Synthesis of Various Glycopolymer Architectures via RAFT Polymerization: From Block Copolymers to Stars

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Well-defined linear poly(acryloyl glucosamine) (PAGA) exhibiting molar masses ranging from 3 to 120 K and low polydispersities have been prepared via reversible addition-fragmentation chain transfer polymerization (RAFT) in aqueous solution without recourse to protecting group chemistry. The livingness of the process was further demonstrated by successfully chain-extending one of these polymers with *N*-isopropylacrylamide affording narrow dispersed thermosensitive diblocks. This strategy of polymerization was finally extended to the preparation of glycopolymer stars from Z designed non-water-soluble trifunctional RAFT agent. After the growth of very short blocks of poly(hydroxyethyl acrylate) ($\overline{DP}_{n\text{branch}} = 10$), AGA was polymerized in aqueous solution in a controlled manner affording well-defined 3-arm glycopolymer stars.

Introduction

Synthetic polymers containing carbohydrate pendant groups, referred to as glycopolymers, are emerging as potentially important materials for a number of applications in medicine and biotechnology notably due to the essential mediating role played by carbohydrates in a wide range of biomolecular recognition events. Because the recognition processes have been proven to be cooperative[1,2] and consequently strongly depend on the spatial distribution of the carbohydrate moieties, synthetic routes affording complex glycopolymer architectures that exhibit well-defined macromolecular structures have been extensively investigated.[3,4] The preparation of linear block or comb-like copolymers,[5,6] hyperbranched glycopolymers,[7] glycodendrimers[8,9] or glycopolymer stars has yet been reported.[10] Glycopolymers with predetermined molar masses and narrow polydispersities have been synthesized via living ionic polymerization,[11−16] ring opening polymerization,[17−19] or ring opening metathesis polymerization.[20−22] Nevertheless, severe disadvantages are associated with these traditional techniques. Indeed, ionic polymerization processes, which are very sensitive to the presence of acidic protons or strongly electrophilic functionalities, require drastic anhydrous reaction conditions and protection of the hydroxy functions while the scope of ring opening metathesis polymerization is strictly limited to strained monomers. Owing to their high compatibility with functional groups, the recent advent of the controlled/living free radical polymerization techniques, such as nitroxide-mediated polymerization (NMP),[23] atom transfer radical polymerization (ATRP),[24,25] or reversible addition-fragmentation chain transfer (RAFT),[26,27] has opened new prospects allowing the preparation of a wide range of tailored glycopolymer architectures.[28−40] However, despite the increasing attention devoted to this family of polymers, very few examples of well-defined glycopolymers have actually been reported without recourse to protecting group chemistry. Narain et al.[41,42] have described the successful preparation of well defined glycopolymers by polymerization of 2-glucoamidoethyl methacrylate and 2-lactobionamidoethyl methacrylate via ATRP. Taking advantage of the very low sensitivity of the RAFT process toward functionalities, Lowe et al.[43] have contributed to the subject by describing the aqueous controlled polymerization of the commercially available 2-methacryloxyethyl glucoside employing a water-soluble dithioester as the RAFT chain transfer agent (CTA) while CAMD has developed low-dispersity poly(6-*O*-vinyladipoyl-D-glucopyranose)[10,44] and poly(methyl 6-*O*-methacryloyl-α-D-glucoside)[45] using xanthate, dithiocarbamate, and dithioester CTAs, respectively. In the present study, we report the synthesis of a suite of well-defined glycopolymer architectures (linear and star polymers) based on the direct "living" polymerization of acryloyl glucosamine (AGA) in aqueous media via trithiocarbonate mediated reversible addition-fragmentation transfer polymerization. The carbohydrate molecule chosen possesses similarities with galactose amine, a sugar moiety used for the molecular recognition of asialoglycoprotein receptor (ASGPR) positive cells, which are largely expressed in hepatocytes.[46] Galactose amine and glucose amine are structural isomers and differ only by the position of the hydroxygroup in 4 position. While Glucose amine does not show the same recognition in biological systems as galactose amine the polymerization should not be affected by this difference.

The homopolymerization of NIPAM using PAGA as macro RAFT resulting in the formation of thermosensitive diblock copolymers is described. Aiming at preparing original hydrophilic sugar-based architectures, the elaboration of glycopolymer and block copolymer stars from a trithiocarbonate trifunctional macromolecular RAFT agent is finally investigated.

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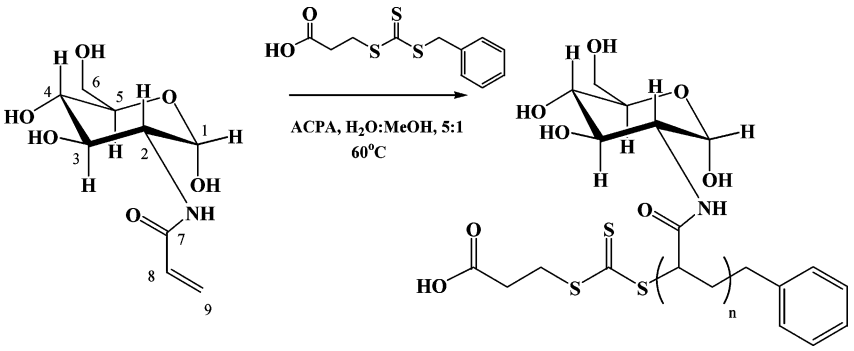
Scheme 1. Aqueous Polymerization of AGA



