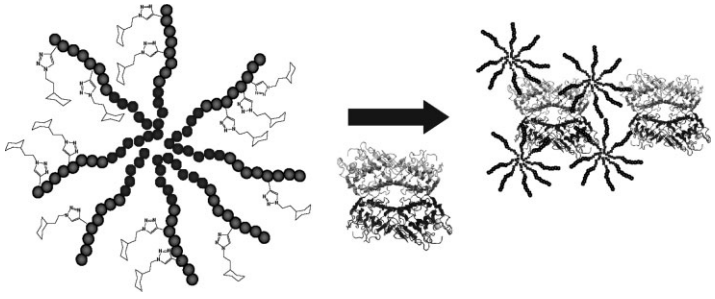
Neoglycopolymers Based on 4-Vinyl-1,2,3-Triazole Monomers Prepared by Click Chemistry[a]

Martin Hetzer, Gaojian Chen, Christopher Barner-Kowollik, Martina H. Stenzel*

The synthesis of a new glycomonomer based on mannose, prepared via CuAAC, is reported. The resulting 1,2,3-triazole linkage between mannose and the polymer backbone ensures the formation of highly stable glycopolymers, which will not undergo hydrolysis. The monomer 2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside was polymerized in the presence of a RAFT agent – 3-benzylsulfanylthiocarbonylsulfanyl propionic acid – to yield well-defined polymers with molecular weights up to 51 500 g mol^{-1} and a PDI of 1.16. The resulting polymer was employed as a macroRAFT agent in the polymerization of NIPAAm in order to generate thermo-responsive block copolymers, which undergo reversible micelle formation at elevated temperatures. The rapid interaction between the polymers prepared and ConA confirms the high affinity of these structures to proteins. While the linear glycopolymers already undergo a fast complexation with ConA, the reported rates have found to be exceeded by the micellar glycopolymer structure presented in the current contribution.



Introduction

Glycopolymers — synthetic polymers with pendant carbohydrates — have received increasing attention not only due to their potential biocompatibility and their good water-solubility. Glycopolymers are even more attractive as bioactive polymers that can readily interact with their biological environment.[1,2] The pendant sugar molecule has a strong affinity to specific proteins, which can result in the formation of stable complexes. An example of a bioactive sugar molecule is mannose, which strongly binds to Concanavalin A (ConA), a tetrameric lectin. This selective recognition is important for cellular signaling events, such as cell adhesion, proliferation, and survival.[3] Several studies have been performed on the binding interactions

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[a] Supporting information for this article is available at the bottom of the article's abstract page, which can be accessed from the journal's homepage at http://www.mbs-journal.de, or from the author.

DOI: 10.1002/mabi.200900199

between ConA and polymers[4–6] containing mannopyranoside repeating units.

Reflecting the rising importance of glycopolymers as materials at the interface between polymer science and biology is the increasing amount of synthesis approaches reported in literature.[7,8] Glycopolymers are obtained either by the polymerization of carbohydrates modified with vinyl functionalities or by the postmodification of reactive polymers as demonstrated on examples such as the Cu(I) catalyzed alkyne and azide 1,3-dipolar *click* cycloaddition (CuAAC), which uses azide containing carbohydrates to react to a polymer backbone[9] or the thiol-ene *click* reaction, which utilizes polymers with pendant vinyl functionalities and glucothiose, a sugar derivative with thiol functionality.[10,11] Most of these approaches have in common that the final polymer structure contain ester functionalities as a linking moiety between the polymer backbone and the pendant sugar group, which can potentially hydrolyze under alkaline conditions or in the presences of hydrolytically active enzymes.[12]

The quest for the polymer chemist is to find a synthetic procedure that is facile without taking recourse to protective chemistry, which also results in stable glycopolymers with high bioactivity. The bioactivity can be hampered by the conjugation of carbohydrates to the polymer backbone as has been demonstrated in the case of mannose. While mannose attached to a polymer backbone via the 1-position shows a strong binding activity towards ConA, poly(6-*O*-methacryloyl mannose) loses its activity.[13] Computational work suggests that free hydroxyl groups at the 3-, 4-, and 6-carbon positions of mannose dictate the binding ability of ConA.[14]

As pointed out above, many glycopolymers are susceptible to hydrolytic activity. To eliminate potentially labile ester functionalities, glycomonomers based only on hydrolytically stable functional groups are attractive in a range of applications. Reports on such glycomonomers, such as a styrene based glycomonomers[15] or polymers with attached mannose to the polymer backbone via amide bonds,[16] are rare.

