Supporting Information

Discovery, crystal structure and atomic force microscopy study of thioether ligated D,L-cyclic antimicrobial peptides against multidrug resistant *Pseudomonas aeruginosa*

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Table S1: Resistance profile of *P. aeruginosa* clinical isolates: R = resistant, I = intermediate, S = susceptible (classification according to CLSI guidelines), diam. = zone diameter in mm of a disk diffusion assay. Susceptibility testing was performed by the Clinical Bacteriology Laboratory of the Geneva University Hospitals.

	PEJ-2.6 S/R	diam.	PEJ-9.1 S/R	diam.
Piperacillin	R	13	I	16
Piperacillin/Tazobactam	R	14	S	22
Ceftazidime	S	18	S	22
Cefepime	S	22	S	22
Imipenem	R	10	R	8
Meropenem	R	15	R	13
Aztreonam	S	24	S	29
Amikacin	S	18	S	18
Gentamicin	R	11	R	12
Tobramycin	S	20	S	21
Norfloxacin	S	18	R	8
Ciprofloxacin	S	22	R	8
Trimethoprim + Sulfamides	R	10	R	11
Polymyxin B	S	20	S	20

	ZEM-1A S/R	diam.	ZEM-9A S/R	diam.
Piperacillin	R	12	I	16
Piperacillin/Tazobactam	R	13	I	18
Ceftazidime	R	6	S	20
Cefepime	R	6	R	14
Imipenem	R	10	R	6
Meropenem	R	8	R	6
Aztreonam	R	6	R	9
Amikacin	S	18	R	6
Gentamicin	S	17	R	7
Tobramycin	S	22	S	17
Norfloxacin	R	10	R	6
Ciprofloxacin	R	13	R	10
Trimethoprim + Sulfamides	R	6	R	6
Polymyxin B	S	21	S	15

Membrane interaction experiment

Preparation: a thin lipid film was prepared by evaporating a solution of 25 mg Egg PG in 1 mL MeOH/$CHCl_3$ 1/1 on a rotary evaporator at room temperature and then in vacuo overnight. The resulting film was hydrated with 1 mL buffer (50 mM CF, 10 mM TIRS, 10 mM NaCl, pH 7.4) for 30 min, subjected to freeze-thaw cycles (7x) and extrusion (15x) through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by gel filtration (Sephadex G-50) with 10 mM TRIS, 107 mM NaCl, pH 7.4. Final conditions: ~ 2.5 mM Egg PG; inside: 50 mM CF, 10 mM TIRS, 10 mM NaCl, pH 7.4; outside: 10 mM TRIS, 107 mM NaCl, pH 7.4.

Experiment: Egg PC or Egg PG stock solutions (37.5 µL) were diluted with a buffer (10mM TRIS buffer, 107 mM NaCl, pH 7.4) and placed in a thermostated fluorescence cuvette and gently stirred (total volume in the cuvette ~3000 µL; final lipid concentration ~31 µM). CF efflux was monitored at λ_{em} 517nm as a function of time after addition of 20µL of peptide in buffer with final concentrations of 1, 5, 7.5, 10, 15, 20 µg/mL at time= 50 seconds and 1.2% Triton X-100 (30 µL, 0.012% final concentration) at time= 300 seconds.
Fluorescence intensities were normalized to fractional emission intensity I(t) using I(t) = (It - I0) / (I∞ - I0) where I0 It at peptide dendrimer addition, I∞ = It at saturation of lysis.

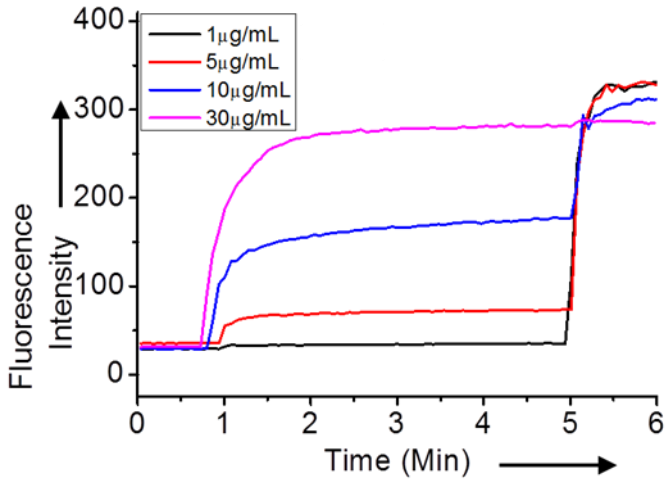


Figure S1. 5(6)-Carboxyfluorescein leakage from phosphatidylglycerol lipid vesicles. Addition of **RH11** to a lipid vesicle solution in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) at 50 s and addition of 1.2% Triton X-100 at 300 s.

Transmission Electron Microscopy (TEM)

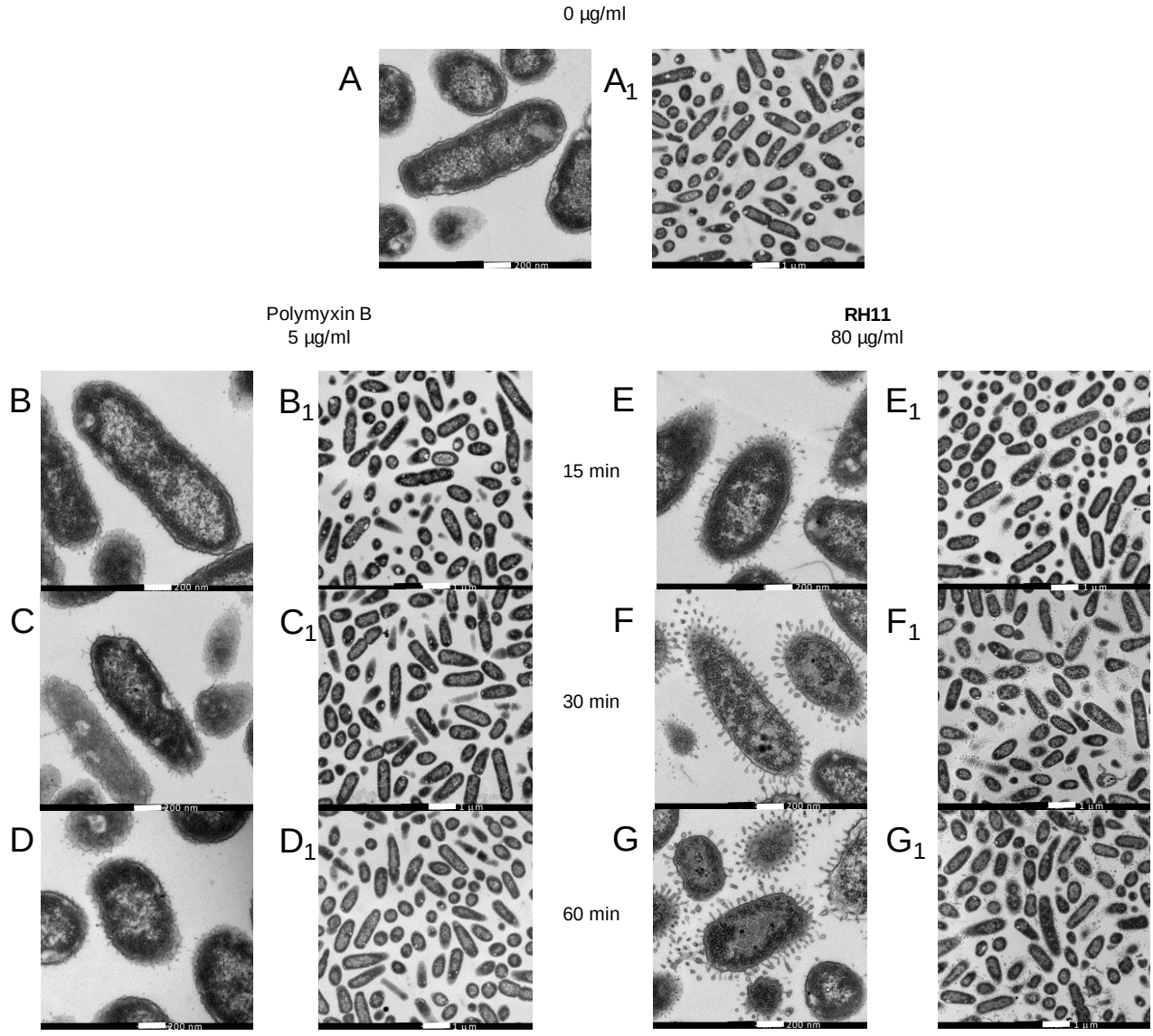


Figure S2. RH11 as membrane disruptive compound similarly to Polymyxin B. TEM pictures of untreated and treated *Pseudomonas aeruginosa*. Perturbations were observed on bacterial surfaces. **A and A_1.** Untreated PAO1**. B and B_1.** Polymyxin B treatment for 15 min. **C and C_1.** Polymyxin B treatment for 30 min. **D and D_1.** Polymyxin B treatment for 60 min. **E and E_1. RH11** treatment for 15 min. **F and F_1. RH11** treatment for 30 min. **G and G_1. RH11** treatment for 60 min.

Dynamic Light Scattering

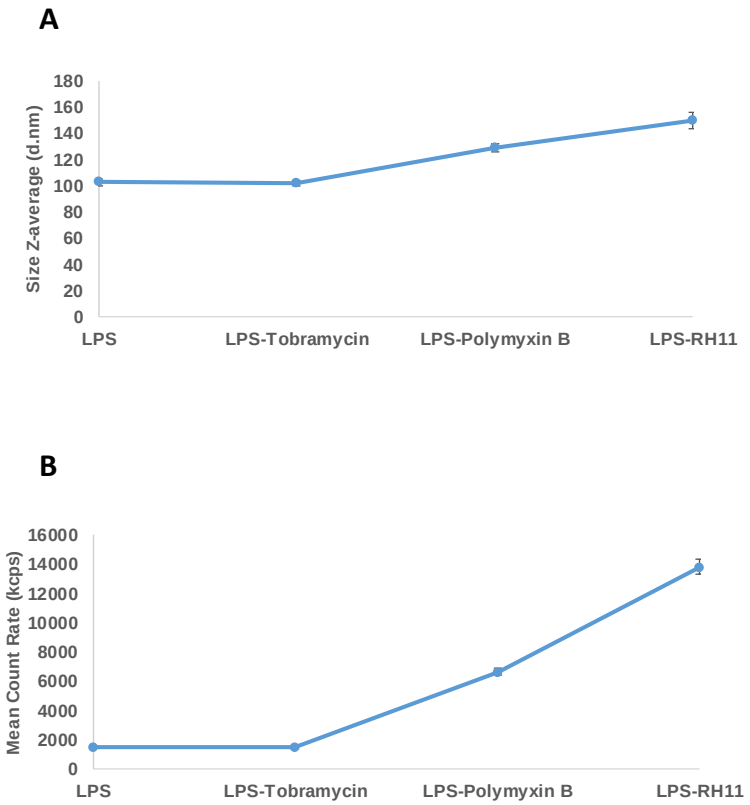


Figure S3. Changes of the hydrodynamic diameter of LPS aggregates in the presence of the compound of interest by using dynamic light scattering. LPS and the compounds were measured at 100 µg/mL. Each sample was measured three times and the experiments were repeated three times. **A.** Size Z-average. **B.** Intensity Polydisperse Index (PDI). **C.** Mean Count Rate.

Atomic Force Microscopy (AFM)

AFM imaging was performed under ambient conditions in air with a Nanosurf easyscan 2 (Nanosurf AG, Switzerland). All measurements were carried out in tapping mode employing PPP-NCHR-W cantilevers from Nanosensors (resonance frequency ~280 kHz, tip radius ~10 nm). The mica substrates (20 × 20 mm^2) were attached to a steel baseplate with Scotch tape and freshly cleaved prior to each new experiment. Peptides (at concentration of 4*MIC) were incubated with bacterial cell (*B.subtilis* OD_{600} = 0.1) in NaCl 0.9% for 10 min. The mixed solution was deposited onto a freshly cleaved mica surface. The solution was removed by gentle rinsing with Milli-Q water after 5 min of incubation time. After dried with Argon gas, the substrate was analyzed with atom force microscopy. Peptide without bacterial treatment and bacterial without peptides were also analyzed by AFM as control.

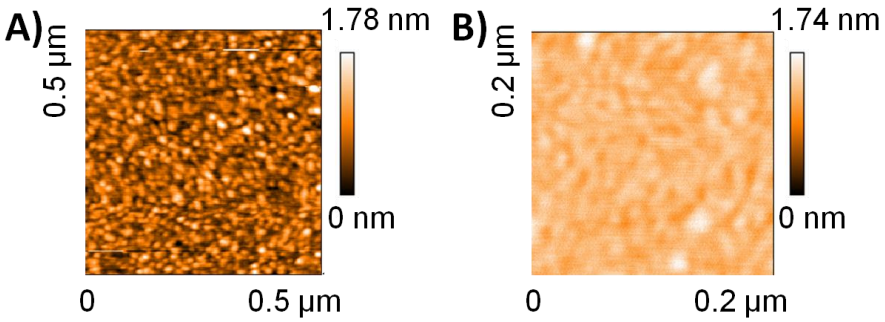


Figure S4. A) and B) are two examples of AFM image of **RH11** alone. Independent AFM of **RH11** analyses were measured more than 200 times, while no aggregated structure was observed.

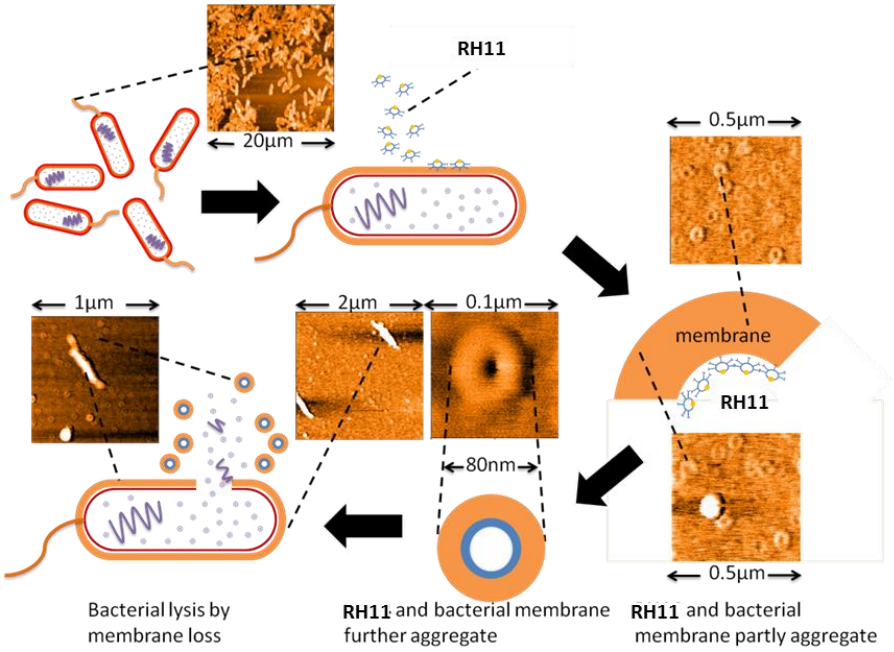


Figure S5. Proposed model of structures formed by aggregation of the peptide at the bacterial membrane followed by detachment when a critical size has been reached, leading to membrane lysis and cell death in the case of bacteria.

Crystallography

Table S2. Crystallographic data.

Structural data	**FdRH11o-**LecB	**FRH11o-**LecB	**FdRH11m-**LecB
Beam line	PXIII	PXIII	PXIII
Wavelength(Å)	1.000030	1.000030	1.036790
Resolution(Å)	47.03 - 1.27	47.43 - 1.55	47.75 - 1.60
Cell dimension			
Space group	P 21 21 21 (No. 19)	C 1 2 1 (No. 5)	P 1 (No. 1)
Unit cell(Å)	45.28, 99.97, 106.61, 90, 90, 90	94.93, 45.75, 103.85, 90, 114.99, 90	45.18, 48.50, 52.54, 84.92, 79.97, 80.58
Measured reflection/unique	399675/120478	121544/53265	91692/51266
Average multiplicity	3.31	2.23	1.78
Completeness (%)	94.1%	86.9%	90.6%
Average I/σ(I)	13.95	12.44	23.48
Correlation CC (1/2) (%)	99.9	99.8	99.9
Wilson B-factor	11.23	14.70	8.58
Refinement			
Resolution range (Å)	47.03 - 1.27	47.43 - 1.55	47.75 - 1.60
R_{work} (%)	0.1628	0.1847	0.1425
R_{free} (%)	0.1845	0.2173	0.1640
Average Biso (Å^2)	15.8	18.4	11.0
All atoms	4296	3828	4005
Solvent atoms	596	393	572
RMSD from ideality angles (°)	1.260	1.052	0.918
Bonds (Å)	0.011	0.008	0.007
Protein Data Bank deposition code	5NF0	5NEY	5NES

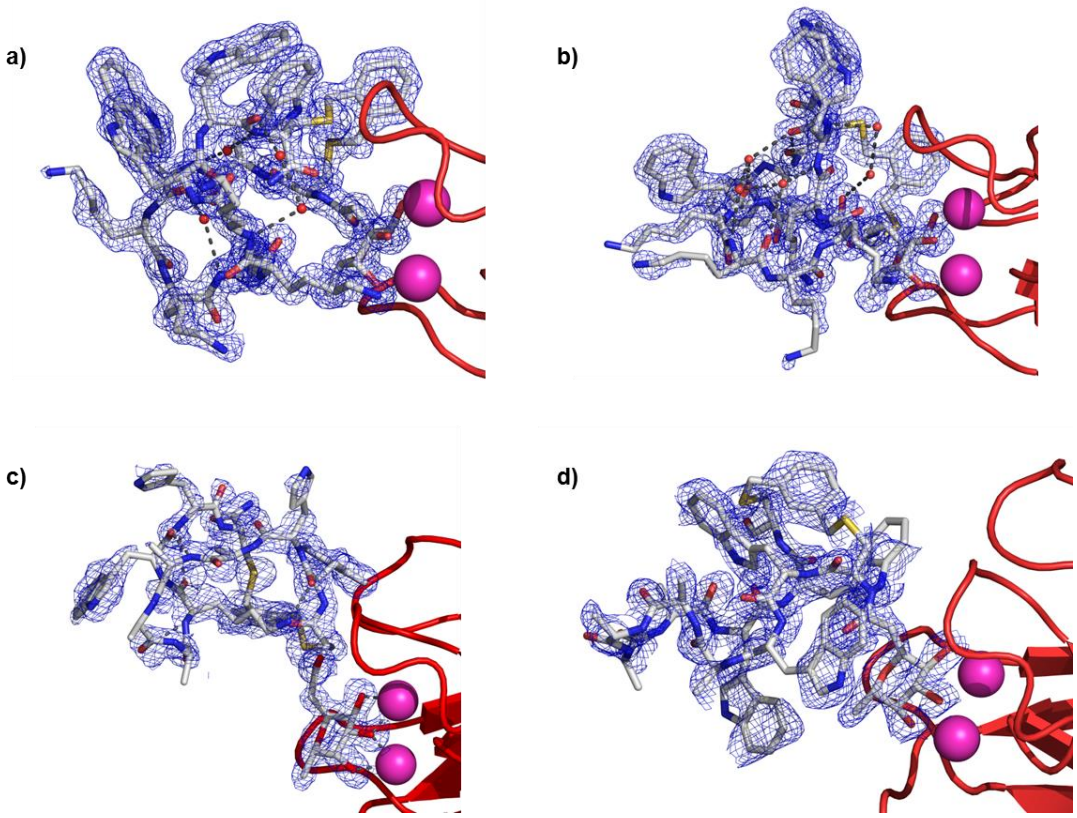


Figure S6. Structures of the different conformations of the monocyclic (in blue dash density). (a) FdRH11o (conformation 2), b) FdRH11o (conformation 3), c) FRH11o and d) FdRH11m.

