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Copolymer Synthesis

Synthesis and Self-Assembly of Rod–Rod Hybrid Poly(γ-benzyl L-glutamate)-*block*-Polyisocyanide Copolymers[**]

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Dedicated to Professor Roeland J. M. Nolte
on the occasion of his 60th birthday

The synthesis and self-assembly of rod–rod block copolymers is still a pristine area in the field of polymer chemistry.[1,2] Nevertheless, it promises applications, for example, as biosensors, scaffolds for tissue engineering, and materials with new properties.[3] To our knowledge only two publications on rod–rod block copolymers have been published till now. Deming and co-workers synthesized polyleucine–poly(hydroxylated glutamate), which formed large vesicular systems in water.[4] This block copolymer was prepared by an elegant method developed by this group for the living polymerization of *N*-carboxy anhydrides of amino acids (NCAs) using a 2,2′-bipyridylnickel(0)(1,5-cyclooctadiene) [Ni(bpy)(cod)] complex as the catalyst.[5] This synthetic method enabled not only the preparation of the above system but also of a host of block polypeptides.[6] The synthesis and self-assembly of hybrid block copolymers containing a π-conjugated polymer and peptide segment was described by Jenekhe and Kong.[7] Upon changing the secondary conformation (that is, helix versus coil) of the peptide segment in a polyfluorene–poly(γ-benzyl glutamate), different types of nanosized assemblies were observed.

Cornelissen and co-workers studied the polymerization and self-assembly of isocyanopeptide polymers[8] and rod–coil block copolymers containing this peptidomimetic segment.[9] These peptide-derived polyisocyanides have unique properties that result from the intramolecular β-sheetlike hydrogen-

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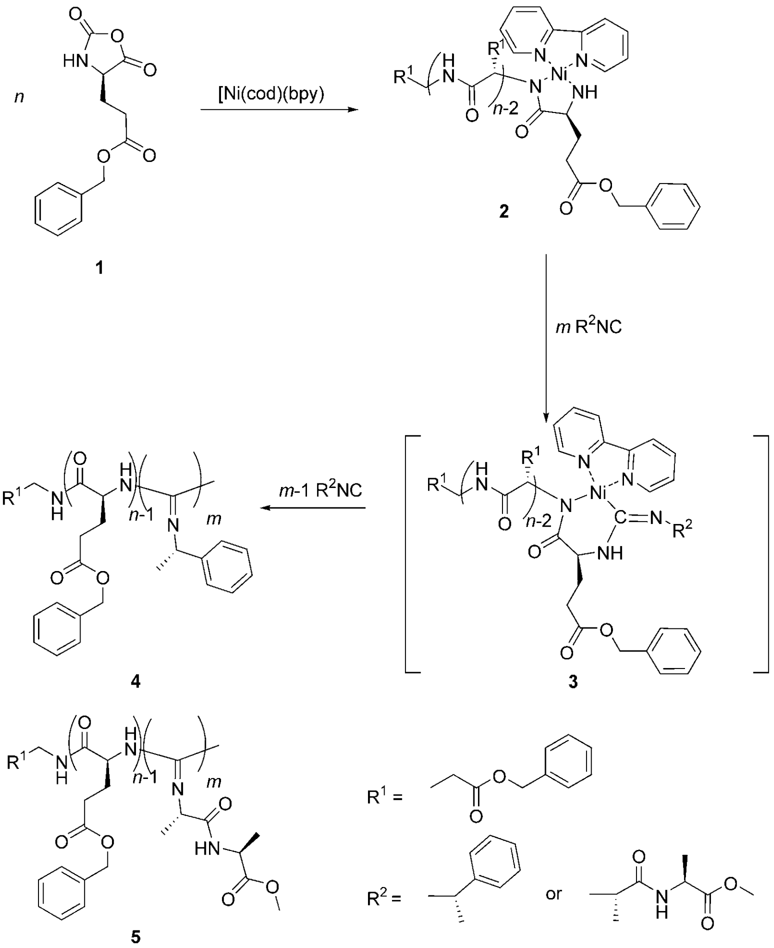
bonding arrays formed between the polymer side arms. The resultant macromolecules are stiffer than DNA, as determined by atomic force microscopy (AFM) studies.[10] The polymerization of isocyanides using nickel salts as a catalyst has been studied in Nolte's research group for several years.[11] From these investigations it was postulated that the polymerization of the isocyanide monomers occurs via a so-called "merry-go-round" mechanism around the central nickel atom resulting in a 4_1 (four repeat units per turn) helix, which is formed as the kinetic product. We have investigated the possibility of using the living polymerization catalyst developed by Deming,[5] which is formed by the action of NCAs on [Ni(bpy)(cod)],[5] as a catalyst for the polymerization of isocyanides. A catalyst that can be applied to two different monomers has great potential, as it allows the synthesis of a whole new class of rod–rod block copolymers with multiple structural motifs (for example, an α-helical peptide segment combined with a β-helical polyisocyanopeptide segment) and consequently unique properties. Herein, we describe the synthesis and self-assembly of hybrid block copolymers composed of a poly(γ-benzyl L-glutamate) block (PBLG) and two different polyisocyanide blocks, namely, poly((*S*)-(−)-α-methylbenzyl isocyanide) (PMBI) and poly(L-isocyanoalanyl-L-alanine methyl ester) (L,L-PIAA).



