

Supporting Information

Structure-activity relationship of synthetic linear KTS-peptides containing meta-aminobenzoic acid as antagonists of $\alpha 1\beta 1$ integrin with antiangiogenic and melanoma' antitumor activities

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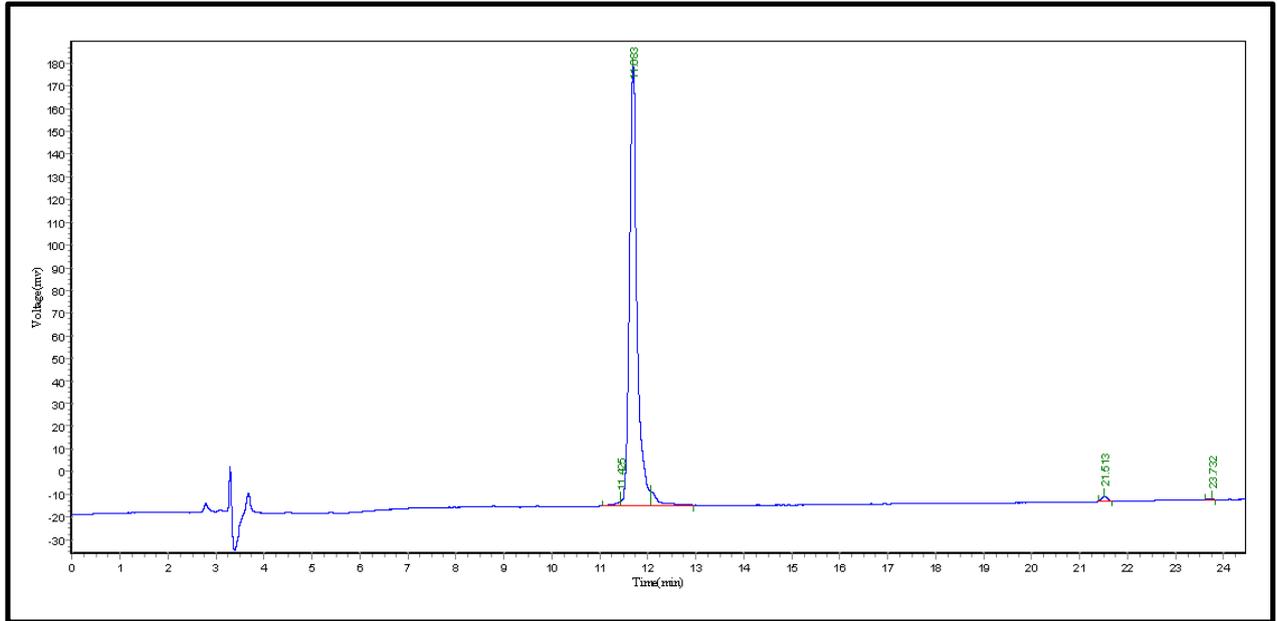
Table S1. Peptides' sequence, molecular weight and purity of synthetic KTS-peptides containing meta-aminobenzoic acid

Peptide Number	Sequence	M _w calcd ¹	M _w obs ²	Purity ³ %
1	H ₂ N-W-KTS-R-TSHY- MABA -TGKSDG-COOH	1830	1829.87	95.51
2	H ₂ N-W-KTS- MABA -R-TSHYGKSDG-COOH	1729	1728.77	95.04
3	H ₂ N-KTS-W-KTS- MABA -R-TSHYGKSDG-COOH	2045	2045.12	97.60
4	H ₂ N-W-KTS- MABA -R- MABA -TSHYGKSDG-COOH	1848	1847.84	96.37
5	H ₂ N-W-KTS- MABA -R-TSHY- MABA -TGKSDG-COOH	1948	1948.94	97.56
6	H ₂ N-W-KTR- MABA -R- MABA -TSHYTGKSDG-COOH	2017	2018.05	95.88
7	H ₂ N-W-KTR- MABA -R- MABA -TSHY- MABA -TGKSDG-COOH	2136	2137.12	95.10
8	H ₂ N-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.

