Synthesis of Rod-Coil Block Copolymers using Two Controlled Polymerization Techniques

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Summary: A double-headed initiator was synthesized yielding two functional groups for the initiation of the nickel mediated ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride and controlled radical polymerization of vinyl monomers via ATRP or NMP. Well-defined block copolymers combining polypeptides and synthetic polymers were obtained.

Keywords: atom transfer radical polymerization; living polymerization; polypeptides; ring-opening polymerization; rod-coil diblock copolymers

Introduction

The combination of bio-inspired structure elements and classical polymer chemistry provides promising opportunities to design polymeric materials with unique solution and solid state properties. Examples are rod-coil type polymers comprising helical polypeptide and flexible vinyl polymer blocks. Block copolymers of this architecture are of interest from both functional and structural points of view. Compared to ''simple'' coil-coil block copolymers the self-assembling of the rod-coil block copolymers is not only controlled by the microphase separation, but also by the tendency to form anisotropic supramolecular assemblies. These competitive processes can lead to morphologies which are different from those commonly observed for block copolymers.[1–5]

We introduced a new synthetic route for well defined pure polypeptide based rod-coil block copolymers combining the controlled ring-opening polymerization of *N*-carboxyanhydrides (NCA) with the controlled radical polymerization techniques via a double-headed initiator (Scheme 1).[6] This combination opens a wide range of possibilities for the controlled synthesis of rod-coil block copolymers by avoiding polymer end group modification. We have chosen a nickel mediated NCA polymerization[7] because this method has proven its potential in the polymerization of a variety of amino acid NCA's.[8] Atom transfer radical polymerization (ATRP) on the other hand, was used for the synthesis of the flexible block due to its robustness and efficiency in macroinitiation. Also the nitroxide mediated polymerization (NMP) was chosen for the combination via a double-headed initiator to show the universal applicability of this method.

Experimental Part

Materials

All solvents were dried and distilled using standard procedures[9] and if necessary degassed by freeze-pump-thaw procedure. Methylmethacrylate (90% Acros) and styrene (Acros) were destilled from CaH_2 under reduced pressure and were stored under nitrogen atmosphere at −30 °C. Cu(I)Br (98% Fluka) was purified according to the published procedure[9]. Hexamethyltriethyltetraamine (HMTETA,

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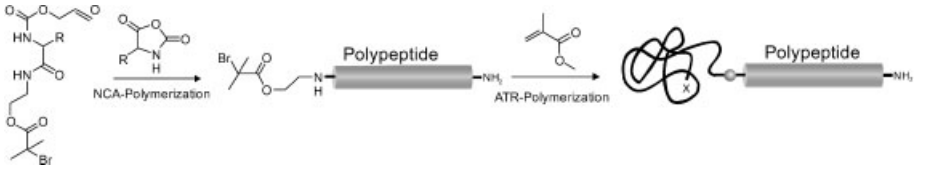


Scheme 1.
Strategy for the combination of two controlled polymerization techniques with a double-headed initiator.

97%, Aldrich) was destilled under reduced pressure and stored under nitrogen, γ-Benzyl-L-glutamic acid-*N*-carboxyanhydride (BLG-NCA) was synthesized[10] and PBLG was polymerized[8] according to the literature.

Combination of NCA Polymerization with ATRP
The synthesis of alloc-L-leucine-*N*-hydroxysuccinimidyl ester **1** and its use for preparation of alloc amides is described in.[8,11] The preparation of the double-headed initiator **4** and the synthesis of PBLG macro-initiator were described before.[6]

Block copolymerization (PBLG-b-PMMA): In a dry round bottomed flask charged Cu(I)Br and macro-initiator were dissolved in DMF (abs.), the solution was degassed by bubbling with nitrogen for 15 minutes. The ligand (HMTETA), MMA and anisole as internal standard were added. The polymerization was done at 80 °C. After the desired polymerization time the catalyst was removes by an alox column and the polymer was precipitated into methanol, isolated and reprecipitated two times.