Experimental Part

Materials. D-Glucosamine hydrochloride (Aldrich, 98%), acryloyl chloride (Aldrich, 96%), 4,4′-azobis(cyanopentanoic acid) (ACPA, Fluka, 98%), sodium carbonate (Chem-Supply, 99.8%), sodium hydrogen carbonate (Ajax Finechem, 99.7%), 2-mercaptopropionic acid (Aldrich, 99%), benzyl bromide (Aldrich, 98%), *N*-isopropylacrylamide (NIPAAm) (Aldrich, 97%), 1,1,1-tris(hydroxymethyl)propane (Aldrich, 99%), 4,4′-azobis(cyanopentanoic acid) (ACPA) (Fluka, 98%), and carbon disulfide (Aldrich, 99,9%) were used without further purification. 2-Hydroxyethyl acrylate (HEA) (Aldrich, 96%) passed through a column of basic aluminum oxide to remove inhibitors. Column chromatography was performed using silica gel (Kiesigel-60, Fluka). All solvents were HPLC grade (Asia Pacific Specialty Chemicals). 3-Benzylsulfanylthiocarbonyl sulfanylpropionic acid (**I**) was prepared as previously described[47] from mercapto propionic acid, carbon disulfide, and benzyl bromide.

Trifunctional RAFT agent (**II**) (Scheme 3) was prepared from **I** and 1,1,1-tris(hydroxymethyl)ethane according to the procedure reported by Hao et al.[48]

Synthesis of Acryloyl Glucosamine (AGA). Acryloyl glucosamine was synthesized as previously reported[49] from D-glucosamine hydrochloride and acryloyl chloride with only minor changes. Typically, 8.48 g of Na_2CO_3 (0.080 mol), 6.72 g of $NaHCO_3$ (0.079 mol), and 17.4 g of glucosamine hydrochloride (0.080 mol) were dissolved in 40 mL of water. The aqueous solution was subsequently cooled in an iced bath. Acryloyl chloride (8 g, 0.088 mol) was added dropwise under vigorous stirring. The temperature was maintained at 0 °C for 2 h and then slowly warmed to room temperature for 1 day. The mixture was further dried under vacuum and freeze-dried. The crude glycomonomer was purified by column chromatography on silica gel with a methanol/ethyl acetate mixture as eluent (20/80 v/v). AGA was finally recrystallized twice in a methanol/ethyl acetate mixture (20/80 v/v) at 4 °C and characterized (NMR, ESI-MS).

White powder, 3.6 g, Yield: 20%. ESI-MS: calculed for $C_9H_{15}NO_6$ (AGA) + Na^+, 256.08; found 256.1. ^{1}H NMR (D_2O) δ (ppm): 6.09−6.24 (H8, H9, anti), 5.67−5.68 (H9, syn), 5.10−5.11 (H1) and 4.63−4.64 (H1), 3.34−3.86 (H2, H3, H4, H5, H6).

Polymerizations. *(a) Preparation of Poly(hydroxyethyl acrylate) (PHEA) 3-Arm Star (Water-Soluble Trifunctional Macromolecular RAFT Agent) (III).* HEA (1.15 mL, 1×10^{-2} mol) was introduced in a Schlenk tube and mixed with a DMSO solution (3 mL) containing 100 mg of **II** (3.34×10^{-4} mol of RAFT functions) and 10.9 mg of AIBN (6.68×10^{-5} mol). The tube was sealed with a rubber septum and degassed with five freeze−pump−thaw cycles and finally transferred to a water bath at 60 °C. The polymerization was stopped by cooling the solution in iced water. After reaction, the polymer was precipitated several times in cyclohexane and finally dried under vacuum.

Yellow paste: 33% of conversion (determined by ^{1}H NMR from relative integration of the peaks corresponding to the protons of the vinyl group and of the polymer backbone). ^{1}H NMR: $\overline{M_n}$ = 4470 g·mol^{-1}, $\overline{DP_{n\ branch}}$ = 10; GPC (DMAc): $\overline{M_n}$ = 3370 g·mol^{-1}; PDI = 1.07. ^{1}H NMR (DMSO-*d*6) δ (ppm): 0.75−0.90 (3H, H-13), 1.2−1.9 (PHEA backbone C*H*$_2$−CH−CO + 2H, H-12), 2.1−2.4 (PHEA backbone CH$_2$−C*H*−CO), 2.7−2.8 (6H, H-8), 3.4−3.6 (PHEA, CH$_2$−*CH*$_2$−OH + 6H, H-7), 3.9−4.1 (PHEA, *CH*$_2$−CH$_2$−OH + 6H, H-10), 7.1−7.4 (15H, Ph).

(b) Linear Glycopolymers. Homopolymerizations of AGA (0.715 mol·L^{-1}) were performed using 4,4′-(azobis(cyanopentanoic acid) ($7.14 \cdot 10^{-4}$ mol·L^{-1}) as the initiator and the trithiocarbonate agent (**I**) given in Scheme 1 as chain transfer agent (CTA) (1.78, 3.57, and 7.14 $\times$ 10^{-3} mol·L^{-1}).

Typically, the aqueous monomer solution (2.5 mL, 2.14×10^{-3} mol) was mixed with a 0.5 mL ethanol solution of ACPA (0.6 mg, 2.14×10^{-6} mol) and **I** (2.91 mg, 1.073×10^{-5} mol) and transferred to Schlenk tubes which were thoroughly deoxygenated by five consecutive freeze−pump−thaw cycles. The tubes were then placed in a constant temperature water bath at 60 °C. Aliquots of solution (250 μL) were drawn at regular intervals (1.5, 3, 4, 5, 6, and 7 h) from the reaction mixture using a gastight syringe purged with nitrogen. The samples were subsequently plunged into iced water and finally freeze-dried overnight. The crude polymer was finally characterized (1H NMR, gel permeation chromatography).

