Supporting Information

I. Methods

1. Peptide syntheses

Automated peptide syntheses were performed on a 24-reactor block SYRO Multiple Peptide Synthesizer equipped with a vortexing unit (Multisyntech, Witten, Germany). Peptides were synthesized by standard Fmoc/tBu strategy using HBTU/DIPEA couplings.

2. Analyses

LC-MS data were collected on an Agilent 1100 Series instrument with a Phenomenex Kinetex C18 100Å column (150 x 4.6 mm, 5 μm at 35 °C) connected to an ESMSD type VL mass detector with a flow rate of 1.5 ml/min was used with the following solvent systems: (A): 0.1% HCOOH in H_2O and (B) MeCN. The column was flushed with 100% A for 2 min, then a gradient from 0 to 100% B over 6 min was used, followed by 2 min of flushing with 100% B. RP-HPLC analyses and purification were performed on an Agilent 1100 Series instrument with a Phenomenex Luna C18 column (250 x 4.6 mm, 5 μm at 35 °C). A flow rate of 1 ml/min was used with the following solvent systems: (A): 0.1% TFA in H_2O and (B): MeCN. The column was flushed for 2 min with 100% A, then a gradient from 0 to 100% B over 15 min was used, followed by 5 min of flushing with 100% B. MALDI-TOF-MS spectra were acquired with a high performance nitrogen laser (337 nm), using the positive and reflector mode with delayed extraction. The matrix solution utilized was as follows: 4-5 mg α-cyano-4-hydroxycinnamic acid in 500μL MeCN, 490μL mQ, 10μL 1M ammoniumcitrate, 1μL TFA. CD spectra were measured using an Aviv 410 CD spectrophotometer equipped with a Peltier temperature control unit

II. Peptide Synthesis and analysis

1. Protocol for deprotection of peptides

Protected peptide-resin is treated with TFA/TIS/mQ = 95:2.5:2.5 (4 mL/100 mg). After 4 hours, the liquid is filtered of and the resin is washed 2 times with neat TFA. The filtrate is evaporated followed by precipitation (twice) by centrifugation at 4000 rpm at 0°C in cold MTBE (-20°C). The peptide was then redissolved in milliQ H_2O for RP-HPLC, LC-MS analysis, RP-HPLC purification or lyophilized for the next step.

2. Syntheses of peptides 1-7

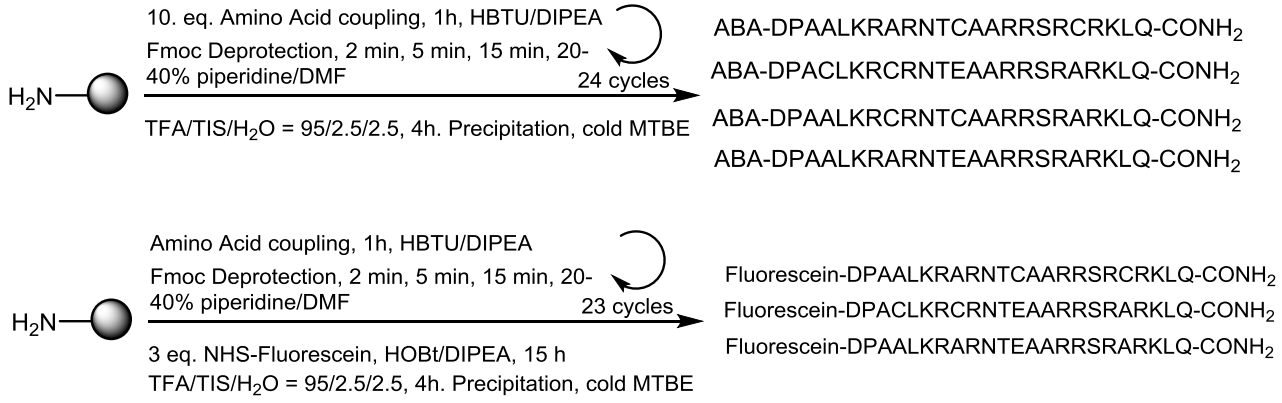


Figure 1: Syntheses of peptides **1-7**

The resin is preswollen in NMP for 10 min and then filtered off. Peptide synthesis is performed on an automated peptide synthesizer using the following protocols for Fmoc deprotection and coupling:

Fmoc deprotection: Fmoc deprotection is performed by adding a solution of 20% piperidine in NMP to the resin and shaking for 2 min, 5 min & 15 min durations. After each addition and shaking cycle, the resin is filtered off and washed with NMP (6 x 45 s).

Coupling: 10 equivalents of a 0.5 M solution of amino acid in NMP, 10 equivalents of a 0.5 M solution of HBTU in DMF and 10 equivalents of a 2.0 M solution of DIPEA in NMP are added to the resin. The reaction mixture is

shaken for 1 hour. The resin is filtered off and washed with NMP (9 x 2 min). The peptide is analyzed according to the above protocol for deprotection of peptides.
Capping: ABA was used for capping and was coupled using the protocol in *coupling* above.
Coupling of Fluorescein: 3 eq. of NHS-fluorescein predissolved in 0.5 mL DMF. 6 eq. of DIPEA and 3 eq. of HOBt predissolved in 0.5 mL DMF and are added successively to the resin. After shaking for 15 h, the reaction mixture was filtered off and the resin washed with 3x DMF, 3x MeOH, 3x DMF and 3x Et_2O.

No.	Sequence	Chemical Formula	Exact Mass	Obsd. Mass
1	ABA-DPAALKRARNTCAARRSRCRKLQ-$CONH_2$	$C_{115}H_{201}N_{47}O_{31}S_2$	2800.50	2801.88
2	ABA-DPACLKRCRNTEAARRSRARKLQ-$CONH_2$	$C_{117}H_{203}N_{47}O_{33}S_2$	2858.51	2859.47
3	ABA-DPAALKRCRNTCAARRSRARKLQ-$CONH_2$	$C_{115}H_{201}N_{47}O_{31}S_2$	2800.50	2801.60
4	ABA-DPAALKRARNTEAARRSRARKLQ-$CONH_2$	$C_{117}H_{203}N_{47}O_{33}$	2794.57	2795.28
I	Fluorescein-DPAALKRARNTCAARRSRCRKLQ-$CONH_2$	$C_{127}H_{204}N_{46}O_{35}S_2$	2997.50	2997.57
II	Fluorescein-DPACLKRCRNTEAARRSRARKLQ-$CONH_2$	$C_{129}H_{206}N_{46}O_{37}S_2$	3055.51	3056.61
IV	Fluorescein-DPAALKRARNTEAARRSRARKLQ-$CONH_2$	$C_{129}H_{206}N_{46}O_{37}$	2991.57	2992.54

Table 1: Calculated and observed masses of peptides

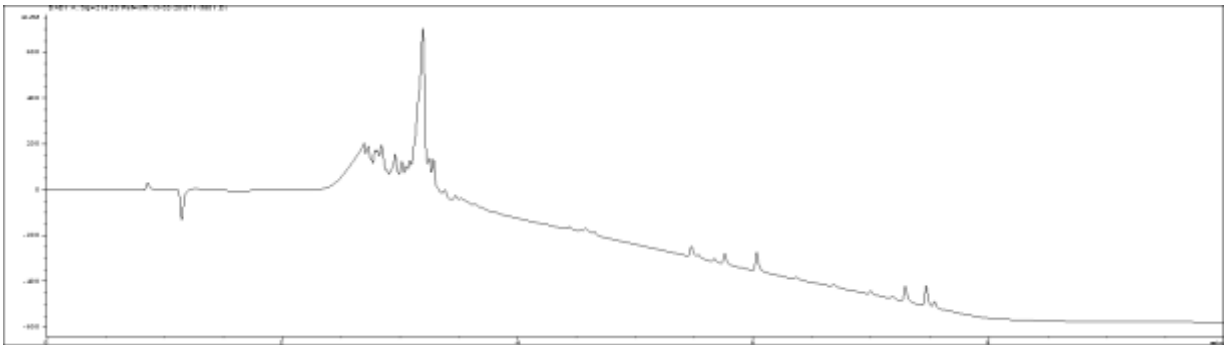


Figure 2: RP-HPLC trace from LC-MS of peptide **1** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

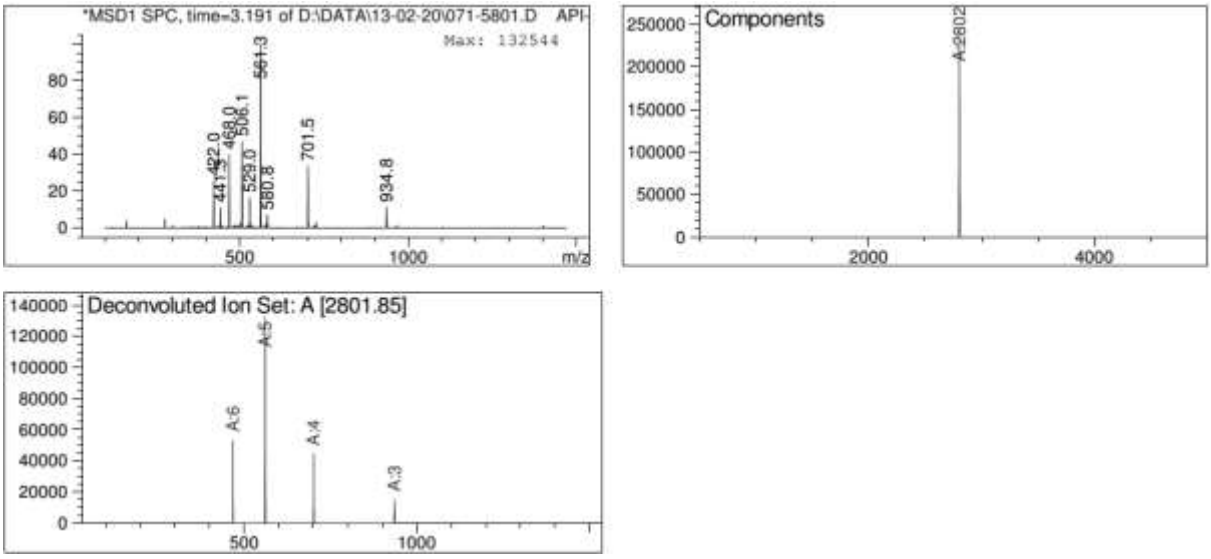


Figure 3: ESI-MS from LC-MS at t = 3.191 min. E.M. Calcd. for $C_{115}H_{201}N_{47}O_{31}S_2$ = 2800.50 and deconvoluted mass found 2801.85

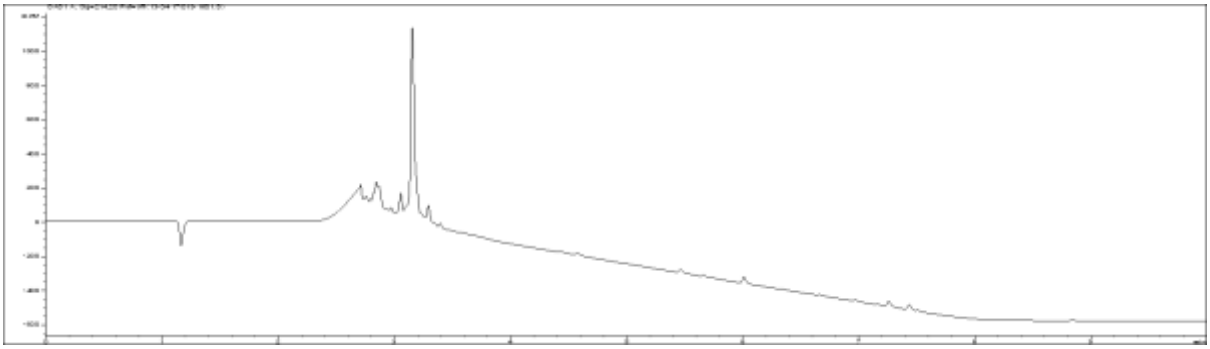


Figure 4: RP-HPLC trace from LC-MS of peptide **2** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

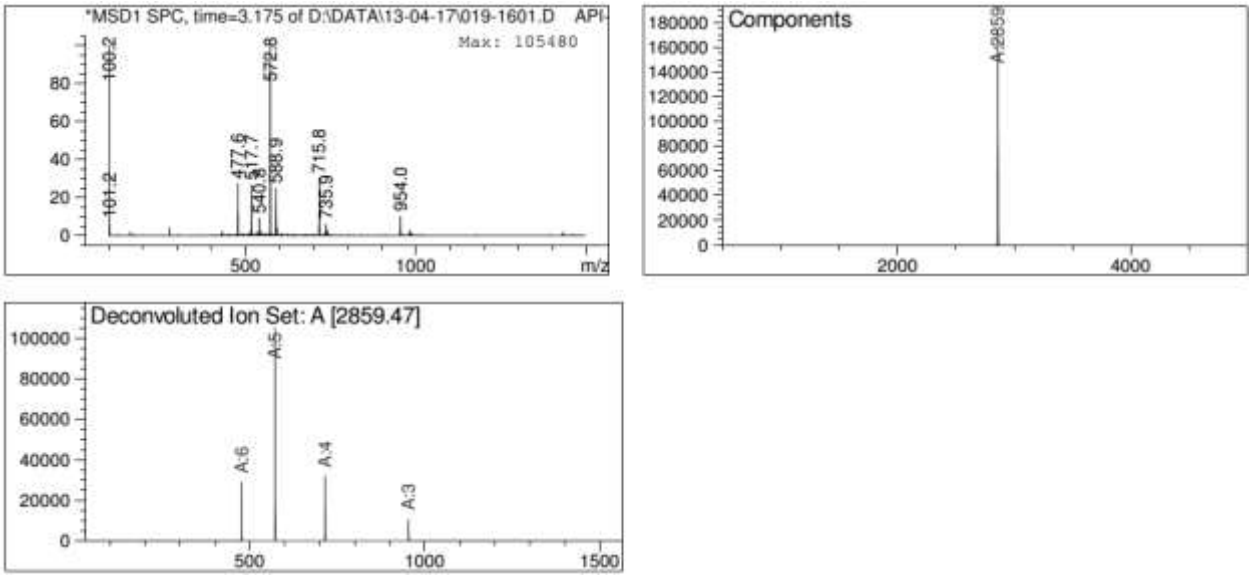


Figure 5: ESI-MS from LC-MS at t = 3.175 min. E.M. Calcd. for $C_{117}H_{203}N_{47}O_{33}S_2$ = 2858.51 and deconvoluted mass found 2859.47

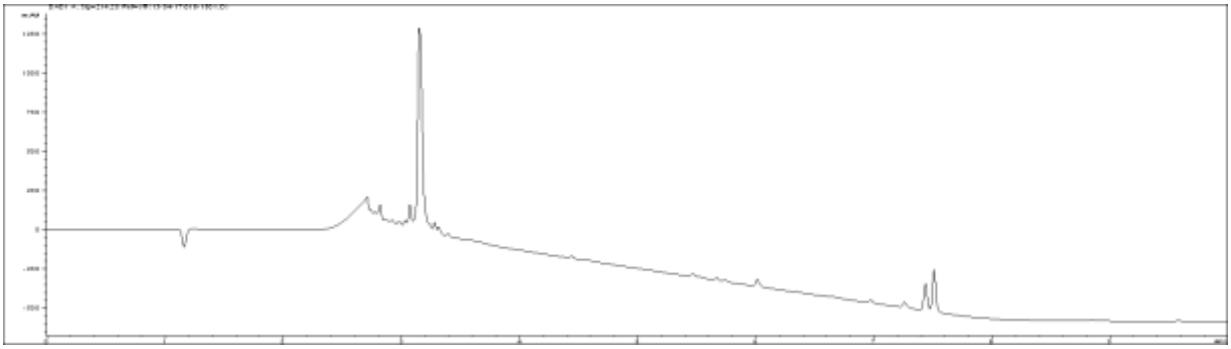


Figure 6: RP-HPLC trace from LC-MS of peptide **3** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

