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# Synthesis and Lectin Recognition of Glyco Star Polymers Prepared by "Clicking" Thiocarbohydrates onto a Reactive Scaffold

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ABSTRACT: Glycopolymers with a four-arm star architecture were prepared from poly(vinyl benzyl chloride) (PVBC) star polymers as reactive scaffold and 1-thio-β-D-glucose sodium salt. The star polymer was prepared via RAFT polymerization using 1,2,4,5-tetrakis(thiobenzoylthiomethyl)benzene as a rate-retarding RAFT agent at a polymerization temperature of 120 °C. The occurrence of known side reactions such as star−star coupling was partly suppressed by optimizing the reaction conditions. The molecular weight distribution in the early stages of the polymerization (< 50% monomer conversion) remained narrow (PDI < 1.3), but side reaction became more pronounced at higher conversions. The polymers were reacted with equimolar amounts of 1-thio-β-D-glucose sodium salt in DMSO in the absence of any catalyst. The rate of reaction was monitored using $^1$H NMR, confirming full conversion after a reaction time of 110 h. Six different glyco star polymers with number of repeating units $N$ ranging from 40 to 680 were tested regarding their ability to bind to Concanavalin A (ConA) using turbidity assay. The rate of reaction and $t_{1/2}$, the time to reach half of the maximum absorption, was found to reach a maximum and minimum, respectively, at a medium molecular weight. The same molecular weight dependency was obtained using precipitation assay, which determines the amount of ConA conjugated to the glycopolymer. Comparison with linear glycopolymers reveals however that the amount of bound ConA and the rate of clustering are not superior in the star architecture.

## Introduction

Carbohydrates play a pivotal role in biological processes such as cell−cell recognition and signaling events. The success of bacteria and viruses in their quest in invading the human body is usually derived from their carbohydrate-filled surface. The key is the ability of carbohydrates to bind selectively to carbohydrate-binding proteins.[1] Surprisingly, the interaction between a single sugar molecule and the protein is rather weak, but the simultaneous binding of numerous sugar molecules can result in strong conjugation. This "glycoside cluster effect"[2] or multivalency effect is determined not only by the thermodynamics between the two participants but also by the architecture.[3] Spherical carbohydrate designs such as dendrimers with their high local carbohydrate concentration accelerate binding to proteins. Therefore, significant effort has been targeted toward the synthesis of synthetic carbohydrates—glycopolymers—where architecture and carbohydrate concentration can be tailored to achieve an optimum outcome. While glycodendrimer and related structures are now well established,[4] star-shaped glycopolymers have long been overlooked as an alternative. In fact, despite the vast variety of literature on the synthesis of glycopolymers[5,6]—synthetic polymer with pendant sugar groups—the number of reports on glyco star polymers is negligible. Only a handful of examples on the synthesis of glyco star polymers, synthesized mainly via ATRP[7−9] or RAFT,[10−12] are known. The need for protective groups, the occurrence of side reactions, or the tedious procedures to synthesize water-soluble initiators for unprotected monomers are a few reasons that might hamper the synthesis of well-defined star structures with a known number of arms. Postfunctionalization emerged in recent years as an alternative to prepare glycopolymers. Different sugar molecules were attached to preformed reactive polymers via efficient and frequently orthogonal reaction such as Cu-catalyzed azide−alkyne click (CuAAC) chemistry,[13,14] amide synthesis using aminosugars,[15−17] thiol−ene "click reaction",[18−20] and the reaction of thiolsugar with bromides.[21]

It is therefore proposed to explore the avenue of postfunctionalization to synthesize glyco star polymers. Cu(I)-catalyzed click reactions always pose the risk of toxic copper impurities while the polymerization of monomers with vinyl pendant groups for thiol−ene reactions can succumb to cross-linking reactions.[22−24] The reaction between the sodium salt of thiolsugars and alkyl chlorides—albeit not as fast as click reaction—represent a welcome alternative since the only side product, NaCl, is not a biological hazard. In order to prepare a reactive scaffold with chloride pendant groups, the living polymerization of vinylbenzyl chloride is encompassed. RAFT polymerization[25] has already been proven to be a successful avenue to prepare well-defined linear poly(vinylbenzyl chloride).[26−30] However, the synthesis of star polymers from this monomer via RAFT or any other technique has yet not been attempted. While RAFT polymerization is a robust technique in terms of functionalities, the synthesis of star polymers is challenged by potential side reactions such as star−star coupling or the nonlinearity of the arm growth with monomer conversion.[31] The type of side reaction is dependent on the attachment of the RAFT agent onto the core, either via R-group or Z-group.[32]

Here, we employed a tetrafunctional RAFT agent using the R-group approach to polymerize vinylbenzyl chloride VBC followed by the nuclephilic substitution with 1-thio-β-D-glucose sodium salt. The polymers were tested regarding their ability to conjugate to proteins.