The conversions were determined by ^{1}H NMR in D_2O by relative integration of vinyl (nonreacted AGA, δ = 5.67−6.24) and CH$_2$−CH glycopolymer backbone protons (δ = 1.3−2.5). Identical results were found with different times of relaxation (t = 1 and 10 s).

The crude homopolymers (PAGA) were subsequently solubilized in water, precipitated twice in methanol to remove nonreacted AGA and finally dried.

The molecular weights of the glycopolymers were finally evaluated by ^{1}H NMR in D_2O from relative integration of the protons of the glycopolymer backbone (C*H*$_2$−C*H*, 3nH, δ = 1.3−2.5 ppm, with n being the degree of polymerization) and of aromatic protons of the benzyl (R group) chain end (5H, δ = 7.24−7.40 ppm) using the following equation:

$$\overline{M_n} = rm_{AGA} + m_{RAFT}$$

with $r = (I_{CH_2-CH}/3)/(I_{Benzyl}/5)$; m_{AGA} and m_{RAFT} are the molar mass of glycomonomer and RAFT agent; I_{CH_2-CH} and I_{Benzyl} are the ^{1}H integration of the glycopolymer backbone and benzyl chain end.

The polymerization of AGA in the presence of **III** was performed following exactly the same procedure.

^{1}H NMR (D_2O) δ (ppm): 1.3−2.5 (3H, H8, H9), 3.3−4.2 (6H, H2, H3, H4, H5, H6), 4.6−4.8 (H1 overlapping with D_2O), 5.1−5.4 (H1), 7.24−7.40 (5H, Phenyl).

See the Supporting Information for NMR spectra

(c) Linear Thermosensitive PAGA-b-PNIPAAm Copolymers. NIPAAm (0.268 g, 2.37×10^{-3} mol) and $PAGA_{180}$ (0.25 g, 5.92×10^{-6} mol) (similar conditions of reaction as run 2, $\overline{M_{nNMR}}$ = 42 200 g·mol^{-1}; $\overline{M_{nGPC}}$ = 52 480 g·mol^{-1}; PDI = 1.10) were dissolved in 1.5 mL of water. After complete dissolution of the macro-RAFT agent, ACPA (0.6 mg, 2.14×10^{-6} mol) was added as DMSO solution (1.5 mL).

The solution was transferred to a Schlenk tube which was thoroughly deoxygenated by five consecutive freeze−pump−thaw cycles. The tube was then placed in a constant temperature water bath at 60 °C. Aliquots of solution (250 μL) were drawn at regular intervals (1, 2, 3, and 4h) from the reaction mixture using a gastight syringe purged with nitrogen. The conversion of NIPAAm was determined by ^{1}H NMR (DMSO-*d*6) from relative integration of vinyl (nonreacted NIPAAm, $\delta = 5.5-6.2$) and H1 glycopolymer protons ($\delta = 4.4-5.4$).

The samples were subsequently plunged into iced water, dialyzed against pure water for 2 days and finally freeze-dried overnight.

The molecular weight of the resulting block copolymer was evaluated by ^{1}H NMR (D_2O) from relative integration of the peaks at 3.3−4.2 ppm corresponding to H2, H3, H4, H5, H6 (PAGA) + PNIPAAm block: 1H, NH−*CH*−CH_3 and 1.07 ppm (PNIPAAm block: 6H, C*H*$_3$−CH−).

^{1}H NMR (D_2O) δ (ppm): 1.07 (PNIPAAm block: 6H, C*H*$_3$−CH−), 1.3−2.5 (PAGA block: 3H, H8,H9; PNIPAAm block: 3h, −C*H*$_2$−CH−CO and −CH_2−C*H*−CO), 3.3−4.2 (PAGA block: 6H, H2, H3, H4, H5, H6; PNIPAAm block: 1H, NH−*CH*−CH_3), 4.6−4.8 (H1 overlapping with D_2O), 5.1−5.4 (H1).

Characterization. ^{1}H NMR spectra were recorded on a Bruker spectrometer (300 MHz) in D_2O. Gel permeation chromatography (GPC) analysis of glycopolymers was performed in *N*,*N*-dimethylacetamide (DMAc) (0.03%w/v LiBr, 0.05% BHT) at 40 °C (flow rate:1 mL/min) using a Shimadzu modular system comprising a DGU-12A solvent degasser, a LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector. The system was equipped with a 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). Calibration was performed with narrow polydispersity polystyrene standards ranging from 500 to 10^6 g/mol.

Electrospray mass spectroscopy (ESI-MS) experiments were carried out using a Thermo Finnigan LCQ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with an atmospheric pressured-ionization source operated in nebulizer-assisted electrospray mode (ESI). The instrument was calibrated with caffeine (Aldrich), MRFA (Thermo Finnigan), Ultramark 1621 (Lancaster), and poly(propylene glycol) (Aldrich, $\overline{M_n}$ = 2700 g·mol^{-1}) in the mass range 195−3822 amu. All spectra were acquired in positive ion mode over the *m*/*z* range 100−1000 with a spray voltage of 5 kV, a capillary voltage of 35 V, a tube lens offset of −30 V, and a capillary temperature of 275 °C (syringe infusion). Nitrogen was used as sheath gas at a flow rate of 0.5 L·min^{-1}, whereas helium was used as the auxiliary gas. The eluent was a 9:1 v/v mixture of H_2O/acetonitrile (1wt % of sodium formate).

