Synthetic ramoplanin analogues are accessible by effective incorporation of arylglycines in solid-phase peptide synthesis. Electronic Supplementary Information

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Supplementary Methods

***Chemicals and reagents*.**

(S)-3,5-dihydroxyphenylglycine (H-L-Dpg-OH, Netchem Inc), (S)/(R) 4-hydroxyphenylglycine (H-L/D-Hpg-OH, Oakwood chemical), (S)-4-fluorophenylglycine (Phg(4F), ABCR Gmbh), Fmoc-OSu (GL Biochem), Na_2CO_3 (Sigma-Aldrich), ACN (Merck), 2-Chlorotrityl chloride resin (1 mmol/g, Merck), DCM (Chem-supply), DMF (Ajax Finechem), hydrazine monohydrate 64-65% (Sigma-Aldrich), MeOH (Scharlau), DIEA (Sigma-Aldrich), Fmoc-AA-OH (Merck, Chem-Impex, Iris-Biotech, GL Biochem), Fmoc-(2S)-2-amino-2-{4-[(tert-butoxy)carbonyl amino]phenyl} acetic acid (Fmoc-L-Phg(NH-Boc)-OH, Netchem), COMU (Merck), TEA (Merck), DIC (Oakwood chemicals), Oxyma (Merck), Boc-Glycine (Sigma-Aldrich), 1,5-lutidine (Sigma-Aldrich), DBU (Sigma-Aldrich), palladium-tetrakis (Sigma-Aldrich), phenylsilane (Sigma-Aldrich), TFA (Sigma-Aldrich), DODT (Sigma-Aldrich), TIS (Sigma-Aldrich), GnHCl (Sigma-Aldrich), NaH_2PO_4 (Sigma-Aldrich), MPAA (Sigma-Aldrich), (4-amino)thiophenol (Sigma-Aldrich), TCEP (Sigma-Aldrich), VA-044 (TCI chemicals), tBuSH (Sigma-Aldrich), EDC (Oakwood chemical), PyBOP (Merck) , DPPA (Merck), TFFH (Iris-Biotech).

Fmoc-amino acid preparations. [1] To a solution of unprotected amino acid (12 mmol) in 1:1 ACN/ 10% Na_2CO_3 (50 mL) stirring at room temperature was added Fmoc-OSu (4.8 g, 14 mmol) in MeCN (50 mL) dropwise over 30 min at 4^{o}C. The solution was stirred overnight before being diluted with 1M HCl (100 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4 and the solvent removed in vacuo after filtration of Na_2SO_4. The crude products were recrystallized from ethyl acetate (50 mL) with hexanes added to the point of precipitation for Fmoc-L/D-Hpg-OH, Fmoc-L-Hpg(3Cl)-OH* and Fmoc-L-Phg(4F)-OH. Fmoc-L-Dpg-OH was obtained after sonication the crude powder into a 1: 9 mixture of diethyl ether/ and hexane to remove the Fmoc derivatives (repeated twice). Fmoc-L/D-Tyr(3Cl)-OH were purified by flash column chromatography using a gradient of 0 - 100% in hexane/ ethyl acetate.

*HCl.H-L-Hpg(3Cl)-OH was obtained using acetic acid/ sulfuryl chloride method applied to L-Hpg. The resulting hydrochloride salt was filtered off and washed with acetic acid (3x) and hexane (3x).[2]

Resin preparations. [3] For compounds **(2a-d, 3, 5a-k, 6)**, 2-chlorotrityl chloride resin (200 mg) was swelled in DCM (8 mL, 30 min), washed with DMF (3x), and incubated with a 5% hydrazine solution in DMF (6 mL, 2x 30 min). The resin was washed with DMF (3x), and a solution of DMF/TEA/MeOH (7:2:1) (4 mL, 15 min) added for capping the unreacted 2-chlorotrityl function. The first Fmoc-protected amino acid (0.075 mmol) was coupled to the resin using DIC (0.075 mmol) and Oxyma (0.15 mmol) overnight at room temperature. In the second step, unreacted hydrazine or amino groups were capped with Boc−glycine (0.15 mmol) that had been activated using DIC (0.15 mmol) and Oxyma (0.3 mmol) for 1 h for the linear GPA precursors containing unprotected phenolic groups **(2a-d, 3)**. The capping of unreacted hydrazine groups at the beginning of

ramoplanin synthesis **(5a-k, 6)** was achieved by a solution of DMF/TEA/acetic anhydride (95:2.5:2.5) (3 mL, 15 min).

For the compounds (**4-S1-3**), The first Fmoc-protected amino acid (0.075 mmol) was coupled to the Rink amide resin using DIC (0.075 mmol) and Oxyma (0.15 mmol) overnight at room temperature. In the second step, unreacted amino groups were capped using a solution of DMF/TEA/acetic anhydride (95:2.5:2.5) (3 mL, 15 min).

For the compounds (**8**, **8(Cl)**, **11-14**), 2-Chlorotrityl chloride resin (200 mg) was swelled in DCM (8 mL, 30 min), washed with DMF (3x), and incubated with Fmoc-Gly-OH (0.075 mmol) and DIEA (0.3 mmol) at room temperature (3mL, 2h). The resin was washed with DMF (3x), and a solution of DMF/TEA/MeOH (7:2:1) (4 mL, 15 min) added for capping the unreacted 2-chlorotrityl function.

Solid phase peptide synthesis. From the Fmoc pre-loaded resins, the iterative cycle of an amino acid was performed using a 1% DBU solution (3 mL, 3 x 30 s) in DMF for Fmoc removal, washing with DMF (3x), coupling of the subsequent Fmoc- or Boc-protected amino acid (0.15 mmol) after activation with DIC (0.15 mmol) and Oxyma (0.3 mmol) for 10 min at 50°C (conventional or microwave heating) and washing with DMF (3x). The Fmoc- or Boc-protected amino acids were activated at 4°C during manual SPPS (followed by conventional heating) and *in situ* while using the Liberty Blue from CEM.

For the compounds (**4-S2-3**), a double coupling strategy (Fmoc-amino acid (0.15 mmol), DIC (0.15 mmol) and Oxyma (0.3 mmol) for 10 min at 50°C microwave heating) was performed twice for the final 4 amino acids.

For the compounds (**8**, **8(Cl)**, **11-14**), the on-resin Alloc removal was achieved with a catalof ytic amount of palladium-tetrakis (10%, 5μmol) using phenylsilane (0.25mmol) as scavenger in DCM (4ml) for 45 minutes. The reaction was repeated twice under nitrogen bubbling.

For compounds **(2a-d, 3, 4-S1-3, 5a-k, 6** and **8** from **9b)**, the cleavage of the hydrazide peptide from resin and removal of protecting groups (tBu, Boc, Trt) was accomplished using a solution of TFA/ TIS/ H_2O (95: 2.5: 2.5 v/ v'/ v'', 5 mL) with shaking at room temperature for 1 h. The cleavage of **6** containing a N-terminal cysteine was accomplished using TFA/ DODT/ TIS/ H_2O (95: 2: 2: 1; v/ v'/ v''/ v''', 5 mL) with shaking at room temperature for 1 h. The cleavage of the partially protected peptide (Scheme 1B – leading to **8**, **8(Cl)**, **11-14**) was performed with a solution of HFIP/DCM (30:70 v/v'; 9 mL) with shaking at room temperature (3 mL, 3 x 30min). All the peptides were concentrated under a stream of nitrogen to ~ 1mL volume and precipitated with ice cold diethyl ether (9 mL). The peptide products were washed by centrifugation three times and collected by centrifugation in a flame-resistant centrifuge.

Ramoplanin cyclization by intramolecular native chemical ligation/ desulfurization.[4] The purified hydrazide peptide (**6**) was dissolved in buffer 1 to a final concentration of 0.75 mM. The solution was cooled to −15 °C

using a salt/ ice bath. Subsequently, 1 M $NaNO_2$ (5 eq) was added and the mixture was stirred for 15 min. MPAA (leading to **7a**) or MPA (leading to **7b**) (40 eq dissolved in buffer 2) was then added to the reaction. After 15 minutes, the pH was slowly adjusted to 7.2 using 1M NaOH. The reaction mixture was stirred on ice for 2 hours, then TCEP (40 eq in dissolved buffer 2 – pH adjusted to 7.4) was added to reduce all the disulfide bridges. After 15 hours at room temperature, another solution of TCEP (40 eq in dissolved buffer 2 – pH adjusted to 7.4) was added and **7a** or **7b** were isolated after RP-HPLC preparative purification.

7a or **7b** were dissolved in buffer 2 containing tBuSH (10%) and TCEP (peptide and TCEP: 2mM) at room temperature. The desulfurization reagent VA-044 dissolved in buffer 2 was added to reach a final concentration of 0.1mM. The temperature of the reaction was set to 35°C. After 4-5hrs, **8** was isolated after RP-HPLC preparative purification.

Buffer 1: GnHCl (6 M) and NaH_2PO_4 (0.2 M), pH 3 (obtained via addition of HCl).

Buffer 2: GnHCl (6 M) and NaH_2PO_4 (0.2 M), pH 7.4 (obtained via addition of NaOH).

Ramoplanin head-to-tail cyclization. The crude peptide (**9**) was dissolved in DMF/ DCM (50: 50 v/ v') to a final concentration of 0.5 mM. At 4°C, TEA (4 eq) was added followed by PyBOP (2eq) dissolved in 1ml of DMF (100 µl addition every 2 minutes), following the portion wise addition of PyBOP the reaction was allowed to return to room temperature. After 2hrs, the solvent was removed under vacuum pressure and the protecting groups were cleaved using a solution of TFA/ TIS/ H_2O (95: 2.5: 2.5 v/ v'/ v'', 5 mL) with shaking at room temperature for 1 h. The crude peptide **8** was isolated as described in ***Solid phase peptide synthesis*** section and purified by RP-HPLC preparative. The same protocol – SPPS, cyclization and TFA cleavage – was applied to the synthesis of the peptides **8(Cl)** and **11-14.**

Ramoplanin aglycone (RAgly) preparation. The protocol was adapted from the reported in the following patent (US5491128A, Aglycons of A/16686 antibiotics). To a 50mL round bottom flask containing DMF (2 mL) and ACN (2 mL), NaI (4 eq, 23.5mg) was added followed by Ramoplanin A2 (99mg, 90% purity) with stirring at room temperature to form a white suspension. TMS-Cl (41 eq, 204µL) was then added, and the suspension became transparent rapidly and slowly turned yellow. The reaction was then heated at 75°C for 3 h. The mixture was then allowed to cool to room temperature and water was added, the pH was then adjusted to 4 with $NaHCO_3$ the reaction mixture was extracted 3 times with n-butanol. The combined butanolic fractions were removed in vacuo with repeated additions of toluene until dry. The dried residue was resolubilised in 4 mL of DMF and purified via preparative RP-HPLC on a gradient of 25-25-80% over 40 minutes. Yield 38.4% (33.2 mg including TFA salt).

LCMS and chiral RP-HPLC-MS characterizations. All LCMS analyses were carried out on a Shimadzu high performance liquid chromatography system coupled to mass spectrometer LCMS-2020 (ESI, operating both in positive and negative mode) equipped with a SPD-20A Prominence photo diode array detector and a LC-20AD solvent delivery module. Analytical separations were performed using an Agilent C18 column (300SB-C18,

5µm, 4.6 x 250 mm) and the detection was performed at 214 nm and 254nm. LCMS-grade solvents were used as such: water + 0.1% FA (solvent A) and ACN + 0.1% FA (solvent B).
Chiral RP-HPLC-MS was carried out on the same system as the LCMS characterization using a Phenomenex chiral column (Lux, 5µm, Cellulose-1, 150 x 10 mm) and the detection was performed at 214 nm and 254nm. LCMS-grade solvents were used as such: water + 0.1% FA (solvent A) and ACN + 0.1% FA (solvent B).
Gradient: 0% of B (5min isocratic), then 0% to 40% of B in 50 min at a flow rate of 2 mL/min.

RP-HPLC preparative. All purifications were performed using a Shimadzu high performance liquid chromatography system equipped with a SPD-M20A Prominence photo diode array detector and two LC-20AP pumps. Preparative separations were performed using a Zorbax SB-C18 column from Agilent (7 µm, 21.2 x 250 mm) with a flow rate of 10 mL/min. The solvents used were water + 0.1% TFA (solvent A) and HPLC-grade ACN + 0.1% TFA (solvent B).

HRMS analysis. Samples were separated on a RSLC 3000 LC system (Thermo) coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific). The LC system consisted of a trap column Acclaim PepMap 100 (100 µm x 2 cm, nanoViper, C18, 5 µm, 100Å; Thermo Scientific) and an Acclaim PepMap RSLC analytical column (75 µm x 50 cm, nanoViper, C18, 2 µm, 100Å; Thermo Scientific). Samples were loaded onto the trap column in uL-pickup mode using 2% acetonitrile, 0.1% TFA transport liquid. The columns were developed with a 30min gradient from 6% to 30% acetonitrile in 0.1% formic acid at 250nL/min coupled to the mass spectrometer nanospray source operated at 1.7kV. The mass spectrometer was operated in data dependent and PRM mode to target the appropriate species. Full scans were acquired at 70k to 500k resolution and MS2 spectra acquired at 35k or 70k resolution, with a 1.5-1.8 m/z isolation window and normalized collision energy between 25 and 33.

Raw data was manually analyzed in XCalibur QualBrowser (Thermo Scientific), with extracted ion chromatograms to the predicted species generated with 10ppm mass tolerance. MS^2 spectra corresponding to the predicted mass were manually characterized for ring closures based on predicted neutral loss peaks of non-crosslinked residues.

NMR analysis.

Data collection: Samples for NMR contained 4 mM of **8** in a 10mM acetate NMR buffer (80% H_2O: 20% DMSO(d6) pH 4.5). All experiments were recorded on a Bruker 600 MHz NMR spectrometer equipped with a cryoprobe and Z axis gradient. 2D 13C HSQC (acquisition times t1 = 4ms, t2 = 53ms) and 2D H2BC experiments (acquisition times t1 = 5.7ms, t2 = 56ms) were recorded using gradients for coherence selection and sensitivity enhancement with additional presaturation of the solvent signal if required. A 2D fast ^{15}N HSQC (Acquisition times t1 = 19ms, t2 = 65ms) was also acquired using a soft watergate element for solvent suppression. 1D 1H and 2D ^{1}H-^{1}H NOESY (with mixing times ranging from 250-450ms) and 2D ^{1}H-^{1}H TOCSY experiments (mixing time 70ms, acquisition times t2 = 425ms, t1 = 32ms) used a DPFGSE sequence for water suppression.[7] Typical

acquisition times for the NOESY experiments were 425ms (t2) and 32ms (t1). Experiments were recorded at 40, 25 and 15 °C which served to overcome some peak overlap which also expedited structural convergence in the calculations. Spectra were processed with nmrPipe[8] and analysed with Xeasy[9] or Spark(https://www.cgl.ucsf.edu/home/sparky).[10] Backbone assignments were made using the standard sequential NOE based assignment strategy using 2DTOCSY and 2D NOESY data and sidechain assignments were further aided using the 2D ^{13}C-^{1}H H2BC experiment.

Structure Calculations: Structures were calculated in a semi-automatic manner using CYANA version 3.0 using the noeassign macro.[11] 200 structures were calculated at each of the seven cycles and the top 20 carried forward to the next cycle. The ^{1}H chemical shift tolerance was set to 0.013 in both dimensions and the 'dref' set to 5.0 Å during automatic calibration of upper bound distance restraints to allow for the effects of spin diffusion. Hydrogen bond restraints were included in cases where several hydrogen bonds were observed in the structure ensemble and when the amide temperature coefficient was markedly lowered (residues D-Thr5, D-Orn10 and Gly14). New CYANA library entries were constructed for the non-standard residues by first building and minimizing the coordinates in Chimera.[12] The library file for the D amido acids was constructed by inverting the sign of the y coordinate of the standard residue within CYANA.

Several, unambiguous trans annular NOEs (HN-HN, HA-HN) were observed consistent with the two stranded anti-parallel sheet observed in ramoplanin as well as a beta turn (residues 8-11). NOESY peaks were assigned using rounds of semi-automatic NOE assignments and structure calculations in torsion angle space using CYANA3. The cyclized amide bond was constructed using upper and lower limit NOEs with a scaling factor of 100 using the 'link' command in CYANA. Structures were calculated with the linkage between L-Dap2 and Hpg17 implemented as either a cis or trans amide. The trans geometry was inferred based on the observed increase in NOE violations for the cis variant as well as an observed hydrogen bond between the sidechain HNγ to the backbone carbonyl of Gly14 which was consistent with a low value of the amide temperature coefficient for the trans (-2.83 ppb/K). In comparison, the sidechain amide was completely solvent exposed in the cis variant ensemble.

Deposition of the chemical shifts, coordinates and restraints: In the deposited chemical shift at the BMRB (STAR file 52188) and the coordinates at the PDB (8V4B), the sequence starts with 6NA as residue 1 ending in D4P 18. Note, during the validation process against the NMR restraints at the PDB, only 252 out of the total of 342 NMR restraints (Table S3) were included in the NMR validation step and the statistics. This is because the NMR restraint statistics software used at the PDB is limited and does not parse NOEs involving pseudo atoms for the non-canonical residues 6NA, GHP and D4P. During publication, correspondence with the PDB to deposit the full NOE list using the older nmr .text format (with a suitable header which can define the pseudoatoms) was unfortunately not successful, meaning that the subset of NOEs (252) converted and stored in the combined nmr data (nef) .str file by the PDB is incomplete and should be viewed as such. The pseudoatom definitions used here are shown under Table S3 for completeness.

Structural statistics and upper distance limits:

Total: 330

short-range, |i-j|<=1: 176

medium-range, 1<|i-j|<5: 50

long-range, |i-j|>=5: 104

Other upper distance restraints: 12

H bond upper restraints: 6

Trans amide cyclisation upper restraints: 6

CYANA target function: 0.31 +/- 0.018 Å2

Average backbone RMSD to mean: 0.09 +/- 0.03 Å (res 1-17), 0.17 +/- 0.07 Å (including lipid)

Average heavy atom RMSD to mean: 0.29 +/- 0.06 Å (res 1-17), 0.49 +/- 0.16 Å (including lipid)

Maximum NOE violation: 0.14 Å

MIC determination and bacterial growth.

Ramoplanin Antimicrobial assay: The antimicrobial activity of the synthetic ramoplanin peptides (**8**, **8(Cl)**, **11-14**) were determined against *MSSA*, *MRSA* and *VISA* alongside vancomycin (**Van**), ramoplanin A2 (**RA2**) and **RAGly**. The assays were conducted in accordance with the Clinical and Laboratory Standards Institute guidelines for microbroth dilution assays, with the addition of absorbance measurements being taken. Stocks of each compound were loaded into 96-well flat bottom polypropylene microtiter plates (Greiner) as two-fold serial dilutions, at 10 times the final required concentration. The stocks were diluted in DMSO, which had a final concentration of 2.0% after addition of the S. aureus at 5 x 10 5 CFU/ml in Cation-Adjusted Mueller Hinton Broth II (Becton Dickson). The plates were covered with BreatheEasy membranes (Diversified Biotech) and a Clariostar plate reader was used to measure the absorbance of each well at 595nm every 4 h for 48 h with orbital averaging after linear shaking (software v5.20R5, BMG Labtech). The minimum inhibitory concentration was determined by the lowest drug concentration that resulted in clear well by eye indicating bacterial growth inhibition. Percentage growth of each well was calculated from the absorbance measurements taken at 20 h relative to the average of the no compound controls on the same plate. GraphPad Prism 9 software was used to plot the average of two biological replicates, each the average of three technical replicates (Table S4). Similarly, the bacterial absorbance ($OD_{600\ nm}$) of each well was measured at 595 nm every 4 h for 48 h (Clariostar plate reader, software v5.20R5, BMG). The percentage growth was calculated relative to the no

compound control and the average of three biological replicates plotted using GraphPad Prism 9 software (Figure S8-10).

Bacterial isolates and media: The S. aureus strains – MSSA (ATCC 29213), MRSA (A8090 – ST5 genotype)[5] and VISA (A8094 – ST5 genotype)[6] – were stored in glycerol broth at -80 °C. The strains were cultured in Brain Heart Infusion broth (Becton Dickson) at 37 °C with shaking and grown in Cation-Adjusted Mueller Hinton Broth II (Becton Dickson) for testing.

Supplementary Tables

Table S1. Summary on the preliminary syntheses of GPA precursors. **1** (vancomycin heptapeptide) and **2a** (teicoplanin heptapeptide) were previously synthesized with COMU/lutidine as coupling reagent. **2b-c** and **3** (corbomycin heptapeptide) were used to validate the benefit of DIC/Oxyma.

Entry	Arylglycine ratio*	Coupling reagent	Temperature (°C)	AA cycle time (min)	Peptide recovery (%)	Peptide purity at 214nm (%)
1	3/ 7	COMU/lutidine	RT	35	46	50
2a	5/ 7		RT	35	32	12
2b	5/ 7	DIC/Oxyma	RT	35	43	47
2c	5/ 7		50 (mw)	15	47	47
2d	5/ 7		50 (ch)	15	54	49
3	6/ 9		50 (mw)	15	61	51

Abb. RT: room temperature; mw: microwave; ch: conventional heating.

Table S2. Macrolactamization optimisation and the effect of the coupling reagent.

Entry	Basis	Solvent	Coupling system	Peptide purity at 214nm (%), 1h	Peptide purity at 214nm (%), 15h
10a	TEA (1eq)	DCM/ DMF (50:50)	EDC (2eq)/ Oxyma (4eq)	nd	10
10b			PyBOP (2eq)/ TEA (2eq)	19	19
10c			DEPBT (2eq)/ TEA (2eq)	1	10
10d			DPPA (2eq)/ TEA (2eq)	1	7
10e			TFFH (2eq)/ TEA (2eq)	6	4

Abb. ch: conventional heating. DEPBT: 3- (Diethylphosphoryloxy)-1,2,3-benzotriazin-4 (3H)-one; DPPA: Diphenylphosphoryl azide. TFFH: N,N,N′,N′-Tetramethylfluoroformamidinium hexafluoro-phosphate.

Table S3. Summary of all dataset NOEs.

NOE Restraints						
0	6NA	QA	15	LEU	HA	5.5
0	6NA	QA	16	DAL	QB	5.5
0	6NA	QA	0	6NA	QD	5.5
0	6NA	QA	0	6NA	QE	5.5
0	6NA	QA	1	ASN	H	5.5
0	6NA	QA	1	ASN	HA	5.5
0	6NA	QA	2	DPP	H	5.5
0	6NA	QB	1	ASN	H	4.92
0	6NA	QB	2	DPP	H	5.5
0	6NA	QD	1	ASN	H	5.5
0	6NA	QE	1	ASN	H	5.5
0	6NA	QG	1	ASN	H	5.5
0	6NA	QB	2	DPP	H	5.5
0	6NA	QG	2	DPP	H	5.5
1	ASN	H	15	LEU	HA	5.13
1	ASN	H	15	LEU	QB	5.5
1	ASN	H	15	LEU	QD1	5.5
1	ASN	H	15	LEU	QD2	5.5
1	ASN	H	16	DAL	H	5.5
1	ASN	H	1	ASN	HB2	4.04
1	ASN	H	1	ASN	HB3	4.07
1	ASN	H	1	ASN	HD21	4.6
1	ASN	H	1	ASN	HD22	4.88
1	ASN	H	2	DPP	HB3	5.44
1	ASN	HA	13	D4P	QD	5.18
1	ASN	HA	15	LEU	H	5.5
1	ASN	HA	15	LEU	HA	4.48
1	ASN	HA	16	DAL	H	5.11
1	ASN	HA	17	D4P	H	5.5
1	ASN	HA	17	D4P	QG	5.5
1	ASN	HA	2	DPP	H	3.35
1	ASN	HA	2	DPP	HB3	5.5
1	ASN	HB2	13	D4P	QD	4.81
1	ASN	HB2	14	GLY	H	4.53
1	ASN	HB2	14	GLY	QA	5.34
1	ASN	HB2	15	LEU	HA	5.5
1	ASN	HB2	2	DPP	H	4.52
1	ASN	HB3	13	D4P	QD	5.1
1	ASN	HB3	14	GLY	H	5.5
1	ASN	HB3	15	LEU	HA	5.5
1	ASN	HB3	2	DPP	H	4.61
1	ASN	HD21	15	LEU	QQD	5.44
1	ASN	HD21	2	DPP	H	5.49
2	DPP	H	13	D4P	QD	5.5
2	DPP	H	15	LEU	HA	5.01
2	DPP	H	15	LEU	QB	5.5

NOE Restraints						
7	GHP	H	9	PHE	H	5.5
7	GHP	H	9	PHE	QD	5.5
7	GHP	H	10	ORD	H	4.29
7	GHP	H	10	ORD	HB2	5.5
7	GHP	H	10	ORD	HB3	5.5
7	GHP	H	11	D4P	HA	5.48
7	GHP	H	11	D4P	QD	5.5
7	GHP	H	11	D4P	QG	5.49
7	GHP	H	7	GHP	QD	4.44
7	GHP	H	7	GHP	QG	3.66
7	GHP	H	8	THR	H	5.25
7	GHP	H	8	THR	QG2	5.5
7	GHP	HA	9	PHE	H	4.58
7	GHP	HA	9	PHE	QD	5.5
7	GHP	HA	9	PHE	QE	5.41
7	GHP	HA	10	ORD	H	5.5
7	GHP	HA	8	THR	H	3.33
7	GHP	HA	8	THR	HA	4.78
7	GHP	HA	8	THR	HB	5.38
7	GHP	HA	8	THR	QG2	4.67
7	GHP	QD	8	THR	H	5.18
7	GHP	QD	8	THR	QG2	5.1
7	GHP	QG	10	ORD	H	5.22
7	GHP	QG	10	ORD	HB3	5.5
7	GHP	QG	8	THR	H	4.18
7	GHP	QG	8	THR	HA	5.13
7	GHP	QG	8	THR	QG2	5.28
8	THR	H	9	PHE	H	4.27
8	THR	H	8	THR	HB	3.89
8	THR	H	8	THR	QG2	4.2
8	THR	HA	9	PHE	QD	5.5
8	THR	HA	10	ORD	H	5.5
8	THR	HA	10	ORD	QG	5.5
8	THR	HA	8	THR	QG2	3.54
8	THR	HB	9	PHE	H	4.55
8	THR	HB	9	PHE	QD	5.42
8	THR	HB	9	PHE	QE	5.47
8	THR	QG2	9	PHE	H	4.96
8	THR	QG2	9	PHE	HZ	4.12
8	THR	QG2	9	PHE	QD	4.67
8	THR	QG2	9	PHE	QE	4.66
8	THR	QG2	10	ORD	H	5.5
9	PHE	H	9	PHE	HB2	3.96
9	PHE	H	9	PHE	HB3	4.03
9	PHE	H	9	PHE	QD	4.05
9	PHE	HA	9	PHE	QD	3.76

