Supporting information for

Foldameric probes for membrane interactions by induced β-sheet folding

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Chemical Communications

Materials and methods

Peptide synthesis and purification. Sequences were synthesized by using a CEM Liberty 1 microwave peptide synthesizer, except for anginex**, 2b** and **2c**, which were synthesized by using a newly developed continuous flow peptide synthesis approach.[1] In both synthetic methods, Tentagel R RAM resin was used as solid support and HATU as coupling reagent. During microwave-enhanced syntheses, couplings were performed in a 3-equivalent amino acid excess at 75 °C for 15 min for α-amino acids, and for 30 min for β residues. Histidine was coupled at 50 °C; arginines were coupled in two cycles. To avoid aspartimide by-product formation between D26 and G27 and the epimerization of D33, Fmoc-Asp(OtBu)-Gly(DMb)-OH (Novabiochem) was used in these couplings. In the continuous flow method, Tentagel resin was filled into a HPLC column and a 1.5 equivalent amino acid excess was used for β-amino acids and Fmoc-Asp(OtBu)-Gly(DMb)-OH couplings. Recirculation of the coupling mixture on the resin was applied for 30 min. For α-amino acids, an excess of 3 equivalents was used without recirculation. All couplings and deprotection cycles were carried out at 50 °C, 70 bar at a flow rate of 0.15 ml min^{-1}, with a 10-min washing step between the cycles. Cleavage was performed with TFA/water/D,L-dithiothreitol/triisopropylsilane (90:5:2.5:2.5), which was followed by precipitation in ice-cold diethyl ether. The resin was washed with acetic acid and water, then followed by filtration, and lyophilization. Peptides were purified with RP-HPLC on a C18 column (Phenomenex Jupiter, 10x250 mm). The HPLC eluents: (A) 0.1% TFA in water and (B) 0.1% TFA, 80% ACN in water, with a gradient from 30% to 60% B in 60 min, at a flow rate of 4 ml min^{-1}. Purity was confirmed by analytical RP-HPLC and ESI MS measurements.

Circular dichroism (CD) measurements. CD measurements were performed with a Jasco J-815 CD Spectrometer. CD spectra were recorded by using a 1 mm thermally jacketed quartz cuvette, from 260 to 190 nm, at a scan speed of 100 nm min^{-1} with 8 accumulations. The peptide concentration was 100 μM in pH 5.6 K-phosphate buffer, with or without 2.5 mM dodecylphosphocholine. For thermal control, a Julabo water thermostat was used with a 10 min equilibration time for each temperature. The solvent baseline measured under identical conditions was subtracted. All spectra were deconvoluted with CCA+ software[2] to three pure components.

NMR experiments. NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer equipped with a 5 mm CP-TCI triple-resonance cryoprobe. Compounds were dissolved at a concentration of 0.5 mM in 20 mM, pH 5.6 d18-HEPES buffer (90% H_2O, 10% D_2O) containing 0.02% NaN_3. For referencing, 4,4-dimethyl-4-silapentane-1-sulfonic acid was used as an external standard. For resonance assignment, 2D homonuclear TOCSY and NOESY, and 2D heteronuclear ^{15}N and ^{13}C HSQC experiments were performed. The NOESY mixing time was 225 ms and the number of scans was 32. TOCSY measurements were carried out with homonuclear Hartman–Hahn transfer, using the DIPSI2 sequence for mixing, with a mixing time of 80 ms; the number of scans was 32. For all the 2D

homonuclear spectra, 2K time domain points and 512 increments were applied. ^{13}C HSQC experiments were performed under the same sample conditions, but the buffer was prepared in D_2O. Signal assignment was based on the 2D NMR spectra obtained at 37 °C. 1D ^{1}H NMR spectra of the 0.5 mM samples were also measured with 12.5 mM d18-DPC. All 1H spectra were acquired with the excitation sculpting solvent suppression pulse scheme.[3] Processing was carried out by using Topspin 3.1 (Bruker), a cosine-bell window function, single-zero filling, and automatic baseline correction. Spectra were analysed by using Sparky 3.114 (T. D. Goddard and D. G. Kneller, University of California, San Francisco, USA).

The secondary structure propensity (SSP)[4] score was calculated by using the ssp software. The refDB random coil reference set was based on the chemical shifts of properly referenced known protein structures.[5]

Viability assay. Murine brain endothelial cell line bEnd.3 was cultured in DMEM (PAN Biotech) supplemented with 10% fetal calf serum (Gibco), penicillin (100 IU ml^{-1}), streptomycin (100 μg ml^{-1}) and L-glutamine (2 mM, all from Sigma Aldrich). The endothelial cells (48 000 cells/well) were plated onto a 24-well tissue culture plate and treated in the presence or absence of different concentrations (5, 10, 25 and 50 μM) of anginex or anginex analogues. Peptides were dissolved in DMSO and diluted in 10 mM HEPES pH 7.3 containing 150 mM NaCl. Cells in control samples were treated with an equivalent amount of the solvent. After incubation for 30 min, samples were harvested with trypsin/EDTA and washed with DMEM, and propidium iodide was then added at a concentration of 10μg ml^{-1} (Sigma Aldrich). The viable cells were assessed by using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson, San Diego, CA, USA). The experiments were repeated three times in duplicate.

Preparation of large unilamellar vesicles and membrane leakage assays

40 mM stock solutions were prepared in chloroform from 1,2-dioleoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Inc.) (DOPC) or from 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG). Aliquots of DOPC alone or DOPC:DOPG mixed in a molar ratio of 8:2 from the stock solutions were dried under nitrogen to obtain thin lipid film and kept under vacuum overnight to remove solvent traces. The lipid films were hydrated with 20 mM HEPES pH 6.5 150 mM NaCl buffer at room temperature for 1 h at 20 mM lipid concentration. For 5,6-carboxyfluorescein (CF) (Sigma)-filled vesicles, the buffer contained 50 mM CF. Lipid solutions were subjected to 10 freeze-thaw cycles and extruded 10 times manually, using a syringe and a 200 nm polycarbonate membrane filter. Size distribution was confirmed by dynamic light scattering (DLS), measurements, which resulted in an average size distribution of 200 nm. Solutions were dialysed until the external CF had been completely removed. Freshly prepared and dialysed lipids were used for the experiments.

Membrane leakage experiments were carried out with 500 μM lipid after a 30 min treatment with 0.5, 2.5, 5, 25 or 50 μM peptide. Fluorescence was measured with an Optima FluoStar plate reader, and CF release was calculated via the following equation:

$$CF\ release\ (\%) = \frac{F_c - F_0}{F_{100} - F_0} \times 100$$

where F_0 is the background fluorescence, F_{100} is the total fluorescence after complete vesicle lysis following the addition of 0.1% Triton X, and F_c is the corrected sample fluorescence. High peptide concentration caused fluorescence quenching. To correct the quenching by the peptides, experiments were repeated with unfilled vesicles and externally added CF in two concentrations, matching the background and the total fluorescence respectively. After similar peptide treatment, a correction factor was calculated for all peptides at all concentrations, based on the sample fluorescence decrease:

$$F_c = F_s \times f_c$$

$$f_c = \frac{I_0 + I_t}{I_{s,0} + I_{s,t}}$$

where f_c is the correction factor, F_s is the sample fluorescence measured with CF filled vesicles, I_0 is the intensity corresponding to the background fluorescence; $I_{s,0}$ is the sample intensity measured at the background fluorescence, I_t is the intensity corresponding to the total fluorescence $I_{s,t}$ is the sample intensity measured at the matching total fluorescence.

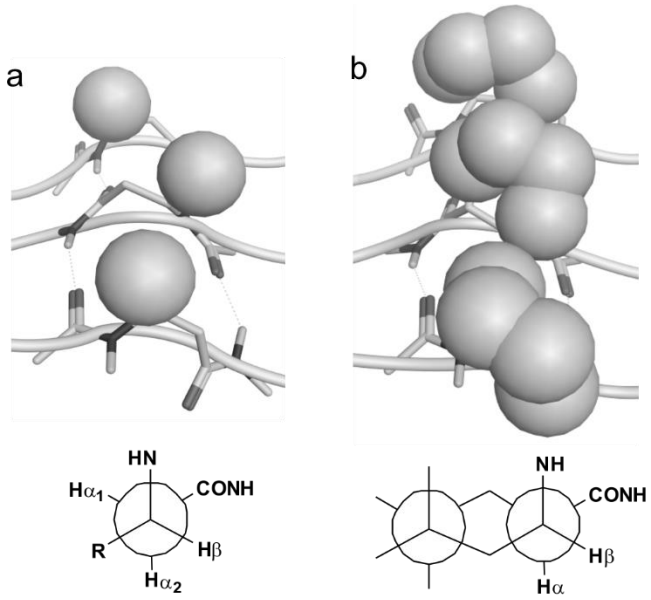


Fig. S1 a Representation of mismatched side-chain overlap due to in-registry β^3-amino acid substitutions. **b** Hydrophobic cluster provided by the constrained cyclic amino acids. The torsion angle θ of all β-amino acids is *gauche* (+) as shown below**,** maintaining the original peptide bond orientation and hydrogen-bond pattern.

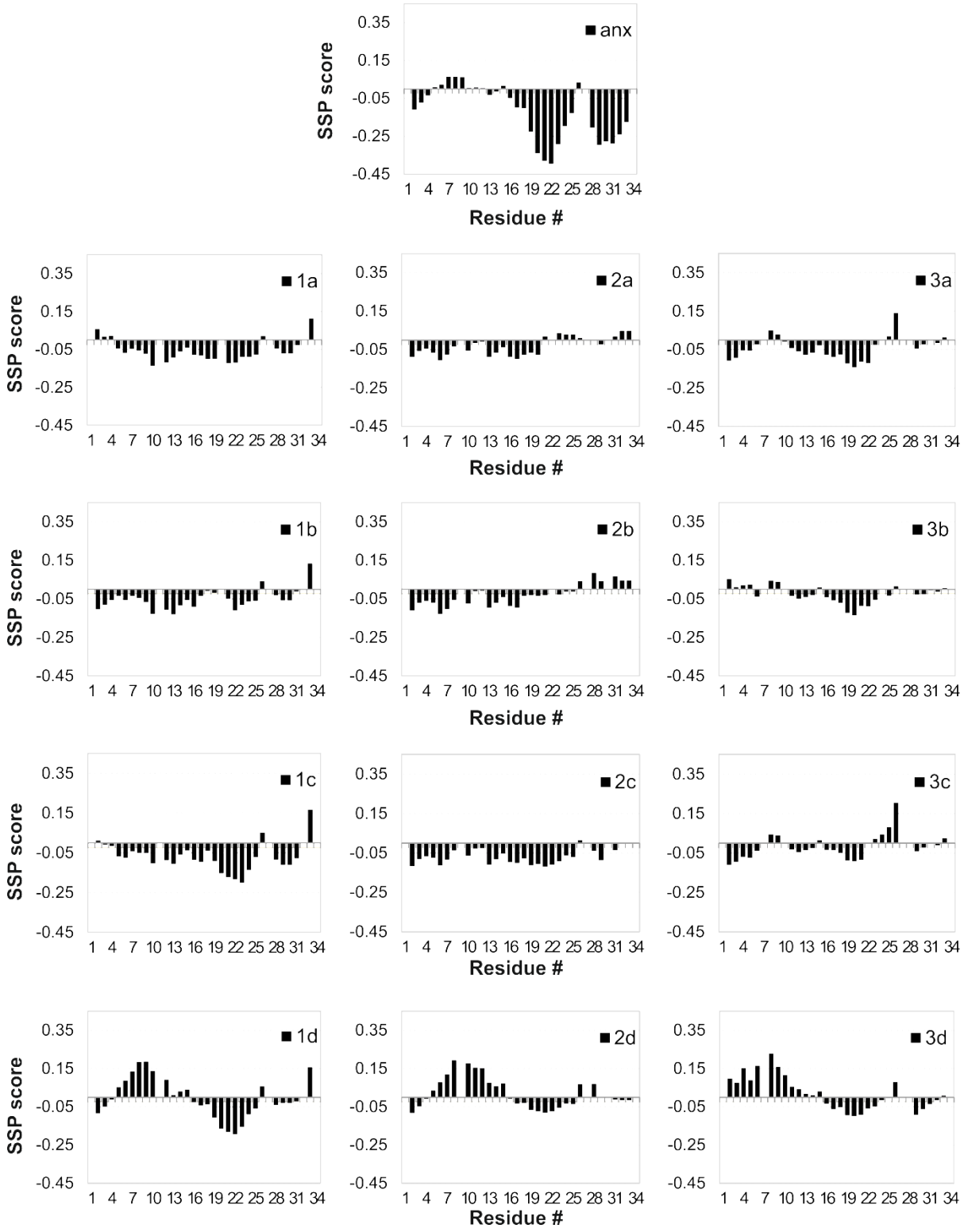


Fig. S2 SSP scores of anginex and analogues. Positive values correspond to a helical, and negative values to a β-sheet propensity. A1, G27 andG34 were excluded from the calculations because of the missing resonances and poor indication of a β-sheet. β-Amino acids were also excluded, due to the lack of reference values.

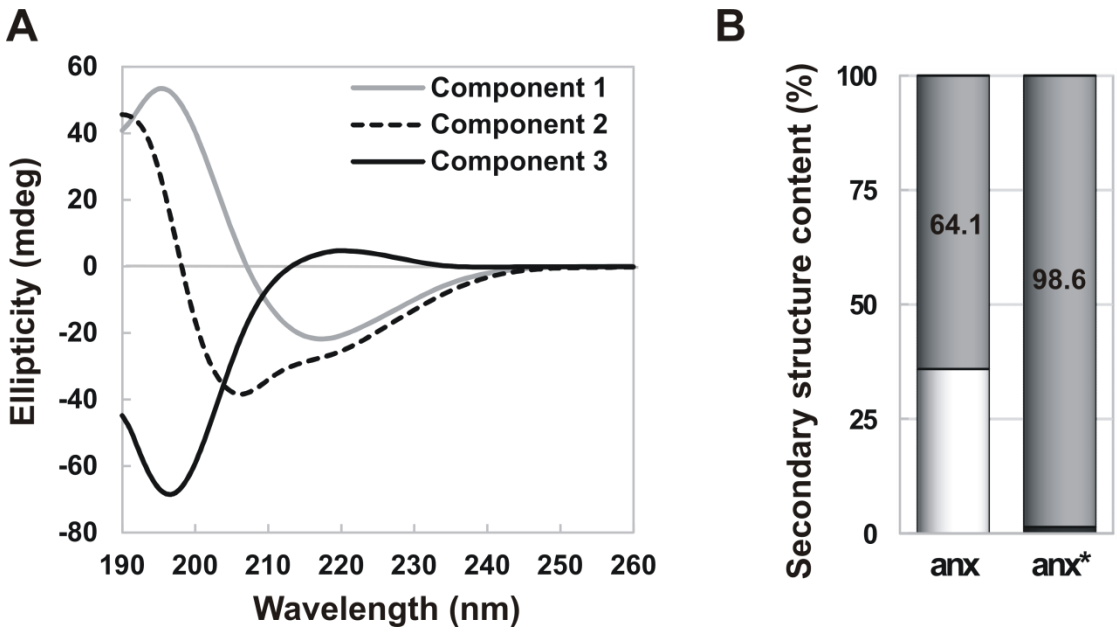


Fig. S3 A Results of the CCA+ deconvolution to 3 components. Components were assigned as follows: component 1: β-sheet; component 2: short helix or turn segments; component 3:random coil. **B** Secondary structure content of anginex (**anx**) before and after induction with 2.5 mM DPC (**anx***). Black, grey and white bars represent random coil, β-sheet, and helix/turn content, respectively.

Table S1 Secondary structure components for all spectra after CCA+ calculation with RMSD error values; Component numbering is as in Figure S2, induction with 2.5 mM DPC is denoted by *.

	T (°C)	Components (%)			NRMSD (%)
		1	2	3	
1a	37	29	8	63	1.1
	37*	41	3	56	10.2
	5	27	0	73	1.2
	10	26	2	72	1.6
	15	27	4	69	1.8
	20	27	5	68	1.0
	25	27	6	67	1.2
	30	29	6	66	0.8
	35	28	7	65	0.6
	40	29	9	63	0.5
	45	30	9	61	0.7
	50	31	10	60	0.7
	55	31	9	60	0.6
	60	32	8	60	1.0
	65	32	10	58	0.8
	70	32	10	58	0.9
	75	33	10	57	1.1
1b	37	23	13	64	0.9
	37*	28	20	53	3.1
	5	18	5	78	0.6
	10	17	7	75	0.5
	15	18	9	73	0.4
	20	19	10	72	1.0
	25	19	12	69	0.5
	30	20	13	68	0.7
	35	21	13	66	1.2
	40	21	14	65	0.8
	45	21	15	64	0.7
	50	23	15	62	1.1
	55	23	15	62	1.2
	60	24	16	60	0.9
	65	25	15	59	1.0
	70	27	15	58	1.3
	75	27	15	57	1.7
1c	37	59	3	38	0.9
	37*	69	1	30	2.1
	5	33	5	62	3.8
	10	33	6	62	2.8
	15	35	5	60	3.4
	20	38	3	59	2.6
	25	40	4	56	3.3
	30	41	7	52	2.8
	35	43	7	50	2.1
	40	45	7	48	1.9
	45	46	8	46	7.1
	50	47	10	44	0.9
	55	50	7	43	2.5
	60	49	9	42	0.5
	65	49	8	43	0.9
	70	48	9	44	0.7
	75	45	10	45	1.1

	T (°C)	Components (%)			NRMSD (%)
		1	2	3	
2a	37	23	15	62	0.6
	37*	21	26	54	1.3
	5	6	17	77	1.6
	10	6	18	76	1.7
	15	8	20	73	2.0
	20	9	20	72	1.6
	25	11	18	71	1.7
	30	12	20	69	1.3
	35	12	21	67	0.9
	40	14	21	65	0.8
	45	14	22	65	1.4
	50	16	22	62	1.1
	55	17	21	61	1.4
	60	18	22	60	1.9
	65	18	23	60	2.4
	70	19	23	58	2.4
	75	20	24	56	1.8
2b	37	33	7	61	4.1
	37*	45	11	44	13.7
	5	2	26	71	3.0
	10	2	27	71	1.8
	15	3	28	69	1.5
	20	6	28	66	1.2
	25	8	27	65	3.5
	30	9	27	64	1.9
	35	10	28	63	3.0
	40	9	30	60	2.2
	45	9	33	59	1.8
	50	10	31	59	3.3
	55	11	30	58	3.6
	60	12	32	56	3.3
	65	13	31	56	4.6
	70	14	32	55	4.5
	75	13	31	56	9.1
2c	37	20	19	61	0.4
	37*	68	7	25	2.1
	5	17	12	71	1.5
	10	17	14	70	1.0
	15	18	15	67	1.6
	20	20	15	66	0.6
	25	21	14	65	0.6
	30	22	16	63	1.2
	35	21	17	62	0.4
	40	21	19	60	2.1
	45	22	19	59	1.1
	50	23	20	57	0.6
	55	24	19	57	1.6
	60	25	19	56	2.1
	65	26	19	56	1.8
	70	27	19	55	2.2
	75	25	21	53	3.0

	T (°C)	Components (%)			NRMSD (%)
		1	2	3	
3a	37	24	30	46	7.7
	37*	51	21	28	1.6
	5	14	15	70	3.5
	10	14	17	69	2.4
	15	17	18	66	3.1
	20	21	20	60	2.8
	25	25	21	55	4.3
	30	26	24	50	4.7
	35	29	22	48	4.2
	40	30	23	47	3.4
	45	32	20	48	2.5
	50	34	18	49	0.9
	55	33	19	49	1.4
	60	36	14	51	2.0
	65	36	12	52	3.1
	70	37	11	53	2.5
	75	37	10	53	2.8
3b	37	13	36	51	16.6
	37*	52	30	18	9.0
	5	23	9	68	0.8
	10	21	14	65	0.5
	15	23	14	63	1.0
	20	24	18	58	1.3
	25	27	17	56	1.0
	30	30	17	53	2.7
	35	30	17	52	3.3
	40	32	17	51	2.5
	45	33	16	51	1.4
	50	33	16	51	1.6
	55	37	15	49	0.5
	60	38	13	49	0.5
	65	38	13	49	0.8
	70	38	13	49	0.7
	75	38	13	49	0.6
3c	37	17	25	58	4.7
	37*	66	14	20	2.2
	5	8	16	77	1.2
	10	8	18	74	1.5
	15	10	18	72	1.7
	20	13	18	69	1.5
	25	16	17	67	1.2
	30	18	17	64	2.1
	35	19	19	62	1.6
	40	20	20	60	2.0
	45	19	22	59	1.4
	50	20	22	58	1.7
	55	21	20	59	2.6
	60	22	21	57	1.5
	65	22	23	55	1.1
	70	23	23	55	1.2
	75	23	21	56	2.9

1d	37	39	17	44	1.5
	37*	58	17	25	0.7
	5	19	19	62	1.5
	10	20	21	59	2.3
	15	20	23	57	2.2
	20	23	22	56	2.1
	25	25	21	55	1.4
	30	27	22	52	1.9
	35	30	21	49	2.1
	40	33	19	48	1.4
	45	34	21	45	1.6
	50	36	18	46	0.3
	55	38	17	45	0.3
	60	38	15	47	1.7
	65	38	14	48	2.1
	70	38	13	49	2.8
	75	37	16	48	2.9
anx	37	64	36	0	4.7
	37*	99	0	1	2.6
	5	42	21	37	1.9
	10	43	21	36	1.6
	15	43	22	34	1.3
	20	45	22	33	1.2
	25	48	21	31	1.2
	30	52	19	29	1.2
	35	55	19	26	0.9
	40	59	17	24	0.7
	45	61	16	23	0.8
	50	64	16	20	0.5
	55	67	16	17	0.4
	60	70	13	18	0.5
	65	69	11	20	0.8
	70	67	8	24	2.3
	75	62	4	34	2.5

2d	37	5	35	60	0.9
	37*	48	26	26	1.6
	5	0	30	70	1.1
	10	1	30	70	0.6
	15	1	31	68	0.2
	20	3	32	65	0.6
	25	4	31	65	0.4
	30	4	34	62	0.8
	35	6	34	61	0.7
	40	6	34	60	0.4
	45	7	34	59	0.5
	50	7	34	58	0.5
	55	9	34	58	0.5
	60	9	34	57	0.8
	65	9	34	57	2.1
	70	9	37	53	2.3
	75	11	33	56	2.5

3d	37	9	34	57	0.4
	37*	51	36	13	3.2
	5	8	24	69	0.2
	10	8	26	67	1.4
	15	8	29	64	0.6
	20	9	28	63	1.2
	25	10	28	62	1.0
	30	13	29	58	0.7
	35	14	30	56	0.9
	40	14	31	55	0.8
	45	14	30	56	1.6
	50	15	32	53	1.6
	55	15	32	53	1.5
	60	17	30	53	2.2
	65	18	31	52	2.2
	70	19	31	50	1.9
	75	19	30	52	3.4

Total average NRMSD: 1.9%

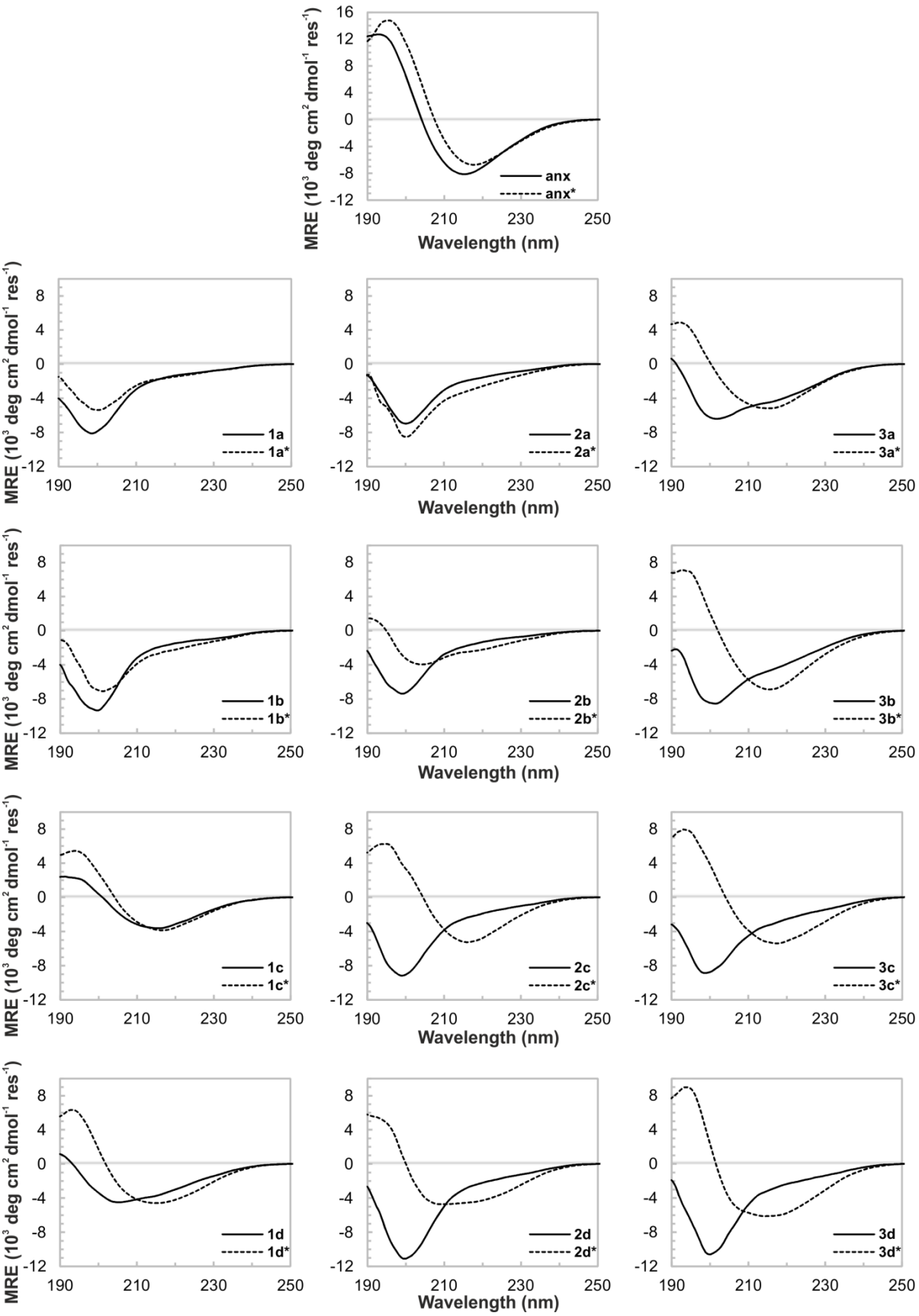


Fig. S4 CD spectra of anginex and analogue in 10 mM K-phosphate pH 5.6 buffer at 37 °C without induction (solid line) and with induction using 2.5 mM DPC (dashed line).

