Supporting Information for:

Designed Cell Penetrating Peptide Dendrimers Efficiently Internalize Cargo Into Cells**

Gabriela A. Eggimann, Emilyne Blattes, Stefanie Buschor, Stephan M. Kammer, Tamis Darbre* and Jean-Louis Reymond*

e-mail: jean-louis.reymond@ioc.unibe.ch

Content:

Figures

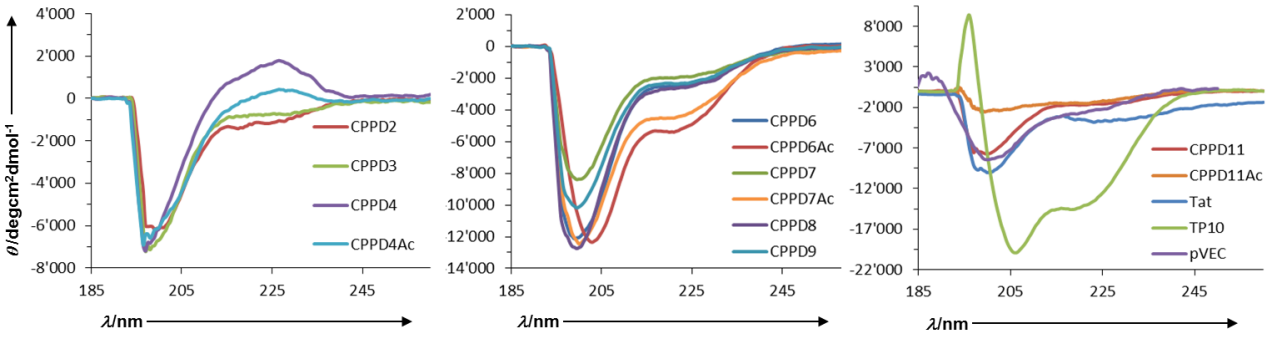


Figure S1. CD measurements of dendritic and linear CPPs in PBS buffer (pH 7.4) at room temperature. For experiment details see experimental procedure 1.8, page 14.

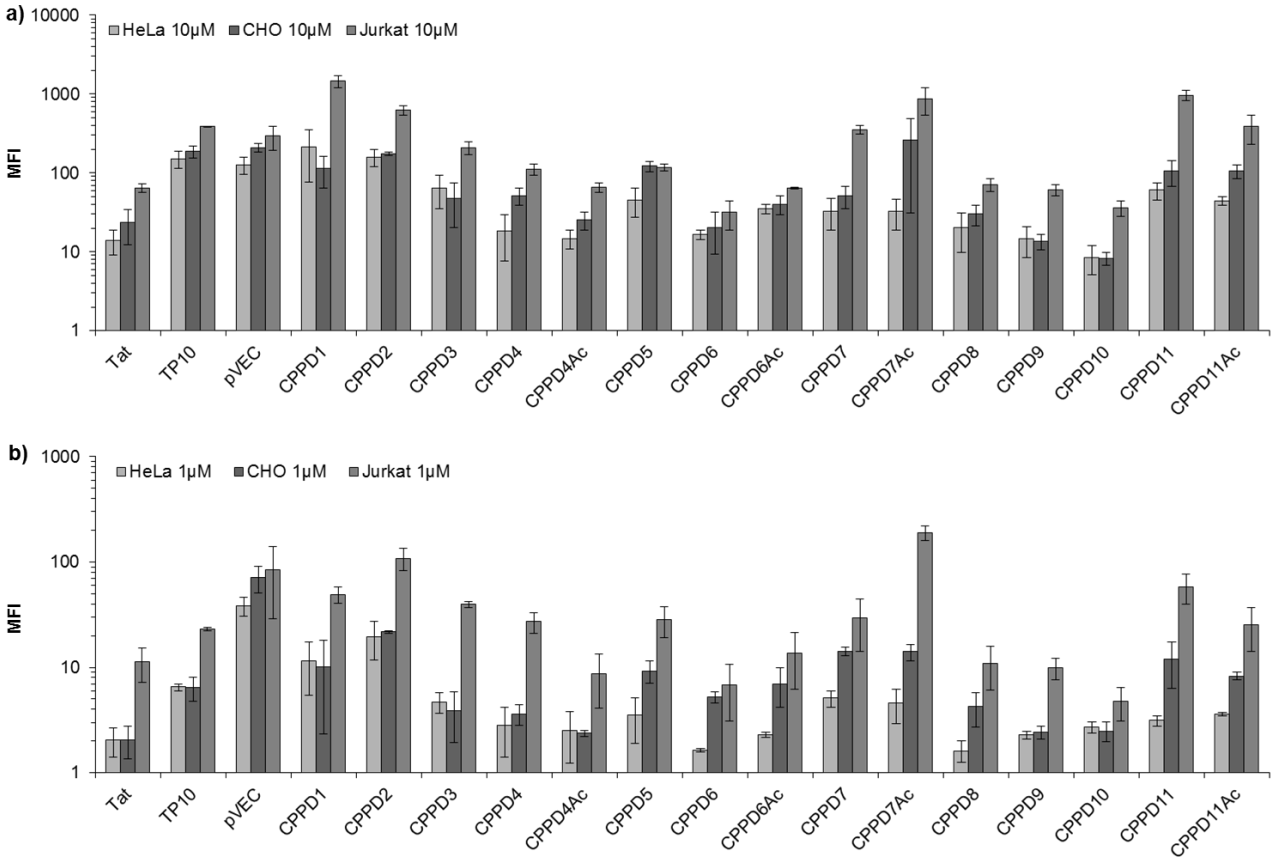


Figure S2. Flow cytometry data of CPPs and CPPDs after 1 h incubation at 37 °C with [a] 10 μM and [b] 1 μM for HeLa (light grey), CHO (dark grey) and Jurkat cells (grey). The Mean Fluorescence Index (MFI) is the ratio of the carboxyfluorescein (CF) fluorescence signal of the treated cells to the fluorescence signal of the untreated cells (blank). The values represent averages of 3 independent experiments performed in duplicates. For experiment details see experimental procedure 1.7, page 13.

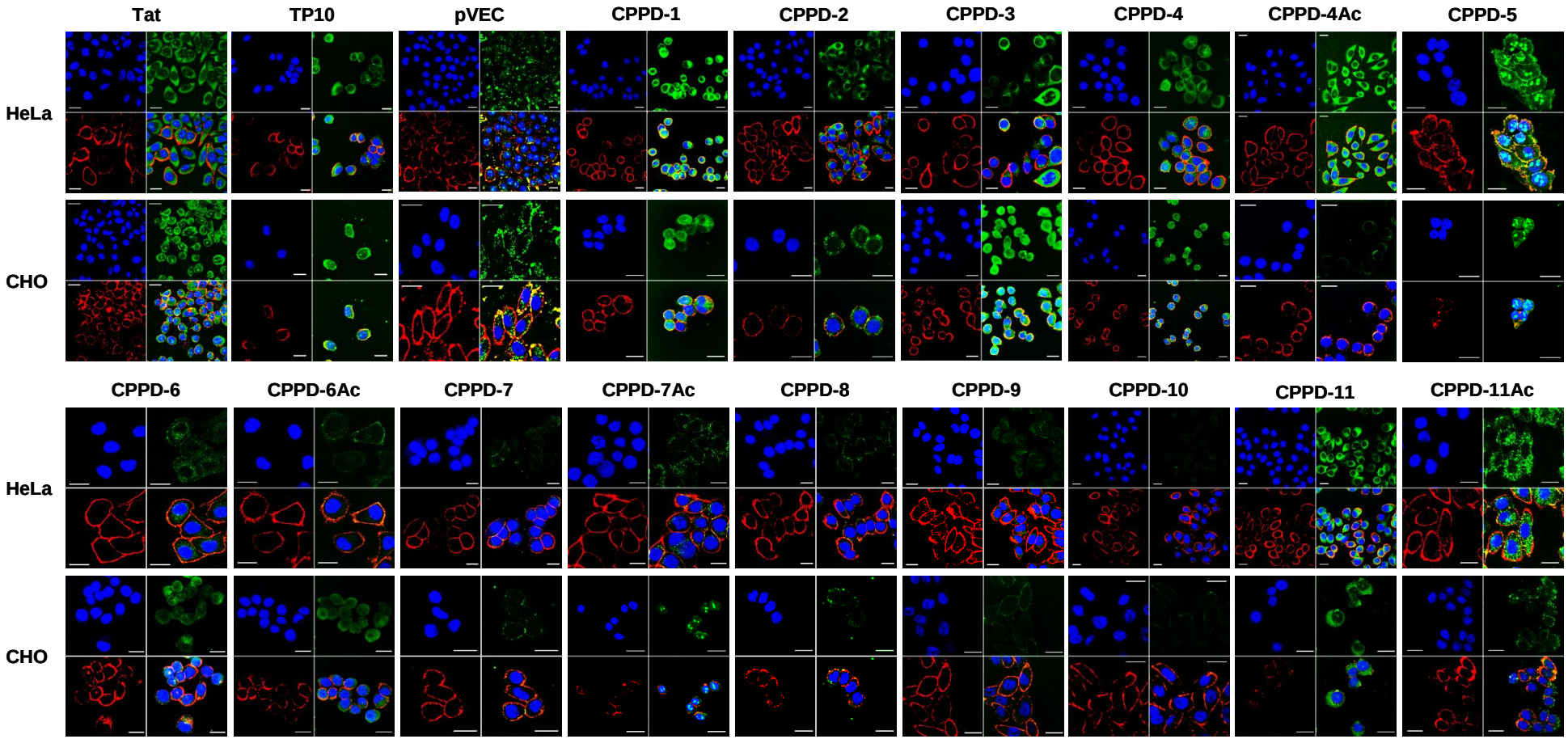


Figure S3. Confocal microscopy images of fixed HeLa and CHO cells after 1 h incubation at 37 °C with 10 µM of linear peptides and peptide dendrimers. Representative images are shown. Blue = DAPI (nucleic acid stain). Green = 5(6)-carboxyfluorescein (compound). Red = Alexa Fluor 594 WGA (wheat germ agglutinin) conjugate (plasma membrane). White bar = 20 µm. For experiment details see experimental procedure 1.7, page 13.

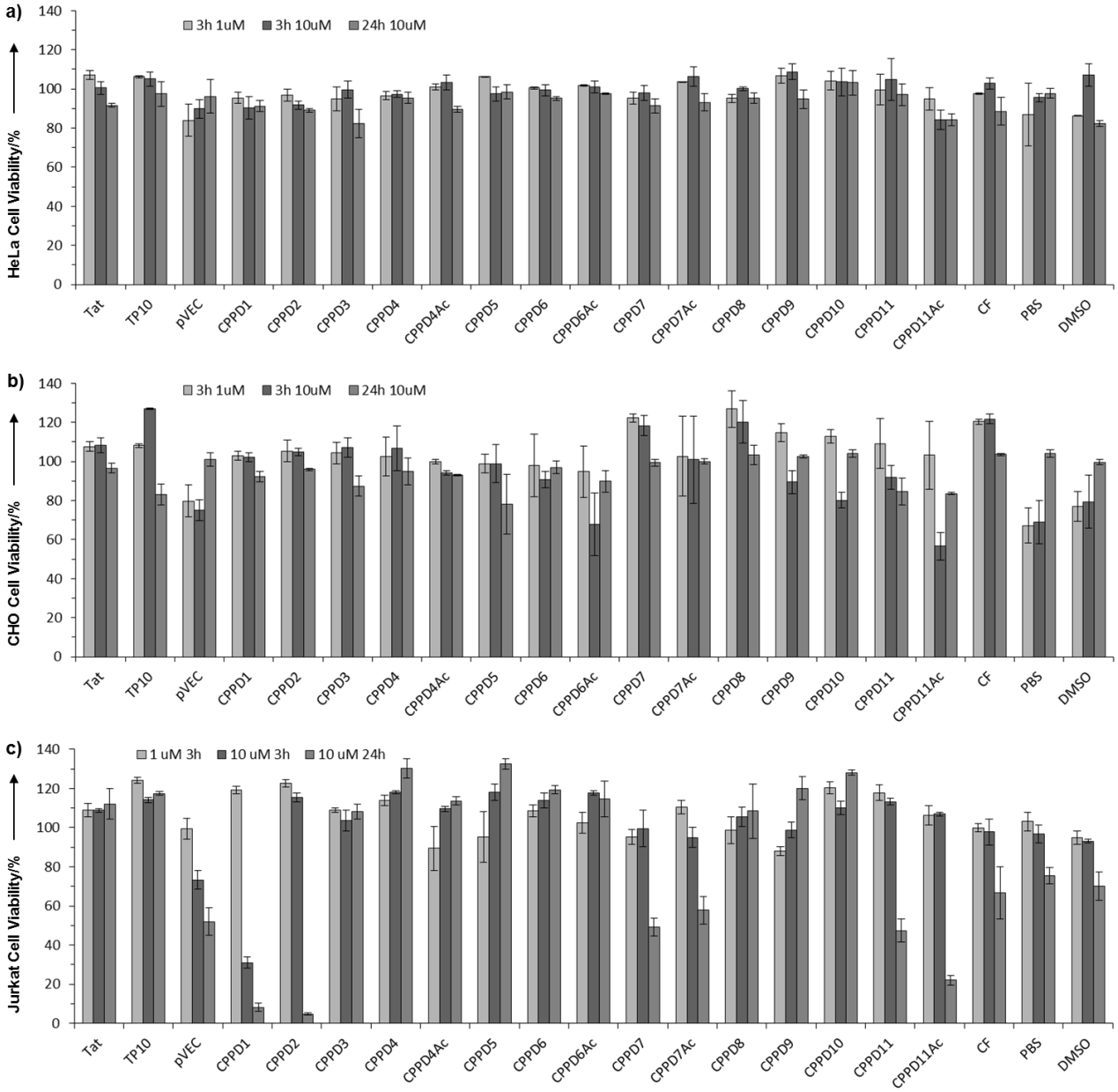


Figure S4. Cytotoxicity of CPPs and CPPDs for [a] HeLa, [b] CHO and [c] Jurkat cells for different incubation times and concentrations: 1 µM for 3 h (light grey), 10 µM for 3 h (dark grey) and 10 µM for 24 h (grey). Cell metabolic activity was evaluated with the WST-8 assay. Untreated cells are defined as 100% viable, wells without cells set as 0% viability. Controls are 5(6)-carboxyfluorescein (CF), DMSO (3 h with 0.1%; 3 h with 1%; 24 h with 1%) and PBS (3 h with 1%; 3 h with 10%; 24 h with 10%). All measurements were done in triplicates. For experiment details see experimental procedure 1.7, page 13.

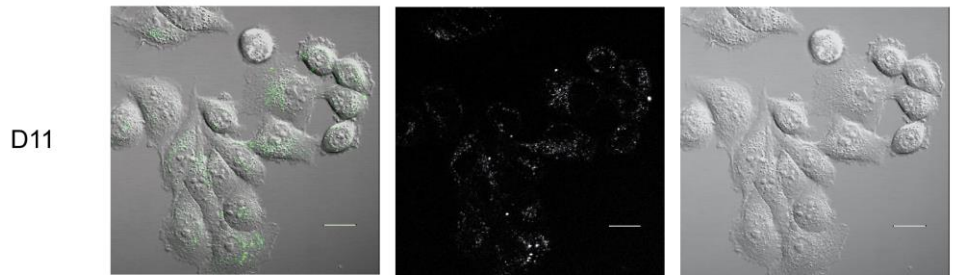


Figure S5. Observed uptake of D11 (10µM) after 1h incubation at 37°C on HeLa cells in live confocal microscopy of the differential interference contrast (DIC, left panel) and the CF fluorescence at 525 nm (middle panel). Left panels are the merged of the DIC and fluorescence pictures. White bar = 20 µm.

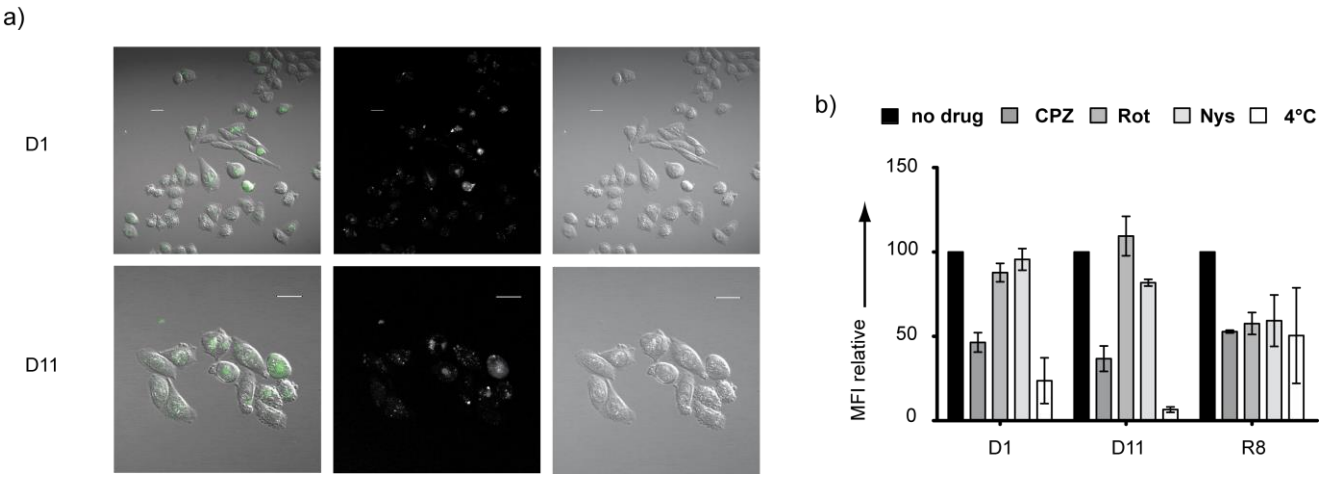


Figure S6. [A] Observed uptake of D1 and D11 (10µM) after 1h incubation at 37°C on CHO cells in live confocal microscopy of the differential interference contrast (DIC, left panel) and the CF fluorescence at 525 nm (middle panel). Left panels are the merged of the DIC and fluorescence pictures. White bar = 20 µm. [B] Uptake mechanism study of D1 and D11 in HeLa cells. Cells were pretreated 30 min with Chlorpromazine 50 µM (CPZ), Rottlerin 20 µM (Rot), Nystatin 25 µg/mL (Nys) or cooled down at 4°C prior to 1 h incubation with 10 µM of the indicated peptide dendrimer or linear peptide (R8) in the continued presence of inhibitor. Error bars represent the SD of three independent experiments. For experiment details see experimental procedure 1.7, page 13.

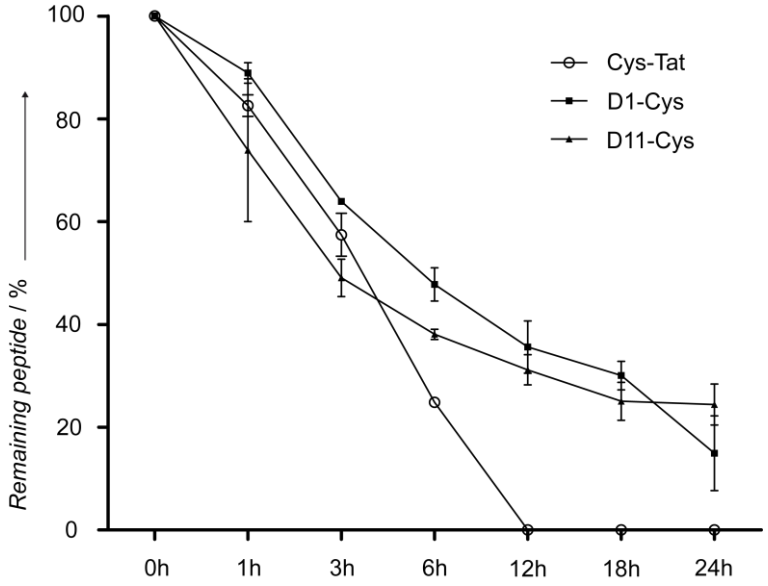


Figure S7. D1, **D11** and **Tat** proteases stability**.** Peptides were incubated with 12.5% of human serum, precipitated, dried and analyzed by LC/MS. Percentages represent the area under peptide peaks compare to the area obtained at 0h time point. Values are normalized according to the signal of the internal standard 3-Hydroxyanisole at 50 µg/mL. Error bars represent the SD of two independent experiments. For experiment details see experimental procedure 1.10, page 14.

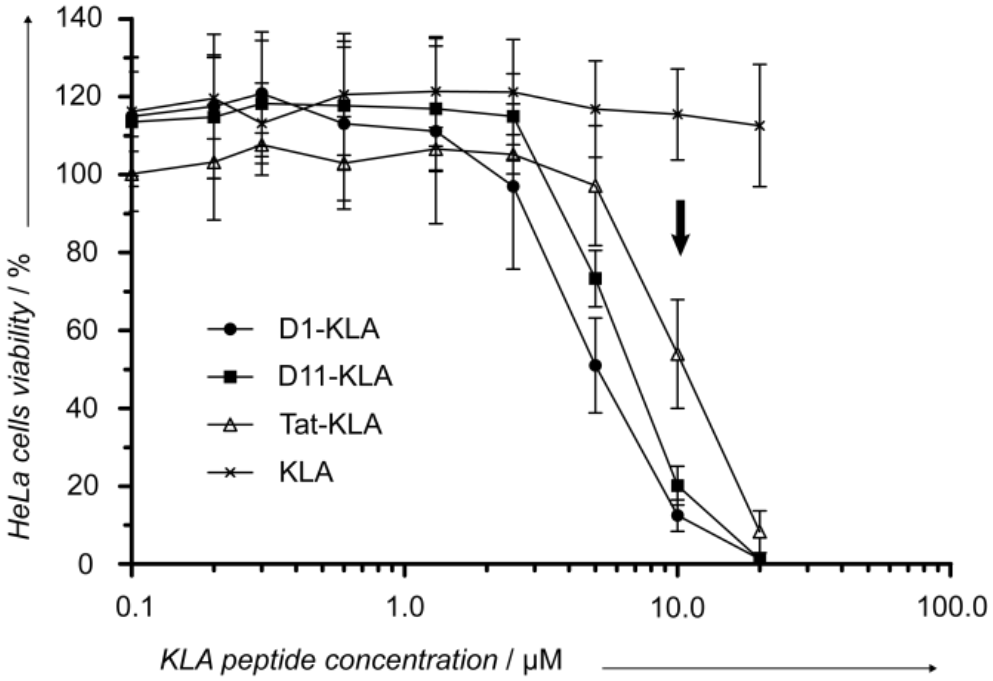


Figure S8. Cytotoxicity of KLA-conjugates on HeLa cells after 24 h of incubation. At 10 µM, p = 0.0002 for D1-KLA and p = 0.0009 for D11-KLA values compare to Tat-KLA (black arrow). One experiment in triplicates out of two is presented here.

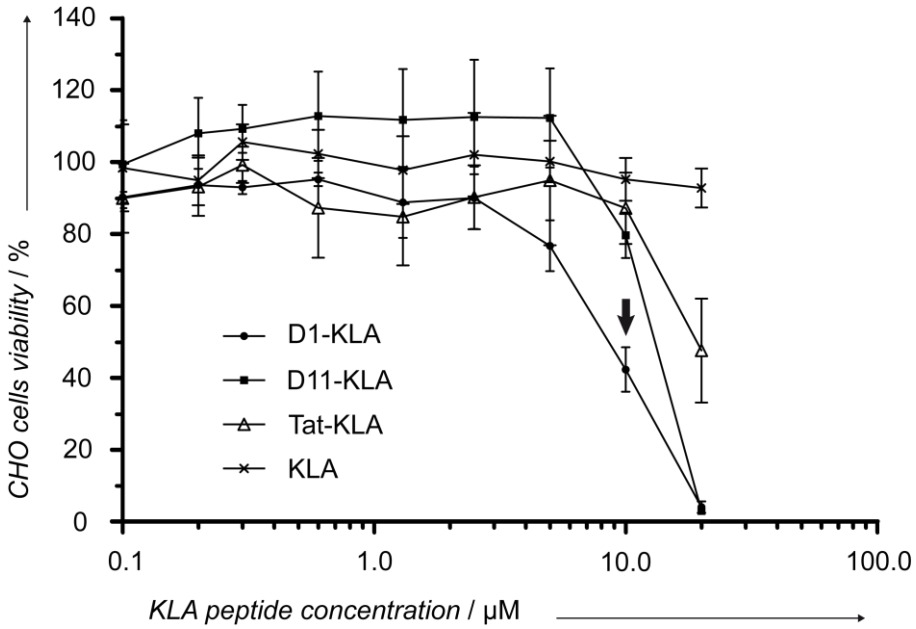


Figure S9. Cytotoxicity of KLA-conjugates on CHO cells after 24 h of incubation. At 10 µM, p = 0.0019 for D1-KLA value compare to Tat-KLA (black arrow). One experiment in triplicates out of two is presented here. For experiment details see experimental procedure 1.7, page 13.

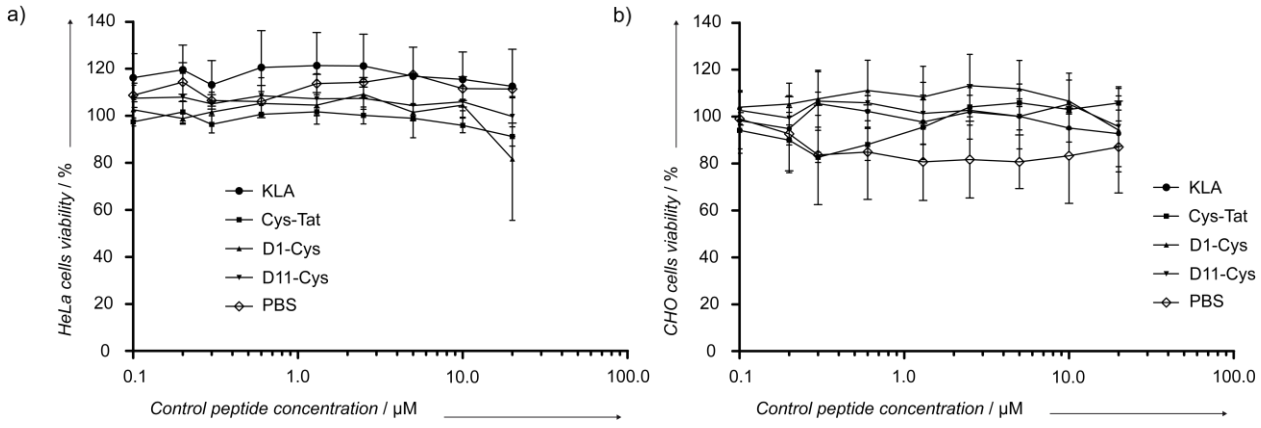


Figure S10. Cytotoxicity of controls for the KLA study in [a] HeLa and [b] CHO cells for 24 h incubation time at different concentrations. Cell metabolic activity was evaluated with the WST-8 assay. All measurements were done in triplicates in 2 independent experiments. For experiment details see experimental procedure 1.7, page 13.

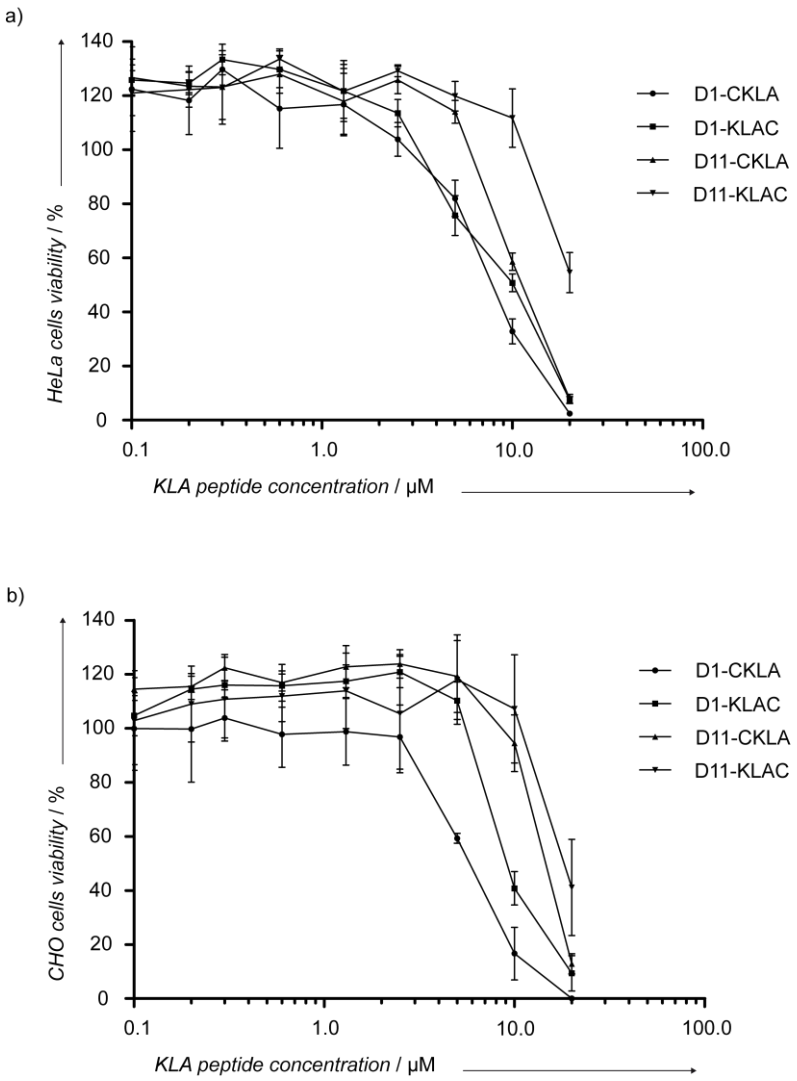


Figure S11. Cytotoxicity of disulfide bridged KLA conjugates in [a] HeLa and [b] CHO cells for 24 h incubation time at different concentrations. Cell metabolic activity was evaluated with the WST-8 assay. All measurements were done in triplicates in 2 independent experiments. For experiment details see experimental procedure 1.7, page 13.

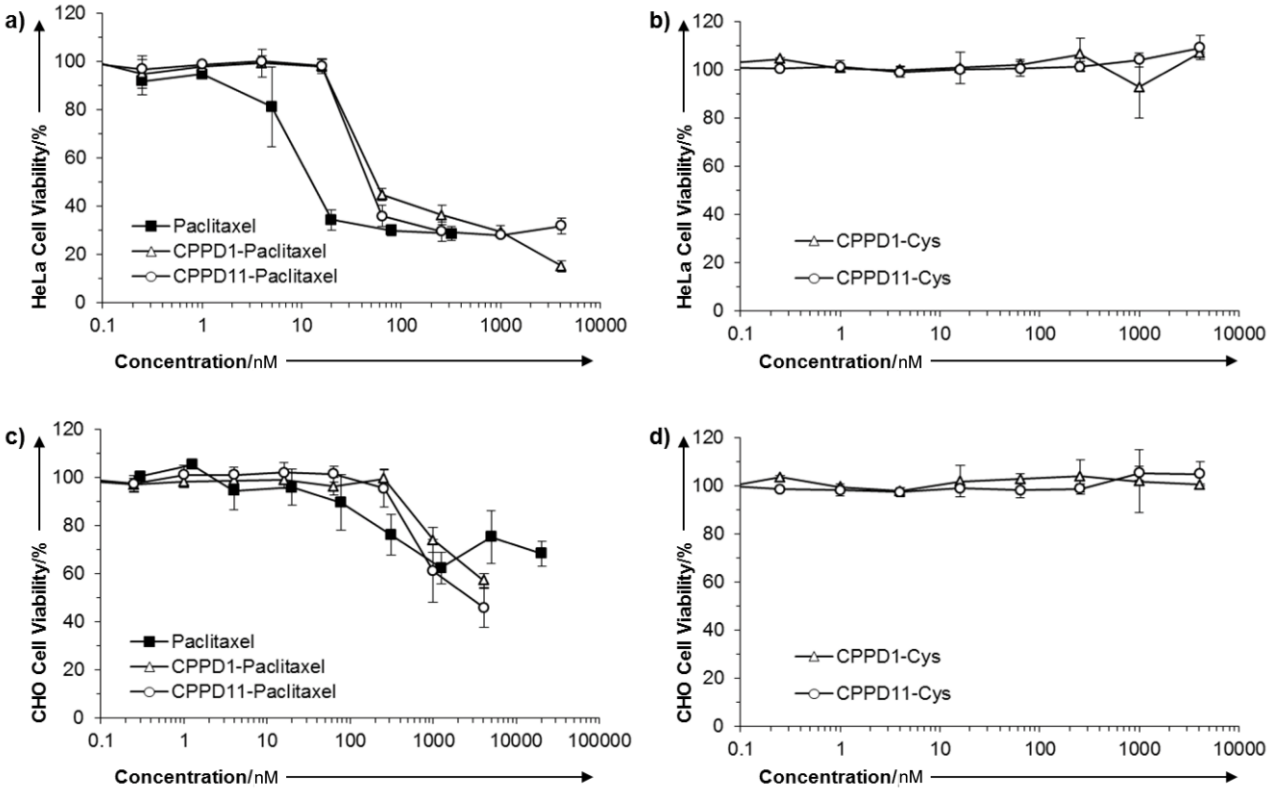


Figure S12. Cytotoxicity of paclitaxel conjugates and controls in [a,b] HeLa and [c,d] CHO cells for 72 h incubation time at different concentrations. Cell metabolic activity was evaluated with the WST-8 assay. All measurements were done in duplicates in 2 independent experiments. For experiment details see experimental procedure 1.7, page 13.

1 Tables

Table S1. Compounds used for the KLA-peptide and the paclitaxel cytotoxicity studies.

Compound	Sequence[a)]	Yield mg (%)[b)]	MS calc/obs
Cys-Tat	CYGRKKRRQRRR	97.8 (43)	1704.0/1704.0
D1-Cys	$(RL)_8(KRL)_4(KKK)_2KGYC$	57.6 (11)	4982.4/4981.4
D11-Cys	$(LI)_8(KRK)_4(KRA)_2KHSC$	48.8 (11)	4644.1/4643.0
KLA	$[KLAKLAK]_2$	7.6 (7)	1523.0/1522.0
ClAc-KLA	ClAc-GG$[KLAKLAK]_2$	27.9 (13)	1713.6/1713.0
Cys-KLA	CGG$[KLAKLAK]_2$	48.6 (21)	1740.3/1739.0
KLA-Cys	$[KLAKLAK]_2$GGC	19.3 (8)	1740.3/1739.0
Tat-KLA	AcC(x-GG$[KLAKLAK]_2$)YGRKKRRQRRR	2.7 (48)	3381.2/3381.1
D1-KLA	$(RL)_8(KRL)_4(KKK)_2KGYC$(x-GG$[KLAKLAK]_2$)	2.5 (38)	6659.5/6659.5
D11-KLA	$(LI)_8(KRK)_4(KRA)_2KHSC$(x-GG$[KLAKLAK]_2$)	2.4 (30)	6321.2/6320.3
D1-CKLA	$(RL)_8(KRL)_4(KKK)_2KGYC$(z-CGG$[KLAKLAK]_2$)	0.4 (6)	6720.7/6719.5
D1-KLAC	$(RL)_8(KRL)_4(KKK)_2KGYC$(z-$[KLAKLAK]_2$GGC)	0.5 (8)	6720.7/6720.5
D11-CKLA	$(LI)_8(KRK)_4(KRA)_2KHSC$(z-CGG$[KLAKLAK]_2$)	0.7 (10)	6382.3/6381.3
D11-KLAC	$(LI)_8(KRK)_4(KRA)_2KHSC$(z-$[KLAKLAK]_2$GGC)	1.3 (17)	6382.3/6381.3
D1-Paclitaxel		0.2 (5)	5922.4/5921.7
D11-Paclitaxel		0.2 (5)	5584.1/5583.5

[a)] one letter codes for amino acids. Branching diamino acids in italics. ClAc = Chloroacetylated N-terminus. x denotes the S-CH_2-CO- bridge between cysteine side-chain and the acetylated N-terminus of the linear peptide. z denotes the disulfide bridge between two cysteine side-chains. All peptides are carboxamide ($CONH_2$) at the C-terminus. [b)] Yields given for RP-HPLC purified products as TFA salts.

