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Synthesis and Characterization of Polymer Networks and Star Polymers Containing a Novel, Hydrolyzable Acetal-Based Dimethacrylate Cross-Linker

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ABSTRACT: An acid-labile dimethacrylate acetal cross-linker, di(methacryloyloxy-1-ethoxy)methane (DMOEM), was synthesized by the reaction of 2-hydroxyethyl methacrylate and paraformaldehyde using *p*-toluenesulfonic acid and toluene as catalyst and solvent, respectively. Group transfer polymerization was employed to use this cross-linker in the preparation of nine hydrolyzable polymer structures: one neat cross-linker network, one randomly cross-linked network of methyl methacrylate (MMA), and seven star-shaped polymers of MMA. Gel permeation chromatography (GPC) in tetrahydrofuran (THF) confirmed the narrow molecular weight distributions of the linear polymer precursors to the stars and demonstrated the increase in molecular weight upon the addition of cross-linker for the formation of star-shaped polymers. Characterization of the star polymers in THF using static light scattering and GPC showed that the molecular weights and the number of arms of each star polymer increased with an increase in the molar ratio of cross-linker to initiator and with a decrease in the molar ratio of monomer to initiator. The star polymers with DMOEM cores bore a smaller number of arms than those cross-linked with the non-hydrolyzable commercial cross-linker ethylene glycol dimethacrylate due to the bulkier structure of DMOEM. All DMOEM-containing polymer networks and star polymers were completely hydrolyzed within 48 h using hydrochloric acid in THF.

Introduction

Polymeric materials with cleavable branches[1−4] are unique materials whose molecular weight (MW) decreases upon branch removal under the appropriate conditions, and hence their properties can change dramatically. Cleavable divinyl cross-linkers represent a common type of labile branching reagent for the synthesis of such materials. These materials can have various applications. For example, hydrolyzable polymer networks can be used as matrices for protein[5] and drug[6] delivery and as biodegradable orthopaedic implants.[7,8] Furthermore, polymer networks that are thermally degradable can be used for the "reworking" of components in the optics and the electronics industries.[9]

Both "living"[10] and "nonliving" polymerization methods can be used for the preparation of cleavable polymers. Free radical polymerization is the most common "nonliving" polymerization method. Using this method, Fréchet and co-workers[5] prepared polyacrylamide networks randomly cross-linked via a diacrylamide methoxybenzaldehyde acetal, cleavable under acidic conditions. Subsequently, these researchers studied the release of protein albumin from these networks upon acidic network cleavage. Using the same polymerization method, Horkay and colleagues[11] synthesized dextran−hydroxyethyl methacrylate hydrogels with labile carbonate bonds. Mikos and co-workers[7,8] prepared networks containing hydrolyzable ester bonds in their main chain by the free radical cross-linking of poly(propylene fumarate) diacrylate. Ober and colleagues[9] obtained highly cross-linked networks by the free radical polymerization of diacrylate and dimethacrylate diesters of di-tertiary diols. These networks were thermolyzed at temperatures above 180 °C to give networks with anhydride cross-links, which were then hydrolyzed in alkaline aqueous solutions to give soluble linear polymers of acrylic or methacrylic acid.

Long and co-workers[12] and Ruckenstein and Zhang[13] used "living" anionic polymerization for the preparation of polymers with hydrolyzable cross-linkers. The former group[12] synthesized core-hydrolyzable star polymers containing the hydrolyzable dimethacrylate cross-linkers 2,5-dimethyl-2,5-hexanediol dimethacrylate and dicumyl alcohol dimethacrylate and the monomers MMA, isobutyl methacrylate, and *tert*-butyl methacrylate. After their preparation, the star polymers were hydrolyzed to the linear arms using *p*-toluenesulfonic acid (*p*TSA) in dioxane at 100 °C. Ruckenstein and Zhang[13] prepared star polymers, branched polymers, and randomly cross-linked polymer networks of MMA using the cross-linker ethylene glycol di(1-methacryloyloxy)ethyl ether (EGDMOEE) which contained an acetal group. The presence of the acetal groups in these polymers gives them the ability to be hydrolyzed in acetone or tetrahydrofuran (THF) by the addition of aqueous hydrochloric acid solutions to give lower MW products.

In a recent report,[14] we used another "living" polymerization technique, group transfer polymerization (GTP),[15−19] for the preparation of hydrolyzable polymer structures. An acid-labile silicon-based dimethacrylate cross-linker, dimethyldi(methacryloyloxy-1-ethoxy)silane (DMDMAES), was used for the synthesis of one neat cross-linker network, one randomly cross-linked network of MMA, two star-shaped polymers of MMA, and two cross-linked star polymer model networks (CSPMNs) of MMA. The cross-linker DMDMAES was very labile and could be hydrolyzed either with hydrochloric acid or even without acid (but more slowly). The purpose of this investigation was the synthesis of another hydrolyzable cross-linker with a lower rate of hydrolysis than the previously synthesized DMDMAES cross-linker, the synthesis by GTP of polymer networks and star-shaped polymers containing this cross-linker, and the examination of their hydrolysis. Thus, the preparation and purification of an acetal dimethacrylate cross-linker were pursued. Acetals are readily hydrolyzable under acidic conditions, but they are stable under basic and neutral conditions.[20,21]

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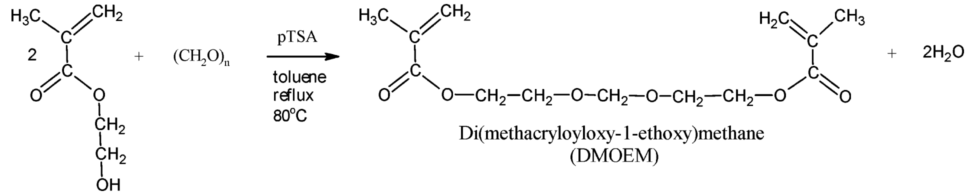

Figure 1. Reaction for the synthesis of the labile cross-linker di(methacryloyloxy-1-ethoxy)methane in the presence of the catalyst *p*-toluenesulfonic acid (*p*TSA).

A dimethacrylate-based hydrolyzable cross-linker with a slower rate of hydrolysis than that of DMDMAES cross-linker would facilitate the investigation of the hydrolysis kinetics of its star polymers if the hydrolysis would occur over a period of hours (DMOEM) rather than a few minutes (DMDMAES).[14] Moreover, DMOEM-based stars, hydrolyzed under acidic conditions in 1−2 days, would be very important for another application of interest in our group: DNA delivery by cationic star polymers.[22] In this application, the DNA-star polymer complexes enter the cell and subsequently go to subcellular organelles called endosomes which are acidic. The process of DNA release from the complex in the endosomes lasts a few hours. The degradation of the core of the star polymers within the same time frame would accelerate this release (the arms of the star are released too) and speed up the process. A similar process with DMDMAES-based cationic star polymers, which degrade almost spontaneously, may result in star polymer degradation before the complex reaches the endosome. Possible applications of networks based on the DMOEM cross-linker are tissue regeneration and drug delivery.

