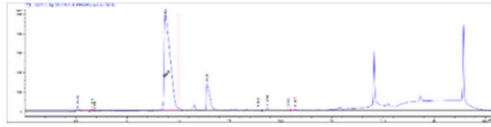
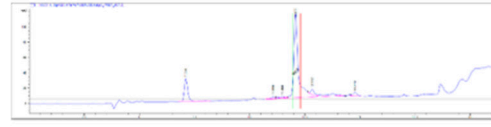


**A**

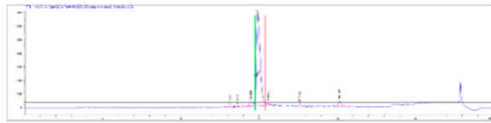
Ova<sub>257-264</sub> ; Native-short  
 H-SIINFEKL-OH  
 R<sub>t</sub> = 6.85 min

**B**

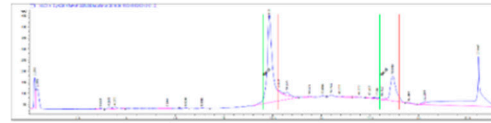
Ova<sub>248-269</sub> ; Native-long  
 H-EVSGLEQLE-SIINFEKL-TEWTS-OH  
 R<sub>t</sub> = 12.08 min

**C**

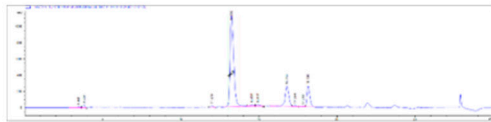
Ova<sub>248-269</sub> (Iva); Isovaline [J]  
 H-EJSGJEQLJ-SIINFEKL-JEJTS-OH  
 R<sub>t</sub> = 14.9 min

**D**

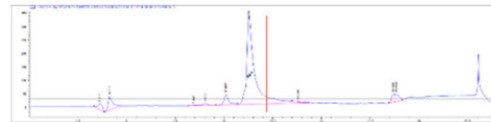
Ova<sub>248-269</sub> (Val); Valine [V]  
 Iva ctrl - H-EVSGVEQLV-SIINFEKL-VEVTS-OH  
 R<sub>t</sub> = 12.31 min

**E**

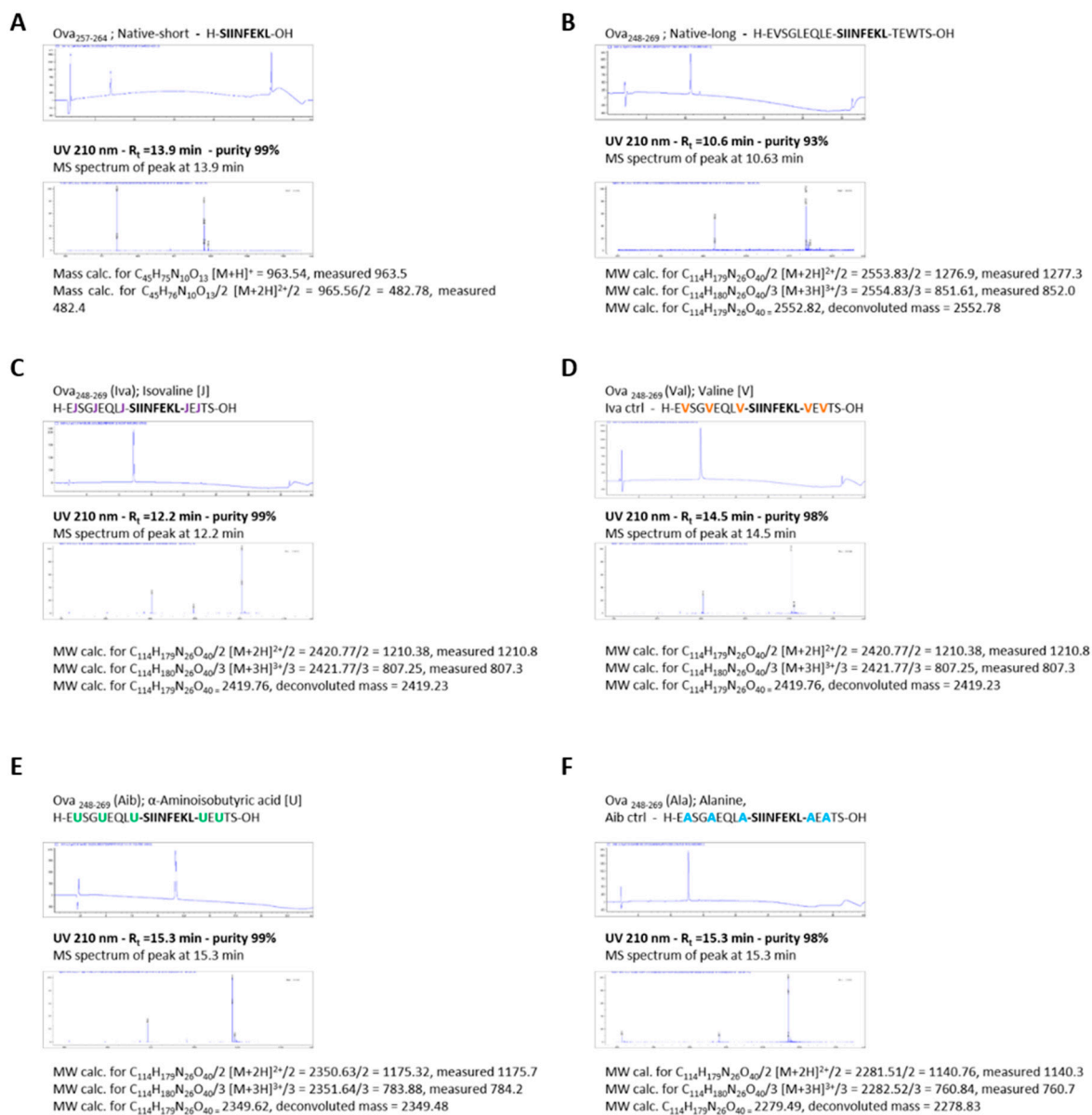
Ova<sub>248-269</sub> (Aib); α-Aminoisobutyric acid [U]  
 H-EUSGUEQLU-SIINFEKL-UEUTS-OH  
 R<sub>t</sub> = 13.23 min

**F**

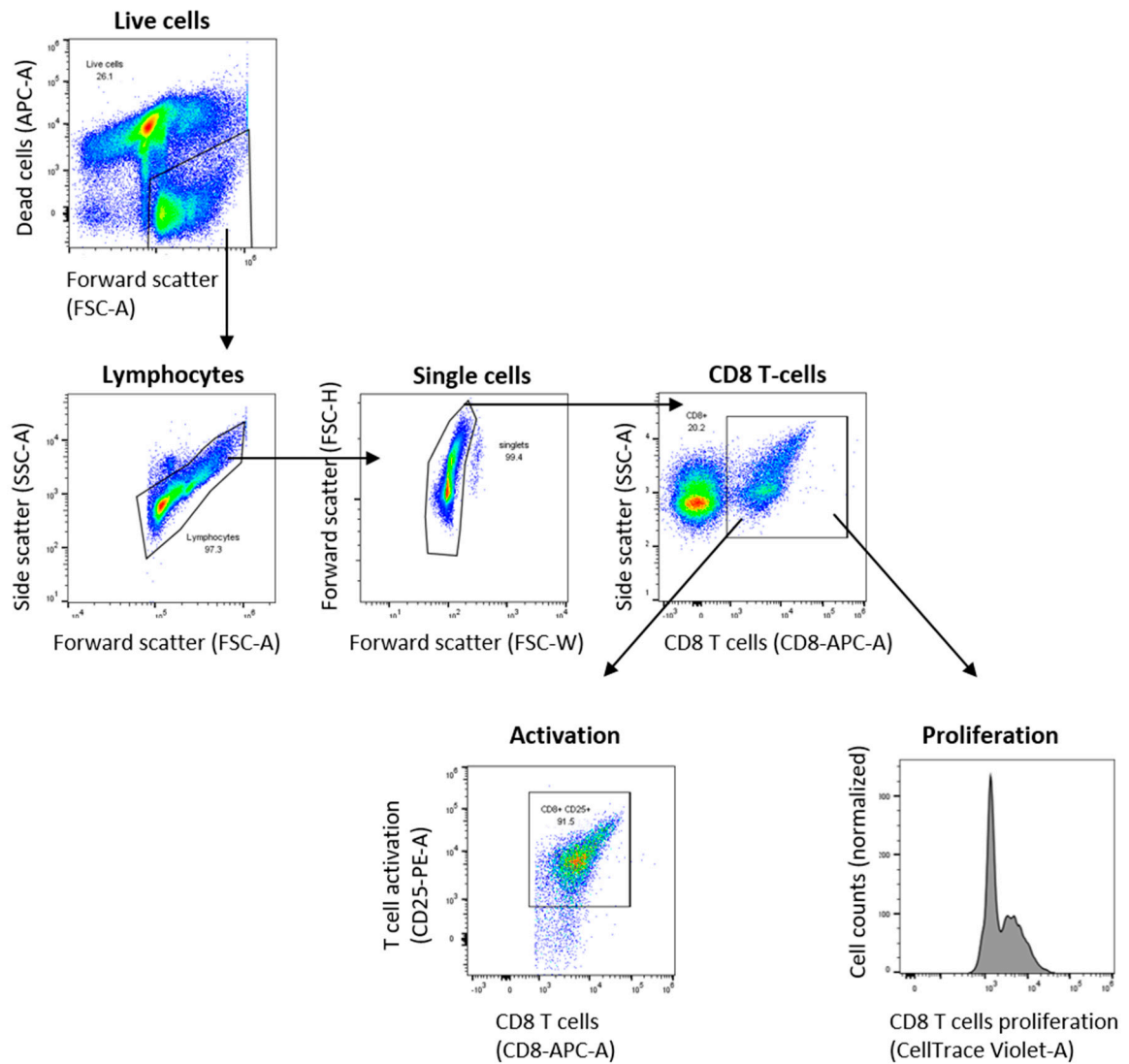
Ova<sub>248-269</sub> (Ala); Alanine [A]  
 Aib ctrl - H-EASGAEQLA-SIINFEKL-AEATS-OH  
 R<sub>t</sub> = 11.27 min



