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Mirror image supramolecular helical tapes formed by the enantiomeric-depsipeptide derivatives of the amyloidogenic peptide amylin(20–29)

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Abstract

Factors that determine the chirality of supramolecular helical tapes formed by a backbone-modified amylin(20–29) depsipeptide and inverso-depsipeptide, were studied by Fourier transform infrared spectroscopy, circular dichroism and transmission electron microscopy. Although β-sheet propensity was absent in both peptides, it was found that the L-depsipeptide formed left-handed and the enantiomeric D-depsipeptide right-handed helical tapes. Moreover, the backbone-modified depsipeptides, showed a certain degree of cross-recognition between both enantiomers, which might have implications in designing amyloid formation inhibitors.

Keywords: Amyloid; Depsipeptide; Peptide nanotubes; Self-assembly; Soft matter

Amyloid formation is the most prominent example of protein self-assembly and represents a self-propagating process responsible for the formation of supramolecular assemblies like amyloid fibrils, amyloidogenic plaques, and proteinaceous aggregates.[1] Amylin consisting of 37 amino acid residues is a highly amyloidogenic peptide and is involved in type 2 diabetes (late onset diabetes) since fibrillar deposits of amylin are cytotoxic for β-cells and subsequently responsible for insulin insufficiency.[2] The sequence of amino acid residues 20–29: Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser **1** (Fig. 1) is recognized as the most amyloidogenic sequence within amylin[3] and rapidly forms amyloid fibrils.

Based on this sequence, we have designed depsipeptide **2** as a potential β-sheet breaker peptide, since the essential amide bonds involved in β-sheet formation have been replaced by ester moieties to disrupt the hydrogen bonding network of the antiparallel β-sheet.[4] However, it turned out that depsipeptide **2** was substantially less potent as a β-sheet breaker than expected since this peptide gave rise to self-assembly leading to helical tapes and peptide nanotubes. Structural investigations by FTIR (Table 1) and CD spectroscopy (Fig. 2) showed that the characteristic properties of a β-sheet secondary structure were absent in the case of depsipeptide **2**. Based on these data it was concluded that the increased hydrophobicity and intrinsic chirality of the depsipeptide were responsible for the self-assembly into helical tapes rather than a hydrogen bond driven process.[4]

In the recent literature, several reports describe the transformation of the molecular chirality of a peptide into the handedness or twist of a supramolecular construct.[5] Peptides, which have a propensity to β-sheet formation, self-assemble into diverse types of twisted morphologies

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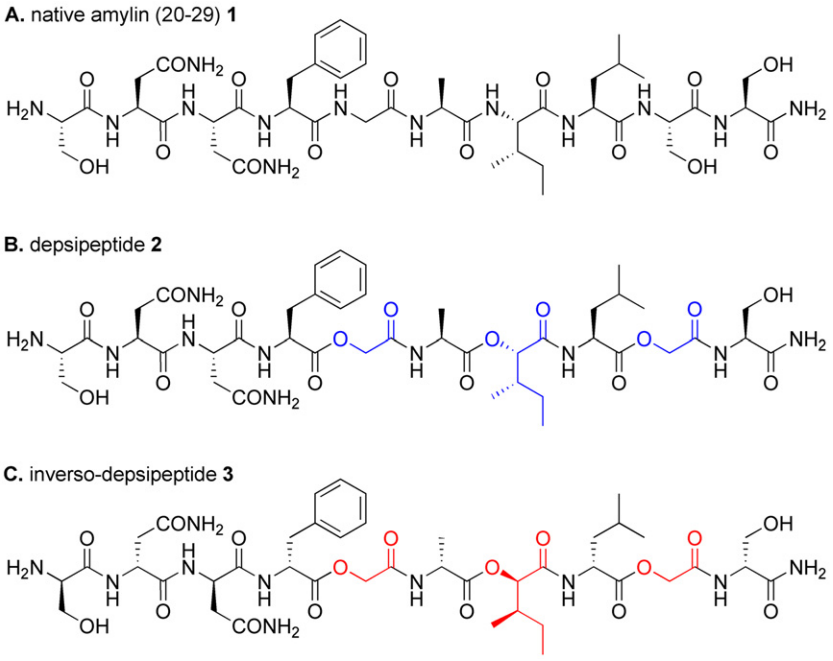


Fig. 1. Structures of native amylin(20–29) (**1**) and the corresponding depsipeptide (**2**) and inverso-depsipeptide (**3**).

