

# Synthesis of Polypeptide Based Rod-Coil Block Copolymers by Using TEMPO Based Bifunctional Initiator

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**Summary:** A bifunctional initiator was synthesized having two functional groups for the initiation of the nickel mediated ring-opening polymerization of  $\gamma$ -benzyl-L-glutamate-N-carboxyanhydride and for the controlled radical polymerization of styrene via NMP. Molecular weights and molecular weight distributions of the polypeptides and block copolymers (PBLG-*b*-PS) were measured by SEC/MALLS. The controlled character of the NMP of styrene was examined by investigation of monomer consumption by gas chromatography.

**Keywords:** bifunctional initiator; nitroxide mediated radical polymerization; polypeptides; ring-opening polymerization; rod-coil block copolymers

## Introduction

Rod-coil block copolymers exhibit a phase separation on the nanometer length scale due to the incompatibility of the dissimilar segments which can lead to interesting morphologies. The self-assembly is not only controlled by the microphase separation of the blocks but also by the tendency of the rigid segments to form anisotropic liquid-crystalline domains. Polypeptide based rod-coil block copolymers consist of vinyl polymers as flexible coil block and a polypeptide block with a rod-like structure that is caused by a stable  $\alpha$ -helical secondary structure. Block copolymers of this architecture are of interest from both functional and structural points of view.<sup>[1–4]</sup>

The synthesis of such block copolymers can be accomplished by means of a bifunctional initiator as described before.<sup>[5–7]</sup> Within this method it is possible to combine two different controlled polymerization

techniques to be conducted consecutively. This combination opens a wide range of possibilities for the controlled synthesis of rod-coil block copolymers by avoiding polymer end group modification. The synthetic route for the preparation of well-defined poly( $\gamma$ -benzyl-L-glutamate)-*b*-poly(styrene) (PBLG-*b*-PS) block copolymers is shown in Scheme 1. To synthesize the peptide block by controlled ring opening polymerization of  $\alpha$ -N-carboxyanhydrides (NCA) via a transition metal catalyzed mechanism as first described by DEMING, functionalized nickel-amido-amidates like molecule **7** can be used as initiators.<sup>[8,9]</sup> After isolation and purification a nitroxide mediated radical polymerization (NMP) of styrene can be initiated using the resulting endfunctionalized PBLG as macroinitiator.

## Experimental Part

### Materials

All solvents were dried and distilled using standard procedures<sup>[10]</sup> and if necessary degassed by freeze-pump-thaw procedure. Styrene (Acros) was distilled from CaH<sub>2</sub> under reduced pressure and was stored under nitrogen atmosphere at –30 °C.

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**Scheme 1.**

Strategy for the synthesis of polypeptide based rod-coil block copolymers.

$\gamma$ -Benzyl-L-glutamic acid-*N*-carboxyanhydride (BLG-NCA) was synthesized and polymerized according to the literature.<sup>[11,12]</sup>

### Synthesis of PBLG-b-PS Block Copolymer

The synthesis of alloc-L-leucine-*N*-hydroxysuccinimidyl ester **5** and its use for preparation of alloc amides has been described previously.<sup>[9,11]</sup> The alkoxyamine **4** 1-(4-aminomethylphenyl)-1-(2,2,6,6-tetramethylpiperidinyloxy)ethyl was synthesized according to literature procedures.<sup>[13]</sup> The preparation of the bifunctional initiator **7** and the synthesis of PBLG macroinitiator were described before.<sup>[5,6]</sup>

### Block Copolymerization (PBLG-b-PS)

A dry round bottomed schlenk flask was charged with the PBLG macroinitiator. It was dissolved in DMF (abs.) and styrene was added. Anisole as internal standard was added. The polymerization was carried out by heating the mixture to 125 °C. After the intended polymerization time the polymer was precipitated with methanol, isolated and reprecipitated from THF with methanol.