## Experimental Part

**Materials.** HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Tris buffer (tris(hydroxymethyl)aminomethane), Concanavalin A (ConA), 1-thio-β-D-glucose sodium

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salt, and dimethyl sulfoxide (anhydrous) were all purchased from Sigma-Aldrich and used as received. Vinyl benzyl chloride (VBC) (mixture of 3- and 4-isomers, 97%, Aldrich) was destabilized via an alumina B column prior to use. The RAFT agent, 1,2,4,5-tetrakis(thiobenzoylthiomethyl)benzene, was prepared according to a procedure described earlier.[34]

**Polymer Synthesis.** *Synthesis of Poly(4-vinylbenzyl chloride).* VBC (5.0 g, 0.033 mol) and 1,2,4,5-tetrakis(thiobenzoylthiomethyl)-benzene 4-arm rate-retarded RAFT agent (30 mg, 0.04 mmol) were added into a polymerization vial and then equally divided and moved into six polymerization vials. The vials were sealed and deoxygenated by nitrogen for 30 min before put into a 120 °C oil bath. Samples were collected and polymerization terminated after 1, 2, 3, 5, 8, and 24 h. The polymerization was stopped by immersing the vials into ice water. The resulting mixtures were then each precipitated in 250 mL of methanol and then collected by centrifugal sedimentation.

Linear poly(4-vinylbenzyl chloride) was prepared in a similar procedure using cumyl dithiobenzoate as controlling agent.

*Modification with 1-Thio-β-D-glucose Sodium Salt.* Each PVBC sample (10.0 mg, $6.57 \times 10^{-5}$ mol repeating units) was mixed with 1-thio-β-D-glucose sodium salt (14.3 mg, $6.57 \times 10^{-5}$ mol) and dissolved in 1.5 mL of DMSO. The mixtures were then transferred into NMR tubes and reacted at 40 °C for 110 h. The resulting mixture was then added into molecular weight 1000 membrane dialyzing tubes and dialyzed against deionized water for 72 h. The purified polymers were then freeze-dried for 48 h to remove any moisture.

***Canavalia ensiformis* Turbidimetry Binding Assay via UV/vis Spectroscopy.** A solution of 0.5 mg mL$^{-1}$ of ConA was prepared in 0.01 M HEPES buffer at pH 7.4. 0.5 mL of this lectin solution was transferred into a masked quartz semimicrocell and placed into the holding block of the Cary 300 UV−vis spectrophotometer for temperature equilibration at 25 °C, and a baseline was subsequently taken. Glycopolymers (1 mg) were dissolved in 0.5 mL of DMSO. HEPES solution (5.5 mL) has been added to result in a solution at a concentration of 0.16 mg mL$^{-1}$. 1 mL of this solution was further diluted with 2 mL of HEPES buffer, leading to a concentration of 0.053 mg mL$^{-1}$. A small volume of this solution (50 μL) was added to the cuvette containing the lectin solution. The solution in the cuvette was thoroughly mixed using a pipet and immediately returned into the holding block where an absorbance at 420 nm was recorded for 20 min. The final glycopolymer−lectin solution (0.55 mL) therefore contained $2.65 \times 10^{-3}$ mg of glycopolymer and 0.25 mg of ConA, which equates to 35 glucose units to 1 ConA molecule.

**Precipitation Assay.** The assay was performed at 15 °C in Tris-HCl buffer (0.1 M, pH = 7.2, containing 0.15 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$) according to a procedure described earlier.[35,36] For each glycopolymer solution, 1 mg of glycopolymer was dissolved in 0.5 mL of DMSO and then diluted with 5.5 mL of Tris-HCl buffer solution, yielding a stock solution containing 534 μM glucose residuals. Varying volumes (from 50 to 1000 μL) of this stock solution were moved into Eppendorf centrifuge tubes and further diluted to 1000 μL to yield glycopolymer solutions containing 26.7−534 μM glucose residuals. Each solution was mixed with Con A (500 μL, 9.6 μM), and the mixtures were allowed to sit for 20 h before centrifuged at 6000 rpm for 10 min. The aqueous phase was removed, and the solid was washed with 1.5 mL of cold buffer three times. Then 500 μL of 1-methylmannose solution (0.1 M, in Tris-HCl buffer) was added to each sample to dissolve the glycopolymer−Con A precipitate. The resulting solutions were diluted with 1 mL of buffer solution, and 50 μL of DMSO was added to dissolve the precipitated glycopolymer. The UV absorbance of these solutions was then measured using UV−vis spectroscopy at a wavelength of 280 nm. In order to determine the absorbance of glycopolymers themselves, a series of control samples were prepared using only glycopolymers without addition of Con A or methylmannose. The absorbance of Con A solutions with various concentrations is measured at 280 nm to determine the extinction coefficient.

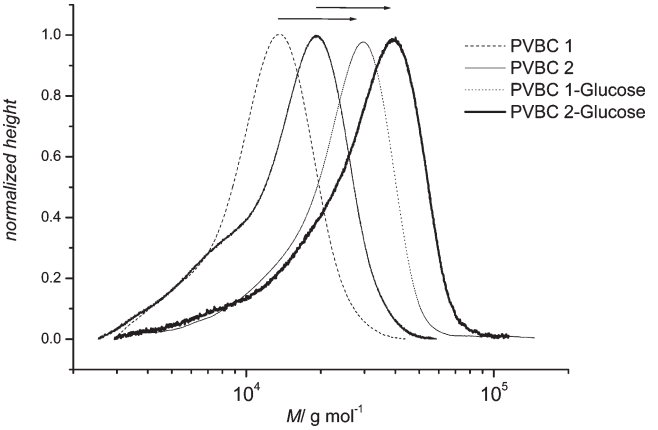



**Figure 1.** Molecular weight distribution of two PVBC star polymers (PVBC1: $N = 96$; PVBC2: $N = 360$) and their corresponding glycopolymer after functionalization with 1-thio-β-D-glucose sodium salt ($N$ = number of repeating units per star polymer).