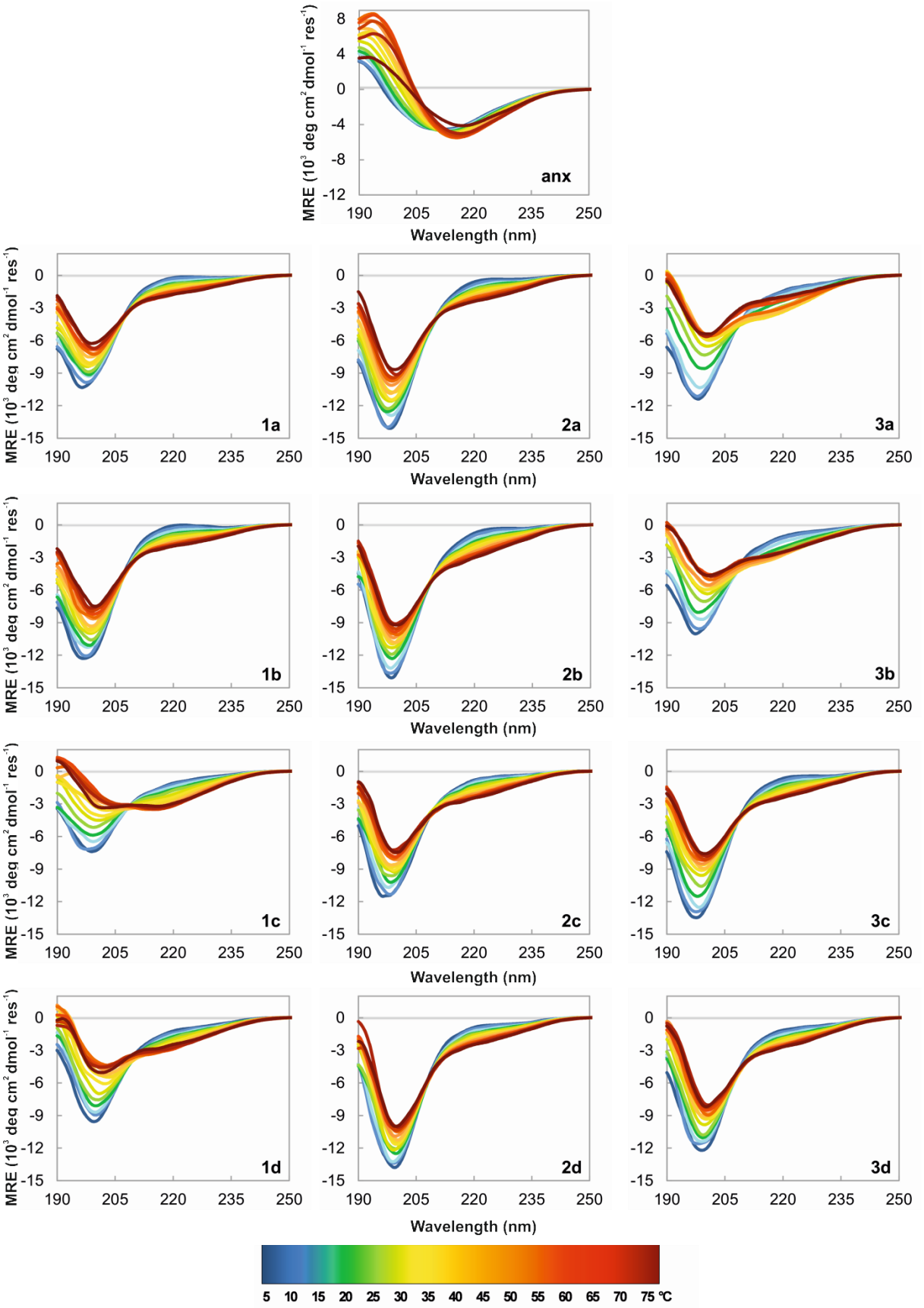


Fig. S5 Temperature-dependent CD curves of anginex and all analogues. Spectra were measured in 10 mM K-phosphate pH 5.6 at 100 μM peptide, in the range 5–75 °C.

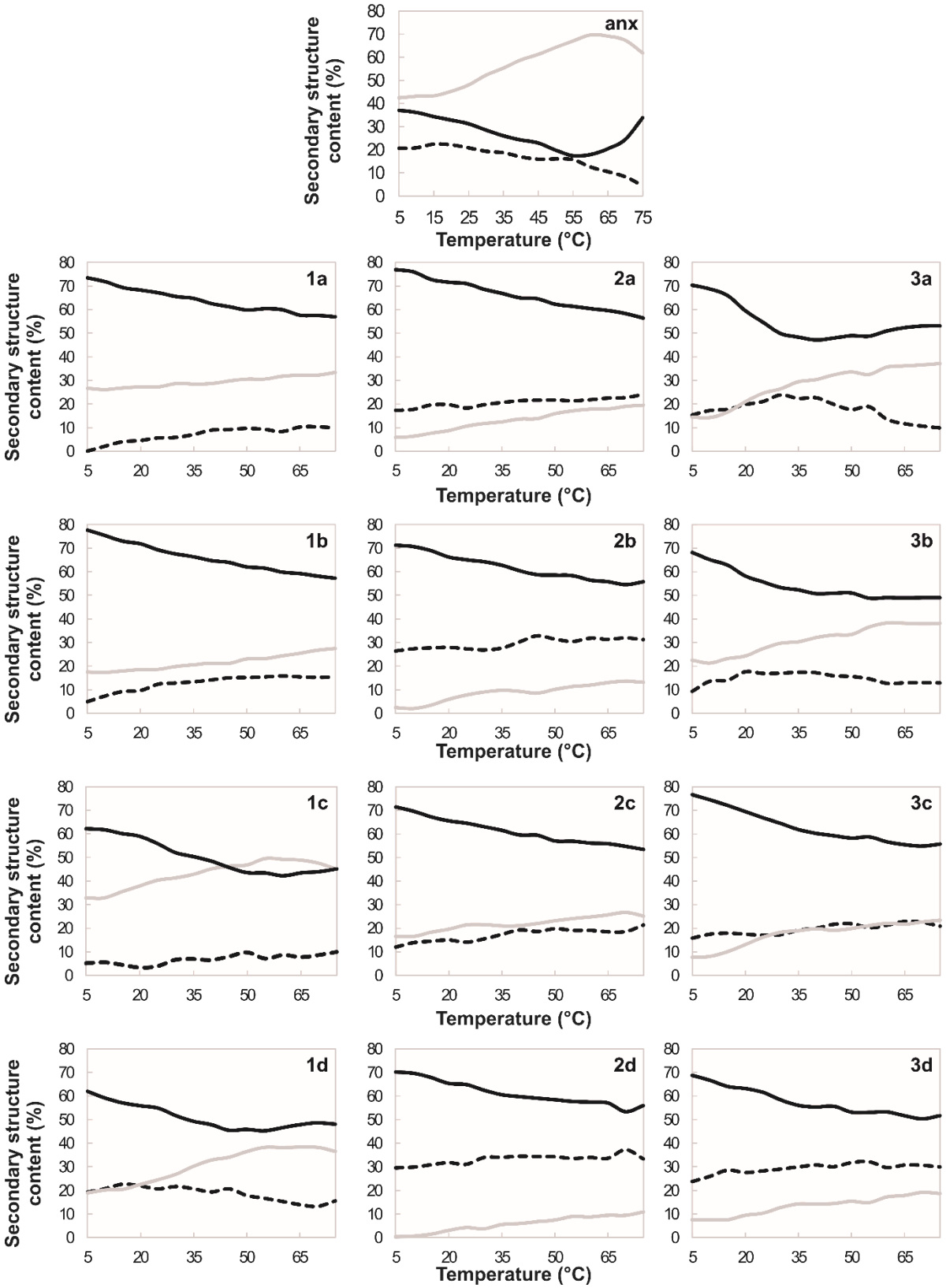


Fig. S6. CCA+ deconvolution results of temperature-dependent CD curves for anginex and analogues. Grey, dashed and black lines indicate β-sheet, helix/turn and random coil secondary structure content, respectively.

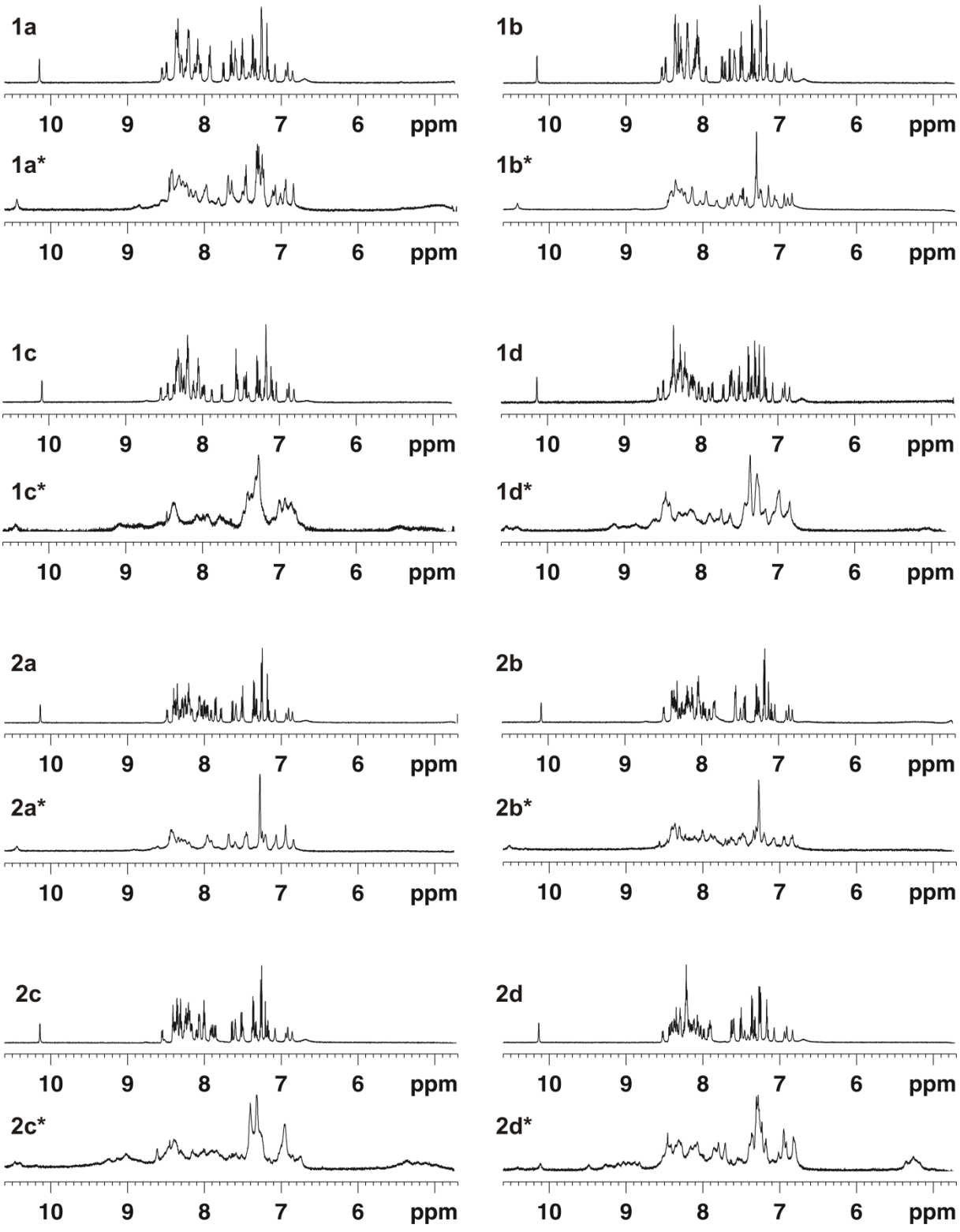


Fig. S7 1D NMR spectra of analogues **1ad** and **2ad** without and with induction with 12.5 mM DPC (denoted by *).

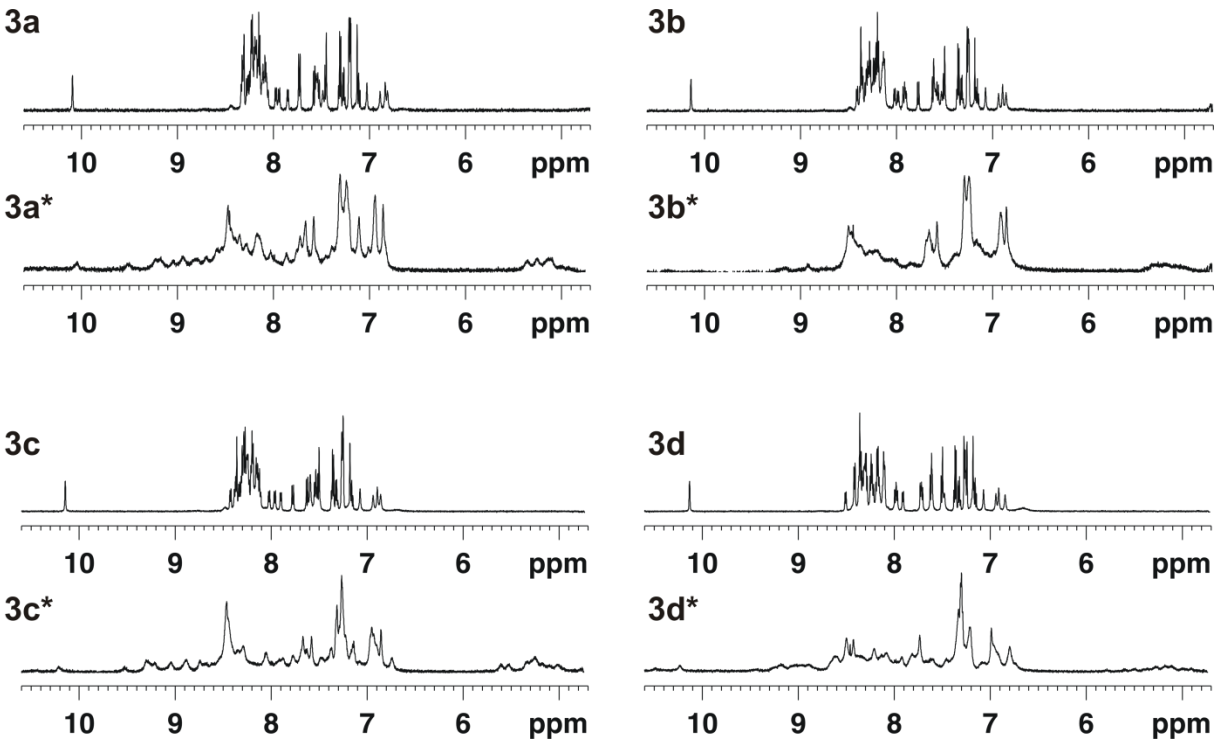


Fig. S8 1D NMR spectra of analogues **3a-d** without and with induction with 12.5 mM DPC (denoted by *).

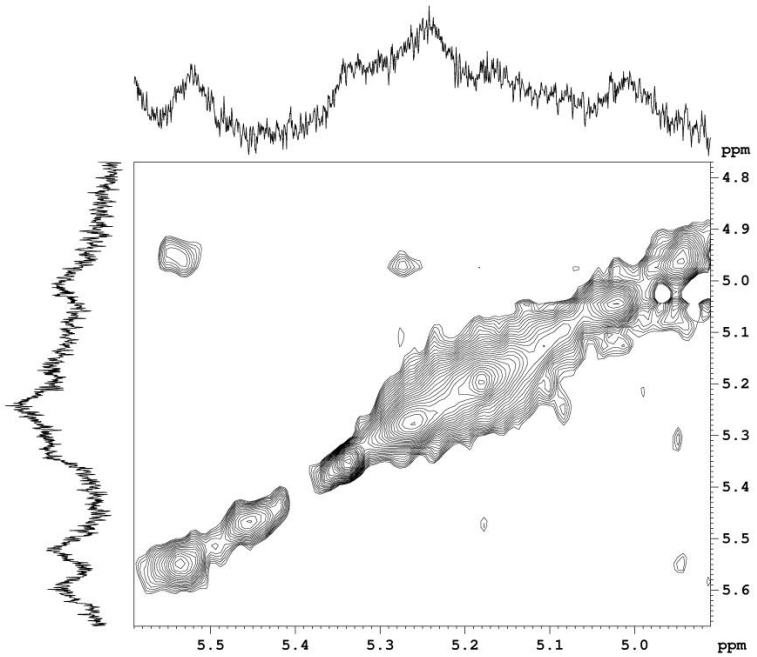


Fig. S9 2D NOESY NMR spectra of **3c** with 12.5 mM DPC, indicating Hα-Hα connections.

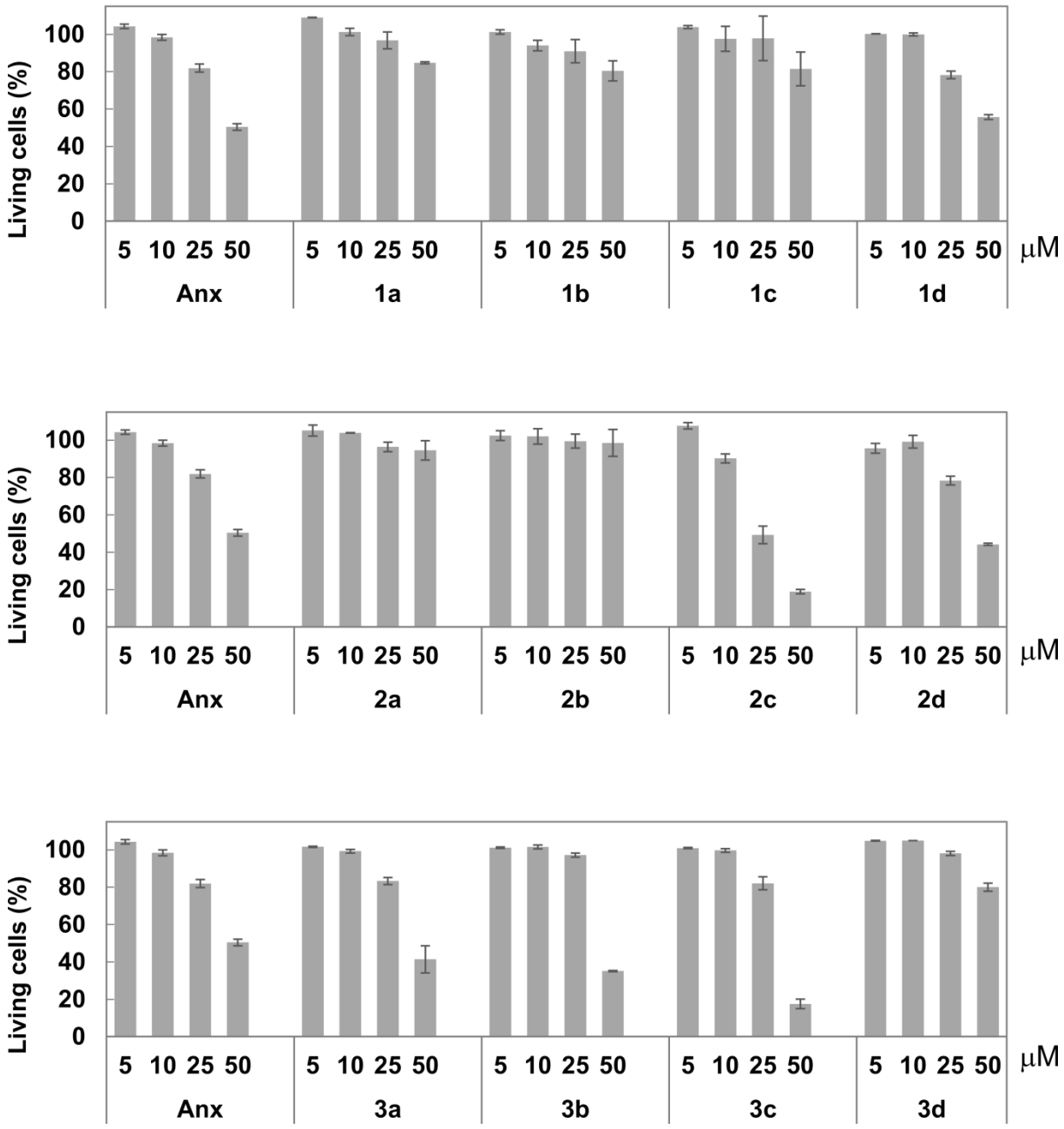


Fig. S10 Effects of anginex and its α/β analogues on the viability of the bEnd.3 mouse brain endothelial cell line. Cells were treated with or without the peptides for 30 min, and the cell viability was then measured by means of viability assay. The results were expressed as percentages of the living cells in the absence of peptides. The diagrams are representative of three independent experiments.

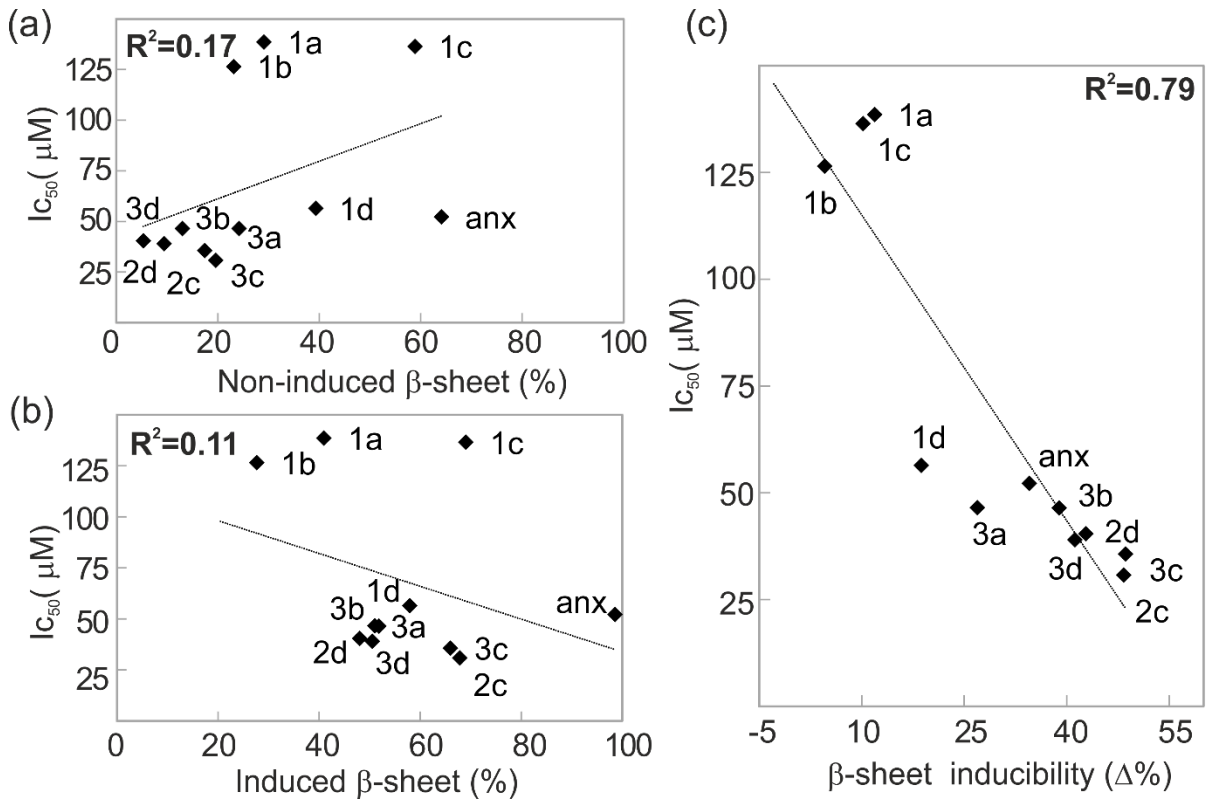


Fig. S11 Correlation between IC_{50} and β-sheet structure. IC_{50} was plotted against β-sheet content % calculated by using the CCA+ software. Correlations are illustrated with (a) the uninduced β-sheet content; (b) the β-sheet content induced by DPC; and (c) the β-sheet inducibility (the difference between the induced and uninduced β-sheet contents, expressed in Δ%).

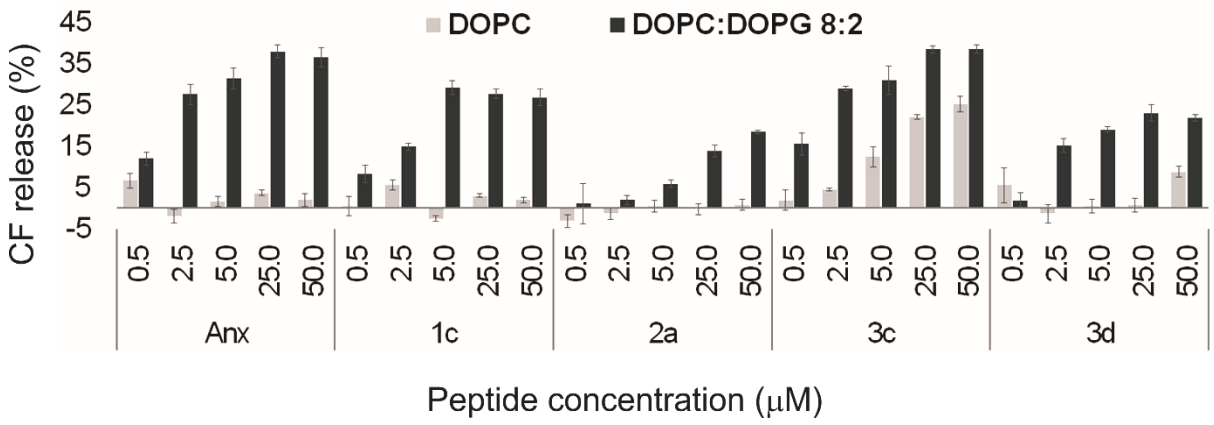


Fig. S12 Membrane leakage experiments. The integrity of the vesicles, indicated by the CF leakage % after peptide treatment of the neutral (DOPC; grey bars) and negatively charged DOPC:DOPG 8:2 (black bars) vesicles.

Peptide characterization

Lyophilized peptides were analysed by ESI MS and by analytical HPLC measurements on an Aeris Peptide XB-C18 4.6 x 250 mm column (Phenomenex) with a gradient from 5% A to 80% B over 25 min at a flow rate of 1.2 $mlmin^{-1}$. Eluents were: A: 0.1% TFA/water; B: 0.1% TFA/20% water/80% ACN. Peptide purity was >95% according to analytical HPLC measurements.