Cheminformatics

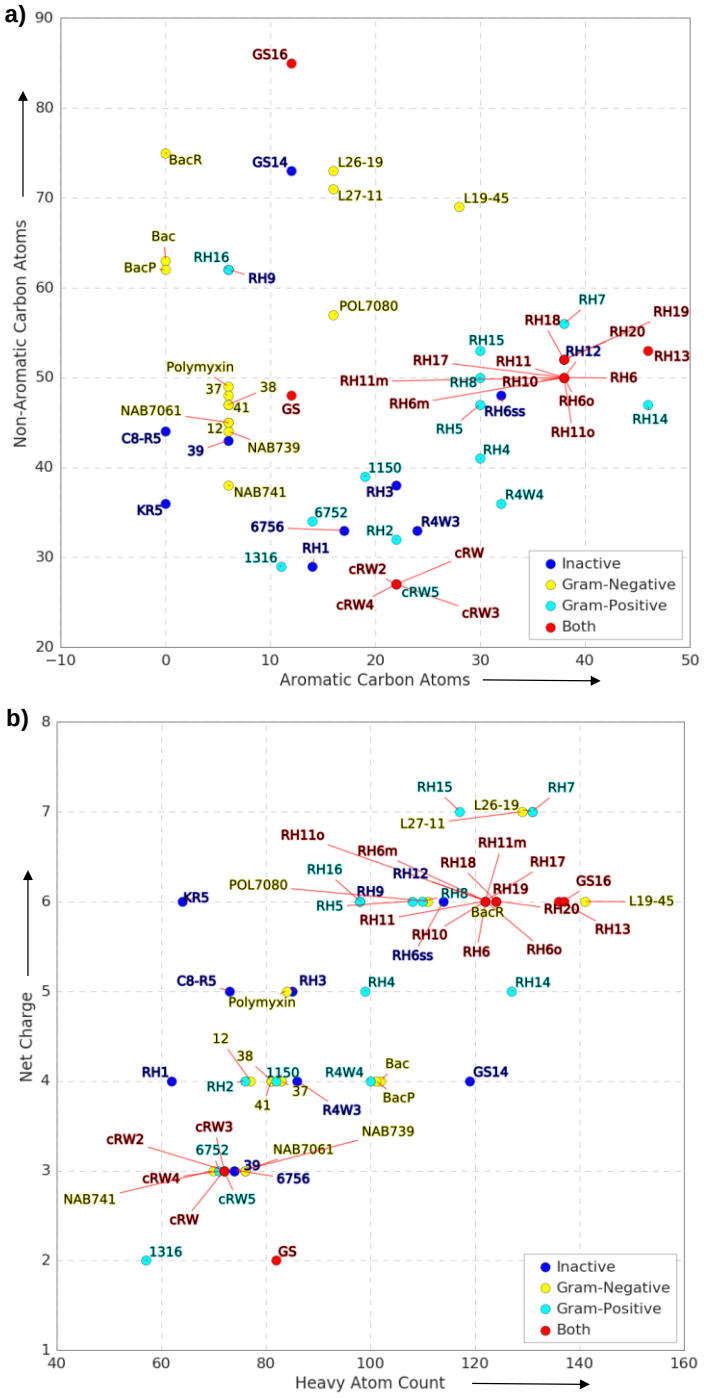
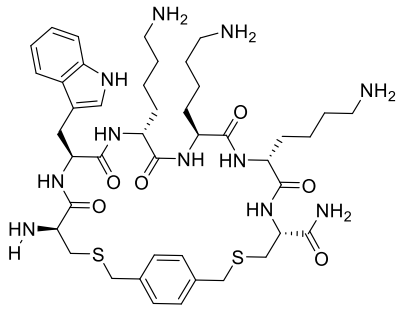
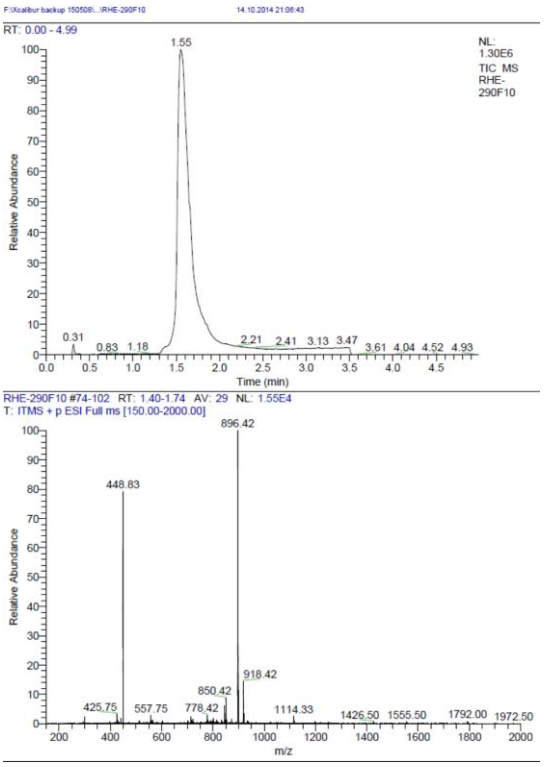


Figure S7. Molecular property analysis of CAMPs collected from the literature (see main text for refs.) and in this work. Compounds codes are given as in the original publications. Polymyxin analogs: 12, 41, 37, 38, 39, NAB7061, NAB739, NAB741; POL7080 analogs: L27-11, L26-19, L18-45; Gramicidin and analogs: GS, GS14, GS16; bactenecin and analogs: Bac, BacR, BacP; all other codes refer to either to antimicrobial cyclic hexa- to octa-peptides, or to CAMPs from this work. Activities are color-coded as inactive (blue) Gram-negative (MIC ≤ 16 µg/mL on *P. aeruginosa* or *E. coli*, yellow), Gram-positive (MIC ≤ 8 µg/mL on *S. aureus* or *B. subtilis*, cyan), or both Gram+ and Gram- (red). The list of cpds with SMILES and compound code is provided in the supporting information. **(a)** Scatter plots of the non-aromatic carbon count versus aromatic carbon count. **(b)** Scatter plot of positive charges versus molecular size (measured as heavy atom count: all non-hydrogen atoms).

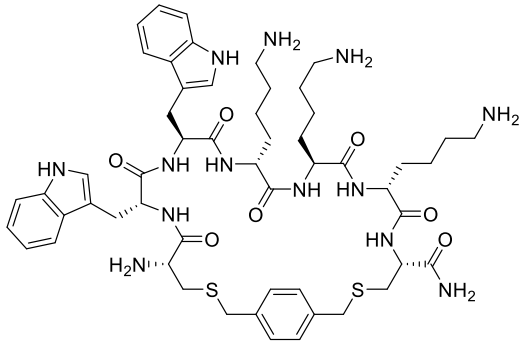
Compound characterization

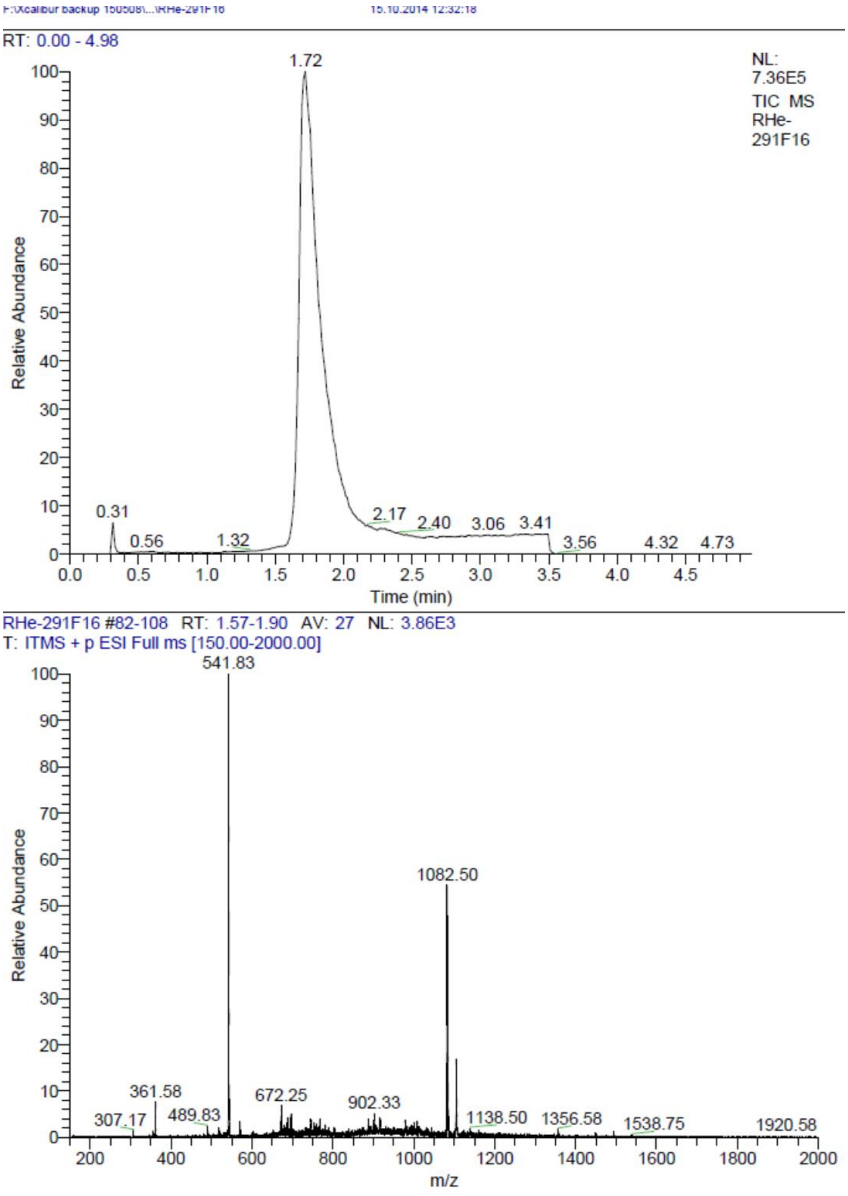
RH1. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH1** was obtained as white solid after preparative RP-HPLC purification (11.0 mg, 12.3 %). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min) Analytical RP-HPLC: t_R = 1.55 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{43}H_{65}N_{11}O_6S_2$ $[M+H]^+$: 896.46, found: 896.42, $[M+Na]^+$: 918.45, found: 918.42.



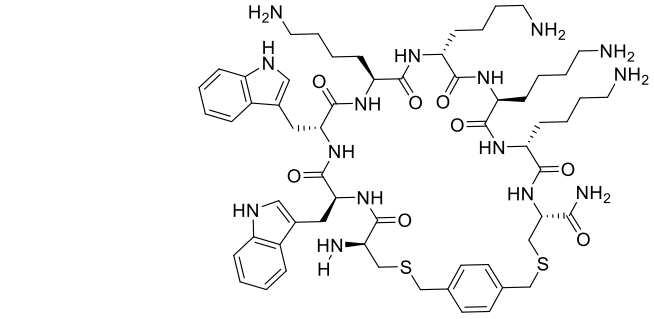


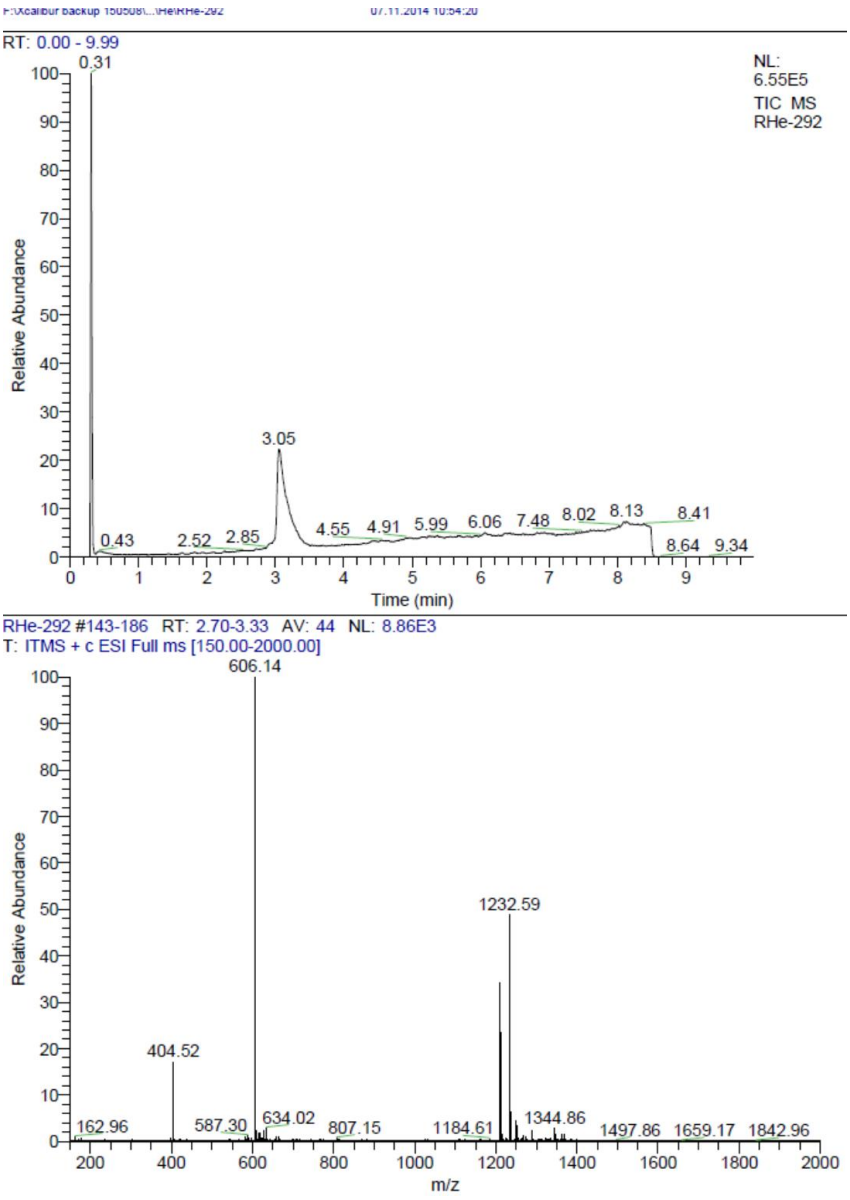
RH2. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH2** was obtained as white solid after preparative RP-HPLC purification (2.2 mg, 2.0 %). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min)**.** Analytical RP-HPLC: t_R = 1.72 min (A/D = 100/0 to 0/100 in 5min, 1.2 mL/min)**.** MS (ESI+) calculated for $C_{54}H_{75}N_{13}O_7S_2$ $[M+H]^+$: 1082.54, found: 1082.50.



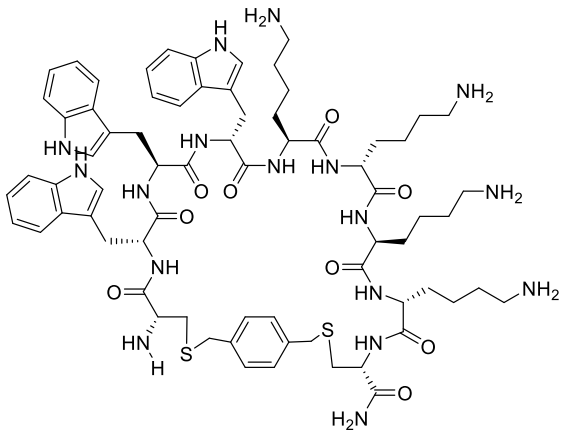


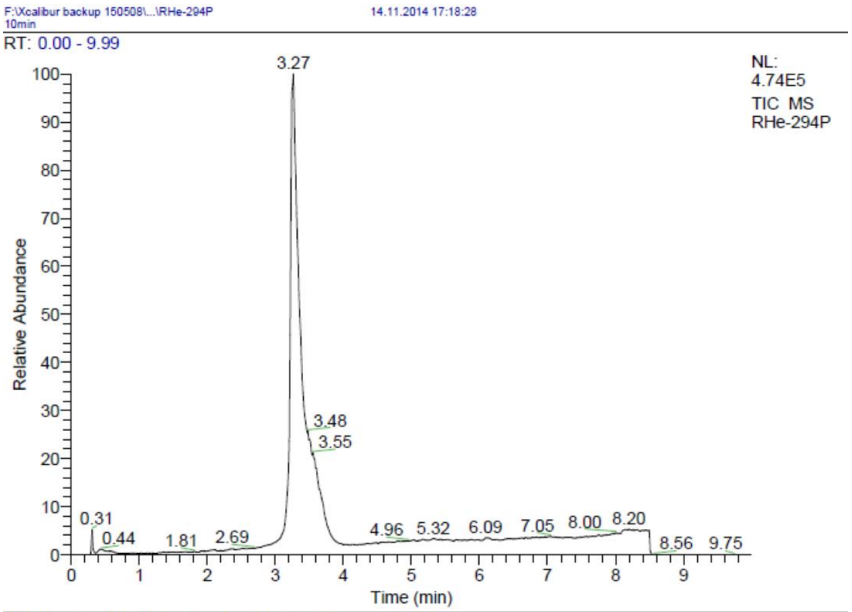
RH3. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH3** was obtained as white solid after preparative RP-HPLC purification (1.8mg, 1.5 %). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.05 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{60}H_{87}N_{15}O_8S_2$ $[M+H]^+$: 1210.63, found: 1210.6, $[M+Na]^+$: 1232.53, found: 1232.59.