A monomer class related to styrene are C-vinyl-heteroaromatic monomers such as 4-vinyl-1,2,3-triazole.[17] 4-Vinyl-1,2,3-triazole shows atypical polymerization kinetics such as non-linear concentration dependence of the rate of polymerization on the monomer concentration.[17] These types of monomer were almost forgotten in recent years, probably because of the elaborate synthesis procedure. With the rise of CuAAC,[18,19] 4-vinyl-1,2,3-triazol monomers experience a renaissance. Hawker and co-workers developed a one-pot and a two-step synthetic procedure based on *click* chemistry to generate 4-vinyl-1,2,3-triazole[20] leading to an array of *N*-functionalized-4-vinyl-1,2,3-triazoles at a fast reaction rate and with high conversions.[21] As depicted in Scheme 1, we adopted this facile synthesis procedure to generate novel glycomonomers based on mannose. Subsequently, reversible addition fragmentation chain transfer (RAFT) polymerization[22] was employed to create well-defined homo- and diblock copolymers, which can self-assemble into micelles. A major point of interest is the bioactivity of the prepared polymers. As mentioned earlier, the presence of the polymeric backbone may have a substantial influence on the selective binding between mannose and the protein ConA.

Experimental Part

Materials

Triethylamine (≥99%), vinyl bromide (98%), ethynyltrimethylsilane (98%), copper(I) iodine, bis(triphenylphosphine)palladium(II) dichloride (purum, ≥98.0%), 2-bromoethanol (95%), D-mannose (powder, cell culture tested), sodium azide (purum p.a., ≥99.0%), amberlite® IR-120, tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, THF), *N*-isopropylacrylamide (NIPAAm) (97%), 4,4′-azobis(4-cyanovaleric acid) (≥75%) were purchased from Sigma-Aldrich and used as received. Technical grade diethyl ether, sodium hydrogen sulfate, sodium hydrogen carbonate, sodium chloride, magnesium sulfate, petrolether 40/60, tetrabutylammonium hydrogen sulfate, magnesium sulfate, methanol, ethyl acetate, sodium ascorbate, copper(II) sulfate, tetrahydrofuran, ethyl acetate, and methanol were used without any further purification. The RAFT agent, 3-benzylsulfanylthiocarbonyltsufanyl propionic acid, was synthesized according to a procedure described elsewhere.[23]

Synthesis of 4-Trimethylsilyl-1-buten-3-yne

To a two-neck flask equipped with Dimroth condenser, 200 mL of dry triethylamine were added. The solution was cooled in an ice bath to 0 °C and degassed with nitrogen for 2 h prior the addition of vinyl bromide (14.89 g, 139.22 mmol) and trimethylsilylacetylene (7.92 g, 80.64 mmol). The colorless reaction mixture was stirred for a further 1 h at 0 °C and was subjected to a single freeze-pump-thaw cycle before the addition of copper(I) iodine (0.169 g, 0.887 mmol) and bis(triphenylphosphine)palladium(II) dichloride (0.257 g, 0.366 mmol). After two additional freeze-pump-thaw cycles, the light yellow reaction mixture was stirred overnight at room temperature and under constant nitrogen flow. Diethyl ether (200 mL) was added to the brown suspension and the organic layer was washed with ice cold 1 M $NaHSO_4$ (5 × 200 mL), saturated $NaHCO_3$ (1 × 200 mL) and saturated NaCl (1 × 200 mL). The ethereal layer was dried over $MgSO_4$, filtered, which gave a clear orange liquid, and concentrated through evaporation, which gave a brown crude product. The crude product was purified by column chromatography on silica gel with a diethyl ether/petrol ether mixture as eluent (1:1 v/v). The product solutions ($R_f = 0.88$) were evaporated as much as possible and the final product was separated with an oil pump into a receiving flask cooled to −78 °C yielding 2.35 g (23.34%) of a colorless liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 0.20$ (s, 9H, Si(CH_3)$_3$), 5.47 (d, $J = 11.1$ Hz, 1H, *trans* CH_2 = CH), 5.65 (d, $J = 2.4$ Hz, 1H, *cis* CH_2=CH),

