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Supporting Information for: Bead Diffusion Assay for Discovering Antimicrobial Cyclic Peptides

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Contents:

Supporting Information Tables

Table S1. Hits against *E. coli.* Beads showing an inhibition disk were picked as hits.

Hit no.[a)]	no+[b)]	noH[c)]	**X^{10}**	**X^{9}**	**X^{8}**	**X^{7}**	**X^{6}**	**X^{5}**	**X^{4}**	**X^{3}**	**X^{2}**	**X^{1}**
E1	1	6	Ile	Val	Thr	Leu	phe	Pro	His	Lys	Thz	glu
E2a	2	7	Ile	Val	Arg	Leu	phe	Pro	Leu	Lys	Thz	glu
E2b			Pro	Val	Arg	Leu	phe	Thz	Leu	Lys	Ile	glu
E3	0	6	Tyr	Val	His	Leu	phe	Thz	His	phe	Asn	glu
E4	2	6	Arg	Val	Arg	Leu	phe	Thi	Tyr	Thi	Leu	glu
E5a	2	5	lys	Val	Arg	Leu	phe	Val	His	phe	Asn	glu
E5b			Arg	Val	His	Leu	phe	Thz	Val	Lys	Asn	glu
E5c			Arg	Val	His	Leu	phe	Val	Phe	Lys	Asn	glu
E5d			lys	Val	Arg	Leu	phe	Val	His	Asn	Thz	glu
E6a	2	6	Arg	Val	His	Leu	phe	Pro	Tyr	Orn	Thz	glu
E6b			Tyr	Val	Arg	Leu	phe	Pro	His	Orn	Thz	glu
E7	0	8	Tyr	Val	Hyp	Leu	phe	Pro	Leu	phe	Ile	glu
E8	1	6	Pro	Val	Thr	Leu	phe	Thz	His	Lys	Ile	glu
E9	3	6	Arg	Val	Orn	Leu	phe	Thi	Phe	Orn	Leu	glu
E10	2	6	lys	Val	Arg	Leu	phe	Thi	Leu	phe	Ile	glu
E10b			Ile	Val	Arg	Leu	phe	Thi	Phe	Lys	Leu	glu
E11a	1	5	Pro	Val	Hyp	Leu	phe	Thi	His	Lys	Asn	glu
E11b			lys	Val	Hyp	Leu	phe	Pro	His	Thi	Asn	glu
E12 a	1	5	lys	Val	His	Leu	phe	Thi	His	Asn	Thz	glu
E12b			lys	Val	His	Leu	phe	Thz	His	Thi	Asn	glu
E13	2	7	lys	Val	Arg	Leu	phe	Val	Val	phe	Ile	glu
E14	2	5	Ile	Val	Orn	Leu	phe	Thz	His	Orn	Ile	glu
E15 a	1	6	Ile	Val	Arg	Leu	phe	Pro	Leu	Thi	Thr	glu
E15b			Arg	Val	Thr	Leu	phe	Pro	Leu	Thi	Ile	glu
E16 a	0	7	Pro	Val	His	Leu	phe	Thi	Val	Lys	Leu	glu
E16b			lys	Val	His	Leu	phe	Pro	Val	Thi	Leu	glu
E17	0	7	Pro	Val	Hyp	Leu	phe	Pro	Leu	Thi	Ile	glu
E18	3	6	lys	Val	Orn	Leu	phe	Thi	Tyr	Orn	Leu	glu

[a)] Sequences were determined from amino acid analysis (AAA) using TAGSFREE decoding. For AAA giving 2 or 4 possible sequences, the sequences are listed as a,b and a, b, c, d. [b)] Number of positively charged residues. [c)] Number of hydrophobic residues. Thz = L-Thiazolidine-carboxylic acid, Thi = β-(2-thienyl)-L-alanine. Orn = L-ornithine. Hyp = 4-hydroxy-L-proline, upper case letters for natural L-amino acids, lower case letter for D-amino acids.

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Table S2. Non-hits against E. coli. Beads showing no inhibition disk were picked as non-hits.

Hit no.[a)]	no+[b)]	noH[c)]	**X^{10}**	**X^{9}**	**X^{8}**	**X^{7}**	**X^{6}**	**X^{5}**	**X^{4}**	**X^{3}**	**X^{2}**	**X^{1}**
NE1	2	7	Arg	Val	Arg	Leu	phe	Thz	Tyr	Thi	Thz	glu
NE2a	0	7	Pro	Val	His	Leu	phe	Thz	Val	Thi	Thr	glu
NE2b	0	7	Pro	Val	Thr	Leu	phe	Val	His	Thi	Thz	glu
NE3	1	5	Arg	Val	His	Leu	phe	Thz	Phe	Asn	Asn	glu
NE4	1	6	lys	Val	Hyp	Leu	phe	Hyp	Leu	Thi	Ile	glu
NE5	2	6	lys	Val	His	Leu	phe	Thi	Val	Lys	Thz	glu
NE6a	1	8	Tyr	Val	Arg	Leu	phe	Pro	Phe	Thi	Ile	glu
NE6b			Pro	Val	Arg	Leu	phe	Thi	Tyr	phe	Ile	glu
NE7a	2	5	lys	Val	Arg	Leu	phe	Thi	His	Asn	Leu	glu
NE7b			Arg	Val	His	Leu	phe	Thi	Leu	Lys	Asn	glu
NE8a	0	7	Pro	Val	Thr	Leu	phe	Val	His	Thi	Thz	glu
NE8b	1	7	Pro	Val	His	Leu	phe	Thz	Val	Thi	Thr	glu
NE9a	1	6	Tyr	Val	Arg	Leu	phe	Val	His	phe	Thr	glu
NE9b			Arg	Val	His	Leu	phe	Val	Tyr	phe	Thr	glu
NE10	2	6	Arg	Val	Arg	Leu	phe	Pro	His	Thi	Leu	glu
NE11	1	6	Tyr	Val	His	Leu	phe	Val	His	Lys	Ile	glu
NE12a	1	6	Tyr	Val	His	Leu	phe	Hyp	Val	Lys	Ile	glu
NE12b			Tyr	Val	Hyp	Leu	phe	Val	His	Lys	Ile	glu
NE13a	0	7	Pro	Val	Hyp	Leu	phe	Val	Phe	Asn	Leu	glu
NE13b			Pro	Val	Hyp	Leu	phe	Val	Leu	phe	Asn	glu
NE14	0	5	Tyr	Val	His	Leu	phe	Hyp	Leu	Asn	Asn	glu
NE15	0	5	Tyr	Val	His	Leu	phe	Hyp	Val	Asn	Asn	glu
NE16	1	4	Ile	Val	His	Leu	phe	Hyp	His	Lys	Thr	glu
NE17	1	6	Arg	Val	Thr	Leu	phe	Val	His	Thi	Leu	glu
NE18a	1	6	Arg	Val	Thr	Leu	phe	Val	His	Thi	Leu	glu
NE18b			Arg	Val	His	Leu	phe	Val	Leu	Thi	Thr	glu
NE19	2	6	lys	Val	Hyp	Leu	phe	Thi	Tyr	Lys	Leu	glu
NE20a	1	7	Tyr	Val	Hyp	Leu	phe	Pro	Phe	Lys	Leu	glu
NE20b			Tyr	Val	Hyp	Leu	phe	Pro	Phe	Lys	Leu	glu
NE21	0	7	Pro	Val	Thr	Leu	phe	Val	His	Thi	Thz	glu
NE22	0	7	Pro	Val	His	Leu	phe	Thz	Val	Thi	Thr	glu
NE23	3	6	lys	Val	Arg	Leu	phe	Thi	Leu	Lys	Ile	glu
NE24	2	5	lys	Val	Hyp	Leu	phe	Hyp	Leu	Orn	Ile	glu
NE25	1	8	Ile	Val	Orn	Leu	phe	Pro	Tyr	Thi	Ile	glu
NE26a	2	5	Pro	Val	Orn	Leu	phe	Thi	His	Orn	Thr	glu

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NE26b	2	6	Ile	Val	Orn	Leu	phe	Thi	Tyr	Lys	Thr	glu
NE27	2	6	lys	Val	Thr	Leu	phe	Thi	Tyr	Orn	Ile	glu
NE28a	3	5	lys	Val	Orn	Leu	phe	Thi	Tyr	Lys	Asn	glu
NE28b			lys	Val	Arg	Leu	phe	Thi	Leu	Lys	Ile	glu
NE29	1	7	Tyr	Val	Hyp	Leu	phe	Val	Phe	Orn	Ile	glu
NE30	1	8	Ile	Val	Arg	Leu	phe	Thz	Val	phe	Ile	glu

[a] Sequences were determined from amino acid analysis (AAA) using TAGSFREE decoding. For AAA giving 2 or 4 possible sequences, the sequences are listed as a,b and a, b, c, d. [b] Number of positively charged residues. [c] Number of hydrophobic residues. Thz = L-Thiazolidine-carboxylic acid, Thi = β-(2-thienyl)-L-alanine. Orn = L-ornithine. Hyp = 4-hydroxy-L-proline, upper case letters for natural L-amino acids, lower case letter for D-amino acids.

Table S3. Hits against *B. subtilis*. Beads showing an inhibition disk were picked as hits.

Hit no.[a]	no+[b]	noH[c]	X^{10}	X^{9}	X^{8}	X^{7}	X^{6}	X^{5}	X^{4}	X^{3}	X^{2}	X^{1}
B1	3	5	lys	Val	Arg	Leu	phe	Pro	Val	Lys	Asn	glu
B2	2	5	Pro	Val	Arg	Leu	phe	Val	His	Lys	Asn	glu
B3	2	5	Arg	Val	His	Leu	phe	Pro	Val	Lys	Asn	glu
B4	0	7	Pro	Val	Hyp	Leu	phe	Pro	Leu	Asn	Ile	glu
B5a	1	6	Ile	Val	Hyp	Leu	phe	Pro	Val	Lys	Asn	glu
B5b			lys	Val	Hyp	Leu	phe	Pro	Val	Asn	Ile	glu
B6a	1	6	Ile	Val	Hyp	Leu	phe	Pro	Val	Lys	Asn	glu
B6b			lys	Val	Hyp	Leu	phe	Pro	Val	Asn	Ile	glu
B7	1	5	lys	Val	Hyp	Leu	phe	Pro	Tyr	Asn	Asn	glu
B8	2	5	lys	Val	Hyp	Leu	phe	Pro	Val	Lys	Asn	glu
B9	1	6	Pro	Val	Hyp	Leu	phe	Pro	Tyr	Lys	Asn	glu
B10	2	4	lys	Val	Hyp	Leu	phe	Pro	His	Lys	Asn	glu
B11	2	5	lys	Val	Hyp	Leu	phe	Pro	Val	Lys	Asn	glu
B12	1	6	Pro	Val	Hyp	Leu	phe	Pro	Tyr	Lys	Asn	glu
B13	2	7	lys	Val	Arg	Leu	phe	Val	Val	phe	Ile	glu

[a] Sequences were determined from amino acid analysis (AAA) using TAGSFREE decoding. For AAA giving 2 or 4 possible sequences, the sequences are listed as a,b and a, b, c, d. [b] Number of positively charged residues. [c] Number of hydrophobic residues. Thz = L-Thiazolidine-carboxylic acid, Thi = β-(2-thienyl)-L-alanine. Orn = L-ornithine. Hyp = 4-hydroxy-L-proline, upper case letters for natural L-amino acids, lower case letter for D-amino acids.

Supplementary Material (ESI) for Chemical Communications

Synthesis

General methods. Reagents were purchased in the highest quality available from Sigma-Aldrich, Advanced ChemTech, Bachem or Novabiochem. TentaGel and AM resins were purchased from Rapp Polymere GmbH. NovaSyn Photocleavable resin was purchased from Novabiochem. The following commercially available amino acids were used in synthesis: Fmoc-Ala-OH, Fmoc-βAla-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Asp(OH)-1-allyl ester, Fmoc-Glu(tBu)-OH, Fmoc-Gly-OH, Fmoc-β-(2-thienyl)-L-alanine, Fmoc-Hyp(tBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH, Fmoc-Thr(tBu)-OH, Fmoc-Val-OH, Fmoc-L-thiazolidine-4-carboxylic acid. All solvents used in reactions were bought in p.a. quality. Solvents for washings were distilled from technical quality. Preparative RP-HPLC was performed with HPLC-grade acetonitrile and milli-Q deionized water in a Waters prepak cartridge 500 g (RP-C18 20 mm, 300 A pore size). Analytical RP-HPLC (flow rate 1.2 mL/min) was performed on Waters 600E systems with a Waters Atlantis (4.6 mm×100 mm, dC18, 5 µm) column, UV detection with Waters 996 photodiode array detector at 214 nm. Eluents for all systems were: A: water + 0.1% TFA; B: Acetonitrile/H_20 (6:4) + 0.1% TFA and C: Acetonitrile/H_20 (9:1) + 0.1% TFA; washing eluent: Acetonitrile/H_20 (1:1). Analytical RP-UPLC was performed on Ultimate 3000 RS from Dionex (autosampler, diode array detector) chromatography system using a Acclaim RSCL 120 Dionex column (C18, 2.2 um, 120 A (Angstrom), 3.0 x 50.0 mm) with a flow rate of 1.2 mL/min. Solid-phase peptide chemistry was performed in polypropylene syringes (BBraun,#2057926) provided with a polyethylene frit and a teflon stopcock and stopper. Photolysis reactions were conducted either with a Hg UV-lamp (100 W) or a UV-Emitter Box equipped with two UV-LED Lamps (90 mW(Ag), lens to bottom distance: 34 mm, with a 110° degree angle of radiation). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 300 (300 MHz) and DRX400 (400 MHz) spectrometers. Chemical shifts (δ) are given in ppm referring to tetramethylsilane (TMS); coupling constants (*J*) in Hertz (Hz). Mass spectra were obtained by electron spray ionization (ES-MS) on a Micromass Autospec Q (Waters/Micromass) instrument in the positive mode and provided by the mass spectrometry service of the Department of Chemistry and Biochemistry, University of Bern.

Silylation procedure for glass vials. Following a standard procedure (T. Naykki, *Anal. Bioanal. Chem*., 2002, **372**, 829), the glassware was placed in a 1 M HCl solution and soaked overnight. The acid solution was decanted and the glassware was rinsed extensively with deionized water followed by a final washing with methanol. The glass vials were dried at 100°C. 5% (v/v) dimethyldichlorosilane in toluene was poured into a large container equipped with a cap. The glassware was placed into the container. The container was sealed and the glassware was allowed to

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stand in these conditions for 10 hours. Then, the glassware was removed, washed with deonized water and dried at 100◦C for 2 hours.

Fmoc-D-Glu-1-allyl ester. The procedure was adapted and simplified from Dumy et al. (E. Garanger, D. Boturyn, O. Renaudet, E. Defrancq and P. Dumy, *J Org Chem*, 2006, **71**, 2402) Commercial Fmoc-D-Glu(OtBu)-OH (5.0 g, 11.8 mmol, 1 eq.) was dissolved in 20 mL of a MeOH: H_2O (20:1, v/v) mixture and a $CsCO_3$ 3 M aq. solution was added dropwise until pH=8. The solution was evaporated to dryness and the white solid taken up with acetonitrile (200 mL). This suspension was added dropwise to a solution of allyl bromide (4.1 mL, 47.2 mmol, 3 eq.) in acetonitrile and stirred at room temperature. After four hours of reaction time, $CsCO_3$ solution (200 µL, 3 M) and allyl bromide (2.0 mL, 24 mmol, 2.0 eq.) were added to the reaction mixture. The reaction was monitored by TLC (CH_2Cl_2:MeOH:AcOH 95:4:1 v/v). After 17 hours of reaction, 100% conversion was achieved and the solution was evaporated to dryness. The white solid was suspended in 30 mL of ethyl acetate and evaporated under reduced pressure. This step was repeated four times. The crude was dissolved in 30 mL of CH_2Cl_2 and added dropwise to 70 mL of a solution of TFA: CH_2Cl_2 5:2 under strong stirring. The reaction was monitored by TLC (CH_2Cl_2:Ethyl acetate 10:1 v/v). After 50 minutes the solution was evaporated to dryness. The residue was sonicated in diethyl ether (5 mL) and evaporated and the procedure was repeated once. A minimum of CH_2Cl_2 was added to solubilize the crude and the solution was precipitated in 300 mL of pentane. Fmoc-D-Glu-1-allyl ester was obtained as a white solid after filtration (4.38 g, 10.7 mmol, 91% yield). ^{1}H NMR (300 MHz, DMSO): δ=12.19 (br. s, 1 H, CO_2H), 7.85 (d, 2 H, $J_{Har.}$=7.3 Hz, $H_{ar.}$), 7.89 (d, 1 H, $J_{NH,CH\alpha}$ =8.0 Hz, NH), 7.72 (d, 2 H, *J*=7.3), 7.46-7.28 (m, 4 H, $H_{ar.}$), 5.98-5.82 (m, 1 H, CH=), 5.31 (dd, 1 H, *J*=1.3 Hz, *J*=17.3 Hz), 5.20 (dd, 1 H, *J*=1.3 Hz, *J*=10.5 Hz), 4.59 (d, 2 H, *J*=5.2 Hz), 4.37-4.19 (m, 3 H, CH_{Fmoc}, CH_{2Fmoc}), 4.20-4.07 (m, 1 H), 2.34 (t, 2 H, *J*= 7.2 Hz), 2.11-1.71 (m, 2 H). ^{13}C NMR (75 MHz, DMSO): δ=173.7 (HOC=O), 171.8 (C=O), 156.2 (C=O), 143.8 (C_{ar}), 143.7 (C_{ar}), 140.8 (C_{ar}), 132.4 (CH=), 127.7 (CH_{ar}), 127.1 (CH_{ar}), 120.1 (CH_{ar}), 117.7 (CH_2=), 65.7 (CH_{2Fmoc}), 64.9 (OCH_2), 53.2 (CH_α), 46.7 (CH_{Fmoc}), 30.0 (CH_2), 26.0 (CH_2).

Library synthesis and screening

Synthesis of photocleavable combinatorial cyclic decapeptides library. The photocleavable cyclic decapeptide library was synthesized on Rapp Polymers TentaGel Macrobeads NH_2 resin (1.0 g, 0.23 mmol/g loading) using the split-and-mix procedure. The resin was first loaded with hydroxyethyl photolinker (4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid) (107 mg, 0.35 mmol, 1.5 eq.), PyBop (195 mg, 0.35 mmol, 1.5 eq.) and DIPEA (115 µL, 0.35 mmol, 3.0 eq.). The reaction was performed under strict light protection and constant stirring. After two hours of reaction time, a small portion of the resin was tested with TNBS and showed no presence of free amines. The resin was washed with MeOH, NMP and CH_2Cl_2 (3×5mL each), sealed in a sylilated glass vial equipped with a septum and dried under high vacuum over night. The first amino acid was loaded by MSNT/MeIm procedure. 1-(Mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT), (408 mg, 1.4 mmol, 6.0 eq.) and Fmoc-D-Glu-1-allyl ester (546 mg, 1.4 mmol, 6.0 eq.) were dried under reduced pressure for 15 hours. The dried resin was swollen in dry CH_2Cl_2 for ten minutes in a sylilated glass vial. MSNT and Fmoc-D-Glu-1-allyl ester were dissolved separately in dry CH_2Cl_2. 1-methyl imidazole (114 µL, 1.4 mmol, 6.0 eq.) was added to the Fmoc-D-glutamate solution and transferred via syringe to the solution of MSNT in CH_2Cl_2. The mixture was immediately transferred to the swollen resin and stirred under argon. After four hours of reaction time, the reagents were washed away with NMP, MeOH and CH_2Cl_2 and the resin was then deprotected with piperidine 20% in NMP (3 mL, 3×10 min). After α-amine deprotection, the resin was splitted in five equal portions according to the split-and-mix procedure and further acylated with 3 equivalents of N-α-Fmoc amino acid in presence of PyBOP (760 mg, 0.69 mmol, 3.0 eq.) and DIPEA (460 µL, 1.38 mmol, 6.0 eq.) in NMP for 60 min. Reagents were washed away (3×5 mL each) with NMP, CH_2Cl_2 and MeOH and controlled by TNBS test. The coupling was repeated and followed by acetylation. Fmoc protecting groups were removed by treatment with piperidine 20% in NMP (3 mL, 3×10 min) and the resin was washed with NMP, CH_2Cl_2 and MeOH. After the last coupling step, the resin bearing the library of decapeptides (1.0 g, 0.23 mmol) was sealed in a polypropylene syringe equipped with a septum and dried under vacuum for one hour, then washed with dry MeOH (10 mL, 2×5 min) and dry CH_2Cl_2 (10 mL, 1×15 min). The resin was treated with $PhSiH_3$ (714 µL, 5.8 mmol, 25 eq.) in dry CH_2Cl_2 (10 mL) for 5 minutes. $Pd(PPh_3)_4$ (70 mg, 0.06 mmol, 0.25 eq.) was then added and the resin was stirred under argon for 15 minutes. The reagents were removed by filtration and the resin washed with dry CH_2Cl_2 (5 mL, 5×1 min). The procedure was repeated twice. The resin was washed with dry CH_2Cl_2 (10 mL, 2×15 min), dioxane:water (9:1, 10 mL, 2×1 min) and NMP (10 mL, 2×1 min). Finally, the last Fmoc protecting group was removed as described above. PyBoP (380 mg, 0.69 mmol, 3.0 eq.) and DIPEA (240 µL, 1.4 mmol, 6.0 eq.)

