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Synthesis, Characterization, and Folding Behavior of β-Amino Acid Derived Polyisocyanides

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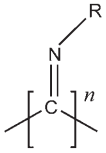
Abstract: Helical polymers of isocyanopeptides derived from β-amino acids have been synthesized and their architectures have been studied in detail. Similar to their α-amino acid analogues, the helical conformation in these macromolecules is stabilized by internal hydrogen-bonding arrays along the polymeric backbone. Unexpectedly, the flexibility of the β-peptide side arms results in a rearrangement of the initial macromolecular architecture, leading to a more stable helical structure possessing a better defined hydrogen-bonding pattern, as was concluded from IR and temperature-dependent circular dichroism studies. Based on these results we propose a dynamic helical model for the β-amino acid derived polyisocyanopeptides; this model is in contrast to the kinetically stable helical macromolecules that are formed upon polymerization of α-amino acid based isocyanopeptides.

Keywords: beta peptides · conformation analysis · helical structures · hydrogen bonds · polyisocyanides

Introduction

The folding and organization of biomacromolecules is of critical importance for life, as has been demonstrated in many landmark papers in the last decades.[1] Likewise, the folding and organization of synthetic macromolecules is a crucial factor in determining the properties of materials in everyday life.[2,3] Some of these synthetic macromolecular systems display folding patterns that are reminiscent of those observed in nature. Polyisocyanides constitute such a class of macromolecules and have been studied extensively in the past.[4–6] These macromolecules contain the repeat unit shown here[7] and can be obtained, among other methods, by polymerization of isocyanides with acid[8] or a Ni^{II} catalyst.[9]



The polymers fold into a 4_1-helical conformation (i.e., residue 5 is on top of residue 1) that can slowly be converted into a random coil structure, depending on the side chain R.[10–12] Polyisocyanides derived from α-amino acids are particularly interesting because their helical backbone is stabilized by the formation of "β-sheet"-like hydrogen-bonding arrays between peptide side chains n and $n+4$;[13] this results in well-defined and very rigid polymers, as reflected by the persistence length, which exceeds that of double-stranded (ds) DNA.[14] This hydrogen-bonded network is even stable in water under ambient conditions, but can be cooperatively disrupted by the addition of strong acid or by heating.[13]

Compared to their α-amino acid analogues, β-amino acids contain an additional C–C bond (Figure 1) around which three conformations can be attained: (+)- and (−)-synclinal (+- and −-*sc*) and antiperiplanar (*ap*). The conformation of β-peptides has been extensively investigated and the β-oligopeptide sequences can adopt helical conformations even if there are as few as six residues.[15,16]

In this article, we report on the synthesis, characterization and folding properties of polymers **1** and **2** derived from isocyano-β-peptides. These novel chiral monomers polymerize

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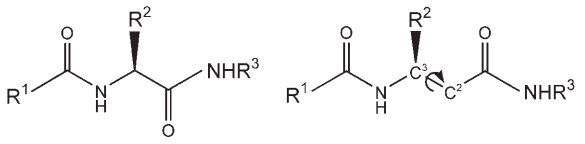
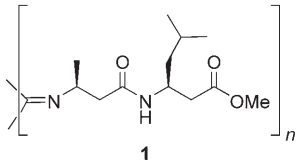


Figure 1. Schematic representation of α- and β^3-amino acid residues. In the latter two types of secondary sheet structures are possible, one in which each residue has an antiperiplanar (*ap*) C^2–C^3 conformation, with the carbonyl groups pointing in the same direction, and another one in which the C^2–C^3 conformation is synclinal (*sc*) or gauche, with the effect that there is no net dipole as in the case of the α-peptides. The *sc* conformation has, so far, only been observed in the sheet section of hair-pin turns containing βhGly (or "β-alanine") residues;[16] on the other hand, the *sc* conformation is typical of β-peptidic helices.



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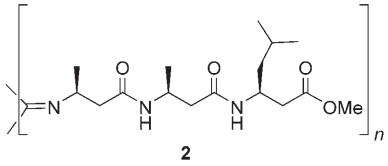

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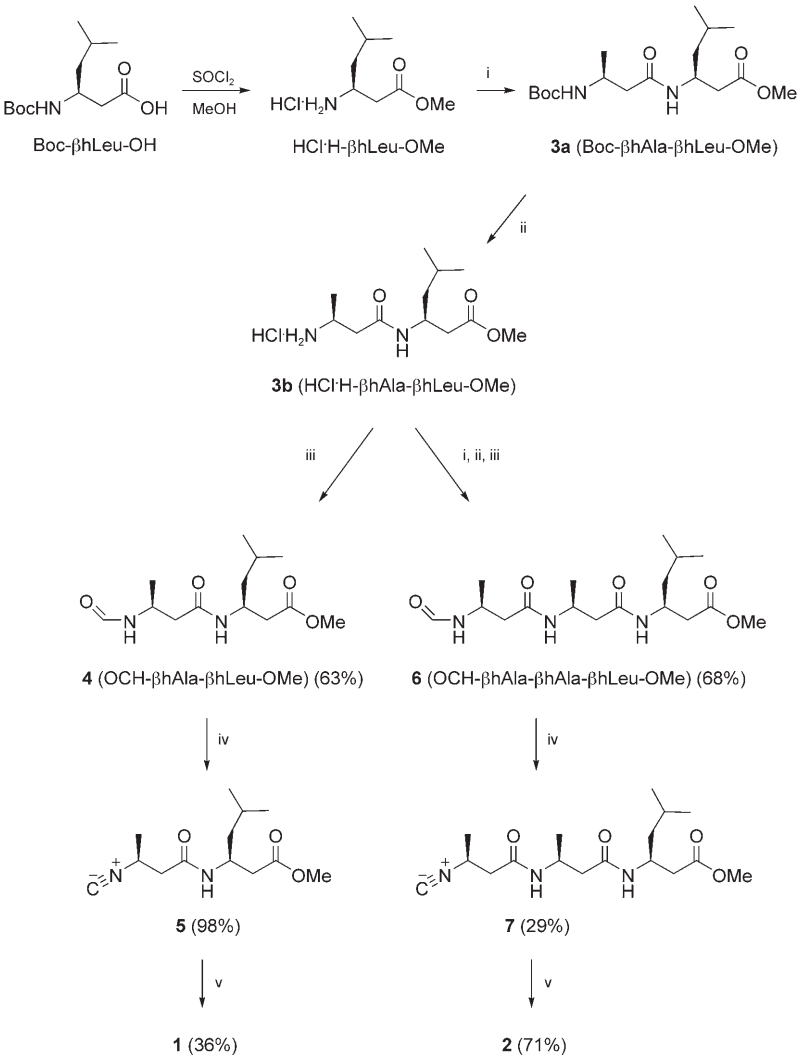
to form helical polymers, with properties that are different from those previously reported for polymers derived from α-amino acids.

