

SUPPORTING INFORMATION

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Materials and methods

Solvents

Solvents for the syntheses were used in the highest quality available (p.a., absolute) and were purchased from SIGMA-ALDRICH (Taufkirchen, Germany), ACROS ORGANICS (Geel, Belgium), FLUKA (Taufkirchen, Germany) or VWR INTERNATIONAL (Fontenay-sous-Bois, France). Extra-dry solvents were supplied by SIGMA-ALDRICH (Taufkirchen, Germany) and ACROS ORGANICS (Geel, Belgium). Analytic and HPLC grade solvents were purchased from FLUKA (Taufkirchen, Germany), VWR INTERNATIONAL (Fontenay-sous-Bois, France), ACROS ORGANICS (Geel, Belgium) and SIGMA-ALDRICH (Taufkirchen, Germany). Solvents for the NMR experiments were provided by DEUTERIO (Kastellaun, Germany). The ultra-pure water (electrical conductivity 18 M Ω -cm) was achieved by purification of demineralised water using the systems SYMPPLICITY from MERCK MILLIPORE (Bedford, UK) and arium® mini from SATORIUS (Göttingen, Germany). Solvents were degassed by passing argon through them.

Reagents

All utilised, commercial available materials and chemicals were purchased in the highest quality available by ABCR (Karlsruhe, Germany), ACROS ORGANICS (Geel, Belgium), ALFA AESAR (Karlsruhe, Germany), BACHEM (Bubendorf, Switzerland), CARL ROTH (Karlsruhe, Germany), FISCHER SCIENTIFIC (Nidderau, Germany), FLUKA (Taufkirchen, Germany), MERCK (Darmstadt, Germany), RIEDEL-DE HAËN (Seelze, Germany), TCI (Eschborn, Germany) or VWR (Darmstadt, Germany) and were used as provided. The Fmoc-protected α -amino acids, coupling reagents and the resins were provided by GL BIOCHEM (Shanghai, China), IRIS (Marktredwitz, Germany), ACROS ORGANICS (Geel, Belgium) and MERCK (Darmstadt, Germany).

Reactions

Air and moisture sensitive reactions were performed in dried laboratory glassware under an argon atmosphere (> 99.996%). The glass apparatus was heated under reduced pressure. After the cooling-down the glassware was purged with dried argon. This procedure was repeated three times. Solids were added in a counter stream of argon and solutions through a septum *via* a syringe equipped with a cannula.

Freeze-Drying

Products were dissolved/suspended in water and were frozen in liquid nitrogen and freeze-dried using a CHRIST ALPHA-2-4 lyophiliser (Osterode am Harz, Germany) equipped with a high vacuum pump. Samples with a volume higher than 2 mL were lyophilised in round bottom flasks, whereas samples with volumes lower than 2 mL in an EPPENDORF safe-lock microcentrifuge tube in speedvac devices RVC 2-18 or RVC 2-18 CD plus of CHRIST (Osterode am Harz, Germany) connected to the lyophiliser.

Flash-Column Chromatography

Flash column chromatography was carried out using silica gel of the type 60 with a particle size of 40–63 μ m provided by MERCK (Darmstadt, Germany) and a pressure of 0.1–1.0 bar. The silica gel was suspended in the elution system and filled in an appropriate glass column equipped with a glass frit. The samples were loaded either by pre-loading on silica gel or as concentrated solution.

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography was performed on JASCO (Gross-Umstadt, Germany) instruments equipped with an analytical column (Nucleodur® RP C-18 analytical HPLC column (250 nm x 4.6 mm, 5 μ m) from MACHEREY-NAGEL (Düren, Germany) with a flow

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of 1.0 mL/min. The compounds were detected by UV absorptions at 215, 254 and 280 nm. Applied elution systems were either A: bi-demineralised H₂O + 0.1% TFA and B: MeOH + 0.1% TFA or B: bi-demineralised H₂O + 0.1% TFA and C: MeCN + 0.1% TFA. Building blocks were investigated on a JASCO system equipped with a MD-2010plus multiwavelength detector, LC-Net II/ADC, CO-2060plus intelligent column thermostat, AS-2055plus intelligent sampler and two PU-2085plus semi-micro HPLC pumps. Peptides were purified with a JASCO system equipped with a MD-2010plus multiwavelength detector, LC-Net II/ADC, a DG-2080-53 3-line degasser and two PU-2086plus intelligent HPLC pumps. The column was heated in a PHARMACIA LKB HPLC column oven 2155. The samples were dissolved in mixtures of bi-demineralised water and either MeOH or MeCN followed by filtering through CHROMAFIL® RC-45/15 MS (MACHERY-NAGEL) filter.

Nuclear Magnetic Resonance (NMR)

NMR experiments were performed on VARIAN (California, USA) instruments (Mercury (VX) 300, Unity 300, Inova-500). The sample temperature was set to either 298 K or 308 K. The effective measuring frequencies are mentioned in the analytic data of the substances. All ¹³C NMR experiments were performed proton-decoupled. The chemical shift δ is indicated in ppm (TMS = 0 ppm). The chemical shift of the solvents served as internal standard [¹H]DMSO: 2.50 ppm (¹H) and 39.52 ppm (¹³C)]. Signal multiplicities are abbreviated as followed s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, m = multiplet and s_{br} = broadened singlet.

Mass Spectrometry

Experiments were performed using the ionisation techniques electrospray ionisation (ESI) on either a BRUKER (Massachusetts, USA) micrOTOF-Q II or a BRUKER maXis ESI-QTOF-MS instrument. The data are presented in mass-to-charge ratio (*m/z*).

UV/Vis Spectroscopy

Concentration determination of the peptides (dissolved in EtOH) was performed on a THERMO SCIENTIFIC device (NanoDrop 2000c, cuvette function [*d* = 1 cm]). The quartz glass cuvette Suprasil® (QS) were supplied by HELMA (Müllheim, Germany). The concentration was calculated using the LAMBERT-BEER law by the means of the absorption of Trp at 280 nm ($\epsilon = 5600 \text{ cm}^{-1} \text{ M}^{-1}$).^[1]

Circular Dichroism (CD) spectroscopy

CD experiments were performed on a J-1500 spectropolarimeter provided by JASCO equipped with a JASCO PTC-510 peltier thermostatted rectangular cell holder and a JULABO F250 thermostat. The device was purged with nitrogen before and during the operations. All experiments were carried out in a 1.0 mm quartz glass cuvette of HELMA (Suprasil® QS) and the temperature was controlled by the sensor in the holder. The following parameters were used for the experiments:

Parameter	MeOH	POPC
Measurement range / nm	260–190	
Data pitch / nm	0.1	
CD scale / mdeg/dOD	200/0.1	
FL scale / mdeg/dOD	200/0.1	
D.I.T. / sec	4	
Bandwidth / nm	1.0	
Scanning speed / nm/min	50	
Accumulation	20	25

Enantiomeric Excess (ee)

The experiments for the determination of the ee value were performed on a SHIMADZU (Kyōto, Japan) or JASCO HPLC system. The SHIMADZU HPLC system was equipped with a DGU-20A3/prominence degasser, two pumps LC-20AD/prominence liquid chromatography, a CBM-20A/prominence communication BUS module, a SPD-M20A/ prominence diode array detector and a SIL-20AC/prominence auto sampler. The JASCO system was equipped with a MD-2010plus multiwavelength detector, LC-Net II/ADC, CO-2060plus intelligent column thermostat, AS-2055plus intelligent sampler and two PU-2085plus semi-micro HPLC pumps. Chiral columns from DAICEL (Mainz, Germany) (Chiralpak® IA, OD) were used.

Continuous Wave (CW)-EPR

All CW-EPR experiments were performed at X-band frequencies and room temperature using a Bruker Elexsys E500 spectrometer equipped with a Bruker super-high Q resonator ER4122SHQE.

Pulsed-EPR [pulsed electron double resonance (PELDOR); electron spin-echo envelope modulation (ESEEM)]

Pulsed-EPR experiments were performed at Q-band frequencies using a commercial Bruker Elexsys E580 pulse X/Q-band spectrometer equipped with a pulsed 170 W Q-band TWT-amplifier (Model 187Ka, Applied Systems Engineering Inc.), a Bruker ENDOR resonator (EN5107D2), a continuous He-flow cryostat (CF95550, Oxford Instruments) and a temperature controller (ITC-5035, Oxford Instruments). The experimental temperature was set to 80 K, during the introduction of the sample into the resonator and stabilized at 50 K for performing EPR experiments.

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Synthesis Fmoc- β^3 -hTOPP-OH (1)3,3,5,5-Tetramethylpiperazine-2,6-dione (9)

3,3,5,5-Tetramethylpiperazine-2,6-dione (9) was synthesised according to literature.^[2]

Cbz-D-Hpg-OH

Cbz-D-Hpg-OH was synthesised according to the procedure described by Stoller *et al.*^[3]

A solution of 4-Hydroxy-D-phenylglycine (2) (5.00 g, 29.9 mmol, 1.00 eq) in an aqueous solution of Na₂CO₃ (10%, 67 mL) was cooled to 0°C and then, CbzCl (4.48 mL, 5.42 g, 31.7 mmol, 1.06 eq) dissolved in toluene (4.48 mL) and dioxane (50.0 mL) was added dropwise. The reaction mixture was stirred at 0°C for 30 min and afterwards at rt for 1 h. After, the organic solvents were evaporated. Ice-water (166 mL) was added to the aqueous phase and it was washed with EtOAc (3 x 50.0 mL). Then, the aqueous phase was acidified with 2 M aq HCl to pH 2 and extracted with EtOAc (3 x 75.0 mL). The combined organic phases were washed with water (50.0 mL), saturated aq NaCl solution (50.0 mL) and dried over MgSO₄. Removing of the solvent under reduced pressure gave Cbz-D-Hpg-OH (8.35 g, 27.7 mmol, 93%) as a colourless solid. ¹H NMR (300 MHz, [D₆]DMSO): δ = 12.51 (s, 1H; COOH), 9.52 (s, 1H; aromatic OH), 7.89 (d, ³J(H,H)=8.0 Hz, 1 H, NH), 7.55–7.26 (m, 5H; aromatic CH), 7.23 (d, ³J(H,H)=8.5 Hz, 2H; aromatic CH), 6.76 (d, ³J(H,H)=8.5 Hz, 2H; aromatic CH), 5.17–4.98 (m, 3H; CH₂, α -CH). ¹³C NMR (75 MHz, [D₆]DMSO, 25°C): δ =172.50 (COOH), 157.19 (C-OH), 155.82 (CONH), 137.00, 128.97, 128.33, 127.80, 127.71, 127.28, 127.18, 115.18 (aromatic C), 65.56 (CH₂), 57.61 (α -C). ESI-MS: m/z =302.1 [M+H]⁺, 324.1 [M+Na]⁺, 625.2 [2M+Na]⁺, 300.1 [M-H]⁻, 601.2 [2M-H]⁻. ESI-HRMS: m/z calculated for C₁₆H₁₅NO₅Na [M+Na]⁺: 324.0842, found: 324.0843.

Cbz-D-Hpg(TBDMS)-OH (3)

Under an argon atmosphere, Cbz-D-Hpg-OH (3.00 g, 9.97 mmol, 1.00 eq) and imidazole (1.70 g, 24.9 mmol, 2.50 eq) were dissolved in dry DMF (6.00 mL). TBDMSCl (1.80 g, 11.9 mmol, 1.20 eq) was added and the reaction mixture was stirred at rt for 25 h. Afterwards, the mixture was diluted with EtOAc (50.0 mL) and H₂O (50.0 mL) and the two resulting phases were separated. The aqueous phase was extracted with EtOAc (3 x 50.0 mL). The combined organic phases were washed with saturated aq NaCl solution (50.0 mL) and dried over MgSO₄. The solvent was evaporated under reduced pressure and purification of the residue by flash-column chromatography (DCM/MeOH/AcOH, 9:1:0.1 \rightarrow 7:1:0.1) led to the pure product 3 (2.70 g, 6.58 mmol, 66%) as a white solid. ¹H NMR (300 MHz, [D₆]DMSO): δ = 7.90 (d, ³J(H,H)= 7.9 Hz, 1H; NH), 7.4–7.24 (m, 7H, aromatic CH), 6.81 (d, ³J(H,H)=8.5 Hz, 2H; aromatic CH), 5.11–5.00 (m, 3H; CH₂, α -CH), 0.95 (s, 9H; CH₃), 0.19 (s, 6H; CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ =172.11 (COOH), 155.65 (Cbz CONH), 154.66 (C-O(TBDMS)), 136.88, 130.14, 128.90, 128.21, 127.67, 127.59, 119.48 (aromatic C), 65.45 (CH₂), 57.49 (α -C), 25.46 (C(CH₃)₃), 17.69 (C(CH₃)₃), -4.63 (CH₃). ESI-MS: m/z =416.2 [M+H]⁺, 438.2 [M+Na]⁺, 853.3 [2M+Na]⁺, 300.1 [M-H]⁻, 601.2 [2M-H]⁻. ESI-HRMS: m/z calculated for C₂₂H₂₉NO₅SiNa [M+Na]⁺: 438.1707, found: 438.1696.