**Nuclear Magnetic Resonance (NMR) Spectroscopy.** All NMR spectra were recorded using a Bruker 300 MHz spectrometer. Deuterated chloroform, deuterium oxide, or deuterated dimethyl sulfoxide were used as solvents at a temperature of 298 K.

**Size Exclusion Chromatography (SEC).** Molecular weight distributions were determined by SEC with a Shimadzu modular system having *N*,*N*-dimethylacetamide (DMAc) (0.03% w/v LiBr, 0.05% BHT stabilizer) at 50 °C with a flow rate of 0.85 mL min$^{-1}$. The system incorporated a DGU-12A solvent degasser, a LC-10AT pump, and a CTO-10A column oven and was equipped with a RID-10A refractive index detector. Polymer Laboratories 5.0 μm bead-size guard column (50 × 7.8 mm) followed by four 300 × 7.8 mm linear PL columns ($10^5$, $10^4$, $10^3$, and 500 Å) were used to separate the samples. The system was calibrated using narrow polystyrene standards ranging from 3000 to $10^6$ g mol$^{-1}$.

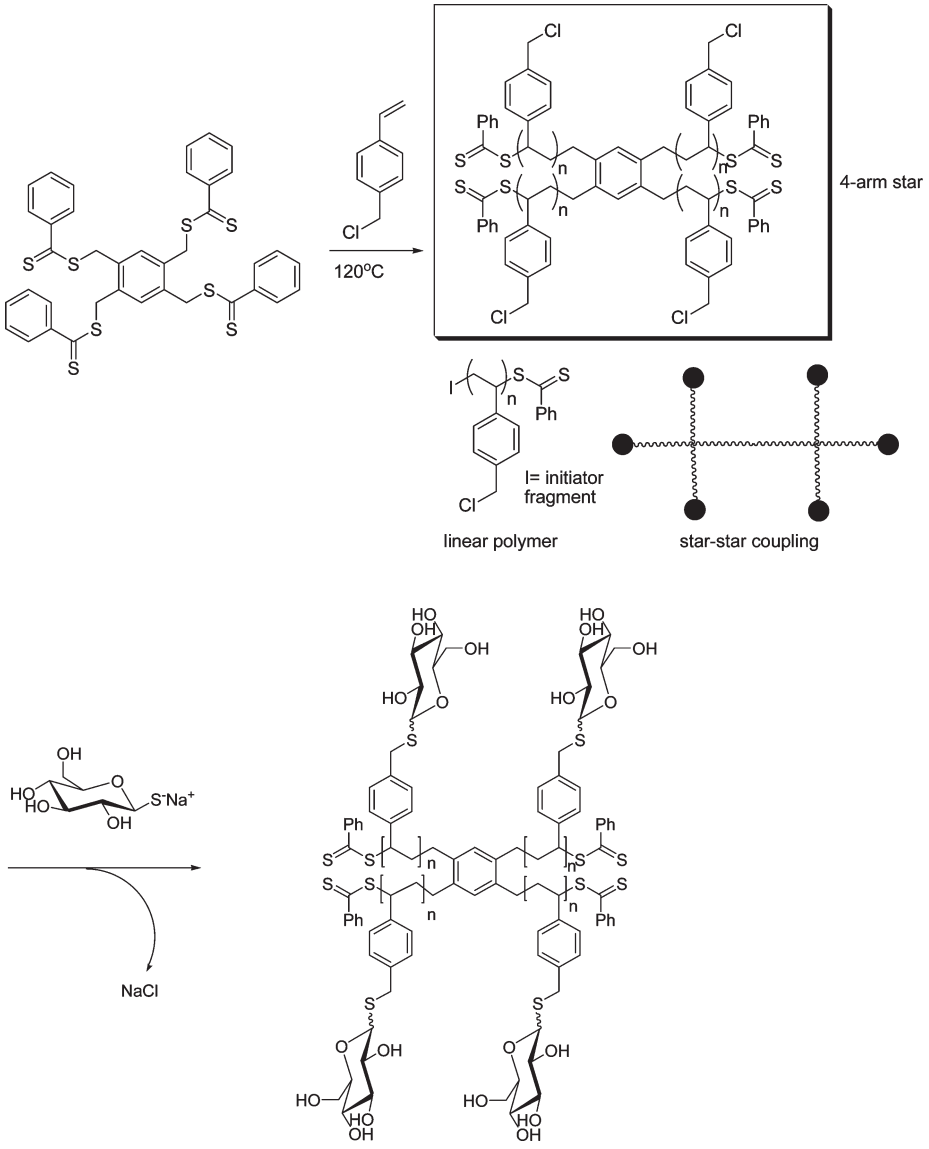
**Fourier-Transform Infrared Spectroscopy (FT-IR).** FT-IR measurements were performed using a Bruker IFS 66/s Fourier transform spectrometer equipped with a tungsten halogen lamp, a KBr beam splitter, a DTGS detector, and an ATR (attenuated total reflectance) unit.

**Light Scattering Analysis.** An ALV 5022F spectrometer equipped with a time correlator (ALV5000) and a He−Ne laser (632.8 nm, 22 mW) was used. Polymers with known concentration dissolved in toluene were measured at an angle of 90°, and the $M_w$ of polymers were calculated using the d$n$/d$c$ value of 0.121.

