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One-Pot Procedure for the Preparation of Rod-Coil Block Copolymers via a Bifunctional Initiator

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Summary: The synthesis of well-defined polypeptide-polyvinyl rod coil block copolymers via the combination of Nickel-mediated ring opening polymerization and atom transfer radical polymerization (ATRP) is described. The strategy for the synthesis is based on a bifunctional initiator. In the first step the poly-benzyl-*L*-glutamate (PBLG) block is prepared which is subsequently used as a macroinitiator for the ATRP of methyl methacrylate (MMA). These two reaction steps are performed either consecutively by isolation of the PBLG-macroinitiator or in a one-pot procedure without isolation of the PBLG-macroinitiator. By the one-pot approach the tedious isolation and purification of the macroinitiator is avoided. Reasonable PMMA-block lengths and low polydispersities indicate a successful synthesis of PBLG-PMMA block copolymers also via the one-pot procedure.

Keywords: atom transfer radical polymerization; one-pot synthesis; polypeptides; ring-opening polymerization; rod-coil diblock copolymers

Introduction

The combination of a polypeptide in its α-helical structure (rod-block) and a synthetic polymer (coil-block) gives rise to interesting morphologies in bulk and solution due to the superposition of both micro phase separation and liquid crystallinity. In the case of using polar or biocompatible polymers as coil-block also promising materials for applications in life science (e.g. drug delivery) can be obtained.

The synthesis is based on a bifunctional initiator which allows to perform two different controlled polymerization techniques consecutively.[1] These are the Nickel mediated polymerization of N-carboxyanhydrides[2] (NCA) yielding the poly-benzyl-*L*-glutamate (PBLG) macroinitiator and the atom transfer radical polymerization (ATRP). It has been shown successfully that it is possible to combine an organo Nickel-complex with an initiating group for the ATRP and to obtain well-defined polypeptides.[1] Since these peptides have an initiating group for the ATRP, they can be used as macroinitiators for a second radical polymerization. These two reaction steps can be performed by isolating the first reaction product, which subsequently is used as macroinitiator for the second polymerization (Figure 1).

The synthesis of the rod-coil block copolymers can be improved regarding the ease of use by the introduction of a one-pot method. After the NCA polymerization the ATRP is directly started in the crude reaction mixture without isolation of the PBLG-macroinitiator. This procedure avoids the tedious isolation and purification of the macroinitiator.

The second block can consist of various monomers due to the versatility of the ATRP. Among the monomers for the coil-block methyl methacrylate is the most studied.[1]

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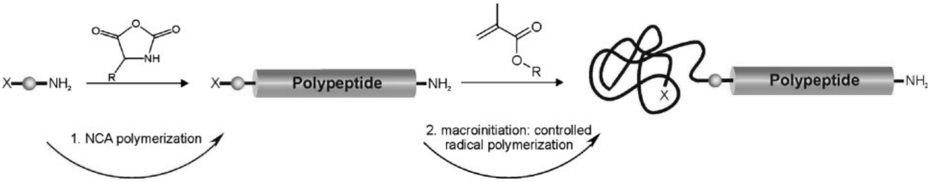

Figure 1.
Synthetic route for the synthesis of rod-coil block copolymers by performing the NCA polymerization and the ATRP of a vinyl monomer consecutively.

Experimental Part

Materials

All solvents were dried and distilled using standard procedures^3 and if necessary degassed by a ‘‘freeze-pump-thaw’’ procedure. Methylmethacrylate (99%, Merck) was distilled 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.[3] Hexamethyltriethyltetraamine (HMTETA, 97%, Aldrich), γ-Benzyl-L-glutamic acid (BLG, >99%, Fluka), phosgene solution (~20% in toluene, purum, Fluka) and (+)-limonene (96%, Fluka) were used as received.

