Well-Defined Glycopolymers from RAFT Polymerization: Poly(methyl 6-*O*-methacryloyl-α-D-glucoside) and Its Block Copolymer with 2-Hydroxyethyl Methacrylate

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ABSTRACT: The glycomonomer methyl 6-*O*-methacryloyl-α-D-glucoside was prepared by lipase-catalyzed transesterification of vinyl methacrylate with methyl α-D-glucoside in dry acetonitrile. The desired 6-*O* regioisomer was obtained in good yield and its structure confirmed by ^{1}H−^{1}H and ^{1}H−^{13}C correlation NMR spectroscopy. Reversible addition−fragmentation chain transfer (RAFT) polymerization of the unprotected monomer was performed directly in aqueous solution using (4-cyanopentanoic acid)-4-dithiobenzoate as the chain transfer agent to give poly(methyl 6-*O*-methacryloyl-α-D-glucoside) with M_n between 16 and 103 kDa (^{1}H NMR) and polydispersities as low as 1.10. Chain extension of one of these polymers with 2-hydroxyethyl methacrylate afforded the novel hydrophilic−hydrophilic block copolymer poly[(methyl 6-*O*-methacryloyl-α-D-glucoside)-*block*-(2-hydroxyethyl methacrylate)].

Introduction

In a general sense, glycopolymers can be defined as synthetic polymers possessing a non-carbohydrate backbone but carrying carbohydrate moieties as pendant or terminal groups.[1,2] Since the pioneering work of Horejsi et al. on the precipitation of lectins by copolymers of acrylamide and allyl glycosides,[3] glycopolymers have raised an ever-increasing interest as artificial materials for a number of biological and biomedical uses. This is mostly due to the expectation that polymers displaying complex functionalities, similar to those found in natural glycoconjugates, might be able to mimic or even exceed their performance in specific applications (biomimetic approach). For instance, studies have been published on the use of glycopolymers as macromolecular drugs,[4−8] drug delivery systems,[9−11] cell culture substrates,[12,13] stationary phase in separation problems[14,15] and bioassays,[16] responsive[17] and catalytic hydrogels,[18] surface modifiers,[19−22] artificial tissues, and artificial organs substrates.[12]

The presence of appropriate functional groups in a glycopolymer though is usually insufficient to bestow it with the biological and physicochemical properties required for a given application. Indeed, control of the macromolecular architecture has proved essential to enable sophisticated functions[6,23] and to draw a correlation between them and the polymer structure itself. For this reason an increasing number of polymer chemists, biochemists, and carbohydrate chemists are approaching the synthesis of novel glycopolymers via precise polymerization techniques.[2,21,24,25] To date, the great majority of reports on the synthesis of well-defined glycopolymers (i.e., glycopolymers with a narrow polydispersity and a predetermined molecular weight) focuses on the use of ring-opening metathesis polymerization (ROMP)[23,26−30] and living anionic[31−35] or living cationic[36−40] vinyl polymerization, while a more limited number of examples exist for living coordination polymerization[41] and living ring-opening polymerization (ROP).[42−46] The appeal of ROMP with Grubbs catalysts (a family of ruthenium−carbine complexes)[47] for the synthesis of glycopolymers lies in the fact that they are tolerant to a variety of polar functionalities (including hydroxyl, carboxylic acid, and sulfate groups) in both the substrate and the solvent mixture, they function at temperatures from ambient to 60 °C, and they allow for the preparation of very short oligomers in a controlled way. Conversely, this technique is limited to the polymerization of strained monomers such as norbornene and cyclobutene, and it imposes a significant synthetic cost for their preparation. Living anionic and living cationic vinyl polymerizations have been widely exploited in glycopolymers' synthesis as well, since they give access to narrow polydispersity homopolymers and block copolymers of predefined length; their use appears to be quite limited in scope though. Both techniques are in fact quite laborious and generally require aprotic solvents, and all reactants must be of the highest purity. Monomers must not contain acidic protons or strongly electrophilic functionalities, and reactions are very sensitive to oxygen and usually require subambient temperatures. As a result, only protected monomers can be used for polymerization, and consequently a fully functional material can be obtained only after a further deprotection step.

Beginning in the 1990s and with the advent of modern living radical polymerization (LRP) techniques,[48−51] a wealth of new possibilities have been disclosed to those pursuing the synthesis of well-defined glycopolymers and complex glycopolymer architectures.[52] LRP processes possess most of the characteristics of a living process but without many of the drawbacks of ionic or coordination polymerization systems.[51,53] For instance, they are compatible with aqueous media (ATRP and RAFT),[54] and a number of monomer functionalities and

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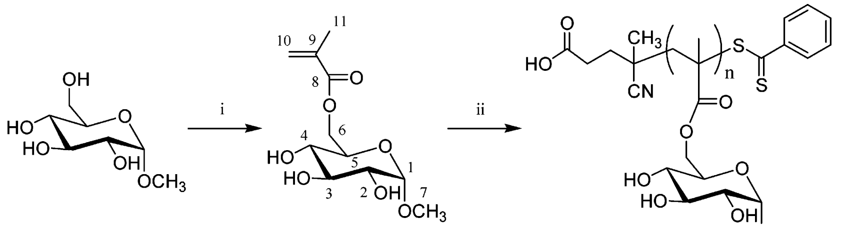
Scheme 1. Lipase-Catalyzed Synthesis of Methyl 6-*O*-Methacryloyl-α-D-glucoside 2 and Its RAFT Polymerization with (4-Cyanopentanoic acid)-4-dithiobenzoate 7 (CPADB)[a]