Abbreviations:

ACHC: (1*R,2S*)-2-aminocyclohexanecarboxylic acid

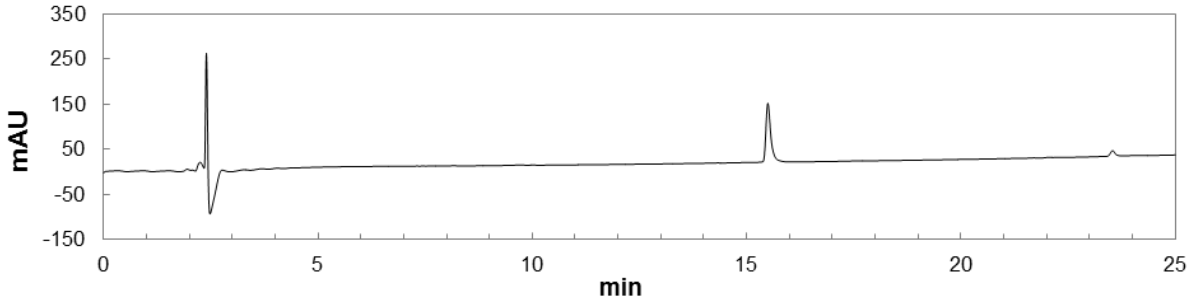
β^3X : β^3 homo amino acids, with single letter abbreviation for the corresponding side-chain in an amino acid

Table S3. Mass spectrometric data of the synthesized compounds

Com-pound	Molecular weight		Expected m/z values (based on monoisotopic mass)		Detected m/z	
	average	monoisotopic			Normal scan rate	Zoom scan – high resolution
anginex	3992.83	3989.31	$[M+3H]^{3+}$	1330.7773	1331.3608	1330.7400
			$[M+4H]^{4+}$	998.3348	999.0249	998.2600
			$[M+5H]^{5+}$	798.8693	799.5126	798.8200
			$[M+6H]^{6+}$	665.8923	666.4899	665.8800
1a	4027.83	4025.32	$[M+3H]^{3+}$	1342.7806	1343.7240	1342.7800
			$[M+4H]^{4+}$	1007.3373	1008.2393	1007.2400
			$[M+5H]^{5+}$	806.0713	807.0386	806.0000
			$[M+6H]^{6+}$	671.8939	672.8059	671.8800
1b	4029.84	4027.34	$[M+3H]^{3+}$	1343.4473	1344.0985	1342.4600
			$[M+4H]^{4+}$	1007.8373	1008.7455	1007.8000
			$[M+5H]^{5+}$	806.4713	807.3943	806.4200
			$[M+6H]^{6+}$	672.2273	673.1611	672.3400
1c	4015.32	4013.37	$[M+3H]^{3+}$	1338.7973	1339.3097	1338.8000
			$[M+4H]^{4+}$	1004.3498	1005.2993	1004.2800
			$[M+5H]^{5+}$	803.6813	805.0835	803.6000
			$[M+6H]^{6+}$	669.9023	671.3417	669.8800
1d	4019.75	4017.36	$[M+3H]^{3+}$	1340.1273	1341.3273	1340.1200
			$[M+4H]^{4+}$	1005.3473	1006.3163	1005.3000
			$[M+5H]^{5+}$	804.4793	805.3724	804.4600
			$[M+6H]^{6+}$	670.5673	671.6081	670.5600
2a	4023.81	4021.38	$[M+3H]^{3+}$	1341.4673	1342.3307	1341.4800
			$[M+4H]^{4+}$	1006.3523	1007.1492	1006.3200
			$[M+5H]^{5+}$	805.2833	806.1402	805.2000
			$[M+6H]^{6+}$	671.2373	672.0614	671.2000
2b	4011.80	4009.38	$[M+3H]^{3+}$	1337.4673	1338.6293	1337.4600
			$[M+4H]^{4+}$	1003.3523	1004.4304	1003.2800
			$[M+5H]^{5+}$	802.8833	803.9897	802.8200
			$[M+6H]^{6+}$	669.2373	670.3300	669.3800
2c	3997.38	3995.37	$[M+3H]^{3+}$	1332.7973	1333.9139	1332.8200
			$[M+4H]^{4+}$	999.8498	1000.9415	999.7800
			$[M+5H]^{5+}$	800.0813	801.3040	800.0400
			$[M+6H]^{6+}$	666.9023	667.8305	666.8800
2d	4019.75	4017.36	$[M+3H]^{3+}$	1340.1273	1341.0403	1340.1600
			$[M+4H]^{4+}$	1005.3473	1006.2889	1005.3000
			$[M+5H]^{5+}$	804.4793	805.4843	804.4200
			$[M+6H]^{6+}$	670.5673	671.5819	670.5600
3a	3998.32	3996.32	$[M+3H]^{3+}$	1333.1139	1333.9195	1333.14
			$[M+4H]^{4+}$	1000.0873	1000.9251	1000.0400
			$[M+5H]^{5+}$	800.2713	801.2392	800.2400
			$[M+6H]^{6+}$	667.0606	668.3106	n.a.
3b	4000.84	3998.34	$[M+3H]^{3+}$	1333.7873	1334.9730	1333.7400
			$[M+4H]^{4+}$	1000.5923	1001.7108	1000.5000
			$[M+5H]^{5+}$	800.6753	801.9253	800.5400
			$[M+6H]^{6+}$	667.3973	671.8740	667.2600
3c	3986.81	3984.32	$[M+3H]^{3+}$	1329.1139	1330.1802	1329.0800
			$[M+4H]^{4+}$	997.0873	998.1621	996.9600
			$[M+5H]^{5+}$	797.8713	798.9590	797.8000
			$[M+6H]^{6+}$	665.0606	666.6560	664.8600
3d	4019.75	4017.36	$[M+3H]^{3+}$	1340.1273	1341.3465	1340.1200
			$[M+4H]^{4+}$	1005.3473	1006.4371	1005.2800
			$[M+5H]^{5+}$	804.4793	805.4998	804.4000
			$[M+6H]^{6+}$	670.5673	671.5690	670.7000

Anginex ANIKLSVQMKLFKRHLKWKRIVKLNDGRELSLDG-NH_2

MW: 3992.80, expected m/z values: **[M+3H]$^{3+}$**: 1331.44; **[M+4H]$^{4+}$**: 998.83; **[M+5H]$^{5+}$**: 799.27; **[M+6H]$^{6+}$**: 666.22; **[M+7H]$^{7+}$**: 571.19



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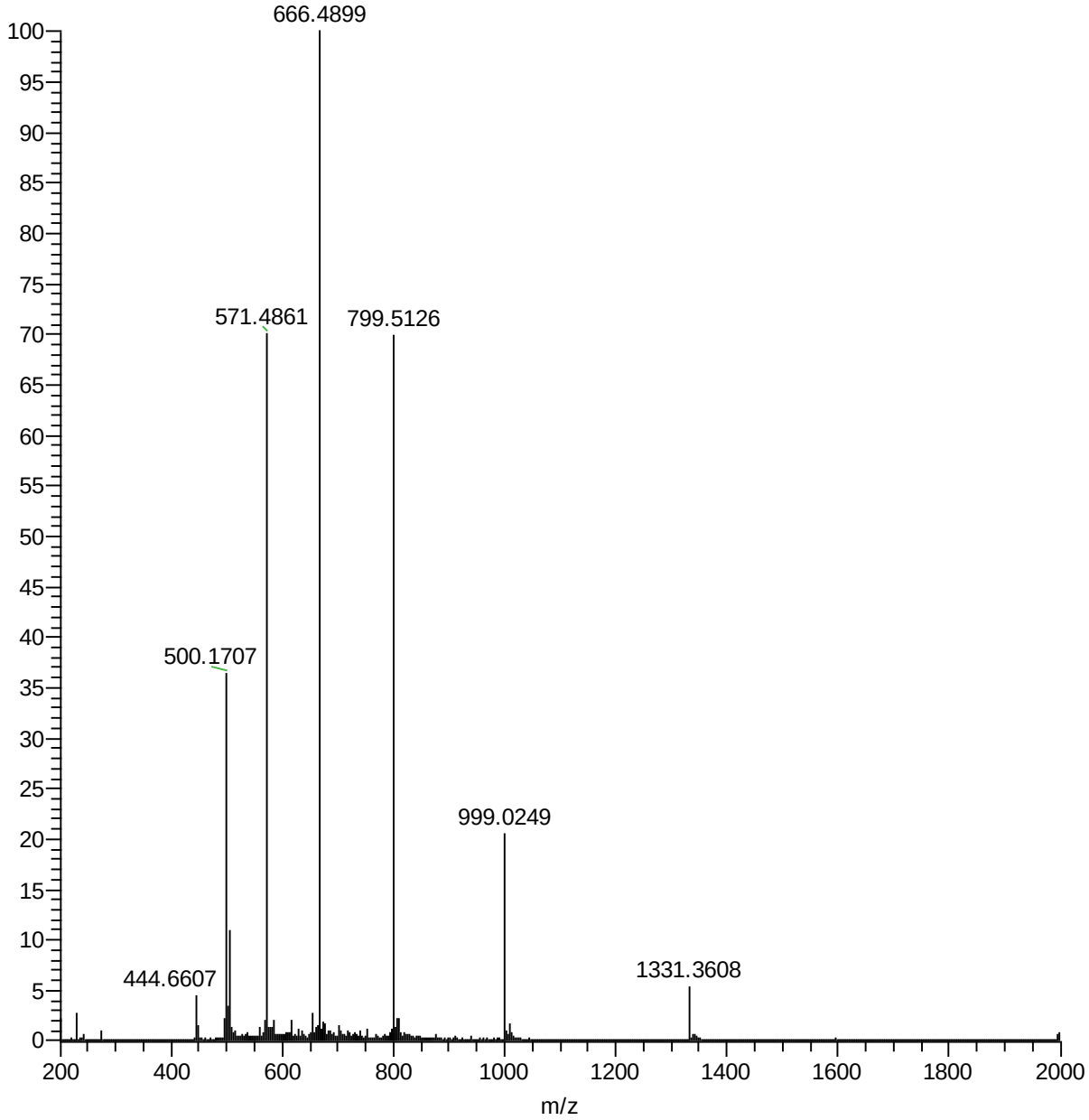
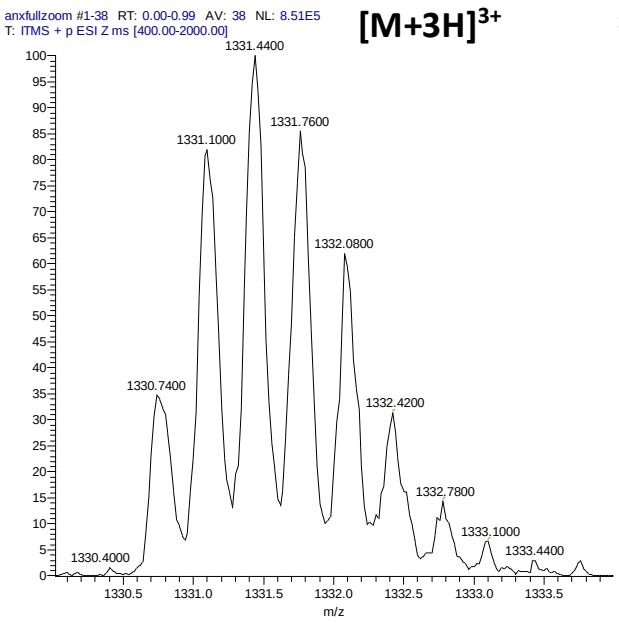
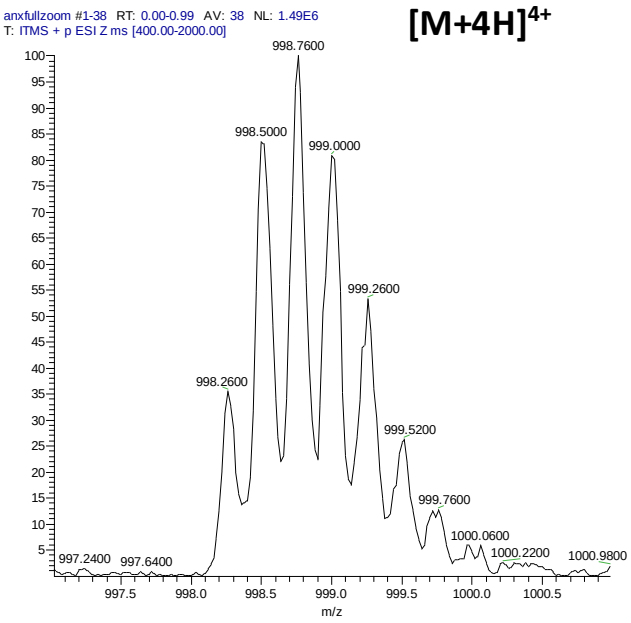
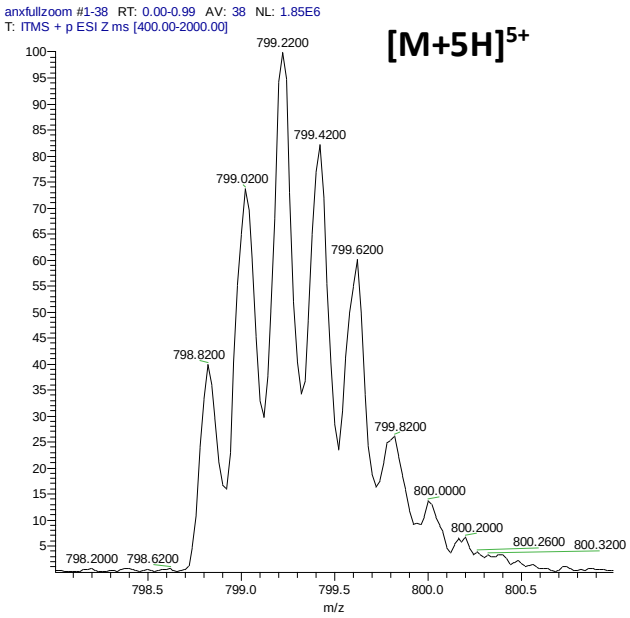
T: ITMS + c ESI Full ms [200.00-2000.00]

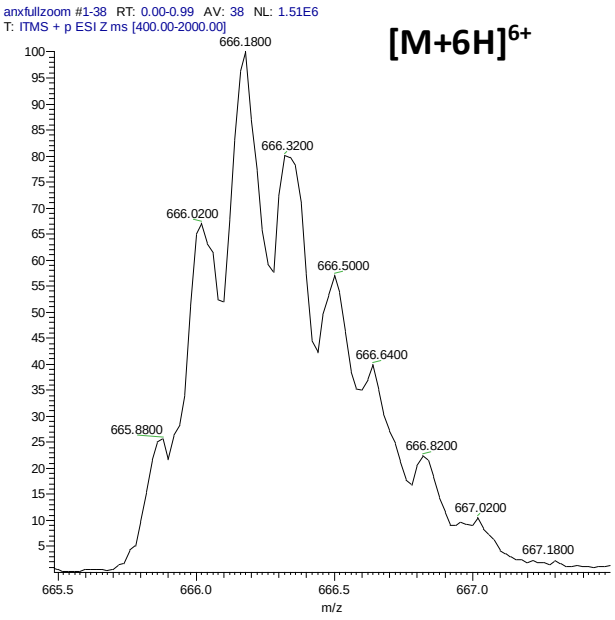


Anginex - High resolution mass spectra



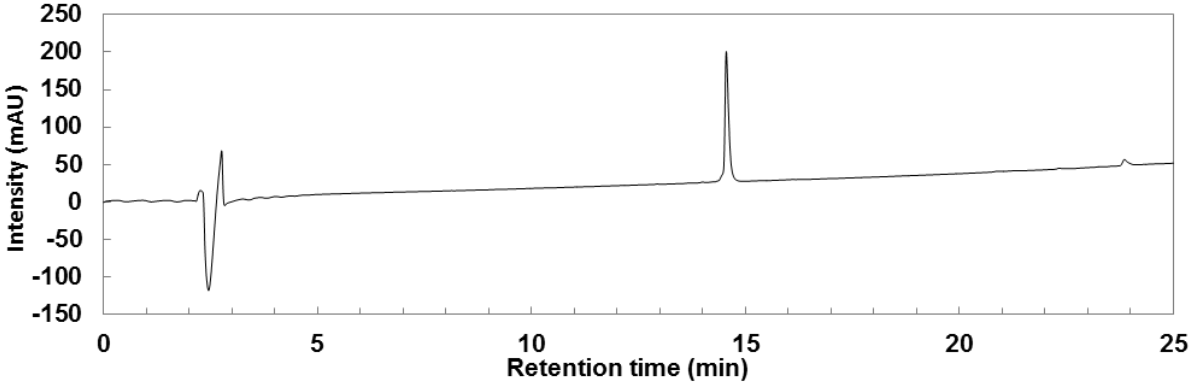


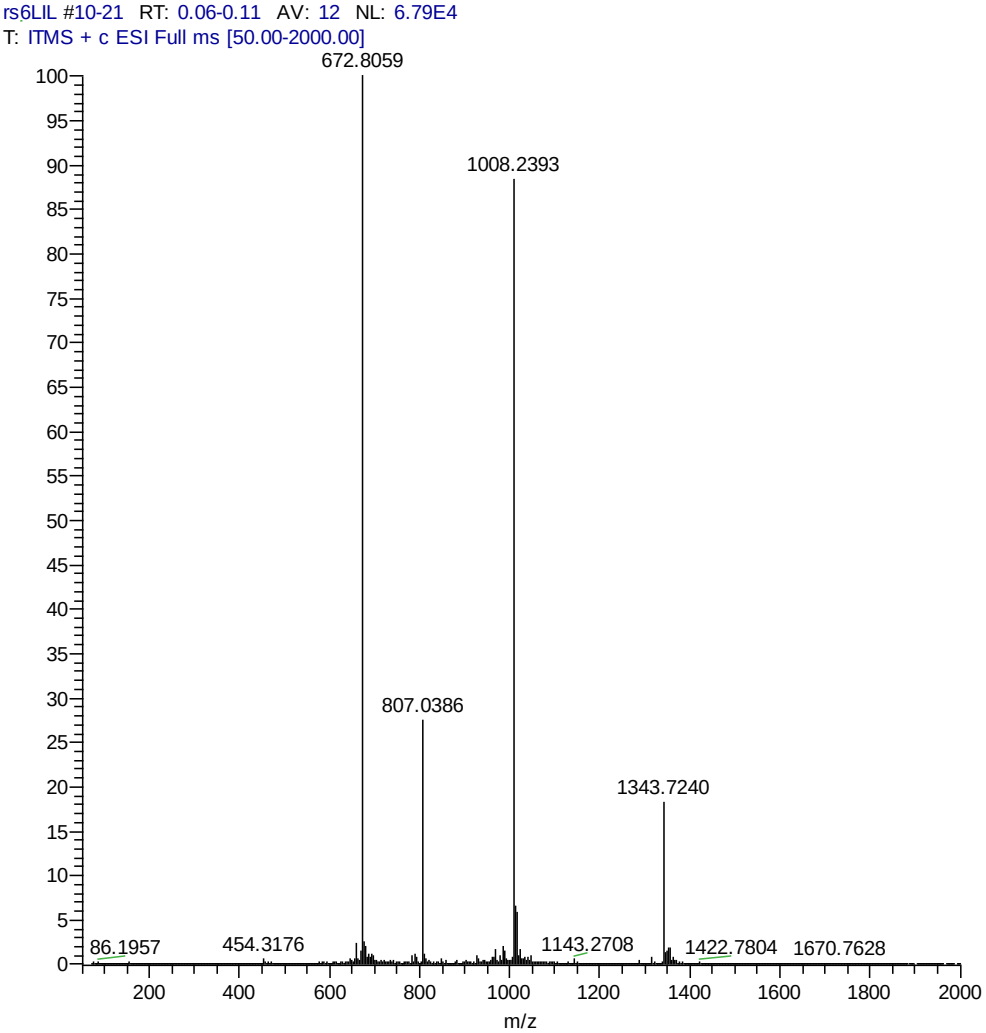




1a ANIKLSVQMK**(ACHC)**FKRHLKWK**(ACHC)**IVKLNDGRELS**(ACHC)**DG-NH_2

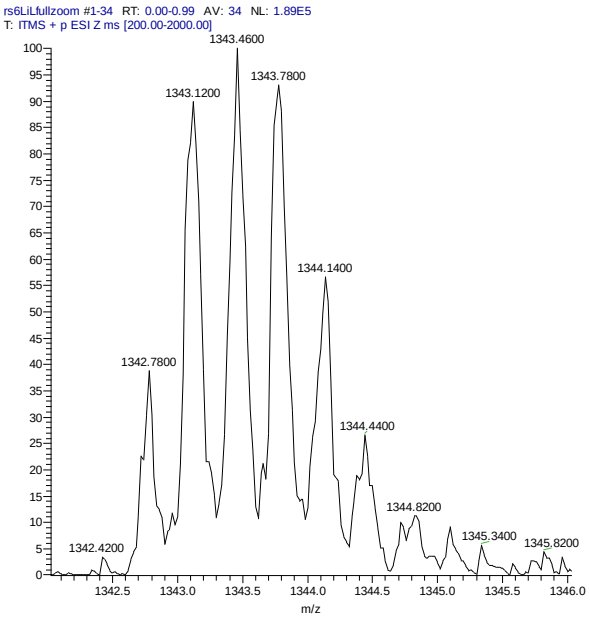
MW: 4027.83, expected m/z values: **[M+3H]$^{3+}$**: 1343.62; **[M+4H]$^{4+}$**: 1007.97; **[M+5H]$^{5+}$**: 806.57; **[M+H]$^{6+}$**: 672.31; **[M+7H]$^{7+}$**: 576.41



