Notes

Preparation of a Comb-Shaped Cholic Acid-Containing Polymer by Atom Transfer Radical Polymerization

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Introduction

Polymethacrylates have found many applications in the biomedical field. Examples include prostheses anchors for joint replacements, bone substitute materials, soft tissue adhesive agents, self-curing material for orthopedics, urinary tract biomaterials,[1−8] etc. Even so, the cytotoxicity and poor biocompatibility of polymethacrylates still cause concern in their use.[1,2] Bile acids are natural compounds biosynthesized from cholesterol in the liver in mammals. They help in the digestion of fats and lipids in food and return to the liver by resorption through the ileum membrane.[9] Cholic acid (CA) is a major bile acid and is considered useful in the synthesis of new prodrugs for liver-specific drug targeting and improved intestinal absorption.[10] The incorporation of a biocompound such as CA into polymers may lead to the improvement of the biological compatibility, activity, and safety of the materials in biomedical applications.[11−18]

Comb-shaped polymers are superbranched macromolecules and have shown unique properties in high intermolecular ordering, self-organization, and liquid crystallinity, which endow the comb-shaped polymers with good thermal and mechanical properties.[19−24] Living polymerization methods such as atom transfer radical polymerization (ATRP) have been used in the preparation of polymers with much better structural control than the conventional radical polymerizations.[23,25−27] To combine the advantages of the comb-shaped polymers, the ATRP method, and the biological origin of bile acids, we have synthesized a comb-shaped CA-containing polymer, poly[methacryloyl tri(ethylene glycol) cholanoate] (PMATCA) with the ATRP method. To the best of our knowledge, this is the first report on the synthesis of a comb-shaped CA-containing polymer.

Experimental Section

Materials. Cholic acid (98%), $FeCl_2$ (99.9%), ethyl-2-bromoisobutyrate (EBIB, 98%), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), tri(ethylene glycol) (TEG), and 1,1′-carbonyl diimidazole (CDI) were purchased from Aldrich. Methacryloyl chloride (from Aldrich) was freshly distilled before use. All reagents were dried to remove water.

Synthesis of the Monomer. The synthesis of the monomer, methacryloyl tri(ethylene glycol) cholanoate (MATCA), involved first the synthesis of tri(ethylene glycol) cholanoate (TEGCA) and then the synthesis of the monomer.

To a solution of CA (20 mmol, 8.16 g) in 100 mL of chloroform, CDI (24 mmol, 3.89 g) was added portion-wise. The mixture was stirred at room temperature for 40 min, followed by the addition of TEG (150 mmol, 20 mL). The mixture was slowly warmed to 60 °C and kept for 24 h under nitrogen. After being cooled to room temperature the content was diluted with chloroform (100 mL), then washed with 10% $NaHCO_3$ (2 × 70 mL), water (2 × 100 mL), 0.1 N HCl (2 × 50 mL), and water (5 × 100 mL), successively. The oil phase was dried over anhydrous Na_2SO_4 overnight, concentrated, and purified by column chromatography with silica gel ($CHCl_3/CH_3OH$ = 19:1, v/v, R_f = 0.26). TEGCA was obtained with a yield of 43%. ^{1}H NMR ($CDCl_3$, δ, ppm, 300 MHz): 0.68 (s, H18), 0.89 (s, H19), 0.98−1.00 (d, H21, J = 5.1 Hz), 3.42−3.45 (m, H3), 3.46−3.75 (m, $COOCH_2CH_2OCH_2CH_2OCH_2CH_2$-OH), 3.85 (s, H7), 3.97 (s, H12), 4.25 (t, $COOCH_2$). MS: m/z = 541.0 ($M + H^+$) by FAB-MS.