Supplementary Material (ESI) for Chemical Communications

were added to the deprotected resin in 5 mL of NMP and the mixture was stirred at room temperature for 3 hours. The reagents were removed by washing with NMP (5 mL, 2×1 min), MeOH (5 mL, 1×1 min) and CH_2Cl_2 (4 mL, 2×1 min). HATU (262 mg, 0.69 mmol, 3.0 eq.) and DIPEA (240 µL, 1.4 mmol, 6.0 eq.) were added to the library and the resin was stirred over night. The mixture was filtered and the resin was further washed with CH_2Cl_2, NMP and MeOH and acylated with acetic anhydride: CH_2Cl_2 (1:1, v/v) for 10 minutes. At the end of the synthesis, the resin was dried and stored at -20°C. Just before screening, the side-chain protecting groups were removed with TFA/TIS/EDT/H_2O (92:5:2:1) (2×2 hours).

Antimicrobial activity screening. Antimicrobial activity screening was started by washing a portion of the fully deprotected library (50 mg, 50% library coverage) with NMP, CH_2Cl_2 and MeOH (5×3 mL each). The beads were swollen in milli-Q water for 1 hour. Water was filtered away and the beads were dried under vacuum for 2 hours. The resin was spread on 10 microscope glass slides and each plate was irradiated under UV light (180 mW) for eight hours and kept Sterile in Petri dishes (φ=9 cm). A single bacterial colony (*E. coli* (Top10) or *B. subtilis* (BR151)) was grown in liquid LB Broth media until reaching at least an OD600 of 0.15. The bacterial suspension was adjusted to OD600=0.132. Agar plates were prepared for the screening half-opened up-side down at 37°C for 20 minutes. Inoculation was carried out by dipping a sterile cotton-wool swab into the suspension. Excess was removed by turning the swab against the side of the tube. Inoculum was evenly spread over the entire surface of the plate by swabbing in three directions. The plate was allowed to dry for 5 minutes before transferring the photocleaved resin beads. The beads were carefully transferred from the glass slide (20×26 mm, Menzel-Gläser) to the freshly inoculated agar plates and incubated at 37°C for 18 hours. The plates were sprayed with a MTT solution (0.1% m/V in H_20) and beads showing an inhibition disk were picked as positive hits. All beads were individually rinsed in ethanol and submitted to AAA.

TAGSFREE and AAA sequence determination of cyclic peptide library members. Single peptide-containing resin beads were hydrolysed with aqueous HCl (6 M) at 110°C for 22 hours and their amino acid composition was determined quantitatively by HPLC after derivatization with phenyl isothiocyanate (PITC). The sequences were then determined by TAGSFREE decoding (J. Kofoed, J.-L. Reymond, *J. Comb. Chem*., 2007, **9**, 1046).

General SPPS procedure for single sequences. The was carried manually or in a PSW 1100 multiple peptide synthesizer (Chemspeed Technologies) equipped with one solid-phase block (16 reactors). Prior to every reaction the resin was swollen in CH_2Cl_2. The Fmoc-protected Tentagel Macrobead RAM resin or Tentagel HL RAM resin was treated twice with a piperidine solution (20% v/v in NMP) for 15 min. Chain elongation: The resin was acylated with each amino acid (3.0 eq.) using PyBop (3.0 eq.) and DIPEA (6.0 eq.) in NMP for 2 h. The completion of the reaction was checked using the TNBS test. In the case of automated synthesis, each acylation step was repeated twice and no free-amine test was performed. The Fmoc protecting groups were removed with a solution of 20% piperidine in NMP (2×15 min). Allyl and N -terminal deprotectionAfter the last coupling, the resin was sealed in a polypropylene syringe (Braun Injekt), equipped with a septum and dried under vacuum for one hour. Then, it was washed with dry MeOH (5 mL, 2×5 min) and swollen in CH_2Cl_2 for 15 min under Argon. $PhSiH_3$ (25 eq.) was diluted in dry CH_2Cl_2, added to the resin and mixed via argon bubbling for 3 min. $Pd(PPh_3)_4$ (0.25 eq.) was then added and the resin was stirred under argon for 15 min. The reagents were removed by filtration, the resin washed with dry CH_2Cl_2 (5 mL, 5×1 min). The procedure was repeated twice. The resin was washed with dry CH_2Cl_2 (10 mL, 2×15 min), dioxane:water (9:1, 10 mL, 2×1 min) and NMP (10 mL, 2×1 min). The last Fmoc protecting group was removed as previously described. On-resin Cyclization: PyBoP (3.0 eq.) and DIPEA (6.0 eq.) were added to the deprotected resin in 3 mL of NMP and the mixture was stirred at room temperature for 3 hours. The reagents were removed by washing with NMP (5 mL, 2×1 min), MeOH (5 mL, 1×1 min) and CH_2Cl_2 (4 mL, 2×1 min). HATU (3.0 eq.) and DIPEA (6.0 eq.) were dissolved in NMP, added to the resin and stirred over night. The mixture was filtered and the resin was washed with CH_2Cl_2, NMP and MeOH and acylated with acetic anhydride: CH_2Cl_2 (1:1, v/v) for 10 min. TFA Cleavage: The cleavage was carried out with TFA/TIS/H_2O (94:5:1) (and TFA/TIS/EDT/H2O (92:5:2:1) for Thi and Thz containing sequences) during 5 hours. The peptide was precipitated with methyl tert-butyl ether, dried under vacuum and then dissolved in a water/acetonitrile mixture. Purification was done by preparative HPLC. The purified peptide was freeze-dried and stored at -20°C.

Cyclic Peptide E1 c(IVTLfPHK*Thz*q). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E1** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (3.3 mg, 6%). Anal. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R=7.5 min; ESI MS(+): Calcd. for $C_{56}H_{87}N_{14}O_{12}S$ ($[M+H]^+$): 1179.6, found: 1179.6.

Cyclic Peptide E3 c(YVHLfThzHfNq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E3** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (3,2 mg, 4%); anal. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R=9.3 min; ESI MS(+): Calcd. for $C_{63}H_{79}N_{15}O_{14}S$ ($[M+H]^+$): 1301,6.7, found: 1301,6.

Cyclic Peptide E4 c(RVRLf*Thi*Y*Thi*Lq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E4** was synthesized in a peptide synthesizer and cylccized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (4.3 mg, 4%); anal. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R=9.3 min; ESI MS(+): Calcd. for $C_{66}H_{96}N_{17}O_{12}S_2$ ($[M+H]^+$): 1382.7, found: 1382.7.

Cyclic Peptide E6 c(RVHLfPY*OrnThz*q). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E6** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (6.5 mg, 9%); anal. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R=8.7 min; ESI MS(+): Calcd. for $C_{60}H_{87}N_{17}O_{12}S$ ($[M+H]^+$): 1269.6, found: 1269.6.

Cyclic Peptide E8 c(PVTLf*Thz*HKIq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E8** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (2.8 mg, 3%); anal. RP-HPLC (93% A, 7% C to 100% C in 12 min): t_R=10.1 min; ESI MS(+): Calcd. for $C_{56}H_{87}N_{14}O_{12}S$ ($[M+H]^+$): 1179.6, found: 1179.6.

Cyclic Peptide E9 c(RV*Orn*Lf*Thi*F*Orn*Lq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E9** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (4.4 mg, 7%); anal. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R=17.0 min; ESI MS(+): Calcd. for $C_{63}H_{97}N_{16}O_{11}S$ ($[M+H]^+$): 1285.7, found: 1285.4.

Cyclic Peptide E13 c(kVRLfVVfIq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E13** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (4.6 mg, 8%); anal. RP-HPLC (80% A, 10% C to 100% C in 10 min): t_R=6.4 min; ESI MS(+): Calcd. for $C_{62}H_{100}N_{15}O_{11}$ ($[M+H]^+$): 1230.8, found: 1230.8.

Cyclic Peptide E14 c(IV*Orn*Lf*Thz*H*Orn*Iq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E14** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (2.8 mg, 5%); anal. RP-HPLC (80% A, 20% C to 20% A, 80% C in 10 min): t_R=7.6 min; ESI MS(+): Calcd. for $C_{57}H_{92}N_{15}O_{11}S$ ($[M+H]^+$): 1194.7, found: 1194.8.

Cyclic Peptide E18 c(kV*Orn*Lf*Thi*Y*Orn*Lq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E18** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (3.0 mg, 5%); anal. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R=8.8 min; ESI MS(+): Calcd. for $C_{63}H_{97}N_{14}O_{12}S$ ($[M+H]^+$): 1273.7, found: 1273.8..

Cyclic Peptide B6 c(IV*Hyp*LfPVKNq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **B6** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (6.9 mg, 13%); anal. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R=16.7 min; ESI MS(+): Calcd. for $C_{56}H_{90}N_{13}O_{13}$ ($[M+H]^+$): 1152.7, found: 1152.6.

Cyclic Peptide B7 c(kV*Hyp*LfPYNNq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **B7** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (7.3 mg, 13%); anal. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R=16.7 min; ESI MS(+): Calcd. for $C_{58}H_{85}N_{14}O_{15}$ ($[M+H]^+$): 1217.6, found: 1217.4.

Cyclic Peptide B10 c(kV*Hyp*LfPHKNq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **B10** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (5 mg, 9%); anal. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R=17.1 min; ESI MS(+): Calcd. for $C_{57}H_{89}N_{16}O_{13}$ ($[(M+2H)/2]^{2+}$): 603.4, found: 603.8.

Cyclic Peptide N9 c(RVHLfVYfTq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **N9** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (6.2 mg, 10%); anal. RP-HPLC (90 A, 10% C to 100% C in 10 min): t_R=8.7 min; ESI MS(+): Calcd. for $C_{64}H_{90}N_{16}O_{13}$ ($[M+2H/2]^+$): 645.9, found: 646.6.

Cyclic Peptide N29 c(YV*Hyp*LfVF*Orn*Iq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **N29** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (7.7 mg , 13%); anal. RP-HPLC (90 A, 10% C to 100% C in 10 min): t_R=8.2 min; ESI MS(+): Calcd. for $C_{64}H_{93}N_{12}O_{13}$ ($[M+H]^+$): 1237.7, found: 1237.8.

Cyclic Peptide E18a c(kV*Orn*LfFY*Orn*Lq). From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E18a** was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (13 mg, 21%); anal. RP-HPLC (80% A, 20% C to 100% C in 11 min): t_R=9.7 min; ESI MS(+): Calcd. for $C_{65}H_{99}N_{14}O_{12}$ ($[(M+2H)/2]^{2+}$): 634.4, found: 634.6 .

Linear Peptide E18b kV*Orn*Lf*Thi*Y*Orn*Lq-NH$_2$. From TentaGel Macrobeads RAM (200 mg, 0.048 mmol), **E18b** was synthesized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (26 mg, 44%); anal. RP-HPLC (100% A to 100% B in 15 min): t_R=11.5 min; ESI MS(+): Calcd. for $C_{63}H_{99}N_{14}O_{13}S$ $[M+H]^+$): 1291.7, found: 1291.8.

Indolicidin $_2$HN-ILPWKWPWWPWRR-COOH Control Peptide C1. Control peptide **C1** was synthesized on hydroxymethyl-photolinker NovaSyn TG resin (250 mg, 0.06 mmol/g). Attachment of the first amino acid to the hydroxymethyl group was done using MSNT as coupling reagent and under an argon atmosphere. The resin was dried under vacuum and purged with Argon in dry CH_2Cl_2 . Dry MSNT (0.30 g, 0.3 mmol, 5 eq.) was dissolved in DCM and minimum of THF was added to the mixture. Fmoc-Arg(Pbf)-OH (649 mg, 0.3 mmol, 5 eq.) was dissolved in DCM and MeIm (53 µL, 0.23 mmol, 3.8 eq.) was added. The mixture was transferred to the MSNT solution. The coupling mixture was then added to the swollen resin and stirred for 2 hours. The procedure was repeated twice. A sample of the resin was removed for determination of the loading by the Fmoc method (loading found: 0.104 mmol/g). Chain elongation was done using PyBop/DIPEA as previously described. The peptide was deprotected by treatment with TFA (TFA:TIS:H_2O 95:2.5:2.5) for 3 hours. 130 mg of resin (0.014 mmol, calcd loading of 0.104 mmol/g) were suspended in mili-Q water and irradiated with UV light (365 nm, Hg Lamp, 100 W) for 4 hours under orbital agitation. HPLC profile showed the peptide in 58% purity in the crude. The solution was recovered, freeze-dried and purifed by preparative RP-HPLC. **C1** was obtained as a colourless foamy solid (12.4 mg, 21% yield); anal. RP-HPLC (95% A, 5% B, isocratic 3 min, then to 10% A, 90% B in 12 min): t_R = 13.8 min; ESI MS(+): Calcd. For $C_{100}H_{131}N_{25}O_{14}$ ($[M+H^{2+}$): 1907.3, ($[M+2H]^{2+}$): 954,2, found: 954,2 ($[M+2H/2)^{2+}$].

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Cyclic Control Peptide C2 c(KYV*Orn*LfPFkN). From TentaGel Macrobeads RAM (300 mg, 0.069 mmol), **C2** (M. A Marques, D. M. Citron, D. M.; C. C. Wang, *Bioorg. Med. Chem*., 2007, **15**, 6667) was synthesized in a peptide synthesizer and cyclized manually. It was obtained as a colourless foamy solid after preparative RP-HPLC purification (24 mg, 28%); anal. RP-HPLC (85% A, 15% C to 100% C in 20 min): t_R=15.0 min; ESI MS(+): Calcd. for $C_{64}H_{95}N_{14}O_{12}$ ($[M+H]^+$): 1251.7, found: 1251.6.

Melittin GIGAVLKVLTTGLPALISWIKRKRQQ-NH_2 Control Peptide C3. From Novasyn TG resin, (500 mg, 0.12 mmol), **Melittin** was manually synthesized and obtained as a colourless foamy solid after preparative RP-HPLC purification (170 mg, 52%); anal. RP-UPLC (100% A, 100% C in 2.2 min): t_R = 1.90 min; ESI MS(+): Calcd. for $C_{131}H_{230}N_{39}O_{31}$ ($[M+H]^+$): 2845.8, found: 2846.0.

Pexiganan GIGKFLKKAKKFGKAFVKILKK-NH_2Control Peptide C4. From Novasyn TG resin, (500 mg, 0.12 mmol) **C4** was manually synthesized and obtained as a colourless foamy solid after preparative RP-HPLC purification (134 mg, 47%); anal. RP-UPLC (100% A, 100% C in 2.2 min): t_R = 1.59 min; ESI MS(+): Calcd. for $C_{122}H_{211}N_{32}O_{22}$ ($[M+H]^+$): 2476.6, found:2477.0.

Biological Assays

Broth microdilution method for antimicrobial peptides. Minimal inhibition concentrations of the peptides against bacterial strains were determined following standard microdilution methods as described by R. Hancock et al (I. Wiegand, K. Hilpert and R. E. Hancock, *Nat Protoc*, 2008, **3**, 163-175.) with two-fold serial dilutions in LB Broth with *B. subtilis* (BR 151) or *E. coli* (Top10). Stock solutions (1.0 mg/mL) were sterilized with a 0.45 µm filter prior to use. 96-well polypropylene no-tissue treated microtiter plates . Each well contained 100 µl (50 µl inoculum plus 50 µl of peptide solution). The final cell density was $1x10^5$ CFU/mL. MIC end-points were determined by visual inspection after incubation at 37°C for 18 to 24 h. The tyrocidine A analogue **C2** c(KYV*Orn*LfPFkN) was used as a positive control and presented a constant MIC value of 8 µl/mL, comparable to the MIC presented in literature for a similar strain of *B. subtilis* (MRL 18734), MIC=2 µl/mL, (M. A. Marques et al. , *Bioorg. Med. Chem.*, 2007, **15**, 6667). Final values are the result of three independent determinations. All dilutions were done in duplicates.

Minimal inhibitory concentration of a series of bacterial strains. MIC values of selected cyclic peptides were tested in Basilea Pharmaceutica Ltd. using the standard broth microdilution method as recommended by the Clinical and Laboratory Standards Institute, protocol M7-A7. The concentration range was 0.06 to 32 µl/mL. The bacterial strains used were: *Staphylococcus aureus* (ATCC25923); *Staphylococcus aureus* (887); *Staphylococcus aureus* (clinical isolate of MRSA,42080); *Staphylococcus epidermidis* (ATCC14990); *Staphylococcus epidermidis* (J147); *Enterococcus faecalis* (ATCC29212); *Enterococcus faecalis* (Van B E808); *Enterococcus faecium* (ATCC19434); *Escherichia coli* (ATCC25922); *Escherichia coli* (HB101(PAT266); *Escherichia coli* (DC2); *Pseudomonas aeruginosa* (ATCC27853); *Pseudomonas aeruginosa* (PAO1 HS); *Pseudomonas aeruginosa* (18S/H); *Pseudomonas aeruginosa* (K799/WT); *Pseudomonas aeruginosa* (K799/61); *Staphylococcus aureus* (ATCC29213).

Hemolysis assay. Serial twofold dilutions of the compounds were prepared in PBS buffer pH 7.4 and 50 µL aliquots were distributed in 96-well plates (Cornstar, untreated). Human red blood cells (RBC) were obtained by centrifuging 1.5 mL of whole blood from friendly donors at 3000 rpm for 15 minutes. Plasma was discarded and the pellet was re-suspended in a 15 mL falcon tube up to 5 mL of PBS Buffer pH 7.4. The washing was repeated three times and the remaining pellet was re-suspended in 10 mL of PBS Buffer pH 7.4 at a final RBC concentration of 5%. The RBC suspension (50 µL) was added to each well and the plate was incubated at room temperature for 4 hours. Minimal haemolytic concentration (MHC) end points were determined by visual inspection of the wells after the incubation period. Controls on each plate included a blank medium control (80

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µl PBS + 80 µl of RBC suspension) and a haemolytic activity control (milli-Q water dilution goingfrom 50% to 0% v/v in RBC suspension). **Pexiganan** and **Melittin** were synthesized and their MHC was measured as control value. (A. E. Barron et al., *PNAS*, 2008, **105**, 8, 2794).

Resistance development assay. MICs of peptide compounds were newly determined as described above using a single culture of *B. subtilis* (BR151) picked from a fresh streak plate. To test for resistance development, MICs of compounds towards *B. subtilis* were determined daily using cells from the well in which the compound concentration was one half the MIC value (1/2 MIC well). For each compound, the 1/2 MIC well from the previous day MIC assay plate, was resuspended, adjusted to a concentration of $5x10^5$ cells/ml in LB Broth (OD600=0.132, diluted 1:100 fold) and used to again determine the MIC of the same compound to which those cells had previously been exposed. All MIC determinations were done in duplicates. The final concentrations of the serial twofold dilutions of the compounds ranged from 0,5 µg/ml to 256 µg/ml. **C2** was used as positive control. After this initial MIC experiment, MICs were then further determinated by the same procedure daily for 15 days. No changes in the MIC values was observed for any of the compounds.