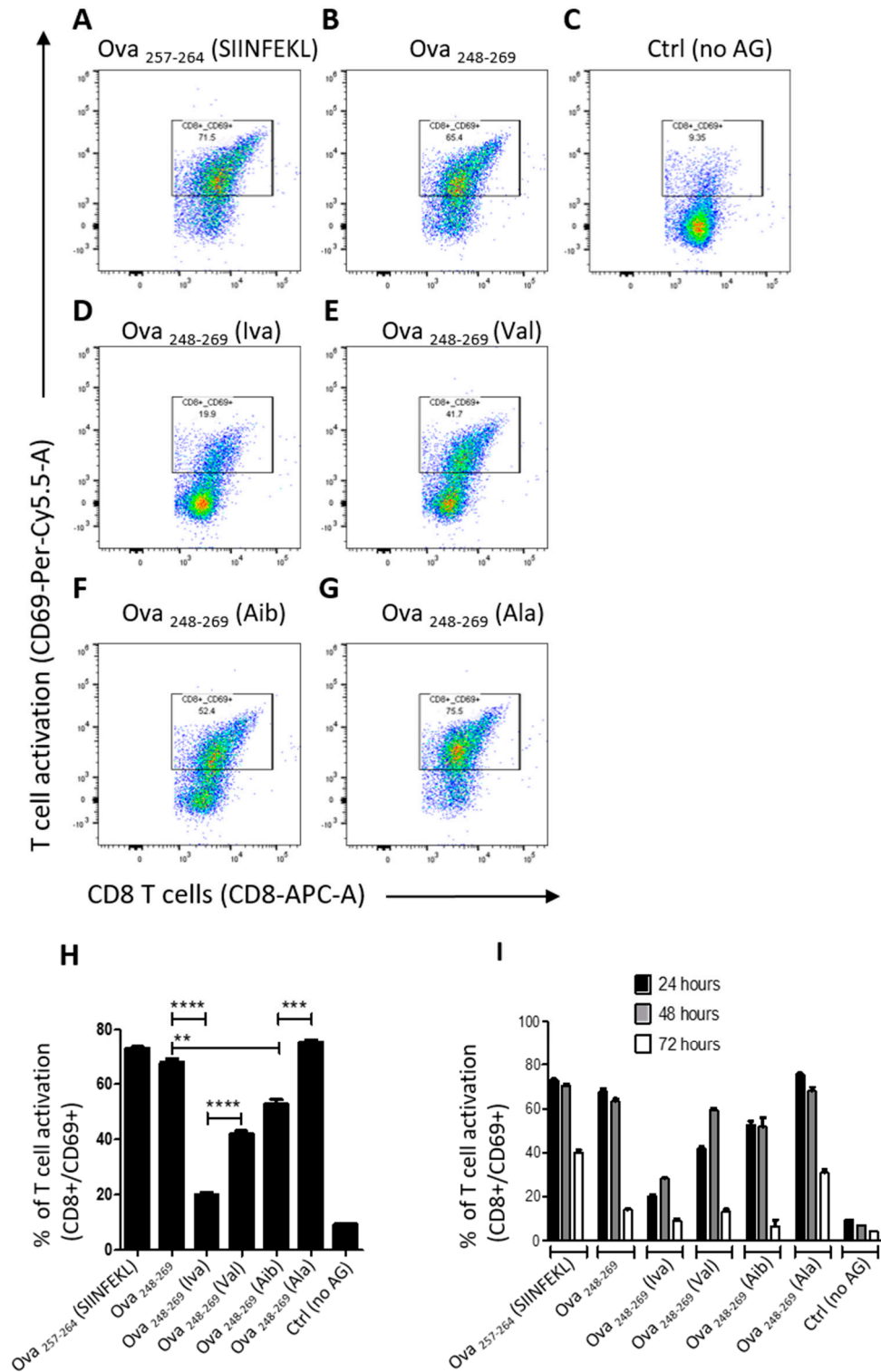
**Supplementary Figure 1. Preparative RP-HPLC methods and traces.** Crude peptides were purified by Preparative RP-HPLC using an Agilent 1260 system and A: Phenomenex Luna C18(2) preparative column (5 μm, 100 Å, 21 mm x 250 mm D x L) or B-F: Phenomenex Jupiter C18 preparative column (5 μm, 300 Å, 21 mm x 250 mm D x L) using an optimised gradient.



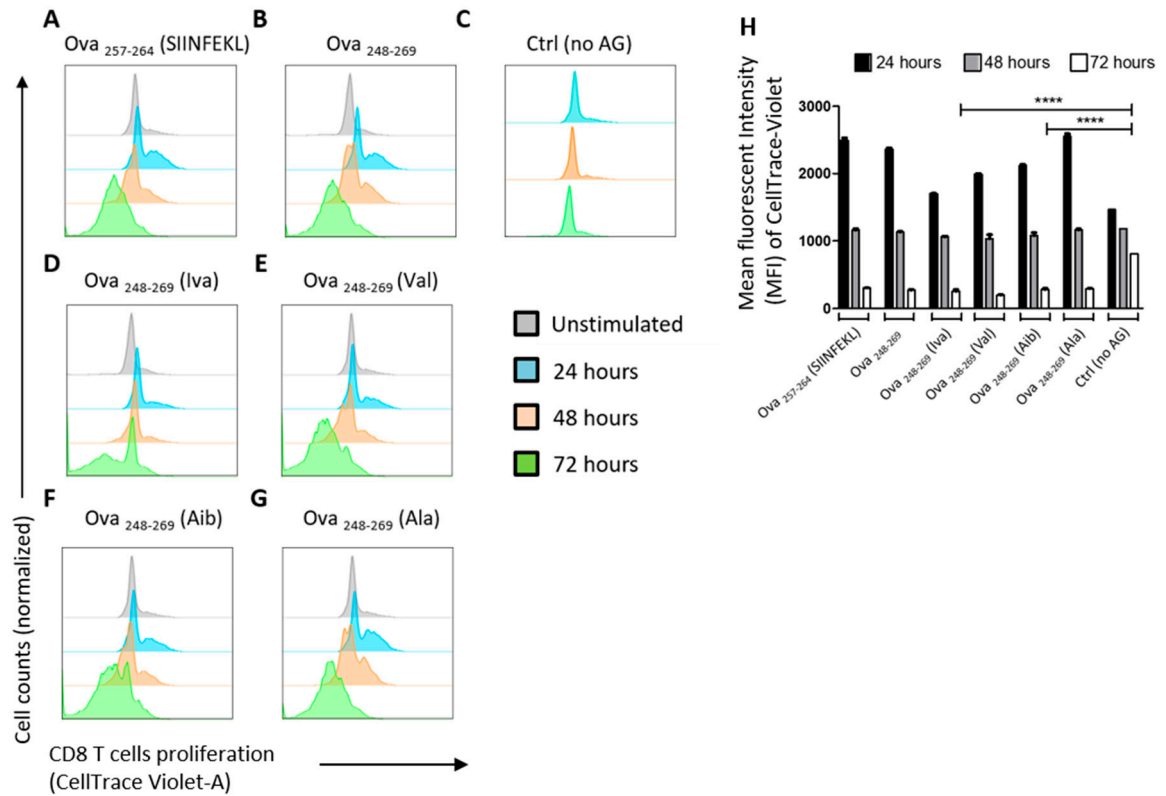
**Supplementary Figure 2. Analytical RP-HPLC-MS traces.** Purity of the peptides was evaluated by HPLC-MS analysis using an Agilent 1200 HPLC equipped with a Diode Array Detector (DAD) and coupled with a single quadrupole mass detector using A: Phenomenex Luna C18 analytical column (5 μm, 100 Å, 4.6 mm x 250 mm D x L) or B-F: Phenomenex Jupiter C18 analytical column (5 μm, 300 Å, 4.6 mm x 150 mm D x L) and the appropriate eluent gradient..