Experimental Procedures

1.1 Materials and Reagents

All reagents were purchased in the highest quality available either from Sigma Aldrich, Bachem, Acors Organics, Invitrogen or TCI Europe. PyBOP, amino acids and their derivatives were purchased from Advanced ChemTech (Giessen, Germany) Novabiochem or IRIS Biotech GmbH. For SPPS amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH. Tenta Gel S RAM® resin (loading: 0.22 to 0.25 $mmol \cdot g^{-1}$) was purchased from Rapp Polymere (Tübingen, Germany). Peptide syntheses were performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper. Ligation reactions were done in solution using standard glass ware. All solvents used in reactions on solid phase and in solution were bought in p.a. quality and distilled prior to use. Analytical RP-HPLC was performed with a DIONEX UltiMate® 3000 RSLC System (DAD-3000 RS Photodiode Array Detector) using a DIONEX Acclaim® RSLC 120 column (C18, 2.2 μm, 3 × 50 mm, flow rate 1.2 $mL \cdot min^{-1}$). Data recording and processing was done with Dionex Chromeleon Management System Version 6.8. The following elution solutions were used for analytical RP-HPLC: A) mQ-deionized H_2O with 0.1% TFA; D) mQ-deionized H_2O/HPLC-grade CH_3CN (10:90) with 0.1% TFA. Preparative RP-HPLC was performed with a Waters Prep LC4000 Chromatography System using a Reprospher 100 (C18-DE, 5 μm, pore size 100 Å, 30 × 100 mm, flow rate 40 $mL \cdot min^{-1}$) from Dr. Maisch GmbH. Compounds were detected by UV absorption at 214 nm using a Waters 486 Tunable Absorbance Detector. The following elution solutions were used for preparative RP-HPLC: A) mQ-deionized H_2O with 0.1% TFA; D) mQ-deionized H_2O/HPLC-grade CH_3CN (40:60) with 0.1% TFA. MS spectra, recorded on either a Thermo Scientific LTQ OrbitrapXL or an AB Sciex QTrap and amino acid analysis were provided by the MS and protein analytical services of the Department of Chemistry and Biochemistry at the University of Bern. The CD spectra were recorded using a Jasco J-715 spectrometer equipped with a PFD-350S temperature controller and a PS-150J power supply. All experiments were measured using a Hellma Suprasil R 100-QS 0.1 cm cuvettes. FACS data were acquired with a BD LSRII special order system and BD FACSDiva software 6.1.3 and analyzed with the FlowJo software. The fixed pictures were taken with an inverted confocal laser microscope LSM 510 META from Zeiss equipped with a 63x objective lens and live pictures with an inverted Nikon TE2000E and a 40x objective lens compatible for Nomarski differential interference contrast (DIC) experiments. For the cytotoxicity assays, the absorbance was read at 450 nm with a Spectra Max 250 from Molecular devices and the program SoftMax Pro 4.0.

1.2 SPPS of linear and dendrimer peptides

Peptides were synthesized by adding 300-500 mg of Tenta Gel S RAM® resin (loading: 0.22-0.25 mmol g^{-1}) in a 10 mL polypropylene syringe fitted with a polyethylene frit, a Teflon stopcock and stopper. The resin was swollen in DCM for 15 min. After removal of the DCM the Fmoc protecting group was removed. Stirring of the reaction mixture at any given step described below was performed by attaching the closed syringe to a rotating axis. The following conditions were used:

Removal of the Fmoc protecting group. At each step the Fmoc protecting group was removed with 5 mL of a piperidine/NMP solution (1:4) for 20 min. The resin was then washed (3x each) with NMP, MeOH and DCM. The effectiveness of the deprotection was monitored by the TNBS test.

Coupling of the Fmoc-protected amino acids. 3.0 x **n** equivalents (**n** = number of reaction site) of Fmoc-protected amino acid and 3.0 x **n** equivalents of PyBOP in 5 mL of NMP were added to the resin. 5.0 x **n** equivalents of DIEA

was added and the reaction was stirred for 1 h for **n** = 1 (0^{th} generation), 2 h for **n** = 2 (1^{st} generation), 3 h for **n** = 4 (2^{nd} generation) and 4 h for **n** = 8 (3^{rd} generation). The resin was then washed (3x each) with NMP, MeOH and DCM. The effectiveness of the coupling was monitored by testing for free amine groups with the 2,4,6-trinitrobenzenesulfonic acid (TNBS) test 1% TNBS in DMF / 10% DIEA in DMF (1:1).

Capping of free amine groups. Because the TNBS test is only a visual test, the resin was acetylated with a solution of acetic acid anhydride/DCM (1:1) for 15 min. The resin was washed (3x each) with NMP, MeOH and DCM.

Deprotection of Lys(Alloc). In dendrimer core a Lys(Alloc) was used to couple the 5(6)-carboxyfluorescein (CF). After the last capping, the syringe was equipped with a septum and dried under vacuum for one hour. The resin was then swollen in dry DCM for 15 min under argon. Alloc protecting group was removed with 0.25 equivalent $Pd(PPh_3)_4$ and 25.0 equivalents of $PhSiH_3$ in dry DCM. The reaction was stirred under argon bubbling for 20 min. The procedure was repeated 3 times and the resin was washed with dry DCM in between the cycles. Finally, the resin was washed with dry DCM (10 mL, 2 x 15 min) and coupled with CF.

Coupling of 5(6)-carboxyfluorescein (CF). 5.0 equivalents of CF and 5.0 equivalents of HOBt in 5 mL of NMP were added to the resin. 5.0 equivalents of DIC were added and the reaction was stirred for 2 h protected from light. The resin was then washed (5 x 5min) with a piperidine/NMP solution (1:4) to remove excess of CF.

N-Acetylation/N-chloroacetylation. When necessary the peptide sequence was chloroacetylated with a solution of chloroacetic acid anhydride (10.0 equivalents per free N-terminus) in 5 mL DCM twice during 15 min. The resin was washed (3x each) with NMP, MeOH and DCM.

TFA cleavage. The cleavage was carried out using a TFA/H_2O/TIS (95:2.5:2.5) solution (if the dendrimer contained Cys a TFA/H_2O/TIS/EDT (94:1:2.5:2.5) solution was used instead) for 5 h under stirring. Peptides were separated from the resin by filtration and precipitated with ice-cold TBME. The crude product obtained after centrifugation (10 min, 3000 g) was dried under vacuum and dissolved in H_2O 0.1% TFA or a H_2O/CH_3CN mixture with 0.1% TFA, purified by preparative RP-HPLC and lyophilized. Yields were calculated for the TFA salts of products.

1.3 Thioether ligation

A solution of the chloroacetylated peptide **ClAc-KLA** (~3-4 mg, 2.0 equivalents) in 500 µL of DMF/H_2O (1:1) was prepared in a pointed glass flask. The mixture was degassed with argon during 5 min. In a second pointed glass flask, 1.0 equivalent of a solid Cys-containing peptide or dendrimer peptide was prepared and the flask was degassed with argon. The **ClAc-KLA** solution was transferred to the glass flask containing the peptide with a gas tight syringe. 55 equivalents of DIEA was added and the solution was stirred at room temperature. The reaction was followed by analytical RP-HPLC. After completion (5-8 h), the reaction was quenched by adding 3 mL of solvent A, filtered and purified by preparative RP-HPLC. Yields were calculated for the TFA salts of the products.

1.4 Heterodimerisation

The monomeric linear peptide X (~2 mg, 1.0 equivalent) was dissolved in 200 µL mQ-deionized H_2O and degassed for 5 min with argon. Then 2.0 equivalents of Aldrithiol (2,2-dithiodipyridine) solution (45.5 mM in MeOH,) was added to the reaction solution. The thiol activation was followed by analytical RP-HPLC. When the reaction was completed, the MeOH was evaporated and the Aldrithiol excess was removed by extraction with DCM (4 times, 500 µL). The water phase was transferred to a pointed glass flask and degassed for 5 min with argon. Then the pH was adjusted to 8.0 by adding $(NH_4)HCO_3$ buffer solution at 400 mM. The monomeric peptide dendrimer Y (~5 mg, 1.0 equivalent) was dissolved in 200 µL degassed mQ-deionized H_2O and added dropwise to the reaction mixture. The solution was stirred

for 30 min under argon at room temperature and then acidified with 3 mL of solvent A. The heterodimer XY was purified by preparative RP-HPLC.

1.5 Preparation of Paclitaxel-SPDP

Paclitaxel (22.5 mg, 1.0 equivalent), SPDP (3-(2-Pyridyldithio)propionic acid N-hydroxysuccinimide ester) (15 mg, 2.0 equivalents) and DMAP (4-(Dimethylamino)-pyridin) (4.5 mg, 1.4 equivalents) were dissolved in 5 mL of dry DCM in a pointed glass flask. The reaction was stirred for 18 h at room temperature. After reaction completion the solvent was evaporated. The crude was dissolved in 0.1 mL DCM and purified by flash chromatography on silica gel (1:1 Ethyl acetate/ hexane) to give pure Paclitaxel-SPDP as a white solid.

1.6 Paclitaxel conjugation

The peptide dendrimer (~5 mg, 1.0 equivalent) and Paclitaxel-SPDP (~4 mg, 5.0 equivalents) were dissolved in 3 mL of DMF in a pointed glass flask. The reaction mixture was degassed with argon for 10 min. Then DIPEA (150 µL, 5% volume excess) was added and the solution was stirred at room temperature under argon. The reaction was monitored via analytical RP-HPLC and after completion (20-30 min) the mixture was evaporated to dryness. The crude was dissolved in Ethyl acetate and extracted twice with H_2O. Aqueous phases were collected and purified by preparative RP-HPLC. The organic phase containing Paclitaxel-SPDP was dried and the recovered solid was reused for another conjugation.

1.7 Cell culture

All equipment, medium and buffer for cell culture were purchased at Invitrogen, Faust, and Sigma Aldrich. HeLa and CHO cells were grown in DMEM (Dulbeccos modified Eagle medium) supplemented with 10% FBS, 1% penicillin/streptomycin, 1% HEPES buffer 1M, and 1% L-glutamine 200 mM. Jurkat cells were grown in RMPI-1640 supplemented with 10% FBS, 1% penicillin/streptomycin, 1% HEPES buffer 1M, 1% sodium pyruvate 100 mM and 2.5% glucose 100 $g \cdot l^{-1}$. Cells were placed in a humidified incubator at 37 °C in presence of 5% CO_2 for culture.

Flow Cytometry Experiments. 20'000 HeLa or CHO cells were seeded on 96 well plates in 100 µL of complete medium and cultured overnight. The day after the medium was removed and 100 µL of peptide solution (1µM or 10µM) or 90 µL of drug (Chlorpromazine (CPZ, 50 µM, Sigma), Rottlerin (Rot, 20µM, Calbiochem) and Nystatin (Nys, 25 µg/mL, Sigma) was added in growth medium. With Jurkat cells, 100'000 cells were seeded on 96 well plates in 50 µL of growth medium 30 min before the assay and 50 µL of peptide solution with double concentration was added. All cell types were incubated 1 h at 37 °C or 4 °C with the peptide solution in presence of drug when indicated. For uptake mechanism studies, the plate with the cells was incubated 30 min with drugs before adding peptides (10 µL at 100 µM). In the case of HeLa and CHO cells, the peptide solution was removed, cells were washed twice with PBS and detached by adding a solution of trypsin / EDTA 0.25% for 3 min. Cells were then collected, centrifuged 5 min at 300 g, washed once with PBS and transferred into FACS tubes. In the case of Jurkat cells, the cells were centrifuged down (5 min, 300 g) and washed twice with PBS. Propidium iodide (0.4 mg/mL in PBS) was added to each sample to stain the dead cells at a final concentration of 0.02 mg/mL. The Mean Fluorescence Intensity (MFI) was obtained by collecting 10'000 events par sample.

Confocal Microscopy. In fixed conditions, 50'000 HeLa or CHO cells were seeded on sterile coverslips (∅ 12 mm, #1 Menzel Gläser) in a 24 well plate with 1 mL of growth medium and cultured overnight. Cells were incubated 1 h at 37°C or 4°C with compounds at the indicated concentration in growth medium. Then they were washed twice with PBS, stained 10 min with wheat germ agglutinin AlexaFluor®594 conjugate (5 µg/mL in HBSS buffer), washed twice with

PBS, fixed with 4% paraformaldehyde in PBS (Affymetrix), stained with DAPI (300 nM in PBS) and finally mounted on microslides in mounting medium (Dako). In live conditions, 20'000 HeLa or CHO cells were seeded on sterile chambered coverglass (Lab-Tek 2, glass thickness 0.17/0.19 µm, 8 wells, Nunc) in 300 µL of growth medium and cultured for 18h. The medium was then removed and 250 µL of live medium mixture (serum free DMEM 1% HEPES 1% BSA) was added. The Koehler illumination of the Nikon TE2000E confocal microscope for DIC pictures record was adjusted at this point. After parameter settings, cells were let recover 15 min at 37°C in 250µL of fresh live medium after what 50µL of peptide solution (60 µM) was gently added for 1h incubation. Cells were then washed twice with serum free DMEM 1% HEPES 3% BSA and directly used to record DIC and fluorescence pictures in live medium. Pictures were afterward treated with Fiji ImageJ software.

WST-8 Cytotoxicity Assay. One day prior to the experiment, 10'000 HeLa or CHO cells were seeded in 100 µL in a 96 well plate and cultured overnight. Jurkat cells were seeded in a 96well u-shaped plate 30 min before the incubation. Cells were then incubated with 90 µL of the peptide solution (CPPDs with free Cys or conjugated with KLA or PTX) at the indicated concentration and for the indicated time. After incubation, 10 µL of WST-8 solution (3.31 mg/mL WST-8 (Ochem Incorporation), 0.074 mg/mL PES (phenazine ethosulfate)) was added and incubated 2 h at 37 °C, and the absorbance was determined at 450 nm.

Statistical Analysis. All graphs involving cells were obtained with GraphPad Prism software. P values were obtained thanks unpaired t-test with 99% of confidence intervals.

1.8 Circular dichroism (CD) spectroscopic measurements

The CD spectra were recorded using a Jasco J-715 spectrometer equipped with a PFD-350S temperature controller and a PS-150J power supply. All experiments were measured using a Hellma Suprasil R 100-QS 0.1 cm cuvettes. Stock solution (1 mg/mL) of peptides and dendrimers were freshly prepared in mQ-deionized water. For the measurement peptides were diluted to a final concentration of 100 µg/mL with degassed PBS. The scan rate was 10 nm/min, pitch 0.5 nm, response 16 sec. and band 1.0 nm. The nitrogen flow was kept around 5 $L \cdot min^{-1}$. Every sample was measured three times successively. The cuvettes were washed (2x each) with 1M HCl, mQ-deionized H_2O and PBS before measurement.

1.9 Hemolysis assay

To determine the minimal hemolytic concentration (MHC), stock solutions of the peptides in mQ-deionized H_2O were prepared (8 mg/mL) and 50 µL were diluted serially by 1/2 in 50 µL PBS in 96 well plates (Nunc, polystyrene, untreated, conical bottom). Human red blood cells (RBC) were obtained by centrifuging 1.5 mL of whole blood from healthy donors at 3'000 rpm for 15 min. Plasma was discarded and the pellet was resuspended in a 15 mL falcon tube up to 5 mL of PBS. The washing was repeated three times and the remaining pellet was resuspended in 10 mL of PBS. The RBC suspension (50 µL) was added to each well and the plate was incubated at room temperature for 4 h. Minimal hemolytic concentration (MHC) end points were determined by visual inspection of the wells after the incubation period.

1.10 Human serum stability

50 µL of a mixture of peptides at 400 µM and internal standard 3-hydroxyanisole at 100 µg/mL in 0.1 M Tris-HCl pH 7.5 buffer were incubated with 50 µL of 25% human serum (human male AB plasma, Sigma) in 0.1 M Tris-HCl pH 7.5, under 300 rpm agitation at 37°C. Human proteins were then precipitated with 100 µL of 100 mM $ZnSO_4$ aqueous solution 10 min in ice and pelleted (10 min, 11 000 g, room temperature). Supernatants were then dried under vacuum,

taken up with 120 µL mQ-deionized H_2O, centrifuged (10 min, 11 000 g, room temperature) and 100 µL of peptide suspension were used for LC/MS analysis. 0h time point gives the maximum of peptide peak intensity (100%) and values are normalized according to the signal of the internal standard.

1.11 Amino acid analysis.

Samples were hydrolyzed with 6M HCl containing 0.1% phenol for 22h 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.

2 Synthesis and Characterization

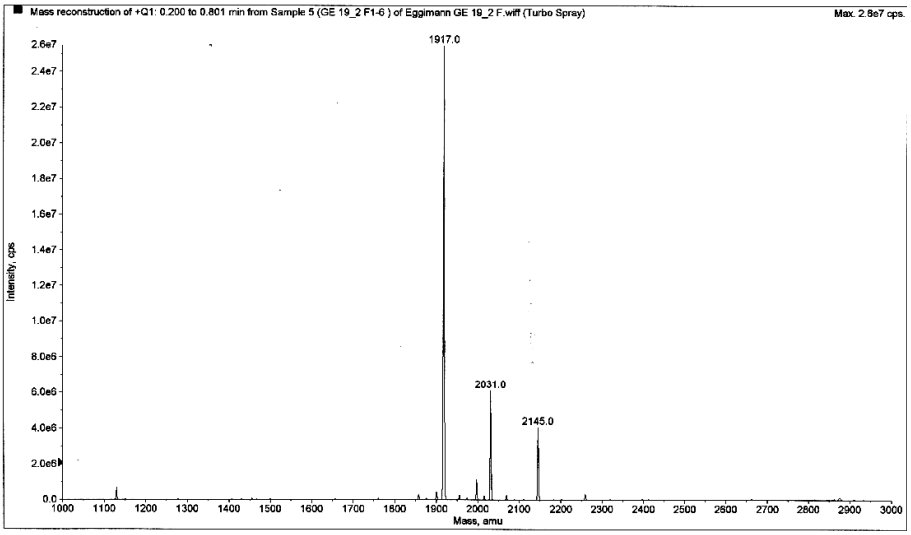
2.1 Linear Peptides.

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.

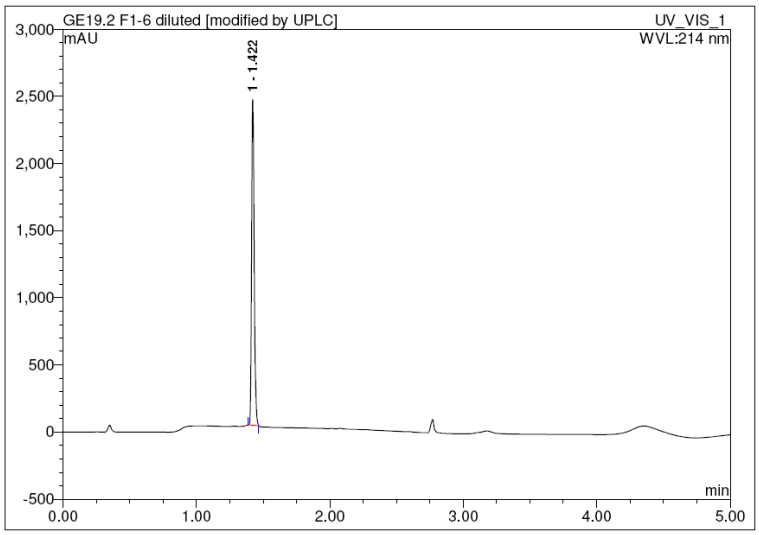
Tat (*-YGRKKRRQRRR).

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+): $C_{85}H_{129}N_{33}O_{19}$ calc/found 1917.1/1917.0 $[M]^+$; 2031.1/2031.0 $[M + TFA]^+$; 2145.1/2145.0 $[M + 2 TFA]^+$.

Mass spectrum, MS (ESI+):



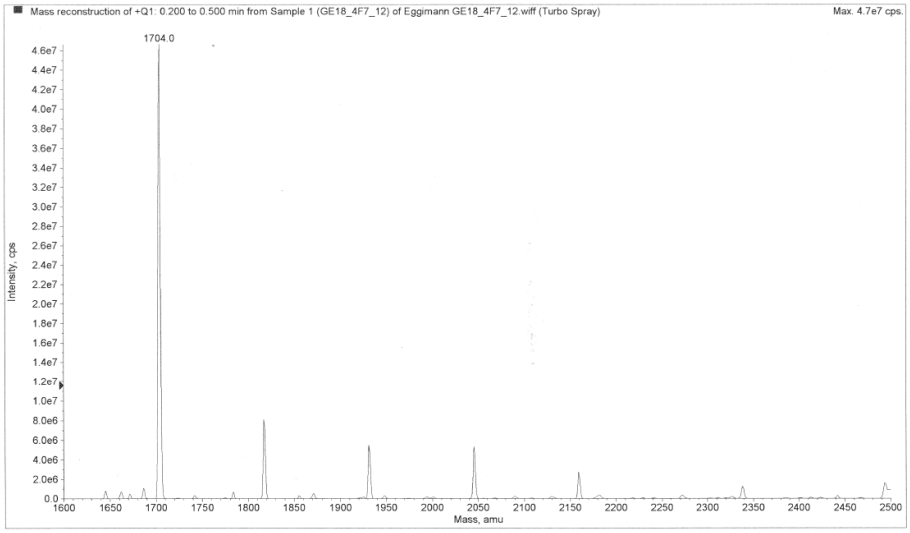
Analytical RP-HPLC chromatogram:



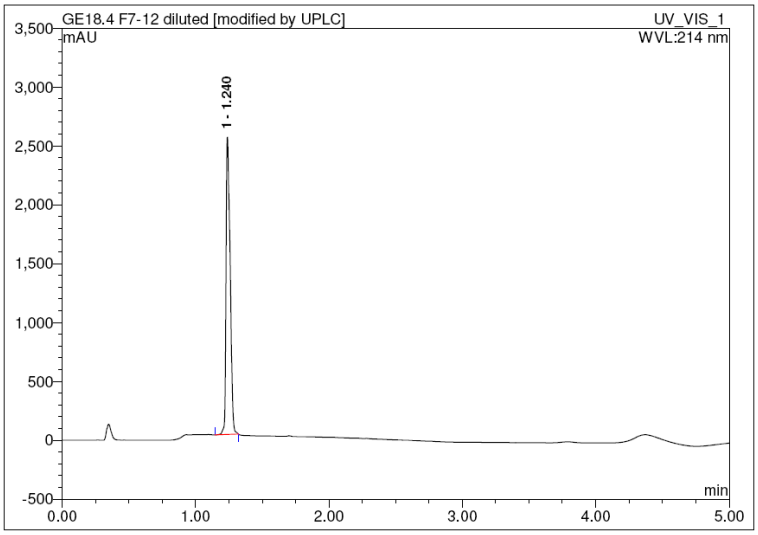
Cys-Tat (AcCYGRKKRRQRRR).

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+): $C_{69}H_{126}N_{34}O_{25}S$ calc/found 1704.0/1704.0 $[M]^+$.

Mass spectrum, MS (ESI+):



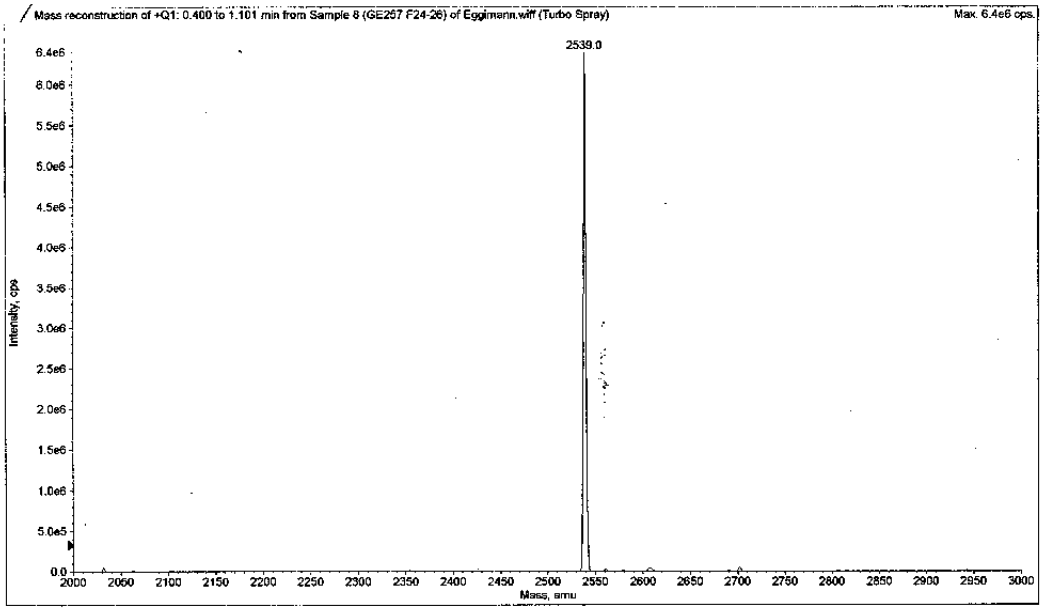
Analytical RP-HPLC chromatogram:



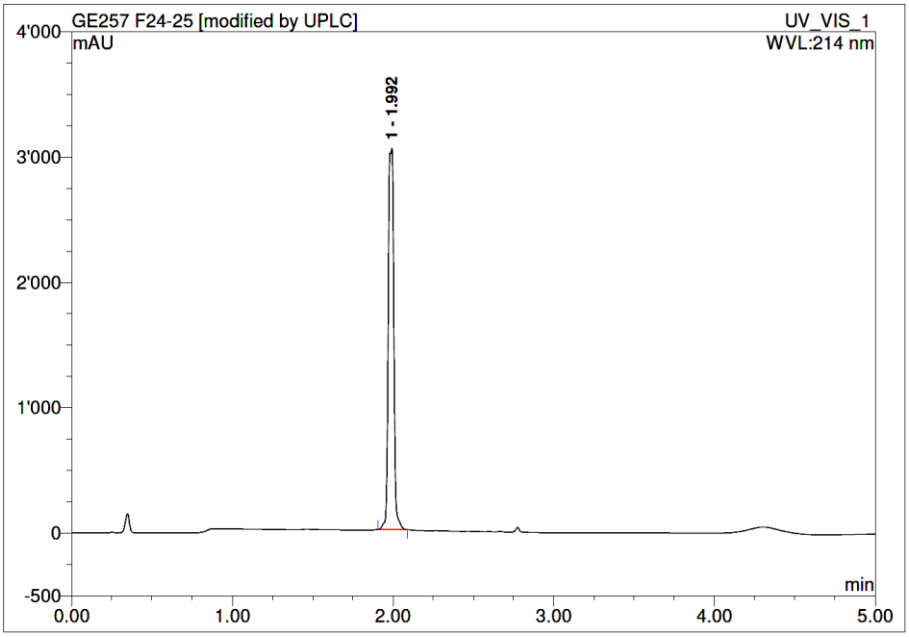
TP10 (*-AGYLLGKINLKALAALAKKIL).

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+): $C_{125}H_{196}N_{27}O_{29}$ calc/found 2541.1/2539.0 $[M]^+$.

Mass spectrum, MS (ESI+):



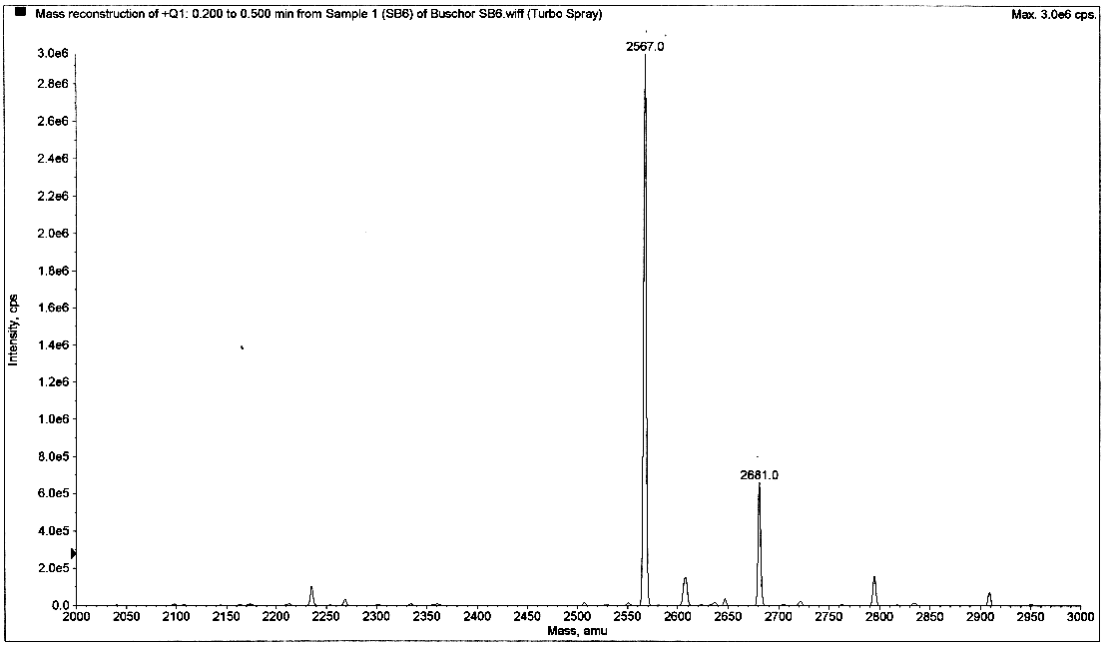
Analytical RP-HPLC chromatogram:



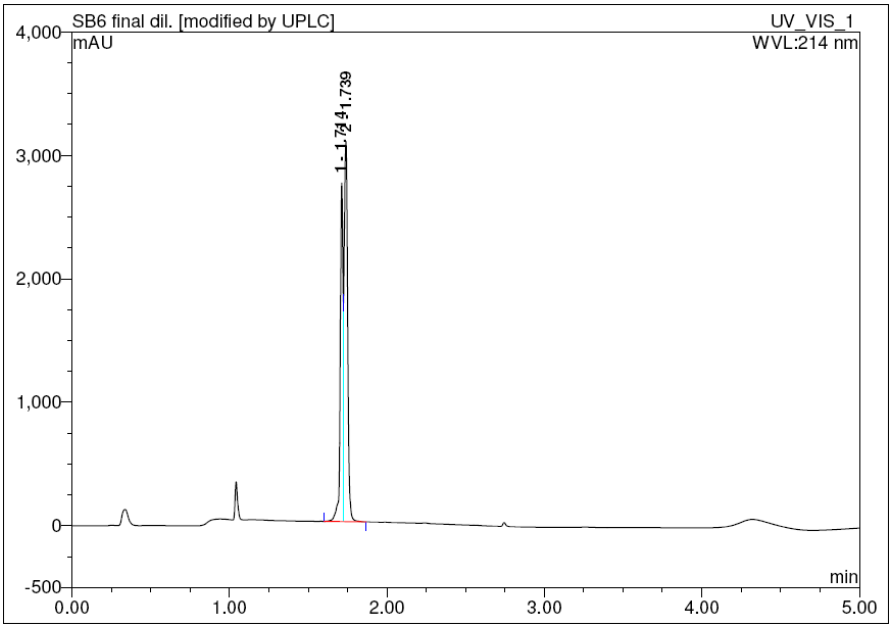
pVEC (*-LLIILRRRIRKQAHAHSK).

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+): $C_{119}H_{188}N_{38}O_{26}$ calc/found 2567.0/2567.0 $[M]^+$; 2681.0/2681.0 $[M + 1\ TFA]^+$.

Mass spectrum, MS (ESI+):



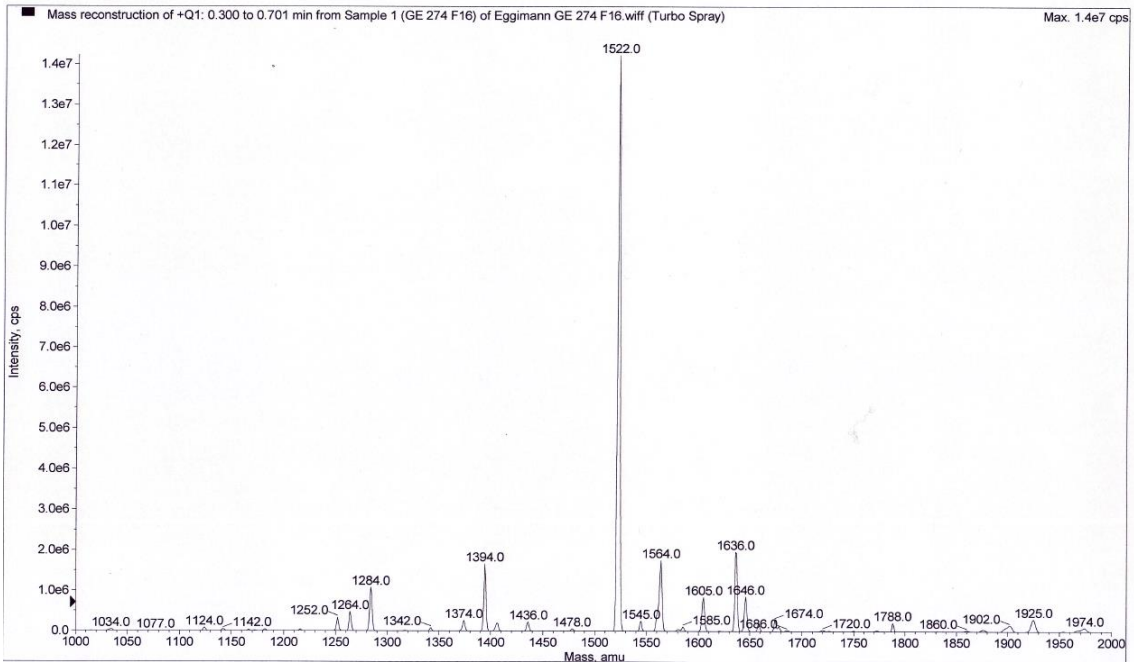
Analytical RP-HPLC chromatogram:



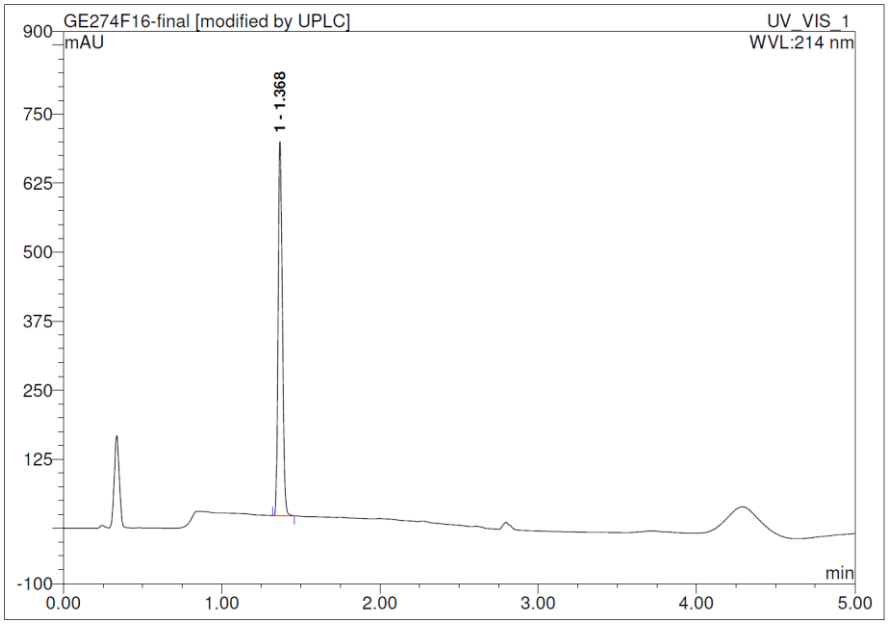
KLA (KLAKLAKKLAKLAK).

From Tenta Gel S RAM® resin (200 mg, 0.23 mmol·g^{-1}), **KLA** was obtained as a foamy colourless solid after preparative RP-HPLC (7.6 mg, 3.3 µmol, 7%). Analytical RP-HPLC: t_R = 1.37 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{72}H_{139}N_{21}O_{14}$ calc/found 1523.0/1522.0 $[M]^+$.