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MS and analytical HPLC traces of purified products

Cyclic Peptide **E1** c(IVTLfPHK*Thz*q)

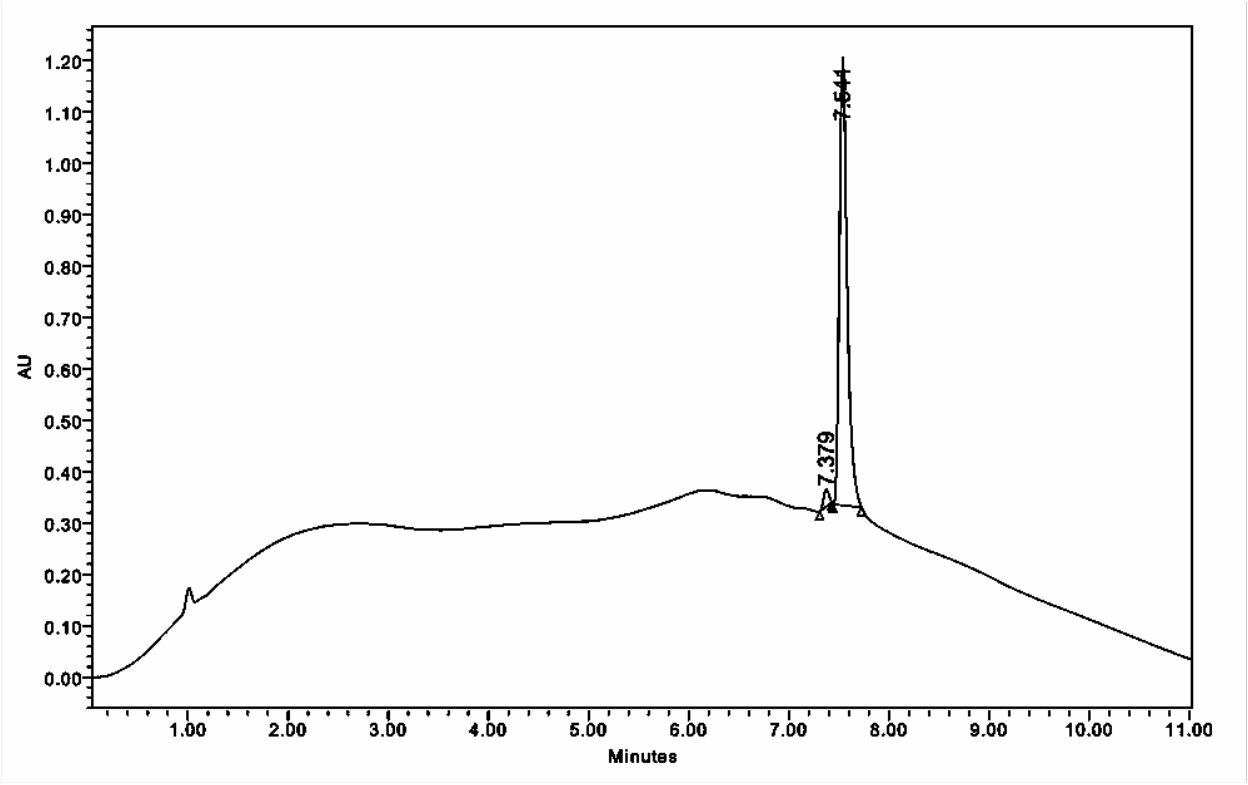

Fig. S1. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R = 7.5 min

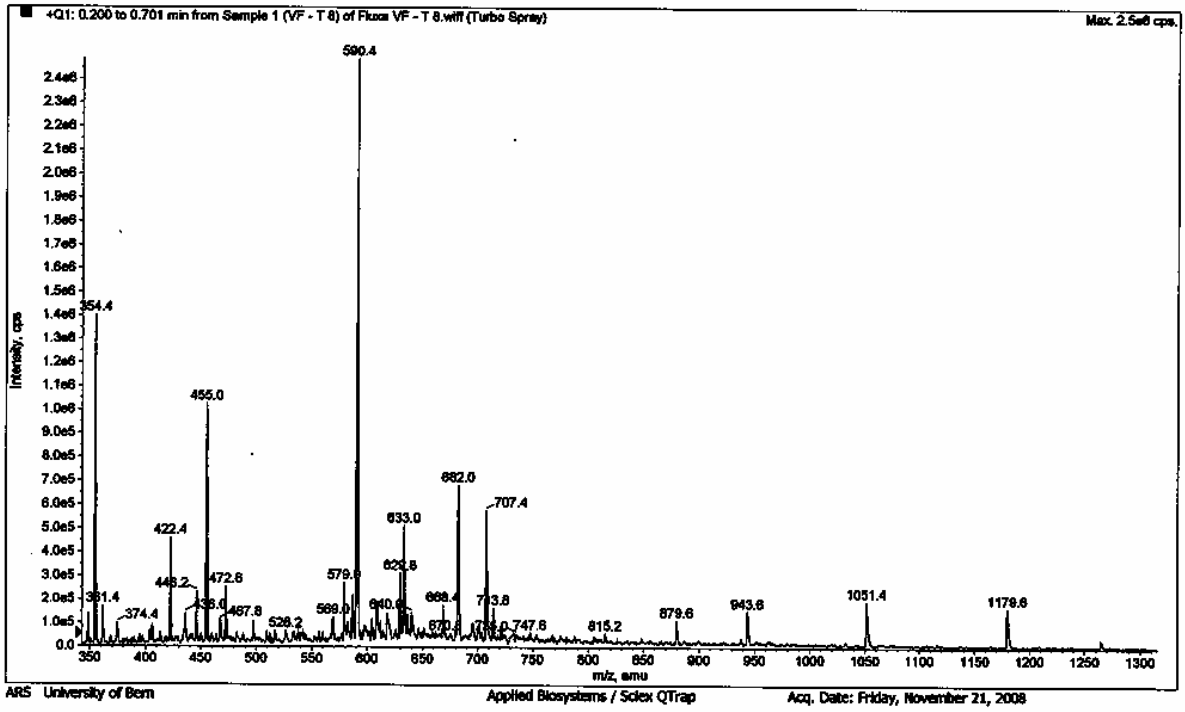

Fig. S2. ESI MS(+): Calcd. for $C_{56}H_{87}N_{14}O_{12}S$ $[M+H]^+$: 1179.6; found: 1179.6 ($[M+H]^+$), found: 590.4 $[(M+2H/2)]^{2+}$.

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Cyclic Peptide **E3** c(**YVHLfThzHfNq**)

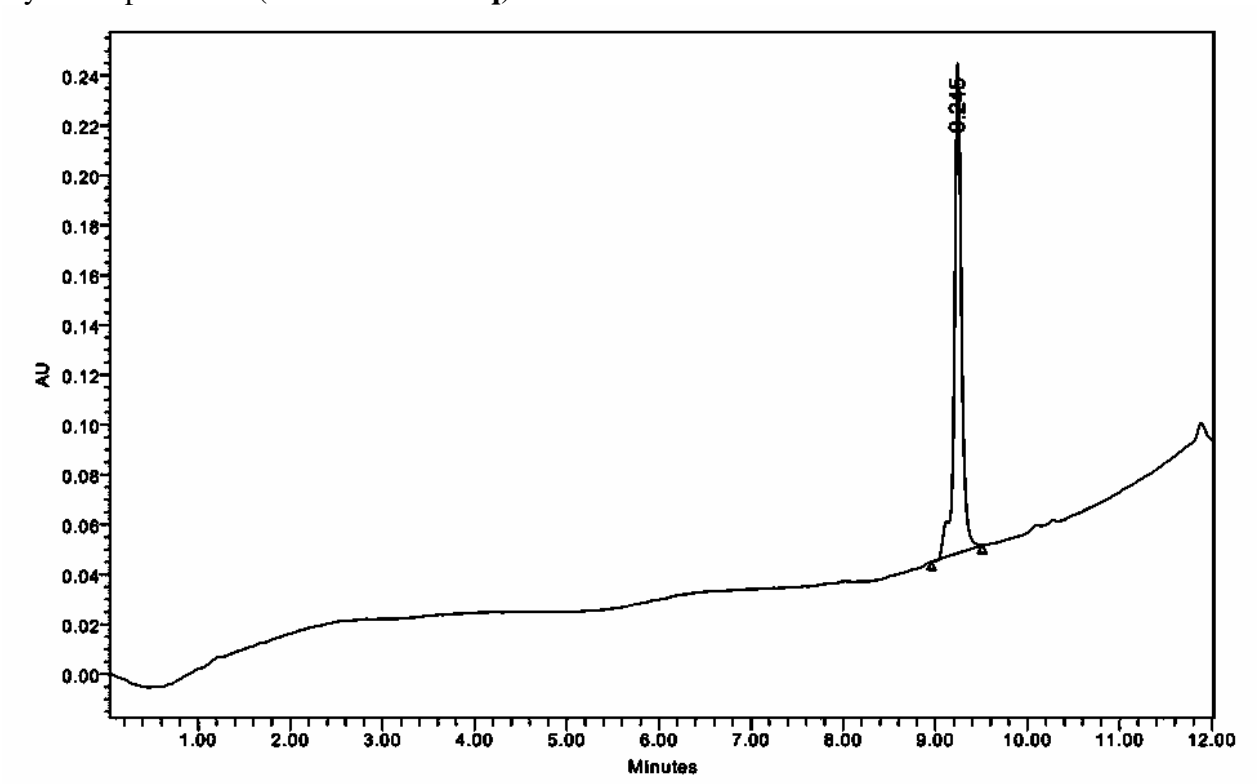

Fig. S3. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R = 9.3 min.

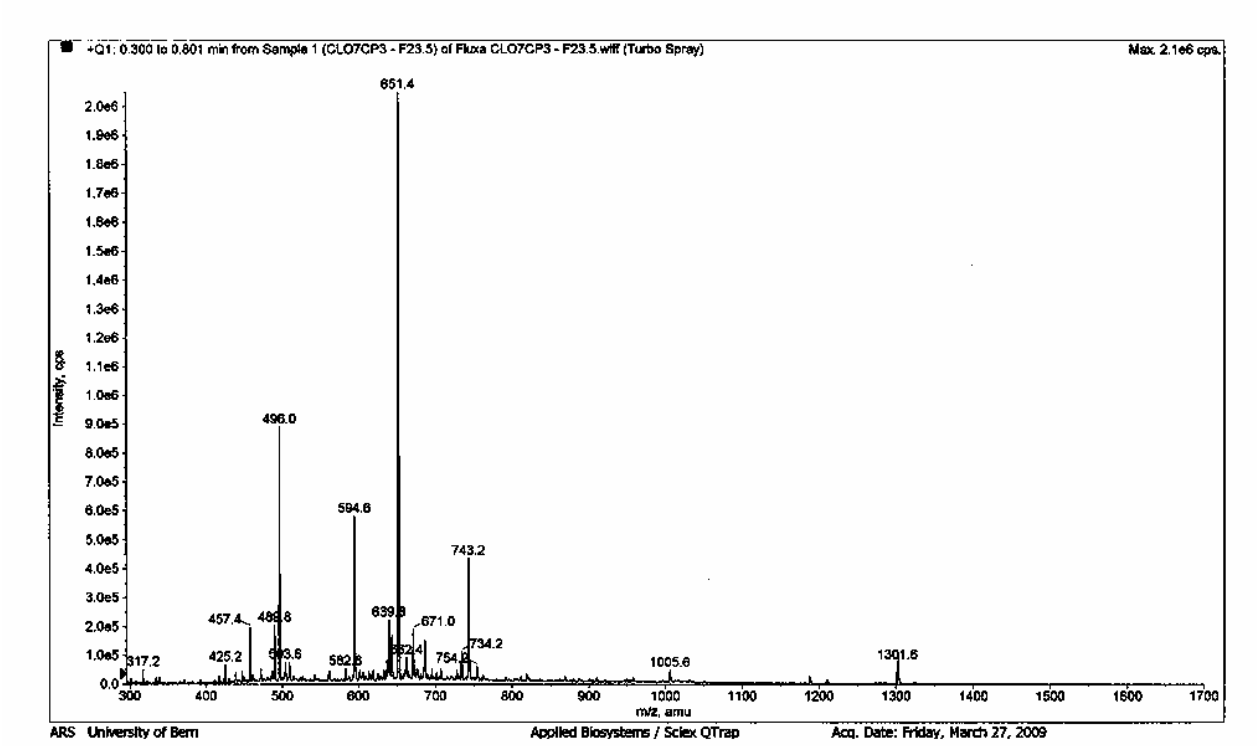

Fig. S4. ESI MS(+): Calcd. for $C_{63}H_{79}N_{15}O_{14}S$ $[M+H]^+$: 1301.6, found: 1301.6, found 651.4 $[(M+2H/2)]^{2+}$.

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Cyclic Peptide **E4** c(RVRLf*Thi*Y*Thi*Lq)

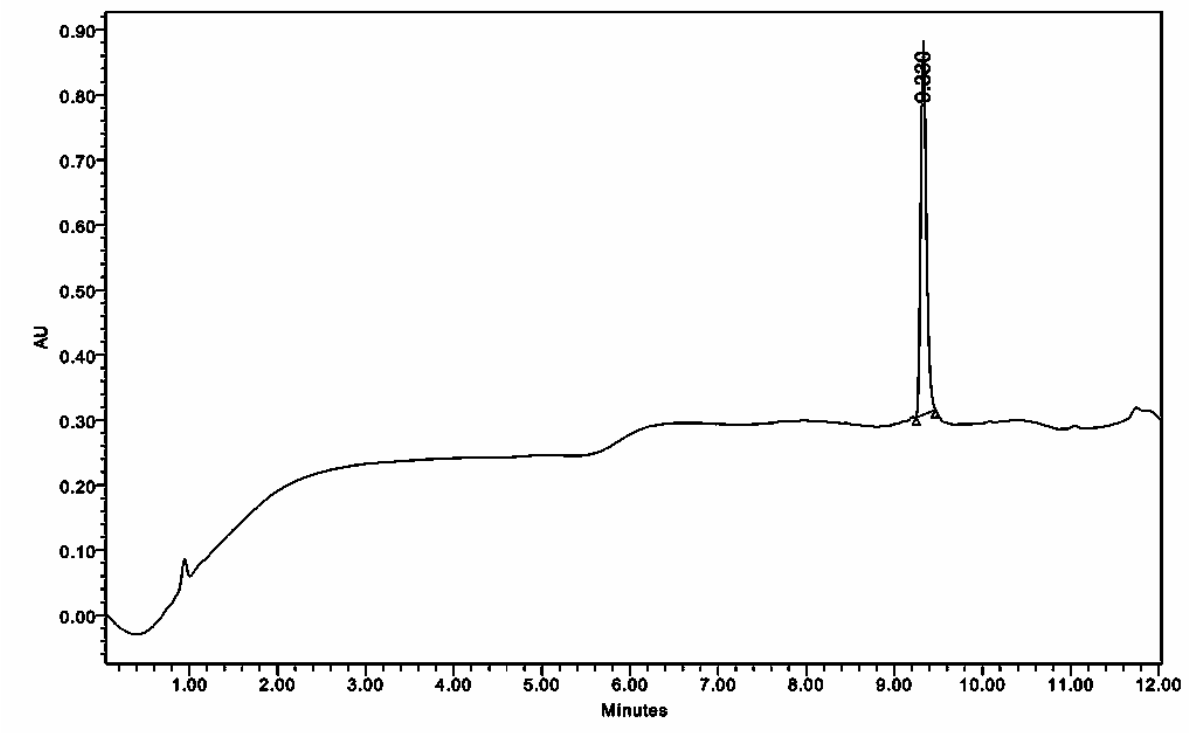

Fig. S5. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R = 9.3 min.

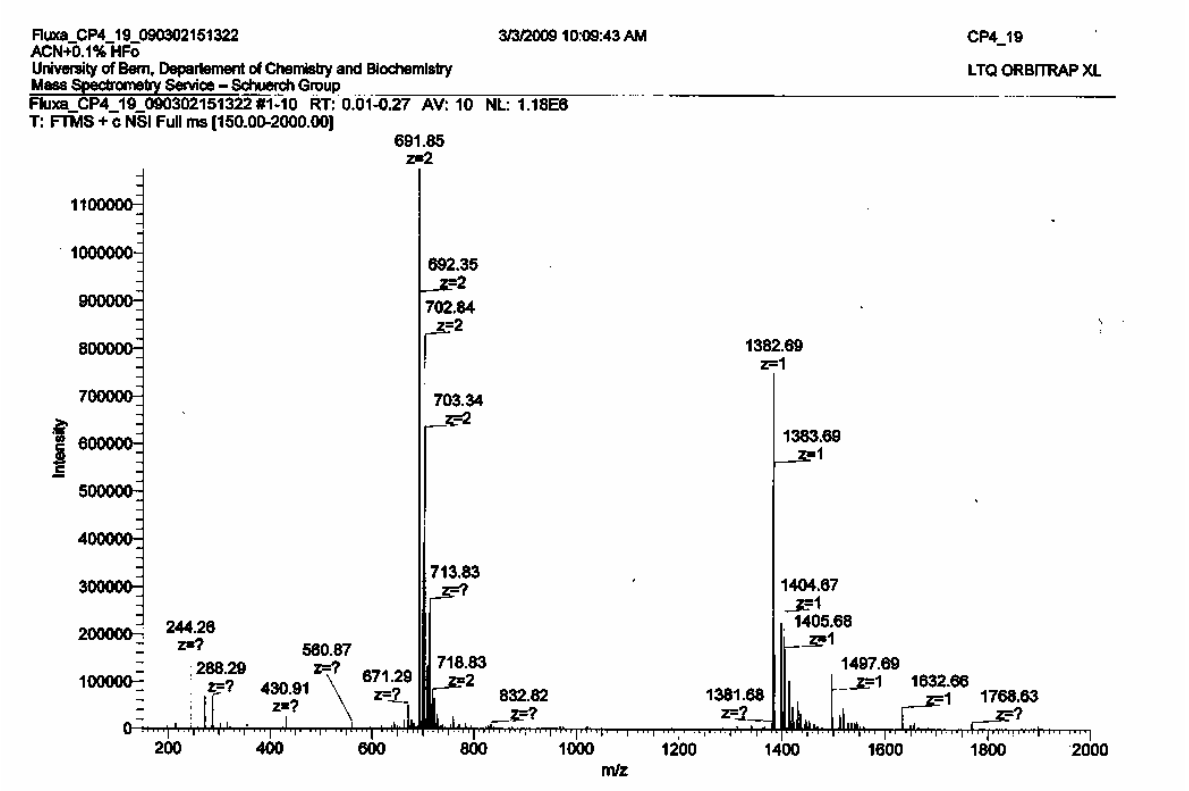

Fig. S6. ESI MS(+): Calcd. for $C_{66}H_{96}N_{17}O_{12}S_2$ $[M+H]^+$: 1382.7, found: 1382.7, found: 691.9 $[(M+2H/2)]^{2+}$.

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Cyclic Peptide **E6** c(RVHLfPY*OrnThz*q)

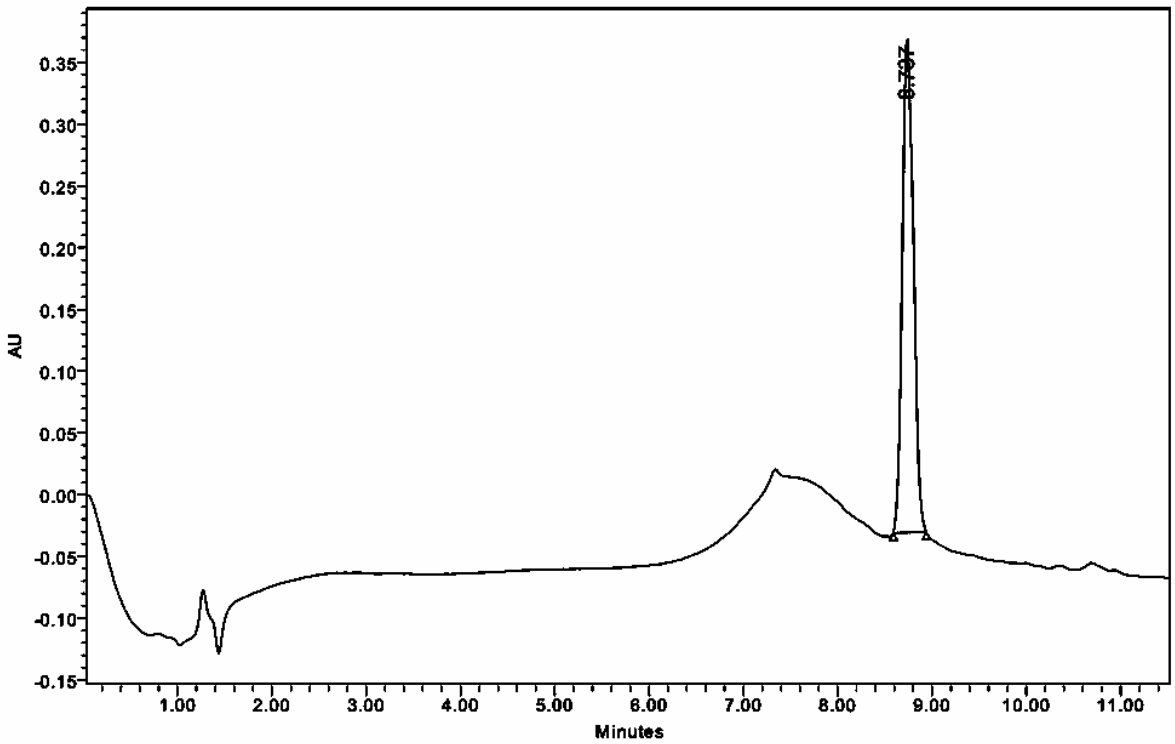

Fig. S7. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R = 8.7 min.

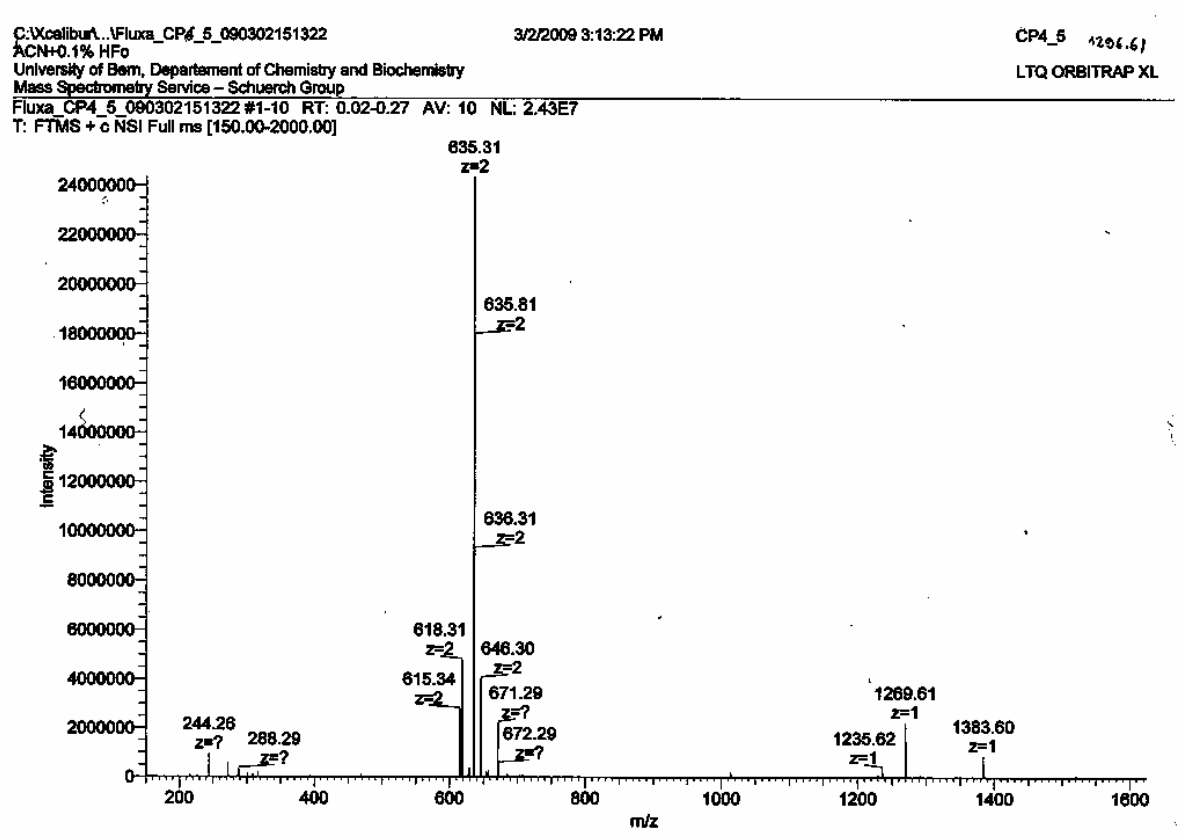

Fig. S8. ESI MS(+): Calcd. for $C_{60}H_{87}N_{17}O_{12}S$ $[M+H]^+$: 1269.6, found: 1269.6, found: 635.31 $[(M+2H/2)]^{2+}$.