Experimental Section

Materials and Methods. 2-Hydroxyethyl methacrylate (HEMA, 97%) was purchased from Merck, Germany. Paraformaldehyde (powder, 95%), tetrabutylammonium hydroxide (40% w/w solution in water), benzoic acid (99.5%), MMA (99%), 1-methoxy-1-trimethylsiloxy-2-methylpropene (MTS, 95%), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH, 95%) and calcium hydride were purchased from Aldrich, Germany. Tetrahydrofuran (99.8%) and toluene (99.5%) were purchased from Labscan, Ireland. THF was used as the mobile phase in chromatography (HPLC grade), and as a solvent (reagent grade) for the polymerizations, and toluene was used as a solvent in the reaction for the synthesis of the cross-linker. *p*TSA was the catalyst in the reaction for the synthesis of the labile cross-linker.

The polymerization solvent, THF, was dried by refluxing it over a potassium−sodium mixture for 3 days and was freshly distilled prior to use. The polymerization catalyst tetrabutylammonium bibenzoate (TBABB) was synthesized by the reaction of tetrabutylammonium hydroxide and benzoic acid in water, following the procedure of Dicker et al.,[17] and was kept under vacuum until use. MMA was passed through a basic alumina column to remove the protic impurities and the polymerization inhibitors. It was subsequently stirred over calcium hydride (to remove the last traces of moisture and protic impurities) in the presence of an added free-radical inhibitor, DPPH, and stored in the refrigerator at ∼5 °C. MMA was freshly distilled under vacuum just before its use and kept under a dry nitrogen atmosphere. The initiator was distilled once prior to the polymerization, but it was neither contacted with calcium hydride nor passed through a basic alumina column because of the risk of hydrolysis. All glassware was dried overnight at 120 °C and assembled hot under dynamic vacuum prior to use.

Cross-Linker Synthesis. The acid-labile acetal cross-linker, di(methacryloyloxy-1-ethoxy)methane (DMOEM), was prepared by the reaction of HEMA with paraformaldehyde in toluene and in the presence of *p*TSA as catalyst under reflux, as shown in Figure 1.

In a typical reaction, a mixture of toluene (600 mL), pTSA (0.3 g, 0.002 mol), HEMA (73.2 mL, 78.3 g, 0.602 mol), and paraformaldehyde (30 g, 1 equiv of $-CH_2O-$ units) was placed in a 1000 mL round-bottom flask, equipped with a mechanical stirrer. The reaction was carried under reflux at 80 °C for 3 h in the presence of added free radical inhibitor DPPH to prevent undesirable thermal polymerization. The reaction mixture was filtered in order to remove the remaining solid paraformaldehyde. The toluene was subsequently removed under reduced pressure using a rotary evaporator at room temperature. The resulting oily residue (yield before column chromatography = 40%) was purified by column chromatography (silica gel/hexane:ethyl acetate = 85:15). The purified product (yield 12.4 g, 15.2%) was stirred over calcium hydride (to remove the last traces of moisture and protic impurities) in the presence of added DPPH, stored in a refrigerator at 5 °C, and then distilled under vacuum. The high purity of the cross-linker was confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopies: ^{1}H NMR ($CDCl_3$, δ): 1.94 [s, $2(CH_3C)$, 6 H], 3.80 [t, $(OCH_2CH_2O)_2CH_2$, 4 H], 4.31 [t, $(OCH_2CH_2O)_2CH_2$, 4 H], 4.74 [s, $(OCH_2CH_2O)_2CH_2$, 2 H], 5.57 (s, olefinic H trans to CO_2, 2 H), 6.12 (s, olefinic H cis to CO_2, 2 H). ^{13}C NMR ($CDCl_3$, δ): 19 [s, $CH_3C(=CH_2)$, 2 C], 64 [s, $(OCH_2CH_2O)_2CH_2$, 2 C], 66 [s, $(OCH_2CH_2O)_2CH_2$, 2 C], 96 (s, $-OCH_2O-$, 1 C), 126 (s, $CH_2=C$, 2 C), 136 [s, $CH_3C(=CH_2)$, 2 C], 168 [s, $OC(=O)C$, 2 C] Anal. ($C_{13}H_{20}O_6$) C, H: calcd 57.35, 7.35: found 57.27, 7.28. The relevant ^{1}H and ^{13}C NMR spectra are displayed in Figure 2.

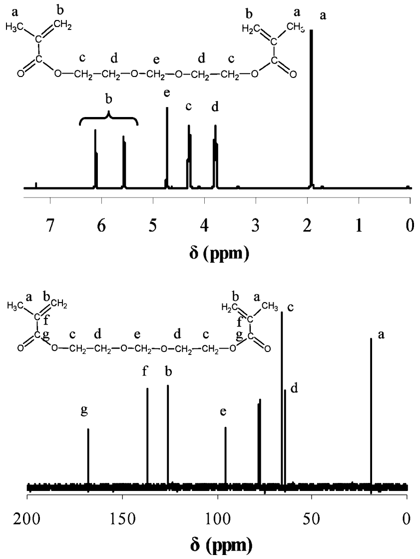

Figure 2. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of the di(methacryloyloxy-1-ethoxy)methane cross-linker in $CDCl_3$.

Polymer Synthesis. Figure 3 shows the chemical structures and names of the main reagents used for the synthesis of the polymer networks and the star polymers.

All syntheses were performed using GTP. The following polymeric materials were prepared: one neat DMOEM network, one MMA−DMOEM randomly cross-linked network, and seven "arm-first" MMA−DMOEM star polymers. The synthetic sequences employed for the preparation of these materials are summarized in Figure 4.

The polymerization reactions were carried out at ambient temperature (25 °C) without thermostating the polymerization reactor. The polymerization exotherms were monitored by a digital thermometer and were used to follow the progress of the reaction.

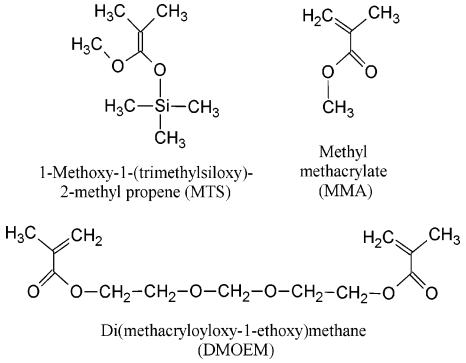

Figure 3. Chemical structures and names of the main reagents used for the polymer synthesis: the monofunctional initiator MTS, the monomer MMA, and the cross-linker DMOEM.