The preparation of the double-headed initiator **1** and the synthesis of PBLG-macroinitiator **3** were performed as previously described.[1]

Synthesis of BLG-NCA

γ-Benzyl-L-glutamic acid-*N*-carboxyanhydride (BLG-NCA) was synthesized according literature procedures[4] with some variations. In a dry three necked flask equipped with a reflux condenser, a nitrogen-inlet and a stop cock 5.09 g (21.4 mmol) of γ-Benzyl-L-glutamic acid (BLG) were suspended in 50 mL dry THF. 14 mL of phosgene solution were added (~20% in toluene, 26.6 mmol, 1.24 eq.). Then the reaction mixture was immersed in an oil bath at 65 °C and stirred under nitrogen-atmosphere for approx. 2 h. Already after 15 min the reaction mixture became clear which indicates the formation of BLG-NCA which is soluble in THF. Then the still hot reaction solution was poured into 100 mL of freshly dried and distilled petroleum ether (PE, low boiling fraction) and it was flushed with nitrogen for 15 min. After crystallization over night at −30 °C the white crystals were isolated and washed with 100 ml of PE and dried under high vacuum. The BLG-NCA was stored under vacuum at −30 °C.

Purification with Acetonitrile[5]

After drying the BLG-NCA, which was synthesized by the above described procedure, it was dissolved in 20 mL dry acetonitrile. Only very few, small particles were filtered off and the acetonitrile was removed under reduced pressure. A yellowish viscous fluid remained which was dried in high vacuum and stored over night at −30 °C. Some white crystals were observed but for a complete crystallization the remainder was dissolved in dry THF again, crystallized from PE and stored over night at −30 °C to assure a complete crystallization. Then the BLG-NCA was isolated, washed with 100 mL of PE, dried in high vacuum and stored under vacuum at −30 °C.

Synthesis in the Presence of (+)-Limonene

The synthesis was analogous to the above described procedure with the difference that 6.2 mL (38.2 mmol, 1.3 eq.) of (+)-limonene were added before the addition of phosgene-solution. In this case it was necessary to use 1.5 eq. of phosgene-solution to obtain a clear solution.

PBLG-Macroinitiated ATRP of MMA

415 mg (0.0075 mmol) of a PBLG-macroinitiator was dissolved in 2.0 mL of

DMF for approx. 2.5 h in a dry Schlenk-flask. Then 3.0 mg (0.021 mmol) CuBr was added which is slightly soluble and a brownish-yellow, turbid solution is formed. After adding 5.0 μL (0.018 mmol) of HMTETA the reaction mixture became clear and green. 1.6 mL (15.0 mmol) of MMA were added and the reaction mixture was immersed into an oil bath thermostated at 80 °C and stirred for 3 d. The polymerization was stopped by air supply and ice-cooling. The reaction mixture was diluted with THF and filtered through an Alox-column. The filtrate is concentrated, precipitated into ice-cooled methanol, isolated and dried under vacuum at RT over night. The polymer is purified by reprecipitation from THF.

One-pot Procedure for the PBLG Macroinitiated ATRP of MMA

1 g (3,8 mmol) of BLG-NCA and 13,3 mg (0,024 mmol) of Ni-initiator **1** were dissolved separately in a total of 7 mL of DMF in dry Schlenk-flasks. The polymerization is started by combing both solutions. It was stirred for 24 h at RT. After the 24 h a sample is taken in order to analyze the conversion and the molecular weight of the PBLG-macroinitiator before starting the ATRP. The sample is concentrated, precipitated into ice-cooled methanol (4 mmol/L HCl), isolated and dried under vacuum at RT over night. To proceed with the ATRP 12.5 μL (0.046 mmol) HMTETA, 7.4 mg (0.052 mmol) CuBr and 1 mL (9.3 mmol) MMA were added and the reaction mixture was immersed into an oil bath thermostated at 80 °C and stirred for 3 d. Within this time the color of the reaction mixture changes from green to brown-green. The polymerization was stopped by air supply and ice-cooling. The reaction mixture was diluted with THF and filtered through an Alox-column. The filtrate is concentrated, precipitated into ice-cooled methanol (4 mmol/L HCl), isolated and dried under vacuum at RT over night. The polymer is purified by reprecipitation from THF.

Characterization

Monomer conversion was determined 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/L) on two PL-gel 5 μm mixed-C columns (Polymer Laboratories, VARIAN) at 80 °C and a flow rate of 0.5 mL/min. Detection was performed with a Shodex RI-101 differential refractive-index detector set at 45 °C and a TriStar MiniDawn light scattering (LS) detector from Wyatt Technology (angles at 30, 90, and 120°).

