Supporting Information

An Antimicrobial Bicyclic Peptide from Chemical Space Against Multidrug Resistant Gram-Negative Bacteria

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1. *Synthesis of bicyclic peptides*

1.1 Material and Methods

Oxyma Pure (hydroxyiminocyanoacetic acid ethyl ester) was purchased from SENN AG, DIC (*N*,*N*'-diisopropyl carbodiimide) was purchased from Iris BIOTECH GMBH, potassium iodide, piperidine, triisopropylsilane, 2,2'-(ethylenedioxy) diethanethiol were purchased from SIGMA ALDRICH, phenylsilane was purchased from TCI (Tokyo Chemical Company), DIEA (*N*-ethyldiisopropylamine) was purchased from Sigma Aldrich, 3,5-bis(chloromethyl)-4-methylbenzoic acid was purchased from Enamine, TFA(trifluoroacetic acid) was purchased from Fluorochem Ltd, chemicals were used as supplied and solvents were of technical grade. Amino acids were used as the following derivatives: Fmoc-Cys(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, and Fmoc-Trp(Boc)-OH. Tentagel S RAM resin was purchased from Rapp Polymere. Cyclic peptide synthesis was performed automatically using a Liberty Blue Automated Microwave Peptide Synthesizer from CEM.

Analytical RP-HPLC was performed with an Ultimate 3000 Rapid Separation LC-MS System (DAD-3000RS diode array detector) using an Acclaim RSLC 120 C18 column (2.2 μm, 120 Å, 3×50 mm, flow 1.2 mL/min) from Dionex. Data recording and processing was done with Dionex Chromeleon Management System Version 6.80 (analytical RP-HPLC). All RP-HPLC were using HPLC-grade acetonitrile and Milli-Q deionized water. The elution solutions were: A MilliQ deionized water containing 0.05% TFA; D MilliQ deionized water/acetonitrile (10:90, v/v) containing 0.05% TFA. Preparative RP-HPLC was performed with a Waters automatic Prep LC Controller System containing the four following modules: Waters2489 UV/Vis detector, Waters2545 pump, Waters Fraction Collector III and Waters 2707 Autosampler. A Dr. Maisch GmbH Reprospher column (C18-DE, 100×30 mm, particle size 5 μm, pore size 100 Å, flow rate 40 mL/min) was used. Compounds were detected by UV absorption at 214 nm using a Waters 248 Tunable Absorbance Detector. Data recording and processing was performed with Waters ChromScope version 1.40 from Waters Corporation. All RP-HPLC were using HPLC-grade acetonitrile and Milli-Q deionized water. The elution solutions were: A MilliQ deionized water containing 0.1% TFA; D MilliQ deionized water/acetonitrile (10:90, v/v) containing 0.1% TFA. MS spectra, recorded on a Thermo Scientific LTQ OrbitrapXL, were provided by the MS analytical service of the Department of Chemistry and Biochemistry at the University of Bern (group PD Dr. Stefan Schürch).

1.2 Synthesis of Bicyclic peptides (Table S1, S2 and S3)

Linear peptides were synthesized using a CEM Liberty Blue using 220 mg resin (Fmoc- Tentagel S RAM resin 0.23 mmol/g). The resin was swollen in DMF/DCM 50:50 for 15 min. at R.T. Then, the Fmoc protecting group was removed. The following conditions were used:

Removal of the Fmoc protecting group – At each step the Fmoc protecting group was removed with 3 mL of piperidine/DMF (1:4, v/v) for 2 min at 75 °C. After filtration the resin was washed 3 times for 7 sec. with DMF.

Coupling of the Fmoc-protected amino acids was performed using 5 eq. of Fmoc-protected amino acid with a concentration of 0.2 M, 5 eq. of Oxima and 6 eq. of DIC, both with a concentration of 0.2 M, were used as coupling reagents in 4.5 mL of DMF. The reaction was stirred for 5 min. at 75°C. The resin was then washed 3 times with DMF for 7 sec.

3,5-Bis(chloromethyl)-4-methylbenzoic acid was coupled using 5 eq. of the linker, 5 eq. of Oxima and 6 eq. of DIC, both with a concentration of 0.2 M, were used as coupling reagents in 4.5 mL of DMF. The reaction was stirred for 5 min. at 75°C and repeated twice. The resin was then washed with DMF for 7 sec. for 3 times.

Cleavage and purification of the linear peptide was carried out by treating the resins with 7 mL of a TFA/DODT/TIS/H_2O (94:2.5:2.5:1, v/v/v/v) solution for 3 h. The peptide solutions were precipitated with 25 mL of TBME, centrifuged for 10 min at 3500 rpm (twice), and the precipitate was dried in high vacuum for 60 min. The crude was then dissolved in a water/acetonitrile mixture and purified by preparative RP-HPLC. The fractions of the crudes were then lyophilized. Yields were calculated for the TFA salts of the products.

Bicyclic peptides were synthesized by submitting peptide dendrimers to high dilution conditions (1 mM) in a mixture of H_2O/MeCN (50:50, v/v). KI (1 eq) was dissolved in the solvent mentioned and DIEA (10 eq.) was added to the mixture and kept under argon atmosphere for 15 min. Peptide dendrimer was added dropwise to the mixture and the reaction was stirred for 60 min under argon atmosphere. The reaction was monitored by LC-MS after 60 min and lyophilized directly. Yields were calculated for the TFA salts.

Table S1: First library of bicyclic peptides.

N	Sequence[a)]	MS calc/obs	MIC BR 151	MIC PAO1	MBIC PAO1	L[b)]	K[c)]
bp1	B[12]LLC[1]KKC[2]L	960.52/961.31	64	128	32	3	2
bp2	B[12]LKLC[1]KKLC[2]L	1201.70/1201.71	32	>256		4	3
bp3	B[12]KKLLKC[1]LKC[2]L	1329.79/1330.43	4	64	16	4	4
bp4	B[12]LLC[1]KLKC[2]KLKL	1442.95/1443.49	64	>256	16	5	4
bp5	B[12]LLC[1]LKKKLKC[2]KL	1570.98/1570.98	32	256	16	5	5
bp6	B[12]KLLLLLLC[1]KKKKC[2]	1683.07/1684.61	8	>256	32	6	5
bp7	B[12]KLKKLKKLC[1]C[2]LKL	1698.08/1699.57	32	128	16	5	6
bp8	B[12]LKLLLLLKC[1]C[2]KKKK	1811.16/1812.96	4	128		6	6
bp9	B[12]LLC[1]LKKKLLKLC[2]KK	1811.16/1812.79	8	128		6	6
bp10	B[12]KLC[1]KLLC[2]LKLLKKKK	1940.25/1940.26	8	256		6	7
bp11	B[12]LKC[1]LLLLKKKC[2]KKKL	1940.25/1940.26	32	>256	>32	6	7
bp12	B[12]LLKKKKC[1]LKLLC[2]LKL	1925.60/1926.67	4	>256	32	7	6
bp13	B[12]LLLKKLLKKC[1]LKC[2]KK	1940.25/1940.26	1	256	32	6	7
bp14	B[12]KLKKLC[1]C[2]LLLLKKLKK	2053.37/2053.34	2	16		7	7
bp15	B[12]LKLKKKC[1]KC[2]LLLKKLL	2053.34/2053.34	2	32		7	7
bp16	B[12]LLC[1]KKLKLKKLC[2]LKKL	2053.37/2053.34	4	>256	32	7	7
bp17	B[12]LKKKKLKLLLKLKC[1]LC[2]	2053.37/2053.34	8	256	32	7	7
bp18	B[12]LKC[1]KC[2]KLLKLLKLKKLK	2181.43/2181.44	2	32		7	8
bp19	B[12]LC[1]KKKLKC[2]LKKLLLLLK	2166.42/2166.43	4	16-32		8	7
bp20	B[12]LLKKLLKLC[1]KKC[2]LLLKK	2166.42/2166.43	4	256	16	8	7
bp21	B[12]LLLKKLLKLKLC[1]LKC[2]KK	2166.42/2166.42	4	256	16	8	7
bp22	B[12]LLLKC[1]KKLKLKKC[2]LKLK	2181.43/2181.43	4	128		7	8
bp23	B[12]KLLLKC[1]LKLLLKKKC[2]LK	2166.42/2166.43	4	>256	32	8	7
bp24	B[12]KKLLLLC[1]LLKC[2]KKLKLK	2166.42/2166.43	2	128		8	7
bp25	B[12]KC[1]KKC[2]LLKLLKKLLKLKL	2294.51/2294.52	2	4		8	8
bp26	B[12]LKLC[1]KKLLLC[2]KLKKKLLK	2294.51/2294.52	2	128	16	8	8
bp27	B[12]LKLLKKLKLKC[1]KC[2]LKLKL	2294.51/2294.52	2	64		8	8
bp28	B[12]LKKLLC[1]KLKLKC[2]LKKKLL	2294.51/2294.53	2	128		8	8
bp29	B[12]LKKKKLLKC[1]KLLLKC[2]LKL	2294.51/2294.52	2	128	32	8	8
bp30	B[12]KLKLC[1]KKLKLLKLKLC[2]LK	2294.51/2294.52	2	128		8	8
bp31	B[12]LLLKKLLLLC[1]KKKKKC[2]KL	2294.51/2294.52	2	256	16	8	8

[a)] Sequences are given using standard one-letter codes for amino acids, capitals = L-amino acids, lower case = D-amino acids, [1] and [2] indicate cyclization points using the SMILES formalism [b)] L = number of leucine residues, [c)] K = number of lysine residues. All MIC (minimal inhibitory concentration)/MBIC (minimal biofilm inhibitory concentration) values reported in µg/mL. MS calc./obs. calculated in Dalton. For **bp1, bp3, bp4, bp6, bp7, bp8, bp9, bp12** the mass is considered $[M+H]^+$, for **bp2, bp5, bp10, bp11, bp13, bp14, bp15, bp16, bp17, bp18, bp19, bp20, bp21, bp22, bp23, bp24, bp25, bp26, bp27, bp28, bp29, bp30, bp31** the mass is considered [M].

Table S2: Second library of bicyclic peptides.

N	Sequence[a)]	MS calc./obs.	MIC BR151	MIC PAO1	MBIC PAO1	L[b)]	K[c)]
bp14	B[12]KLKKLC[1]C[2]LLLLKKLKK	2053.37/2053.34	1	16	>128	7	7
bp32	B[12]KKLKLC[1]C[2]LLLLKKLKK	2053.16/2053.34	1	>64	32	7	7
bp33	B[12]LKKLK C[1]C[2]LLLLKKLKK	2053.16/2053.34	1	64	32	7	7
bp34	B[12]LKLKK C[1]C[2]LLLLKKLKK	2053.16/2053.34	2	>64	16	7	7
bp35	B[12]KLKKC[1]LC[2]LLLLKKLKK	2053.16/2053.34	1	>64	>32	7	7
bp36	B[12]LKC[1]KKC[2]LLLLLKKLKK	2053.16/2053.34	2	16	>32	7	7
bp37	B[12]KLKKL C[1]C[2]LLLLKKLKKL	2166.42/2166.43	1	16	32	8	7
bp38	B[12]C[1]KLKKC[2]LLLLLKKLKK	4106.68/4106.71	16	64	>32	7	7
bp39	B[12]KKKLL C[1]C[2]LLLLKKLKK	2053.16/2053.34	1	>64	32	7	7
bp40	B[12]KKLKC[1]LLC[2]LLLLKKLKK	2166.41/2166.42	1	16	16	8	7
bp41	B[12]KKLKLC[1]LC[2]LLLLKKLKK	2166.41/2166.42	2	>64	32	8	7
bp15	B[12]LKLKKKC[1]KC[2]LLLKKLL	2053.34/2053.34	2	32	>128	7	7
bp42	B[12]LKKKKLC[1]KC[2]LLKLKLL	2053.16/2053.34	2	64		7	7
bp43	B[12]LKLKKKC[1]LC[2]KLLKKLL	2053.16/2053.34	4	8	32	7	7
bp44	B[12]LKLKKLC[1]KC[2]LLKKKLL	2053.16/2053.34	2	>64	16	7	7
bp45	B[12]LKKKLKC[1]KC[2]LLLKKLL	2053.16/2053.34	2	32		7	7
bp46	B[12]LKLKKLC[1]LC[2]KLKKKLL	2053.16/2053.34	2	>64	16	7	7
bp47	B[12]LKLKKKC[1]KC[2]LLLKKLLL	2166.42/2166.43	1	8	32	8	7
bp48	B[12]LLKKLKC[1]LC[2]KLKKKLL	2053.16/2053.34	2	>64		7	7
bp49	B[12]C[1]KLKKKLKC[2]LLLKKLL	4106.68/4105.70	4	32		7	7
bp50	B[12]LKKKLKC[1]LC[2]KLLKKLL	2053.34/2053.34	2	4	16	7	7
bp51	B[12]LKLKKKC[1]KC[2]LLLKKL	1940.26/1941.85	2	>64	16	6	7

[a)] Sequences are given using standard one-letter codes for amino acids, capitals = L-amino acids, lower case = D-amino acids, [1] and [2] indicate cyclization points using the SMILES formalism [b)] L = number of leucine residues, [c)] K = number of lysine residues. All MIC/MBIC vales reported in µg/mL. MS calc./obs. calculated in Dalton. The mass is considered [M] for all the AMBPs and for **bp51** the mass is considered $[M+H]^+$. **bp38** and **bp49** contain the "BC" sequence which cannot cyclize intramolecularly and were obtained as dimers.

Table S3: Third library of bicyclic peptides.

N	Sequence[a)]	MS calc./obs.	MIC BR 151	MIC PAO1	MBIC PAO1	L[b)]	K[c)]
bp50	B[12]LKKKLKC[1]LC[2]KLLKKLL	2053.34/2053.34	2	4	16	7	7
bp52	B[12]lKkKlKc[1]Lc[2]KlLkKlL	2053.34/2053.34	2	8	8	7	7
bp53	B[12]LkKkLkC[1]lC[2]kLlKkLl	2053.34/2053.34	2	8	8	7	7
bp54	B[12]lKKKlKC[1]lC[2]KllKKll	2053.34/2053.35	16	32	16	7	7
bp55	B[12]LkkkLkC[1]LC[2]kLLkkLL	2053.34/2053.34	8	16	16	7	7
bp56	B[12]lkkklkc[1]lc[2]kllkkll	2053.34/2053.34	1	4	16	7	7
bp57	B[12]FKKKFKC[1]FC[2]KFFKKFF	2291.25/2291.24	2	8	16	7	7
bp58	B[12]WKKKWKC[1]WC[2]KWWKKWW	2564.31/2564.31	2	32	>16	7	7

[a)] Sequences are given using standard one-letter codes for amino acids, capitals = L-amino acids, lower case = D-amino acids, [1] and [2] indicate cyclization points using the SMILES formalism [b)] L = number of leucine residues (L, F, W), [c)] K = number of lysine residues. All MIC/MBIC values reported in µg/mL. MS calc./obs. calculated in Dalton.

2. Serum Stability Assay

Human serum was diluted in DMEM (1:4, v/v). Selected peptide was diluted in TRIS buffer to a concentration of 400 µM. Aliquots of peptide solution (50 µL) were added to aliquots of serum (50 µL) in sterile Eppendorf tubes, to reach a peptide concentration of 200 µM during the assay. Samples were incubated at 37 °C with stirring at 350 rpm. Different samples (triplicates) were quenched at different time points (0/1/3/6/24 h) by precipitating serum proteins through the addition of (0.1 M) $ZnSO_4$/ ACN (1:1) (100 µL) and cooling down in ice bath. Protein precipitates were pelleted under centrifugation and the supernatants were sampled and evaporated to dryness in a centrifugal evaporator. Samples were re-suspended in a H_2O/ ACN (4:1, v/v) mixture and centrifuged again to remove residual protein precipitate. Supernatants were then sampled and analyzed by LC-MS. Experiment controls included a precipitation control for each peptide, to test their resistance to the protein precipitation conditions, and serum blanks, to check reproducibility over different serum batches. Two peaks originating from DMEM, surviving the incubation in serum and the protein precipitation conditions were used as internal standard.

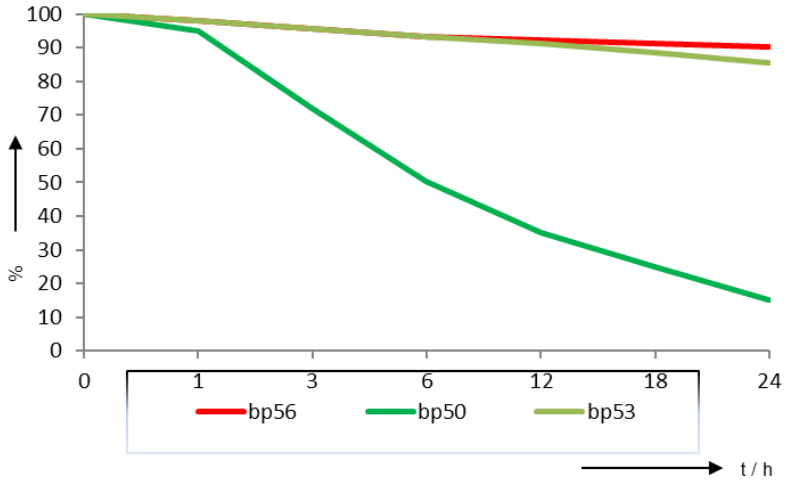


Figure S1: Serum stability measurement of bicyclic peptides **bp50**, **bp53** and **bp56**.

3. Antimicrobial activity

3.1 Broth Microdilution Method

Bicyclic peptides cytotoxicity was assayed against *Pseudomonas aeruginosa* PAO1, *Pseudomonas aeruginosa* PA14 , *P. aeruginosa* ZEM 1.A, *P. aeruginosa* ZEM 9.A, *P. aeruginosa* PEJ 2.6, *P. aeruginosa* PEJ 9.1, *Pseudomonas aeruginosa* 16060789, *Pseudomonas aeruginosa* 16051788 , *Pseudomonas aeruginosa* 16050914, *Pseudomonas aeruginosa* X1604603, *Pseudomonas aeruginosa* X1605418, *Acinetobacter baumannii* (ATCC19606), *Acinetobacter baumannii* 16050008, X1605034, 16050079, 16050179, X1605062, *Staphylococcus aureus* (clinical isolate of MRSA), *Bacillus Subtilis* BR151, *K. pneumoniae*-S, *E. coli* W3110. To determine the Minimal Inhibitory Concentration (MIC), Broth Microdilution method was used. A colony of bacteria from glycerol stock was grown in LB medium overnight at 37 °C and 180 rpm shaking. The compounds were prepared as stock solutions of 8 mg/mL in sterilized milliQ deionized water, added to the first well of 96-well sterile, polypropylene round bottom microtiter plates (TPP, untreated) and diluted serially by ½. The concentration of the bacteria was quantified by measuring absorbance at 600 nm and diluted to an OD_{600} of 0.022 in MH medium. The sample solutions (150 µL) were mixed with 4 µL diluted bacterial suspension with a final inoculation of about $5x10^5$ CFU. For each test, two columns of the plate were kept for sterility control (MH medium only), growth control (MH medium with bacterial inoculum, no compound). The positive control, Polymyxin B (starting with a concentration of 64 µg/mL) in MH medium with bacterial inoculums, was introduced in the two first lines of the plate. The plates were incubated at 37 °C for ~18 hours under static conditions. 15 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (1 mg/mL in sterilized milliQ deionized water) were added to each well and the plates were incubated for 20-30 minutes at room temperature. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the dendrimer that inhibits the visible growth of the tested bacteria (yellow) with the unaided eye. For Broth Microdilution assay, Polymyxin B was used as references.

3.2 Biofilm Inhibition and Dispersal on Polystyrene Microtiter Plates

96-well sterile, U-bottomed polystyrene microtiter plates (TPP Switzerland) were prepared by adding 200 µL of sterile deionized water to the peripheral wells to decrease evaporation from test wells. Aliquots of 180 µL of culture medium (M63 medium broth enriched with 20% glycerol, 1M $MgSO_4$ and potassium hydroxide) containing desired concentration of the test compound were added to the internal wells. Compound containing solutions were sterile filtered (pore size 0.22 µm) prior to addition to the wells. Inoculum of *Pseudomonas aeruginosa* strain PAO1, *E. coli* W3110 and *Acinetobacter baumannii* (ATCC19606) were prepared from 5 mL overnight culture grown in LB broth overnight at 37 °C and 180 rpm shaking. Aliquots of 20 µL of overnight cultures, pre-washed in 0.25% (w/v) nutrient broth and normalized to an OD_{600} of 1.5,

were inoculated into the test wells. Plates were incubated in a humid environment for 24-25 hours at 37 °C under static conditions. Wells were washed twice with 200 μL sterile deionized water before staining with 200 μL M63 broth containing 0.5 mM WST-8 and 20 μM phenazine ethosulfate for 4 hours at 37 °C under static conditions. Afterwards, the well supernatants were transferred to a polystyrene flat bottomed 96-well plate (TPP Switzerland) and the absorbance was measured at 450 nm with a plate reader (SpectraMax250 from Molecular Devices).

For biofilm dispersal, biofilm was formed as described above but in the absence of compound for 24 hours. Wells were washed twice with 200 μL sterile deionized water before adding 200 μL 0.25% (w/v) nutrient broth containing the desired concentration of compound. Compound containing solutions were sterile filtered (pore size 0.22 μm) prior to addition to the wells. After another 24 hours of incubation at 37 °C under static conditions, the well supernatants were discarded and the wells were washed twice with 200 μL sterile deionized water. The biofilm was stained with 200 μL of 0.25% (w/v) nutrient broth containing 0.5 mM WST-8 and 20 μM phenazine ethosulfate for 2.5-3 hours at 37 °C under static conditions. The resulting absorbance was measured as in the biofilm inhibition experiment. For *E. coli* W3110 biofilms, crystal violet solution was used to stain the biofilm and the absorbance was measured at 590 nm.

Table S4: MBIC and Dispersal of **bp56** and Polymyxin B on PAO1, *E. Coli* and *A. baumannii*.

Compounds	MBIC PAO1	Dispersal PAO1	MBIC *E. Coli*	Dispersal *E. Coli*	MBIC *A. baumannii*	Dispersal *A. baumannii*
bp56	16	32	16	32	16	32
Polymyxin B	4	8	2	8	2	4

3.3 Biofilm Inhibition with *P. aeruginosa* strains from CF patients

Bacterial strains. Eight strains of *Pseudomonas aeruginosa*, isolated from respiratory specimens collected from CF patients, were tested. Three strains (PaPh26, PaPh29 and PaPh32) were "multi-drug resistant" because resistant to at least three of the following groups of antibiotics: β-lactams with or without β-lactamase inhibitor, aminoglycosides, fluoroquinolones, folate-pathway inhibitors (trimethoprim-sulphamethoxazole), tetracyclines, and macrolides. *P. aeruginosa* ATCC27853 reference strain was used as quality control strain in MIC assays. Strains were stored at -80 °C in a Microbank system (Biolife Italiana S.r.l., Milan, Italy) and subcultured in Tryptone Soy broth (TSB) (Oxoid Srl, Milan, Italy) then twice on Muller-Hinton agar (MHA) (Oxoid Srl) prior to the use in this study.

Standardization of the bacterial inoculum. Some colonies from an overnight 37 °C growth onto MHA were resuspended in cation-adjusted Muller-Hinton broth (CAMHB) (Oxoid srl) to an optical density measured at 550 nm (OD_{550}) of 1.0 (corresponding to 1-5 x 10^9 CFU/mL). This standardized bacterial suspension was then diluted accordingly to use.

In vitro **activity against planktonic bacteria.** MICs and MBCs of both bicyclic peptide **bp56** and Tobramycin were determined by microdilution technique, in accordance with CLSI guidelines, with some modifications. Briefly, serial two-fold dilutions of both compounds - ranging from 0.25 to 128 μg/mL - were prepared in CAMHB at a volume of 100 μL/well in 96-well microtiter plates (Kartell LabWare, Noviglio, Italy). Each well was then inoculated with 5 μL of the standardized inoculum, corresponding to a final test concentration of about 0.5-1 x 10^5 CFU/well. After incubation at 37 °C for 20 h, the MIC was read as the lowest concentration of the test agent that completely inhibited visible growth. To measure the MBC, 100 μL of broth from clear wells were plated on TSA plates and incubated at 37 °C for 24 h. MBC was defined as the lowest concentration of the test agent killing of at least 99.99% of the original inoculum.

In vitro **activity against biofilm formation**. In each well of a 96-well flat-bottom polystyrene tissue-culture microtiter plate (Iwaki; Bibby-Sterilin Italia Srl), 5 μL of a standardized inoculum (1-5 × 10^7 CFU/mL) were added to 100 μL of CAMHB containing test agent at concentrations equal to ½, ¼, and ⅛ MIC. After incubation at 37 °C for 24 h, non-adherent bacteria were removed by washing once with 100 μL sterile PBS (pH 7.2; Sigma-Aldrich Srl, Milan, Italy). Slime and adherent cells were fixed by incubating for 1 h at 60°C and stained for 5 min at room temperature with 100 μL of 1% crystal violet solution. The wells were then rinsed with distilled water and dried at 37 °C for 30 min. Biofilms were destained by treatment with 100 μL of 33% glacial acetic acid (Sigma-Aldrich Srl) for 15 min, and the OD_{492} was then measured. The low cut-off was represented by approximately 3 standard deviations above the mean OD_{492} of control wells (containing medium alone without bacteria).

Statistical analysis. All experiments were performed in triplicate and repeated on two different occasions. Differences between frequencies were assessed by Fisher's exact test. Statistical analysis of results was conducted with GraphPad Prism version 4.00 (GraphPad software Inc.; San Diego, CA, USA), considering as statistically significant a p value of < 0.05.

In vitro **activities of bicyclic peptide R92 and Tobramycin against planktonic cells of *P. aeruginosa* strains from CF patients**. MIC and MBC values were obtained by broth microdilution technique, in accordance with CLSI guidelines, and expressed as μg/mL. Each experiment was performed in triplicate and repeated on two different occasions (n = 6).

Effect of bp56 and Tobramycin on *P. aeruginosa* biofilm formation. Biofilms were allowed to form (37 °C, 24 h) in each well of a 96-well microtiter plate in the presence of **A**) **bp56** or **B**) Tobramycin tested at several sub-MICs (1/2xMIC 1/4xMIC 1/8xMIC). Biofilm biomass was then measured by crystal violet assay. The effect of test compound against biofilm formation is shown as the percentage of biofilm biomass after exposure, compared to control (100%, black line). Control samples were infected but not exposed to the test agent. Dotted green and red lines indicate a significant reduction or increase in biofilm formation, respectively (≤70% or ≥130% compared to control sample; $p<0.05$, Fisher's exact test). Each experiment was carried out in single and repeated on two different occasions (n = 6).

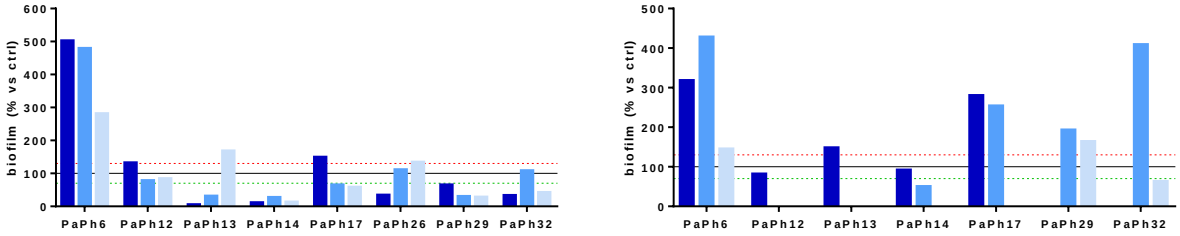


Figure S2: Effect of **bp56** (left) and Tobramycin (right) on *P. aeruginosa* strains from CF patients biofilm formation.

4. Hemolysis Assay

To determine the minimal hemolytic concentration (MHC) stock solutions of 8 mg/mL of the peptide in H_2O were prepared and 50 µL were diluted serially by ½ in 50 µL PBS (pH 7.4) in 96-well plate (Costar or Nunc, polystyrene, untreated). Human red blood cells (hRBC) were obtained by centrifugation of 1.5 mL of whole blood, from the blood bank of Bern, at 3000rpm for 15 minutes at 4 °C. Plasma was discarded and the pellet was re-suspended in 5 mL of PBS. The washing was repeated three times and the remaining pellet was re-suspended in 10 mL of PBS at a final hRBC concentration of 5%. The hRBC suspension (50 µL) was added to each well and the plate was incubated at room temperature for 4 hours. Minimal hemolytic concentration (MHC) end points were determined by visual determination of the wells after the incubation period. Controls on each plate included a blank medium control (50 µL PBS + 50 µL of hRBC suspension) and a hemolytic activity control (mQ-deionized water 50 µL + 50 µL hRBC suspension).

MHC values: **bp52** > 2000 µg/mL, **bp53** > 2000 µg/mL, **bp56** = 500 µg/mL.

5. Circular dichroism spectroscopy

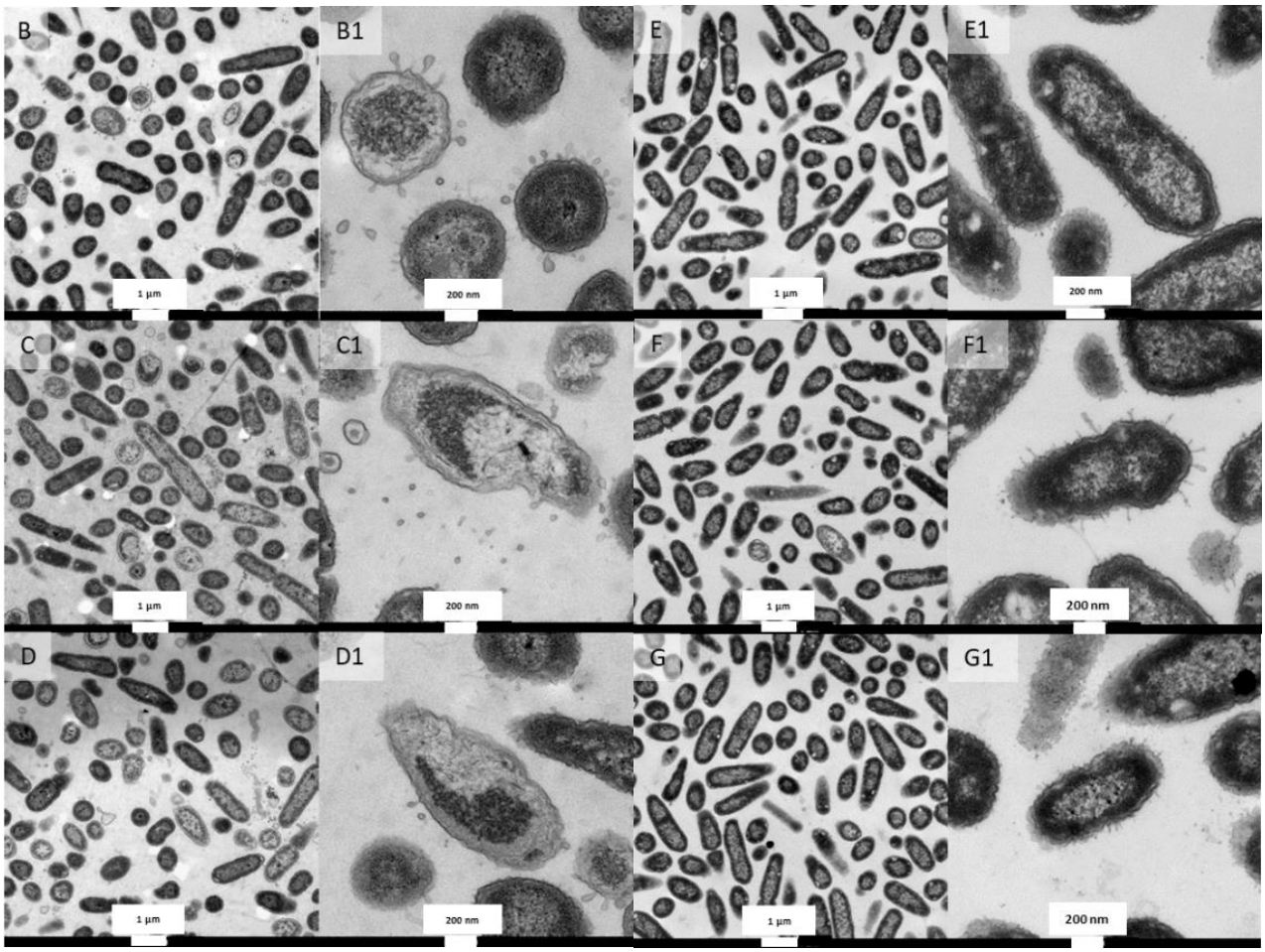
Circular dichroism (CD) experiments were measured on a Jasco J-715 Spectropolarimeter. All the experiments were performed using Hellma Suprasil ® 110-QS 0.1 cm cuvettes. For each peptide, the measurements were performed in phosphate buffer (PB, pH=7.0, 10 mM), 5%, 10%, 15% and 20% of 2,2,2-trifluoroethanol (TFE) in PBS (pH=7.0, 10 mM) respectively and in 5 mM dodecylphosphocholine. The concentration of the peptides was 0.20 mg/mL and each sample was measured using one accumulation. The scan rate was 10 nm/min, pitch 0.5 nm, response 16 sec and bandwidth 1.0 nm. The nitrogen flow was kept >8.5 L/min. After each measurement, the cuvettes were washed successively with 1 M HCl, milli-Q H_2O and PB buffer.

6. TEM Transmission Electron Microscopy

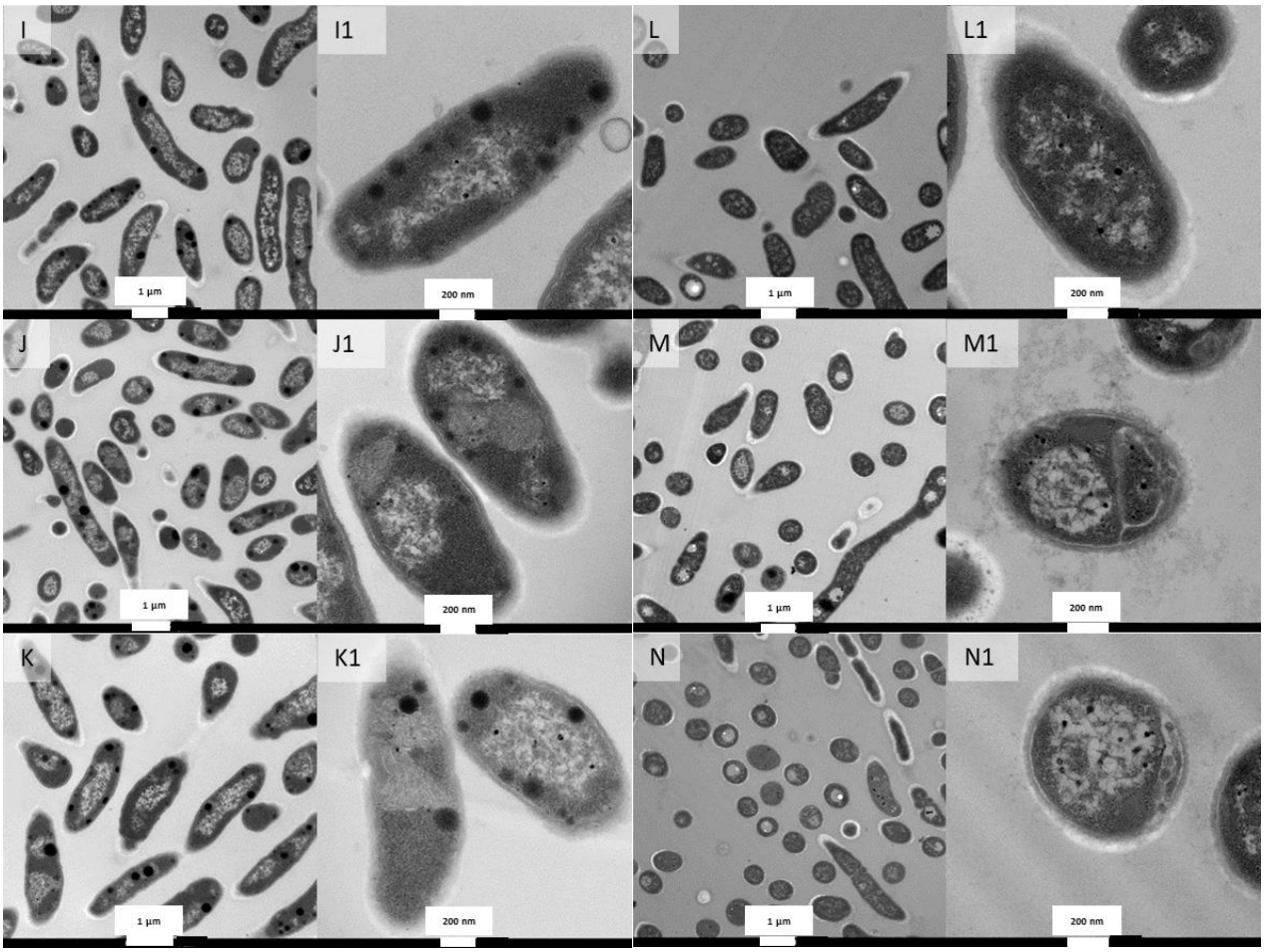
Exponential phase of *Pseudomonas aeruginosa* PAO1, *Acinetobater baumannii* and *Escherichia Coli* were washed with PBS and treated with 40 μg/mL of **bp56** and 5 μg/mL of Polymyxin B in M63 minimal medium. Each time, 1 ml of the bacteria were centrifuged after 15, 30 and 60 min at 12'000 rpm for 3 min and fixed overnight with 2.5% glutaraldehyde (Agar Scientific, Stansted, Essex, UK) in 0.15 M HEPES (Fluka, Buchs, Switzerland) with an osmolarity of 670 mOsm and adjusted to a pH of 7.35. The next day, PAO1 were washed with 0.15 M HEPES three times for 5 min, postfixed with 1% OsO_4 (SPI Supplies, West Chester, USA) in 0.1 M Na-cacodylate-buffer (Merck, Darmstadt, Germany) at 4 °C for 1 h. Thereafter, bacteria cells were washed in 0.1 M Na-cacodylate-buffer three times for 5 min and dehydrated in 70, 80, and 96% ethanol (Alcosuisse, Switzerland) for 15 min each at room temperature. Subsequently, they were immersed in 100% ethanol (Merck, Darmstadt, Germany) three times for 10 min, in acetone (Merck, Darmstadt, Germany) two times for 10 min, and finally in acetone-Epon (1:1) overnight at room temperature. The next day, bacteria cells were embedded in Epon (Fluka, Buchs, Switzerland) and hardened at 60 °C for 5 days.