Results and Discussion

Synthesis of Linear Homoglycopolymers. Kinetic studies on the homopolymerization of the glycomonomer (AGA) via the RAFT process were conducted in water-ethanol mixtures (5:1; v:v) in the presence of 3-benzylsulfanylthiocarbonylsulfanylpropionic acid (**I**) as CTA, with ethanol ensuring the solubility of the CTA in the solution, see Scheme 1. The homopolymerization of AGA was investigated at three different concentrations of **I** (1.78×10^{-3}, 3.57×10^{-3}, and 7.14×10^{-3} mol·L^{-1}) at 60 °C in the presence of ACPA (7.14×10^{-4} mol·L^{-1}). Conversion versus time plots (runs 1−3) for the polymerization of AGA mediated with **I** are given in Figure 1. After an initial period of inhibition depending on the CTA concentration (up to 3 h with the highest concentration of RAFT agent, run 3), the homopolymerization of AGA was proven to proceed with pseudo-first-order kinetics consistent with a constant radical concentration. Given the low concentration in monomer (~ 0.71 M), the polymerization rates appeared to be quite fast (89.5 and 71.4% in 7h for the runs 1 and 2 respectively). It is worth noting that, although runs 1 and 2 exhibited high polymerization rates, a significantly higher polymerization inhibition was induced by run 3 which was conducted with the highest concentration of CTA (Figure 1). Extended inhibition periods at higher RAFT agent concentrations might be caused by slow fragmentation of the intermediate of the initial addition step of the macroradical onto the RAFT agent. Additionally, the stability of the leaving group determines the reinitiation, thus, can delay the onset of the polymerization.[50]

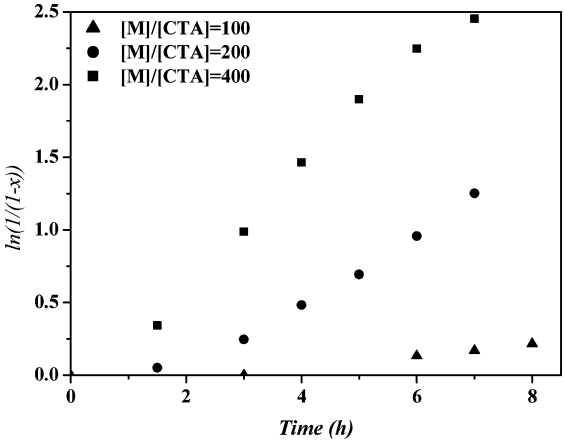


Figure 1. Pseudo-first-order plots obtained by ^{1}H NMR for the polymerization of AGA in H_2O/ethanol (5/1, v/v) at 60 °C with various concentrations of **I**. [AGA] = 0.715 mol·L^{-1}, [ACPA] = 7.14×10^{-4} mol·L^{-1}, [RAFT] = 1.78×10^{-3} mol·L^{-1} (square, run 1), 3.57×10^{-3} mol·L^{-1} (circle, run 2), 7.14×10^{-3} (triangle, run 3).

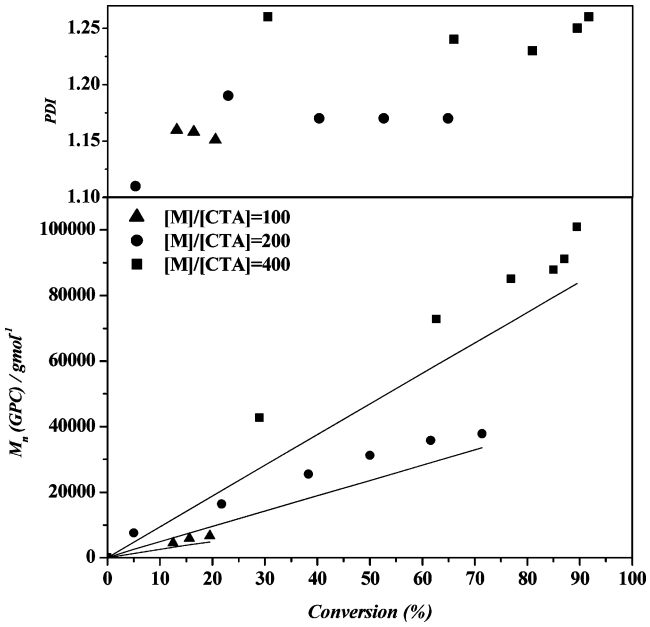


Figure 2. Evolution of the molecular weight ($\overline{M_n}$, GPC) and polydispersity with conversion for the RAFT polymerization of AGA in H_2O/ethanol (5/1, v/v) at 60 °C in the presence of **I**. [AGA] = 0.715 mol·L^{-1}, [ACPA] = 7.14×10^{-4} mol·L^{-1}, [RAFT] = 1.78×10^{-3} mol·L^{-1} (square, run 1), 3.57×10^{-3} mol.L^{-1} (circle, run 2), 7.14×10^{-3} (triangle, run 3). The straight line indicates the theoretically expected molecular weight.

