RAFT Polymerization and Thio-Bromo Substitution: An Efficient Way towards Well-Defined Glycopolymers

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Received 15 June 2017; accepted 18 July 2017; published online 00 Month 2017
DOI: 10.1002/pola.28745

ABSTRACT: Despite an increasing effort to design well-defined glycopolymers, the convenient synthesis of polymers with higher DPs (>100) and without tedious protection and deprotection steps remains a challenge. Combining the reversible addition fragmentation transfer (RAFT) polymerization and the efficient substitution of primary bromo groups by thiols, we were able to synthesize a set of well-defined glycopolymers with DPs of up to 115. With the polymerization of the highly reactive monomer (2-bromoethyl)-acrylate polymers with low dispersities were obtained that could efficiently be functionalized with various sugar thiol(ate)s. In particular, derivatives of D-glucose, D-galactose, and D-mannose gave excellent degrees of functionalization close to quantitative conversion using only a slight excess of the thiol. This atom efficient synthesis can even be applied for copolymers with acid or base labile components due to the use of unprotected sugar moieties and, hence, the lack of further deprotection steps. Binding studies with the lectin concanavalin A and the subsequent competition studies with α-D-methyl-mannopyranose (αMeMan) proved the effective binding of these derivatives and revealed a DP- and carbohydrate-dependent clustering and dissolution. © 2017 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2017**, *00*, 000–000

KEYWORDS: carbohydrates; glycosylation; lectins; nucleophilic substitution; polymers; postmodification; RAFT

INTRODUCTION Synthetic, carbohydrate-functionalized polymers gained increased interest in biological sciences due to the crucial role of natural glycoconjugates in cell-cell recognition processes. The main interactions of living organisms are saccharide-protein, protein-protein, and protein-antibody interactions and include binding possibilities in a multivalent manner caused by hydrophobic and electrostatic interactions as well as by hydrogen bonding.[1] The saccharide-protein interactions were found to play important roles in cell adhesion and cell differentiation, but also in inflammations, viral replications, and parasitic infections.[2]

Biological events are often related to the interaction between carbohydrates and lectins. Lectins are binding proteins with high stereo-specificity for carbohydrates. A single saccharide reveals only a low affinity for its natural ligand, whereas multivalent interactions between a single or more lectins with one or more of the corresponding carbohydrate units are highly prevalent in nature.[3] The so-called "cluster-effect"[4] strongly influences the design of well-defined glycopolymer architectures.

Early glycopolymer synthesis was conducted by Kobayashi *et al*. in 1985, who prepared lactose and D-maltose conjugated styrene polymers, offering strong interactions with hepatocytes and D-galactose selective lectins.[5] Since then, various polymers with defined architectures can be obtained due to the use of modern polymerization techniques and available sugar derivatives. In general, there are two ways to obtain carbohydrate-conjugated polymers: Polymerization of carbohydrate-conjugated monomers (glycomonomers) or postpolymerization conjugation of a suitable, reactive polymer. Many attempts were performed to polymerize glycomonomers in a controlled manner by anionic[6] and cationic,[7] controlled radical,[8] ring-opening,[9] and other polymerization techniques.[10] However, the synthesis of glycomonomers usually requires various steps and the functional groups of unprotected glycomonomers reveal incompatibility with most controlled polymerization techniques.[11] Additionally, high molar mass polymers with a narrow distribution are still challenging to obtain by polymerizing glycomonomers. To postmodify polymers with carbohydrates, a reactive

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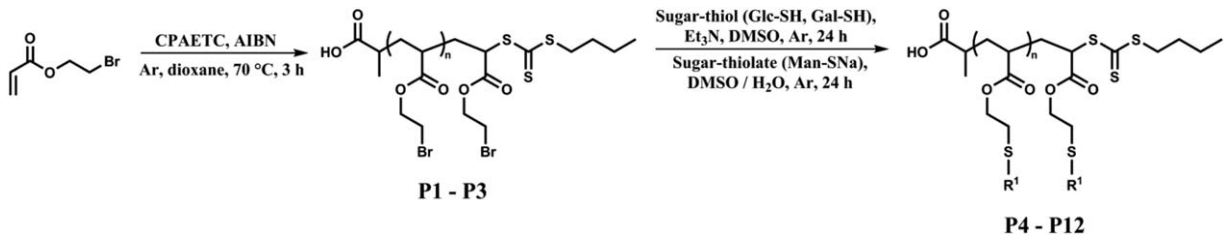

FIGURE 1 Schematic representation of the polymers **P1** to **P12** obtained via RAFT with subsequent postglyosylation.

polymer backbone is required. For this purpose, many functional groups are reported, including 4-nitrophenyl carbonate,[12] *para*-fluoro-phenyl,[13] alkinyl-,[14] alkenyl,[15] and others.[11]

A relatively unexplored reaction for the postmodification of polymers represents the nucleophilic substitution of various alkyl halides with suitable nucleophiles. Mainly used in metal-catalyzed polymerizations, the use of halide-containing polymers obtained by reversible deactivation radical polymerization (RDRP) techniques is limited. This is primarily attributed to the sensitivity of alkyl halides towards abstraction by radicals, which is intentionally used in iodine transfer polymerization.[16] However, only a few examples using RDRP for alkyl halide monomers are reported and even less are applied for the synthesis of glycopolymers. Stenzel and coworkers reported the synthesis of star shaped glycopolymers derived from poly(vinyl benzylchloride) (PVBC) via reversible addition-fragmentation polymerization (RAFT).[17] The polymers were reacted with equimolar amounts of 1-deoxy-1-thio-β-D-glucopyranose sodium salt in DMSO for 110 h without any catalyst. Also poly(epichlorhydrin) obtained via cationic ring-opening polymerization (CROP) was postmodified with 1-deoxy-1-thio-β-D-glucopyranose to obtain bristle-like polymers with moderate dispersity ($Đ = 1.68$).[18] Recently, the Perrier group demonstrated the versatility of poly(bromoethyl acrylate) (PBEA), which was polymerized via RAFT.[19] Various nucleophiles were used to yield efficient substitution of the bromides, which resulted in polymers with a wide range of functionalities including already a D-glucose derivative. However, a rather large excess of the nucleophile 1-deoxy-1-thio-β-D-glucopyranose sodium salt was used to form the D-glucosylated acrylate. To the best of our knowledge, there is no systematic study, which shows the synthesis of glyco-homopolymers by controlled radical polymerization techniques with low dispersities and DPs exceeding 100 units comparing different sugar residues.

Based on these results, we attempted to further exploit the reaction based on PBEA to synthesize a whole library of various glycopolymers and to test their suitability for selective binding to well-known lectins (Fig. 1). For this purpose, polymers with lengths ranging from 45 to 115 repeating units were prepared by RAFT polymerization and subsequently modified with various sugar residues (D-glucose, D-galactose, D-mannose). All these polymers were finally tested for their affinity to the binding-protein concanavalin A (Con A).