Scheme 1. Synthesis of PBLG-*block*-PMBI (**4**) and PBLG-*block*-L,L-PIAA (**5**) by a combination of *N*-carboxyanhydride ring-opening and isocyanide polymerization.

The diblock copolymers were synthesized by the nickel-catalyzed living polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride (Bn-Glu NCA, **1**) according to the procedure of Deming and co-workers (Scheme 1),[5,6] followed by the addition of (*S*)-(−)-α-methylbenzyl isocyanide (MBI) or L-isocyanoalanyl-L-alanine methyl ester (L,L-IAA) to the reaction mixture. Upon addition of the isocyanide to the PBLG homopolymer with the active nickel complex still attached (intermediate **2**, Scheme 1),[5] a color change from red to yellow was observed, which indicated an alteration in the coordination sphere of the metal. From previous studies by Deming and co-workers[12] it is known that the N-terminus of the growing polypeptide chain can be terminated when **2** is treated with an electrophile. An isocyanide coordinated to a nickel(II) center is an electrophilic species. We therefore expect that the coordinated primary amine is able to react with an incoming isocyanide, likely through the temporary formation of a five-coordinate complex,[13] to yield a carbene-like initiator complex for the isocyanide polymerization.[9,11] This polymerization subsequently commences through the coordination of free monomers to the formally nickel(II) center (possibly helped by the partial dissociation of the bpy ligand) and their insertion in the growing polyisocyanide chain. More detailed experiments to elucidate the precise polymerization mechanism are currently in progress.

During polymerization of the L,L-IAA monomer, gelation of the reaction mixture was observed within minutes after addition of the isocyanide, which indicates its rapid polymerization and the formation of high-molecular-weight material. After workup, block copolymers free from residual homopolymers were isolated by selective solvent extractions. The block copolymers were characterized by ^{1}H NMR, FTIR, and CD spectroscopy and gel permeation chromatography (GPC; see the Supporting Information for details).

PBLG and the copolymers were first studied by GPC in THF, which provided direct evidence of the formation of the block copolymers. PBLG aggregates in this solvent, and thus gives rise to a broad peak.[14] The resultant block copolymer had a smaller elution volume, which indicates a higher molecular weight than the PBLG homopolymer. However, the rigid nature of the copolymers prevented calibration with standards such as polystyrene. The polymers were subsequently studied in 0.1 M LiBr in DMF at 65 °C. While PBLG is completely soluble without any aggregation in this solvent, the block copolymers did not dissolve, thus indicating the absence of any residual homopolymer. GPC analysis at 65 °C in this solvent provided the molecular weight parameters of the PBLG block isolated from a sample taken before addition of the isocyanide monomers, which allows the determination of the degree of polymerization of the block copolymers by ^{1}H NMR spectroscopy (Table 1). The degree of polymeri-

Table 1: Molecular weight data for PBLG-*block*-polyisocyanide polymers.

Entry	Polymer	PBLG segment[a] [kg mol^{-1}]	DP[b]	PBLG[a] M_w/M_n	Block ratio[c] (DP[b])	Polyisocyanide[d] [kg mol^{-1}]	Block copolymer[d] [kg mol^{-1}]
1	PBLG-*block*-PMBI	30	146	1.30	0.89 (130)	17	47
2	PBLG-*block*-PMBI	25	122	1.20	1.27 (155)	26	52
3	PBLG-*block*-L,L-PIAA	35	170	1.43	7.3 (1244)	229	264
4	PBLG-*block*-L,L-PIAA	46	225	1.47	5.2 (1163)	214	260

[a] Molecular weight data were obtained by GPC at 65 °C by using 0.1 M LiBr in DMF as the solvent and polystyrene standards. [b] Degree of polymerization. [c] Determined by integration of the ^{1}H NMR spectrum measured in $CDCl_3$ or CD_2Cl_2. [d] Determined by using GPC data of the PBLG block and ^{1}H NMR data of the block copolymer.

zation (DP) of the polyisocyanide block was determined by analysis of the integrals of the protons of the methyl groups in the case of L,L-PIAA, or the phenyl group in the case of PMBI, relative to the benzylic protons of the PBLG segment. Further evidence for the formation of block copolymers came from AFM studies. A relatively short contour length was observed for the polypeptide homopolymer upon deposition of the sample on mica (not shown), while significantly longer individual polymer chains could be visualized for the block copolymers (Figure 1).