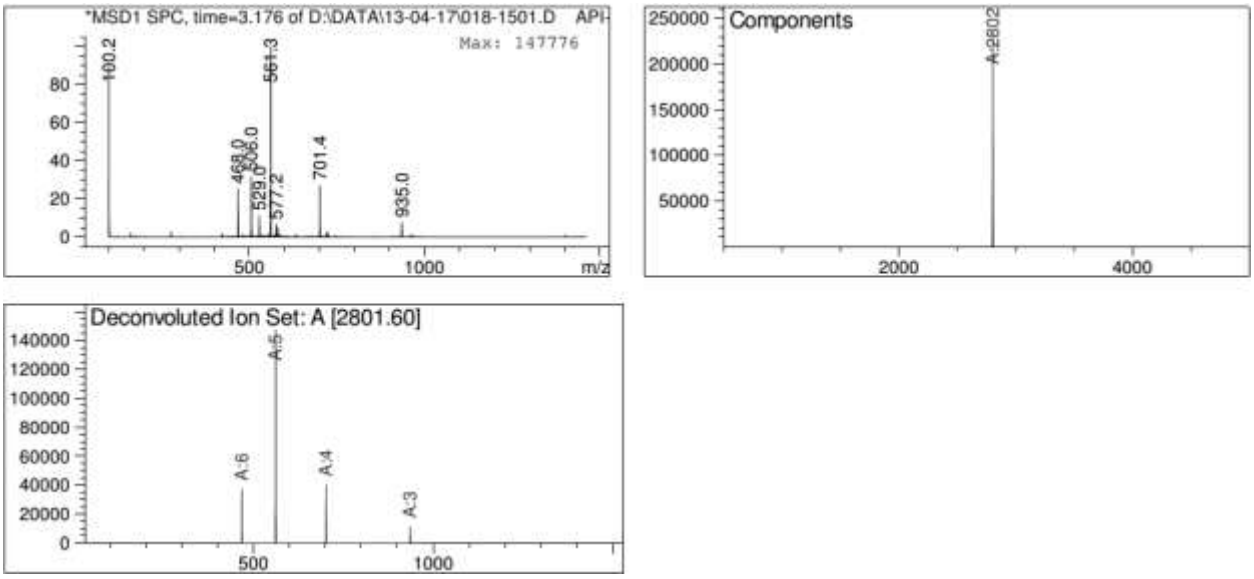


Figure 7: ESI-MS from LC-MS at t = 3.176 min. E.M. Calcd. for $C_{115}H_{201}N_{47}O_{31}S_2$ = 2800.50 and deconvoluted mass found 2801.60

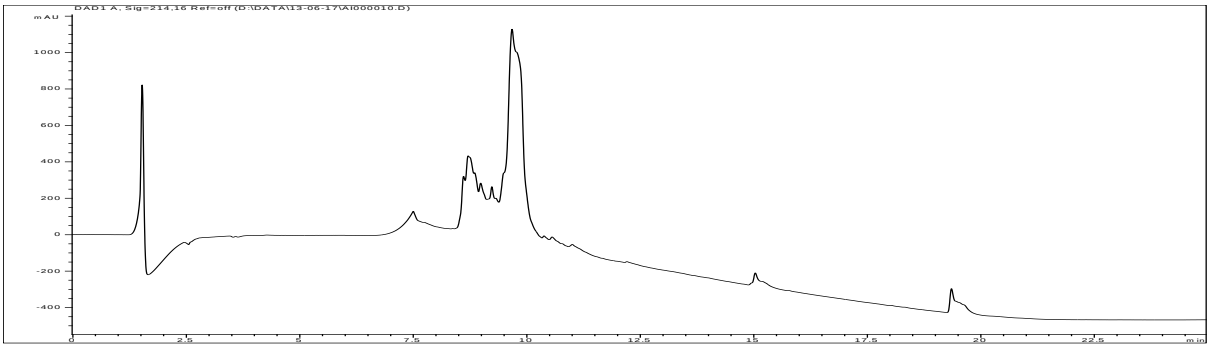


Figure 8: RP-HPLC trace of peptide **4** (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 μm, at 35 °C)

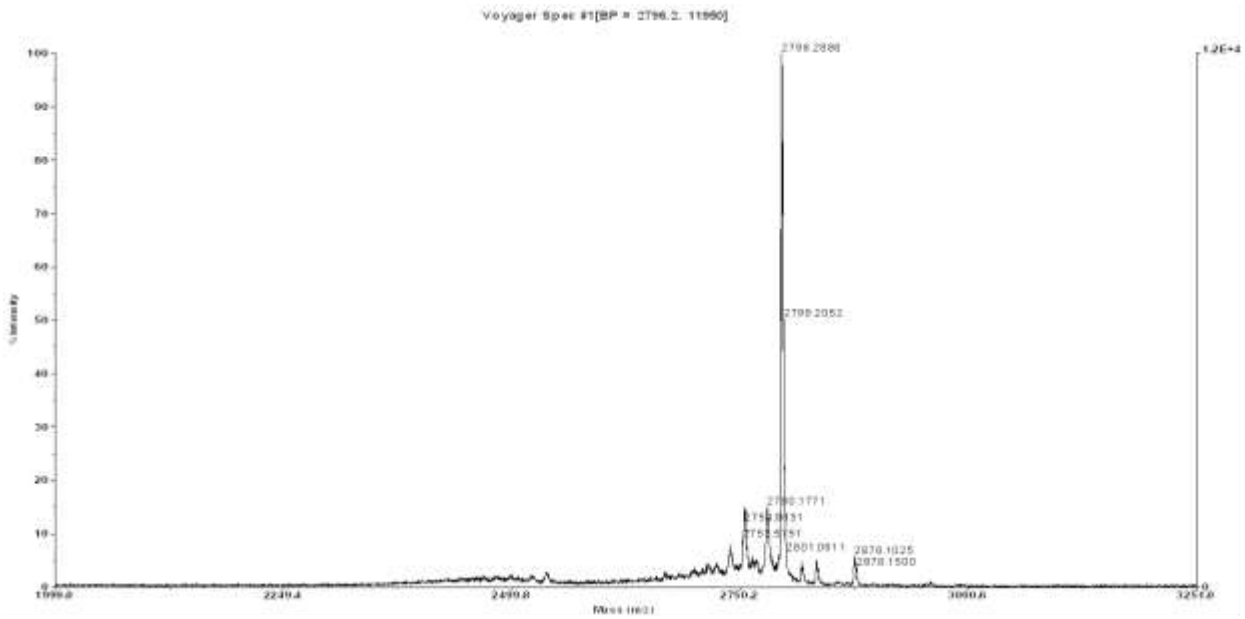


Figure 9: MALDI-MS spectrum of peak at t_R= 9.681. E.M. Calcd. for $C_{115}H_{201}N_{47}O_{31}S_2$ = 2794.57 and mass found $M+H^+$= 2796.28

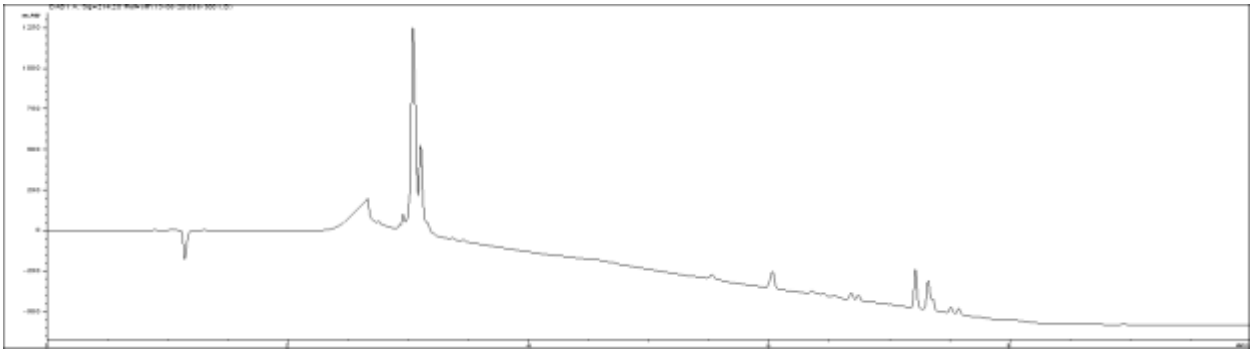


Figure 10: RP-HPLC trace from LC-MS of HPLC purified peptide **4** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

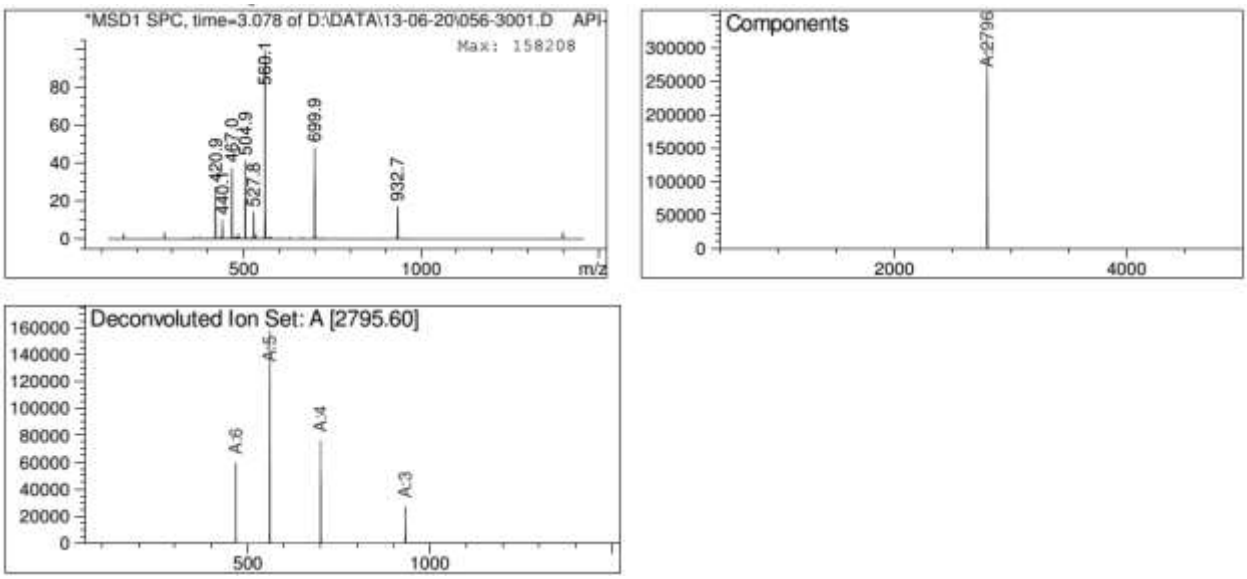


Figure 11: ESI-MS from LC-MS at t = 3.078 min. E.M. Calcd. for $C_{115}H_{201}N_{47}O_{31}S_2$ = 2794.57 and deconvoluted mass found 2795.60

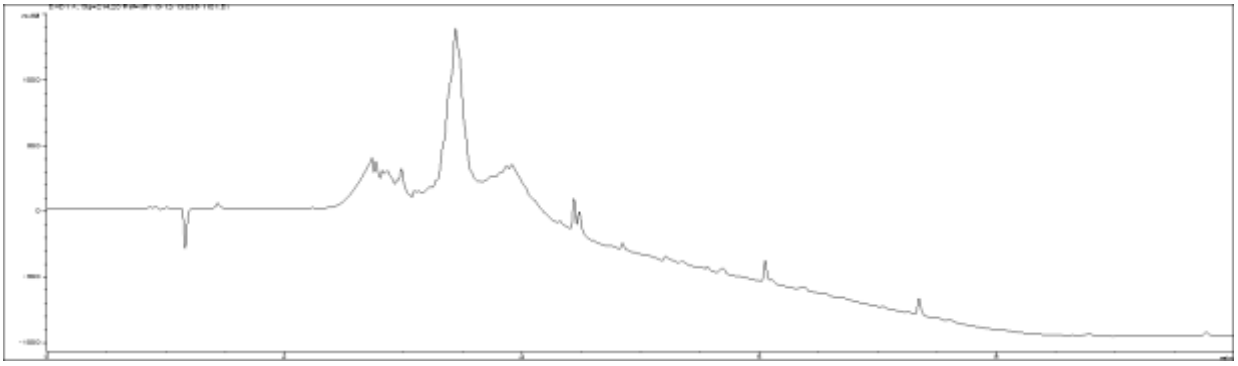


Figure 12: RP-HPLC trace from LC-MS of peptide **I** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

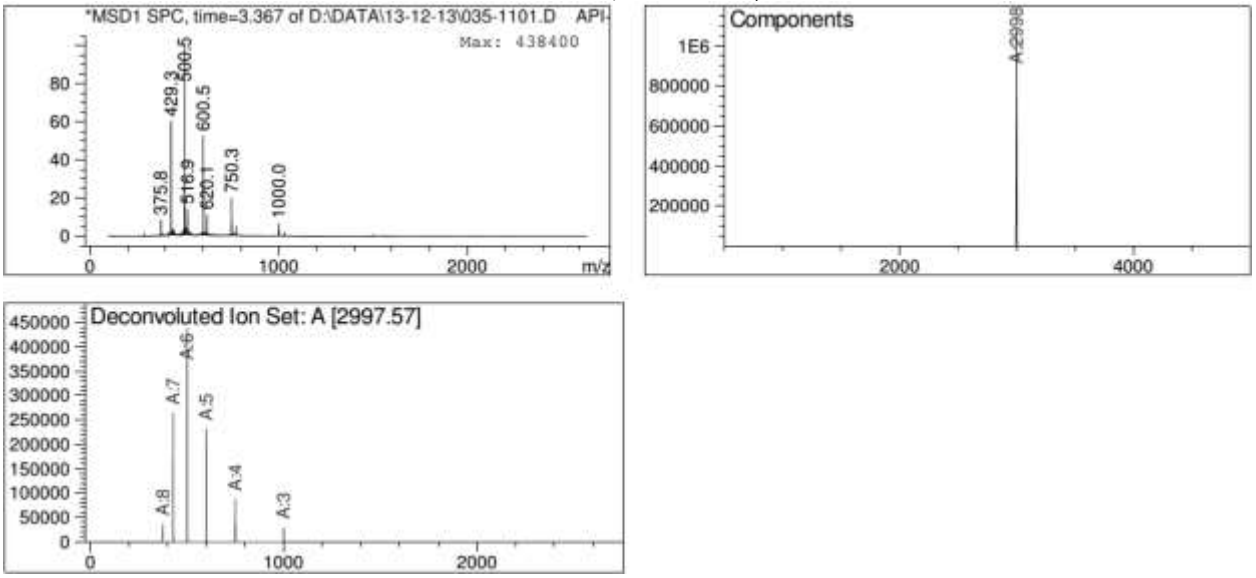


Figure 13: ESI-MS from LC-MS at t = 3.367 min. E.M. Calcd. for $C_{127}H_{204}N_{46}O_{35}S_2$ = 2997.50 and deconvoluted mass found 2997.57

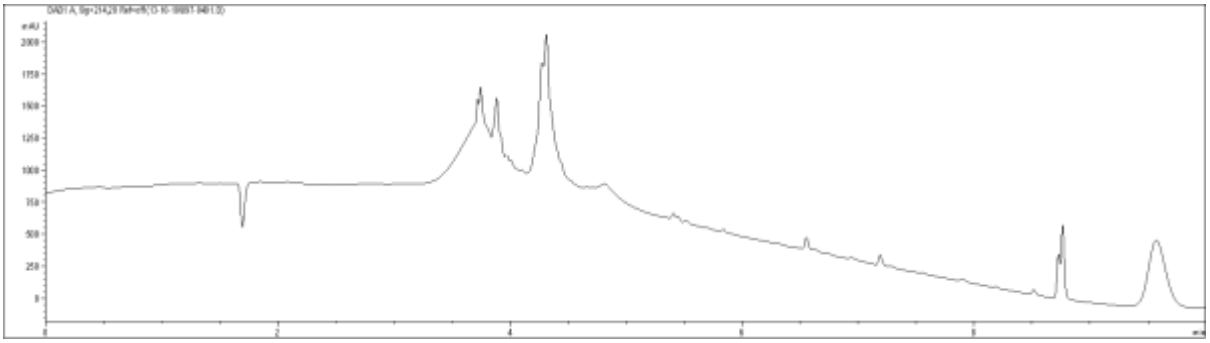


Figure 14: RP-HPLC trace from LC-MS of peptide **II** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