Sections were produced with an ultramicrotome UC6 (Leica Microsystems, Vienna, Austria), first semithin sections (1 μm) for light microscopy which were stained with a solution of 0.5% toluidine blue O (Merck, Darmstadt, Germany) and then ultrathin sections (70-80 nm) for electron microscopy. The sections, mounted on single slot copper grids, were stained with uranyl acetate and lead citrate with an ultrostainer (Leica Microsystems, Vienna, Austria). Sections were then examined with a Tecnai Spirit transmission electron microscope equipped with two digital cameras (Olympus-SIS Veleta CCD Camera, FEI Eagle CCD Camera).

PAO1 **bp56** (40 μg/mL) and Polymyxin B (5 μg/mL)



A. baumannii **bp56** (40 μg/mL) and Polymyxin B (5 μg/mL)



E.Coli **bp56** (40 µg/mL) and Polymyxin B (5 µg/mL)

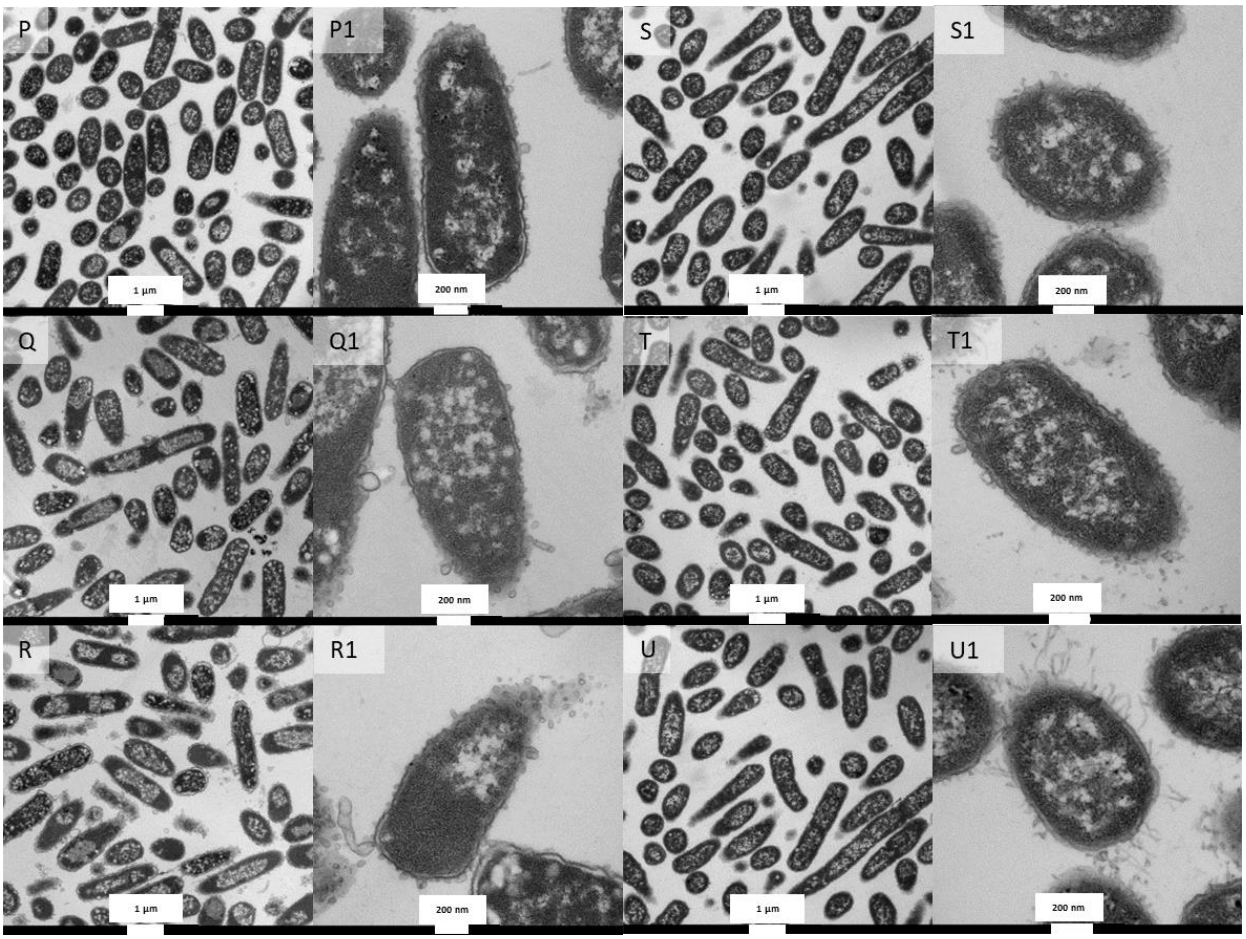
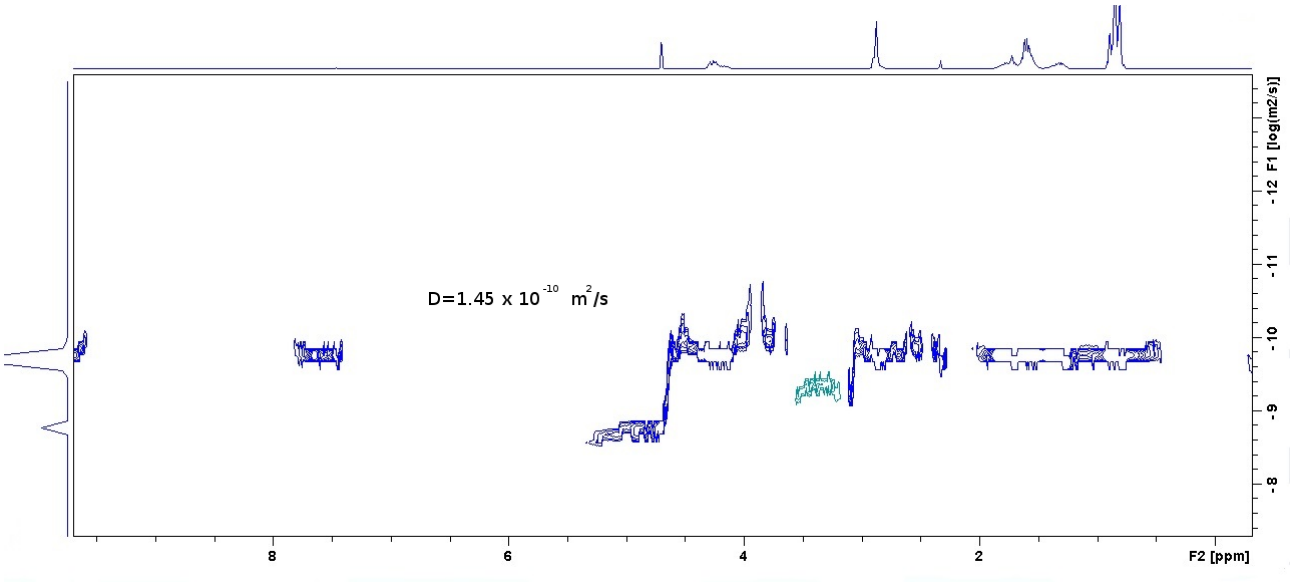


Figure S3: TEM of bicyclic peptides **bp56** and Polymyxin B on PAO1 and *A. baumannii*. **B and B$_1$. bp56** treatment to *P. aeruginosa* for 15 min. **C and C$_1$. bp56** treatment for 30 min. **D and D$_1$.** bp56 treatment for 60 min. **E and E$_1$.** Polymyxin B treatment for 15 min. **F and F$_1$.** Polymyxin B treatment for 30 min. **G and G$_1$.** Polymyxin B treatment for 60 min. **I and I$_1$. bp56** treatment to *A. baumanii* for 15 min. **J and J$_1$. bp56** treatment for 30 min. **K and K$_1$. bp56** treatment for 60 min. **L and L$_1$.** Polymyxin B treatment for 15 min. **M and M$_1$.** Polymyxin B treatment for 30 min. **N and N$_1$.** Polymyxin B treatment for 60 min. **P and P$_1$. bp56** treatment to *E. coli* for 15 min. **Q and Q$_1$. bp56** treatment for 30 min. **R and R$_1$. bp56** treatment for 60 min. **S and S$_1$.** Polymyxin B treatment for 15 min. **T and T$_1$.** Polymyxin B treatment for 30 min. **U and U$_1$.** Polymyxin B treatment for 60 min.

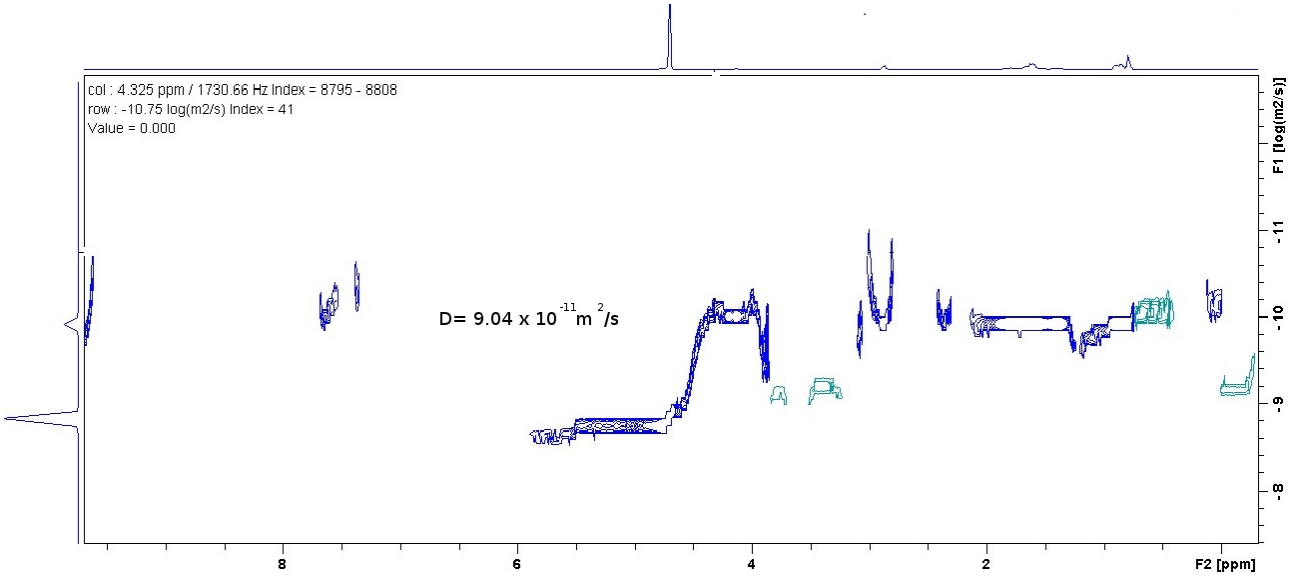
7. DOSY NMR

Standard diffusion NMR experiments were performed using a Bruker DRX500 with diluted solutions of dendrimer (5-10 mg·mL^{-1}) in D_2O (pD 7.8, at 303 K) and D_2O/20%TFE (pD 7.8, at 303 K). The gradient with a maximum strength of $50 \cdot 10^{-4}$ T·cm^{-1} was calibrated using the HOD proton signal in D_2O (99.997%). The diffusion time Δ was either 80 ms or 100 ms, 120 ms, 150 ms and the gradient duration δ was 7 ms. Data analysis was performed by using the Bruker Simfit software and the diffusion coefficient D [$m^2 \cdot s^{-1}$] was derived from peak integrals or intensities of several isolated signals. In both conditions, **bp56** was present as a single species in solution with diffusion coefficients in the typical range for monomers of compounds with similar molecular weight.

bp56 DOSY NMR (400 MHz, D_2O, pD 7.8)

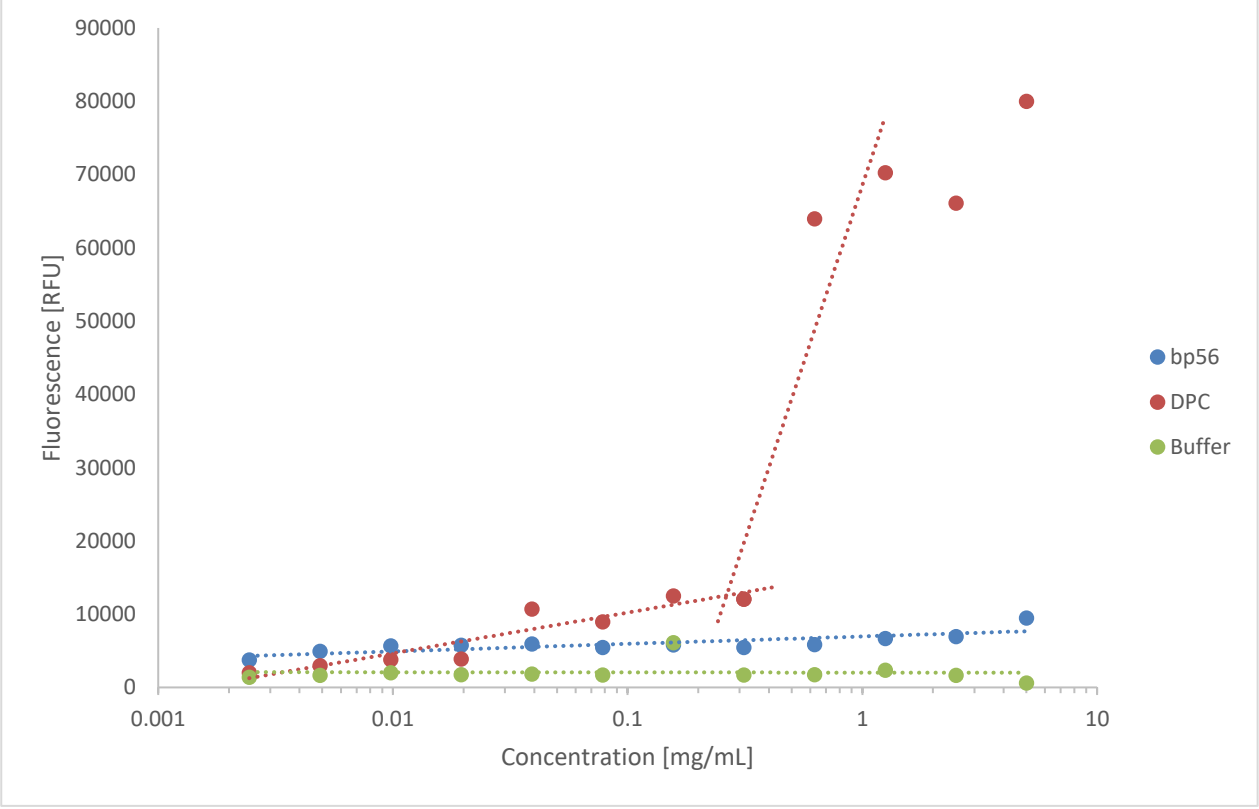


bp56 DOSY NMR (400 MHz, D_2O/20%TFE, pD 7.8)



8. Micelle Formation Assay

The critical micellar concentration (CMC) was measured using the Nile red method. CMC was determined by serial dilution of a stock solution of 10 mg·mL^{-1} of the peptide dendrimers in PBS (pH 7.4) to a final concentration range between 10 mg·mL^{-1} and 4.9 µg·mL^{-1}. 5 µL of Nile red in methanol (2 µM) and was added to each well of a 96 well plate and dried under the fume hood air flow at room temperature for 1 h to obtain final concentration of Nile red 0.2 µM. 50 µL of the compound stock solutions were diluted serially by ½ with 50 µL of PBS and 50 µL was added to the TPP 96 well plate. Dodecylphosphocholine (DPC) was used as a positive control. The plate was incubated 3 h and subjected to fluorescence measurement at excitation wave length at 540 nm and emission wavelength range from 580 nm to 660 nm was recorded on a Tecan Infinite M1000 Pro plate reader. Analysis of the data was performed using the signal at 642 nm. For dodecylphosphocholine CMC was obtained as 0.3125 mg·mL^{-1}. Corresponding to 0.89 mM in agreement with literature data (0.9 mM).



9. Molecular Dynamics

9.1 Preparation of the model

Molecular dynamics (MD) simulations were performed for the bicyclic peptide **bp56** using GROMACS software version 2016.3 and the Gomos53a6 force field. The starting topology was built by adding the non-natural bonds including the bis(chloromethyl)-4-methylbenzoic acid linker to the corresponding linear peptide and the initial coordinates were generated using the CORINA software. A dodecahedral box was created around the protein 1.0 nm from the edge of the protein and filled with extended simple point charge water molecules. Sodium and chloride ions were added to produce an electroneutral solution at a final concentration of 0.15 M NaCl. The energy was minimized using a steepest gradient method to remove any close contacts before the system was subjected to a two-phase position-restrained MD equilibration procedure. The system was first allowed to evolve for 100 ps in a canonical NVT (*N* is the number of particles, *V* the system volume, and *T* the temperature) ensemble at 300 K before pressure coupling was switched on and the system was equilibrated for an additional 100 ps in the NPT (*P* is the system pressure) ensemble at 1.0 bar.

9.2 Simulated annealing MD

This pre-equilibrated system was used as the starting point for the simulated annealing molecular dynamics (SA-MD) run. The system was heated to 450 K and high energy conformers were sampled at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ns. Each of the conformers was then cooled down from 450 K to 300 K over 50 ns and allowed to evolve for an additional 200 ns. Typically, the last 100 ns (150 → 250 ns) of each SA-MD run were used in the subsequent analysis. All bond lengths were constrained to their equilibrium values by using the LINCS algorithm. The neighbor list for the calculation of nonbonded interactions was updated every five time steps with a cutoff of 1.0 nm with a step size of 2 fs. A twin range cutoff of 1.0 nm was used for both Coulomb and Lennard-Jones interactions. The system was split into two groups, “Protein” and “Non-Protein”, which were coupled separately to a temperature bath using the V-rescale algorithm with a time constant of 0.1 ps while the pressure coupling was conducted using an isotropic Parrinello − Rahman barostat with a time constant of 2.0 ps. The PyMol Molecular Graphics System, version 1.8.x (Schrödinger, LLC), was used for visualization.

Table S5. Clustering of MD structures of **bp56** for the 10 trajectories of the simulated annealing. The cutoff (0.05 nm) was adjusted to obtain a large number of clusters while preserving a significant number of structures in the main cluster. The center of the main cluster of each run was used in subsequent analyses.

Starting structure	**# of clusters**	**# structures in main cluster out of 10001 total**
SA2	3988	5155
SA4	2721	4043
SA6	567	5794
SA8	1530	7739
SA10	798	9029
SA12	471	9418
SA14	3409	4654
SA16	1287	5125
SA18	201	9582
SA20	136	9851

9.3 Parameters for the non-natural residues

The model for the bicyclic peptide was built by adding the bis(chloromethyl)-4-methylbenzoic acid linker (DBZ) to the N-terminus of the linear peptide. Bonds were added between the alkylated cysteine residues (CYX) and the corresponding methyl groups of the DBZ residue leading to the final desired topology. The parameters for the 2 non-natural residues of the Gromos53a6 force field (aminoacids.rtp) were derived from the existing amino acids. They were defined as follows.

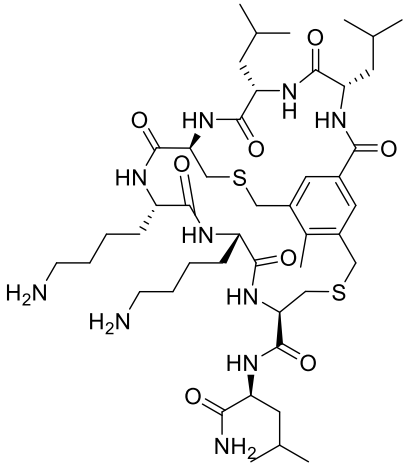
```
[ DBZ ]  ; derived from PHE, small positive charge to match CYX
 [ atoms ]
   CA   CH3     0.00000     0
   CG     C     0.00000     0
  CE1     C    -0.14000     1
  HE1    HC     0.14000     1
  CE2     C    -0.14000     2
  HE2    HC     0.14000     2
  CD1     C     0.00000     3
  CB1   CH2     0.24100     4
  CD2     C     0.00000     5
  CB2   CH2     0.24100     6
   CZ     C     0.00000     7
    C     C       0.450     8
    O     O      -0.450     8
 [ bonds ] ;
   CA    CG    gb_27  ; similarly to CB-CG in PHE
   CG   CD1    gb_16
   CG   CD2    gb_16
  CD1   CE1    gb_16
  CD2   CE2    gb_16
  CD1   CB1    gb_27  ; single bond to C
  CD2   CB2    gb_27  ; idem
  CE1   HE1    gb_3
  CE1    CZ    gb_16
  CE2   HE2    gb_3
  CE2    CZ    gb_16
   CZ     C    gb_27  ; probably close enough to normal CHn-C
    C     O    gb_5
    C    +N    gb_10
 [ exclusions ] ; aromatics exclude repulsion penalty 1-4
;  ai    aj
   C    HE1
   C    HE2
   C    CD1
   C    CD2
   CZ   CB1
   CZ   CB2
   CZ    CG
  CE1    CA
  CE1   CD2
  CE1   HE2
  HE1   CE2
  HE1   CB1
  HE1    CG
  CE2   CD1
  CE2    CA
  HE2   CB2
  HE2    CG
  CD1   CB2
  CB1   CD2
  CB1    CA
  CB2    CA
 [ angles ]
;  ai    aj    ak   gromos type
   CA    CG   CD1     ga_27
   CA    CG   CD2     ga_27
  CD1    CG   CD2     ga_27
   CG   CD1   CE1     ga_27
   CG   CD2   CE2     ga_27
   CG   CD1   CB1     ga_27
   CG   CD2   CB2     ga_27
  CB1   CD1   CE1     ga_27
  CB2   CD2   CE2     ga_27
  CD1   CE1   HE1     ga_25
```

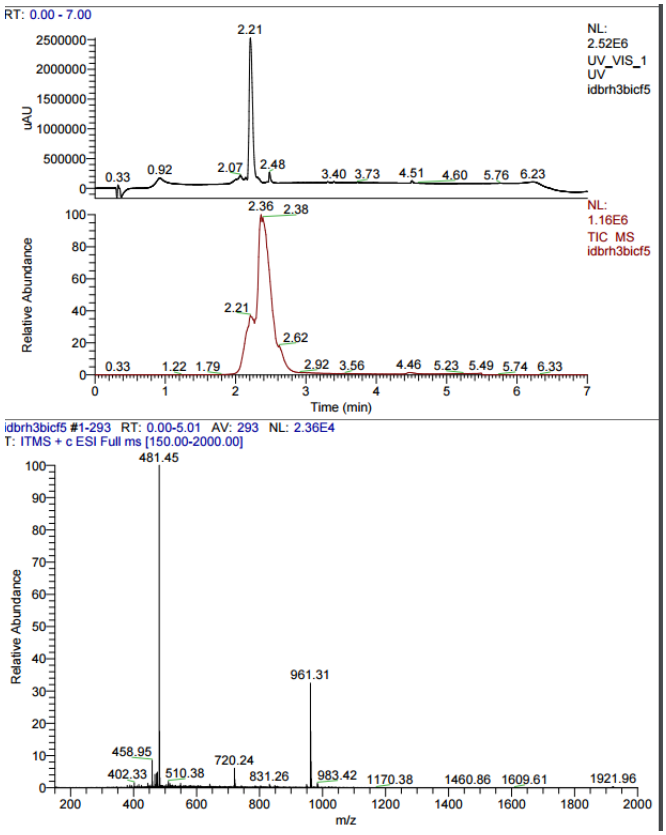
```
  CD1   CE1    CZ     ga_27
  HE1   CE1    CZ     ga_25
  CD2   CE2   HE2     ga_25
  CD2   CE2    CZ     ga_27
  HE2   CE2    CZ     ga_25
  CE1    CZ   CE2     ga_27
  CE1    CZ     C     ga_27
  CE2    CZ     C     ga_27
   CZ     C     O     ga_30
   CZ     C    +N     ga_19
    O     C    +N     ga_33
 [ impropers ]
;  ai    aj    ak    al   gromos type
   CG   CD1   CD2    CA     gi_1
   CG   CD1   CE1    CZ     gi_1
   CG   CD2   CE2    CZ     gi_1
  CD1    CG   CD2   CE2     gi_1
  CD1    CG   CE1   CB1     gi_1
  CD1   CE1    CZ   CE2     gi_1
  CD2    CG   CD1   CE1     gi_1
  CD2    CG   CE2   CB2     gi_1
  CD2   CE2    CZ   CE1     gi_1
  HE1   CD1    CZ   CE1     gi_1
  HE2   CD2    CZ   CE2     gi_1
   CZ   CE1   CE2     C     gi_1
    C    CZ    +N     O     gi_1
 [ dihedrals ]
;  ai    aj    ak    al   gromos type
   CZ     C    +N   +CA     gd_14
  CE1    CZ     C    +N     gd_33  ; HC-C-C-

[ CYX ]  ; derived from CYS1 and MET topologies, note that the charge is non-zero since it is
intended to be connected to DBZ residue and match the charge
 [ atoms ]
    N     N    -0.31000     0
    H     H     0.31000     0
   CA   CH1     0.00000     1
   CB   CH2     0.24100     2
   SG     S    -0.48200     3
    C     C       0.450     4
    O     O      -0.450     4
 [ bonds ]
    N     H    gb_2
    N    CA    gb_21
   CA    CB    gb_27
   CA     C    gb_27
   CB    SG    gb_32
    C     O    gb_5
    C    +N    gb_10
 [ angles ]
;  ai    aj    ak   gromos type
   -C     N     H     ga_32
   -C     N    CA     ga_31
    H     N    CA     ga_18
    N    CA    CB     ga_13
    N    CA     C     ga_13
   CB    CA     C     ga_13
   CA    CB    SG     ga_16
   CA     C     O     ga_30
   CA     C    +N     ga_19
    O     C    +N     ga_33
 [ impropers ]
;  ai    aj    ak    al   gromos type
    N    -C    CA     H     gi_1
   CA     N     C    CB     gi_2
    C    CA    +N     O     gi_1
 [ dihedrals ]
;  ai    aj    ak    al   gromos type
  -CA    -C     N    CA     gd_14
   -C     N    CA     C     gd_39
    N    CA    CB    SG     gd_34
    N    CA     C    +N     gd_40
```

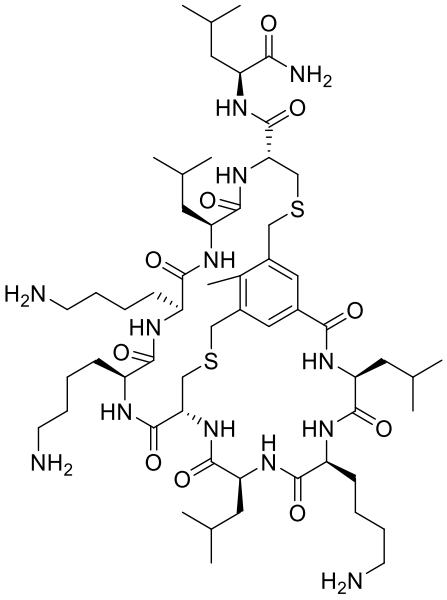
10. HPLC and MS Data

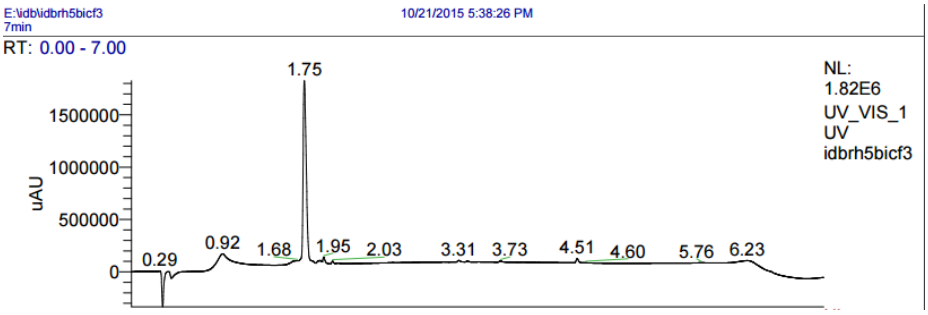
B[12]LLC[1]KKC[2]L (bp1) was obtained as foamy white solid after preparative RP-HPLC (24.4 mg, 23.5 %). Analytical RP-HPLC: t_R = 2.21 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS(ESI$^+$): $C_{46}H_{76}N_{10}O_8S_2$ calc./obs. 960.52/961.31 Da $[M+H]^+$.

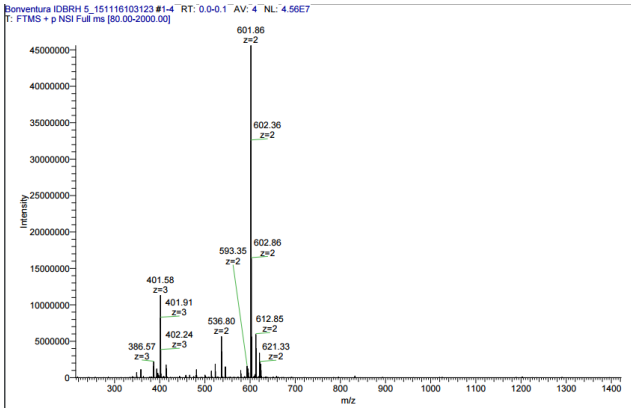


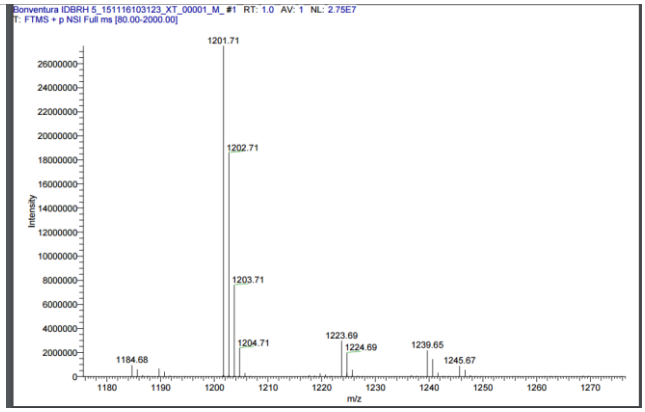


B[12]LKLC[1]KKLC[2]L (bp2) was obtained as foamy white solid after preparative RP-HPLC (18.1 mg, 13.9 %). Analytical RP-HPLC: t_R = 1.75 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS(ESI+): $C_{58}H_{99}N_{13}O_{10}S_2$ calc./obs. 1201.70/1201.71 Da [M], 1223.69/1223.70 Da [M+Na], 1239.70/1239.65 Da [M+K].