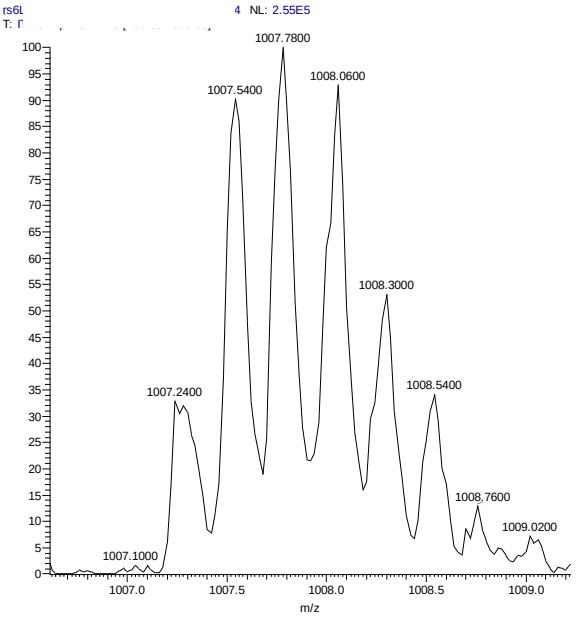


1a – High resolution mass spectra

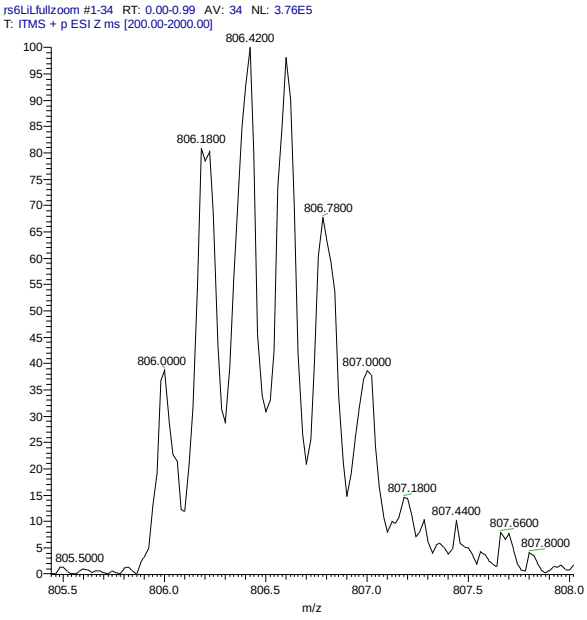
$[M+3H]^{3+}$



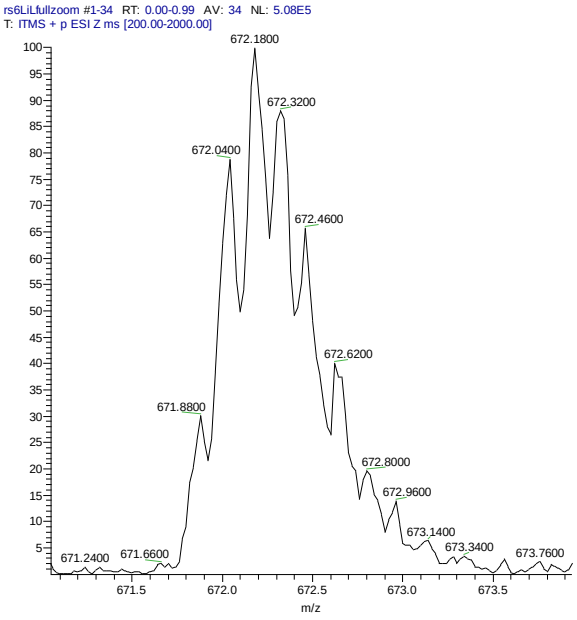
$[M+4H]^{4+}$



$[M+5H]^{5+}$

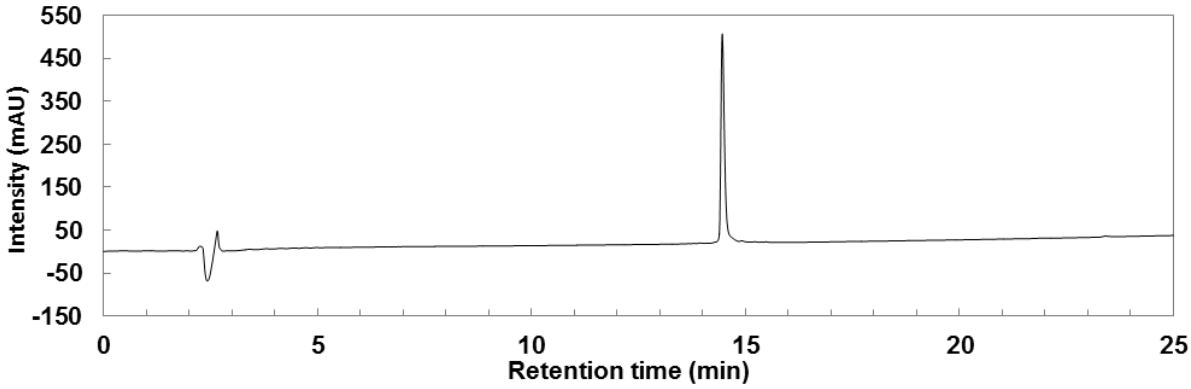


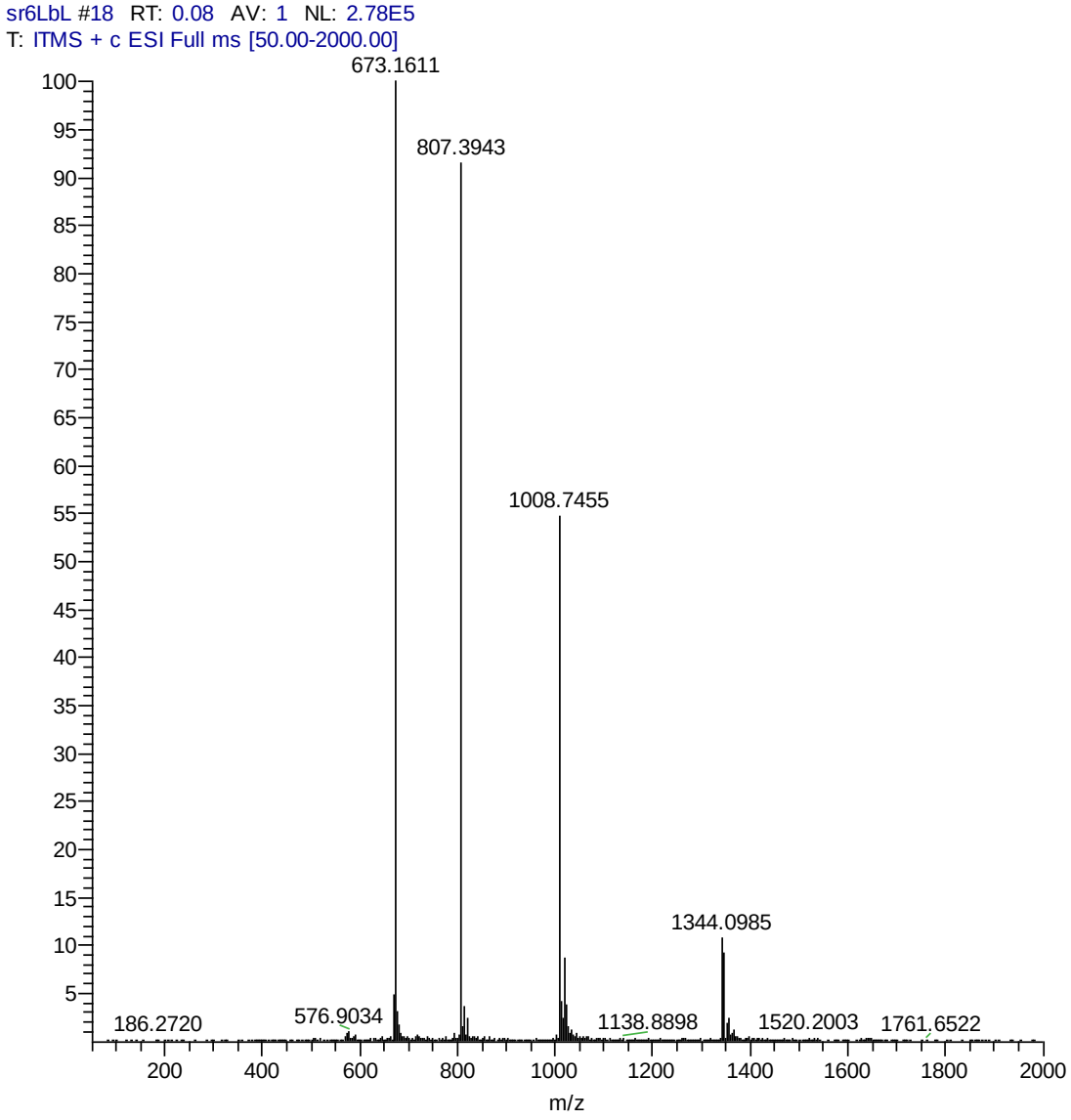
$[M+6H]^{6+}$



1b ANIKLSVQMK**(ACHC)**FKRHLKWK**β³**IIVKLNDGRELS**(ACHC)**DG-NH_2

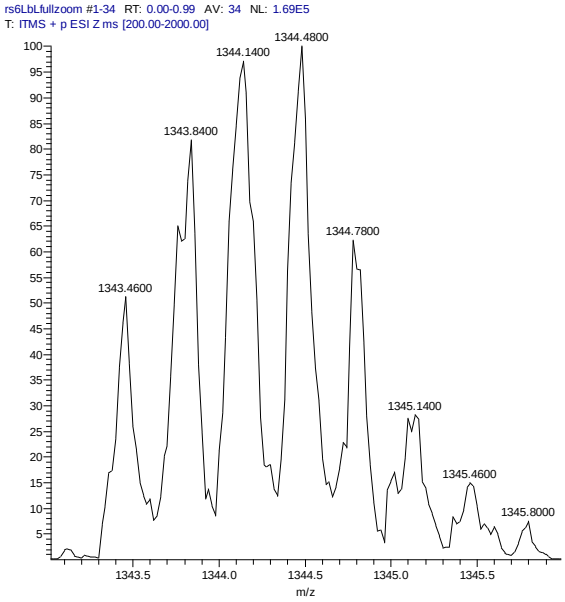
MW: 4029.84, expected m/z values: **[M+3H]³⁺**: 1344.29; **[M+4H]⁴⁺**: 1008.47; **[M+5H]⁵⁺**: 806.98; **[M+6H]⁶⁺**: 672.65; **[M+7H]⁷⁺**: 576.70



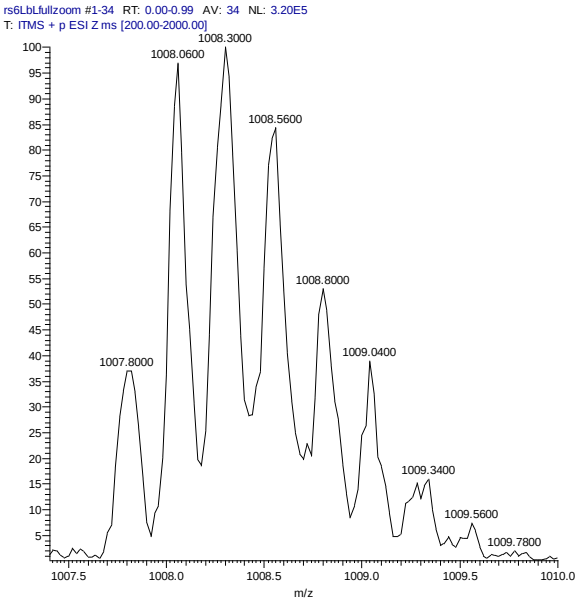


1b – High resolution mass spectra

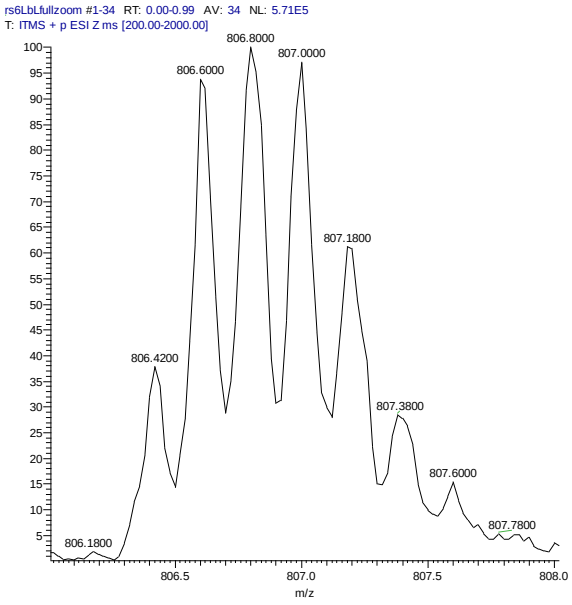
$[M+3H]^{3+}$



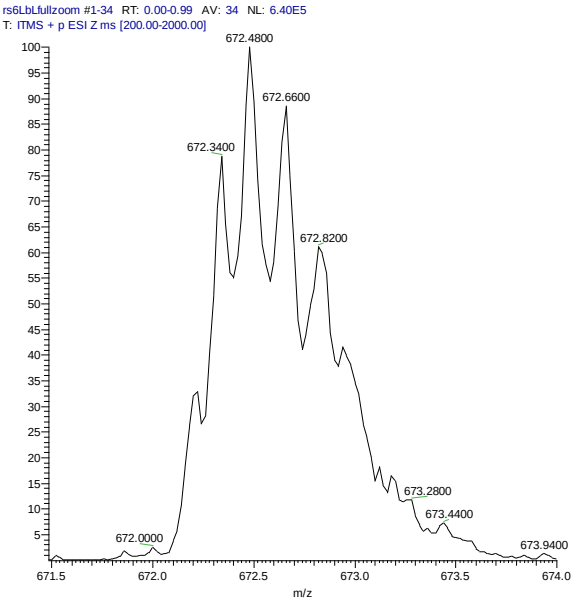
$[M+4H]^{4+}$



$[M+5H]^{5+}$

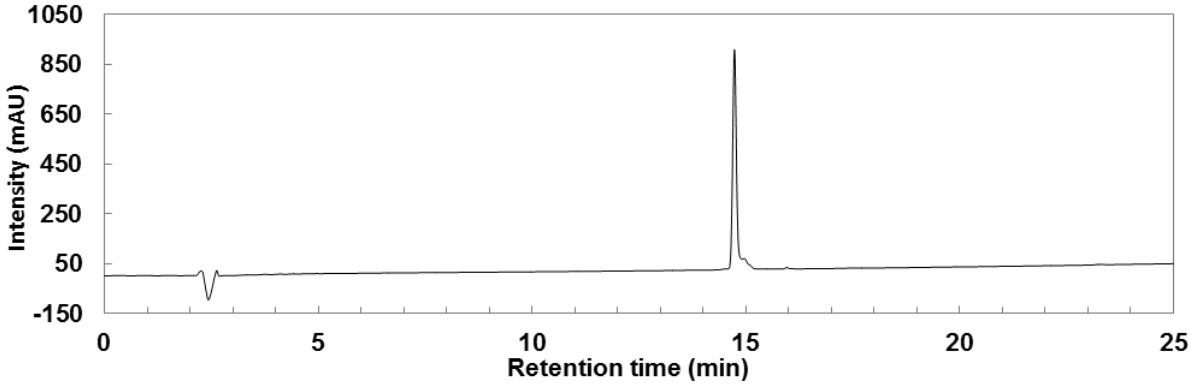


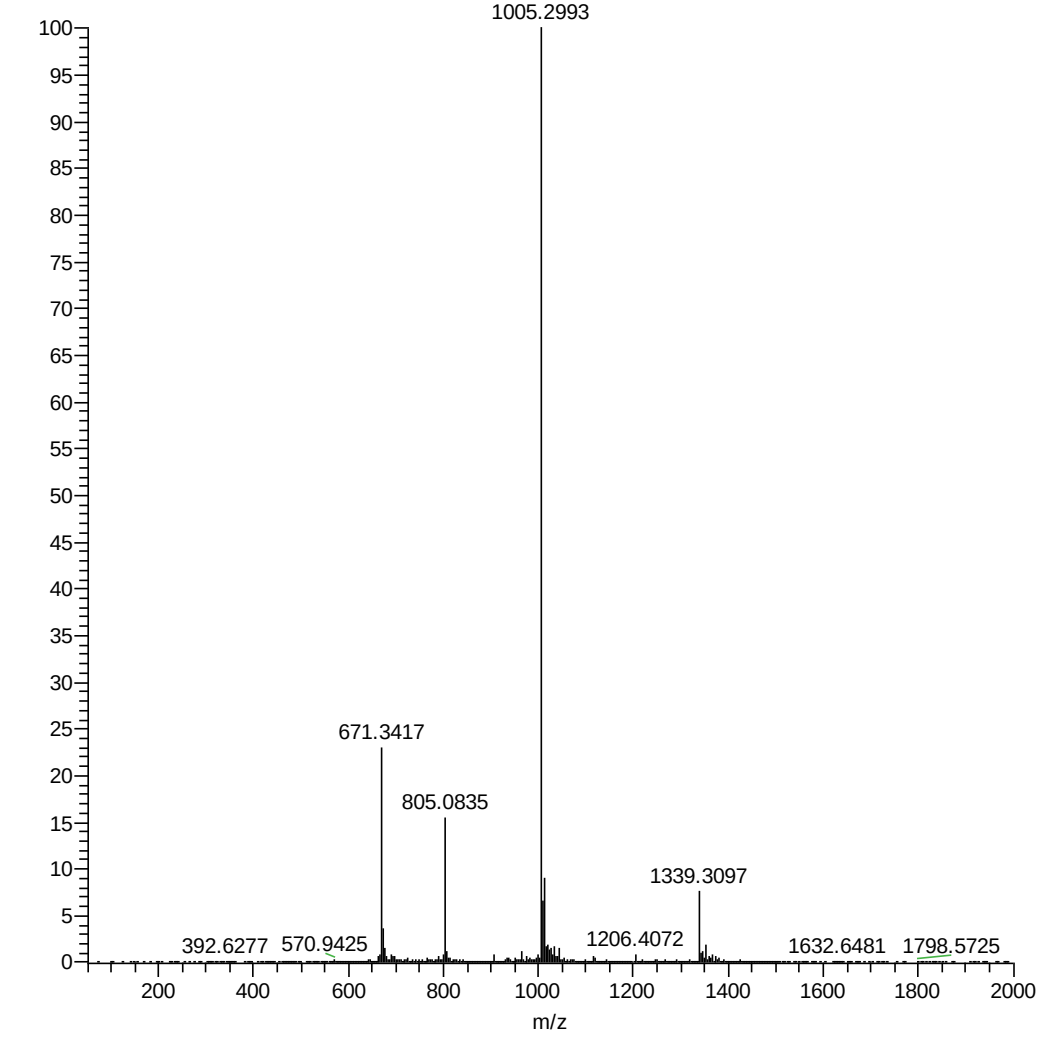
$[M+6H]^{6+}$



1c ANIKLSVQMK**(ACHC)**FKRHLKWKIIVKLNDGRELS**(ACHC)**DG-NH_2

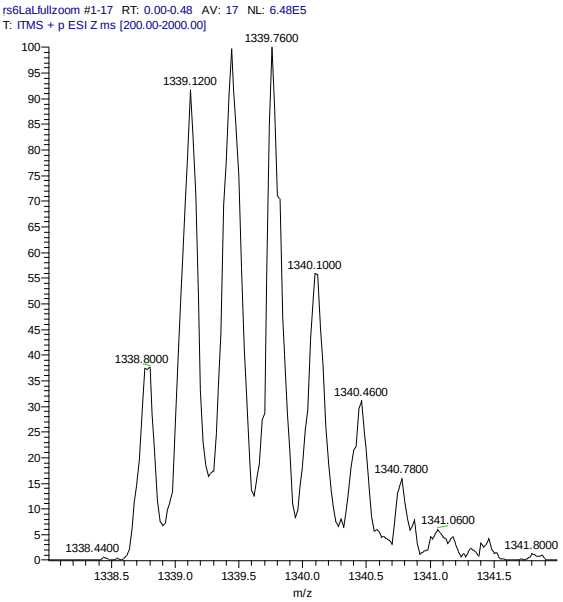
MW: 4015.32, expected m/z values: **[M+3H]$^{3+}$**: 1339.45; **[M+4H]$^{4+}$**: 1004.84; **[M+5H]$^{5+}$**: 804.07; **[M+6H]$^{6+}$**: 670.23; **[M+7H]$^{7+}$**: 574.62



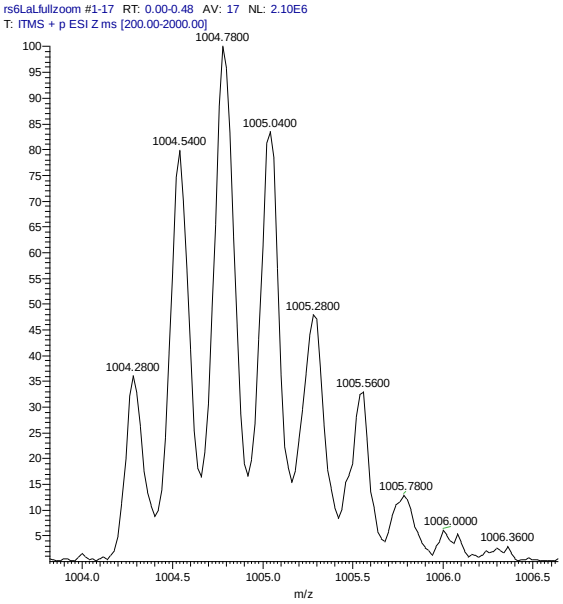


1c – High resolution mass spectra

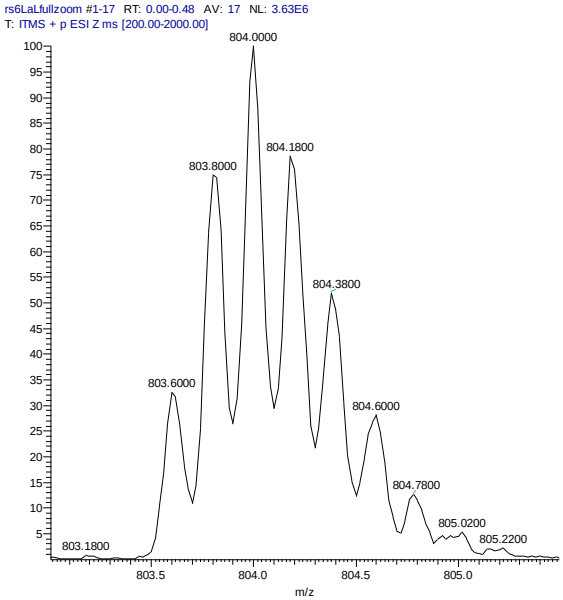
$[M+3H]^{3+}$



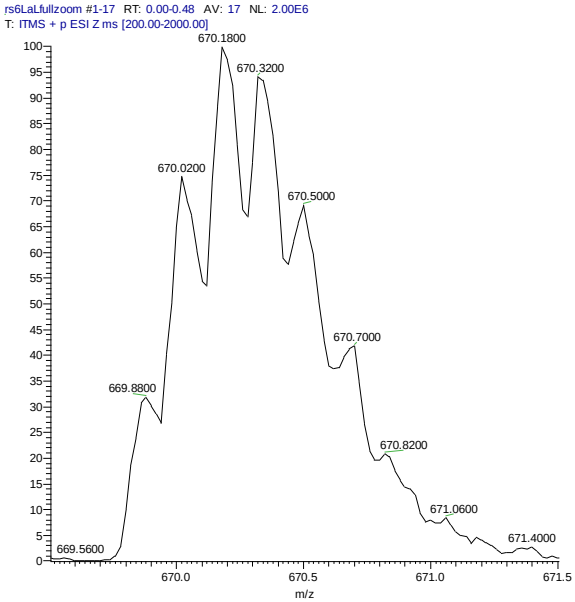
$[M+4H]^{4+}$



$[M+5H]^{5+}$

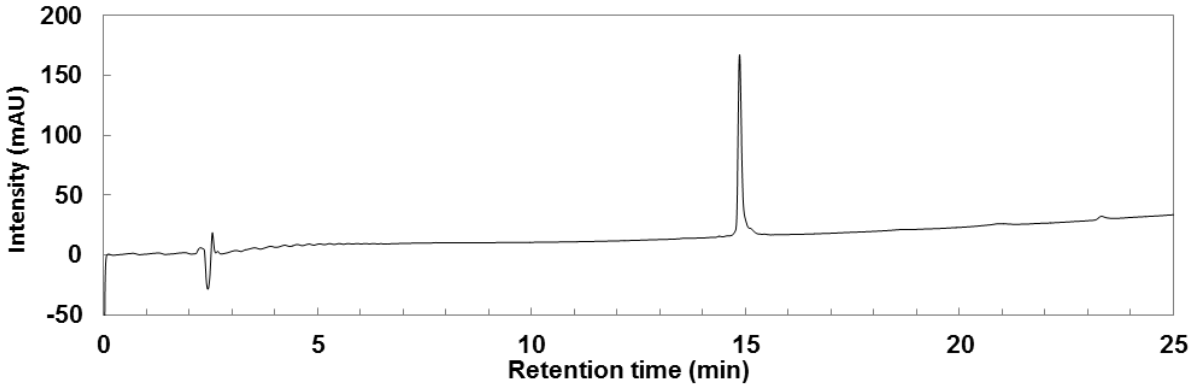


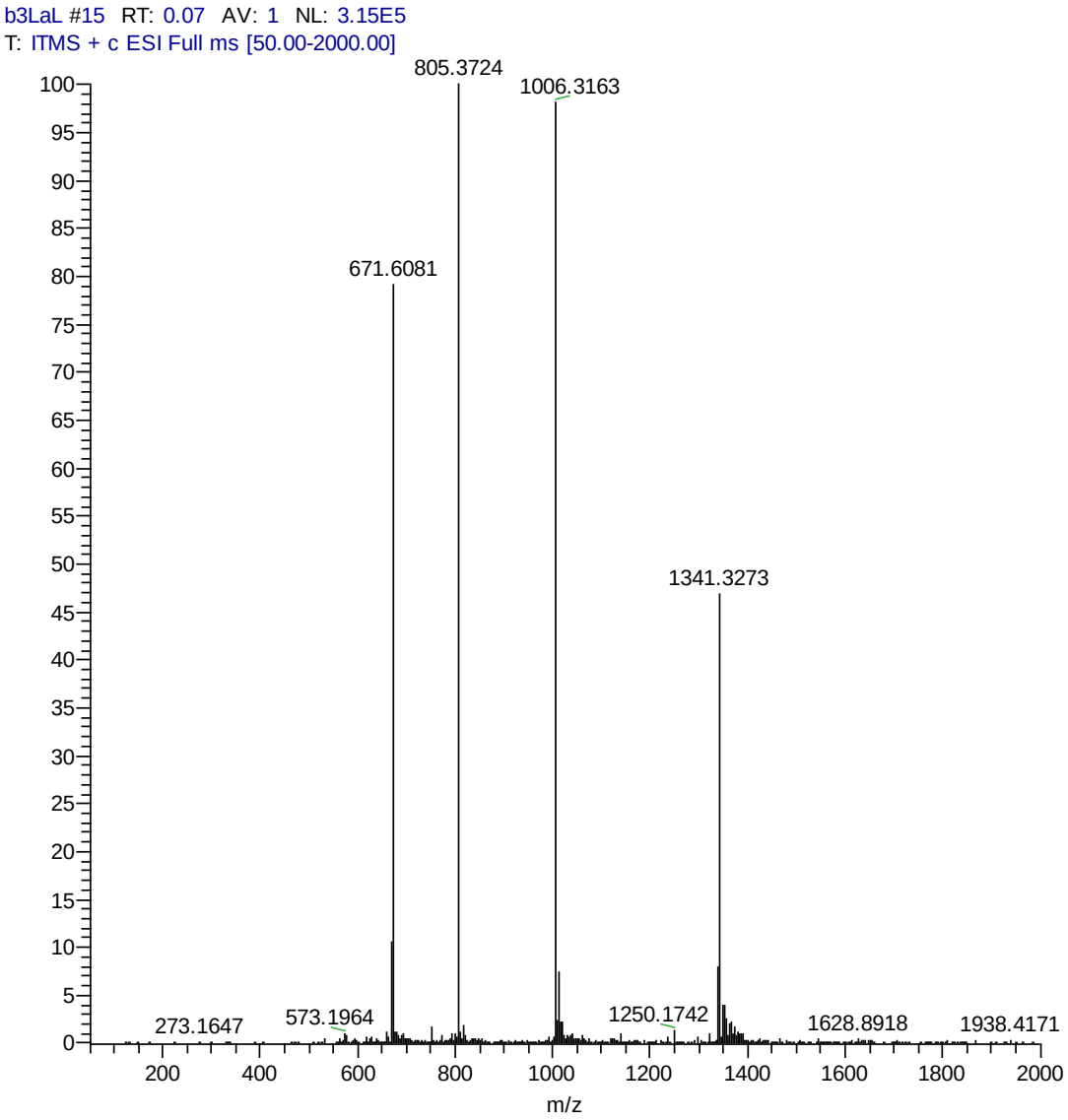
$[M+6H]^{6+}$



1d ANIKLSVQMKβ^3LFKRHLKWKIIVKLNDGRELSβ^3LDG-NH_2

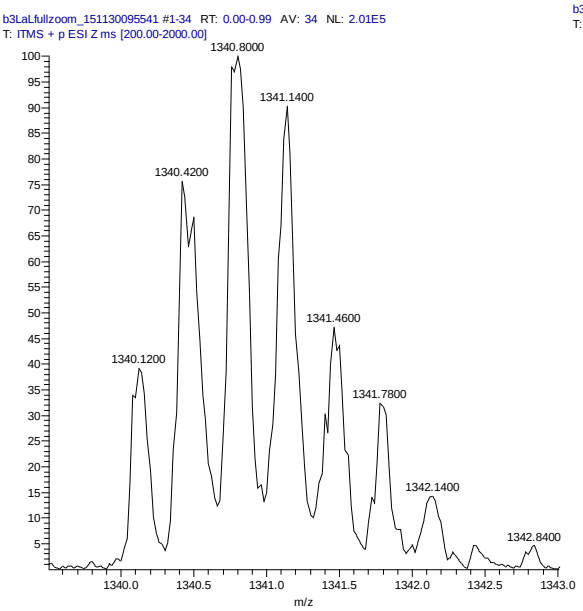
MW: 4020.07, expected m/z values: **[M+3H]$^{3+}$**: 1341.03; **[M+4H]$^{4+}$**: 1006.03; **[M+5H]$^{5+}$**: 805.02; **[M+6H]$^{6+}$**: 671.02; **[M+7H]$^{7+}$**: 575.30



