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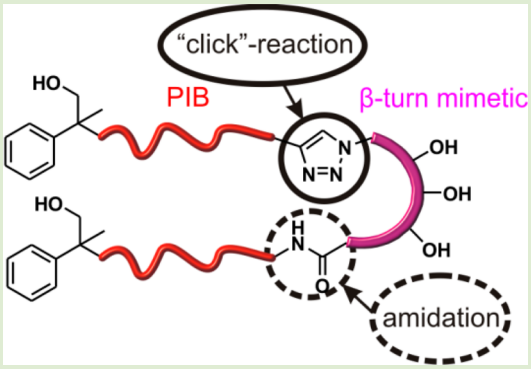
Synthesis of an Amphiphilic β-Turn Mimetic Polymer Conjugate

Marlen Malke, Haitham Barqawi, and Wolfgang H. Binder*

Chair of Macromolecular Chemistry, Institute of Chemistry, Division of Technical and Macromolecular Chemistry, Faculty of Natural Sciences II (Chemistry, Physics and Mathematics), Martin-Luther-University Halle-Wittenberg, von-Danckelmann-Platz 4, Halle D-06120, Germany

Supporting Information

ABSTRACT: A new biomimetic polymer containing a beta-turn mimetic element (**1**) was synthesized, using a combination of living carbocationic polymerization (LCCP), amidation, and "click" chemistry. Two different α-ω-functionalized polyisobutylenes (PIBs **3** and **5**) bearing either an alkyne group (PIB **3**) or a primary amine group (PIB **5**) were directly synthesized via LCCP. The linking of the two PIB strands with the closely positioned carboxyl/azido moieties of a β-turn dipeptide (BTD) **2** was achieved via a sequence of amidation reaction and the Cu^{I}-mediated azide/alkyne "click" reaction. By means of size exclusion chromatography (SEC), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), NMR spectroscopy, and LC/MALDI-TOF MS, a detailed structural proof of the β-turn mimetic PIB conjugate (**1**) was possible.



Design of the three-dimensional structure of (bio-)polymers has largely been accomplished by peptidic and proteinic elements, rationally shaped in their sequence by synthetic methods. Thus, "peptidomimetic" chemistry has gained ground, enabling us to mimic proteins or even complex structuring elements via artificial (shape-constrained) polymers[1] such as peptoids,[2,3] foldamers,[2,3] or polymeric nucleic acids (PNAs).[4] As peptides are known to constitute useful drugs but often are poorly bioavailable or limited in their stability in vivo,[5] modulation of just the necessary structural elements of a protein (such as an α-helix, β-turn, or a β-strand) has become important, with β-turn mimetics being one of the intensely researched areas emerging due to their highly functional and pharmaceutical role.[6] Numerous studies have demonstrated that rigid, nonpeptidic, conformationally constrained compounds such as carbohydrates,[7,8] steroids,[9] or aromatic compounds (bi- and terphenyls)[10] can most efficiently copy a β-turn characterized by specific torsion angles.[11]

We here report on the synthesis of a biocompatible amphiphilic hybrid molecule (**1**), consisting of the artificial β-turn structure (**2**) and two linked polymer chains (**3**, **5**) (see Scheme 1). The concept follows a hydrophilic β-turn mimetic based on a bicyclic carbohydrate,[12] which can act as a folding element within, e.g., a lipid bilayer membrane, forcing two hydrophobic polymers into a restricted space. In the past there have been numerous publications dealing with β-turn structures purely geometrically constrained or stabilized via supramolecular interactions.[11,13−18] Out of this big repertoire we have chosen a carbohydrate-based, hydrophilic 7,5-bicyclic thiazolidine lactam (β-turn dipeptide (BTD)) first synthesized by Geyer et al.,[12] whose conformation is close to the natural β-turn structural fold: both X-ray analysis and NMR spectroscopy have proven the rigidity and fixed stereochemistry of this β-turn mimetic even in aqueous environment.[12,19] Furthermore, this BTD could be easily synthesized and modified to achieve **2**, making it suitable for linking with a biocompatible polymer such as poly(isobutylene) (PIB).

