Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for

Convergent synthesis and cellular uptake of multivalent cell penetrating peptides derived from Tat, Antp, pVEC, TP10 and SAP

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Content:

Figures

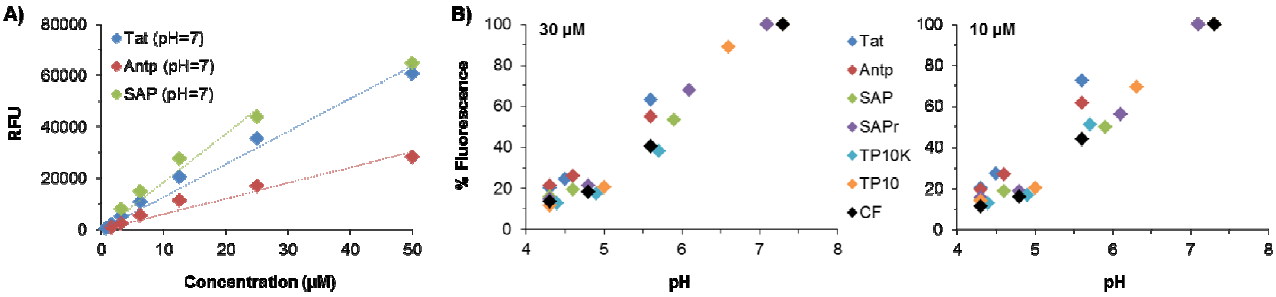


Figure S1. Fluorescence measurement of linear CF-labeled CPPs. (A) Relative fluorescence signal (RFU) at 530 nm (excitation 480 nm) for different compound concentrations. The signals show linear correlation up to a concentration of 50 µM. (B) Fluorescence signal at 530 nm (excitation 480 nm) for 30 and 10 µM at different pH values. The fluorescence signal at pH 7 was set to 100% and the other signals relative to this reference. The fluorescence signals between pH 5.5-6.5 were reduced by 30-50% compared to pH 7. At acidic pH of 4.5-5, the remaining signal was 20%. All measurements were done in PBS buffer at room temperature.

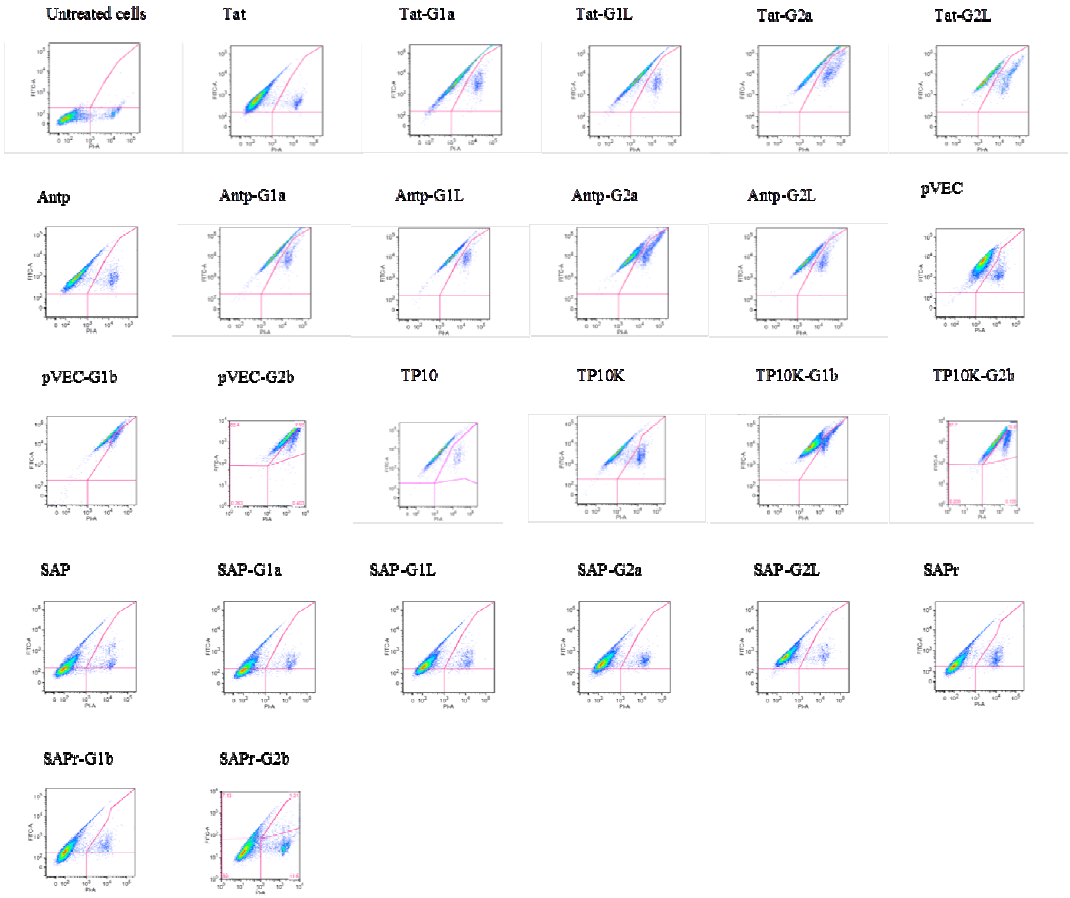


Figure S2. Primary flow cytometry data of HeLa cells after incubation with linear and multivalent CPPs at 10 µM at 37 °C.

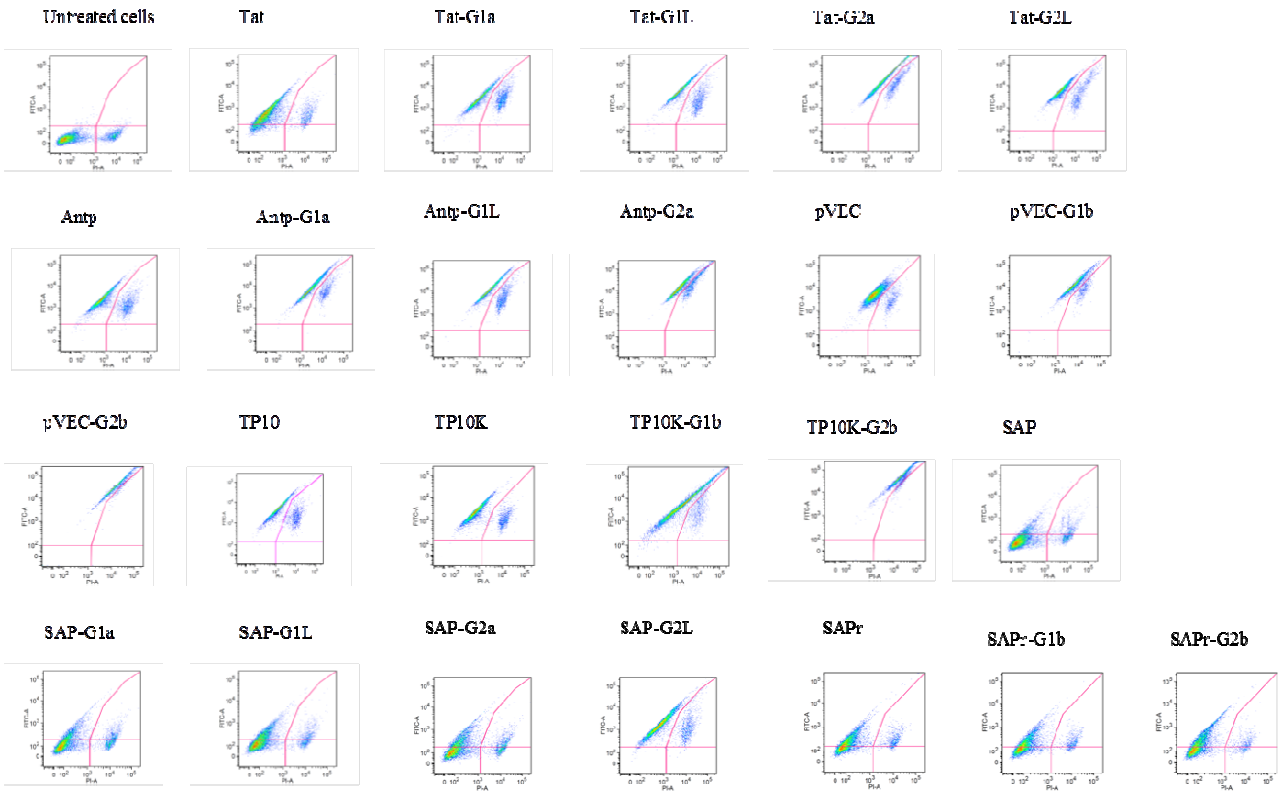
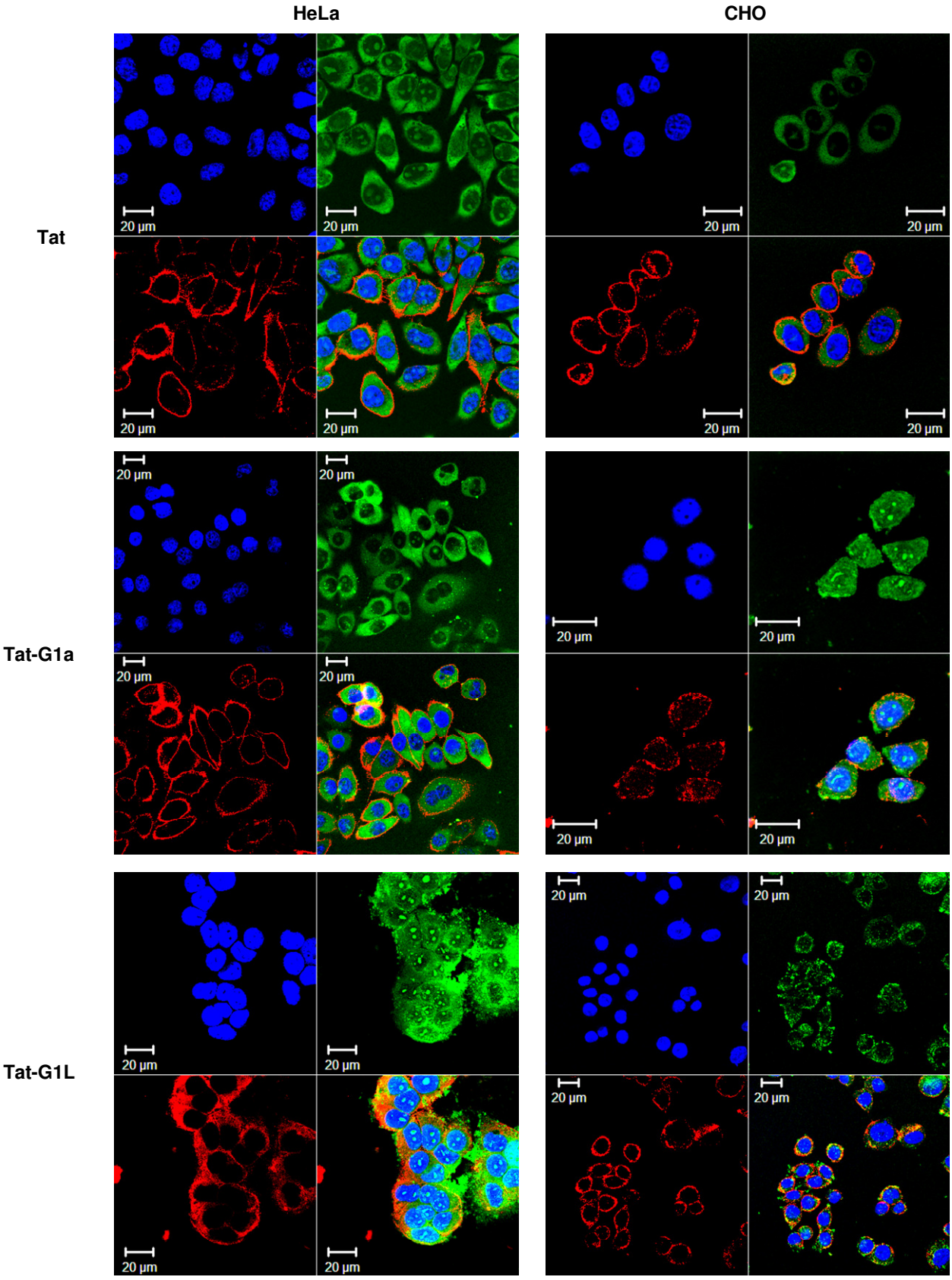
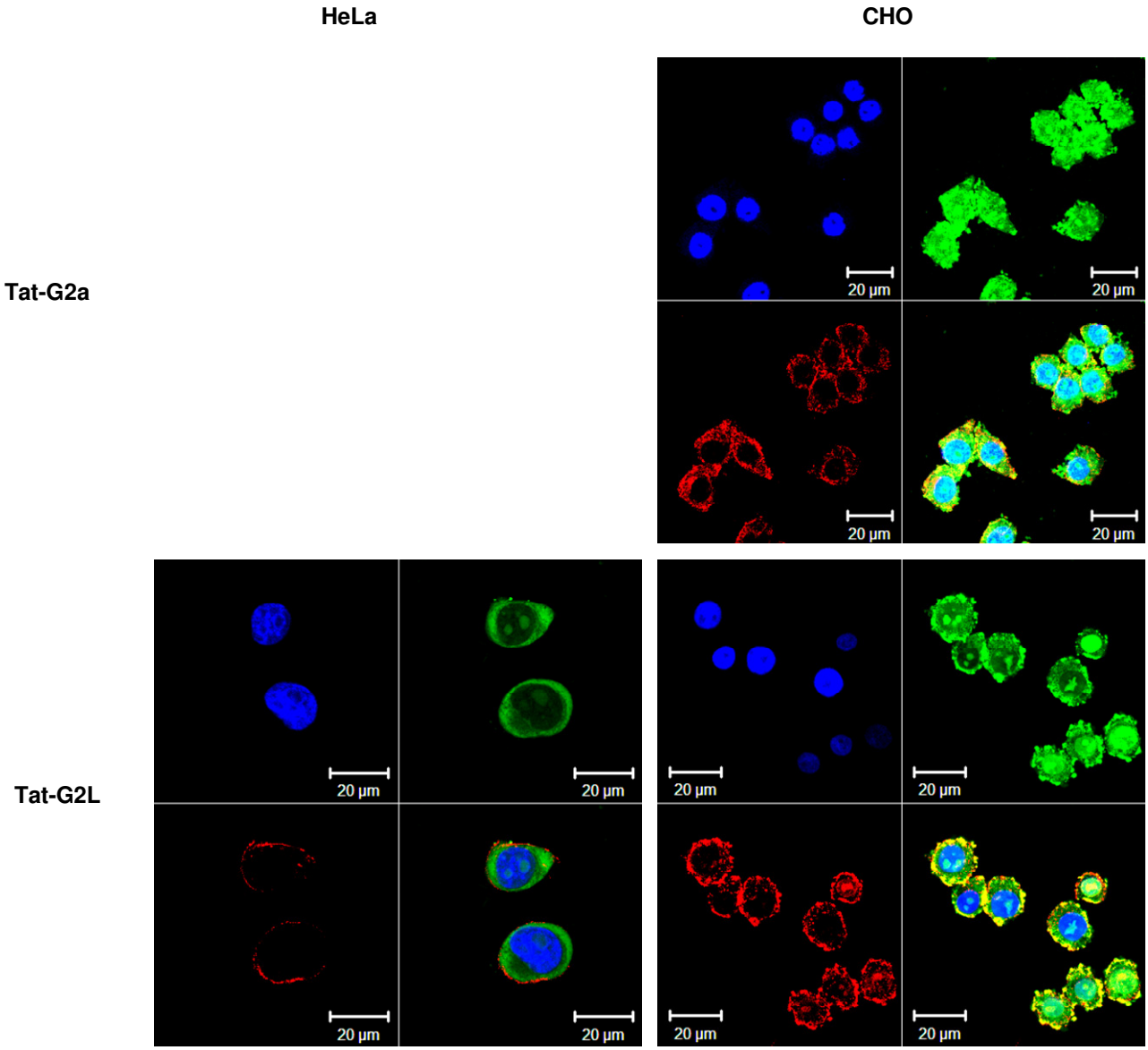
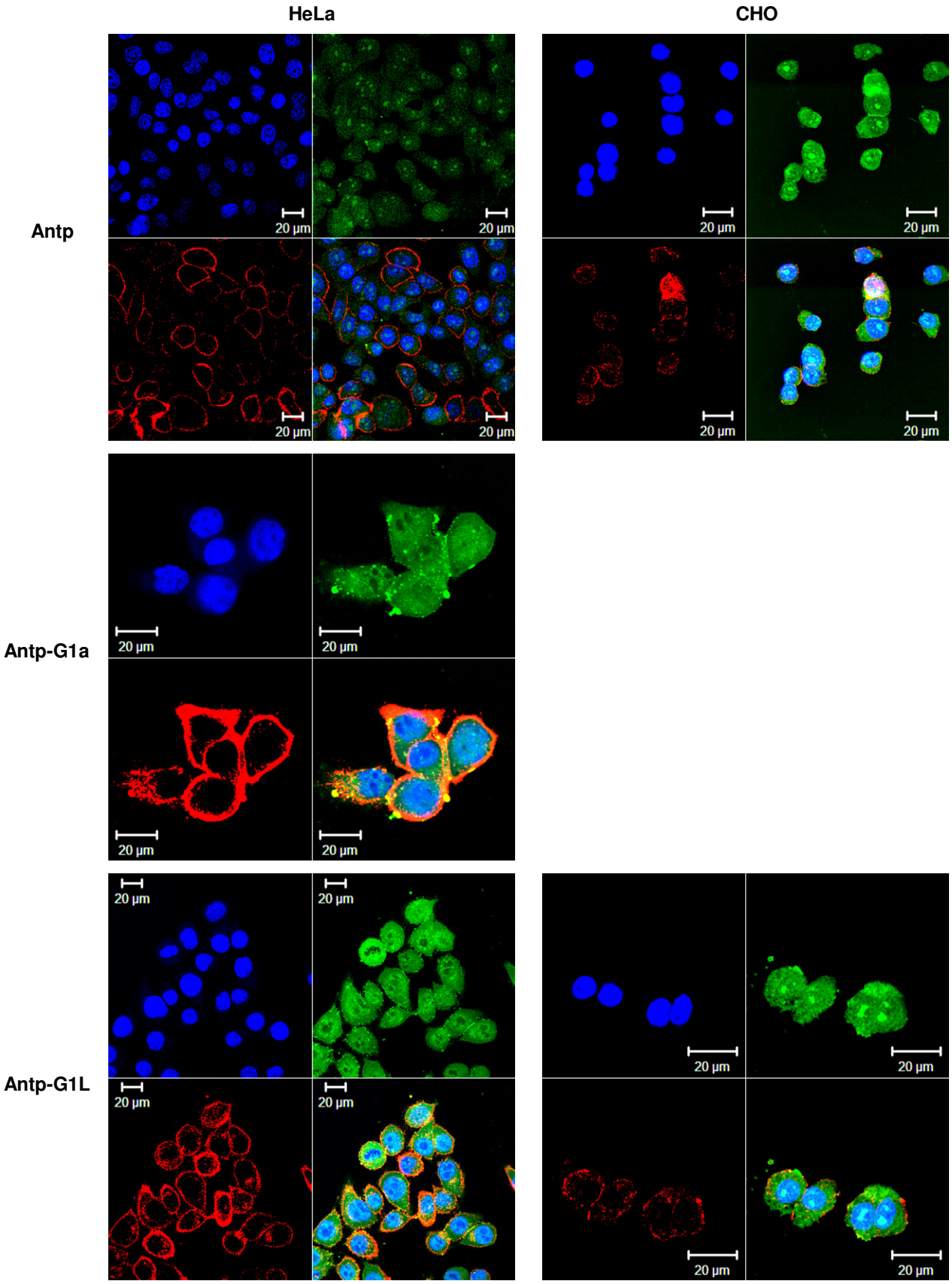


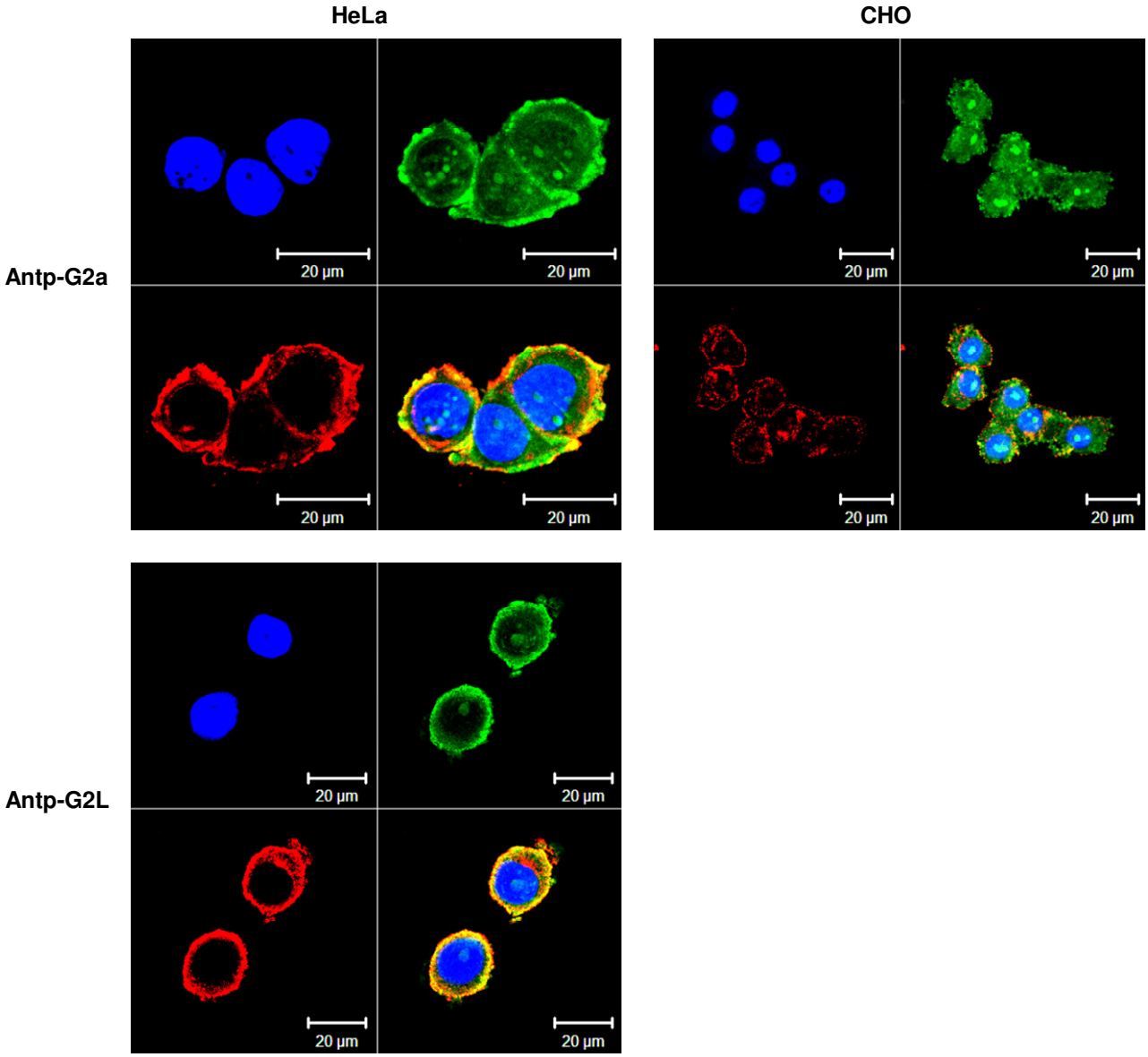
Figure S3. Primary flow cytometry data of CHO cells after incubation with linear and multivalent CPPs at 10 µM at 37 °C.

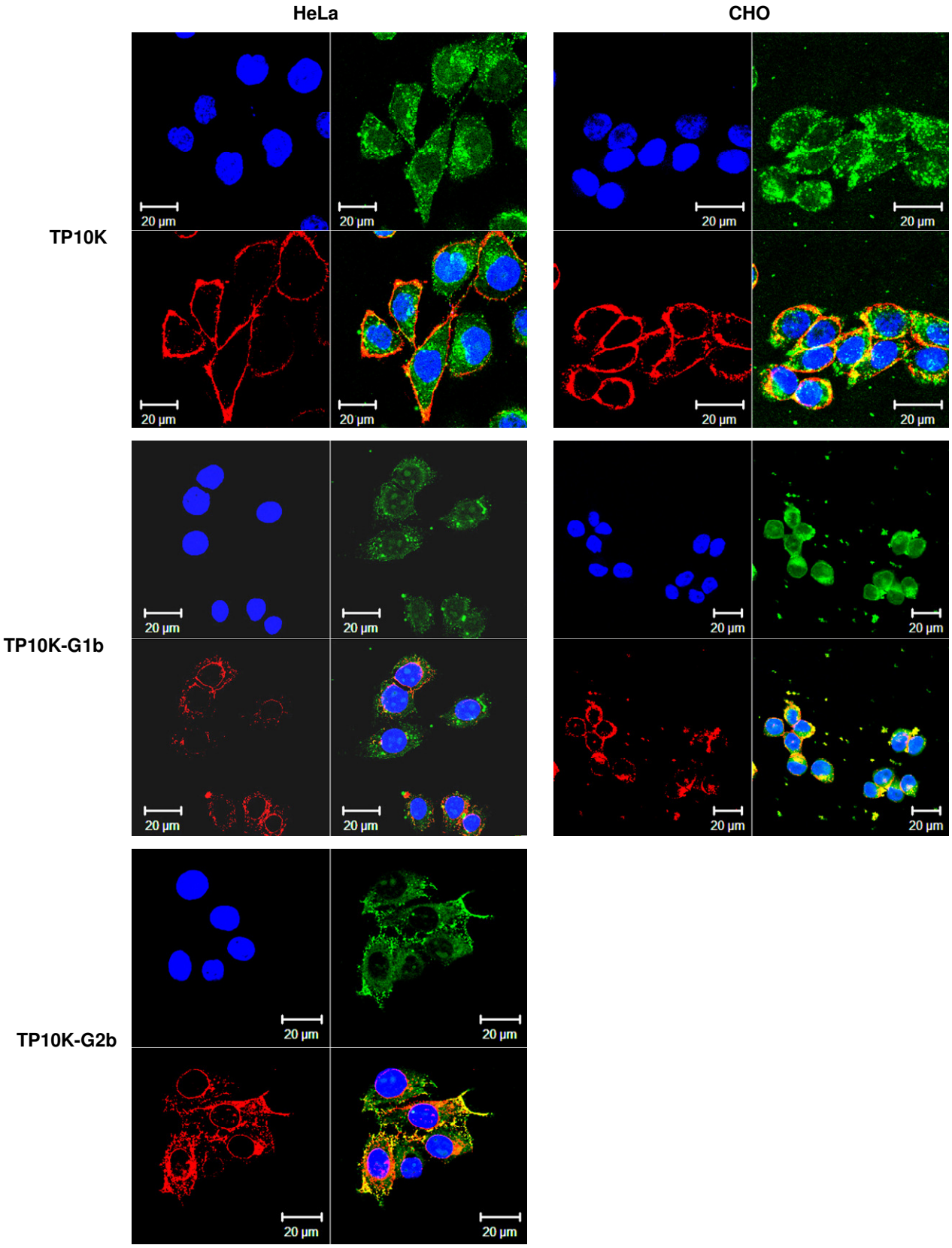
Figure S4 (next nine pages). Confocal microscopy images of fixed HeLa and CHO cells after 1 hour incubation at 37 °C with 10 µM of linear and multivalent CPPs. **pVEC-G2b** and **TP10K-G2b** in HeLa cells were measured with 1 µM. Representative images are shown. Blue = DAPI (nucleic acid stain). Green = 5(6)-carboxyfluorescein (compound). Red = Alexa Fluor® 594 WGA (wheat germ agglutinin) conjugate (plasma membrane).

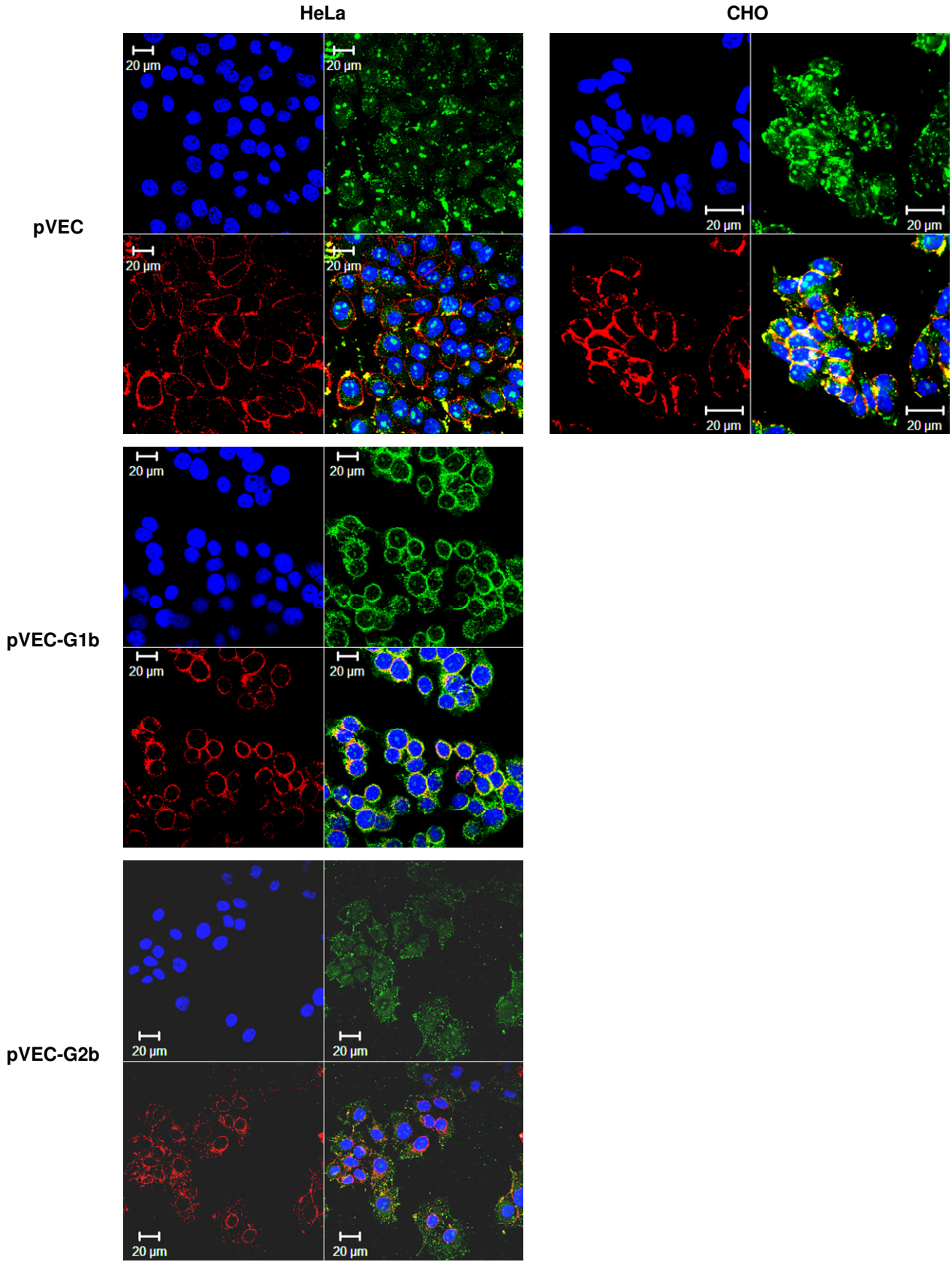

HeLa
CHO
Tat
Tat-G1a
Tat-G1L
20 μm

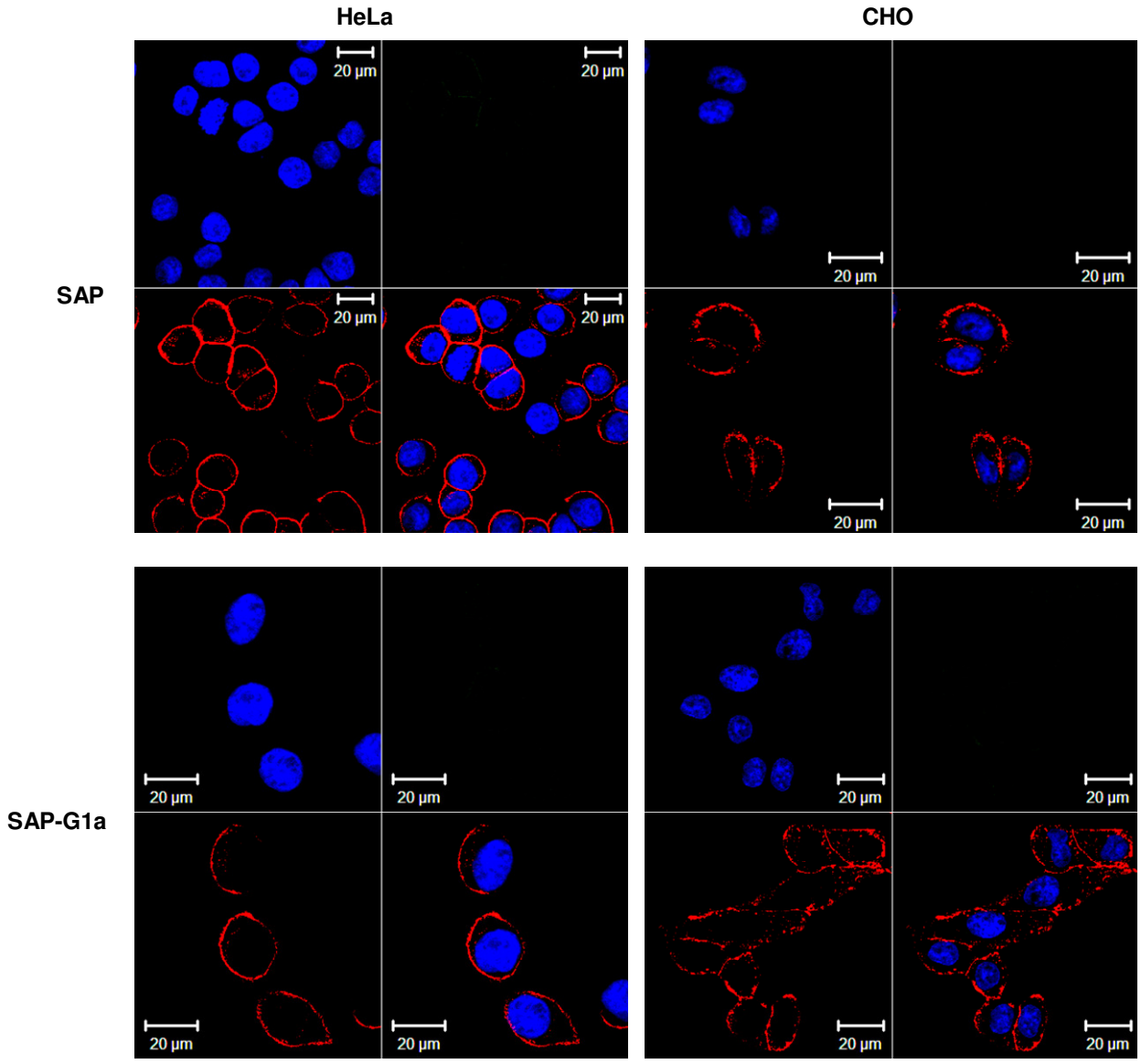


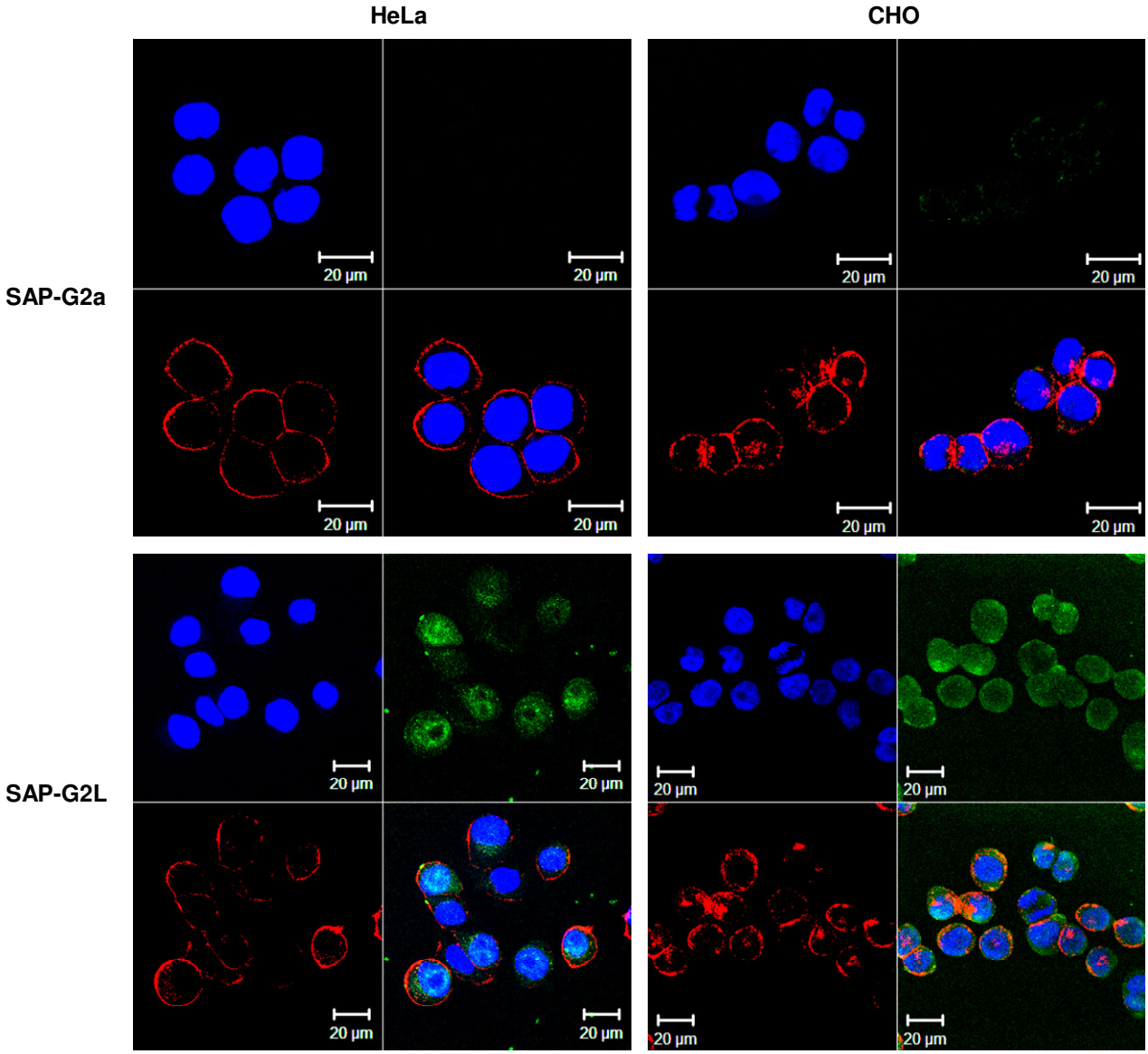


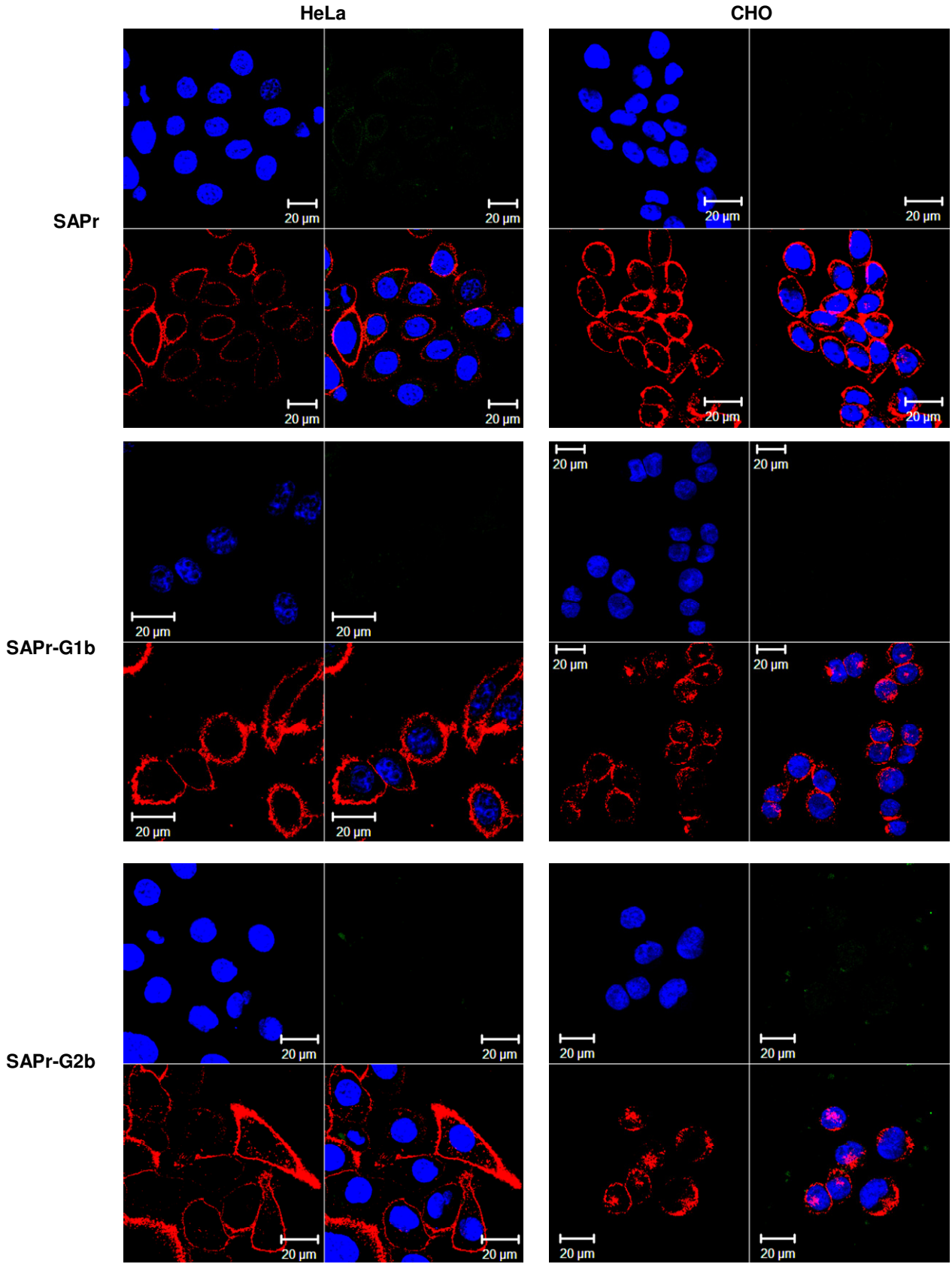









HeLa
CHO
SAP-G2a
SAP-G2L
20 μm


HeLa
CHO
SAPr
SAPr-G1b
SAPr-G2b
20 μm

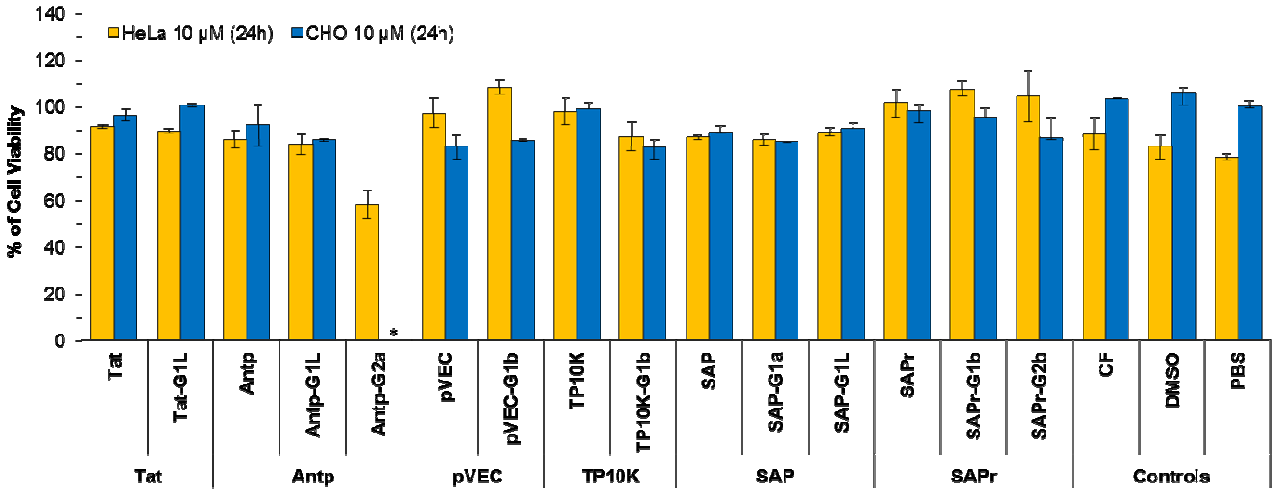


Figure S5. Cytotoxicity of linear and multivalent CPPs after 24 hour incubation at 10 µM: HeLa (yellow) and CHO (blue). Cell metabolic activity was evaluated with the WST8 assay. Untreated cells are defined as 100% viable, wells without cells set as 0% viability. Controls are 5(6)-carboxyfluorescein (CF), DMSO (1%) and PBS (10%). All measurements were done in duplicate. * = not determined.