Fig. S1-Peptide 1

HPLC



MS spectrum

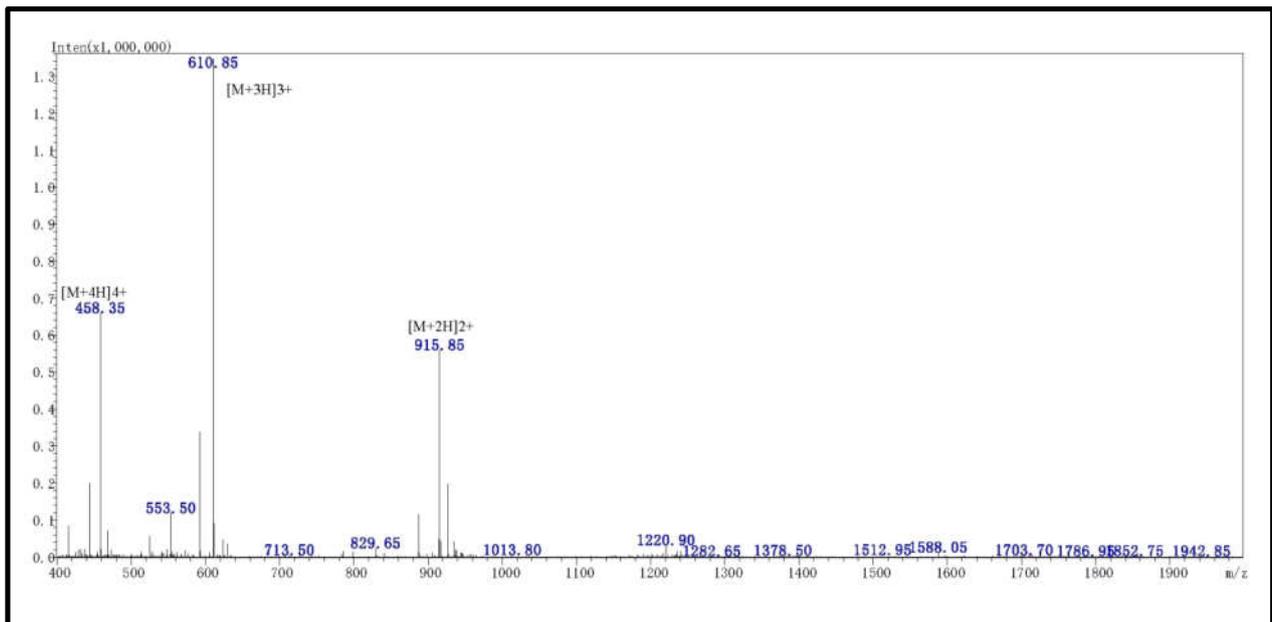
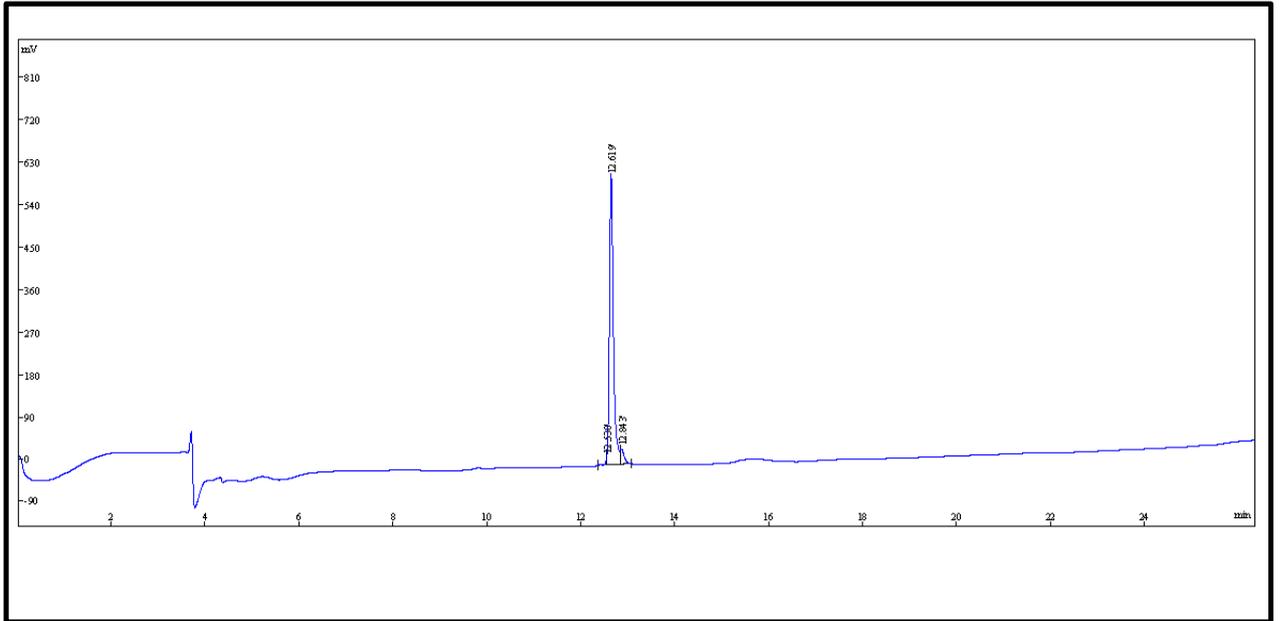


Fig. S2-Peptide 2

HPLC



MS spectrum

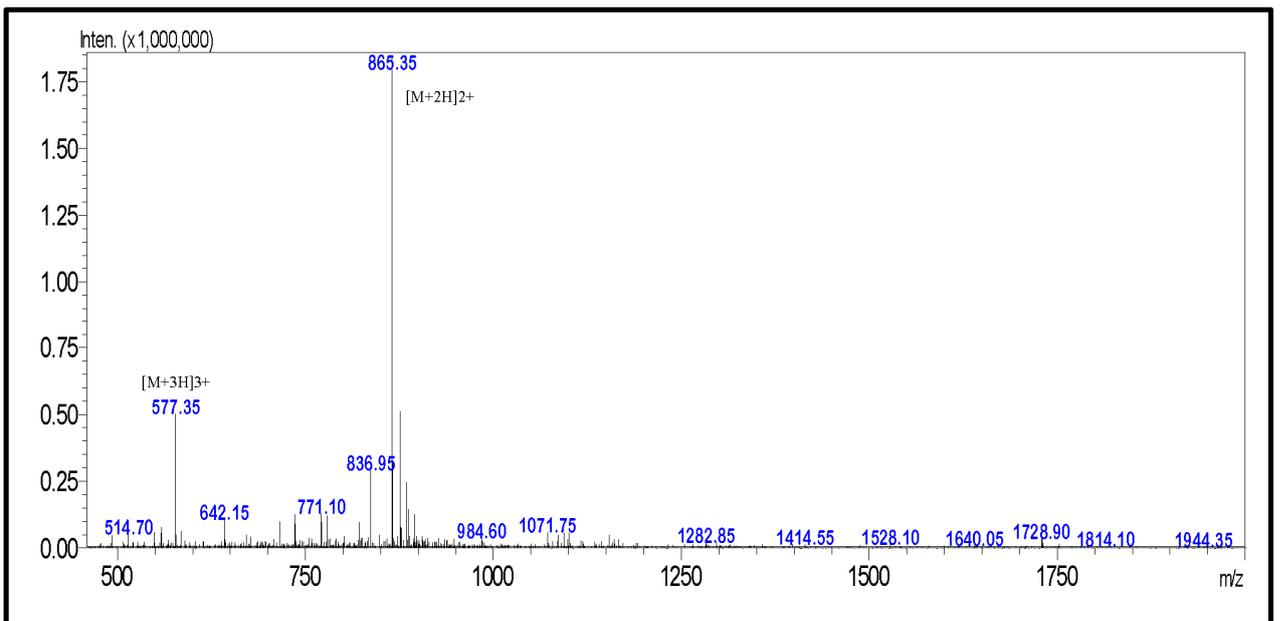
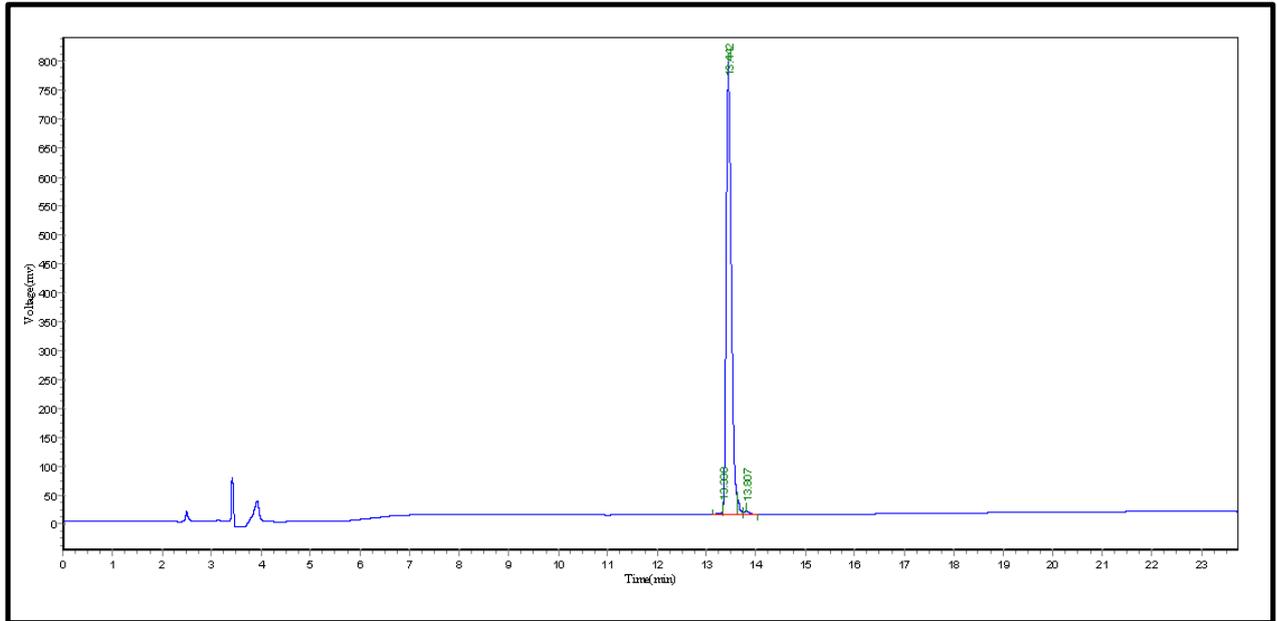