Cbz-D-Hpg(TBDMS)-CHN₂ (4)

Under an argon atmosphere, a solution of Cbz-D-Hpg(TBDMS)-OH (3) (3.99 g, 9.60 mmol, 1.00 eq) dissolved in dry THF (48.0 mL) was cooled to -15°C. Then, Et₃N (1.07 mL, 1.46 g, 10.6 mmol, 1.10 eq) and isobutyl chloroformate (1.44 mL, 1.37 g, 10.6 mmol, 1.10 eq) were added and the mixture was stirred at -15°C for 45 min. Afterwards, the reaction was warmed up to 0°C and treated with diazomethane (0.60 M in Et₂O, 32.0 mL, 2.00 eq) under light exclusion. The reaction mixture was stirred at 0°C for 30 min. After, the reaction mixture was warmed up to rt and stirred at rt for 5 h, the reaction was quenched with AcOH (2.20 mL, 2.30 g, 53.2 mmol, 4.00 eq). Then, 6% aq NaHCO₃ solution (100 mL) and EtOAc (50.0 mL) were added. The phases were separated, and the aqueous phase was extracted with EtOAc (2 x 50.0 mL). The combined organic phases were washed with saturated aq NH₄Cl solution (3 x 50.0 mL), saturated aq NaCl solution (3 x 50.0 mL) and dried over MgSO₄. The organic solvent was removed under reduced pressure. Purification by flash-column chromatography (pentane/EtOAc, 7:1 \rightarrow 3:1) led to the desired product 4 (2.98 g, 6.78 mmol, 61%) as a yellow oil. ¹H NMR (300 MHz, [D₆]DMSO): δ =8.04 (d, ³J(H,H)=8.2 Hz, 1H; NH), 7.43–7.23 (m, 7H; aromatic CH), 6.82 (d, ³J(H,H)=8.5 Hz, 2H; aromatic CH), 6.11 (s, 1H; CH), 5.22 (d, ³J(H,H)=8.2 Hz, 1H; β -CH), 5.06 (s, 2H; CH₂), 0.95 (s, 9H; CH₃), 0.19 (s, 6H; CH₃). ¹³C NMR (75 MHz, [D₆]DMSO): δ =192.38 (COCHN₂), 155.65 (Cbz CONH), 154.85 (C-O(TBDMS)), 136.77, 129.64, 129.13, 128.21, 127.70, 127.63, 119.66 (aromatic C), 65.62 (CH₂), 61.42 (α -C), 53.52 (COCHN₂), 25.43 (C(CH₃)₃), 17.79 (C(CH₃)₃), -4.65 (CH₃). ESI-MS: m/z = 462.2 [M+Na]⁺, 901.3 [2M+Na]⁺, 438.2 [M-H]⁻. ESI-HRMS: m/z calculated for C₂₃H₂₉N₃O₄SiNa [M+Na]⁺: 462.1820, found: 462.1819.

Cbz-D- β^3 -hHpg-OBn

Cbz-D-Hpg(TBDMS)-CHN₂ (4) (5.55 g, 12.6 mmol, 1.00 eq) was dissolved in dry THF/BnOH (9:1, 24.0 mL), cooled to 0°C and treated with silver(I)-benzoate (230 mg, 1.01 mmol, 0.08 eq) under light exclusion and sonication at rt for 2 h. Then, H₂O (100 mL) and EtOAc (100 mL) were added and the aqueous phase was acidified with 2 M aq HCl solution to a pH of 2. The aqueous phase was extracted with EtOAc (3 x 75.0 mL) and the combined organic phases were washed with saturated aq NaCl solution (3 x 50.0 mL), dried over MgSO₄. The solvent was removed under reduced pressure to give the Cbz-D- β^3 -hHpg(TBDMS)-OBn (5). The crude intermediate product 5 containing residual BnOH was dissolved in MeOH (142 mL) and conc. HCl (37%, 15.8 mL) was added. The reaction mixture was stirred at rt for 1 h. Afterwards, H₂O (100 mL) and EtOAc (100 mL) were added, the phases were separated, and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with saturated aq NaCl solution (3 x 50.0 mL) and dried over MgSO₄. The solvent was removed under reduced pressure and the residual was purified by flash-column chromatography (pentane/EtOAc, 3:1 \rightarrow 1:1). The desired TBDMS deprotected product (3.57 g, 8.82 mmol, 70%) was isolated as a

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colourless solid. ¹H NMR (300 MHz, [D₆]DMSO): δ=9.29 (s, 1H; aromatic OH), 7.80 (d, ³J(H,H)=8.9 Hz, 1H; NH), 7.42–7.20 (m, 10H; aromatic CH), 7.13 (d, ³J(H,H)=8.5 Hz, 2H; aromatic CH), 6.70 (d, ³J(H,H)=8.5 Hz, 2H; aromatic CH), 5.14–4.83 (m, 5H; β-CH, CH₂), 2.94–2.61 (m, 2H; CH₂). ¹³C NMR (75 MHz, [D₆]DMSO): δ=169.98 (COOBn), 156.39 (aromatic C-OH), 155.14 (Cbz CONH), 136.77, 135.95, 132.47, 128.22, 128.19, 127.76, 127.65, 127.59, 127.56, 127.41, 127.34, 114.92 (aromatic C), 65.35, 65.22 (CH₂), 51.13 (β-CH), 41.13 (CH₂).

ESI-MS: *m/z*=406.2 [M+H]⁺, 428.2 [M+Na]⁺, 833.3 [2M+Na]⁺, 404.2 [M-H]⁻. ESI-HRMS: *m/z* calculated for C₂₄H₂₃NO₅Na [M+Na]⁺: 428.1468, found: 428.1459.

Cbz-D-β³-hPpg(Tf)-OBn (6)

A solution of Cbz-D-β³-hPpg-OBn (4.79 g, 11.8 mmol, 1.00 eq) and pyridine (2.75 mL, 2.69 g, 35.4 mmol, 3.0 eq) in dry DCM (36.0 mL) was cooled to a 0°C. Afterwards, Tf₂O (5.00 g, 17.7 mmol, 1.50 eq) was added slowly and the reaction mixture was stirred at 0°C for 15 min. Then, the mixture was warmed up to rt and stirred for 20 min. The reaction was quenched with saturated aq NaHCO₃ solution. The resulting aqueous phase was extracted with DCM (3 x 50.0 mL). The combined organic phases were washed with saturated aq NaCl solution (3 x 50.0 mL) and dried over MgSO₄. Then, the solvent was removed under reduced pressure and pyridine was removed as an azeotropic mixture with toluene to give the product **6** (6.26 g, 11.6 mmol, 99%) as a brown solid. ¹H NMR (300 MHz, [D₆]DMSO): δ=8.03 (d, ³J(H,H)=8.5 Hz, 1H; NH), 7.53 (d, ³J(H,H)=8.8 Hz, 2H; aromatic CH), 7.43 (d, ³J(H,H)=8.8 Hz, 2H; aromatic CH), 7.39–7.17 (m, 10H; aromatic CH), 5.15–4.94 (m, 5H; β-CH, CH₂), 2.98–2.74 (m, 2H; CH₂). ¹³C NMR (125 MHz, [D₆]DMSO): δ=169.71 (COOBn), 155.30 (Cbz CONH), 148.11, 143.23, 136.82, 135.88, 128.69, 128.34, 128.25, 128.23, 127.87, 127.74, 127.65, 121.28 (aromatic C), 118.20 (q, ¹J(C,F)=318.8 Hz, CF₃), 65.59, 65.48 (CH₂), 51.35 (β-C), 40.50 (CH₂). ¹⁹F NMR (282 MHz, [D₆]DMSO): δ=-72.88 (s, 3F; CF₃). ESI-MS: *m/z*=560.1 [M+Na]⁺, 1097.3 [2M+Na]⁺, 536.1 [M-H]⁻. ESI-HRMS: *m/z* calculated for C₂₅H₂₂F₃NO₇Na [M+Na]⁺: 560.0961, found: 560.0961.

Cbz-4-pinacolboronyl-D-β³-hPhg-OBn (7)

Under argon atmosphere, Cbz-D-β³-hPpg(Tf)-OBn (**6**) (1.68 g, 3.12 mmol, 1.00 eq) was dissolved in degassed dioxane (20.0 mL) and bis(pinacolato)diboron (948 mg, 3.74 mmol, 1.20 eq), KOAc (925 mg, 9.42 mmol, 3.02 eq), PdCl₂(dppf) (228 mg, 312 μmol, 0.10 eq) and dppf (173 mg, 132 μmol, 0.10 eq) were added. The reaction mixture was stirred at 80°C for 7 h. Afterwards, EtOAc (300 mL) was added to the suspension. The organic phase was washed with saturated aq NaCl solution (3 x 100 mL) and dried over MgSO₄. Then, the solvent was removed under reduced pressure and the residual was purified by flash-column chromatography (pentane/EtOAc, 5:1 → 2:1) to give the pure product **7** (1.55 g, 3.01 mmol, 96%) as a light yellow solid. ¹H NMR (300 MHz, [D₆]DMSO): δ=7.96 (d, ³J(H,H)=8.6 Hz, 1H; NH), 7.62 (d, ³J(H,H)=8.0 Hz, 2H; aromatic CH), 7.42–7.15 (m, 12H; aromatic CH), 5.10–4.93 (m, 5H; CH₂, β-CH), 2.93–2.73 (m, 2H; CH₂), 1.29 (s, 12H; CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ=169.74 (COOBn), 155.19 (Cbz CONH), 145.51, 136.85, 135.86, 134.42, 128.18, 127.75, 127.65, 127.59, 125.82 (aromatic C), 83.49 (C(CH₃)₂), 65.43, 65.30 (CH₂), 51.69 (β-C), 40.68 (CH₂), 24.54 (C(CH₃)₂). ESI-MS: *m/z*=516.3 [M+H]⁺, 538.3 [M+Na]⁺, 1053.5 [2M+Na]⁺. ESI-HRMS: *m/z* calculated for C₃₀H₃₄BNO₆Na [M+Na]⁺: 538.2377, found: 538.2369.

Cbz-4-dihydroxyborane-D-β³-hPhg-OBn (8)

Cbz-4-pinacolboronyl-D-β³-hPhg-OBn (**7**) (3.74 g, 7.26 mmol, 1.00 eq) was dissolved in acetone (330 mL) and H₂O (291 mL). NH₄OAc (1.68 g, 21.8 mmol, 3.00 eq) and NaIO₄ (4.81 g, 22.5 mmol, 3.10 eq) were added and the mixture was stirred at rt for 2 d. Then, the organic solvent was removed under reduced pressure. The aqueous phase was extracted with Et₂O (3 x 100 mL) and the combined organic phases were washed with saturated aq NaCl solution (100 mL) and dried over MgSO₄. Afterwards, evaporation of the organic solvent in vacuo led to the product **8** (2.83 g, 6.54 mmol, 90%) as a white solid. ¹H NMR (300 MHz, [D₆]DMSO): δ(ppm)=8.06–7.88 (m, 3H; NH, aromatic CH), 7.76 (d, ³J(H,H)=8.0 Hz, 2H; aromatic CH), 7.41–7.24 (m, 10H; aromatic CH), 5.11–4.89 (m, 5H; CH₂, β-CH), 2.93–2.73 (m, 2H; CH₂). ¹³C NMR (126 MHz, [D₆]DMSO): δ=169.93 (COOBn), 155.29 (Cbz CONH), 144.00, 136.95, 135.93, 134.14, 128.26, 128.23, 127.83, 127.71, 127.66, 125.35 (aromatic C), 65.49, 65.34 (CH₂), 51.71 (β-C), 40.68 (CH₂). ESI-MS: *m/z*=448.2 [M+H]⁺, 470.2 [M+Na]⁺, 917.4 [2M+Na]⁺. ESI-HRMS: *m/z* calculated for C₂₄H₂₄BNO₆Na [M+Na]⁺: 470.1750, found: 470.1740.

Cbz-4-(3,3,5,5-tetramethyl-2,6-dioxopiperazine-1-yl)-D-β³-hPhg-OBn (10)

Under an oxygen atmosphere, Cbz-4-dihydroxyborane-D-β³-hPhg-OBn (**8**) (2.80 g, 6.47 mmol, 1.00 eq) was dissolved in DMSO (135 mL) and 3,3,5,5-tetramethylpiperazine-2,6-dione (**9**) (1.10 g, 6.47 mmol, 1.00 eq), Cu(OAc)₂ (1.17 g, 6.47 mmol, 1.00 eq), Et₃N (1.26 mL, 920 mg, 9.06 mmol, 1.40 eq) and powdered 4 Å molecular sieve (4.00 g) were added. The resulting suspension was stirred at rt in the presence of oxygen for 14 d. Then, the mixture was filtrated over Celite® and the filtrate was diluted with EtOAc (100 mL), H₂O (100 mL) and 2 M aq HCl (100 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with saturated aq NaCl solution (3 x 100 mL), dried over MgSO₄ and the solvent was removed under reduced pressure. Purification by flash-column chromatography (pentane/EtOAc, 1:1 → 1:3) led to the final product **13** (2.58 g, 4.62 mmol, 71%) as a white solid. ¹H NMR (300 MHz, [D₆]DMSO): δ=7.98 (d, ³J(H,H)=8.7 Hz, 1H; NH), 7.41 (d, ³J(H,H)=8.3 Hz, 2H; aromatic CH), 7.37–7.28 (m, 10H; aromatic CH), 7.05 (d, ³J(H,H)=8.3 Hz, 2H; aromatic CH), 5.14–4.94 (m, 5H; CH₂, β-CH), 2.91–2.82 (m, 2H; CH₂), 1.39 (s, 12H; CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ=176.50 (CONR₂), 169.85 (COOBn), 155.26 (Cbz CONH), 141.98, 136.82, 135.87, 134.93, 128.46, 128.25, 128.20, 127.81, 127.72, 127.67, 126.70 (aromatic C), 65.54, 65.39 (CH₂), 59.61 (C(CH₃)₂), 51.17 (β-C), 40.67 (CH₂), 27.95 (C(CH₃)₂). ESI-MS: *m/z*=558.3 [M+H]⁺, 580.3 [M+Na]⁺, 1137.5 [2M+Na]⁺. ESI-HRMS: *m/z* calculated for C₃₂H₃₅N₃O₆Na [M+Na]⁺: 580.2418, found: 580.2411.