1 **2** **3**

[a] For clarity, the position numbering of 2 used for NMR assignment is also reported (see Experimental Section). Conditions: (i) vinyl methacrylate, Novozym 435, acetonitrile, 50 °C, 5 days; (ii) 4,4′-azobis(cyanopentanoic acid), (4-cyanopentanoic acid)-4-dithiobenzoate, water-ethanol 9:1, 70 °C.

can be carried out under normal radical polymerization conditions. Also, they apply to a variety of vinyl monomers (and subsequent polymer backbones)[55] and can be readily adapted to surface functionalization.[56,57] Particularly interesting are the reports by R. Narain and S. P. Armes on the atom transfer radical polymerization of 2-gluconamidoethyl methacrylate and 2-lactobionamidoethyl methacrylate in water/methanol mixtures, since they describe the preparation of low-polydispersity glycopolymers without resorting to protective groups' chemistry.[58−60] More recently, the first example of reversible addition−fragmentation chain transfer (RAFT) polymerization of a sugar monomer was described by A. B. Lowe et al., who successfully polymerized the commercially available 2-methacryloxyethyl glucoside directly in water and in the presence of a water-soluble dithioester,[61] while our group has successfully synthesized the first narrow-polydispersity, poly(vinyl ester)-like glycopolymer via the xanthate- and dithiocarbamate-mediated RAFT polymerization of 6-*O*-vinyladipoyl-D-glucopyranose.[62]

As part of our systematic investigation on the chemical and enzymatic synthesis of unprotected vinyl glycomonomers and on their controlled radical polymerization to materials of desired architecture, we now report the lipase-catalyzed synthesis of methyl 6-*O*-methacryloyl-α-D-glucoside (6-*O*-MMAGlc) and its living radical polymerization in water via reversible addition−fragmentation chain transfer (RAFT, Scheme 1). The synthesis of the novel hydrophilic−hydrophilic block copolymer poly[(methyl 6-*O*-methacryloyl-α-D-glucoside)-*block*-(2-hydroxyethyl methacrylate)] is also described.

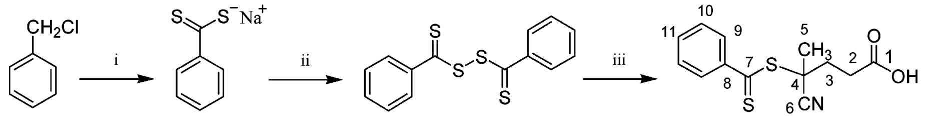
Experimental Section

Materials and Methods. Unless otherwise specified, all chemicals were reagent grade. Methyl α-D-glucoside (Aldrich, 99%), lithium bromide (Aldrich, 99+%), vinyl methacrylate (Aldrich, 98%), 4,4′-azobis(cyanopentanoic acid) (ACPA, Fluka, 98%), and deuterium oxide (99.9%, Cambridge Isotopes) were used as received. All solvents were HPLC grade (Asia Pacific Specialty Chemicals), and acetonitrile was dried for 48 h on activated 4 Å molecular sieves prior to use. Novozym 435 was kindly donated by Novozymes A/S. 2-Hydroxyethyl methacrylate (97%, Aldrich) was distilled under vacuum prior to use. Flash chromatography was performed with a 76 mm o.d. glass column loaded with 200 g of silica gel (60 Å, ≤63 μm, Fluka) and eluted with ethyl acetate/hexane/ethanol 7:2:1 at flow rate of 5 cm min^{-1}. The same eluant mixture was used for TLC analysis, which was performed on glass-backed silica gel plates (60 Å, 5−17 μm, Macherey-Nagel). Following solvent evaporation, the developed plates were immersed in a 20% H_2SO_4/ethanol solution and heated at 80 °C for 30 min for spots detection.

Analysis. NMR experiments were conducted on Bruker Avance DMX300 and DMX500 spectrometers (magnetic field strength of 300.2, 500.1 MHz for ^{1}H nuclei and 75.5, 125.8 MHz for ^{13}C nuclei, respectively). Molecular weights and molecular weight distributions were measured by size exclusion chromatography (SEC) on a Shimadzu modular LC system comprising a DGU-12A solvent degasser, a LC-10AT pump, a SIL-10AD autoinjector, a CTO-10A column oven, and a RID-10A refractive index detector. The system was equipped with a 50 × 7.8 mm guard column and four 300 × 7.8 mm linear columns (Phenomenex 500, 10^3, 10^4, and 10^5 Å pore size; 5 μm particle size). *N*,*N*-Dimethylacetamide (HPLC, 0.03% w/v LiBr, 0.05% BHT) was used as eluant at a flow rate of 1 mL min^{-1} while the columns temperature was maintained at 40 °C. Polymer solutions (3−5 mg mL^{-1}) were injected in 50 μL volumes. Calibration was performed with narrow polydispersity polystyrene standards (Polymer Laboratories) in the range 0.5−1000 kDa, and SEC traces were elaborated with Cirrus 2.0 software (PL). LC-MS analysis was performed with a ThermoMAT high-pressure liquid chromatography system consisting of a solvent degasser, a quaternary pump, an autoinjector, and a dual-wavelength UV detector and equipped with a C8 Luna column (Phenomenex, 150 × 4.6 mm, 100 Å pore size, 5 μm particle size). The system was interfaced to a Thermo Finnigan LCQ Deca ion-trap mass spectrometer (Thermo Finnigan, San José, CA) equipped with an atmospheric pressure-ionization source operated in nebulizer-assisted electrospray mode (ESI). The instrument was calibrated with caffeine (Aldrich), MRFA (tetrapeptide, Thermo Finnigan), Ultramark 1621 (Lancaster), and poly(propylene glycol) (M_n 2700, Aldrich) in the mass range 195−3822 amu. All spectra were acquired in positive ion mode over the m/z range 100−1000 or 500−2000 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) or 350 °C (HPLC-MS). Nitrogen was used as sheath gas at a flow rate of 0.5 L min^{-1} and helium as the dumping gas. Vibrational spectra were collected in the range 500−4000 cm^{-1} with a Bruker IFS66/S FT-IR operated in transmittance mode. Unless otherwise specified, melted samples were placed between NaCl plates.

