Sugar-Coated Amphiphilic Block Copolymer Micelles from Living Radical Polymerization: Recognition by Immobilized Lectins

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ABSTRACT: Block copolymers poly[((ethylene glycol)methyl ether methacrylate)-*b*-(benzyl methacrylate)] containing protected sugar groups were synthesized by transition-metal-mediated living radical polymerization. Sugar-derived initiators were prepared by esterification of the hydroxyl group of isopropylidene-protected galactose and glucose with 2-bromoisobutyryl bromide. Both protected galactose and glucose-derived initiators were respectively used to initiate the hydrophilic monomer (PEGMA) and the hydrophobic monomer (BzMA) in both one-pot and two-step polymerizations. In both cases the polymerization occurred with good first-order kinetics, producing AB blocks with molecular weights close to that targeted and with low polydispersity (1.1−1.2). Following removal of the isopropylidene protective groups via acidolysis with 50% trifluoroacetic acid (TFA) at ambient temperature, block copolymer micelles were obtained by a dialysis solvent exchange process. The size and polydispersity of the polymer micelle were estimated by dynamic light scattering, and the *Z*-averaged hydrodynamic diameters were found to be between 35 and 41 nm with a unimodal size distribution. Furthermore, the binding ability of a galactose bearing copolymer micelles was confirmed by recognition with an HPLC column packed with immobilized RCA-1 lectin.

Introduction

The self-association of amphiphilic block copolymers into supramolecular assemblies has been well-documented in the scientific literature and is attracting a growing interest for application as functional nanofabricated materials.[1−5] Amphiphilic block copolymers can form "polymeric micelles" and "polymeric aggregates", in selective solvents consisting of a core formed by the solvent insoluble part of the macromolecule surrounded by a shell of solvated "blocks" which offer potential as carrier systems for hydrophobic drugs and other active molecules.[6−9]

A further challenge in this area is cellular-specific targeting, which would allow for the recognition and binding of the micelle/aggregate host to the target site. Recent progress in glyco-biology shows that cell surface oligosaccharides play essential roles in various biological recognition processes.[10] These recognition processes are often based on carbohydrate−protein interaction and are expected to be one of the most promising routes in cellular-specific drug targeting. In this respect, synthetic polymers incorporating sugar residues are being developed from different synthetic routes.[11] Among these glycopolymers, amphiphilic block copolymers containing sugar residues have been synthesized via the living cationic polymerization of isobutyl vinyl ethers and vinyl ethers having *N*-acetyl-D-glucosamine residues[12] and by atom transfer radical polymerization (ATRP) using sugar-carrying methacrylate and acrylate monomers as the hydrophilic section and styrene as the hydrophobic segment.[13,14]

An elegant method has been proposed by Kataoka et al. using suitably derivatized simple sugars as initiators for the ring-opening polymerization of both ethylene oxide and lactides.[15] This attracted our attention, and we set out to extend this idea to living radical polymerization that would allow for flexibility in the design of amphiphilic block copolymers using the available methacrylate and acrylate monomers. Copper-mediated living radical polymerization, often called atom transfer radical polymerization (ATRP), can proceed with almost an absence of irreversible chain transfer and chain termination.[16] This type of polymerization allows for the synthesis of well-defined block copolymers of (meth)-acrylates.[17] In particular, it is relatively easy to prepare amphiphilic block copolymers.[18] Diblock and triblock copolymers with different hydrophobic segments[19] have been reported, which exhibit self-assembly behavior into polymeric micelles in aqueous media. A powerful aspect of this type of polymerization is the potential to introduce α-functionality via the initiator. Simple condensation of an alcohol precursor with 2-bromoisobutyryl bromide leads to a diverse array of functional initiators and subsequently to functional polymers.[20,21]

Herein we describe the synthesis of glucose and galactose derived monofunctional initiators, which have been used as initiators for the preparation of amphiphilic block copolymers by living radical polymerization. The hydrophilic monomer poly(ethylene glycol)methyl ether methacrylate and the hydrophobic monomer, benzyl methacrylate, were used to synthesize amphiphilic block copolymers. These block copolymers have been demonstrated to form polymeric micelles in water, which are recognized by an appropriate lectin.

Experimental Section

Materials. Poly(ethylene glycol) methyl ether methacrylate (M_n = 475 g mol^{-1}) (MeO-PEGMA) and benzyl methacrylate (BzMA), supplied by Aldrich, were degassed before use. CuBr (Aldrich, 98%), triethylamine (BDH, 99%), 2-bromoisobutyryl bromide (Lancaster, 98%), 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (Aldrich, 98%), 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose glucofuranose (Aldrich, 98%), trifluoroacetic acid, toluene, dichloromethane, and tetrahydrofuran were used as received. *N*-(*n*-Propyl)-2-pyridylmethanimine ligand was synthesized as previously described.[22] Immediately prior to po-

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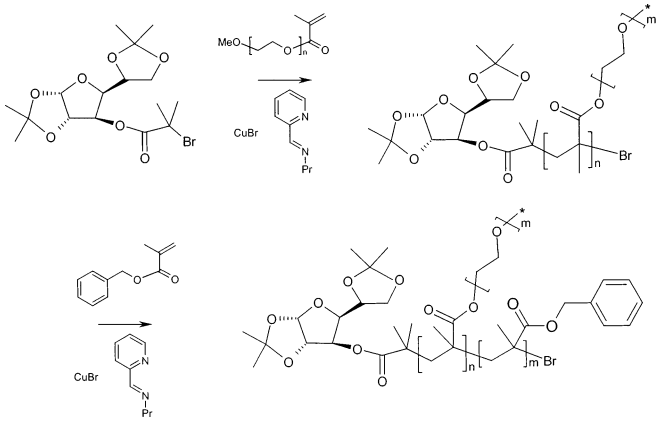


Figure 1. Synthesis of glucose and galactose derived initiators **1** and **2**.

lymerization, all solvents, monomers, and other reagents were degassed via a minimum of three freeze−pump−thaw cycles. All manipulations were carried out under a nitrogen atmosphere using standard Schlenk or syringe techniques.

