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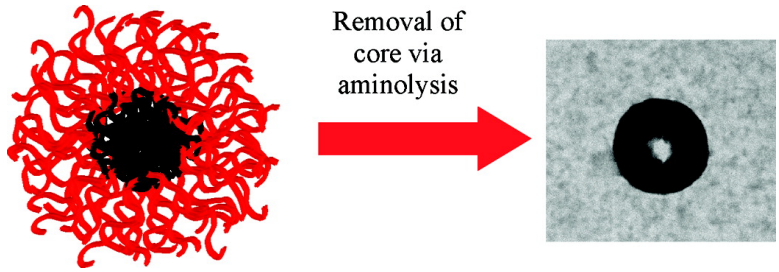

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Polygalactose Containing Nanocages: The RAFT Process for the Synthesis of Hollow Sugar Balls

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Hollow poly(6-*O*-acryloyl-α-D-galactopyranose) (PAGP) nanospheres were prepared in a facile manner using the RAFT (reversible addition fragmentation chain transfer) process. Initially, an amphiphilic block copolymer, poly(lactide)-*block*-poly(6-*O*-acryloyl-α-D-galactopyranose) (PLA-*b*-PAGP), was synthesized using a poly(lactide) (PLA) macroRAFT agent. It was attained in high yields and displayed low PDI values. The block copolymers self-assembled in aqueous solution to form micelles with pendent galactose moieties covering the surface. By using hexandiol diacrylate the micelles were cross-linked at the nexus of the copolymer, creating stable aggregates. Aminolysis with hexylamine allowed the removal of the PLA core without any detrimental effect on the glycopolymer units to produce hollow nanocages. Characterization of these hollow "sugar balls" with transmission electron microscopy (TEM) showed the cross-linked micelles with a central void due to the removal of the hydrophobic block. These micelles are advantageous in drug delivery applications, especially those involving the liver, thanks to the pendent galactose functionalities covering the surfaces of the nanocages.

Introduction

Carbohydrates play a significant role in biological recognition events such as cell−cell interactions or signaling events.[1] It is therefore not surprising that carbohydrates and their synthetic analogues, glycopolymers, are receiving increased attention. Glycopolymers are synthetic polymers with pendant carbohydrate moieties. They have been investigated in an array of diverse applications from macromolecular drugs to drug delivery systems.[2]

Significant efforts in recent years have focused on the synthesis of well-defined glycopolymers with control over both the molecular weight and architecture of the final macromolecules. Various polymerization techniques have been utilized to synthesize glycopolymers in a controlled fashion; these include living ionic polymerization,[3] Huisgen 1,3-dipolar cycloaddition (also known as "click chemistry"),[4,5] ring opening metathesis polymerization (ROMP),[6] atom transfer radical polymerization (ATRP),[7−11] and reversible addition-fragmentation chain-transfer (RAFT) polymerization.[12−24]

In many biological recognition events, the mediating role of glycopolymers was shown to be cooperative.[25] Macromolecular species that exhibited spherical structures were described as advantageous when ligand receptor interactions were concerned. Comb-like copolymers,[26] hyperbranched glycopolymers,[9] glycol starpolymers,[19,27] or glycodendrimers[28,29] show an enhanced so-called multivalency effect. Micelles are versatile polymeric structures with unique core−shell morphologies; they represent an additional glycopolymer architecture.[24,30,31] The self-assembly properties of amphiphilic polymers to form micelles only occurs under specific conditions and many approaches have been developed to cross-link the aggregates to prevent disintegration into unimers. The cross-linking of a micelle[32−34] can be carried out within the core,[24,32−38] the shell,[38−42] or along the interface between both blocks of the copolymer.[43,44]

The most interesting approach is via the shell-cross-linked method, despite a higher likelihood of intermicellar cross-linking. One advantage of shell cross-linking is that the loading of the final drug carrier is not compromised as with core-cross-linked micelles, that is, higher concentrations of drugs can be incorporated into the vessel. Furthermore, shell-cross-linked micelles allow the removal of the inner core resulting in nanosized hollow spheres, also called nanocages.[45−48] Degradation of the inner core has been achieved through a variety of methods including enzyme-catalyzed reactions,[47,48] ozonolysis,[46] or by introducing potentially labile metal complexes as the connecting moieties between the hydrophilic and hydrophobic blocks of the copolymers.[45]

RAFT polymerization[49−52] has been shown to be a versatile tool in the synthesis of well-defined polymer architectures,[53] especially for the formation of amphiphilic block copolymers.[54] The RAFT process is a very robust technique, tolerant toward a range of functional groups and, therefore, negates the need for additional synthetic pathways to install protecting groups on the monomers being used. This is especially advantageous in the case of the synthesis of glycopolymers. Via the RAFT process, well-defined glycopolymer structures can be obtained despite the presence of the numerous free sugar hydroxy groups. However, using unprotected sugars limits the choice of solvents that can successfully used for the polymerizations. Protecting the pendent sugar groups allows a broader range of solvents to choose from,[21,55,56] which in turn often coincides with a better controlled RAFT process.[54]

The application of a RAFT agent (typically a trithiocarbonate or dithioester) allows for the formation of complex polymer architectures with pendant sugar moieties including the synthesis of block copolymers for micelles.[54] It can also be employed to cross-link the resulting self-assembled structure.[24,38,43,57] As a result of the RAFT mechanism, the final polymers carry a thiocarbonylthio functionality as reactive group. The incorporation of this group allows the chain extension of the polymer upon the addition of a new monomer. For cross-linking, the

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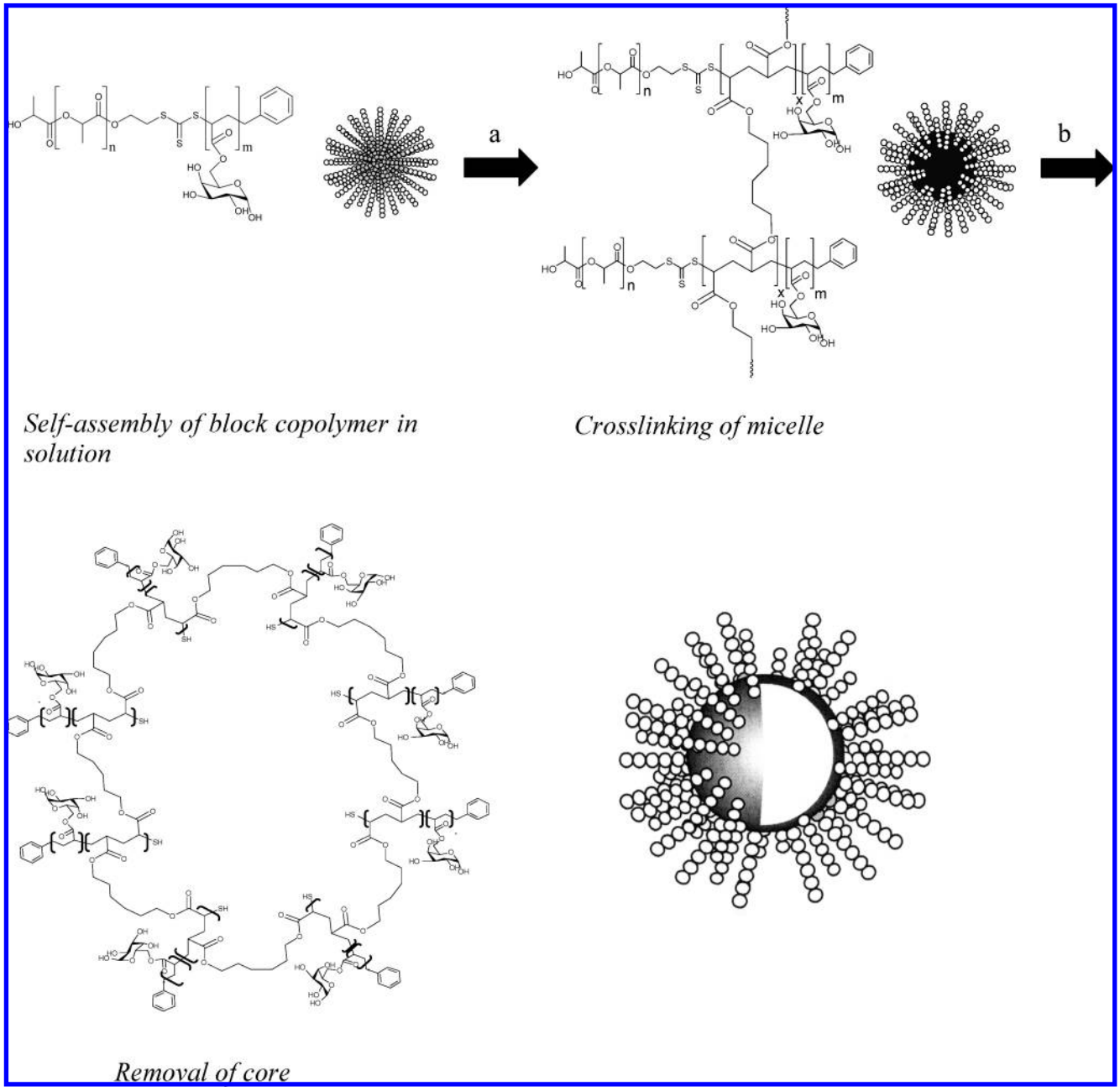
Scheme 1. Schematic Approach to the Synthesis of Poly(6-*O*-acryloyl-α-D-galactopyranose) (PAGP) Sugar Balls[a]



[a] Using the RAFT process followed by aminolysis. (a) Hexandiol diacrylate/water/AIBN/60°C; (b) hexylamine.