The retrosynthetic concept is shown in Scheme 1, based on an orthogonal sequence of "click"-based chemistry and amidation reactions, both enabling us to link the heterotelechelic PIB strands **3** and **5** sequentially to the BTD (**2**). A significant synthetic challenge was posed by the close proximity of the two reactive moieties forming the β-turn structure (the carboxylic acid moiety and the azide moiety), which according to the X-ray structure are only 7 Å apart.[20]

Starting from 7,5-bicyclic thiazolidine lactam methyl ester, which could be prepared according to the literature,[19,21] ester hydrolysis via LiOH furnished the BTD (**2**), bearing an azide functionality on one side and a carboxylic acid moiety on the other side for linkage of the two individual PIB polymer chains (for synthesis and characterization see Supporting Information). Previously prepared, heterotelechelic (α-ω-functionalized) PIBs bearing either hydroxymethyl/alkyne moieties on one side (compound **3**, M_n = 4700 g/mol; PDI = 1.39) or hydroxymethyl/amine moieties (compound **5**, M_n = 5000 g/mol, PDI = 1.30) were obtained via living carbocationic polymerization (LCCP) of isobutylene by combining the synthetic methods of Olubummo et al.[22] and Morgan et al.[23] (for syntheses and characterization see Supporting Information). Subsequently, two approaches[24] for the synthesis of the

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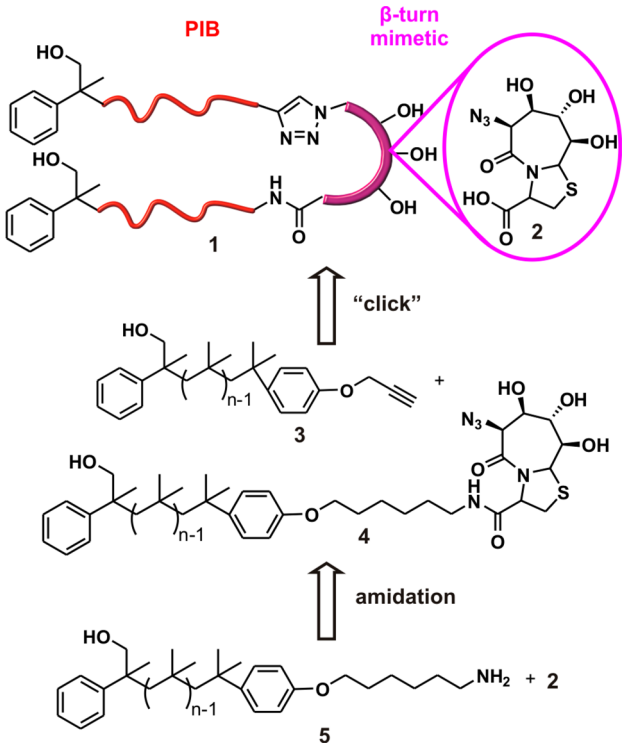
Scheme 1. Retrosynthetic Route Towards the Biomimetic Polymer Containing a *β*-Turn Mimetic Element (1) by Linkage of the Two PIB Strands 3 and 5 with the *β*-Turn Mimetic BTD 2



final *β*-turn mimetic PIB conjugate (**1**) were probed: in the successful first approach the fragments **2** and **5** were connected via an amidation reaction using (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and *N*-methlymorpholine (NMM) as a peptide-coupling system, proving the successful coupling and the purity of the resulting PIB strand **4** via NMR spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (for synthesis and characterization see Supporting Information). In a second step the two PIB strands **3** and **4** were linked via a Cu^{I}-mediated azide/alkyne "click" reaction[25] resulting in the final compound **1** (for synthesis see Supporting Information). Due to the constrained conformation of both the BTD (**2**) and **4**, conformational space at the linkage sites (carboxyl and azido moiety) is reduced resulting in a strong kinetic hindrance and thus an extremely low reactivity of both moieties. Therefore, *N*,*N*-diisopropylethylamine (DIPEA) as a base with reduced steric demand was used; when using *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) as the significantly bulkier base, no product was formed proving that indeed steric factors significantly influence the success of this reaction.

