Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

β^{3R3}-Peptides: design and synthesis of novel peptidomimetics and their self-assembling properties at the air/water interface

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Supporting Information

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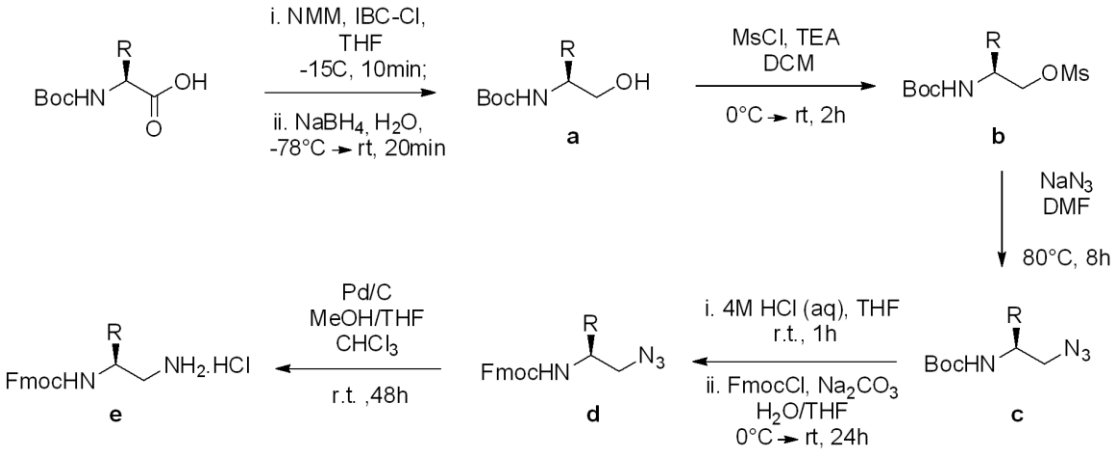
Contents

1. General experimental details

Commercial grade reagents and solvents were used as purchased without further purification, except as indicated below. All solvents were HPLC grade. Regarding DCM, amylene-stabilized HPLC grade was chosen. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian 400-MR spectrometer. The proton signal of residual, non-deuterated solvent (δ 7.26 ppm for $CHCl_3$, δ 2.50 ppm for DMSO, δ 3.31 ppm for MeOD) was used as an internal reference for ^{1}H NMR spectra. For 13C NMR spectra, the chemical shifts are reported relative to the carbon signal of the solvent (δ 77.16 ppm for $CDCl_3$, δ 39.52 ppm for DMSO, δ 49.00 ppm for MeOD). Coupling constants are reported in Hertz (Hz). The following abbreviations are used to indicate the multiplicities: s, singlet, d, doublet; t, triplet; m multiplet. Reversed-phase HPLC (RP-HPLC) was performed with 214 nm UV detection on a C18 (Agilent Eclipse, 4.6 × 100 mm) analytical column at 60 °C and a flow rate of 1 mL/min. Eluent: (A) water + 0.1% TFA; (B) acetonitrile + 0.1% TFA. The purity was determined by integration of the UV-signal with the software ChemStation for LC from Agilent Technologies. The solid-support resin was purchased from Rapp Polymers. Solid phase reactions were performed on an automated Activotec P11 Peptide Synthesizer.

2. Building blocks synthesis and characterization

2.1 Procedure for the synthesis of the diamine monomers



Scheme S1. Synthesis of the diamine monomers FmocNH-*v*Xaa-NH_2.HCl (R = $CH(CH_3)_2$, **e1**; $CH_2CH(CH_3)_2$, **e2**).

The following synthesis is based on protocols presented in literature.[3]

General procedure for the preparation of compounds **a1-2**.

N-methylmorpholine (NMM) (1.1 equiv) and isobutylchloroformate (1.1 equiv) were added to a cold (-15°C) solution of Boc-L-aminoacid (1 equiv) in dry THF (0.8 mM) under Ar. After 10 min, the precipitated NMM hydrochloride salt was removed by filtration; washed with THF and cooled to -78°C. A solution of sodium borohydride ($NaBH_4$) (1.5 equiv) in water (2.6 M) was added at once, resulting in strong hydrogen gas formation. The reaction mixture was stirred 20 min at room temperature and the solvent was evaporated at 40°C. The solid was dissolved in DCM and 5% aqueous citric acid. The organic layer was washed with brine, saturated $NaHCO_3$ solution and again brine, dried over Mg_2SO_4, filtered and evaporated to give a solid. The final product was isolated as a colorless solid after drying in high vacuum.

Boc-Protected-(*S*)-Valine Alcohol (**a1**) (R= $CH(CH_3)_2$)

Compound **a1** was prepared from Boc-L-Valine (30g, 138 mmol) and isolated as a white solid (Yield: 24.42g, 120 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.77 (d, *J* = 8.4 Hz, 1H), 3.70 – 3.54 (m, 2H), 3.49 – 3.28 (m, 1H), 2.85 (s, 1H), 1.89 – 1.70 (m, 1H), 1.42 (s, 9H), 0.94 – 0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.95, 79.60, 64.15, 58.13, 29.39, 28.49, 19.62, 18.61; *m/z* (ESI-HRMS) found $[M + Na]^+$, 226.1429 $C_{10}H_{21}NNaO_3$ requires $[M + Na]^+$, 226.1414.

Boc-Protected-(*S*)-Leucine Alcohol (**a2**) (R= $CH_2CH(CH_3)_2$)

Compound **a2** was prepared from Boc-L-Leucine hydrate (30g, 120 mmol) and isolated as a white solid (Yield: 22.23g, 102 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.70 (d, *J* = 6.8 Hz, 1H), 3.74 – 3.57 (m, 2H), 3.53 – 3.40 (m, 1H), 2.98 (b s, 1H), 1.70 – 1.56 (m, 1H), 1.42 (s, 9H), 1.29 (dd, *J* = 8.2, 6.3 Hz, 2H), 0.91 – 0.88 (m, 6H); ^{13}C NMR (100 MHz, $cdcl_3$): δ 156.65, 79.63, 66.43, 51.02, 40.64, 28.49, 24.90, 23.15, 22.32; *m/z* (ESI-HRMS) found $[M + Na]^+$, 240.1589 $C_{11}H_{23}NNaO_3$ requires $[M + Na]^+$, 240.1570.

General procedure for the preparation of compounds **b1-2.**

Under Ar, to a solution of compound 1 (1 equiv) in dry DCM cooled to 0°C, triethylamine (1 equiv) and methanesulfonyl chloride (MsCl) (1 equiv) were added dropwise. The reaction mixture was stirred at 0°C for 1h and then 1h at room temperature. Ice-water was added and the organic layer was washed with water, 5% citric acid, brine, $NaHCO_3$ and again brine, dried over Mg_2SO_4, filtered and evaporated. The final product was isolated after drying in high vacuum.

Boc-Protected-(*S*)-Valine methanesulfonate (**b1**)

Compound **b1** was prepared from compound **a1** (24.42g, 120 mmol) and isolated as a white crystalline solid (Yield: 33.4g, 119 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.62 (d, *J* = 8.2 Hz), 4.26 (d, *J* = 4.2 Hz), 3.62 (b s, *J* = 6.5 Hz), 3.03 (s), 1.90 – 1.81 (m), 1.45 (s), 0.98 (dd, *J* = 10.2, 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.76, 69.85, 55.05, 37.55, 29.23, 28.49, 19.54, 18.60; *m/z* (ESI-HRMS) found $[M + Na]^+$, 304.1192 $C_{11}H_{23}NNaO_5S$ requires $[M + Na]^+$, 304.1189.

Boc-Protected-(*S*)-Leucine methanesulfonate (**b2)**

Compound **b2** was prepared from compound **a2** (22.23g, 102 mmol) and isolated as a white crystalline solid (Yield: 28.6g, 97 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.54 (d), 4.26 (dd), 4.14 (dd, *J* = 10.0, 4.1 Hz), 3.92 (b s), 3.02 (s), 1.72 – 1.62 (m), 1.44 (s), 0.93 (dd, *J* = 6.6, 3.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.41, 71.74, 48.07, 40.28, 37.44, 28.48, 24.77, 23.05, 22.16; *m/z* (ESI-HRMS) found $[M + Na]^+$, 318.1339 $C_{12}H_{25}NNaO_5S$ requires $[M + Na]^+$, 318.1346.

General procedure for the preparation of compounds **c1-2.**

To a solution of compound **b** (1 equiv) in DMF sodium azide (NaN_3) (3 equiv) was added and the reaction mixture was stirred at 80°C for 8h. The solvent was removed under reduced pressure and the residue was re-dissolved in hexane and washed with water, 5% citric acid and brine, dried over Mg_2SO_4, filtered and evaporated to give colorless oil.

Boc-Protected-(*S*)-Valine Azide (**c1**)

Compound **c1** was prepared from compound **b1** (33.4g, 119 mmol) and isolated as a white crystalline solid (Yield: 19.64g, 86 mmol, 72.3%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.55 (d, *J* = 7.3 Hz), 3.59 – 3.45 (m), 3.41 (d, *J* = 4.5 Hz), 1.86 – 1.73 (m), 1.45 (s), 0.93 (t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.69, 79.71, 55.68, 53.20, 29.94, 28.50, 19.63, 18.53; *m/z* (ESI-HRMS) found $[M + Na]^+$, 251.1496 $C_{10}H_{20}N_4NaO_2$ requires $[M + Na]^+$, 251.1478 .

Boc-Protected-(*S*)-Leucine Azide (**c2**)

Compound **c2** was prepared from compound **b2** (28.6g, 97 mmol) and isolated as a white crystalline solid (Yield: 19.71g, 81 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.46 (s, 1H), 3.80 (s, 1H), 3.44 (dd, *J* = 8.5 Hz, 1H), 3.31 (dd, *J* = 12.1, 3.9 Hz, 1H), 1.64 (dt, *J* = 18.3, 5.6 Hz, 1H), 1.45 (s, 9H), 1.38 – 1.25 (m, 2H), 0.93 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.38, 79.75, 55.33, 48.67, 41.46, 28.50, 24.89, 23.08, 22.28; *m/z* (ESI-HRMS) found $[M + Na]^+$, 265.1647 $C_{11}H_{22}N_4NaO_2$ requires $[M + Na]^+$, 265.1635.

General Procedure for the preparation of compounds **d1-2.**

A mixture of THF: HCl(aq) (12M) = 10:4 (0.2 M) cooled to 0°C, was slowly added to compound **c**. The reaction mixture was stirred for 1h and then the solvent was evaporated *in vacuo* and then lyophilized to afford the hydrochloride salt as a crystalline residue. The residue in was dissolved in THF:water = 3:5 (0.1 M) and treated with Na_2CO_3 (5 eq). The resulting suspension was cooled to 0°C and fluorenyl chloroformate (FmocCl) (1 eq) was added. The reaction mixture was stirred 24h at room temperature and then THF was removed under reduced pressure. The resulting yellow solid was dissolved by adding Et_2O. The organic layer was washed with water, 5% citric acid and brine. The solvent was concentrated and precipitation in Hexane afforded compound **d**.

Fmoc-(*S*)-Valine Azide (**d1**)

Compound **d1** was prepared from compound **c1** (19.64g, 86 mmol) and isolated as a white solid (Yield: 29.5, 84 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 4.82 (d, *J* = 9.2 Hz, 1H), 4.45 (d, *J* = 6.8 Hz, 2H), 4.24 (t, *J* = 6.8 Hz, 1H), 3.64 – 3.52 (m, 1H), 3.44 (d, *J* = 4.8 Hz, 2H), 1.90 – 1.73 (m, 1H), 0.94 (dd, *J* = 10.5, 6.8 Hz, 6H), 0.81 (b, *J* = 4.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.21, 143.97, 143.95, 141.44, 127.80, 127.16, 127.14, 125.12, 120.09, 120.08, 66.75, 56.30, 53.12, 47.42, 29.83, 19.58, 18.64; *m/z* (ESI-HRMS) found $[M + H]^+$, 351.1851 $C_{20}H_{23}N_4O_2$ requires $[M + H]^+$, 351.1816.

Fmoc-(*S*)-Leucine Azide (**d2**)

Compound **d2** was prepared from compound **c2** (19.71g, 81mmol) and isolated as a white solid (Yield: 28.3g, 78 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (td, *J* = 7.5, 1.1 Hz, 2H), 4.76 (d, *J* = 8.7 Hz, 1H), 4.45 (d, *J* = 6.8 Hz, 2H), 4.23 (t, *J* = 6.8 Hz, 1H), 3.97 – 3.76 (m, 1H), 3.45 (dd, *J* = 12.3, 4.3 Hz, 1H), 3.33 (dd, *J* = 12.3, 4.4 Hz, 1H), 1.68 – 1.56 (m, 1H), 1.48 – 1.37 (m, 1H), 1.35 – 1.27 (m, 1H), 0.93 (dd, *J* = 6.5, 1.0 Hz, 6H), 0.79 (b, *J* = 20.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.88, 143.95, 143.92, 141.43, 127.79, 127.15, 127.13, 125.10, 120.09, 120.08, 66.67, 55.31, 49.15, 47.40, 41.31, 24.81, 23.09, 22.15; *m/z* (ESI-HRMS) found $[M + Na]^+$, 387.1834 $C_{21}H_{24}N_4O_2$ requires $[M + Na]^+$, 387.1791 .

General Procedure for the preparation of compounds **e1**-**2**.