Combination of NCA polymerization with NMP
The nitroxide **5** with the spacer X_1 (**5a**: 2,2,5 Trimethyl-3-(1-p-6-aminohexanoic acid methylphenylethoxy)-4-phenyl-3-azahexan) and X_2 (**5b**: 2,2,5 Trimethyl-3-(1-p-aminomethylphenylethoxy)-4-phenyl-3-azahexane) were prepared according to literature[12,13] and converted with alloc-L-leucine-*N*-hydroxysuccinimidyl ester **1** following the same procedure as described for the combination of NCA polymerization with ATRP.[6]

The synthesis of the double-headed initiator **(phen)Ni(amido-amidate)-NMP complex 7** was performed following a procedure similar to the method published by DEMING:[8] 86 mg (0.477 mmol) 1, 10 phenanthroline (phen) (dissolved in 4 mL DMF (abs.)) were added under a nitrogen atmosphere to 136 mg (0.494 mmol) $Ni(COD)_2$ suspended in 10 ml DMF (abs.). The mixture was stirred at room temperature for two hours to form a (phen)Ni(COD) solution and subsequently 269 mg (0.487 mmol) Alloc-L-leucin-NMP **6** (dissolved in 4 mL DMF (abs.)) were added. The mixture was allowed to react over night at room temperature. The product was isolated by precipitation into 50 mL diethyl ether (abs.). After drying in vacuum the solid product was obtained (**7a**: it was not possible to determine the yield due to a very low conversion; **7b**: 0.43 g (0.061 mmol), 12% yield). **IR** (KBr pellet, in cm^{-1}): 3385 (N–H, valence), 3051 (C–H-valence, aromatic), 2961 (C–H, valence aliphatic), 1715 (C=O, ester), 1655 (amide I, C=O-valence), 1516 (amide II, C=O-valence)

Synthesis of NMP macro-initiator: Polymerization of **γ-benzylglutamate-*N*-carboxyanhydride** (macro-initiator). γ-BLG-NCA was dissolved in DMF (abs.) and transferred with a syringe to the initiator **7** (dissolved in (DMF (abs.)) under nitrogen atmosphere. The mixture was stirred for 16 hours at room temperature. The polymer solution was precipitated with cool methanol (0 °C) with a small concentration of HCl (4 mM HCl) to destroy the nickel complex. The polymer was isolated and reprecipitated two times from THF with methanol.

Block copolymerization (PBLG-b-PS): A dry round bottomed flask was charged with the macro-initiator. It was dissolved in DMF (abs.) and styrene was added. The polymerization by heating the mixture to 130 °C. After the desired polymerization time the polymer was precipitated with methanol, isolated and reprecipitated two times.

Characterization

Polymer conversions were determined by investigation of monomer consumption by gas chromatography. Molecular weights and molecular weight distributions were measured by SEC/MALLS combination in DMF (membrane filtered and degassed) containing LiBr (0.1 mol%) on two PL-gel 5μm mixed-C columns (Polymer Laboratories) at 80 °C and a flow rate of 0.5 mL/min. Detection was performed with a Melz LCD201 differential refractive-index detector (set at 35 °C), a Thermo Separation Products UV150 Spectraseries UV-visible light detector set at 270nm, and a TriStar MiniDawn light scattering detector from Wyatt Technology (angles at 30, 90, and 120 °).