Characterization. ^{1}H NMR and ^{13}C NMR were carried out on Bruker-DPX 300 and 500 MHz instruments. Molecular weight and molecular weight distribution of polymers were measured by size exclusion chromatography on a system equipped with a guard column 2 mixed D columns (Polymer Laboratories, mixed pore size), with both DRI and UV detectors, and eluted with tetrahydrofuran at 1 mL min^{-1}. Molecular weights were calculated against narrow PMMA standards. FTIR spectra were recorded on a Bruker VECTOR 22 instrument with fitted an attenuated total reflection (ATR) cell. Hydrodynamic diameters of the polymer micelles and aggregates were estimated by dynamic light scattering (DLS) at 25 °C, using a Zetasizer 3000 HS_A with a polarized incident beam at 633 nm supplied by a 10 mW He−Ne laser (Malvern Instruments). A scattering angle of 90° was used in this study with the CONTIN algorithm as supplied by Malvern Instruments.

Interaction between the sugar end chain and RCA-1 Lectin was determined by passing the polymer into a column packed with RCA-1 immobilized beads (Shodex Afpak ARC 894, Showa Denko) where the commercial column has the lectin covalently attached to the support precluding elution during analysis. Measurements were performed at ambient temperature at a flow rate of 0.8 mL min^{-1} using a liquid chromatograph (JASCO) equipped with UV detector (JASCO, $\lambda = 254$ nm). A 1/15 M phosphate buffer (pH = 7.4) containing 0.15 M NaCl was used as eluent.

Synthesis of 3-Isobromobutyryl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (1) (Figure 1). 1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose (10 g, 0.038 mol) and 75 mL of dichloromethane were placed in a 250 mL three-neck flask, equipped with a magnetic stirrer. The solution was cooled to 0 °C with an ice/water bath prior to the addition of 6.3 mL of triethylamine (1.2 equiv), followed by dropwise addition of 5.7 mL of 2-bromoisobutyryl bromide (1.2 equiv). The solution was stirred at ambient temperature overnight. The resulting yellow solution was washed with water, K_2CO_3, and finally water. The organic layer was dried with $MgSO_4$, filtered, and solvent removed in vacuo to give a yellow oil. Yield 78% (12.3 g) ($C_{16}H_{25}BrO_7$: M = 409.29 g mol^{-1}); purity determined by ^{1}H NMR.

^{1}H NMR ($CDCl_3$, 300 MHz) δ_{ppm}: 5.84 (s, 1H, C*H*), 5.83 (s, 1H, C*H*), 4.43−3.95 (m, 5H, CH_2 and C*H*), 1.87 (s, 6H, (CH_3)$_2$-Br), 1.46−1.25 (m, 12H, CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_{ppm}: 170.3 (C=O), 112.88 and 109.84 (quaternary carbons), 105.57 (*C*H), 83.09, 80.49, 77.84, 72.65, 68.01 (*C*H$_2$), 55.66 (*C*−Br), 30.97 ((*C*H$_3$)$_2$−C−Br) 27.15, 26.61, 25.6 ((*C*H$_3$)$_2$−C−O). Mass spectra (EI): molecular peak at $M = 409$ m/z, M − 80 = 329 (Br), M − 150 = 259 (CO−C(CH$_3$)$_2$Br). Accurate mass (EI): MH^+ theoretical = 409.086; MH^+ measured = 409.102. FT-IR (ATR) ν_{max}/cm^{-1}: 1736 υ(C=O).

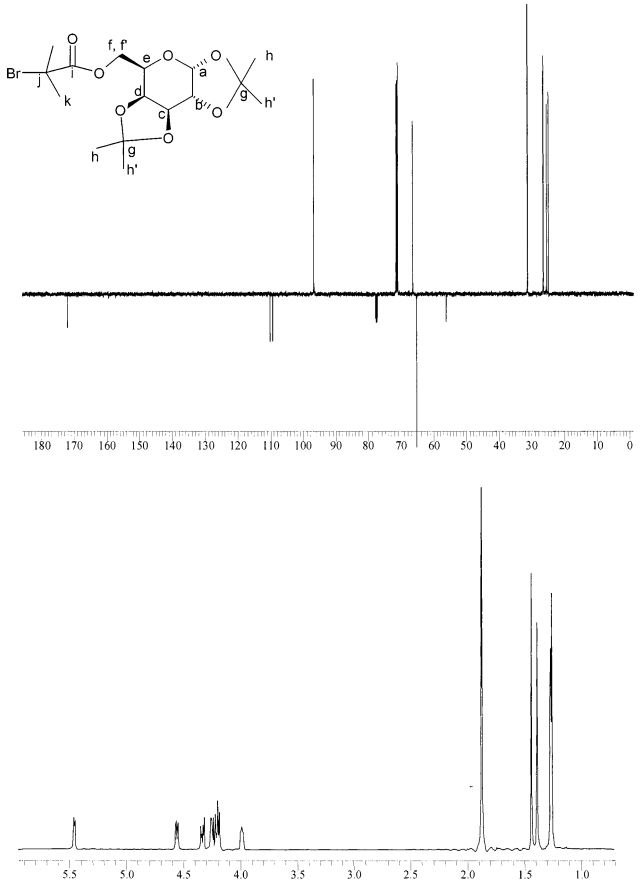


Figure 2. ^{13}C and ^{1}H NMR spectra (500 MHz) of 6-isobromobutyryl-1,2:3,4-di-*O*-isopropylidene-galactopyranose, **2**.

Synthesis of 6-Isobromobutyryl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (2) (Figure 1). The synthesis was identical to that described for **1**. The organic layer was dried with $MgSO_4$ and filtered, and the solvent was removed in vacuo to give a yellow solid. Yield 67% (10.5 g) ($C_{16}H_{25}BrO_7$: M = 409.29 g mol^{-1}).

