# Supplementary Information

# Conformational interplay in hybrid peptide-helical aromatic foldamer macrocycles

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**This PDF file includes:**

# 1. Materials and methods for chemical synthesis

## 1.1. General

Fmoc-$Q^{Sul}$-OH, Fmoc-$Q^{Ala}$-OH, Fmoc-$Q^{Asp(tBu)}$-OH, and P monomer were prepared by following the reported synthetic protocols.[1,2,3,4] The synthetic procedure for Fmoc-$Q^{Sem}$-OH will be reported in due course. If not otherwise mentioned, chemical reagents were purchased from Sigma-Aldrich, and solvents from Fisher Scientific and used without further purification. Anhydrous tetrahydrofuran (THF) and anhydrous dichloromethane (DCM) were obtained from MBRAUN SPS-800 solvent purification system. Anhydrous chloroform ($CHCl_3$) and *N,N*-diisopropylethylamine (DIPEA) were distilled over $CaH_2$ prior to use. Exclusively ultrapure water was used. DMF and NMP (peptide grade) were purchased from Carlo Erba. Rink amide MBHA, Cl-MPA ProTide®, and low-loading preloaded Fmoc-Gly-Wang resins were purchased from CEM. Low-loading Wang and cysteamine 2-chlorotrityl resins were purchased from Sigma. Fmoc-*N*-protected amino acids, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium-hexafluorophosphate (HBTU) were purchased from IRIS. α-Chymotrypsin and Trypsin were obtained from bovine pancreas; Pronase E from Streptomyces Griseus and were all purchased from Sigma-Aldrich.

## 1.2. Methods for Solid-Phase Synthesis

### Solid Phase Peptide Synthesis

The peptide segment was assembled by using a Liberty Blue CEM® synthesizer at a minimum scale of 50 μmol. Microwave couplings were performed twice at 75 °C for 10 min with N-Fmoc-α-amino acid (10 equiv. relative to the resin loading), HBTU (9 equiv.), and DIPEA (12 equiv.) in DMF. Fmoc deprotection was performed twice with 20% piperidine in DMF at 75 °C (1 × 30 sec. and 1 × 180 sec.). Wang and Rink amide resins were washed with DMF (2 × 2 mL) after each deprotection step and one time 3 mL after each coupling step. When ProTide® resin was

[1] B. Baptiste, C. Douat-Casassus, K. Laxmi-Reddy, F. Godde and I. Huc, *J Org Chem*, 2010, **75**, 7175-7185.
[2] J. Buratto, C. Colombo, M. Stupfel, S. J. Dawson, C. Dolain, B. Langlois d'Estaintot, L. Fischer, T. Granier, M. Laguerre, B. Gallois and I. Huc, *Angew Chem Int Ed*, 2014, **53**, 883-887.
[3] M. Vallade, P. Sai Reddy, L. Fischer and I. Huc, *Eur. J. Org. Chem*, **2018**, 5489-5498.
[4] X. Hu, S. J. Dawson, P. K. Mandal, X. de Hatten, B. Baptiste and I. Huc, *Chem Sci*, 2017, **8**, 3741-3749.

used, after the Fmoc deprotection the resin was extensively washed with DMF (4 × 4 mL) and one time with 4 mL DMF after the coupling step.

## Solid Phase Foldamer Synthesis

The microwave-assisted solid-phase synthesis (SPS) of foldamer-peptide hybrids was carried out on a Discover-Bio CEM® microwave oven in an open vessel mode. The temperature of the reaction mixture within the reactor vessel was monitored with an optical fiber probe.

Two different solid-phase synthesis approaches of foldamer-peptide hybrids have been implemented. Linear oligomers **1a**, **3a-5a**, **8a**, **9a, 13a, 14a, 15a** were synthesized by iterative couplings of Fmoc-P-OH and Fmoc-Q-OH on corresponding resin-bound peptides. Linear oligomers **2a**, **6a**, **7a**, **10a,** and **11a** were built by relying on a fragment condensation approach of Fmoc-foldamer-OH segments on resin-bound H-Gly-peptides by using BOP/DIPEA as coupling reagents.

Sequences **9a**-**11a** were assembled on ProTide® resin because the PEG-polystyrene resin matrix appeared to give much better yield and crude purity of the resulting foldamer-peptide hybrids.

✓ **Fmoc Deprotection**. The resin was suspended at room temperature in a solution of 20% piperidine in DMF for 1 × 3 min and 1 × 7 min with one DMF washing in between the two cycles. To note, in the case of $Q^{Sul}$-rich sequences, after the Fmoc deprotection of the first installed $Q^{Sul}$ residue, the resin was incubated for 3 × 10 minutes with a solution of 20% DIPEA in NMP to substitute the piperidine salt formed on the sulfonic acid side chain.[4] This process was next performed until the end of the SPFS. The resin was finally washed with dry THF (3 × 3 mL) prior to perform the in situ coupling.

✓ **In situ Coupling of Fmoc-Q-OH or Fmoc-P-OH on resin-bound H-Q-oligomers.** To the pre-swollen resin in dry THF (1.25 mL) was added *2*,*4*,*6*-Collidine (9.0 equiv. relative to the resin loading). Concurrently, the Fmoc-aromatic monomer (*i.e.* Fmoc-Q-OH or Fmoc-P-OH, 3 equiv.) was suspended in 1.25 mL anhydrous $CHCl_3$ together with triphenylphosphine (8 equiv). Trichloroacetonitrile (TCAN) was quickly added and the reaction mixture was vigorously mixed before to be added to the suspended resin within 30-60 sec. The reaction vessel was then placed under microwave irradiation (25 W, ramp to 50 °C over 5 min, then hold at 50 °C for 15 min). The resin was filtered off and washed with anhydrous THF (2 × 3 mL). The coupling step was repeated once. The resin was then filtered off and washed with THF (3 × 3 mL) and DMF (2 × 3 mL).[5]

[5] X. Hu, S. J. Dawson, Y. Nagaoka, A. Tanatani and I. Huc, *J Org Chem*, 2016, **81**, 1137-1150.

✓ **HBTU coupling to resin-bound amine-P-oligomer or resin-bound amine-Gly-peptide.** To the pre-swollen resin in DMF (2.5 mL), Fmoc-Q-OH (3 equiv.), HBTU (2.9 equiv.), and DIEA (6 equiv.) were successively added. The reaction vessel was next placed in the microwave oven and heating was applied (25 W, ramp to 50 °C over 5 min, then hold at 50 °C for 10 min). The resin was filtered off, rinsed twice with DMF (3 mL) and the coupling step was repeated once. The resin was then filtered off and washed with and DMF (3 x 3 mL).

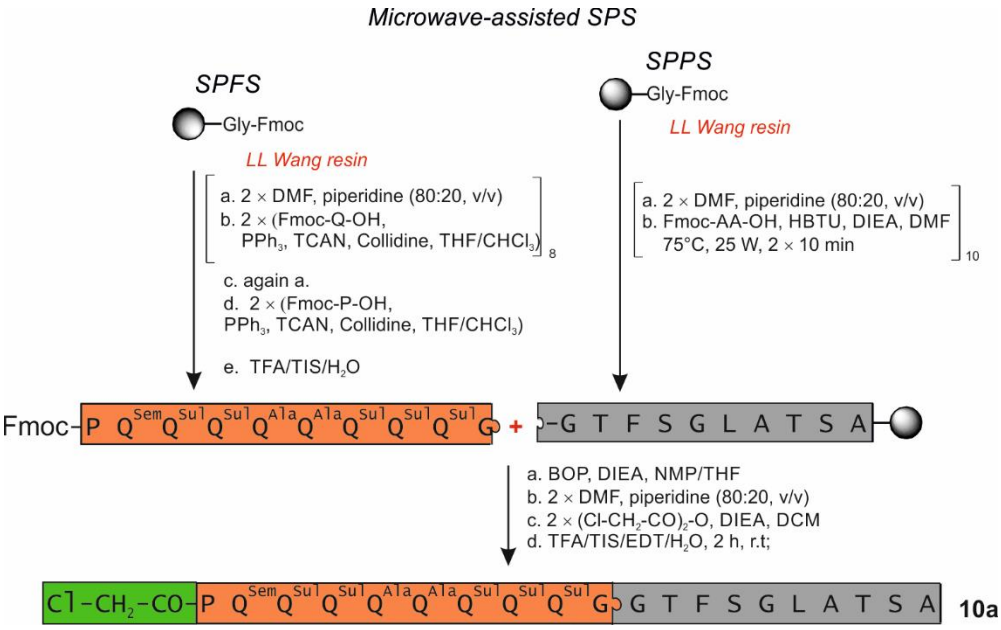



**Figure S1.** Schematic representation of the fragment condensation approach between a pre-build Fmoc-foldamer-OH segment and a resin-bound peptide.

✓ **Fragment condensation.** Prior to use, the crude Fmoc-foldamer-OH (1 equiv. relative to resin-bound H-Gly-peptide) was lyophilized to remove any remaining moisture. The freeze-dried solid was dissolved in a mixture of anhydrous NMP and anhydrous THF (50:50, v/v, 250 µL) directly followed by the addition of anhydrous DIPEA (4.5 equiv.) and BOP (1.5 equiv.). After 3 minutes of pre-activation, the mixture was added to resin-bound H-Gly-peptide under nitrogen atmosphere. The mixture was stirred for four days at r.t. To note after one day, 1.5 equiv. of BOP and 4.5 equiv of DIPEA were added immediately followed by one additional equiv. of resin-bound H-Gly-peptide to drive the reaction to completion. The progress of the reaction was monitored by analyzing the reaction mixture via RP-HPLC. The disappearance of the Foldamer from the supernatant indicated the progress of the reaction. Afterward, the resin was filtered off, washed with DMF (3 × 3 mL) and any remaining resin-bound free amine peptide was acetylated by using an acetic anhydride mixture in DCM (see conditions below).

✓ **N-capping of truncated sequences**

Any remaining free amine (aromatic or aliphatic) resulting from an incomplete coupling step was acetylated by incubating the resin with a solution of acetic anhydride in DCM (50:50, v/v) for 10 minutes at r.t. The resin was then filtered off and washed with DCM (3 × 3 mL) and DMF (3 × 3 mL).

✓ **General procedure for N-terminal chloroacetylation**

Before performing the chloroacetylation step on the resin-bound $NH_2$-oligomer, the resin was transferred to a 5 mL syringe equipped with a filter and washed with DCM (3 × 3 mL). Chloroacetic anhydride (10 equiv. in respect to N-terminal amine) was dissolved together with DIPEA (20 equiv.) in DCM. The reaction mixture was directly added to the resin and shaken for 15 minutes at r.t. This coupling step was repeated once without any washing in between. The resin was then filtered off, washed with DCM (3 × 3 mL) and dried briefly under a nitrogen stream.

✓ **Cleavage of resin-bound oligomers**

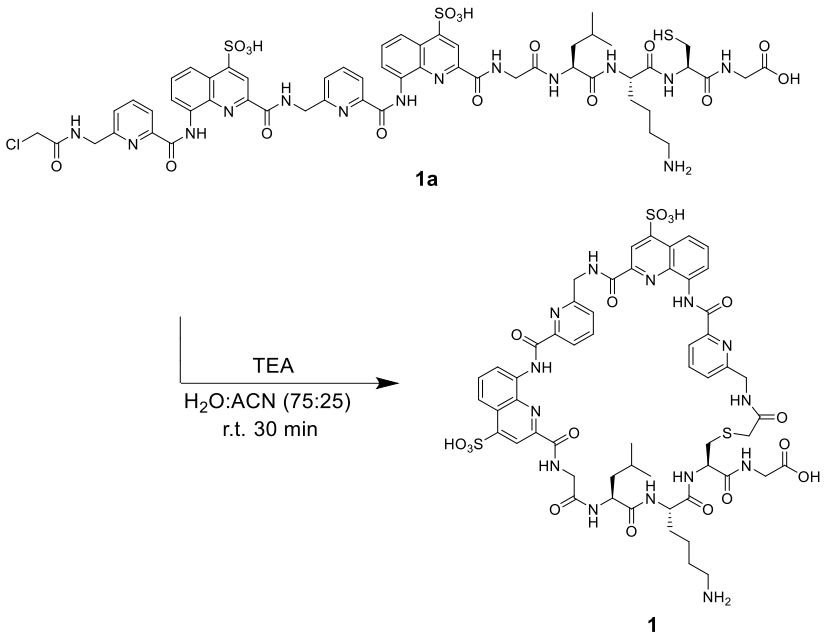
Sidechain deprotection and cleavage were performed simultaneously by treatment of the resin with a cleavage cocktail (10-15 mL per gram of resin) consisting of TFA/TIS/EDT/$H_2O$ (92.5:2.5:2.5:2.5, v/v/v/v) for 120 min at r.t for Cys-containing foldamer-peptides and TFA/TIS/$H_2O$ (95:2.5:2.5, v/v/v) for Fmoc-foldamer-Gly-OH segments. Foldamer-peptide hybrids (or Fmoc-foldamer-Gly-OH) were precipitated by adding cold $Et_2O$ and centrifugation. The precipitate was dried under a nitrogen stream, suspended in an acetonitrile/water mixture containing 0.1 % TFA, filtered, and lyophilized to obtain the crude linear oligomers as a yellowish powder.

In the case of $Q^{Sul}$-rich oligomers, incubating the LL-Wang resin with 1 mL of DMF after TFA cleavage for several hours could increase the yield.

## Foldamer-peptide hybrid macrocyclization

A diluted solution of Cl-$CH_2$-CO-foldamer-peptide-OH oligomer was incubated either in a 0.5 M $Et_3N$/DMF or a 0.5 M $Et_3N$/water/$CH_3CN$ solution at room temperature. Completion of the macrocyclization was monitored by RP-HPLC and the crude mixture was then lyophilized before to be purified by semi-preparative RP-HPLC to give the expected macrocycle in high purity and good yield.

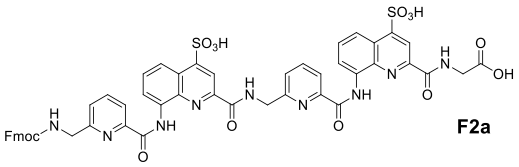
# 2. Experimental procedures for chemical synthesis



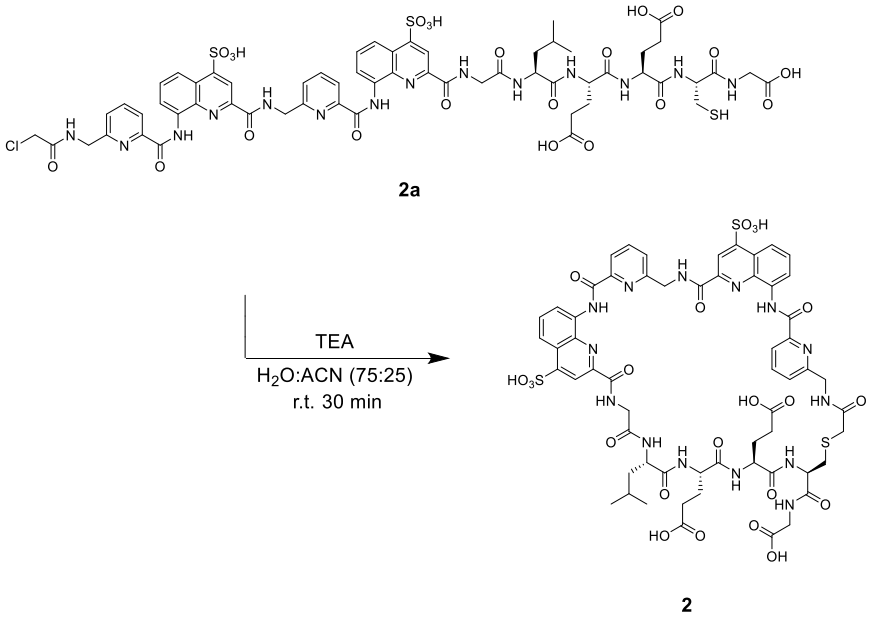

**Compound 1a**: Foldamer-peptide **1a** was built by relying on the CEM Liberty Blue microwave automated peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After chloroacetylation, the resin was cleaved with TFA/TIS/$H_2O$ and the crude product (57 mg, 86%) was directly used in the macrocyclization reaction. HRMS ($ESI^+$) *m/z* calcd for $C_{55}H_{62}ClN_{14}O_{17}S_3$ $[M+H]^+$ 1321.3268 found 1321.3064.

**Compound 1:** Compound **1a** was dissolved in 5 mL of a water/$CH_3CN$ 75:25 (v/v) solvent mixture to a 7.5 mM dilution and TEA (352 µL, 0.5 M) was added. After 30 min at r.t. without agitation, the solvents were evaporated by freeze drying and the crude macrocycle was purified by using semi-preparative RP-HPLC (gradient: from 10% to 40% solvent D over 15 minutes at 50 °C) to give **1** as a light yellow solid (35.0 mg, 61%). $^1H$ NMR (500 MHz, acetonitrile-$d_3$) δ 11.85 (s, 1H), 11.82 (s, 1H), 9.79 (s, 1H), 8.70 (s, 1H), 8.61 – 8.55 (m, 1H), 8.52 (d, *J* = 8.5 Hz, 1H), 8.45 (d, *J* = 9.6 Hz, 1H), 8.35 (m, 3H), 8.21 (dd, *J* = 26.7, 8.0 Hz, 3H), 8.05 – 7.80 (m, 8H), 7.71 (t, *J* = 9.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 4.80-4-40 (water suppression region), 4.07 – 3.86 (m, 1H), 3.73 (q, *J* = 11.8, 9.7 Hz, 2H), 3.17 (q, *J* = 7.9 Hz, 4H), 3.07 (m, 7H), 1.80 – 1.70 (m, 2H), 1.54 (m, 6H), 1.27 (t, *J* = 7.9 Hz, 5H), 0.81 (d, *J* = 7.0 Hz, 3H), 0.67 (d, *J* = 6.8 Hz, 3H). HRMS ($ESI^-$) *m/z* calcd for $C_{55}H_{59}N_{14}O_{17}S_3$ $[M-H]^-$ 1284.3423 found 1283.3613

**Compound *D*-1:** For compound ***D*-1** the synthesis, purification and analyses was repeated as described for **1** respectively **1a** by using *D*-amino acids instead of *L*-amino acids in the peptide segment. Similar yields and purities were obtained.



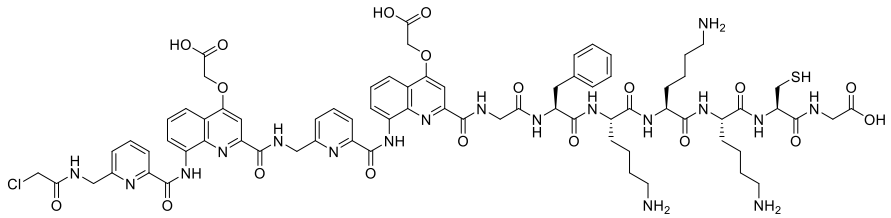

**Foldamer fragment F2a**: **F2a** was synthesized on a preloaded Fmoc-Gly-Wang resin (loading: 0.31 mmol/g) using general SPFS procedure on a 100 µmol scale. **F1a** was recovered in 65% yield (70 mg) after cleavage using 4 mL of TFA/water 95:5 (v/v) mixture for 2h at r.t and was used without any further purification. HRMS ($ESI^-$) *m/z* calcd for $C_{51}H_{38}N_9O_{14}S_2$ $[M-H]^{-1}$ 1064.1985 found 1064.2127.



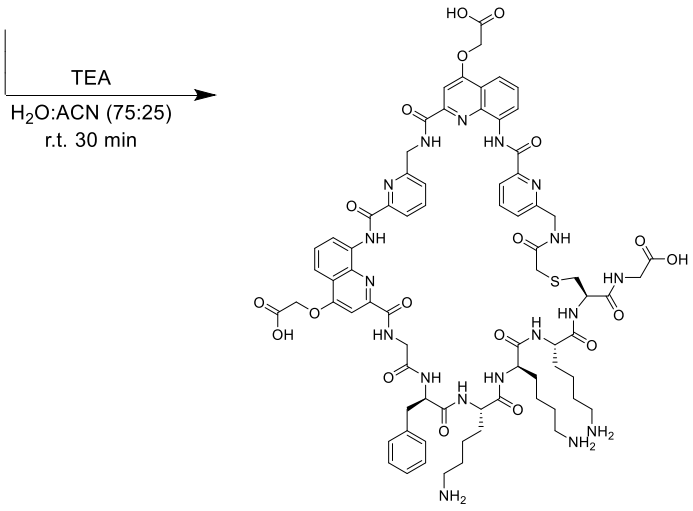

**Compound 2a**: Peptide Fmoc-Gly-Leu-Glu(*t*Bu)-Glu(*t*Bu)-Cys(Trt)-Gly was loaded on a Fmoc-Gly-Wang resin (0.32 mmol/g) using CEM Liberty Blue microwave automated peptide synthesizer on a 50 µmol scale. After Fmoc deprotection, **F2a** was coupled by fragment condensation on an 8 µmol scale. After final chloroacetylation and TFA cleavage, crude **2a** (8 mg, 61%) was directly used in the macrocyclization reaction without further purification.

**Compound 2**: Compound **2a** was dissolved in 1.1 mL water/$CH_3CN$ 70:30 (v/v) to a 7.5 mM dilution and TEA (480 µL, 0.5 M) was added. After 30 min at r.t. without agitation and monitoring the reaction by HPLC, the reaction mixture was acidified using TFA. The solvents were evaporated by freeze drying and the crude macrocycle was purified by using semi-preparative RP-HPLC with a gradient from 5% to 35% solvent D over 10 minutes to give **2** as

a light yellow solid (2.4 mg, 30%).$^{1}$H NMR was performed in 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$. $^{1}$H NMR (500 MHz, acetonitrile-$d_3$) δ 11.83 (s, 1H), 11.66 (s, 1H), 9.78 (s, 1H), 9.10 (d, *J* = 7.6 Hz, 1H), 8.77 (d, *J* = 7.9 Hz, 2H), 8.50 (d, *J* = 9.4 Hz, 2H), 8.39 (dd, *J* = 27.5, 9.4 Hz, 2H), 8.31 (d, *J* = 10.0 Hz, 1H), 8.25 (t, *J* = 8.7 Hz, 1H), 8.10 – 7.92 (m, 8H), 7.87 (t, *J* = 9.2 Hz, 1H), 7.81 (d, *J* = 9.3 Hz, 1H), 7.74 (s, 1H), 7.63 (t, *J* = 9.3 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 4.80-4-40 (water suppression region), 3.94 (d, *J* = 15.9 Hz, 1H), 3.89 – 3.76 (m, 1H), 3.73 (dd, *J* = 14.8, 8.6 Hz, 3H), 3.53 – 3.43 (m, 1H), 3.26 (d, *J* = 16.3 Hz, 1H), 3.21 – 2.93 (m, 4H), 2.48 (dq, *J* = 24.2, 9.1, 8.2 Hz, 4H), 2.33 – 2.09 (m, 5H), 1.85 – 1.72 (m, 1H), 1.60 – 1.47 (m, 2H), 1.47 – 1.34 (m, 1H), 1.26 (dd, *J* = 16.6, 7.9 Hz, 2H), 1.21 – 1.08 (m, 1H), 0.89 (s, 1H), 0.83 (d, *J* = 7.7 Hz, 3H), 0.68 (d, *J* = 7.4 Hz, 3H). HRMS (ESI$^{-}$) *m/z* calcd for $C_{59}H_{61}N_{14}O_{22}S_3$ $[M-H]^{-1}$ 1413.3325 found 1413.3066.



**3a**

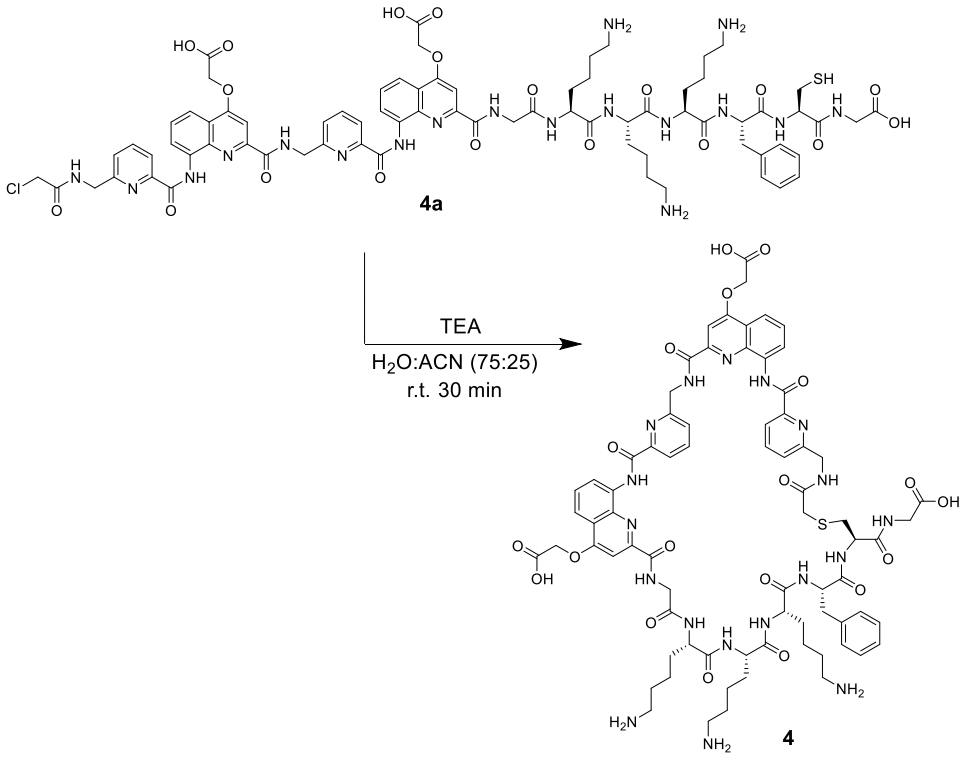



**3**

**Compound 3a:** Foldamer-peptide **3a** was synthesized by relying on the CEM Liberty Blue microwave automated peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. The crude product (74 mg, 93%) was directly engaged in the macrocyclization step without further purification. HRMS (ESI$^{+}$) *m/z* calcd for $C_{74}H_{88}ClN_{18}O_{19}S$ $[M+H]^{+}$ 1599.5877 found 1599.5671.

**Compound 3:** Compound **3a** was dissolved in 6.2 mL of water/acetonitrile mixture 75:25 (v/v) to a reach a 7.5 mM concentration and TEA (433 µL, 0.5 M) was added. After 30 min at r.t. without agitation, the reaction mixture was lyophilized and the crude macrocycle was purified

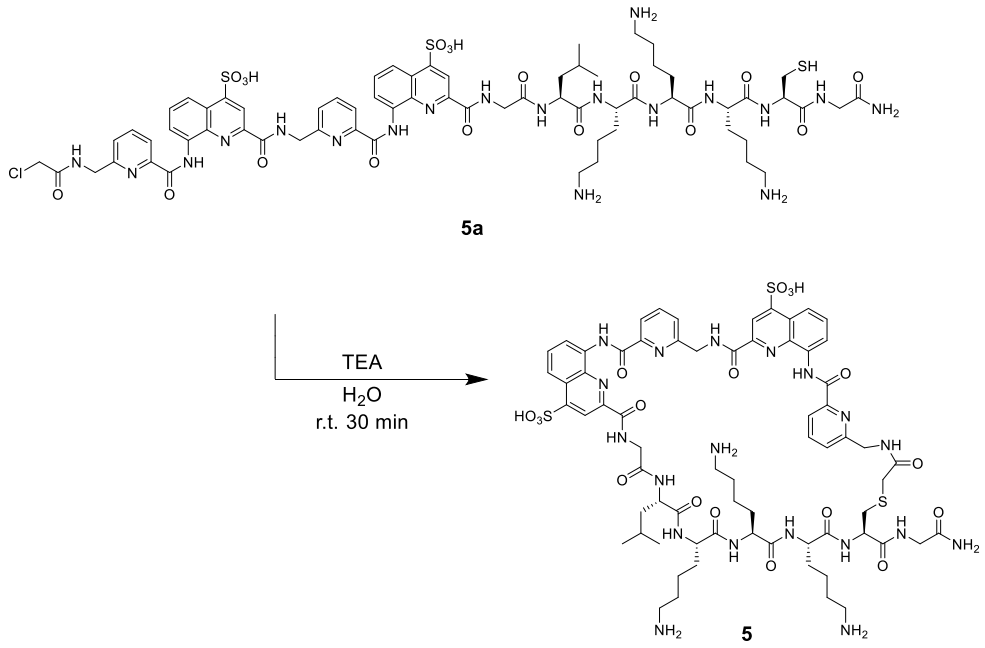
by using semi-preparative RP-HPLC with a gradient from 10% to 40% solvent D over 15 minutes at 50 °C to give **3** as a colorless solid (31 mg, 42%). $^{1}$H NMR spectrum was performed in a water/acetonitrile-$d_3$ 1:1 (v/v). $^{1}$H NMR (500 MHz, acetonitrile-$d_3$) δ 11.40 (s, 1H), 11.15 (s, 1H), 9.65 (d, *J* = 6.5 Hz, 1H), 9.05 (d, *J* = 7.8 Hz, 1H), 8.63 (d, *J* = 7.0 Hz, 1H), 8.38 (d, *J* = 10.1 Hz, 1H), 8.28 (p, *J* = 6.7, 5.3 Hz, 3H), 8.10 – 7.81 (m, 10H), 7.57 (t, *J* = 8.8 Hz, 2H), 7.46 (s, 1H), 7.34 (d, *J* = 8.7 Hz, 2H), 7.27 – 7.11 (m, 5H), 6.59 (s, 1H), 5.22 (dd, *J* = 18.1, 6.9 Hz, 1H), 4.89 (m, 1H), 3.75 (dd, *J* = 16.9, 6.3 Hz, 1H), 3.53 (d, *J* = 17.9 Hz, 1H), 3.48 – 3.32 (m, 4H), 3.25 (q, *J* = 8.2 Hz, 3H), 3.08 (q, *J* = 6.8, 6.2 Hz, 6H), 3.02 – 2.91 (m, 1H), 2.81 (m, 1H), 2.25 (dd, *J* = 23.2, 10.7 Hz, 2H), 2.07 (s, 2H), 1.95 (m, 8H), 1.79 (m, 6H), 1.60 (m, 4H), 1.42 – 1.33 (m, 2H).HRMS (ESI$^-$) *m/z* calcd for $C_{74}H_{85}N_{18}O_{19}S$ $[M-H]^-$ 1561.5959 found 1561.6431.