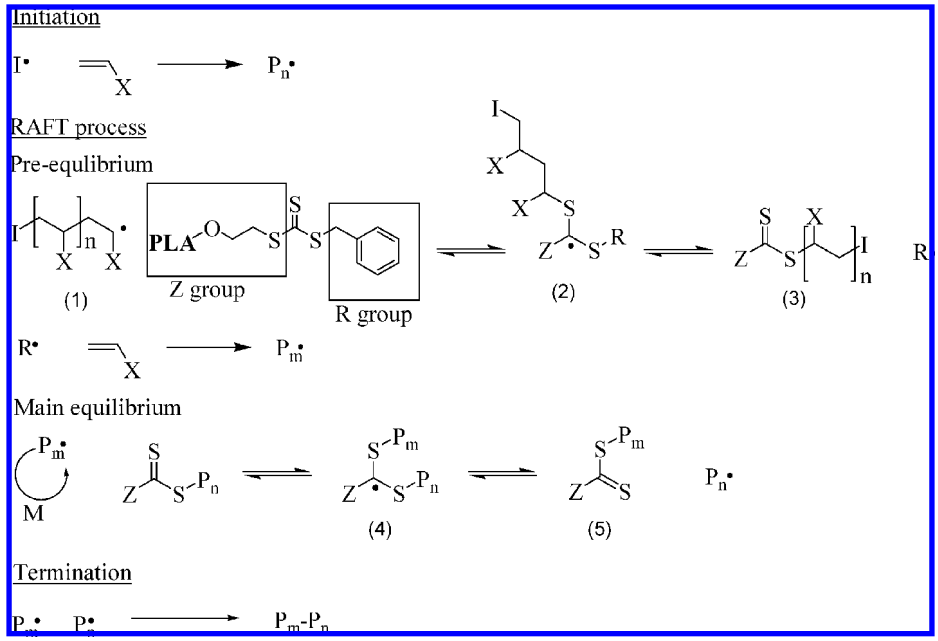
chain extension of block copolymers that have already self-assembled into micelles with a monomer incorporating a divinyl functionality leads to the formation stabilized structures.

Herein, the synthesis of hollow nanosized sugar cages is discussed. These sugar cages were generated by a combination of RAFT polymerization, cross-linking, and core-removal (Scheme 1). Initially, a block copolymer based on D,L-poly(lactide) (the degradable block) and the glycopolymer 6-*O*-acryloyl-α-D-galactopyranose (AGP) was generated followed by a cross-linking step. The cross-linking took place at the nexus between the hydrophilic shell and hydrophobic core of the self-assembled micelle. The poly(lactide) (PLA) core was then degraded through aminolysis resulting in the formation of glycopolymer nanocages, as outlined in Scheme 1.

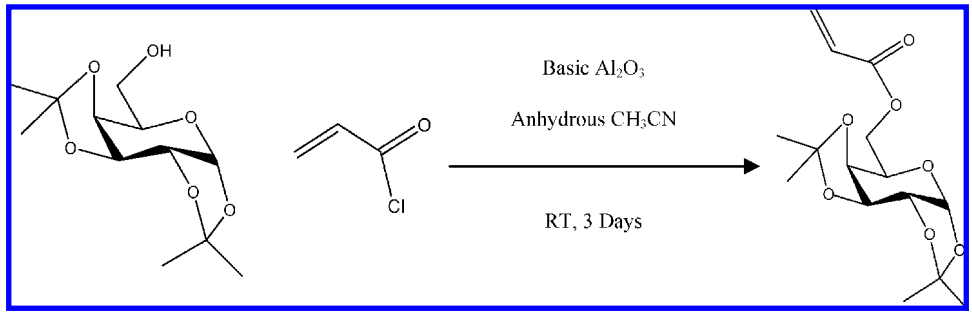
The pathway presented in Scheme 1 prevents intermicelle cross-linking since the cross-linking takes place only at the nexus between the hydrophilic and hydrophobic block. This approach is only possible using the RAFT process and not via any of the other controlled/living radical polymerization techniques. The RAFT agent needs to be connected to the PLA block via the so-called "Z-group" of the RAFT agent. The Z-group is permanently attached to the active RAFT group, here a trithiocarbonate, and will not fragment in the presence of radicals (Scheme 2).[54] Therefore, the chain extension will always take place between the PLA block and the block prepared by the RAFT process, here the glycopolymer chain, resulting in the insertion of further blocks at the nexus between PLA and glycopolymer (Scheme 2).

While the concept of hollow nanospheres (or nanocages) has been widely explored, this is, to our knowledge, the first report regarding the formation of hollow glycopolymer nanocages. These nanocages can possibly be employed as a drug delivery carrier with an increased loading capacity.

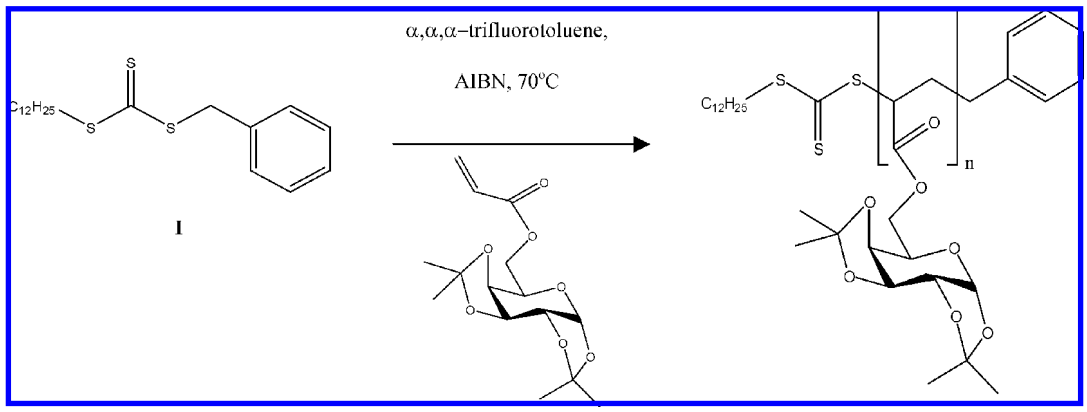
Scheme 2. RAFT Process in the Presence of Poly(lactide) Functionalized RAFT Agent Attached via the Z-Group[43,72]



Scheme 3. Synthesis of 1,2:3,4-Di-*O*-isopropylidene-6-*O*-acryloyl-α-D-galactopyranose (AIpGP) from 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose and Acryloyl Chloride



Scheme 4. Homopolymerisation of 1,2:3,4-Di-*O*-isopropylidene-6-*O*-acryloyl-α-D-galactopyranose (AIpGP) Using a Trithiocarbonic Acid Benzyl Ester Dodecyl Ester RAFT Agent



Experimental Section

Materials. 1-Dodecanethiol, potassium hydroxide, carbon disulfide (99%), benzyl bromide, acetonitrile, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, acryloyl chloride, aluminum(III) oxide, and *N*,*N*-dimethylacetamide were purchased from Aldrich and used without further purification. Technical grade methanol, hexylamine, α,α,α-trifluorotoluene and formic acid (UniLab, 90%) was used as received. Hexandiol diacrylate (Aldrich) was destabilized by passing it through basic alumina and used immediately. 2,2′-Azobis(isobutyronitrile) (Fluka, 98%) was recrystallized from methanol.

