

Supplementary Methods

Acronyms used

- **aa:** amino acid
- **¹³C-NMR:** Carbon NMR
- **¹⁹F-NMR:** Fluorine NMR
- **¹H-NMR:** Proton NMR
- **2D NMR:** 2-Dimensional NMR
- **COSY:** Correlation spectroscopy
- **DHB:** 2,5-Dihydroxybenzoic acid
- **DIPEA:** *N,N*-diisopropylamine
- **DMF:** *N,N*-dimethylformamide
- **DMSO:** Dimethyl sulfoxide
- **DMSO-d₆:** Hexadeuterodimethyl sulfoxide
- **Et₂O:** Diethylether
- **EtOAc:** Ethyl acetate
- **Fmoc-:** Fluorenylmethoxycarbonyl-
- **HFIP:** 1,1,1,3,3,3-Hexafluoroisopropanol
- **HFIP-d₂:** Dideutero-1,1,1,3,3,3-hexafluoroisopropanol
- **HMBC:** Heteronuclear multiple bond correlation spectroscopy
- **HMQC:** Heteronuclear multiple-quantum correlation spectroscopy
- **HPLC:** High performance liquid chromatography
- **HRMS:** High resolution mass spectrometry
- **HSQC:** Heteronuclear single quantum coherence spectroscopy
- **MALDI:** Matrix assisted laser desorption/ionization
- **MALDI-TOF:** Matrix assisted laser desorption/ionization time of flight mass spectrometry
- **NHS-ester:** *N*-hydroxysuccinimide ester
- **NMR:** Nuclear magnetic resonance spectroscopy
- **NOESY:** Nuclear Overhauser effect spectroscopy
- **PDA detector:** Photo diode array detector
- **QCI-F Probe:** Quadruple resonance cryoprobe
- **SEC:** Size exclusion chromatography
- **SQ detector:** Single quadrupole detector
- **TFA:** Trifluoroacetic acid
- **UPLC:** Ultraperformance liquid chromatography

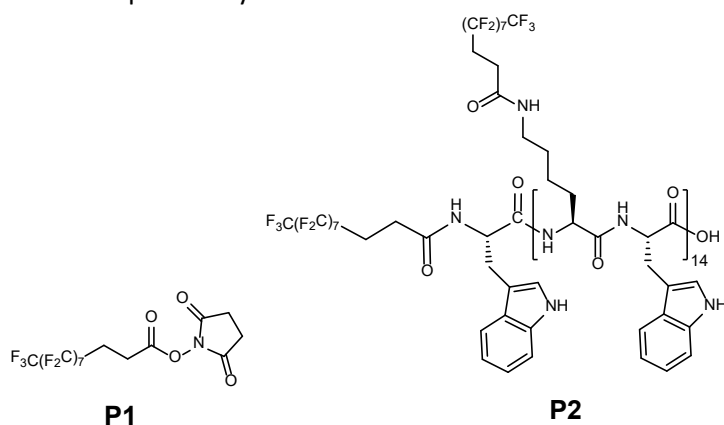
Synthetic procedures and characterization of compounds

Overall strategy

Peptides were assembled with standard solid phase peptide synthesis methods (for details see general procedure 1 below) employing Fmoc-chemistry while long coupling times and a high excess of coupling reagents were applied to realize a near quantitative conversion.¹ Amidation reactions for the introduction of perfluoroalkylchains at lysine side chains and the N-terminus of the assembled peptide were performed after cleavage from the resin with NHS ester **P1** (for details see general procedure 2).²

General Information

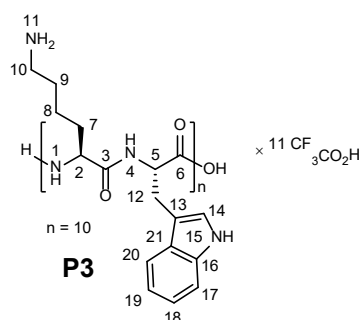
Chemicals were purchased from Sigma Aldrich, Fluorochem, Novabiochem or Bachem and used as received unless otherwise noted. UPLC experiments were performed with an Acquity UPLC-H Class Bio from Waters equipped with a PDA and a SQ detector 2 with the following column: ACQUITY UPLC, HSS T3 1.8 μm , 2.1 $\times$ 100 mm. Solvents for UPLC analysis were water and acetonitrile, each containing 0.1 % formic acid, later on referred to as (A) and (B). The flow rate was set to 0.61 ml/min and the column temperature to 40 °C. Method: 0 min – 100 % A; 1 min – 100 % A; 4 min – 100 % B; 4.5 min – 100 % B; 5.0 min 100% A; 5.5 min – 100 % A. Mass detection for UPLC analysis was performed in scan mode for positive ions (cone voltage 40 V, desolvation temperature: 600°C). A Varian PrepStar with an Ultimate 3000 RS Variable Wavelength Detector was used for preparative HPLC with the following column: Dr. Maisch Reprospher 100 C18-DE, 5 μm , 150 $\times$ 40 mm. Ultrapure water was prepared by means of an Ultra Ionic system from Milli-Q. Size exclusion chromatography was performed with a Shodex GPC HFIP-803 column, 10 μm , 300 $\times$ 8 mm, in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a flow rate of 0.75 ml/min and 40°C column temperature. HRMS experiments were conducted on a Bruker maXis 4G. MALDI experiments were carried out on a Bruker microflex LRF, which was calibrated with Bruker protein calibration standard 1 and 2 (1: insulin, ubiquitin I, cytochrom C, myoglobin; 2: trypsinogen, protein A, albumin-bovine). NMR experiments were performed at 25 °C on Bruker Avance III NMR spectrometers operating at 500 or 600 MHz proton frequency. The NMR spectrometers were equipped with inverse or direct observe broadband probe heads or with a four-channel cryogenic QCI-F probe (600 MHz) all with self-shielded z-gradients. ¹³C shifts were determined by 2D NMR experiments (HMBC and HMQC). ¹H and ¹³C signals were assigned by 2D NMR (COSY, NOESY, HMBC, HMQC). Chemical shifts are reported in δ values (ppm) and are relative to the solvent residual signal (for samples in HFIP-d₂, $\delta(^1\text{H})$ = 4.41 ppm; $\delta(^{13}\text{C})$ = 68.07 ppm; DMF-d₇, $\delta(^1\text{H})$ = 2.75 ppm; $\delta(^{13}\text{C})$ = 29.76 ppm; for samples in CDCl₃, $\delta(^1\text{H})$ = 7.26 ppm; $\delta(^{13}\text{C})$ = 77.0 ppm; for samples in DMSO-d₆ $\delta(^1\text{H})$ = 2.50 ppm; $\delta(^{13}\text{C})$ = 39.5 ppm). The trifluoroacetic acid (TFA) content of peptides with free amino groups was determined by comparison of the integrals of the TFA signal in the ¹⁹F{¹H} NMR spectra of samples before and after spiking with a defined amount of TFA. The preparation of **P1** and **P2** has been described previously.³



General procedure 1 – Synthesis of the Lys-Trp oligomers and related compounds

2-chlorotrityl chloride resin (200 mg) was placed in a plastic syringe equipped with a filter frit. The resin was washed with CH_2Cl_2 (~2 ml). Fresh CH_2Cl_2 (~2 ml) was added and DIPEA (20 μl , 0.12 mmol, 2.4 eq.), and Fmoc-amino acid (1.0 eq., 50 μmol) were added. After the sample was agitated on a shaker for 2 h liquids were removed by filtration and the resin washed with CH_2Cl_2 (~4 ml) and DMF (~4 ml). The unreacted binding sites on the resin were capped by treatment with a capping mix (5 ml, 15 % MeOH, 5 % DIPEA, 80 % CH_2Cl_2) under agitation for 30 minutes. Peptide coupling steps were performed using a peptide synthesizer (intavis multi pep RSI) employing Fmoc-amino acids (5.0 eq.), PyBOP (5.0 eq.) and DIPEA (5.0 eq.) with a reaction time of first 45 min followed by a 2nd coupling step with identical reagent loadings for 90 min. For peptides longer than 15 amino acids (**P4**, **P5**) the coupling times were extended to 90 minutes and 180 minutes respectively for aa > 15. Fmoc-deprotection was performed with piperidine (20 % piperidine in DMF, 3 cycles (1 ml) of 5 min. each). The resin was washed with CH_2Cl_2 (5 $\times$ ~2 ml) before the cleavage solution (5 ml, 96 % TFA, 3 % triisopropylsilane, 3 % H_2O) was added and the mixture agitated for 1 h. Subsequently, the cleavage solution was removed by filtration and the resin washed with TFA (1 ml). The combined TFA solutions were concentrated under reduced pressure until the mixture started to become viscous. Ice cold Et_2O (50 ml) was added and the resulting precipitate collected by centrifugation (4650 g). The precipitate was washed with Et_2O (3 $\times$ 20 ml) and the product obtained after drying as a colorless solid.

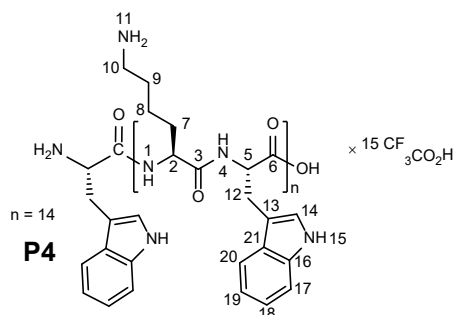
H-(Lys-Trp)₁₀-OH $\times$ 11 $\text{CF}_3\text{CO}_2\text{H}$ (**P3**)



P3 was isolated as a white solid (122 mg, 27.6 μmol , 55 %).

UPLC-MS: T_R = 2.75 min; MS (ES+) m/z : 452.9 [100 %, $M+7H^+$], 528.0 [100 %, $M+6H^+$], 633.5 [60 %, $M+5H^+$]. HRMS: calculated for: $\text{C}_{170}\text{H}_{227}\text{N}_{40}\text{O}_{21}^{5+}$: 632.9579; found: 632.9586. ^1H -NMR (DMSO- d_6 , 500 MHz, 293 K) δ 10.85-10.66 (m, 10 H, H-15), 8.36-7.66 (m, 52 H, H-1, H-4 and H-11), 7.65-7.45 m, 10 H, H-20), 7.35-7.25 (m, 10 H, H-17), 7.17- 7.06 (m, 10 H, H-14), 7.06-6.96 (m, 10 H, H-18), 6.96-6.83 (m, 10 H, H-19), 4.64-4.46 (m, 9 H, H-5), 4.43-3.94 (m, 11 H, 10 H-2 and H-5), 3.25-2.90 (m, 20 H, H-12), 2.70-2.55 (m, 20 H, H-10), 1.70-1.30 (m, 40 H, H-7 and H-9), 1.25-1.10 (m, 20 H, H-8). ^{13}C -NMR (chemical shifts determined by 2D NMR, C=O was not detected) δ 136 (C-16), 127 (C-21), 123 (C-14), 121 (C-18), 118 (C-20 and C-19), 111 (C-17), 110 (C-13), 53 (C-2), 52 (C-5), 38 (C-10), 31 (C-7), 27 (C-12 and C-9), 22 (C-8).

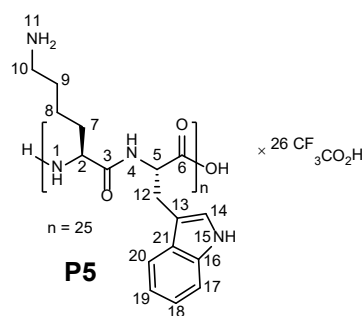
H-Trp-(Lys-Trp)₁₄-OH × 15 CF₃CO₂H (P4)



P4 was isolated as a pale yellow solid (175 mg, 28.2 μmol, 56 %).

UPLC-MS: T_R = 2.78 min; MS (ES+) m/z: 513.0 [80 %, M+9H⁺], 576.8 [100 %, M+8H⁺], 659.0 [60 %, M+7H⁺]. HRMS: calculated for: C₂₄₉H₃₂₈N₅₈O₃₀⁸⁺: 576.3235; found: 576.3225. ¹H-NMR (DMSO-d₆, 500 MHz, 293 K) 10.95 (s, 1 H, H-15), 10.85-10.78 (m, 2 H, H-15), 10.78-10.66 (m, 12 H, H-15), 8.35-7.60 (m, 73 H, H-1, H-4 and H-11), 7.60-7.48 (m, 15 H, H-20), 7.37-7.26 (m, 15 H, H-17), 7.20-6.81 (m, 45 H, H-14, H-18 and H-19) 4.73-4.48 (m, 14 H, H-5), 4.34-4.10 (m, 14 H, H-2), 3.75 (b, 1 H, H-5), 3.16-2.88 (m, 30 H, H-12), 2.77-2.60 (m, 28 H, H-10), 1.70-1.30 (m, 56 H, H-7 and H-9), 1.30-1.07 (m, 28 H, H-8). ¹³C-NMR (chemical shifts determined by 2D NMR experiments, only CH_n (n=1-3) resonances detected) δ 123 (C-14), 121 (C-18), 118 (C-20 and C-19), 111 (C-17), 53 (C-2), 52 (C-5), 38 (C-10), 31 (C-7), 27 (C-12 and C-9), 22 (C-8).

H-(Lys-Trp)₂₅-OH × 26 CF₃CO₂H (P5)

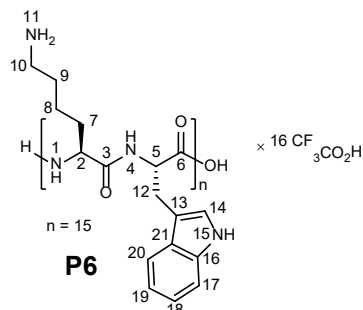


P5 was isolated as a yellow solid (220 mg, 20.3 μmol, 41 %) after precipitation from ether and was either used as such or further purified by preparative HPLC. Following lyophilization H-(Lys-Trp)₂₅-OH × 26 CF₃CO₂H was isolated as a white powder (85 mg, 7.8 μmol, 16 % yield after purification by preparative HPLC).

UPLC-MS: T_R = 2.86 min; MS (ES+) m/z: 788.5 [50 %, M+10H⁺], 876.2 [70 %, M+9H⁺], 985.5 [80 %, M+8H⁺], 1126.1 [100 %, M+7H⁺], 1313.8 [30 %, M+6H⁺]. HRMS: calculated for: C₄₂₅H₅₆₃N₁₀₀O₅₁¹¹⁺: 716.7683 ; found 716.6757. ¹H-NMR (DMSO-d₆, 500 MHz, 293 K) δ 10.88-10.80 (m, 4 H, H-15), 10.80-10.66 (m, 21 H, H-15), 8.59 (d, ³J_{H-H} = 7.5 Hz, 1 H, H-1 or H-4), 8.31 (d, ³J_{H-H} = 7.8 Hz, 1 H, H-1 or H-4), 8.21-7.70 (m, 125 H, H-1, H-4 and H-11), 7.66 (d, ³J_{H-H} = 8.0 Hz, 1 H, H-20), 7.60-7.51 (m, 24 H, H-20), 7.35-7.25 (m, 25 H, H-17), 7.19- 6.83 (m, 75 H, H-14 , H-18 and H-19) 4.64-4.46 (m, 24 H, H-5), 4.34-4.10 (m, 24 H, H-2), 4.06-4.02 (m, 1 H, H-5), 3.75-3.70 (m, 1 H, H-2), 3.25-2.90 (m, 50 H, H-12), 2.77-2.60 (m, 50 H, H-10), 1.70-1.35 (m, 100 H, H-7 and H-9), 1.32-1.07 (m, 50 H, H-8). ¹³C-NMR (chemical

shifts determined by 2D NMR experiments, only CH_n (n=1-3) resonances detected) δ 123 (C-14), 121 (C-18), 118 (C-20 and C-19), 111 (C-17), 53 (C-2), 52 (C-5), 38 (C-10), 31 (C-7), 27 (C-12 and C-9), 22 (C-8).

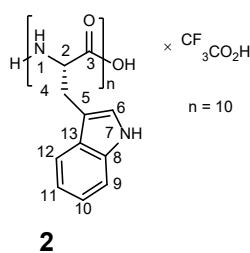
H-(Lys-Trp)₁₅-OH $\times$ 16 CF₃CO₂H (P6)



P6 was isolated as a pale yellow solid (164 mg, 25.0 μ mol, 50 %).

UPLC-MS: T_R = 2.79 min; MS (ES+) m/z: 527.1 [80 %, M+9H⁺], 592.9 [100 %, M+8H⁺], 677.3 [80 %, M+7H⁺], 791.5 [30 %, M+6H⁺]. HRMS: calculated for: C₂₅₅H₃₃₉N₆₀O₃₁⁷⁺: 676.8108; found: 676.8109. ¹H-NMR (DMSO-d₆, 500 MHz, 293 K) 10.88-10.80 (m, 3 H, H-15), 10.80-10.66 (m, 12 H, H-15), 8.59 (d, ³J_{H-H} = 7.4 Hz, 1 H, H-1 or H-4), 8.31 (d, ³J_{H-H} = 7.9 Hz, 1 H, H-1 or H-4), 8.21-7.70 (m, 75 H, H-1, H-4 and H-11), 7.66 (d, ³J_{H-H} = 7.9 Hz, 1 H, H-20), 7.60-7.51 (m, 14 H, H-20), 7.35-7.25 (m, 15 H, H-17), 7.19- 6.83 (m, 45 H, H-14, H-18 and H-19) 4.64-4.46 (m, 14 H, H-5), 4.34-4.10 (m, 14 H, 10 H-2), 4.03 (q, ³J_{H-H} = 7.0 Hz, 1 H, H-5), 3.71 (b, 1 H, H-2), 3.25-2.90 (m, 30 H, H-12), 2.77-2.60 (m, 30 H, H-10), 1.70-1.35 (m, 60 H, H-7 and H-9), 1.32-1.07 (m, 30 H, H-8). ¹³C-NMR (chemical shifts determined by 2D NMR experiments, C=O was not detected) δ 123 (C-14), 121 (C-18), 118 (C-20 and C-19), 111 (C-17), 53 (C-2), 52 (C-5), 38 (C-10), 31 (C-7), 27 (C-12 and C-9), 22 (C-8).

Decatryptophan $\times$ CF₃CO₂H (2 $\times$ CF₃CO₂H)

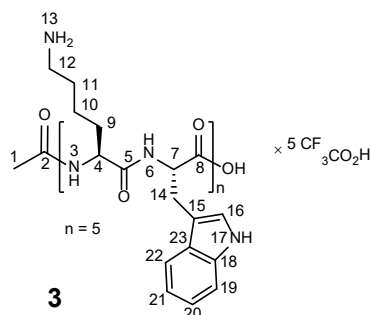


Decatryptophan (**2**) was prepared as its trifluoroacetate salt according to general procedure 1 with the following modifications: a resin loading of 125 μ mol/g resin was chosen and peptide coupling times on the peptide synthesizer were extended to 2 hours and 4 hours, respectively. The final peptide product was precipitated from the cleavage solution by addition of Et₂O without prior concentration.

UPLC-MS: T_R = 3.84 min; MS (ES+) m/z: 941.1 [100 %, M+2 H⁺], 1881.1 [50 %, M+1 H⁺], 1903.1 [20 %, M+1 Na⁺]. HRMS: calculated for: C₁₁₉H₁₀₃N₂₀O₁₁⁺: 1879.8110 ; found 1879.8084. ¹H-NMR (DMF-d₇, 500 MHz, 293 K) δ 11.02 (d, ³J_{H-H} = 2.4 Hz, 1 H, H-7) 10.84-10.81 (m, 2 H, H-7), 10.79 (d, ³J_{H-H} = 2.4 Hz, 1 H, H-7), 10.77 (d, ³J_{H-H} = 2.4 Hz, 1 H, H-7), 10.74 (d, ³J_{H-H} = 2.4 Hz, 1 H, H-7), 10.71 (d, ³J_{H-H} = 2.4 Hz, 1 H, H-7), 10.70-10.66 (m, 3 H, H-7), 9.09 (d, ³J_{H-H} = 7.5 Hz, 1 H, H-1), 8.43 (b, 3 H, H-1 (N-terminus), 8.33 (d,

$^3J_{H-H} = 7.5$ Hz, 1 H, H-1), 8.21-7.98 (m, 7 H, H-1), 7.76 (d, $^3J_{H-H} = 7.9$ Hz, 1 H, H-12) 7.66-7.52 (m, 9 H, H-12), 7.46-7.33 (m, 10 H, H-9), 7.27-7.10 (m, 10 H, H-6), 7.09- 6.90 (m, 20 H, H-10, H-11), 4.88-4.64 (m, 9 H, H-2), 4.46-4.40 (m, 1 H, H-2), 3.34-3.06 (m, 20 H, H-4). ^{13}C -NMR (chemical shifts were determined by 2D NMR experiments; not all carbon atoms were detected) δ 124 (C-6), 121 (C-10), 119 (C-11 and C-12), 111 (C-9), 54.6 ($3 \times \text{C-2}$), 54.5 ($3 \times \text{C-2}$), 54.1 (C-2), 53.8 (C-2), 53.5 (C-2), 28 (C-4).

Ac-(Lys-Trp)₅-OH $\times$ 5 CF₃CO₂H (3×5 CF₃CO₂H)



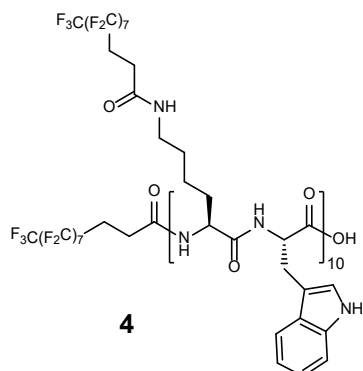
Ac-(Lys-Trp)₅-OH (**3**) $\times$ 5 CF₃CO₂H was prepared according to general procedure 1 with the following modification: Following the final Fmoc-deprotection the N-terminus was capped with acetic anhydride (4 ml, 10 % in DMF with 1 % DIPEA) for 15 min.