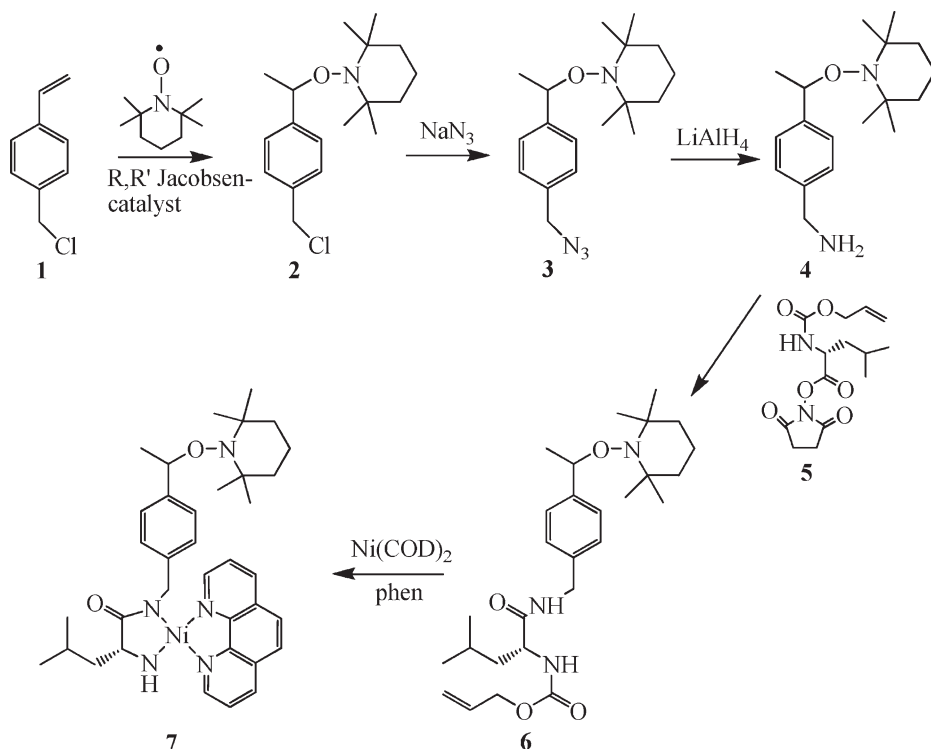
### Characterization

Monomer conversion was determined by gas chromatography. Molecular weight and molecular weight distribution were measured by SEC/MALLS combination in DMF (membrane filtered and degassed) containing LiBr (0.1 mol%) on two PL-gel 5  $\mu$ m mixed-C columns (Polymer Laboratories) at 80 °C and a flow rate of 0.5 mL/min. Detection was performed with a SHOEDX RI -101 differential refractive-index detector (set at 35 °C) and a TriStar MiniDawn light scattering detector from Wyatt Technology (angles at 30, 90, and 120°).

## Results and Discussion

The application of controlled polymerization techniques is particularly suitable for the preparation of well-defined rod-coil block copolymers as they provide good control over the end groups. For the combination of both polymerization techniques - NMP and nickel mediated NCA polymerization - a corresponding bifunctional initiator has to be synthesized, which contains the functionalities of an amido-amidate group on one side and a nitroxide group on the other side. The synthetic route for such an initiator is shown in Scheme 2. The nitroxide group 2,2,5-trimethyl-3-(1-p-aminomethylphenylethoxy)-4-phenyl-3-azahexane (TIPNO) has already been described as one side of the bifunctional initiator<sup>[5]</sup> and is applicable to a variety of monomers. However 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) and its derivatives are typically used for styrene polymerization. Furthermore, the synthesis of TIPNO based nitroxide groups is more complex and laborious. The TEMPO based alkoxyamine as initiating group for NMP of styrene was synthesized according to literature procedures.<sup>[13]</sup> Subsequently this alkoxyamine **4** was converted with alloc-L-leucine-*N*-hydroxysuccinimidyl ester **5** into the direct precursor **6** for the bifunctional initiator. The purity of **6** was verified by MALDI-ToF mass spectrometry and NMR analysis. This compound can be transformed into the initiating complex **7** by reaction with nickel cyclooctadiene complex (Ni(COD)<sub>2</sub>) and phenantroline as ligand (yield 21%).