Synthesis. *Synthesis of Trithiocarbonic Acid Benzyl Ester Dodecyl Ester RAFT Agent (I).* 1-Dodecanethiol (30.0 g, 1.49×10^{-1} mol) was suspended in distilled water (200 mL) and cooled in an ice bath. This was followed by the slow addition of potassium hydroxide (8.52 g, 1.49×10^{-1} mol) before carbon disulfide (30.0 mL) was introduced dropwise. The addition of the carbon disulfide resulted in the formation of a yellow emulsion. Finally, benzyl bromide (23.4 g, 1.49×10^{-1} mol) was added, after which the reaction vessel was heated to 80 °C for 12 h. After cooling, the water phase was separated from the organic phase and washed with methylene chloride (3 × 50 mL).

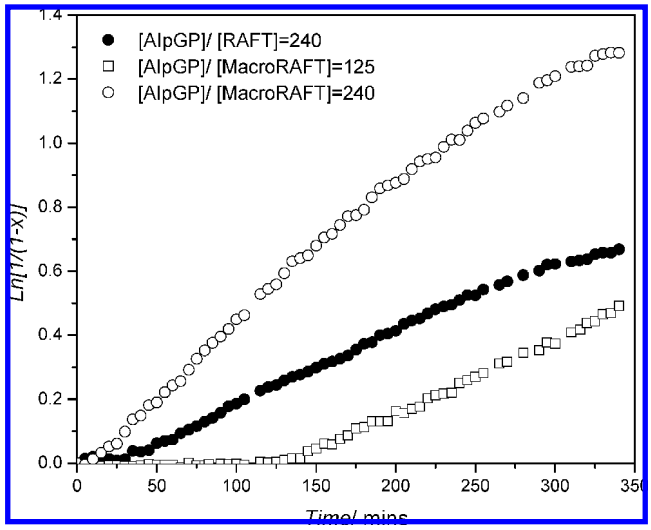


Figure 1. Pseudo-first-order plot of the RAFT polymerizations of 1,2:3,4-di-*O*-isopropylidene-6-*O*-acryloyl-α-D-galactopyranose (AlpGP) at 70 °C in α,α,α-trifluorotoluene in the presence of D,L-PLA macroRAFT agent or RAFT agent **I**. [AlpGP] = 7.65 × 10^{-1} mol L^{-1}, [AIBN] = 6.40 × 10^{-4} mol L^{-1}, [macroRAFT] = 3.19 × 10^{-3} mol L^{-1} (○), 6.10 × 10^{-3} mol L^{-1} (□), or [RAFT **I**] = 3.19 × 10^{-3} mol L^{-1} (●).

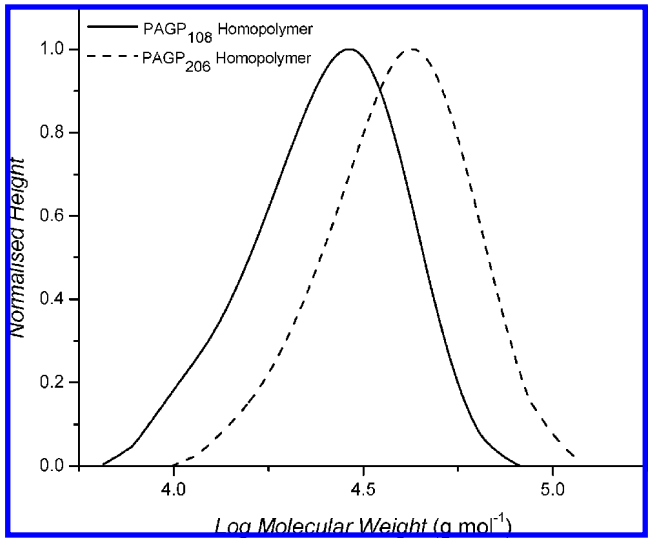


Figure 2. The SEC curves for PAGP after the hydrolysis of the AlpGP homopolymers, synthesized with the trithiocarbonic acid benzyl ester dodecyl ester RAFT agent **I** at 70 °C in α,α,α-trifluorotoluene, [AlpGP] = 7.65 × 10^{-1} mol L^{-1}, [AIBN] = 6.38 × 10^{-4} mol L^{-1}. $PAGA_{206}$: [RAFT] = 3.19 × 10^{-3} mol L^{-1}, 8 h, 86% conversion. $PAGP_{108}$: [RAFT] = 6.12 × 10^{-3}mol L^{-1}, 20 h, 86% conversion.

The combined organic phases were dried over anhydrous magnesium sulfate. After filtration, the solvent was removed under reduced pressure and dried in vacuo, yielding a bright yellow solid (79%). Melting point 29.52 °C (determined via DSC); ESI-MS (CH_2Cl_2) calcd for $C_{20}H_{32}S_3$ + Ag^+ *m/z*, 475.07; found, *m/z* 475.13; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 0.89 (t, 3H, C*H*$_3$), 1.26 (s, 18H, CH_3-C$_9$*H*$_{18}$-(CH$_2$)$_2$-S), 1.76 (m, 2H, C*H*$_2$-CH$_2$-S), 3.37 (t, 2H, C*H*$_2$-S), 4.61 (t, 2H, S-C*H*$_2$-Ph), 7.32 (m, 5H, Ar*H*); ^{13}C{^{1}H} NMR ($CDCl_3$, 75.4 MHz) δ (ppm) 14.04 (1C, *C*H$_3$), 22.60 (1C, CH$_3$-*C*H$_2$), 27.90, 28.83, 29.03, 29.26, 29.35, 29.47 (8C, *C*$_8$H$_{16}$-CH$_2$-S), 31.83 (1C, CH$_3$-CH$_2$-*C*H$_2$), 37.00 (1C, *C*H$_2$-S), 41.27 (1C, S-*C*H$_2$-Ar), 127.63, 128.59, 129.16 (3C, Ar*C*), 135.04 (1C, Ar*C*), 223.70 (1C, *C*═S).

Synthesis of D,L-Poly(lactide) MacroRAFT Agent (II). The macroRAFT Agent **II** was synthesized as described by Hales et al.[43] 3,6-Dimethyl-1,4-dioxane-2,5-dione (15 g, 1.04 × 10^{-1} mol) and 2-(benzylsulfanylthiocarbonylsulfanyl) ethanol (8.97 × 10^{-1}g, 3.68 × 10^{-3} mol) were mixed and stirred under vacuum at 120 °C heat for 12 h. After purging with nitrogen degassed $SnOct_2$ (55.9 mg, solution in 1 mL toluene) was added to the yellow mixture. The temperature was increased to 140 °C and the mixture was left to react for 5 h. At the increased temperature, the mix became a golden liquid, which when cooled, formed a hard glassy mass. After cooling, dichloromethane was added to extract the cooled polymer mix from the flask. Under stirring, the dichloromethane solution was added dropwise to an excess of chilled methanol. The precipitated polymer was dried under vacuum.

The concentration of trithiocarbonate end-groups in the macroRAFT agent **II** was determined on a Cary 300 UV−vis spectrophotometer. The concentration of thiocarbonylthio groups in the macroRAFT agents were calculated using the absorption peak maximum from the RAFT agent **I** at 433 nm ($\epsilon = 58$ L·mol^{-1}·cm^{-1}) in chloroform. The molecular weight of the macroRAFT agents were derived from $M_n = (m_{sample} \times \epsilon_{488nm} \times d)/(V \times E)$, where m_{sample} is the weight of the polymer sample (in g), ϵ_{488nm} is the molar absorptivity at λ= 488 nm (in L·mol^{-1}·cm^{-1}), *d* is the length of the pathway (in cm), *V* is the volume (in L) of the sample, and *A* is the measured absorbance.

The monomer conversion was found to be >90%. The molecular weight using UV/vis was calculated to be 12000 g mol^{-1}, which is in good agreements with the value obtained by SEC (12500 g mol^{-1}, PDI = 1.25). The deviation from the theoretical molecular weight (M_n = 56 × 0.9 × 72 = 3690 g mol^{-1}) is explained by the low efficiency of the RAFT agent to initiate a ring-opening polymerization.

