Supporting Information

Structure–Activity Relationship of Synthetic Linear KTS-Peptides Containing Meta-Aminobenzoic Acid as Antagonists of α1β1 Integrin with Anti-Angiogenic and Melanoma Anti-Tumor Activities

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Table S1. Peptides' sequence, molecular weight and purity of synthetic KTS-peptides containing meta-aminobenzoic acid

Peptide Number	Sequence	M_w calcd[1]	M_w obs[2]	Purity[3] %
1	H_2N-W-*KTS*-R-TSHY-**MABA-**TGKSDG-COOH	1830	1829.87	95.51
2	H_2N-W-*KTS*- **MABA**-R-TSHYGKSDG-COOH	1729	1728.77	95.04
3	H_2N-*KTS*-W-*KTS*-**MABA**-R-TSHYGKSDG-COOH	2045	2045.12	97.60
4	H_2N-W-*KTS*-**MABA**-R-**MABA-**TSHYGKSDG-COOH	1848	1847.84	96.37
5	H_2N-W-*KTS*-**MABA**-R-TSHY-**MABA**-TGKSDG-COOH	1948	1948.94	97.56
6	H_2N-W-*KTR*-**MABA**-R-**MABA-**TSHYTGKSDG-COOH	2017	2018.05	95.88
7	H_2N-W-*KTR*-**MABA**-R-**MABA**-TSHY-**MABA**-TGKSDG-COOH	2136	2137.12	95.10
8	H_2N-W-*KTS*-R-TSHY-TGKSDG-COOH	1710	1710.80	96.68

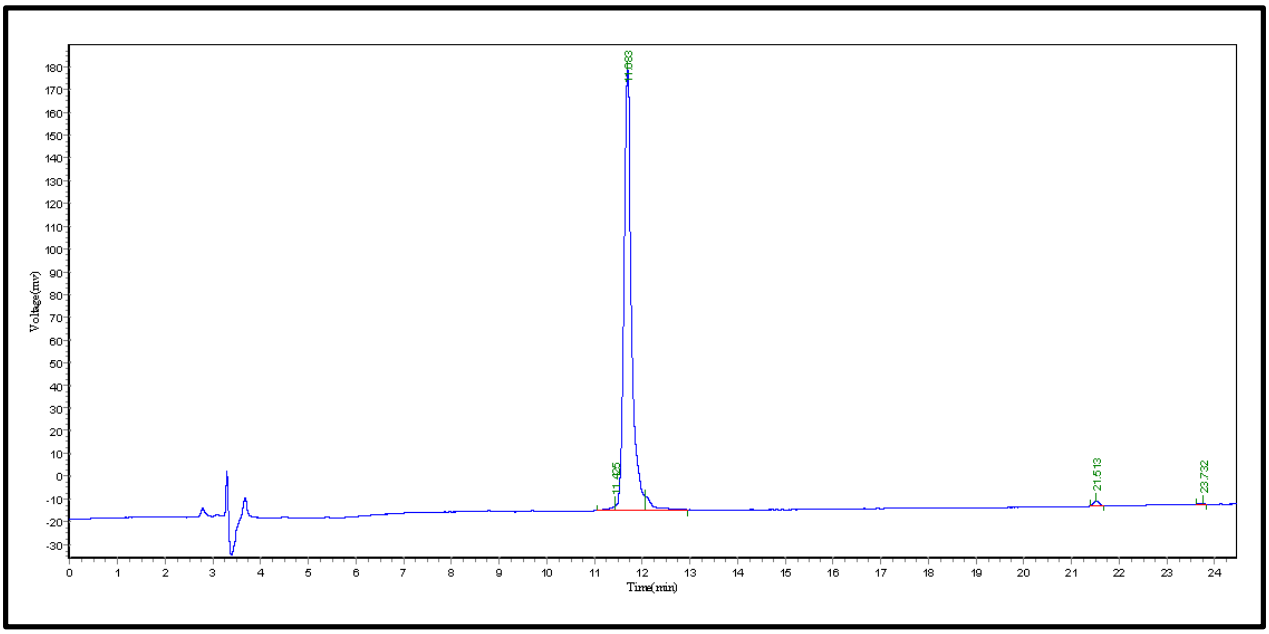
1. Calculated from theoretical sequence, amino acids in capital letters.

2. Based on exact mass calculation for $[M+H]^+$.

3. According to HPLC data.

Figure S1-Peptide 1

HPLC



MS spectrum

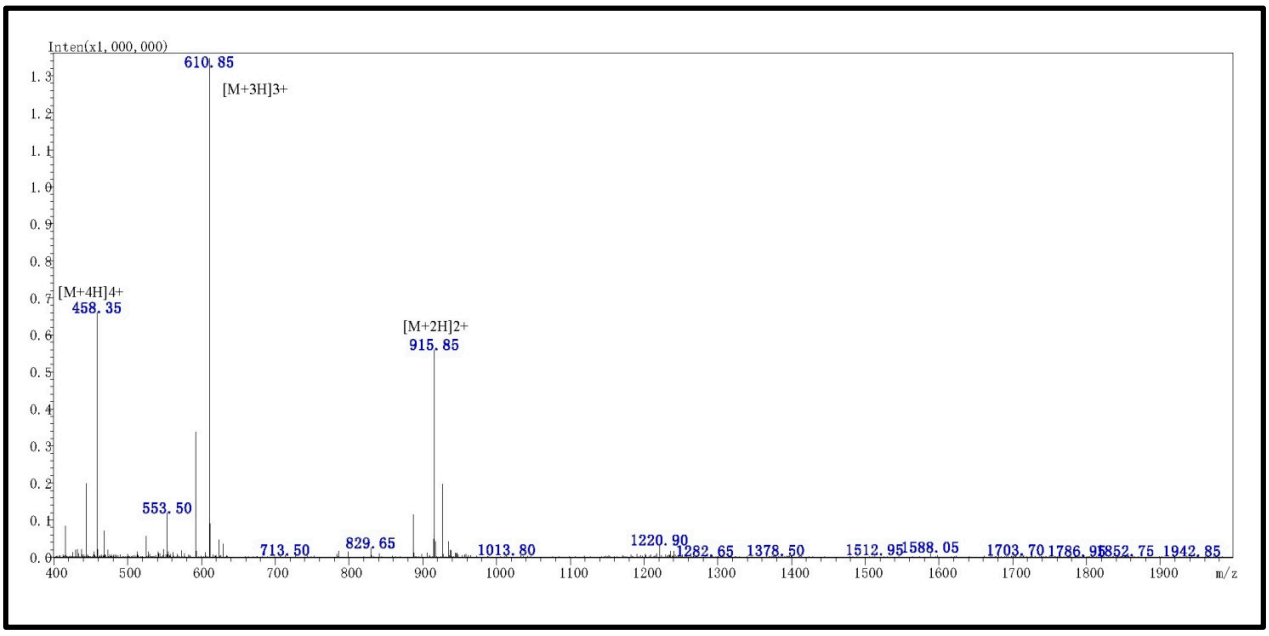
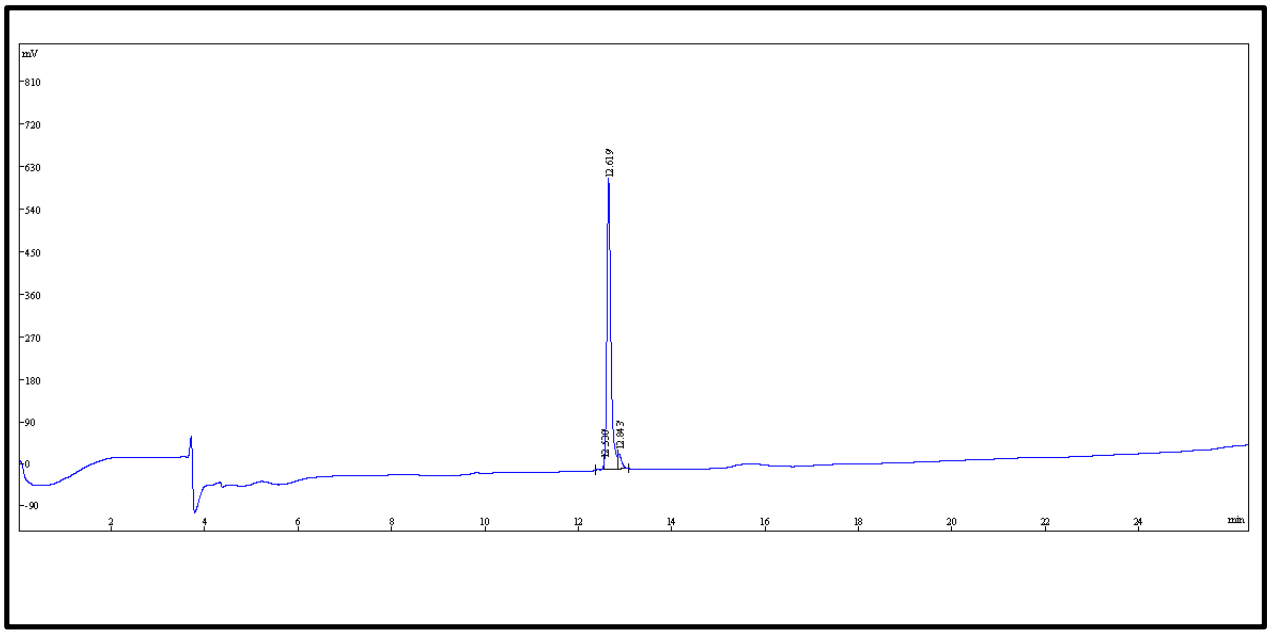