2	DPP	H	15	LEU	QQD	5.44
2	DPP	H	16	DAL	H	5.5
2	DPP	H	16	DAL	QB	5.5
2	DPP	H	17	D4P	H	5.22
2	DPP	H	17	D4P	QG	5.5
2	DPP	H	2	DPP	HB2	3.9
2	DPP	H	2	DPP	HG1	4.08
2	DPP	H	3	GHP	H	4.81
2	DPP	HA	3	GHP	HA	5.5
2	DPP	HA	3	GHP	QD	5.5
2	DPP	HA	3	GHP	QG	5.5
2	DPP	HB2	17	D4P	H	5.5
2	DPP	HB2	17	D4P	QG	5.5
2	DPP	HB2	3	GHP	H	4.67
2	DPP	HB2	3	GHP	HA	5.5
2	DPP	HB2	3	GHP	QG	4.81
2	DPP	HB3	15	LEU	HA	5.5
2	DPP	HB3	17	D4P	H	5.5
2	DPP	HB3	17	D4P	HA	5.5
2	DPP	HB3	17	D4P	QG	5.5
2	DPP	HB3	3	GHP	H	4.9
2	DPP	HG1	16	DAL	H	5.19
2	DPP	HG1	16	DAL	HA	5.5
2	DPP	HG1	16	DAL	QB	5.5
2	DPP	HG1	17	D4P	H	4.19
2	DPP	HG1	17	D4P	HA	4.47
2	DPP	HG1	17	D4P	QG	4.58
2	DPP	HG1	3	GHP	HA	5.5
3	GHP	H	13	D4P	HA	5.5
3	GHP	H	14	GLY	H	5.5
3	GHP	H	3	GHP	QD	5.09
3	GHP	H	3	GHP	QG	3.56
3	GHP	HA	13	D4P	H	5.5
3	GHP	HA	13	D4P	HA	3.9
3	GHP	HA	13	D4P	QD	5.5
3	GHP	HA	13	D4P	QG	5.46
3	GHP	HA	14	GLY	H	5.06
3	GHP	HA	17	D4P	QD	5.28
3	GHP	HA	17	D4P	QG	5.18
3	GHP	HA	4	ORD	HA	5.5
3	GHP	HA	4	ORD	HB2	5.5
3	GHP	HA	4	ORD	HB3	5.5
3	GHP	HA	5	DTH	H	4.94
3	GHP	HO4	9	PHE	H	5.5
3	GHP	HO4	7	GHP	HA	5.5
3	GHP	QD	9	PHE	H	5.01
3	GHP	QD	9	PHE	HA	5.45
3	GHP	QD	9	PHE	HB2	4.86
9	PHE	HA	9	PHE	QE	4.84
9	PHE	HA	10	ORD	QG	5.5
9	PHE	HB2	10	ORD	H	4.88
9	PHE	HB2	17	D4P	HA	5.5
9	PHE	HB2	17	D4P	QG	5.5
9	PHE	HB3	10	ORD	H	4.77
9	PHE	HB3	17	D4P	HA	4.93
9	PHE	HB3	17	D4P	QD	5.46
9	PHE	HB3	17	D4P	QG	5.32
9	PHE	QD	9	PHE	HZ	3.86
9	PHE	QD	17	D4P	HA	5.5
10	ORD	H	10	ORD	HB3	4.04
10	ORD	H	10	ORD	QD	5.5
10	ORD	H	10	ORD	QG	4.4
10	ORD	H	11	D4P	H	5.02
10	ORD	HA	11	D4P	H	3.39
10	ORD	HA	11	D4P	QG	5.5
10	ORD	HB2	11	D4P	H	4.51
10	ORD	HB2	11	D4P	QD	5.11
10	ORD	HB2	11	D4P	QG	4.79
10	ORD	HB3	11	D4P	H	4.96
10	ORD	HB3	11	D4P	QG	5.47
10	ORD	QG	11	D4P	H	5.35
10	ORD	QG	11	D4P	QD	5.5
11	D4P	H	11	D4P	QD	4.19
11	D4P	H	11	D4P	QG	4.49
11	D4P	HA	12	DTH	H	3.53
11	D4P	HA	12	DTH	QG2	5.35
11	D4P	QD	12	DTH	H	5
11	D4P	QD	12	DTH	QG2	4.58
11	D4P	QG	12	DTH	H	3.72
11	D4P	QG	12	DTH	QG2	4.65
12	DTH	H	12	DTH	QG2	4.21
12	DTH	HA	12	DTH	QG2	3.75
12	DTH	HA	13	D4P	H	3.52
12	DTH	HA	13	D4P	QG	4.77
12	DTH	HB	13	D4P	H	4.21
12	DTH	HB	13	D4P	QG	4.73
12	DTH	QG2	13	D4P	H	4.41
12	DTH	QG2	13	D4P	HA	5.5
12	DTH	QG2	13	D4P	QD	4.62
12	DTH	QG2	13	D4P	QG	4.36
13	D4P	H	13	D4P	QD	5.47
13	D4P	H	13	D4P	QG	3.86
13	D4P	H	14	GLY	H	5.08
13	D4P	H	17	D4P	QD	5.5
13	D4P	HA	14	GLY	H	3.36
13	D4P	HA	14	GLY	HA2	5.45

3	GHP	QD	9	PHE	HB3	4.91
3	GHP	QD	10	ORD	H	4.36
3	GHP	QD	10	ORD	HB2	5.43
3	GHP	QD	10	ORD	HB3	5.5
3	GHP	QD	10	ORD	QG	5.5
3	GHP	QD	11	D4P	H	4.64
3	GHP	QD	12	DTH	H	5.01
3	GHP	QD	5	DTH	H	4.92
3	GHP	QD	7	GHP	H	3.87
3	GHP	QD	7	GHP	HA	4.77
3	GHP	QD	8	THR	H	5.5
3	GHP	QD	8	THR	QG2	5.5
3	GHP	QG	9	PHE	HB2	5.5
3	GHP	QG	13	D4P	HA	5.5
3	GHP	QG	17	D4P	QD	6
3	GHP	QG	4	ORD	H	4.43
3	GHP	QG	4	ORD	HA	5.5
3	GHP	QG	5	DTH	HA	5.5
3	GHP	QG	6	D4P	HA	5.49
3	GHP	QG	7	GHP	HA	5.5
4	ORD	H	13	D4P	HA	4.45
4	ORD	H	13	D4P	QG	4.65
4	ORD	H	14	GLY	H	5.46
4	ORD	H	4	ORD	HB2	4.09
4	ORD	H	4	ORD	QD	5.5
4	ORD	H	5	DTH	H	4.01
4	ORD	H	5	DTH	HA	5.5
4	ORD	H	5	DTH	HB	5.5
4	ORD	HA	13	D4P	HA	5.18
4	ORD	HA	13	D4P	QD	5.5
4	ORD	HA	4	ORD	QD	5.49
4	ORD	HA	4	ORD	QG	4.11
4	ORD	HB2	12	DTH	H	5.5
4	ORD	HB2	13	D4P	HA	5.48
4	ORD	HB2	13	D4P	QD	4.82
4	ORD	HB2	13	D4P	QG	4.78
4	ORD	HB2	4	ORD	QD	4.18
4	ORD	HB2	5	DTH	H	4.47
4	ORD	HB2	5	DTH	HB	5.5
4	ORD	HB3	13	D4P	HA	5.5
4	ORD	HB3	13	D4P	QD	4.87
4	ORD	HB3	13	D4P	QG	4.75
4	ORD	HB3	14	GLY	H	5.5
4	ORD	HB3	5	DTH	H	5.5
4	ORD	QD	13	D4P	QD	4.21
4	ORD	QD	13	D4P	QG	5.5
4	ORD	QD	5	DTH	H	5.5
4	ORD	QD	5	DTH	QG2	5.5
13	D4P	HA	14	GLY	HA3	5.45
13	D4P	HA	17	D4P	QD	5.31
13	D4P	HA	17	D4P	QG	5.5
13	D4P	QD	14	GLY	H	4.63
13	D4P	QD	14	GLY	HA2	5.5
13	D4P	QD	14	GLY	HA3	5.5
13	D4P	QD	14	GLY	QA	4.81
13	D4P	QG	14	GLY	H	4.59
13	D4P	QG	14	GLY	QA	5.34
14	GLY	H	15	LEU	H	4.42
14	GLY	H	15	LEU	HA	5.5
14	GLY	H	15	LEU	QQD	5.44
14	GLY	H	17	D4P	QD	5.34
14	GLY	H	17	D4P	QG	5.31
14	GLY	HA2	15	LEU	QB	5.24
14	GLY	HA2	17	D4P	QD	5.16
14	GLY	HA2	17	D4P	QG	5.5
14	GLY	HA3	15	LEU	QB	5.24
14	GLY	HA3	17	D4P	QD	5.16
14	GLY	HA3	17	D4P	QG	5.5
14	GLY	QA	15	LEU	QQD	5.28
14	GLY	QA	17	D4P	QD	4.54
15	LEU	H	15	LEU	HG	4.21
15	LEU	H	15	LEU	QB	3.54
15	LEU	H	15	LEU	QD1	5.5
15	LEU	H	15	LEU	QD2	5.5
15	LEU	H	15	LEU	QQD	4.54
15	LEU	H	16	DAL	H	5.35
15	LEU	HA	15	LEU	HG	4.03
15	LEU	HA	15	LEU	QD1	4.29
15	LEU	HA	15	LEU	QD2	4.29
15	LEU	HA	15	LEU	QQD	3.59
15	LEU	HA	16	DAL	QB	4.56
15	LEU	HA	17	D4P	H	5.29
15	LEU	HG	16	DAL	H	5.14
15	LEU	QB	15	LEU	QD1	4.05
15	LEU	QB	15	LEU	QD2	4.05
15	LEU	QB	16	DAL	H	4.42
15	LEU	QB	16	DAL	HA	5.5
15	LEU	QB	17	D4P	H	5.5
15	LEU	QD1	16	DAL	H	5.5
15	LEU	QD2	16	DAL	H	5.5
15	LEU	QQD	16	DAL	H	4.5
16	DAL	H	16	DAL	QB	3.63
16	DAL	H	17	D4P	H	4.44
16	DAL	HA	17	D4P	QG	5.5
16	DAL	QB	17	D4P	H	4.72
16	DAL	QB	17	D4P	HA	5.5

4	ORD	QG	12	DTH	H	5.5
4	ORD	QG	13	D4P	HA	5.5
4	ORD	QG	13	D4P	QD	4.68
4	ORD	QG	13	D4P	QG	5.5
4	ORD	QG	5	DTH	H	5.5
4	ORD	QG	5	DTH	HB	5.5
5	DTH	H	11	D4P	HA	5.5
5	DTH	H	12	DTH	H	4.27
5	DTH	H	12	DTH	QG2	5.14
5	DTH	H	13	D4P	HA	4.98
5	DTH	H	5	DTH	QG2	4.79
5	DTH	H	6	D4P	H	4.8
5	DTH	HA	12	DTH	H	5.5
5	DTH	HA	5	DTH	QG2	3.53
5	DTH	HA	6	D4P	H	3.29
5	DTH	HA	6	D4P	QD	5.5
5	DTH	HA	6	D4P	QG	4.54
5	DTH	HB	11	D4P	QD	5.48
5	DTH	HB	11	D4P	QG	5.39
5	DTH	HB	12	DTH	H	5.31
5	DTH	HB	12	DTH	QG2	5.19
5	DTH	HB	6	D4P	H	4.2
5	DTH	HB	6	D4P	QD	5.5
5	DTH	HB	6	D4P	QG	4.74
5	DTH	QG2	11	D4P	QD	4.97
5	DTH	QG2	11	D4P	QG	4.48
5	DTH	QG2	12	DTH	H	5.24
5	DTH	QG2	6	D4P	H	4.15
5	DTH	QG2	6	D4P	QD	5.32
5	DTH	QG2	6	D4P	QG	4.44
6	D4P	H	11	D4P	QG	5.25
6	D4P	H	6	D4P	QD	4.69
6	D4P	H	6	D4P	QG	3.58
6	D4P	HA	10	ORD	H	5.5
6	D4P	HA	11	D4P	H	5.5
6	D4P	HA	11	D4P	HA	3.95
6	D4P	HA	11	D4P	QD	5.08
6	D4P	HA	11	D4P	QG	4.42
6	D4P	HA	12	DTH	H	4.77
6	D4P	HA	7	GHP	HA	5.5
6	D4P	QD	7	GHP	H	5.15
6	D4P	QG	11	D4P	QG	4.17
6	D4P	QG	12	DTH	H	4.99
6	D4P	QG	12	DTH	QG2	5.19
17	D4P	H	17	D4P	QD	5.23
17	D4P	H	17	D4P	QG	4.34
H bonds upper restraints						
2	DPP	O	14	GLY	H	2
2	DPP	O	14	GLY	N	3
5	DTH	H	12	DTH	O	2
5	DTH	N	12	DTH	O	3
10	ORD	H	7	GHP	O	2
10	ORD	N	7	GHP	O	3
H bonds lower restraints						
3	DPP	O	15	GLY	N	2.7
6	DTH	O	13	DTH	N	2.7
11	ORD	O	8	GHP	N	2.7
Cyclic peptide upper restraints						
2	DPP	NG	17	D4P	C	1.33
2	DPP	HG1	17	D4P	C	2
2	DPP	CB	17	D4P	C	2.43
2	DPP	NG	17	D4P	CA	2.43
2	DPP	NG	17	D4P	O	2.25
2	DPP	HG1	17	D4P	O	3.1
cyclic peptide lower restraints						
3	DPP	NG	18	D4P	C	1.33
3	DPP	HG1	18	D4P	C	2
3	DPP	CB	18	D4P	C	2.43
3	DPP	NG	18	D4P	CA	2.43
3	DPP	NG	18	D4P	O	2.25

Pseudoatom definitions of the non-canonical amino acids:

QG is for the ortho pair ie H2/H6 for D4P and HC2/H6 for GHP.

QD is for the meta pair ie H3/H5 for D4P and H3/H5 for GHP.

Lipid pseudoatoms are QA, QB, QG, QD, QE along the chain as expected.

Table S4. Summary of the antimicrobial activity.

	MW (HCl salt)	MSSA (uM)	MSSA (ug/ mL)	MRSA (uM)	MRSA (ug/ mL)	VISA (uM)	VISA (ug/ mL)
Van	1485.71	1	1.5	1	1.5	8	11.9
RA2	2554.1	1	2.6	1	2.6	1	2.6
RAgly	2373.9	0.5	1.2	0.5	1.2	1	2.5
8	2339.9	2	4.7	2	4.7	4	9.4
8(Cl)	2455.9	2	4.7	2	4.7	4	9.5
11	2246.9	1	2.2	1	2.2	2	4.5
12	2230.9	4	8.9	2	4.5	4	8.9
13	2248.9	>16		>16		>16	
14	2245.9	>16		>16		>16	

Supplementary Figures

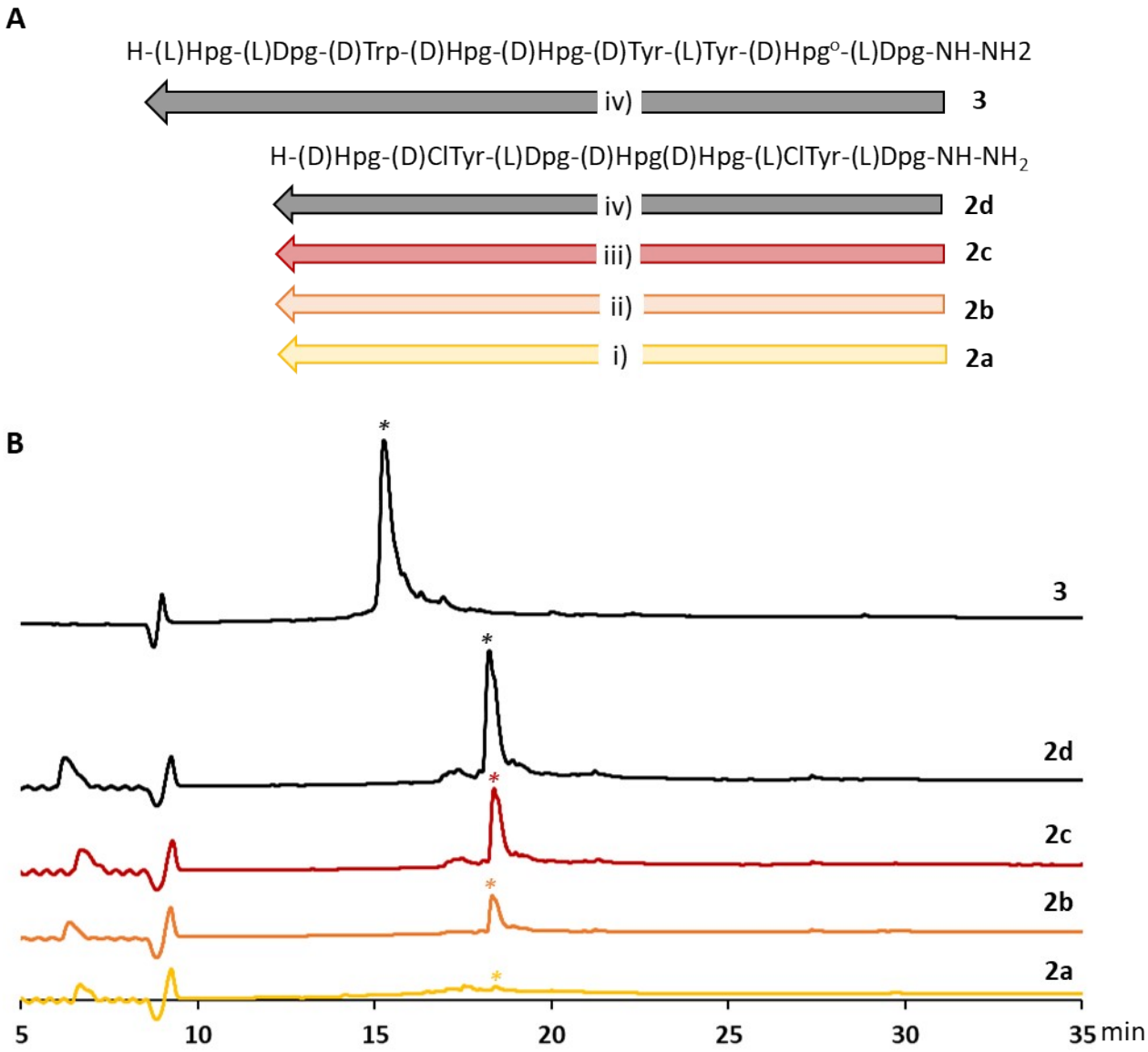


Figure S1. Optimization of the SPPS targeting linear GPA peptide precursors in link with Table 1. (**A**) SPPS optimization of the teicoplanin precursor (**2a-d**) and its application to the corbomycin precursor (**3**); (**B**) RP-HPLC chromatogram at 214nm of the crude peptides. The Fmoc removal is identical in every SPPS: DBU/ DMF (v/ v'; 1: 99), 3 x 30s, RT. The coupling condition was modified as following: i) Fmoc-AA-OH (3eq), COMU (3eq), 1,5-lutidine (3eq), DMF, 30min, RT (yellow color); ii) Fmoc-AA-OH (3eq), DIC (3eq), Oxyma (6eq), DMF, 30min, RT (orange color); iii) Fmoc-AA-OH (3eq), DIC (3eq), Oxyma (6eq), DMF, 30min, 50^0C – conventional heating (red color); iv) Fmoc-AA-OH (3eq), DIC (3eq), Oxyma (6eq), DMF, 30min, 50^0C (30w) – microwave heating (black color). °The original sequence has a D-Dpg at this position.

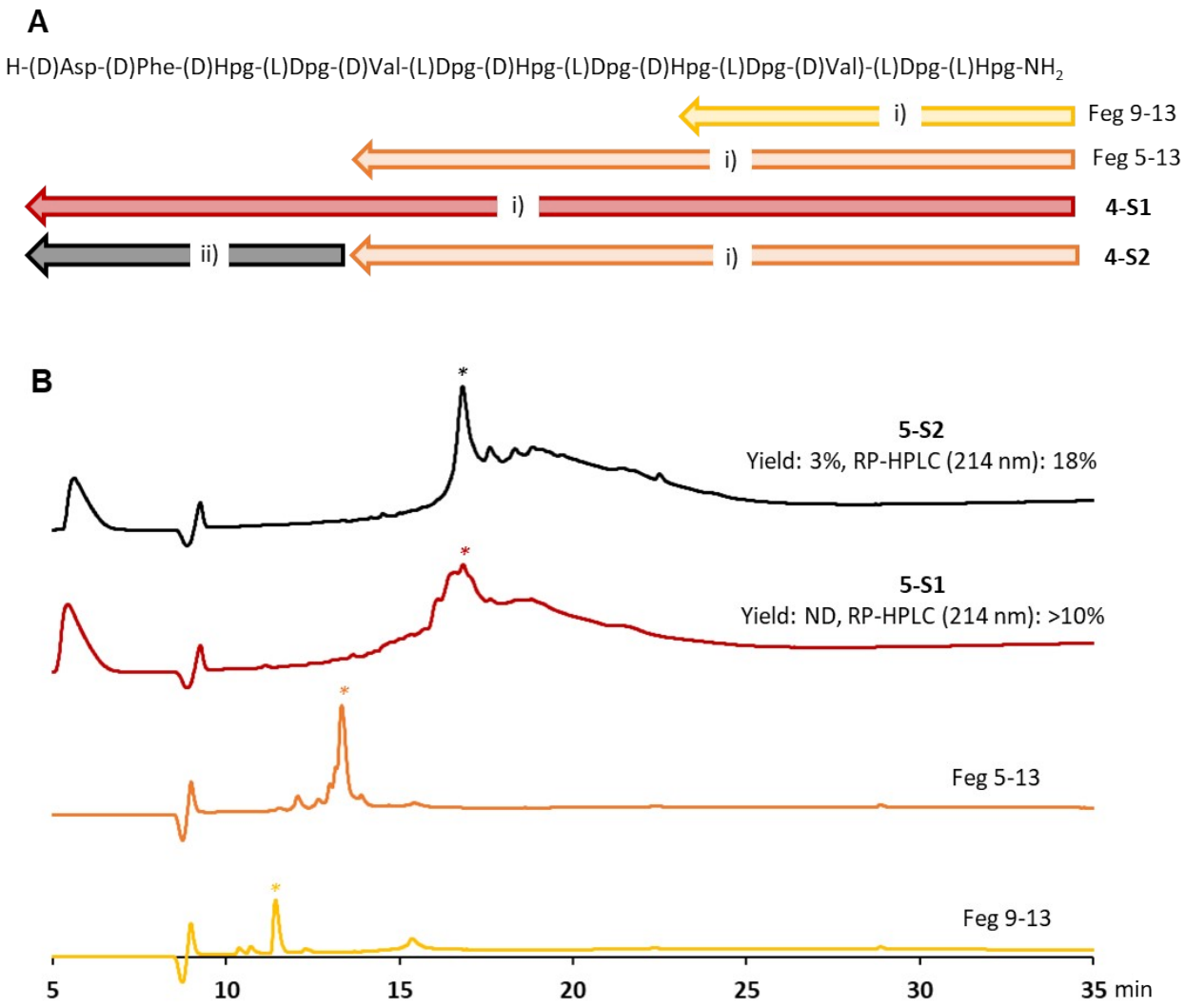


Figure S2. Optimization of the SPPS targeting linear retro-inverso feglymycin variant (**4**). (**A**) SPPS monitoring Feg 9-13 and 5-13) and optimization (**4-S1-2**); (**B**) RP-HPLC chromatogram at 214nm of the crude peptides. The Fmoc removal is identical in every SPPS: DBU/ DMF (v/ v'; 1: 99), 3 x 30s, RT. The coupling condition was modified as following: i) Single coupling, Fmoc-AA-OH (3eq), DIC (3eq), Oxyma (6eq), DMF, 30min, 50^0C – conventional heating; ii) Double coupling, Fmoc-AA-OH (3eq), DIC (3eq), Oxyma (6eq), DMF, 30min, 50^0C – conventional heating.

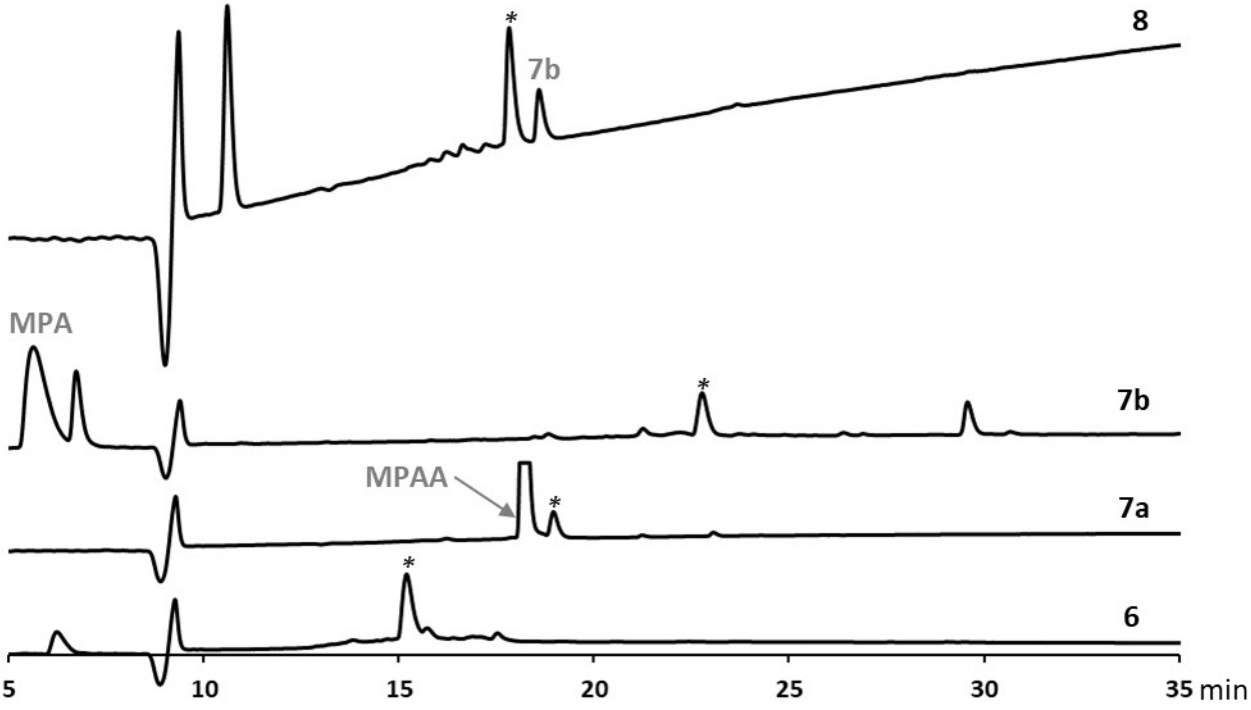
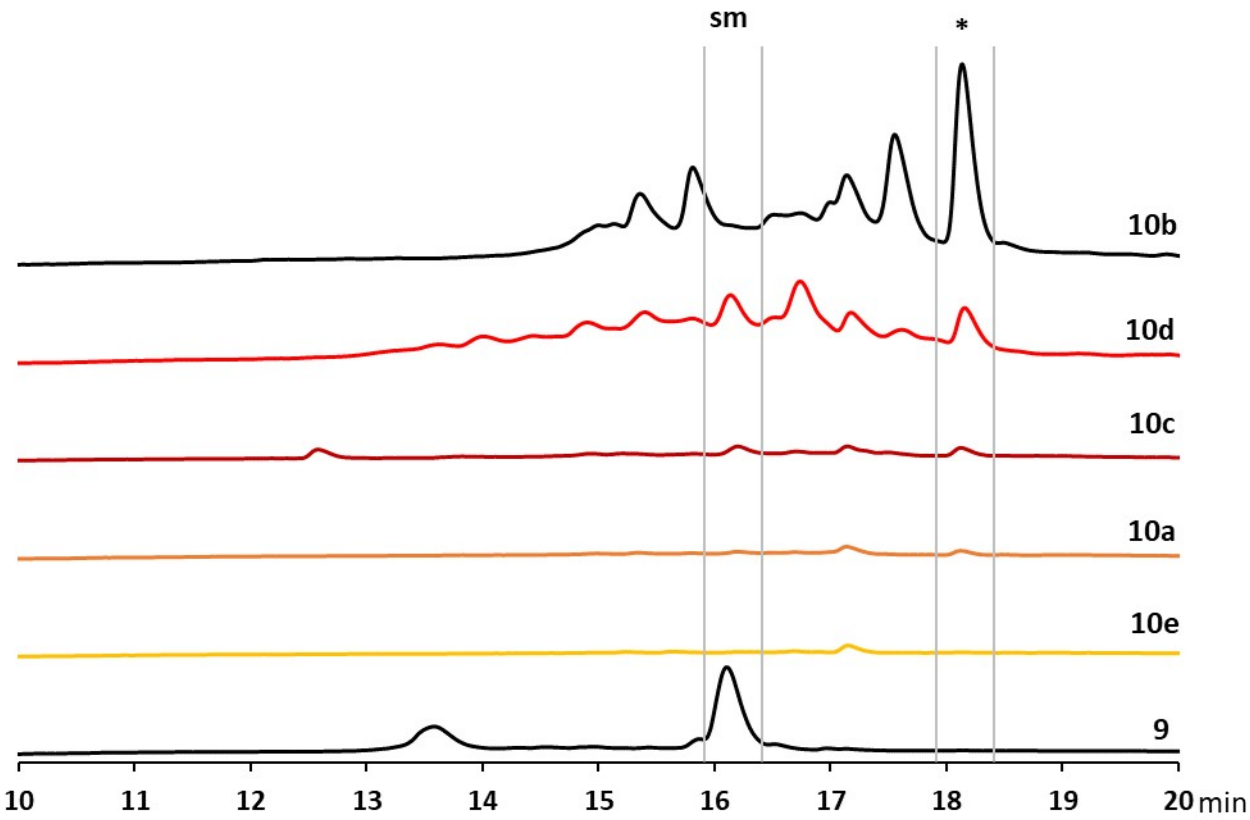


Figure S3. Ramoplanin aglycone by intramolecular native chemical ligation followed by desulfurization. RP-HPLC chromatogram at 214nm of the linear precursor **6**, the monitoring of the cyclization (**7a-b**) by NCL using MPAA and MPA and the desulfurization (4h) leading to **8**.