Compound **d** (1 equiv) dissolved in MeOH:THF:$CHCl_3$ = 40:10:1 (0.2 M), was hydrogenated in the presence of 10% Pd/C (10% w/w) at room temperature till RP-HPLC indicates completion of the reaction (approximately 48 h). The solution was filtered over GHP membrane (0.2 µm). The filtrate was evaporated to afford a solid. The solid was dissolved in a minimal amount of hot EtOH and precipitated by dropwise addition to cold diethyl ether to yield compound **5** as white powder.

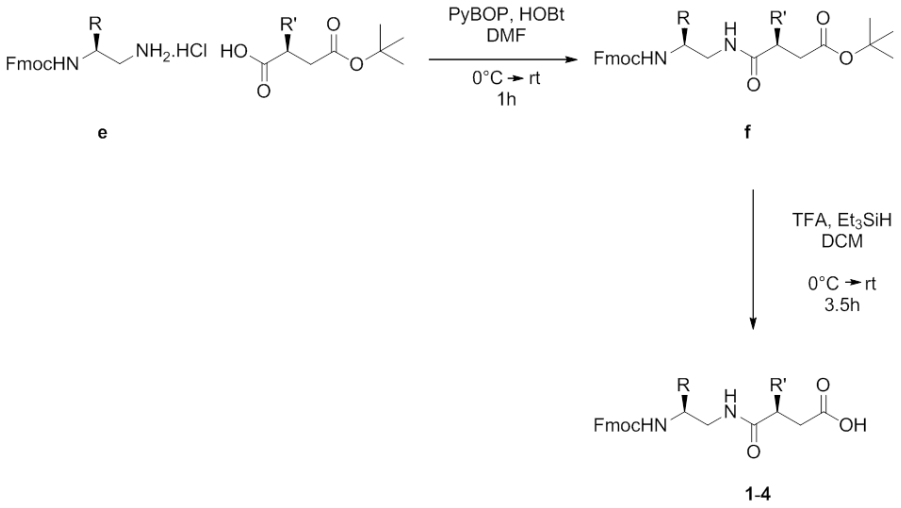
Fmoc-(*S*)-Valine Amine Hydrochloride Salt (**e1**) (FmocNH-*v*Val-NH_2.HCl)

Compound **e1** was prepared from compound **d1** (29.5g, 84 mmol) and isolated as a white solid (Yield: 26.4g, 73.1 mmol, 87%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, *J* = 7.5 Hz), 7.70 (dd, *J* = 7.5, 0.9 Hz), 7.41 (t, *J* = 7.4 Hz), 7.33 (t, *J* = 7.4 Hz), 4.61 (dd, *J* = 10.6, 6.4 Hz), 4.36 (dd, *J* = 10.6, 6.3 Hz), 4.25 (t, *J* = 6.4 Hz), 3.63 – 3.57 (m), 3.15 (dd, *J* = 13.0, 3.2 Hz), 2.85 (dd, *J* = 12.9, 10.8 Hz), 1.84 – 1.76 (m), 0.94 (dd, *J* = 13.7, 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 157.78, 143.95, 143.70, 141.24, 127.36, 126.68, 124.73, 124.61, 119.52, 119.50, 66.30, 54.91, 41.65, 30.70, 18.13, 17.07; *m/z* (ESI-HRMS) found $[M - Cl]^+$, 325.1920 $C_{20}H_{25}N_2O_2$ requires $[M - Cl]^+$, 325,1911; RP-HPLC analysis 5% to 95% MeCN in 10 min, T_R = 7.1 min.

Fmoc-(*S*)-Leucine Amine Hydrochloride Salt (**e2**) (FmocNH-*v*Leu-NH_2.HCl)

Compound **e2** was prepared from compound **d2** (28.3g, 78 mmol) and isolated as a white solid (Yield: 26.6g, 71 mmol, 91%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, *J* = 7.6 Hz, 2H), 7.68 (dd, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (tt, 2H), 4.63 (dd, *J* = 10.7, 6.6 Hz, 1H), 4.36 (dd, *J* = 10.7, 6.3 Hz, 1H), 4.25 (t, *J* = 6.4 Hz, 1H), 3.70 – 3.62 (m, 1H), 3.15 (dd, *J* = 13.0, 3.0 Hz, 1H), 2.84 (dd, *J* = 12.9, 11.0 Hz, 1H), 1.60 – 1.53 (m, 1H), 1.50 – 1.43 (m, 1H), 1.20 – 1.14 (m, 1H), 0.96 – 0.89 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 145.38, 142.70, 128.81, 128.11, 126.00, 120.94, 67.62, 45.70, 42.20, 25.66, 23.52, 21.91; *m/z* (ESI-HRMS) found $[M - Cl]^+$, 339.2071 $C_{21}H_{27}N_2O_2$ requires $[M + Na]^+$, 339,2067; RP-HPLC analysis 5% to 95% MeCN in 10 min, T_R = 7.4 min.

2.2 Procedure for the synthesis of the dimer building blocks (1-5)



Scheme S2. Synthesis of the dimer buiding blocks FmocHN-*v*Xaa-*s*Xaa-OH (**1**, R=$CH(CH_3)_2$, R'=CH_3; **2**, R=$CH_2CH(CH_3)_2$, R'=CH_3; **3**, R=$CH(CH_3)_2$, R'=$CH_2C_6H_5$; **4**, R=$CH_2CH(CH_3)_2$, R'=$CH_2C_6H_5$).

General procedure for the preparation of compounds f1-4.

To a solution of the diacid *tert*-butyl mono-ester[4] (1 equiv) in dryDMF (0.1 M) stirred at 0°C under Ar was added PyBOP (1 equiv), HOBt.H_2O and DIEA (3 equiv). The solution was stirred at 0 °C for 5min and then compound **e** was added. The solution was stirred at 0 °C for 15min and then allowed to warm to room temperature. After 1h water was added via a syringe till the reaction mixture got turbid and then put at 4°C overnight. The white precipitated powder was filtered, washed with water, dissolved with DCM and washed with brine (x3); dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield a vitreous solid which was precipitated from AcOEt/Hexane mixture. The precipitate powder was filtered and dry *in vacuo* to yield compound **f**.

FmocHN-*v*Val-*s*Ala-O*t*Bu (**f1**, R=$CH(CH_3)_2$, R'=CH_3)

Compound **f1** was prepared from **e1** (7.86g, 21.78 mmol) and (*R*)-4-(*tert*-Butoxy)-2-methyl-4-oxobutanoic acid[4] ((*S*)-(*s*Ala)-O*t*Bu) (4.1g, 21.78 mmol) and isolated as white powder (Yield: 9.37g, 18.94 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.32 (td, *J* = 7.4, 0.9 Hz, 2H), 6.18 (b s, 1H), 5.02 (d, *J* = 4.7 Hz, 1H), 4.46 (dd, *J* = 10.6, 7.1 Hz, 1H), 4.35 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.22 (t, *J* = 6.9 Hz, 1H), 3.61 – 3.48 (m, 1H), 3.47 – 3.23 (m, 2H), 2.67 – 2.54 (m, 2H), 2.26 (dd, *J* = 16.0, 5.1 Hz, 1H), 1.83 – 1.74 (m, 1H), 1.39 (s, 9H), 1.14 (d, *J* = 6.7 Hz, 3H), 0.95 (dd, *J* = 14.7, 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.12, 171.96, 157.17, 144.06, 144.04, 141.46, 127.82, 127.19, 125.26, 125.19, 120.09, 120.09, 80.92, 66.88, 57.25, 47.43, 41.93, 39.52, 37.22, 30.68, 28.17, 19.40, 18.47, 17.49; *m/z* (ESI-HRMS) found $[M + Na]^+$, 517.2700 $C_{29}H_{38}N_2NaO_5$ requires $[M + Na]^+$, 517.2673.

FmocHN-*v*Leu-*s*Ala-O*t*Bu (**f2**, R=$CH_2CH(CH_3)_2$, R'=CH_3)

Compound **f2** was prepared from **e2** (7.37g, 19.66 mmol) and (*R*)-4-(*tert*-Butoxy)-2-methyl-4-oxobutanoic acid[4] ((*S*)-(*s*Ala)-O*t*Bu) (3.7g, 19.66 mmol) and isolated as white powder (Yield: 9.10g, 17.89 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.57 (dd, *J* = 7.5, 0.7 Hz, 2H), 7.35 (t, *J* = 7.3 Hz, 2H), 7.27 (t, *J* = 7.4 Hz, 2H), 6.58 (t, *J* = 5.0 Hz, 1H), 5.40 (d, *J* = 8.6 Hz, 1H), 4.43 (dd, *J* = 10.5, 7.2 Hz, 1H), 4.31 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.17 (t, *J* = 6.8 Hz, 1H), 3.89 – 3.72 (m, 1H), 3.38 – 3.18 (m, 2H), 2.68 – 2.58 (m, 2H), 2.25 (dd, *J* = 15.4, 4.9 Hz, 1H), 1.69 – 1.58 (m, 1H), 1.37 (s, 9H), 1.28 – 1.22 (m, 1H), 1.13 (d, *J* = 6.5 Hz, 3H), 0.77 (d, *J* = 23.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.90, 171.74, 156.70, 143.89, 143.86, 141.23, 127.61, 126.96, 125.06, 125.00, 119.89, 80.56, 66.49, 49.84, 47.22, 44.18, 41.69, 39.24, 37.09, 27.99, 24.72, 23.00, 22.12, 17.51; *m/z* (ESI-HRMS) found $[M + H]^+$, 509.3032 $C_{30}H_{41}N_2O_5$ requires $[M + H]^+$, 509.3010.

FmocHN-*v*Val-*s*Phe-O*t*Bu (**f3**, R=$CH(CH_3)_2$, R'=$CH_2C_6H_5$)

Compound **f3** was prepared from **e1** (7.65g, 21.19 mmol) and (*R*)-4-(*tert*-Butoxy)-4-oxo-2-(1-phenylmethyl)butanoic acid[4] ((*S*)-(*s*Phe)-O*t*Bu) (5.6g, 21.19 mmol) and isolated as white powder (Yield: 11.25g, 19.70 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.27 (t, *J* = 7.4 Hz, 2H), 7.21 – 7.16 (m, 2H), 7.15 – 7.09 (m, 3H), 6.26 (b s, 1H), 5.22 (d, *J* = 8.5 Hz, 1H), 4.46 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.27 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.18 (t, *J* = 6.9 Hz, 1H), 3.41 – 3.11 (m, 3H), 2.91 (dd, *J* = 12.5, 8.6 Hz, 1H), 2.87 – 2.76 (m, 1H), 2.74 – 2.57 (m, 2H), 2.34 (dd, *J* = 16.8, 5.2 Hz, 1H), 1.58 – 1.48 (m, 1H), 1.36 (s, 9H), 0.84 (dd, *J* = 15.4, 6.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.46, 171.45, 156.60, 143.85, 143.79, 141.13, 138.93, 128.94, 128.84, 128.28, 127.49, 126.87, 126.34, 126.25, 125.03, 124.91, 119.77, 80.55, 66.32, 60.19, 56.88, 47.11, 44.86, 40.97, 38.11, 37.40, 29.61, 27.85, 19.18, 18.21; *m/z* (ESI-HRMS) found $[M + H]^+$, 571.3173 $C_{35}H_{43}N_2O_5$ requires $[M + H]^+$, 571.3166.

FmocHN-*v*Leu-*s*Phe-O*t*Bu (**f4**, R=$CH_2CH(CH_3)_2$, R'=$CH_2C_6H_5$)

Compound **f4** was prepared from **e2** (7.37g, 19.67 mmol) and (*R*)-4-(*tert*-Butoxy)-4-oxo-2-(1-phenylmethyl)butanoic acid[4] ((*S*)-(*s*Phe)-O*t*Bu) (5.2g, 19.67 mmol) and isolated as white powder (Yield: 11.04g, 18.88 mmol, 96%). ^{1}H NMR (400 MHz, CDC_3): δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 6.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.33 (tdd, *J* = 9.6, 6.9, 2.9 Hz, 2H), 7.23 – 7.15 (m, 3H), 7.15 – 7.11 (m, 2H), 5.72 (b s, 1H), 4.59 (d, *J* = 7.8 Hz, 1H), 4.51 (dd, *J* = 10.7, 6.6 Hz, 1H), 4.38 (dd, *J* = 10.5, 6.6 Hz, 1H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.64 – 3.43 (m, 1H), 3.36 – 3.22 (m, 1H), 3.16 – 3.05 (m, 1H), 2.91 – 2.82 (m, 1H), 2.80 – 2.63 (m, 3H), 2.37 (dd, *J* = 16.6, 4.2 Hz, 1H), 1.58 – 1.46 (m, 1H), 1.41 (d, *J* = 4.4 Hz, 9H), 1.22 – 1.11 (m, 1H), 1.04 (ddd, *J* = 13.8, 8.2, 5.6 Hz, 1H), 0.85 (dd, *J* = 9.8, 6.7 Hz, 6H); ^{13}C NMR (100 MHz, $CHCl_3$): δ 174.37, 171.78, 156.28, 144.19, 144.03, 141.51, 141.47, 139.27, 129.28, 129.15, 128.66, 128.47, 127.78, 127.17, 127.11, 126.63, 125.24, 125.14, 120.06, 120.04, 81.00, 66.26, 49.90, 47.54, 45.46, 43.50, 41.19, 38.53, 37.79, 28.21, 28.19, 24.78, 23.02, 22.17; *m/z* (ESI-HRMS) found $[M + H]^+$, 585.3325 $C_{36}H_{45}N_2O_5$ requires $[M + H]^+$, 585.3323.

General procedure for the preparation of compounds **1-4**.