The bifunctional initiator **7** was used to initiate the polymerization of  $\gamma$ -benzyl-L-glutamate *N*-carboxyanhydride ( $\gamma$ -BLG-NCA)

**Scheme 2.**

Synthesis of bifunctional initiator **7** for combination of NCA polymerization with NMP.

yielding the polypeptide block. The NCA polymerization was carried out at ambient temperature in DMF as solvent. After isolation and purification PBLG macroinitiators were obtained having a molecular weight in a range from  $M_n = 17\,000$  g/mol to  $44\,000$  g/mol with an average polydispersity of 1.32 as determined by size exclusion chromatography with light scattering detector (SEC/MALLS) (see Table 1).

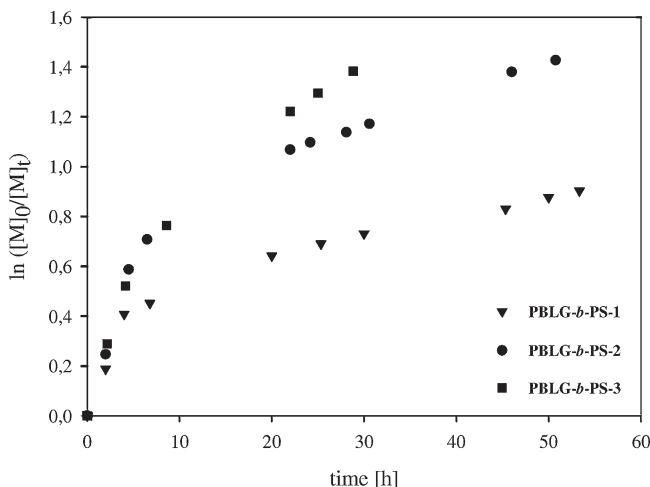
PBLG macroinitiators **PBLG-2** ( $M_n = 24\,000$  g/mol) and **PBLG-4** ( $M_n = 44\,000$  g/mol) were used in the NMP of styrene in DMF. To demonstrate the controlled character of the NMP-polymerization, kinetic measurements were carried out by determining the monomer consumption via gas chromatography (GC). In order to observe the kinetic behaviour different ratios of styrene to macroinitiator were used. All experiments

**Table 1.**

SEC/MALLS results for Ni-catalyzed NCA-polymerization with **7** as initiator.

No. <sup>a)</sup>	$M_{n(\text{theo})} \cdot p$ [g/mol] <sup>b)</sup>	$M_{n(\text{exp.})}$ [g/mol] <sup>c)</sup>	PD <sup>c)</sup>	conversion <sup>d)</sup>	time
<b>PBLG-1</b>	9 000	17 000	1.34	71%	16 h
<b>PBLG-2</b>	28 500	24 000	1.30	78%	16 h
<b>PBLG-3</b>	44 000	28 500	1.32	71%	16 h
<b>PBLG-4</b>	77 000	44 000	1.29	60%	19 h

<sup>a)</sup> polymerization at 25 °C in DMF; <sup>b)</sup> calculated; <sup>c)</sup> measured by SEC-MALLS (eluent DMF/LiBr); <sup>d)</sup> gravimetrically determination.



