

Supporting information

**Enhancing Chemical Stability through Structural Modification of Antimicrobial Peptides with Non-Proteinogenic Amino Acids**

Takahito Ito,<sup>1,2,†</sup> Natsumi Matsunaga,<sup>1,†</sup> Megumi Kurashima,<sup>1</sup> Yosuke Demizu,<sup>1,2,\*</sup> and Takashi Misawa<sup>1,\*</sup>

<sup>1</sup>National Institute of Health Sciences, 3-25-26, Tonomachi, Kawasaki-ku, Kawasaki-shi, Kanagawa 210-9501 Japan

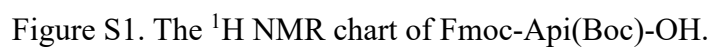
<sup>2</sup>Graduate School of Medical Life Science, Yokohama City University, 1-7-29 Yokohama, Kanagawa 230-0045, Japan

\*Correspondence: misawa@nihs.go.jp (T.M.), demizu@nihs.go.jp (Y. D.); Tel.: +81-44-270-6580 (T.M.)

<sup>†</sup> The authors contributed equally to this work.

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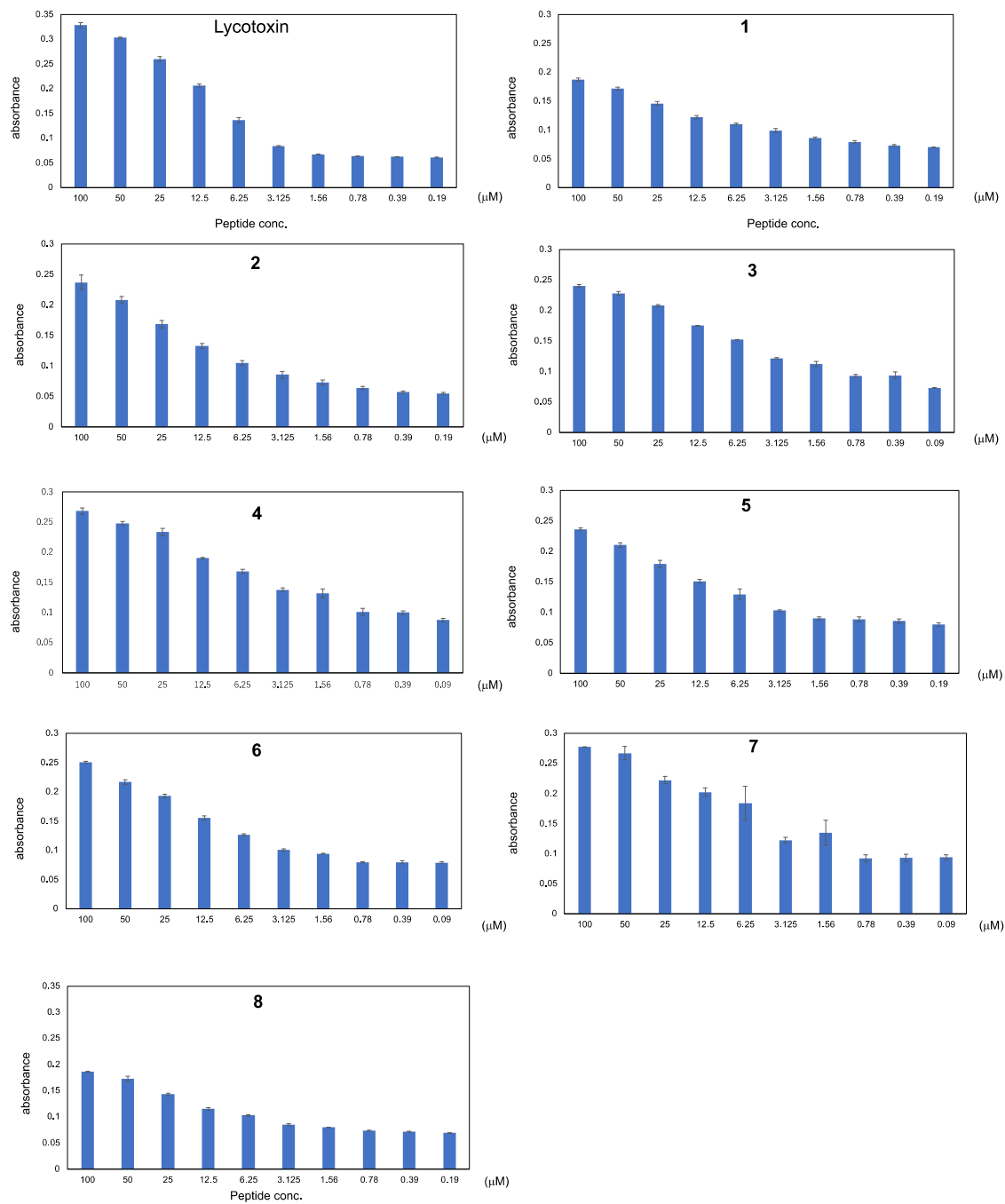
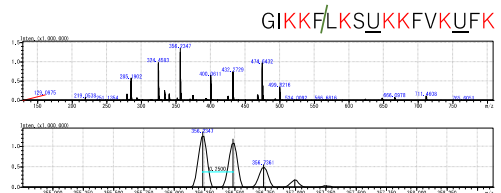
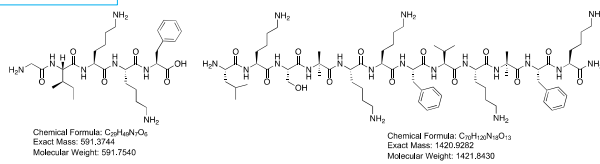
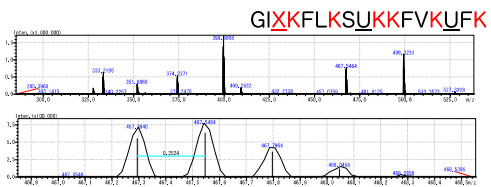
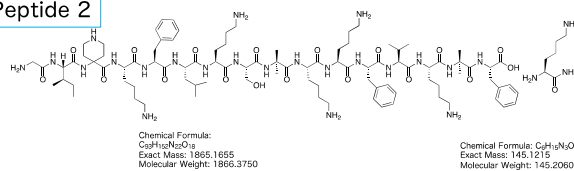