Synthesis of 1,2:3,4-Di-O-isopropylidene-6-O-acryloyl-α-D-galactopyranose (AlpGP). To a stirred suspension of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (5.00 g, 1.92 × 10^{-2} mol) and basic alumina (3.90 g, 8.82 × 10^{-2} mol) in distilled acetonitrile (50 mL), acryloyl chloride (8.00 mL, 9.89 × 10^{-2} mol) was added at room temperature. The reaction mixture was stirred for 3 days. The solids were removed by filtration though a micromembrane. Reaction completion was monitored using thin layer chromatography with hexane and ethyl acetate (2:1) as the solvent. The solvent was removed under reduced pressure to yield a light yellow oil, which was purified via column chromatography (hexane/ethyl acetate, 2:1). A viscous light yellow oil was obtained (56%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.33−1.53 (m, 12H, C*H*$_3$), 4.10 (m, 1H, sugar moiety C*H*), 4.13 (m, 4H, sugar moiety 1C*H*H + 3C*H*), 4.61 (m, 1H, sugar moiety CH*H*), 5.53 (d, 1H, anomeric C*H*), 5.84 (d, 1H, C*H*H═CH), 6.17 (dd, 1H, CHH═C*H*), 6.40 (d, 1H, CH*H*═CH). ^{13}C{^{1}H} NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 24.36, 24.83, 25.85 (4C, *C*H$_3$), 63.40 (1C, *C*H$_2$, sugar C6), 65.90, 70.35, 70.47, 70.96 (4C, *C*H, sugar C2−C5), 96.17 (1C, *C*H, anomeric *C*, sugar C1), 108.67 (1C, *C*(CH3)$_2$), 109.57 (1C, *C*(CH$_3$)$_2$), 128.08 (1C, CH$_2$═*C*H-C(O)), 130.95 (1C, *C*H$_2$═CH-C(O)), 165.95 (1C, CH$_2$═CH-*C*(O)).

Polymerization of 1,2:3,4-Di-O-isopropylidene-6-O-acryloyl-α-D-galactopyranose with I and II. AlpGP (2.40 g, 7.65 × 10^{-3} mol) was dissolved in 10 mL of α,α,α-trifluorotoluene and either RAFT **I** (1.15 × 10^{-2} g, 3.19 × 10^{-5} mol) or macroRAFT **II** (3.82 × 10^{-1} g, 3.19 × 10^{-5} mol) was added to the sugar solvent mixture. AIBN (1.05 × 10^{-3} g, 6.38 × 10^{-6} mol) was introduced and the mixture was divided into five sample vials. Each of the vials was sealed with a rubber septa. The vials were kept chilled in a cold water bath and purged with nitrogen for 20 min. The vials were then placed in an oil bath at 70 °C and removed from the bath after allotted times. The polymer conversions were determined using ^{1}H NMR and FT-NIR in separate experiments. Conversions by ^{1}H NMR were obtained by evaporating the resulted reaction mixtures and diluting the polymer in $CDCl_3$.

The homopolymerization resulted in 86% monomer conversion after a reaction time of 8 h. After deprotection, SEC analysis gave a molecular weight of M_n (SEC) = 51000 g mol^{-1}, PDI = 1.17 (M_n (theo) = 46000 g mol^{-1}).

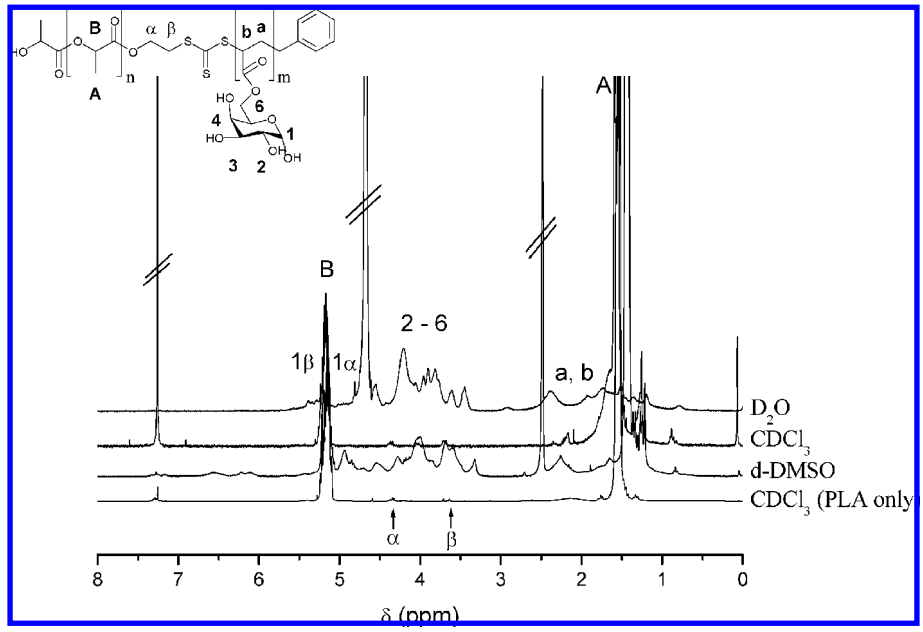


Figure 3. ^{1}H NMR spectra for PLA_{170}-*b*-$PAGP_{62}$ in different deuterated solvents and PLA macroRAFT agent in $CDCl_3$.

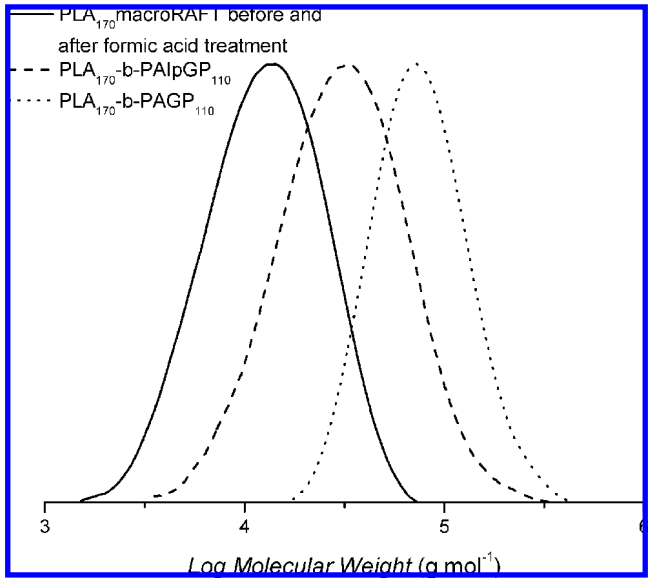


Figure 4. The SEC chromatograms of the PLA_{170} macroRAFT agent (before and after treatment with formic acid), PLA_{170}-*b*-$PAIpGP_{110}$ (protected sugar), and PLA_{170}-*b*-$PAGP_{110}$ (deprotected sugar; only the graph after formic acid treatment is shown here because it is identical to the SEC curves before treatment).

The block copolymerization reached 73% conversion after a reaction time of 6 h. The molecular weight analysis after deprotection gave a molecular weight of M_n (SEC) = 52000 g mol^{-1}, PDI = 1.20 (M_n (theo) = 73000 g mol^{-1}).

Deprotection of the D,L-PLA-block-PAIpGP Copolymer To Form D,L-PLA-block-PAGP and the Self-Assembly Procedure Used To Form Micelles. D,L-PLA-*b*-PAIpGP (120 mg) was dissolved in 4 mL of formic acid, 90%. The solution was stirred at 60 °C in an oil bath for 1 h. The resultant solution was dialysed against distilled water for 2 days with a water change every 6 h to remove the acid. Self-assembly into particles occurred during the course of purifying the copolymer D,L-PLA-*b*-PAGP.

Hydrolysis of PLA Using the D,L-PLA-block-PAGP Copolymer. D,L-PLA_{170}-*b*-$PAGP_{216}$ (10 mg) was added to 2 mL of hexylamine and left shaking overnight. The resulting mixture was dialyzed against methanol to remove the remaining hexylamine and cleaved polymers. After dialysis, the samples were placed under high vacuum to remove methanol. ^{1}H NMR with deuterated dimethyl sulfoxide as the solvent and size exclusion chromatography (SEC) were used for the analysis of the hydrolyzed products.