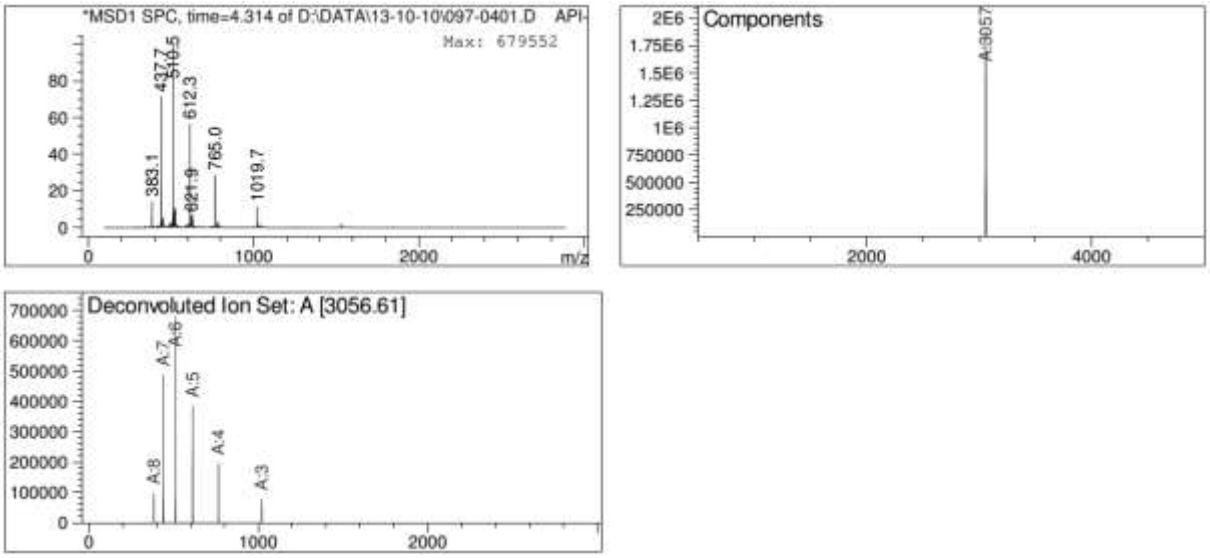


Figure 15: ESI-MS from LC-MS at t = 4.314 min. E.M. Calcd. for $C_{129}H_{206}N_{46}O_{37}S_2$ = 3055.51 and deconvoluted mass found 3056.61

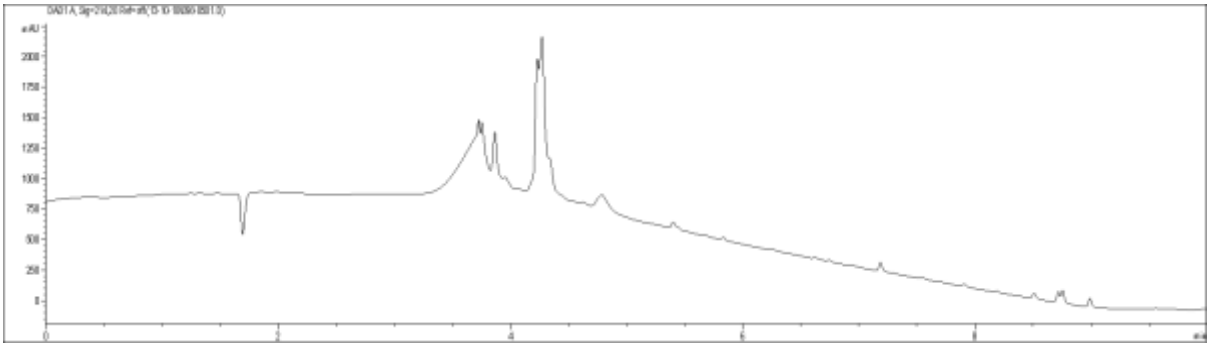


Figure 16: RP-HPLC trace of crude peptide **IV** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

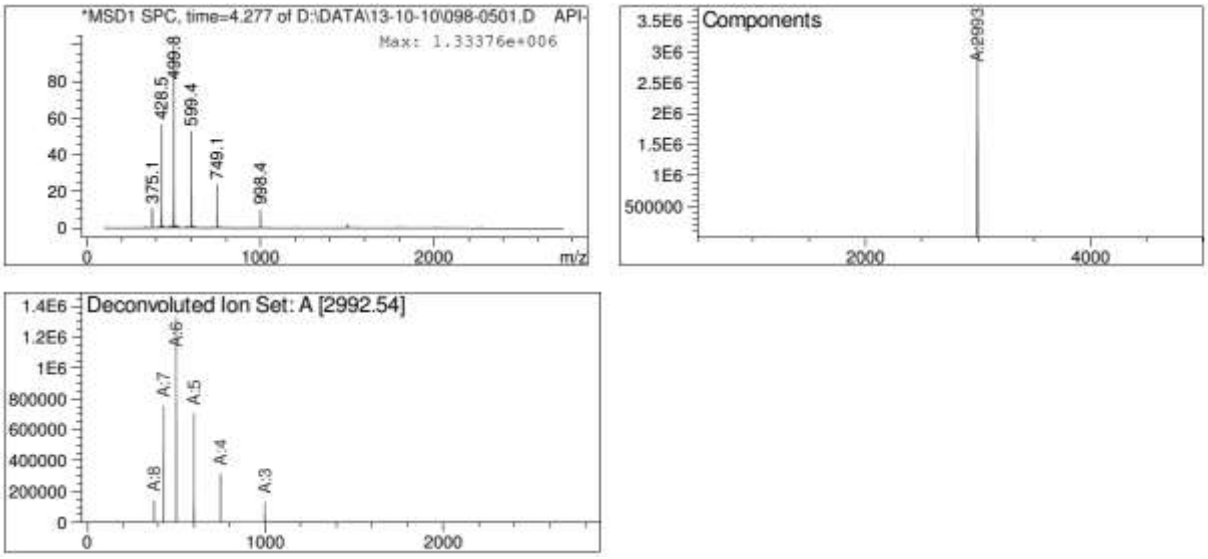


Figure 17: ESI-MS from LC-MS at t = 4.277 min for crude compound **7**. E.M calcd. for $C_{129}H_{206}N_{46}O_{37}$ = 2991.57 and deconvoluted mass found 2992.54.

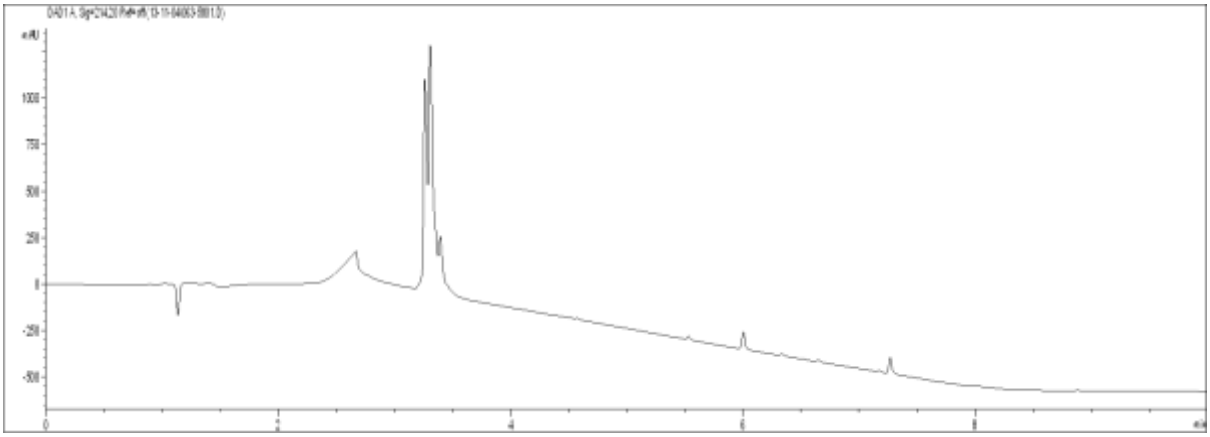


Figure 18: RP-HPLC trace of HPLC purified peptide **IV** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

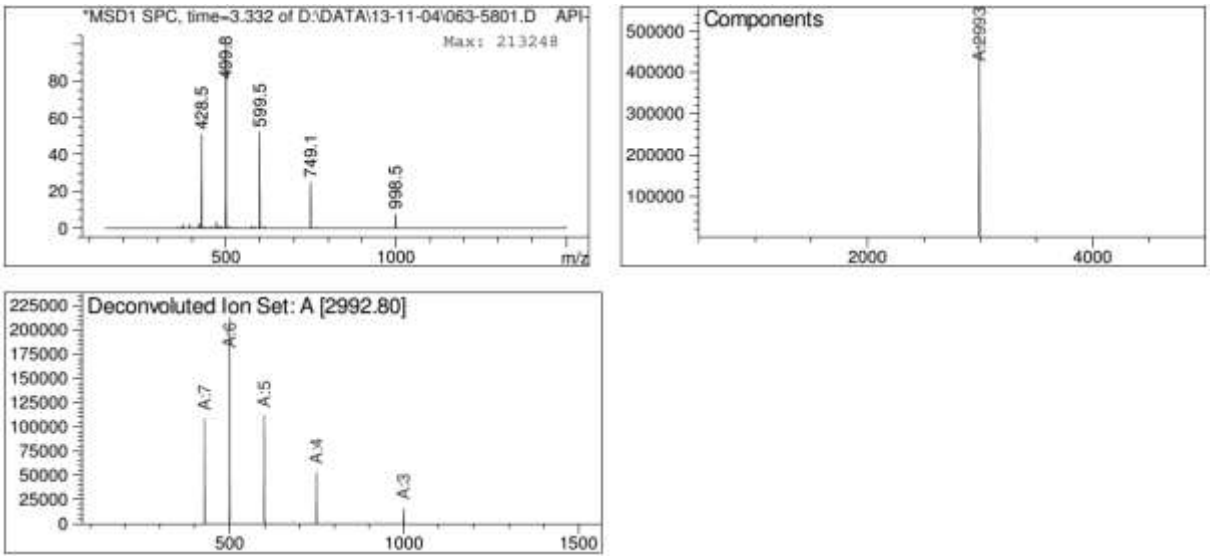
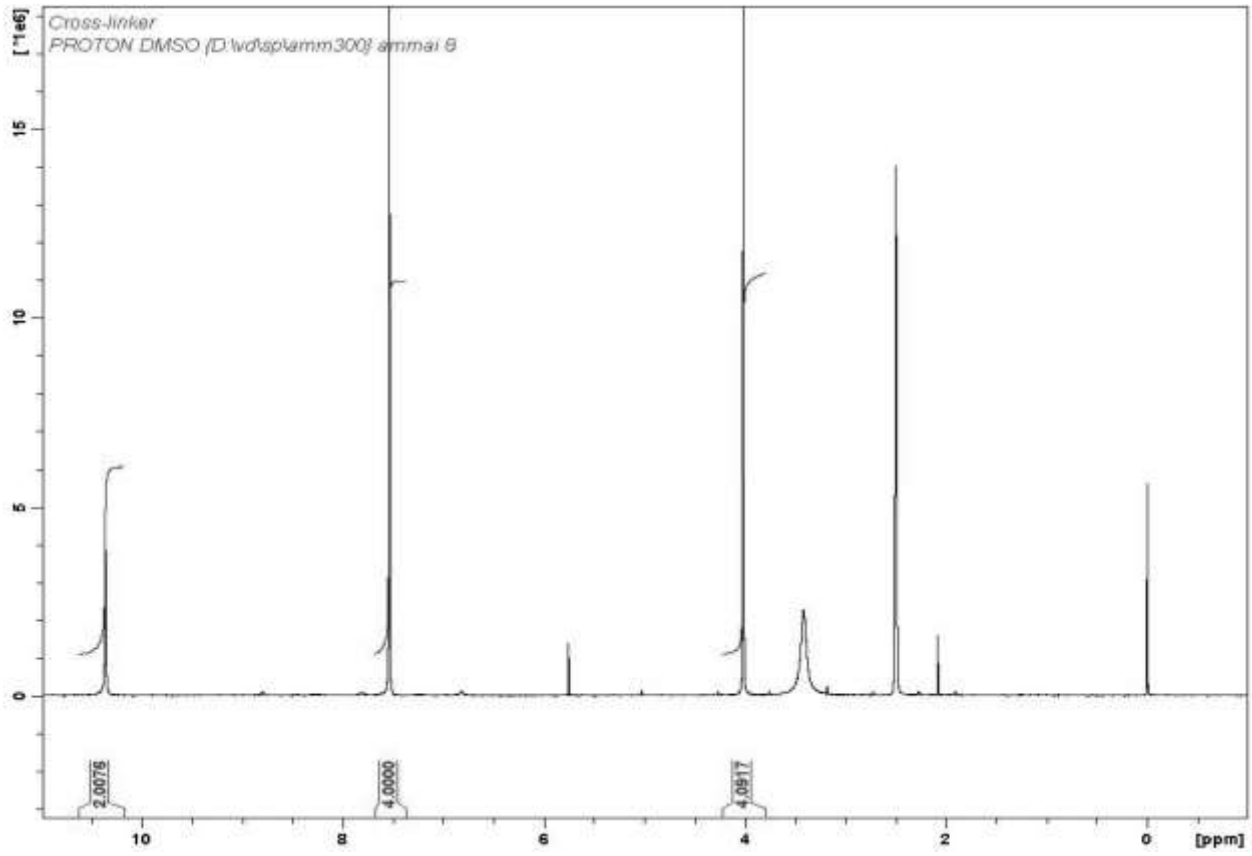


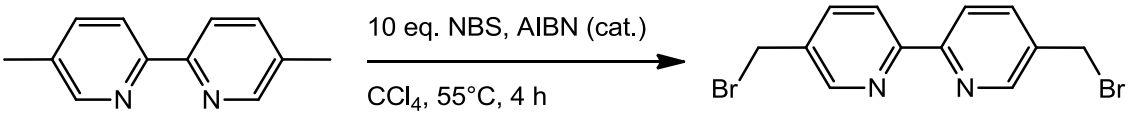
Figure 19: ESI-MS from LC-MS at t = 3.332 min. E.M calcd. for HPLC purified peptide **7** $C_{129}H_{206}N_{46}O_{37}$ = 2991.57 and deconvoluted mass found 2992.80.