As expected for a controlled/living process, the molecular weights increased linearly with conversion (Figure 2). GPC traces of PAGA exhibited monodisperse peaks, and no high molecular weight impurity resulting from termination reactions could be detected even at high conversion (71%), see Figure 3. Similar to literature results,[43] the experimental molecular weights determined by GPC in DMAc were to some extent higher than

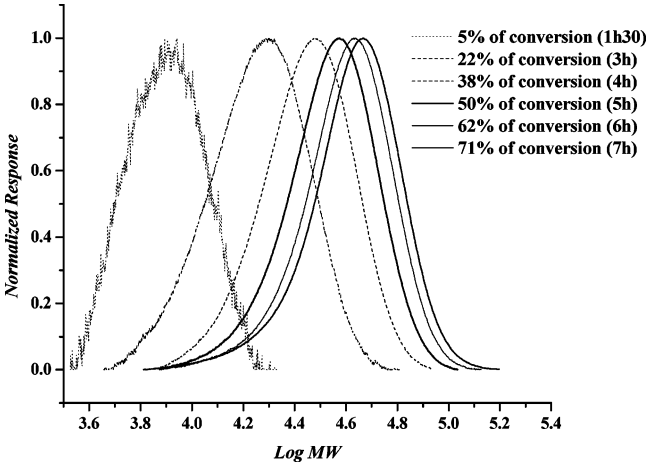


Figure 3. Evolution of the GPC traces for the RAFT polymerization of AGA in H_2O/ethanol (5/1, v/v) at 60 °C in the presence of **I**. [AGA] = 0.715 mol·L^{-1}, [**I**] = 3.57 × 10^{-3} mol·L^{-1}, [ACPA] = 7.14 × 10^{-4} mol·L^{-1}. From left to right, $T_{Polymerization}$ = 1.5, 3, 4, 5, 6, and 7 h.

Table 1. Summary of the Homopolymerization Experiments in the Presence of **I** at 60 °C in H_2O/Ethanol (5/1, v/v) and Main Characteristics of the Resulting Homoglycopolymers

run no.	[RAFT] (mM)	reaction time (h)	conversion (%)	$\overline{M}_{nth}$[a]	$\overline{M}_{nNMR}$[b]	$\overline{M}_{nGPC}$[c]	PDI[c]
1	1.78	7	89.5	83700	116700	100800	1.26
2	3.57	7	71.4	33550	32550	37800	1.19
3	7.14	8	19.5	4800	5900	6600	1.15

[a] Determined from conversion of AGA. [b] Determined from relative integration of the aromatic chain end group peaks and polymer backbone peak. [c] Determined from DMAc GPC (PS calibration).

the theoretical values which can be attributed to the use of a PS calibration. After the nonreacted monomer and remaining initiator were removed by precipitation in ethanol, the molecular weights of the glycopolymers were finally evaluated by ^{1}H NMR in D_2O from relative integration of the protons of the glycopolymer backbone and of aromatic protons of the benzyl chain end.

As shown in Table 1, the calculated molecular weights were in reasonable agreement with the theoretical values and is consistent with a controlled/living process. The accuracy using ^{1}H NMR for molecular weight calculations should be considered when comparing these results. Especially at high [M]/[RAFT] ratio and high conversions, the resolution of the RAFT agent may be limited (run 1). Furthermore, NMR studies do not address the problem that RAFT terminated polymer cannot be distinguished from a termination product. A further evidence of the growth of the glycopolymer in a controlled fashion was given by the polydispersity index of the glycopolymers which typically comprised of values between 1.1 and 1.3 with molar masses ranging from 3 to 120 K. Polydispersity values should only be carefully evaluated since the polystyrene calibration might present a distorted picture of the reality. However, the symmetric peaks showing a narrow molecular weight distribution might indeed confirm an excellent control over the polymerization.

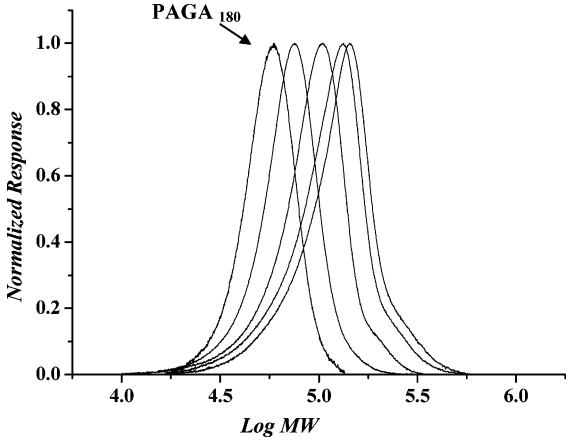
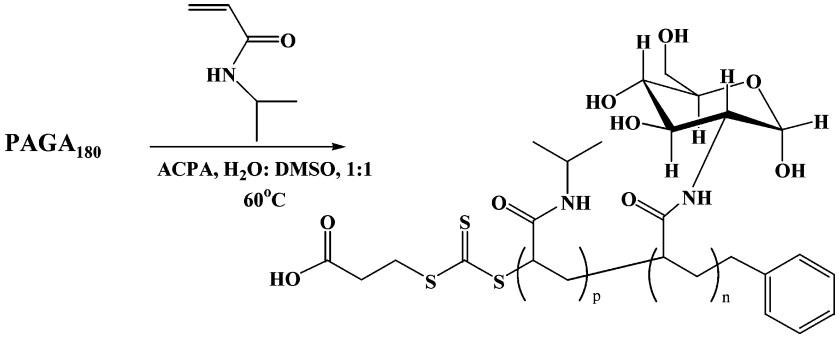


Figure 4. Evolution of the GPC traces for the chain extension of PAGA with NIPAAm at 60 °C in H_2O/DMSO (1/1, v/v) [NIPAAm] = 0.79 mol·L^{-1}, [macroRAFT] = 1.97 × 10^{-3} mol·L^{-1}, [ACPA] = 7.14 × 10^{-4} mol·L^{-1}. From left to right, $T_{Polymerization}$ = 0, 1, 2, 3, and 4 h.