**Supplementary Figure 3: Flow cytometry gating pathway for T cell activation and proliferation markers.** Plots are showing the relationship of different fluorescence intensity of T cells labeled with antibodies against specific markers, measured in arbitrary units in log scale (see method section for details). Initial gating was set on live cells before lymphocytes were isolated using SSC-A vs FSC-A. After elimination of doublets the gate was set on CD8 T cells using the SSC-A vs CD8-APC-A. For T cell activation, a gate was set within CD8 T cells that expresses the cell surface marker CD25 (CD8<sup>+</sup>/CD25<sup>+</sup>). For cell proliferation, histograms were created using the number of CD8<sup>+</sup> T cells against the (reduction over time of) cell proliferation staining (CellTrace Violet).



**Supplementary Figure 4: Assessment of cell activation via measuring the expression of the surface marker CD69 on CD8 T cells after stimulation with Ova-derived peptide epitopes.** A-G: Representative flow cytometry dot plots show CD8 T cell activation after 24 h stimulated with indicated peptides. H: Frequency of CD69 positive single CD8 cells after 24 h I: Frequency of CD69 positive single CD8 cells over time. Statistical significance (\*\*\*\*: P value <0.0001; \*\*\*: P value <0.001; \*\*: P value <0.01).



**Supplementary Figure 5: CD8 T cell proliferation dynamics.** CellTrace Violet labelled and LPS activated OT-1 mouse splenocytes stimulated with the tested peptides: the native ova-control peptides (A: short Ova<sub>257-264</sub> (SIINFEKL) or B: longer Ova<sub>248-269</sub>), unstimulated (C) or with the ova peptides substituted with exo-amino acids (D: Ova<sub>248-269</sub> (Iva) and F: Ova<sub>248-269</sub> (Aib)) or peptides substituted natural amino acids (E: Ova<sub>248-269</sub> (Val) and G: Ova<sub>248-269</sub> (Ala)). Cell proliferation was assessed via Flow cytometry by measuring reduction of the cell proliferation staining amongst CD8 T cell population after 24-, 48-, or 72 hours. A-G: Representative images show a histogram shift over time (blue: 24h, orange: 48h, green: 72h) that represent the reduction of the cell proliferation (CellTrace Violet) dye intensity amongst CD8 T cell population against the unstimulated control cells (grey histogram). H: Geometric Mean (CellTrace) of CD8 T cells. Three technical replicas were performed for each of the three biological replicas.

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## Peptide Synthesis protocols

### Ova<sub>257-264</sub> ; Native-short - H-SIINFEKL-OH

Amino acid	Specific Derivative	Cycle
	Final Deprotection	0.10-Final Deprotection (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Asn - N	Fmoc-Asn(Trt)-OH	0.10-Single Coupling (HS) (UV)
Phe - F	Fmoc-Phe-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Lys - K	Fmoc-Lys(Boc)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Resin Swelling (HS)

### Ova<sub>248-269</sub> ; Native-long - H-EVSGLEQLE-SIINFEKL-TEWTS-OH

Amino acid	Specific Derivative	Cycle
	Final Deprotection	0.10-Final Deprotection (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
Val - V	Fmoc-Val-OH	0.10-Double Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Gly - G	Fmoc-Gly-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Gln - Q	Fmoc-Gln(Trt)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)

Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Asn - N	Fmoc-Asn(Trt)-OH	0.10-Single Coupling (HS) (UV)
Phe - F	Fmoc-Phe-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Lys - K	Fmoc-Lys(Boc)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Thr - T	Fmoc-Thr(tBu)-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Trp - W	Fmoc-Trp(Boc)-OH	0.10-Single Coupling (HS) (UV)
Thr - T	Fmoc-Thr(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-Wang	0.10-Resin Swelling (HS)

**Ova<sub>248-269</sub> (Iva); Isovaline - H-EXSGXEQ LX-SIINF EKL-XEXTS-OH**

Amino acid	Specific Derivative	Cycle
	Final Deprotection	0.10-Final Deprotection (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
EX2 - X	Fmoc-Isovaline-OH	0.10-Double Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Double Coupling (HS) (UV)
Gly - G	Fmoc-Gly-OH	0.10-Double Coupling (HS) (UV)
EX2 - X	Fmoc-Isovaline-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Gln - Q	Fmoc-Gln(Trt)-OH	0.10-Double Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Double Coupling (HS) (UV)
EX2 - X	Fmoc-Isovaline-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)



Asn - N	Fmoc-Asn(Trt)-OH	0.10-Single Coupling (HS) (UV)
Phe - F	Fmoc-Phe-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Lys - K	Fmoc-Lys(Boc)-OH	0.10-Double Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Double Coupling (HS) (UV)
EX2 - X	Fmoc-Isovaline-OH	0.10-Double Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
EX2 - X	Fmoc-Isovaline-OH	0.10-Single Coupling (HS) (UV)
Thr - T	Fmoc-Thr(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-Wang	0.10-Resin Swelling (HS)

**Ova** <sub>248-269</sub> (Val); Valine, Iva ctrl - H-EVSGVEQLV-SIINFEKL-VEVTS-OH

Amino acid	Specific Derivative	Cycle
	Final Deprotection	0.10-Final Deprotection (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
Val - V	Fmoc-Val-OH	0.10-Double Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Gly - G	Fmoc-Gly-OH	0.10-Single Coupling (HS) (UV)
Val - V	Fmoc-Val-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Gln - Q	Fmoc-Gln(Trt)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Val - V	Fmoc-Val-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Asn - N	Fmoc-Asn(Trt)-OH	0.10-Single Coupling (HS) (UV)

Phe - F	Fmoc-Phe-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Lys - K	Fmoc-Lys(Boc)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Val - V	Fmoc-Val-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Val - V	Fmoc-Val-OH	0.10-Single Coupling (HS) (UV)
Thr - T	Fmoc-Thr(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-Wang	0.10-Resin Swelling (HS)

**Ova<sub>248-269</sub> (Aib);  $\alpha$ -Aminoisobutyric acid - H-EYSGYEQLY-SIINFEKL-YEYTS-OH**

Amino acid	Specific Derivative	Cycle
	Final Deprotection	0.10-Final Deprotection (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
EX1 - B	Fmoc-Aib-OH	0.10-Double Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Double Coupling (HS) (UV)
Gly - G	Fmoc-Gly-OH	0.10-Double Coupling (HS) (UV)
EX1 - B	Fmoc-Aib-OH	0.10-Single Coupling 4 min (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Gln - Q	Fmoc-Gln(Trt)-OH	0.10-Double Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Double Coupling (HS) (UV)
EX1 - B	Fmoc-Aib-OH	0.10-Single Coupling 4 min (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Asn - N	Fmoc-Asn(Trt)-OH	0.10-Single Coupling (HS) (UV)
Phe - F	Fmoc-Phe-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)

Lys - K	Fmoc-Lys(Boc)-OH	0.10-Double Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Double Coupling (HS) (UV)
EX1 - B	Fmoc-Aib-OH	0.10-Double Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
EX1 - B	Fmoc-Aib-OH	0.10-Single Coupling 4 min (HS) (UV)
Thr - T	Fmoc-Thr(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-Wang	0.10-Resin Swelling (HS)