Optimization of the NCA-Polymerization

DEMING found that the Nickel-amido-amidate initiated NCA-polymerization yielded polypeptides with narrow molecular weight distribution (polydispersity lower than 1.20) and a wide range of molecular weights from 500 g/mol to 500,000 g/mol.[6] The NCA-polymerization which is performed to prepare the PBLG-macroinitiator is shown in Figure 2. The bifunctional initiator **1** bearing the bromo-isobutyric acid group for the initiation of the ATRP and the

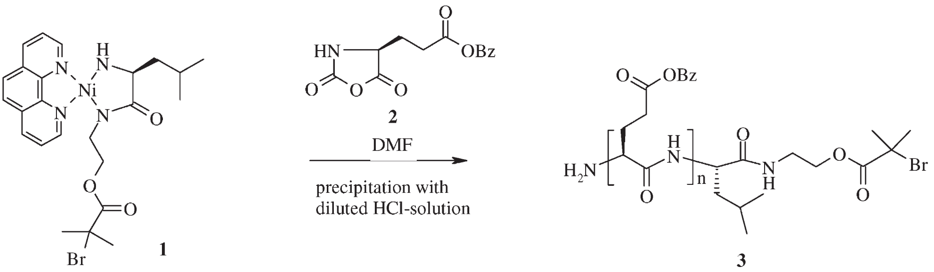

Figure 2.
NCA-polymerization via Deming method[7] at RT for 24 h in DMF to yield the PBLG-macroinitiator **3**.

Nickel-amido-amidate group, which starts the polymerization of the γ-Benzyl-L-glutamic acid-NCA **2** (BLG-NCA), was used. After a reaction time of 24 h at RT in DMF and destruction of the Nickel-complex at the polymer chain end, the PBLG-macroinitiator **3** is obtained.

For the synthesis of well-defined block copolymers the adjustability of the molecular weight and the realization of a low polydispersity (PD) for the PBLG-macroinitiator is crucial. In previous experiments[1] it was found that the experimental molecular weights were much higher than theoretically expected (see also entries 1 and 2, Table 1). Among other things these deviations were explained by impurities present in the Nickel-initiator **1** which were detected by elemental analysis.[1] However, from the dimensions of the deviations it was assumed that there had to be additional reasons. Therefore, the purification of the BLG-NCA from HCl impurities was pursued. During the synthesis of BLG-NCA from BLG and phosgene HCl is produced as byproduct. HCl can form a BLG-NCA adduct in which it is bound to the amide group. When performing the NCA-polymerization the HCl is released and destroys the Nickel-initiator. The concentration of the active initiator is reduced and the [M]/[I] ratio is increased. Hence, significantly higher molecular weights than theoretically expected are obtained for the PBLG-macroinitiator.

Different methods were applied to remove HCl traces in the BLG-NCA. These were the use of a smaller excess of phosgene and flushing the reaction mixture with nitrogen (see entries 3 and 4, Table 1), purification by acetonitrile[5] (see entries 7–10, Table 1) and addition of a non-basic HCl-scavenger like limonene (see entries 5–6, Table 1).

For PBLG-1 and 2 the deviations between theoretical and experimental molecular weights are very high and not reproducible. Only for PBLG-2 the PD is within the range for a controlled polymerization.

For entries PBLG-3 and 4 it is remarkable that only small variations in the synthesis of the BLG-NCA (reducing the amount of phosgene and flushing with nitrogen during the workup of BLG-NCA) have such a great impact on the results of the NCA-polymerization. The deviations in molecular weights were reduced to an average of 66% and the PDs were both within the range for a controlled polymerization.

For PBLG-5 and 6 BLG-NCAs were applied which were synthesized in the presence of the non-basic HCl-scavenger (+)-limonene. The deviations in molecular weights account for an average of only 31%. The results of PBLG-5 were not reproducible and the BLG-NCA synthesized in the presence of (+)-limonene contained some more impurities. Therefore this method was not further applied.