Synthesis of Methyl 6-*O*-Methacryloyl-α-D-glucoside (6-*O*-MMAGlc, 2). In a typical experiment, methyl α-D-glucoside **1** (8.0 g, 0.041 mol), Novozym 435 (4.0 g), and vinyl methacrylate (4.4 g, 0.039 mol) were introduced into a conical flask and suspended in acetonitrile (40 mL). The flask was sealed with a rubber septum and shaken at 200 rpm and 50 °C for 5 days before stopping the reaction by filtering off the enzyme. The filtrate was washed with methanol (100 mL), and the collected organic phases were rotary evaporated to dryness to yield a yellow-brown syrup. The gross product was then purified by flash chromatography, and the collected fractions were checked by TLC for the presence of the product (R_f 0.39). The pooled fractions were then rotary evaporated to afford the title product as a clear syrup (yield 70% with respect to methyl α-D-glucoside). ESI-MS: calcd for $C_{11}H_{22}O_7N$, 280.14; found 280.07 ($M + NH_4^+$); calcd for $C_{11}H_{18}NaO_7$, 285.10; found 285.13 ($M + Na^+$). ^{1}H NMR (300 MHz, CD_3OD, 30 °C) δ (ppm): 1.84 (dd, 3 H, J = 1.5, 1.1 Hz, H-11), 3.30 (dd, 1 H, J = 10.1, 8.7

Scheme 2. Synthesis of the RAFT Agent (4-Cyanopentanoic acid)-4-dithiobenzoate 7 (CPADB)[63,a]



4 **5** **6** **7**

[a] For clarity, the position numbering of 7 used for NMR peaks assignment is also reported (see Experimental Section). Conditions: (i) elemental sulfur, sodium methoxide, methanol, 70 °C, 10 h; (ii) potassium ferricyanide, water, r.t., 1 h; (iii) 4,4′-azobis(cyanopentanoic acid), ethyl acetate, 70 °C, 28.5 h.

Table 1. Summary of Polymerization Experiments

run no.	monomer (M)	RAFT agent (mM)	reaction time (min)	conversion (%)[c]	M_n (kDa) theory	M_n (kDa) NMR[d]	M_n (kDa) SEC[e]	M_w/M_n SEC	polymer
1	**2** (0.87)[a]	CPADB (2.0)	100	99	113 700	103 000	70 700	1.17	$\mathbf{3}_{(391)}$
2	**2** (0.87)[a]	CPADB (4.2)	140	100	54 300	58 600	41 600	1.18	$\mathbf{3}_{(222)}$
3	**2** (0.87)[a]	CPADB (8.1)	103	98	27 500	22 100	22 100	1.10	$\mathbf{3}_{(83)}$
4	**2** (0.85)[a]	CPADB (17)	220						
5	HEMA (0.88)[b]	$\mathbf{3}_{(83)}$ (1.4)	75				45 000	1.20	8

[a] Initiator concentration: 3.8 mM. [b] Initiator concentration: 2.0 mM. [c] SEC, calculated from the relative area of monomer and polymer peaks. [d] ^{1}H NMR, calculated from the ratio between the RAFT agent end-of-chain aromatic protons and the H-1 proton of the repeating unit. [e] Polystyrene equivalents.

H-4), 3.39 and 3.40 (4H, s, H-7 and dd, $J = 9.2$, 3.8 Hz, H-2), 3.62 (dd, 1 H, $J = 9.2$, 9.2 Hz, H-3), 3.75 (ddd, 1 H, $J = 9.9$, 6.4, 1.9 Hz, H-5), 4.25 (dd, 1 H, $J = 11.8$, 6.7 Hz, H-6), 4.45 (dd, 1 H, $J = 11.8$, 2.1 Hz, H-6), 4.65 (d, 1 H, $J = 3.7$ Hz, H-1), 5.62 (m, 1 H, $J = 1.6$ Hz, H_Z-10), 6.11 (dd, 1 H, $J = 1.6$, 1.0 Hz, H_E-10). ^{13}C NMR (CD_3OD) δ (ppm): 18.37 (C-11), 55.50 (C-7), 65.19 (C-6), 71.09 (C-5), 71.96 (C-4), 73.50 (C-2), 75.07 (C-3), 101.26 (C-1), 126.26 (C-10), 137.72 (C-9), 168.70 (C-8). FTIR (NaCl plates) ν (cm^{-1}): 3378 (vs, br, $\nu_{\text{O-H}}$), 2930, 2838 (s, $\nu_{\text{C-H}}$), 1720 (vs, $\nu_{\text{C=O}}$), 1637 (m, $\nu_{\text{C=C}}$). After characterization, the monomer was dissolved in water (HPLC grade, pH 7) to give a 1.00 M solution that was then stored in a freezer.