EXPERIMENTAL

Materials and Methods

All reagents and solvents were commercial products purchased from Sigma-Aldrich (triethylamine, dioxan, AIBN, Con A), Roth (DMSO, Zellutrans dialysis tube), or Carbosynth (α-D-thiomannose sodium salt) and were used without further purification. (4-Cyanopentanoic acid)ylethyl trithiocarbonate (CPAETC) was synthesized as previously reported.[19,20] HBS buffer (0.10 M HEPES, 0.9 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$) was prepared with Milli-Q water as the solvent.

Equipment

^{1}H NMR spectra were measured with a Bruker spectrometer (300 MHz). Elemental analysis was performed with a Leco CHN-932. Size-exclusion chromatography (SEC) of polymers **P1** to **P3** was performed on a Agilent system (series 1200) equipped with a G1310A pump, a G1362A refractive index detector and a PSS GRAM column with DMAc (+ 0.21 wt % LiCl) as eluent. The column oven was set to 40 °C and a polystyrene (PS) standard was used for calibration. SEC of polymers **P4** to **P12** was performed on a Jasco system equipped with a PU-980 pump, a RI-2031 Plus refractive index detector and a PSS SUPREMA column with H_2O (+ 0.1 M $NaNO_3$ and 0.05% NaN_3) as eluent. The column oven was set to 30 °C and a pullulan standard was used for calibration. UV/Vis absorbance spectra were measured with an Analytik Jena AG Specord250 spectrometer. Quartz cuvettes were purchased from Hellma Analytics.

Syntheses of the Thio-Sugars

1-Deoxy-1-thio-β-D-glucopyranose and 1-deoxy-1-thio-β-D-galactopyranose were synthesized according to literature procedures.[21]

Synthesis of 2-Bromoethyl Acrylate (BEA)

The BEA monomer was synthesized according to a modified literature procedure.[19] Thirty-eight milliliter of acryloyl chloride (0.54 mol) were dissolved in 250 mL dry CH_2Cl_2 under an Ar atmosphere and 82 mL of Et_3N (0.59 mol) were added. The mixture was cooled to 0 °C and 53 mL of 2-bromoethanol (0.66 mol) dissolved in 40 mL CH_2Cl_2 were added dropwise. The reaction mixture was allowed to stir for 18 h at room temperature. Subsequently, the solution was filtered and the organic layer was washed thrice with saturated, aqueous $NaHCO_3$ solution. Additionally, the organic layer was stirred with 250 mL of 0.1 M $NaOH_{(aq)}$

solution for 24 h separated and dried with Na_2SO_4. The solution was filtered and the organic solvent was evaporated *in vacuo* to obtain the crude product as a colorless oil. Ten milligram of 2,6-di-tert-butyl-4-methylphenol were added and the product was subjected a fractionated destillation under vacuum to yield 34.97 g (0.2 mol) of the desired product in highest purity and 37% yield. bp 55 °C (4.5 mmHg). δH (300 MHz; $CDCl_3$) 6.49 (m, 1H, CH_2═CH), 6.19 (m, 1H, CH_2═CH), 5.90 (m, 1H, CH_2═CH), 4.48 (t, 2H, ^3J 6.2, C$H_2$$CH_2$Br), and 4.48 (t, 2H, ^3J 6.2, CH_2CH_2Br).

General Procedure for the Syntheses of PBEA Homopolymers

CPAETC, BEA, and AIBN were dissolved in dioxane and the reaction mixture was deoxygenated with Ar for 10 min and stirred at 65 °C for 4 h. After completion, the solution was cooled to room temperature, opened to air, and precipitated in diethyl ether to give PBEA homopolymers **P1** to **P3**.

P1

^{1}H NMR (300 MHz; $CDCl_3$, δ ppm) 4.99–4.88 (1 H, m), 4.42 (2*n* H, br s), 3.57 (2*n* H, br s), 3.40 (2 H, t, ^{3}J 7.4), 2.49 (2*n* H, br s), 2.05 (2*n* H, br s), 1.94–1.50 (2*n* + 1 H, m), 1.48–1.41 (3 H, m), 0.95 (3 H, t, ^3J 7.3). SEC (DMAc + 0.21 wt % LiCl, PS standard): M_n = 8,800 g mol^{-1}, M_w = 9,600 g mol^{-1}, $Đ$ = 1.10.

P2

^{1}H NMR (300 MHz; $CDCl_3$, δ ppm) 4.98–4.88 (1 H, m), 4.43 (2*n* H, br s), 3.57 (2*n* H, br s), 3.39 (2 H, t, ^{3}J 7.2), 2.47 (2*n* H, br s), 2.15–1.97 (2*n* H, br s), 1.90–1.39 (2*n* H, m), 0.96 (3 H, t, ^{3}J 7.4). SEC (DMAc + 0.21 wt % LiCl, PS standard): M_n = 11,200 g mol^{-1}, M_w = 13,500 g mol^{-1}, $Đ$ = 1.21.

P3

^{1}H NMR (300 MHz; $CDCl_3$, δ ppm) 4.98–4.88 (1 H, m), 4.42 (2*n* H, br s), 3.57 (2*n* H, br s), 3.38 (2 H, t, ^{3}J 6.4), 2.47 (2*n* H, br s), 2.14–1.96 (2*n* H, m), 1.92–1.39 (2*n* H, m), 0.95 (3 H, t, ^{3}J 7.3). SEC (DMAc + 0.21 wt % LiCl, PS standard): M_n = 18,000 g mol^{-1}, M_w = 20,000 g mol^{-1}, $Đ$ = 1.11.

General Procedure for the Postpolymerization Modification with Glc and Gal

One hundred milligram of the precursor polymer was dissolved in 1 mL DMSO and a solution of the desired carbohydrate (DP·1.1 equiv.) in 1 mL DMSO was added. The solution was deoxygenated with Ar for 30 min and 1 equiv. (based on the carbohydrate) dry triethylamine was added dropwise. The reaction mixture was stirred at room temperature for one day and dialyzed against water for one week (CE, MWCO: 3.5 kDa). The dialyzed solution was freeze-dried to obtain the desired polymer.

P4

^{1}H NMR (300 MHz; D_2O, δ ppm) 4.49 (*n* H, d, ^3J 9.8, H_{Glc}-1), 4.24 (2*n* H, br s), 3.85–3.69 (*n* H, m), 3.69–3.55 (*n* H, m), 3.47–3.17 (4*n* H, m), 3.07–2.74 (2*n* H, m), 2.33 (2*n* H, br s), 2.03–1.00 (2*n* + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): M_n = 10,000 g mol^{-1}, M_w = 11,800 g mol^{-1}, $Đ$ = 1.19. Anal. calcd for $C_{503}H_{824}O_{317}S_{48}$: C, 44.81; H, 6.16; S, 11.41; Br, 0. Found: C, 43.87; H, 6.17; S, 10.81; Br, 0.89.

P5

^{1}H NMR (300 MHz; D_2O, δ ppm) 4.44 (*n* H, d, ^{3}J 9.3, H_{Gal}-1), 4.25 (2*n* H, br s), 3.89 (*n* H, s), 3.82–3.41 (5*n* H, m), 3.16–2.72 (2*n* H, m), 2.36 (2*n* H, br s), 2.04–1.00 (2*n* + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): M_n = 9,900 g mol^{-1}, M_w = 12,000 g mol^{-1}, $Đ$ = 1.22. Anal. calcd for $C_{503}H_{824}O_{317}S_{48}$: C, 44.81; H, 6.16; S, 11.41; Br, 0. Found: C, 41.88; H, 6.13; S, 11.99; Br, 1.99.