Fig. S3-Peptide 3

HPLC



MS spectrum

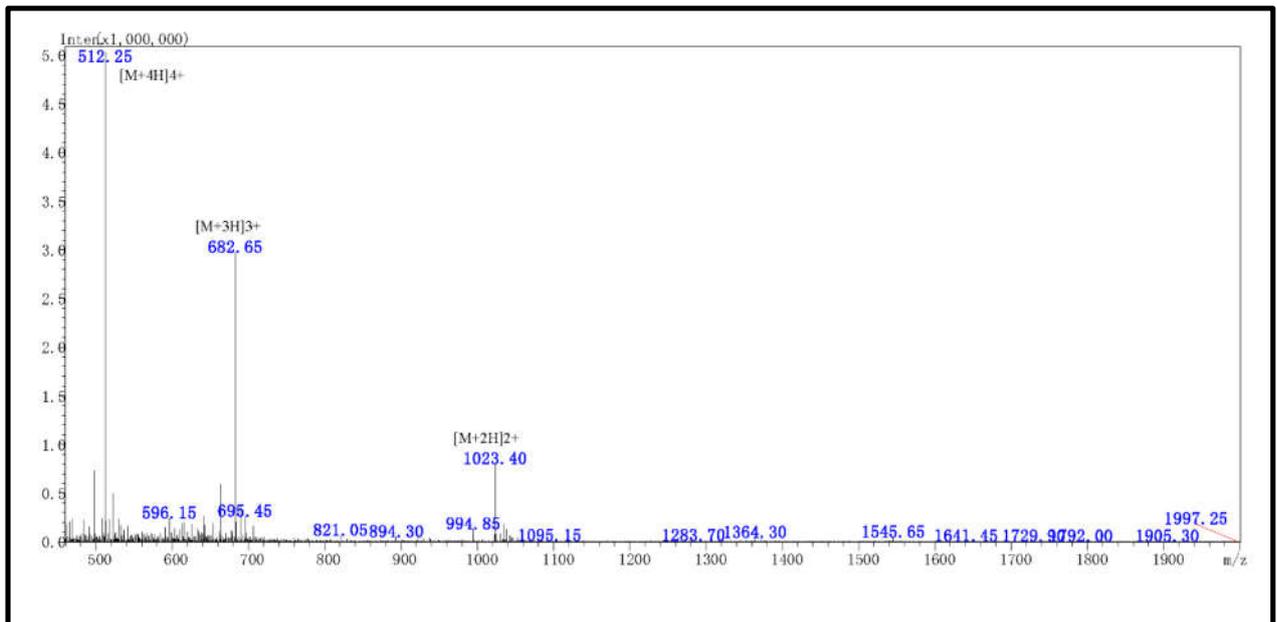
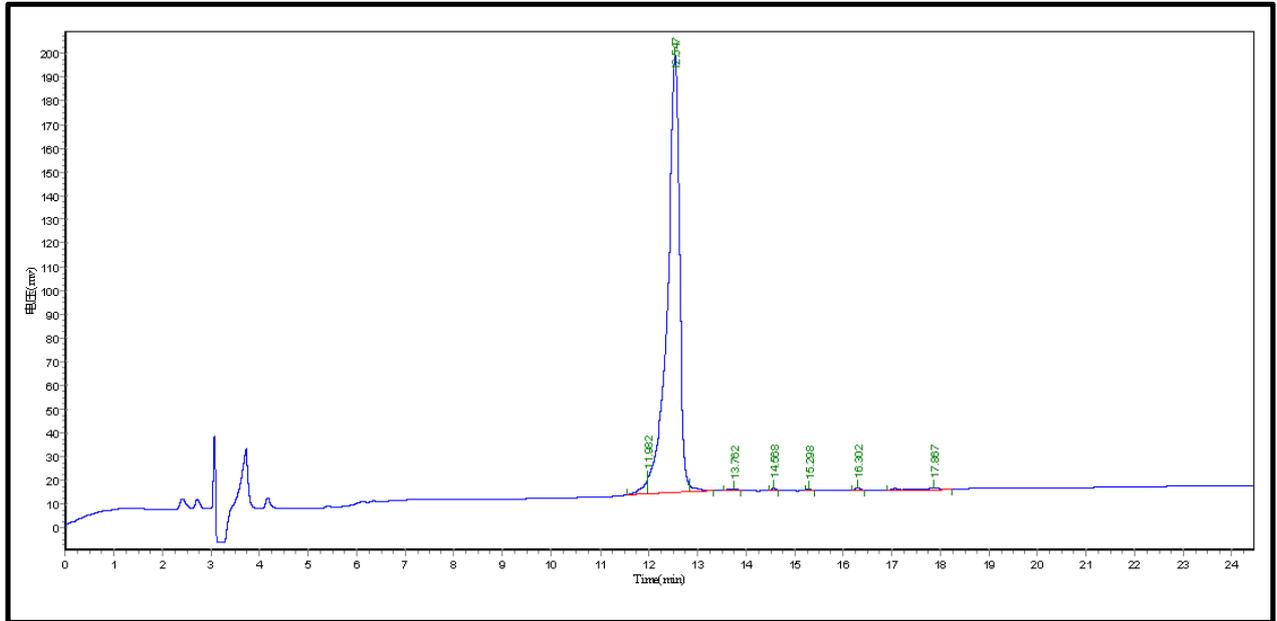