Synthesis of (4-Cyanopentanoic acid)-4-dithiobenzoate (CPADB, 7). The RAFT agent was prepared according to the method described in the literature[63] with only minor changes, and its structure was confirmed via ^{1}H and ^{13}C NMR (Scheme 2). Overall yield 39% (based on the initial moles of benzyl chloride). ^{1}H NMR ($CDCl_3$) δ (ppm): 10.16 (br, 1 H, OH), 7.87 (m, 2 H, H-9), 7.53 (tt, 1 H, H-11), 7.36 (m, 2 H, H-10), 2.34−2.74 (m, 4 H, H-2, H-3), 1.93 (s, 3 H, H-5). ^{13}C NMR ($CDCl_3$) δ (ppm): 24.04 (C-5), 29.44 (C-2), 33.00 (C-3), 118.28 (C-6), 126.57 (C-10), 128.47 (C-9), 132.93 (C-8), 144.42 (C-11), 176.50 (C-1), 222.14 (C-7). FTIR (NaCl plates) ν (cm^{-1}): ∼3000 (s, br, $\nu_{\text{O-H}}$), 1711 (vs, $\nu_{\text{C=O}}$), 1230 ($\nu_{\text{C=S}}$), 1048 (m, $\rho_{\text{C-H ring}}$).

Homopolymerization of 6-*O*-MMAGlc. In a typical experiment (run 3, Table 1) the monomer solution (3.0 mL, 3.0 × 10^{-3} mol) was introduced in a Schlenk tube and mixed with an ethanol solution of 4,4′-azobis(4-cyanopentanoic acid) (ACPA, 5.98 × 10^{-2} M, 220 μL, 1.32 × 10^{-5} mol) and (4-cyanopentanoic acid)-4-dithiobenzoate (CPADB, 1.20 × 10^{-1} M, 235 μL, 2.81 × 10^{-5} mol). The tube was then sealed with a rubber septum, degassed with four freeze−evacuate−thaw cycles, and transferred to an oil bath preheated to 70 °C. Aliquots of solution (200 μL) were drawn every 10 min from the reaction mixture using a gastight syringe prepurged with nitrogen and fitted with a 0.72 mm o.d. needle. The sampled solution was split in two, quenched in ice−water, and stored in a refrigerator. At the end of the polymerization, all collected samples were freeze-dried for 2 h and redissolved in DMAc 0.05% BHT for SEC analysis. The remaining polymer was recovered (159 mg) by precipitation in excess methanol followed by centrifugation and freeze-drying. Total reaction time: 103 min. Conversions were calculated directly from the SEC chromatograms using the formula

$$x = \frac{A_{\text{polymer}}/\kappa}{A_{\text{polymer}}/\kappa + A_{\text{mono}}} \tag{1}$$

where A_{polymer} and A_{monomer} are the area of the polymer and monomer peaks, respectively, and κ is the ratio between the specific refractive index of polymer and monomer.

$$\kappa = \frac{(\mathrm{d}n/\mathrm{d}c)_{\text{polymer}}}{(\mathrm{d}n/\mathrm{d}c)_{\text{monomer}}} \tag{2}$$

A value of 1.19 was determined for κ from repeated SEC injections of narrow polymer and monomer samples of known concentration. Final conversion: 98%. M_n (SEC) 22 100; PDI 1.10. ^{1}H NMR (500 MHz, D_2O, 40 °C) δ (ppm): 0.98 and 1.10 (CH_2 chain), 1.9 (11-H), 3.39 (4-H), 3.46 (H-7), 3.57 (2-H), 3.67 (3-H), 3.81 (5-H), 4.07 (6-H), 4.36 (6-H), 4.81 (1-H), 7.55 (H_{meta} arom), 7.73 (H_{para} arom), 7.73 (H_{orto} arom). ^{13}C NMR (500 MHz, D_2O, 40 °C) δ (ppm): 17.38 (C-11), 45.5 (C-9), 54.5 (C-10), 55.75 (C-7), 65.22 (C-6), 69.78 (C-5), 70.62 (C-4), 71.89 (C-2), 73.82 (C-3), 99.86 (C-1), 179.5 (C-8).

Chain Extension of Poly(6-*O*-MMAGlc) with 2-Hydroxyethyl Methacrylate. HEMA (426 mg, 3.28 × 10^{-3} mol) and poly(6-*O*-MMAGlc) $\mathbf{3}_{83}$ (116 mg, 1.75 × 10^{-3} mol) were weighed into a 10 mL flask and diluted with a 50% water−ethanol solution (HPLC grade, 3.0 mL). Once the macro-RAFT agent was completely dissolved, 4,4′-azobis(4-cyanopentanoic acid) was added as ethanol solution (4.44 × 10^{-2} M, 300 μL, 1.33 × 10^{-5} mol). The resulting stock solution was divided into six Schlenk tubes, degassed with three cycles of freeze−evacuate−thaw, and transferred to a water bath preheated to 60 °C. Every 15 min, a tube was removed from the bath and plunged into ice−water for 5 min. At the end of the polymerization, an aliquot (200 μL) was drawn from each sample and diluted with DMAc 0.05% BHT (2.00 mL) for SEC analysis. Final M_n (SEC) 45 000; PDI 1.20.