Mass spectrum, MS (ESI+):



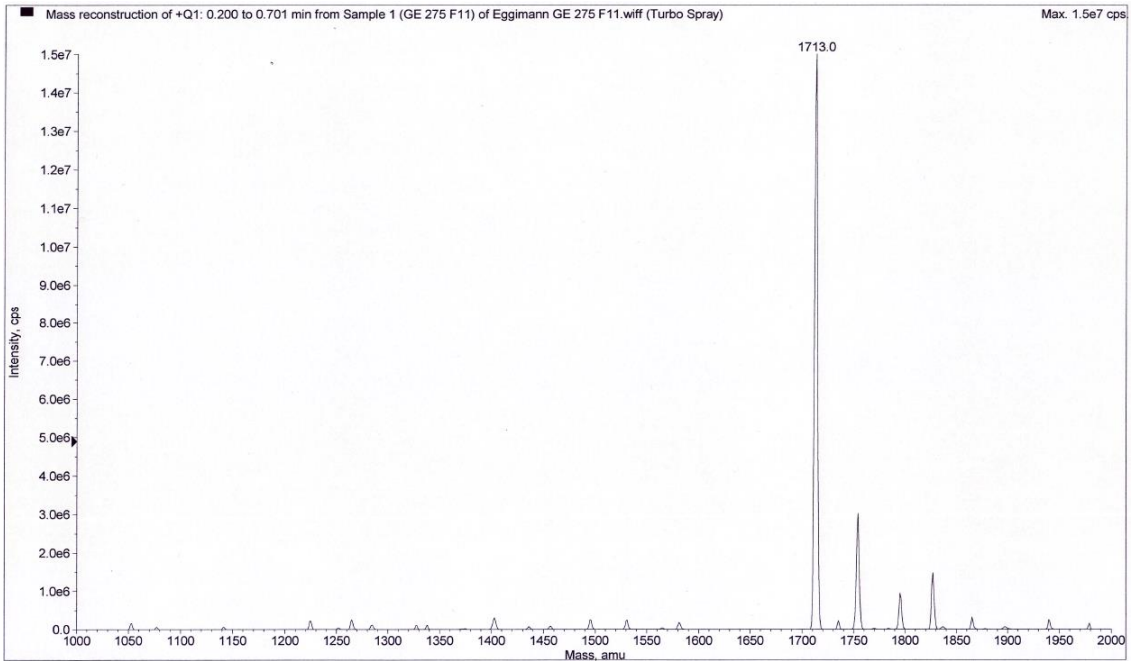
Analytical RP-HPLC chromatogram:



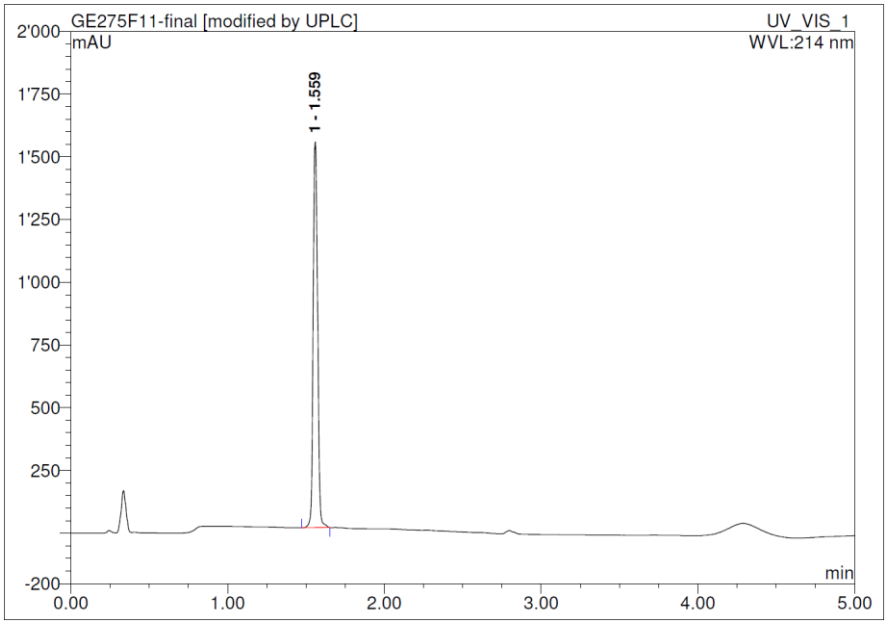
ClAc-KLA (ClAc-GGKLAKLAKKLAKLAK).

From Tenta Gel S RAM® resin (400 mg, 0.23 mmol·g^{-1}), **ClAc-KLA** was obtained as a foamy colourless solid after preparative RP-HPLC (27.9 mg, 11.6 µmol, 13%). Analytical RP-HPLC: t_R = 1.56 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{78}H_{146}ClN_{23}O_{14}$ calc/found 1713.6/1713.0 $[M]^+$.

Mass spectrum, MS (ESI+):



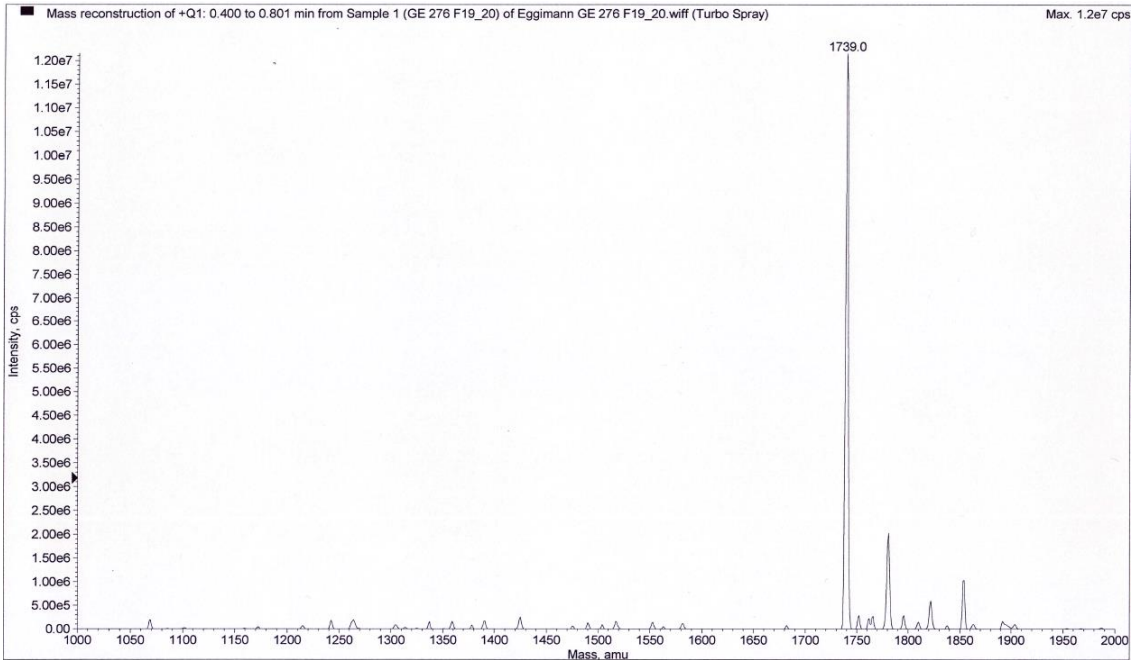
Analytical RP-HPLC chromatogram:



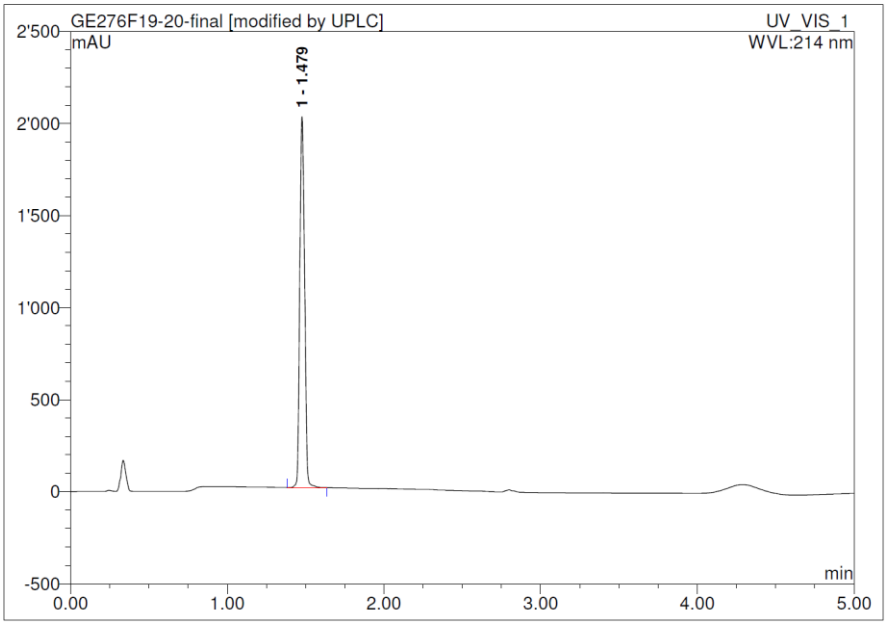
C-KLA (CGGKLAKLAKKLAKLAK).

From Tenta Gel S RAM® resin (400 mg, 0.23 mmol·g^{-1}), **C-KLA** was obtained as a foamy colourless solid after preparative RP-HPLC (48.6 mg, 19.1 µmol, 21%). Analytical RP-HPLC: t_R = 1.48 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{79}H_{150}N_{24}O_{17}S$ calc/found 1740.3/1739.0 $[M]^+$.

Mass spectrum, MS (ESI+):



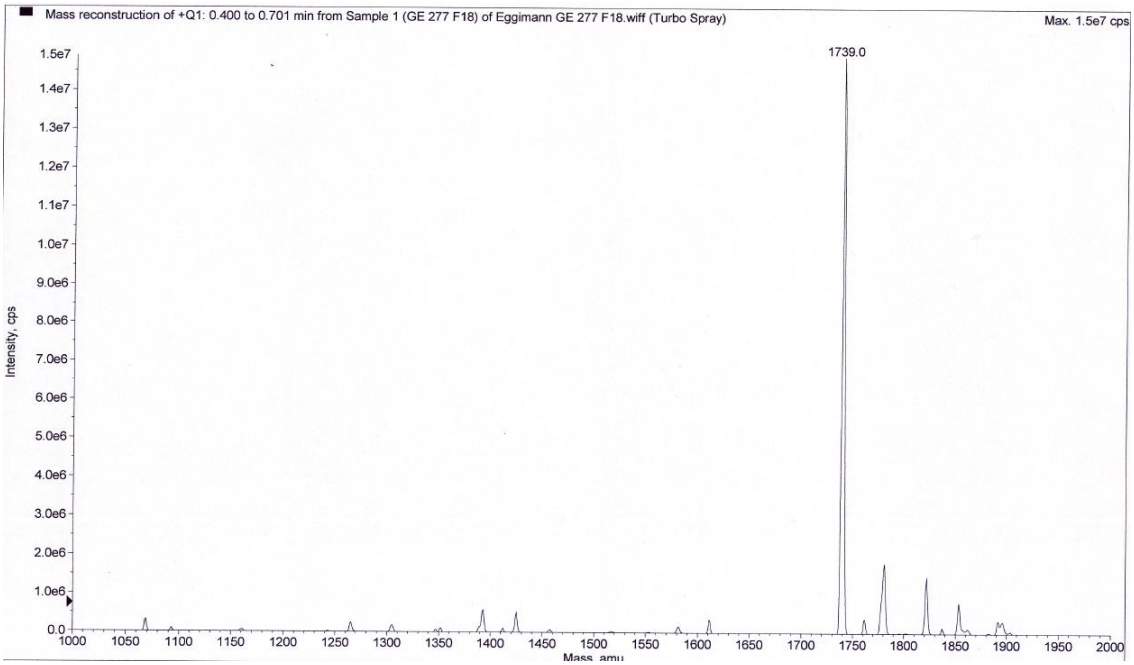
Analytical RP-HPLC chromatogram:



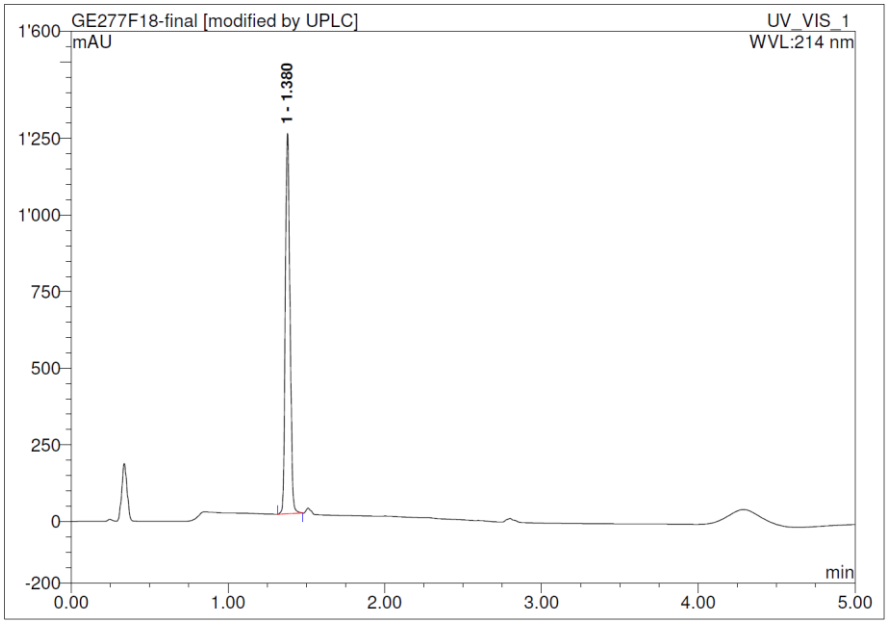
KLA-C (KLAKLAKKLAKLAKGGC).

From Tenta Gel S RAM® resin (400 mg, 0.23 mmol·g^{-1}), **KLA-C** was obtained as a foamy colourless solid after preparative RP-HPLC (19.3 mg, 7.6 µmol, 8%). Analytical RP-HPLC: t_R = 1.38 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{79}H_{150}N_{24}O_{17}S$ calc/found 1740.3/1739.0 $[M]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

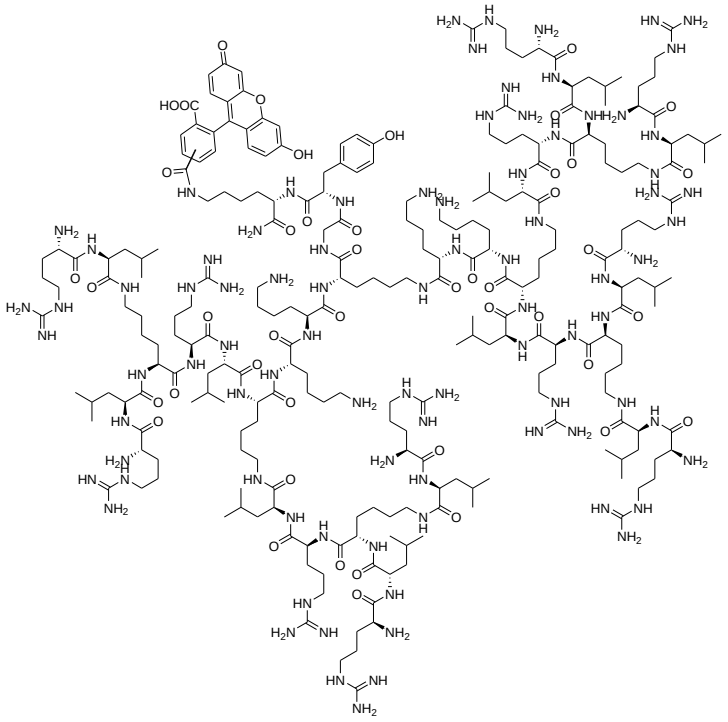


2.2 Peptide Dendrimers.

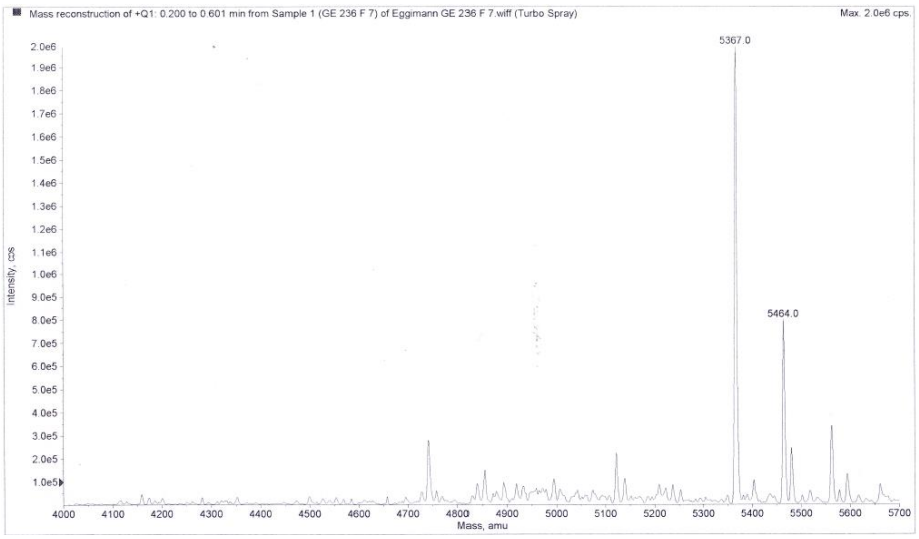
Ac denotes an acetyl group attached to a free amine group. * denotes 5(6)-carboxyfluorescein attached to a free amine group through an amide bond. Italics *K* denotes a branching lysine residues.

CPPD1 ((RL)$_8$(*K*RL)$_4$(*K*KK)$_2$*K*GYK*).

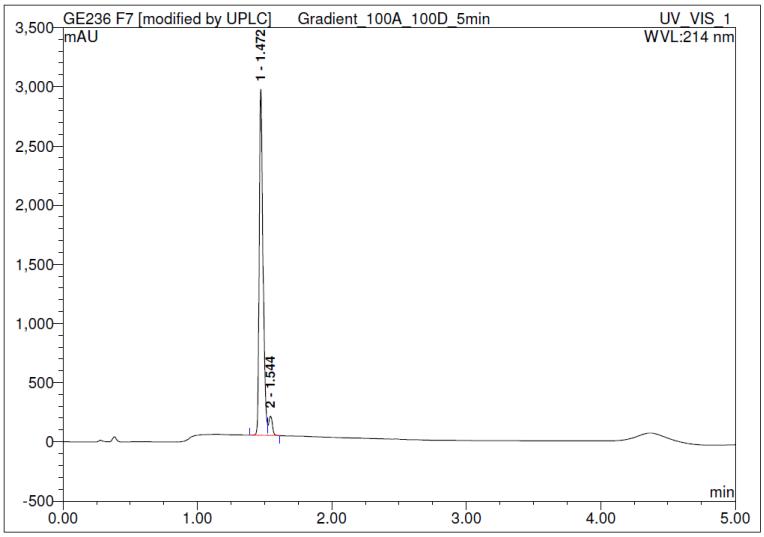
From Tenta Gel S RAM® resin (300 mg, 0.23 mmol·g^{-1}), **CPPD1** was obtained as a foamy yellow solid after preparative RP-HPLC (14.7 mg, 1.8 µmol, 3%). Analytical RP-HPLC: t_R = 1.47 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{248}H_{445}N_{87}O_{45}$ calc/found 5365.7/5367.0 $[M]^+$; 5463.7/5464.0 $[M + 2\ K + 1\ Na]^+$.



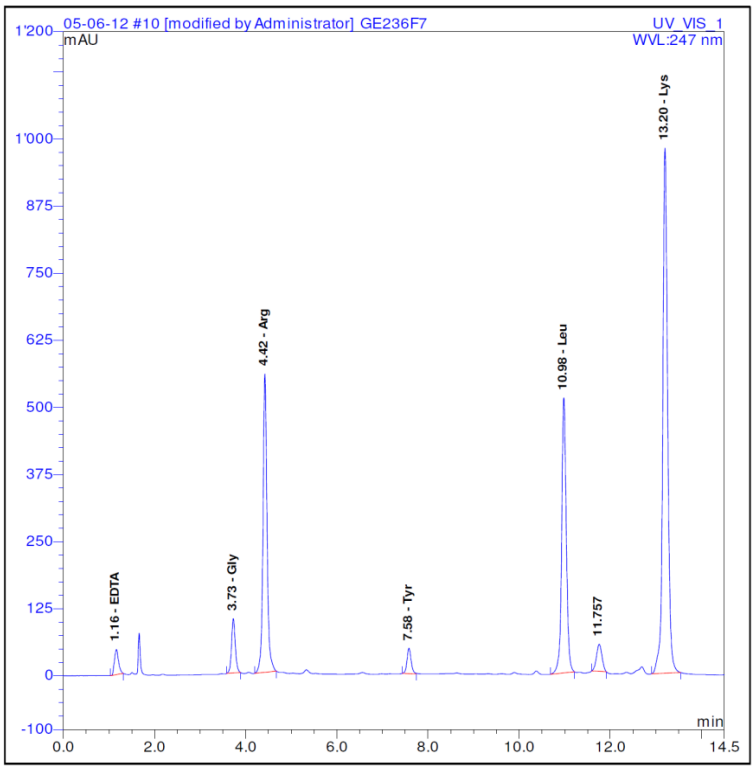
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

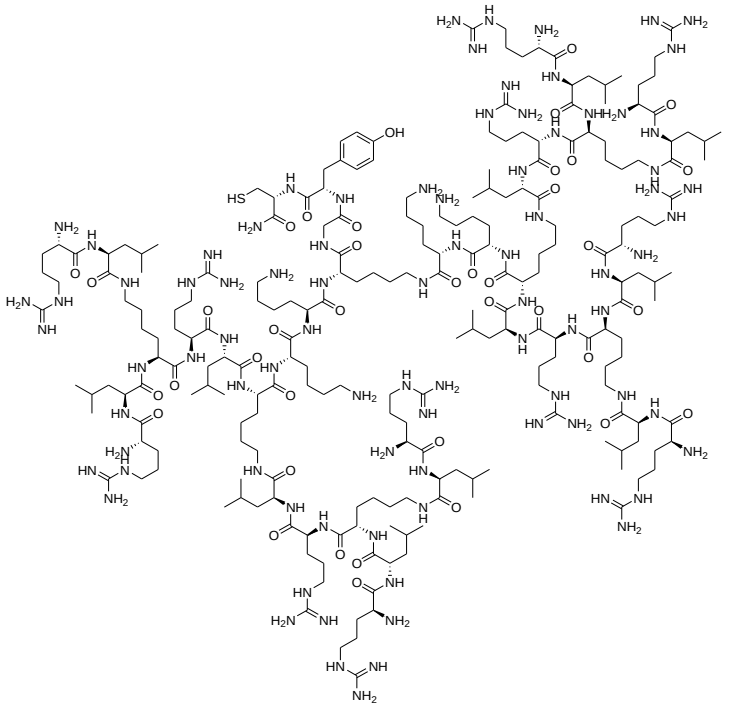


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.098	4.92	46.99		403.95	EDTA
3.73	3.74	0.085	9.58	101.04		692.39	Gly
4.42	4.44	0.088	56.93	555.54		3772.13	Arg
7.58	7.63	0.094	4.96	47.22		326.32	Tyr
10.98	11.03	0.103	60.02	512.46		3597.28	Leu
13.20	13.24	0.109	121.87	978.09		3790.83	Lys
Total:						12582.91	

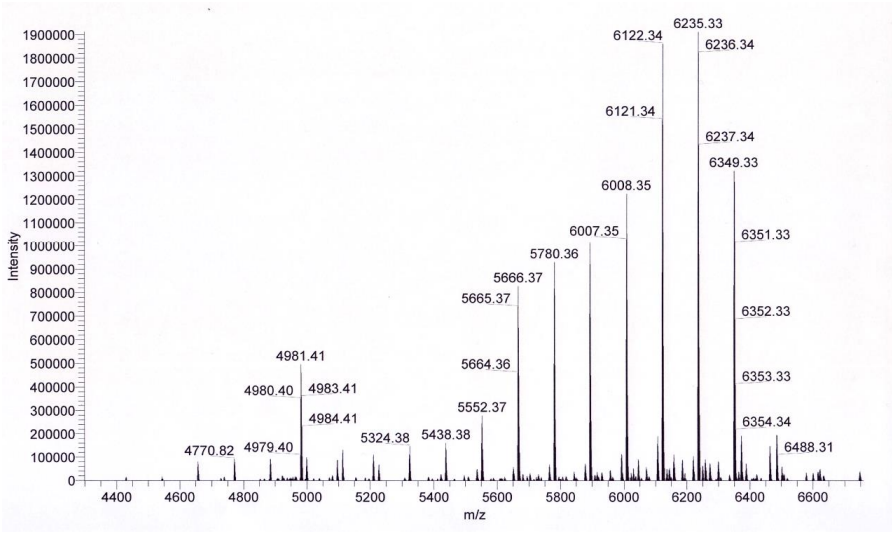
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	3772.1	12	11.8
Gly	692.4	1	2.2
Leu	3597.3	12	11.2
Lys	3790.8	12	11.8
Tyr	326.3	1	1.0

CPPD1-Cys ((RL)$_8$(*K*RL)$_4$(*K*KK)$_2$*K*GYC).

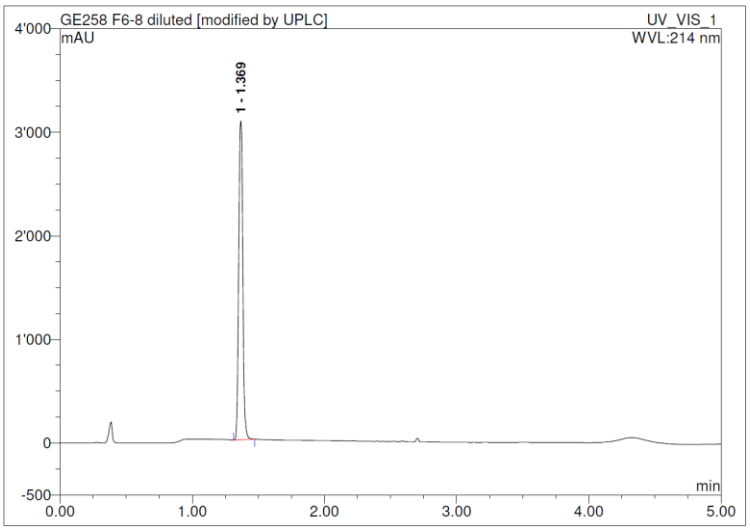
From Tenta Gel S RAM® resin (300 mg, 0.23 mmol·g^{-1}), **CPPD1-Cys** was obtained as a foamy colourless solid after preparative RP-HPLC (57.6 mg, 7.5 µmol, 11%). Analytical RP-HPLC: t_R = 1.37 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). HRMS (NSI+): $C_{224}H_{428}N_{86}O_{39}S$ calc/found 4982.4/4981.4 $[M]^+$; 5324.5/5324.4 $[M + 3\ TFA]^+$; 5438.5/5438.4 $[M + 4\ TFA]^+$; 5552.5/5552.4 $[M + 5\ TFA]^+$; 5666.5/5666.4 $[M + 6\ TFA]^+$; 5780.6/5780.4 $[M + 7\ TFA]^+$; 6008.6/6008.4 $[M + 9\ TFA]^+$; 6122.6/6122.3 $[M + 10\ TFA]^+$; 6236.6/6235.3 $[M + 11\ TFA]^+$; 6350.7/6349.3 $[M + 12\ TFA]^+$.



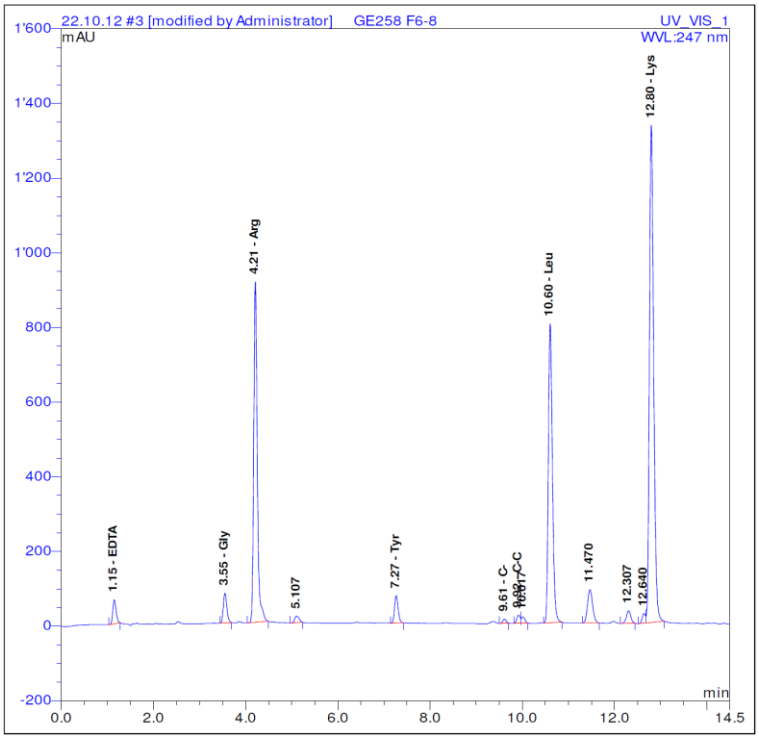
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:


GE258 F6-8 diluted [modified by UPLC]
UV_VIS_1
mAU
WVL:214 nm
1 - 1.369
4'000
3'000
2'000
1'000
0
-500
min
0.00
1.00
2.00
3.00
4.00
5.00

Analytical RP-HPLC chromatogram of amino acid analysis:

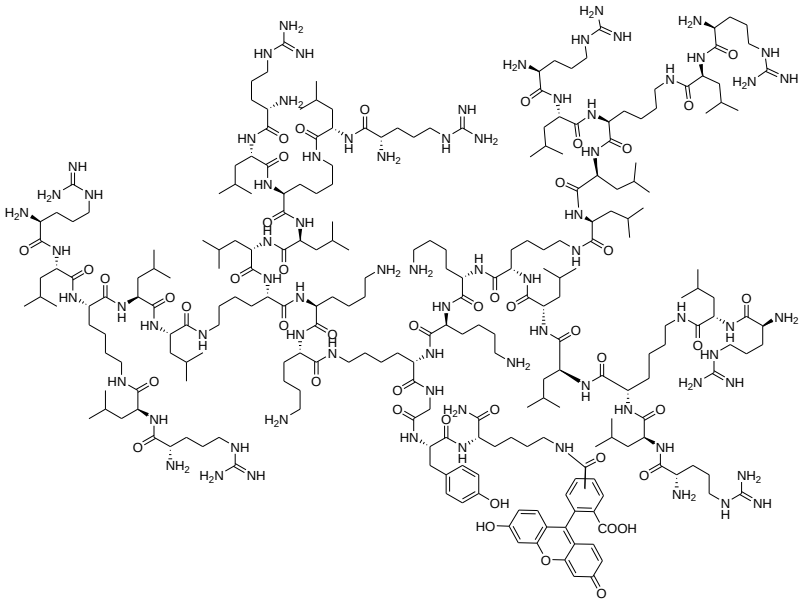


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.15	0.077	5.34	64.04		408.15	EDTA
3.55	3.56	0.081	6.96	79.49		503.08	Gly
4.21	4.22	0.078	81.01	911.96		5811.50	Arg
7.27	7.31	0.084	6.68	73.55		459.00	Tyr
9.61	9.65	0.080	0.89	10.68		54.64	C-
9.92	9.96	n.a.	1.84	21.64		110.71	C-C
10.60	10.67	0.094	81.47	800.73		5137.77	Leu
12.80	12.85	0.098	142.38	1331.62		5301.66	Lys
Total:						17786.52	

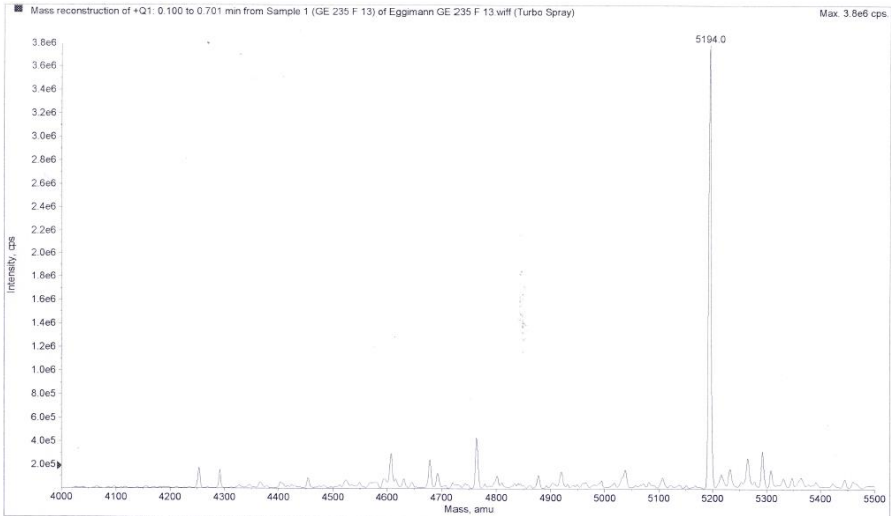
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	5811.5	12	12.4
Gly	503.1	1	1.1
Leu	5137.8	12	10.9
Lys	5301.7	11	11.3
Tyr	459.0	1	1.0

CPPD2 ((RL)$_8$(*K*LL)$_4$(*K*KK)$_2$*K*GYK*).

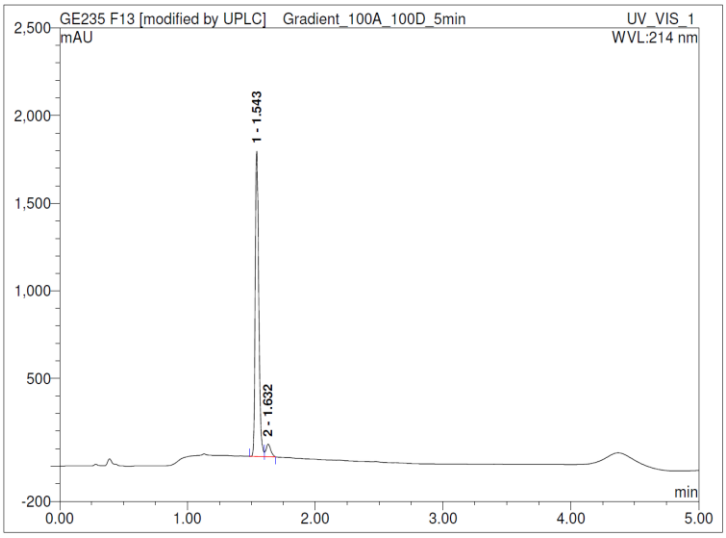
From Tenta Gel S RAM® resin (300 mg, 0.23 mmol·g^{-1}), **CPPD2** was obtained as a foamy yellow solid after preparative RP-HPLC (9.6 mg, 1.3 µmol, 2%). Analytical RP-HPLC: t_R = 1.54 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{248}H_{441}N_{75}O_{45}$ calc/found 5193.6/5194.0 $[M]^+$.



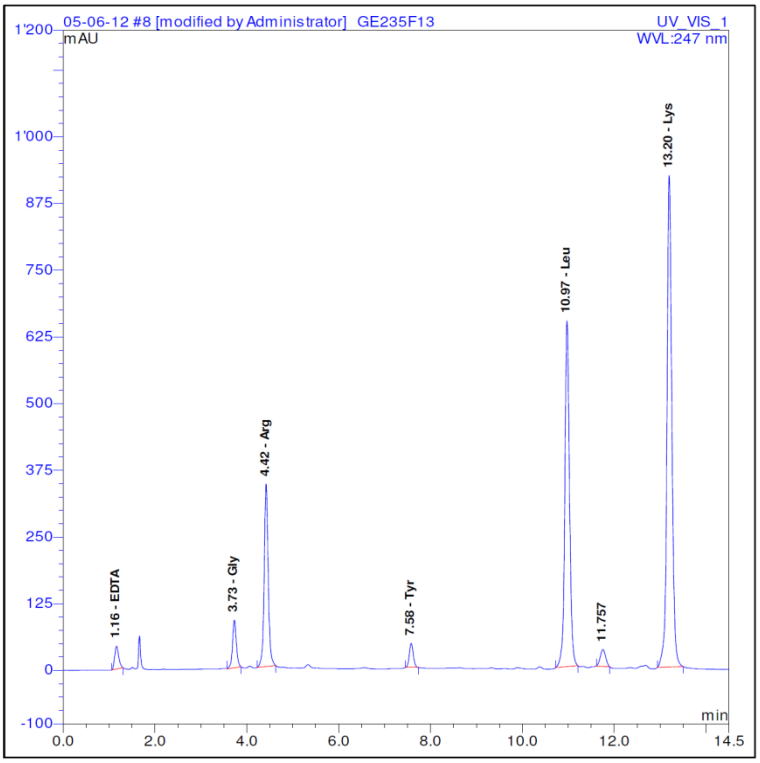
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

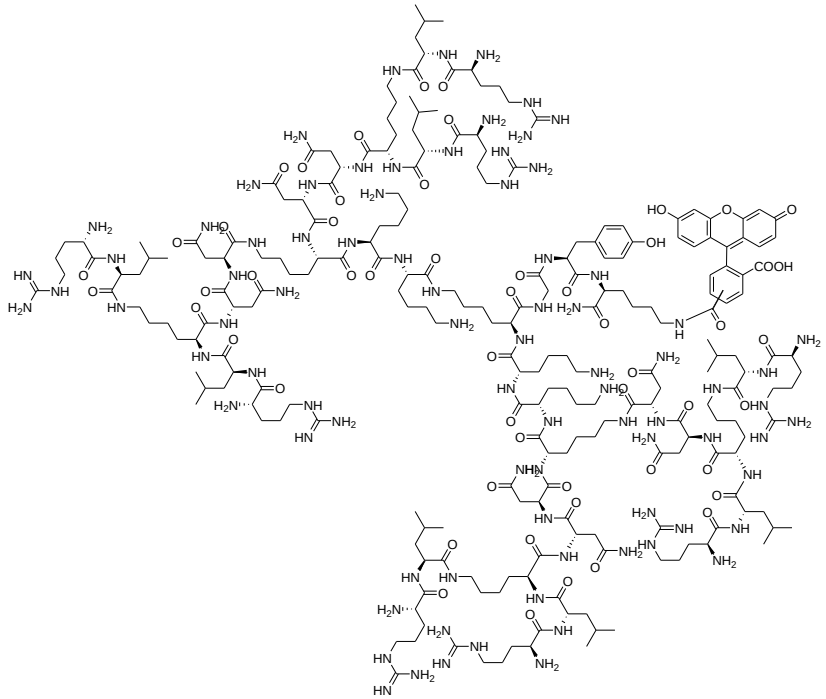


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.16	1.16	0.097	4.41	42.86		368.46	EDTA
3.73	3.74	0.085	8.64	89.46		613.06	Gly
4.42	4.44	0.088	34.47	342.05		2322.56	Arg
7.58	7.63	0.092	4.51	45.19		312.28	Tyr
10.97	11.03	0.104	76.31	648.04		4549.00	Leu
13.20	13.24	0.109	113.69	921.68		3572.20	Lys
Total:						11737.56	

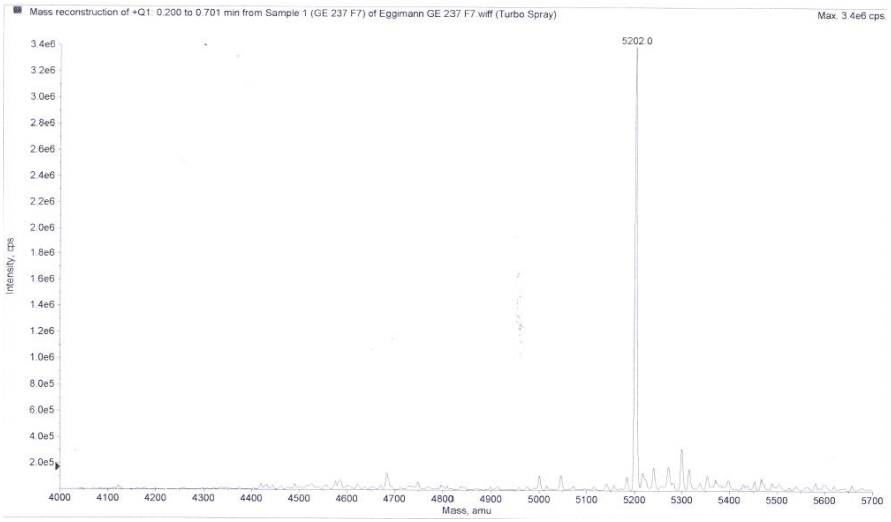
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	2322.6	8	7.8
Gly	613.1	1	2.1
Leu	4549.0	16	15.2
Lys	3572.2	12	11.9
Tyr	312.3	1	1.0

CPPD3 ((RL)$_8$(*K*NN)$_4$(*K*KK)$_2$*K*GYK*).