The polymerizations were carried out at a final solids concentration of 25% v/v. A typical polymerization, yielding the "arm-first" core-hydrolyzable MMA star polymer MMA_{10}-*b*-$DMOEM_4$-*star*, is detailed in the following. TBABB catalyst (~10 mg, ~20 μmol), freshly distilled THF (9.1 mL), and MTS initiator (0.2 mL, 0.98 mmol) were added, in this order, to a 100 mL round-bottom flask, kept under a dry nitrogen atmosphere, and sealed with a rubber septum. Subsequently, MMA (1.1 mL, 10 mmol) was slowly added with a syringe to the solution under stirring, giving a polymerization exotherm (26.7−44.4 °C), which abated within a few seconds. A sample of 0.1 mL was extracted from the reaction solution for gel permeation chromatography (GPC) analysis (full monomer conversion, polymer $M_n = 935$ g mol^{-1}, and $M_w/M_n = 1.09$), and DMOEM (1.0 mL, 3.9 mmol) was added. The reaction temperature rose from 36.5 to 45.5 °C, and a sample was withdrawn again for GPC analysis (full cross-linker conversion, apparent star polymer $M_n = 6890$ g mol^{-1} and $M_w/M_n = 3.05$). Various star polymers were synthesized by using different amounts of monomer or cross-linker. For the syntheses of the polymer networks, the initiator was added last, after the cross-linker (and monomer) had been transfer to the reactor.

Polymer Hydrolysis. The neat DMOEM network, the MMA−DMOEM randomly cross-linked network and the "arm-first" star polymers were hydrolyzed successfully in THF in the presence of hydrochloric acid, at room temperature. For instance, 0.13 g of the vacuum-dried star polymer MMA_{20}-*b*-$DMOEM_8$ (0.27 mmol of DMOEM units) was dissolved in 15 g of THF and was hydrolyzed by the addition of 0.26 mL of concentrated HCl, 12 M (0.31 g of HCl solution, 3 mmol of neat HCl), corresponding to a 5-fold molar excess of HCl with respect to the DMOEM units. Samples were withdrawn regularly and analyzed using GPC. The hydrolysis reaction was left to proceed for 28.5 h, at which point it reached completion (100% conversion by ^{1}H NMR: theoretically expected mol % HEMA = 44.4, experimental = 43.7; theoretically expected MW = 4180 g mol^{-1}, $M_n = 2810$ g mol^{-1}, $M_w/M_n = 1.21$; M_n is the number-average MW, M_w is the weight-average MW, and M_w/M_n is the polydispersity index, PDI). To ensure their complete hydrolysis, all other polymer samples were hydrolyzed using a higher HCl concentration (a 10-fold rather than a 5-fold molar excess) and for a longer time period (48 h rather than 29 h). The final hydrolysis products were analyzed using GPC and ^{1}H NMR. For example, 0.16 g of the vacuum-dried star polymer MMA_{20}-*b*-$DMOEM_4$ (0.20 mmol of DMOEM units) was dissolved in 9 g of THF and was hydrolyzed by the addition of 0.34 mL of concentrated HCl, 12 M (0.41 g of HCl solution, 4 mmol of neat HCl), corresponding to a 10-fold molar excess of HCl with respect to the DMOEM units. After 48 h of hydrolysis reaction, analyses provided the following results: 100% conversion by ^{1}H NMR: theoretically expected mol % HEMA = 28.6, experimental = 27.4; theoretically expected MW = 3140 g mol^{-1}, $M_n = 2560$ g mol^{-1}, $M_w/M_n = 1.20$).

Polymer Characterization. *Gel Permeation Chromatography.* Molecular weights (MWs) and molecular weight distributions (MWDs) of the star polymers and their linear precursors and of the hydrolyzed polymer products were determined at ambient temperature (25 °C) by GPC using a single Polymer Laboratories PL-Mixed "D" column (bead size = 5 μm; pore sizes = 100, 500, 10^3, and 10^4 Å). The mobile phase was THF (flow rate 1 mL min^{-1}), delivered using a Polymer Laboratories PL-LC1120 isocratic pump. The refractive index signal was measured using an ERC-7515A refractive index detector also supplied by Polymer Laboratories. The calibration curve was based on eight narrow MW (630, 4250, 13 000, 28 900, 50 000, 128 000, 260 000, and 520 000 g mol^{-1}) linear polyMMA standards supplied by Polymer Laboratories, which provided accurate MW calculations for the linear precursors but only qualitative estimates of the MWs of the star polymers. In particular, the following quantities were calculated: M_ns, M_ws, PDIs, and the peak MWs, M_ps, which are the MWs at the peak maximum.

^{1}H NMR Spectroscopy. The compositions of the star polymers and of the hydrolyzed polymer products were determined by ^{1}H NMR spectroscopy in deuterated chloroform ($CDCl_3$) or in deuterated dimethyl sulfoxide (d_6-DMSO). The spectra were recorded using a 300 MHz Avance Bruker spectrometer equipped with an Ultrashield magnet.

Static Light Scattering. Absolute M_ws of the star polymers were measured using static light scattering (SLS) in a GPC configuration. Thus, the scattering intensity and the refractive index were monitored simultaneously. A Brookhaven molecular weight analyzer, BI-MwA, equipped with a 30 mW red diode laser emitting at 673 nm and a multiangle detector determining the intensity of scattered light at seven different angles, 35°, 50°, 75°, 90°, 105°,

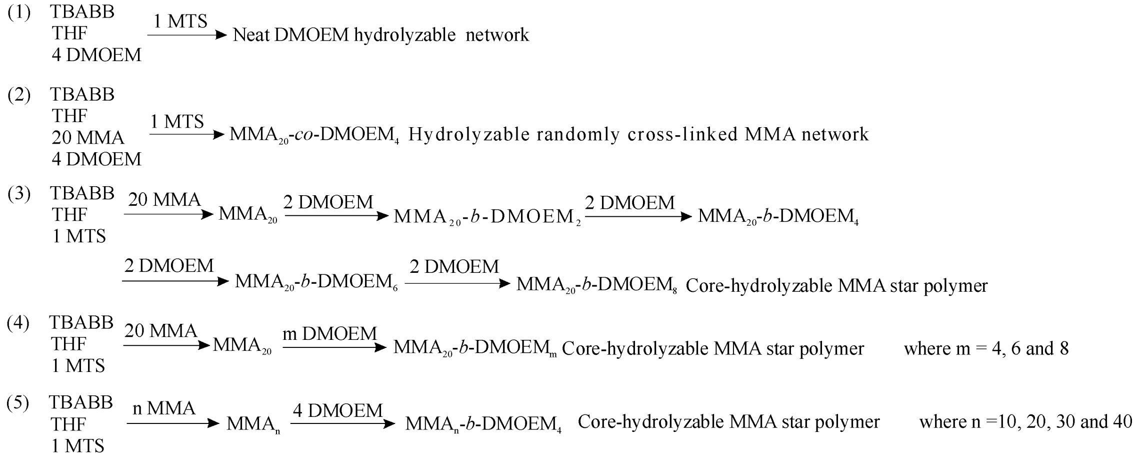

Figure 4. Synthetic sequences employed for the preparation of the polymers of this study. TBABB = tetrabutylammonium bibenzoate, polymerization catalyst; THF = tetrahydrofuran, polymerization solvent; DMOEM = di(methacryloyloxy-1-ethoxy)methane, hydrolyzable cross-linker (prepared in this study); MTS = 1-methoxy-1-(trimethylsiloxy)-2-methylpropene, monofunctional initiator; MMA = methyl methacrylate, monomer.