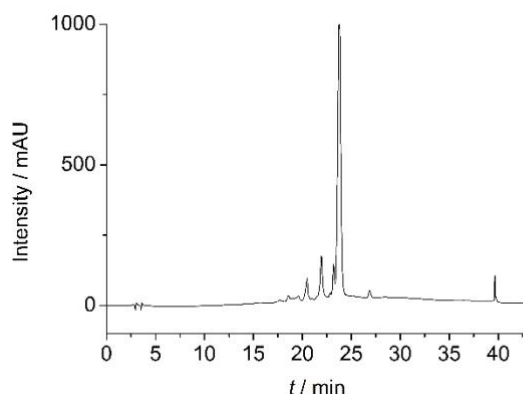
SUPPORTING INFORMATION

Fmoc-4-(3,3,5,5-tetramethyl-2,6-dioxopiperazine-1-yl)-D-β³-hPhg-OH (**11**)

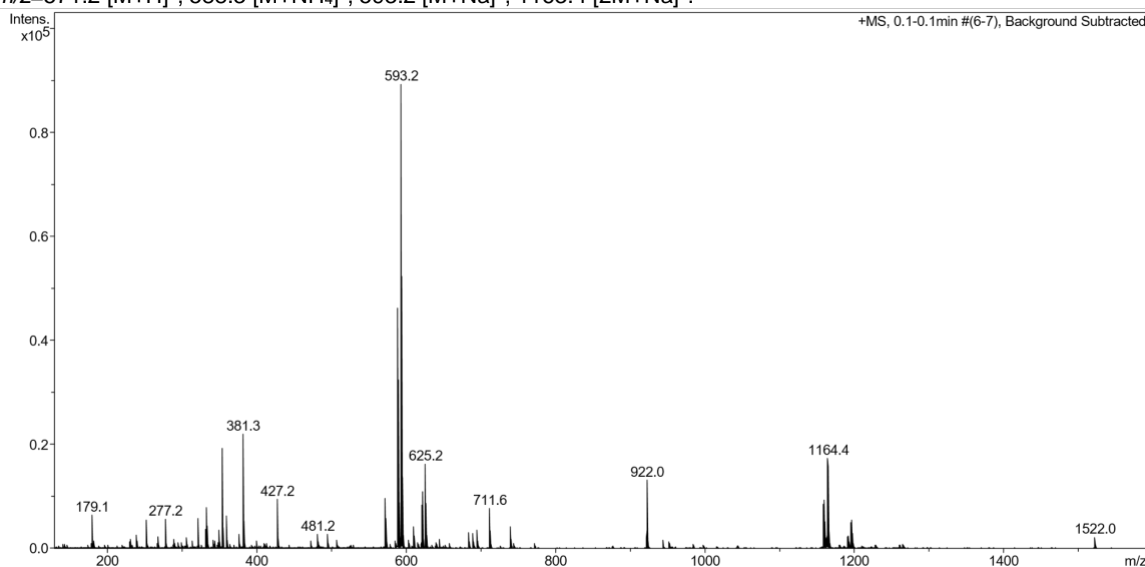
To a solution of Cbz-4-(3,3,5,5-tetramethyl-2,6-dioxopiperazine-1-yl)-D-β³-hPhg-OBn (**10**) (500 mg, 897 μmol, 1.00 eq) in MeOH (21.2 mL) and DCM (3.00 mL) Pd(OH)₂/C (50% H₂O, 101 mg, 717 μmol, 0.80 eq) was added. Hydrogen was passed through the suspension at rt for 1 h, and then, it was stirred at rt under a hydrogen atmosphere for 21 h. Afterwards, the mixture was first filtered through a pleated filter and then the filtrate was passed through a micron syringe filter. The solvent was removed in vacuo to give the crude unprotected amino acid. The crude intermediate product and NaHCO₃ (152 mg, 1.79 mmol, 2.00 eq) were dissolved in DMF (5.23 mL) and Fmoc-OSu (303 mg, 897 μmol, 1.00 eq) was added. Then, the reaction mixture was stirred at rt for 20 h. After, H₂O was added to the reaction and the aqueous phase was acidified to pH 2 with 2 M aq HCl. The phase was extracted with EtOAc (3 x 50.0 mL). The combined organic phases were washed with saturated aq NaCl solution (50.0 mL) and dried over MgSO₄. The solvent was evaporated, and the residue was purified by flash-column chromatography (DCM/MeOH/AcOH, 100:0:0.1 → 94:6:0.1) to provide the pure product **11** (419 mg, 750 μmol, 84%) as a white solid. ¹H NMR (300 MHz, [D₆]DMSO): δ=8.00 (d, ³J(H,H)=8.7 Hz, 1H; NH), 7.42 (d, ³J(H,H)=8.4 Hz, 2H; aromatic CH), 7.39–7.29 (m, 8H; aromatic CH), 7.06 (d, ³J(H,H)=8.4 Hz, 2H; aromatic CH), 5.08–4.96 (m, 1H; CH), 4.32–4.17 (m, 3H; CH₂, β-CH), 2.77–2.65 (m, 2H; CH₂), 1.41 (s, 12H; CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ=176.52 (CONR₂), 171.54 (COOH), 155.27 (Fmoc CONH), 143.77, 143.73, 142.50, 140.61, 134.78, 128.38, 127.49, 126.98, 126.70, 125.07, 119.97, 119.20 (aromatic C), 65.38 (CH₂), 55.31 (C(CH₃)₂), 51.19 (β-C), 46.65 (CH), 40.83 (CH₂), 27.96 (C(CH₃)₂). ESI-MS: *m/z*=556.3 [M+H]⁺, 578.3 [M+Na]⁺, 1111.5 [2M+H]⁺. ESI-HRMS: *m/z* calculated for C₃₂H₃₃N₃O₆Na [M+Na]⁺: 578.2262, found: 578.2242.

Fmoc-4-(3,3,5,5-Tetramethyl-2,6-dioxo-4-oxylpiperazine-1-yl)-d-β³-hPhg-OH (**1**)

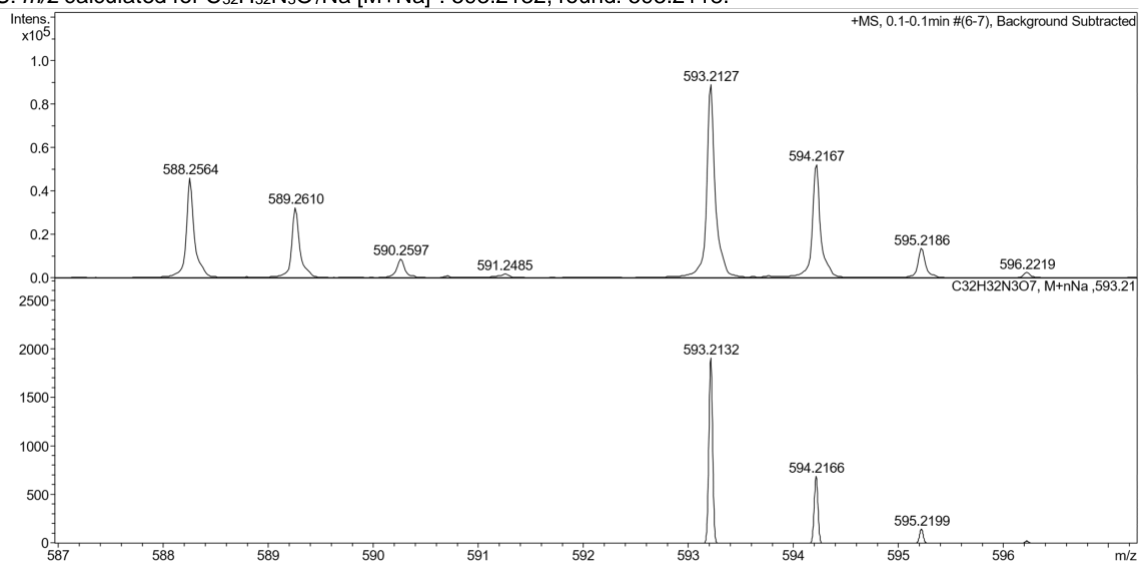
A solution of Fmoc-4-(3,3,5,5-tetramethyl-2,6-dioxopiperazine-1-yl)-d-β³-hPhg-OH (**11**) (413 mg, 743 μmol, 1.00 eq) dissolved in DCM (99.0 mL) was cooled to 0°C and treated with *m*-CPBA (70%, 366 mg, 2.12 mmol, 2.00 eq). The reaction mixture was stirred at 0°C for 15 min followed by stirring at rt for 5 h. The solvent was removed in vacuo. Purification of the crude product by flash-column chromatography (DCM/MeOH/AcOH, 100:0:0.1 → 98:2:0.1) led to the product **1** (288 mg, 505 μmol, 68%) as an orange solid. HPLC: (gradient 10 → 100% C in 30 min): *t_R* = 23.76 min.



ESI-MS: *m/z*=571.2 [M+H]⁺, 588.3 [M+NH₄]⁺, 593.2 [M+Na]⁺, 1163.4 [2M+Na]⁺.



SUPPORTING INFORMATION

ESI-HRMS: m/z calculated for $C_{32}H_{32}N_3O_7Na [M+Na]^+$: 593.2132, found: 593.2118.

SUPPORTING INFORMATION

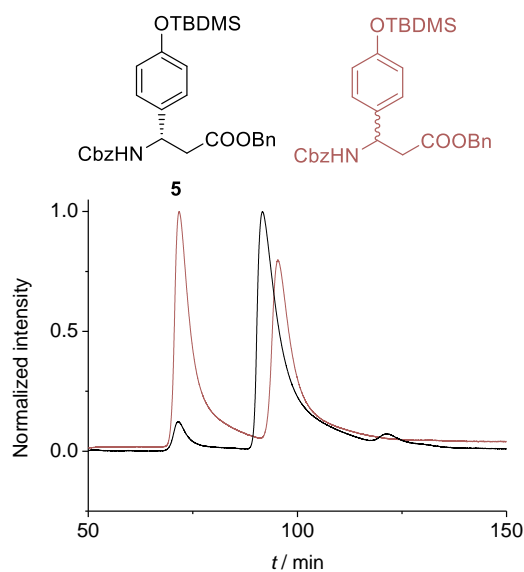
Racemization study β^3 -hTOPP

Figure S1. Investigation of compound **5** regarding optical purity. The optical purity was determined on a Shimadzu HPLC system. HPLC was performed using a Chiralpak® IA column and hexane/isopropanol as eluent (isocratic 92:8, flow 0.6 mL/min).

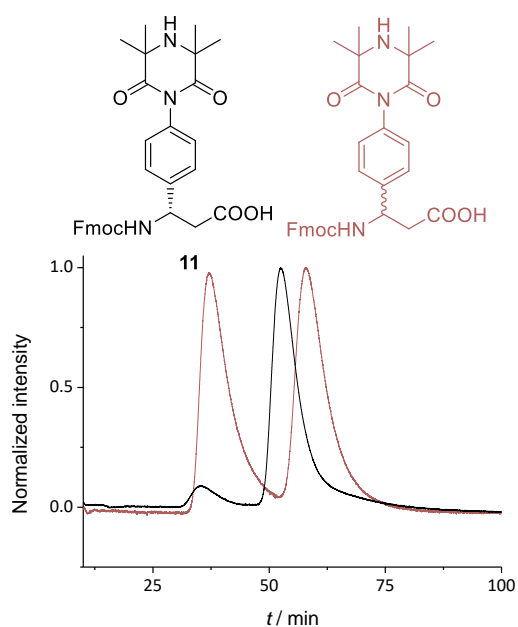


Figure S2. Investigation of compound **11** regarding optical purity. The optical purity was determined on a JASCO HPLC system. HPLC was performed using a Chiralcel® OD column and hexane/isopropanol.

SUPPORTING INFORMATION

Synthesis of the β^3 -amino acids

The Fmoc-protected D- β^3 -amino acids were synthesized according to the procedure described by Seebach and Diederichsen:^[4] Under an Ar atmosphere, a solution of the Fmoc-protected D- α -amino acid Fmoc-D-Lys(Boc)-OH, Fmoc-D-Trp(Boc)-OH or Fmoc-D-Val-OH (15.5 mmol, 1.00 eq) in dry THF (73.0 mL) was cooled to 0°C and Et₃N (1.10 eq) and *i*-BuOCOCI (1.10 eq) were added. The reaction mixture was stirred at 0°C for 30 min. Afterwards, diazomethane (0.6 M in Et₂O, 2.00 eq) was added under light exclusion. The mixture was warmed up to rt and stirred for 5 h. After, the reaction was quenched with AcOH (4.00 eq), 6% aq NaHCO₃ (100 mL) and EtOAc (100 mL) were added and the phases were separated. The aqueous phase was extracted with EtOAc (2 x 100 mL). The combined organic phases were washed with a saturated aq NH₄Cl solution (2 x 100 mL) and a saturated aq NaCl solution (2 x 100 mL), dried over MgSO₄ and the solvent was evaporated to give the desired diazo ketone. The crude diazo ketone was used without further purification steps. The diazo ketone was dissolved in THF/H₂O (9:1, 94.0 mL) and treated with AgOCOPh (0.10 eq) under light exclusion and sonication in an ultrasound bath at rt for 2 h. Then, H₂O and EtOAc were added and the aqueous phase was acidified with 2 M HCl solution to a pH of 2. The aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with saturated aq NaCl solution (3 x 50.0 mL) and dried over MgSO₄. Removal of the solvent in vacuo led to the desired crude D- β^3 -amino acid.

For purification the crude amino acids Fmoc-D- β^3 -hLys(Boc)-OH and Fmoc-D- β^3 -hTrp(Boc)-OH were dissolved in DCM (10 mL) and added slowly to cold pentane (-22°C, 1 L). The precipitation was filtered off and washed with cold pentane.

The crude Fmoc-D- β^3 -hVal-OH was purified via flash-column chromatography (DCM/MeOH/AcOH, 40:1:0.1).

Table S1. Yields of the synthesised β^3 -amino acids.