Cross-Linking of Self-Aggregated Structure and Core Hydrolysis. D,L-PLA_{170}-*b*-$PAGP_{216}$ (10 mg) was dissolved in 5 mL of dimethyl sulfoxide and dialysed against distilled water for 2 days with a water change every 6 h. The resultant dialyzed polymer solution was diluted to 10 mL with distilled water. Hexandiol diacrylate (HDA; 3.50×10^{-4} g, 1.55×10^{-6} mol) was suspended in 1 mL of distilled water and from this 100 μL was pipetted and eventually suspended in the dialysis mixture. AIBN (7.00×10^{-3} g, 4.27×10^{-5} mol) was dissolved in 10 mL of methanol and 10 μL of the AIBN methanol solution was pipetted into the reaction mixture, followed by nitrogen degassing for 30 min. The reaction mixture was placed in an oil bath at 60 °C for 24 h with fast stirring. Aliquots were taken to monitor the conversion of the polymerized HDA. The unreacted HDA was extracted by mixing deuterated $CDCl_3$ with the aliquots to collect HDA in the organic phase. ^{1}H NMR spectra of unreacted HDA were taken at different reaction times. Trimethylsilane was used as an internal standard with 10 μL being added to the 600 μL of extracted HDA in $CDCl_3$. The cross-linked polymers were purified by freeze-drying. The cross-linked aggregates underwent a further treatment with hexylamine using the same conditions mentioned in the above PLA hydrolysis section.

Analysis. *Nuclear Magnetic Resonance (NMR) Spectroscopy.* All NMR spectra were recorded using a Bunker 300 MHz spectrometer. The RAFT agents were analyzed using deuterated chloroform and the polymers were examined using deuterated chloroform, deuterium oxide or deuterated dimethyl sulfoxide.

Fourier Transform Near-Infrared Red (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 stretching overtone of the monomer at $\upsilon = 6164$ 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 8000−4000 cm^{-1} and were obtained from the added interferograms of 12 scans with the resolution of 4 cm^{-1}. The conversion was determined by selecting a linear baseline between 6173.99 and 6155.99 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

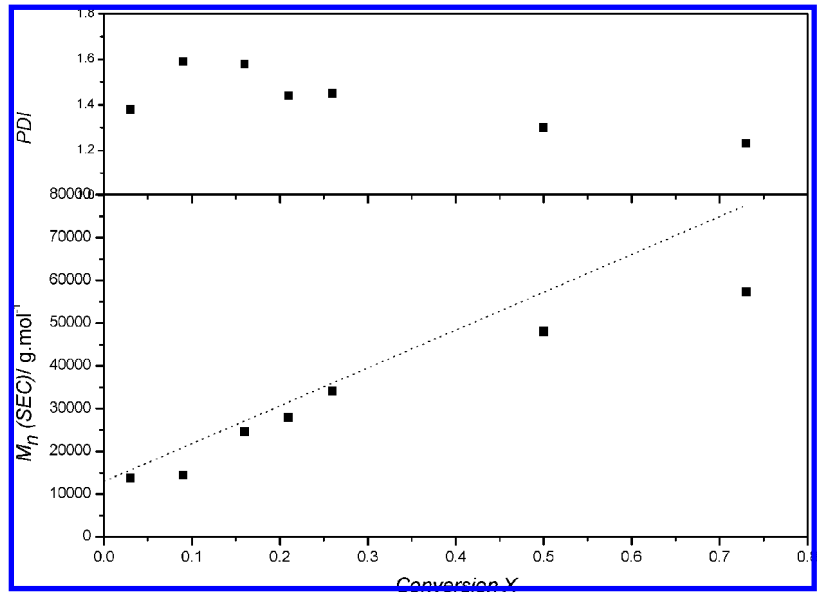


Figure 5. The evolution of molecular weight and polydispersity index (PDI) for D,L-PLA-*b*-PAGP with conversion (*X*) versus the molecular weight obtained by SEC with the polymerization of AIpGP at 70 °C in α,α,α-trifluorotoluene in the presence of a polylactide macroRAFT agent. [AIpGP] = 6.73 × 10^{-1} mol L^{-1}, [macroRAFT] = 2.80 × 10^{-3} mol L^{-1}, [AIBN] = 5.60 × 10^{-4} mol L^{-1}.

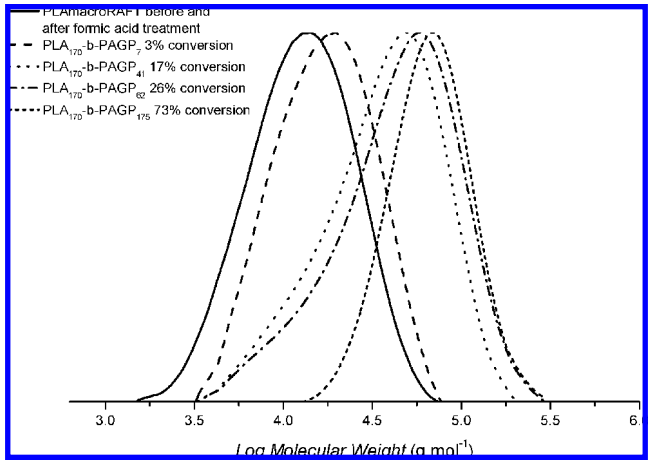


Figure 6. SEC chromatograms of PLA_{170} macroRAFT agent and PLA_{170}-*b*-$PAGP_{7,\ 38,\ 62,\ 175}$ block copolymers obtained at various conversions at 70 °C followed by deprotection. [AIpGP] = 6.73 × 10^{-1} mol L^{-1}, [macroRAFT] = 2.80 × 10^{-3} mol L^{-1}, [AIBN] = 5.60 × 10^{-4} mol L^{-1}.

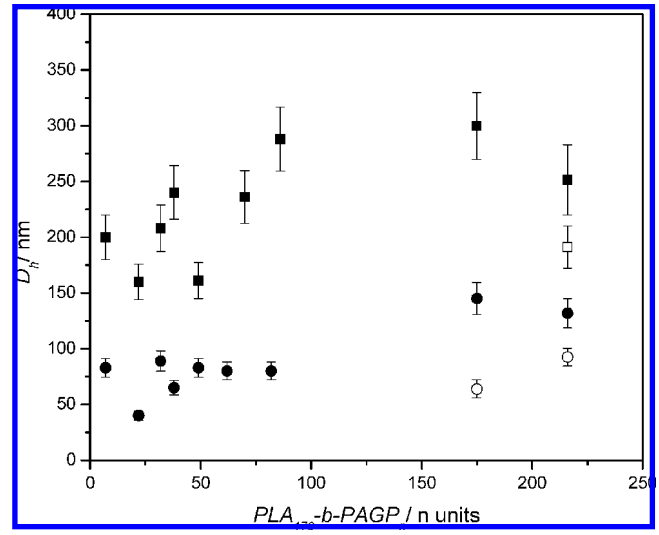


Figure 7. Hydrodynamic diameter (D_h) of micelles with increasing number of repeating units of PAGP block at 25 °C in water (■) and methanol (●); the open symbol represent the diameter of the cross-linked micelle in water (□) and methanol (○).

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 500 to 10^6 g mol^{-1}. A similar system was available with THF as the mobile phase to determine the molecular weight of the D,L-PLA using the Mark−Houwink parameters $K = 25.9 \times 10^{-5}$ dL·g^{-1}, α = 0.689 to calculate absolute molecular weights.

Dynamic Light Scattering (DLS). The hydrodynamic diameters (D_h) of the aggregates were determined using a Brookhaven Zetaplus particle size analyzer (1 angle: 90 °). 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 by dialyzing the PLA-*b*-PAGP samples with either distilled water, methanol, or *N*,*N*-dimethylacetamide.

Transmission Electron Microscopy (TEM). The TEM micrographs were obtained using a JEOL 1400 transmission electron microscope. The samples were prepared by casting an aqueous or methanol solution of the PLA-*b*-PAGP polymer (1 g L^{-1}) onto a copper grid. No staining was applied.

Results and Discussion

Prior to the investigation of the synthesis of the block copolymer, we evaluated the RAFT process of the glycomonomer in the absence of the PLA block to obtain vital information on the suitability of the RAFT agent and solvent for the chosen glycomonomer.