The BLG-NCAs which were purified by the use of acetonitrile (PBLG-7 to PBLG-10) were also synthesized with the lower amount of phosgene compared to PBLG-3 and 4. The purification with acetonitrile was done according literature[5] in order to remove the BLG-NCA-HCl adduct which

Table 1.
Results of polymerizations of BLG-NCA using different preparation and purification methods for the BLG-NCA.

entry	NCA-method	$M_{n,\ th.}$ (g/mol)	$M_{n,\ SEC}$ (g/mol)	deviation (%)	PD
PBLG-1	1.4 eq. $COCl_2$	10 200	53 400	+ 425	1.52
PBLG-2	1.4 eq. $COCl_2$	15 200	96 100	+ 534	1.34
PBLG-3	1.3 eq. $COCl_2$	22 500	38 000	+ 69	1.23
PBLG-4	1.3 eq. $COCl_2$	28 400	46 200	+ 63	1.31
PBLG-5	limonene	29 600	31 500	+ 6	1.37
PBLG-6	limonene	56 500	88 000	+ 56	1.29
PBLG-7	CH_3CN	29 400	28 500	− 3	1.15
PBLG-8	CH_3CN	34 300	44 100	+ 29	1.40
PBLG-9	CH_3CN	48 500	55 200	+ 22	1.46
PBLG-10	CH_3CN	58 500	79 500	+ 36	1.44

is not soluble in acetonitrile in contrast to the pure BLG-NCA. The PBLG-macroinitiators (entries 7 to 10, Table 1) synthesized by the use of the acetonitrile purified BLG-NCA show only molecular weight deviations of an average of 23%. PBLG-7 shows a very good agreement in molecular weights and a polydispersity below 1.20. For PBLG-8 to 10 these results could not be reproduced yet. Therefore further investigations are under way in which additional parameters influencing the polymerization like for example the BLG-NCA concentration are varied.

Nevertheless the purification method of the BLG-NCA with acetonitrile showed the most promising results for a controlled NCA-polymerization. In the following syntheses of PBLG-macroinitiators the acetonitrile purified BLG-NCA has been employed.

One-Pot Synthesis of PBLG-b-PMMA

The isolation and purification of the PBLG-macroinitiator is a tedious and time-consuming procedure. It has to be reprecipitated two times and after this purification it has to be dissolved again (2–3 h) for the macroinitiated ATRP. Therefore a one-pot method should be investigated to shorten the whole procedure. In this way only the complete block copolymer has to be purified which is technically easier than the purification of the PBLG-macroinitiator. With the above described optimization of the NCA-polymerization the molecular weights of the PBLG-macroinitiator are better predictable. Thus, it is no longer required to isolate the macroinitiator and determine its molecular weight before starting the ATRP. A one-pot synthesis with all the reagents simultaneously in the reaction mixture appeared not to be promising because of a wide variety of possible side reactions and incompatible reaction conditions for the preparation of both blocks at a time. Hence, a one-pot approach with the sequential addition of monomers and reagents and changing the reaction conditions was chosen.

In Figure 3 the reaction scheme is depicted. First the PBLG-macroinitiator is synthesized for 24 h at RT and then the ATRP is started in the crude reaction mixture by adding the copper-ligand system and the monomer and stirring the reaction mixture at 80 °C for 3d.

First the influence of the conditions of the ATRP (3d, 80 °C) on the PBLG-macroinitiator was investigated (see Table 2).

Entry 1 in Table 2 shows an experiment where a PBLG-macroinitiator was stirred for 3d at 80 °C. After the reaction the macroinitiator was isolated and purified in order to determine the resulting weight and its molecular weight. The changes of the amount, molecular weight and polydispersity are all within the error of the analytical methods. Therefore it can be concluded that the PBLG-macroinitiator is not affected by the conditions for the ATRP (stirring for 3d at 80 °C).

A variation of the above described experiment is shown as entry 2 in Table 2. The NCA-polymerization was performed for 24 h at RT. With regard to the aspired one-pot procedure the reaction mixture was not worked up but it was stirred at 80 °C for another 3d. Before starting the reaction period under the temperature conditions of the ATRP a sample was taken in order to analyze the conversion of the NCA-polymerization and the molecular weight of the PBLG-macroinitiator. The conversion did not change within the limit of experimental errors. This result was not unexpected due to previous experiments in which the extension of the reaction time of a NCA-polymerization at RT for 8 h did not influence the conversion. About 70% conversion proved to be the limit. The reason for this might be the deactivation of a certain amount of monomer by traces of humidity and air. However, the SEC analysis shows an increase of molecular weight of about 29,000 g/mol due to the time keeping the reaction mixture at elevated temperatures, whereas the polydispersity within the error remains unchanged. The increase in molecular weight accounts for 32% of the molecular