To prove the successful synthesis of the *β*-turn mimetic PIB conjugate (**1**) extensive spectroscopic, spectrometric, and chromatographic characterizations were conducted.

First the *β*-turn mimetic PIB conjugate **1** was analyzed by high-field NMR spectroscopy. Figure 1 shows the (600 MHz) ^{1}H NMR of **1**, enabling a full assignment of all peaks with the aid of 2D NMR spectroscopy (HH−COSY, HSQC) as well as by the use of the $^{1}H/^{13}C$ NMR spectra of the starting compounds **2**, **3**, **4**, and **5** (see Supporting Information) and an overlay of the ^{1}H NMR spectra of **3**, **4**, and **1** (see Supporting Information Figure S7). A clear proof of the polymer/BTD linkage is given by the appearance of the resonance of the newly formed triazole moiety at 8.16 ppm as well as the shift of the methine group in vicinity to the former azide to 6.20 ppm, confirming the successful linking of the two single PIB strands **3** and **4** via the azide/alkyne "click" reaction. Furthermore, the disappearance of the resonance of the former alkyne moiety at 2.50 ppm (CH_2-C-CH) together with the shift of the doublet of the methylene group in vicinity to the former alkyne to 5.16 ppm evidenced the formation of **1**. Since all signals can be assigned to the structure and the integrals of the PIB initiators (assignment b′ in Figure 1), the PIB quenchers (assignments f and u in Figure 1) and the *β*-turn resonances matched, and the chemical identity of **1** could be proven. The integration of the region from 2 to 0.5 ppm of the polymer backbone gave a calculated $M_{n(NMR)}$ of 10.400 g/mol, indicative of the linkage of both PIB chains (**3** and **4**) to the BTD (**2**).

Size exclusion chromatography (SEC) was done for the single strands **3** and **4** and of the resultant *β*-turn mimetic PIB