Results and Discussion

The monomers used in this study were prepared from β-amino acids, nowadays easily accessible[15] and even commercially available. The synthetic route to the monomers and polymers derived from the β-di- and tripeptides is outlined in Scheme 1. The starting Boc-βhLeu-OH was deprotected at the N terminus and esterified at the C terminus in a one-pot reaction by using thionyl chloride in methanol as the reagent. Subsequent coupling with Boc-βhAla-OH, to give the dipeptide derivative **3a**, was carried out using benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent and diisopropylethylamine (DIPEA) as the base. N-Terminal deprotection was achieved with HCl (2 M) in ethyl acetate (→**3b**), and the same coupling-deprotection sequence was used to attach a second βhAla residue to give a β-tripeptide. The *N*-formyl peptides **4** and **6** were prepared by allowing a mixture of the corresponding β-peptide HCl salt and sodium formate in ethyl formate to react at 80 °C. Finally, the isocyano derivatives **5** and **7** were obtained from the formylated precursors by a dehydration reaction with diphosgene and base in chloroform at −30 °C.[17] With the exception of the conversion of the formyl peptide **6** to the isocyano derivative **7**, all reactions gave reasonable-to-good yields; the relatively low yield of **7** is the result of loss of material during the difficult purification step.

The monomeric isocyanodi- and tripeptides **5** and **7** were polymerized with 3 mol% $Ni(ClO_4)_2 \cdot 6H_2O$ in CH_2Cl_2. A small amount of ethanol was added to dissolve the Ni^{II} cata-



Scheme 1. Synthesis of polyisocyanides **1** and **2**, derived from β-amino acids: i) Boc-βhAla-OH, BOP, DIPEA, ethyl acetate; ii) HCl/ethyl acetate; iii) ethyl formate, sodium formate, 80 °C; iv) diphosgene, *N*-methylmorpholine, $CHCl_3$, −30 °C; v) 1/30 equiv $Ni(ClO_4)_2 \cdot 6H_2O$, CH_2Cl_2/EtOH.

lyst and to act as the initiator in the polymerization reaction.[6a] Selected IR and ^{1}H NMR data of the prepared monomers and polymers are shown in Table 1.

Table 1. Selected IR [ν in cm^{-1}] and ^{1}H NMR [measured in $CDCl_3$; δ in ppm] data of monomers and polymers of isocyano-β-peptides **1** and **2**.

	5		**1**		**7**		**2**	
	($CHCl_3$)	(solid)	($CHCl_3$)	(solid)	($CHCl_3$)	(solid)	($CHCl_3$)	(solid)
ν_{NH}	3430	3296	3272	3280	3429	3284	3286	3279
$\nu_{amide\ I}$	1674	1649	1638	1637	1666	1645	1644	1639
$\nu_{amide\ II}$	1516	1547	1549	1543	1506	1551	1546	1543
δ_{NH}	6.3	–	7.9	–	6.1/7.1	–	8.3	–

The table reveals that the IR stretching vibrations of the polymers in solution are similar to those observed in the solid state; this fact implies that the hydrogen-bonding motif is similar in both cases. This result was confirmed by ^{1}H NMR spectroscopy, which showed a strong downfield shift of the amide protons in the polymers, indicative of a strong hydrogen-bonded network. We propose, therefore, that just like in the case of the polyisocyano-α-peptides, the helical backbone formed during polymerization is stabilized by a sheet-like organization between the peptide side chains, which leads to a more stable and more rigid polymer (Figure 2).[13] It is likely that one set of hydrogen bonds is present between the dipeptide side chains of **1** and two hydrogen bonds between the tripeptide side chains of **2**. In the latter case, the amide hydrogen-bonding arrays are probably unidirectional, as was also observed in the X-ray structure of β-amino acid tripeptide parallel pleated sheets reported by Seebach et al.[18]

The molecular weight of the polymers was determined with the help of atomic force microscopy (AFM) measurements. Due to their rigidity, the individual macromolecules could be easily observed on a mica surface and their contour lengths were measured (Figure 3). This procedure was used previously by Prokhorova et al.[19] to determine the molecular weight of polymethacrylates and polystyrenes with bulky substituents and has also been applied to polyisocyano-α-peptides.[20] The measured average heights of polymers **1** and **2** amounted to 0.60 nm and 0.72 nm, respectively, which is smaller than would be expected from model calculations (2.2 nm for **1**, 3.0 nm for **2**). This is likely the result of the fact that the polymers adopt a collapsed conformation on the mica surface.[21] Histograms of the measured length distributions are shown in Figure 4 and the calculated molar mass data are summarized in Table 2. In these analyses, the degree of polymerization was derived from the contour lengths, assuming a 4_1-type helix with a helical pitch of 4.6 Å. A similar approach has been followed in the case of the polyisocyano-α-peptides.[13,20]

The sensitivity of the polymerization reaction to the structure of the monomer is reflected in molar mass and polydispersity data, which is significantly different for the two types of monomers. Interestingly, a concentrated solution of the polymer with β-dipeptide side chains in $C_2H_2Cl_4$ gave rise to a cholesteric lyotropic meso-

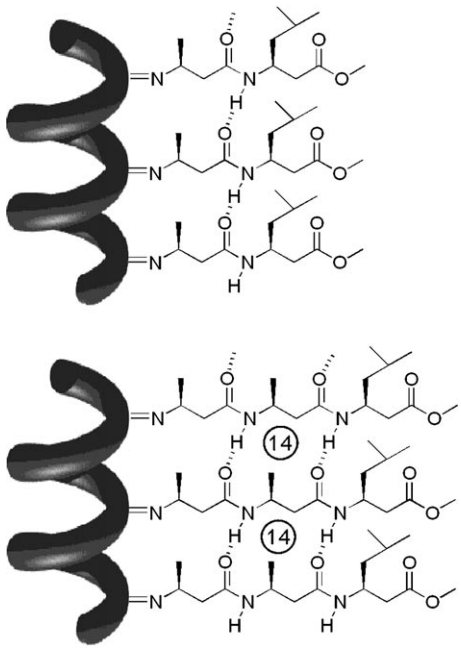


Figure 2. Schematic representation of hydrogen-bonding arrays between the side-chain β-peptide portions of the polymers **1** (top) and **2** (bottom). In **2** a 14-membered hydrogen-bonded ring is formed.