## Results and Discussion

According to theory, star−star coupling as well as the formation of linear RAFT-terminated polymer chains can occur. Both side products are a function of the radical concentration. Recommendation to suppress these side reactions therefore includes a high ratio of RAFT agent to initiator concentration, a small number of arms, a high addition rate of the macroradical to the RAFT agent $k_\alpha$, and a high transfer of the linear macroradical to a starbound RAFT group $k_{trStar}$, a highly retarded system and a fast propagating monomer.[34,37] Earlier work on the synthesis of polystyrene star revealed that sufficiently high polymerization temperatures, which increases $k_p$, result in a better polymerization outcome with obtained molecular weights closer to theoretical values.[33] Therefore, we chose a ratio between monomer and RAFT group concentration of 200 to 1 (maximum 800 repeating units per star) and an elevated temperature of 120 °C and self-initiation of vinylbenzyl chloride (mixture of isomers 3-VBC and 4-VBC). The polymerization is rapid after an inhibition period of

**Scheme 1. Synthesis of 4-Arm Glycopolymer Stars (Including Their Potential Side Products) with Pendant Glucose Units via RAFT Polymerization Followed by the Biocompatible Reaction between Chloride and Thiols[a]**




[a] The detailed structure of the star−star coupling product can be found in earlier publications.[31,33]

~1 h, with almost complete conversion in 24 h (see Supporting Information, Figure S1). A short inhibition period is commonly observed in RAFT polymerization and can possibly be assigned to the slow fragmentation of the pre-equilibrium. The reader is referred to more detailed literature on the mechanism of the RAFT process.[38,39] The molecular weight distributions at lower conversions were unimodal with PDIs below 1.3 (Figure 1), but significant broadening is observed at higher conversions. The potential side reactions with the formation of low molecular weight products as depicted in Scheme 1 can become more pronounced at higher conversions (see Supporting Information, Figure S2). Indeed, detailed analysis of the SEC curves (see Supporting Information, Figure S3) reveals the broadening of the molecular weight at higher monomer conversions with a visible low molecular weight shoulder emerging, which can be assigned to linear macroRAFT agents. Simultaneously, the PDI increases steadily from 1.13 (5% monomer conversion) to 1.9 (85% monomer conversion). The molecular weight, as measured using SEC equipped with a RI detector only, evolves close to the calculated value with conversion. However, at higher conversion a deviation from the expected value is noticeable, which can be assigned not only to the smaller hydrodynamic diameter of stars compared to linear chains but also to the emergence of linear side products. To test this hypothesis, the polymers were tested using light scattering analysis, demonstrating a good agreement between the theoretical molecular weight calculated using conversion and the weight-average molecular weight (Table 1).

A set of 4-arm stars with 5, 12, 24, 45, 60, and 85% monomer conversion (which is equivalent to a 40, 96, 192, 360, 480, and 680 VBC repeating units per star) were purified and modified with 1-thio-$\beta$-D-glucose sodium salt in DMSO as solvent (Table 2). In contrast to earlier work,[40−42] no catalyst was employed in order to avoid toxic impurities while equimolar amounts of VBC repeating units and 1-thio-$\beta$-D-glucose sodium salt were used. The reaction was monitored online using $^1$H NMR to determine the end point of the reaction. The doublet signal of the anomeric proton at 4.26 ppm decreases with proceeding reaction (Figure 2). Meanwhile, the methylene group of the polymer adjacent to Cl undergoes a shift from 4.6 to ~3.2 ppm. The integral for the anomeric proton at 4.26 ppm was employed to quantify the rate of reaction (Figure 2, inset), confirming that 100% conversion can be achieved after an extended reaction period of 110 h at low

**Table 1. Comparison of Theoretical Molecular Weight with Molecular Weight Obtained via SEC and LLS**

| time/h | monomer conversion | $M_n$(theo)/g mol$^{-1}$ | $M_w$(LLS)[a]/g mol$^{-1}$ | $M_n$(SEC)/g mol$^{-1}$ | $M_w$(SEC)/g mol$^{-1}$ |
|---|---|---|---|---|---|
| 1 | 0.048 | 6 636.8 | 6 950 | 5 580 | 6 030 |
| 2 | 0.123 | 15 756.8 | 14 870 | 13 600 | 15 800 |
| 3 | 0.225 | 28 160 | 25 700 | 20 000 | 25 400 |
| 24 | 0.85 | 104 160 | 102 010 | 51 400 | 97 600 |

[a] ALV5000 (He/Ne laser 632.8 nm, polymer dissolved in toluene).

**Table 2. Molecular Weights and PDI's of Polymers Prepared Determined via SEC (RI Detector)**

| number of repeating units | PVBC before functionalization | | PVBC after functionalization | |
|---|---|---|---|---|
| | $M_n$(SEC)/g mol$^{-1}$ | PDI | $M_n$(SEC)/g mol$^{-1}$ | PDI |
| 40 | 6 000 | 1.08 | 6 000 | 1.43 |
| 96 | 13 000 | 1.16 | 24 000 | 1.27 |
| 192 | 20 000 | 1.27 | 34 000 | 1.4 |
| 360 | 36 000 | 1.29 | 43 000 | 1.57 |
| 480 | 42 000 | 1.34 | 47 000 | 1.6 |
| 680 | 51 000 | 1.9 | 53 000 | 2.4 |