D- β^3 -amino acids	yield
Fmoc-D- β^3 -hLys(Boc)-OH	82%
Fmoc-D- β^3 -hTrp(Boc)-OH	79%
Fmoc-D- β^3 -hVal-OH	68%

Fmoc-D- β^3 -hLys(Boc)-OH

¹H NMR (300 MHz, [D₆]DMSO): δ =12.10 (s_{br}, 1H; COOH), 7.88 (d, ³J(H,H)=7.5 Hz, 2H; Fmoc CH_{Ar}), 7.69 (d, ³J(H,H)=7.2 Hz, 2H; Fmoc CH_{Ar}), 7.48–7.28 (m, 4H; Fmoc CH_{Ar}), 7.14 (d, ³J(H,H)=8.6 Hz, 1H; Fmoc NH), 6.75–6.61 (m, 1H; Boc CH), 4.35–4.15 (m, 3H; Fmoc CH, Fmoc CH₂), 3.85–3.67 (m, 1H; β -CH), 2.88 (q, ³J(H,H)=6.6 Hz, 2H; ζ -CH), 2.41–2.24 (m, 2H; α -CH₂), 1.49–1.12 (m, 15H; γ -CH₂, δ -CH₂, ε -CH₂, Boc CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ =172.13 (COOH), 155.30, 155.26 (Boc CONH, Fmoc CONH), 143.73, 143.60, 140.49 (aromatic C), 127.39, 127.34, 126.80, 126.78, 124.96, 124.93, 119.85 (aromatic CH), 77.15 (C(CH₃)₃), 65.02 (Fmoc CH₂), 47.82 (β -CH), 46.75 (Fmoc CH), 33.82 (ε -CH₂), 29.23 (γ -CH₂), 28.20 (C(CH₃)₃), 22.69 (ζ -CH₂). ESI-MS: *m/z*=505.3 [M+Na]⁺, 987.5 [2M+Na]⁺, 481.3 [M-H]⁻. ESI-HRMS: *m/z* calculated for C₂₇H₃₄N₂O₆Na [M+Na]⁺: 505.2309, found: 505.2311.

Fmoc-D- β^3 -hTrp(Boc)-OH

¹H NMR (300 MHz, [D₆]DMSO): δ =12.30 (s_{br}, 1H; COOH), 8.03 (d, ³J(H,H)=8.2 Hz, 1H; aromatic CH), 7.87 (d, ³J(H,H)=7.6 Hz, 2H; aromatic CH), 7.70 (d, ³J(H,H)=7.8 Hz, 2H; aromatic CH), 7.65–7.58 (m, 2H; aromatic CH), 7.51 (s, 1H; aromatic CH), 7.46–7.15 (m, 7H; NH, aromatic CH), 4.30–4.00 (m, 4H; Fmoc CH, Fmoc CH₂, β -CH), 2.92–2.81 (m, 2H; α -CH₂), 2.53–2.44 (γ -CH₂, overlapped with the DMSO signal), 1.55 (s, 9H; CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ =172.13 (COOH), 155.24 (Fmoc CONH), 148.78 (Boc CONH), 143.57, 140.44, 134.57, 132.54, 130.54, 129.01, 128.29, 127.33, 126.75, 124.93, 124.89, 124.04, 123.45, 122.25, 119.83, 119.05, 117.17, 114.50 (aromatic C), 83.26 (C(CH₃)₃), 65.24 (Fmoc CH₂), 47.95 (β -CH), 46.66 (Fmoc CH), 40.11–39.02 (α -CH₂ overlapped with DMSO signal), 29.54 (γ -CH₂), 27.59 (C(CH₃)₃). ESI-MS: *m/z*=541.2 [M+H]⁺, 563.2 [M+Na]⁺, 1103.4 [2M+Na]⁺, 539.3 [M-H]⁻. ESI-HRMS: *m/z* calculated for C₃₂H₃₂N₂O₆Na [M+Na]⁺: 563.2153, found: 523.2141.

Fmoc-D- β^3 -hVal-OH

¹H NMR (300 MHz, [D₆]DMSO): δ =12.07 (s_{br}, 1H; COOH), 7.87 (d, ³J(H,H)=7.4 Hz, 2H; aromatic CH), 7.77–7.64 (m, 2H; aromatic CH), 7.41 (t, ³J(H,H)=7.4 Hz, 2H; aromatic CH), 7.36–7.27 (m, 2H; aromatic CH), 4.38–4.11 (m, 3H; Fmoc CH, Fmoc CH₂), 3.82–3.67 (m, 1H; β -CH), 2.46–2.23 (m, 2H; α -CH₂), 1.80–1.65 (m, 1H; CH), 0.83 (d, ³J(H,H)=6.7 Hz, 6H; CH₃). ¹³C NMR (126 MHz, [D₆]DMSO): δ =172.75 (COOH), 155.28 (Fmoc CONH), 143.95, 143.77, 140.66, 140.65 (Fmoc C_{Ar}), 127.52, 126.99, 126.95, 125.19, 125.16, 120.01, 120.00 (Fmoc CH_{Ar}), 65.16 (Fmoc CH₂), 53.00 (β -CH), 46.78 (Fmoc CH), 36.69 (α -CH₂), 31.70 (CH), 18.81, 17.96 (CH₃). ESI-MS: *m/z*=354.2 [M+H]⁺, 376.1 [M+Na]⁺, 729.3 [2M+Na]⁺, 352.2 [M-H]⁻, 705.4 [2M-H]⁻. ESI-HRMS: *m/z* calculated for C₂₁H₂₃NO₄Na [M+Na]⁺: 376.1519, found: 376.1518.

SUPPORTING INFORMATION

Synthesis of the β^3 -peptides

Peptides **P1–P5** were synthesised using manual microwave-assisted Fmoc SPPS on a NovaPEG Rink Amide resin LL (0.19 mmol/g, 50.0 μ mol, 263 mg) using a Discover microwave (CEM, North Carolina, USA). In a BD Discardit II syringe (Becton Dickinson, Fraga, Spain) equipped with a PE frit, the resin (1.00 eq) was swollen in DCM at rt for 30 min. Then, it was washed with NMP (5 x) followed by microwave-assisted Fmoc deprotection with 20% piperidine in NMP (1: 50°C, 25 W, 30 s; 2: 50°C, 25 W, 3 min). Between the two deprotection steps the resin was washed with NMP (3 x) and afterwards with NMP, DCM, DMF (10 x each) and NMP (3 x). For the loading of the resin a solution of Fmoc-D- β^3 -hLys(Boc)-OH (5.00 eq), HOBt (5.00 eq) and DIC (5.00 eq) in NMP was added and the coupling was performed by microwave irradiation (60 °C, 35 W, 15 min). Double coupling was carried out. Between the coupling steps the resin was washed with NMP (3 x) and after final coupling thoroughly with NMP, DCM, DMF (10 x each). Afterwards, a solution of Ac₂O/2,6-lutidine/NMP (1:2:7, v/v/v) was added to the swelled resin and the mixture was shaken at rt for 5 min to acetylate free amine functions. The capping solution was removed, and the procedure was repeated. After removing of the reaction mixture, the resin was washed thoroughly with NMP, DCM (10 x each) and DMF (5 x).

Each coupling cycle was started with microwave-assisted double Fmoc deprotection by adding 20% piperidine in NMP (1: 50°C, 25 W, 30 s; 2: 50°C, 25 W, 3 min) and washing between the deprotection steps with NMP (3 x) and afterwards with NMP and DCM (10 x each), DMF (5 x) and NMP (3 x). The relevant β -amino acid (Fmoc-D- β^3 -hLys(Boc)-OH, Fmoc-D- β^3 -hTrp(Boc)-OH or Fmoc-D- β^3 -hVal-OH) (4.00 eq) and the activation reagents HOAt/HATU (4.00 eq/3.90 eq) were placed in a small sample vessel and a solvent mixture of NMP/DMF/DMSO (600 μ L, 1:0.8:0.2, v/v/v) was added and the solution was sonicated. Then, the activation bases (2,6-lutidine/DIEA, 4.80 eq/3.20 eq) were added and the mixture was subjoined with the resin. The coupling was performed using microwave irradiation (60°C, 25 W, 15 min). Each amino acid was double coupled (3.00 eq amino acid; 2,6-lutidine/DIEA, 3.60 eq/2.40 eq) and the resin was washed between the coupling steps with NMP (3 x) and afterwards thoroughly with NMP and DCM (10 x each), DMF (5 x) and NMP (3 x). The coupling conditions described above were changed after the 7th coupled amino acid. Then, the solvent mixture contained 0.8 M LiCl in NMP/DMF/DMSO (1:0.8:0.2, v/v/v), the power was reduced to 15 W and the reaction time was increased to 30 min (for every 4th amino acid after the β^3 -hTOPP it was increased to 35 min and for the last 5 amino acids to 40 min coupling time). Upon completion of the sequence, the resin was dried in vacuo. The peptide cleavage from the resin and simultaneous deprotection of the protecting groups were performed at rt for 2 h in a mixture of TFA/H₂O/TIS (95:2.5:2.5, v/v/v). After cleavage of the peptide from the resin, the resulting solution was concentrated in a nitrogen stream and the addition of ice-cold Et₂O led to precipitation of the peptide. The resulting suspension was centrifuged at -5°C followed by decanting of the supernatant and washing of the peptide pellet with ice-cold Et₂O (3 x). The crude peptide was dried in vacuo.

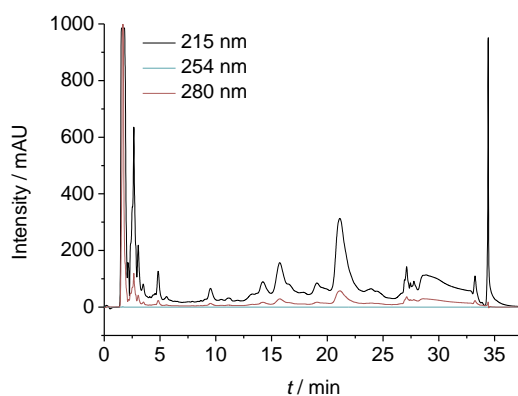
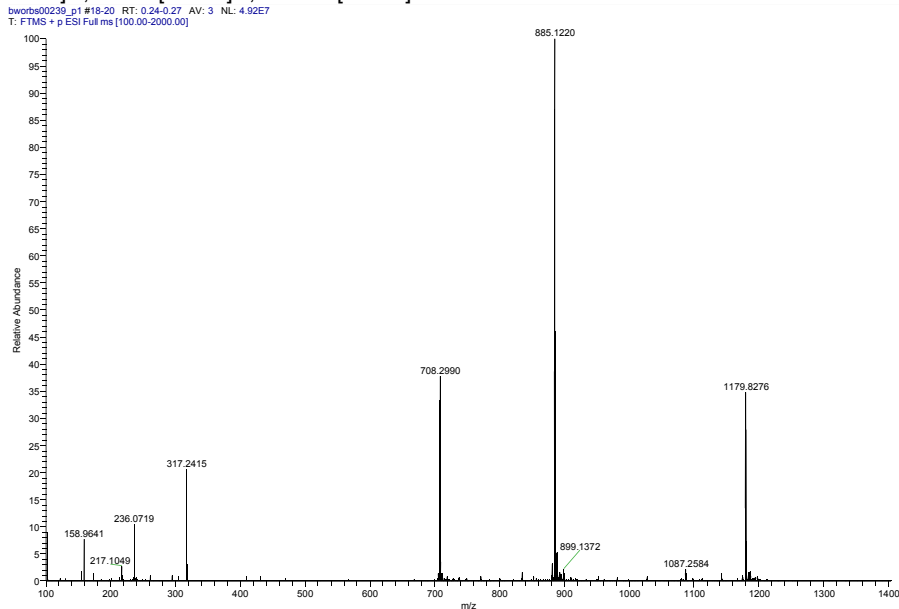
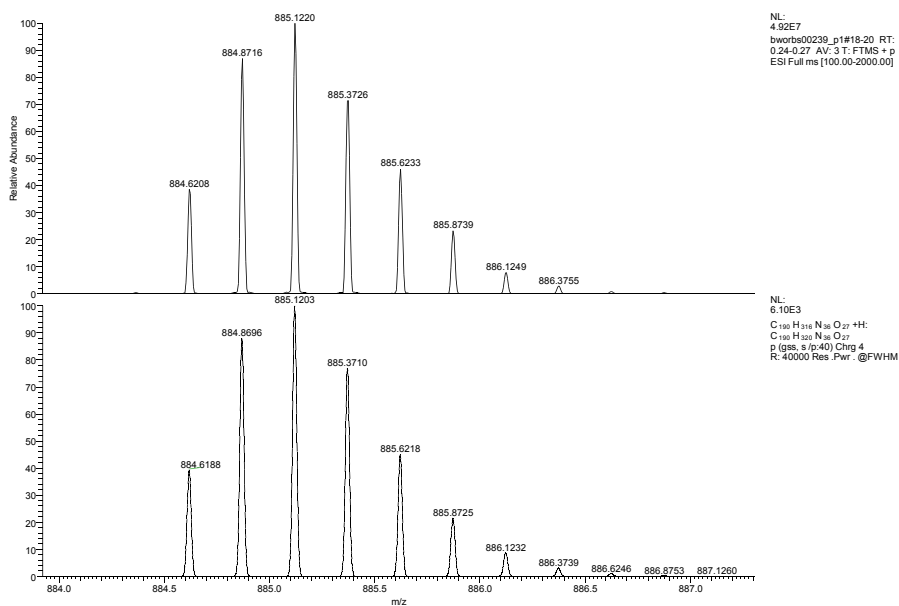
Coupling of the β^3 -hTOPP

The first β^3 -hTOPP label was coupled using 2.00 eq of Fmoc-d- β^3 -hTOPP-OH (**1**), the activation reagents HOAt/HATU (2.00 eq/1.90 eq) and the activation bases 2,6-lutidine/DIEA (2.40 eq/1.60 eq) in a solvent mixture of NMP/DMF/DMSO (1:0.8:0.2, v/v/v) and microwave irradiation (60°C, 25 W, 15 min). The coupling of the second label was carried out in a solvent mixture of 0.8 M LiCl in NMP/DMF/DMSO (1:0.8:0.2, v/v/v) and by microwave irradiation (60°C, 15 W, 35 min) using the same amount of reagents like in the case of the first label. Afterwards, the resin was successively washed with NMP and DCM (10 x each), DMF (5 x) and NMP (3 x).