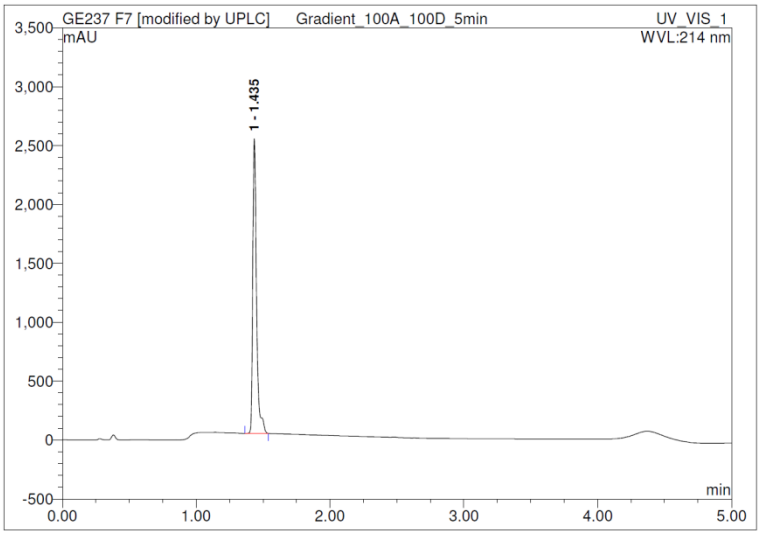
From Tenta Gel S RAM® resin (300 mg, 0.23 mmol·g^{-1}), **CPPD3** was obtained as a foamy yellow solid after preparative RP-HPLC (23.3 mg, 3.1 µmol, 5%). Analytical RP-HPLC: t_R = 1.44 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{232}H_{401}N_{83}O_{53}$ calc/found 5201.2/5202.0 $[M]^+$.



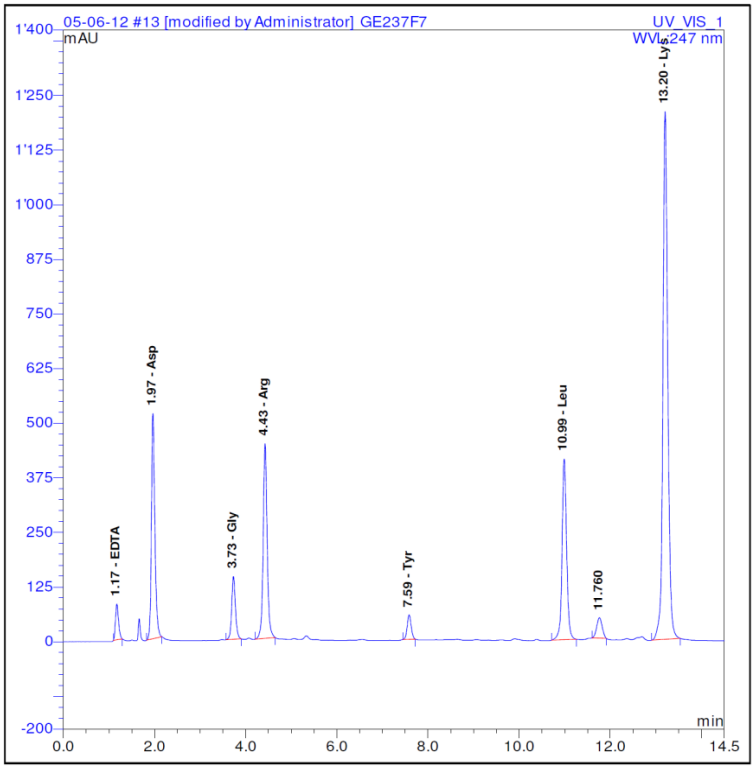
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



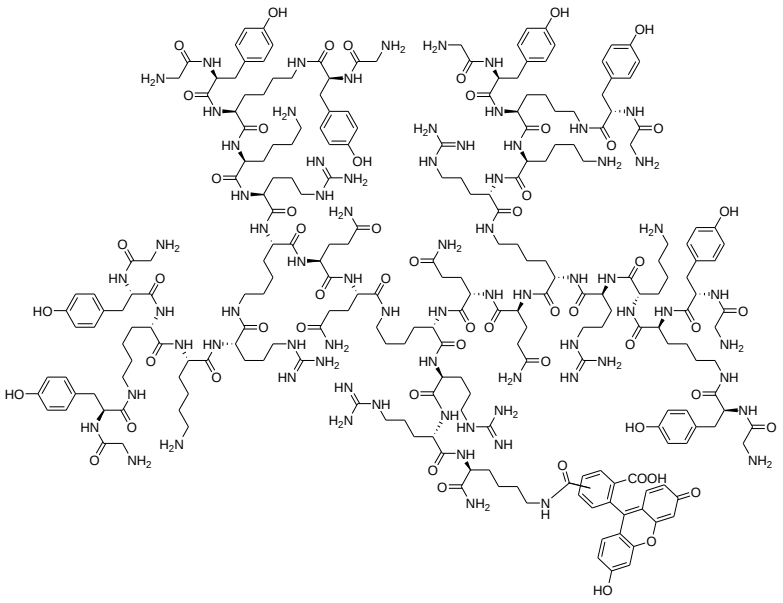
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.17	1.16	0.073	6.43	81.82		703.37	EDTA
1.97	1.96	0.077	44.95	515.07		3010.92	Asp
3.73	3.74	0.083	13.48	143.38		982.54	Gly
4.43	4.44	0.088	45.10	445.95		3028.01	Arg
7.59	7.63	0.093	5.67	56.09		387.61	Tyr
10.99	11.03	0.102	47.96	413.81		2904.77	Leu
13.20	13.24	0.109	149.93	1207.59		4680.32	Lys
Total:						15697.54	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	3028.0	8	7.8
Asn[a)]	2800.7	8	7.2
Gly	982.5	1	2.5
Leu	2904.8	8	7.5
Lys	4680.3	12	12.0
Tyr	387.6	1	1.0

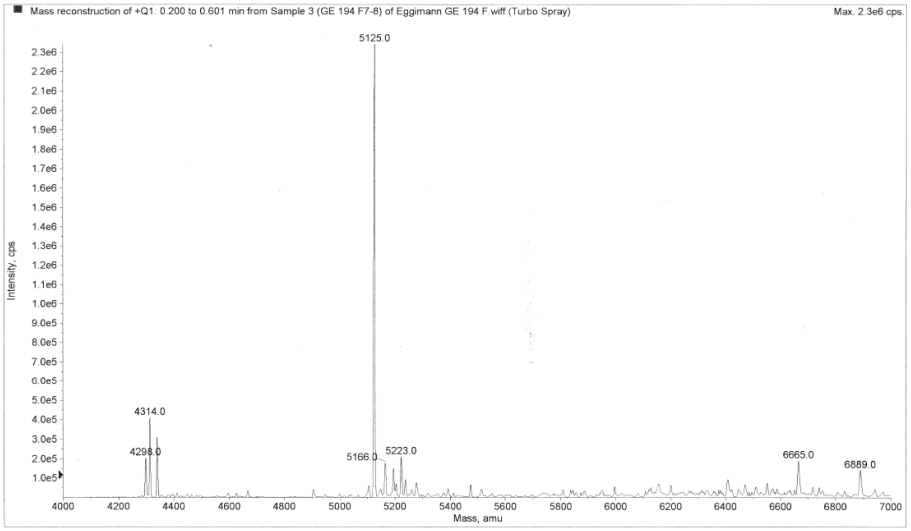
[a)] Detected as aspartic acid.

CPPD4 ((GY)$_8$(*K*KR)$_4$(*K*QQ)$_2$*K*RRK*).

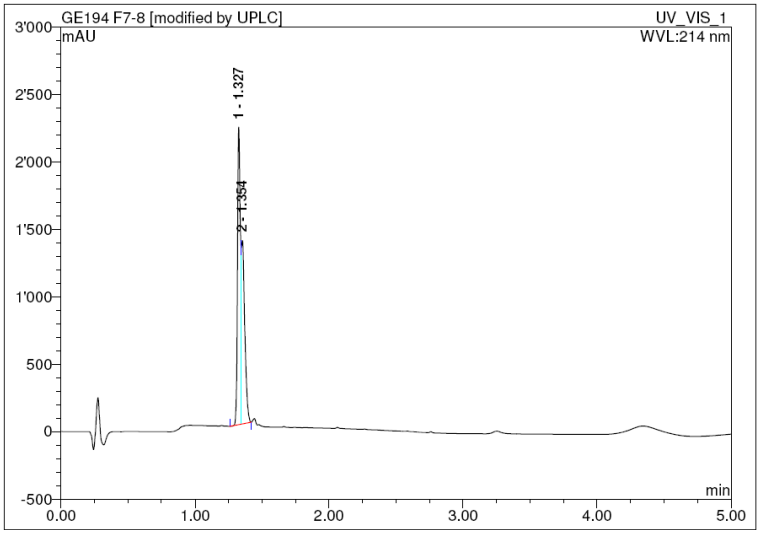
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD4** was obtained as a foamy yellow solid after preparative RP-HPLC (13.2 mg, 1.8 µmol, 2%). Analytical RP-HPLC: t_R = 1.33 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{237}H_{357}N_{73}O_{56}$ calc/found 5124.8/5125.0 $[M]^+$.



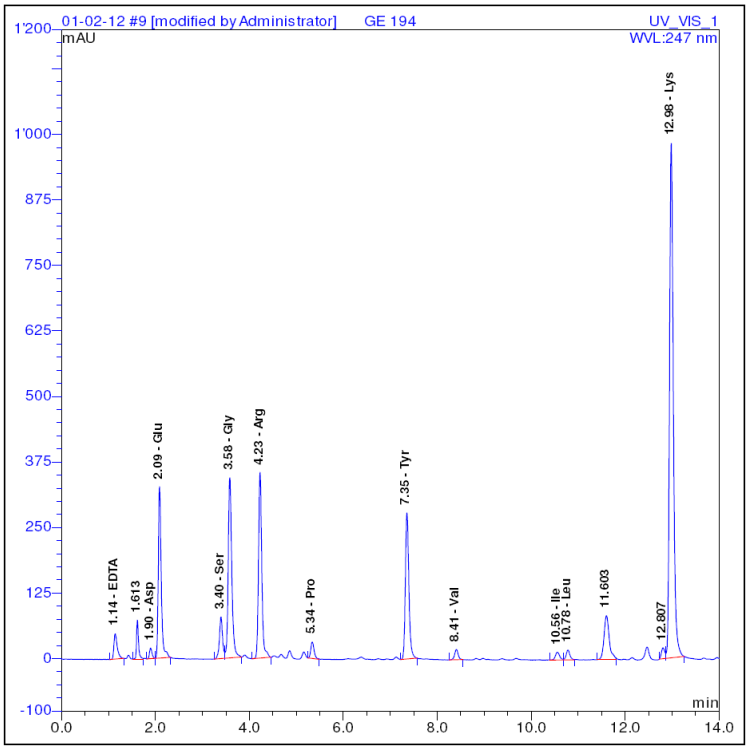
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



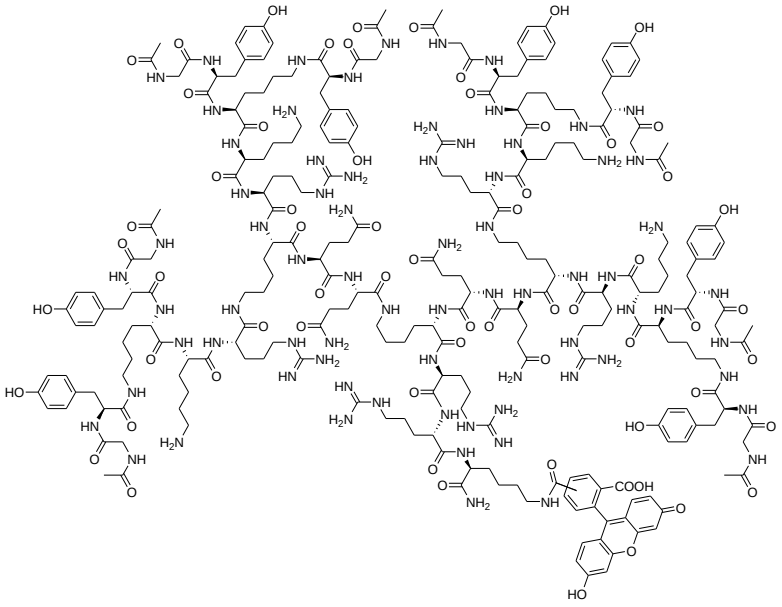
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.14	1.14	0.072	3.93	48.01		213.10	EDTA
1.90	1.90	0.058	1.28	20.32		91.34	Asp
2.09	2.09	0.063	23.86	327.03		1573.13	Glu
3.40	3.34	0.068	6.16	79.33		465.09	Ser
3.58	3.59	0.077	30.15	343.58		1999.72	Gly
4.23	4.22	0.071	28.44	354.23		1906.48	Arg
5.34	5.34	0.075	2.59	31.45		152.33	Pro
7.35	7.37	0.078	24.30	278.91		1560.05	Tyr
8.41	8.42	0.078	1.69	19.56		107.82	Val
10.56	10.56	0.095	1.55	14.70		82.09	Ile
10.78	10.80	0.083	1.75	18.81		109.76	Leu
12.98	13.02	0.084	92.03	981.21		3174.62	Lys
Total:						11435.53	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	1906.5	6	7.1
Gln[a)]	1573.1	4	5.9
Gly	1999.7	8	7.4
Lys	3174.6	12	11.8
Tyr	1560.1	8	5.8

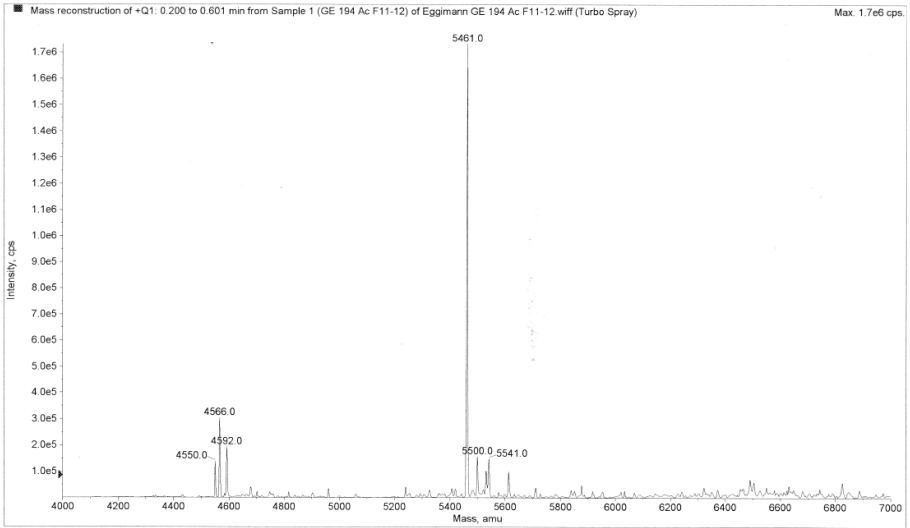
[a)] Detected as glutamic acid.

CPPD4Ac ((AcGY)$_8$(*K*KR)$_4$(*K*QQ)$_2$*K*RRK*).

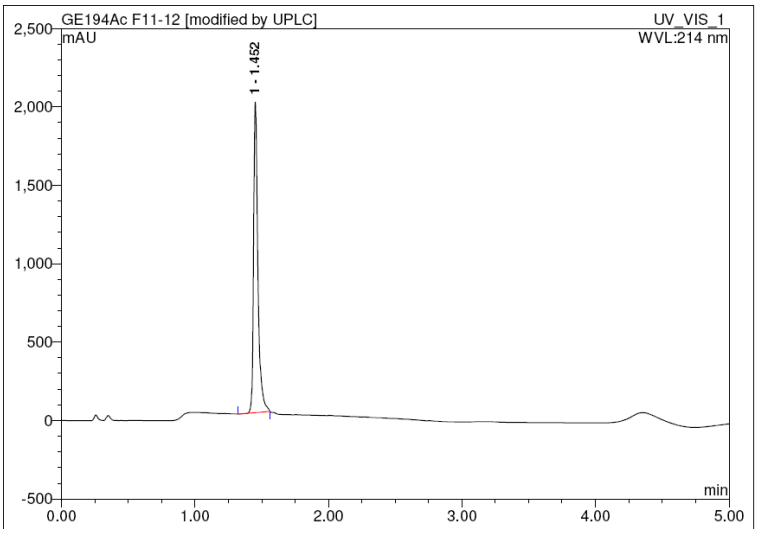
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD4Ac** was obtained as a foamy yellow solid after preparative RP-HPLC (4.7 mg, 0.7 µmol, 1%). Analytical RP-HPLC: t_R = 1.45 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{253}H_{373}N_{73}O_{64}$ calc/found 5461.1/5461.0 $[M]^+$.



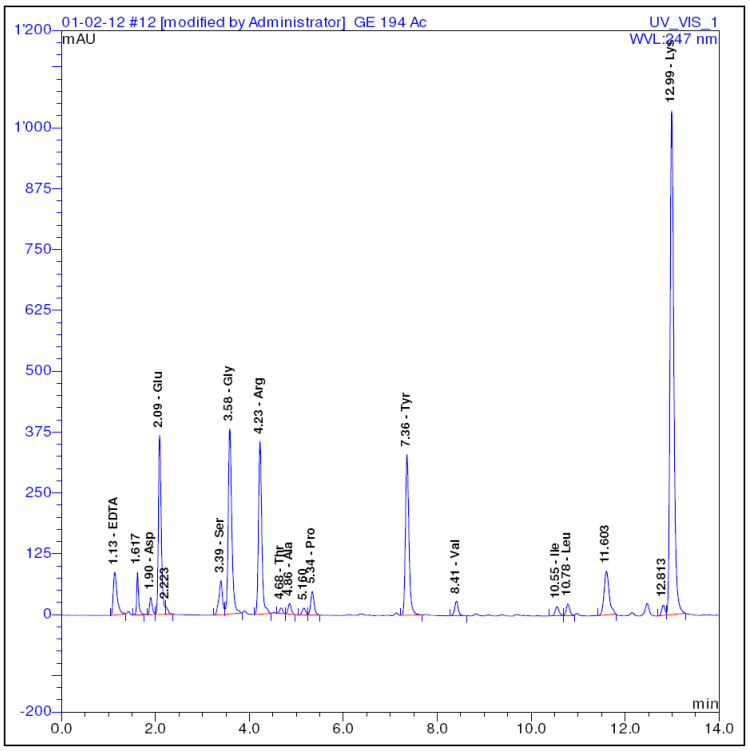
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



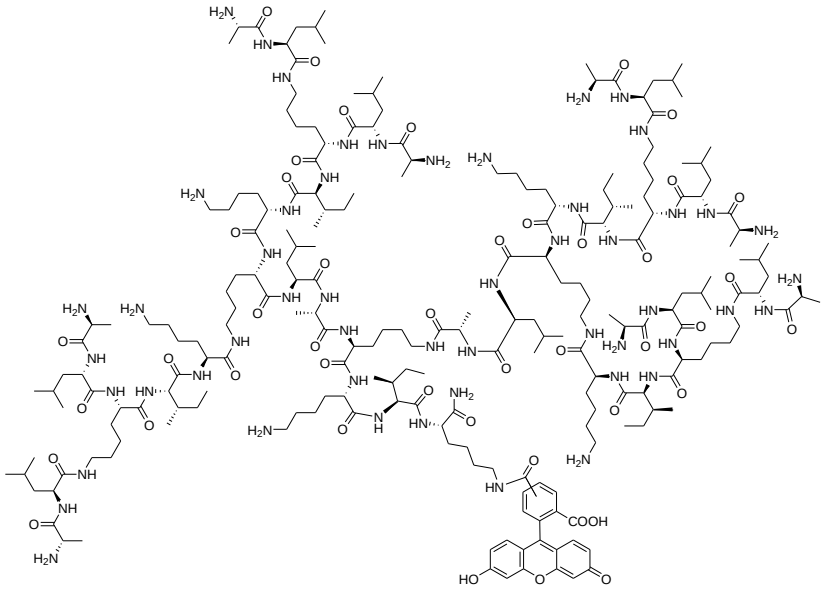
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.13	1.14	0.081	8.10	87.34		387.71	EDTA
1.90	1.90	0.058	2.34	35.68		160.35	Asp
2.09	2.09	0.063	26.10	368.12		1770.75	Glu
3.39	3.34	0.078	6.29	69.58		407.94	Ser
3.58	3.59	0.077	33.28	380.16		2212.61	Gly
4.23	4.22	0.071	28.61	354.74		1909.24	Arg
4.68	4.68	0.072	0.87	11.66		78.78	Thr
4.86	4.86	0.072	1.68	21.99		122.34	Ala
5.34	5.34	0.076	4.04	47.97		232.33	Pro
7.36	7.37	0.078	28.99	329.91		1845.28	Tyr
8.41	8.42	0.080	2.76	30.17		166.30	Val
10.55	10.56	0.088	1.88	18.75		104.68	Ile
10.78	10.80	0.080	2.23	24.53		143.19	Leu
12.99	13.02	0.084	96.22	1034.85		3348.15	Lys
Total:						12889.65	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	1909.2	6	6.9
Gln[a)]	1170.8	4	4.2
Gly	2212.6	8	8.0
Lys	3348.2	12	12.1
Tyr	1845.3	8	6.7

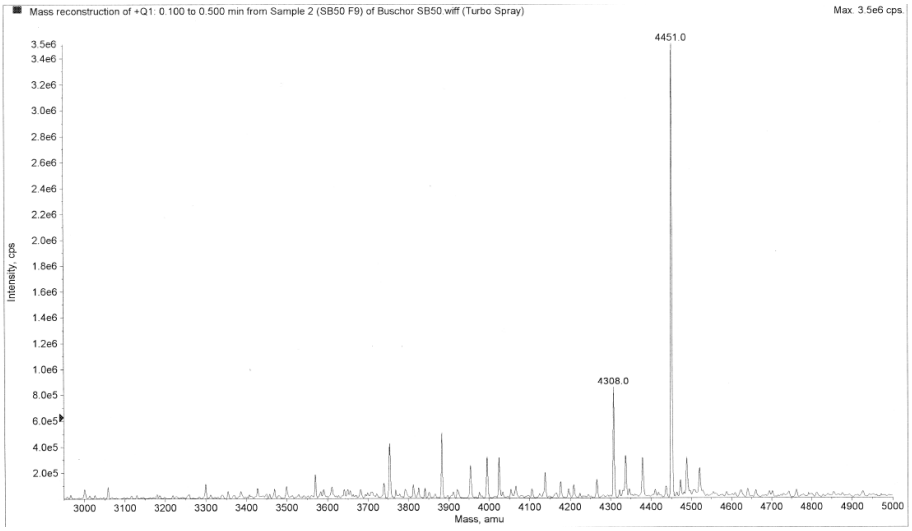
[a)] Detected as glutamic acid.

CPPD5 ((AL)$_8$(*K*IK)$_4$(*K*LA)$_2$*K*KIK*).

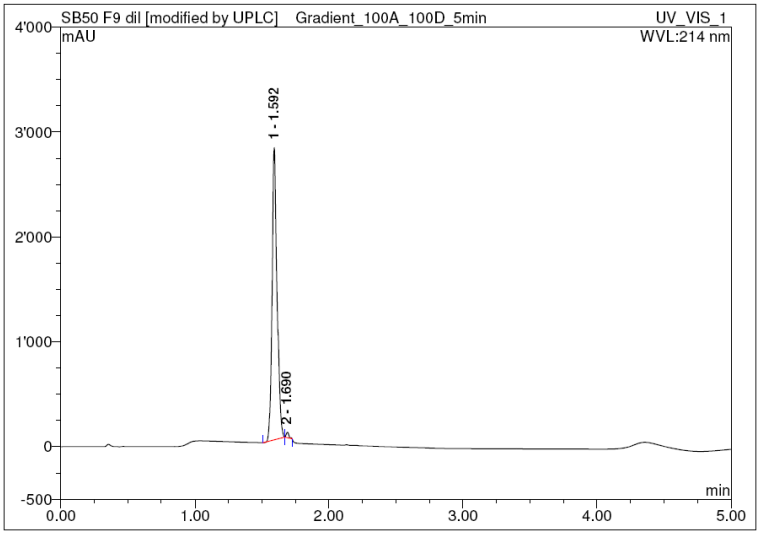
From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **CPPD5** was obtained as a foamy yellow solid after preparative RP-HPLC (33.4 mg, 5.6 µmol, 5%). Analytical RP-HPLC: t_R = 1.59 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{219}H_{394}N_{52}O_{44}$ calc/found 4449.7/4451.0 $[M]^+$.



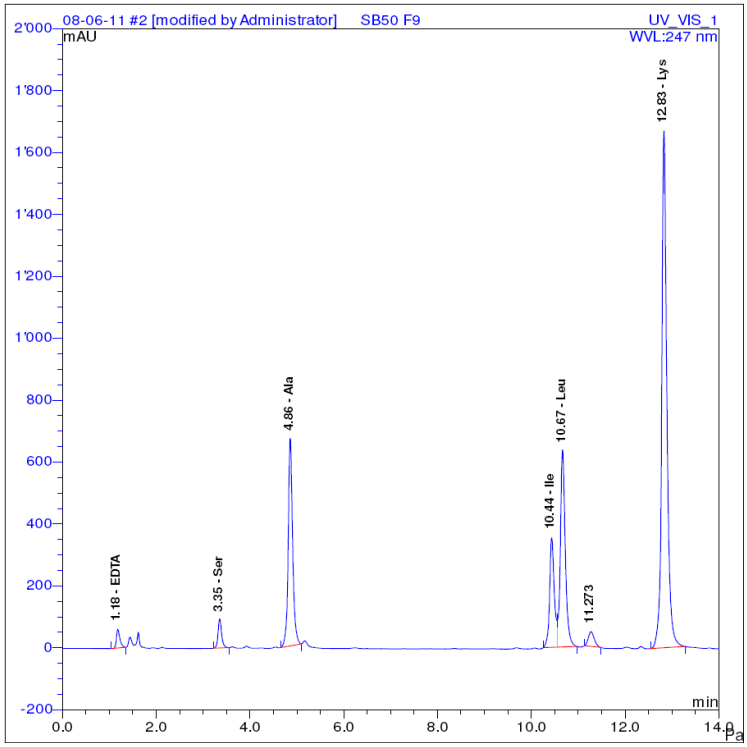
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

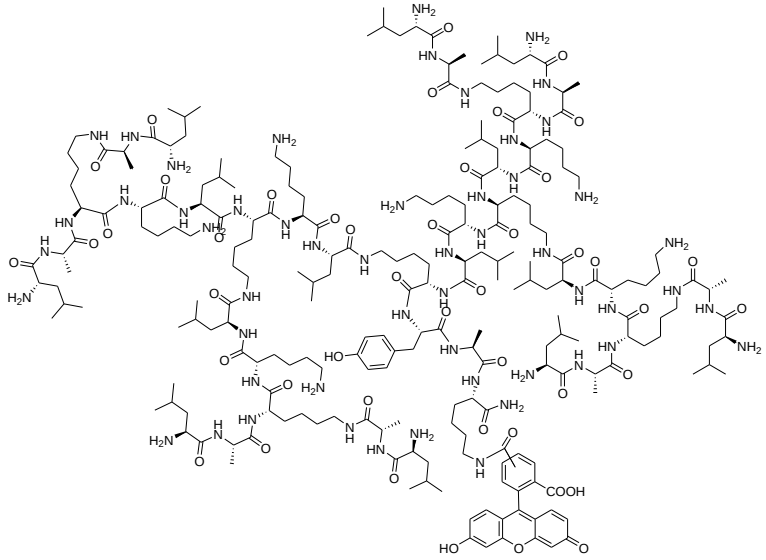


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.18	0.075	5.11	59.78		534.06	EDTA
3.35	3.37	0.077	8.25	93.60		706.05	Ser
4.86	4.85	0.093	72.87	670.06		5007.27	Ala
10.44	10.44	0.100	40.47	353.79		2769.51	Ile
10.67	10.67	0.105	78.20	636.27		5145.18	Leu
12.83	12.85	0.108	213.74	1669.10		7194.43	Lys
Total:						21356.50	

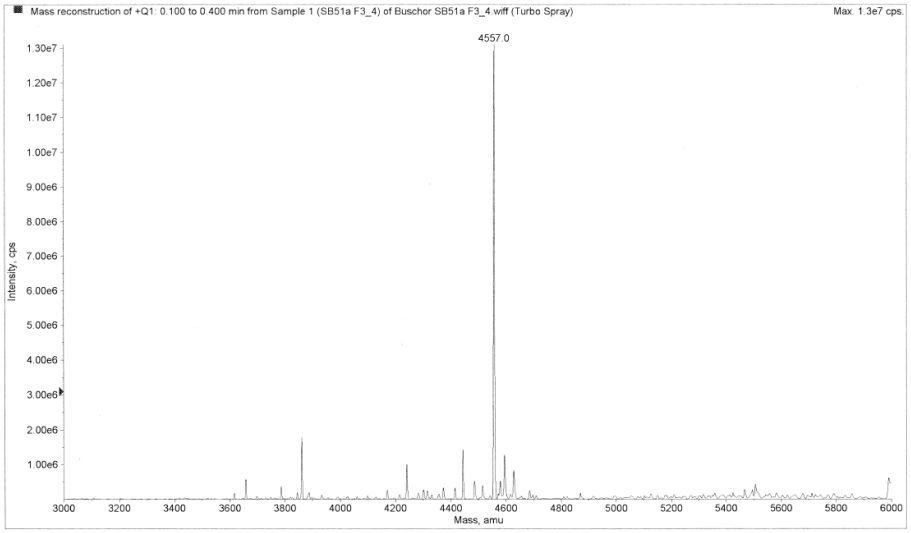
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	5007.3	10	9.5
Ile	2769.5	5	5.2
Leu	5145.2	10	9.7
Lys	7194.4	13	13.6

CPPD6 ((LA)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*YAK*).

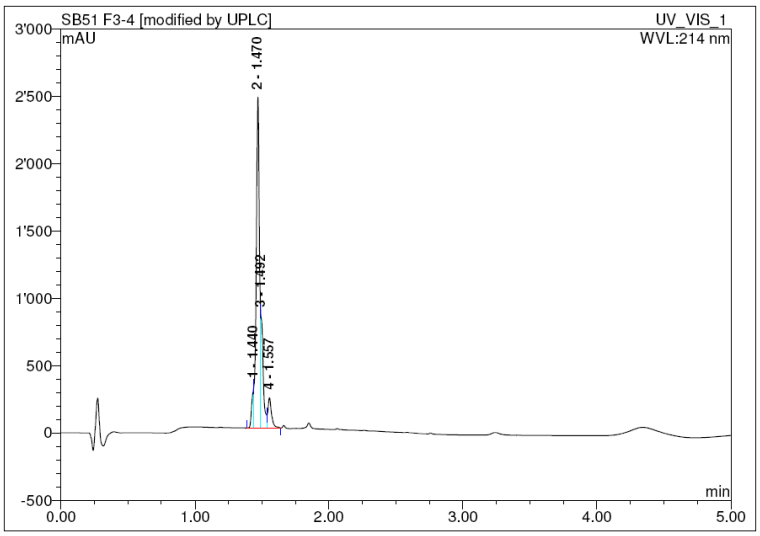
From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **CPPD6** was obtained as a foamy yellow solid after preparative RP-HPLC (55.1 mg, 9.0 µmol, 8%). Analytical RP-HPLC: t_R = 1.47 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{225}H_{389}N_{53}O_{45}$ calc/found 4556.8/4557.0 $[M]^+$.