Supplementary Figure 4. Ramoplanin aglycone by head-to-tail macrocyclization. RP-HPLC chromatogram at 214nm of the linear precursor **9**, the monitoring of the cyclization after 15hrs.

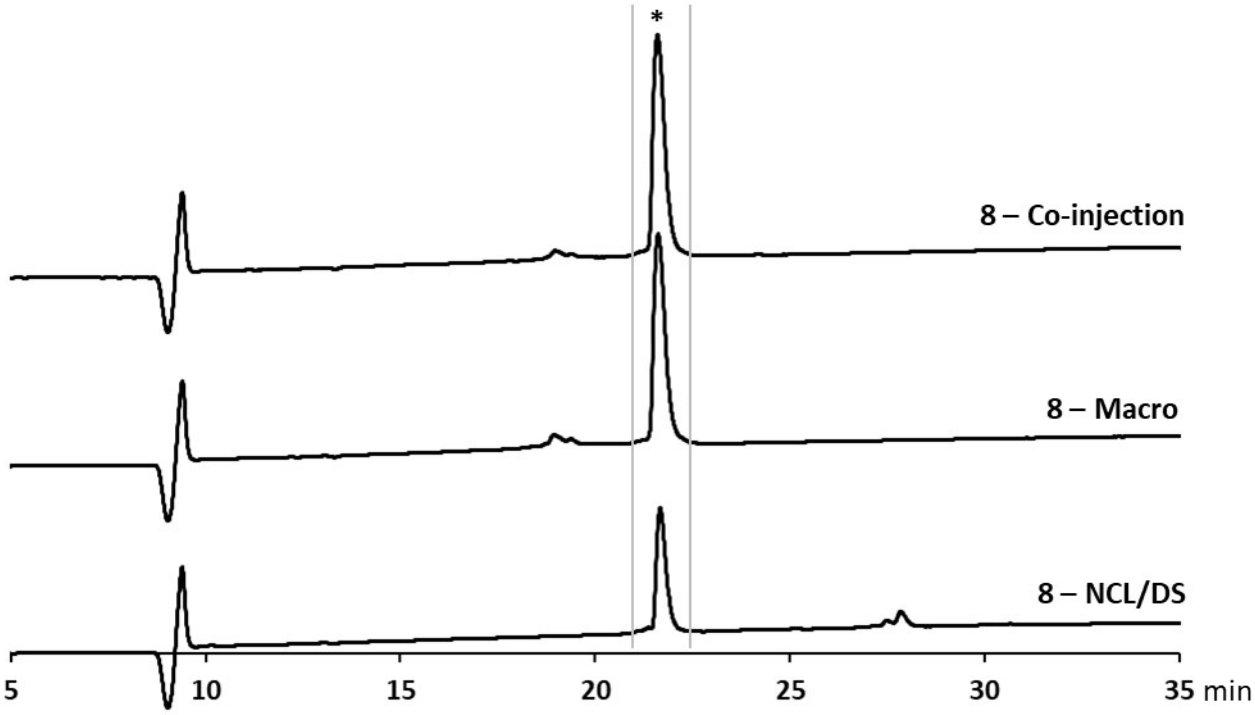
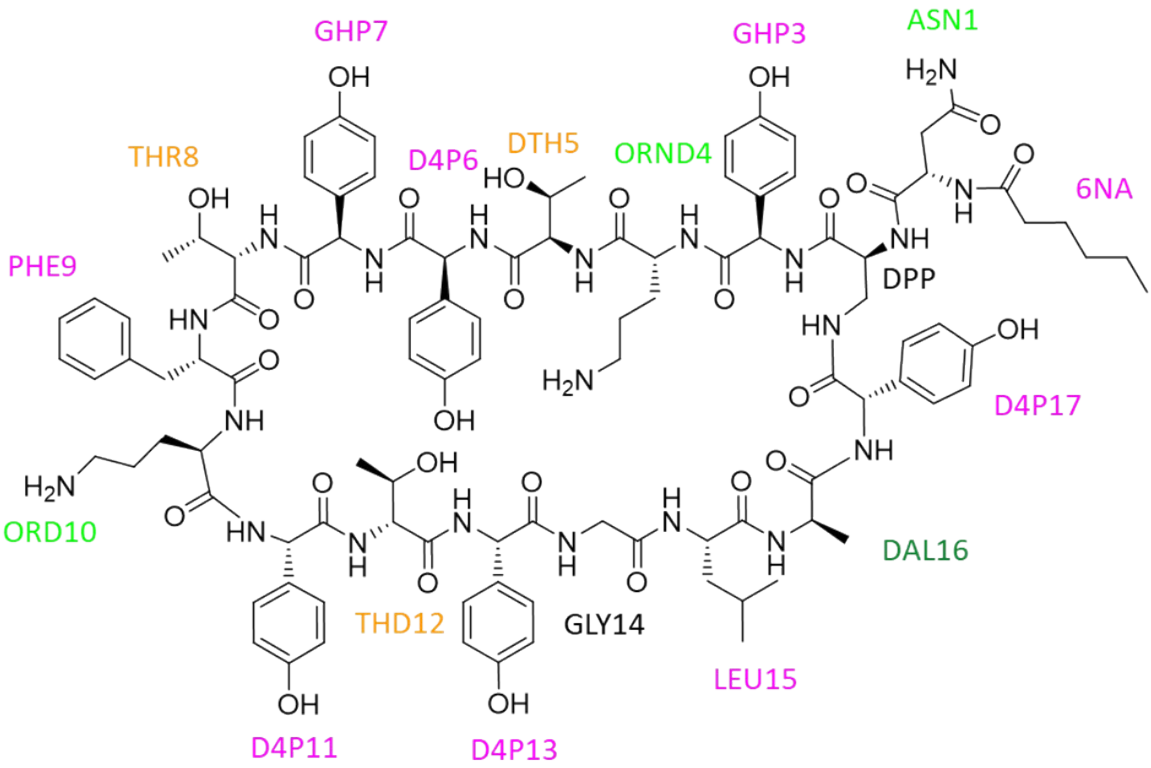


Figure S5. Co-injection of the compounds obtained by NCL/ desulfurization and head-to-tail cyclization. RP-HPLC chromatogram at 214nm showing that both products are identical.

H bonds (Å):
DPP (O) GLY14 (H): 2.00
DPP (O) GLY14 (N): 3.00
GHP7 (O) ORD10 (H): 2.00
GHP7 (O) ORD10 (N): 3.00
DTH5 (H) DTH12 (O): 2.00
DTH5 (N) DTH12 (O): 3.00

Trans amide linker upper limit restraints:
Scaled x5 in CYANA
DPP (NG) D4P17 (C): 1.33
DPP (HG1) D4P17 (C): 2.00
DPP (CB) D4P17 (C): 2.43
DPP (NG) D4P17 (CA): 2.43
DPP (NG) D4P17 (O): 2.25
DPP (HG1) D4P17 (O): 3.1



Amide temperature coefficients (-ppb/k) bold values:

ASN1: 8.12
DPP: **0.92, 2.832**
GHP3: 10.40
ORD4: 8.33
DTH5: **2.68**
D4P6: 9.94
GHP7: 9.39
THR8: 4.68
PHE9: 5.16

ORD10: **1.80**
D4P11: 8.41
DTH12: 9.73
D4P13: 9.13
GLY14: **0.06**
LEU15: 10.82
DAL16: 11.16
D4P17: 1.3

Figure S6. Amino acid naming used in NMR data analysis and additional data collected other than NOEs supplemented in Table S3.

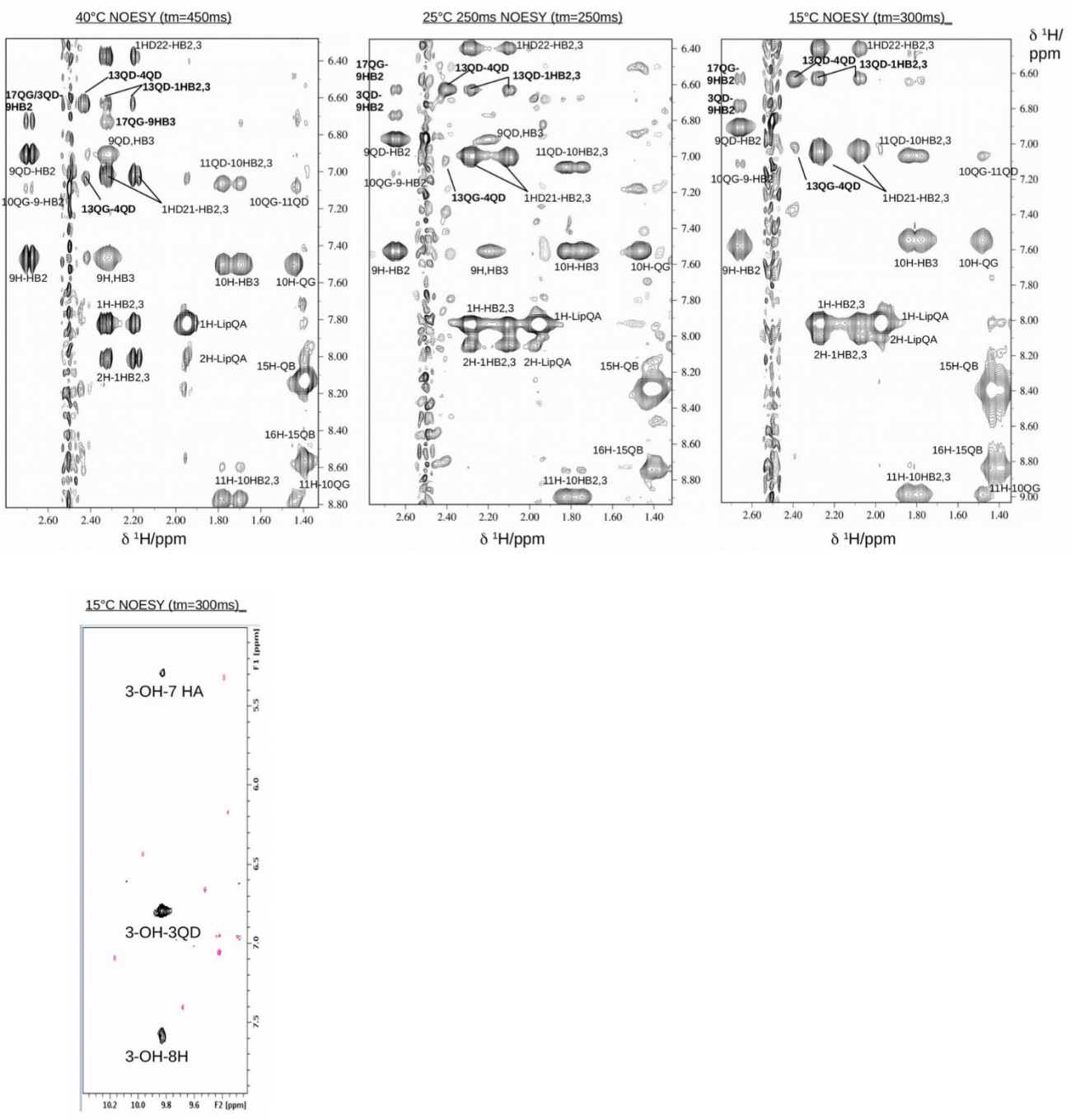


Figure S7. Region of 2D NOESY spectra recorded at different temperatures. It is showing the quality of the data and the identification of some long-range NOEs (in bold) between the sidechains of residues D-Hpg3, Hpg17 and Phe9 in the hydrophobic core and between the sidechains of Hpg13 and D-Orn4 and also between Hpg13 and Asn1 across the sheet. NOEs were observed between the broadened OH of D-Hpg3 to the backbone of HN of Thr8 and to the HA of D-Hpg7 are shown in the bottom spectrum.

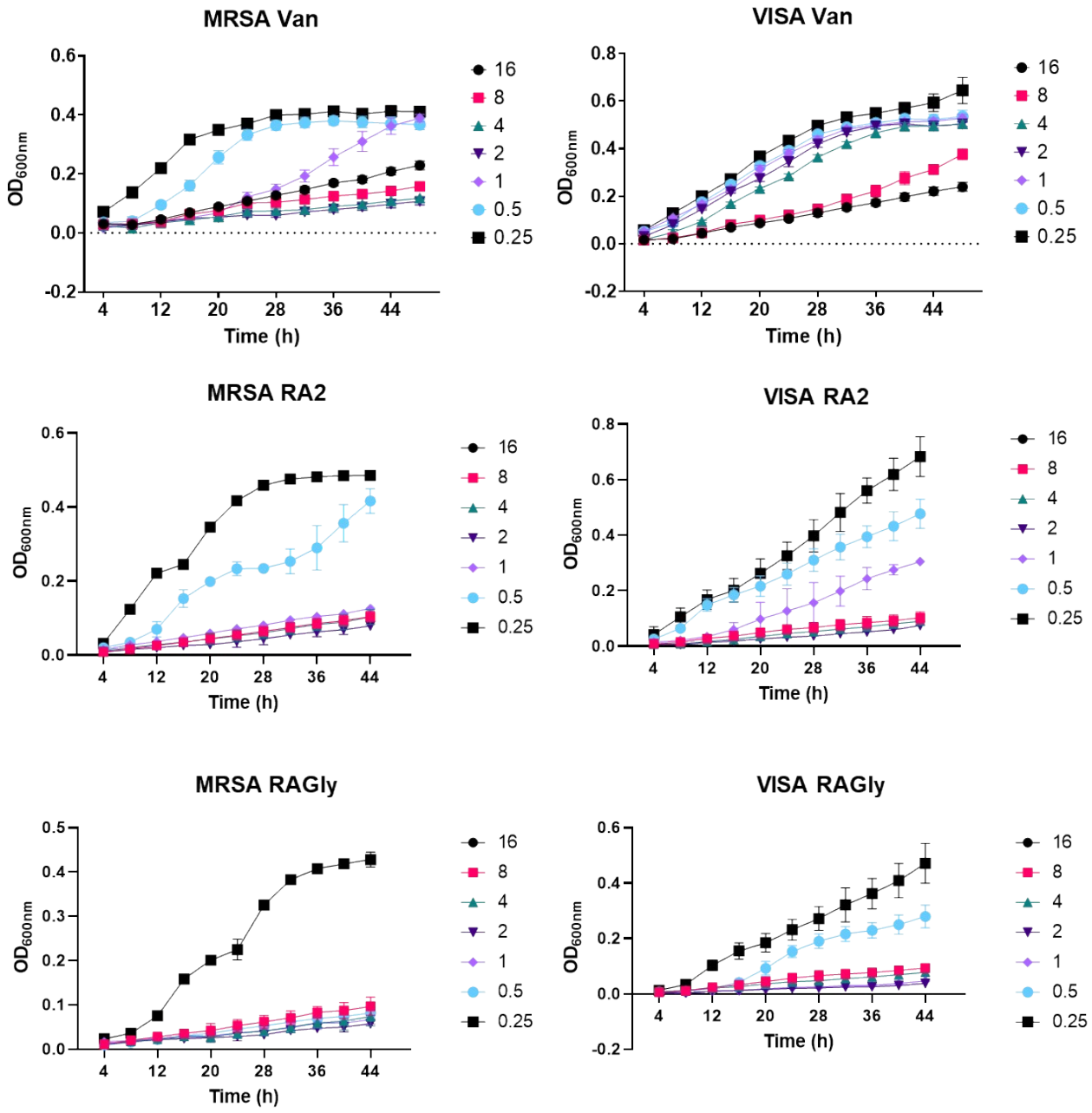


Figure S8. The growth of the clinical isolates MRSA and VISA at 6 concentrations of the 3 controls **Van**, **RA2** and **RAGly** determined by OD $_{600nm}$ monitoring.

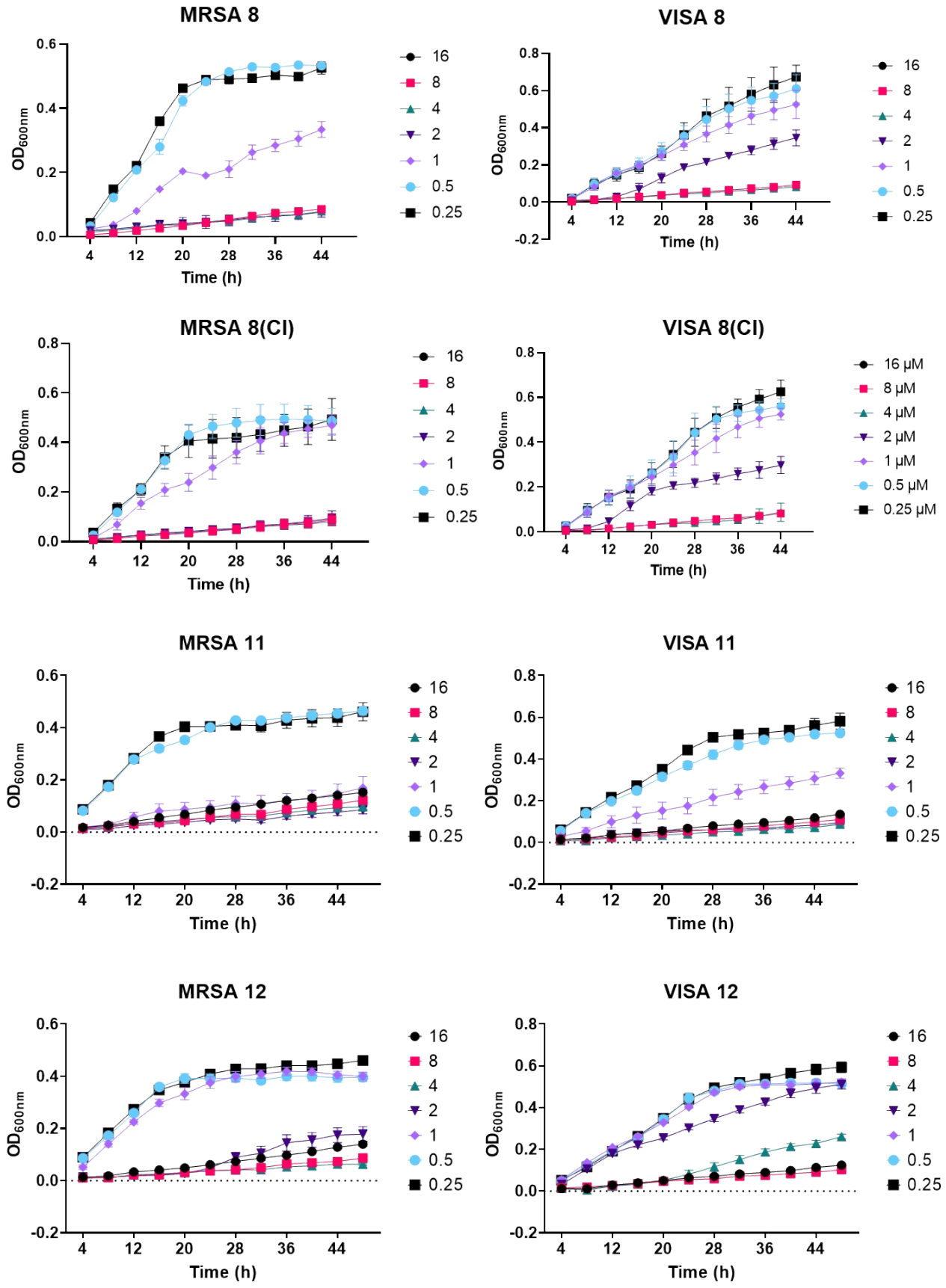


Figure S9. The growth of the clinical isolates MRSA and VISA at 6 concentrations of the 4 bioactive synthetic ramoplanin **8**, **8(Cl)**, **11** and **12** determined by OD_{600nm} monitoring.

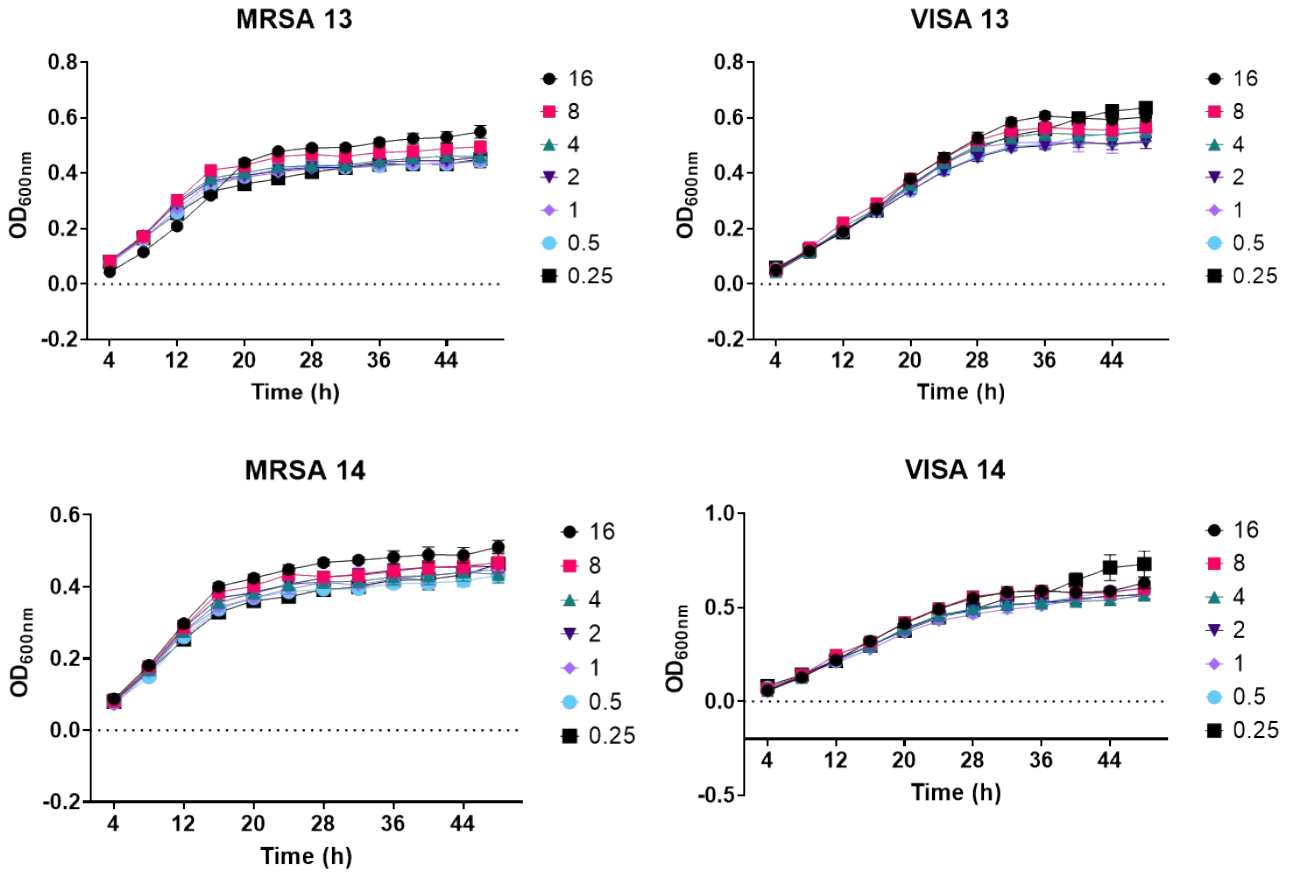
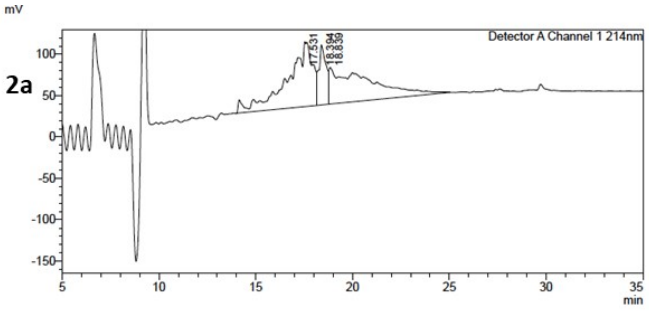
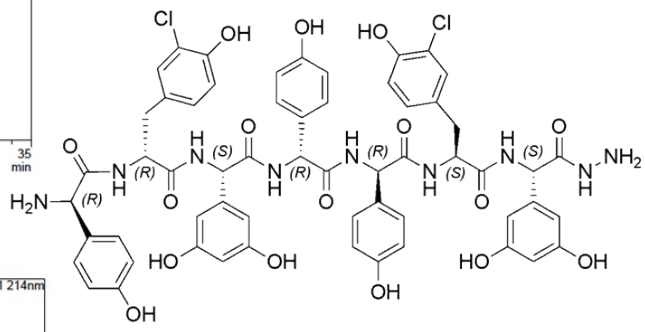


Figure S10. The growth of the clinical isolates MRSA and VISA at 6 concentrations of the 2 inactive synthetic ramoplanin **13** and **14** determined by OD_{600nm} monitoring.