Oxidation of the reduced label

The label was re-oxidized according to a similar procedure described in literature.^[3] The crude peptide (1.00 eq) was dissolved in MeCN/MeOH (1:1, 100 μ L for 2 mg), Cu(OAc)₂ (3.00 eq for each TOPP label) was added and the resulting mixture was stirred at rt for 2 h followed by purification via HPLC.

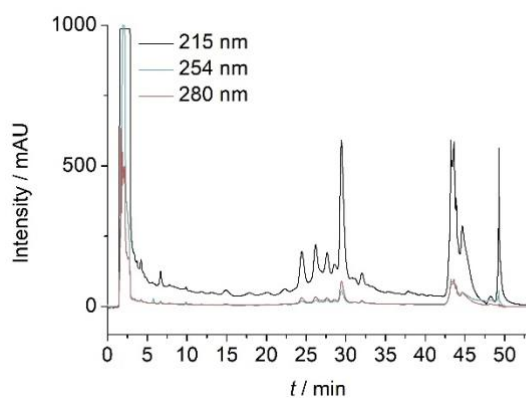
SUPPORTING INFORMATION

H-hLys₂-hTrp₂-hVal₁₉-hTrp₂-hLys₂-NH₂ (**P1**)HPLC: (60°C, gradient 70 → 90% C in 30 min): $t_R = 21.09$ min.ESI-MS: $m/z=708.3$ [M+5H]⁵⁺, 885.1 [M+4H]⁴⁺, 1179.8 [M+3H]³⁺.ESI-HRMS: m/z calculated for C₁₉₀H₃₂₀N₃₆O₂₇ [M+4H]⁴⁺: 885.1203, found: 885.1220.

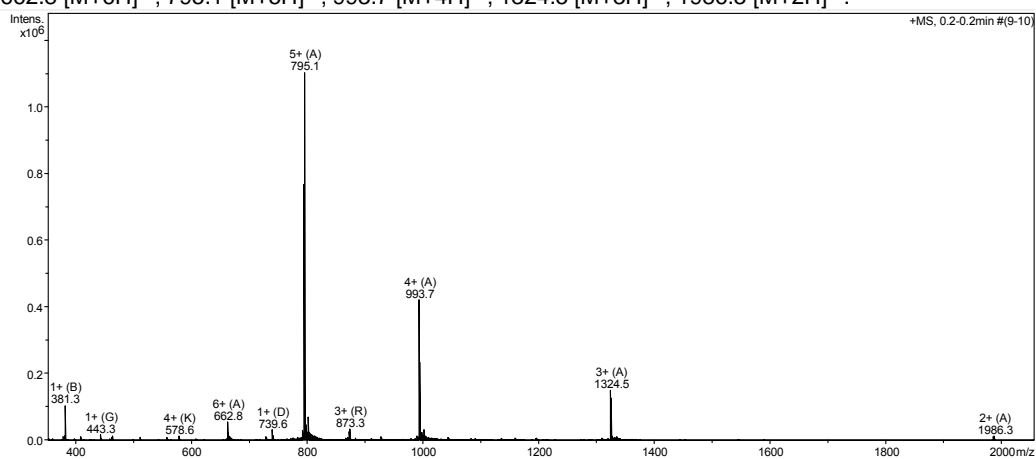
SUPPORTING INFORMATION

H-hLys₂-hTrp₂-hVal₄-hTOPP-hVal₁₂-hTOPP- hVal-hTrp₂-hLys₂-NH₂ (**P2**)

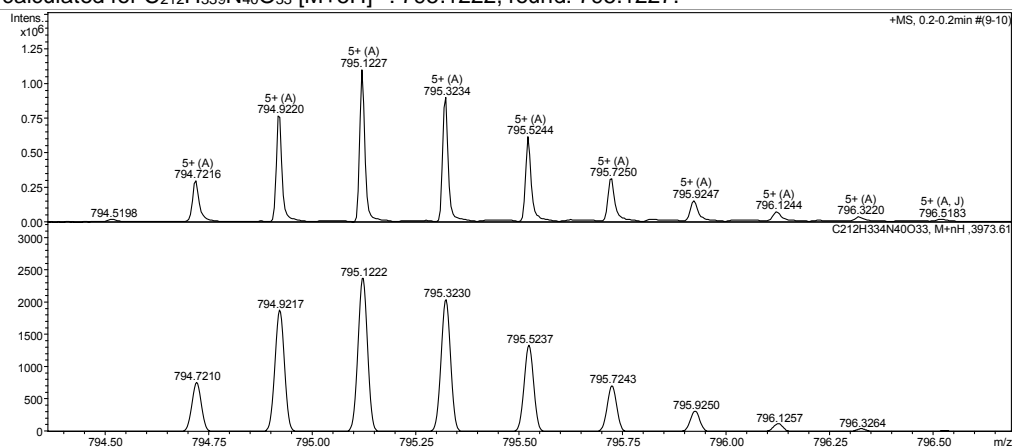
HPLC: (60°C, gradient 68 → 80% C in 40 min): $t_R = 29.43$ min.



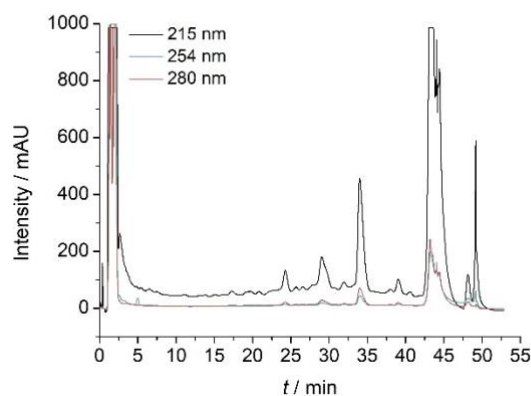
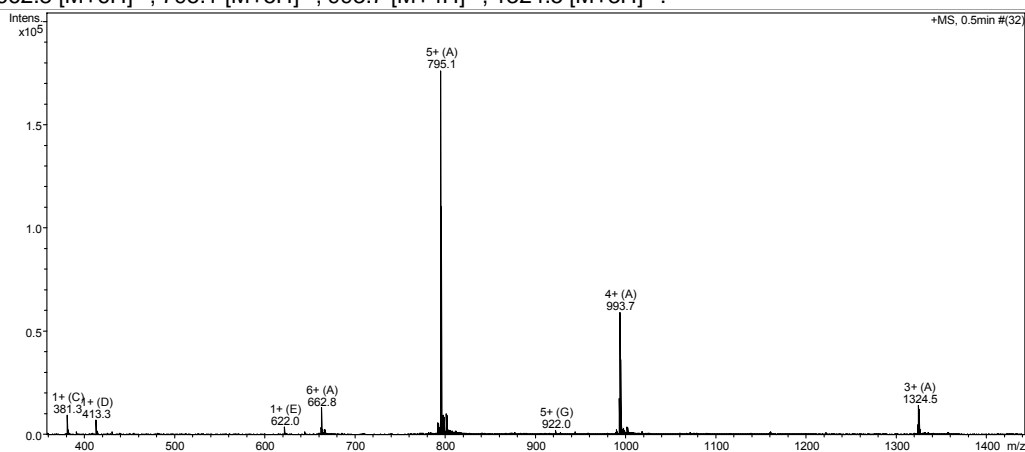
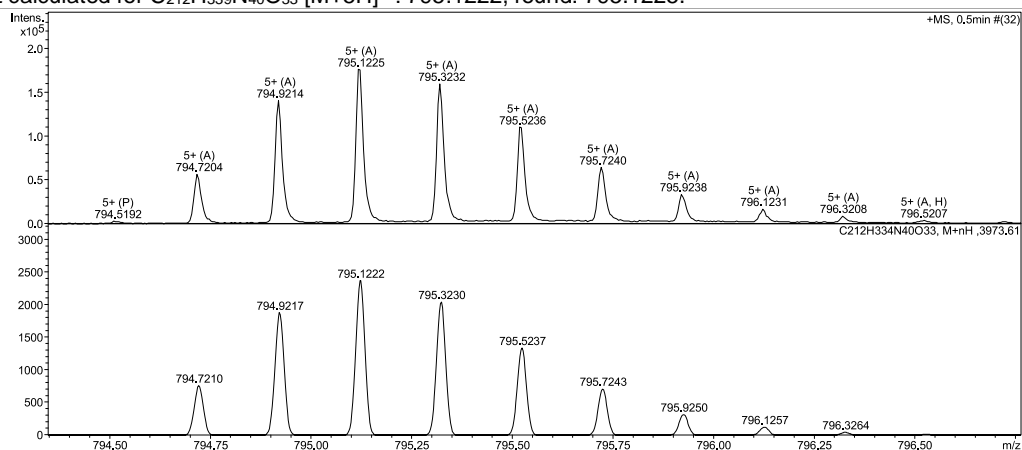
ESI-MS: $m/z = 662.8$ $[M+6H]^{6+}$, 795.1 $[M+5H]^{5+}$, 993.7 $[M+4H]^{4+}$, 1324.5 $[M+3H]^{3+}$, 1986.3 $[M+2H]^{2+}$.



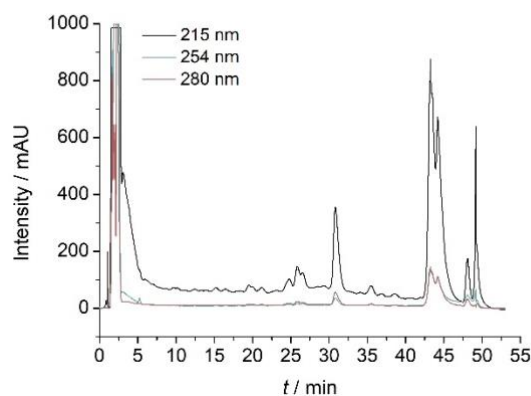
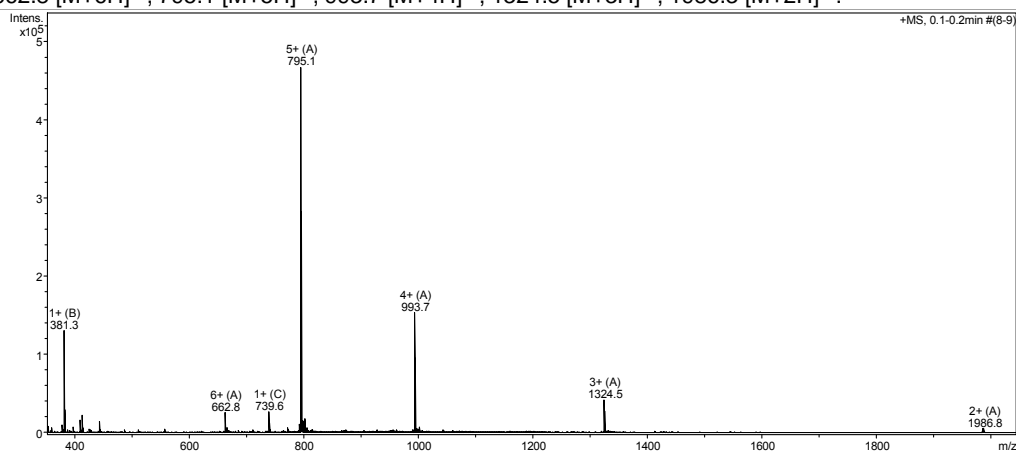
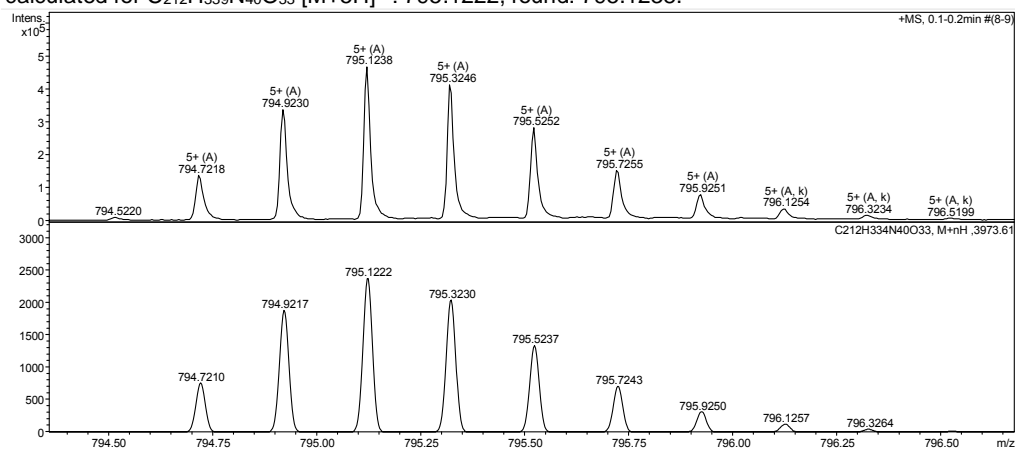
ESI-HRMS: m/z calculated for $C_{212}H_{339}N_{40}O_{33}$ $[M+5H]^{5+}$: 795.1222, found: 795.1227.



