Macromolecules **2006,** *39,* 3967−3970 **3967**

Poly(2-isopropyl-2-oxazoline)−Poly(L-glutamate) Block Copolymers through Ammonium-Mediated NCA Polymerization

Matthias Meyer and Helmut Schlaad*

Max Planck Institute of Colloids and Interfaces,
Colloid Department, Am Mühlenberg 1,
14476 Potsdam-Golm, Germany

Received March 9, 2006
Revised Manuscript Received March 29, 2006

Introduction

Stimuli-responsive polymers and their bioconjugates have raised considerable attention because of their promising potential in biomedical applications. The most thoroughly studied “smart” systems are those responding to a change in temperature and/or pH in aqueous solution,[1−4] but also field- or molecule-specific sensitive materials have been described.[5]

Important thermosensitive polymers are poly(N-substituted acrylamide) and poly(2-alkyl-2-oxazoline), which have a lower critical solution temperature (LCST) in aqueous solution. Such polymers are soluble in water below the LCST and become insoluble when the temperature is raised above the LCST. Ideally, for biomedical applications, the LCST should be near the human body temperature. Other polymers respond to a change in pH, like biodegradable polyesters or weak polyelectrolytes. For polyelectrolytes, the phase transition occurs at a pH around the pK_a, at which the degree of ionization is 50%. Poly(acrylic acid) and poly(methacrylic acid), the most frequently studied polyacids, have a pK_a value of around 5−6. In the case of polypeptide-based polyacids, like poly(L-glutamate), the phase transition is accompanied by a change of the secondary structure of the chains.[6] Chains adopt an (insoluble) α-helical conformation at $pH < pK_a$ and a (soluble) random coil conformation at a higher pH. This feature makes polypeptides very interesting not only for biomedical applications but also for the bioinspired generation of complex superstructures.[7,8]

Here, we report a convenient route for the synthesis of a diblock copolymer made of a thermosensitive poly(2-isopropyl-2-oxazoline) (LCST ∼ 40 °C)[9,10] and a pH-sensitive poly(L-glutamate). We preferred the cationic polymerization of oxazolines over the controlled radical or anionic polymerization of acrylamides/acrylates because the products can be of higher quality with respect to molecular weight distribution and functionality.[11] A primary amino-end-functionalized poly(2-isopropyl-2-oxazoline) was prepared and then used as a macroinitiator for the ring-opening polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride (NCA). The intermediate and final products were characterized by various techniques, including ^{1}H NMR spectroscopy and analytical ultracentrifugation (AUC).

Experimental Part

Chemicals. Chemicals and solvents were purchased from various suppliers and used, unless otherwise noted, as received. Sigma-Aldrich: isobutyronitrile (99.6%), 2-aminoethanol (99+%), cadmium acetate dihydrate (98%), methyl *p*-tosylate (98%), acetanhydride (99+%); Acros Organics: 4-(*N*-Boc-amino)-piperidine (Boc = *tert*-butyloxycarbonyl) (98%), trifluoroacetic acid (99%); Bachem: γ-benzyl L-glutamate (>99%); Iris Biotech: triphosgene (98.3%); Merck: ethyl acetate (>99.5%), hexane (≥99%), acetonitrile (99.9%); Fluka-Riedel-deHaën: dichloromethane (>99.9%), diethyl ether (≥99.5%), tetrahydrofuran (>99.5%); Roth: *N*-methylpyrrolidone (99.8%). 2-Isopropyl-Δ^2-oxazoline and γ-benzyl L-glutamate NCA were prepared according to established procedures described in the literature.[12,13]