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Cyclic Peptide **E8** c(PVTLf*Thz*HKIq)

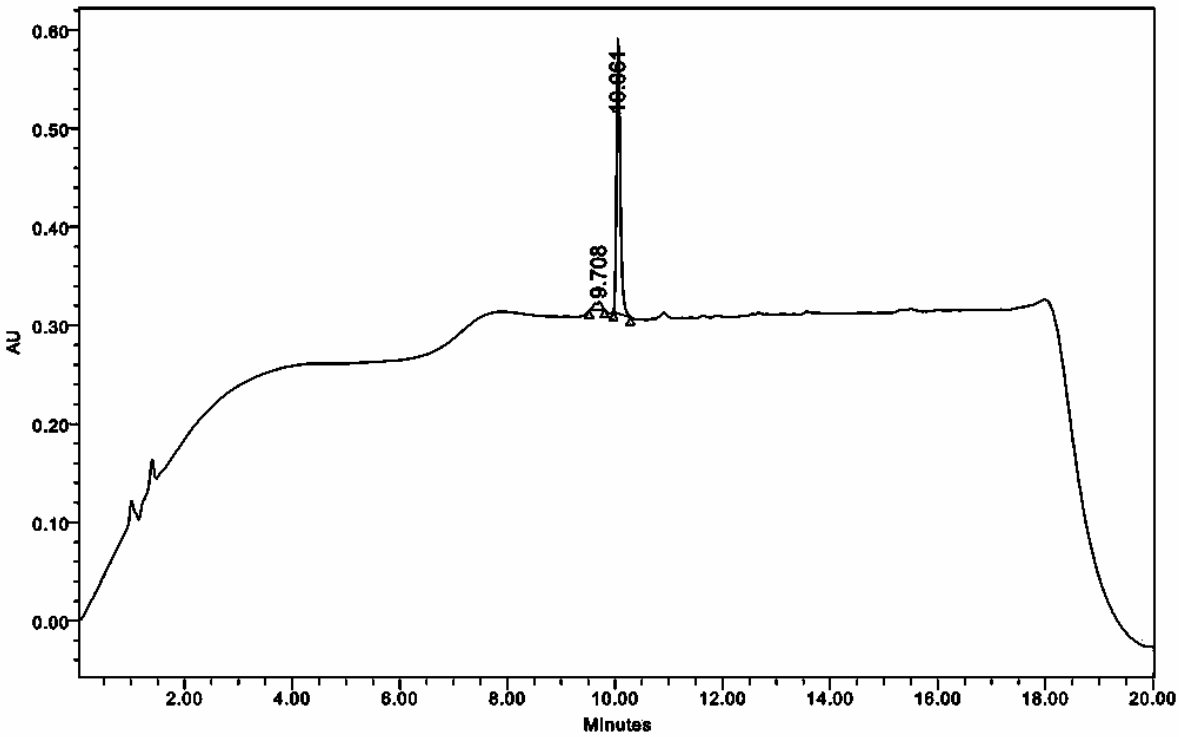

Fig. S9. RP-HPLC (93% A, 7% C to 100% C in 12 min): t_R = 10.1 min.

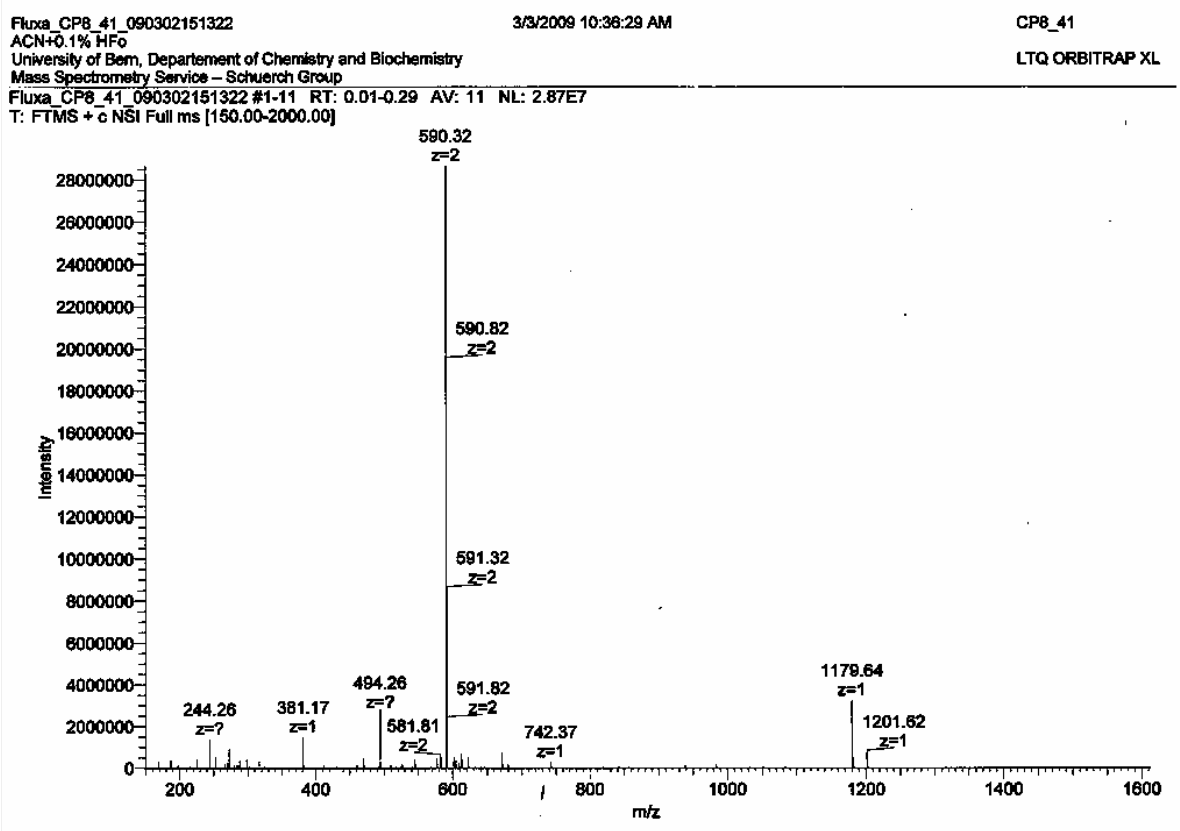

Fig. S10. ESI MS(+): Calcd. for $C_{56}H_{87}N_{14}O_{12}S$ $[M+H]^+$: 1179.6, found: 1179.6, found: 590.3 $[(M+2H/2)]^{2+}$.

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Cyclic Peptide **E9** c(RV*Orn*Lf*Thi*F*Orn*Lq)

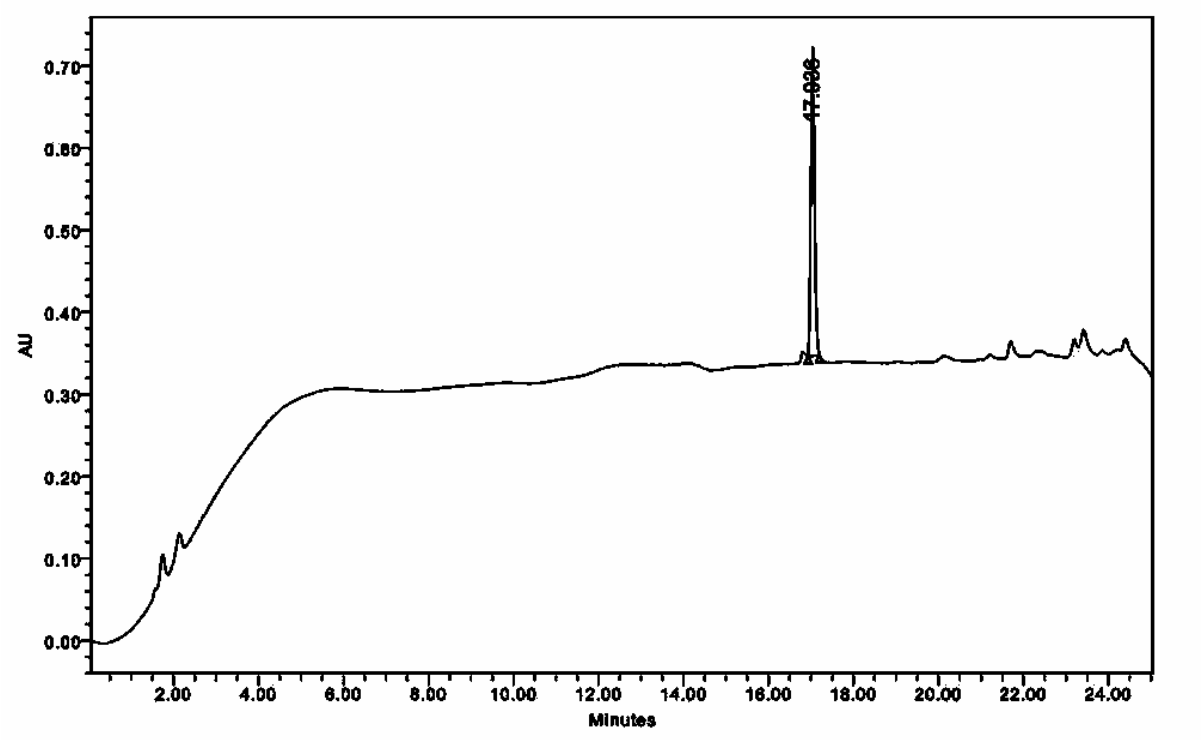

Fig. S11. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R = 17.0 min.

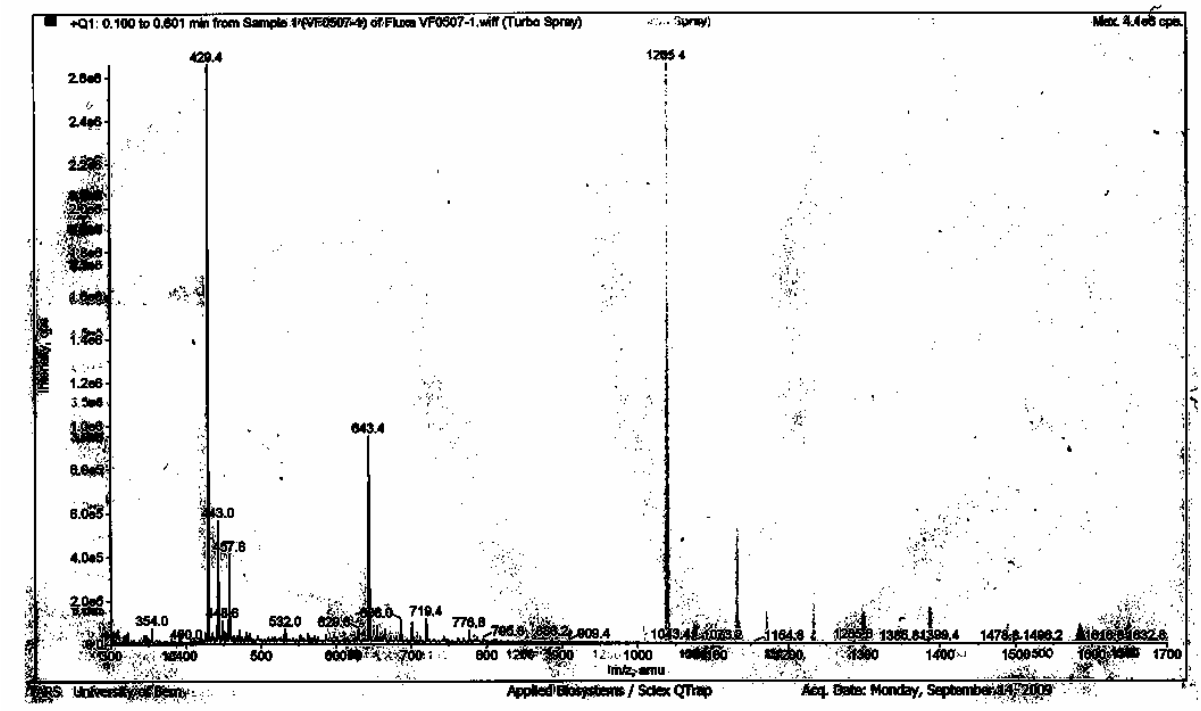

Fig. S12. ESI MS(+): Calcd. for $C_{63}H_{97}N_{16}O_{11}S$ $[M+H]^+$: 1285.7, found: 1285.4, found: 643.4 $[(M+2H/2)]^{2+}$, found: 429.4 $[(M+3H/3)]^{3+}$.

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Cyclic Peptide **E13** c(kVRLfVVfIq)

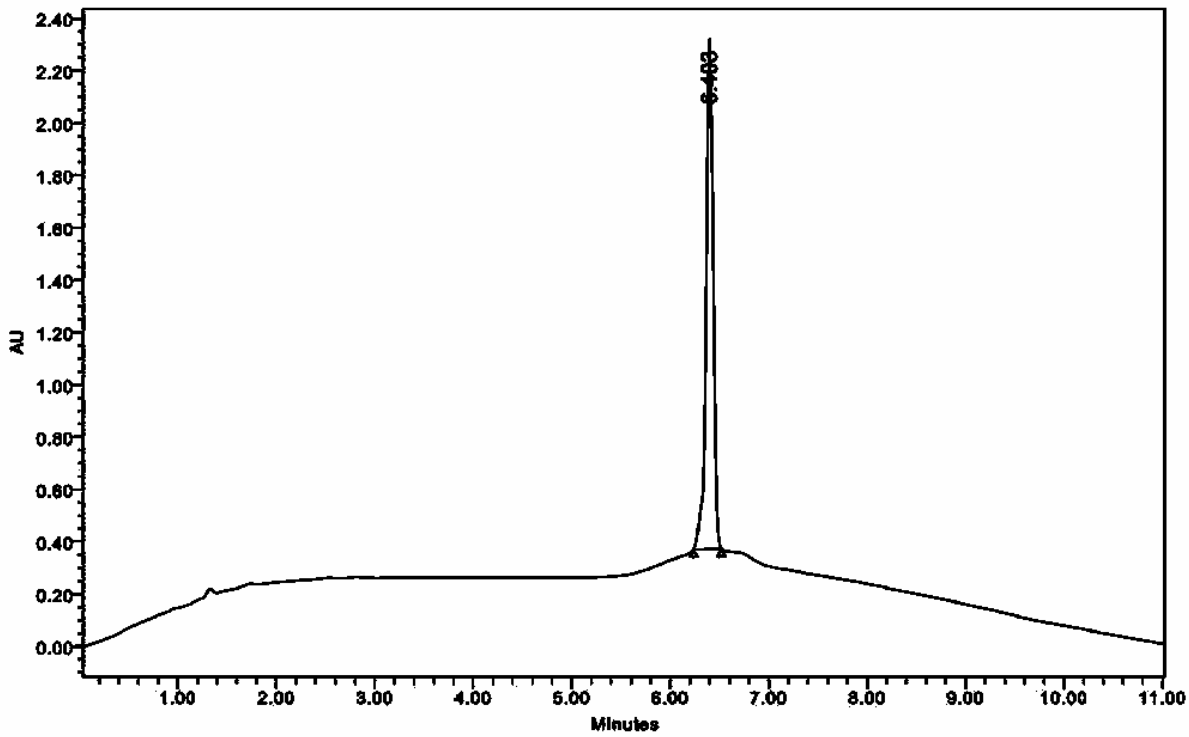

Fig. S13. RP-HPLC (80% A, 10% C to 100% C in 10 min): t_R = 6.4 min.

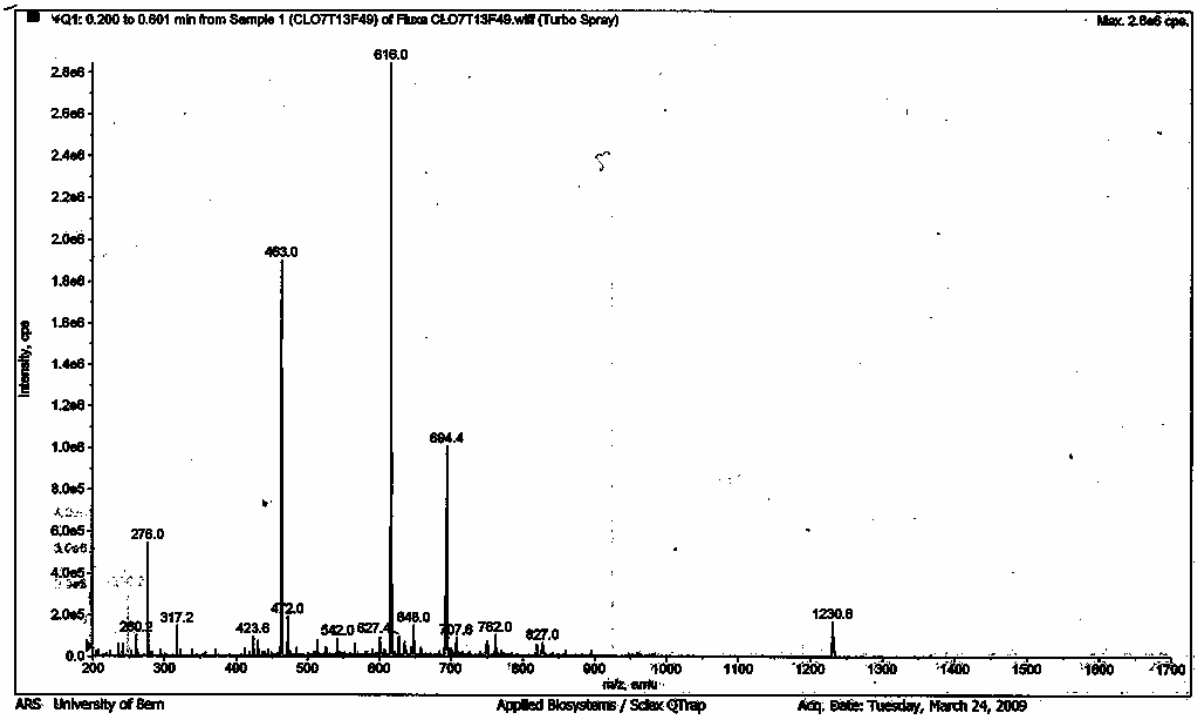

Fig. S14. SI MS(+): Calcd. for $C_{62}H_{100}N_{15}O_{11}$ $[M+H]^+$: 1230.8, found: 1230.8, found: 616.0 $[(M+2H/2)]^{2+}$, found: 694.4 $[(M+2K/2)]^{2+}$.

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Cyclic Peptide **E14** c(IV*Orn*Lf*Thz*H*Orn*Iq)

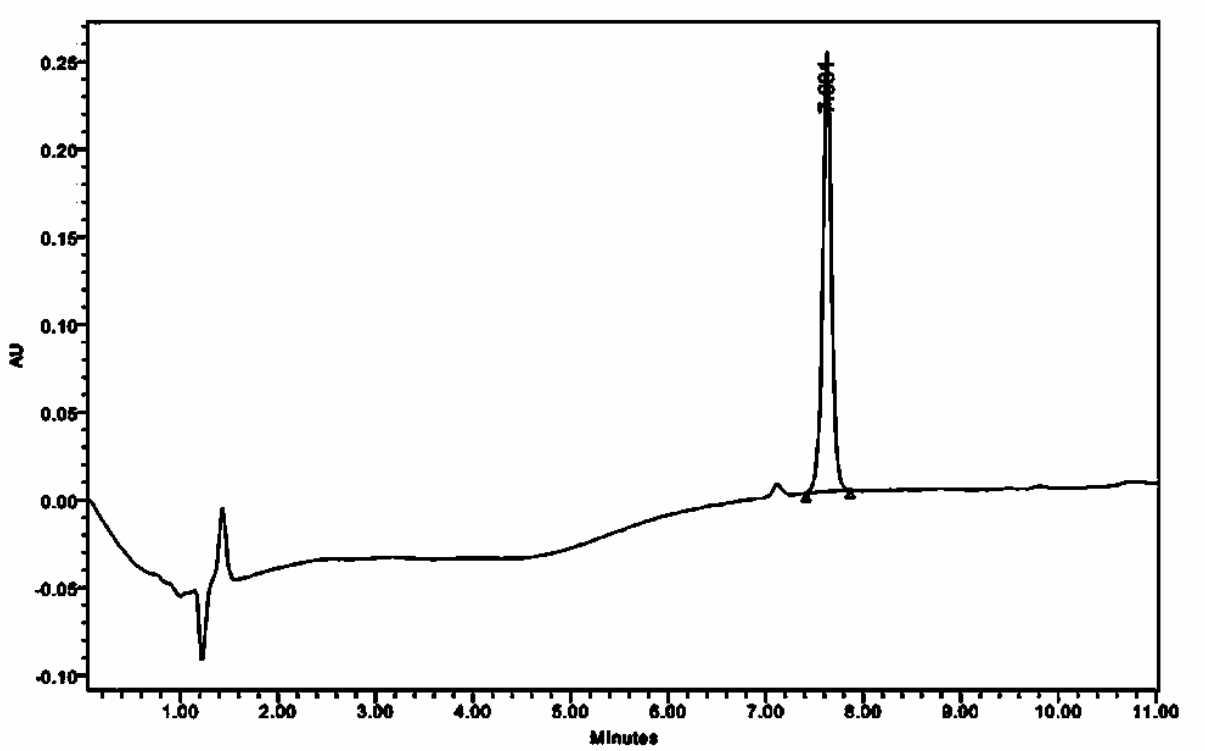

Fig. S15. RP-HPLC (80% A, 20% C to 20% A, 80% C in 10 min): t_R = 7.6 min.

