# Facile synthesis of 1,4-cis-polyisoprene–polypeptide hybrids with different architectures

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# Facile Synthesis of 1,4-*cis*-Polyisoprene-Polypeptide Hybrids with Different Architectures†

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The synthesis of polypeptide hybrids by controlled/living ring-opening polymerization of N-carboxyanhydrides (NCA) using Schreiner's thiourea catalyst and amino-alcohol terminated poly(1,4-*cis*-isoprene)s as initiators was demonstrated for γ-benzyl-L-glutamate (BLG) and ε-tert-butyloxycarbonyl-L-lysine (BLL) NCAs. One-pot synthesis of amino-alcohol terminated macroinitiators from heterotelechelic keto/aldehyde polyisoprene by reductive amination of carbonyl(s) was presented. Selection of amines allowed to obtain mono-, di- and tri-functional macroinitiators that were used for the synthesis of polyisoprene-polypeptide hybrids of different architecture: AB, BAB, AB2, BAB2, CBABC. Due to the living character of the polymerization, the molar mass of the polypeptide blocks could be controlled by the monomer to initiator ratio ($6000<M_n<44000$ g/mol), while sequential monomer addition allowed the synthesis of a pentablock terpolymer: poly(BLG-b-BLL-b-isoprene-b-BLL-b-BLG)

## Introduction

During the last three decades a lot of efforts were put on the synthesis of hybrid block copolymers based on peptides and other synthetic polymers,[1-3] as polypeptide materials are of great interest due to their ability to form different secondary structures (α-helix, β-sheets)[4] which can lead to self-assembly and have effects on chemical and mechanical properties.[2] Hybrid materials constituted of one polypeptide block and one non-polypeptide block may combine the self-assembly property of polypeptide and the properties of the second block (solubility, elasticity, melt processability, etc.).[1] Hybrids can be synthesized either by coupling of two previously synthetized blocks (*grafting to* method), by polymerization of a second monomer using the first block as a macroinitiator (MI) (*grafting from* method) or by (co)polymerization of a macromonomer (*grafting through* method). The latter two methods are considered to be easier because they do not face solubility issues as could exist in the *"grafting to"* pathway.

Ring-opening polymerization (ROP) of α-amino acid N-carboxyanhydrides (NCA) is a powerful method to obtain well-defined homopolypeptides and (block)-copolymers of different amino acids.[2] First examples of hybrids synthesis by NCA polymerization were reported in the mid-70s by Gallot[5] and Yamashita.[6] In both cases, primary amine terminated polymers (polybutadiene or polystyrene) were used to initiate the polymerization of NCA. Using this method many different hybrids were synthesized.[7] Nevertheless, control over polypeptide block synthesis was not achieved yet and all hybrids required tedious purification-fractionation procedure to remove homopolymers. Starting from the late 90s, four main methods were developed to control NCA polymerization: (i) use of transition metal (cobalt or nickel) complexes as initiators,[8-10] (ii) use of ammonium salts as initiators,[11-13] and (iii) use of primary amines to initiate the polymerization in DMF at 0°C[14] or (iv) under high vacuum conditions.[15,16] All of these methods were later employed for polypeptide hybrids synthesis.[11,17-29]

Recently, Pahovnik suggested the use of OH-terminated PEO and polystyrene as macroinitiators in the presence of methanesulfonic acid which could catalyze the addition of the first monomeric units and protonate the terminal generated $NH_2$-group, preventing the propagation to occur.[30] After complete initiation, propagation was started by addition of a tertiary amine that partly deprotonate the ammonium groups, leading to (co)polymers with narrow dispersity (≤1.17) for β-benzyl-L-aspartate NCA, whereas with γ-benzyl-L-glutamate (BLG) NCA, Đ in the range 1.4-1.6 were observed.[30]

Also recently, Hadjichristidis suggested the use of amino-alcohols of the type $RN(R')CH_2CH_2OH$ for NCA ROP in the presence of N,N'-bis[3,5-bis(trifluoromethyl)phenyl]thiourea (TPT) as the catalyst and as a polymerization controlling agent.[31, 32] Alcohols without tertiary nitrogen in the molecule did not initiate the polymerization of BLG-NCA while amino-alcohols led to linear or star shaped poly(BLG) with controlled molar mass and very narrow dispersity (≤1.08). Similar results were obtained for the (co)polymerization of lysine(Z)-NCA and γ-propargyl-L-glutamate NCA. Polypeptides with controlled molar masses up to 180 000 g/mol were thus obtained in few hours. To the best of our knowledge aminoalcohol/TPT initiating system has not been applied for polypeptide hybrids synthesis.

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Polypeptide hybrids based on polyisoprene could be of particular interest as they could mimic natural rubber (NR) that consists of polyisoprene with 100% 1,4-*cis* configuration of monomer units, containing small quantities of proteins which play an important role in the unique physical properties of NR.[33,34] The first example of poly(isoprene-*b*-peptide) synthesis was reported by Hayashi in 1994, using di-amino telechelic polyisoprene as a macroinitiator for BLG-NCA ROP in DMF at room temperature.[34] Extraction purification was needed to remove admixture of homopolymers. Poly(BLG) block exhibited a α-helix conformation while polyisoprene conformation is a random coil, leading to microheterophase structure of the material.[35,36] These hybrids were used as membranes, potentially for biodegradable medical applications.[37] The tensile test showed that Young's modulus decreases and the value of elongation at the breaking point increases with an increase in the content of the PI block.[38] Finally, they tested compatibility of poly(BLG-*b*-isoprene-*b*-BLG) with rabbit blood and tissue demonstrating that this material had good biocompatibility.[39] Later, Lecommandoux used the same method as in ref.[34] to obtain poly(isoprene-*b*-lysine(Z)) and poly(isoprene-*b*-lysine).[40,41] In solution, stimuli-responsive micelles as a function of pH and ionic stress were obtained.[40]

Interestingly, 1,4-*cis*-monomer units content in the described PI-polypeptide hybrids never excided 66%,[34-41] when it is known, that even small quantity of non-1,4-*cis*-units can change dramatically the physical properties of PI.

In this work, a facile method to synthesize pure 1,4-*cis*-poly(isoprene) macroinitiators from NR with different aminoalcohols at the chain-end(s) is described. Starting from these macroinitiators, hybrids of different architectures (AB, BAB, $AB_2$, $BAB_2$, CBABC) were obtained (Figure 1).

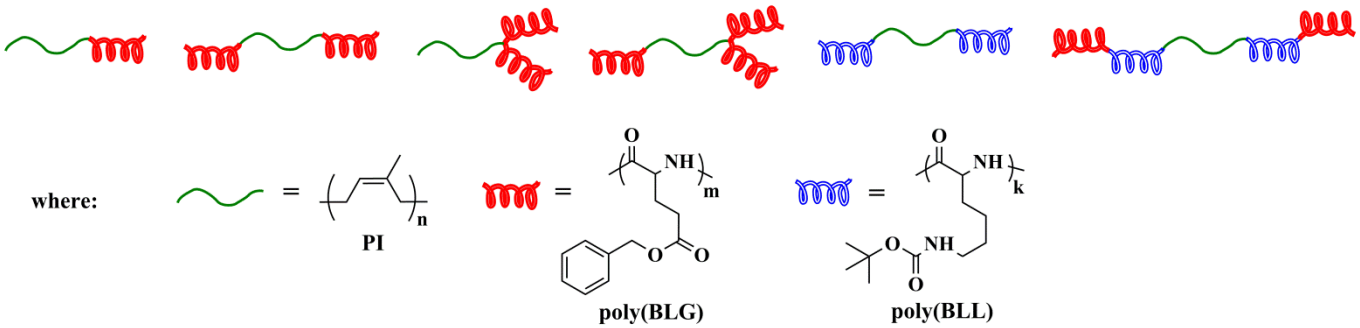



Figure 1 Polyisoprene-polypeptide hybrids synthesized in this work.