**Compound 4a**: Foldamer-peptide **4a** was synthesized by relying on the CEM Liberty Blue microwave automated peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After final chloroacethylation and TFA cleavage, the crude product (95 mg, 98%) was directly used in the macrocyclization reaction without further purification. HRMS (ESI$^+$) *m/z* calcd for $C_{74}H_{88}ClN_{18}O_{19}S$ $[M+H]^+$ 1599.5882 found 1599.5824.

**Compound 4**: Compound **4a** was dissolved in 6.0 mL of a water/acetonitrile solvent mixture 75:25 (v/v) to reach a concentration of 7.5 mM. TEA (433 µL, 0.5 M) was then added. After 30 min at r.t. without agitation, the reaction mixture was freeze dried and the obtained crude

macrocycle was purified by using semi-preparative RP-HPLC with a gradient from 20% to 45% solvent B over 15 minutes at 50 °C to give **4** as a colorless solid (25 mg, 26%). $^1$H NMR was performed in a water/acetonitrile-$d_3$ 1:1 (v:v). $^1$H NMR (500 MHz, acetonitrile-$d_3$) δ 11.57 (s, 1H), 11.37 (s, 1H), 9.74 (d, *J* = 6.4 Hz, 1H), 8.73 (d, *J* = 7.9 Hz, 1H), 8.65 (d, *J* = 6.7 Hz, 1H), 8.40 (d, *J* = 9.2 Hz, 1H), 8.26 – 7.73 (m, 15H), 7.71 – 7.55 (m, 4H), 7.55 – 7.21 (m, 13H), 6.68 (s, 1H), 5.16 (dd, *J* = 18.2, 6.8 Hz, 1H), 5.01 (q, *J* = 15.9 Hz, 1H), 4.90 – 4.70 (m, 1H), 3.93 – 3.79 (m, 1H), 3.62 (d, *J* = 19.1 Hz, 1H), 3.46 (t, *J* = 7.9 Hz, 1H), 3.28 – 3.02 (m, 9H), 2.84 (m, 9H), 2.39 (t, *J* = 8.9 Hz, 1H), 1.98 (t, *J* = 10.5 Hz, 2H), 1.89 – 1.48 (m, 14H), 1.49 – 1.13 (m, 6H).HRMS (ESI$^+$) *m/z* calcd for $C_{74}H_{88}N_{18}O_{19}S$ [M+2H]$^{+2}$ 782.3091 found 782.3081.

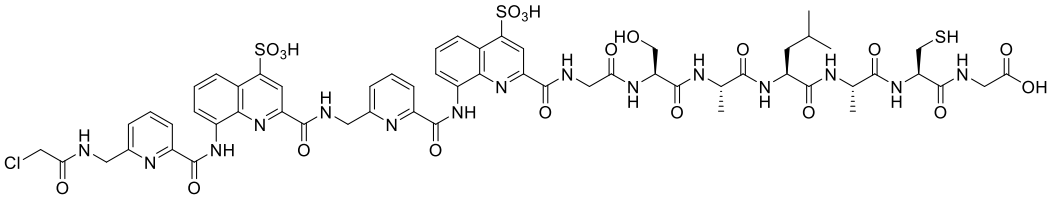



**5a** **5**

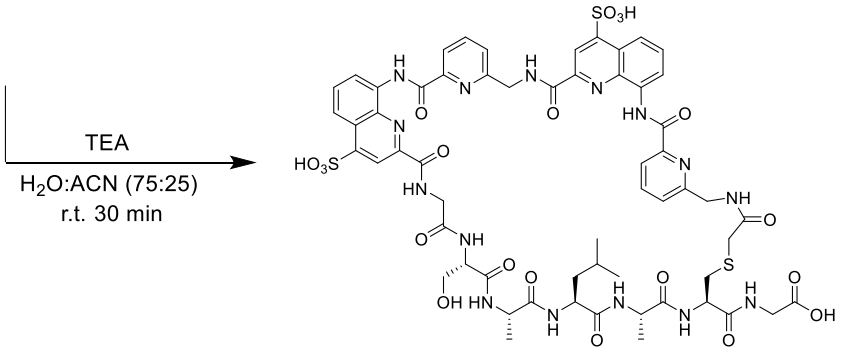
**Compound 5a**: Foldamer-peptide **5a** was built on Rink Amide MBHA resin (0.33 mmol/g) by relying on the CEM Liberty Blue peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After chloroacetylation the resin was cleaved with TFA/TIS/$H_2O$ and the crude product (39 mg, 52%) was directly used in the macrocyclization reaction without further purification. MALDI-TOF *m/z* calcd for $C_{67}H_{85}ClN_{19}O_{18}S_3$ [M-H]$^-$ 1574.5 found 1572.9

**Compound 5:** Compound **5a** was dissolved in 3.5 mL water to a 7.5 mM dilution and TEA (230 µL, 0.5 M) was added. After 30 min at r.t. without agitation, the solvents were evaporated by freeze drying and the obtained crude macrocycle was purified by using semi-preparative RP-HPLC (general procedure 3) with a gradient from 0% to 40% solvent D over 25 minutes to give **5** as a light yellow solid (5 mg, 12%). $^1$H NMR was performed in 12.5 mM ammoniumacetate buffer at pH 8.5 with 25% acetonitrile-$d_3$. $^1$H NMR (500 MHz, acetonitrile-

$d_3$) δ 11.56 (s, 1H), 11.49 (s, 1H), 9.85 (s, 1H), 8.68 (s, 1H), 8.42 (d, *J* = 9.5 Hz, 1H), 8.29 (d, *J* = 9.4 Hz, 2H), 8.18 (t, *J* = 8.5 Hz, 2H), 8.03 (t, *J* = 9.9 Hz, 3H), 7.95 (t, *J* = 8.2 Hz, 1H), 7.91 – 7.70 (m, 4H), 7.59 (t, *J* = 8.9 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 4.80-4.40 (water suppression region), 3.86 (t, *J* = 14.5 Hz, 2H), 3.74 (d, *J* = 18.5 Hz, 1H), 3.53 (d, *J* = 16.0 Hz, 1H), 3.40 (d, *J* = 16.2 Hz, 1H), 3.24 (q, *J* = 15.0 Hz, 2H), 3.12 (q, *J* = 11.7, 8.5 Hz, 2H), 2.97 (m, 4H), 2.84 (m, 4H), 1.84 – 1.27 (m, 25H), 0.78 (d, *J* = 7.2 Hz, 3H), 0.66 (d, *J* = 6.9 Hz, 3H). Amino acid amide resonances between 8.4 ppm and 7.7 ppm partially suppressed. HRMS (ESI$^-$) *m/z* calcd for $C_{67}H_{84}N_{19}O_{18}S_3$ [M-H]$^-$ 1538.5482 found 1538.5360.



**6a**

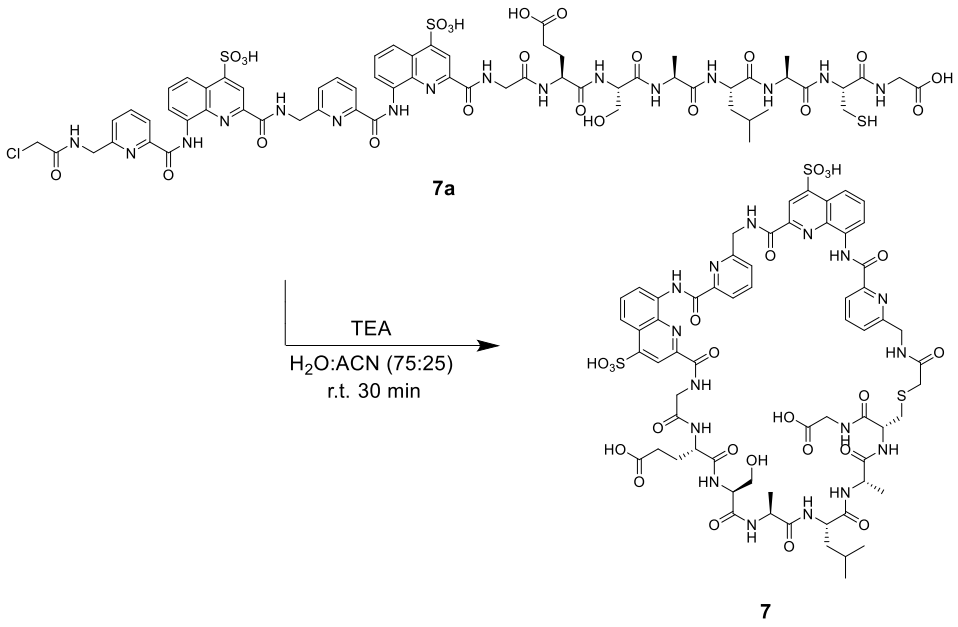



**6**

**Compound 6a**: Peptide Fmoc-Ser(*t*Bu)-Ala-Leu-Ala-Cys(Trt)-Gly was loaded on Fmoc-Gly-Wang resin (0.31 mmol/g) using CEM Liberty Blue peptide synthesizer. Fragment **F2a** was next condensed on the resin-bound amine-free peptide on a 10 µmol scale. After final chloroacetylation followed by TFA cleavage the crude product (12 mg, 86%) was directly used in the cyclization reaction. HRMS (ESI$^+$) *m/z* calcd for $C_{58}H_{65}ClN_{15}O_{20}S_3$ [M+H]$^+$ 1422.3380 found 1422.3610

**Compound 6:** Compound **6a** was dissolved in 34 mL of water/acetonitrile 75:25 (v/v) solution mixture and TEA (1.7 mL, 0.5 M) was added. After 3h at r.t. without agitation, the solvents were evaporated by freeze drying and the crude macrocycle was purified by using semi-preparative RP-HPLC with a gradient from 10% to 30% solvent D over 20 minutes to give **6** as a light yellow solid (2.3 mg, 20%). $^1$H NMR was performed in 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$. $^1$H NMR (400 MHz, acetonitrile-$d_3$) δ 11.68 (s, 1H), 11.58 (s, 1H), 9.99 (d, *J* = 5.2 Hz, 1H), 8.77 (s, 1H), 8.72 (s, 1H), 8.55 – 8.47 (m, 2H), 8.37 – 8.32 (m, 1H), 8.32 – 8.19 (m, 4H), 8.06 (td, *J* = 7.6, 5.6 Hz, 4H), 7.95 – 7.82 (m, 7H), 7.66 (t,

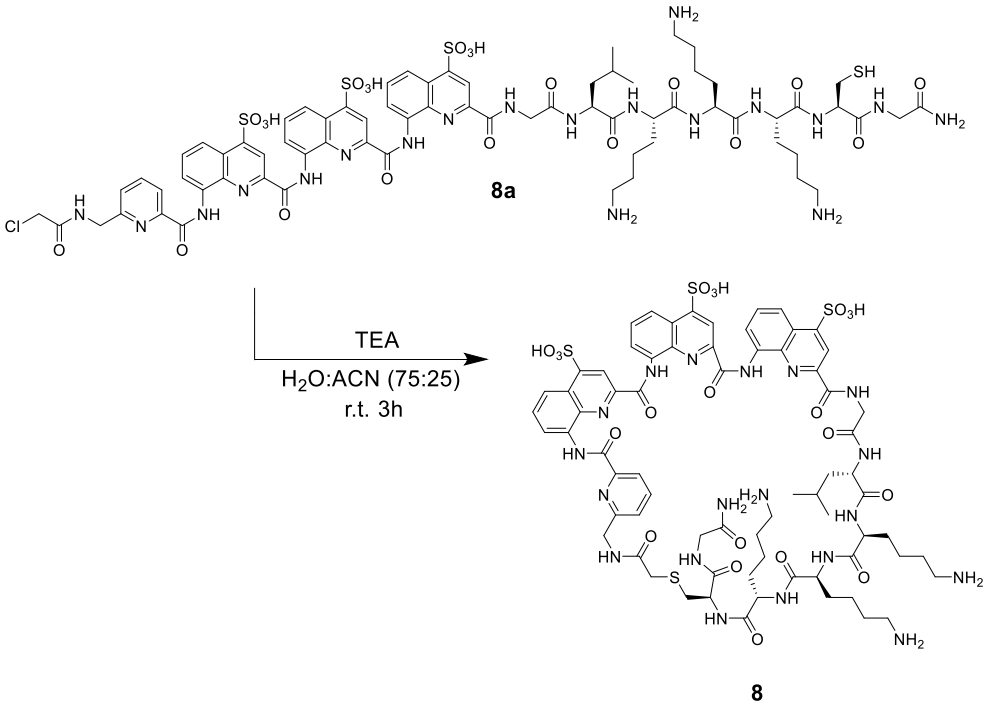
*J* = 8.5 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 4.80-4 – 40 (water suppression region), 3.96 (td, *J* = 13.6, 12.6, 4.3 Hz, 1H), 3.85 (dd, *J* = 25.4, 4.1 Hz, 1H), 3.78 – 3.69 (m, 4H), 3.68 – 3.61 (m, 1H), 3.27 (d, *J* = 23.3 Hz, 3H), 3.01 (dd, *J* = 14.1, 5.6 Hz, 1H), 2.90 (dd, *J* = 14.0, 8.0 Hz, 1H), 1.67 (d, *J* = 7.3 Hz, 3H), 1.45 (d, *J* = 7.1 Hz, 4H), 1.38 (d, *J* = 6.9 Hz, 1H), 1.27 (t, *J* = 7.6 Hz, 6H), 0.96 – 0.84 (m, 2H), 0.78 (d, *J* = 6.9 Hz, 3H), 0.62 (d, *J* = 6.9 Hz, 3H). HRMS ($ESI^-$) *m/z* calcd for $C_{58}H_{62}N_{15}O_{20}S_3$ $[M-H]^-$ 1384.3457 found 1384.3375.




**Compound 7a**: The peptide fragment was synthesized on a preloaded Fmoc-Gly-Wang resin (loading: 0.31 mmol/g) using CEM Liberty Blue peptide synthesizer. Fragment **F2a** was next condensed on the resin-bound amine-free peptide by relying on the fragment condensation approach on a 10 µmol scale. After final chloroacetylation followed by TFA cleavage the crude product (13 mg, 87%) was directly engaged in the macrocyclization reaction. HRMS ($ESI^+$) *m/z* calcd for $C_{63}H_{72}ClN_{16}O_{23}S_3$ $[M+H]^+$ 1551.3801 found 1551.4003.

**Compound 7**: Compound **7a** (0.25 mM) was dissolved in 36 mL of water/acetonitrile 75:25 (v/v) solution mixture and TEA (1.7 mL, 0.5 M) was added. After 3h at r.t. without agitation, the solvents were evaporated by freeze drying. The crude macrocycle was next purified by using semi-preparative RP-HPLC with a gradient from 5% to 28% solvent D over 20 minutes to give **7** as a light yellow solid (2.6 mg, 20%). $^1H$ NMR was performed in 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$. $^1H$ NMR (400 MHz, acetonitrile-$d_3$) δ 11.67 (s, 1H), 11.56 (s, 1H), 9.99 (s, 1H), 8.73 (s, 1H), 8.58 – 8.47 (m, 3H), 8.42 (d, *J* = 8.3 Hz, 1H), 8.38 – 8.28 (m, 3H), 8.25 (t, *J* = 7.9 Hz, 1H), 8.12 – 7.85 (m, 11H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.75 (t, *J* = 5.8 Hz, 1H), 7.66 (t, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 4.80-4-40 (water suppression region), 4.10 – 3.94 (m, 1H), 3.85 (td, *J* = 17.1, 16.4, 4.9 Hz, 1H), 3.77 – 3.68 (m, 3H), 3.63 (dd, *J* = 17.3, 5.4 Hz, 1H), 3.24 (d, *J* = 5.2 Hz, 2H), 3.17 (q, *J* = 7.5 Hz, 4H), 3.04 (d,

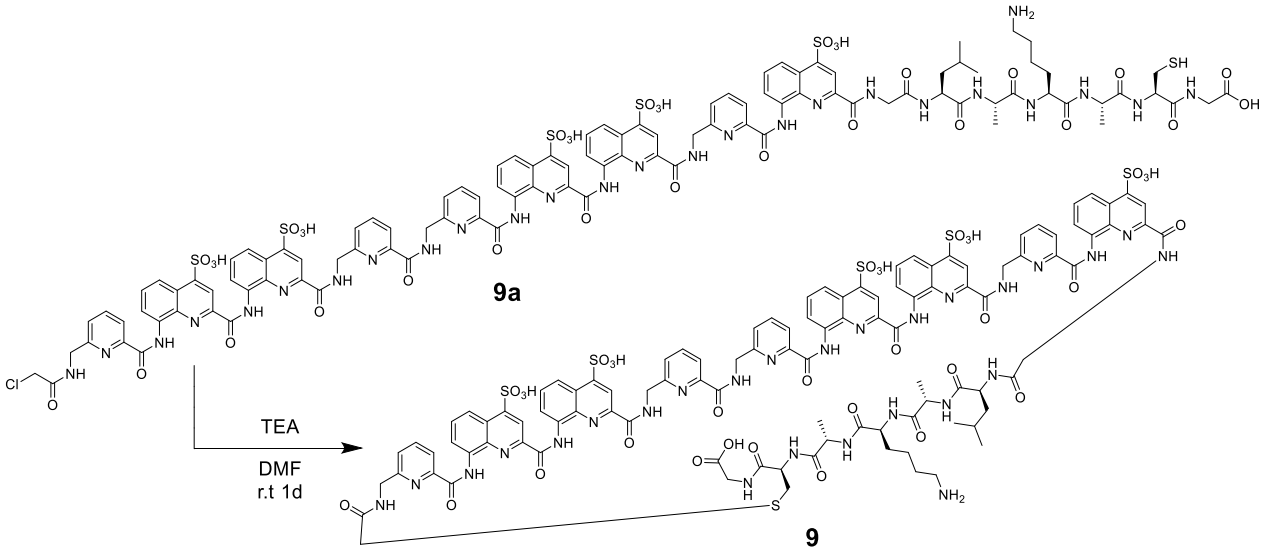
*J* = 5.2 Hz, 1H), 2.86 (dd, *J* =f 14.2, 8.3 Hz, 1H), 2.17 (d, *J* = 7.6 Hz, 2H), 1.60 (d, *J* = 6.1 Hz, 3H), 1.43 (d, *J* = 7.2 Hz, 3H), 1.38 (d, *J* = 7.1 Hz, 3H), 1.26 (t, *J* = 7.5 Hz, 6H), 0.86 (dd, *J* = 6.2, 4.5 Hz, 6H). HRMS (ESI$^-$) *m/z* calcd for $C_{63}H_{69}N_{16}O_{23}S_3$ [M-H]$^-$ 1513.3883 found 1515.3924.




**Compound 8a**: Foldamer-peptide **8a** was built on Rink Amide MBHA resin (loading: 0.33 mmol/g) by relying on the CEM Liberty Blue peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After final chloroacethylation and TFA cleavage the crude product (80 mg, 97%) was directly used in the macrocyclization reaction without further purification. HRMS (ESI$^-$) *m/z* calcd for $C_{70}H_{85}ClN_{19}O_{21}S_4$ [M-H]$^{-1}$ 1690.4738 found 1690.4170.

**Compound 8:** Compound **8a** was dissolved in 6.3 mL of a water/acetonitrile solvent mixture 75:25 (v:v) to reach a 7.5 mM dilution and TEA (0.4 mL, 0.5 M) was added. After 3h at r.t. without agitation, the solvents were evaporated by freeze drying and the obtained crude macrocycle was purified by using semi-preparative RP-HPLC with a gradient from 0% to 40% solvent D over 25 minutes to give compound **8** as a light yellow solid (8.2 mg, 10%). $^1$H NMR was performed in 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$ performing water suppression experiment. $^1$H NMR (500 MHz, acetonitrile-$d_3$) δ 12.47 (s, 1H), 11.81 (s, 1H), 11.15 (s, 1H), 8.88 (d, *J* = 8.1 Hz, 1H), 8.76 (s, 1H), 8.60 (d, *J* = 9.4 Hz, 2H), 8.50 (m, 2H), 8.39 (s, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.26 (d, *J* = 9.1 Hz, 1H), 8.11 (d, *J* = 10.0 Hz, 1H), 8.01 (q, *J* = 8.9, 8.4 Hz, 3H), 7.90 – 7.83 (m, 4H), 7.77 (t, *J* = 4.0 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.45 (t, *J* = 8.6 Hz, 1H), 7.35 (d, *J* = 9.2 Hz, 2H), 6.96 (s, 1H), 4.80-4-40 (water

suppression region) 3.86 (t, *J* = 15.7 Hz, 2H), 3.69 (d, *J* = 20.1 Hz, 1H), 3.64 – 3.53 (m, 2H), 3.30 (d, *J* = 14.8 Hz, 1H), 3.18 – 3.04 (m, 7H), 2.96 (m, 2H), 2.87 (d, *J* = 3.9 Hz, 1H), 2.70 (dd, *J* = 14.9, 10.0 Hz, 1H), 2.41 (d, *J* = 20.2 Hz, 1H), 2.22 (d, *J* = 4.6 Hz, 2H), 1.88 – 1.73 (m, 10H), 1.64 – 1.39 (m, 11H), 1.23 – 1.13 (m, 1H), 0.99 (s, 1H), 0.55 (d, *J* = 7.0 Hz, 3H), 0.14 (d, *J* = 7.1 Hz, 3H). HRMS (ESI$^-$) *m/z* calcd for $C_{70}H_{83}N_{19}O_{21}S_4$ [M-2H]$^{-2}$ 826.7452 found 826.7511

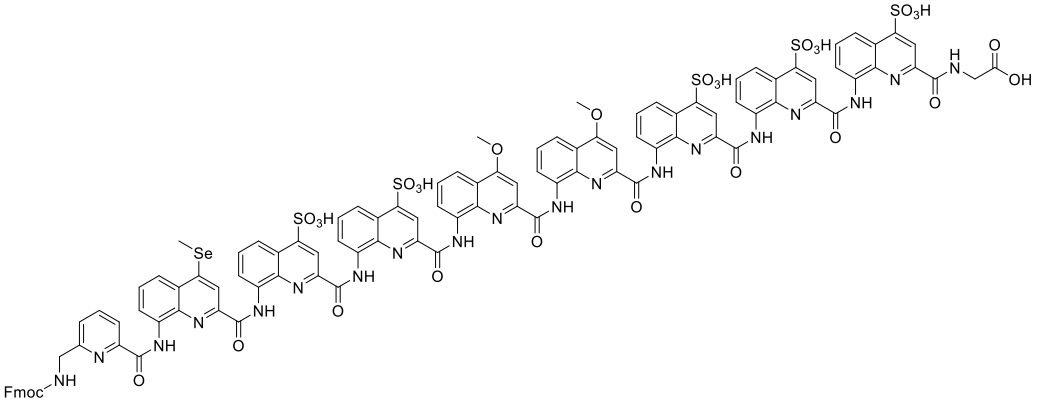



**Compound 9a**: Foldamer-peptide **9a** was synthesized on the CEM Liberty Blue peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part. Half of the resin was cleaved in the presence of TFA/TIS/$H_2O$/EDT and the crude product (30 mg, 47%) was directly engaged in the macrocyclization reaction without further purification. HRMS (ESI$^-$) *m/z* calcd for $C_{105}H_{99}ClN_{26}O_{33}S_6$ [M-2H]$^{-2}$ 1239.7473 found 1239.7576

**Compound 9:** Compound **9a** was dissolved in 48 mL DMF containing triphenylphosphine (3.1 mg, 12 µmol) at a concentration of 0.25 mM. TEA (3.3 mL, 0.5 M) was added under nitrogen atmosphere and the reaction mixture was kept for 36 h at r.t. without any stirring. The solvents were next evaporated under reduced pressure, remaining traces of DMF were removed by lyophilizing the sample. The obtained crude macrocycle was purified by using semi-preparative RP-HPLC with a gradient from 8% to 32% solvent D over 20 minutes to give compound **9** as a light yellow solid (3.4 mg, 11%). $^1$H NMR was performed in 12.5 mM ammonium acetate buffer at pH 8.5 with 10% $D_2O$. $^1$H NMR (500 MHz, TPS) δ 11.70 (s, 1H), 11.60 (s, 1H), 11.10 (s, 1H), 11.07 (s, 1H), 10.72 (s, 1H), 8.72 (d, *J* = 9.3 Hz, 2H), 8.54 (dd, *J* = 16.4, 8.7 Hz, 2H), 8.46 – 8.23 (m, 8H), 8.17 – 8.08 (m, 3H), 8.04 – 7.27 (m, 27H), 7.12 (d, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.99 (t, *J* = 9.1 Hz, 1H), 4.80-4-40 (water suppression region), 3.94 – 3.75 (m, 4H), 3.71 – 3.50 (m, 4H), 3.26 – 3.14 (m, 5H), 2.93 (m, 2H), 2.85 –

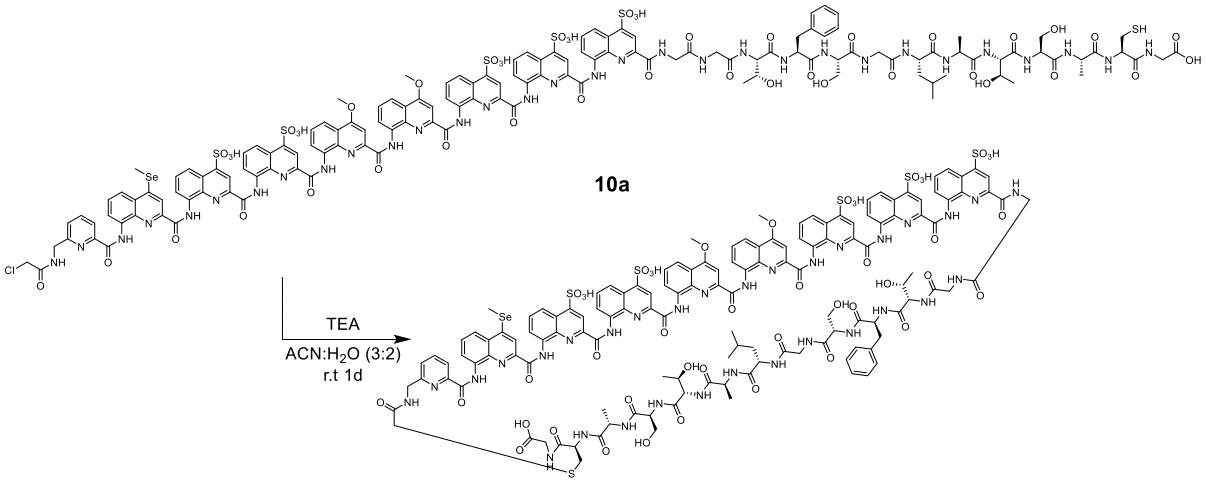
2.64 (m, 4H), 2.12 (d, *J* = 8.3 Hz, 1H), 1.86 – 1.78 (m, 3H), 1.71 (m, 5H), 1.30 (m, 10H), 0.88 (dd, *J* = 21.0, 6.6 Hz, 2H), 0.78 (d, *J* = 6.9 Hz, 3H), 0.65 (d, *J* = 6.8 Hz, 3H).HRMS ($ESI^-$) *m/z* calcd for $C_{105}H_{98}N_{26}O_{33}S_6$ $[M-2H]^{-2}$ 1221.7590 found 1221.7500.

**Compound *D*-9:** For compound ***D*-9** the synthesis, purification, and analyses were repeated as described for **9** respectively **9a** by using *D*-amino acids instead of *L*-amino acids in the peptide segment. Similar yields and purities were obtained.



**F10a**

**Compound F10a**: Foldamer segment **F10a** was synthesized on a preloaded Fmoc-Gly Wang resin (0.31 mmol/g) on a 100 µmol scale. After TFA cleavage **F10a** (200 mg, 85%) was used in subsequent reactions without any further purification. HRMS ($ESI^-$) *m/z* calcd for $C_{107}H_{73}N_{19}O_{30}S_5Se$ $[M-2H]^{-2}$ 1172.1303 found 1172.1785.