^{1}H NMR ($CDCl_3$) (Figure 2) δ: 5.45 (d, J_{ab} = 4.58 Hz, 1H, Ha), 4.55 (dd, J_{cb} = 17 Hz, J_{cd} = 6.75 Hz, 1H, Hc), 4.33 (dd, $J_{ff'}$ = 9.75 Hz, J_{ef} = 3.75 Hz, 1H, Hf), 4.27−4.18 (m, 3H, Hb, Hf′, Hd), 3.98 (td, J_{ed} = 1.5 Hz, 1H, He), 1.88 (s, 6H, Hk), 1.45 (s, 3H, Hh′), 1.39 (s, 3H, Hh″), 1.28 (d, 6H, Hh). ^{13}C NMR ($CDCl_3$) (Figure 2) δ: 171.9 (Ci), 110.0 (Cg), 109.2 (Cg′), 96.6 (Ca), 71.4 (Cd), 71.1 (Cc), 70.8 (Cb), 66.3 (Ce), 65.1 (Cf), 56.0 (Cj), 31.2 (Ck), 26.4 (Ch), 25.4 (Ch′), 24.8 (Cb″). Mass spectra; electrospray ionization (ESI) in 25 μm in 1:1 chloroform: methanol, MH^+ theoretical = 409.085 60, MH^+ measured = 409.086 16, MNH_4^+ theoretical = 426.112 19, MNH_4^+ measured = 426.111 80, MNa^+ theoretical = 431.067 58, MNa^+ measured = 431.067 17. FT-IR (ATR) ν_{max}/cm^{-1}: 1733 υ(C=O).

Synthesis of Poly[((ethylene glycol)methyl ether methacrylate)-*b*-(benzyl methacrylate)] Terminated by Protected Sugar Group in Two Steps. *Polymerization of first block*, poly(ethylene glycol)methyl ether methacrylate with (**2**) as initiator; [M]/[I]/[Cu]/[ligand] = 20.6/1/1/2. Cu^{I}Br (0.17 g, 1.22 mmol) and protected sugar initiator (**2**) (0.5 g, 1.22 mmol) were placed with a magnetic stirrer bar in a dry Schlenk flask. The solution was evacuated and flushed with nitrogen three times. Poly(ethylene glycol)methyl ether methacrylate (12.22 g, 0.025 mol), *n*-propyl-2-pyridinalmethanimine (0.73 g, 2.44 mmol), and anhydrous toluene (12 mL, 50 wt %) were added to the Schlenk tube. The solution was subsequently deoxygenated by three freeze−pump−thaw cycles and placed in an oil bath at 60 °C. The reaction was left for a period of 7 h, reaching a conversion of 80%, prior to being quenched at ambient temperature and purified by passing the solution through a short column of basic alumina. The polymer was recovered by evaporation of the volatiles under vacuum. The

conversion was determined by ^{1}H NMR of samples removed periodically by comparison of the integration peaks from the monomers (COOCH_2, 4.35 ppm) with the corresponding of the peaks from the polymer backbone (COOCH_2, 4.20 ppm). The *absolute* M_n was determined by ^{1}H NMR of the purified polymer by comparison of the integration peaks of the initiator (CH_3 between 1.5 and 1.3 ppm) with the peak corresponding to the polymer backbone (CH_3 between 1.2 and 0.6 ppm). The ^{1}H NMR also allowed checking for residual monomer in the final product.

Polymerization of the second block, poly(benzyl methacrylates); [M]/[I]/[Cu]/[ligand] = 11.4/1/1/2. Cu^{I}Br (0.037 g, 0.246 mmol) and the first block (synthesized above) (2 g, 0.246 mmol) were placed with a magnetic stirrer bar in a dry Schlenk flask. The solution was evacuated and flushed with nitrogen three times. Benzyl methacrylate (0.51 g, 2.89 mmol), *n*-propyl-2-pyridinalmethanimine (0.076 g, 0.492 mmol), and anhydrous toluene (3 mL, 50 wt %) were added to the Schlenk flask using degassed syringes. The solution was subsequently deoxygenated by three freeze−pump−thaw cycles and placed in an oil bath at 90 °C. The reaction was left for a period of 7 h, reaching a conversion of 70%, prior to being quenched to ambient temperature and purified by passing the solution over basic alumina. The polymer was recovered by evaporation of the volatiles. Conversion was determined by ^{1}H NMR on samples removed periodically by comparison of the integration peaks of the monomers (COOCH_2, 5.25 ppm) with the corresponding peaks of the polymer backbone (COOCH_2, 4.90 ppm). The molar ratio of the block was determined by^{1}H NMR of the purified polymer by comparison of the integration peaks corresponding to the block poly[((ethylene glycol)methyl ether methacrylate) (COOCH_2, 4.20 ppm) with the peak corresponding to the block poly(benzyl methacrylate) (COOCH_2, 4.90 ppm). The NMR also allowed checking for residual monomer in the final product.

^{1}H NMR ($CDCl_3$, 500 MHz) (Figure 9) δ: 7.281 (H_t), 4.909 (H_s), 4.088 (H_n), 3.660 (H_p), 3.577 (H_i), 3.392 ($H_{p'}$), 2.077, 1.892, and 1.792 ($H_l + H_q$), 1.528 and 1.318 ($H_h + H_{h'}$), 0.927 and 0.735 ($H_m + H_r$) all broad singlets.

Synthesis of Copolymer Poly[((ethylene glycol)methyl ether methacrylate)-*b*-(benzyl methacrylate)] Terminated by Protected Sugar Group in One Pot Step. [M]/[I]/[Cu]/[ligand] = 38.71/1/2. Cu^{I}Br (0.14 g, 0.98 mmol) and a protected sugar initiator (**2**) (0.4 g, 0.98 mmol) were placed with a magnetic stirrer bar in a dry Schlenk flask. The solution was evacuated and flushed with nitrogen three times. Poly(ethylene glycol)methyl ether methacrylate (4.89 g, 10.1 mmol), *n*-propyl-2-pyridinalmethanimine (0.29 g, 1.96 mmol), and anhydrous toluene (5 mL, 50 wt %) were added to the Schlenk using degassed syringes. The solution was subsequently deoxygenated by three freeze−pump−thaw cycles and placed in a oil bath at 60 °C. The reaction was left for 10 h, reaching nearly quantitative monomer conversion. The conversion was determined by ^{1}H NMR on samples removed periodically by comparison of the integration peaks from the monomers (COOCH_2, 4.35 ppm) with those corresponding to the polymer backbone (COOCH_2, 4.20 ppm). The absolute M_n was determined by ^{1}H NMR by comparison of the integration peaks from the initiator derived α-terminus (CH_3 between 1.5 and 1.3 ppm) with the peak corresponding to the polymer backbone (CH_3 between 1.2 and 0.6 ppm). Immediately following the polymerization of MeO-PEGMA, the oil bath was heated to 90 °C and benzyl methacrylate (4.98 g, 27.83 mmol) was added to the Schlenk flask by syringe under nitrogen. After 15 h, the solution was cooled to ambient temperature and passed two times through a short alumina column. The final block copolymer was recovered by removal of the volatiles. The conversion was determined by ^{1}H NMR on samples removed periodically by comparison of the integration peaks of the monomers (COOCH_2, 5.25 ppm) with the corresponding peak from the polymer backbone (COOCH_2, 4.90 ppm). The molar ratio of the block copolymer composition was determined by ^{1}H NMR of the purified polymer by comparison of the integration peaks corresponding to the block poly((ethylene glycol)-methyl ether methacrylate) (COOCH_2 at 4.20 ppm) with the peak corresponding to the block poly(benzyl methacrylate) (COOCH_2 at 4.90 ppm).