To a solution of **f** (1 equiv) in 70:30 (v/v) DCM/Trifluoroacetic Acid (0.1 M) stirred at 0'C was added Triethylsilane (4 equiv). The solution was stirred at r.t till TLC revelead completion of the reaction (approximately 3.5h) and toluene was added; the contents were concentrated *in vacuo*, precipitated and deeply washed with cold Et_2O. The filtrate was evaporated *in vacuo* to afford the product.

FmocHN-*v*Val-*s*Ala-OH (**1**, R=$CH(CH_3)_2$, R'=CH_3)

Compound **1** was prepared from **f1** (9.37g, 18.94 mmol) and isolated as white powder (Yield: 7.39g, 16.85 mmol, 89%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.04 (b s, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.76 (t, *J* = 5.6 Hz, 1H), 7.71 (dd, *J* = 7.1, 5.1 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (tt, 2H), 6.99 (d, *J* = 9.4 Hz, 1H), 4.36 – 4.30 (m, 1H), 4.26 – 4.19 (m, 2H), 3.44 – 3.39 (m, 1H), 3.26 – 3.18 (m, 1H), 3.03 – 2.94 (m, 1H), 2.67 – 2.60 (m, 1H), 2.49 – 2.44 (m, 1H), 2.16 (dd, *J* = 16.3, 6.6 Hz, 1H), 1.75 – 1.64 (m, 1H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.80 (dd, 6H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 174.67, 173.15, 156.17, 143.99, 143.81, 140.71, 127.57, 127.01, 125.21, 120.07, 65.19, 55.54, 46.81, 40.44, 37.54, 35.86, 29.28, 19.48, 17.84, 17.80; *m/z* (ESI-HRMS) found $[M + H]^+$, 439.2258 $C_{25}H_{31}N_2O_5$ requires $[M + H]^+$, 439.2227; RP-HPLC analysis 5% to 95% MeCN in 10 min + 95% MeCN for 4 min, T_R = 8.5 min.

FmocHN-*v*Leu-*s*Ala-OH (**2**, R=$CH_2CH(CH_3)_2$, R'=CH_3)

Compound **2** was prepared from **f2** (9.10g, 17.89 mmol) and isolated as white powder (Yield: 7.37g, 16.28 mmol, 91%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.03 (b s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.84 (t, *J* = 5.8 Hz, 1H), 7.69 (dd, *J* = 7.3, 4.2 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (tdd, *J* = 7.4, 2.5, 0.9 Hz, 2H), 7.00 (d, *J* = 9.0 Hz, 1H), 4.36 – 4.18 (m, 3H), 3.65 – 3.51 (m, 1H), 3.18 – 3.03 (m, 1H), 2.98 – 2.86 (m, 1H), 2.69 – 2.57 (m, 1H), 2.49 – 2.43 (m, 1H), 2.16 (dd, *J* = 16.3, 6.5 Hz, 1H), 1.61 – 1.47 (m, 1H), 1.31 – 1.20 (m, 1H), 1.20 – 1.12 (m, 1H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.83 (dd, *J* = 15.5, 6.6 Hz, 6H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 174.66, 173.13, 155.78, 144.01, 143.79, 140.72, 140.71, 127.56, 127.00, 126.97, 125.15, 120.08, 120.07, 65.03, 48.69, 46.84, 43.01, 40.79, 37.52, 35.89, 24.21, 23.29, 21.76, 17.90; *m/z* (ESI-HRMS) found $[M + H]^+$, 453.2429 $C_{26}H_{32}N_2O_5$ requires $[M + H]^+$, 453.2384; RP-HPLC analysis 5% to 95% MeCN in 10 min + 95% MeCN for 4 min, T_R = 9.0 min.

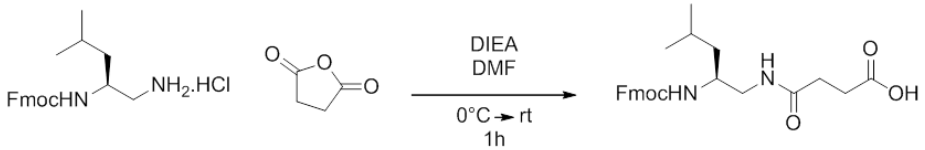
FmocHN-*v*Val-*s*Phe-OH (**3**, R=$CH(CH_3)_2$, R'=$CH_2C_6H_5$)

Compound **3** was prepared from **f3** (11.25g, 19.70 mmol) and isolated as white powder (Yield: 9.12g, 17.73 mmol, 90%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.07 (b s, 1H), 7.88 (d, *J* = 5.3 Hz, 1H), 7.84 (t, *J* = 5.7 Hz, 1H), 7.69 (t, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.4, 0.6 Hz, 1H), 7.26 – 7.22 (m, 2H), 7.19 – 7.14 (m, 3H), 6.92 (d, *J* = 9.4 Hz, 1H), 4.36 – 4.28 (m, 1H), 4.25 – 4.18 (m, 2H), 3.40 – 3.34 (m, 1H), 3.24 – 3.15 (m, 1H), 3.00 – 2.91 (m, 1H), 2.89 – 2.79 (m, 2H), 2.54 (dd, *J* = 12.5, 6.8 Hz, 1H), 2.46 (d, *J* = 8.5 Hz, 1H), 2.14 – 2.06 (m, 1H), 1.62 – 1.49 (m, 1H), 0.77 (dd, *J* = 9.5, 6.8 Hz, 3H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 173.32, 173.05, 156.13, 144.00, 143.78, 140.70, 139.27, 128.87, 128.13, 127.56, 127.00, 126.07, 125.19, 120.06, 65.20, 55.38, 46.80, 43.25, 40.43, 37.88, 35.43, 28.81, 19.53, 17.52; *m/z* (ESI-HRMS) found $[M + H]^+$, 515.2598 $C_{31}H_{34}N_2O_5$ requires $[M + H]^+$, 515.2540; RP-HPLC analysis 5% to 95% MeCN in 10 min + 95% MeCN for 4 min, T_R = 9.5 min.

FmocHN-*v*Leu-*s*Phe-OH (**4**, R=$CH_2CH(CH_3)_2$, R'=$CH_2C_6H_5$)

Compound **4** was prepared from **f4** (11.04g, 18.88 mmol) and isolated as white powder (Yield: 9.28g, 17.55 mmol, 93%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.05 (b s, 1H), 7.93 (t, *J* = 5.6 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.67 (t, *J* = 7.9 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.24 (t, 2H), 7.19 – 7.14 (m, 3H), 6.91 (d, *J* = 9.0 Hz, 1H), 4.36 – 4.18 (m, 3H), 3.60 – 3.50 (m, 1H), 3.13 – 3.03 (m, 1H), 2.95 – 2.81 (m, 3H), 2.54 (dd, *J* = 14.5, 8.9 Hz, 1H), 2.46 (d, *J* = 8.4 Hz, 1H), 2.09 (dd, *J* = 16.7, 4.6 Hz, 1H), 1.56 – 1.45 (m, 1H), 1.26 – 1.12 (m, 1H), 1.09 – 1.02 (m, 1H), 0.80 (dd, *J* = 21.8, 6.5 Hz, 6H);^{13}C NMR (100 MHz, d_6-DMSO): δ 173.32, 173.03, 155.74, 144.02, 143.76, 140.72, 139.34, 128.87, 128.16, 127.55, 126.99, 126.09, 125.12, 120.08, 65.04, 48.61, 46.83, 43.24, 43.08, 40.58, 37.88, 35.38, 24.14, 23.26, 21.65; *m/z* (ESI-HRMS) found $[M + Na]^+$, 529.2730 $C_{32}H_{37}N_2O_5$ requires $[M + Na]^+$ 529.2697; RP-HPLC analysis 5% to 95% MeCN in 10 min + 95% MeCN for 4 min, T_R = 9.9 min.

Preparation of FmocHN-*v*Val-*s*Ala-OH (**5**, R=$CH(CH_3)_2$, R'=H)

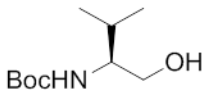


e2 **5**

Scheme S3. Synthesis of the dimer buiding blocks FmocHN-*v*Leu-*s*Gly-OH (**5**).

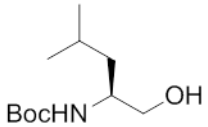
To a solution of **e2** (8g, 21.34 mmol, 1 equiv) in dryDMF (0.1 M) stirred at 0 °C under Ar was added Succinic anhydride (2.135g, 21.34 mmol, 1 eq.) and DIEA (11.18ml, 64 mmol, 3 equiv). The solution was stirred at 0 °C for 15min and then allowed to warm to room temperature. After 1h water was added via a syringe till the reaction mixture got turbid and then stored at 4°C overnight. The white precipitated powder was filtered, washed with water, dissolved with DCM and washed with 5% HCl(aq) (x3); dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield a vitreous solid which was precipitated from $CHCl_3$/Hexane mixture. The precipitate powder was filtered and dry *in vacuo* to yield compound **5** as white powder (Yield: 7.86g, 17.92 mmol, 84%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 11.97 (b s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.79 – 7.60 (m, 3H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (td, *J* = 7.4, 3.1 Hz, 2H), 7.02 (d, *J* = 9.1 Hz, 1H), 4.49 – 3.99 (m, 3H), 3.48 – 3.38 (m, 1H), 3.26 – 3.16 (m, 1H), 3.09 – 2.97 (m, 1H), 2.40 (t, *J* = 7.0 Hz, 2H), 2.30 (t, *J* = 6.8 Hz, 2H), 1.53 – 1.25 (m, 2H), 1.09 – 0.99 (m, 1H), 0.86 – 0.71 (m, 6H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 173.79, 171.08, 155.80, 144.01, 143.79, 140.72, 127.56, 127.00, 126.98, 125.17, 120.08, 120.07, 65.05, 48.76, 46.84, 43.10, 40.81, 30.09, 29.21, 24.22, 23.27, 21.74; *m/z* (ESI-HRMS) found $[M + H]^+$, 439.2242 $C_{25}H_{31}N_2O_5$ requires $[M + H]^+$, 439.2233; RP-HPLC analysis 5% to 95% MeCN in 10 min + 95% MeCN for 4 min, T_R = 8.7 min.

2.3 NMR Spectra of the building blocks synthesis intermediates and products



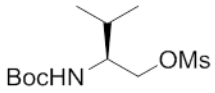
a1

^{1}H (400 MHz, $CDCl_3$)



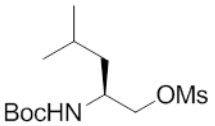
a2

^{1}H (400 MHz, $CDCl_3$)



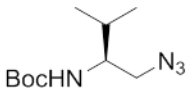
b1

^{1}H (400 MHz, $CDCl_3$)



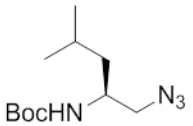
b2

^{1}H (400 MHz, $CDCl_3$)



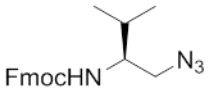
c1

^{1}H (400 MHz, $CDCl_3$)



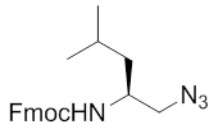
c2

^{1}H (400 MHz, $CDCl_3$)



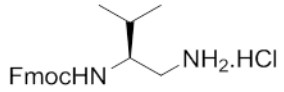
d1

^{1}H (400 MHz, $CDCl_3$)



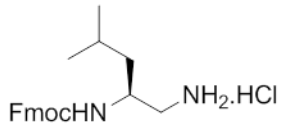
d2

^{1}H (400 MHz, $CDCl_3$)



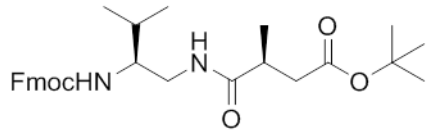
e1

^{1}H (400 MHz, MeOD)



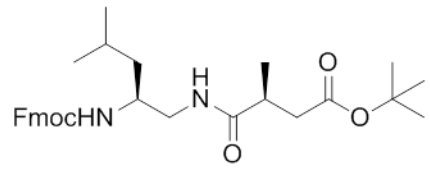
e2

^{1}H (400 MHz, MeOD)



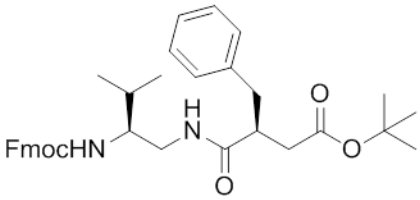
f1

^{1}H (400 MHz, $CDCl_3$)



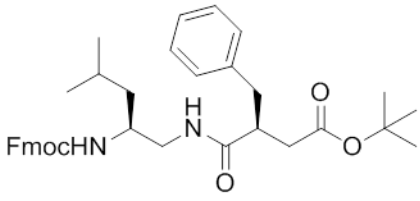
f2

^{1}H (400 MHz, $CDCl_3$)



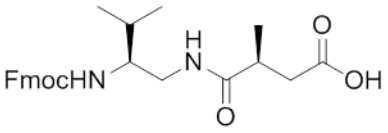
f3

^{1}H (400 MHz, $CDCl_3$)



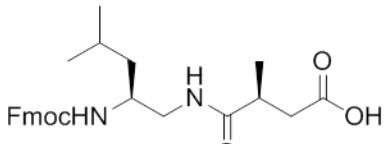
f4

^{1}H (400 MHz, $CDCl_3$)



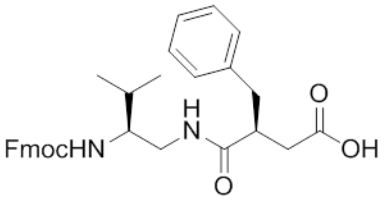
1

^{1}H (400 MHz, d_6-DMSO)