A solution of TEGCA (10 mmol, 5.40 g) and triethylamine (12 mmol, 1.7 mL) in THF (75 mL) was first cooled to 0 °C. A solution of methacryloyl chloride (12 mmol, 1.2 mL) in THF (25 mL) was added dropwise over a period of 2 h under stirring. The mixture was then gradually warmed to room temperature and stirred for 12 h. The salt was filtered off, and the concentrated product was purified on a chromatographic column of silica gel using ethyl acetate as the eluent (R_f = 0.34). The monomer MATCA was obtained with a yield of 65%. ^{1}H NMR ($CDCl_3$, δ, ppm, 300 MHz): 0.61 (s, 3H18), 0.80 (s, 3H19), 0.89−0.95 (d, 3H21, J = 6.1 Hz), 1.98 (s, $C(CH_3)=CH_2$), 3.38 (s, H3), 3.58−3.71 (m, $COOCH_2CH_2OCH_2CH_2OCH_2CH_2OCO-$), 3.78 (s, H7), 3.90 (s, H12), 4.12−4.19 (t, $COOCH_2CH_2$, J = 9.0 Hz), 4.21−4.28 (t, $CH_2CH_2OCOC(CH_3)=CH_2$, J = 9.9 Hz), 5.51(s, $C(CH_3)=CH$), 6.07 (s, $C(CH_3)=CH$). MS: m/z = 609.1 ($M + H^+$) by FAB-MS.

Synthesis of the Polymer. The polymerization was performed under ultrahigh-purity grade argon using a standard Schlenk apparatus. To a Schlenk flask, $FeCl_2$ (0.038 mmol, 4.8 mg) and PMDETA (0.076 mmol, 0.016 mL) in 0.1 mL of DMF were added under argon and stirred at room temperature for 1 h. MATCA (1.37 mmol, 0.83 g) in 1.0 mL of DMF and EBIB (0.038 mmol, 5.6 μL) in 0.1 mL of DMF were then added successively to the solution. After being degassed by three "freeze−pump−thaw" cycles the flask was sealed under vacuum and was then placed in an oil bath thermostated at 70 °C and maintained for 36 h. The flask was then opened at room temperature, and the content was diluted with THF and passed through a short chromatographic column filled with alumina. The eluted solution was concentrated on a rotary evaporator and poured into cold diethyl ether to precipitate out the polymer. After vacuum-drying for 24 h at 40 °C, PMATCA was obtained.

Characterization of the Materials. NMR spectra of the monomer and polymers were recorded on an AV-300 spectrometer from Bruker operating at 300.0 MHz for ^{1}H in deuterated chloroform or dimethyl sulfoxide. The monomer conversion of the polymerization was determined by the ratio of the vinyl signals to the nonchanging signal of the monomer in the ^{1}H NMR spectra. FT-IR spectra were recorded on an FT-IR FTS-135 (Bio-Rad) with samples prepared from ground polymer powders mixed with KBr. Mass spectrometry was performed on an MS ZAB-HS apparatus (VG Company). Thermal analyses were done on a DSC 204 calorimeter (TA Instruments), scanning from 25

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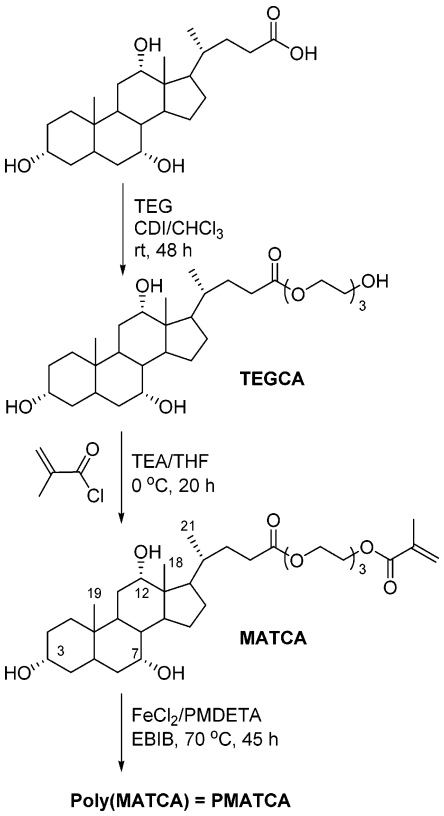

Figure 1. Synthesis of the monomer MATCA and the polymer PMATCA from cholic acid.

to 300 °C at a rate of 10 °C/min. The melting temperature (T_m) was determined as the onset of the melting endotherm. The molecular weights of the polymers were determined with a model 410 gel permeation chromatograph (Waters) with THF as the eluent (1.0 mL/min) and polystyrene standard. X-ray diffraction analysis was performed on a Rigaku D/Max-2500 X-ray apparatus under Cu 40 kV 100 mA (JPG).