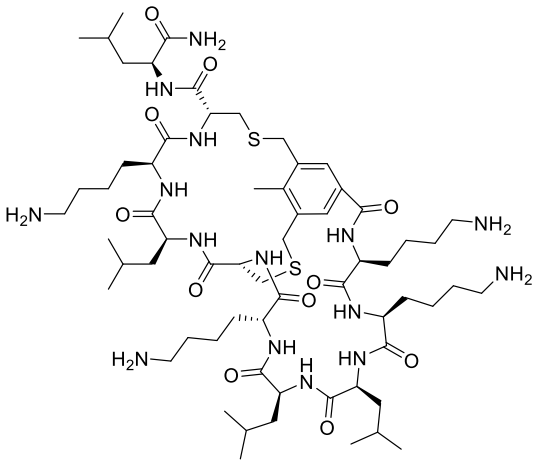


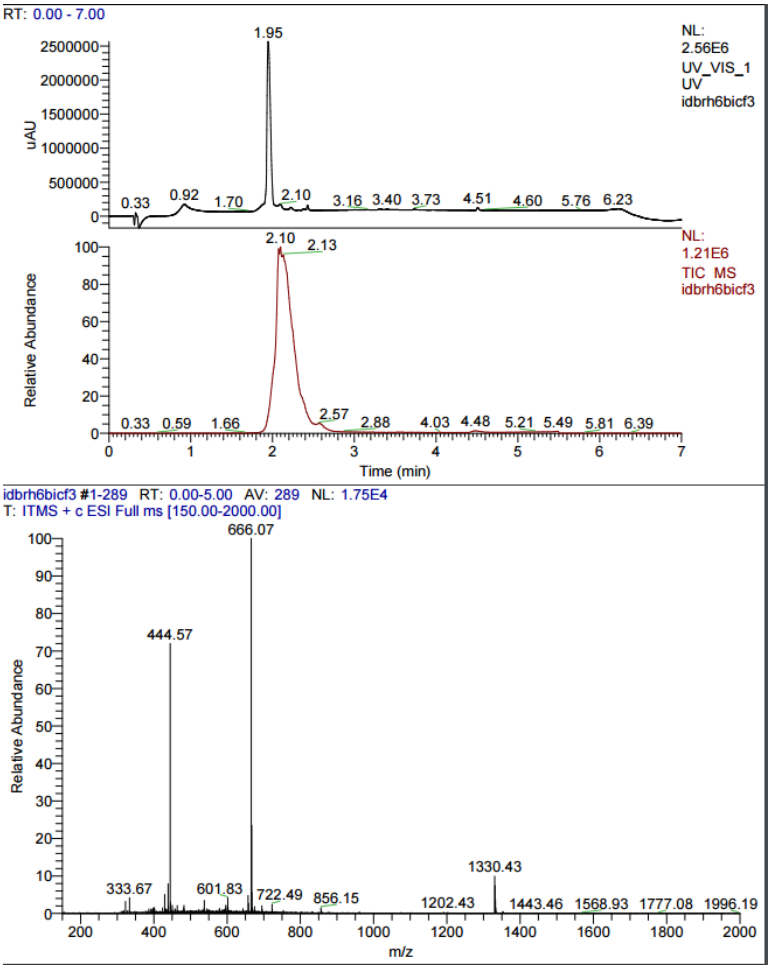




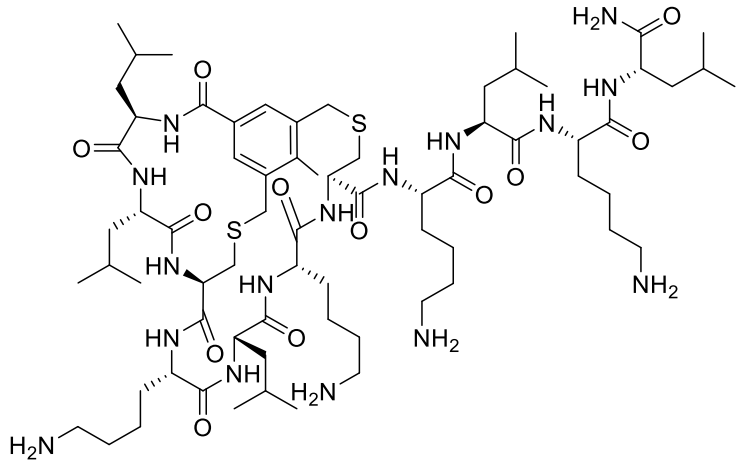


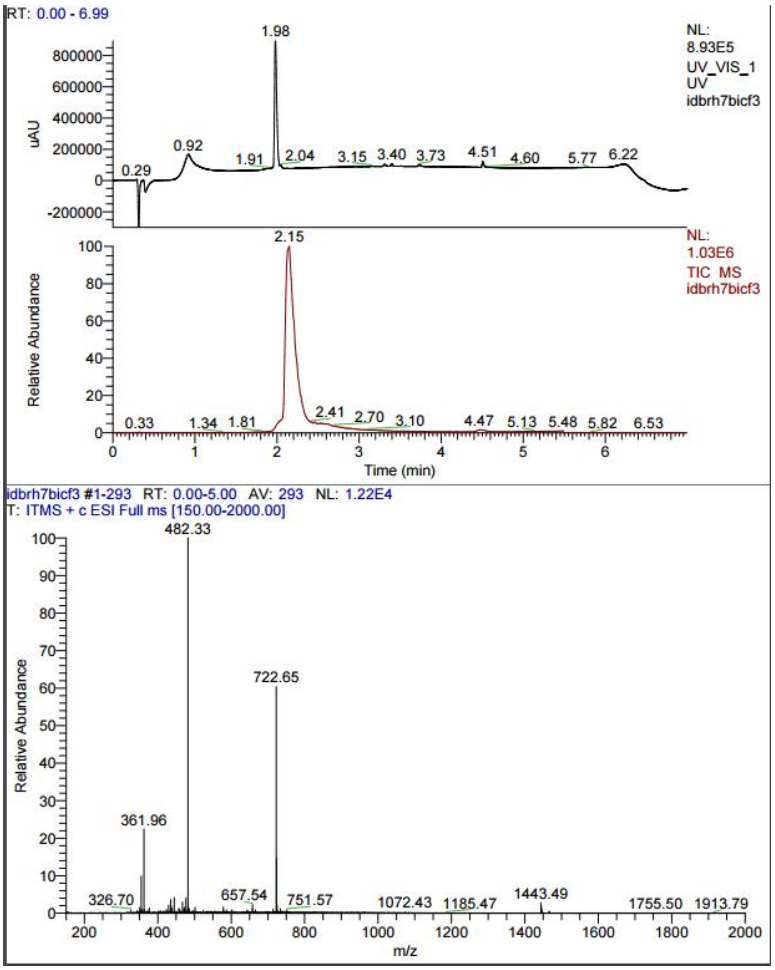
B[12]KKLLKC[1]LKC[2]L (bp3) was obtained as foamy white solid after preparative RP-HPLC (21.6 mg, 15.0 %). Analytical RP-HPLC: t_R = 1.95 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI+): $C_{64}H_{111}N_{15}O_{11}S_2$ calc./obs.1329.79/1330.43 Da $[M+H]^+$.



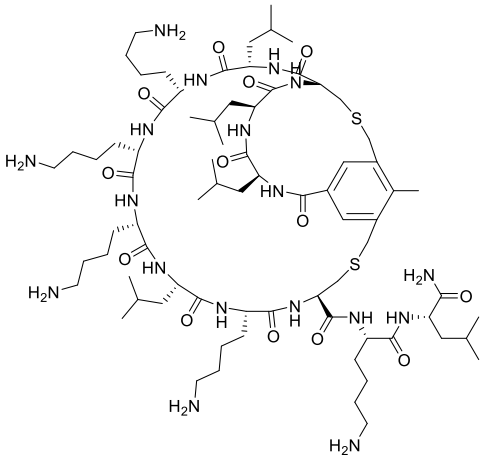


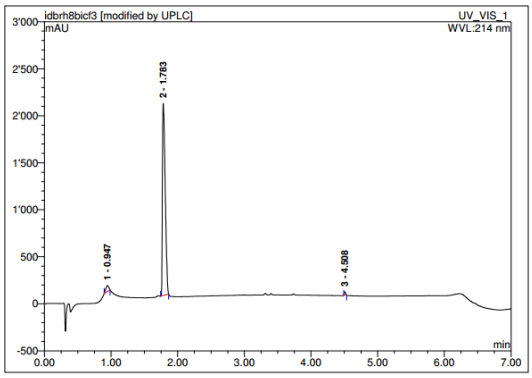
B[12]LLC[1]KLKC[2]KLKL (bp4) was obtained as foamy white solid after preparative RP-HPLC (35.0 mg, 22.4 %). Analytical RP-HPLC: t_R = 1.98 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS(ESI+): $C_{70}H_{122}N_{16}O_{12}S_2$ calc./obs.1442.95/1443.49 Da $[M+H]^+$.

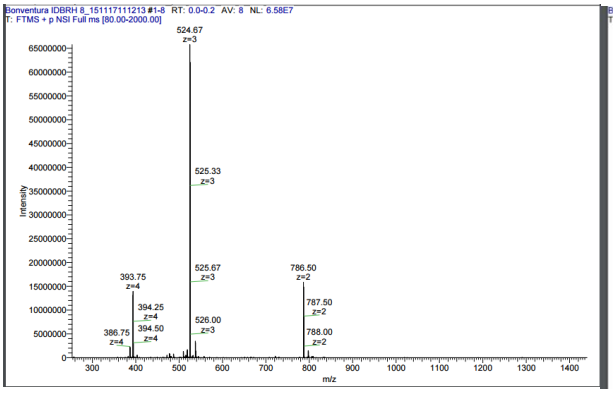


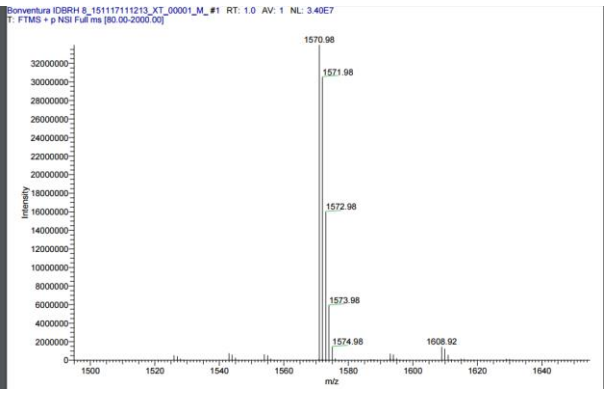


B[12]LLC[1]LKKKLKC[2]KL (bp5) was obtained as foamy white solid after preparative RP-HPLC (17.9 mg, 9.8 %). Analytical RP-HPLC: t_R = 2.17 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI+): $C_{76}H_{134}N_{18}O_{13}S_2$ calc./obs. 1570.98/1570.98 Da [M], 1608.92/1608.92 Da [M+K].

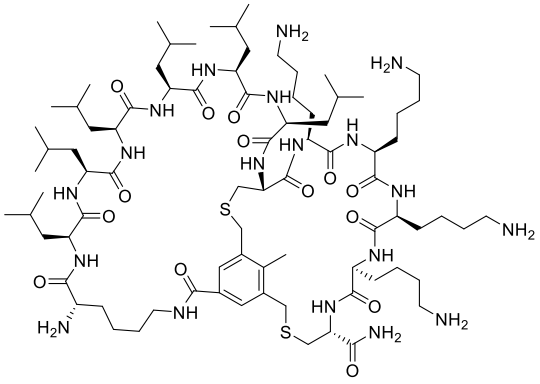


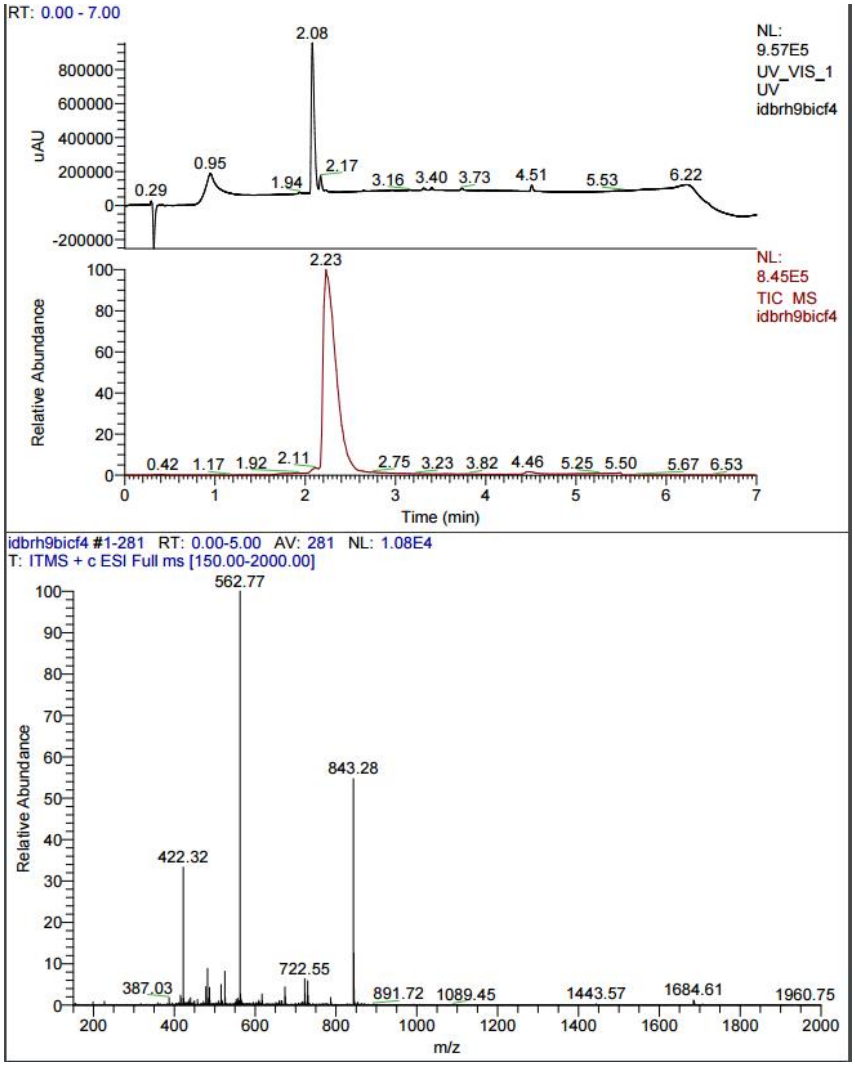




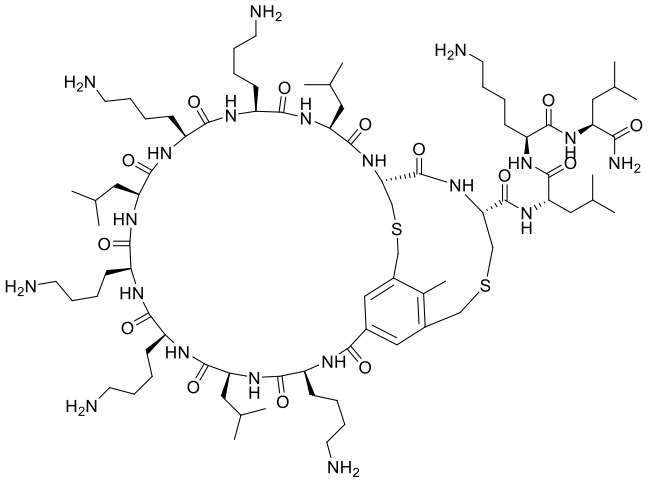


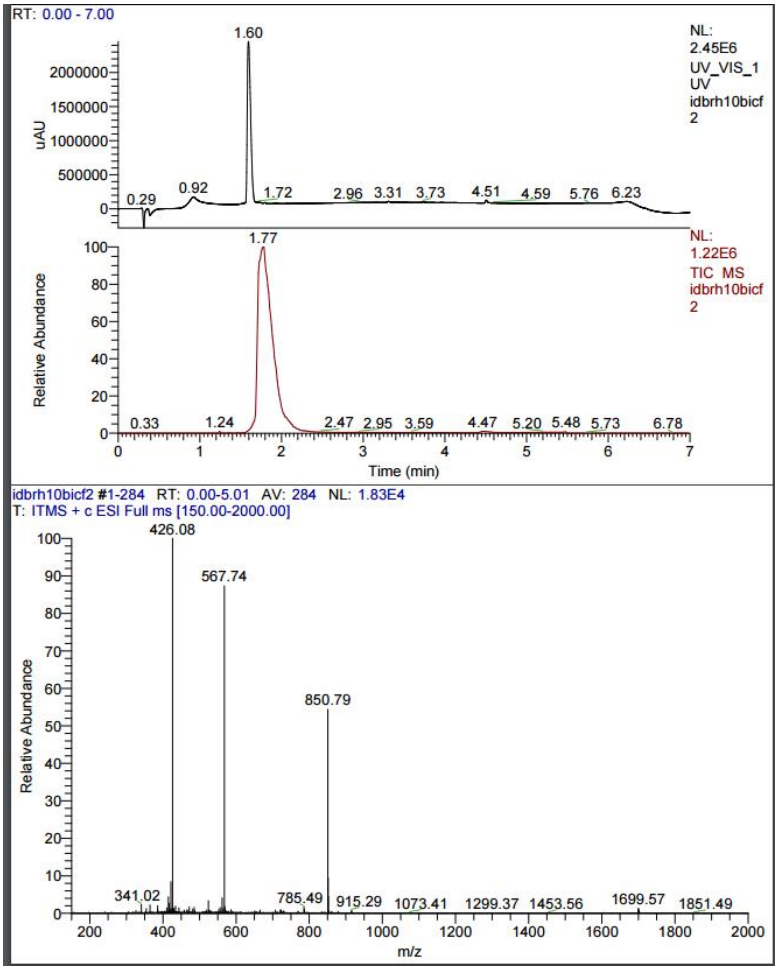
B[12]KLLLLLLC[1]KKKKC[2] (bp6) was obtained as foamy white solid after preparative RP-HPLC (9.6 mg, 5.2 %). Analytical RP-HPLC: t_R = 2.08 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI+): $C_{82}H_{145}N_{19}O_{14}S_2$ calc./obs. 1683.07/1684.61 Da $[M+H]^+$.



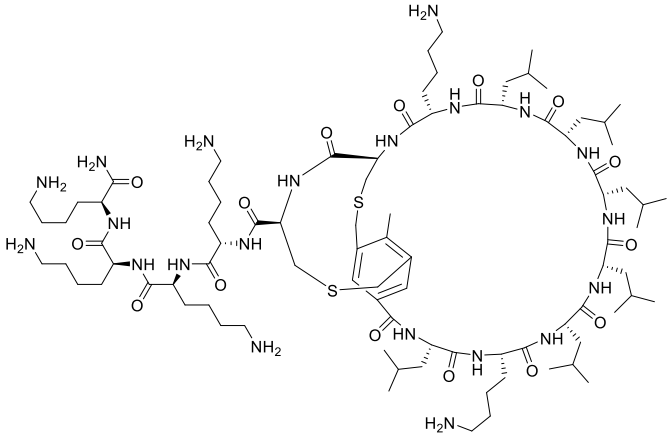


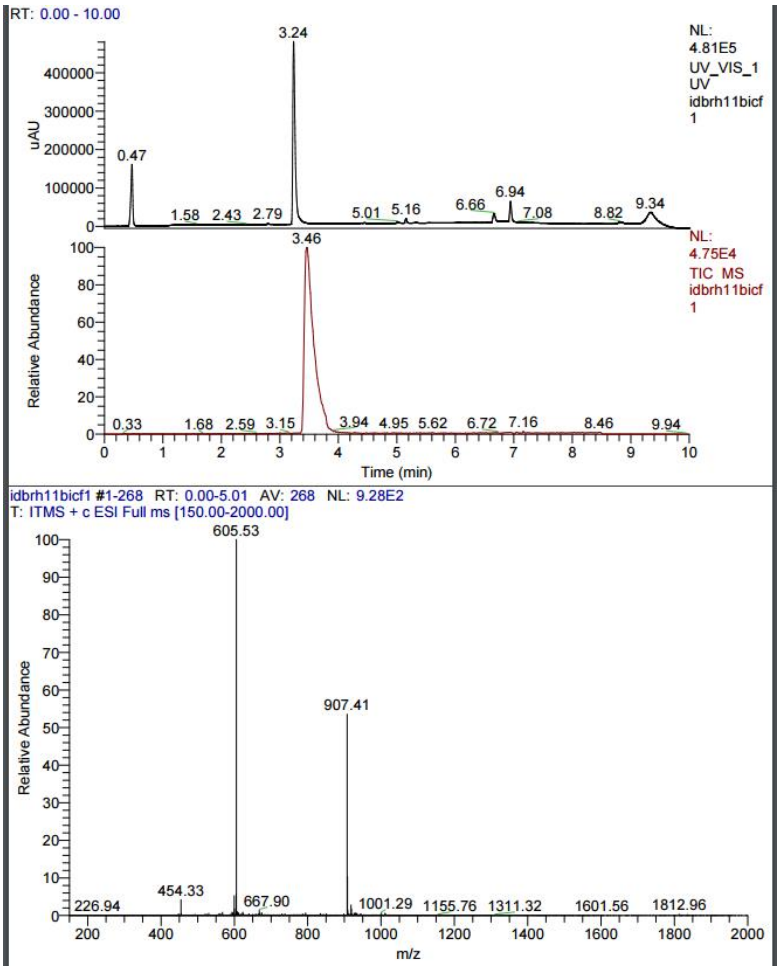
B[12]KLKKLKKLC[1]C[2]LKL (bp7) was obtained as foamy white solid after preparative RP-HPLC (16.0 mg, 8.7 %). Analytical RP-HPLC: t_R = 1.60 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI+): $C_{82}H_{146}N_{20}O_{14}S_2$ calc./obs. 1698.08/1699.57 Da $[M+H]^+$.



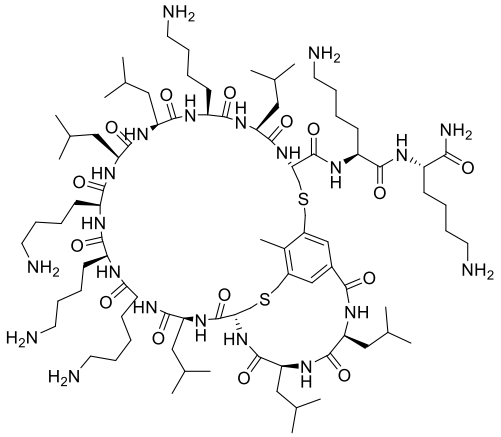


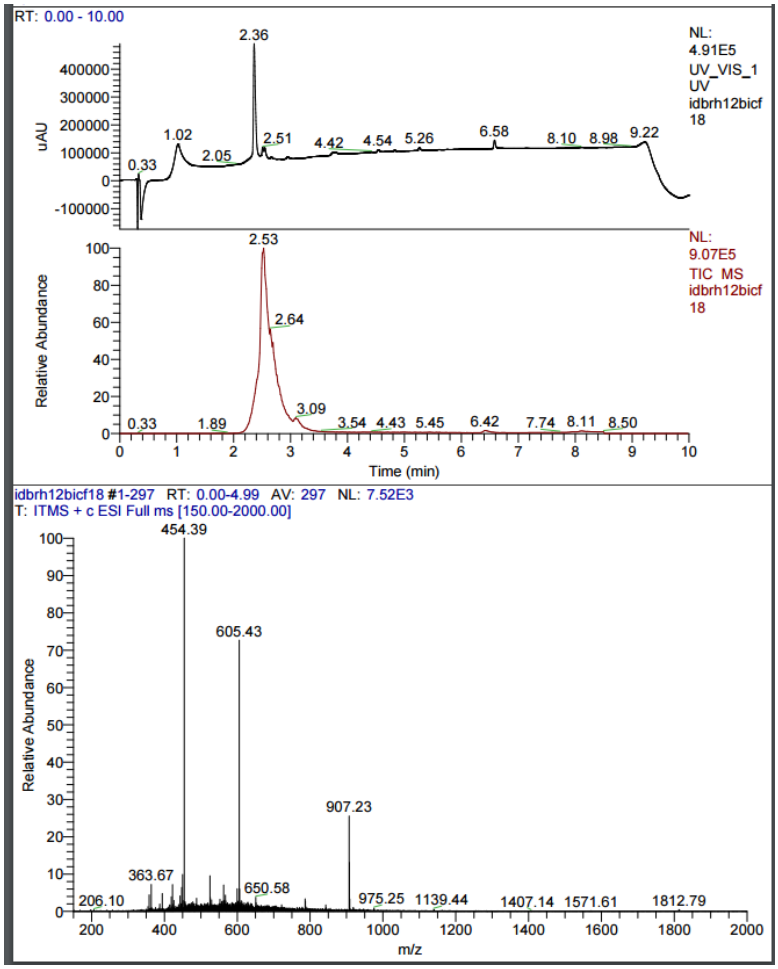
B[12]LKLLLLLKC[1]C[2]KKKK (bp8) was obtained as foamy white solid after preparative RP-HPLC (13.8 mg, 7.0 %). Analytical RP-HPLC: t_R = 3.24 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS(ESI$^+$): $C_{88}H_{157}N_{21}O_{15}S_2$ calc./obs.1811.16/1812.96 Da $[M+H]^+$.



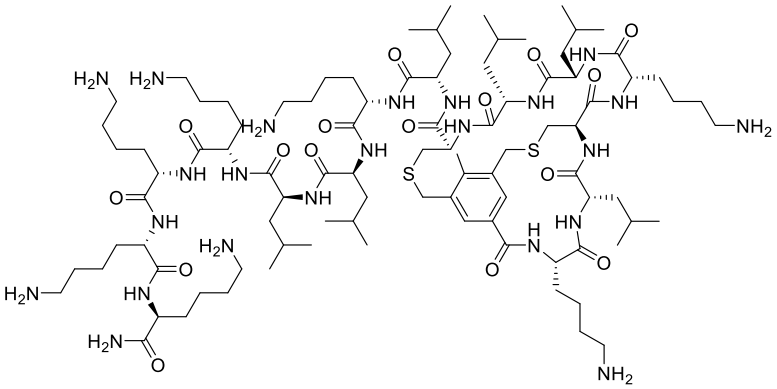


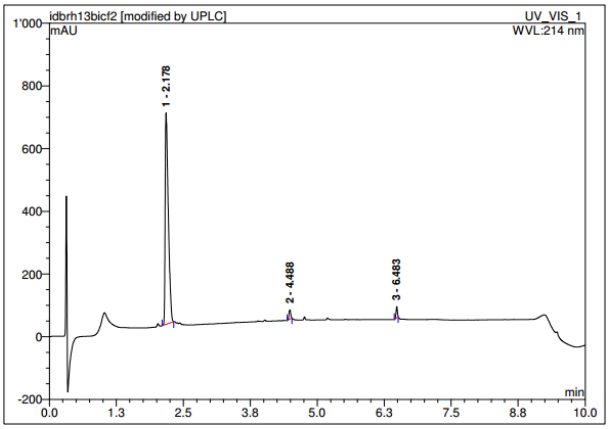
B[12]LLC[1]LKKKLLKLC[2]KK (bp9) was obtained as foamy white solid after preparative RP-HPLC (19.5 mg, 9.9 %). Analytical RP-HPLC: t_R = 2.36 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS(ESI$^+$): $C_{88}H_{157}N_{21}O_{15}S_2$ calc./obs.1811.16/1812.79 Da $[M+H]^+$.

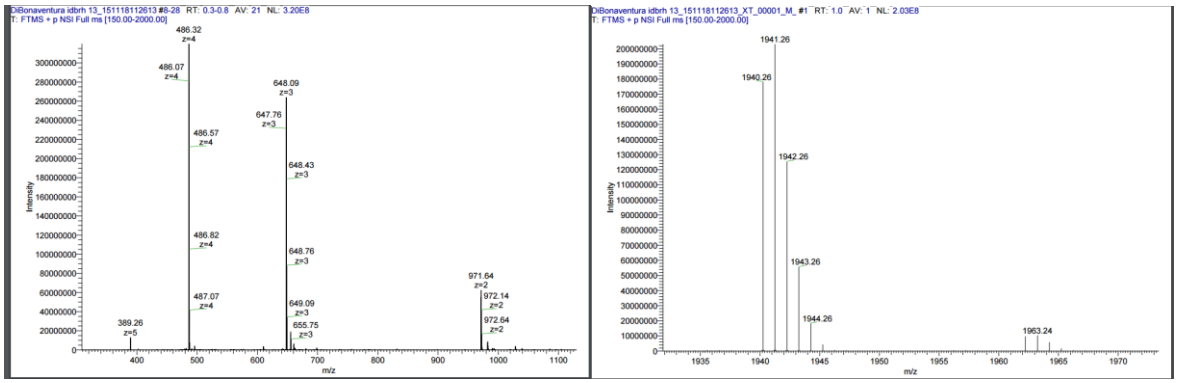




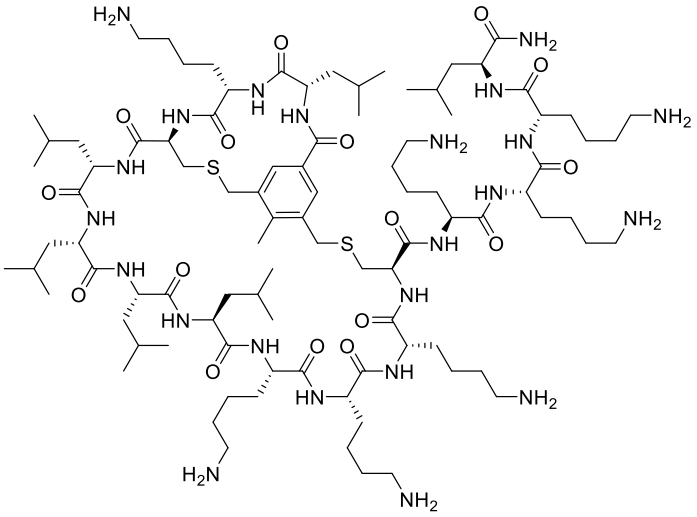
B[12]KLC[1]KLLC[2]LKLLKKKK (bp10) was obtained as foamy white solid after preparative RP-HPLC (9.0 mg, 4.2 %). Analytical RP-HPLC: t_R = 2.17 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS (ESI+): $C_{94}H_{169}N_{23}O_{16}S_2$ calc./obs.1940.25/1940.26 Da [M].

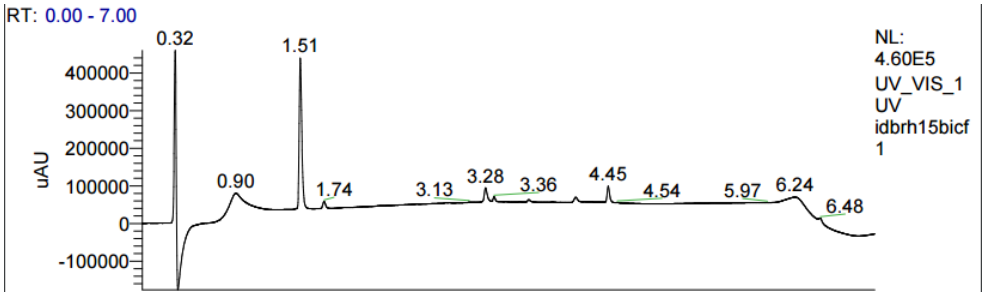


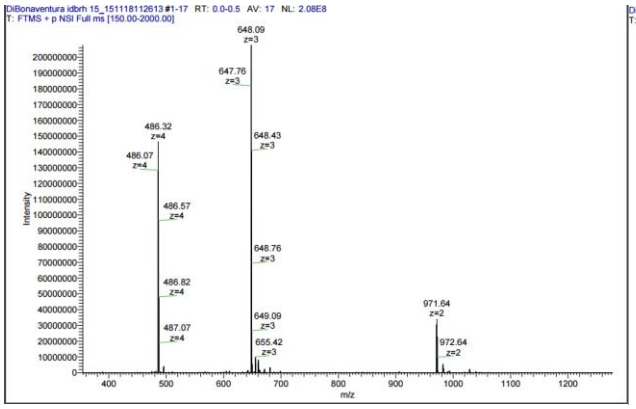


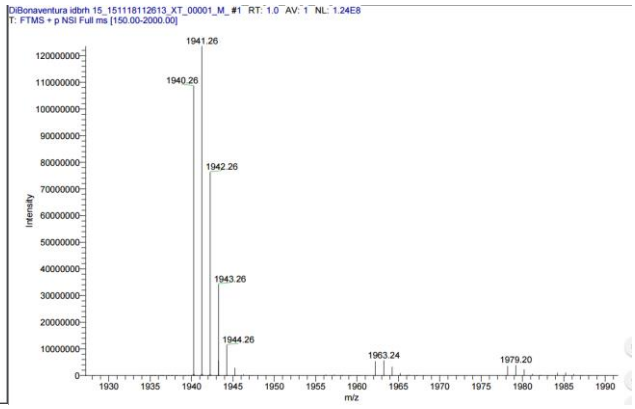


B[12]LKC[1]LLLLKKKC[2]KKKL (bp11) was obtained as foamy white solid after preparative RP-HPLC (20.0 mg, 9.5 %). Analytical RP-HPLC: t_R = 1.51 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI+): $C_{94}H_{169}N_{23}O_{16}S_2$ calc./obs.1940.25/1940.26 Da [M].

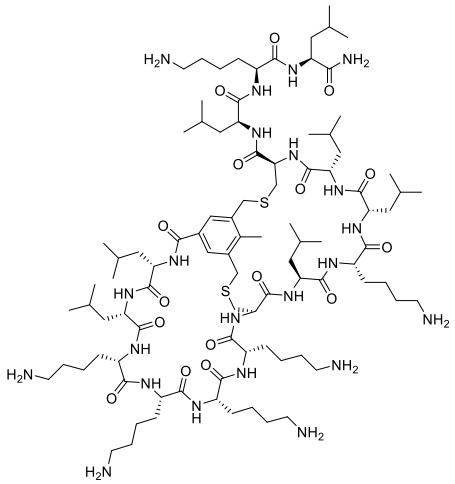


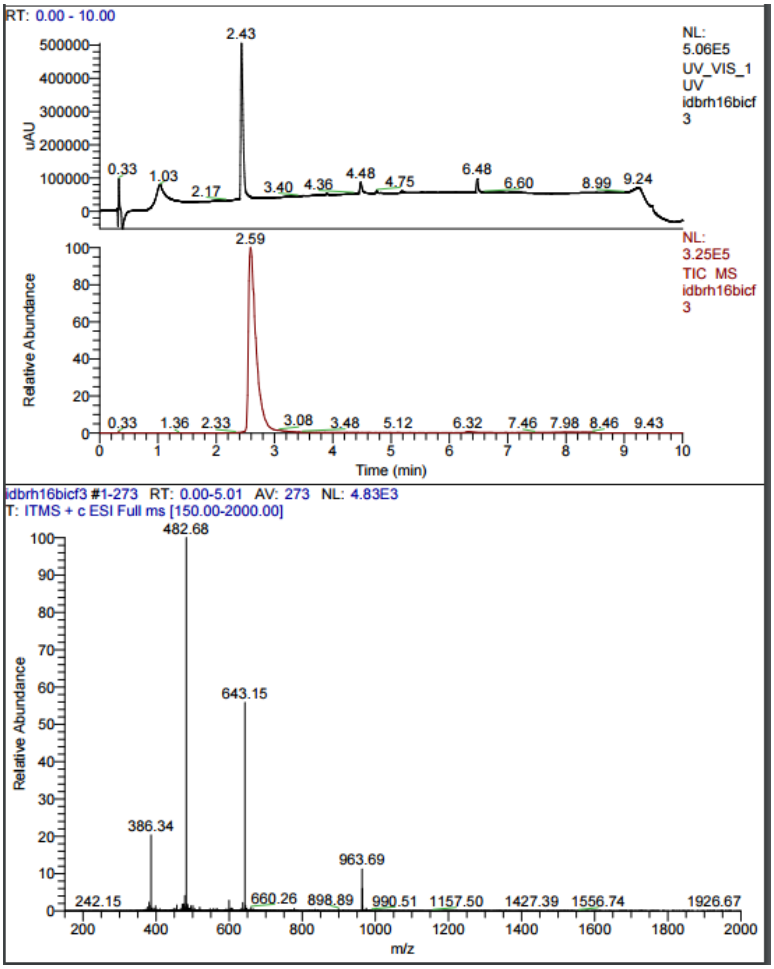




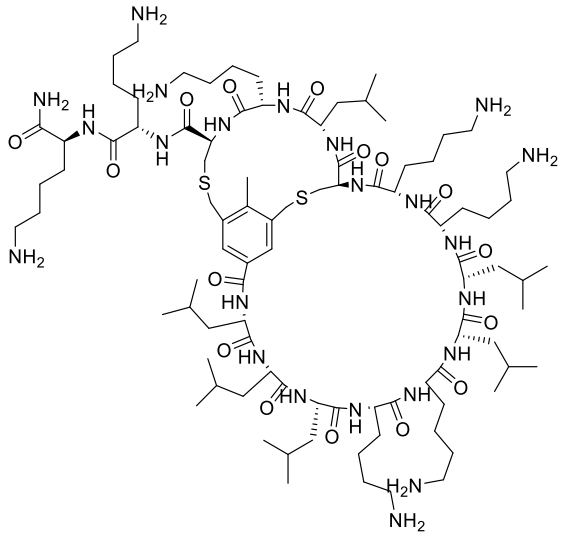


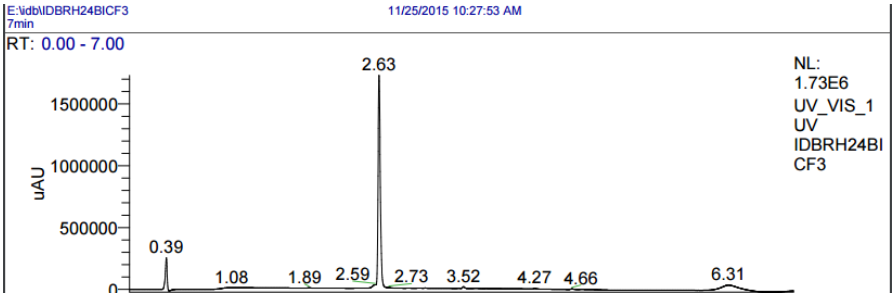
B[12]LLKKKKC[1]LKLLC[2]LKL (bp12) was obtained as foamy white solid after preparative RP-HPLC (12.3 mg, 5.4 %). Analytical RP-HPLC: t_R = 2.43 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS (ESI+): $C_{94}H_{168}N_{22}O_{16}S_2$ calc./obs.1925.60/1926.67 Da $[M+H]^+$.