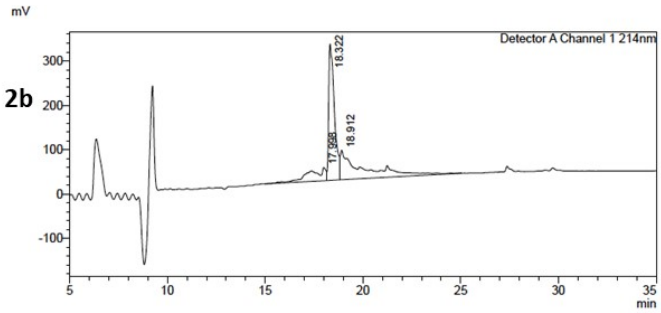
Compound characterizations



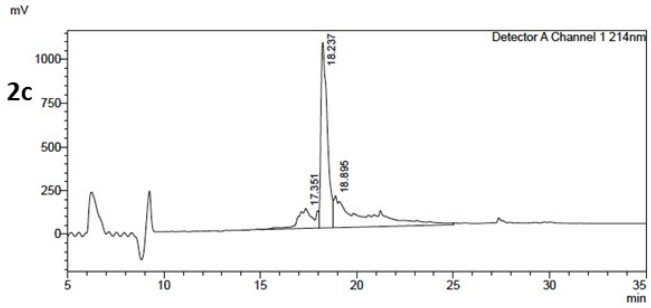
2a



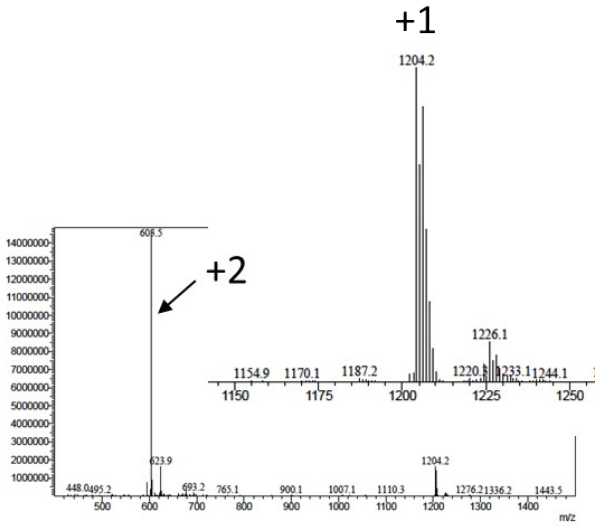
Chemical Formula: $C_{58}H_{55}Cl_2N_9O_{16}$
Exact Mass: 1203.31
Molecular Weight: 1205.03

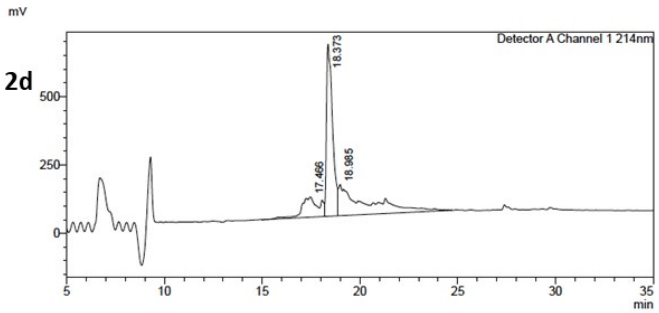


2b



2c

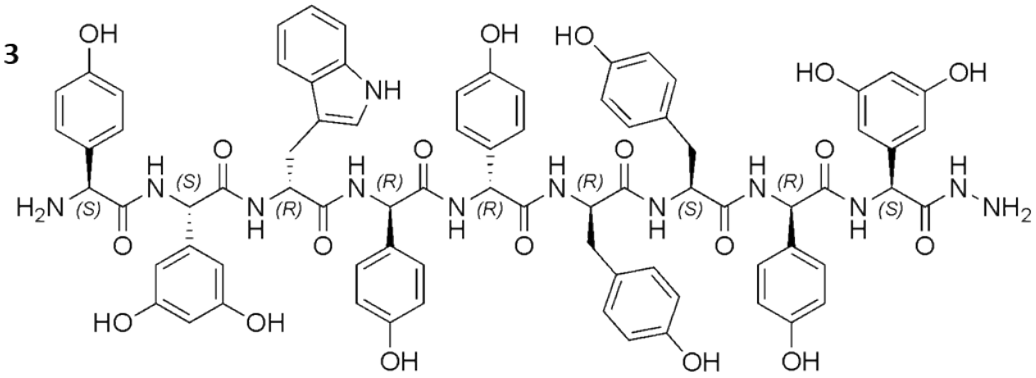




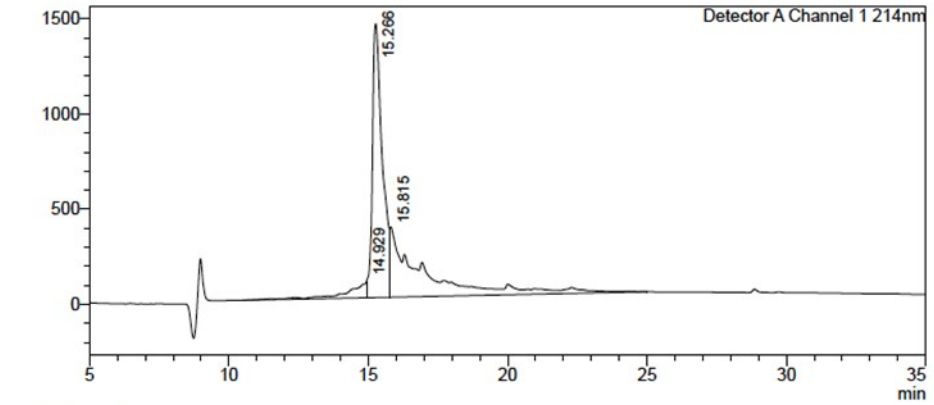
2d

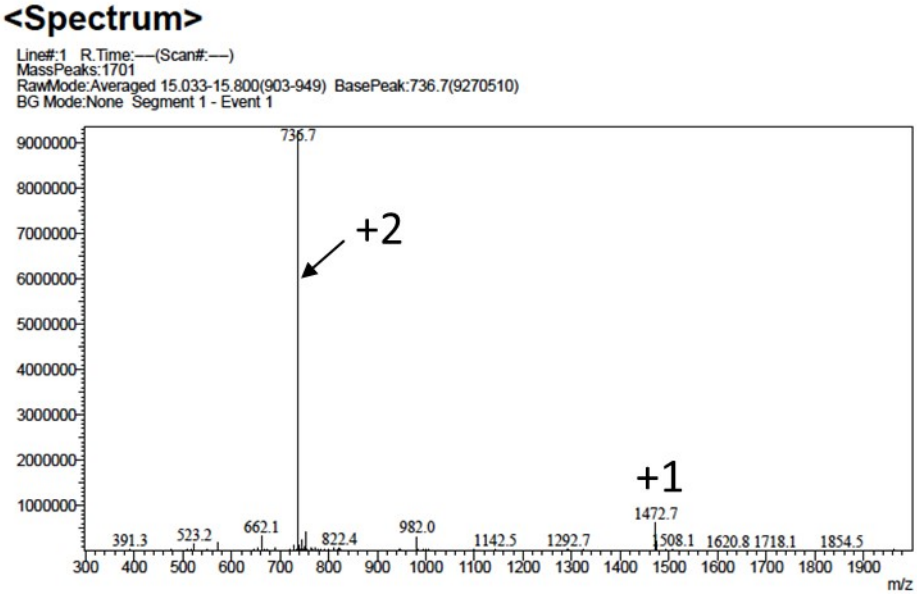
Compound characterizations

3



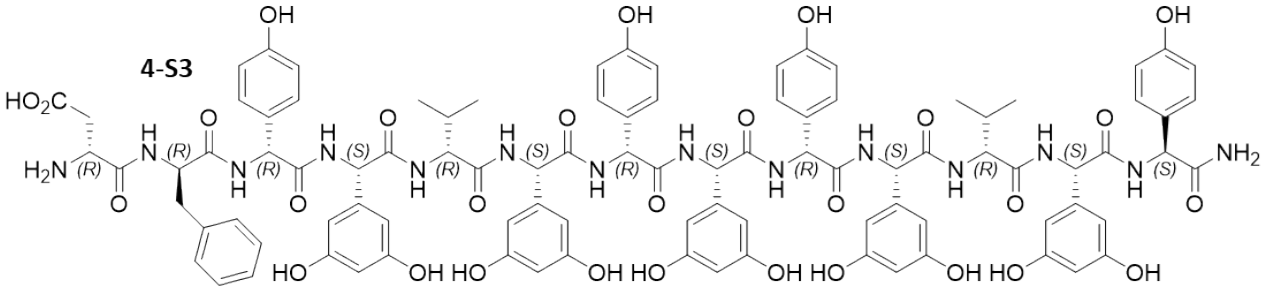
Chemical Formula: $C_{77}H_{74}N_{12}O_{19}$
Exact Mass: 1470.52
Molecular Weight: 1471.50





Compound characterizations

4-S3



Chemical Formula: $C_{95}H_{98}N_{14}O_{29}$
Exact Mass: 1898.6624
Molecular Weight: 1899.8980

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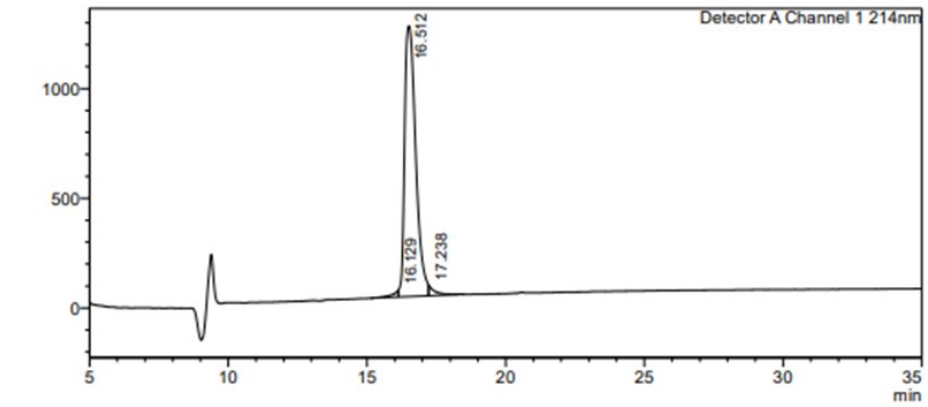
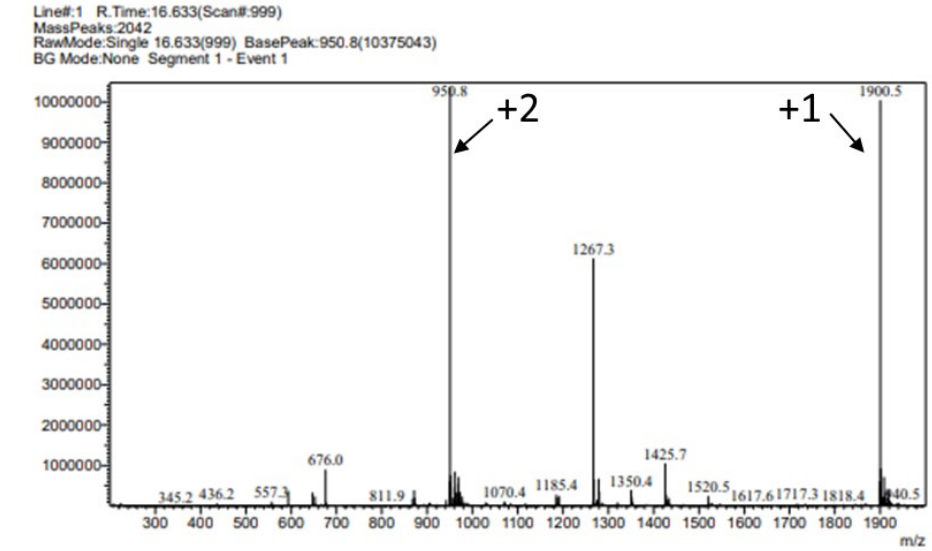
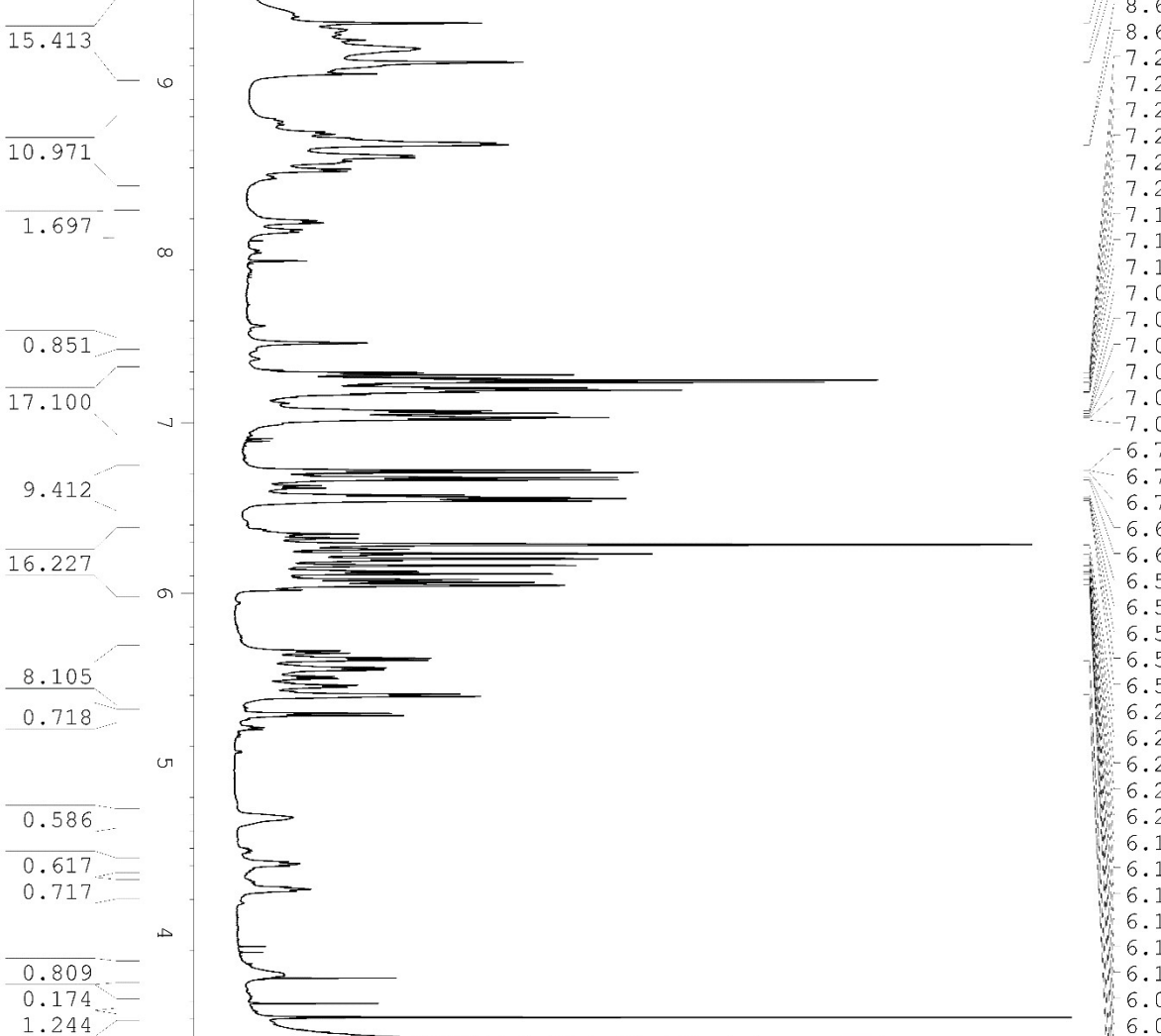


<Spectrum>

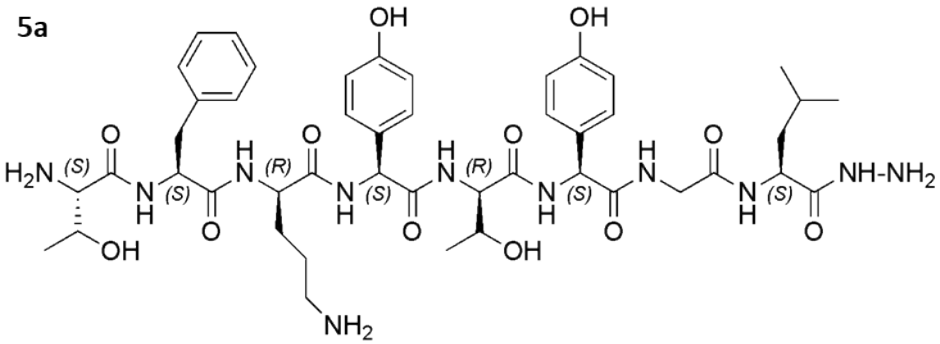




Proton NMR of Feglymycin (**4-S3**) retro inverso, recorded in d6-DMSO (calibrated to DMSO residual).

Compound characterizations

5a



Chemical Formula: $C_{46}H_{65}N_{11}O_{12}$
Exact Mass: 963.48
Molecular Weight: 964.09

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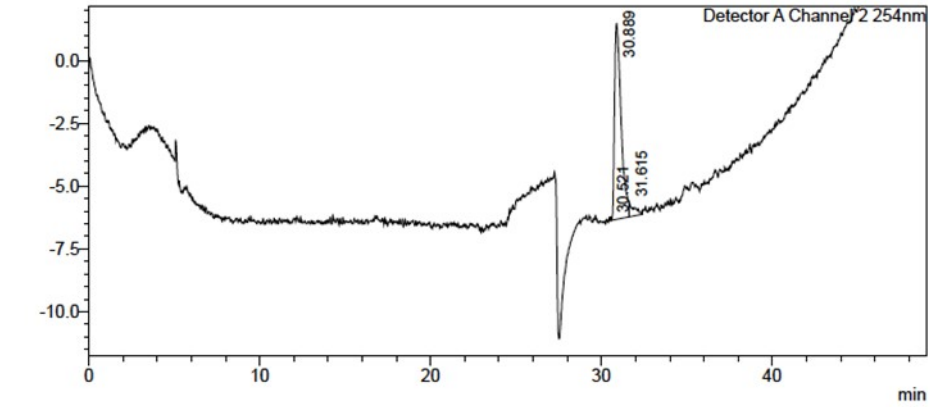

Peak Table

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	30.521	541	129	0.247		M		0.247
2	30.889	206370	7791	94.029		VM		94.029
3	31.615	12564	603	5.725		VM		5.725
Total		219475	8523					100.000

<Spectrum>

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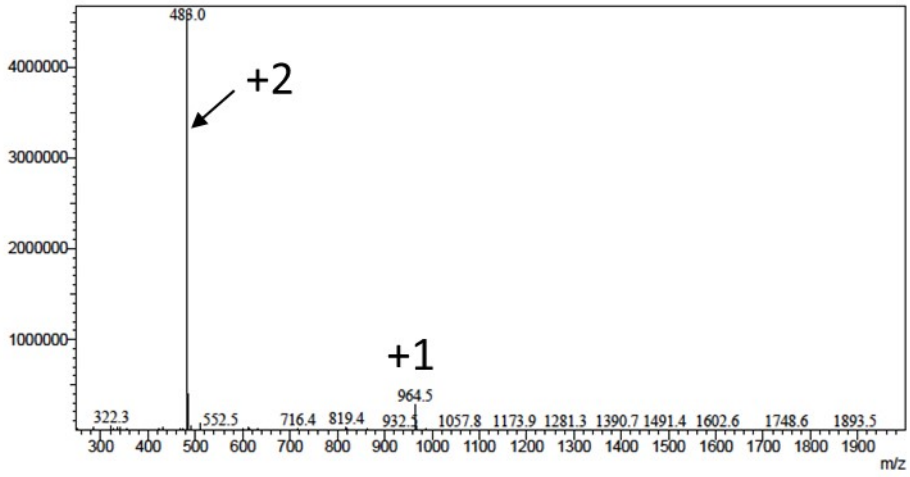
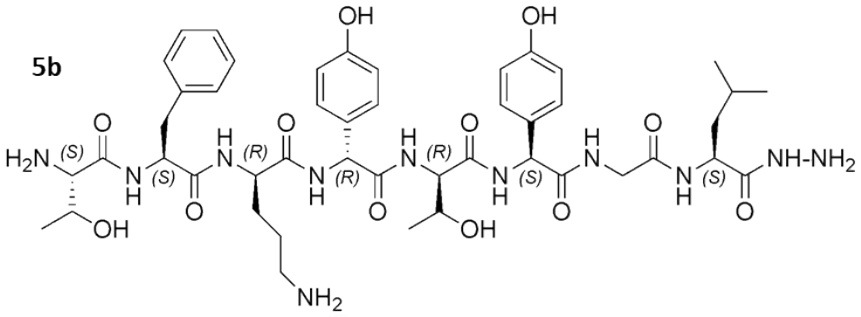
RawMode:Averaged 30.467-31.600(1829-1897) BasePeak:483.0(4632200)
BG Mode:None Segment 1 - Event 1



Compound characterizations

5b



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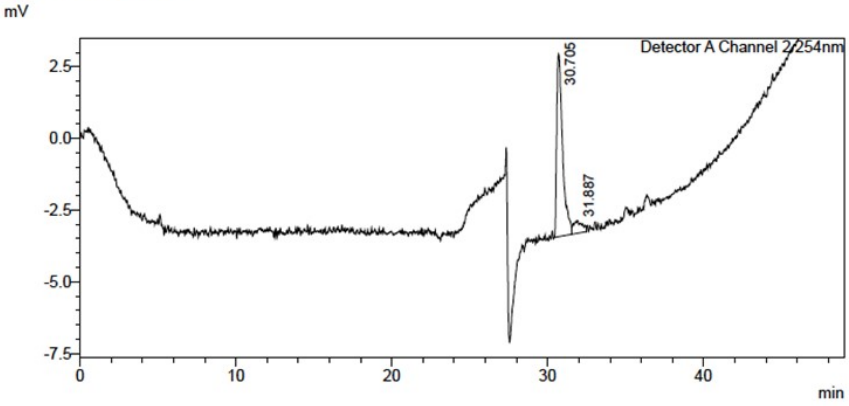
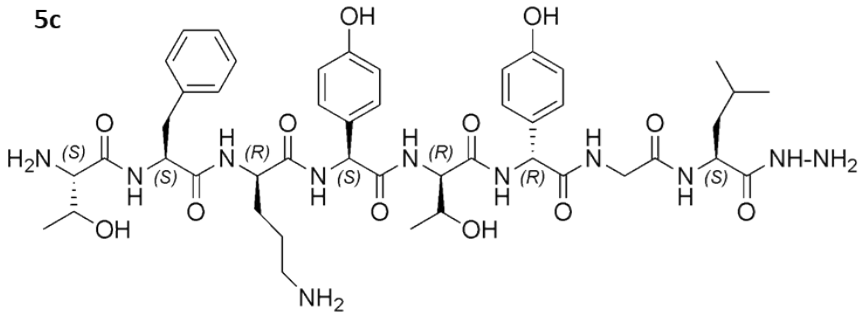


Peak Table

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	30.705	166521	6365	90.481		M		90.481
2	31.887	17518	447	9.519		VM		9.519
Total		184039	6812					100.000

5c



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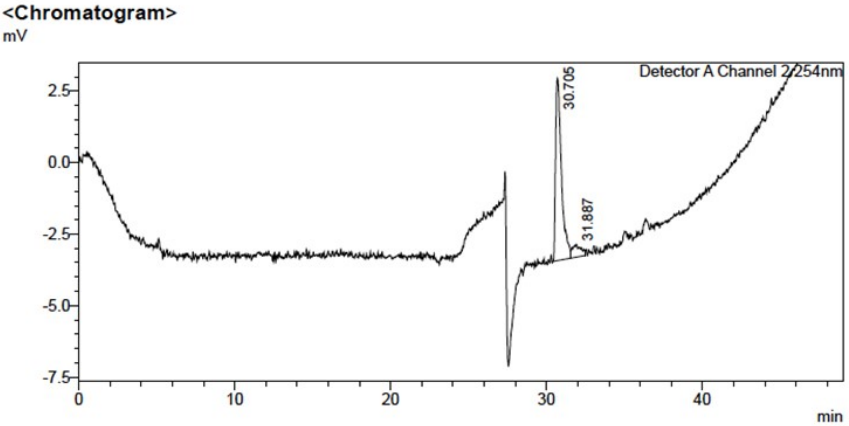


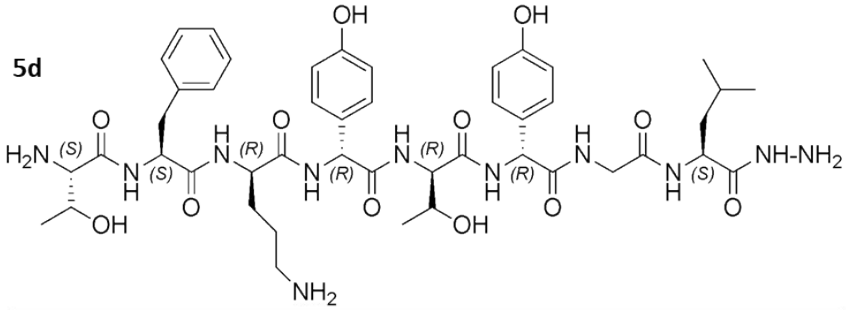
Peak Table

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	30.705	166521	6365	90.481		M		90.481
2	31.887	17518	447	9.519		VM		9.519
Total		184039	6812					100.000

Compound characterizations

5d



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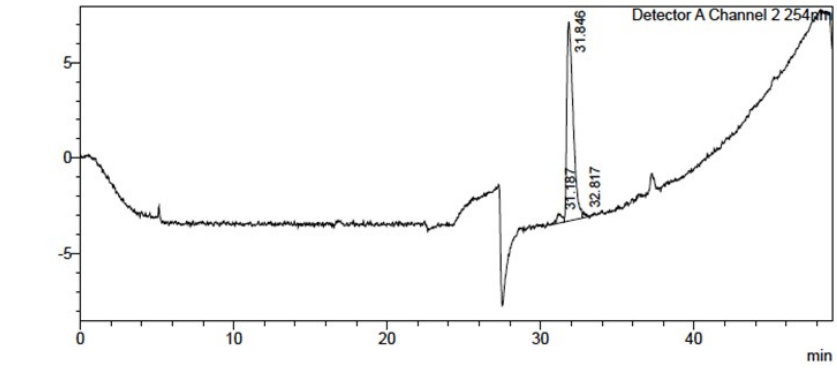
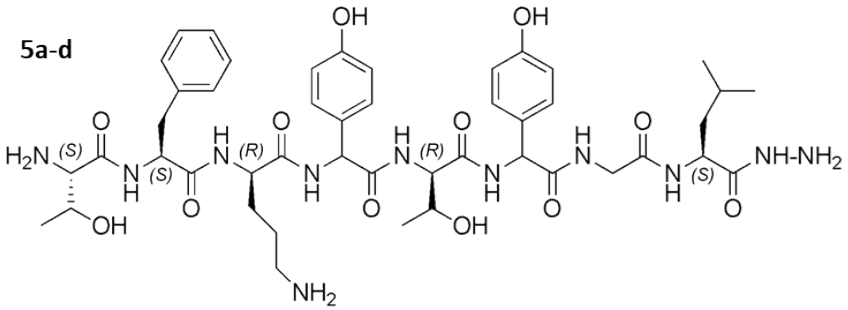