IV. Synthesis of N,N'-(1,4-phenylene)bis(2-bromoacetamide)

To a solution of p-Phenylenediamine **5** (54 mg, 0.5 mmol) in CH_2Cl_2 (5 mL), dry pyridine (96 μL, 1.2 mmol) and 4-(dimethylamino)pyridine (1.5 mg) was added bromoacetyl bromide (96 μL, 1.1 mmol) at 0°C, and the mixture was stirred at 25°C for 3h. The resulting precipitates were collected by filtration and washed with CH_2Cl_2 (3 x 5 mL) to give N,N'-(1,4-phenylene)bis(2-bromoacetamide) **b** (145 mg, 0.41 mmol, 82%) as a white solid. ^{1}H NMR (DMSO-d6, 300 MHz): δ 4.02 (s, 4H), δ 7.54 (s, 4H), δ 10.43 (s, 2H) ESI-MS: Calcd. for $C_{10}H_{10}Br_2N_2O_2$: 350.01, obsd. $M+H^+$= 350.8

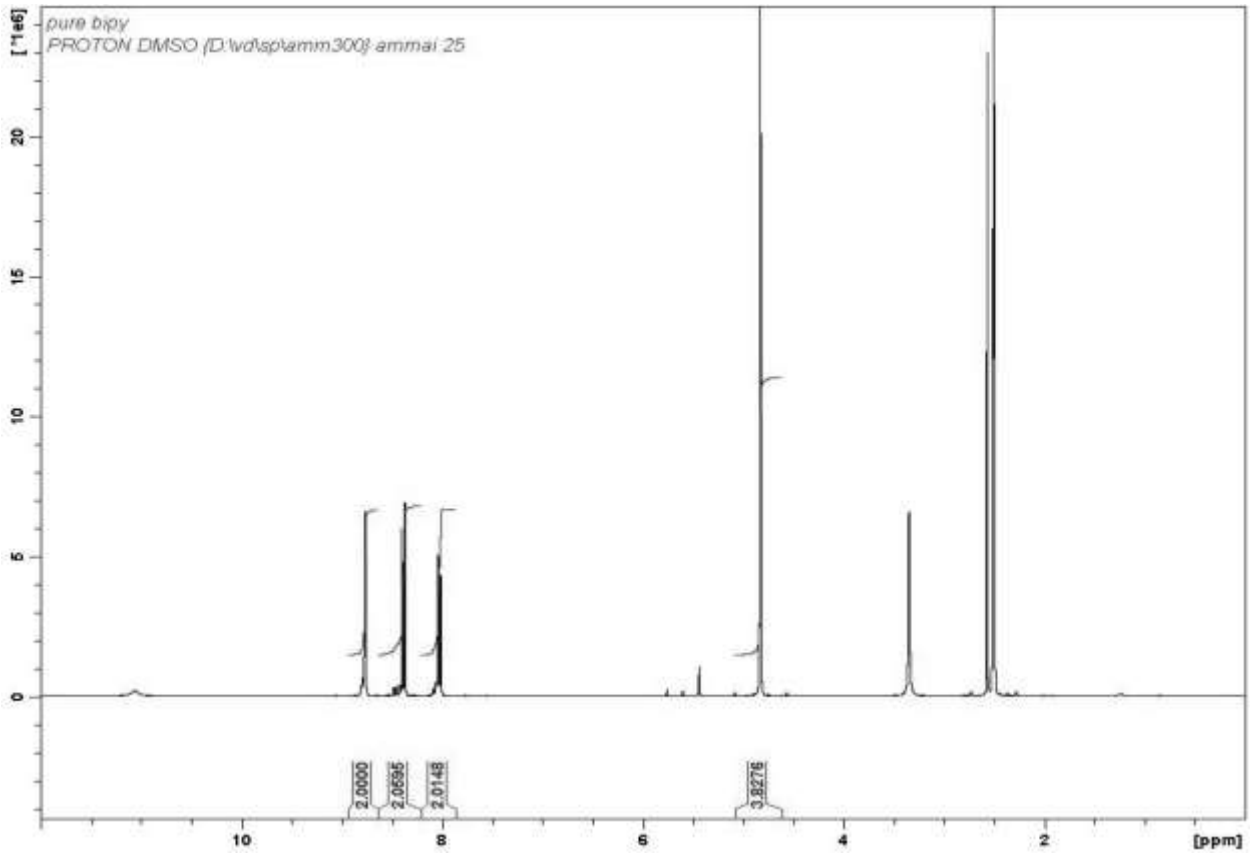


V. Synthesis of 5,5'-bis(bromomethyl)-2,2'-bipyridine



6 **c**

5, 5'-dimethyl-2,2' bipyridine **6** (2 mmol, 368 mg) was dissolved in 20 mL dry CCl_4 together with NBS (10 mmol, 1.78 g) and AIBN (20 mg) was stirred under reflux for 4 hrs at 55°C. The reaction mixture was then dried under vacuum and purified by silica gel column chromatography using EtOAc/Hexane (1:1) to obtain pure 5,5'-bis(bromomethyl)-2,2'-bipyridine **c**. ^{1}H NMR (DMSO-d6, 300 MHz): δ 8.85 (d, 2H), δ 8.43 (d, 2H), δ 8.10 (d, 2H), δ 4.88 (s, 4H), δ 2.63 (s, 1.5H) ESI-MS: Calcd. for $C_{12}H_{10}Br_2N_2$: 342.01, obsd. $M+H^+$= 342.9



VI. Stapled peptides

The following stapled peptides were synthesized:

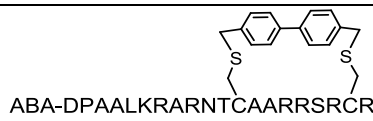
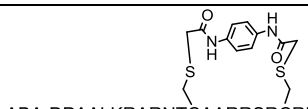
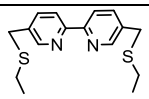
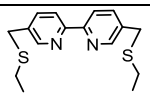
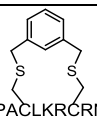
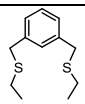
No.	Peptide	Chemical Formula	Exact Mass [M]	Observed
1a	 ABA-DPAALKRARNTCAARRSRCRKLQ-CONH$_2$	$C_{129}H_{211}N_{47}O_{31}S_2$	2978.58	M + H$^+$ = 2980.88
Ia	 Fluorescein-DPAALKRARNTCAARRSRCRKLQ-CONH$_2$	$C_{141}H_{214}N_{46}O_{35}S_2$	3175.58	M + H$^+$ = 3178.31
1b	 ABA-DPAALKRARNTCAARRSRCRKLQ-CONH$_2$	$C_{125}H_{209}N_{49}O_{33}S_2$	2988.56	M + H$^+$ = 2991.49
Ib	 Fluorescein-DPAALKRARNTCAARRSRCRKLQ-CONH$_2$	$C_{137}H_{212}N_{48}O_{37}S_2$	3185.56	3186.73
1c	 ABA-DPAALKRARNTCAARRSRCRKLQ-CONH$_2$	$C_{127}H_{209}N_{49}O_{31}S_2$	2980.57	2981.74
Ic	 Fluorescein-DPAALKRARNTCAARRSRCRKLQ-CONH$_2$	$C_{139}H_{212}N_{48}O_{35}S_2$	3177.57	3178.83
2d	 ABA-DPACLKRCRNTEAARRSRARKLQ-CONH$_2$	$C_{125}H_{209}N_{47}O_{33}S_2$	2960.56	M + H$^+$ = 2962.74
IId	 Fluorescein-DPACLKRCRNTEAARRSRARKLQ-CONH$_2$	$C_{137}H_{212}N_{46}O_{37}S_2$	3157.56	M + H$^+$ = 3161.14
3d	 ABA-DPAALKRCRNTCAARRSRARKLQ-CONH$_2$	$C_{123}H_{207}N_{47}O_{31}S_2$	2902.55	M + H$^+$ = 2904.71

Table 2: Calculated and observed masses of stapled peptides.

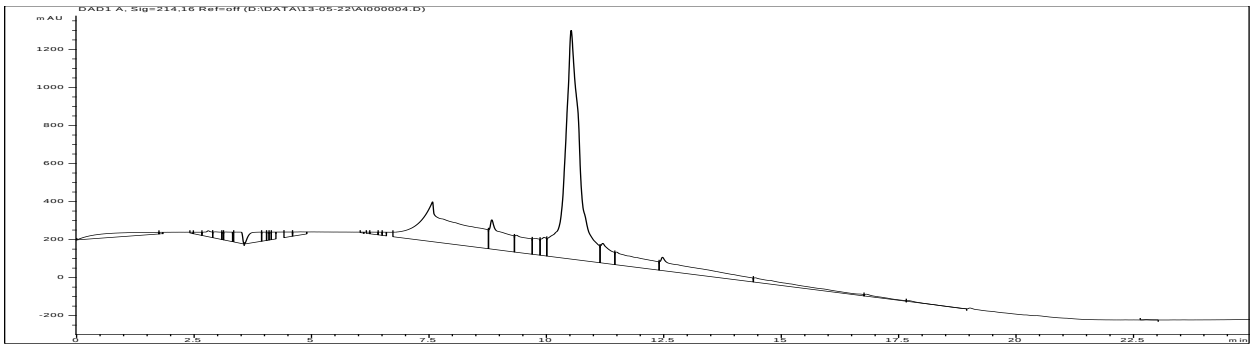


Figure 20: RP-HPLC trace of HPLC purified peptide **1a** with t_R= 10.526 min (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 µm, at 35 °C).

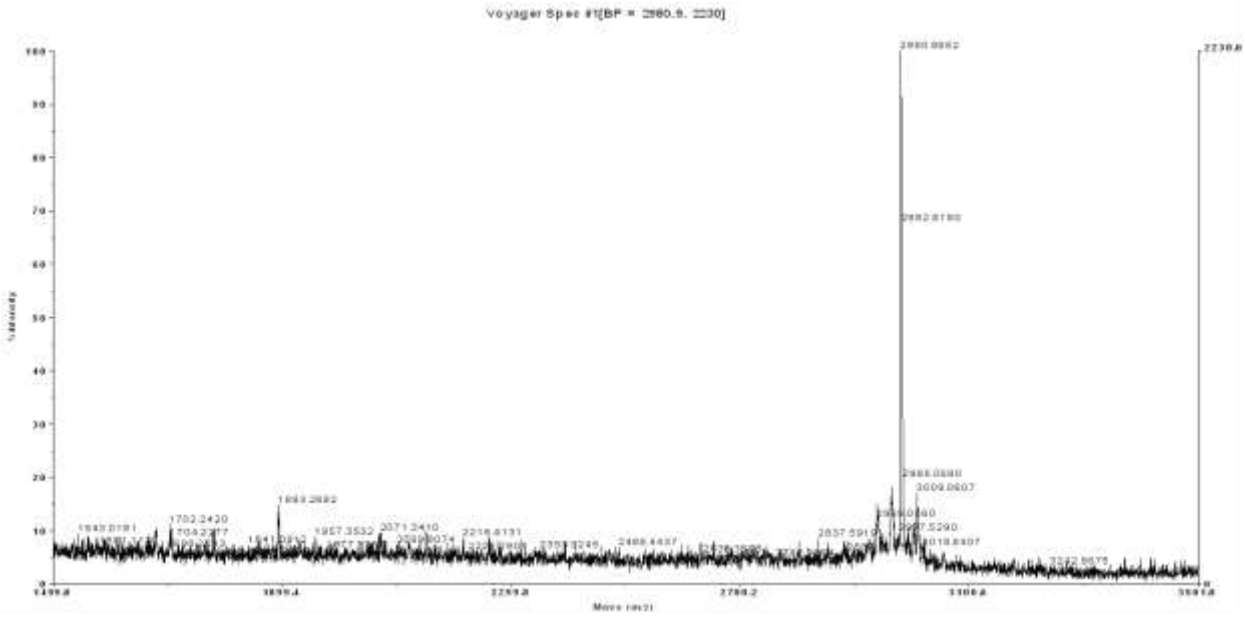


Figure 21: MALDI-MS spectrum of RP-HPLC purified peptide **1a**. E.M. Calcd. for $C_{129}H_{211}N_{47}O_{31}S_2$ = 2978.58 and mass found $M+H^+$= 2980.88

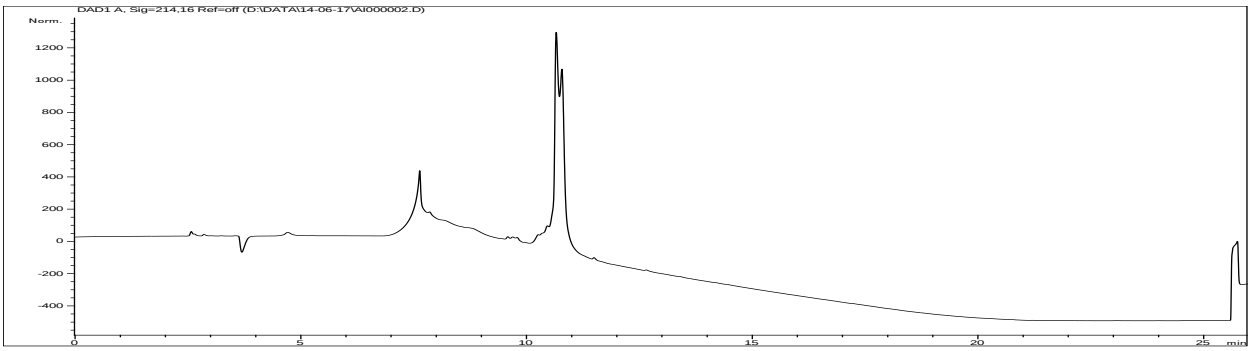


Figure 22: RP-HPLC trace of HPLC purified peptide **Ia** with t_R= 10.526 min (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 μm, at 35 °C)

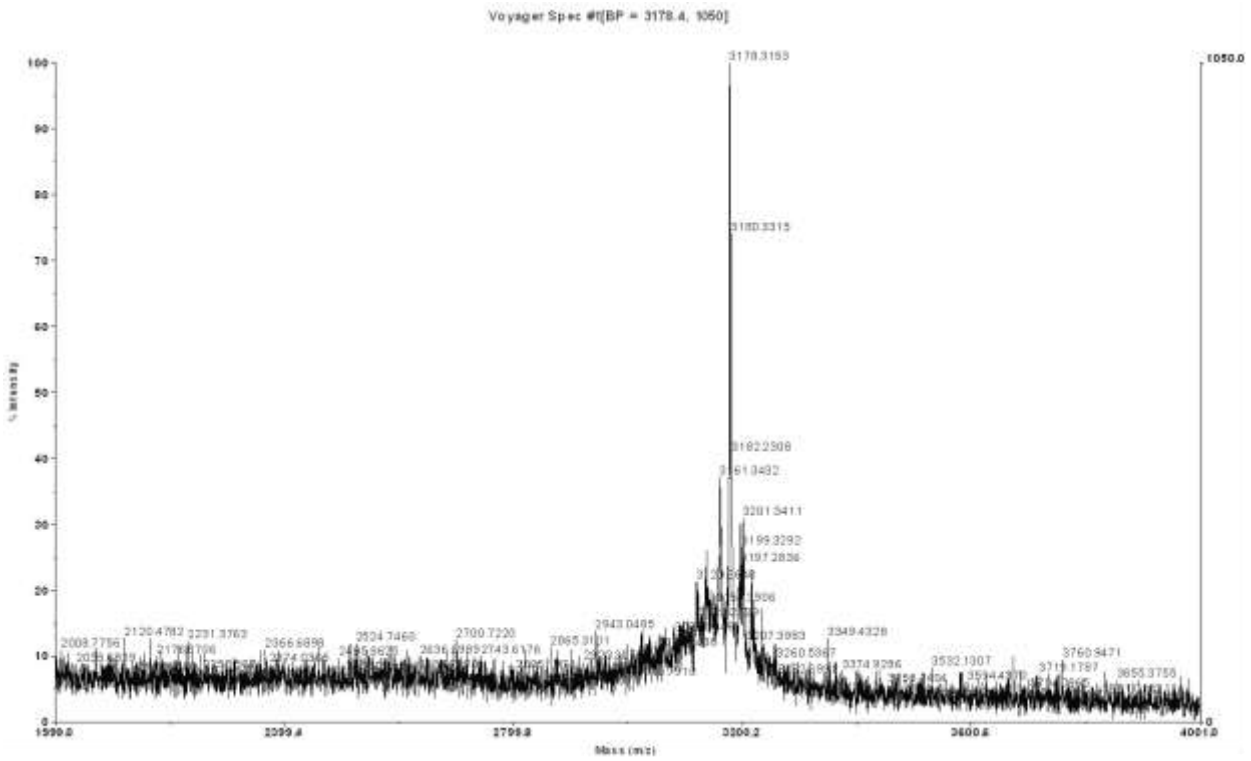


Figure 23: MALDI-MS spectrum of RP-HPLC purified peptide **Ia**. E.M. Calcd. for $C_{141}H_{21}4N_{46}O_{35}S_2$ = 3175.58 and mass found $M+H^+$= 3178.31