Experimental Procedures

Amino acid analysis. Samples were hydrolyzed in the gas phase with 6M HCl containing 0.1% phenol (v/v) for 22h (or 48h) at 115°C under N_2 vacuum according to Chang and Knecht.[1] The liberated amino acids were coupled with phenylisothiocyanate (PITC), and the resulting phenylthiocarbamoyl (PTC) amino acids were analyzed by RP-HPLC on a Nova Pack C18 column (4 µm, 3.9 mm × 150 mm, Waters) with a Dionex Summit® HPLC system with an automatic injection system according to Bidlingmeyer et al.[2] The corresponding ammonium acetate buffer replaced the 0.14 M sodium acetate buffer, pH 6.3. Cysteine was detected as carboxymethyl cysteine (CMCys).

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). To cast the gels (84 × 73 × 0.75 mm), Bio-Rad short plates/spacer plates were filled with resolving gel until 2 cm under the glass rim and covered with miliQ-deionized H_2O. When polymerization was finished (~ 20 min) the water was removed and stacking gel was poured on top of the resolving gel. The pocket forming comb was inserted. Polymerization of the stacking gel was generally finished after 5 min. The gels were used in electrophoresis boxes purchased from Bio-Rad. After the addition of electrophoresis buffer, the gel pockets were washed with the latter and compounds (6-36 µg dissolved in a mixture of 12.5 µL miliQ-deionized H_2O and 2.5 µL sample buffer) or molecular marker (2.5 µL of the prepared solution) were added using a 50 µL glass syringe. Power was supplied by a Consort E452 (200 V) during 0.7 to 1.3 h. To develop the gels, they were removed from the glass plates and stained in a staining/fixation bath for 1h on a shaker followed by a background destaining bath (2h). The gels were washed in a mixture of miliQ-deionized H_2O/glycerol. Pictures of the gels were obtained by the use of a flat-bed scanner.

Preparation. All stock solutions and buffers were filtered prior to use.

Resolving gel 20% (for two gels). 1.5 M Tris·Base pH 8.7 (2.5 mL), aq. solution of SDS (10%, 0.1 mL), aq. solution of acrylamide (Rotiphorese Gel A®, 30%, 6.65 mL), aq. solution of bisacrylamide (Rotiphorese Gel B®, 2%, 0.325 mL), aq. solution of APS (10%, 33 µL), TMED (5 µL).

Stacking gel 4.3% (for two gels). miliQ-deionized H_2O (2 mL), 0.25 M Tris·Base pH 6.8 with 0.2% of SDS and Coomassie® Brilliant Blue R-250 (2.5 mL), aq. solution of acrylamide (30%) and bisacrylamide (0.8%) (0.75 mL), aq. solution of APS (10%, 50 µL), TMED (10 µL).

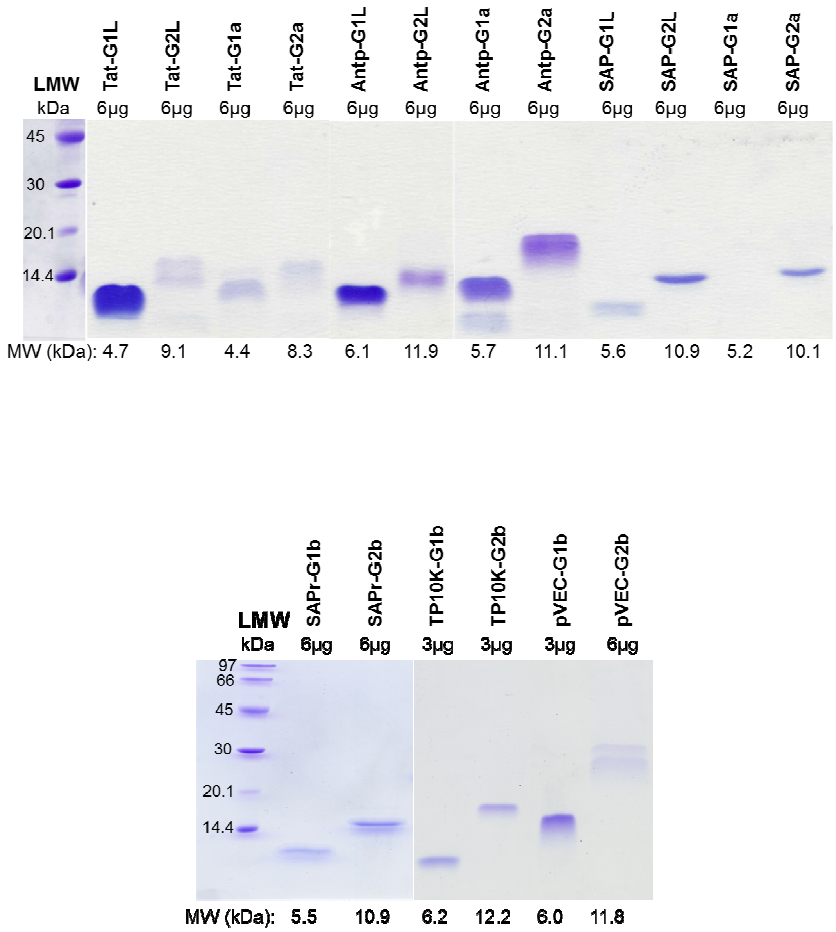
Sample buffer. SDS (5 g), 1M Tris·Base pH 6.8 (15 mL), glycerol (22.5 mL), β-mercaptoethanol (12.5 mL), bromphenol blue.

Molecular Marker. Calibration Kit 17-0446-01 purchased from GE Healthcare was used as molecular marker. The protein mixture containing phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) was dissolved in miliQ-deionized H_2O (160 µL) and sample buffer (40 µL). The solution was heated at 100 °C for 5 min and cooled back down to room temperature prior to use on the gel.

Electrophoresis buffer. To a solution of glycine (72 g) and Tris·Base (15 g) in miliQ-deionized H_2O (975 mL) a solution of SDS (10%, 25 mL) was added.

Staining/fixation bath. MeOH (500 mL), acetic acid (99%, 125 mL), miliQ-deionized H_2O (625 mL), Coomassie® Brilliant Blue R-250 (2.5 g).

Background destaining bath. MeOH (2.5 L), acetic acid (99%, 0.5 L), miliQ-deionized H_2O (2.5 L).



LMW: low molecular weight marker

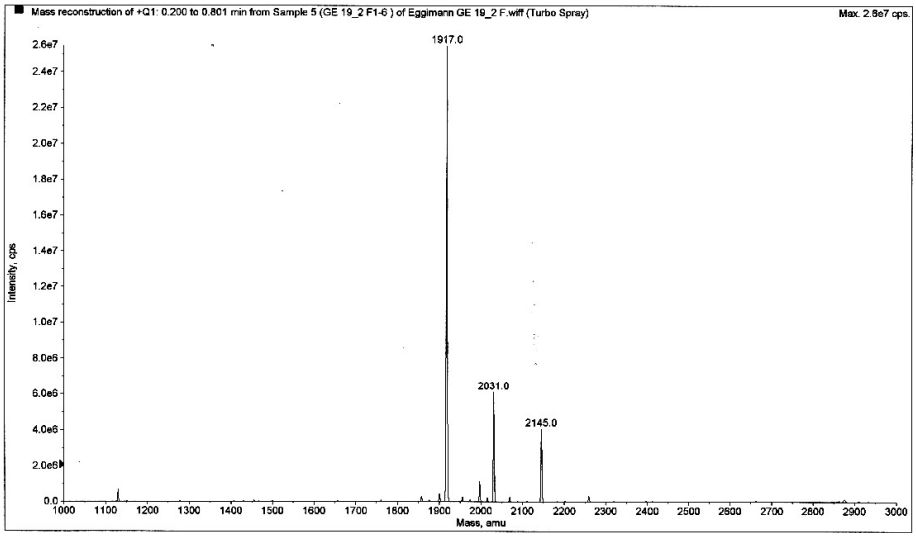
Figure S6. SDS PAGE analysis of multivalent CPPs.

Synthesis and Characterization

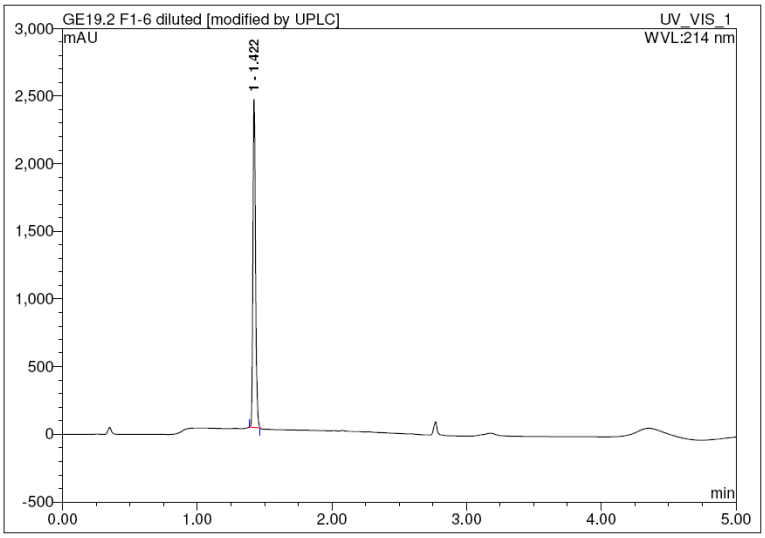
Linear Peptides. Ac denotes an acetyl group attached to a free amine group. ClAc denotes a chloroacetyl group attached to a free amine group. * denotes 5(6)-carboxyfluorescein attached to a free amine group through an amide bond.

Tat (*-YGRKKRRQRRR). From Tenta Gel S RAM® resin (500 mg, 0.25 mmol·g^{-1}), **Tat** was obtained as a foamy yellow solid after preparative RP-HPLC (90.9 mg, 32.1 µmol, 26%). Analytical RP-HPLC: t_R = 1.42 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{85}H_{129}N_{33}O_{19}$ found/calc. 1917.0/1917.1 $[M]^+$; 2031.0/2031.1 $[M + TFA]^+$; 2145.0/2145.1 $[M + 2 TFA]^+$.

Mass spectrum, MS (ESI+):

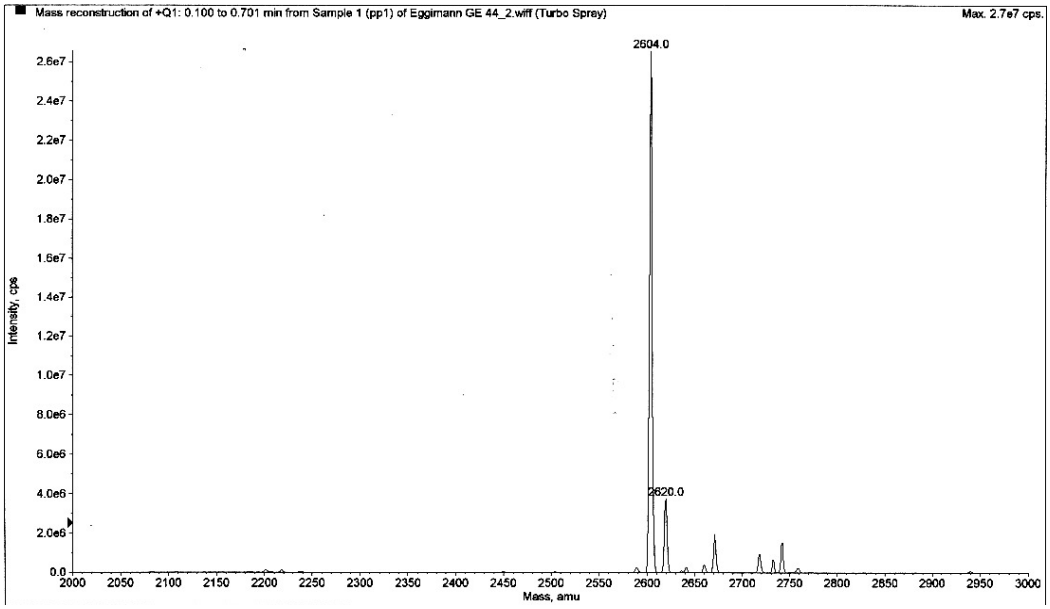


Analytical RP-HPLC chromatogram:

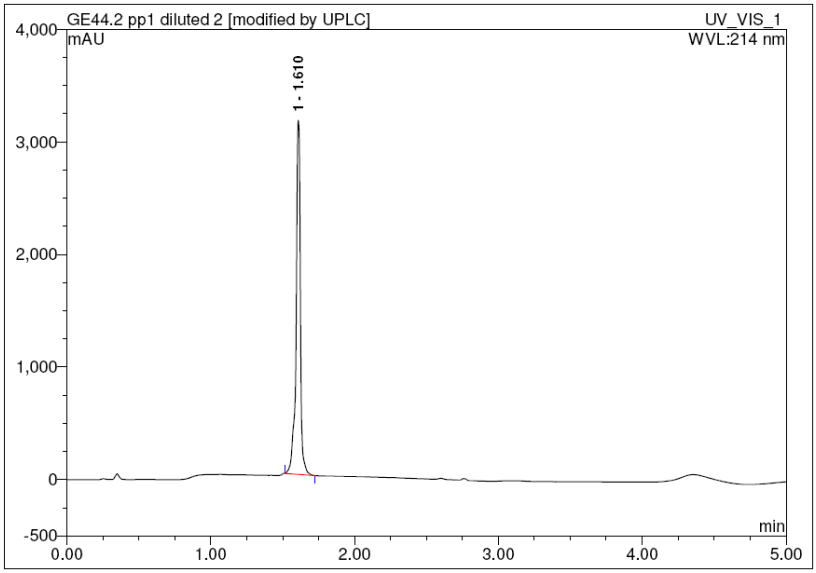


Antp (*-RQIKIWFQNRRMKWKK). From Tenta Gel S RAM® resin (500 mg, 0.25 mmol·g^{-1}), **Antp** was obtained as a foamy yellow solid after preparative RP-HPLC (119.0 mg, 45.7 µmol, 28%). Analytical RP-HPLC: t_R = 1.61 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{125}H_{179}N_{35}O_{25}S$ found/calc. 2604.0/2604.0 $[M]^+$.

Mass spectrum, MS (ESI+):

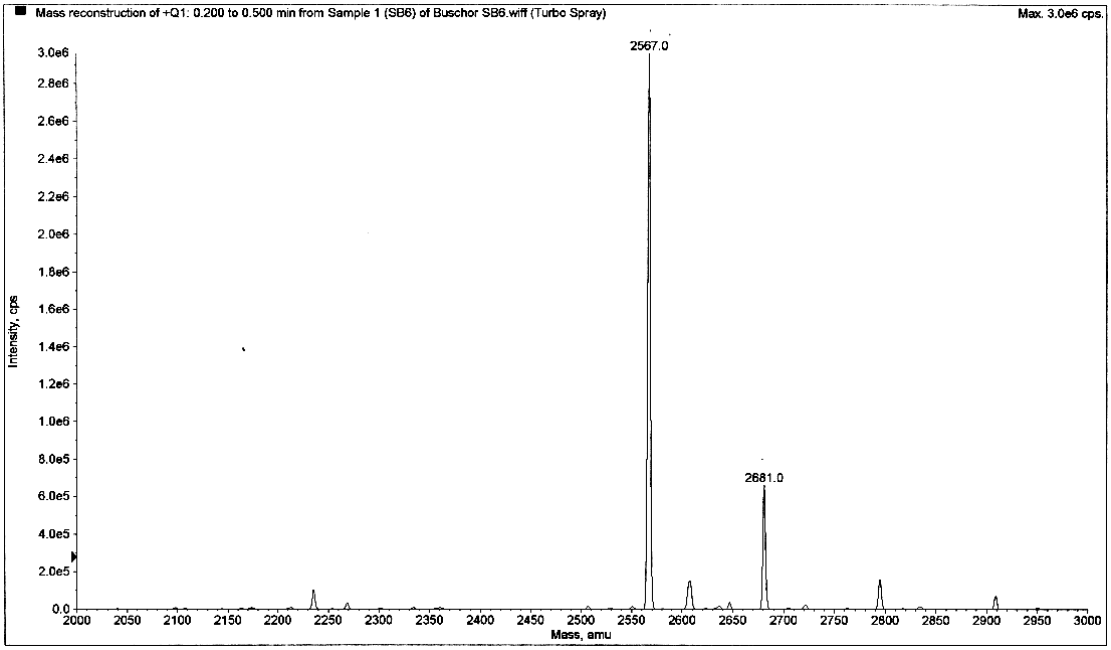


Analytical RP-HPLC chromatogram:

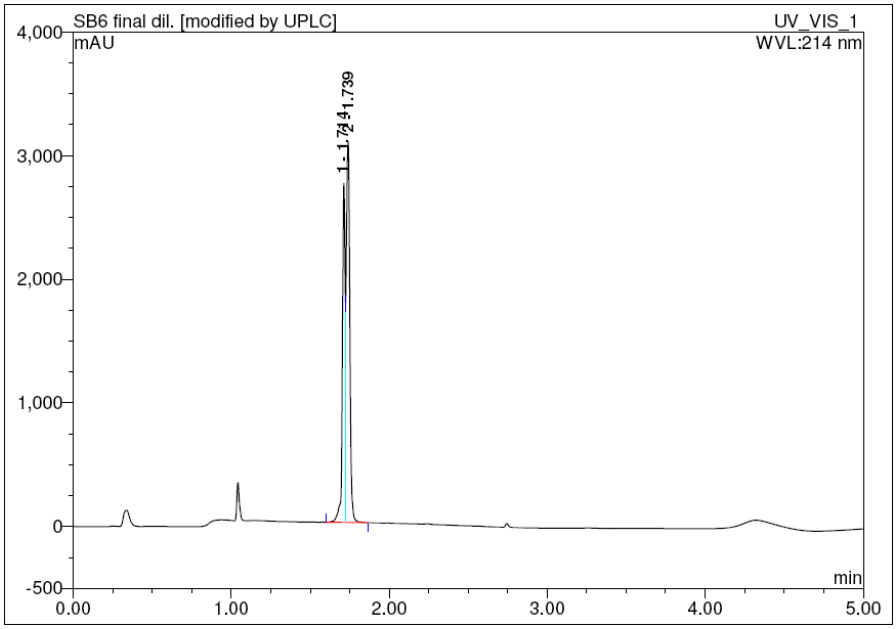


pVEC (*-LLIILRRRIRKQAHAHSK). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **pVEC** was obtained as a foamy yellow solid after preparative RP-HPLC (94.3 mg, 29.0 µmol, 26%). Analytical RP-HPLC: t_R = 1.71 and 1.74 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{119}H_{188}N_{38}O_{26}$ found/calc. 2567.0/2567.0 $[M]^+$; 2681.0/2681.0 $[M + TFA]^+$.

Mass spectrum, MS (ESI+):

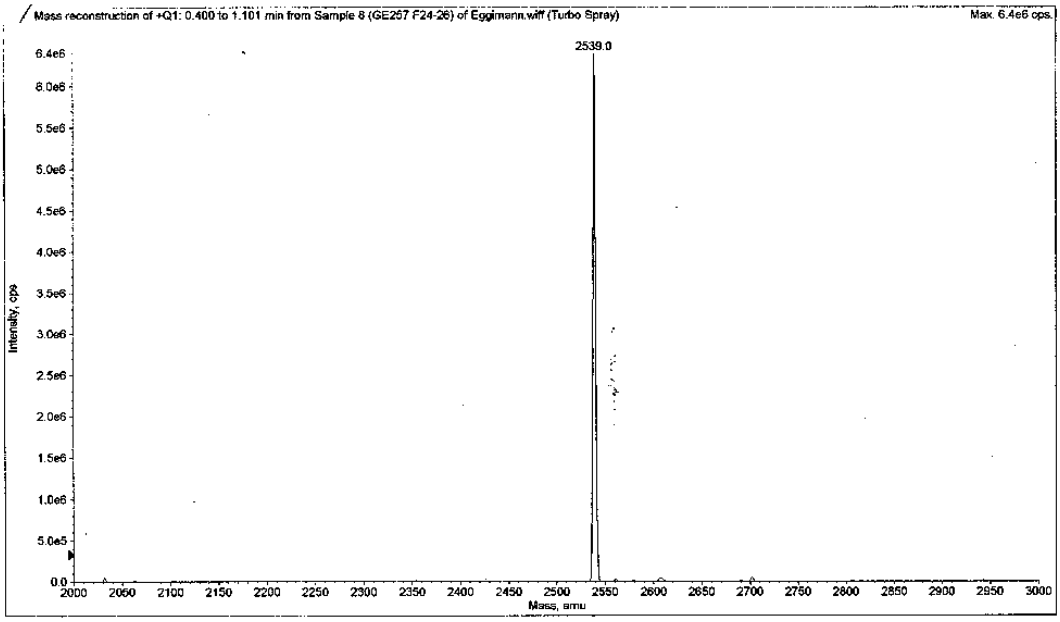


Analytical RP-HPLC chromatogram:

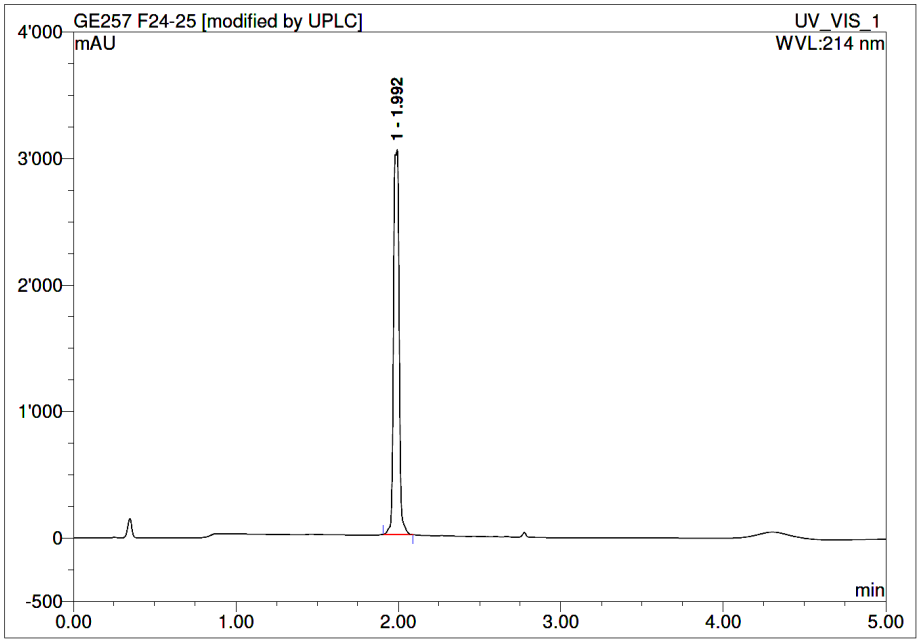


TP10 (*-AGYLLGKINLKALAALAKKIL). From Tenta Gel S RAM® resin (500 mg, 0.23 mmol·g^{-1}), **TP10** was obtained as a foamy yellow solid after preparative RP-HPLC (51.3 mg, 17.1 µmol, 15%). Analytical RP-HPLC: t_R = 1.99 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{125}H_{196}N_{27}O_{29}$ found/calc. 2539.0/2541.1 $[M]^+$.

Mass spectrum, MS (ESI+):

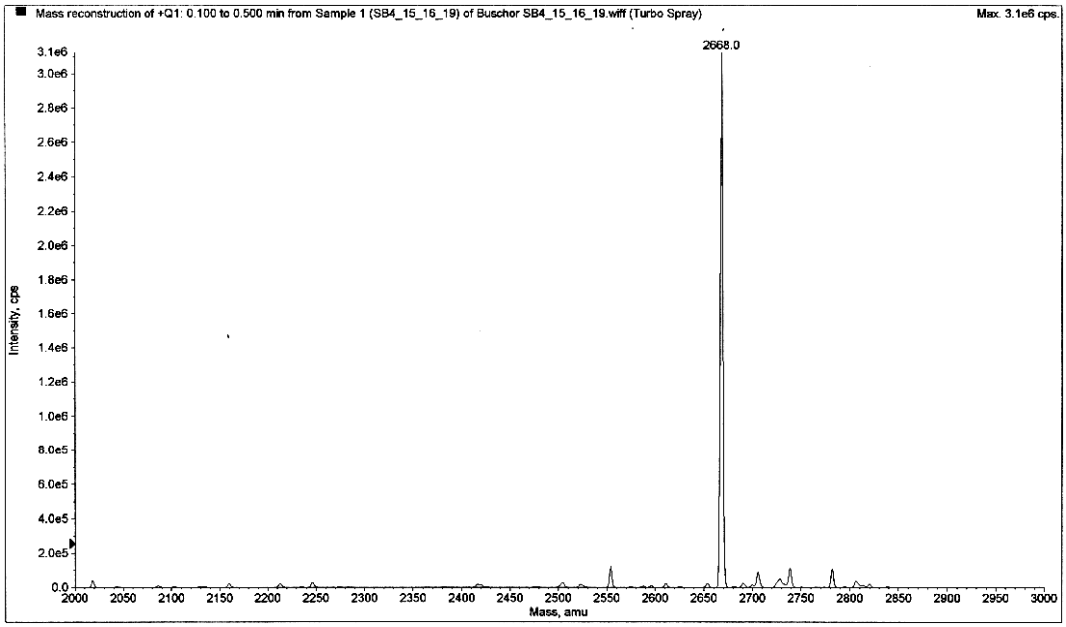


Analytical RP-HPLC chromatogram:

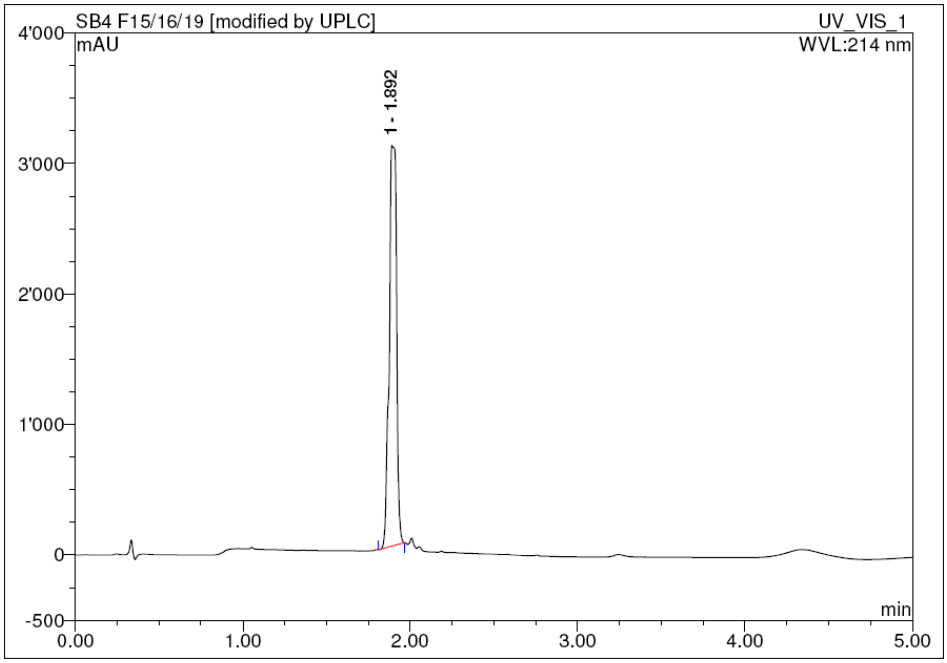


TP10K (*-AGYLLGKINKLKALAALAKKIL). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **TP10K** was obtained as a foamy yellow solid after preparative RP-HPLC (55.6 mg, 17.2 µmol, 16%). Analytical RP-HPLC: t_R = 1.89 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{131}H_{207}N_{29}O_{30}$ found/calc. 2668.0/2668.2 $[M]^+$.

Mass spectrum, MS (ESI+):

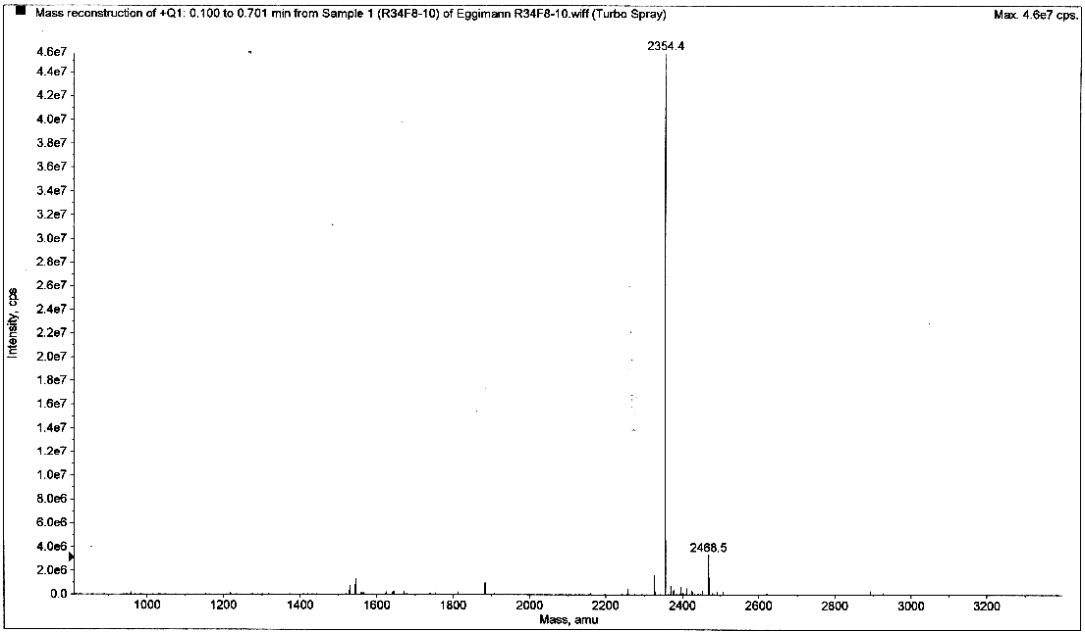


Analytical RP-HPLC chromatogram:



SAP (*-[VRLPPP]$_3$). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **SAP** was obtained as a foamy yellow solid after preparative RP-HPLC (78.3 mg, 29.0 µmol, 43%). Analytical RP-HPLC: t_R = 1.72 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{117}H_{172}N_{28}O_{24}$ found/calc. 2354.4/2354.8 $[M]^+$; 2468.5/2468.8 $[M + TFA]^+$.

Mass spectrum, MS (ESI+):



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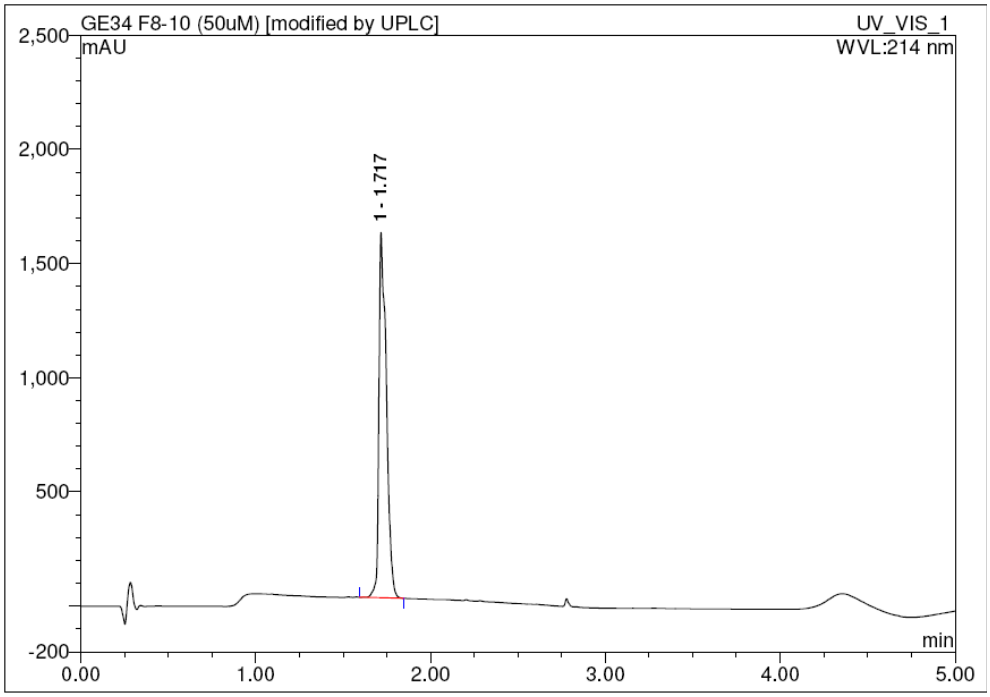
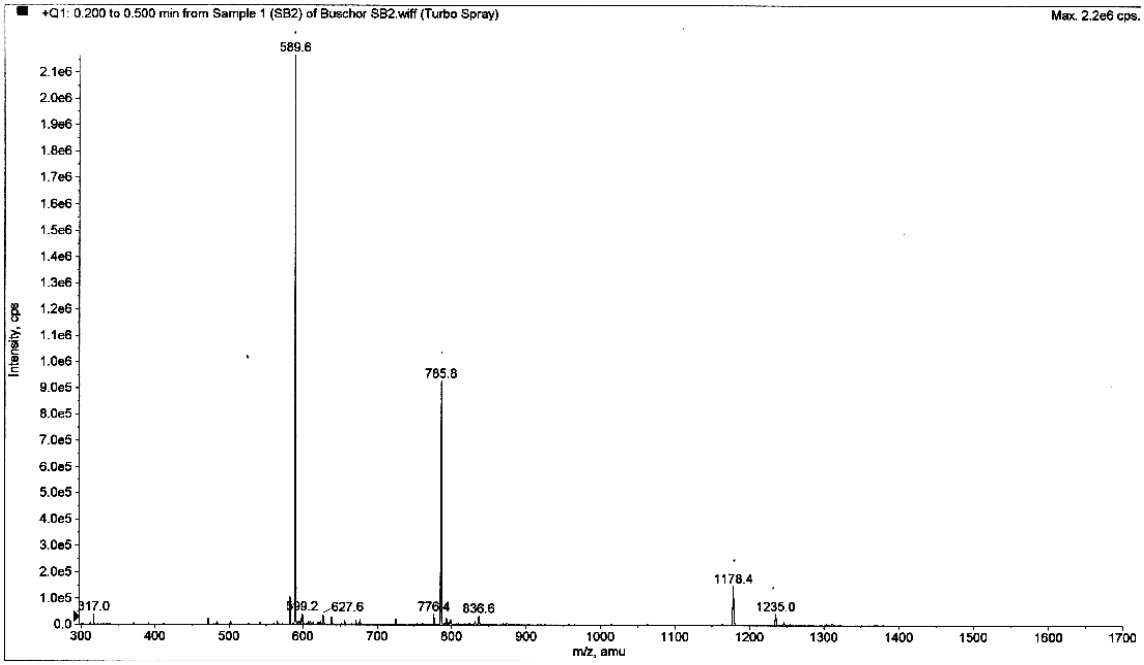
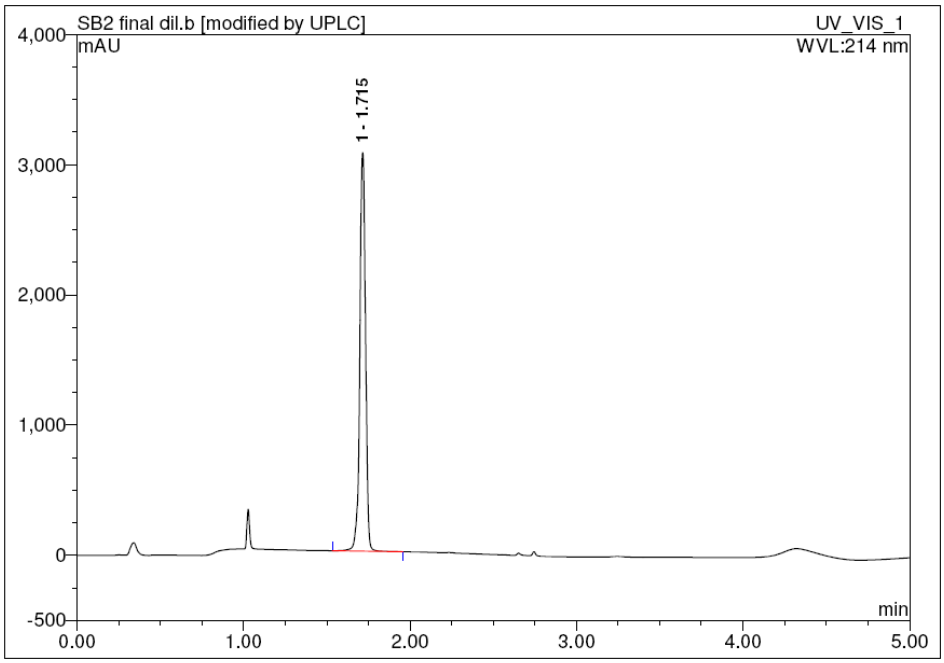


SAPr (*-[PPPLRV]$_3$). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **SAPr** was obtained as a foamy yellow solid after preparative RP-HPLC (121.7 mg, 45.1 µmol, 36%). Analytical RP-HPLC: t_R = 1.72 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{117}H_{172}N_{28}O_{24}$ found/calc. 2354.5/2354.8 $[M]^+$.