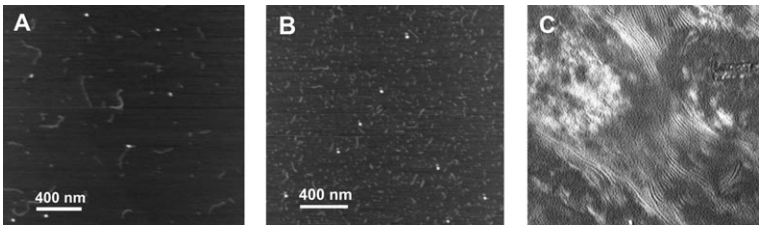


Figure 3. AFM micrographs of A) polymer **1**, B) polymer **2**, and C) LC phase of **1**.

phase, highlighting the rigid rod character of the polymer (Figure 3C). For the tripeptide-based polymer **2** the formation of a similar lyotropic mesophase under the same conditions was less pronounced, probably because of the combination of a lower molecular weight and a reduced solubility.

The configuration of the chirality center closest to the polymer backbone is known to play a dominant role in de-

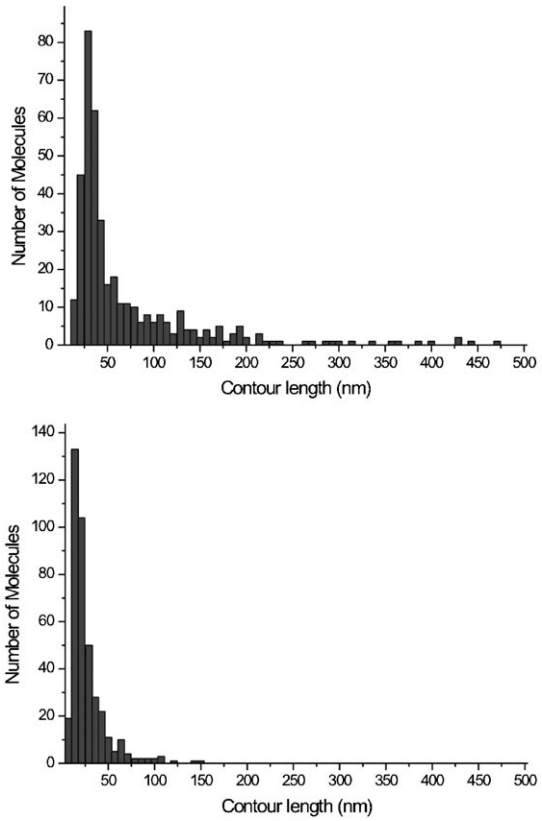


Figure 4. Histograms showing the length distributions of molecular chains of polymer **1** (top) and polymer **2** (bottom) as derived from AFM.

Table 2. Molar mass data of polyisocyano-β-peptides as determined by AFM.

	M_n [kg mol^{-1}]	M_w [kg mol^{-1}]	DP[a]	D[b]
1	147	332	580	2.3
2	72	90	210	1.3

[a] DP = Degree of polymerization. [b] D = Dispersity.

termining the screw sense of the polyisocyanide backbone.[22] In the two polymers **1** and **2**, these chirality centers have both the *S* configuration, and hence we expect that a right-handed or *P* helix is formed during polymerization. In the circular dichroism (CD) spectra of both polymers, we observed a single negative Cotton effect at $\lambda = 313$ nm originating from the n–π* transitions of the imine chromophores. These CD spectra are similar to those measured for polyisocyanides derived from α-alanine dipeptides, confirming that the amide groups in a hydrogen bond have a similar effect on the n–π* transitions of the imine chromophores.[23] In contrast to polyisocyano-α-alaninepeptides, the assumed right-handed or *P* screw sense results in a negative Cotton effect and the magnitude of the effect is smaller. The first observation can be tentatively rationalized by the opposite direction of the first amide group, whereas the smaller magnitude is likely caused by the larger distance to the polymer backbone as is clear from the schematic representation in Figure 5.

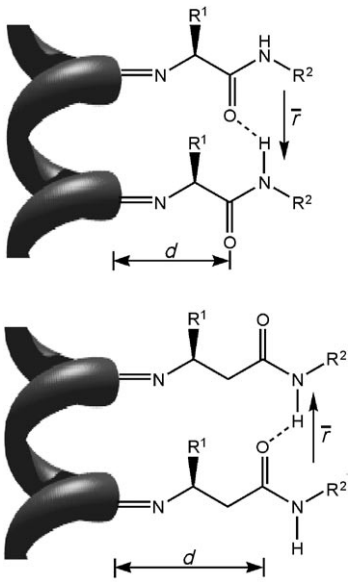


Figure 5. Schematic representation of hydrogen bonding arrays of a polyisocyano-α-peptide (top) and a polyisocyano-β-peptide (bottom), based on reported X-ray structures of β-peptidic sheets of this type.[18] The additional methylene group in the latter leads to a larger distance *d* from the helical backbone to the amide groups and a different direction $\bar{r}$.

In the case of the polyisocyano-α-dialanines, the 4_1-helical conformation that results from the Ni^{II}-catalyzed reaction is retained in solution for prolonged periods of time, as was demonstrated by, amongst other techniques, CD spectroscopy.[13] Surprisingly, in the case of the β-peptide derivatives **1** and **2**, we observed a slow disappearance of the negative Cotton effect at $\lambda = 313$ nm and the evolvement of a new positive band at $\lambda = 285$ nm (Figure 6). These spectral changes were irreversible and dilution studies showed that they are not the result of aggregation processes, but are inherent to the macromolecular architecture. The isodichroic point observed at $\lambda = 250$ nm revealed that this change likely involved a transition between two discrete conformations, a kinetically controlled structure formed upon polymerization ("native" polymer), and a thermodynamically more stable structure ("altered" polymer). The optical rotations at the D-line showed the same time-dependent trend upon standing of the polymer in solution, see Table 3. Although similar sign inversions found for other helical polymers have been ascribed to helicity inversions,[24] we presume that in the present case they are caused by a change in the arrangement of the amide groups coupled to a possible change of the helical pitch of the right-handed polymer (vide supra).

At room temperature the conformational change proceeds more rapidly with the β-dipeptide derivative **1** than with the β-tripeptide derivative **2**, and in both cases the transformation is accelerated at elevated temperatures. The rate constants of the structural changes were determined at four different temperatures, and from these measurements the transition state parameters could be calculated using either the Eyring or Arrhenius equation (Table 4).