Results and Discussion

Monomer Synthesis. The lipase-catalyzed acylation of methyl α-D-glucoside **1** with vinyl methacrylate was performed in neat acetonitrile at 50 °C (Scheme 1). Novozym 435 (a commercially available immobilized lipase) was chosen as the catalyst because of its ability to catalyze regioselective esterification and transesterification reactions in a number of organic solvents (i.e., THF, acetone, acetonitrile, pyridine)[64−66] and because it can be easily recovered from the reaction medium by filtration. After 5 days of shaking, unreacted methyl α-D-glucoside could still be seen at the bottom of the reaction flask, while TLC analysis showed that a new compound with R_f 0.39 was present in the reaction mixture. This indicates that the reaction proceeds at a much slower

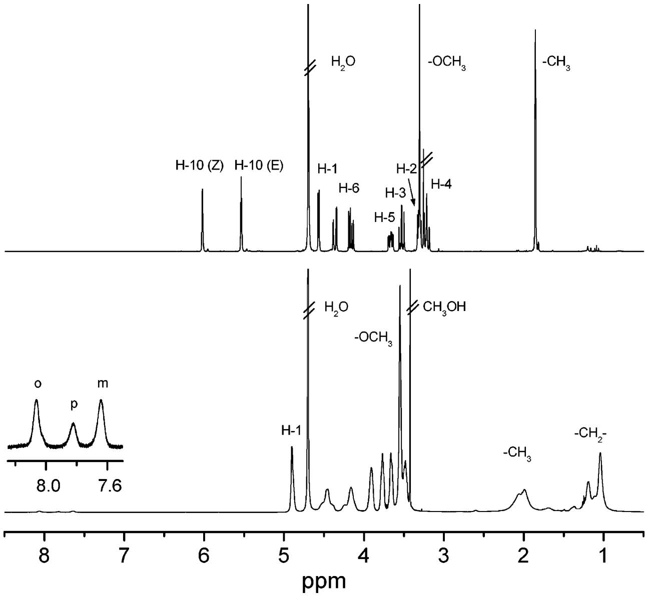


Figure 1. ^{1}H NMR spectrum of methyl 6-*O*-methacryloyl-α-D-glucoside **2** (top, 300 MHz, CD_3OD, 30 °C) and poly(methyl 6-*O*-methacryloyl-α-D-glucoside) $\mathbf{3}_{(83)}$ (bottom, 500 MHz, D_2O, 40 °C). See Scheme 1 for nuclei numbering.

rate than previously observed for the acylation of **1** with divinyladipate under identical conditions. In the latter case, quantitative consumption of the substrate occurred within 24 h of reaction.[67] Such observation is consistent with a report by T. Kobayashi on the selectivity for various carboxylic acids of lipase B from *Candida antarctica*.[68] The study shows how both the presence of conjugation with the carboxyl group and of branching in the non-carboxylic region independently lower the $V_{\max}/K_M$ value by an order of 1, where $V_{\max}$ is the maximum reaction rate and K_M is the Michaelis constant. Following chromatographic purification **2** was recovered in high purity with a 70% yield, and its structure was confirmed by correlation NMR experiments (^{1}H−^{1}H COSY, ^{1}H−^{13}H HMQC, and HMBC). The top panel of Figure 1 shows the ^{1}H NMR spectrum of the purified product, in which the two vinyl protons and the α-anomeric proton can be clearly distinguished. For clarity, the nuclei numbering used in peaks assignment is also reported. Comparison between the ^{13}C spectrum of **2** and that of the starting methyl α-D-glucoside reveals that the greatest change in chemical shift occurred for C-5 (−2.43 ppm) and C-6 (+2.49 ppm), while other signals of the sugar ring were virtually unchanged. Both sign and entity of these shifts are consistent with the studies of Y. Tsuda et al. on the positional isomers of *O*-acylglucopyranosides[69] and confirm that the acylation took indeed place in position 6.

RAFT Polymerization of 6-*O*-MMAGlc. Vinyl glycomonomer **2** was polymerized directly in water−ethanol mixtures without the need to resort to protective group chemistry. Addition of about 10% v/v of ethanol to the reaction medium completely dissolved of the RAFT agent without the need to alter the solution pH and with no noticeable effect on the resulting polymers' solubility. This approach was favored over the use of a slightly basic pH since the latter accelerates (4-cyanopentanoic acid)-4-dithiobenzoate (CPADB) hydrolysis as well and depending on the specific system investigated can lead to a loss of control over the polymerization process.[70] Thus, four experiments were conducted using different RAFT agent concentrations while holding all other parameters constant, and their results are summarized in Table 1 (runs 1−4).

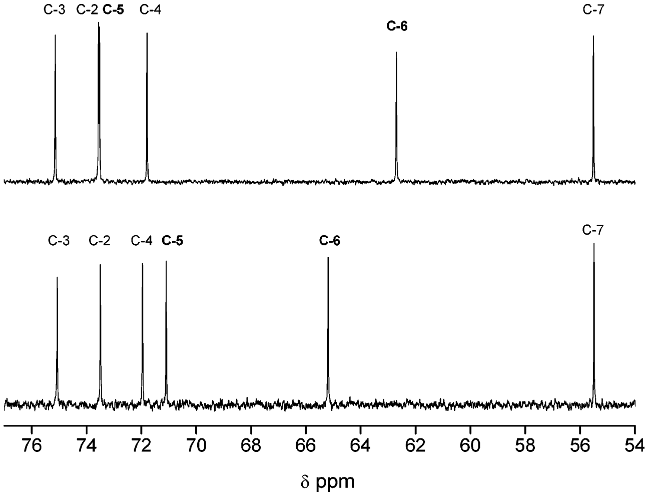


Figure 2. Expanded region of the ^{13}C NMR spectrum of α-D-glucopyranose (top) and methyl 6-*O*-methacryloyl-α-D-glucoside **2** (bottom). Both spectra were collected in CD_3OD at 300 MHz. See Scheme 1 for nuclei numbering.