Peak Table

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	31.187	12603	471	4.052		M		4.052
2	31.846	295159	10408	94.906		VM		94.906
3	32.817	3241	302	1.042		VM		1.042
Total		311003	11182					100.000

5a-d



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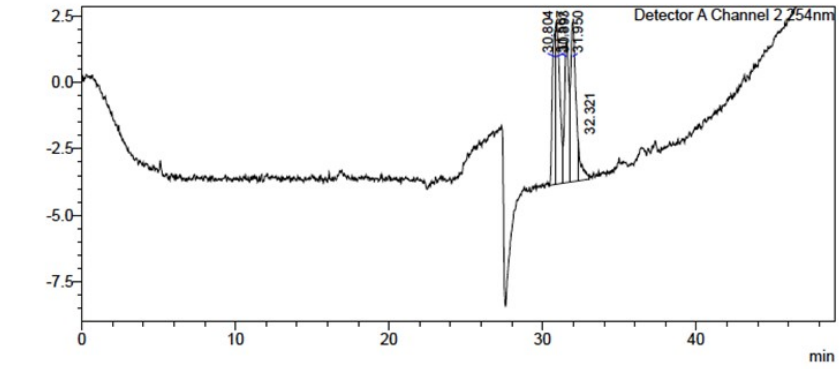


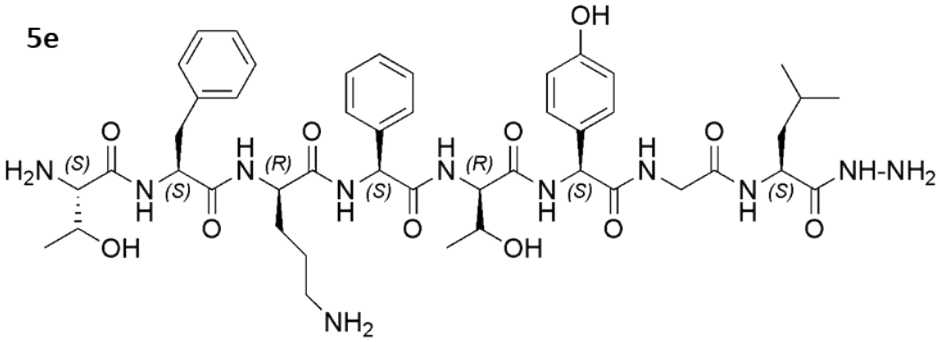
Peak Table

Detector A Channel 2 254nm

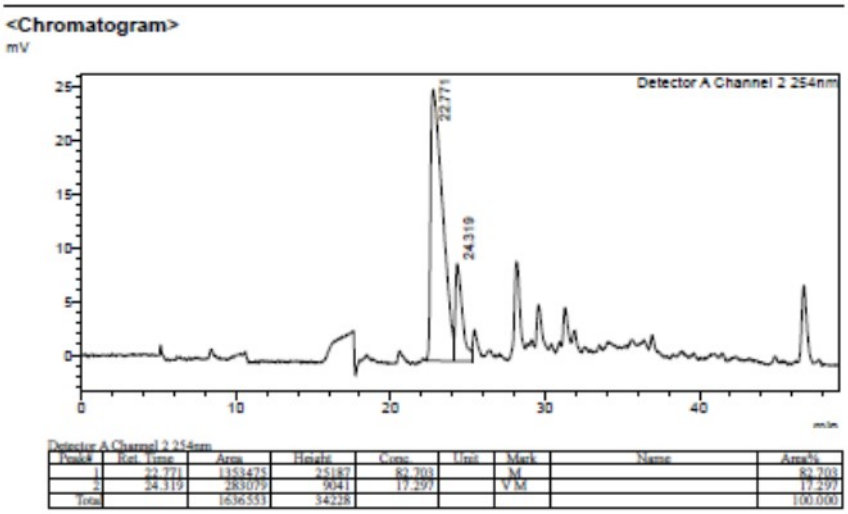
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	30.804	69667	5718	15.358		M		15.358
2	30.893	126847	6086	27.964		VM		27.964
3	31.567	100958	5340	22.256		VM		22.256
4	31.950	136524	5960	30.097		VM		30.097
5	32.321	19617	1515	4.325		VM		4.325
Total		453614	24619					100.000

Compound characterizations

5e

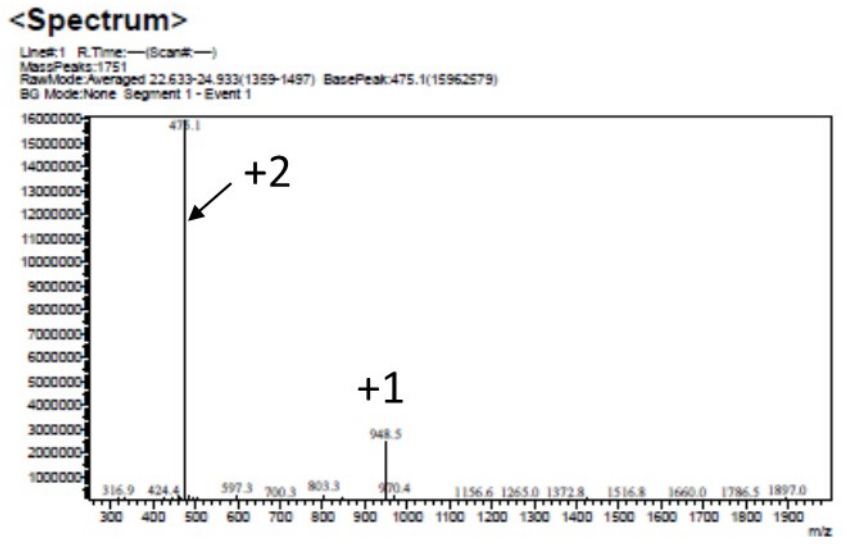


Chemical Formula: $C_{46}H_{65}N_{11}O_{11}$
Exact Mass: 947.4865
Molecular Weight: 948.0920



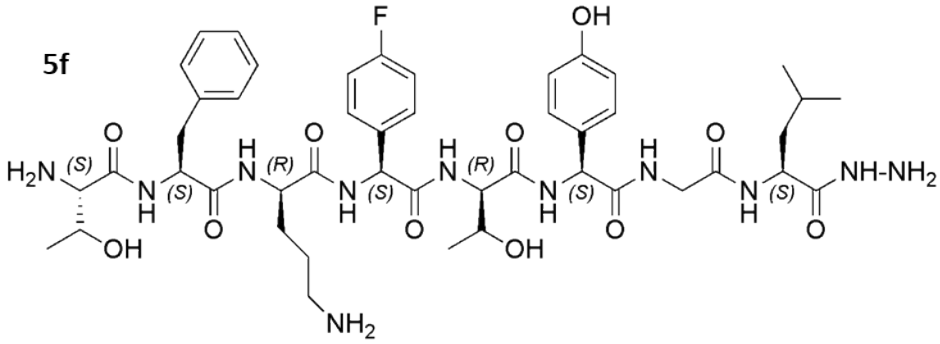
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	22.771	1353475	25187	82.703		M		82.703
2	24.319	283079	9041	17.297		VM		17.297
Total		1636553	34228					100.000



Compound characterizations

5f



Chemical Formula: $C_{46}H_{64}FN_{11}O_{11}$
Exact Mass: 965.4771
Molecular Weight: 966.0824

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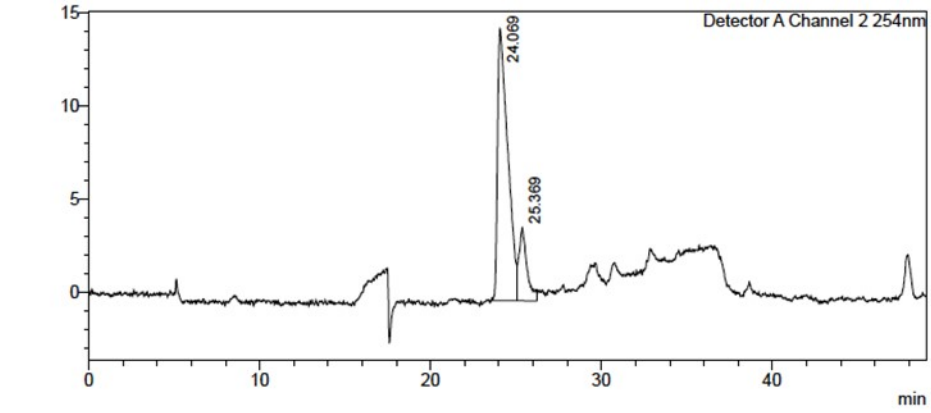
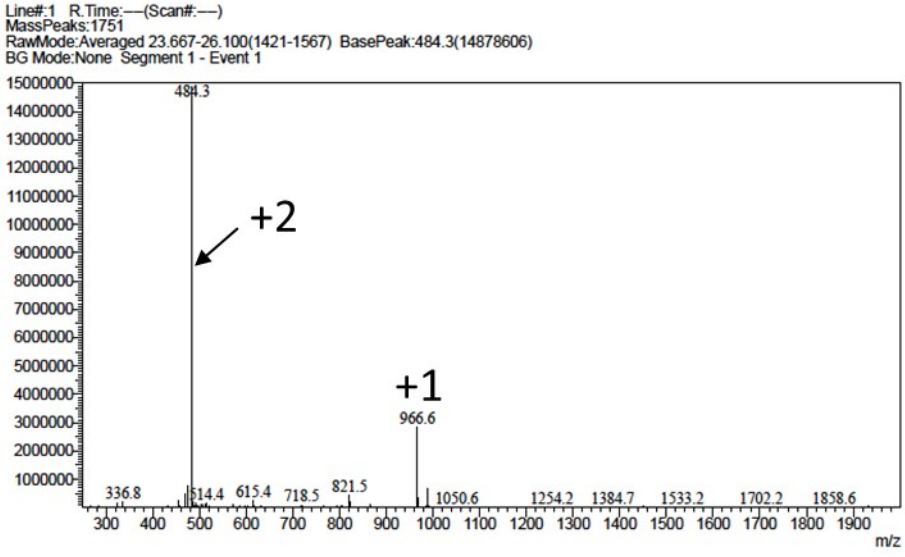


Peak Table

Detector A Channel 2 254nm

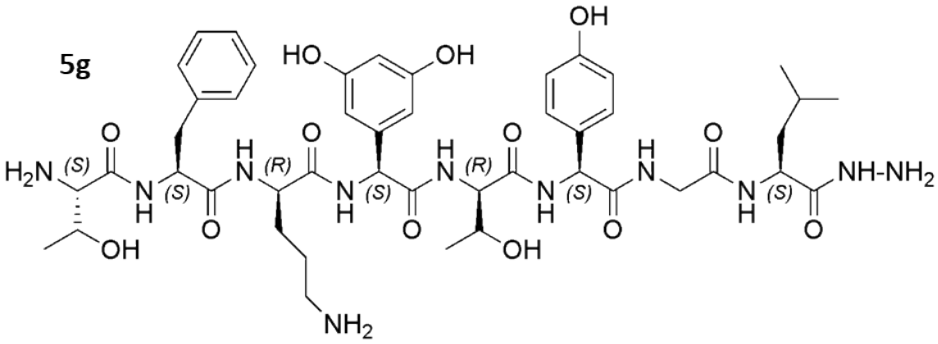
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	24.069	604707	14565	82.565		M		82.565
2	25.369	127691	3910	17.435		VM		17.435
Total		732398	18476					100.000

<Spectrum>



Compound characterizations

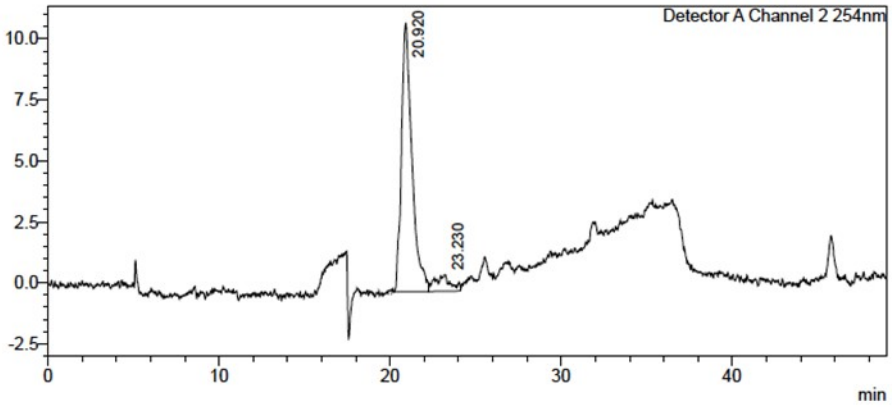
5g



Chemical Formula: $C_{46}H_{65}N_{11}O_{13}$
Exact Mass: 979.4763
Molecular Weight: 980.0900

<Chromatogram>

mV



Peak Table

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	20.920	469301	10991	91.770		M		91.770
2	23.230	42087	677	8.230		VM		8.230
Total		511388	11668					100.000

<Spectrum>

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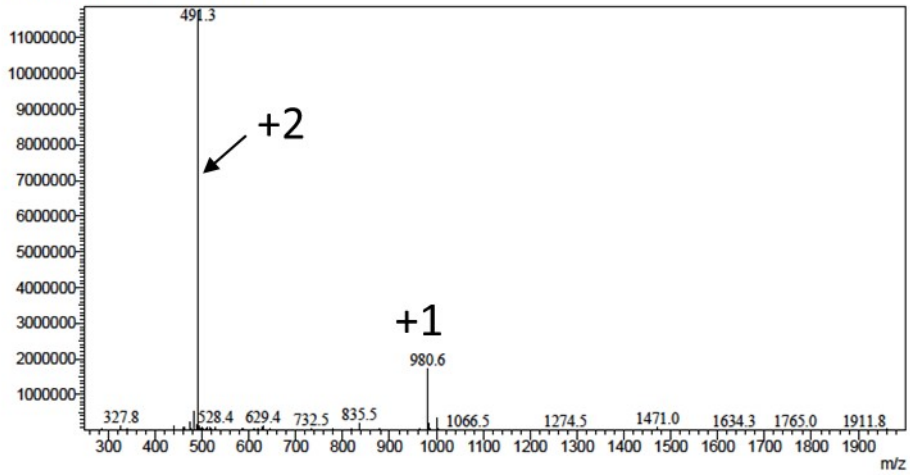
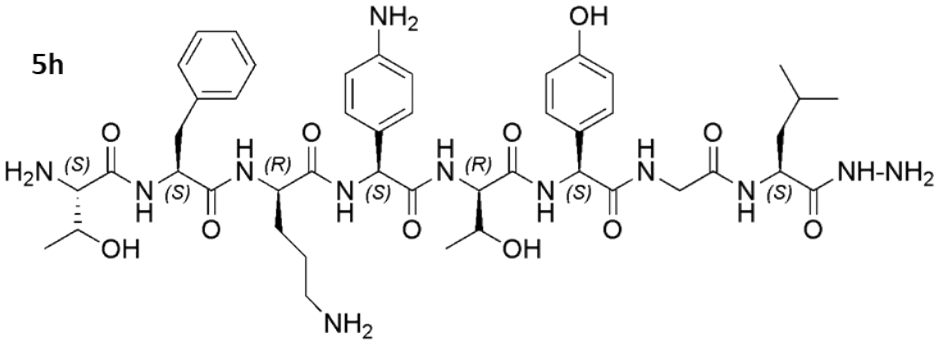
BG Mode:None Segment 1 - Event 1



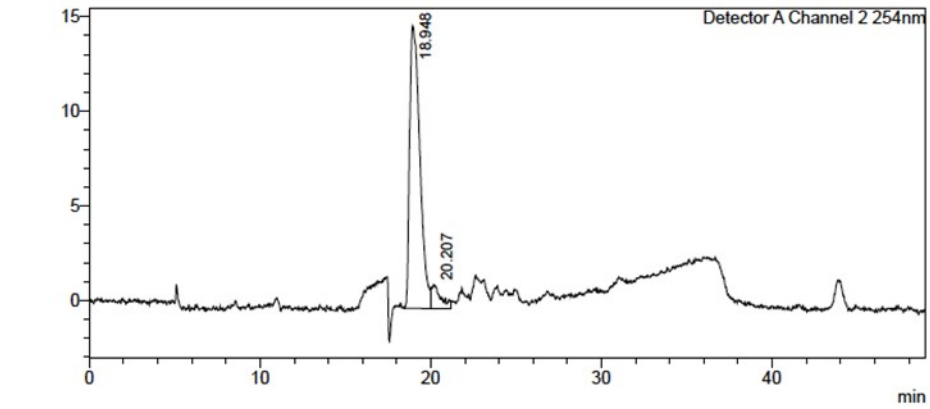
Compound characterizations

5h



Chemical Formula: $C_{46}H_{66}N_{12}O_{11}$
Exact Mass: 962.4974
Molecular Weight: 963.1070

<Chromatogram>



Peak Table

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	18.948	616010	14939	92.486		M		92.486
2	20.207	50047	1259	7.514		V M		7.514
Total		666057	16198					100.000

<Spectrum>

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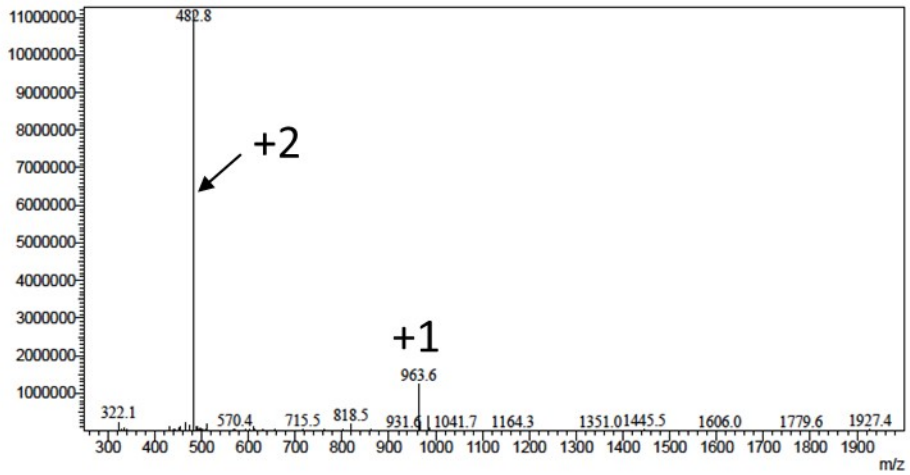
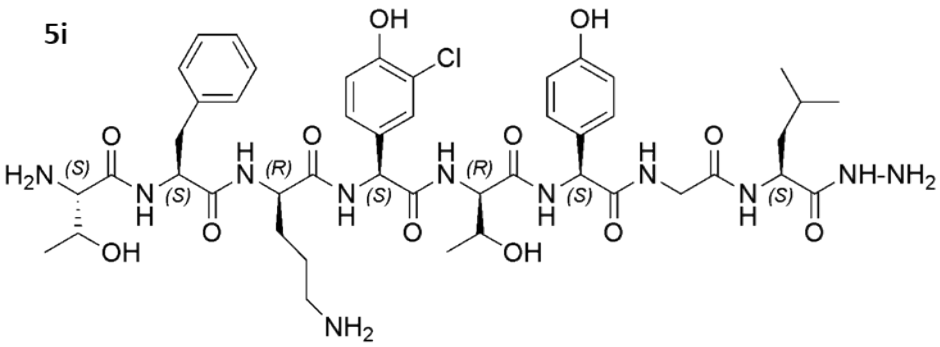
BG Mode:None Segment 1 - Event 1

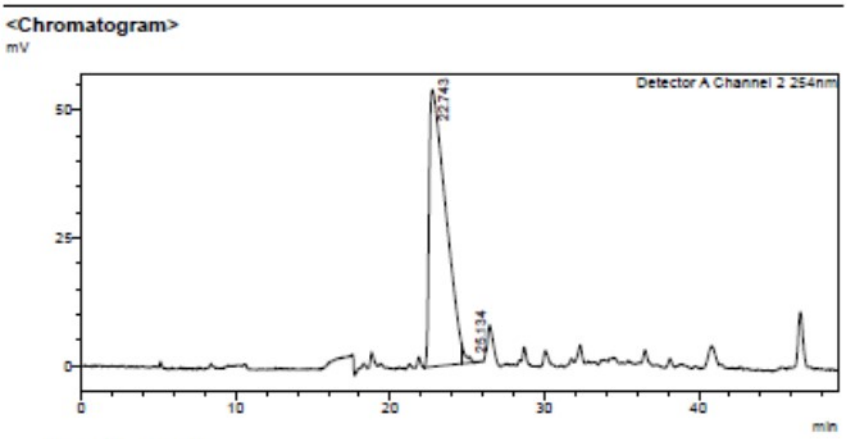


Compound characterizations

5i

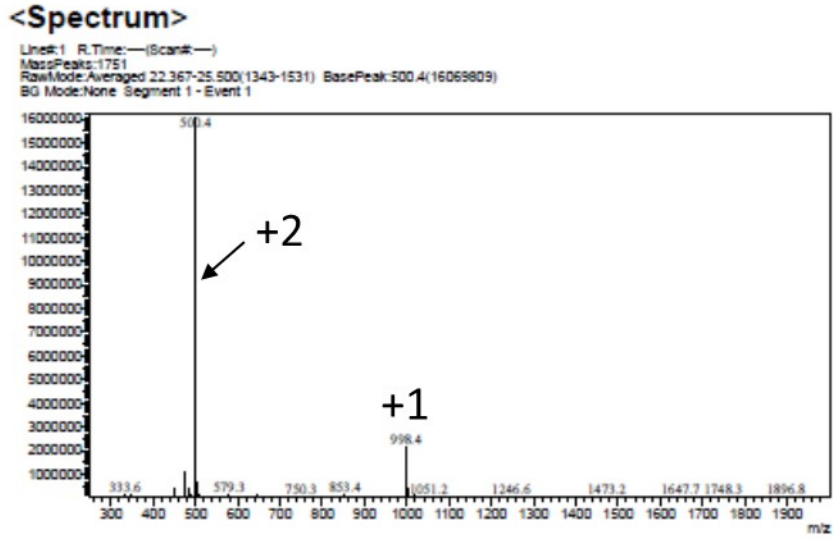


Chemical Formula: $C_{46}H_{64}ClN_{11}O_{12}$
Exact Mass: 997.44
Molecular Weight: 998.53



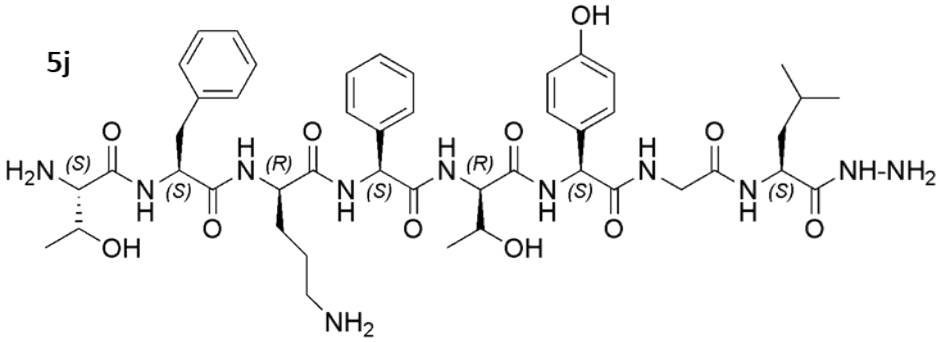
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	22.743	4019779	54054	98.456		M		98.456
2	25.134	63034	1207	1.544		VM		1.544
Total		4082814	55261					100.000

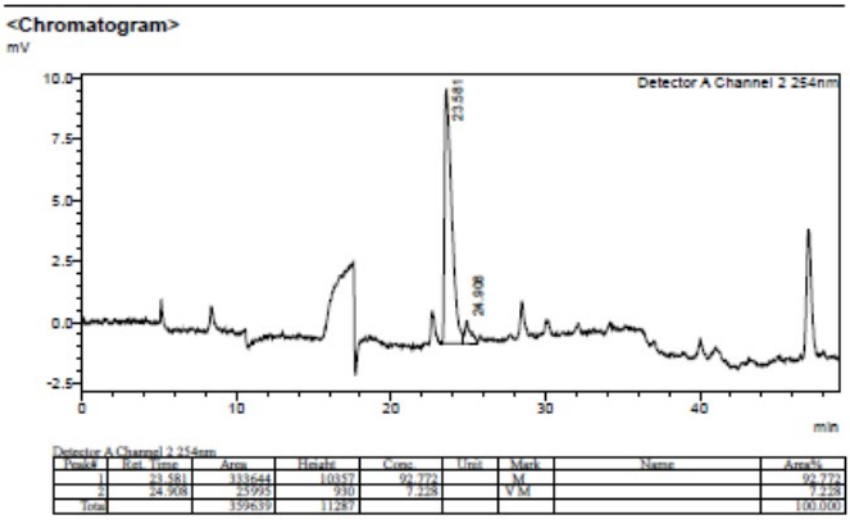


Compound characterizations

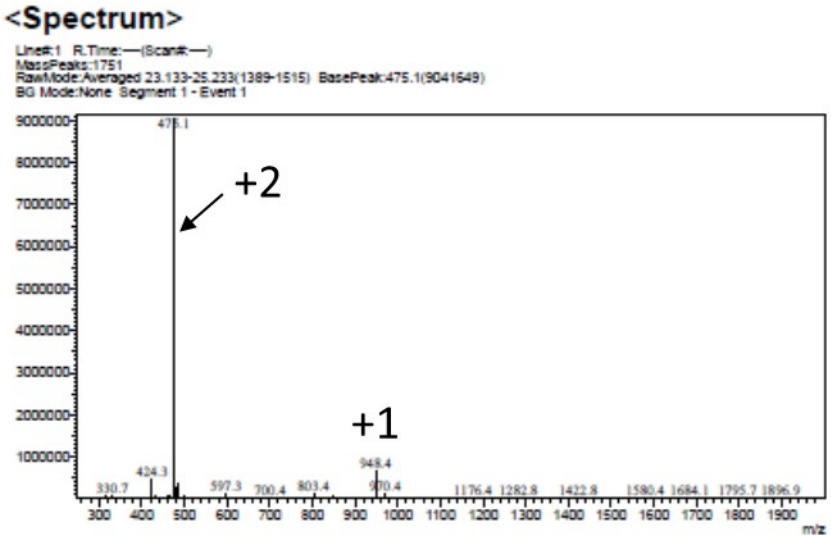
5j



Chemical Formula: $C_{46}H_{65}N_{11}O_{11}$
Exact Mass: 947.4865
Molecular Weight: 948.0920