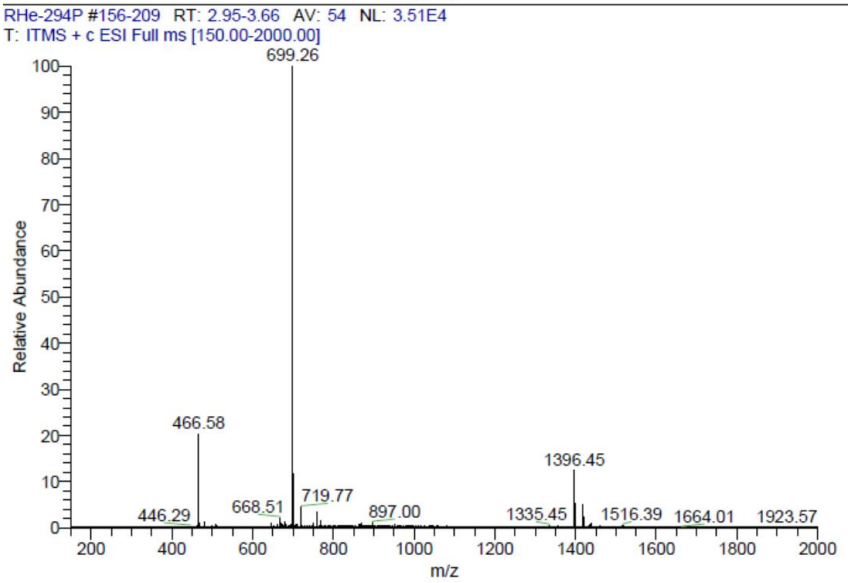




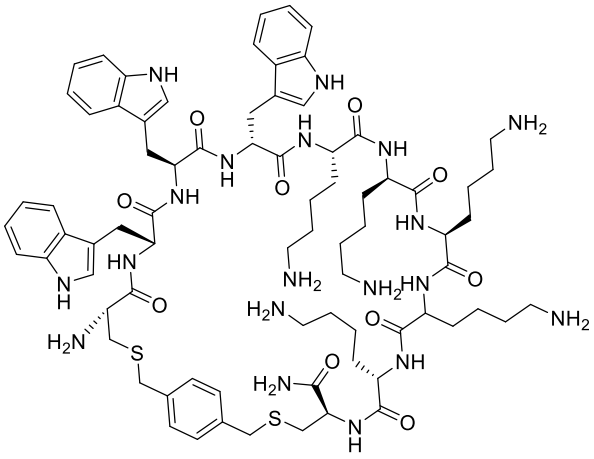
RH4. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH4** was obtained as white solid after preparative RP-HPLC purification (17.1mg, 12.2 %). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.27 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{71}H_{97}N_{75}O_9S_2$ $[M+H]^+$: 1396.71, found: 1396.45.

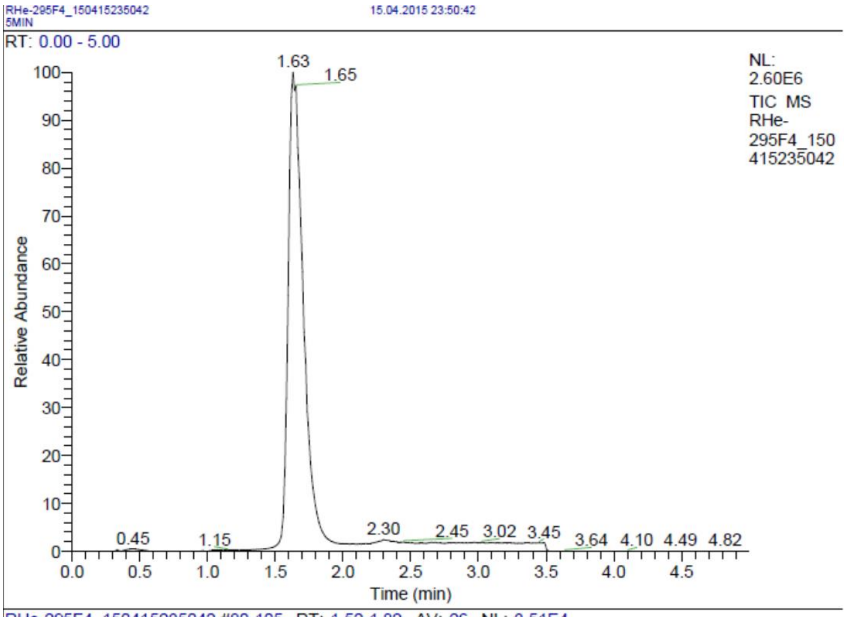




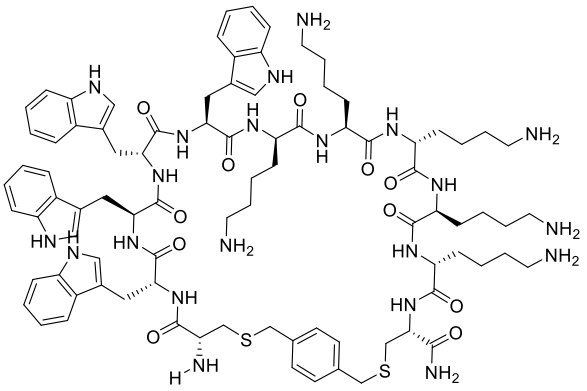


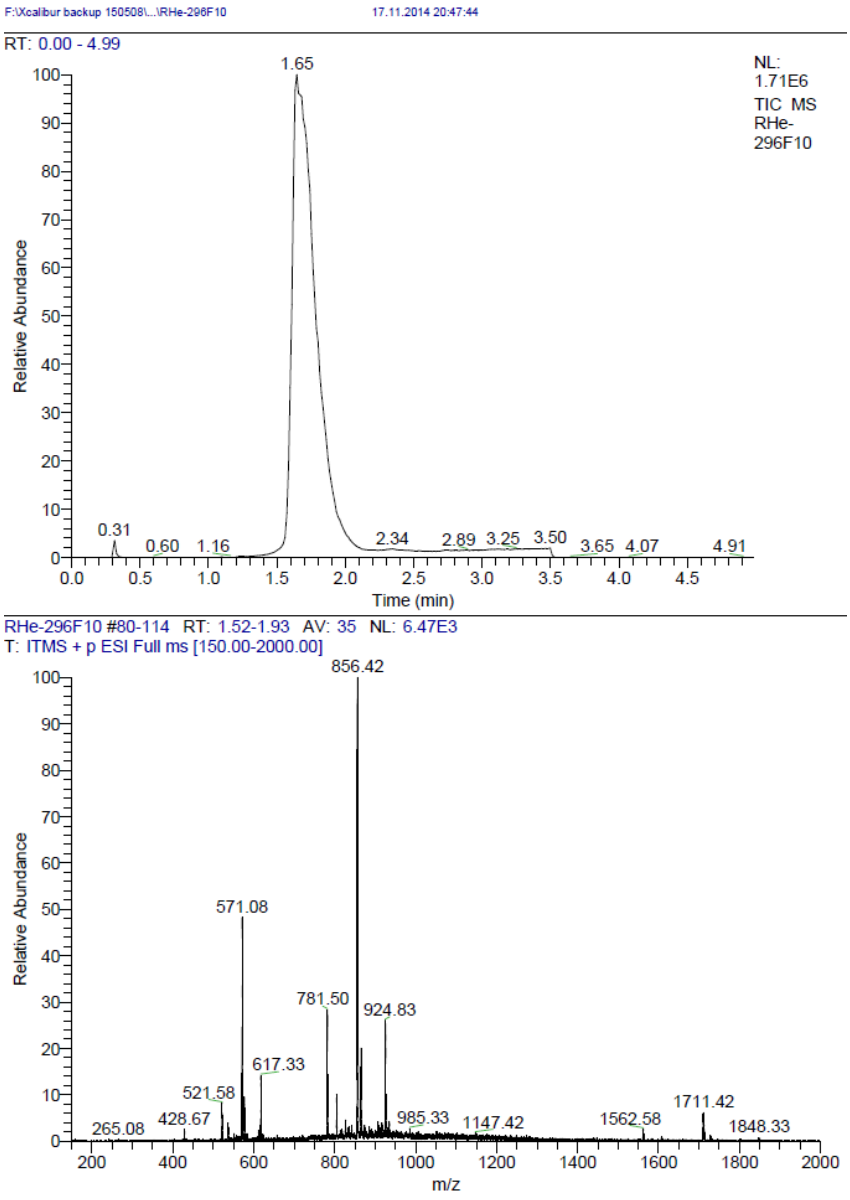
RH5. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH5** was obtained as white solid after preparative RP-HPLC purification (8.7mg, 5.6 %). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.63 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{77}H_{109}N_{19}O_{10}S_2$ $[M+H]^+$: 1524.80, found: 1524.33.



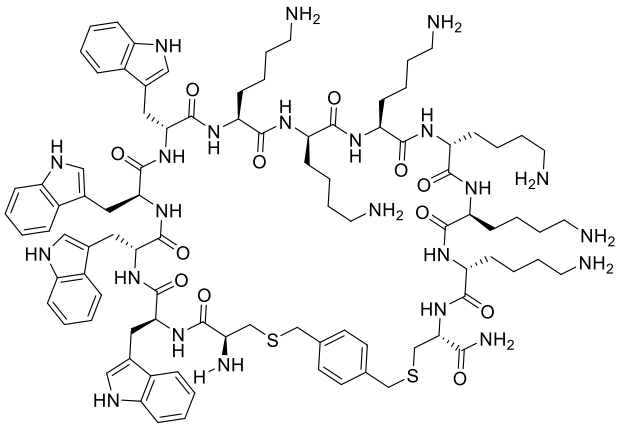


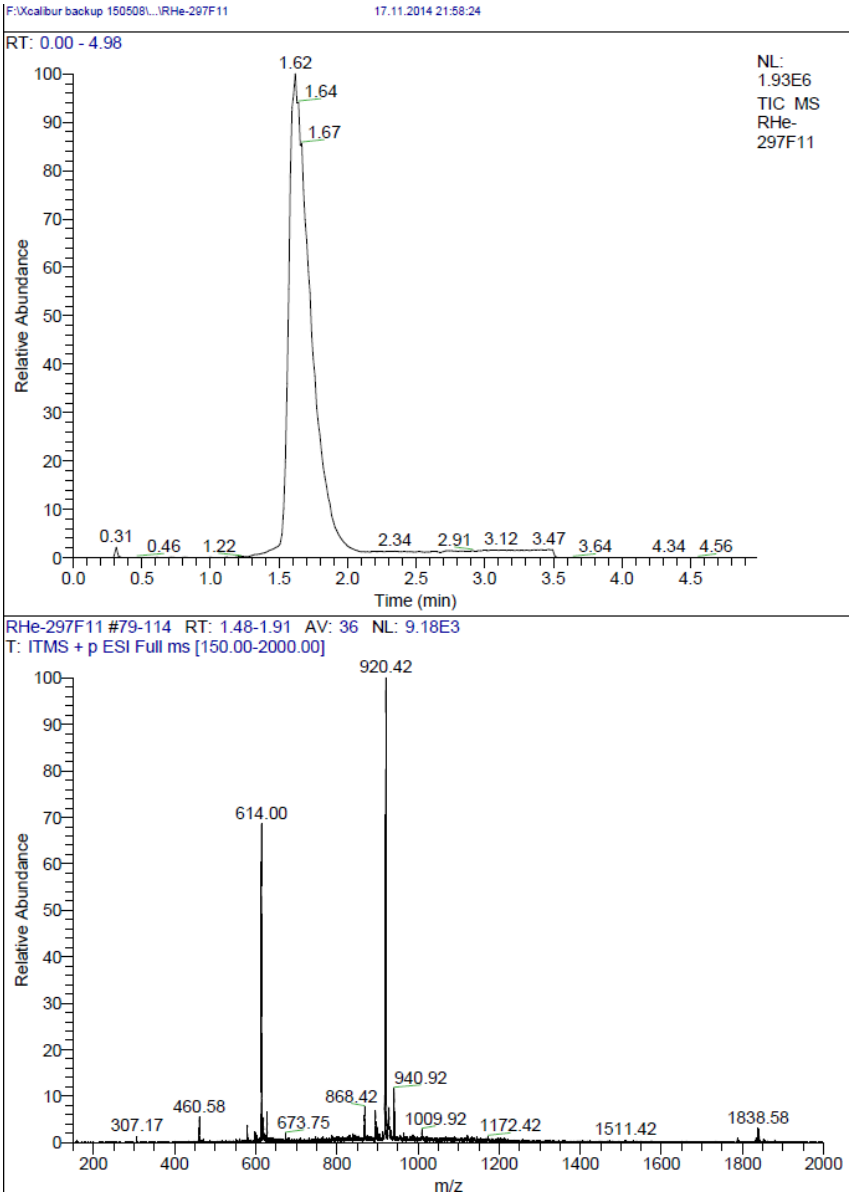
RH6. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH6** was obtained as white solid after preparative RP-HPLC purification (11.4mg, 6.7 %). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min)**.** Analytical RP-HPLC: t_R = 1.65 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min)**.** MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.55, found: 1711.42.



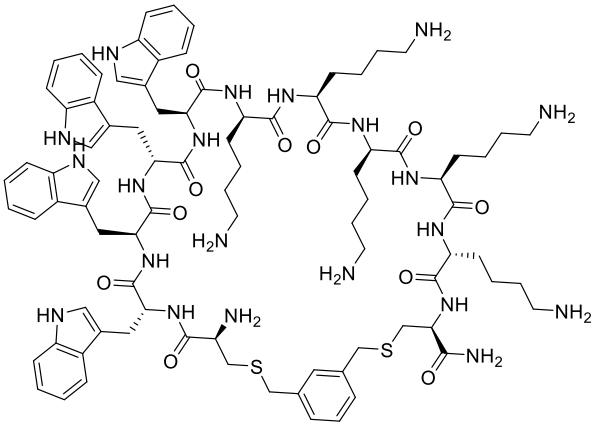


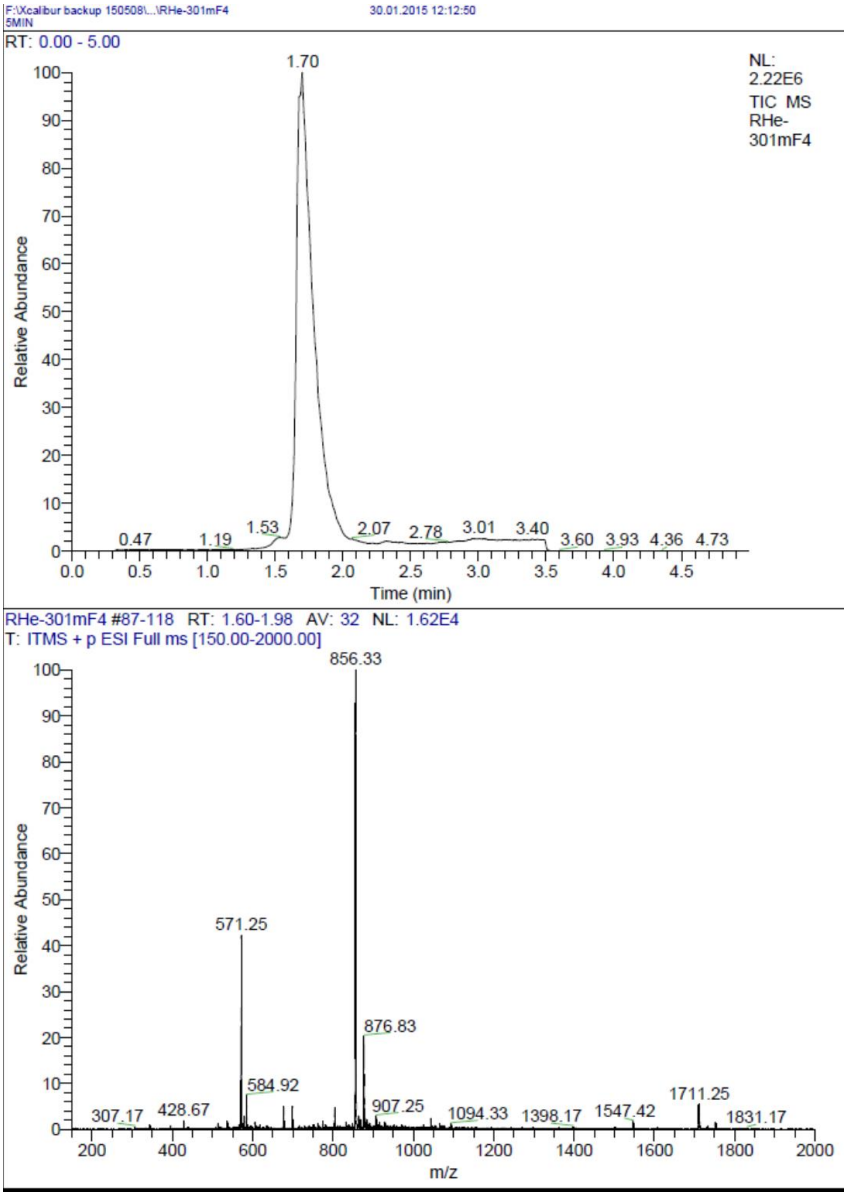
RH7. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH7** was obtained as white solid after preparative RP-HPLC purification (17.5mg, 9.5%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.62 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{94}H_{131}N_{23}O_{12}S_2$ $[M+H]^+$: 1838.98, found: 1838.58.