Results and Discussion

Synthesis. The synthetic route of the comb-shaped CA-containing polymer is shown in Figure 1. Tri(ethylene glycol) (TEG) was selected as the spacer between the methacryloyl group and CA to increase the reactivity of the monomer during polymerization as well as the hydrophilicity of the final polymer PMATCA.

The structures of TEGCA and MATCA were confirmed with FT-IR and ^{1}H NMR spectroscopy. The IR spectrum of TEGCA showed characteristic absorptions of the carbonyl C═O (1727 cm^{-1}) and oxo −O− (1196 cm^{-1}) groups. The IR spectrum of MATCA also showed the carbonyl group C═O (1720 cm^{-1}) and the double bond CH$_2$═CH− (1668 cm^{-1}). The small downward shift of the carbonyl absorption was attributed to the conjugation of the C═C double bond with the carbonyl group in the monomer. The ^{1}H NMR spectrum of the comb-shaped polymer PMATCA showed the disappearance of the double bonds with retention of the other characteristic resonance signals of the monomer (data not shown).

Control of Polymerization. A frequently used catalyst complex is the bipyridine (bPy) complex with cuprous chloride, CuCl·bPy.[27] The chelate ligand bPy is generally satisfactory, but its toxicity[28] makes the complex undesirable in the preparation of polymers for biomedical applications. Therefore, the ferrous complex[29] of the chelate amine ligand PMDETA[30] was used instead. The catalyst complex FeCl$_2$·PMDETA was formed in situ to simplify the synthetic operations.

Table 1. Influence of the Preparation Method of the Catalyst Complex and the Amount of Added Ligand in the ATRP of MATCA[a]

method	$[L]_0/[FeCl_2]_0$ (mol ratio)	time (h)	conversion (%)	$M_{n,th}$	$M_{n,GPC}$	PDI
A[b]	1.0	36.0	67.5	1.48×10^4	1.08×10^4	1.29
B[c]	1.0	36.0	70.9	1.55×10^4	1.64×10^4	1.24
B	2.0	36.0	72.5	1.59×10^4	1.62×10^4	1.27

[a] Polymerization in DMF at 70 °C. L = PMDETA, $[MATCA]_0/[EBIB]_0$ = 36.0. [b] Method A: feeding sequence, EBIB−$FeCl_2$−MATCA−PMDETA/15 min. [c] Method B: feeding sequence, $FeCl_2$−PMDETA/60 min, then MATCA−PMDETA.

Table 2. Effects of Solvent and Temperature on the ATRP of MATCA Catalyzed by $FeCl_2$PMDETA[a]

solvent	temp (°C)	conversion (%)	$M_{n,th}$	$M_{n,GPC}$	PDI
EtAc	70.0	33.3	7.30×10^3	6.00×10^3	1.21
EtAc/DMF (1:1)	70.0	70.8	1.55×10^4	1.41×10^4	1.27
DMF	70.0	70.9	1.55×10^4	1.64×10^4	1.24
DMF	60.0	62.5	1.37×10^4	1.49×10^4	1.27
DMF	40.0	29.2	6.39×10^3	5.00×10^3	1.17

[a] Polymerization for 36 h. $[MATCA]_0/[EBIB]_0/[FeCl_2]_0/[PMDETA]_0$ = 36:1:1:2.