Fig. S4-Peptide 4

HPLC



MS spectrum

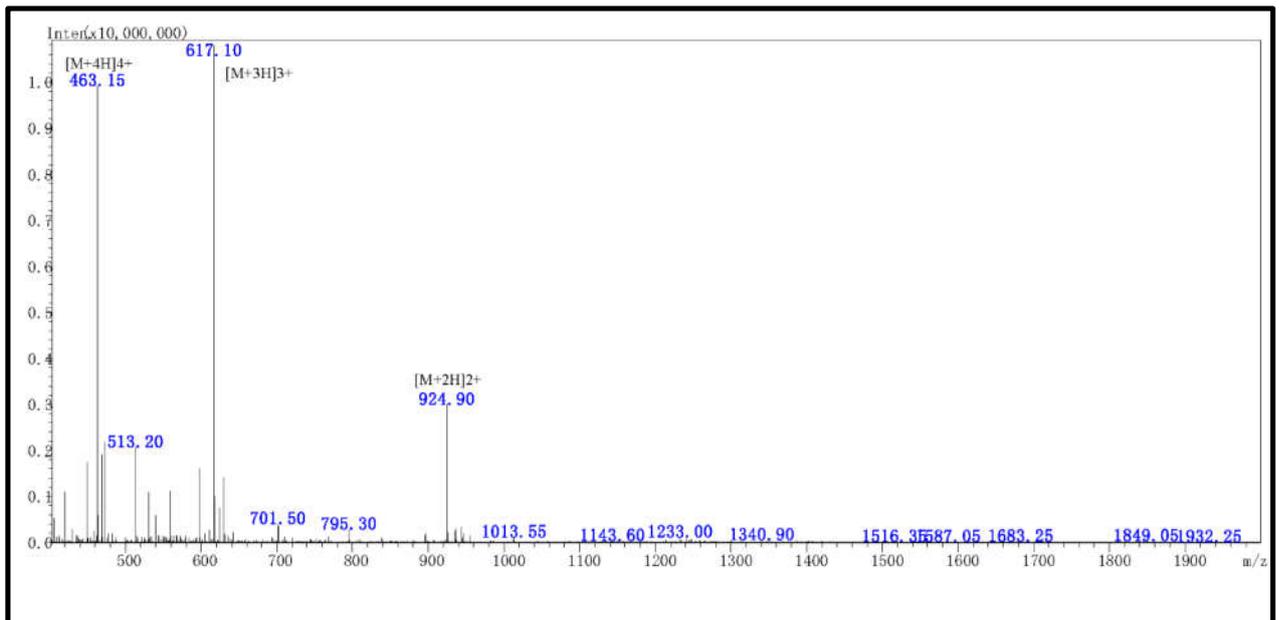
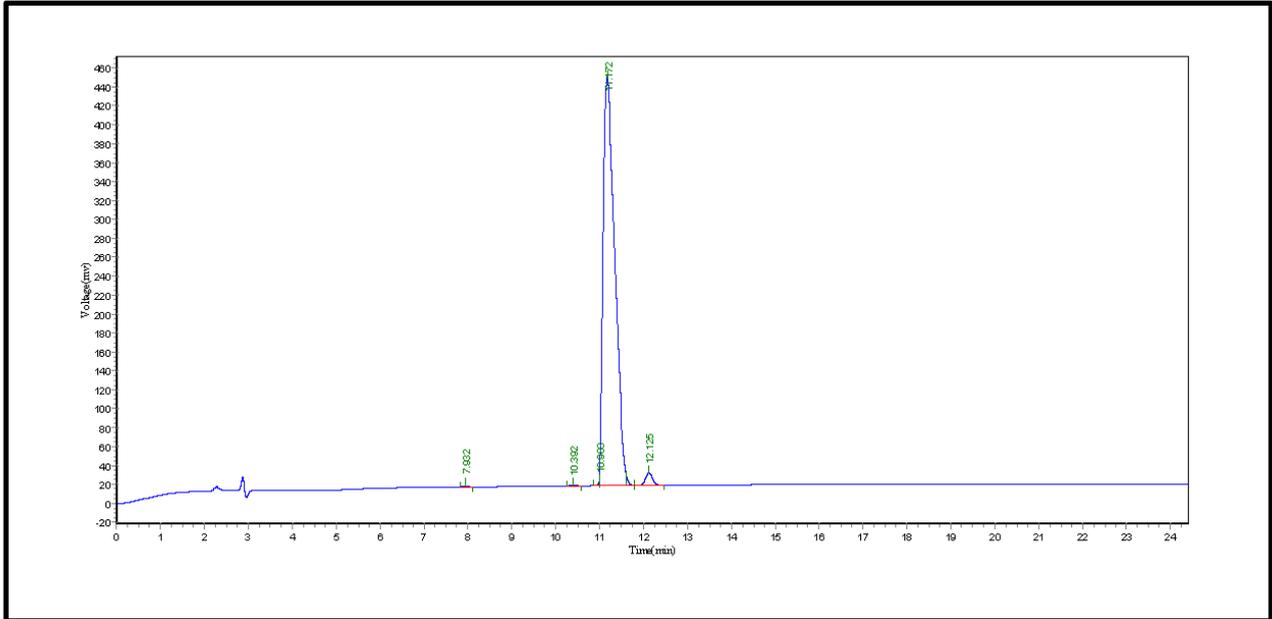