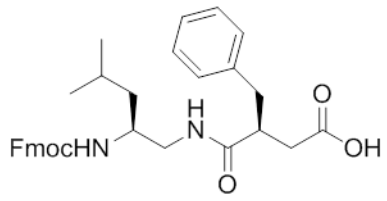
2

^{1}H (400 MHz, d_6-DMSO)



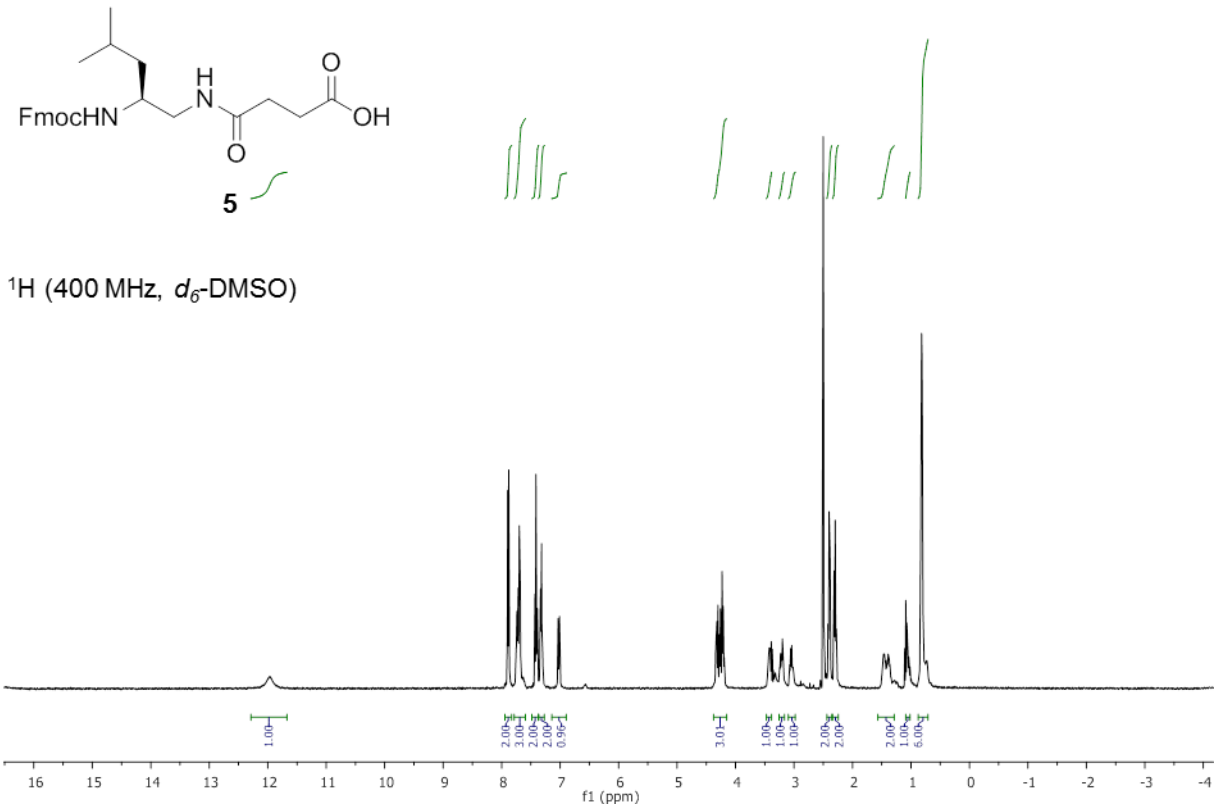
3

^{1}H (400 MHz, d_6-DMSO)



4

^{1}H (400 MHz, d_6-DMSO)



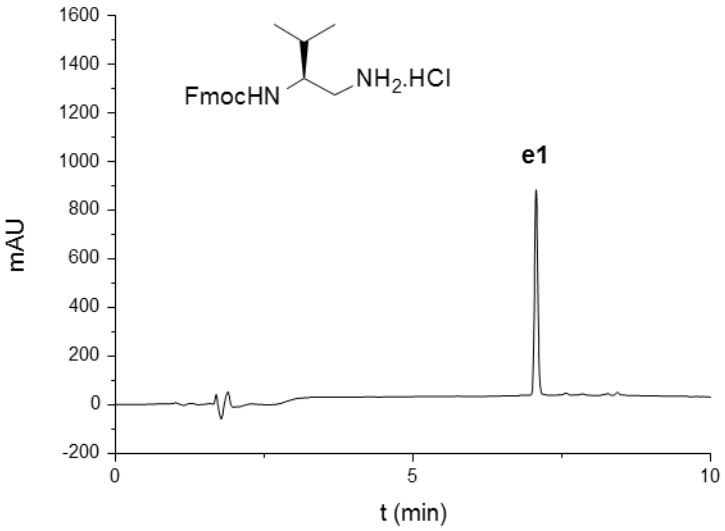
2.4 RP-HPLC spectra of the building blocks synthesis key intermediates and products

RP-HPLC analysis of **e1**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min + 95% B for 4 min.

T_R = 7.1 min.

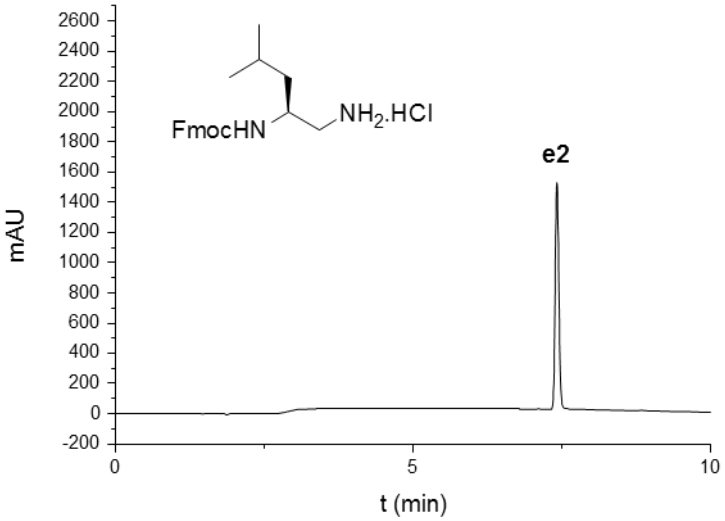


RP-HPLC analysis of **e2**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min.

T_R = 7.4 min.

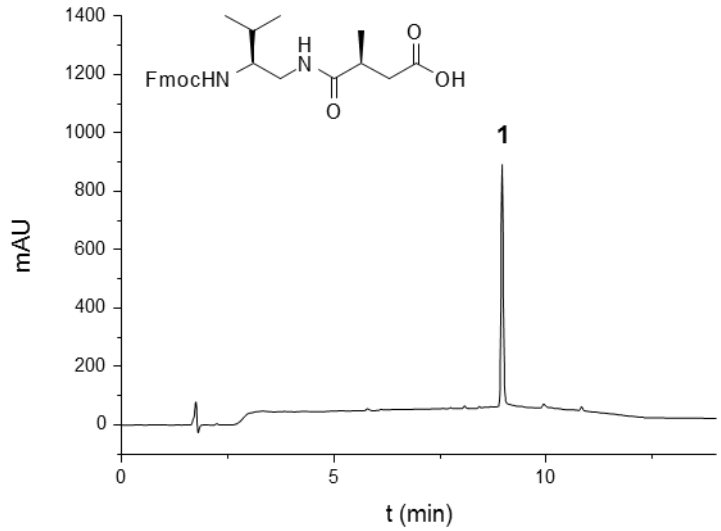


RP-HPLC analysis of **1**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min + 95% B for 4 min.

T_R = 8.5 min.

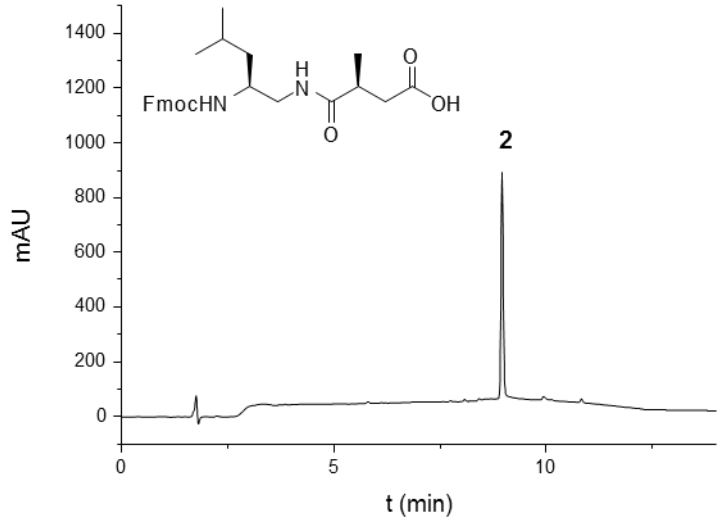


RP-HPLC analysis of **2**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min + 95% B for 4 min.

T_R = 9.0 min.

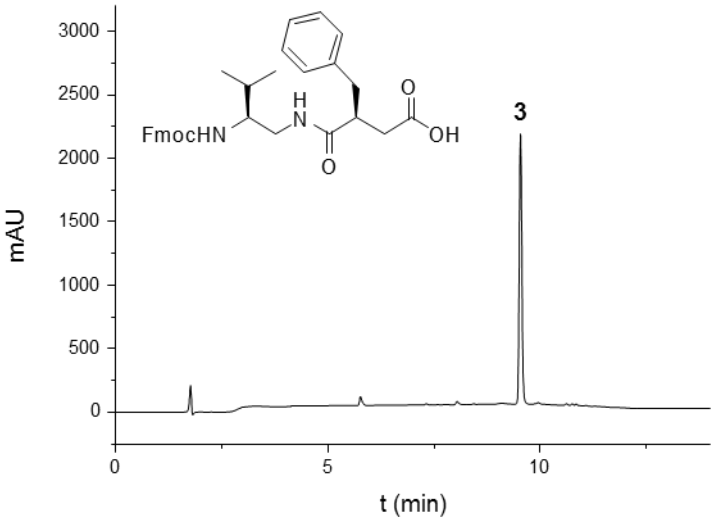


RP-HPLC analysis of **3**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min + 95% B for 4 min.

T_R = 9.5 min.

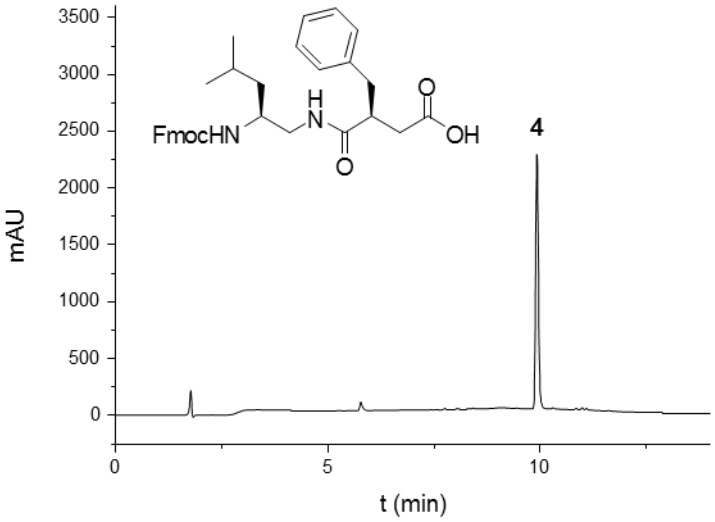


RP-HPLC analysis of **4**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min + 95% B for 4 min.

T_R = 9.9 min.

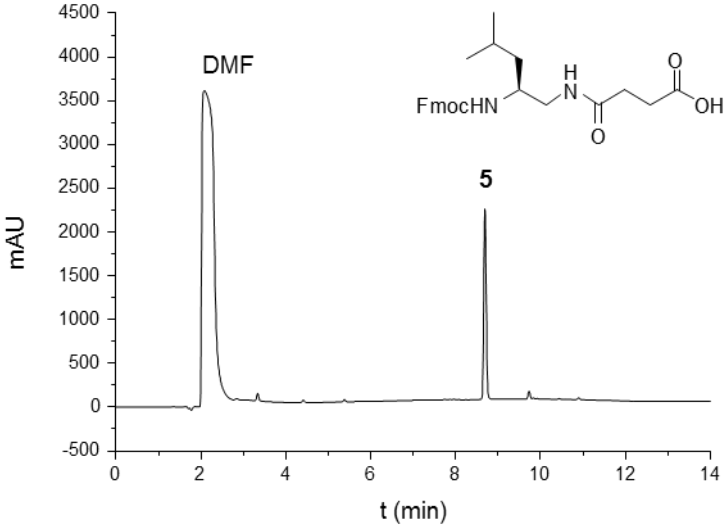


RP-HPLC analysis of **5**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 10 min + 95% B for 4 min.

T_R = 8.7 min.



3. Oligomers synthesis and characterization

3.1 Procedure for the synthesis of the oligomers (6-11)

Solid-Phase Synthesis.