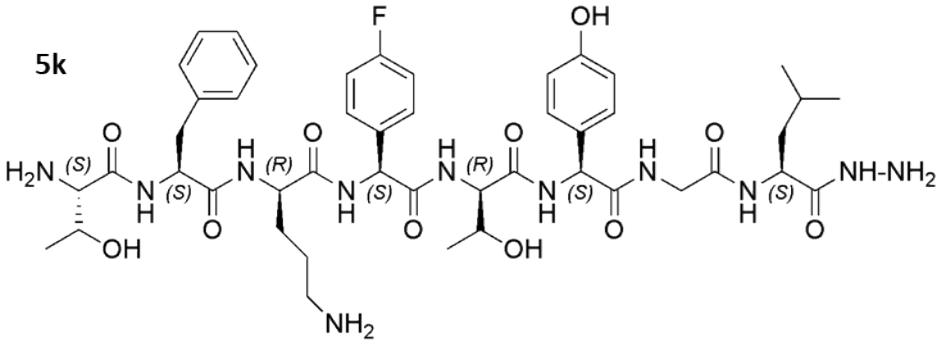


Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	23.581	333644	10357	92.772		M		92.772
2	24.908	25995	930	7.228		VM		7.228
Total		359639	11287					100.000



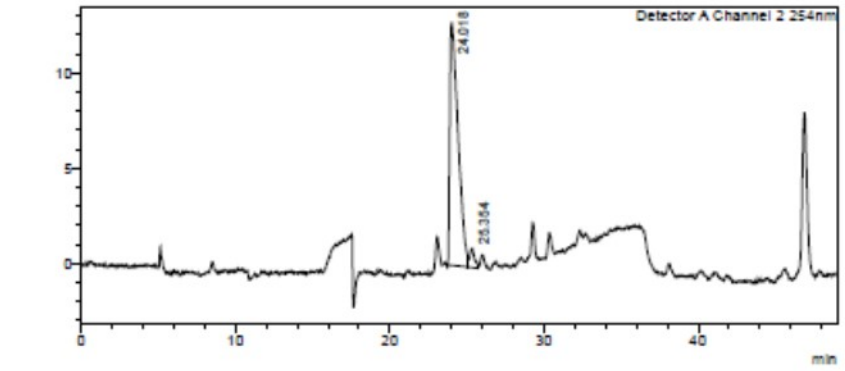
Compound characterizations

5k



Chemical Formula: $C_{46}H_{64}FN_{11}O_{11}$
Exact Mass: 965.4771
Molecular Weight: 966.0824

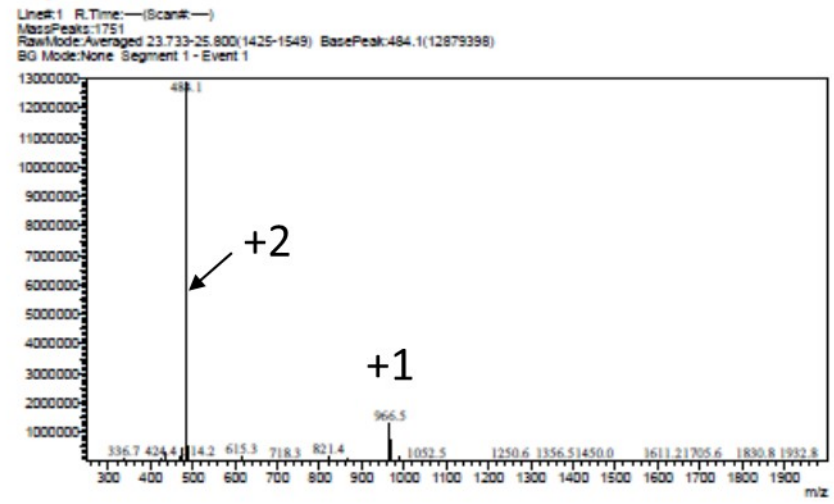
<Chromatogram>



Detector A Channel 2 254nm

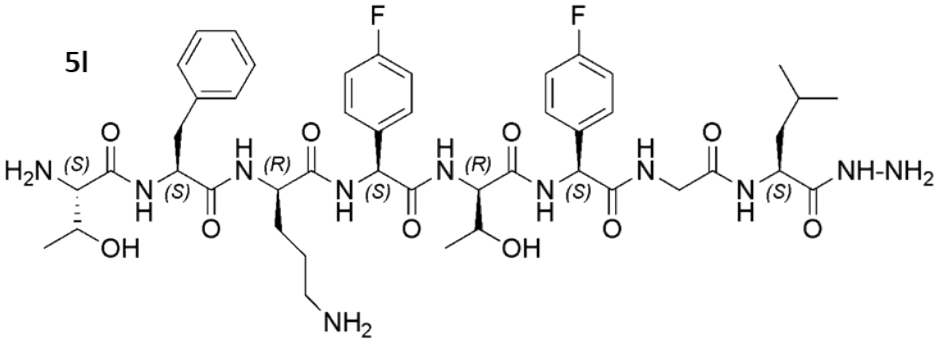
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	24.018	472953	12737	95.561		M		95.561
2	25.354	21968	1019	4.439		VM		4.439
Total		494921	13756					100.000

<Spectrum>



Compound characterizations

5l



Chemical Formula: $C_{46}H_{63}F_2N_{11}O_{10}$
Exact Mass: 967.47
Molecular Weight: 968.07

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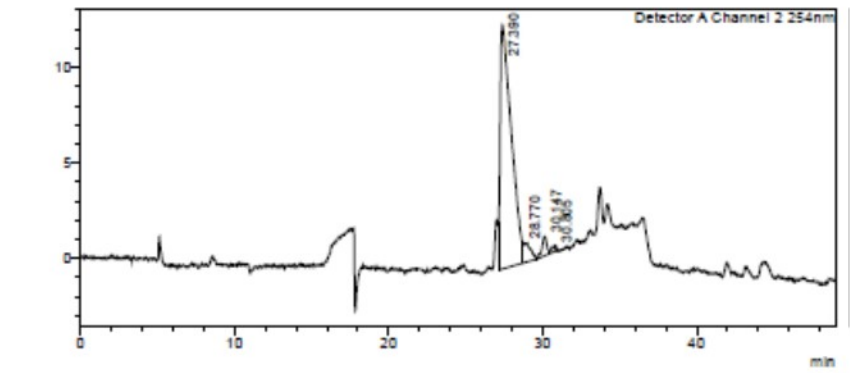
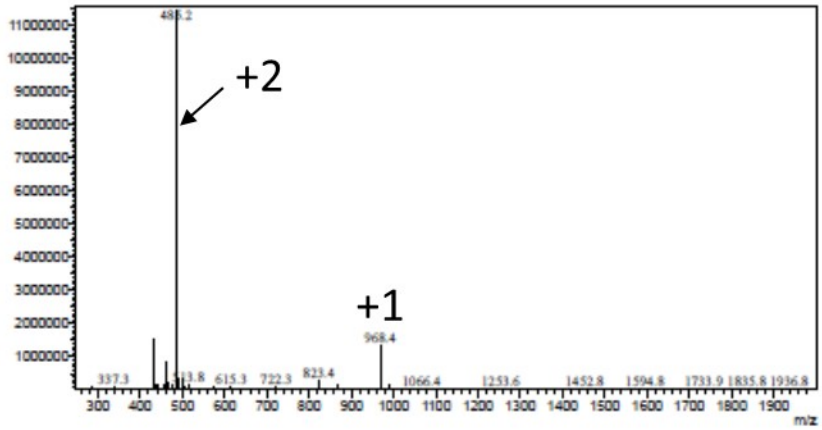


Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	27.390	637299	12777	91.183		M		91.183
2	28.770	33079	1055	4.733		V M		4.733
3	30.147	22318	1006	3.193		M		3.193
4	30.805	6230	301	0.891		V M		0.891
Total		698926	15139					100.000

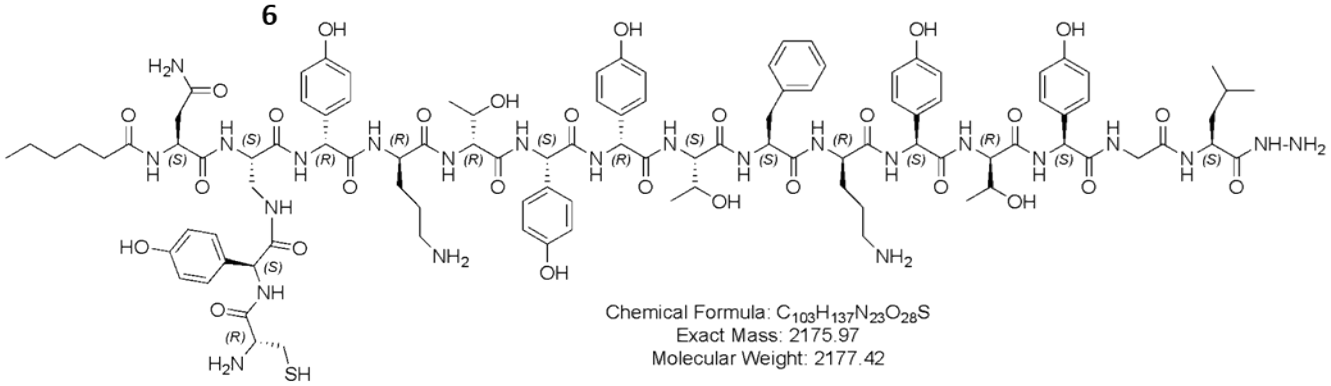
<Spectrum>

Line#:1 R.Time:—(Scan#:—)
MassPeaks:1751
RawMode:Averaged 26.967-31.033(1619-1863) BasePeak:485.2(11446563)
BG Mode:None Segment 1 - Event 1



Compound characterizations

6



Chemical Formula: $C_{103}H_{137}N_{23}O_{28}S$
Exact Mass: 2175.97
Molecular Weight: 2177.42

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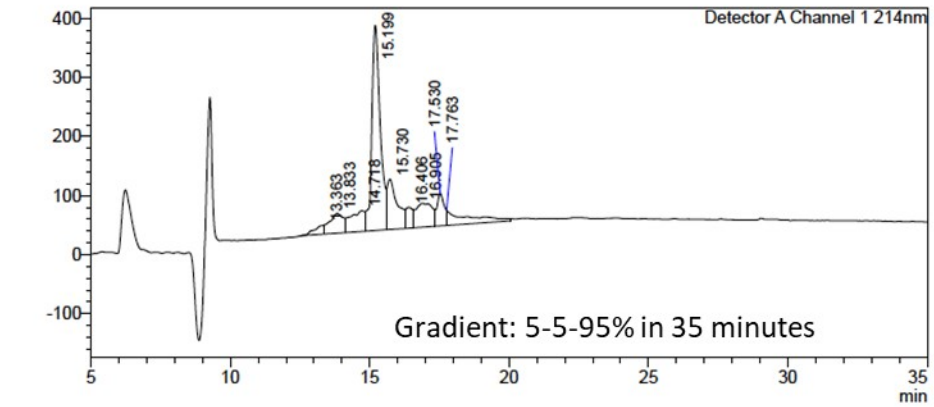

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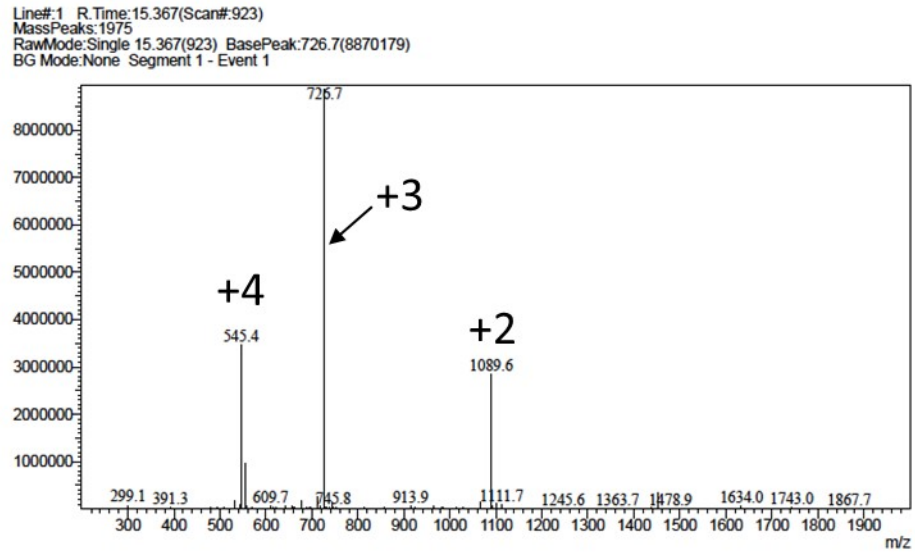
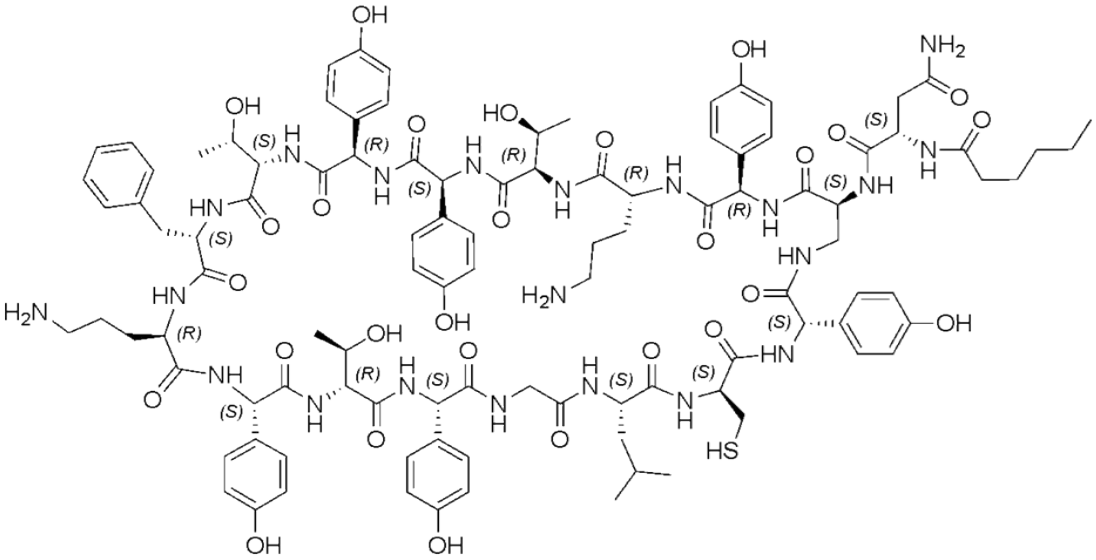
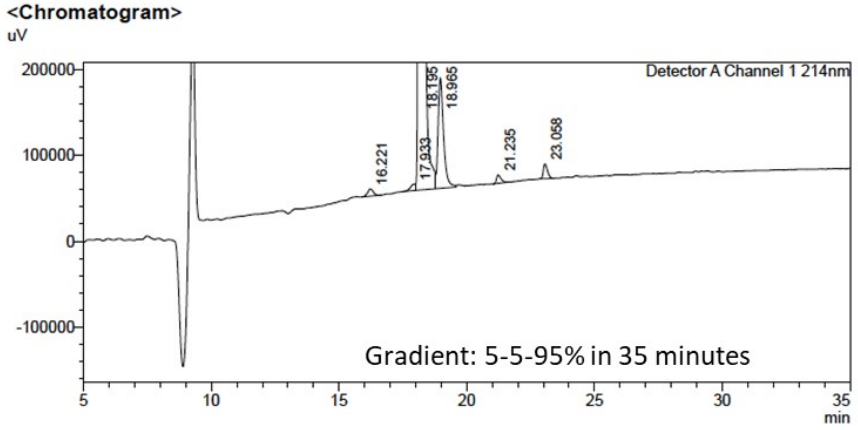


Compound characterizations



Chemical Formula: $C_{103}H_{133}N_{21}O_{28}S$
Exact Mass: 2143.9350
Molecular Weight: 2145.3760

7a



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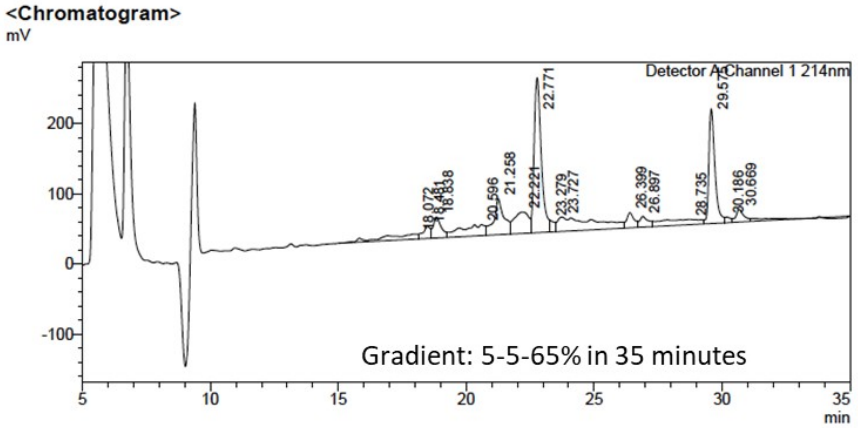
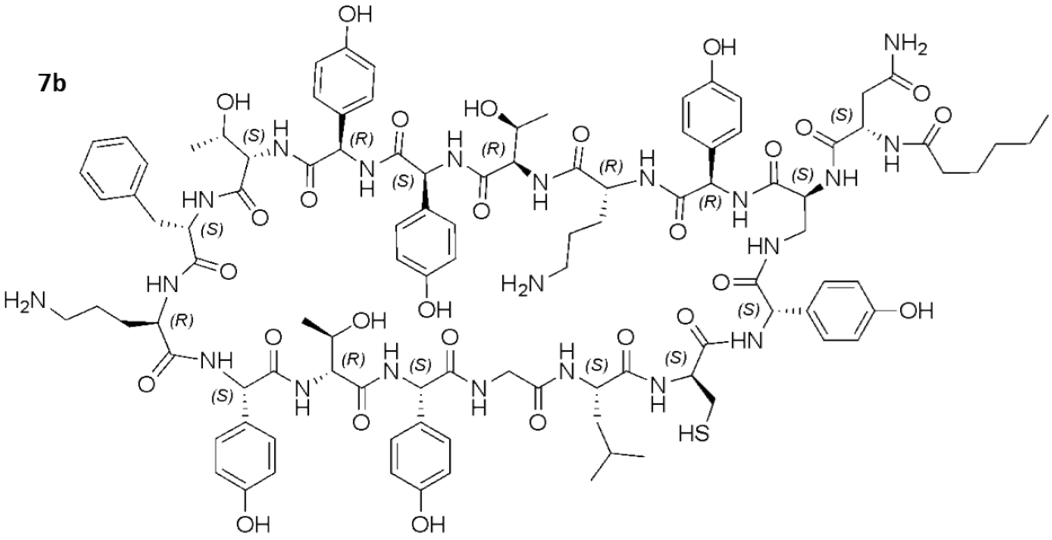
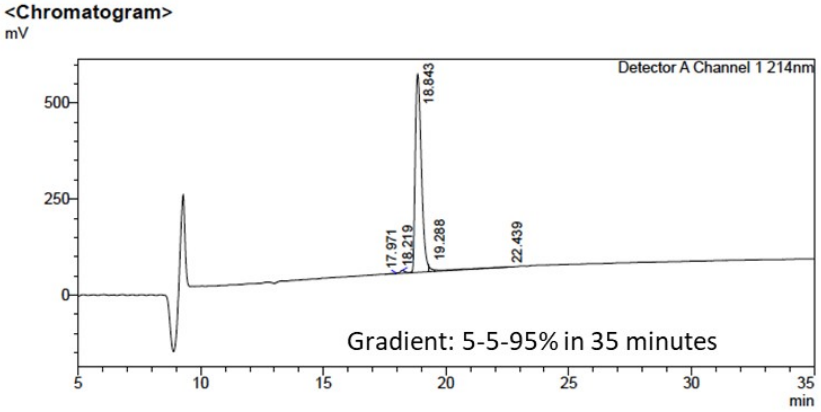


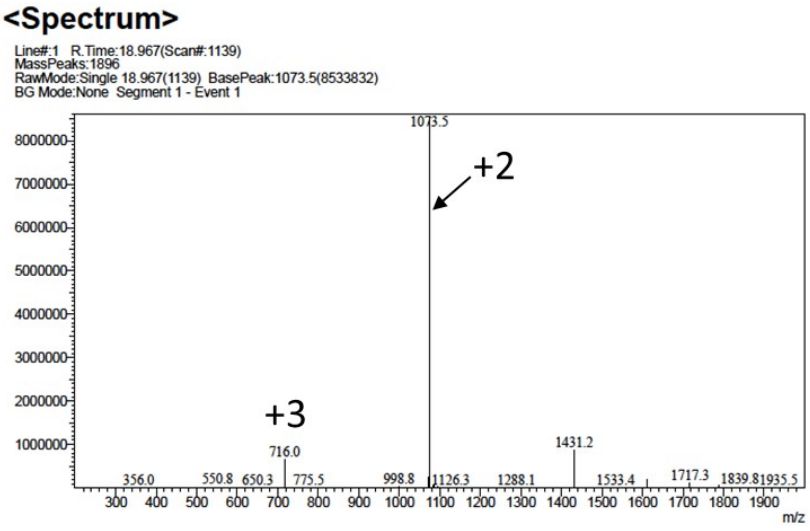
Compound characterizations

7b

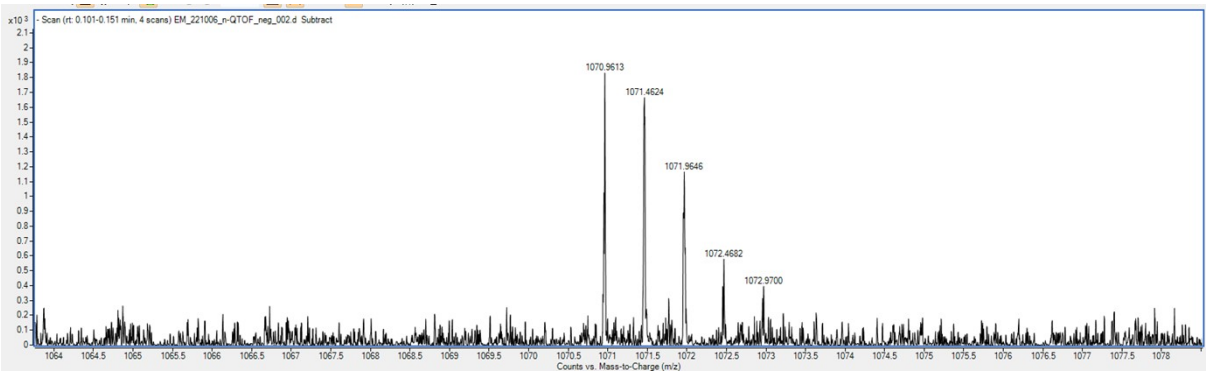


Chemical Formula: $C_{103}H_{133}N_{21}O_{28}S$
Exact Mass: 2143.9350
Molecular Weight: 2145.3760





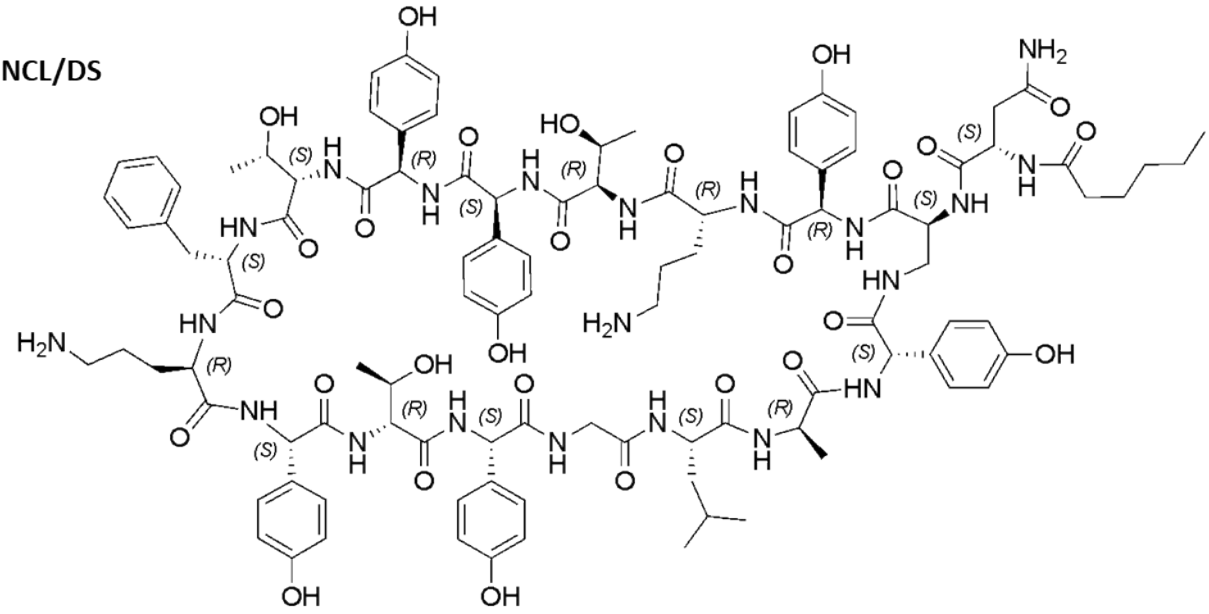
Compound characterizations



$C_{103}H_{133}N_{21}O_{28}S$ $[M-2H]^{-2}$ calculated: 1071.4619 Found: 1070.4624

Compound characterizations

8 – NCL/DS



Chemical Formula: $C_{103}H_{133}N_{21}O_{28}$
Exact Mass: 2111.96
Molecular Weight: 2113.32

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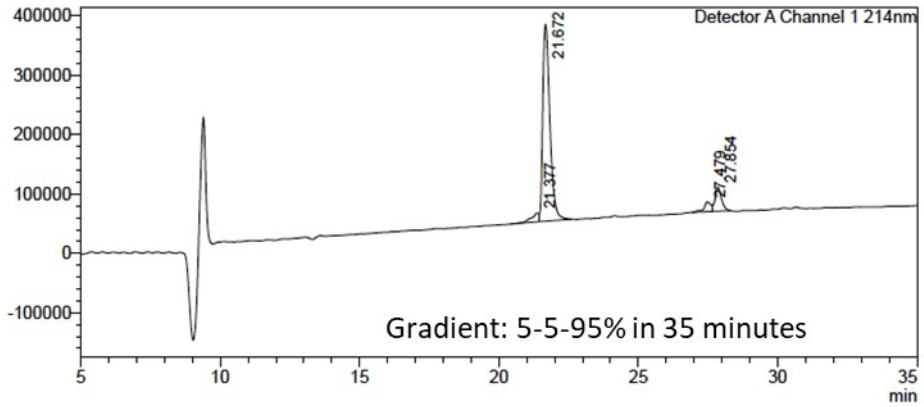