Figure S2. The hemolytic activity of Lycotoxin and peptides 1-8. The human red blood cells were treated with indicated concentration of each peptide.

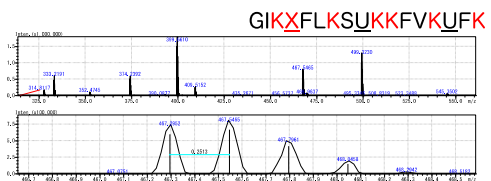
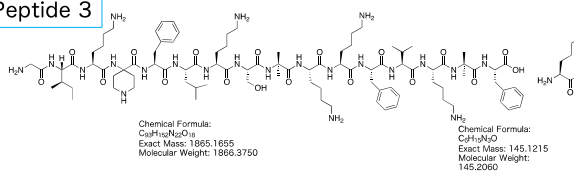
### Peptide 1



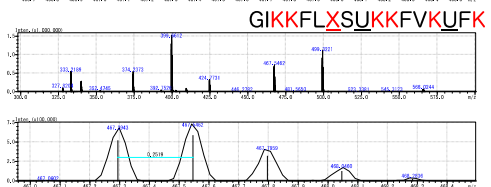
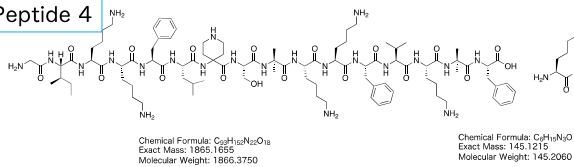
### Peptide 2