## Experimental

### Materials.

Natural rubber (NR) RRIM600 was kindly provided by UMR iATE in Thailand. 3-Chloroperoxybenzoic acid (mCPBA) (70-75 %, Acros), periodic acid ($H_5IO_6$) (≥ 99 %, Aldrich), acetic acid (99%, Aldrich), sodium triacetoxyborohydride ($NaBH(OAc)_3$) (97%, Aldrich), diethanolamine (DEA) (99%, Alfa Aesar), N-methylethanolamine (≥ 98%, Aldrich), ethanolamine (≥ 99.5 %, Aldrich), acetaldehyde (≥ 99.5 %, Aldrich), $Na_2CO_3$ (>99.5%, Sigma), $CaH_2$ (93%, Acros), $CD_2Cl_2$ (99.9 %, Euriso-top), Celite® (R566, Aldrich) and toluene (Reag. Ph. Eur., VWR Chemicals) were used without further purification. Chloroform (99.2%, VWR Chemicals) and i-$Pr_2NEt$ (99.5%, Acros) were kept over $CaH_2$ and cryo-distilled under reduce pressure before use. Methane sulfonic acid (99.5%, Aldrich) was dried overnight by 4 Å molecular sieves before use. N,N'-bis[3,5-bis(trifluoromethyl)phenyl]thiourea (TPT) (>98 %, TCI), γ-Benzyl-L-glutamate N-carboxyanhydride (BLG-NCA) (96 %, Isochem) and Boc-L-Lysine N-carboxyanhydride (BLL-NCA) (97 %, Isochem) were kept in a glovebox and used as received. Tetrahydrofuran (THF) and dichloromethane (DCM) were purified using solvent purification system PureSolv MD7 from INERT (unless otherwise mentioned). Methanol and diethyl ether (reagent grade, Aldrich) were used as received.

### Synthesis of functionalized PI

All syntheses (except keto/aldehyde PI) were conducted in Ar atmosphere in vacuum-dried 15 mL tubes.

**Synthesis of heterotelechelic keto/aldehyde PI.** 5 g of NR was dissolved overnight in 250 mL of THF in round-bottom flask under vigorous stirring. Then 50 mL of mCPBA (0.14 g, 0.81 mmol) solution in THF were added dropwise at room temperature. After 2 h the solution of 0.3 g of periodic acid (2.2 eq towards mCPBA, 1.78 mmol) in 50 mL of THF was added dropwise to the epoxidized NR solution. The reaction was carried out for 2 h and then 0.5 g of $Na_2CO_3$ were added and stirred for 20 min to neutralize acids. The reaction mixture was filtered on glass filter with Celite® affording yellow solution which was then concentrated under reduced pressure to ~10 mL and precipitated into a large excess of cold methanol. The polymer was separated by decantation, then dissolved in diethyl ether (~ 50 mL) and filtered on Celite® a second time. Concentration and precipitation steps were repeated and polymer was dissolved in DCM. The final product was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording a yellowish and transparent viscous liquid. *Yield:* 80 %, $M_n$ = 10540 g/mol, Đ = 1.6. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 9.74 (t, 1H, -$CH_2$*CH*O), 5.14 (t (broad), 159H -$CH_2$*CH*=), 2.47 (t, 2H, -*CH*$_2$CHO), 2.42 (t, 2H, -*CH*$_2$COCH$_3$), 2.33 (t, 2H, -*CH*$_2$CH$_2$CHO), 2.00 – 2.12 (m (broad), 603H, -*CH*$_2$CH= and -*CH*$_2$C(CH$_3$)=), 1.68 (s (broad), 446H -(*CH*$_3$)C=CH-).

**Synthesis of heterotelechelic keto/hydroxyl PI (PI-OH).** 2 g of keto/aldehyde PI (0.2 mmol) were dissolved in 5 mL of dry THF. 0.19 g (4 eq, 0.8 mmol) of $NaBH(OAc)_3$ and 8 µL (1.3 eq, 0.13 mmol) of acetic acid were added to the reaction. The mixture was stirred at 40

°C overnight and the product was separated by two successive precipitations into a large excess of cold methanol, then dissolved in $Et_2O$, filtered on a glass filter with Celite® and dried overnight at 40°C under dynamic vacuum. *Yield*: 90 %, $M_n$ = 8780 g/mol, Đ = 1.6. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 159H -$CH_2$*CH*=), 3.58 (t, 2H, -C*H*$_2$OH), 2.42 (t, 2H, -C*H*$_2$COCH$_3$), 2.00 – 2.12 (m (broad), 603H, -*CH*$_2$CH= and -*CH*$_2$C(CH$_3$)=), 1.68 (s (broad), 446H -(*CH*$_3$)C=CH-).

**Synthesis of heterotelechelic keto/N-methyl(2-hydroxyethyl)amine PI (MI1).** 1.0 g of keto/aldehyde PI (0.1 mmol) were dissolved in 2.8 mL of dry THF and 27 mg (3.5 eq, 0.37 mmol) of N-methylethanolamine were added to the solution and the reaction mixture was stirred at 40°C for 2 h. Finally, 71 mg (3.2 eq, 0.33 mmol) of NaBH(OAc)$_3$ and 8 µL (1.3 eq, 0.13 mmol) of acetic acid were added to the reaction. The heterogeneous medium was stirred at 40°C for 2 h and then directly precipitated into a large excess of methanol under vigorous stirring. The polymer was separated by decantation and then dissolved in diethyl ether, filtered on a glass filter with Celite®, concentrated under reduced pressure to ~3 mL and precipitated again in methanol. After removal of methanol by decantation, the polymer was dissolved in DCM. The final **MI1** was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording colorless viscous liquid. Yield: 89 %, $M_n$ = 10090 g/mol, Đ = 1.49. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 148H -$CH_2$*CH*=), 3.53 (t, 2H, -*CH*$_2$OH), 2.51 (t, 2H, -N(CH$_3$)*CH*$_2$CH$_2$OH), 2.37 – 2.45 (m, 4H, -C*H*$_2$N< and -C*H*$_2$COCH$_3$), 2.24 (s, 3H, -N(*CH*$_3$)-), 2.00 – 2.12 (m (broad), 603H, -*CH*$_2$CH= and -*CH*$_2$C(CH$_3$)=), 1.68 (s (broad), 446H -(*CH*$_3$)C=CH-).

**Synthesis of heterotelechelic keto/di(2-hydroxyethyl)amine PI (MI2).** 1.0 g of keto/aldehyde PI (0.1 mmol) were dissolved in 2.8 mL of dry THF and 39 mg (3.5 eq, 0.37 mmol) of diethanolamine were added to the solution and the reaction mixture was stirred at 40°C for 2 h. Finally, 71 mg (3.2 eq, 0.33 mmol) of NaBH(OAc)$_3$ and 8 µL (1.3 eq, 0.13 mmol) of acetic acid were added to the reaction. The heterogeneous medium was stirred at 40°C for 2 h and then directly precipitated into a large excess of methanol under vigorous stirring. The polymer was separated by decantation and then dissolved in diethyl ether, filtered on a glass filter with Celite®, concentrated under reduce pressure to ~3 mL and precipitated again in methanol. After removal of methanol by decantation, the polymer was dissolved in DCM. The final **MI2** was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording colorless viscous liquid. Yield: 85 %, $M_n$ = 9790 g/mol, Đ = 1.56. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 138H -$CH_2$*CH*=), 3.57 (t, 4H, -*CH*$_2$OH), 2.64 (t, 4H, -N(*CH*$_2$CH$_2$OH)$_2$), 2.52 (t, 2H, -C*H*$_2$N<), 2.42 (t, 2H, -C*H*$_2$COCH$_3$), 2.00 – 2.12 (m (broad), 603H, -*CH*$_2$CH= and -*CH*$_2$C(CH$_3$)=), 1.68 (s (broad), 446H -(*CH*$_3$)C=CH-).

**Synthesis of telechelic di-(N-ethyl(2-hydroxyethyl)amine) PI (MI3).** 1.0 g of keto/aldehyde PI (0.1 mmol) were dissolved in 3.6 mL of dry THF and 52 µL (5 eq, 0.5 mmol) of ethanolamine were added to the solution and the reaction mixture was stirred at 40 °C. After 2 h, 0.19 g (9 eq, 0.9 mmol) of NaBH(OAc)$_3$ and 8 µL (1.3 eq, 0.13 mmol) of acetic acid were added to the reaction. The heterogeneous medium was stirred at 40°C overnight and then it was cooled down to 0 °C for the addition of 0.11 mL (20 eq, 2 mmol) of acetaldehyde to the medium to alkylate newly formed secondary amino chain-ends as well as remaining excess of ethanolamine. The reaction was left for 2 h at 40 °C and finally 0.43 g (20 eq, 2 mmol) of NaBH(OAc)$_3$ were added to complete reduction (the temperature was reduced to 0 °C during addition to avoid evaporation of acetaldehyde). The reaction was finished after additional 2 h at 40 °C and the mixture was directly precipitated into large excess of methanol under vigorous stirring. The polymer was separated by decantation and then dissolved in diethyl ether, filtered on a glass filter with Celite®, concentrated under reduced pressure to ~3 mL and precipitated again in methanol. After removal of methanol by decantation, the polymer was dissolved in DCM. The final **MI3** was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording yellow viscous liquid. Yield: 84 %, $M_n$ = 12190 g/mol, Đ = 1.52. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 163H -$CH_2$*CH*=), 3.36 – 3.50 (m, 4H, -*CH*$_2$OH), 2.73 (m, 1H, -*CH*(CH$_3$)-N<), 2.34 – 2.64 (m, 10H, -N(*CH*$_2$CH$_3$)*CH*$_2$CH$_2$OH and -C*H*$_2$N<), 2.00 – 2.12 (m (broad), 603H, -*CH*$_2$CH= and -*CH*$_2$C(CH$_3$)=), 1.68 (s (broad), 446H -(*CH*$_3$)C=CH-), 1.02 (m, 6H, *CH*$_3$CH$_2$-), 0.95 (d, 3H, -CH(*CH*$_3$)N<).