Monomer Syntheses. (a) 2-Isopropyl-Δ^2-oxazoline: 2-Aminoethanol (204 g, 3.34 mol) was added drop-by-drop to a suspension of isobutyronitrile (218 g, 3.16 mol) and cadmium acetate dihydrate (42.6 g, 0.16 mol) at 130 °C. The solution was stirred for 24 h at that temperature and then fractionated by vacuum distillation. Yield: 264 g (2.34 mol, 74%). ^{1}H NMR ($CDCl_3$): δ/ppm = 0.93 (d, 6H, ^3J = 7.1 Hz, CH_3), 2.30 (m, 1H, ^3J = 7.1 Hz, CH), 3.55 (t, 2H, ^3J = 9.6 Hz, CH_2), 3.96 (t, 2H, ^3J = 9.6 Hz, CH_2). (b) γ-Benzyl L-glutamate NCA: A mixture of γ-benzyl L-glutamate (10.0 g, 42 mmol) and dry ethyl acetate (300 mL, freshly distilled from CaH_2) was heated to reflux under a dry argon atmosphere. Triphosgene (4.2 g, 14 mmol) was added, and the mixture was stirred for 7 h under reflux and 17 h at room temperature. Then, the solution was cooled to 0 °C, filtered, and washed with cold water and aqueous $NaHCO_3$ solution. After drying of the solution with anhydrous $NaSO_4$ and concentration to 1/3 of its original volume, the product was precipitated with hexane, filtered, recrystallized twice from ethyl acetate, and dried in a vacuum. Yield: 6.0 g (23 mmol; 54%). ^{1}H NMR ($CDCl_3$): δ/ppm = 2.05−2.30 (m, 2H, CH_2), 2.58 (t, 2H, ^3J = 7.25 Hz, CH_2), 4.38 (t, 1H, ^3J = 5.98 Hz, CH), 5.13 (s, 2H, OCH_2), 6.86 (s, 1H, NH), 7.29−7.42 (m, 5H, arom H).

Polymer Syntheses. (a) ω-Aminopoly(2-isopropyl-2-oxazoline), **1**: A solution of methyl *p*-tosylate (0.06 mL, 0.4 mmol) and 2-isopropyl-Δ^2-oxazoline (4.7 g, 41 mmol; twice distilled and dried over CaH_2) in dry acetonitrile (10 mL; freshly distilled from CaH_2) was stirred for 2 days at 80 °C under an argon atmosphere. 4-(*N*-Boc-amino)-piperidine (0.25 g, 1.2 mmol) was then added at room temperature, and the mixture was stirred for another 3 days at 80 °C. After evaporation of the solvent in a vacuum, the solid residue was dissolved in water, dialyzed against bidistilled water (molecular weight cutoff: 3.5 kDa), and freeze-dried. Isolated yield: 3.1 g (63%); molecular weight: 16.8 kg/mol (MALDI−TOF MS). The polymer (2.8 g) was then dissolved in dichloromethane (22 mL) and stirred with trifluoroacetic acid (5.4 mL) for 50 min at room temperature. The reaction mixture was partially neutralized with aqueous NaOH, and the polymer was extracted with dichloromethane. After evaporation of the solvent, the polymer was dissolved in water, dialyzed against bidistilled water, and freeze-dried. Isolated yield: 1.3 g (45%). ^{1}H NMR (D_2O): δ/ppm = 1.11 (br, 6H, CH_3), 2.75, 2.95 (br, 1H, CH), 3.50−3.69 (br, 4H, NCH_2). (b) Poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate), **2**: Solutions of **1** (0.34 g) in *N*-methylpyrrolidone (8 mL; freshly distilled from CaH_2) and of NCA (1.38 g, 5.24 mmol) in *N*-methylpyrrolidone (8 mL) were combined at room temperature, and the mixture was stirred for 4 days at 50 °C under an argon atmosphere. Acetanhydride was then added, and the copolymer was precipitated into diethyl ether (three times), filtered, and dried in a vacuum. Isolated yield: 0.4 g (23%); molecular weight: 75.6 kg/mol (NMR). The copolymer (0.28 g) was then dissolved in tetrahydrofuran (14 mL) and stirred with 1 N aqueous NaOH solution (2.9 mL) for 20 h at room temperature. The solution was dialyzed against bidistilled water and freeze-dried. Isolated yield: 0.12 g (42%). ^{1}H NMR (D_2O): δ/ppm = 1.02−1.06 (br, CH_3), 1.94−2.32 (br, CH_2), 2.71, 2.92 (br, CH), 3.46−3.65 (m, NCH_2); 4.33 (br, NCH).