**Ova<sub>248-269</sub> (Ala); Alanine, Aib ctrl - H-EASGAEQLA-SIINFEKL-AEATS-OH**

Amino acid	Specific Derivative	Cycle
	Final Deprotection	0.10-Final Deprotection (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Double Coupling (HS) (UV)
Ala - A	Fmoc-Ala-OH	0.10-Double Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Gly - G	Fmoc-Gly-OH	0.10-Single Coupling (HS) (UV)
Ala - A	Fmoc-Ala-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Gln - Q	Fmoc-Gln(Trt)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Ala - A	Fmoc-Ala-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Ile - I	Fmoc-Ile-OH	0.10-Single Coupling (HS) (UV)
Asn - N	Fmoc-Asn(Trt)-OH	0.10-Single Coupling (HS) (UV)
Phe - F	Fmoc-Phe-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)

Lys - K	Fmoc-Lys(Boc)-OH	0.10-Single Coupling (HS) (UV)
Leu - L	Fmoc-Leu-OH	0.10-Single Coupling (HS) (UV)
Ala - A	Fmoc-Ala-OH	0.10-Single Coupling (HS) (UV)
Glu - E	Fmoc-Glu(OtBu)-OH	0.10-Single Coupling (HS) (UV)
Ala - A	Fmoc-Ala-OH	0.10-Single Coupling (HS) (UV)
Thr - T	Fmoc-Thr(tBu)-OH	0.10-Single Coupling (HS) (UV)
Ser - S	Fmoc-Ser(tBu)-Wang	0.10-Resin Swelling (HS)

## CEM liberty Blue synthesis cycle

### 0.10-Resin Swelling (HS)

Step	Operation	Parameters	Pause
1	Swell Resin	Main solvent volume: 15 Time: 300	False

### 0.10-Final Deprotection (HS) (UV)

Step	Operation	Parameters	Pause
1	Deprotection	ID: Standard Deprotection Deprotection volume: 4.5	False
2	Wash	Main solvent volume: 4 Time: 5.0	False
3	Wash	Drain type: UVDrain Main solvent volume: 4 Time: 5.0	False
4	Wash	Drain type: UVDrain Main solvent volume: 4 Time: 5.0	False
5	Wash	Drain type: Default Main solvent volume: 4 Time: 5.0 Drain type: Default	False

### 0.10-Single Coupling (HS) (UV)

Step	Operation	Parameters	Pause
1	Deprotection	ID: Standard Deprotection Deprotection volume: 4.5	False
2	Wash	Main solvent volume: 4 Time: 15	False
3	Wash	Drain type: Default Main solvent volume: 4 Time: 5	False
4	Wash	Drain type: Default Main solvent volume: 4 Time: 5	False
5	Wash	Drain type: UVDrain Main solvent volume: 4 Time: 3	False
6	Coupling	Drain type: Default ID: Standard Coupling Bottle position: (from method) Bottle position volume: 2.5 Activator bottle position: PositionACT Activator volume: 1 Activator base bottle position: PositionACTB Activator base volume: 0.5 Delay reagent time: 0 Delay reagent bottle position: PositionACTB Delay reagent volume: 0 Main solvent volume: 2 Value1: 1	False
7	Wash Thru Manifold	Main solvent volume: 4 Time: 5 Drain type: Default	False

8	Wash	Main solvent volume: 4 Time: 5 Drain type: Default	False
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## 0.10-Double Coupling (HS) (UV)

Step	Operation	Parameters	Pause
1	Deprotection	ID: Long Standard Deprotection Deprotection volume: 4.5	False
2	Wash	Main solvent volume: 4 Time: 15 Drain type: Default	False
3	Wash	Main solvent volume: 4 Time: 5 Drain type: Default	False
4	Wash	Main solvent volume: 4 Time: 5 Drain type: UVDrain	False
5	Wash	Main solvent volume: 4 Time: 5.0 Drain type: Default	False
6	Coupling	ID: Long Standard Coupling Bottle position: (from method) Bottle position volume: 2.5 Activator bottle position: PositionACT Activator volume: 1 Activator base bottle position: PositionACTB Activator base volume: 0.5 Delay reagent time: 0 Delay reagent bottle position: PositionACTB Delay reagent volume: 0 Main solvent volume: 2 Value1: 1	False
7	Coupling	ID: Long Standard Coupling Bottle position: (from method) Bottle position volume: 2.5 Activator bottle position: PositionACT Activator volume: 1 Activator base bottle position: PositionACTB Activator base volume: 0.5 Delay reagent time: 0 Delay reagent bottle position: PositionACTB Delay reagent volume: 0 Main solvent volume: 2 Value1: 1	False
8	Wash Thru Manifold	Main solvent volume: 4 Time: 5.0 Drain type: Default	False
9	Wash	Main solvent volume: 4 Time: 5.0 Drain type: Default	False

## 0.10-Single Coupling 4 min (HS) (UV)

Step	Operation	Parameters	Pause
1	Deprotection	ID: Standard Deprotection Deprotection volume: 4.5	False
2	Wash	Main solvent volume: 4 Time: 15	False

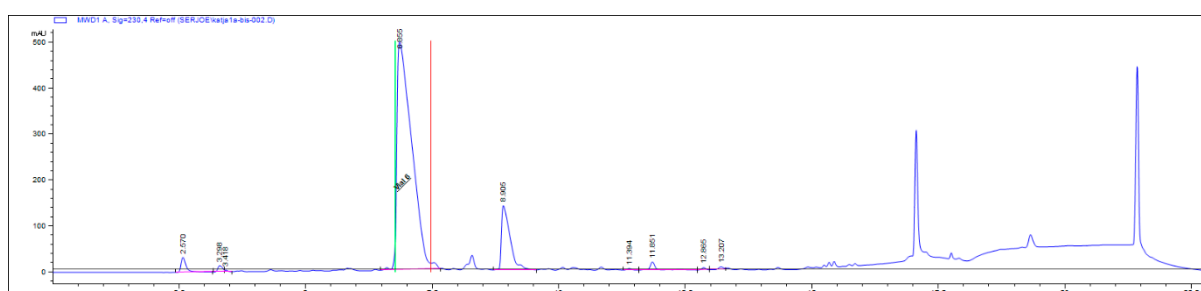
3	Wash	Drain type: Default Main solvent volume: 4 Time: 5	False
4	Wash	Drain type: Default Main solvent volume: 4 Time: 5	False
5	Wash	Drain type: UVDrain Main solvent volume: 4 Time: 3	False
6	Coupling	Drain type: Default ID: Long Standard Coupling Bottle position: (from method) Bottle position volume: 2.5 Activator bottle position: PositionACT Activator volume: 1 Activator base bottle position: PositionACTB Activator base volume: 0.5 Delay reagent time: 0 Delay reagent bottle position: PositionACTB Delay reagent volume: 0 Main solvent volume: 2 Value1: 1	False
7	Wash Thru Manifold	Main solvent volume: 4 Time: 5	False
8	Wash	Drain type: Default Main solvent volume: 4 Time: 5 Drain type: Default	False

## Preparative RP-HPLC methods and traces

### Ova<sub>257-264</sub> ; Native-short - H-SIINFEKL-OH

Column: Phenomenex Luna C18(2) preparative column (5 µm, 100 Å, 21mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	75	25	20
12	50	50	20
15	0	100	20
18	0	100	20
21	75	25	20

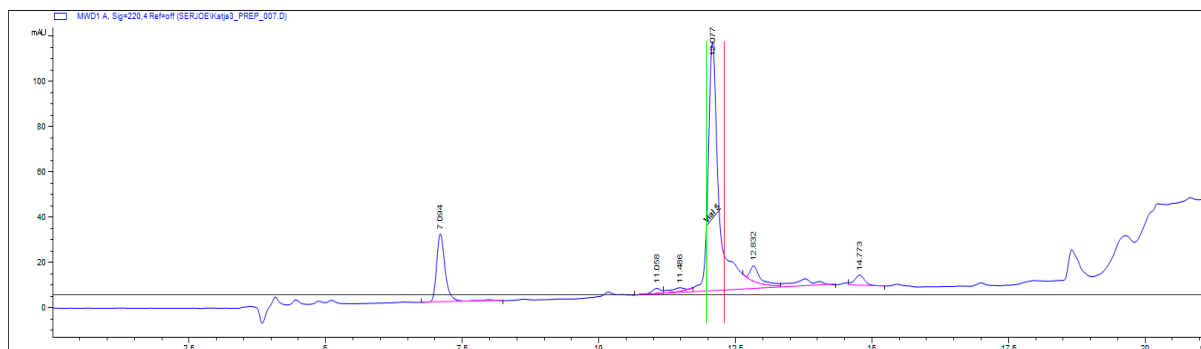


**R<sub>t</sub> = 6.85 min**

### Ova<sub>248-269</sub> ; Native-long - H-EVSGLEQL-SIINFEKL-TEWTS-OH

Column: Phenomenex Jupiter C18 preparative column (5 µm, 300 Å, 21mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	75	25	20
15	50	50	20
16	0	100	20
19	0	100	20
21	75	25	20