Synthesis of Thermosensitive Block Copolymers PAGA-*b*-PNIPAAm. To confirm the "livingness" of the process, we investigated the ability to chain-extend the glycopolymers to yield block copolymers consisting of one water-soluble glycopolymer block and one thermo-responsive block (PNIPAAm). With this aim, a PAGA macroRAFT agent with a narrow molecular weight distribution ($PAGA_{180}$, see the Experimental Section) was prepared and precipitated twice in MeOH to remove traces of monomer and initiator. The polymer was subsequently used as a RAFT agent for the growth of the thermosensitive PNIPAAm second block, see Scheme 2. The growth of the second block was conducted in a DMSO/water mixture (1/1, v/v) to overcome the water solubility problem of PNIPAAm at 60 °C. The polymerization was very fast (39, 63.7, 81.6, and 88% of conversion after 1, 2, 3, and 4 h of reaction respectively). As shown in Figure 4, chain extension of $PAGA_{180}$ resulted in a clear shift of the GPC peaks toward higher molecular weights with time indicating the growth of a PNIPAAm block. The experimental molecular weight of the purified final diblock (4 h) determined by ^{1}H NMR ($\overline{M}_{nNMR}$ = 88 400 g·mol^{-1}, $PAGA_{180}$-*b*-$PNIPAAm_{408}$) was in good agreement with the theoretical value ($\overline{M}_{nth}$ = 82 000 g·mol^{-1}). The molecular weight distribution of the resulting block copolymers

Scheme 2. Reaction Scheme for the Block Extension of PAGA with *N*-Isopropyl Acrylamide



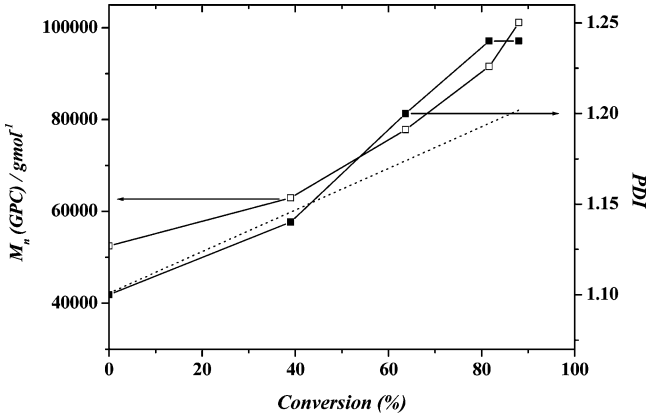
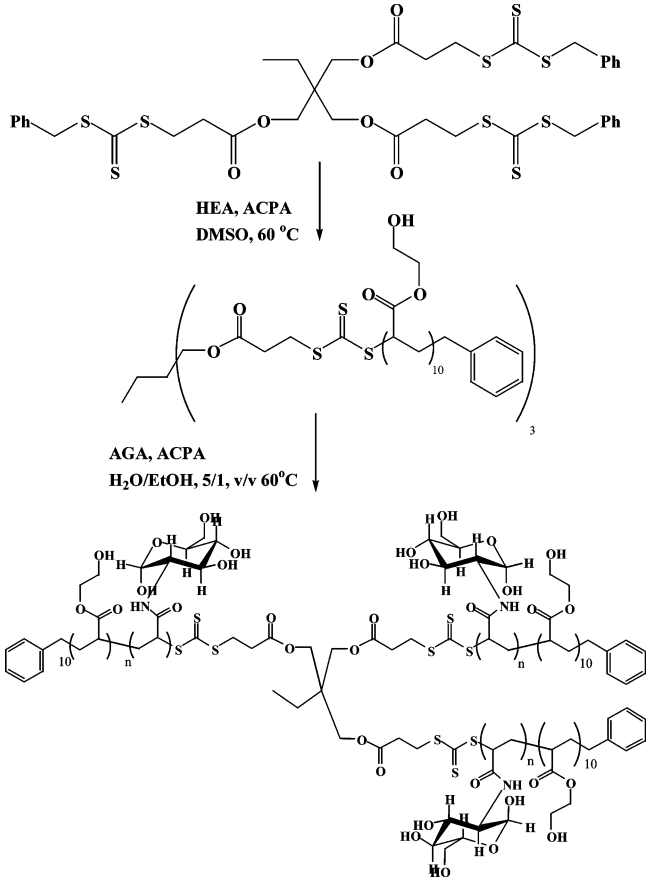


Figure 5. Evolution of the molecular weight ($\overline{M}_n$, GPC) (open symbols) and polydispersity (closed symbols) with conversion for the chain extension of PAGA with NIPAAm in H_2O/DMSO (1/1, v/v) at 60 °C. [NIPAAm] = 0.79 mol·L^{-1}, [macroRAFT] = 1.97 × 10^{-3} mol·L^{-1}, [ACPA] = 7.14 × 10^{-4} mol·L^{-1}. The dotted line indicates the theoretically expected molecular weight.

Scheme 3. Reaction Scheme for the Preparation of 3-Arm Glycopolymer Stars



was narrow (Figure 5). These results clearly corroborate the chain extension of PAGA in a controlled manner even though a slight tail was observed at lower molecular weights suggesting the presence of nonreactivated PAGA chains. All of the copolymers were soluble in water at 20 °C.