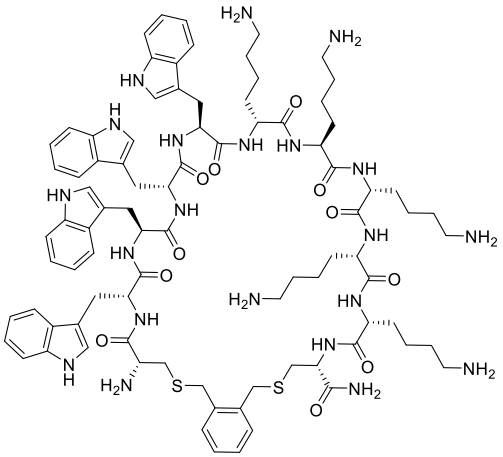


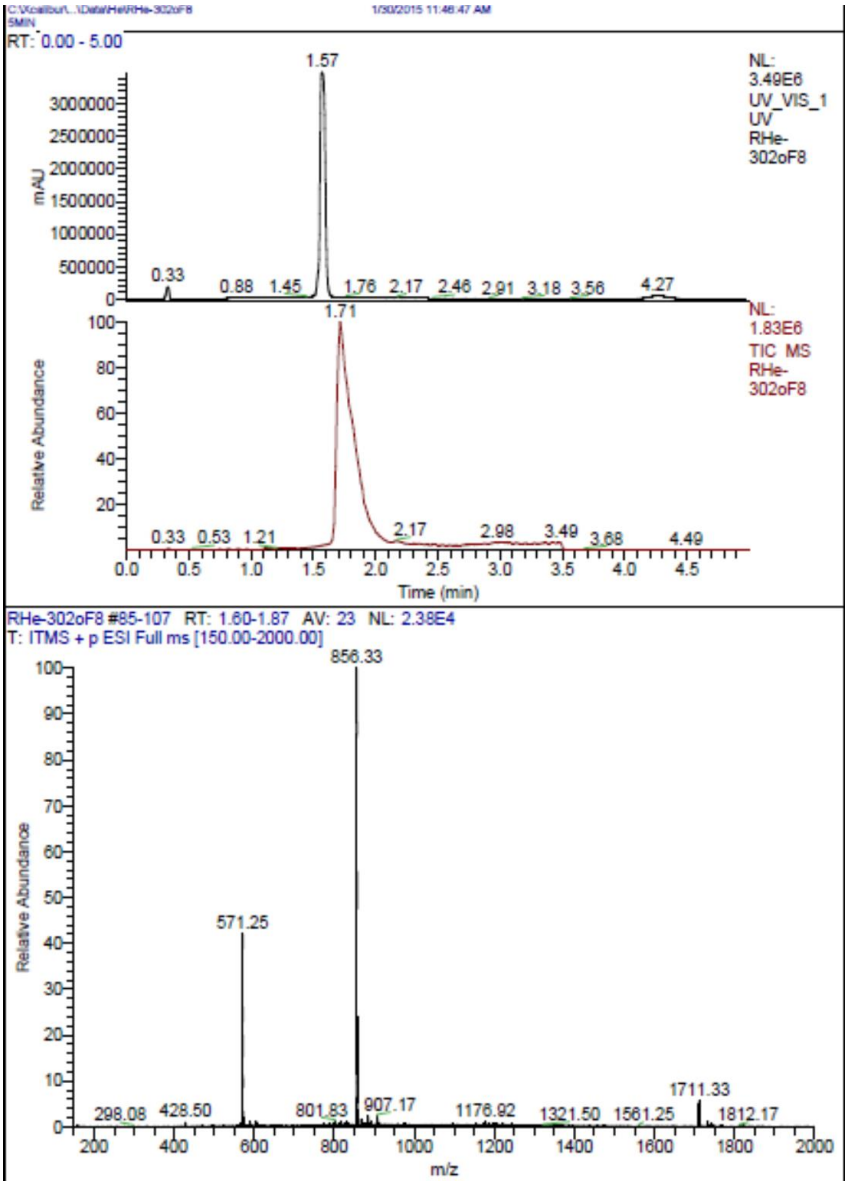
RH6m. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH6m** was obtained as white solid after preparative RP-HPLC purification (8.7mg, 5.1%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.70 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.25.



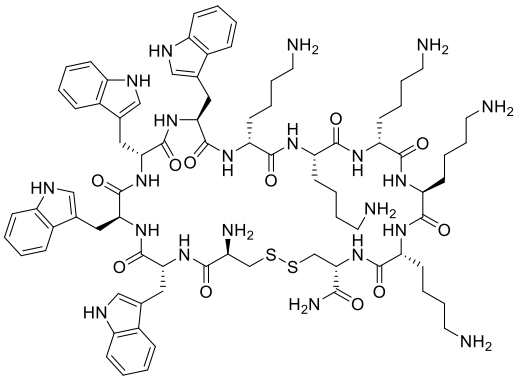


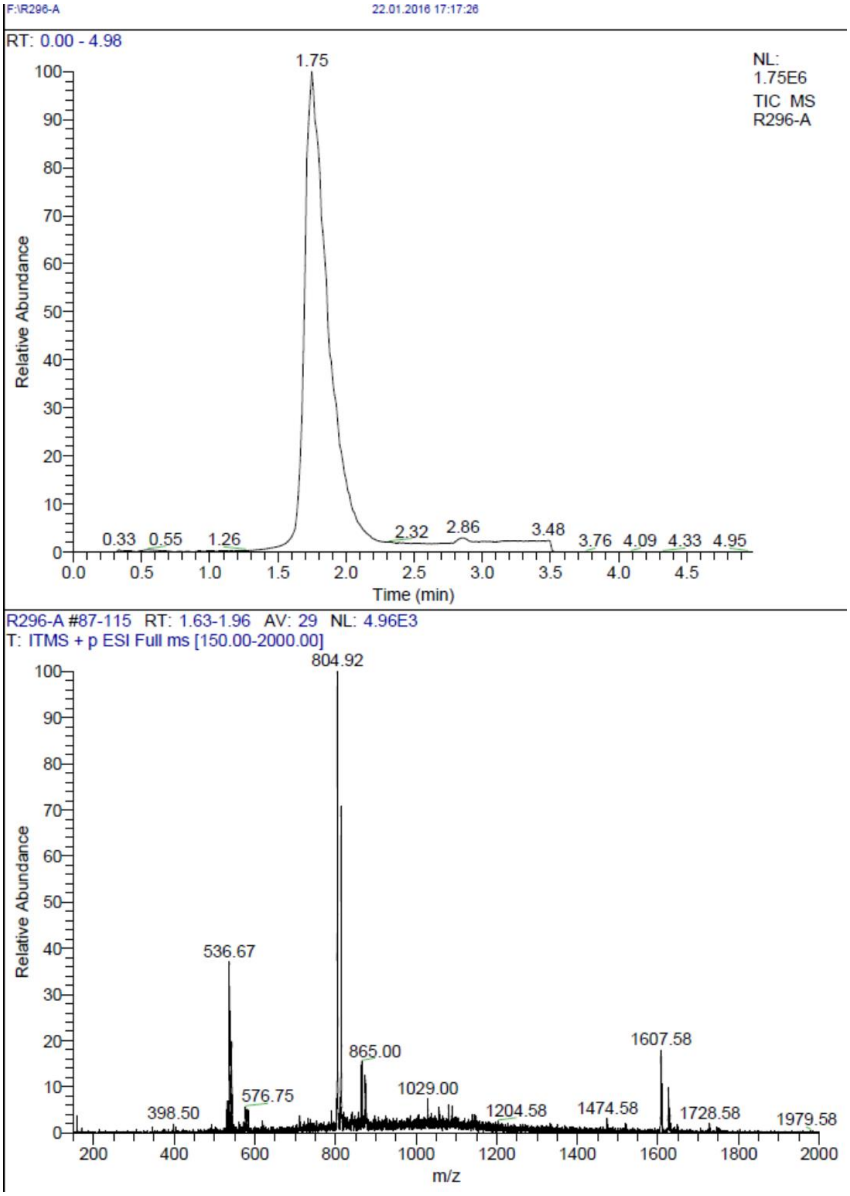
RH6o. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH6o** was obtained as white solid after preparative RP-HPLC purification (9.6mg, 5.6%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.57 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.33.



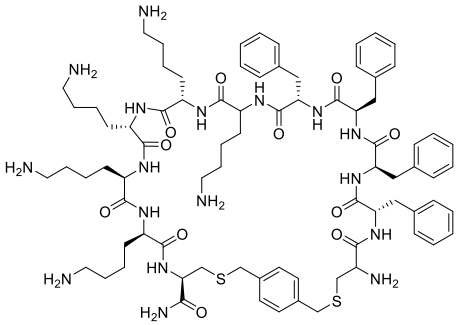


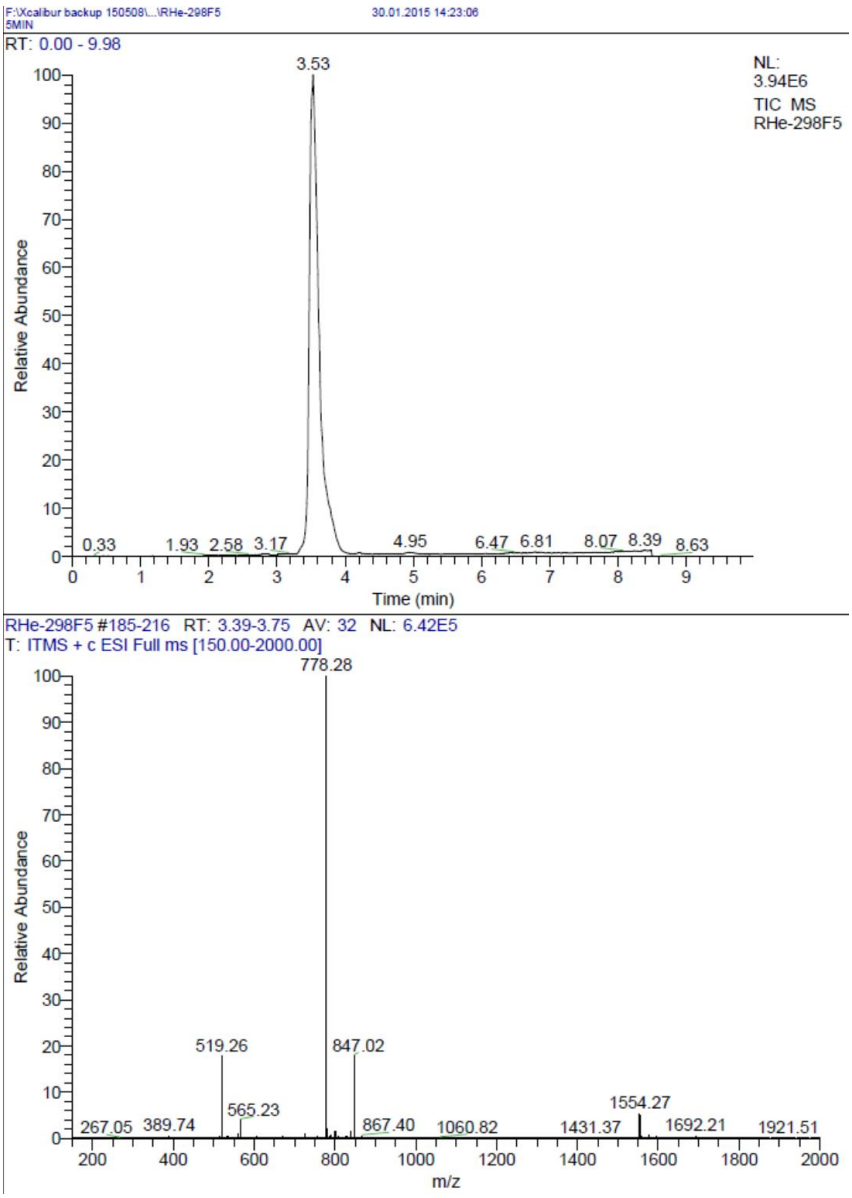
RH6ss. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH6ss** was obtained as white solid after preparative RP-HPLC purification (17.5mg, 10.9%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min)**.** Analytical RP-HPLC: t_R = 1.75 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min)**.** MS (ESI+) calculated for $C_{80}H_{111}N_{21}O_{11}S_2$ $[M+H]^+$: 1607.01, found: 1607.58.



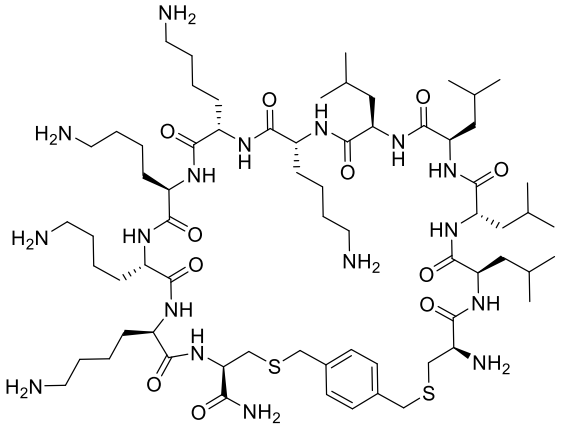


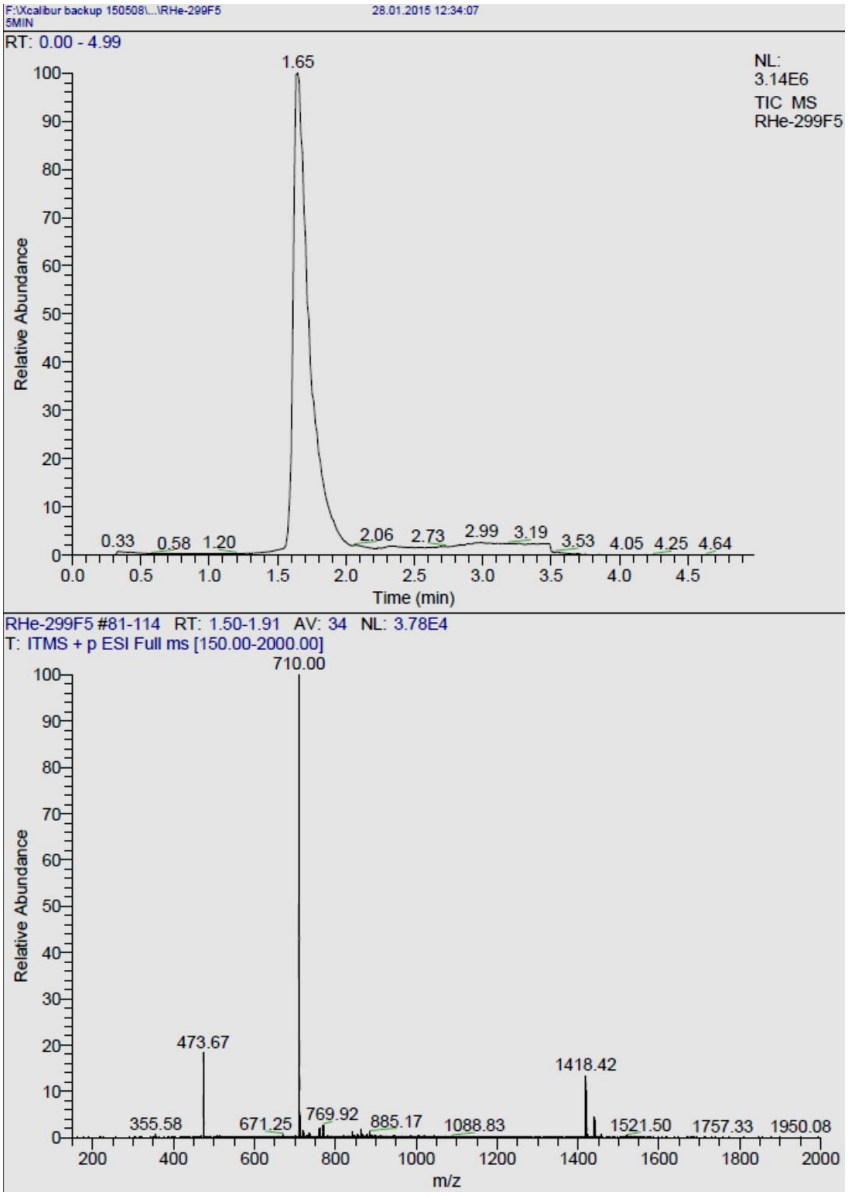
RH8. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH8** was obtained as white solid after preparative RP-HPLC purification (7.7mg, 5.0%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.35 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{80}H_{115}N_{17}O_{11}S_2$ $[M+H]^+$: 1554.84, found: 1554.27.



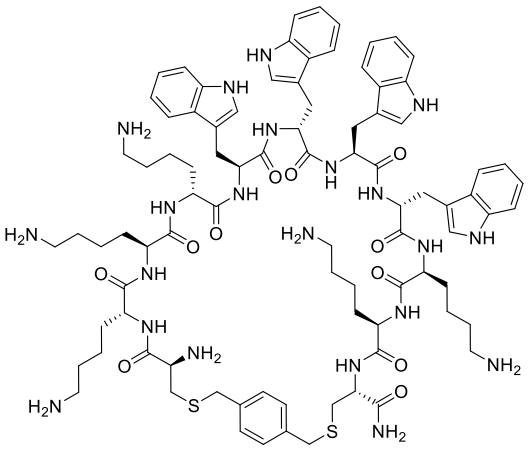


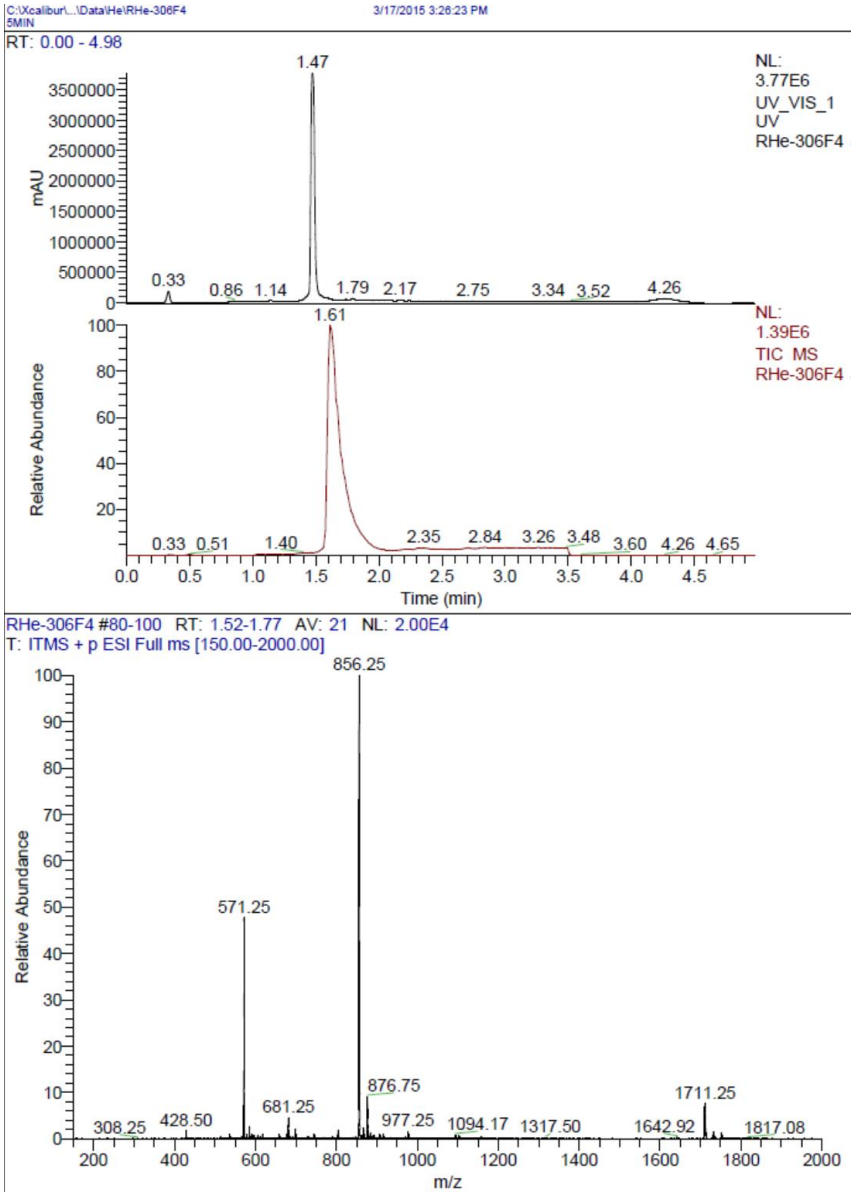
RH9. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH9** was obtained as white solid after preparative RP-HPLC purification (28.8, 20.3%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.65 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{68}H_{123}N_{17}O_{11}S_2$ $[M+H]^+$: 1418.90, found: 1418.42.