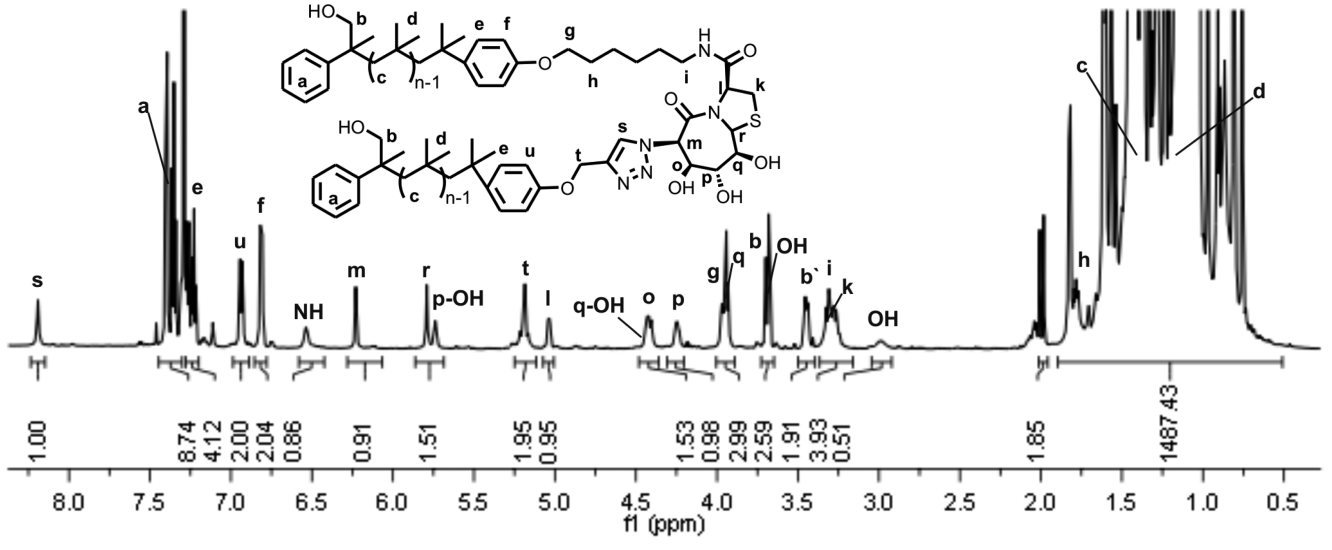


Figure 1. ^{1}H NMR spectrum of the *β*-turn mimetic polymer conjugate **1**.

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conjugate (**1**) in THF. Figure 2 shows the normalized refractive index (RI) response over the retention volume, determining

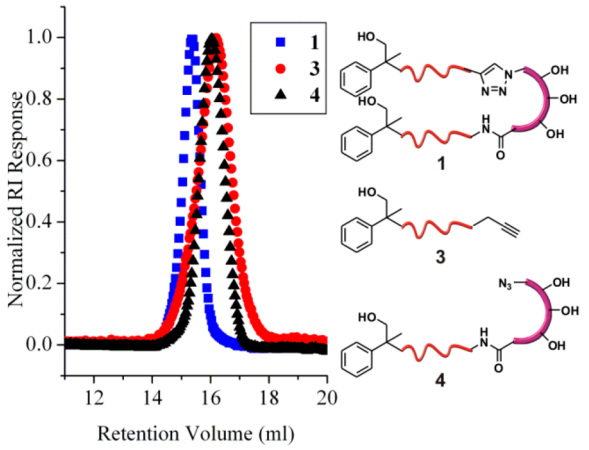

Figure 2. Normalized SEC RI traces (1 mL/min, THF) of the fully linked product **1** (M_n = 10 100 g/mol, PDI = 1.16) and of the PIB strands **3** (M_n = 4700 g/mol, PDI = 1.39) and **4** (M_n = 5300 g/mol, PDI = 1.20) on the basis of PIB standards for calibration.

molecular weights (M_n) and the molecular weight distributions (M_w/M_n) on the basis of narrow PIB standards for calibration. The analysis of the SEC trace for **3** shows a monomodal distribution with a M_n of 4700 g/mol and a molecular weight distribution (M_w/M_n) of 1.39. Similar results could be found for **4** with a monomodal distribution, M_n, of 5300 g/mol and a relatively narrow molecular weight distribution of 1.20. The SEC trace for the final β-turn mimetic PIB conjugate (**1**) showed a clear shift to shorter retention time and thus to higher molecular weights, together with a monomodal distribution (PDI = 1.16) and no shoulder formation. Moreover, the measured $M_{n(SEC)}$ of 10 100 g/mol corresponded to the sum of the single PIB strands **3** and **4**, which was in good agreement with the calculated M_n of 10 400 g/mol resulting from the ^{1}H NMR spectroscopy. This clearly showed the successful conversion to product **1** bearing two polymer chains connected to the β-turn mimetic and its purity.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was also carried out to finally prove the structure of the complete β-turn mimetic PIB conjugate (**1**), specifically addressing the presence of both polymer chains on the BTD (**2**). Figure 3a shows the MALDI-TOF MS of **1** with a mass distribution from 5500 to 9000 g/mol and a maximum at 7000 g/mol. The chemical identity of **1** could be proven by the distance between two peaks indicating the repetitive unit of 56.1 g/mol for PIB (Figure 3b) for all of the peaks of the three different series. Figure 3c shows the expanded spectrum of the β-turn mimetic PIB conjugate (**1**) with a listed view of the simulated peaks. All three peaks could be assigned to the desired product **1** with 110 units of isobutylene and different salts. The signal at 7057.79 g/mol agreed with the calculated value of 7058.30 g/mol as a Li adduct, and the signal at 7074.67 g/mol corresponded with the simulated value of 7074.28 g/mol for the Na adduct. The third signal at 7092.75 g/mol showed different ionization behavior, matching with the calculated value of 7092.30 g/mol by exchange of three protons with three Li ions and one Na ion. This phenomenon could be explained by the nature of the β-turn **2**, where the three protons of the three hydroxyl groups