Mass spectrum, MS (ESI+):

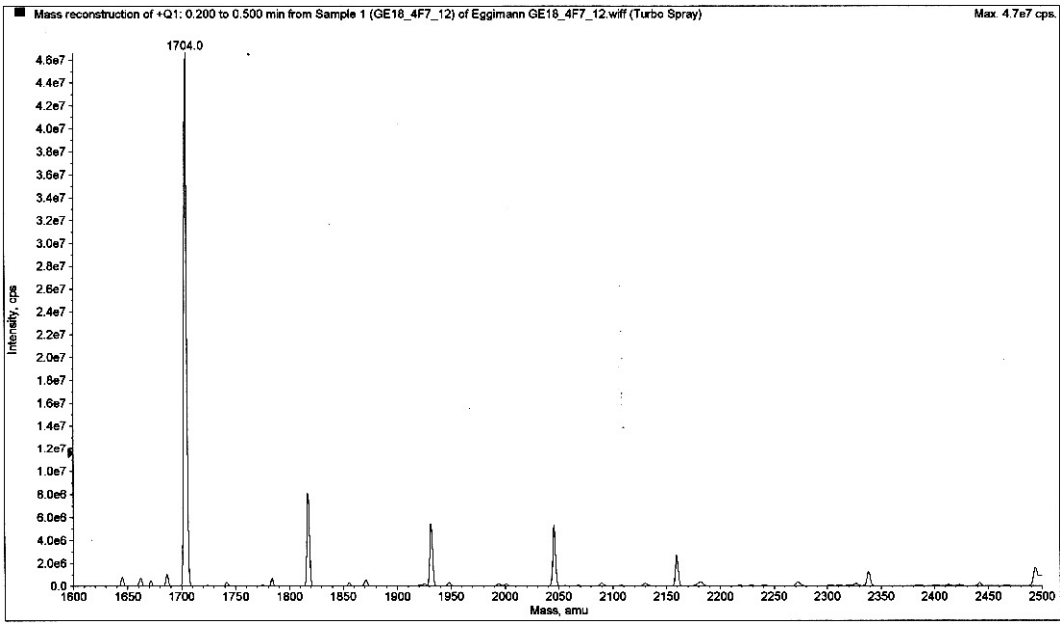


Analytical RP-HPLC chromatogram:

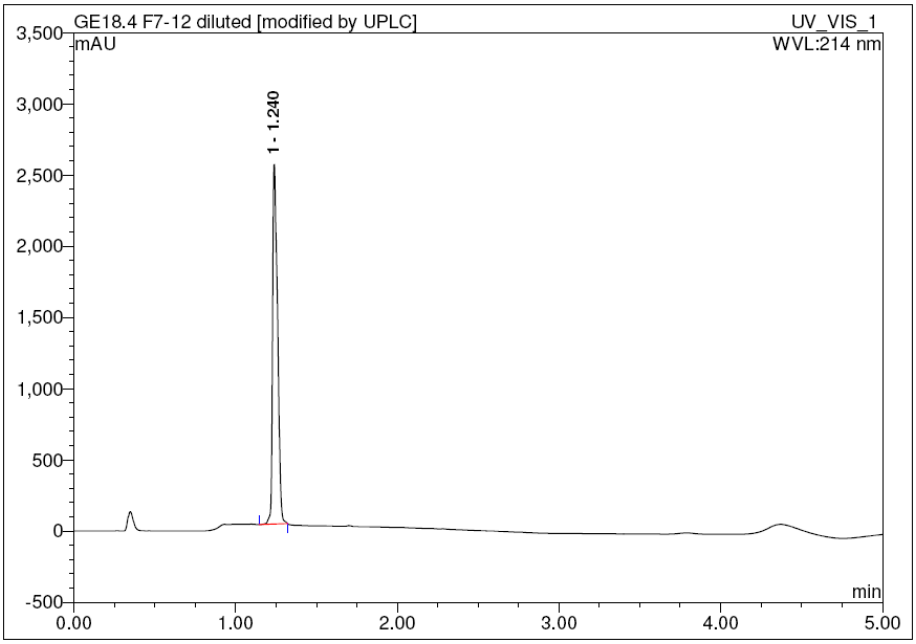


Cys-Tat (AcCYGRKKRRQRRR). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **Cys-Tat** was obtained as a foamy colourless solid after preparative RP-HPLC (97.8 mg, 37.4 µmol, 43%). Analytical RP-HPLC: t_R = 1.24 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{69}H_{126}N_{34}O_{25}S$ found/calc. 1704.0/1704.0 $[M]^+$.

Mass spectrum, MS (ESI+):

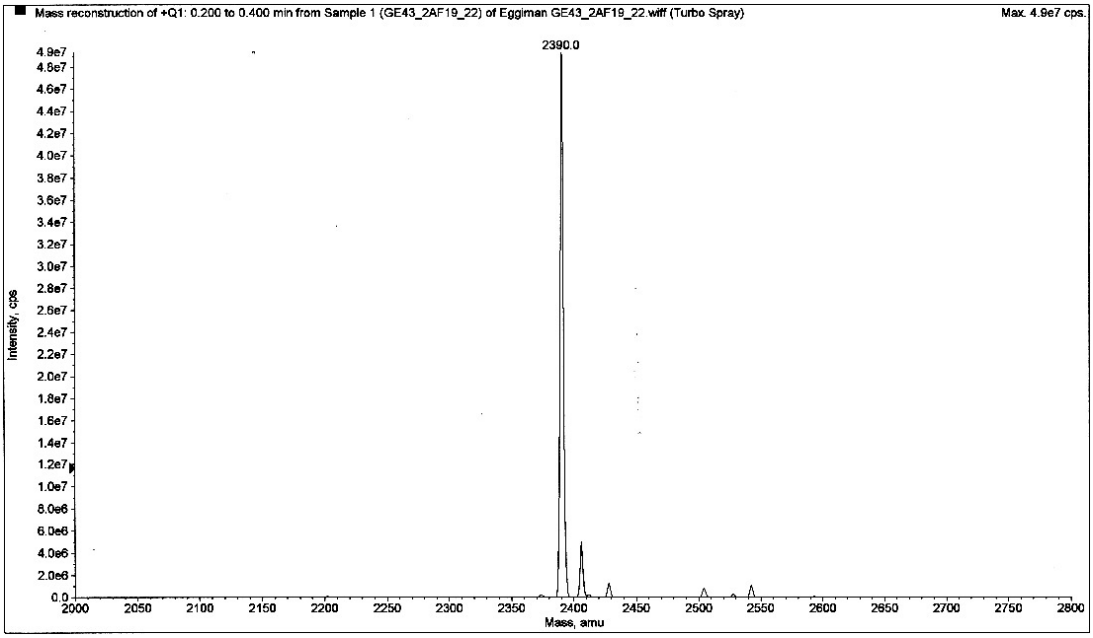


Analytical RP-HPLC chromatogram:

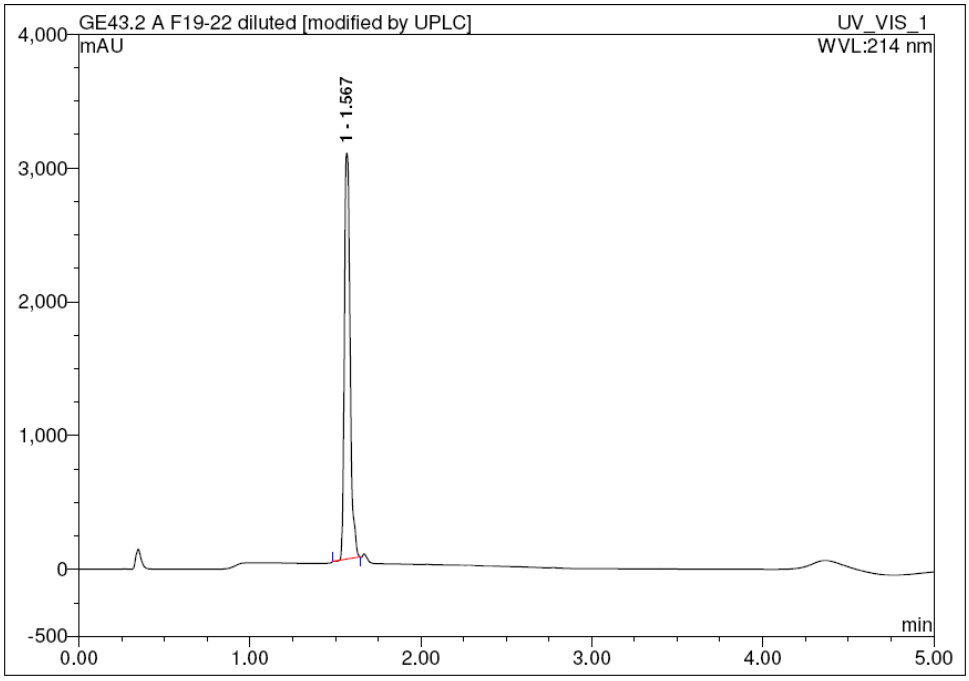


Cys-Antp (AcCRQIKIWFQNRRMKWKK). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **Cys-Antp** was obtained as a foamy colourless solid after preparative RP-HPLC (119.5 mg, 37.5 µmol, 34%). Analytical RP-HPLC: t_R = 1.57 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{109}H_{179}N_{36}O_{21}S_2$ found/calc. 2390.0/2390.92 $[M]^+$.

Mass spectrum, MS (ESI+):

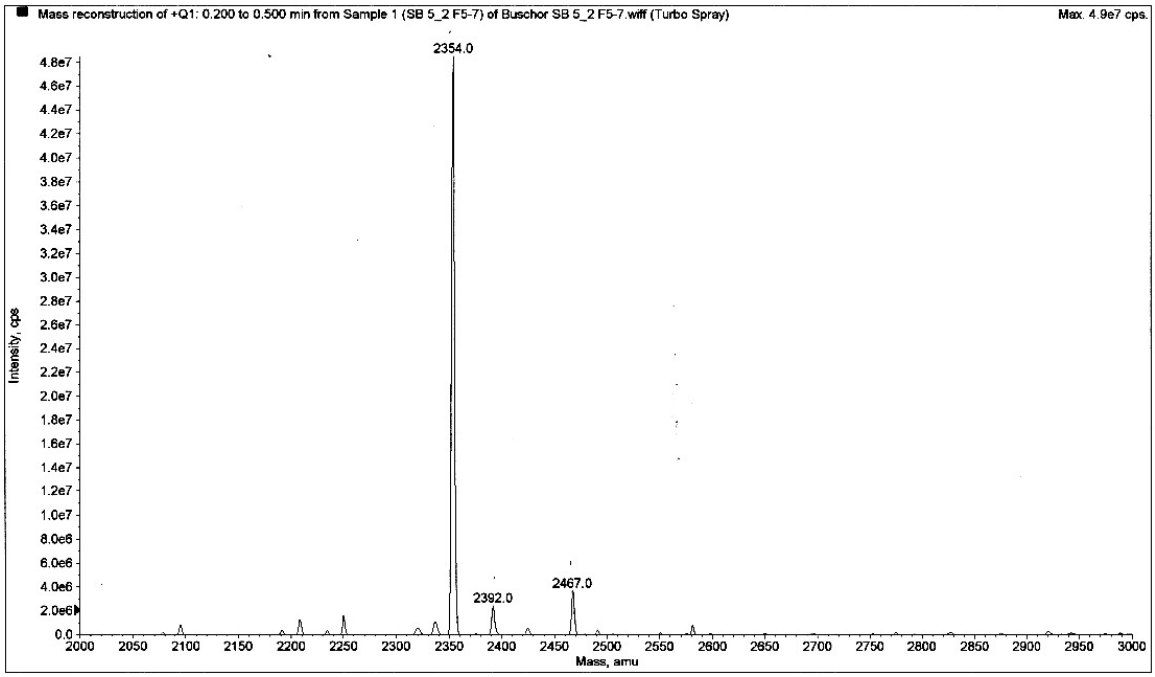


Analytical RP-HPLC chromatogram:

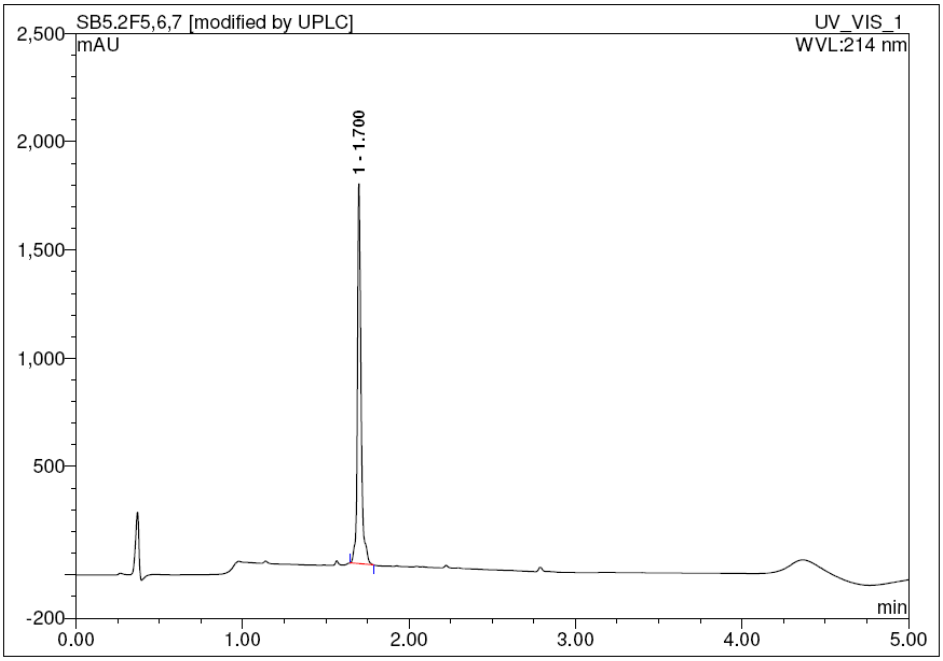


Cys-pVEC (AcCLLIILRRRIRKQAHAHSK). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **Cys-pVEC** was obtained as a foamy colourless solid after preparative RP-HPLC (87.3 mg, 28.7 µmol, 26%). Analytical RP-HPLC: t_R = 1.70 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{103}H_{184}N_{39}O_{22}S$ found/calc. 2354.0/2353.88 $[M]^+$; 2392.0/2392.9 $[M + K]^+$; 2467.0/2467.9 $[M + TFA]^+$.

Mass spectrum, MS (ESI+):

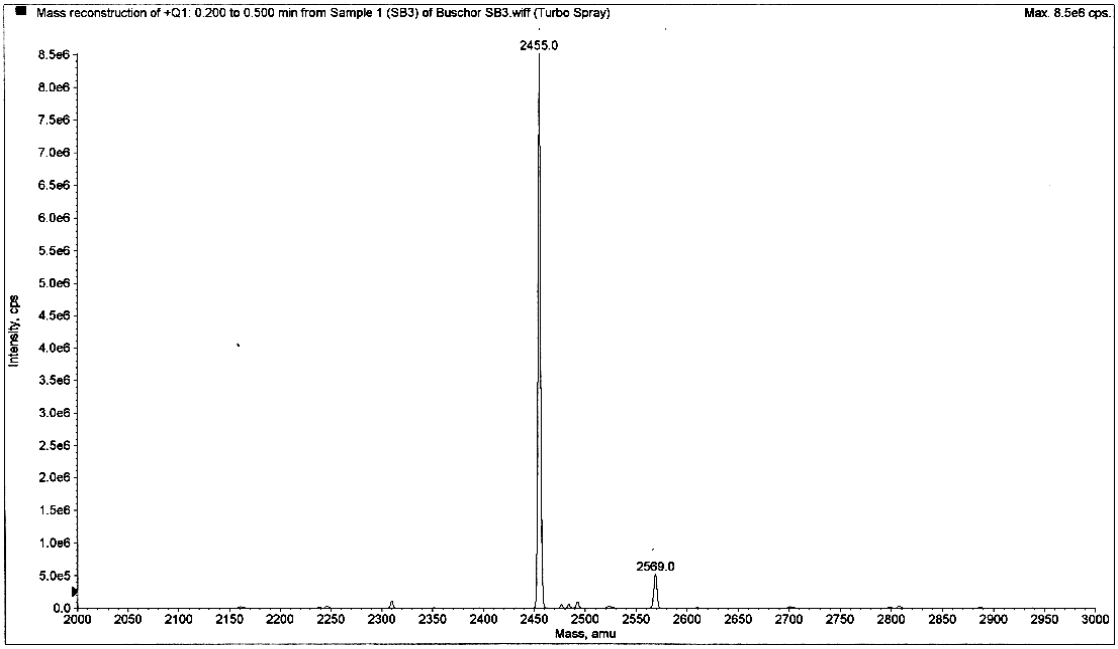


Analytical RP-HPLC chromatogram:

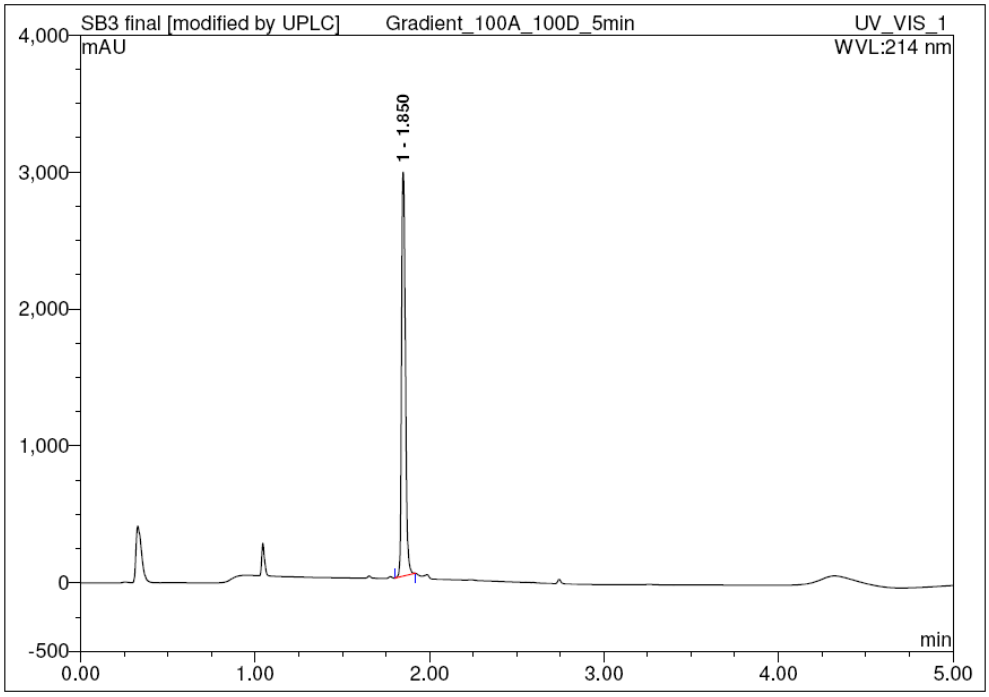


Cys-TP10K (AcCAGYLLGKINKLKALAALAKKIL). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **Cys-TP10K** was obtained as a foamy colourless solid after preparative RP-HPLC (73.8 mg, 24.4 µmol, 22%). Analytical RP-HPLC: t_R = 1.85 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{115}H_{204}N_{30}O_{26}S$ found/calc. 2455.0/2455.1 $[M]^+$; 2569.0/2569.1 $[M + TFA]^+$.

Mass spectrum, MS (ESI+):

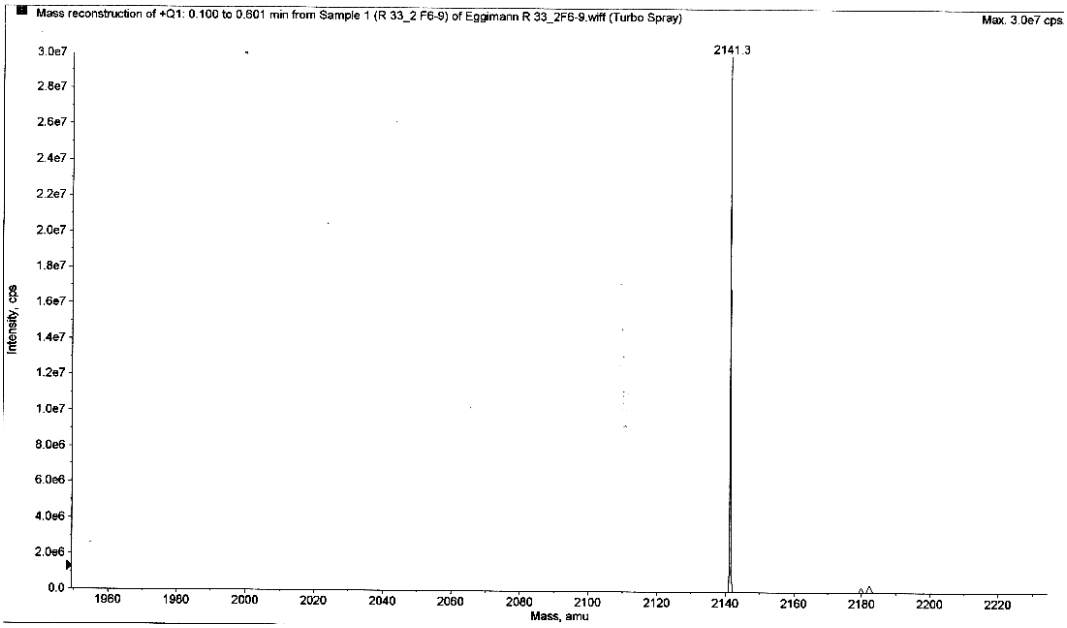


Analytical RP-HPLC chromatogram:

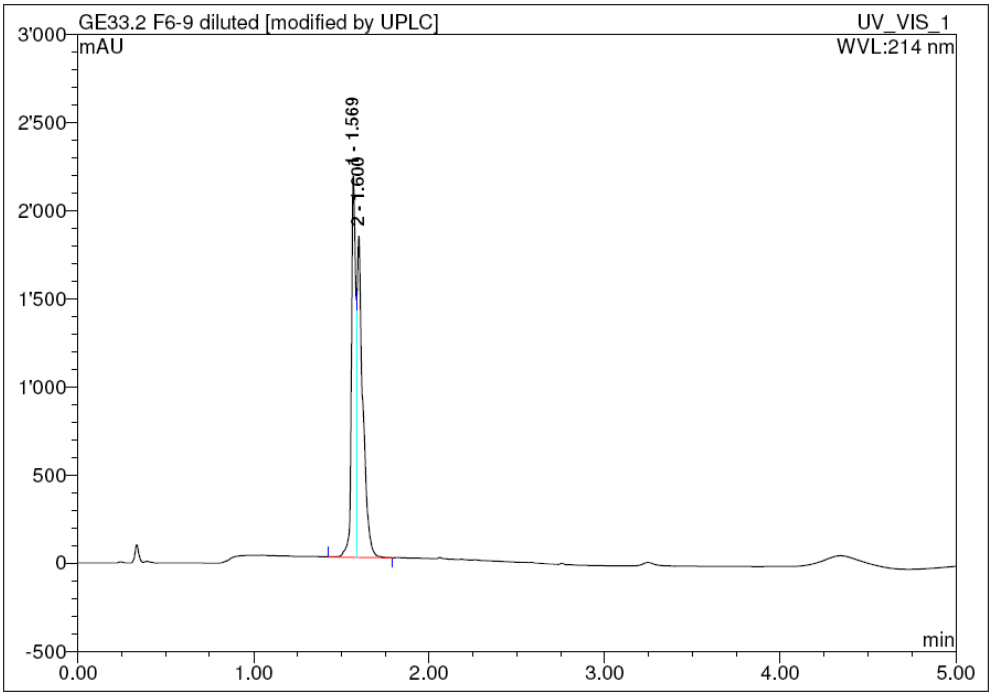


Cys-SAP (AcC[VRLPPP]$_3$). From Tenta Gel S RAM® resin (2 g, 0.39 mmol·g^{-1}), **Cys-SAP** was obtained as a foamy colourless solid after preparative RP-HPLC (193.8 mg, 78.0 µmol, 10%). Analytical RP-HPLC: t_R = 1.57 and 1.60 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{101}H_{169}N_{29}O_{20}S$ found/calc. 2141.3/2141.7 $[M]^+$.

Mass spectrum, MS (ESI+):

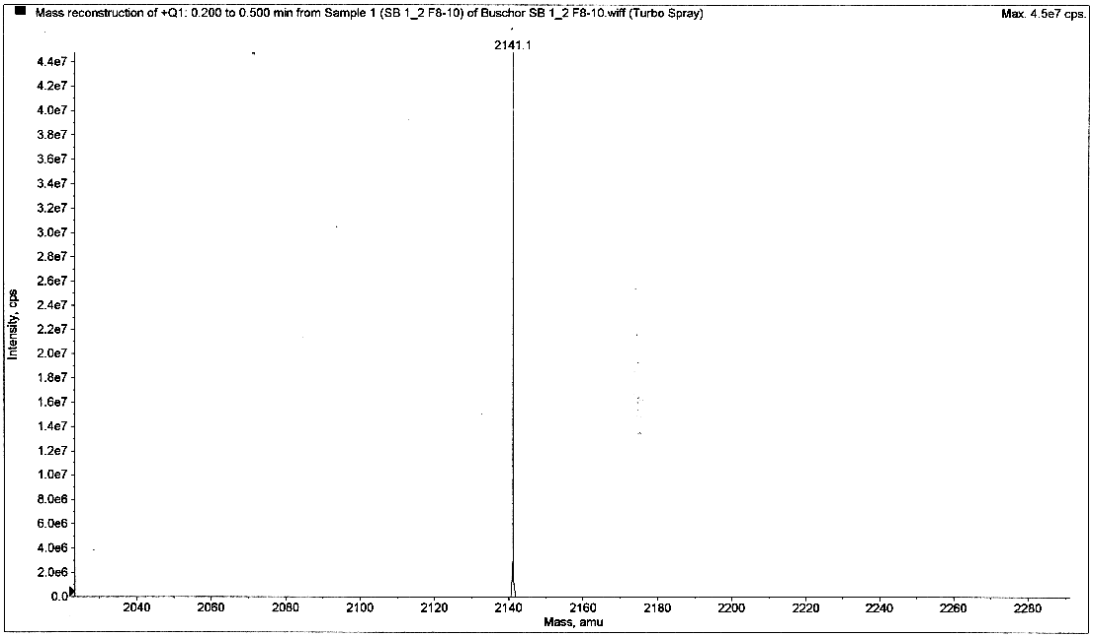


Analytical RP-HPLC chromatogram:

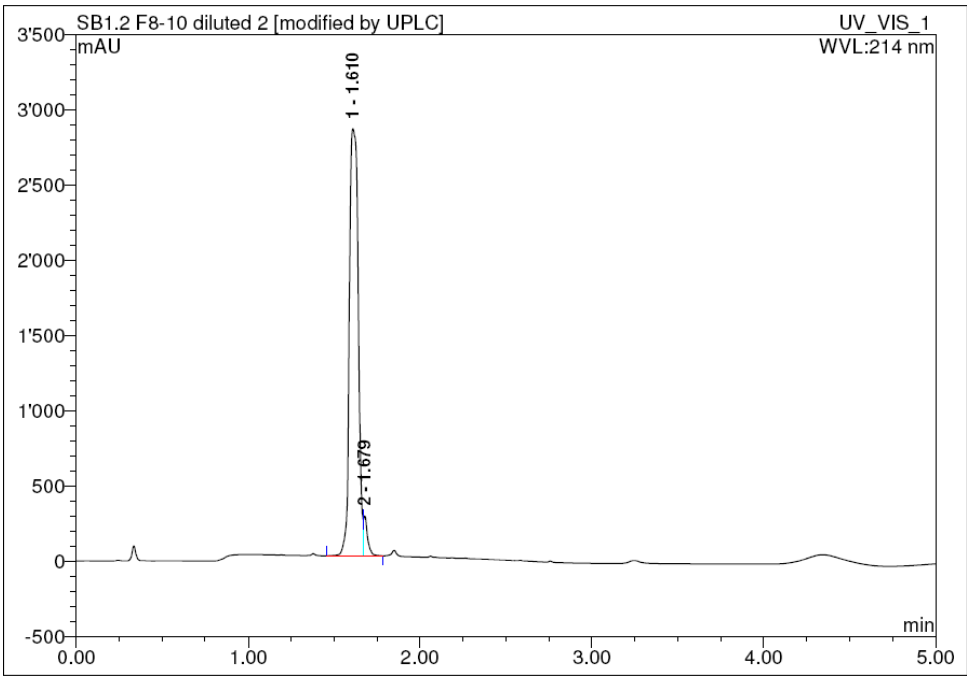


Cys-SAPr (AcC[PPPLRV]$_3$). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **Cys-SAPr** was obtained as a foamy colourless solid after preparative RP-HPLC (73.7 mg, 29.7 µmol, 27%). Analytical RP-HPLC: t_R = 1.61 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{101}H_{169}N_{29}O_{20}S$ found/calc. 2141.1/2141.7 $[M]^+$.

Mass spectrum, MS (ESI+):

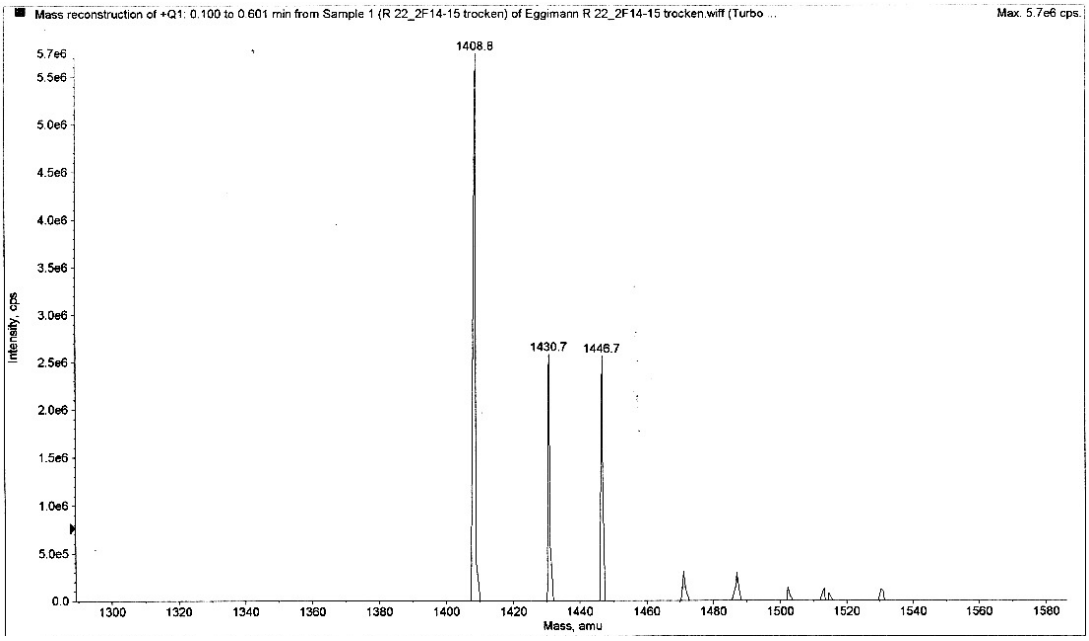


Analytical RP-HPLC chromatogram:

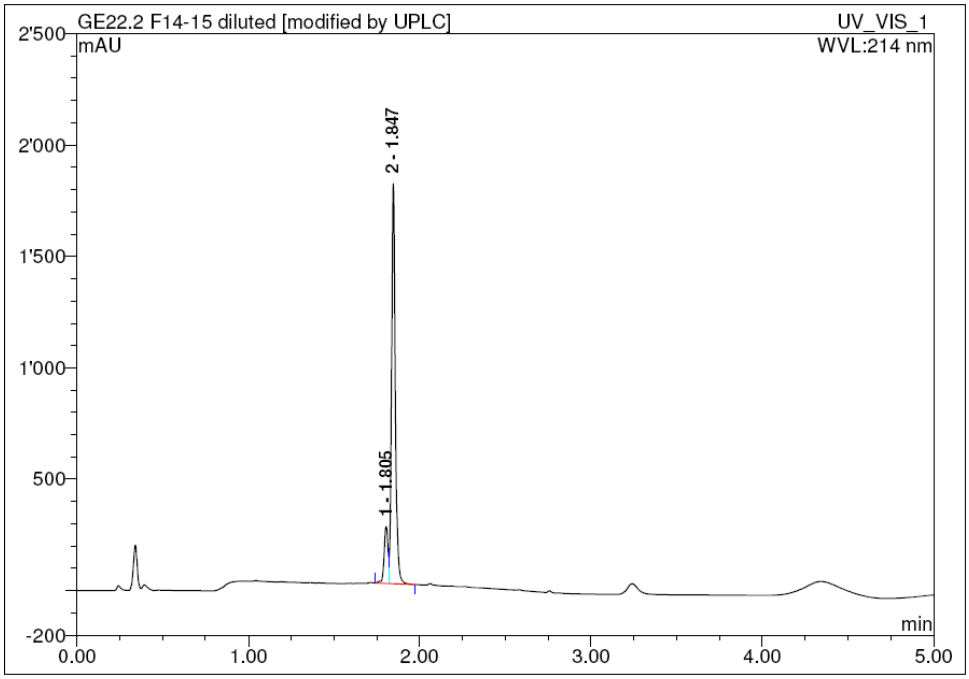


G1L (*-[K(ClAc)LAQ]$_2$). From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G1L** was obtained as a foamy yellow solid after preparative RP-HPLC (27.4 mg, 19.4 µmol, 16%). Analytical RP-HPLC: t_R = 1.85 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{65}H_{87}Cl_2N_{13}O_{18}$ found/calc. 1408.8/1409.4 $[M]^+$; 1430.7/1432.3 $[M + Na]^+$; 1446.7/1448.5 $[M + K]^+$.

Mass spectrum, MS (ESI+):

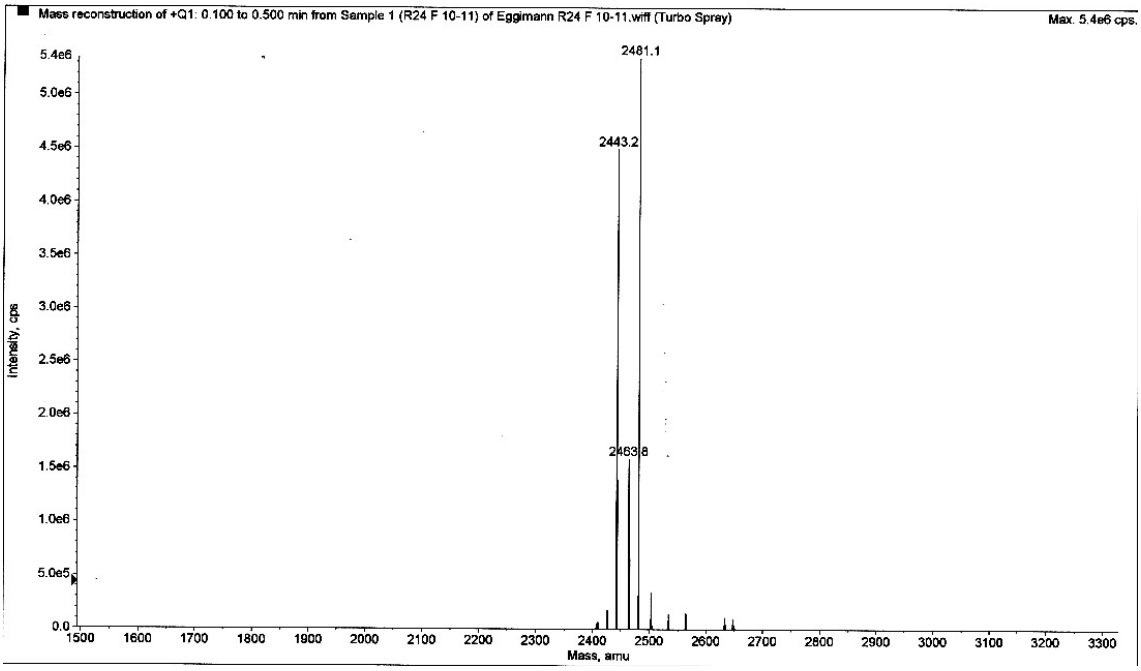


Analytical RP-HPLC chromatogram:

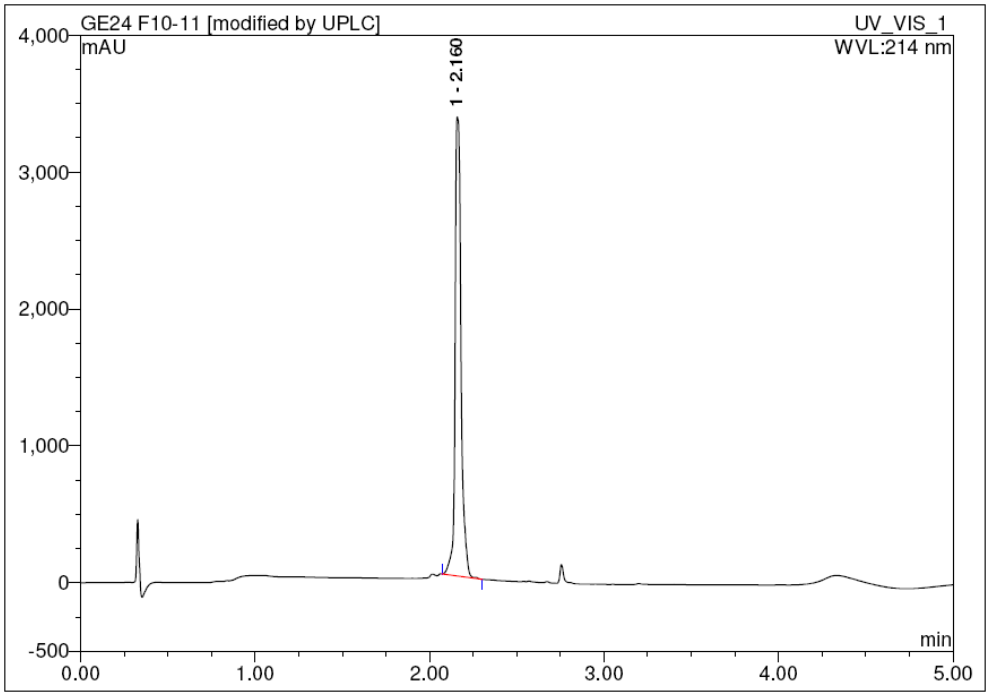


G2L (*-[K(ClAc)LAQ]$_4$). From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2L** was obtained as a foamy yellow solid after preparative RP-HPLC (60.3 mg, 24.7 µmol, 20%). Analytical RP-HPLC: t_R = 2.16 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{109}H_{161}Cl_4N_{25}O_{30}$ found/calc. 2443.2/2443.4 $[M]^+$; 2463.8/2466.3 $[M + Na]^+$; 2481.1/2482.5 $[M + K]^+$.