Several factors may affect the control of the ATRP and the properties of the polymers. In the practical implementation of an ATRP, it is an accepted way to use a catalyst complex formed in situ. A habitual practice[31−37] is to use the so-called successive feeding technique, i.e., the metal salt ($FeCl_2$ in this case), the ligand/solvent, monomer, and initiator were added successively into a reactor (method A). As both the monomer (MATCA) and the initiator (EBIB) may have a certain ability to coordinate with the catalytic central metal cation, one should consider whether the catalyst complex can be formed effectively in this case.

In this study, a different procedure was used: the ligand (PMDETA) and the metal salt ($FeCl_2$, in DMF) were first mixed, and they were allowed to react under continuously stirring for a sufficiently long duration (1 h), and then the monomer and initiator were added to the catalyst complex-containing solution (method B). The results in Table 1 showed that method B adopted in this study is superior to method A. In method B the difference between $M_{n,GPC}$ and $M_{n,th}$ is smaller and the MWD is narrower (Table 1). The formation of the catalyst complex in the case of method A may not be as efficient.

It is worth noting that in the case of method B the excess of the ligand had no significant influence on the polymerization control and on the property of the resultant polymer (Table 1). PMDETA is a three-dentate chelate ligand. According to the results in Table 1, it is postulated that 1 mol of PMDETA reacts with 1 mol of $FeCl_2$ to form the catalyst complex. A small excess of PMDETA could suppress the dissociation of the catalyst complex so as to increase the polymerization rate and the monomer conversion to a certain extent (Table 1).

Table 2 shows the effect of solvents and temperature on the ATRP of MATCA. In a less polar solvent, ethyl acetate (EtAc), the polymerization was slow (only 33.3% conversion after 36 h) and the MW of the polymer was low ($M_{n,GPC} = 6.0 \times 10^3$). This is possibly due to the poor solubility of both the catalyst complex and the polymer in this solvent. Adding the more polar DMF to EtAc improved the polymerization, and in pure DMF, the ATRP of MATCA showed good controllability. The MW of the polymer ($M_{n,GPC} = 1.64 \times 10^4$) was close to the theoretically calculated value ($M_{n,th} = 1.55 \times 10^4$).