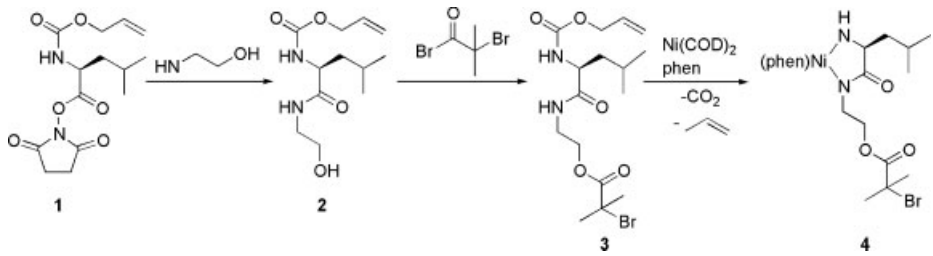
Results and Discussion

Combination of Nickel Mediated NCA Polymerization with ATRP

The synthesis of the double-headed initiator **4** (Scheme 2) for the combination of nickel mediated NCA polymerization with ATRP has already been described.[6] It was synthesized as depicted in Scheme 2 with an overall yield of 23%. First alloc-L-leucin-*N*-hydroxysuccinimidyl ester **1**[14] was reacted with aminoethanol to yield alloc-L-leucin-(2-hydroxyethyl)amide **2**. Subsequently the ATRP initiator moiety α-bromoisobutyrate was introduced by esterification of the hydroxyl group with acid bromide. Converting **3** with nickel cyclooctadien complex ($Ni(COD)_2$) and phenanthroline as ligand the initiating complex **4** was received. **4** can be isolated and stored under nitrogen.

Both polymerization mechanisms include transition metal complexes, and Nickel complexes are used for ATR-polymerizations, too.[15] Thus one question was whether or not the ATRP initiator is stable under the conditions of the last step in the initiator synthesis and the polymerization of the NCA itself.

Previous approaches towards peptide containing rod-coil block copolymers all used the coil polymer as macro-initiator for the NCA polymerization.[1–5] The double-headed initiator can be used for both sequences – peptide first or vinyl polymer first. In the later case it is necessary to perform the ATR-polymerization with the initiator in the alloc-amide form, thus before the activation with $Ni(COD)_2$ (see scheme 2). The activation step then has to be done after the polymerization, thus this sequence requires polymer end group modification. On the other hand if the NCA polymerization is done first no end group modifications are necessary and the peptide block is used as macro-initiator.



Scheme 2.
Synthesis of the double-headed initiator for combination of Nickel mediated NCA polymerization with ATRP.[6]

The NCA polymerization of γ-benzyl-L-glutamate N-carboxyanhydride (γ–BLG-NCA) was carried out in DMF as solvent with initiator **4**. The monomer to initiator ratio was varied and different initiator batches were used. The results of these experiments reveal a reasonably linear increase of the molecular weight with the monomer to initiator ratio for each individual initiator batch as determined by size exclusion chromatography with light scattering detector (SEC/MALLS).[6] The variations of the experimental molecular weight obtained from every individual initiator batch resulted from inactive impurities which were found in the initiator.[6] Similar effects have been reported by Deming for nickel amido-amidate initiators.[16] The polymerization is well controlled and poly(γ-benzyl-L-glutamate) (PBLG) with a narrow molecular weight distribution can be obtained (polydispersity 1.2–1.4). Taking into account the effect due to the inactive impurities, it is possible to get well controlled polymers with adjusted molecular weight.

These polymers all have an intact bromo isobutyrate end group as evidenced by MALDI-TOF investigation.[6] Thus the treatment with $Ni(COD)_2$ and the NCA polymerization did not destroy the ATRP initiator and well defined macro-initiators were obtained.