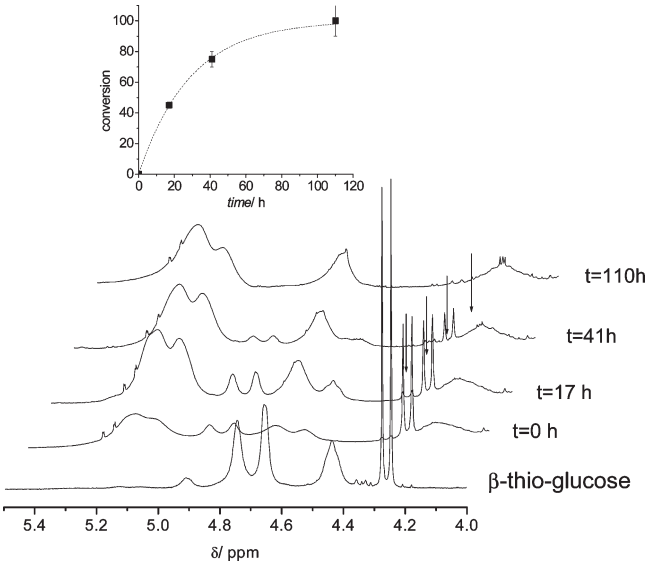



**Figure 2.** $^1$H NMR in DMSO-$d_6$ recorded at different time intervals of the reaction mixture of PVBC ($M_n$(SEC) = 36 000 g mol$^{-1}$, PDI = 1.29, $N$ = 360) and 1-thio-$\beta$-D-glucose sodium salt at 40 °C. The arrows indicate the anomeric signal used for rate determination pointing to the position at 4.26 ppm.

temperatures with only NaCl as side product. The quantitative rate determination is only possible using the anomeric signal of 1-thio-$\beta$-D-glucose sodium salt while the signals relating to the polymer shift during the course of the reaction in intensity and sometimes in position.

The final product was subsequent purified via dialysis against water; NMR of the 4-arm glycopolymer shows an excellent agreement between the integral intensity of the backbone signals at 5.9−7.5 ppm (4H) and the anomeric proton (1H) at 4.1 ppm, confirming the full conversion. The assignment of the signals was attempted using 2D-NMR spectra (COSY, NOESY) (see Supporting Information, Figures S4 and S5); however, the detailed assignment of the carbohydrate signals could not be confidently carried out. It is concluded that for the thiol−chloride modification approach a 1:1 ratio between VBC repeating unit and 1-thio-$\beta$-D-glucose sodium salt is therefore sufficient to achieve full functionalization (Figure 3).

FT-IR spectroscopic analysis of the products is a further indication of the successful reaction. The sharp signal at 1260 cm$^{-1}$,

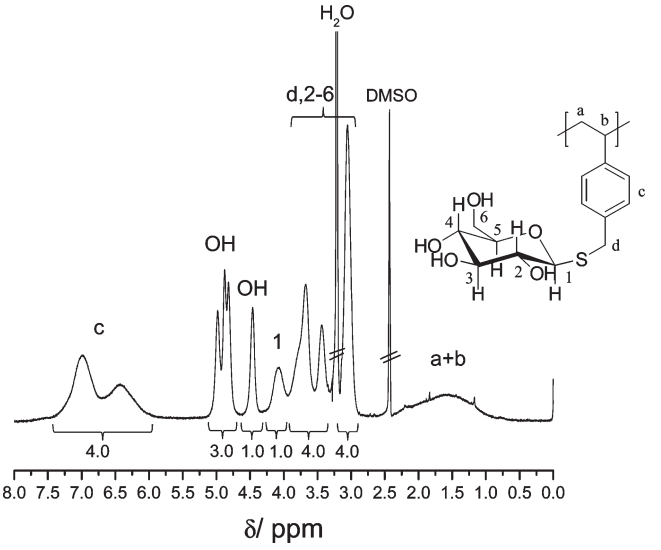



**Figure 3.** $^1$H NMR of the reaction product after dialysis against water ($N$ = 680).

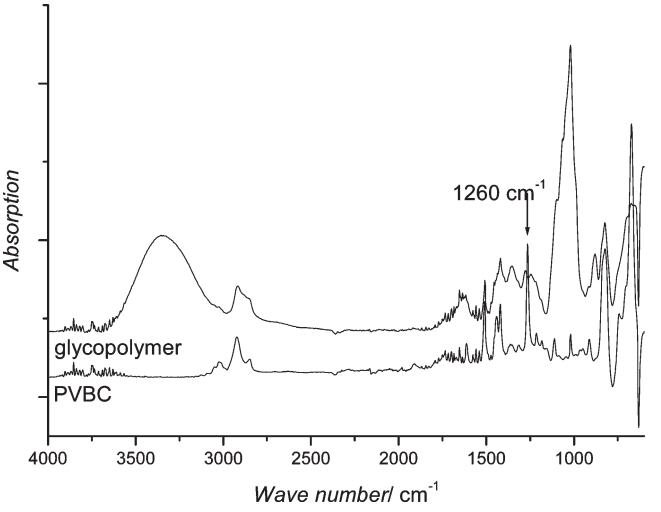



**Figure 4.** FT-IR spectrum of PVBC and glycopolymer ($N$ = 680).

which is pinpointing to the $CH_2$−Cl bond,[43] disappears while the presence of OH and C−O groups is visible in the region at 3100−3700 cm$^{-1}$ and 900−1150 cm$^{-1}$, respectively (Figure 4).