**Synthesis of heterotelechelic N-ethyl(2-hydroxyethyl)amine/di(2-hydroxyethyl)amine PI (MI4).** 1.0 g of keto/aldehyde PI (0.1 mmol) were dissolved in 2.8 mL of dry THF and 39 mg (3.5 eq, 0.37 mmol) of diethanolamine were added to the solution and the reaction mixture was stirred at 40°C for 2 h. Then 71 mg (3.2 eq, 0.33 mmol) of NaBH(OAc)$_3$ and 8 µL (1.3 eq, 0.13 mmol) of acetic acid were added to the reaction and medium was stirred at 40°C. After 2 h 0.8 mL of dry THF, 52 µL (5 eq, 0.5 mmol) of ethanolamine, 0.15 g (7 eq, 0.7 mmol) of NaBH(OAc)$_3$ and 20 µL (3.2 eq, 0.32 mmol) of acetic acid were added to the reaction and the mixture was stirred at 40°C overnight. Then it was cooled down to 0 °C for the addition of 0.11 ml (20 eq, 2 mmol) of acetaldehyde to the medium to alkylate all primary and secondary amines present in the system. The reaction was left for 2 h at 40 °C and finally 0.43 g (20 eq, 2 mmol) of NaBH(OAc)$_3$ were added to complete reduction (the temperature was reduced to 0 °C during addition to avoid evaporation of acetaldehyde). The reaction was finished after additional 2 h at 40 °C and the mixture was directly precipitated into large excess of methanol under vigorous stirring. The polymer was separated by decantation and then dissolved in diethyl ether, filtered on a glass filter with Celite®, concentrated under reduced pressure to ~3 mL and precipitated again in methanol. After removal of methanol by decantation, the polymer was dissolved in DCM. The final **MI4** was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording colorless viscous liquid. Yield: 75 %, $M_n$ = 9450 g/mol, Đ = 1.70. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 159H -$CH_2$*CH*=), 3.57 (t, 4H, -N(CH$_2$*CH*$_2$OH)$_2$) 3.36 – 3.50 (m, 2H, -N(C$_2$H$_5$)CH$_2$*CH*$_2$OH), 2.73 (m, 1H, -*CH*(CH$_3$)-N<), 2.64 (t, 4H, -N(*CH*$_2$CH$_2$OH)$_2$), 2.34 – 2.60 (m, 6H, -N(*CH*$_2$CH$_3$)*CH*$_2$CH$_2$OH and -C*H*$_2$N<), 2.00 – 2.12 (m (broad), 639H, -*CH*$_2$CH= and -*CH*$_2$C(CH$_3$)=), 1.68 (s (broad), 477H -(*CH*$_3$)C=CH-), 1.02 (t, 3H, *CH*$_3$CH$_2$-), 0.95 (d, 3H, -CH(*CH*$_3$)N<).

**Synthesis of heterotelechelic 2-hydroxyethylamine/hydroxy PI.** 1.0 g of PI-OH (0.1 mmol) were dissolved in 3.6 mL of dry THF and 52 µL (5 eq, 0.5 mmol) of ethanolamine were added to the solution and the reaction mixture was stirred at 40°C. After 2 h, 0.19 g (9 eq, 0.9 mmol) of NaBH(OAc)$_3$ and 8 µL (1.3 eq, 0.13 mmol) of acetic acid were added to the reaction. The heterogeneous medium was stirred at 40°C overnight. Then the mixture was directly precipitated into large excess of methanol under vigorous stirring. The polymer was separated by decantation and then dissolved in diethyl ether, filtered

on a glass filter with Celite®, concentrated under reduced pressure to ~3 mL and precipitated again in methanol. After removal of methanol by decantation, the polymer was dissolved in DCM. The final product was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording viscous liquid. Yield: 74 %, $M_n$ = 11570 g/mol, Đ = 1.52. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 145H -$CH_2$*CH*=), 3.51 – 3.61 (m, 4H, -*$CH_2$*OH), 2.60 – 2.83 (m, 3H, -NH*$CH_2$*$CH_2$OH and -C*H*($CH_3$)NH-), 2.00 – 2.12 (m (broad), 582H, -*$CH_2$*CH= and -*$CH_2$*C($CH_3$)=), 1.68 (s (broad), 458H -(*$CH_3$*)C=CH-), 1.06 (d, 3H, -CH(*$CH_3$*)NH-).

**Synthesis of heterotelechelic N-ethyl(2-hydroxyethyl)amine/hydroxy PI (MI5).** 1.0 g of 2-hydroxyethylamine/hydroxy PI (0.1 mmol) were dissolved in 3.6 mL of dry THF and 55 µL (10 eq, 1 mmol) of acetaldehyde were introduced to the medium and the reaction mixture was stirred at 40°C. After 2 h 0.43 g (20 eq, 2 mmol) of $NaBH(OAc)_3$ and 16 µL (2.6 eq, 0.26 mmol) of acetic acid were added to the reaction (the temperature was reduced to 0 °C during addition to avoid evaporation of acetaldehyde). The heterogeneous medium was stirred at 40 °C overnight. Then the mixture was directly precipitated into large excess of methanol under vigorous stirring. The polymer was separated by decantation and then dissolved in diethyl ether, filtered on a glass filter with Celite®, concentrated under reduced pressure to ~3 mL and precipitated again in methanol. After removal of methanol by decantation, the polymer was dissolved in DCM. The final **MI5** was obtained by evaporation of DCM under reduced pressure and drying overnight at 40°C in vacuum affording viscous liquid. Yield: 62 %, $M_n$ = 11660 g/mol, Đ = 1.50. $^1$H NMR ($CD_2Cl_2$) δ (ppm): 5.14 (t (broad), 145H -$CH_2$*CH*=), 3.58 (t, 2H, -C*$H_2$*OH), 3.36 – 3.51 (m, 2H, -N($C_2H_5$)$CH_2$*$CH_2$*OH), 2.73 (m, 1H, -*CH*($CH_3$)-N<), 2.32 – 2.67 (m, 4H, -N(*$CH_2$*$CH_3$)*$CH_2$*$CH_2$OH), 2.00 – 2.12 (m (broad), 583H, -*$CH_2$*CH= and -*$CH_2$*C($CH_3$)=), 1.68 (s (broad), 427H -(*$CH_3$*)C=CH-), 1.02 (t, 3H, *$CH_3$*$CH_2$-), 0.95 (d, 3H, -CH(*$CH_3$*)N<).

**Polypeptide hybrids synthesis.**

**BLG-NCA ROP initiated by aminoalcohol terminated PI.** The polymerizations were typically carried out as follows (Run 1 in Table 1): 20 mg of TPT were dissolved in 3 mL of dry DCM and then 0.2 g (0.02 mmol) of **MI1** were added as a solution in 1 mL of DCM. The polymerization was started by addition of 1 mL of BLG-NCA solution in DCM, containing 0.13 g (0.48 mmol) of the monomer. The mixture was stirred at 25 °C and after 1.3 h the polymer was precipitated in 37 mL of methanol containing 2 mL of $H_2O$. The mixture was kept for 40 minutes at room temperature to hydrolyze NCA in case of not complete monomer conversion. Then methanol was removed by evaporation and the sample was dried in vacuum at room temperature overnight. Crude polymer was analyzed by NMR and SEC.