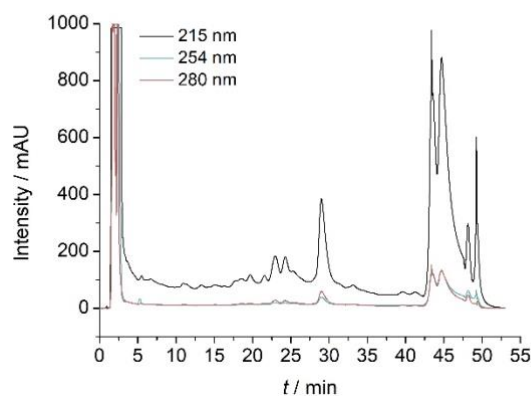
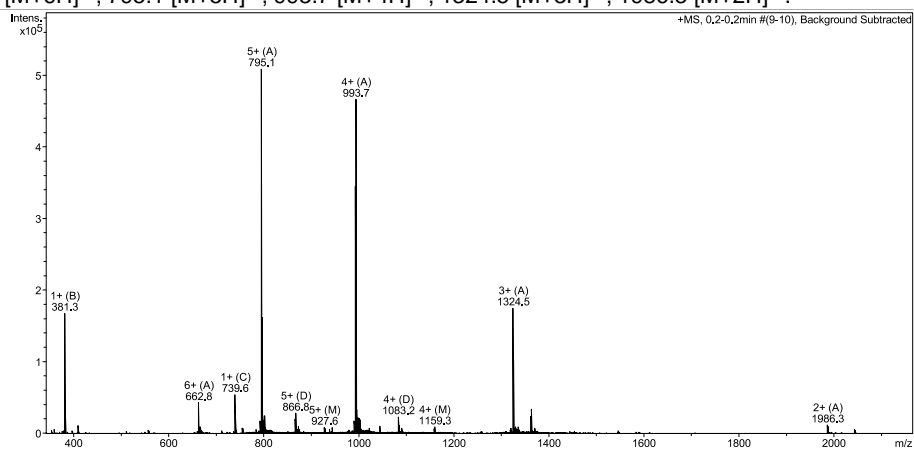
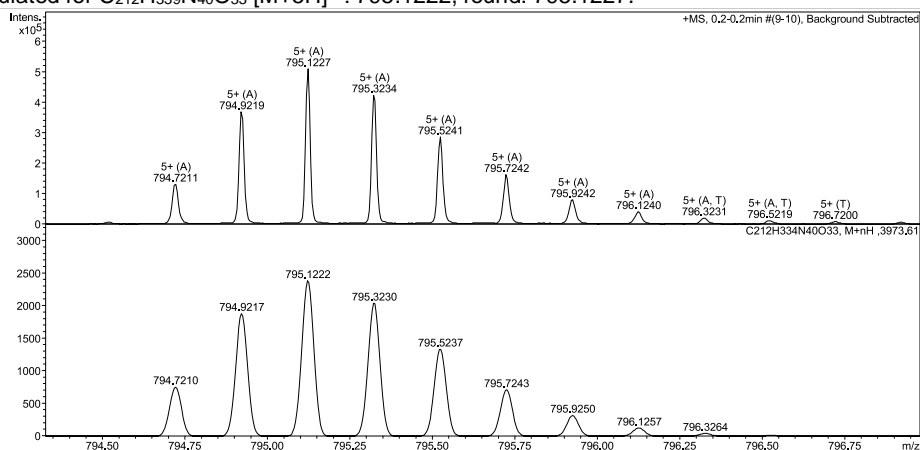
SUPPORTING INFORMATION

H-hLys₂-hTrp₂-hVal₃-hTOPP-hVal₁₃-hTOPP- hVal-hTrp₂-hLys₂-NH₂ (**P3**)HPLC: (60°C, gradient 69 → 79% C in 40 min): $t_R = 33.93$ min.ESI-MS: $m/z = 662.8$ [M+6H]⁶⁺, 795.1 [M+5H]⁵⁺, 993.7 [M+4H]⁴⁺, 1324.5 [M+3H]³⁺.ESI-HRMS: m/z calculated for C₂₁₂H₃₃₉N₄₀O₃₃ [M+5H]⁵⁺: 795.1222, found: 795.1225.

SUPPORTING INFORMATION

H-hLys₂-hTrp₂-hVal₂-hTOPP-hVal₁₄-hTOPP-hVal-hTrp₂-hLys₂-NH₂ (**P4**)HPLC: (60°C, gradient 68 → 80% C in 40 min): $t_R = 30.80$ min.ESI-MS: $m/z = 662.8$ [M+6H]⁶⁺, 795.1 [M+5H]⁵⁺, 993.7 [M+4H]⁴⁺, 1324.5 [M+3H]³⁺, 1986.3 [M+2H]²⁺.ESI-HRMS: m/z calculated for C₂₁₂H₃₃₉N₄₀O₃₃ [M+5H]⁵⁺: 795.1222, found: 795.1238.

SUPPORTING INFORMATION

H-hLys₂-hTrp₂-hVal-hTOPP-hVal₁₅-hTOPP-hVal-hTrp₂-hLys₂-NH₂ (**P5**)HPLC: (60°C, gradient 68 → 80% C in 40 min): $t_R = 29.01$ min.ESI-MS: $m/z = 662.8$ [M+6H]⁶⁺, 795.1 [M+5H]⁵⁺, 993.7 [M+4H]⁴⁺, 1324.5 [M+3H]³⁺, 1986.3 [M+2H]²⁺.ESI-HRMS: m/z calculated for C₂₁₂H₃₃₉N₄₀O₃₃ [M+5H]⁵⁺: 795.1222, found: 795.1227.

SUPPORTING INFORMATION

CD experiments

Measurements in solution (TFE and MeOH) were performed at 10°C using a peptide concentration of 10 μM . Experiments in lipids (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)) were performed using a peptide/lipid (P/L) ratio of 1/20 in a 50 mM sodium phosphate buffer (pH 7.5) and a peptide concentration of 20 μM at 20°C. For the preparation of the SUVs, solutions of the peptide in MeOH and the lipid in CHCl_3 were mixed followed by removing of the solvents in a nitrogen stream. The resulting lipid film was dried over night in vacuo at 50°C. The buffer was added, and the film swelled at rt for 2 h, followed by vortexing the mixture for 1 min at 5 min intervals (3 \times). To form SUVs the mixture was treated with ultrasound sonifier sonoplus HD2076 (BANDELIN, Berlin, Germany; 30 min, Cycle 4, 60% power).

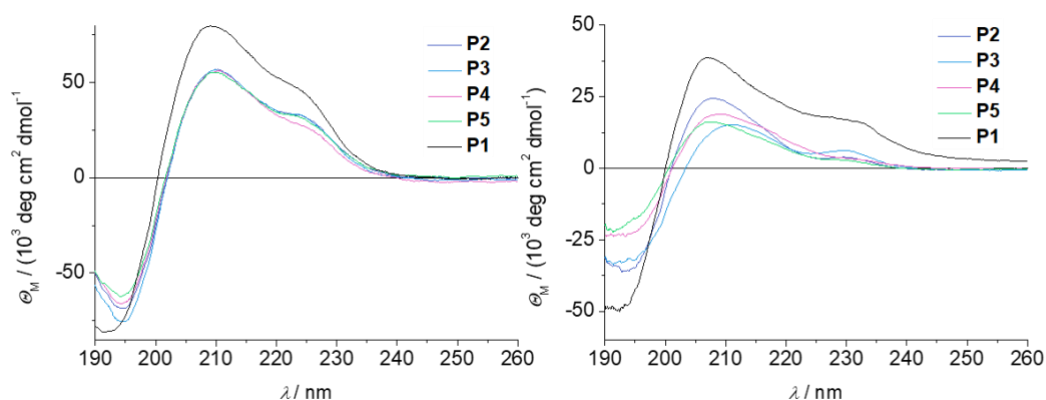


Figure S3. CD investigation of the β^3 -peptides **P2**, **P3**, **P4**, **P5** and the reference peptide **P1** in TFE and DOPC. Left: CD spectra recorded in TFE. Right: CD spectra recorded in DOPC. The data illustrate an average of three spectra (**P2**, **P3** and **P4**) and of two spectra (**P5**) (see **Figure S4**).

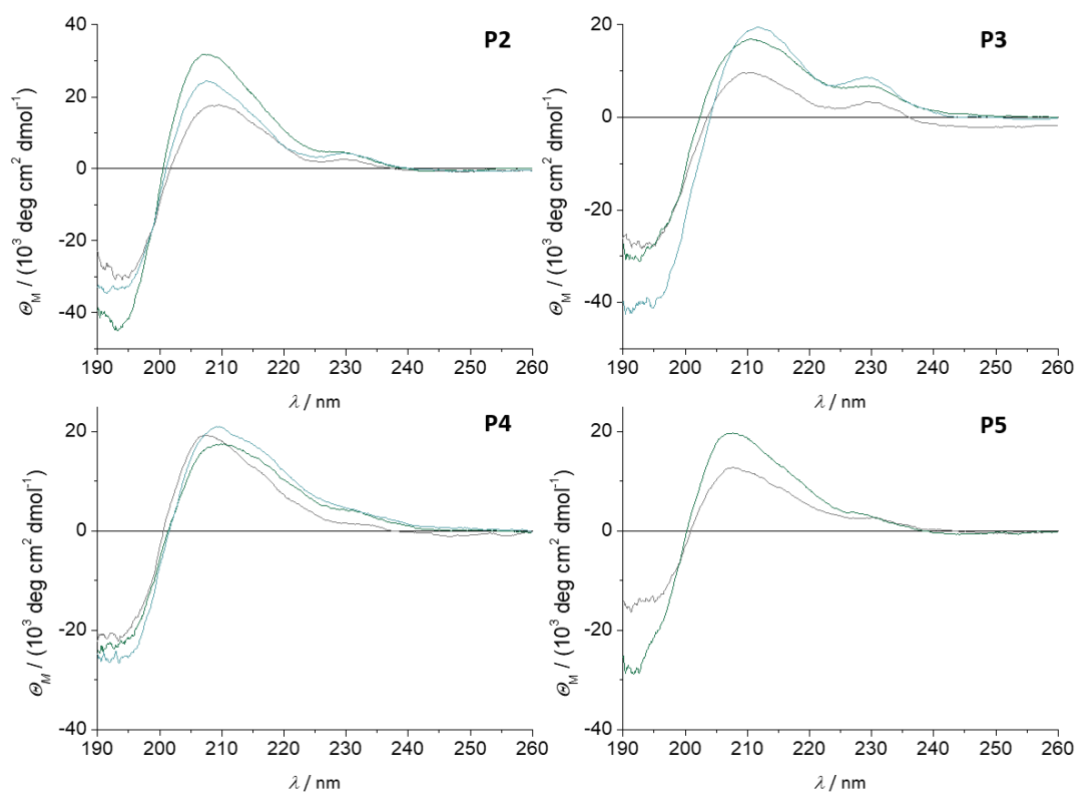


Figure S4. Individual CD spectra of the β^3 -peptides **P2**, **P3**, **P4** and **P5** in DOPC.

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Preparation of EPR samples

Samples for EPR experiments on β -TOPP labeled peptides in methanol were prepared by dissolving the peptide in MeOH containing 20% glycerol. Peptide concentrations, as determined by UV absorbance of the tryptophans, were adjusted to 50 μ M. Labelling efficiency was estimated between 50-75 % based on comparison with modulation depths of samples with 100% labelling efficiency.^[5] Sample volumes of 20 μ L were transferred into EPR quartz tubes (1.6 mm O.D., 1 mm I.D.) to perform continuous wave (CW)-EPR experiments. Samples of the peptides in multilamellar vesicles (MLVs) of POPC in TrisHCl buffer (20 mM, pH = 7.4) were prepared either using D₂O-buffer and protonated lipid (POPC) or H₂O-buffer and deuterated lipid (D31-POPC). Stock solutions of POPC or D31-POPC (25 mg/mL) in chloroform and the β^3 -hTOPP-labeled peptide in methanol were mixed yielding a spin/phospholipid molar ratio of approximately 1/3000. Solvents were removed under a stream of nitrogen followed by 3 h vacuum drying. TrisHCl buffer (20 mM, pH = 7.4) was added to the dry β -peptide/phospholipid mixture yielding spin concentrations of about 40 μ M. For the diluted sample of **P2**, additional stock solutions of D31-POPC (25 mg/mL) in chloroform and the β^3 -hTOPP-labeled peptide in methanol were mixed yielding a spin/phospholipid molar ratio of approximately 1/6000 (corresponding P/L = 1/3000) and a spin concentration of 20 μ M. After 1 h of incubation, 20 μ L of sample were transferred into an EPR quartz tube (1.6 mm O.D., 1 mm I.D.) and used for CW-EPR experiments. All EPR samples were frozen in liquid nitrogen before performing pulse-EPR experiments.

Continuous wave (CW) EPR experiments

CW-EPR spectra of β^3 -hTOPP labeled peptides in MeOH (20% glycerol) were recorded using sample volumes of 20 μ L at a nominal peptide concentration of 50 μ M.

To distinguish whether the nitroxide spin labels are exposed to the solution or to the lipid environment, CW-EPR spectra of the four β^3 -hTOPP labeled peptides in MLV were recorded. In comparison with CW-EPR spectra recorded in methanol, which already display the intrinsically restricted mobility of the label (high field hyperfine line is strongly reduced in intensity) in MLV the three nitroxide EPR lines display additional broadening, particularly at the high field site (Figure S5). The effect can be clearly assigned to an additional restriction in mobility of the β^3 -hTOPP spin label due to the lipid environment.