Deprotection of the Isopropylidene Sugar Residue in the Block Copolymers. Deprotection of groups from the sugar residue in the block copolymer was achieved by washing with a 50% v/v mixture of trifluoroacetic acid (TFA) and water. The solution was stirred at ambient temperature for 1 h. The clear solution was subsequently transferred into a dialysis tubing membrane (Sygma, cellulose tubing cutoff molecular weight, 2000) for dialysis against water. The dialysate was exchanged every 2 h over 2 days. The polymer was recovered by freeze-drying following dialysis. The deprotection was followed by monitoring the disappearance of protons from the isopropylidene group at 1.32 and 1.5 ppm and appearance of the hydroxyl protons obtained after their removal at δ = 2.5 ppm by ^{1}H NMR (Figure 7).

Preparation of Polymer Aggregates/Micelles. Aqueous solution of blocks copolymers were prepared following the method described by Zhang et al.[25] using solvent exchange. A solution of 2 wt % of copolymer in DMF was prepared and filtered through a 0.45 μm filter (Durapore membrane filter, Millipore). Distilled water was added to the solution at a rate of 1 drop every 10 s until a concentration of 25 wt % water was achieved. The resulting micellar dispersion was placed in a dialysis tubing (Sygma, cellulose tubing cutoff molecular weight, 2000) for dialysis against water. The dialysate was exchanged every 2 h over a period of 2 days.

Results and Discussion

Synthesis of Glucose and Galactose Derived Monofunctional Initiators. The carbohydrate-derived initiators, **1** and **2**, were prepared in one step from commercially available monohydroxyl functional isopropylidene-protected precursors via the esterification of the hydroxyl group with 2-bromoisobutyryl bromide in the C-3 position (glucose derived) or in the C-6 position (galactose derived) (Figure 1). The tertiary bromide functionality was chosen as it has already been found to be an efficient initiator for copper(I)-mediated living radical polymerization (ATRP) of various methacrylates in conjunction with pyridinal methanimine ligands.[22] The isopropylidene-protected precursors reduce the possibility of complexation and/or competitive coordination at the copper by free hydroxyl groups on the initiator, which have been found to have a dramatic effect on the rate of polymerization and thus termination reactions. The condensation reaction was followed by ^{1}H NMR (Figure 2) with the product showing a sharp singlet at δ = 1.88 ppm, assigned to the two methyl groups in the α-position of the bromide on the ^{1}H NMR and signals at 171.9 ppm (C_i), 59.0 ppm (C_j), and 31.2 ppm (C_k) in the ^{13}C NMR.

Synthesis of Poly[(ethylene glycol)methyl ether methacrylate-*b*-(benzyl methacrylate)] Terminated by a Protected Sugar Group (Scheme 1). Copper(I)-mediated living radical polymerization (ATRP) in the presence of oxyethylene groups has been previously reported by our laboratory.[23] It was shown that polymerization of poly(ethylene glycol)methyl methacrylate monomer (MeO-PEGMA, M_n = 480 g mol^{-1}) in toluene solution, mediated by copper(I) bromide/*N*-(*n*-propyl)-2-pyridylmethanimime as catalyst, is fast relative to benzyl methacrylate (BzMA) under similar conditions. This high rate of polymerization is ascribed to complexation of the oxyethylene groups at the copper in a dynamic equilibrium with the pyridyl methanimine ligand complexation, resulting in a more active catalyst.[23]

A consequence of this effect is a significant curvature of the first-order rate plot at 90 °C at high conversion

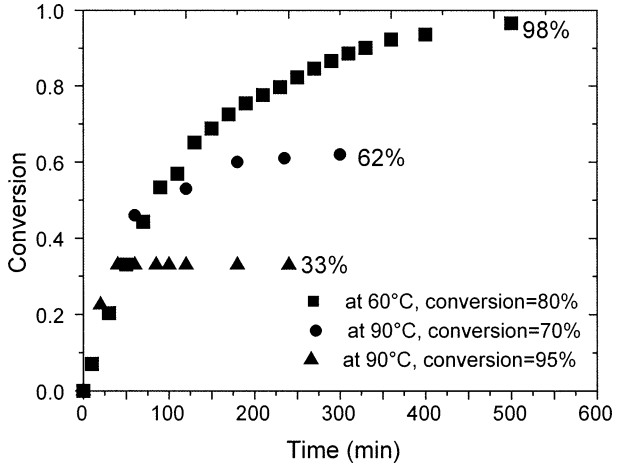


Figure 3. Conversion vs time for copolymerization of BzMA on **2**-P(MeO-PEGMA) macroinitiator polymerized under different reaction conditions.

(>90%). Different explanations have been given for explain this behavior, and in order to determine whether the behavior is the result of a reduction of the concentration of active species with an associated loss of living character, the polymerization of the first block was been carried out at both 90 and 60 °C with the galactose-derived initiator, **2**, and the reaction was stopped at different conversions to give macroinitiators. The macroinitiator was used for the reinitiation of benzyl methacrylate monomer (BzMA) at 90 °C (with a ratio of [I]/[Cu]/[ligand] = 1/1/2). The conversion/time plot (Figure 3) shows that the first block (the macroinitiator for step 2) synthesized at 90 °C does not reinitiate BzMA to more than 30−50% conversion, and the SEC of the block copolymer product showed a bimodal mass distribution. This suggests a decrease in the concentration of active species during the polymerization of the first block at this temperature. Conversely, polymerization of MeO-PEGMA at 60 °C with both **1** and **2** proceeded with very good first-order kinetics, indicative of a constant concentration of active species and living character of the polymerization with k_p[Pol*] = 5.78 × 10^{-3} (1) and 6.72 × 10^{-3} (2) (where Pol* = active propagating polymer and k_p = propagation rate constant) (Figure 4). Moreover, the number-average molar