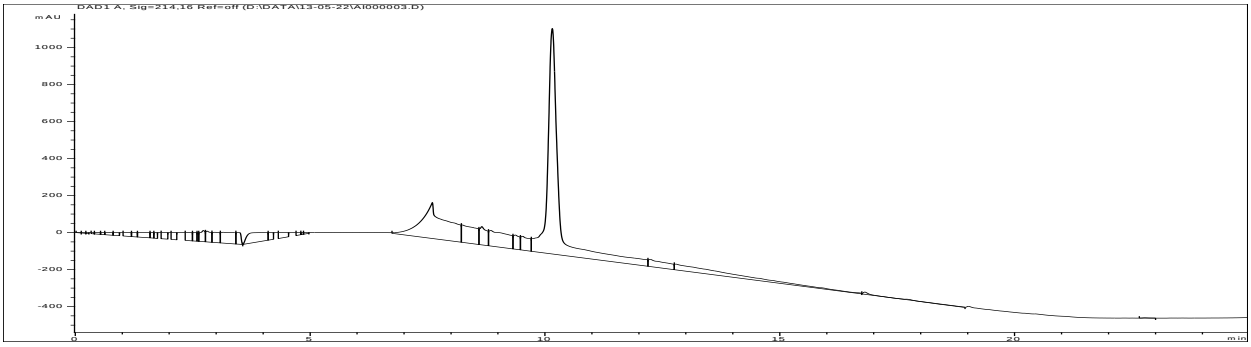


Figure 24: RP-HPLC trace of HPLC purified peptide **1b** with t_R= 10.155 min (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 μm, at 35 °C)

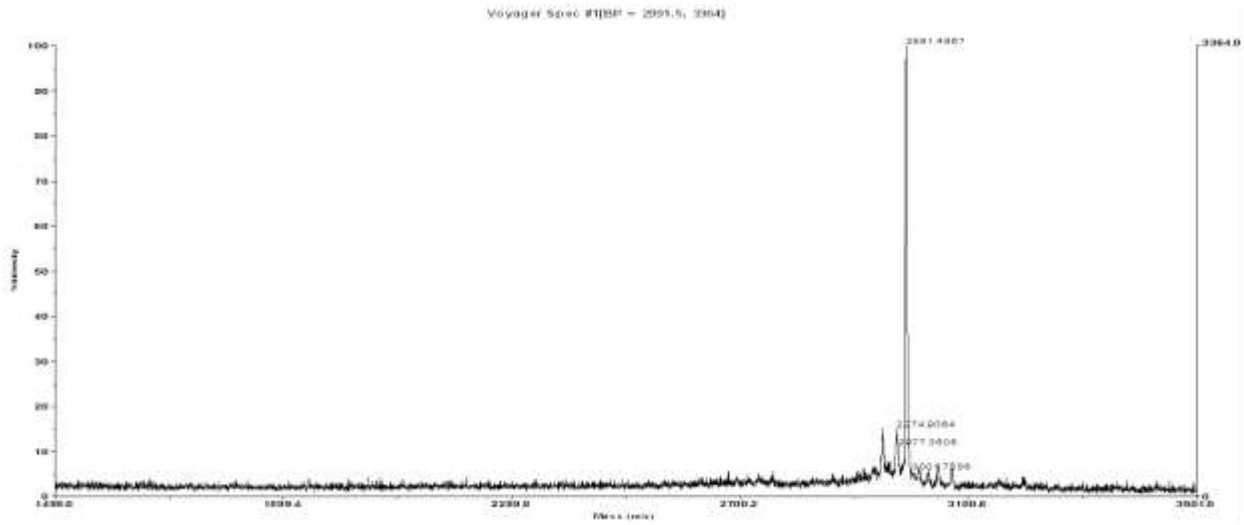


Figure 25: MALDI-MS spectrum of RP-HPLC purified peptide **1b**. E.M. Calcd. for $C_{125}H_{209}N_{49}O_{33}S_2$ = 2988.56 and mass found M+H$^+$= 2991.49

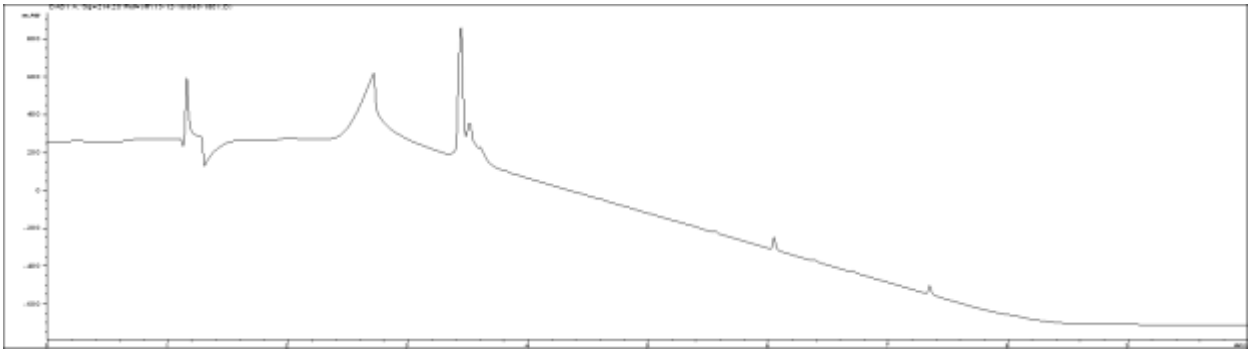


Figure 26: RP-HPLC trace of HPLC purified peptide **Ic** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

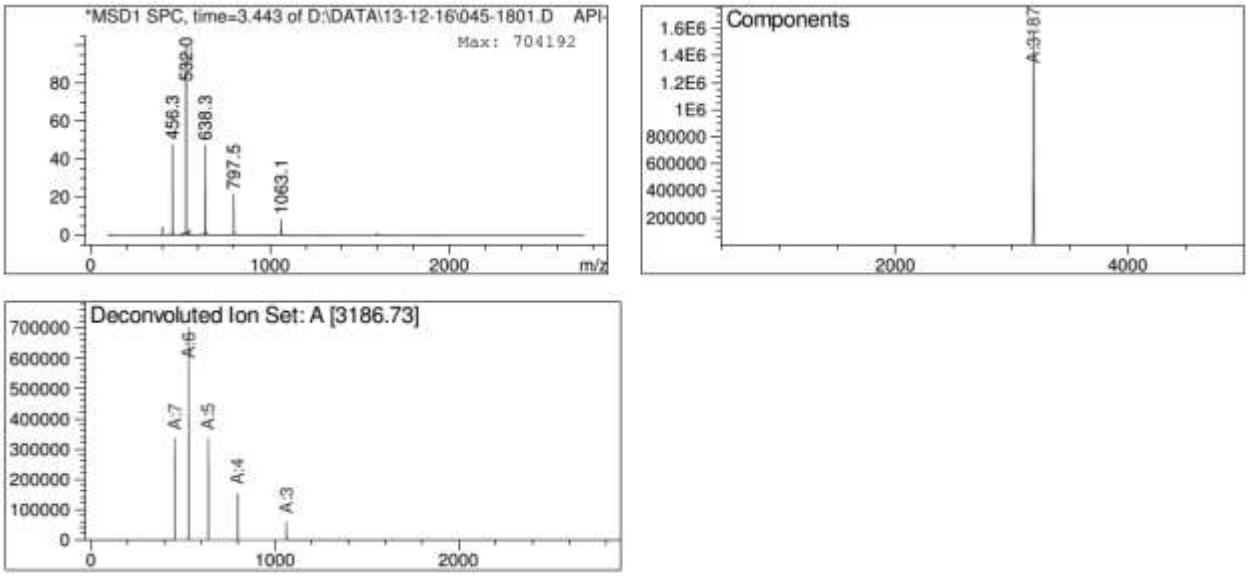


Figure 27: ESI-MS from LC-MS at t = 3.443 min. E.M calcd. for HPLC purified peptide **Ic** $C_{137}H_{212}N_{48}O_{37}S_2$ = 3185.56 and deconvoluted mass found 3186.73.

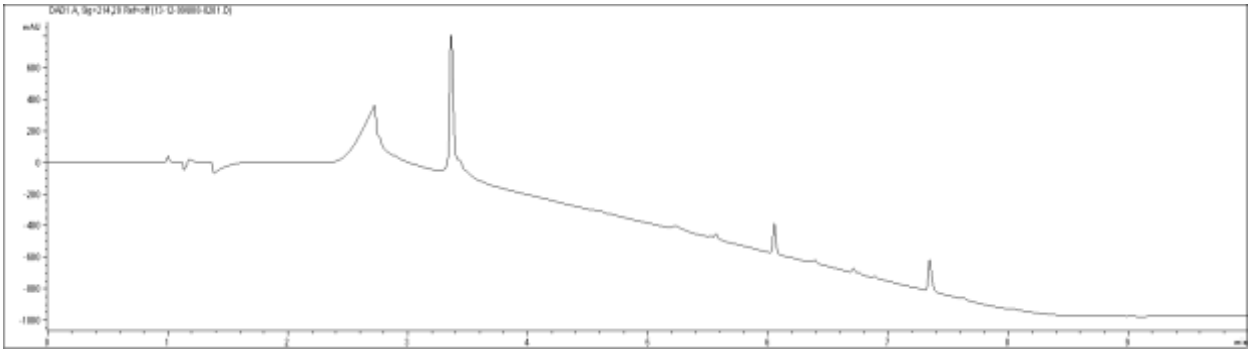


Figure 28: RP-HPLC trace of HPLC purified peptide **1c** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

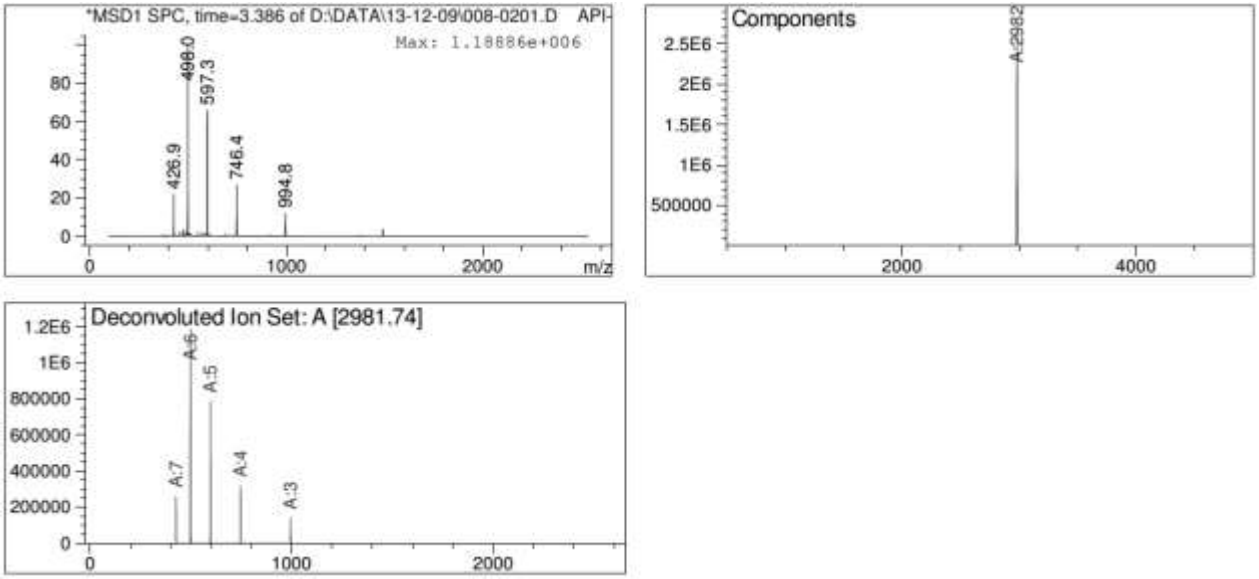


Figure 29: ESI-MS from LC-MS at t = 3.386 min. E.M calcd. for HPLC purified peptide **1c** $C_{127}H_{209}N_{49}O_{31}S_2$ = 2980.57 and deconvoluted mass found 2981.74.

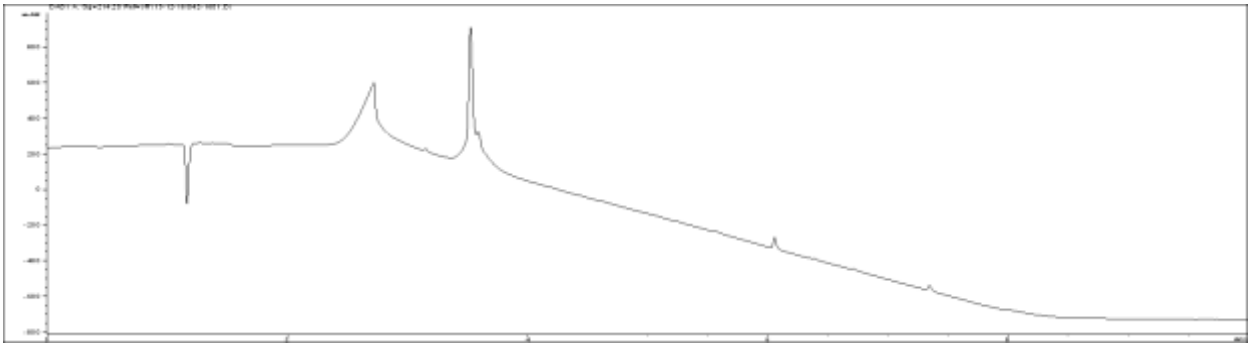


Figure 30: RP-HPLC trace of HPLC purified peptide **Ic** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 μm, at 35 °C)

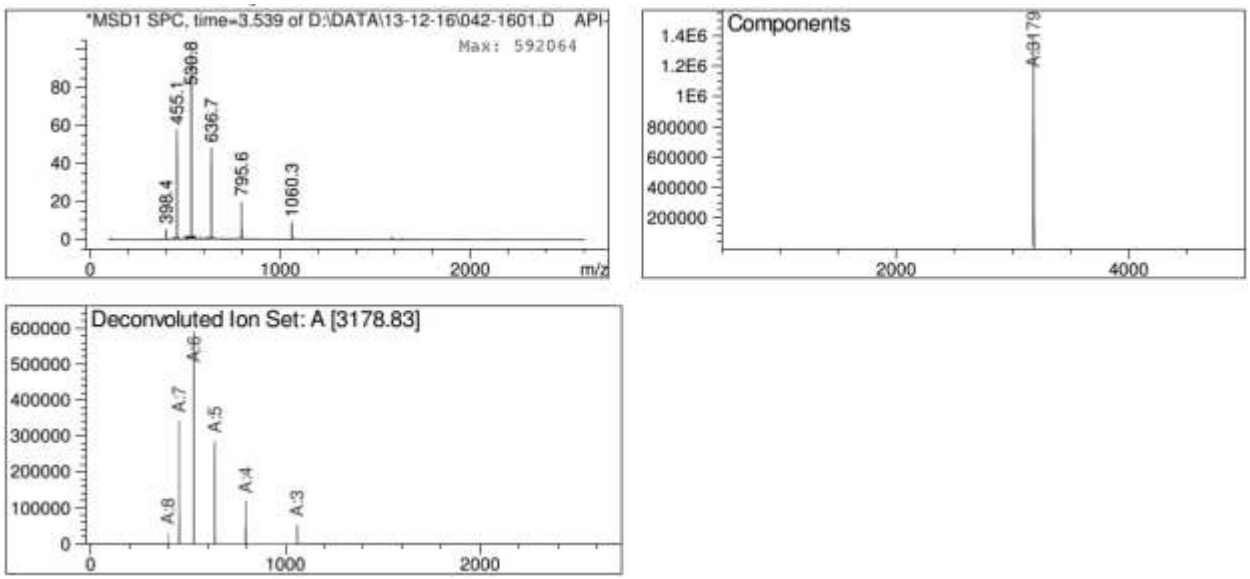


Figure 31: ESI-MS from LC-MS at t = 3.539 min. E.M calcd. for HPLC purified peptide **Ic** $C_{139}H_{212}N_{48}O_{35}S_2$ = 3177.57 and deconvoluted mass found 3178.83.