Mass spectrum, MS (ESI+):

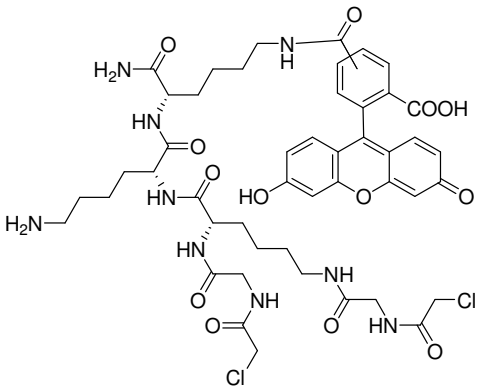


Analytical RP-HPLC chromatogram:

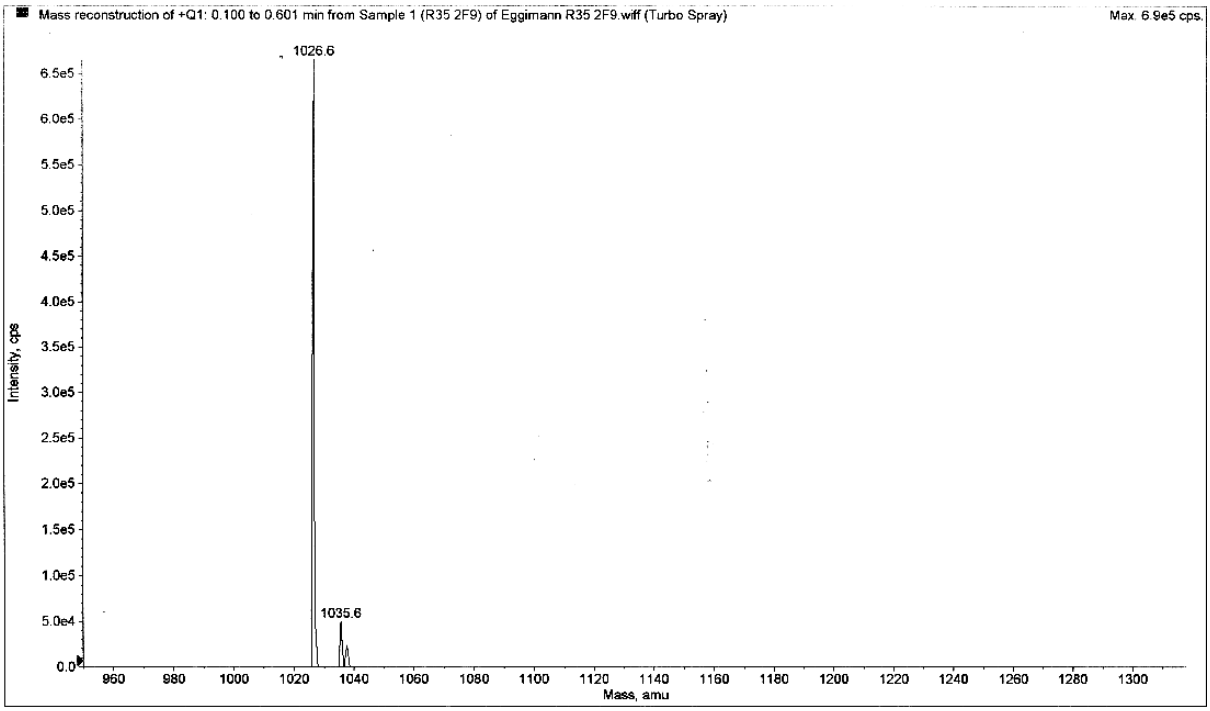


Peptide Dendrimers. Ac denotes an acetyl group attached to a free amine group. ClAc denotes a chloroacetyl group attached to a free amine group. * denotes 5(6)-carboxyfluorescein attached to a free amine group through an amide bond. Italics *K* detones a branching lysine residues.

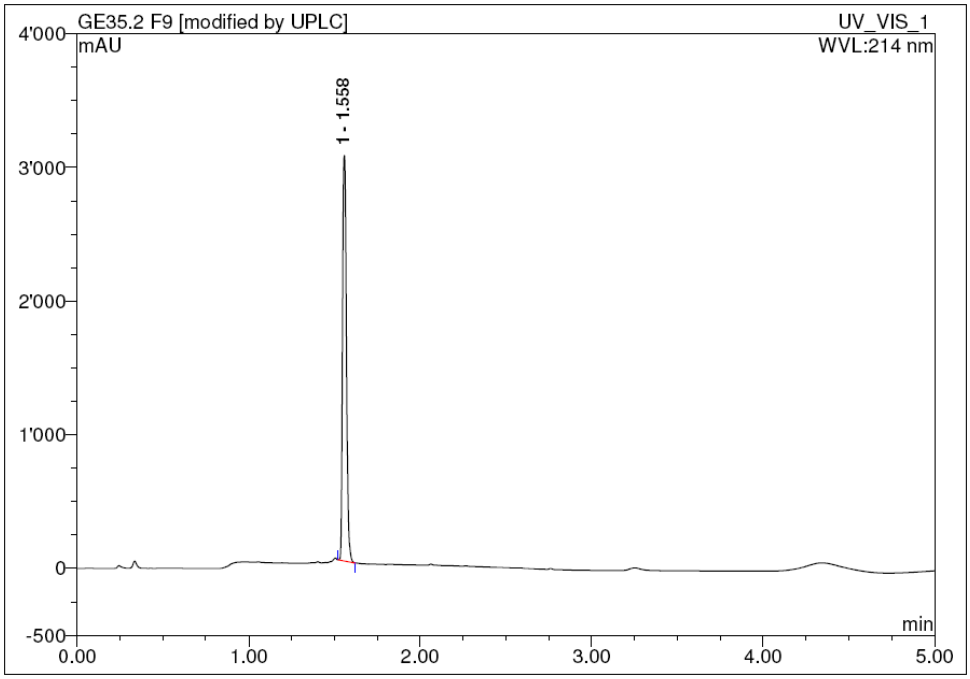
G1a ((ClAcG)$_2$*K*kK*). From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G1a** was obtained as a foamy yellow solid after preparative RP-HPLC (27.3 mg, 23.9 µmol, 20%). Analytical RP-HPLC: t_R = 1.56 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{47}H_{57}Cl_2N_9O_{13}$ found/calc. 1026.6/1026.9 $[M]^+$.



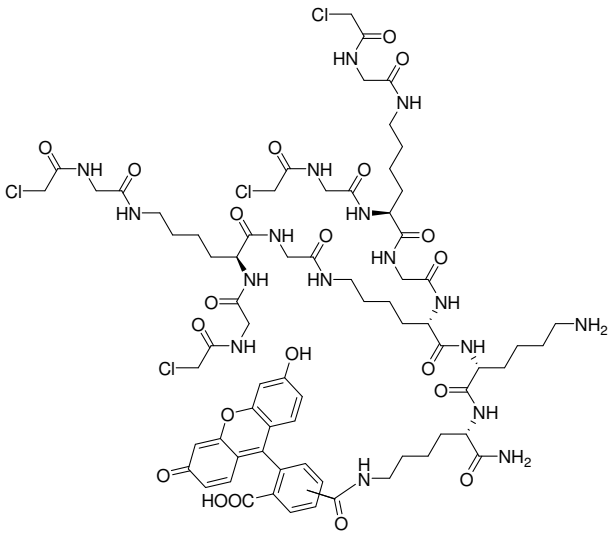
Mass spectrum, MS (ESI+):



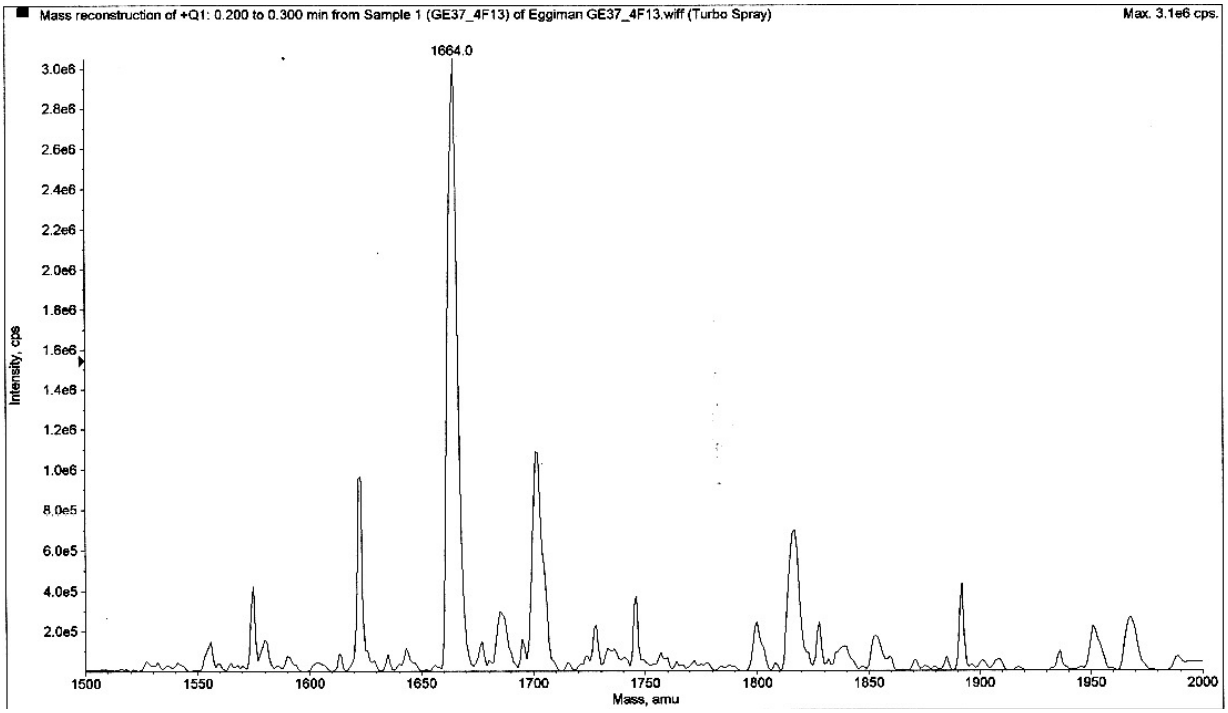
Analytical RP-HPLC chromatogram:



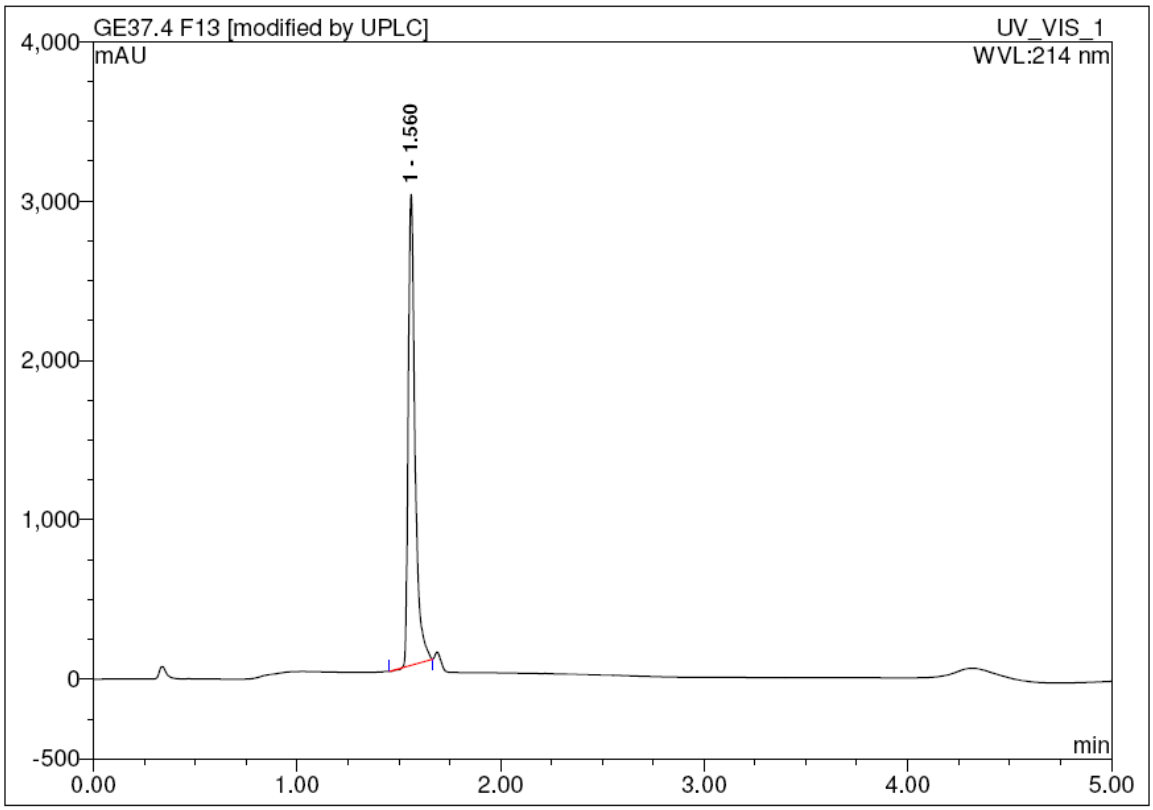
G2a ((ClAcG)$_4$(*K*G)$_2$*K*kK*). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **G2a** was obtained as a foamy yellow solid after preparative RP-HPLC (19.9 mg, 11.2 µmol, 10%). Analytical RP-HPLC: t_R = 1.56 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{71}H_{95}Cl_4N_{17}O_{21}$ found/calc. 1664.0/1664.4 $[M]^+$.



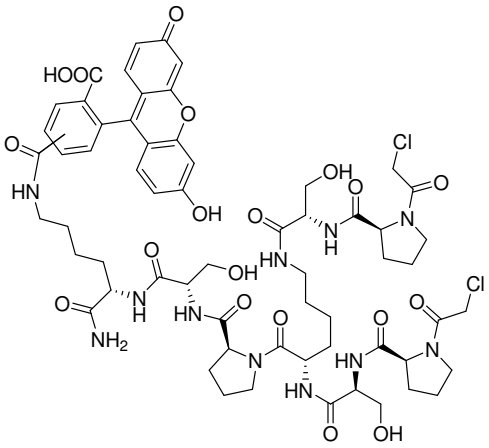
Mass spectrum, MS (ESI+):



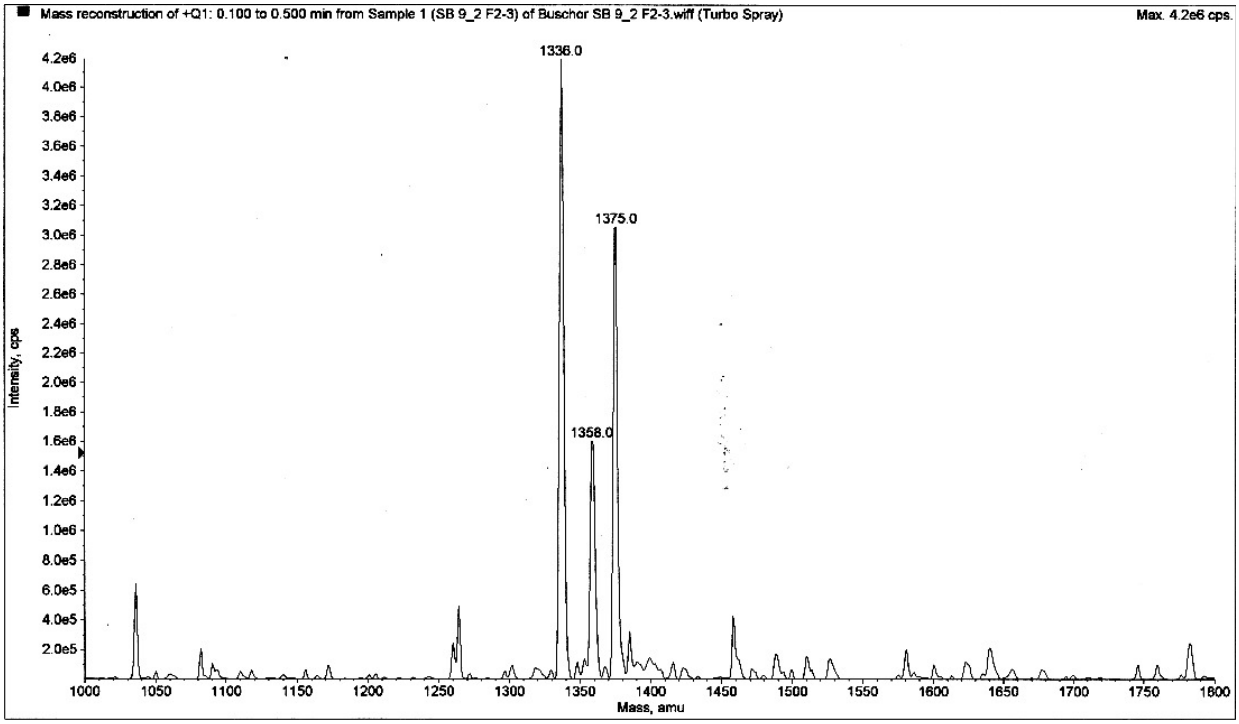
Analytical RP-HPLC chromatogram:



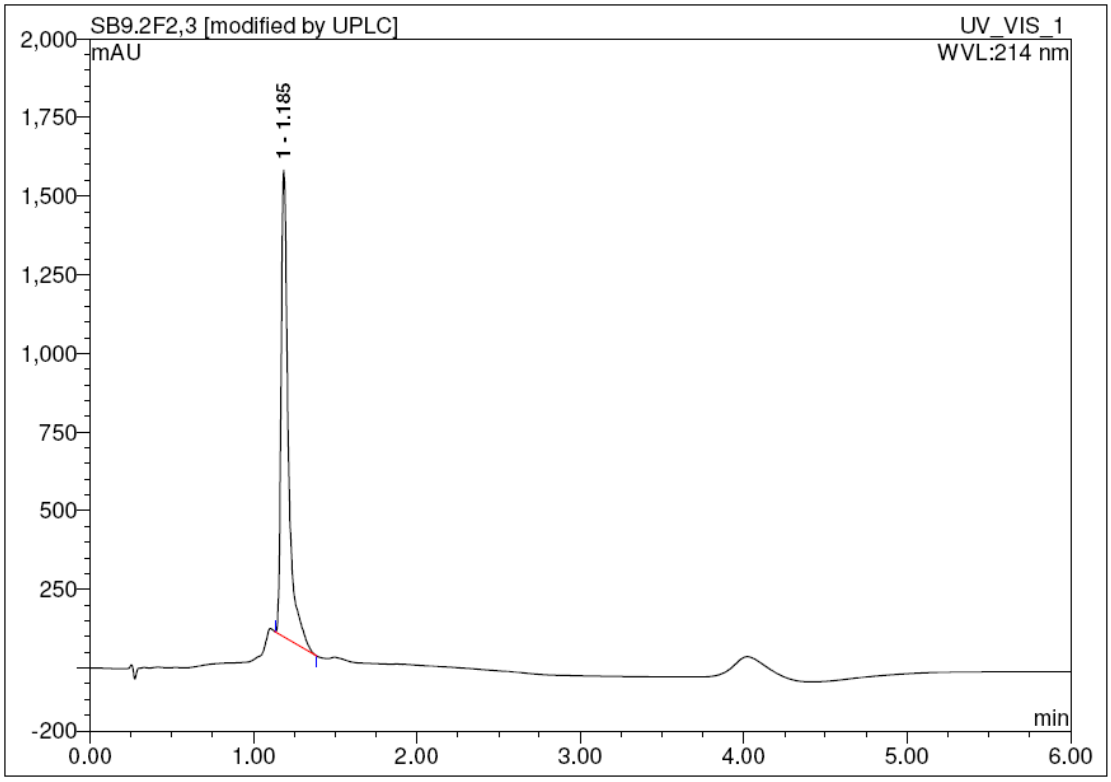
G1b ((ClAcPS)$_2$*K*PSK*). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **G1b** was obtained as a foamy yellow solid after preparative RP-HPLC (51.1 mg, 38.2 µmol, 35%). Analytical RP-HPLC: t_R = 1.19 min (A/D 80/20 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{61}H_{75}Cl_2N_{11}O_{19}$ found/calc. 1336.0/1337.2 $[M]^+$; 1358.0/1360.2 $[M + Na]^+$; 1375.0/1376.3 $[M + K]^+$.



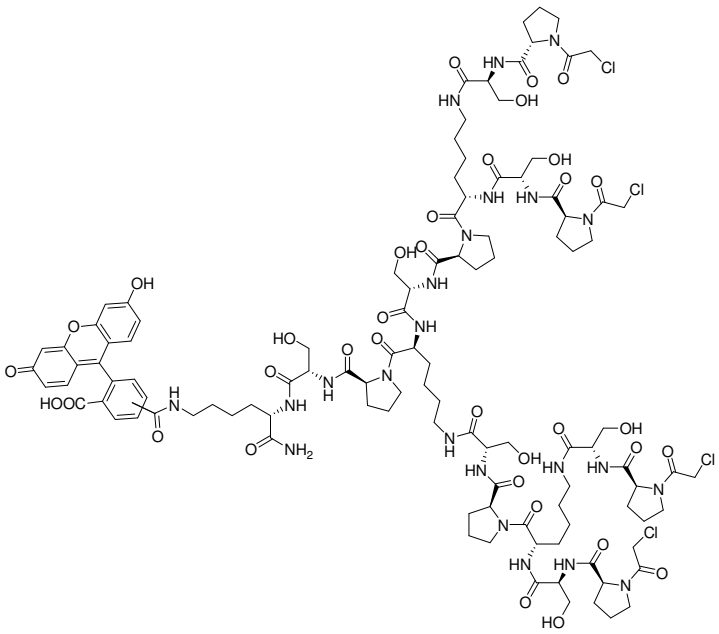
Mass spectrum, MS (ESI+):



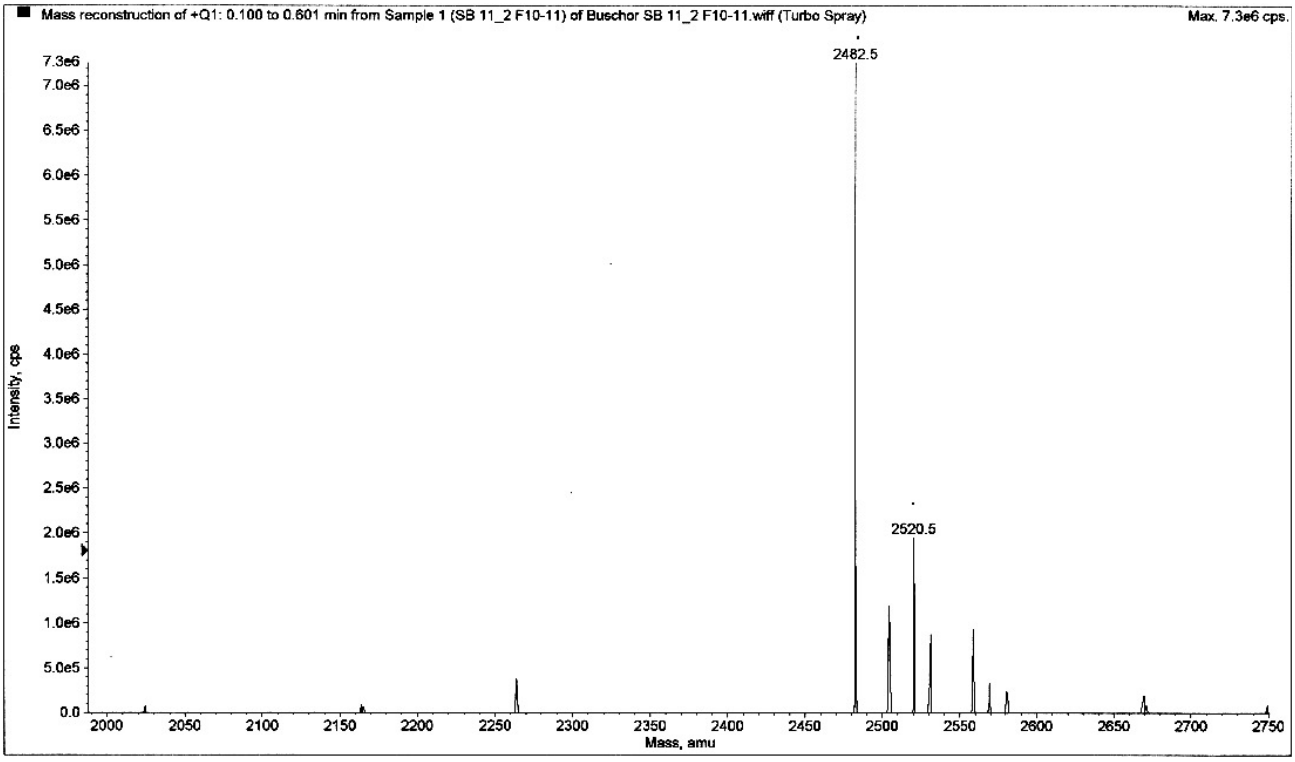
Analytical RP-HPLC chromatogram:



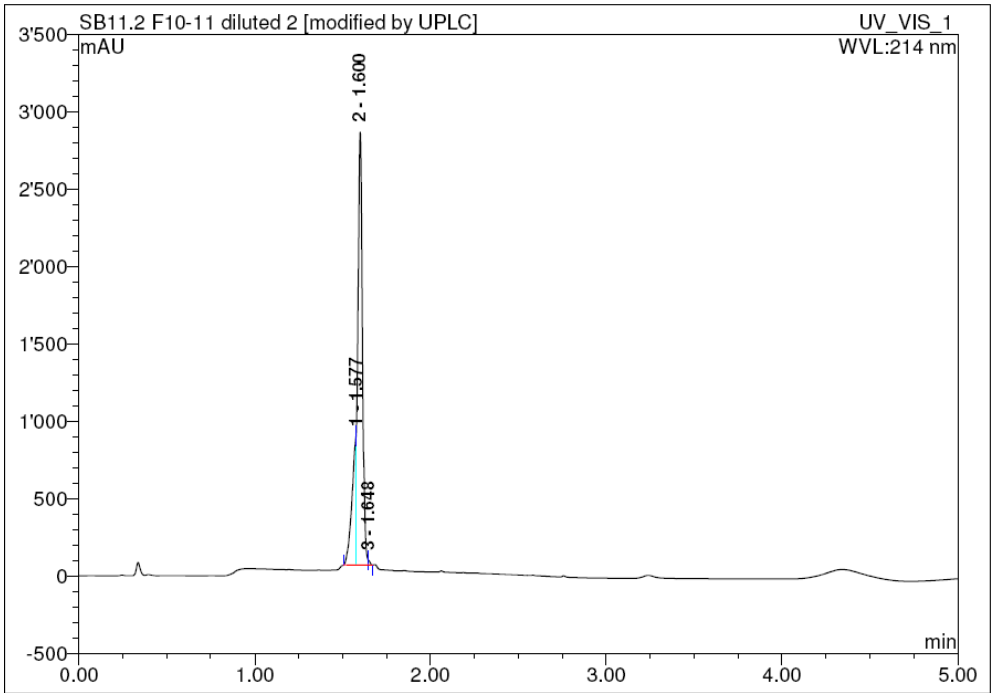
G2b ((ClAcPS)$_4$(*K*PS)$_2$*K*PSK*). From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **G2b** was obtained as a foamy yellow solid after preparative RP-HPLC (60.2 mg, 24.3 µmol, 22%). Analytical RP-HPLC: t_R = 1.60 min (A/D 80/20 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{109}H_{149}Cl_4N_{23}O_{35}$ found/calc. 2482.5/2483.3 $[M]^+$; 2520.5/2522.4 $[M + K]^+$.



Mass spectrum, MS (ESI+):

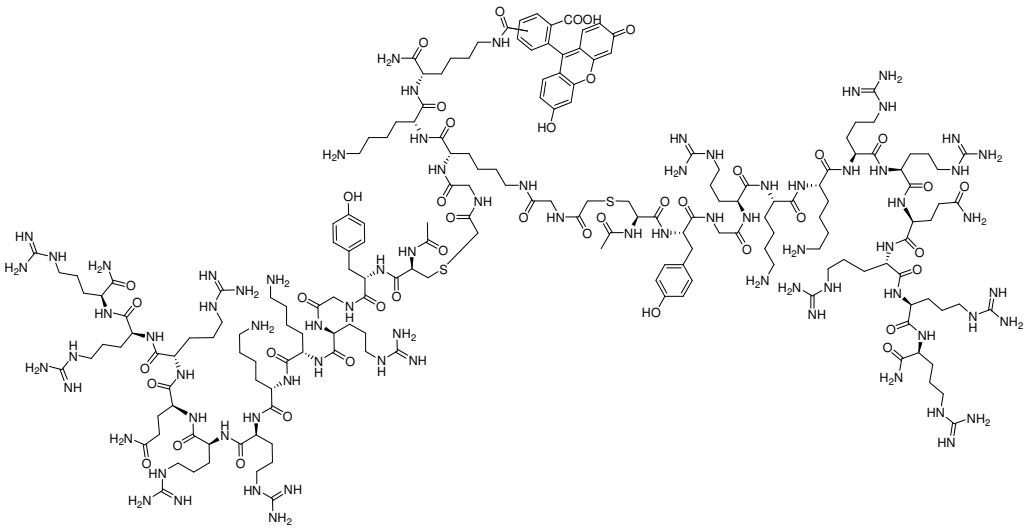


Analytical RP-HPLC chromatogram:

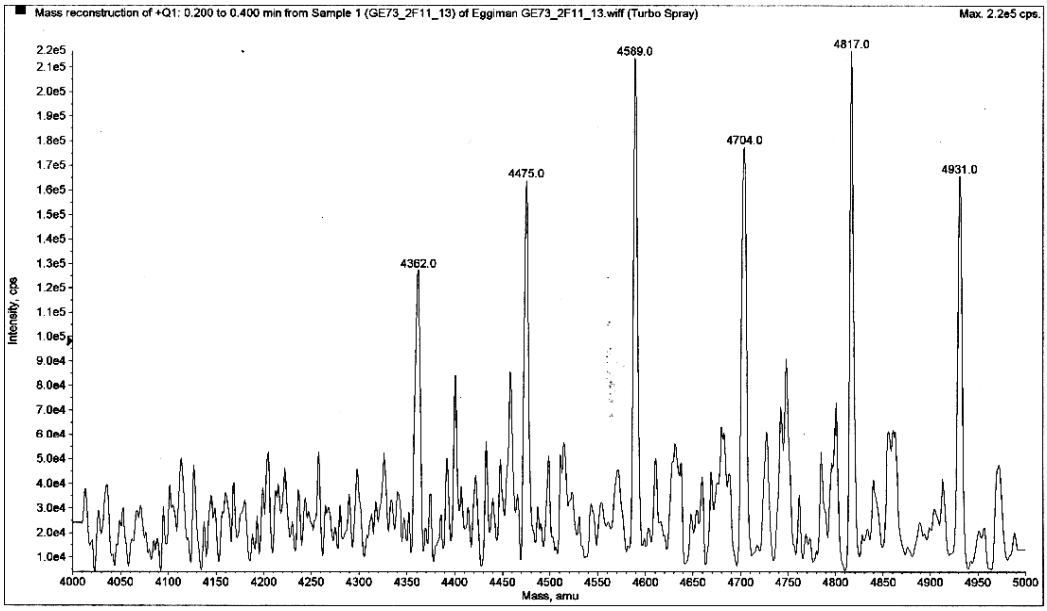


Thioether Ligation. Ac denotes an acetyl group attached to a free amine group. x denotes the S-CH_2-CO- bridge between cysteine side-chain and the N-terminus of the dendrimer or the lysine side-chain. * denotes 5(6)-carboxyfluorescein attached to a free amine group through an amide bond.

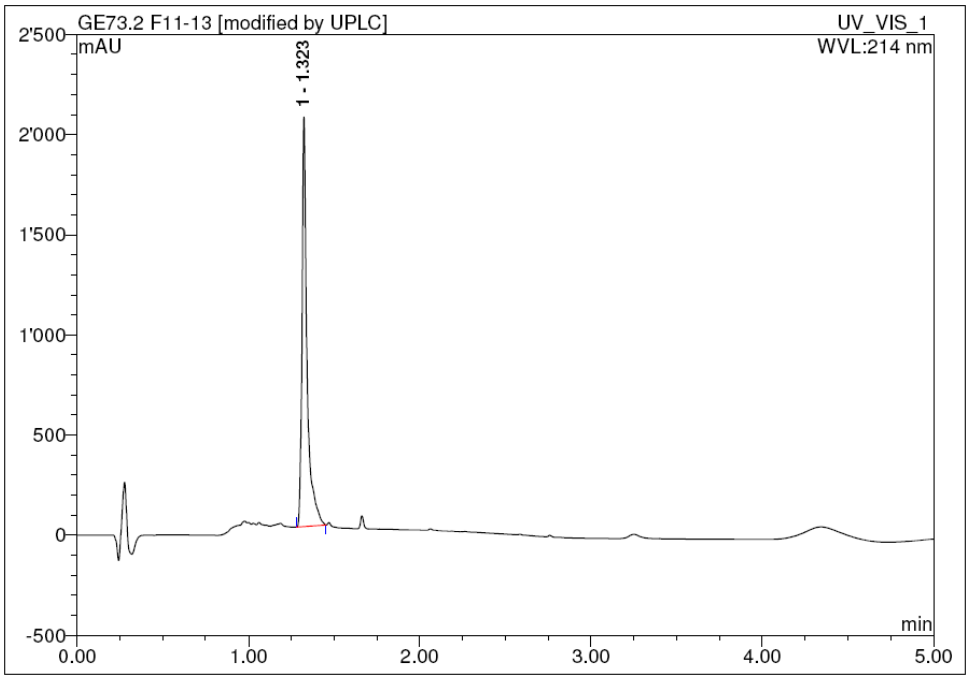
Tat-G1a ((AcC(YGRKKRRQRRR-NH_2)xG)$_2$*K*kK*). From starting materials **G1a** and **Cys-Tat** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **Tat-G1a** was obtained as a foamy yellow solid after preparative RP-HPLC (2.4 mg, 0.4 µmol, yield 16%). Analytical RP-HPLC: t_R = 1.32 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{185}H_{307}N_{77}O_{43}S_2$ found/calc. 4362.0/4362.0 $[M]^+$; 4475.0/4476.0 $[M + TFA]^+$; 4589.0/4590.0 $[M + 2TFA]^+$; 4704.0/4704.1 $[M + 3TFA]^+$; 4817.0/4818.1 $[M + 4TFA]^+$; 4931.0/4932.1 $[M + 5TFA]^+$.



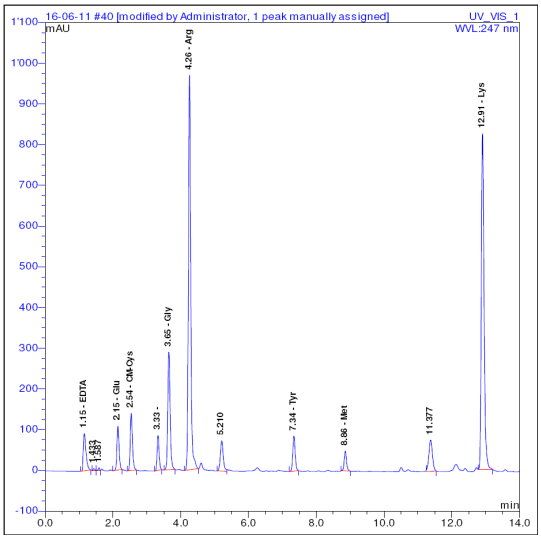
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

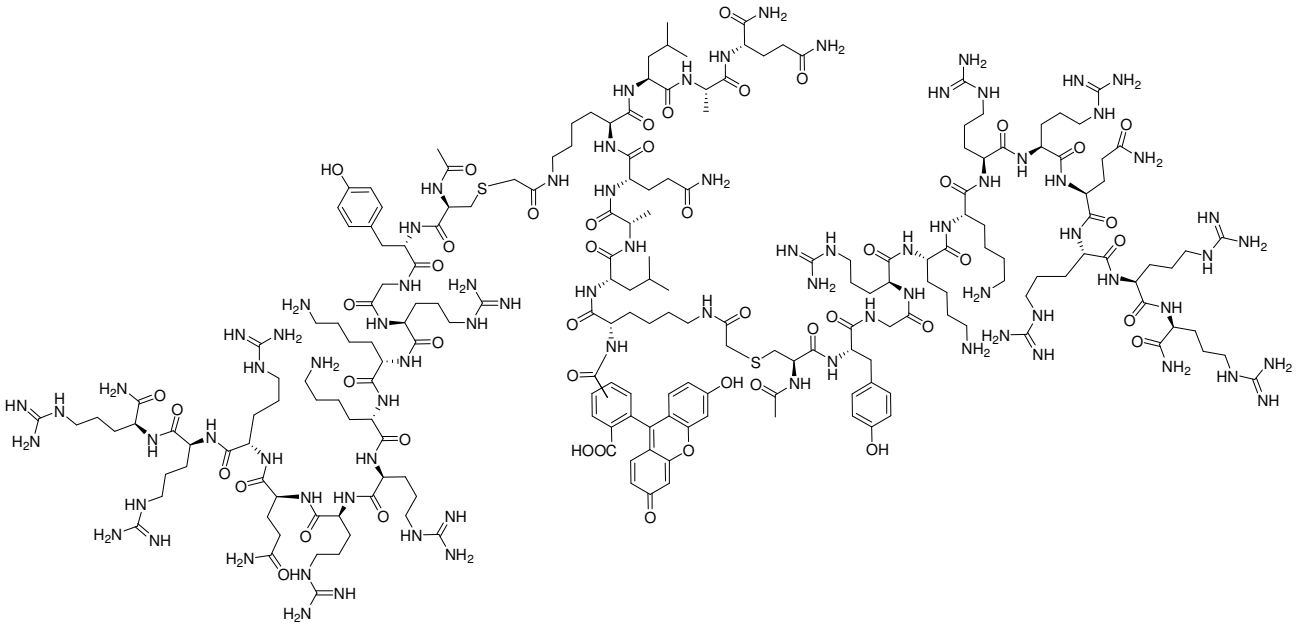


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.16	0.085	8.56	91.42		358.16	EDTA
2.15	2.14	0.067	7.92	107.86		614.77	Glu
2.54	2.53	0.068	10.58	140.28		747.41	CM-Cys
3.65	3.65	0.073	23.44	289.17		1699.57	Gly
4.26	4.25	0.070	77.31	969.14		5775.98	Arg
7.34	7.34	0.076	7.08	85.97		501.22	Tyr
8.86	8.82	0.070	3.81	48.64		311.65	Met
12.91	12.92	0.085	77.25	825.30		2839.61	Lys
Total:						12848.37	

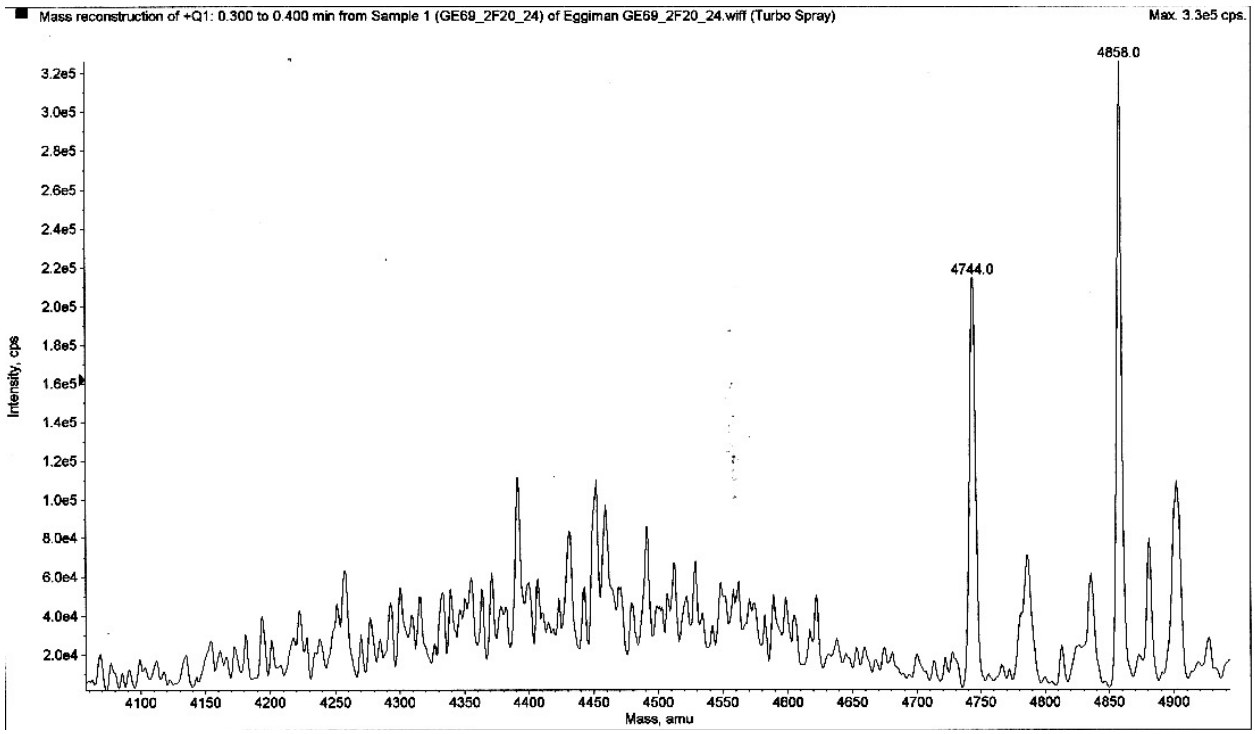
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	5776.0	12	13.8
CM-Cys[a)]	747.4	2	1.8
Gln[b)]	614.8	2	1.5
Gly	1699.6	4	4.1
Lys	2839.6	7	6.8
Tyr	501.2	2	1.2

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

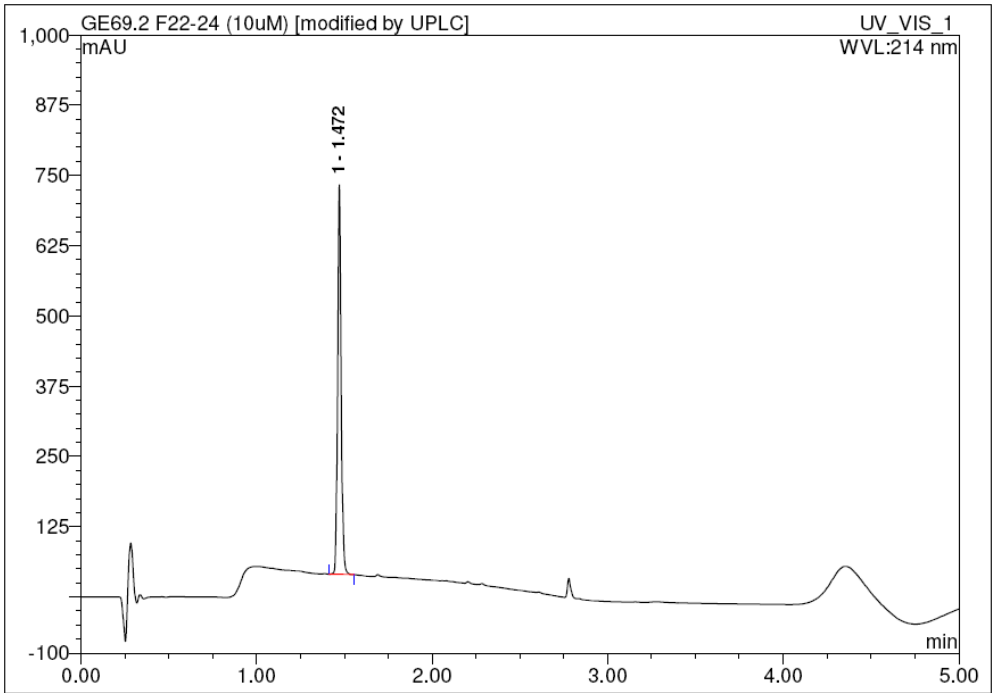
Tat-G1L (*-[K(x-(AcCYGRKKRRQRRR-NH$_2$))LAQ]$_2$). From starting materials **G1L** and **Cys-Tat** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **Tat-G1L** was obtained as a foamy yellow solid after preparative RP-HPLC (7.8 mg, 1.2 µmol, yield 60%). Analytical RP-HPLC: t_R = 1.47 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{203}H_{337}N_{81}O_{48}S_2$ found/calc. 4744.0/4744.5 $[M]^+$; 4858.0/4858.5 $[M + TFA]^+$.