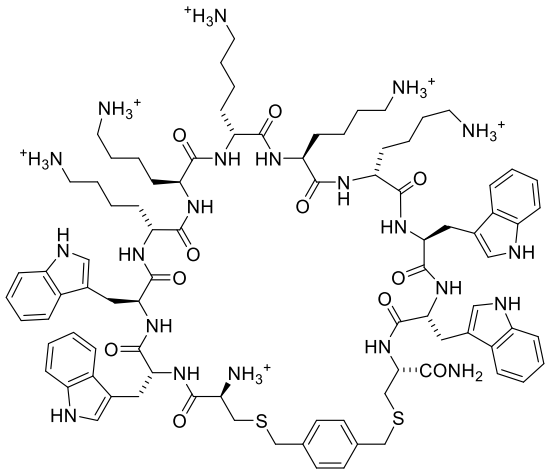


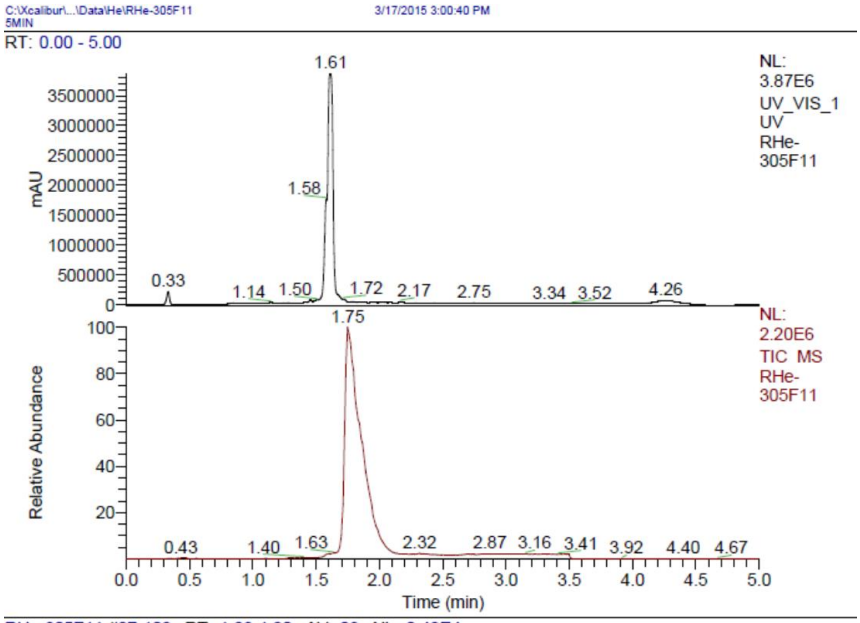
RH10. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH10** was obtained as white solid after preparative RP-HPLC purification (13.7mg, 8.0%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.47 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.25.

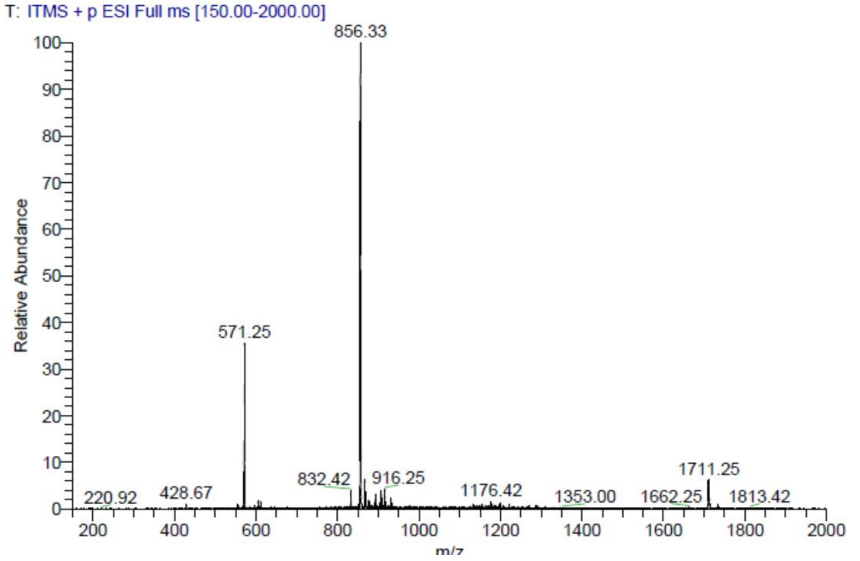




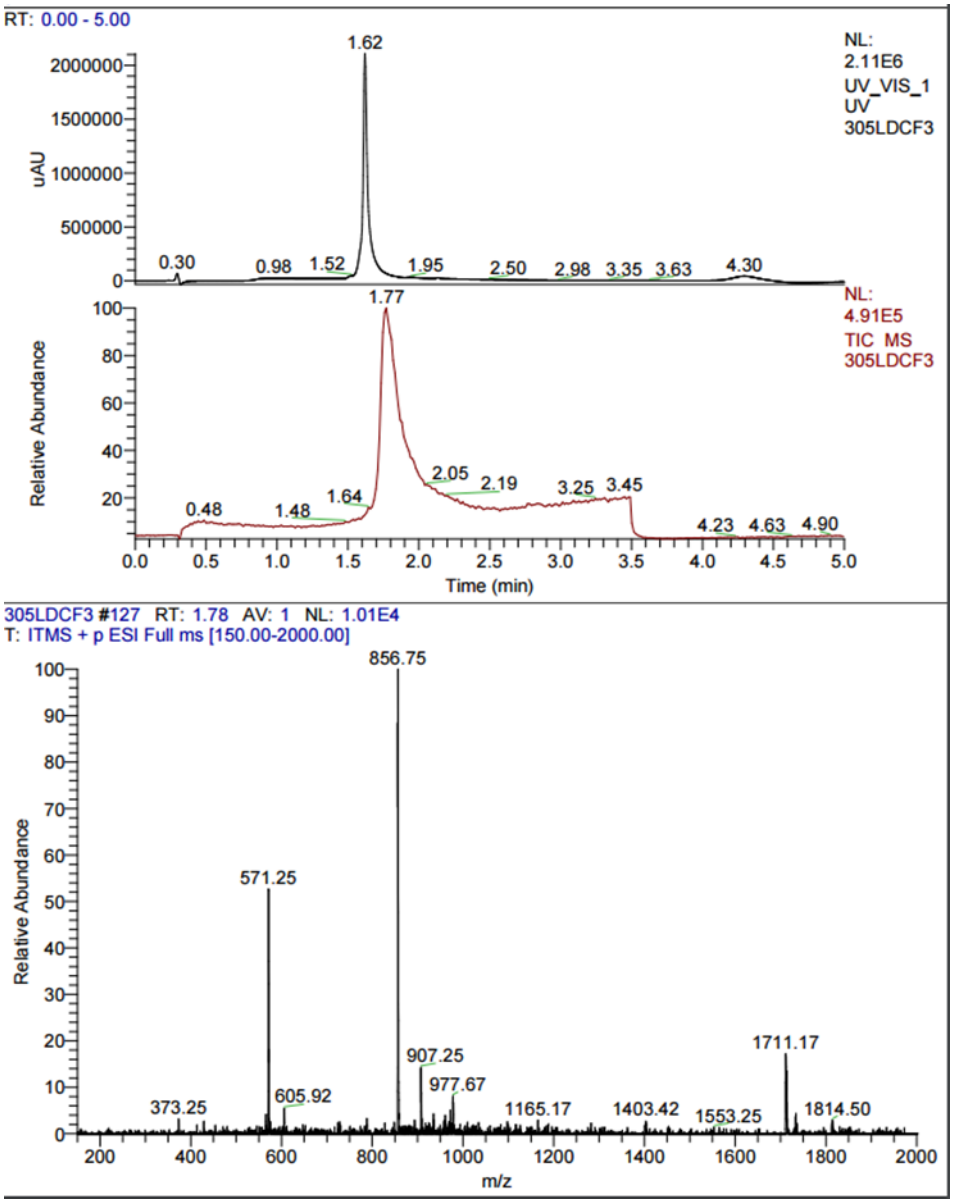
RH11. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH11** was obtained as white solid after preparative RP-HPLC purification (11.2mg, 6.5%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.61 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.25.



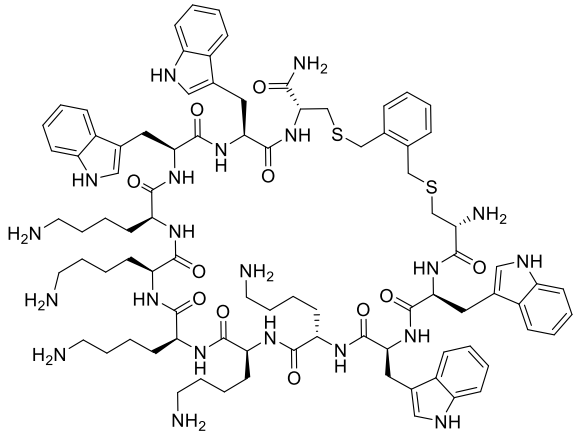


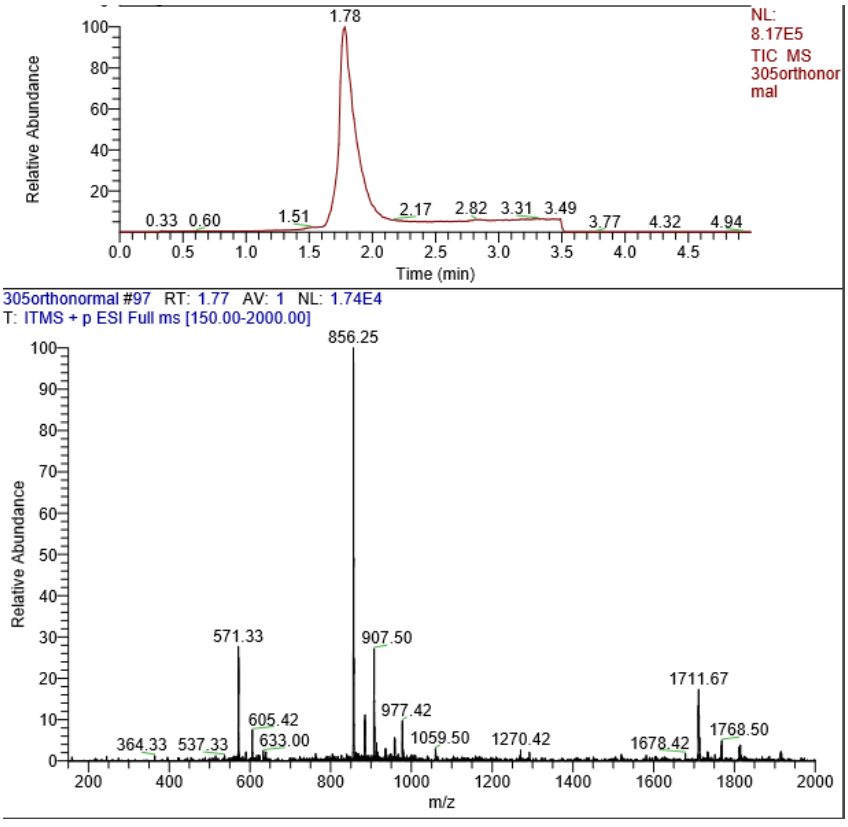


dRH11. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide d**RH11** was obtained as white solid after preparative RP-HPLC purification (8.2 mg, 4.5%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.62 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.17.

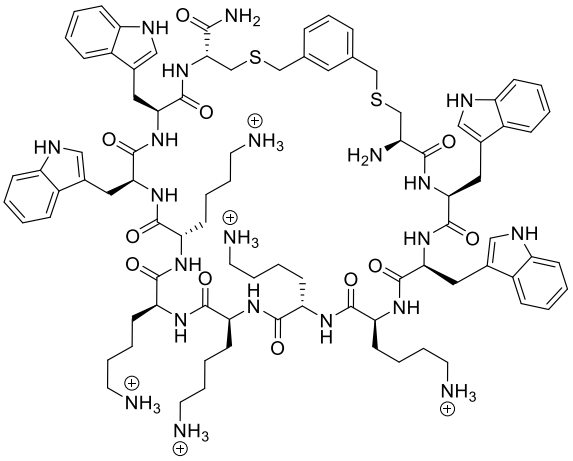


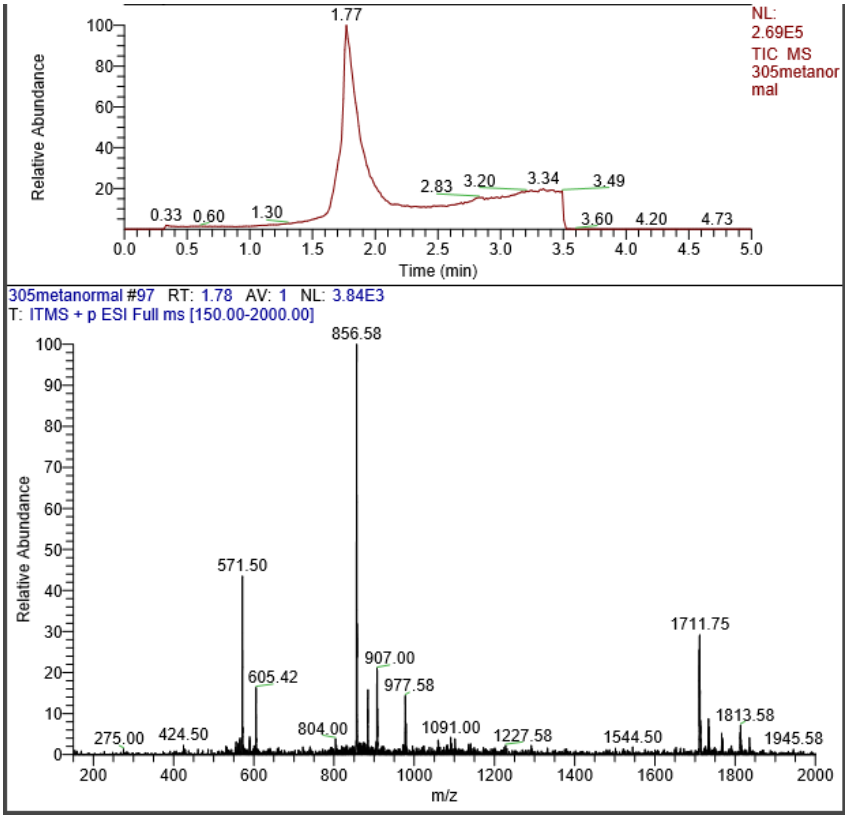
RH11o. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH11o** was obtained as white solid after preparative RP-HPLC purification (8.1mg, 4.7%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.78 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.67.



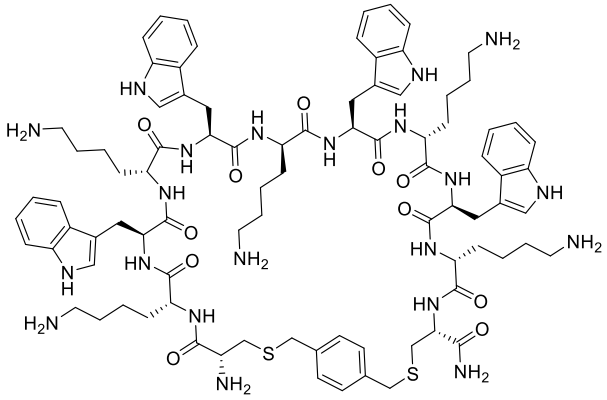


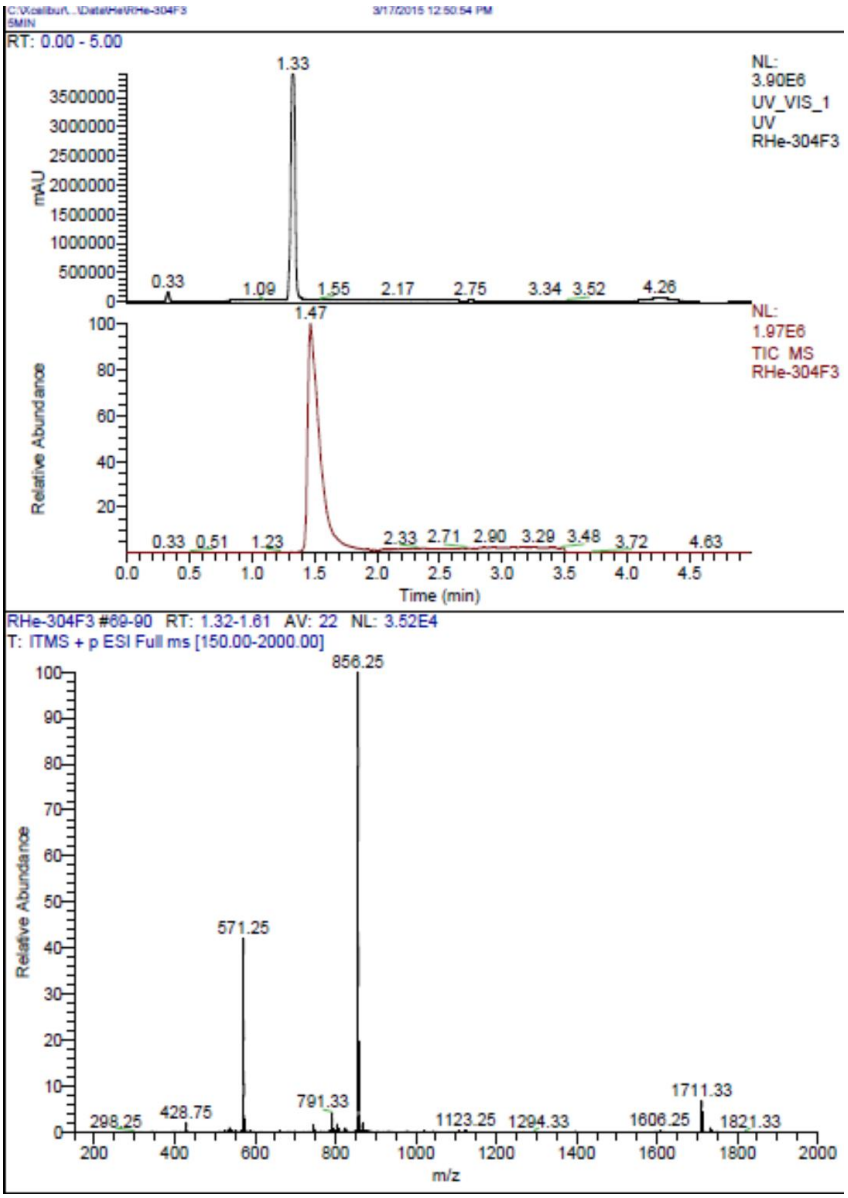
RH11m. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH11m** was obtained as white solid after preparative RP-HPLC purification (9.1mg, 5.2%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.77 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.75.