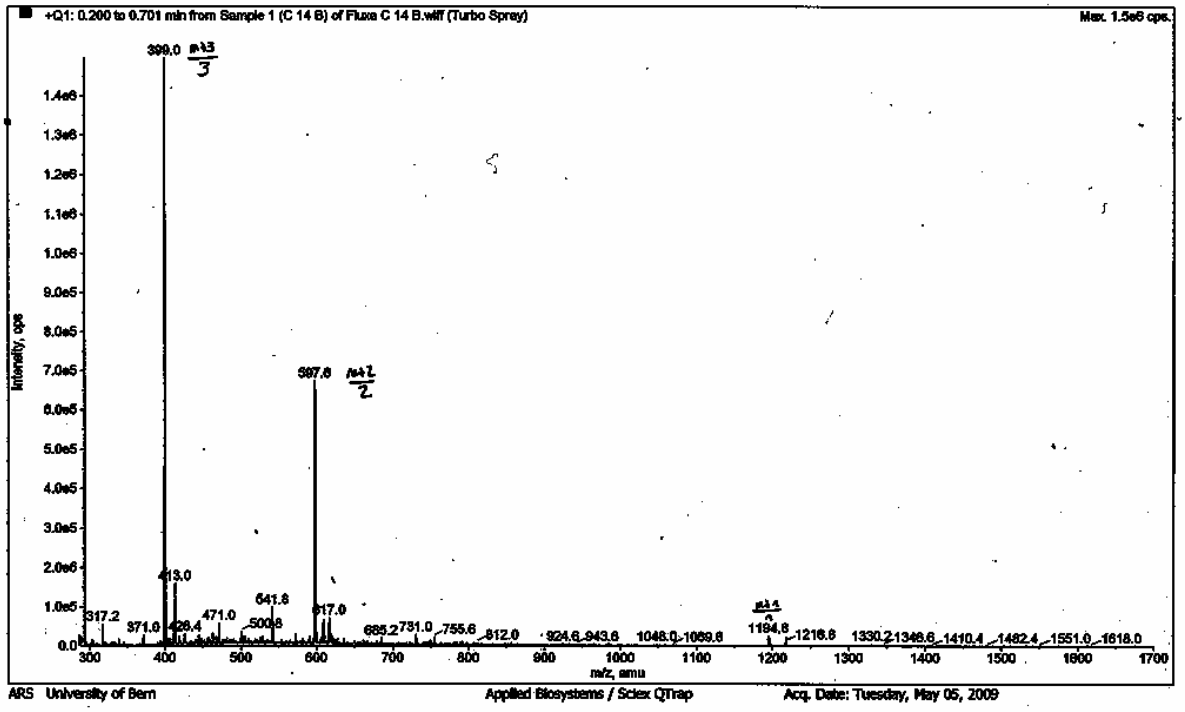

Fig. S16. ESI MS(+): Calcd. for $C_{57}H_{92}N_{15}O_{11}S$ $[M+H]^+$: 1194.7, found: 1194.8, found: 597.6 $[(M+2H/2)]^{2+}$, found: 399.0 $[(M+3H/3)]^{3+}$.

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Cyclic Peptide **E18** c(kV*Orn*Lf*Thi*Y*Orn*Lq)

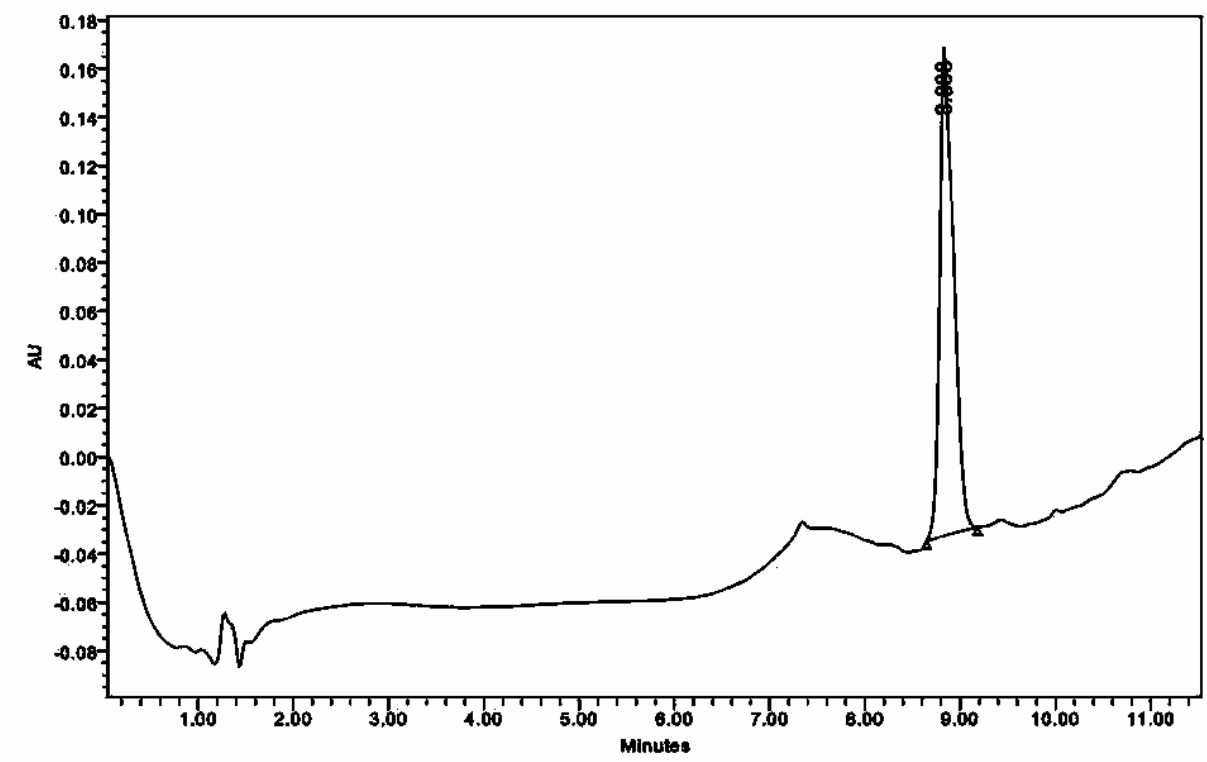

Fig. S17. RP-HPLC (90% A, 10% C to 100% C in 10 min): t_R = 8.8 min.

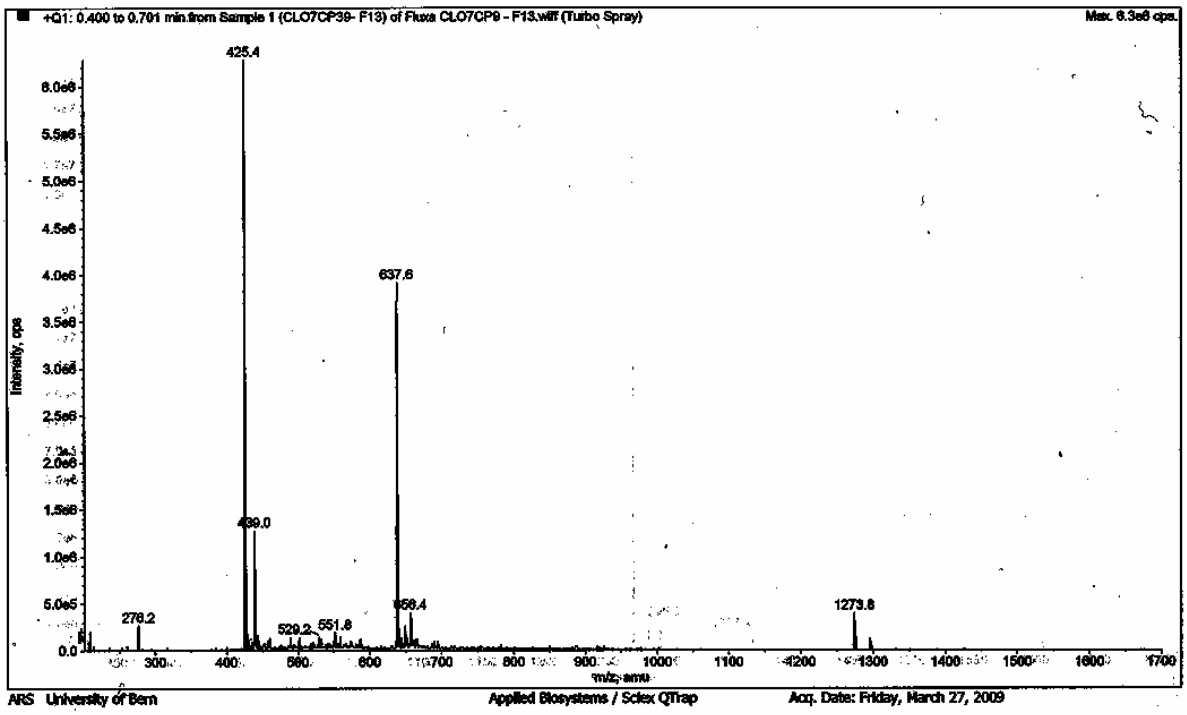

Fig. S18. ESI MS(+): Calcd. for $C_{63}H_{97}N_{14}O_{12}S$ $[M+H]^+$: 1273.7, found: 1273.8, found: 637.6 $[(M+2H/2)]^{2+}$, found: 656.4.6 $[(M+H+K/2)]^{2+}$, found: 425.4 $[(M+3H/3)]^{3+}$.

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Cyclic Peptide **B6** c(IV*Hyp*LfPVKNq)

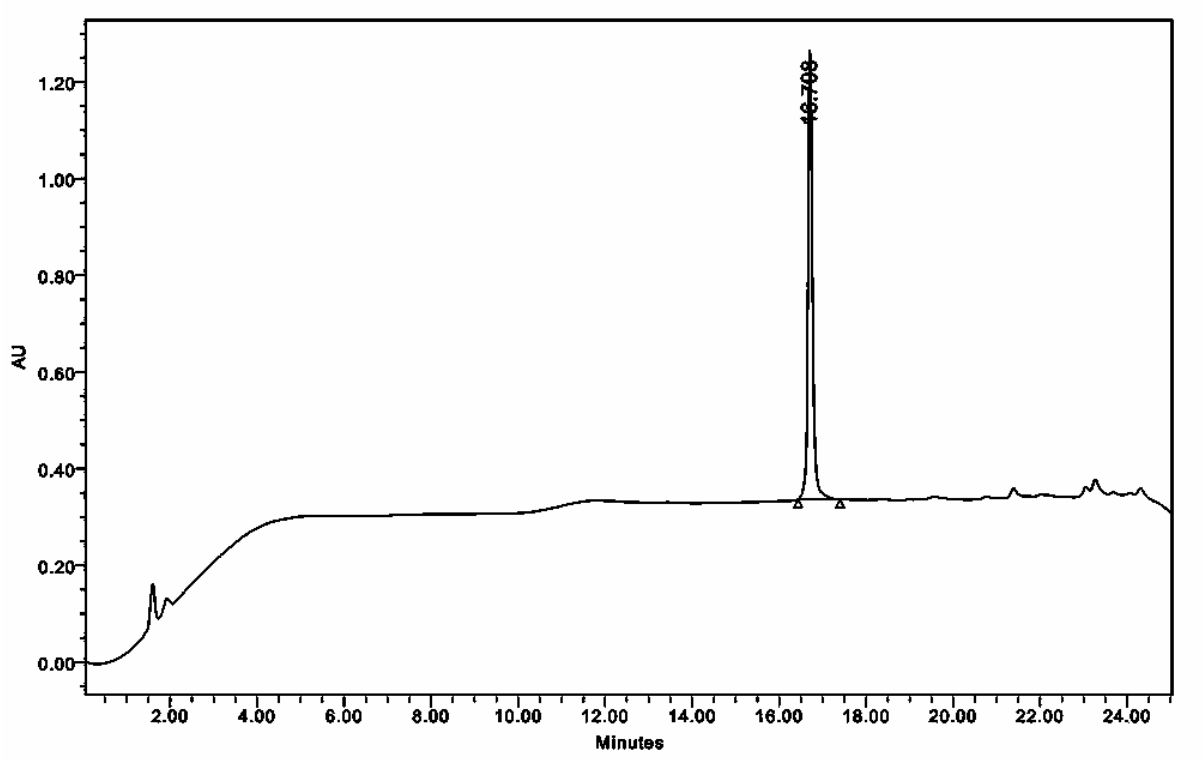

Fig. S19. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R = 16.7 min.

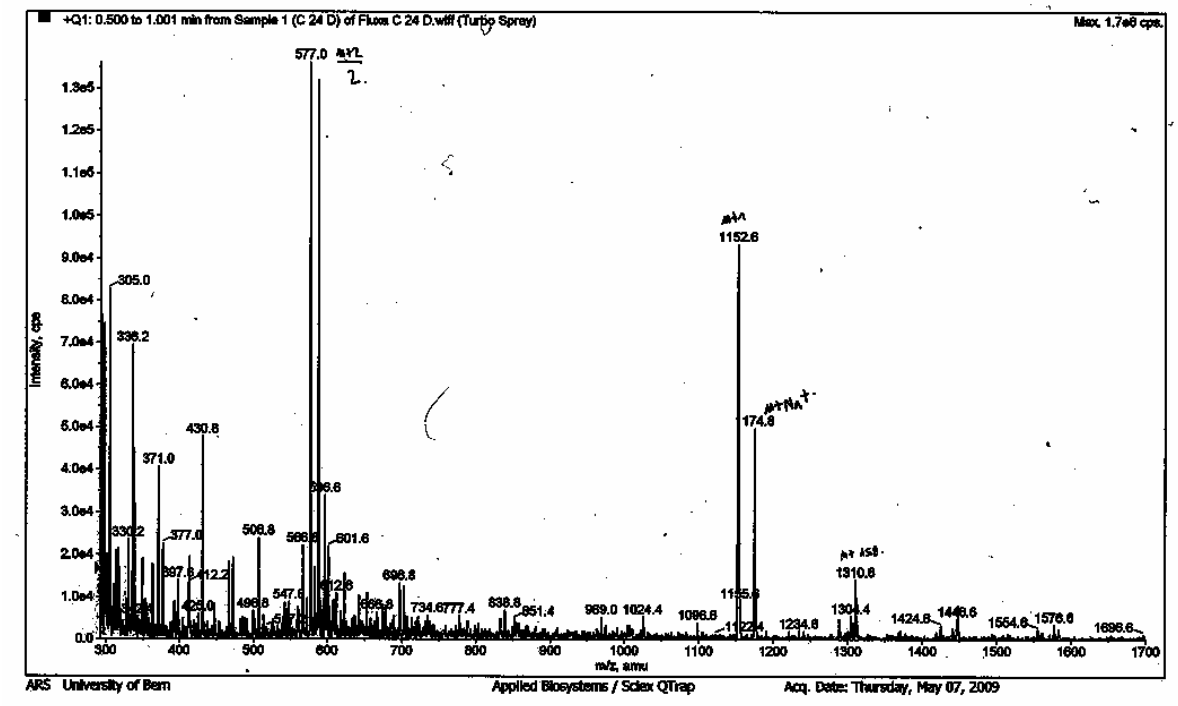

Fig. S20. ESI MS(+): Calcd. for $C_{56}H_{90}N_{13}O_{13}S$ $[M+H]^+$: 1152.7, found: 1152.6, found: 1310.6 $[M+1H+HFo+TFA]^+$,found: 1174.8 $[M+Na]^+$, found: 577.0 $[(M+2H/2)]^{2+}$.

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Cyclic Peptide **B7** c(kV*Hyp*LfPYNNq)

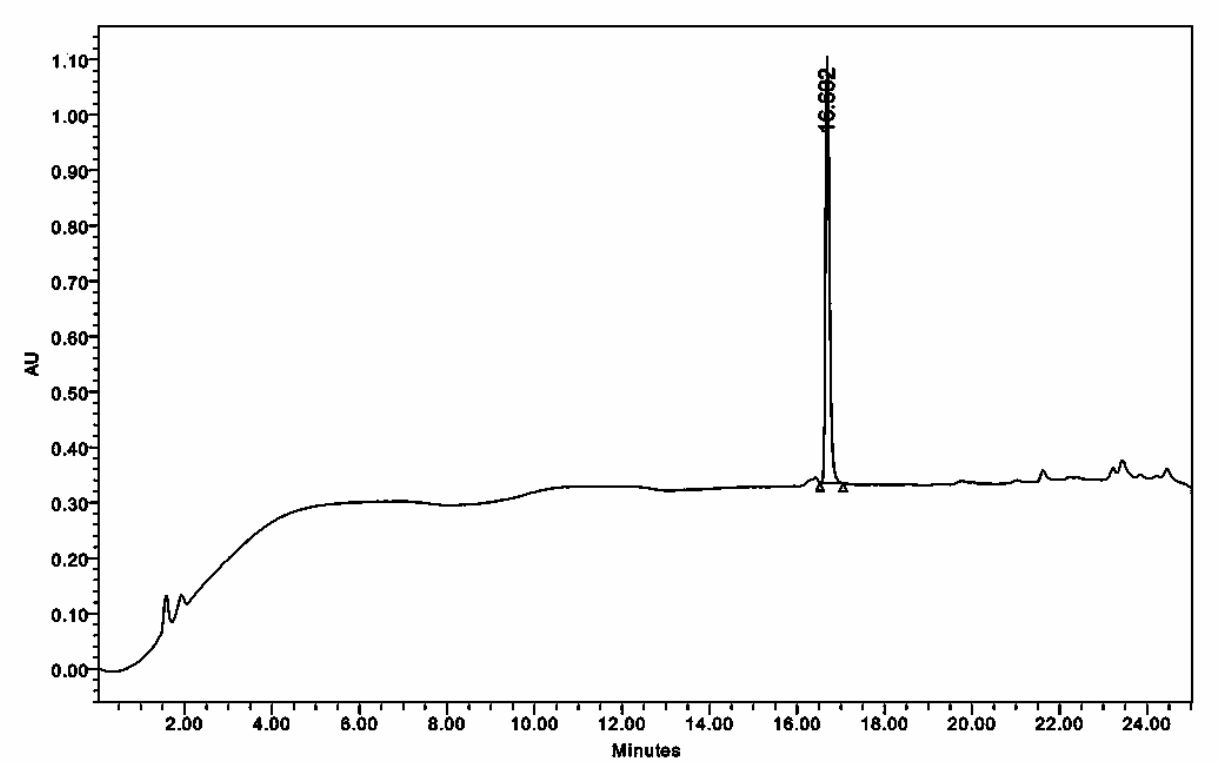

Fig. S21. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R = 16.7 min.

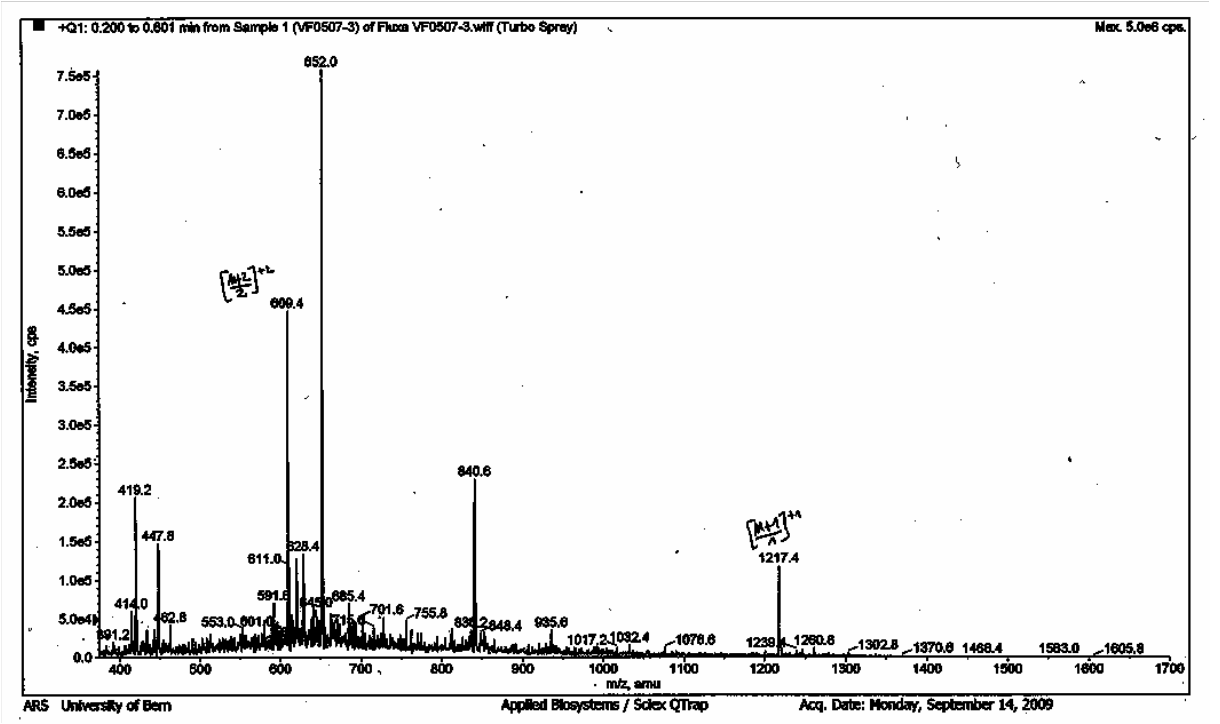

Fig. S22. ESI MS(+): Calcd. for $C_{58}H_{85}N_{14}O_{15}$ $[M+H]^+$: 1217.6, found: 1217.4, found: 609.4 $[(M+2H/2)]^{2+}$, found: 419.2 $[(M+2H+K/3)]^{3+}$.