Analytical Methods. ^{1}H NMR measurements were carried out at room temperature on a Bruker DPX-400 spectrometer operating at 400.1 MHz. ^{19}F NMR was recorded on a Bruker AV-300 at 282.4 MHz. FT-IR spectroscopy was performed on a BioRad 6000 FT-IR spectrometer equipped with a single reflection diamond ATR.

---

* Corresponding author: Ph ++49.331.567.9514; Fax ++49.331.567.9502; e-mail schlaad@mpikg-golm.mpg.de.

10.1021/ma060523c CCC: $33.50

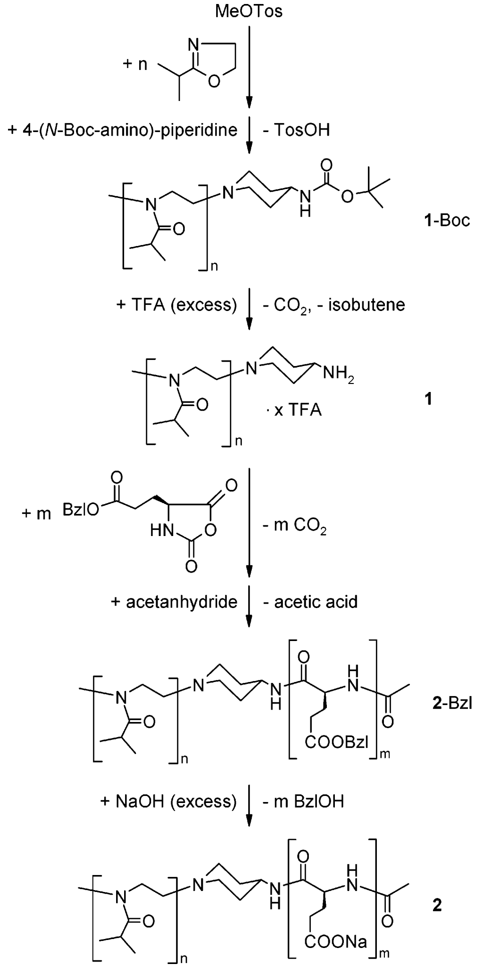
C/H/N specific elemental analysis (EA) was carried out on a Vario EL elemental analyzer. Size-exclusion chromatography (SEC) using tetrahydrofuran as the eluent was performed at room temperature at a flow rate of 1.0 mL/min. The column set consisted of two 300 × 8 mm PSS SDV, 5 μm, 10^3 and 10^5 Å (PSS GmbH, Mainz, Germany); calibration was done with polystyrene standards. Differential refractive index (RI) and UV detectors were used. Analytical ultracentrifugation (AUC) was performed on an Optima XL-I ultracentrifuge (Beckman-Coulter, Palo Alto, CA) equipped with Rayleigh interference and UV/vis absorption optics. Sedimentation-velocity runs of ~0.15 wt % polymer solutions were performed with 60 000 rpm at 25 °C. Data were evaluated with the SEDFIT software (Peter Schuck, http://www.analyticalultracentrifugation.com). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis (MALDI−TOF MS) was done on a Bruker Reflex III operating with a nitrogen laser source (λ = 337 nm) in the linear mode; 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile was used as the matrix and sodium trifluoroacetate as the cation source (analyte/matrix/salt = 1:250:1 w/w/w). Calibration was done with poly(ethylene oxide). Static light scattering (SLS) was performed at 25 °C with an ALV goniometer (ALV GmbH, Langen, Germany) and a He−Ne laser light source (λ = 633 nm); data were evaluated by a standard Zimm analysis. An NFT-Scanref differential refractometer was used for measurement of the refractive index increment.