The glycopolymer used in this work possessed galactose as the pendant side groups. Galactose moieties play an important part in many biological processes such as the selective targeting of hepatocytes using the unique interaction between the asialoglycoprotein receptor (ASGPR) and hepatocytes.[58] The synthesis of AIpGP was reported earlier employing esterification reactions on the protected sugar with (meth)acryloyl chloride

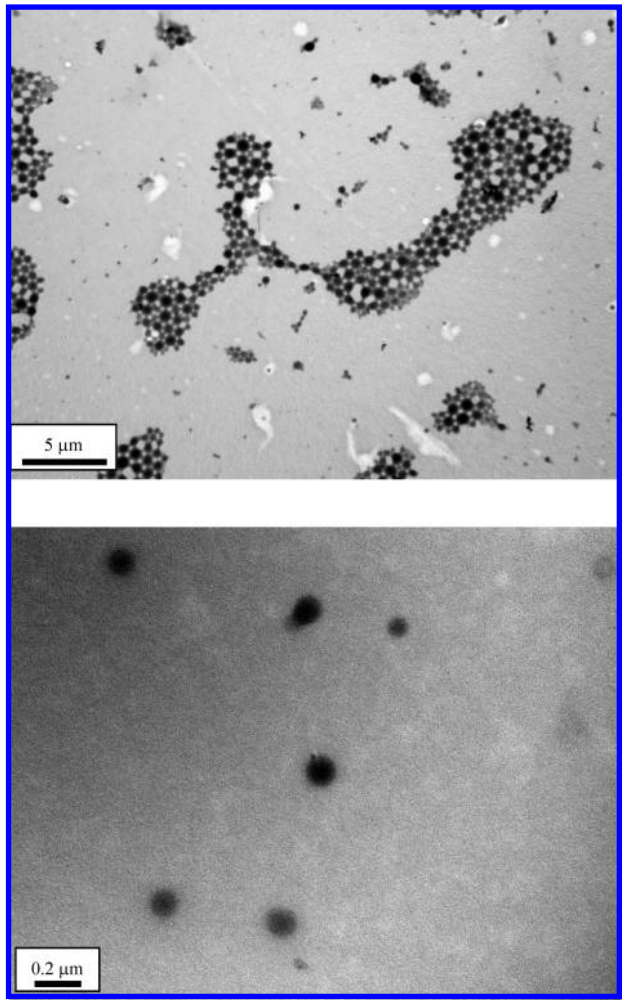


Figure 8. TEM micrographs of micelles obtained from PLA_{170}-*b*-$PAGP_{216}$ (top) and PLA_{170}-*b*-$PAGP_{41}$ (bottom) from water solution.

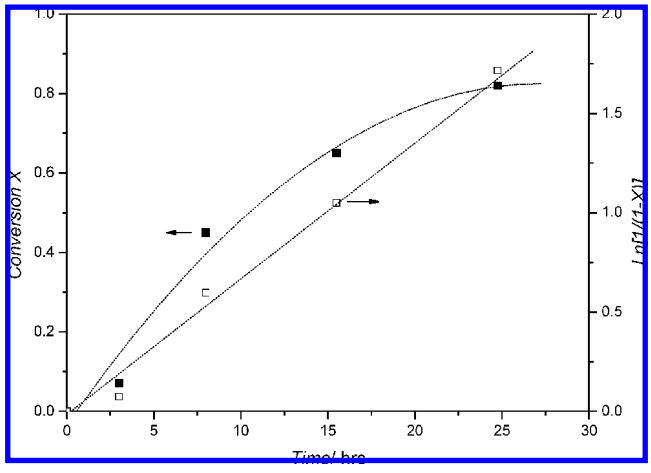


Figure 9. Pseudo-first-order kinetics and conversion versus time plots of the cross-linking reaction of PLA_{170}-*b*-$PAGP_{216}$ with HDA at 60 °C in distilled water yielding PLA_{170}-*b*-$PHDA_8$-*b*-$PAGP_{216}$. [HDA] = 1.53 × 10^{-4} mol L^{-1}, [PLA_{170}-*b*-$PAGP_{216}$] = 1.57 × 10^{-5} mol L^{-1}, [AIBN] = 4.22 × 10^{-6} mol L^{-1}.

or anhydride.[59−61] Amines, such as pyridines, were employed as catalysts, which introduced elements of high toxicity into the system. Therefore, an alternative synthesis route was adapted using finely dispersed aluminum oxide as the catalyst. High conversions of more than 90% in combination with high purity and very short reaction times were reported when modifying

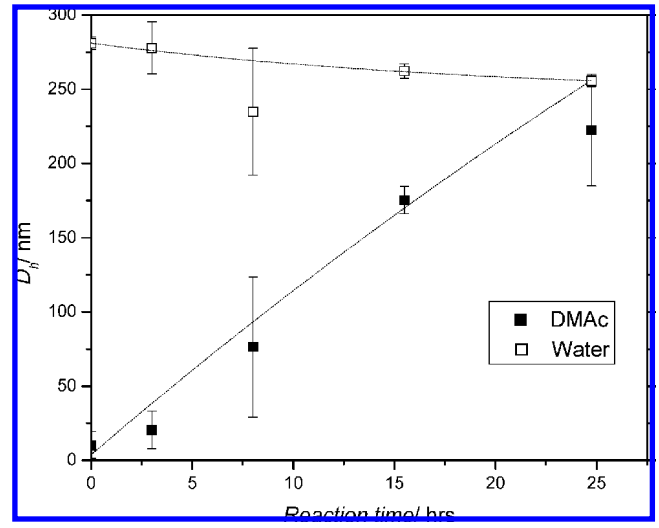


Figure 10. Dynamic light scattering measurements for the cross-linked particles during the polymerization of PLA_{170}-*b*-$PAGP_{216}$ with HDA. The measurements were taken at different reaction times using water and DMAc as the solvents.

several carbohydrates with acetyl chloride.[62] Unfortunately, this approach could not fully be transferred to acryloyl chloride and an extended reaction period was required while the product yield was around 56% after purification via column chromatography (Scheme 3).

In the next step, the investigation of the RAFT homopolymerization of AIpGP under various conditions was conducted. This step was necessary before evaluating the influence of the PLA on the RAFT process. The RAFT agent - trithiocarbonic acid benzyl ester dodecyl ester - was employed to find suitable conditions for the homopolymerization (Scheme 4).

A primary concern was the choice of solvent that could dissolve both the glycomonomer and PLA. Many high boiling polar solvents such as DMSO and *N*,*N*-dimethyl acetamide prevented the efficient control of the polymerization of the AIpGP in the presence of trithiocarbonic acid benzyl ester dodecyl ester. Therefore, α,α,α-trifluorotoluene was chosen as a polymerization solvent. It was able to dissolve PLA in high concentrations while allowing optimum control over the RAFT polymerization of AIpGP. The pseudo-first order kinetic plot displayed in Figure 1 shows a linear relationship up to high conversion for the homopolymerization (Figure 1, closed circle). Only at reaction times after 250 min does the reaction slow down, indicating the loss of some of the radicals.

SEC analysis of the protected glyco homopolymer was inconclusive. To confirm the livingness of the RAFT process, a deprotection step was carried forming poly(6-*O*-acryloyl-α-D-galactopyranose) PAGP. The resulting SEC diagrams show narrow molecular weight distributions with the molecular weight shifting to higher values with respect to conversion (Figure 2). Moreover, low polydispersity indices (PDI) of around 1.15 were observed.

After the reaction conditions were optimized for the homopolymerization, similar reaction parameters were then applied to the synthesis of block copolymers (Scheme 5). The poly(lactide) macroRAFT agent was synthesized according to a previously published procedure.[43] A hydroxyfunctionalized RAFT agent was employed to initiate the ring-opening polymerization of 3,6-dimethyl-1,4-dioxane-2,5-dione, resulting in the formation of the PLA macroRAFT agent (**II**). The reaction temperature of 140 °C was well below the decomposition temperature. Trithiocarbonates were found to disintegrate into

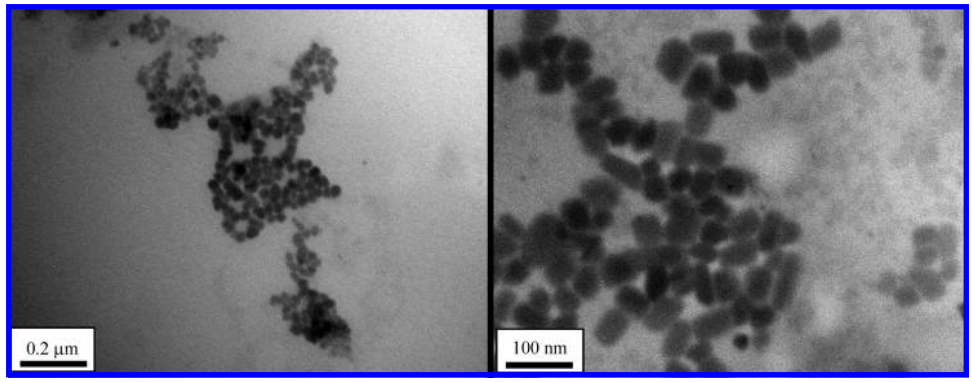


Figure 11. TEM micrographs of cross-linked micelles formed from PLA_{170}-*b*-$PHDA_8$-*b*-$PAGP_{216}$.