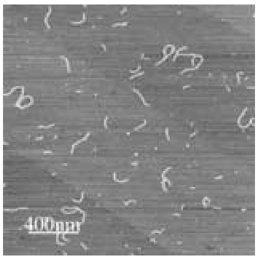


Figure 1. AFM image of isolated PBLG-*block*-L,L-PIAA strands. The sample was prepared from a 0.01 g L^{-1} polymer solution in $CHCl_3$.

FTIR spectroscopic studies were carried out on films cast from a suitable solvent. A selection of the IR vibrations is given in Table 2. The PBLG homopolymer adopts a typical α-helical structure as is evident from the amide I and amide II positions. No indications were found for β-sheet secondary structures. The block copolymers showed a shoulder ($\tilde{\nu} = 1614\ \text{cm}^{-1}$) on the amide I vibration, indicative of an imine IR vibration. In the case of PBLG-*block*-PMBI, an amide I vibration was observed at 1650 cm^{-1}, showing the stability of the α-helical PBLG. A shift was observed in PBLG-*block*-L,L-PIAA, which indicates that a mixture of the PBLG α-helix and the β-sheet of L,L-PIAA was present.

Table 2: Selected IR spectroscopic data [cm^{-1}].

Polymer	$\tilde{\nu}_{\text{amide I}}$	$\tilde{\nu}_{\text{amide II}}$	$\tilde{\nu}_{\text{amide A}}$
PBLG	1650.0	1548.0	3292.0
L,L-PIAA	1657.0	1532.0	3276.0
PBLG-*block*-L,L-PIAA	1654.0	1530.3	3270.5
PBLG-*block*-PMBI	1650.0	1548.0	3291.0

CD analysis confirmed the existence of conformationally ordered block copolymers, in agreement with the FTIR studies. The helical structure of the PBLG segment gave a double minimum at 222 and 210 nm. Furthermore, the chiral polyisocyanopeptide displayed a single Cotton effect centered around ≈315 nm, which is typical for the n–π* transition of the C=N chromophores in these materials.[8] Together the FTIR and CD spectra revealed that the PBLG-*block*-L,L-PIAA has three secondary structural motifs within one macromolecule, that is, an α-helical polypeptide segment and a polyisocyanide helix with side arms organized in a parallel β-sheet, which to our knowledge is unprecedented.

Preliminary self-assembly studies were performed in organic solutions. The formation of spherical objects was observed upon fast drying of a solution of PBLG-*block*-L,L-PIAA (Figure 2). Confocal laser scanning microscopy revealed that these objects were actually polymersomes with a uniform diameter of 7.5 μm (standard deviation of 0.9 μm).[15] Slow evaporation resulted in the formation of closed films, which suggests that the polymersomes are a kinetically trapped architecture. Polarized optical microscopy studies showed that the polymers were aligned and confirmed that the polymersomes were hollow (Figure 2). However, PBLG-*block*-PMBI was unable to assemble into spheres and only formed a continuous film. The absence of the stabilizing hydrogen-bonding array along the polymeric backbone of the polyisocyanide segment in this block copolymer will likely result in a less well-defined helix, which in return will affect the self-organizing properties of the macromolecule.[3] This finding may explain why no defined aggregates are formed from PBLG-*block*-PMBI under the applied conditions.

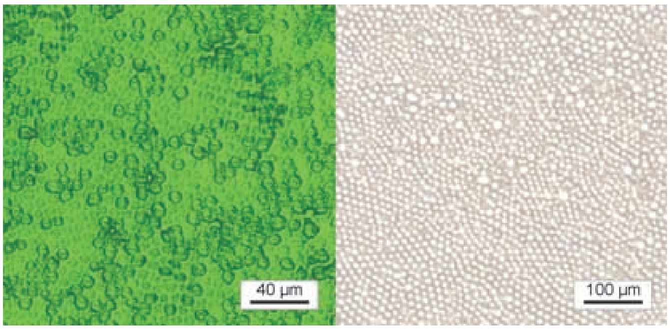


Figure 2. Confocal laser scanning (left) and optical microscope (right) images showing the typical morphologies of PBLG-*block*-L,L-PIAA. The sample was prepared by spreading drops of dilute chloroform solutions (1 mg mL^{-1}) on glass slides.