Results and Discussion

The target block copolymer, poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate), was made through a combination of a cationic ring-opening polymerization of 2-isopropyl-Δ^2-oxazoline and an anionic ring-opening polymerization of γ-benzyl L-glutamate NCA (see also Okada et al.[14]). The key step in the procedure outlined in Scheme 1 is the synthesis of an ω-primary amine−poly(2-isopropyl-2-oxazoline), from which later the polypeptide chain was grown. Not following an established route (amino-functionalization of polyoxazoline through Mitsunobu reaction or treatment with ammonia, degree of functionalization: ≥70%),[10,15] we chose 4-(*N*-Boc-amino)piperidine as the functionalization agent because piperidines are highly efficient quenching agents of the polymerization of oxazolines.[16] By this, one introduces a primary and a tertiary amino group at the chain end of the poly(2-isopropyl-2-oxazoline). Both can initiate the polymerization of the NCA, either through a nucleophilic attack of the NCA at the C-5 position (primary amine; "amine mechanism") or through generation of a deprotonated NCA nucleophile (tertiary amine; "activated monomer mechanism").[17,18] The problem of dual initiation can be avoided by applying the concept of "ammonium-mediated NCA polymerization", which was recently introduced by Schlaad et al.[19] (see also refs 20 and 21).

Synthesis of ω-Primary Amino-Poly(2-isopropyl-2-oxazoline), 1. 2-Isopropyl-Δ^2-oxazoline was polymerized in acetonitrile at 80 °C using methyl *p*-tosylate as the initiator; the molar ratio of monomer over initiator was about 100:1. Under these conditions, monomer conversion should come to completion within 48 h.[22] Polymerization was quenched with 4-(*N*-Boc-amino)piperidine (3-fold excess with respect to initiator), and the product **1**-Boc was isolated by freeze-drying after exhaustive dialysis. The expected chemical structure of **1**-Boc was confirmed by ^{1}H NMR analysis (Figure 1A), with the three methyl groups of the Boc protecting group showing at δ = 1.43 ppm as a singlet; the methylene protons of the piperidine cycle appeared at 2.33 ppm. According to MALDI−TOF MS (Supporting Information, SI), the absolute number-average molecular weight ($\bar{M}_n$) of **1**-Boc is 16.8 kg/mol, corresponding to an average number of $\bar{n}$ = 147 repeating units. The deviation between $\bar{n}$ and the theoretical degree of polymerization is attributed to a nonaccurate volumetric measure of the initiator rather than a less than quantitative initiator efficiency.[10] Evaluation of the integrals of ^{1}H NMR signals *4* and *1* (ratio *1*/*4* = 0.0104) indicates that the ω-(*N*-Boc-amino)-functionalization of the polymer chains was quantitative (calculated ratio *1*/*4* = 9H/(147 × 6H) = 0.0102).

Scheme 1. Synthesis of Poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate)[a]



[a] Tos = tosylate, Boc = *tert*-butyloxycarbonyl, TFA = trifluoroacetic acid, Bzl = benzyl.

SEC analysis of **1**-Boc showed a monomodal and narrow molecular weight distribution (SI). Based on a polystyrene calibration curve, the apparent polydispersity index, i.e., the ratio of the apparent weight over the number-average molecular weight, is $\bar{M}_w^{app}/\bar{M}_n^{app}$ = 1.05. The molecular weight averages obtained by SEC ($\bar{M}_n^{app}$ = 17.5 kg/mol, $\bar{M}_w^{app}$ = 18.3 kg/mol) were in reasonable agreement with the absolute values determined by MALDI−TOF MS ($\bar{M}_n$ = 16.8 kg/mol) and by SLS ($\bar{M}_w$ ~ 16 kg/mol; **1**-Boc in 0.15 M saline solution, d*n*/d*c* = 0.1867 mL/g).

The polymerization of 2-isopropyl-Δ^2-oxazoline proceeded in a controlled manner at 80 °C (cf. Kataoka et al.[10]) only when the monomer had been double-distilled and dried over CaH_2. Otherwise, the polyoxazolines had a broad molecular weight distribution with a pronounced tailing toward low molecular weights.