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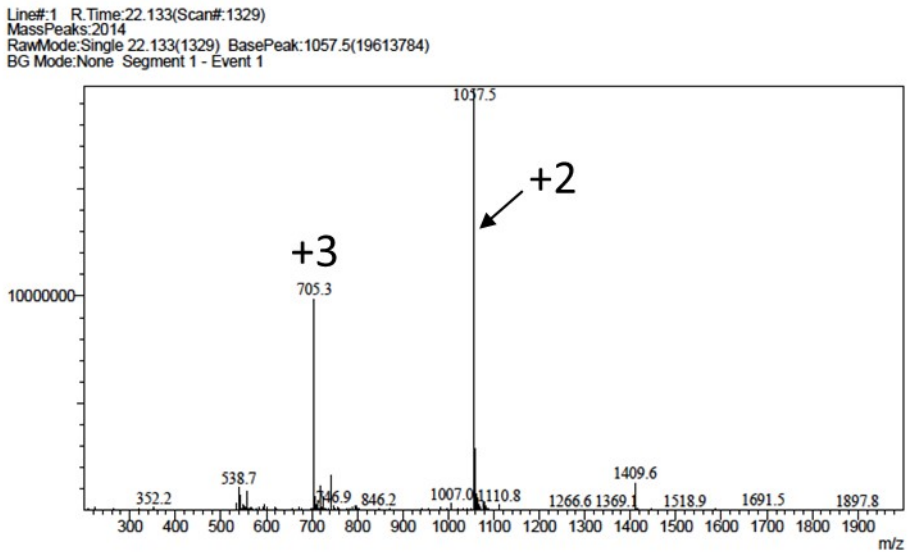
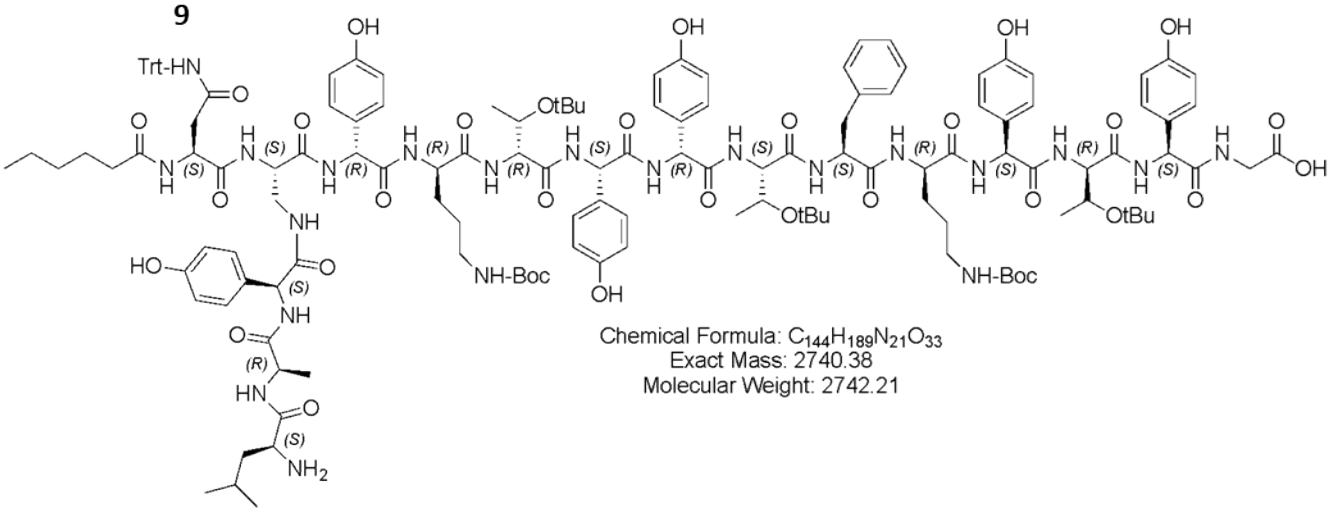


Compound characterizations

9



Chemical Formula: $C_{144}H_{189}N_{21}O_{33}$
Exact Mass: 2740.38
Molecular Weight: 2742.21

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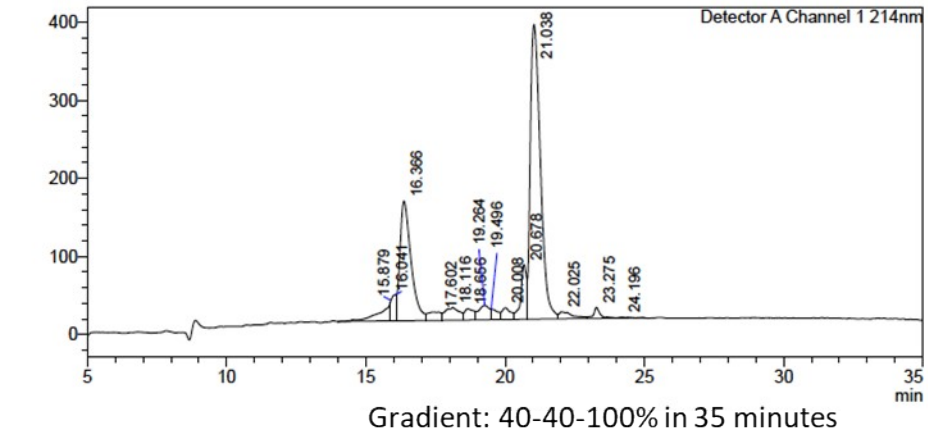
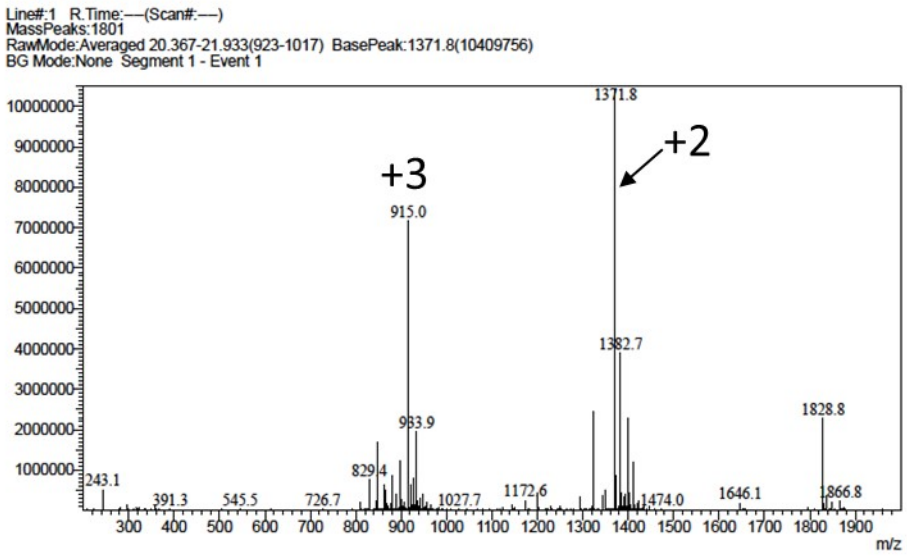


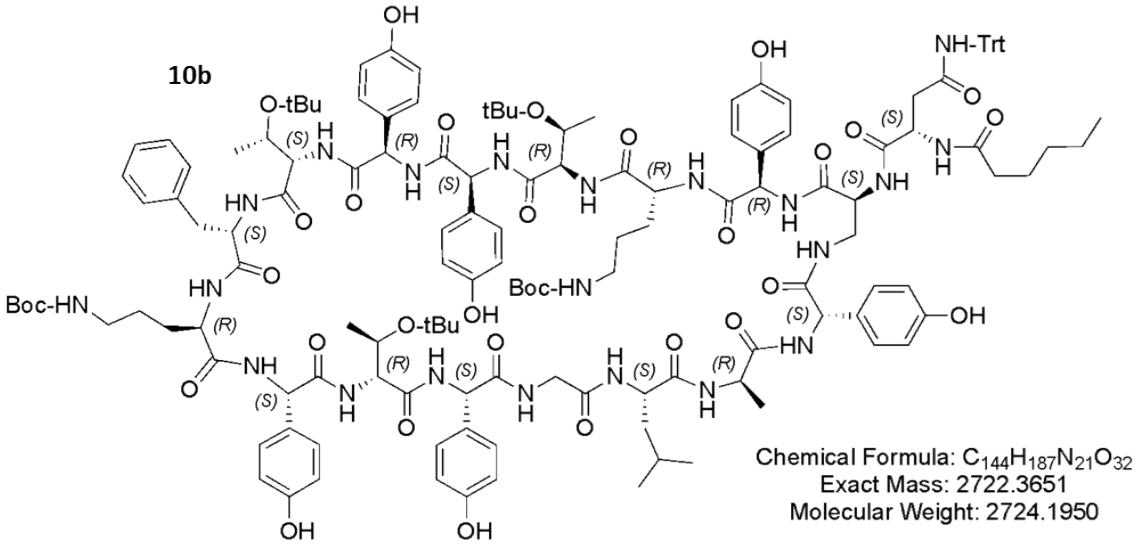
Gradient: 40-40-100% in 35 minutes

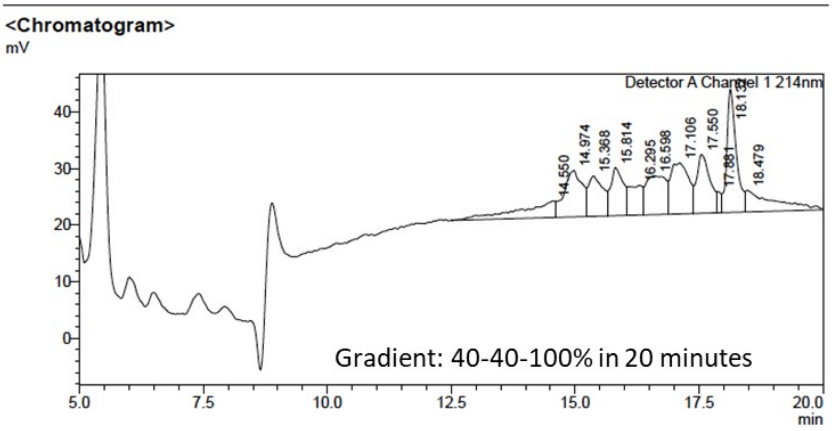
<Spectrum>

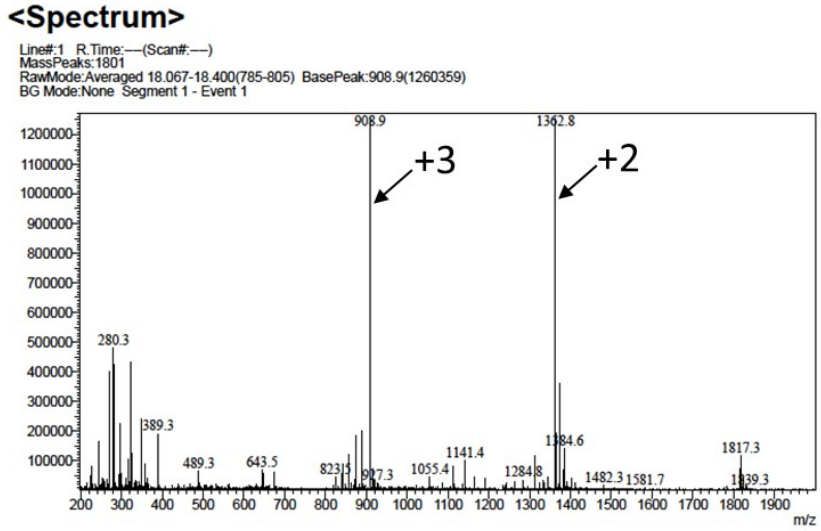


Compound characterizations

10b

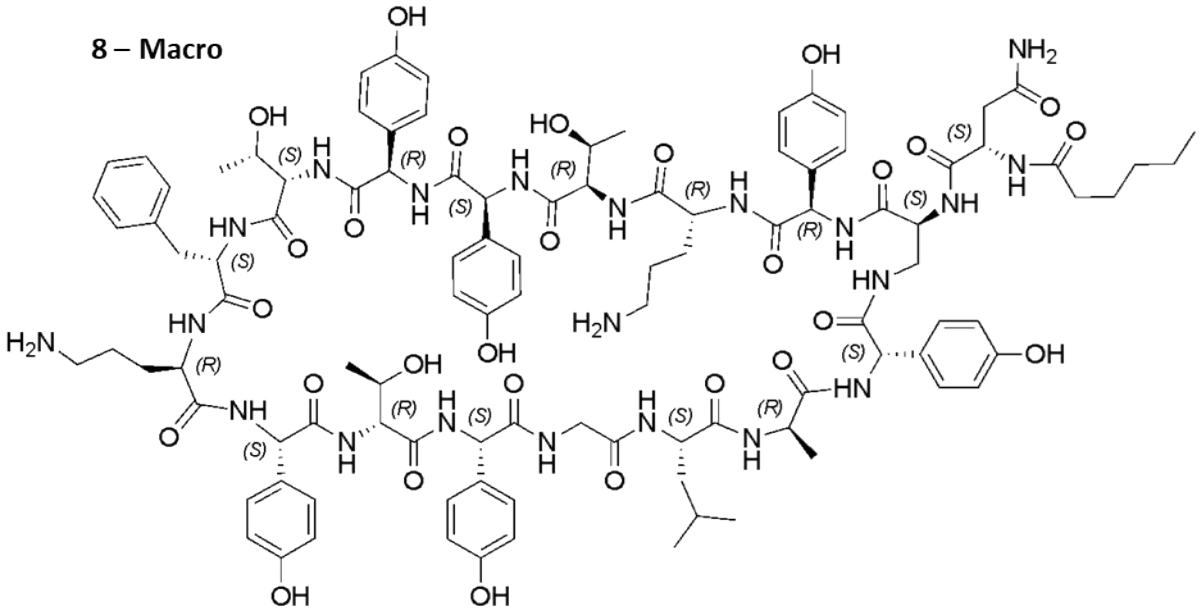






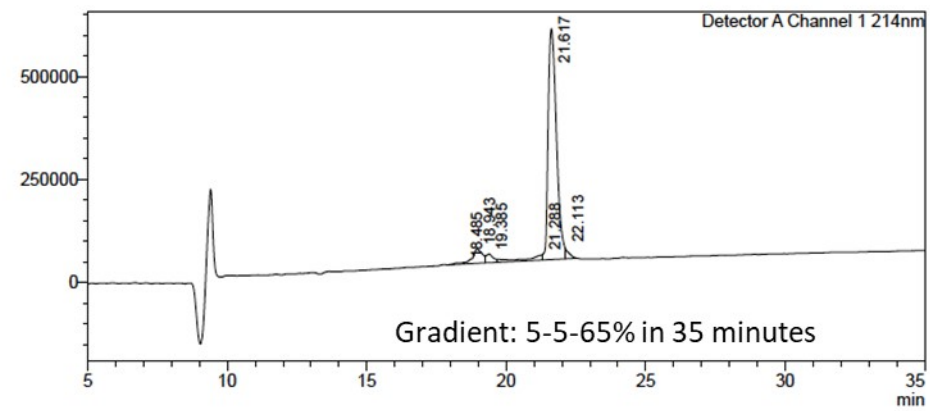
Compound characterizations

8 – Macro

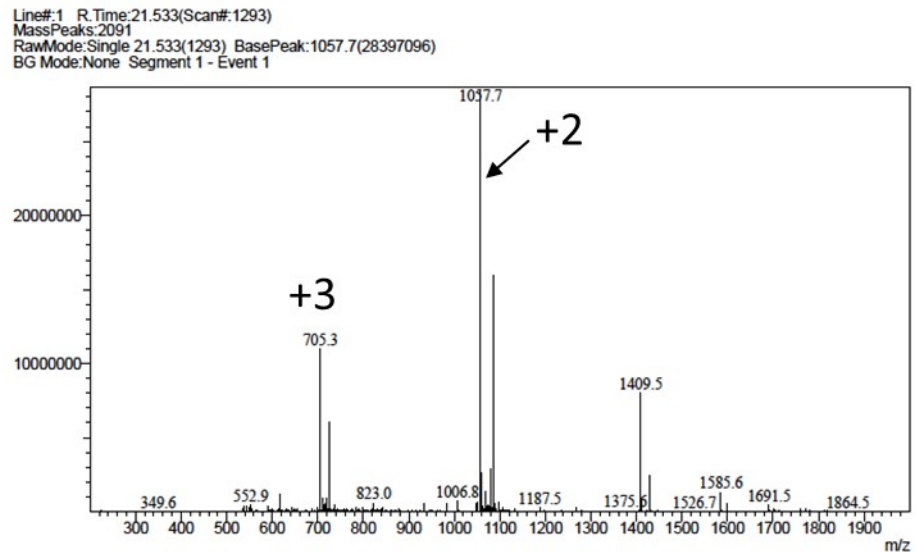


Chemical Formula: $C_{103}H_{133}N_{21}O_{28}$
Exact Mass: 2111.96
Molecular Weight: 2113.32

<Chromatogram>

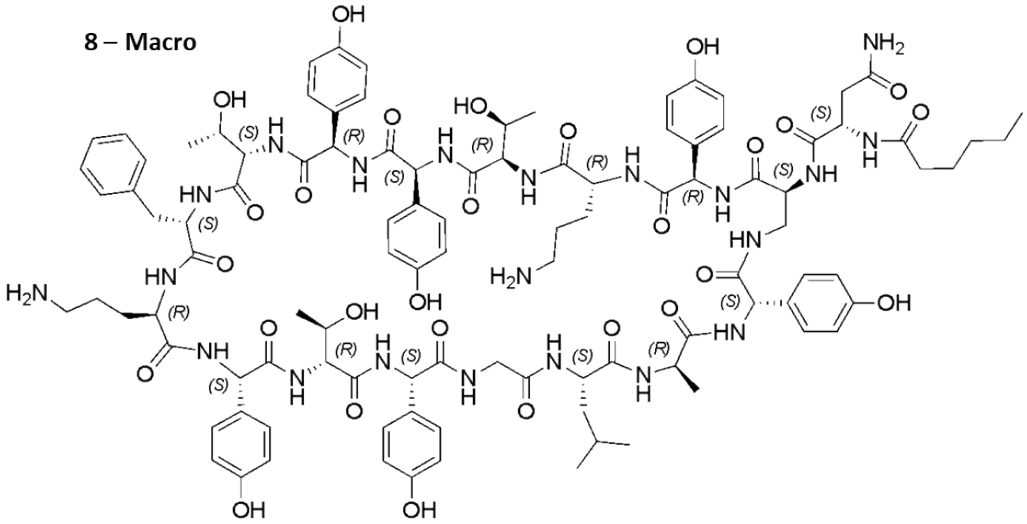


<Spectrum>

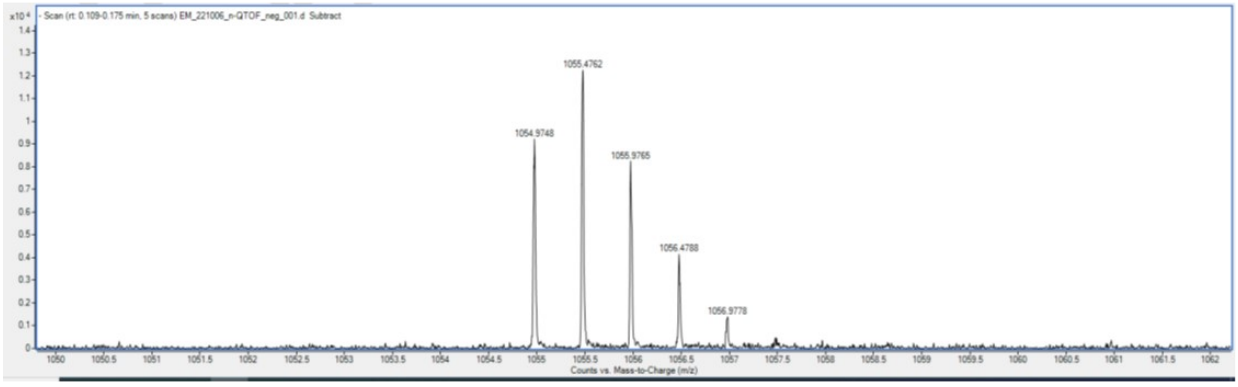


Compound characterizations

8 – Macro



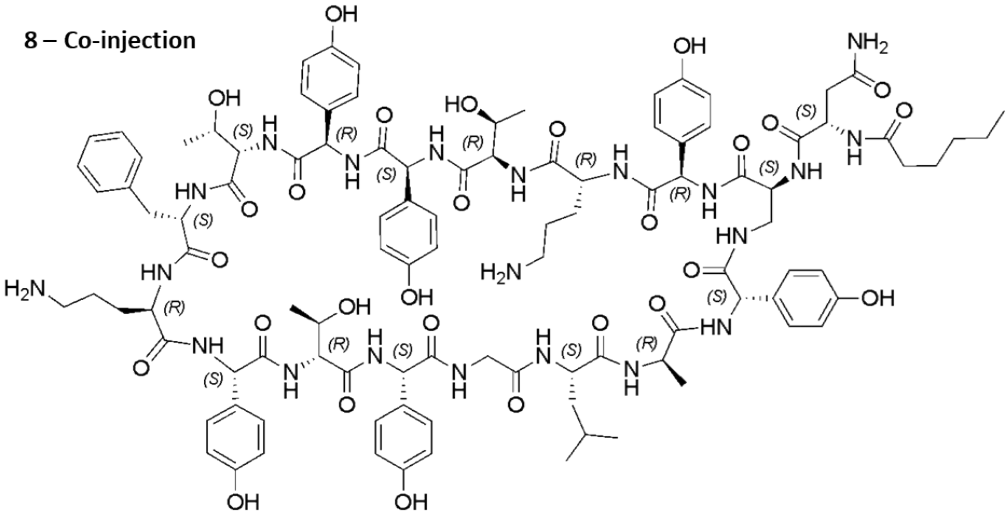
Chemical Formula: $C_{103}H_{133}N_{21}O_{28}$
Exact Mass: 2111.96
Molecular Weight: 2113.32



HRMS: m/z calcd for $[M-2H]^{2-}$ $C_{103}H_{131}N_{21}O_{28}^{2-}$ 1055.4758 Da, found 1054.9748 Da.
Calculated exact mass: 2111.94 Da.

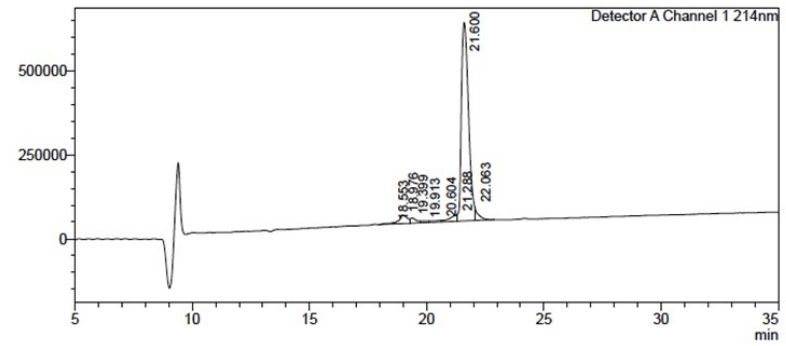
Compound characterizations

8 – Co-injection



Chemical Formula: $C_{103}H_{133}N_{21}O_{28}$
Exact Mass: 2111.96
Molecular Weight: 2113.32

<Chromatogram>



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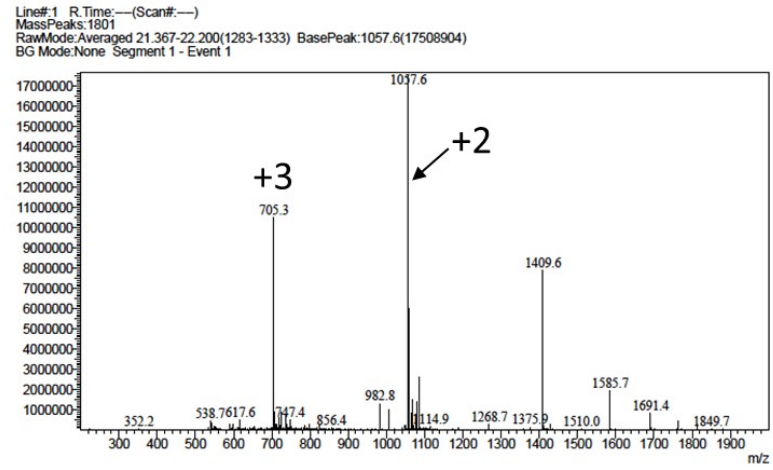
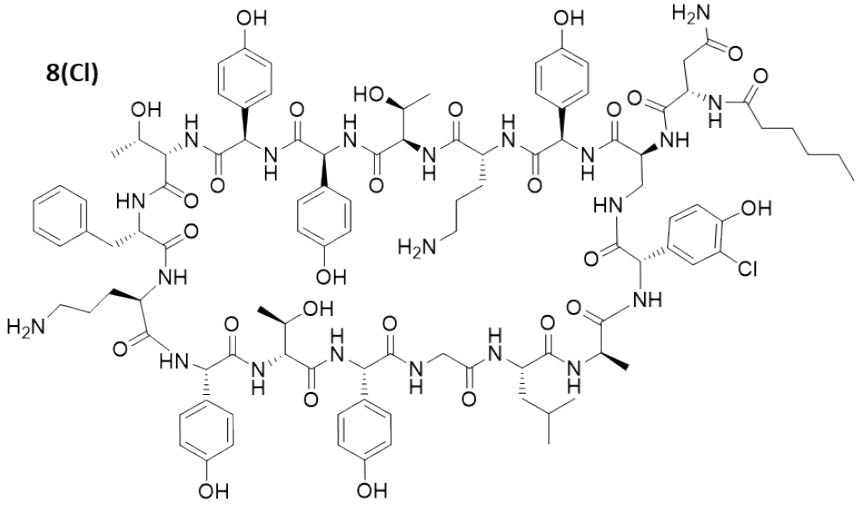
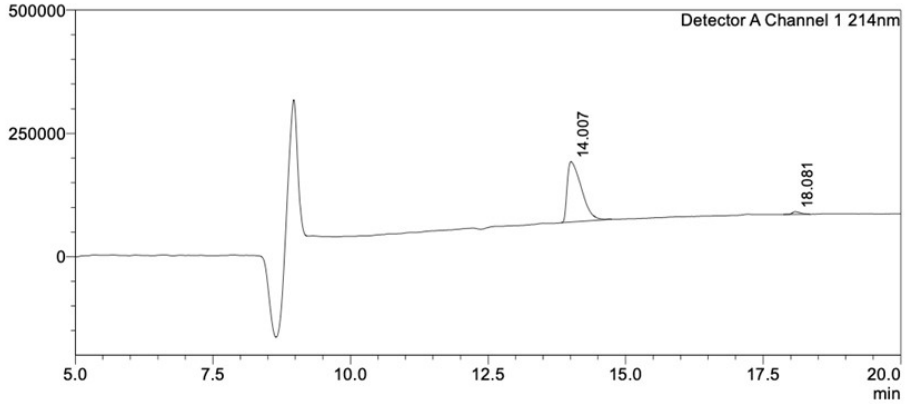


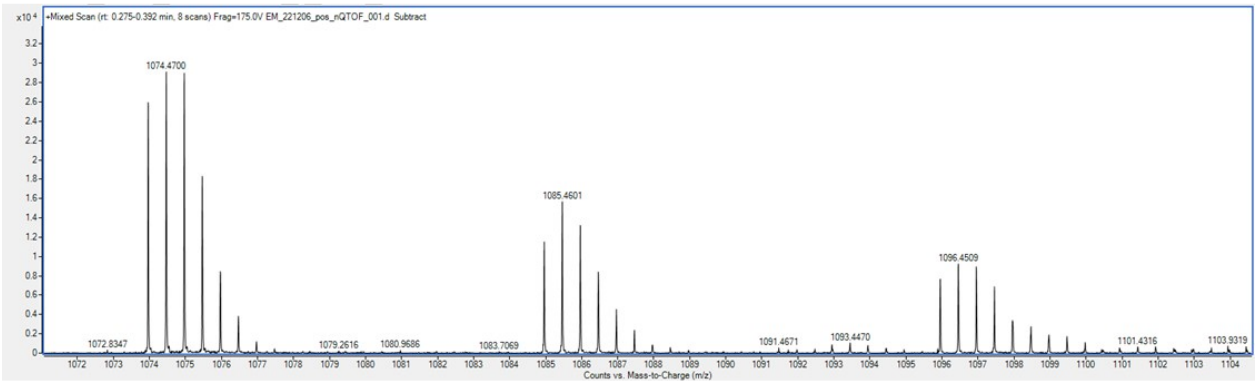
Compound characterizations

8(Cl)