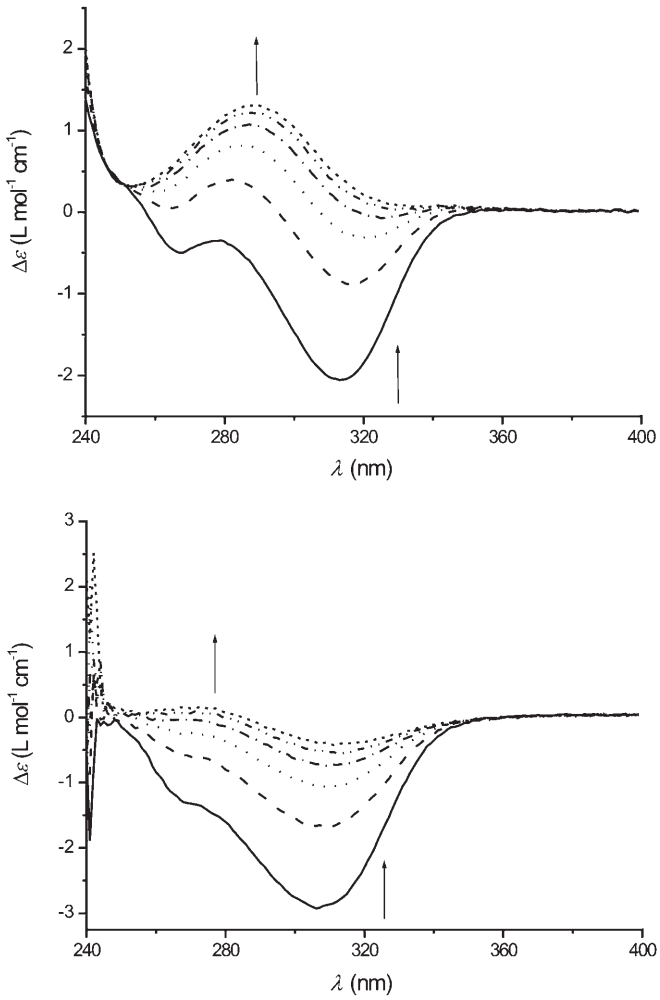


Figure 6. Conformational changes monitored by CD-spectroscopy at 10 min intervals upon standing in solution of **1** (top) in $CHCl_3$ at 25 °C and **2** (bottom) in $C_2H_2Cl_4$ at 55 °C.

Table 3. Optical rotation data [° cm^2 g^{-1}] measured in $CHCl_3$.

	$[\alpha]_D$ monomer	$[\alpha]_D$ "native" polymer	$[\alpha]_D$ "altered" polymer
1	−15.8	−87.5	27.8
2	−64.0	−160.5	7.5

Table 4. Thermodynamic parameters describing the conformational changes of polyisocyano-β-peptides as determined by CD spectroscopy.

	$\Delta H^{\neq}$ [kJ mol^{-1}]	$\Delta S^{\neq}$ [J mol^{-1} K^{-1}]	$\Delta G^{\neq}_{298}$ [kJ mol^{-1}]	E_a [kJ mol^{-1}]
1[a]	73.2	−56.7	90.1	75.6
2[b]	61.8	−116.0	96.4	64.6

[a] Measured in $CHCl_3$, temperature range 5–35 °C. [b] Measured in $C_2H_2Cl_4$, temperature range 45–75 °C. For the measurements and the calculations it is assumed that a linear relationship exists between $\Delta\varepsilon$ at $\lambda =$ 313 nm and the fraction of native polymer in solution.

The fact that the process occurs at rather low temperatures is reflected in the relatively low Arrhenius activation energies. The highly negative $\Delta S^{\neq}$ value measured for polymer **2**, relative to **1**, suggests that the former loses more degrees of freedom in the transition state than the latter, as is expected since it contains more sterically crowded side chains and has an additional array of hydrogen bonds. This more negative entropy of activation is the main reason for the lower annealing rate of the β-tripeptide derivative.

Huang and Euler have calculated an Arrhenius activation energy of 70 kJ mol^{-1} (CH_2Cl_2) for the unfolding process of poly(phenylisocyanide) into a random-coil-like structure.[11] Although this number is of similar magnitude as the E_a values for the β-amino acid derived polyisocyanides in Table 4, it is more likely that in the present case the chiroptical changes are associated with a change in side-chain conformation. The thermal unfolding of polyisocyano-α-dipeptides, macromolecules that also have the stabilizing hydrogen-bonding array along the polymeric backbone, is associated with an substantially higher activation energy of 90 kJ mol^{-1}.[25] This indicates that more energy is needed to break the hydrogen bonds in order to disrupt or racemize the helical structure.

IR studies on the annealing of native **1** revealed that the hydrogen-bonded network remained intact (Figure 7) and

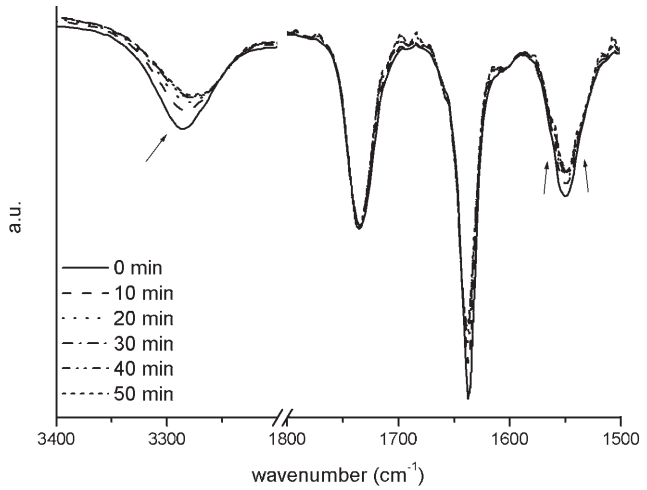


Figure 7. Conformational change of the *β*-dipeptidylpolyisocyanide **1** upon standing in $CHCl_3$ (4 mg mL^{-1}) at room temperature, as monitored by IR spectroscopy at 10 min intervals.

no significant difference in the NMR spectra of the two polymers was observed. AFM studies and the fact that the lyotropic liquid crystalline (LC) phase of this polymer remained present after annealing, with only a minimal change in cholesteric pitch, suggested that the rigid-rod-like character of the polymer did not alter (Figure 8). AFM indicated no significant change in chain dimensions in going from the native to the altered polymer.