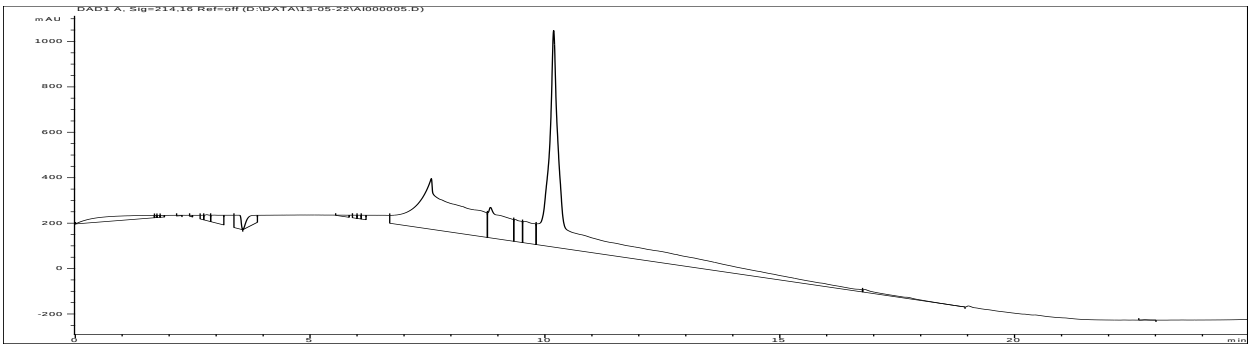


Figure 32: RP-HPLC trace of HPLC purified peptide **2d** with t_R= 10.185 min (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 μm, at 35 °C)

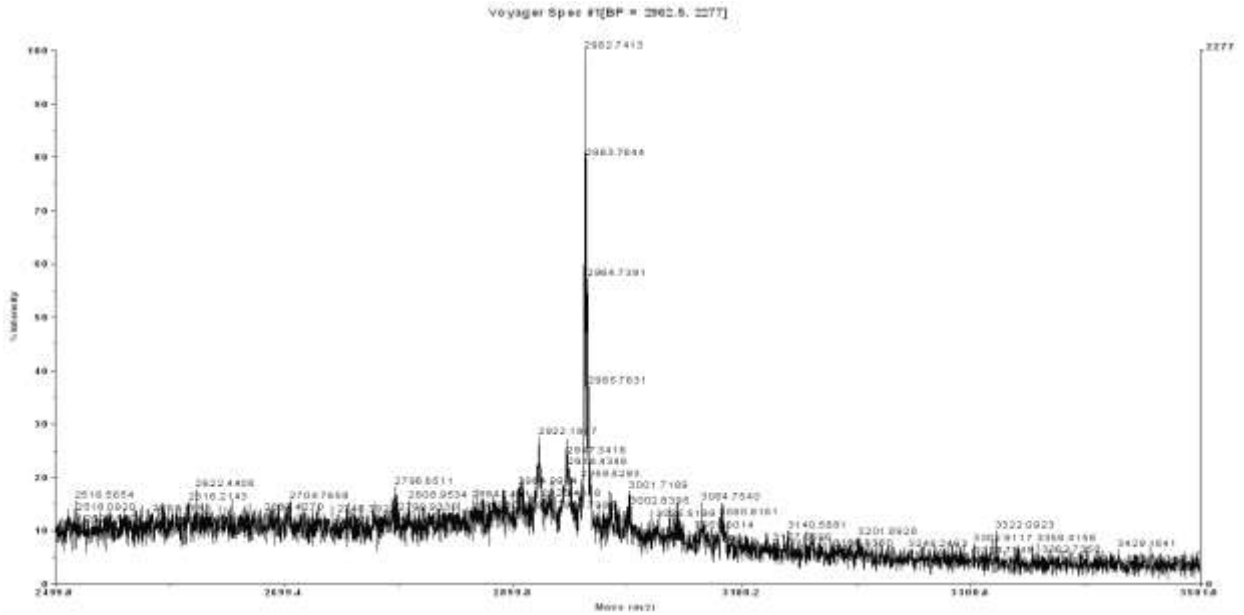


Figure 33: MALDI-MS spectrum of RP-HPLC purified peptide **2d**. E.M. Calcd. for $C_{125}H_{209}N_{47}O_{33}S_2$ = 2960.56 and mass found M+H$^+$= 2962.74

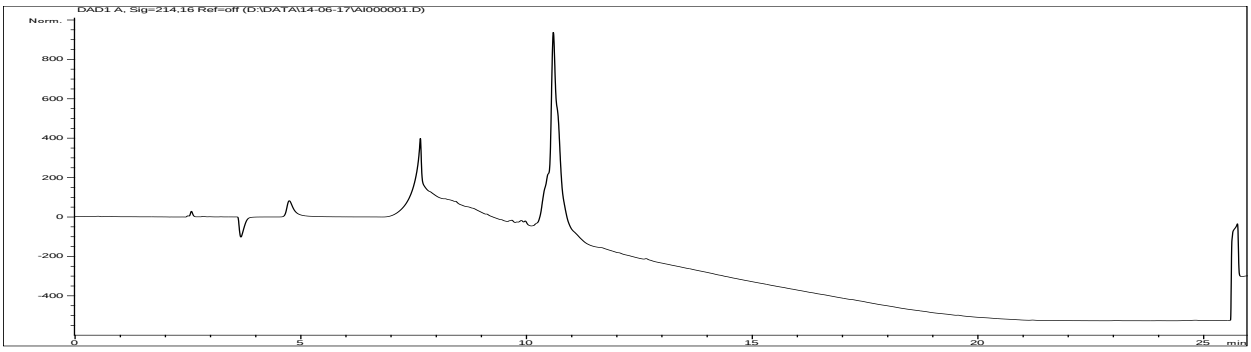


Figure 34: RP-HPLC trace of HPLC purified peptide **IId** with t_R= 10.492 min (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 µm, at 35 °C)

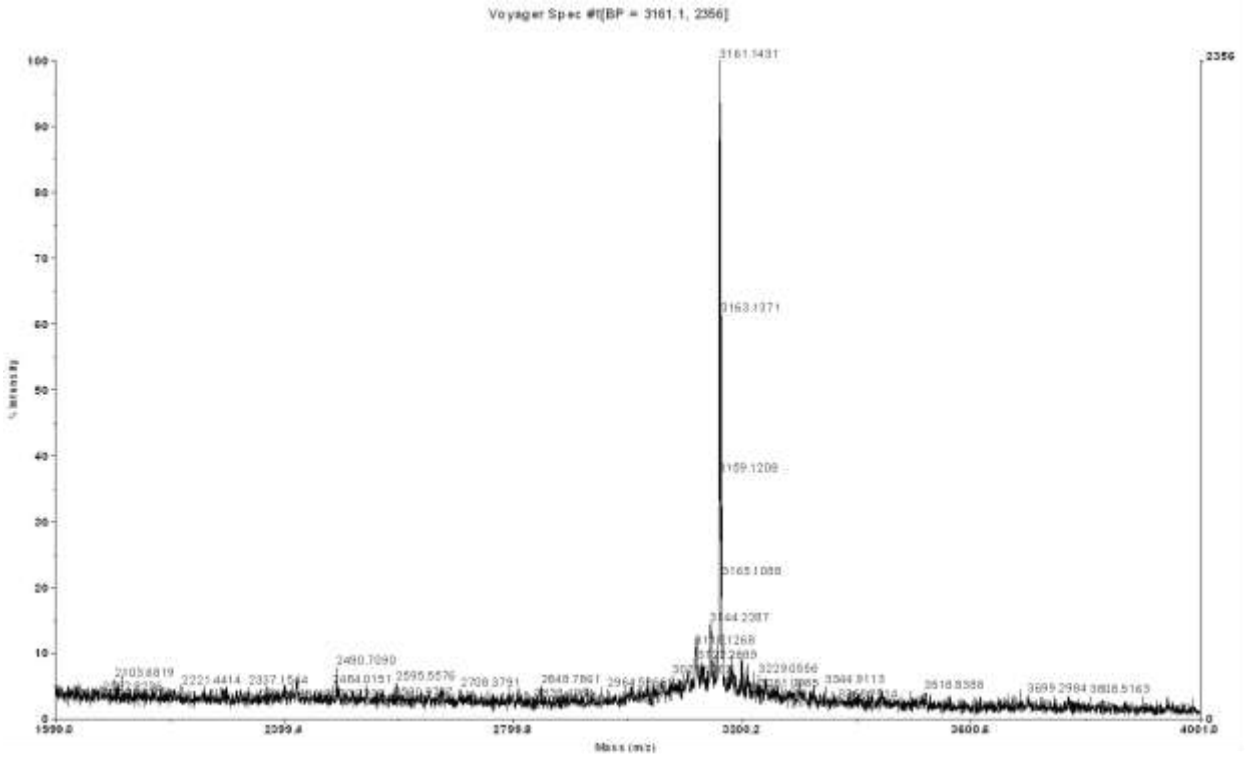


Figure 35: MALDI-MS spectrum of RP-HPLC purified peptide **IId**. E.M. Calcd. for $C_{137}H_{212}N_{46}O_{37}S_2$ = 3157.56 and mass found $M+H^+$= 3161.14

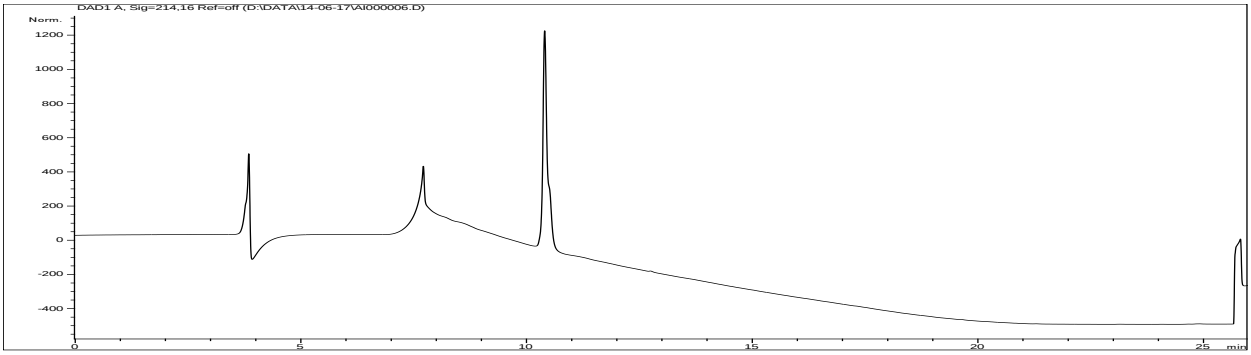


Figure 36: RP-HPLC trace of HPLC purified peptide **3d** with t_R = 10.397 min (0-100% ACN in 15 min on Luna C18(2) 100 Å, 250 x 2.1 mm, 2.6 μm, at 35 °C)

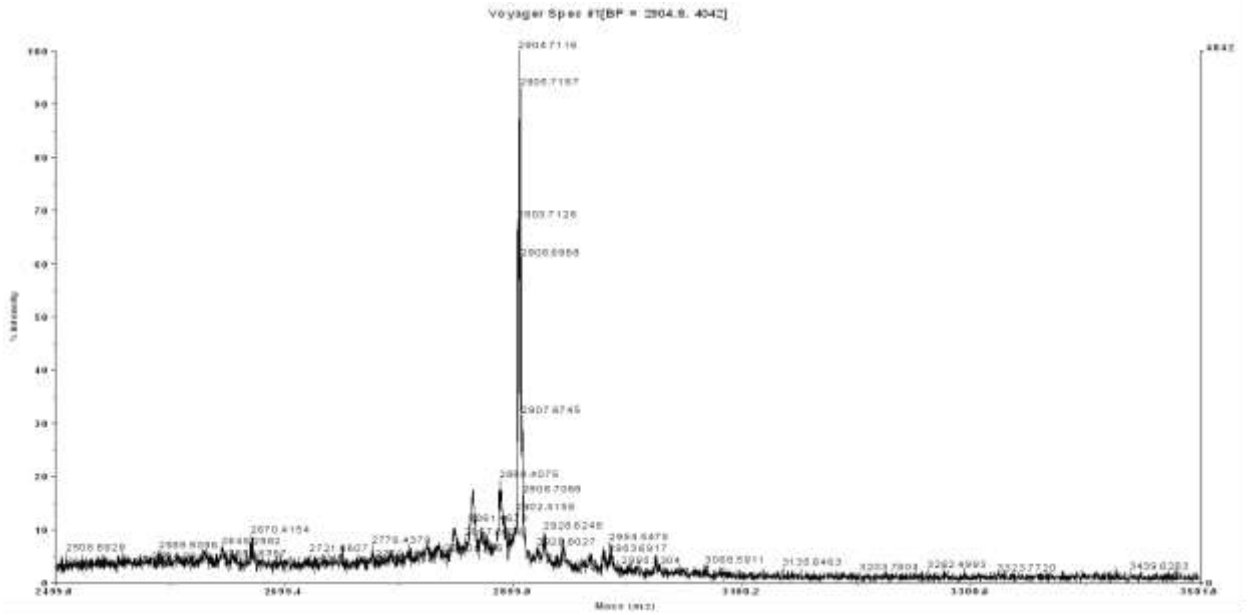


Figure 37: MALDI-MS spectrum of RP-HPLC purified peptide **3d**. E.M. Calcd. for $C_{123}H_{207}N_{47}O_{31}S_2$ = 2902.55 and mass found $M+H^+$= 2904.71

VII. Circular Dichroism studies

All HPLC purified peptide solutions were prepared at 5 μM in 50 mM phosphate buffer (pH 7.0) without TFE. CD studies were conducted at 25 °C on an Aviv 410 CD spectrophotometer equipped with a Peltier temperature control unit. Results (fig. 38) showed that the uncross-linked GCN4 sequence **4** shows almost no helical character whereas cross-linking with Biphenyl as in the case of **1a** shows most helical character. Surprisingly, cross-linking with Bipyridine does not improve the helicity significantly as can be seen from the curve of **1c**. Among the i, i+4 stapled peptides **2d** is more helical than **3d** which would explain its better DNA binding. In general the i, i+7 stapled peptides **1a** & **1b** show more helical character than the i, i+4 ones **2d** & **3d** which is expected given the two turn stabilization in case of the former.