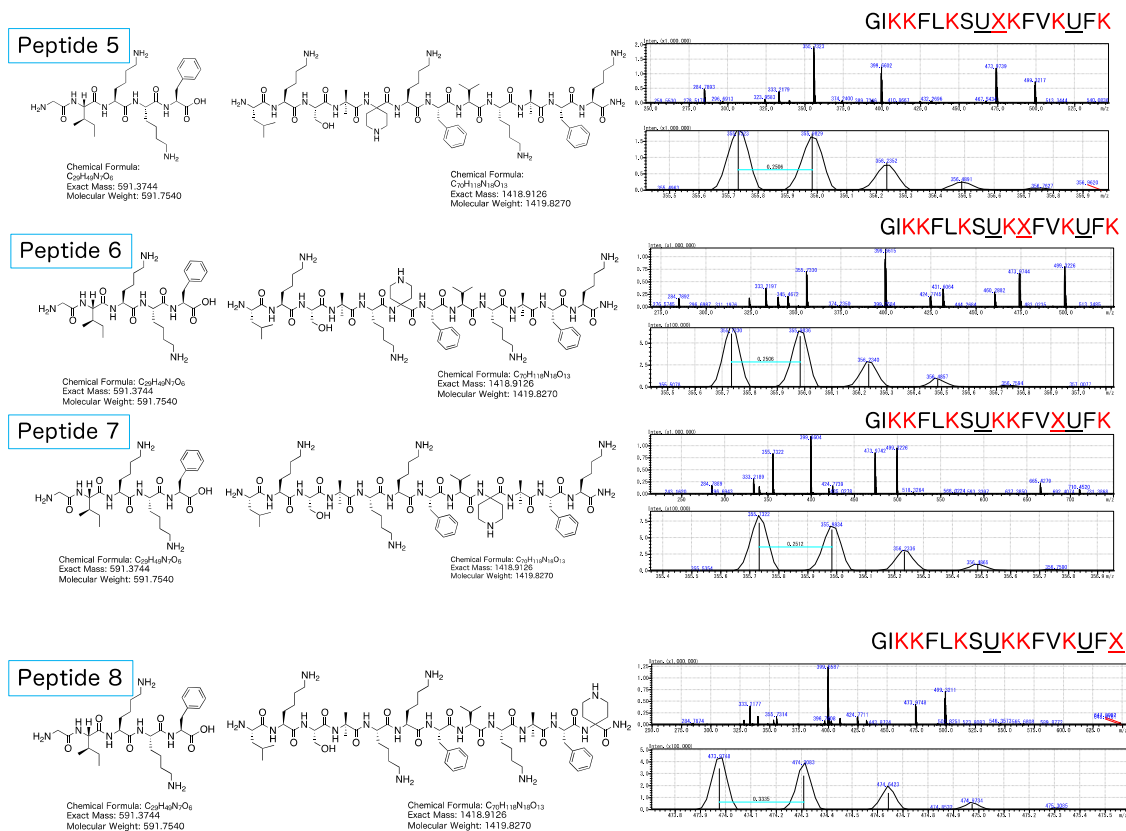


### Peptide 3



### Peptide 4





**Figure S3.** The digestion assay of **peptides 2-8**. The LC/MS analysis of the peptide fragments by proteinase K at 37 °C after 24 hour incubation.

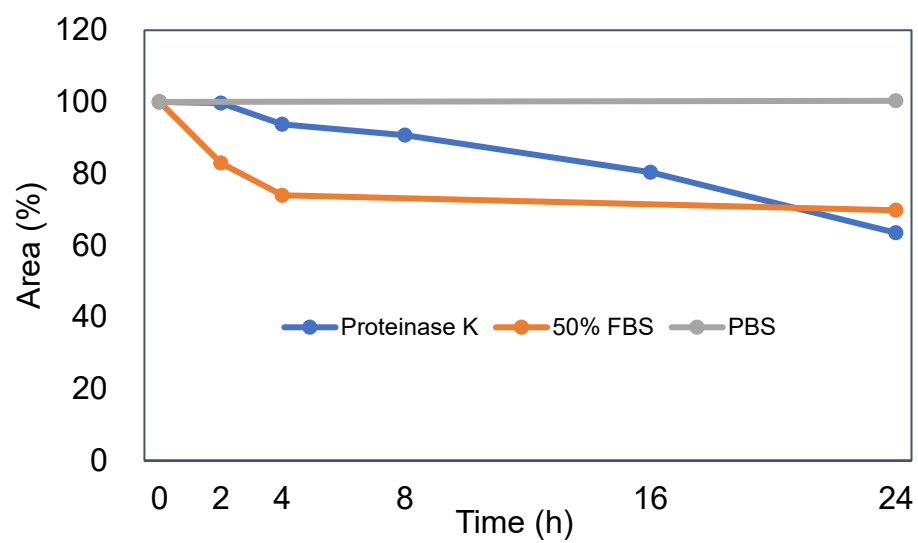


Figure S4. The chemical stability of peptide **2** against PBS, proteinase K, and 50% FBS. The remains of peptide **2** were evaluated by HPLC analysis.