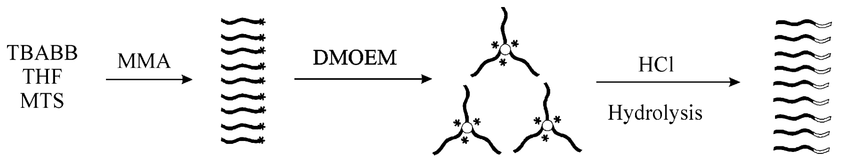

Figure 5. Schematic representation of the synthesis and hydrolysis of an “arm-first” star polymer with a core composed of a hydrolyzable cross-linker. TBABB = tetrabutylammonium bibenzoate, polymerization catalyst; THF = tetrahydrofuran, polymerization solvent; DMOEM = di(methacryloyloxy-1-ethoxy)methane, hydrolyzable cross-linker (prepared in this study); MTS = 1-methoxy-1-(trimethylsiloxy)-2-methylpropene, monofunctional initiator; MMA = methyl methacrylate, monomer; HCl = hydrochloric acid, hydrolysis reagent.

Table 1. Molecular Weights and Compositions of the Hydrolysis Products of the Neat DMOEM Network and the Randomly Cross-Linked MMA−DMOEM Network

			GPC results		composition (mol % HEMA)	
initial network	hydrolysis product	theoretical MW[a]	M_n	M_w/M_n	theoretical	by ^{1}H NMR
DMOEM$_4$	HEMA$_8$	1140	1390	1.54	100.0	100.0
MMA$_{20}$-*co*-DMOEM$_4$	MMA$_{20}$-*co*-HEMA$_8$	3140	5580	1.09	28.6	23.6

[a] The molecular weight of the initiator fragment of 100 g mol^{-1} has also been included in the calculation.

130°, and 145°, was used. A Polymer Laboratories PL-LC1120 isocratic pump was used to deliver the THF mobile phase at a flow rate of 1 mL min^{-1}. The refractive index signal was measured using a PL-RI 800 refractive index detector while a PL-Mixed “D” column was used, both supplied by Polymer Laboratories. The analysis for the calculation of the absolute M_ws was conducted using the PSS-WinGPC 7 SLS-flow software. Polymer samples were dissolved in HPLC-grade THF at 2% w/v polymer concentrations and were filtered through 0.45 μm pore size syringe filters. The refractive index increments (d*n*/d*c*) of the polymer solutions in THF were determined using an ABBE refractometer.

Results and Discussion

Preparation of Cross-Linker. The acid-sensitive cross-linker DMOEM was prepared by a reaction between HEMA and paraformaldehyde in the presence of *p*TSA catalyst in toluene.[23,24] A 3-fold molar excess of paraformaldehyde relative to the reactive hydroxy groups of HEMA was used to ensure a high conversion of the latter. Use of excess of HEMA was avoided as a larger amount of unreacted HEMA in the final reaction mixture would make more difficult the purification of the DMOEM cross-linker by column chromatography. Typical yields of the reaction for the DMOEM preparation were 40%, while overall yields after column chromatography and distillation were 15%.

Preparation and Hydrolysis of the Neat Cross-Linker Network and the Randomly Cross-Linked Network of MMA. To prove the efficiency of the hydrolyzable DMOEM cross-linker in forming networks via GTP, we performed preliminary experiments in which we prepared two simple network structures: first, a DMOEM “homopolymer” (neat cross-linker) network and, second, a randomly cross-linked MMA−DMOEM network. As shown in reactions 1 and 2 of Figure 4, for these syntheses, the MTS initiator was added last. In both cases, successful gelation took place a few seconds after the MTS addition, demonstrating the action of DMOEM as cross-linker.

Next, to prove the hydrolyzability of the DMOEM cross-linker, samples from both of these networks were subjected to hydrolysis in THF solution after the addition of excess HCl. Within 48 h, both network samples were dissolved in THF, giving transparent solutions. The hydrolysis of the networks based on this cross-linker was easier than that of the tertiary esters of Long and co-workers[12] (0.5 g of star polymer, 40 mL of dioxane, 0.1 g of *p*TSA monohydrate, at 100 °C in 12 h) and Ober et al.[9] (thermal decomposition between 180 and 200 °C) where high temperatures were used for their decomposition, but more difficult than that of our[14] siloxy-DMDMAES cross-linker (0.3 g of gel, 0.25 mL of THF, one drop of concentrated HCl, 0.48 mmol, in 5 min) and the acetal-based cross-linkers of Ruckenstein and Zhang[13] (1.0 g of polymer gel, 30 mL of solvent, 0.2 M HCl solution, in 5 min) and Fréchet and co-workers[5] (network, acid hydrolysis at pH 5, in 12 h) since a longer time was needed for the hydrolysis of networks containing DMOEM under similar conditions.

The resulting soluble polymer samples were recovered by precipitation in water and filtration and were vacuum-dried at room temperature. They were subsequently characterized in terms of their MWs and compositions by GPC and ^{1}H NMR, respectively. Each DMOEM cross-linker unit was expected to be converted upon hydrolysis to two HEMA units. Thus, the neat DMOEM network should yield a linear HEMA homopolymer, while the randomly cross-linked MMA−DMOEM network should be converted to a linear MMA−HEMA random copolymer. Given that the DMOEM/initiator molar ratio upon both syntheses was 4/1, the former polymer should be the linear 8-mer of HEMA, while the latter should have the formula MMA$_{20}$-*co*-HEMA$_8$. Table 1 displays the experimental MWs and compositions of the hydrolysis products, which appear to be along the lines expected from the basis of the “livingness” of GTP and the full hydrolysis of DMOEM. The M_n of the hydrolyzed neat DMOEM network was a little higher than the theoretically expected MW, indicating some initiator deactivation during the polymerization, while its PDI was sufficiently low (~1.5) and within the limits for a “living” polymerization, especially for these low MWs. The M_n of the hydrolyzed randomly cross-linked MMA−DMOEM network was also higher than the theoretically expected, indicating partial initiator deactivation during synthesis, too, while its PDI was satisfactorily low (~1.1). The HEMA content (calculated from the relative areas of the peaks of the hydroxy protons of HEMA at 4.8 ppm and the methoxy protons of MMA at 3.6 ppm) of the hydrolysis products was close to the theoretically expected.