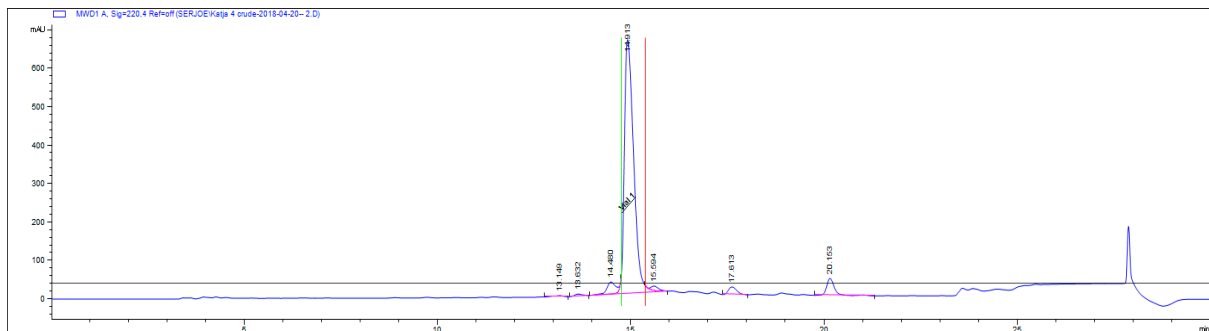
**R<sub>t</sub> = 12.08 min**



**Ova<sub>248-269</sub> (Iva); Isovaline - H-EXSGXEQIX-SIINFEKL-XEXTS-OH**

Column: Phenomenex Jupiter C18 preparative column (5 µm, 300 Å, 21mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	75	25	20
20	50	50	20
21	0	100	20
24	0	100	20
25	75	25	20

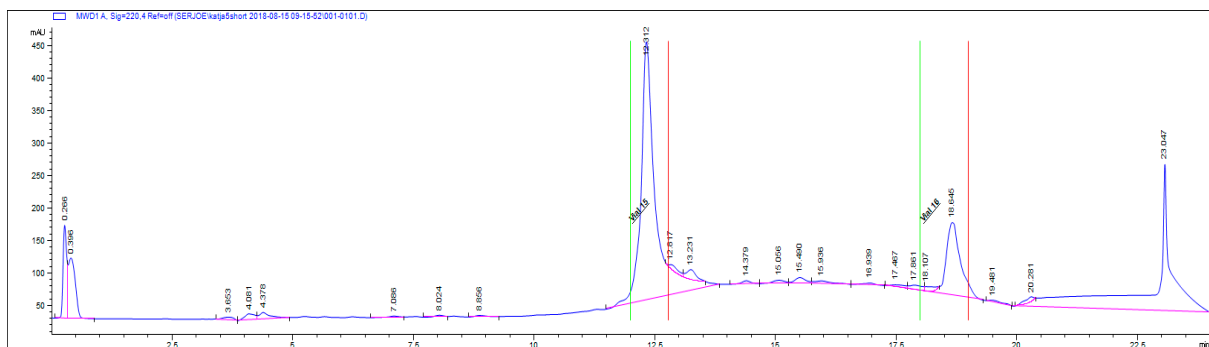


**R<sub>t</sub> = 14.9 min**

**Ova<sub>248-269</sub> (Val); Valine, Iva ctrl - H-EVSGVEQLV-SIINFEKL-VEVTS-OH**

Column: Phenomenex Jupiter C18 preparative column (5 µm, 300 Å, 21mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	75	25	20
15	50	50	20
16	0	100	20
19	0	100	20
21	75	25	20

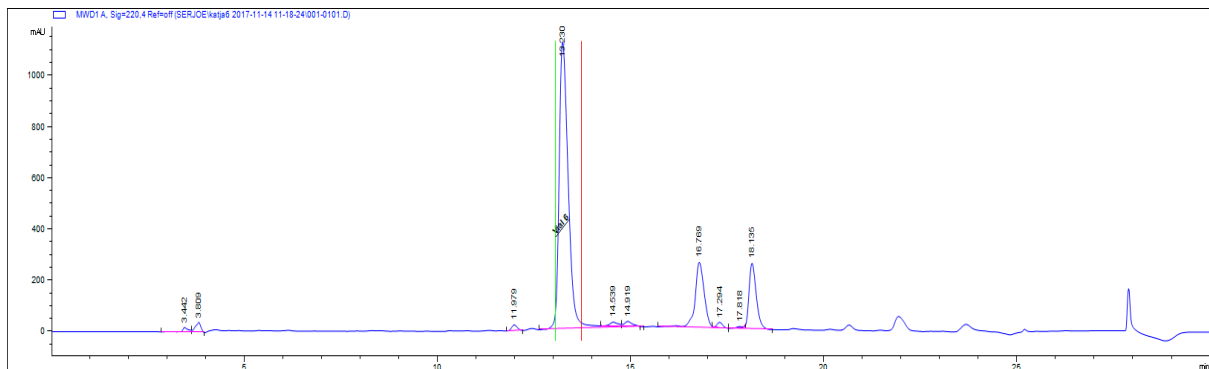


**R<sub>t</sub> = 12.31 min**

**Ova<sub>248-269</sub> (Aib);  $\alpha$ -Aminoisobutyric acid - H-EYSGYEQLY-SIINFEKL-YEYTS-OH**

Column: Phenomenex Jupiter C18 preparative column (5  $\mu$ m, 300 Å, 21mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	75	25	20
20	50	50	20
21	0	100	20
24	0	100	20
25	75	25	20

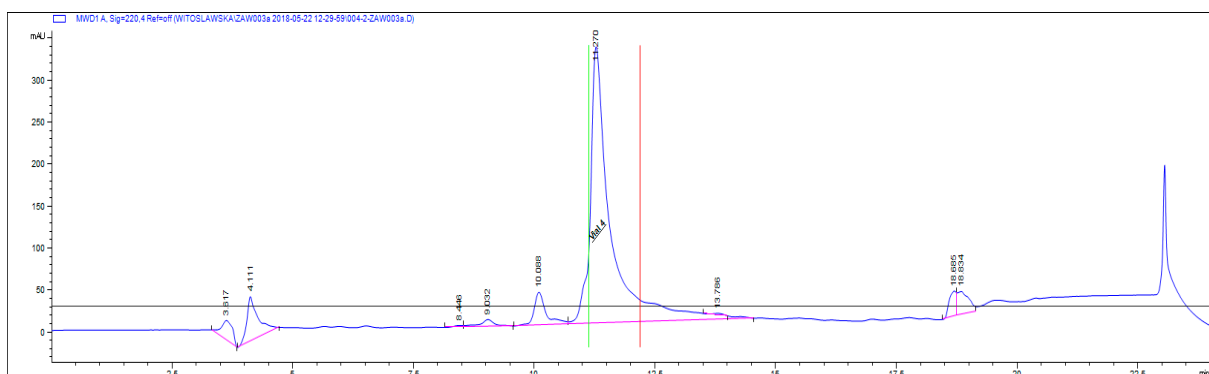


**R<sub>t</sub> = 13.23 min**

**Ova<sub>248-269</sub> (Ala); Alanine, Aib ctrl - H-EASGAEQLA-SIINFEKL-AEATS-OH**

Column: Phenomenex Jupiter C18 preparative column (5  $\mu$ m, 300 Å, 21mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	75	25	20
15	50	50	20
16	0	100	20
19	0	100	20
21	75	25	20