UPLC-MS: $T_R = 2.87$ min; MS (ES+) m/z : 327.5 [50 %, $M+5 \text{ H}^+$], 545.1 [100 %, $M+4 \text{ H}^+$], 545.1 [20 %, $M+3 \text{ H}^+$], 816.6 [10 %, $M+2 \text{ H}^+$]. HRMS: calculated for: C₈₇H₁₁₆N₂₀O₁₂²⁺: 816.4535; found 816.4543. ^1H -NMR (DMSO, 500 MHz, 293 K) δ 10.80 (s, 1 H, H-17) 10.78-10.73 (m, 3 H, H-17), 10.71 (s, 1 H, H-17), 8.17- 7.59 (m, 25 H, H-3, H-6 and H-13), 7.58-7.50 (m, 5 H, H-22), 7.33-7.26 (m, 5 H, H-19), 7.15- 7.07 (m, 5 H, H-16), 7.06-7.00 (m, 5 H, H-20), 6.98-6.88 (m, 5 H, H-21), 4.61-4.48 (m, 9 H, H-7), 4.30-4.02 (m, 11 H, 10 $\times$ H-3 and 1 $\times$ H-7), 3.25-2.93 (m, 10 H, H-14), 2.70-2.60 (m, 10 H, H-12), 1.81 (s, 3 H, H-1), 1.69-1.30 (m, 20 H, H-9 and H-11), 1.25-1.09 (m, 10 H, H-10). ^{13}C -NMR (chemical shifts were determined by 2D NMR experiments, C=O was not detected) δ 136 (C-18), 127 (C-23), 123 (C-16), 121 (C-20), 118 (C-22 and C-21), 111 (C-19), 110 (C-15), 53 (C-4), 52 (C-7), 38 (C-12), 31 (C-9), 27 (C-14) 26 (C-11), 22.1 (C-1), 22 (C-10).

General procedure 2 – Amidation of Lys-Trp oligomers with NHS-fluoroalkyl-ester **P1**

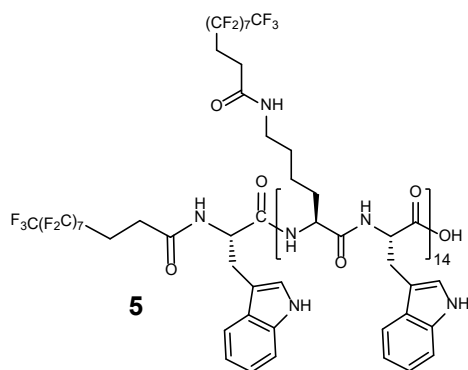
The lysine-tryptophan oligomer (1.00 eq) and NHS-ester **P1** (2.00 eq for every NH₂-group of the peptide) were dissolved in DMF (200 ml). DIPEA (4.0 eq. for every NH₂-group of the peptide) was added and the mixture stirred for 56 hours at 50 °C. Subsequently the mixture was concentrated under reduced pressure. The formed precipitate was isolated by filtration, washed with cold DMF (2 $\times$ 25 ml), EtOAc (2 $\times$ 25 ml), Et₂O (2 $\times$ 25 ml) and dried under reduced pressure.

(2*H*,2*H*,3*H*,3*H*-perfluoroundecanoyl)-(Lys(2*H*,2*H*,3*H*,3*H*-perfluoroundecanoyl)-Trp)₁₀-OH (4)



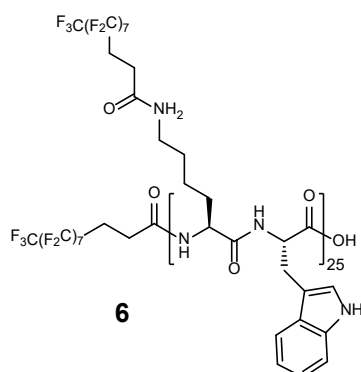
P3 (1.00 eq., 250 mg, 56.6 μmol) was converted according to general procedure 2. **5** was obtained as an orange solid (295 mg, 35.2 μmol , 62 %). SEC HFIP: T_r : 11.1 min; MALDI: 8399.1 $[\text{M} + \text{Na}^+]$. Unambiguous assignment of the NMR-data was not possible with a reasonable effort due to signal overlap in 1D- ^1H and 2D-NMR spectra. A representative 1D- ^1H , as well as HSQC, NOESY and COSY NMR spectra, a size exclusion chromatography trace and a MALDI-TOF MS spectrum are shown in Supplementary Figures 14-21.

(2*H*,2*H*,3*H*,3*H*-perfluoroundecanoyl)-Trp-(Lys(2*H*,2*H*,3*H*,3*H*-perfluoroundecanoyl)-Trp)₁₄-OH (5)



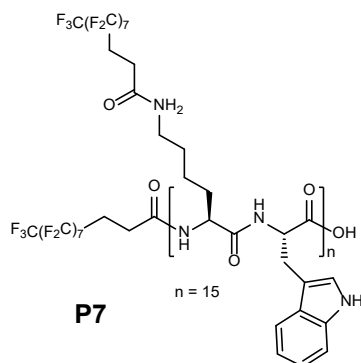
P4 (1.00 eq., 300 mg, 65.1 μmol) was converted according to general procedure 2. **5** was obtained as a brown solid (385 mg, 32.9 μmol , 51 %). SEC HFIP: T_r : 10.1 min; MALDI: 11740 $[\text{M} + \text{Na}^+]$. Unambiguous assignment of the NMR-data was not feasible with a reasonable effort. A representative size exclusion chromatography trace and a MALDI-TOF MS spectrum are shown in Supplementary Figures 22-25.

(2H,2H,3H,3H-perfluoroundecanoyl)-(Lys(2H,2H,3H,3H-perfluoroundecanoyl)-Trp)₂₅-OH (6)



P5 (1.00 eq., 300 mg, 27.7 μmol) was converted according to general procedure 2. **6** was obtained as a brown solid (280 mg, 13.9 μmol , 50 %). A second batch was prepared with H-(Lys-Trp)₂₅-OH $\times$ 26 CF₃CO₂H, **P5** that was purified by preparative HPLC (1.00 eq., 75 mg, 6.9 μmol) following the same procedure but employing only half volumes of all solvents. The product was obtained as a brown solid (115 mg, 5.7 μmol , 83 %). Both batches were tested in laser desorption/postionization studies and the second batch was used for characterization. SEC HFIP: T_r : 9.5 min; MALDI: 20224 [M+Na⁺]. Unambiguous assignment of the NMR-data was not feasible with a reasonable effort. A representative 1D-¹H NMR spectrum, a size exclusion chromatography trace and a MALDI-TOF MS spectrum are shown in Supplementary Figures 26-29.

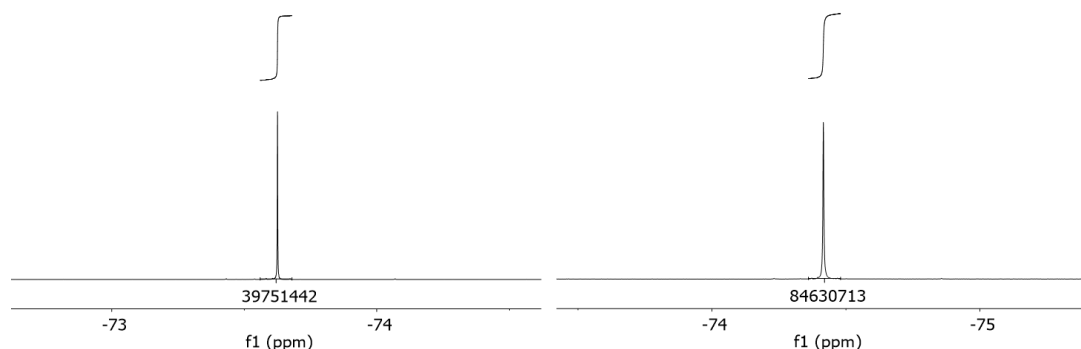
(2H,2H,3H,3H-perfluoroundecanoyl)-(Lys(2H,2H,3H,3H-perfluoroundecanoyl)-Trp)₁₅-OH (P7)



P6 (1.00 eq., 300 mg, 45.7 μmol) was converted according to general procedure 2. **P7** was obtained as a brown solid (330 mg, 26.8 μmol , 59 %). SEC HFIP: T_r : 10.2 min; MALDI: 12342 [M+Na⁺]. Unambiguous assignment of the NMR-data was not feasible with a reasonable effort. 1D-¹H NMR spectrum, a size exclusion chromatography trace and a MALDI-TOF MS spectrum are shown in Supplementary Figures 30-32.

Supplementary Note 1. Determination of the TFA content in peptide products

A comparison of the integral values for the trifluoroacetate signal in the ^{19}F -NMR spectrum of the respective compound (4.0 mg) in DMSO-d_6 (0.5 mL) before and after spiking with a defined amount of trifluoroacetic acid (1.0 μl , 13 μmol) was used to estimate the trifluoroacetate equivalents α in the original sample (compound name $\times \alpha \text{ CF}_3\text{CO}_2\text{H}$) as follows and is here exemplified for compound **P3**.



Supplementary Figure 1. ^{19}F -NMR (DMSO-d_6 , 471 MHz, 298 K, D1 = 4s) of **P3** $\times \alpha \text{ CF}_3\text{CO}_2\text{H}$ (4.0 mg) in DMSO-d_6 (0.50 ml) before (left) and after (right) addition of trifluoroacetic acid (1.0 μl , 13 μmol).

$$\alpha = \frac{n_{\text{CF}_3\text{CO}_2\text{H}} \cdot M_{w(\text{P2free})}}{m - n_{\text{CF}_3\text{CO}_2\text{H}} \cdot M_{w(\text{CF}_3\text{CO}_2\text{H})}} \quad (1)$$

with

$$n_{\text{CF}_3\text{CO}_2\text{H}} = \frac{I_{bs} \cdot n_{\text{CF}_3\text{CO}_2\text{H}(s)}}{(I_{as} - I_{bs})} \quad (2)$$

where

$n_{\text{CF}_3\text{CO}_2\text{H}}$ is the amount of substance of $\text{CF}_3\text{CO}_2\text{H}$ before spiking;

I_{bs} is the integral of the $\text{CF}_3\text{CO}_2\text{H}$ signal *before* spiking;

$n_{\text{CF}_3\text{CO}_2\text{H}(s)}$ is the amount of substance of $\text{CF}_3\text{CO}_2\text{H}$ added for spiking (13 μmol);

I_{as} is the integral of the $\text{CF}_3\text{CO}_2\text{H}$ signal *after* spiking;

$M_{w(\text{P2free})}$ is the theoretical molecular weight of **P3**, which is free of $\text{CF}_3\text{CO}_2\text{H}$ (3161.91 g/mol);

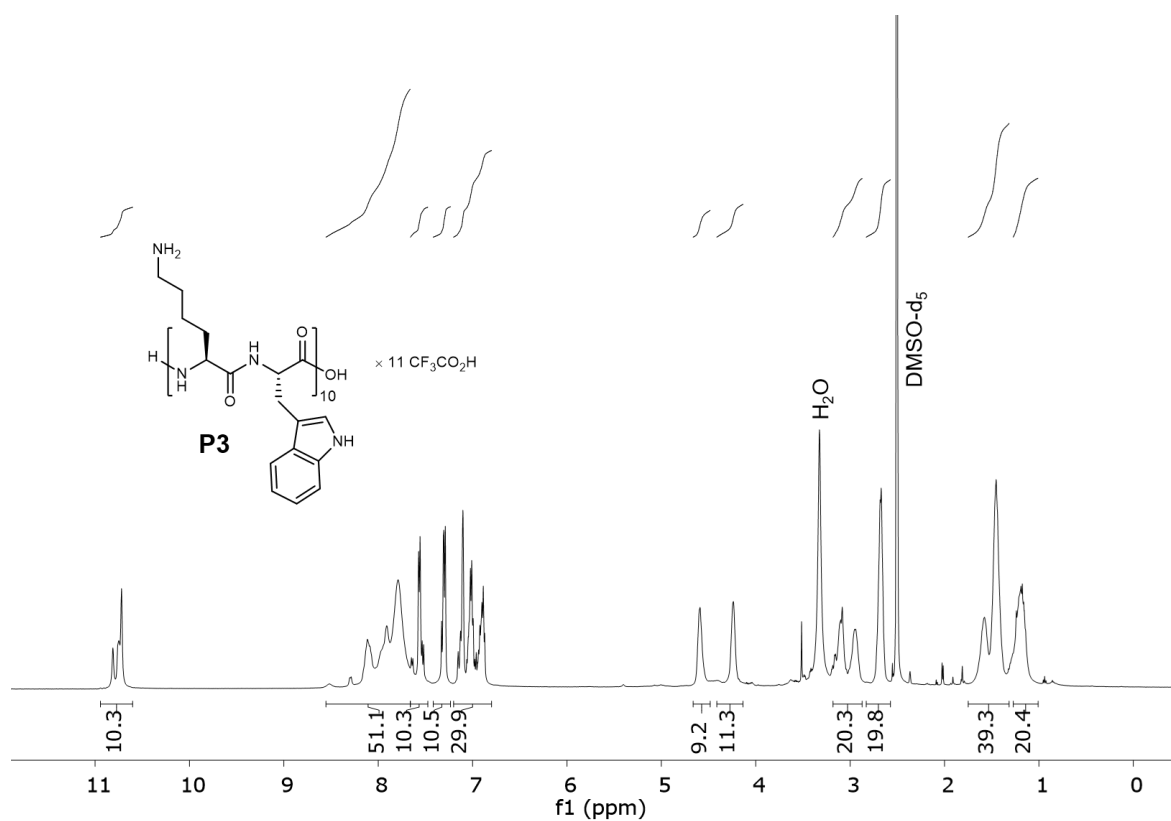
m is the weight of **P3** $\times \alpha \text{ CF}_3\text{CO}_2\text{H}$ employed in the experiment (4.0 mg);

$M_{w(\text{CF}_3\text{CO}_2\text{H})}$ is the molecular weight of $\text{CF}_3\text{CO}_2\text{H}$ (114.02 g/mol);

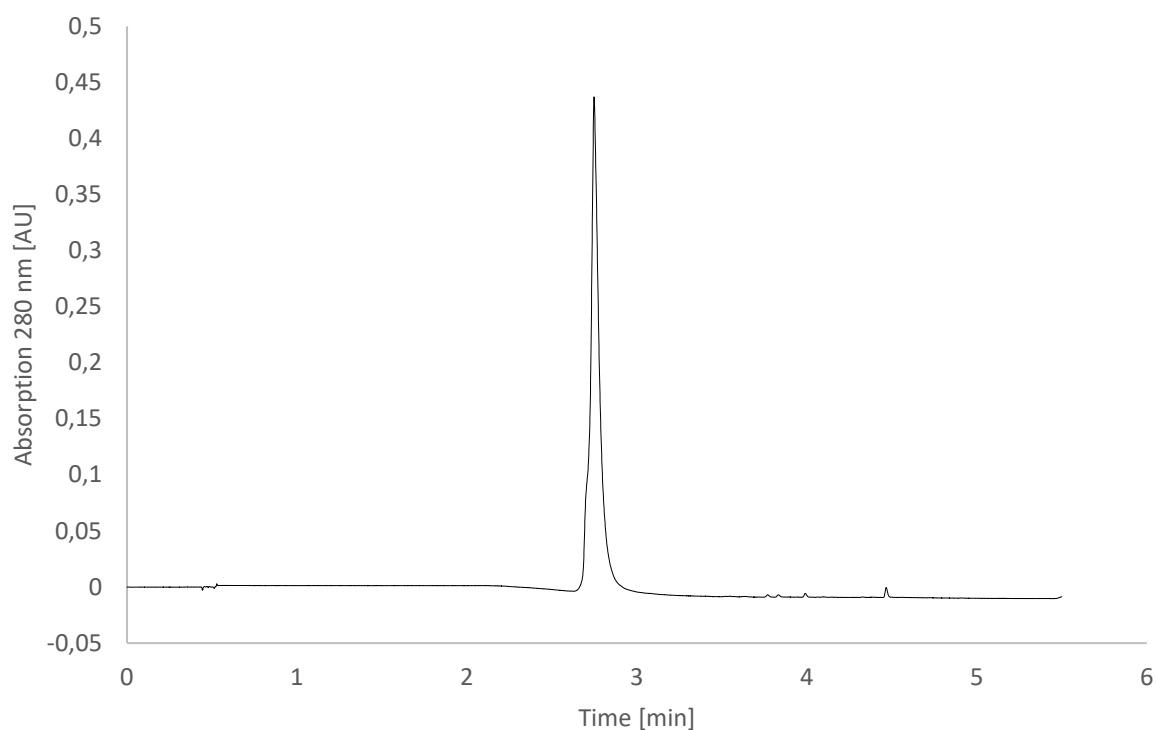
The results for compounds **3**, **P3-P5** are given in Supplementary Table 1.

compound	theoretical M_w of $\text{CF}_3\text{CO}_2\text{H}$ free compound	α calculated	number of basic amine units in molecule
3	1632.00 g/mol	6.3	5
P3	3161.91 g/mol	13.6	11
P4	4605.67 g/mol	17.4	15
P5	7877.74 g/mol	28.0	26

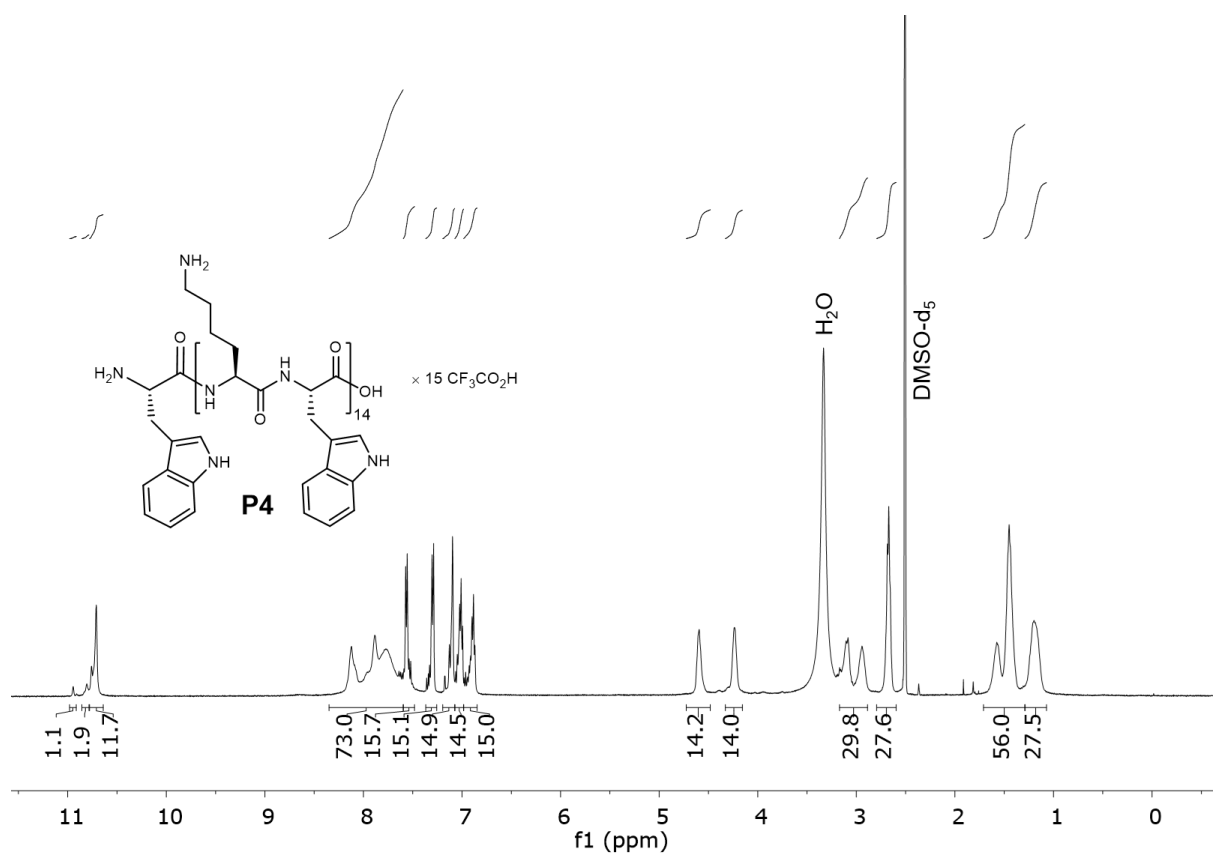
Supplementary Table 1. $\text{CF}_3\text{CO}_2\text{H}$ equivalents contained per formular unit for compounds **3**, **P3 - P5**. The calculated values match reasonably well with the number of basic amine units of the molecules.