These combined data suggest that both the sheet-like arrangement of the polymer side chains and the rigid helical

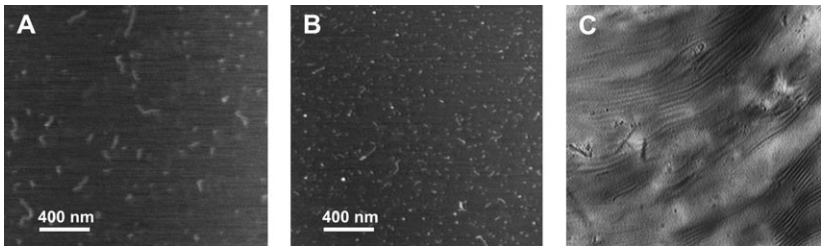


Figure 8. AFM micrographs of polymers **1** (A) and **2** (B), and LC phase of **1** (C), all in the "altered" state.

nature of the polymer are conserved during annealing. Possibly, during polymerization the monomers are fixed in a sheet-like arrangement in which a large number of β-amino acid side chains have an *anti* C^2–C^3 conformation. This will result in a relatively large local dipole moment that would decrease in magnitude when the amino acid side chains change conformation from an *ap*-C^2–C^3 arrangement into an *sc*-C^2–C^3 conformation that might lead to a "better" hydrogen-bonding array. The change in amide bond directionality would tentatively explain the observed sign inversion of the CD-spectra (Figure 6). The proposed rearrangement is also in agreement with the sharpening of the IR bands and the small shift of the NH-stretch vibration to lower wavenumbers (Figure 7). Isomerization of the helix from *P* to *M* can only be achieved if multiple hydrogen bonds are simultaneously disrupted. This is expected to result in a broadening of the NH-stretch vibration. The sharpening and shift of this band also implies that the altered hydrogen-bonded network is stronger, more favorable from an enthalpic point of view. The possible change in conformation around the C^2–C^3 bonds in the altered structure is supported by molecular mechanics calculations from which structures are obtained in which a number of amino acids are in the (+)-*sc* conformation reversing the direction of the amide hydrogen bonding (Figure 9).

Whereas the helical conformation of polyisocyano-α-peptides could be disrupted by adding strong acid or by heating,[13] the altered conformation of polyisocyano-β-peptides turned out to be more stable. Heating a solution of polymer **1** or **2** up to 110 °C in tetrachloroethane resulted in only minimal changes of the CD spectra. Acidification of a solution of either native or altered polymer **1** with trifluoroacetic acid (TFA) resulted in complete loss of chiroptical activity, suggesting unfolding of the helical polymer likely induced by disruption of the hydrogen-bonded network (Figure 10). However, when these solutions were neutralized with triethylamine, the chiroptical activity of the altered polymer **1** was restored, while the CD spectrum of native **1** became the same as that of the altered **1** (Figure 11). This observation supports our hypothesis that the altered conformation is the energetically more stable one. Acidification/neutralization of altered polymer **2** showed the same trend, the difference being that even at high concentrations of TFA (~25 %) some chiroptical activity remained.

Similar results were recently reported by Yashima et al. on the reversible folding/unfolding of achiral poly(4-carboxyphenylisocyanide) in the presence of chiral amines.[26] Driving force for the refolding process was the formation of hydrogen bonds between chiral ammonium ions and the carboxylate groups that are covalently attached to the polyisocyanide backbone. In our case, both the hydrogen-bonding

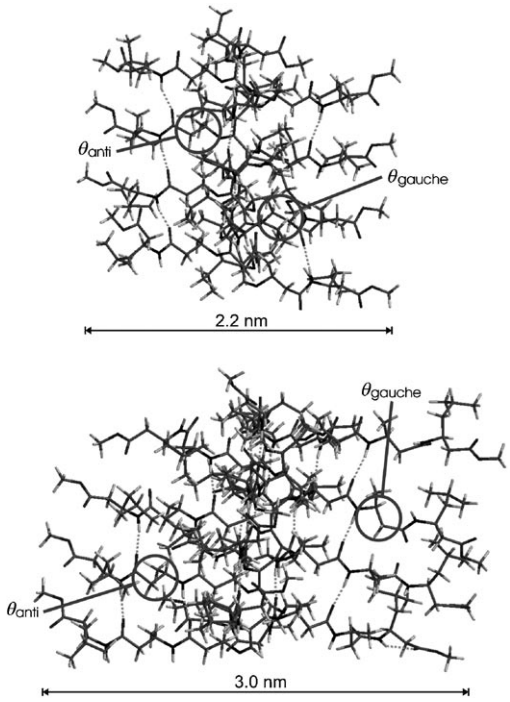


Figure 9. Possible structure of the hydrogen-bonding network as revealed by molecular mechanics calculations by using the force field method for polymer **1** (top) and polymer **2** (bottom).

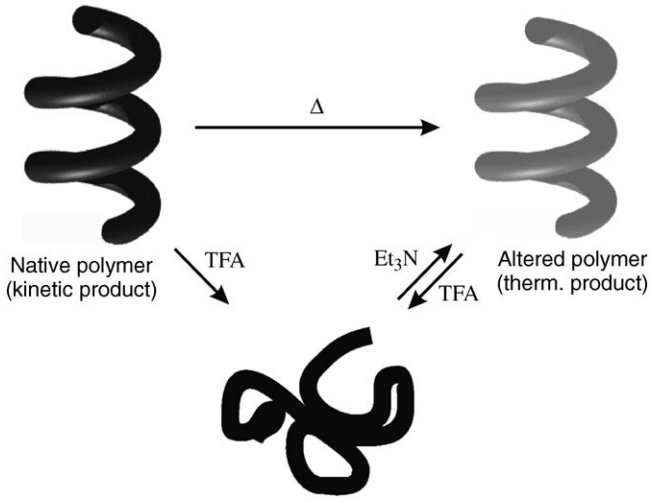


Figure 10. Schematic representation of proposed different conformations of polymer **1**.

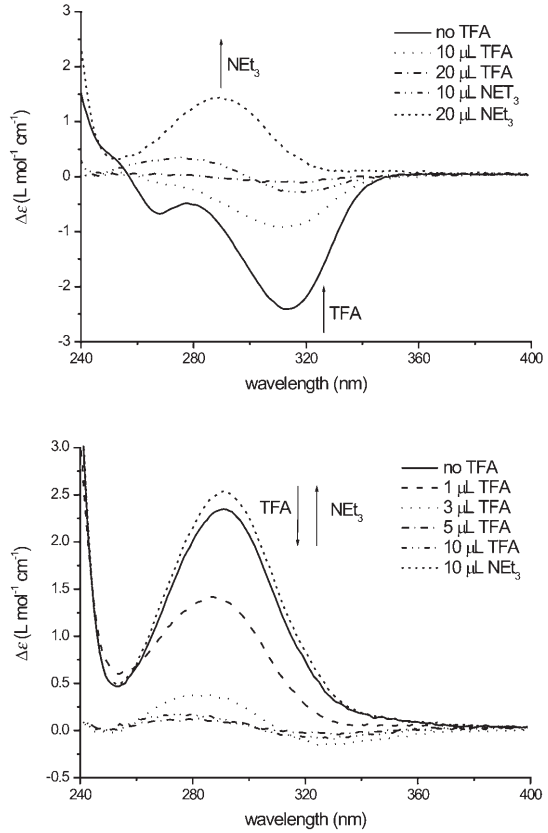


Figure 11. Changes of the CD spectra of native **1** (top) and altered **1** (bottom) upon acidification and subsequent neutralization.

moieties and the chiral groups are present in the macromolecules.