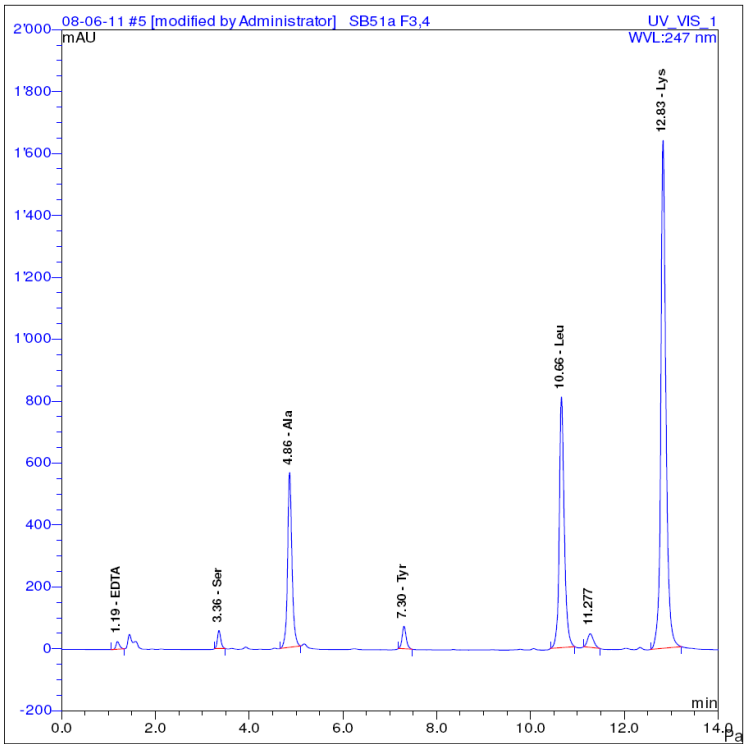
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

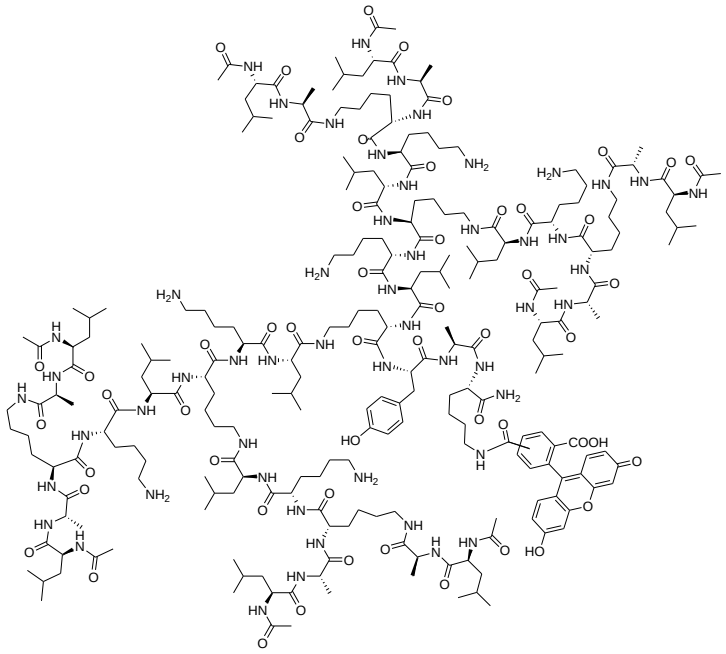


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.19	1.18	0.078	2.13	24.56		219.45	EDTA
3.36	3.37	0.075	4.77	58.20		439.06	Ser
4.86	4.85	0.093	61.24	564.45		4218.05	Ala
7.30	7.29	0.090	7.27	72.44		548.34	Tyr
10.66	10.67	0.105	99.72	809.64		6547.10	Leu
12.83	12.85	0.108	208.51	1639.73		7067.83	Lys
Total:						19039.83	

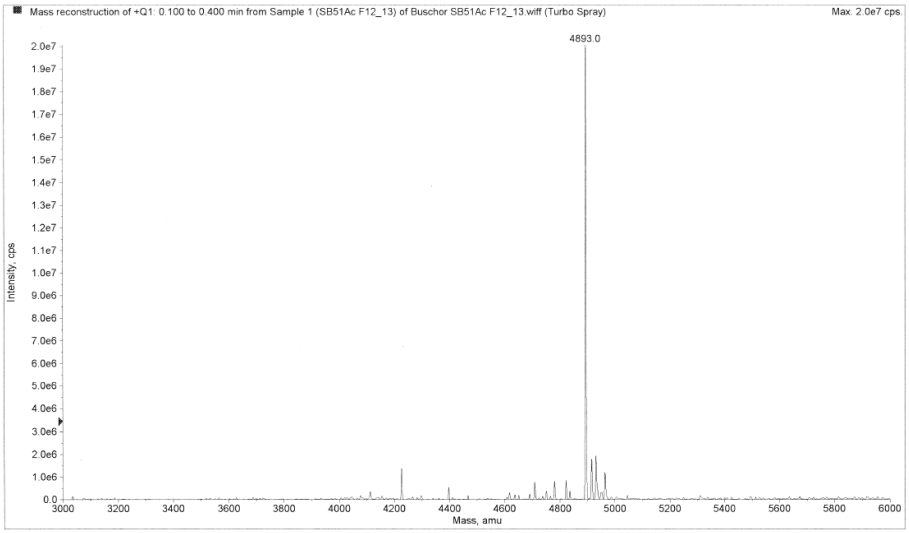
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	4218.1	9	8.7
Leu	6547.1	14	13.5
Lys	7067.8	14	14.6
Tyr	548.3	1	1.1

CPPD6Ac ((AcLA)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*YAK*).

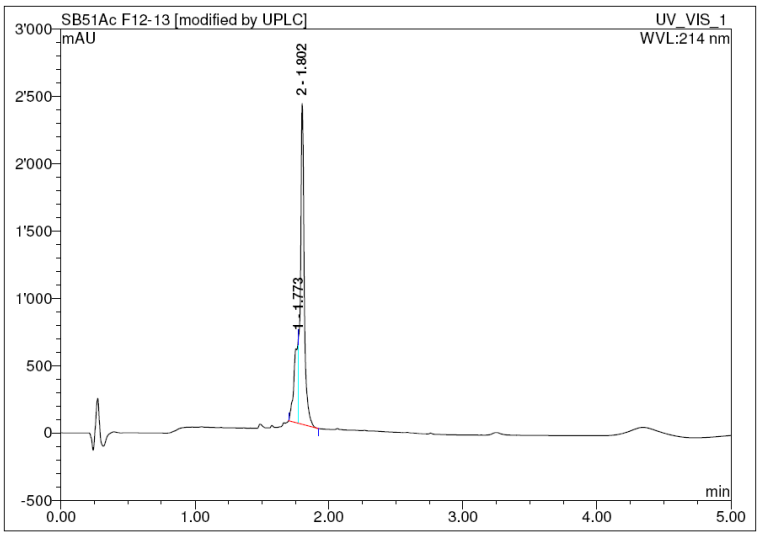
From Tenta Gel S RAM® resin (100 mg, 0.22 mmol·g^{-1}), **CPPD6Ac** was obtained as a foamy yellow solid after preparative RP-HPLC (9.0 mg, 1.6 µmol, 7%). Analytical RP-HPLC: t_R = 1.80 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{241}H_{405}N_{53}O_{53}$ calc/found 4893.1/4893.0 $[M]^+$.



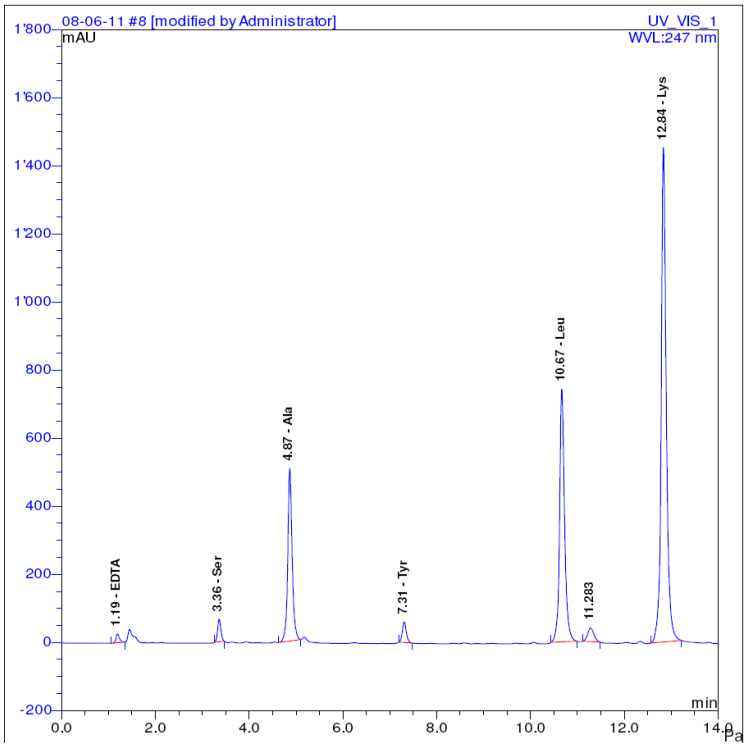
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

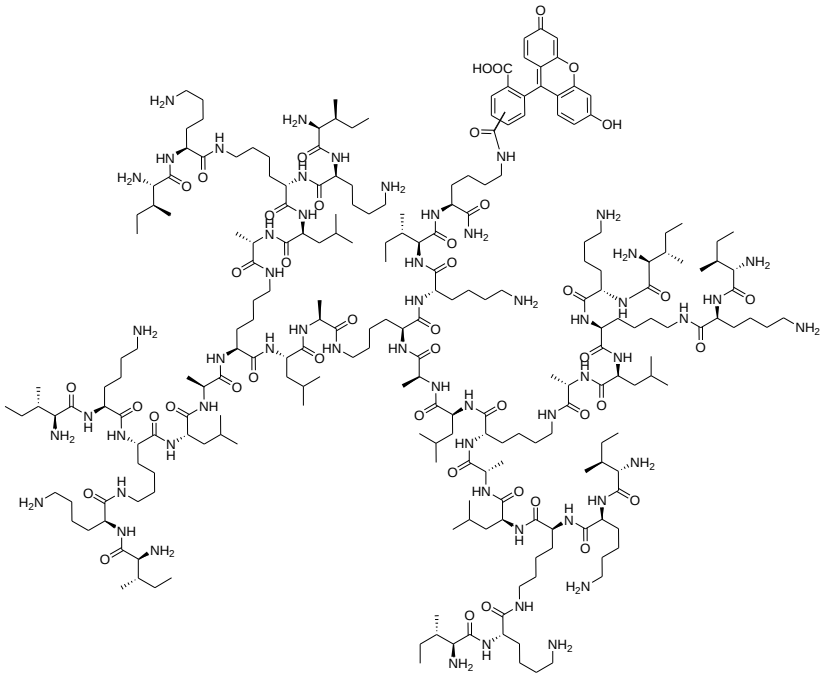


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.19	1.18	0.078	2.24	25.71		229.68	EDTA
3.36	3.37	0.074	5.34	66.40		500.88	Ser
4.87	4.85	0.093	54.93	506.52		3785.12	Ala
7.31	7.29	0.090	5.94	59.81		452.76	Tyr
10.67	10.67	0.104	90.64	741.92		5999.52	Leu
12.84	12.85	0.106	182.46	1451.44		6256.25	Lys
Total:						17224.21	

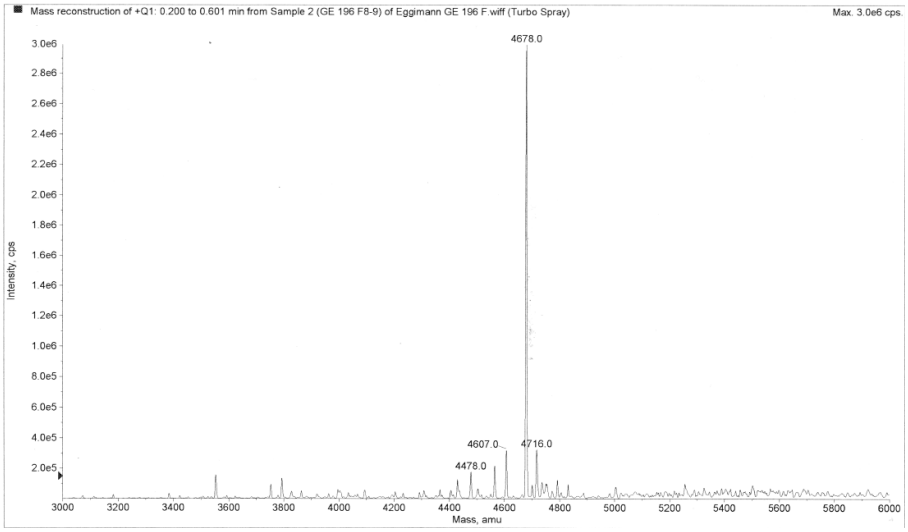
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	3785.1	9	8.7
Leu	5999.5	14	13.8
Lys	6256.3	14	14.4
Tyr	452.8	1	1.0

CPPD7 ((IK)$_8$(*K*LA)$_4$(*K*LA)$_2$*K*KIK*).

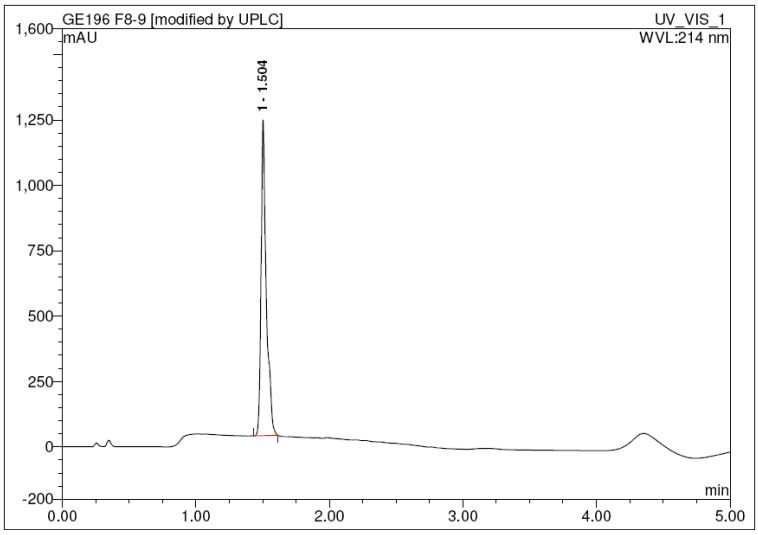
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD7** was obtained as a foamy yellow solid after preparative RP-HPLC (6.4 mg, 1.0 µmol, 1%). Analytical RP-HPLC: t_R = 1.50 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{231}H_{412}N_{56}O_{44}$ calc/found 4678.1/4678.0 $[M]^+$.



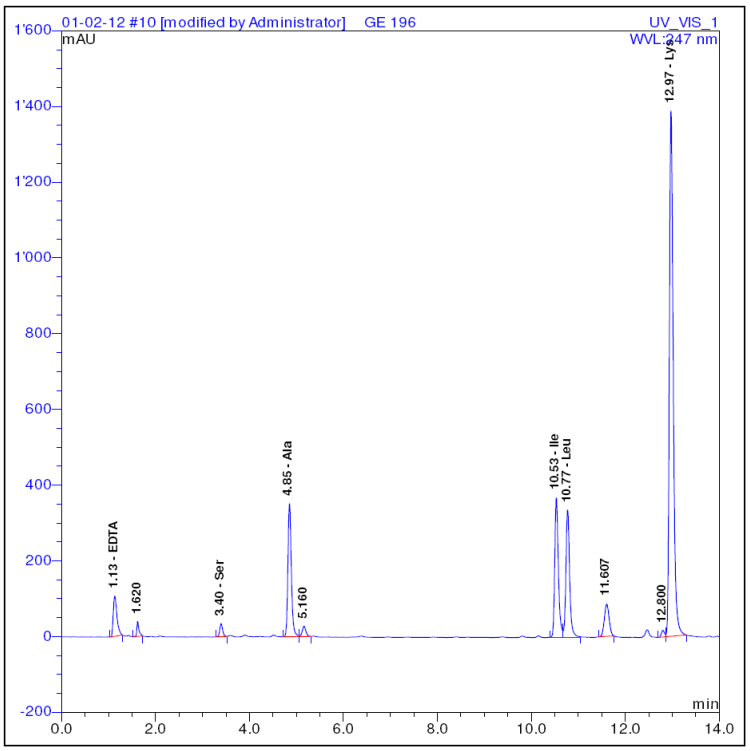
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

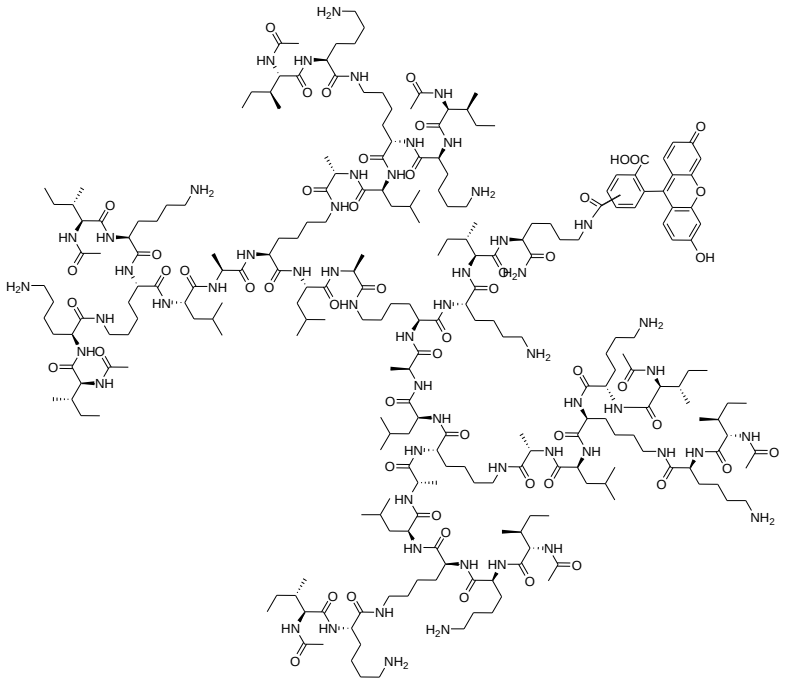


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.13	1.14	0.082	9.59	105.88		469.98	EDTA
3.40	3.34	0.065	2.45	34.24		200.74	Ser
4.85	4.86	0.076	29.83	351.25		1953.88	Ala
10.53	10.56	0.080	33.04	368.70		2058.52	Ile
10.77	10.80	0.081	31.09	336.56		1964.35	Leu
12.97	13.02	0.085	132.33	1387.95		4490.58	Lys
Total:						11138.06	

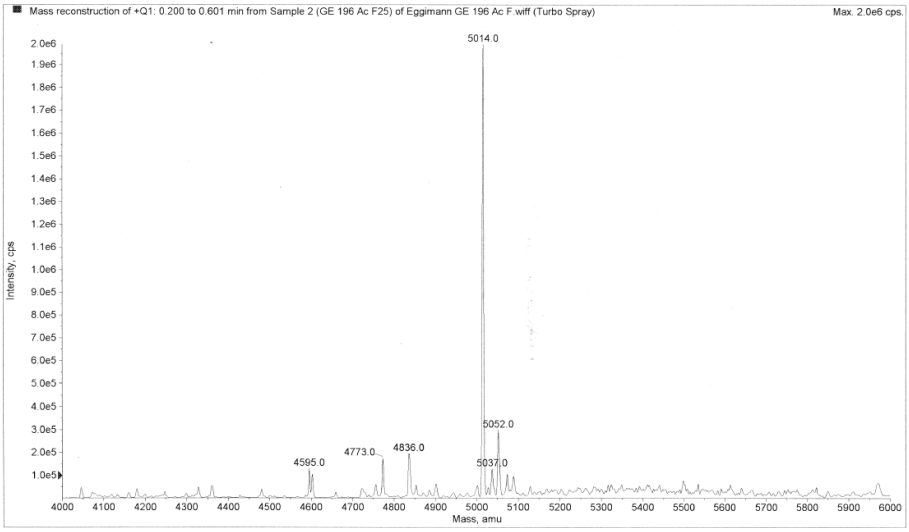
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1953.9	6	7.1
Ile	2058.5	9	7.5
Leu	1964.4	6	7.1
Lys	4490.6	17	16.3

CPPD7Ac ((AcIK)$_8$(*K*LA)$_4$(*K*LA)$_2$*K*KIK*).

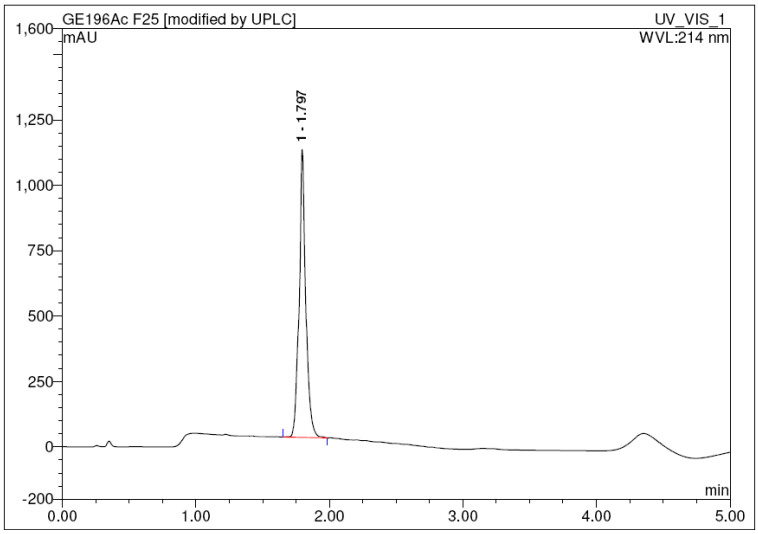
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD7Ac** was obtained as a foamy yellow solid after preparative RP-HPLC (3.0 mg, 0.5 µmol, 1%). Analytical RP-HPLC: t_R = 1.80 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{247}H_{428}N_{56}O_{52}$ calc/found 5014.4/5014.0 $[M]^+$.



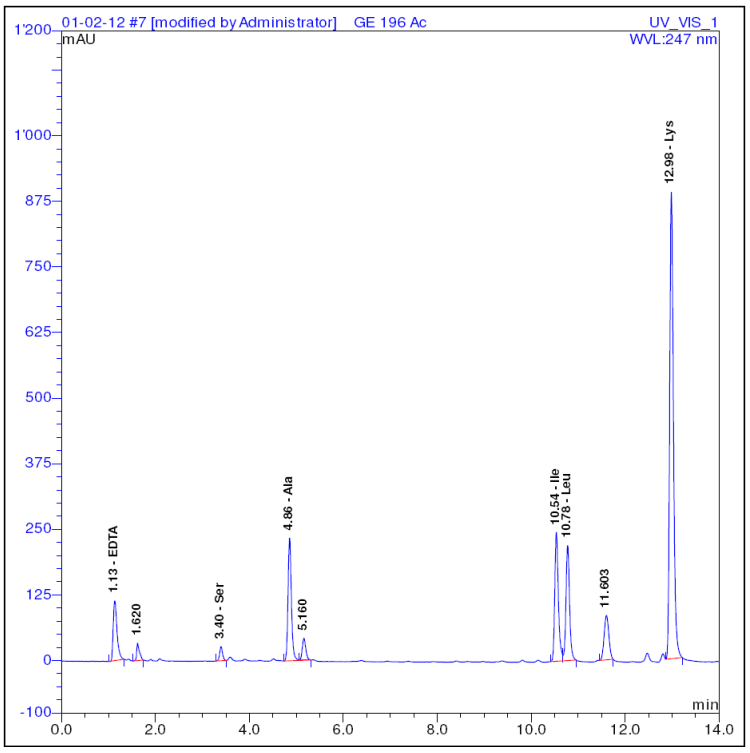
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

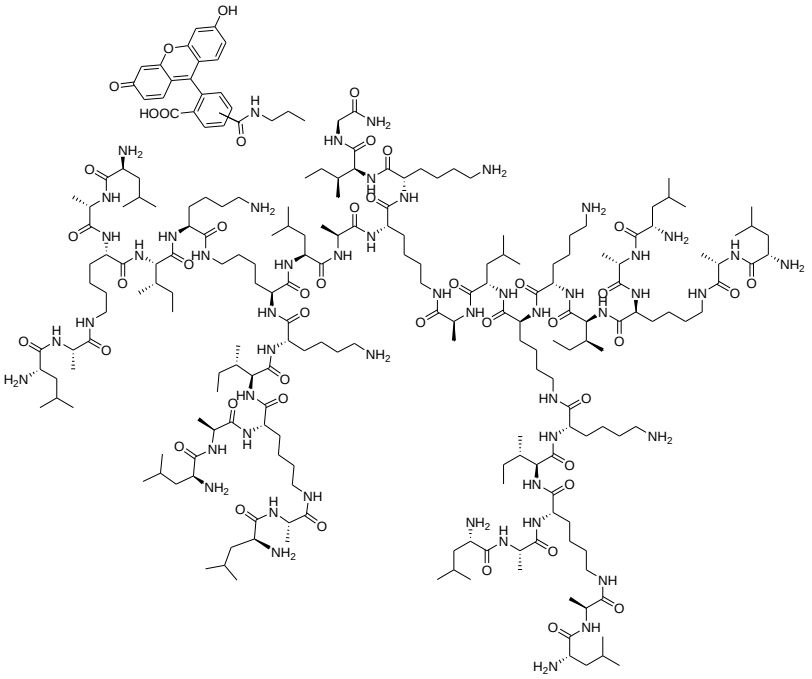


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.13	1.14	0.084	10.65	113.94		505.77	EDTA
3.40	3.34	0.064	1.93	27.00		158.31	Ser
4.86	4.86	0.075	19.63	233.92		1301.18	Ala
10.54	10.56	0.079	21.94	245.54		1370.90	Ile
10.78	10.80	0.080	19.54	219.37		1280.34	Leu
12.98	13.02	0.084	82.09	888.89		2875.91	Lys
Total:						7492.41	

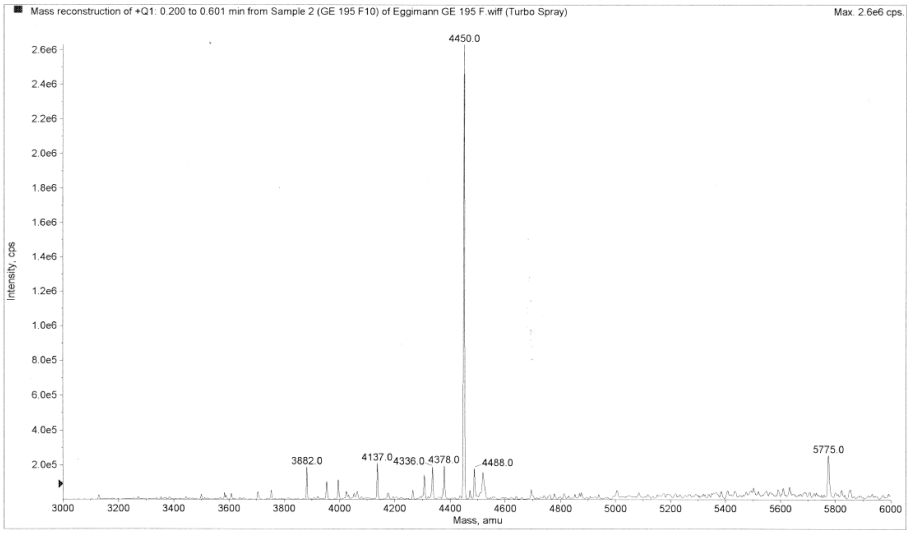
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1301.2	6	7.2
Ile	1370.9	9	7.6
Leu	1280.3	6	7.1
Lys	2875.9	17	16.0

CPPD8 ($(LA)_8(KIK)_4(KLA)_2KKIK^*$).

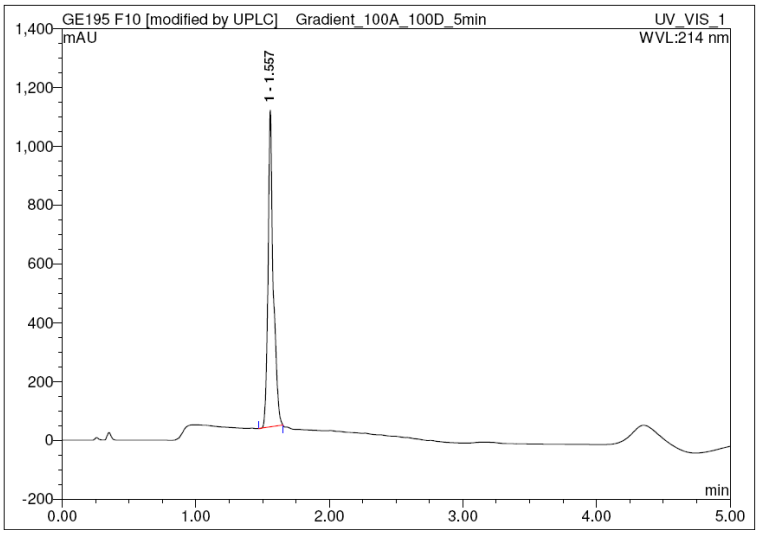
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD8** was obtained as a foamy yellow solid after preparative RP-HPLC (11.1 mg, 1.9 µmol, 2%). Analytical RP-HPLC: t_R = 1.56 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{219}H_{384}N_{52}O_{44}$ calc/found 4449.7/4450.0 $[M]^+$.



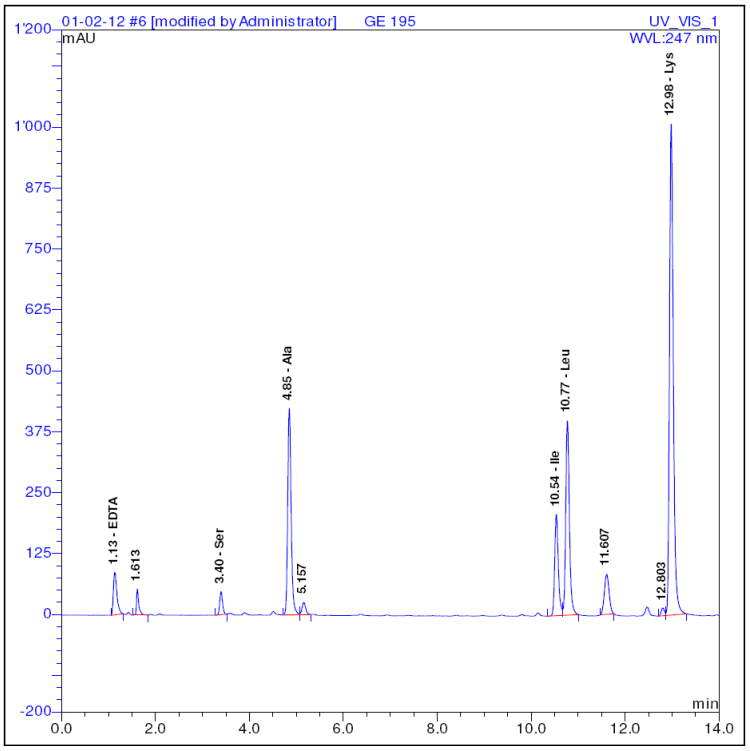
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

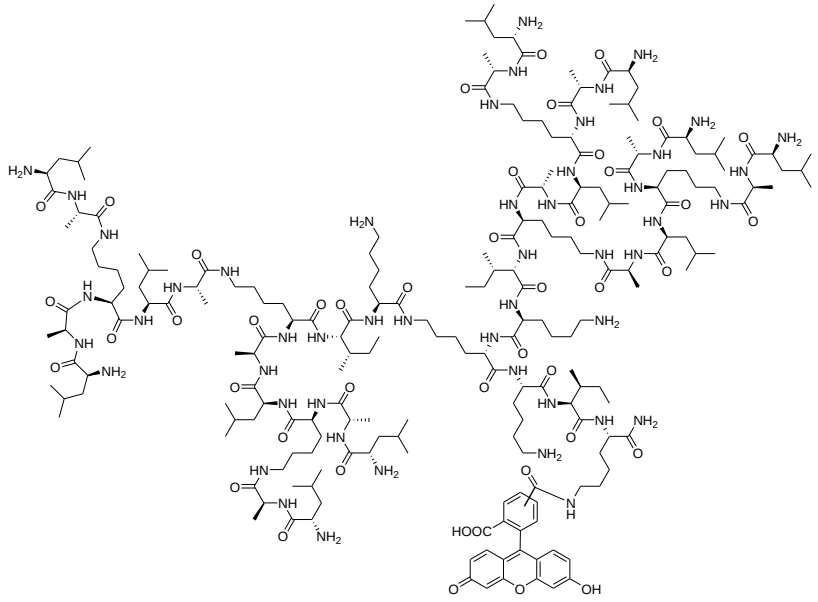


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.13	1.14	0.078	7.62	87.39		387.90	EDTA
3.40	3.34	0.063	3.26	47.00		275.55	Ser
4.85	4.86	0.076	35.95	423.51		2355.83	Ala
10.54	10.56	0.079	18.60	207.81		1160.24	Ile
10.77	10.80	0.081	36.30	398.55		2326.15	Leu
12.98	13.02	0.084	94.75	1007.31		3259.06	Lys
Total:						9764.72	

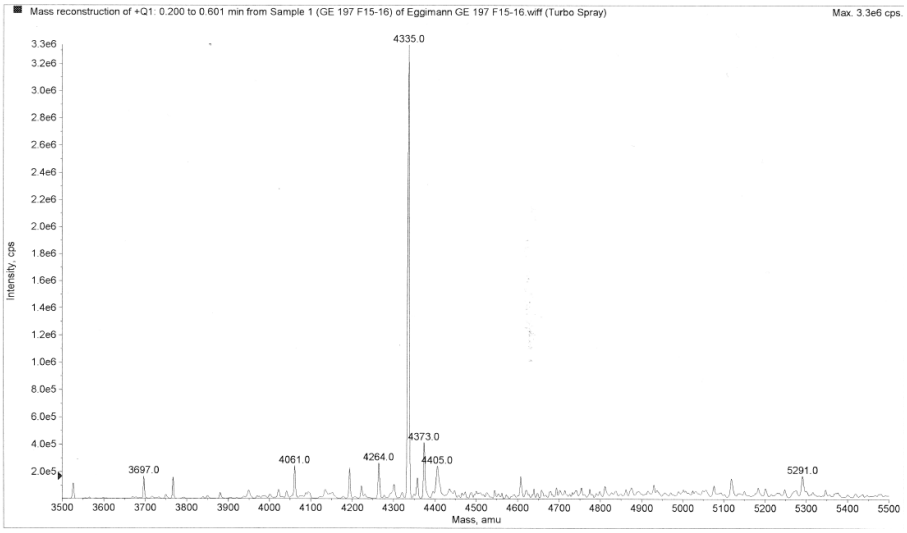
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	2355.8	10	9.8
Ile	1160.2	5	4.8
Leu	2326.2	10	9.7
Lys	3259.1	13	13.6

CPPD9 ((LA)$_8$(*K*LA)$_4$(*K*IK)$_2$*K*KIK*).

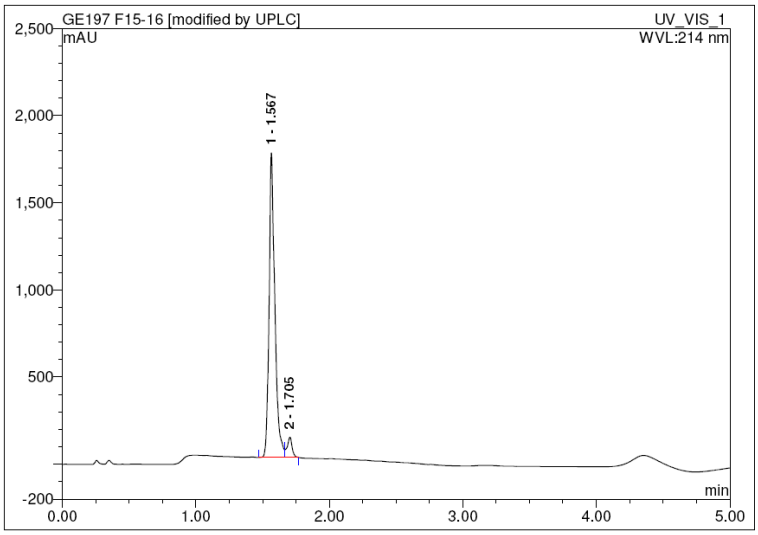
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD9** was obtained as a foamy yellow solid after preparative RP-HPLC (8.2 mg, 1.5 µmol, 2%). Analytical RP-HPLC: t_R = 1.57 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{213}H_{370}N_{50}O_{44}$ calc/found 4335.5/4335.0 $[M]^+$.



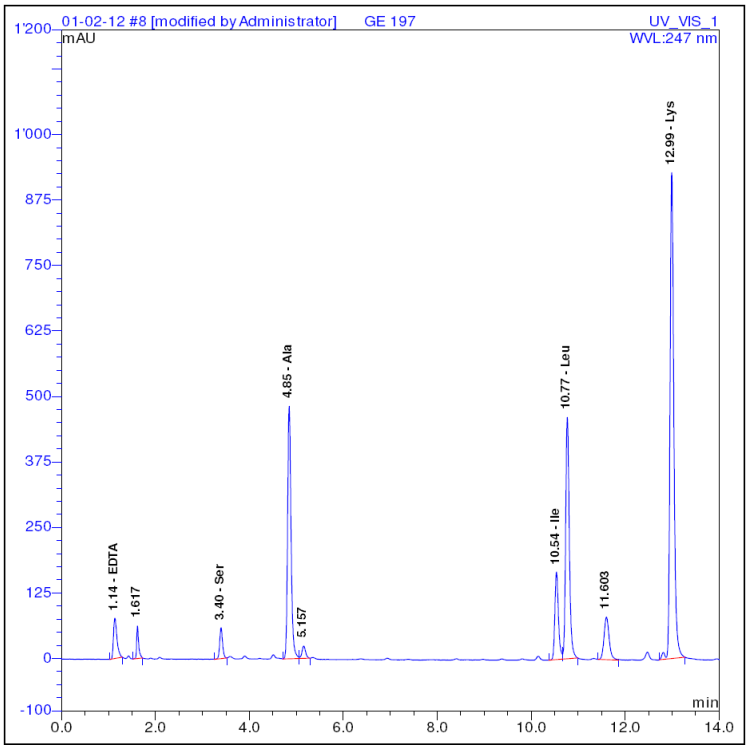
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

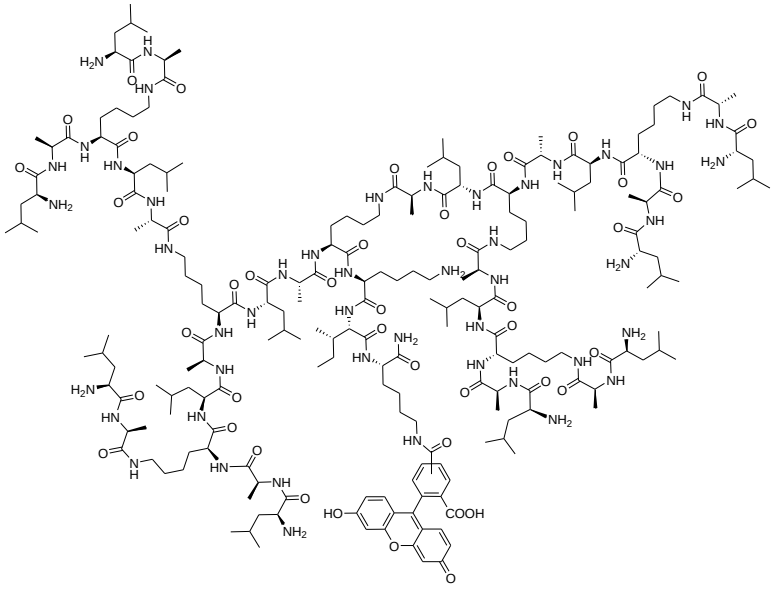


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.14	1.14	0.077	6.57	76.60		340.03	EDTA
3.40	3.34	0.064	4.11	58.67		343.98	Ser
4.85	4.86	0.076	40.92	481.92		2680.73	Ala
10.54	10.56	0.079	14.66	167.01		932.47	Ile
10.77	10.80	0.081	41.56	460.42		2687.27	Leu
12.99	13.02	0.084	87.22	927.35		3000.36	Lys
Total:						9984.83	

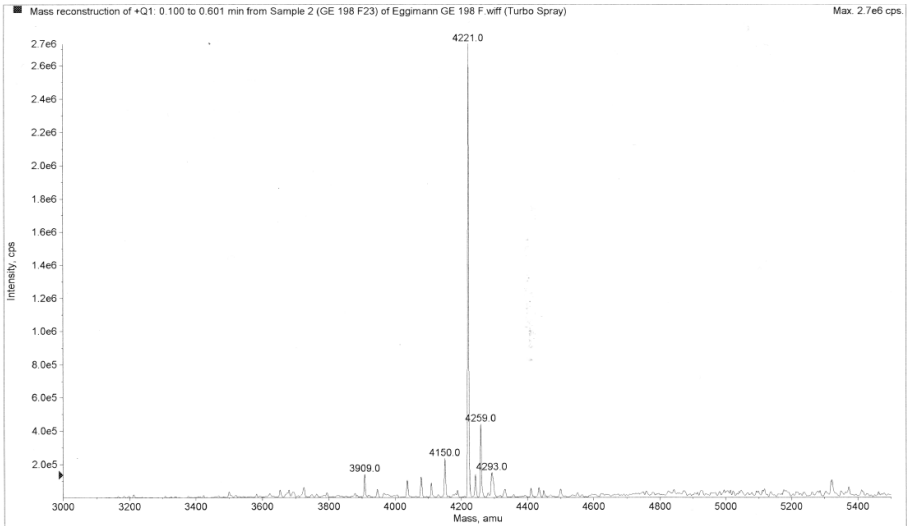
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	2680.7	12	11.0
Ile	932.5	3	3.8
Leu	2687.3	12	11.0
Lys	3000.4	11	12.3

CPPD10 ((LA)$_8$(*K*LA)$_4$(*K*LA)$_2$*K*KIK*).