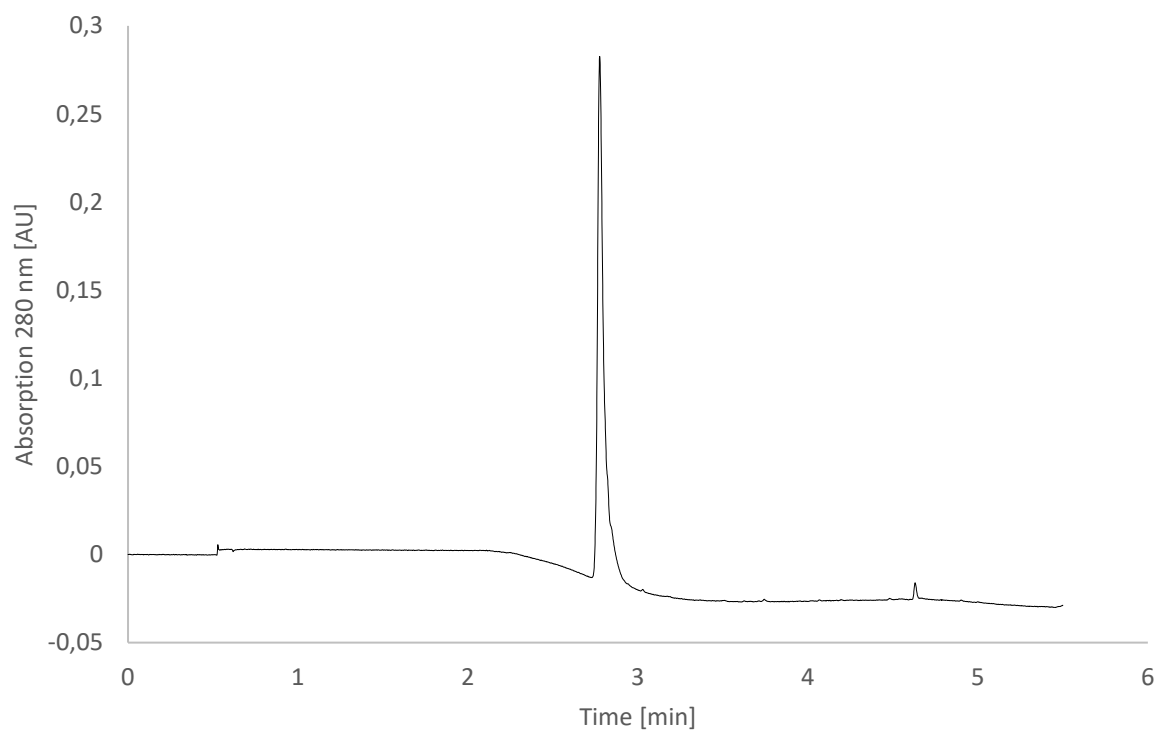
Supplementary Figure 2. ^1H -NMR spectrum of compound **P3** $\times 11 \text{ CF}_3\text{CO}_2\text{H}$: DMSO- d_6 , 500 MHz, 298 K.



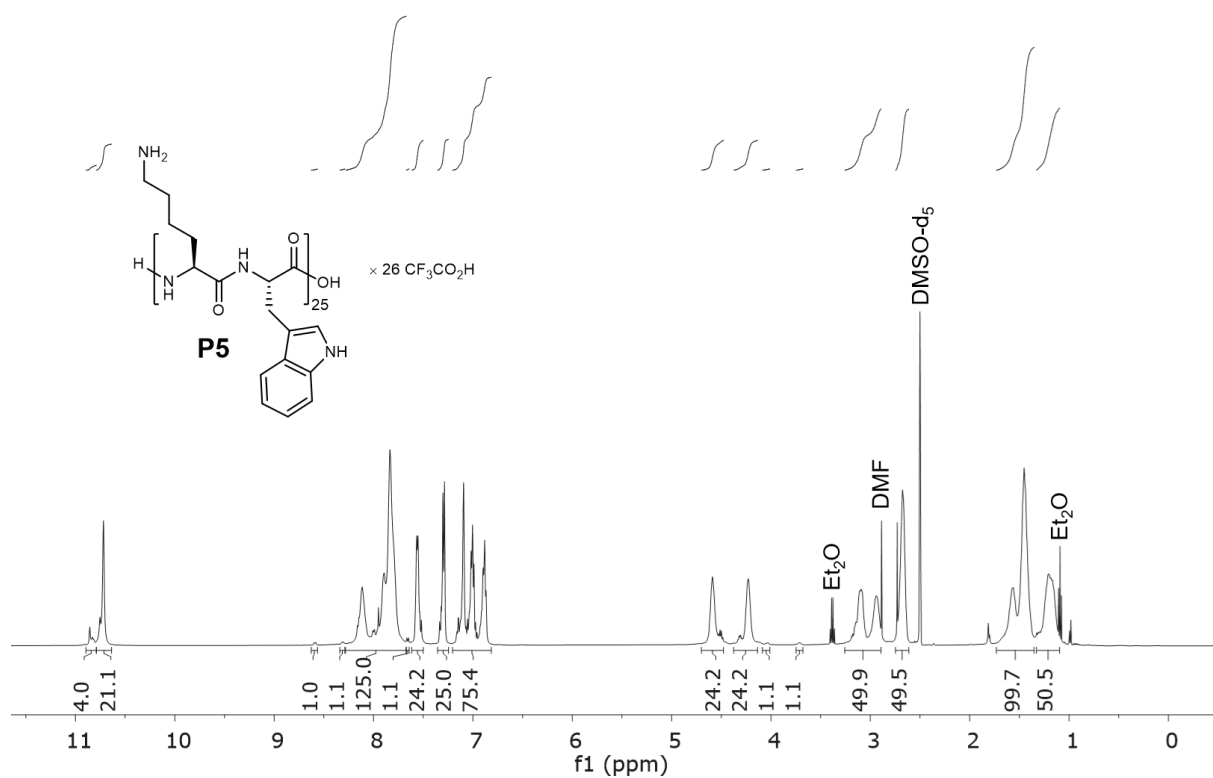
Supplementary Figure 3. UPLC-chromatogram for compound **P3**.



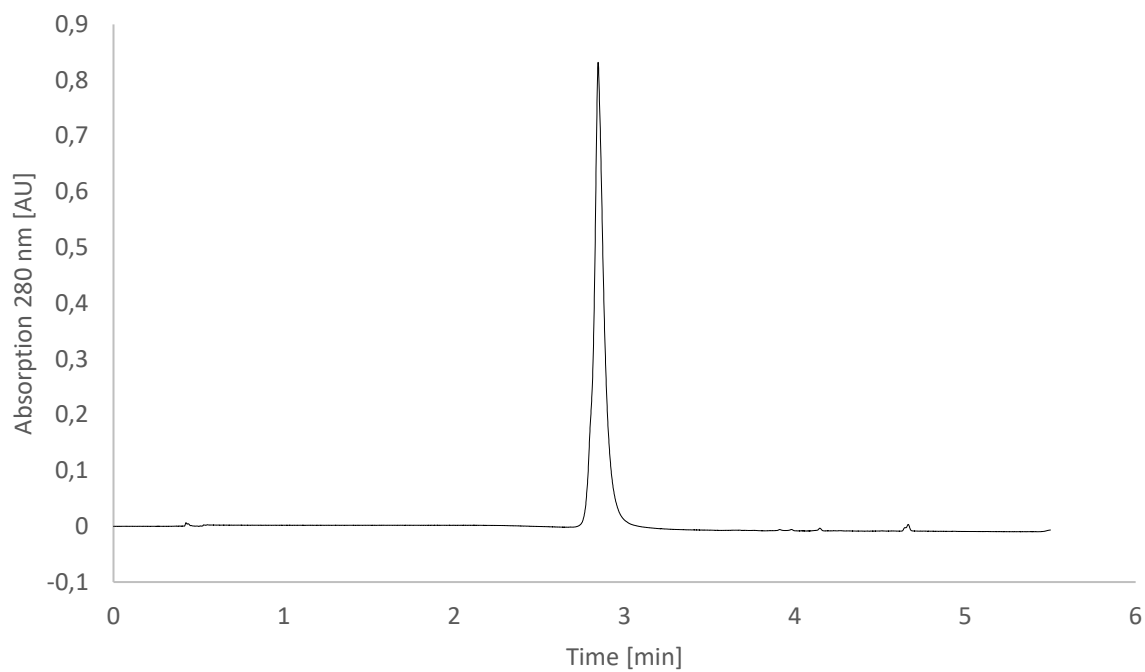
Supplementary Figure 4. $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz, 298 K), compound **P4** $\times 16 \text{ CF}_3\text{CO}_2\text{H}$.



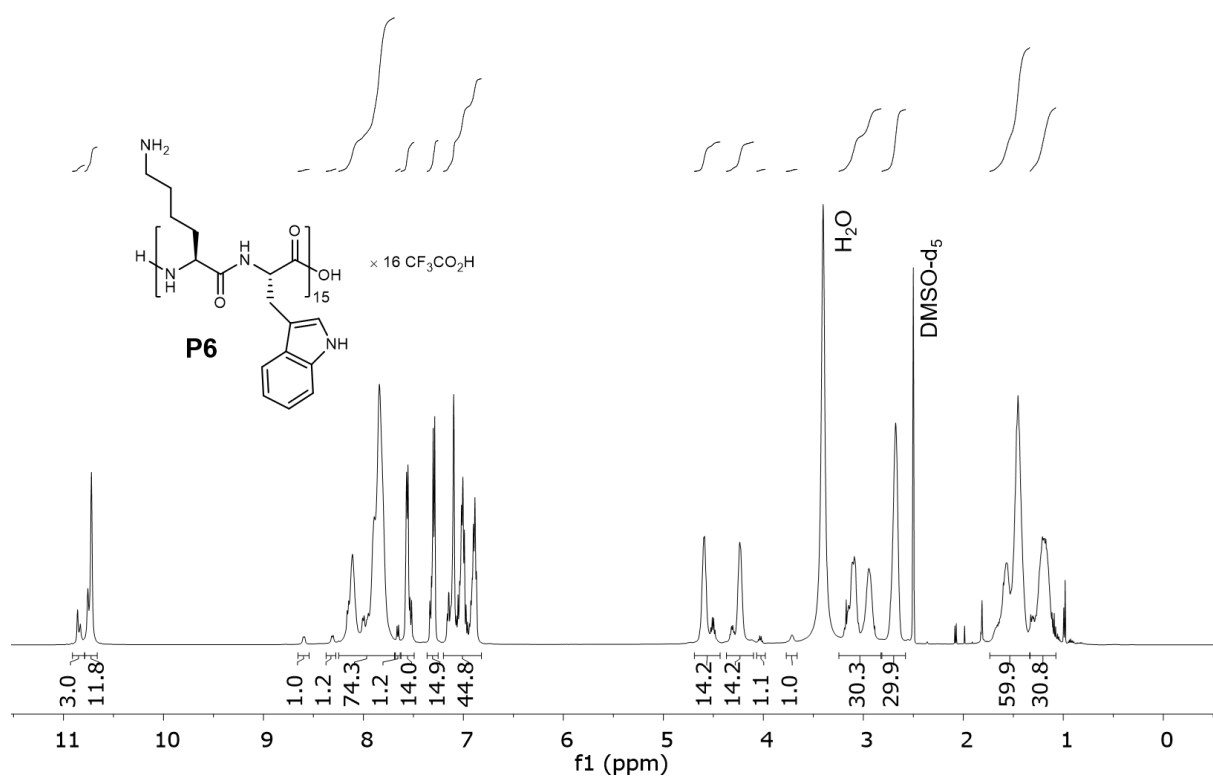
Supplementary Figure 5. UPLC-chromatogram for compound **P4**.



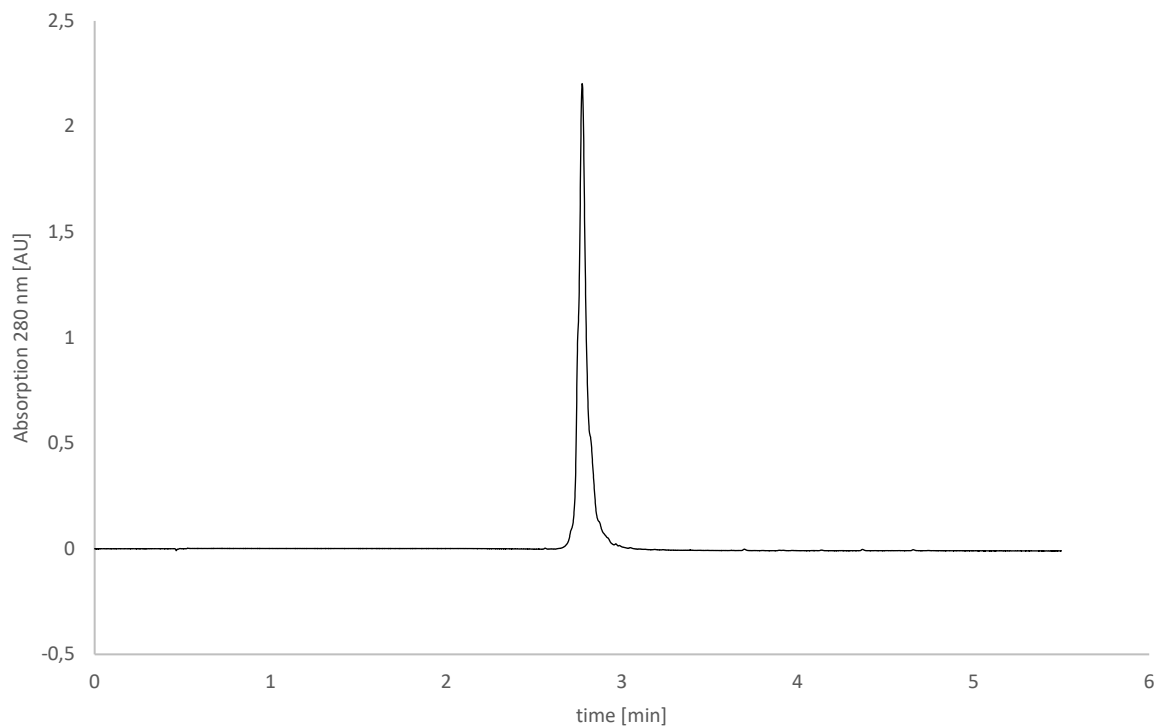
Supplementary Figure 6. ¹H-NMR (DMSO-d₆, 500 MHz, 298 K) of compound **P5** × 26 CF₃CO₂H.



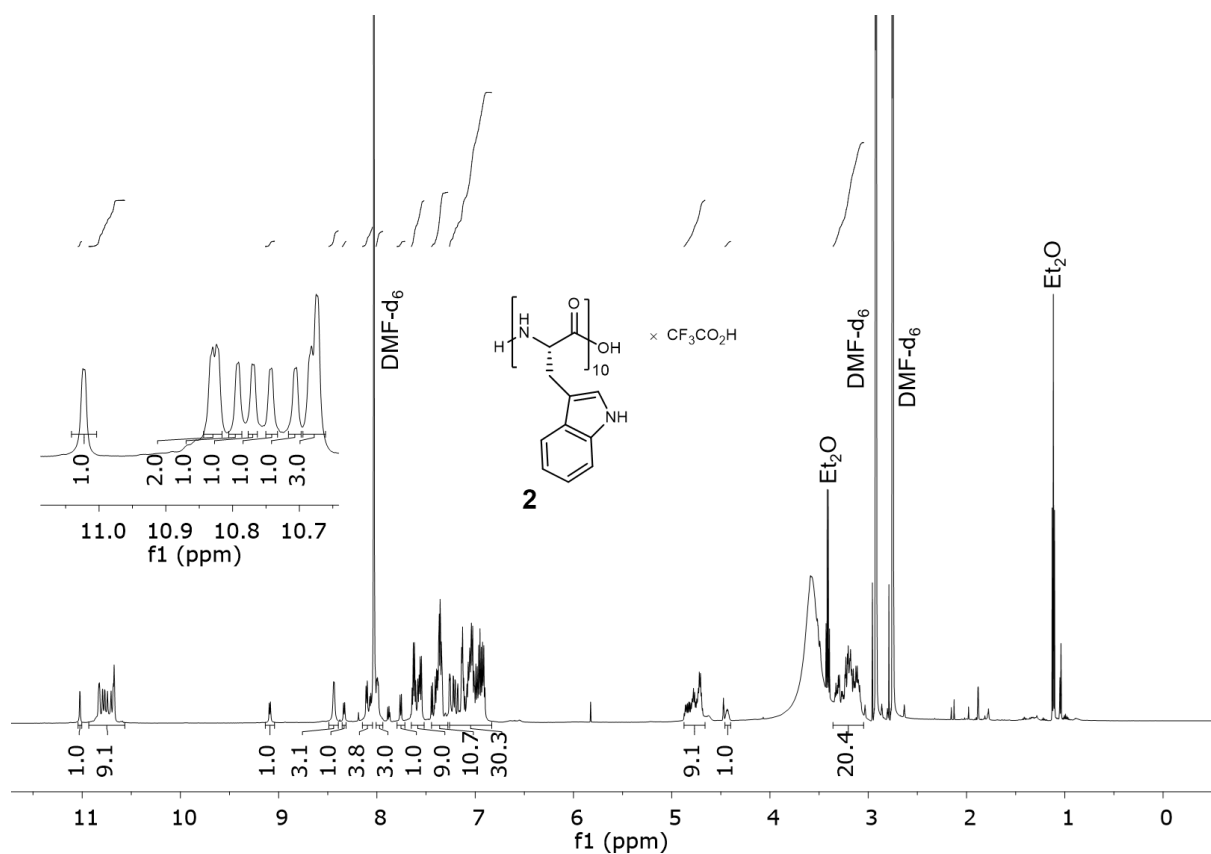
Supplementary Figure 7. UPLC-chromatogram for compound **P5**.



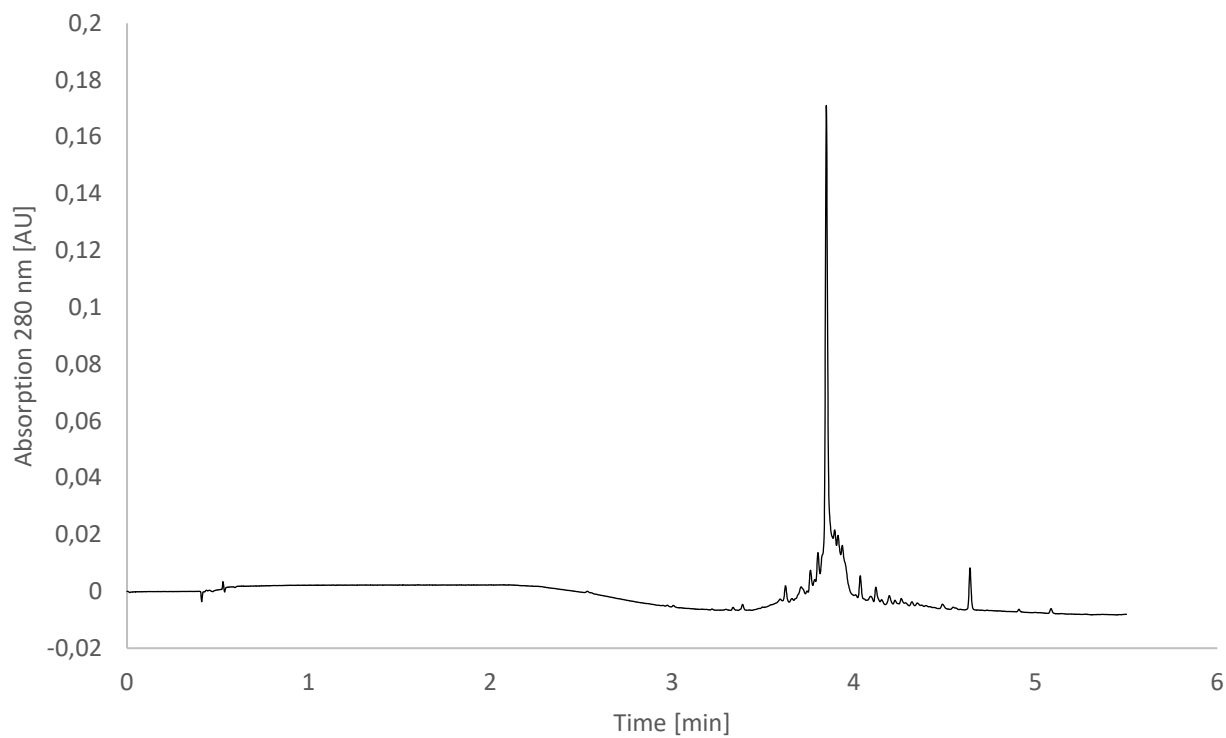
Supplementary Figure 8. ¹H-NMR (DMSO-d₆, 500 MHz, 298 K) of compound **P6** $\times$ 16 CF₃CO₂H.



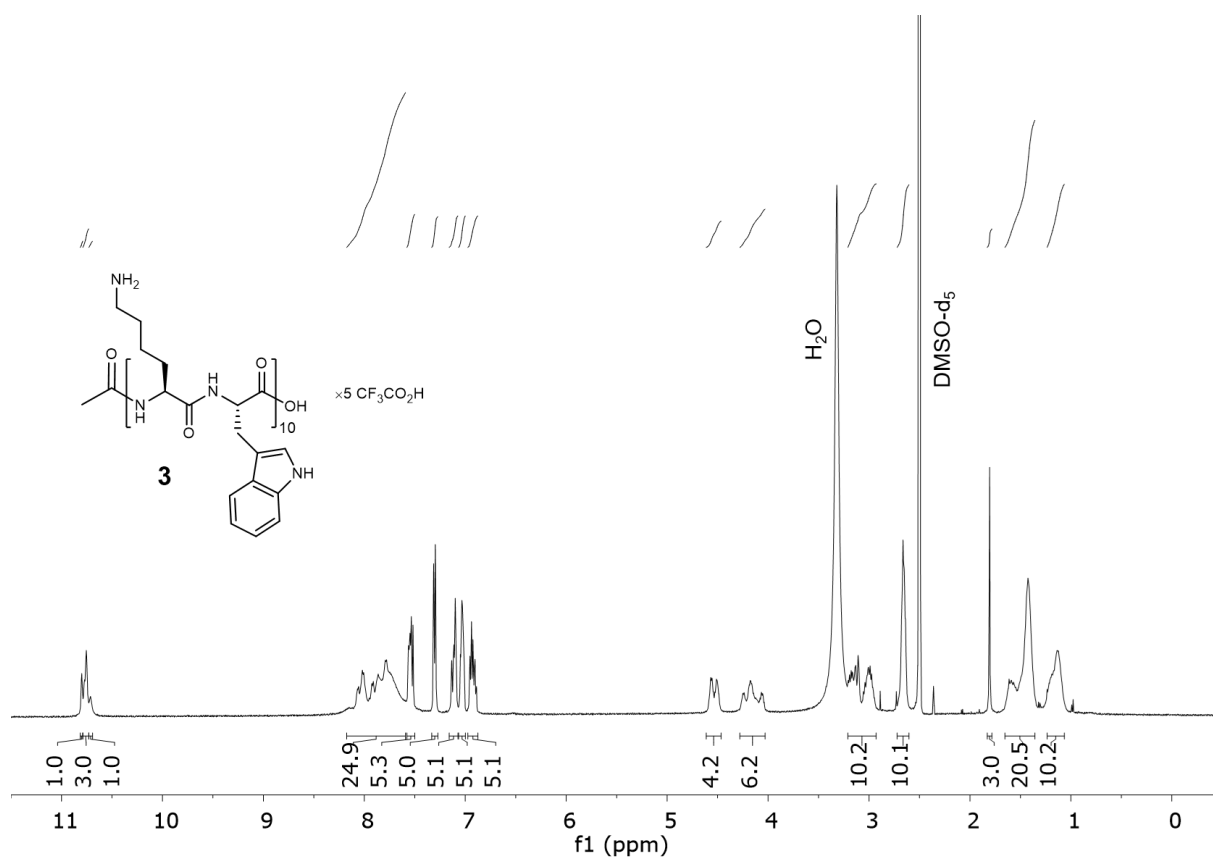
Supplementary Figure 9: UPLC-chromatogram for compound **P6**.