Synthesis of 3-Arm Glycopolymer Stars. Finally, we investigated the possibility to generate 3-arm PAGA stars from a Z-designed trifunctional RAFT agent (**II**, see Scheme 3) derived from 3-benzylsulfanylthiocarbonylsulfanylpropionic acid.[52] The trifunctional RAFT agent is not water-soluble, and no control of the polymerization of AGA could be achieved in

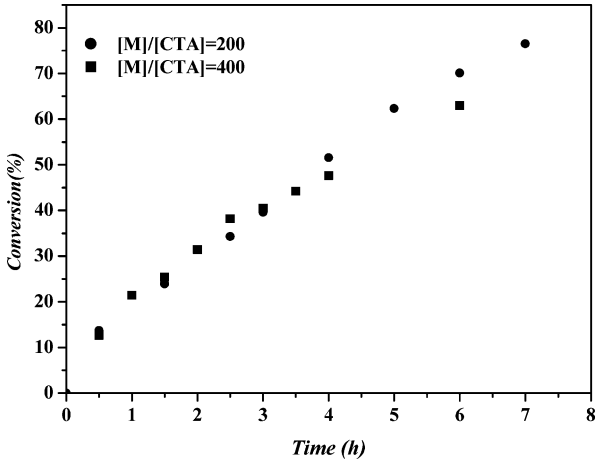


Figure 6. Conversion vs time plots obtained by ^{1}H NMR for the aqueous polymerization of AGA at 60 °C in H_2O/ethanol (5/1, v/v) with various concentrations of poly(hydroxyethyl acrylate) 3-arm star macroRAFT agent. [AGA] = 0.715 mol·L^{-1}, [ACPA] = 7.14 × 10^{-4} mol·L^{-1}, [RAFT functions] = 1.78 × 10^{-3} mol·L^{-1} (square, run 4), 3.57 × 10^{-3} mol·L^{-1} (circle, run 5).

the presence of DMAc, DMF, or DMSO. The molecular weights did not evolve linearly with conversion and the molecular weight distribution exceeded 1.5. Therefore, we first grew short blocks of hydrophilic PHEA from **II** in order to prepare a water-soluble trifunctional RAFT agent **III**. After polymerization and purification of the polymer (to remove traces of initiator and monomer), a well-defined PHEA star (**III**:GPC (DMAc): $\overline{M}_n$ = 3370 g·mol^{-1}; PDI = 1.07) with arms exhibiting a degree of polymerization of 10 (^{1}H NMR:$\overline{M}_n$ = 4470 g ·mol^{-1}) was obtained.

Similar to the experiments carried out with 3-benzylsulfanylthio carbonylsulfanylpropionic acid, the homopolymerization of AGA in the presence of **III** was conducted in water-ethanol mixtures (5:1; v:v). The kinetics of the homopolymerization of AGA were investigated at two different concentrations of RAFT agent **III** (1.78 × 10^{-3} and 3.57 × 10^{-3} mol·L^{-1}) at 60 °C in the presence of ACPA (7.14 × 10^{-4} mol·L^{-1}). Polymerizations proceeded reasonably fast (about 60−70% in 6 h, see Figure 6) and no inhibition period was observed with **III**. Although the polymerizations in the presence of **I** and **III** were carried out under exactly the same reaction conditions, it has to be noted the leaving group has now been transformed from a benzyl group to a PHEA polymer (Scheme 3). This might confirm earlier findings that the decreased stability of the leaving group when using an acrylate instead of a benzyl leaving groups can indeed erase any inhibition periods in acrylate polymerizations.[51] The conversions increased steadily with time. The polymerization rates were independent of the RAFT agent concentration and appeared to be quite similar to the rates observed in the presence of **I**. The evolution of molecular weights and polydispersity with conversion (investigated by GPC analysis in DMAc) is given in Figure 7 and Table 2. The molecular weights of the 3-arm stars increased with conversion but not in a linear fashion as expected. Due to the PS calibration, experimental molecular weights of the stars evaluated by GPC were slightly higher than the theoretical molecular weights (calculated from the conversion by ^{1}H NMR) at low and moderate conversions, whereas above 50% of conversion, the experimental molecular weights appeared to be systematically below the theoretical molecular weights. This trend which differs from the MW versus conversion plots of the linear chains (Figure 2) is probably a consequence of the branched character of the polymers exhibit-

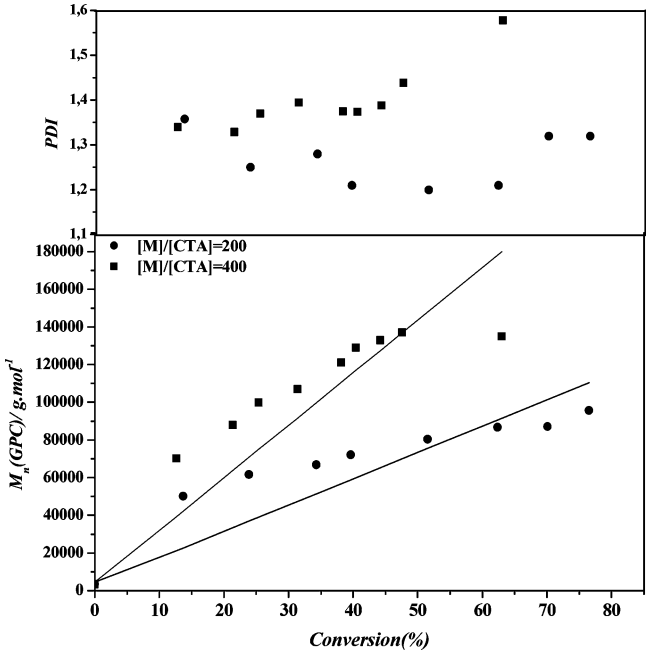


Figure 7. Evolution of the molecular weight ($\overline{M}_n$, GPC) and polydispersity with conversion for the RAFT polymerization of AGA at 60 °C in the presence of poly(hydroxyethyl acrylate) 3-arm star macroRAFT agent. [AGA] = 0.715 mol·L^{-1}, [ACPA] = 7.14 × 10^{-4} mol·L^{-1}, [RAFT functions] = 1.78 × 10^{-3} mol·L^{-1} (square, run 4), 3.57 × 10^{-3} mol·L^{-1} (circle, run 5). The straight line indicates the theoretically expected molecular weight.