Figure S2-Peptide 2

HPLC



MS spectrum

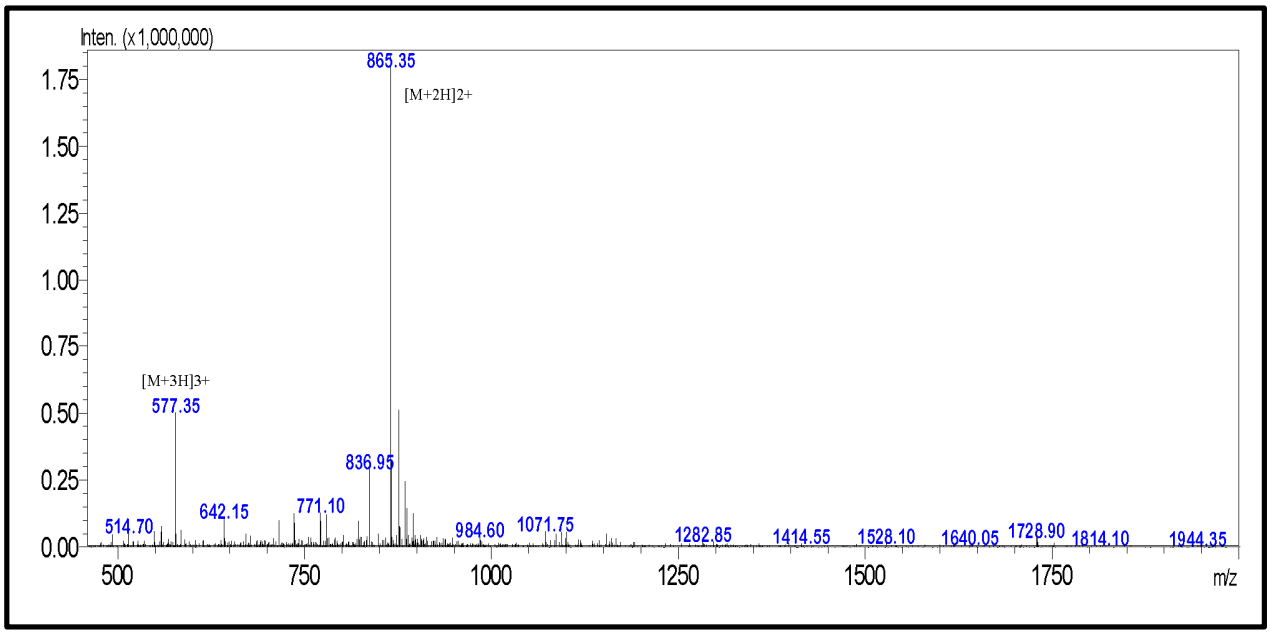
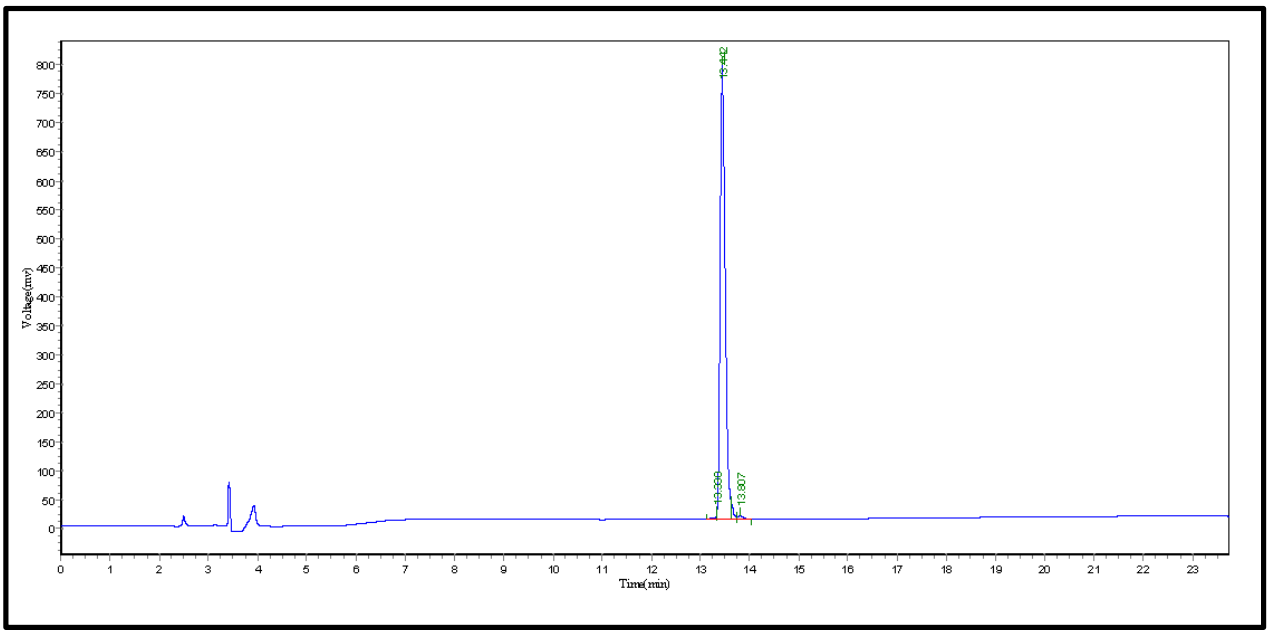