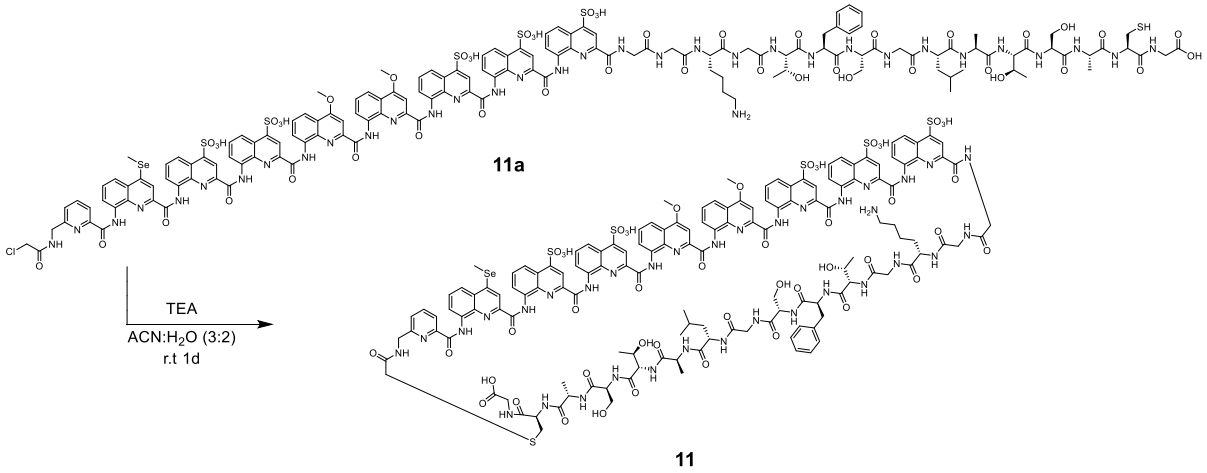



**10**

**Compound 10a**: The peptide fragment was synthesized on Cl-MPA ProTide® (LL) resin (0.23 mmol/g) on a 50 µmol scale. For the initial loading step, Fmoc-Gly-OH (149 mg, 0.5 mM, 10 equiv.) was dissolved in 2.5 mL DMF and added to the resin together with a solution containing CsI (32 mg, 0.125 mM, 2.5 equiv.) and DIPEA (174 µL, 1mM, 20 equiv.) in 1 mL DMF. The suspension was heated to 50 °C by microwave irradiation for 15 min at 25W. The peptide was

elongated by using CEM Liberty Blue peptide synthesizer. **F10a** was next engaged to the fragment condensation on the resin-bound free amine peptide in a 10 µmol scale. After final chloroacethylation and TFA cleavage, the obtained crude product was directly engaged in the macrocyclization reaction without further purification (22 mg, 75%). HRMS ($ESI^-$) *m/z* calcd for $C_{138}H_{132}ClN_{31}O_{45}S_6Se$ $[M-2H]^{-2}$ 1625.3119 found 1625.3047

**Compound 10:** With the increase of size and sequence complexity, we decided to perform all the subsequent macrocylizations in an oxygen-free media. Compound **10a** was dissolved in a 67 mL of a degassed water/acetonitrile mixture 40:60 (v/v) to reach a final 0.1 mM concentration and TEA (4.5 mL, 0.5 M) was added under positive nitrogen pressure. After 24h under nitrogen atmosphere at r.t. without stirring, the solvents were removed by rotary evaporation and remaining water was removed by lyophilization. The obtained crude macrocycle was purified by using semi-preparative RP-HPLC on C8 column at 50 °C with a gradient from 10% to 25% solvent D over 15 minutes to afford compound **10** (2.0 mg, 10%) as a diastereomeric mixture. $^1H$ NMR was performed in 12.5 mM ammonium acetate buffer at pH 8.5 with 50% acetonitrile-$d_3$. $^1H$ NMR (500 MHz, Acetonitrile-$d_3$) δ 11.39 (s, 1H), 11.37 (s, 1H), 11.30 (s, 1H), 11.26 (s, 1H), 11.09 (s, 1H), 11.06 (s, 1H), 11.00 (s, 1H), 10.96 (m, 5H), 10.52 (m, 4H), 8.42 (m, 7H), 8.34 – 8.14 (m, 12H), 8.14 – 7.90 (m, 12H), 7.90 – 7.59 (m, 22H), 7.59 – 7.39 (m, 23H), 7.39 – 6.91 (m, 27H), 6.79 (d, *J* = 38.9 Hz, 2H), 6.53 (d, *J* = 5.8 Hz, 2H), 6.31 (d, *J* = 9.8 Hz, 2H), 4.80-4-40 (water suppression region), 3.75 – 2.28 (m, 51H), 1.78 – 1.61 (m, 13H), 1.46 – 1.15 (m, 51H), 1.09 – 0.75 (m, 29H). HRMS ($ESI^-$) *m/z* calcd for $C_{138}H_{130}N_{31}O_{45}S_6Se$ $[M-3H]^{-3}$ 1071.3410 found 1071.2499.



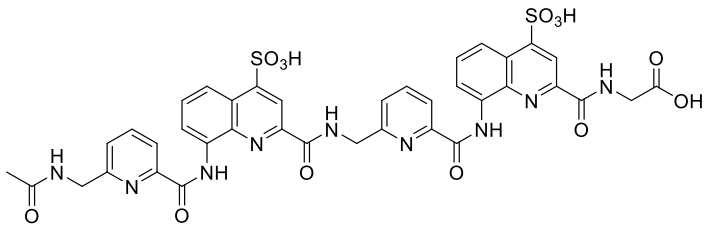

**Compound 11a:** The peptide fragment was synthesized on Cl-MPA ProTide® (LL) resin (0.23 mmol/g) on a 50 µmol scale by using CEM Liberty Blue peptide synthesizer. Foldamer segment **F10** was next engaged in the fragment condensation on the resin-bound free amine peptide on a 17 µmol scale. After final chloroacethylation and TFA cleavage, the crude product

(18 mg, 29%) was directly used in the macrocyclization step without further purification. HRMS ($ESI^+$) *m/z* calcd for $C_{146}H_{147}ClN_{34}O_{47}S_6Se$ $[M-2H]^{-2}$ 1717.8690 found 1718.4410.

**Compound 11**: Compound **11a** was dissolved in 45 mL of an oxygen-free water/acetonitrile mixture 40:60 (v/v) to reach a 0.1 mM concentration and TEA (3.5 mL, 0.5 M) was added under nitrogen atmosphere. The reaction mixture was kept one day under nitrogen atmosphere at r.t. without agitation. Solvents were then removed by rotary evaporation and the remaining water was removed by freeze-drying. The obtained crude macrocycle was purified by using semi-preparative RP-HPLC on C8 column at 50 °C with a gradient from 10% to 25% solvent D over 15 minutes to furnish ***(P)*-11** (0.6 mg, 4%) and ***(M)*-11** (0.6 mg, 4%) as solids. $^1$H NMR spectra were recorded in 12.5 mM ammonium acetate buffer at pH 8.5 with 50% acetonitrile-$d_3$ performing water suppression experiment.

**Compound *(M)*-11:** $^1$H NMR(500 MHz, acetonitrile-$d_3$) δ 11.40 (s, 1H), 11.30 (s, 1H), 11.11 (s, 1H), 11.02 (s, 1H), 11.00 (s, 1H), 10.96 (s, 1H), 10.58 (s, 1H), 10.52 (s, 1H), 8.48 (d, *J* = 10.8 Hz, 2H), 8.41 (d, *J* = 10.2 Hz, 1H), 8.34 – 7.90 (m, 13H), 7.90 – 7.62 (m, 11H), 7.62 – 7.26 (m, 22H), 7.22 (d, *J* = 9.2 Hz, 1H), 7.14 (d, *J* = 9.3 Hz, 2H), 7.02 (d, *J* = 9.3 Hz, 1H), 6.57 (s, 1H), 6.35 (s, 1H), 4.50-4.20 (water suppression range), 4.01 – 3.34 (m, 9H), 3.33 – 2.86 (m, 7H), 2.60 (m, 5H), 2.43 – 2.27 (m, 2H), 2.16 (m, 2H), 1.81 – 1.52 (m, 12H), 1.51 – 1.15 (m, 25H), 1.15 – 0.89 (m, 16H). HRMS ($ESI^-$) *m/z* calcd for $C_{146}H_{145}N_{34}O_{47}S_6Se$ $[M-3H]^{-3}$ 1133.0818 found 1132.9717.

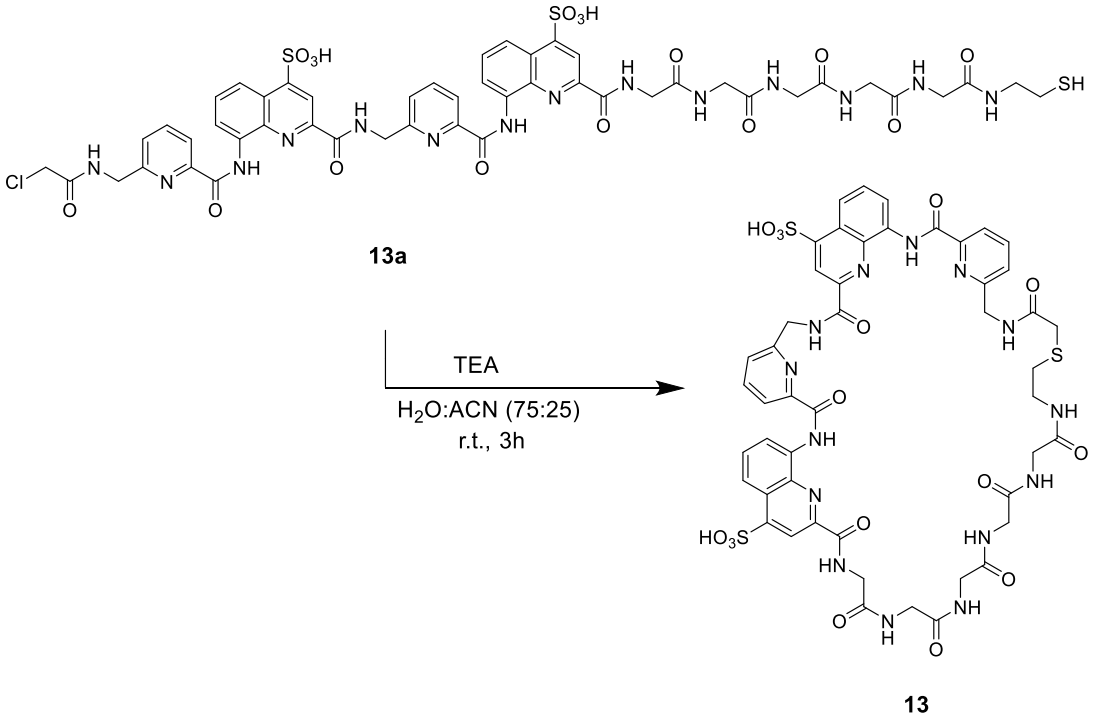
**Compound *(P)*-11:** $^1$H NMR(500 MHz, acetonitrile-$d_3$) δ 11.39 (s, 1H), 11.35 (s, 1H), 11.11 (s, 1H), 11.05 (s, 1H), 11.02 (s, 1H), 10.97 (s, 1H), 10.55 (s, 1H), 10.52 (s, 1H), 8.52 – 8.33 (m, 4H), 8.33 – 7.96 (m, 12H), 7.87 – 7.65 (m, 12H), 7.59 – 7.26 (m, 22H), 7.22 (d, *J* = 9.1 Hz, 1H), 7.13 (d, *J* = 9.1 Hz, 1H), 7.09 – 7.00 (m, 2H), 6.60 (s, 1H), 6.33 (s, 1H), 4.50 – 4.20 (water suppression range), 4.00 – 3.31 (m, 8H), 3.31 – 2.83 (m, 8H), 2.60 (d, *J* = 15.8 Hz, 1H), 2.54 (m, 4H), 2.50 – 2.16 (m, 3H), 1.82 – 1.50 (m, 14H), 1.47 – 1.15 (m, 26H), 1.15 – 0.87 (m, 20H). HRMS ($ESI^-$) *m/z* calcd for $C_{146}H_{145}N_{34}O_{47}S_6Se$ $[M-3H]^{-3}$ 1133.0818 found 1132.9702.




**12**

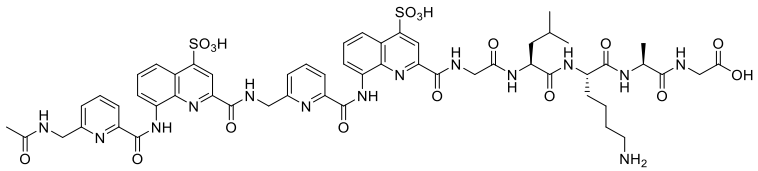
**Compound 12**: **12** was synthesized on a preloaded Fmoc-Gly-Wang resin (loading: 0.31 mmol/g) using general SPFS procedure on a 25 µmol scale. After final acetylation, **12** was recovered in 73% yield (16 mg) after cleavage using 2 mL of TFA/water (95:5, v/v) mixture for

2h at r.t. This crude product was further purified on semi-preparative RP-HPLC with a gradient from 5% to 15% solvent D over 15 min at 50 °C. **12** was obtained as a light yellow powder (4 mg, 25%) after lyophilization. $^{1}$H NMR (400 MHz, Deuterium Oxide) δ 11.64 (s, 1H), 11.38 (s, 1H), 9.93 (d, *J* = 4.0 Hz, 1H), 8.65 (s, 1H), 8.35 (d, *J* = 9.4 Hz, 1H), 8.25 (d, *J* = 9.3 Hz, 1H), 8.17 (dt, *J* = 8.1, 4.0 Hz, 2H), 8.02 (t, *J* = 5.1 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.87 – 7.74 (m, 5H), 7.66 (s, 1H), 7.64 – 7.52 (m, 2H), 7.19 (d, *J* = 8.6 Hz, 1H), 3.42 (s, 1H), 3.20 (q, *J* = 6.4, 4.8 Hz, 1H). HRMS (ESI$^{-}$) *m/z* calcd for $C_{38}H_{30}N_9O_{13}S_2$ [M-H]$^{-1}$ 884.1409 found 884.2050.



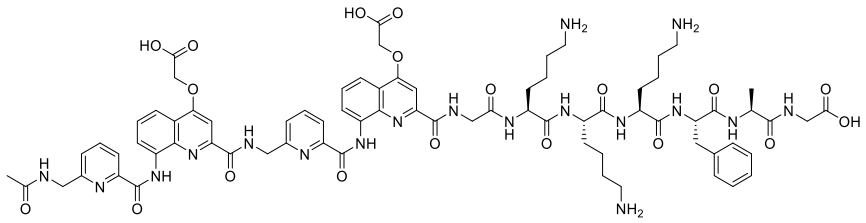

**Compound 13a**: Foldamer-peptide 1**3a** was built on cysteamine 2-chlorotrityl resin (loading: 1.63 mmol/g) by relying on the CEM Liberty Blue peptide synthesizer for the peptide segment and on a 100 µmol scale. After Fmoc deprotection, **F2a** was coupled by fragment condensation on an 50 µmol scale. After final chloroacetylation and TFA cleavage, crude **13a** (18 mg, 30%) was directly used in the macrocyclization reaction without further purification

**Compound 13**: Compound **13a** was dissolved in 60 mL of a water/$CH_3CN$ solvent mixture 75:25 (v/v) to a 0.5 mM dilution and TEA (2 mL, 0.5 M) was added. After 3 h at r.t. without agitation and monitoring the reaction by HPLC, the reaction mixture was acidified using TFA. The solvents were evaporated by freeze drying and the crude macrocycle was purified by using semi-preparative RP-HPLC with a gradient from 10% to 30% solvent D over 15 minutes to give **13** as a light yellow solid (0.5 mg, 3%). HRMS (ESI$^{-}$) *m/z* calcd for $C_{48}H_{45}N_{14}O_{16}S_3$ [M-H]$^{-1}$ 1169.2054 found 1169.2305.



**14**

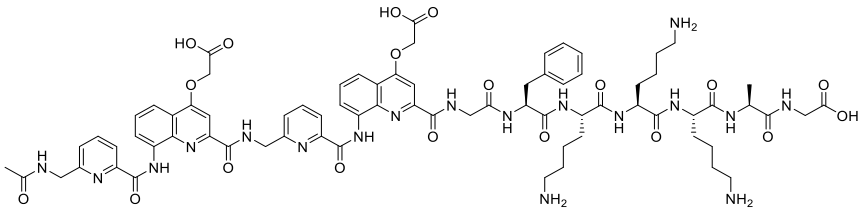
**Compound 14**: Foldamer-peptide **14** was synthesized on a preloaded Fmoc-Gly-Wang resin (0.31 mmol/g) using the CEM Liberty Blue peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After final acetylation and TFA cleavage the crude product was recovered as light white powder (61 mg, 45µmol, 97%) was purified on semi-preparative RP-HPLC (with a gradient from 10% to 40% solvent D at 50 °C. **14** was obtained as light orange powder (32.0 mg, 52%) after freeze drying. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 12.26 (s, 2H), 9.59 (t, *J* = 6.4 Hz, 1H), 9.12 (t, *J* = 6.1 Hz, 1H), 8.94 (ddd, *J* = 18.0, 7.7, 1.3 Hz, 2H), 8.63 – 8.56 (m, 3H), 8.50 (s, 1H), 8.28 (t, *J* = 6.1 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.14 (dd, *J* = 7.7, 1.1 Hz, 1H), 8.10 – 8.05 (m, 2H), 8.01 (t, *J* = 7.7, 7.2 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.72 – 7.69 (d, 1H), 7.38 (dd, *J* = 7.8, 1.1 Hz, 1H), 5.02 (d, *J* = 6.5 Hz, 2H), 4.39 – 3.99 (m, 8H), 3.79 – 3.62 (m, 2H), 2.72 (t, *J* = 7.1 Hz, 2H), 1.57 (s, 3H), 1.53 – 1.46 (m, 4H), 1.41 – 1.21 (m, 5H), 1.17 – 1.15 (m, 3H), 0.67 (dd, *J* = 12.5, 6.5 Hz, 6H). HRMS (ESI$^-$) *m/z* calcd for $C_{55}H_{61}N_{14}O_{17}S_2$ $[M-H]^-$ 1253.3786 found 1253.4049.



**15**

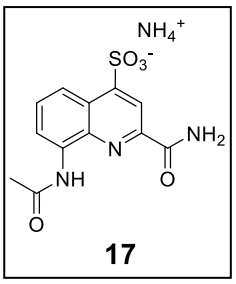
**Compound 15**: Foldamer-peptide **15** was synthesized on a preloaded Fmoc-Gly-Wang resin (0.31 mmol/g) using the CEM Liberty Blue peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After final acetylation and TFA cleavage the crude product was recovered as a light white powder (29 mg, 19 µmol, 76%). After purification on semi-preparative RP-HPLC with a gradient from 25% to 35% solvent B at 50 °C within 20 minutes, **15** was obtained as a colorless powder (6.2 mg, 21%). $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 11.77 (s, 1H), 11.65 (s, 1H), 9.60 (s, 1H), 8.44 (s, 2H), 8.38 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 8.16 (t, *J* = 7.7 Hz, 1H), 8.13 – 8.05 (m, 4H), 8.02 – 7.96 (m, 2H), 7.92 – 7.84 (m, 5H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.76 – 7.59 (m, 11H), 7.57 (t, *J*

= 8.1 Hz, 1H), 7.36 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.21 (m, 5H), 7.18 – 7.12 (m, 1H), 6.94 (s, 1H), 5.14 (s, 2H), 4.95 (m, 4H), 4.51 (td, *J* = 8.8, 4.6 Hz, 1H), 4.34 – 4.21 (m, 2H), 4.13 (d, *J* = 6.4 Hz, 2H), 3.86 – 3.67 (m, 4H), 3.08 – 3.01 (m, 1H), 2.80 – 2.61 (m, 9H), 1.66 (s, 3H), 1.57 – 1.36 (m, 11H), 1.32 – 1.10 (m, 13H). HRMS (ESI$^+$) *m/z* calcd for $C_{74}H_{90}N_{18}O_{19}$ $[M+2H]^{+2}$ 767.3309 found 767.3392.



**16**

**Compound 16**: Foldamer-peptide **16** was synthesized on a preloaded Fmoc-Gly-Wang resin (0.31 mmol/g) using CEM Liberty Blue peptide synthesizer and the Liberty Bio for the stepwise assembly of the foldamer part on a 50 µmol scale. After final acetylation and TFA cleavage, a crude light white powder was recovered in 91% yield (70 mg). This crude product was further purified on semi-preparative RP-HPLC with a gradient from 20% to 50% solvent D at 50 °C. **16** was obtained as a colorless powder (40.0 mg, 57%) after lyophilization. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 11.84 (s, 1H), 11.81 (s, 1H), 9.40 (s, 1H), 8.69 (m, 2H), 8.60 (dd, *J* = 16.1, 7.5 Hz, 2H), 8.23 (d, *J* = 6.2 Hz, 2H), 8.09 (m, 3H), 8.03 (s, 1H), 7.97 (t, *J* = 7.7 Hz, 1H), 7.94 – 7.87 (m, 4H), 7.74 (dd, *J* = 7.0, 1.7 Hz, 1H), 7.58 (m, 3H), 7.43 (s, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.16 – 7.08 (m, 3H), 7.01 (t, *J* = 7.5 Hz, 2H), 6.90 (t, *J* = 7.3 Hz, 1H), 5.05 – 4.85 (m, 2H), 4.73 – 4.54 (m, 4H), 4.35 (d, *J* = 7.2 Hz, 1H), 4.24 – 4.09 (m, 4H), 4.01 (dd, *J* = 26.1, 13.2 Hz, 1H), 3.85 (dd, *J* = 15.1, 5.9 Hz, 1H), 3.76 – 3.57 (m, 2H), 2.97 (d, *J* = 12.4 Hz, 1H), 2.76 (dd, *J* = 36.8, 10.4 Hz, 5H), 1.89 (s, 3H), 1.68 (s, 6H), 1.64 – 1.21 (m, 18H), 1.18 (t, *J* = 9.0 Hz, 6H. HRMS (ESI$^-$) *m/z* calcd for $C_{74}H_{87}N_{18}O_{19}$ $[M-H]^-$ 1531.6394 found 1531.6647.



**17**

**Compound 17**: Compound **17** was synthesized on solid support using Rink amide MBHA resin on a 33 µmol scale. Fmoc-Q$^{Sul}$-OH (47 mg, 96 µmol, 3 equiv) was attached on solid support using PyBOP (48 mg, 96 µmol, 3 equiv), DIPEA (33 µL, 192 µmol, 6 equiv) in 1 mL DMF for 1h at r.t. After Fmoc removal, the amine was acetylated using acetic anhydride in DCM 50:50 (v:v) for 30 min at rt. The crude compound was obtained after cleavage from the resin (general procedure 2.6) and TFA was removed under reduced pressure. **17** was obtained after purification on semi-preparative HPLC (general procedure 3) using a gradient from 0% to 40% solvent D over 25 minutes as a yellow solid (7 mg, 69%). $^1$H

NMR (500 MHz, DMSO-$d_6$) δ 10.33 (s, 1H), 9.14 (s, 1H), 8.72 (dd, *J* = 7.8, 1.3 Hz, 1H), 8.51 (s, 1H), 8.48 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.81 (s, 1H), 7.62 (dd, *J* = 8.6, 7.8 Hz, 1H), 7.05 (s, 4H), 2.33 (s, 3H). HRMS ($ESI^-$) *m/z* calcd for $C_{12}H_{10}N_3O_5S$ $[M-H]^{-1}$ 308.0347 found 308.0349.

# 3. Materials and Methods for HPLC, MS, UV, NMR and CD

## 3.1. HPLC

RP-HPLC analyses as well as semi-preparative purification were performed on an Ultimate 3000 HPLC System (ThermoFisher Scientific). For analytical analysis, a Nucleodur C18 Gravity column (4 x 100 mm, 5 µm, Macherey-Nagel) was used, and semi-preparative purifications were performed on a Nucleodur C18 Gravity column (10 x 250 mm, 5 µm, Macherey-Nagel). A Nucleodur C8 Gravity column (4 x 50 mm, 5 µm, Macherey-Nagel) was also used in analytic mode and for semi-preparative purification, the Nucleodur C8 Gravity column (10 x 100 mm, 5 µm, Macherey-Nagel) was employed. Semi-preparative purification was performed with an automated fraction collector system from ThermoFisher Scientific. When using acidic conditions 0.1% TFA was added to aqueous mobile phase (referred to as mobile phase A) and to acetonitrile (referred to as mobile phase B). When using basic conditions, a $NH_4OAc$-$NH_4OH$ buffer (12.5 mM, pH 8.5) was used as the aqueous mobile phase (referred to as mobile phase C) in combination with pure acetonitrile (referred to as mobile phase D). For analytical RP-HPLC analysis, a flow rate of 1.0 mL/min was applied; semi-preparative purification on RP-HPLC was performed at a flow rate of 5.0 mL/min. The column eluent was monitored by UV detection at 214, 254, and 300 nm with a diode array detector.

## 3.2. High-resolution electrospray mass (HR-MS)

HR-MS spectra were recorded on a Bruker microTOF II by direct infusion from aqueous media in either positive or negative ionization mode. The instrument was calibrated in positive and negative mode by direct infusion of a calibration solution (Agilent Technologies ESI-L Low Concentration Tuning Mix).

## 3.3. UV calibration curve to determine the molar extension coefficient of $Q^{Sul}$ residue .

Compound **15** (7.0 mg) was dissolved in $NH_4OAc$-$NH_4OH$ buffer (12.5 mM pH 8.5) to reach a 0.3 mM concentration. This reference solution was next used to prepare seven diluted solution at concentrations 0.3 mM, 0.15 mM, 0.1 mM, 0.075 mM, 0.050 mM, 0.025 mM, and 0.0125

mM. The UV absorbance at 375 nm was recorded on a Nanodrop One instrument (Thermo Fisher Scientific) by using a 1 cm quartz cuvette. The absorbance values were plotted against the concentration to generate a calibration curve. The molar extinction coefficient was calculated from the slope and a value for **ε**= 2678 L.mol$^{-1}$.cm$^{-1}$ at 375 nm was found. This **ε** value was used for determining the concentration of Q$^{Sul}$-rich foldamer-peptide hybrid solutions. To note, this **ε** is in good agreement with the previously $\varepsilon_{370\ nm}$ value of a (Q$^{Sul}$)$_8$ octamer of 20959 L.mol$^{-1}$.cm$^{-1}$.[4]

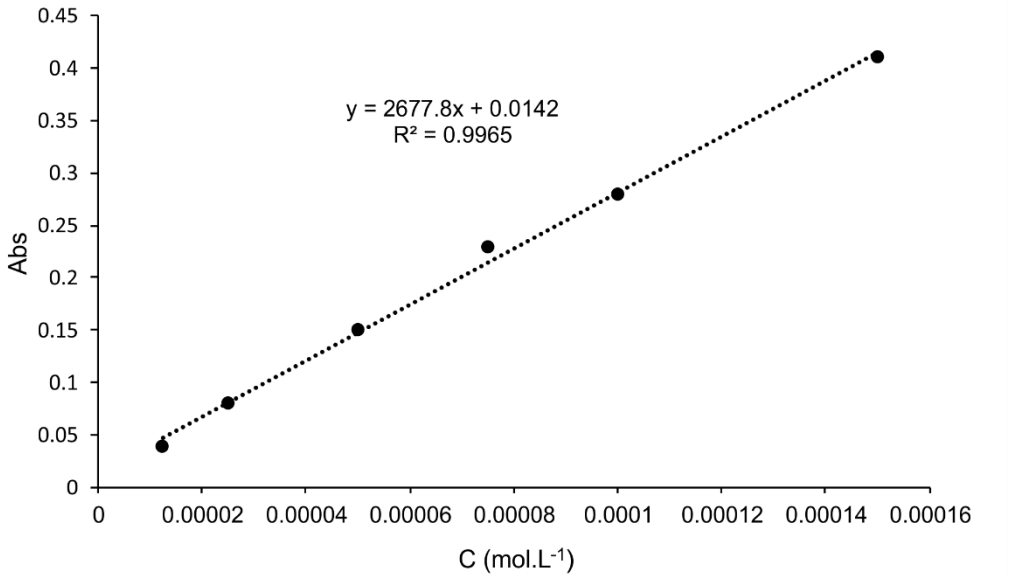



**Figure S2.** UV calibration curve at 375 nm of the Ac-Q$^{sul}$-NH$_2$ residue (**15**) to get access of the $\varepsilon_{375nm}$ value.

## 3.4. Nuclear Magnetic Resonance (NMR)

$^1$H NMR spectra were recorded on Avance III HD 400 MHz Bruker BioSpin and Avance III HD 500 MHz Bruker BioSpin spectrometers. All chemical shifts are reported in ppm and calibrated against residual solvent signals of DMSO-$d_6$ (δ 2.50 ppm) and $CD_3CN$ (δ 2.05) (δ value in an $NH_4OAc/CD_3CN$ 3:1 (v/v). In the case of $^1$H NMR spectra recorded in $H_2O/D_2O$ 90:10 (v/v), 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt (TPS) was added (δ 0.00 ppm) to the medium. Coupling constants (*J*) are reported in Hz. Signal multiplicities were abbreviated as *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet, and *m*, multiplet.