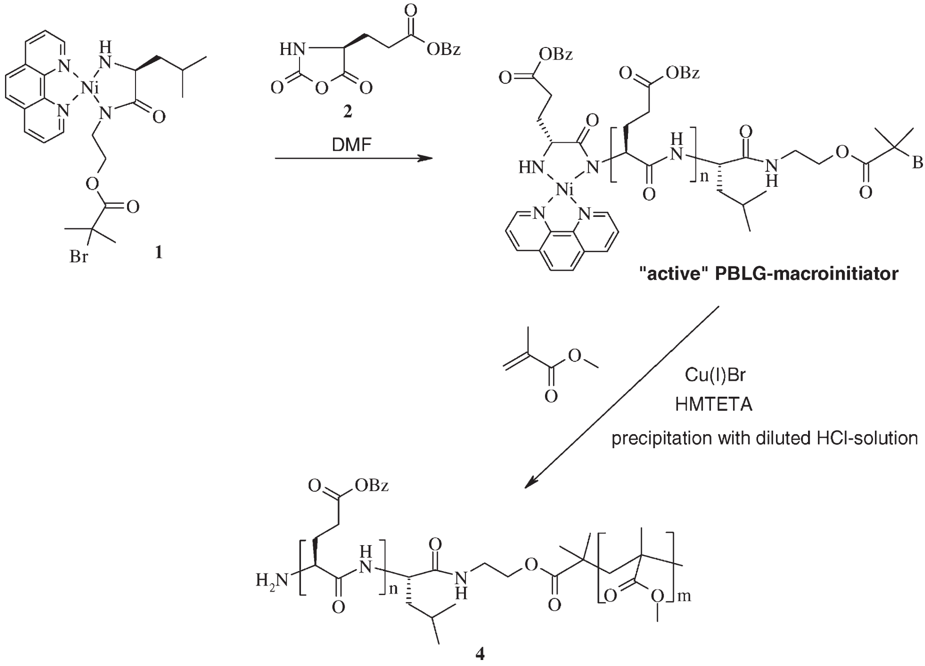

Figure 3.
Reaction scheme for the preparation of PBLG-b-PMMA **4** via the one-pot method (sequential addition of monomers).

weight of the PBLG-macroinitiator. The increase in molecular weight without a further conversion can be related to some sort of coupling reactions of active (Nickel-complex terminated) PBLG-chains. As the polydispersity does not change this has to be a random process (coupling of PBLG chains independent from their chain lengths).

Thus, in summary the preliminary experiments show that the isolated PBLG-macroinitiator is stable under the ATRP conditions but the active PBLG-macroinitiator appears to undergo some sort of coupling reactions.

Despite these problems the one-pot procedure was investigated.

Table 3 summarizes the results of two one-pot syntheses (entries 3–4). As a comparison the results of two block copolymerizations (entries 1–2) are included where the PBLG-macroinitiator was isolated and purified before starting the ATRP.

Applying the conventional method only quite small PMMA-blocks were obtained which were significantly smaller than expected from the monomer-to-initiator ratios. These results are in accordance with previous investigations.[1]

Table 2.
Experiments to confirm the stability of the PBLG-macroinitiator under "ATRP-conditions" (3d at 80 °C).

entry	PBLG-macroinitiator			PBLG-macroinitiator after 3d at 80 °C		
	initial weight/ conversion	$M_{n,\ SEC}$ (g/mol)	PD	resulting weight/ conversion	$M_{n,\ SEC}$ (g/mol)	PD
1	188.7 mg	88 000	1.29	180.4 mg (96%)	86 300	1.33
2	74.8%	90 100	1.54	74.9%	118 700	1.50

Table 3.

SEC-results for PBLG-PMMA block copolymers. Entries 1 and 2: PBLG-macroinitiated ATRP of MMA with isolated PBLG-macroinitiator and [M]/[I] = 1990 for entry 1 and 2820 for entry 2; Entries 3 and 4: One-pot syntheses: 1) 24 h NCA-polymerization at RT; 2) 3d at 80 °C PBLG-macroinitiated ATRP of MMA using CuBr/HMTETA as ATRP-catalyst with Ni-initiator/BLG-NCA/MMA = 1:160:390.