P7

^{1}H NMR (300 MHz; D_2O, δ ppm) 4.49 (*n* H, d, ^3J 9.7, H_{Glc}-1), 4.24 (2*n* H, br s), 3.85–3.70 (*n* H, m), 3.70–3.56 (*n* H, m), 3.55–3.18 (4*n* H, m), 3.11–2.70 (2*n* H, m), 2.37 (2*n* H, br s), 2.11–1.00 (2*n* + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): M_n = 17,400 g mol^{-1}, M_w = 21,800 g mol^{-1}, $Đ$ = 1.25. Anal. calcd for $C_{866}H_{1418}O_{548}S_{81}$: C, 44.84; H, 6.16; S, 11.20; Br, 0. Found: C, 44.15; H, 6.18; S, 9.48; Br, 2.14.

P8

^{1}H NMR (300 MHz; D_2O, δ ppm) 4.44 (*n* H, d, ^3J 8.9, H_{Gal}-1), 4.24 (2*n* H, br s), 3.90 (*n* H, s), 3.83–3.41 (5*n* H, m), 3.17–2.71 (2*n* H, m), 2.38 (2*n* H, br s), 2.04–0.99 (2*n* + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): M_n = 16,400 g mol^{-1}, M_w = 21,000 g mol^{-1}, $Đ$ = 1.28. Anal. calcd for $C_{866}H_{1418}O_{548}S_{81}$: C, 44.84; H, 6.16; S, 11.20; Br, 0. Found: C, 43.28; H, 6.18; S, 9.31; Br, 3.09.

P10

^{1}H NMR (300 MHz; D_2O, δ ppm) 4.49 (*n* H, d, ^3J 9.7, H_{Glc}-1), 4.24 (2*n* H, br s), 3.86–3.69 (*n* H, m), 3.69–3.55 (*n* H, m), 3.55–3.17 (4*n* H, m), 3.08–2.71 (2*n* H, m), 2.36 (2*n* H, br s), 2.06–1.00 (2*n* + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): M_n = 23,300 g mol^{-1}, M_w = 29,300 g mol^{-1}, $Đ$ = 1.26. Anal. calcd for $C_{1273}H_{2084}O_{807}S_{118}$: C, 44.86; H, 6.16; S, 11.10; Br, 0. Found: C, 43.53; H, 6.20; S, 10.20; Br, 1.54.

P11

^{1}H NMR (300 MHz; D_2O, δ ppm) 4.44 (*n* H, d, ^3J 9.1, H_{Gal}-1), 4.24 (2*n* H, br s), 3.89 (*n* H, s), 3.83–3.41 (5*n* H, m), 3.22–2.72 (2*n* H, m), 2.37 (2*n* H, br s), 2.09–1.00 (2*n* + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): M_n = 23,300 g mol^{-1}, M_w = 29,900 g mol^{-1}, $Đ$ = 1.28. Anal. calcd for $C_{1273}H_{2084}O_{807}S_{118}$: C, 44.86; H, 6.16; S, 11.10; Br, 0. Found: C, 41.73; H, 6.05; S, 10.58; Br, 2.52.

General Procedure for the Postpolymerization Modification with Man

One hundred milligram of the precursor polymer was dissolved in 1 mL DMSO and a solution of Man-SNa (DP·1.1 equiv.) in 0.5 mL H_2O was added. The solution was deoxygenated with Ar for 30 min, stirred at room temperature for one day and dialyzed against water for one week (CE, MWCO: 3.5

TABLE 1 Summary of BEA Polymerization

Abbrev.	$[M]_0$/CTA	[CTA]/I_0	Conv.[a] (%)	$M_{n,th}$[b] (g/mol)	$M_{n,NMR}$[c] (g/mol)	$M_{n,SEC}$[d] (g/mol)	$Đ$
P1	60	10	69	7,600	8,300	8,800	1.10
P2	150	10	58	15,700	14,200	11,200	1.21
P3	300	5	42	22,700	20,800	18,000	1.11

[a] Determined from ^{1}H NMR of the polymerization mixture before precipitation.
[b] Calculated from monomer conversion.
[c] Determined from ^{1}H NMR end-group analysis [calculated from signal intensity of the proton of the tertiary C-atom next to trithiocarbonate ($\delta = 4.93$ ppm) in comparison to the proton signal of the C1 atom of the acryl ester ($\delta = 4.43$ ppm) before postmodification]. These ratios were used to calculate the DP.
[d] SEC: DMAc + 0.21 wt % LiCl, PS calibration.

kDa). The dialyzed solution was freeze-dried to obtain the desired polymer.

P6

^{1}H NMR (300 MHz; D_2O, δ ppm) 5.30 (n H, s, H_{Man}-1), 4.49–4.07 (3n H, m), 3.97 (n H, s), 3.93–3.49 (4n H, m), 2.88 (2n H, s), 2.38 (2n H, br s), 2.11–0.93 (2n + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): $M_n = 3{,}300$ g mol^{-1}, $M_w = 4{,}800$ g mol^{-1}, $Đ = 1.44$. Anal. calcd for $C_{503}H_{824}O_{317}S_{48}$: C, 44.81; H, 6.16; S, 11.41; Br, 0. Found: C, 43.76; H, 6.27; S, 10.36; Br, 0.

P9

^{1}H NMR (300 MHz; D_2O, δ ppm) 5.30 (n H, s, H_{Man}-1), 4.50–4.06 (3n H, m), 3.97 (n H, s), 3.92–3.47 (4n H, m), 2.88 (2n H, s), 2.38 (2n H, br s), 2.12–0.93 (2n + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): $M_n = 9{,}400$ g mol^{-1}, $M_w = 12{,}600$ g mol^{-1}, $Đ = 1.34$. Anal. calcd for $C_{866}H_{1418}O_{548}S_{81}$: C, 44.84; H, 6.16; S, 11.20; Br, 0. Found: C, 43.80; H, 6.14; S, 10.45; Br, 0.

P12

^{1}H NMR (300 MHz; D_2O, δ ppm) 5.30 (n H, s, H_{Man}-1), 4.47–4.06 (3n H, m), 3.98 (n H, s), 3.93–3.46 (4n H, m), 2.88 (2n H, s), 2.38 (2n H, br s), 2.15–0.97 (2n + 1 H, m). SEC (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, pullulan standard): $M_n = 15{,}900$ g mol^{-1}, $M_w = 21{,}200$ g mol^{-1}, $Đ = 1.33$. Anal. calcd for $C_{1273}H_{2084}O_{807}S_{118}$: C, 44.86; H, 6.16; S, 11.10; Br, 0. Found: C, 43.83; H, 6.16; S, 10.42; Br, 0.36.