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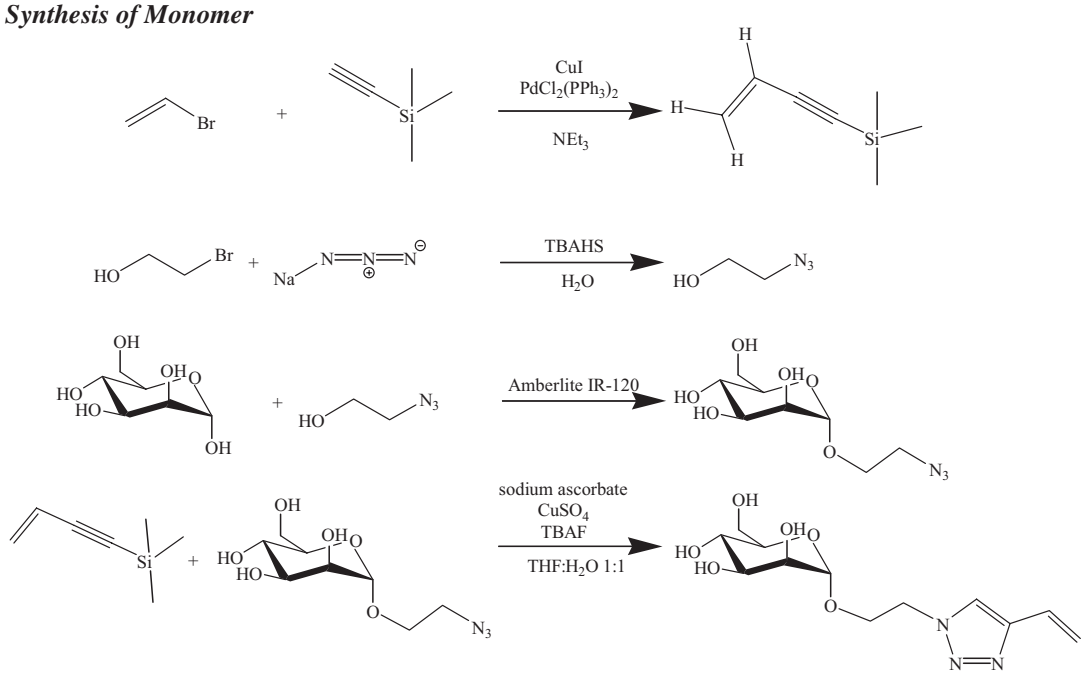
DOI: 10.1002/mabi.200900199



Scheme 1. Synthesis of 2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside and the subsequent RAFT polymerization to prepare thermo-responsive block copolymers.

5.82 (dd, $J = 17.6$ Hz, 11.1 Hz, 1H, $CH_2{=}CH$). ^{13}C NMR ($CDCl_3$, 300 MHz): $\delta = 0.25$, 95.4, 104.1, 117.6, 128.3.

Synthesis of 2-Azidoethanol

2-Bromoethanol (20.76 g, 0.166 mol), sodium azide (21.58 g, 0.332 mol), and tetrabutylammonium hydrogen sulfate (1.00 g, 2.95 mmol) were added to a round bottom flask filled with 40 mL distilled water. The reaction mixture was stirred for 2 d, giving a white suspension with orange droplets on the surface. Diethyl ether (150 mL) was added and the organic layer was washed with distilled H_2O (3 × 20 mL). The organic phases were collected, dried over $MgSO_4$ and filtrated. The reaction mixture was evaporated to dryness and the product further dried under vacuum yielding 6.08 g (21.03%) of a slightly yellow liquid.

^1H NMR ($CDCl_3$, 300 MHz): $\delta = 3.78$ (t, $J = 5.1$ Hz, 2H, $O{-}CH_2$), 3.45 (t, $J = 4.9$ Hz, 2H, $N_3{-}CH_2$).

CAUTION! Although we had never experienced any adverse events when working with these products, organic azides are potentially explosive compounds and they should be handled with utmost care!!

Synthesis of 2′-Azidoethyl-*O*-α-D-mannopyranoside

The procedure was described earlier by Haddleton et al.[24] Amberlite IR-120 (1.24 g) and 2-azidoethanol (6.08 g, 69.82 mmol) were added to a round bottom flask equipped with a condenser and heated to 90 °C. After 30 min D-mannose (1.24 g, 6.9 mmol) was added in a single portion. After the reaction mixture was stirred at 90 °C for a further 2.5 h, the suspension was filtered through a very short cotton wool pad and the filtered solid was washed with methanol. 3.5 g silica were added to the slightly yellow solution, stirred for 1 h and the remaining solvent removed through evaporation. The crude product dispersed on the silica gel was purified by column chromatography using an ethyl acetate/methanol mixture as eluent (19:1 v/v) to yield 0.86 g (50.6%) of a slightly yellow powder.