The final molecular weights of polymers from runs 1 to 3 as determined by ^{1}H NMR are in excellent agreement with the theoretical values calculated from the formula

$$M_n = M_M x \frac{[M]_0}{[RAFT]_0} + M_{RAFT} \tag{3}$$

where M_M and M_{RAFT} are the molecular weights of monomer and RAFT agent, respectively, x is the conversion, and $[M]_0$ and $[RAFT]_0$ are the initial concentrations of monomer and RAFT agent.[71] Average molecular weights measured by size exclusion chromatography (SEC) were considerably lower instead, most probably due to the inadequacy of polystyrene standards to approximate the hydrodynamic volume of poly(6-*O*-MMAGlc) in *N*,*N*-dimethylacetamide. Figure 1 (bottom) shows the ^{1}H NMR of $\mathbf{3}_{(83)}$ in D_2O at 40 °C. A slightly higher temperature was used to collect polymer spectra in order to reduce samples viscosity and improve resolution. All the glucoside signals can be identified in the region 3.3−5.0 ppm and easily correlated with those of the starting monomer (top). Enlargement of the aromatic area clearly displays three signals from the end-of-chain dithiobenzoyl group, while polymer backbone alkenyl protons can be seen at 0.98 and 1.10 ppm. The shifting to lower fields of the anomeric proton signal (about 0.16 ppm) is entirely due to the change in solvent and is not observed when the polymer spectrum in D_2O is compared to that of methyl α-D-glucoside in the same solvent (see Supporting Information).

Intermediate molecular weight distributions and conversions were obtained from SEC analysis of samples collected at increasing reaction times using eq 1. The evolution of the molecular weight distribution with time for run 3 is shown in Figure 3. The distribution shifts to higher molecular weights and becomes narrower as the polymerization proceeds. For all experiments, no high molecular weight shoulder appeared in the acquired SEC traces even at 100% conversion. When the SEC M_n values were plotted against conversion, the former linearly increased up to quantitative monomer consumption as expected for a well-controlled living

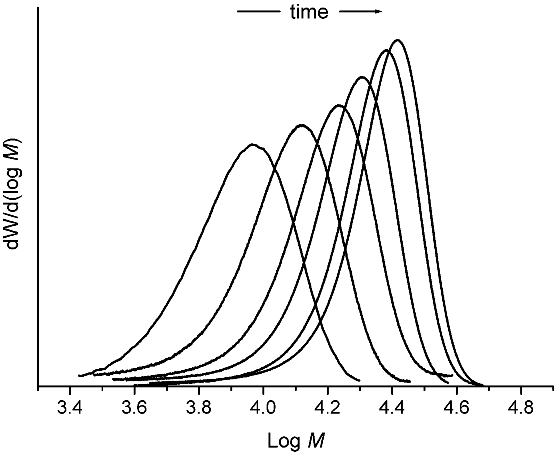


Figure 3. Example of evolution of the molecular weight distribution with conversion for the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside **2** (run 3 in Table 1). From left to right, the curves correspond to 16%, 39%, 62%, 70%, 89%, and 95% conversion, respectively. Normalized areas; SEC, polystyrene equivalents.

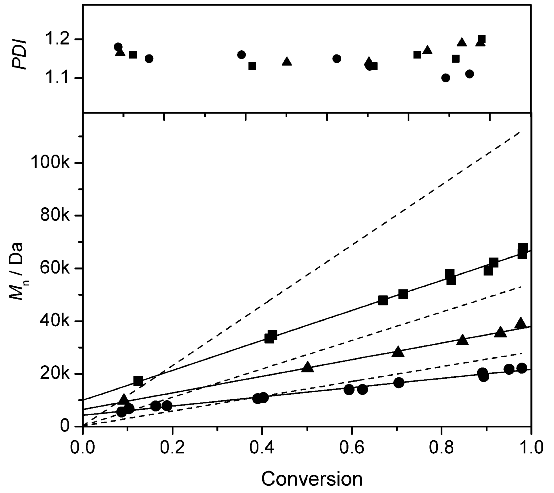


Figure 4. Evolution of the molecular weight and polydispersities with conversion for the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside **2**. [RAFT] (mM) = 2.0 (■), 4.2 (▲), and 8.1 (●), all other parameters being identical. Full line: linear regression of experimental values; dashed line: theoretical value.

process (Figure 4, bottom panel). However, the experimental trend lines are systematically lower than the theoretical lines above ~20−40% conversion and systematically higher than the theoretical lines below this range; moreover, the extrapolated lines cross the M_n axis between 4000 and 10 000 Da at zero conversion. This observation might suggest hybrid behavior between conventional and living free radical polymerization for our system,[72,73] but at this stage we think that it is rather the result of the use of a polystyrene calibration. Similar problems with polystyrene-equivalent molecular weights of glycopolymers have been previously reported in the literature.[74−77] The polydispersity index remained lower than 1.2 throughout the polymerization in all cases, with final values in the range 1.10−1.18. When a 8.1 mM concentration of RAFT agent was used (run 3), PDI values showed a slightly decreasing trend with conversion, whereas with lower concentrations of RAFT agent a decrease of the polydispersity index in the first half of the polymerization was followed by an increase of comparable magnitude in the second half (Figure 4, top panel).