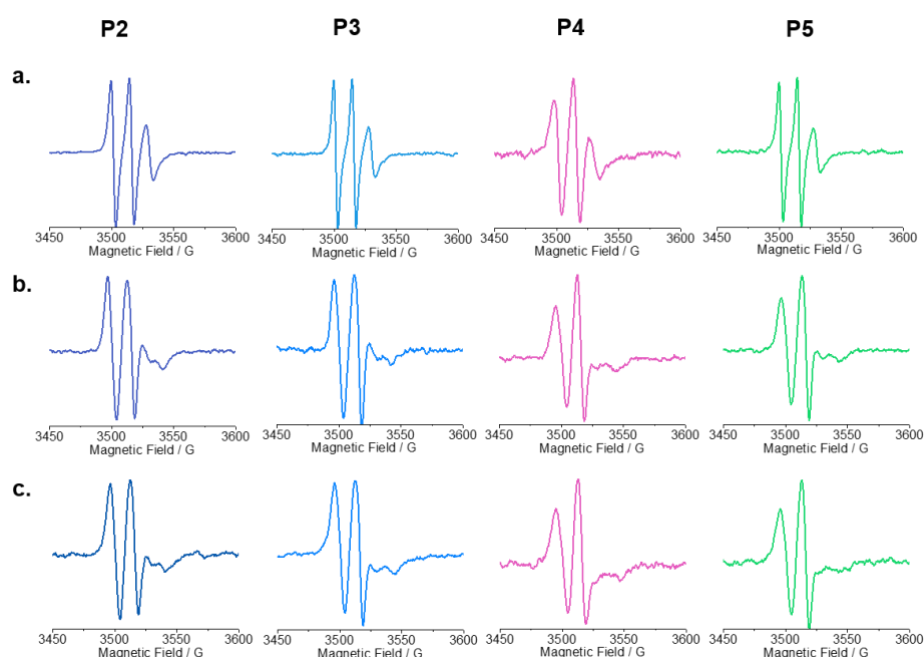


Figure S5. Room temperature, continuous wave (CW)-EPR spectra recorded at X-band frequencies. β^3 -hTOPP peptide samples were prepared in **a.** MeOH (20% glycerol); **b.** MLVs with TrisHCl buffer, using D31-POPC and H₂O-buffer; **c.** MLVs with TrisHCl buffer, using POPC and D₂O-buffer. Instrumental settings were the following: $\nu=9.874$ GHz; center field=3520 G; sweep width=200 G; microwave power=2 mW; modulation frequency=100 kHz; modulation amplitude=1 G; conversion time=5.12 ms; sweep time=5.24 s. The number of scans varied in a range from 100 to 500 according to the intensity of the EPR signal.

Pulsed EPR/ESEEM experiments

Electron spin echo envelope modulation (ESEEM) experiments were performed at Q-band (34 GHz) using the three-pulse sequence $\frac{\pi}{2} - \tau - \frac{\pi}{2} - T - \frac{\pi}{2}$ with a $\pi/2$ pulse length of 8–10 ns. In order to avoid *blind spots*, a 2D experiment was carried out. In the x-axis 512 points were acquired with increasing the delay time T between the second and the third $\frac{\pi}{2}$ pulse with a time-step of 4 ns. In the y-axis the delay

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time τ between the first and the second $\frac{\pi}{2}$ pulse was increased with a time-step of 16 ns starting from 254 ns to 398 ns. A 4-step phase cycle was applied to suppress artefacts due to an overlap between echoes. The area of the stimulated echo was integrated fixing a symmetric gate around the center of the EPR signal with a length between 8 and 14 ns. 20 shots per points were acquired with a shot repetition time of 3 ms. The microwave frequency was set to the center of the resonator dip and the magnetic field adjusted to the maximum of the EPR signal. The number of scans varied between 15 and 20. Each spectrum was converted from the time domain to the frequency domain applying Fourier transformation. Firstly, the experimental data were background corrected using a 1st order polynomial function. Then, a zero-filling procedure was used achieving 2048 points in order to improve the resolution of the Fourier transformation procedure. A Hamming windowing function allowed for smoothing the exponential decay of the stimulated echo till zero. Fourier transformation was applied focusing on a range of frequencies from 5 to 60 MHz including the Larmor frequencies of deuterons and protons at Q-band frequencies. The strongest deuterium modulation was observed at $\tau = 318$ ns. This effect was very strong if the peptides were prepared in D31-POPC and H₂O-buffer. If POPC and D₂O-buffer were used, only a residual deuterium ESEEM was observed, which is in agreement with some penetration of water molecules into the lipid bilayer. In conclusion the ESEEM experiments suggest a proper integration of the peptides into the lipid bilayer (Figure S6). Sample temperature was 50 K.

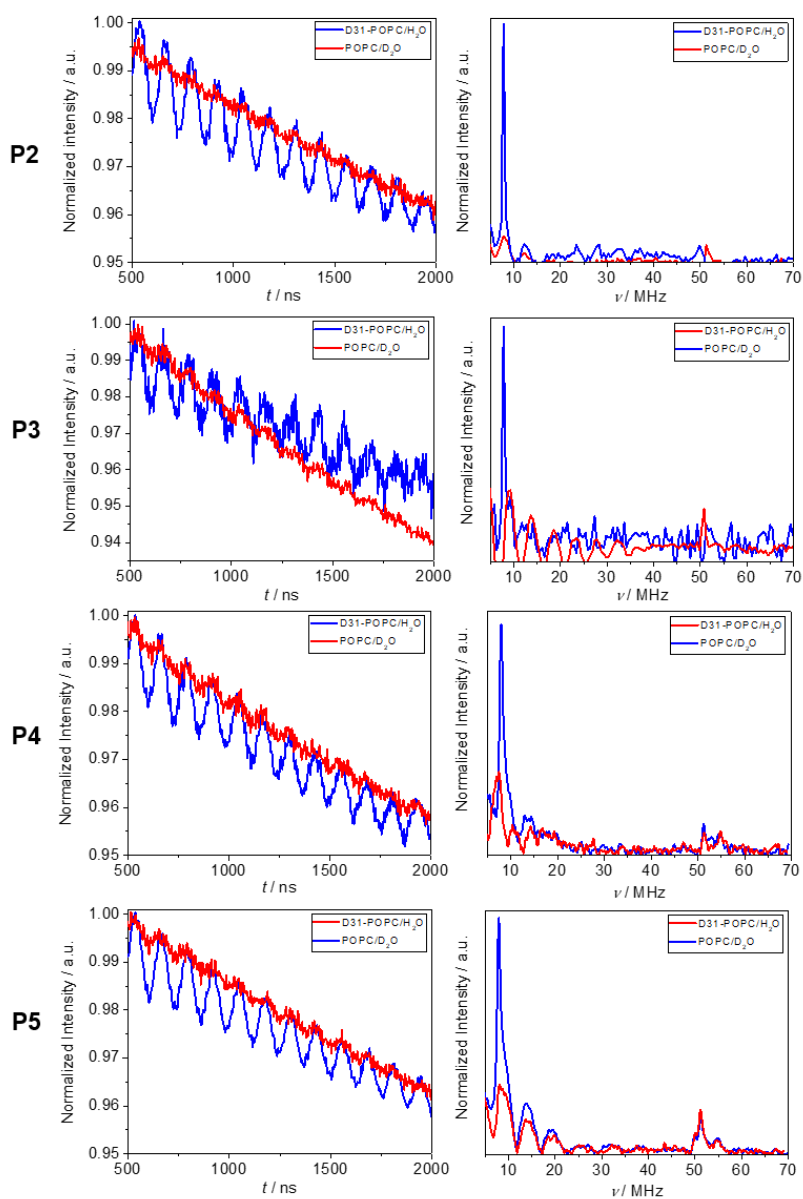


Figure S6. 3-pulse ESEEM spectra of β^3 -hTOPP labeled peptides prepared in MLVs of POPC in D₂O-buffer (red line) and D31-POPC in H₂O-buffer (blue line). The shown EPR spectra were recorded using $\tau = 318$ ns and are reported in time domain (left) and in frequency domain (right) after Fourier transformation. At Q-band frequencies, deuterons show a Larmor frequency of $\nu_D = 7.8$ MHz; for protons, $\nu_H = 50.9$ MHz.

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All PELDOR/DEER experiments were performed at a temperature of 50 K under strong resonator overcoupling conditions in order to ensure a broad resonance dip. The standard 4-pulse DEER sequence (see references in main text) was adopted fixing ν_{pump} 30-50 MHz over the center of the dip in the maximum of the nitroxide line and ν_{detect} 90 MHz below ν_{pump} . This set-up allows to generate short pulses with large bandwidths for both pumping and detection frequencies. The large bandwidth of the pulses suppresses orientation selection effects as we showed in a previous detailed study.^[5] Samples in MeOH were measured with typical π -pulse length of 12 ns at ν_{pump} and 20/22 ns at ν_{detect} . The typical π -pulse length was 12/14 ns at ν_{pump} and 14/16 ns at ν_{detect} for the samples in MLV. Time delay between the first $\frac{\pi}{2}$ and the second π detection pulses was set to 400 ns. The dipolar evolution time between the Hahn-echo sequence and the third detection pulse varied in a range between 1.2 and 4.3 μs . The pump π pulse was applied at 500 ns and moved with a time-step of 4-8 ns with the last pulse applied 100 ns before the last detection π pulse to avoid an overlap between pump and detection pulses. However, some artifacts appeared in the last 100-800 ns of the experimental PELDOR/DEER trace. Therefore, the last part of the trace was cut during data analysis. The area of the refocused echo was integrated fixing a symmetric gate around the center of the EPR signal with a length between 20 and 34 ns to reduce the noise of the PELDOR/DEER trace. 50 shots per points were acquired with a shot repetition time of 4 ms. Typical acquisition times were on the order 10 to 22 h. The number of scans recorded per hour varied between 39 and 95 per hour depending on the length of the respective PELDOR/DEER trace. For data analysis, the program DeerAnalysis^[6] was employed: dipolar traces were background corrected using either a mono-exponential or a second-order polynomial function. Distance distributions were extracted from the experimental data, using a fitting procedure based on Tikhonov regularization (Figure S7a). Normalized frequency distributions obtained from the PELDOR traces in MeOH show the expected intensity ratio of the components for $\nu(\theta=0^\circ)$ and $\nu(\theta=90^\circ)$ (Figure S7b), which is consistent with simulations of full Pake patterns for Gaussian distance distributions of HWHM of 0.1 nm (right) and 0.2 nm (left). This indicates that the obtained distributions do not show visible distortions from orientation selection. The intensity components around zero frequencies are attributed to slight baseline distortions given by the background correction.

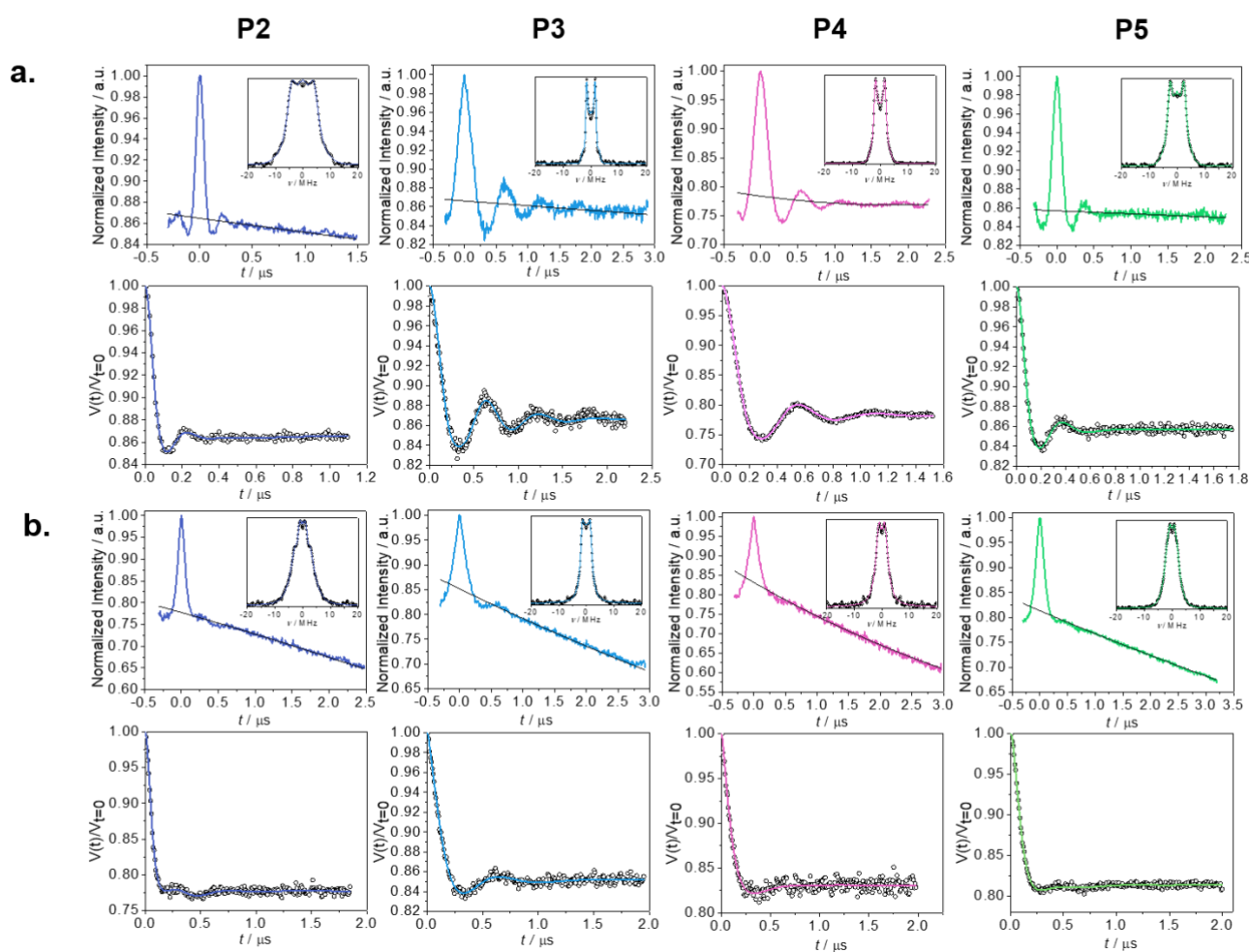


Figure S7a. Experimental PELDOR/DEER traces of β^3 -hTOPP labeled peptides in **a.** MeOH (top: time traces; bottom: background correction time traces) and **b.** MLVs of D31-POPC in H_2O -buffer (top: time traces; bottom: background correction time traces) and corresponding Pake pattern (insets) obtained after background correction. The fitted background function is displayed on top of the traces.