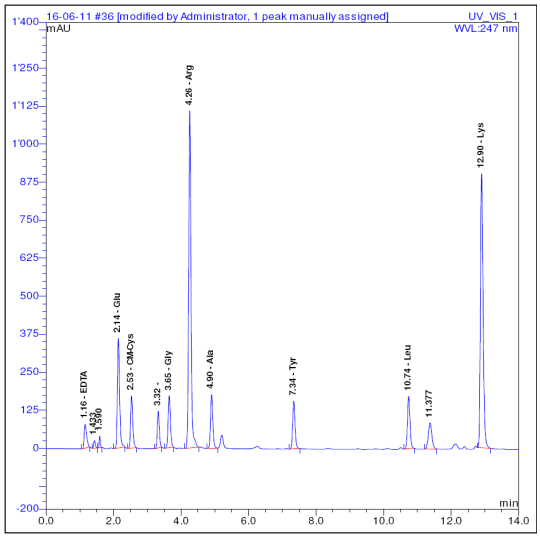
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

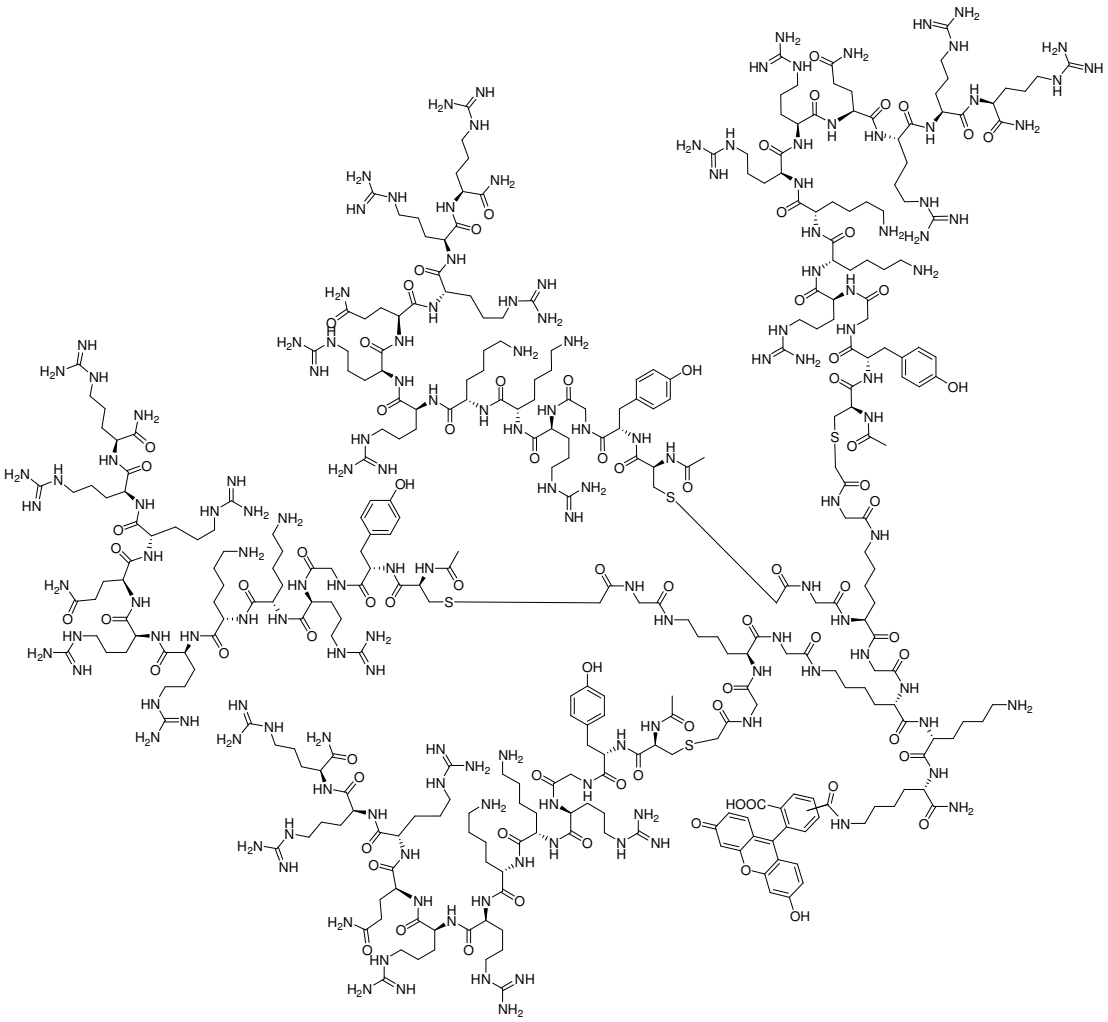


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.080	6.66	77.92		305.27	EDTA
2.14	2.14	0.068	27.39	360.53		2054.85	Glu
2.53	2.53	0.068	12.87	171.46		913.54	CM-Cys
3.65	3.65	0.072	13.40	170.69		1003.21	Gly
4.26	4.25	0.070	88.26	1108.94		6609.17	Arg
4.90	4.90	0.073	14.18	176.48		1022.58	Ala
7.34	7.34	0.075	12.95	156.53		912.62	Tyr
10.74	10.74	0.079	14.74	171.96		1057.60	Leu
12.90	12.92	0.085	83.10	901.35		3101.27	Lys
Total:						16980.12	

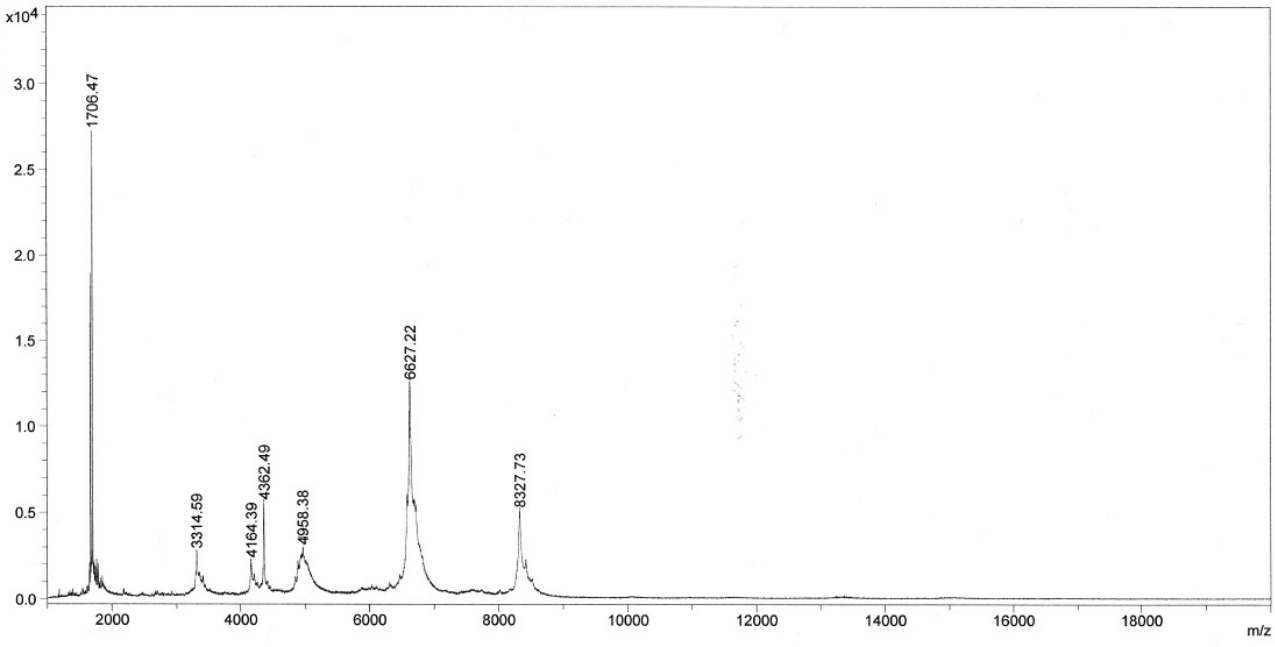
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1022.6	2	2.0
Arg	6609.2	12	12.7
CM-Cys[a)]	913.5	2	1.8
Gln[b)]	2054.9	4	3.9
Gly	1003.2	2	1.9
Leu	1057.6	2	2.0
Lys	3101.3	6	6.0
Tyr	912.6	2	1.8

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

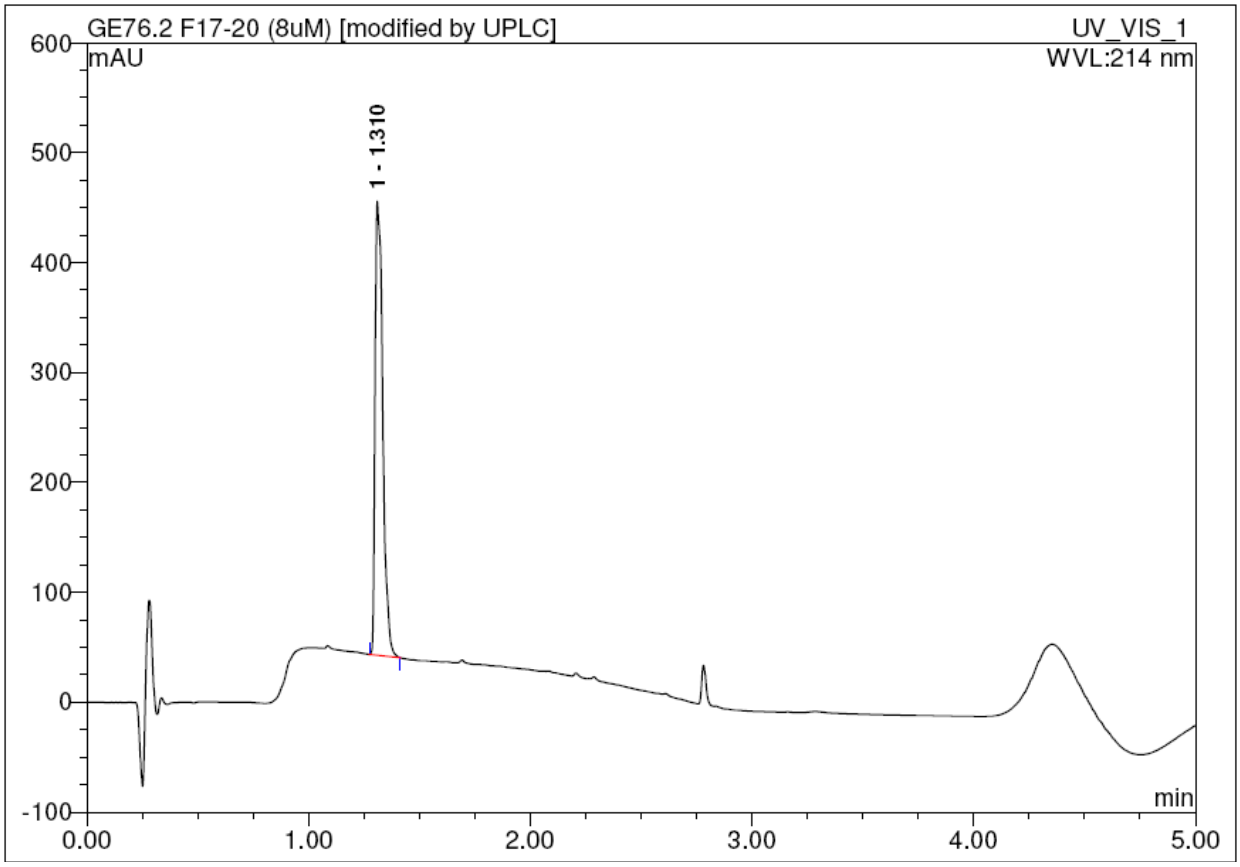
Tat-G2a ((AcC(YGRKKRRQRRR-NH_2)xG)$_4$(*K*G)$_2$*K*kK*). From starting materials **G2a** and **Cys-Tat** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **Tat-G2a** was obtained as a foamy yellow solid after preparative RP-HPLC (6.2 mg, 0.5 µmol, yield 41%). Analytical RP-HPLC: t_R = 1.31 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (MALDI-TOF+): $C_{347}H_{595}N_{153}O_{81}S_4$ found/calc. 8327.7/8334.7 $[M]^+$; $C_{278}H_{471}N_{119}O_{66}S_3$ (3-fold ligation product) found/calc. 6627.2/6632.7 $[M]^+$; $C_{209}H_{346}ClN_{85}O_{51}S_2$ (2-fold ligation product with one unreacted chloroacetyl group) found/calc. 4958.4/4965.1 $[M]^+$.



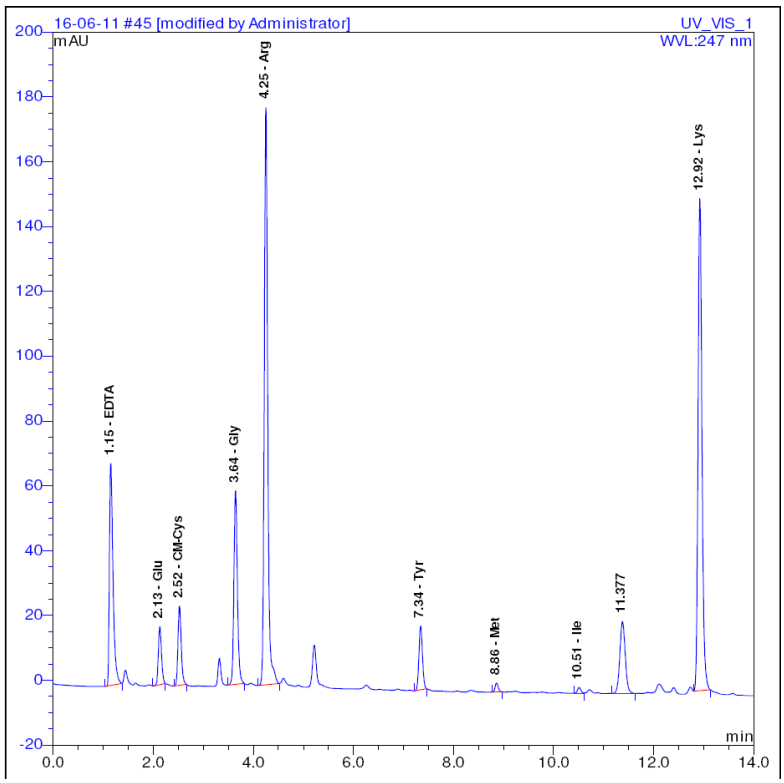
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

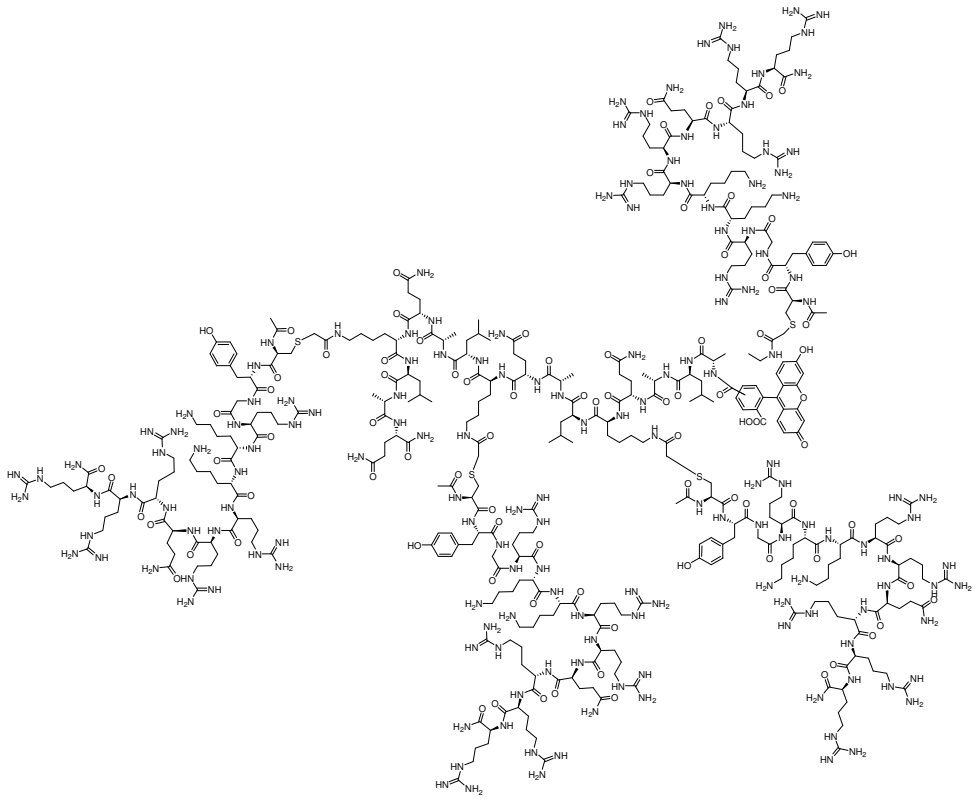


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.16	0.073	5.66	68.50		268.36	EDTA
2.13	2.14	0.069	1.33	17.89		101.95	Glu
2.52	2.53	0.068	1.83	24.41		130.07	CM-Cys
3.64	3.65	0.073	4.84	59.80		351.46	Gly
4.25	4.25	0.071	14.30	178.00		1060.89	Arg
7.34	7.34	0.076	1.60	19.68		114.74	Tyr
8.86	8.82	0.068	0.20	2.73		17.47	Met
10.51	10.50	0.074	0.14	1.73		10.32	Ile
12.92	12.92	0.082	13.74	151.85		522.46	Lys
Total:						2577.72	

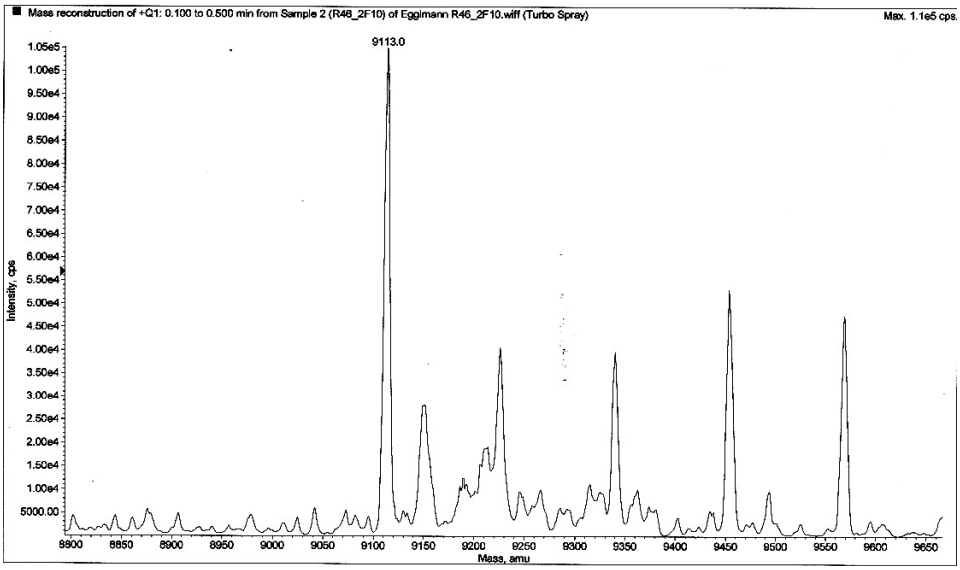
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	1060.9	24	27.4
CM-Cys[a)]	130.1	4	3.4
Gln[b)]	102.0	4	2.6
Gly	351.5	10	9.1
Lys	522.5	13	13.5
Tyr	114.7	4	3.0

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

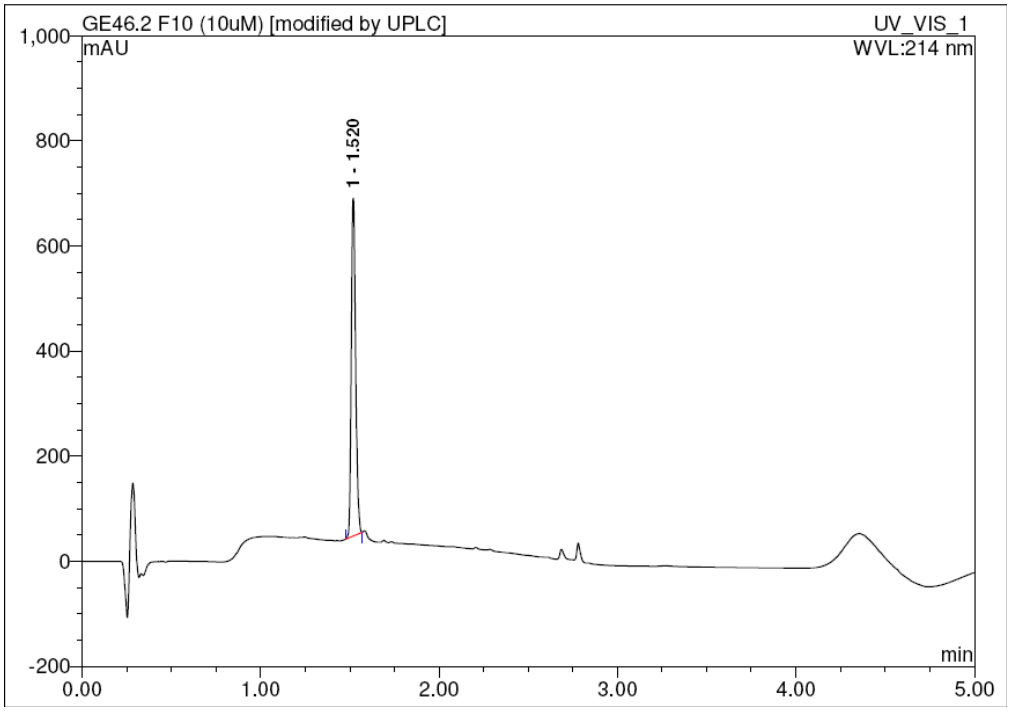
Tat-G2L (*-[K(x-(AcCYGRKKRRQRRR-NH$_2$))LAQ]$_4$). From starting materials **G2L** and **Cys-Tat** using the general procedure described above (solvent: 0.5 M $NaHCO_3$ buffer pH 8.0/CH_3CN (2/1, v/v)), **Tat-G2L** was obtained as a foamy yellow solid after preparative RP-HPLC (2.4 mg, 0.2 µmol, yield 33%). Analytical RP-HPLC: t_R = 1.52 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{385}H_{661}N_{161}O_{90}S_4$ found/calc. 9113.0/9113.7 $[M]^+$.



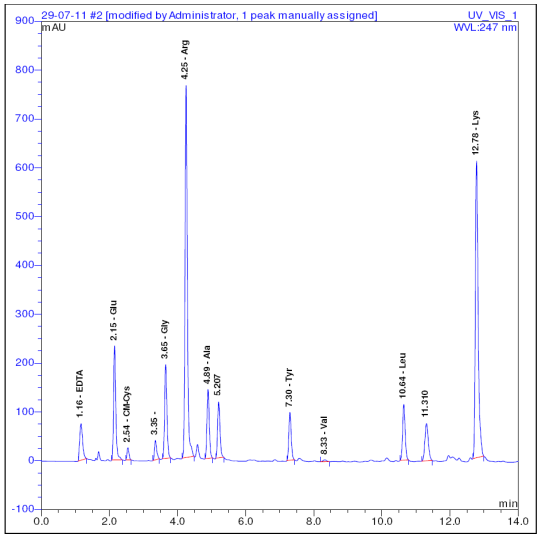
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

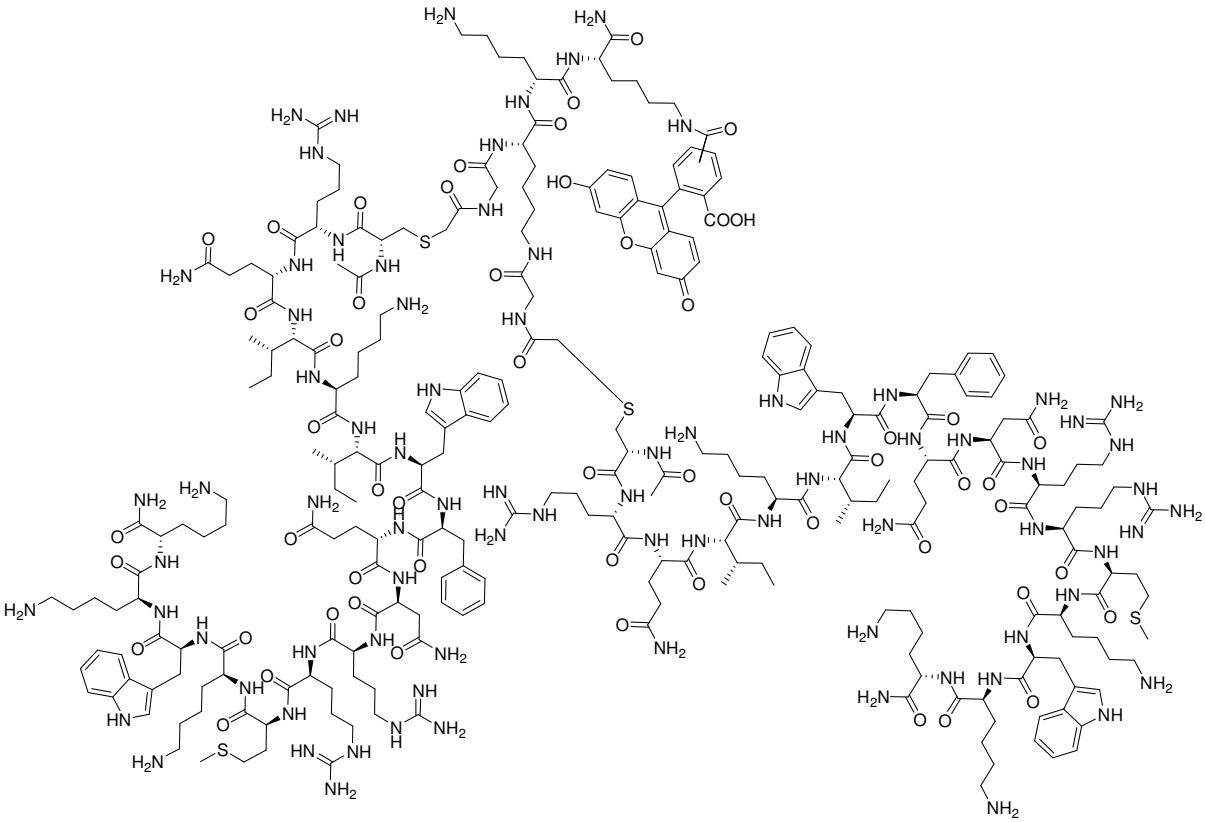


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.17	0.095	7.51	74.77		608.23	EDTA
2.15	2.15	0.066	17.55	233.58		1291.05	Glu
2.54	2.54	0.063	1.66	24.78		130.03	CM-Cys
3.65	3.65	0.072	15.01	192.06		1107.95	Gly
4.25	4.24	0.070	60.46	761.89		4512.48	Arg
4.89	4.89	0.072	10.91	140.92		806.56	Ala
7.30	7.30	0.075	7.96	98.07		562.69	Tyr
8.33	8.35	0.118	0.44	3.61		20.75	Val
10.64	10.64	0.079	9.85	114.25		688.58	Leu
12.78	12.78	0.084	57.61	607.64		2002.58	Lys
Total:						11730.92	

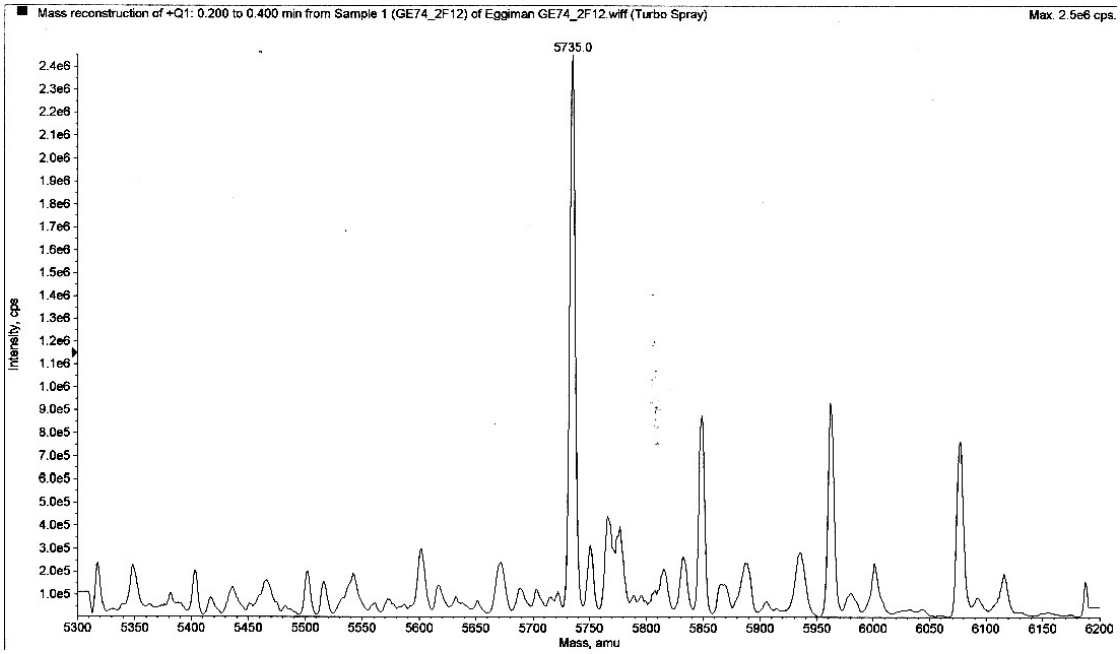
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	806.6	4	4.7
Arg	4512.5	24	26.0
CM-Cys[a)]	130.0	4	0.8
Gln[b)]	1291.1	8	7.4
Gly	1108.0	4	6.4
Leu	688.6	4	4.0
Lys	2002.6	12	11.5
Tyr	562.7	4	3.2

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

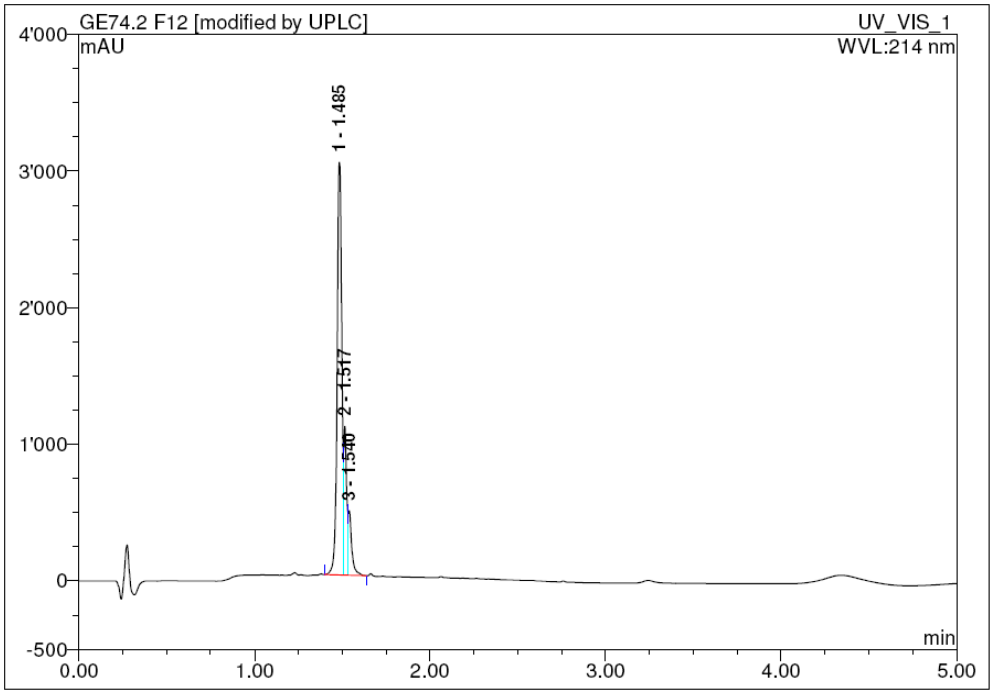
Antp-G1a ((AcC(RQIKIWFQNRRMKWKK-NH_2)xG)$_2$*KkK).** From starting materials **G1a** and **Cys-Antp** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **Antp-G1a** was obtained as a foamy yellow solid after preparative RP-HPLC (4.5 mg, 0.6 µmol, yield 26%). Analytical RP-HPLC: t_R = 1.49 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{265}H_{407}N_{81}O_{55}S_4$ found/calc. 5735.0/5735.8 $[M]^+$.



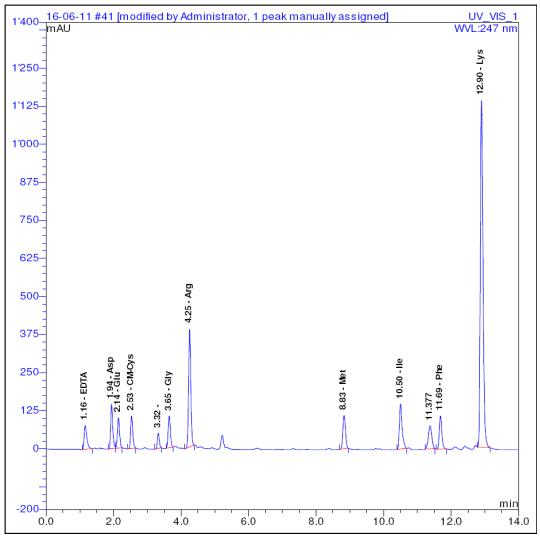
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

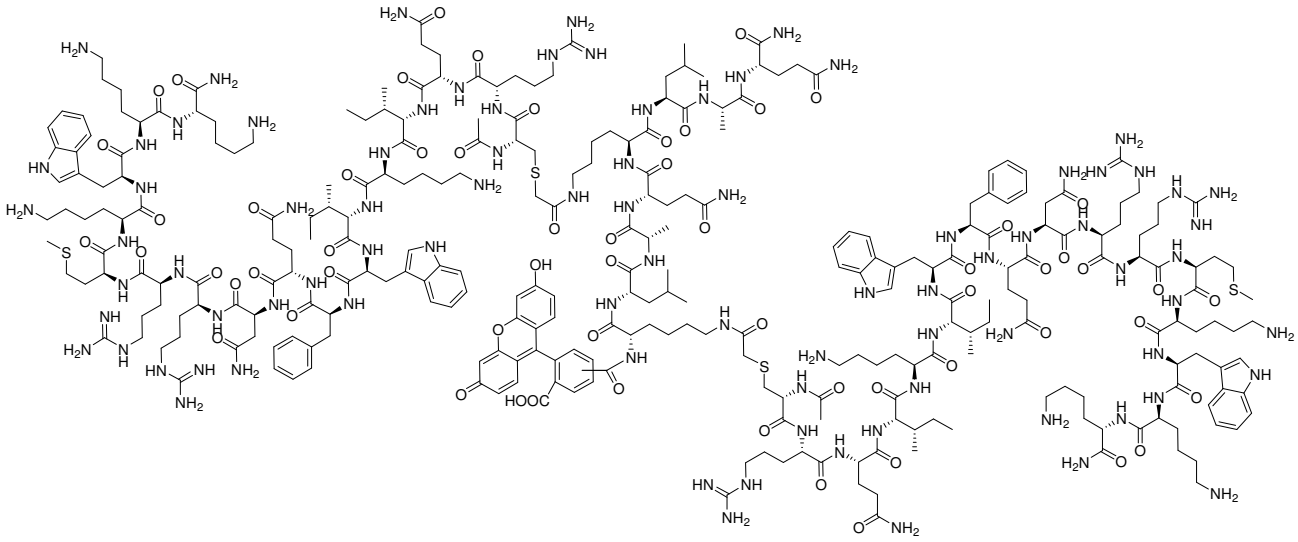


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.083	7.16	77.23		302.57	EDTA
1.94	1.94	0.064	10.46	145.73		780.03	Asp
2.14	2.14	0.068	7.33	99.59		567.64	Glu
2.53	2.53	0.068	7.96	107.24		571.37	CM-Cys
3.65	3.65	0.072	7.97	103.85		610.39	Gly
4.25	4.25	0.070	29.26	386.94		2306.11	Arg
8.83	8.82	0.087	9.95	108.94		697.99	Met
10.50	10.50	0.080	13.88	147.93		880.62	Ile
11.69	11.68	0.079	9.41	108.52		686.06	Phe
12.90	12.92	0.086	106.43	1138.70		3917.92	Lys
Total:						11320.69	

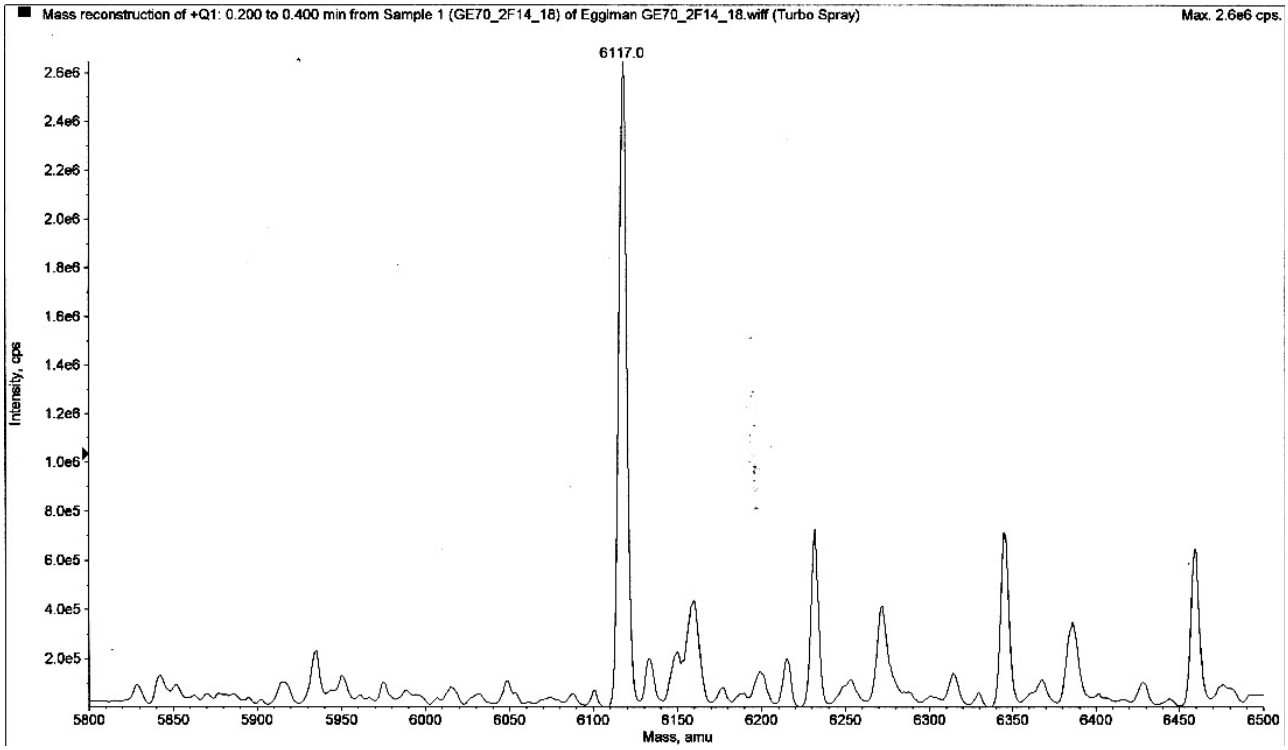
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	2306.1	6	7.3
Asn[a)]	780.0	2	2.5
CM-Cys[b)]	571.4	2	1.8
Gln[c)]	567.6	4	1.8
Gly	610.4	2	1.9
Ile	880.6	4	2.8
Lys	3917.9	11	12.5
Met	698.0	2	2.2
Phe	686.1	2	2.2

[a)] Detected as Asp. [b)] CM-Cys = carboxymethyl cysteine. [c)] Detected as Glu.