Table 1
Physicochemical properties of the amylin(20–29) derivatives

Compound	R_t (min)	ESMS $[M+H]^+$ found (calcd)	FTIR[a] (cm^{-1})	Morphology[b]
1[4,13]	17.15	1008.65 (1008.50)	1631 (s)	Typical amyloid fibrils
L-Depsipeptide **2**	17.57	981.45 (981.45)	1663 (s)	Left-handed helical tapes
			1640 (m)	Helical tapes progressing into tubes
			1740 (w)	
D-Depsipeptide **3**	17.60	981.45 (981.45)	1663 (s)	Right-handed helical tapes
		$[M+Na]^+$ 1002.75	1640 (m)	Closed tubes
			1739 (w)	
2 + **3** (1:1 w/w)	—	—	—	Flat ribbons

[a] Typical amide I absorption frequency (cm^{-1}), 1630: aggregated β-sheets; 1640: unordered structure; 1675: antiparallel β-sheet; 1740 carbonyl in ester bond, according to Ref. 14.
[b] As observed by TEM.

such as helical tapes, twisted ribbons, fibrils, and fibers.[6,7] The twist supposedly stems from the intrinsic chirality of the L-amino acid building blocks of the peptide. Theoretical studies on the origin of β-sheet twisting demonstrate the right-handed twist of a β-strand,[8] which gives rise to a left-handed twist around the long axis of the tape.[6]

To obtain more insight into the self-assembly of depsipeptide **2**, and especially with respect to the handedness of a supramolecular helical tapes, the enantiomer of **2**, inverso-depsipeptide **3**, was synthesized. The synthesis of **3** started with the conversion of (2*R*,3*R*)-2-amino-3-methylpentanoic acid (D-isoleucine, **4**) into its corresponding α-hydroxy derivative (H-Ilec-OH) in the presence of $NaNO_2/H_2SO_4$ according to the procedure described by Shin et al.[9] followed by the esterification with allylbromide/K_2CO_3 to give α-hydroxy ester **5**[10] in 54% overall yield (Scheme 1). Then, Fmoc-D-Ala-OH was coupled with DCC/DMAP as coupling reagents and the protected depsidipeptide was obtained in 70% yield. Finally, the allyl group was removed by treatment with $Pd(PPh_3)_4$/phenylsilane to give Fmoc-D-Ala-D-Ilec-OH (**6**)[11] in 95% yield. Building block **6** was used in the solid phase synthesis of inverso-depsipeptide **3**[12] analogously as previously described for its depsipeptide **2**.[4]

Native amylin(20–29) **1** was used as the reference peptide. A solution of **1** (10 mg/mL in 0.1% TFA/H_2O, pH 1) rapidly formed an opaque gel since gelation is an indication of peptide fibrillization at sufficiently high concentration. The formation of amyloid fibrils was verified by transmission electron microscopy (TEM),[4,13] FTIR (Table 1),[14] and CD spectroscopy (Fig. 2; Note: a peptide solution of 1 mg/mL in 0.1% TFA/H_2O, pH 1 was used for the CD measurements otherwise a turbid CD sample was obtained. Moreover, as a control, TEM- and FTIR studies verified the presence of amyloid fibrils at this peptide concentration (data not shown)). Depsipeptide **2** was dissolved in 0.1% TFA/H_2O (10 mg/mL, pH 1) and rapidly (within 10 min) gelled the solution upon standing at 4 °C. TEM studies

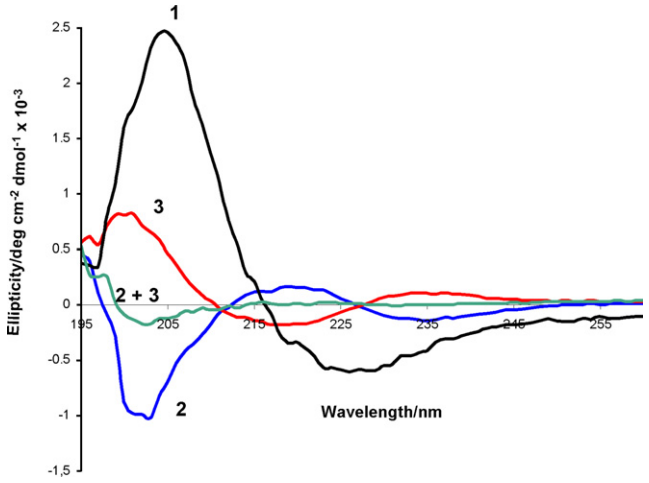
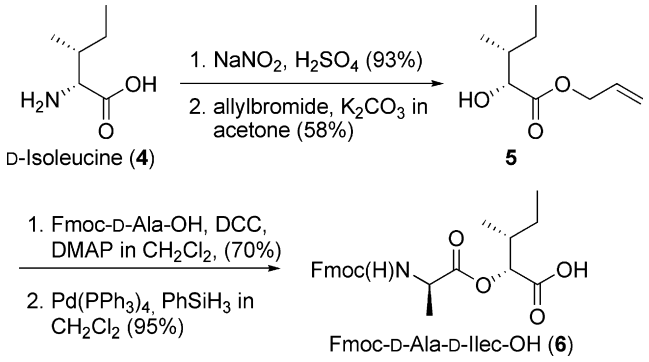