^{1}H NMR (300 MHz, D_2O): $\delta = 3.54$ (t, $J = 7.2$ Hz, 2H, N_3-CH_2), 3.65–3.99 (m, 8H), 4.92 (d, $J = 1.7$ Hz, 1H, anomeric H).

Synthesis of 2′-(4-Vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside

2′-Azidoethyl-*O*-α-D-mannopyranoside (0.73 g, 2.93 mmol), sodium ascorbate (0.0960 g, 0.485 mmol), $CuSO_4 \cdot 5\ H_2O$ (0.0583 g, 0.234 mmol), 4.4 mL of tetrabutylammonium fluoride (1 M in THF) and 4-tetramethylsilyl-1-buten-3-yne (0.47 g, 3.73 mmol) were weighed into a small round bottom flask, which was filled up with 4.4 mL of distilled water. The flask was fitted with a rubber septum and the solution was allowed to stir for 2 d. The now dark brown reaction mixture was transferred into a round bottom flask. THF was removed through evaporation and the remaining solution freeze-dried overnight. The crude monomer was purified by column chromatography on silica gel with ethyl acetate/methanol mixture as eluent (19:1 v/v) in order to obtain 0.332 g (36.5%) of a slightly orange solid.

^{1}H NMR (300 MHz, D_2O): $\delta = 3.6$–3.72 (m, 4H, H4, H3, H6), 3.86 (m, 1H, H5) 3.92 (m, 1H, H2), 4.09 (m, 2H, O$-CH_2-CH_2$-triazole), 4.63 (m, 2H, O$-CH_2-CH_2$-triazole), 4.86 (s, 1H, anomeric H), 5.42 (d, $J = 10.4$ Hz, 1H, *trans* CH_2=CH), 5.90 (d, $J = 17.8$ Hz, 1H, *cis* CH_2=CH), 6.70 (dd, $J = 29.1$ Hz, 11.3 Hz, 1H, CH_2=CH), 8.06 (s, 1H, triazole). ^{13}C NMR (300 MHz, D_2O): $\delta = 51.8$ (CH_2 CH_2N), 62.4 (CH_2CH_2N), 67.3 (C4), 68.1 (C6), 71.5 (C3), 72.1 (C5), 74.5 (C2), 101.2 (anomeric C), 119.0 (CH_2=CH), 124.7 (C$-CH_2-$N, triazole), 126.4 (CH_2=CH), 148.0 (C$-CH_2-$N, triazole).

ESI-MS $[C_{12}H_{19}N_3O_6Na]^+$: m/z (theo) = 324.12, m/z (exp) = 324.39;

FT-IR: 3320, 2923, 1641, 1360, 1228, 1133, 1090, 1049, 1027, 976, 917, 873, 806, 670 cm^{-1}.

Synthesis of Poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside) via RAFT Polymerization

Homopolymerisations of 2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside (1.106 mol · L^{-1}) were performed using 4,4′-azobis(4-cyanovaleric acid) (ACPA), (1.38×10^{-3} mol · L^{-1}) as the initiator and 3-benzylsulfanylthiocarbonylsulfanyl propionic acid as chain transfer agent (2.77×10^{-3} mol · L^{-1}). Typically, the monomer (100 mg, 0.3319 mmol) was dissolved in 0.1 mL of distilled water and mixed with 0.1 mL of ACPA (0.116 mg, 4.149×10^{-4} mmol) also dissolved in water and RAFT agent (0.226 mg, 8.298×10^{-4} mmol) dissolved in methanol. The reaction mixture was transferred to a Schlenck tube and thoroughly deoxygenated by three consecutive freeze-pump-thaw cycles. The reaction mixture was then transferred into an IR-cuvette and purged with nitrogen for a further 45 min, before being placed in the FT-IR for a 24 h polymerization at 60 °C. The molecular weight of the resulting polymer after 24 h was measured to be $\overline{M}_n = 58\,500$ g · mol^{-1} by NMR and $\overline{M}_n = 51\,500$ g · mol^{-1} by GPC with a polydispercity index (PDI) of 1.16.