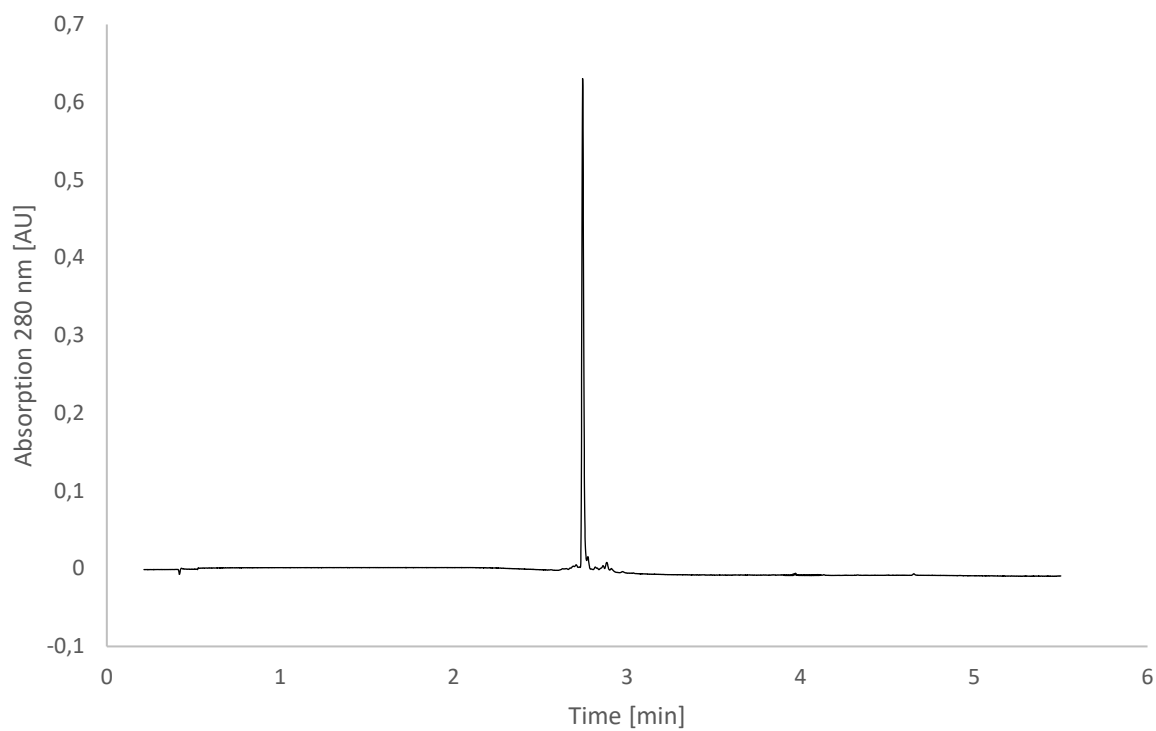
Supplementary Figure 10. ^1H -NMR (DMF-d_7 , 600 MHz, 298 K) of decatryptophan **2** $\times \text{CF}_3\text{CO}_2\text{H}$. The insert shows the signals of the NH protons of the indole rings.



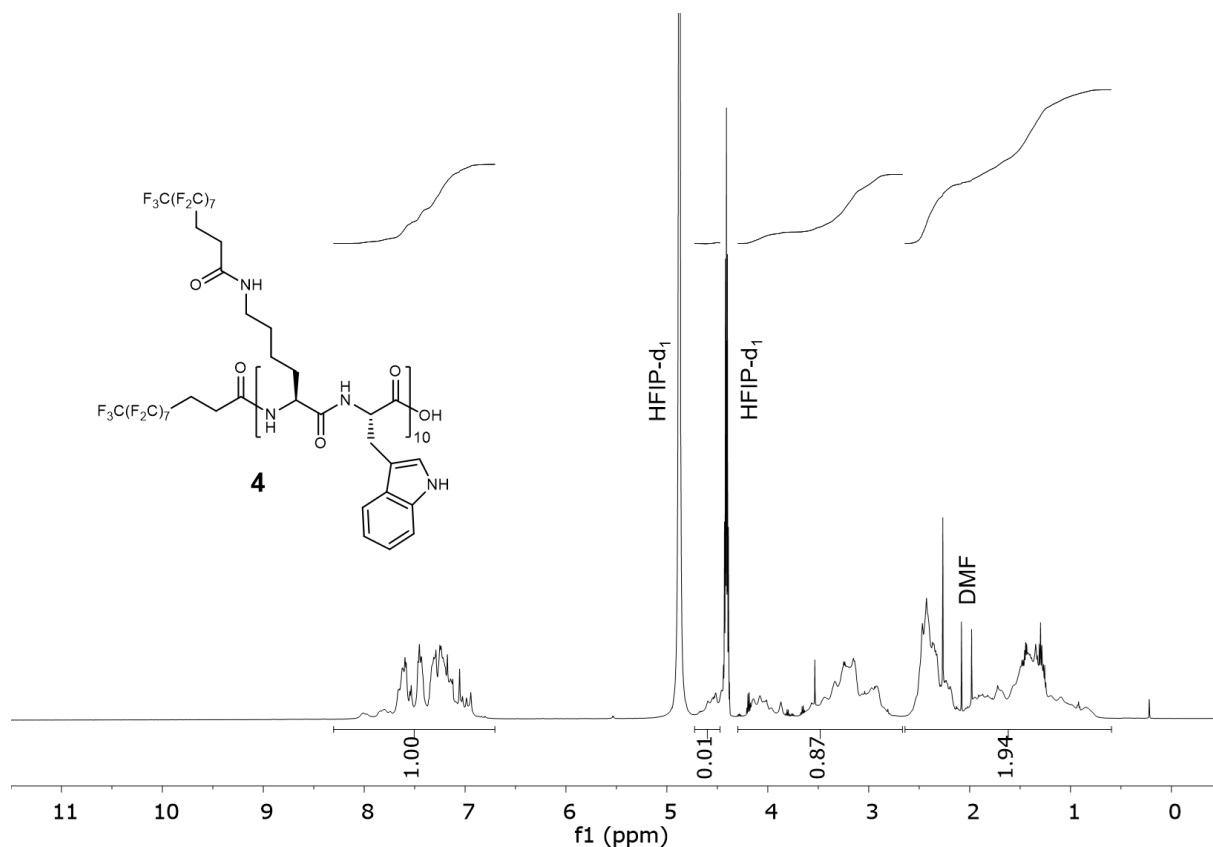
Supplementary Figure 11. UPLC-chromatogram for decatryptophan **2**.



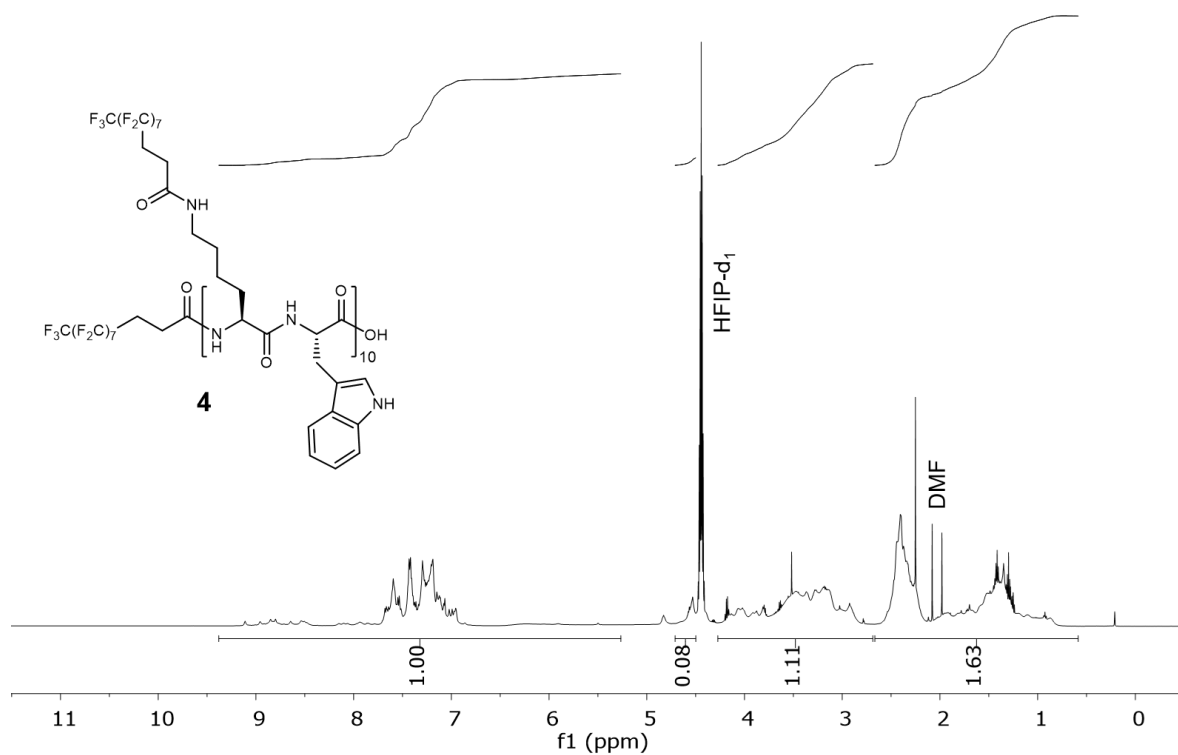
Supplementary Figure 12. ^1H -NMR (DMSO- d_6 , 500 MHz, 298 K) of compound **3** $\times 5 \text{ CF}_3\text{CO}_2\text{H}$.



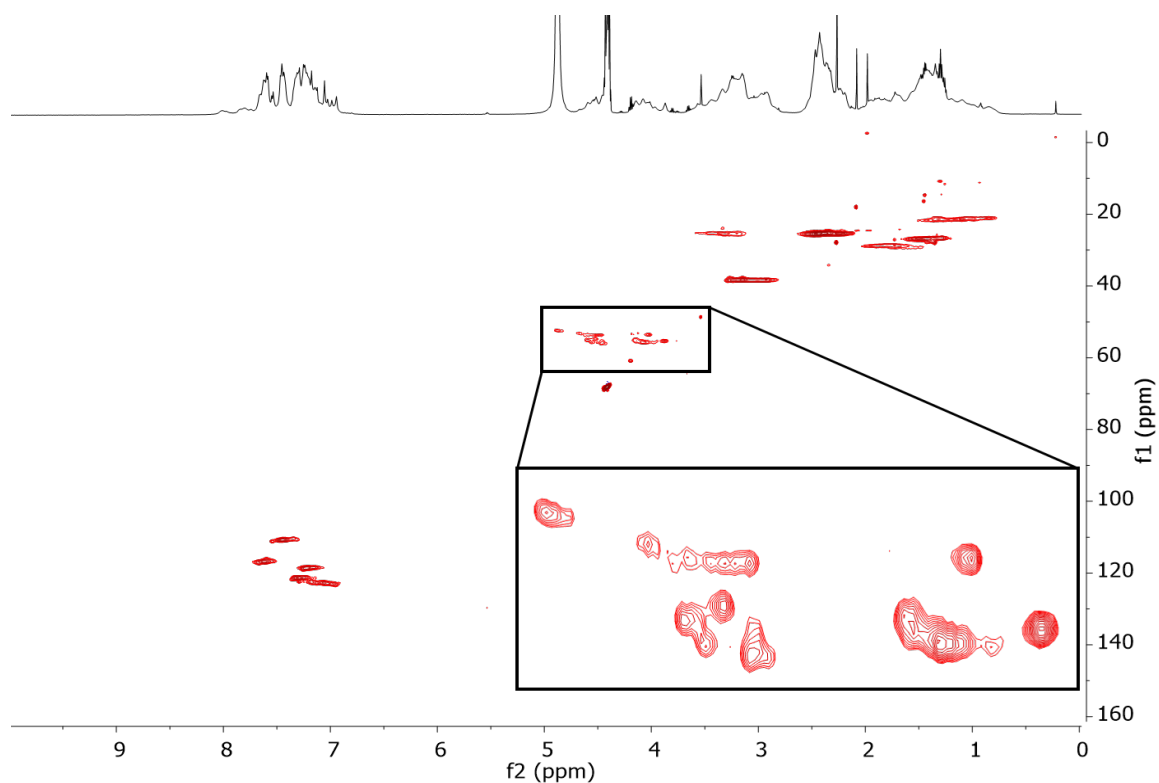
Supplementary Figure 13. UPLC-chromatogram for compound **3**.



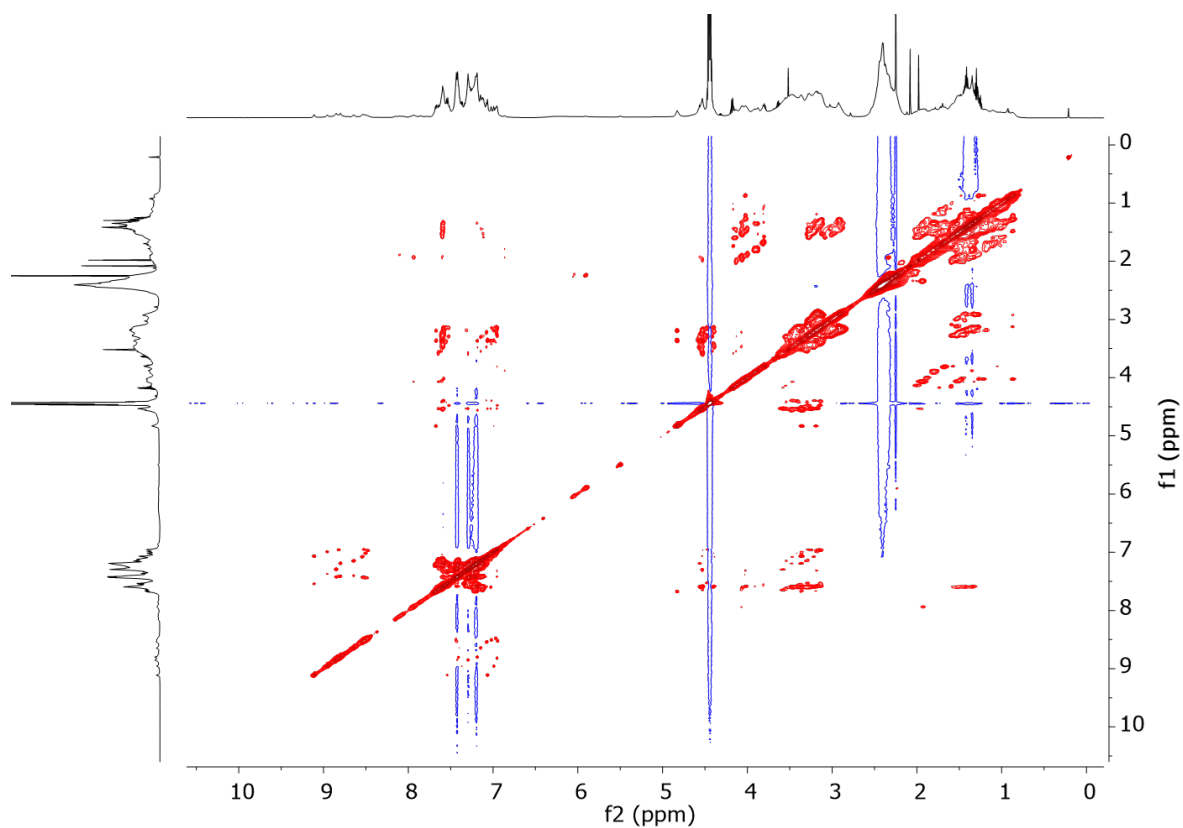
Supplementary Figure 14. ^1H -NMR (HFIP- d_2 , 600 MHz, 298 K) of compound **4**.



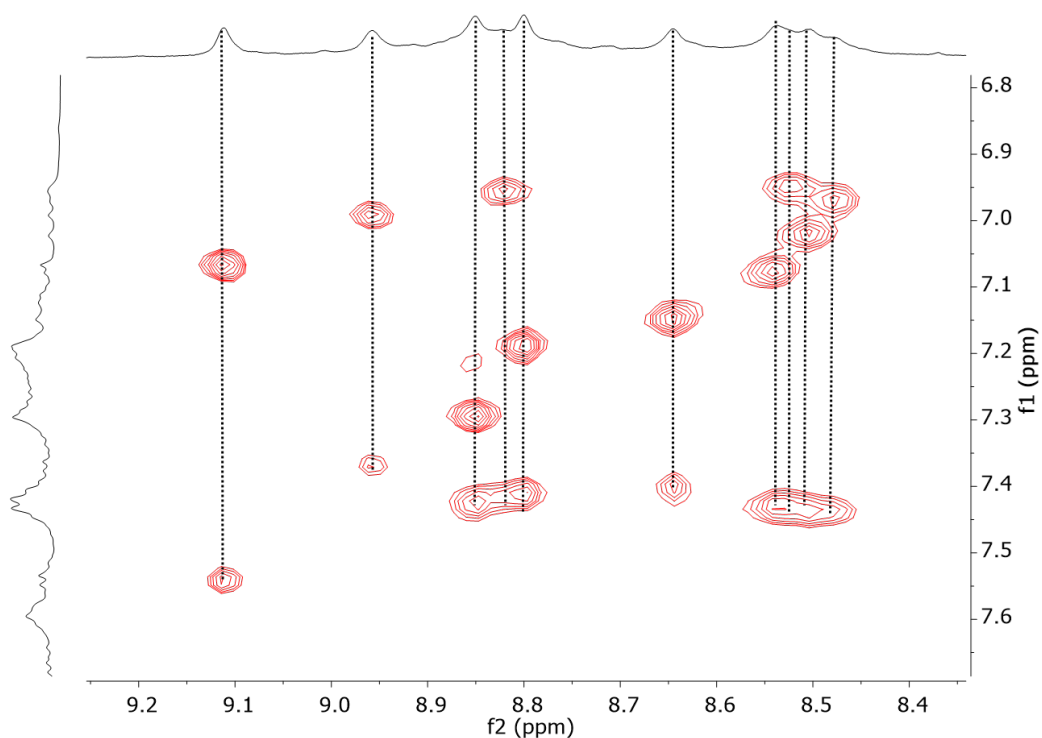
Supplementary Figure 15. ^1H -NMR (HFIP- d_2 , 600 MHz, 298 K), compound **4** after addition of 10% H_2O recorded with presaturation (water and $\text{HO-CD}(\text{CF}_3)_2$ signal suppression). The addition of H_2O enables observation of the NH-signals. Note that the deuterium to protium exchange is only partial.



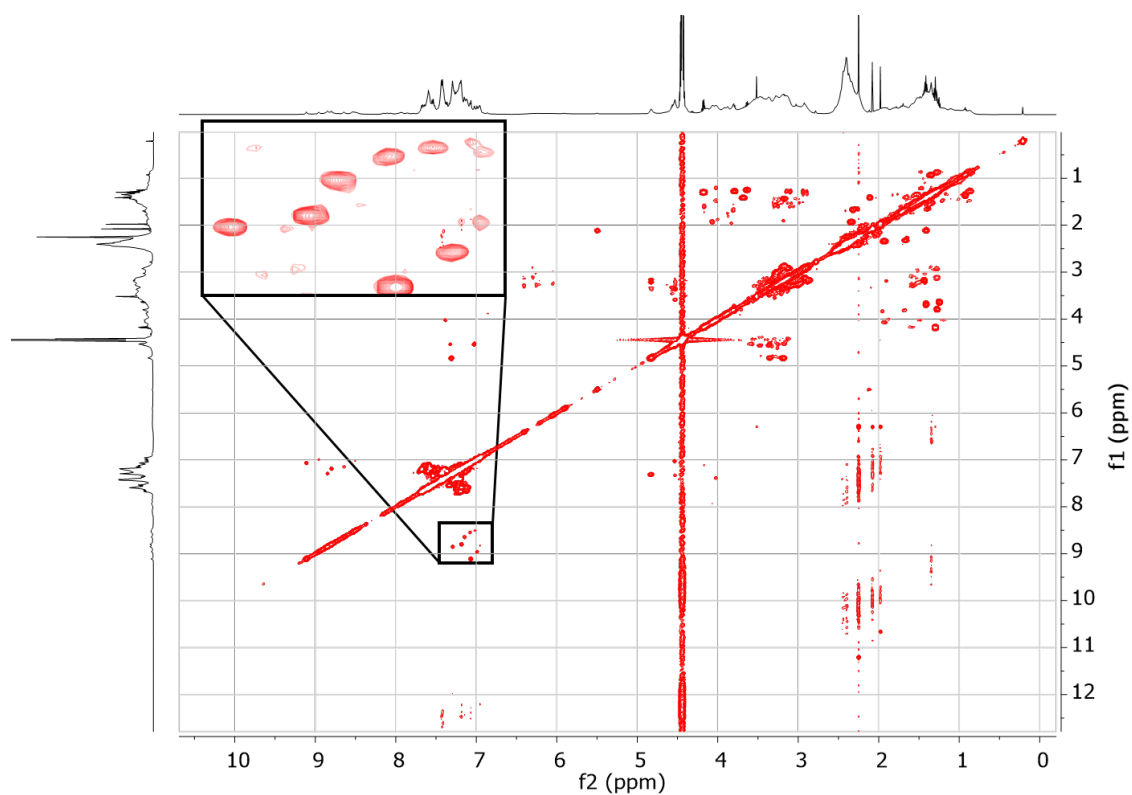
Supplementary Figure 16. HSQC of compound **4** in HFIP- d_2 . The inset shows a zoom into the C_α -region. However, a conclusive determination of the number of individual C_α -atoms and accordingly amino acid units was not possible with this experiment due to overlapping signals.