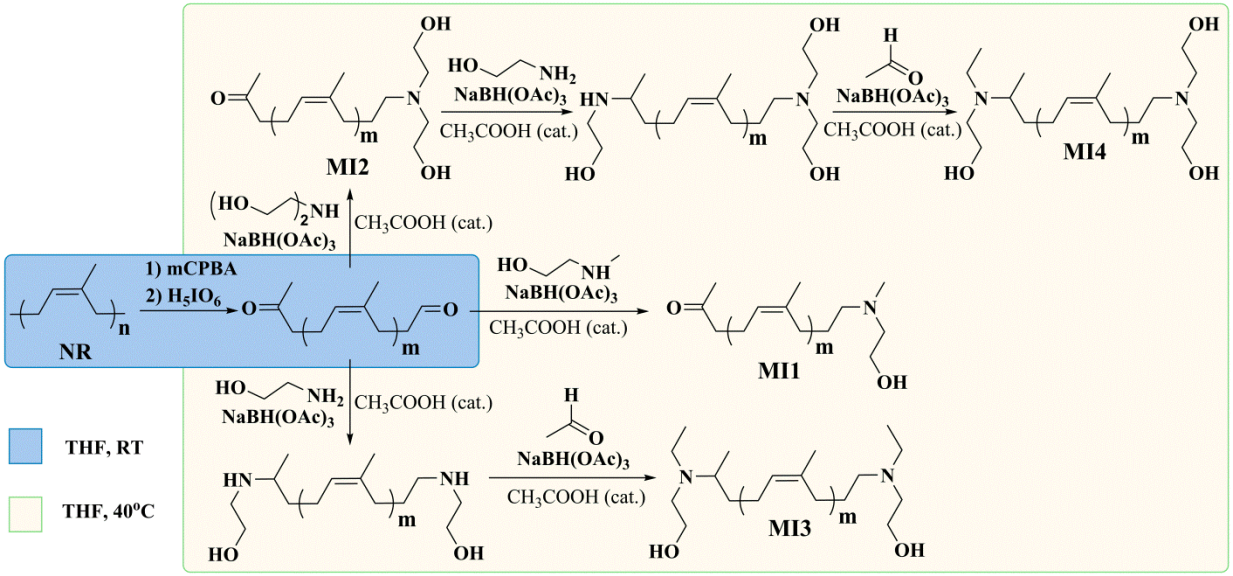
**Poly(BLL-b-isoprene-b-BLL) and poly(BLG-b-BLL-b-isoprene-b-BLL-b-BLG) synthesis.** The polymerization was started by the addition of solution containing 4.4 mL of DCM, 0.034 g ($6.8 \cdot 10^{-2}$ mmol) of TPT, and 0.16 g ($1.29 \cdot 10^{-2}$ mmol) of **MI3** into the tube with 0.10 g (0.38 mmol) of BLL-NCA. The mixture was stirred for 5.5 h at 25 °C and after that small sample (~0.4 mL) was taken and poured into 37 mL of methanol containing 2 mL of $H_2O$. Directly after that the solution of 0.11 g (0.39 mmol) of BLG in 0.8 mL of DCM was introduced to the remaining reaction mixture. After additional 2 h of polymerization the reaction was stopped by precipitation in methanol. The first sample and the final mixture were kept for at least 40 minutes at room temperature to hydrolyze NCA in case of not complete monomer conversion. Then methanol was removed by evaporation and the samples were dried in vacuum at room temperature overnight. The full monomer conversion was evidenced by absence of monomer (or products of its methanolysis/hydrolysis) signals in $^1$H NMR spectra

**Methods.**

$^1$H, HSQC and HMBC NMR (400 MHz) spectra were recorded in $CD_2Cl_2$ at 25°C on a Bruker AC-400 spectrometer calibrated relatively to the solvent peak in reference to tetramethylsilane. All DOSY (Diffusion Ordered Spectroscopy) measurements were performed at 298K on a Bruker Avance III 400 spectrometer operating at 400.33 MHz and equipped with a 5 mm Bruker multinuclear z-gradient direct cryoprobe-head capable of producing gradients in the z direction with strength 53.5 G $cm^{-1}$. Each sample was dissolved in 0.4 mL of $CD_2Cl_2$ for internal lock and spinning was used to minimize convection effects. The DOSY spectra were acquired with the *ledbpgp2s* pulse program from Bruker topspin software. The duration of the pulse gradients and the diffusion time were adjusted in order to obtain full attenuation of the signals at 95 % of maximum gradient strength. The values were 1.2 ms for the duration of the gradient pulses and 100 ms for the diffusion time. The gradients strength was linearly incremented in 16 steps from 5 % to 95 % of the maximum gradient strength. A delay of 8 s between echoes was used. The data were processed using 8192 points in the F2 dimension and 128 points in the F1 dimension with the Bruker topspin software. Field gradient calibration was accomplished at 25°C using the self-diffusion coefficient of $H_2O$+$D_2O$ at 19.0 x $10^{-10}$ $m^2$ $s^{-1}$. Polymer molar masses were determined by Size Exclusion Chromatography (SEC). If not otherwise mentioned, solution of bis(trifluoromethane)sulfonimide lithium salt (10 mmol/L) in THF was used as eluent. Measurements were performed on Ultimate 3000 system from Thermoscientific equipped with diode array detector. The system also included a multi-angle light scattering detector (MALS), a viscometer and differential refractive index detector (dRI) from Wyatt technology. Polymers were separated on a PSS SDV Linear S gel columns (300 x 8 mm) (exclusion limits from 1000 g/mol to 150 000 g/mol) at a flow rate of 1 mL/min. Columns temperature was held at 36 °C. Using the differential refractive index detector dn/dc values were determined for **MI1**, poly(BLG) and their block-copolymer (Run 2 in Table 1): 0.1147, 0.1204 and 0.1177 correspondingly. Taking into consideration proximity of these values, dn/dc = 0.1177 was used for all PI-poly(BLG) hybrids. While for poly(BLL-*b*-isoprene-*b*-BLL) and poly(BLG-*b*-BLL-*b*-isoprene-*b*-BLL-*b*-BLG) dn/dc values were quite different (0.0951 and 0.1036 respectively) and were used only for these hybrids. When mentioned, THF as eluent was used for SEC. Measurements in THF were performed on an Ultimate 3000 system from Thermoscientific equipped with diode array detector. The system also included a multi-angles light scattering detector and differential refractive index detector from Wyatt technology. Polymers were separated on three G2000, G3000 and G4000 TOSOH HXL gel columns (300 x 7.8 mm)

(exclusion limits from 1000 g/mol to 400000 g/mol) at a flow rate of 1 mL/min. Columns temperature was held at 40 °C. TA Instrument RSA3 was used to study dynamic mechanical properties of poly(BLG-b-isoprene-b-BLG). The sample was analyzed under air atmosphere from – 80 °C to 60 °C at heating rate of 4 °C/min. The measurements were performed in tensile mode at a frequency of 1 Hz, an initial static force of 0.1 N and strain sweep of 0.06 %. TEM analysis was performed using a HITACHI H600 apparatus operating at an accelerating voltage of 75 kV. A small drop of the sample (in suspension) was deposited on copper grids covered by a Formavar film and conductive carbon. Differential scanning calorimetry (DSC) measurements were performed using a DSC Q100 $LN_2$ apparatus from TA Instruments. The samples were cooled to -100 °C and heated back to 100 °C at the rate of 10 °C $min^{-1}$. Consecutive cooling and heating run were also performed at 10 °C $min^{-1}$. The analyses were carried out in a helium atmosphere with aluminum pans.




Scheme 1 Synthesis of amino-hydroxy macroinitiators

## Results and discussion

### Synthesis of macroinitiators.

The chemical pathway to mono-, di-, and tri-functional macroinitiators is presented on Scheme 1. NR was selected as the starting material because it is the only source of poly(1,4-*cis*-isoprene) with 100 % 1,4-*cis* configuration.[33] The first step was partial epoxidation of NR with m-CPBA followed by cleavage of the formed epoxide units by periodic acid ($H_5IO_6$) to obtain a polymer of desired molar mass with aldehydic and ketonic chain-ends. This method was reported to be able to produce polyisoprene with 2000 g/mol ≤$M_n$≤ 20 000 g/mol.[42] It was decided to focus our study on 10 000 g/mol as it permits to monitor chain-end modification by NMR spectrometry and at the same time the chains are long enough to influence the properties of the final material.

The synthesis of heterotelechelic keto-diol polyisoprene (bearing –$N(CH_2CH_2OH)_2$ end-group) from keto-aldehyde PI has already been described in the literature[43] and was recently slightly modified.[44] Using N-methylethanolamine and the same reductive amination procedure, mono functional macroinitiator (**MI1**) was obtained (Scheme 1). The full conversion of chain-end was confirmed by the disappearance of the –CH=O signal at 9.74 ppm (Figure 2) and by the appearance of new signals of the –$N(CH_3)CH_2CH_2OH$ group (signals 18 – 21 in Figure 2). The values of the integrals of these signals were in good agreement with molar mass determined by SEC also indicating the absence of side reaction on ketone chain-end.

Following the described procedure, reductive amination of aldehyde chain-end with diethanolamine was conducted to obtain di-functional macroinitiator (**MI2**) (Scheme 1). $^1H$ NMR analysis (Figure 2) revealed full conversion of aldehyde (signal 14 in Figure 2) to amino dialcohol (signals 15 – 17 in Figure 2), while the signal of $CH_2$ group linked to ketone (signal 6 in Figure 2) remained unchanged and the value of its integral corresponded to the molar mass of the polymer.