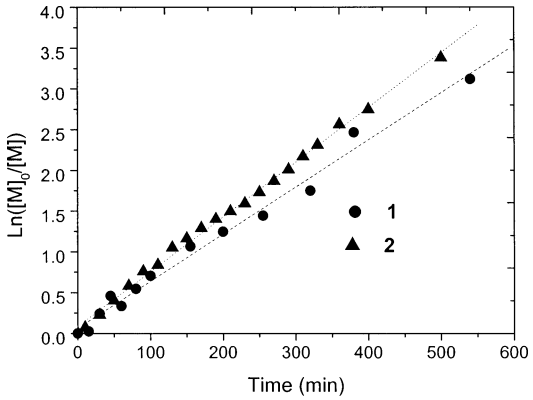
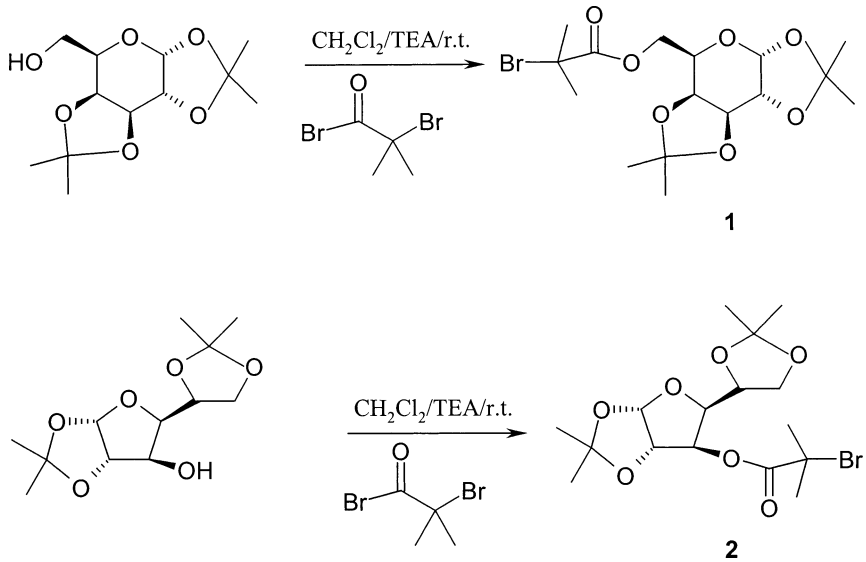


Figure 4. First-order kinetic plots for the polymerization initiated by **2** and **1** at 60 °C with [I]/[MeO-PEGMA]/[CuBr]/[ligand] = 1/20.6/1/2; lines are regression fit for the data.

mass, M_n, of the product increased linearly with monomer conversion, in close agreement with the theoretical M_n with the polydispersity remaining narrow during the polymerization (PDI < 1.2) (Figure 5).

Amphiphilic block copolymers were prepared using BzMA as the hydrophobic second block (Scheme 1). Benzyl methacrylate was chosen so as to allow observation by a UV detector in the SEC. Polymerization of the second block was carried out at 90 °C (the reaction rate is always much slower in nonpolar media), with good first-order kinetics (Figure 6) and with the final M_n of the product increasing linearly with monomer conversion in close agreement with the theoretical M_n with polydispersity remaining narrow during the polymerization (PDI < 1.2) (Figure 7). In the case of the one-pot reaction, when the monomer had been consumed, the second, BzMA, was added directly into the reaction vessel and the temperature raised to 90 °C. The resulting copolymers showed a very low PDI (Table 1). The AB blocks are prepared with a range of molecular weight (5000−11 000 g mol^{-1}, measured by ^{1}H NMR) and were very close to that targeted mass with all molar ratios of monomer used. The formation of block copolymers was indicated by dual detection SEC with both differential refractive index and UV detection (Figure 8). Both detectors gave nearly identical chromatograms, when the interdetector delay is taken into account, giving good evidence for block copolymer formation.

Scheme 1



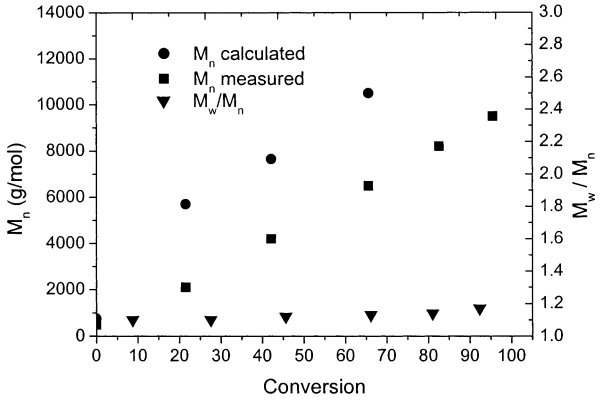


Figure 5. Evolution of M_n and M_w/M_n as a function of MeO-PEGMA conversion for polymerization at 60 °C with [**1**]/[MeO-PEGMA]/[CuBr]/[ligand] = 1/20.6/1/2.

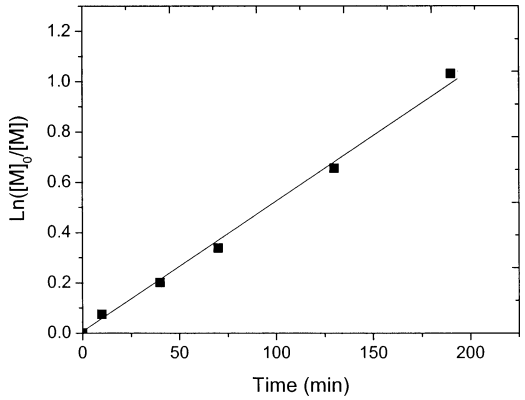


Figure 6. First-order kinetic plot for the copolymerization of BzMA using **2**-P(MeO-PEGMA) macroinitiator at 90 °C with [I]/[BzMA]/[CuBr]/[ligand] = 1/11.4/1/2.