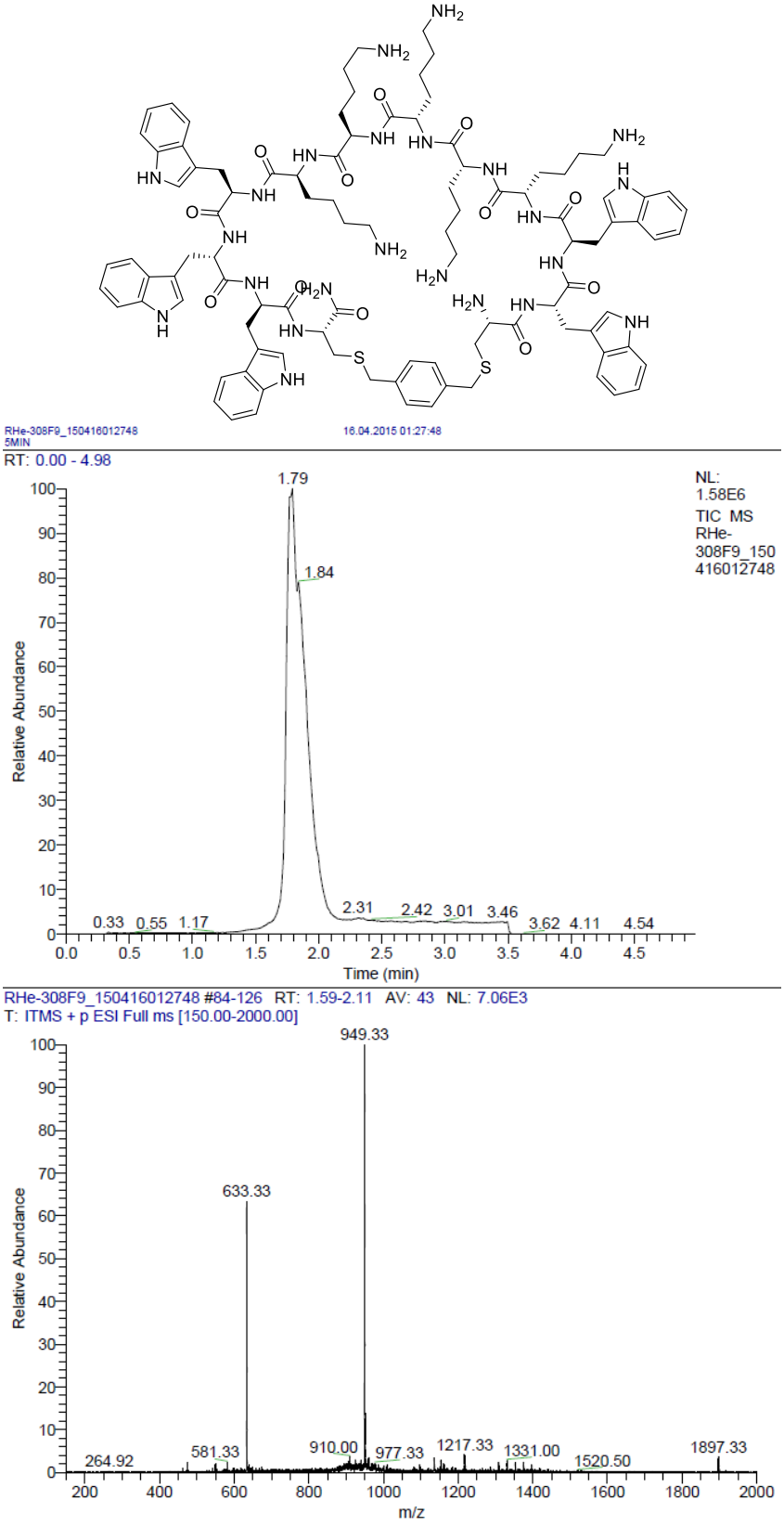


RH12. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH12** was obtained as white solid after preparative RP-HPLC purification (14.1mg, 8.2%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.33 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.33.

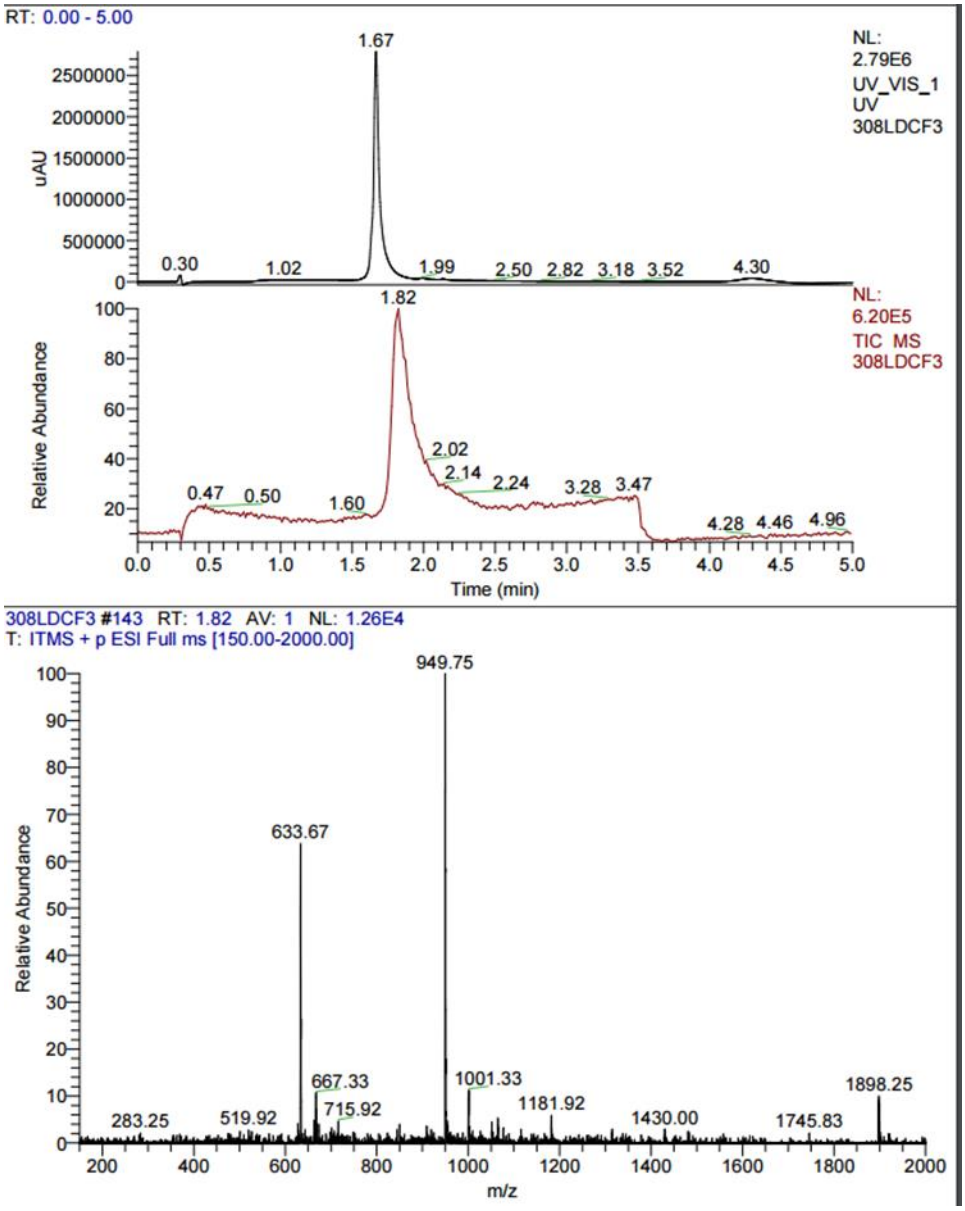




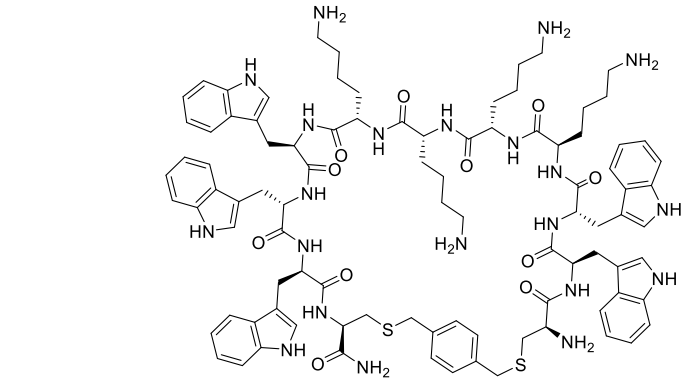
RH13. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH13** was obtained as white solid after preparative RP-HPLC purification (12.2mg, 6.4%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.79 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{99}H_{129}N_{23}O_{12}S_2$ $[M+H]^+$: 1897.36, found: 1897.33.

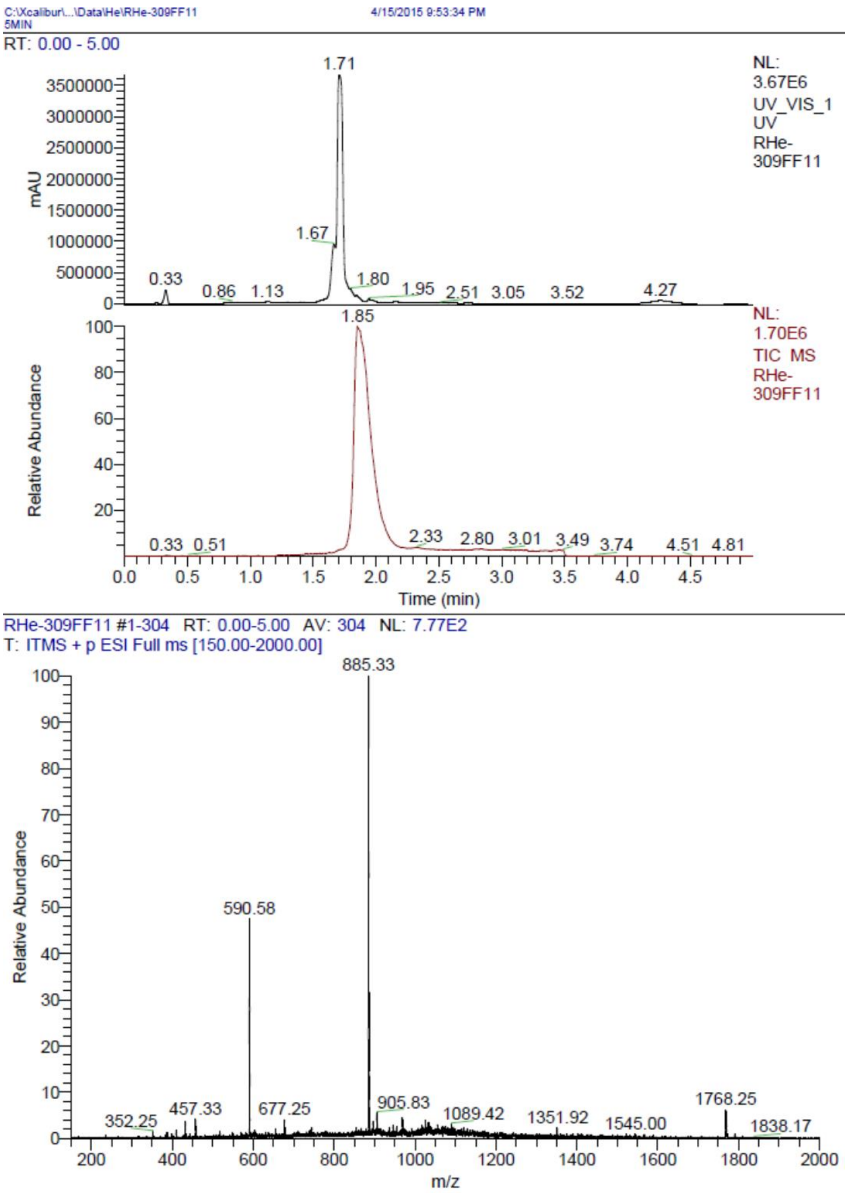


dRH13. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide d**RH13** was obtained as white solid after preparative RP-HPLC purification (6.1 mg, 3.4%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.67 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{99}H_{129}N_{23}O_{12}S_2$ $[M+H]^+$: 1897.36, found: 1898.25.

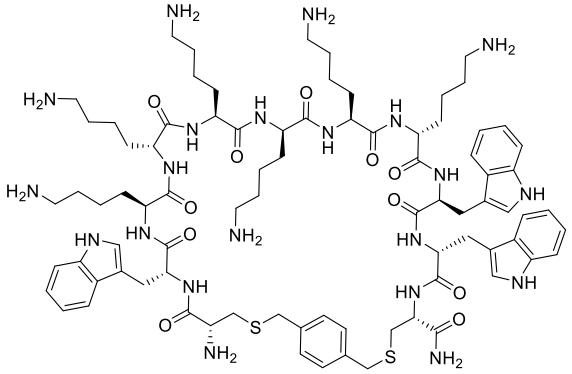


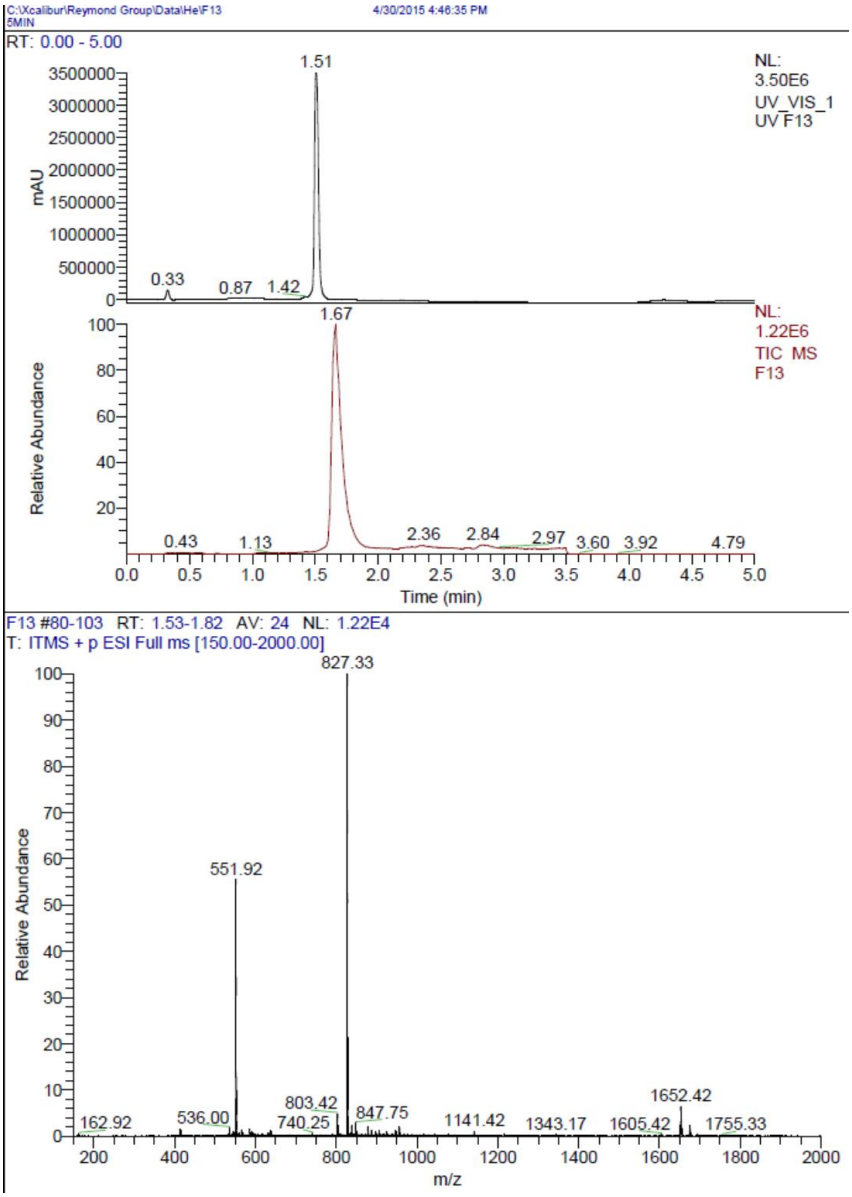
RH14. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH14** was obtained as white solid after preparative RP-HPLC purification (10.5mg, 5.9%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min)**.** Analytical RP-HPLC: t_R = 1.71 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min)**.** MS (ESI+) calculated for $C_{93}H_{117}N_{21}O_{11}S_2$ $[M+H]^+$: 1768.87, found: 1768.25.