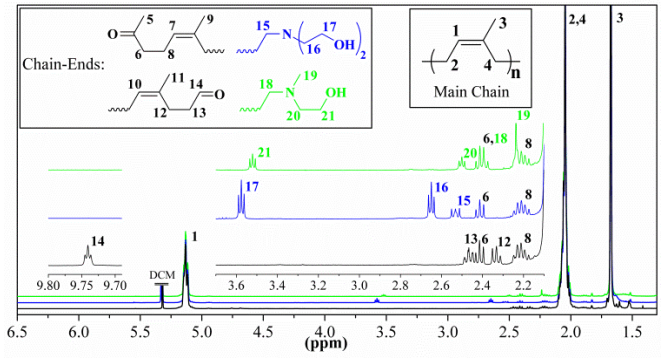



Figure 2 $^1H$ NMR spectra of keto-aldehyde PI (black line), **MI1** (green line) and **MI2** (blue line).

In contrast to the previously discussed secondary amines, primary amines react both with aldehydes and ketones.[43-45] This afforded the synthesis of symmetrical di-functional macroinitiator (**MI3**) and tri-

functional macroinitiator (**MI4**) (Scheme 1). Reductive amination of keto-aldehyde PI by ethanolamine led to 2-hydroxyethylamino telechelic PI, which cannot be used directly for the controlled ROP of NCA, secondary amine groups promoting side reactions.[31] Secondary amines were then converted into tertiary ones by achieving the reductive amination of acetaldehyde by the polymer chain-ends (in other words reductive alkylation of secondary amine by acetaldehyde[46]) was performed. Interestingly, as this last step required the same conditions as the previous one (THF, 40°C, $NaHB(OAc)_3$), the two steps were conducted as a one-pot synthesis: after completion of the first step, an excess of acetaldehyde and a new amount of $NaHB(OAc)_3$ were added to the reaction mixture to alkylate all amines present in the reaction medium (both chain-ends and excess of ethanolamine). $^1$H NMR spectrum of **MI3** is presented in Figure 3. Disappearance of the signal at 9.74 ppm indicated the full conversion of the aldehyde chain-end. New signals, corresponding to the amino-alcohol end-groups appeared at 2.38 – 2.78 and 3.47 ppm. A new signal corresponding to $CH_3$ group (signal 6 in Figure 3) appeared at 0.94 ppm, as well as a peak at 1.04 ppm (signals 4, 14, in Figure 3), the integration of these signals showed good agreement with molar mass of **MI3**, confirming the full conversion of the ketone chain-end into an amino-alcohol group.

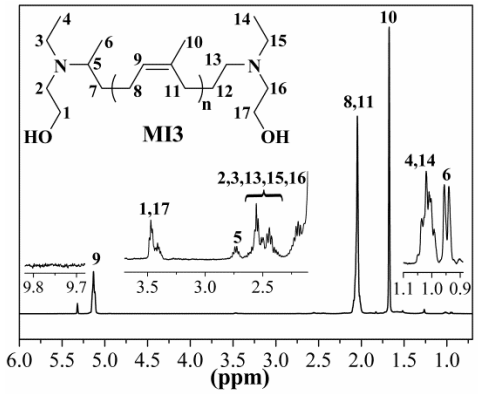



Figure 3 $^1$H NMR spectrum of **MI3**.

**MI4** was synthesized by reductive amination of aldehyde chain-end with diethanolamine followed by reductive amination of ketone chain-end by ethanol amine and subsequent alkylation by acetaldehyde (Scheme 1). All three steps can be done as a one-pot synthesis. $^1$H NMR spectrum of **MI4** is shown in Figure 4. Signals 1 – 6 have the same chemical shifts as the signals of the same group in **MI3** (1 – 6 in Figure 3), while signals 13 – 15 (Figure 4) are similar to the signals 15 – 17 (**MI2**) in Figure 2, confirming the synthesis of a tri-functional macroinitiator by successive modifications of both aldehyde and ketone chain-ends.

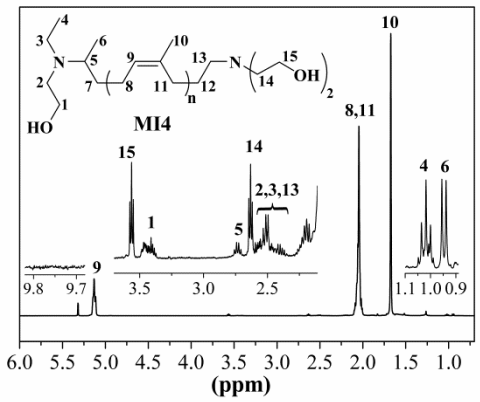



Figure 4 $^1$H NMR spectrum of **MI4**.

**Polypeptide hybrids synthesis.**

It was first attempted the synthesis of poly(1,4-*cis*-isoprene-*b*-BLG) using OH-terminated polyisoprene (**PI-OH**) as the macroinitiator in the presence of methanesulfonic acid similarly to the procedure reported recently by Pahovnik.[30] Analysis of the products revealed that this initiating system is not suitable for polyisoprene-polypeptide synthesis due to interaction of strong acid with PI double bonds (See Supporting Information for details). That is why the synthesis of amino-alcohol terminated macroinitiators (**MI1**, **MI2**, **MI3**, **MI4**) were done for the NCA ROP catalyzed by TPT as described by Hadjichristidis.[31] Table 1 summarizes the results of BLG-NCA polymerization in DCM at room temperature using amino-alcohol terminated macroinitiators. Polymerization reactions were monitored by NMR and stopped after complete consumption of the monomer (polymerization time indicated in the Table 1). First, **MI1** was used to obtain linear poly(isoprene-*b*-BLG) (Scheme 2) of two different molar masses (Runs 1 and 2 in Table 1) by varying the monomer to macroinitiator ratio. In both cases $M_n$ was close to the calculated values indicating that the polymerization is controlled. Moreover, the molar mass distribution of the hybrids decreased with the increase of the molar mass and was even narrower than that of the macroinitiator. Such behavior was already observed previously for polystyrene-polypeptide hybrids.[25] Interestingly, the SEC-trace of high molar mass copolymer (Figure 5, blue line) completely shifted to lower retention time without the presence of unreacted **MI1** indicating the formation of block-copolymer and high efficiency of **MI1**. Nevertheless, a small shoulder can be noticed at lower retention time corresponding to macromolecules with approximately twice the molar mass of the main peak (Figure 5). This can be explained by intermolecular reaction of terminal $NH_2$ group with pendant ester group of another molecule (Scheme S2). To check this hypothesis, a model reaction between *i*$BuNH_2$ and benzyl acetate was conducted. The formation of N-*i*-butylacetamide and benzyl alcohol was detected (Figure S4), indicating that coupling reactions between the polypeptide chains could occur. On the contrary, for hybrids with lower molar masses (Figure 5, red line), only one side of

the peak (lower molar mass) shifted to lower retention time. This might indicate that only short polyisoprene chains initiated the polymerization. To clarify this phenomenon, other analyses were performed. $^1$H NMR (Figure 6A) analysis resulted in broad signals of polypeptide protons due to hydrogen bonding. The use of $CDCl_3$/$CF_3COOD$ mixture (usually utilized for polypeptide NMR), was not possible due to reactivity of PI double bonds with strong acids, that made impossible precise quantification by $^1$H NMR. DOSY NMR spectrum showed that the signals corresponding to both blocks were aligned with a diffusion coefficient (D) $2.45 \cdot 10^{-11}$ m$^2$/s, while D(**MI1**) = $5.29 \cdot 10^{-11}$ m$^2$/s, indicating that polyisoprene and poly(BLG) are probably linked. Nevertheless, this analysis is not sufficient as the polymers can be linked physically due to possible aggregation. To confirm absence of unreacted macroinitiator, additional experiments were conducted on example of **MI2** (see below). Assuming the high efficiency of **MI1**, the shape of chromatogram (Figure 5 red line) could be explained by peculiar hydrodynamic behavior of the hybrid composed of a polyisoprene block with relatively broad dispersity (Đ = 1.49) and a poly(BLG) block with narrow dispersity (according to the literature[31] dispersity of the poly(BLG) obtained with this initiating system was ≤1.05) in the solvent used for SEC (10 mM solution of $LiN(SO_2CF_3)_2$ in THF). The poly(BLG) block might have a great influence on the hydrodynamic volume of the hybrids with shorter polyisoprene block, but the influence of relatively short poly(BLG) on larger hybrid molecule might be negligible. Nevertheless, the analysis indicates the controlled synthesis of poly(isoprene-*b*-BLG) block-copolymers.