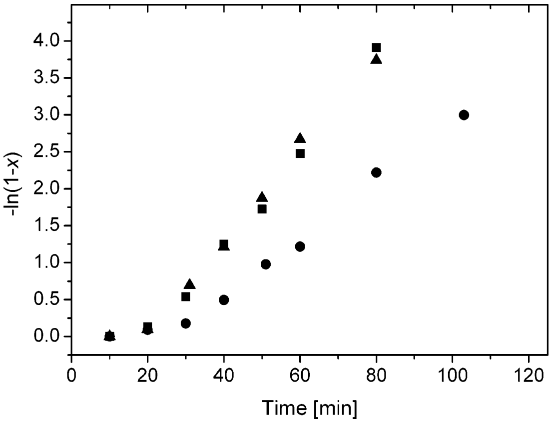


Figure 5. First-order plot for the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside **2** with various RAFT agent concentrations. [CPADB] (mM) = 2.0 (■), 4.2 (▲), and 8.1 (●), all other parameters being identical.

Reactions in runs 1−3 were very fast, especially if the relatively low monomer concentration is taken into account (~0.9 M), and reached completion in about 80−100 min. When −ln(1 − *x*) was plotted against the reaction time, it clearly showed that after an initial inhibition period of 20−30 min all reactions proceeded with pseudo-first-order kinetics. While little or no difference could be observed between runs 1 and 2 ([RAFT] = 2.0 and 4.2 mM, respectively), a higher RAFT agent concentration induced a longer inhibition period (30 min) and a slower overall polymerization rate (Figure 5).

Retardation and inhibition in dithiobenzoate-mediated RAFT polymerization experiments are well-known phenomena, and their origin is still the subject of a lively debate.[78−84] In this case, to further investigate this effect an experiment was conducted using twice the CPADB concentration as in run 3 ([CPADB] = 17 mM, run 4 in Table 1). In addition to the protocol followed for previous polymerizations, an extra 100 μL of reaction mixture was sampled at increasing times, diluted with 2.0 mL of 50:50 water−acetonitrile/1 mM $NH_4^+HCOO^-$ and subjected to electrospray ionization mass spectrometry (ESI-MS). As a result, no polymer peak appeared in the SEC chromatograms throughout the nearly 4 h of reaction, and dropwise addition of the remaining solution to excess methanol did not afford any precipitate; complete inhibition of the polymerization process seemed to have occurred. Electrospray ionization mass spectrometry (ESI-MS) has already been propitiously used in our group for end group assignment and mechanistic elucidation of the RAFT process.[85−87] Figure 6 shows the mass spectrum obtained after 138 min: when isotopic effects are discounted, a series of six evenly spaced peaks 262 atomic units apart can be clearly distinguished, a value exactly matching the monomer mass (262.11 Da). Also, labeled peaks can be assigned to $M{\cdot}NH_4^+$ ions of **3** with a degree of polymerization ranging from 1 (*m*/*z* 559.1, calculated 559.17) to 6 (*m*/*z* 1869.4, calculated 1869.74), thus proving the production of low molecular weight oligomers. This result clearly indicates that the polymerization process had not been inhibited but rather dramatically slowed down, an effect consistent with slow reinitiation during the preequilibrium of the RAFT process.[81] In a recent paper, A. B. Lowe et al. have also described the use of CPADB **7** as RAFT agent for the polymerization of 2-methacryloxyethyl glucoside (a methacrylate-type glycomonomer)

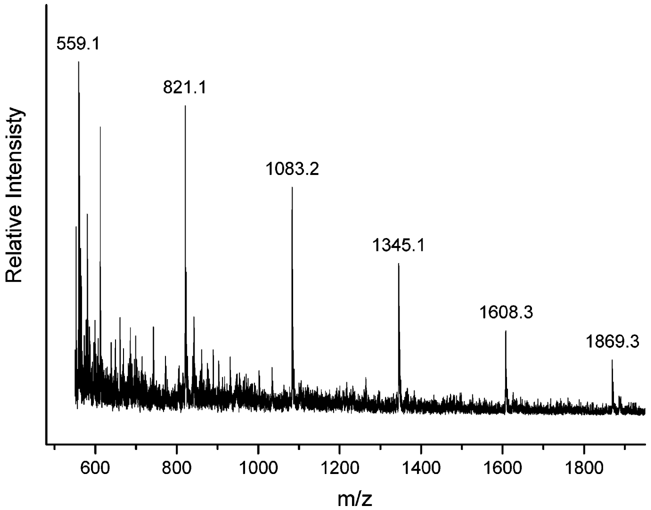
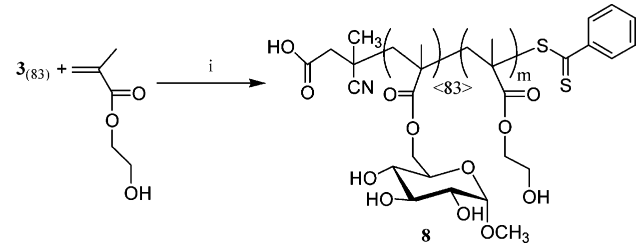


Figure 6. ESI-MS spectrum of methyl 6-*O*-methacryloyl-α-D-glucoside oligomers obtained from run 4 (see Table 1). The labeled peaks correspond to NH_4^+ molecular ions of **3** with increasing degrees of polymerization (Scheme 1).