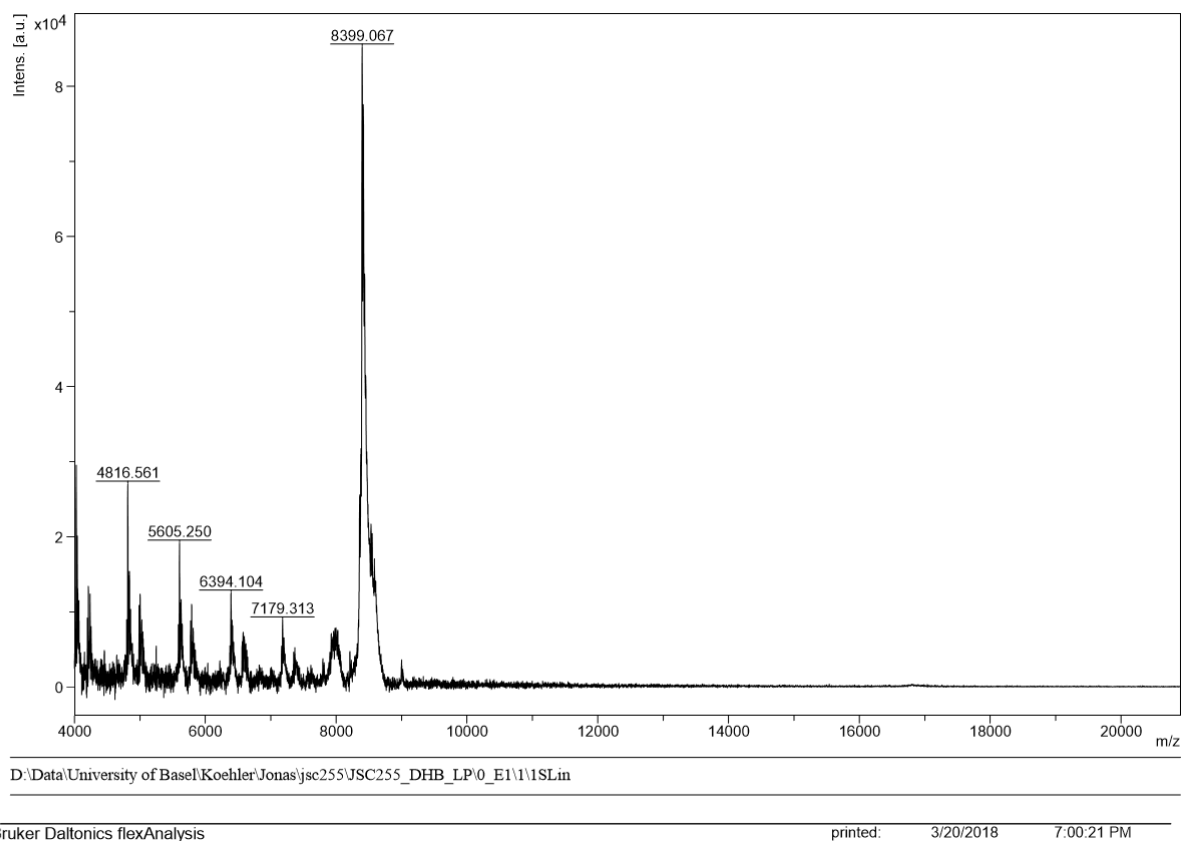
Supplementary Figure 17. NOESY of compound **4** in HFIP- d_2 after addition of 10% H_2O recorded with presaturation (water and $HO-CD(CF_3)_2$ signal suppression).



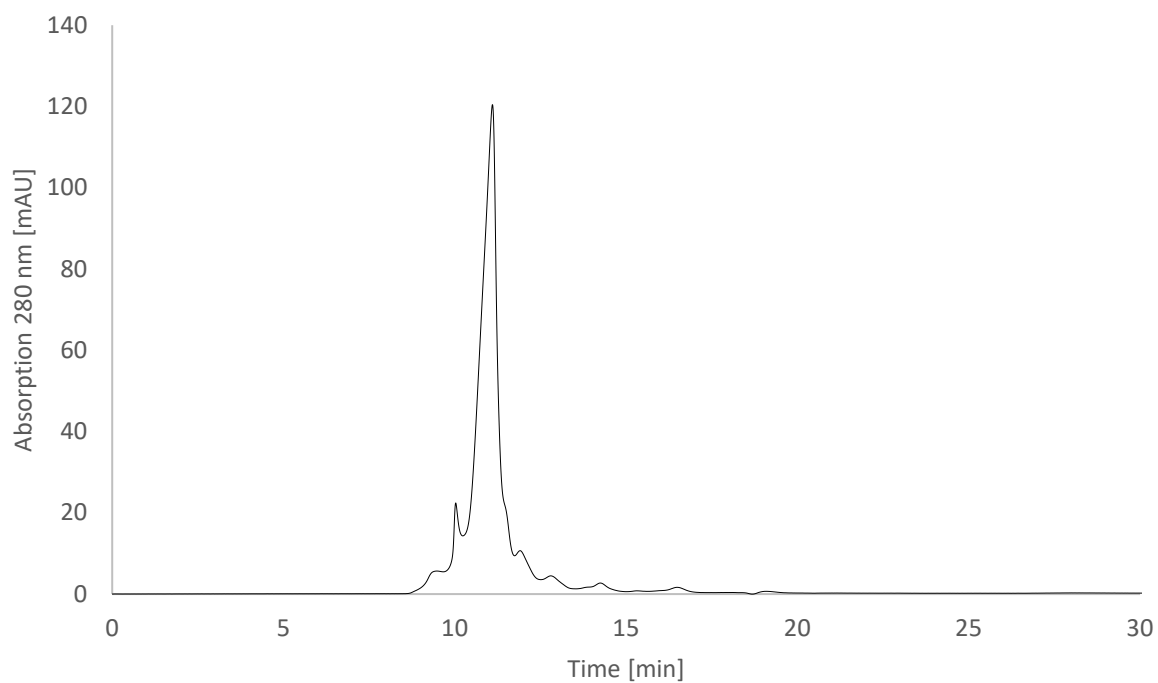
Supplementary Figure 18. Zoom into the indole NH region of NOESY spectrum of compound **4** shown in Supplementary Figure 17. 10 different indol NH moieties can be distinguished by indol CH correlations.



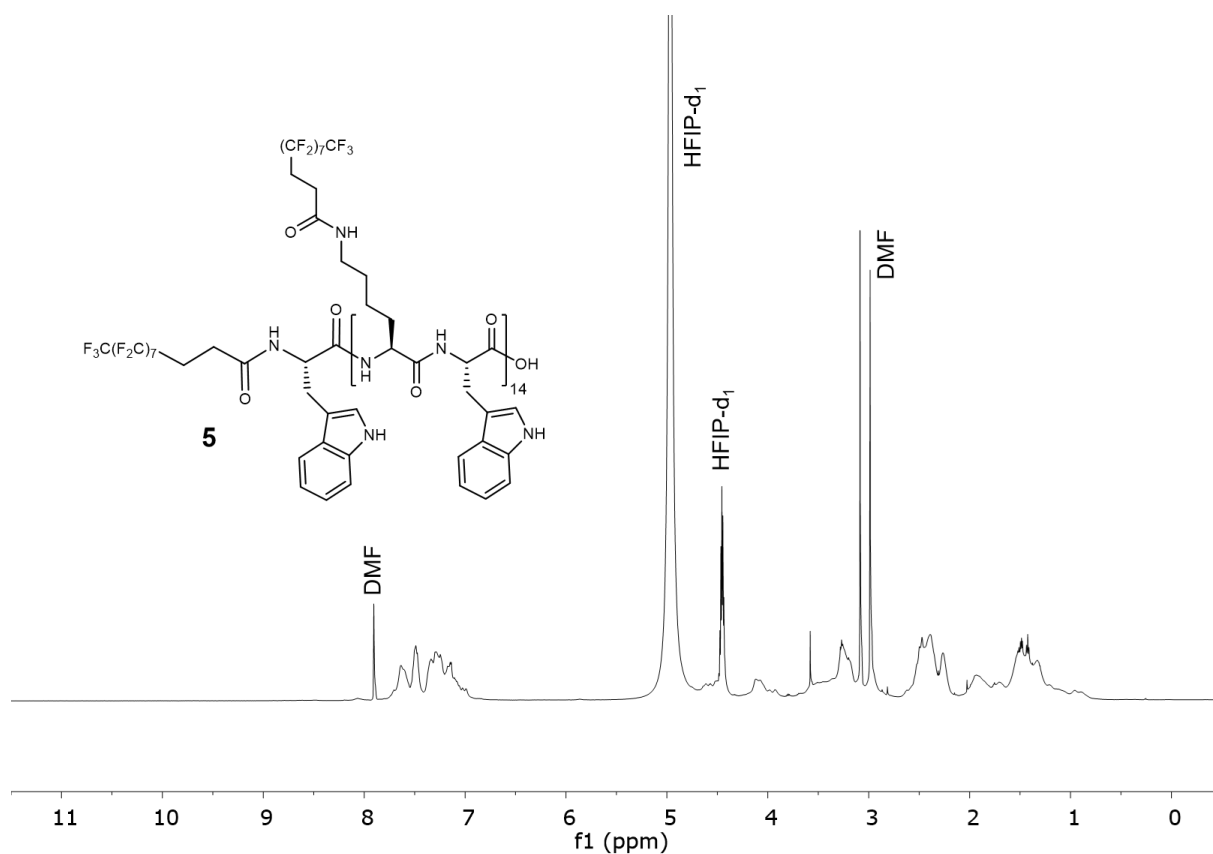
Supplementary Figure 19. COSY of compound **4** in HFIP- d_2 after addition of 10% H_2O , recorded with presaturation (water and $HO-CD(CF_3)_2$ signal suppression). The inset shows a zoom of the indole NH region revealing 10 individual indol NH-groups.



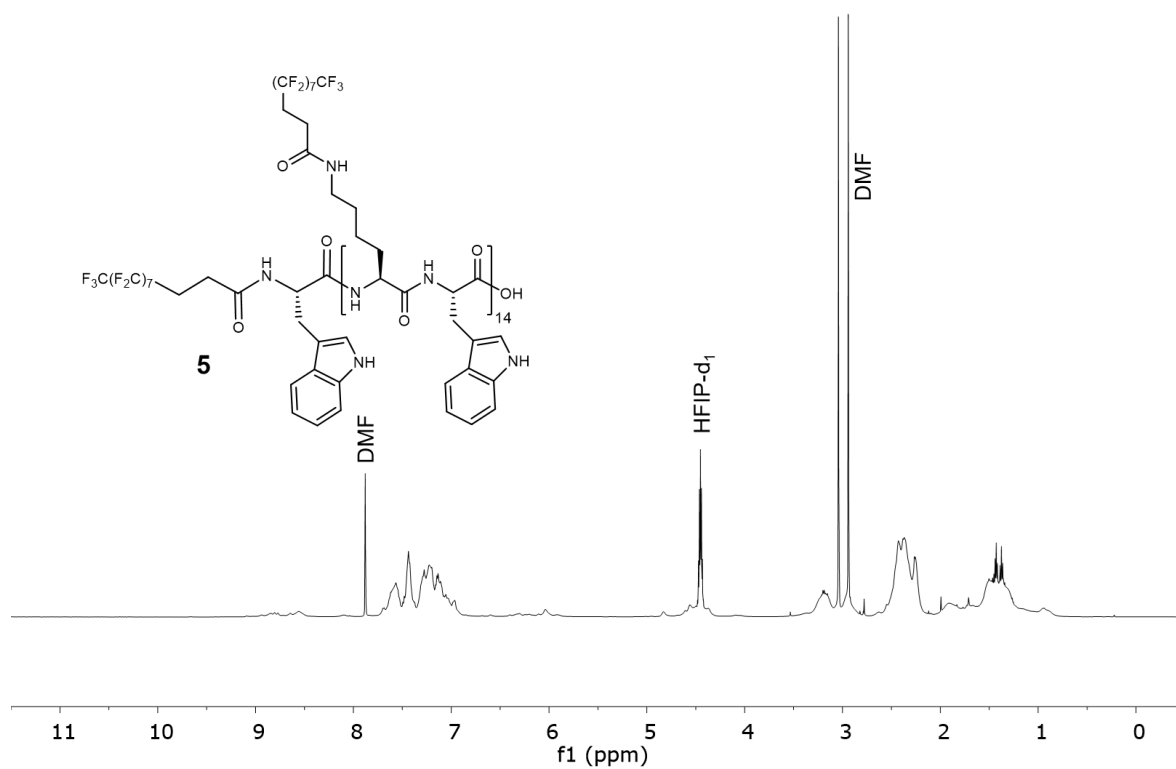
Supplementary Figure 20. MALDI-TOF spectra of compound **4** recorded with DHB as matrix.



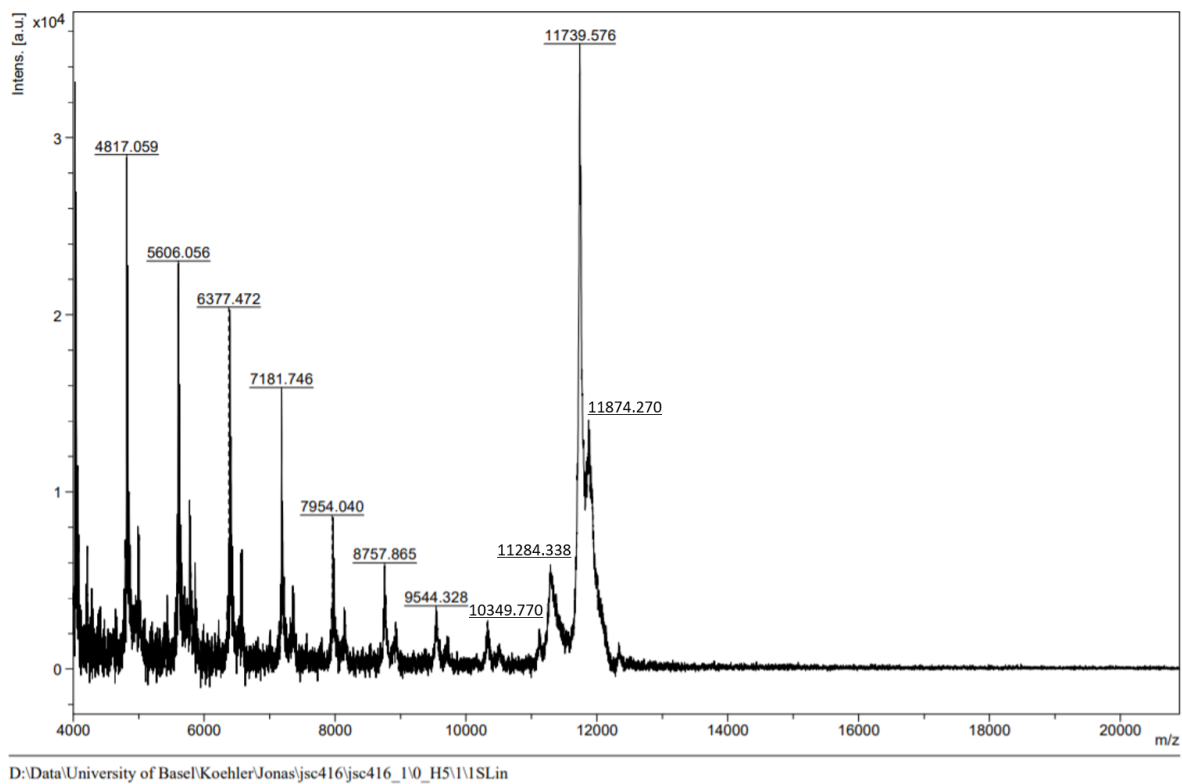
Supplementary Figure 21. Size exclusion chromatogram of compound **4**.



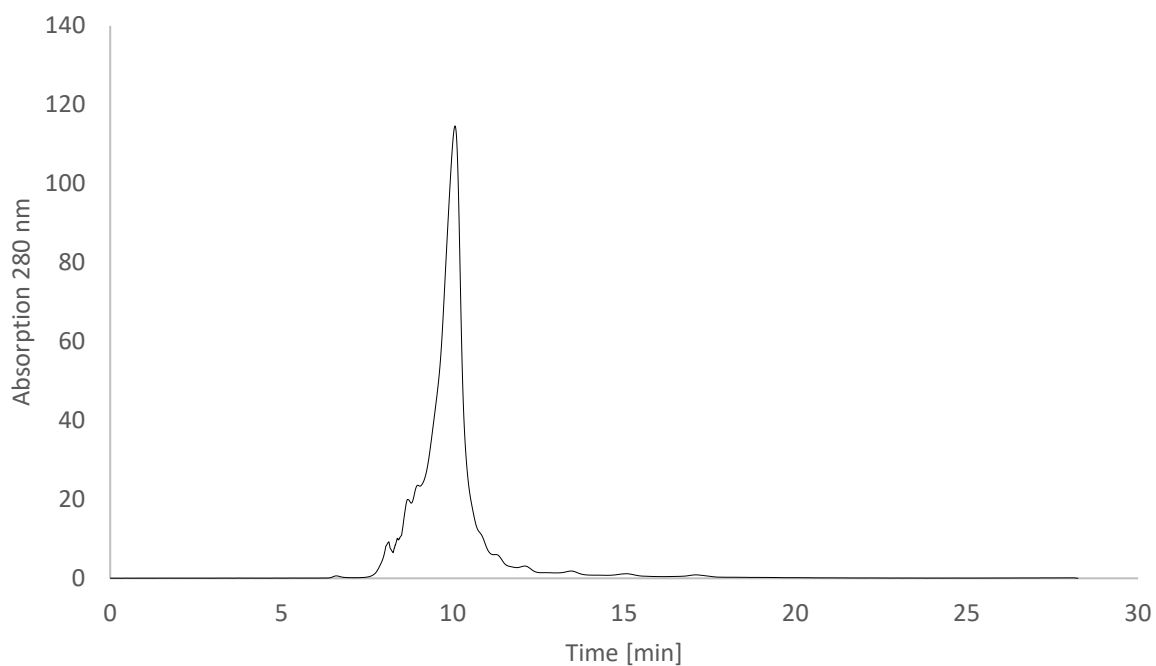
Supplementary Figure 22. ^1H -NMR (HFIP- d_2 , 600 MHz, 298 K) of compound **5**.



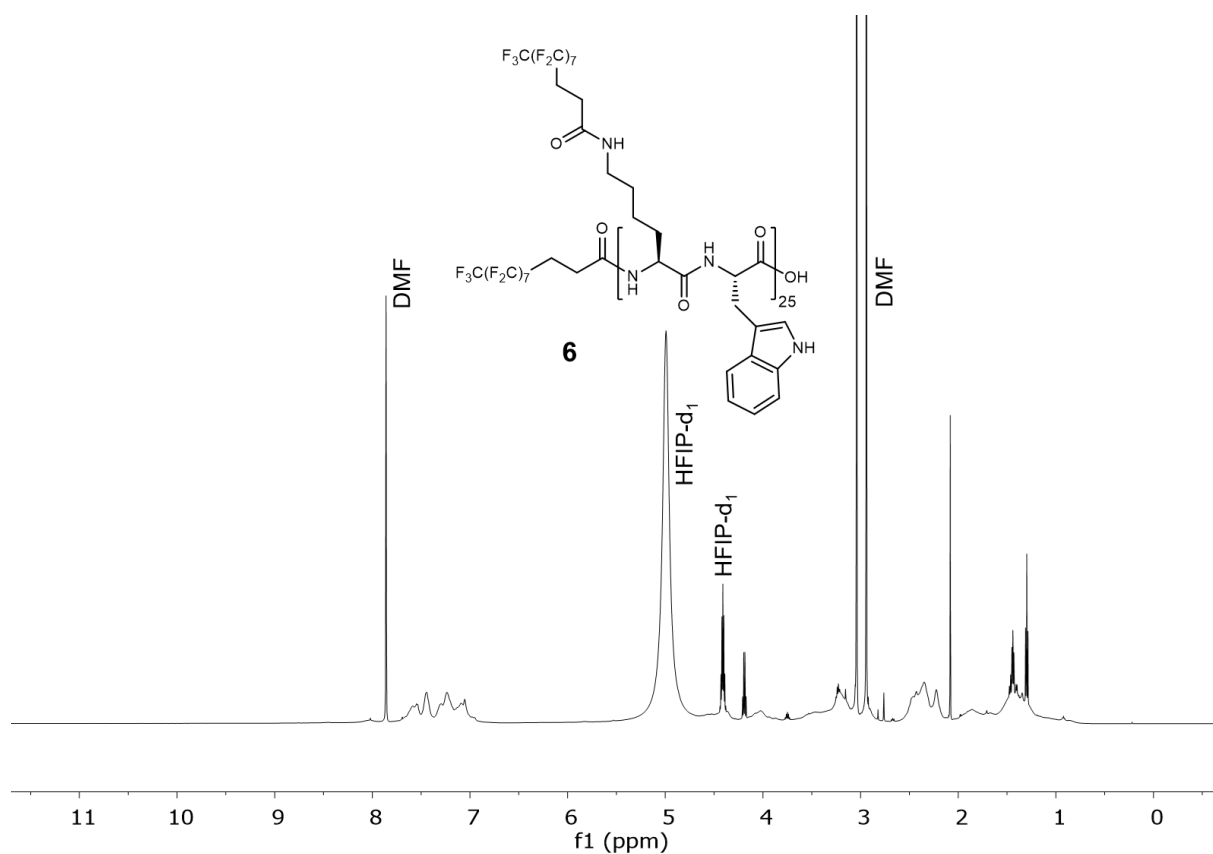
Supplementary Figure 23. ^1H -NMR (HFIP- d_2 , 600 MHz, 298 K), compound **5** after addition of 10% H_2O recorded with presaturation (water and $\text{HO-CD}(\text{CF}_3)_2$ signal suppression). The addition of H_2O enables observation of the NH-signals. Note that the deuterium to protium exchange is only partial.