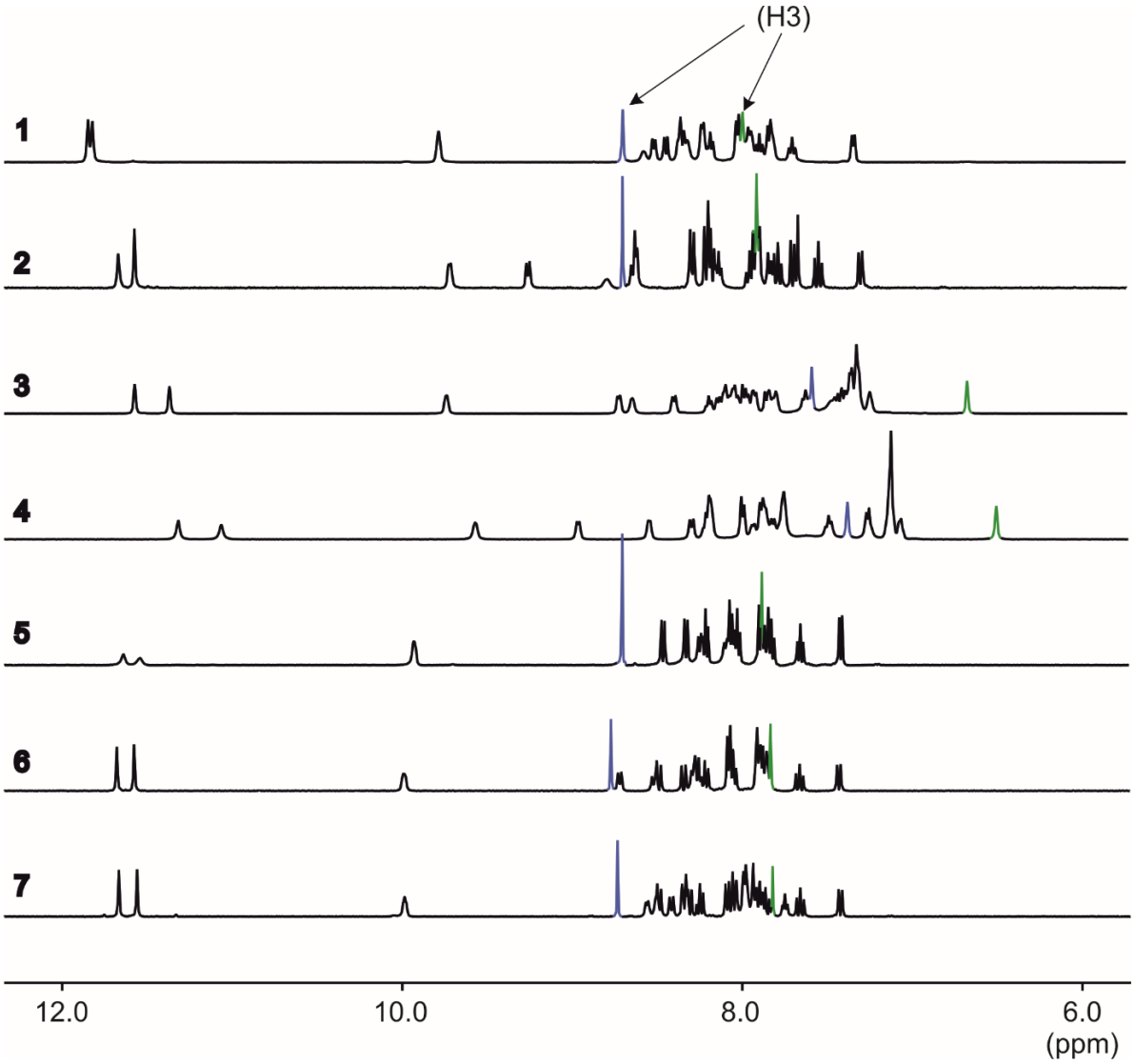



**Figure S3**:[1]H NMR Spectrum overlay of **1**-**7**. (500 MHz, in water:acetonitrile-$d_3$ 75:25 (v:v), water supression at 25 °C. Quinoline H3 protons marked in blue and green.

## 3.5. Circular dichroism (CD)

All CD curves were recorded on a Jasco J-810 spectrometer with 2 mm quartz cuvette. Following parameters were used: Wavelength range from 500 to 250 nm (for PQPQ containing sequences) and 650 to 250 nm (consecutive Q containing oligomers), scan speed: 50 nm/min, accumulation: 2, response time: 1.0 s, bandwidth: 2, temperature: 20 °C, sensitivity: standard, data pitch: 0.1 nm, nitrogen gas flow rate: 500L/h. If not otherwise mentioned, the sample solution was prepared in degassed ultrapure water/acetonitrile solvent mixture 75:25 (v/v). $\Delta\varepsilon$ values were obtained by using the formula: $\Delta\varepsilon = m°/(C.l.32980)$; $\Delta\varepsilon = cm^2.mmol^{-1}$, m°= CD value in milli degrees, l = cuvette pathlength in cm, C = sample concentration in mol/L.

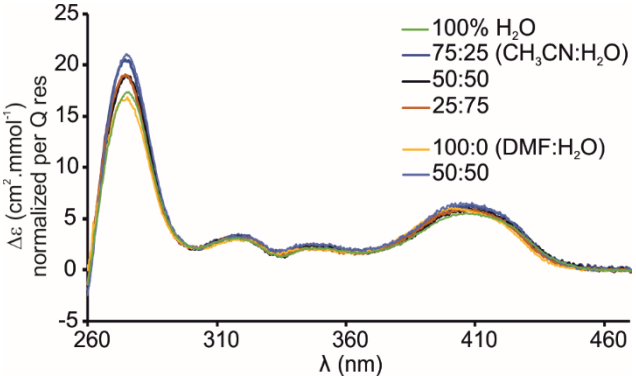



**Figure S4.** CD spectra of **8** in different acetonitrile:water mixtures. The composition of the solvent did not induce a change of handedness control of the Foldamer.

## 4. 2D TOCSY and variable temperature NMR spectra

The two-dimensional TOCSY experiments were recorded at different temperatures (50 °C, 25 °C, 0 °C and -5 °C) at Avance III NMR spectrometers (Bruker BioSpin GmbH) operating at a spectrometer frequency of 400 MHz equipped with 5-mm direct PABBO/BB/19F-1H/D probes with single axis Z gradient capabilities. Processing was performed with MestReNova (v.12.0.0) NMR processing software from Mestrelab Research.

2D TOCSY spectra were recorded with a phase-sensitive pulse sequence using composite pulse scheme MLEV with water suppression employing an excitation sculpting element (mlevesgpph) from the Bruker pulse program library. Data acquisition was performed with 2048 (F2) x 256 (F1) data points in States-TPPI mode. The recycling delay was 2.0 s and 8 transients per increment were applied at a sweep width of 8 kHz in both dimensions resulting in an acquisition time of 0.1283 s. The TOCSY mixing time was set to 80 ms. Special acquisition parameters regarding the water suppression element of the pulse sequence were adopted from the optimized parameter set of the respective one-dimensional experiment. A 90° shifted sine-square multiplication and an exponential window of 1.0 Hz in both dimensions prior to FT was applied. Zero filling and forward LP with MIST algorithm in F1 has been used to yield a final matrix of 1K x 1K real points. Automatic phase correction as well as baseline correction was applied in both dimensions. 1D Traces of the 2D plot showing the TOCSY correlation from the respective NH resonance to the methylene Hs were made by extracting slices from the respective cross peaks in F1.

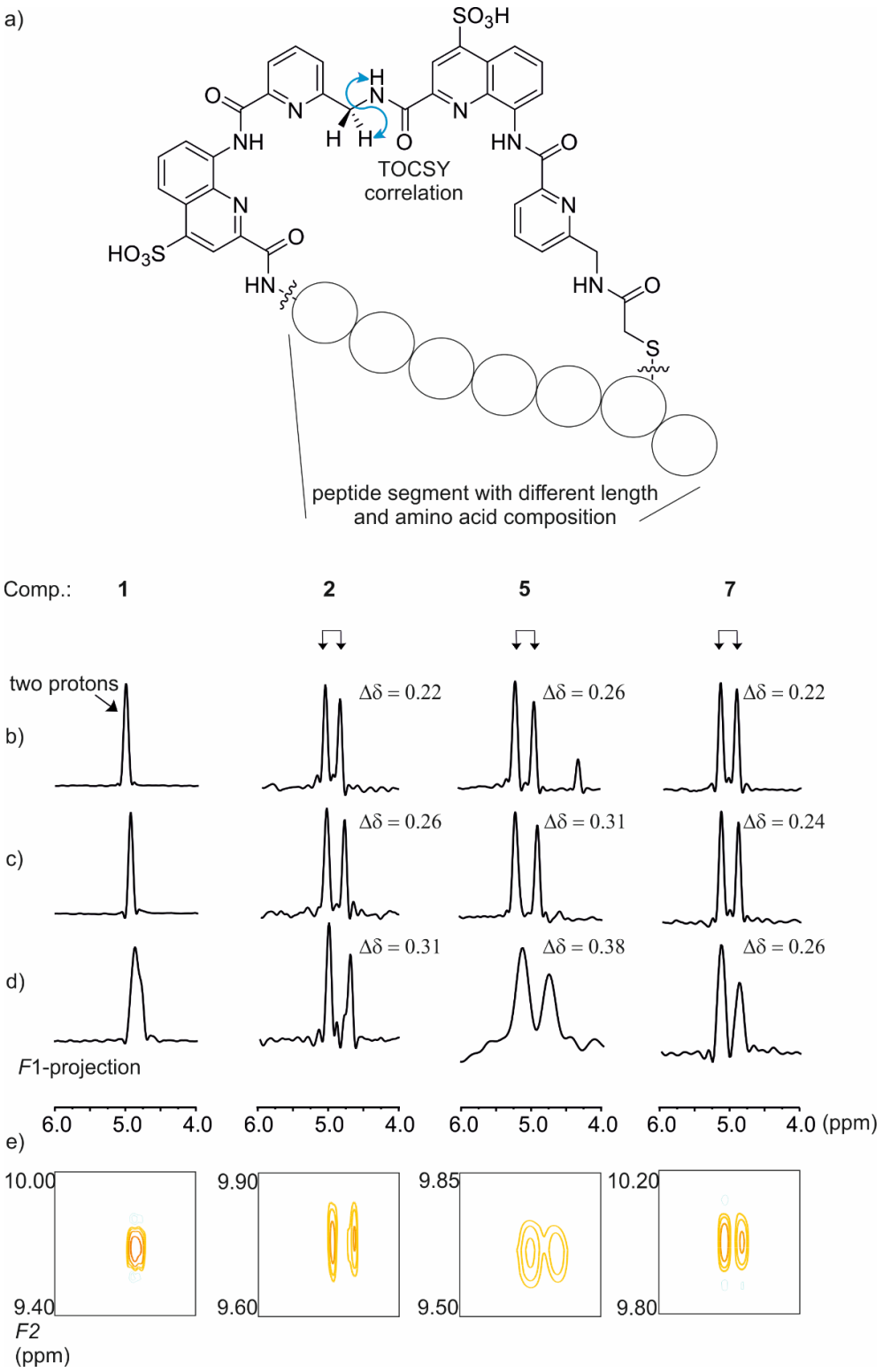



**Figure S5:** Schematic chemical representation of PQPQ-Foldamer-peptide macrocycle (a). *F*1 projection of traces derived from TOCSY correlations between the amide adjacent to the methylene group protons of **1, 2, 5** and **7** at 50 °C (b), 25 °C (c) and 0 °C (d). Excerpts of 2D TOCSY at 0 °C (e).

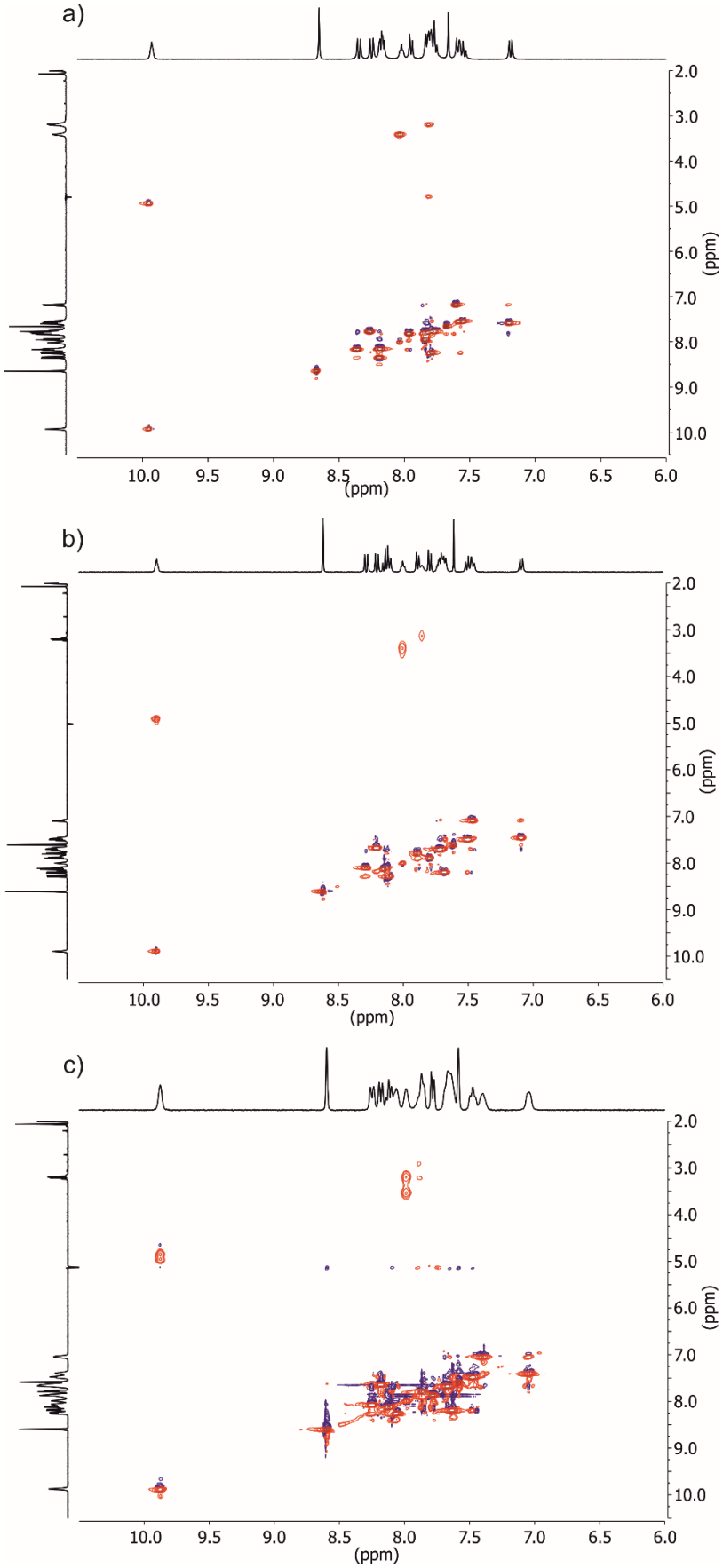



**Figure S6:** Zoom of TOCSY spectra (400 MHz, 50 mM $NaHCO_3$ in $H_2O$/10% $D_2O$, water supression) of 12 at 25 °C (a), 0 °C (b) and -5 °C (c).

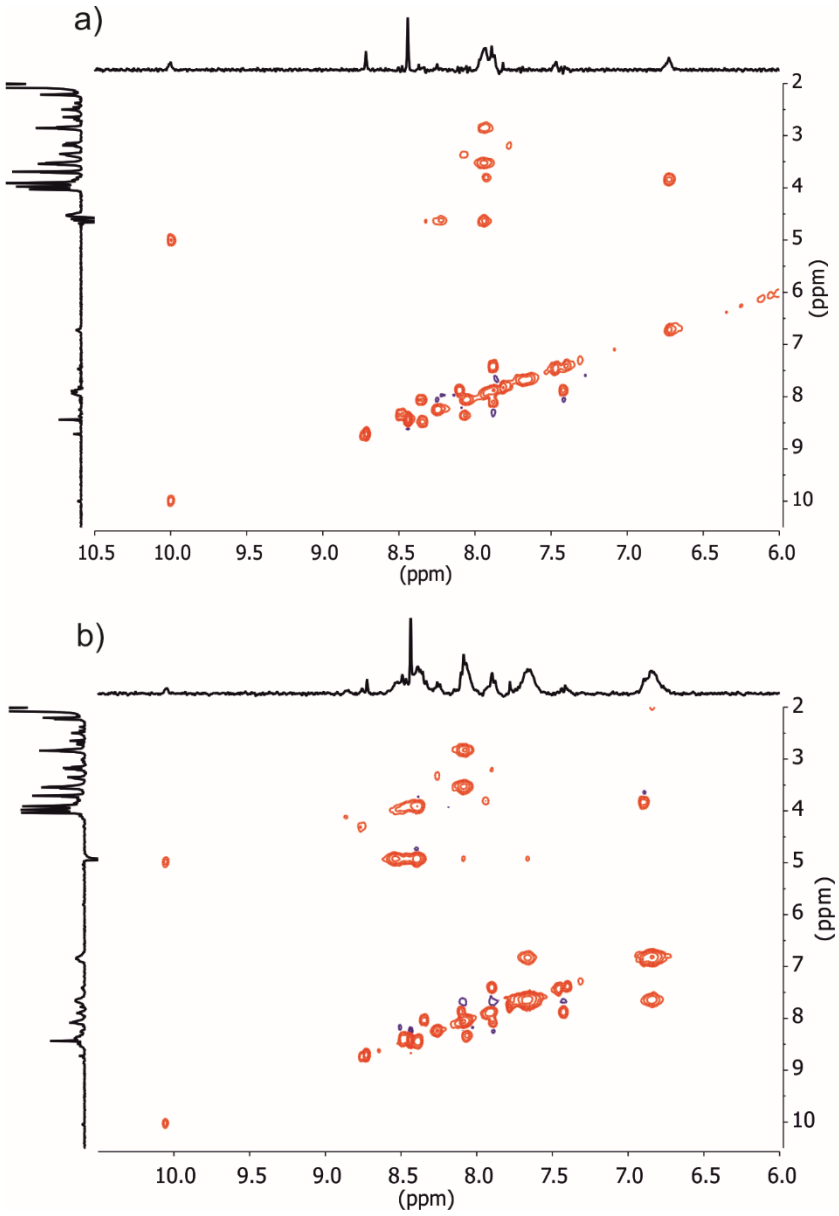



**Figure S7:** Zoom of TOCSY spectra (400 MHz, 12.5 mM $NH_4OAc$ in $H_2O$/25% $CD_3CN$, water supression) of 13 at 25 °C (a) and 0 °C (b).

## 5. Crystallization and X-ray diffraction measurements

In the following section (bio)crystallization using vapor diffusion method, X-ray diffraction measurement at Synchrotron Radiation facility, structure determination and refinement of compounds **1**, **7**, and **9** are described. All three crystal structures were determined using the

dual space method in structure solution software *ShelxD*[6] and refined by performing full-matrix least-squares method on $F^2$ with *ShelxI-2014*[7] within the *Olex2*[8] suite.

### 5.1. Crystallization and X-ray diffraction of compounds **1**, **7** and **9**

For crystallization, the lyophilized powder of hybrid macrocycles was dissolved in ultrapure water. Screening trials were performed using sparse matrix screen JBScreen Basic 1 - 3, Wizard 1 - 4, PACT ++4, and NucPro1 from Jena Bioscience on 96-well plate by sitting drop vapor diffusion at 25 °C. For each screening condition, 0.8 µL of the compound solution was mixed with 0.8 µL of crystallization reagent. The crystallization screening hit was optimized by means of increasing drop size and hanging drop vapor diffusion method in a 24-well Linbro-style plate.

Crystallization of compound **9** as an enantiopure solution lead to crystals that diffracted poorly. Consequently, the *D*-enantiomer of **9** was synthesized and the lyophilized powder was dissolved in ultrapure water. A racemic mixture of *L*- and *D*-enantiomers was prepared by mixing in a 1:1 ratio the solutions of enantiopure *L*- and *D*-compound **9**. The mixture was again lyophilized and redissolved in ultrapure water to a final concentration of 10 mM.

For mounting, a single crystal was fished from the drop with a micro-loop, quickly soaked in cryo-protectant solution, and flash-frozen in liquid nitrogen.

For compound *D*-**1**, diffraction measurements at atomic resolution were obtained in the X10SA (PXII) beamline, Swiss Light Source, with a Pilatus 6M detector.[9] Diffraction data were measured at T = -173 °C and λ = 0.8000 Å. The crystal was exposed for 0.5 s and 0.5° oscillation per frame. Diffraction data were processed with *CrysAlis*$^{Pro}$ suite version 39.46.[10]

For compounds **7** and **9**, diffraction measurements at atomic resolution were obtained in the P13 beamline operated by EMBL Hamburg at the Petra III storage ring (DESY, Hamburg), with a Pilatus 6M detector.[11] Diffraction data were measured at T = -173 °C and λ = 0.97625

[6] G. M. Sheldrick, *Acta Crystallogr A Found Adv*, 2015, **71**, 3-8.
[7] G. M. Sheldrick, *Acta Crystallogr C Struct Chem*, 2015, **71**, 3-8.
[8] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *Journal of Applied Crystallography*, 2009, **42**, 339-341.
[9] M. R. Fuchs, C. Pradervand, V. Thominet, R. Schneider, E. Panepucci, M. Grunder, J. Gabadinho, F. S. Dworkowski, T. Tomizaki, J. Schneider, A. Mayer, A. Curtin, V. Olieric, U. Frommherz, G. Kotrle, J. Welte, X. Wang, S. Maag, C. Schulze-Briese and M. Wang, *J Synchrotron Radiat*, 2014, **21**, 340-351.
[10] Rigaku Oxford Diffraction, 2015, CrysAlisPro Software System, (Version 1.171, Rigaku Corporation,Oxford, UK)

[11] M. Cianci, G. Bourenkov, G. Pompidor, I. Karpics, J. Kallio, I. Bento, M. Roessle, F. Cipriani, S. Fiedler and T. R. Schneider, *J Synchrotron Radiat*, 2017, **24**, 323-332.

Å. The crystal was exposed for 0.04 s and 0.1° oscillation per frame. Diffraction data were processed with *CrysAlis*$^{Pro}$ suite version 39.46.[10]

**Table 1**: Crystallization conditions of compound **1**, **7** and **9**

| Compound | Conc. (in $H_2O$) | Crystallization reagent (reservoir solution) | Crystallogenesis Duration | Cryo-protectand |
|---|---|---|---|---|
| *D*-**1** | 7 mM | 28% polyethylene glycol 400, 100 mM HEPES, pH 7.5, 200 mM $CaCl_2$ | 7 days | 50% v/v PEG 400 solution |
| *L*-**7** | 5mM | 1.6 M $NaH_2PO_4$, 0.4M $K_2HPO_4$, 0.1 M Sodium phosphate citrate, pH 4.2 | 7 days | Glycerol + reservoir solution (1:1) |
| **9** | 10mM | 15% PEG 400, 0.1 TRIS, 0.08 M $CaCl_2$, and 15 mM NaCl at pH 8.5 | 4 days | Glycerol + reservoir solution (1:1) |

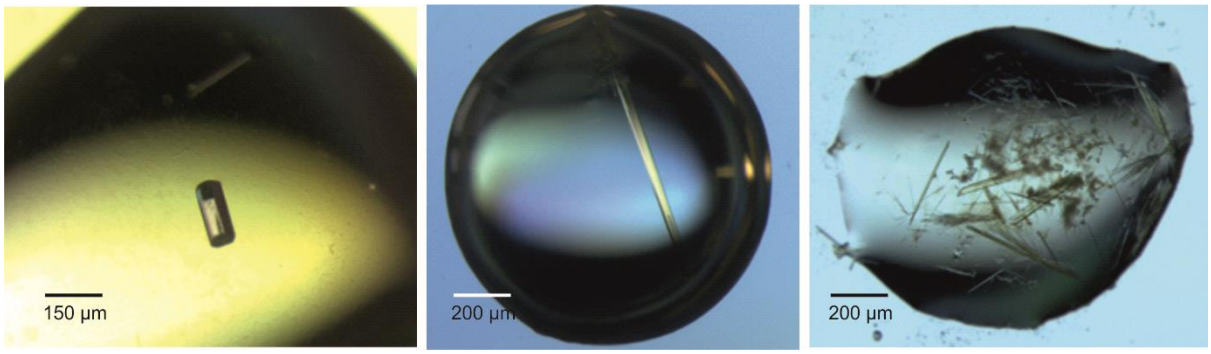



**Figure S8.** Crystals of **1**, **3** and **7** from left to right in the crystallization medium under the microscope. Drop size 1.5 µL to 2.0 µL.

### 1. Structure determination and refinement:

The crystal of *D*-**1** belonged to space group $P6_5$ with one molecule in the asymmetric unit. The structure was solved by dual space solution method using *ShelxD*[6] and refined by full-matrix least-squares method on $F^2$ with *ShelxI-2014*[7] within *Olex2*.[8] After each refinement step, visual inspection of the model and electron density was carried out in *Coot*.[12] Except for the C-terminal Gly residue, all non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted by using HFIX into idealized positions with a riding model. After several failed attempts to model disordered water molecules, the

[12] P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 486-501

PLATON/SQUEEZE protocol was performed.[13] DFIX, SADI, and FLAT instructions were used to improve the geometry of the molecules and their displacement parameters. One calcium ion from the crystallization solution could be identified together with five coordination oxygens which were anisotropically refined.

The structure of **7** was resolved in the triclinic space group P1 with eight molecules in the asymmetric unit. The structure was solved by dual space solution method using *ShelxD*[6] and refined by full-matrix least-squares method on F2 with *ShelxI-2014*[7] within *Olex2*.[8] After each refinement step, visual inspection of the model and electron density was carried out in *Coot*.[12] All non-hydrogen atoms of the Foldamer backbone as well as peptide main chain could be refined with anisotropic displacement parameters. Peptide side-chain atoms were left isotropic. Medium resolution diffraction patterns as well as incompleteness of data led to imprecisions in the electron density map. Thus, the peptide side chains for glutamic acid and leucine could only be refined partially. Hydrogen atoms were inserted by using HFIX into idealized positions and refined with a riding model. The PLATON/SQUEEZE[13] procedure was implemented after several attempts to model disordered water molecules. Shelx compatible restraints and constraints (FLAT, DFIX, RIGU, AFIX, EADP) instructions were performed to improve the 3D geometry of the model and temperature parameters.

The crystal of **9** belonged to space group P1 with six molecules in the asymmetric unit. The structure was solved by dual space solution method using *ShelxD*[6] and refined by full-matrix least-squares method on $F^2$ with *ShelxI-2014*[7] within *Olex2*.[8] After each refinement step, visual inspection of the model and electron density was carried out in *Coot*.[12] The majority of all non-hydrogen atoms of the Foldamer backbone as well as peptide main chain could be refined with anisotropic displacement parameters. Atoms with a high degree of disorder were refined with isotropic displacement parameters. Medium resolution diffraction patterns as well as incompleteness of data led to imprecisions in the electron density map. Thus, the peptide side chains lysine and C-terminal glycine could only be refined partially. Four calcium atoms from the crystallization drop with their hydration shell could be identified and modeled with full occupancy. Hydrogen atoms were inserted by using HFIX into idealized positions and refined with a riding model. The PLATON/SQUEEZE[13] procedure was implemented after several attempts to model disordered water molecules. Shelx compatible restraints and constraints FLAT, DFIX, SIMU, RIGU, SADI, AFIX, and EADP instructions were performed to improve the geometry of the molecule and thermal parameters.

The obtained cif file for compound **1** was examined using IUCr´s *checkcif* algorithm. After several failed attempts to upload the CIF files of compounds **7** and **9** into IUCr´2 *checkcif*

[13] A. L. Spek, *Acta Crystallogr D Biol Crystallogr*, 2009, **65**, 148-155.

algorithm, the PLATON „Validation Mode“ tool was performed to examine the structures. All level A and B alerts are listed.

**Table 2**: Group 1 alerts in crystal structures of **1**, **7** and **9**

| Compound | Alert |
|---|---|
| *D*-**1** | |
| | PLAT234_ALERT_4_A Large Hirshfeld Difference N76 --C75 . 0.40 Ang. |
| | This alert concerns the side-chain lysine residue and is inherent to the quality of the data. It does not reflect wrong assignment. |
| Compound **7** | Alert |
| | 023_ALERT_3_A Resolution (too) Low [sin(theta)/Lambda < 0.6].. 27.42 Degree |
| | 029_ALERT_3_A _diffrn_measured_fraction_theta_full value Low . 0.877 Why? |
| | 201_ALERT_2_A Isotropic non-H Atoms in Main Residue(s) ..... 18 Report<br>O593 N594 C563 C564 C580 C581<br>etc. |
| | Amino acid side chain atoms have been left isotropic due to their disorder. These alerts are inherent to the quality of the data. |
| | 375_ALERT_2_A Strange C-O-H Geometry (C-O > 1.45 Ang) ..... O570 Check |
| | 375_ALERT_2_A Strange C-O-H Geometry (C-O > 1.45 Ang) ..... O169 Check |
| Compound **9** | Alert |
| | 023_ALERT_3_A Resolution (too) Low [sin(theta)/Lambda < 0.6].. 24.66 Degree |
| | 029_ALERT_3_A _diffrn_measured_fraction_theta_full value Low . 0.910 Why? |
| | 201_ALERT_2_A Isotropic non-H Atoms in Main Residue(s) ..... 18 Report<br>O363 O964 N350 N364 N951 N965 etc. |
| | Peptide side chains have been left isotropic due to their disorder. Mentioned alerts are inherent to the quality of the data. |
| | 412_ALERT_2_A Short Intra XH3 .. XHn H73F ..H733 . 1.65 Ang.<br>x,y,z = 1_555 Check |

413_ALERT_2_A Short Inter XH3 .. XHn H33C ..H52 . 1.88 Ang.
x,y,z = 1_555 Check

414_ALERT_2_A Short Intra D-H..H-X H34E ..H35E 1.77 Ang.
x,y,z = 1_555 Check

414_ALERT_2_A Short Intra D-H..H-X H960 ..H965 1.76 Ang.
x,y,z = 1_555 Check

430_ALERT_2_A Short Inter D...A Contact O3X ..O30 . 2.50 Ang.
-1+x,y,1+z = 1_456 Check

Short oxygen-oxygen inter distance contact represents two symmetry-related atoms and does not reflect wrong assignment.

**Table 3:** Group 2 alerts in crystal structures of 1, 7 and 9

| Compound *D*-**1** | Alert |
|---|---|

PLAT084_ALERT_3_B High wR2 Value (i.e. > 0.25) ..... 0.36 Report

This alert is inherent to the quality of the data.

PLAT201_ALERT_2_B Isotropic non-H Atoms in Main Residue(s) ..... 3 Report
O86 O87 C85

This alert concerning a side chain COOH group. ANIS displacement parameters could not be performed for mentioned atoms due to their disorder.

PLAT234_ALERT_4_B Large Hirshfeld Difference O88 --C82 . 0.30 Ang.
PLAT234_ALERT_4_B Large Hirshfeld Difference C79 --C82 . 0.26 Ang.

These alerts concerning disordered cys-gly side-chain atoms and are inherent to the quality of the data.

PLAT241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C84 Check
PLAT242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of Ca89 Check
PLAT341_ALERT_3_B Low Bond Precision on C-C Bonds ..... 0.02051 Ang.
PLAT430_ALERT_2_B Short Inter D...A Contact O54 ..O95 . 2.55 Ang.
1+x-y,x,-1/6+z = 2_454 Check
PLAT430_ALERT_2_B Short Inter D...A Contact O86 ..O93 . 2.66 Ang.
1+y,-x+y,1/6+z = 6_655 Check

Alerts 2B concerns side-chain atoms and calcium hydration shell atoms. However, they do not indicate an incorrect atom-type assignment.