Turbidimetry Assay

The aggregation studies with Con A were conducted as previously reported.[22] Con A was fully dissolved in HBS buffer ($\sim$ 1 mg mL^{-1}) and diluted to a 1 μM stock solution. 1 mL of the Con A stock solution was added to a quarz glass cuvette, which was placed in the UV–Vis spectrometer. One milliliter of a 50 μM (50 μM per sugar unit) stock solution of the polymer in HBS buffer was appended with a pipette to the ground of the glass cuvette and the absorbance of the mixture was immediately recorded at $\lambda = 420$ nm for 30 min every 0.5 s. The interaction rate was calculated by using the slope of the linear fit of the steepest portion. Every experiment was conducted thrice.

Reversal Aggregation Assay

The competition experiments with α-D-methyl-mannopyranose were carried out according to literature procedure.[22] The solutions of the turbidimetry assay were allowed to rest for 2 h at room temperature. Following the addition of 0.2 mL of a 54 mM stock solution of methyl-α-D-mannopyranoside in HBS buffer, the absorbance at $\lambda = 420$ nm was immediately recorded for 60 min every 0.5 s.

RESULTS AND DISCUSSION

RAFT Polymerization of BEA

Similar to the previously published procedure, the BEA monomer was polymerized using CPAETC as chain transfer agent (CTA) and AIBN as radical initiator for the RAFT process. Optimizing the synthesis and purification procedure for the monomer (see experimental section for details) we were able to increase the final DP of the polymers without the loss of control. As a consequence, various DPs could be addressed by changing the ratio [monomer]/[CTA] (Table 1).

For all polymers, low dispersity ($Đ \leq 1.2$) and narrow mono-modal distributions were obtained, emphasizing the capabilities to polymerize halide-containing monomers by RDRP. In addition, we analyzed the end group fidelity using NMR analysis. The intensities of the signals at $\delta = 0.95$ ppm for the terminal CH_3 group and of the signal at $\delta = 3.38$ ppm for the CH_2 group next to the trithiocarbonate match well with the expectations proving the excellent retention of the RAFT end group. Recent studies showed the synthesis of a similar polymer named poly(2-bromoethyl methacrylate) by postmodification of poly(2-hydroxyethyl methacrylate) with a mixture of various chemicals.[23] Our study represents a more effective approach due to the lack of postbromination procedures and the introduction of the bromide directly to the monomer circumventing the requirement for additional tedious modification steps.

Postglycosylation

With the defined precursor polymers at hand, the various carbohydrates were examined for the next step. For this purpose, the thiolated carbohydrates (1-deoxy-1-thio-β-D-glucopyranose,[21] 1-deoxy-1-thio-β-D-galactopyranose,[21] and commercially available 1-deoxy-1-thio-β-D-mannopyranose sodium salt) were reacted with precursor polymers **P1** to **P3** in a S_N2 reaction (Table 2). These carbohydrates were chosen for

TABLE 2 Overview of the Glycopolymers **P4** to **P12** Prepared and Tested in This Study

Abbrev.	DP	Attached Sugar (R^1)	$M_{n,th}$[a]	$M_{n,SEC}$[b]	$Đ$
P4	45	Glc	13,500	10,000	1.19
P5	45	Gal	13,500	9,900	1.22
P6	45	Man	13,500	3,300	1.44
P7	78	Glc	23,200	17,400	1.25
P8	78	Gal	23,200	16,400	1.28
P9	78	Man	23,200	9,400	1.34
P10	115	Glc	34,100	23,300	1.26
P11	115	Gal	34,100	23,300	1.28
P12	115	Man	34,100	15,900	1.33

[a] Calculated by assuming polymers with the respective DP and full substitution of the bromide groups.
[b] SEC: H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, Pullulan calibration.

different reasons. D-galactose (Gal) conjugated polymers were shown to bind to hepatocytes via a receptor-mediated mechanism and, therefore, representing useful targeting moieties for the development of polymers used in various biomedical fields, such as tissue engineering and drug-loaded nanoparticles.[24] D-Mannose (Man) is in the focus of the scientific community due to its strong interactions with the lectin Con A and with macrophages, which overexpress mannose-receptors on their surface.[25] D-Glucose (Glc) interacts with transport proteins like with the GLUT1 transporter, which is shown to be overexpressed in various types of cancers, such as renal cell carcinoma[26] or non-small cell lung carcinoma.[27]

D-Galactose and D-glucose were attached using only 1.1 equivalents of the deprotected β-1-thiol derivatives. The S_N2 reactions with polymers **P1** to **P3** were conducted in DMSO and the thiol functionalities of the carbohydrates were deprotonated with one equivalent of triethyl amine. The reactions were stopped after 24 h and purified by dialysis against H_2O (MWCO: 3.5 kDa) to remove low-molar mass impurities and side-products such as DMSO and formed triethyl ammoniumbromide. In the case of D-mannose attachment, 1.1 equivalents of the commercially available sodium salt of the α-1-thiol derivative of D-mannose were used. Therefore, no base is required and the formation of sodium bromide as a side-product represents the driving force for the substitution reaction. The mannosylated polymers were purified in a similar manner to the gluco- and galactosylated ones. The success of the reaction can be shown by various NMR techniques as depicted for polymer **P12** in Figure 2, representative for all other polymers. All spectra of polymers **P1** to **P12** are available in the Supporting Information (Fig. S1–S47).

The substitution of the bromides in **P3** against the sugar-thiolate results in the formation of a thio-ether. This is clearly visible in the shift of the ethyl acrylate signals from $\delta = 4.42$ ppm in **P3** to $\delta = 4.22$ ppm in **P12,** respectively, from $\delta = 3.57$ ppm in **P3** to $\delta = 2.89$ ppm in **P12** in the ^{1}H NMR spectra. Additionally, the signal of the C1 proton appears nicely isolated at $\delta = 5.30$ ppm, whereas the other D-mannose proton signals are visible between $\delta = 3.5$ and 4.5 ppm. As also previously shown,[28] the attachment of carbohydrates result in water-soluble polymers **P4** to **P12**, whereas precursor polymers **P1** to **P3** revealed nonsolubility in water. Additionally, by binding D-mannose *S*-glycosidically to the polymer backbone, a thioether is formed, which possesses a lower rate of hydrolysis of the thioglycosidic bond by enzymatic cleavage relative to *O*-glycosydically coupled sugar residues.[29]