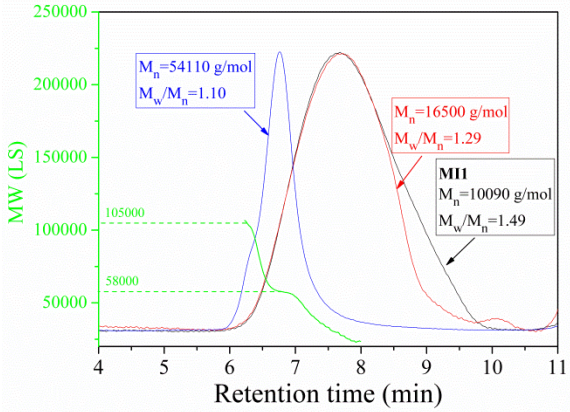
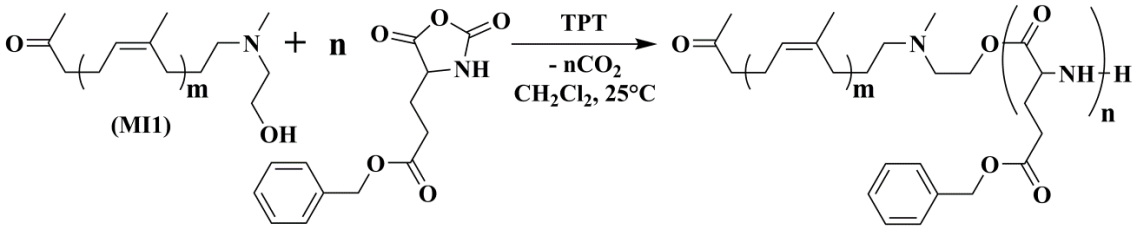



Figure 5 SEC traces of **MI1** (black line) and poly(isoprene-b-BLG)s (Runs 1 (red line) and 2 (blue line) in Table 1) and dependence of molar mass vs time (green line, left axis) for Run 2.

Table 1 Polymerization of BLG-NCA using **MI1**, **MI2**, **MI3**, and **MI4**[a]

| Run | MI | | | [BLG]/[TPT]/[MI] | Time (h)[b] | Hybrid | | |
|---|---|---|---|---|---|---|---|---|
| | compound | $M_n$ (g/mol) | Đ | | | $M_{n\ calc}$ (g/mol) | $M_{n\ SEC}$ (g/mol) | Đ |
| 1 | **MI1** | 10090 | 1.49 | 24/2/1 | 1.3 | 15350 | 16500 | 1.29 |
| 2 | | | | 203/2/1 | 4.0 | 54550 | 54110 | 1.10 |
| 3 | **MI2** | 9790 | 1.56 | 24/2/1 | 1.0 | 15050 | 16020 | 1.27 |
| 4 | | | | 47/2/1 | 3.0 | 20080 | 21190 | 1.10 |
| 5 | | | | 197/2/1 | 20.0[c] | 52930 | 48900 | 1.10 |
| 6 | **MI3** | 12190 | 1.52 | 29/4/1 | 2.0 | 18540 | 19020 | 1.28 |
| 7[d] | | | | 58/4/1 | 3.0 | 24890 | 27520 | 1.17 |
| 8 | | | | 116/4/1 | 3.0 | 37590 | 36550 | 1.13 |
| 9 | **MI4** | 9450 | 1.70 | 37/4/1 | 3.5 | 17550 | 20920 | 1.13 |

[a] Polymerization conditions: [BLG] = 0.1 mol/L, DCM, 25 °C. [b] Time for full monomer conversion evidenced by $^1$H NMR. [c] Polymerization left overnight, time was not optimized. [d] Polymerization was done in DCM without purification.




Scheme 2 Synthesis of linear poly(isoprene-b-BLG) using **MI1**

It is known that macromolecular architecture can have a large influence on polymer self-assembly and properties of the final material,[47] that is why **MI2** was then employed to synthesize 3-miktoarm hybrids (Scheme 3) of three different molar masses (Runs 3 – 5 in Table 1). In all cases, $M_n$s of the obtained polymers were close to the theoretical values and molar mass distributions were much lower than that of the macroinitiator. Like for linear block copolymers, the complete shift of the trace on the SEC chromatograms was observed for the highest molar mass hybrid indicating full conversion of macroinitiator (Figure 7). For the hybrids with $M_n$≤22000 g/mol, only shift of low molar mass side of the peaks was observed. To confirm that all polyisoprene is reacted the sample of the hybrid (Run 4 in Table 1) and a mixture of poly(BLG) with **MI2** were injected into SEC

using THF without any additive as eluent (in contrast to 10 mM solution of $LiN(SO_2CF_3)_2$ in THF usually used in this study) (Figure S5). Without added salt, poly(BLG) blocks strongly interact with the column resulting in very broad signal of low intensity mainly in low molecular region of the chromatogram. In contrast, addition of homo poly(BLG) to **MI2** does not change the shape of the peak and poly(BLG) does not come out of the column during the time of analysis. This confirms that even for hybrids with $M_n \leq 22000$ g/mol all **MI2** participates in block-copolymer formation. To the best of our knowledge, this is the first example of 3-miktoarm star polyisoprene-(poly(BLG))$_2$ synthesis, as well as only the second case of such architecture of polypeptide–(conventional polymer) hybrid.[48,49]

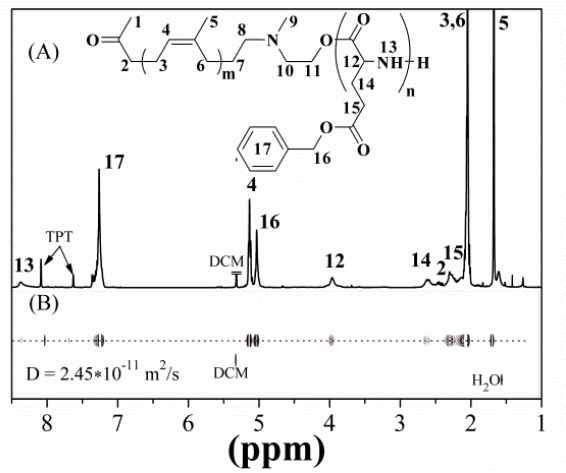
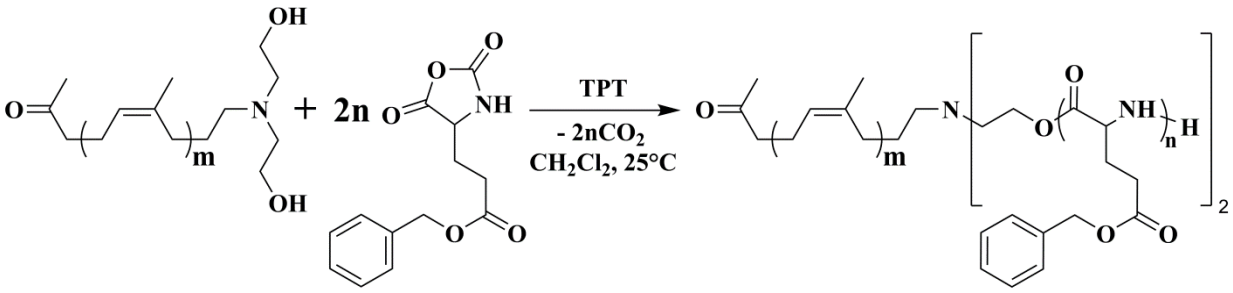
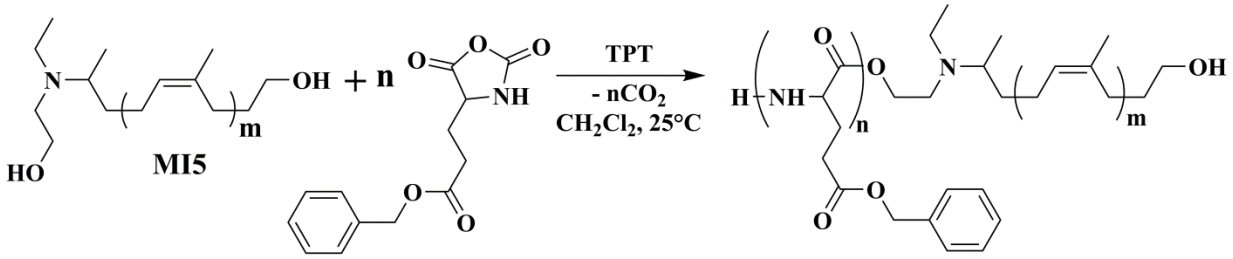



Figure 6 $^1$H (A) and DOSY (B) NMR spectra of poly(isoprene-b-BLG) (Run 1 in Table 1).




Scheme 3 Synthesis of 3-miktoarm star poly(isoprene-(b-BLG)$_2$) using **MI2**.