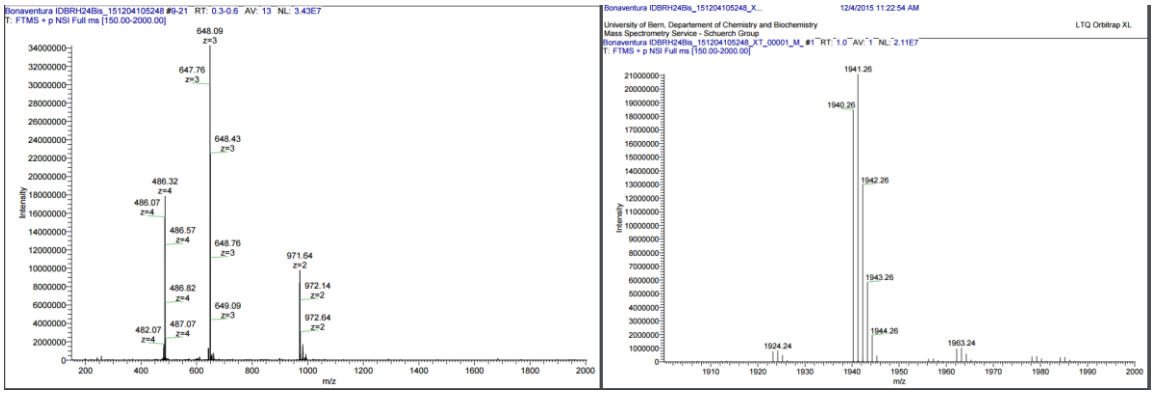




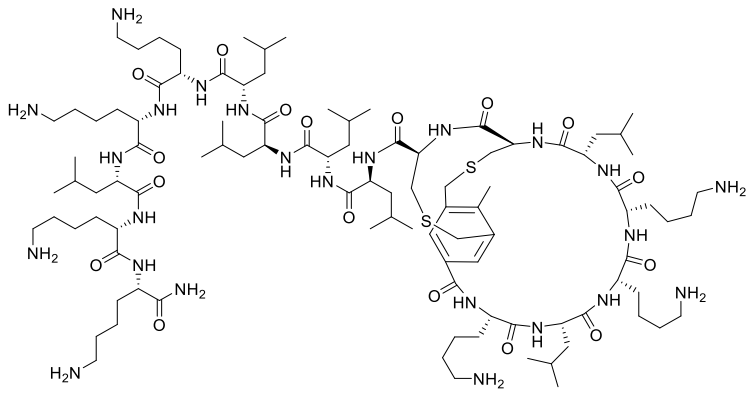
B[12]LLLKKLLKKC[1]LKC[2]KK (bp13) was obtained as foamy white solid after preparative RP-HPLC (34.4 mg, 16.4 %). Analytical RP-HPLC: t_R = 2.63 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{94}H_{169}N_{23}O_{16}S_2$ calc./obs.1940.25/1940.26 Da [M].

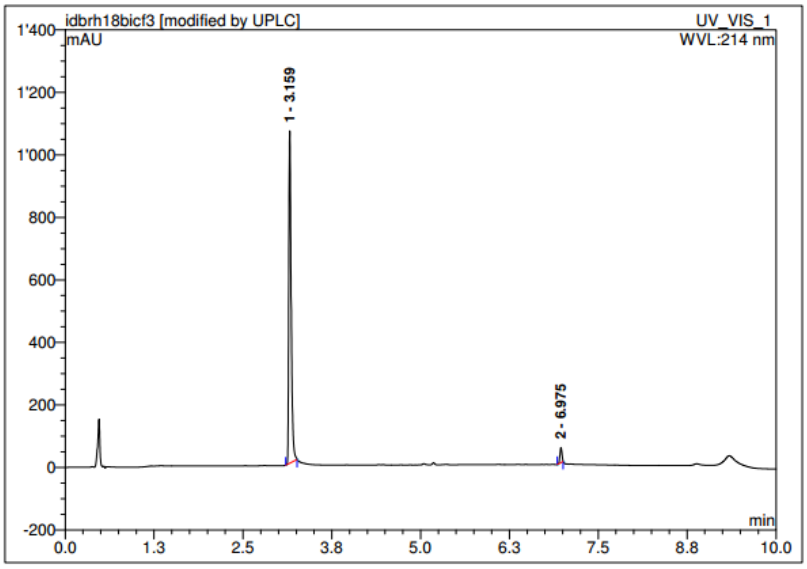


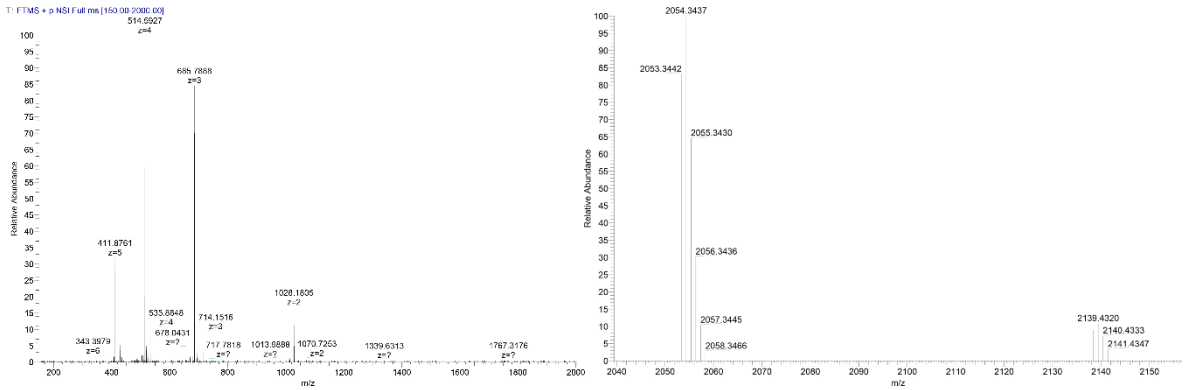




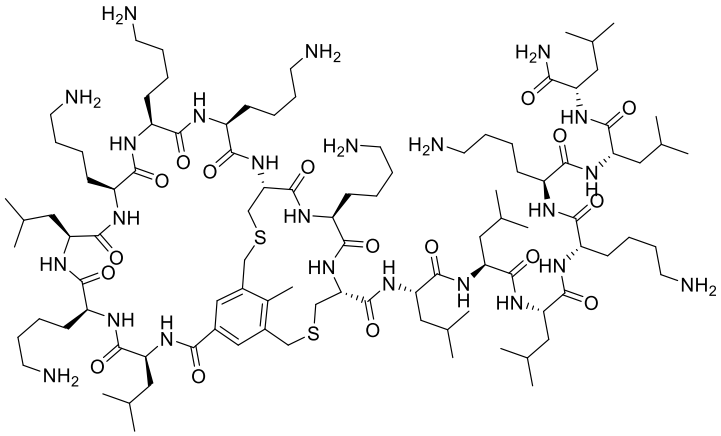
B[12]KLKKLC[1]C[2]LLLLKKLKK (bp14) was obtained as foamy white solid after preparative RP-HPLC (39.4 mg, 17.7 %). Analytical RP-HPLC: t_R = 3.16 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.37/2053.34 Da [M].

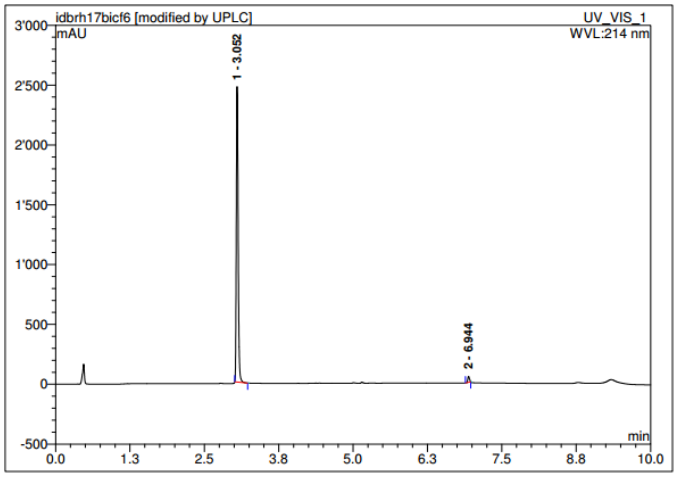


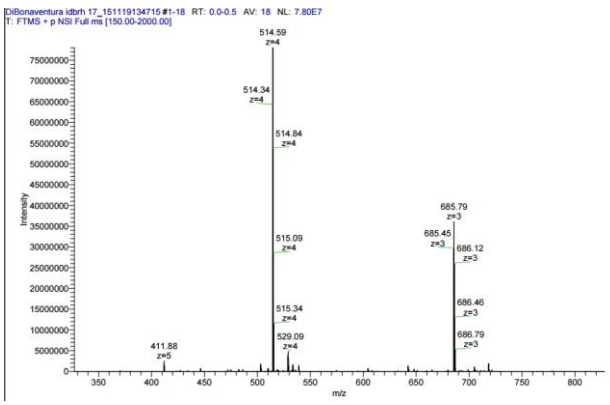


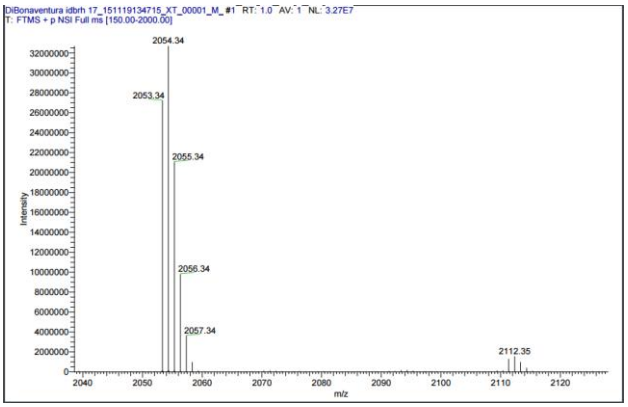


B[12]LKLKKKC[1]KC[2]LLLKKLL (bp15) was obtained as foamy white solid after preparative RP-HPLC (18.4 mg, 8.3 %). Analytical RP-HPLC: t_R = 3.05 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS (ESI+): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

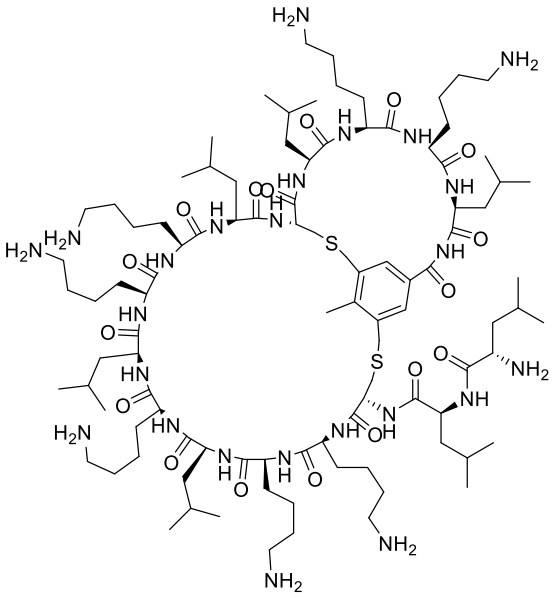


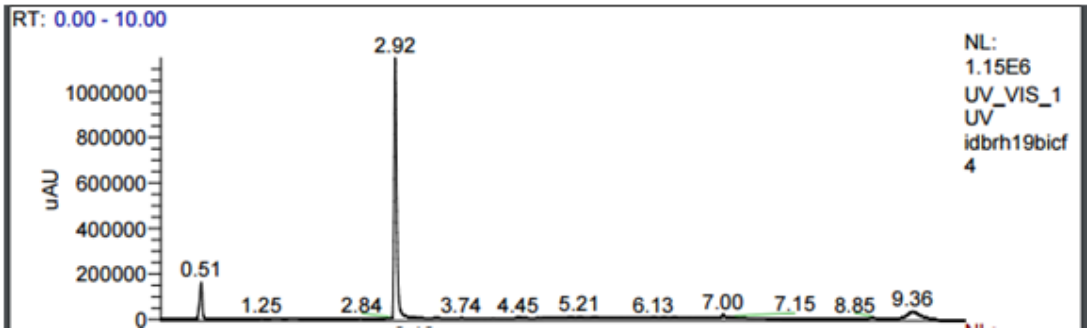


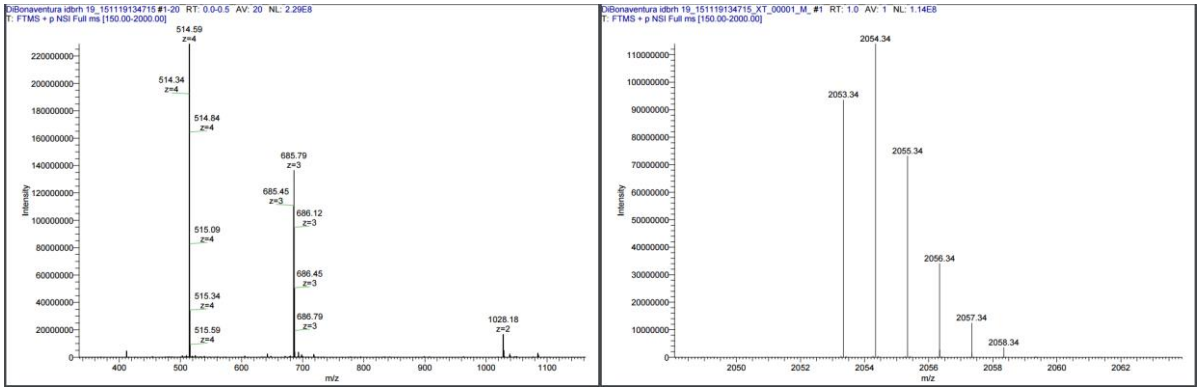




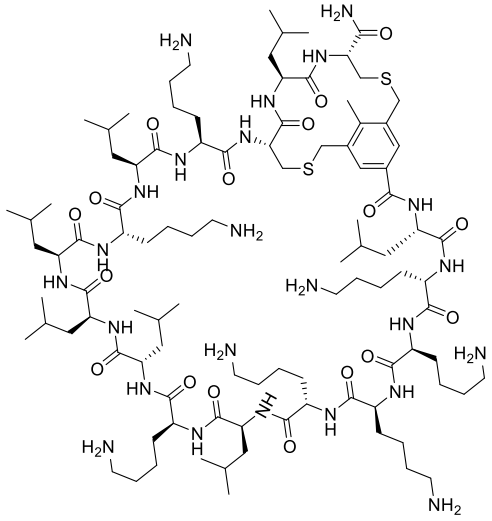
B[12]LLC[1]KKLKLKKLC[2]LKKL (bp16) was obtained as foamy white solid after preparative RP-HPLC (15.7 mg, 7.0 %). Analytical RP-HPLC: t_R = 2.92 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS (ESI+): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.37/2053.34 Da [M].

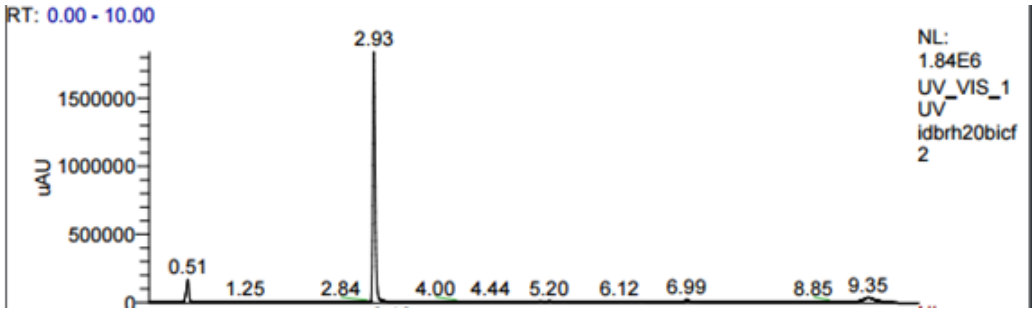


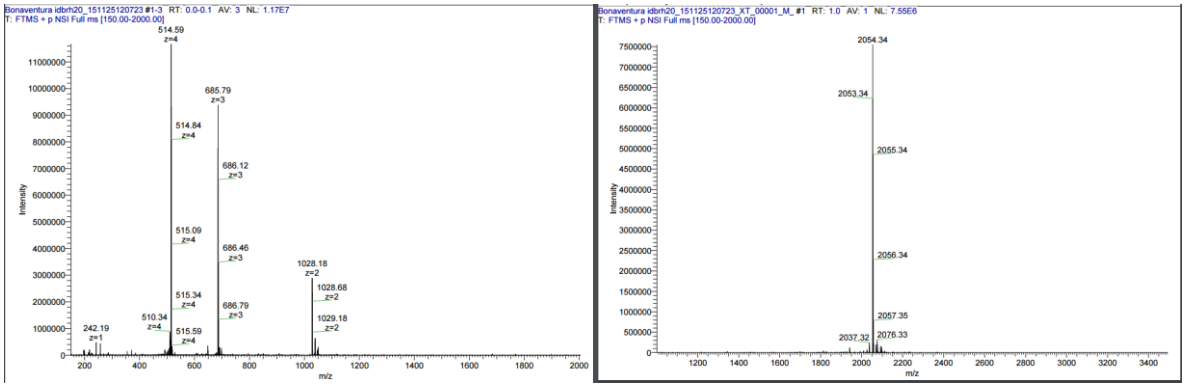




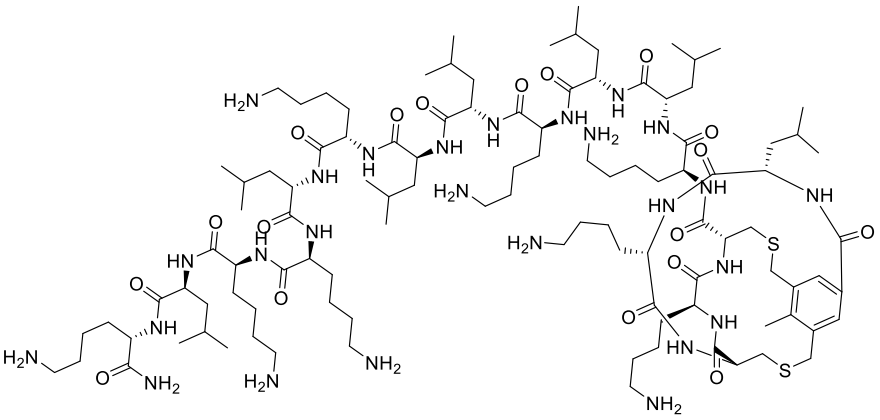
B[12]LKKKKLKLLLKLKC[1]LC[2] (bp17) was obtained as foamy white solid after preparative RP-HPLC (12.1 mg, 5.4 %). Analytical RP-HPLC: t_R = 2.93 min (A/D 100:0 to 0:100 in 10.00 min, λ = 214 nm). MS (ESI+): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.37/2053.34 Da [M].

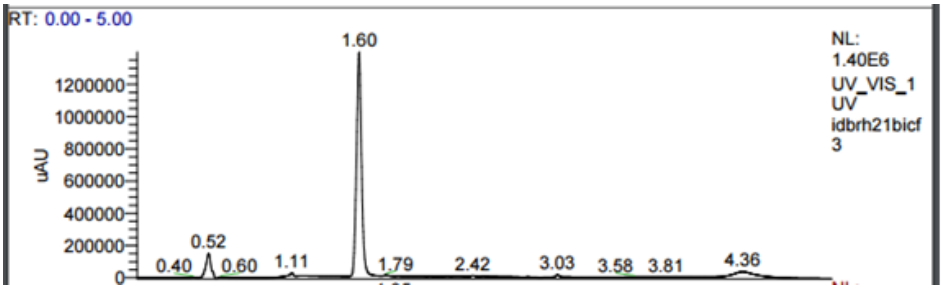


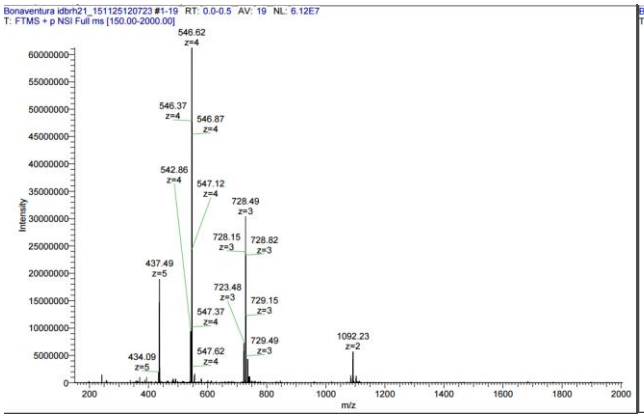


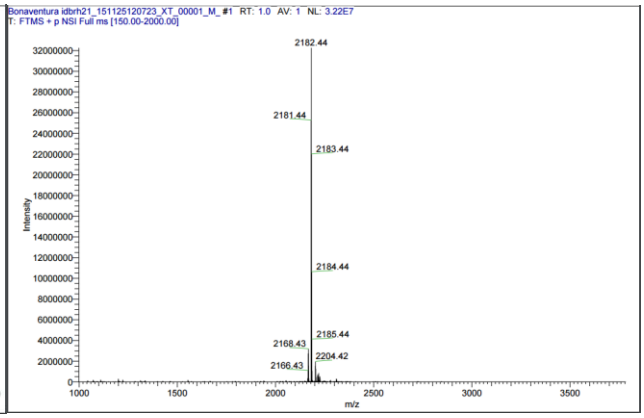


B[12]LKC[1]KC[2]KLLKLLKLKKLK (bp18) was obtained as foamy white solid after preparative RP-HPLC (11.5 mg, 4.8 %). Analytical RP-HPLC: t_R = 1.60 min (A/D 100:0 to 0:100 in 5.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{192}N_{26}O_{18}S_2$ calc./obs. 2181.43/2181.44 Da [M].

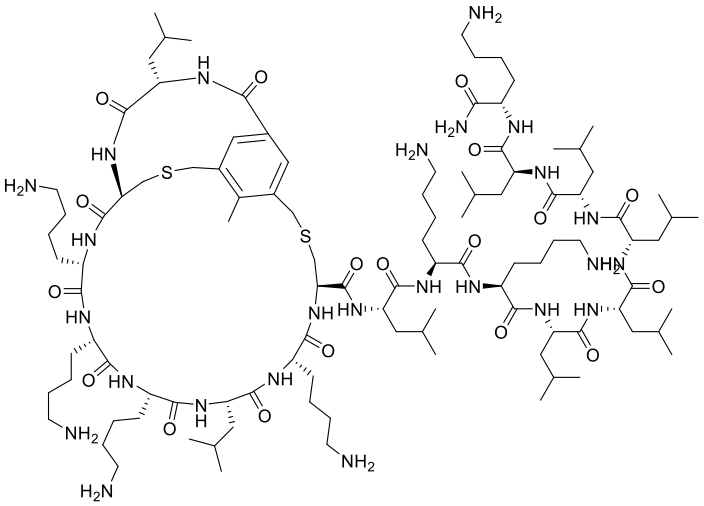


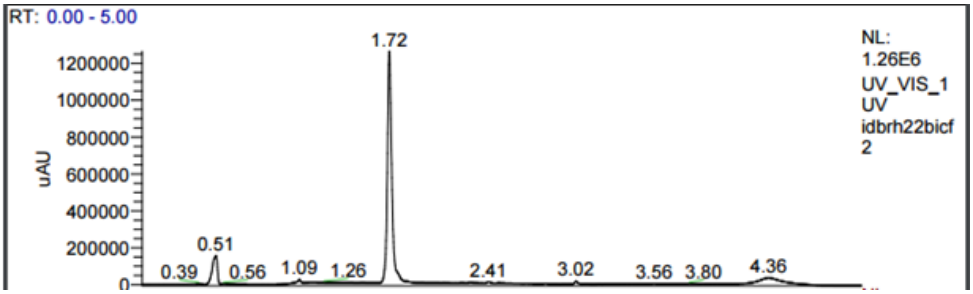


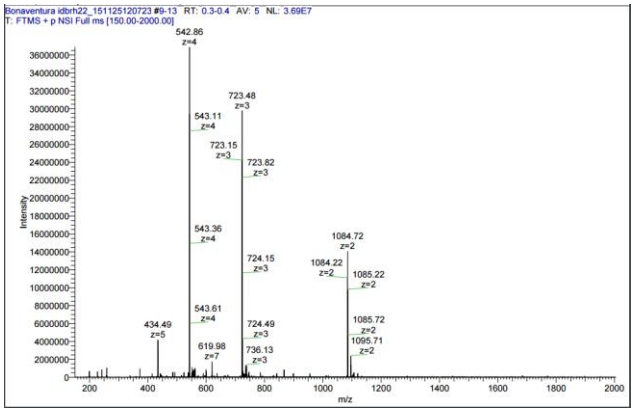


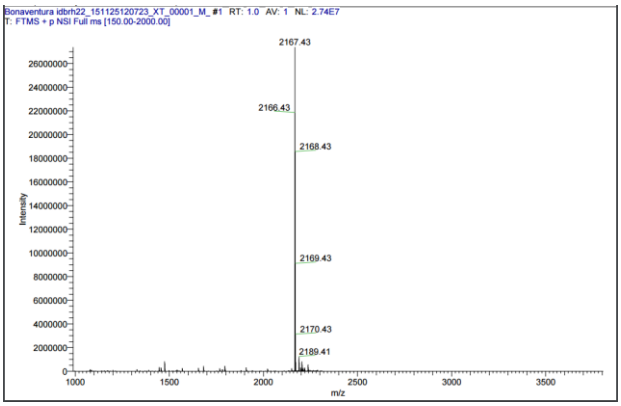


B[12]LC[1]KKKLKC[2]LKKLLLLLK (bp19) was obtained as foamy white solid after preparative RP-HPLC (22.9 mg, 9.7 %). Analytical RP-HPLC: t_R = 1.72 min (A/D 100:0 to 0:100 in 5.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.42/2166.43 Da [M].

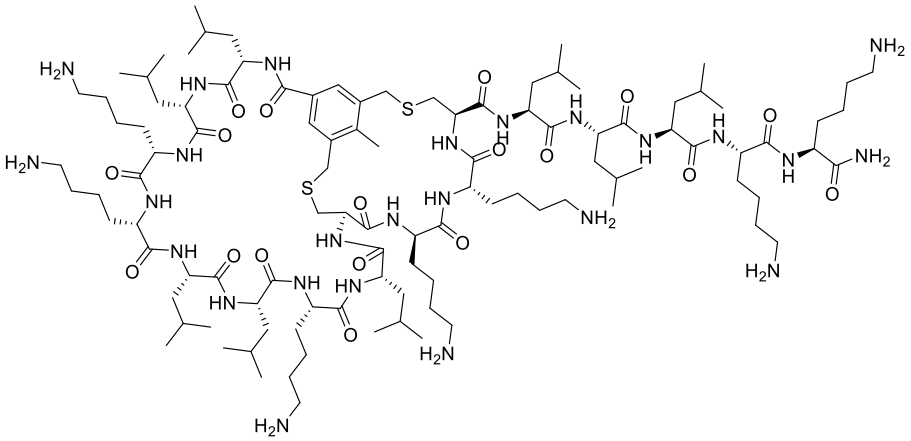


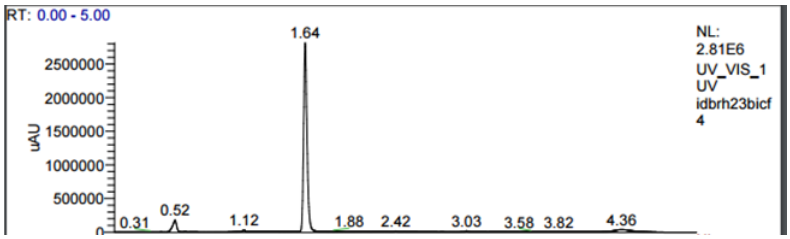


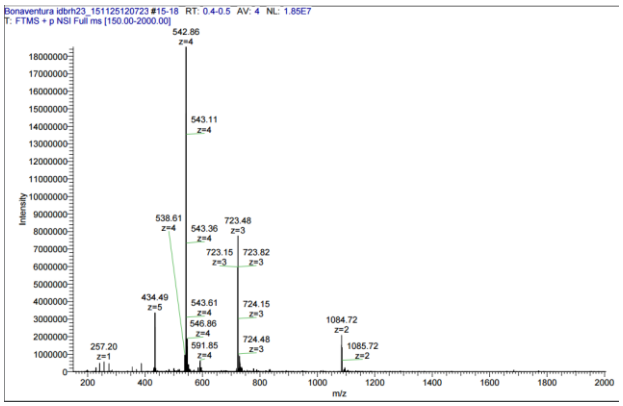


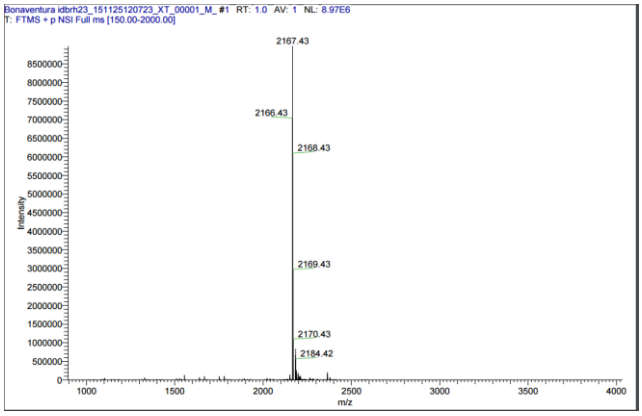


B[12]LLKKLLKLC[1]KKC[2]LLLKK (bp20) was obtained as foamy white solid after preparative RP-HPLC (35.3 mg, 15.1 %). Analytical RP-HPLC: t_R = 1.64 min (A/D 100:0 to 0:100 in 5.00 min, λ = 214 nm). MS (ESI+): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.42/2166.43 Da [M].

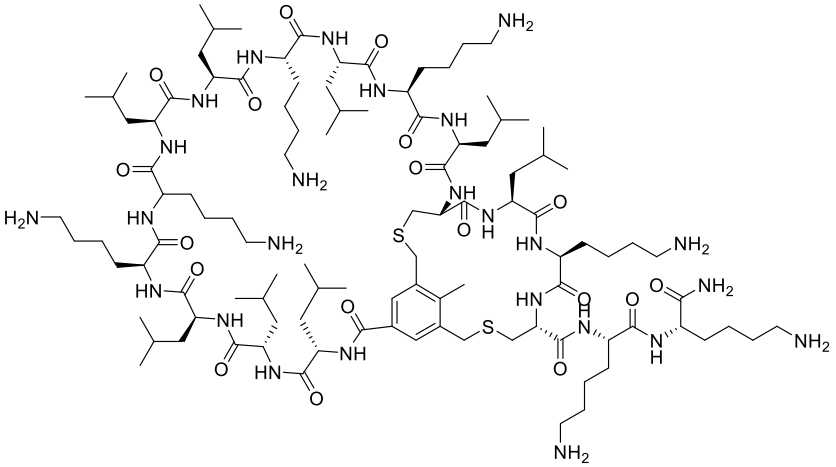


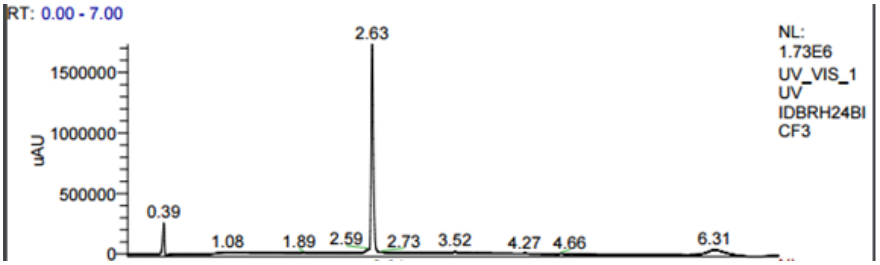


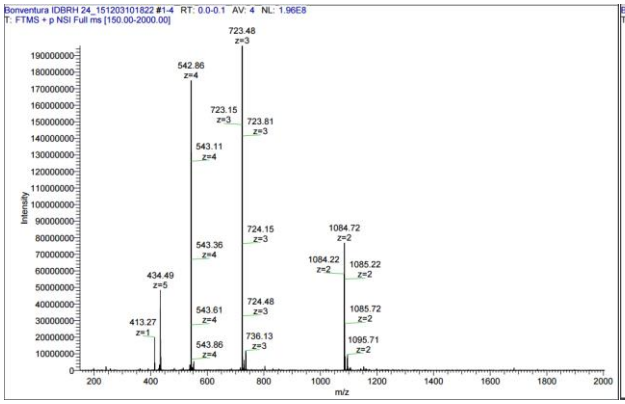


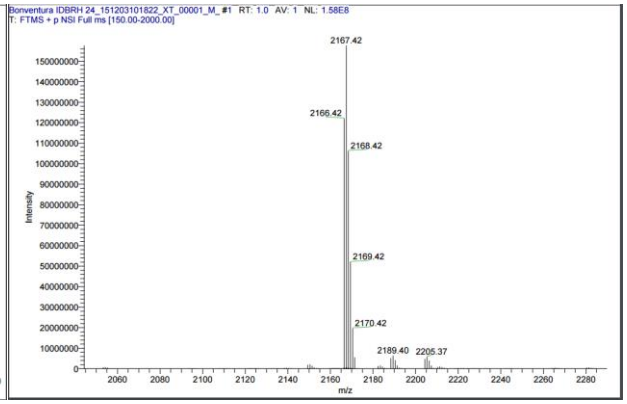


B[12]LLLKKLLKLKLC[1]LKC[2]KK (bp21) was obtained as foamy white solid after preparative RP-HPLC (21.5 mg, 9.1 %). Analytical RP-HPLC: t_R = 2.63 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.42/2166.42 Da [M].

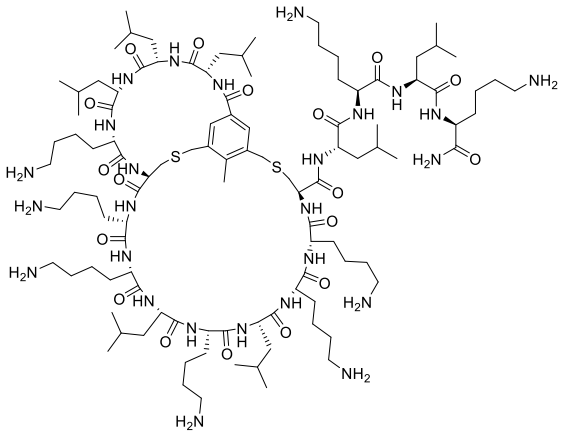


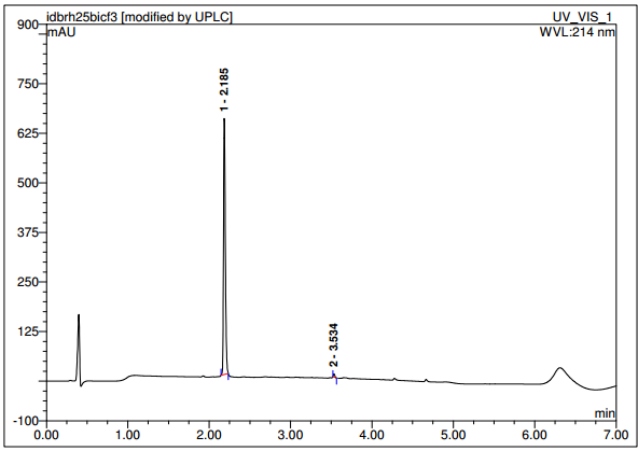


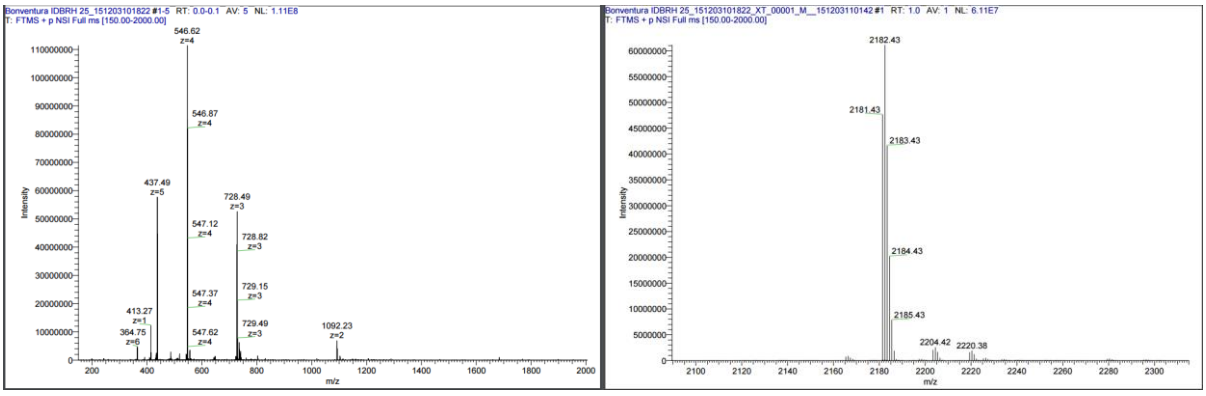




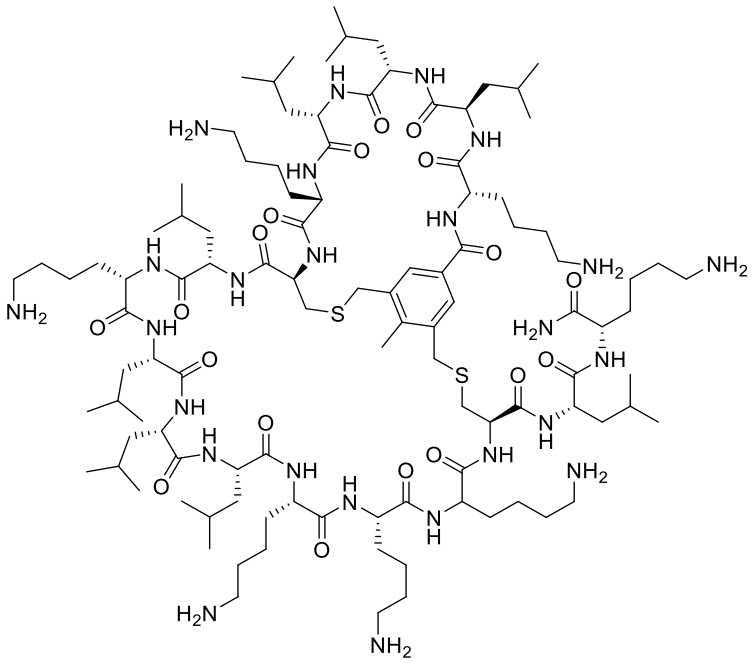
B[12]LLLKC[1]KKLKLKKC[2]LKLK (bp22) was obtained as foamy white solid after preparative RP-HPLC (16.9 mg, 7.1 %). Analytical RP-HPLC: t_R = 2.19 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{192}N_{26}O_{18}S_2$ calc./obs. 2181.43/2181.43 Da [M].