These PBLG macro-initiators were used in the ATRP of MMA. Due to the low solubility of polypeptides in conventional ATRP solvents, suitable polymerization conditions (catalyst, ligand, temperature, concentration, etc.) had to be identified. Cu(I)/HMTETA in DMF at 80 °C was found to be an appropriate system for the ATRP of MMA with the PBLG macro-initiator.[6] In addition, the influence of the PBLG as macro-initator on the kinetics of the ATR-polymerization was investigated. As shown in Figure 1 the reaction follows a first order kinetics under the macro-initiation conditions. After three days a conversion of 90% was reached. However, if α-bromo isobutyric acid ethyl ester was used as initiator instead of the PBLG macro-initiator the reaction starts very fast, but stops after a short time (approximately 1 h). Thus under these conditions only low conversion can be reached. It can be speculated, that the catalyst system in DMF is too active and thus the radical concentration too high resulting in termination reactions. The addition of PBLG without a functional end group has a big influence to the monomer conversion, too. The polymerization starts fast but after a

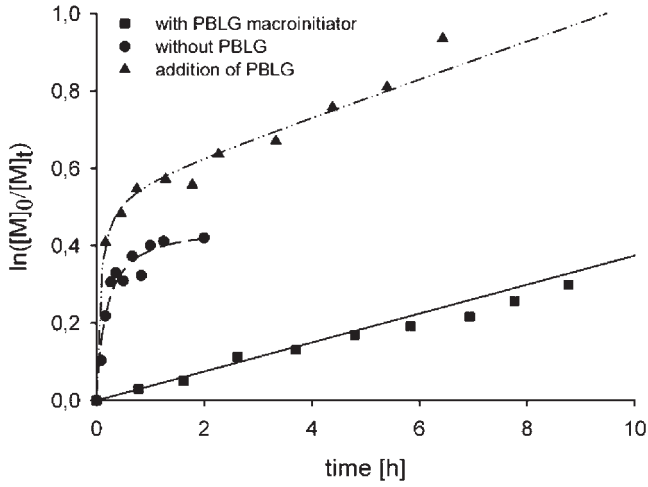


Figure 1.
$\ln([M]_0/[M]_t)$ as a function of time with macro-initiator, without macro-initiator and with addition of PBLG, ATRP of MMA at 80 °C with Cu(I)Br, HMTETA, DMF as solvent and anisole as internal standard.

short time $\ln[M]_0/[M]_t$ becomes linear. The linear part is almost parallel to the curve obtained for the polymerisation employing the macro-initiator (see Figure 1). It is supposed that the polyamide backbone of the peptide shows interactions with the Cu-catalyst system and thus influences the equilibration of the ATRP.[17] Probably the the complexation of the Cu with PBLG reduces the activity of the initiating complex. The results are evidence that it is not possible to simply transfer the conditions used for low molecular weight initiators to a polymerization of the same monomer with the PBLG macro-initiators, but a screening for suitable reaction conditions is necessary.

The rod-coil block copolymers obtained by the controlled nickel mediated NCA polymerization and subsequent ATRP (PBLG-b-PMMA) were investigated by XRD measurements and AFM after storage in THF vapour. The XRD measurements (not shown) reveal that before treatment with THF vapour there were peaks attributed to both α-helical and β-sheet material. After the treatment only peaks resulting from α-helical material were observed. The AFM pictures reveal a lamellar structure of the block copolymer (Figure 2).

Combination of Nickel Mediated NCA Polymerisation with NMP

For many vinyl monomers, especially for styrene, the nitroxide mediated polymerization (NMP) is an alternative technique for a controlled radical polymerization which is less sensitive to variations in parameters like solvent polarity, concentration etc.

Therefore the possibility of combining NMP and nickel mediated NCA polymerization was investigated by synthesizing the double headed initiator **7** with an amido-amidate group and a nitroxide group (Scheme 3). A nitroxide **5** (synthesis according to literature[18]) was converted with alloc-L-leucine-*N*-hydroxysuccinimidyl