Preparation and Hydrolysis of Star Polymers of MMA. After demonstrating the cross-linking ability of DMOEM from the network formation mentioned above, we pursued the synthesis of “arm-first” star polymers by the sequential GTP of MMA and DMOEM, shown in reactions 4 and 5 of Figure 4. The synthesis and hydrolysis of an “arm-first” star polymer are depicted schematically in Figure 5.

The synthesis of the star polymer is based on the ability of this polymerization method to produce linear “living” polymers upon the addition of a methacrylate monomer to a THF solution

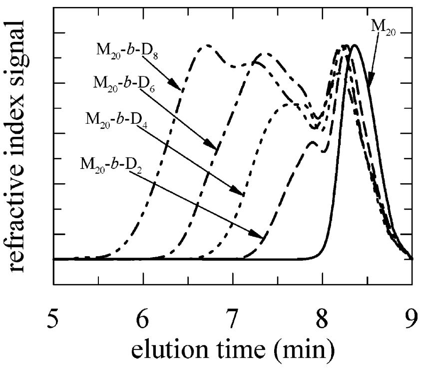

Figure 6. GPC chromatograms of hydrolyzable "arm-first" star polymers of methyl methacrylate (MMA or M) with different loadings of the di(methacryloyloxy-1-ethoxy)methane (DMOEM or D) hydrolyzable cross-linker. The GPC chromatogram of the linear polyMMA precursor is also shown. The star polymers MMA_{20}-*b*-$DMOEM_m$-*star*, with $m = 2$, 4, 6, and 8, were synthesized by the sequential addition of the cross-linker DMOEM.

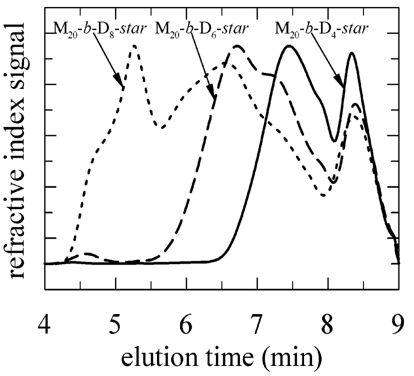

Figure 7. GPC chromatograms of "arm-first" star polymers of methyl methacrylate (MMA or M) with different loadings of the di(methacryloyloxy-1-ethoxy)methane (DMOEM or D) hydrolyzable cross-linker. The star polymers MMA_{20}-*b*-$DMOEM_m$-*star*, with $m = 4$, 6 and 8, were synthesized by one single addition of the cross-linker DMOEM.

of monofunctional initiator and catalyst and the conversion of these linear polymers to star polymers upon the addition of a dimethacrylate cross-linker. The labile nature of the present cross-linker leads to its hydrolysis and its separation into two HEMA units, thus eliminating the interconnection between different linear chains and converting the star polymers into linear polymers.

To optimize the amount of hydrolyzable cross-linker necessary for the synthesis of the star polymers, the synthesis of a star polymer was performed, MMA_{20}-*b*-$DMOEM_8$-*star*, where the molar ratio of DMOEM to initiator was varied successively. Thus, in a single reactor, after the formation of linear MMA homopolymer with 20 units, amounts of DMOEM were sequentially added so that the final DMOEM/MTS molar ratio was 2/1, 4/1, 6/1, and 8/1. After the polymerization of each successive amount of cross-linker, samples were withdrawn for GPC analysis. The GPC chromatograms of the analyzed samples are overlaid in Figure 6, and the results of the MW calculation are summarized in Table 2.

Figure 6 shows that the linear homopolymer MMA_{20}, eluting at ~8.5 min, had a unimodal and narrow MWD. Table 2 lists the M_n and PDI of this polymer, which had values 2280 g mol^{-1} and 1.10, respectively. With each successive addition of DMOEM cross-linker, the MWs of the polymer products grew and their MWDs broadened significantly, presenting up to four individual peaks. In particular, as the DMOEM/MTS ratio increased from 2 to 8, Table 2 shows that the M_ps of the highest MW peaks of the polymers increased from approximately 7300 to 58 000 g mol^{-1}. The increase in MW was associated with the interconnection of the linear chains to form star polymers, which were further interconnected and got larger by each successive addition of cross-linker. From the relative peak areas, the linear polymers contributed 42% and 34% to the total mass of the samples with 4/1 and 6/1 DMOEM/MTS molar ratio, respectively. Thus, by increasing the DMOEM/MTS ratio from 4/1 to 6/1, the incorporation of the linear polymer into the star was not too significant. Athough it contained only 25% of linear polyMMA, the star sample with a DMOEM/MTS ratio of 8/1 was not considered to be the best for the formation of star polymers because of its very high PDI (= 4.4) and the large amount of cross-linker necessary for its synthesis. At the other extreme, the use of such small ratios as 2/1 led to an unacceptably low M_p value of the star polymer of 7300 g mol^{-1} and a large percentage (65%) of unattached linear polymer.

We also synthesized the star polymers MMA_{20}-*b*-$DMOEM_m$-*star* with $m = 4$, 6, and 8, in which all the appropriate amount of cross-linker was added at once (reaction 4 in Figure 4), whose GPC chromatograms and MWs are presented in Figure 7 and Table 3, respectively. The MWs of these star polymers with the higher cross-linker/initiator molar ratios were very high. In particular, star polymer MMA_{20}-*b*-$DMOEM_8$-*star* presented a multimodal size distribution with the largest MW peak (eluting at ~5.2 min) having an M_p of 825 000 g mol^{-1}. Star polymer MMA_{20}-*b*-$DMOEM_6$-*star* displayed fewer peaks, but its largest MW peak (the small peak eluting at ~4.5 min) had an even higher MW with an M_p of 2 540 000 g mol^{-1}. The very high MWs of these samples resulted from the relatively high cross-linker/initiator molar ratios and the relatively short arms which allowed the further interconnection of the initially formed star polymers leading to very viscous, almost gel-like, solutions. The M_p of star polymer MMA_{20}-*b*-$DMOEM_8$-*star* synthesized by the addition of the DMOEM cross-linker in four steps was found to be much lower (57 800 g mol^{-1}) than that of the same polymer synthesized by the one-step addition of the same total amount of cross-linker, found to be 825 000 g mol^{-1}. The lower MW of the star polymer prepared by the stepwise addition of cross-linker can be attributed to the small star polymers formed initially (by the addition of the first feed of cross-linker), which can only be partially interconnected by the subsequent additions of cross-linker due to the large steric hindrance associated with star−star approach. In contrast, the growth of the star polymers

Table 2. Molecular Weights and Unattached Linear Fraction

polymer sample	theoretical MW[a]	GPC results M_n	GPC results M_w/M_n	GPC results M_p	% linear polymer
MMA_{20}	2100	2280	1.10	2940	100.0
MMA_{20}-*b*-$DMOEM_2$	N/A[b]	3280	1.43	3640, 7260	65.0
MMA_{20}-*b*-$DMOEM_4$	N/A[b]	5210	1.85	3640, 9990	41.9
MMA_{20}-*b*-$DMOEM_6$	N/A[b]	6760	2.56	3840, 8520, 17900	34.3
MMA_{20}-*b*-$DMOEM_8$	N/A[b]	9300	4.42	3840, 9480, 22200, 57800	25.3

[a] The molecular weight of the initiator fragment of 100 g mol^{-1} has also been included in the calculation. [b] Not applicable.