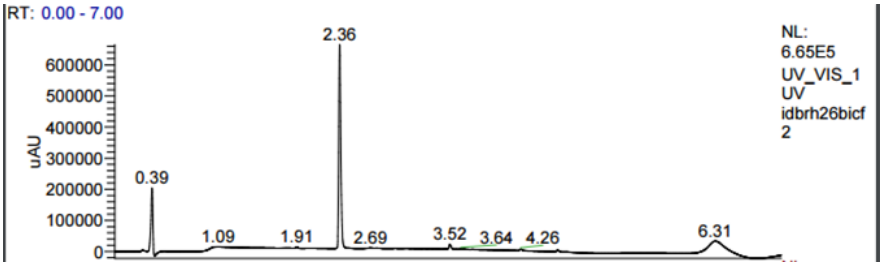


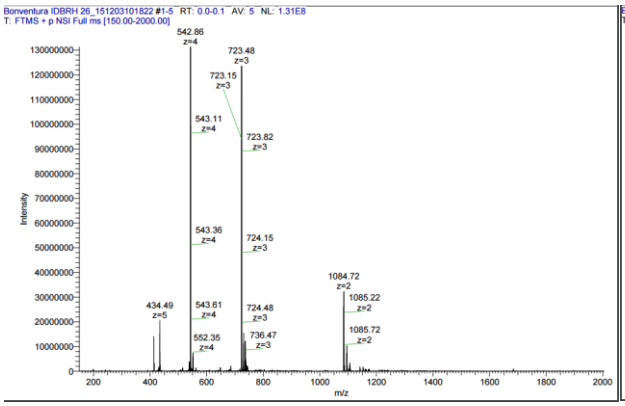


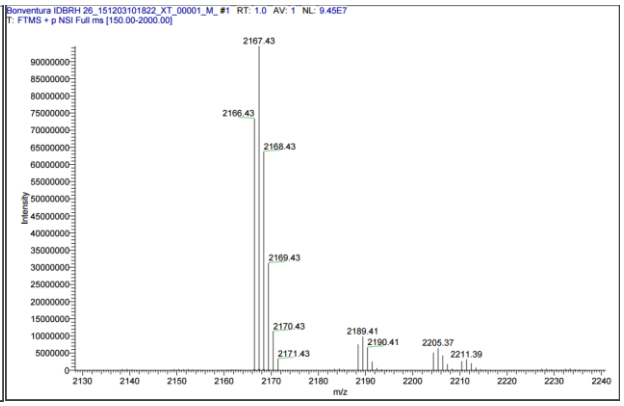


B[12]KLLLKC[1]LKLLLKKKC[2]LK (bp23) was obtained as foamy yellow solid after preparative RP-HPLC (22.7 mg, 9.6 %). Analytical RP-HPLC: t_R = 2.36 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.42/2166.43 Da [M].

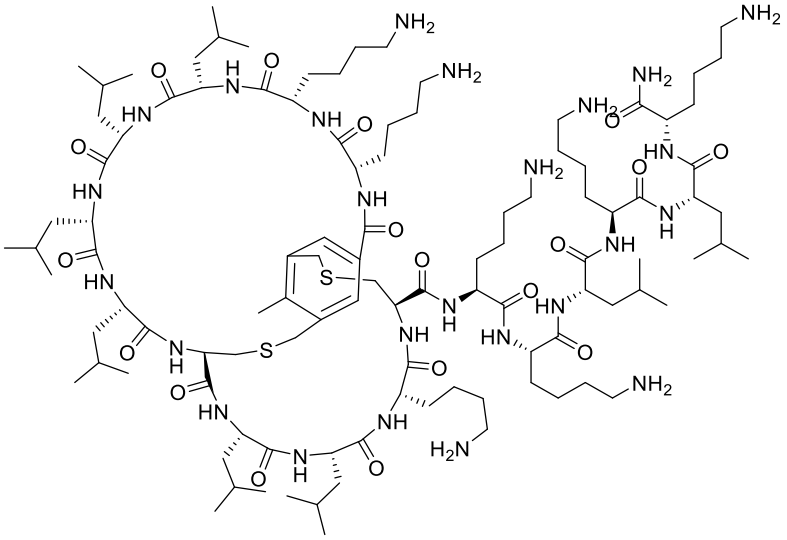


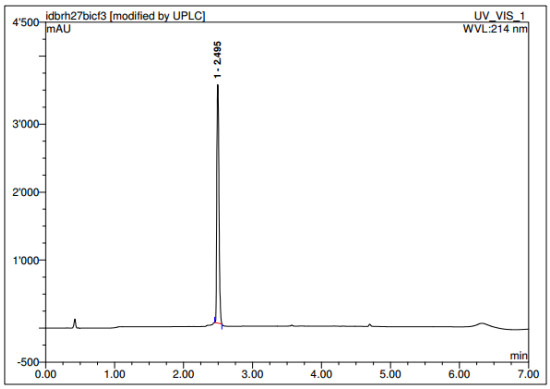


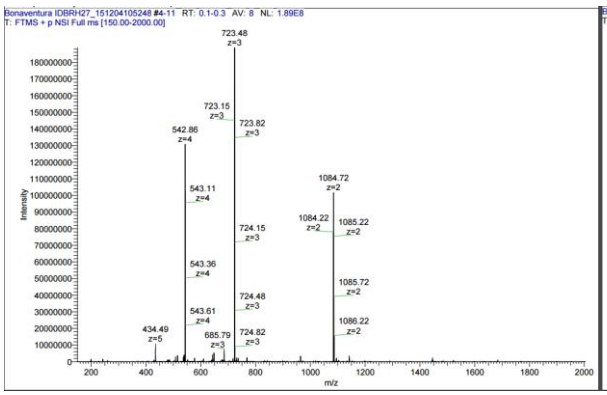


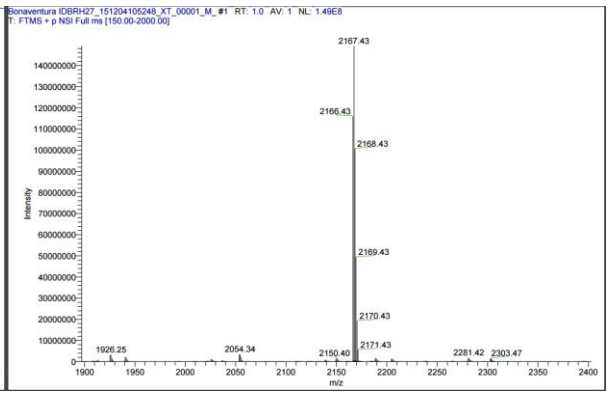


B[12]KKLLLLC[1]LLKC[2]KKLKLK (bp24) was obtained as foamy white solid after preparative RP-HPLC (17.8 mg, 7.6 %). Analytical RP-HPLC: t_R = 2.49 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$calc./obs. 2166.42/2166.43 Da [M].

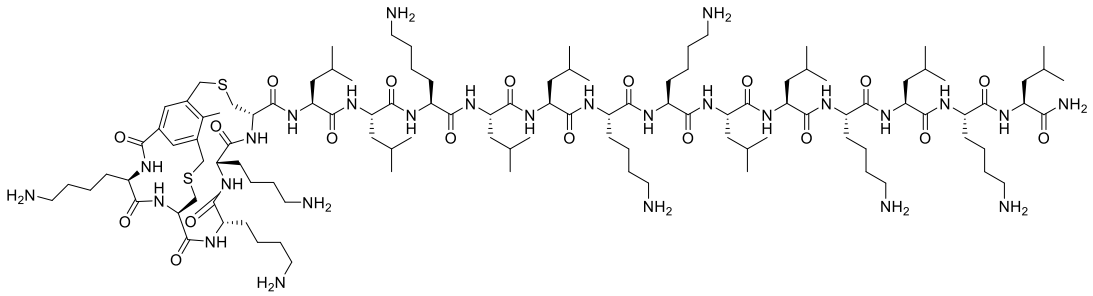


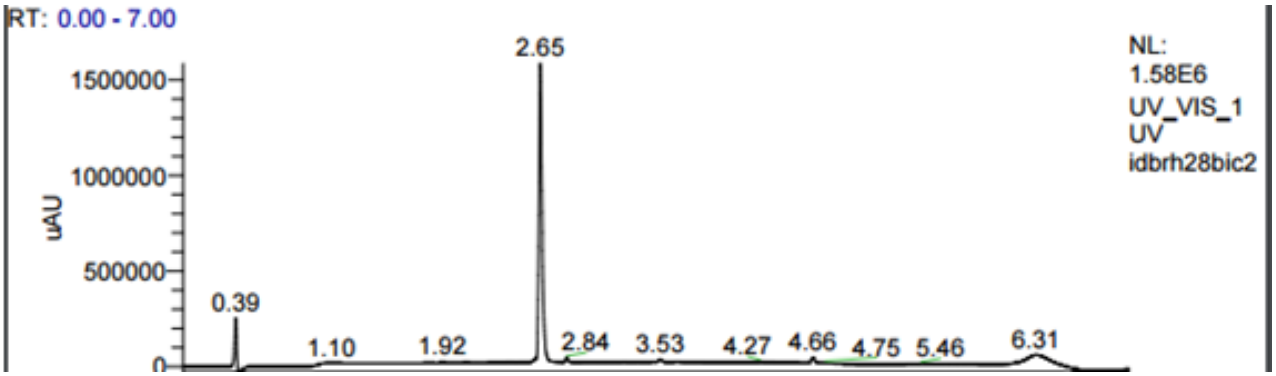


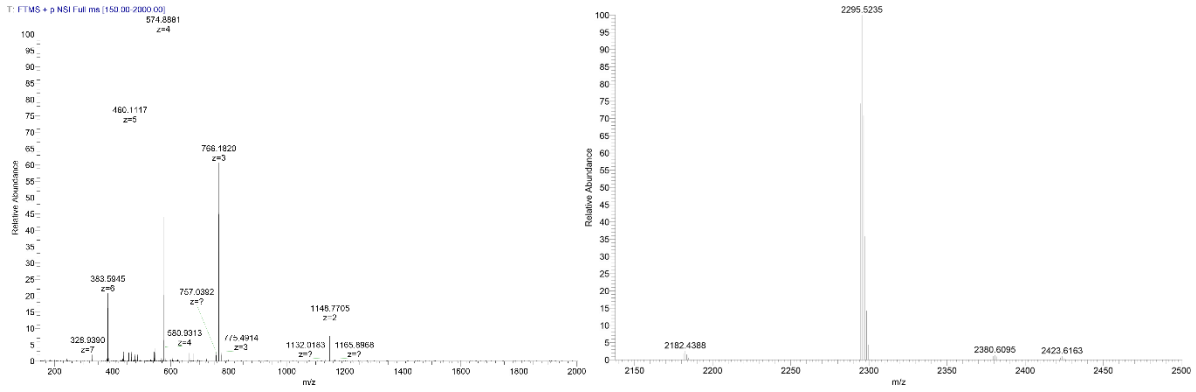


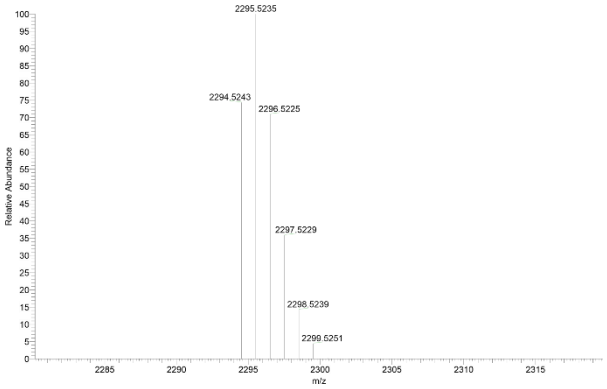


B[12]KC[1]KKC[2]LLKLLKKLLKLKL (bp25) was obtained as foamy white solid after preparative RP-HPLC (10.6 mg, 4.2 %). Analytical RP-HPLC: t_R = 2.65 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.52/2294.52 Da [M].

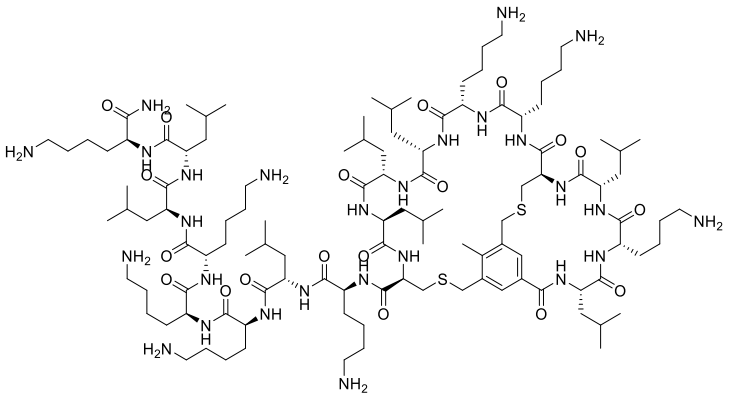


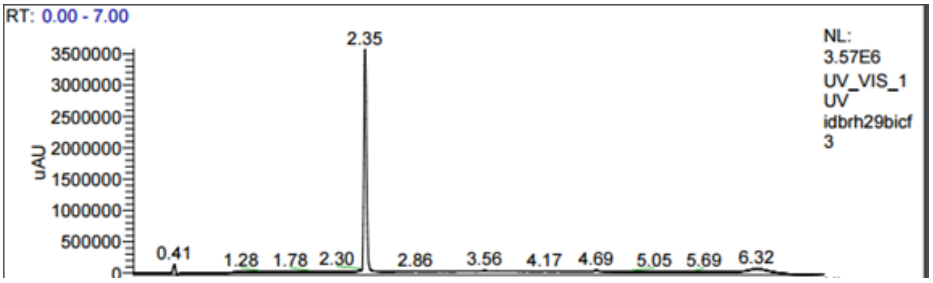


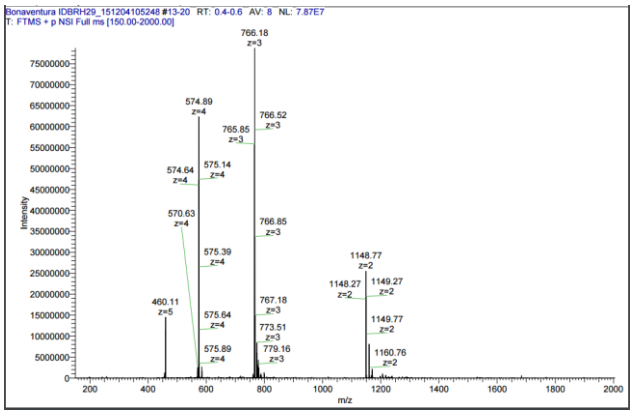


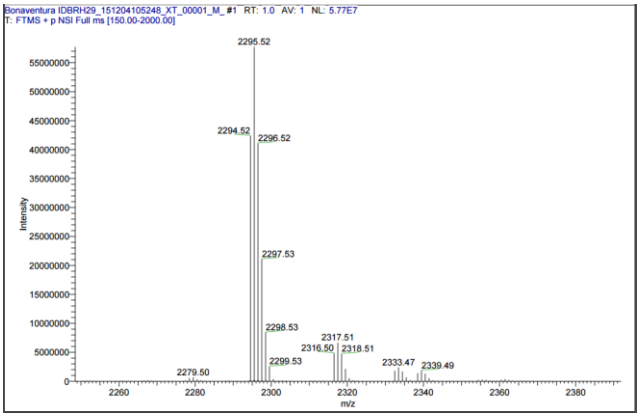


B[12]LKLC[1]KKLLLC[2]KLKKKLLK (bp26) was obtained as foamy white solid after preparative RP-HPLC (35.8 mg, 14.6 %). Analytical RP-HPLC: t_R = 2.35 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.51/2294.52 Da [M].

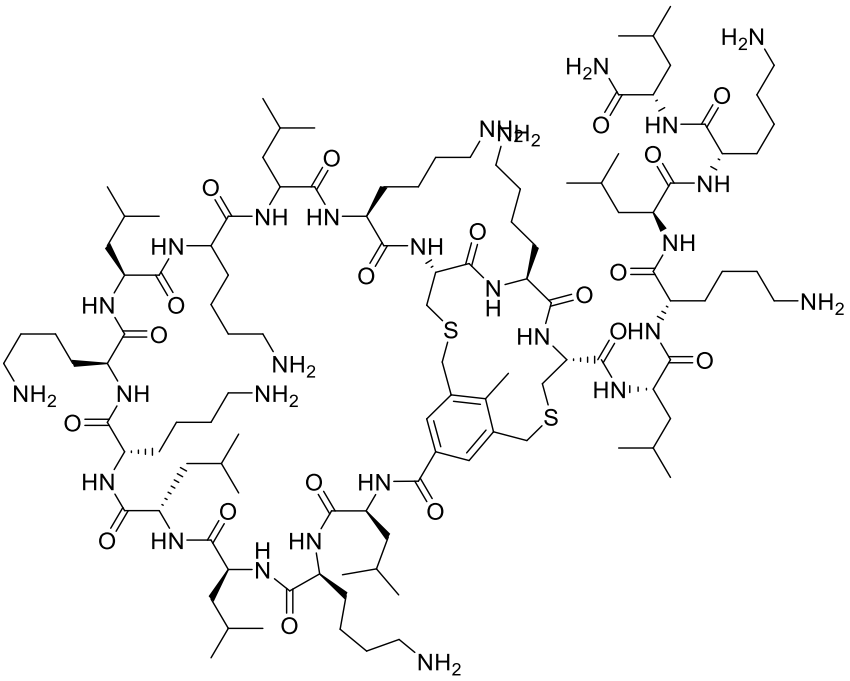


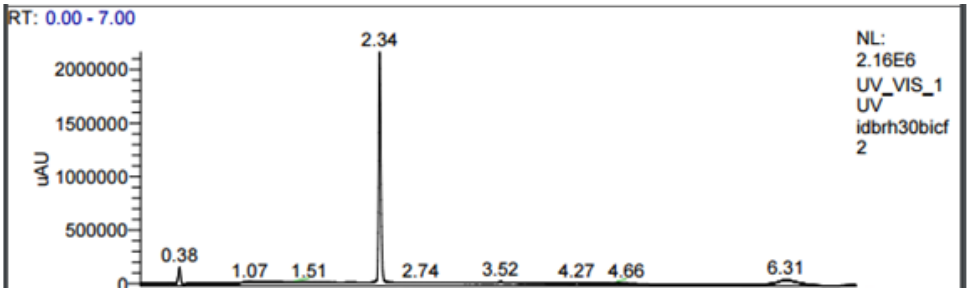


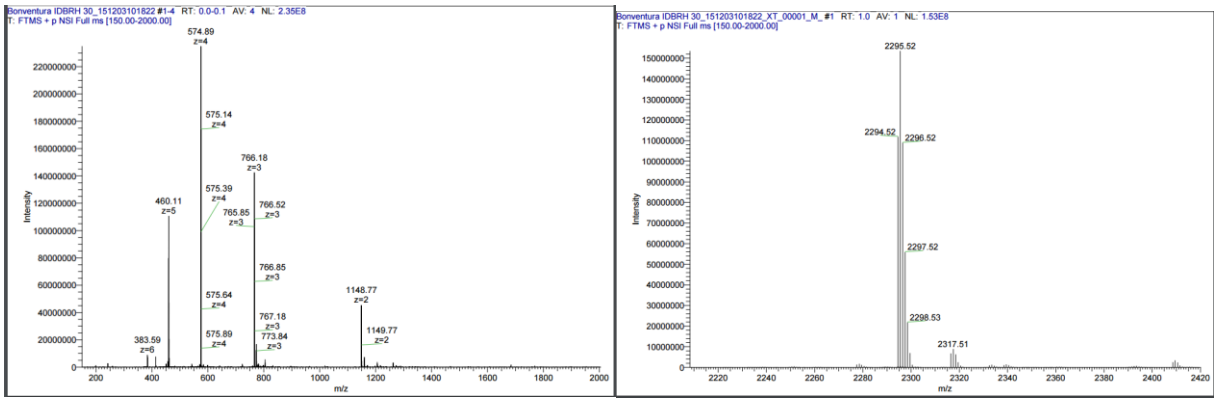




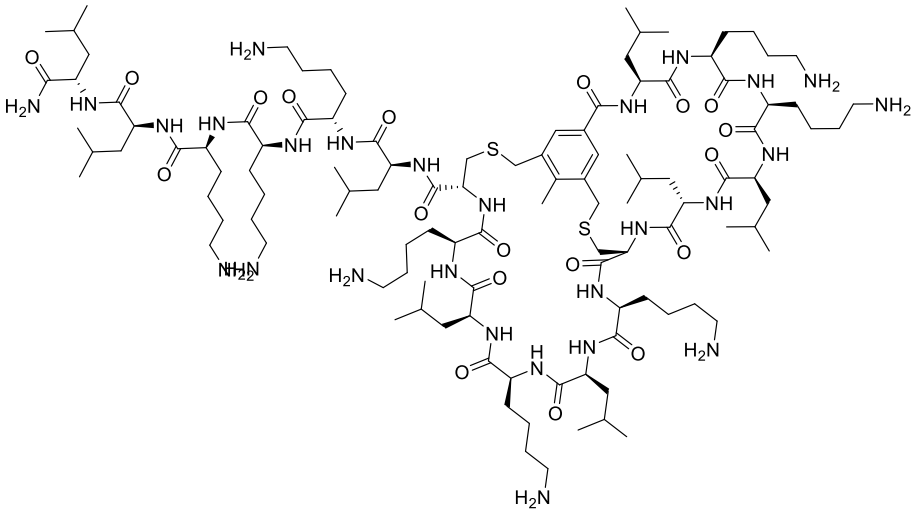
B[12]LKLLKKLKLKC[1]KC[2]LKLKL (bp27) was obtained as foamy white solid after preparative RP-HPLC (23.4 mg, 9.4 %). Analytical RP-HPLC: t_R = 2.34 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.51/2294.52 Da [M].

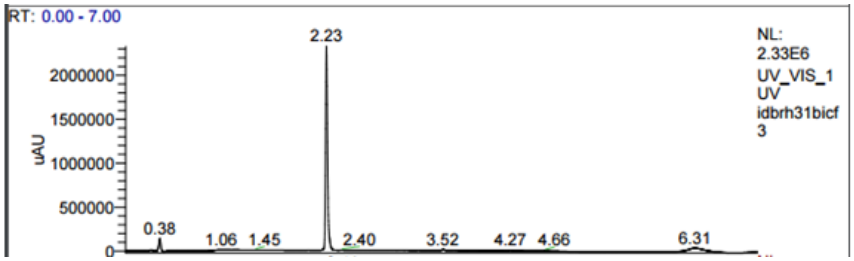


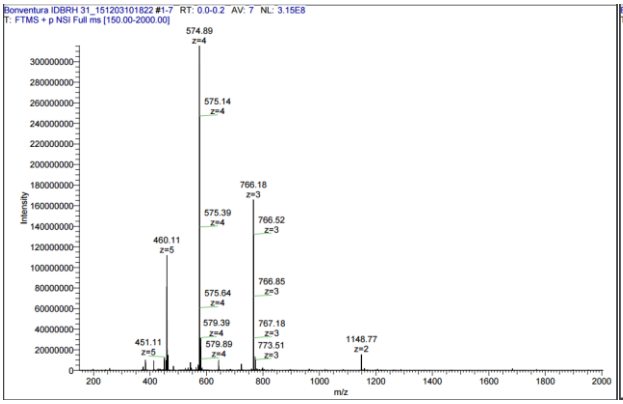


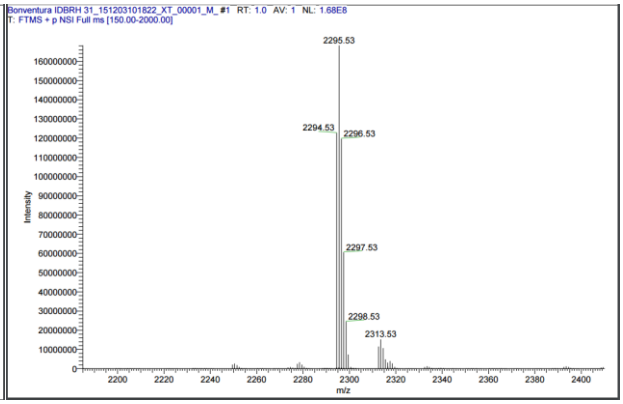


B[12]LKKLLC[1]KLKLKC[2]LKKKLL (bp28) was obtained as foamy white solid after preparative RP-HPLC (34.8 mg, 14.0%). Analytical RP-HPLC: t_R = 2.23 min (A/D 100:0 to 0:100 in 7.0 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.51/2294.53 Da [M].

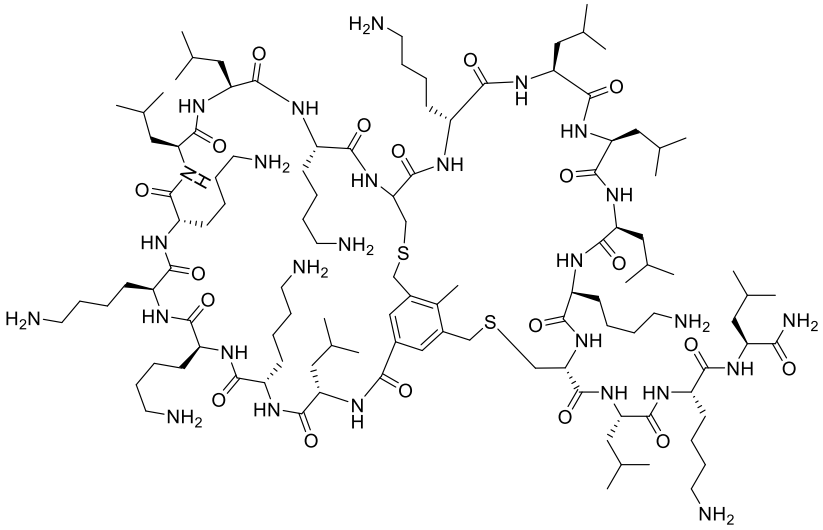


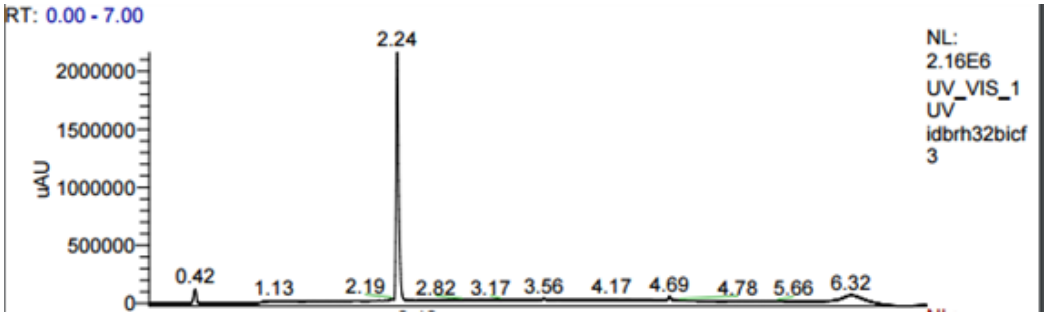


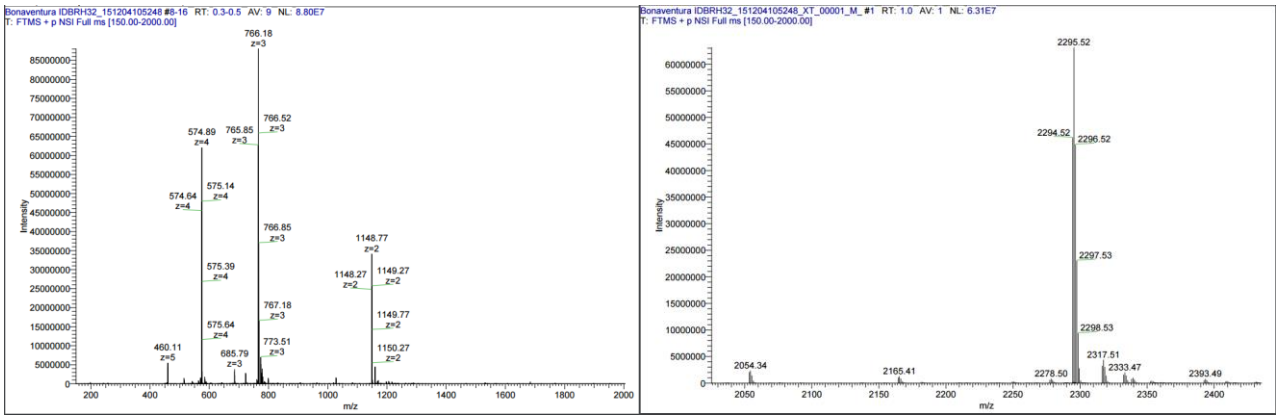




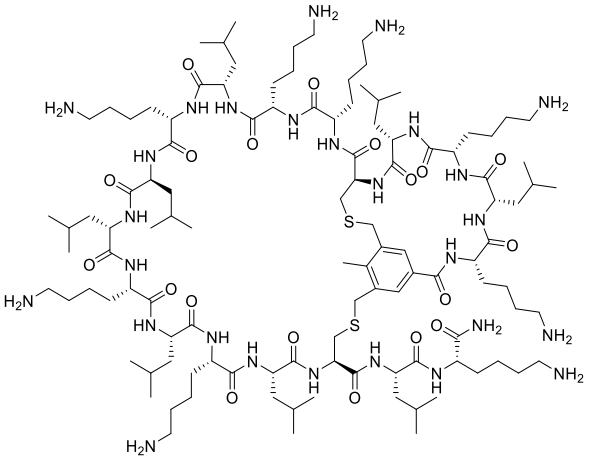
B[12]LKKKKLLKC[1]KLLLKC[2]LKL (bp29) was obtained as foamy white solid after preparative RP-HPLC (28.4 mg, 11.4 %). Analytical RP-HPLC: t_R = 2.24 min (A/D 100:0 to 0:100 in 7.0 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.51/2294.52 Da [M].

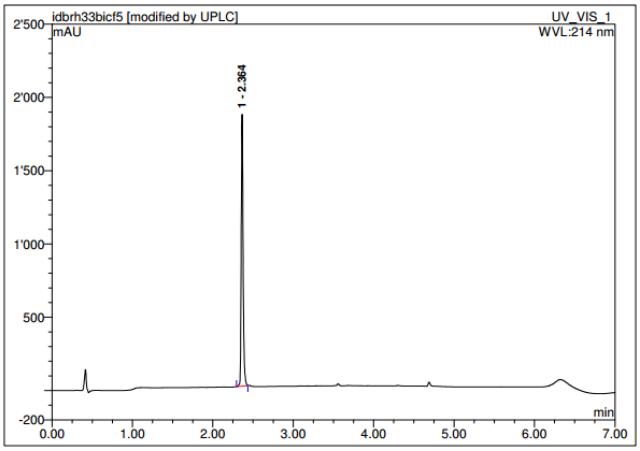


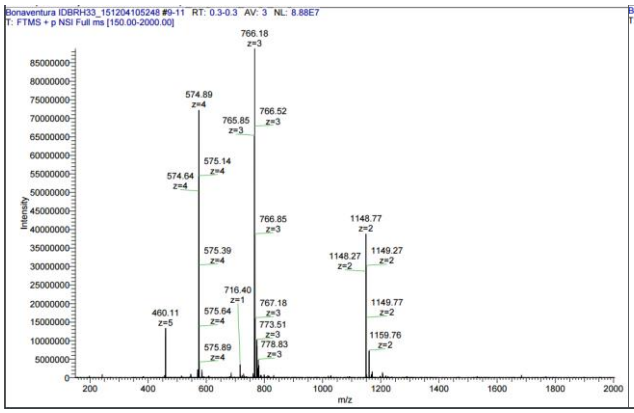


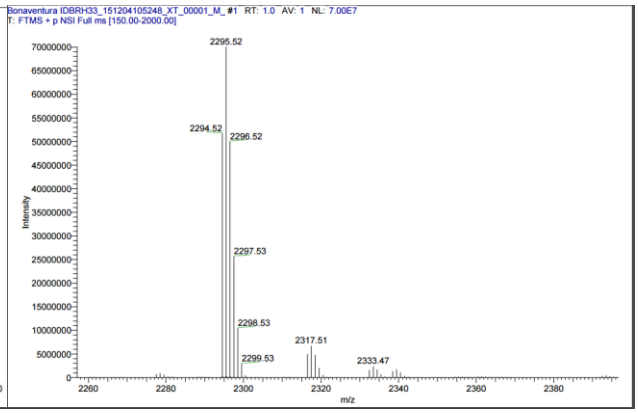


B[12]KLKLC[1]KKLKLLKLKLC[2]LK (bp30) was obtained as foamy white solid after preparative RP-HPLC (21.8 mg, 8.8%). Analytical RP-HPLC: t_R = 2.36 min (A/D 100:0 to 0:100 in 7.0 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.51/2294.52 Da [M].

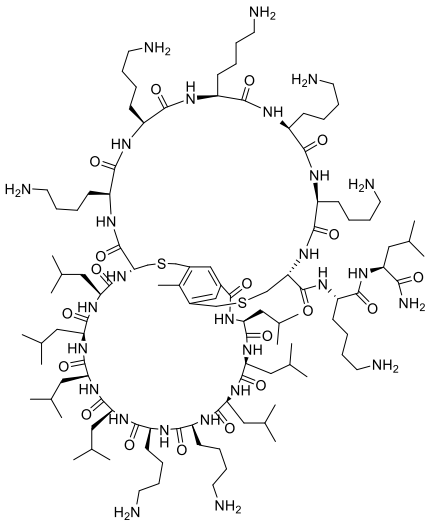


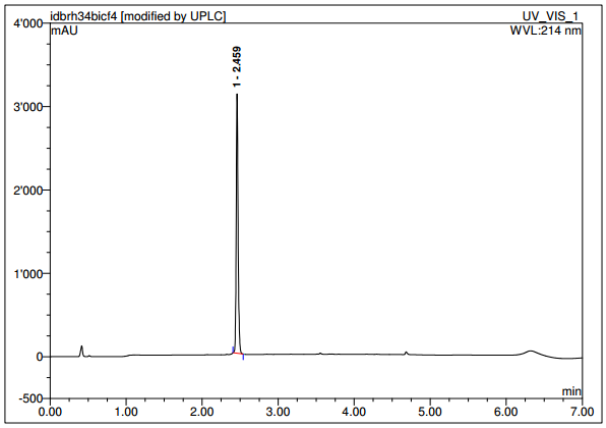


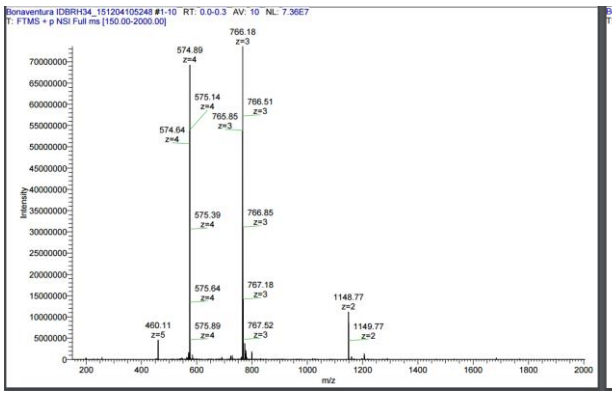


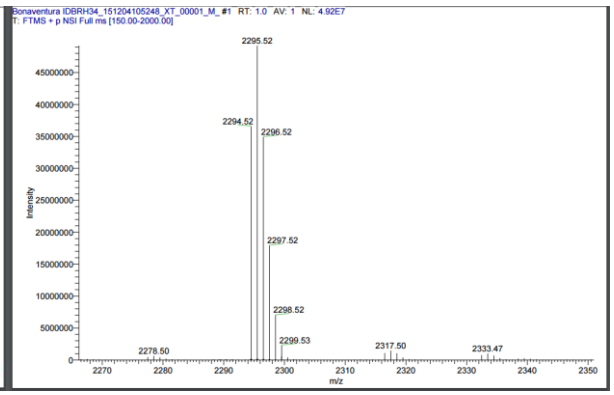


B[12]LLLKKLLLLC[1]KKKKKC[2]KL (bp31) was obtained as foamy white solid after preparative RP-HPLC (25.9 mg, 10.4%). Analytical RP-HPLC: t_R = 2.42 min (A/D 100:0 to 0:100 in 7.0 min, λ = 214 nm). MS (ESI$^+$): $C_{112}H_{203}N_{27}O_{19}S_2$ calc./obs. 2294.51/2294.52 Da [M].