Figure S3-Peptide 3

HPLC



MS spectrum

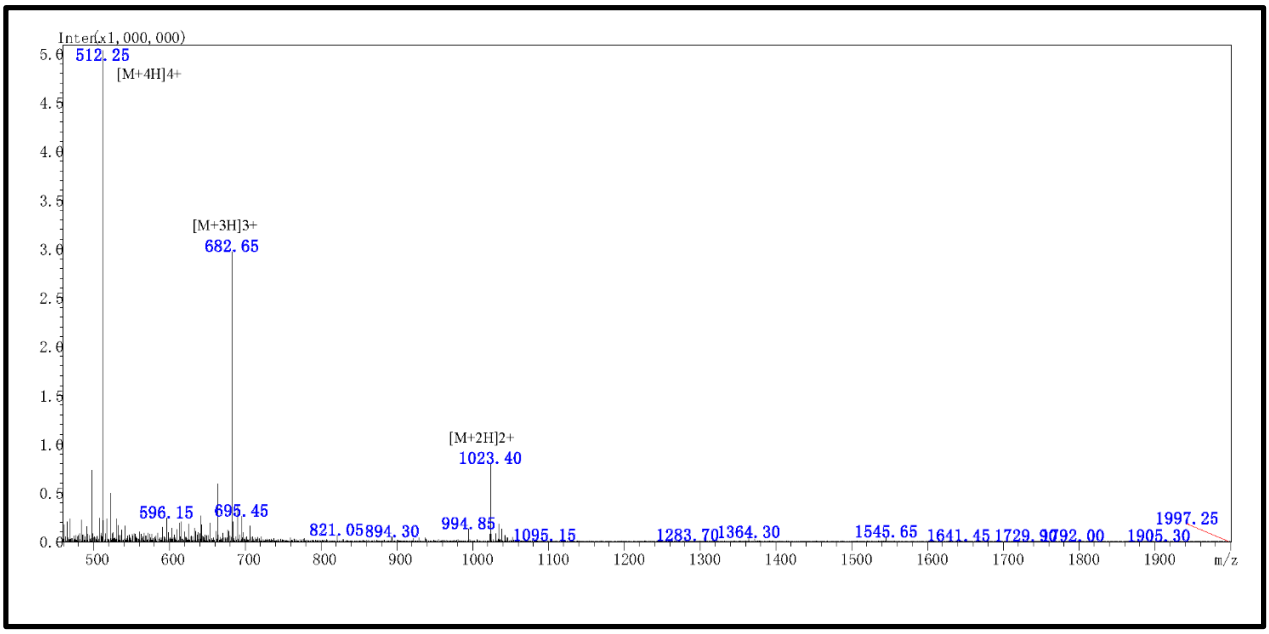
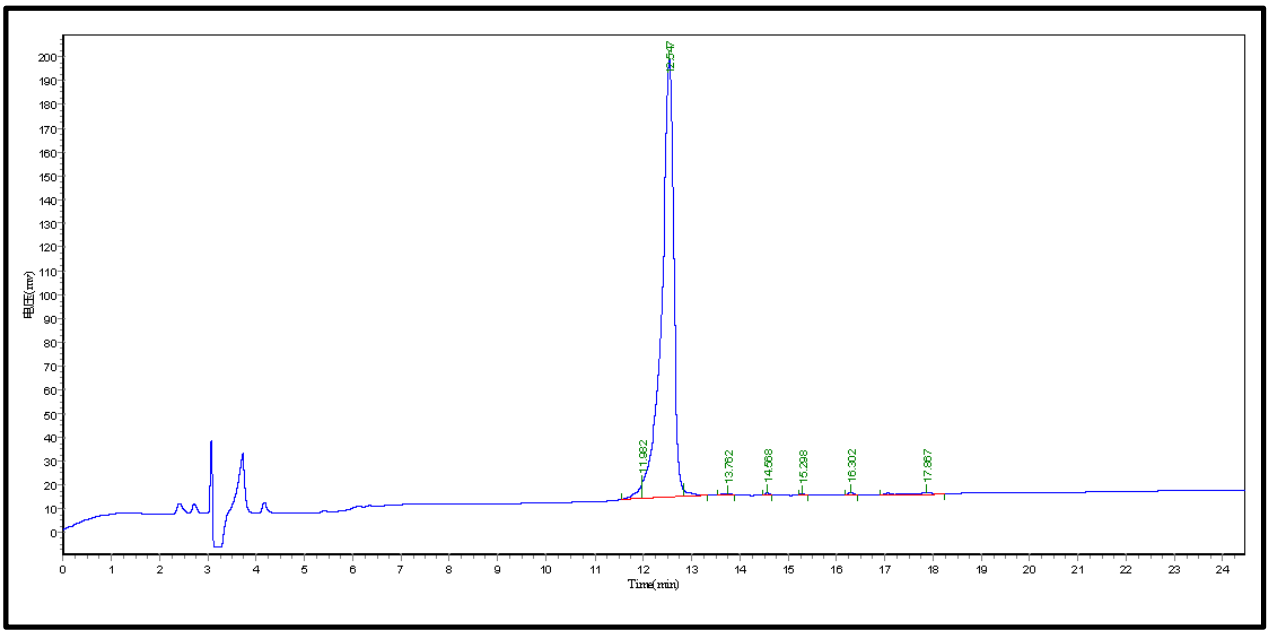