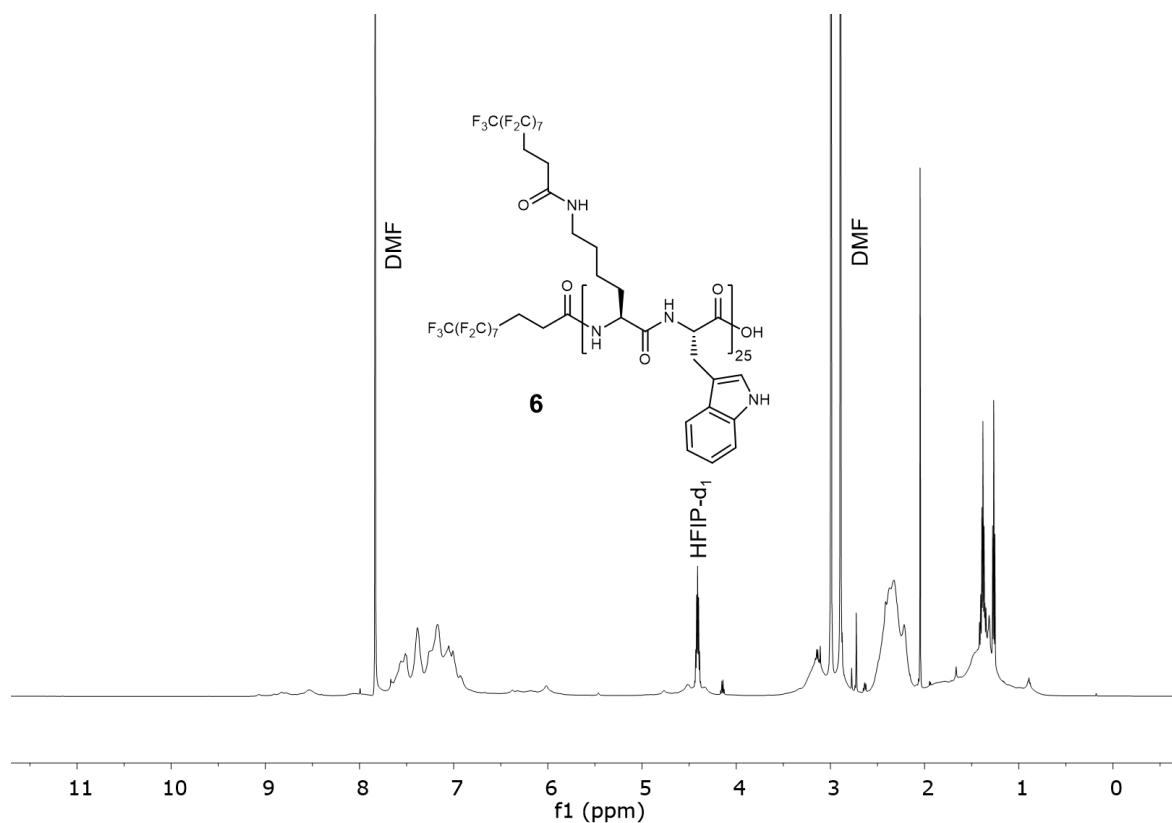
Supplementary Figure 24. MALDI-TOF Mass spectrum of compound **5** recorded with DHB as a matrix.



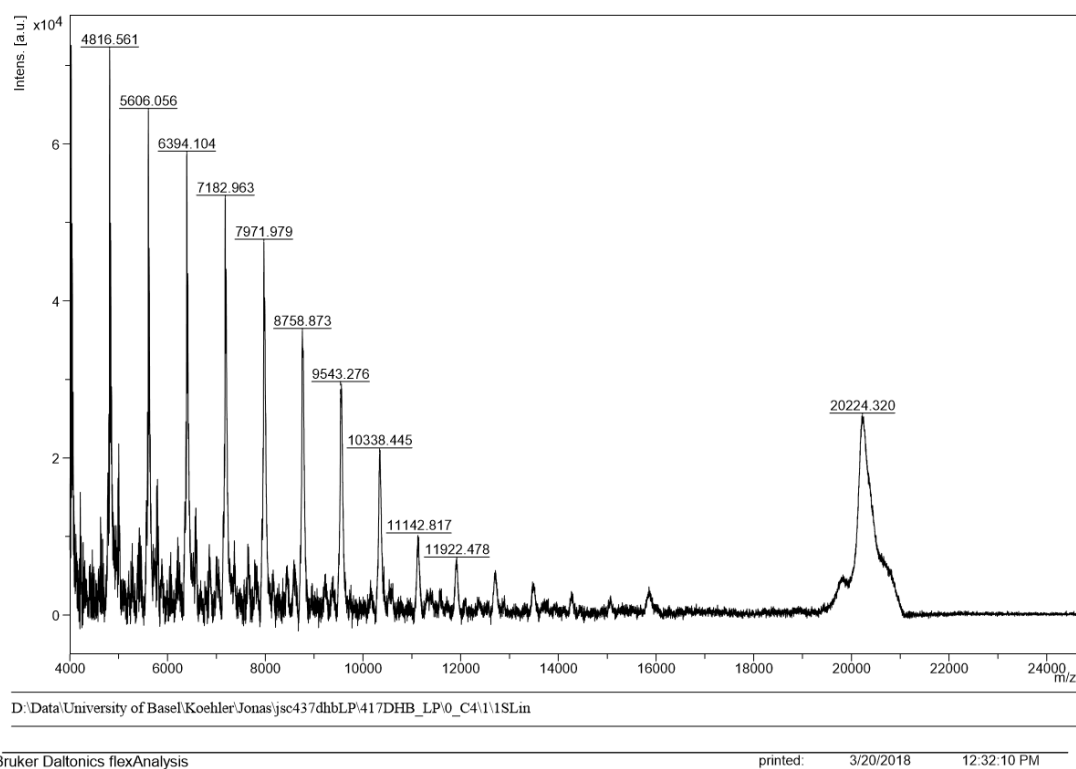
Supplementary Figure 25. Size exclusion chromatogram of compound **5**.



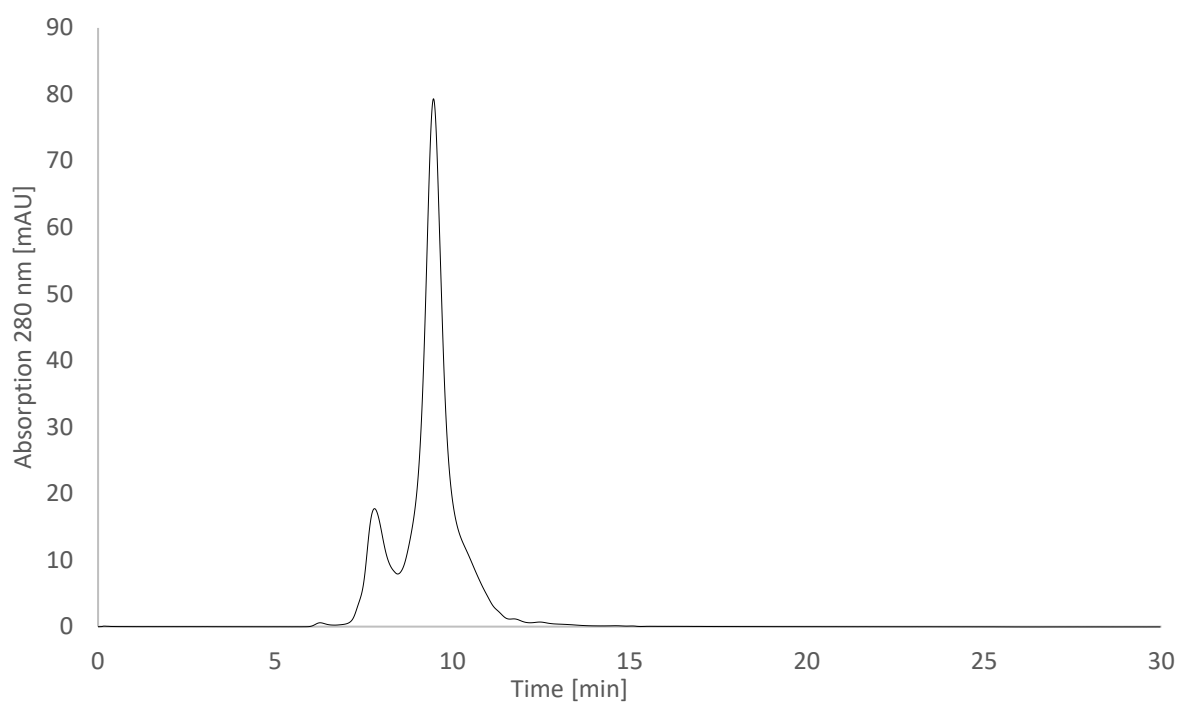
Supplementary Figure 26. ^1H -NMR (HFIP- d_2 , 600 MHz, 298 K) of compound **6**.



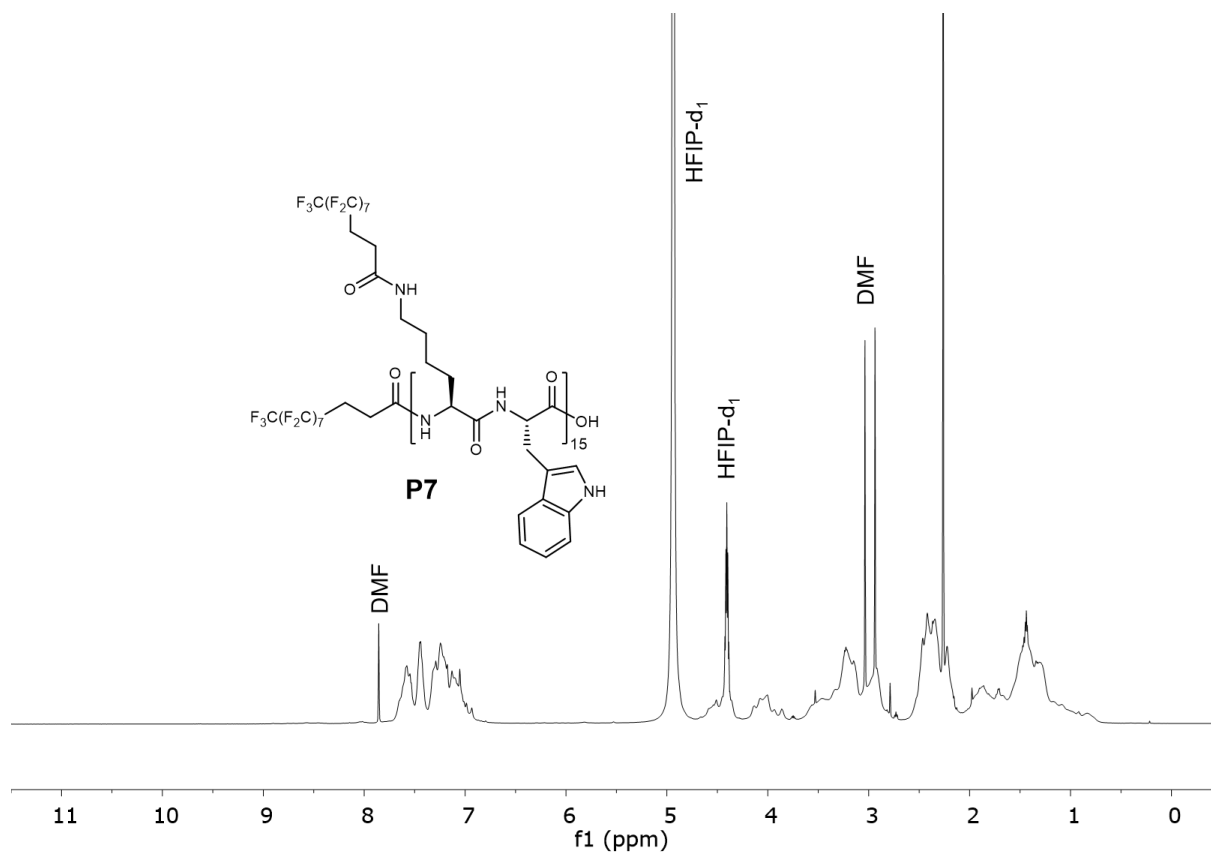
Supplementary Figure 27. ^1H -NMR (HFIP- d_2 , 600 MHz, 298 K), compound **6** after addition of 10% H_2O recorded with presaturation (water and $\text{HO-CD}(\text{CF}_3)_2$ signal suppression). The addition of H_2O enables observation of the NH-signals. Note that the deuterium to protium exchange is only partial.



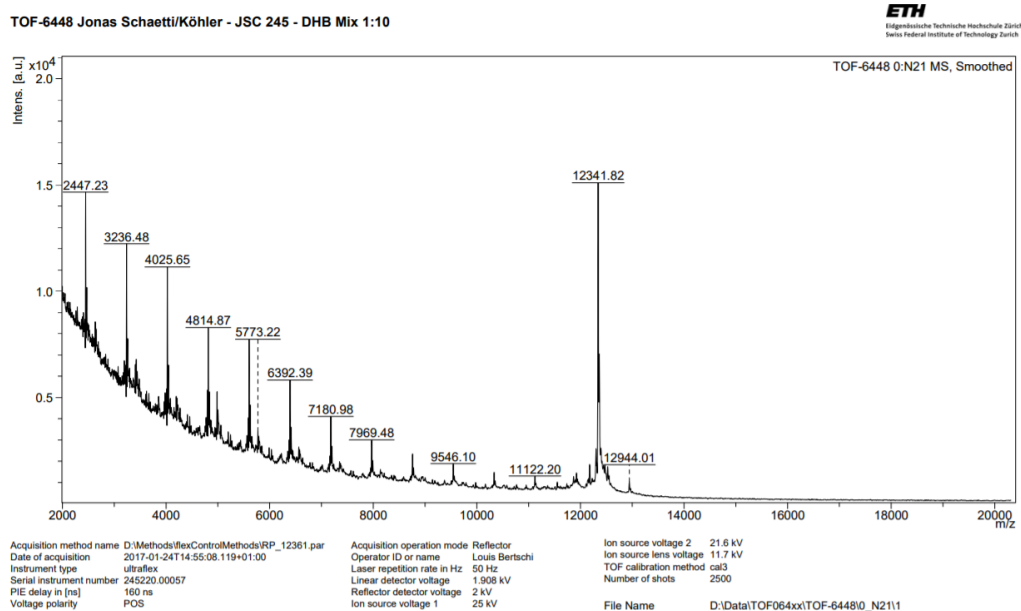
Supplementary Figure 28. MALDI-TOF spectrum of compound **6** recorded with DHB as a matrix.



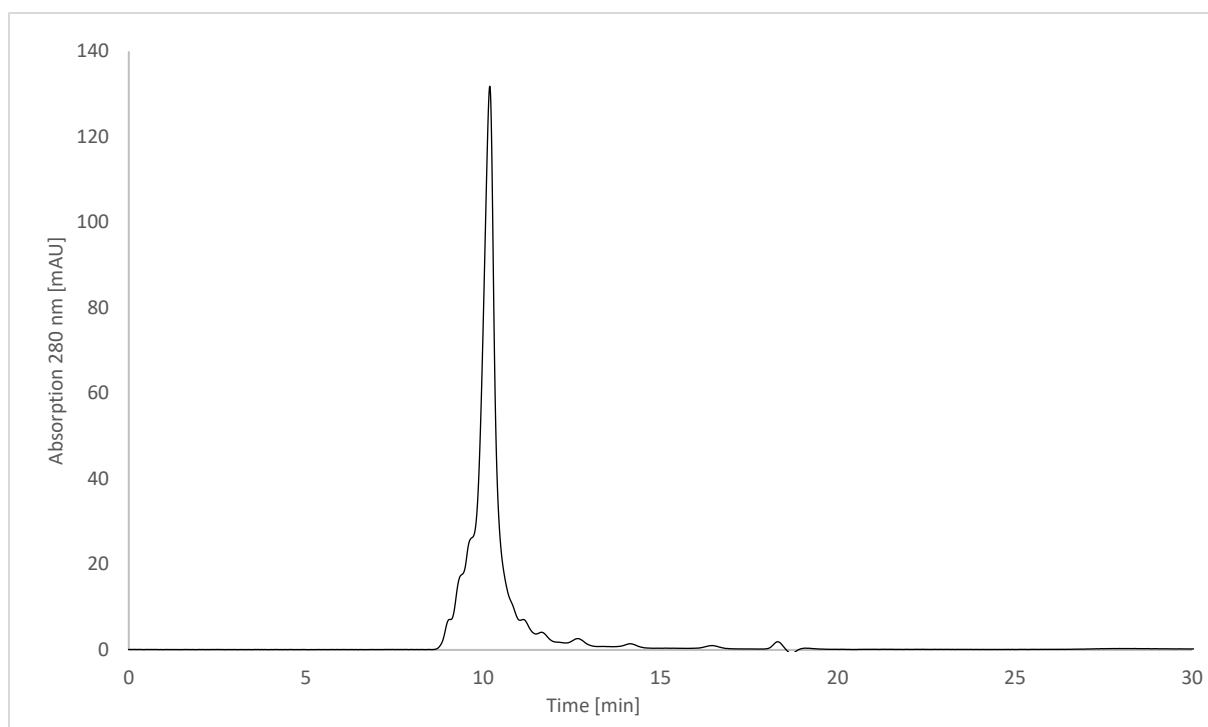
Supplementary Figure 29. Size exclusion chromatogram of compound **6**.



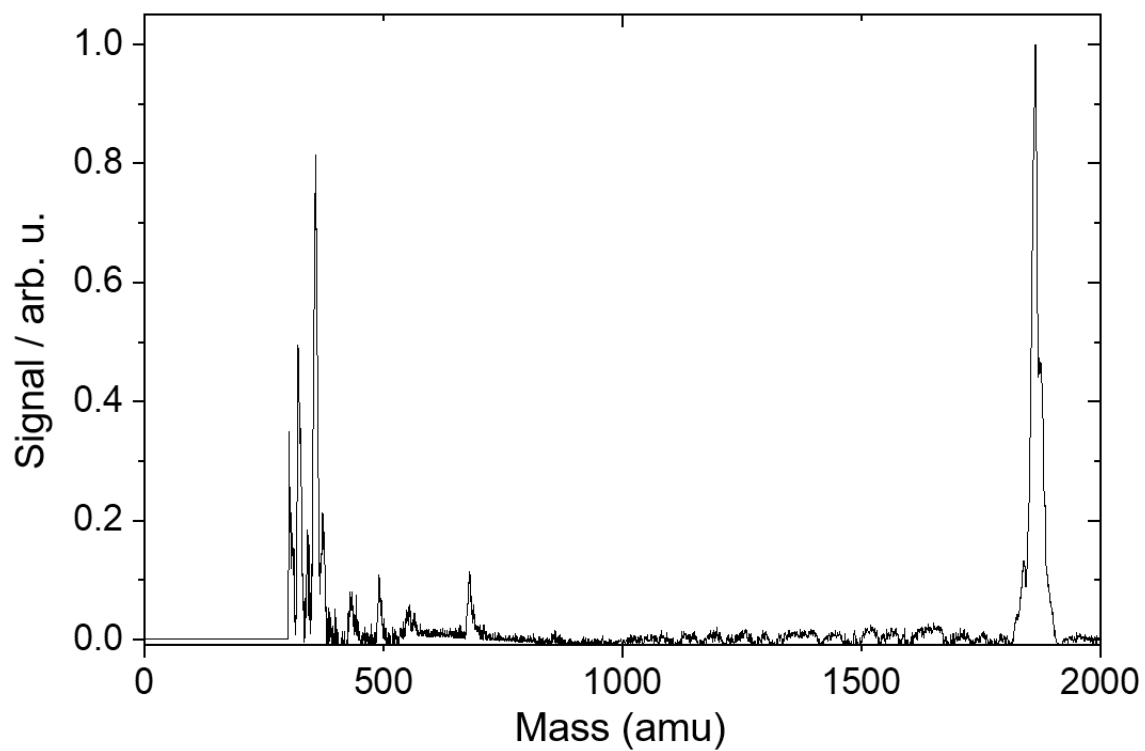
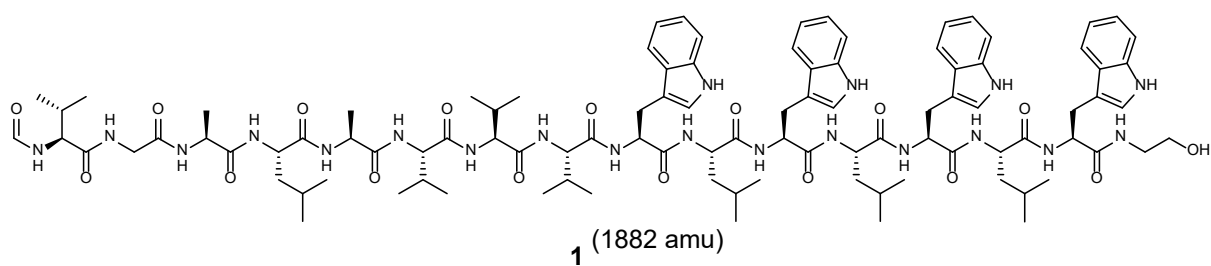
Supplementary Figure 30. ¹H-NMR (HFIP-d₂, 600 MHz, 298 K) of compound **P7**.