Synthesis of Poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside)-*block*-poly(*N*-isopropyl acrylamide) via RAFT Polymerization

NIPAAm (32 mg, 2.83×10^{-4} mol) and macroRAFT agent (9 mg, 2.3×10^{-7} mol) were dissolved in 0.5 mL *N*,*N*-dimethylacetamide (DMAc). A stock solution of 2.95 mg 2,2′-azoisobutyronitrile (AIBN) in 2 mL *N*,*N*-dimethylformamide (DMF) was prepared. 20 μL of this solution was added to the monomer macroRAFT agent mixture to yield a concentration ratio of [NIPAAm]:[macroRAFT]:[AIBN] = 1 200:1:0.5. The vial was sealed and thoroughly degassed via freeze-pump thaw and heated for 24 h at 60 °C. The mixture was then dialysed against distilled water (molecular weight cut-off 10 kDa) and freeze-dried. The molecular weight of the resulting polymer was measured to be $\overline{M}_n = 32\,400$ g · mol^{-1} (PDI = 1.12). From NMR, the composition of the polymer was calculated to be poly-(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside)$_{129}$-*block*-poly(*N*-isopropylacrylamide)$_{93}$.

Turbidimetry Assay

A solution of 1 μM ConA in pH 7.4 HEPES-buffered saline (HBS) was made fresh before the assay. Turbidity measurements were performed by adding 500 μL of the ConA solution to a dry quartz microcuvette and put into the holder of a Cary 300 UV-VIS Spectrophotometer at a certain temperature for 1 min. A solution of the ligand in HBS buffer was then added (50 μL at 500 μM per mannose residue). Upon addition, the solution was mixed vigorously for 5 s using a pipette. Absorbance data were recorded at 420 nm for 10 min at 1.2 Hz.

NMR Spectroscopy

All NMR spectrums were recorded using a Bruker 300 MHz spectrometer. Deuterated chloroform, deuterium oxide or deuterated dimethyl sulfoxide were used as solvent.

Fourier Transform Near-Infrared (FT-NIR) Spectroscopy

Reaction mixtures were deoxygenated by purging with nitrogen for 30 min. Monomer conversions were determined via online FT-NIR spectroscopy by observing the decrease of the vinylic

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DOI: 10.1002/mabi.200900199

stretching overtone of the monomer at $\nu = 6\,165\,cm^{-1}$. A Bruker IFS\S Fourier transform spectrometer equipped with a tungsten halogen lamp, a CaF_2 beam splitter and a liquid nitrogen cooled InSb detector were used for the FT-NIR measurements. The spectra were recorded in the spectral region of 8 000–4 000 cm^{-1} and were obtained from the added interferograms of 16 scans with the resolution of 4 cm^{-1}. The conversion was determined by selecting a linear baseline between 6 193.05 and 6 130.30 cm^{-1}. The absorbance was integrated between the two points and monomer to polymer conversions were calculated via Beer-Lambert's law.

Size Exclusion Chromatography (SEC)

Molecular weight distributions were determined by SEC with a Shimadzu modular system having 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 mm × 7.8 mm) followed by four 300 mm × 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 3 000 to 10^6 g · mol^{-1}.

Dynamic Light Scattering (DLS)

The hydrodynamic diameters (D_h) of the aggregates were determined using a Malvern Nano-Zetasizer. The mean diameter was obtained from the arithmetic mean using the relative intensity of each particle size. The solution (0.5 g · L^{-1}) was prepared in distilled water and was filtered through 0.45 μm filters before analysis. Samples were incubated in the holder for 5 min at certain temperatures before the instrument start to measure the particle size.

Electrospray Ionization Mass Spectrometry (ESI-MS)

ESI-MS measurements were performed on a Thermo Finnigan LCQ Deca quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electrospray mode which was used in positive ion mode. Monomer samples (0.1–0.2 mg · mL^{-1}) were dissolved in 6:4 v/v mixture of methanol/water. Mass calibration was performed using caffeine, Met-Arg-Phe-Ala acetate salt (MRFA, Sigma-Aldrich) and Ultramark 1621 (Sigma-Aldrich) in the *m/z* range 195–1 822. All spectra were acquired within the *m/z* range of 150–2 000 with a spray voltage of 5 kV, a capillary voltage of 39 V, and a capillary temperature of 275 °C. Nitrogen was used as a sheath gas (flow: 40% of maximum) and helium was used as the auxiliary gas (flow: 5% of maximum). The instrumental resolution of the employed experimental set-up is 0.2 amu.