Fig. S5-Peptide 5

HPLC



MS spectrum

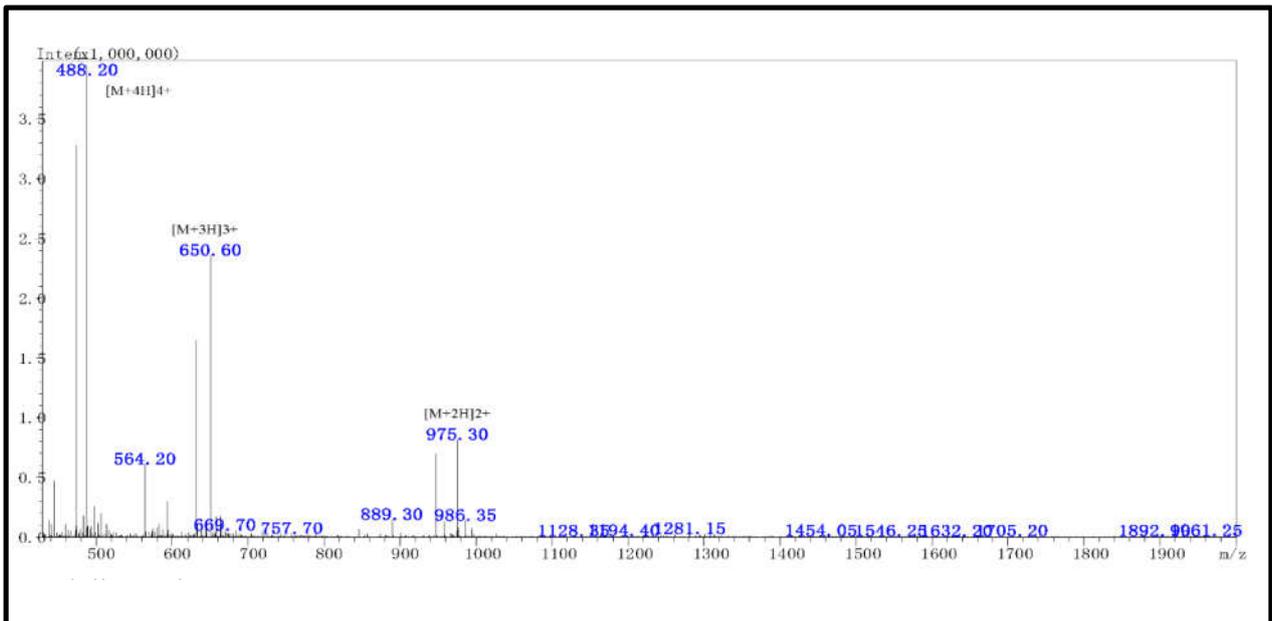
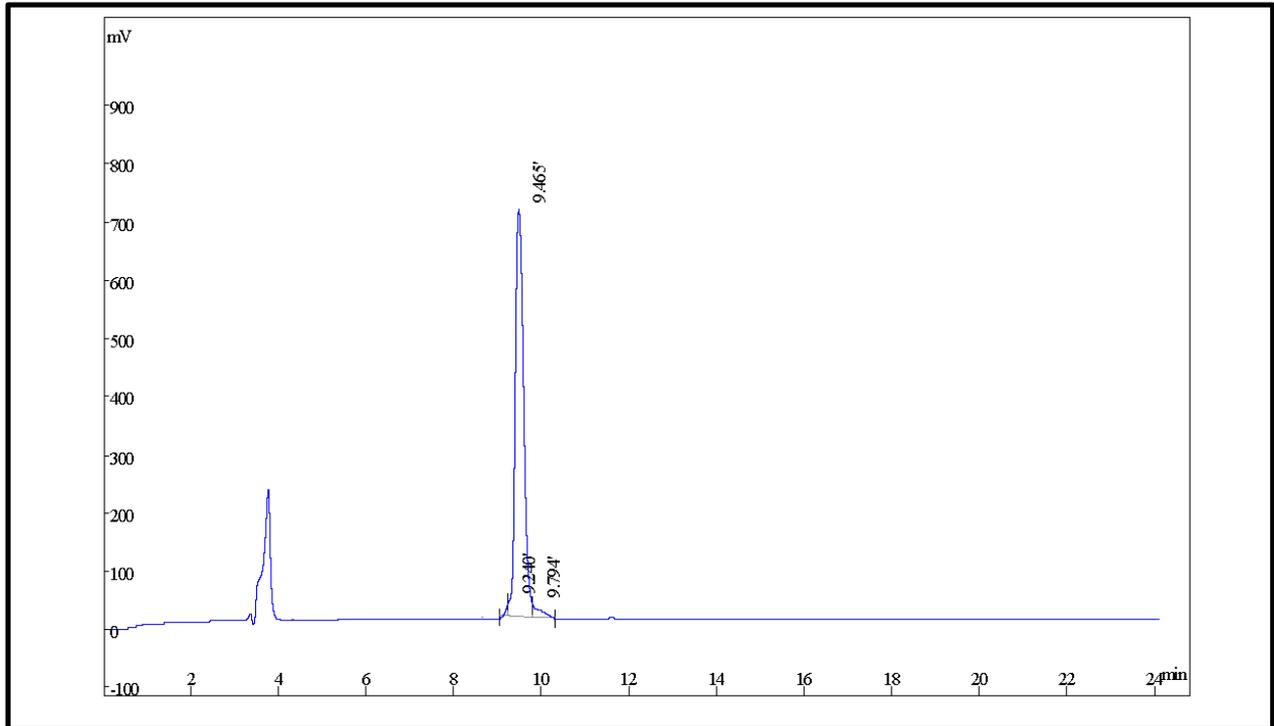