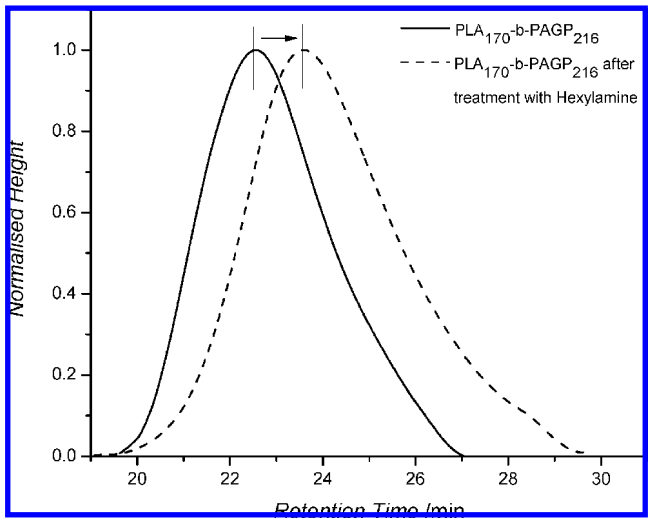


Figure 12. SEC chromatograms showing PLA_{170}-*b*-$PAGP_{216}$ before and after the hydrolysis reaction using hexylamine for the degradation of PLA.

CS_2 at temperatures above 200 °C.[63] The activity and the concentration of active RAFT end groups was confirmed using UV/vis spectroscopy. The resulting polymer showed a small molecular weight distribution and a molecular weight measured using SEC, which is in good agreement with the molecular weight calculated using UV/vis results. This indicates that every polymer chain is terminated with a trithiocarbonate functionality. However, the molecular weight is significantly higher than the theoretical molecular weight, indicative for a low activity of the RAFT agent to initiate the ring-opening polymerization. Unreacted RAFT agent was removed during the precipitation of the polymer into methanol. Investigation of the solution via UV/vis revealed the presence of the low molecular weight RAFT agent.

The PLA macroRAFT agents were then employed in the RAFT polymerization of the glycomonomer 1,2:3,4-di-*O*-isopropylidene-6-*O*-acryloyl-α-D-galactopyranose (AIpGP) (Scheme 5).

The PLA macroRAFT agent was readily dissolved in α,α,α-trifluorotoluene and the polymerization was carried out using similar concentrations to that of the homopolymer. The block copolymerization was found to have a higher rate of polymerization (Figure 1), which is in good agreement with earlier findings.[64] In addition, an experiment with an increased amount of PLA macroRAFT agent was carried out showing an inhibition period of around 130 min coupled with retardation in the rate of polymerization. The reduced rate of polymerization in RAFT polymerizations is usually assigned to a slow fragmentation of the intermediate RAFT agent (species (2) in Scheme 2). However, retardation is usually not observed in trithiocarbonates and the reduced rate of polymerization can rather be assigned to increased viscosity, which can lead to lower initiator efficiencies.[65]

SEC analysis of PLA-*b*-PAIpGP was more conclusive with the molecular weight increasing linearly with conversion while the associated PDI values were low. However, since the homopolymers were analyzed after removal of the protective groups, we attempted the removal of the protective group for the purpose of comparison between homopolymer and block copolymer. Care should be taken during this procedure to fine-tune the conditions to avoid the degradation of PLA and the destruction of the RAFT agent (which would cleave the block copolymer) and also make sure the ester bond between the polymeric backbone and galactose units are not severed.

The subsequent reaction step to remove the protective groups was therefore subject to a detailed investigation (Scheme 5). Initially, trifluoroacetic acid (TFA) was utilized in order to obtain PAGP. However, the aggressive environment led to the destruction of the block copolymers. Instead of the TFA treatment, the block copolymer was heated with formic acid for a short period of time. ^{1}H NMR measurements confirmed the disappearance of methyl signals at $\delta = 1.30$ ppm while the PLA block was fully intact. The ratio between sugar and PLA signals confirmed that the developed technique did not destroy the hydrolysis sensitive PLA block nor did it cleave the ester bond on the glycomonomer. Unfortunately, the precise quantitative analysis of the deprotection step was hampered by the overlay of PLA methyl signal ($\delta = 1.25-1.50$ ppm) and methyl signals of the isopropylidene protective group ($\delta = 1.33-1.53$ ppm; Figure 3).

The RAFT process is a robust system that is tolerant to impurities that may be present in the polymerization system.[66] Even so, it was important to make sure that the RAFT agent was not destroyed or removed in the presence of the formic acid during the deprotection step. To test this, the trithiocarbonic acid benzyl ester dodecyl ester RAFT agent was dissolved in neat formic acid and left stirring overnight at 60 °C (see Supporting Information). The reaction was monitored through UV−vis spectroscopy and no decrease in the absorbance was observed. After removing the formic acid under reduced pressure, ^{1}H NMR showed that the RAFT agent had remained intact.

Additional control experiments tested the stability of the PLA block in the acid system. SEC diagrams of the PLA macroRAFT agent before and after treatment with formic acid did not show any changes, including color changes, confirming the stability of the block copolymer (Figure 4). From these experiments it

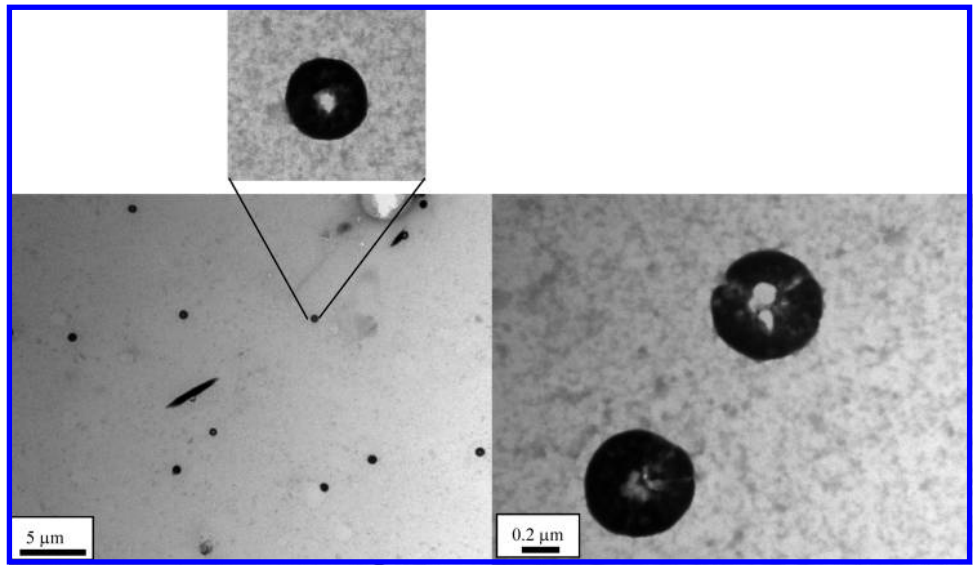
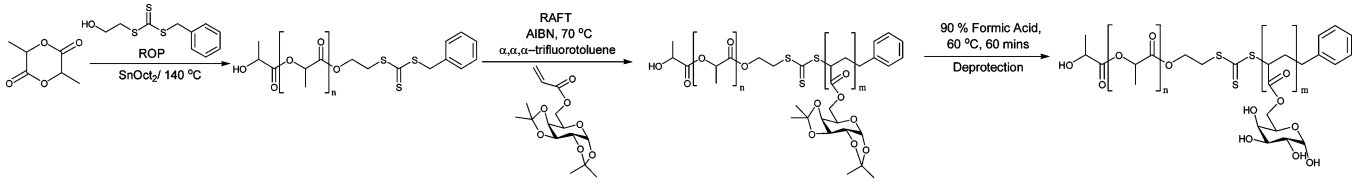


Figure 13. TEM micrographs of the hollow poly(6-*O*-acryloyl-α-D-galactopyranose) nanocages, formed after the removal of the PLA core.

Scheme 5. Synthesis of D,L-Polylactide-*block*-poly(6-*O*-acryloyl-α-D-galactopyranose)



can be inferred that only the protective groups on the sugar moieties were cleaved under these conditions, while the polymer backbone did not show any signs of degradation.