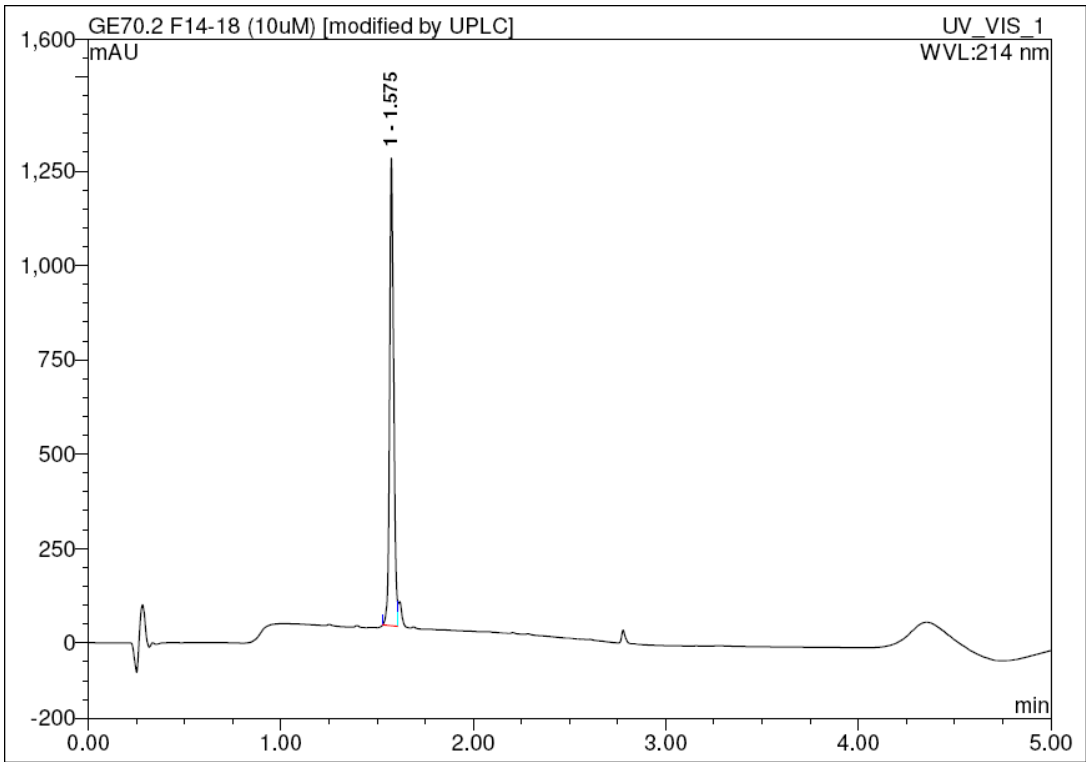
Antp-G1L (*-[K(x-[(AcCRQIKIWFQNRRMKWKK-NH$_2$))LAQ]$_2$). From starting materials **G1L** and **Cys-Antp** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **Antp-G1L** was obtained as a foamy yellow solid after preparative RP-HPLC (9.8 mg, 1.3 µmol, yield 64%). Analytical RP-HPLC: t_R = 1.58 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{283}H_{437}N_{85}O_{60}S_4$ found/calc. 6177.0/6118.3 $[M]^+$.



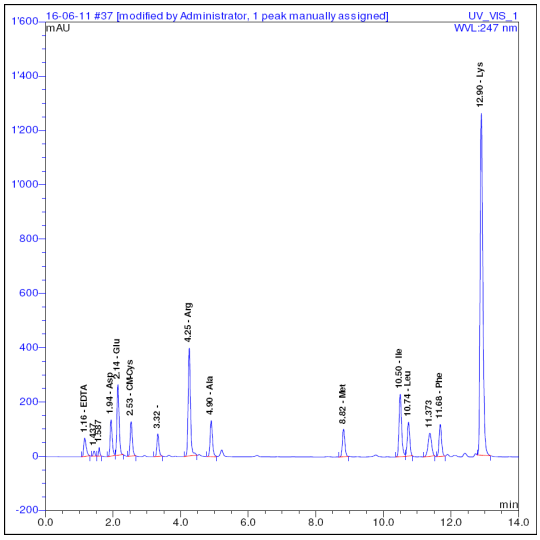
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

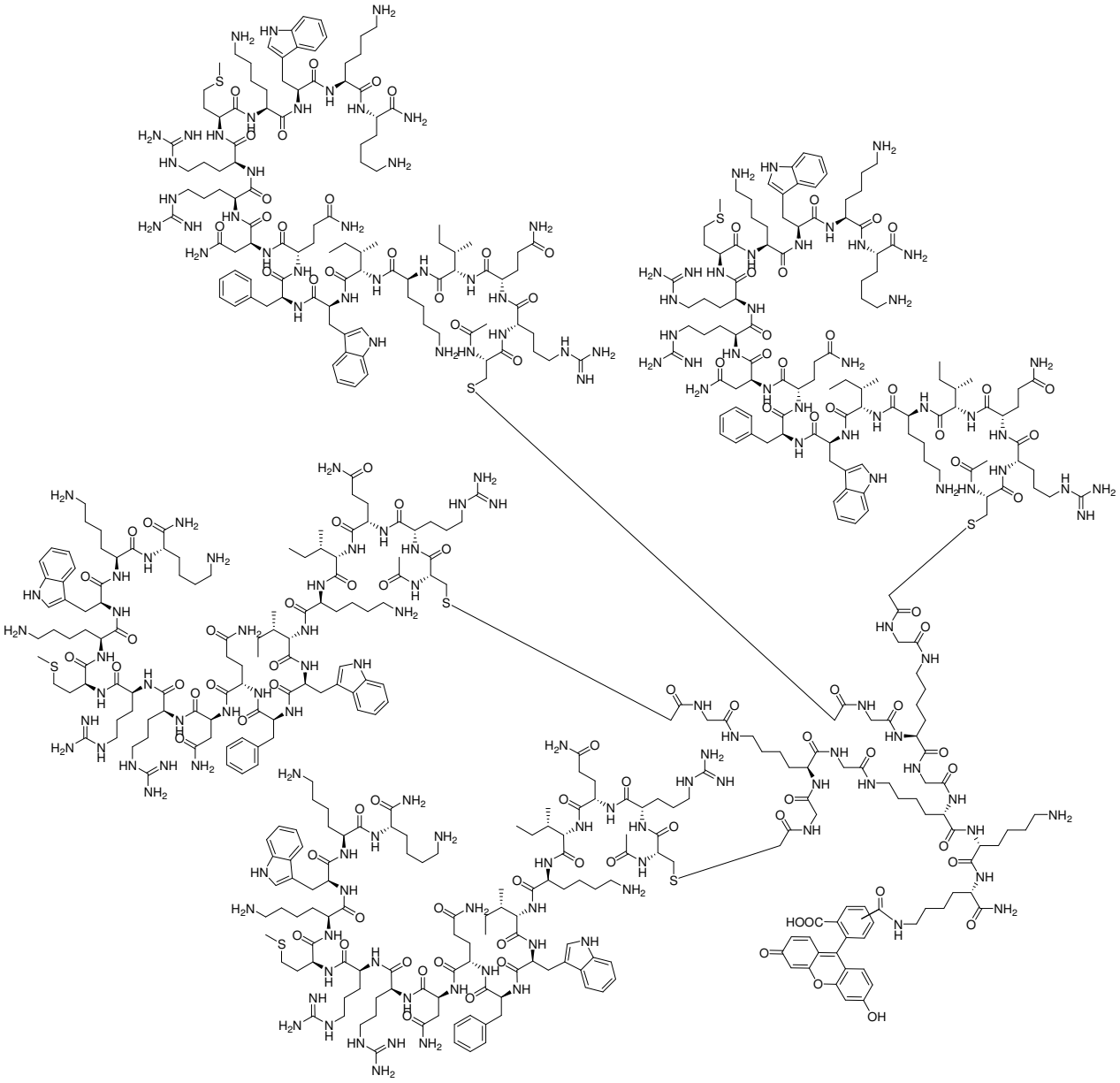


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.078	5.70	67.07		262.78	EDTA
1.94	1.94	0.064	9.49	133.88		716.61	Asp
2.14	2.14	0.068	19.36	259.78		1480.65	Glu
2.53	2.53	0.068	9.47	126.77		675.44	CM-Cys
4.25	4.25	0.070	31.49	397.23		2367.44	Arg
4.90	4.90	0.074	10.84	132.47		767.56	Ala
8.82	8.82	0.078	8.55	101.47		650.14	Met
10.50	10.50	0.079	20.18	230.12		1369.93	Ile
10.74	10.74	0.079	10.59	124.81		767.66	Leu
11.68	11.68	0.079	10.00	117.60		743.51	Phe
12.90	12.92	0.087	118.32	1258.45		4329.95	Lys
Total:						14131.66	

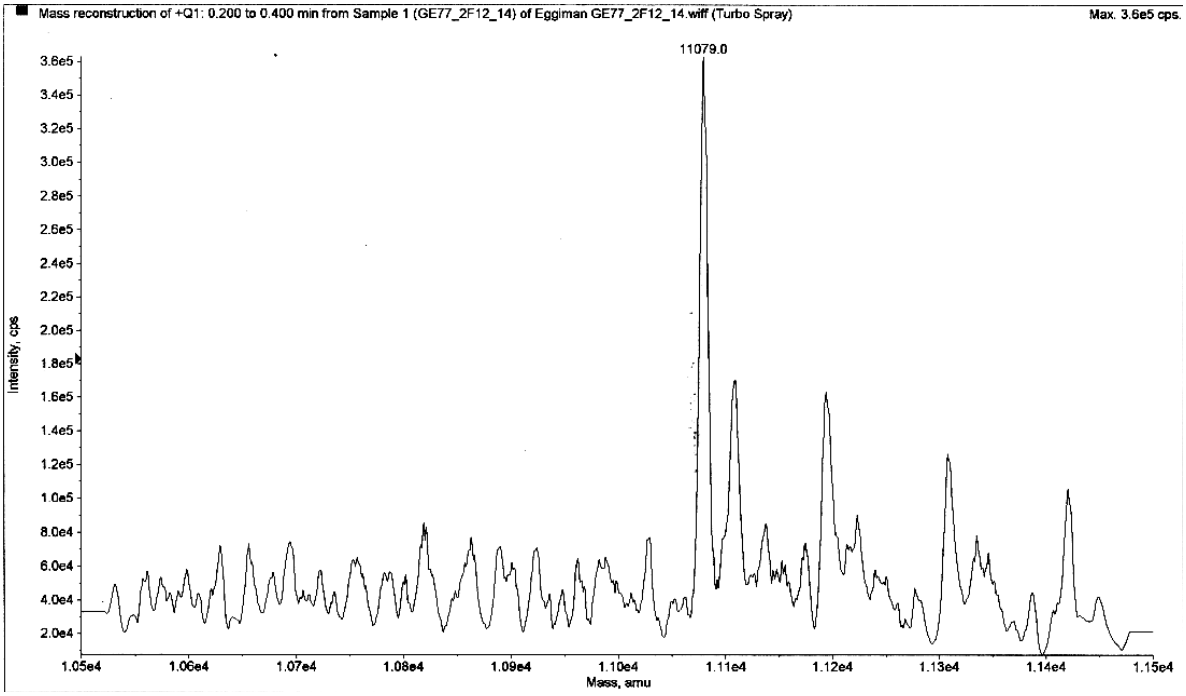
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	767.6	2	2.1
Arg	2367.4	6	6.5
Asn[a)]	716.6	2	2.0
CM-Cys[b)]	675.4	2	1.9
Gln[c)]	1480.7	6	4.1
Ile	1369.9	4	3.8
Leu	767.7	2	2.1
Lys	4330.0	10	11.9
Met	650.1	2	1.8
Phe	743.5	2	2.0

[a)] Detected as Asp. [b)] CM-Cys = carboxymethyl cysteine. [c)] Detected as Glu.

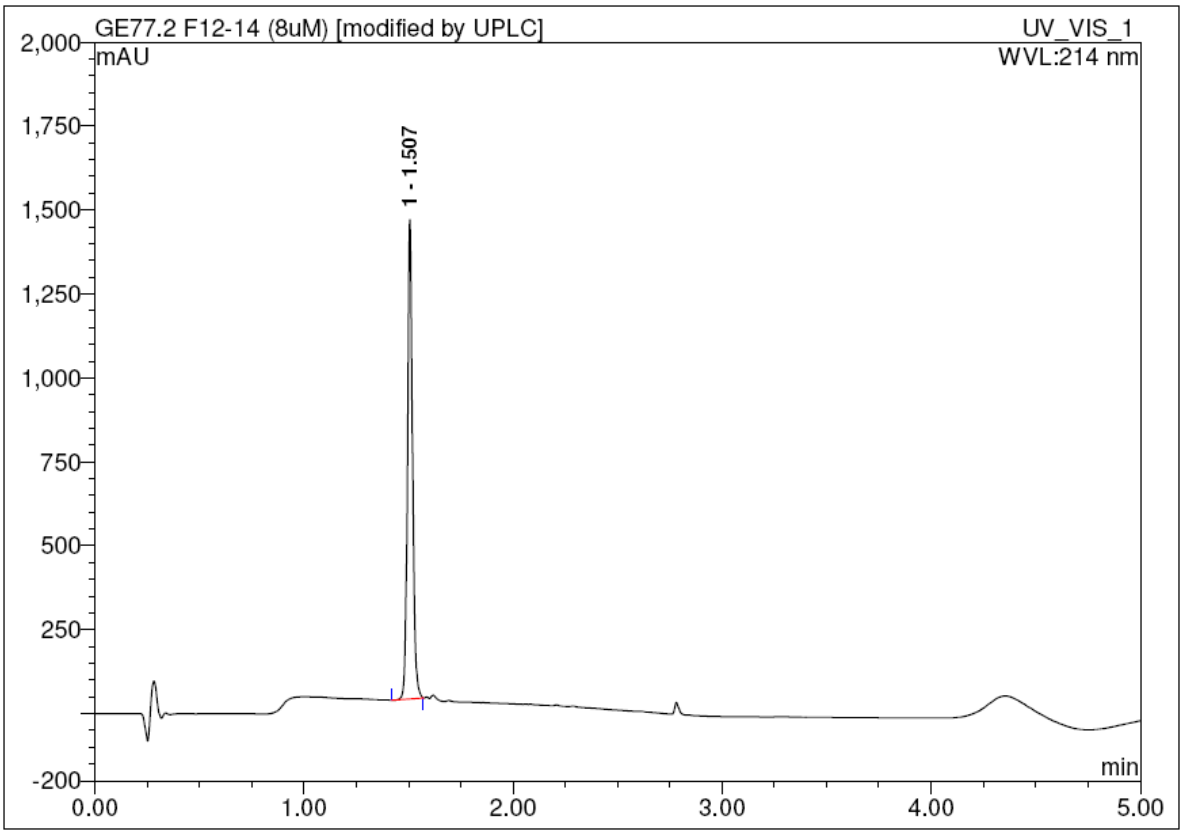
Antp-G2a ((AcC(RQIKIWFQNRRMKWKK-NH_2)xG)$_4$(*K*G)$_2$*K*kK*). From starting materials **G2a** and **Cys-Antp** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **Antp-G2a** was obtained as a foamy yellow solid after preparative RP-HPLC (9.7 mg, 0.7 µmol, yield 50%). Analytical RP-HPLC: t_R = 1.51 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{507}H_{795}N_{161}O_{105}S_8$ found/calc. 11079.0/11082.3 $[M]^+$.



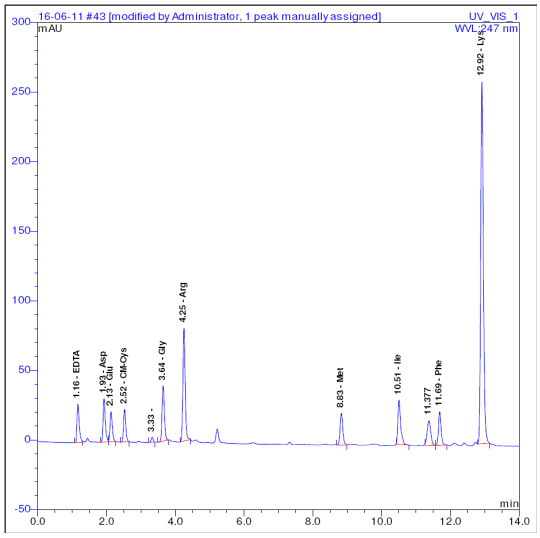
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

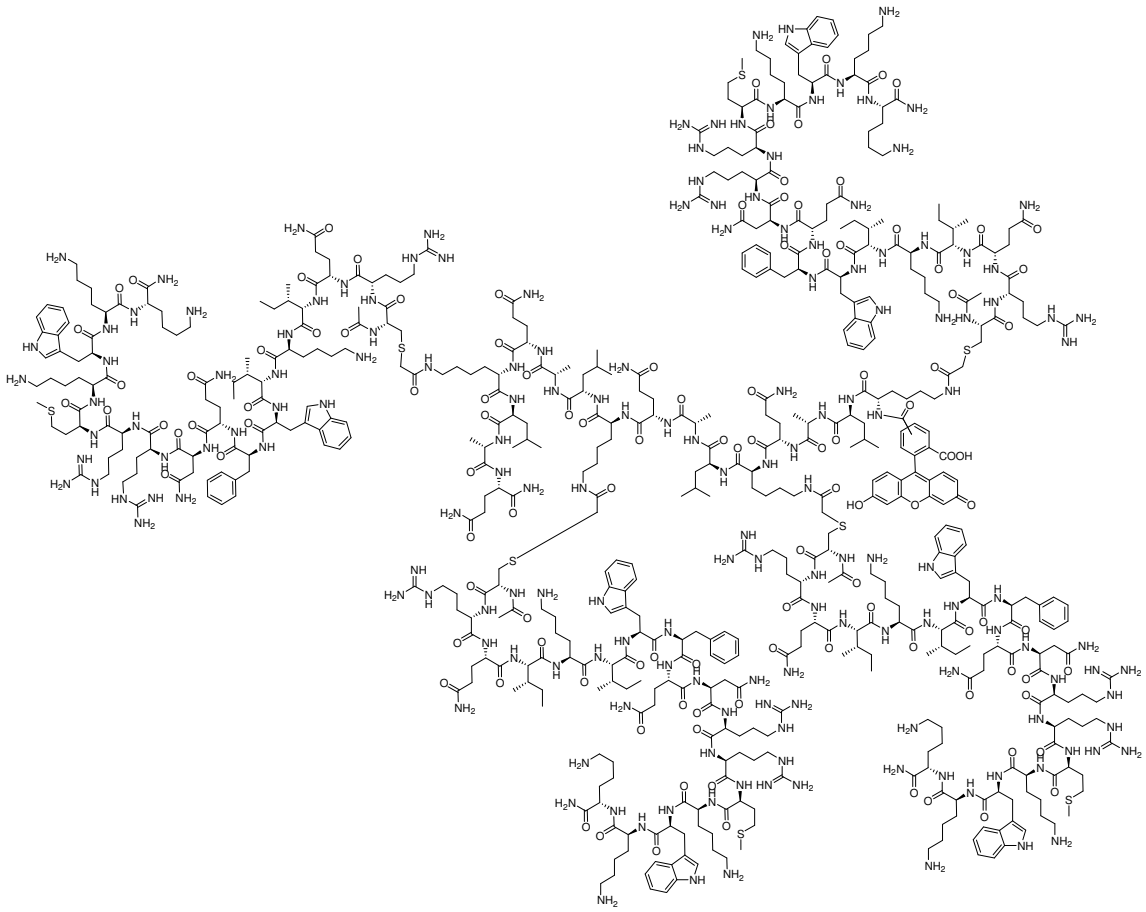


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.068	2.08	27.46		107.58	EDTA
1.93	1.94	0.067	2.32	31.14		166.70	Asp
2.13	2.14	0.069	1.64	21.52		122.66	Glu
2.52	2.53	0.068	1.75	23.37		124.54	CM-Cys
3.64	3.65	0.072	3.14	39.85		234.24	Gly
4.25	4.25	0.070	6.29	81.06		483.13	Arg
8.83	8.82	0.084	2.11	22.82		146.19	Met
10.51	10.50	0.080	3.01	31.75		189.01	Ile
11.69	11.68	0.080	2.11	23.94		151.38	Phe
12.92	12.92	0.083	23.56	259.97		894.49	Lys
Total:						2619.91	

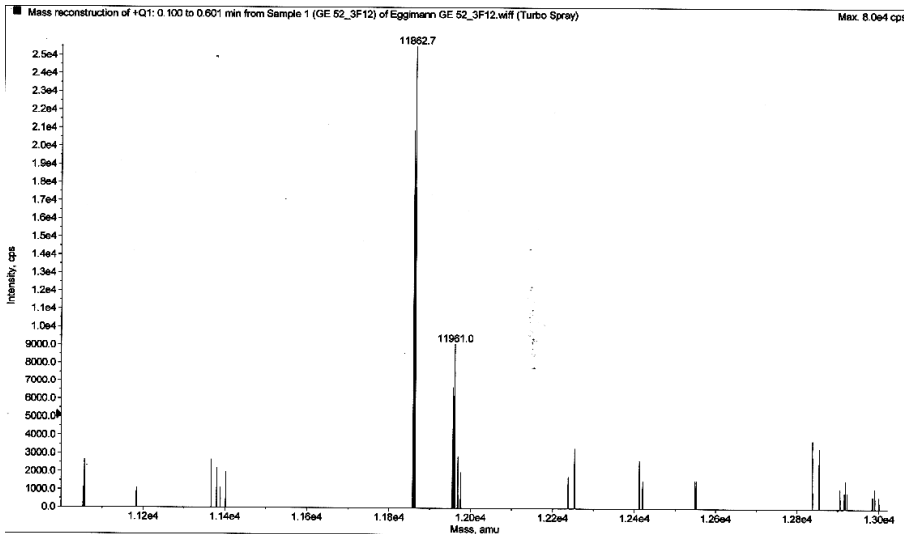
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	483.1	12	13.7
Asn[a)]	166.7	4	4.7
CM-Cys[b)]	124.5	4	3.5
Gln[c)]	122.7	8	3.5
Gly	234.2	6	6.6
Ile	189.0	8	5.3
Lys	894.5	21	25.3
Met	146.2	4	4.1
Phe	151.4	4	4.3

[a)] Detected as Asp. [b)] CM-Cys = carboxymethyl cysteine. [c)] Detected as Glu.

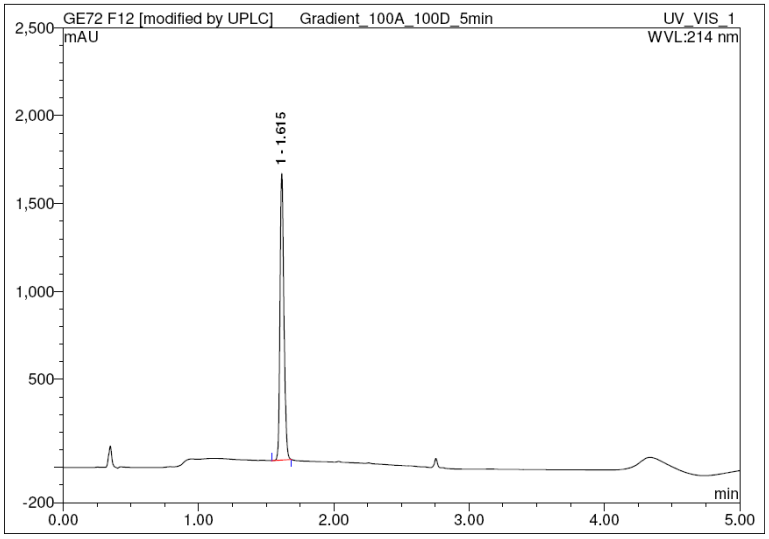
Antp-G2L (*-[K(x-[(AcCRQIKIWFQNRRMKWKK-NH$_2$))LAQ]$_4$). From starting materials **G2L** and **Cys-Antp** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v)), **Antp-G2L** was obtained as a foamy yellow solid after preparative RP-HPLC (2.2 mg, 0.2 µmol, yield 28%). Analytical RP-HPLC: t_R = 1.62 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{545}H_{861}N_{169}O_{114}S_8$ found/calc. 11862.7/11861.3 $[M]^+$; 11961.0/11962.3 $[M + 2K + Na]^+$.



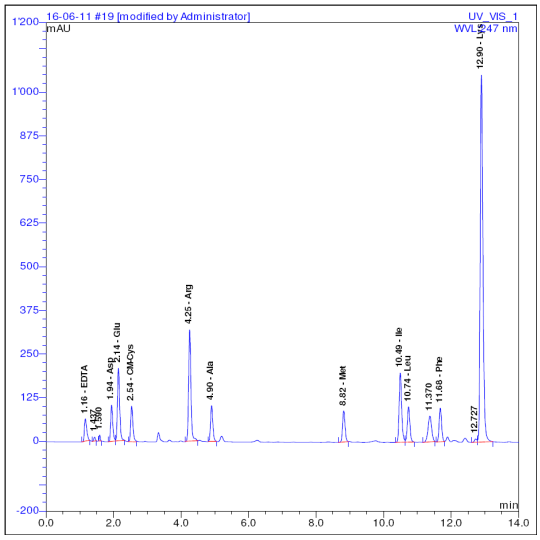
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

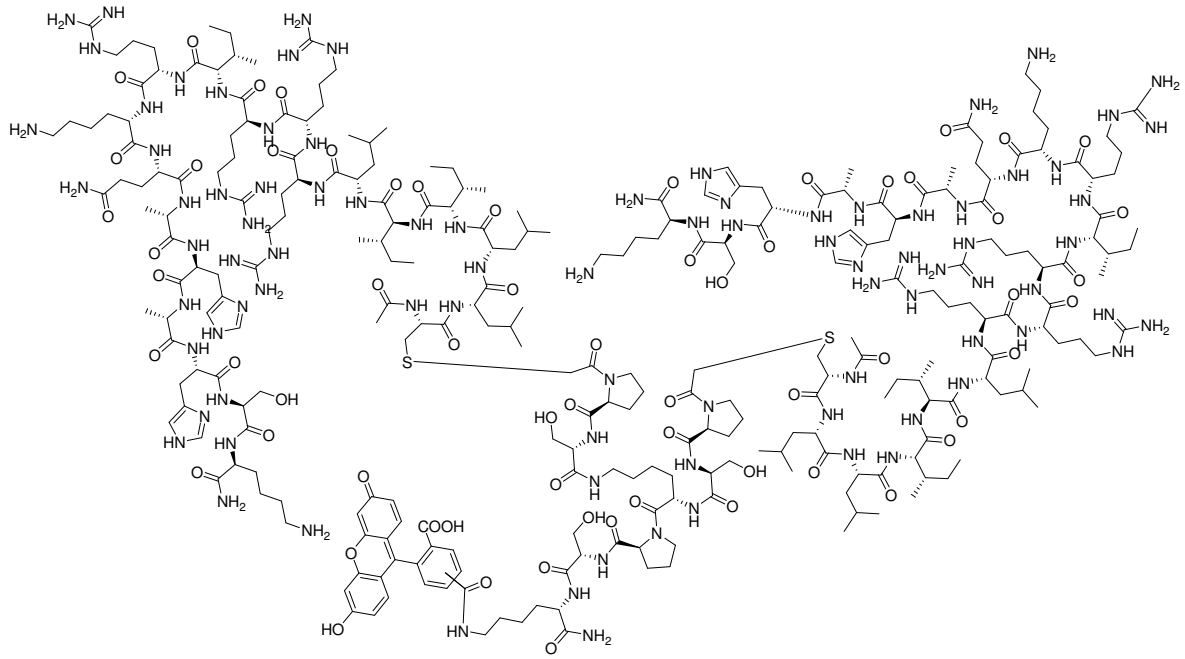


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.074	5.05	63.76		249.80	EDTA
1.94	1.94	0.064	7.44	103.99		556.64	Asp
2.14	2.14	0.068	15.72	207.85		1184.63	Glu
2.54	2.53	0.069	7.85	101.68		541.75	CM-Cys
4.25	4.25	0.071	25.29	318.60		1898.84	Arg
4.90	4.90	0.073	8.13	102.35		593.05	Ala
8.82	8.82	0.078	7.47	89.26		571.89	Met
10.49	10.50	0.079	17.42	198.55		1182.01	Ile
10.74	10.74	0.080	8.89	101.31		623.10	Leu
11.68	11.68	0.078	8.07	96.44		609.74	Phe
12.90	12.92	0.085	98.63	1051.25		3617.02	Lys
Total:						11628.44	

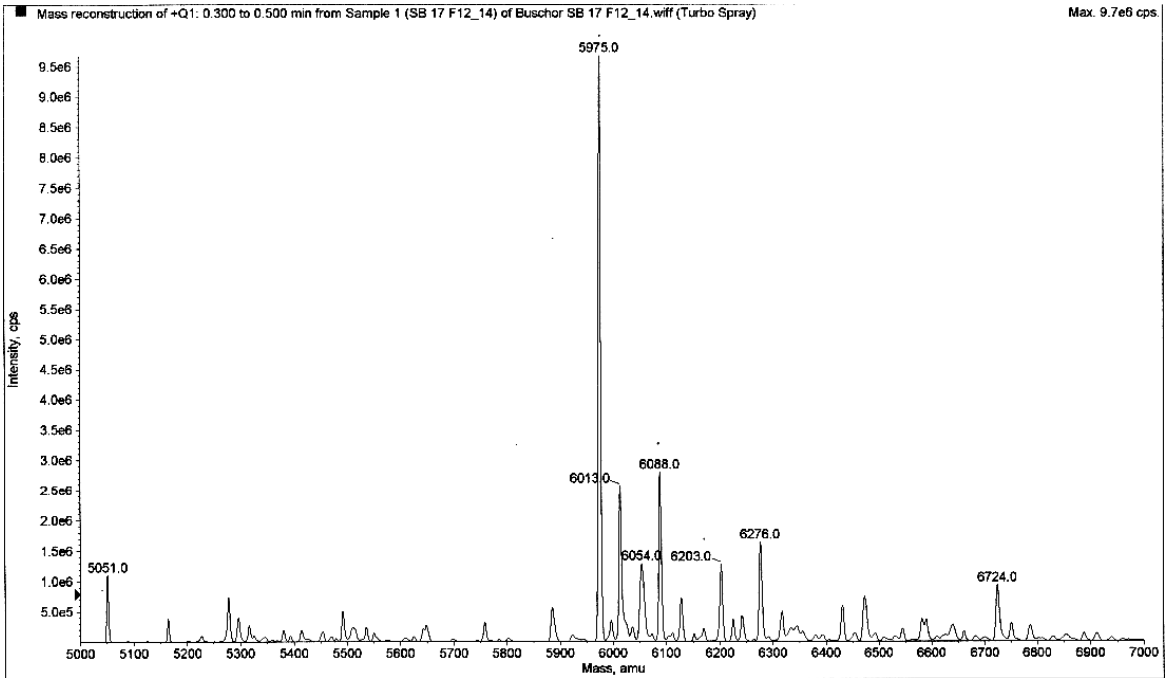
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	593.1	4	4.0
Arg	1898.8	12	12.7
Asn[a)]	556.6	4	3.7
CM-Cys[b)]	541.8	4	3.6
Gln[c)]	1184.6	12	7.9
Ile	1182.0	8	7.9
Leu	623.1	4	4.2
Lys	3617.0	20	24.2
Met	571.9	4	3.8
Phe	609.7	4	4.1

[a)] Detected as Asp. [b)] CM-Cys = carboxymethyl cysteine. [c)] Detected as Glu.

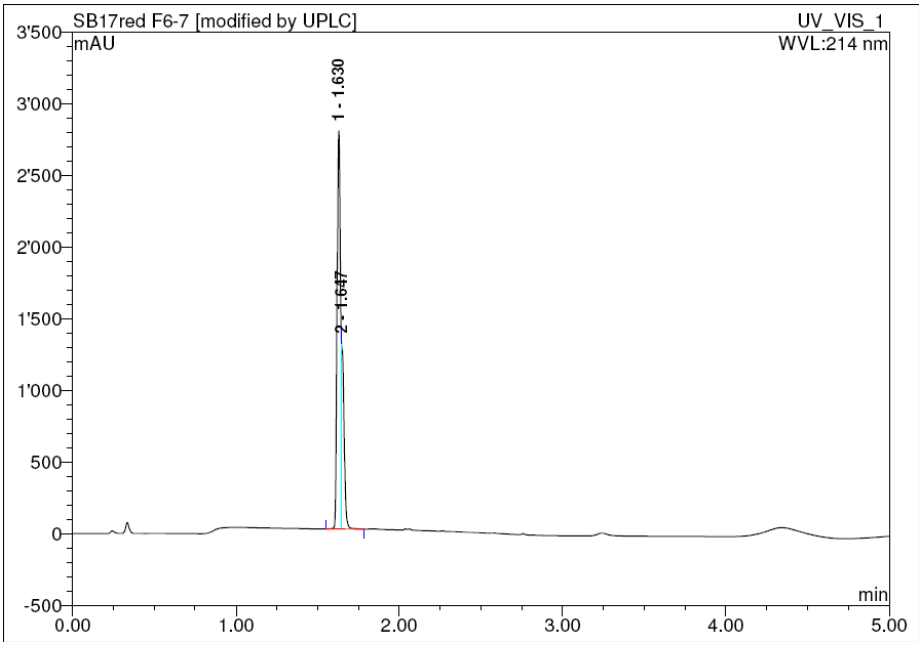
pVEC-G1b ((AcC(LLIILRRRIRKQAHAHSK-NH_2)xPS)$_2$*K*PSK*). From starting materials **G1b** and **Cys-pVEC** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **pVEC-G1b** was obtained as a foamy yellow solid after preparative RP-HPLC (11.3 mg, 1.8 µmol, yield 81%). Analytical RP-HPLC: t_R = 1.63 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{267}H_{443}N_{89}O_{63}S_2$ found/calc. 5975.0/5972.1 $[M]^+$; 6013.0/6011.2 $[M + K]^+$; 6054.0/6050.3 $[M + 2K]^+$; 6088.0/6086.1 $[M + TFA]^+$; 6203.0/6200.1 $[M + 2TFA]^+$; 6276.0/6278.3 $[M + 2K + 2TFA]^+$.