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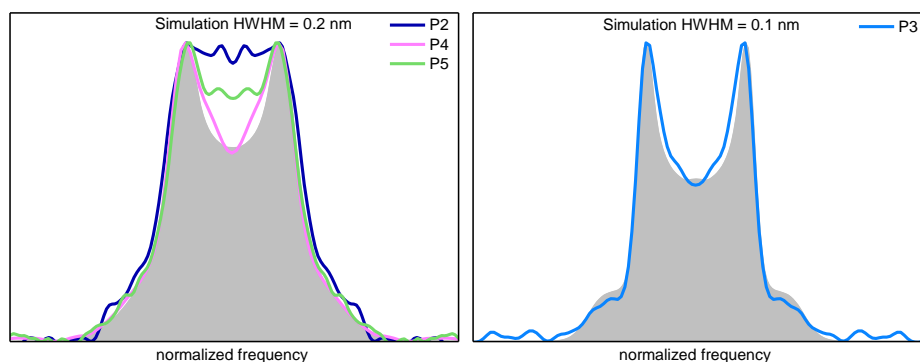


Figure S7b. Calculated full Pake patterns for Gaussian single peak distance distributions at $r = 2.95$ nm of 0.1 nm (right) and 0.2 nm (left) width (HWHM) as compared to the FT of the PELDOR signals after baseline correction.

The extracted distance distributions were validated employing the statistical validation tool of DeerAnalysis 2013. For all traces the background starting point was varied in a range from 240 ns up to 1000 ns, performing 11 trials. Additionally, the influence of the noise on the distance distributions was estimated carrying out other 10 trials where the noise was increased of a factor of 2 by adding artificially random points to the experimental data. Combining all data sets achieved from each attempt for both parameters under investigation, in total 110 trials were carried out followed by pruning of the trial results with a prune factor of 1.15. This procedure excludes the data sets which exceed more than 15% the lowest root mean square deviation (rmsd). The resulting distance distributions, shown in figure S7c present a 2σ confidential interval; the grey bars correspond to an error of ± 2 times the rmsd for each point of the distance distribution.

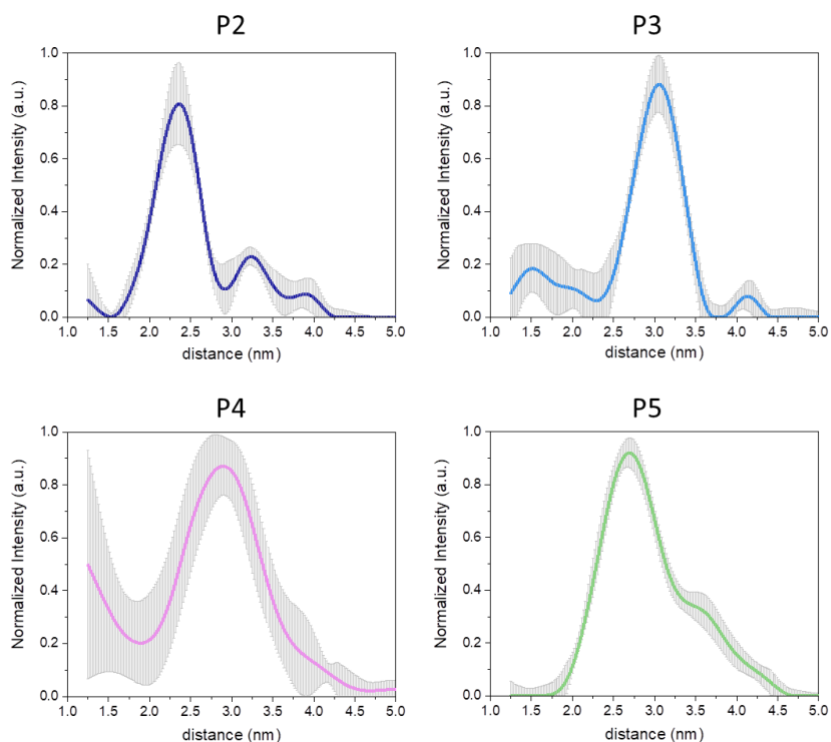


Figure S7c. Validation of the distance distributions extracted from the experimental PELDOR/DEER traces of the samples P2, P3, P4 and P5 in MLVs of D31-POPC.

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Table S2. Peak distances and half width of half maximum (HWHM) of the distance distributions in MeOH.

Peptide	P2	P3	P4	P5
<i>r</i> / nm	2.17	3.09	2.95	2.55
HWHM / nm	0.24	0.15	0.21	0.21

Table S3. Peak distances and half width of half maximum (from plots in Fig. S7a) of the distance distributions in D31-POPC.

Peptide	P2	P3	P4	P5
<i>r</i> / nm	2.37	3.07	2.92	2.74
HWHM / nm	0.34	0.33	0.64	0.45

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Inter-Spin Distances from modeled β^3 -Peptides

Simplified models of the peptides were generated from two different backbone torsion angle sets (see Table S4).

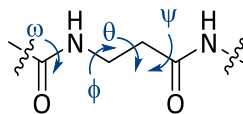


Table S4. Backbone torsion angle sets of the 3_{14} -helix. Angles are given in degrees.

helix	Ψ	θ	ϕ	ω
3.25₁₄ ^[7]	139.9	-60	134.3	-180
3.0₁₄ ^[8]	139.3	-55	123.4	-180

First the peptide backbones were generated according to the angular data using the molecular editor Avogadro^[9]. For simplification of the modelling, valine and lysine side-chains were dismissed. Next, two DFT optimized (planar) TOPP residues were attached to each model. Therefore, the peptide backbone and the TOPP geometry were kept fixed, yet their mutual orientation about the connecting bond was adjusted. A more reasonably adjusted orientation of the residues was achieved by applying the MERCK molecular force field (MMFF94).

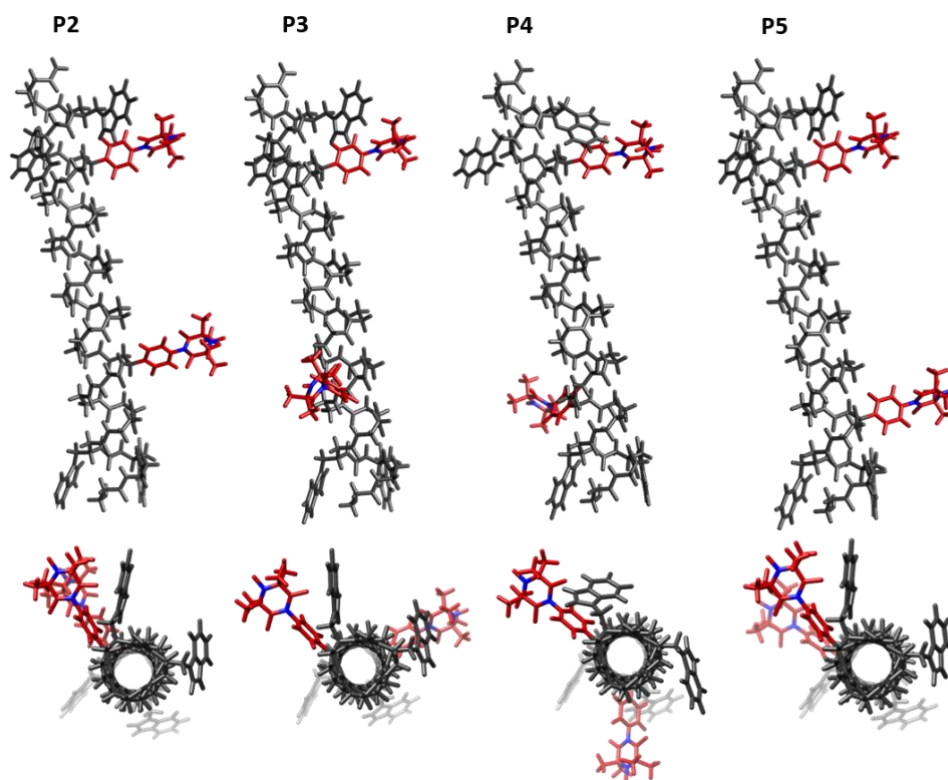


Figure S8. Models created according to backbone angles of set **3.25₁₄**.

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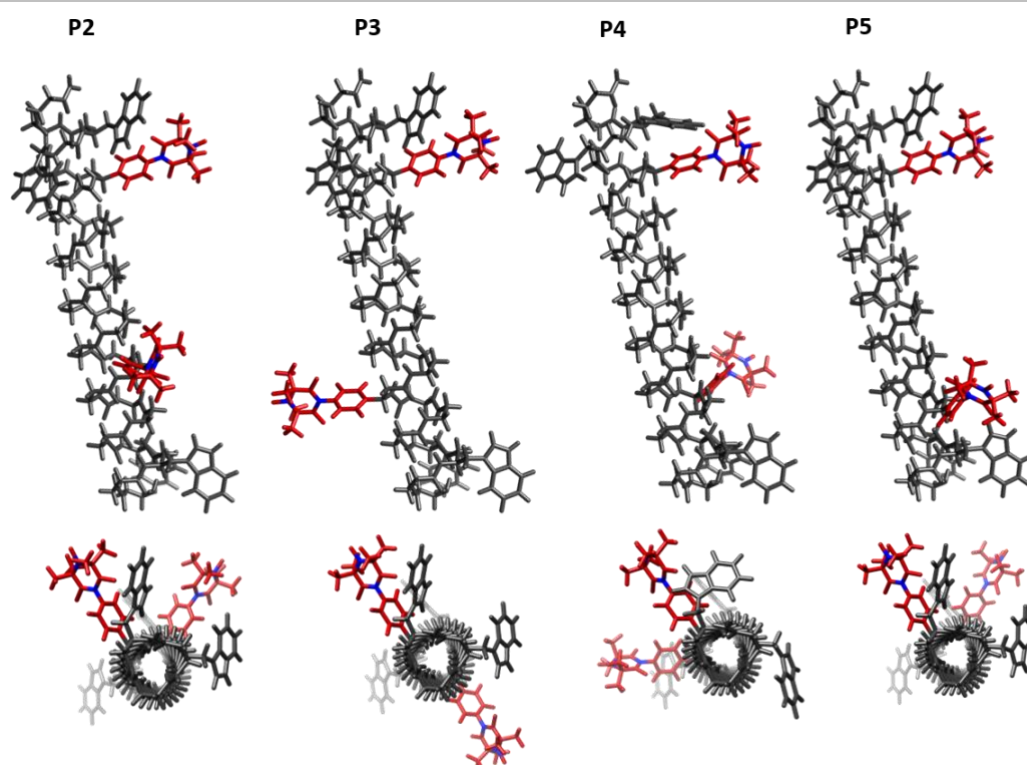


Figure S9. Models created according to backbone angles of set **3.0₁₄**.

Inter-spin distances were determined as average of the distances $O_{NO}-O_{NO}$, $O_{NO}-N_{NO}$, $N_{NO}-O_{NO}$ and $N_{NO}-N_{NO}$, since the unpaired electron is delocalized between the oxygen and nitrogen atom of the nitroxide radical. All single values and the average are given in the following tables.

Table S5. Determined inter-spin distances r in nm from the theoretical model of β^3 -peptide **P2**.

	3.25₁₄	3.0₁₄
$r(O-O)$	2.0525	2.2133
$r(O-N)$	2.0461	2.1609
$r(N-O)$	2.0753	2.1912
$r(N-N)$	2.0597	2.1337
$r(\text{average})$	2.0584	2.1748

Table S6. Determined inter-spin distances r in nm from the theoretical model of β^3 -peptide **P3**.

	3.25₁₄	3.25₁₄
$r(O-O)$	2.9592	3.0241
$r(O-N)$	2.8830	2.9099
$r(N-O)$	2.8843	2.9277
$r(N-N)$	2.8084	2.8161
$r(\text{average})$	2.8837	2.9195

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Table S7. Determined inter-spin distances r in nm from the theoretical model of β^3 -peptide **P4**.

	3.25₁₄	3.0₁₄
$r(\text{O-O})$	3.0211	2.1480
$r(\text{O-N})$	2.9533	2.1420
$r(\text{N-O})$	2.9345	2.1241
$r(\text{N-N})$	2.8688	2.1129
$r(\text{average})$	2.9444	2.1318

Table S8. Determined inter-spin distances r in nm from the theoretical model of β^3 -peptide **P5**.

	3.25₁₄	3.0₁₄
$r(\text{O-O})$	2.5312	2.4616
$r(\text{O-N})$	2.5383	2.4261
$r(\text{N-O})$	2.5234	2.4456
$r(\text{N-N})$	2.5234	2.4066
$r(\text{average})$	2.5291	2.4350

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Author Contributions

J. Wegner developed and synthesized the β^3 -hTOPP label, developed peptide design, performed the synthesis and characterization of the peptides, and generated the peptide models.

G. Valora performed all CW and PELDOR experiments in lipid bilayers and the ESEEM experiments.

K. Halbmaier performed the CW and PELDOR experiments in MeOH.

A. Kehl prepared and characterized the peptide samples for EPR and performed some of the pulse EPR experiments.

B. Worbs helped with synthesizing the peptides.

M. Bennati helped in analyzing the EPR data and supervised the EPR part of the project.

U. Diederichsen helped by the development of the β^3 -hTOPP label, designing the peptide experiments and wrote the article.

All authors contributed in writing the article.