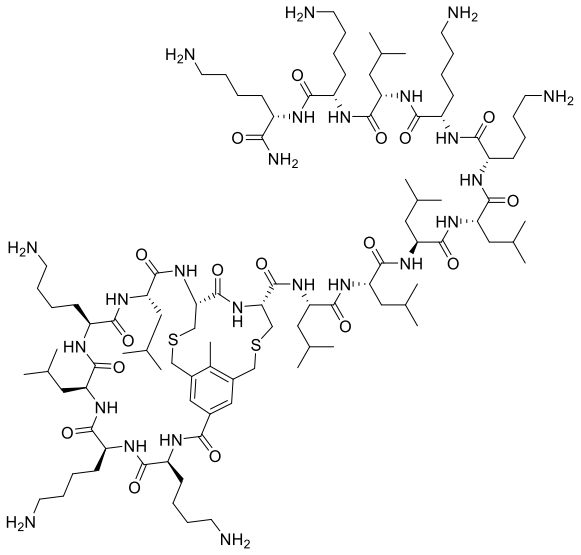


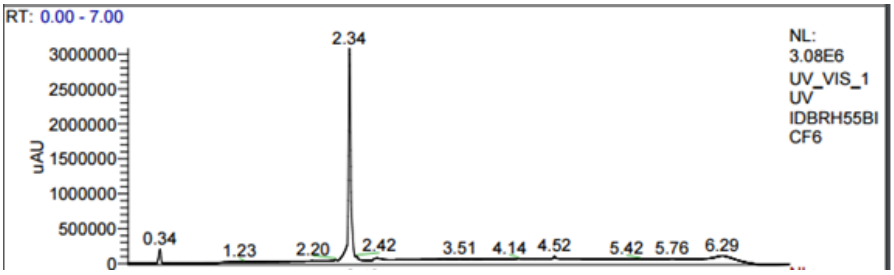


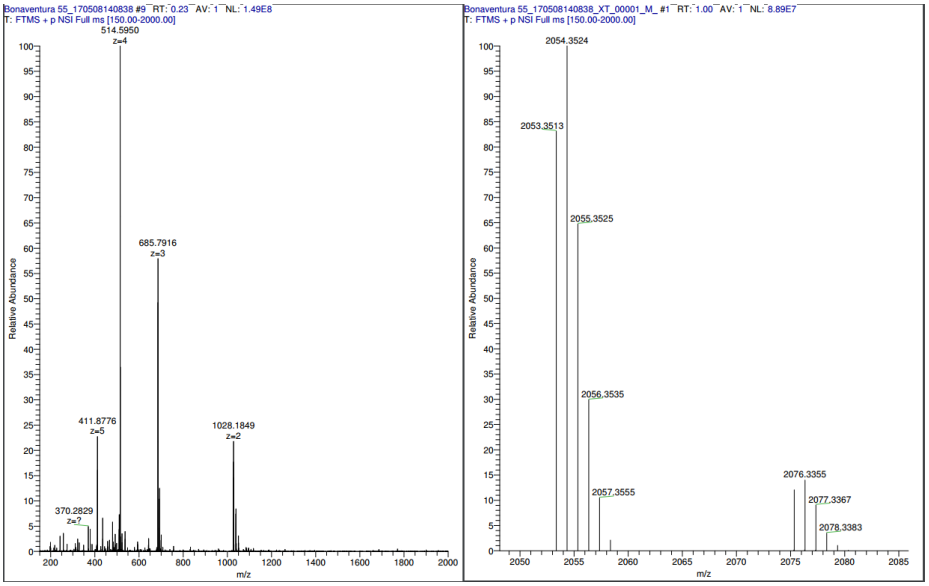




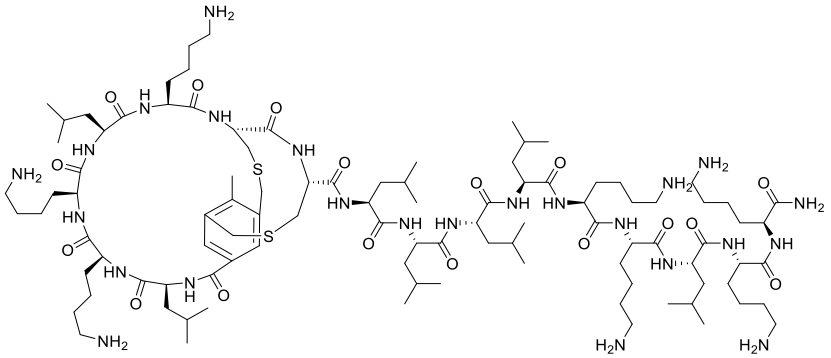
B[12]KKLKLC[1]C[2]LLLLKKLKK (bp32) was obtained as foamy white solid after preparative RP-HPLC (7.5 mg, 3.3 %). Analytical RP-HPLC: t_R = 2.34 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

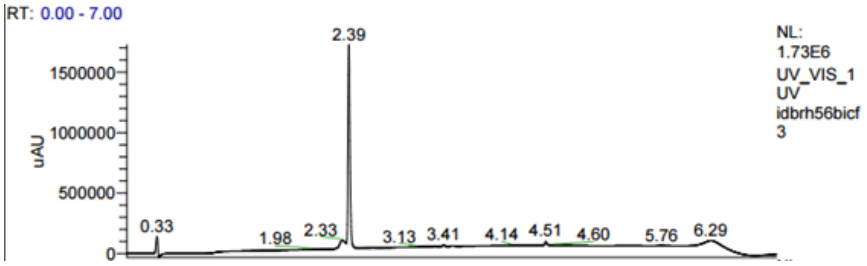


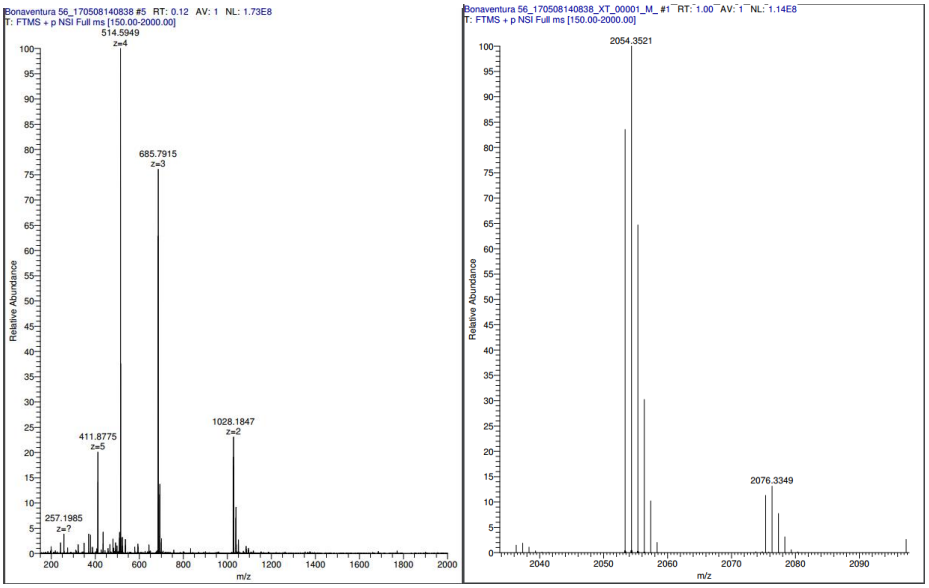




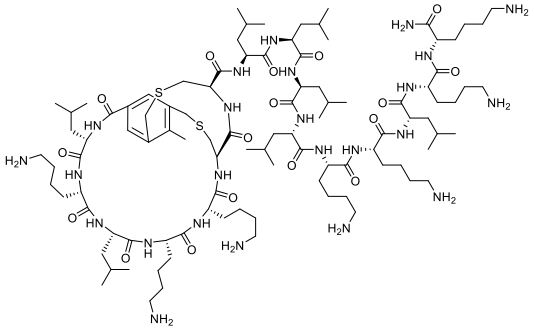
B[12]LKKLKC[1]C[2]LLLLKKLKK (bp33) was obtained as foamy white solid after preparative RP-HPLC (8.5 mg, 3.8 %). Analytical RP-HPLC: t_R = 2.39 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

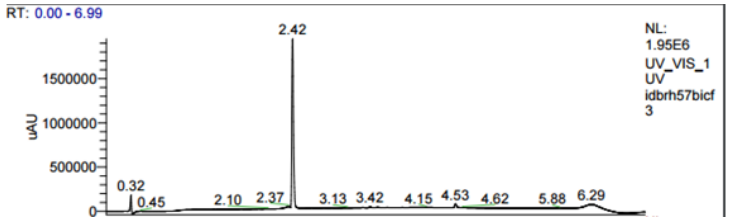


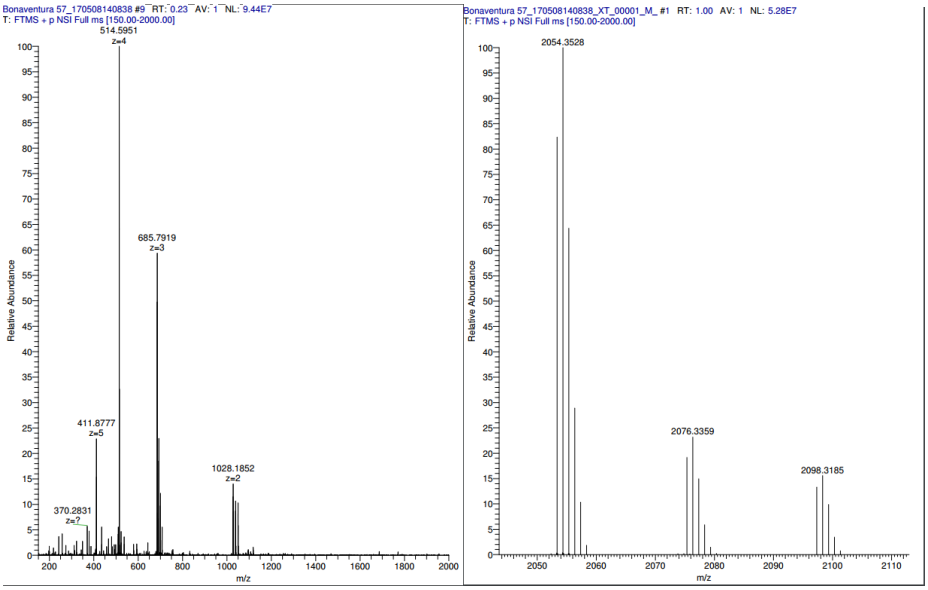




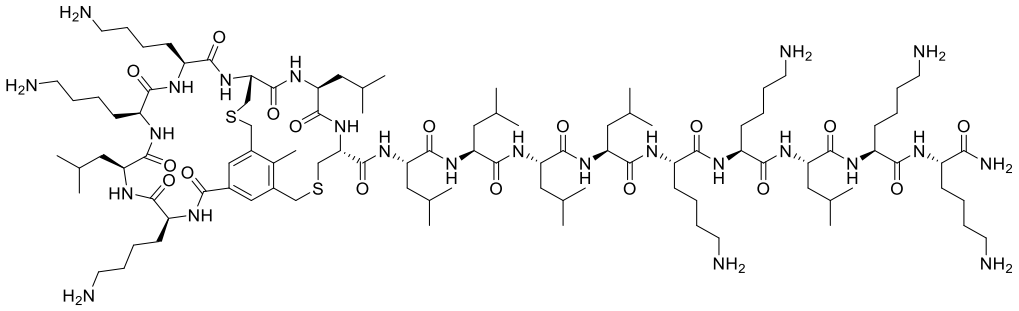
B[12]LKLKKC[1]C[2]LLLLKKLKK (bp34) was obtained as foamy white solid after preparative RP-HPLC (9.2 mg, 4.1 %). Analytical RP-HPLC: t_R = 2.42 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

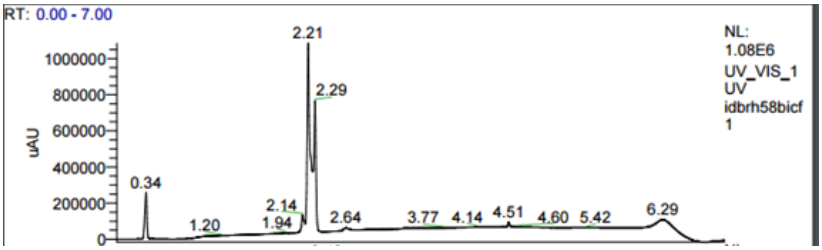


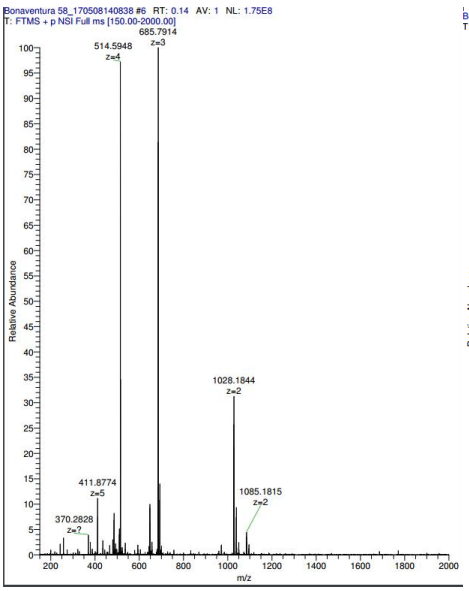


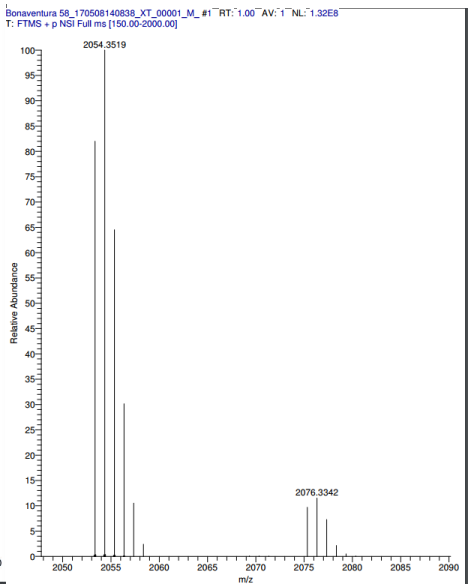


B[12]KLKKC[1]LC[2]LLLLKKLKK (bp35) was obtained as foamy white solid after preparative RP-HPLC (10.2 mg, 4.5 %). Analytical RP-HPLC: t_R = 2.21 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

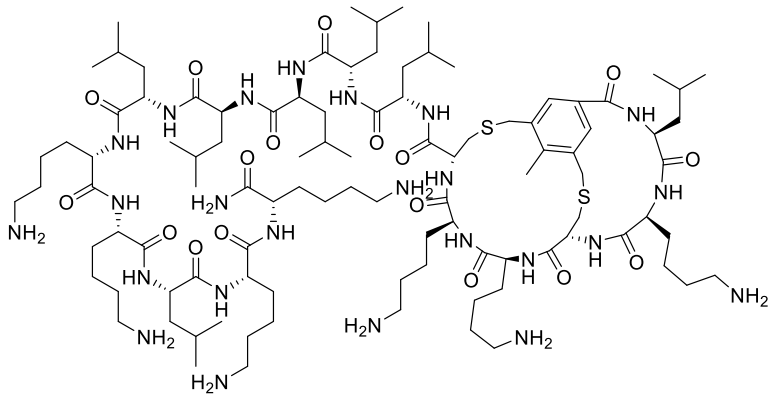


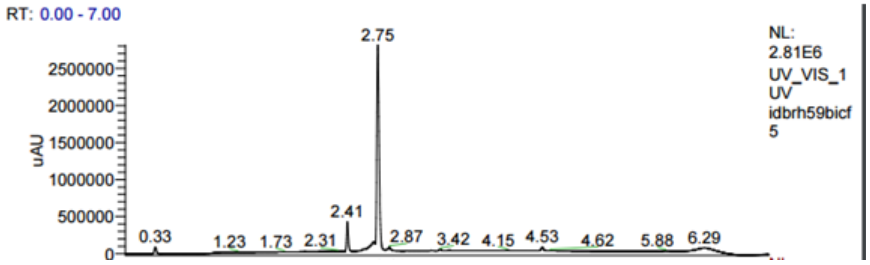


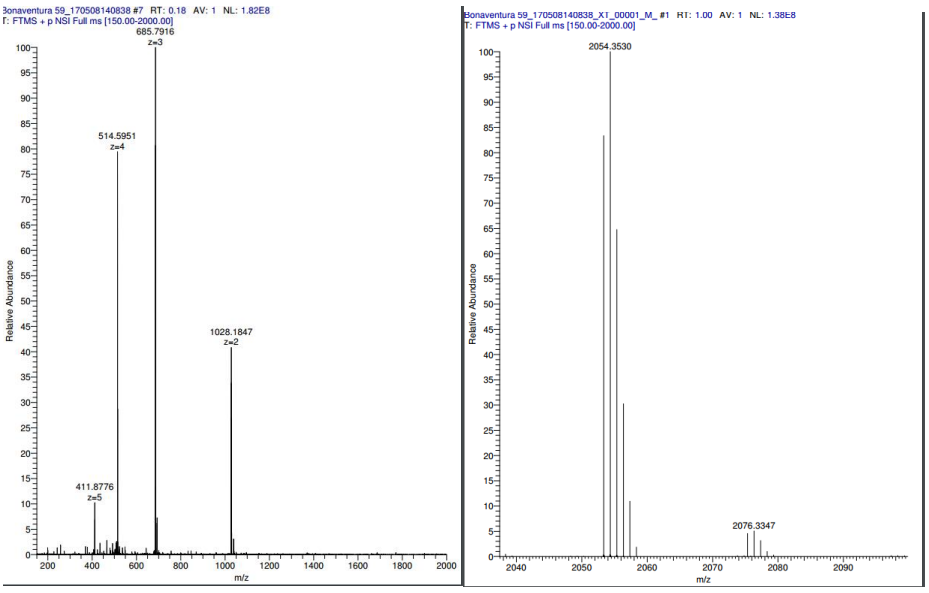




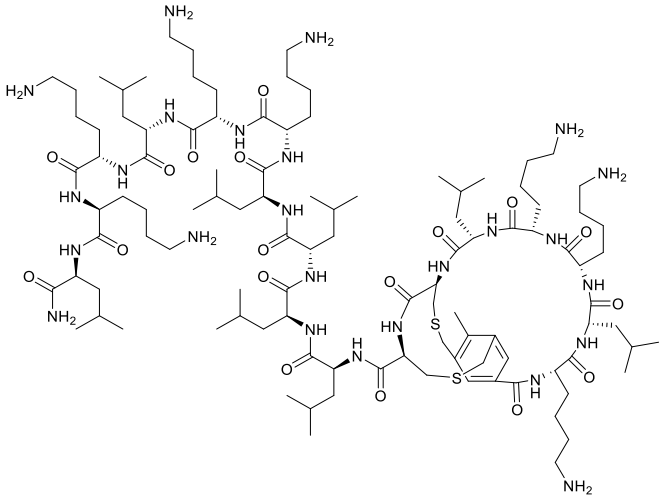
B[12]LKC[1]KKC[2]LLLLLKKLKK (bp36) was obtained as foamy white solid after preparative RP-HPLC (11.0 mg, 4.9 %). Analytical RP-HPLC: t_R = 2.75 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

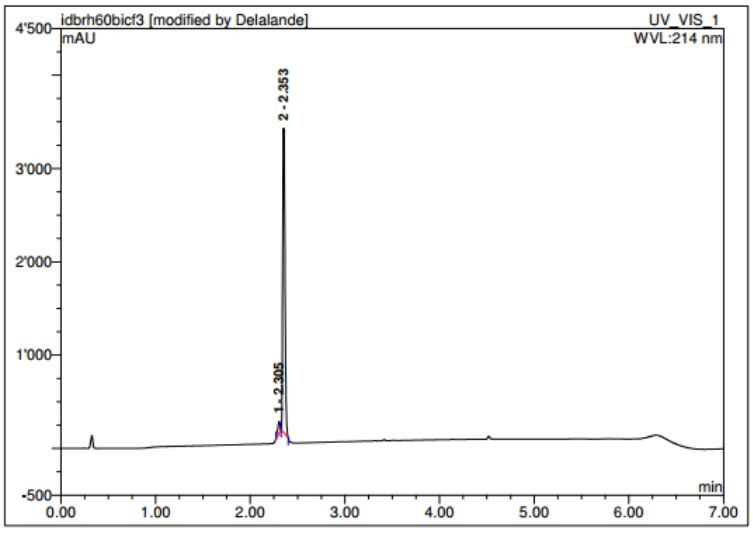


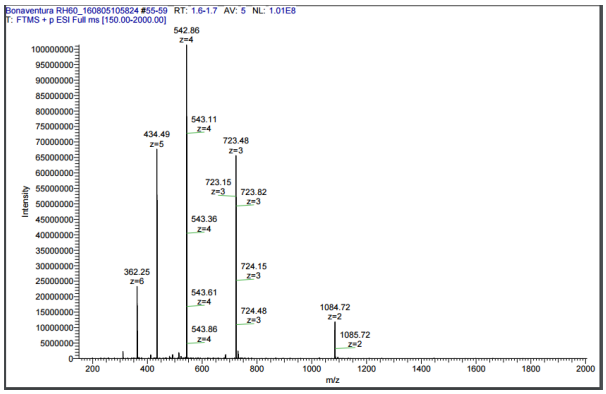


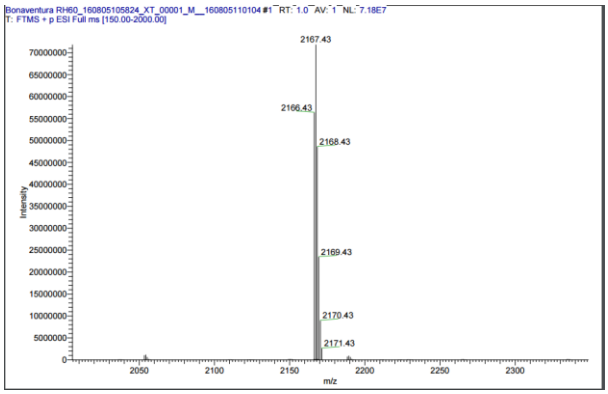


B[12]KLKKLC[1]C[2]LLLLKKLKKL (bp37) was obtained as foamy white solid after preparative RP-HPLC (11.7 mg, 5.0 %). Analytical RP-HPLC: t_R = 2.35 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.42/2166.43 Da [M].

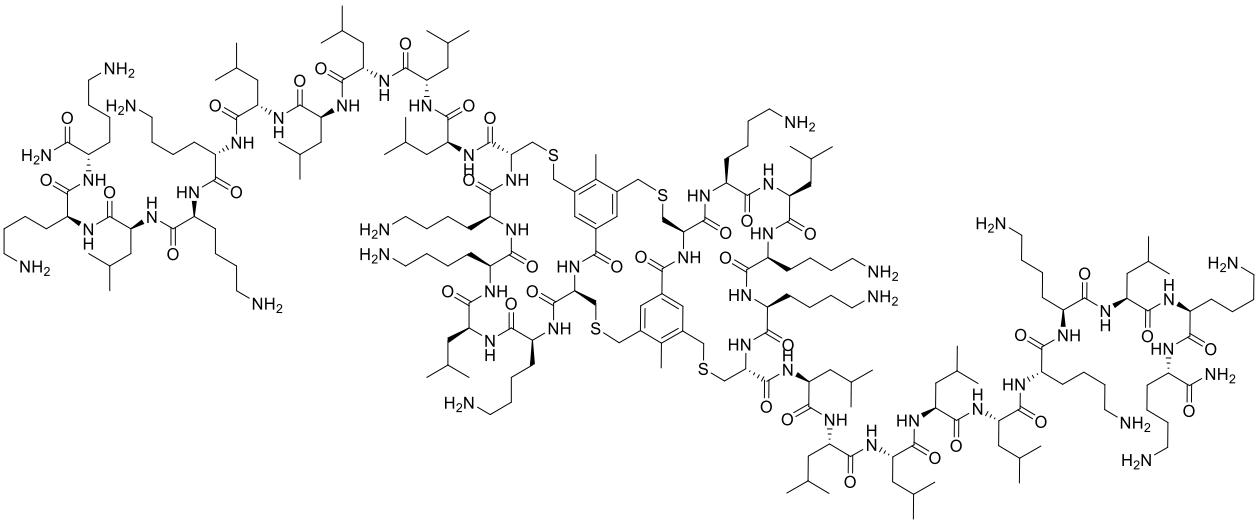


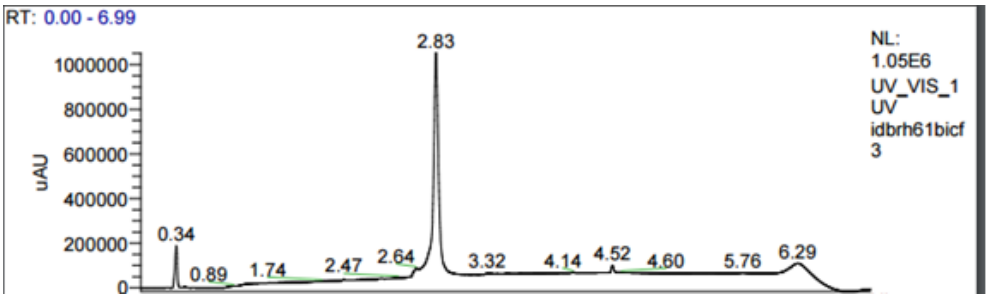


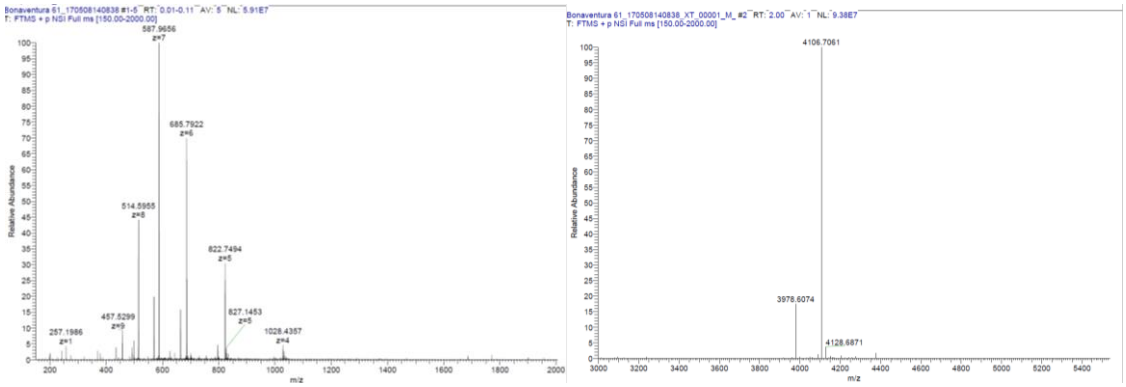




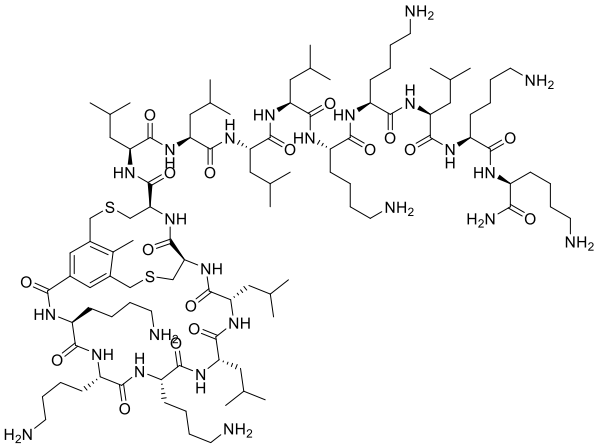
B[12]C[1]KLKKC[2]LLLLLKKLKK (bp38) was obtained as foamy white solid after preparative RP-HPLC (13.0 mg, 5.8 %). Analytical RP-HPLC: t_R = 2.83 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 4106.68/4106.71 Da [M].

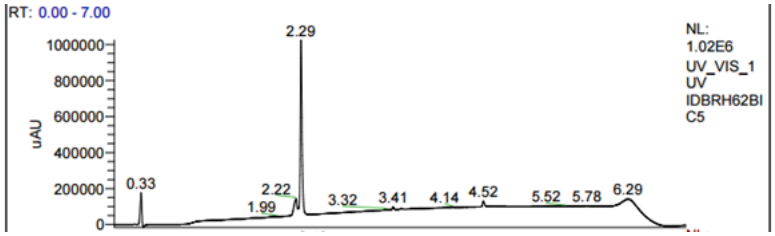


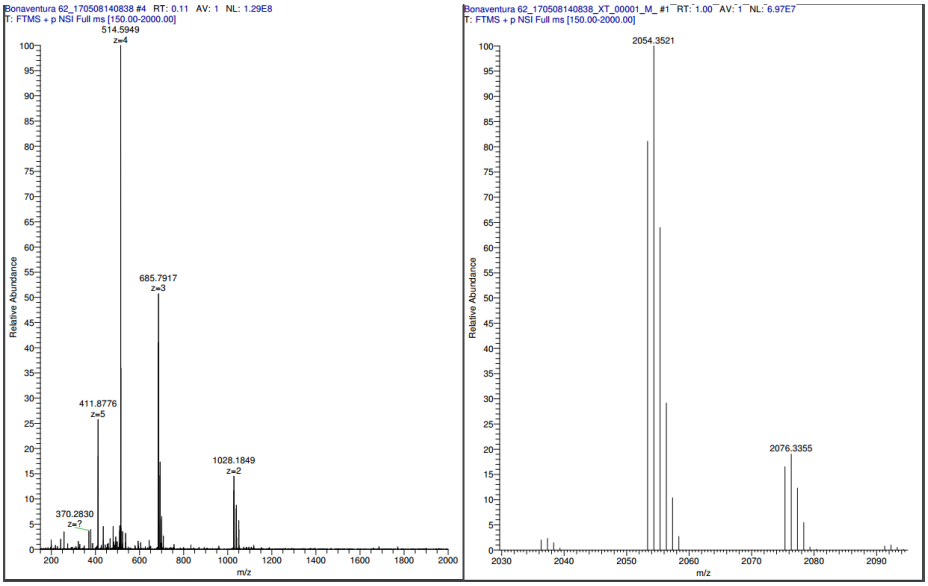




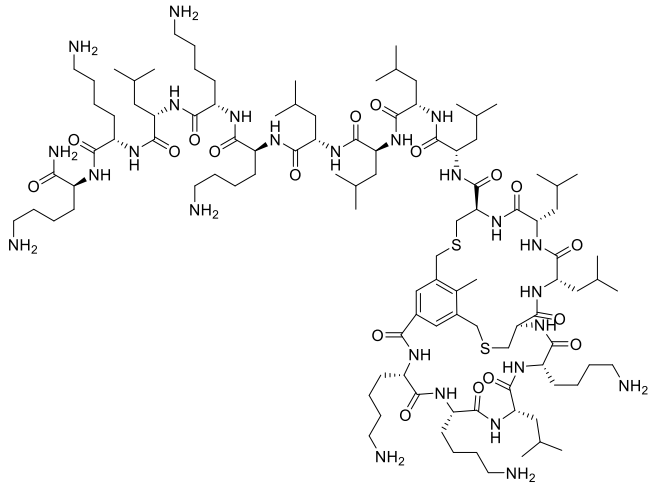
B[12]KKKLLC[1]C[2]LLLLKKLKK (bp39) was obtained as foamy white solid after preparative RP-HPLC (12.5 mg, 5.6 %). Analytical RP-HPLC: t_R = 2.29 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

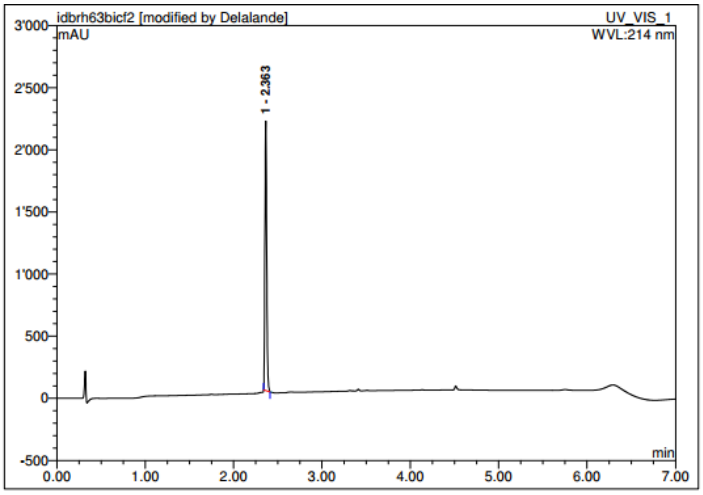


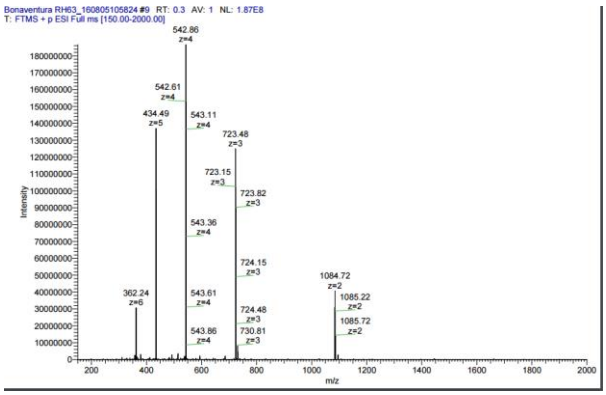


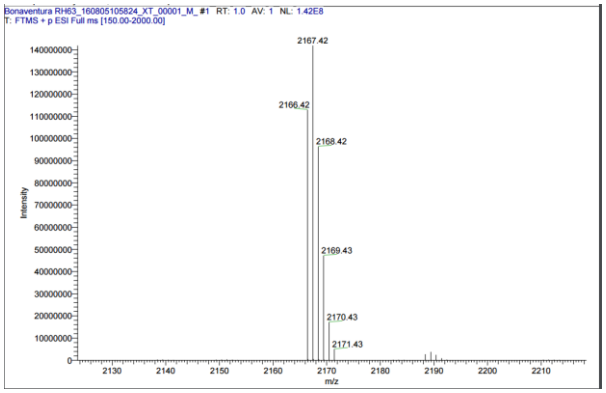


B[12]KKLKC[1]LLC[2]LLLLKKLKK (bp40) was obtained as foamy white solid after preparative RP-HPLC (14.2 mg, 6.0 %). Analytical RP-HPLC: t_R = 2.36 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.41/2166.42 Da [M].