Table 3. Molecular Weights, Compositions, and Fraction of Unattached Arms of the Polymers

		GPC results				SLS results[d]		composition (mol % DMOEM)	
sample formula	theor MW[a]	M_n	M_w/M_n	M_p	% linear polymer	M_w	no. of arms	theor	by ^{1}H NMR
MMA_{20}	2100	2440	1.10	2940	25.3	210 000	78	28.6	24.9
MMA_{20}-*b*-$DMOEM_8$ (four successive additions of cross-linker)	N/A[b]	9300	4.42	3840, 9480, 22200, 57800					
MMA_{20}	2100	2280	1.10	2640					
MMA_{20}-*b*-$DMOEM_8$ (all cross-linker added at once)	N/A[b]	41500	2.16	3450, 21000, **75400**,[e] 825000	17.3	1 350 000	540	28.6	22.9
MMA_{20}	2100	1640	1.20	2140					
MMA_{20}-*b*-$DMOEM_6$	N/A[b]	8670	5.37	2870, 7070, 24000, **59000**,[e] 2540000[c]	20.9	108 160	55	23.1	22.4
MMA_{20}	2100	1450	1.39	2280					
MMA_{20}-*b*-$DMOEM_4$	N/A[b]	5480	2.36	3100, **16100**[e]	33.0	32 400	16	16.7	15.6
MMA_{10}	1100	935	1.09	1730					
MMA_{10}-*b*-$DMOEM_4$	N/A[b]	6890	3.05	2030, **17000**[e]	19.0	56 100	41	28.6	28.5
MMA_{30}	3100	2860	1.10	3270					
MMA_{30}-*b*-$DMOEM_4$	N/A[b]	5210	1.72	3840, **11700**[e]	49.5	17 600	6	11.8	9.5
MMA_{40}	4100	4110	1.09	4750					
MMA_{40}-*b*-$DMOEM_4$	N/A[b]	6310	1.65	5000, **12400**[e]	57.2	24 200	5	9.1	6.5

[a] The molecular weight of the initiator fragment of 100 g mol^{-1} has also been included in the calculation. [b] Not applicable. [c] Very small peak, indicating some interlinking of star polymers. [d] Calculated for the main star peak. [e] Main star peak (indicated in bold).

formed by the addition of all cross-linker at once is less sterically hindered because it requires only the star−linear chain rather star−star approach.

The M_p of star polymer MMA_{20}-*b*-$DMOEM_4$-*star* of Figure 7 (all cross-linker added at once) was 16 100 g mol^{-1} (Table 3) and higher than that of star polymer MMA_{20}-*b*-$DMOEM_4$-*star* of Figure 6 (cross-linker added in two additions of equal amount) which was 9990 g mol^{-1} (Table 2). Moreover, in the former case there was a lower fraction of unattached linear polymer (33%, Table 3) than in the latter (42%, Table 2). Thus, it was decided to use for the subsequent syntheses of star polymers a 4/1 cross-linker/initiator molar ratio. As described above, molar ratios of cross-linker/initiator higher than 4/1 were to be avoided because they resulted in complex polymer products containing a proportion of multistar entities.

Note that the optimal cross-linker/initiator molar ratio may differ from cross-linker to cross-linker. In particular, for the more effective EGDMA cross-linker, a molar ratio of EGDMA/MTS of 4/1 was also determined to be best,[25,26] whereas for the less effective silicon-based DMDMAES cross-linker, a molar ratio of DMDMAES/MTS of 6/1 was found to be optimal.[14] On the other hand, an 8/1 cross-linker/initiator molar ratio seemed to be optimal for the synthesis of star polymers using the EGDMOEE hydrolyzable cross-linker of Ruckenstein and Zhang,[13] while an even higher (10−15) cross-linker/initiator molar ratio was required for the *tert*-butyl acrylate star polymer synthesis using EGDMA as cross-linker.[27]

Figure 8 compares the GPC chromatogram of star polymer MMA_{20}-*b*-$DMOEM_4$-*star* in which all DMOEM was added at once during synthesis and that of another star polymer bearing also 20 MMA units, but cross-linked with the commercially available non-hydrolyzable cross-linker EGDMA, with structure MMA_{20}-*b*-$EGDMA_4$-*star*.

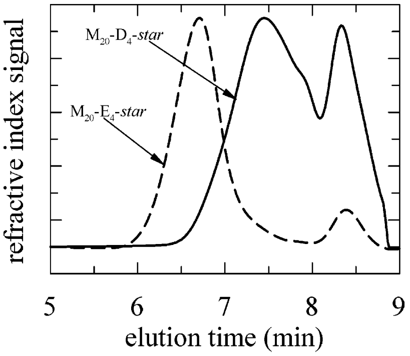

Figure 8. GPC chromatograms of "arm-first" star polymers of methyl methacrylate (MMA or M) with the hydrolyzable di(methacryloyloxy-1-ethoxy)methane (DMOEM or D) cross-linker and the nonhydrolyzable ethylene glycol dimethacrylate (EGDMA or E) cross-linker.

The GPC chromatogram of star polymer MMA_{20}-*b*-$EGDMA_4$-*star* was taken from our previous work.[14] The EGDMA-containing star polymer exhibited a star polymer peak (higher MW region) with a MW higher than that of the DMOEM-based star polymer. The GPC chromatograms of both star polymers exhibited bimodal MWDs. However, the lower MW peak of the EGDMA-based star polymer, corresponding to unattached linear polymer, had a smaller relative area than the lower MW peak of the DMOEM-based star polymer. Thus, DMOEM is a less efficient cross-linker than EGDMA, probably due to the bulkier structure of the former, leading to a greater percentage of unreacted methacrylate groups in the case of DMOEM than EGDMA.