All solid-phase reactions were performed on an automated standard peptide synthesizer in 0.025 mmol scale referring to the following general solid-phase protocols. As solid support, Tentagel S RAM resin (loading 0.23 mmol/g) (Fmoc-protected) was used for the synthesis of oligomers **6**, **7** and **8**. A commercially available trityl-tentagel−OH resin was modified with ethylenediamine (EDA) as linker for the synthesis of the oligomers **9**, **10** and **11**. Commercially available trityl-tentagel-OH resin was converted to trityl-tentagel chloride resin by addition of freshly distilled acetylchloride and heating the mixture at 60°C in toluene for 3 h. After cooling down, ethylenediamine was added and shaken for 48 h to obtain Trityl-tentagel-ethylenediamine resin (Trt-EDA resin). Loading of Trt-EDA resin was determined by standard loading test giving a loading of 0.2 mmol/g.[5] The resin was swollen twice for 15 min in DCM before starting the initial Fmocdeprotection.

General Procedure. Coupling/Fmoc-Deprotection Protocol.

The selected building block **1-5** (5 equiv) and O-(7-azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate (HATU) (46.5 mg, 4.9 equiv) were placed as powder in the amino acid vial and placed in the peptide synthesizer. The solids were dissolved in 2 mL of DMF with a gentle nitrogen stream. Then 10 equiv of 1 M DIEA solution in DMF was added. Preactivation was carried out for 3 min in the amino acid vial before the solution was transferred to the resin. Afterward the resin with the coupling solution was shaken carefully for 1 h, the reaction vessel was emptied and washed with DMF. Then the whole procedure was repeated once. Fmoc deprotection was performed using a solution of 25% piperidine in DMF. The deprotection was initially carried out for 5 min and checked by UV monitoring for the fluorenyl piperidine adduct at 301 nm. This step was repeated with increasing deprotection time (10, 20, 30, 40, 60 min) until the deprotection was complete. The deprotection time was considerably reduced by the use of 0.4M LiCl in 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), Pip and DMF mixture.

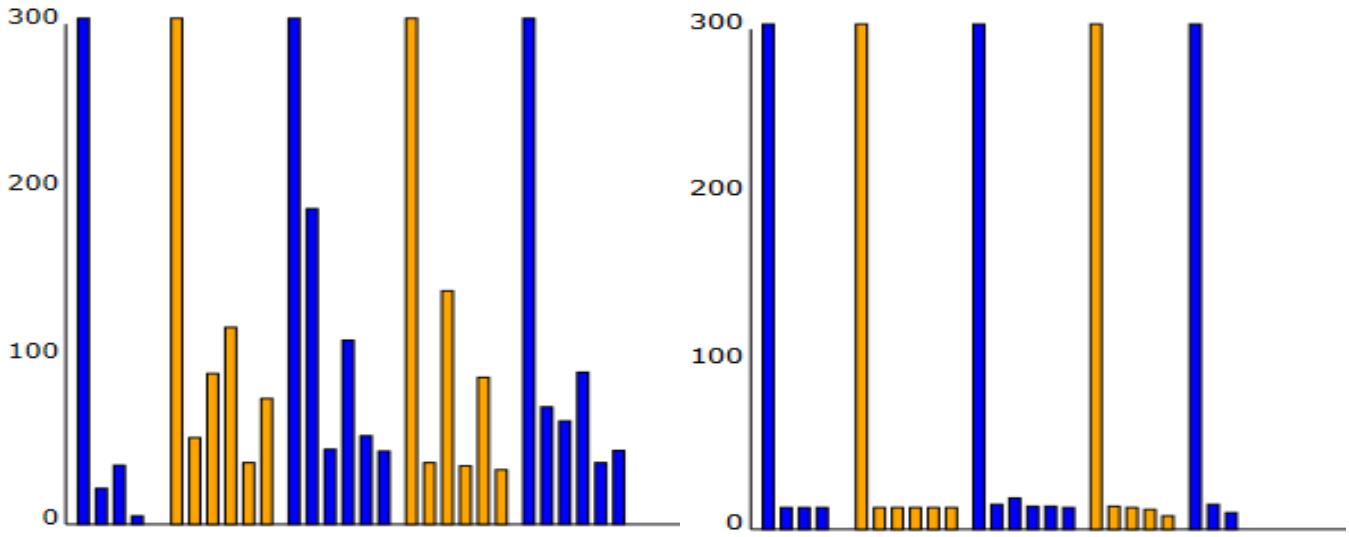


Fig S1. Fmoc cleavage UV-patterns of oligomer **6** with 25% piperidine in DMF (left) and 0.4M LiCl in DBU, Pip and DMF (right) as mixture for deprotection. The signals derived from the deprotection of the resin and the four coupling steps with dimer building blocks.

Final Cleavage from Solid Support.

The final cleavage was performed in two different ways based on the resin. For Tentagel S RAM resin, the cleavage cocktail (95% TFA, 2.5% triisopropylsilane, and 2.5% water 1 mL/50 mg resin) was added to the resin and allowing it to react for 70 min. In the case of Trt-EDA resin, the cleavage was performed by the addition of a solution of 30% TFA in DCM and shaking for 30 min.The cleavage solution was filtered and purged into ice-cold diethyl ether. The precipitate was isolated. The residue was dissolved in MeOH, diluted with water and lyophilized overnight, giving the final product and the corresponding yield.

H_2N-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sGly-NH_2 (6)

Compound **6** (17 mg, 0.019 mmol) was obtained with a yield of 76%.

ESI-HRMS calcd for $C_{47}H_{82}N_9O_8^+$ $[M+H]^+$ 900.6281, found 900,6296; RP-HPLC analysis 5% to 95% MeCN in 60 min, T_R = 21.5 min.

H_2N-vLeu-sPhe-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sGly-NH_2 (7)

Compound **7** (23 mg, 0.019 mmol) was obtained with a yield of 77%.

ESI-HRMS calcd for $C_{64}H_{106}N_{11}O_{10}^+$ $[M+H]^+$ 1188.8119, found 1188.8106; RP-HPLC analysis 5% to 95% MeCN in 60 min, T_R = 28.2 min.

H_2N-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sPhe-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sGly-NH_2 (8)

Compound **8** (39 mg, 0.021 mmol) was obtained with a yield of 83%.

ESI-HRMS calcd for $C_{101}H_{166}N_{17}O_{16}^+$ $[M+H]^+$ 1873.2693 found 1873.2819; RP-HPLC analysis 5% to 95% MeCN in 60 min, T_R = 51.0 min.

H_2N-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sGly-vGly-NH_2 (9)

Compound **9** (18 mg, 0.019 mmol) was obtained with a yield of 76%.

ESI-HRMS calcd for $C_{49}H_{87}N_{10}O_8^+$ $[M+H]^+$ 943.6703, found 943.6656; RP-HPLC analysis 5% to 90% MeCN in 30 min, T_R = 12.6 min.

H_2N-vVal-sAla-vLeu-sPhe-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sGly-vGly-NH_2 (10)

Compound **10** (23 mg, 0.016 mmol) was obtained with a yield of 64.3%.

ESI-HRMS calcd for $C_{76}H_{129}N_{14}O_{12}^+$ $[M+H]^+$ 1429.9909, found 1429.9905; RP-HPLC analysis 5% to 90% MeCN in 30 min, T_R = 18.4 min.

H_2N-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sPhe-vLeu-sAla-vVal-sPhe-vVal-sAla-vLeu-sGly-vGly-NH_2 (11)

Compound **11** (24 mg, 0.017 mmol) was obtained with a yield of 73.4%.

ESI-HRMS calcd for $C_{103}H_{171}N_{18}O_{16}^+$ $[M+H]^+$ 1916.3115, found 1916.3096; RP-HPLC analysis 5% to 95% MeCN in 60 min, T_R = 43.5 min.

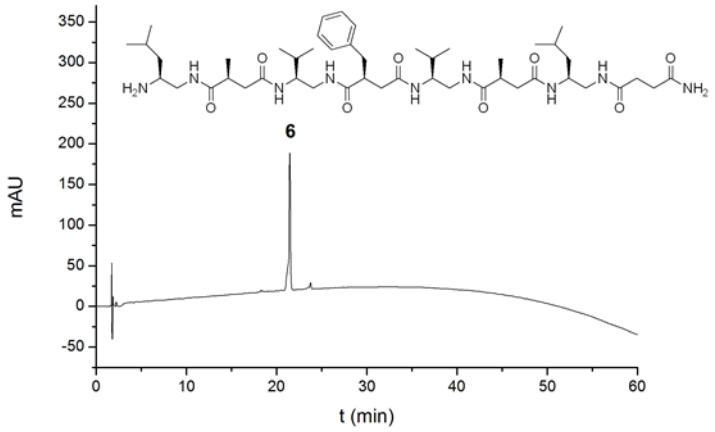
3.2 RP-HPLC spectra of the oligomers (6-11)

RP-HPLC analysis of **6**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 60 min.

T_R = 21.5 min.

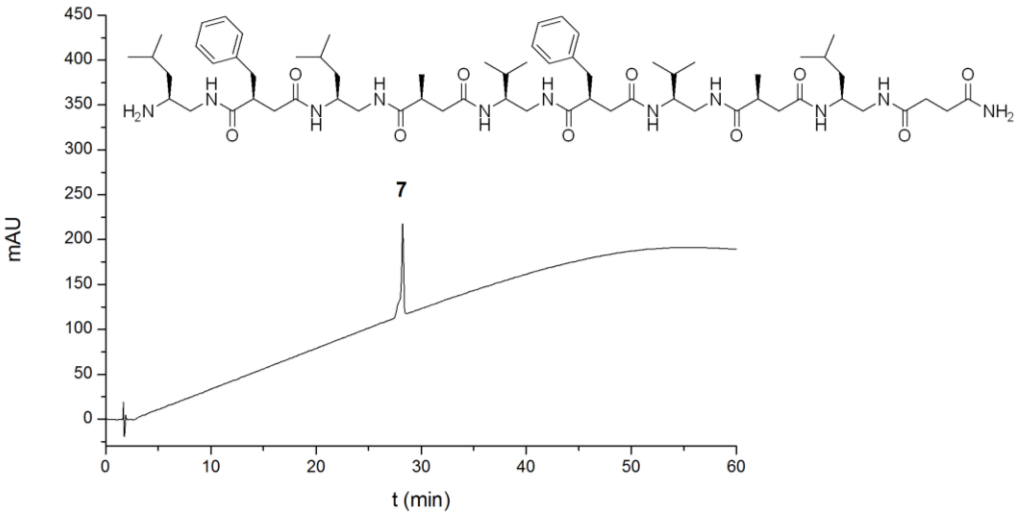


RP-HPLC analysis of **7**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 60 min.

T_R = 28.2 min.

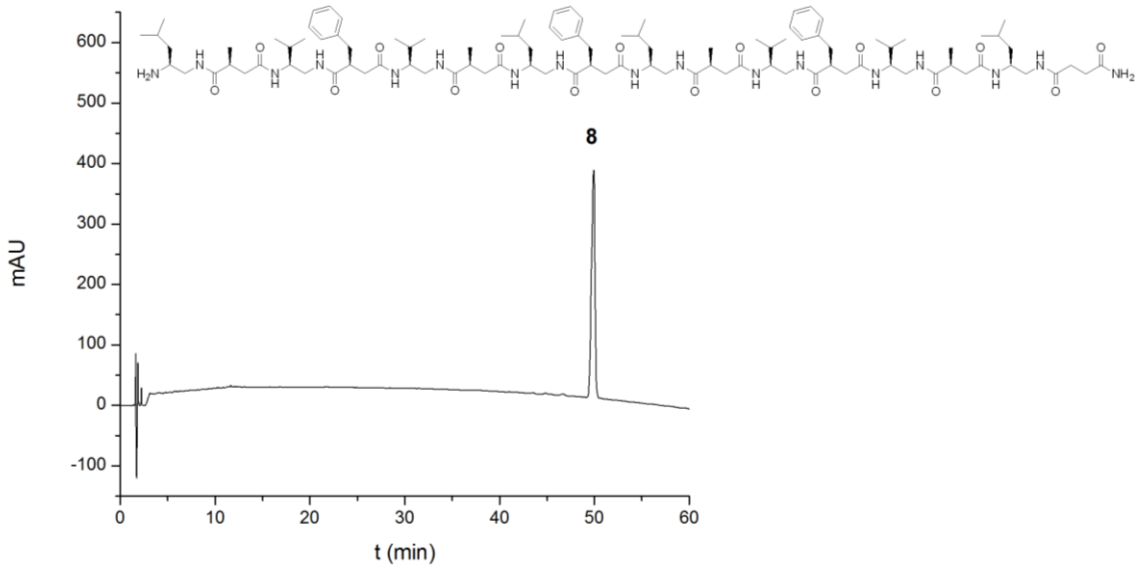


RP-HPLC analysis of **8**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 60 min.

T_R = 51.0 min.

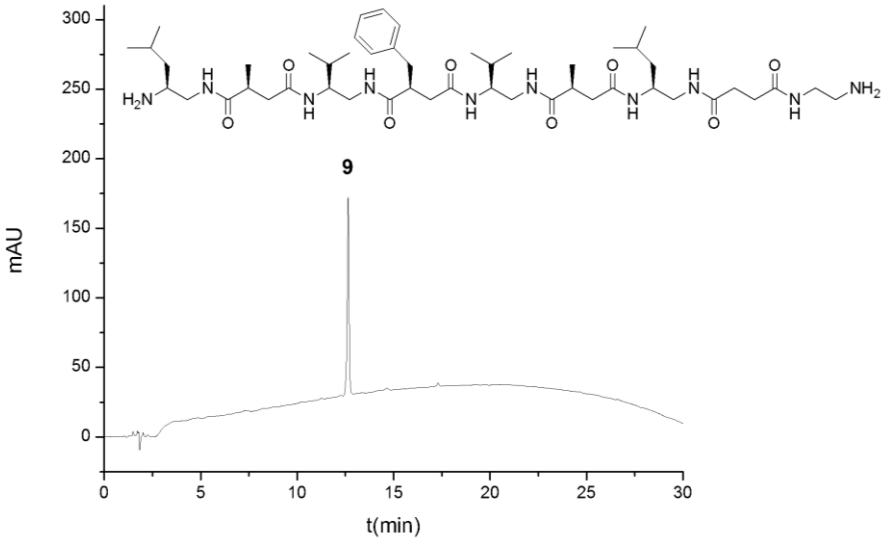


RP-HPLC analysis of **9**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 30 min.