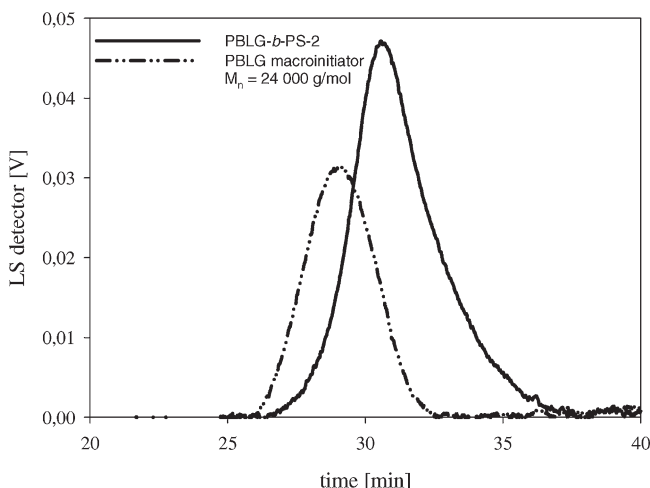
**Figure 1.**

$\ln([M]_0/[M]_t)$  vs time plot of NMP of styrene in DMF ( $T = 125^\circ\text{C}$ ) with PBLG as macroinitiator: **PBLG-*b*-PS-1**  $[M]/[I] = 927$ , ( $M_n(\text{PBLG}) = 24\,000\text{ g/mol}$ ); **PBLG-*b*-PS-2**  $[M]/[I] = 1150$ , ( $M_n(\text{PBLG}) = 44\,000\text{ g/mol}$ ), **PBLG-*b*-PS-3** ( $125^\circ\text{C}$ )  $[M]/[I] = 935$  ( $M_n(\text{PBLG}) = 44\,000\text{ g/mol}$ ).

of NMP-polymerizations result in a similar trend as depicted in Figure 1. At the beginning of the NMP (up to 4 h) a linear relationship between  $\ln([M]_0/[M]_t)$  and time with a monomer consumption of 9%/h can be noticed whereas in the further process the polymerization rate decelerates ( $\sim 1\%/h$ ).

The SEC chromatogram (Figure 2) shows a shift of the block copolymer to

higher elution times compared to the PBLG macroinitiator ( $M_n = 24\,000\text{ g/mol}$ ), indicating a decreasing hydrodynamic volume for the block copolymer compared to the macroinitiator. However, SEC-MALLS results show an increase of the molecular weights (Table 2). On the other hand gradient polymer elution chromatography (GPEC) measurements revealed an only minor proportion of block copolymer.



**Figure 2.**

SEC-MALLS chromatogram of PBLG-*b*-PS block copolymers prepared by NMP with PBLG- macroinitiator ( $M_n = 24\,000$ ).

**Table 2.**Molecular weight and PD of PBLG-*b*-PS block copolymers.

No	$M_{n(\text{exp.})}$ [g/mol] <sup>a,b)</sup>	$P_G^c) = \frac{[M]_0}{[I]_0}$	PBLG- <i>b</i> -PS $M_{n(\text{exp.})}$ [g/mol] <sup>b)</sup>	PD <sup>b)</sup>	conversion <sup>d)</sup>
<b>PBLG-<i>b</i>-PS-1</b>	24 000	927	49 000	1.18	60%
<b>PBLG-<i>b</i>-PS-2</b>	44 000	1150	85 000	1.24	75%
<b>PBLG-<i>b</i>-PS-3</b>	44 000	935	74 000	1.21	84%

<sup>a)</sup> molecular weight of PBLG macroinitiator; <sup>b)</sup> measured by SEC-MALLS (eluent DMF/LiBr); <sup>c)</sup> calculated; <sup>d)</sup> determined by GC.

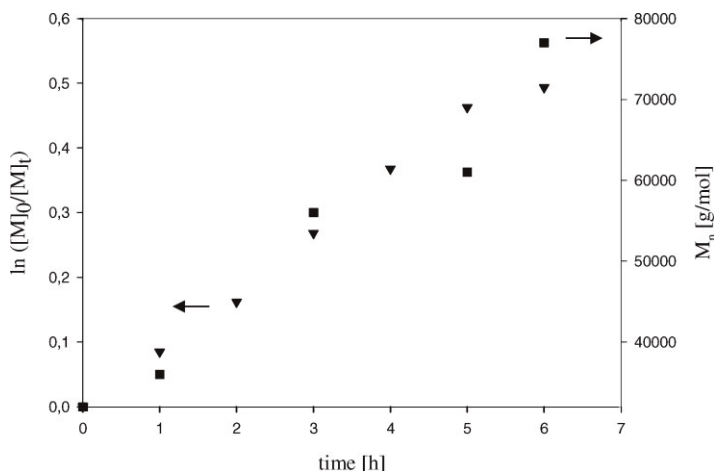
Even if the coil block does not contribute significantly to the hydrodynamic volume as proposed by SCHLAAD et al.,<sup>[3]</sup> these results are unexpected and an alternative explanation has to be found.