To investigate the effect of arm length on the synthesis of DMOEM-based star polymers, more star polymers were prepared at the 4/1 optimal DMOEM/MTS molar ratio by varying the MMA/MTS molar ratio (degree of polymerization of the arm) from 10/1 to 40/1. The GPC chromatograms of these star polymers are shown in Figure 9, and the results of the related calculations are listed in Table 3. As the degree of polymerization of the arm increased, the M_p of the main peak in these samples decreased and the percentage of unattached arm (linear polymer) increased, reflecting the greater steric hindrance caused by longer arms.

The star polymer compositions in cross-linker were measured using ^{1}H NMR by ratioing the areas of the characteristic peaks of the cross-linker and the monomer. The peak of the acetal protons of DMOEM at 3.7 ppm and that of the three methoxy protons of MMA at 3.6 ppm were used as characteristic peaks. These compositions are shown in Table 3 along with the theoretical ones. There was a good agreement between the two, with the ^{1}H NMR compositions being systematically slightly lower than the theoretical, reflecting the broadening of the peaks

Table 4. Molecular Weights and Compositions of the Final Hydrolysis Products of the Star Polymers

polymer sample	hydrolyzed polymer	theor MW[a]	GPC results			composition (mol % HEMA)	
			M_n	M_w/M_n	M_p	theor	by ^{1}H NMR
MMA$_{20}$-*b*-DMOEM$_8$-*star*[b]	MMA$_{20}$-*b*-HEMA$_{16}$	4180	2810	1.21	3720	44.4	43.7
MMA$_{20}$-*b*-DMOEM$_8$-*star*[c]	MMA$_{20}$-*b*-HEMA$_{16}$	4180	3280	1.43	3950	44.4	42.5
MMA$_{20}$-*b*-DMOEM$_6$-*star*	MMA$_{20}$-*b*-HEMA$_{12}$	3660	2540	1.21	3310	37.5	34.1
MMA$_{10}$-*b*-DMOEM$_4$-*star*	MMA$_{10}$-*b*-HEMA$_8$	2140	2100	1.19	2390	44.4	39.6
MMA$_{20}$-*b*-DMOEM$_4$-*star*	MMA$_{20}$-*b*-HEMA$_8$	3140	2560	1.20	3210	28.6	27.4
MMA$_{30}$-*b*-DMOEM$_4$-*star*	MMA$_{30}$-*b*-HEMA$_8$	4140	3260	1.18	4070	21.1	19.3
MMA$_{40}$-*b*-DMOEM$_4$-*star*	MMA$_{40}$-*b*-HEMA$_8$	5140	4370	1.17	5300	16.7	17.1

[a] The molecular weight of the initiator fragment of 100 g mol^{-1} has also been included in the calculation. [b] Four successive additions of cross-linker. [c] All cross-linker added at once.

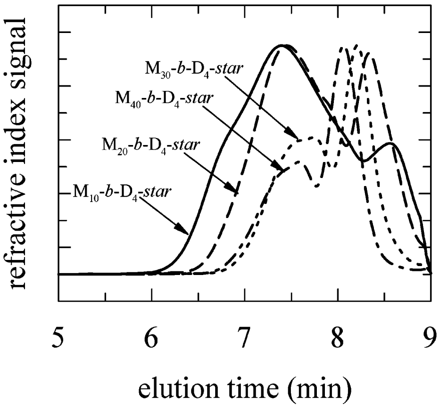

Figure 9. GPC chromatograms of "arm-first" star polymers of the hydrolyzable cross-linker di(methacryloyloxy-1-ethoxy)methane (DMOEM or D) with different loadings of the monomer methyl methacrylate (MMA or M). The star polymers MMA$_n$-*b*-DMOEM$_4$-*star*, with n = 10, 20, 30, and 40, were synthesized by one single addition of the DMOEM cross-linker.

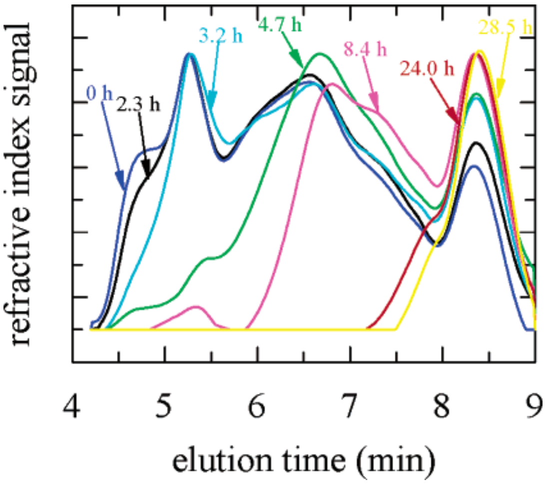

Figure 10. GPC chromatograms of the products of the hydrolysis of the star polymer MMA$_{20}$-*b*-DMOEM$_8$ at various times of the hydrolysis reaction.

of DMOEM units which were immobilized in the core of the stars.

SLS on THF solutions of the star polymers allowed the determination of their absolute M_ws and weight-average number of arms, also presented in Table 3. The M_ws of the star polymers determined by SLS were found to be greater than the corresponding GPC M_ws, calculated using a MW calibration based on linear polyMMA standards, due to the compact nature of the star structure. The weight-average number of arms was calculated by dividing the M_ws of the star polymer determined by SLS by the M_ws of their arms determined by GPC. For the star polymers with structure MMA$_n$-*b*-DMOEM$_4$-*star*, Table 3 shows that the calculated numbers of arms decreased from 41 to 5, as n increased from 10 to 40, respectively, manifesting the effect of steric hindrance of the arm on star polymer formation. The numbers of arms calculated for the above star polymers were at the lower end of the spectrum of values for numbers of arms of "arm-first" star polymers with EGDMA cores also prepared by GTP with reported values of 20,[28] 30,[29] 50,[30] and 15−40.[31]

Table 3 shows that as the cross-linker/initiator molar ratio (m) of the MMA$_{20}$-*b*-DMOEM$_m$-*star* star polymers increased from 4 to 8, the M_ws increased from 32 400 to 1 350 000 g mol^{-1}, and the calculated number of arms also increased from 8 to 540. Thus, the number of arms in star polymers also increases with the ratio of cross-linker to initiator when the ratio of monomer to initiator is kept constant. The number of arms of 540 indicates extensive interconnection between stars driven by the large amount of cross-linker used in the synthesis of MMA$_{20}$-*b*-DMOEM$_8$-*star*.