PLAT934_ALERT_3_B Number of (Iobs-Icalc)/Sigma(W) > 10 Outliers .. 4 Check

| Compound **7** | Alert |
|---|---|
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C18 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C104 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C159 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C278 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C392 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C417 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of S694 Check |
| | 241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C689 Check |
| | 242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N103 Check |
| | 242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N277 Check |
| | 242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C279 Check |
| | 242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of S451 Check |
| | 242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N691 Check |
| | 242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C600 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C564 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C595 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C766 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C65 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C81 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C163 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C264 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C293 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C364 Check |
| | 315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C464 Check |

315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C664 Check

Alerts concerning atoms with missing neighbor carbon atoms. This reflects partially or not refined amino acid side-chains carbon-carbon bonds and those atoms have been left without hydrogen atoms.

340_ALERT_3_B Low Bond Precision on C-C Bonds ..... 0.02216 Ang.
416_ALERT_2_B Short Intra D-H..H-D H772 ..H783 . 1.56 Ang.
x,y,z = 1_555 Check
416_ALERT_2_B Short Intra D-H..H-D H270 ..H281 . 1.52 Ang.
x,y,z = 1_555 Check
416_ALERT_2_B Short Intra D-H..H-D H208 ..H485 . 1.73 Ang.
x,y,z = 1_555 Check
420_ALERT_2_B D-H Without Acceptor O71 --H71 . Please Check
420_ALERT_2_B D-H Without Acceptor O169 --H169 . Please Check
420_ALERT_2_B D-H Without Acceptor O370 --H370 . Please Check
420_ALERT_2_B D-H Without Acceptor O670 --H670 . Please Check
910_ALERT_3_B Missing # of FCF Reflection(s) Below Theta(Min). 11 Note
911_ALERT_3_B Missing FCF Refl Between Thmin & STh/L= 0.472 4936 Report

| Compound **9** | Alert |
|---|---|

082_ALERT_2_B High R1 Value ..... 0.16 Report
084_ALERT_3_B High wR2 Value (i.e. > 0.25) ..... 0.43 Report
090_ALERT_3_B Poor Data / Parameter Ratio (Zmax > 18) ..... 4.35 Note

These alerts are inherent to the quality of the data.

241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of S962 Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of N301 Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C655 Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C665 Check

241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of N31A Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C59A Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of S96 Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C20 Check
241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C59 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C348 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C360 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of S695 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N667 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C699 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C756 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C762 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N555 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C440 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C536 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C564 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N58A Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N85A Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C29A Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C53B Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of N58 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C15 Check
242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C95 Check

Alerts are a consequence of anisotropic refinement and are inherent to the quality of the data.

315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C966 Check
315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C747 Check

315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C567 Check
315_ALERT_2_B Singly Bonded Carbon Detected (H-atoms Missing). C148 Check

Alerts concerning atoms with missing neighbor carbon atoms. This reflects partially or not refined amino acid side-chains carbon-carbon bonds and those atoms have been left without hydrogen atoms.

341_ALERT_3_B Low Bond Precision on C-C Bonds ..... 0.05004 Ang.
410_ALERT_2_B Short Intra H...H Contact H49F ..H51A . 1.82 Ang.
x,y,z = 1_555 Check
412_ALERT_2_B Short Intra XH3 .. XHn H33A ..H33F . 1.72 Ang.
x,y,z = 1_555 Check
413_ALERT_2_B Short Inter XH3 .. XHn H33G ..H54C . 1.97 Ang.
x,1+y,z = 1_565 Check
413_ALERT_2_B Short Inter XH3 .. XHn H52E ..H73H . 1.91 Ang.
1+x,1+y,z = 1_665 Check
430_ALERT_2_B Short Inter D...A Contact O1X ..O26 . 2.74 Ang.
-1+x,y,1+z = 1_456 Check
430_ALERT_2_B Short Inter D...A Contact O4X ..O764 . 2.59 Ang.
x,y,z = 1_555 Check
430_ALERT_2_B Short Inter D...A Contact O5X ..O881 . 2.74 Ang.
x,y,z = 1_555 Check
430_ALERT_2_B Short Inter D...A Contact O5X ..O765 . 2.78 Ang.
x,y,z = 1_555 Check
430_ALERT_2_B Short Inter D...A Contact O6X ..N162 . 2.63 Ang.
-1+x,y,1+z = 1_456 Check
430_ALERT_2_B Short Inter D...A Contact O6X ..O7X . 2.78 Ang.
x,y,z = 1_555 Check
430_ALERT_2_B Short Inter D...A Contact O9X ..O281 . 2.57 Ang.
-1+x,y,1+z = 1_456 Check
911_ALERT_3_B Missing FCF Refl Between Thmin & STh/L= 0.427 2967 Report

Atomic coordinates and structure factors have been uploaded to the Cambridge Crystallographic Data Centre (CCDC) with accession code 2002474, 2010173 and 2010131 are available free upon request.

**Table 4**: Crystal data and refinement details for compound **1**, **7** and **9**.

| Compound | **1** | **7** | **9** |
|---|---|---|---|
| Formula | $C_{55}H_{57}CaN_{14}O_{22}S_3$ | $C_{58.3}H_{54.5}N_{15.6}O_{18.8}S_3$ | $C_{102.7}H_{88.7}Ca_{0.7}N_{25.7}O_{35.2}S_6$ |
| M [g/mol] | 1659.17 | 1371.56 | 2463.73 |
| *d*min [Å] | 0.8 | 1.06 | 1.17 |
| Crystal system | Hexagonal | Triclinic | Triclinic |
| Space group | $P6_5$ | P1 | P1 |
| a [Å] | 17.90824(9) | 26.4540(2) | 26.6387(9) |
| b [Å] | 17.90824(9) | 27.9371(2) | 28.6958(6) |
| c [Å] | 46.5216(2) | 30.9544(3) | 36.1958(12) |
| α [°] | 90 | 90.943(6) | 84.231(2) |
| β [°] | 90 | 92.550(6) | 71.432(3) |
| y [°] | 120 | 91.431(6) | 87.031(2) |
| Volume [$Å^3$] | 12920.9 (11) | 22843.4 (3) | 26089.9(14) |
| Z | 6 | 8 | 6 |
| $\rho$/g $mm^{-3}$ | 1.279 | 0.798 | 0.941 |
| Color and Shape | yellow hexagonal prism | Yellow needle | Yellow needle |
| Size (mm) | 0.15 x 0.02 x 0.02 | 0.4 x 0.04 x 0.02 | 0.04 x 0.04 x 0.15 |
| μ/$mm^{-1}$ | 0.262 | 0.270 | 0.406 |
| Total reflections | 160150 | 95050 | 110283 |
| Unique data [Fo > 2σFo] | 17481 | 44786 | 25313 |
| $R_{int}$ | 0.057 | 0.0354 | 0.1016 |
| Parameters/restraints | 842/20 | 5927/559 | 7146/5020 |
| R1, wR2 | 0.1215, 0.3576 | 0.0699, 0.2171 | 0.1634, 0.4346 |
| Goodness-of-fit | 1.714 | 0.984 | 1.142 |
| Total potential solvent accessible void volume from SQUEEZE/ $Å^3$ | 4276.5 | 11665 | 11531 |
| CCDC number | 2002474 | 2010173 | 2010131 |

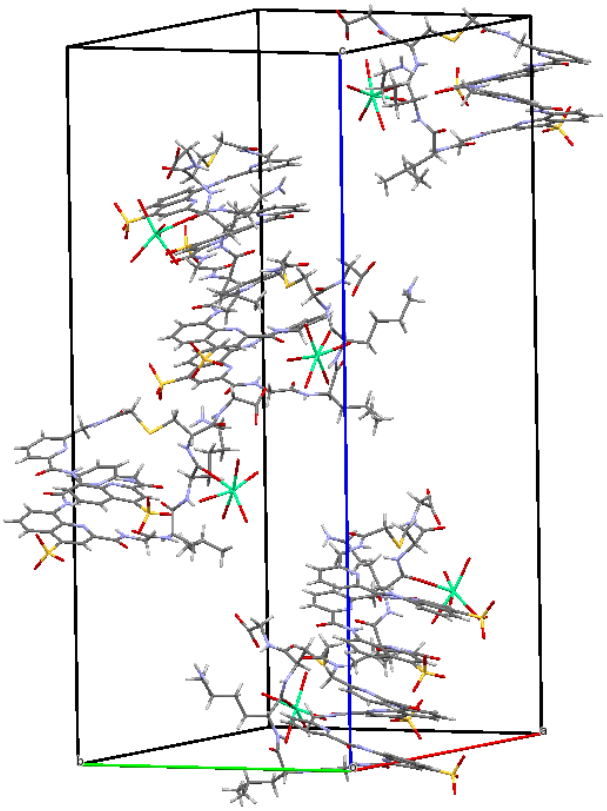


**Figure S9.** Crystal packing arrangement of six identical molecules of compound *D*-**1** in the unit cell.

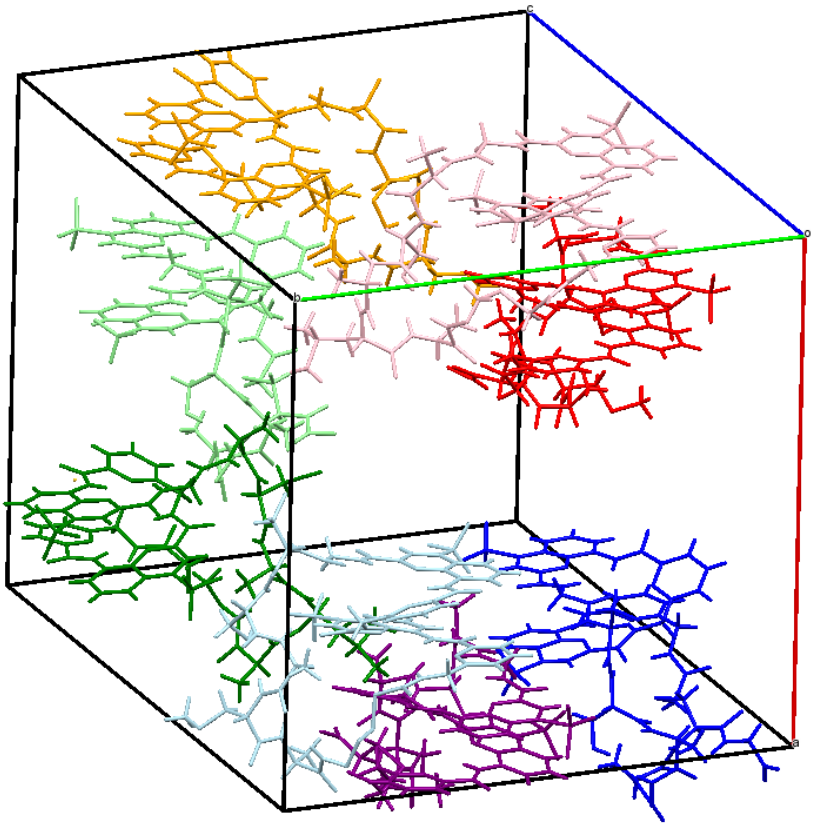


**Figure S10.** Crystal packing arrangement of eight individual molecules of compound **7** in one unit cell. For visualization, the different conformers have been stained in different colors.

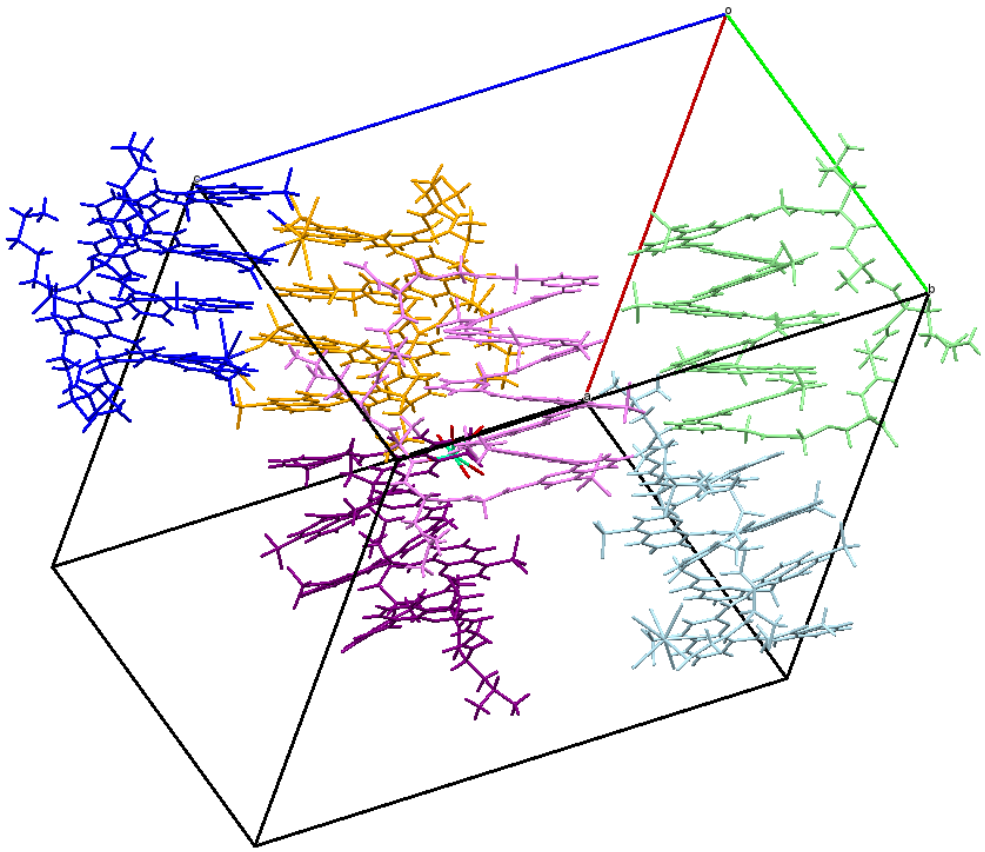


**Figure S11.** X-ray structure of the cocrystallization of compound *L*-**9** and *D*-**9** showing six individual molecules in one unit cell. For visualization, the conformers have been stained in different colors. Compound *L*-**9** (*P*-helicity) is shown in green, light blue and purple. Compound *D*-**9** (*M*-helicity) is shown in dark blue, orange and pink.

**Table 1**: Peptide loop dihedral angles (phi and psi) obtained from peptide-Foldamer-hybrid macrocycle x-ray structures from N to C terminus (top to bottom).

| **Comp.** | **1** | | | **3** | | | **7** | | | **9** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Aa* | Dihedral-angle (deg) | | *Aa* | Dihedral-angle (deg) | | *Aa* | Dihedral-angle (deg) | | *Aa* | Dihedral-angle (deg) | |
| | | ϕ | ψ | | ϕ | ψ | | ϕ | ψ | | ϕ | ψ |
| N | *Leu* | -75.2 | -61.4 | *Phe* | -95.2 | 6.00 | *Glu* | -148 | 58.5 | *Leu* | -119 | 7.2 |
| ↓ | *Lys* | -149 | 130 | *Lys* | -63.4 | 149 | *Ser* | -89.2 | 112 | *Ala* | -79.2 | 174 |
| | *Cys* | -71.4 | -50.0 | *Lys* | -66.1 | 150 | *Ala* | -56.3 | -50.9 | *Lys* | -124 | -20.9 |
| | | | | *Lys* | -56.5 | 145 | *Leu* | -68.5 | -28.1 | *Ala* | -136 | 142 |
| | | | | *Cys* | -56.0 | 142 | *Ala* | -85.1 | -38.6 | *Cys* | -78.4 | 127 |
| C | | | | | | | *Cys* | -137 | 176 | | | |

# 5. Biodegradation assay of foldamer-peptide hybrids in the presents of Pronase E, Trypsin or alpha-Chymotrypsin

## 5.1. Methods

The stability of foldamer-peptide hybrids in protease-rich medium was tested in a 96-well polypropylene plate at room temperature. Therefore, the linear and the cycle sequences were incubated with pronase E, trypsin or α-chymotrypsin respectively over a monitored range of time. To each well, an aqueous buffer (40 µL; 10 mM TRIS, pH 7.5 for pronase E and trypsin or 10 mM TRIS pH 8.0 for α-chymotrypsin), an enzyme solution (1.7 µL, 1.0 mg/mL pronase E, 0.1 mg/mL for trypsin and α-chymotrypsin) and a solution of foldamer-peptide-hybrid (8.3 µL; 2.0 mM in the enzyme-specific buffer) was added in that order.[14,15] The concentration of the foldamer-peptide-hybrid solution was determined by relying on UV calibration curve and UV absorbance.

As blank experiments, two control wells were performed meaning in one well no foldamer-peptide hybrid stock solution was added and in the second well, the enzyme was missing. During the incubation time, the 96-well plate was recovered with aluminum foil to prevent fast evaporation.

At defined time points, the reaction was quenched with a 5% aqueous TFA solution (200 µL), and the progress of foldamer-peptide hybrid's degradation was monitored by using RP-HPLC.

Compounds **3** and **16** were analyzed on a C18-analytical column (linear gradient 15% to 65% mobile phase B, 7 min, 50 °C). **1** and **14** were analyzed on a C8-analytical column (linear gradient 10% to 35% D, 35% to 100% in 3 min, 7 min, 50 °C, $NH_4OAc$-$NH_4OH$ buffer 15 mM pH 5.5). **4** and **15** were analyzed on a C18-analytical column (linear gradient 15% to 45% mobile phase B for 7 min, 45% to 100% in 3 min, 50 °C).

All RP-HPLC analyses were performed in duplicates. Peaks and their chromatographic parameters were analyzed by Chromeleon 7 software with the Cobra Wizard data processing tool. The relative area in % for the intact, undigested foldamer-peptide-hybrid was plotted against the incubation time.

[14] J. D. Sadowsky, J. K. Murray, Y. Tomita and S. H. Gellman, *Chembiochem*, 2007, **8**, 903-916.
[15] E. Teyssieres, J. P. Corre, S. Antunes, C. Rougeot, C. Dugave, G. Jouvion, P. Claudon, G. Mikaty, C. Douat, P. L. Goossens and G. Guichard, *J Med Chem*, 2016, **59**, 8221-8232.

## 5.2. LC-MS studies for foldamer-peptide-hybrids after incubation with proteases

To identify the fragments formed after incubation with proteases, the 96-well plate used for enzyme degradation studies was placed on the LC autosampler and each well was analyzed by RP-LCMS. 5 µL of the in well solution was injected on a C18 column Kinetex® (2.1 x 50 mm, 2.6 µm, Phenomenex) (5% for 1 min, 5 to 100% B in 5 min, 50 °C; mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile, 0.5 mL/min). The corresponding total ion count (TIC) profile from the TOF-MS detector gave the corresponding *m/z* values which allowed the identification of the foldamer-peptide hybrid fragments.

In the case of compound **16**, the starting material and a degradation product co-eluted. The quantification was thus carried out by measuring the extracted ion count (EIC) on the RP-LCMS. Therefore, the sample solutions after incubation with a given protease were injected on the LC-MS and the EIC ratio between signal 1533.6800 +- 0.5 *m/z* (starting material) and 1405.6220 +- 0.5 *m/z* (degradation product) was measured. By combining the UV trace ($\lambda$ = 254 nm) of the signal (which corresponded to the sum of the UV signal of the two compounds) and selectively quantifying the two compounds by EIC, the degradation of compound **16** over time could be monitored.

microTOF II parameters:

| | | |
|---|---|---|
| Source | End Plate Offset | 500 V |
| | Capillary | 4500 V |
| | Nebulizer | 3.0 bar |
| | Dry Gas | 8.0 L/min |
| | Dry Temp | 200 °C |
| Tune | Capillary Exit | 240.0 V |
| | Skimmer 1 | 80.0 V |
| | Hexapole | 23.0 V |
| | Hexapole RF | 450.0 V |
| | Skimmer 2 | 23.0 V |

For the reference experiment, a sample solution (40 µl) (after protease degradation, 40 min incubation time), was enriched with starting compound (5 µL, 2.0 mM solution). The mixture sample was analyzed by RP-LCMS with the above-mentioned parameters and the two EIC signals were compared with a reference solution (40 µL protease degradation sample + 5 µL

water). After calculating the correlation between UV signal, enriched UV signal, EIC signal, and enriched EIC signal, a correcting factor was obtained which was used when determining the quantity of starting material in the degradation experiment.

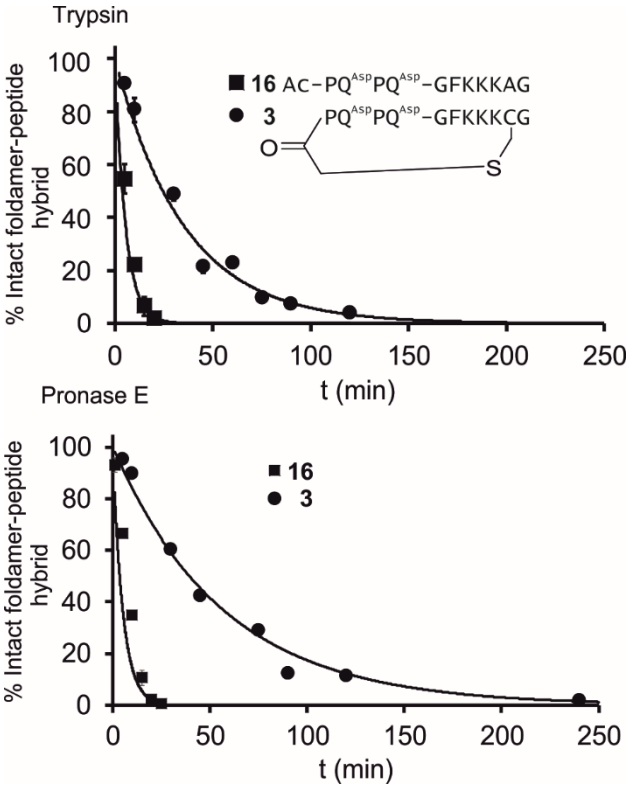



**Figure S12.** Enzymatic degradation of foldamer-peptide-hybrid compounds in the presence of Trypsin or Pronase E. The decreasing abundance of intact macrocyclic compound **3** (circles) is shown with its non-cyclic reference compound **16** (squares).

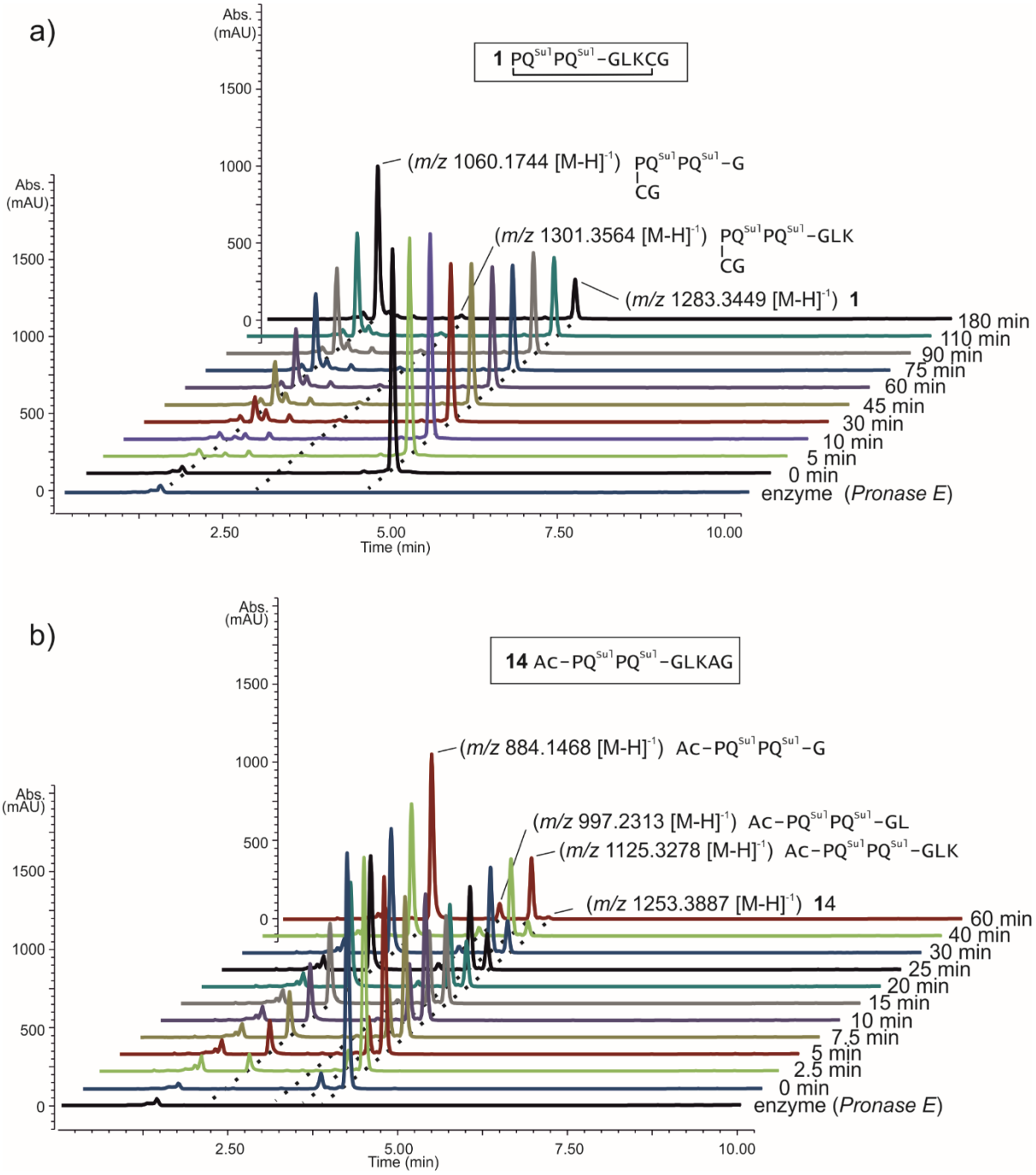



**Figure S13.** Overlaid chromatograms during the incubation of compound **1** in the presence of Pronase E (a) and **14** (b) Analytical HPLC (linear gradient 10% to 35% D in 7 min, 35% to 100% in 3 min, 50 °C, $NH_4OAc$-$NH_4OH$ buffer 15 mM pH 5.5, λ=254 nm). Peptide-foldamer-hybrid fragments formed during the degradation process determined by LC-MS.

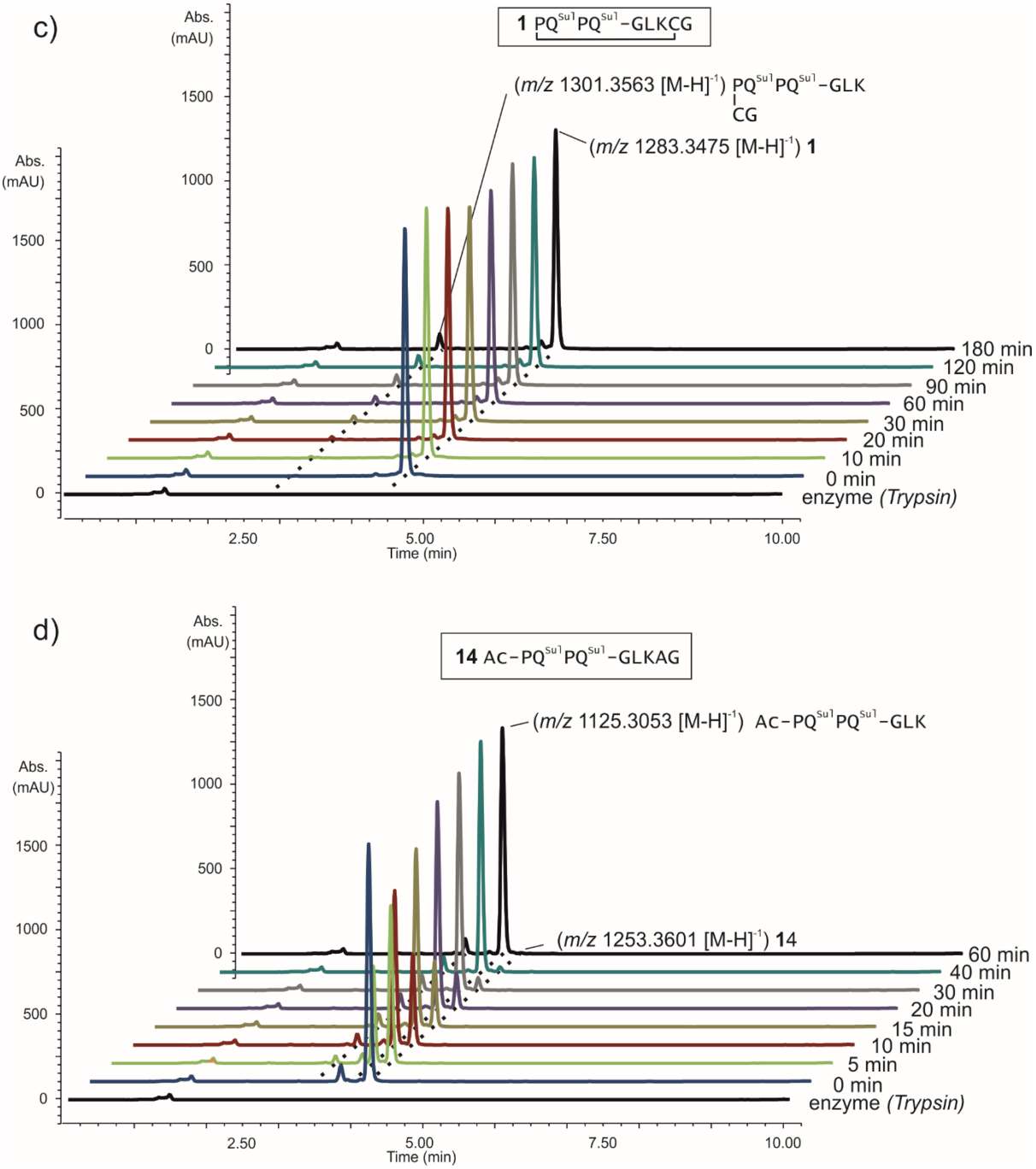



**Figure S14.** Overlaid chromatograms during the incubation of compound **1** in the presence of Trypsin (c) and **14** (d) Analytical HPLC (linear gradient 10% to 35% D in 7 min, 35% to 100% in 3 min, 50 °C, $NH_4OAc$-$NH_4OH$ buffer 15 mM pH 5.5, λ=254 nm). Peptide-foldamer-hybrid fragments formed during the degradation process and determined by LC-MS.