Finally, SEC investigations of the deprotected block copolymers were carried out. Analysis of the PLA-*b*-PAGP led to higher molecular weights compared to PLA-*b*-PAIpGP, which is in contrast to the expected weight loss (Figure 4). It should be noted here that the molecular weights of the protected block copolymers, the PLA-*b*-PAIpGP samples, were much lower than the predicted theoretical values, while the deprotected block copolymers were close to the expected molecular weights (Figure 5). In this work, poly(styrene) standards were employed for the SEC calibration. It has been repeatedly reported that these standards are not suitable for glycopolymers since the hydrodynamic radii of glycopolymers are significantly higher.[15,17,55] The result of these experiments was the successful synthesis of a range of amphiphilic block copolymers, PLA-*b*-PAGP, with narrow molecular weight distributions and only slight molecular weight tailing (Figure 6). This low molecular weight tailing, which makes the SEC curves slightly asymmetric, is derived from dead polymers, which is generated during the course of the polymerization by termination reactions. The molecular weight increased linearly with conversion, very close to the theoretical molecular weight value, which is presented as the dotted line in Figure 5. A deviation at higher conversion could be assigned to termination reactions, resulting in the formation of dead polymer (Scheme 2). Termination reactions are usually accompanied by the broadening of the molecular weight distribution, which is not the case here. In addition, the SEC curve displayed in Figure 6 does not show any obvious tailing. The decrease in molecular weight at higher conversion could therefore only be explained by the altered hydrodynamic radius of the block copolymer with conversion.

The amphiphilic character of the resulting PLA-*b*-PAGP block copolymer was immediately visible with the appearance and disappearance of certain NMR signals in various solvents. DMSO, which fully dissolves both blocks, revealed the full signal intensity of both polymer chains. In contrast, the use of water results in the formation of aggregates and as a consequence the PLA signals at 5.10 ppm disappeared. Due to the aggregate formation in water, the movement of PLA is limited affecting the NMR relaxation time, thus the signal intensity is reduced. When chloroform was used as the solvent the opposite effect was observed with the PLA being fully soluble while PAGP cannot be dissolved; thus generating inverse aggregates. Consequently, the sugar signals disappeared and the NMR spectrum resembled pure PLA (Figure 3).[67]

Dynamic light scattering (DLS) was employed to determine the hydrodynamic diameter of the PLA_{170}-*b*-$PAGP_x$ block copolymers with different block lengths in methanol and water. A slight increase in diameter can be observed as the length of the hydrophilic block increases (Figures 7 and 8).[68] The aggregates obtained in water were observed to have significantly higher hydrodynamic diameters, which could be due to the increased swelling of the PAGP shell in water (and possibly some changes in aggregation number). The tendency of these micelles to aggregate in water could be a further reason for the increased hydrodynamic diameter.

TEM micrographs confirmed the spherical shape of the micelles. The diameter obtained from micelles in the aqueous solution was found to be smaller than the particle size measured using DLS indicative of the dehydration of the shell under the examination conditions. It could, however, also indicate as mentioned before that the DLS value is the result of some aggregation. A strong tendency to aggregate during the drying process can be seen. The likelihood of this happening is usually more pronounced for micelles with a small size distribution.[69]

Cross-linking was carried out in a diluted aqueous polymer solution (1 mg mL^{-1}) to maintain the micellar structure and to work well below the overlap concentration. The divinyl cross-

linker hexamethylene diacrylate (HDA) and oil-soluble radical initiator (AIBN) were added to stabilize the structures. The polymerization procedure displayed in Scheme 1 was carried out at 60 °C. The stability of the micelles at elevated temperatures was tested prior to the polymerization using DLS. The hydrodynamic radius does not seem to be significantly affected by temperature changes with the diameter at 60 °C being only around 10% smaller.

The rate of polymerization proceeded according to first-order kinetics with more than 80% of the cross-linker being consumed after 25 h (Figure 9). With a cross-linker to RAFT agent ratio of 10 and 86% conversion after a reaction time of 25 h, more than eight molecules (16 vinyl groups) of cross-linker were added per polymer chain resulting in a triblock structure PLA_{170}-*b*-$PHDA_8$-*b*-$PAGP_{216}$.

The development of the hydrodynamic diameter of the product (after purification) at different reaction times was monitored using different solvents (Figure 10). For this purpose, the (cross-linked) micelles were isolated as described in the experimental section and redissolved in either water or *N*,*N*-dimethyl acetamide (DMAc). The aggregates size in water remained unaltered, which is not surprising considering that micelles exist in the early stages of the polymerization. A slight contraction may be observed at higher cross-linker conversions, which could be assigned to the tighter packing of polymer chains.[38,57] In contrast, DMAc is a good solvent for both blocks. The measured diameter at the early stages of the cross-linking polymerization is close to the typical size of a block copolymer below 10 nm. With increasing cross-linker conversion, the diameter increases, indicating permanent cross-linking of block copolymers. With increasing consumption of cross-linker, more and more block copolymers are cross-linked, resulting in an increase in hydrodynamic diameter. The cross-linked micelle obtained after a reaction time of 25 h had a similar hydrodynamic radius in water and DMAc.

Analysis of the cross-linked sample via DLS using methanol results in significantly smaller particles (Figure 7). This shows that water and DMAc are clearly better solvents for the glycopolymer block when compared to methanol, allowing significant swelling of the shell. The micelles in the dry state undergo significant shrinkage and take on a slight egg-shaped appearance (Figure 11).

Prior to the degradation of the PLA in the core of the cross-linked micelle as suggested in Scheme 1, the hydrolysis was studied using uncrosslinked micelles. Several alkaline solutions were employed ranging from NaOH to K_2CO_3.[70] NMR analysis of the treated product revealed, however, that either PLA had not been degraded or that the ester bond between the galactose unit and polymeric backbone had been cleaved. The degradation of the core using hexylamine (aminolysis)[71] was found to be the most successful approach to producing the nanocages. A quick study on the initial RAFT agent used to construct the macroRAFT agent (2-(benzylsulfanylthiocarbonylsulfanyl) ethanol) showed the conversion of the compound into thiol containing species was fast under the aminolysis conditions. UV−vis and NMR spectroscopy confirmed the destruction of the trithiocarbonate. The block copolymer was shaken with hexylamine and the resulting product was dialysed against water. ^{1}H NMR analysis confirmed the disappearance of the PLA, while all the galactose pendant chains were still present on the chain ends of the polymer. In addition, a decline in molecular weight, equivalent to that of the degraded PLA chain, was observed using SEC (Figures 12 and 13).

The chosen conditions were therefore suitable to degrade the PLA block without affecting the glycopolymer. Cross-linked micelles of PLA_{170}-*b*-$PHDA_8$-*b*-$PAGP_{216}$ were subjected to the same aminolysis treatment. The occurrence of a thiol endgroup after aminolysis as displayed in Scheme 1 is rather unlikely because thiols will form disulfide bridges under these conditions. However, this remains to be confirmed. Following dialysis, the aqueous solution was cast onto a copper grid and analyzed using TEM. The hollow spheres were clearly visible confirming not only the removal of the core, but also the successful cross-linking process. The diameter of the particles was now significantly enlarged since the contracting force of the PLA core had been removed. The increase in diameter has been utilized in earlier publications as proof for the successful nanocage formation.[39,45]

Conclusions

Hollow sugar balls were successfully prepared via RAFT polymerization. A block copolymer based on D,L-polylactide-*block*-poly(6-*O*-acryloyl-α-D-galactopyranose) was self-assembled in aqueous solution and cross-linked via RAFT chain extension using HDA. Subsequent degradation of the core resulted in glycopolymer nanocages. Challenges in the preparation of hollow sugar balls included the choice of solvent for the RAFT polymerization to allow the preparation of the amphiphilic block copolymers. Furthermore, sugar deprotection and the degradation of the PLA core needed to be carried out selectively without cleaving other sensitive bonds. Formic acid for the deprotection step of sugar and hexylamine for the degradation of the PLA block were utilized to generate well-defined nanocages. The next step is the application of these nanocages as drug delivery vessels. The galactose shell makes it possible to the nanocages to successfully target hepatocytes allowing for the delivery of the contents of the cage to the liver. This can be advantageous for the delivery of medicines to a specific site in the body, for example, the transportation of platinum based drugs for the treatment of liver cancer. Various compounds can be incorporated within the nanocage and this is the subject of our current investigations.

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Supporting Information Available. Stability testing of the RAFT agent under acidic conditions using UV/vis and NMR. Also included is the NMR spectra of the block copolymer prior and after deprotection. This material is available free of charge via the Internet at http://pubs.acs.org.

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