Conclusion

Polymerization of isocyanides derived from β-peptides results in the formation of rigid helical macromolecules, of which the backbones are stabilized by hydrogen bonds between the peptide side chains. Unlike their polyisocyano-α-peptide counterparts, the initially formed product of the polymerization reaction readily changes structure and another hydrogen-bond-stabilized conformation is adopted ("altered" polymer). This transformation most likely comprises a change in the sheet-like structure of the peptide side groups, possibly from an *ap*-C^2–C^3 to a *sc*-C^2–C^3 conformation. Upon acidification, both the native and altered β-amino-acid dipeptide-based polymers lose chiroptical activity, indicating that the macromolecules unfold into a nonhelical conformation. When the solutions are subsequently neutralized, only the energetically more favorable altered conformation of the polymers is regained.

These novel polyisocyanido-β-peptides display properties that are different from those previously reported for their α-peptidic analogues. These differences are believed to originate from the increased flexibility of the β-peptidic substituents on the polymer backbone, when compared to the α-peptidic ones. This flexibility allows for a rearrangement of the hydrogen-bonding network in the polymer, resulting in a more dynamic structure of the polymer chains.

Experimental Section

General methods and materials: Dichloromethane, chloroform, and ethyl acetate were purified by distillation from CaH_2, $CaCl_2$, and P_2O_5, respectively. Tetrachloroethane and *N*-methylmorpholine were distilled under reduced pressure from $CaCl_2$ and sodium, respectively. All other chemicals were commercial products and were used as received. Flash chromatography was performed using silica gel (0.035–0.070 mm) purchased from Acros and TLC-analyses were carried out on silica 60 F_{254} coated glass either from Merck or Acros. Compounds were visualized by spraying with Cl_2/TDM or $Ni(ClO_4)_2 \cdot 6H_2O$ in EtOH. ^{1}H NMR spectra were recorded on Bruker WM-200 and Bruker AC-300 instruments at 297 K; ^{13}C NMR spectra were acquired on a Bruker AC-300 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane ($\delta =$ 0.00 ppm) as an internal standard. FT-IR spectra were recorded on Bio-Rad FTS 25 and Anadis IR300 instruments (resolution 1 cm^{-1}). CD spectra were measured on a JASCO 810 instrument. Melting points were measured on a Jeneval THMS 600 microscope equipped with a Linkam 92 temperature control unit and are reported uncorrected. Mass spectrometry (EI) was performed on a VG 7070E instrument. Elemental analyses were determined on a Carlo Erba 1180 instrument. Optical rotations were measured on a Perkin–Elmer 241 Polarimeter.

Atomic force microscopy: Imaging was carried out on a Nanoscope III instrument from Digital Instruments operating in the tapping mode at room temperature. Samples were prepared by spin-coating (1400 rpm) a 1 mg L^{-1} polymer solution in chloroform on freshly cleaved mica.

Preparation of cholesteric LC phases: Concentrated solutions of polymer **1** in 1,1,2,2,-tetrachloroethane were prepared by allowing solvent to make contact with the polymer, which was crushed between two microscope cover plates. Imaging was subsequently carried out on a Jeneval THMS 600 microscope.

Annealing experiments: Solutions of **1** in $CHCl_3$ (4 mg mL^{-1}) and of **2** in 1,1,2,2-tetrachloroethane (2 mg mL^{-1}) were prepared and 300 μL of this solution was transferred to a quartz cuvette with a 1 mm path length. By using the Peltier temperature controller unit of the CD instrument, the solution was brought to the desired temperature and the annealing of the polymers was monitored using interval scan measurements at desired time-intervals. The resulting annealed polymers were subsequently used for other spectroscopic and microscopic investigations. 100 μL of the solution of **1** was transferred to a liquid cell and an IR-spectrum was taken at desired time intervals.

Acid-induced denaturation: Solutions of polyisocyano-β-peptides in $CHCl_3$ (2 mg·mL^{-1}) were prepared and transferred to quartz cuvettes with a 1 mm path length. To these solutions 5 μL of a 20% solution of TFA in $CHCl_3$ was added stepwise until all chiroptical activity had been lost. The processes were monitored by CD spectroscopy.

HCl·H-βhAla-βhLeu-OMe (3b): Methanol (5 mL) was cooled to −70 °C by using acetone/CO_2 and thionyl chloride (0.45 mL, 0.61 mmol) was added dropwise. Whilst stirring the solution, Boc-βhLeu-OH (500 mg, 2.0 mmol) was added and the solution was warmed up slowly to 70 °C and refluxed for 1 h at this temperature. The slightly yellow solution was concentrated, redissolved and again concentrated in EtOH and subsequently in *t*BuOH (2×); the resulting product was dried in vacuo. The yellow solid was dissolved in ethyl acetate (10 mL) and Boc-βhAla-OH (0.41 mg, 2.2 mmol) and DIPEA (0.82 mL) were added subsequently. The solution was cooled in ice and BOP (1.14 g, 2.6 mmol) was added. The solution was brought slowly to room temperature and was stirred for an additional 20 h. The organic layer was washed with aqueous saturated sodium bicarbonate solution (2 mL), 10% citric acid (2 mL), aqueous saturated sodium chloride solution (2 mL), and water (2 mL), and dried

over Na_2SO_4. The solvent was evaporated and the white solid (**3a**) was redissolved in HCl (20 mL) saturated ethyl acetate and stirred for 1 h. HCl was removed by repetitive solution/evaporation by using EtOH, *t*BuOH (2×), and CH_2Cl_2 as solvents in succession. The residual yellow oil was dried in vacuo. The product **3b** was directly used in the next step of the reaction. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.40$ (s, 3H; $NH_3^+Cl^-$), 7.28 (d, $J = 8.5$ Hz, 1H; NH), 4.32 (m, 1H; CH ala), 3.78 (m, 1H; CH leu), 3.68 (s, 3H; OCH_3), 2.55 (m, 4H; 2 CH_2), 1.58 (m, 2H; CH_2CH-$(CH_3)_2$), 1.51 (d, $J = 6.5$ Hz, 3H; CH_3), 1.29 (m, 1H; $CH_2CH(CH_3)_2$), 0.91 ppm (d, $J = 6.4$ Hz, 6H; $CH_2CH(CH_3)_2$).