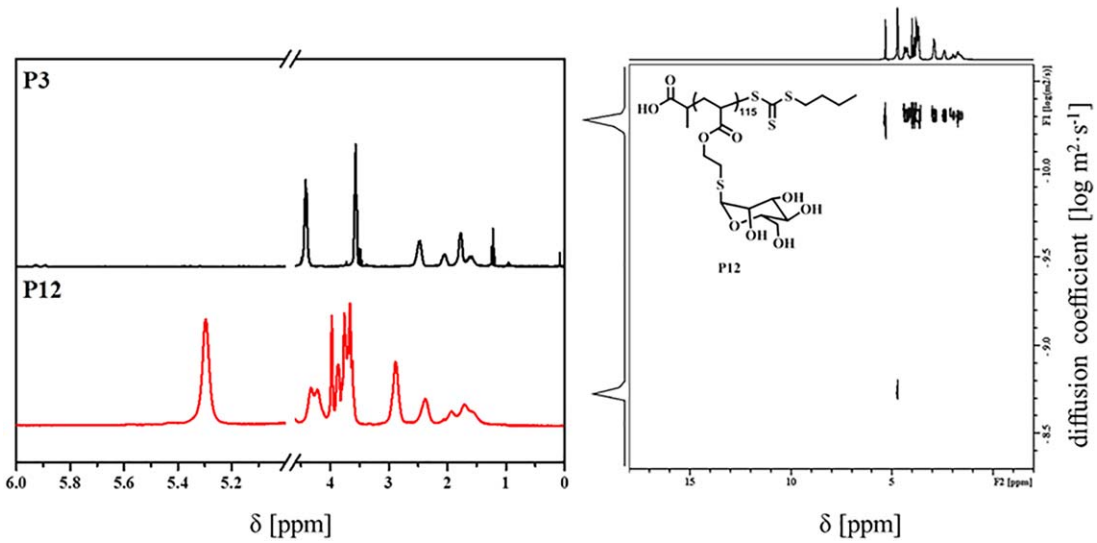

FIGURE 2 Left: Comparison of ^{1}H NMR of compounds **P3** (measured in $CDCl_3$) and **P12** (measured in D_2O) between $\delta = 0$ and 6 ppm. Intermittent axis between $\delta = 4.6$ and 5 ppm was used due to solvent residue signal in the spectrum of **P12**. Right: ^{1}H diffusion-ordered NMR (DOSY) at $T = 25$ °C of **P12**. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Elemental Compositions of Polymers **P4** to **P12**; the Calculated DFs are Based on Theoretical Br Content of Polymers **P1** to **P3**

Abbrev.	DP	Saccharide	Elemental Composition[a] (%)				DF (%)
			C	H	S	Br	
P1	45	–	**33.74**	**4.00**	**1.16**	**43.35**	–
P4	45	Glc	43.87	6.17	10.81	0.89	97.9
P5	45	Gal	41.88	6.13	11.99	1.99	95.4
P6	45	Man	43.76	6.27	10.36	0	100
P2	78	–	**33.66**	**3.97**	**0.68**	**43.89**	–
P7	78	Glc	44.15	6.18	9.48	2.14	95.1
P8	78	Gal	43.28	6.18	9.31	3.09	93
P9	78	Man	43.80	6.14	10.45	0	100
P3	115	–	**33.63**	**3.96**	**0.46**	**44.12**	–
P10	115	Glc	43.53	6.20	10.20	1.54	96.5
P11	115	Gal	41.73	6.05	10.58	2.52	94.3
P12	115	Man	43.83	6.16	10.42	0.36	99.2

[a] Elemental composition of starting material polymers **P1** to **P3** was calculated assuming one polymer species with the depicted DP.

^{1}H diffusion-ordered NMR (DOSY) investigations of **P12** revealed the appearance of all signals (except the signal for the solvent residue) at high diffusions constants typical for polymers ($D = 5{\cdot}10^{-11}$ m^2 s^{-1}), indicating the successful attachment of D-mannose units without the appearance of any side-products.

In contrast to the previous polymers **P1** to **P3** the end group fidelity of the RAFT group could not be determined after postpolymerization functionalization due to the overlap of the important signals in the NMR with the signals of the sugar moiety. Reinitiation experiments, however, indicated a degradation of the trithiocarbonate as no block formation is observed.

To further evaluate the quality of the substitution reaction, the elemental composition of polymers **P4** to **P12** was analyzed. The determined halogen content represents the remaining bromoethyl ester content and can be used to calculate the degree of functionalization (DF) with the following equation (Table 3).

$$\mathrm{DF} = 1 - \frac{\text{Br content (sugar polymer)}}{\text{Br content (PBEA)}} \times 100$$

The DF ranges from 93 to 100% and some general tendencies are noticeable. D-Mannose functionalized polymers **P6**, **P9,** and **P12** showed remaining Br content between 0 and the lower detection limit (0.36%), whereas the other polymers revealed Br content between 0.89 and 3.09%. Therefore, the use of the sodium salt of the corresponding thiol-sugars, instead of the thiol-sugars and a suitable base, seems to be beneficial for the substitution reaction. This might be attributed to the decreased solubility of the formed sodium bromide in the solvent mixture of D-mannosylated polymers in comparison to the solubility of the formed triethyl ammoniumbromide of the other polymers in DMSO. Additionally, the DF with D-galactose is generally decreased in comparison to D-glucose, which might be caused by the existence of small amounts of 1-deoxy-1-thio-α-D-galactopyranose, which could be sterically hindered during the substitution reaction. Due to the well resolved signals from the C1 protons of the D-mannose units in ^{1}H NMR, they can be used to validate the DF by comparing their intensity to that of the backbone signal at $\delta = 2.9$ ppm. The obtained data is in good agreement with the results obtained from the elemental analysis of the polymers.

The polymers **P4** to **P12** were also investigated via SEC to obtain information about the increase of the molar mass of the polymers and their dispersities (Fig. 3).

The traces still show a mono-modal distribution and the dispersity remains narrow below 1.5, indicating no formation of macromolecular side-products during the S_N2 reaction and the absence of previously observed chain-chain coupling due to the removal of the CTA and the subsequent disulfide formation or other side reactions.[19] The latter can certainly be attributed to the reduced excess of thio-sugar molecules used for the substitution. The symmetric broadening of the SEC traces and the slight increase of the dispersities relative to the precursor polymers **P1** to **P3** is most probably related to the difference in the SEC system applied for the analysis of the polymers and potential interactions of the attached sugar moieties with the column material. An indication for this phenomenon is further the later elution of D-mannosylated polymers **P6**, **P9,** and **P12** in comparison to the corresponding D-glucosylated or D-galactosylated polymers, respectively, although the absolute molar masses should be very similar. An additional effect for this shift in the elution

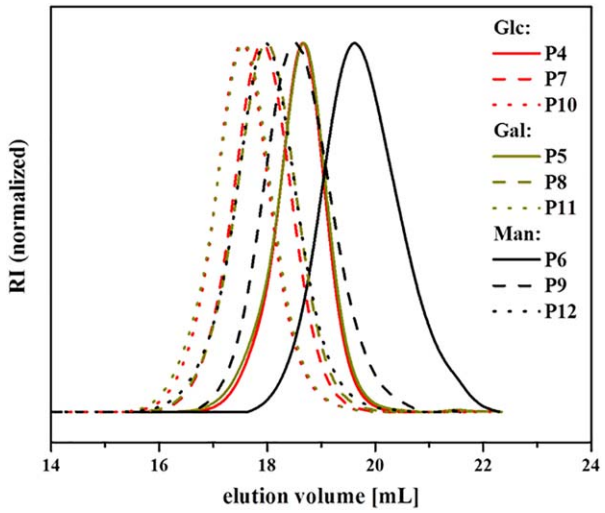

FIGURE 3 SEC traces of polymers **P4** to **P12** (H_2O, 0.1 M $NaNO_3$, 0.05% NaN_3, Pullulan standard). [Color figure can be viewed at wileyonlinelibrary.com]

volume might be the interaction between the axial C2 hydroxyl groups of the D-mannose units and the carboxylic ester functionalities on the polymer backbone, which decrease the resulting hydrodynamic radius, whereas D-glucose and D-galactose possess equatorial hydroxyl groups at their C2 atoms. Another reason could be the stronger interaction of D-mannose with the column material in comparison to the other sugars. D-Glucose conjugated polymers roughly eluted after the same volume than the D-galactolysated polymers with the same DP.