Fig. 2. CD spectra of native amylin(20–29) (**1**), depsipeptide (**2**), inverso-depsipeptide (**3**), and a racemic mixture (1:1 wt/wt) of depsipeptide (**2**)/inverso-depsipeptide (**3**). The peptides were dissolved in 0.1% TFA/H_2O (1 mg/mL, pH 1) and aged for one week (at 4 °C) prior to analysis (see also Supplementary data: at 1 mg/mL, the peptide solution is not depleted by precipitation or aggregation of peptide **2** for at least two weeks aging at 4 °C as judged by HPLC analysis).

on the supramolecular morphology of depsipeptide **2** showed a left-handed twist (Fig. 3A). Furthermore, helical tapes progressing into a peptide nanotube (Fig. 3B) and closed peptide nanotubes, up to 10 μm in length (Fig. 3C), were observed. Although FTIR and CD spectroscopy showed that depsipeptide **2** did not assume a β-sheet, the macroscopic left-handed twist of the helical tape corresponded to that of a regular β-sheet peptide.[6] The CD experiments were performed at a peptide concentration of 1 mg/mL to ensure a translucent solution. Furthermore, it was shown by HPLC (see Supplementary data) that the solution was not depleted by precipitation or aggregation of depsipeptide **2**, which was an indication that the CD signal corresponded to the conformation of depsipeptide **2** and not to a small remainder of the soluble form. Similar to depsipeptide **2**, the enantiomeric depsipeptide **3** also rapidly turned the solution into a gel (10 mg/mL in 0.1% TFA/H_2O, pH 1). Likewise, inverso-depsipeptide **3** did not show characteristic β-sheet features



Scheme 1. Synthesis of Fmoc-D-Ala-D-Ilec-OH (**6**).

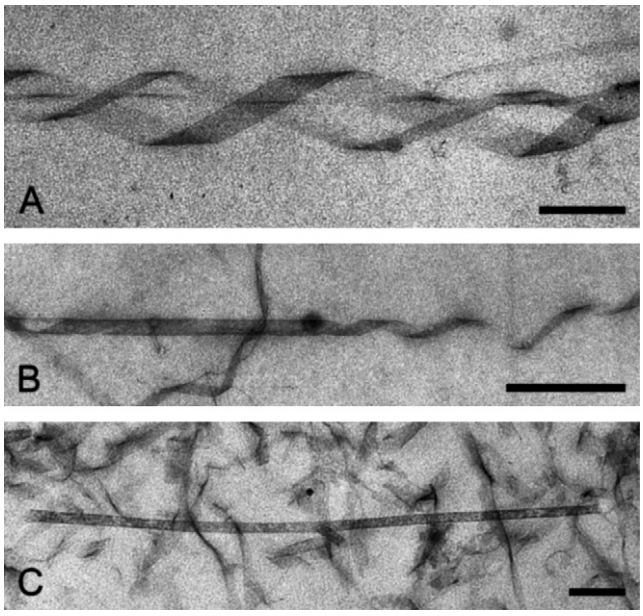


Fig. 3. TEM-images of depsipeptide (**2**): (A) Intertwined self-assembled helical ribbons; (B) Helical ribbons progressing into a peptide nanotube; (C) A self-assembled peptide nanotube. Scale bars represent 1 μm (conditions: 10 mg peptide/mL 0.1% TFA/H_2O, pH 1).

(Table 1 and Fig. 2) either and displayed the expected opposite Cotton effect of depsipeptide **2** of the CD curve. Inspection by TEM evidenced the presence of a right-handed helical tape (Fig. 4A), which progressed into closed peptide nanotubes (Fig. 4B).