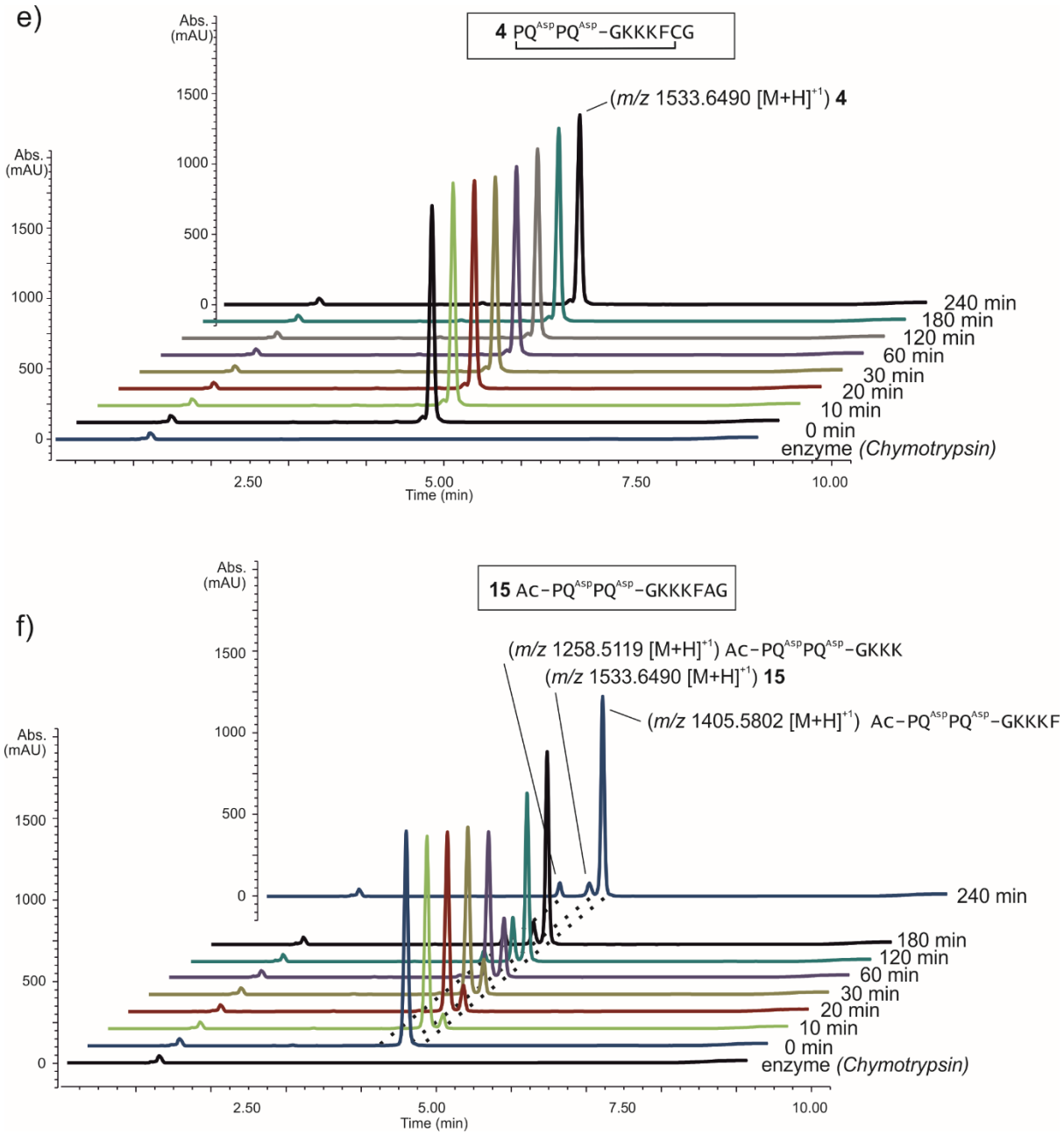



**Figure S15.** Overlaid chromatograms during the incubation of compound **4** in the presence of α-Chymotrypsin (e) and **15** (f). Analytical HPLC (linear gradient 15% to 45% B in 7 min, 45% to 100% in 3 min, 50 °C, 0.1% TFA, λ=254 nm). Peptide-foldamer-hybrid fragments formed during the degradation process and determined by LC-MS.

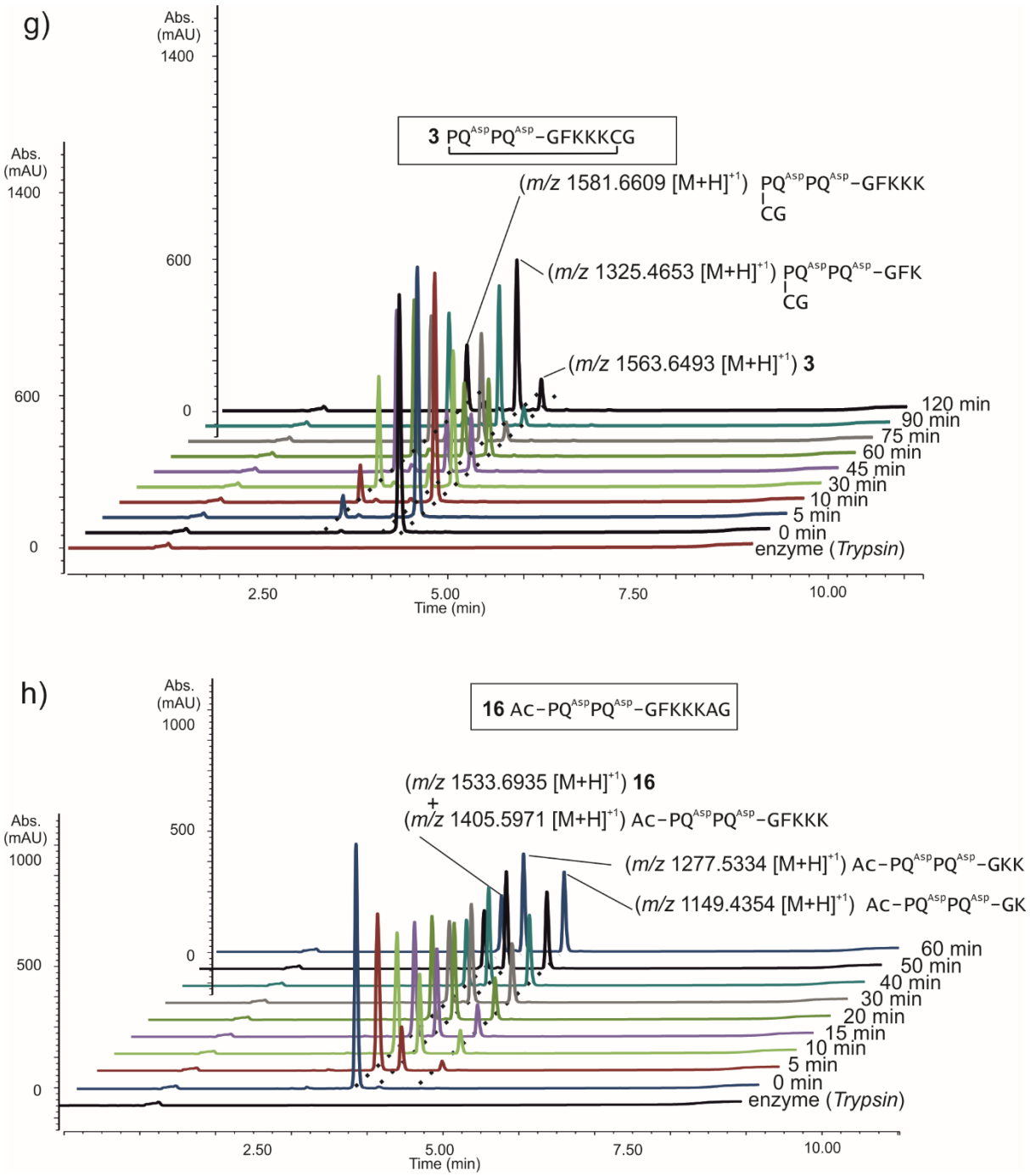



**Figure S16.** Overlaid chromatograms during the incubation of compound **3** in the presence of Trypsin (g) and **16** (h). Analytical HPLC (linear gradient 15% to 65% B in 7 min, 65% to 100% in 2 min, 50 °C, 0.1% TFA, λ=254 nm). Peptide-foldamer-hybrid fragments formed during the degradation process and determined by LC-MS.

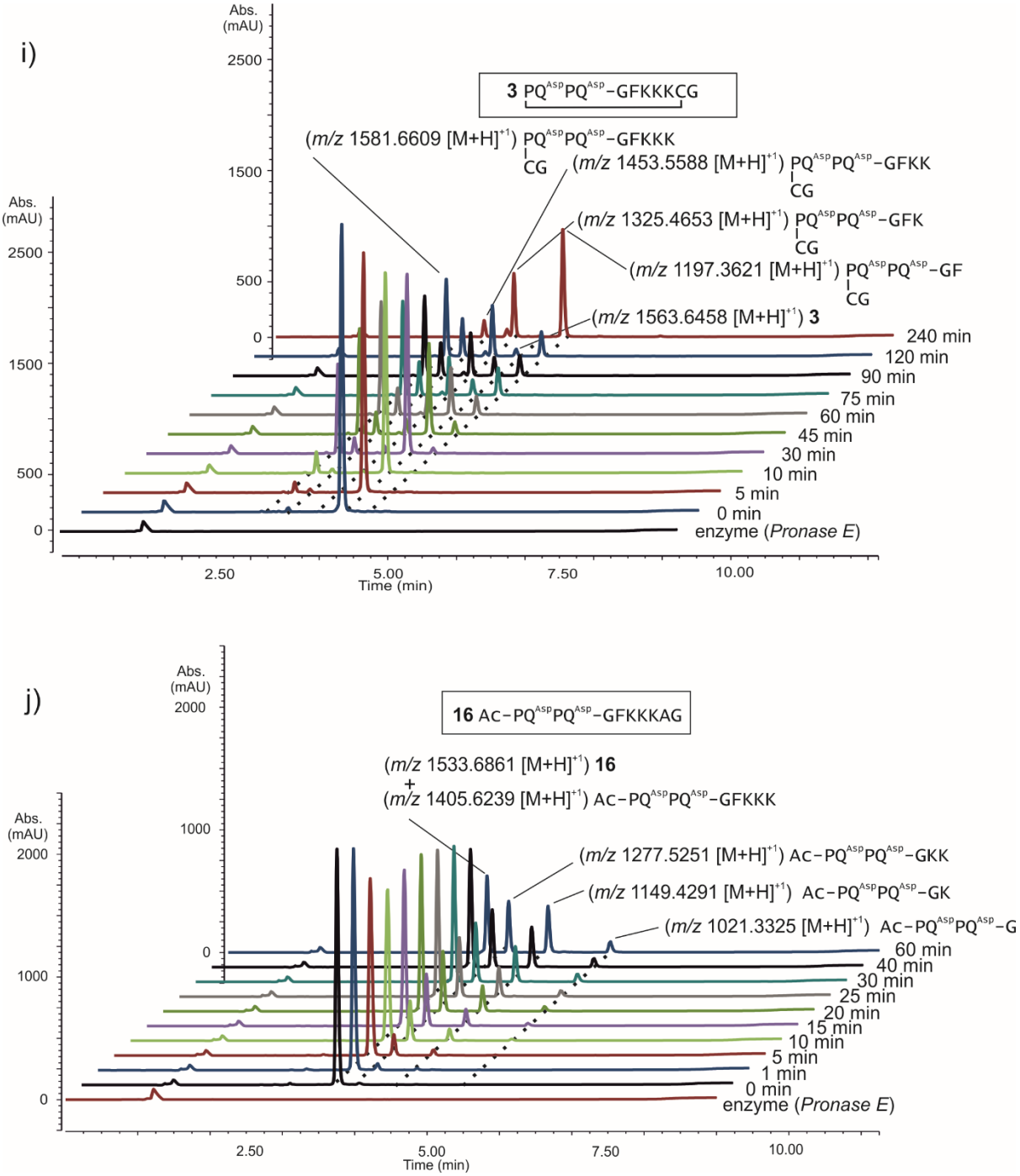
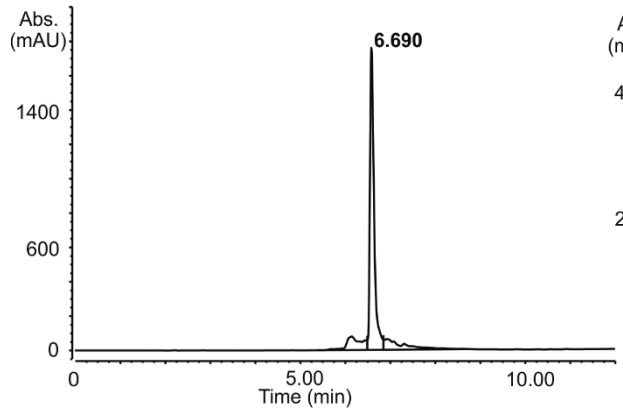


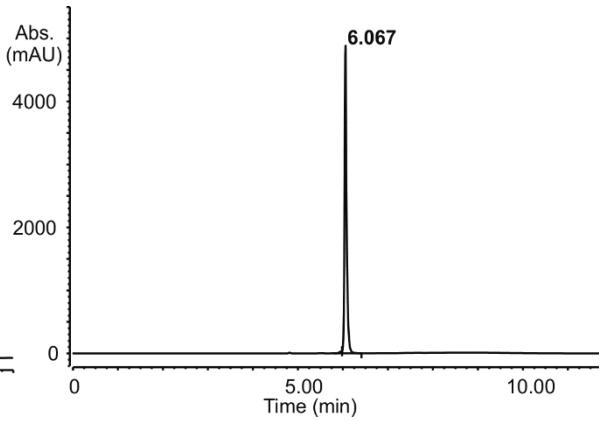

**Figure S17.** Overlaid chromatograms during the incubation of compound **3** (*i*) and **16** (*j*) in the presence of Pronase E. Analytical HPLC (linear gradient 15% to 65% B in 7 min, 65% to 100% in 2 min, 50 °C, 0.1% TFA, λ=254 nm). Peptide-foldamer-hybrid fragments formed during the degradation process and determined by LC-MS.

# 6. $^{1}$H NMR spectra and HPLC profiles

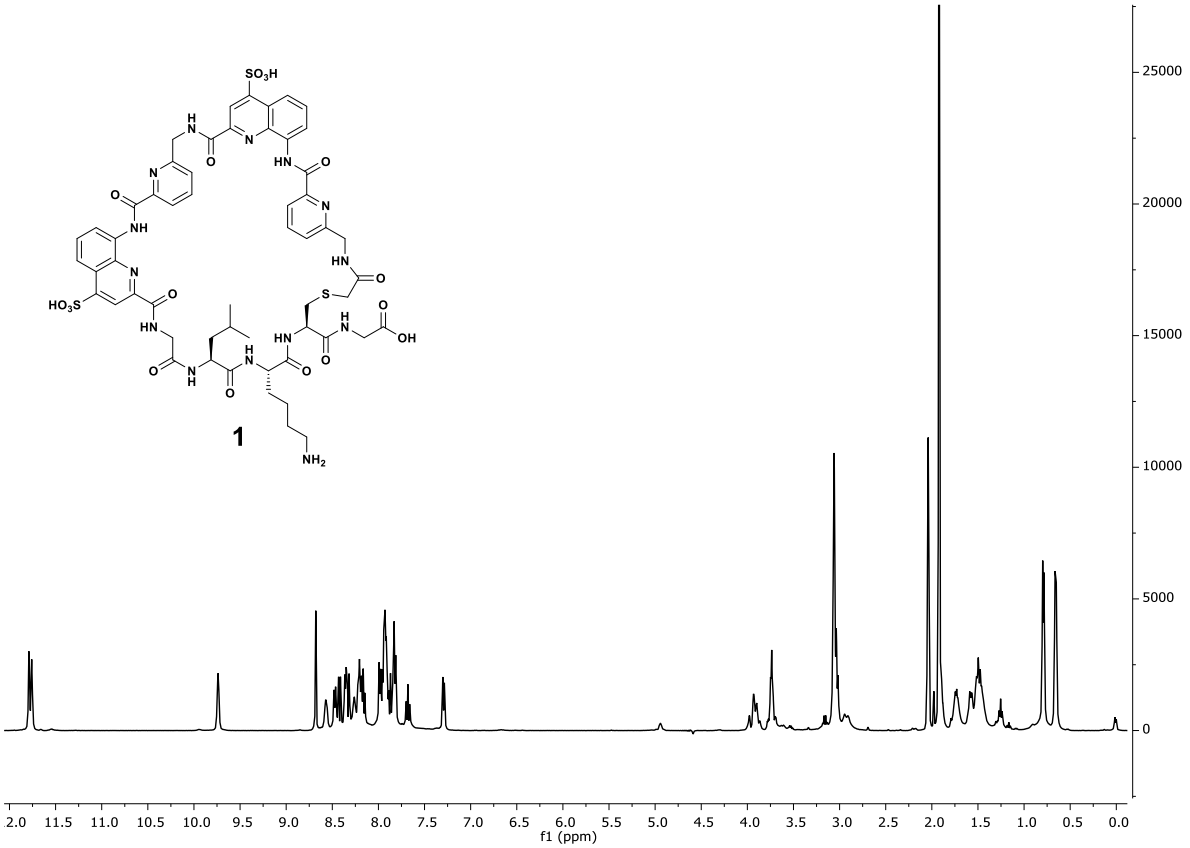
Compound **1**.




Analytical RP-HPLC profile of **1** crude from SPS. (20 to 80 vol. % B over 10 min, 25 °C, λ=300 nm)

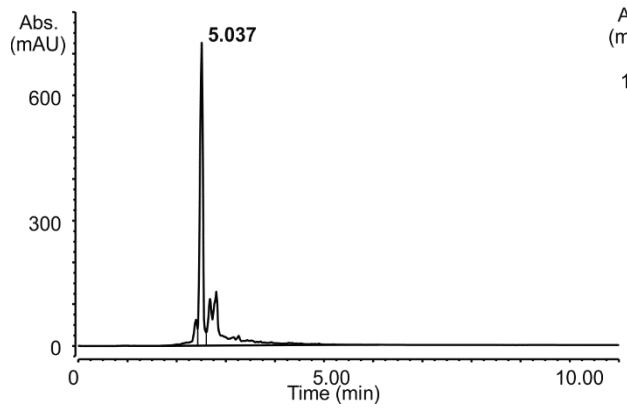



Analytical RP-HPLC profile of **1**. (10 to 80 vol. % B over 10 min, 25 °C, λ=254 nm)

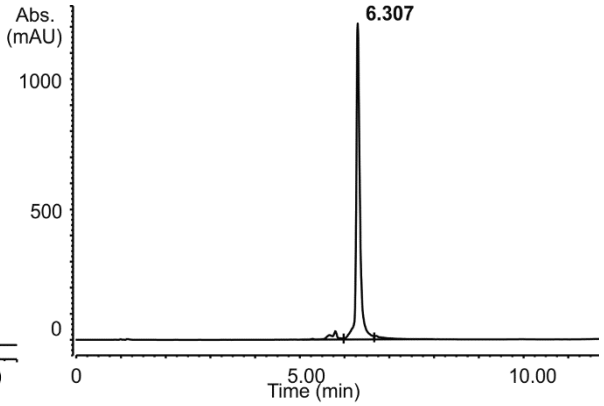



$^{1}$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$, water supression, 25 °C) of **1**.

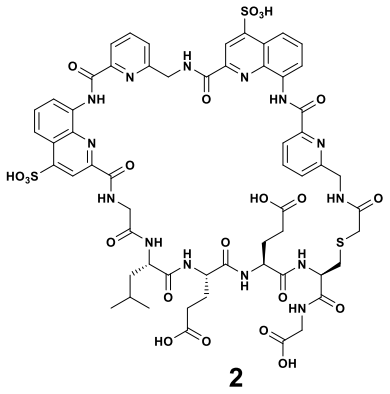
Compound **2**.




Semi-preparative RP-HPLC profile of **2** crude from SPS. (5 to 35 vol. % B over 10 min, 25 °C, λ=300 nm)




Analytical RP-HPLC profile of **2**. (10 to 45 vol. % B over 10 min, 25 °C, λ=254 nm)

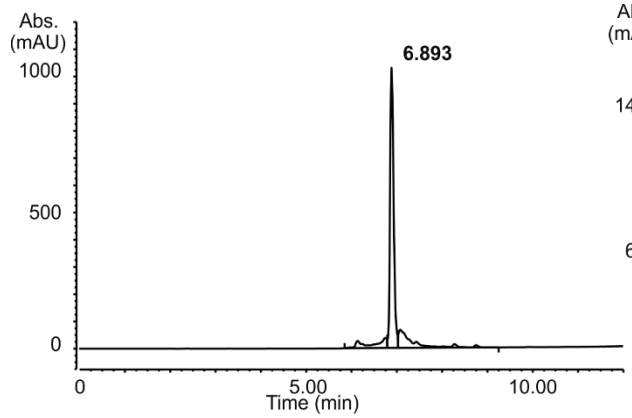


**2**

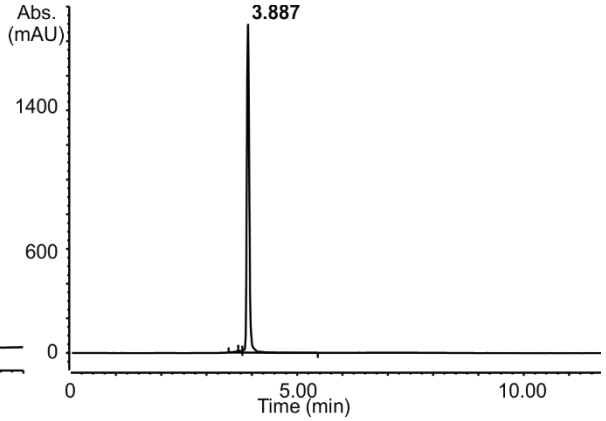



$^{1}$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-*d*$_3$, water supression, 25 °C) of **2**.

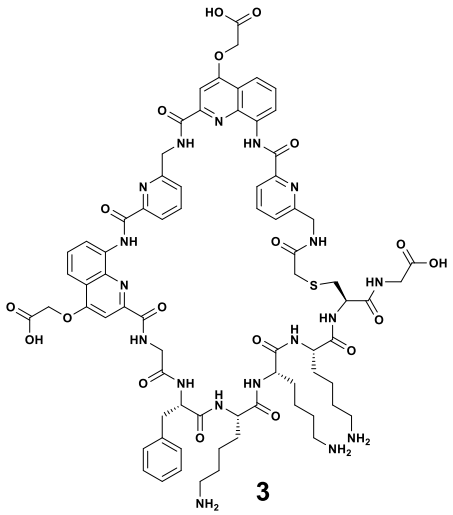
Compound **3**.




Analytical RP-HPLC profile of **3** crude from SPS. (20 to 80 vol. % D over 10 min, 25 °C, λ=254 nm)




Analytical RP-HPLC profile of **3**. (10 to 80 vol. % D over 10 min, 25 °C, λ=254 nm)

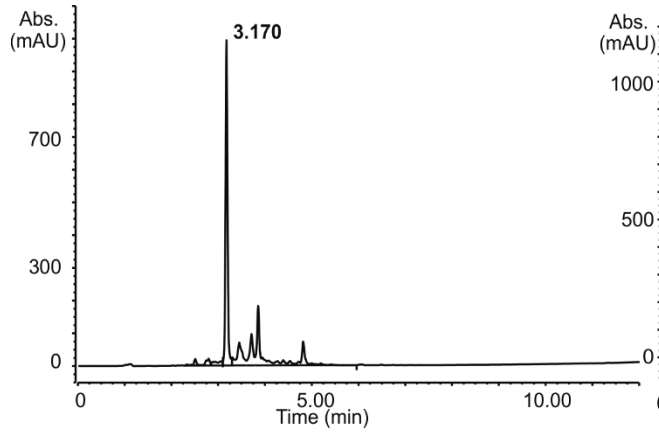


**3**

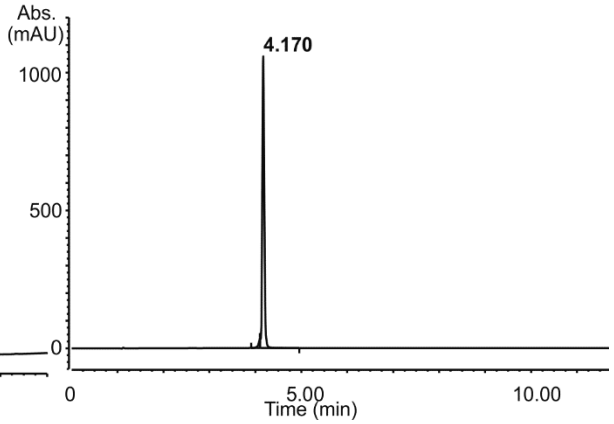



$^{1}$H NMR Spectrum (500 MHz, water/acetonitrile-$d_3$ (3:1, v/v), water supression, 25 °C) of **3**.

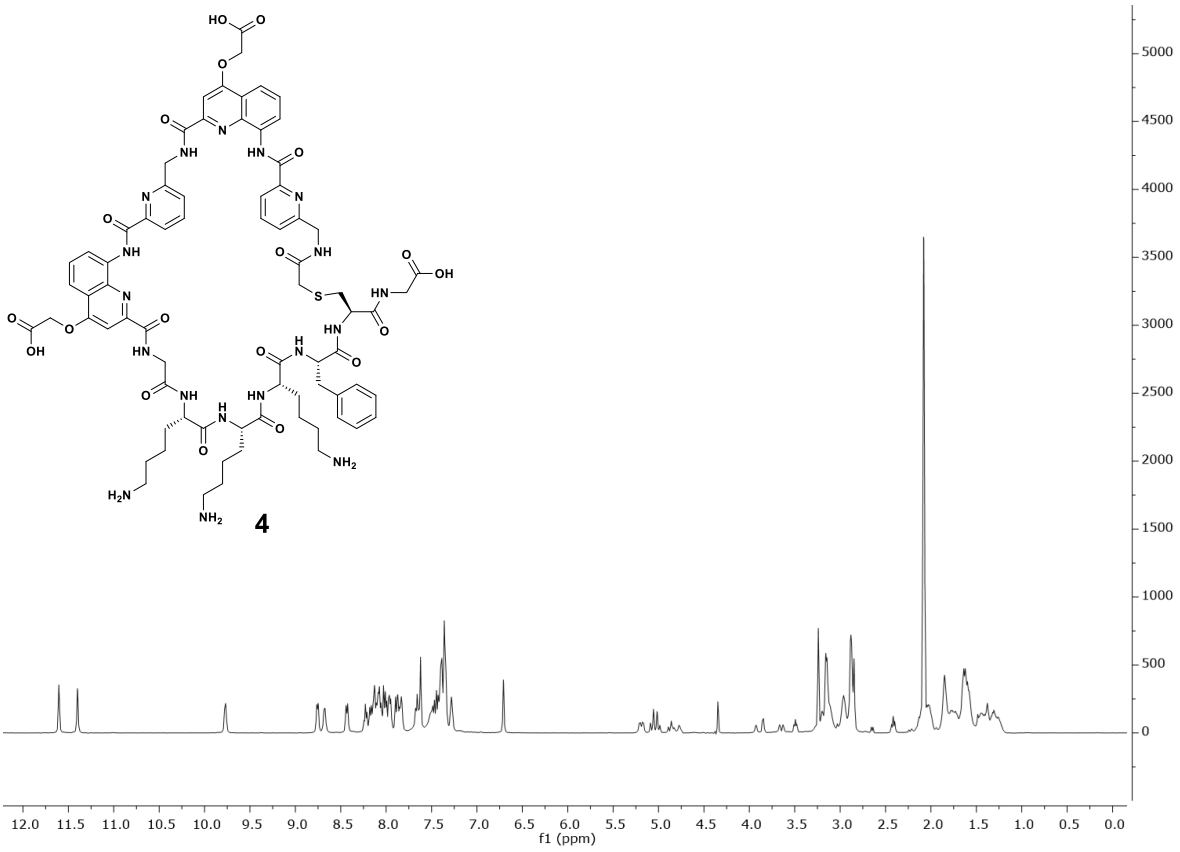
Compound **4**.




Analytical RP-HPLC profile of **4** crude from SPS. (20 to 80 vol. % B over 10 min, 50 °C, λ=254 nm)

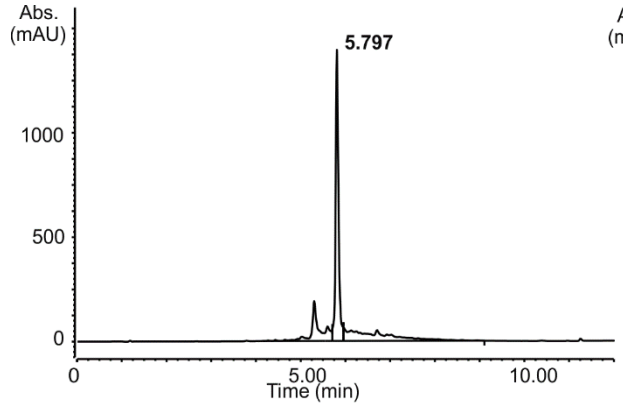



Analytical RP-HPLC profile of **4**. (10 to 80 vol. % D over 10 min, 50 °C, λ=300 nm)

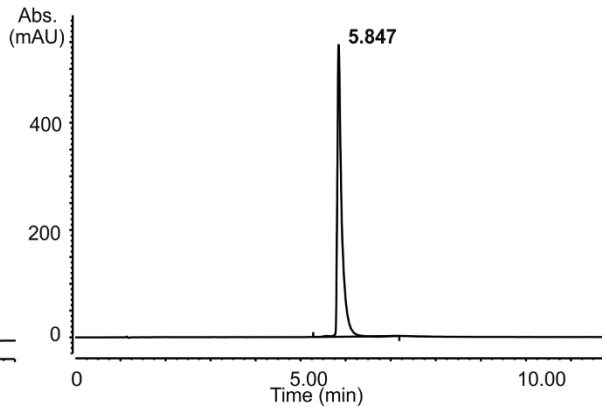



$^{1}$H NMR Spectrum (500 MHz, water/acetonitrile-$d_3$ (3:1, v/v), water supression, 25 °C) of **4**.