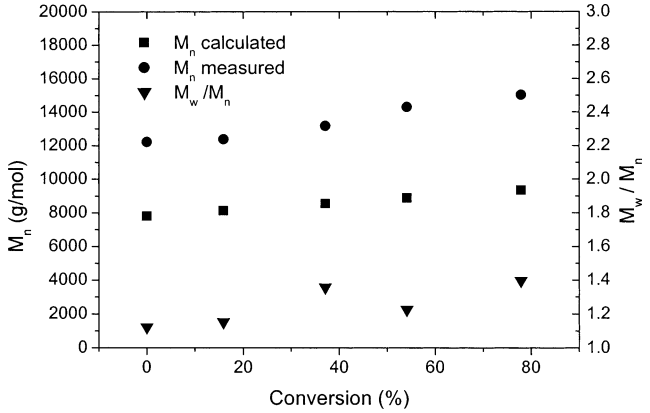


Figure 7. Evolution of M_n and M_w/M_n as a function of BzMA conversion for copolymerization using **2**-P(MeO-PEGMA) macroinitiator at 90 °C with [I]/[BzMA]/[CuBr]/[ligand] = 1/11.4/1/2.

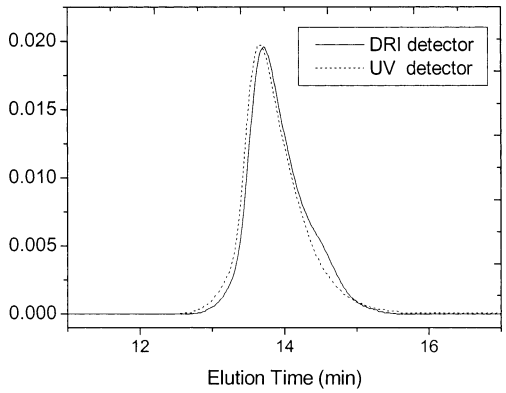


Figure 8. SEC traces with both UV and DRI detectors for **2**-P((MeO-PEGMA)-*b*-(BzMA)), C.

Deprotection of the Sugar Residues in the Block Copolymer. Deprotection of the sugar residues present in the α-terminus of the block copolymer was achieved by reaction with 50% trifluoroacetic acid (TFA) at ambient temperature for 1 h.[24] 50% reaction at ambient temperature for 1 h gave optimum conditions to remove the protective group from the sugar residue with no detectable cleavage of the sugar from the copolymer. It has been previously been shown that reaction with 90% TFA for 20 min caused significant hydrolysis of the ester functionality in the main chain.[15] Figures 9 and 10 show typical ^{1}H NMR spectra taken before and after the TFA treatment. The isopropylidene protons (1.2−1.4 ppm) have disappeared, and the protons from the sugar residue are still present. The molar ratio of PPEGMA to PBzMA was found to remain constant after deprotection (^{1}H NMR) with the polydispersity indices remaining low (Table 2), indicating that the main chain did not undergo random scission with no acidolysis of the ester function of the methacrylate group occurring.

Preparation and Characterization of Sugar-Coated Amphiphilic Block Copolymer Micelles. Block copolymer micelles were prepared by the dialysis solvent exchange process following the procedure de-

Table 1. Copolymers Synthesized in This Work

	first block P(MeO−PEGMA)		second block P(BzMA)		block copolymer
	[M]/[I]	M_n (g mol^{-1})[a]	[M]/[I]	M_n (g mol^{-1})[c]	M_w/M_n [b]
2-P((MeO-PEGMA)-*b*-(BzMA)), A[d]	20.6	9600	11.4	1400	1.18
1-P((MeO-PEGMA)-*b*-(BzMA), B[d]	20.6	9000	11.4	1500	1.21
2-P((MeO-PEGMA)-*b*-(BzMA)), C[e]	10.3	3500	28.4	2800	1.20
1-P((MeO-PEGMA)-*b*-(BzMA), D[e]	5.15	2500	14.2	2300	1.13

[a] Determined by ^{1}H NMR. [b] Determined by SEC. [c] Determined by molar ratio of the block copolymer in ^{1}H NMR. [d] Synthesized by a two-step polymerization. [e] Synthesized by a one-step polymerization.

Table 2. Molecular Characteristics of Block Copolymers after TFA Treatment (50% v/v in Water)

	before TFA treatment		after TFA treatment	
	mol % of PPEGMA/PBzMA	M_w/M_n of the block copolymers	mol % of PPEGMA/PBzMA	M_w/M_n of the block copolymers
6-(*i*Bi*P*Gal)-P((MeO-PEGMA)-*b*-(BzMA)), A	71/29	1.18	72/28	1.20
3-(*i*Bi*P*Glu)-P((MeO-PEGMA)-*b*-(BzMA), B	69/31	1.21	60/40	1.15
6-(*i*Bi*P*Gal)-P((MeO-PEGMA)-*b*-(BzMA)), C	31/69	1.20	33/67	1.15
3-(*i*Bi*P*Glu)-P((MeO-PEGMA)-*b*-(BzMA), D	29/71	1.13	26/74	1.11

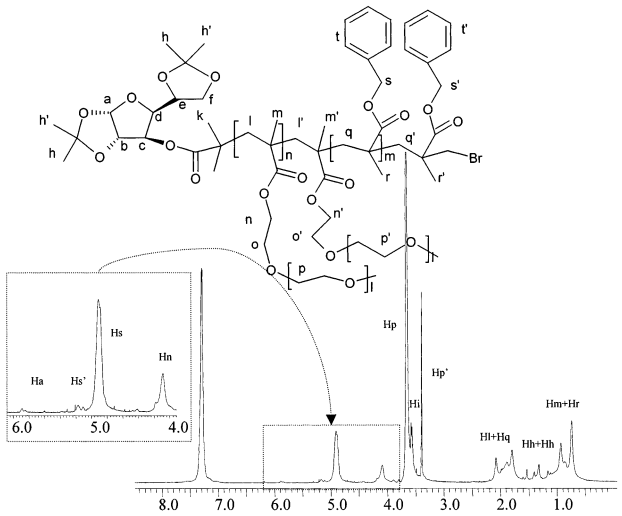


Figure 9. ^{1}H NMR ($CDCl_3$) for **1**-P((MeO-PEGMA)-*b*-(BzMA), D, prior to deprotection.