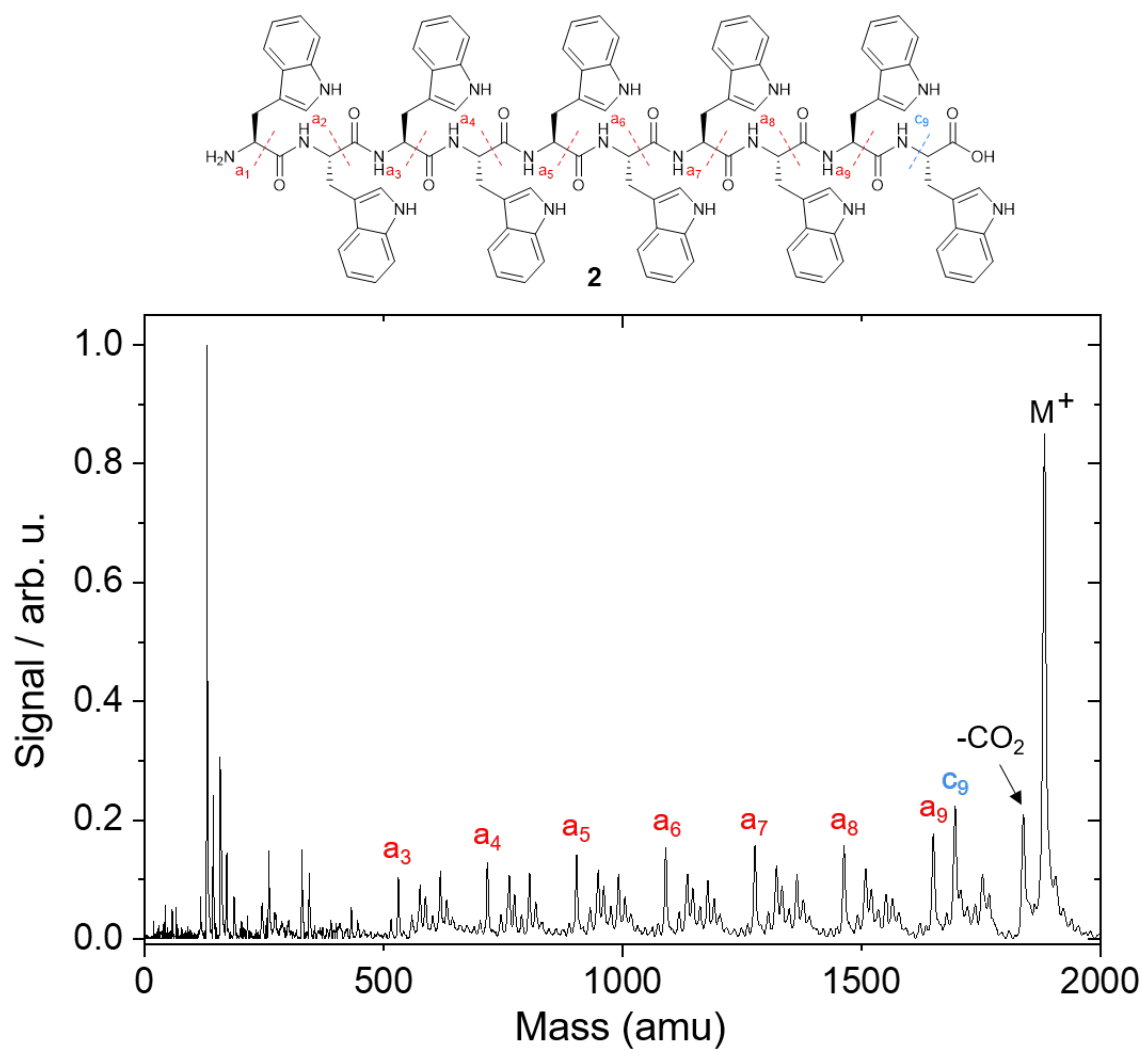
Supplementary Figure 31. MALDI-TOF Mass spectrum of compound **P7** recorded with DHB as a matrix.



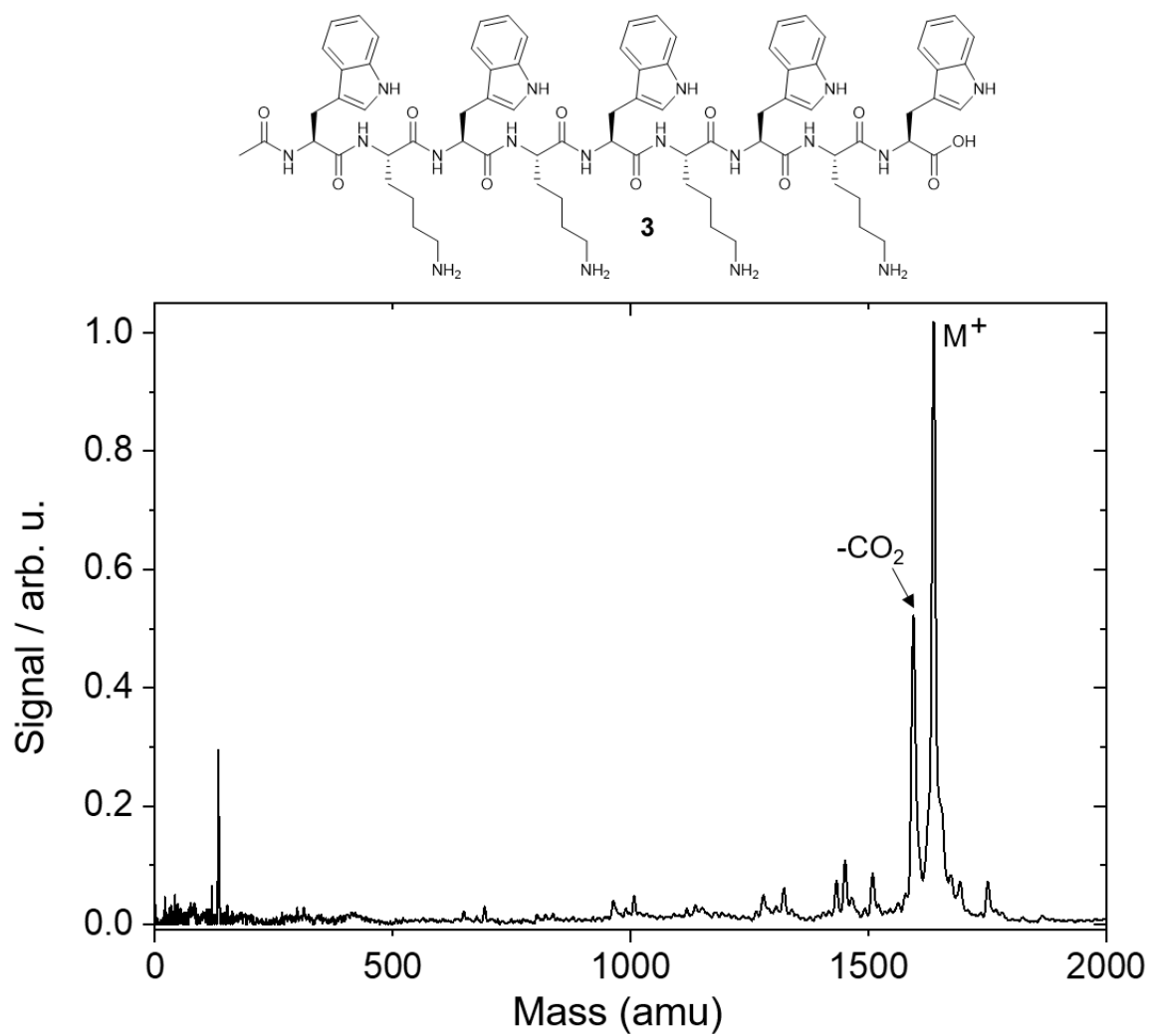
Supplementary Figure 32. Size exclusion chromatogram of compound **P7**.



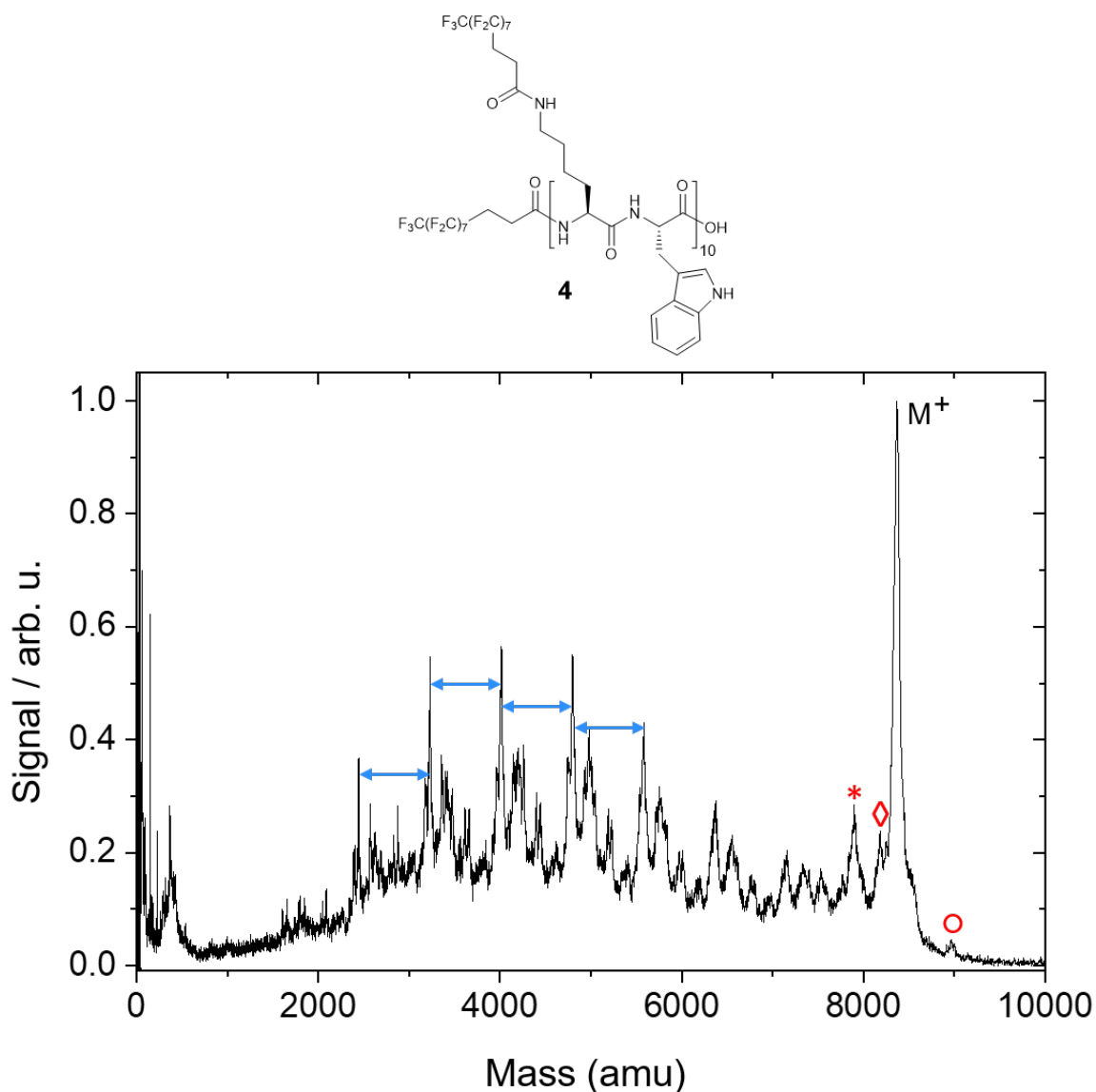
Supplementary Figure 33. Laser desorption/photoionization TOF mass spectrum of commercial Gramicidin D (Sigma Aldrich, purity as delivered, main component A1), recorded after shortpulse infrared laser desorption (7 ns, 1064 nm). The peaks below 500 amu are noise, unrelated to the desorbed molecule.



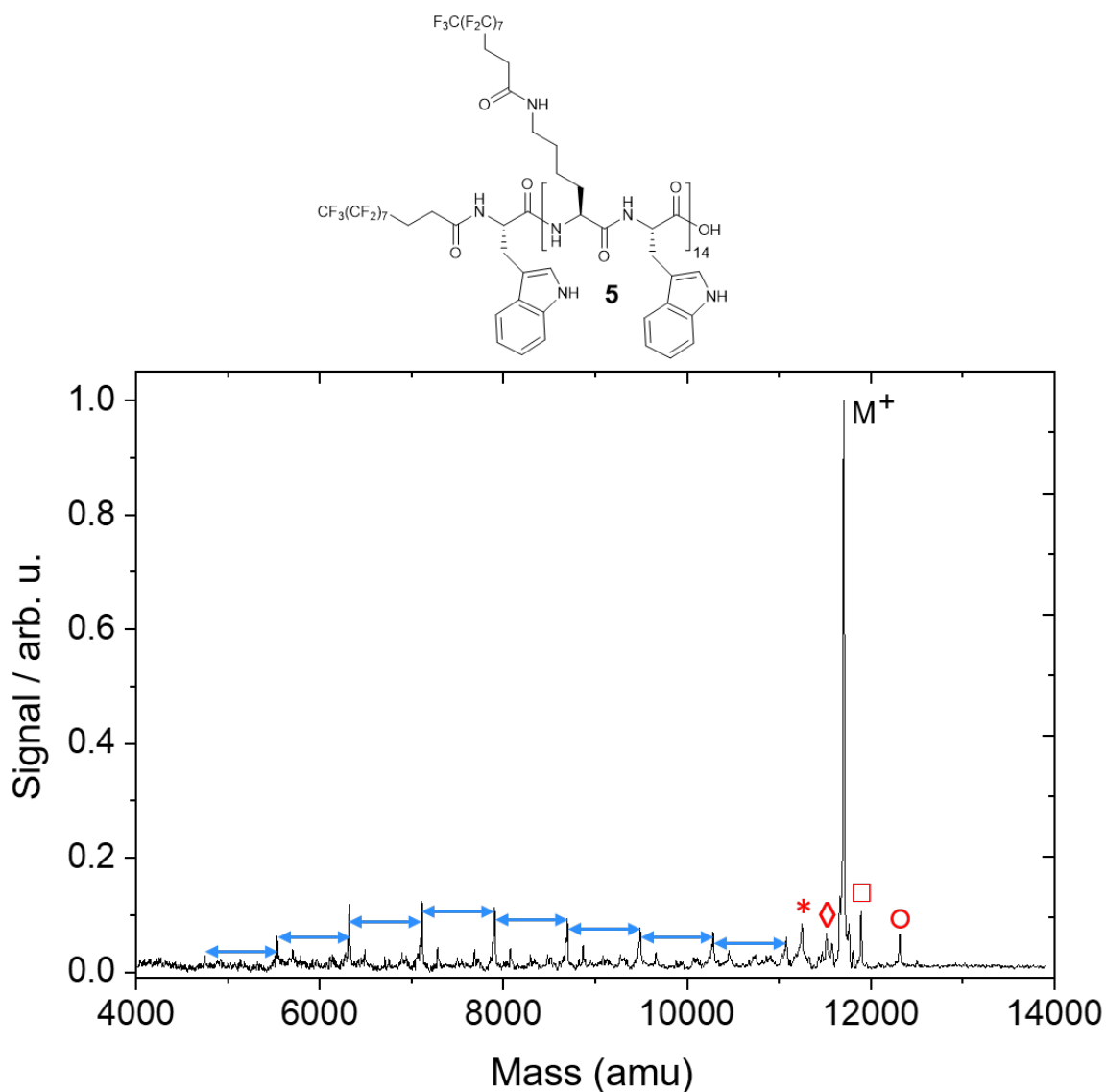
Supplementary Figure 34. Laser desorption/photoionization TOF mass spectrum of the TFA salt of deca-tryptophan **2** recorded after shortpulse infrared laser light desorption (7 ns, 1064 nm).



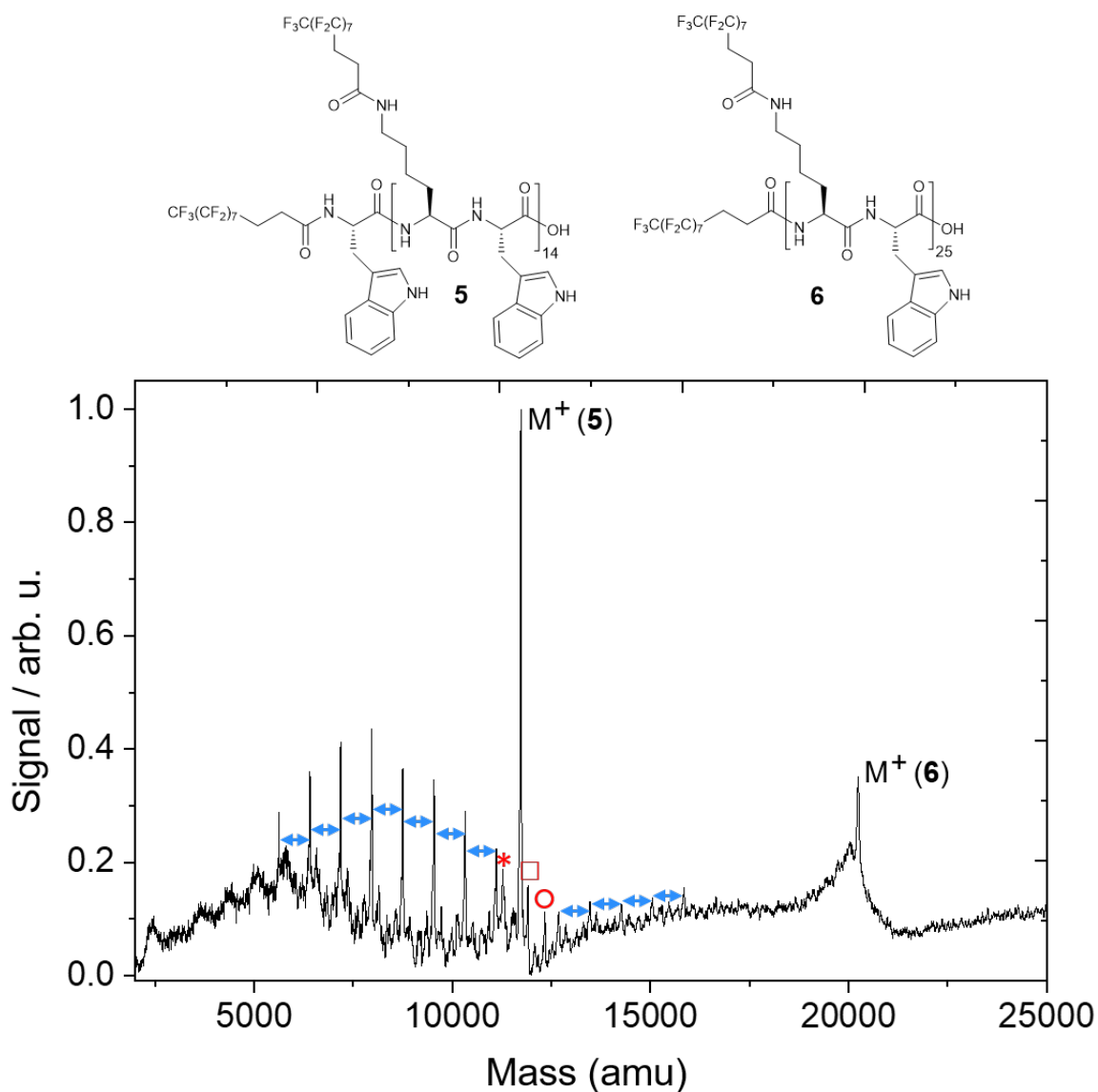
Supplementary Figure 35. Laser desorption/photoionization TOF mass spectrum of the TFA salt of compound **3** recorded after short-pulse infrared laser light desorption (7 ns, 1064 nm).