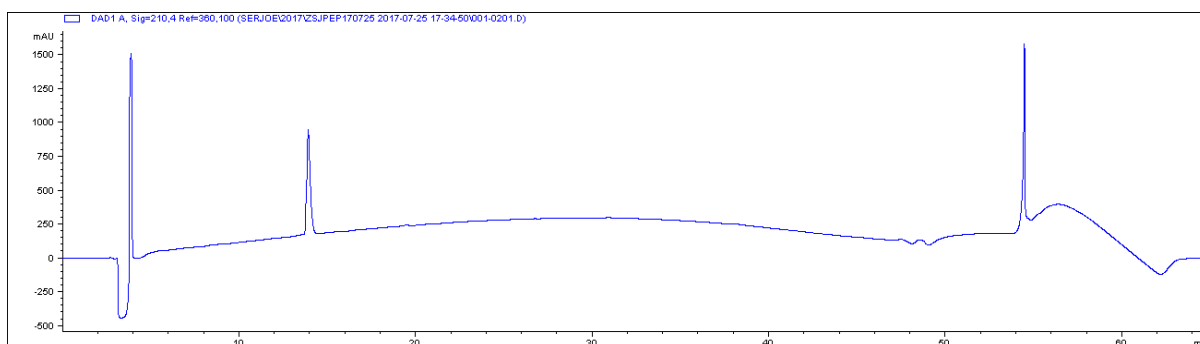
**R<sub>t</sub> = 11.27 min**

## Analytical RP-HPLC-MS traces

Ova<sub>257-264</sub> ; Native-short - H-SIINFEKL-OH

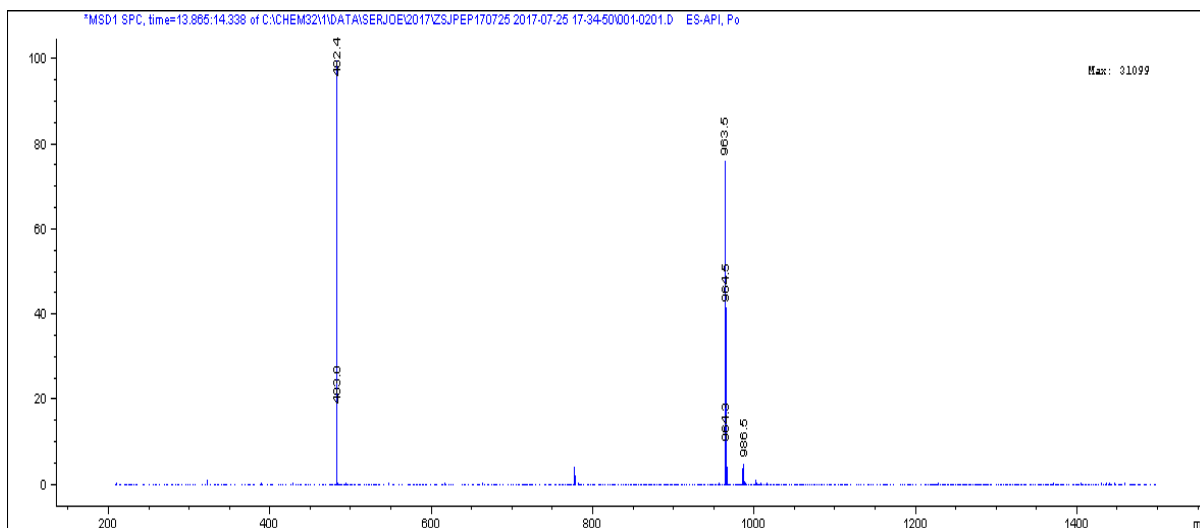
Column: Phenomenex Luna C18(2) analytical column (5 µm, 100 Å, 4.6mm x250 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	85	15	1
45	0	100	1
50	0	100	1
58	85	15	1



UV 210 nm - R<sub>t</sub> =13.9 min - purity 99%

MS spectrum of peak at 13.9 min



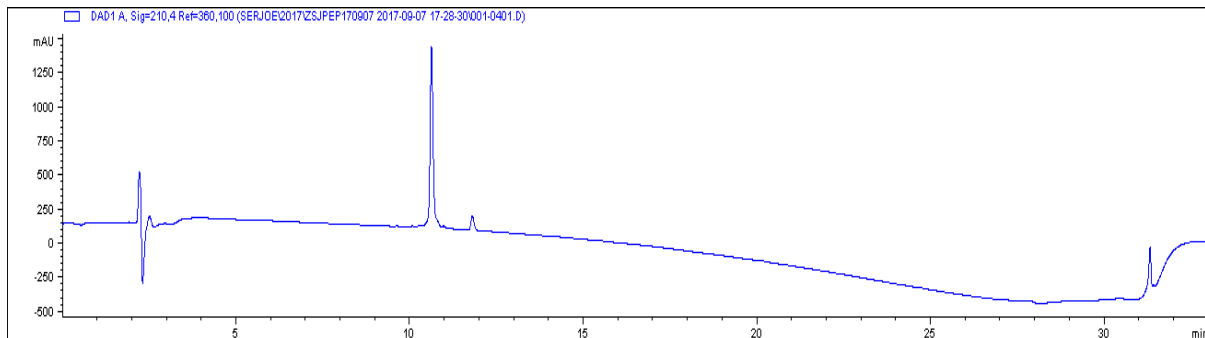
Mass calculated for C<sub>45</sub>H<sub>75</sub>N<sub>10</sub>O<sub>13</sub> [M+H]<sup>+</sup> = 963.54, measured 963.5

Mass calculated for C<sub>45</sub>H<sub>76</sub>N<sub>10</sub>O<sub>13</sub>/2 [M+2H]<sup>2+</sup>/2 = 965.56/2 = 482.78, measured 482.4

**Ova<sub>248-269</sub> ; Native-long - H-EVSGLEQLE-SIINFEKL-TEWTS-OH**

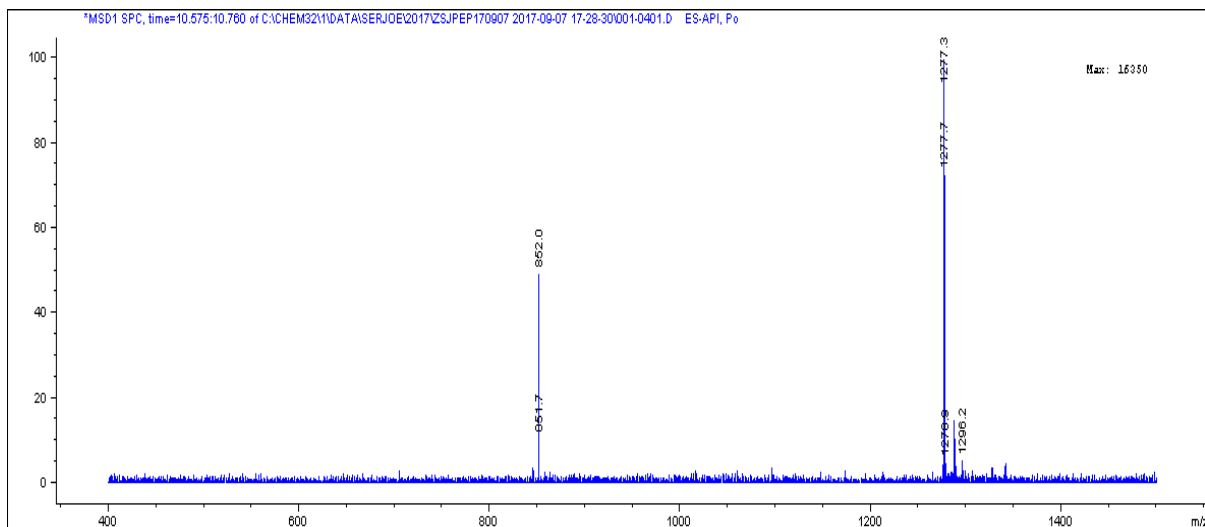
Column: Phenomenex Jupiter C18 analytical column (5 µm, 300 Å, 4.6mm x150 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	85	15	1
30	0	100	1
33	0	100	1
36	85	15	1



**UV 210 nm - R<sub>t</sub> =10.6 min - purity 93%**

MS spectrum of peak at 10.63 min



Molecular weight calculated for  $C_{114}H_{179}N_{26}O_{40}/2 [M+2H]^{2+}/2 = 2553.83/2 = 1276.9$ , measured 1277.3

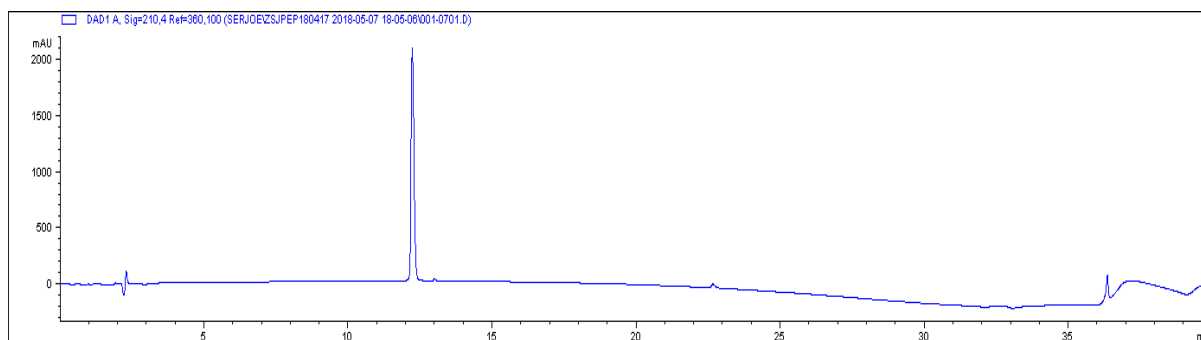
Molecular weight calculated for  $C_{114}H_{180}N_{26}O_{40}/3 [M+3H]^{3+}/3 = 2554.83/3 = 851.61$ , measured 852.0