The expected molecular weight shift upon functionalization was confirmed using SEC (Figure 1). Apart from the molecular weight shift, SEC reveals the absence of high molecular weight shoulders indicating that no side products such as cross-linked polymers are formed.

Only selected results are shown here, but NMR, FT-IR, and SEC studies were carried out on all products leading to similar results independent from the molecular weight.

The ability of the glycopolymers prepared to conjugate to Concanavalin A (ConA), a lectin that is known to specifically bind to $\alpha$-D-mannosyl and $\beta$-D-glucosyl residues, was tested using

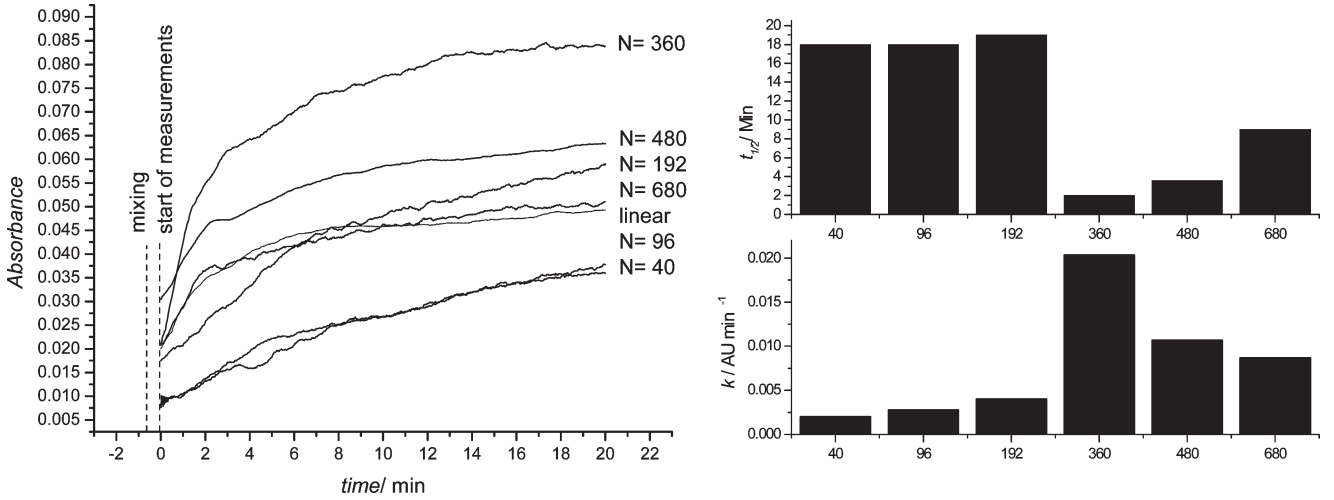



**Figure 5.** Left: absorption vs time of the reaction between 4-arm glyco star polymers with various molecular weight ($N$ = number of repeating units of star) and a linear glyco polymer with $N$ = 180 with ConA as monitored using UV/vis spectroscopy. Right: rate of reaction and $t_{1/2}$ (time required to reach half of maximum absorption) in dependency of $N$, the number of repeating units of each 4-arm star.

turbidity assay. ConA has four binding sites for these sugar molecules allowing ConA to act like a cross-linker when interacting with glycopolymer. With the specific binding of glycopolymer to ConA, cluster formation takes place and the originally clear polymer solution turns turbid, which can conveniently be monitored using an UV/vis spectrometer. Kiessling and co-workers investigated in detail the interaction of multivalent ligands with receptors highlighting frequently the influence of the polymer architecture and the epitope density as one of many parameters influencing the multivalent effect.[44−47] A variety of architectures have been tested ranging from linear polymers to micelles and dendrimers. To our knowledge, this is the first report on the binding of lectins to star polymers. (An amphiphilic star polymer was tested earlier,[48] but the binding architecture was not a free star, but a self-assembled structure.) To test the rate of binding of the glycopolymers synthesized with ConA, a solution with 0.055 mg mL$^{-1}$ of polymer were prepared. To aid the solubility of the star polymer, a small amount of DMSO was added (< 1 vol %). While higher molar ratios of more than 30 mol % of DMSO in water can lead to significant unfolding of ConA, the small amount used here does not affect the secondary protein structure.[49] The clear polymer solution in HEPES buffer was mixed with a solution of ConA, which immediately forms precipitate. A very fast conjugation was observed with higher polymer concentration, which led to immediate strong precipitation resulting in maximum cluster formation before the sample could be inserted into the UV/vis spectrometer. The measured absorption vs reaction time is displayed in Figure 5. The dependency of the absorption on the molecular weight of the star polymer becomes immediately evident. Further analysis of the rate of reaction (obtained from the slope of the initial stages of the reaction)[41] and the time $t_{1/2}$ required to reach half of the maximum absorption[51] discloses a maximum for the rate and a minimum for $t_{1/2}$ at a medium molecular weight of the star polymer. It therefore seems that higher molecular weights do not always enhance binding with proteins, and molecular weight optimization may be necessary. Kiessling and co-workers carried out detailed studies on molecular weight dependency and observed as well that the interaction with lectins increases with increasing molecular weight, but then reached a plateau once a certain molecular weight was achieved.[50] In other studies by Kiessling's group a small drop in activity at very high molecular weight was observed, which is in agreement with our current study.[46] To confirm the results obtained via turbidity assay, more detailed analysis was carried out using precipitation assay.[36] For that purpose, glycopolymer solutions of a