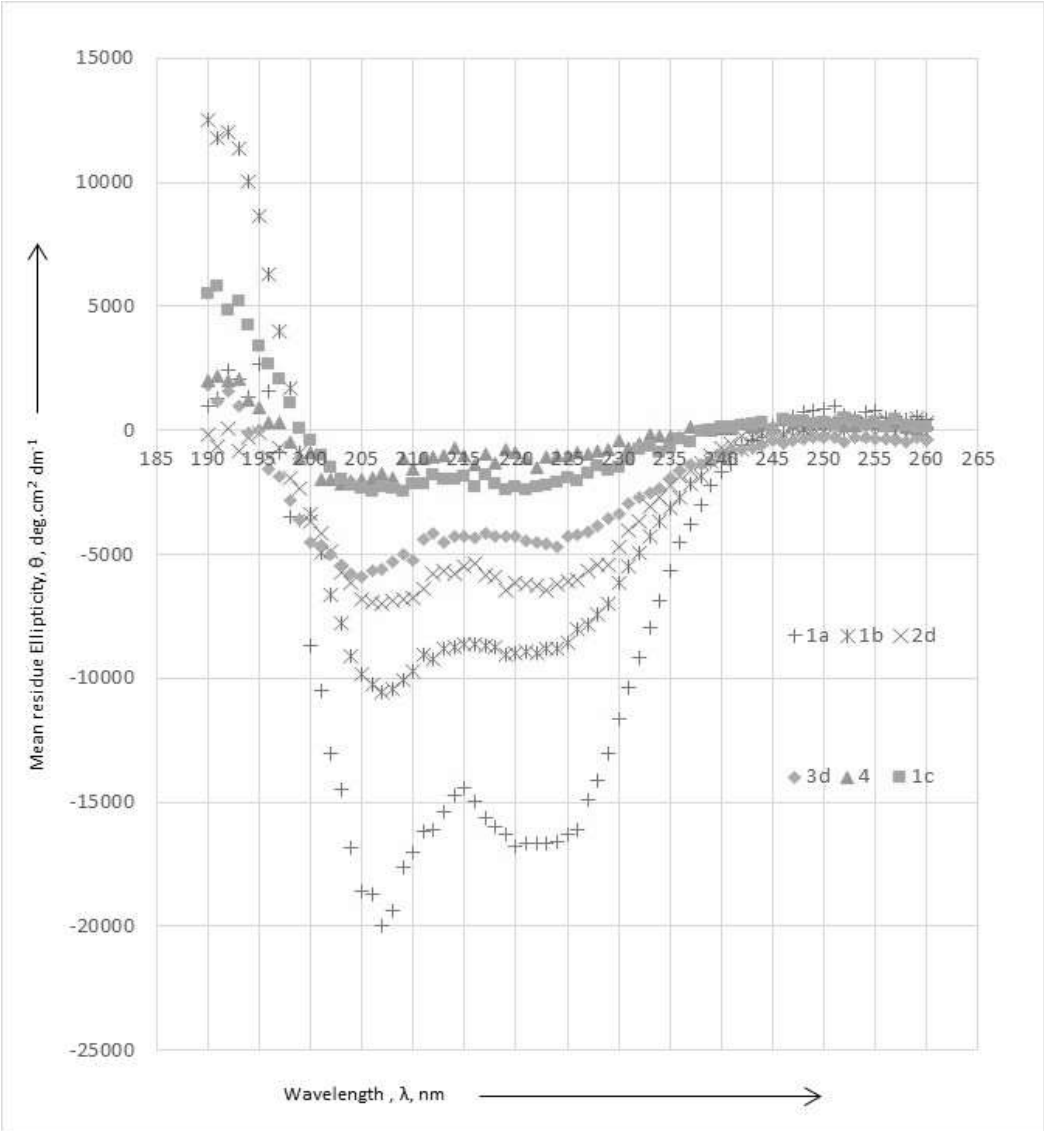


Figure 38: Plotted data obtained from Circular Dichroism studies showing Mean residue Ellipticity, θ, in deg.cm^2 dm^{-1} on the Y axis and Wavelength λ, in nm on the X axis for peptides **4**, **1a**, **1b**, **1c** and **2d**.

VIII. Electrophoretic Mobility Assay

Full gels and gels using random dsDNA are shown in figure 39-41. Figure 42 shows the gel of an additional gel to confirm that the black dot of peptide 3d at 2.5 μM (fig. 40, right) is an artifact. Experimental procedure is described in the article.

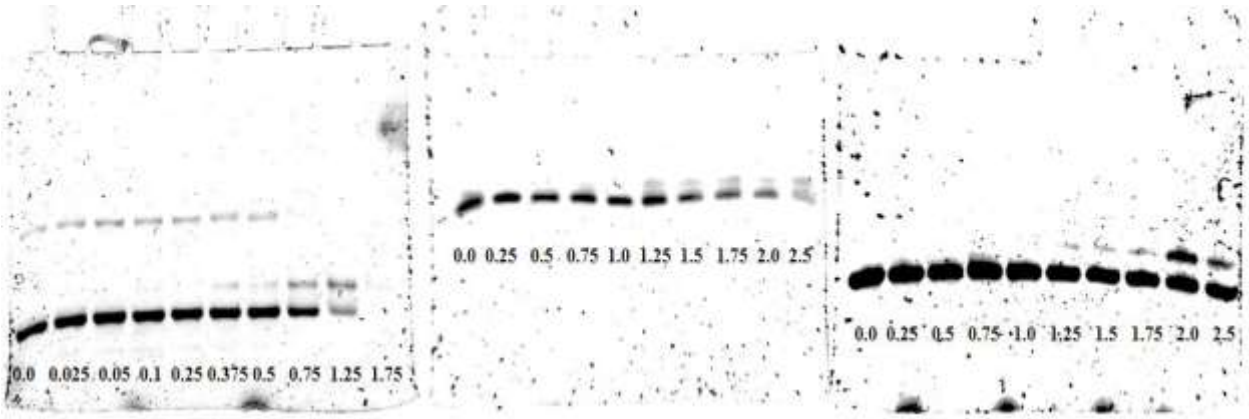


Figure 39: Full gels obtained from EMSA for peptides **1a**, **1b** and **1c** (from left to right) respectively using CRE DNA. DNA concentration = 167 nM for all gels. Peptide concentrations in μM (from left to right) are indicated below each gel.

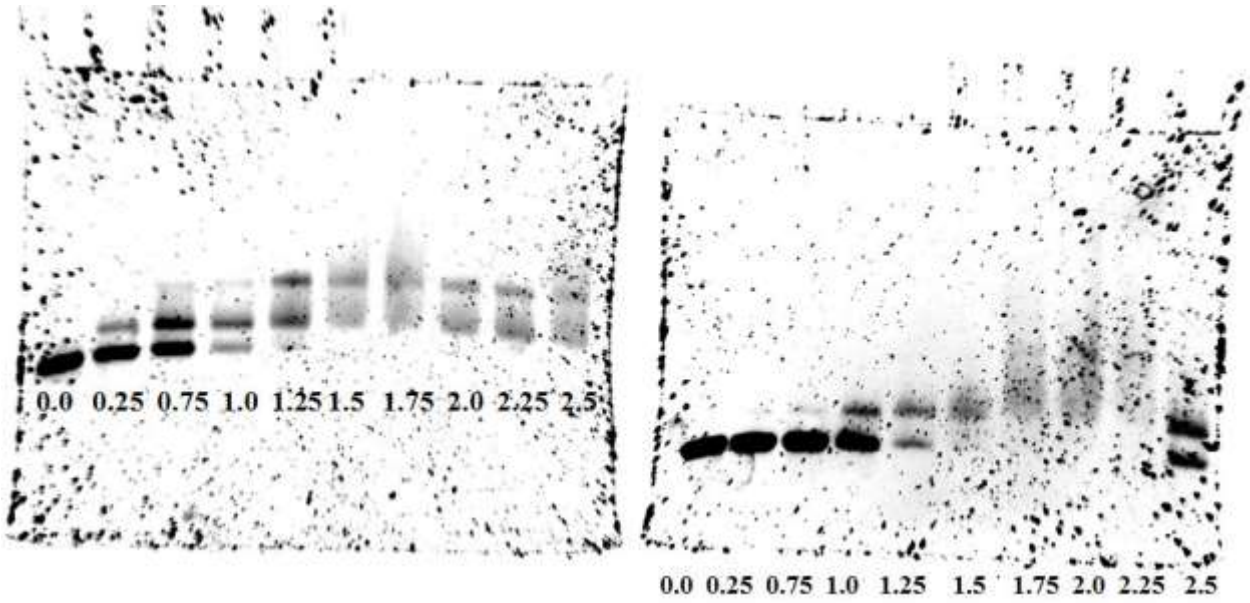


Figure 40: Full gels obtained from EMSA for peptides **2d** and **3d** (from left to right) respectively using CRE DNA. DNA concentration = 167 nM for all gels. Peptide concentrations in μM (from left to right) are indicated below each gel.

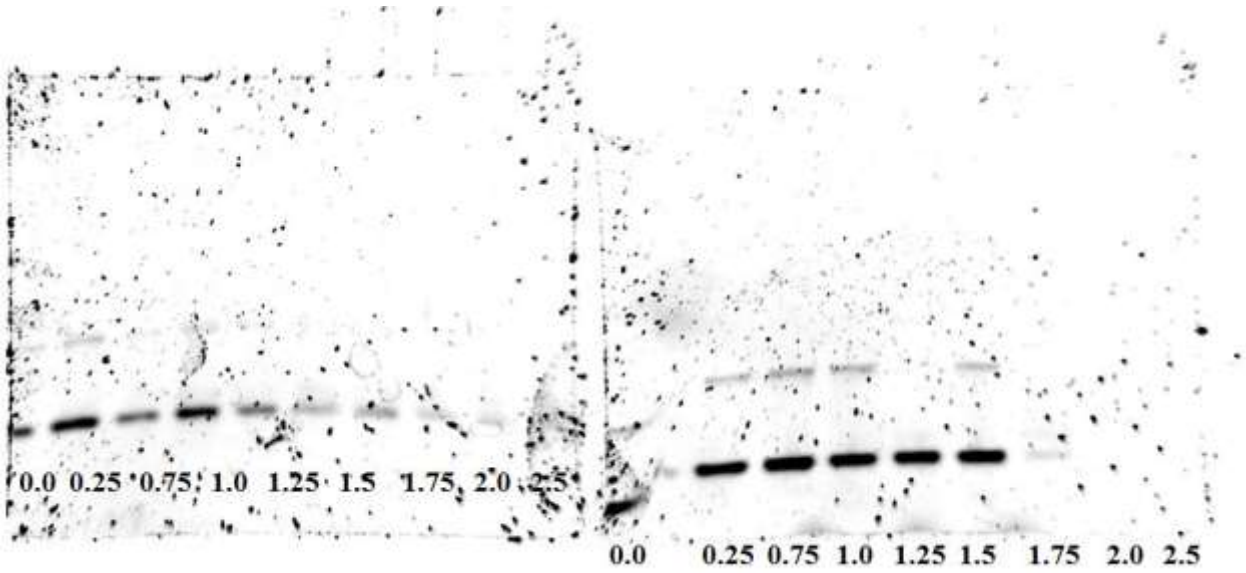


Figure 41: Full gels obtained from EMSA for peptides **4** using CRE DNA (left) and **2d** using random dsDNA (right). DNA concentration = 167 nM for both gels. Peptide concentrations in μM (from left to right) are indicated below each gel.

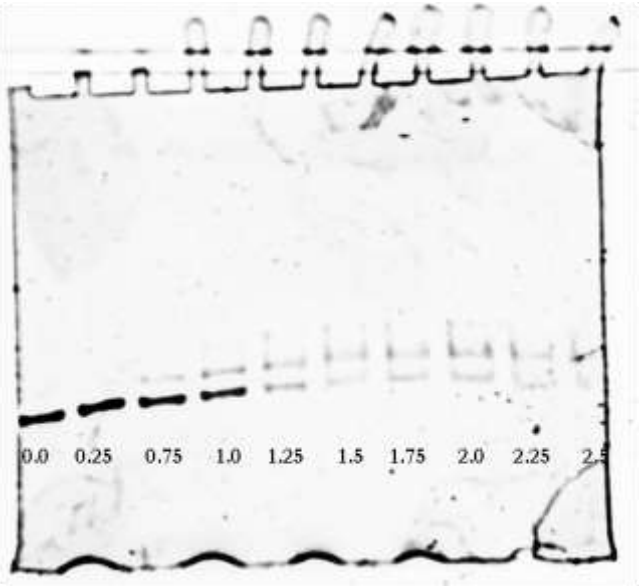


Figure 42: Full gel obtained from EMSA for peptides **3d** using CRE DNA (concentration = 167 nM). Peptide concentrations in μM (from left to right) are indicated below each gel. Gelred was used as staining agent instead of the previous used Sybr Gold. Therefore intensities are lower than in other gels.

IX. DLS-measurement

In order to investigate the aggregation of DNA and peptide, leading to precipitation and a subsequent decrease in intensity of the band, the dynamic light scattering (DLS) is measured of peptides **1a** and **1c** in the same mixture as the loading mixture in the gel electrophoresis. DLS measurement was performed in fivefold on a Zetasizer Nano series, Malvern in a 100 µL cuvette. The results are shown in figure 43. On the left, the volume distribution displays the aggregation of peptides **1a** with DNA at higher concentration as a 'particle' with larger size is present. Also when looking to the count rate on the right of fig. 43, a higher amount of particles is present at high concentration which can be explained by aggregation. These results are conform the visual observation of the gels where peptide **1a** shows a decreased intensity of the band at higher concentration. Also a decreased intensity of the band of **1a** at higher concentration compared to peptide **1c** at 2 µM is observed.

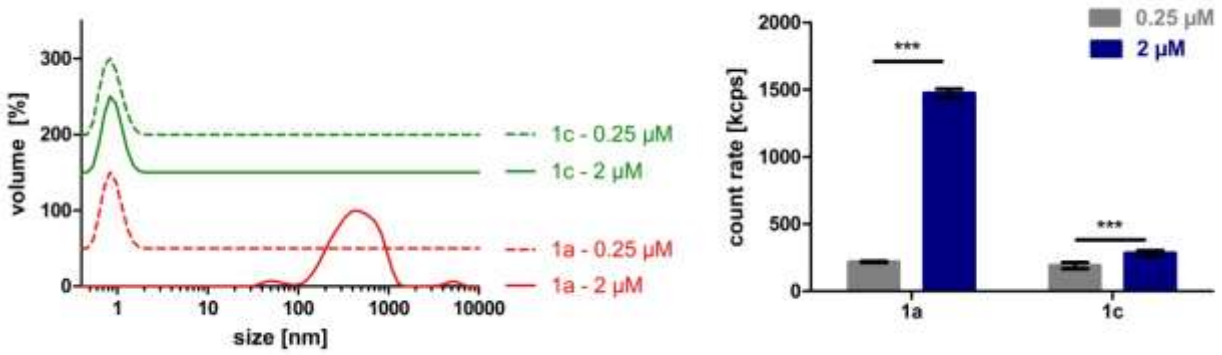


Figure 43: DLS measurement of peptides **1a** and **1c** at a peptide concentration of 0.25 µM and 2 µM and a DNA concentration of 167nM. On the left, the volume distribution is shown in function of the size of the measured particles. On the right, the count rate of each samples in shown.

X. Flow cytometry

RAW264.7 cells cultivated in DMEM (10^5/well, 1 ml) were plated in a 24-well plates and incubated overnight at 37 °C and 5 % CO_2. Peptides were added in an overall concentration of 0.25 μM and 1 μM and incubated for 2 h at the same conditions or on ice. Cells were washed with PBS and detached with Na_4EDTA. Cells were re-suspended in PBS and added to the BD Accuri flow cytometer. Experiments were carried out in duplicate. The amount of peptide associated to the cell was measured by flow cytometry. Blank cells were measured and the signal received corresponded to auto-fluorescence. A threshold was chosen to separate cells emitting light due to auto-fluorescence from cells containing fluorescently labeled peptides. The area under the histograms beyond this threshold correspond to the number of cells which actually emit sufficient intensity of fluorescence to make sure fluorescently labeled peptides are present. This number is further referred to as the percent of peptide positive cells (fig. 44, right). The percent of peptide positive cells for two peptides may be 100 % yet one may better due to its higher mean fluorescence value. If a peptide is not taken up by all cells, the percentage of peptide positive cells is lower than 100 % proportional to the colored area under the curve. It is therefore important to both compare the mean fluorescence and the amount of peptide positive cells. The raw data, represented as histograms, can be found in fig. 45-49.