Lectin Binding

One critical requirement for the application of glycopolymers, for example, in targeted drug delivery, is their ability to selectively bind to lectins for the respective type of sugar. To examine the binding efficacy of the glycopolymers, turbidimetry assays were performed for the polymers **P4** to **P12** using the lectin Con A. Con A consists of aggregates of 25,000 g mol^{-1} size. While existing at pH range 5–5.6 as a dimer, Con A is predominantly aggregating into tetramers above pH = 7.[30] Each monomer unit is known to selectively bind to one unit of α-gluco- or α-mannopyranose, but no binding should be observed in the case of the galactopyranose.[31] The rate of clustering was monitored in real-time by measuring the absorbance at $\lambda = 420$ nm over time after mixing the lectin and polymer solutions. The change in turbidity is related to the rate of receptor-receptor associations caused by the sugar-units of the polymers.[32] The slope of the steepest portion of the initial curve was used to represent the clustering rate, expressed in arbitrary units per second (a.u. s^{-1}).[22] The initial values of the curves are correlated to the formation of isolated Con A polymer clusters, whereas interactions between the clusters occur at later points. The formation of cross-linked clusters of higher order increases over the time and, therefore, the analysis is limited to the initial portion of the curve.[33] The experimental results are summarized in Figure 4. All spectra of the triplicate measurements including the linear fits are available in the Supporting Information (Fig. S9, S14, S18, S23, S28, S32, S37, S42, and S46).

Polymers **P5**, **P8,** and **P11** bearing D-galactose residues did not show any aggregation with Con A over a time period of 30 min, which is in accordance to previously reported D-galactolysated polymers.[14] D-Glucosylated and D-mannosylated polymers exhibited the formation of Con A clusters with varying clustering rates. As a general trend, the polymers grafted with D-mannose residues revealed higher rates of Con A clustering than their D-glucosylated polymers with the same DP. This might be attributed to the higher binding affinity of the Con A tetramers to α-D-mannose in comparison to D-glucose residues. Another reason could be the decreased hydrodynamic radius of D-mannosylated polymers relative to the D-glucosylated ones, which could offer beneficial shape or length of the polymers in solution in terms of Con A binding. The absorbance of D-mannose bearing polymers reached

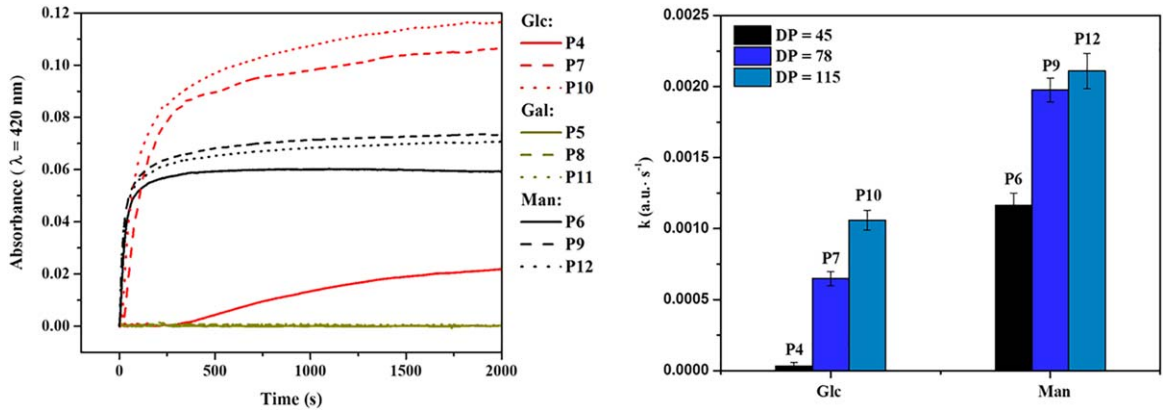

FIGURE 4 Results of turbidimetry measurements. Left: Absorbance ($\lambda = 420$ nm) curves after adding 1 mL solution of polymers **P4** to **P12** (50 μM per sugar unit) to 1 μM solutions of Con A in HBS buffer. Right: Calculated rates of clustering between Con A tetramers and D-glucosyl- or D-mannosylated polymers obtained by a linear fit of the steepest portions of the curves, *k* values represent the average of three replicates. [Color figure can be viewed at wileyonlinelibrary.com]

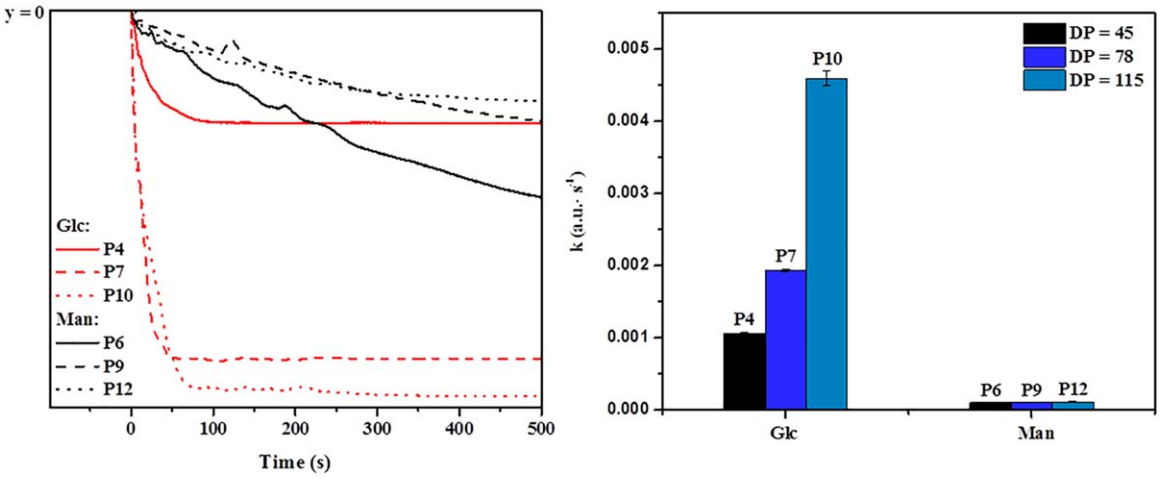

FIGURE 5 Results of reversal aggregation measurements. Left: Absorbance ($\lambda = 420$ nm) curves after adding 0.2 mL solution of αMeMan (54 mM) to the polymer solutions (50 μM per sugar unit) in HBS buffer. Right: Calculated rates of the reverse interaction between Con A aggregates and the competitor αMeMan obtained by a linear fit of the steepest portions of the curves. [Color figure can be viewed at wileyonlinelibrary.com]

very fast a plateau and stayed almost constant at this level for the remaining measurement, which is attributed to a rapid precipitation of most of the Con A tetramers (Fig. 4). In contrast, D-glucosylated polymers exhibited a continuous, but slower increase of the absorbance, indicating secondary interactions such as cross-linked clusters or partially soluble conjugates. In particular, the shortest D-glucose bearing polymer **P4** exhibited different clustering rates compared to all other polymers. One reason is the general decreased affinity of D-glucose to Con A relative to D-mannose. Another one is certainly the overall length of the polymer. Considering a fully stretched chain the length of **P4** can be estimated to be ~80 Å taking into account the binding angles. The distance between two binding sites of the Con A tetramer is around 72 Å and,[34] therefore, the length of the polymer, which is just above the distance between the binding sites, paired with the low affinity of Con A towards D-glucose did not result in fast agglutination of the Con A clusters. In the light of this, the existence of a critical polymer length for high clustering rates of Con A aggregates is assumed, which is also in accordance to literature reports.[35]