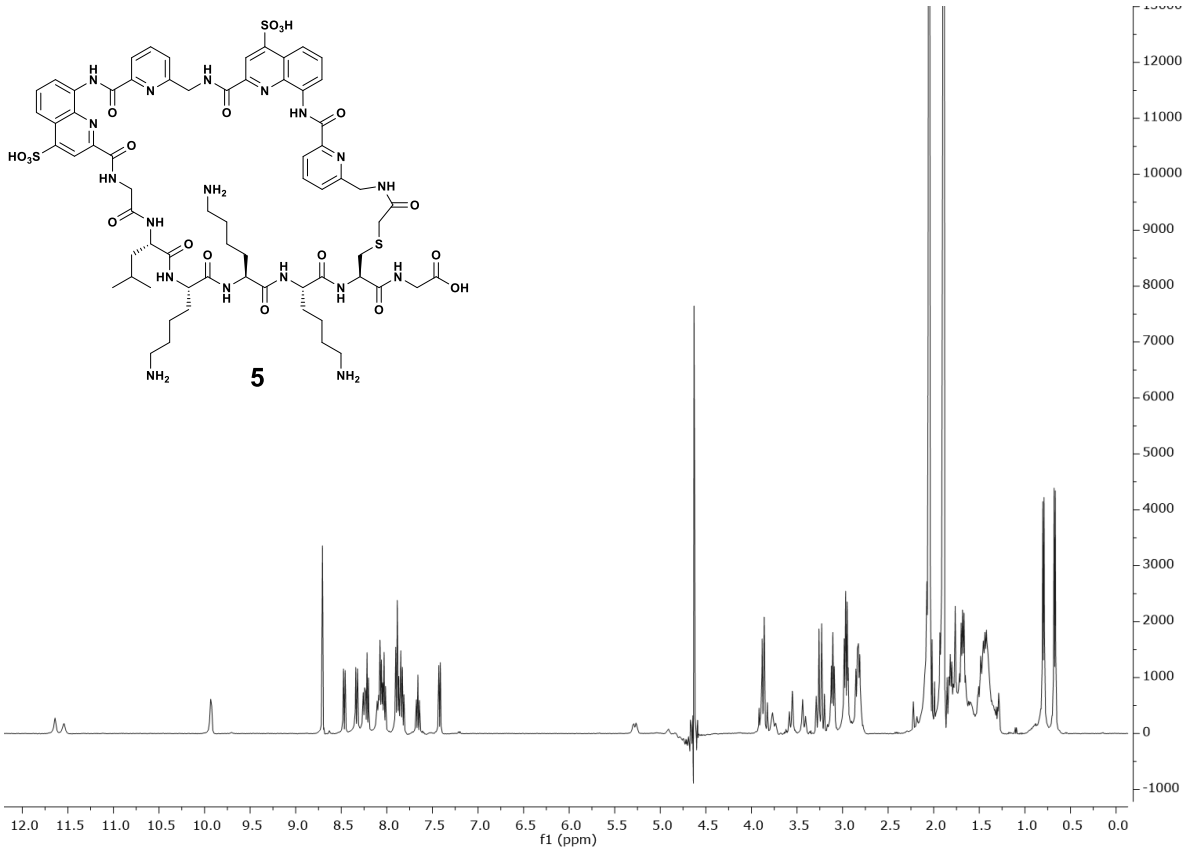
Compound **5**.




Analytical RP-HPLC profile of **5** crude from SPS. (10 to 45 vol. % B over 10 min, 25 °C, λ=254 nm)

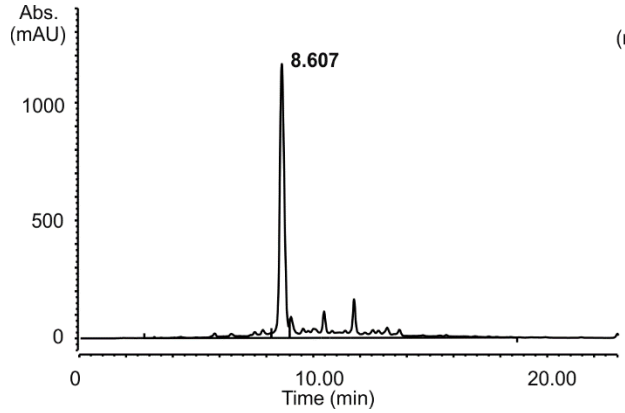



Analytical RP-HPLC profile of **5**. (10 to 50 vol. % D over 10 min, 25 °C, λ=254 nm)

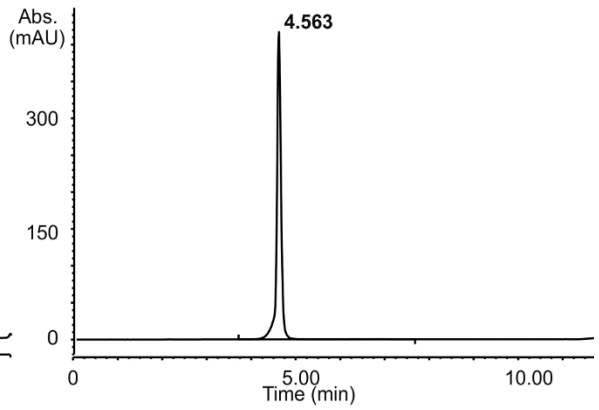



$^1$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$, water suppression, 25 °C) of **5**.

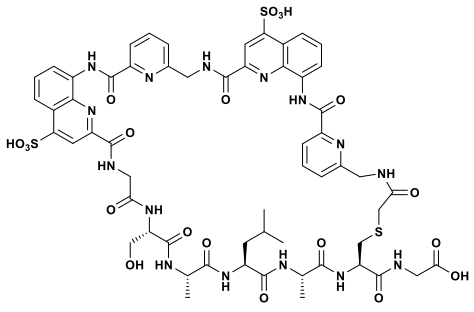
Compound **6**.




Semi-preparative RP-HPLC profile of **6** crude from SPS. (10 to 28 vol. % D over 20 min, 25 °C, λ=300 nm)




Analytical RP-HPLC profile of **6**. (10 to 30 vol. % D over 10 min, 25 °C, λ=254 nm)

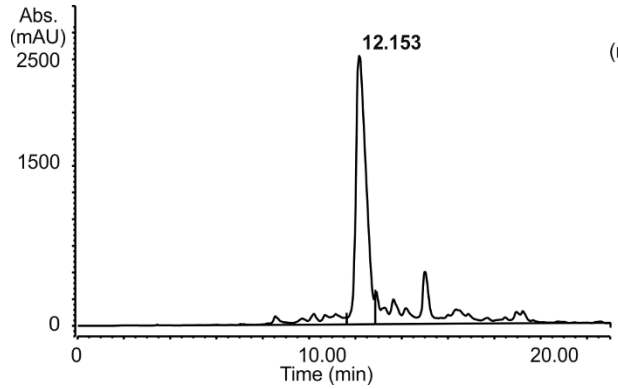


**6**

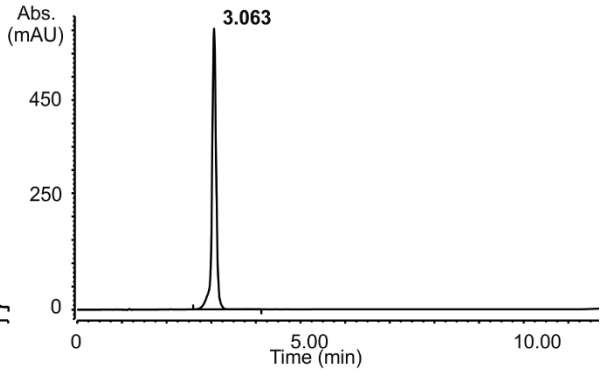



$^{1}$H NMR Spectrum (400 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile-$d_3$, water suppression, 25 °C) of **6**.

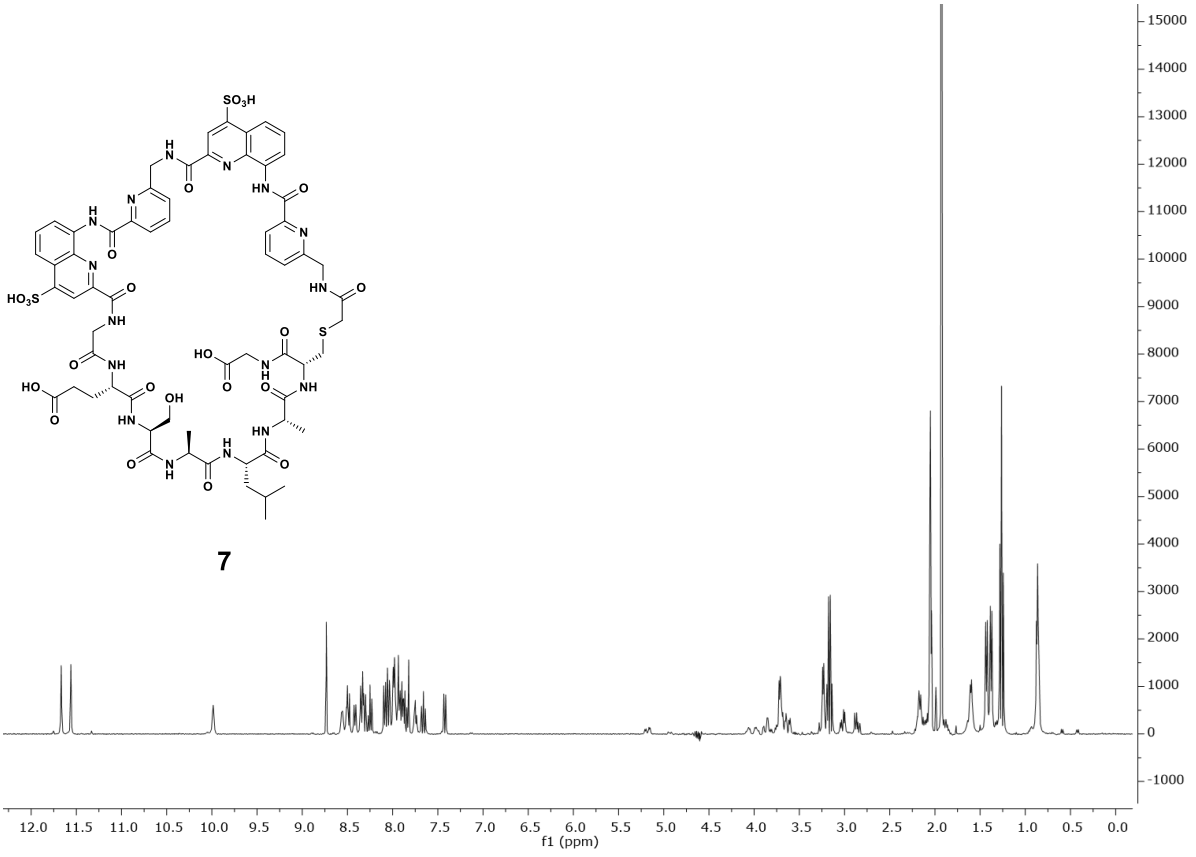
Compound **7**.




Semi-preparative RP-HPLC profile of **7** crude from SPS. (5 to 20 vol. % D over 20 min, 25 °C, λ=300 nm)



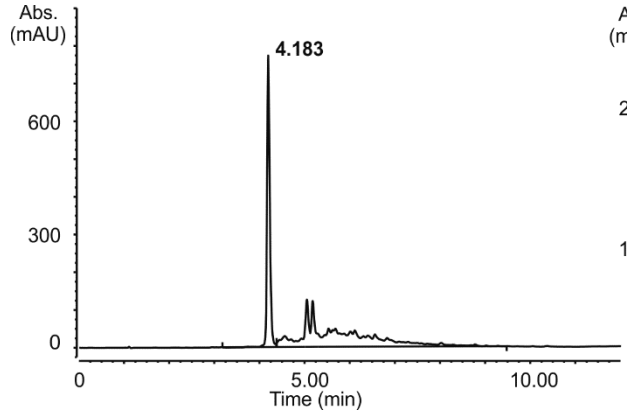

Analytical RP-HPLC profile of **7**. (10 to 30 vol. % D over 10 min, 25 °C, λ=254 nm)



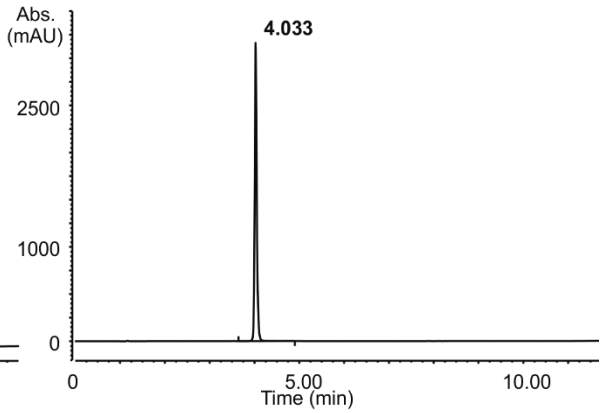

**7**

$^1$H NMR Spectrum (400 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile $d_3$, water suppression, 25 °C) of **7**.

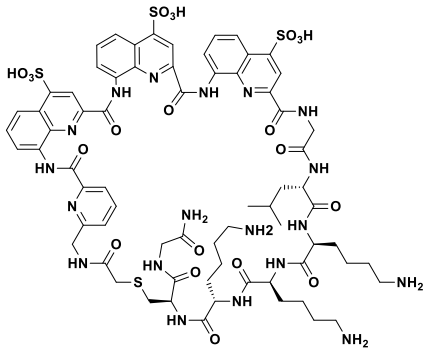
Compound **8**.




Analytical RP-HPLC profile of **8** crude from SPS. (10 to 45 vol. % B over 10 min, 25 °C, λ=254 nm)




Analytical RP-HPLC profile of **8**. (10 to 45 vol. % B over 10 min, 25 °C, λ=254 nm)

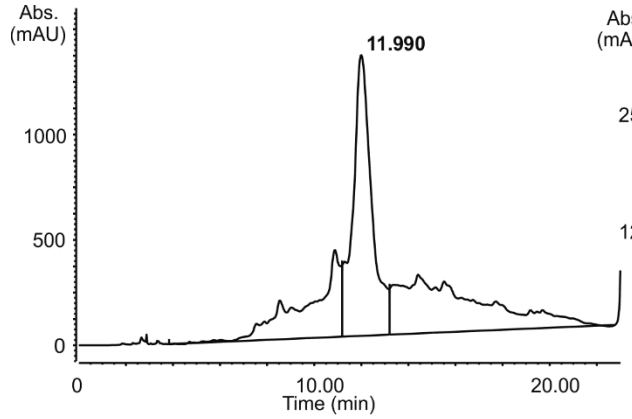


**8**

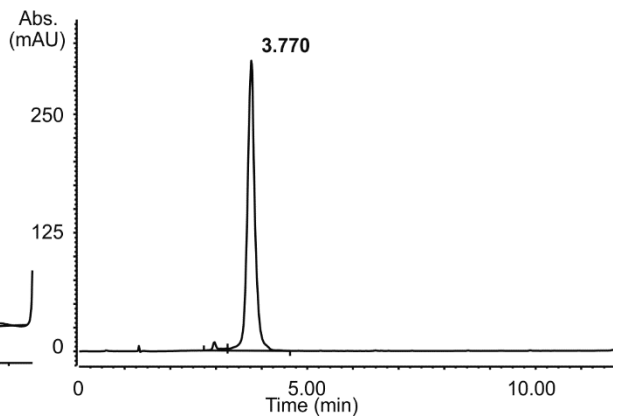



$^1$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile $d_3$, water suppression, 25 °C) of **8**.

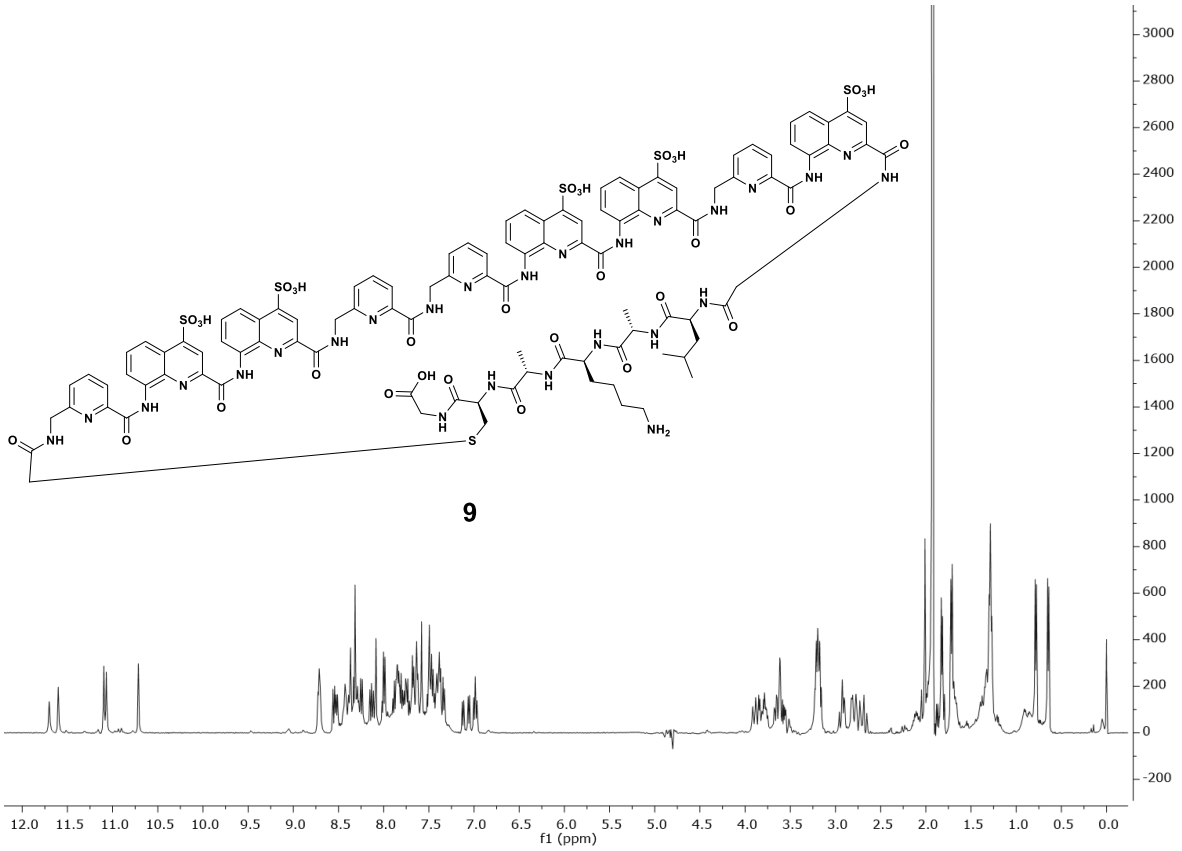
Compound **9**.




Semi-preparative RP-HPLC profile of **9** crude from SPS. (8 to 25 vol. % D over 20 min, 25 °C, λ=300 nm)



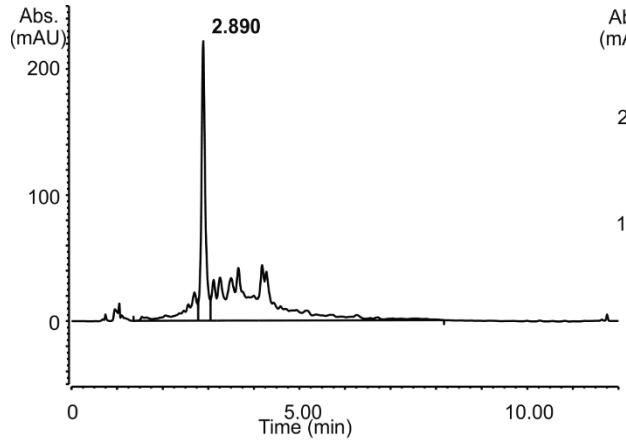

Analytical RP-HPLC profile of **9**. (5 to 50 vol. % D over 10 min, 25 °C, λ=254 nm)



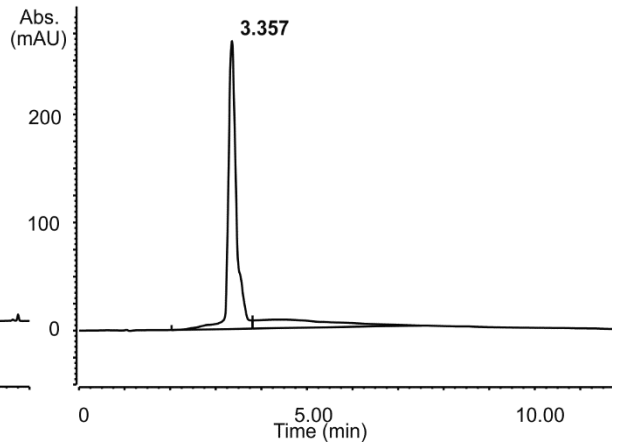

**9**

$^1$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 10% $D_2O$, water suppression, 25 °C) of **9**.

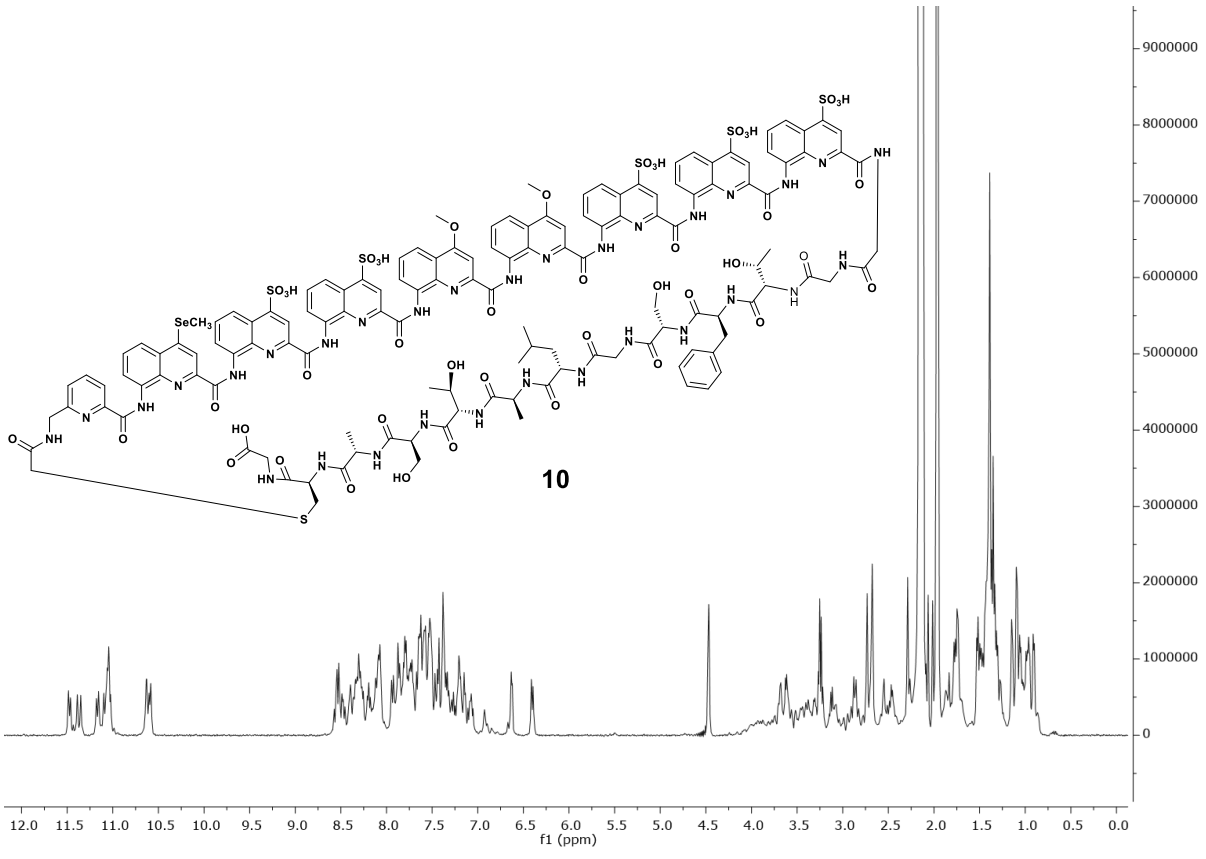
Compound **10**.




Analytical RP-HPLC profile of **10** crude from SPS. (10 to 45 vol. % D over 10 min, 50 °C, λ=254 nm)

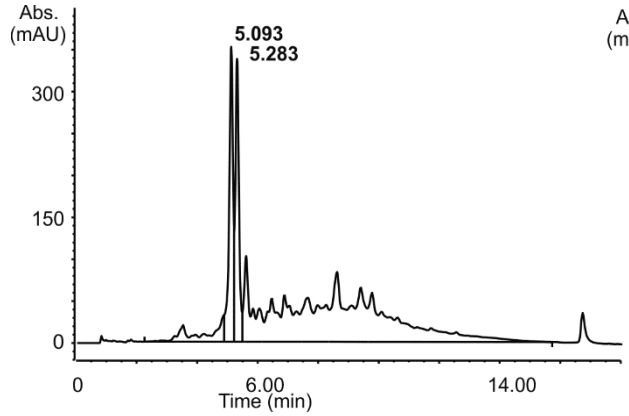



Analytical RP-HPLC profile of **10**. (10 to 80 vol. % D over 10 min, 50 °C, λ=254 nm)

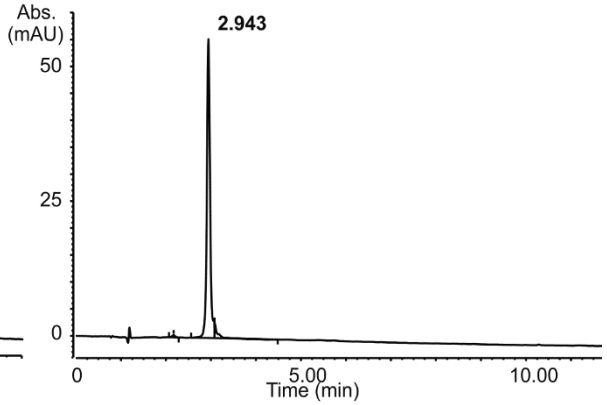



$^{1}$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 50% acetonitrile $d_3$, water suppression, 25 °C) of **10**.

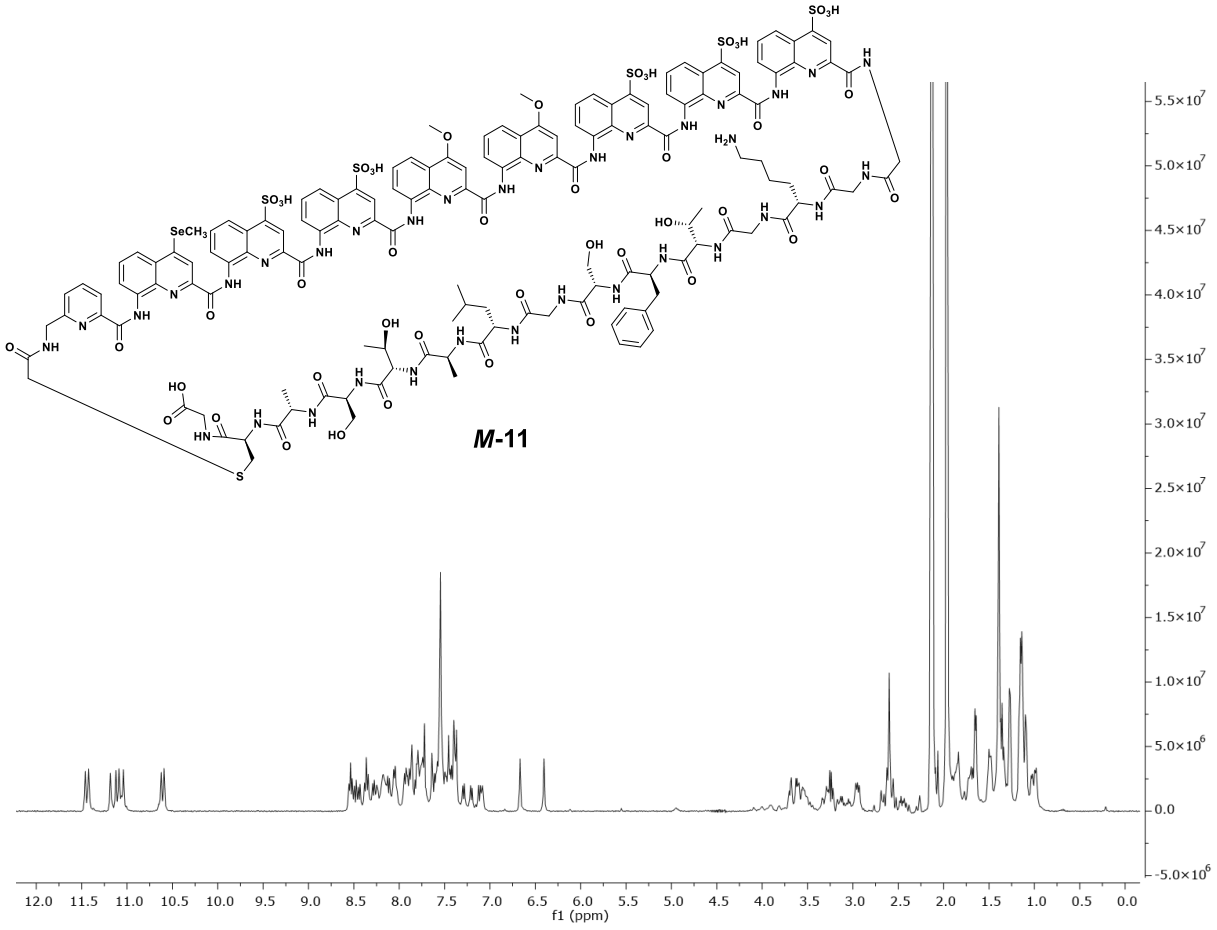
Compound ***M*-11**.