Molecular weight calculated for  $C_{114}H_{179}N_{26}O_{40} = 2552.82$ , deconvoluted mass = 2552.78

**Ova<sub>248-269</sub> (Iva); Isovaline - H-EXSGXEQIX-SIINFKFL-XEXTS-OH**

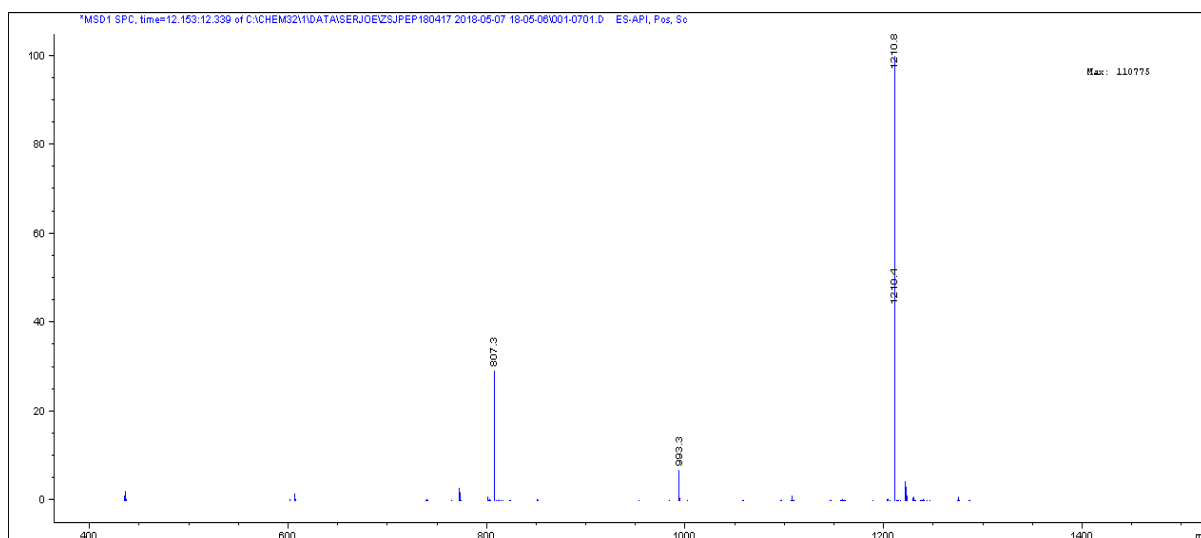
Column: Phenomenex Jupiter C18 analytical column (5 µm, 300 Å, 4.6mm x150 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	85	15	1
30	0	100	1
33	0	100	1
36	85	15	1



**UV 210 nm - R<sub>t</sub> =12.2 min - purity 99%**

MS spectrum of peak at 12.2 min



Molecular weight calculated for  $C_{114}H_{179}N_{26}O_{40}/2 [M+2H]^{2+}/2 = 2420.77/2 = 1210.38$ , measured 1210.8

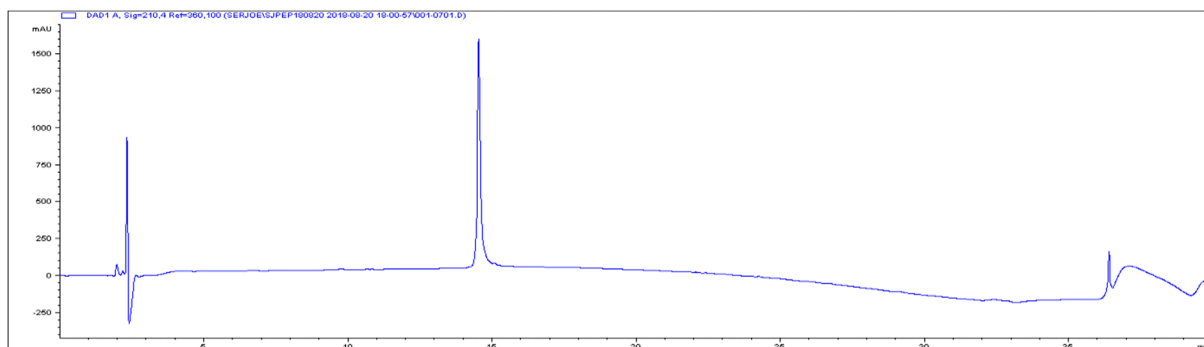
Molecular weight calculated for  $C_{114}H_{180}N_{26}O_{40}/3 [M+3H]^{3+}/3 = 2421.77/3 = 807.25$ , measured 807.3

Molecular weight calculated for  $C_{114}H_{179}N_{26}O_{40} = 2419.76$ , deconvoluted mass = 2419.23

Ova<sub>248-269</sub> (Val); Valine, Iva ctrl - H-EVSGVEQLV-SIINFEKL-VEVTS-OH

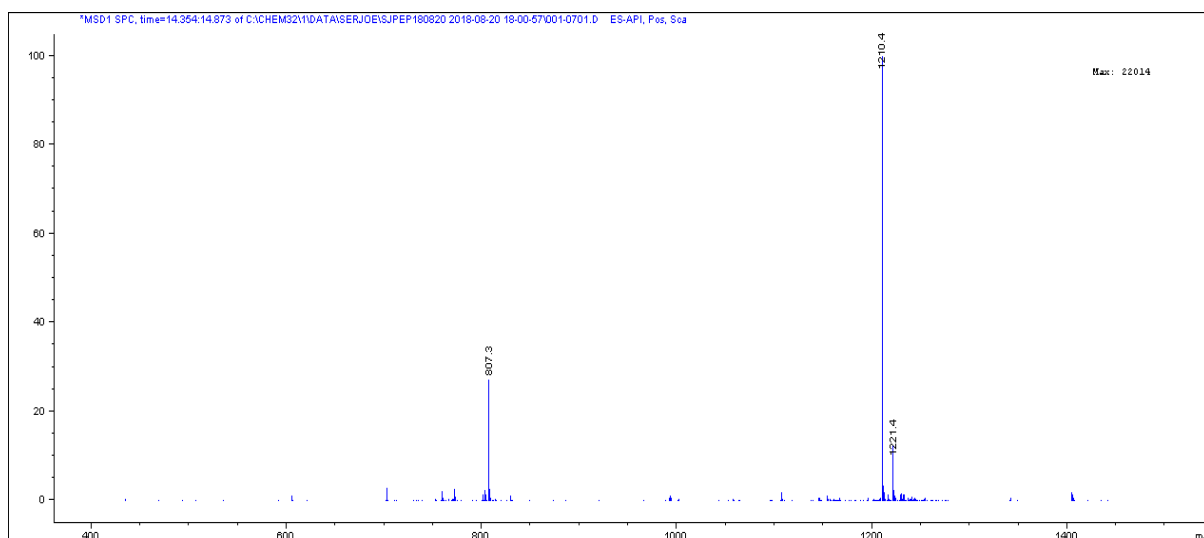
Column: Phenomenex Jupiter C18 analytical column (5 µm, 300 Å, 4.6mm x150 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	95	5	1
30	0	100	1
33	0	100	1
36	95	5	1



UV 210 nm - R<sub>t</sub> =14.5 min - purity 98%

MS spectrum of peak at 14.5 min



Molecular weight calculated for C<sub>114</sub>H<sub>179</sub>N<sub>26</sub>O<sub>40</sub>/2 [M+2H]<sup>2+</sup>/2 = 2420.77/2 = 1210.38, measured 1210.8