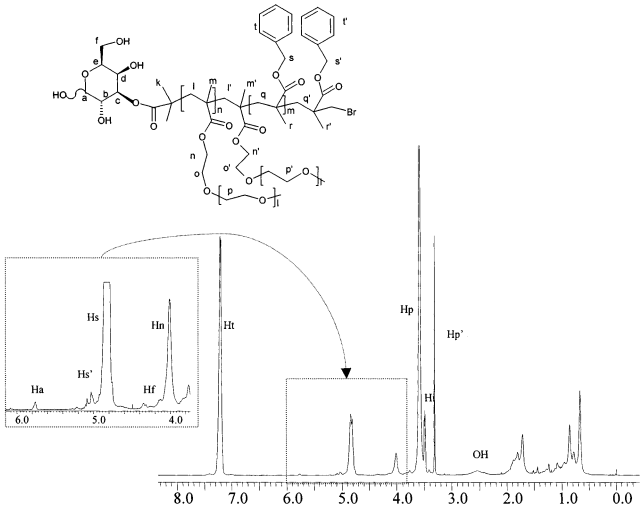


Figure 10. ^{1}H NMR ($CDCl_3$) of **1**-P((MeO-PEGMA)-*b*-(BzMA), D, following TFA deprotection.

Table 3. Size of 6-(*i*BGal)-P((MeO-PEGMA)-*b*-(BzMA)), A, and 6-(*i*BGal)-P((MeO-PEGMA)-*b*-(BzMA)), C, Micelles Determined by DLS

copolymers	*Z*-averaged hydrodynamic diameter (nm)	mol % of PEGMA/ PBzMA
6-(*i*BGal)-P((MeO-PEGMA)-*b*-(BzMA)), A	34.2	71/29
6-(*i*BGal)-P((MeO-PEGMA)-*b*-(BzMA)), C	44.8	31/69

scribed by Eisenberg et al.[25] in order to form stable micelles/aggregates in a completely aqueous environment. Copolymers were first dissolved in DMF (a good solvent for both PPEGMA and PBzMA blocks), and deionized water was subsequently added slowly to obtain a 25 wt % water mixture. The DMF was then removed by dialysis against distilled water over a period of at least 4 days. The critical micelle concentration (cmc) was found to be 0.01 g L^{-1} by fluorimetry.[27] The size and polydispersity of the micelles/aggregates obtained by dialysis were estimated by dynamic light scattering. The intensity size distribution of the **2**-P((MeO-PEGMA)-*b*-(BzMA)), C (Figure 11), gave a single peak indicating good polydispersity. If the micelles were not of uniform size, i.e., not monomodal, this would be usually observed by the presence of a tail or a second peak. To test this possibility, the intensity distribution

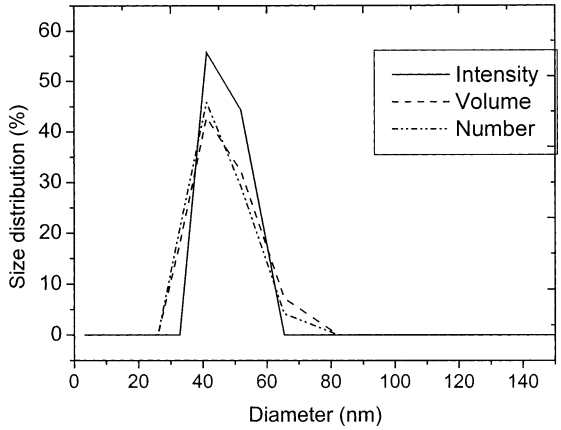


Figure 11. Size distribution plots for **2**-P((MeO-PEGMA)-*b*-(BzMA)), C; micelles determined by DLS (histogram analysis).

was converted to a volume distribution using the MIE theory[26] with 1.59 as the sample refractive index (real RI) with absorbency of the particle = 0 (considering as clear transparent solution). This increases the intensity of the peaks of d^3 (volume of a sphere = $^4/_3\pi(d/2)^3$). This was also converted into an intensity distribution using the Rayleigh approximation[26] with an intensity of scattering proportional to d^6 (mean diameter based on the intensity of the light). In both cases the **2**-P((MeO-PEGMA)-*b*-(BzMA)), C, showed micelles with a unimodal size distribution (Figure 11). This is excellent evidence for the presence of only one family of micelle sizes.

The micelle size was estimated using the CONTIN algorithm for evaluation of the correlation function, Table 3. The *Z*-averaged hydrodynamic diameters were 34.2 nm for the **2**-P((MeO-PEGMA)-*b*-(BzMA)), A, and 41.8 nm for **2**-P((MeO-PEGMA)-*b*-(BzMA)), C, with a larger size for the copolymer having the higher mol % of the hydrophobic block. Thus, the copolymers display polymeric micelles in aqueous solution with a suitable size range and the narrow dispersity required for potential carrier systems for hydrophobic drug or other active molecule delivery.

Interaction between Sugar-Coated Amphiphilic Block Copolymer Micelles and RCA-1 Lectin. The binding ability of the sugar-terminated block copolymer micelles was assayed by passing aqueous solutions through an HPLC column packed with immobilized RCA-1 lectin. RCA-1 lectin is a glycoprotein isolated from plants, which selectively recognizes β-D-galactose and β-*N*-galactosamine. Figure 12 shows that the copolymers containing deprotected glucose (derived from **1**) or protected galactose (**2**) display one sharp peak at an elution time of 2 min, indicating that no interaction of the micelles or unimers with the RCA-1 lectin. Conversely, the major fraction of the copolymer containing deprotected galactose micelles (from initiator **2**) is retained in the column and displays a broad peak at an elution time = 12.5 min. The copolymer shows a strong interaction with the RCA-1 lectin, confirming the existence of galactose residue coated the amphiphilic block copolymer micelle.

