **2004,** *126*, 6599−6607.
(46) Bontempo, D.; Li, R. C.; Ly, T.; Brubaker, C. E.; Maynard, H. D. *Chem. Commun.* **2005,** 4702−4704.
(47) Ladmiral, V.; Monaghan, L.; Mantovani, G.; Haddleton, D. M. *Polymer* **2005,** *46*, 8536−8545.
(48) Whelan, S. A.; Hart, G. W. *Circ. Res.* **2003,** *93*, 1047−1058.
(49) Khidekel, N.; Ficarro, S. B.; Peters, E. C.; Hsieh-Wilson, L. C. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13132−13137.
(50) Slawson, C.; Hart, G. W. *Curr. Opin. Struct. Biol.* **2003**, *13*, 631−636.
(51) Takasu, A.; Niwa, T.; Itou, H.; Inai, Y.; Hirabayashi, T. *Macromol. Rapid Commun.* **2000**, *21*, 764−769.
(52) Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966**, *5*, 41−44.
(53) Nishimura, S. I.; Furuike, T.; Matsuoka, K.; Maruyama, K.; Nagata, K.; Kurita, K.; Nishi, N.; Tokura, S. *Macromolecules* **1994**, *27*, 4876−4880.
(54) Presumably due to the limited solubility of the unprotected glycopolymer in methanol, gelation of the polymer was observed at later stages of the polymerization.

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