HCL·H-βhAla-βhAla-βhLeu-OMe: Compound **3b** (0.28 g, 1.0 mmol) was dissolved in ethyl acetate (10 mL) and Boc-βhAla-OH (0.22 g, 1.1 mmol) and DIPEA (0.52 mL) were added in succession. The solution was cooled in ice and BOP (0.49 g, 1.1 mmol) was added. The solution was brought slowly to room temperature and was stirred for 20 h. The white precipitate was filtered off, dissolved in $CHCl_3$ (25 mL), and filtered, and the filtrate was concentrated and dried in vacuo. The product was redissolved in HCl saturated ethyl acetate (20 mL) and stirred for 1 h. HCl was removed by repetitive solution/evaporation by using EtOH, *t*BuOH (2×), and CH_2Cl_2 as solvents in succession. A white solid with satisfactory purity for use in the following reaction steps was obtained in an overall yield of 76% (starting from Boc-βhLeu-OH). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.35$ (s, 3H; $NH_3^+Cl^-$), 7.97 (d, $J = 7.3$ Hz, 1H; NH ala), 7.14 (s, 1H; NH leu), 4.28 (m, 2H; 2CH), 3.72 (m, 1H; CH), 3.66 (s, 3H; OCH_3), 2.42 (m, 6H; 3CH_2), 1.53 (m, 2H; CH_2CH$(CH_3)_2$), 1.44 (m, 3H; CH_3 *ala*-ala-leu), 1.28 (m, 1H; $CH_2CH(CH_3)_2$), 1.21 (d, $J = 5.9$ Hz, 3H; CH_3 ala-*ala*-leu), 0.90 (d, $J = 5.8$ Hz, 6H; $CH_2CH(CH_3)_2$).

OCH-βhAla-βhLeu-OMe (4): Compound **3b** (133 mg, 0.47 mmol) was dissolved in ethyl formate (25 mL) and sodium formate (100 mg, 1.47 mmol) was added. The solution was heated at reflux overnight at 80°C. The solvent was evaporated and the white solid was redispersed in CH_2Cl_2 (10 mL). The mixture was heated and filtered while firmly shaking, and the residue was washed three times with CH_2Cl_2 (5 mL). The filtrate was concentrated and the obtained yellow oil was purified by column chromatography (silica gel, eluent 5% MeOH in $CHCl_3$) to yield **4** (80 mg, 63%) as white crystalline product. M.p. 103°C; $[\alpha]_D = -56.8°$ ($c = 5.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 8.10$ (s, 1H; HCO), 6.92 (d, J = 5.0 Hz, 1H; NH ala), 6.17 (d, $J = 8.6$ Hz, 1H; NH leu), 4.39 (m, 1H; CH), 4.31 (m, 1H; CH), 3.69 (s, 3H; OCH_3), 2.50 (m, 4H; 2 CH_2), 1.54 (m, 2H; CH_2CH$(CH_3)_2$), 1.29 (m, 1H; $CH_2CH(CH_3)_2$), 1.24 (d, 3H; CH_3, $J = 6.8$ Hz), 0.92 ppm (d, $J = 6.4$ Hz, 6H; $CH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 172.2$, 170.3 (C=O), 160.5 (HC=O), 51.7 (OCH_3), 44.3 (CH_2CH(CH_3)2), 43.2 (CH_2 leu), 41.6, 41.3 (CH), 38.8 (CH_2 ala), 25.0 ($CH_2CH(CH_3)_2$), 22.8, 22.0 ($CH_2CH(CH_3)_2$), 19.8 ppm (CH_3); FT-IR (KBr): $\tilde{\nu} = 3282$ (NH), 1745, 1730 (C=O ester), 1653, 1634 (amide I), 1554, 1535 cm^{-1} (amide II); EI-MS: *m/z* :272 $[M]^+$; elemental analysis calcd (%) for $C_{13}H_{24}N_2O_4$: C 57.33, H 8.88, N 10.29; found: C 57.54, H 8.91, N 10.17.

OCH-βhAla-βhAla-βhLeu-OMe (6): This compound was prepared starting from HCl·H-βhAla-βhAla-βhLeu-OMe and by following the same procedure as described above for **4**. The product **6** was obtained as a white crystalline product, which formed a gel at very low concentrations (yield: 68%). M.p. 216°C; $[\alpha]_D = -32.8°$ ($c = 0.5$ in MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 8.10$ (s, 1H; HCO), 7.0 (dd, $J = 7.8$, 16.9 Hz, 2H; 2NH ala), 6.17 (d, $J = 8.9$ Hz, 1H; NH leu), 4.32 (m, 3H; 3CH), 3.70 (s, 3H; OCH_3), 2.47 (m, 6H; 3 CH_2), 1.55 (m, 2H; CH_2CH$(CH_3)_2$), 1.29 (m, 1H; $CH_2CH(CH_3)_2$), 1.23 (m, 6H; 2 CH_3 ala), 0.92 ppm (d, $J = 6.4$ Hz, 6H; $CH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 171.9$, 170.1, 169.8 (C=O), 160.1 (HC=O), 51.8 (OCH_3), 44.4 (CH_2CH$(CH_3)_2$), 43.3 (CH_2 leu), 42.9 (CH leu), 41.6 (CH ala), 41.5 (2) (2 CH_2 ala), 38.9 (CH ala), 25.2 ($CH_2CH(CH_3)_2$), 23.0, 22.3 ($CH_2CH(CH_3)_2$), 20.2, 19.9 ppm (2CH_3 ala); FT-IR (KBr): $\tilde{\nu} = 3285$ (NH), 1733 (C=O ester), 1637 (amide I), 1542 cm^{-1} (amide II); EI-MS: *m/z* :357 $[M]^+$, 326 $[M-OCH_3]^+$l; elemental analysis calcd (%) for $C_{17}H_{31}N_3O_5$: C 57.12, H 8.74, N 11.76; found: C 56.31, H 8.72, N 11.28.

Isocyanide 5 from formyl derivative 4: To prevent early polymerization, all glassware was rinsed three times with an aqueous 0.5% HF solution and then with an aqueous saturated sodium bicarbonate solution. Under a N_2 atmosphere, compound **4** (120 mg, 0.44 mmol) was dissolved in $CHCl_3$ (10 mL). Freshly distilled *N*-methylmorpholine (121 μL, 1.10 mmol) was added and the solution was cooled to −30°C using acetone/CO_2. Over a period of 1 h, diphosgene (26.5 μL, 0.22 mmol) in $CHCl_3$ (5 mL) was added dropwise, while maintaining $T = -30$°C and then more diphosgene (8 μL) was added directly. The solution was stirred for an additional 10 min at this temperature and then brought to 0°C. An ice-cold aqueous saturated sodium bicarbonate solution (2 mL) was added and the mixture was stirred vigorously for 5 min. The organic layer was separated, extracted once with water (2 mL), and dried on Na_2SO_4. The solvent was evaporated and the product was dried in vacuum to yield **5** (110 mg, 98%) as colorless sticky material. M.p. 370°C (decomp); $[\alpha]_D = -15.8°$ ($c = 0.3$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 6.24$ (d, $J = 8.9$ Hz, 1H; NH), 4.36 (m, 1H; CH ala), 4.16 (m, 1H; CH leu), 3.70 (s, 3H; OCH_3), 2.50 (m, 4H; 2 CH_2), 1.60 (m, 2H; CH_2CH-$(CH_3)_2$), 1.43 (m, 3H; CH_3 ala), 1.32 (m, 1H; $CH_2CH(CH_3)_2$), 0.92 (d, $J = 6.4$ Hz, 6H; $CH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 172.0$, 167.1, (C=O), 155.5 (CN), 51.8 (OCH_3), 47.3 (CH ala), 44.4 (CH_2CH-$(CH_3)_2$), 44.1, 43.0 (2 CH_2), 38.6 (CH leu), 25.3 ($CH_2CH(CH_3)_2$), 23.0, 22.2 ($CH_2CH(CH_3)_2$), 21.6 ppm (CH_3); FT-IR: $\tilde{\nu} = 3296$ (NH), 2139 (CN), 1738 (C=O ester), 1649 (amide I), 1547 cm^{-1} (amide II). Because of the sticky nature of this compound no elemental analyses could be carried out. MS: : *m/z* calcd for $C_{13}H_{22}N_2O_3$: 254.1631; found: 254.1632.