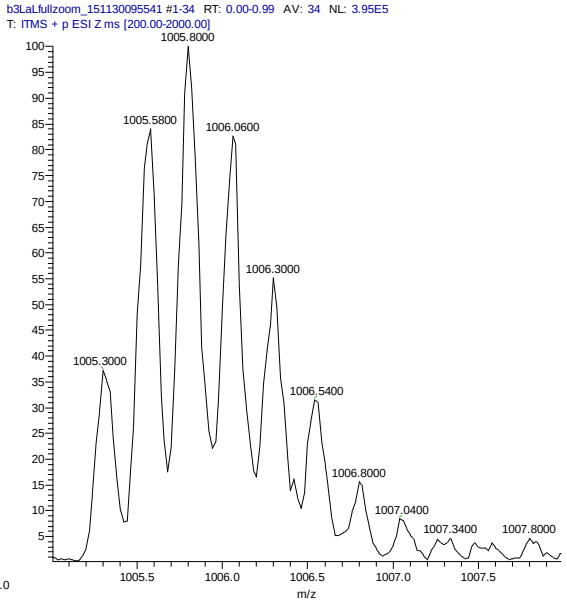


1d – High resolution mass spectra

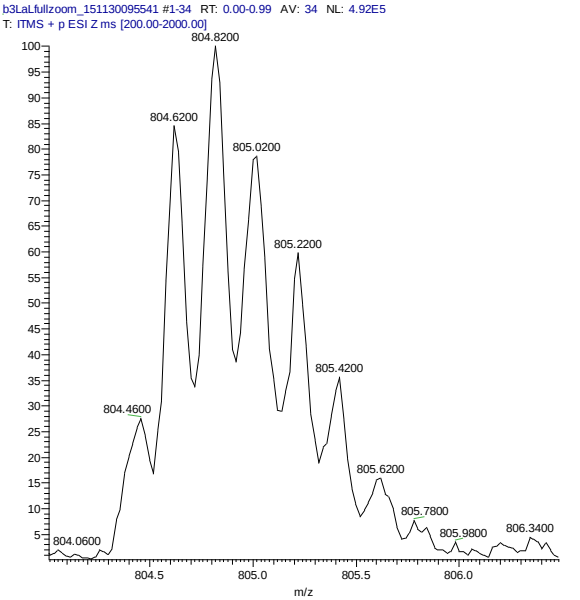
$[M+3H]^{3+}$



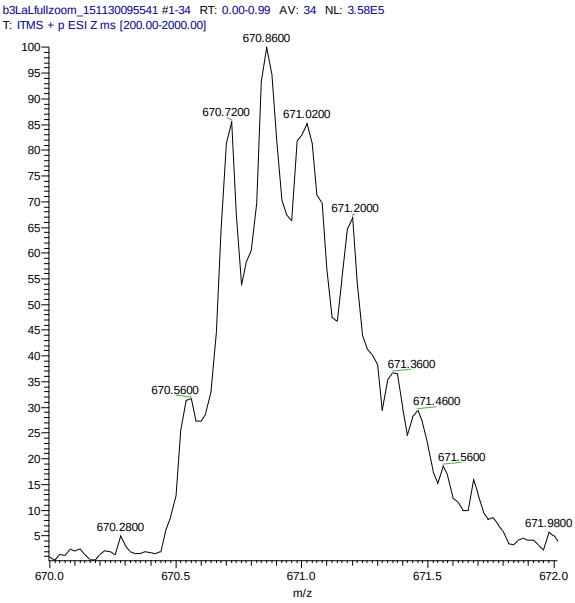
$[M+4H]^{4+}$



$[M+5H]^{5+}$

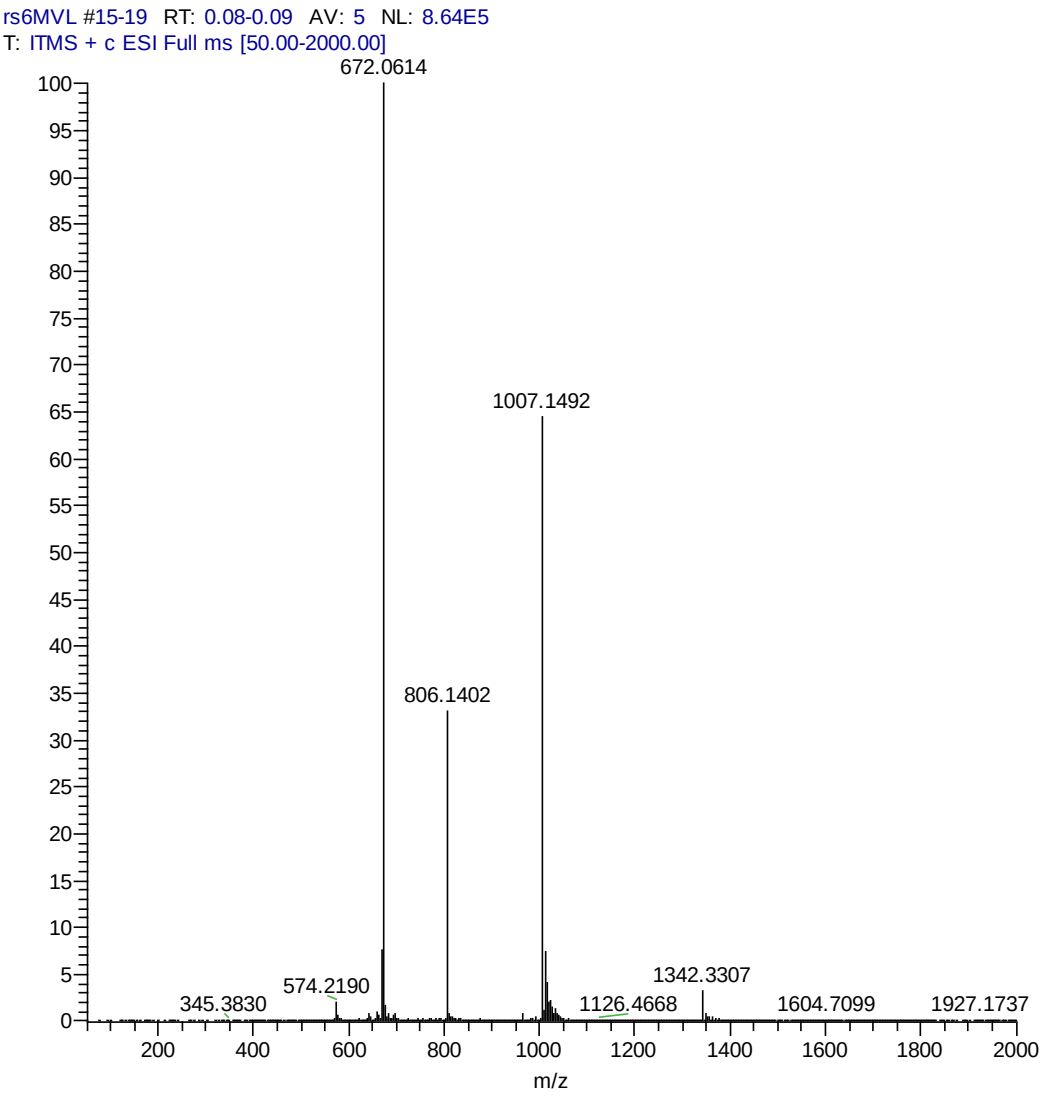


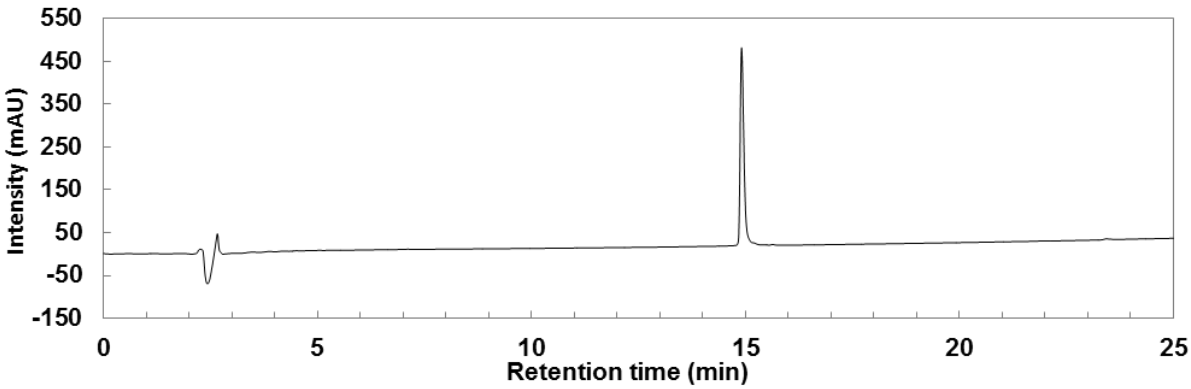
$[M+6H]^{6+}$



2a ANIKLSVQ**(ACHC)**KLFKRHLKWKII**(ACHC)**KLNDGRE**(ACHC)**SLDG-NH_2

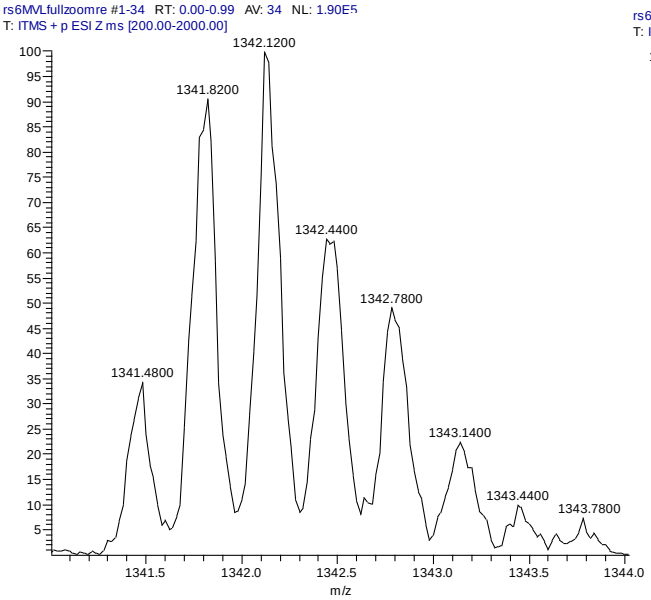
MW: 4023.81, expected m/z values: **[M+3H]$^{3+}$**: 1342.28; **[M+4H]$^{4+}$**: 1006.96; **[M+5H]$^{5+}$**: 805.77; **[M+6H]$^{6+}$**: 671.64; **[M+7H]$^{7+}$**: 575.84



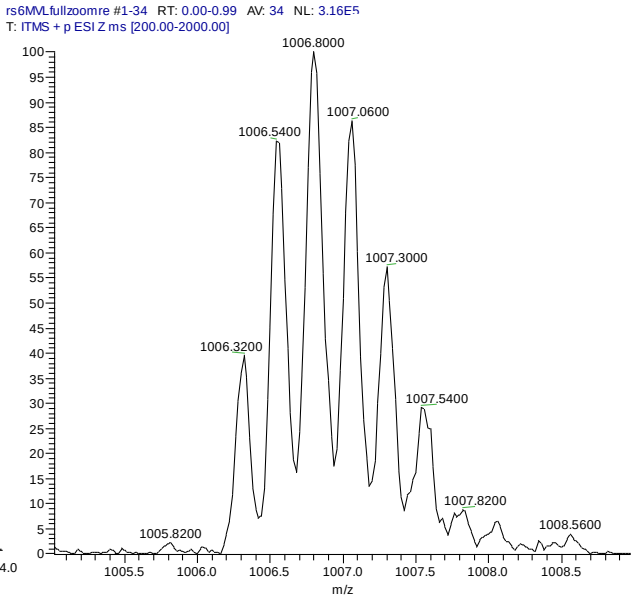


2a – High resolution mass spectra

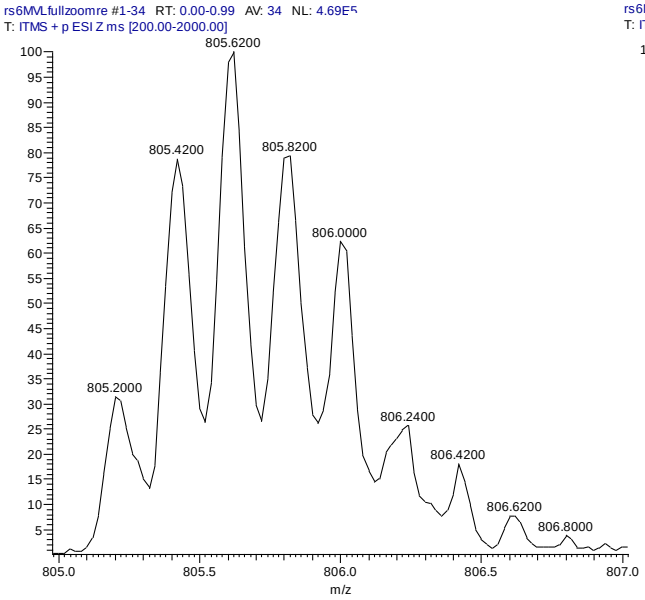
$[M+3H]^{3+}$



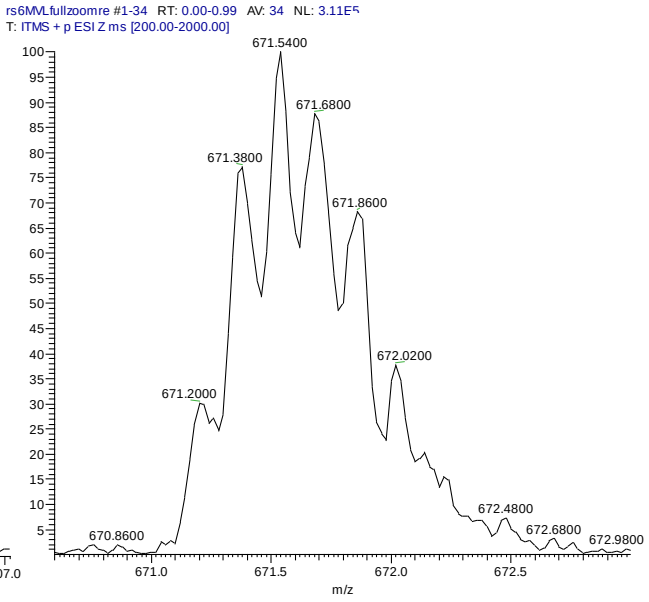
$[M+4H]^{4+}$



$[M+5H]^{5+}$

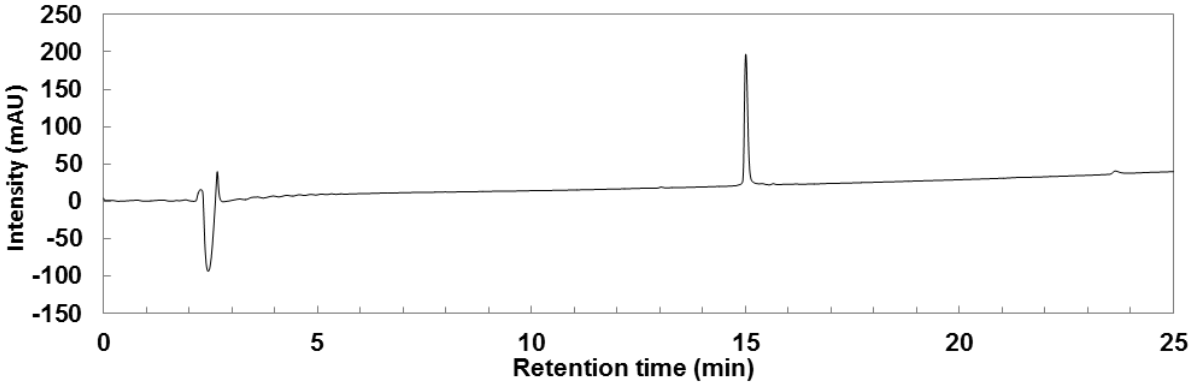


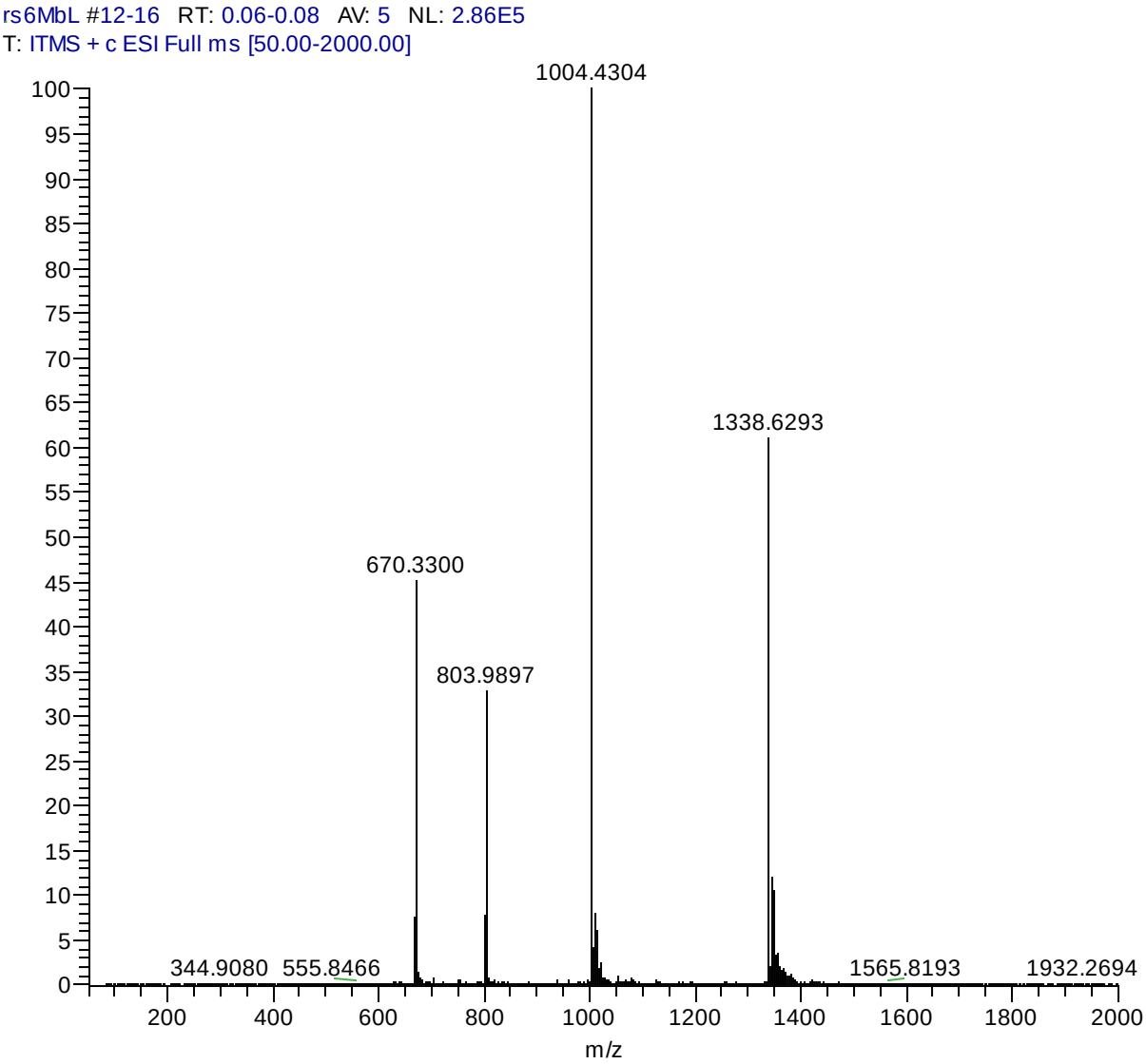
$[M+6H]^{6+}$



2b ANIKLSVQ**(ACHC)**KLFKRHLKWKII**β³V**KLNDGRE**(ACHC)**SLDG-NH_2

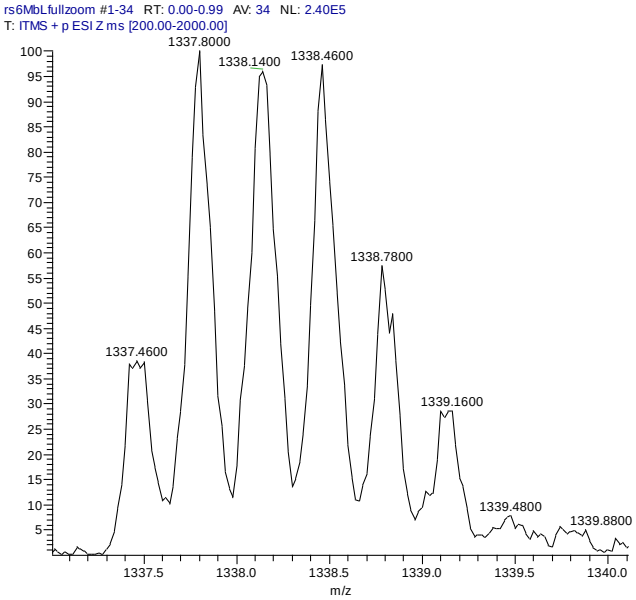
MW: 4011.80, expected m/z values: **[M+3H]$^{3+}$**: 1338.27; **[M+4H]$^{4+}$**: 1003.96; **[M+5H]$^{5+}$**: 803.37; **[M+6H]$^{6+}$**: 669.64; **[M+7H]$^{7+}$**: 574.12



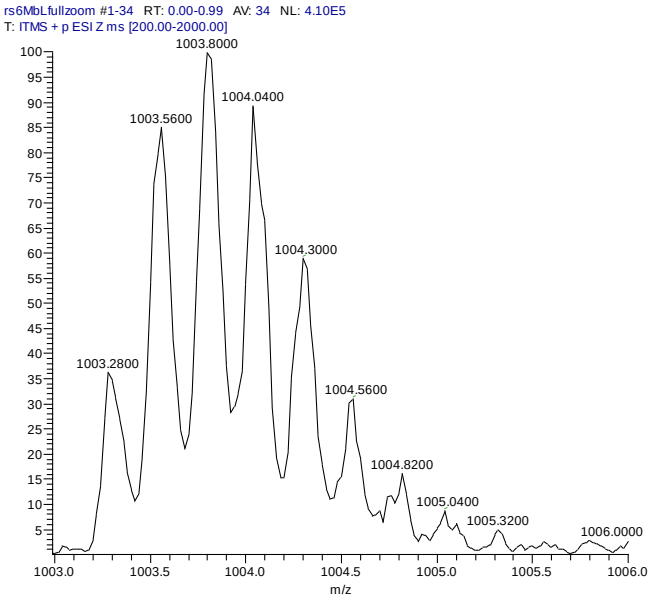


2b – High resolution mass spectra

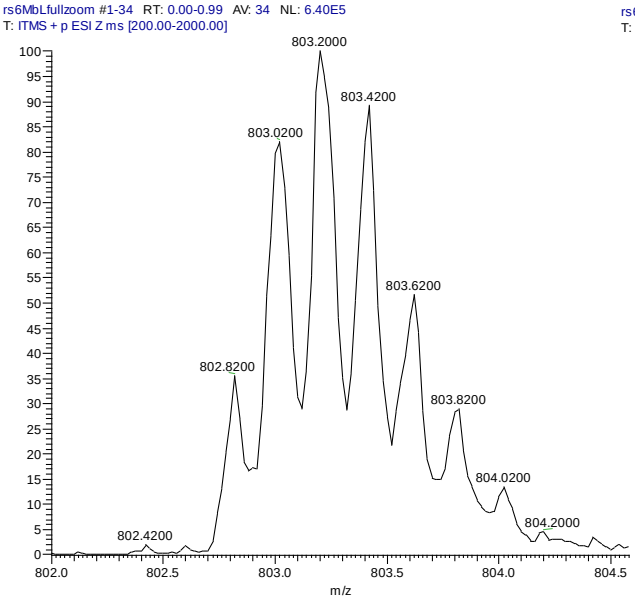
$[M+3H]^{3+}$



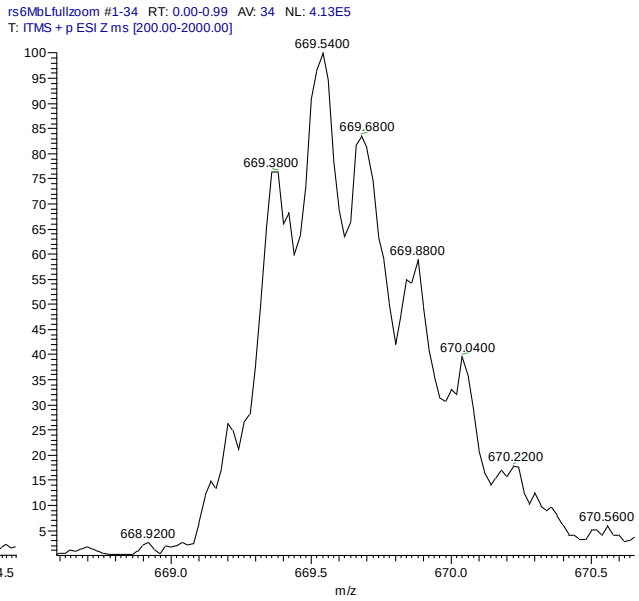
$[M+4H]^{4+}$



$[M+5H]^{5+}$

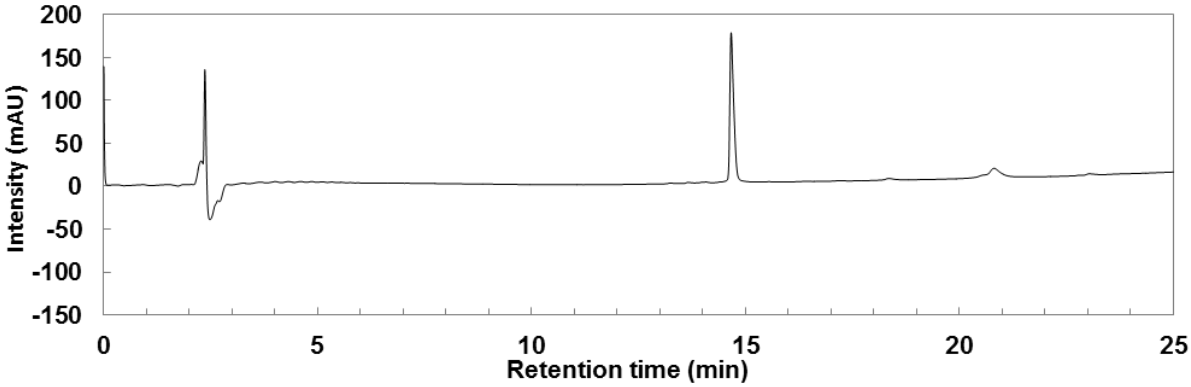


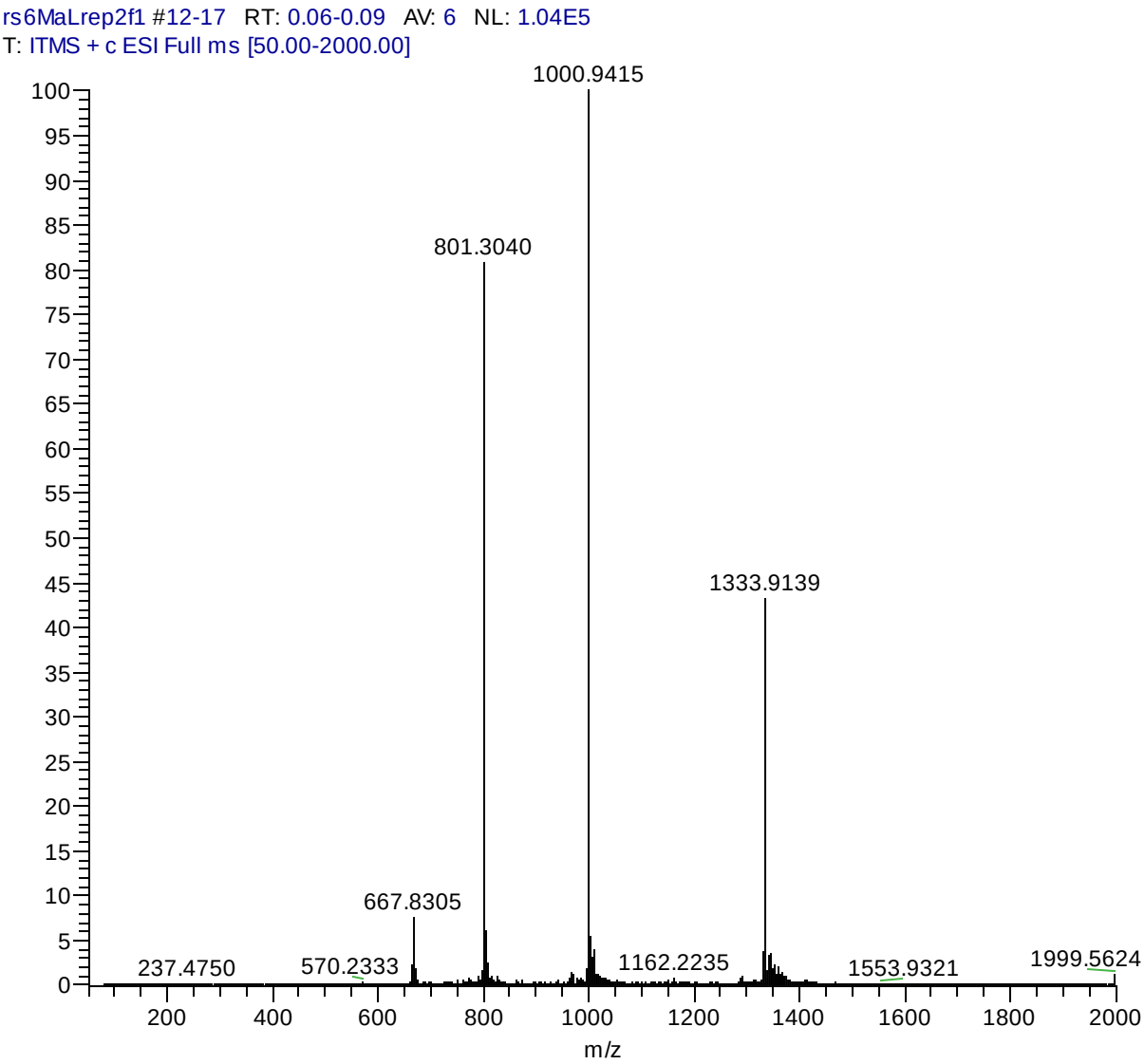
$[M+6H]^{6+}$



2c ANIKLSVQ**(ACHC)**KLFKRHLKWKIIVKLNDGRE**(ACHC)**SLDG-NH_2

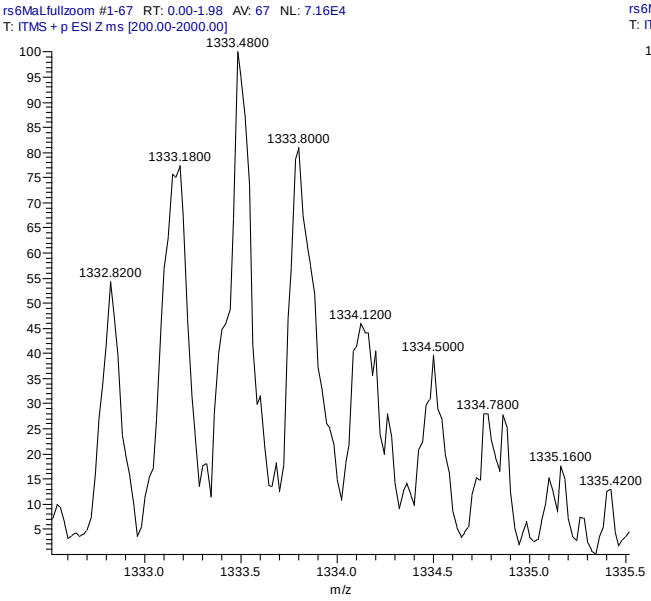
MW: 3997.38, expected m/z values: **[M+3H]$^{3+}$**: 1333.47; **[M+4H]$^{4+}$**: 1000.35; **[M+5H]$^{5+}$**: 800.48; **[M+6H]$^{6+}$**: 667.24; **[M+7H]$^{7+}$**: 572.06