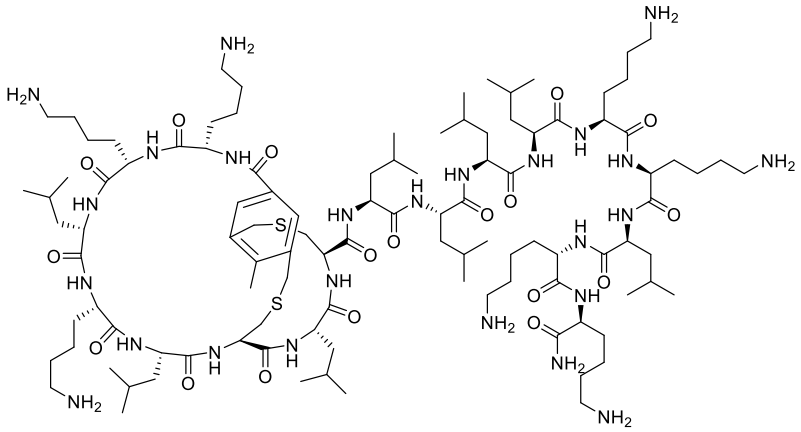


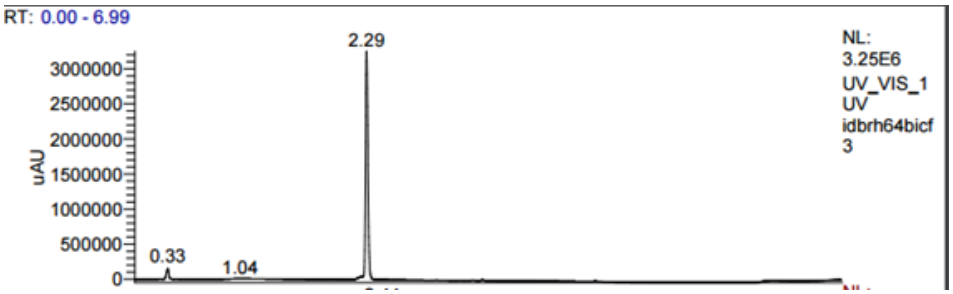


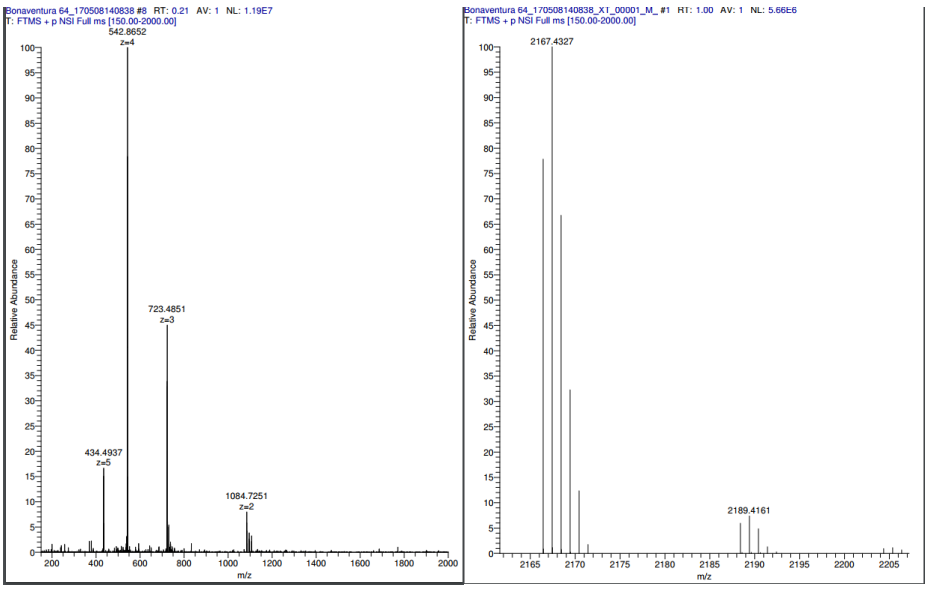




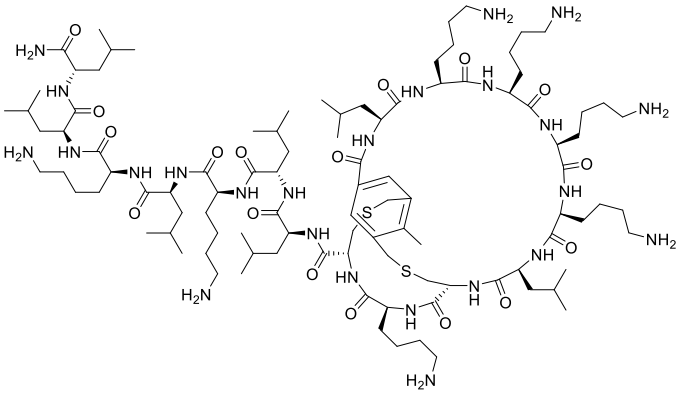
B[12]KKLKLC[1]LC[2]LLLLKKLKK (bp41) was obtained as foamy white solid after preparative RP-HPLC (15.2 mg, 6.4 %). Analytical RP-HPLC: t_R = 2.29 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{106}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.44/2166.43 Da [M].

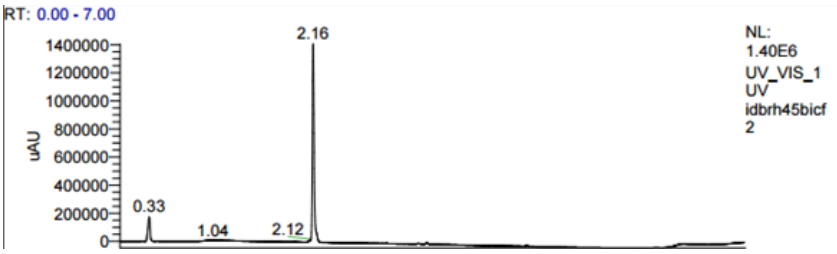


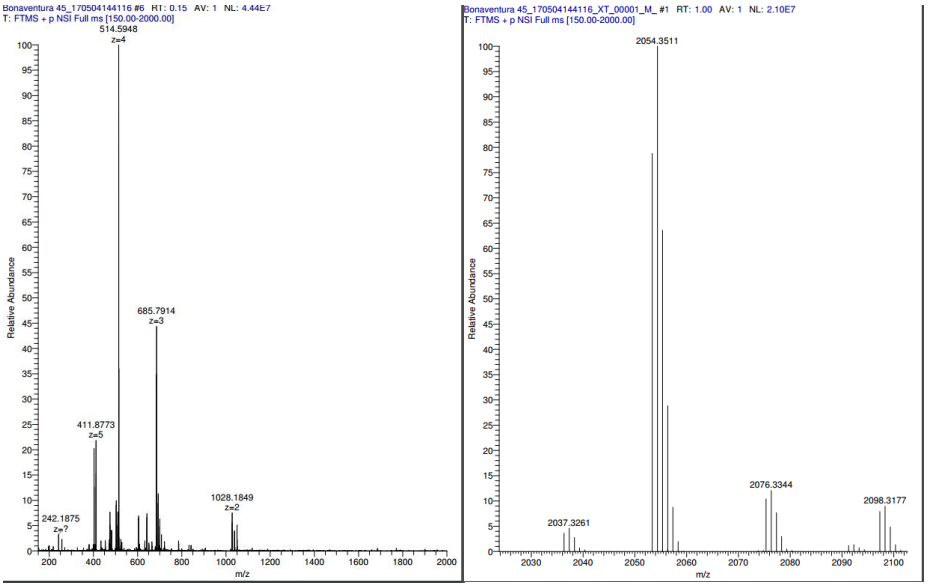




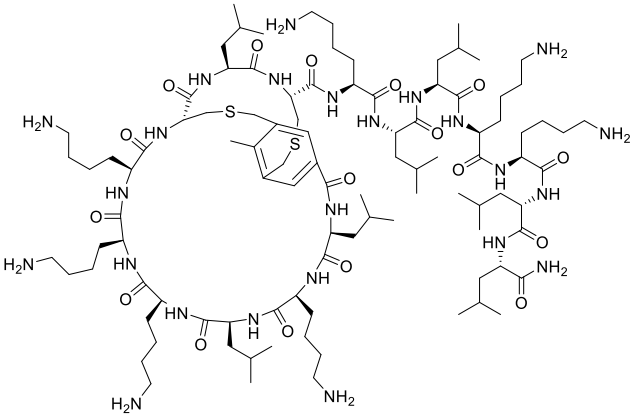
B[12]LKKKKLC[1]KC[2]LLKLKLL (bp42) was obtained as foamy white solid after preparative RP-HPLC (21.5 mg, 9.5 %). Analytical RP-HPLC: t_R = 2.16 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.16/2053.34 Da [M].

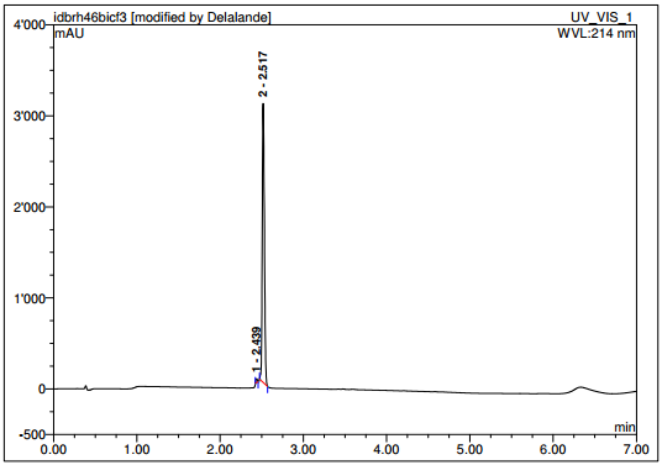


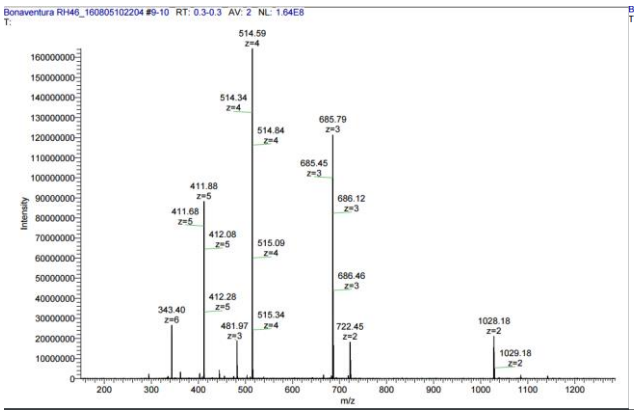


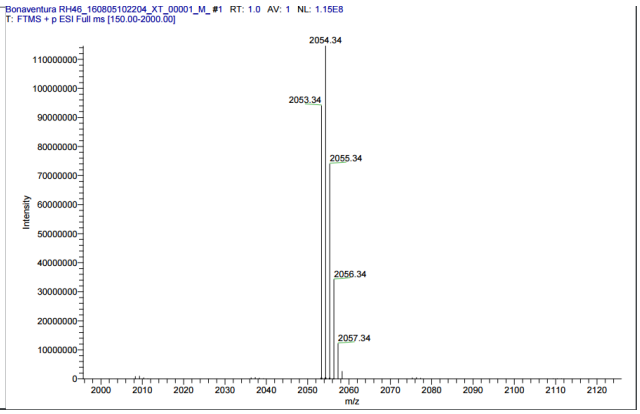


B[12]LKKKKLC[1]KC[2]LLKLKLL (bp43) was obtained as foamy white solid after preparative RP-HPLC (22.8 mg, 10.2 %). Analytical RP-HPLC: t_R = 2.46 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.16/2053.34 Da [M].

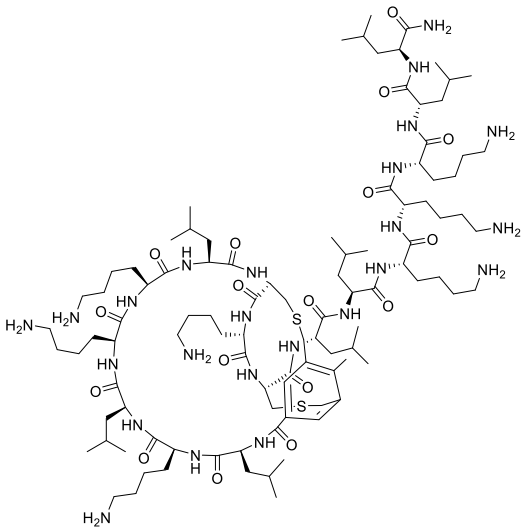


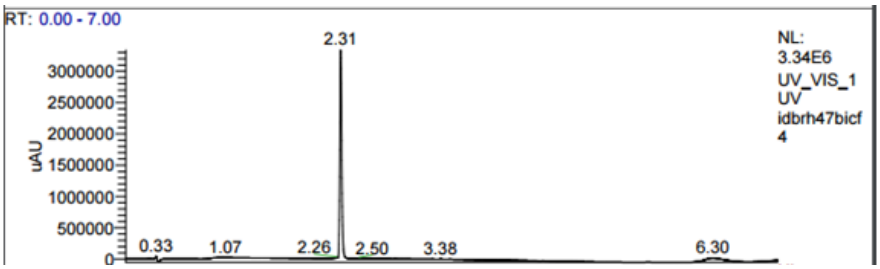


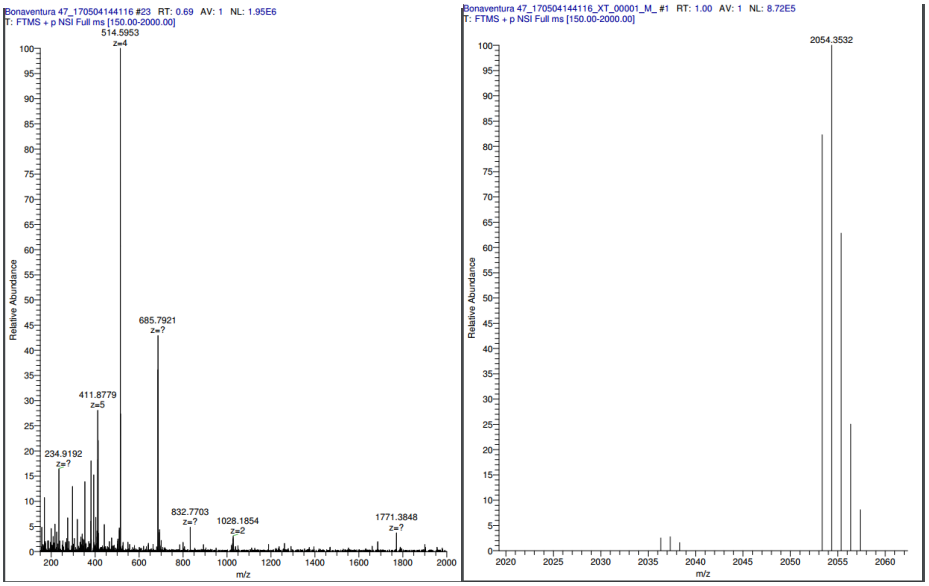




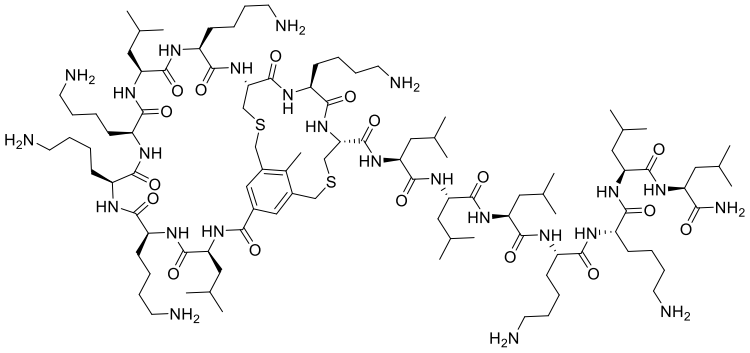
B[12]LKLKKLC[1]KC[2]LLKKKLL (bp44) was obtained as foamy white solid after preparative RP-HPLC (18.8 mg, 8.4 %). Analytical RP-HPLC: t_R = 2.31 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.16/2053.34 Da [M].

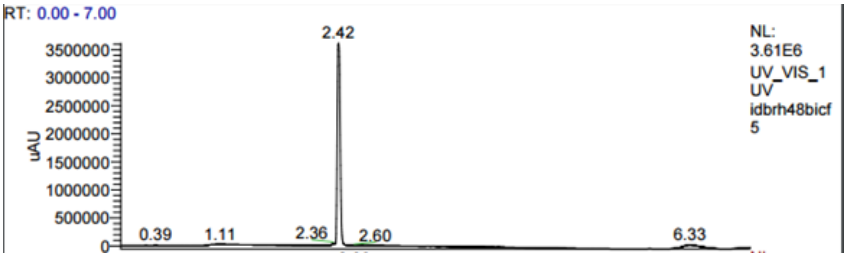


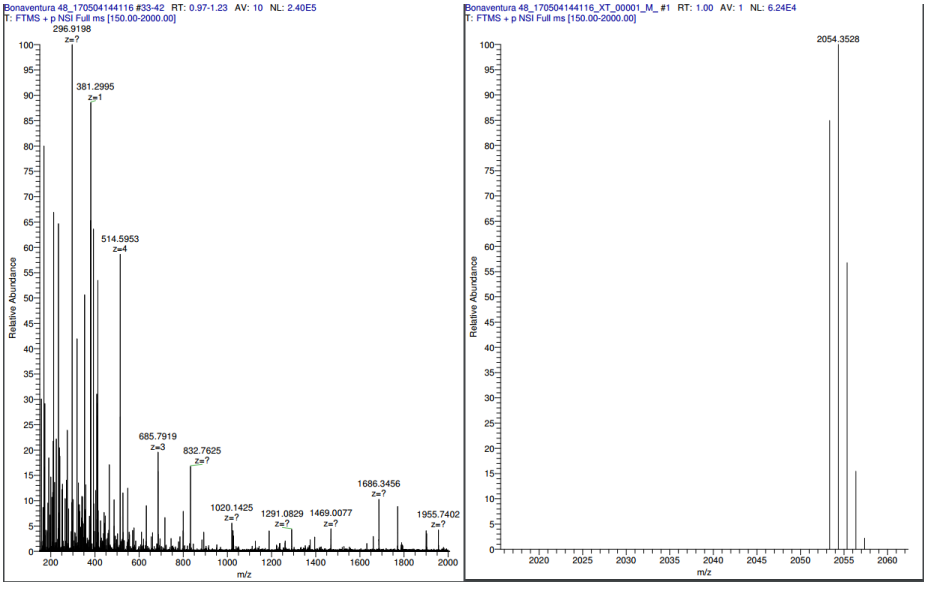




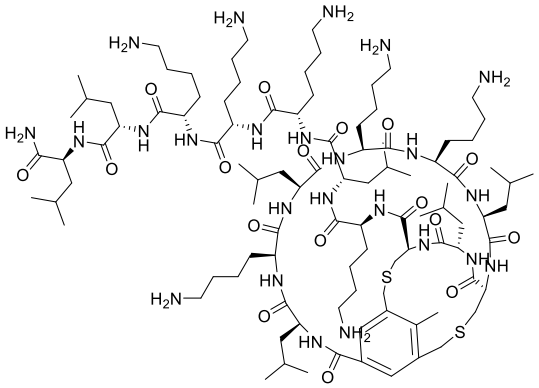
B[12]LKKKLKC[1]KC[2]LLLKKLL (bp45) was obtained as foamy white solid after preparative RP-HPLC (26.2 mg, 11.8 %). Analytical RP-HPLC: t_R = 2.42 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.16/2053.34 Da [M].

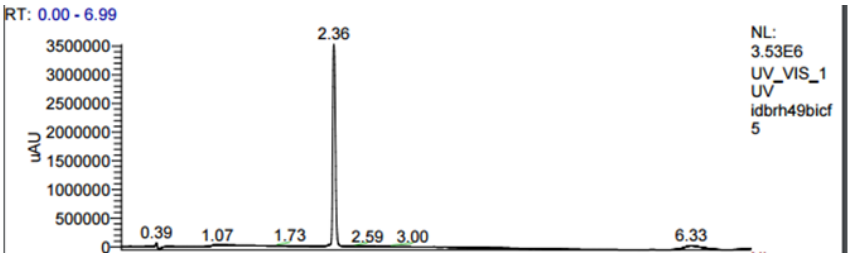


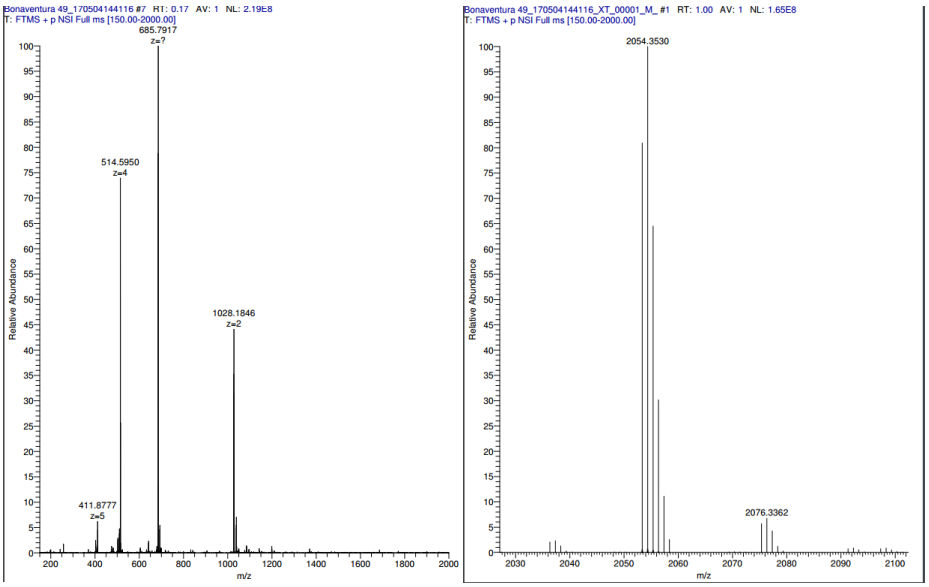




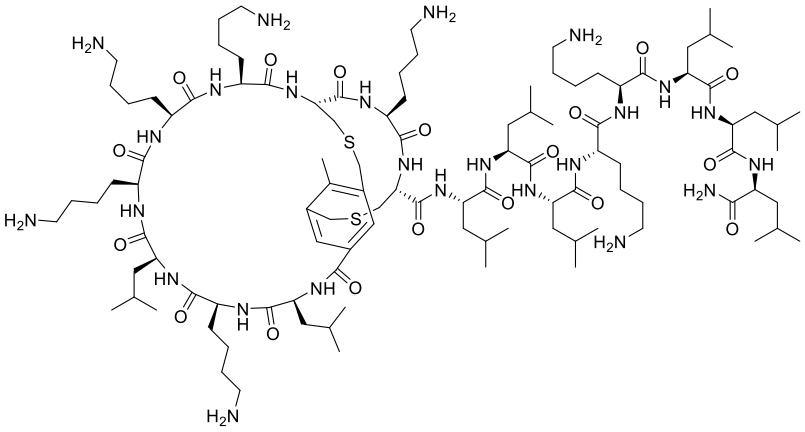
B[12]LKLKKLC[1]LC[2]KLKKKLL (bp46) was obtained as foamy white solid after preparative RP-HPLC (21.2 mg, 9.5 %). Analytical RP-HPLC: t_R = 2.36 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.16/2053.34 Da [M].

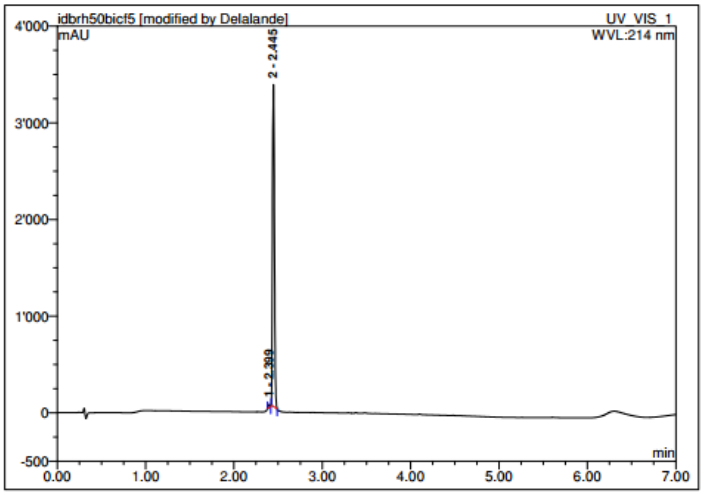


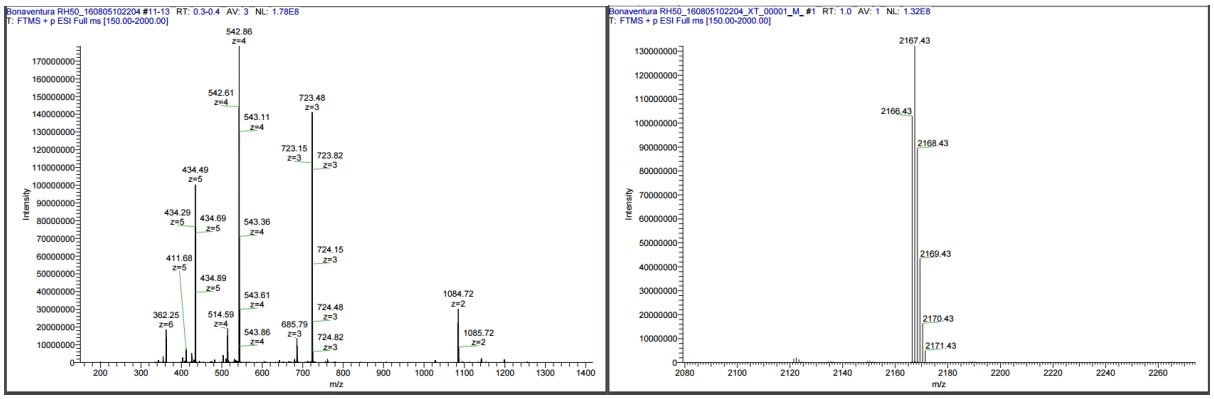




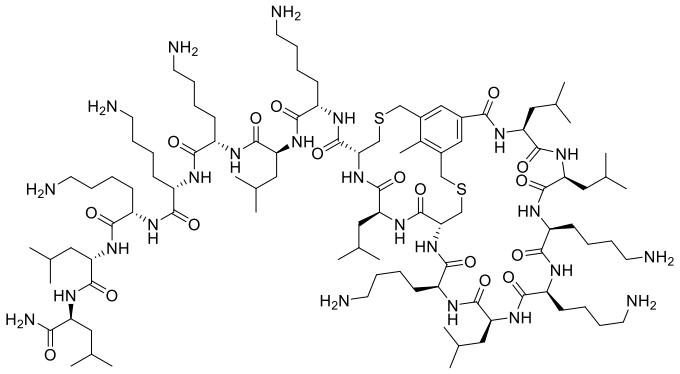
B[12]LKLKKKC[1]KC[2]LLLKKLLL (bp47) was obtained as foamy white solid after preparative RP-HPLC (11.2 mg, 4.8 %). Analytical RP-HPLC: t_R = 2.40 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{108}H_{191}N_{25}O_{18}S_2$ calc./obs. 2166.42/2166.43 Da [M].

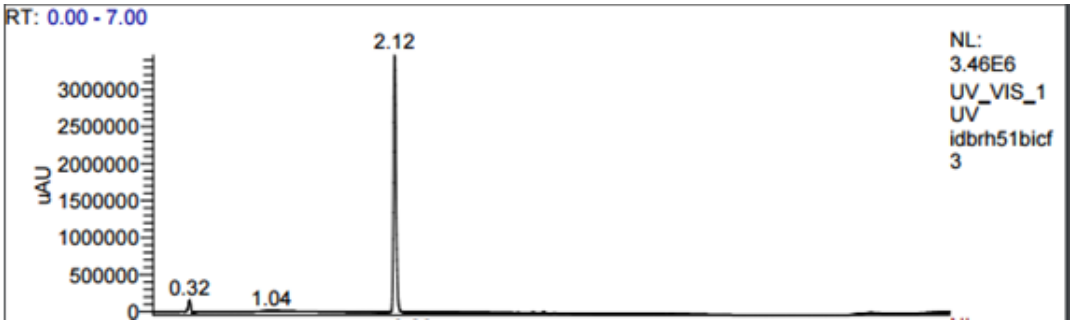


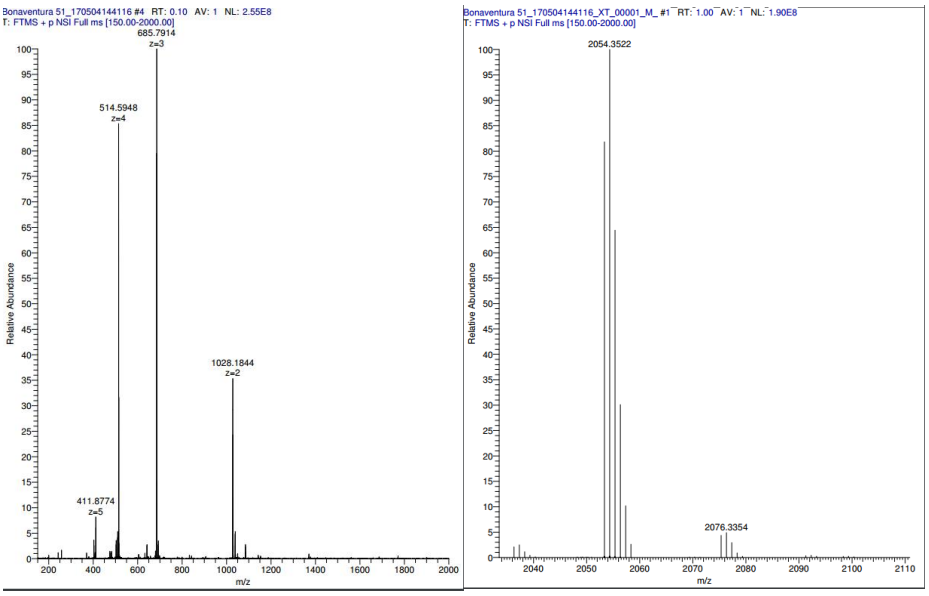




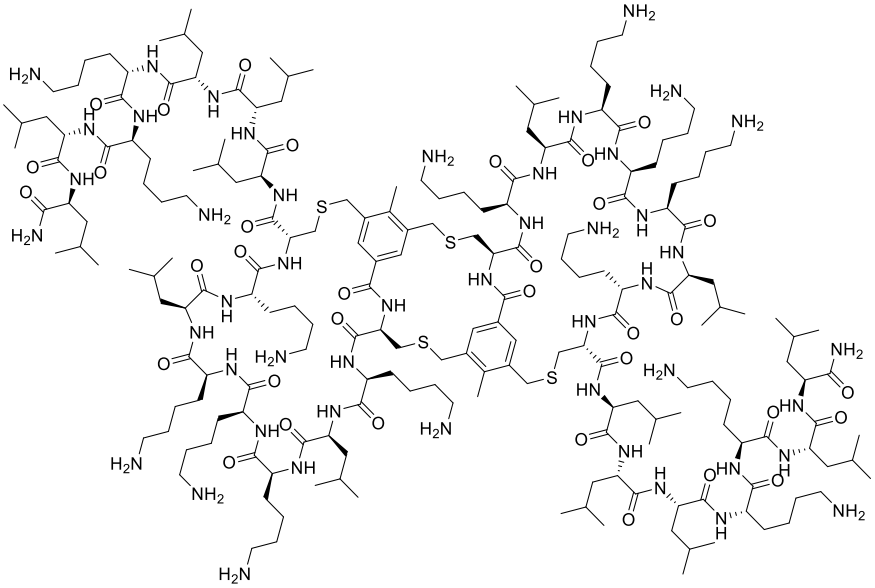
B[12]LLKKLKC[1]LC[2]KLKKKLL (bp48) was obtained as foamy white solid after preparative RP-HPLC (17.2 mg, 7.7 %). Analytical RP-HPLC: t_R = 2.12 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.16/2053.34 Da [M].

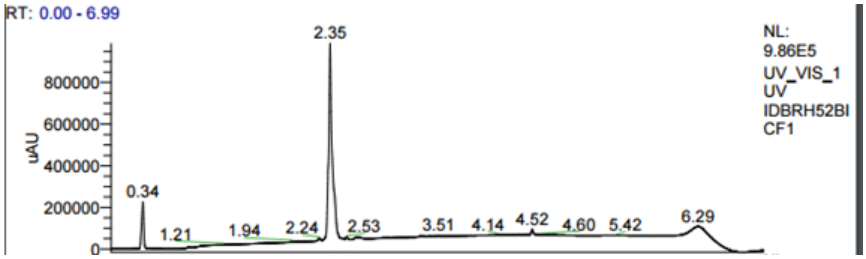




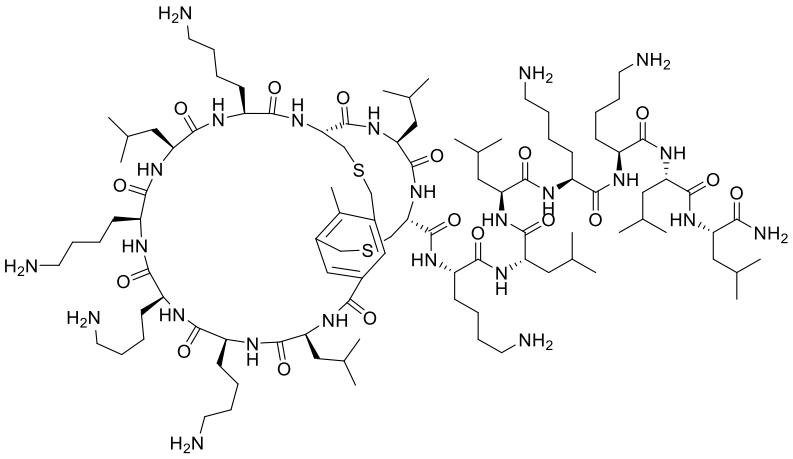


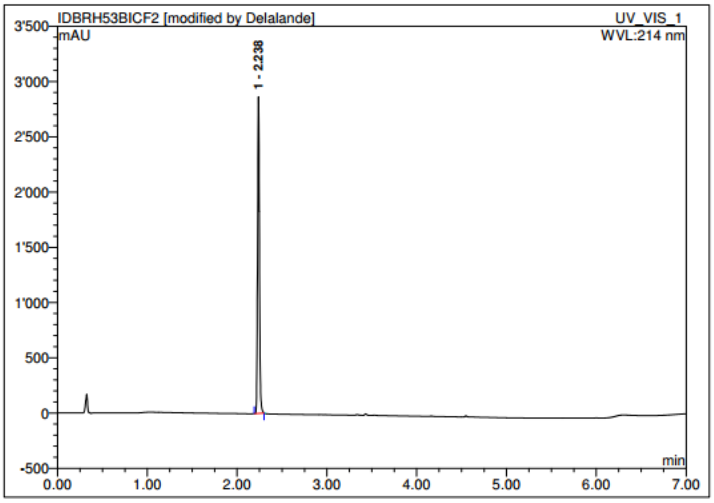
B[12]C[1]KLKKKLKC[2]LLLKKLL (bp49) was obtained as foamy white solid after preparative RP-HPLC (18.7 mg, 8.4 %). Analytical RP-HPLC: t_R = 2.35 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 4106.68/4105.70 Da [M].