The reaction conditions for the NMP are drastic, and it is possible that the PBLG degrades at the temperatures and reaction times necessary for the TEMPO mediated polymerization. In order to verify the instability of the PBLG block a control experiment has been carried out. PBLG was tested under the NMP conditions (125 °C) without addition of styrene. In this experiment no PBLG could be isolated after keeping the DMF solution at 125 °C for a typical reaction time of 54 hours.

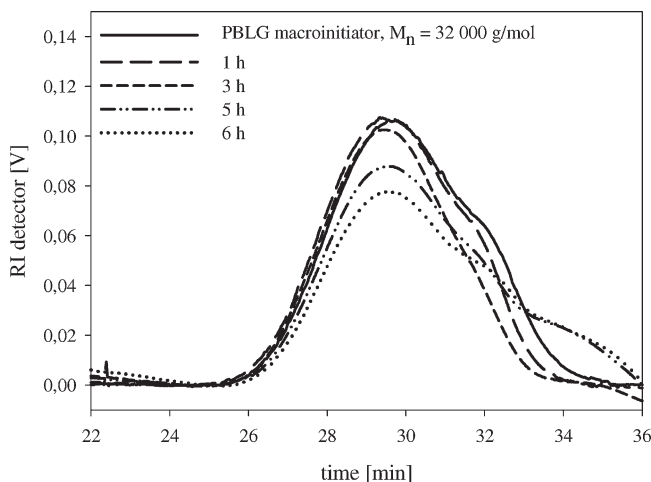
Thus we assume that the PBLG block is degraded, while styrene is polymerized. The PS formed has a higher molecular weight but lower hydrodynamic volume

compared to the PBLG macroinitiator due to the difference between coil (PS) and rigid rod (PBLG). The degradation seems to start when the polymerization kinetics decelerates abruptly (~ 4–5 h) (see Figure 1). Up to this point a conversion of approximately 40% has been reached. We assume that DMF partially decomposes into amines at these high temperatures and that the resulting amine products as nucleophiles attack the PBLG resulting in the degradation.

Thus in another experiment the polymerization kinetics was investigated only within the first 6 hours (Figure 3). The relationship between  $\ln([M]_0/[M]_t)$  and time shows a linear increase of the conversion for the first 5 hours. In addition, the linear increase of the molecular weight, which is another indication for the controlled character of the NMP could be established.

**Figure 3.**

Kinetic plot of the NMP of styrene with PBLG macroinitiator ( $M_n = 32\,000$  g/mol) and evolution of the molecular weight with polymerization time.



**Figure 4.**

Evolution of SEC-MALLS chromatograms of PBLG-*b*-PS during the polymerization time prepared by NMP with PBLG macroinitiator ( $M_n = 32\,000$ ) and  $[M]/[I] = 1100$ .

The SEC chromatograms (Figure 4) of the polymers obtained from this experiment demonstrate almost the same hydrodynamic volume as the macroinitiator. This is the expected behaviour, since the coil does not contribute much to the hydrodynamic volume.<sup>[3]</sup> Only after 5 and 6 hours the molecular weight distribution is shifted slightly to higher elution times<sup>[7]</sup> as stated before. Therefore we assume that up to a conversion of 40% of the NMP PBLG-*b*-PS block copolymers can be synthesized.

## Conclusion

The synthesis of polypeptide based rod-coil block copolymers is possible by means of a bifunctional initiator which combines two controlled polymerization techniques the ring-opening polymerization of *N*-carboxyanhydrides and the controlled radical polymerization. It was started with the NCA-polymerization initiated by the nickel-amido-amidate group according to DEMING yielding PBLG macroinitiators. Subsequently styrene was polymerized by NMP resulting in PBLG-*b*-PS block copolymers. Block copolymers could only be prepared by limiting the reaction times, i.e.

if the PBLG is exposed to temperatures of 125 °C for less than 4 hours. After 5 to 6 hours the PBLG block is instable under these drastic conditions and degrades.

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