Results and Discussion

Several pathways are available to generate effectively 4-vinyl-1,2,3-triazole monomers.[20,21] Key is the formation of 1,2,3-triazole derivatives by an efficient and highly stereoselective process, the Cu(I) catalyzed *click* reaction between alkynes and azides. *Click* reactions are *generally* fast reactions frequently leading to complete conversions. The introduction of the vinyl functionality in 4-position can be achieved using Wittig reactions or by applying elimination steps.[21] However, the presence of mannose hampered the success of these reactions leading potentially to the destruction of the sugar molecule and to a broad array of products. In an alternative strategy, the synthesis of the proposed glycomonomer, 2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside, was carried out using the procedure outlined in Scheme 1. The synthesis of 4-trimethylsilyl-1-buten-3-yne was observed to be the most critical step. The volatility of the reactants limit the yield of the reaction resulting in fluctuating outcomes with yields as low as 23%. The following Cu(I) catalyzed reaction of 4-trimethylsilyl-1-buten-3-yne with 2′-azidoethyl-*O*-α-D-mannopyranoside lead to a dark brown product, from which – upon purification via column chromatography – the final product can be isolated at high purity. The yield of only 36% is not an indication of the inefficiency of the click reaction, but more the result of thorough purification of the resulting monomer with some monomer being lost on the column. ^{1}H-NMR analysis confirms the structure of the monomer and the high regio-selectivity of the *click* reaction as evidenced by the single peak at $\delta = 8.06$ ppm corresponding to the 1,2,3-triazole ring (see electronic supporting information). The molecular weight of the monomer was measured via ESI-MS analysis and is in close agreement with the theoretical value (see electronic supporting information).

The following quest for a suitable RAFT agent for the controlled polymerization of the monomer synthesized was facilitated by earlier studies on reactivity ratios of the copolymerization of 4-vinyl-1,2,3-triazole with styrene and methyl methacrylate, respectively.[17] The reactivity ratios suggest that these types of monomers have reactivities comparable to acrylates and acrylamides. Both types of monomers were successfully polymerized using trithiocarbonate-type RAFT agents. Therefore, 3-benzylsulfanylthiocarbonylsulfanyl propionic acid was employed as the chain transfer agent in a mixture of monomer and 4,4′-azobis(4-cyanovaleric acid) as initiator in a water/ethanol mixture. The rate of polymerization was subsequently followed by the declining intensity of the vinylic bond using FT-NIR. A monomer conversion of 47% was reached after a polymerization time of 24 h (see electronic supporting information). It is recommended for further block copolymer synthesis to stop the polymerization at an early stage to ensure a high percentage of RAFT endgroups. High monomer conversions can lead to the formation of dead polymer, thus the formation of bimodal distributions in the subsequent block copolymer synthesis.

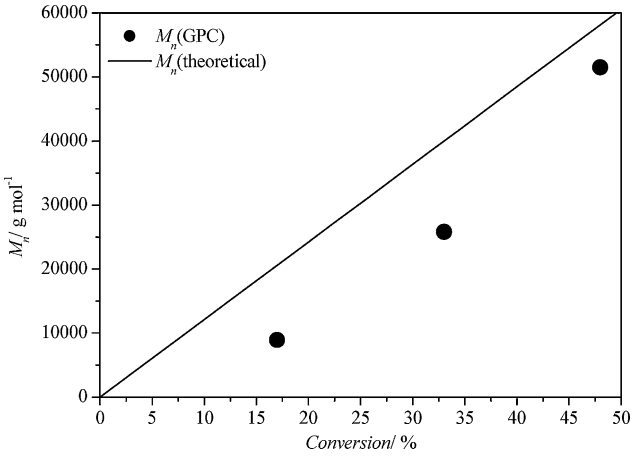

Figure 1. Molecular weight versus conversion of the polymerization of 2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside in water/methanol in the presence of 3-benzylsulfanylthiocarbonylsulfanyl propionic acid as RAFT agent. $[M]_o = 1.106\ mol \cdot L^{-1}$, $[ACPA] = 1.38 \times 10^{-3}\ mol \cdot L^{-1}$, $[RAFT] = 2.77 \times 10^{-3}\ mol \cdot L^{-1}$.