T_R = 12.6 min.

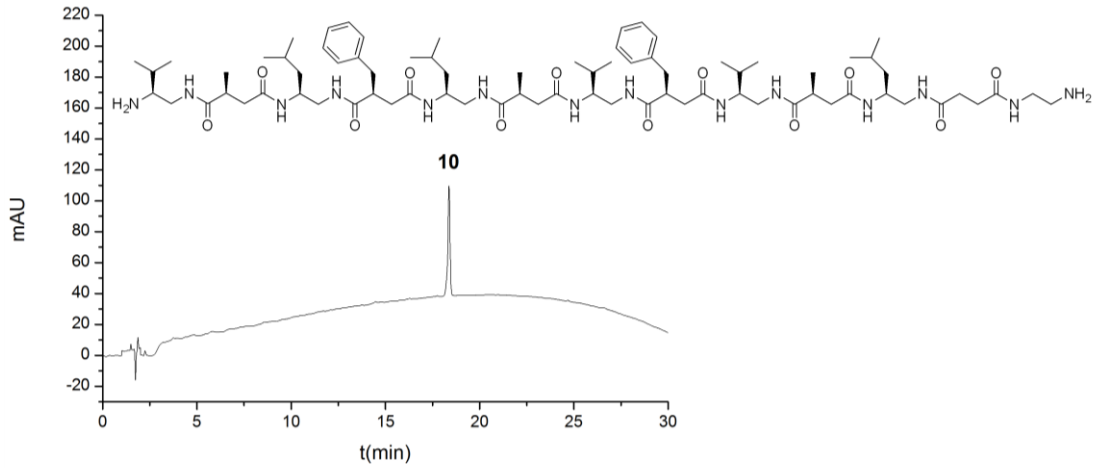


RP-HPLC analysis of **10**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

Gradient: Linear 5-95% B in 30 min.

T_R = 18.4 min.

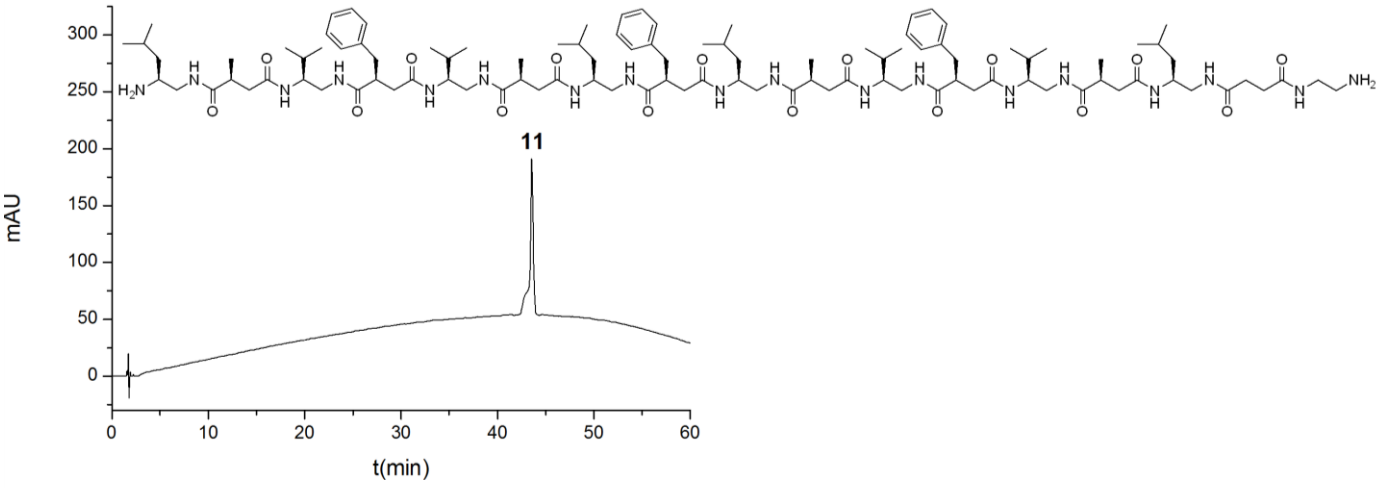


RP-HPLC analysis of **11**.

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA

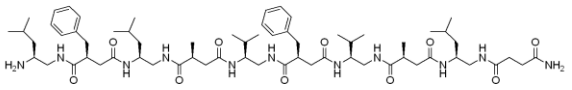
Gradient: Linear 5-95% B in 60 min.

T_R = 43.5 min.



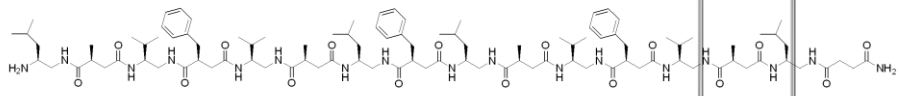
3.3 ^{1}H-NMR spectra of the oligomer 7, 8 and 11

The complexity and the chain length of the β^{3R3}-oligomers investigated here does not allow for characterization in bulk using NMR as conventional high-resolution methods. Indeed, the complexity and the chain length of the β^{3R3}-oligomers investigated here resulted in preliminary NMR results being uninformative. Only the incorporation of ^{13}C and/or ^{15}N labels in defined positions might allow for making assignments which is a basic prerequisite for the interpretation of 2D NOESY/ROESY spectra demanded for conformational analysis. The synthesis of different ^{13}C labelled β^{3R3}-peptides sequences is ongoing.



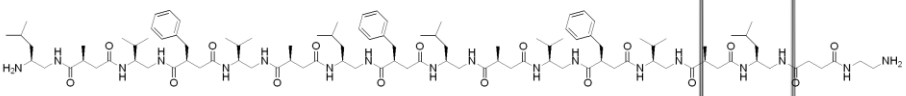
7

^{1}H (400 MHz, CD_3OD)



8

^{1}H (400 MHz, CD_3OD)



11

^{1}H (400 MHz, CD_3OD)

4. Oligomers Structure Analysis

4.1 Circular Dichroism Spectroscopy (CD)

Circular Dichroism (CD) spectra in methanol were recorded on a Jasco J-715 (Japan) spectrometer by using a quartz cuvette with an optical path length of 0.1 cm. The spectra were recorded in the wavelength interval from 190 to 260 nm, with 0.5 nm step resolution. 3 scans were accumulated for one spectrum. The peptide concentration was 100 µM. The spectra of the pure subphase were subtracted. The measured CD signal was transformed to mean residue molar ellipticity [θ] and divided by the number of peptide bonds to obtain $[\theta]_{RES}$.

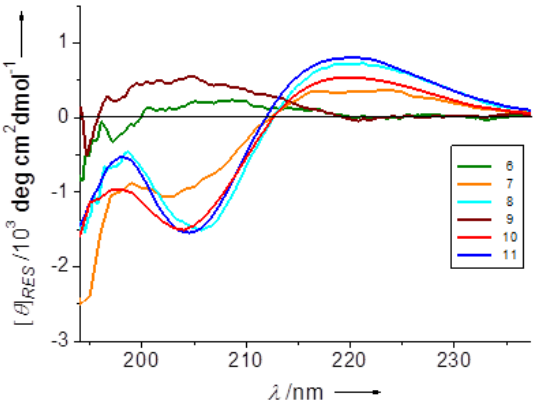


Fig. S2. CD spectra in methanol (c = 0.1 mM). The oligomers **7**, **8**, **10** and **11** in MeOH exhibit two minima around 193 and 204 nm, which hint at the existence of chiral secondary structures. Interestingly, these traces would be attributable to nonpolar strand conformations according to literature data on β-peptides.[2,6] Nevertheless, the different backbones of β-peptides and α-peptides lead to different conformational geometries which result in different CD spectra.[7] Similarly, the different intramolecular hydrogen bond patterns available to β^{3R3}-peptides and β-peptides can lead to alternative geometries and different CD spectra. Differently, oligomers **6** and **9** show strongly decreased CD signals indicating non-specific aggregation.[8]

4.2 Infrared Reflection-Absorption-Spectroscopy (IRRAS)

Infrared Reflection-Absorption (IRRA) spectra were recorded with a Vertex 70 FT-IR spectrometer (Bruker, Germany) coupled with a Langmuir film balance (Riegler & Kirstein, Potsdam, Germany). The trough and the external reflectance unit (XA 511) were flushed with dry air in a sealed container to obtain a stable atmosphere. To measure the surface pressure, a filter paper as a Wilhelmy plate was used. The peptide was spread from a methanol solution onto the subphase (10 mM PBS, pH 7.4, 20 °C) to obtain high surface pressure (referred as 'single shot method'[9]), which corresponds to a theoretical bulk solution of 0.2 µM. The film was compressed symmetrically by two barriers with a velocity of 8.2 cm^2/min. After relaxation for one hour, the reflected IR beam was detected at the same angles as incidence (40°) with a liquid nitrogen cooled MCT (Mercury Cadmium Telluride) detector with a resolution of 8 cm^{-1}. 800 scans were made for a p-polarization beam to obtain a sample spectrum R (scanner velocity 20 kHz), apodized using Blackman-Harris 3-term function and fast Fourier transformed after one level of zero filling. Due to the shuttle technique, a pure subphase spectrum R_0 is recorded before each sample spectrum is measured. The reflectance-absorbance RA is plotted as $-\lg(R/R_0)$. Therefore, the strong absorption bands from water can be eliminated. Details of the technique can be found in the works of Flach and Mendelsohn et al.[10, 11]

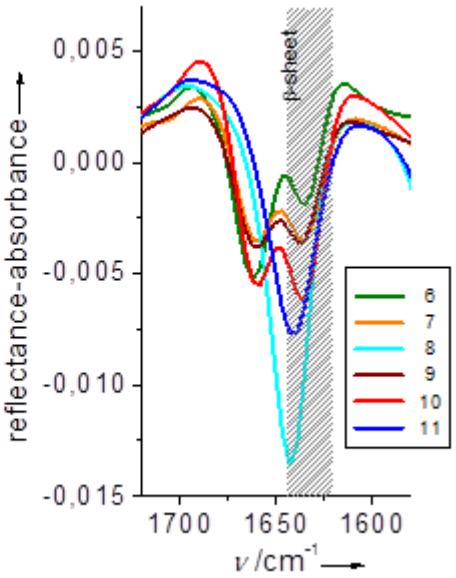


Fig. S3. Amide I band from IRRA spectra at the air/water interface after compression of the film. The oligomers **6**, **7**, **9**, and **10** show the maximum in the Amide I band around 1635 cm-1 indicating the formation of a strong intermolecular hydrogen bond network typical for crystalline β-sheets at the air/water interface.[2,12,13,14] A second band appears at high frequency (around 1662 cm-1) which is attributable to amide CO groups not involved as acceptors in H-bonding,[2] and can derive from non-specific aggregation.[15,16] The intensity ratio of these two bands changes along with the oligomer length. This indicates a changing ratio between the crystalline and non-crystalline parts of sheet monolayers. The shortest oligomer **6** has the largest tendency to form non-specific aggregates. The longest oligomers **8** and **11** exhibit a single and quite broad band around 1645 cm^{-1} which is characteristic for non-aggregated strands in a less ordered intermolecular network, as reported for both α-peptides[15,16] and β-peptides.[2]

4.3 Grazing Incidence X-Ray Diffraction (GIXD)

The grazing incidence X-ray diffraction measurements were carried out at the undulator beamline BW1 using the liquid surface diffractometer at HASYLAB, DESY (Hamburg, Germany). The experimental setup and evaluation procedures have been described in detail elsewhere.[17] The setup is equipped with a temperature controlled Langmuir trough (Riegler and Kirstein, Potsdam, Germany), which is enclosed in a sealed, helium-filled container. The synchrotron X-ray beam is monochromated to a wavelength of 1.304 Å and is adjusted to strike the helium/water interface at a grazing incidence angle $\alpha_i = 0.85\alpha_c$ ($\alpha_c = 0.13°$ is the critical angle for total reflection) illuminating approximately 2×50 mm^2 of the monolayer surface. A MYTHEN detector system (PSI, Villigen, Switzerland) measures the diffracted signal and is rotated to scan the in-plane Q_{xy} component of the scattering vector. A Soller collimator in front of the MYTHEN restricted the in-plane divergence of the diffracted beam to 0.09°. The vertical strips of the MYTHEN measure the out-of-plane Q_z component of the scattering vector between 0.0 and 0.75 Å^{-1}. The diffraction data consist of Bragg peaks at diagnostic Q_{xy} values obtained by summing the diffracted intensity over a defined vertical angle or Q_z-window. The in-plane lattice repeat distances d of the ordered structures in the monolayer are calculated from the Bragg peak positions: $d = 2\pi/Q_{xy}$. To estimate the extent of the crystalline order in the monolayer, the in-plane coherence length L_{xy}, is approximated from the full-width at half-maximum (fwhm) of the Bragg peaks using $L_{xy} \sim 0.9(2\pi)/\text{fwhm}(Q_{xy})$ using the measured fwhm(Q_{xy}) corrected for the instrumental resolution. Integrating the diffracted intensity normal to the interface over the Q_{xy} window of the diffraction peak yields the corresponding Bragg rod. The thickness of the scattering unit is estimated from the fwhm of the Bragg rod using $0.9(2\pi)/\text{fwhm}(Q_z)$. All experiments have been performed at the air/water interface at 20 °C and 20 mN/m.