In summary, we have found a route to synthesize rigid rod–rod block copolymers comprising a peptide block and a polyisocyanide block. These compounds assemble upon drying into ordered layers of hollow capsules with potential applications in the fields of therapeutics or biomaterials. All protected amino acids and isocyanides are in principle compatible with the block copolymer synthesis, which allows a wealth of hybrid materials to be synthesized. As the secondary structure of the polymer segments can be controlled, for example, during the synthesis or by external stimuli, it is expected that aggregate formation can also be controlled.[4,9] In-depth investigations of the self-assembly of the block copolymers and the polymerization mechanism are in progress.

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[1] For examples of self-assembling coil–coil diblock copolymers, see: a) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967 – 973, and references therein; b) N. S. Cameron, M. K. Corbierre, A. Eisenberg, *Can. J. Chem.* **1999**, *77*, 1311 – 1326.

[2] For examples of self-assembling rod–coil diblock copolymers, see: a) S. A. Jenekhe, X. L. Chen, *Science* **1998**, *279*, 1903 – 1907; b) S. A. Jenekhe, X. L. Chen, *Science* **1999**, *283*, 372 – 375; c) M. Muthukumar, C. K. Ober, E. L. Thomas, *Science* **1997**, *277*, 1225 – 1231; d) F. Chécot, S. Lecommandoux, Y. Gnanou, H.-A. Klok, *Angew. Chem.* **2002**, *114*, 1395 – 1399; *Angew. Chem. Int. Ed.* **2002**, *41*, 1339 – 1343.

[3] R. A. L. Jones, *Nat. Mater.* **2004**, *3*, 209 – 210.

[4] E. G. Bellomo, M. D. Wyrsta, L. Pakstis, D. J. Pochan, T. J. Deming, *Nat. Mater.* **2004**, *3*, 244 – 248.

[5] a) T. J. Deming, *Nature* **1997**, *390*, 386 – 389; b) T. J. Deming, S. A. Curtin, *J. Am. Chem. Soc.* **2000**, *122*, 5710 – 5717.

[6] a) J. N. Cha, G. D. Stucky, D. E. Morse, T. J. Deming, *Nature* **2000**, *403*, 289 – 292; b) A. P. Nowak, V. Breedveld, L. Pakstis, B. Ozbas, D. J. Pine, D. Pochan, T. J. Deming, *Nature* **2002**, *417*, 424 – 428.

[7] X. Kong, S. A. Jenekhe, *Macromolecules* **2004**, *37*, 8180 – 8183.

[8] J. J. L. M. Cornelissen, J. J. J. M. Donners, R. de Gelder, W. S. Graswinckel, G. A. Metselaar, A. E. Rowan, N. A. J. M. Sommerdijk, R. J. M. Nolte, *Science* **2001**, *293*, 676 – 680.

[9] a) J. J. L. M. Cornelissen, M. Fischer, N. A. J. M. Sommerdijk, R. J. M. Nolte, *Science* **1998**, *280*, 1427 – 1430; b) D. M. Vriezema, A. Kros, R. de Gelder, J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, *Macromolecules* **2004**, *37*, 4736 – 4739.

[10] P. Samorì, C. Ecker, I. Gossl, P. A. J. de Witte, J. J. L. M. Cornelissen, G. A. Metselaar, M. B. J. Otten, A. E. Rowan, R. J. M. Nolte, J. P. Rabe, *Macromolecules* **2002**, *35*, 5290 – 5294.

[11] J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, N. A. J. M. Sommerdijk, *Chem. Rev.* **2001**, *101*, 4039 – 4070.

[12] K. R. Brzezinska, S. A. Curtin, T. J. Deming, *Macromolecules* **2002**, *35*, 2970 – 2976.

[13] A comparable insertion mechanism for isoelectronic CO has been postulated based both on experimental and theoretical studies. See: a) C. S. Shultz, J. M. DeSimone, M. Brookhart, *J. Am. Chem. Soc.* **2001**, *123*, 9172 – 9173; b) F. De Angelis, A. Sgamellotti, N. Re, *Organometallics* **2002**, *21*, 2036 – 2040.

[14] H. Block, *Poly(γ-benzyl L-glutamate) and Other Glutamic Acid Containing Polymers*, Gordon & Breach, New York, **1983**, p. 59.

[15] Large polymersomes were also observed upon assembly of rod–coil diblock copolymers; see ref. [2a].