Scheme 4 Synthesis of linear poly(isoprene-b-BLG) using **MI5**.

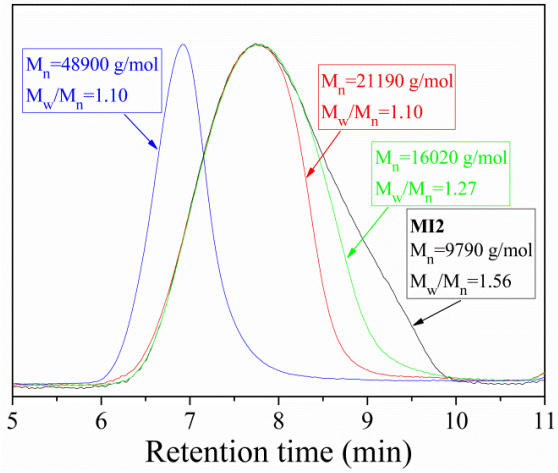



Figure 7 SEC traces of **MI2** (black line) and poly(isoprene-(b-BLG)$_2$)s (Runs 3 (green line), 4 (red line), 5 (blue line) in Table 1).

In a last step, initiators **MI3** and **MI4**, which contain aminoalcohol groups at both chain-ends (Scheme 1) were used as macroinitiators. The group obtained by reductive amination of the ketone chain-end contains secondary-alkyl-substituted amino group. To make sure that this group can initiate NCA polymerization, monofunctional initiator (**MI5**) containing (2-hydroxyethyl)ethyl amino group at one end and hydroxyl at the other end was synthesized by reductive amination of **PI-OH** ketone group. BLG-NCA ROP with **MI5** as initiator (Scheme 4) showed that the molar mass of the obtained block-copolymer increased in comparison to **MI5** and that the molar mass distribution narrowed (Figure 8) similarly to the hybrids described above. Moreover, NMR analysis showed that the –OH group of **MI5** remained intact after NCA ROP (Figure S6). It can then be concluded that amino alcohol group obtained from ketone chain-end is suitable for controlled NCA polymerization.

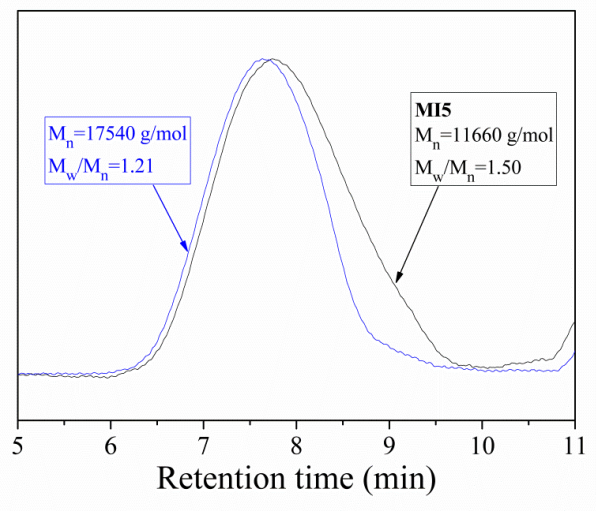



Figure 8 SEC traces of **MI5** (black line) and poly(isoprene-b-BLG) (blue line) obtained on **MI5**.

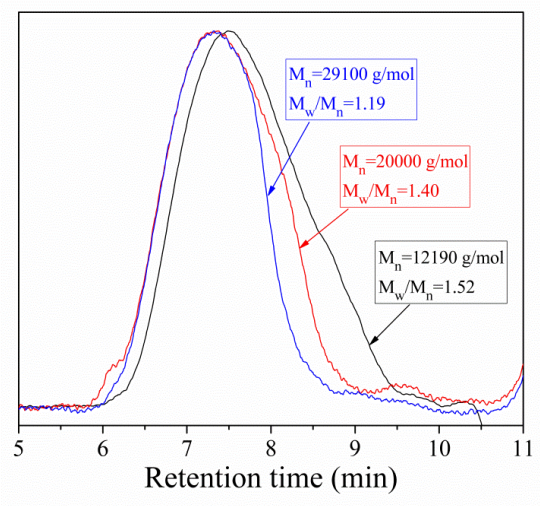
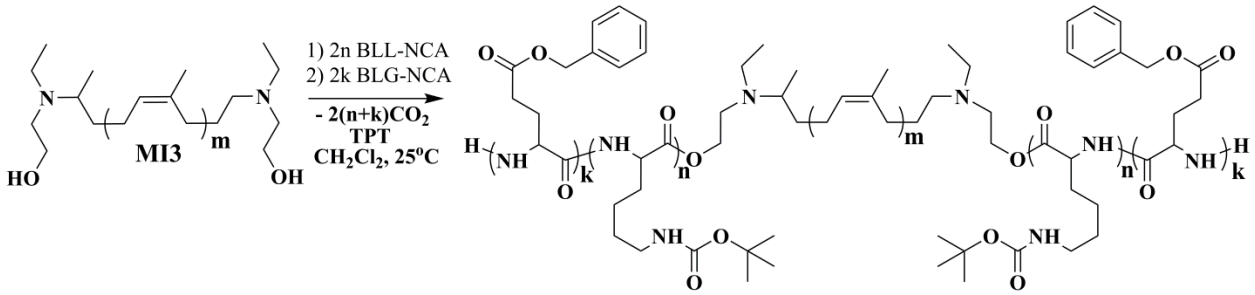



Figure 9 SEC traces of **MI3** (black line), poly(BLL-b-isoprene-b-BLL) (red line) and poly(BLG-b-BLL-b-isoprene-b-BLL-b-BLG) (blue line) obtained by sequential monomer addition (theoretical $M_n$s are 19290 and 28320 g/mol respectively).

**MI3** should lead to the synthesis of symmetric BAB type block-copolymers. Runs 6 – 8 in Table 1 demonstrate that the molar mass of these copolymers can be controlled by [BLG]/[**MI3**] and that the dispersity decreased with the increase of poly(BLG) block size. To test the sensitivity of the initiating system to impurities, one experiment was conducted in commercial DCM without any purification (Run 7 in Table 1). The molar mass of the copolymer was close to the calculated value and the dispersity was narrow (Figure S7), indicating that this system is not too sensitive to impurities (particularly water) that are present in the solvent. In contrast, for polymerization in DMF at room temperature, high purity conditions were required.[25] Polymerization in DMF needs also 2 to 4 days to reach high conversion, whereas for run 7 (Table 1) only 3 h were needed. Taking into consideration the fact that the reaction of NCA with water is not instantaneous,[50] it can be assumed that propagation is quick enough in the given conditions compared to NCA hydrolysis. **MI4** allowed the synthesis of asymmetrical miktoarm star $BAB_2$ type block-copolymers (Run 9 in Table 1), with again well-controlled molar mass and a low dispersity. To the best of our knowledge this is the first example of such architecture for polypeptide-polymer hybrid.

One of the most important advantages of living polymerization is the possibility to synthesize block-copolymers by sequential monomer addition.[51] That is why it was tried the polymerization of ε-tert-butyloxycarbonyl-L-lysine-NCA (BLL-NCA) initiated by **MI3** followed by BLG-NCA polymerization (Scheme 5). The SEC analysis of poly(BLL-*b*-isoprene-*b*-BLL) is shown on Figure 9. In contrast to PI-poly(BLG) hybrids, the chromatogram of poly(BLL-*b*-isoprene-*b*-BLL) was shifted towards smaller retention time at both sides of the peak. This could be explained by a different behavior in solution of the different polypeptide hybrids. Nevertheless, the molar mass of poly(BLL)-PI conjugate is close to the calculated value and the dispersity became narrower, indicating that the aminoalcohol macroinitiator can initiate not only BLG but also other NCA ROP. After addition of BLG-NCA, the chromatogram changed only at low molar mass region as for other BLG hybrids. $M_n$ of obtained pentablock terpolymer was close to the calculated value, which indicated the formation of the expected product. The synthesis of poly(BLG-*b*-BLL-*b*-isoprene-*b*-BLL-*b*-BLG) demonstrated the possibility of multi-block copolymer synthesis by sequential co-monomer addition.




Scheme 5 Synthesis of poly(BLG-b-BLL-b-isoprene-b-BLL-b-BLG) by sequential monomer addition with **MI3**.

Block copolymers behavior was further investigated. Figure 10 shows the dependence of intrinsic viscosity vs. molar mass for 3-miktoarm star polyisoprene-$(poly(BLG))_2$ and poly(BLG)-polyisoprene-poly(BLG) triblock-copolymer with different length of the poly(BLG) block, measured by the viscosimeter detector of the SEC. As expected, in all cases, hybrids are characterized by viscosity values in between the one of **MI1** and poly(BLG). Surprisingly, the comparison of Mark-Houwink-Sakurada plots of hybrids with similar $M_n$ of BLG-block and different architectures (Figure 11) did not reveal any difference in intrinsic viscosity, this can be explained by insufficient sensitivity of the SEC viscometer detector.