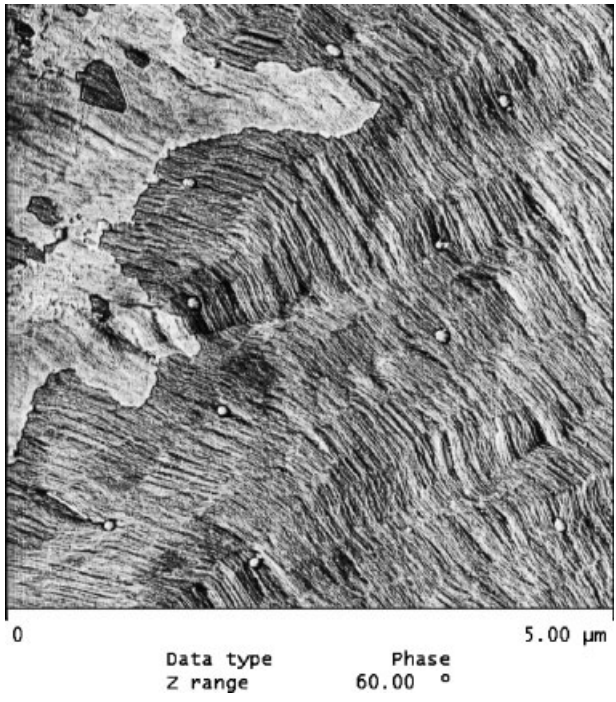
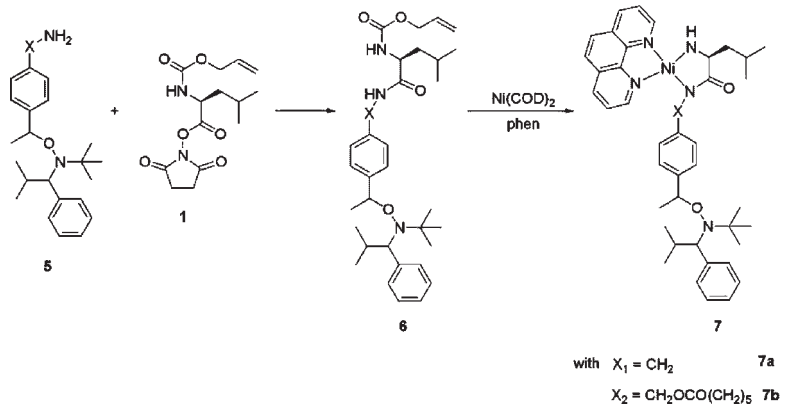


Figure 2.
AFM picture of PBLG-b-PMMA block copolymer (M_n of PBLG block 85000 g/mol, M_n of PMMA block 50500 g/mol) after storage in THF vapour.



Scheme 3.
Synthesis of double-headed initiators **7** for combination of NCA polymerization with NMP.

ester **1** into the corresponding ester **6** and reacted with $Ni(COD)_2$ and phenanthroline to yield the double-headed initiator **7**(yield 12%). We used two different spacers (group X) between the amido-amidate group and the nitroxide.

Both initiators **7a** and **7b** were used to initiate the polymerization of γ–BLG-NCA. PBLGs were obtained having a molecular weight of $M_n = 42\,000$ g/mol (PBLG-X_1) and 23 000 g/mol (PBLG-X_2) and a polydispersity of 1.5 and 1.4 respectively. The presence of the nitroxide end group in the PBLG macroinitiator was confirmed by MALDI-ToF measurements. These PBLGs macro-initiators with nitroxide end groups were used in the NMP of styrene in DMF under reaction conditions as described in reference.[13] The results of the block copolymerizations are summarized in Table 1.

For the block copolymers obtained with PBLG-X_1 as macro-initiator (Table 1/entry 1 and 2) a shoulder in the SEC chromatogram (Figure 3) was observed. This shoulder is supposed to be due to PS-homopolymer resulting from the styrene autopolymerization. The investigation of the polymerizations kinetics via GC (see Figure 4) shows a