The protecting group at the ω-chain end of **1**-Boc was removed by treatment with trifluoroacetic acid at room temperature. The reaction was complete after 50 min, as indicated by ^{1}H NMR analysis of the dialyzed product **1** (SI). The terminal amino groups are in the form of ammonium trifluoroacetates,

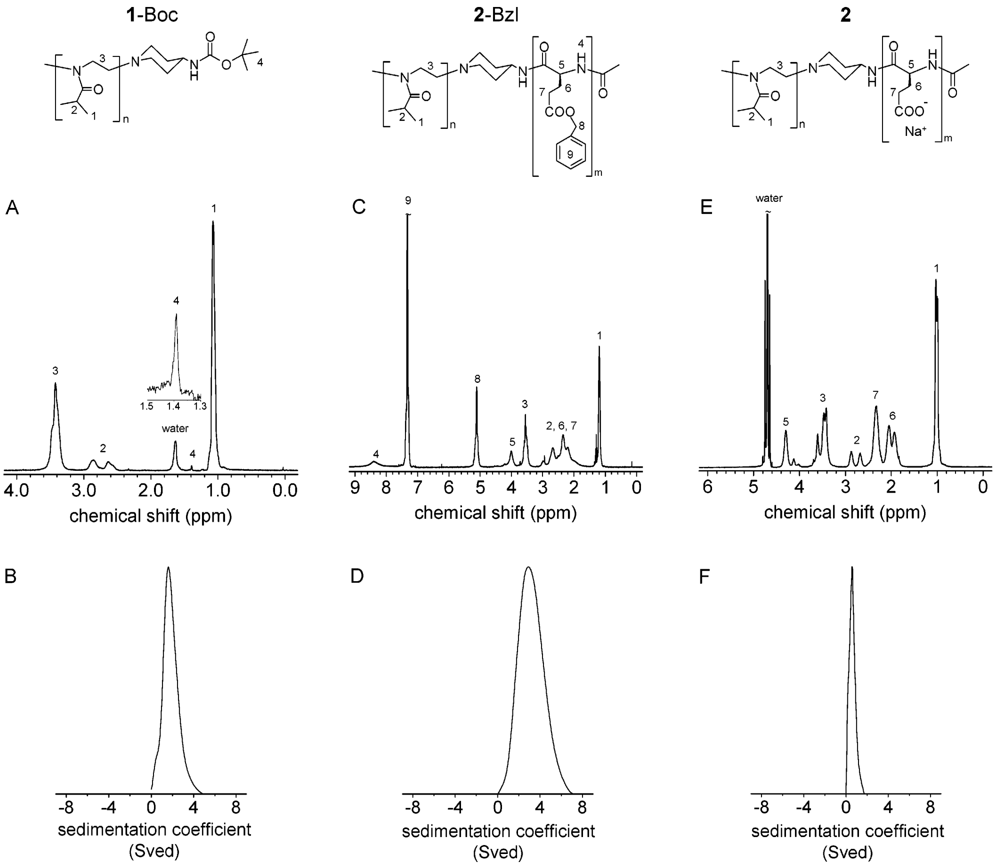


Figure 1. ^{1}H NMR and AUC data of samples **1**-Boc (A: NMR, $CDCl_3$), **1** (B: AUC, THF), **2**-Bzl (C: NMR, $CDCl_3$; D: AUC, THF), and **2** (E: NMR, D_2O; F: AUC, 0.1 N $NaNO_3$). AUC = analytical ultracentrifugation, THF = tetrahydrofuran.

which is supported by ^{19}F NMR ($CDCl_3$, signal at $\delta = -76$ ppm). According to EA, **1** contains 61.1% C and 12.8% N (**1**-Boc: 61.6% C, 12.4% N; calculated: 63.7% C, 12.4% N), indicating that the poly(2-isopropyl-2-oxazoline) chain was not affected during the exposure to the acid. The molecular weight distribution also did not change (SEC, SI).