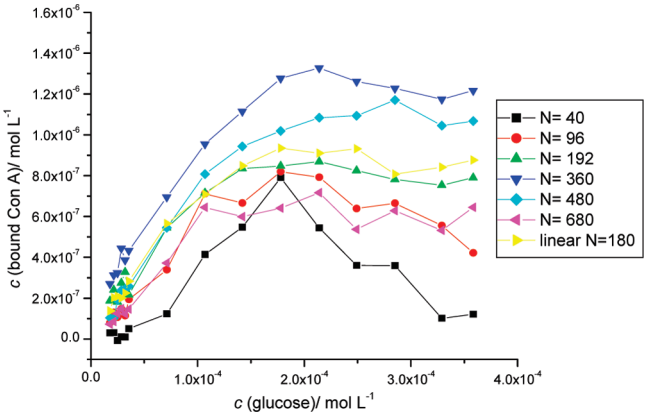



**Figure 6.** Concentration of ConA bound by glycopolymers in correlation with the concentration of glucose repeating units in the aqueous solution (initial ConA concentration: 9.6 $\mu$M).

wide range of concentrations were incubated with an excess of ConA. The glycopolymer−ConA precipitate was isolated and redissolved using 1-methylmannose, a competitive ligand that with its strength to bind to ConA easily dissolves the network. The amount of ConA in the network was determined using UV/vis taking the absorption of the glycopolymer itself into account (Figure 6). The overall amount of ConA bound is rather small compared to the more powerful binding between mannose and ConA. Only around 12% of available ConA is bound using the most efficient polymer at the highest concentration ($N$ = 360). Further increase of the glycopolymer concentration is not possible due to the low solubility of the polymer in water. However, the point of interest in this study is the molecular weight dependency of the binding events. The amount of ConA conjugated evidently increases with number of repeating units $N$ until a maximum at $N$ = 360 has been reached. Further increase of molecular weight results then in a decrease of the amount of ConA bound. The results of the precipitation assay are therefore in excellent agreement with the turbidity assay, confirming that binding goes through a maximum. It should be pointed out though that both assays measure different activity. While the turbidity assay determines the rate of cluster formation, the precipitation assay allows the calculation of the amount of ConA per polymer.[46] Please note that the bell-shaped curve for very low molecular weights such as obtained for $N$ = 40 have been observed earlier.[35]

A question of importance is the performance of star-shaped polymers in comparison to linear glycopolymers. Therefore, the equivalent glycopolymer was obtained using a similar procedure but with cumyl dithiobenzoate as controlling agent. Interestingly, both assays reveal an activity of the linear glycopolymer with $N = 180$ repeating units similar to a star with $N = 192$ repeating units. It therefore seems that there is no noticeable advantage of a star-shaped polymer in this study. Considering that dendrimers are advantageous structures when it comes to their ability to bind to lectins, it is surprising to see that star polymers cannot be found in the same category. With the set of results presented here it is too early to draw any conclusions. More experiments are needed.

## Conclusion

Postfunctionalization of reactive 4-arm star polymers led to 4-arm glycopolymers with high biological activity. The reaction between poly(vinylbenzyl chloride) PVBC and with 1-thio-$\beta$-D-glucose sodium salt at 40 °C was slow, but this disadvantage is outweighed by the absence of any hazardous side products an toxic catalyst such as amines. This approach was demonstrated here using the commercially available 1-thio-$\beta$-D-glucose sodium salt, but to generate polymers with high bioactivity, the same pathway can be employed using mannose derivatives. The biological activity was observed to be dependent on the molecular weight, and both turbidity assay and precipitation assay revealed a maximum activity at medium molecular weights. The amount of bound ConA is rather low as expected from glucose polymer, and it is recommended to employ thiol-functionalized mannose to generate more bioactive polymers. Surprising in this study is the comparison test with linear polymer. The star polymer seems not to be advantageous when it comes to its bioactivity. Further studies are warranted.