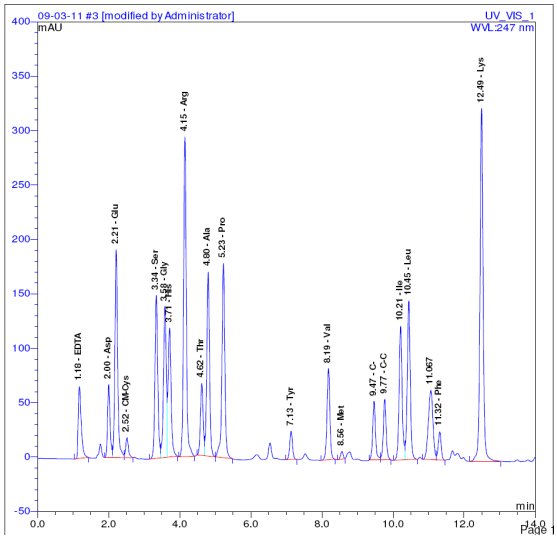
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

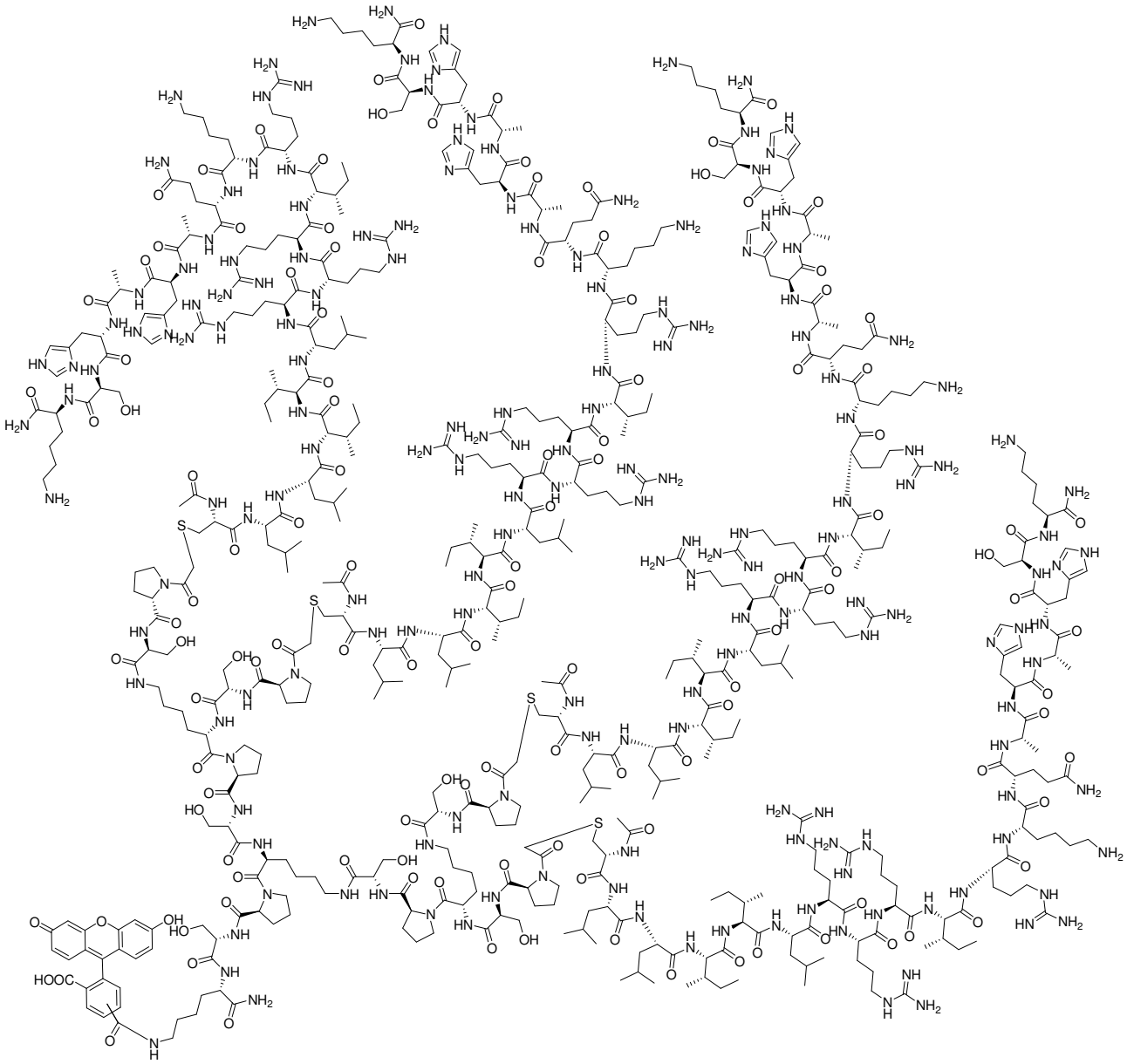


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.17	0.089	6.52	65.72		481.7	EDTA
2.00	2.00	0.072	5.39	66.86		388.2	Asp
2.21	2.21	0.078	17.09	190.97		1173.8	Glu
2.52	2.51	0.078	1.62	18.20		106.3	CM-Cys
3.34	3.34	0.083	13.92	149.66		989.2	Ser
3.58	3.58	0.089	12.89	138.58		890.6	Gly
3.71	3.72	0.097	11.53	118.36		796.5	His
4.15	4.14	0.078	26.26	293.48		1925.4	Arg
4.62	4.62	0.082	5.88	66.08		504.5	Thr
4.80	4.80	0.083	15.44	169.02		1091.4	Ala
5.23	5.23	0.082	17.08	178.43		1000.9	Pro
7.13	7.13	0.083	2.37	26.07		163.9	Tyr
8.19	8.20	0.086	8.01	83.94		536.7	Val
8.56	8.57	0.080	0.58	7.01		48.9	Met
9.47	9.48	0.080	4.96	53.84		187.0	C-
9.77	9.78	0.081	5.13	55.70		193.4	C-C
10.21	10.22	0.088	12.29	122.76		782.7	Ile
10.45	10.45	0.088	14.38	145.77		967.2	Leu
11.32	11.32	0.087	2.46	25.85		172.1	Phe
12.49	12.50	0.089	33.22	324.26		1154.0	Lys
Total:						13554.31	

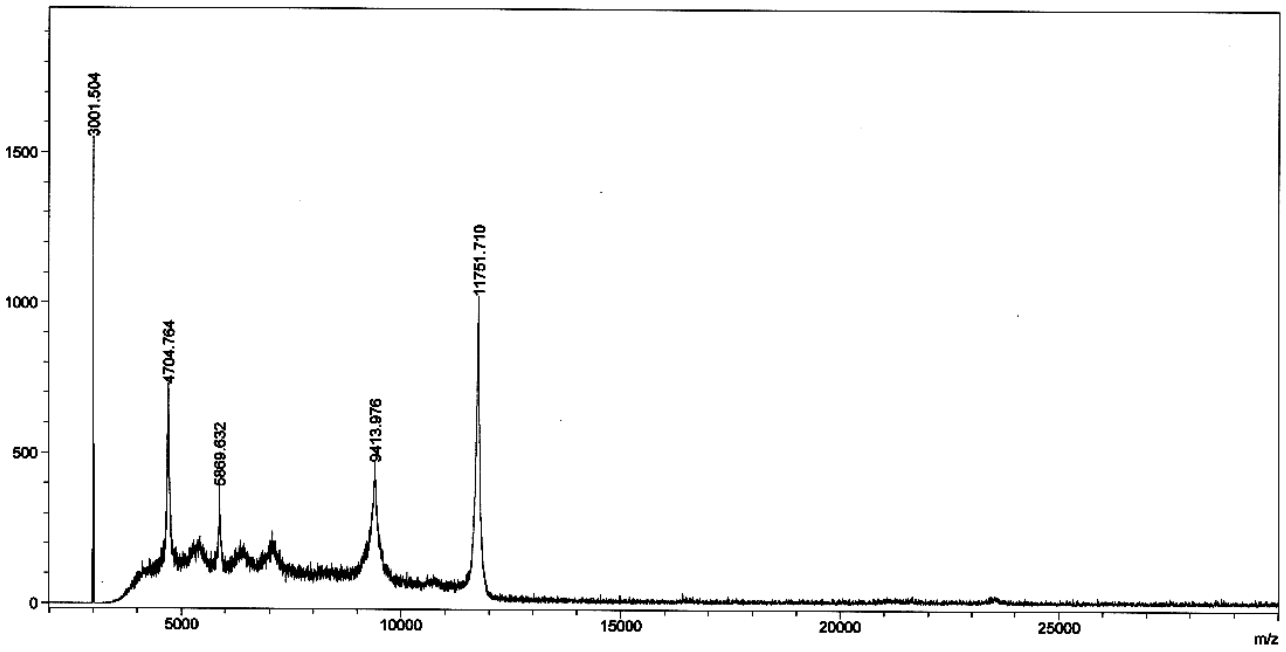
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1091.4	4	4.8
Arg	1925.4	8	8.5
CM-Cys[a)]	106.3	2	0.5
Gln[b)]	1173.8	2	5.2
His	796.5	4	3.5
Ile	782.7	6	3.5
Leu	967.2	6	4.3
Lys	1154.0	6	5.1
Pro	1000.9	3	4.4
Ser	989.2	5	4.4

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

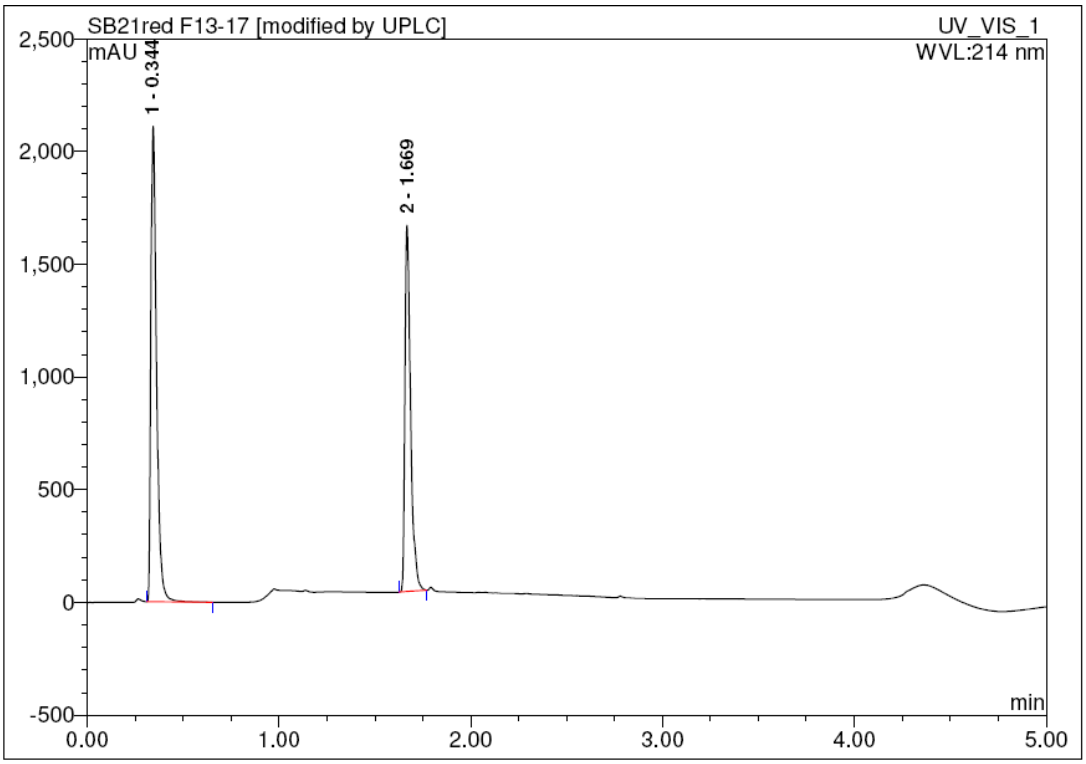
pVEC-G2b ((AcC(LLIILRRRIRKQAHAHSK-NH_2)xPS)$_4$(*K*PS)$_2$*K*PSK*). From starting materials **G2b** and **Cys-pVEC** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **pVEC-G2b** was obtained as a foamy yellow solid after preparative RP-HPLC (3.1 mg). Analytical RP-HPLC: t_R = 1.67 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (MALDI-TOF+): $C_{521}H_{885}N_{179}O_{123}S_4$ found/calc. 11751.7/11753.0 $[M]^+$; $C_{418}H_{701}ClN_{140}O_{101}S_3$ (3-fold ligation product with one unreacted chloroacetyl group) found/calc. 9414.0/9435.6 $[M]^+$.



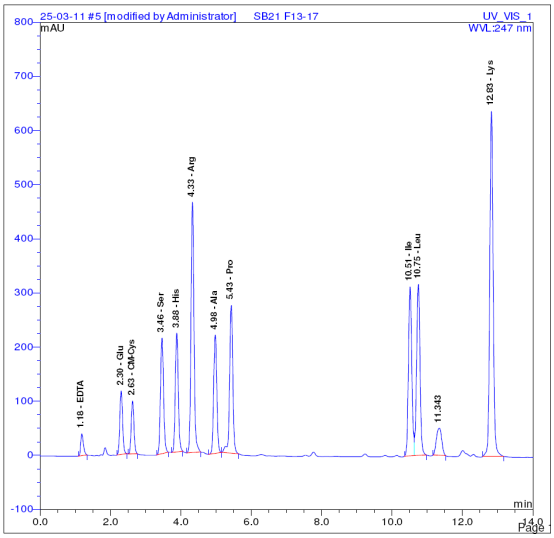
Mass spectrum, MS (MALDI-TOF+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

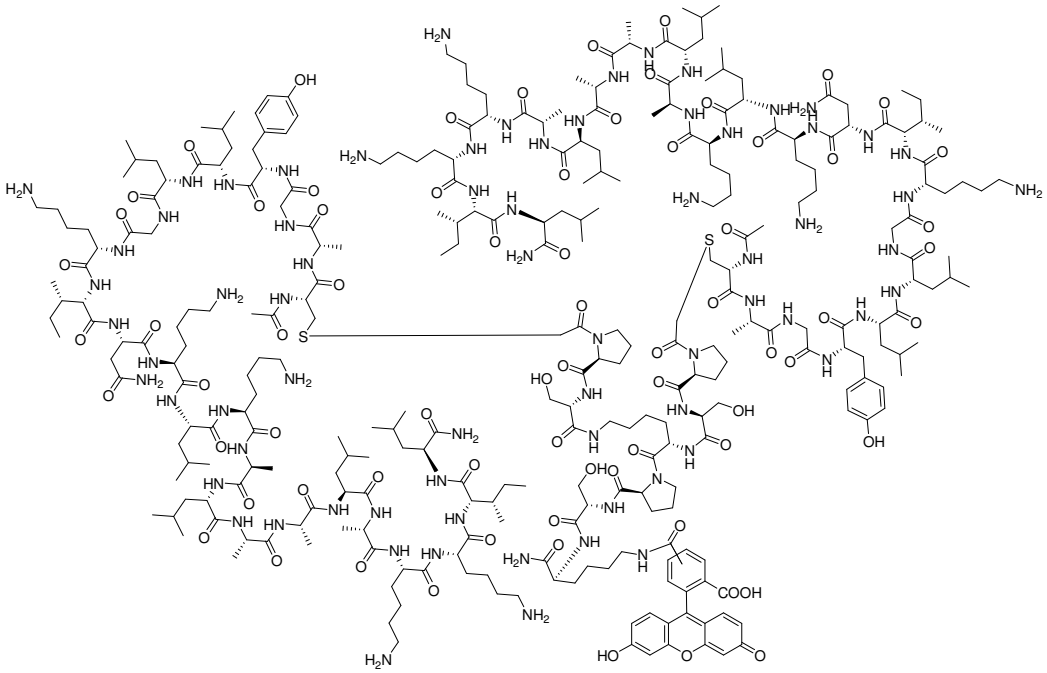


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.18	0.082	3.57	40.17		362.8	EDTA
2.30	2.31	0.086	10.87	117.23		733.4	Glu
2.63	2.64	0.085	8.85	97.65		576.1	CM-Cys
3.46	3.47	0.095	21.85	213.70		1515.4	Ser
3.88	3.89	0.091	21.61	219.39		1538.4	His
4.33	4.34	0.090	45.80	462.40		3201.5	Arg
4.98	4.99	0.097	22.58	218.71		1553.9	Ala
5.43	5.45	0.095	29.24	272.80		1669.5	Pro
10.51	10.52	0.103	34.68	312.07		2163.9	Ile
10.75	10.75	0.105	35.94	315.88		2276.8	Leu
12.83	12.82	0.102	71.34	637.01		2407.9	Lys
Total:						17999.56	

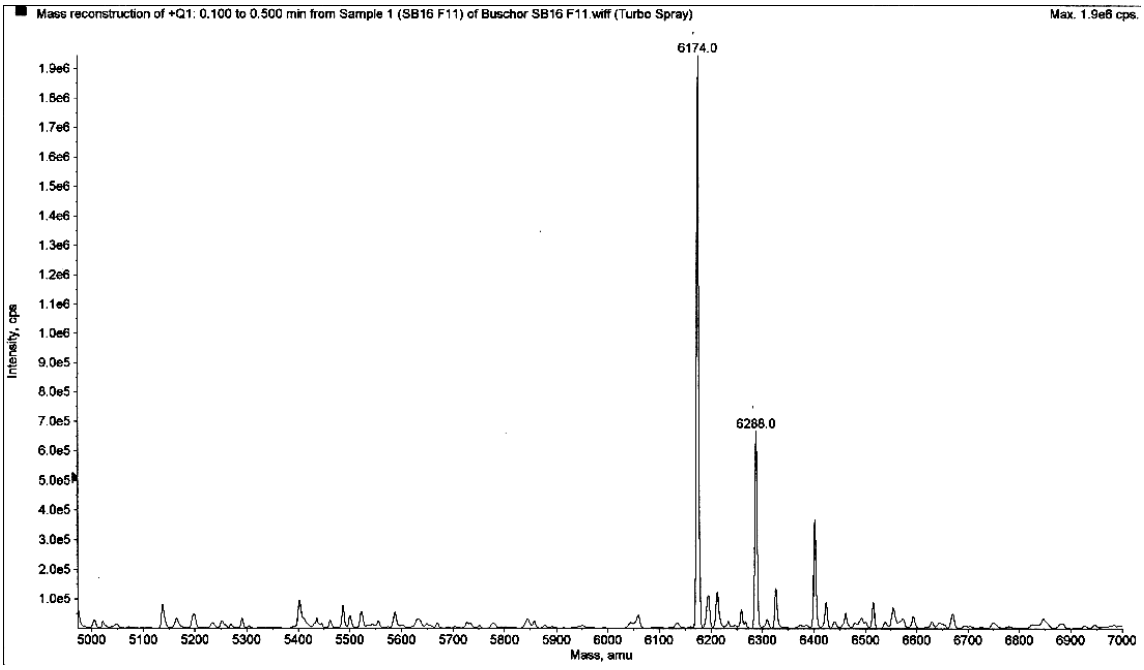
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1553.9	8	8.3
Arg	3201.5	16	17.1
CM-Cys[a)]	576.1	4	3.1
Gln[b)]	733.4	4	3.9
His	1538.4	8	8.2
Ile	2163.9	12	11.5
Leu	2276.8	12	12.1
Lys	2407.9	12	12.8
Pro	1669.5	7	8.9
Ser	1515.4	11	8.1

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

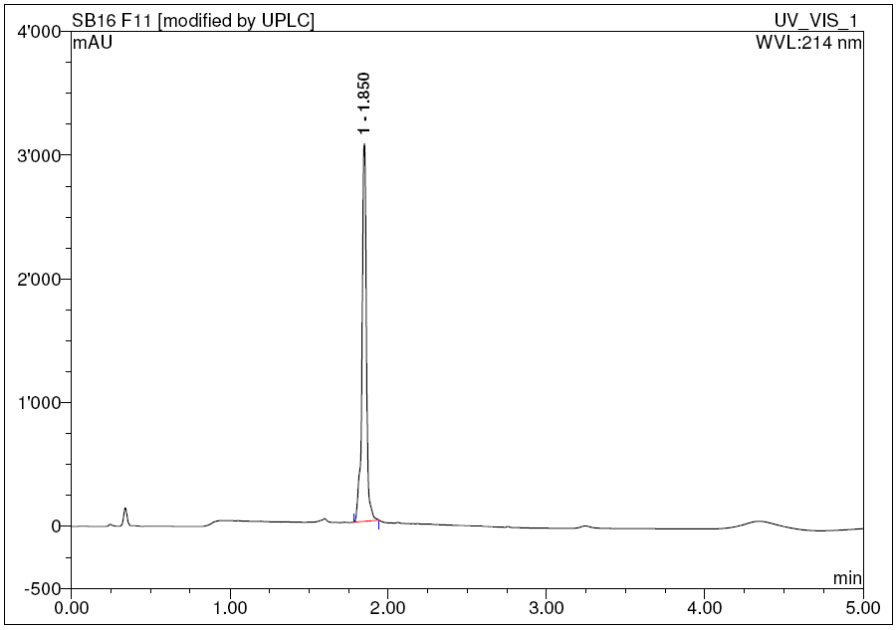
TP10K-G1b ((AcC(AGYLLGKINKLKALAALAKKIL-NH_2)xPS)$_2$*KPSK*). From starting materials **G1b** and **Cys-TP10K** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **TP10K-G1b** was obtained as a foamy yellow solid after preparative RP-HPLC (9.3 mg, 1.4 µmol, yield 61%). Analytical RP-HPLC: t_R = 1.85 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{291}H_{481}N_{71}O_{71}S_2$ found/calc. 6174.0/6174.5 $[M]^+$; 6288.0/6288.5 $[M + TFA]^+$.



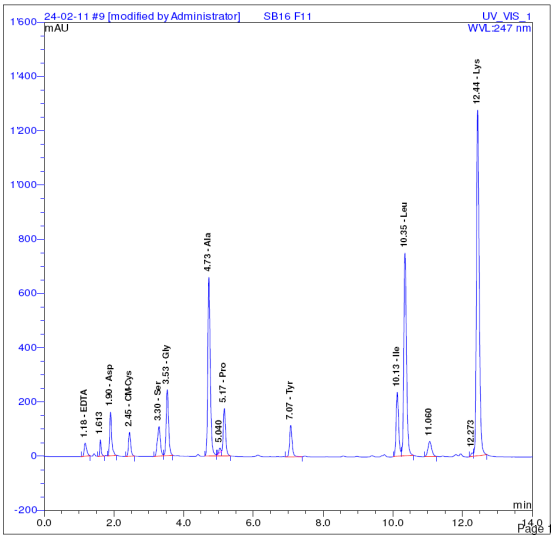
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

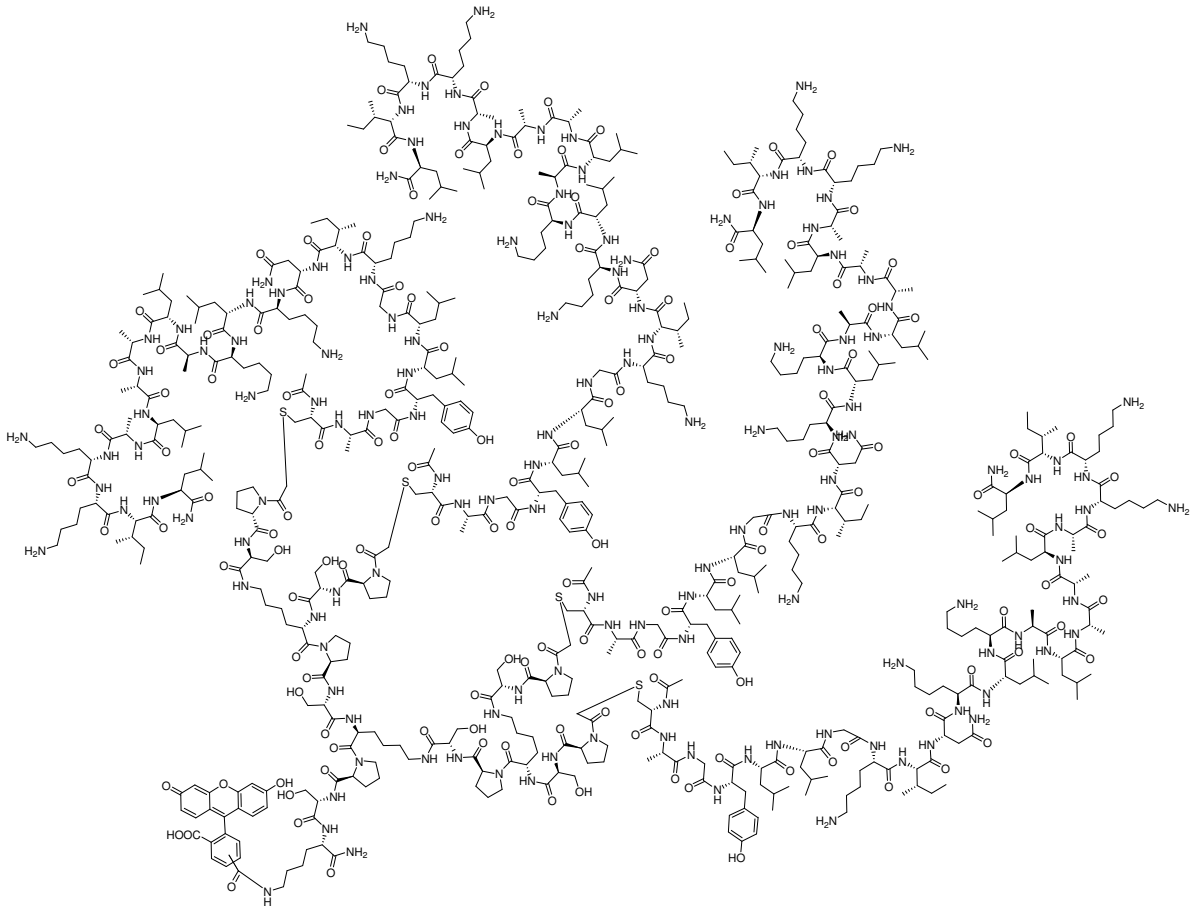


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.17	0.076	3.97	48.14		359.0	EDTA
1.90	1.90	0.059	11.04	160.43		760.0	Asp
2.45	2.45	0.066	6.48	88.46		453.6	CM-Cys
3.30	3.30	0.095	10.88	108.96		640.0	Ser
3.53	3.53	0.072	19.44	243.11		1402.6	Gly
4.73	4.73	0.074	54.85	659.02		3797.3	Ala
5.17	5.17	0.072	15.69	174.97		865.3	Pro
7.07	7.08	0.074	9.79	116.17		659.1	Tyr
10.13	10.14	0.076	19.70	235.65		1337.4	Ile
10.35	10.37	0.082	67.27	747.30		4404.9	Leu
12.44	12.47	0.083	116.83	1274.26		4102.1	Lys
Total:						18781.44	

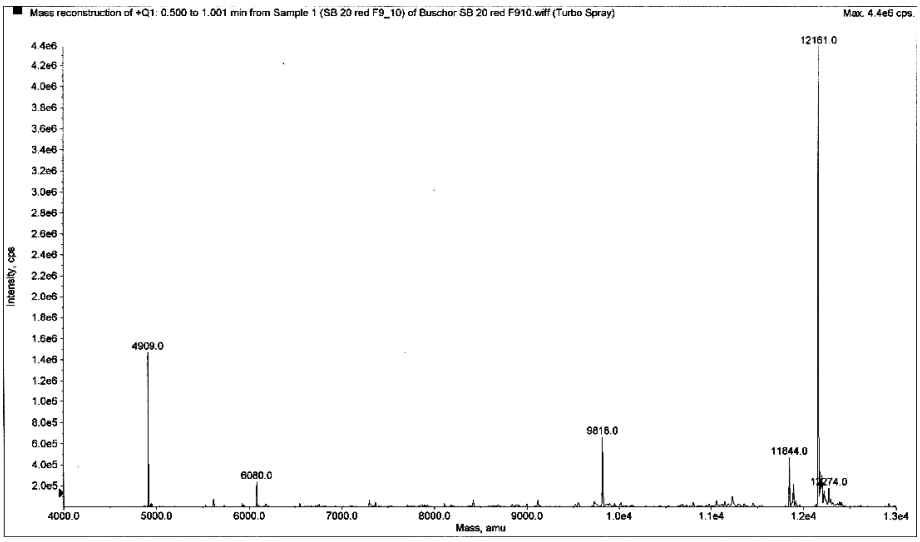
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	3797.3	10	11.1
Asn[a)]	760.0	2	2.2
CM-Cys[b)]	453.6	2	1.3
Gly	1402.6	4	4.1
Ile	1337.4	4	3.9
Leu	4404.9	12	12.9
Lys	4102.1	12	12.0
Pro	865.3	3	2.5
Ser	640.0	3	1.9
Tyr	659.1	2	1.9

[a)] Detected as Asp. [b)] CM-Cys = carboxymethyl cysteine.

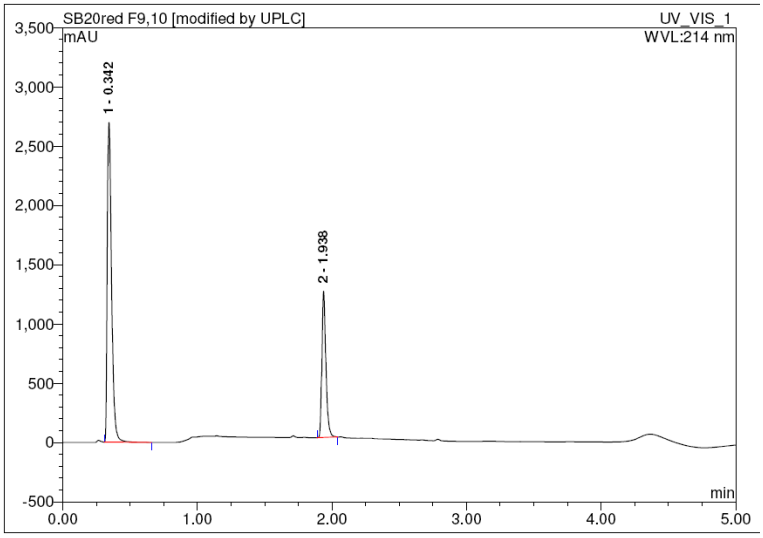
TP10K-G2b ((AcC(AGYLLGKINKLKALAALAKKIL-NH_2)xPS)$_4$(*K*PS)$_2$*K*PSK*). From starting materials **G2b** and **Cys-TP10K** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **TP10K-G2b** was obtained as a foamy yellow solid after preparative RP-HPLC (2.0 mg, 0.2 µmol, yield 13%). Analytical RP-HPLC: t_R = 1.94 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{569}H_{961}N_{143}O_{139}S_4$ found/calc. 12161.0/12157.9 $[M]^+$.



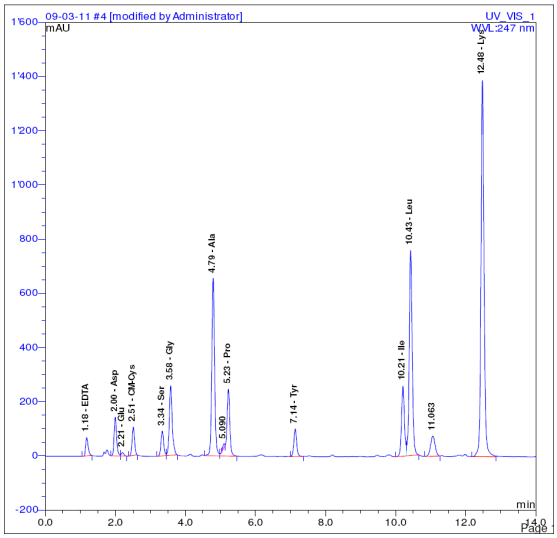
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

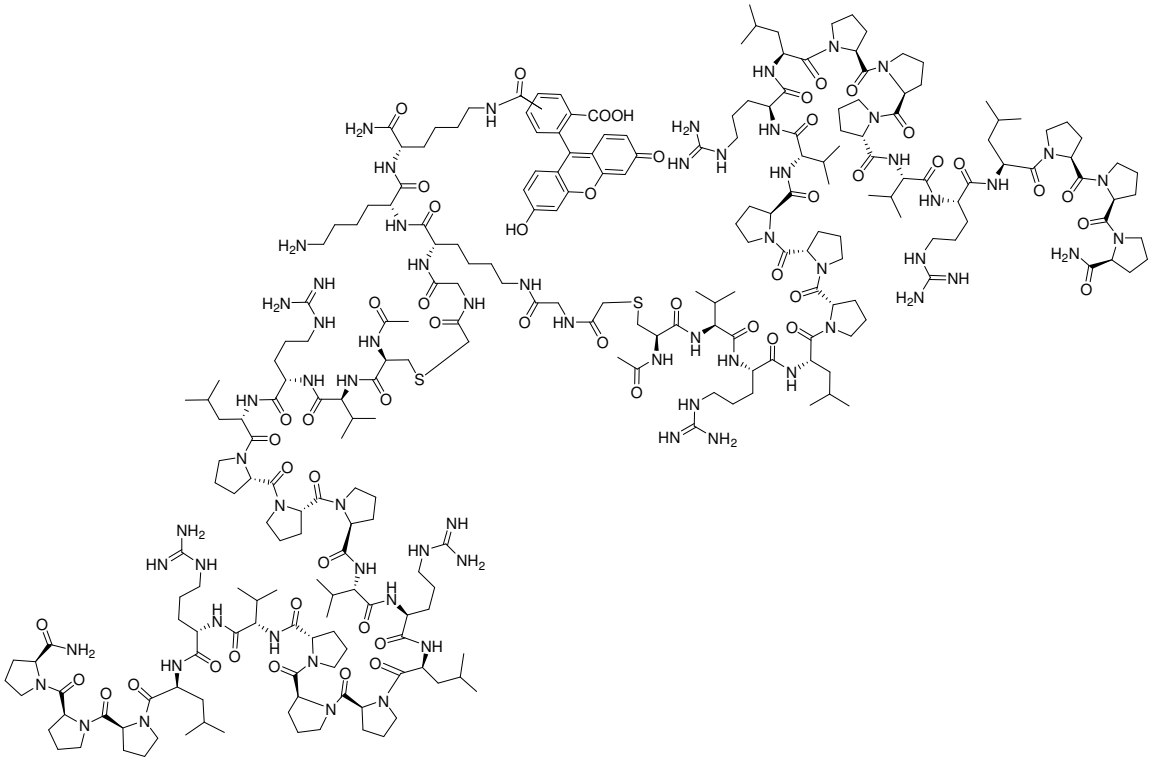


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.17	0.077	5.68	67.55		495.1	EDTA
2.00	2.00	0.072	11.53	142.64		828.2	Asp
2.21	2.21	0.083	1.10	12.46		76.6	Glu
2.51	2.51	0.075	8.49	104.60		611.1	CM-Cys
3.34	3.34	0.081	8.18	91.43		604.3	Ser
3.58	3.58	0.084	24.95	256.63		1649.2	Gly
4.79	4.80	0.083	61.19	655.29		4231.5	Ala
5.23	5.23	0.083	26.35	246.99		1385.5	Pro
7.14	7.13	0.085	9.63	101.89		640.6	Tyr
10.21	10.22	0.087	24.92	257.77		1643.5	Ile
10.43	10.45	0.089	75.18	756.30		5018.2	Leu
12.48	12.50	0.090	142.87	1387.39		4937.4	Lys
Total:						22121.29	

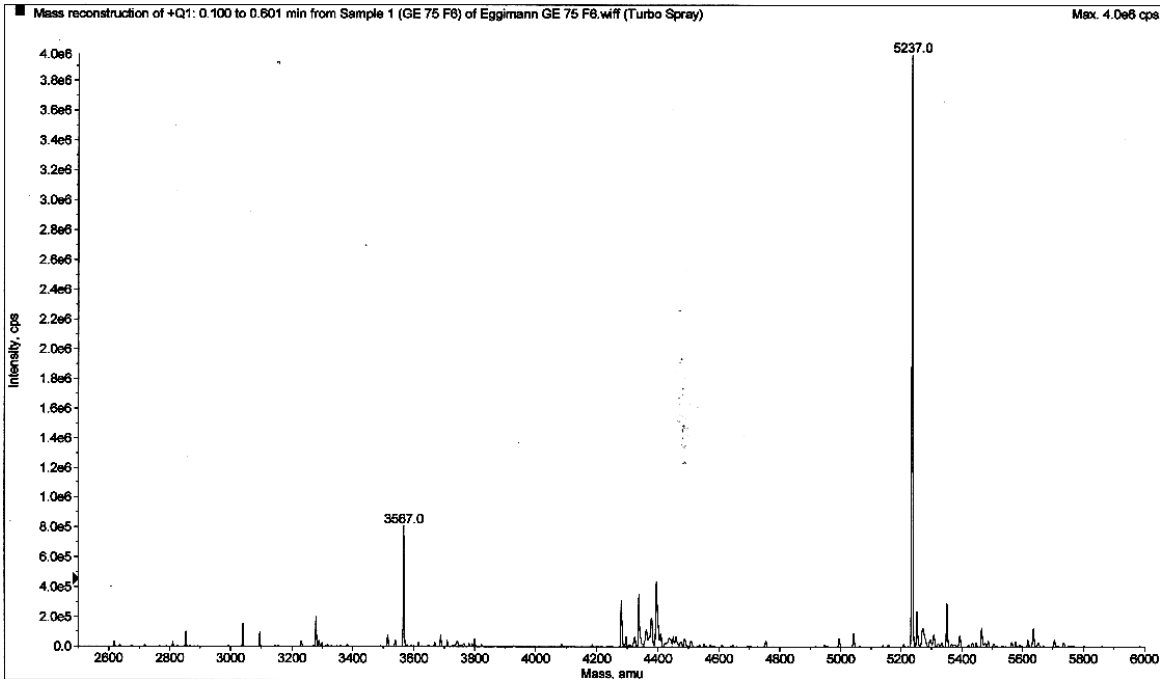
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	4231.5	20	21.6
Asn[a)]	828.2	4	4.2
CM-Cys[b)]	611.1	4	3.1
Gly	1649.2	8	8.4
Ile	1643.5	8	8.4
Leu	5018.2	24	25.6
Lys	4937.4	24	25.2
Pro	1385.5	7	7.1
Ser	604.3	7	3.1
Tyr	640.6	4	3.3

[a)] Detected as Asp. [b)] CM-Cys = carboxymethyl cysteine.

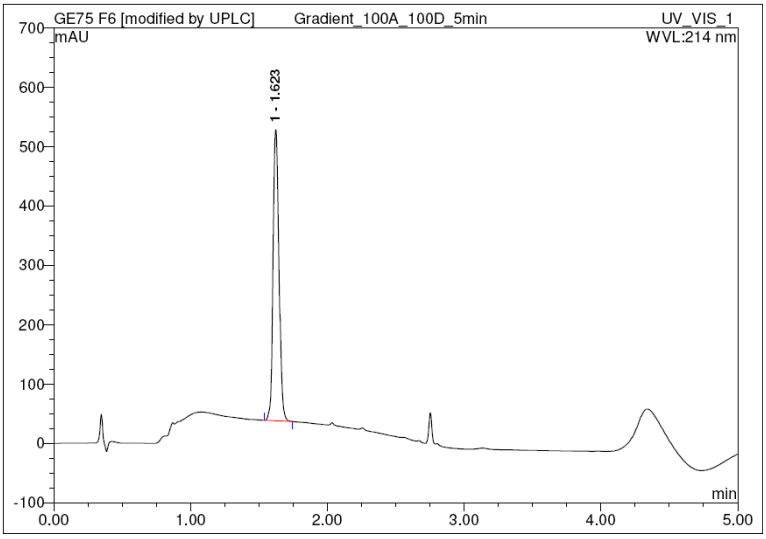
SAP-G1a ((AcC(VRLPPPVRLPPPVRLPPP-NH_2)xG)$_2$*K*kK*). From starting materials **G1a** and **Cys-SAP** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v)), **SAP-G1a** was obtained as a foamy yellow solid after preparative RP-HPLC (3.7 mg, 0.6 µmol, yield 65%). Analytical RP-HPLC: t_R = 1.62 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{249}H_{393}N_{76}O_{53}S_2$ found/calc. 5237.0/5237.3 $[M]^+$.



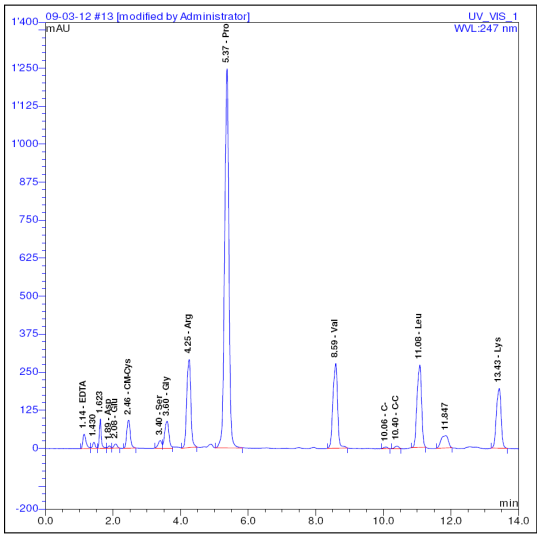
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

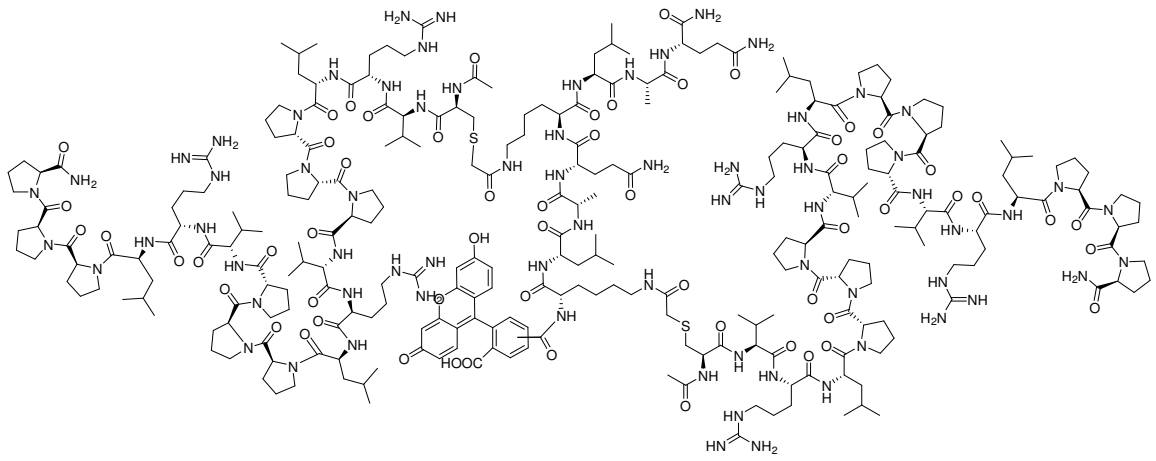


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.14	1.15	0.098	5.20	47.37		361.87	EDTA
1.89	1.90	0.088	0.69	7.44		40.03	Asp
2.08	2.08	0.105	1.51	13.79		82.73	Glu
2.46	2.46	0.112	11.01	91.97		608.84	CM-Cys
3.40	3.35	0.124	2.95	24.35		187.12	Ser
3.60	3.60	0.135	12.53	87.92		654.55	Gly
4.25	4.25	0.132	40.60	289.71		2201.38	Arg
5.37	5.40	0.136	181.30	1246.50		8303.88	Pro
8.59	8.59	0.151	44.13	278.58		2237.18	Val
10.06	10.09	0.137	0.78	5.73		29.05	C-
10.40	10.39	0.134	1.07	7.90		40.05	C-C
11.08	11.08	0.153	42.16	271.14		2321.99	Leu
13.43	13.43	0.156	31.59	196.27		907.34	Lys
Total:						17976.02	

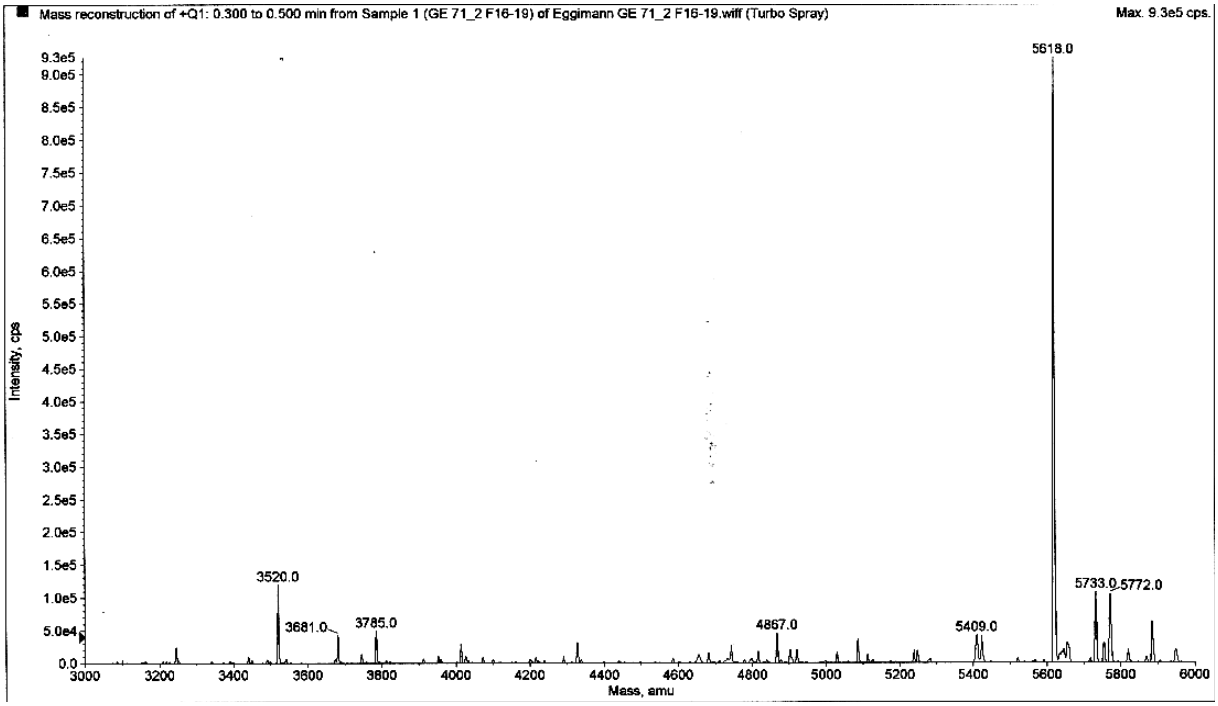
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	2201.4	6	5.5
CM-Cys[a)]	608.8	2	1.5
Gly	654.6	2	1.6
Leu	2322.0	6	5.8
Lys	907.3	3	2.3
Pro	8303.9	18	20.7
Val	2237.2	6	5.6

[a)] CM-Cys = carboxymethyl cysteine.