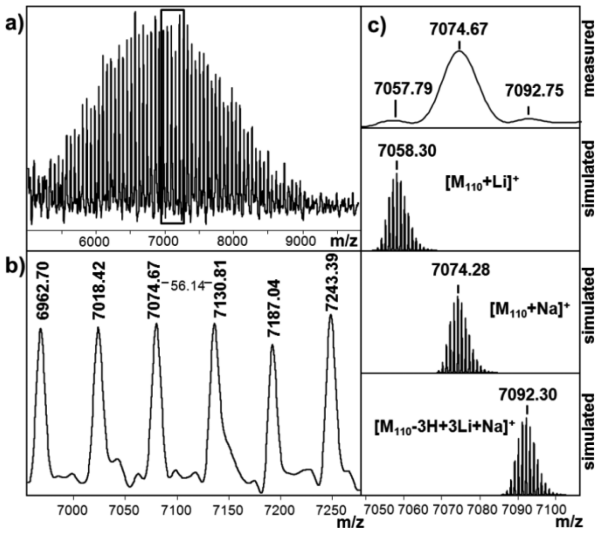

Figure 3. MALDI-TOF MS of **1** of (a) the region from 5000 to 10 000 Da, (b) expanded spectrum according to the highlighted region in (a), and (c) expanded spectrum with a listed view of the simulated peaks.

could be exchanged with alkali metal ions. A doubling of the molecular weight of the β-turn mimetic PIB conjugate (**1**) as the result of the connection of the two single PIB strands (**3** and **4**) with similar molecular weights could be observed, which agreed well with the SEC results. An overlay of **4** with **1** (see Supporting Information Figure S5) demonstrated the shift of the molecular weight and the disappearance of **4**.

To achieve a full picture of the linking process and the ultimate purity of the final product, we conducted LC/MALDI-TOF MS. On the basis of the successful application of the hyphenated methodology in the past in our group,[26] where the separation of telechelic and block copolymers via polarity (liquid chromatography under critical conditions (LCCC)) and hydrodynamic volume (SEC) was achieved, we modified this method by coupling LC with MALDI-TOF MS to prove the successful attachment of both PIB fragments (**3** and **4**) onto the central BTD unit (**2**). Figure 4 shows the results of LC/MALDI-TOF MS of **1** with (a) the LC trace of **1**, **3**, and **4** measured at critical conditions of PIB (MTBE/MeOH = 14.8:85.2 (v/v)) at a flow rate of 0.3 mL/min, T = 30 °C, and (b) the MALDI-TOF MS spectra of the directly correlated fractions of **1**. Looking solely at the first dimension (Figure 4a) it might be concluded that the final product **1** consists of unremoved traces of **3** and **4** due to the shoulder formation of the peak. However, the correlated spectra of the MALDI-TOF MS (Figure 4b) clearly show just one series from 6000 to 10 000 Da and no additional series smaller than 5000 Da as they would be observed for the MALDI-TOF MS spectra of the single PIB strands **3** and **4**, thus excluding the presence of unremoved traces of **3** and **4**. To demonstrate the separation efficiency of the applied LC/MALDI-TOF MS method, a mixture of **1** and **4** (80:20 = w/w) was subjected to LC/MALDI-TOF MS, clearly demonstrating the separation efficiency of the used LC technique by showing two separated series for **4** (4000−6000 Da) and **1** (6000−9000 Da) (see Figure S6 of the Supporting Information together with an overlay of the LC traces of **1** and the mixture of **1** and **4**, together with the correlated MALDI-TOF MS spectra for **1** and the mixture of **1** and **4**). The shoulder formation of all peaks is explainable as a SEC effect,[26] as the linkage of the two single