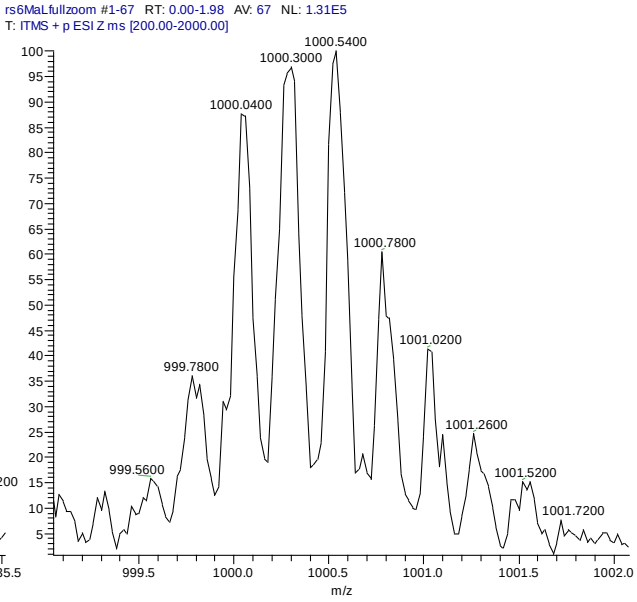


2c – High resolution mass spectra

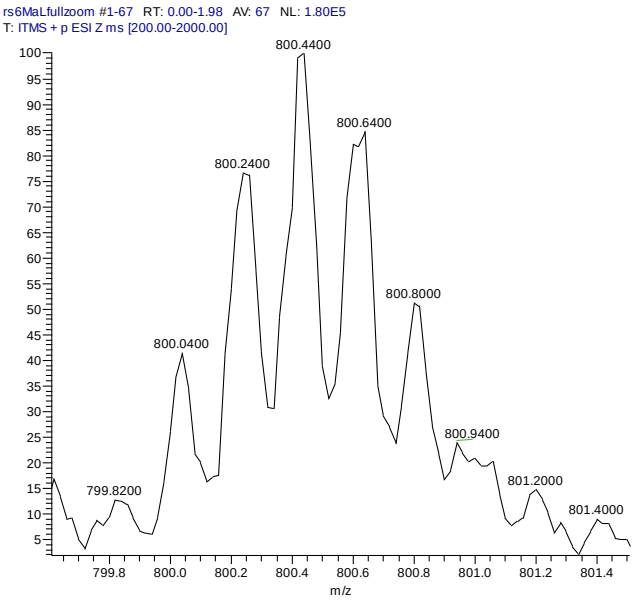
$[M+3H]^{3+}$



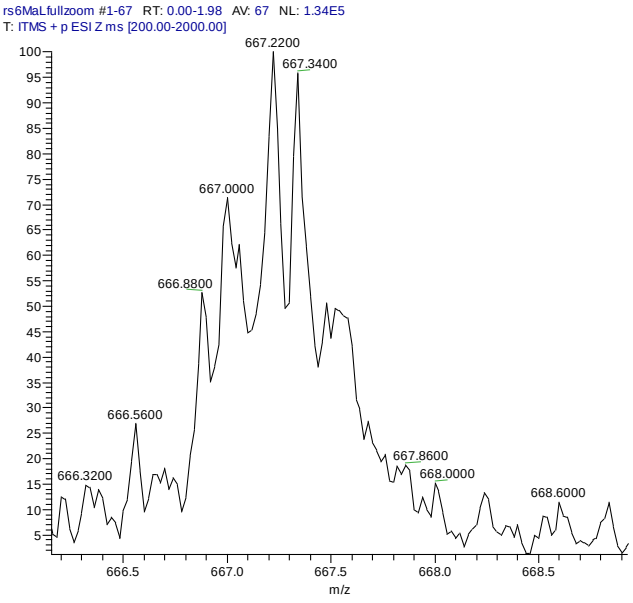
$[M+4H]^{4+}$



$[M+5H]^{5+}$

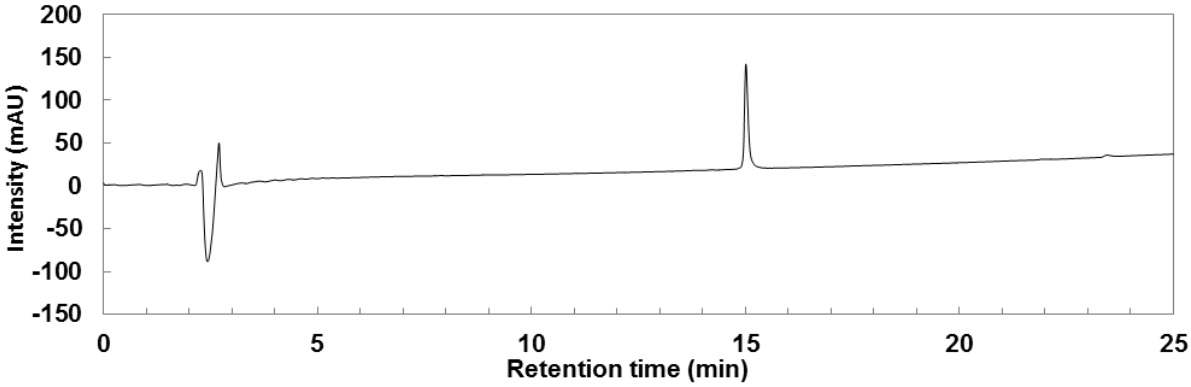


$[M+6H]^{6+}$

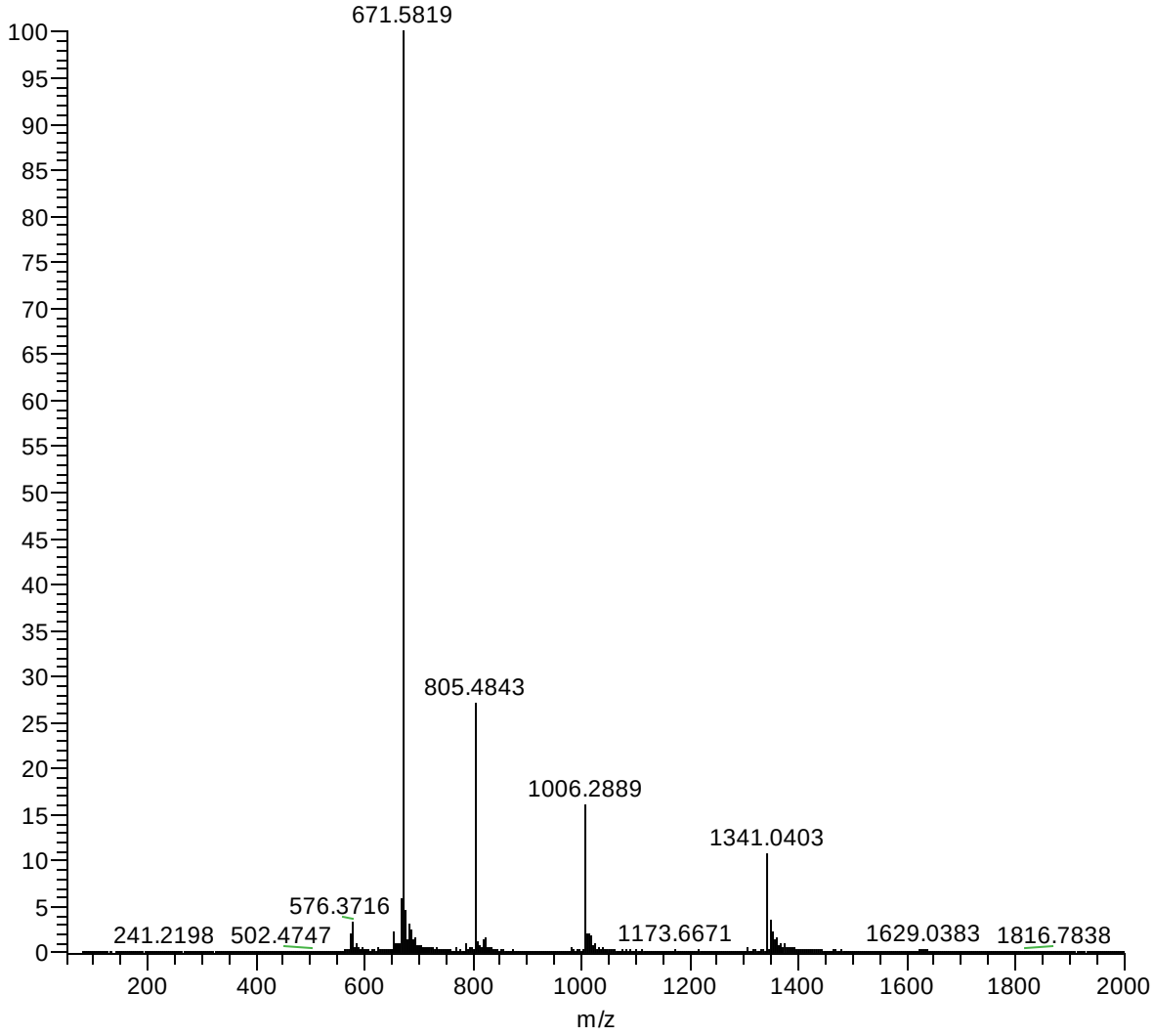


2d ANIKLSVQβ^3**M**KLFKRHLKWKIIVKLNDGREβ^3**L**S**L**DG-NH_2

MW: 4020.07, expected m/z values: **[M+3H]$^{3+}$**: 1341.03; **[M+4H]$^{4+}$**: 1006.03; **[M+5H]$^{5+}$**: 805.02; **[M+6H]$^{6+}$**: 671.02; **[M+7H]$^{7+}$**: 575.30

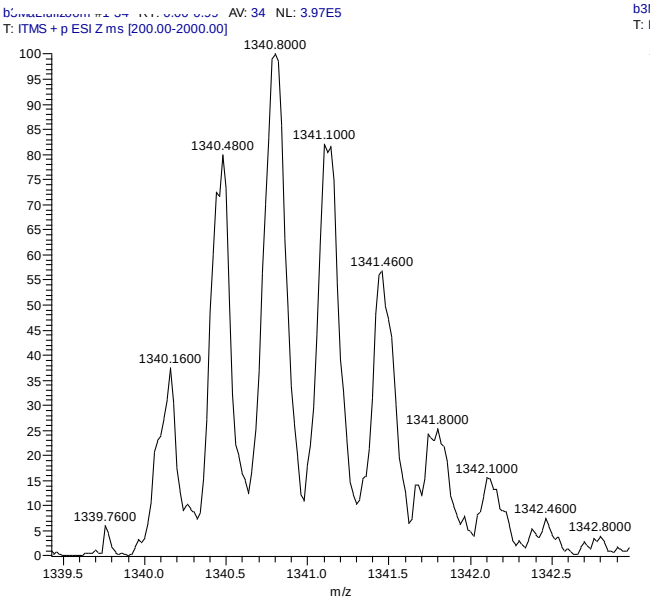


b3MaL_10uM #13-18 RT: 0.07-0.09 AV: 6 NL: 5.70E5
T: ITMS + c ESI Full ms [50.00-2000.00]

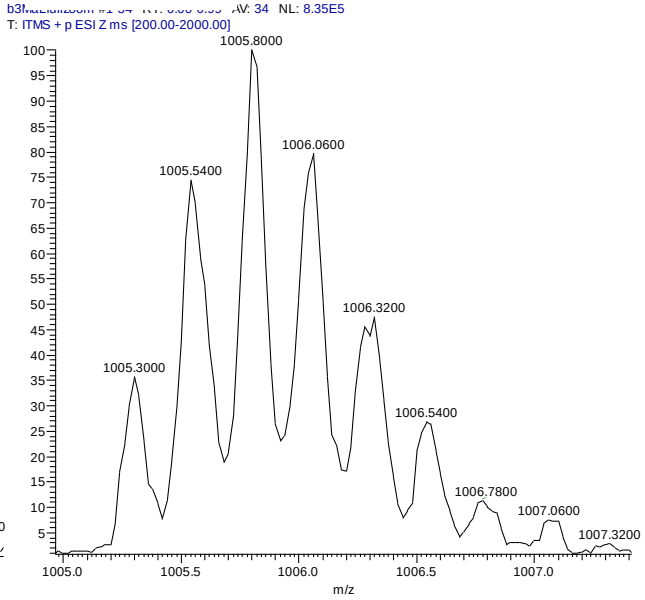


2d – High resolution mass spectra

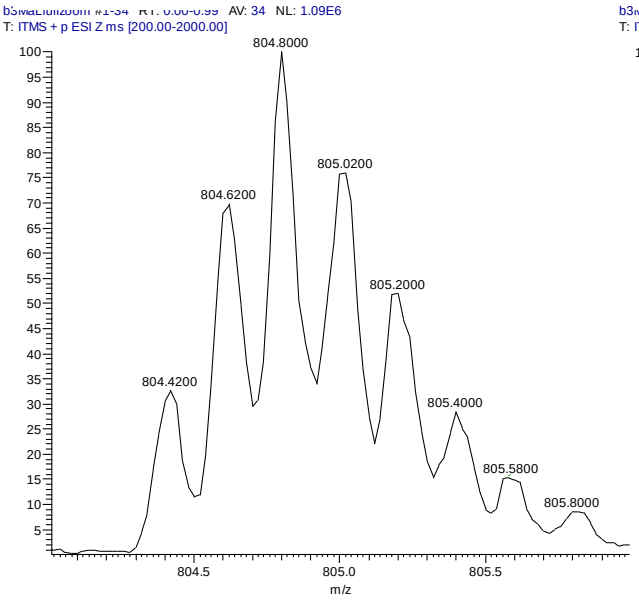
$[M+3H]^{3+}$



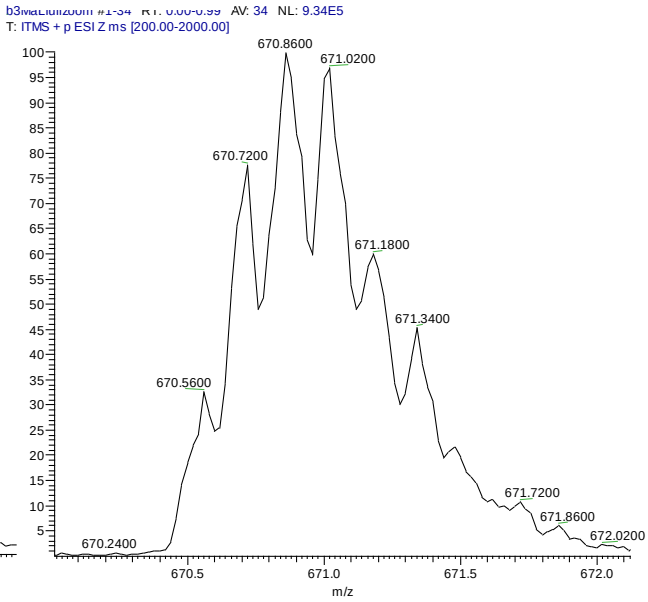
$[M+4H]^{4+}$



$[M+5H]^{5+}$

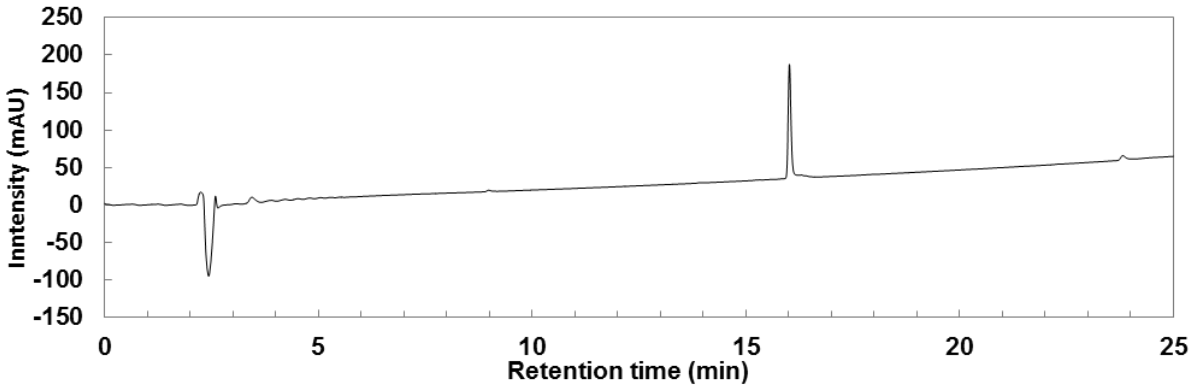


$[M+6H]^{6+}$

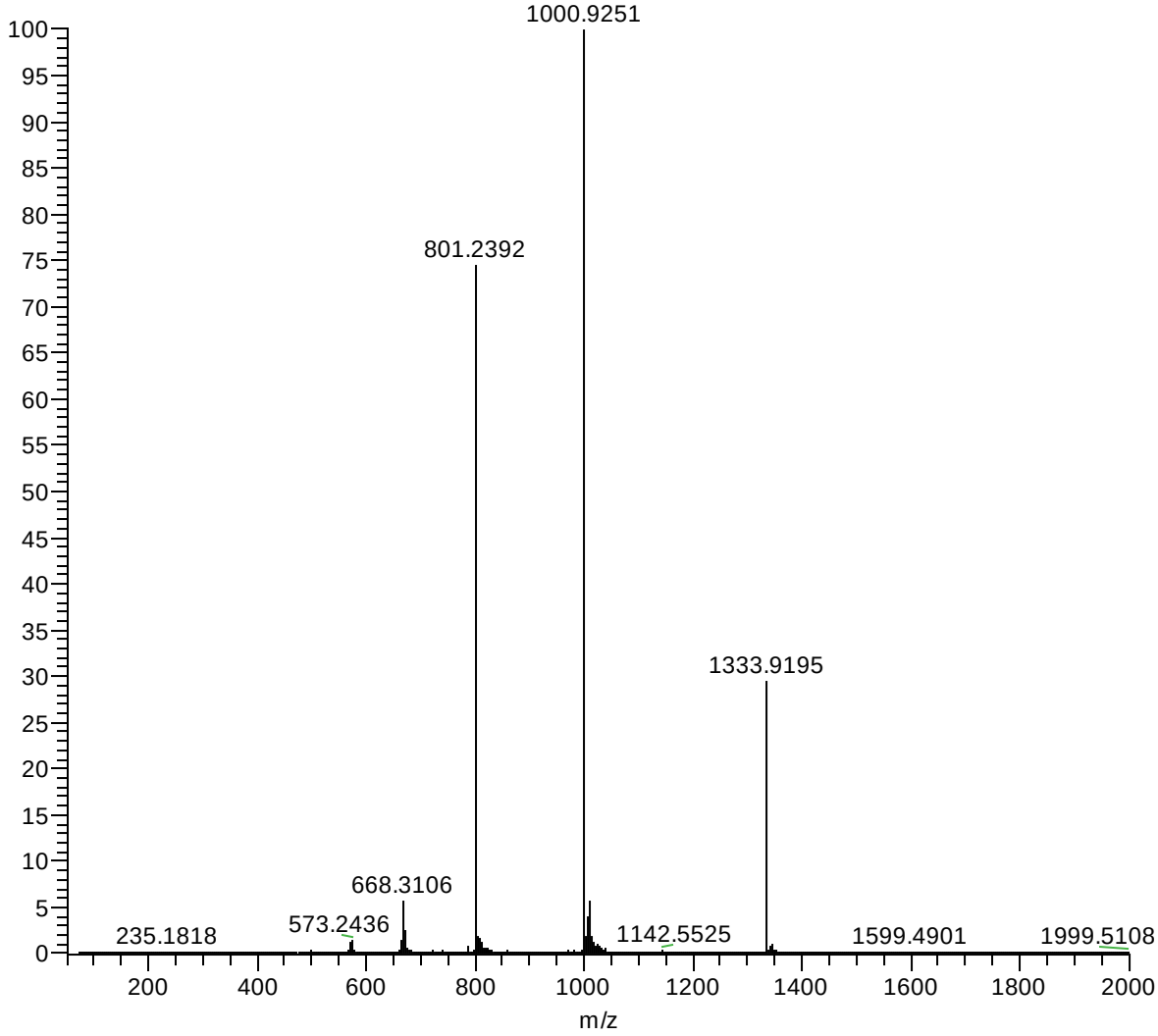


3a ANIKLS**(ACHC)**QMKLFKRHLKWKIIVK**(ACHC)**NDG**(ACHC)**ELSLDG-NH_2

MW: 3998.32, expected m/z values: **[M+3H]$^{3+}$**: 1333.78; **[M+4H]$^{4+}$**: 1000.59; **[M+5H]$^{5+}$**: 800.67; **[M+6H]$^{6+}$**: 667.39; **[M+7H]$^{7+}$**: 572.20

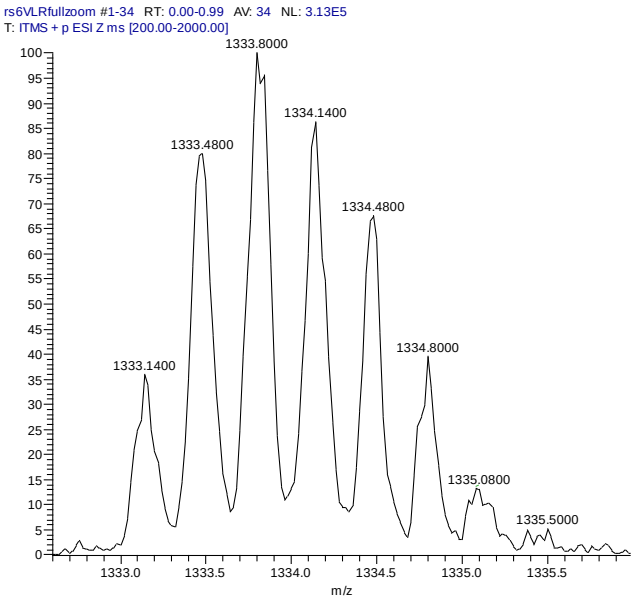


rs6VLR #47-65 RT: 0.26-0.34 AV: 19 NL: 2.39E5
T: ITMS + c ESI Full ms [50.00-2000.00]