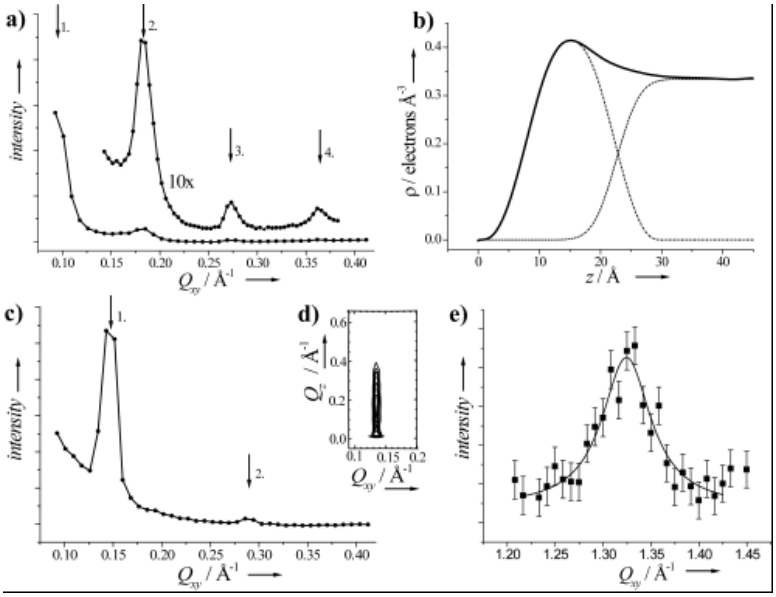


Fig. S4. a) Corrected X-ray intensities versus the in-plane scattering vector component Q_{xy} for **11**. No Bragg peak has been observed in the wide-angle region in good agreement with the IRRAS data showing the amide I band only at a high wavenumber of 1641 cm-1. This non-aggregated strand structure is characterized by the lack of long range correlation between the strands. The layer of oligomer **11** exhibits two-dimensional smectic order (Bragg peaks in the small-angle region). Thereby, oligomer **11** exhibits high order in the direction of the long repeat distance. 4 Bragg peaks have been observed at *Qxy* values of 0.092, 0.182, 0.273 and 0.366 Å-1 corresponding to a d(01) spacing of 68.7 Å and its higher order reflections. The long repeat distances determined after compression of the film are shorter than expected for elongated strands (these distances were obtained by the addition of bond lengths with the structures presenting an all-trans linear conformation and were calculated by Chem3D Pro). The difference between the observed and the calculated values increases with increasing oligomer length. Specifically, a length of 68.7 Å for oligomer **11** (86% of the calculated extended strand length of 80.2 Å) ruling out any helical or hairpin conformation and thus was assigned to a bended strand like secondary structure b) Electron density profile ρ(z) of **11** versus the vertical *z* coordinate. The Fwhm of the Gaussian-shaped curve amounts to 14.3 Å which translates directly into the thickness of the corresponding films, proving the bending of the strands. c) Corrected X-ray intensities versus the in-plane scattering vector component Q_{xy} for **7**. The layers of oligomer **7** also exhibits two-dimensional smectic order (Bragg peaks in the small-angle region). Two Bragg peaks are found at 0.144 and 0.287 $Å^{-1}$ (Fig 4c) corresponding to a $d_{(01)}$ spacing of 43.6 Å. A length of 43.6 Å was measured for oligomer **7** (89% of the calculated extended strand length of 49.1 Å). d) Contour plot of the corrected X-ray intensities as a function of in-plane (Q_{xy}) and out-of-plane (Q_z) scattering vector components of **7**. The Bragg rods show that the out-of-plane component of the scattering vector has clearly maximum intensity above the horizon which suggests that the strands are not lying entirely flat at the surface and more likely bend upon compression. e) Corrected X-ray intensities versus the in-plane scattering vector component Q_{xy} for **7**. The short oligomer **7** exhibits Bragg peaks in the wide- as well as in the small-angle regions. The weak Bragg peak at 1.327 $Å^{-1}$ (Fig 4e) corresponds to the interstrand distance of 4.735 Å defined by the hydrogen bonds in crystalline β-sheets (proved by IRRAS experiments showing a band at 1635 cm^{-1}).[12,13,14] The correlation length in transversal direction amounts to 97 Å, corresponding to 20 molecular repeats. This excludes either any helical or unordered conformation. Interestingly, oligomer **8** does not give any signal in GIXD thus indicating neither translational nor longitudinal long-range correlation between the strands typical of amorphous intermolecular networks. This is in agreement with IRRAS data where oligomer **8** exhibits a single band around 1645 cm^{-1} indicating the presence of strand conformations which do not assemble into sheets with a strong, crystalline intermolecular network.[15,16,2]

4.4 X-ray Reflectivity (XR)

X-ray reflectivity measurements were carried out at the same beamline as GIXD experiments. The experimental setup and evaluation procedures are described in detail elsewhere.[18] The specular X-ray reflectivity (XR) data collection was performed by using a NaI scintillation detector. The X-ray reflectivity was measured with the geometry, $\alpha_i = \alpha_f = \alpha$, where α_i is the vertical incidence angle and α_f is the vertical exit angle of the reflected X-rays. XR data were collected as a function of the incidence angle, α_i, varied in the range of 0.06° - 3.5°, corresponding to a range of 0.01 $Å^{-1}$ - 0.6 $Å^{-1}$ of the vertical scattering vector component Q_z. The background scattering from the subphase was measured at $2\theta_{xy} = 0.7°$ and subtracted from the signal measured at $2\theta_{xy} = 0$.

The electron density profile has been obtained with a model-independent method.[19] From the experimentally observed reflectivity curve, the corresponding profile correlation function is estimated via indirect Fourier transformation. For this profile correlation function the matching electron density profile is then derived by square-root deconvolution. No a priori assumptions on the shape of the electron-density profile have to be made.

The X-ray reflectivity can be inverted to yield the laterally averaged electron density ρ(z) of the monolayer as a function of the vertical z coordinate. For both oligomers, the profile can be described by one symmetrical electron density distribution assuming a root-mean-square roughness of 3 Å. The full-width at half-maximum (Fwhm) of the Gaussian-shaped curve amounts to 14.3 Å for **11** and 13.9 Å for **7**, translating directly into the thickness of the corresponding films. This thickness is larger than expected for a layer of β-strands lying flat at the air/water interface supporting the model of strand bending.

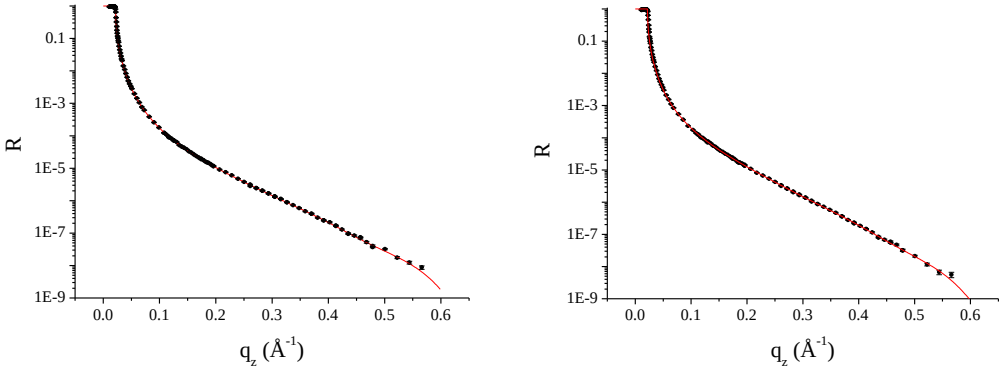


Fig. S5. Specular X-ray reflectivity, *R*, of monolayers of oligomer **7** (left) and **11** (right) on water together with the best fits to the data (solid red lines).

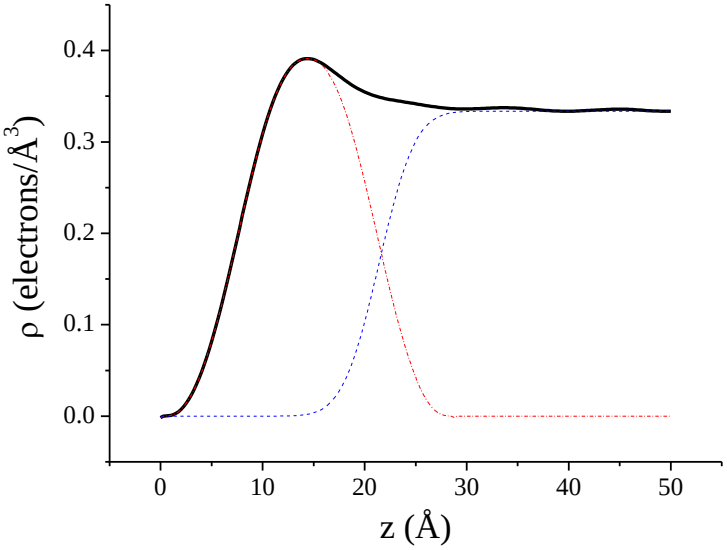


Fig. S6. Electron density profiles along the surface normal z of a monolayer of **7** on water derived from the corresponding reflectivity curve. The profile has been described by a symmetrical electron density distribution (red dotted line) and using a roughness of 3 Å (blue dotted line).