Figure S4-Peptide 4

HPLC



MS spectrum

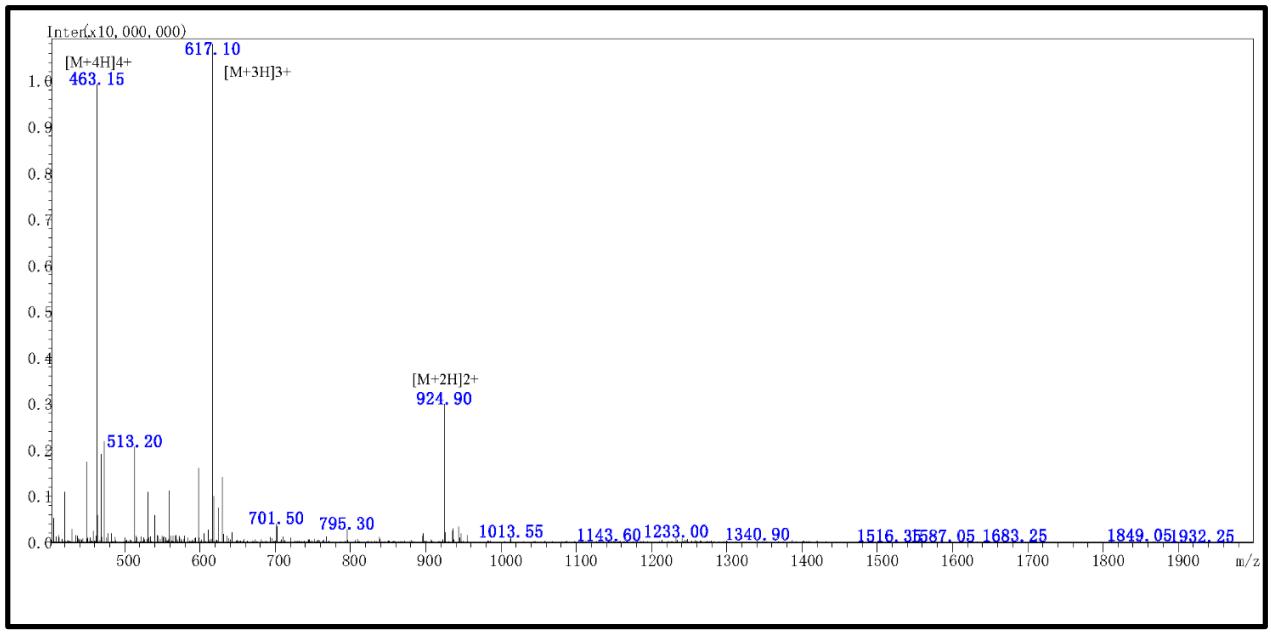
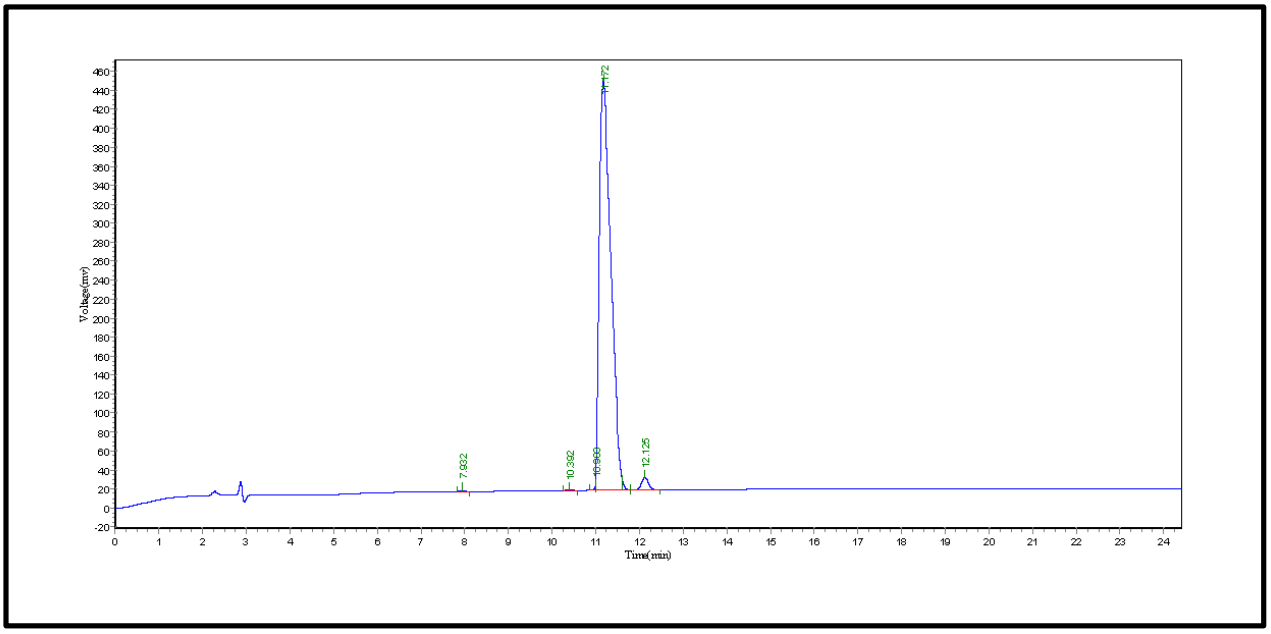