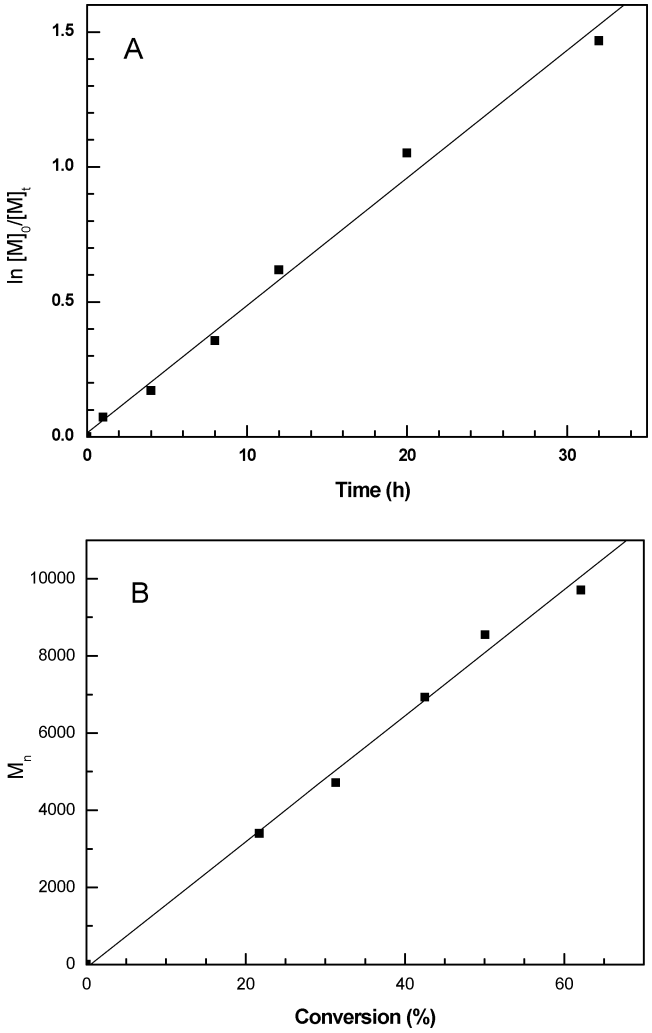

Figure 2. (A) Semilogarithmic plot of the MATCA conversion with time and (B) variation of the molecular weight of the polymer with the monomer conversion during the ATRP of MATCA with EBIB as the initiator and $FeCl_2$(PMDETA) as the catalyst (70 °C, $[M]_0/[I]_0$ = 36.0:1, $[FeCl_2]_0/[PMDETA]_0$ = 1:2).

To avoid undesirable side reactions such as various chain transfers and terminations, the living polymerization is usually conducted at a temperature as low as possible. As shown in Table 2, at a lower temperature (e.g., 40 °C), the PDI of the polymer seems to be lower, but the monomer conversion is low due to the slow polymerization rate. Increased temperature improved the monomer conversion. At 70 °C, the MW ($M_{n,GPC}$) of the polymer was close to the expected value ($M_{n,th}$), and the conversion was also much higher.

Kinetics of Polymerization. The kinetics of the ATRP of MATCA in DMF with EBIB as an initiator and $FeCl_2$(PMDETA) as the catalyst was investigated (70 °C, $[MATCA]_0/[EBIB]_0$ = 36.0). The polymerization, monitored with ^{1}H NMR spectroscopy, was found to be first order with respect to the monomer concentration at a monomer conversion less than 75%, as supported by the linear relationship of $\ln[M]_0/[M]_t$ (where $[M]_0$ and $[M]_t$ are the molar concentration of the monomer at time 0 and time t, respectively) versus the polymerization time (Figure 2A) and M_n (number-average MW) of the polymer versus conversion (Figure 2B). Above 75% monomer conversion, an obvious deviation of PMATCA from linearity was observed, which implies the possible occurrence of the chain-transfer side reactions.[38,39]

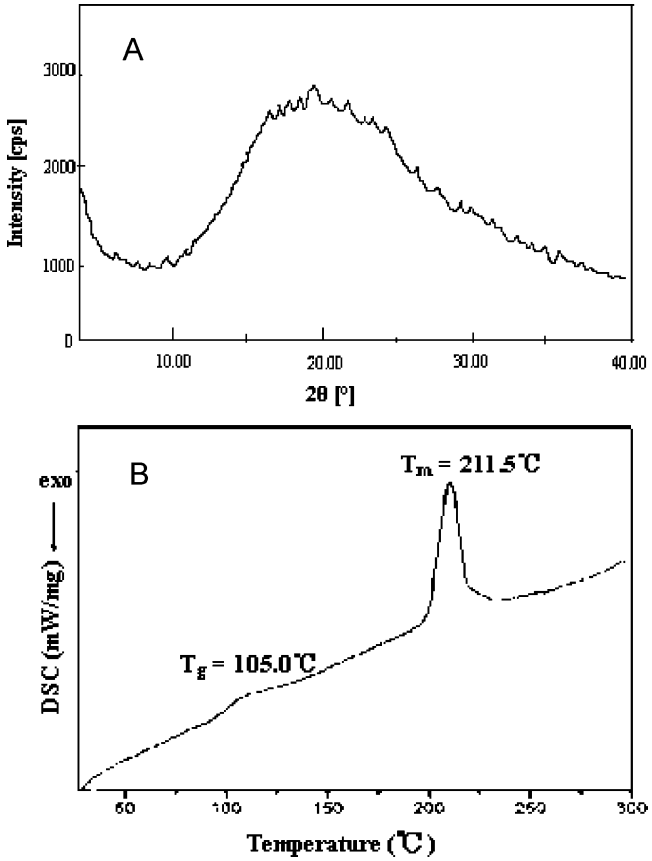

Figure 3. X-ray diffraction diagram (A) and DSC thermogram (B) of PMATCA (M_n = 1.6 × 10^4, PDI = 1.27).