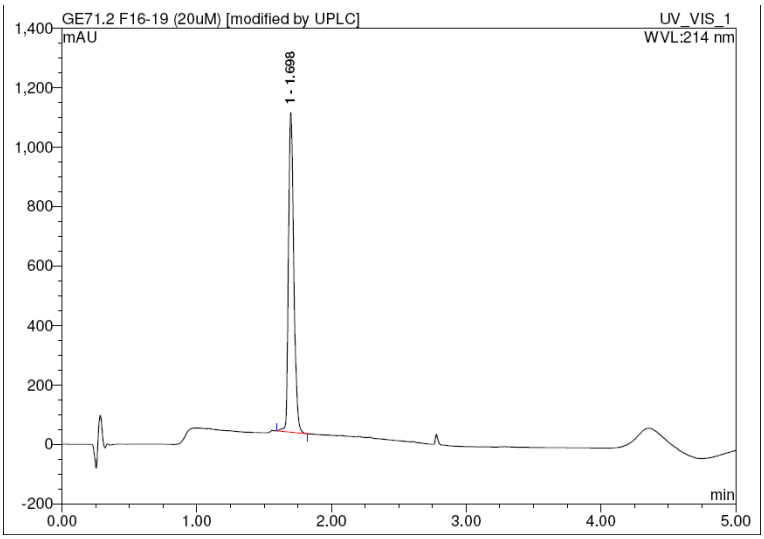
SAP-G1L (*-[K(x-(AcCVRLPPPVRLPPPVRLPPP-NH$_2$))LAQ]$_2$). From starting materials **G1L** and **Cys-SAP** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **SAP-G1L** was obtained as a foamy yellow solid after preparative RP-HPLC (7.5 mg, 1.2 µmol, yield 61%). Analytical RP-HPLC: t_R = 1.70 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{267}H_{423}N_{71}O_{58}S_2$ found/calc. 5618.0/5619.8 $[M]^+$.



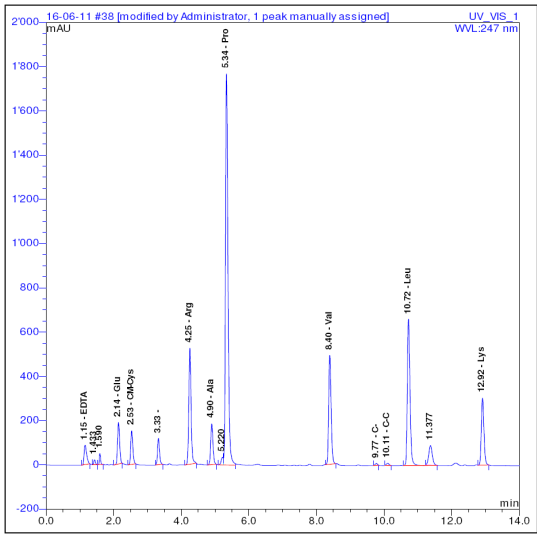
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

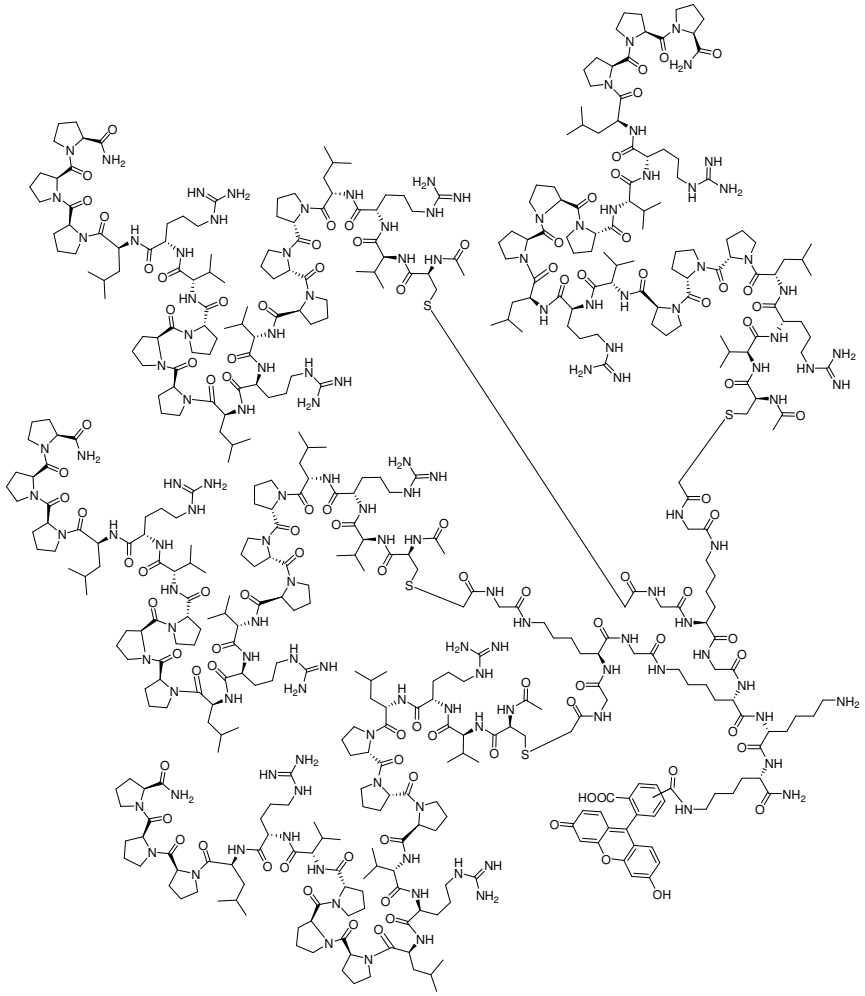


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.16	0.082	7.69	87.65		343.39	EDTA
2.14	2.14	0.066	13.27	187.71		1069.84	Glu
2.53	2.53	0.066	10.96	151.86		809.14	CM-Cys
4.25	4.25	0.070	40.92	523.92		3122.49	Arg
4.90	4.90	0.073	14.61	184.61		1069.66	Ala
5.34	5.36	0.078	152.82	1767.72		8799.46	Pro
8.40	8.41	0.078	41.56	492.27		2898.04	Val
9.77	9.76	0.072	0.59	8.00		28.30	C-
10.11	10.07	0.086	0.81	9.16		32.39	C-C
10.72	10.74	0.083	60.52	661.26		4067.03	Leu
12.92	12.92	0.082	27.16	303.06		1042.74	Lys
Total:						23282.48	

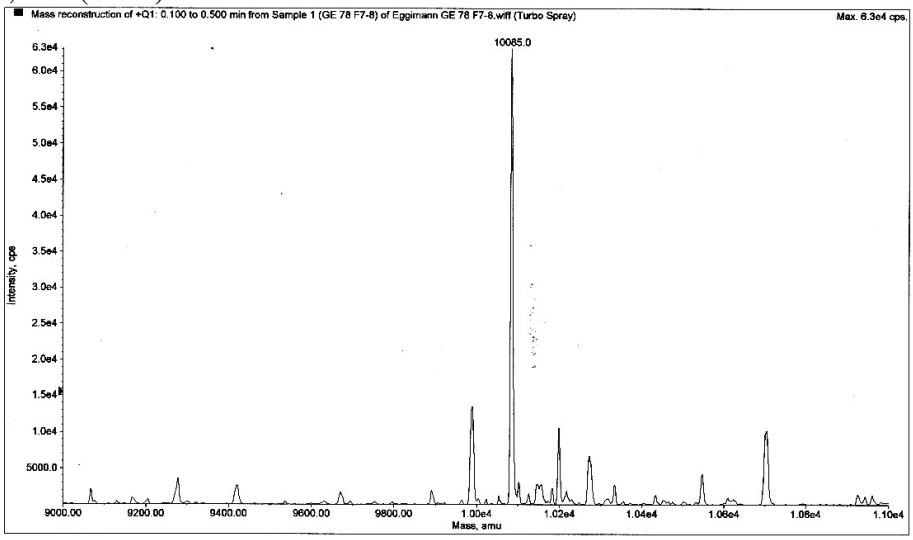
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1069.7	2	2.2
Arg	3122.5	6	6.3
CM-Cys[a)]	809.1	2	1.6
Gln[b)]	1069.8	2	2.2
Leu	4067.0	8	8.2
Lys	1042.7	2	2.1
Pro	8799.5	18	17.7
Val	2898.0	6	5.8

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

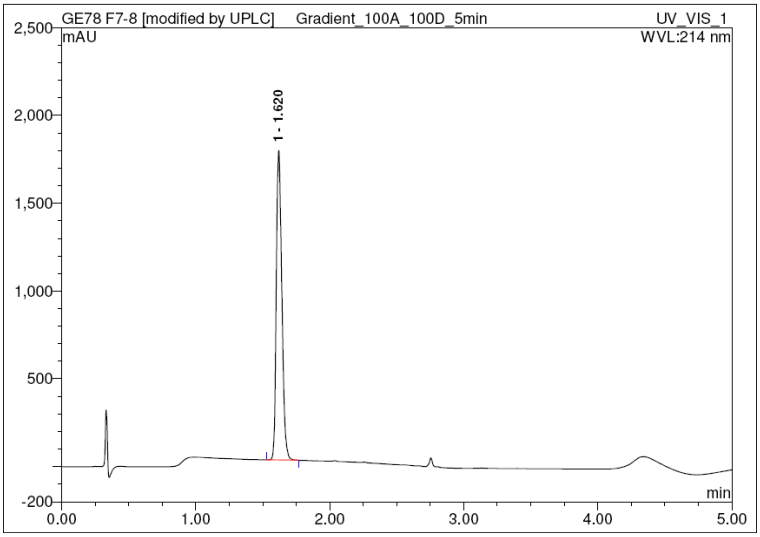
SAP-G2a ((AcC(VRLPPPVRLPPPVRLPPP-NH_2)xG)$_4$(*K*G)$_2$*K*kK*). From starting materials **G2a** and **Cys-SAP** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v)), **SAP-G2a** was obtained as a foamy yellow solid after preparative RP-HPLC (5.8 mg, 0.5 µmol, yield 92%). Analytical RP-HPLC: t_R = 1.62 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{475}H_{767}N_{133}O_{101}S_4$ found/calc. 10085.0/10085.3 $[M]^+$.



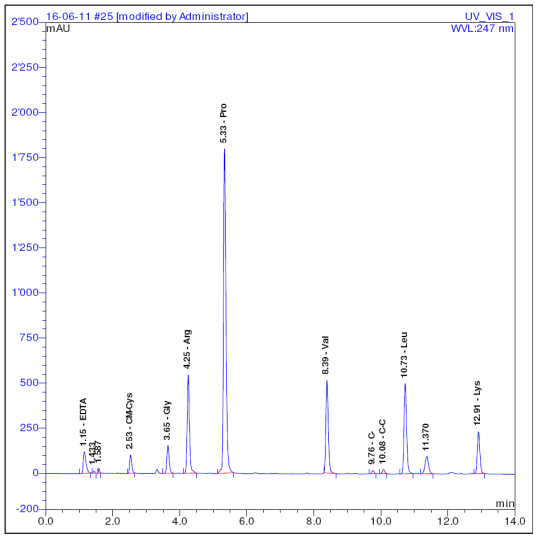
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Amino acid analysis:

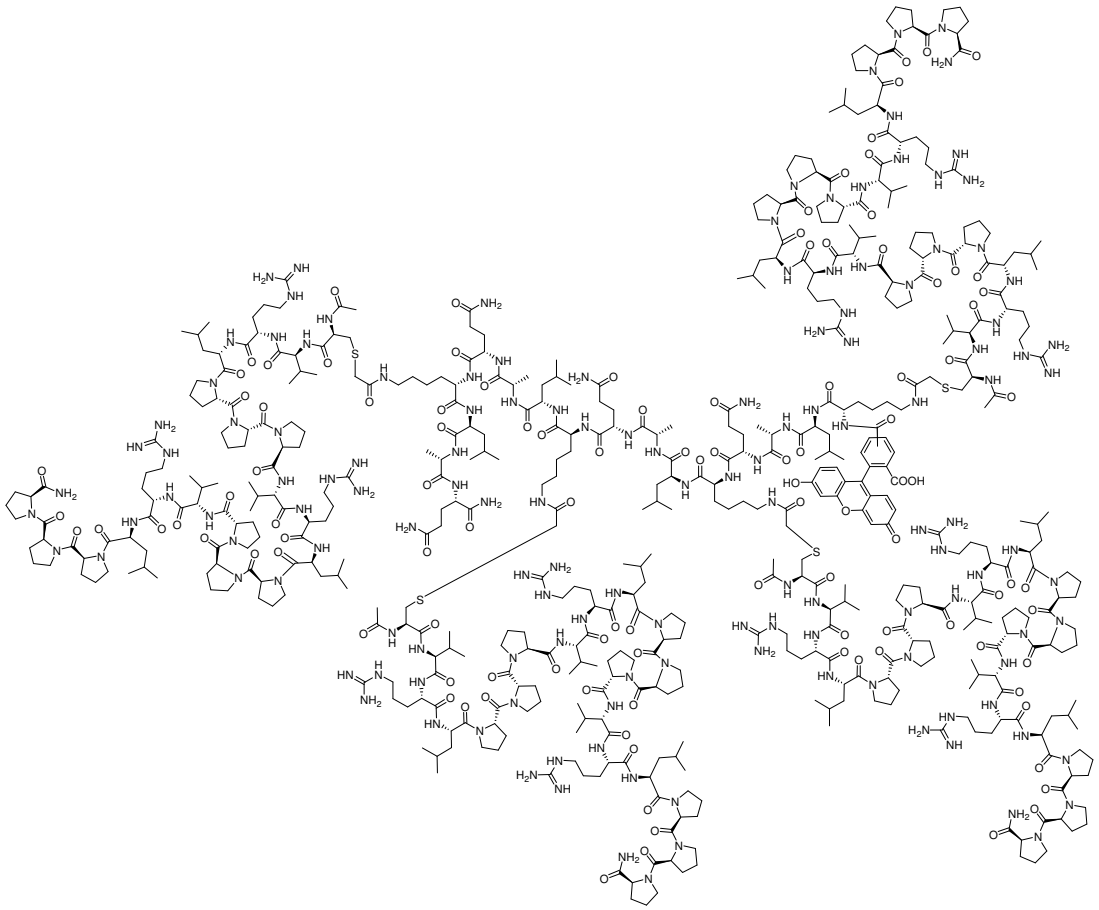


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.16	0.086	11.21	119.47		468.07	EDTA
2.53	2.53	0.068	7.62	102.46		545.93	CM-Cys
3.65	3.65	0.072	12.21	154.31		906.91	Gly
4.25	4.25	0.070	43.12	545.39		3250.44	Arg
5.33	5.36	0.078	155.49	1798.04		8950.41	Pro
8.39	8.41	0.078	43.48	513.15		3021.02	Val
9.76	9.76	0.073	1.43	18.32		64.77	C-
10.08	10.07	0.081	2.05	24.18		85.50	C-C
10.73	10.74	0.081	44.44	500.16		3076.20	Leu
12.91	12.92	0.082	20.72	232.64		800.44	Lys
Total:						21169.69	

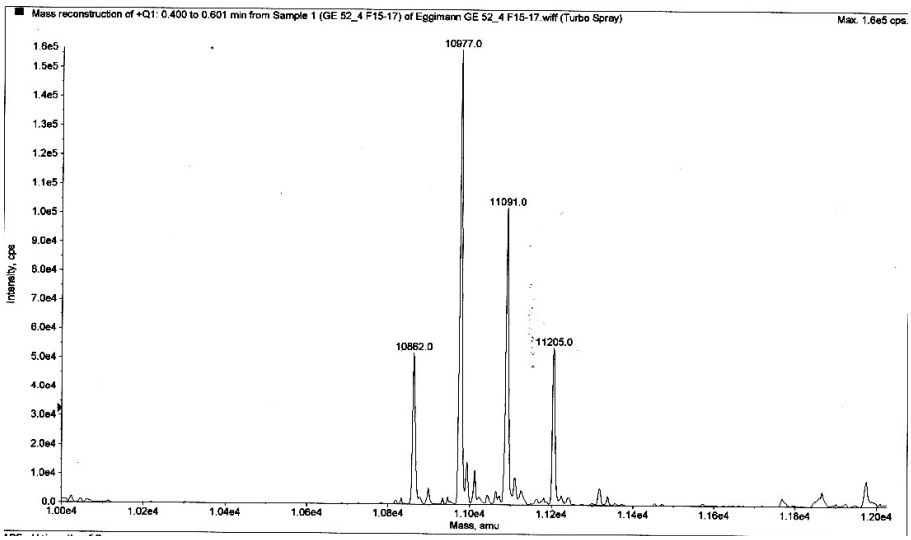
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	3250.4	12	13.8
CM-Cys[a)]	545.9	4	2.3
Gly	906.9	6	3.8
Leu	3076.2	12	13.0
Lys	800.4	5	3.4
Pro	8950.4	36	37.9
Val	3021.0	12	12.8

[a)] CM-Cys = carboxymethyl cysteine.

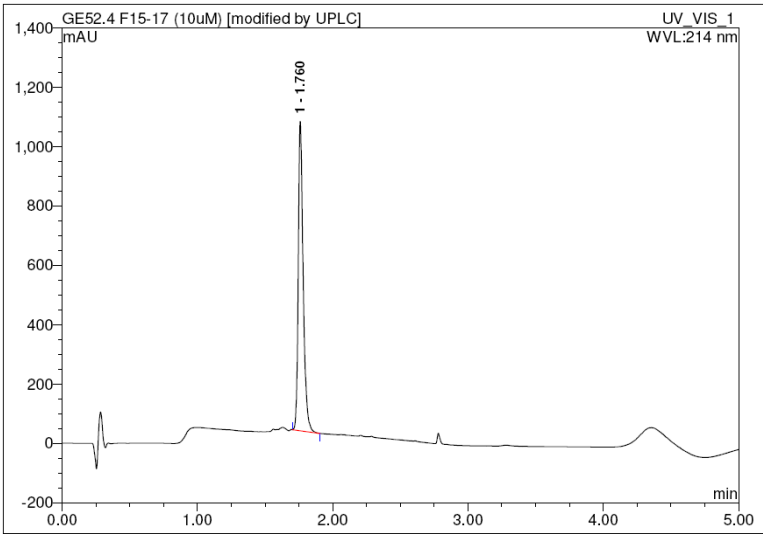
SAP-G2L (*-[K(x-(AcCVRLPPPVRLPPPVRLPPP-NH_2))LAQ]$_4$). From starting materials **G2L** and **Cys-SAP** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **SAP-G2L** was obtained as a foamy yellow solid after preparative RP-HPLC (8.6 mg, 0.7 µmol, yield 58%). Analytical RP-HPLC: t_R = 1.76 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{513}H_{833}N_{141}O_{110}S_4$ found/calc. 10862.0/10864.2 $[M]^+$; 10977.0/10978.2 [M + TFA]$^+$; 11091.0/11092.2 [M + 2TFA]$^+$; 11205.0/11206.3 [M + 3TFA]$^+$.



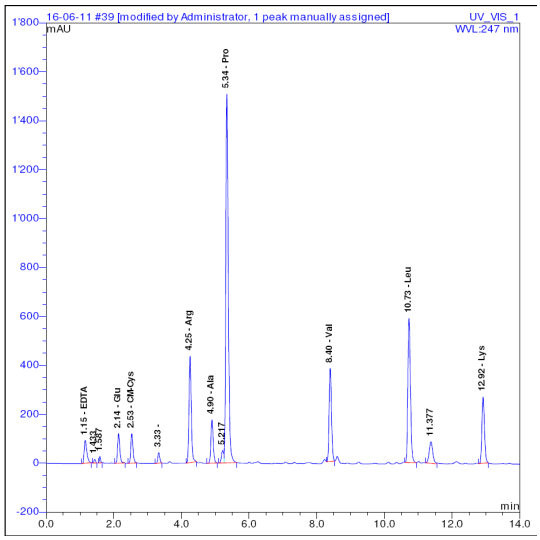
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

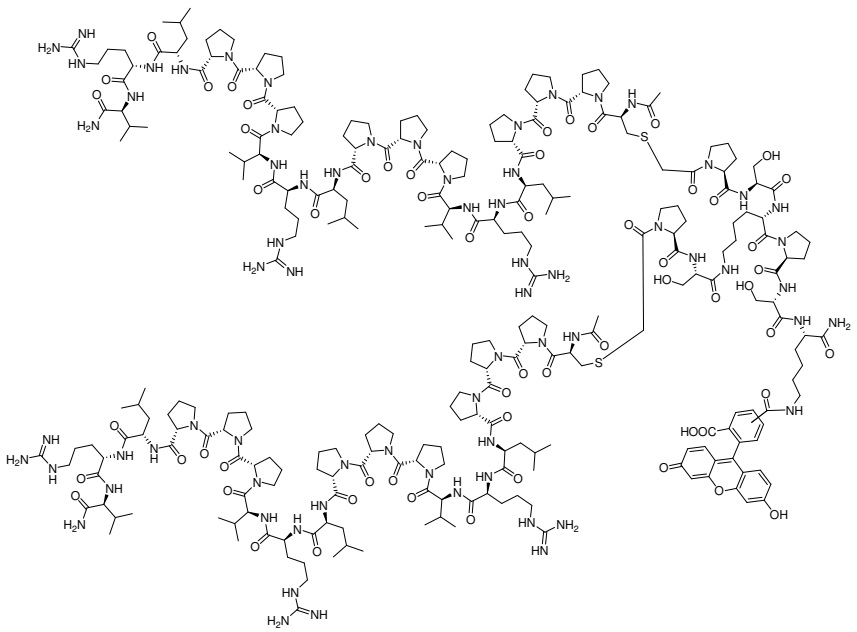


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.16	0.083	8.45	93.20		365.12	EDTA
2.14	2.14	0.067	9.03	120.64		687.56	Glu
2.53	2.53	0.068	9.02	120.44		641.72	CM-Cys
4.25	4.25	0.070	34.02	434.52		2589.68	Arg
4.90	4.90	0.073	14.12	176.85		1024.73	Ala
5.34	5.36	0.078	129.35	1507.22		7502.76	Pro
8.40	8.41	0.077	31.19	381.05		2243.30	Val
10.73	10.74	0.082	52.20	589.53		3625.87	Leu
12.92	12.92	0.082	23.89	270.41		930.38	Lys
Total:						19611.12	

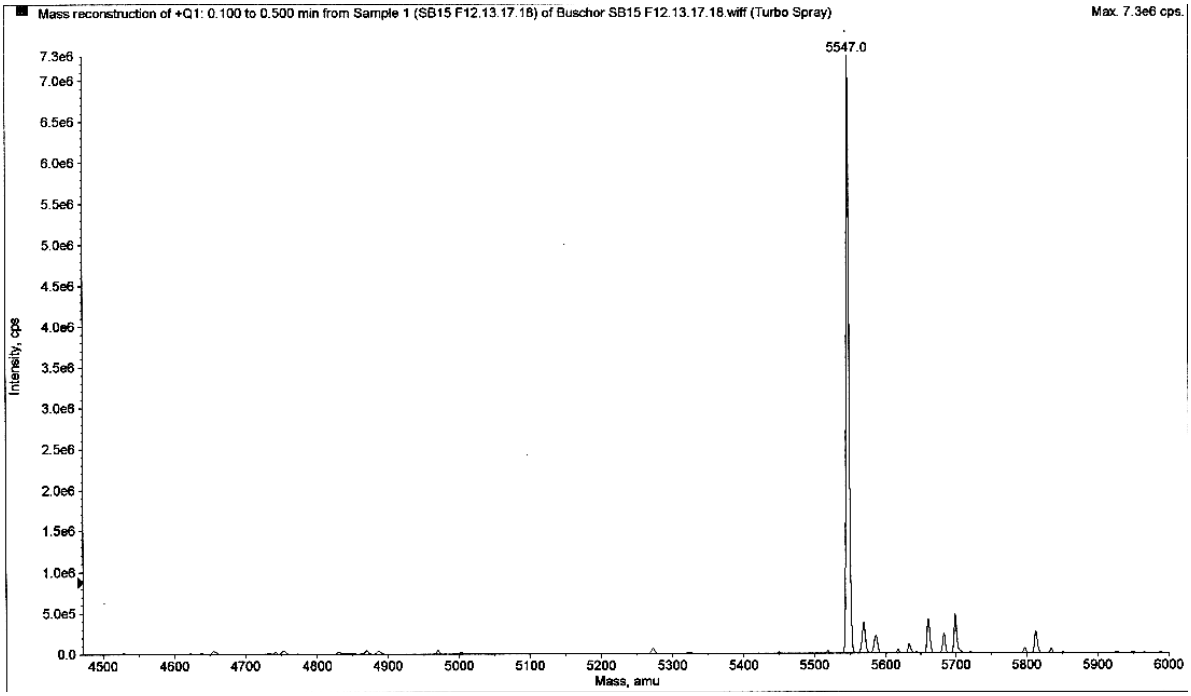
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1024.7	4	4.9
Arg	2589.7	12	12.4
CM-Cys[a)]	641.7	4	3.1
Gln[b)]	687.6	4	3.3
Leu	3625.9	16	17.3
Lys	930.4	4	4.5
Pro	7502.8	36	25.9
Val	2243.3	12	10.7

[a)] CM-Cys = carboxymethyl cysteine. [b)] Detected as Glu.

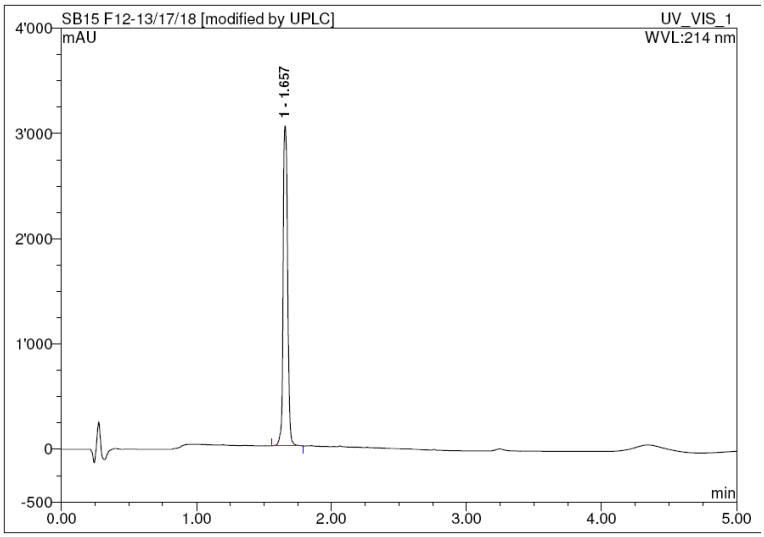
SAPr-G1b ((AcC(PPPLRVPPPLRVPPPLRV-NH_2)xPS)$_2$*K*PSK*). From starting materials **G1b** and **Cys-SAPr** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **SAPr-G1b** was obtained as a foamy yellow solid after preparative RP-HPLC (7.9 mg, 1.4 µmol, yield 60%). Analytical RP-HPLC: t_R = 1.66 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{263}H_{411}N_{69}O_{59}S_2$ found/calc. 5547.0/5547.6 $[M]^+$.



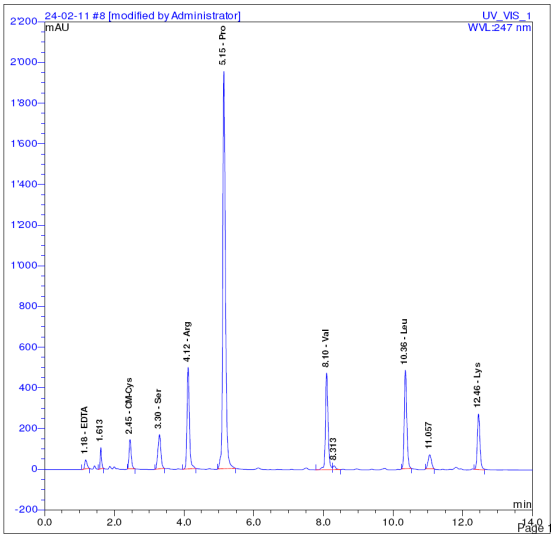
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

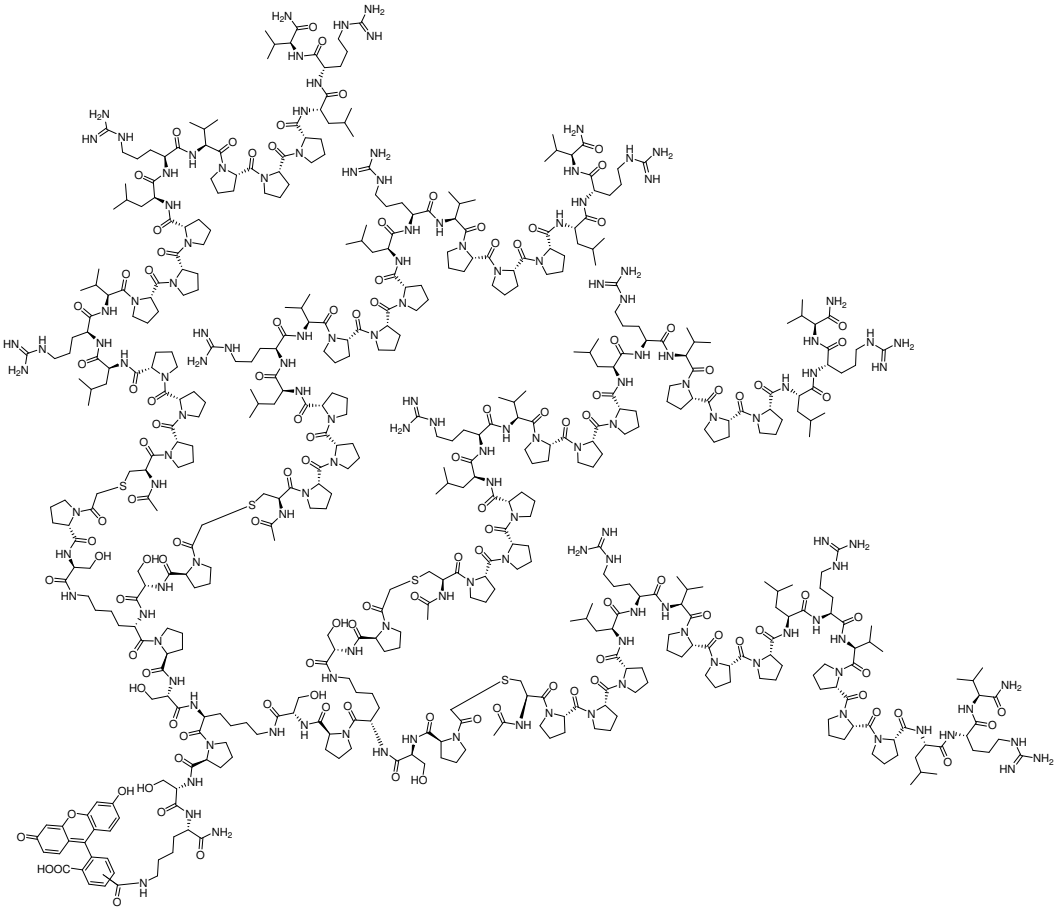


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.17	0.072	3.36	44.29		330.4	EDTA
2.45	2.45	0.065	10.39	143.96		738.2	CM-Cys
3.30	3.30	0.094	16.58	168.09		987.4	Ser
4.12	4.11	0.070	39.58	499.31		2921.1	Arg
5.15	5.17	0.079	174.44	1952.67		9657.1	Pro
8.10	8.11	0.077	41.44	474.83		2679.6	Val
10.36	10.37	0.078	41.16	483.39		2849.3	Leu
12.46	12.47	0.079	23.86	273.00		878.8	Lys
Total:						21041.91	

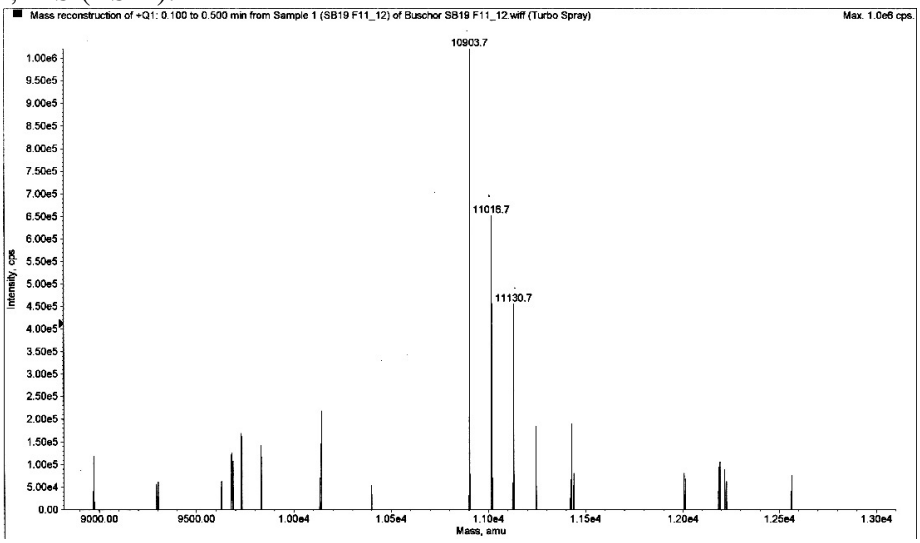
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	2921.1	6	6.5
CM-Cys[a)]	738.2	2	1.6
Leu	2849.3	6	6.3
Lys	878.8	2	2.0
Pro	9657.1	21	21.5
Ser	987.4	3	2.2
Val	2679.6	6	6.0

[a)] CM-Cys = carboxymethyl cysteine.

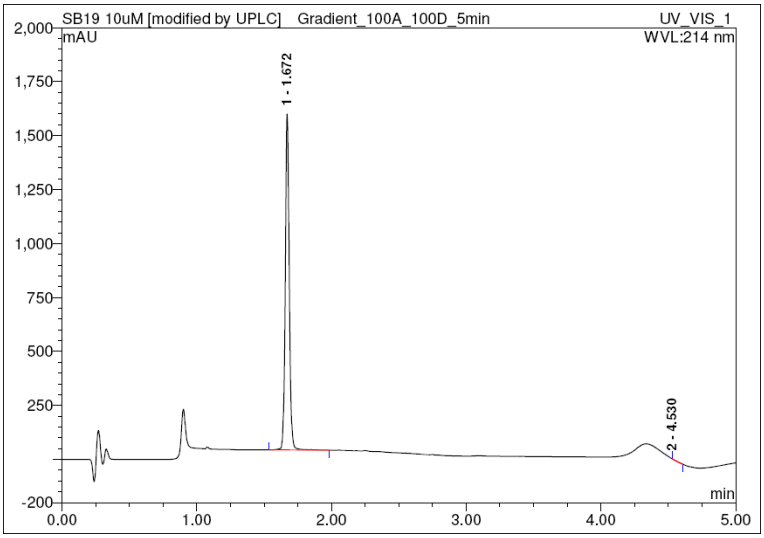
SAPr-G2b ((AcC(PPPLRVPPPLRVPPPLRV-NH_2)xPS)$_4$(*K*PS)$_2$*K*PSK*). From starting materials **G2b** and **Cys-SAPr** using the general procedure described above (solvent: DMF/H_2O (1/1, v/v), 20 equivalents KI), **SAPr-G2b** was obtained as a foamy yellow solid after preparative RP-HPLC (9.1 mg, 0.8 µmol, yield 65%). Analytical RP-HPLC: t_R = 1.70min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{513}H_{821}N_{139}O_{115}S_4$ found/calc. 10903.7/10904.1 $[M]^+$; 11016.7/11018.1 $[M + TFA]^+$; 11130.7/11132.1 $[M + 2TFA]^+$.



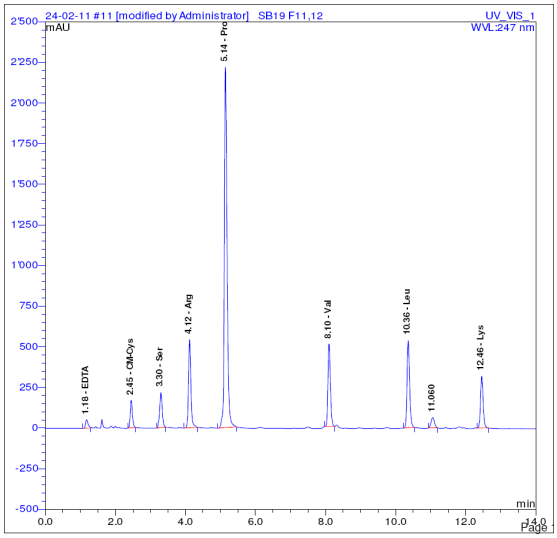
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.17	0.077	4.41	52.21		389.5	EDTA
2.45	2.45	0.065	12.07	168.34		863.2	CM-Cys
3.30	3.30	0.076	18.49	215.12		1263.6	Ser
4.12	4.11	0.069	42.86	541.96		3170.7	Arg
5.14	5.17	0.079	199.53	2217.92		10968.9	Pro
8.10	8.11	0.076	41.70	507.64		2864.8	Val
10.36	10.37	0.078	45.95	535.33		3155.4	Leu
12.46	12.47	0.079	27.56	318.49		1025.3	Lys
Total:						23701.27	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	3170.7	12	12.8
CM-Cys[a)]	863.2	4	3.5
Leu	3155.4	12	12.8
Lys	1025.3	4	4.1
Pro	10968.9	43	44.2
Ser	1263.6	7	5.1
Val	2864.8	12	11.6

[a)] CM-Cys = carboxymethyl cysteine.

References

1. Chang, J. Y.; Knecht, R., Direct Analysis of the Disulfide Content of Proteins - Methods for Monitoring the Stability and Refolding Process of Cystine-Containing Proteins. *Analytical Biochemistry* **1991,** *197* (1), 52-58.

2. Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L., Rapid analysis of amino acids using pre-column derivatization. *Journal of Chromatography B: Biomedical Sciences and Applications* **1984,** *336* (1), 93-104.