Figure S5-Peptide 5

HPLC



MS spectrum

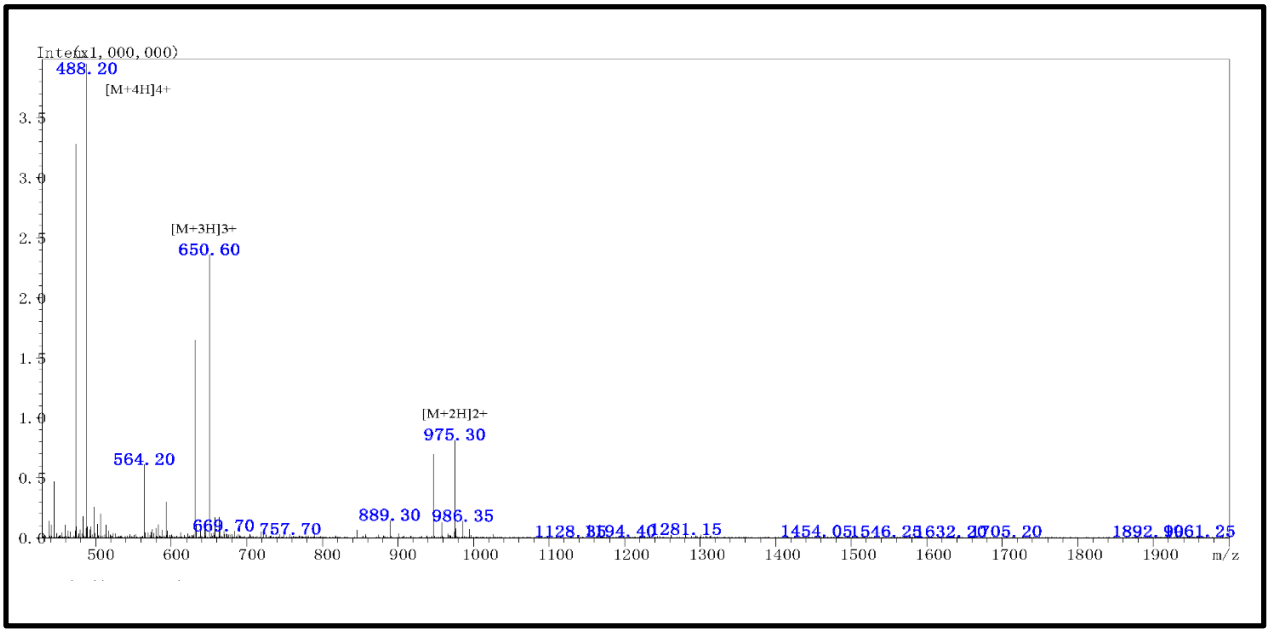
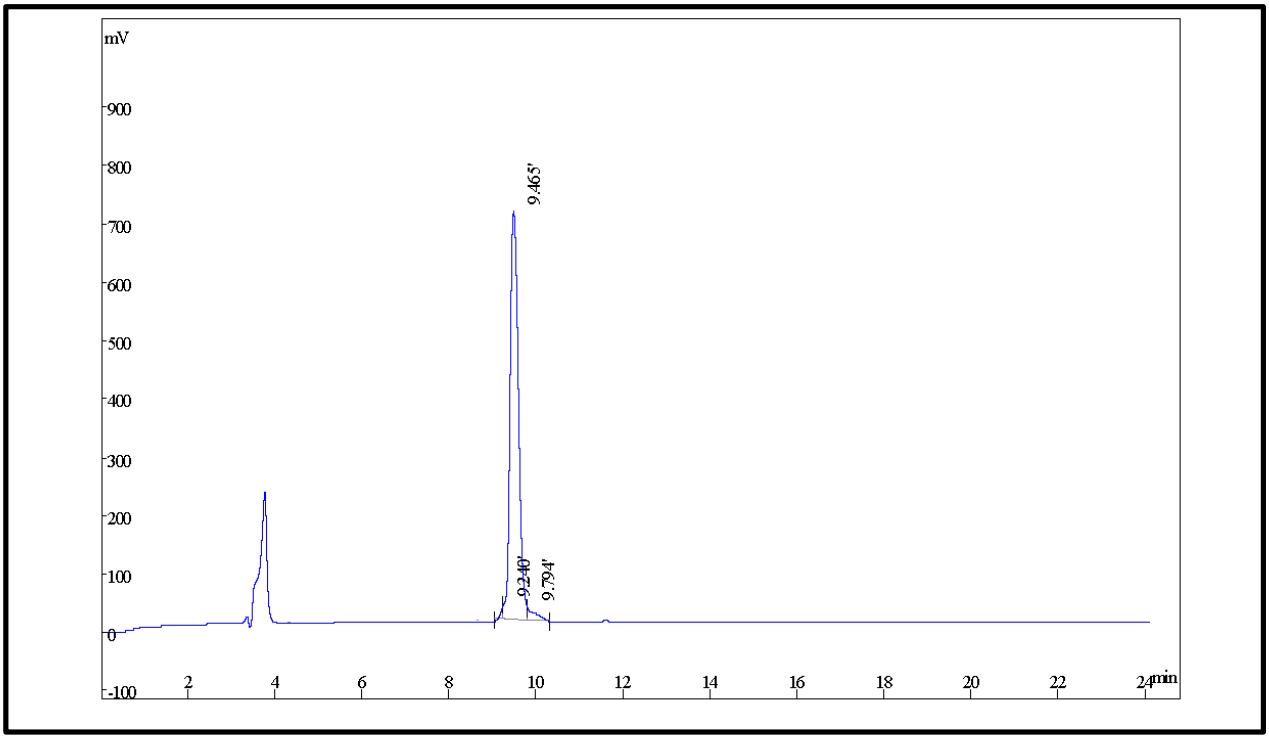


Figure S6-Peptide 6

HPLC



MS spectrum

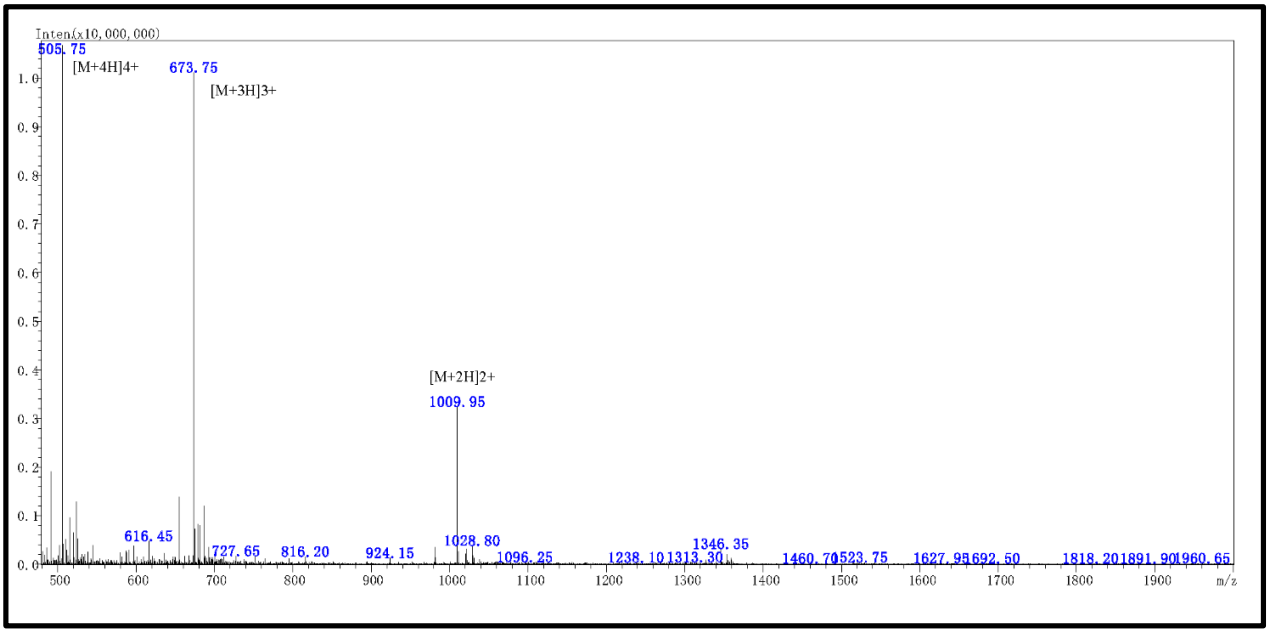
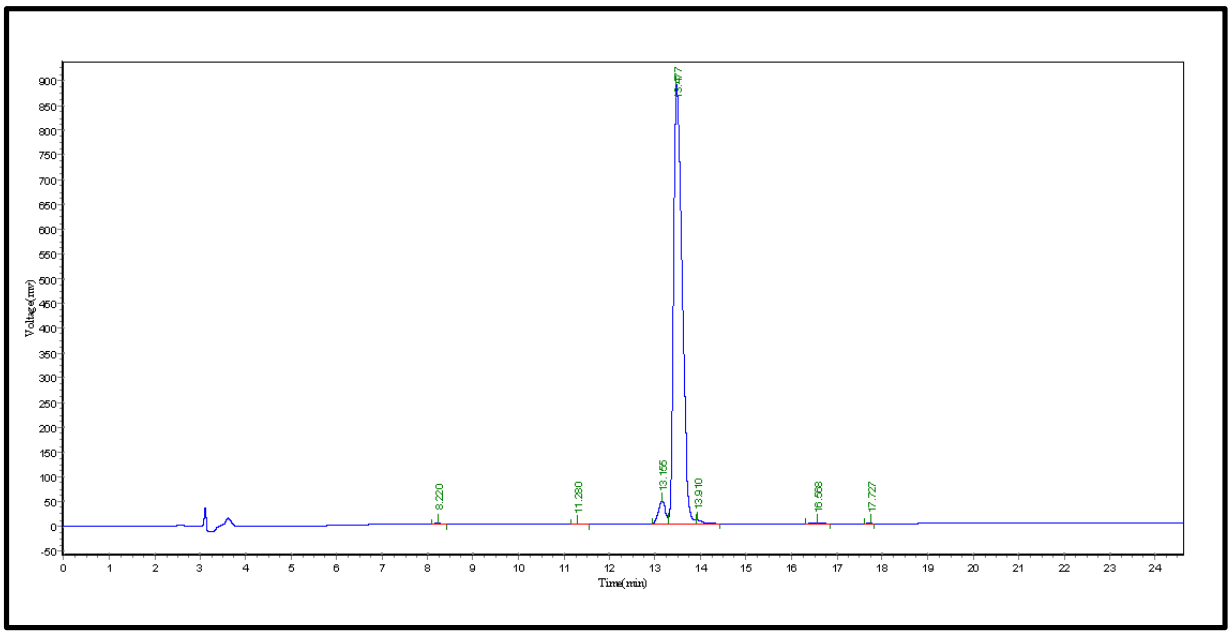