A racemic mixture formed by an equimolar amount of **2** and **3** was still able to gel the solvent after dissolution in 0.1% TFA/H_2O (10 mg/mL, pH 1) and showed—as expected—a baseline-like absorption by CD spectroscopy

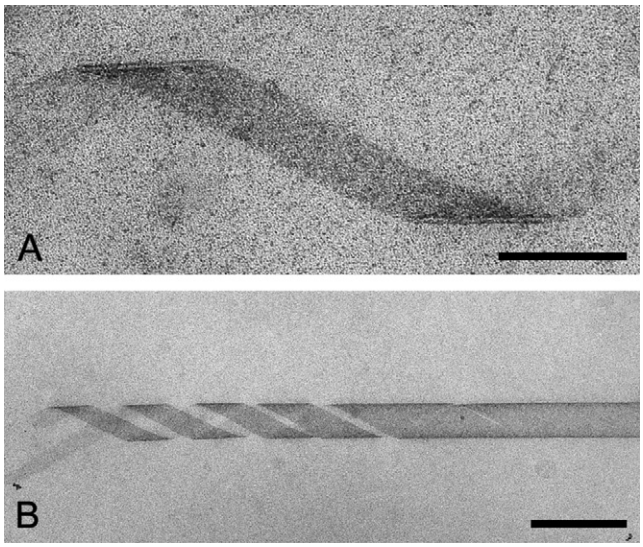


Fig. 4. TEM-images of inverso-depsipeptide (**3**): (A) Self-assembled helical ribbons; (B) Two helical ribbons progressing into a peptide nanotube. Scale bars represent 100 nm (A) and 500 nm; (B) (conditions: 10 mg peptide/mL 0.1% TFA/H_2O, pH 1).

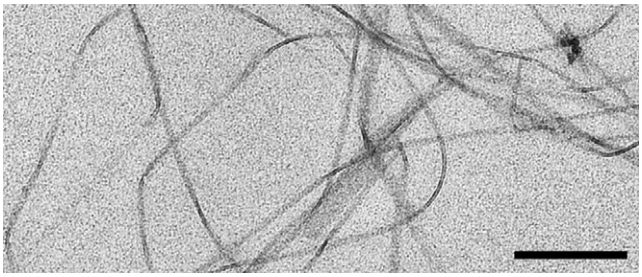


Fig. 5. TEM-images of a racemic mixture of depsipeptide (**2**) and inverso-depsipeptide (**3**). Scale bar represents 500 nm (conditions: 10 mg peptide/mL 0.1% TFA/H_2O, pH 1).

(Fig. 2). However, TEM images did not show the presence of helical entities, rather flat ribbons were observed (Fig. 5), which might be indicative of 'racemate' ribbons through cross-recognition of depsipeptide **2** and inverso-depsipeptide **3** during the process of self-assembly.

It is known from the literature that cross-recognition between L- and D-peptides—to inhibit β-sheet- or amyloid formation—is not often observed,[15] probably due to a less efficient hydrogen bonding network between both enantiomers.[5e] However, the racemic mixture of **2** and **3** did not lead to self-sorting into individual left-handed (L-depsipeptide, **2**) and right-handed (D-depsipeptide = inverso-depsipeptide, **3**) nanostructures as was observed by Stupp and co-workers in the case of their chiral dendron rodcoils.[5d] Apparently, our backbone-modified depsipeptides, showed a certain degree of cross-recognition between both enantiomers leading to the formation of supramolecular tapes, possibly because the intermolecular interactions are dominated by hydrophobic interactions of the amino acid side chains rather than intermolecular hydrogen bonding. We believe that insight into the driving forces of these recognition processes will be valuable for the design of supramolecular bionanomaterials[16] and for the development of potential amyloid formation inhibitors.

In conclusion, we described the synthesis of the enantiomeric depsipeptide derived from the highly amyloidogenic peptide sequence amylin(20–29) and reported on the ability of these enantiomeric homochiral depsipeptides to direct the handedness of the resulting supramolecular tapes.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.12.021.