After their synthesis and characterization, the star polymers were subjected to hydrolysis using a solution of HCl in THF. A 10-fold molar excess of HCl relative to the cross-linker was used for the hydrolysis. After 2 days of hydrolysis, the resulting polymer samples were recovered by precipitation in *n*-hexane and were vacuum-dried at room temperature. The results of the GPC and ^{1}H NMR characterization of the hydrolysis product of all the star polymers along with the theoretically expected results are summarized in Table 4. GPC showed that, upon hydrolysis, the M_n decreased, approaching the MW value of the theoretically expected hydrolysis product, a MMA−HEMA diblock copolymer, also listed in Table 4. The PDIs of the hydrolysis products of the star polymers varied between 1.17 and 1.43, within the limits for a "living" polymerization. ^{1}H NMR confirmed the presence of the expected percentage of HEMA in the hydrolyzed product. Both the experimental MWs and compositions of the hydrolysis products appeared to be along the lines expected from the basis of the "livingness" of GTP and the full hydrolysis of DMOEM.

Figure 10 shows the GPC chromatograms at various stages of the hydrolysis of star polymer MMA$_{20}$-*b*-DMOEM$_8$-*star* prepared by one single addition of the DMOEM cross-linker. According to the stoichiometry, the final hydrolysis product should be MMA−HEMA diblock copolymers with 20 units of MMA and 16 units of HEMA. The figure shows that the largest particles, which were multistar assemblies (eluting at 4−5.5 min), were almost completely hydrolyzed during the first 4 h of the reaction. The star polymers with smaller MWs (eluting from 5.5 to 7.5 min) were hydrolyzed between the 4th and 10th hours of the reaction, while complete hydrolysis of the sample (including the dimers eluting at 8 min) required more than 24 h. Thus, under acidic conditions, the hydrolysis of these star polymers requires ∼1 day, similar to the time frame of DNA transfection in which exogenous DNA is transferred into the cell as a complex with a cationic polymer. Development of cationic star polymers with hydrolyzable DMOEM cores may accelerate the DNA transfer process by speeding up the complex breakup in the acidic endosome via the hydrolysis of the star polymer.

Conclusions

A novel hydrolyzable acetal-based dimethacrylate cross-linker was synthesized and copolymerized with MMA using GTP to prepare polymer networks and star polymers. The relative bulkiness of this cross-linker did not allow as large a number of arms at the cores as with the more traditional dimethacrylate cross-linker EGDMA. The number of arms in DMOEM-based star polymers decreased with an increase in the length of the arm of the star due to increased steric hindrances. The number of arms increased with the amount of cross-linker used for the formation of the star polymer. Polymers containing this cross-linker were hydrolyzed in THF using hydrochloric acid to give lower MW polymer products. Complete hydrolysis to linear arms was achieved within 2 days.

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References and Notes

(1) Inoki, M.; Akutsu, F.; Yamaguchi, H.; Naruchi, K.; Miura, M. *Macromol. Chem. Phys.* **1994**, *195*, 2799−2804.
(2) Hawker, C. J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1456−1459.
(3) Kambouris, P.; Hawker, C. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, *22*, 2717−2721.
(4) Yamagishi, H.; Saito, K.; Furusaki, S.; Sugo, T.; Hosoi, F.; Okamoto, J. *J. Membr. Sci.* **1993**, *85*, 71−80.
(5) Murthy, N.; Thng, Y. X.; Schuck, S.; Xu, M. C.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 12398−12399.
(6) Ulbrich, K.; Ŝubr. V.; Podperová, P.; Bure×f0ová, M. *J. Controlled Release* **1995**, *34*, 155−165.
(7) Timmer, M. D.; Shin, H.; Horch, R. A.; Ambrose, C. G.; Mikos, A. G. *Biomacromolecules* **2003**, *4*, 1026−1033.
(8) Timmer, M. D.; Carter, C.; Ambrose, C. G.; Mikos, A. G. *Biomaterials* **2003**, *24*, 4707−4714.
(9) Ogino, K.; Chen, J.-S.; Ober, C. K. *Chem. Mater.* **1998**, *10*, 3833−3838.
(10) Webster, O. W. *Science* **1991**, *251*, 887−893.
(11) Stubbe, B. G.; Horkay, F.; Amsden, B.; Hennink, W. E.; De Smedt, S. C.; Demeester, J. *Biomacromolecules* **2003**, *4*, 691−695.
(12) Kilian, L.; Wang, Z.-H.; Long, T. E. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 3083−3093.
(13) Ruckenstein, E.; Zhang, H. *Macromolecules* **1999**, *32*, 3979−3983.
(14) Themistou, E.; Patrickios, C. S. *Macromolecules* **2004**, *37*, 6734−6743.
(15) Webster, O. W.; Hertler, W. R.; Sogah, D. Y.; Farnham, W. B.; RajanBabu, T. V. *J. Am. Chem. Soc.* **1983**, *105*, 5706−5708.
(16) Sogah, D. Y.; Hertler, W. R.; Webster, O. W.; Cohen, G. M. *Macromolecules* **1987**, *20*, 1473−1488.
(17) Dicker, I. B.; Cohen, G. M.; Farnham, W. B.; Hertler, W. R.; Laganis, E. D.; Sogah, D. Y. *Macromolecules* **1990**, *23*, 4034−4041.
(18) Webster, O. W. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 2855−2860.
(19) Webster, O. W. *Adv. Polym. Sci.* **2004**, *167*, 1−34.
(20) Bruice, P. Y. *Organic Chemistry*; Prentice Hall: Englewood Cliffs, NJ, 1995; pp 788−791.
(21) Carey, F. A. *Organic Chemistry*, 3rd ed.; McGraw-Hill: New York, 1996; pp 696−700.
(22) Georgiou, T. K.; Vamvakaki, M.; Patrickios, C. S.; Yamasaki, E. N.; Phylactou, L. A. *Biomacromolecules* **2004**, *5*, 2221−2229.
(23) Goethals, E. J.; De Clercq, R. R.; De Clercq, H. C.; Hartmann, P. J. *Makromol. Chem., Macromol. Symp.* **1991**, *47*, 151−162.
(24) De Clercq, R. R.; Goethals, E. J. *Macromolecules* **1992**, *25*, 1109−1113.
(25) Simmons, M. R.; Yamasaki, E. N.; Patrickios, C. S. *Macromolecules* **2000**, *33*, 3176−3179.
(26) Simmons, M. R.; Yamasaki, E. N.; Patrickios, C. S. *Polymer* **2000**, *41*, 8523−8529.
(27) Held, D.; Müller, A. H. E. *Macromol. Symp.* **2000**, *157*, 225−237.
(28) Haddleton, D. M.; Crossman, M. C. *Macromol. Chem. Phys.* **1997**, *198*, 871−881.
(29) Vamvakaki, M.; Patrickios, C. S. *Chem. Mater.* **2002**, *14*, 1630−1638.
(30) Vamvakaki, M.; Hadjiyannakou, S. C.; Loizidou, E.; Patrickios, C. S.; Armes, S. P.; Billingham, N. C. *Chem. Mater*. **2001**, *13*, 4738−4744.
(31) Simms, J. A. *Rubber Chem. Technol.* **1991**, *64*, 139−151.

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