Fig. S6-Peptide 6

HPLC



MS spectrum

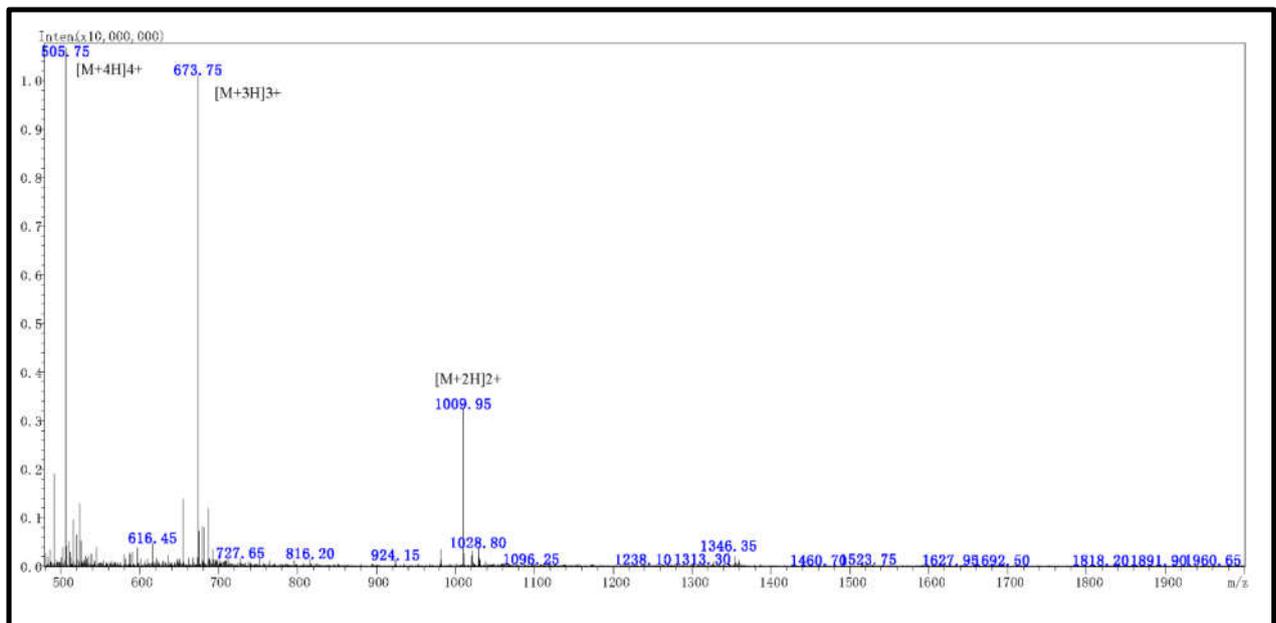
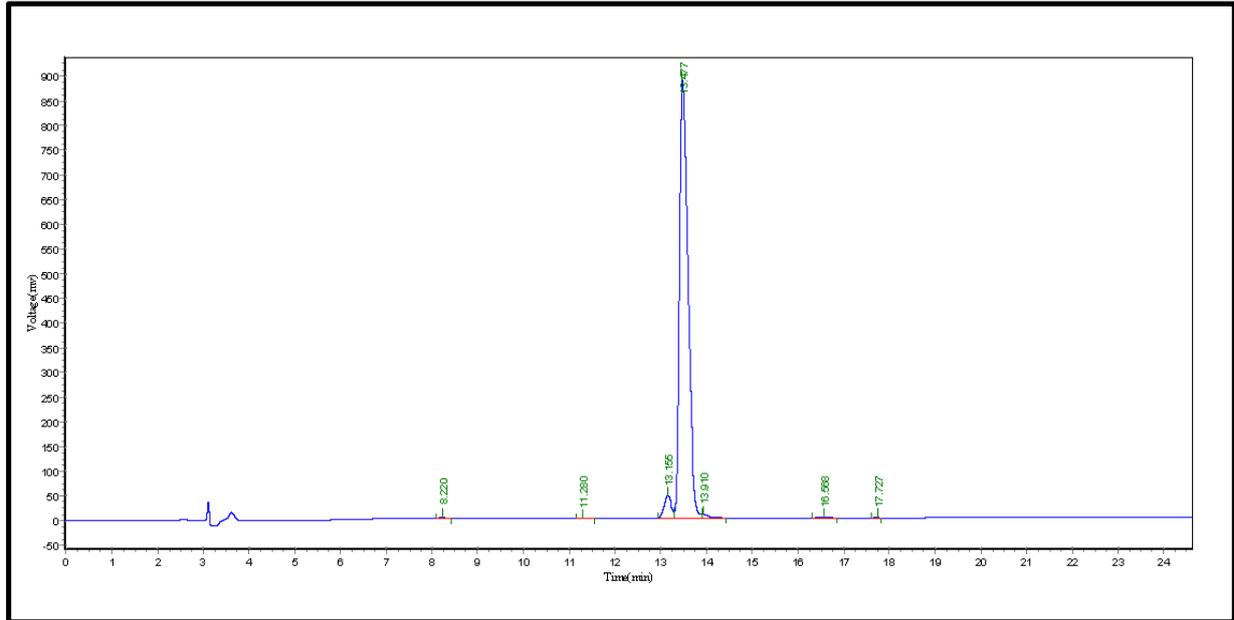


Fig. S7-Peptide 7

HPLC



MS spectrum

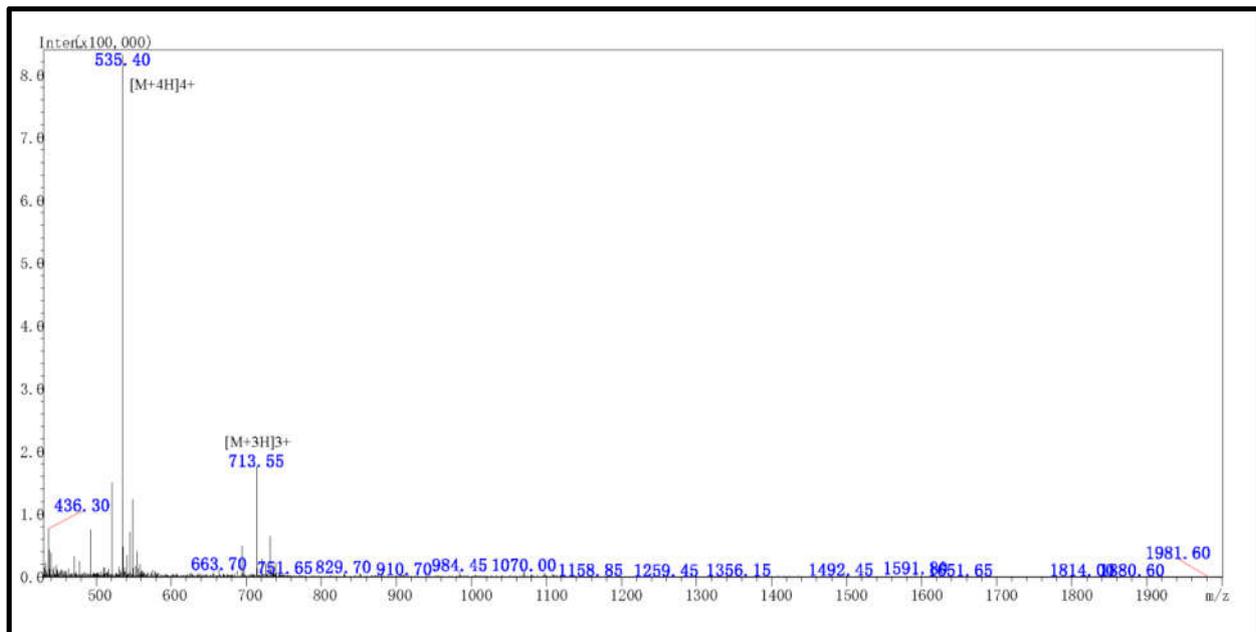
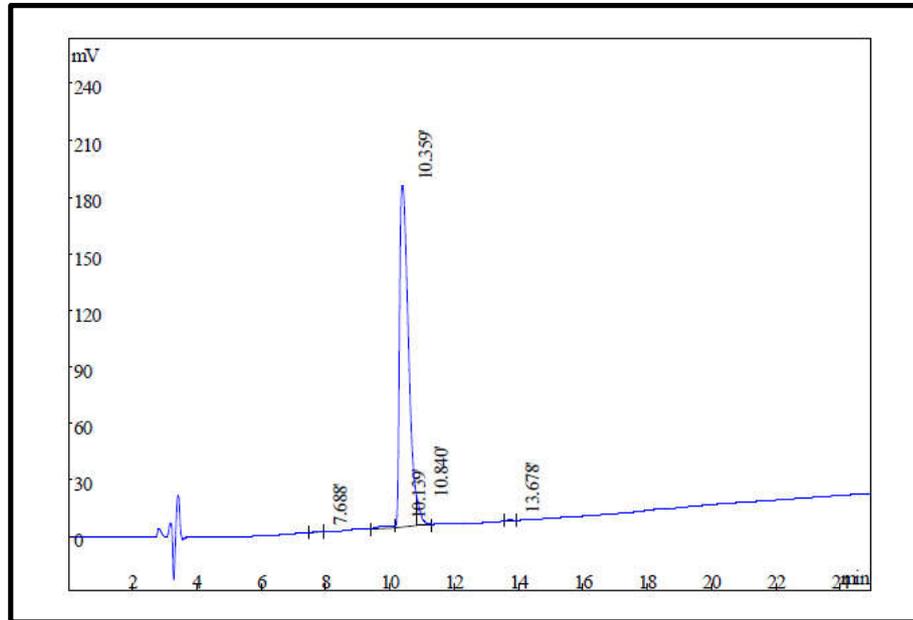