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Cyclic Peptide **B10** c(kV*Hyp*LfPHKNq)

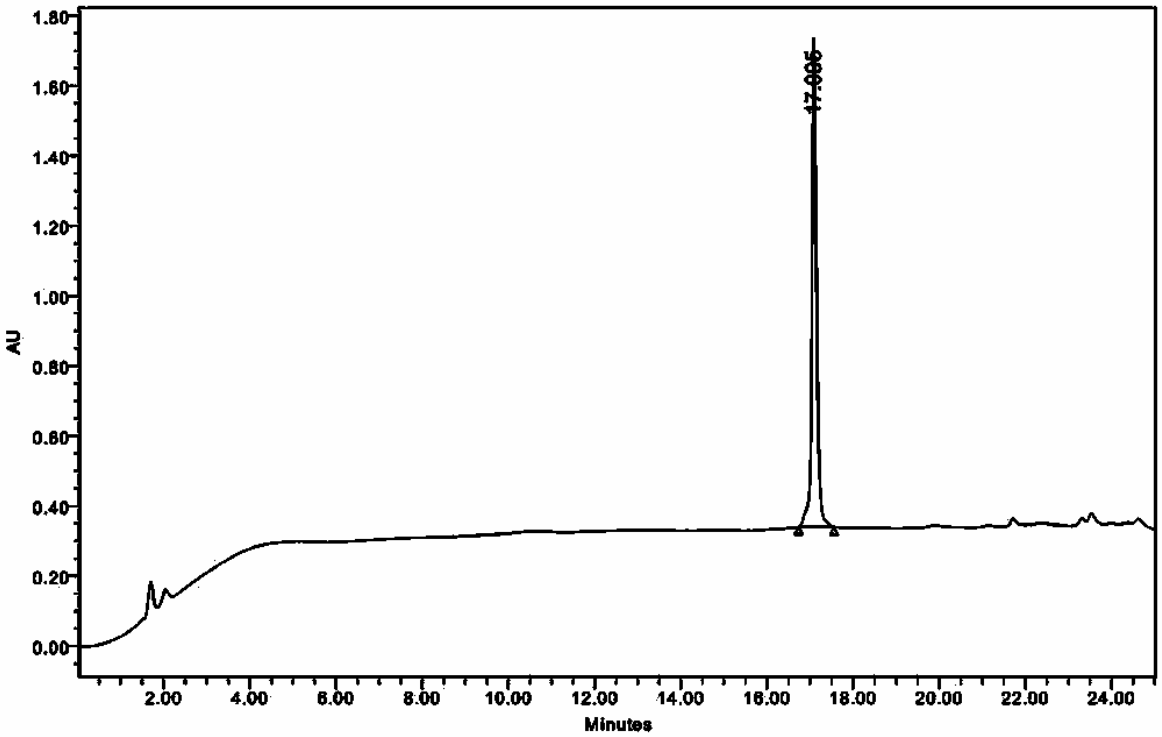

Fig. S23. RP-HPLC (85% A, 15% C to 10% A, 90% C in 18 min): t_R = 17.1 min.

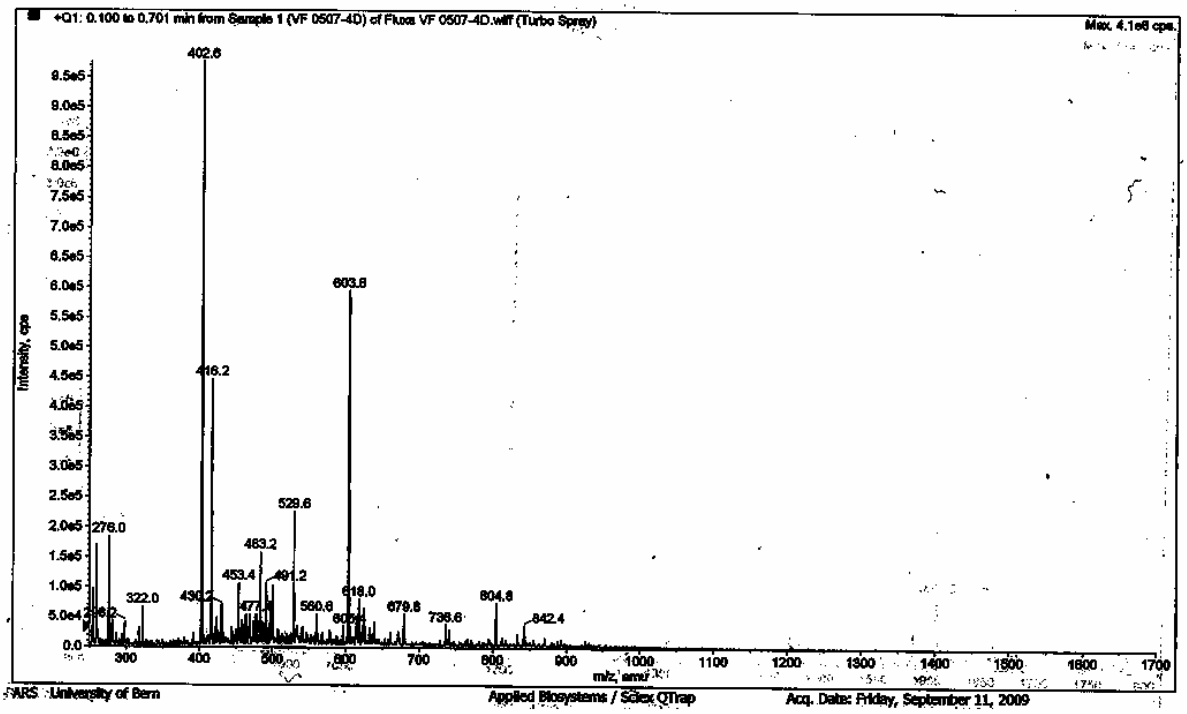

Fig. S24. ESI MS(+): Calcd. for $C_{57}H_{89}N_{16}O_{13}S$ $[M+H]^+$: 1205.7, found: 603.6 $[(M+2H/2)]^{2+}$, found: 402.6 $[(M+3H/3)]^{3+}$.

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Cyclic Peptide **N9** c(RVHLfVYFTq)

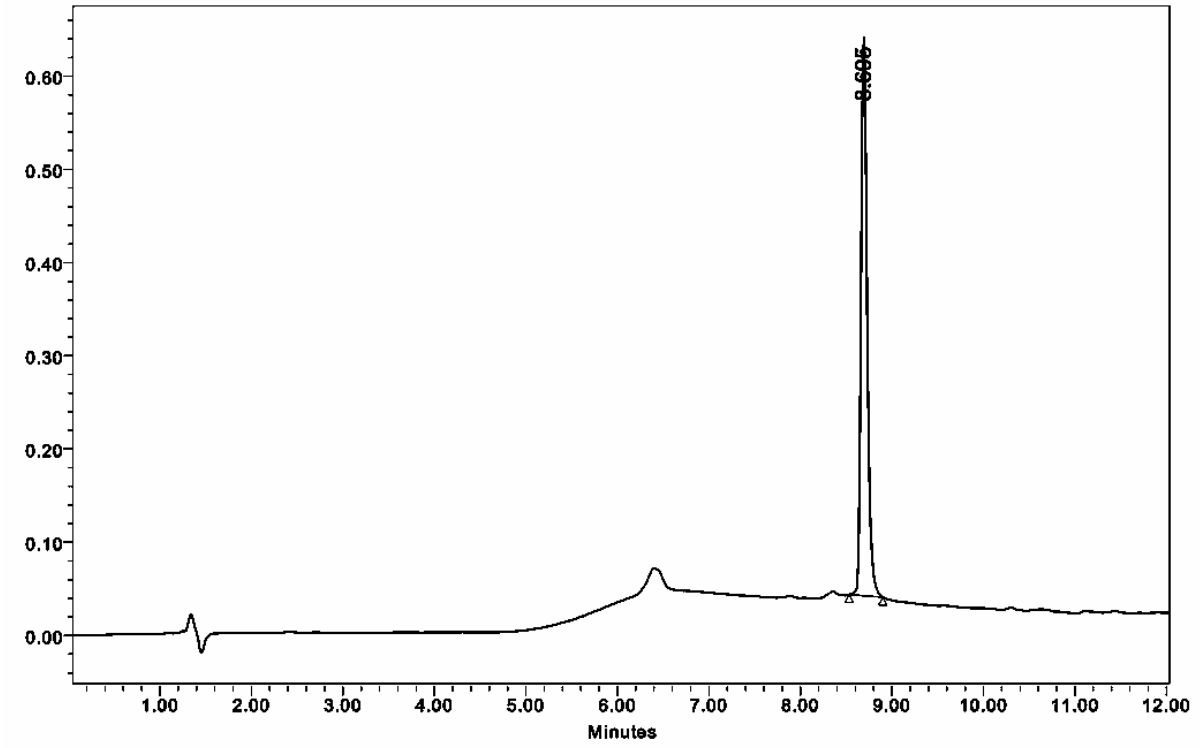

Fig. S25. RP-HPLC (90 A, 10% C to 100% C in 10 min): t_R = 8.7 min.

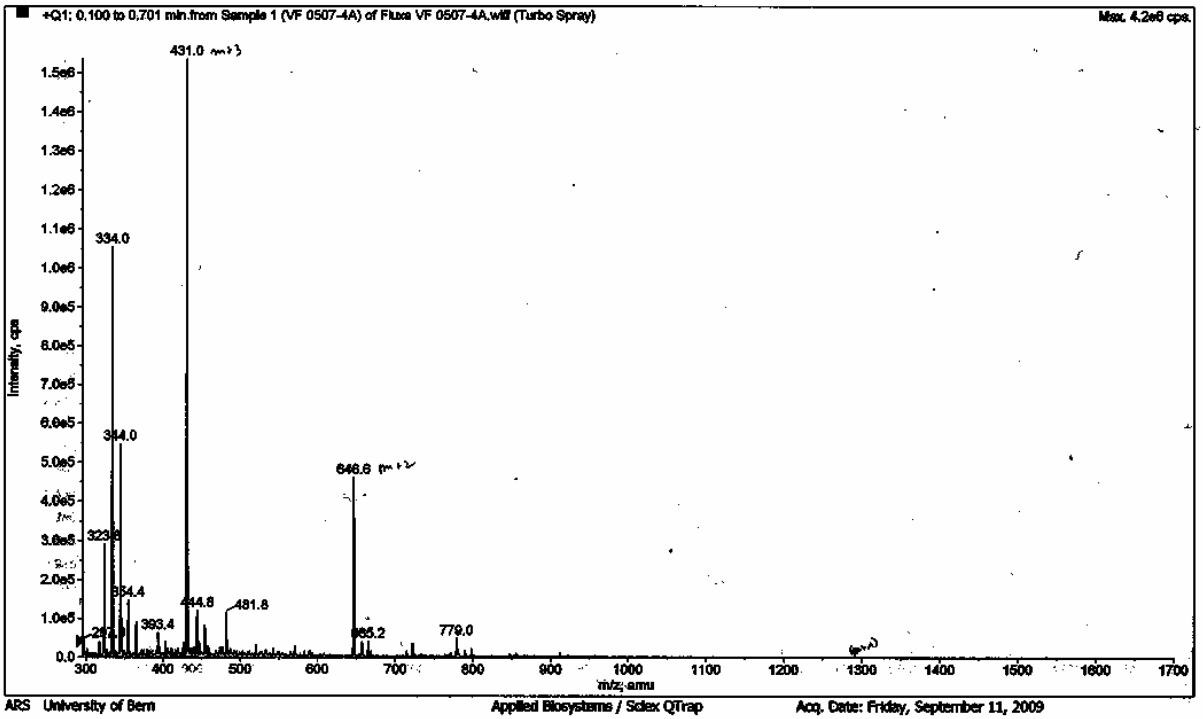

Fig. S26. ESI MS(+): Calcd. for $C_{64}H_{90}N_{16}O_{13}$ $[M+H]^+$: 1290.4, found: 646.6 $[(M+2H/2)]^{2+}$, found: 431.0 $[(M+3H/3)]^{3+}$, found: 323.6 $[(M+4H/4)]^{4+}$.

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Cyclic Peptide **N29** c(YV*Hyp*LfVF*Orn*Iq)

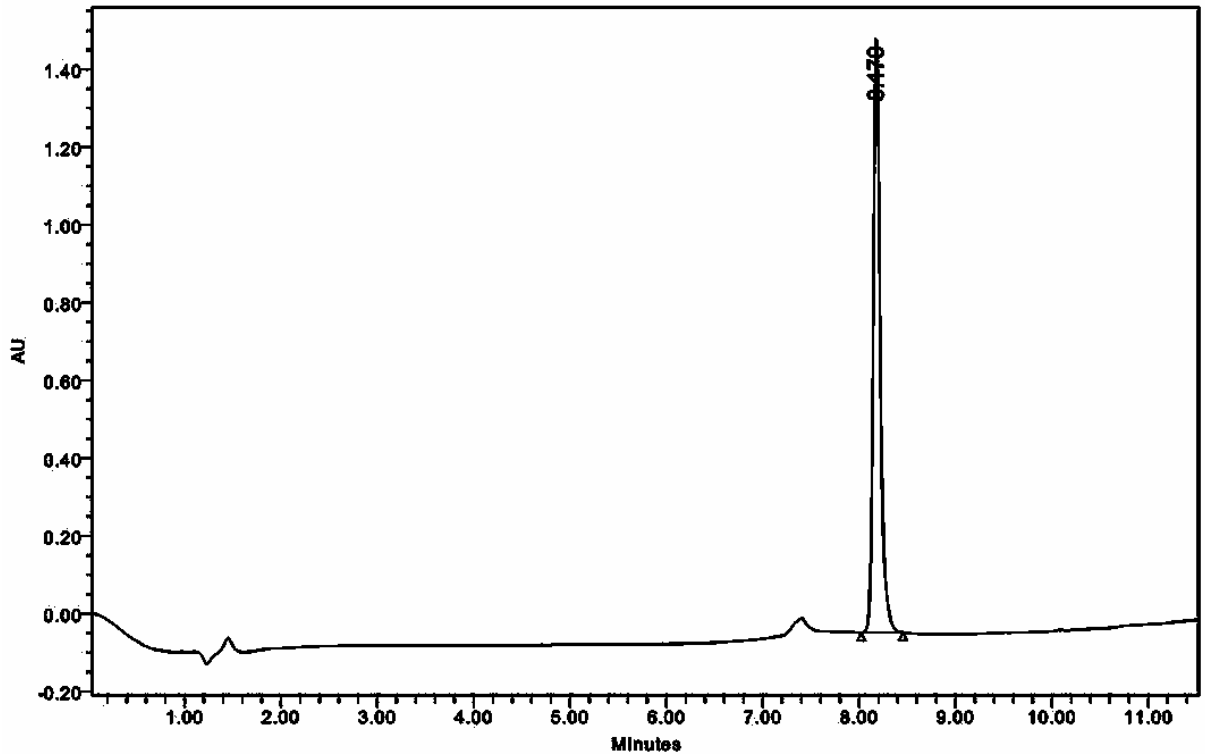

Fig. S27. RP-HPLC (90 A, 10% C to 100% C in 10 min): t_R = 8.2 min.

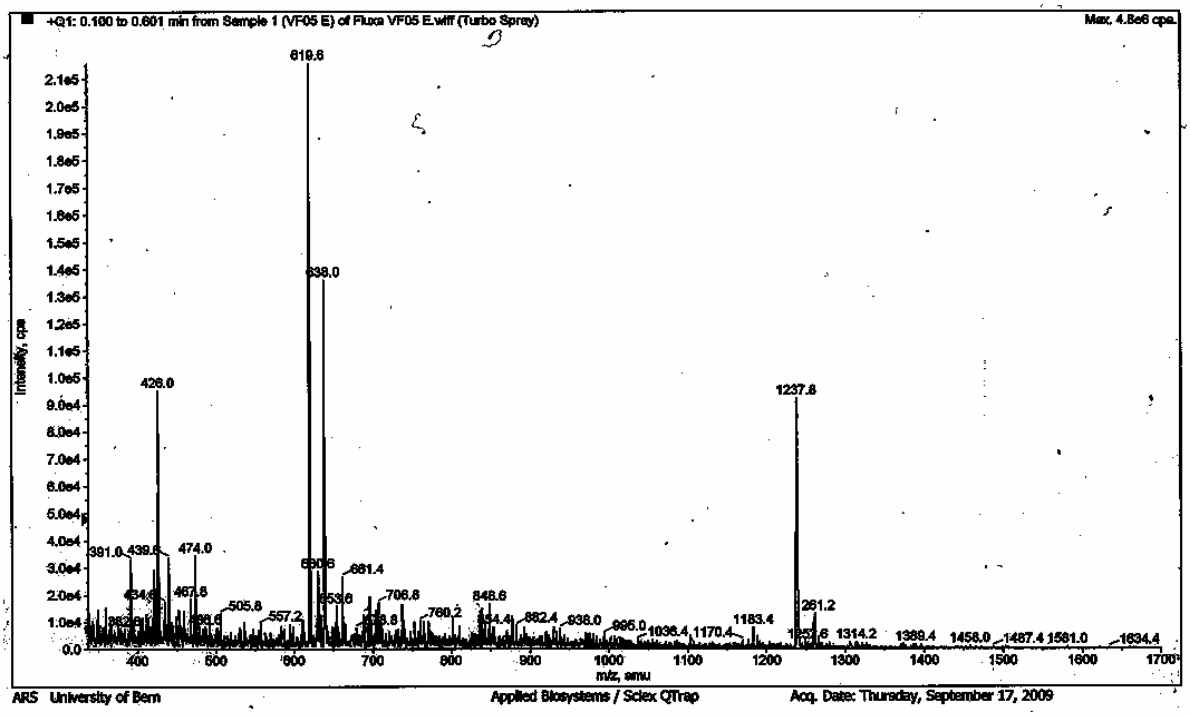

Fig. S28. ESI MS(+): Calcd. for $C_{64}H_{93}N_{12}O_{13}$ $[M+H]^+$: 1237.7, found: 1237.8, found: 619.6 $[(M+2H/2)]^{2+}$, found: 638.0 $[(M+H+K/2)]^{2+}$, found: 426.0 $[(M+2H+K/3)]^{3+}$.

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Cyclic Peptide **E18a** c(kV*Orn*LfFY*Orn*Lq)

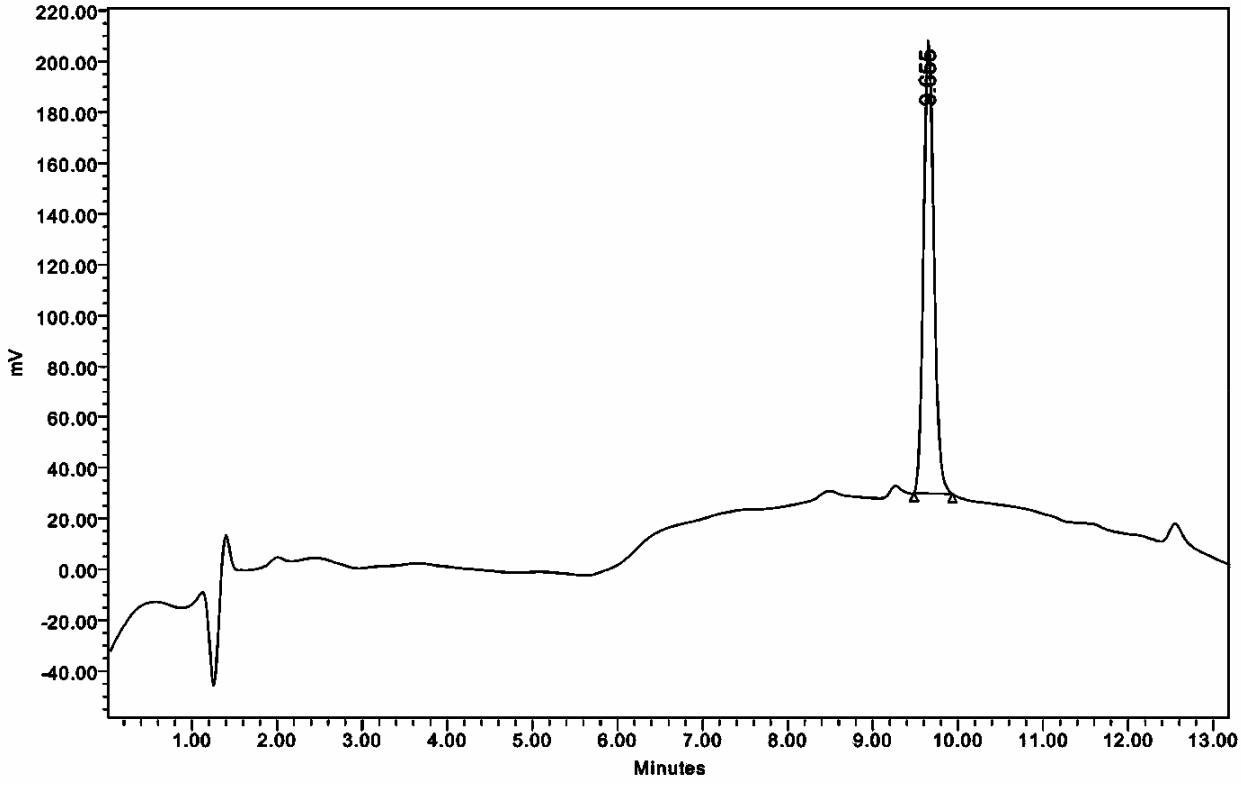

Fig. S29. RP-HPLC (80% A, 20% C to 100% C in 11 min): t_R = 9.7 min.