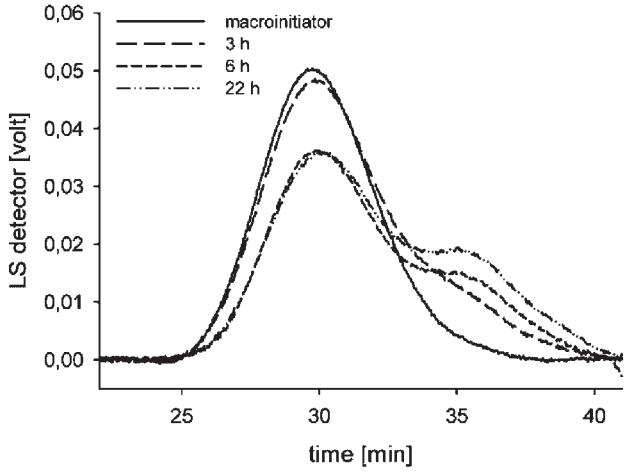


Figure 3.
SEC-MALLS chromatogram of PBLG-PS block copolymers prepared by NMP with the PBLG-X_1 macro-initiator (Table 1/entry 2).

Table 1.
Molecular weight of PBLG-b-PS block copolymers.

Entry	macroinitiator	$PG^{b)} = \frac{[M]_0}{[I]_0}$	block copolymer M_n [g/mol]$^{c)}$	PD$^{c)}$	PS block M_n [g/mol]$^{c)}$
1$^{a)}$	PBLG-X_1	815	150 000	1.2	108 000
2$^{a)}$	PBLG-X_1	870	130 000	1.2	88 000
3$^{a)}$	PBLG-X_2	960	73 000	1.5	50 000

a) polymerization at 130 °C in DMF.
b) calculated.
c) Measured by SEC-MALLS (eluent DMF/LiBr)

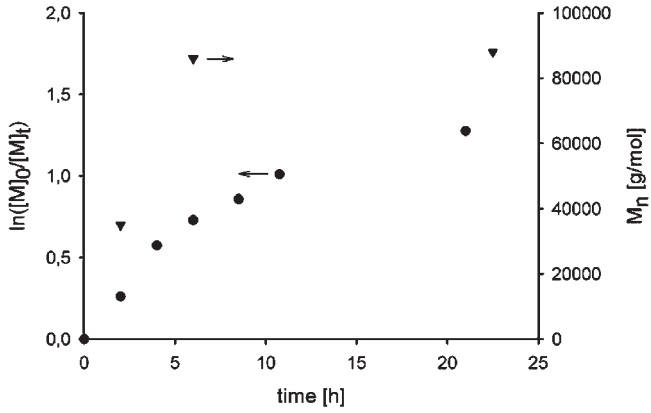


Figure 4.
Kinetics of the NMP of styrene with PBLG-X_1 (Table 1/entry 2) and evolution of the molecular weight.

linear increase of the conversion and the molecular weight only for approximately the first 8 hours. After this time the polymerization kinetics is no longer first order and the molecular weight does not increase with the conversion anymore.

On the other hand for the macro-initiator PBLG-X_2 having only a small spacer between the two functional groups of the double-headed initiator (Table 1/entry 3) the formation of PS homopolymer during the polymerisation was not observed. The

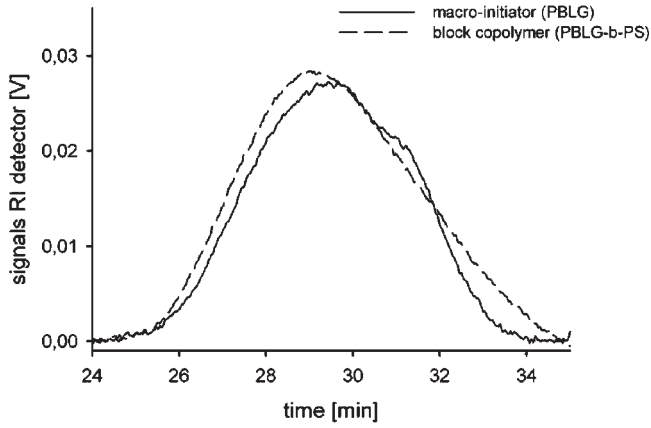


Figure 5.
SEC-MALLS chromatogram of PBLG-PS block copolymers prepared by NMP with PBLG-X_2 macro-initiators (Table 1/enrty 3).