Figure S7-Peptide 7

HPLC



MS spectrum

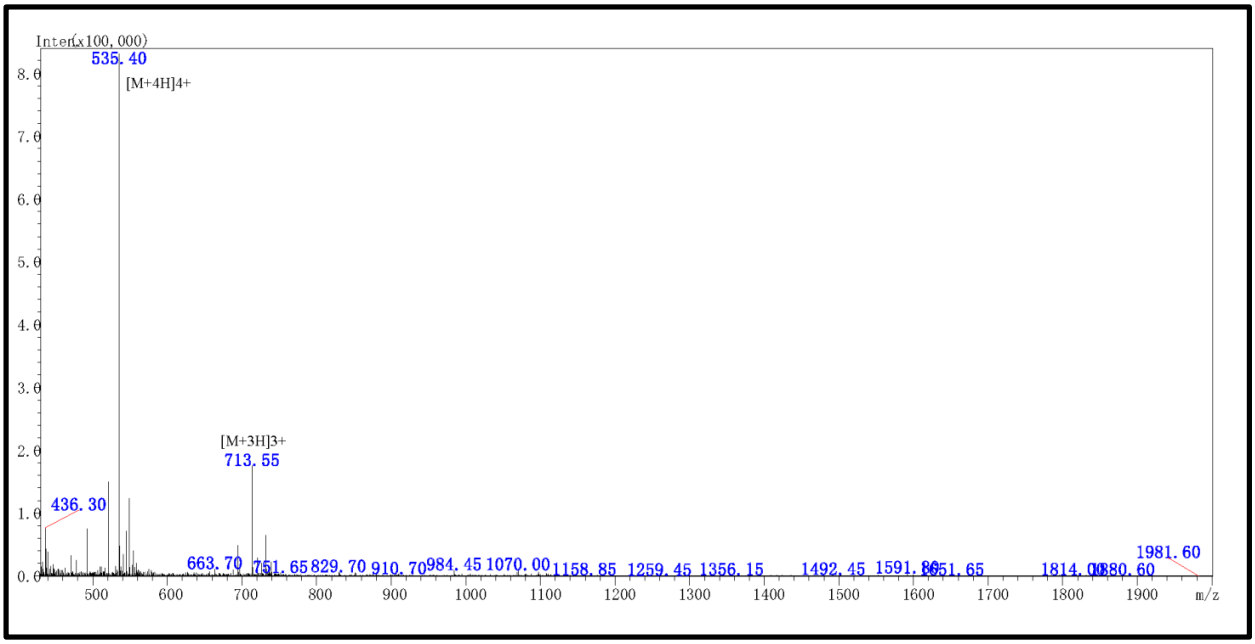
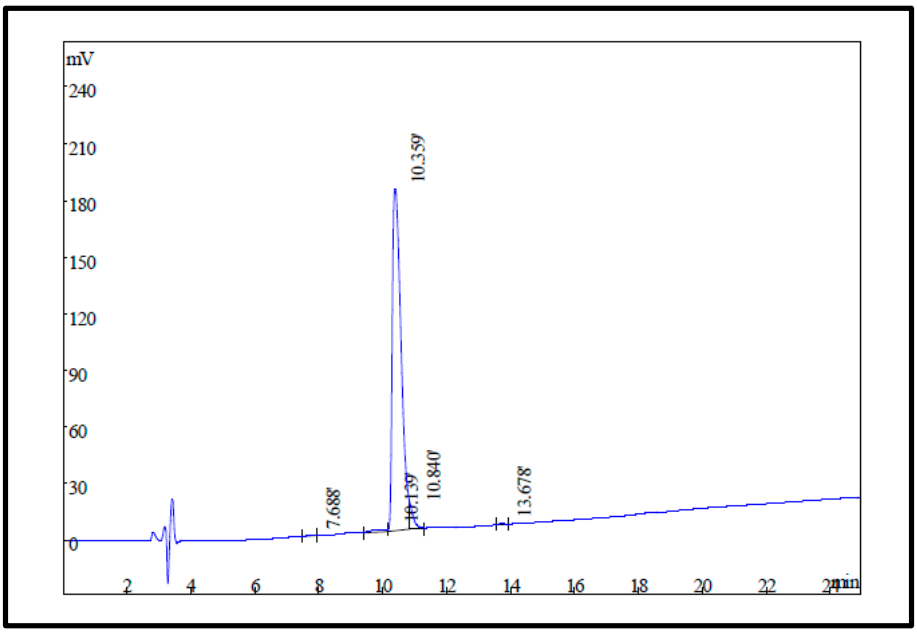