In summary, living radical polymerization has been used to prepare amphiphilic block copolymers containing α-terminal sugar residues derived from both glucose and galactose. The amphiphilic block copolymers form aggregates/micelles in aqueous media retaining their binding properties toward appropriate lectins. This

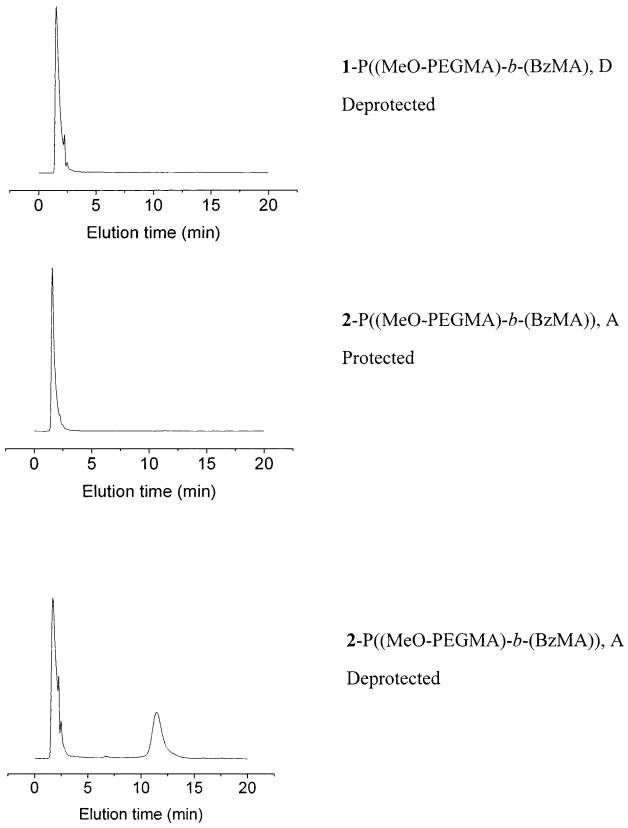


Figure 12. HPLC traces of protected and deprotected sugar-bearing block copolymer micelles.

offers scope to amplify the binding by multisite recognition by micelles and/or polymeric aggregates with a hydrophibic interior which may be used for carrying guest molecules. Living radical polymerization increases the availability and applicability of a multitude of different functionality within polymers used in this way. This approach lends itself to glyco-receptor targeting of appropriate active molecules.

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References and Notes

(1) Zhang, L.; Yu, K.; Eisenberg, A. *Science* **1996**, *272*, 1777.
(2) Jenekhe, S. A.; Chen, X. L. *Science* **1998**, *279*, 1903.
(3) Harada, A.; Kataoka, K. *Science* **1999**, *283*, 65.
(4) Znbarev, E. R.; Pralle, M. U.; Stupp, S. I. *Science* **1999**, *283*, 523.
(5) Won, Y. Y.; Davis, H. T.; Bates, F. S. *Science* **1999**, *283*, 960.
(6) Nakanishi, T.; Fukushima, S.; Okamoto, K.; Suzuki, M.; Matsumura, Y.; Yokoyama, M.; Sakurai, Y.; Kataoka, K. *J. Controlled Release* **2001**, *74* (1−3), 295.
(7) Yasugi, K.; Nagasaki, Y.; Kato, M.; Kataoka, K. *J. Controlled Release* **1999**, *62*, 89.
(8) Zhu, L.; Xiangzhou, Y.; Shilin, J. *J. Appl. Polym. Sci.* **1990**, *39*, 1.
(9) Piskin, E.; Kaitian, X.; Denkbas, E. B.; Kucukyavuz, Z. *J. Biomater. Sci., Polym. Ed.* **1995**, *7*, 359.
(10) Miyata, T.; Nakamae, K. *Trends Polym. Sci.* **1997**, *5*, 198.
(11) Okada, M. *Prog. Polym. Sci.* **2001**, *26*, 67.
(12) Yamada, K.; Minoda, M.; Miyamoto, T. *Macromolecules* **1999**, *32*, 3553.
(13) Ohno, K.; Tsujii, Y.; Fukuda, T. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 2473. Chen, Y. M.; Wulff, G. *Macromol. Chem. Phys.* **2001**, *202*, 3273. Chen, Y. M.; Wulff, G. *Macromol. Chem. Phys.* **2001**, *202*, 3426. Gotz, H.; Harth, E.; Schiller, S. M.; Frank, C. W.; Knoll, W.; Hawker, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 3379. Li, Z. C.; Liang, Y. Z.; Chen, G. Q.; Li, F. M. *Macromol. Rapid Commun.* **2000**, *21*, 375. Ohno, K.; Fukuda, T.; Kitano, H. *Macromol. Chem. Phys.* **1998**, *199*, 2193.
(14) Li, Z. C.; Liang, Y. Z.; Chen, G. Q.; Li, F. M. *Macromol. Rapid Commun.* **2000**, *21*, 375.
(15) Yasugi, K.; Nakamura, T.; Nagasaki, Y.; Kato, M.; Kataoka, K. *Macromolecules* **1999**, *32*, 8024.
(16) *Controlled Radical Polymerization*; Matyjaszewski, K., Ed.; American Chemical Society: Washington, DC, 1998; Vol. 685. *Controlled/Living Radical Polymerization: Progress in ATRP, NMP and RAFT*; Matyjaszewski, K., Ed.; American Chemical Society: Washington, DC, 2000, Vol. 768.
(17) Wang, J. S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614. Wang, J.-S.; Greszta, D.; Matyjaszewski, K. *Polym. Mater. Sci. Eng.* **1995**, *73*, 416. Granel, C.; Dubois, P.; Jerome, R.; Teyssie, P. *Macromolecules* **1996**, *29*, 8576. Kotani, Y.; Kato, M.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **1996**, *29*, 6979. Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689.
(18) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921.
(19) Jankova, K.; Chen, X. Y.; Kops, J.; Batsberg, W. *Macromolecules* **1998**, *31*, 538.
(20) (a) Huan, K.; Bes, L.; Haddleton, D. M.; Khosdel, E. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 1833. (b) Bes, L.; Huan, K.; Haddleton, D. M. Khosdel to be published in Chapter ACS book.
(21) Haddleton, D. M.; Waterson, C. *Macromolecules* **1999**, *32*, 8732.
(22) Haddleton, D. M.; Edmonds, R.; Heming, A. M.; Kelly, E. J.; Kukulj, D. *New J. Chem.* **1999**, *23*, 477.
(23) Haddleton, D. M.; Crossman, M. C.; Dana, B. H.; Duncalf, D. J.; Heming, A. M.; Kukulj, D.; Shooter, A. J. *Macromolecules* **1999**, *32*, 2110.
(24) Haddleton, D. M.; Perrier, S.; Bon, S. A. F. *Macromolecules* **2000**, *33*, 8246.
(25) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organics Synthesis*, 2nd ed.; Wiley-Interscience: New York, 1991.
(26) Zhang, L.; Barlow, J.; Eisenberg, A. *Macromolecules* **1995**, *28*, 6055.
(27) Hiemenz, P. C. *Principle of Colloid and Surface Chemistry*, 2nd ed.; Marcel Dekker: New York, 1986.
(28) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587.

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