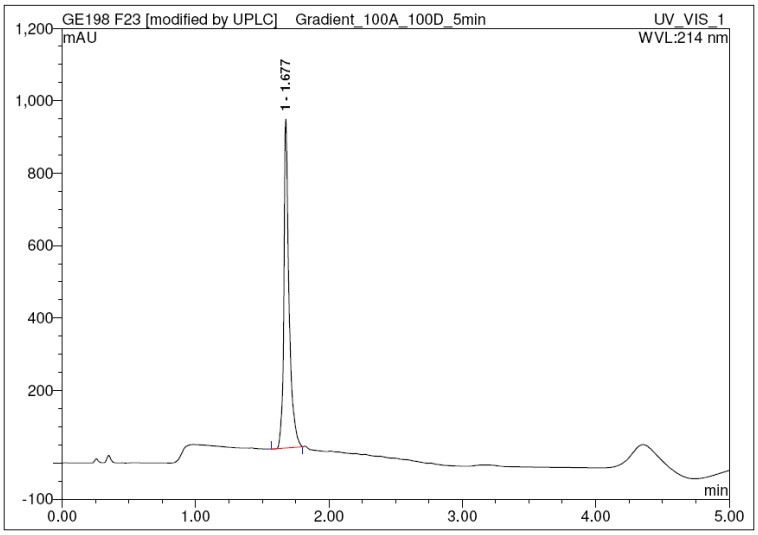
From Tenta Gel S RAM® resin (200 mg, 0.25 mmol·g^{-1}), **CPPD10** was obtained as a foamy yellow solid after preparative RP-HPLC (12.3 mg, 2.3 µmol, 3%). Analytical RP-HPLC: t_R = 1.68 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{207}H_{356}N_{48}O_{44}$ calc/found 4221.3/4221.0 $[M]^+$.



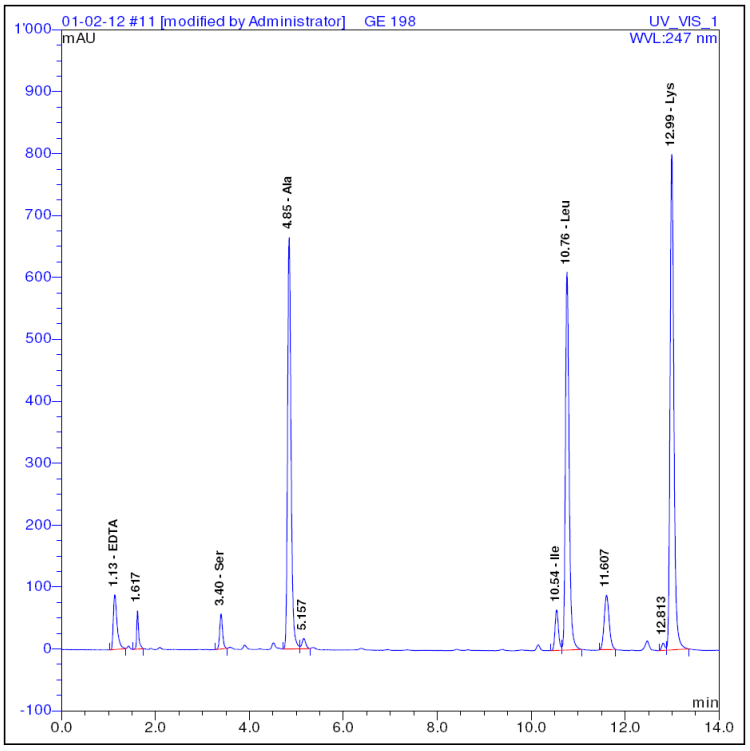
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

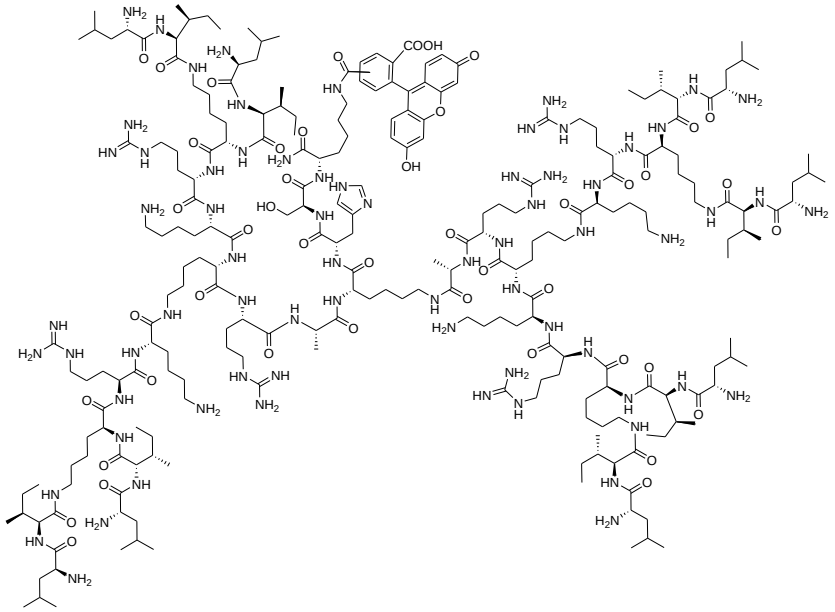


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.13	1.14	0.080	8.18	88.42		392.47	EDTA
3.40	3.34	0.064	4.02	56.67		332.24	Ser
4.85	4.86	0.077	56.85	664.41		3695.85	Ala
10.54	10.56	0.078	5.60	65.50		365.72	Ile
10.76	10.80	0.082	55.92	610.28		3561.90	Leu
12.99	13.02	0.083	74.88	800.78		2590.85	Lys
Total:						10939.03	

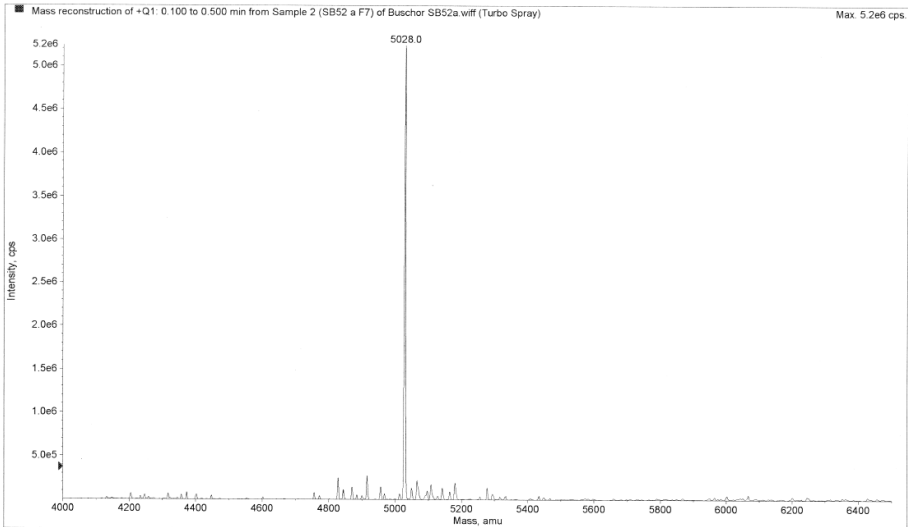
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	3695.9	14	13.8
Ile	365.7	1	1.4
Leu	3561.9	14	13.3
Lys	2590.9	9	9.6

CPPD11 ((LI)$_8$(*K*RK)$_4$(*K*RA)$_2$*K*HSK*).

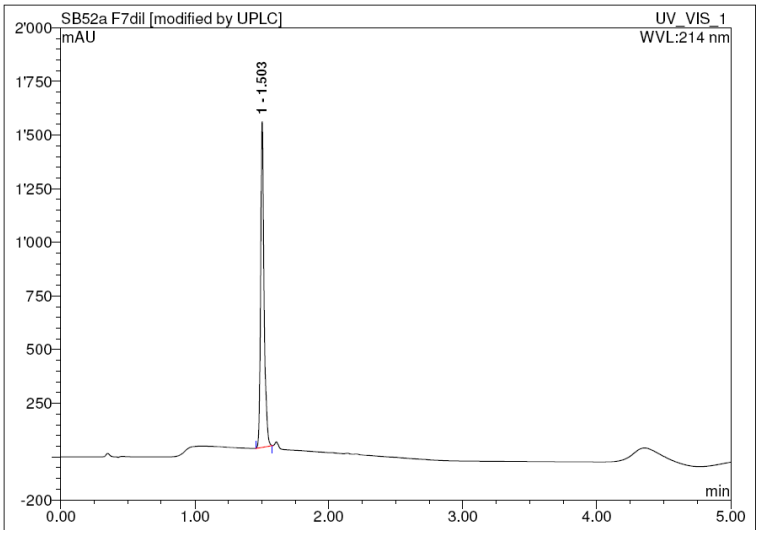
From Tenta Gel S RAM® resin (500 mg, 0.22 mmol·g^{-1}), **CPPD11** was obtained as a foamy yellow solid after preparative RP-HPLC (15.0 mg, 2.1 µmol, 2%). Analytical RP-HPLC: t_R = 1.50 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{240}H_{427}N_{71}O_{45}$ calc/found 5027.4/5028.0 $[M]^+$.



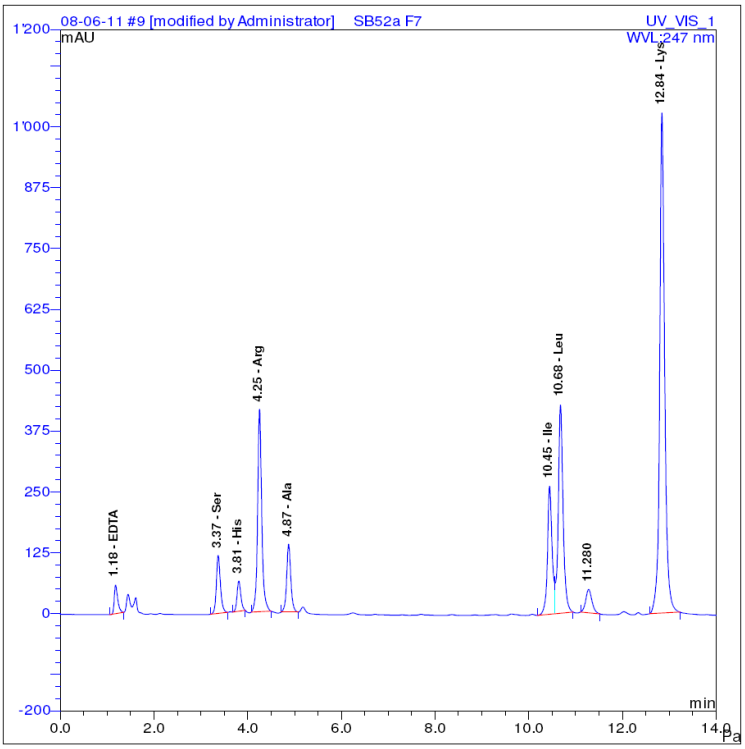
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

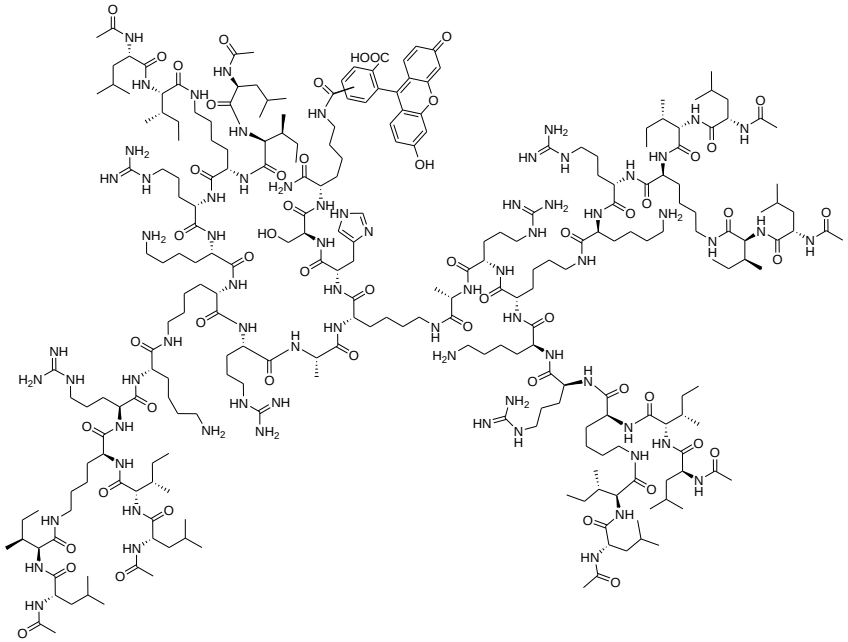


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.18	1.18	0.075	5.01	58.75		524.91	EDTA
3.37	3.37	0.088	11.98	119.13		898.68	Ser
3.81	3.79	0.085	5.77	61.87		484.51	His
4.25	4.23	0.087	42.49	416.04		3168.09	Arg
4.87	4.85	0.090	14.33	139.40		1041.68	Ala
10.45	10.44	0.100	30.50	262.78		2057.07	Ile
10.68	10.67	0.101	50.96	427.76		3459.09	Leu
12.84	12.85	0.104	127.84	1027.67		4429.65	Lys
Total:						16063.68	

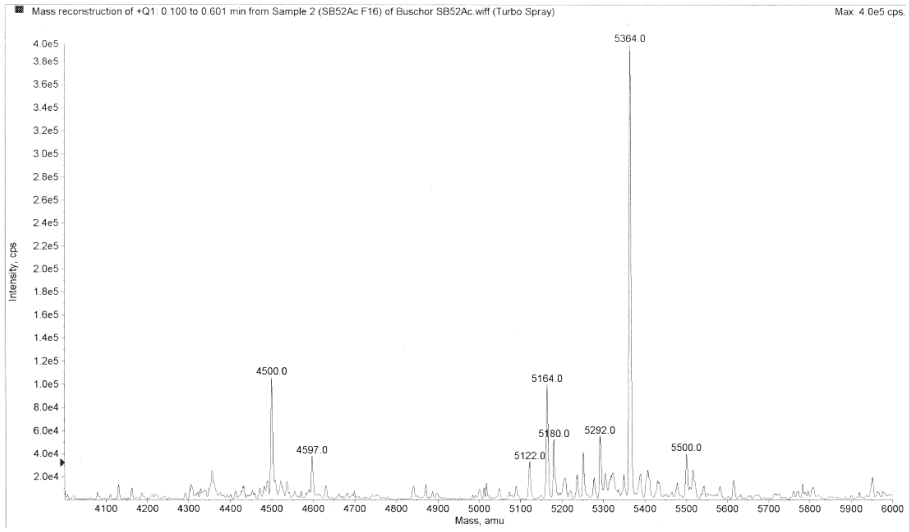
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1041.7	2	2.6
Arg	3168.1	6	7.8
His	484.5	1	1.2
Ile	2057.1	8	5.0
Leu	3459.1	8	8.5
Lys	4429.7	12	10.8
Ser	898.7	1	2.2

CPPD11Ac ((AcLI)$_8$(*K*RK)$_4$(*K*RA)$_2$*K*HSK*).

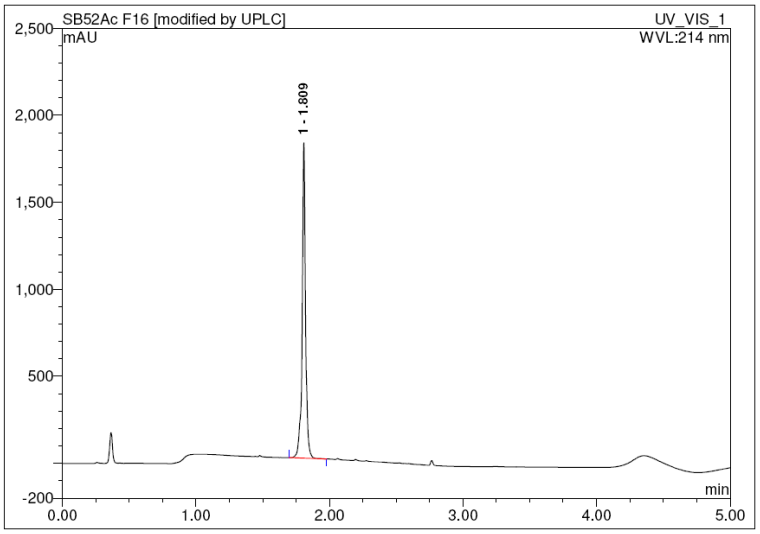
From Tenta Gel S RAM® resin (100 mg, 0.22 mmol·g^{-1}), **CPPD11Ac** was obtained as a foamy yellow solid after preparative RP-HPLC (7.0 mg, 1.1 µmol, 5%). Analytical RP-HPLC: t_R = 1.81 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{256}H_{443}N_{71}O_{53}$ calc/found 5363.7/5364.0 $[M]^+$.



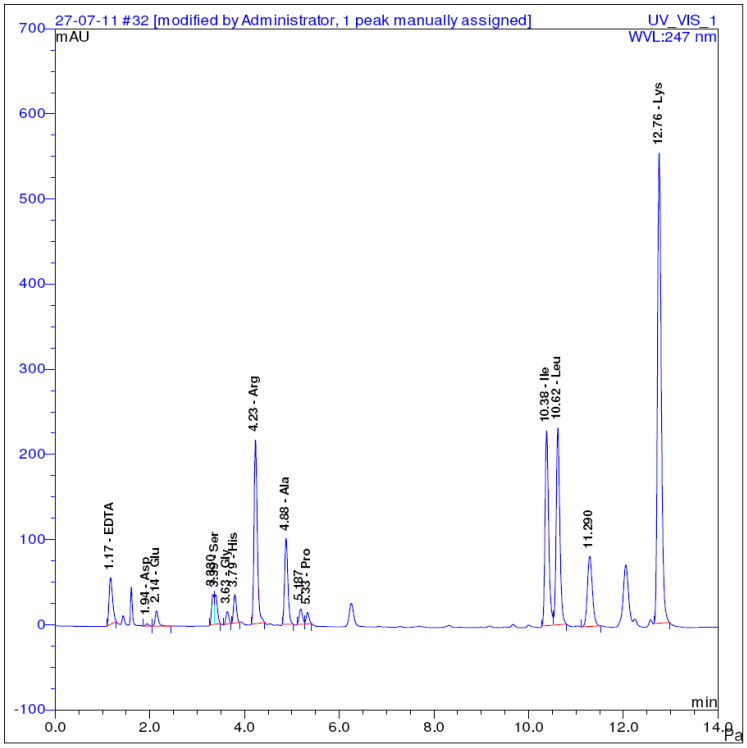
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

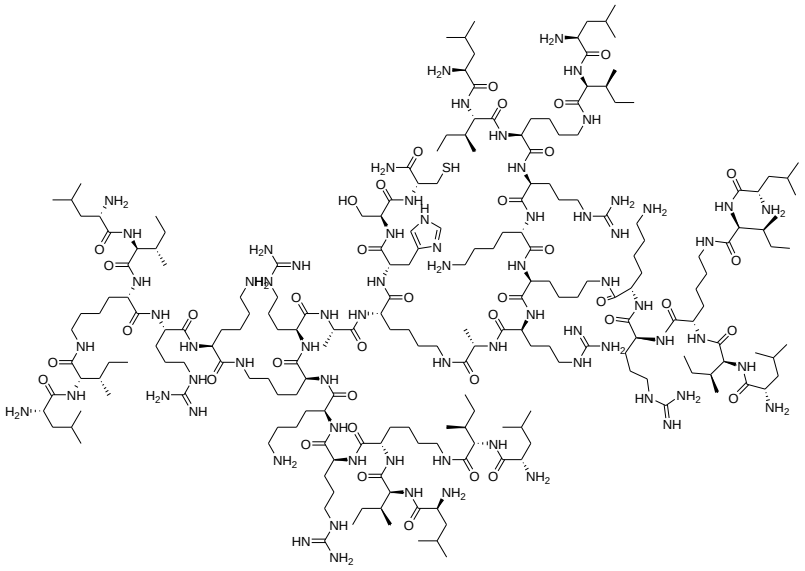


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.17	1.17	0.081	4.73	54.71		420.19	EDTA
1.94	1.94	0.062	0.18	2.65		13.61	Asp
2.14	2.14	0.067	1.41	17.80		97.46	Glu
3.39	3.40	n.a.	2.49	34.74		206.44	Ser
3.63	3.64	0.067	1.00	14.39		82.42	Gly
3.79	3.80	0.069	2.40	33.36		208.71	His
4.23	4.23	0.071	17.11	215.59		1298.85	Arg
4.88	4.88	0.074	8.04	100.82		591.57	Ala
5.33	5.34	0.068	0.97	13.34		66.44	Pro
10.38	10.42	0.080	20.34	228.71		1382.36	Ile
10.62	10.65	0.081	20.71	231.27		1426.58	Leu
12.76	12.79	0.085	51.13	552.18		1891.82	Lys
Total:						7686.44	

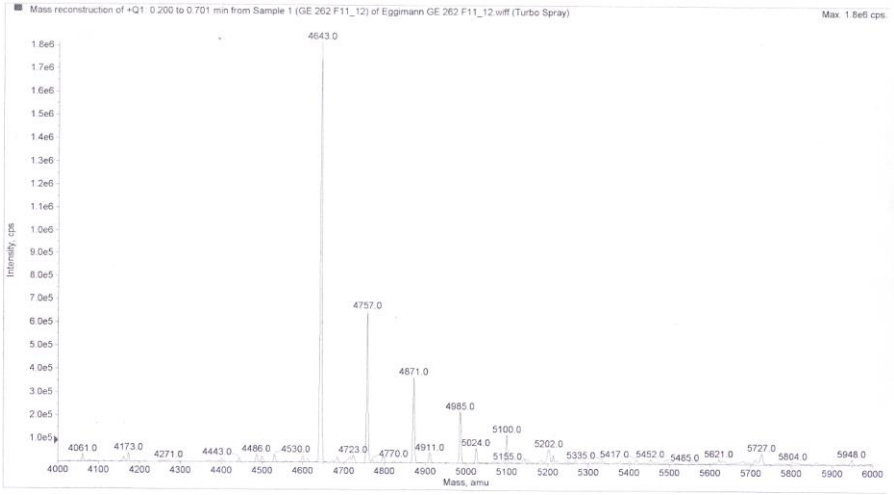
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	591.6	2	3.2
Arg	1298.9	6	7.0
His	208.7	1	1.1
Ile	1382.4	8	7.5
Leu	1426.6	8	7.7
Lys	1891.8	12	10.3
Ser	206.4	1	1.1

CPPD11-Cys ((LI)$_8$(*K*RK)$_4$(*K*RA)$_2$*K*HSC).

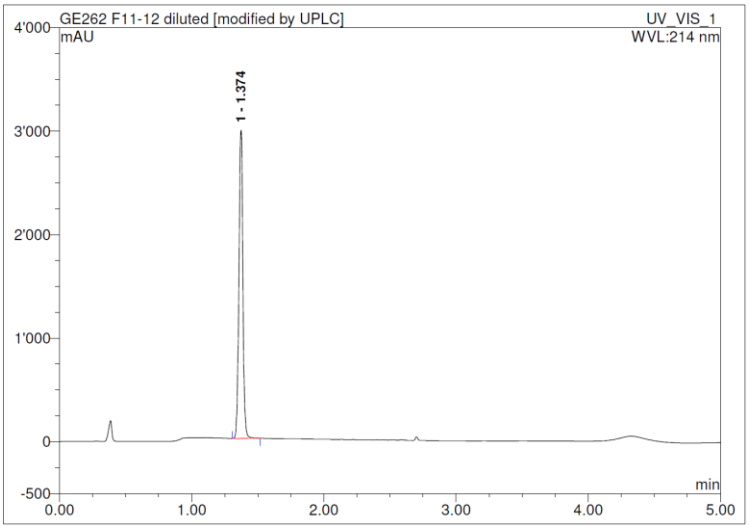
From Tenta Gel S RAM® resin (300 mg, 0.23 mmol·g^{-1}), **CPPD11-Cys** was obtained as a foamy colourless solid after preparative RP-HPLC (48.8 mg, 7.3 µmol, 11%). Analytical RP-HPLC: t_R = 1.37 min (A/D 100/0 to 0/100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{216}H_{410}N_{70}O_{39}S$ calc/found 4644.1/4643.0 $[M]^+$; 4758.1/4757.0 $[M + 1\ TFA]^+$; 4872.1/4871.0 $[M + 2\ TFA]^+$; 4986.1/4985.0 $[M + 3\ TFA]^+$; 5100.2/5100.0 $[M + 4\ TFA]^+$.



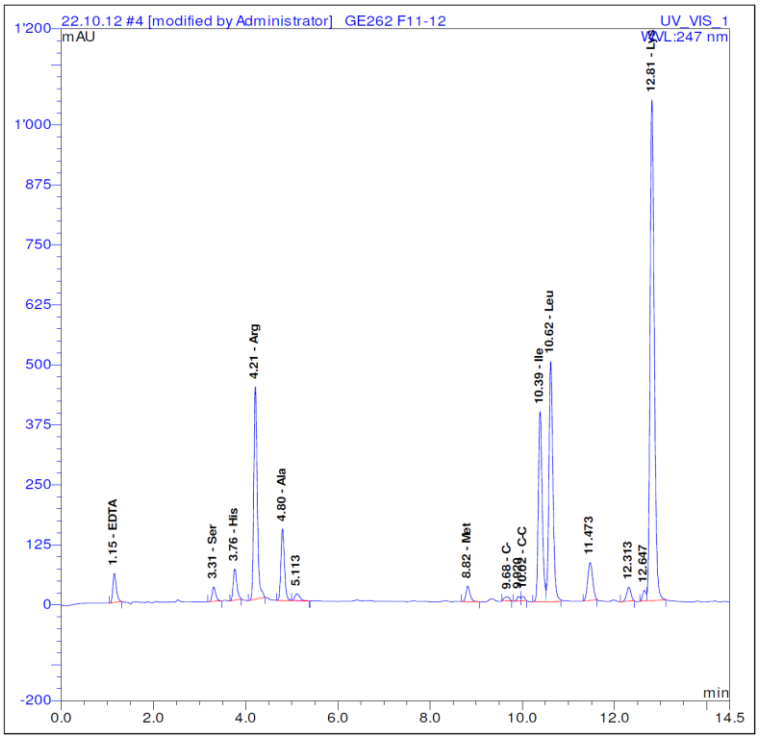
Mass spectrum, MS (ESI+):



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
1.15	1.15	0.078	5.21	60.27		384.14	EDTA
3.31	3.31	0.081	2.70	30.15		198.91	Ser
3.76	3.77	0.082	5.90	64.99		458.96	His
4.21	4.22	0.079	38.81	442.86		2822.12	Arg
4.80	4.82	0.082	13.53	149.60		918.05	Ala
8.82	8.75	0.090	3.20	31.91		223.75	Met
9.68	9.65	0.136	1.10	8.55		43.73	C-
10.02	9.96	n.a.	0.82	9.95		50.87	C-C
10.39	10.44	0.089	38.49	396.43		2438.57	Ile
10.62	10.67	0.091	50.11	499.89		3207.45	Leu
12.81	12.85	0.097	110.25	1043.63		4105.22	Lys
Total:						14851.76	

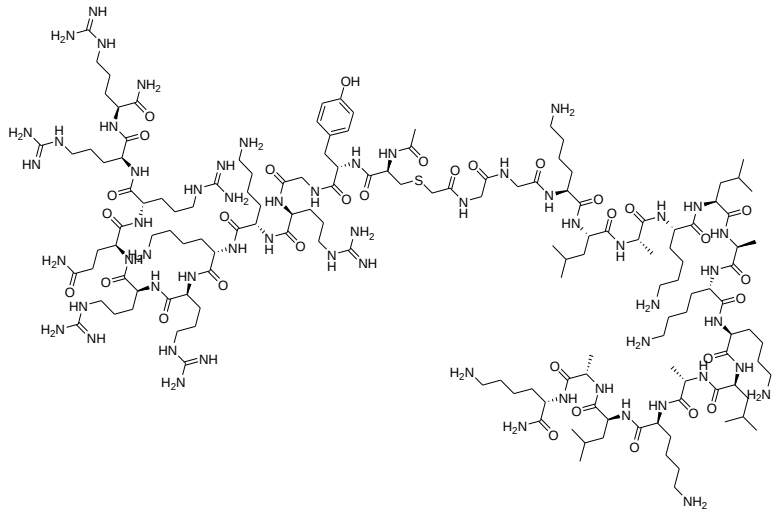
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	918.1	2	2.4
Arg	2822.1	6	7.3
His	459.0	1	1.2
Ile	2438.6	8	6.3
Leu	3207.5	8	8.3
Lys	4105.2	11	10.7
Ser	198.9	1	0.5

Thioether Ligation.

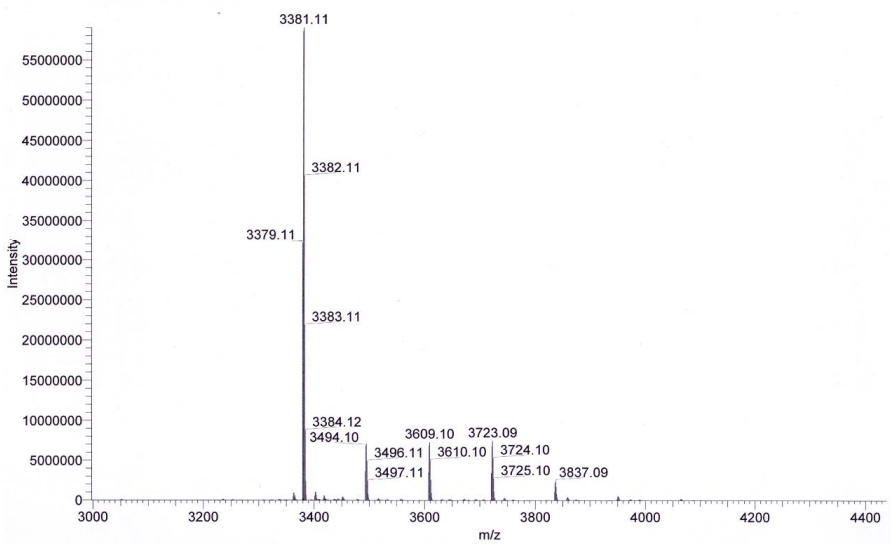
x denotes the S-CH_2-CO- bridge between cysteine side-chain and the acetylated N-terminus of the linear peptide **ClAc-KLA**.

(Tat/KLA)x (AcC(x-GG[KLAKLAK]$_2$)YGRKKRRQRRR).

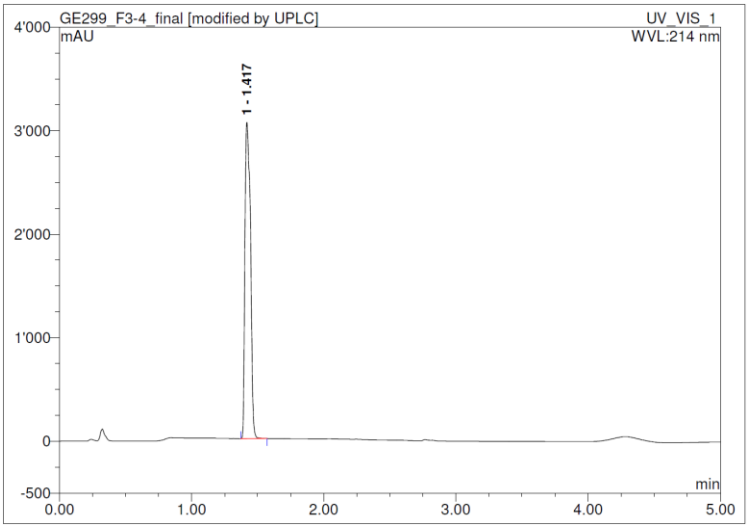
From starting materials **Cys-Tat** and **ClAc-KLA** using the general procedure described (solvent: DMF/H2O (1/1, v/v)), **(Tat/ClAc-KLA)x** was obtained as a foamy colourless solid after preparative RP-HPLC (2.7 mg, 0.6 µmol, yield 48%). Analytical RP-HPLC: t_R = 1.42 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{147}H_{271}N_{57}O_{32}S$ calc/found 3381.2/3381.1 $[M]^+$; 3495.2/3494.1 $[M + 1\ TFA]^+$; 3609.2/3609.1 $[M + 2\ TFA]^+$; 3723.2/3723.1 $[M + 3\ TFA]^+$; 3837.2/3837.1 $[M + 4\ TFA]^+$.