Fig. S8-Peptide 8

HPLC



MS spectrum

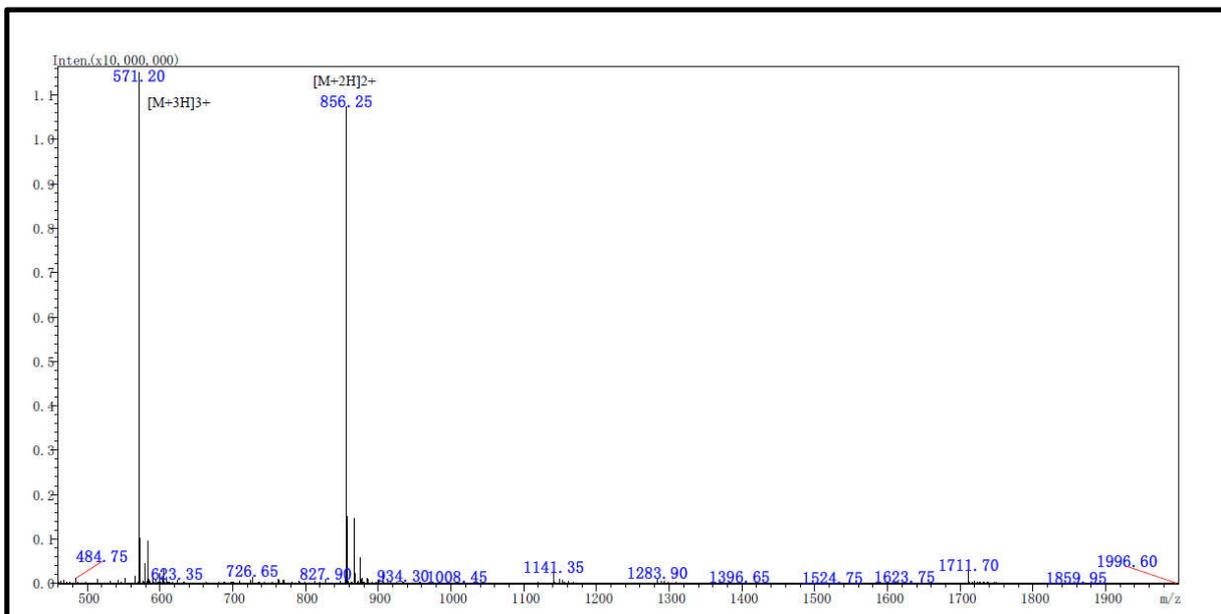


Fig. S9- Protocol of synthesis of peptide 7

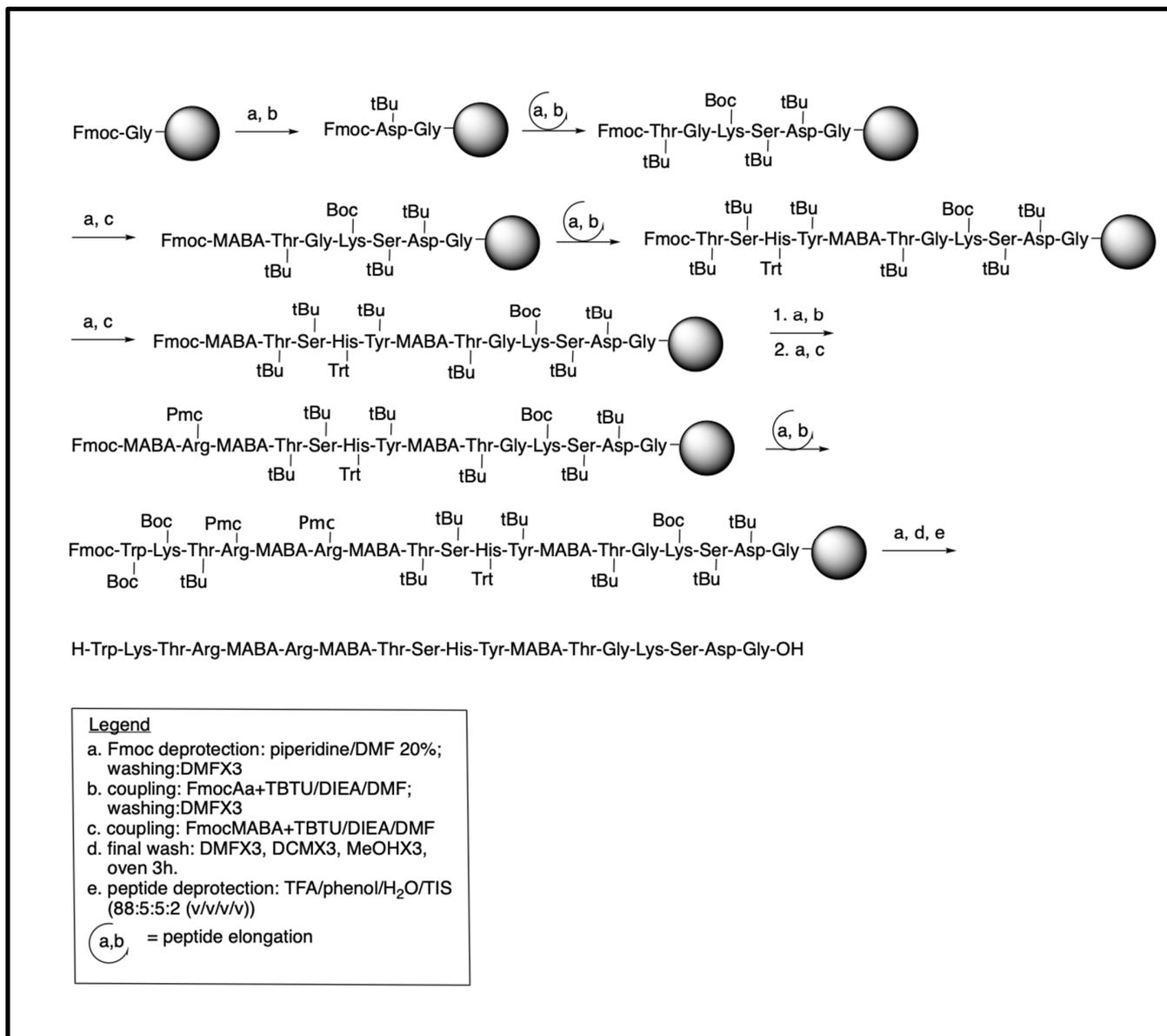


Table S2. Selectivity of the inhibitory effects of MABA-peptide 4 compared to Viperistatin on various integrins in cell adhesion assay.