SEC chromatogram (Figure 5) shows a shift of the block copolymer to higher molecular weights upon styrene polymerization without the formation of an additional shoulder. Further investigations copolymerisation of the BLG-NCA and styrene using double-headed initiators with amido-amidate and NMP groups are necessary.

Conclusion

Synthesis of well defined polypeptide based rod-coil block copolymers is possible via the combination of the ring-opening polymerization of *N*-carboxyanhydrides and controlled radical polymerization employing doubled-headed initiators. Two bifunctional initiators were synthesized having a nickel amido-amidate group for NCA polymerization and an ATRP group or an NMP group respectively. The nickel-amido-amidate group was in both cases used to initiate the polymerization of benzyl-L-glutamate-NCA, yielding macro-initiators for the controlled radical polymerization. Subsequent ARTP of MMA yielded well defined block copolymers. However, the ATRP requires fine tuning of the reaction conditions for each monomer, in order to adjust the reactivity of the chains and to ensure control. NMP using PBLG macro-initiators seems to be less sensitive.

Acknowledgements: The authors thank the Deutsche *Forschungsgemeinschaft (DFG)* and the *Netherlands Organisation for Scientific Research (NWO)* for financial support and Dr. Ralf Kleppinger (DSM) for the XRD and AFM measurements. This project is associated with the European Graduate School ''Microstructural Control in Free-Radical Polymerization''.

[1] H. A. Klok, J. F. Langenwalter, S. Lecommandoux, *Macromolecules* **2000**, *33*, 7819.
[2] R. Yoda, S. Komatsuzaki, E. Nakanishi, T. Hayashi, *Eur. Polym. J.* **1995**, *31*, 335.
[3] H. Schlaad, B. Smarsly, M. Losik, *Macromolecules* **2004**, *37*, 2210.
[4] M. Losik, S. Kubowicz, B. Smarsly, H. Schlaad, *Eur. Phys. J. E.* **2004**, *15*, 407.
[5] H. A. Klok, S. Lecommandoux, *Adv. Mater.* **2001**, *13*, 1217.
[6] S. Steig, F. Cornelius, P. Witte, B. B. P. Staal, C. E. Koning, A. Heise, H. Menzel, *Chem. Comm.* **2005**, 5420.
[7] T. J. Deming, *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 3011.
[8] S. A. Curtin, T. J. Deming, *J. Am. Chem. Soc.* **1999**, *121*, 7427.
[9] W. L. F. Armarego, D. D. Perrin, *Purification of Laboratory Chemicals*, 4th ed.,Butterworth-Heinemann, Oxford, **1996**.
[10] W. D. Fuller, M. S. Verlander, M. Goodman, *Biopolymers* **1976**, *15*, 1869.
[11] K. R. Brzezinska, T. J. Deming, *Macromolecules* **2001**, *34*, 4348.
[12] J. Dao, D. Benoit, C. J. Hawker, *J. Polym. Sci. A* **1998**, *36*, 2161.
[13] A. W. Bosman, R. Vestberg, A. Heumann, J. M. J. Frechet, C. J. Hawker, *J. Am. Chem. Soc* **2003**, *125*, 715.
[14] K. R. Brzezinska, T. J. Deming, *Macromol. Biosci.* **2004**, *4*, 566.
[15] M. Kamigaito, T. Ando, M. Sawamoto, *Chem. Rev.* **2001**, *101*, 3689.
[16] T. J. Deming, S. A. Curtin, *J. Am. Chem. Soc.* **2000**, *122*, 5710.
[17] H. Rettig, E. Krause, H. G. Börner, *Macromol. Rapid Commun.* **2004**, *25*, 1251.