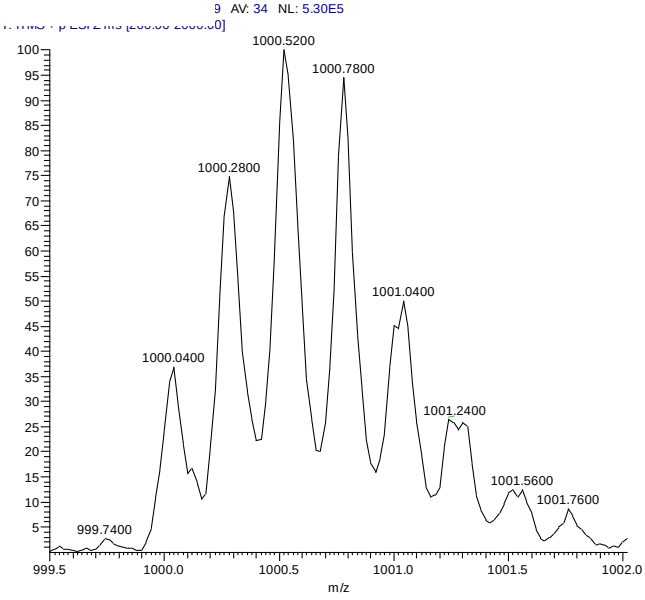


3a – High resolution mass spectra

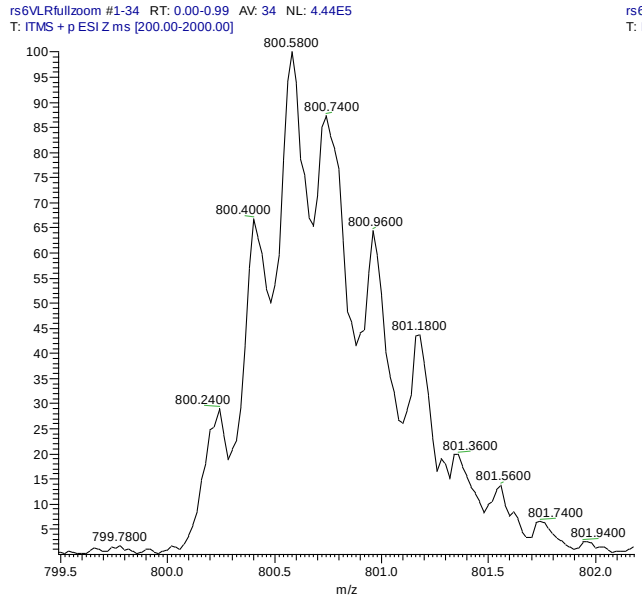
$[M+3H]^{3+}$



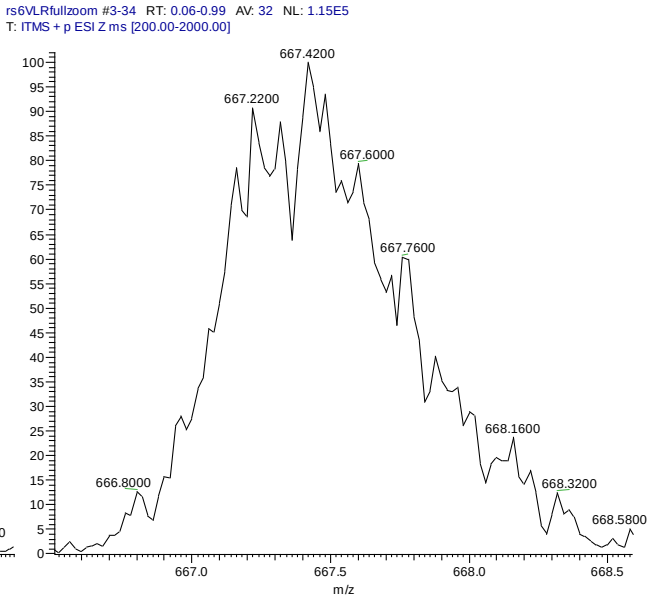
$[M+4H]^{4+}$



$[M+5H]^{5+}$

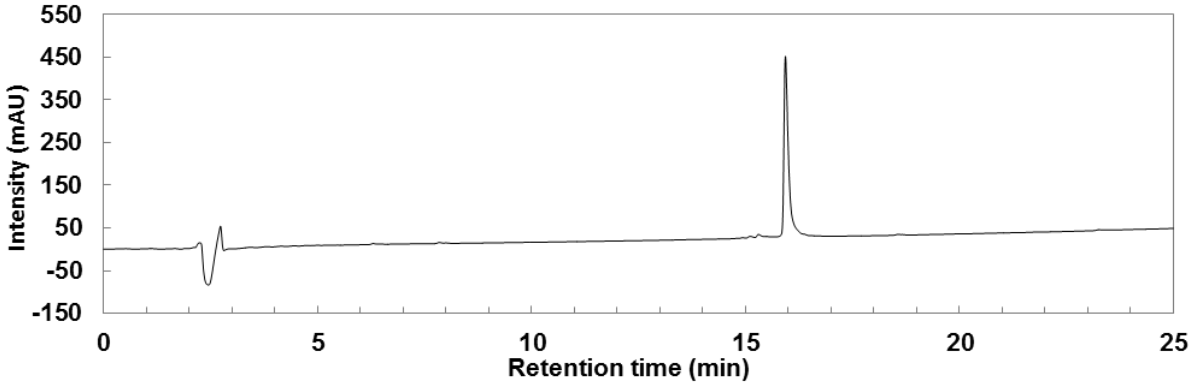


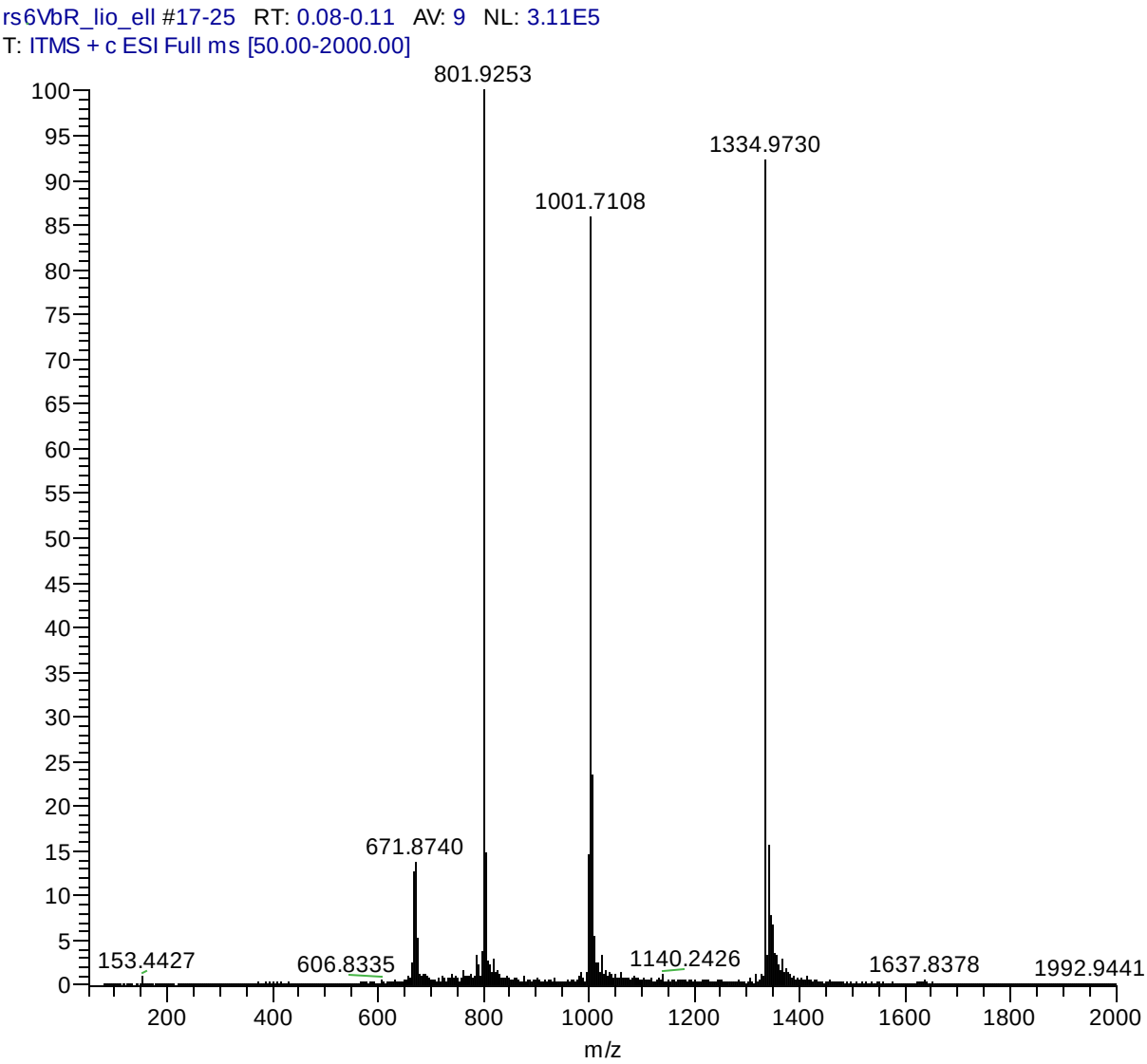
$[M+6H]^{6+}$



3b ANIKLS**(ACHC)**QMKLFKRHLKWKIIVK**β³L**NDG**(ACHC)**ELSLDG-NH_2

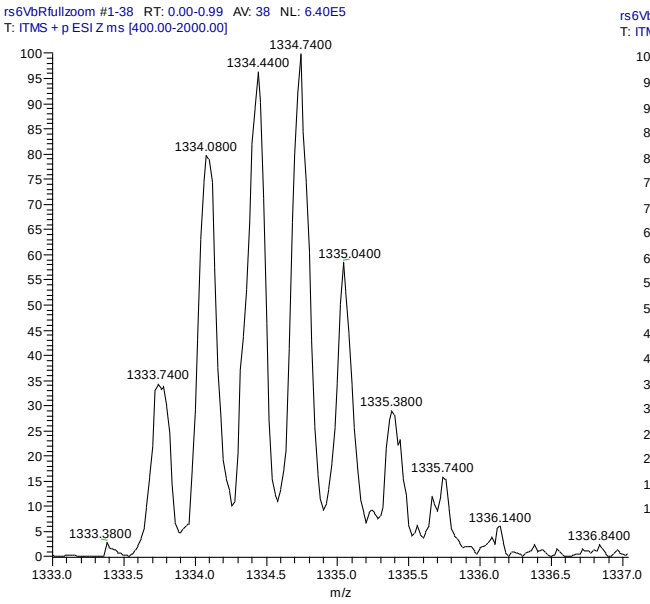
MW: 4000.84, expected m/z values: **[M+3H]$^{3+}$**: 1334.62; **[M+4H]$^{4+}$**: 1001.22; **[M+5H]$^{5+}$**: 801.18; **[M+6H]$^{6+}$**: 667.81; **[M+7H]$^{7+}$**: 572.56



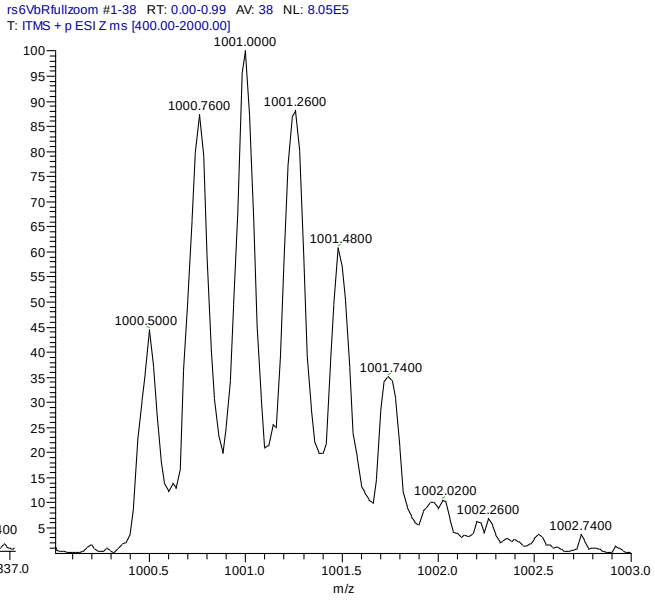


3b – High resolution mass spectra

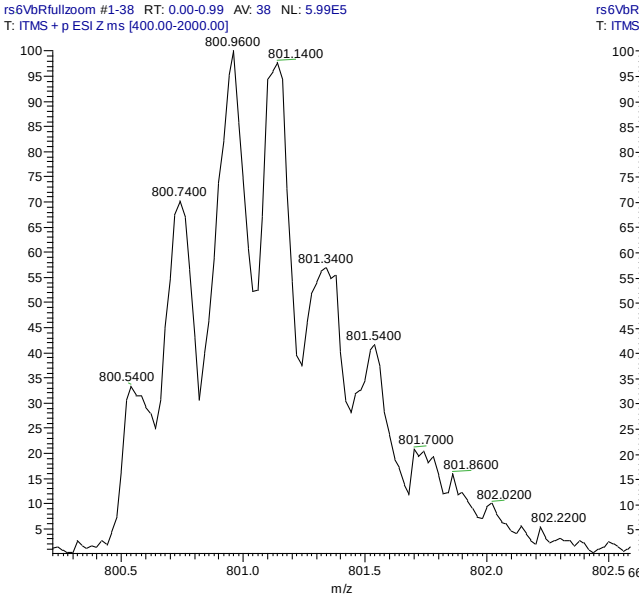
$[M+3H]^{3+}$



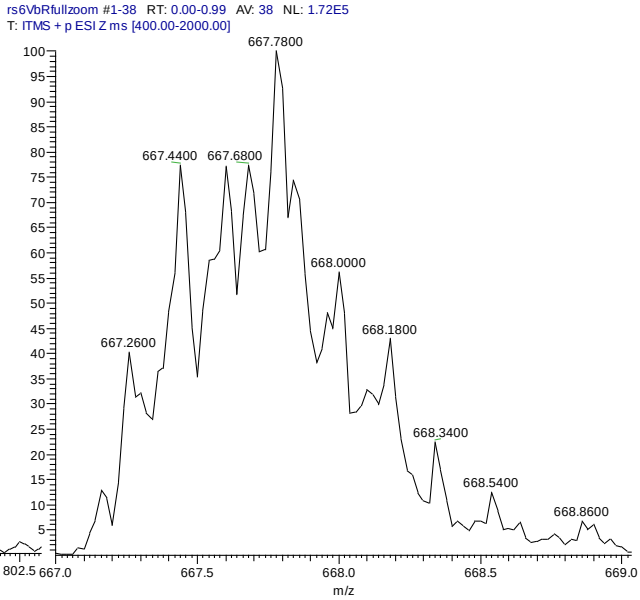
$[M+4H]^{4+}$



$[M+5H]^{5+}$

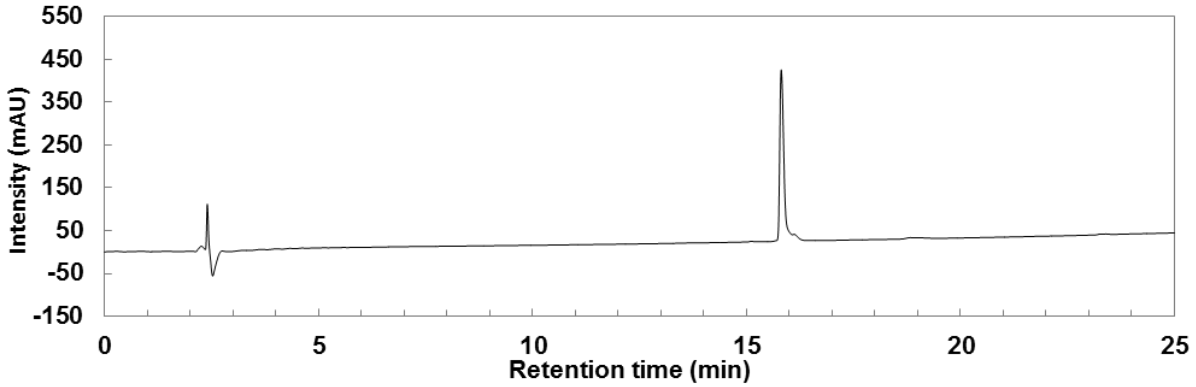


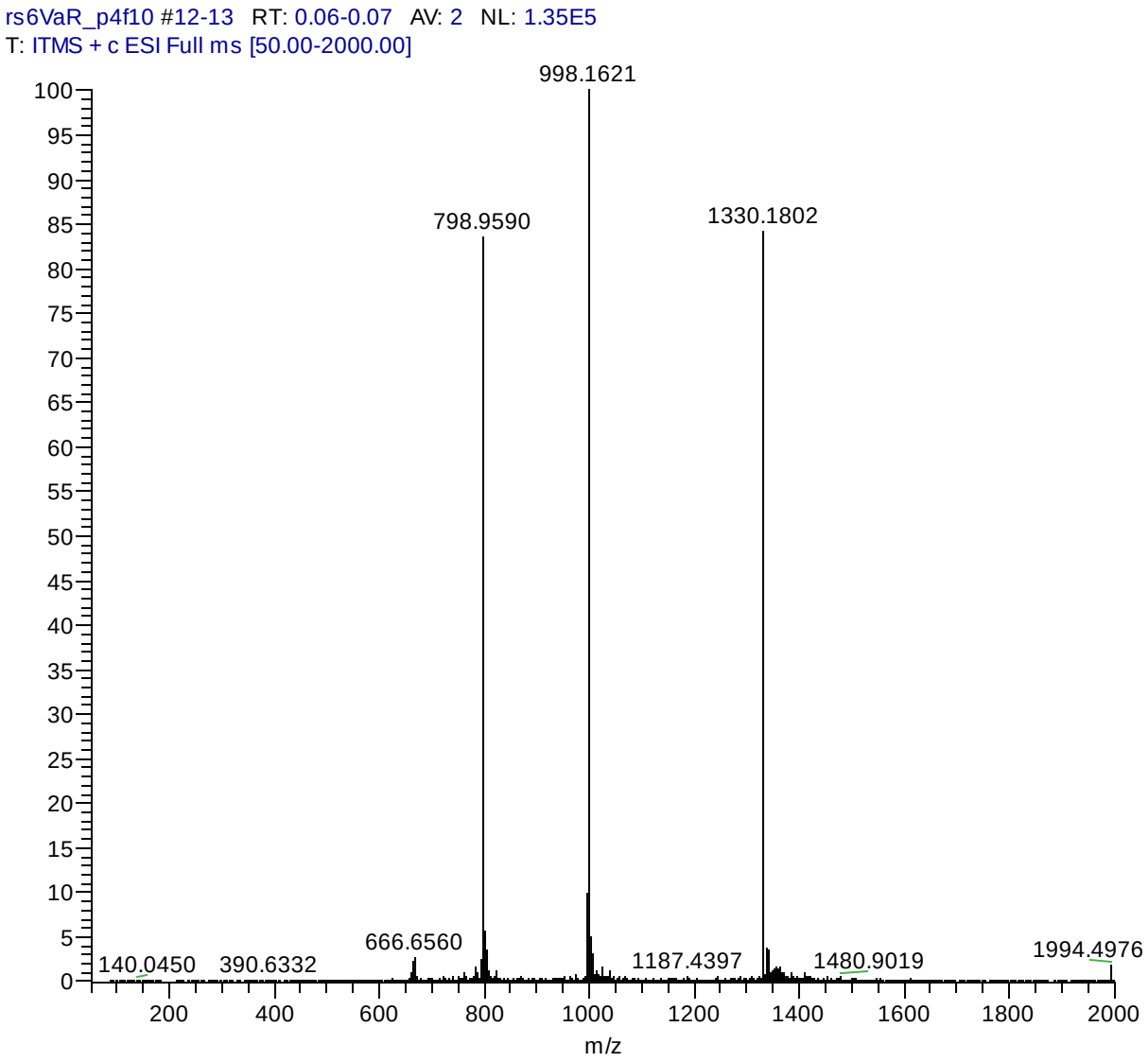
$[M+6H]^{6+}$



3c ANIKLS**(ACHC)**QMKLFKRHLKWKIIVKLNDG**(ACHC)**ELSLDG-NH_2

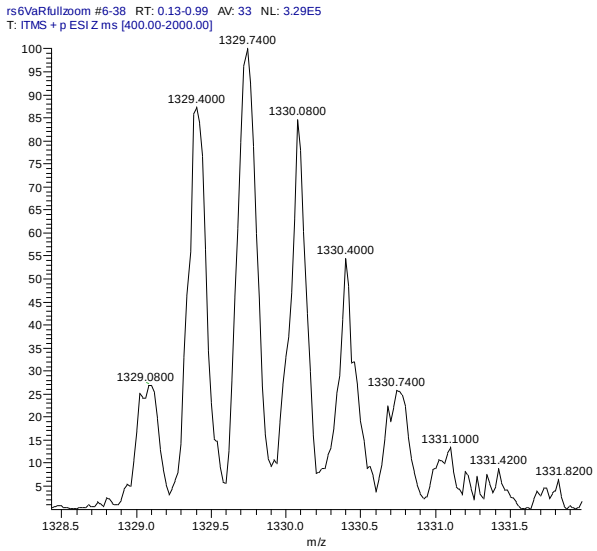
MW: 3986.81, expected m/z values: **[M+3H]$^{3+}$**: 1329.94; **[M+4H]$^{4+}$**: 997.71; **[M+5H]$^{5+}$**: 798.37; **[M+6H]$^{6+}$**: 665.48; **[M+7H]$^{7+}$**: 570.55



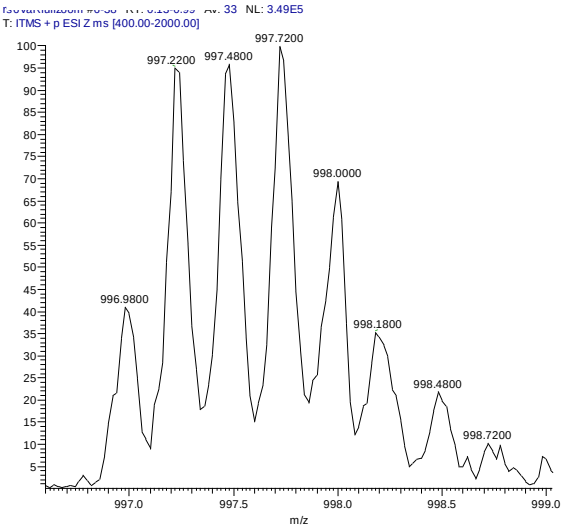


3c – High resolution mass spectra

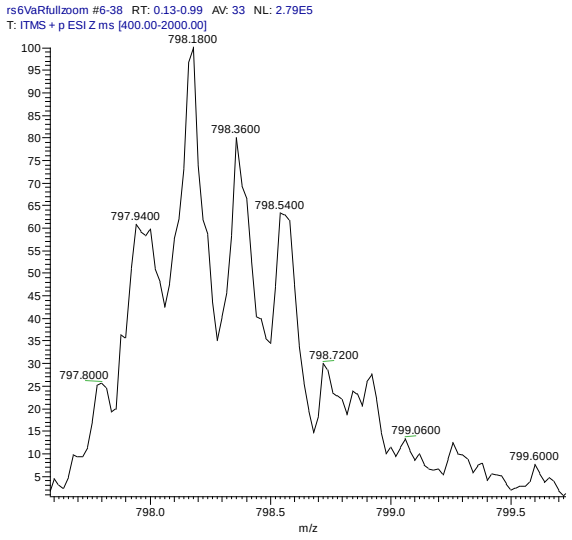
$[M+3H]^{3+}$



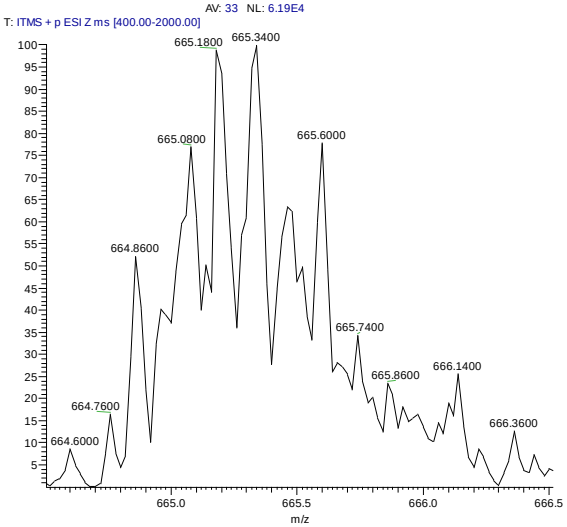
$[M+4H]^{4+}$



$[M+5H]^{5+}$

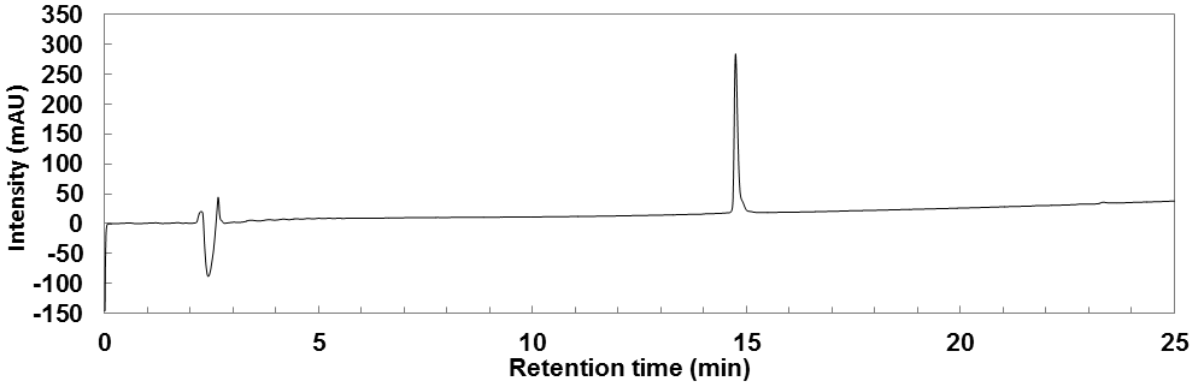


$[M+6H]^{6+}$

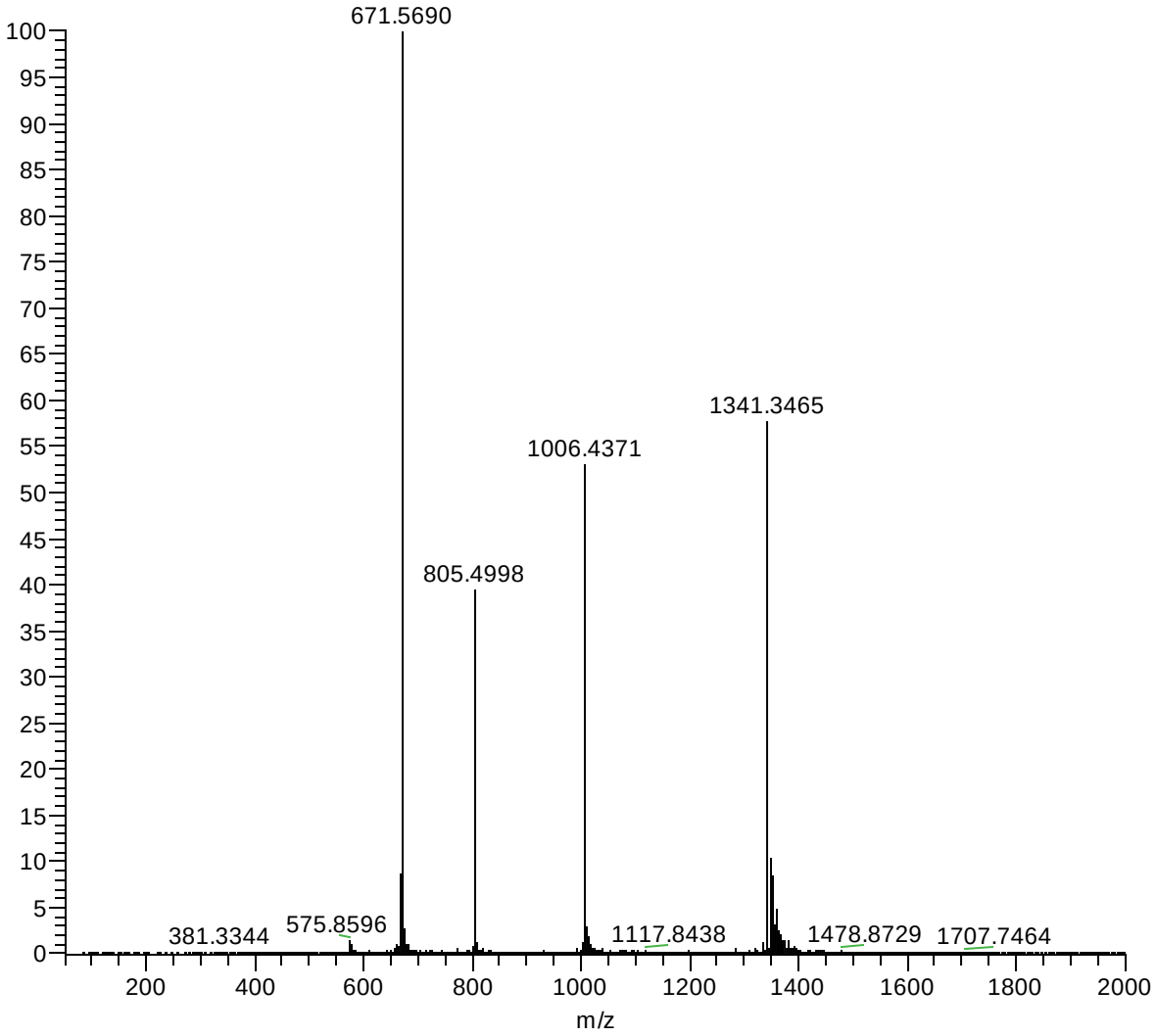


3d ANIKLSβ^3**V**QMKLFKRHLKWKIIVKLNDGβ^3**R**ELSLDG-NH_2

MW: 4020.07, expected m/z values: **[M+3H]$^{3+}$**: 1341.03; **[M+4H]$^{4+}$**: 1006.03; **[M+5H]$^{5+}$**: 805.02; **[M+6H]$^{6+}$**: 671.02; **[M+7H]$^{7+}$**: 575.30