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 $\lambda_{ex} = 485$ nm and $\lambda_{em} = 530$ 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;

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

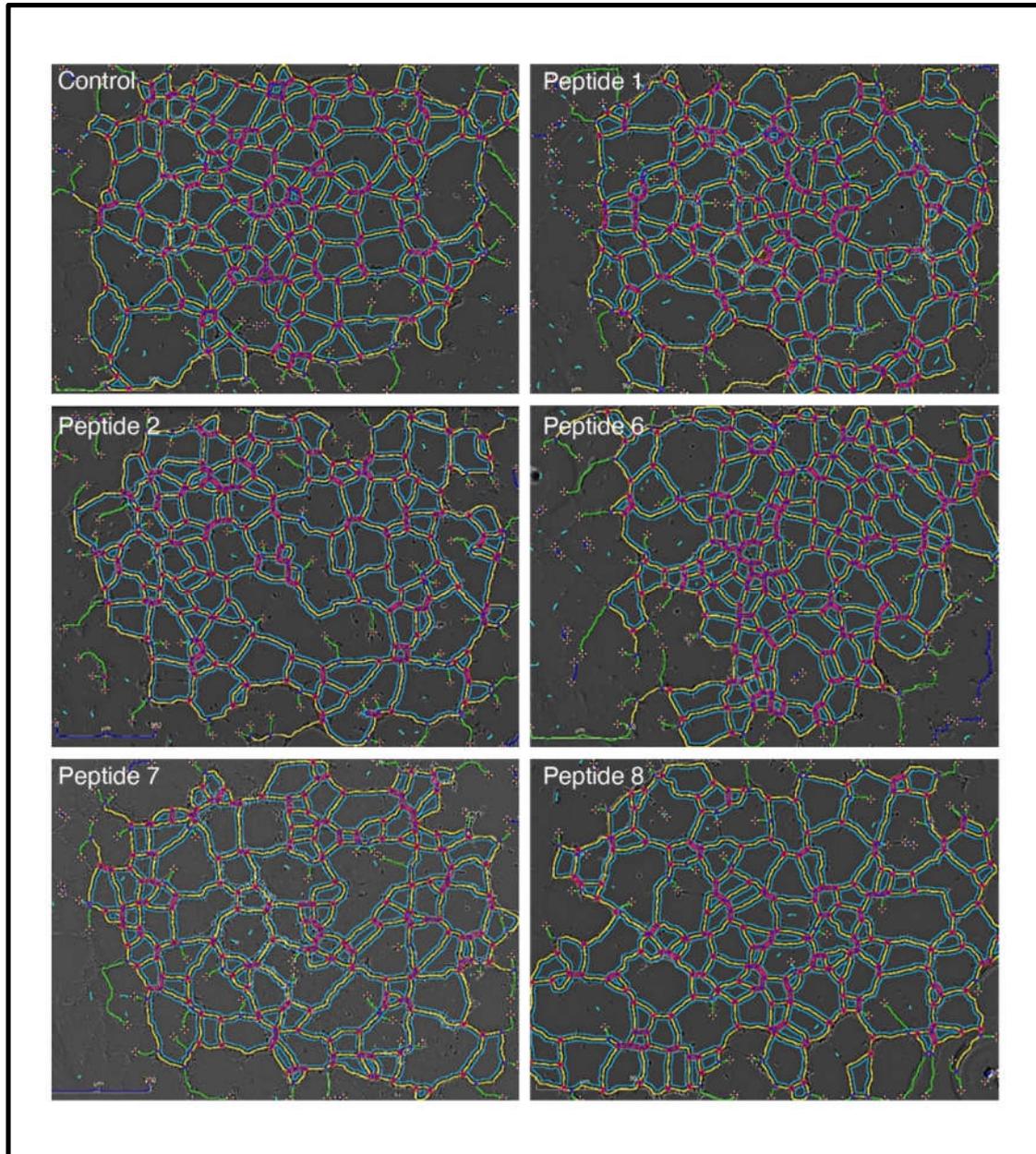


Table S3- Median survival of groups of B16 melanoma mice treated with peptides in comparison to control group of sick mice.

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

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

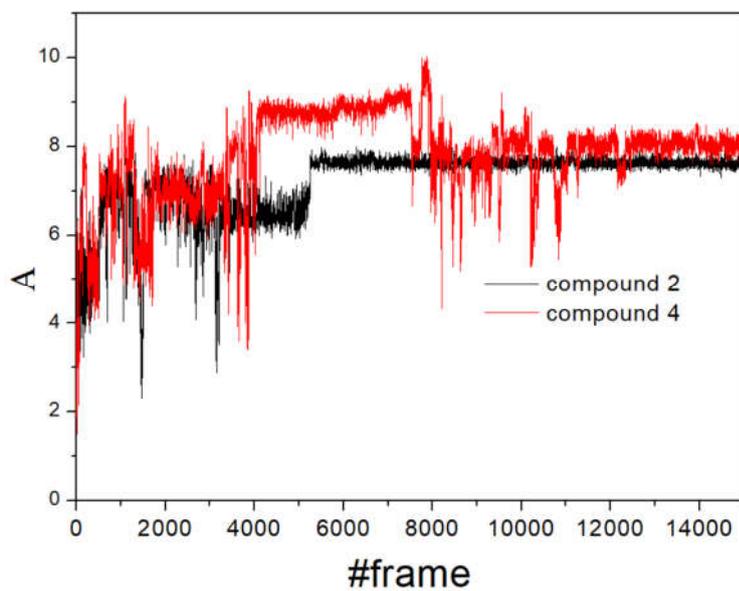


Figure S12- The central structures of the biggest cluster after Molecular Dynamic simulations of compounds 2 (left) and 4 (right). Carbon atoms of compounds 2 and 4 are colored in green and blue respectively.