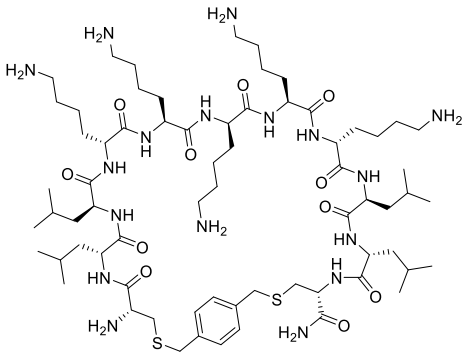


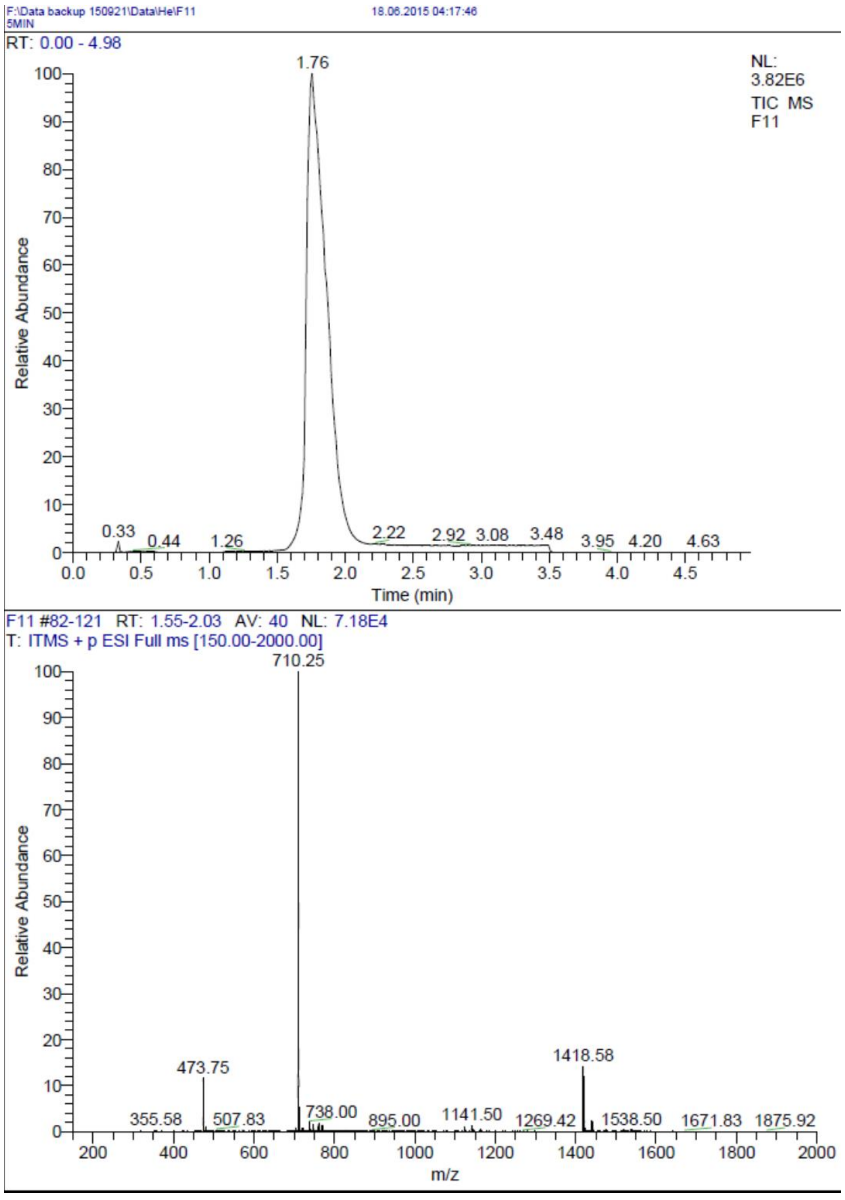
RH15. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH15** was obtained as white solid after preparative RP-HPLC purification (14.8mg, 9.0%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.51 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{83}H_{121}N_{21}O_{11}S_2$ $[M+H]^+$: 1652.90, found: 1652.42.



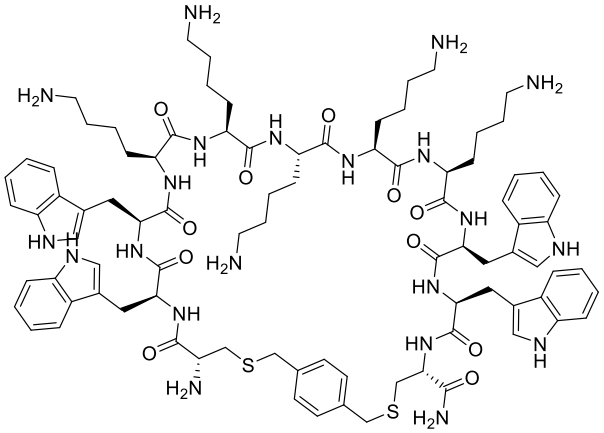


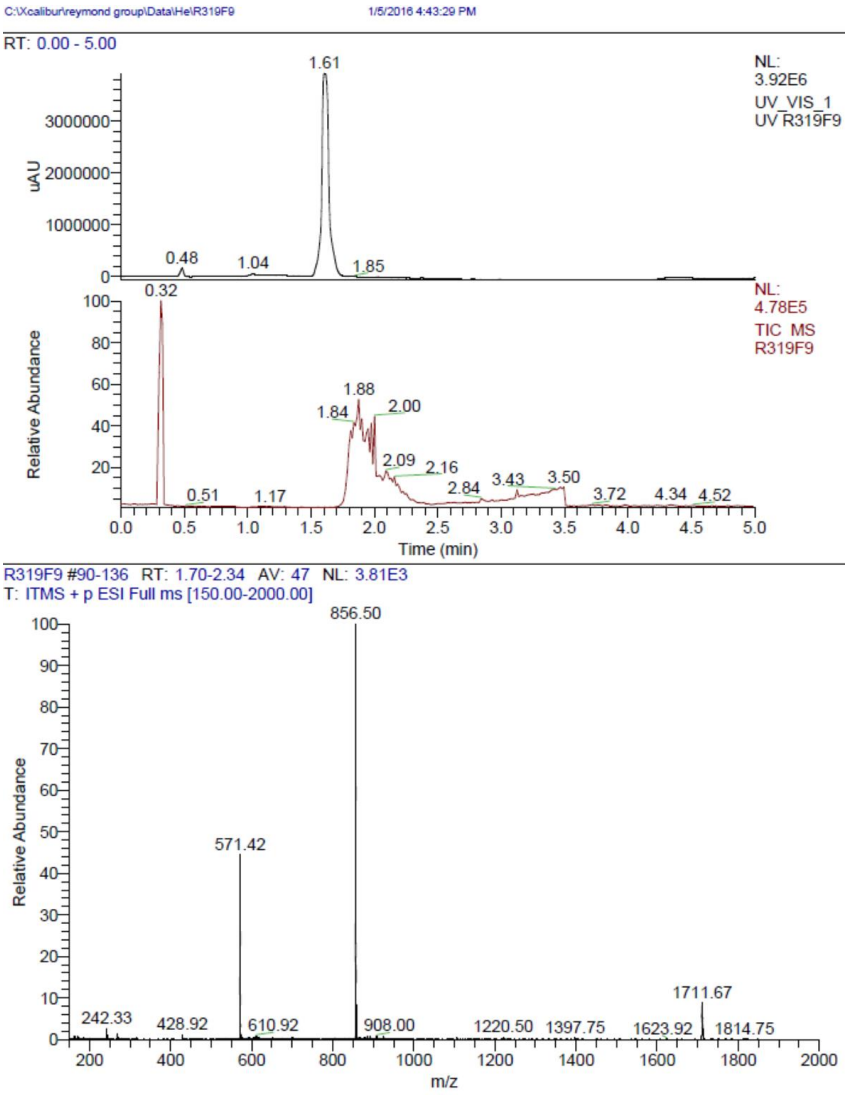
RH16. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH16** was obtained as white solid after preparative RP-HPLC purification (35.7mg, 25.2%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min)**.** Analytical RP-HPLC: t_R = 1.76 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min)**.** MS (ESI+) calculated for $C_{68}H_{123}N_{17}O_{11}S_2$ $[M+H]^+$: 1418.9, found: 1418.58.



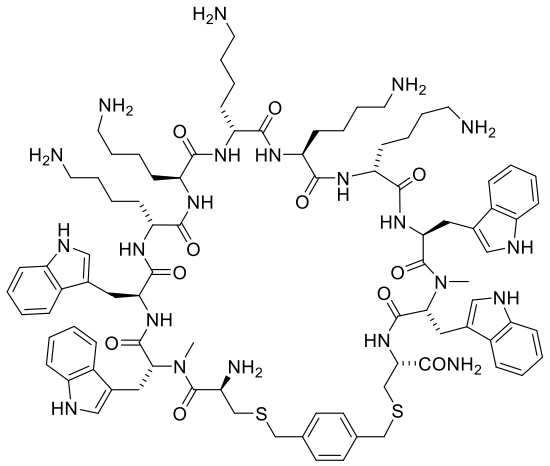


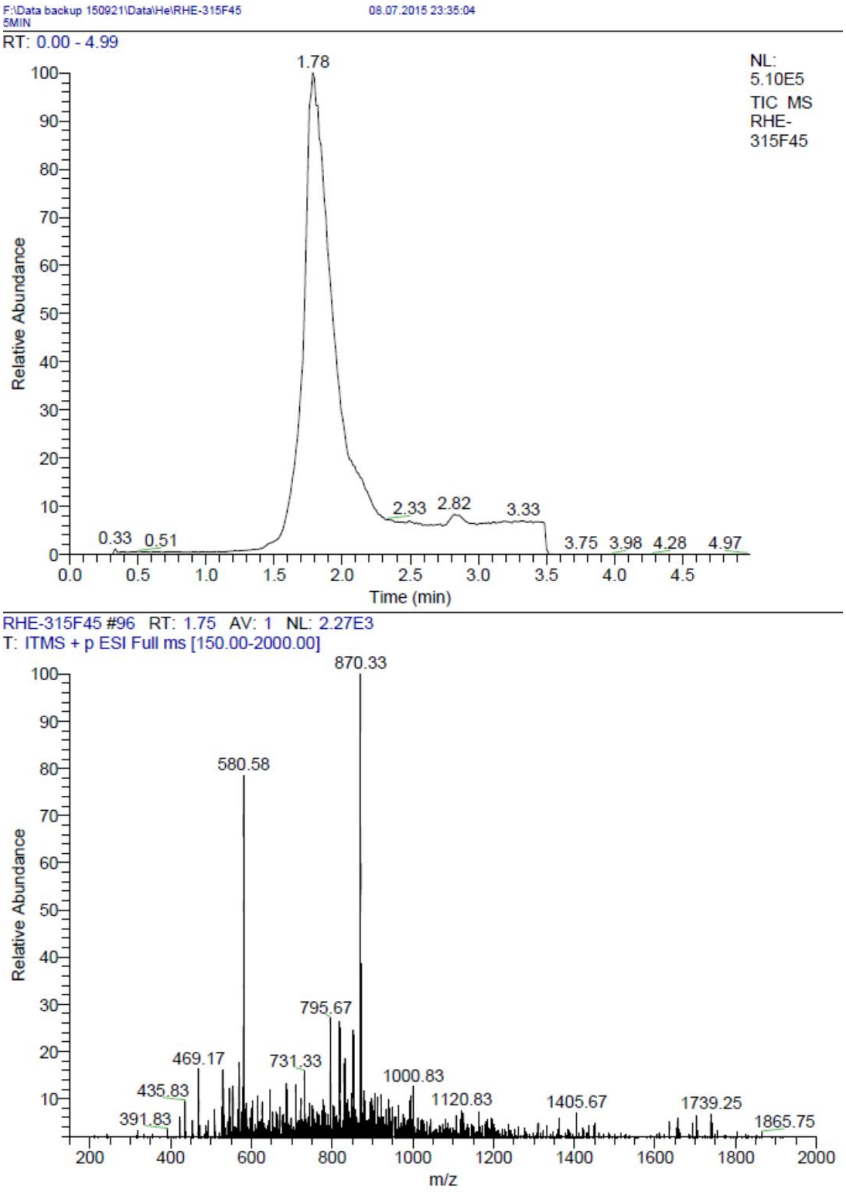
RH17. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH17** was obtained as white solid after preparative RP-HPLC purification (19.6mg, 11.5%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min)**.** Analytical RP-HPLC: t_R = 1.61 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min)**.** MS (ESI+) calculated for $C_{88}H_{119}N_{21}O_{11}S_2$ $[M+H]^+$: 1711.15, found: 1711.67.



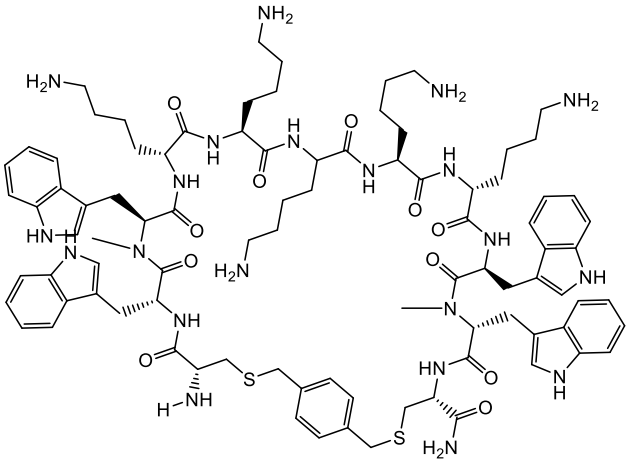


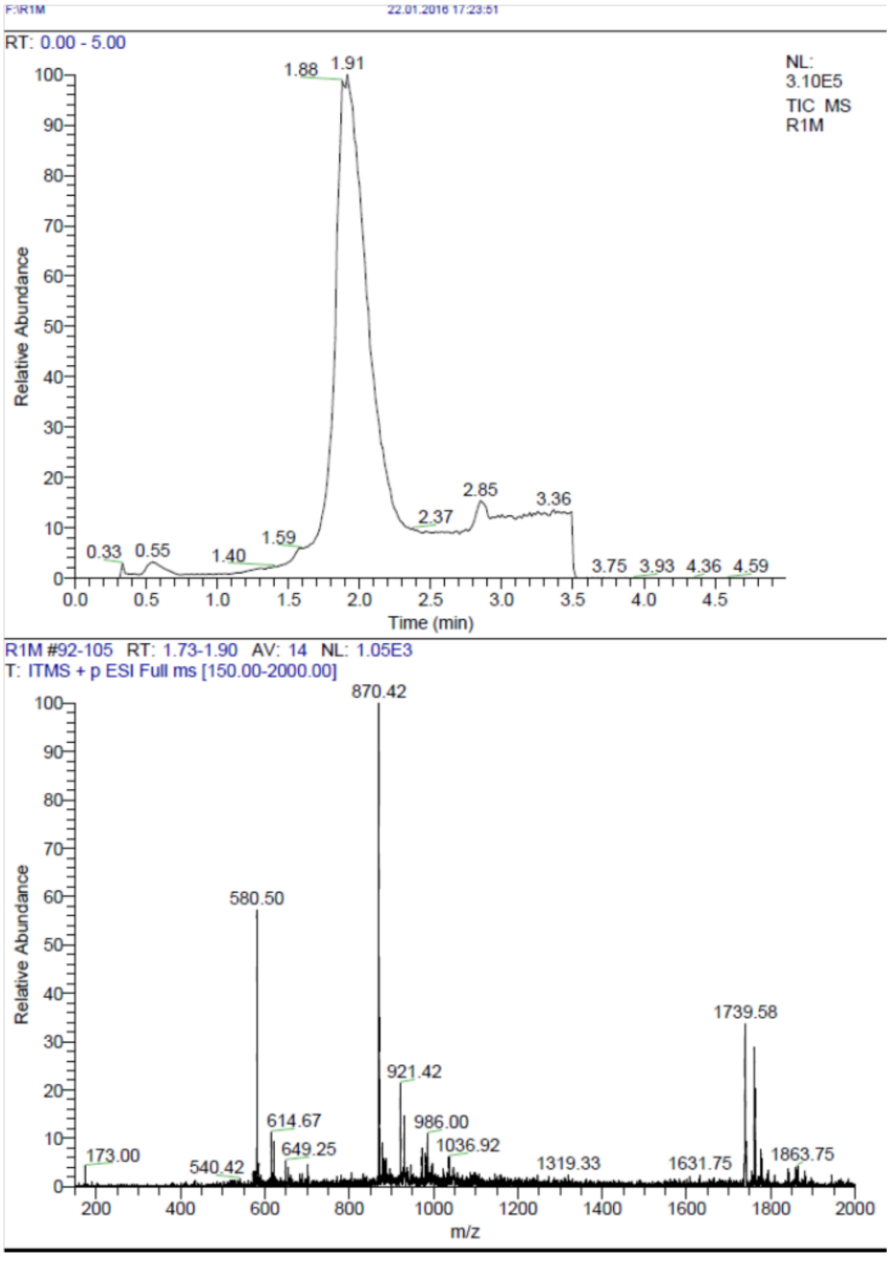
RH18. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH18** was obtained as white solid after preparative RP-HPLC purification (6.5mg, 3.7%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.78 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{90}H_{123}N_{21}O_{11}S_2$ $[M+H]^+$: 1739.20, found: 1739.25.