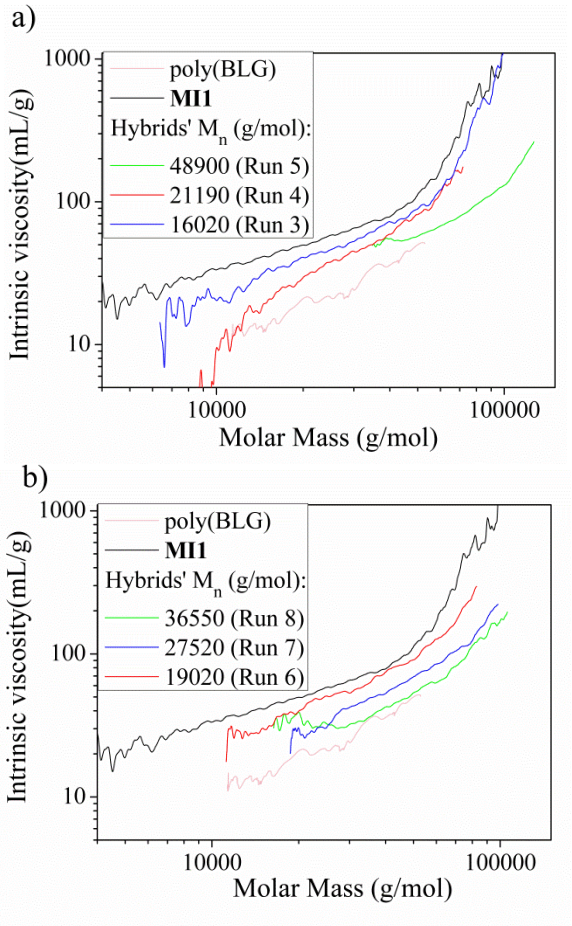



Figure 10 Mark-Houwink-Sakurada plots of **MI1**, poly(BLG), poly(isoprene-(b-BLG)$_2$) (Runs 3, 4, 5 in Table 1) (a) and poly(BLG-b-isoprene-b-BLG) (Runs 6, 7, 8 in Table 1) (b).

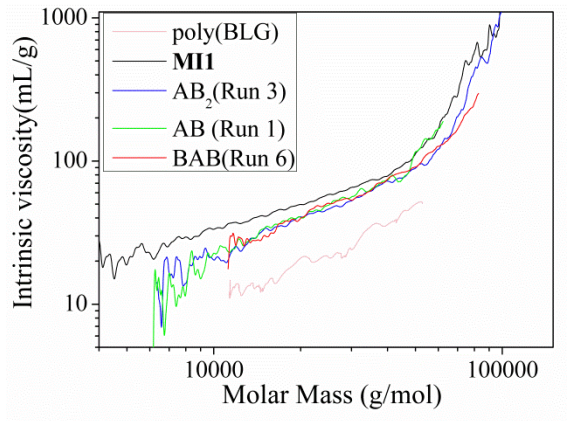



Figure 11 Mark-Houwink-Sakurada plots of **MI1**, poly(BLG), poly(isoprene-(b-BLG)$_2$) (Runs 3 in Table 1), poly(isoprene-b-BLG) (Runs 1 in Table 1) and poly(BLG-b-isoprene-b-BLG) (Runs 6 in Table 1).

It is known that poly(BLG)[52] and its block-copolymers with polydimethylsiloxane[54] can form physical organogels in toluene due to fibers formation by self-assembly. To find out whether the obtained polyisoprene-polypeptide hybrids can form such organogels, poly(isoprene-*b*-BLG) and poly(isoprene-(*b*-BLG)$_2$) were dissolved in toluene at 60°C for 5 minutes and then cooled down to room temperature. Both hybrids formed gels when hybrids with long poly(BLG) blocks were used (Figure 12 a and b), in contrast, hybrids with short polypeptide chains were soluble in toluene and did not form any gels (Figure 12 c and d).


Figure 12 Vial inversion tests of 20 g/L organogels of poly(isoprene-(b-BLG)$_2$) (Runs 5 and 3 in Table 1) (a and c respectively) and poly(isoprene-b-BLG) (Runs 1 and 2 in Table 1) (b and d respectively) in toluene.

The gel of linear hybrid (Runs 2 in Table 1) was analyzed by TEM (Figure S8). Similarly to previously reported BLG organogels,[52,53] fibers can be seen on the image, indicating that the presence of PI block does not prevent poly(BLG) self-assembly.

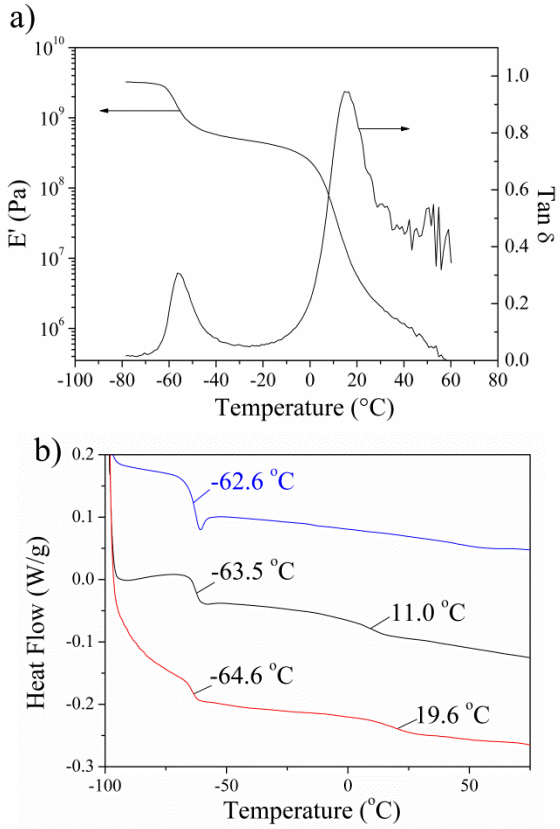



Figure 13 DMA traces of poly(BLG-b-isoprene-b-BLG) (Run 6 in Table 1) (a) and DSC thermogram of **MI2** (blue line) and poly(BLG-b-isoprene-b-BLG) (Run 6 (black line) and 8 (red line) in Table 1) (b).

AB and AB$_2$ hybrids are solid or soft sticky substance depending on PI - polypeptide ratio, whereas films can be prepared with BAB and BAB$_2$ hybrids. They were analyzed by DMA (Figure 13a for poly(BLG-*b*-isoprene-*b*-BLG)). A clear glass transition can be noticed at -56 °C for the polyisoprene block and a β relaxation process of poly(BLG) block[36,54] at 15 °C. After 50 °C the film became too soft and the analysis had to be stopped. DSC analysis showed similar transitions (Figure 13b) at slightly lower temperatures. Moreover, $T_g$s of these hybrids were very close to the $T_g$ of **MI2** (Figure 13b) whatever the length of poly(BLG) block indicating that polyisoprene block was not affected by NCA ROP. Interestingly, the temperature of β relaxation process

increased with $M_n$ of poly(BLG) block (compare black and red lines in Figure 13b). It can be noticed the capacity to form films using a 10 000 g/mol PI which normally behaves as a viscous liquid.

## Conclusions

In this work, a new approach to synthesize polypeptide hybrids by controlled ROP of NCA using TPT/aminoalcohol initiating system was presented. Several poly(1,4-*cis*-isoprene) macroinitiators were synthesized by two-pot synthesis starting from NR. These initiators were employed to synthesize hybrids of poly(BLG) and poly(1,4-*cis*-isoprene) with different architectures: linear (AB, BAB) and 3-miktoarm star ($AB_2$, $BAB_2$) copolymers. This route allowed to circumvent the sometimes complicated synthesis of primary-amino terminated macroinitiators. Moreover, ROP of NCA required only few hours in contrast to most of the previously described systems taking several days for full monomer conversion. Due to this high propagation rate, it was shown that the system is not extremely sensitive to impurities and can be conducted even in commercial DCM without purification. These macroinitiators were also suitable for BLL-NCA polymerization, as well as for the synthesis of the pentablock poly(BLG-*b*-BLL-*b*-isoprene-*b*-BLL-*b*-BLG). The properties of such hybrids are still under investigation and could help to understand the enhanced properties of NR. Moreover, this route should open the possibility to synthesize new polypeptide hybrids, as polybutadiene[44,55] and polystyrene[48] with amino-alcohol chain-ends were already described.

## Conflicts of interest

There are no conflicts to declare.

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