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**Supporting Information Available:** Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Mammen, M.; Chio, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37* (20), 2755–2794.
(2) Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **2001**, *17* (7/8/9), 543–551.
(3) Mulder, A.; Huskens, J.; Reinhoudt, D. N. *Org. Biomol. Chem.* **2004**, *2* (23), 3409–3424.
(4) Chabre, Y. M.; Roy, R. *Curr. Top. Med. Chem.* **2008**, *8* (14), 1237–1285.
(5) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40* (3), 431–449.
(6) Spain, S. G.; Gibson, M. I.; Cameron, N. R. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45* (11), 2059–2072.
(7) Dai, X.-H.; Zhang, H.-D.; Dong, C.-M. *Polymer* **2009**, *50* (19), 4626–4634.
(8) Muthukrishnan, S.; Plamper, F.; Mori, H.; Muller, A. H. E. *Macromolecules* **2005**, *38* (26), 10631–10642.
(9) Qiu, S.; Huang, H.; Dai, X. H.; Zhou, W.; Dong, C. M. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47* (8), 2009–2023.
(10) Zhang, L.; Stenzel, M. H. *Aust. J. Chem.* **2009**, *62* (8), 813–822.
(11) Bernard, J.; Favier, A.; Zhang, L.; Nilasaroya, A.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Macromolecules* **2005**, *38* (13), 5475–5484.
(12) Bernard, J.; Hao, X.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Biomacromolecules* **2005**, *7* (1), 232–238.
(13) Geng, J.; Mantovani, G.; Tao, L.; Nicolas, J.; Chen, G.; Wallis, R.; Mitchell, D. A.; Johnson, B. R. G.; Evans, S. D.; Haddleton, D. M. *J. Am. Chem. Soc.* **2007**, *129* (49), 15156–15163.
(14) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128* (14), 4823–4830.
(15) Baek, M.-G.; Roy, R. *Macromol. Biosci.* **2001**, *1* (7), 305–311.
(16) Boyer, C.; Davis, T. P. *Chem. Commun.* **2009**, No. 40, 6029–6031.
(17) Martinez, G.; Fernandez-Garcia, M.; Sanchez-Chaves, M. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43* (1), 18–27.
(18) Chen, G.; Amajjahe, S.; Stenzel, M. H. *Chem. Commun.* **2009**, No. 10, 1198–1200.
(19) Nurmi, L.; Lindqvist, J.; Randev, R.; Syrett, J.; Haddleton, D. M. *Chem. Commun.* **2009**, *19*, 2727–2729.
(20) Diehl, C.; Schlaad, H. *Macromol. Biosci.* **2009**, *9* (2), 157–161.
(21) Xue, C.; Donuru, V. R. R.; Liu, H. *Macromolecules* **2006**, *39* (17), 5747–5752.
(22) Ma, J.; Cheng, C.; Sun, G. R.; Wooley, K. L. *Macromolecules* **2008**, *41* (23), 9080–9089.
(23) Ma, J.; Cheng, C.; Wooley, K. L. *Aust. J. Chem.* **2009**, *62* (11), 1507–1519.
(24) Valade, D.; Boyer, C.; Davis, T. P.; Bulmus, V. *Aust. J. Chem.* **2009**, *62* (10), 1344–1350.
(25) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2009**, *62* (11), 1402–1472.
(26) Quinn, J. F.; Chaplin, R. P.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40* (17), 2956–2966.
(27) Strube, O. I.; Schmidt-Naake, G. *Macromol. Symp.* **2008**, *275*, 13–23.
(28) Fu, G. D.; Xu, L. Q.; Yao, F.; Zhang, K.; Wang, X. F.; Zhu, M. F.; Nie, S. Z. *ACS Appl. Mater. Interfaces* **2009**, *1* (2), 239–243.
(29) Yeh, K. M.; Chen, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44* (18), 5362–5377.
(30) Zhang, H. J.; Deng, J. J.; Lu, L. C.; Cai, Y. L. *Macromolecules* **2007**, *40* (26), 9252–9261.
(31) Barner-Kowollik, C.; Davis, T. P.; Stenzel, M. H. *Aust. J. Chem.* **2006**, *59* (10), 719–727.
(32) Stenzel, M. H. *Macromol. Rapid Commun.* **2009**, *30* (19), 1603–1624.
(33) Stenzel-Rosenbaum, M.; Davis, T. P.; Chen, V.; Fane, A. G. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39* (16), 2777–2783.
(34) Chaffey-Millar, H.; Stenzel, M. H.; Davis, T. P.; Coote, M. L.; Barner-Kowollik, C. *Macromolecules* **2006**, *39* (19), 6406–6419.
(35) Khan, M. I.; Mandal, D. K.; Brewer, C. F. *Carbohydr. Res.* **1991**, *213*, 69–77.
(36) Wolfenden, M. L.; Cloninger, M. J. *Bioconjugate Chem.* **2006**, *17* (4), 958–966.
(37) Chaffey-Millar, H.; Busch, M.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *Macromol. Theory Simul.* **2005**, *14* (3), 143–157.
(38) Feldermann, A.; Coote, M. L.; Stenzel, M. H.; Davis, T. P.; Barner-Kowollik, C. *J. Am. Chem. Soc.* **2004**, *126* (48), 15915–15923.
(39) Konkolewicz, D.; Hawkett, B. S.; Gray-Weale, A.; Perrier, S. *Macromolecules* **2008**, *41* (17), 6400–6412.
(40) Durette, P. L.; Shen, T. Y. *Carbohydr. Res.* **1980**, *81* (2), 261–274.
(41) Morais, G. R.; Humphrey, A. J.; Falconer, R. A. *Tetrahedron* **2008**, *64* (30−31), 7426–7431.
(42) Encinas, L.; Chiara, J. L. *Eur. J. Org. Chem.* **2009**, *2009* (13), 2163–2173.
(43) Lee, Y. S.; Byoun, Y. S. *Bull. Korean Chem. Soc.* **2002**, *23* (12), 1833–1835.
(44) Strong, L. E.; Kiessling, L. L. *J. Am. Chem. Soc.* **1999**, *121* (26), 6193–6196.
(45) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124* (8), 1615–1619.
(46) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124* (50), 14922–14933.
(47) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem., Int. Ed.* **2006**, *45* (15), 2348–2368.
(48) Dai, X.-H.; Dong, C.-M. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46* (3), 817–829.
(49) Jackson, M.; Mantsch, H. H. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1991**, *1078* (2), 231–235.
(50) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119* (41), 9931–9932.
(51) $t_{1/2}$ was determined from the absorption vs ln(time) graph. The maximum of the first derivative of the sigmoidal curve corresponds to the absorption at $t_{1/2}$.