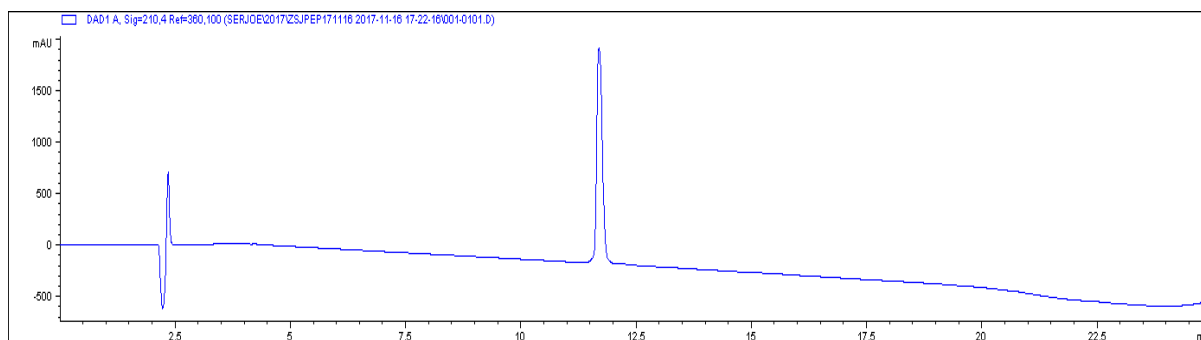
Molecular weight calculated for C<sub>114</sub>H<sub>180</sub>N<sub>26</sub>O<sub>40</sub>/3 [M+3H]<sup>3+</sup>/3 = 2421.77/3 = 807.25, measured 807.3

Molecular weight calculated for C<sub>114</sub>H<sub>179</sub>N<sub>26</sub>O<sub>40</sub> = 2419.76, deconvoluted mass = 2419.23

**Ova<sub>248-269</sub> (Aib);  $\alpha$ -Aminoisobutyric acid - H-EYSGYEQLY-SIINFEKL-YEYTS-OH**

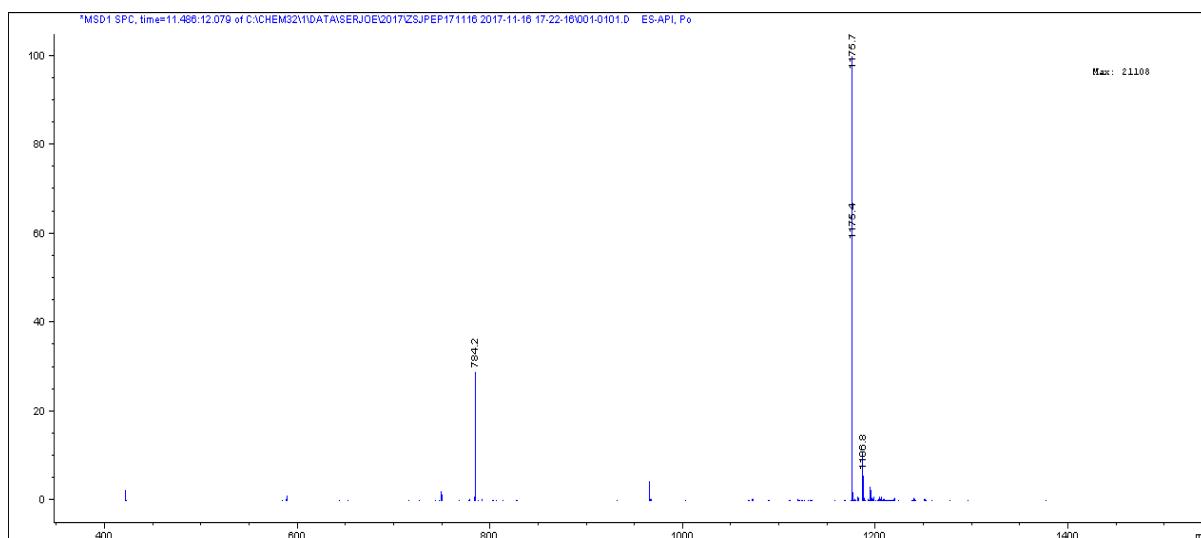
Column: Phenomenex Jupiter C18 analytical column (5  $\mu$ m, 300 Å, 4.6mm x150 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	85	15	1
30	0	100	1
33	0	100	1
36	85	15	1



**UV 210 nm -  $R_t$  =15.3 min - purity 99%**

MS spectrum of peak at 15.3 min



Molecular weight calculated for  $C_{114}H_{179}N_{26}O_{40}/2 [M+2H]^{2+}/2 = 2350.63/2 = 1175.32$ , measured 1175.7

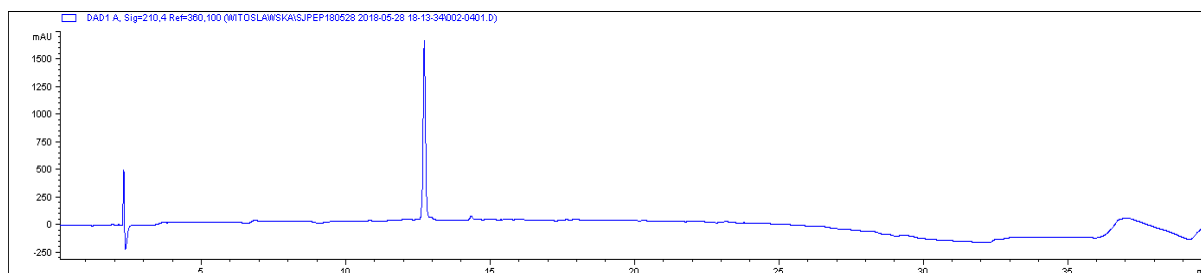
Molecular weight calculated for  $C_{114}H_{180}N_{26}O_{40}/3 [M+3H]^{3+}/3 = 2351.64/3 = 783.88$ , measured 784.2

Molecular weight calculated for  $C_{114}H_{179}N_{26}O_{40} = 2349.62$ , deconvoluted mass = 2349.48

Ova<sub>248-269</sub> (Ala); Alanine, Aib ctrl - H-EASGAEQLA-SIINFEKL-AEATS-OH

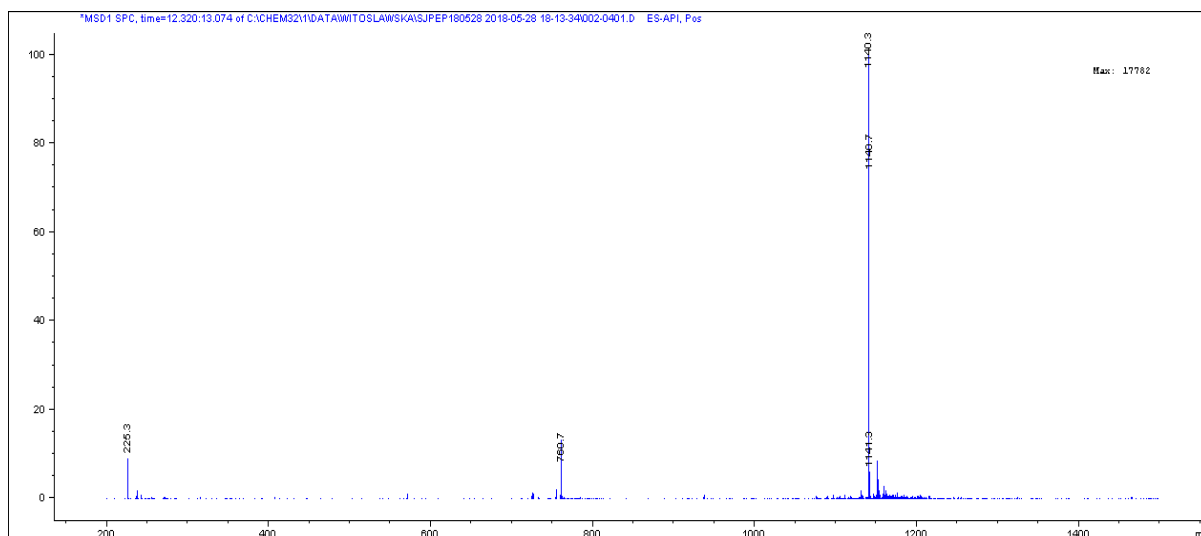
Column: Phenomenex Jupiter C18 analytical column (5 μm, 300 Å, 4.6mm x150 mm D x L)

Time min	A - Water+ 0.1 % TFA %	B - ACN+ 0.1 % TFA %	Flow mL/min
0	95	5	1
30	0	100	1
33	0	100	1
36	95	5	1



UV 210 nm - R<sub>t</sub> =15.3 min - purity 98%

MS spectrum of peak at 15.3 min



Molecular weight calculated for C<sub>114</sub>H<sub>179</sub>N<sub>26</sub>O<sub>40</sub>/2 [M+2H]<sup>2+</sup>/2 = 2281.51/2 = 1140.76, measured 1140.3

Molecular weight calculated for C<sub>114</sub>H<sub>180</sub>N<sub>26</sub>O<sub>40</sub>/3 [M+3H]<sup>3+</sup>/3 = 2282.52/3 = 760.84, measured 760.7

Molecular weight calculated for C<sub>114</sub>H<sub>179</sub>N<sub>26</sub>O<sub>40</sub> = 2279.49, deconvoluted mass = 2278.83