Scheme 3. Chain Extension of Poly(methyl 6-*O*-methacryloyl-α-D-glucoside) $3_{(83)}$ with 2-Hydroxyethyl Methacrylate and Block Copolymer Obtained Thereof[a]



[a] Conditions: (i) 4,4′-azobis(4-cyanopentanoic acid), water−ethanol 1:1, 60 °C, 75 min.

under experimental conditions very similar to those used in this study ($[M]_0 = 1.63$ M, $[ACPA]_0 = 1.6$ mM, $[CPADB]_0 = 8.0$ mM, aqueous solution, 70 °C, syringe sampling every ~20 min). In that case, no inhibition was observed, maybe because of the higher initial monomer concentration. Unfortunately, the effect of a systematic variation in the RAFT agent concentration was not investigated.

Synthesis of Poly[(6-*O*-MMAGlc)-*b*-(HEMA)]. To further corroborate the livingness of the glycopolymers obtained from our experiments, the reprecipitated polymer from run 3 was used as macro-RAFT agent for the polymerization of HEMA (Scheme 3). Figure 7 shows the evolution of the SEC polymer traces with time for samples reacted between 15 and 75 min; as a reference, the chromatogram at 0 min corresponding to the initial macro-RAFT agent is also displayed. The peak molecular weight constantly shifted to higher values at the proceeding of the reaction, while a small shoulder with the same retention time as the initial block can still be seen around 1850 s. Also, no high molecular weight shoulder appears in the SEC traces, indicating a low occurrence of bimolecular termination. Clearly, chain extension took place in a living fashion, although some of the initial polymer was not reinitiated.

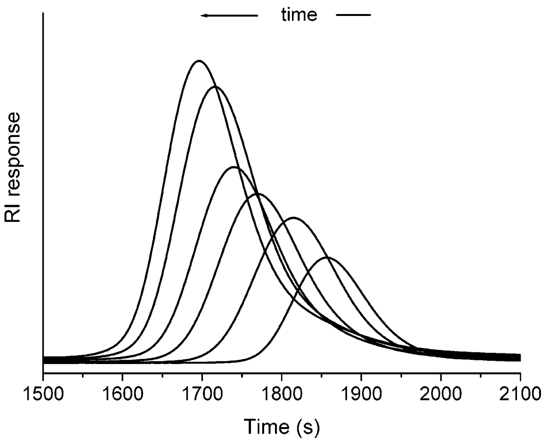


Figure 7. Evolution of the SEC traces with time for the chain extension of poly(methyl 6-*O*-methacryloyl-α-D-glucoside) $3_{(83)}$ with HEMA (polymer region). From right to left, the curves correspond to reaction times of 0, 15, 30, 45, 60, and 75 min, respectively.

This was probably due to the macroRAFT agent having been polymerized to high conversion (98%), thus decreasing the percentage of end-of-chain dithiobenzoyl groups present in the final polymer. Above ~95% conversion, the residual monomer concentration is low enough to allow competition between propagation and termination of the macroradicals. Since termination is chain length dependent and its rate coefficient decreases with chain length,[88,89] the majority of termination reactions occur between growing macroradicals and the primary radicals constantly supplied by the initiator decomposition. This has little effect on the molecular weight or polydispersity of resulting polymer but significantly reduces its chain end functionality and its ability to form copolymers.[90] Also, dead chains may have been produced during the purification of the polymer, as already noticed in the case of dithiobenzoyl-terminated poly(methyl methacrylate).[91] The final value for M_n was 45 000 Da (polystyrene equivalent) with a polydispersity index of 1.20. At the end of the polymerization, all samples were completely soluble, and no cross-linked material could be detected even after 75 min of reaction. This is consistent with what reported by S. P. Armes et al., who have shown that the use of methanol or water−methanol mixtures suppresses cross-linking in the living polymerization of HEMA via ATRP.[92]

Conclusion

The novel glycomonomer methyl 6-*O*-methacryloyl-α-D-glucoside was synthesized in high yield from the lipase-catalyzed, regioselective acylation of methyl α-D-glucoside with vinyl methacrylate. Living radical polymerization of the unprotected monomer was directly performed in water−ethanol mixtures using (4-cyanopentanoic acid)-4-dithiobenzoate as the RAFT agent and well-defined poly(methyl 6-*O*-methacryloyl-α-D-glucoside)s with molecular weights in the range 22−70 kDa were obtained in a quantitative way and with a polydispersity as low as 1.10. When RAFT agent concentrations up to 8.1 mM were used, all reactions achieved completion within 100 min, while a higher concentration rendered the process impractically slow. Finally, chain extension with 2-hydroethyl methacrylate of one of the obtained glycopolymers afforded hydrophilic−hydrophilic block copolymers with various HEMA contents and without any noticeable cross-linking. In conclusion, RAFT polymerization proves to be a viable method for the direct synthesis of well-defined glycopolymers in aqueous media without the need to resort to protective group chemistry. Our group is now studying the synthesis of new glycomonomers of biological relevance as well as the preparation of new, well-defined glycopolymer architectures for medical applications.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of methyl α-D-glucoside (D_2O and CD_3OD), methyl 6-*O*-methacryloyl-α-D-glucoside (CD_3OD), (4-cyanopentanoic acid)-4-dithiobenzoate ($CDCl_3$), $\mathbf{3}_{(83)}$ (D_2O), $\mathbf{3}_{(222)}$ (D_2O), and $\mathbf{3}_{(391)}$ (D_2O). This material is available free of charge via the Internet at http://pubs.acs.org.

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