Entry	$M_{n,\ SEC}$ [PBLG-macroini.]	$M_{n,\ SEC}$ (g/mol) [PBLG-b-PMMA]	$M_{n,\ SEC}$ (g/mol) [PMMA-block]	PD
PBLG-b-PMMA 1	44 100	60 800	16 700	1.27
PBLG-b-PMMA 2	88 000	103 600	15 600	1.26
PBLG-b-PMMA 3	59 500	93 100	33 600	1.36
PBLG-b-PMMA 4	68 100	118 200	50 100	1.27

The polymers resulting from the one-pot syntheses were analyzed by NMR-spectroscopy to confirm the existence of the PMMA-block. For all block copolymers the PMMA-block was clearly detectable. Figure 4 shows the SEC-traces of the PBLG-macroinitiator and of the resultant rod-coil block copolymer PBLG-b-PMMA 3. The molecular weight increases of PBLG-b-PMMA 3 and 4 account for 56% and 74% of the molecular weight of the PBLG-macroinitiator. Additionally the polydispersities for PBLG-b-PMMA 3 and 4 are within the range for a controlled polymerization. However, in some experiments the one-pot synthesis failed and only a very small increase in molecular weights was obtained.

Before starting the ATRP in the crude reaction mixture of the previous NCA-polymerization a PBLG-sample was taken in order to analyze the conversion of the polymerization and the molecular weight of the PBLG-macroinitiator. It can be seen that the SEC-trace for the block copolymer is shifted to lower elution volumes. This indicates a larger hydrodynamic volume for the block copolymer compared to the macroinitiator. The fact that the shift is relatively small can be explained by the different morphologies of the PBLG- and PMMA-block. Whereas the PBLG-block forms a stretched α-helical conformation the PMMA-block adopts a random coiled structure. Therefore the hydrodynamic volume of the block copolymer is mainly determined by the PBLG-block and hence the shift in elution volumes is small.

The significant molecular weight increases and low polydispersities found

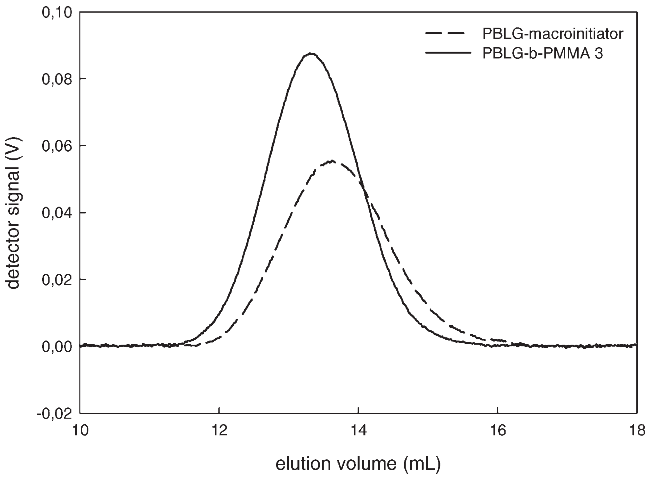

Figure 4.

SEC-traces (detector signal of LS (90°)-detector above elution volume) of PBLG-macroinitiator (PBLG-sample was taken before starting the ATRP) and PBLG-b-PMMA 3 synthesized by one-pot method.

for PBLG-b-PMMA 3 and 4 are promising for a successful one-pot procedure. Further experiments have to be performed in order to improve the reproducibility of the one-pot syntheses and to realize adjustable molecular weights for the PMMA-block.

Conclusion

In previous experiments[1] the successful synthesis of well-defined PBLG-PMMA rod-coil block copolymers was shown. As further development and simplification of the synthetic procedure the one-pot method was introduced. A first step toward the one-pot process was the optimization of the NCA-polymerization to produce well-defined PBLG-macroinitiators. Employing specially purified NCA monomer ("HCl-free") a far better adjustment of molecular weights was accomplished. Using the one-pot method PBLG-PMMA block copolymers with reasonable PMMA-block lengths and low polydispersities can be obtained. Nevertheless there are still problems related to the one-pot method, e.g. the influence of the ATRP-conditions on the NCA-polymerization and the possible formation of PMMA-homopolymer by ATRP of MMA initiated by unreacted Nickel-initiator.

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