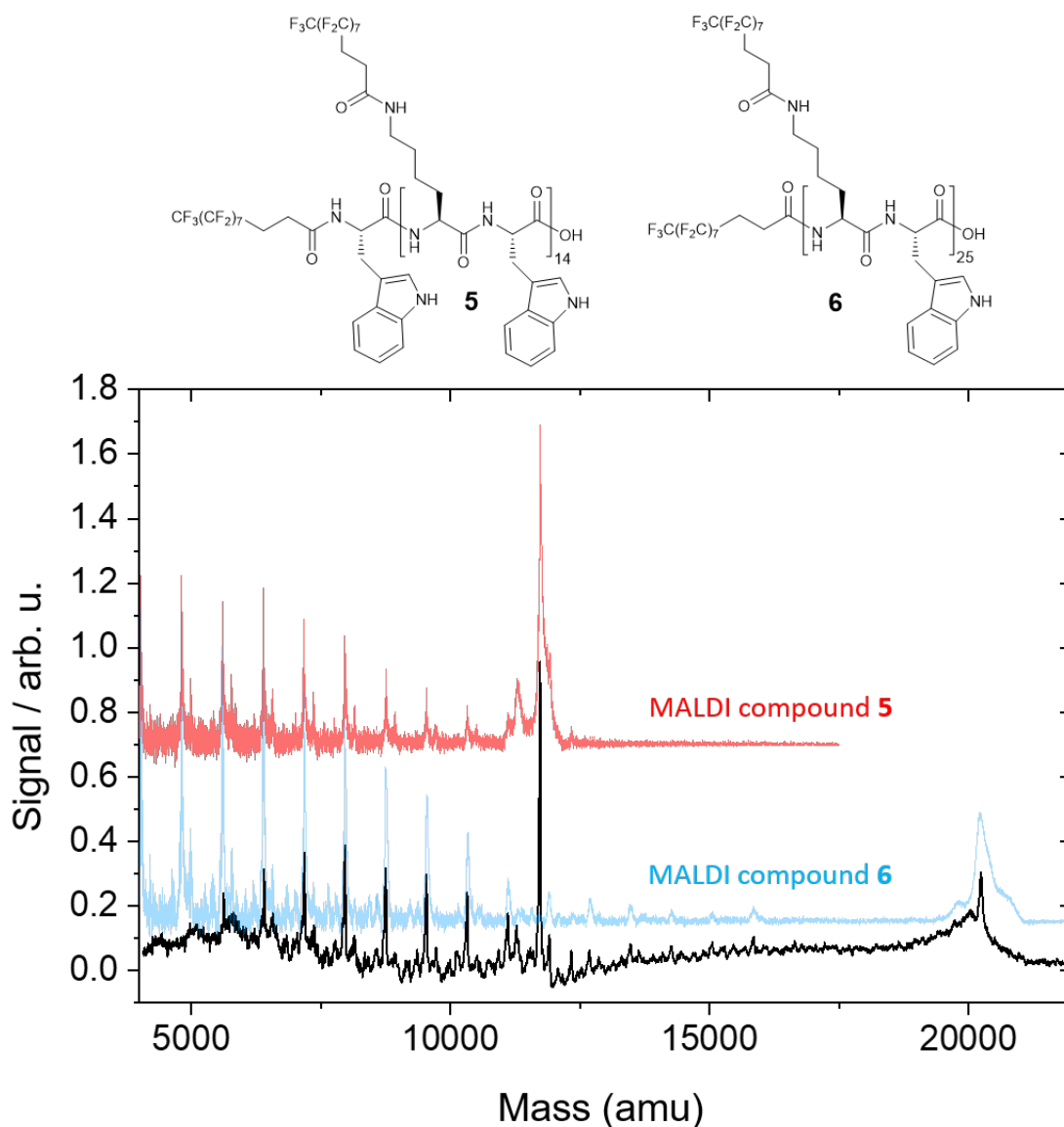
Supplementary Figure 36. Laser desorption/photoionization TOF mass spectra of compound (**4**), using 266 nm laser desorption from Trp matrix and carried by a T = 115 K Argon supersonic gas jet. The blue arrows indicate the mass difference of Trp-Lys(2H,2H,3H,3H-perfluoroundecanoyl) units (788 amu) between fragments. * corresponds to the loss of a 2H,2H,3H,3H-perfluoroundecanoyl unit (475 amu); ◊ corresponds to the loss of the C-terminal tryptophan unit (188 amu); ○ is likely caused by an impurity which results from incorporation of an additional Lys(2H,2H,3H,3H-perfluoroundecanoyl) unit (602 amu) during synthesis.



Supplementary Figure 37. Laser desorption/photoionization TOF mass spectra of compound **5** recorded after 343 nm laser desorption ($E = 15 \mu\text{J}$, $\tau = 292 \text{ fs}$) from Trp matrix at $T = 300 \text{ K}$. The blue arrows indicate the mass difference of Trp-Lys(2H,2H,3H,3H-perfluoroundecanoyl) units (788 amu) between fragments. * corresponds to the loss of a 2H,2H,3H,3H-perfluoroundecanoyl unit (475 amu); ◊ corresponds to the loss of the C-terminal tryptophan unit (188 amu); ◻ and ○ are likely caused by impurities which result from incorporation of an additional Trp (186 amu) or Lys(2H,2H,3H,3H-perfluoroundecanoyl) unit (602 amu) during synthesis.



Supplementary Figure 38. Laser desorption/photoionization TOF mass spectra of a mixture of compound 5 and 6 (ratio 3 : 28) recorded after 343 nm laser desorption ($E = 15 \mu\text{J}$, $\tau = 292 \text{ fs}$). The blue arrows indicate the mass difference of Trp-Lys(2H,2H,3H,3H-perfluoroundecanoyl) units (788 amu) between fragments. * corresponds to the loss of a 2H,2H,3H,3H-perfluoroundecanoyl unit (475 amu) from 5; □ and ○ are likely caused by impurities which result from incorporation of an additional Trp (186 amu) or Lys(2H,2H,3H,3H-perfluoroundecanoyl) unit (602 amu) during synthesis.



Supplementary Figure 39. Calibration of the high mass spectra. Overlay of MALDI-TOF mass spectra for compounds **5** and **6** and laser desorption/VUV postionization experiment depicted in Supplementary Figure 38, which was conducted with a mixture of compound **5** and **6**. An offset has been applied to highlight the match between the observed signals in the three spectra. Comparing the spectra allowed unambiguous calibration of the laser desorption/VUV postionization experiment. The MALDI instrument was calibrated with protein standards as specified in the synthesis section of the Supplementary Methods section.

Supplementary Note 2. Computational details

A single molecule of the artificial protein (**6**) was simulated in GROMACS 2018.1 using a vacuum setup, i.e. periodic boundary conditions switched off, the box is ignored, and no distance cut-offs employed when evaluating interatomic interactions. The GROMOS force-field version 54B7 was used as the force-field of the Lysine and Tryptophan residues,^{4,5} while the force-field for the fluoroalkyl chains was obtained using the Automated Topology Builder methodology, based on automated parametrization employing the GROMOS force-field and optimal partial charge assignment aided by ab-initio quantum calculations.^{6,7} Canonical-ensemble (NVT) molecular dynamics simulations were performed over the temperature range 10-400K in steps of 10K. The temperature coupling method employed was velocity rescaling with a stochastic term.⁸ The length of the bonds involving hydrogen atoms was constrained with the LINCS algorithm.⁹ The leapfrog integrator with a time step of 1 fs was employed. At each temperature, 200 runs of 2 ns each were performed. Each run started from the same stretched configuration, but with different initial velocities randomly assigned from a Maxwell distribution at each given temperature. Thus, simulations at each temperature spanned a combined length of 400 ns. For each temperature, the quantities of interest were averaged over the last 100 ps of each run, and then the pool of averages were re-averaged and the standard deviation was calculated. The average end-to-end distance, $\langle R_e \rangle$, was calculated between the terminus of the amino acids at the ends of the backbone. The average solvent surface accessible area, $\langle \text{SASA} \rangle$, was calculated using the method described in Ref. 10.¹⁰

Compound	(1)	(2)	(3)	(4)	(5)	(6)	(P2)	(P7)
MW / amu	1882	1880	1631	8377	11716	20201	3832	12320
Molecular beam								
Backing gas	Ar/Ne/Ne/Ne	Argon	Argon	Argon	Argon	Argon	Argon	Neon
Backing pressure /bar	20 / 28 / 28 / 28	20	30	48	48	48	48	48
Gas temperature / K	300	300	300	90	300	300	300	115
Source pressure/mbar	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	10 ⁻⁵	10 ⁻⁵	10 ⁻⁴	10 ⁻⁴
Trp. admixture	none	none	none	1:20	none	none	none	1:20
Desorption								
Desorption λ / nm	1064 / 266 / 1040 / 343	1064	1064	266	343	343	1064	266
Desorption τ	7 ns / 7 ns / 290 fs / 290 fs	7 ns	7 ns	7 ns	290 fs	290 fs	7 ns	7 ns
Desorption E	32 mJ / 4 mJ / 15 μ J / 15 μ J	32 mJ	32 mJ	1.77 mJ, 2.64 mJ, 4 mJ, 5.44 mJ	15 μ J	15 μ J	32 mJ	1.77 mJ, 2.64 mJ, 4 mJ, 5.44 mJ, 8.53 mJ
Circular spot diameter	3 mm / 3 mm / 100 μ m / 100 μ m	3 mm	3 mm	3 mm	100 μ m	100 μ m	3 mm	3 mm
Repetition rate / Hz	10 / 10 / 20 & 100 / 20 & 100	10	10	10	100	100	10	10
Ionization								
Wavelength λ / nm	157.6	157.6	157.6	157.6	157.6	157.6	157.6	157.6
Pulse duration τ / ns	10	10	10	10	10	10	10	10
Energy E / mJ	0.8	0.8	0.8	0.528, 0.8	1.6	1.6	0.8	0.528, 0.8
Spot size / mm x mm	3 x 1	3 x 1	3 x 1	3 x 1	3 x 1	3 x 1	3 x 1	3 x 1
Averaging / shots	500	500	300	500	5000	5000	600	1000

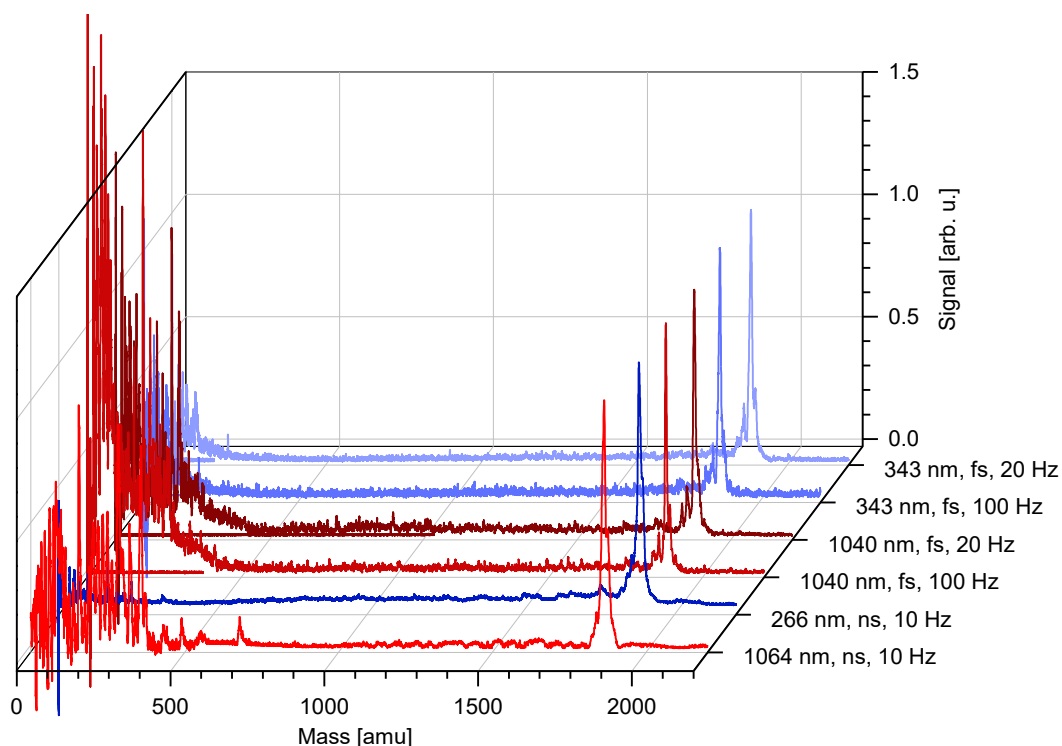
Supplementary Table 2. Desorption and ionization efficiency. Experimental parameters used in the laser desorption/photoionization experiments.

Supplementary Note 3. The influence of the desorption laser wavelength and pulse length on the character of the mass spectrum

Throughout this work, various laser wavelengths and pulse durations were explored to optimize the desorption conditions. As illustrated in Figure 5 of the main text, the final mass spectra for compound **(5)** resemble each other in almost all detail, even when comparing nanosecond and femtosecond desorption. The process differs, however, in the efficiency of the molecular individualization.

Here we complement this information by comparing six different desorption settings for gramicidin D, namely *nanosecond* desorption 1064 nm and 266 nm as well as *femtosecond* desorption with 1030 nm and 343 nm and repetition rates of 10, 20 and 100 Hz. This is shown in Supplementary Figure 40. While one finds different absolute numbers of molecules, the overall mass spectrum remains largely unchanged for gramicidin D.

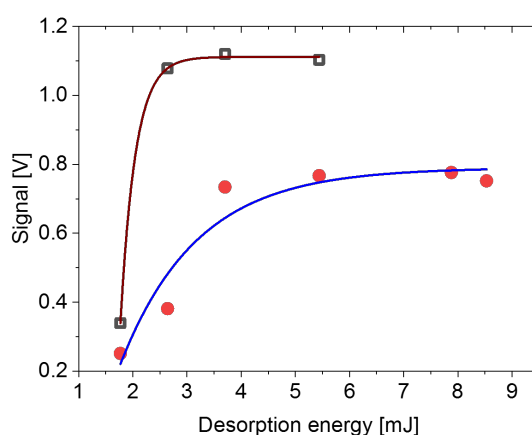
However, UV desorption becomes important for high-mass compounds, such as **(4)** and **(5)**, and the 20 kDa polypeptides **(6)** could only be seen after fs desorption at 343 nm. Both shorter wavelength and shorter pulses go along with a reduced penetration depth of the laser light. This is consistent with the observation of fewer molecular aggregates in the vacuum chamber.



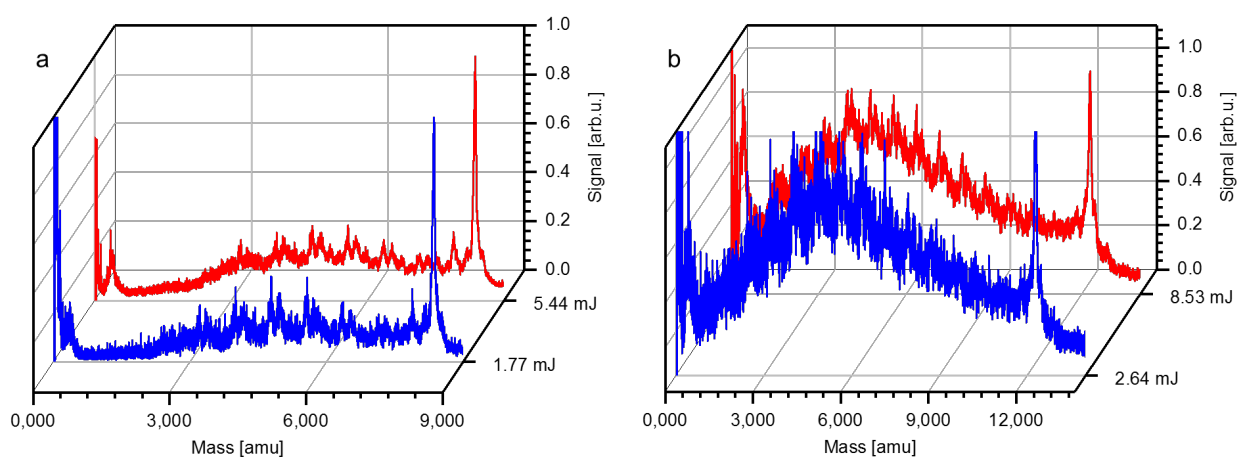
Supplementary Figure 40. Mass spectra of gramicidin D, recorded for different laser conditions as tabulated in Supplementary Table 2. Even under vastly different desorption conditions – from pulse durations between 290 fs to 7 ns and, energies between 40 μ J and 30 mJ and wavelengths between 343 nm and 1064 nm, the changing laser settings *significantly influence the number of released molecules but much less their mass spectrum*. The signals are normalized to the gramicidin peak. The noise below 500 amu is also present without the molecular beam. As described in the main text, the key difference between the different laser conditions is in the number of aggregates that are released by the pulses. They decide about the economy of the desorption process, but are too massive to be detected by the TOF-MS.

Supplementary Note 4. The influence of the desorption energy/fluence

When varying the desorption laser power, we observe the threshold behavior and saturation seen in Supplementary Figure 41. It suggests that all accessible material has been volatilized, that high power light facilitates the occurrence of large aggregates – which would not show up in TOF-MS - or that fragmentation processes start to compete with the intact volatilization process. The latter can be essentially excluded, when comparing signals differing more than 3-fold in desorption energy in Supplementary Figure 42.



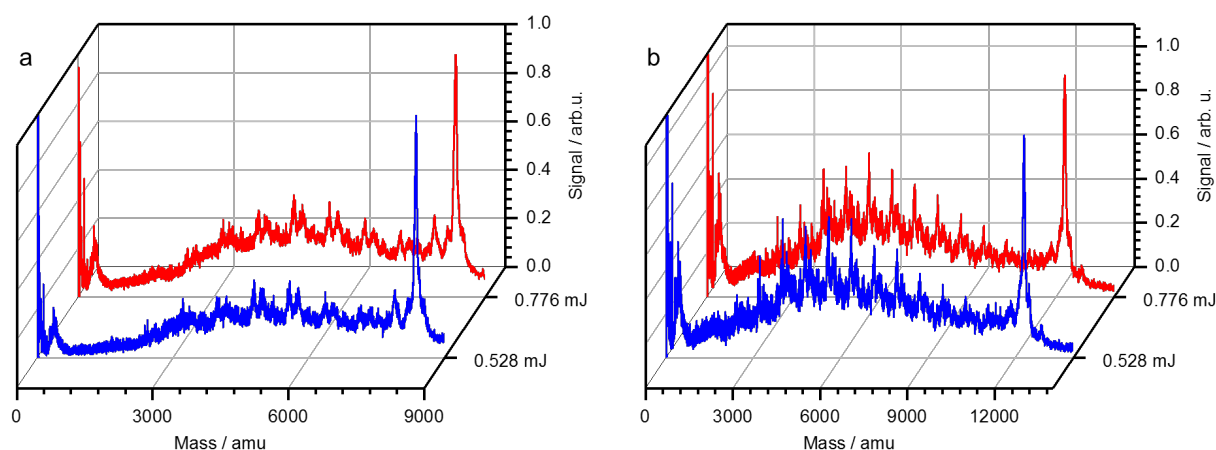
Supplementary Figure 41. The signal increases with **increasing desorption laser energy**, as is illustrated here for the high-mass polymers **(4)** with 8377 amu and **(P7)** with 12320 amu. They are represented by grey squares and red circles, respectively. The experimental data are fitted by exponential growth curves to guide the eye. The compounds were desorbed using 266 nm light with 7 ns pulse duration at 10 Hz repetition rate and ionized with 157.6 nm and 0.8 mJ pulse energy (see Supplementary Table 2).



Supplementary Figure 42. Mass spectra of (a) compound **(4)** and (b) compound **(P7)** for different desorption energies. The mass spectra for low desorption energies have been scaled to show that the desorption process does not induce any additional major fragmentation even when the energy is increased by a factor of 3. The compounds were desorbed using 266 nm light with 7 ns pulse duration at 10 Hz repetition rate and ionized with 157.6 nm and 0.8 mJ pulse energy (see Supplementary Table 2).

Supplementary Note 5. The influence of the ionization energy/fluence on the normalized mass peak

The ionization laser pulse energy obviously affects the ion signal of the principle mass and to first order one would expect a linear dependence for a single-photon ionization processes, as observed for cold clusters of tryptophan¹¹. On the other hand, it is justified to assume that the total absorption cross section grows linearly with the number of chromophores, i.e. here with the length of the peptide chain.



Supplementary Figure 43. Mass spectra of (a) compound (**4**) and (b) compound (**P7**) for different ionization energies. The mass spectra for low ionization energy have been scaled by a factor of 1.4. Within the 50% power variation, the overall molecular beam intensity follows linearly the laser energy while the mass peak distribution does not change substantially. The data are shown without adaptive baseline correction. The experimental conditions are given in Supplementary Table 2 with 4 mJ desorption pulse energy.

Supplementary References

- [1] Chan WC, White PD. *Fmoc Solid Phase Peptide Synthesis*. Oxford University Press, Oxford (2000).
- [2] Malik L, Nygaard J, Hoiberg-Nielsen R, Arleth L, Hoeg-Jensen T, Jensen KJ. Perfluoroalkyl chains direct novel self-assembly of insulin. *Langmuir* **28**, 593-603 (2012).
- [3] Schätti J, Sezer U, Pedalino S, Cotter JP, Arndt M, Mayor M, Köhler V. Tailoring the volatility and stability of oligopeptides. *J Mass Spectrom* **52**, 550-556 (2017).
- [4] Poger D, Mark AE, van Gunsteren WF. A new force field for simulating phosphatidylcholine bilayers. *J Comput Chem* **31**, 1117-1125 (2010).
- [5] Schmid N, Eichenberger AP, Choutko A, Riniker S, Winger M, Mark AE, van Gunsteren WF. Definition and testing of the GROMOS force-field versions 54A7 and 54B7. *Eur Biophys J* **40**, 843-856 (2011).

- [6] Malde AK, Zuo L, Breeze M, Stroet M, Poger D, Nair PC, Oostenbrink C, Mark AE. An Automated force field Topology Builder (ATB) and repository: version 1.0. *J Chem Theory Comput* **7**, 4026-4037 (2011).
- [7] Koziara KB, Stroet M, Malde AK, Mark AE. Testing and validation of the Automated Topology Builder (ATB) version 2.0: prediction of hydration free enthalpies. *J Comput Aid Mol Des* **28**, 221-233 (2014).
- [8] Bussia G, Donadio D, Parrinello M. Canonical sampling through velocity rescaling. *J Chem Phys* **126**, 014101 (2007).
- [9] Hess B, Bekker H, Berendsen HJC, Fraaije JGEM. LINCS: A linear constraint solver for molecular simulations. *J Comput Chem* **18** 1463-1472 (1997).
- [10] Eisenhaber F, Lijnzaad P, Argos P, Sander, C, Scharf M. The double cubic lattice method: Efficient approaches to numerical integration of surface area and volume and to dot surface contouring of molecular assemblies. *J Comput Chem* **16**, 273-284 (1995).
- [11] M. Marksteiner, P. Haslinger, M. Scalfani, H. Ulbricht, M. Arndt, UV and VUV Ionization of Organic Molecules, Clusters, and Complexes, *J. Phys. Chem. A* **113**, 9952-9957 (2009).
- [12] S. Gerlich, S. Eibenberger, M. Tomandl, S. Nimmrichter, K. Hornberger, P. Fagan, J. Tüxen, M. Mayor, M. Arndt, Quantum interference of large organic molecules, *Nature Commun* **2**, 263 (2011).