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10. Synthesis of (2*R*,3*R*)-allyl-2-hydroxy-3-methylpentanoate (**5**): The synthesis of (2*R*,3*R*)-2-hydroxy-3-methylpentanoic acid starting from D-isoleucine was performed according to the procedures of Shin et al.[9] Optical rotation and spectroscopic data were in agreement with the literature. Crude (2*R*,3*R*)-2-hydroxy-3-methylpentanoic acid (1.81 g, 13.5 mmol) was dissolved in acetone (50 mL) and K_2CO_3 (2.85 g, 20.3 mmol) followed by the addition of allylbromide (2.4 mL, 27 mmol) and the obtained reaction mixture was stirred for 24 h. After concentration in vacuo, the residue was redissolved in EtOAc (50 mL) and the solution was washed with 5% aqueous $NaHCO_3$ (2 × 25 mL) and brine (25 mL), dried (Na_2SO_4) and concentrated in vacuo to obtain (2*R*,3*R*)-allyl-2-hydroxy-3-methylpentanoate (**5**) as a colourless oil in 58% yield (1.34 g). R_f 0.76 (CH_2Cl_2/MeOH 98/2 v/v); $[\alpha]_D$ −5.8 (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t (*J* 7.4 Hz), 3H, δCH_3), 0.99 (d (*J* 6.9 Hz), 3H, $\gamma' CH_3$), 1.32–1.39 (double m, 2H, γCH_2), 1.83 (m, 1H, βCH), 2.77 (br s, 1H, OH), 4.11 (d (*J* 3.9 Hz), 1H, αCH), 4.69 (m, 2H, ∼O–CH_2), 5.26–5.39 (m, 3H, C=CH_2), 5.93 (m, 1H, C=C*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.7, 15.4, 23.7, 39.1, 66.0, 74.8, 119.0, 131.5, 174.7.
11. Synthesis of Fmoc-D-Ala-D-Ilec-OH (**6**): The synthesis was carried out as described for its enantiomer Fmoc-L-Ala-L-Ilec-OH.[4] Based on allyl ester **5** (930 mg, 5.4 mmol), compound **6** was obtained as a white solid in 67% overall yield (403 mg). R_f 0.40 (CH_2Cl_2/MeOH 95/5 v/v); $[\alpha]_D$ +11.5 (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (t (*J* 7.4 Hz), 3H, δCH_3 Ilec), 1.01 (d (*J* 6.9 Hz), 3H, $\gamma' CH_3$ Ilec), 1.28–1.55 (5H, γCH_2 Ilec) (2m)/βCH$_3$ Ala (d (*J* 6.9 Hz)), 2.04 (m, 1H, βCH Ilec), 4.23 (t (*J* 6.8 Hz), 1H, CH Fmoc), 4.39 (d (*J* 6.8 Hz), 2H, CH_2

Fmoc), 4.48 (m, 1H, αCH Ala), 5.05 (d (*J* 3.9 Hz), 1H, αCH Ilec), 5.34 (d (*J* 7.4 Hz), 1H, NH), 7.13–7.77 (br m, 8H, arom Fmoc); ^{13}C (75 MHz, $CDCl_3$): δ 11.6, 15.3, 18.4, 24.4, 36.5, 47.1, 49.5, 67.1, 76.6, 120.0, 125.1, 127.1, 127.7, 141.3, 143.7, 155.8, 172.8, 173.8.

12. Solid phase synthesis of inverso-depsipeptide **3** (the synthesis was performed analogously as described for **2**[4]). Tentagel resin functionalized with a Rink Amide linker was used to obtain the C-terminal peptide amide. Fmoc-protected amino acids were coupled with HBTU/HOBt/DIPEA as coupling reagents and the reaction was monitored with the Kaiser test.[17] Amino acid couplings to an alcohol moiety were performed with DIC in the presence of a catalytic amount of DMAP. Depsidipeptide Fmoc-D-Ala-D-Ilec-OH (**6**) was introduced by using HATU/HOAt/DIPEA to ensure a rapid and complete coupling. After completion of the synthesis, the peptide was deprotected and detached from the resin by treatment with TFA, purified by preparative HPLC and characterized by analytical HPLC and mass spectrometry. Purification was performed by dissolving 50 mg of the crude peptide in a minimum amount of buffer A and loaded onto an Adsorbosphere XL C8 column (90 Å pore size, 10 μm particle size, 2.2 × 25 cm). The peptide was eluted with a flow rate of 10 mL/min using a linear gradient of buffer B (100% in 40 min) from 100% buffer A (buffer A: 0.1% TFA in CH_3CN/H_2O 5/95 v/v, buffer B: 0.1% TFA in CH_3CN/H_2O 95/5 v/v). Compound **3** was obtained in 32% yield (16 mg). The purity was evaluated by analytical HPLC on an Adsorbosphere XL C8 column (90 Å pore size, 5 μm particle size, 0.46 × 25 cm) at a flow rate of 1.0 mL/min using a linear gradient of buffer B (100% in 20 min) from 100% buffer A. The peptide was characterized using electrospray mass spectrometry (ESMS), which was performed on a Shimadzu LCMS-QP8000 single quadruple bench top mass spectrometer operating in a positive ionization mode.
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