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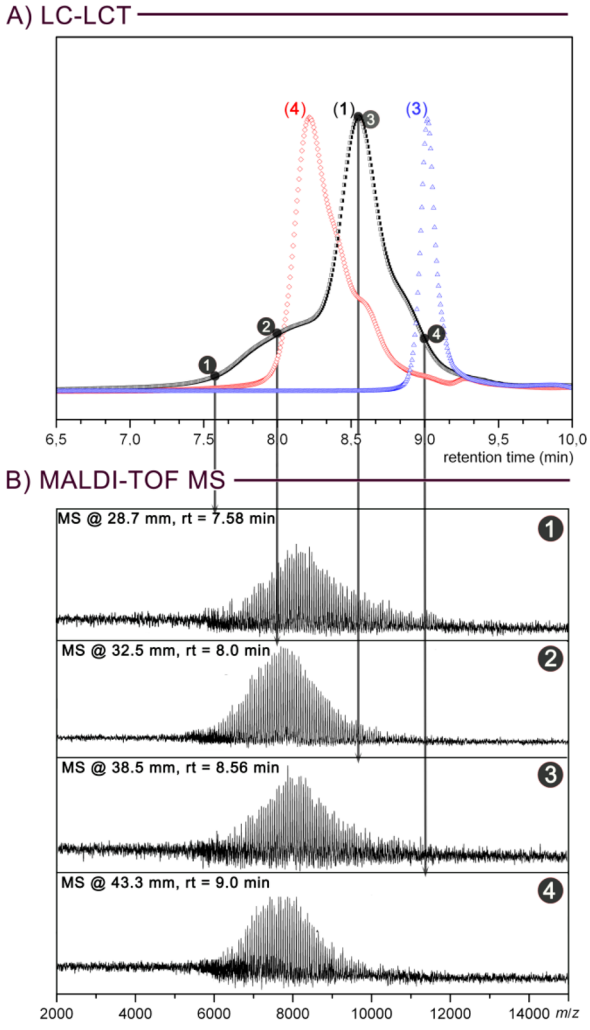

Figure 4. LC/MALDI-TOF MS of **1**. (A) LC trace of **1**, **3**, and **4** measured at critical conditions of PIB (MTBE/MeOH = 14.8:85.2 (v/v)) at a flow rate of 0.3 mL/min, T = 30 °C. B) MALDI-TOF MS spectra of the correlated fractions of **1** proving the purity of the final amphiphilic hybrid molecule.

PIB strands **3** and **4** to achieve the β-turn mimetic PIB conjugate (**1**) changes both the polarity and the molecular weight, thus modifying the LCCCs. Hence, the chromatogram of **1** showed SEC behavior, which was reflected in the correlated MALDI-TOF MS spectra (Figure 4b) due to the small shift from higher molecular weights to lower molecular weights according to retention time of the first dimension. Therefore, the purity of the final amphiphilic hybrid molecule **1** could be proven via LC/MALDI-TOF MS.

In conclusion, for the first time we have demonstrated the successful attachment of two polymer chains (PIB strands **3** and **4**) to yield a complete β-turn mimetic.

As the synthesis of this amphiphilic β-turn mimetic PIB conjugate **1** was successful in yielding ultrapure material, the behavior of the system will be investigated in terms of membrane incorporation and the constraint imposed by the artificial folding element. As the turn itself showed membrane activity[27] and PIB–PEO block copolymers have been well investigated using DPPC, DOPC, and DPPC/DOPC as membrane systems,[28−33] this amphiphilic β-turn mimetic PIB conjugate **1** promises interesting effects for secondary structure formation in mixed lipid and polymeric membranes.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, spectroscopic methods, chromatographic methods, spectroscopic methods, coupling technique, and synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: wolfgang.binder@chemie.uni-halle.de.

Notes

The authors declare no competing financial interest.

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