Chemical Formula: $C_{103}H_{132}ClN_{21}O_{28}$
Exact Mass: 2145.92
Molecular Weight: 2147.76

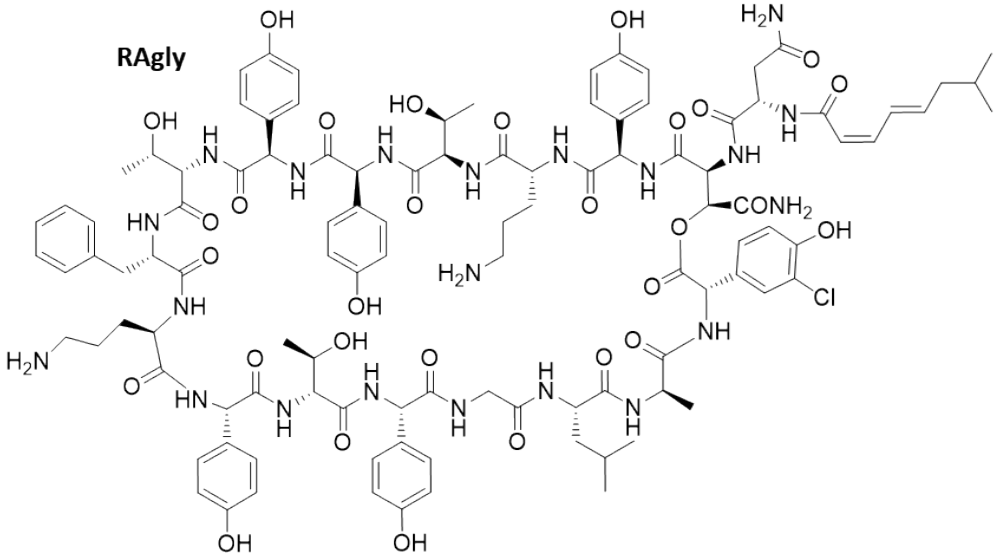




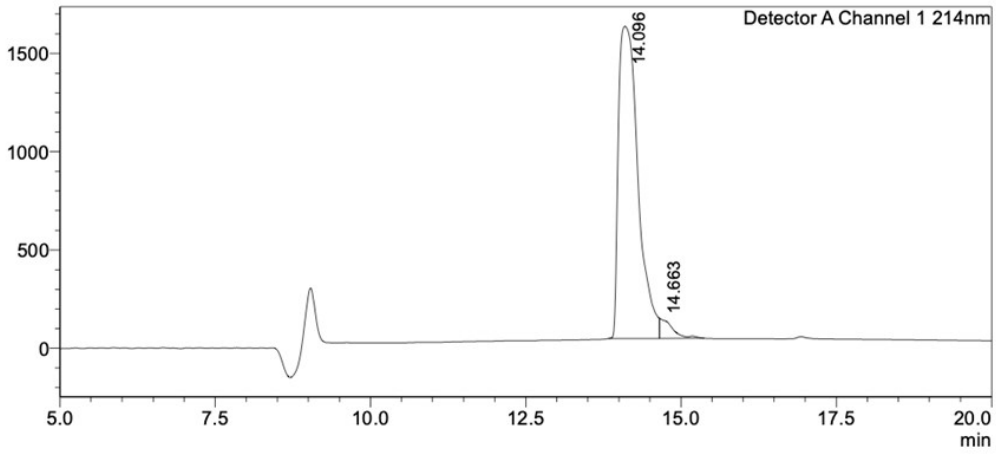
HRMS: m/z calcd for $[M+2H]^{2+}$ $C_{103}H_{134}ClN_{21}O_{28}{}^{2+}$ 1074.4709 Da, found 1074.4700 Da.
Calculated exact mass: 2146.94 Da.

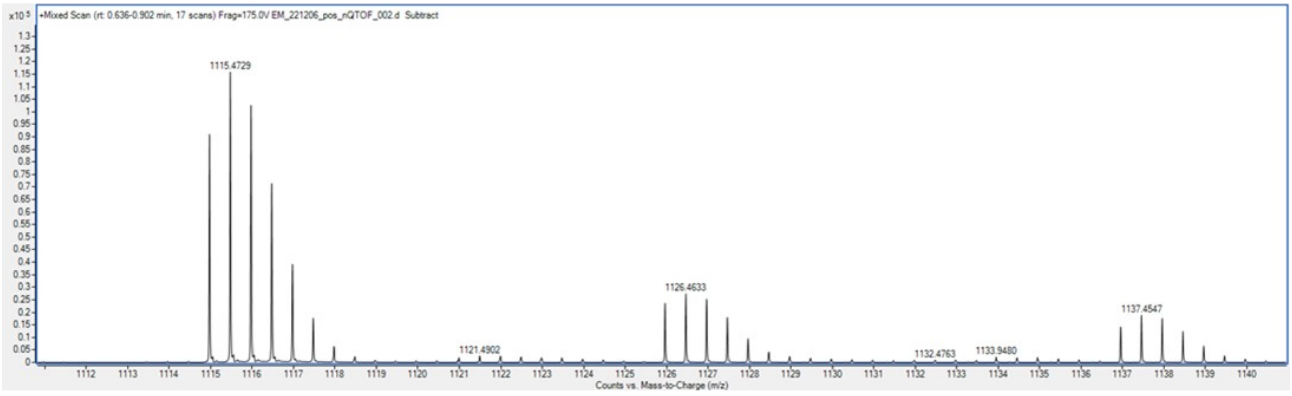
Compound characterizations

RAgly



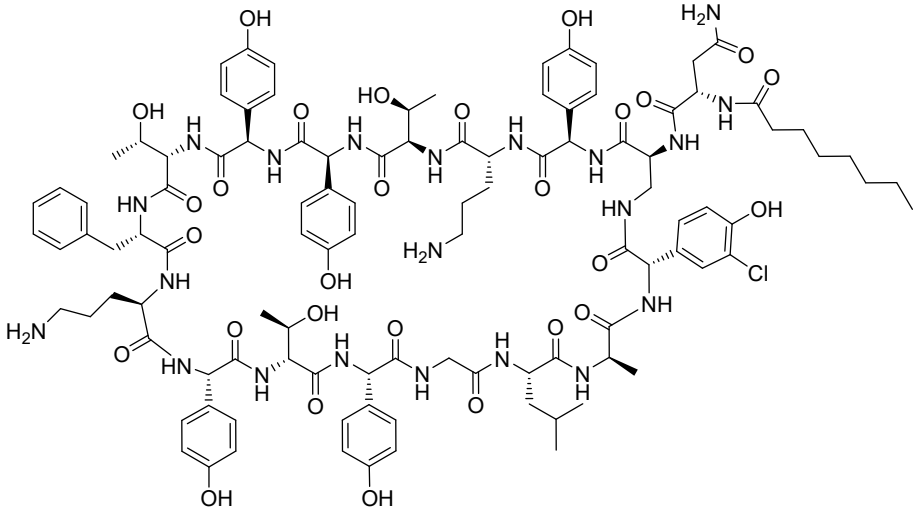
Chemical Formula: $C_{107}H_{134}ClN_{21}O_{30}$
Exact Mass: 2227.93
Molecular Weight: 2229.82



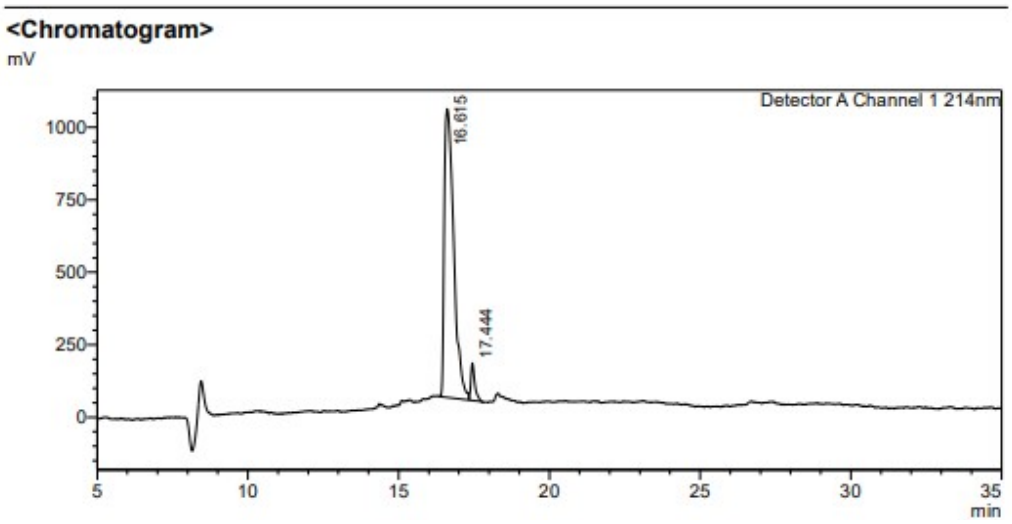


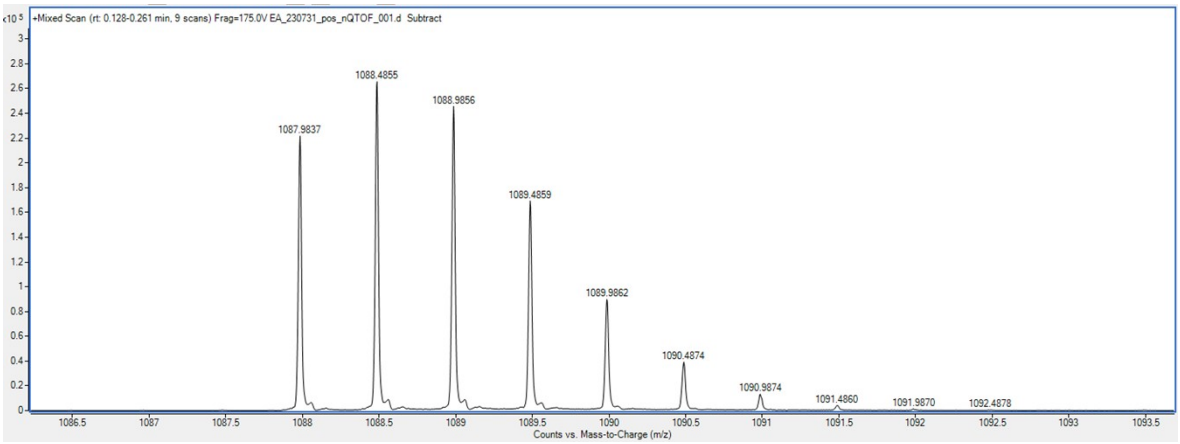
HRMS: m/z calcd for $[M+2H]^{2+}$ $C_{107}H_{136}ClN_{21}O_{30}{}^{2+}$ 1115.4737 Da, found 1115.4729 Da.
Calculated exact mass: 2228.94 Da.

Compound characterizations



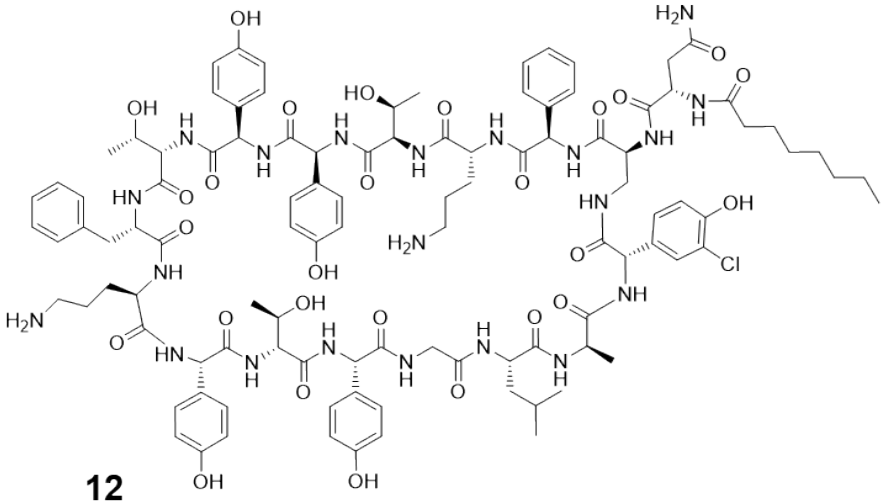
11 Chemical Formula: $C_{105}H_{136}ClN_{21}O_{28}$
Exact Mass: 2173.9552





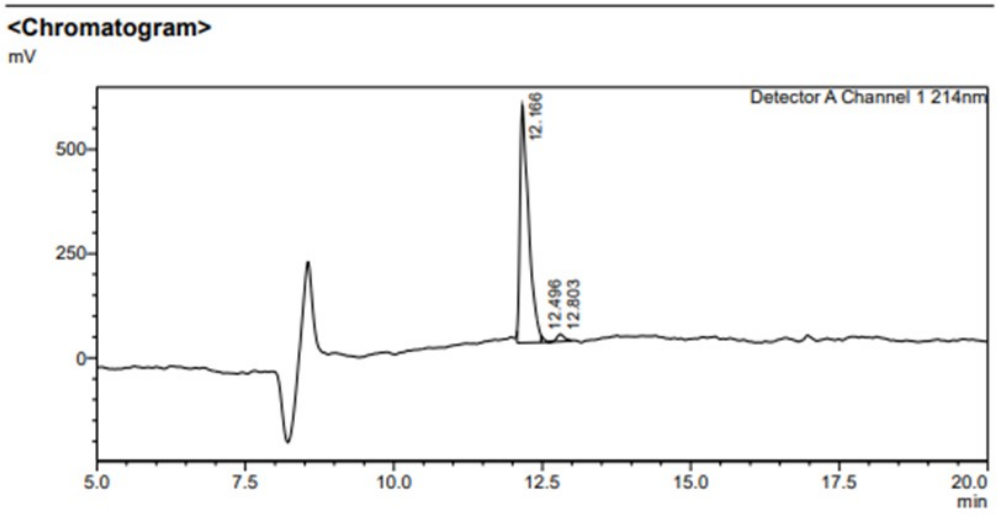
$C_{105}H_{138}ClN_{21}O_{28}$ $[M+2H]^{+2}$ calculated: 1088.4866 Found: 1088.4855

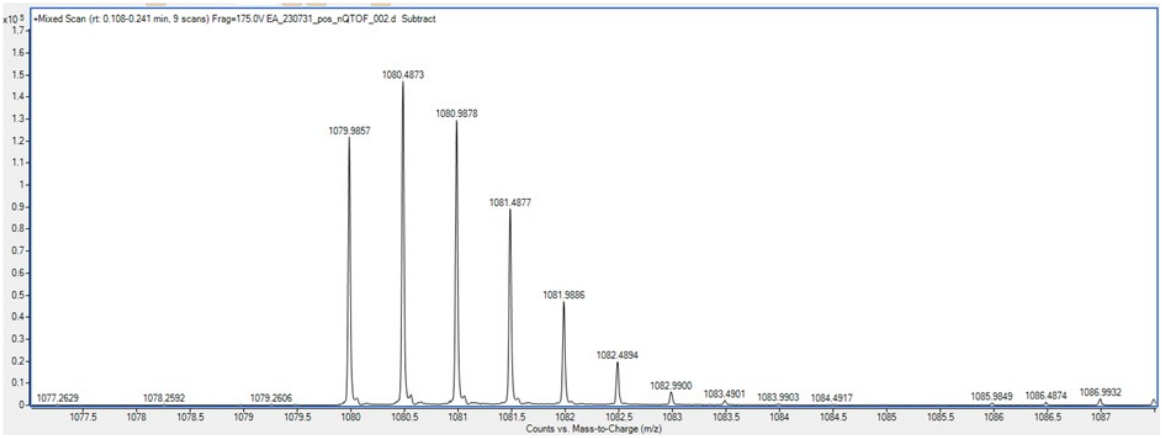
Compound characterizations



12

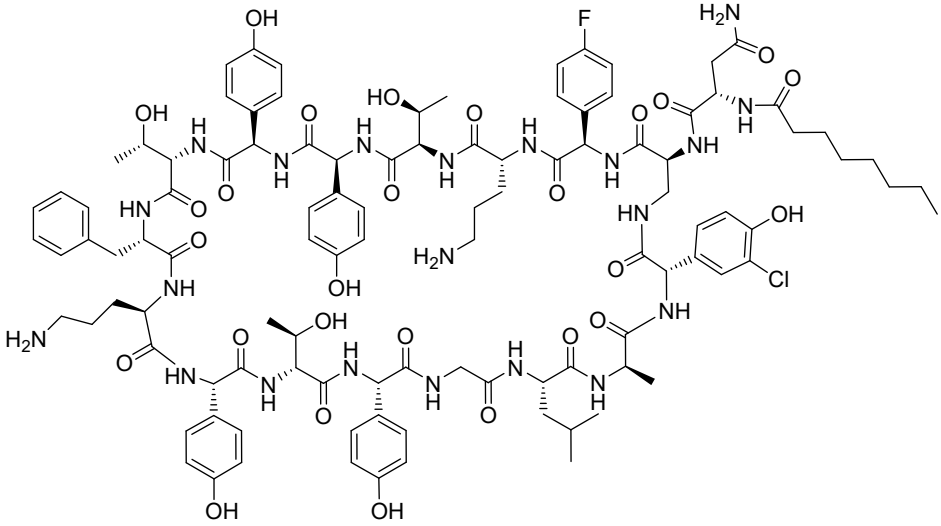
Chemical Formula: $C_{105}H_{136}ClN_{21}O_{27}$
Exact Mass: 2157.9603



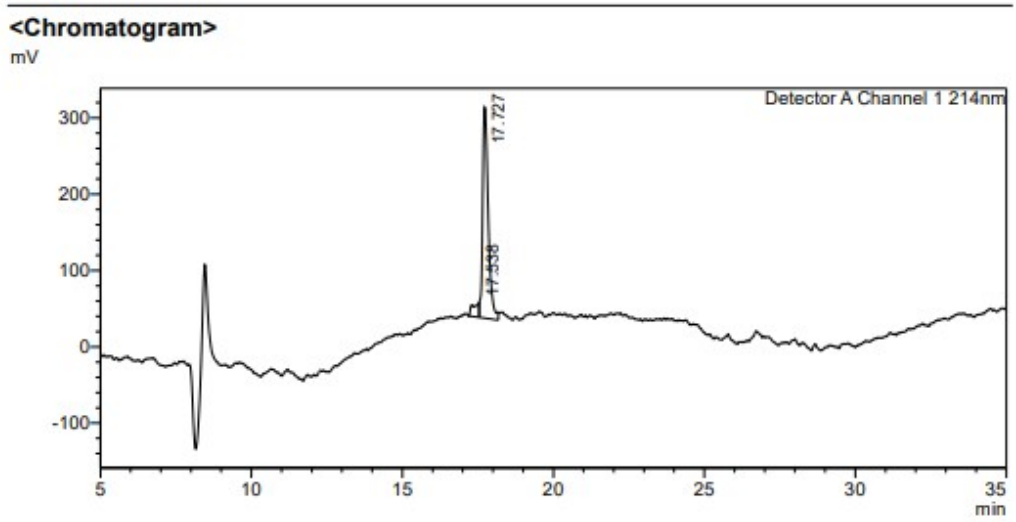


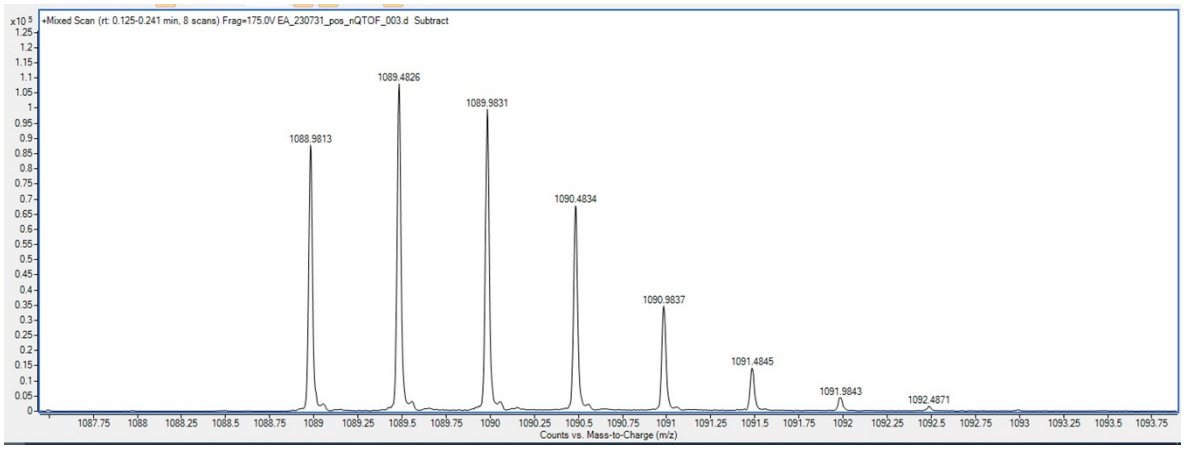
$C_{105}H_{136}ClN_{21}O_{27}$ $[M+2H]^{+2}$calculated: 1080.4891 Found: 1080.4873

Compound characterizations



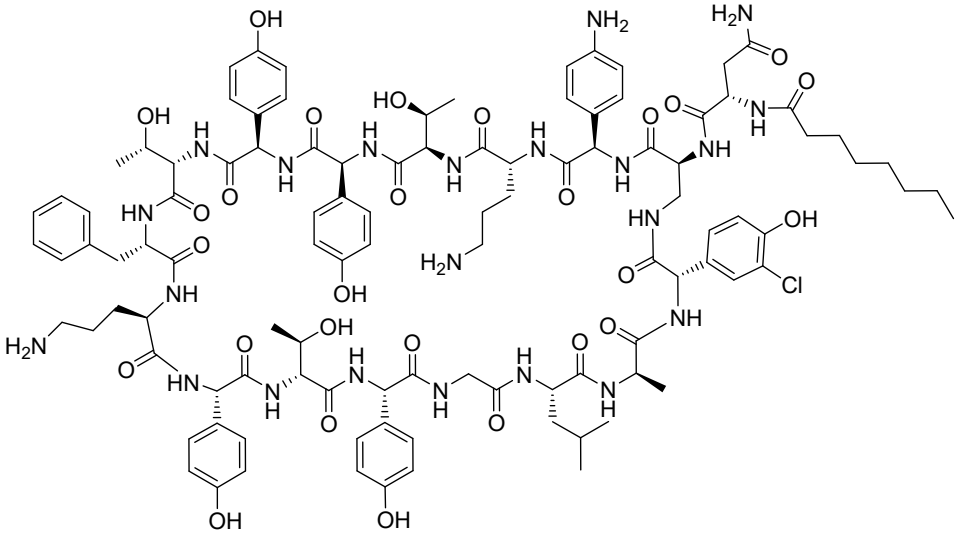
13 Chemical Formula: $C_{105}H_{135}ClFN_{21}O_{27}$
Exact Mass: 2175.9509



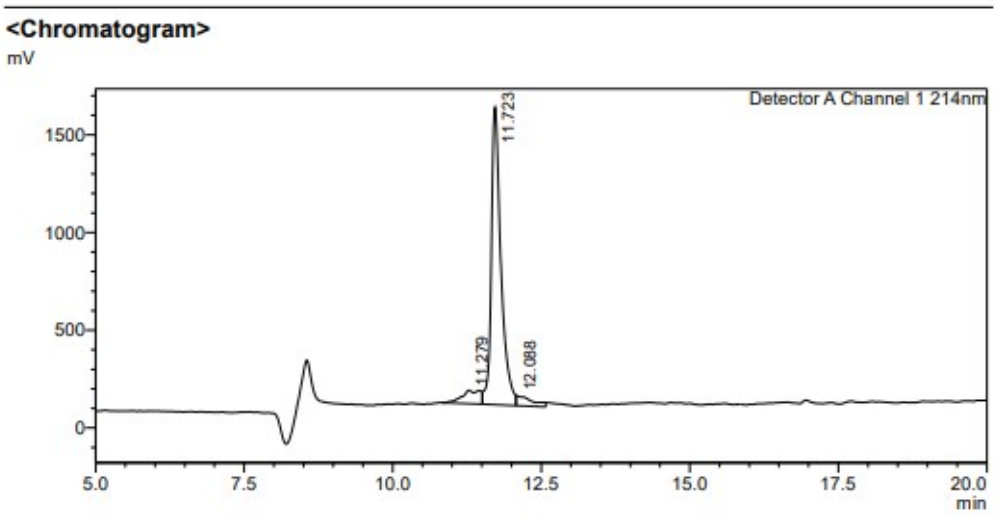


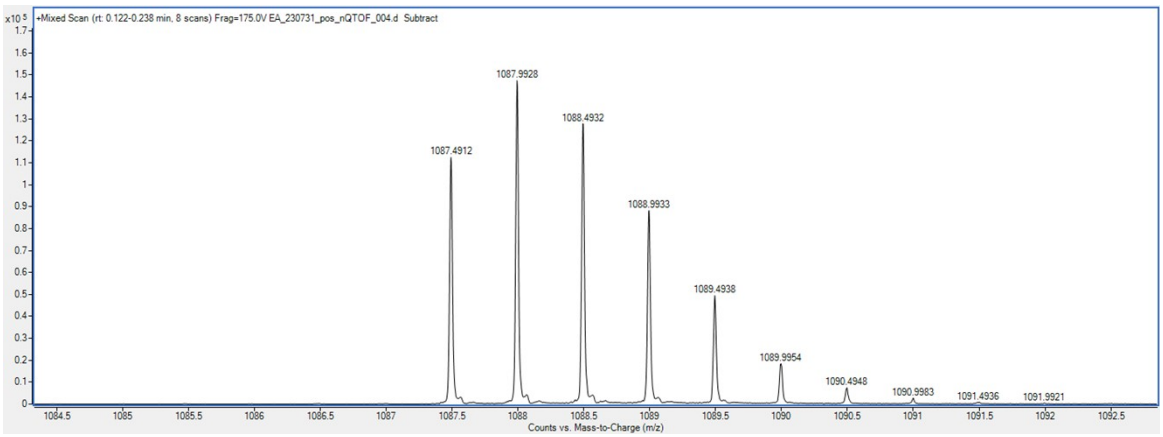
Chemical Formula: $C_{105}H_{135}ClFN_{21}O_{27}$ $[M+2H]^{+2}$ calculated: 1089.4844 Found: 1089.4826

Compound characterizations



14 Chemical Formula: $C_{105}H_{137}ClN_{22}O_{27}$
Exact Mass: 2172.9712





Chemical Formula: $C_{105}H_{137}ClN_{22}O_{27}$ $[M+2H]^{+2}$ calculated: 1087.9946 Found: 1087.9928

Supplementary references

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