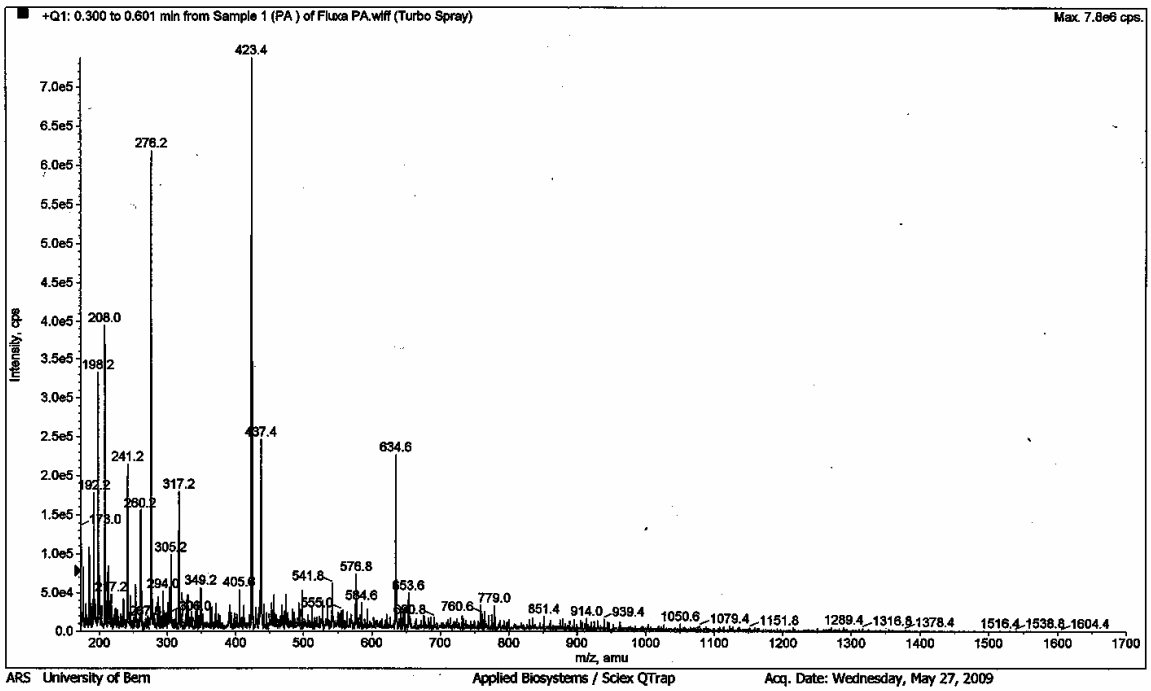

Fig. S30. ESI MS(+): Calcd. for $C_{65}H_{99}N_{14}O_{12}$ $[M+H]^+$: 1267.7, found: 634.6 $[(M+2H/2)]^{2+}$, found: 423.4 $[(M+3H/3)]^{3+}$.

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Linear Peptide **E18b** kV*Orn*Lf*Thi*Y*Orn*Lq-NH_2

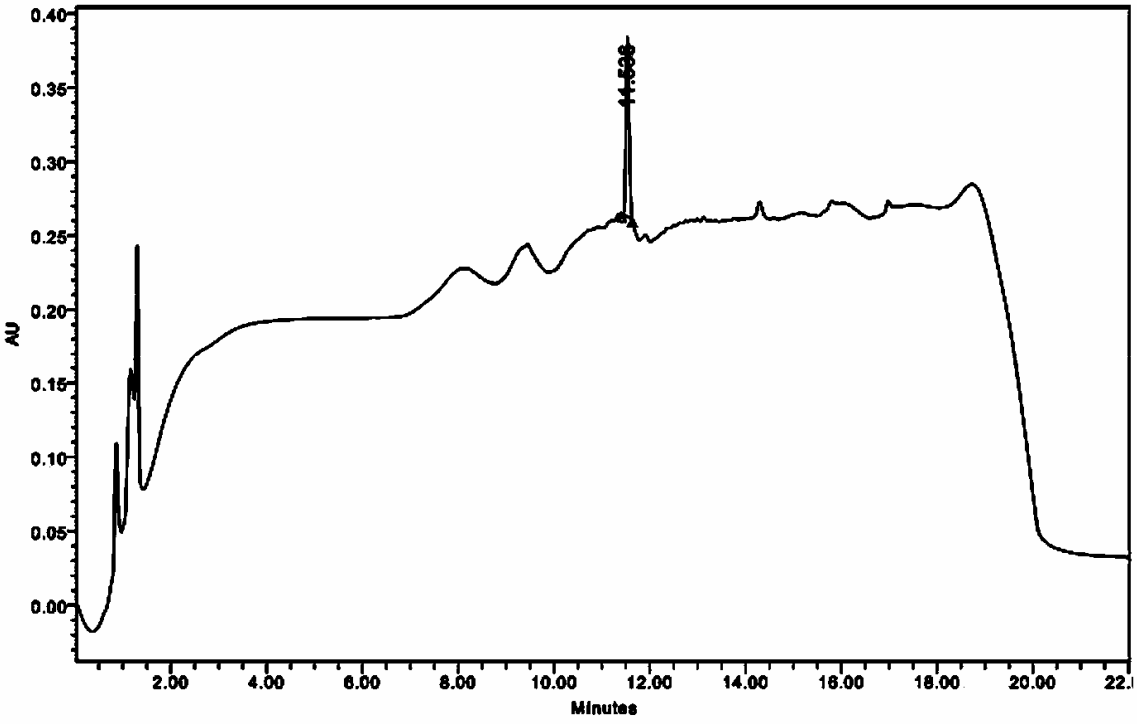

Fig. S31. RP-HPLC (100% A to 100% B in 15 min): t_R = 11.5 min.

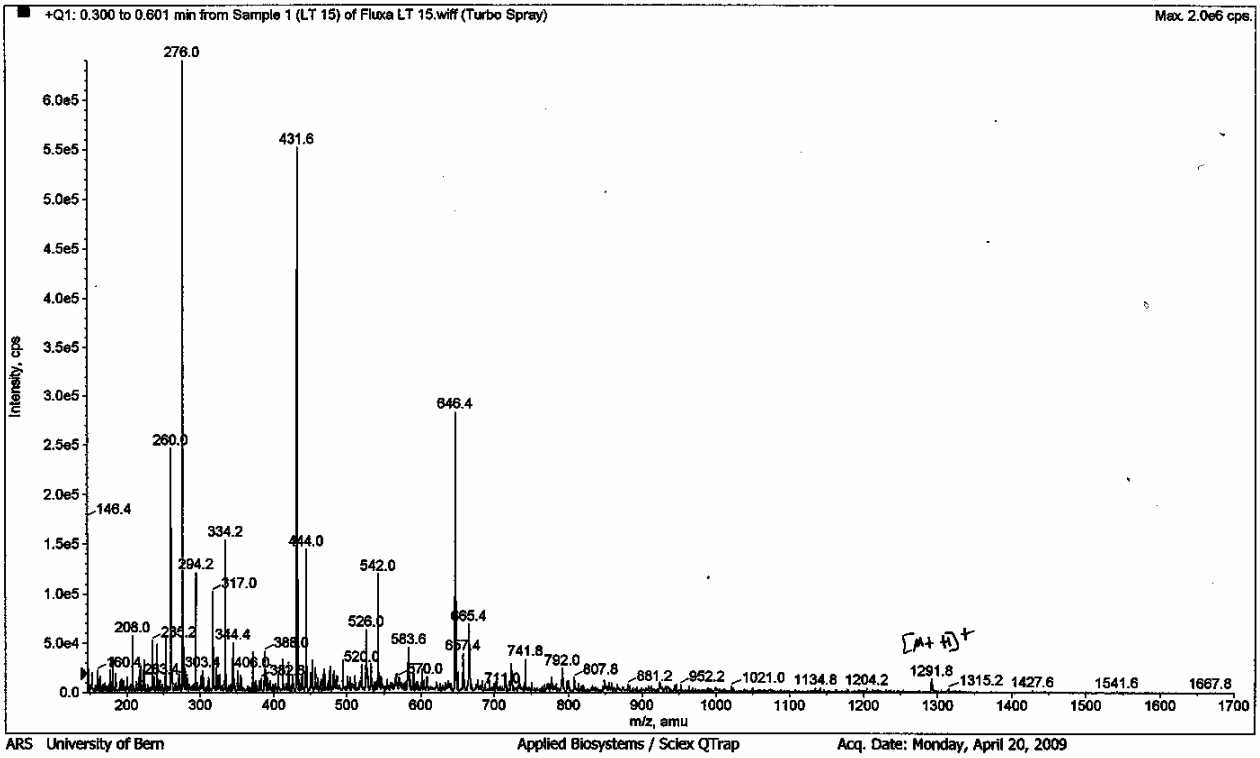

Fig. S32. ESI MS(+): Calcd. for $C_{63}H_{99}N_{14}O_{13}S$ $[M+H]^+$: 1291.7, found: 1291.8, found:646.4 $[(M+2H/2)]^{2+}$, found: 431.6 $[(M+3H/3)]^{3+}$.

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Cyclic Control Peptide **C1** $_2$HN-ILPWKWPWWPWRR-COOH

a) b)

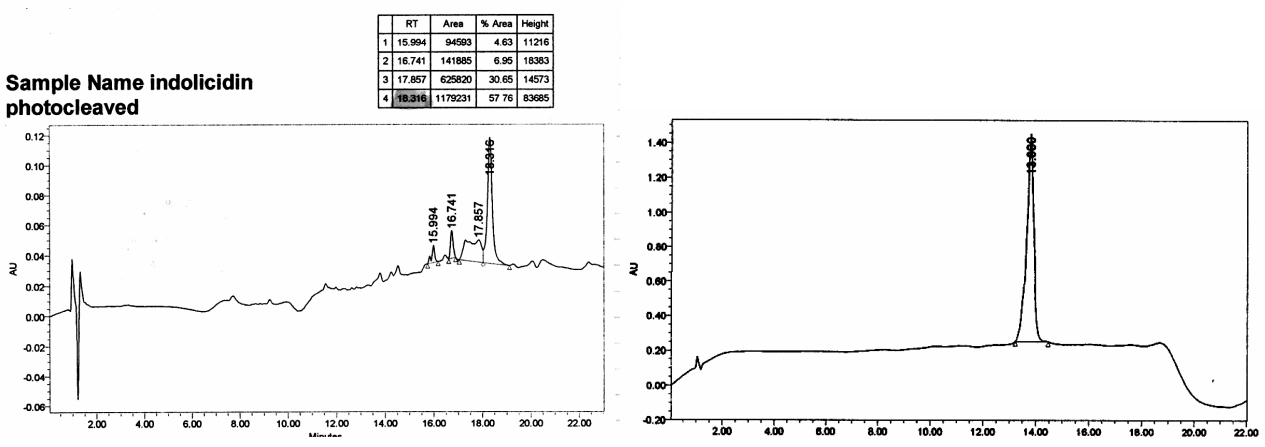

Fig. S33. a) RP-HPLC of photocleaved crude from peptide **C1** (2 min. iso. 100% A, then to 100% B in 18 min): t_R = 18.3 min, 58% of area at 214 nm. b) RP-HPLC of purified peptide (3 min. iso. 95% A, 5% B then to 10% A, 90% B in 12 min): t_R = 13.8 min.

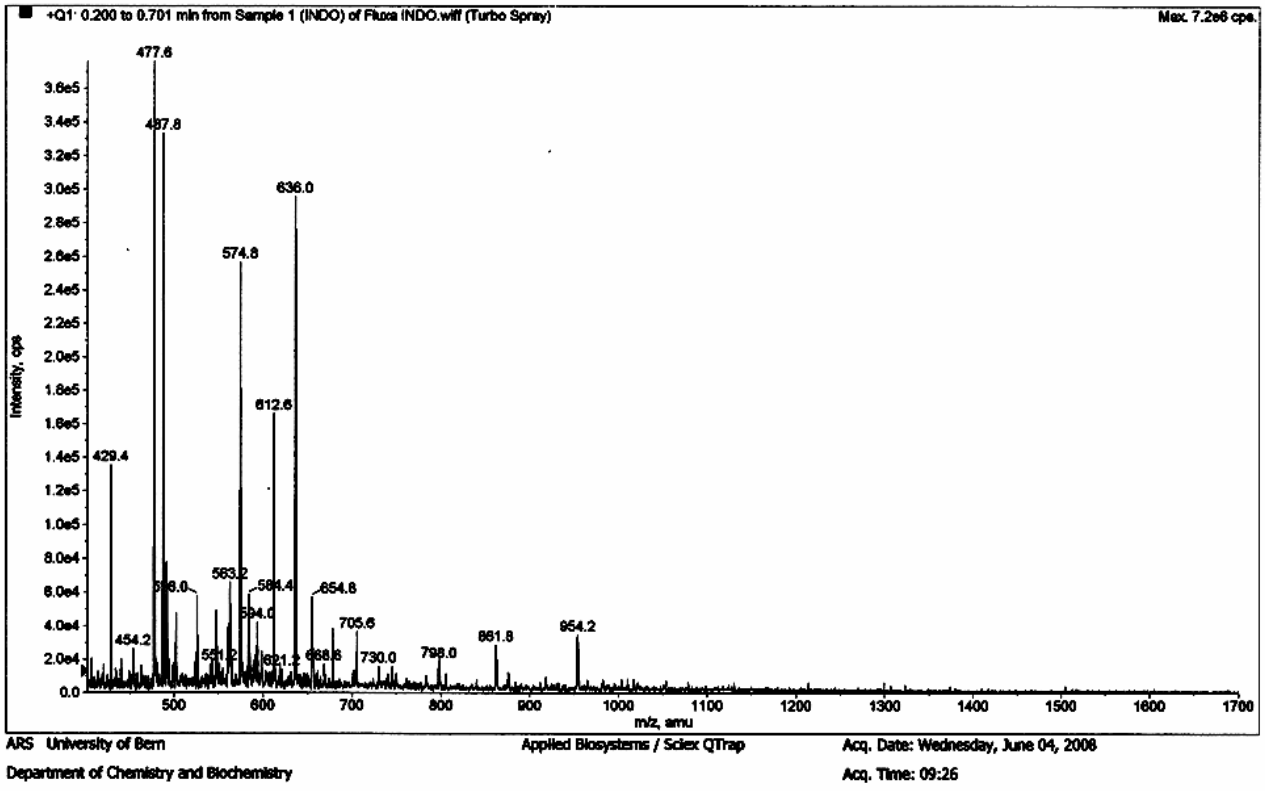

Fig. S34. ESI MS(+): Calcd. for $C_{100}H_{133}N_{26}O_{13}$ $[M+H]^+$: 1907.4, found: 954.2 $[(M+2H/2)]^{2+}$, found: 636.0 $[(M+3H/3)]^{3+}$, found: 477.6 $[(M+4H/4)]^{4+}$.

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Cyclic Control Peptide **C2** c(KYV*Orn*LfPFkN)

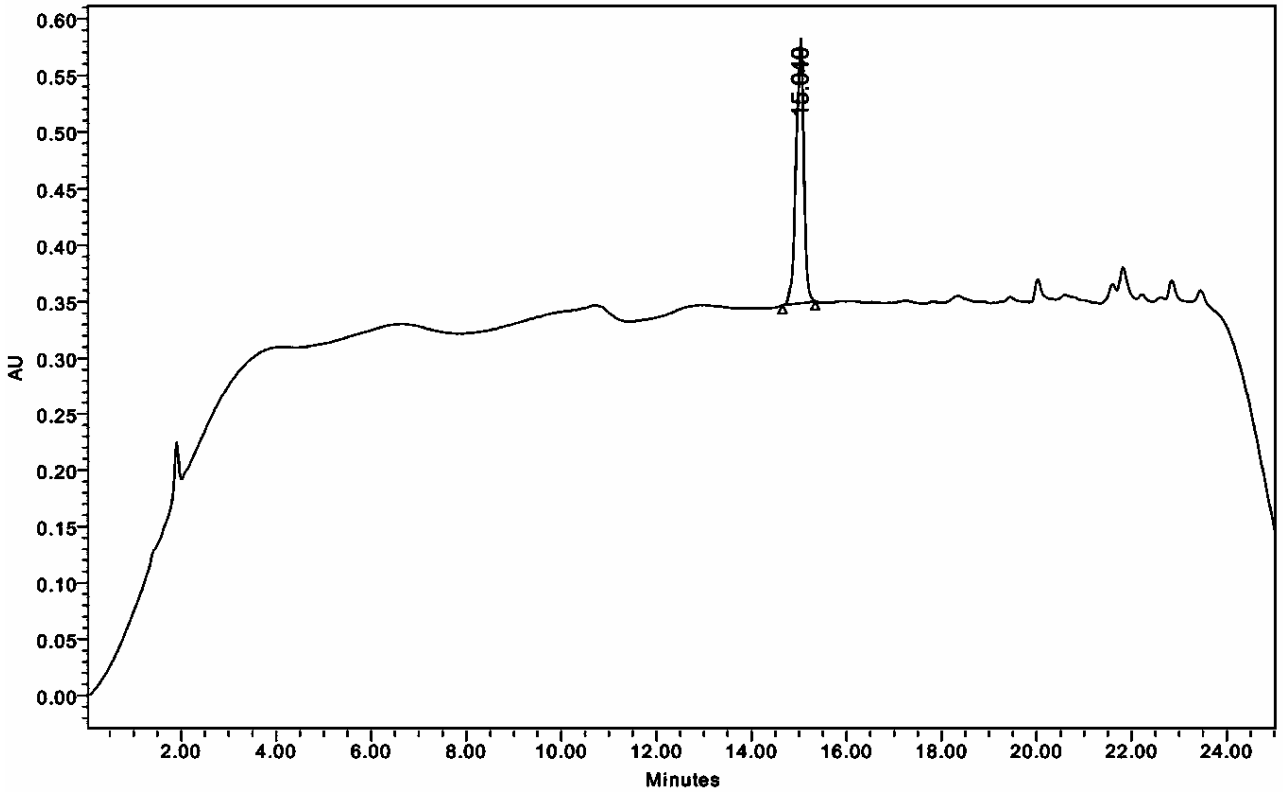

Fig. S35. RP-HPLC (85% A, 15% C to 100% C in 20 min): t_R = 15.0 min

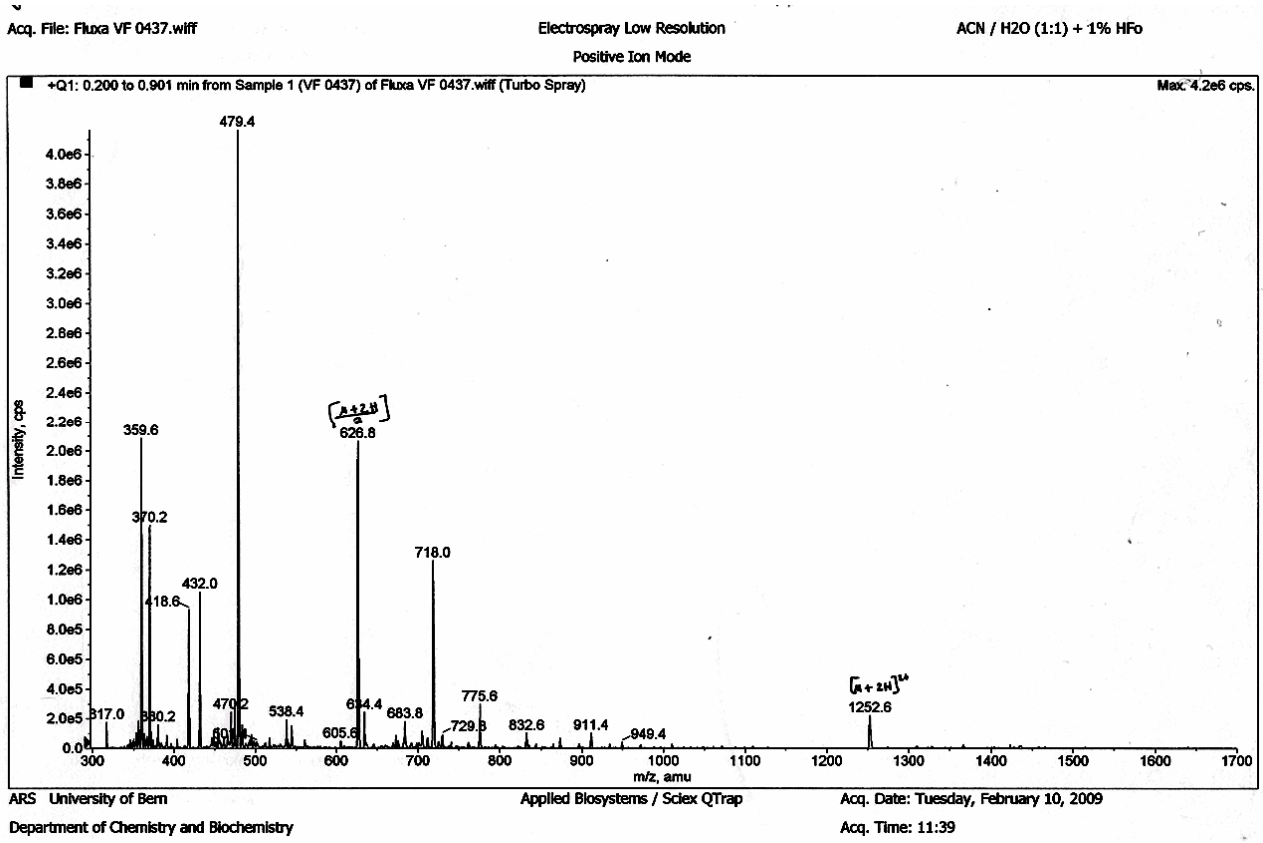

Fig. S36. ESI MS(+): Calcd. for $C_{64}H_{95}N_{14}O_{12}$ $[M+H]^+$: 1251.7, found: 1252.6, found: 626.8 $[(M+2H/2)]^{2+}$.