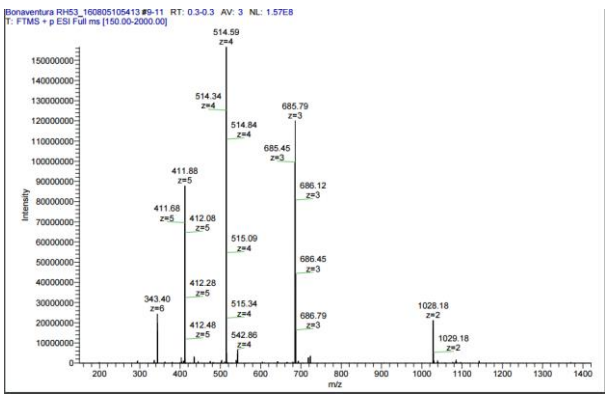


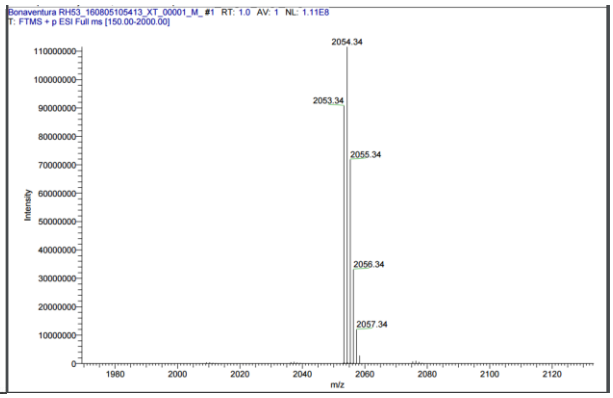


B[12]LKKKLKC[1]LC[2]KLLKKLL (bp50) was obtained as foamy white solid after preparative RP-HPLC (13.7 mg, 6.1 %). Analytical RP-HPLC: t_R = 2.24 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

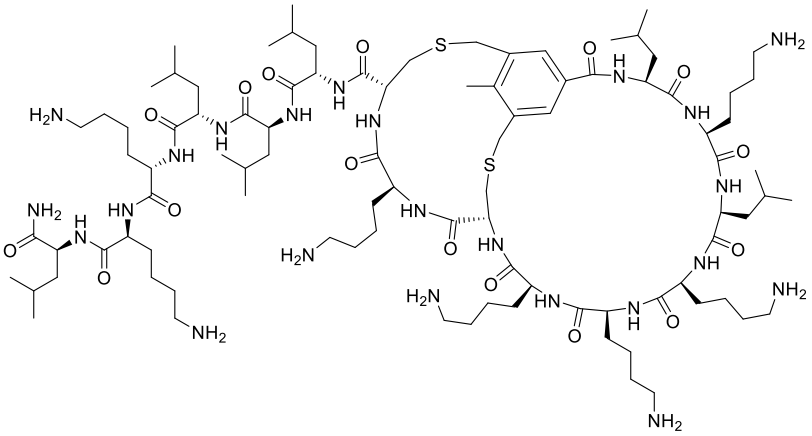


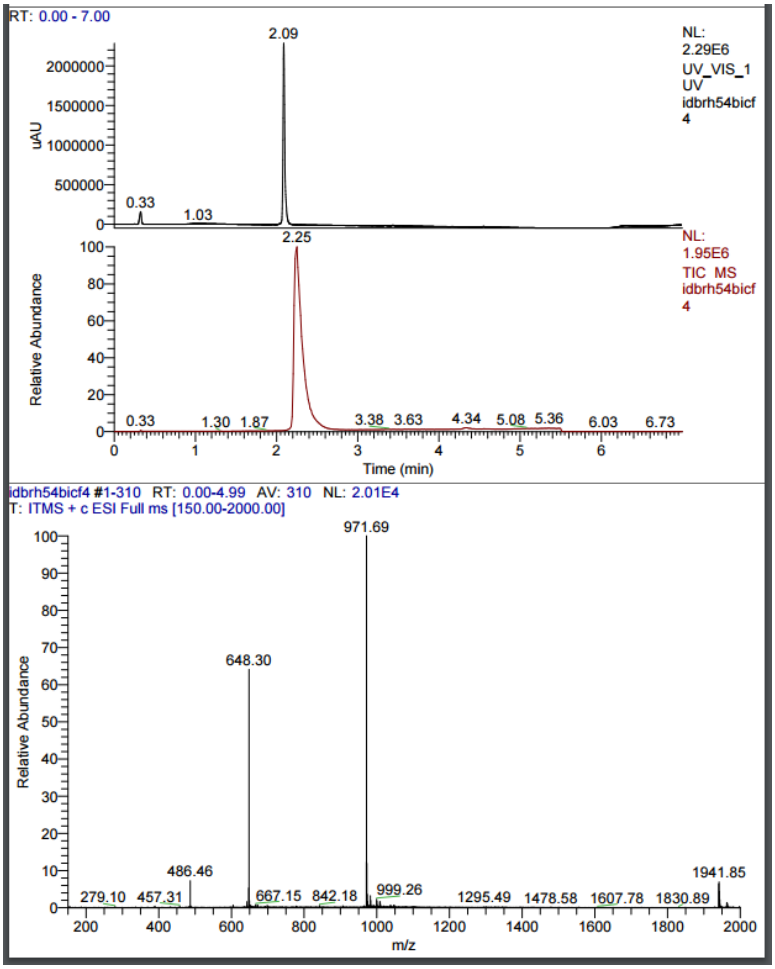




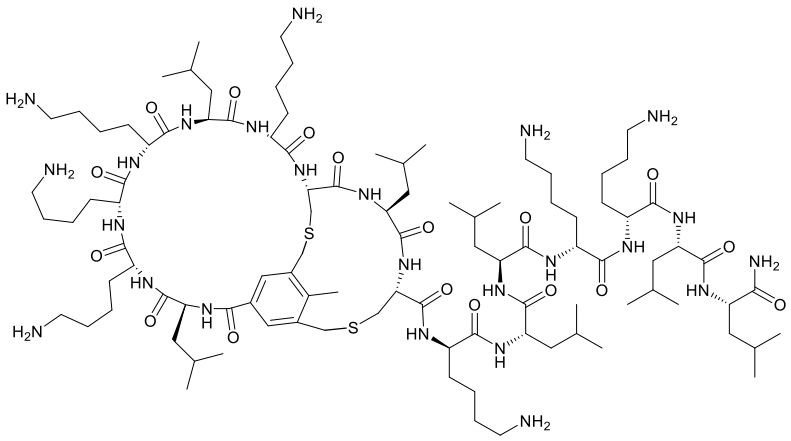


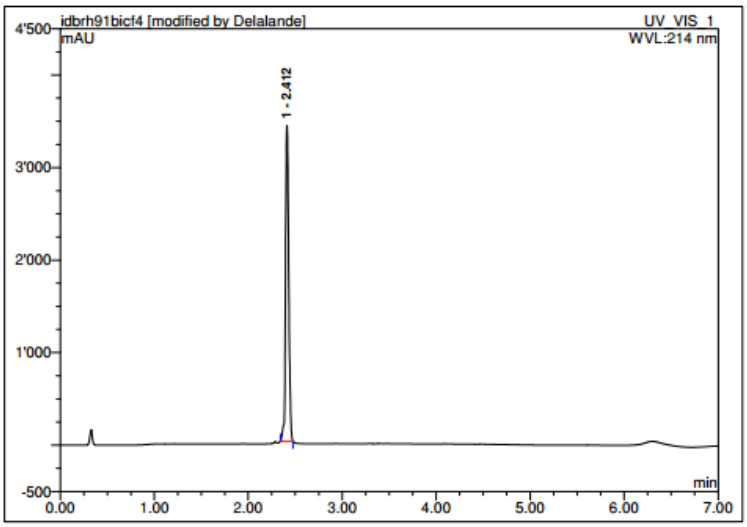
B[12]LKLKKKC[1]KC[2]LLLKKL (bp51) was obtained as foamy white solid after preparative RP-HPLC (21.7 mg, 10.4 %). Analytical RP-HPLC: t_R = 2.09 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{94}H_{159}N_{23}O_{16}S_2$ calc./obs. 1940.26/1941.85 Da $[M+H]^+$, 970.34/971.69 Da $[M+2H]^{2+}$, 647.12/648.30 Da $[M+3H]^{3+}$, 485.13/486.46 Da $[M+4H]^{4+}$.

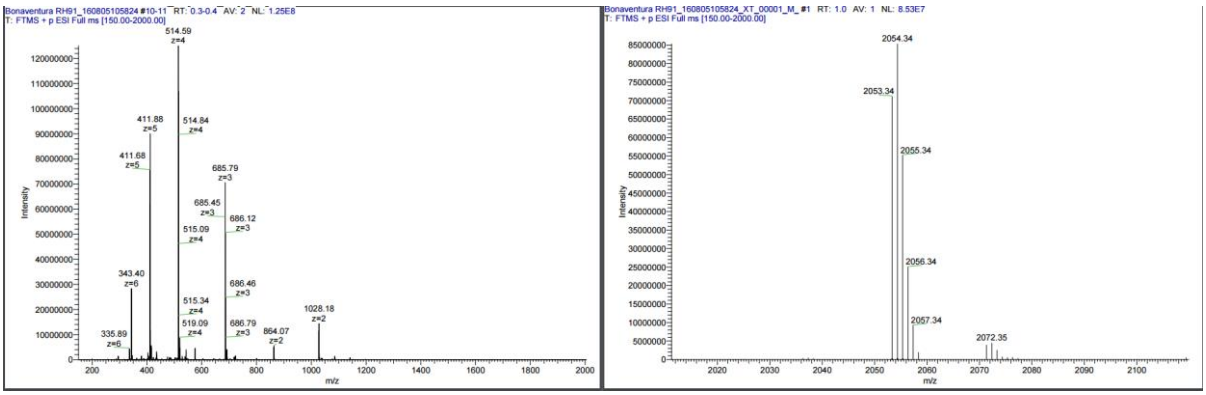




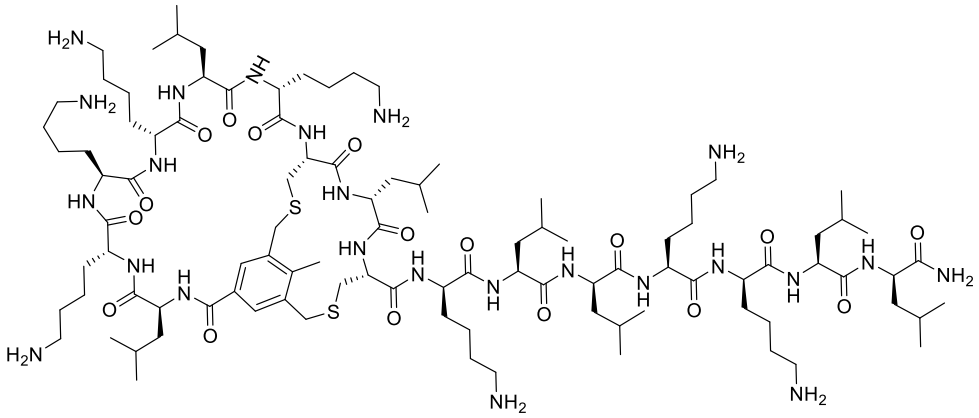
B[12]lKkKlKc[1]Lc[2]KlLkKlL (bp52) was obtained as foamy white solid after preparative RP-HPLC (23.5 mg, 10.5 %). Analytical RP-HPLC: t_R = 2.41 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

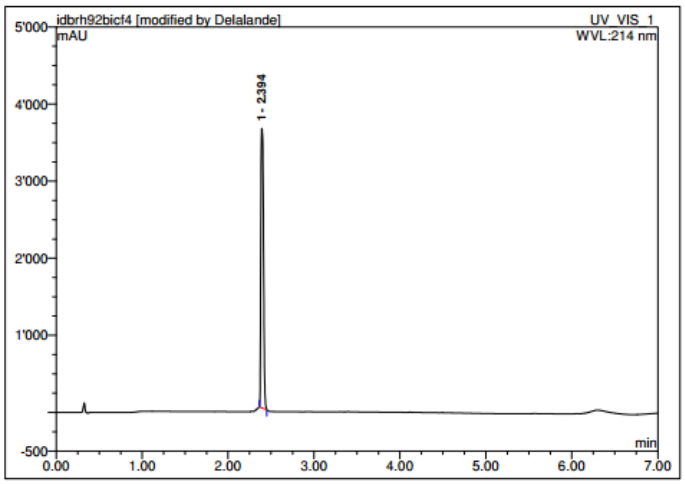


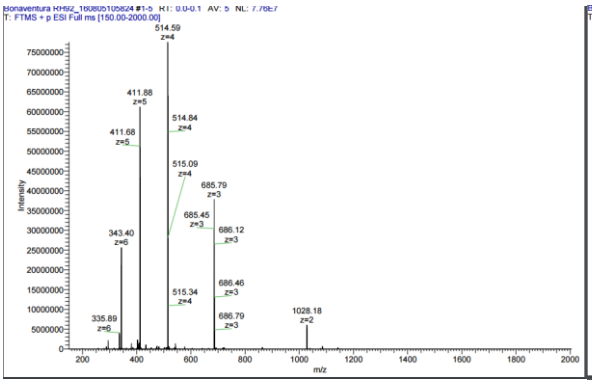


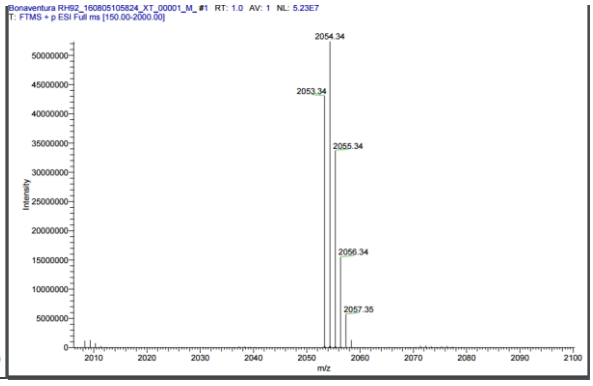


B[12]LkKkLkC[1]lC[2]kLlKkLl (bp53) was obtained as foamy white solid after preparative RP-HPLC (26.5 mg, 11.9 %). Analytical RP-HPLC: t_R = 2.39 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

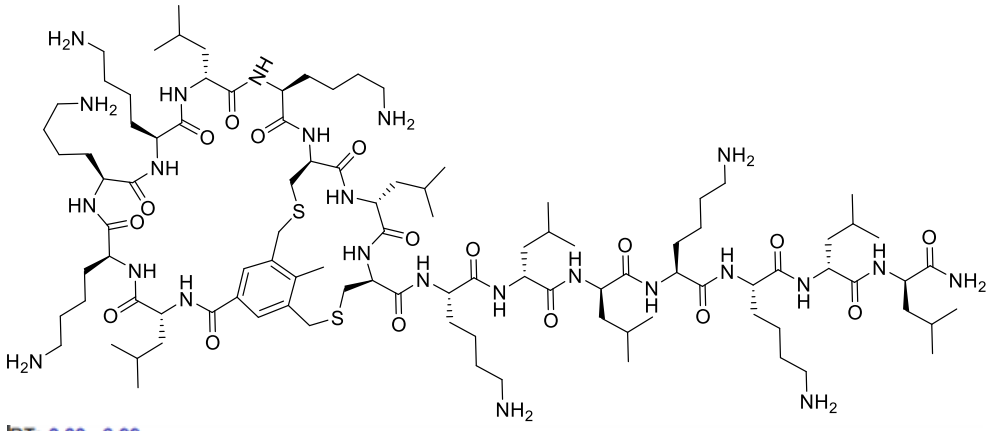


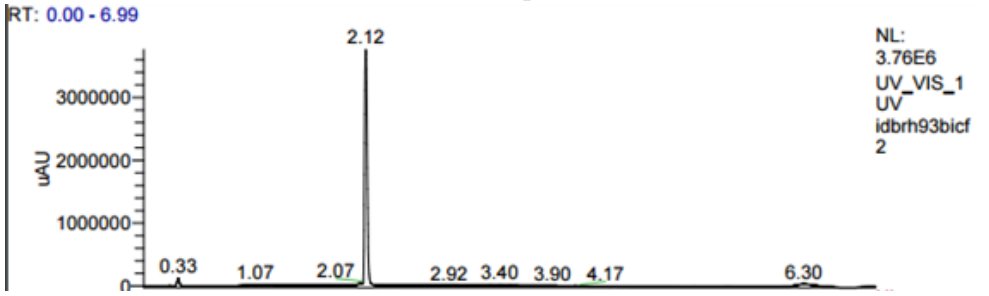


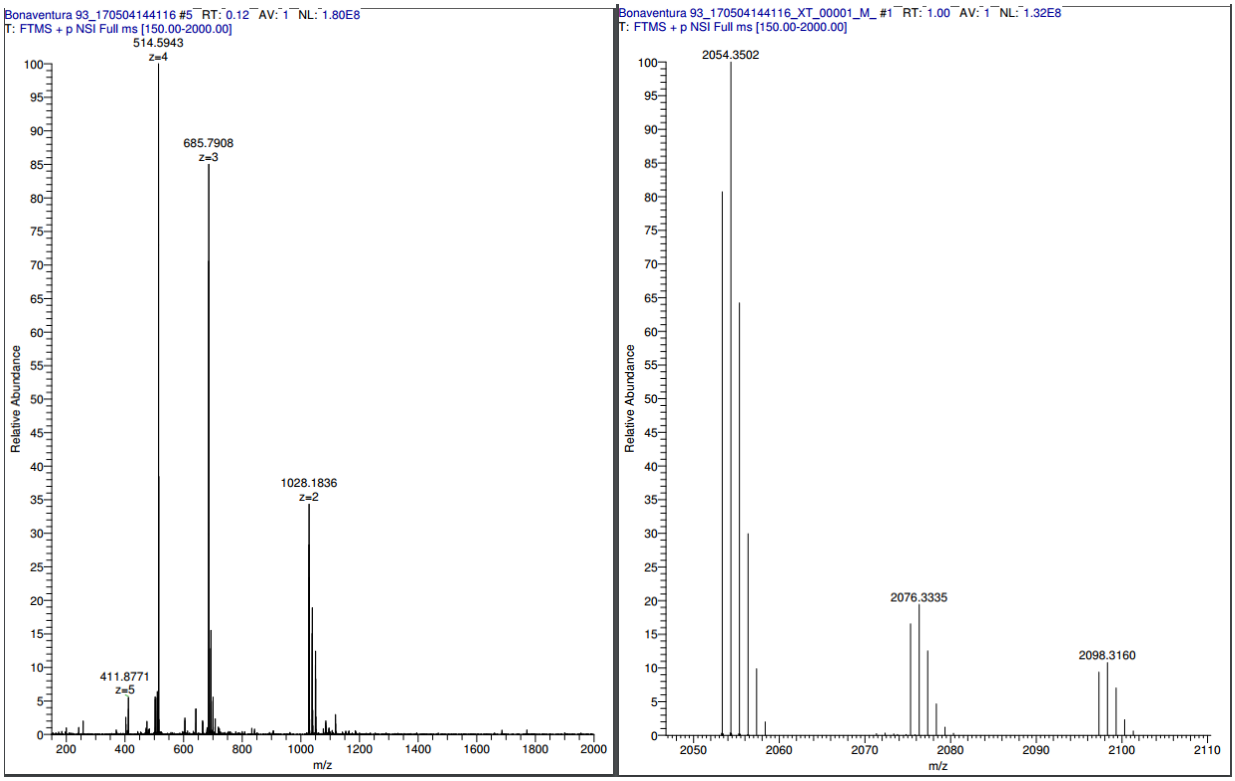




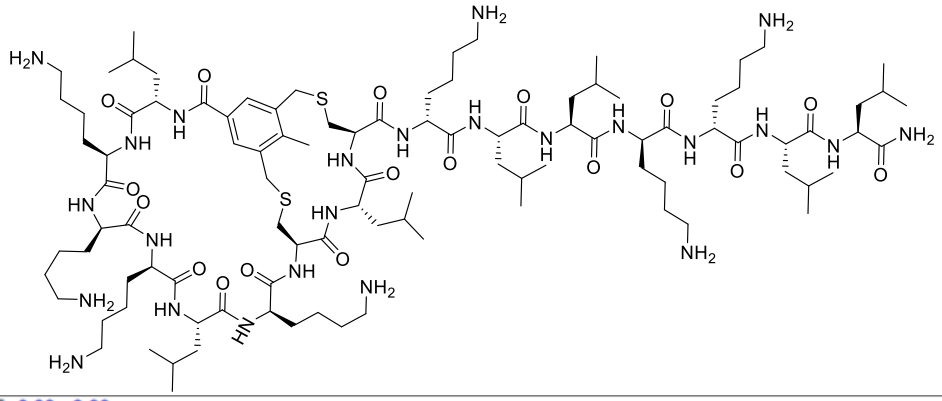
B[12]lKKKlKC[1]lC[2]KllKKll (bp54) was obtained as foamy white solid after preparative RP-HPLC (24.5 mg, 11.0 %). Analytical RP-HPLC: t_R = 2.12 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.35 Da [M].

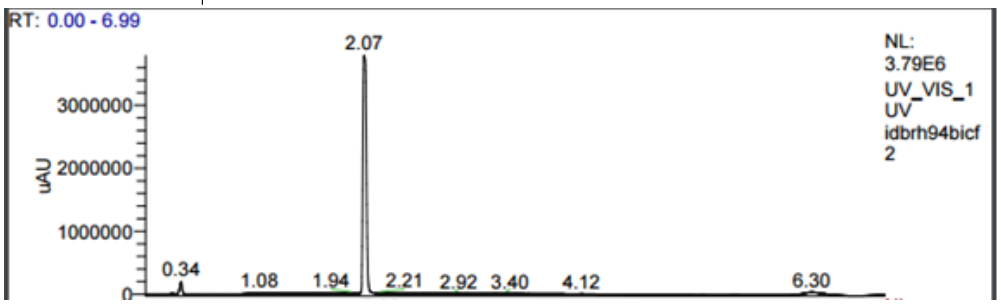


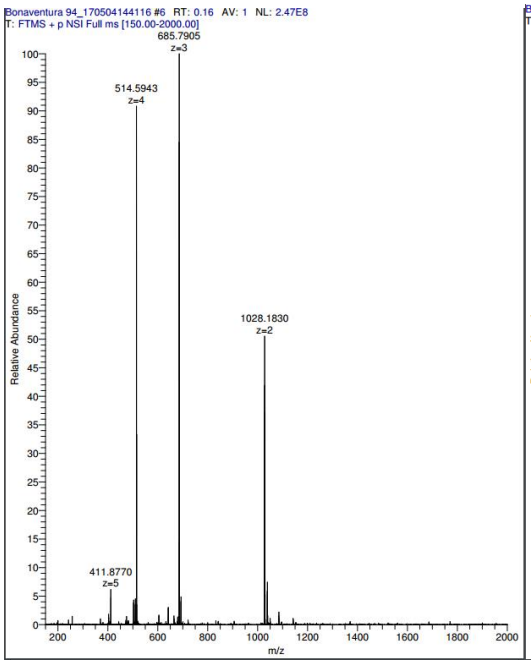


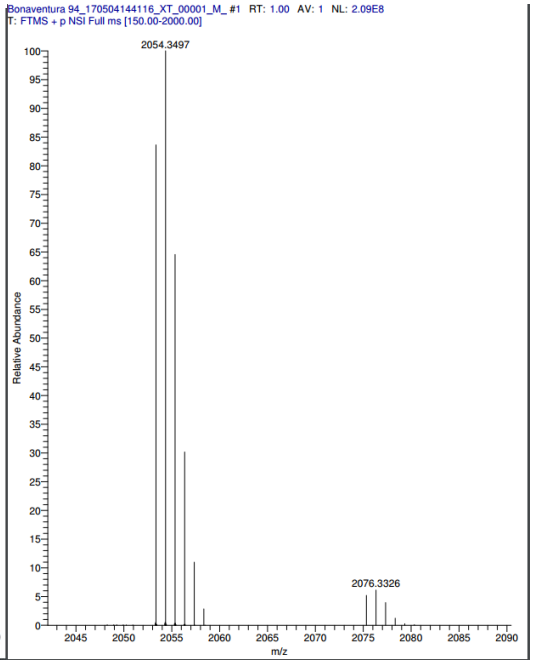


B[12]LkkkLkC[1]LC[2]kLLkkLL (bp55) was obtained as foamy white solid after preparative RP-HPLC (24.8 mg, 11.1 %). Analytical RP-HPLC: t_R = 2.07 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

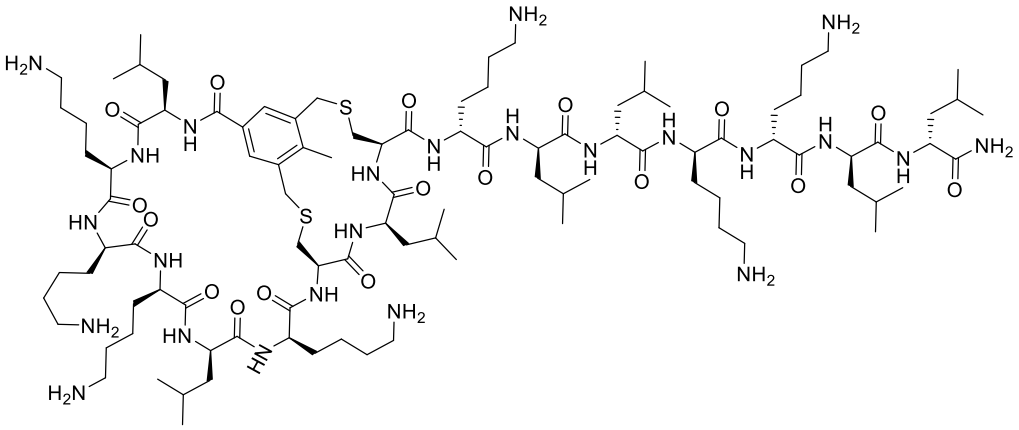


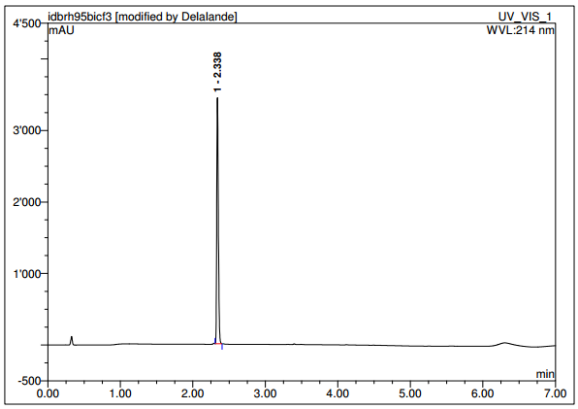


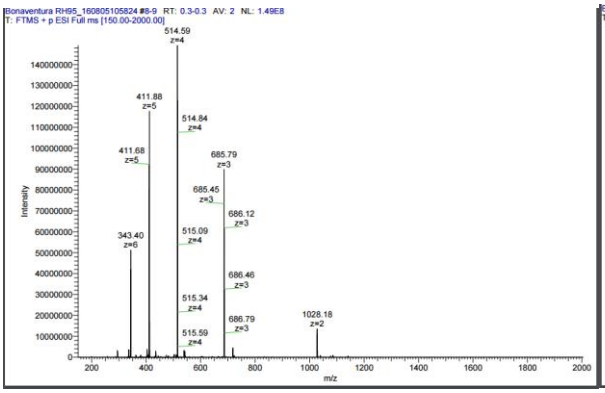


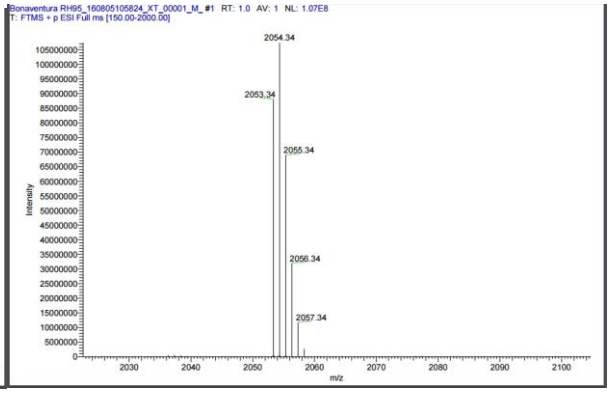


B[12]lkkklkc[1]lc[2]kllkkll (bp56) was obtained as foamy white solid after preparative RP-HPLC (34.8 mg, 15.6 %). Analytical RP-HPLC: t_R = 2.34 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{100}H_{180}N_{24}O_{17}S_2$ calc./obs. 2053.34/2053.34 Da [M].

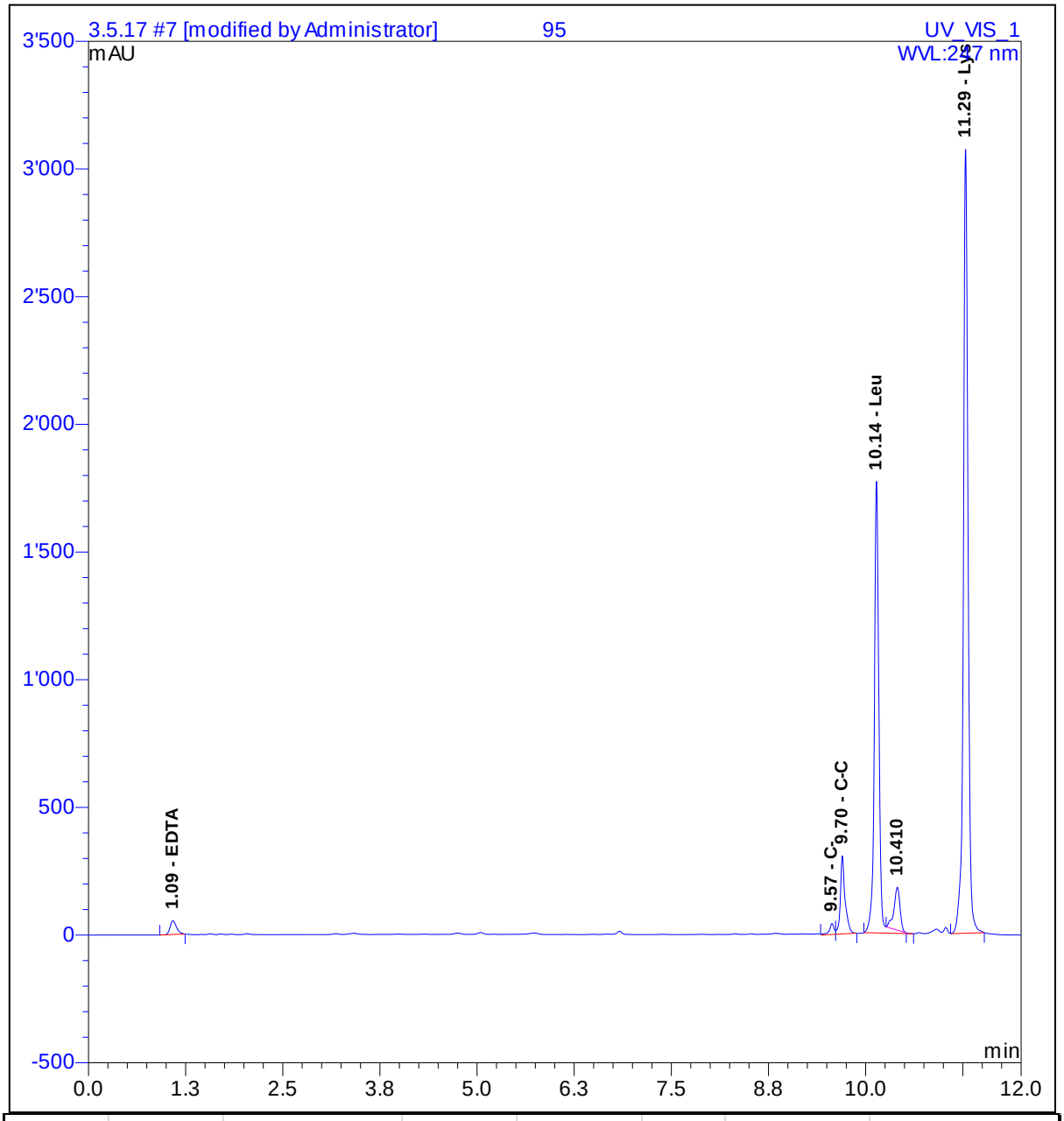






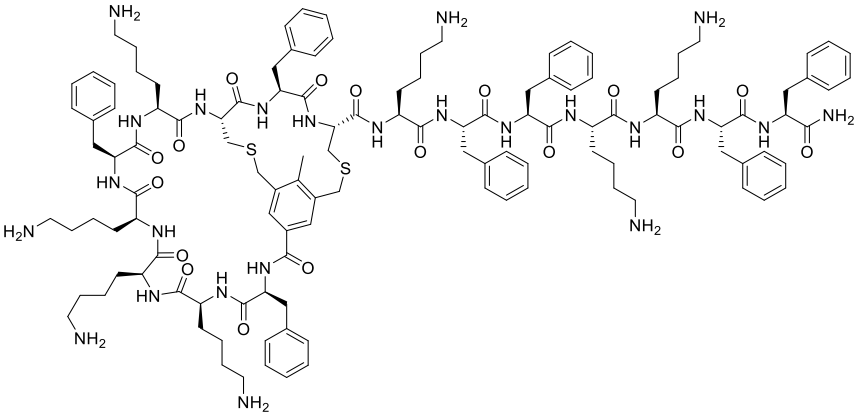


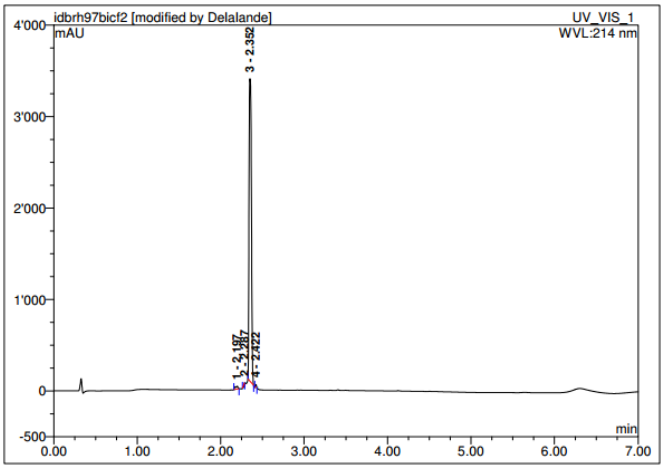
Amino acids analysis of compound **bp56**

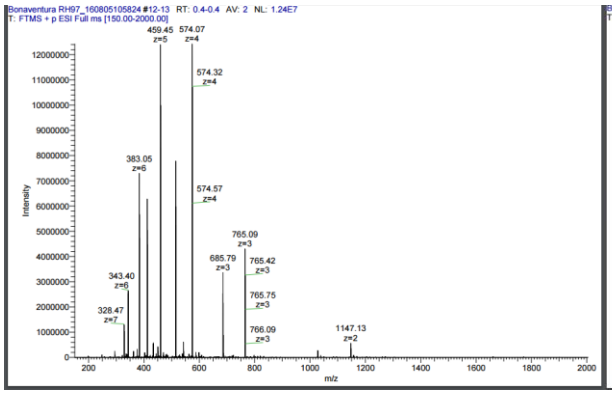


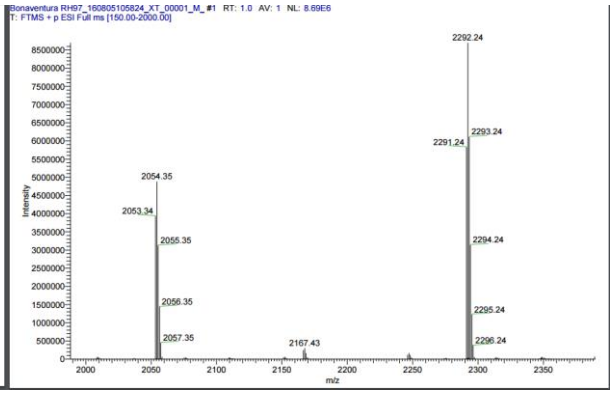
RT	RT (STD)	PW(50%)	Area	Height	n.a.	Amount	Peak Name
min	min	min	mAU*min	mAU	n.a.	pmol	
1.09	1.06	0.087	5.09	53.56		408.96	EDTA
9.57	9.51	0.056	3.02	42.83		113.69	C-
9.70	9.71	0.054	21.25	305.57		811.04	C-C
10.14	10.13	0.058	121.56	1767.88		7711.83	Leu
11.29	11.27	0.059	212.07	3069.32		7663.99	Lys
Total:						16709.51	

B[12]FKKKFKC[1]FC[2]KFFKKFF (bp57) was obtained as foamy white solid after preparative RP-HPLC (31.2 mg, 12.7 %). Analytical RP-HPLC: t_R = 2.35 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{121}H_{166}N_{24}O_{17}S_2$ calc./obs. 2291.25/2291.24 Da [M].









B[12]WKKKWKC[1]WC[2]KWWKKWW (bp58) was obtained as foamy white solid after preparative RP-HPLC (23.2 mg, 8.3 %). Analytical RP-HPLC: t_R = 2.37 min (A/D 100:0 to 0:100 in 7.00 min, λ = 214 nm). MS (ESI$^+$): $C_{135}H_{173}N_{31}O_{17}S_2$ calc./obs. 2564.31/2564.31 Da [M].