Properties of PMATCA. The tacticity[38,40] of PMATCA was estimated from the ^{1}H NMR spectrum. The ratio of integrals of the signals corresponding to the syndiotactic (rr), atactic (rm), and isotactic segments (mm) was found to be 14.6:39.4:46.0. The isotacticity (46%), i.e., fraction of isotactic units, of PMATCA is much higher than that of PMMA synthesized via ATRP (rr/rm/mm = 58:38:4).[34] This confirms the comb-shaped structure of the CA-containing polymer made by ATRP. This is further evidenced by the wide-angle X-ray diffraction (WAXD) and DSC studies of PMATCA (Figure 3). The obtuse peak at around 20° (2θ) is an evidence of the semicrystalline structure[41] of the polymer (Figure 3A), which also explains the melting peak of PMATCA at 211.5 °C in the DSC trace (Figure 3B). The DSC shows that PMATCA has a high T_g of 105 °C. The polymer chain of PMATCA contains bulky and hydroxy-containing CA residues in high density, factors that tend to raise the T_g of polymers. The rigidity of the main chain, as evidenced by the high T_g, is also characteristic of the comb-shaped polymers.

Conclusions

A comb-shaped CA-containing polymer was successfully synthesized by ATRP for the first time. The polymerization in DMF produced a polymer with a moderate molecular weight and a low polydispersity. The formation of the catalyst complex prior to the addition of the monomers was shown to be a better way in the ATRP polymerization of the monomer. This may be applicable to other systems. The kinetic characteristics of the polymerization are typical of a controllable living polymerization at a conversion below 75% under the optimized conditions. The formation of the comb-shaped polymer is evidenced by the semicrystalline nature and the high T_g of the polymer.