Synthesis of Poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate), 2. Polymer **1** was used as a macroinitiator for the ammonium-mediated polymerization of γ-benzyl L-glutamate NCA ([NCA]/[**1**] ~ 250:1) in *N*-methylpyrrolidone solution at 50 °C. The polymerization was performed for 4 days to ensure quantitative conversion of the NCA. As indicated by AUC (Figure 1D), the obtained poly(2-isopropyl-2-oxazoline)-*block*-poly(γ-benzyl L-glutamate), **2**-Bzl, exhibits a monomodal sedimentation coefficient distribution, $g^*(s)$ (s = sedimentation coefficient; maximum at $s_{max} = 2.9$ Sv), and thus molecular weight distribution, and is free of homopolymer (Figure 1B: $s_{max} = 1.6$ Sv). The apparent polydispersity index was calculated to be $\bar{s}_w^{app}/\bar{s}_n^{app} \sim 1.5$, which is considered too high because $g^*(s)$ had not been corrected with respect to diffusion effects. Also, the sedimentation process might be affected by the fact that **2**-Bzl has a random coil-disrupted α-helical conformation.[23] SEC analysis of **2**-Bzl failed, regardless of which eluent (tetrahydrofuran or *N*-methylpyrrolidone) or column material (PSS SDV or PSS GRAM) was used.

Based on the integrals of the ^{1}H NMR resonances *1* (147 × 6H) and *8* ($\bar{m}$ × 2H) (Figure 1C), the average number of γ-benzyl L-glutamate in the copolymer was determined to be $\bar{m} = 268$ (**2**-Bzl: $\bar{M}_n = 75.6$ kg/mol). Hence, the efficiency of **1** was greater than 90%. Any residual traces of the macroinitiator had been extracted during the precipitation of the crude block copolymer into diethyl ether.

The benzyl protecting groups of **2**-Bzl were removed by alkaline hydrolysis (NaOH) at room temperature. The complete disappearance of benzyl groups after 20 h was confirmed by ^{1}H NMR (Figure 1E) and FT-IR spectroscopy (SI).[6] Quantitative evaluation of the integrals of the NMR resonances suggests that degradation of the polymer backbone did not happen during hydrolysis; the ratio of integrals *1/3* = 1.49 (2-isopropyl-2-oxazoline: 1.50) and *1/7* = 0.59 (**2**-Bzl: 0.55). Racemization of L-glutamate units was avoided, as indicated by FT-IR analysis of **2** after acidification with HCl, the characteristic amide II band of a polypeptide α-helix appearing at $\tilde{\nu} = 1544$ cm^{-1}.[24]

AUC indicated a monomodal distribution of **2** in a 0.1 N aqueous $NaNO_3$ solution (Figure 1F) with $s_{max} = 0.6$ Sv and $\bar{s}_w^{app}/\bar{s}_n^{app} \sim 1.2$ (upper limit). Under neutral pH conditions, both segments of **2** adopt a random coil conformation, which might explain the apparent discrepancy between the sedimentation coefficient distributions of **2** and **2**-Bzl.

In every step, however, considerable amounts of material were lost (isolated yields were below 65%) during the exhaustive workup procedures, especially dialysis.

In summary, we described a convenient procedure for the synthesis of a well-defined poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate) through combined cationic/anionic ring-opening polymerization. This concept may be extended to other polyoxazoline−polypeptide block copolymers. The key intermediate step is the preparation of an ω-(ammonium trifluoroacetate)-poly(2-isopropyl-2-oxazoline) by quantitative functionalization of the polyoxazoline chain end with 4-(*N*-Boc-amino)-piperidine and subsequent hydrolysis of the Boc protecting group with trifluoroacetic acid. This polymer was then used as a macroinitiator for an ammonium-mediated polymerization of γ-benzyl L-glutamate NCA. The alkaline hydrolysis of the benzyl protecting groups was quantitative without causing degradation or racemization. The final product ($\bar{M}_n = 68.0$ kg/mol, sodium salt) has a narrow molecular weight distribution with an apparent polydispersity index of about 1.2.

The thermo- and pH-responsive aggregation behavior of poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate) in aqueous solution is currently being investigated.