Figure S8-Peptide 8

HPLC



MS spectrum

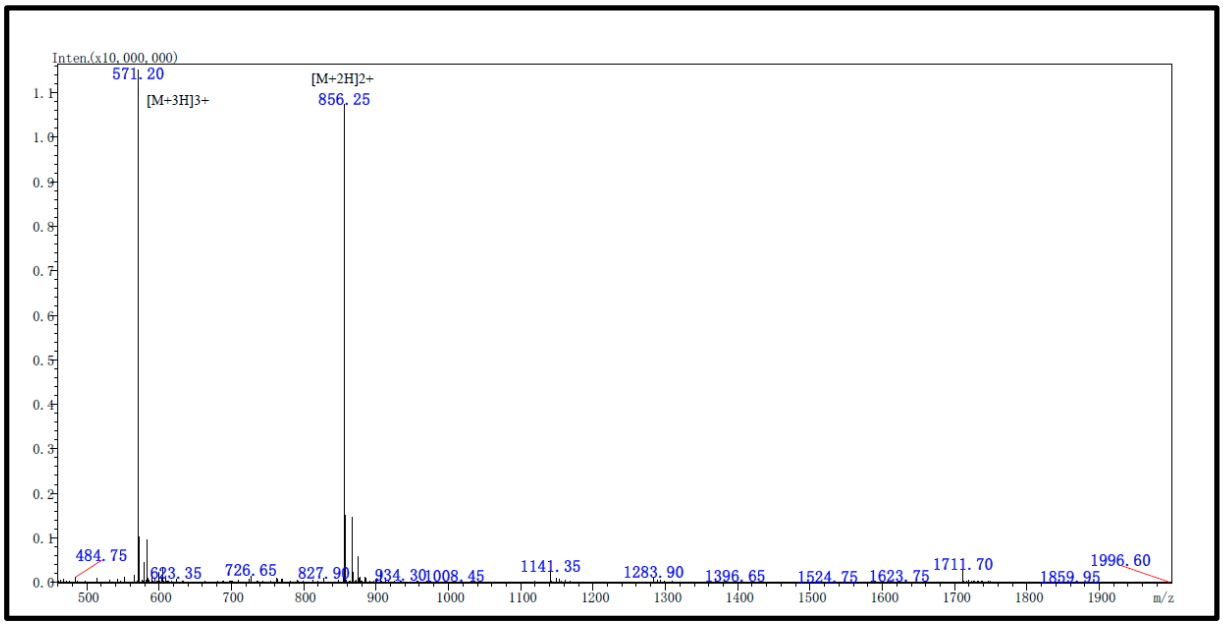


Figure S9- Protocol of synthesis of peptide 7

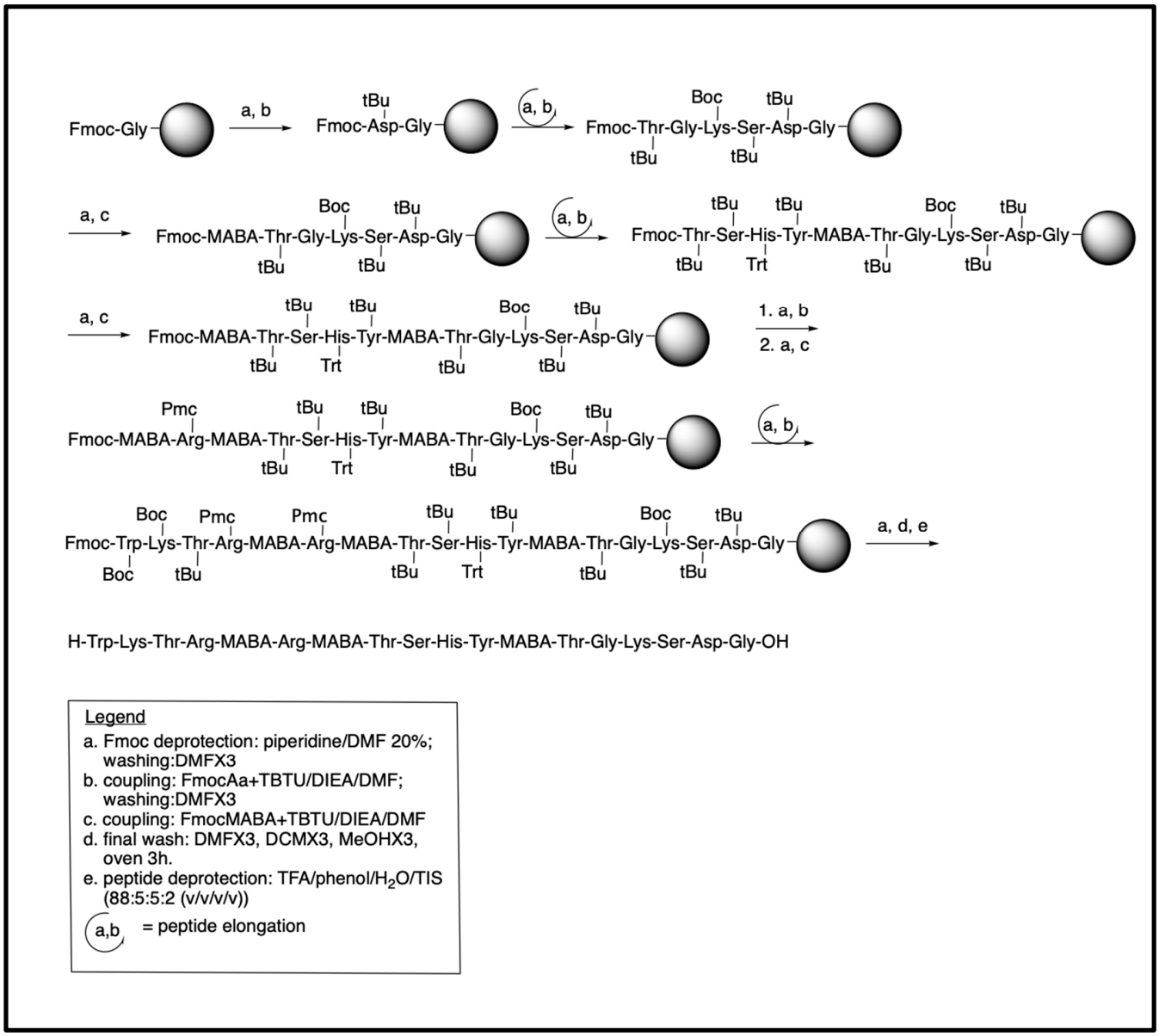


Table S2. Selectivity of the inhibitory effects of MABA-peptide 4 compared to Viperistatin on various integrins in cell adhesion assay.

Cell Type	Integrin	Ligand	MABA-peptide 4 IC_{50} (μM)	Viperistatin IC_{50} (nM)
α1K562	α1β1	Collagen IV	550	0.3
α2K562	α2β1	Collagen I	3,000	>10,000
α6K562	α6β1	Laminin	>10,000	>10,000
K562	α5β1	Fibronectin	>5,000	>5,000
Jurkat	α4β1	VCAM-1	>5,000	>10,000
SW480α9	α9β1	VCAM-1	>10,000	>10,000
Platelets	αIIbb3	Fibrinogen	>10,000	>10,000
JY-B cells	αvb3	Vitronectin	>10,000	>10,000

Peptides binding activity was evaluated for anti-adhesion effect using cell lines expressing different major integrin subunits. Before the day of the experiment, each well of a 96-well plate was coated with 1 μg/ 0.1 ml ligands. Cells were labeled with the fluorescent tag CMFDA. Labeled cells (1×10^5 cells/well) were added to each well in the absence or presence of inhibitor and incubated at 37°C for 60 min. Unbound cells were removed by washing the wells three times and bound cells were lysed by the addition of 0.5% Triton X-100 and the amount of fluorescence was quantified, at λ_{ex} = 485 nm and λ_{em} = 530 nm. To determine the number of adhered cells from the fluorescence values, a standard curve was generated by serial dilutions of known numbers of CMFDA-labeled cells. The data represent means of the results of three experiments. VCAM-1, vascular cell adhesion molecule-1; α1, α2, α6 K562, K562 cells transfected with α1, α2 or α6 integrins; SW480α9 = SW480 cells transfected with α9 integrin;

Figure S10- Phase contrast images taken 6 h of the treated HUVECs on Matrigel to measure tube formation after treatment with MABA-peptides.