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Melittin GIGAVLKVLTTGLPALISWIKRKRQQ-CONH$_2$

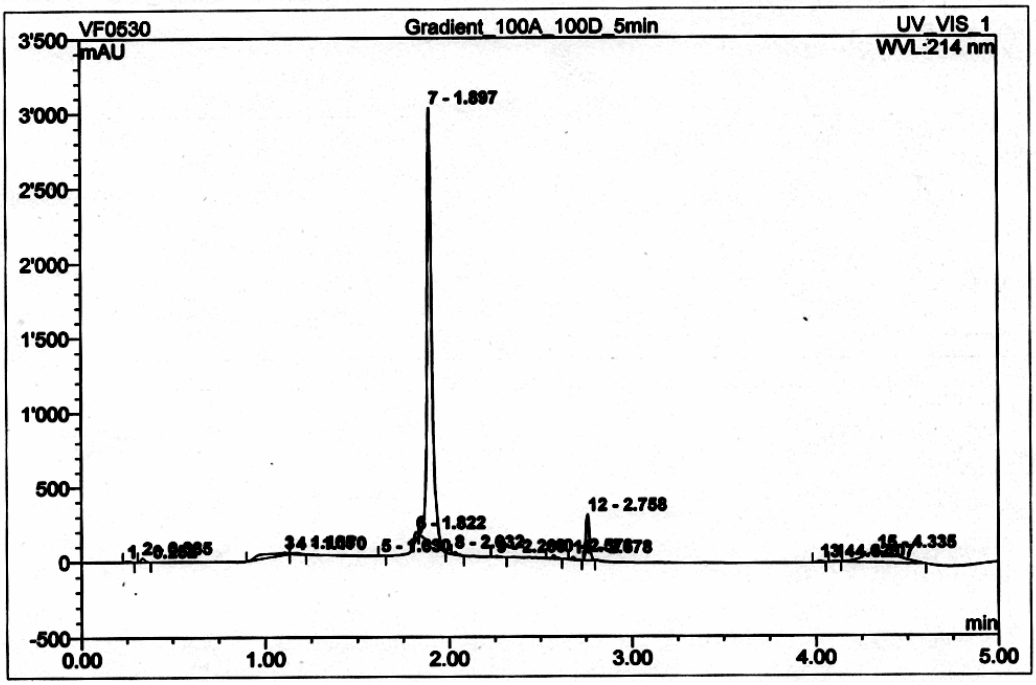

Fig. S37. RP-UPLC (100% A, 100% C in 2.2 min): t_R = 1.90 min

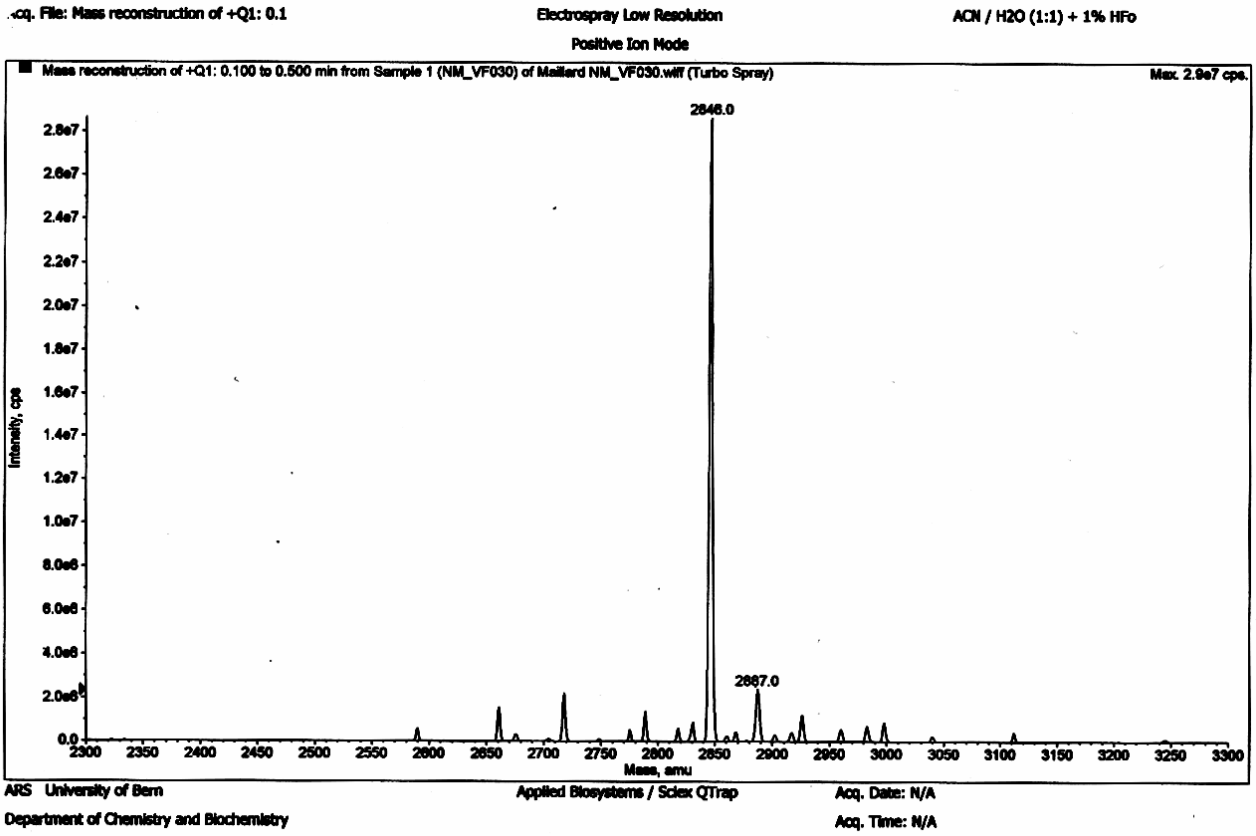

Fig. S38. ESI MS(+): Calcd. for $C_{131}H_{230}N_{39}O_{31}$ ([M+H]$^+$): 2845.8, found: 2846.0.

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Pexiganan GIGKFLKKAKKFGKAFVKILKK-CONH$_2$

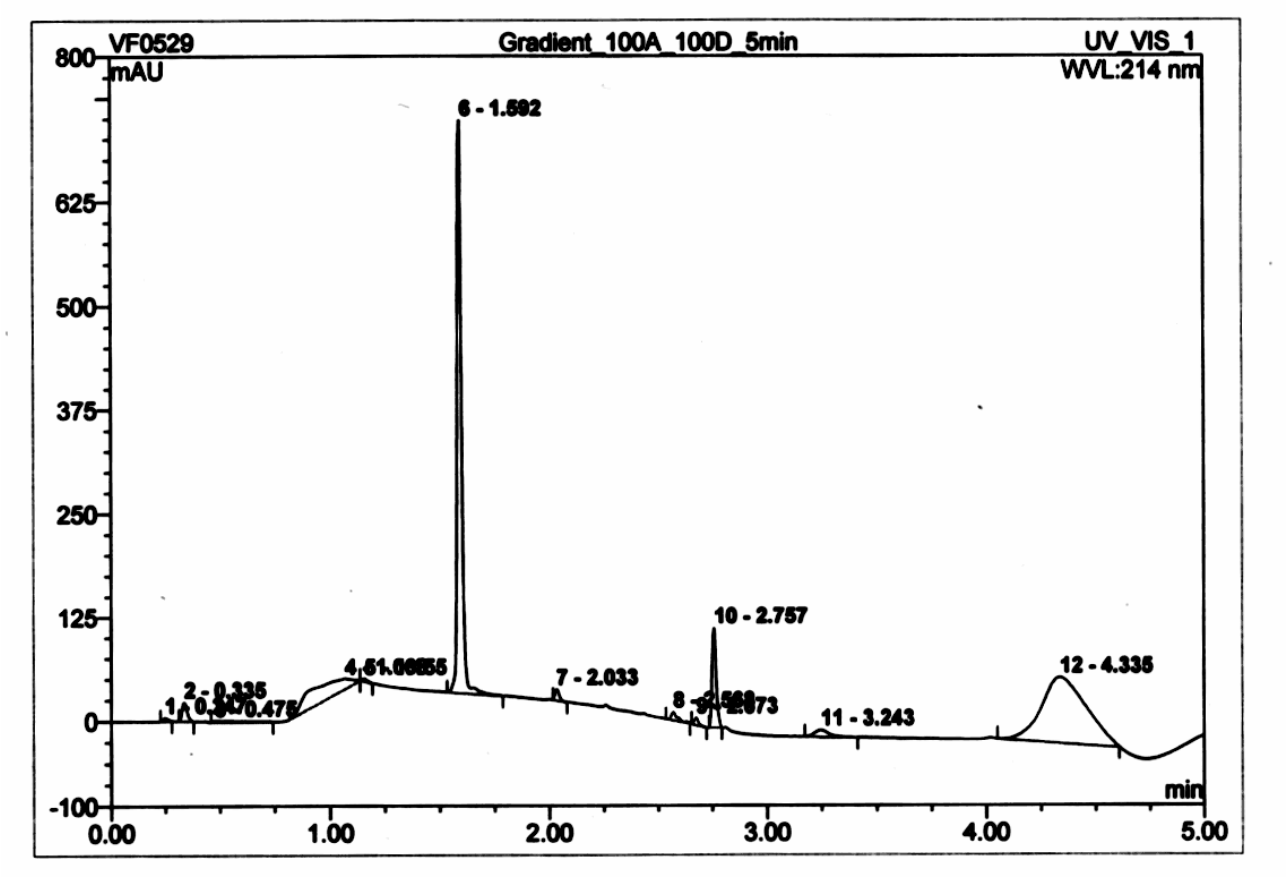

Fig. S39. RP-UPLC (100% A, 100% C in 2.2 min): t_R = 1.59 min

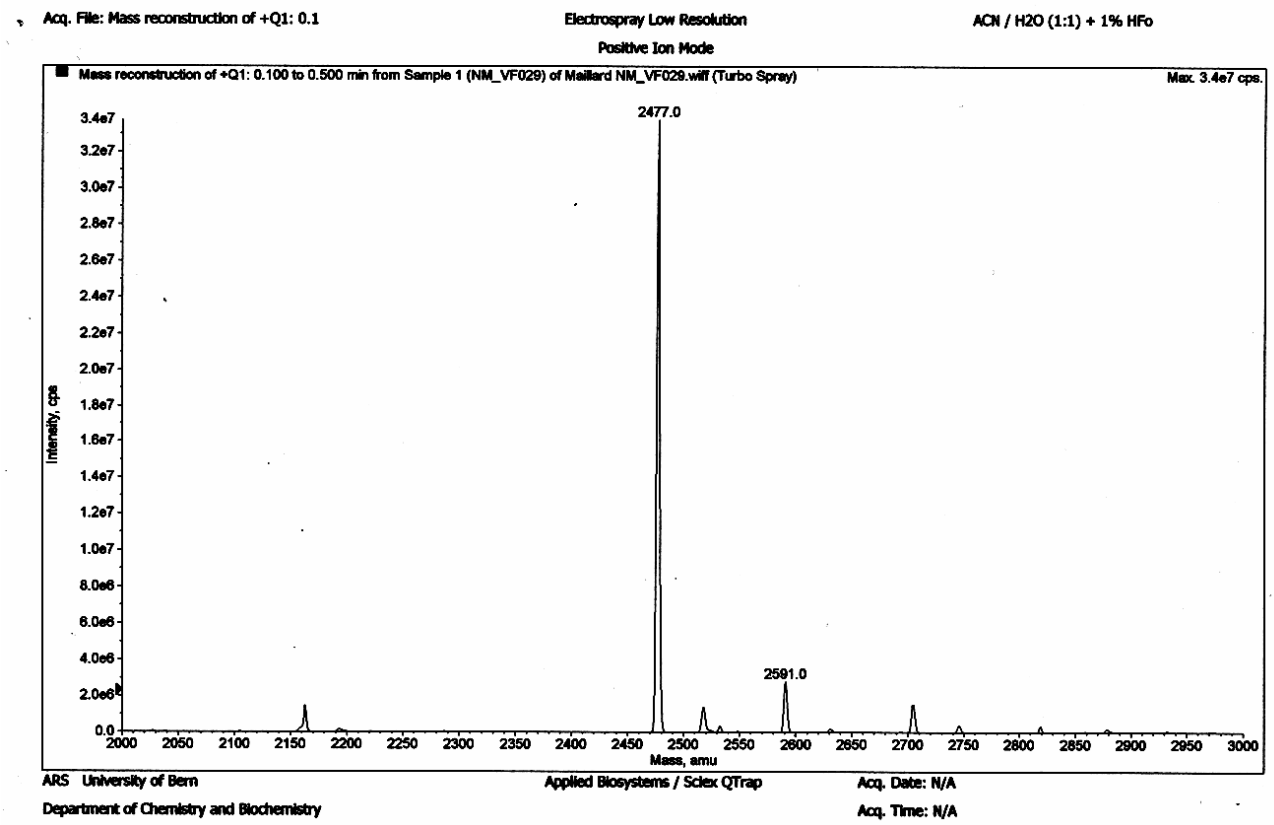

Fig. S40. ESI MS(+): Calcd. for $C_{122}H_{211}N_{32}O_{22}$ ([M+H]$^+$): 2476.6, found:2477.0.

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NMR spectra of Fmoc-D-Glu-1-Allyl ester

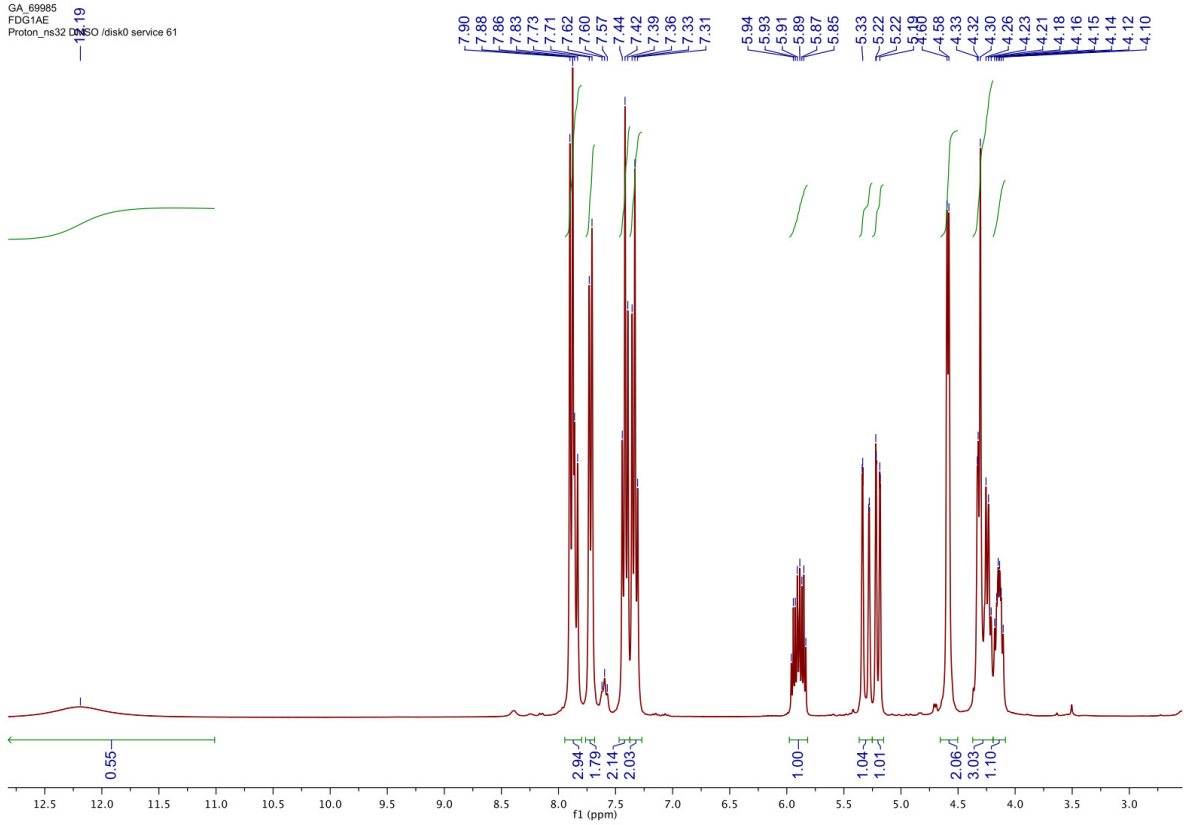

Fig. S41. [1]HNMR spectra of Fmoc-D-Glu-1-Allyl ester

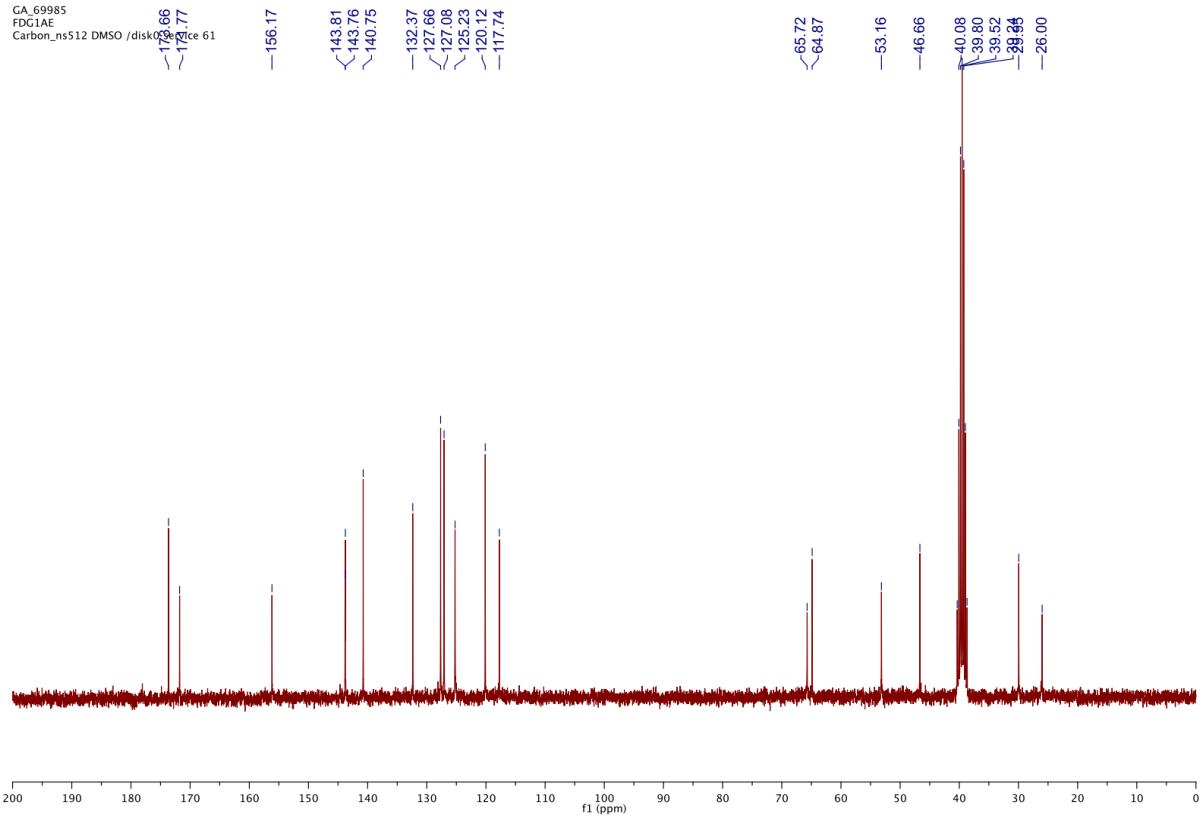

Fig. S42. [13]CNMR spectra of Fmoc-D-Glu-1-Allyl ester