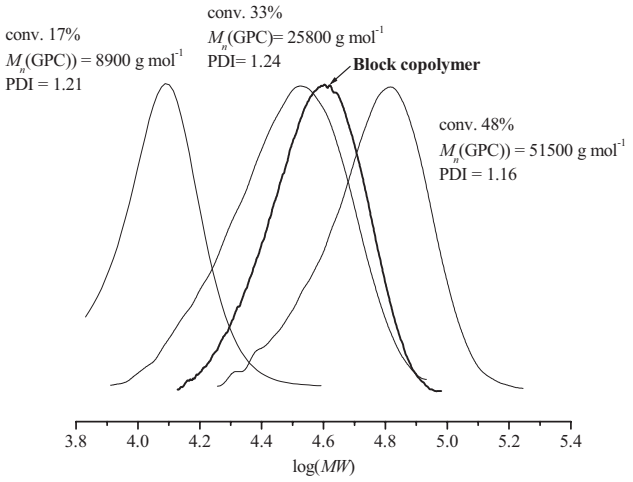

Figure 2. GPC curves of poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside) and poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside)$_{129}$-*block*-poly(*N*-isopropyl acrylamide)$_{93}$ prepared via RAFT polymerization.

The living characteristic of the polymerization was validated by confirming the linear relationship between conversion and molecular weight. Separate samples were prepared and isolated at a preset reaction time. The molecular weight was observed to increase with conversion in close proximity to the calculated values. Deviations from the theoretical molecular weight are assigned to the polystyrene calibration of the GPC system (Figure 1).

In addition, the molecular weight distributions were found to be narrow without any shoulder formation or significant low molecular weight tailing, which would be indicative of substantial termination reactions (see Figure 2). It can therefore be concluded that trithiocarbonates can control the polymerization of 4-vinyl-1,2,3-triazole monomers effectively.

The presence of the RAFT endgroup can be verified via chain extension and UV/Vis analysis after intense purification of the polymer using dialysis. It should be noted here that the RAFT endgroup can potentially undergo hydrolysis in aqueous solution at elevated pH values.[25] Suitable pH values for the dialysis of RAFT polymers can be obtained by the presence of CO_2 in the water. The presence of RAFT endgroups were also confirmed via UV/Vis analysis employing the peak maximum at 305 nm, which correlates to the $\pi-\pi^*$ absorption of the yellow RAFT agent. The UV/Vis spectrum of the polymer has been compared to the spectrum of the RAFT agent confirming the presence of the RAFT group as a terminal unit at each polymer chain.

The existence of the RAFT endgroup is the key to the successful formation of block copolymers.[26] Poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside) prepared via RAFT polymerization was chain extended using NIPAAm in order to prepare thermo-responsive block copolymers. A glycopolymer based macroRAFT agent with the molecular weight of 25 800 $g \cdot mol^{-1}$ was therefore polymerized in the presence of NIPAAm in DMA to yield a block copolymer with a number average molecular weight of 32 400 $g \cdot mol^{-1}$. As displayed in Figure 2, the resulting block copolymer was found to have a symmetric molecular weight distribution with a PDI of 1.12. The thermoresponsive block copolymer was further investigated using DLS. Both blocks are fully water-soluble at room temperature displaying therefore with 10.1 nm only the size of a unimers (a single block copolymer) in the DLS analysis. At the elevated temperature of 40 °C above the lower critical solution temperature (LCST) of NIPAAm (32 °C), the block copolymer takes on an amphiphilic character resulting in the formation of micelles indicative of the increased hydrodynamic diameter D_h of 22.5 nm (see the supporting information). Upon cooling of the solution, the micelles start to dissociate again.

The purpose of the current study is the development of a stable glycopolymers with pendant sugar groups of high bioactivity. As mentioned earlier, the presence of the polymer backbone can hamper the binding of mannose to ConA. Mannose containing compounds are known to prevent the clustering of red blood cells, which is caused by the strong binding between surface carbohydrates on these cells and ConA, since mannose will compete for the binding sites on ConA.[27] While the binding between one mannose molecule and ConA is rather weak, the combined effect of multiple mannose ligands increases the affinity to ConA significantly. It is not only the amount of mannose that determines the binding to ConA. The affinity to ConA can furthermore be enhanced by carefully chosen polymer architectures.[27] To test the binding ability of the