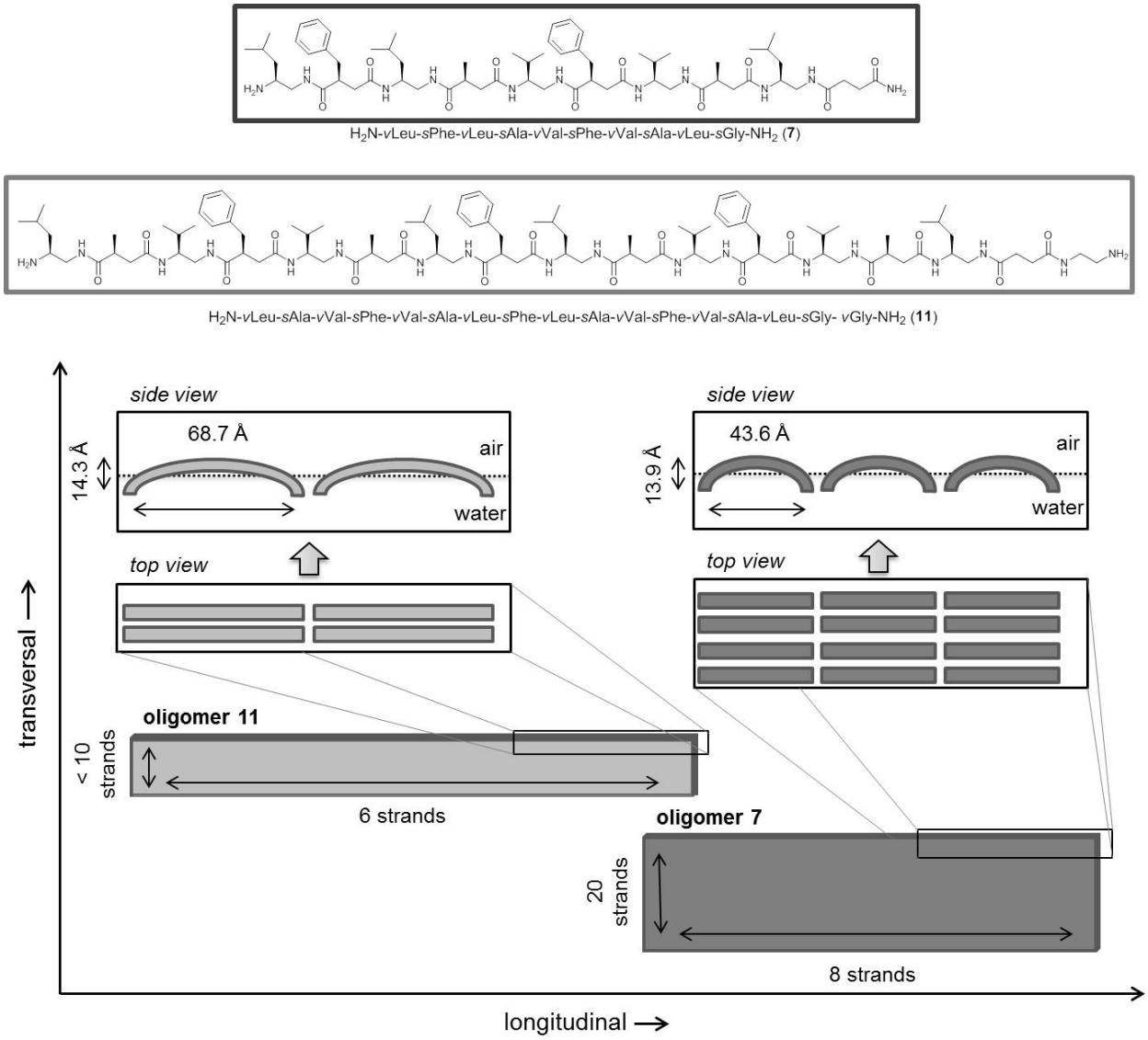


Fig. S7. Structural model for strand aggregation of oligomer **7** and **11** at the air/water interface to form sheets as derived from IRRAS and GIXD data. The short oligomer **7** exhibits Bragg peaks in the wide- as well as in the small-angle regions (Fig S5). The weak Bragg peak at 1.327 $Å^{-1}$ (Fig S5c) corresponds to the interstrand distance of 4.735 Å defined by the hydrogen bonds in crystalline β-sheets (proved by IRRAS experiments showing a band at 1635 cm^{-1}).[12,13,14] The correlation length in transversal direction amounts to 97 Å, corresponding to 20 molecular repeats (Fig. 5). This excludes either any helical or unordered conformation. For the layer of oligomer **11**, no Bragg peak has been observed in this region in good agreement with the IRRAS data showing the amide I band only at a high wavenumber of 1641 cm^{-1}. This non-aggregated strand structure is characterized by the lack of long range correlation between the strands. The layers of oligomers **7** and **11** additionally exhibit two-dimensional smectic order (Bragg peaks in the small-angle region, Fig. S5 a and c). Thereby, oligomer **11** exhibits high order in the direction of the long repeat distance. 4 Bragg peaks have been observed at Q_{xy} values of 0.092, 0.182, 0.273 and 0.366 $Å^{-1}$ corresponding to a $d_{(01)}$ spacing of 68.7 Å and its higher order reflections (Fig S5a). For oligomer **7**, two Bragg peaks are found at 0.144 and 0.287 $Å^{-1}$ (Fig. S5c) corresponding to a $d_{(01)}$ spacing of 43.6 Å. The long repeat distances determined after compression of the film are shorter than expected for elongated strands (these distances were obtained by the addition of bond lengths with the structures presenting an all-trans linear conformation and were calculated by Chem3D Pro). The difference between the observed and the calculated values increases with increasing oligomer length. Specifically, a length of 43.6 Å was measured for oligomer **7** (89% of the calculated extended strand length of 49.1 Å), and 68.7 Å for oligomer **11** (86% of the calculated extended strand length of 80.2 Å) ruling out any helical or hairpin conformation and thus was assigned to a bended strand like secondary structure. There are several possible arrangements of the oligomers that lead to a repeat distance shorter than the full length of the elongated molecule. The relative shorter length of **11** compared to **7** could be explained by the addition of the terminal *v*Gly which is flexible and hydrophilic and therefore tends to dive into the water. In addition, the analysis of the Bragg rods shows that the out-of-plane component of the scattering vector has clearly maximum intensity above the horizon (Fig. S5d) which suggests that the strands are not lying entirely flat at the surface and more likely bend upon compression. To prove the bending, the layer thickness has been determined by X-ray

reflectivity experiments. The Fwhm of the Gaussian-shaped curve amounts to 14.3 Å for oligomer **11** (Fig. S6) and 13.9 Å for oligomer **7** (Fig. S6), which translates directly into the thickness of the corresponding films. This thickness is larger than expected for a layer of β-sheets lying flat at the air/water interface and the bending rigidity is smaller for the longer oligomer. The correlation lengths in the direction of the long repeat distance are in the order of 400 Å, corresponding to 6 (oligomer **11**) or 8 (oligomer **7**) aligned strands. These results support a structure model with bended strands which self-assemble into sheets with different crystallinity, depending on the chain length and chemical properties of terminal residues. Interestingly, specific residues at the terminal positions also promote alignment of α/β-peptides molecules along the H-bond direction within two dimensional sheet assemblies.[20] Additionally, in D/L α-peptides strands all the side chains are on one face and give rise to bending of sheets,[21] and therefore the disposition of all side chains on one face might also explain the bending of the β^{3R3}-peptides.

5 Enzymatic Stability Assays

The MALDI measurements were carried out using a Bruker Autoflex MALDI TOF system and as Matrix DHB (2,5-dihydroxybenzoic acid). Oligomer **11** (**m/z**=1917.683) was dissolved in 40µl total volume (1pmol/µl) of buffer (30% MeOH 25mM Tris HCl , 3mM $CaCl_2$) and incubated at 37°C with 100ng of Pronase**.** The mixture was analyzed via MALDI at 0h, 24h, 48h and 8 days. A peptide with the sequence NVFVHPNYSK (m/z=1219.6106) was used as control.

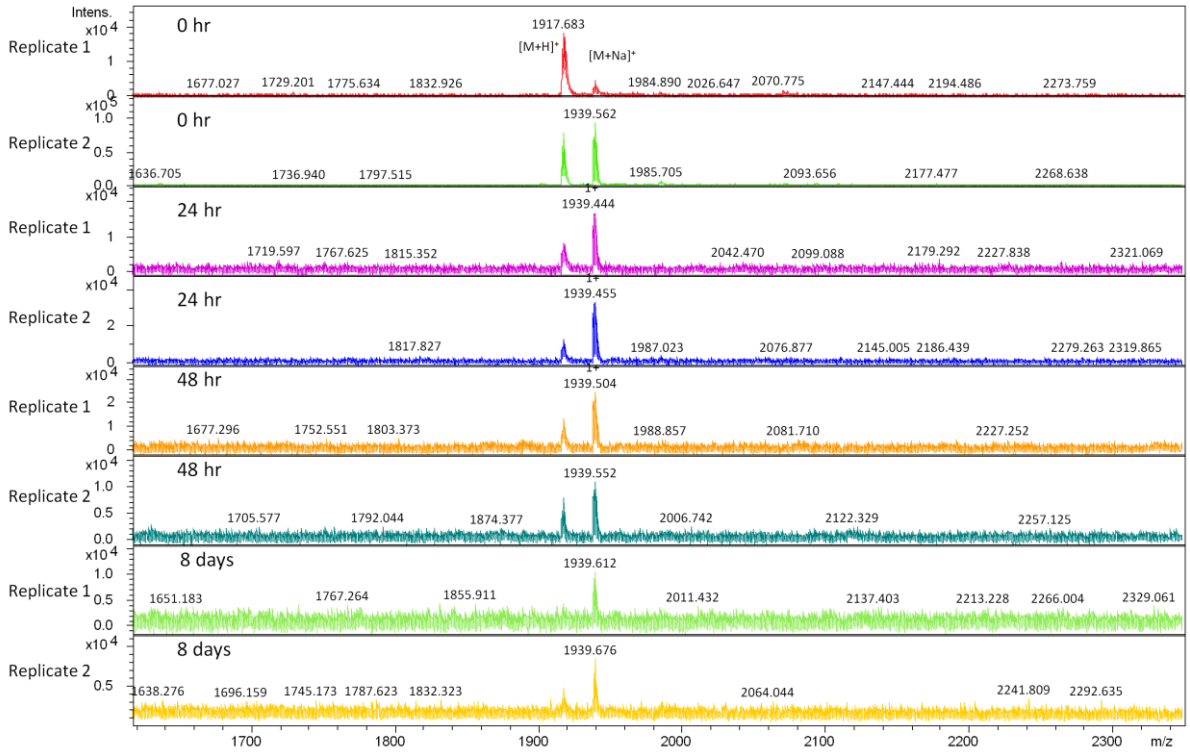


Fig. S8. MALDI spectra of Pronase digestion of oligomer **11** in 30% MeOH and 25mM Tris HCl, 3mM $CaCl_2$ aqueous buffer.

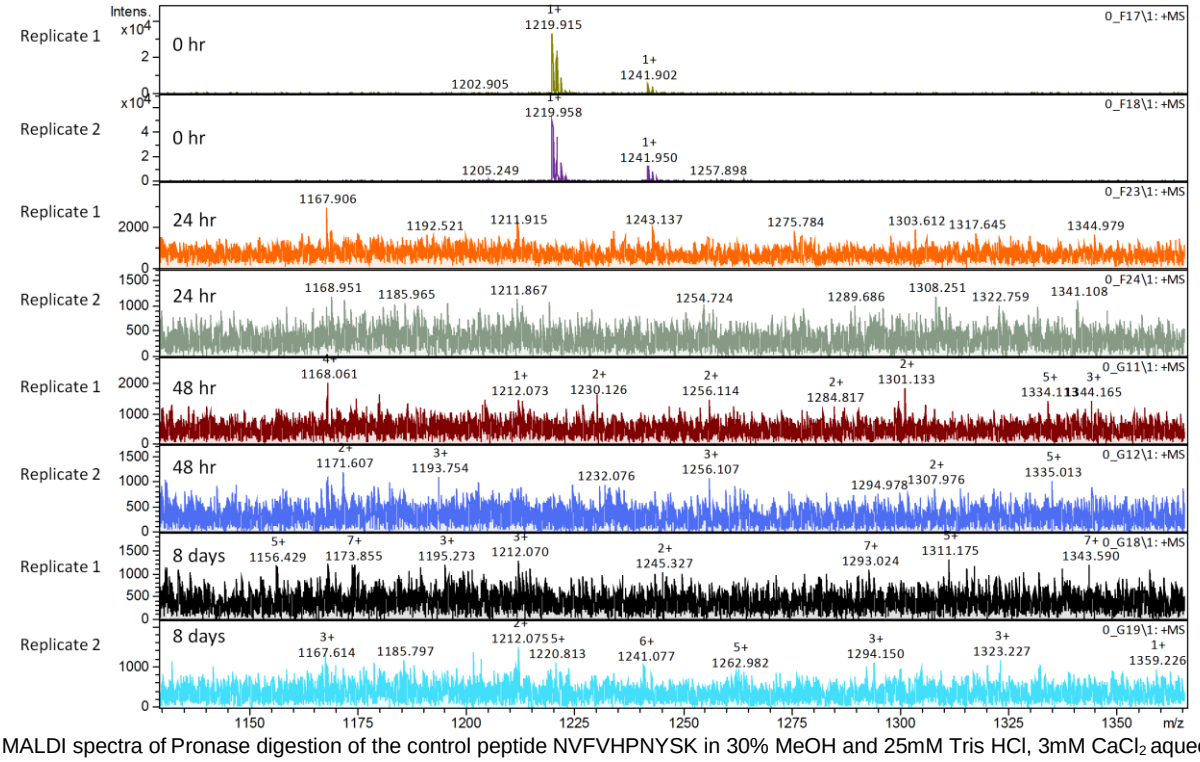


Fig. S9. MALDI spectra of Pronase digestion of the control peptide NVFVHPNYSK in 30% MeOH and 25mM Tris HCl, 3mM $CaCl_2$ aqueous buffer.

6. References

1. D. Seebach, D. F. Hook and A. Glattli, *Biopolymers*, 2006, **84**, 23-37.
2. T. A. Martinek, G. K. Toth, E. Vass, M. Hollosi and F. Fulop, *Angew. Chem.-Int. Edit.*, 2002, **41**, 1718-+.
3. S. Mosca, F. Wojcik and L. Hartmann, *Macromol. Rapid Commun.*, 2011, **32**, 197-202.
4. D. A. Evans, L. D. Wu, J. J. M. Wiener, J. S. Johnson, D. H. B. Ripin and J. S. Tedrow, *J. Org. Chem.*, 1999, **64**, 6411-6417.
5. M. Amblard, J. A. Fehrentz, J. Martinez and G. Subra, *Mol. Biotechnol.*, 2006, **33**, 239-254.
6. F. Rua, S. Boussert, T. Parella, I. Diez-Perez, V. Branchadell, E. Giralt and R. M. Ortuno, *Org. Lett.*, 2007, **9**, 3643-3645.
7. W. F. DeGrado, J. P. Schneider and Y. Hamuro, *J. Pept. Res.*, 1999, **54**, 206-217.
8. C. Dannehl, O. G. Travkova and G. Brezesinski, *Chem. Lett.*, 2012, **41**, 1178-1180.
9. D. Dieudonne, A. Gericke, C. R. Flach, X. Jiang, R. S. Farid and R. Mendelsohn, *J. Am. Chem. Soc.*, 1998, **120**, 792-799.
10. C. R. Flach, J. W. Brauner, J. W. Taylor, R. C. Baldwin and R. Mendelsohn, *Biophys. J.*, 1994, **67**, 402-410.
11. R. Mendelsohn, G. R. Mao and C. R. Flach, *Biochim. Biophys. Acta-Biomembr.*, 2010, **1798**, 788-800.
12. E. Maltseva, A. Kerth, A. Blume, H. Mohwald and G. Brezesinski, *ChemBioChem*, 2005, **6**, 1817-1824.
13. C. Olak, A. Muenter, J. Andrae and G. Brezesinskia, *J. Pept. Sci.*, 2008, **14**, 510-517.
14. M. Hoernke, B. Koksch and G. Brezesinski, *Biophys. Chem.*, 2010, **150**, 64-72.
15. M. Jackson and H. H. Mantsch, *Crit. Rev. Biochem. Molec. Biol.*, 1995, **30**, 95-120.
16. L. K. Tamm and S. A. Tatulian, *Q. Rev. Biophys.*, 1997, **30**, 365-429.
17. M. Lepere, C. Chevallard, G. Brezesinski, M. Goldmann and P. Guenoun, *Angew. Chem.-Int. Edit.*, 2009, **48**, 5005-5009.
18. K. Kjaer, J. Alsnielsen, C. A. Helm, P. Tippmankrayer and H. Mohwald, *J. Phys. Chem.*, 1989, **93**, 3200-3206.
19. J. S. Pedersen and I. W. Hamley, *J. Appl. Crystallogr.*, 1994, **27**, 36-49.
20. S. Segman, M. R. Lee, V. Vaiser, S. H. Gellman and H. Rapaport, *Angew. Chem.-Int. Edit.*, 2010, **49**, 716-719.
21. O. S. Andersen, *Annu. Rev. Physiol.*, 1984, **46**, 531-548.