Table 2. Summary of the Homopolymerization Experiments in the Presence of Poly(hydroxyethyl acrylate) 3-Arm Star MacroRAFT Agent at 60 °C in H_2O/Ethanol (5/1, v/v) and Main Characteristics of the Resulting Glycopolymer Stars

run no.	[RAFT] (mM)	reaction time (h)	conversion (%)	$\overline{M}_{nth}$[a]	$\overline{M}_{nNMR}$[b]	$\overline{M}_{nGPC}$[c]	PDI
4	1.78	3	40.5	117030	179400	131400	1.37
5	3.57	3	39.6	59320	101000	72220	1.21

[a] Determined from conversion of AGA. [b] Determined from relative integration of the aromatic chain end group peaks and polymer backbone peak. [c] Determined from DMAc GPC (PS calibration).

ing lower hydrodynamic volumes than the equivalent linear polymers. Similar to previous work involving Z-designed multifunctional RAFT agent[10,52], the molecular weight distribution of the stars was shown to be strongly influenced by the initial [M]/[CTA] ratio. As such, the molecular weight distribution of the stars prepared at a higher concentration of RAFT functions ([M]/[CTA] = 200) was quite narrow throughout the polymerization (PDI ≤ 1.32) while the polydispersity of the stars prepared at a lower concentration ([M]/[CTA] = 400) targeting higher molecular weights tended to increase significantly at high conversions reaching 1.6 at 63% of conversion. Upon closer inspection of the GPC traces of the stars synthesized at a lower concentration of CTA revealed the presence of a tail at low molecular weights (Figure 8, chromatograms on the top) suggesting the presence of dead polymer chains. This result probably stems from the arm growth process that occurs at the nexus of the core and the arm in the case of the Z approach. The increase in steric congestion around the trithio carbonate groups with conversion affects the interaction of the macroradical growing arms and the RAFT groups, resulting in termination reactions between the growing arms and consequently broadens the molecular weight distribution of the stars. This behavior has been investigated and discussed in detail

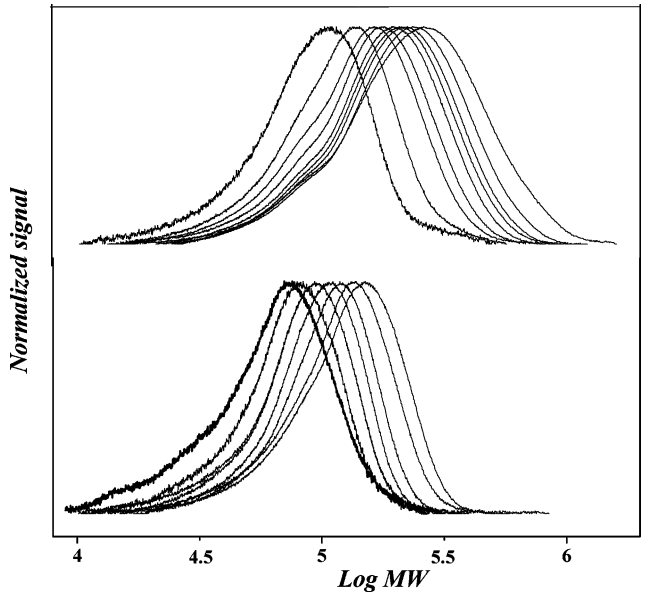


Figure 8. Evolution of the GPC traces for the RAFT polymerization of AGA at 60 °C in the presence of poly(hydroxyethyl acrylate) 3-arm star macroRAFT agent. [RAFT functions] = 1.78 × 10^{-3} (top) and 3.57 × 10^{-3} mol·L^{-1} (bottom), [ACPA] = 7.14 × 10^{-4} mol·L^{-1}.

elsewhere.[52] By cleavage of arms from the core further information on the evolution of the molecular weight of the arm with conversion and hence the amount of termination products was obtained.[52]

Conclusions

RAFT polymerization was exploited for the preparation of narrow dispersed AGA-based glycopolymers in aqueous solution without recourse of protecting group chemistry. Linear poly(acryloyl glucosamine) homopolymers with molar masses ranging from 3 to 120 K and PDI comprised between 1.1 and 1.3 have been synthesized. The glycopolymers were subsequently chain-extended with NIPAAm giving birth to well-defined thermosensitive PAGA-*b*-PNIPAAm diblocks confirming the living/controlled character of the polymerization. Finally, glycopolymer stars were elaborated from a water-soluble 3-arm PHEA macro RAFT agent via the Z approach. The polymerization of AGA proceeded in a controlled fashion with molecular weight increasing linearly with conversion. However, similar to previous work describing the preparation of stars via the Z approach[10,52], the polymerization at high [M]/[RAFT] ratio (400) resulted in some loss of control at high conversions due to the increasing steric congestion (around the RAFT groups) inherent to this strategy.

The self-assembly in water of the thermosensitive PAGA-*b*-PNIPAAm diblocks above the LCST of PNIPAAm and the stabilization of the resulting nano-objects by cross-linking are now under investigation.

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Supporting Information Available. NMR spectra of the compounds studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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