Acknowledgment. Ines Below, Marlies Gräwert, Olaf Niemeyer, Silvia Pirok, Birgit Schonert, Antje Völkel, Erich C., Dr. Matthias Heydenreich (U Potsdam), and Dr. Steffen Weidner (BAM, Berlin) are gratefully thanked for their contributions to this work. Financial support was given by the Max Planck Society and the German Research Foundation (Sfb 448: "Mesoscopically organized composites").

Supporting Information Available: MALDI−TOF MS, ^{1}H NMR, FT-IR, and SEC data. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Liu, S.; Billingham, N. C.; Armes, S. P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2328−2331.
(2) Arotcaréna, M.; Heise, B.; Ishaya, S.; Laschewsky, A. *J. Am. Chem. Soc.* **2002**, *124*, 3787−3793.
(3) Schilli, C. M.; Zhang, M. F.; Rizzardo, E.; Thang, S. H.; Chong, Y. K.; Edwards, K.; Karlsson, G.; Müller, A. H. E. *Macromolecules* **2004**, *37*, 7861−7866.
(4) André, X.; Zhang, M.; Müller, A. H. E. *Macromol. Rapid Commun.* **2005**, *26*, 558−563.
(5) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173−1222.
(6) Kukula, H.; Schlaad, H.; Antonietti, M.; Förster, S. *J. Am. Chem. Soc.* **2002**, *124*, 1658−1663.
(7) Gallot, B. *Prog. Polym. Sci.* **1996**, *21*, 1035−1088.
(8) Schlaad, H.; Antonietti, M. *Eur. Phys. J. E* **2003**, *10*, 17−23.
(9) Uyama, H.; Kobayashi, S. *Chem. Lett.* **1992**, 1643−1646.
(10) Park, J.-S.; Akiyama, Y.; Winnik, F. M.; Kataoka, K. *Macromolecules* **2004**, *37*, 6786−6792.
(11) Weberskirch, R.; Preuschen, J.; Spiess, H. W.; Nuyken, O. *Macromol. Chem. Phys.* **2000**, *201*, 995−1007.
(12) Witte, H.; Seeliger, W. *Liebigs Ann. Chem.* **1974**, 996−1009.
(13) Poché, D. S.; Moore, M. J.; Bowles, J. L. *Synth. Commun.* **1999**, *29*, 843−854.
(14) Tsutsumiuchi, K.; Aoi, K.; Okada, M. *Macromolecules* **1997**, *30*, 4013−4017.
(15) Kobayashi, S.; Masuda, E.; Shoda, S.; Shimano, Y. *Macromolecules* **1989**, *22*, 2878−2884.
(16) Nuyken, O.; Gross, A.; Maier, G. *Macromol. Chem. Phys.* **1996**, *197*, 83−95.
(17) Kricheldorf, H. R. *Alpha-Aminoacid-N-Carboxyanhydrides and Related Heterocycles*; Springer: Berlin, 1987.
(18) Deming, T. J. *Adv. Mater.* **1997**, *9*, 299−311.
(19) Dimitrov, I.; Schlaad, H. *Chem. Commun.* **2003**, 2944−2945.
(20) Lutz, J.-F.; Schütt, D.; Kubowicz, S. *Macromol. Rapid Commun.* **2005**, *26*, 23−28.
(21) Dimitrov, I. V.; Berlinova, I. V.; Vladimirov, N. G. *Macromolecules* **2006**, *39*, 2423−2426.
(22) Wiesbrock, F.; Hoogenboom, R.; Leenen, M. A. M.; Meier, M. A. R.; Schubert, U. S. *Macromolecules* **2005**, *38*, 5025−5034.
(23) Dimitrov, I.; Kukula, H.; Cölfen, H.; Schlaad, H. *Macromol. Symp.* **2004**, *215*, 383−393.
(24) Klok, H.-A.; Langenwalter, J. F.; Lecommandoux, S. *Macromolecules* **2000**, *33*, 7819−7826.

MA060523C