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References and Notes

(1) Bettencourt, A.; Calado, A.; Amaral, J.; Vale, F. M.; Rico, J. M. T.; Monteiro, J.; Castro, M. *Int. J. Pharm.* **2001**, *219*, 89−93.
(2) Kim, S. B.; Kim, Y. J.; Yoon, T. L.; Park, S. A.; Cho, I. H.; Kim, E. J.; Kim, I. A.; Shin, J. W. *Biomaterials* **2004**, *25*, 5717−5723.
(3) Silikas, N.; Al-Kheraif, A.; Watts, D. C. *Biomaterials* **2005**, *26*, 197−204.
(4) Schiling, A. F.; Linhart, W.; Filke, S.; Gebauer, M.; Schinke, T.; Rueger, J. M.; Amling, M. *Biomaterials* **2004**, *25*, 3963−3972.
(5) Tunnery, M. M.; Jones, D. S.; Gorman, S. P. *Int. J. Pharm.* **1997**, *151*, 121−126.
(6) Elfick, A. P. D. *Biomaterials* **2002**, *23*, 4463−4467.
(7) Ramage, G.; Tunney, M. M.; Patrick, S.; Gorman, S. P.; Nixon, J. R. *Biomaterials* **2003**, *24*, 3221−3227.
(8) Legeais, J. M.; Werner, L.; Briat, B.; Thevenin, D.; Renard, G.; Pouliquen, Y. *Vision Res.* **1995**, *35*, 125.
(9) Zhu, X. X.; Nichifor, M. *Acc. Chem. Res.* **2002**, *35*, 539−546.
(10) Kramer, W.; Wess, G.; Enhsen, A.; Falk, E.; Hoffmann, A.; Nechermann, G.; Schubert, G.; Urman, M. *J. Controlled Release* **1997**, *43*, 17−30.
(11) Benrebouh, A.; Zhang, Y. H.; Zhu, X. X. *Macromol. Rapid Commun.* **2000**, *21*, 685−690.
(12) Zhu, X. X.; Avoce, D.; Deslauriers, V.; Liu, H. Y.; Song, Z.; Nichifor, M.; Benrehouh, A.; Gouin, S. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **2002**, *43*, 629−630.
(13) Denike, J. K.; Zhu, X. X. *Macromol. Rapid Commun.* **1994**, *15*, 459−465.
(14) Zhu, X. X.; Moskova, M.; Denike, J. K. *Polymer* **1996**, *37*, 493−498.
(15) Zhang, Y. H.; Akram, M.; Liu, H. Y.; Zhu, X. X. *Macromol. Chem. Phys.* **1998**, *199*, 1399−1404.
(16) Zuluaga, F.; Valderruten, N. E.; Wagener, K. B. *Polym. Bull.* **1999**, *42*, 41−46.
(17) Hu, X. Z.; Zhang, Z.; Zhang, X.; Li, Z. Y.; Zhu, X. X. *Steroids* **2005**, *70*, 531−537.
(18) Liu, H. Y.; Avoce, D.; Song, Z. J.; Zhu, X. X. *Macromol. Rapid Commun.* **2001**, *22*, 675−680.
(19) Plate, N. A.; Shibaev, V. P. *Comb-Shaped Polymers and Liquid Crystals*; Plenum Press: New York, 1987.
(20) Li, H.; Zhang, W. F.; Wang, Y. Q.; He, B. L. *Polym. Adv. Technol.* **2003**, *14*, 226−231.
(21) Jabbarzadeh, A.; Athinson, J. D.; Tanner, R. I. *Macromolecules* **2003**, *36*, 5020−5031.
(22) O'Donnell, P. M.; Brzezinska, K.; Powell, D.; Wagener, K. B. *Macromolecules* **2001**, *34*, 6845−6849.
(23) Chang, C.; Pugh, C. *Macromolecules* **2001**, *34*, 2027−2039.
(24) Daniels, D. R.; Mcleish, T. C. B.; Crosby, B. J.; Young, R. N.; Fernyhough, C. M. *Macromolecules* **2001**, *34*, 7025−7033.
(25) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.
(26) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689−3745.
(27) Wang, J. S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614−5615.
(28) Zhu, S. M.; Yan, D. Y. *Macromol. Rapid Commun.* **2000**, *21*, 1209−1213.
(29) Gibson, V. C.; O'Reilly, R. K.; Wass, D. F.; White, A. J. P.; Williams, D. J. *Macromolecules* **2003**, *36*, 2591−2593.
(30) Nanda, A. K.; Matyjaszewski, K. *Macromolecules* **2003**, *36*, 1487−1493.
(31) Jakubowski, W.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 4139−4146.
(32) Nguyen, S.; Marchessault, R. H. *Macromolecules* **2005**, *38*, 290−296.
(33) Gibson, V. C.; O'Reilly, R. K.; Wass, D. F.; White, A. J. P.; Williams, D. J. *Dalton Trans.* **2003**, 2824−2830.
(34) Matyjaszewski, K.; Wei, M. L.; Xia, J. H.; McDermott, N. E. *Macromolecules* **1997**, *30*, 8161−8164.
(35) Zhu, S. M.; Yan, D. Y. *Macromolecules* **2000**, *33*, 8233−8238.
(36) Zhu, S. M.; Yan, D. Y.; Zhang, G. S.; Li, M. *Macromol. Rapid Commun.* **2000**, *201*, 2666−2669.
(37) Neugebauer, D.; Matyjaszewski, K. *Macromolecules* **2003**, *36*, 2598−2603.
(38) Ando, T.; Kamigaito, M.; Sawamoto, M.; *Macromolecules* **1997**, *30*, 4507−4510.
(39) Gobelt, B.; Matyjaszewski, K. *Macromol. Chem. Phys.* **2000**, *201*, 1919−1624.
(40) Uegaki, H.; Kotani, Y.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **1997**, *30*, 2249−2253.
(41) Guo, K.; Chu, C. C.; Chkhaidze, E.; Katsarava, R. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 1463−1477.

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