Another trend is obtained by comparing the rate of clustering caused by the polymers with the same sugar residues, but with different DPs. With higher DPs (for D-glucose as well as for D-mannose bearing polymers) the precipitation of Con A clusters is promoted more rapidly. Therefore, we assume a dependency of the speed of clustering with the epitope density, which was also reported for other glycopolymers.[33,35] Additionally, the rate of clustering for polymers with D-mannose residues seems to approximate a constant level in dependency of the DP. A lower increase of the clustering rates for D-mannosylated polymers with higher DPs relative to **P12** in comparison to the increase between **P9** to **P12** is expected. To exactly determine the required DP, more turbidimetry investigations with D-mannosylated polymers of higher DPs are necessary. The described polymerization and postglycosylation could be used to synthesize these polymers in future studies.

Reversal Aggregation

In addition to the previous binding assays, the strengths and efficiencies of the interaction between glycopolymer and lectin can be evaluated by competition experiments. For this purpose, the aggregates formed during turbidimetry assays were allowed to rest for around 2 h to finish the formation of higher-order aggregates and were subsequently treated with an excessive amount of α-D-methyl-mannopyranose (αMeMan), a competitor for the binding sites of Con A. The absorbance at $\lambda = 420$ nm over the time was monitored and the results are summarized in Figure 5 for D-manno- and D-glucosylated polymers. The D-galactose bearing polymers were omitted due to the inefficient binding to the lectin Con A and all spectra are available in the Supporting Information (Fig. S10, S19, S24, S33, S38, and S47).

The results of the reversal aggregation assay revealed carbohydrate-specific and length-dependent tendencies. The rates of the dissolution of the D-glucosylated polymers are strongly enhanced relative to those of the D-mannosylated polymers when treated with the monovalent competitor αMeMan in large excess. The turbidity was rapidly reduced to a constant level, indicating a rather weak interaction between Con A and the D-glucose bearing polymers. Additionally, the dissolution of the D-glucose Con A clusters happened faster with increasing DP of the polymers. The dissolution of the cluster of D-mannosylated polymers was slow but continuous to a constant level, indicating higher binding affinity of the Con A tetramers to D-mannosylated polymers. Other tendencies are observable when comparing the difference of the highest and the lowest absorbance ($\lambda = 420$ nm) after addition of Con A to the difference

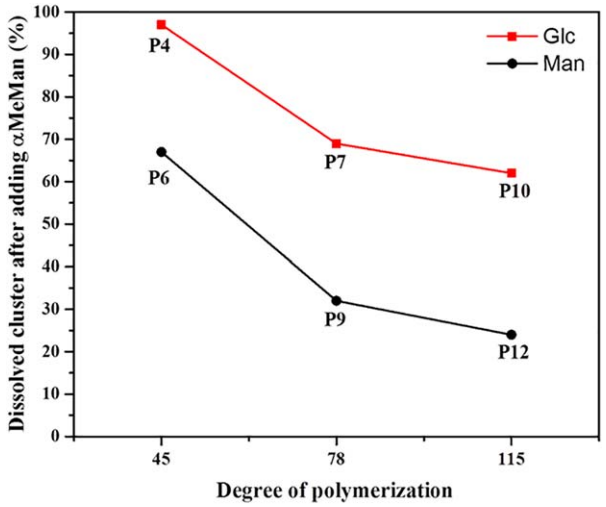

FIGURE 6 Ratio of the dissolved Con A polymer clusters after adding αMeMan relative to the DP. The values were calculated by the ratio of the moduli of the difference between the highest and lowest absorbance after adding αMeMan respectively Con A to the polymer solutions. [Color figure can be viewed at wileyonlinelibrary.com]

obtained after addition of αMeMan. First of all, the Con A aggregates of D-mannosylated polymers (**P6**, **P9**, **P12**) were not completely dissolved after adding the competitor and a clear trend is that with higher DP, the amount of undissolved Con A clusters remaining in solution increases (Fig. 6, Supporting Information Table S1).

Comparing the different sugar moieties (except for the D-galactolysated polymers) all aggregates with D-glucosylated polymers revealed decreased stability relative to the respective clusters of the D-mannosylated polymers with the same DP. This is in accordance with the results we obtained for the reversal aggregation assay showing higher dissolution rates for increasing DPs of the polymers (**P4** < **P7** < **P10**). It is further noteworthy to mention that we observe an increased total stability of the D-glucosylated polymers **P7** (69%) and **P10** (62%) although Con A clusters of D-glucose are commonly fully dissolved after adding αMeMan.[22] For the D-mannosylated polymers an even higher stability is observed despite the addition of almost 1000 equivalents of the competitor αMeMan, which has the same binding motif. This could indicate beneficial properties of the presented polymers in terms of their length, the flexibility of the polymeric backbone and way of attachment of the pendant sugar moieties, which may yield in an improved binding strength to the lectin.

CONCLUSION

In summary, this work demonstrates the high potential of the combination of RAFT polymerization and the bromo-thio substitution to create different, well-defined sugar-conjugated polymers. The basis for the reactive scaffold is the (2-bromo-ethyl)-acrylate monomer that enables excellent control in the RAFT polymerization and high DPs of up to 115 repeating units. The bromides were readily substituted in a post-glycosylation procedure by addition of almost equal amounts of sugar-thiol(ate)s, in particular D-glucose, D-galactose, and D-mannose, in an S_N2 reaction. The substitution was performed directly with unprotected carbohydrates, emphasizing the versatility of this method towards potential copolymers or linker structures, which are labile under the basic or acidic conditions usually applied for the deprotection of sugar units. Furthermore, the reactions are highly atom efficient and create only nontoxic side-products (triethyl ammoniumbromide, sodium bromide). The analytics of the novel polymers revealed mono-modal distributions with narrow dispersities and excellent DF of up to 100%, which was confirmed by elemental analysis. Binding studies of Con A with the synthesized polymers as multivalent ligands prove the selective binding ability of the sugar moieties as expected for the small molecules. D-Mannosylated polymers revealed higher clustering rates than the D-glucosylated polymers, whereas D-galactose bearing polymers showed no formation of cluster with Con A at all. With the linear, high molar mass polymers at hand, we could also demonstrate that the precipitation of Con A clusters is promoted more rapidly with increasing DP of the polymers. Reverse results were obtained by treating the Con A clusters with monovalent competitor α-D-methyl-mannopyranose (αMeMan). The turbidity of the D-glycosylated polymer clusters was rapidly reduced to a constant level, whereas the D-mannosylated ones decreased slower but continuous. This indicates a weak interaction between the Con A binding site and the multivalent D-glucose bearing polymers. Additionally, the remaining undissolved clusters of D-glucosylated and D-mannosylated polymers indicate an excellent binding affinity of these new types of polymers, which surpasses most other reported polymers with respective sugar groups attached.