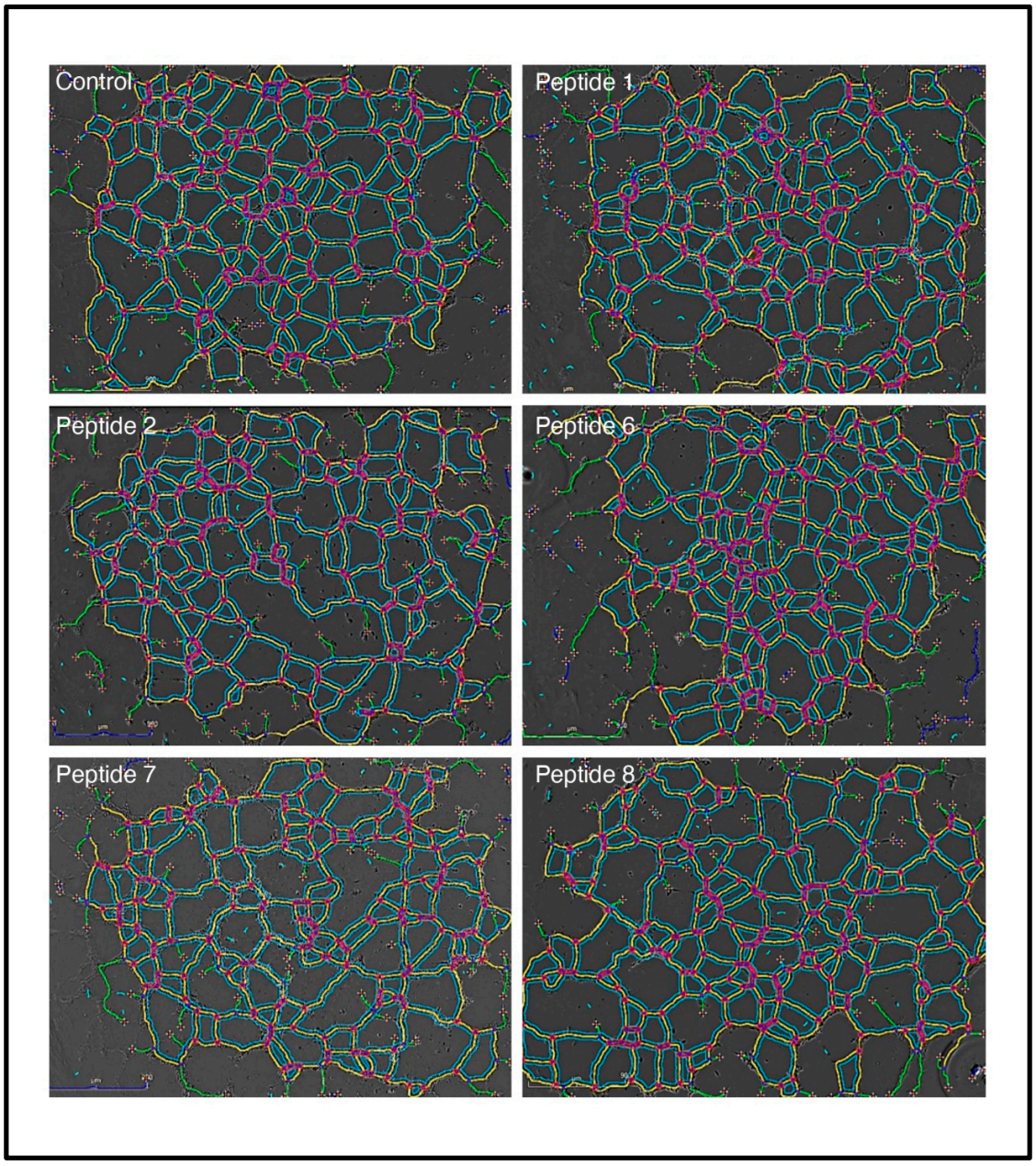


Table S3. Median survival of groups of B16 melanoma mice treated with peptides in comparison to control group of sick mice.

Group (n=10)	**1**	**2**	**3**	**4**	**5**	**6**
Treatment	**Control Sick-saline**	**Control sick-untreated**	**Peptide 3**	**Peptide 4**	**Peptide 5**	**Peptide 6**
Median survival	32	35.5	50	73	35	38
Log-rank (Mantel-Cox) test						
Chi square	0.1699	1.745	0.4813	15.48	1.123	0.5095
df	1	1	1	1	1	1
P value	0.6802	0.1865	0.4878	≤0.0001	0.2894	0.4753
P value summary	ns	ns	ns	***	ns	ns
Are the survival curves significantly different?	No	No	No	**Yes**	No	No
Log-rank test for trend						
Chi square	0.9317	1.161	0.03682	13.54	1.049	0.3005
df	1	1	1	1	1	1
P value	0.3344	0.2812	0.8478	0.0002	0.3058	0.5836
P value summary	ns	ns	ns	***	ns	ns
Significance trend	No	No	No	**Yes**	No	No
Log-rank (Mantel-Cox) test	0.1699	1.745	0.4813	15.48	1.123	0.5095

*** $p < 0.001$

Figure S11- RMSD plots for compound 2 and 4 from the Molecular Dynamic simulation.

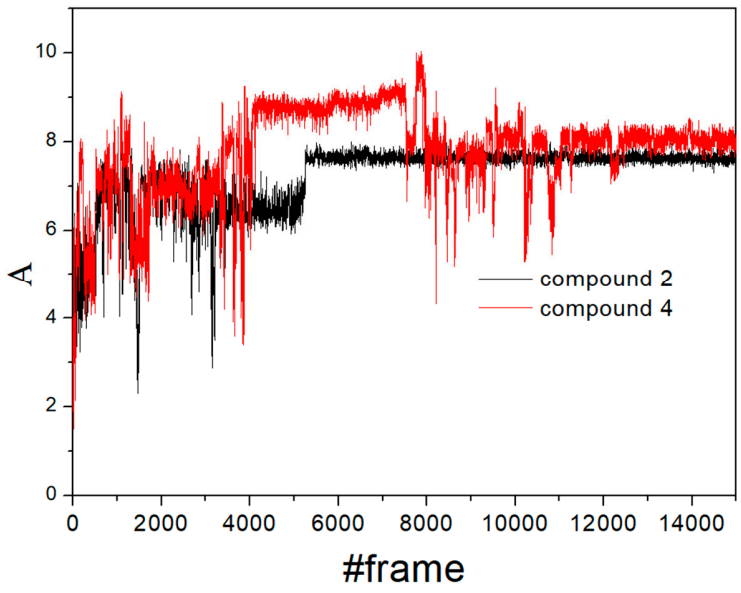


Figure S12- The central structures of the biggest cluster after Molecular Dynamic simulations of compounds 2 (left) and 4 (right). Carbon atoms of compounds 2 and 4 are colored in green and blue respectively.