Macromol. Biosci. **2010**, *10*, 119–126

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DOI: 10.1002/mabi.200900199

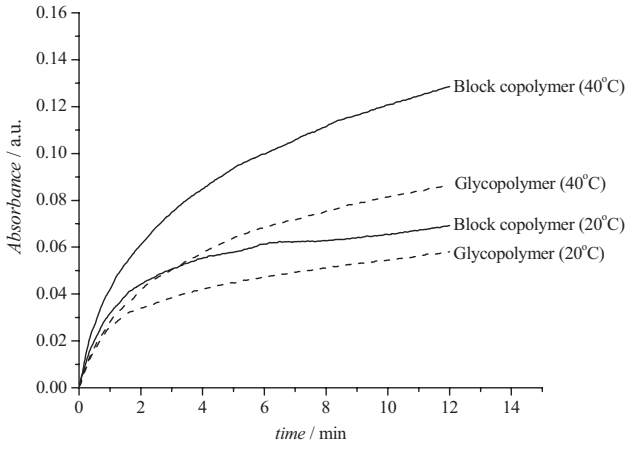

Figure 3. Turbidity measurements via UV/Vis spectroscopy to monitor the cluster formation between poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside)$_{129}$ or poly-(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside)$_{129}$-*block*-poly(*N*-isopropyl acrylamide)$_{93}$ and ConA at different temperatures.

synthesized glycopolymer to ConA, a solution of polymer was mixed with ConA. With the interaction between ConA and the glycopolymer big clusters are formed, which will significantly scatter light. The subsequent turbidity of the solution – monitored via UV/Vis spectroscopy – is an indication of occurring binding events. As seen in Figure 3, the binding of the homopolymer to ConA takes place within a few seconds. Elevated temperature accelerated the reaction slightly. Interestingly, the reaction rate of the block copolymer at 40 °C is faster than the block copolymer. The rate and turbidity is exceeding all the other structures. It needs to be considered that at 40 °C, which is above the LCST of PNIPAAm, the formation of micelles occurs. As a result, the glycopolymer in the micelle takes on a more brush-like conformation, while unimeric chains in contrast are expected to take on a coil structure. It has been reported earlier that rigid scaffolds for sugars are entropically favorable to enhance binding.[28] The binding between a mannose containing polymer and ConA can be reversed by adding excess 1-methyl-D-mannopyranoside to the solution, which acts as a competitive ligand.[29] 1-methyl-D-mannopyranoside was therefore added to the turbid solution of poly(2′-(4-vinyl-[1,2,3]-triazol-1-yl)ethyl-*O*-α-D-mannopyranoside) and ConA. An immediate decline in turbity was observed confirming that binding between polymer and ConA was specifically caused by the presence of mannose (see ESI). The specific interaction between the glycopolymer prepared and ConA was in addition tested by using PNA, a lectin from *Arachis Hypogaea*, which is usually selective to galactose. Mixing polymer and PNA does not result in increased turbity, interaction between the polymer and other proteins is therefore absent (see ESI).

Conclusion

In conclusion, we demonstrated in this communication that *click* chemistry is not only a versatile tool for the design of complex polymer architectures, but it can also be employed to generate bioactive and stable glycomonomers. The resulting glycomonomers can be polymerized via RAFT using a RAFT agent that creates an intermediate radical of medium stability such as trithiocarbonates. The resulting polymers bind readily to proteins, therefore the binding ability is not hampered by the backbone.

Acknowledgements: M. H. S. and C. B.-K. acknowledge the *Australian Research Council* (ARC) for generous funding. The authors thank Dr. *Sadik Amajjahe* for helpful discussions. The authors thank Mr. *Edgar Wong* for the ESI-MS measurements and Mr. *Steve Jacenyik* for the excellent management of CAMD. M. H. S acknowledges the *University of New South Wales* (UNSW) for financial support. C. B.-K. acknowledges funding from the *Karlsruhe Institute of Technology* (KIT) in the context of the *Excellence Initiative* for leading German universities. M. H. thanks the *Dr. Jost Henkel Stiftung* for a scholarship.

Received: June 5, 2009; Revised: July 10, 2009; Published online: September 3, 2009; DOI: 10.1002/mabi.200900199

Keywords: click chemistry; glycopolymer; micelles; reversible addition fragmentation chain transfer (RAFT)

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Macromolecular Journals
DOI: 10.1002/mabi.200900199