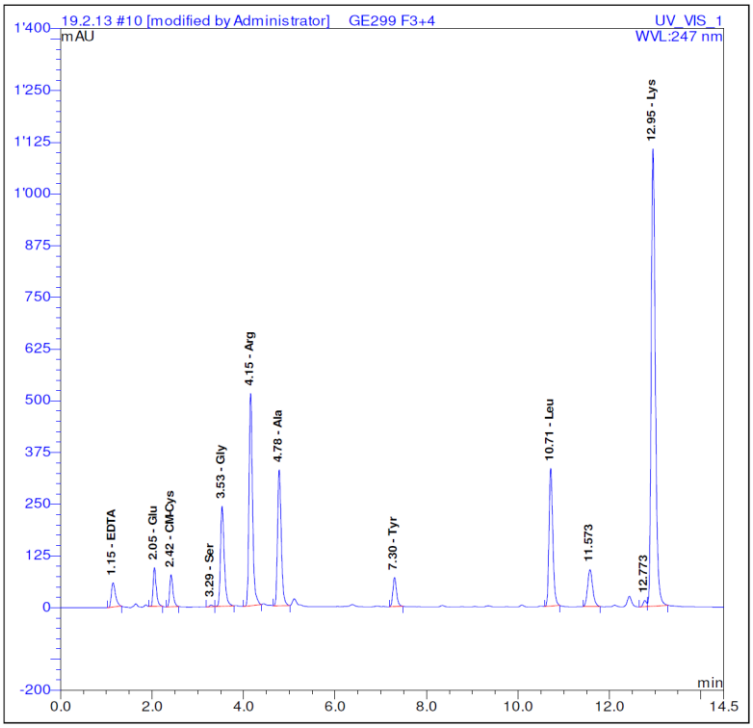
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



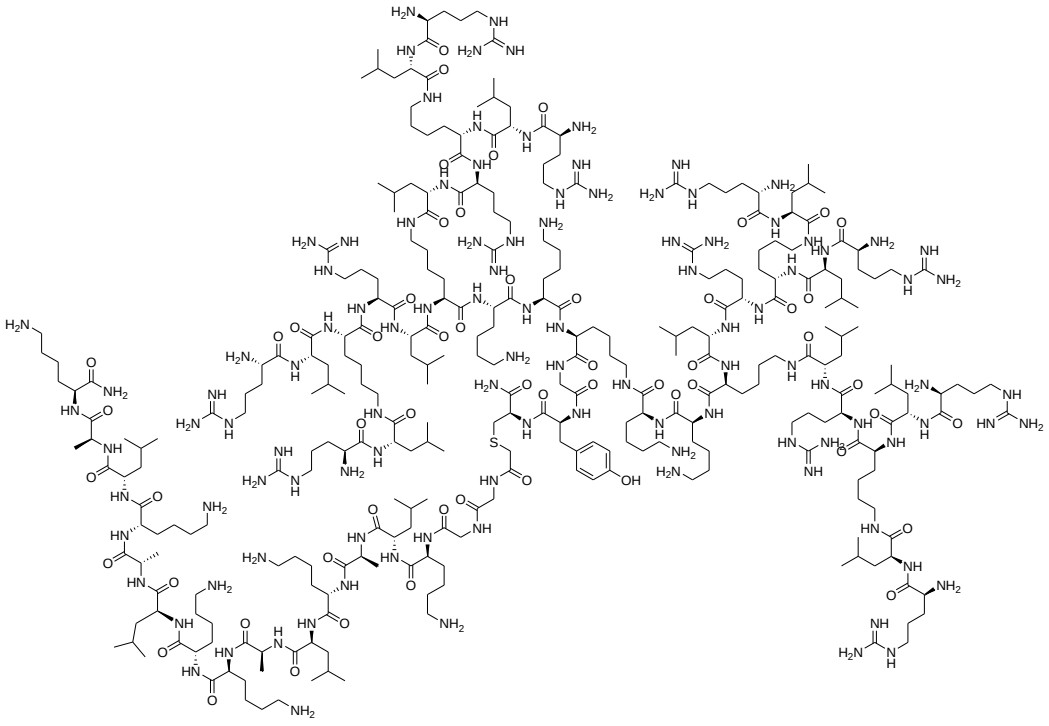
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.19	0.095	6.12	58.46		512.19	EDTA
2.05	2.06	0.071	7.42	93.43		645.68	Glu
2.42	2.42	0.074	6.41	77.38		482.38	CM-Cys
3.29	3.31	0.076	0.33	4.16		33.87	Ser
3.53	3.55	0.085	23.25	242.11		1857.73	Gly
4.15	4.17	0.079	45.47	513.19		3751.96	Arg
4.78	4.81	0.085	30.91	328.42		2381.28	Ala
7.30	7.35	0.085	6.60	70.18		528.15	Tyr
10.71	10.76	0.087	31.68	332.50		2504.16	Leu
12.95	12.98	0.096	116.54	1105.33		5103.67	Lys
Total:						17801.07	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	2381.3	4	3.9
Arg	3752.0	6	6.1
Cys[a)]	482.4	1	0.8
Gln[b)]	645.7	1	1.1
Gly	1857.7	3	3.0
Leu	2504.2	4	4.1
Lys	5103.7	8	8.3
Tyr	528.2	1	0.9

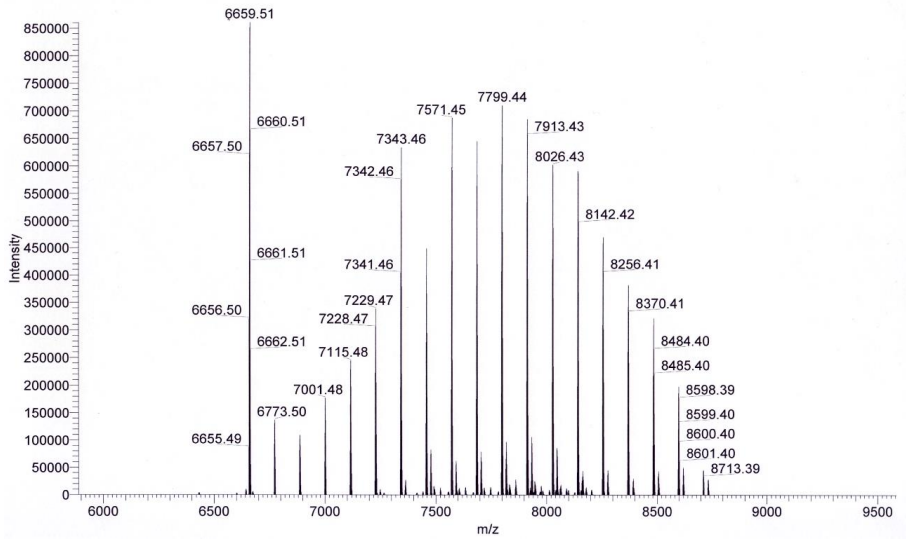
[a)] Detected as carboxymethyl cysteine (CMCys). [b)] Detected as glutamic acid.

(CPPD1/KLA)x ((RL)$_8$(*K*RL)$_4$(*K*KK)$_2$*K*GYC(x-GG[KLAKLAK]$_2$)).

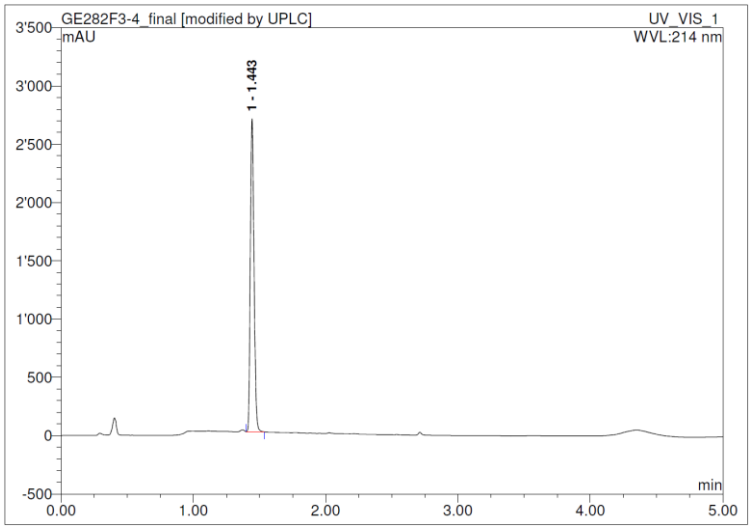
From starting materials **CPPD1-Cys** and **ClAc-KLA** using the general procedure described (solvent: DMF/H2O (1/1, v/v)), **(CPPD1/ClAc-KLA)x** was obtained as a foamy colourless solid after preparative RP-HPLC (2.5 mg, 0.3 µmol, yield 38%). Analytical RP-HPLC: t_R = 1.44 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{302}H_{573}N_{109}O_{56}S$ calc/found 6659.5/6659.5 $[M]^+$; 6773.6/6773.5 [M + 1 TFA]$^+$; 7001.6/7001.5 [M + 3 TFA]$^+$; 7115.6/7115.5 [M + 4 TFA]$^+$; 7229.6/7229.5 [M + 5 TFA]$^+$; 7343.7/7343.5 [M + 6 TFA]$^+$; 7571.7/7571.5 [M + 8 TFA]$^+$; 7799.7/7799.4 [M + 10 TFA]$^+$; 7913.8/7913.4 [M + 11 TFA]$^+$; 8027.8/8026.4 [M + 12 TFA]$^+$.



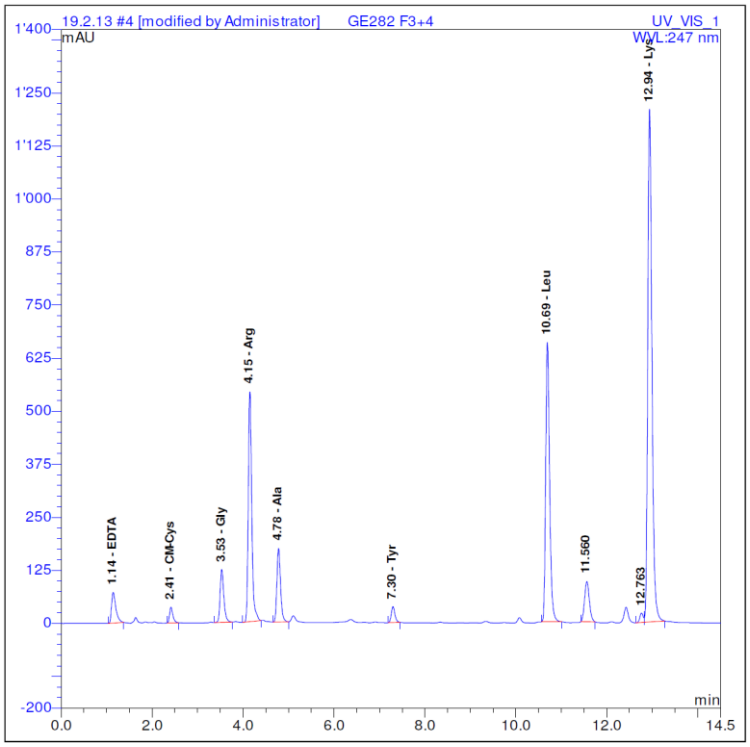
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



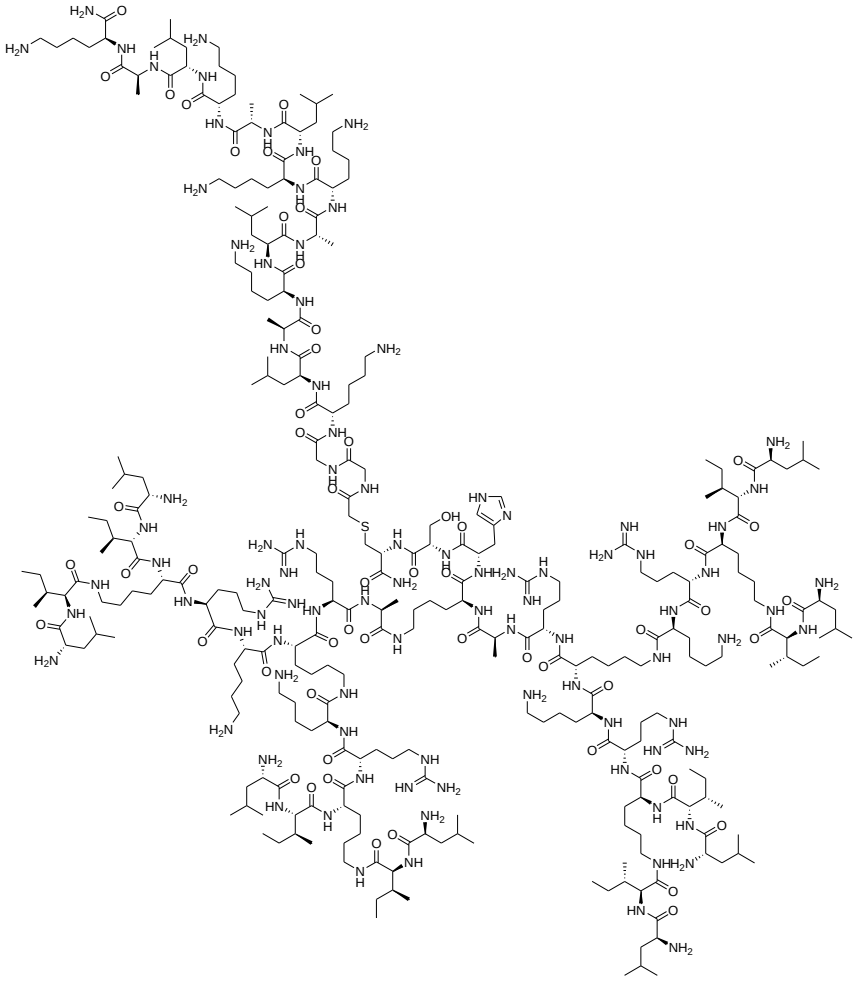
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.14	1.19	0.098	7.95	72.23		632.87	EDTA
2.41	2.42	0.074	3.00	36.34		226.53	CM-Cys
3.53	3.55	0.084	11.89	124.99		959.01	Gly
4.15	4.17	0.078	47.85	541.92		3961.97	Arg
4.78	4.81	0.084	16.03	173.23		1256.00	Ala
7.30	7.35	0.084	3.42	37.42		281.60	Tyr
10.69	10.76	0.090	65.74	658.55		4959.79	Leu
12.94	12.98	0.097	129.32	1208.19		5663.48	Lys
Total:						17941.25	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1256.0	4	3.9
Arg	3962.0	12	12.4
Cys[a)]	226.5	1	0.7
Gly	959.0	3	3.0
Leu	4959.8	16	15.5
Lys	5663.5	17	17.7
Tyr	281.6	1	0.9

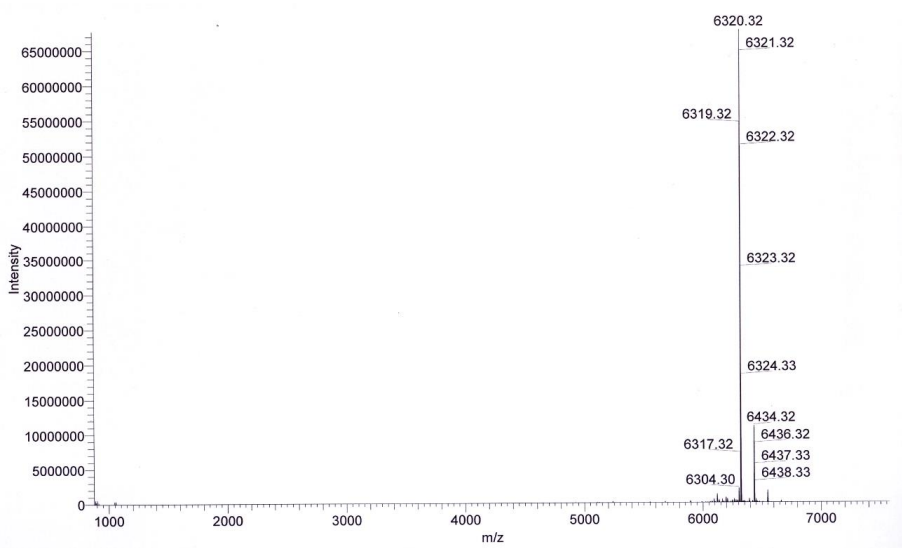
[a)] Detected as carboxymethyl cysteine (CMCys).

(CPPD11/KLA)x ((LI)$_8$(*K*RK)$_4$(*K*RA)$_2$*K*HSC(x-GG[KLAKLAK]$_2$)).

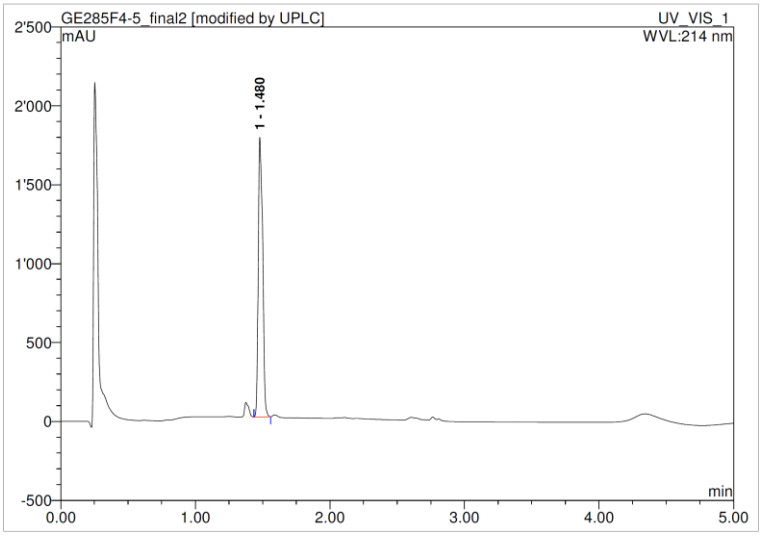
From starting materials **CPPD11-Cys** and **ClAc-KLA** using the general procedure described (solvent: DMF/H2O (1/1, v/v)), **(CPPD11/ClAc-KLA)x** was obtained as a foamy colourless solid after preparative RP-HPLC (2.4 mg, 0.3 µmol, yield 30%). Analytical RP-HPLC: t_R = 1.48 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm; traces of DMF detected). HRMS (NSI+): $C_{294}H_{555}N_{93}O_{56}S$ calc/found 6321.2/6320.3 $[M]^+$.



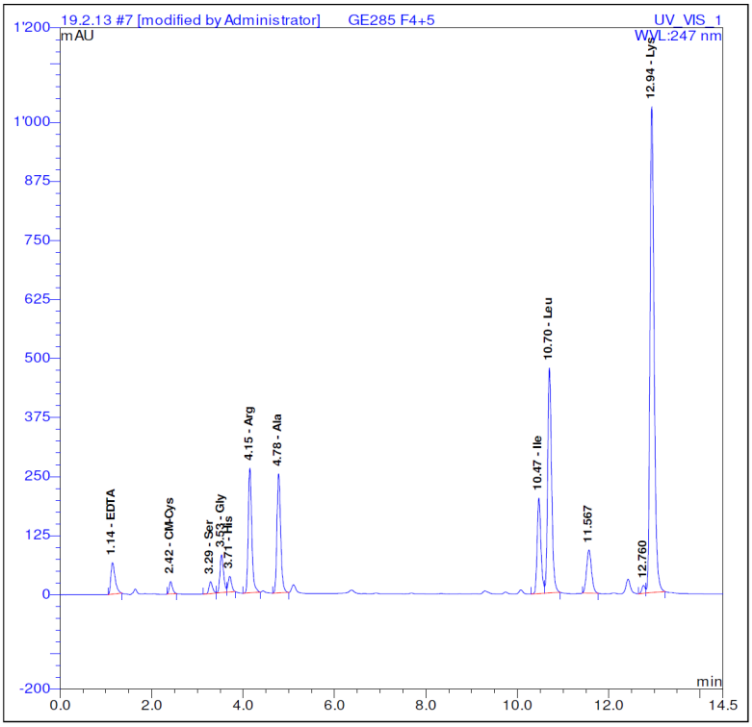
Mass spectrum, HRMS (NSI+):



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
1.14	1.19	0.097	7.22	66.82		585.46	EDTA
2.42	2.42	0.074	2.09	26.03		162.26	CM-Cys
3.29	3.31	0.081	2.19	24.87		202.59	Ser
3.53	3.55	0.085	7.32	79.97		613.61	Gly
3.71	3.73	0.082	2.91	33.18		264.04	His
4.15	4.17	0.080	23.55	264.01		1930.15	Arg
4.78	4.81	0.086	23.81	251.96		1826.89	Ala
10.47	10.52	0.086	19.22	202.47		1493.93	Ile
10.70	10.76	0.090	47.30	476.29		3587.10	Leu
12.94	12.98	0.096	108.62	1028.97		4757.01	Lys
Total:						15423.03	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1826.9	6	6.7
Arg	1930.2	6	7.0
Cys[a)]	162.3	1	0.6
Gly	613.6	2	2.2
His	264.0	1	1.0
Ile	1493.9	8	5.4
Leu	3587.1	12	13.1
Lys	4757.0	17	17.3
Ser	202.6	1	0.7

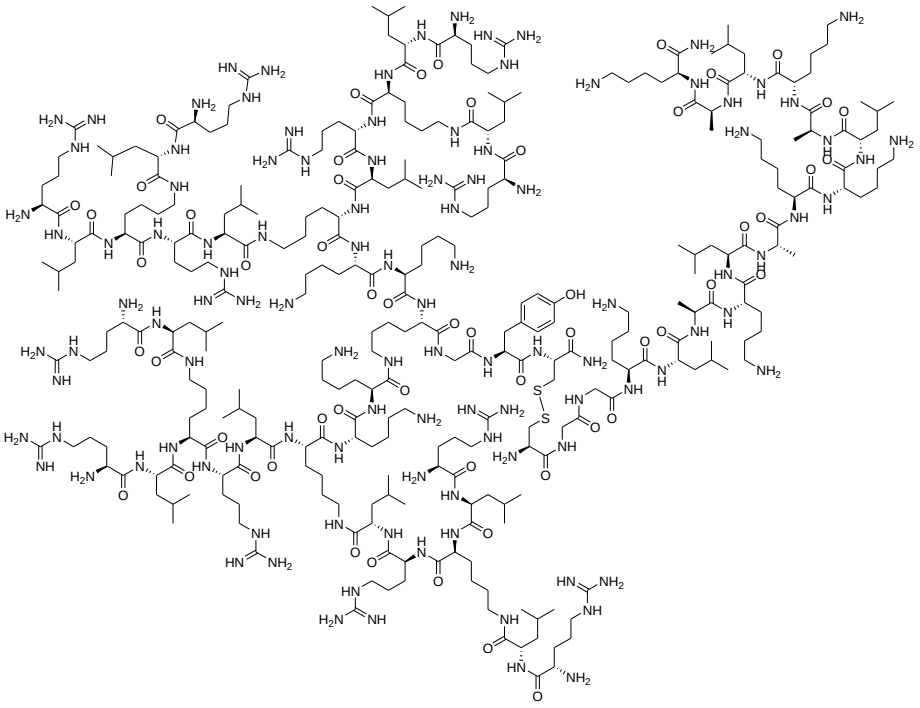
[a)] Detected as carboxymethyl cysteine (CMCys).

Heterodimers.

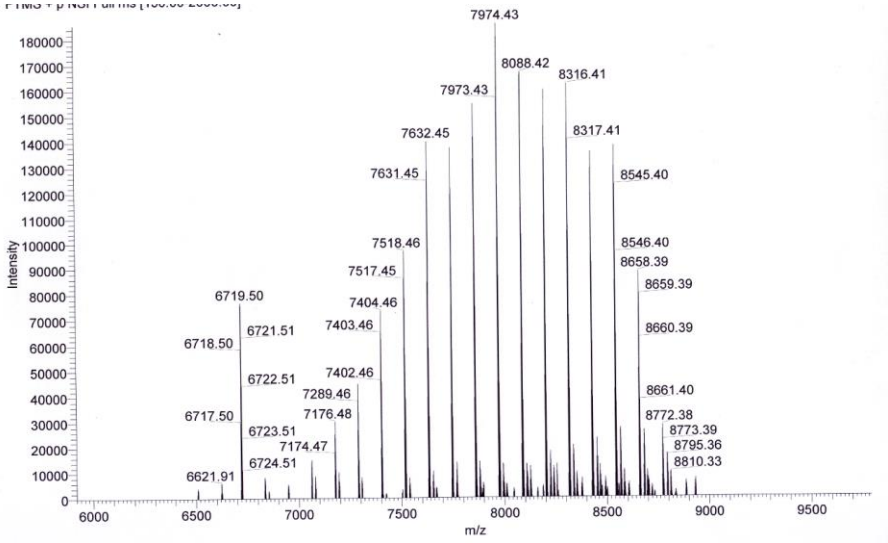
z denotes the disulfide bridge between two cysteine side-chains.

(CPPD1/KLA)z ((RL)$_8$(*K*RL)$_4$(*K*KK)$_2$*K*GYC(z-CGG[KLAKLAK]$_2$)).

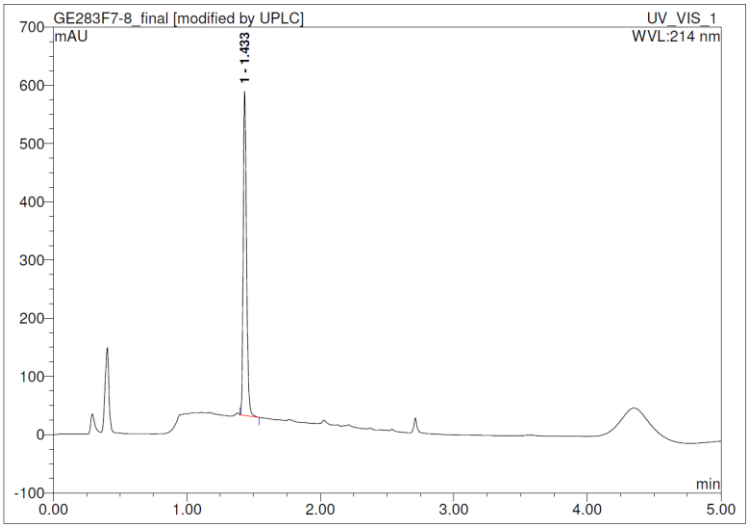
From starting materials **CPPD1-Cys** and **Cys-KLA** using the general procedure described, **(CPPD1/KLA)z** was obtained as a foamy colourless solid after preparative RP-HPLC (0.4 mg, 0.04 µmol, yield 6%). Analytical RP-HPLC: t_R = 1.43 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{303}H_{576}N_{110}O_{56}S_2$ calc/found 6720.7/6719.5 $[M]^+$; 7176.7/7176.5 $[M + 4\ TFA]^+$; 7404.8/7404.5 $[M + 6\ TFA]^+$; 7518.8/7518.5 $[M + 7\ TFA]^+$; 7632.8/7632.5 $[M + 8\ TFA]^+$; 7974.9/7974.4 $[M + 11\ TFA]^+$; 8088.9/8088.4 $[M + 12\ TFA]^+$; 8316.9/8316.4 $[M + 14\ TFA]^+$; 8659.0/8658.4 $[M + 17\ TFA]^+$; 8773.0/8772.4 $[M + 18\ TFA]^+$.



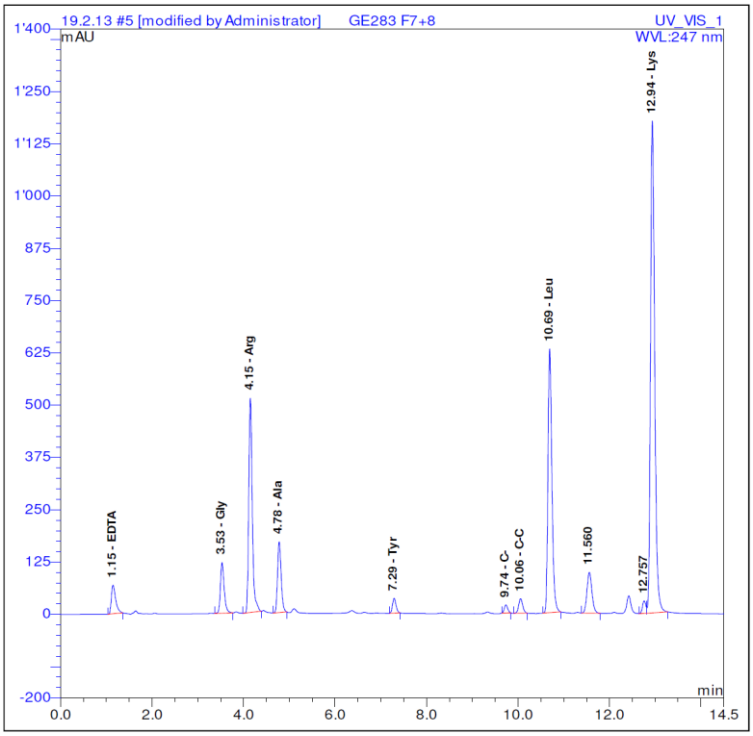
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

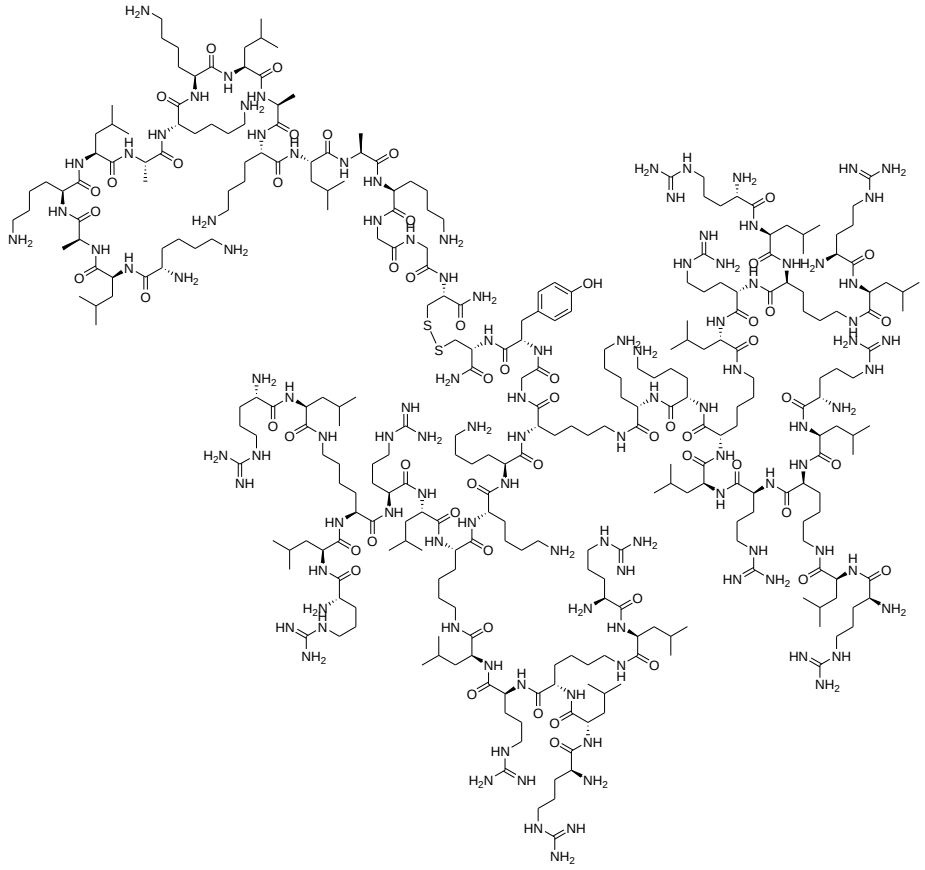


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.19	0.097	7.43	68.41		599.39	EDTA
3.53	3.55	0.085	11.60	121.19		929.87	Gly
4.15	4.17	0.078	45.41	512.27		3745.21	Arg
4.78	4.81	0.084	15.40	169.00		1225.39	Ala
7.29	7.35	0.083	3.14	35.25		265.31	Tyr
9.74	9.79	0.081	1.66	19.36		96.43	C-
10.06	10.09	0.095	3.60	35.09		174.79	C-C
10.69	10.76	0.090	62.66	630.54		4748.79	Leu
12.94	12.98	0.097	125.24	1176.06		5484.59	Lys
Total:						17269.77	

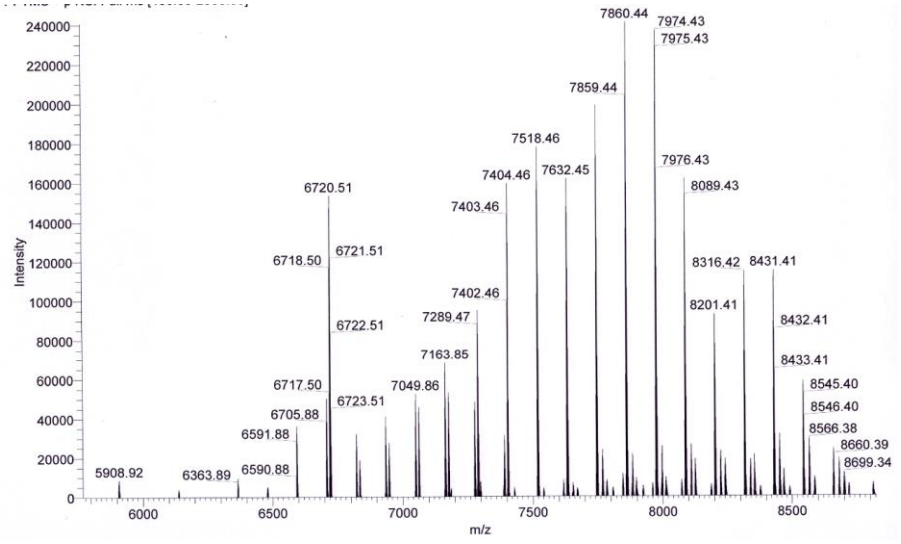
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1225.4	4	4.0
Arg	3745.2	12	12.1
C-	96.4	1	0.9
C-C	174.8		
Gly	929.9	3	3.0
Leu	4748.8	16	15.4
Lys	5484.6	17	17.8
Tyr	265.3	1	0.9

(KLA/CPPD1)z ((RL)$_8$(*K*RL)$_4$(*K*KK)$_2$*K*GYC(z-[KLAKLAK]$_2$GGC)).

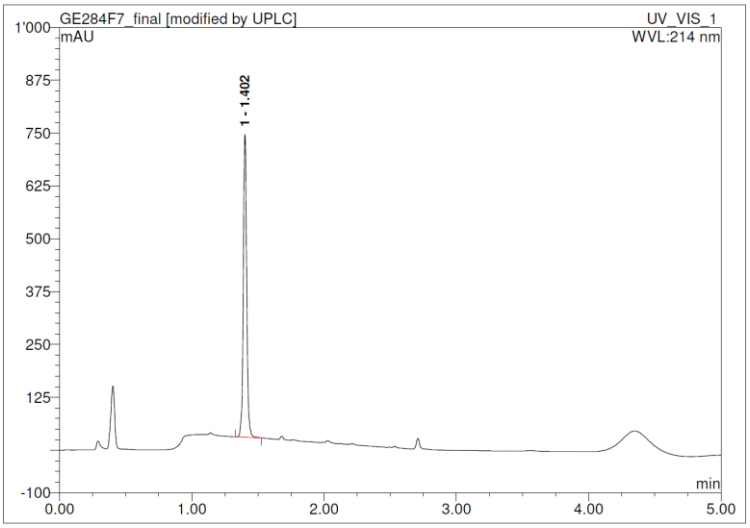
From starting materials **CPPD1-Cys** and **KLA-Cys** using the general procedure described, **(KLA/CPPD1)z** was obtained as a foamy colourless solid after preparative RP-HPLC (0.5 mg, 0.05 µmol, yield 8%). Analytical RP-HPLC: t_R = 1.40 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{303}H_{576}N_{110}O_{56}S_2$ calc/found 6720.7/6720.5 $[M]^+$; 7404.8/7404.5 $[M + 6\ TFA]^+$; 7518.8/7518.5 $[M + 7\ TFA]^+$; 7632.5/7632.5 $[M + 8\ TFA]^+$; 7860.9/7860.4 $[M + 10\ TFA]^+$; 7974.9/7974.4 $[M + 11\ TFA]^+$; 8202.9/8201.4 $[M + 13\ TFA]^+$; 8316.9/8316.4 $[M + 14\ TFA]^+$; 8431.0/8431.4 $[M + 15\ TFA]^+$.