This work demonstrated the use of RAFT for the polymerization of bromide-containing monomer BEA with high control and DPs higher than 100 as well as the subsequent post-glycosylation with almost quantitative DFs by versatile S_N2 reaction with thiol(ate) derivatives of various sugars. The D-mannose bearing polymers via thioether functionality revealed the formation of very stable clusters with the lectin Con A.

ACKNOWLEDGMENTS

The authors gratefully thank Gabi Sentis and Peter Bellstedt for the conducted DOSY NMR measurements and Beate Lentvogt and Sandra Köhn for the performed determination of the elemental compositions. The funding of the collaborative research center ChemBioSys (SFB 1127) by the Deutsche Forschungsgemeinschaft (DFG) is highly acknowledged. J.C. Brendel further thanks the DFG for support (Return Grant, BR 4905/2–1).

REFERENCES

1 Y. Miura, Y. Hoshino, H. Seto, *Chem. Rev.* **2016**, *116*, 1673.

2 (a) Y. Miura, Polym. J. **2012**, *44*, 679; (b) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683.

3 (a) R. J. Pieters, Org. Biomol. Chem. **2009**, *7*, 2013; (b) A. Ghadban, L. Albertin, *Polymers* **2013**, *5*, 431.

4 J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555.

5 (a) K. Kobayashi, H. Sumitomo, Y. Ina, Polym. J. **1985**, *17*, 567; (b) A. Kobayashi, T. Akaike, K. Kobayashi, H. Sumitomo, *Macromol. Rapid Commun.* **1986**, *7*, 645.

6 S. Loykulnant, A. Hirao, *Macromolecules.* **2000**, *33*, 4757.

7 M.-P. Labeau, H. Cramail, A. Deffieux, *Macromol. Chem. Phys.* **1998**, *199*, 335.

8 (a) D. M. Haddleton, R. Edmonds, A. M. Heming, E. J. Kelly, D. Kukulj, New J. Chem. **1999**, *23*, 477; (b) K. Ohno, Y. Tsujii, T. Fukuda, *J. Polym. Sci. Part A: Polym. Chem.* **1998**, *36*, 2473.

9 K. Aoi, K. Tsutsumiuchi, M. Okada, *Macromolecules.* **1994**, *27*, 875.

10 V. Ladmiral, E. Melia, D. M. Haddleton, *Eur. Polym. J.* **2004**, *40*, 431.

11 J. A. Burns, M. I. Gibson, C. R. Becer, Functional Polymers by Post-Polymerization Modification; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, **2012**; pp. 237.

12 M. C. García-Oteiza, M. Sánchez-Chaves, F. Arranz, *Macromol. Chem. Phys.* **1997**, *198*, 2237.

13 C. R. Becer, K. Babiuch, D. Pilz, S. Hornig, T. Heinze, M. Gottschaldt, U. S. Schubert, *Macromolecules.* **2009**, *42*, 2387.

14 V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin, D. M. Haddleton, *J. Am. Chem. Soc.* **2006**, *128*, 4823.

15 G. Chen, S. Amajjahe, M. H. Stenzel, *Chem. Commun.* **2009**, 1198.

16 G. David, C. Boyer, J. Tonnar, B. Ameduri, P. Lacroix-Desmazes, B. Boutevin, *Chem. Rev.* **2006**, *106*, 3936.

17 Y. Chen, G. Chen, M. H. Stenzel, *Macromolecules.* **2010**, *43*, 8109.

18 J. C. Kim, Y. Rho, G. Kim, M. Kim, H. Kim, I. J. Kim, J. R. Kim, M. Ree, *Polym. Chem.* **2013**, *4*, 2260.

19 T. R. Barlow, J. C. Brendel, S. Perrier, *Macromolecules.* **2016**, *49*, 6203.

20 C. J. Ferguson, R. J. Hughes, D. Nguyen, B. T. T. Pham, R. G. Gilbert, A. K. Serelis, C. H. Such, B. S. Hawkett, *Macromolecules.* **2005**, *38*, 2191.

21 A. Bruneau, M. Roche, A. Hamze, J.-D. Brion, M. Alami, S. Messaoudi, *Chem. Eur. J.* **2015**, *21*, 8375.

22 Y. Gou, J. Geng, S.-J. Richards, J. Burns, C. Remzi Becer, D. M. Haddleton, *J. Polym. Sci. Polym. Chem.* **2013**, *51*, 2588.

23 H. Zhou, Y. Chen, C. M. Plummer, H. Huang, Y. Chen, *Polym. Chem.* **2017**, *8*, 2189.

24 (a) Y. Wang, G. Jiang, T. Qiu, F. Ding, Drug Dev. Ind. Pharm. **2012**, *38*, 1039; (b) C. S. Cho, S. J. Seo, I. K. Park, S. H. Kim, T. H. Kim, T. Hoshiba, I. Harada, T. Akaike, *Biomaterials* **2006**, *27*, 576.

25 (a) S. Kawakami, A. Sato, M. Nishikawa, F. Yamashita, M. Hashida, Gene Ther. **2000**, *7*, 292; (b) H. Kitano, Y. Takahashi, K. Mizukami, K. Matsuura, *Colloids Surf. B* **2009**, *70*, 91.

26 A. Lidgren, A. Bergh, K. Grankvist, T. Rasmuson, B. Ljungberg, *BJU Int.* **2008**, *101*, 480.

27 M. Younes, R. W. Brown, M. Stephenson, M. Gondo, P. T. Cagle, *Cancer.* **1997**, *80*, 1046.

28 E. L. Dane, S. L. Chin, M. W. Grinstaff, *ACS Macro Lett.* **2013**, *2*, 887.

29 J. R. Rich, A. Szpacenko, M. M. Palcic, D. R. Bundle, *Angew. Chem. Int. Ed.* **2004**, *43*, 613.

30 S. M. Dimick, S. C. Powell, S. A. McMahon, D. N. Moothoo, J. H. Naismith, E. J. Toone, *J. Am. Chem. Soc.* **1999**, *121*, 10286.

31 K. H. Mortell, R. V. Weatherman, L. L. Kiessling, *J. Am. Chem. Soc.* **1996**, *118*, 2297.

32 A. M. Puertas, F. J. de las Nieves, *J. Phys.: Condens. Matter.* **1997**, *9*, 3313.

33 C. W. Cairo, J. E. Gestwicki, M. Kanai, L. L. Kiessling, *J. Am. Chem. Soc.* **2002**, *124*, 1615.

34 P. N. Kanellopoulos, K. Pavlou, A. Perrakis, B. Agianian, C. E. Vorgias, C. Mavrommatis, M. Soufi, P. A. Tucker, S. J. Hamodrakas, *J. Struct. Biol.* **1996**, *116*, 345.

35 C. Xiao, C. Zhao, P. He, Z. Tang, X. Chen, X. Jing, *Macromol. Rapid Commun.* **2010**, *31*, 991.