Semi-preparative RP-HPLC profile of **11** crude from SPS. (10 to 35 vol. % D over 15 min, 50 °C, λ=300 nm)

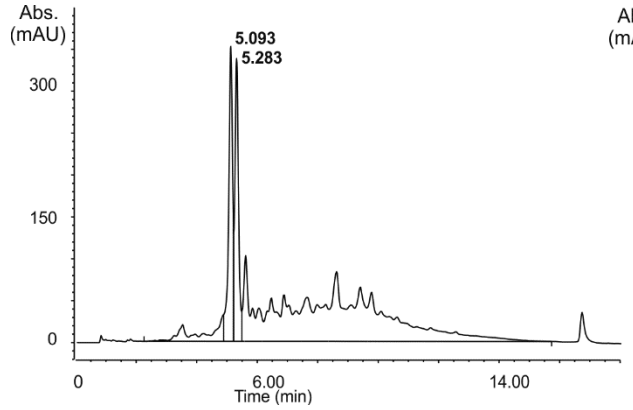



Analytical RP-HPLC profile of ***M*-11**. (10 to 45 vol. % D over 10 min, 50 °C, λ=300 nm)

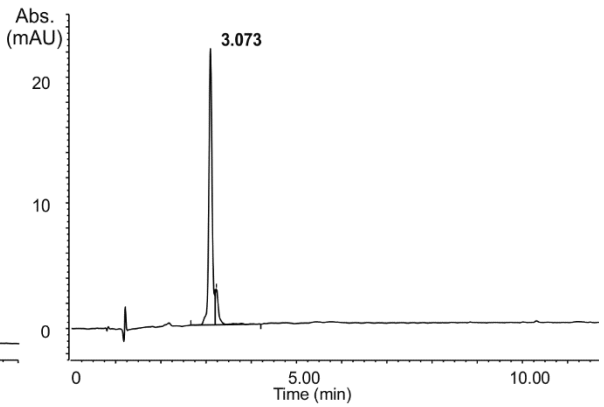



$^{1}$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 50% acetonitrile $d_3$, water suppression, 25 °C) of ***M*-11**.

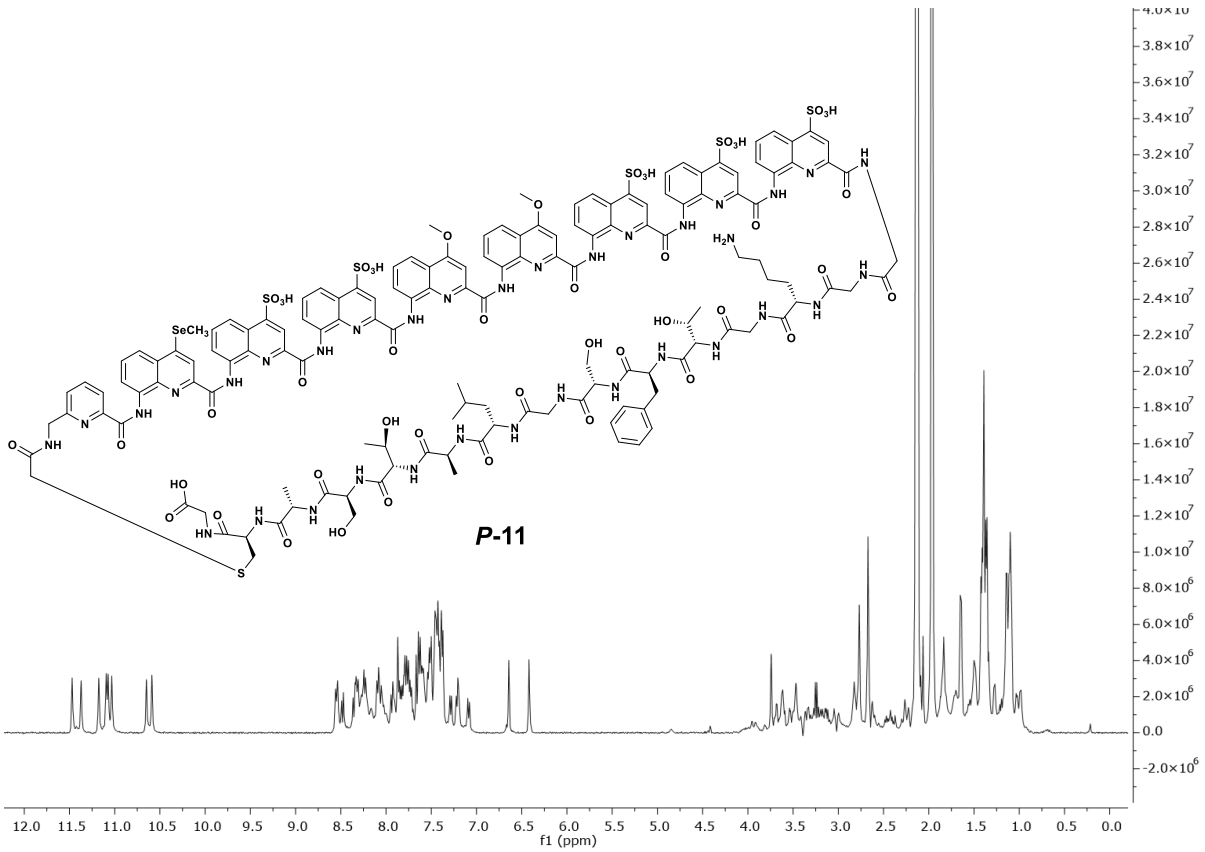
Compound ***P*-11**.




Semi-preparative RP-HPLC profile of **11** crude from SPS. (10 to 35 vol. % D over 15 min, 50 °C, λ=300 nm)

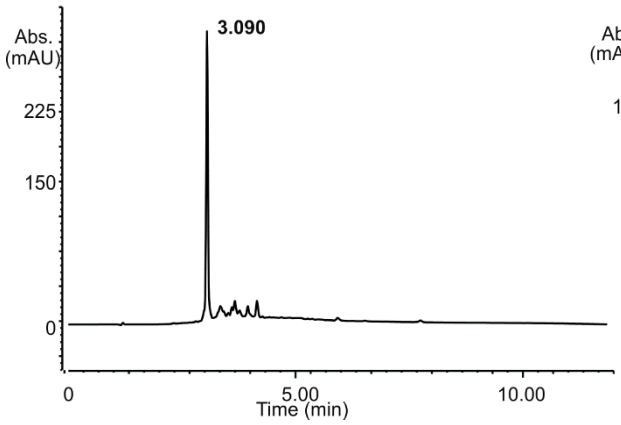



Analytical RP-HPLC profile of ***P*-11**. (10 to 45 vol. % D over 10 min, 50 °C, λ=300 nm)

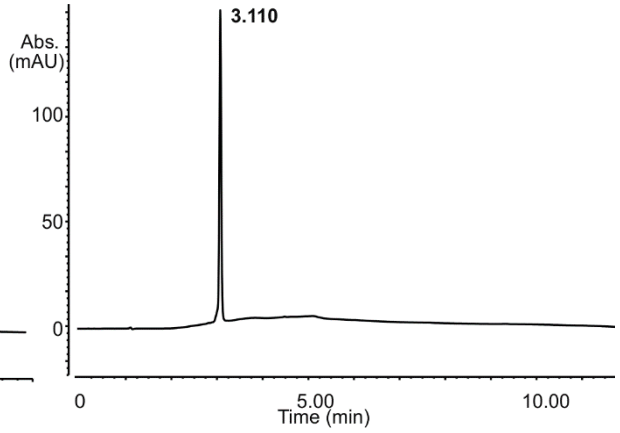



$^1$H NMR Spectrum (500 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 50% acetonitrile $d_3$, water suppression, 25 °C) of ***P*-11**.

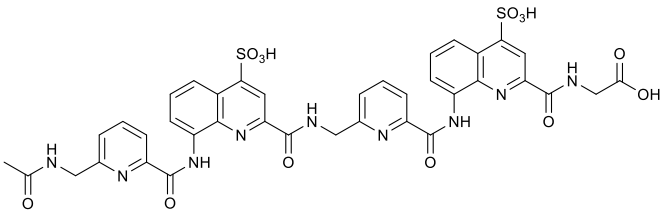
Compound **12**.




Analytical RP-HPLC profile of **12** crude from SPS. (10 to 100 vol. % D over 10 min, 25 °C, λ=300 nm)




Analytical RP-HPLC profile of **12**. (10 to 100 vol. % D over 10 min, 25 °C, λ=300 nm)

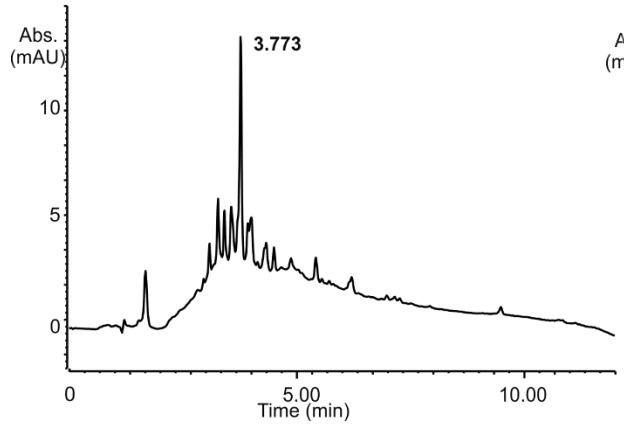


**12**

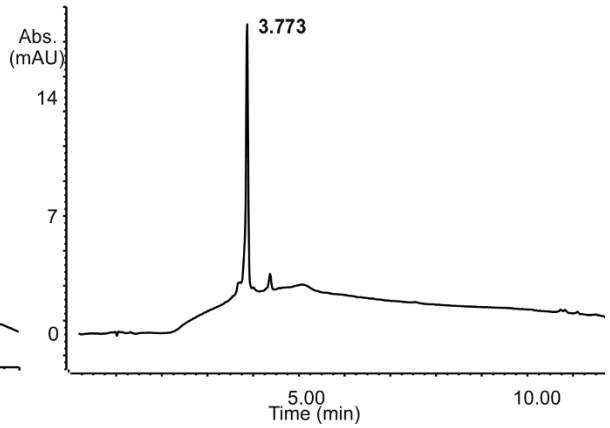



$^1$H NMR Spectrum (400 MHz, 50 mM $NaHCO_3$ buffer with 10% $D_2O$, water suppression, 25 °C) of **12**.

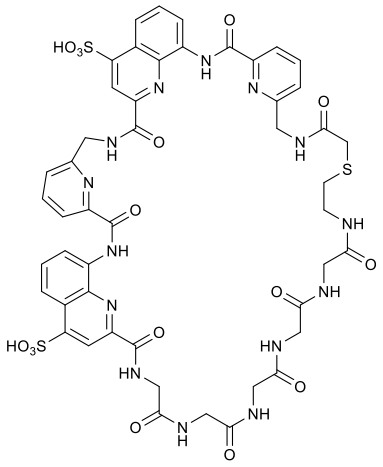
Compound **13**.




Analytical RP-HPLC profile of **13** crude from SPS. (10 to 100 vol. % D over 10 min, 25 °C, λ=300 nm)




Analytical RP-HPLC profile of **13**. (10 to 100 vol. % D over 10 min, 25 °C, λ=300 nm)

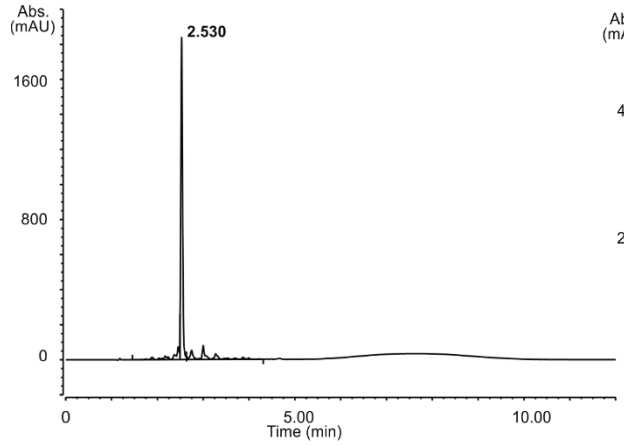


**13**

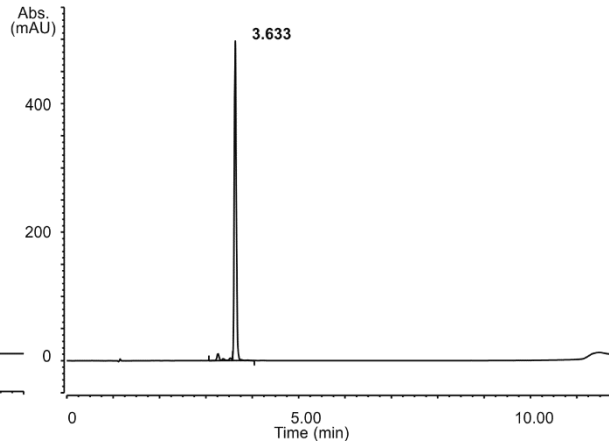



$^1$H NMR Spectrum (400 MHz, 12.5 mM ammonium acetate buffer at pH 8.5 with 25% acetonitrile $d_3$, water suppression, 25 °C

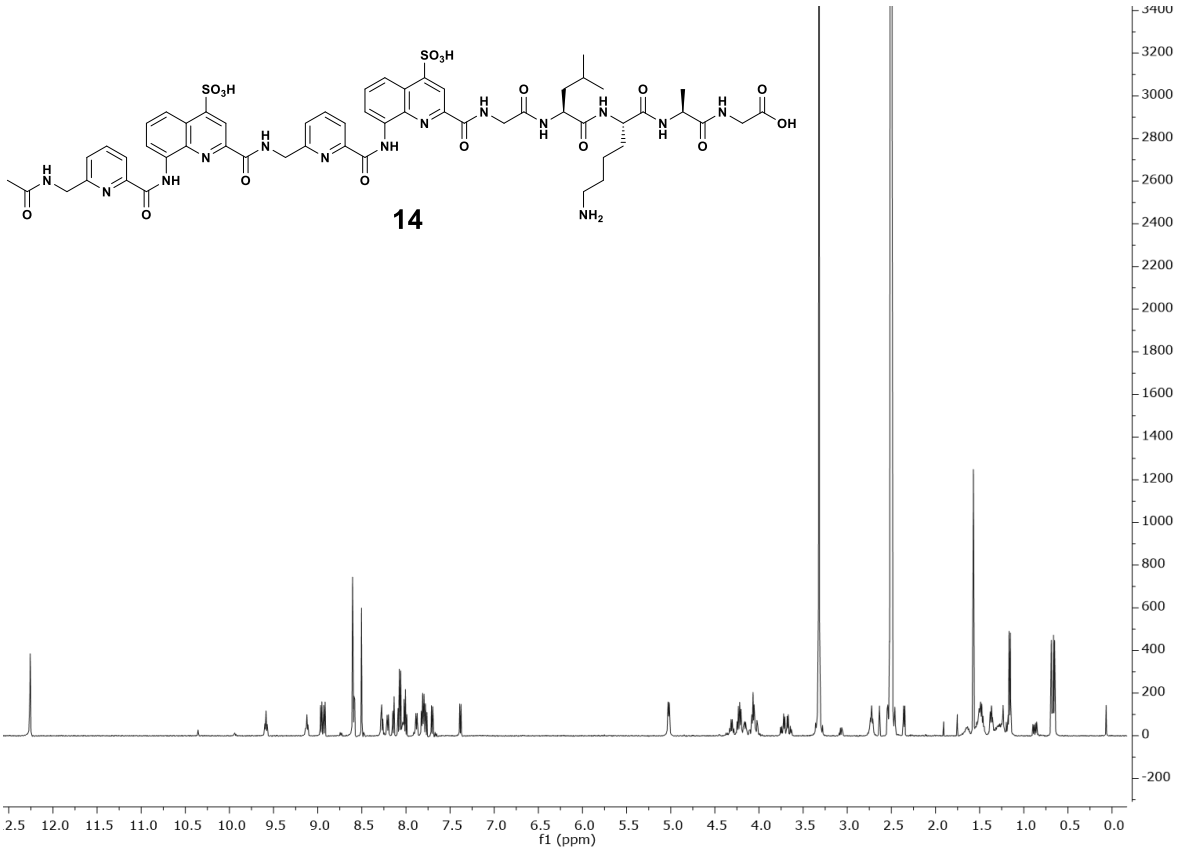
Compound **14**.




Analytical RP-HPLC profile of **14** crude from SPS. (10 to 100 vol. % D over 15 min, 25 °C, λ=300 nm)

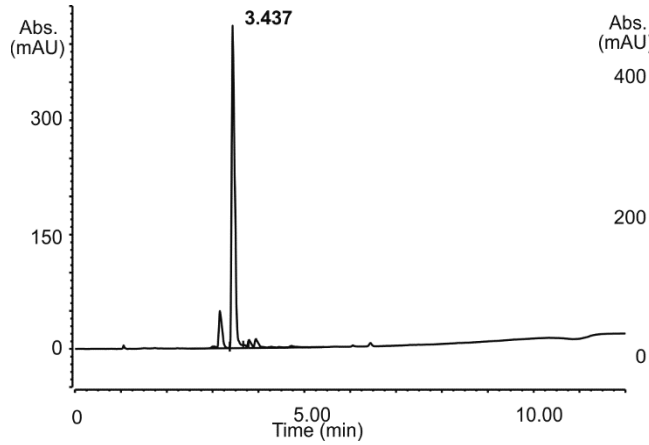



Analytical RP-HPLC profile of **14**. (10 to 45 vol. % D over 10 min, 25 °C, λ=300 nm)

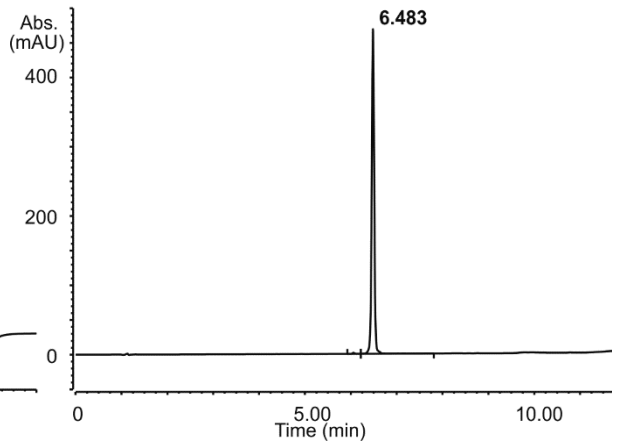



$^{1}$H NMR Spectrum (500 MHz, DMSO-$d_6$, 25 °C) of **14**.

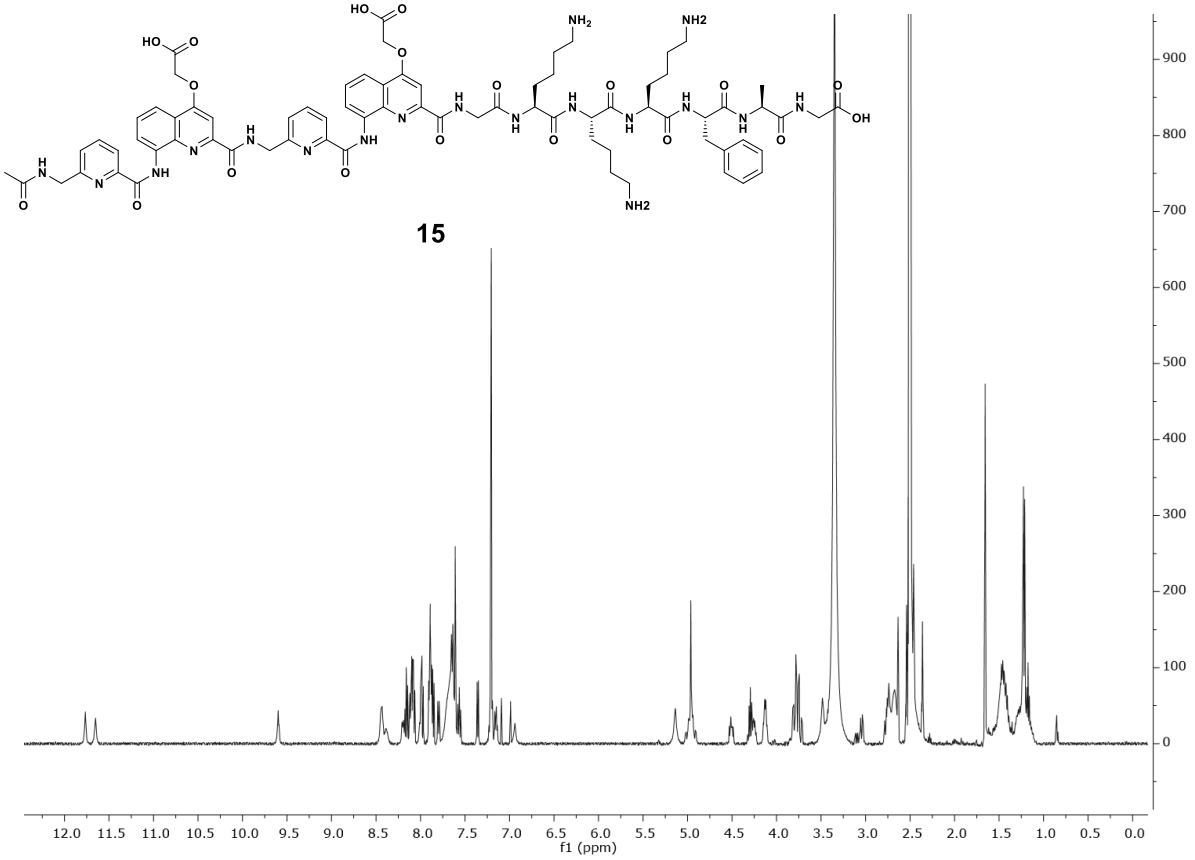
Compound **15**.




Analytical RP-HPLC profile of **15** crude from SPS. (10 to 100 vol. % B over 10 min, 50 °C, λ=254 nm)

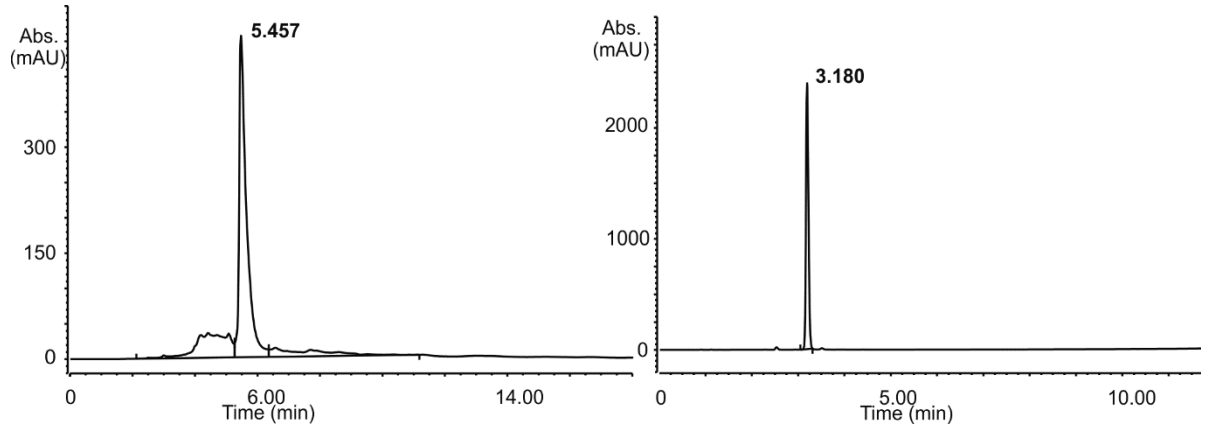



Analytical RP-HPLC profile of **15**. (10 to 45 vol. % B over 10 min, 50 °C, λ=254 nm)



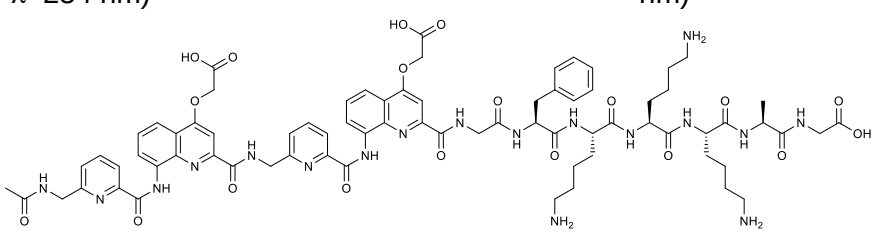

$^{1}$H NMR Spectrum (500 MHz, DMSO-$d_6$, 25 °C) of **15**.

Compound **16**.




Analytical RP-HPLC profile of **16** crude from SPS. (20 to 50 vol. % D over 10 min, 50 °C, λ=254 nm)

Analytical RP-HPLC profile of **16**. (20 to 80 vol. % B over 10 min, 50 °C, λ=254 nm)

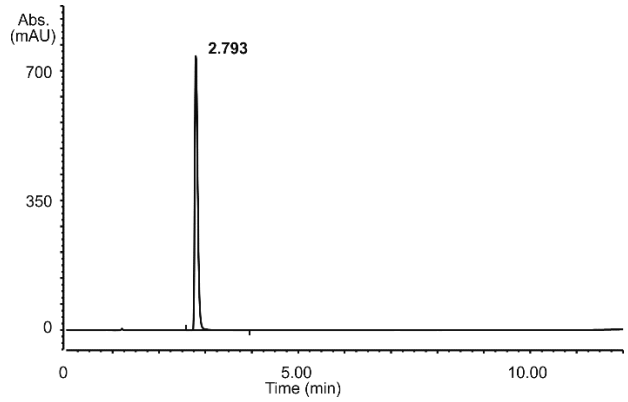


**16**

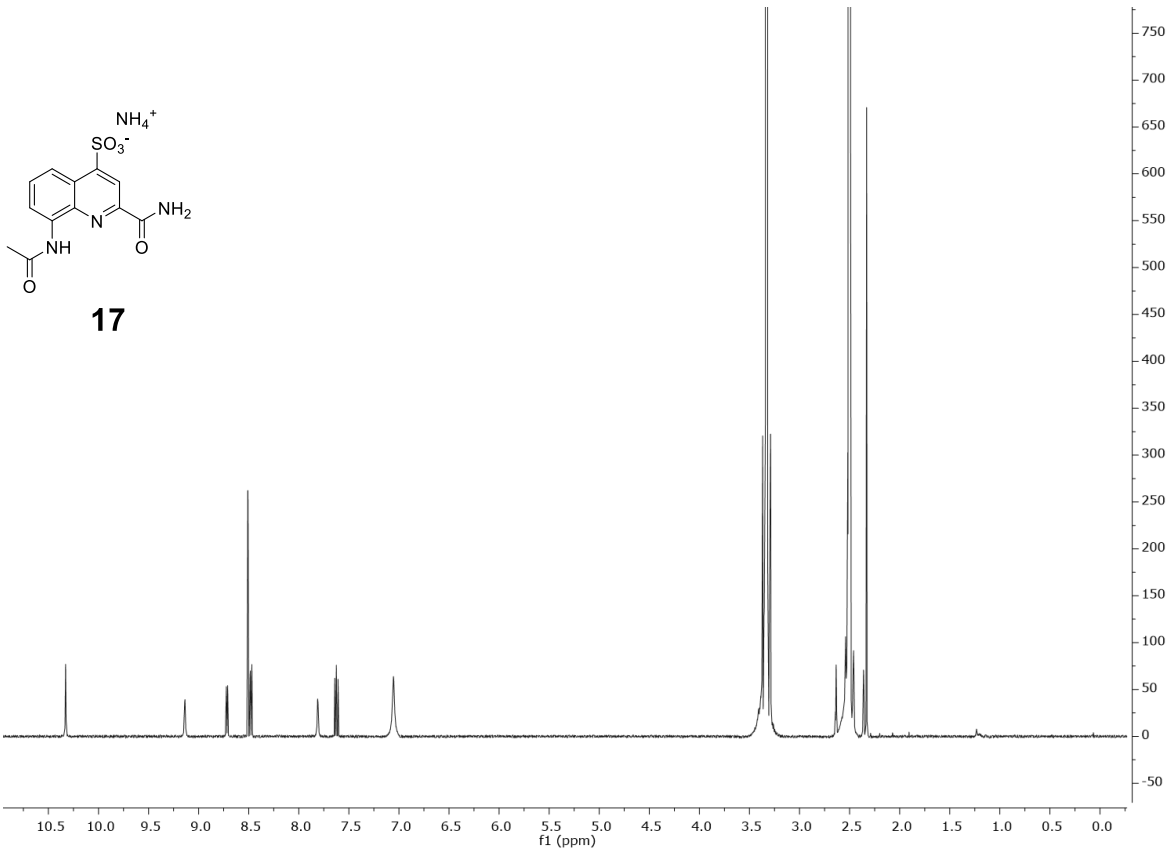



$^{1}$H NMR Spectrum (500 MHz, DMSO-$d_6$, 25 °C) of **16**.

Compound **17**.




Analytical RP-HPLC profile of **17**. (5 to 50 vol. % D over 10 min, 50 °C, λ=254 nm)




$^{1}$H NMR Spectrum (500 MHz, DMSO-$d_6$, 25 °C) of **17**.