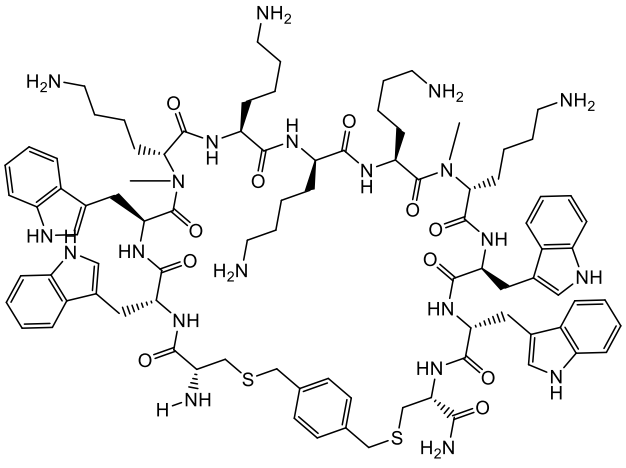


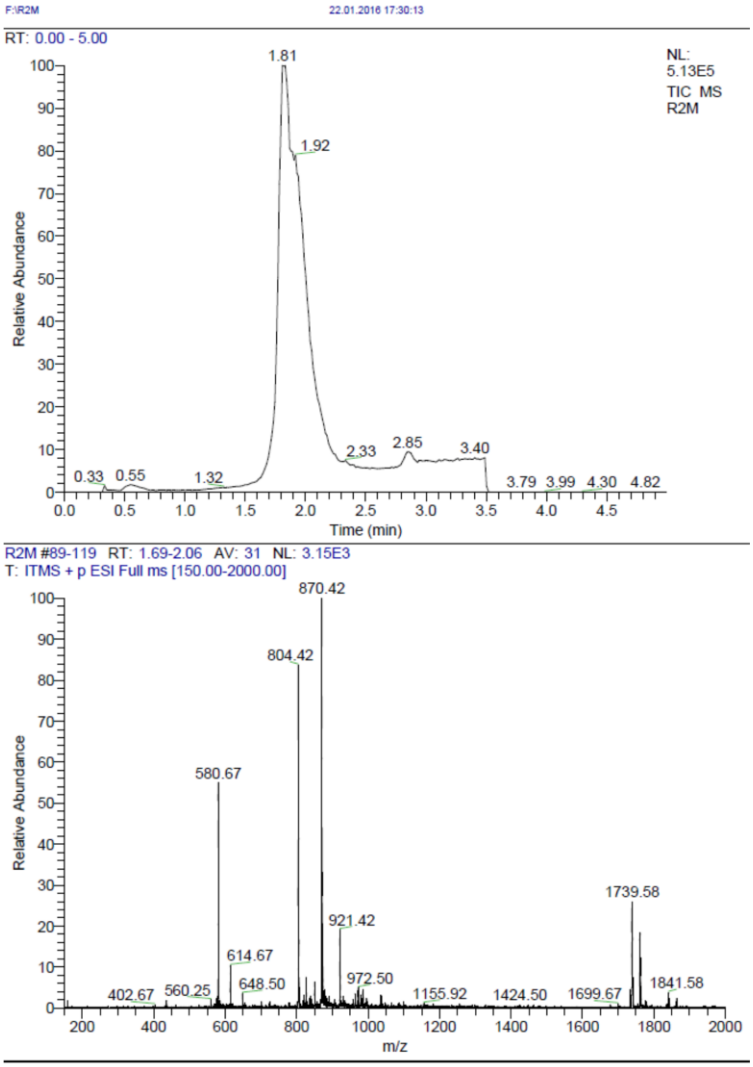
RH19. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH19** was obtained as white solid after preparative RP-HPLC purification (3.6mg, 2.1%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.91 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{90}H_{123}N_{21}O_{11}S_2$ $[M+H]^+$: 1738.9, found: 1739.58



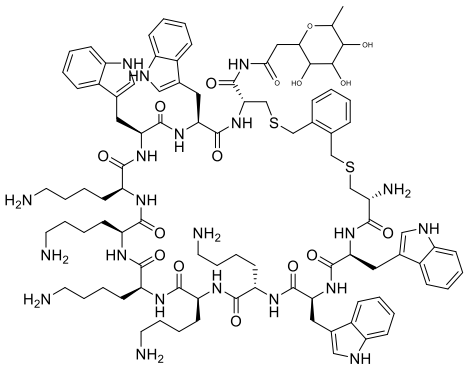


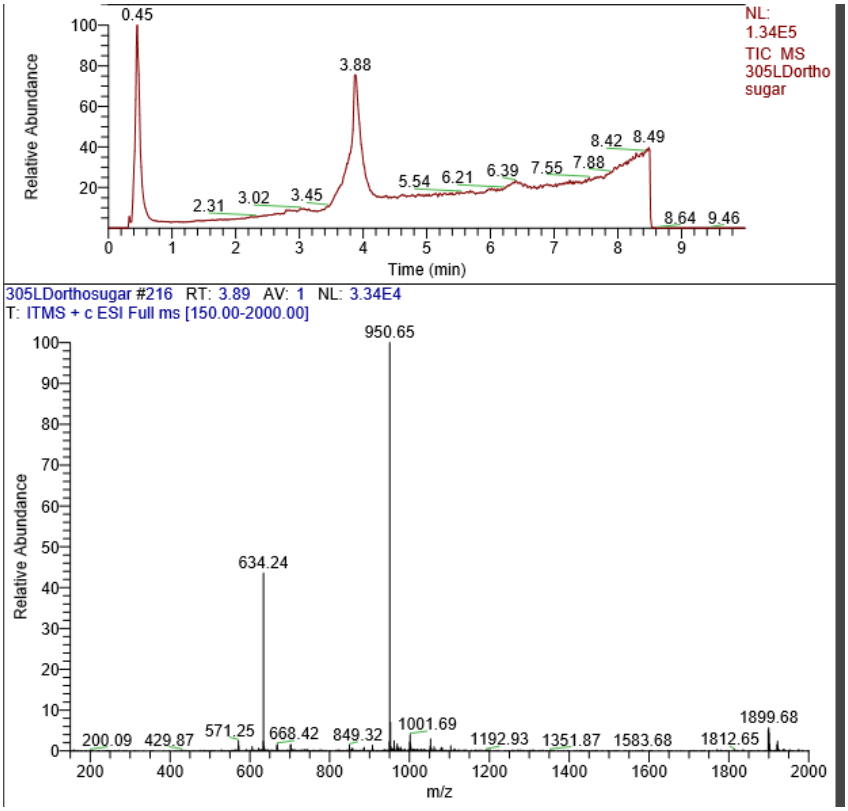
RH20. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **RH20** was obtained as white solid after preparative RP-HPLC purification (3.8mg, 2.2%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 1.61 min (A/D = 100/0 to 0/100 in 5 min, 1.2 mL/min). MS (ESI+) calculated for $C_{90}H_{123}N_{21}O_{11}S_2$ $[M+H]^+$: 1738.9, found: 1739.58





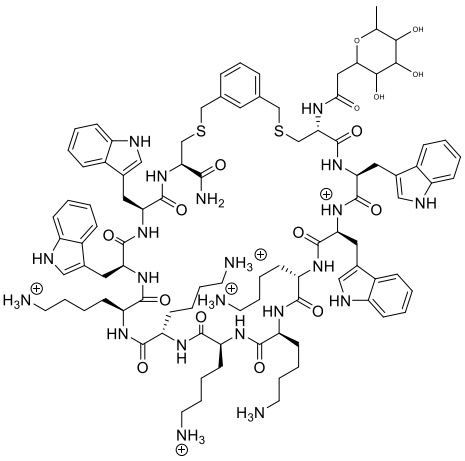
FRH11o. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FRH11o** was obtained as white solid after preparative RP-HPLC purification (8.3mg, 4.8%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.88 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.68.

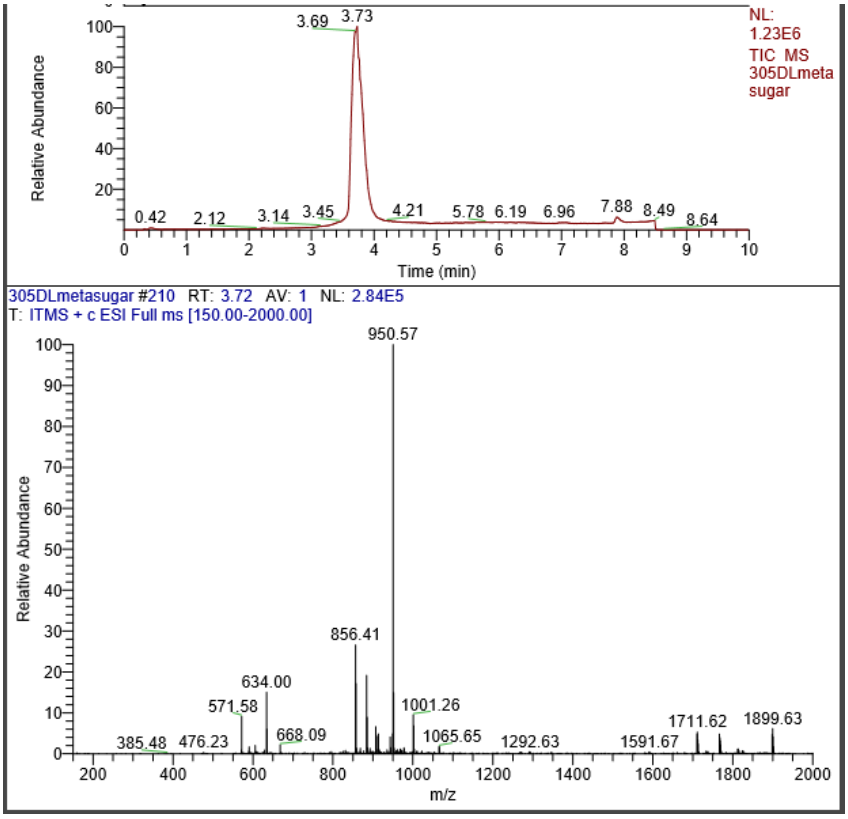




FdRH11o. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FdRH11o** was obtained as white solid after preparative RP-HPLC purification (8.1mg, 4.7%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.88 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.68.

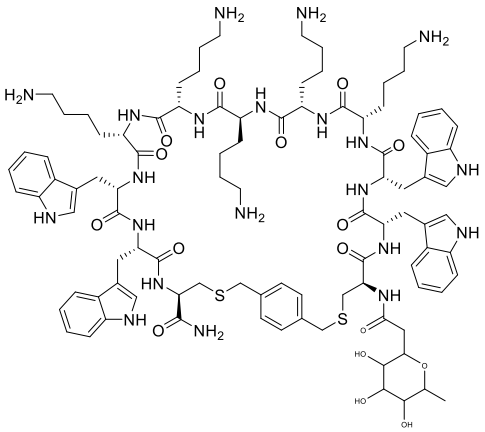
FdRH11m. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FdRH11m** was obtained as white solid after preparative RP-HPLC purification (5.9mg, 3.4%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.73 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.63.

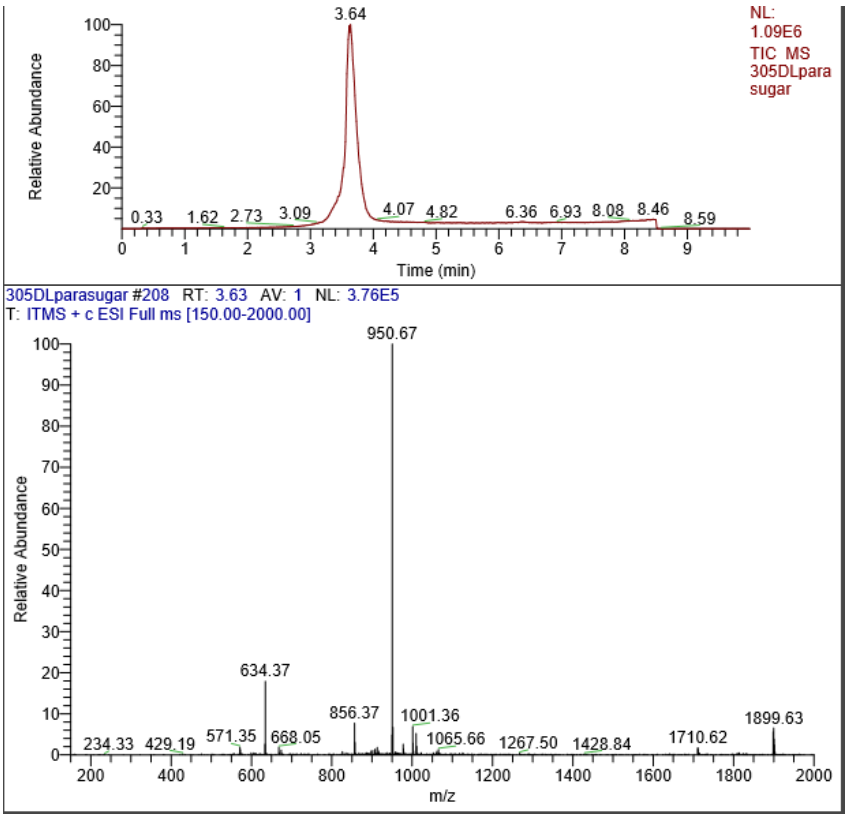




FRH11m. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FRH11m** was obtained as white solid after preparative RP-HPLC purification (6.2mg, 3.6%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.73 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.63.

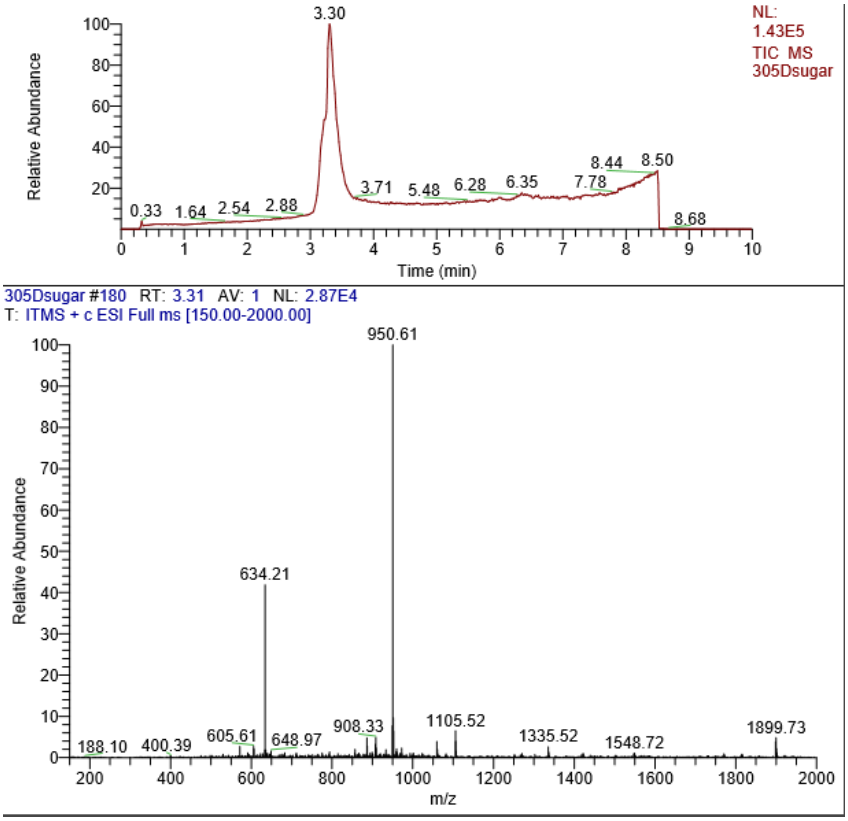
FdRH11p. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FdRH11p** was obtained as white solid after preparative RP-HPLC purification (7.0mg, 4.0%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.64 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.63.





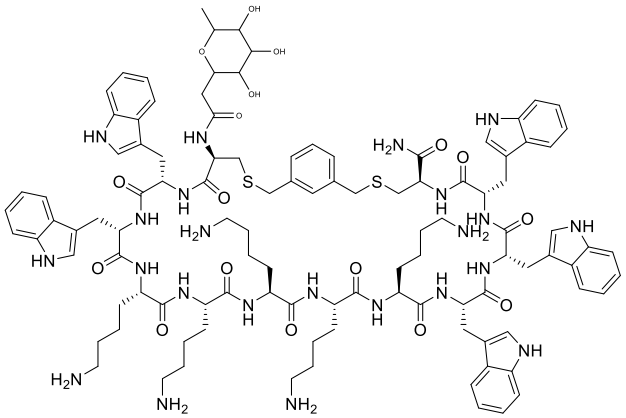
FRH11p. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FRH11p** was obtained as white solid after preparative RP-HPLC purification (6.3mg, 3.6%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.64 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.63.

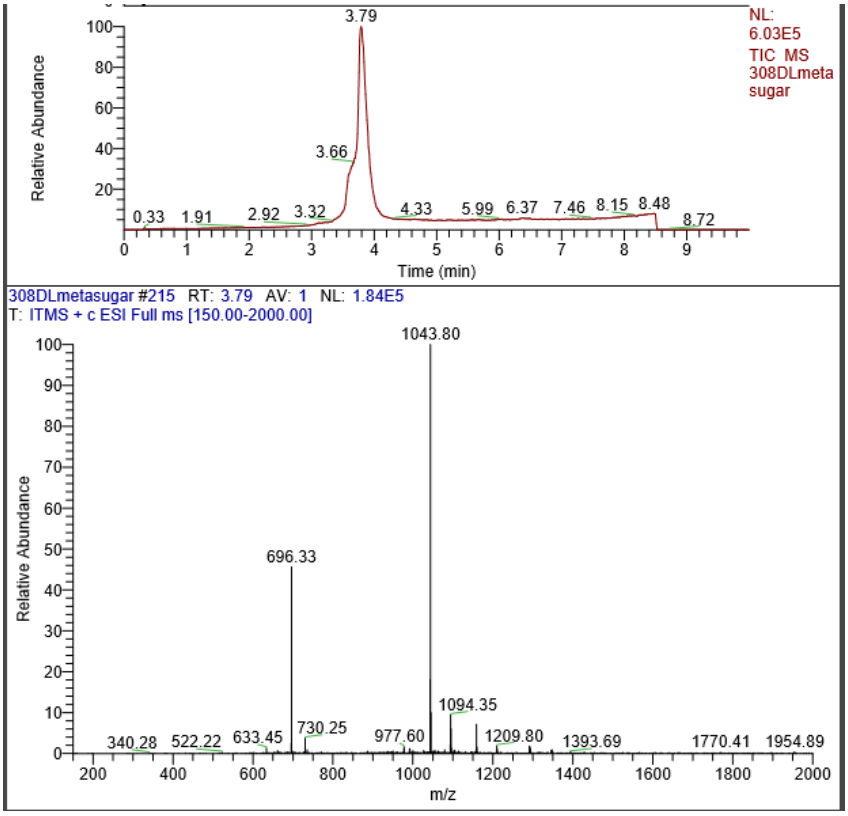
FddRH11p. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FddRH11p** was obtained as white solid after preparative RP-HPLC purification (8.8mg, 5.1%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.30 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{96}H_{131}N_{21}O_{16}S_2$ $[M+H]^+$: 1899.32, found: 1899.73.



FRH13o. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FRH13o** was obtained as white solid after preparative RP-HPLC purification (3.2mg, 1.6%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.79 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{107}H_{141}N_{23}O_{17}S_2$ $[M+H]^+$: 2085.53, found: 2085.33.

FRH13m. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FRH13m** was obtained as white solid after preparative RP-HPLC purification (3.5mg, 1.8%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.79 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{107}H_{141}N_{23}O_{17}S_2$ $[M+H]^+$: 2085.53, found: 2085.33.





FRH13p. Starting with 400 mg of Tenta Gel S RAM (0.25 mmol/g). Peptide **FRH13p** was obtained as white solid after preparative RP-HPLC purification (3.3mg, 1.6%). Prep. RP-HPLC (A/D = 100/0 to 0/100 in 60 min, 60 mL/min). Analytical RP-HPLC: t_R = 3.82 min (A/D = 100/0 to 0/100 in 10 min, 1.2 mL/min). MS (ESI+) calculated for $C_{107}H_{141}N_{23}O_{17}S_2$ $[M+H]^+$: 2085.53, found: 2085.33.