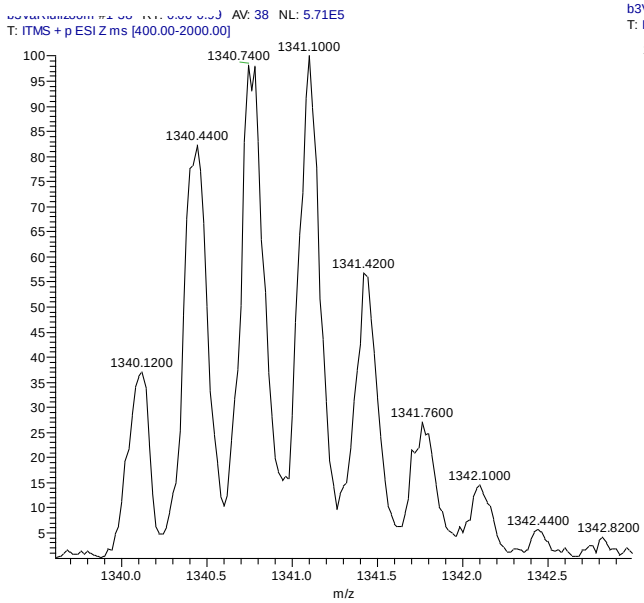


b3VaR #18-21 RT: 0.09-0.10 AV: 4 NL: 2.00E5
T: ITMS + c ESI Full ms [50.00-2000.00]

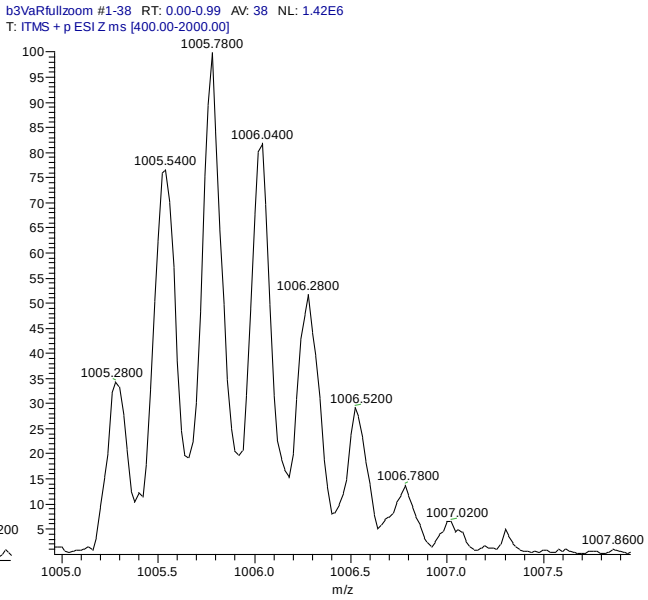


3d – High resolution mass spectra

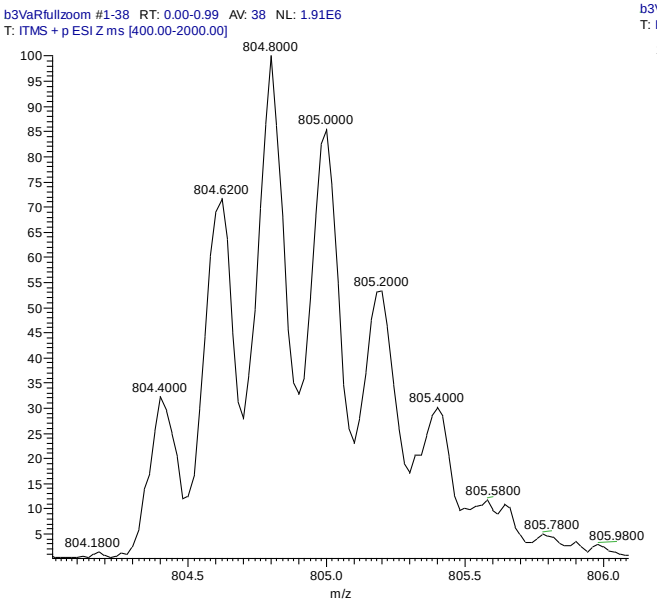
$[M+3H]^{3+}$



$[M+4H]^{4+}$



$[M+5H]^{5+}$



$[M+6H]^{6+}$

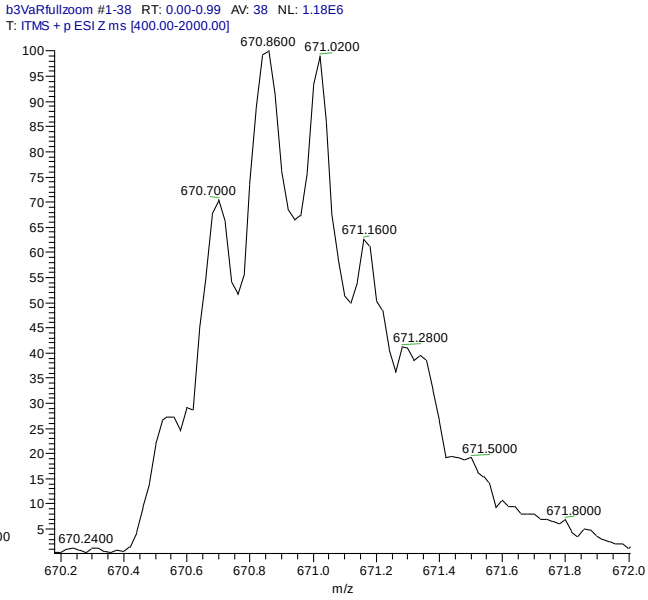


Table S4. Assigned chemical shifts for anginex and analogues, at 310K and peptide concentration of 500 μM in 10 mM pH 5.6 d18-HEPES referenced to external DSS.

1	NH	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	**[a]**	[a]	[a]
	Hα	4.07[b]	4.09	4.08	4.07	4.08	4.08	4.08	4.09	4.07	4.08	4.08	4.07	4.07
	Cα	51.76	50.91	51.67	51.66	51.68	51.67	51.68	51.68	51.68	51.69	51.68	51.66	51.68
	Cβ	19.56	19.27	19.25	19.25	19.27	19.28	19.25	19.26	19.26	19.26	19.26	19.25	19.28
2	NH	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
	Hα	4.89[b]	4.79	4.78	4.77	4.78	4.78	4.78	4.79	4.79	4.78	4.78	4.78	4.78
	Cα	53.03	53.02	53.03	53.02	53.00	53.03	53.02	53.03	53.01	53.04	53.03	53.02	52.98
	Cβ	38.80	38.77	38.75	38.78	38.79	38.79[c]	38.76	38.76[b]	38.78	38.80	38.79	38.77	38.73
3	NH	8.16	8.15	8.15	8.15	8.15	8.15	8.13	8.14	8.16	8.15	8.13	8.12	8.18
	Hα	4.18	4.17	4.16	4.16	4.16	4.16	4.16	4.17	4.16	4.16	3.70	4.17	4.17
	Cα	61.16	61.11	61.11	61.10	61.17	61.07	61.08	61.08	61.14	61.11	61.09	61.09	61.27
	Cβ	39.07	39.02	39.05	39.03	39.00	39.05	39.02	39.03	39.01	39.06	39.05	39.04	38.97
4	NH	8.32	8.32	8.31	8.31	8.30	8.31	8.30	8.31	8.30	8.31	8.30	8.30	8.30
	Hα	4.32	4.32	4.31	4.31	4.30	4.31	4.32	4.32	4.30	4.32	4.32	4.32	4.29
	Cα	56.28	56.14	56.15	56.13	56.31	56.09	56.10	56.09	56.32	56.21	56.11	56.09	56.48[b]
	Cβ	32.95	32.90	32.89	32.92	32.90	32.91	32.92	32.92[b]	32.87	32.91	32.92	32.89	32.79
5	NH	8.17	8.16	8.15	8.16	8.14	8.16	8.14	8.15	8.14	8.16	8.14	8.14	8.11
	Hα	4.37	4.38	4.37	4.36	4.36	4.37	4.37	4.38	4.36	4.38	4.39	4.39	4.32
	Cα	55.27	55.03	55.05	54.98	55.24	54.95	54.96	54.96	55.09	54.99	54.97	54.95	55.13
	Cβ	42.68[b]	42.76	42.69	42.67	42.64	42.26	42.30[b]	42.32	42.36	42.77[d]	42.76	42.74[b]	42.53
6	NH	8.24	8.25	8.24	8.24	8.21	8.26	8.25	8.25	8.24	8.19	8.17	8.16	8.10
	Hα	4.45	4.47	4.46	4.45	4.43	4.47	4.47	4.48	4.44	4.42	4.42	4.42	4.31
	Cα	58.40	58.17	58.15	58.17	58.28	58.04	58.04	58.06	58.39	58.20	58.19	58.19	59.07
	Cβ	63.68	63.70	63.69	64.04	63.67	63.71	63.69	63.72	63.63	63.79	64.03	64.02	63.71
7	NH	8.01	8.03	8.02	8.03	8.03	8.01	8.00	8.00	8.00	7.73	7.71	7.71	7.64
	Hα	4.07	4.12	4.11	4.11	4.03	4.14	4.14	4.15	4.06	2.67	2.68	2.68	2.39/2.56
	Cα	62.88	62.35	62.37	62.32	63.19	61.97	61.98	61.98	62.96	48.15[b]	48.15	48.14	41.52
	Cβ	32.71	32.81	32.80	32.81	32.55	32.97	32.98	32.98	32.67	50.57	50.58	50.57	55.71
8	NH	8.27	8.32	8.30	8.31	8.24	8.30	8.28	8.29	8.16	8.15	8.13	8.12	8.23
	Hα	4.27	4.32	4.31	4.31	4.21	4.32	4.32	4.32	4.16	4.16	4.16	4.16	4.13

	Cα	56.38	55.81	55.81	55.80	56.60	55.74	55.78	55.77	56.73	56.05	56.05	56.05	57.19
	Cβ	29.32	29.45	29.43	29.44	29.12	29.72	30.38	29.83	29.47	29.53	29.53	29.52	29.46
9	NH	8.23	8.28	8.27	8.28	8.14	7.80	7.79	7.79	7.83	8.33	8.31	8.31	8.35
	Hα	4.41	4.44	4.44	4.43	4.39	2.68	4.28	2.69	2.49/2.49	4.39	4.39	4.39	4.37
	Cα	55.99	55.47	55.48	55.45	55.99	47.84	47.86	47.86	44.02	55.82	55.84	55.82	56.28
	Cβ	33.01	33.14	33.17	33.15	32.91	50.75	50.73	50.73	49.10	32.81	32.79	32.78	32.65
10	NH	8.13	8.17	8.16	8.17	8.05	8.02	8.00	8.01	8.16	8.23	8.21	8.21	8.04
	Hα	4.23	4.24	4.23	4.22	4.14	4.08	4.08	4.09	4.09	4.22	4.22	4.22	4.21
	Cα	56.33[b]	56.22[b]	56.25	56.24	57.13	56.42	56.41	56.43	57.66	56.46[c]	56.43[c]	56.45[c]	56.30
	Cβ	33.01[b]	33.13[b]	33.14[b]	33.13[b]	32.93[b]	33.09[b]	33.06[c]	33.10[c]	32.88[b]	33.01[b]	32.95[d]	32.98[c]	33.03[b]
11	NH	7.95	7.44	7.43	7.44	7.64	8.01	7.99	8.00	8.08	7.97	7.95	7.95	7.91
	Hα	4.30	2.68	2.67	2.65	2.29/2.40	4.31	4.32	4.32	4.27	4.30	4.30	4.31	4.26
	Cα	55.11	47.57	47.87	47.56	44.56	54.78	54.82	54.79	55.40	54.93	54.93	54.93	55.21[b]
	Cβ	42.49	51.67	50.90	50.87	48.19	42.50	42.52	42.60	42.16	42.57	42.54	42.53	42.42
12	NH	8.07	8.02	8.01	8.02	8.19	8.14	8.12	8.13	8.05	8.10	8.08	8.08	8.04
	Hα	4.58	4.51	4.51	4.50	4.55	4.58	4.58	4.59	4.53	4.59	4.60	4.60	4.57
	Cα	57.62	57.45	57.49	57.46	58.34	57.49	57.51	57.52	58.14	57.54	57.55	57.55	57.77
	Cβ	39.68	39.88	39.88	39.88	39.62	39.73	39.76	39.77	39.75	39.78	39.76	39.75	39.59
13	NH	8.07	8.09	8.08	8.08	8.22	8.11	8.09	8.10	8.04	8.11	8.09	8.09	8.05
	Hα	4.21	4.24	4.22	4.22	4.19[b]	4.21	4.21	4.23	4.18	4.22	4.22	4.23	4.21
	Cα	56.31[b]	55.95	55.95	55.96	56.20[b]	56.01	56.01	56.01	56.40	56.11[c]	56.11[c]	56.14[c]	56.24[c]
	Cβ	32.93[b]	33.20	33.15	33.11[b]	32.93[c]	33.13[b]	33.11[c]	33.13[c]	33.02	33.23[b]	33.18	33.14	33.02[b]
14	NH	8.07	8.15	8.14	8.15	8.01	8.11	8.10	8.10	8.00	8.11	8.08	8.09	8.03
	Hα	4.21	4.21	4.20	4.19	4.19	4.19	4.19	4.20	4.18	4.20	4.21	4.21	4.19
	Cα	56.27[b]	56.04	56.02	56.08	56.47[b]	56.20[c]	56.05	56.06	56.29	56.09[c]	56.12[b]	56.11	56.21[c]
	Cβ	30.88	31.06	31.09	31.06	30.91	31.00	31.01	31.05[c]	30.91	31.05	31.05	30.98	30.95
15	NH	8.38	8.46	8.43	8.43	8.36	8.43	8.43[b]	8.45	8.33	8.44	8.42	8.41	8.37
	Hα	4.66	4.64	4.62	4.60	4.59	4.63	4.63	4.63	4.60	4.60[c]	4.63	4.63	4.61
	Cα	56.31	55.36	55.31	55.44	55.48	55.28	55.41	55.23	55.50	55.38	55.44	55.45	55.50
	Cβ	31.03	29.58	29.51	29.62	29.25	29.49	29.65	29.32[b]	29.46	29.51	29.57	29.58	29.54
16	NH	8.04	8.15	8.13	8.13	8.06	8.15	8.13	8.15	8.06	8.14	8.12	8.12	8.09
	Hα	4.26	4.28	4.28	4.26	4.27	4.27	4.27	4.28	4.25	4.27	4.27	4.27	4.26
	Cα	55.29[d]	55.04	55.05	55.08	55.12	55.05	55.10	55.10	55.20	55.13	55.15	55.13	55.22[c]

	Cβ	42.43	42.60	42.65	42.56	42.61	42.53	42.57	42.55	42.54	42.56	42.55	42.53	42.55
17	NH	8.18	8.20	8.21	8.21	8.17	8.20	8.19	8.20	8.15	8.20	8.18	8.19	8.17
	Hα	4.23	4.21	4.21	4.19	4.20	4.20	4.20	4.21	4.21	4.20	4.21	4.20	4.21
	Cα	56.73	56.34[b]	56.38	56.42	56.48	56.37	56.39	56.42	56.44	56.46[c]	56.44[b]	56.45	56.43[c]
	Cβ	32.98	32.91[b]	32.83[c]	32.81[b]	32.77	32.98[b]	32.96[c]	33.02[c]	32.78[b]	32.84	32.83	32.80	32.82
18	NH	7.86	7.87	7.90	7.85	7.84	7.86	7.85	7.86	7.84	7.85	7.84	7.83	7.84
	Hα	4.72	4.70	4.69	4.68	4.68	4.68	4.68	4.70	4.68	4.70	4.70	4.69	4.69
	Cα	56.80	56.75	56.92	56.75	56.84	56.73	56.81	56.76	56.86	56.73	56.78	56.81	56.83
	Cβ	30.06	30.10	29.98	30.05	30.07	30.11	30.07	30.06	30.02	30.11	30.12	30.05	30.06
19	NH	8.06	8.07	8.04	8.04	8.02	8.04	8.00	8.04	8.01	8.06	8.06	8.04	8.05
	Hα	4.35	4.22	4.11	4.24	4.23	4.23	4.23	4.24	4.23	4.24	4.25	4.24	4.24
	Cα	56.09	56.28[b]	56.81	56.24	56.32	56.25	56.25	56.26	56.34	56.21	56.24[c]	56.42[c]	56.28
	Cβ	33.57	33.49	33.14	33.28	33.23	33.34	33.42	33.23[b]	33.25	33.31	33.30	33.18	33.32
20	NH	8.09	7.59	7.66	7.97	7.92	7.93	7.91	7.93	7.92	7.94	7.92	7.89	7.92
	Hα	4.20	2.72	2.35/2.51	4.12	4.12	4.10	4.09	4.13	4.12	4.14	4.14	4.14	4.13
	Cα	60.77	47.42	40.02	60.88	60.94	60.82	60.88	60.87	60.89	60.87	60.91	60.92	60.91
	Cβ	39.31	51.12	54.31	38.92	38.91	38.91	38.25	38.94	38.92	38.97	38.98	38.95	38.93
21	NH	8.18	7.88	8.01	8.17	8.11	8.17	8.07	8.17	8.14	8.16	8.14	8.13	8.16
	Hα	4.37	4.00	4.10	4.22	4.25	4.11	4.00	4.18	4.15	4.19	4.19	4.19	4.18
	Cα	60.31	60.95	61.04	60.59	60.62	60.93	61.02	60.75	60.86	60.83	60.83	60.83	60.81
	Cβ	38.85	38.78	38.94	38.81	38.90	38.18	38.82	38.60	38.54	38.71	38.65	38.62	38.53
22	NH	8.36	7.88	8.06	8.21	8.21	7.73	8.01	8.13	8.10	8.08	8.07	8.08	8.12
	Hα	4.19	4.11	4.10	4.12	4.13	2.69	2.58/2.41	4.11	4.09	4.11	4.10	4.11	4.10
	Cα	61.60	61.82	62.01	61.88	61.89	47.52	40.37	62.12	62.14	61.95	62.05	62.08	62.10
	Cβ	33.55	33.22	33.15	33.20	33.25	50.85	54.84	33.05	33.03	33.04	33.03	33.05	33.02
23	NH	8.31	8.28	8.24	8.28	8.25	7.91	8.08	8.25	8.23	8.23	8.15	8.23	8.25
	Hα	4.51	4.37	4.39	4.41	4.43	4.17	4.37	4.39	4.38	4.31	4.23	4.33	4.34
	Cα	55.64	55.91	55.88	55.79	55.83	56.22	55.51	55.81	55.94	56.15	56.18[c]	56.00	56.02
	Cβ	33.39	33.29	33.39	33.36	33.29	33.46	33.05[c]	33.43[b]	33.32[b]	33.16[b]	33.29	33.21	33.23
24	NH	8.44	8.30	8.30	8.35	8.33	8.24	8.34	8.35	8.27	7.73	7.86	8.23	8.24
	Hα	4.42	4.36	4.35	4.36	4.37	4.33	4.26	4.36	4.35	2.69	2.47/2.44	4.32	4.32
	Cα	54.75	54.98	55.05	54.99	54.94	55.07	55.48	55.39	55.33[b]	47.88	43.99	55.32	55.54
	Cβ	42.68[b]	42.63	42.60	42.59	42.58	42.51	42.65[b]	42.69	42.58	50.58	48.32[b]	42.74	42.53

25	NH	8.56	8.50	8.48	8.51	8.49	8.43	8.45	8.49	8.45	8.18	8.29	8.36	8.44
	Hα	4.60	4.66	4.65	4.62	4.62	4.67	4.68	4.66	4.65	4.57	4.68	4.66	4.68
	Cα	54.30	53.66	53.65	53.84	53.90	53.35	53.35	53.60	53.63	53.43	53.27	53.35	53.41
	Cβ	38.40	38.61	38.64	38.52	38.52	38.69	38.63	38.65[b]	38.61	39.22	39.39	38.76	38.56
26	NH	8.06	8.15	8.14	8.11	8.08	8.20	8.14	8.18	8.12	8.32	8.34	8.18	8.11
	Hα	4.56	4.57	4.56	4.55	4.56	4.55	4.55	4.56	4.56	4.61	4.62	4.61	4.54
	Cα	53.90	54.09	54.11	54.02	54.03	54.20	54.33	54.08	54.13	54.18	54.37	54.41	54.20
	Cβ	40.87	40.98	40.97	40.90	40.89	41.00	41.07	40.83	41.03	41.04	41.14	41.11	41.10
27	NH	8.26	8.33	8.31	8.29	8.28	8.33	8.32	8.29	8.31	8.20	8.23	8.18	8.30
	Hα2	4.10	4.04	4.03	4.05	4.05	3.99	3.97	4.02	4.03	3.84	3.89	3.87	3.92
	Hα3	3.80	3.85	3.83	3.80	3.81	3.86	3.88	3.84	3.85	3.88	3.83	3.86	3.76
	Cα	45.45	45.55	45.53	45.49	45.53	45.56	45.55	45.52	45.57	45.63	45.62	45.62	45.73
28	NH	7.95	8.00	7.99	7.97	7.96	7.98	7.99	7.95	7.96	7.53	7.50	7.48	7.65
	Hα	4.31	4.31	4.30	4.29	4.29	4.33	4.34	4.35	4.29	2.67	2.67	2.67	2.49/2.49
	Cα	56.65	56.36	56.37	56.43	56.57	56.02	56.02	56.00	56.52	47.70[b]	47.71	50.73	43.70
	Cβ	31.19	30.94	30.93	30.99	30.96	30.92	30.95	31.03	30.87	50.81	50.80	47.74	49.69
29	NH	8.44	8.44	8.43	8.43	8.43	8.34	8.33	8.33	8.34	8.08	8.06	8.06	8.35
	Hα	4.54	4.37	4.36	4.41	4.42	4.27	4.28	4.32	4.27	4.23	4.25	4.24	4.29
	Cα	56.03[d]	56.32	56.31	56.17	56.30	56.46	56.49	56.08	56.54	56.15[c]	56.24[c]	56.20[c]	56.38[b]
	Cβ	31.02	30.13	30.08	30.30	30.23	30.34	29.75	30.19	30.13	30.08	30.18	30.16	30.53
30	NH	8.43	8.24	8.23	8.29	8.28	7.80	7.79	7.83	7.86	8.22	8.22	8.21	8.27
	Hα	4.46	4.41	4.40	4.42	4.38	2.71	2.70	2.73	2.45/2.45	4.38	4.39	4.39	4.38
	Cα	54.65[d]	54.94	54.92	54.84	55.04	47.60	47.66	47.56	44.16	54.97	54.93	54.95	55.11
	Cβ	43.46[d]	42.75	42.80	43.01	42.94	50.73	50.73	50.76	48.29	42.74	42.39[b]	42.73	42.68[c]
31	NH	8.25	8.17	8.16	8.18	8.12	7.95	7.93	7.94	8.15	8.25	8.24	8.24	8.25
	Hα	4.65[b]	4.44	4.44	4.48	4.42	4.37	4.38	4.40	4.47	4.46	4.46	4.47	4.48
	Cα	57.74[d]	58.14	58.17	58.04	58.35	57.80	57.80	57.73	57.97	57.95	57.95	57.94	57.87
	Cβ	63.86	64.02	64.01	63.70	63.75	63.97	64.00	64.02	64.14	64.04	63.79	63.77	63.79
32	NH	8.44	7.70	7.70	7.72	7.79	8.35	8.34	8.29	8.37	8.27	8.26	8.26	8.28
	Hα	4.43	2.68	2.67	2.65	2.44/2.44	4.34	4.34	4.36	4.36	4.35	4.36	4.36	4.36
	Cα	54.94	47.87	47.58	47.86	44.31	55.41	55.44	54.99	55.34[b]	55.35	55.32	55.32	55.32
	Cβ	43.14[d]	50.58	50.57	50.60	48.32	42.63	42.54	42.62	42.66	42.36[d]	42.75	42.40	42.40[c]
33	NH	8.25	8.04	8.02	8.03	8.20	8.24	8.22	8.24	8.22	8.22	8.21	8.21	8.22

	Hα	4.60	4.50	4.49	4.48	4.55	4.56	4.57	4.58	4.58	4.57	4.58	4.58	4.58
	Cα	54.40	54.21	54.19	54.17	54.39	54.28	54.29	54.22	54.29	54.28	54.27	54.27	54.28
	Cβ	41.46	41.23	41.20	41.28	41.33	41.03	41.13	40.89	41.11	41.09	41.15	41.11	41.15
34	NH	8.20	8.32	8.30	8.30	8.30	8.19	8.18	8.19	8.18	8.20	8.17	8.19	8.17
	Hα2	3.84	3.88	3.86	3.84	3.87	3.87	3.86	3.86	3.85	3.88	3.86	3.86	3.86
	Hα3	3.81	3.88	3.86	3.84	3.87	3.87	3.86	3.86	3.85	3.88	3.86	3.86	3.86
	Cα	45.05	45.10	45.10	45.09	45.12	45.14	45.09	45.09	45.10	45.12	45.11	45.11	45.09

a Missing resonance/the resonance could not be assigned due to chemical exchange.
b The error of the assignment is +/-0.05 ppm due to overlap
c The error of the assignment is +/-0.15 ppm due to overlap
d The error of the assignment is +/-0.25 ppm due to overlap

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