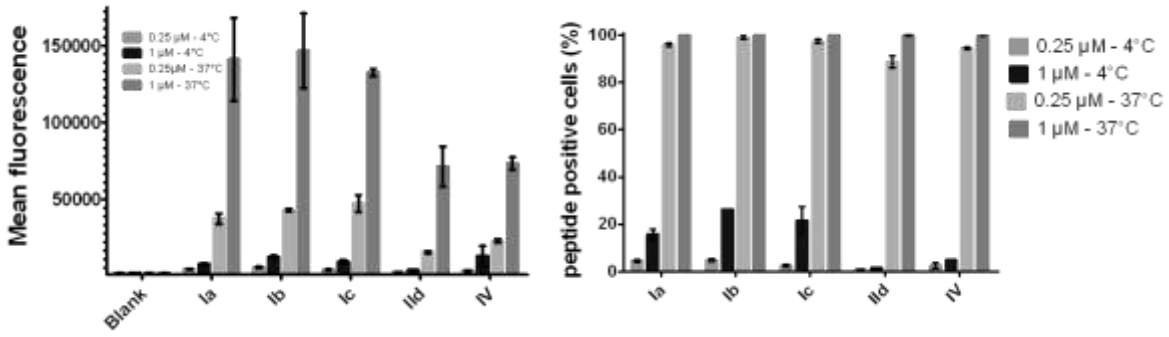
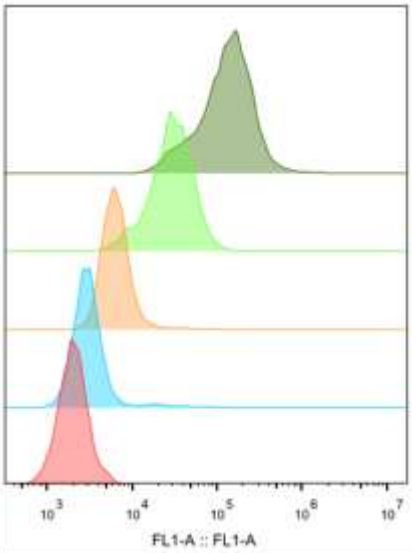
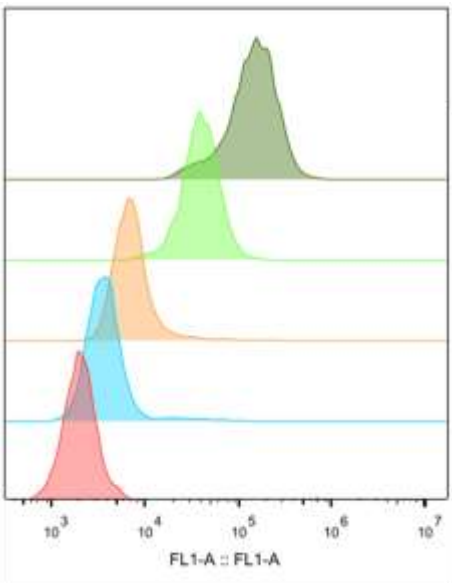


Figure 44: Mean fluorescence of fluorescently labelled peptides **Ia**, **Ib**, **Ic**, **IId** and **IV** (left) and peptide positive cells (right) using the following concentrations and incubation conditions: 0.25 μM at 4°C, 1 μM at 4°C, 0.25 μM at 37°C and 1 μM at 37°C.



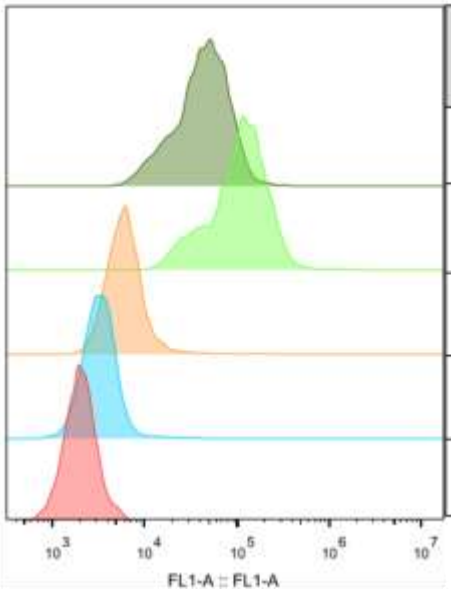
Conditions Peptide Ia	Count	Mean (FL1-A)	FL1-A+ (Freq. of parent)
37 °C 1 µM	5155	160044	100
37 °C 0.25 µM	5464	35170	95.5
4 °C 1 µM	4111	8361	17.4
4 °C 0.25 µM	3408	4786	5.05
Blank	23248	2207	0

Figure 45: Histograms responding to the uptake of peptide Ia under different conditions and compared with the blank measurement. 'Count' represents the cell count during each measurement. FL1-A+ represents the peptide positive cells.



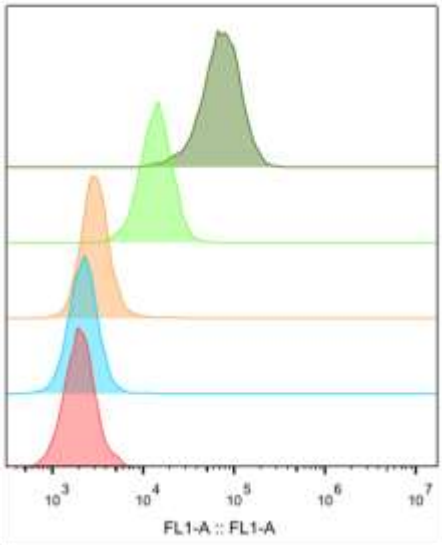
Conditions Peptide Ib	Count	Mean (FL1-A)	FL1-A+ (Freq. of parent)
37 °C 1 µM	6219	163849	100.0
37 °C 0.25 µM	7245	43630	99.5
4 °C 1 µM	4723	13203	26.4
4 °C 0.25 µM	5337	6165	5.17
Blank	23248	2207	0

Figure 46: Histograms responding to the uptake of peptide Ib under different conditions and compared with the blank measurement. 'Count' represents the cell count during each measurement. FL1-A+ represents the peptide positive cells.



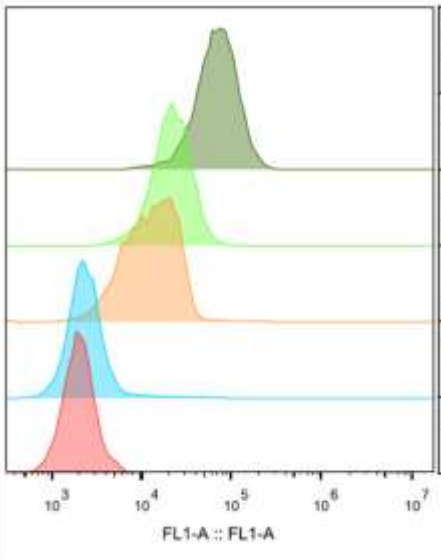
Conditions Peptide Ic	Count	Mean (FL1-A)	FL1-A+ (Freq. of parent)
37 °C 0.25 µM	5674	51197	98.1
37 °C 1 µM	4882	134245	100
4 °C 1 µM	3596	8395	17.4
4 °C 0.25 µM	3667	4140	2.26
Blank	23248	2207	0

Figure 47: Histograms responding to the uptake of peptide Ic under different conditions and compared with the blank measurement. 'Count' represents the cell count during each measurement. FL1-A+ represents the peptide positive cells.



Conditions Peptide IId	Count	Mean (FL1-A)	FL1-A+ (Freq. of parent)
37 °C 1 µM	10280	80470	100.0
37 °C 0.25 µM	8989	15141	86.9
4 °C 1 µM	6245	3738	1.55
4 °C 0.25 µM	6582	2604	0.77
Blank	23248	2207	0

Figure 48: Histograms responding to the uptake of peptide IId under different conditions and compared with the blank measurement. 'Count' represents the cell count during each measurement. FL1-A+ represents the peptide positive cells.



Conditions Peptide IV	Count	Mean (FL1-A)	FL1-A+ (Freq. of parent)
37 °C 1 µM	8194	76430	99.7
37 °C 0.25 µM	8793	23989	94.9
4 °C 1 µM	5124	17755	69.0
4 °C 0.25 µM	5437	3740	3.27
Blank	23248	2207	0

Figure 49: Histograms responding to the uptake of peptide Ia under different conditions and compared with the blank measurement. 'Count' represents the cell count during each measurement. FL1-A+ represents the peptide positive cells.

XI. Peptide stability

The peptide stability was assessed using a trypsin digest with a trypsin to peptide ratio of 1:1000 (wt%). The test is described in further detail in the experimental section. The degradation in function of time shows that the stapled peptides are more stable than the control peptide IV (fig. 50). Figures 51-54 displays the degradation of the peptide in function of time.

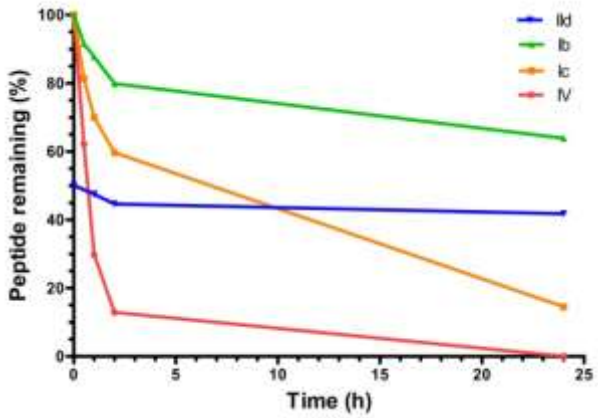
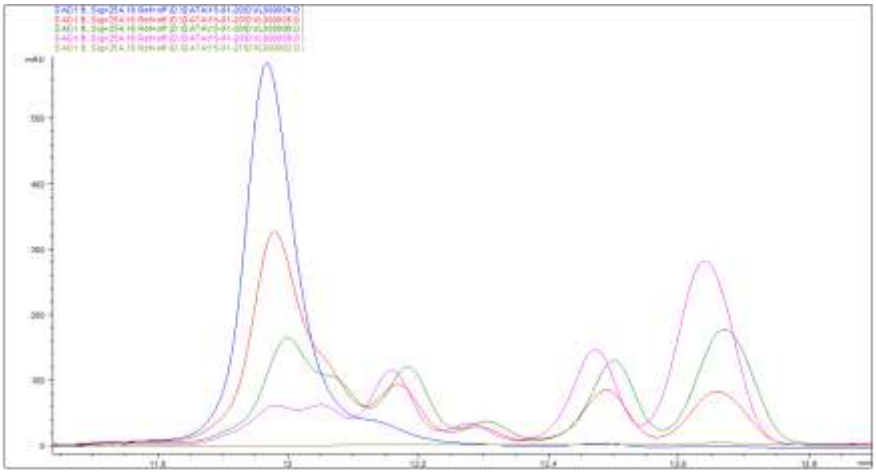
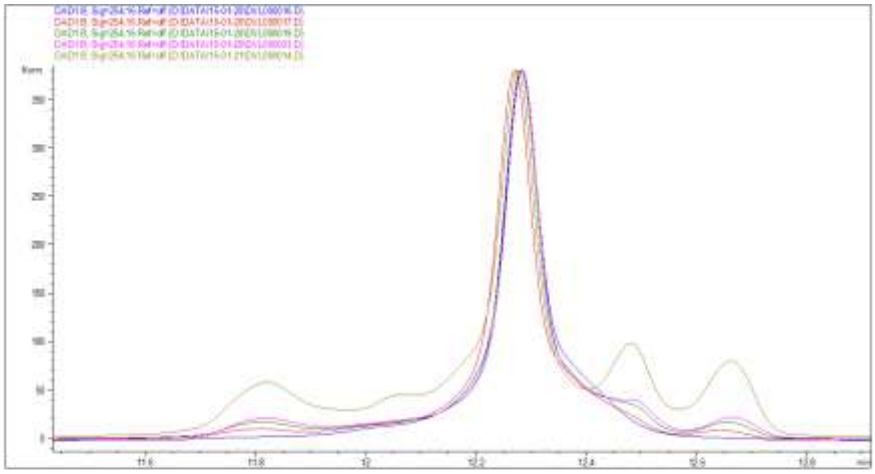


Figure 50: Chromatograms of RP-HPLC of samples of peptide IV taken after 0 min (blue), 30 min (red), 1 h (green), 2 h (pink),



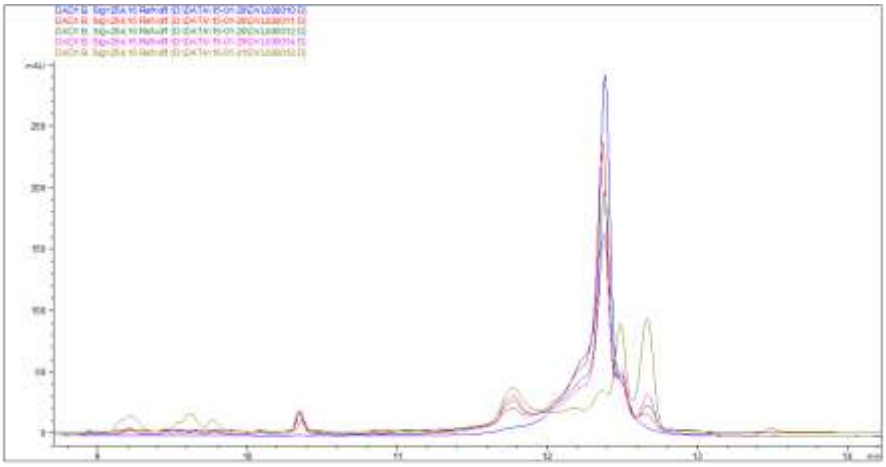
Peak (min)	MW (g/mol)	Peptide fragment
11.95	2995	Fluorescein-DPAALKRARNTEAARRSRARKLQ-NH_2
12.16	2154	Fluorescein-DPAALKRARNTEAARR-OH
12.29	1998	Fluorescein-DPAALKRARNTEAAR-OH
12.48	1355	Fluorescein-DPAALKRAR-OH
12.67	1129	Fluorescein-DPAALKR-OH

Figure 51: Chromatograms of RP-HPLC of samples of peptide IV taken after 0 min (blue), 30 min (red), 1 h (green), 2 h (pink).



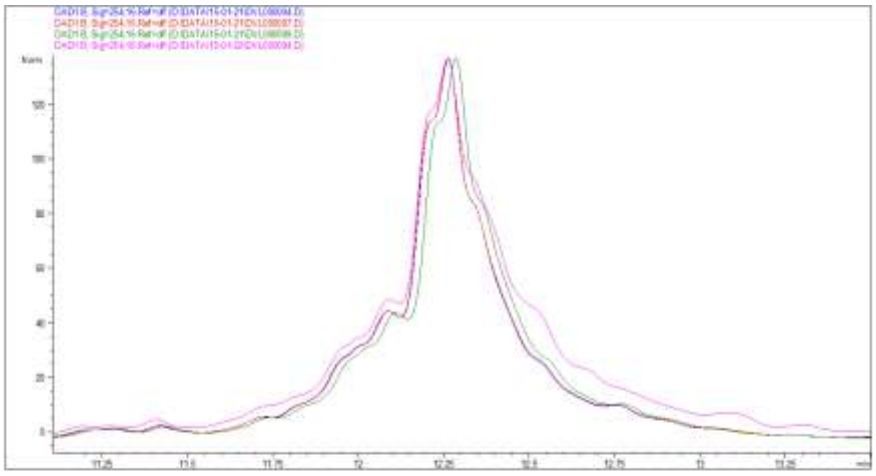
Peak (min)	MW (g/mol)	Peptide fragment
12.28	3189	Fluorescein-DPAALKRARNTC*AARRSRC*RKLQ-NH_2
12.47	1355	Fluorescein-DPAALKRAR-OH
12.67	1129	Fluorescein-DPAALKR-OH

Figure 52: Chromatograms of RP-HPLC of samples of peptide Ib taken after 0 min (blue), 30 min (red), 1 h (green), 2 h (pink).



Peak (min)	MW (g/mol)	Peptide fragment
12.38	3181	Fluorescein-DPAALKRARNTC*AARRSRC*RKLQ-NH_2
12.49	1357	Fluorescein-DPAALKRAR-OH
12.67	1129	Fluorescein-DPAALKR-OH

Figure 53: Chromatograms of RP-HPLC of samples of peptide Ic taken after 0 min (blue), 30 min (red), 1 h (green), 2 h (pink).



Peak (min)	MW (g/mol)	Peptide fragment
12.10	3162	Fluorescein-DPAC*LKRC*RNTEAARRSRARKLQ-NH_2
12.25	3162	Fluorescein-DPAC*LKRC*RNTEAARRSRARKLQ-NH_2
12.55	2566	Fluorescein-DPAC*LKRC*RNTEAARRSR-OH
12.75	2166	Fluorescein-DPAC*LKRC*NTEAAR-OH

Figure 54: Chromatograms of RP-HPLC of samples of peptide IId taken after 0 min (blue), 30 min (red), 1 h (green), 2 h (pink).