Isocyanide 7 from formyl peptide ester 6: This compound was synthesized starting from **6**, by using the procedure described for the conversion of **4** to **5**. The product **6** was purified by chromatography using a short column (silica gel, eluent $CHCl_3$/acetone, 2:1 v/v; a trace amount of triethylamine was added). Unfortunately, due to the unstable nature of the compound a large quantity of product was lost during the purification procedure. Compound **6** was obtained as a white crystalline solid in 29% yield. M.p. 157°C; $[\alpha]_D = -64.0°$(c=0.5 CHCl3); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.07$ (d, $J = 7.1$ Hz, 1H; NH ala), 6.08 (d, $J = 8.8$ Hz, 1H; NH leu), 4.30 (m, 2H; 2CH ala), 4.15 (m, 1H; CH leu), 3.68 (s, 3H; OCH_3), 2.41 (m, 6H; 3CH_2), 1.55 (m, 2H; CH_2CH$(CH_3)_2$), 1.41 (m, 3H; CH_3 *ala*-ala-leu), 1.30 (m, 1H; $CH_2CH(CH_3)_2$), 1.21 (d, $J = 6.7$ Hz, 3H; CH_3 ala-*ala*-leu), 0.92 ppm (d, $J = 6.4$ Hz, 6H; $CH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 171.9$, 170.1, 166.9 (C=O), 155.4 (CN), 51.8 (OCH_3), 47.2 (CH *ala*-ala-leu), 44.5 (CH_2CH$(CH_3)_2$), 44.0 (CH2 *ala*-ala-leu), 43.2 (CH_2 leu), 43.0 (CH leu), 41.2 (CH ala-*ala*-leu), 38.9 (CH_2 ala-*ala*-leu), 25.2 ($CH_2CH(CH_3)_2$), 23.0, 22.3 ($CH_2CH(CH_3)_2$), 21.6, 19.8 ppm (CH_3 ala); FT-IR: $\tilde{\nu} = 3284$ (NH), 2141 (CN), 1740 (C=O ester), 1645 (amide I), 1551 cm^{-1} (amide II); EI-MS: *m/z* :339 $[M]^+$, 308 $[M-OCH_3]^+$, 282 $[M-OCH_3-CN]^+$; *m/z* calcd for $C_{17}H_{29}N_3O_4$: 339.2158; found: 339.2157; elemental analysis calcd (%) for $C_{17}H_{29}N_3O_4$: C 60.15, H 8.61, N 12.38; found: C 59.33, H 8.57, N 12.18.

Polymer 1 from isocyanide 5: A solution of **5** (110 mg, 0.43 mmol) in of CH_2Cl_2 (5 mL) was added dropwise to a stirred solution of $Ni(ClO_4)_2$·-$(H_2O)_6$ (5.3 mg, 0.014 mmol) in CH_2Cl_2 (5 mL) and absolute ethanol (0.10 mL). The solution turned immediately yellowish/brown and IR spectroscopy showed the complete consumption of the isocyanide monomer after 90 min. The solvent was evaporated and the resulting brown solid was redissolved in a small amount of $CHCl_3$. The polymer was precipitated by dropping this solution into methanol/water (10 mL, 3:1, v/v) under vigorous stirring. The product was filtered off and washed extensively with methanol/water (3:1, v/v), until the filtrate remained colorless, and then once with methanol. A slightly yellow crystalline product **1** was obtained, which was dried in vacuo. Yield 40 mg of **1** (36%). $[\alpha]_D$ = see Table 3; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.2$–7.5 (brs, 1H; NH), 4.29 (brs, 2H; 2CH), 3.62, 3.54 (s, 3H; OCH_3), 2.7–2.2 (brs, 4H; 2CH_2), 1.9–1.3 (brs, 6H; $CH_2CH(CH_3)_2$, CH_3 ala), 0.92 ppm (brs, 6H; CH_2CH-$(CH_3)_2$); FT-IR: $\tilde{\nu} = 3280$ (NH), 1736 (C=O ester), 1637 (amide I), 1543 cm^{-1} (amide II); elemental analysis calcd (%) for $C_{13}H_{22}N_2O_3$: C 61.39, H 8.72, N 11.01; found: C 60.79, H 8.67, N 10.77.

Polymer 2 from isocyanide 7: This polymer was prepared from **7** (85 mg, 0.22 mmol) by using the same procedure as described for polymer **1**. After precipitation of the product in methanol/water (3:1, v/v) the solution was centrifuged at 3400 rpm for 5 min. The pellet was redispersed in methanol/water (3:1, v/v) and this procedure was repeated twice. The

product was finally redispersed in methanol, collected by centrifugation and dried in vacuo. Yield 60 mg of **2** (71 %). $[\alpha]_D$ = see Table 3; ^{1}H NMR ($CDCl_3$, 200 MHz): 8.9–7.7 (brs, 2H; NH), 4.39 (brs, 3H; 3CH), 3.61, 3.51 (s, 3H; OCH_3), 2.8–2.2 (brs, 6H; $3CH_2$), 2.0–1.4 (brs, 6H; CH_2CH-$(CH_3)_2$, CH_3 *ala*-ala-leu), 1.26 (br, 3H; CH_3 ala-*ala*-leu), 0.91 ppm (brs, 6H; $CH_2CH(CH_3)_2$); FT-IR: $\tilde{\nu}$ = 3279 (NH), 1737 (C=O ester), 1639 (amide I), 1543 cm^{-1} (amide II); elemental analysis calcd (%) for $C_{17}H_{29}N_3O_4 \cdot 0.5\,H_2O$: C 58.60, H 8.68, N 12.06; found: C 58.53, H 8.56, N 11.93.

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