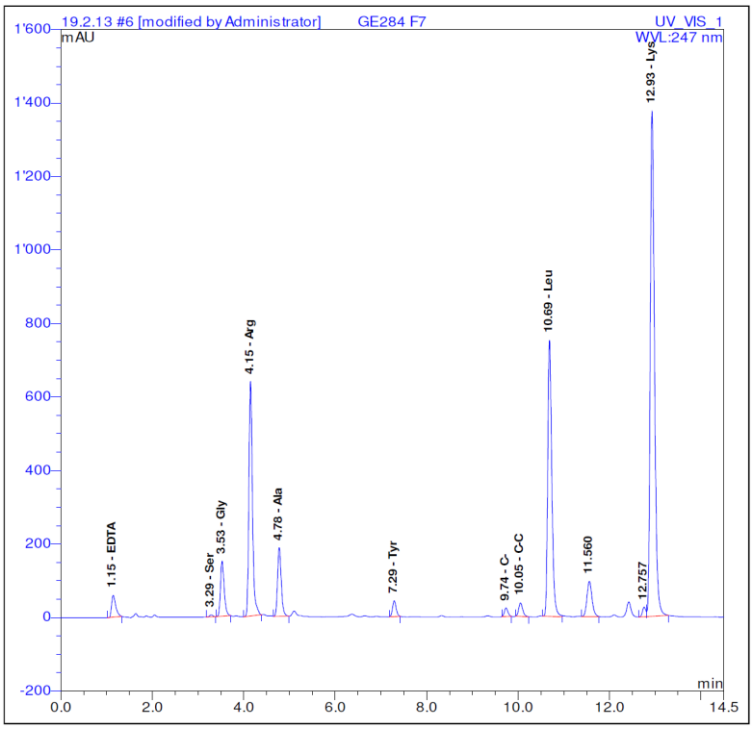
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

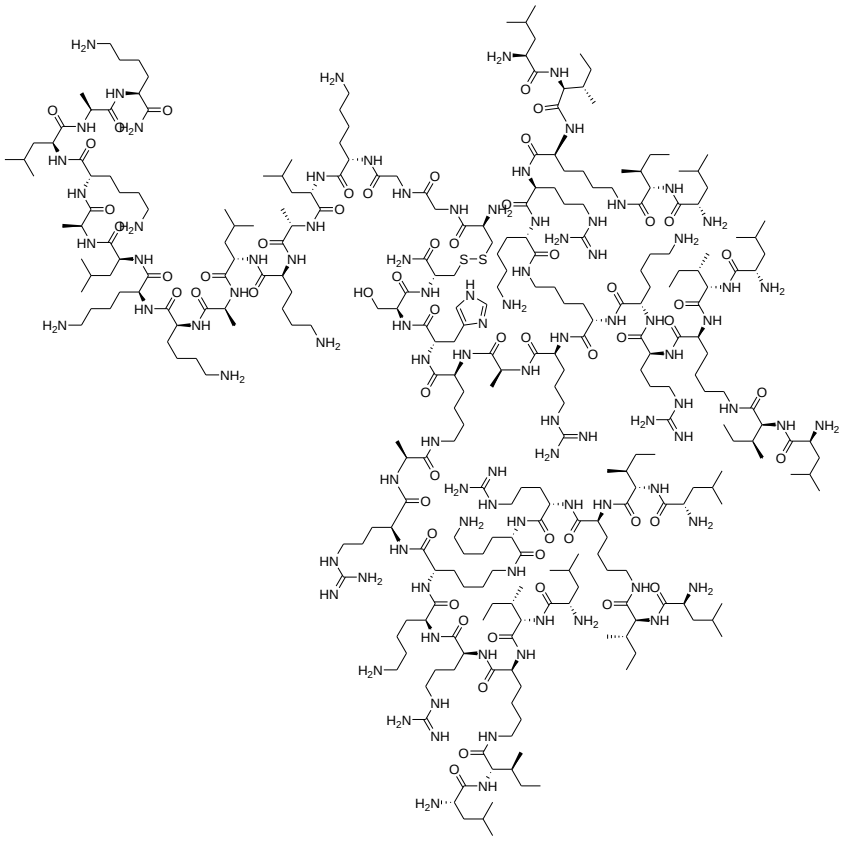


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.19	0.095	6.15	58.97		516.72	EDTA
3.29	3.31	0.076	0.43	5.39		43.91	Ser
3.53	3.55	0.085	13.97	149.55		1147.52	Gly
4.15	4.17	0.079	56.96	638.19		4665.83	Arg
4.78	4.81	0.085	17.44	186.80		1354.40	Ala
7.29	7.35	0.085	3.94	43.05		323.97	Tyr
9.74	9.79	0.083	2.04	22.91		114.10	C-
10.05	10.09	0.094	3.73	36.56		182.13	C-C
10.69	10.76	0.092	76.81	751.49		5659.73	Leu
12.93	12.98	0.099	149.31	1375.24		6538.84	Lys
Total:						20547.15	

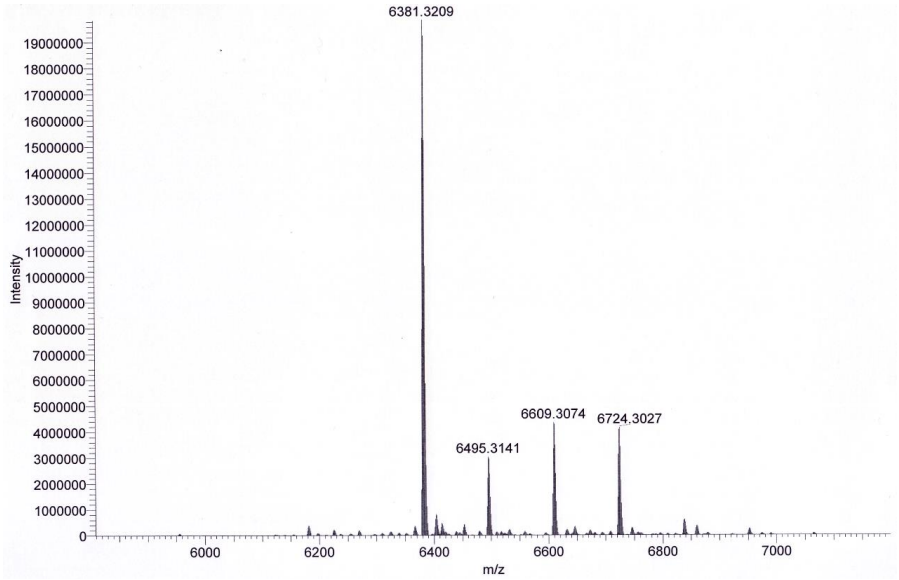
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1354.4	4	3.7
Arg	4665.8	12	12.6
C-	114.1	1	0.8
C-C	182.1		
Gly	1147.5	3	3.1
Leu	5659.7	16	15.3
Lys	6538.8	17	17.7
Tyr	324.0	1	0.9

(CPPD11/KLA)z ((LI)$_8$(*K*RK)$_4$(*K*RA)$_2$*K*HSC(z-CGG[KLAKLAK]$_2$)).

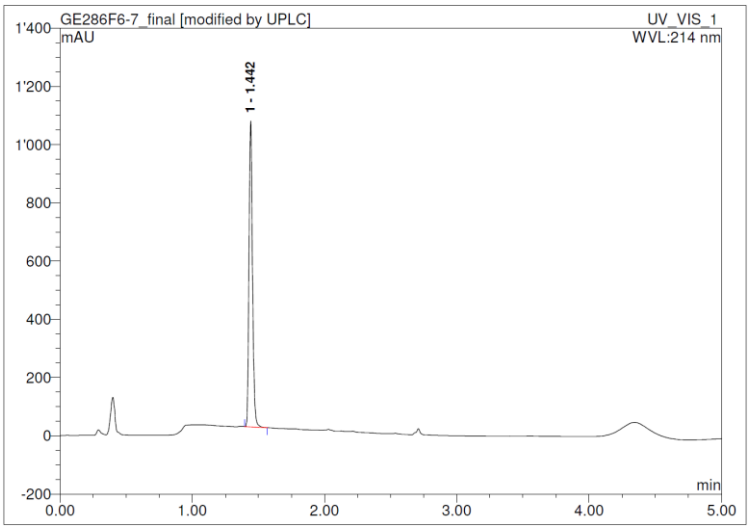
From starting materials **CPPD11-Cys** and **Cys-KLA** using the general procedure described, **(CPPD11/KLA)z** was obtained as a foamy colourless solid after preparative RP-HPLC (0.7 mg, 0.08 µmol, yield 10%). Analytical RP-HPLC: t_R = 1.44 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{295}H_{558}N_{94}O_{56}S_2$ calc/found 6382.3/6381.3 $[M]^+$; 6496.3/6495.3 $[M + 1\ TFA]^+$; 6610.3/6609.3 $[M + 2\ TFA]^+$; 6724.4/6724.3 $[M + 3\ TFA]^+$.



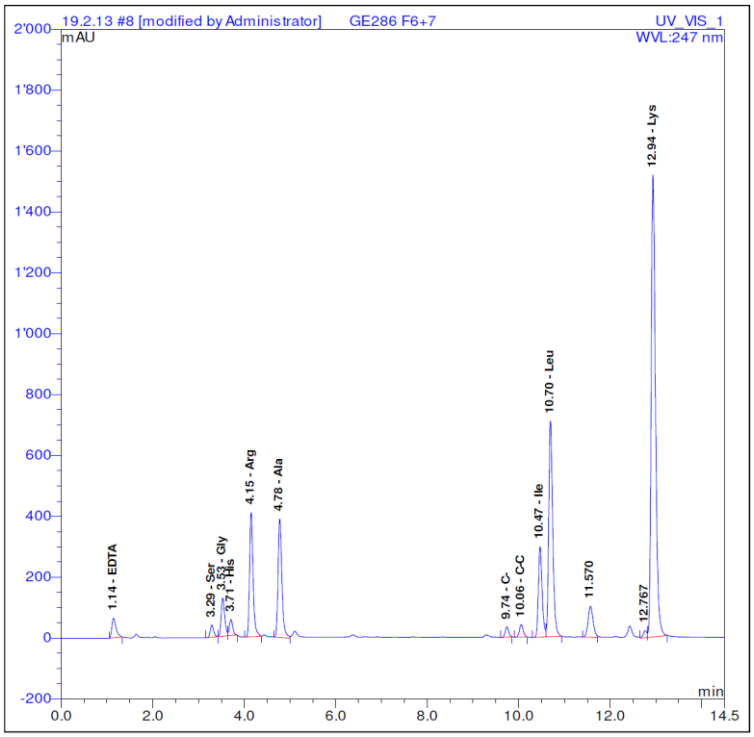
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

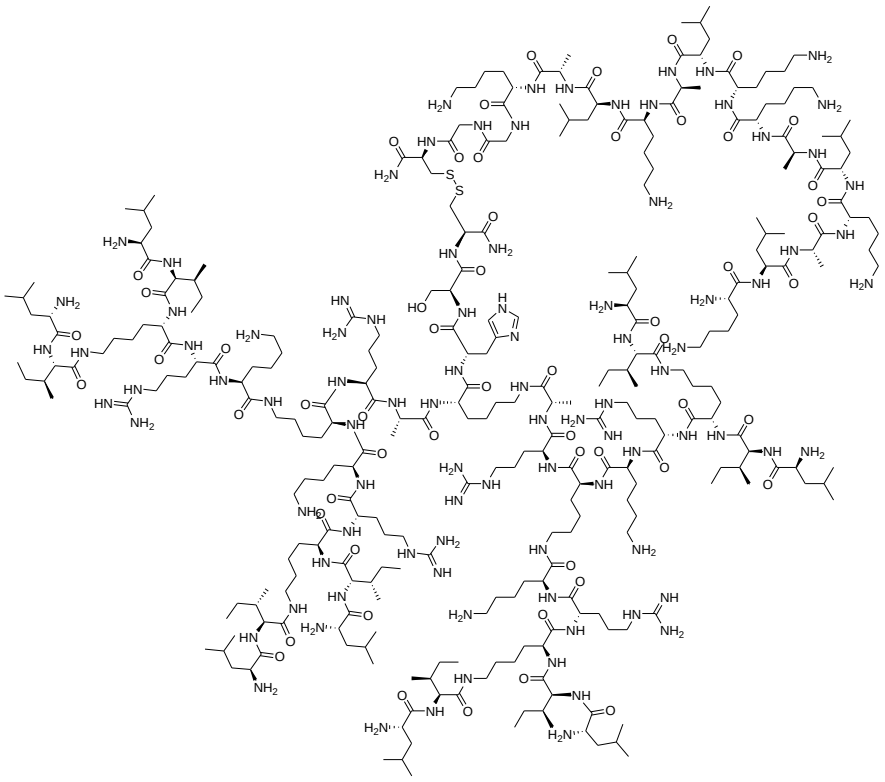


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.14	1.19	0.095	6.83	64.23		562.76	EDTA
3.29	3.31	0.080	3.46	39.83		324.47	Ser
3.53	3.55	0.083	11.28	125.63		963.96	Gly
3.71	3.73	0.080	4.56	52.89		420.95	His
4.15	4.17	0.079	36.10	408.13		2983.82	Arg
4.78	4.81	0.086	37.55	388.53		2817.11	Ala
9.74	9.79	0.083	2.98	33.37		166.21	C-
10.06	10.09	0.090	4.04	41.49		206.67	C-C
10.47	10.52	0.086	27.99	296.42		2187.14	Ile
10.70	10.76	0.091	71.54	708.22		5333.86	Leu
12.94	12.98	0.099	164.70	1516.49		7212.56	Lys
Total:						23179.50	

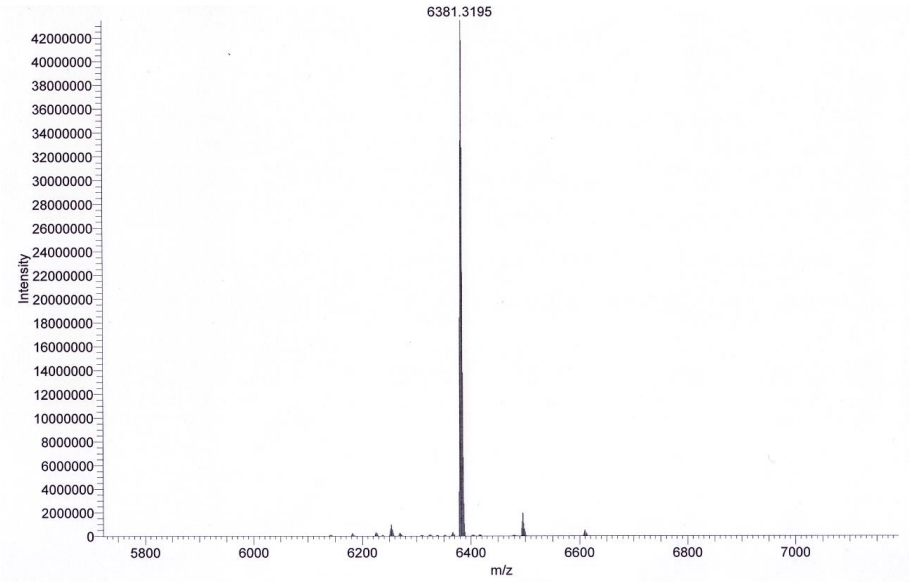
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	2817.1	6	6.7
Arg	2983.8	6	7.1
C-	166.2	1	0.9
C-C	206.7		
Gly	964.0	2	2.3
His	421.0	1	1.0
Ile	2187.1	8	5.2
Leu	5333.9	12	12.7
Lys	7212.6	17	17.2
Ser	324.5	1	0.8

(KLA/CPPD11)z ((LI)$_8$(*K*RK)$_4$(*K*RA)$_2$*K*HSC(z-[KLAKLAK]$_2$GGC)).

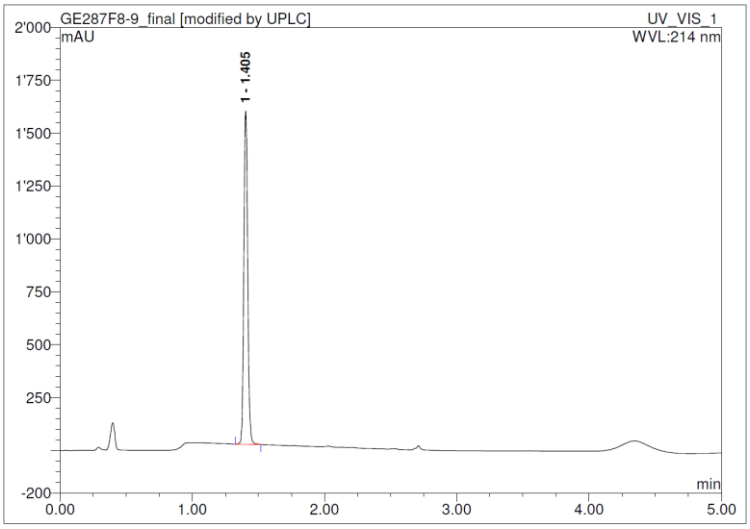
From starting materials **CPPD11-Cys** and **KLA-Cys** using the general procedure described, **(KLA/CPPD11)z** was obtained as a foamy colourless solid after preparative RP-HPLC (1.3 mg, 0.1 µmol, yield 17%). Analytical RP-HPLC: t_R = 1.41 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{295}H_{558}N_{94}O_{56}S_2$ calc/found 6382.3/6381.3 $[M]^+$.



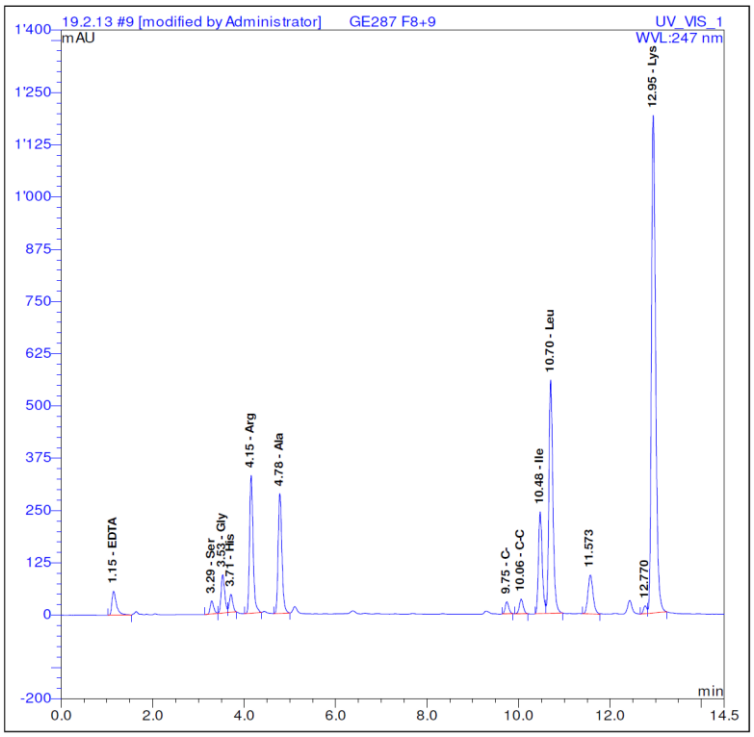
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:



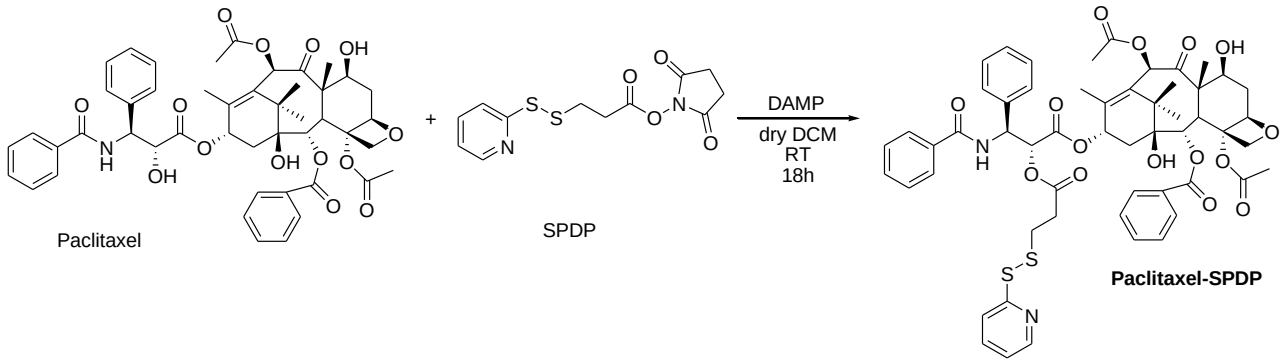
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.19	0.098	6.67	57.12		500.48	EDTA
3.29	3.31	0.081	2.74	31.08		253.16	Ser
3.53	3.55	0.085	8.37	91.82		704.51	Gly
3.71	3.73	0.082	3.78	42.92		341.57	His
4.15	4.17	0.080	29.39	329.35		2407.85	Arg
4.78	4.81	0.087	27.26	286.91		2080.28	Ala
9.75	9.79	0.083	2.56	28.51		142.01	C-
10.06	10.09	0.091	3.45	35.02		174.45	C-C
10.48	10.52	0.086	23.00	243.11		1793.79	Ile
10.70	10.76	0.091	55.89	558.04		4202.82	Leu
12.95	12.98	0.097	126.81	1190.27		5553.20	Lys
Total:						18154.11	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	2080.3	6	6.4
Arg	2407.9	6	7.4
C-	142.0	1	1.0
C-C	174.5		
Gly	704.5	2	2.2
His	341.6	1	1.0
Ile	1793.8	8	5.5
Leu	4202.8	12	12.9
Lys	5553.2	17	17.0
Ser	253.2	1	0.8

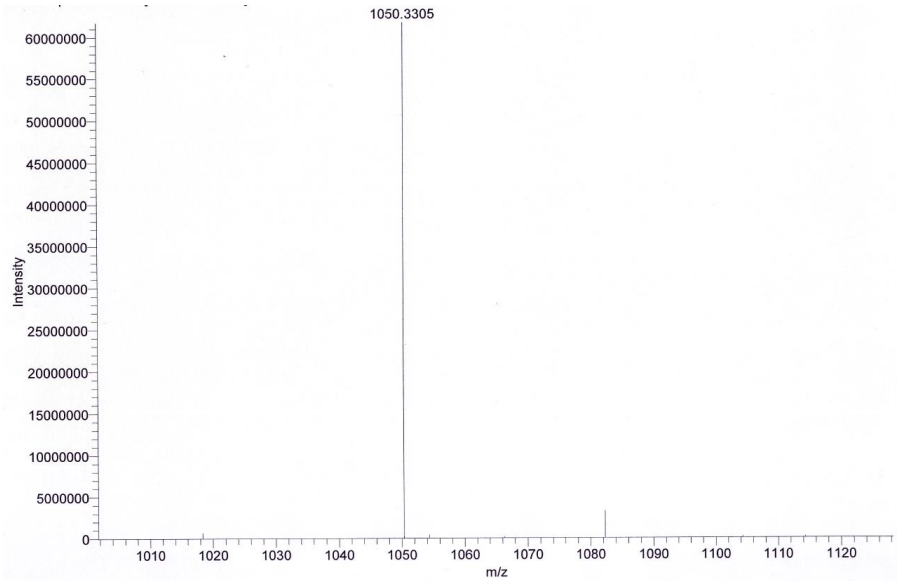
2.3 Paclitaxel conjugates.

Paclitaxel-SPDP.

From starting materials **Paclitaxel** and **SPDP** using the general procedure described, **Paclitaxel-SPDP** was obtained as amorphous solid after column flash chromatography (7.8 mg, 7.4 µmol, yield 28%). HRMS (NSI+): $C_{55}H_{58}N_2O_{15}S_2$ calc/found 1051.2/1050.3 $[M]^+$.

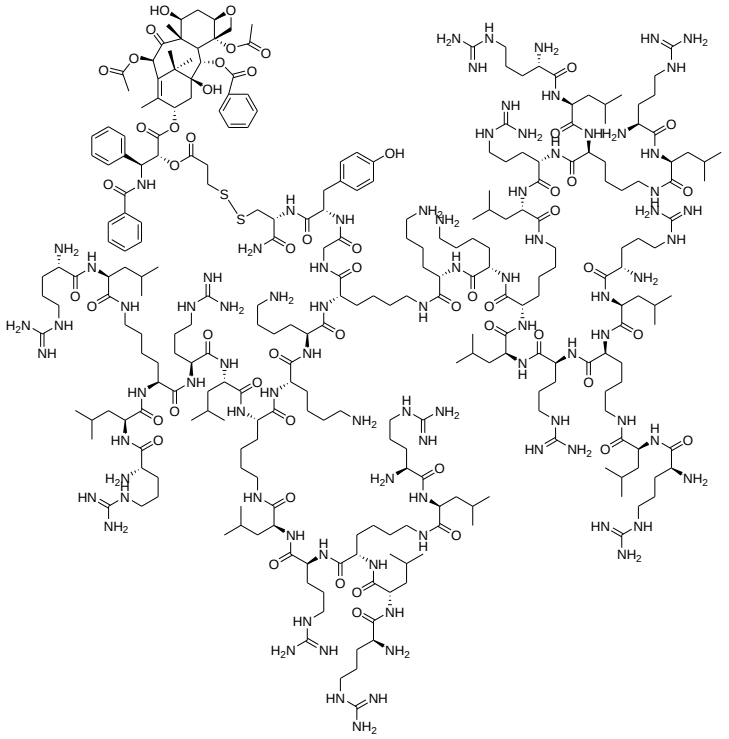


Mass spectrum, HRMS (NSI+):

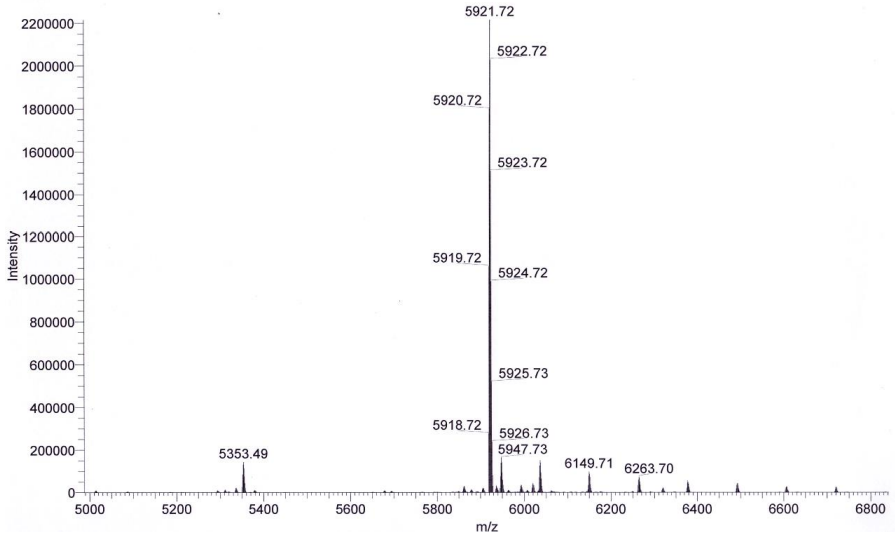


CPPD1-Paclitaxel.

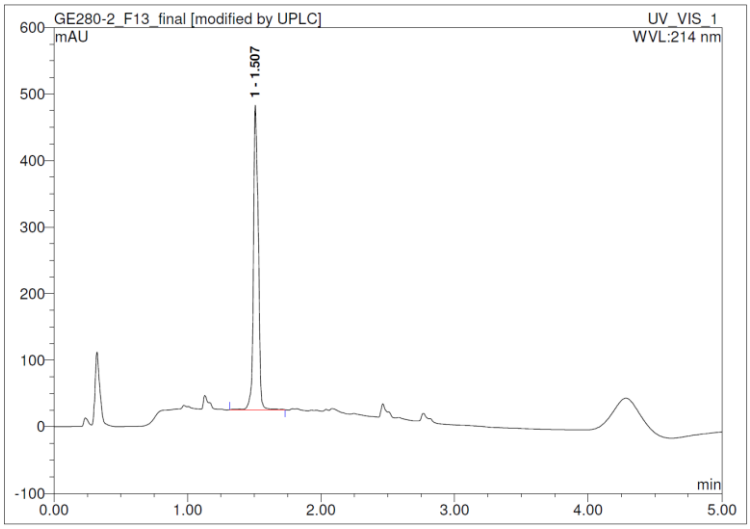
From starting materials **CPPD1-Cys** and **Paclitaxel-SPDP** using the general procedure described, **CPPD1-Paclitaxel** was obtained as a colourless solid after preparative RP-HPLC (0.2 mg, 0.03 µmol, yield 5%). Analytical RP-HPLC: t_R = 1.51 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{274}H_{481}N_{87}O_{54}S_2$ calc/found 5922.4/5921.7 $[M]^+$.



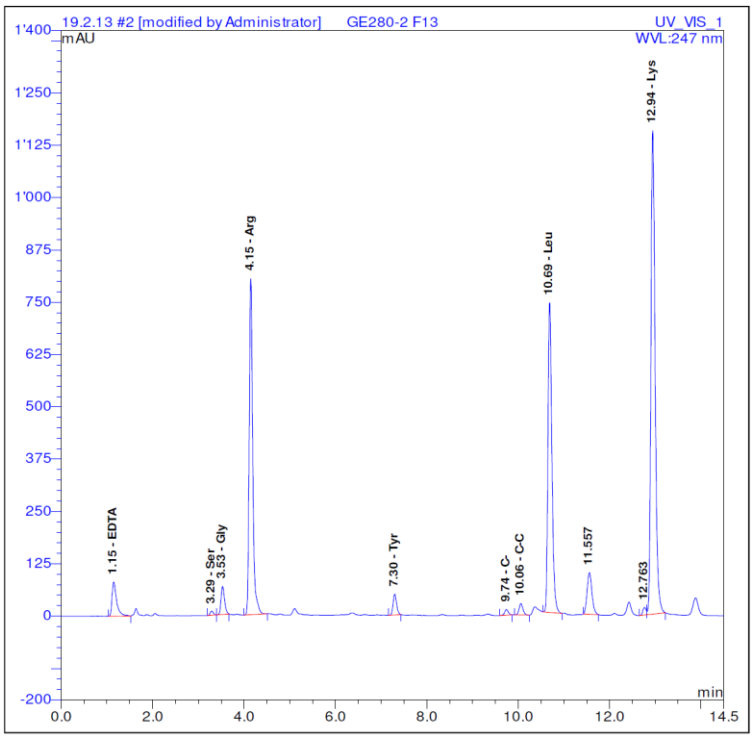
Mass spectrum, HRMS (NSI+):



Analytical RP-HPLC chromatogram:



Analytical RP-HPLC chromatogram of amino acid analysis:

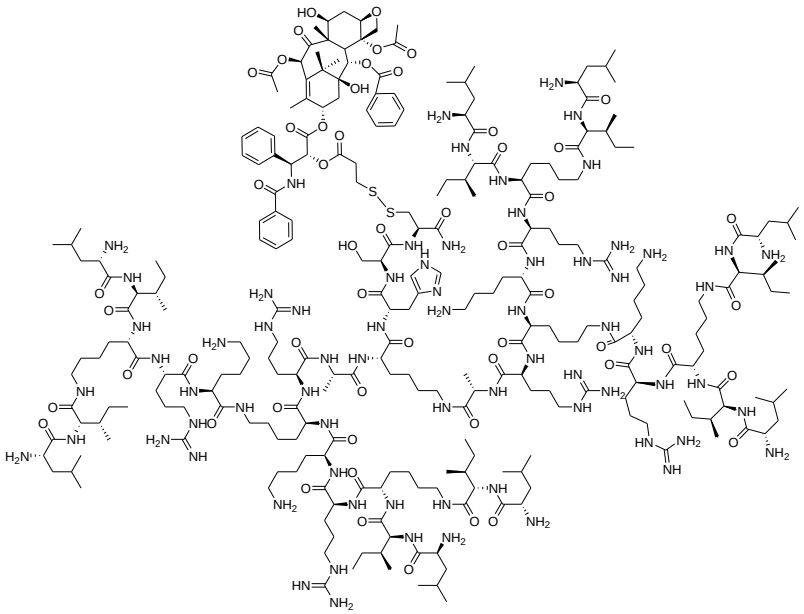


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.15	1.19	0.098	9.48	81.48		713.94	EDTA
3.29	3.31	0.076	0.78	9.64		78.56	Ser
3.53	3.55	0.082	5.97	67.02		514.28	Gly
4.15	4.17	0.077	71.03	803.36		5873.39	Arg
7.30	7.35	0.083	4.46	49.81		374.84	Tyr
9.74	9.79	0.082	1.22	13.49		67.18	C-
10.06	10.09	0.091	2.72	27.50		136.99	C-C
10.69	10.76	0.091	73.84	739.93		5572.64	Leu
12.94	12.98	0.097	122.60	1155.55		5369.21	Lys
Total:						18701.01	

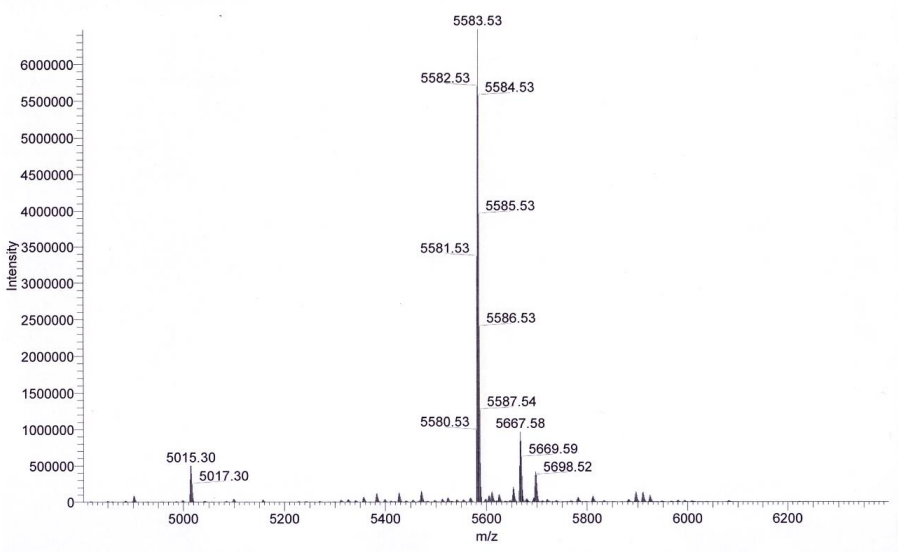
Amino Acid	Amount pmol	Quantity calc	Quantity obs
Arg	5873.4	12	12.4
C-	67.2	1	0.4
C-C	137.0		
Gly	541.3	1	1.2
Leu	5572.6	12	11.8
Lys	5369.2	11	11.4
Tyr	374.8	1	0.8

CPPD11-Paclitaxel.

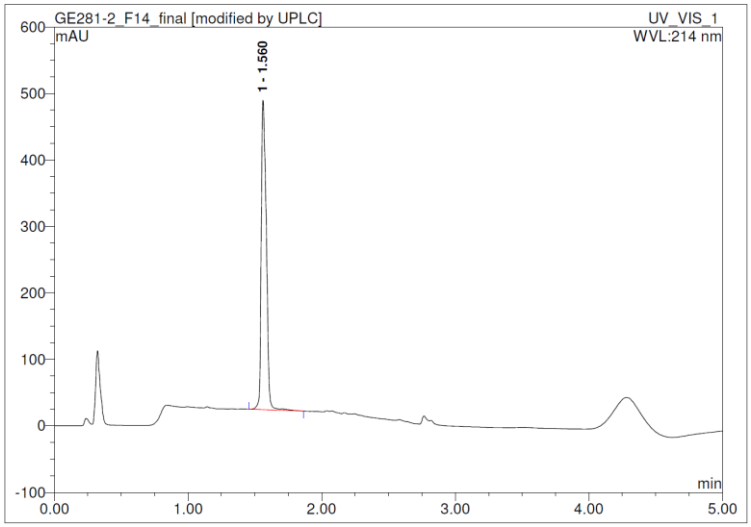
From starting materials **CPPD11-Cys** and **Paclitaxel-SPDP** using the general procedure described, **CPPD11-Paclitaxel** was obtained as a colourless solid after preparative RP-HPLC (0.2 mg, 0.03 µmol, yield 5%). Analytical RP-HPLC: t_R = 1.56 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). HRMS (NSI+): $C_{266}H_{463}N_{71}O_{54}S_2$ calc/found 5584.1/5583.5 $[M]^+$.



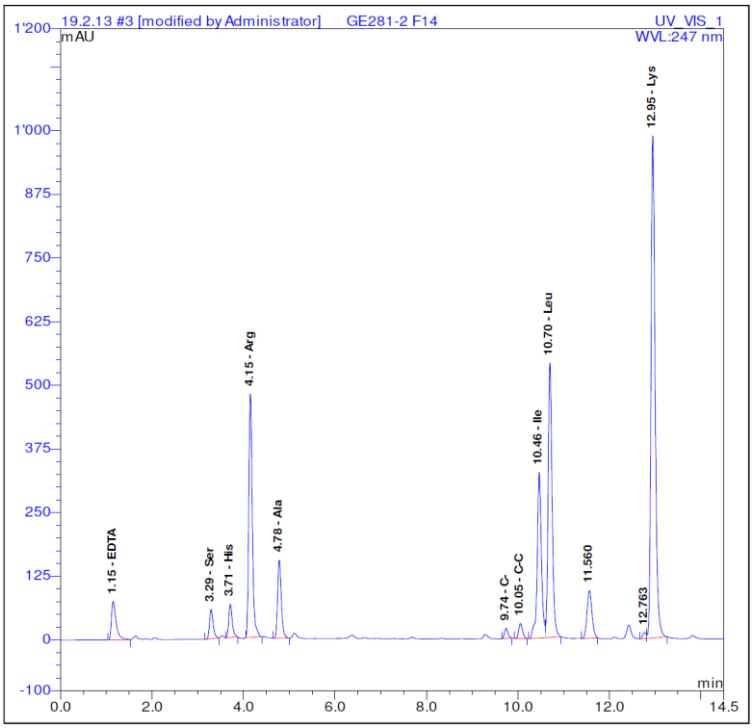
Mass spectrum, HRMS (NSI+):



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	1.19	0.100	8.93	75.39		660.58	EDTA
3.29	3.31	0.083	5.20	57.44		467.87	Ser
3.71	3.73	0.081	5.80	65.28		519.53	His
4.15	4.17	0.079	42.46	478.88		3501.08	Arg
4.78	4.81	0.085	14.29	153.50		1112.99	Ala
9.74	9.79	0.082	1.74	19.87		98.97	C-
10.05	10.09	0.089	2.87	29.82		148.55	C-C
10.46	10.52	0.089	34.23	325.58		2402.28	Ile
10.70	10.76	0.090	53.88	539.09		4060.10	Leu
12.95	12.98	0.095	103.40	986.55		4528.27	Lys
Total:						17500.22	

Amino Acid	Amount pmol	Quantity calc	Quantity obs
Ala	1113.0	2	2.5
Arg	3501.1	6	7.9
C-	99.0	1	0.6
C-C	148.6		
His	519.5	1	1.2
Ile	2402.3	8	5.4
Leu	4060.1	12	9.2
Lys	4528.3	17	10.2
Ser	467.9	1	1.1

3 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.