## HPLC analytical method

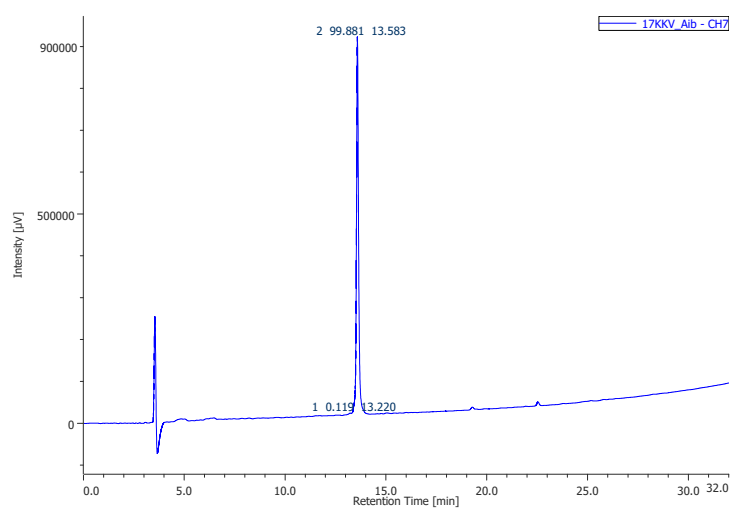
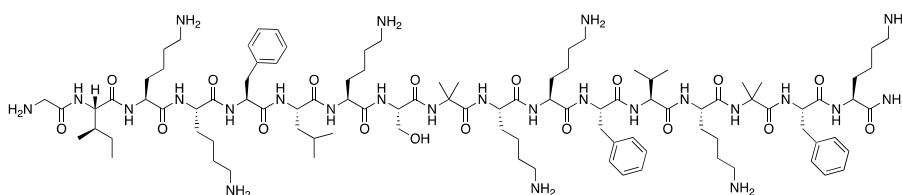
Column	: Preparative column	XBridge BEH C18 OBD Prep Column 5 $\mu$ m 19 $\times$ 250 mm (Waters)
	Analysis column	Inertsil WP300 C18 5 $\mu$ M 150 $\times$ 3.0 mm (GL Sciences)
Conditions	: solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH <sub>3</sub> CN	
Flow rate	: preparation	10 mL/min
	analysis	1.0 mL/min
Gradient	: 10–90% gradient of solvent B	30 min



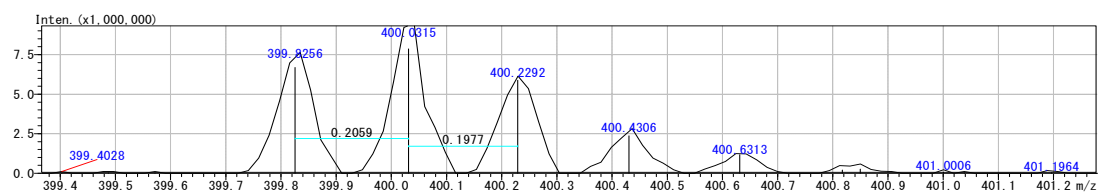
## Pep-1

H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Aib-Lys-Lys-Phe-Val-Lys-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 99.9%



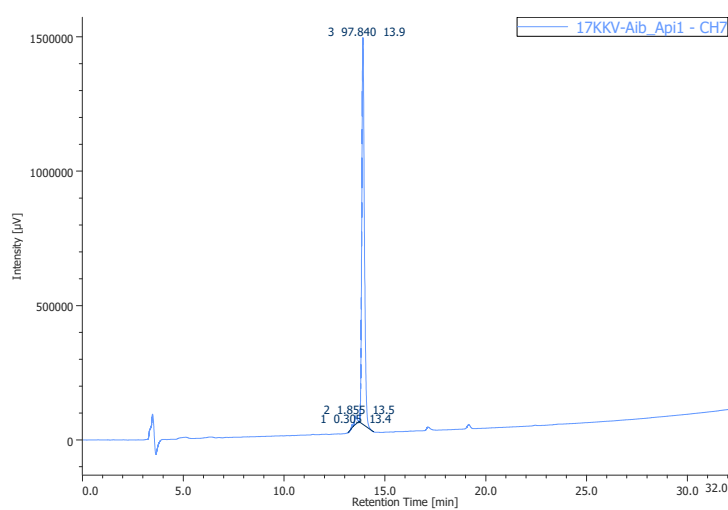
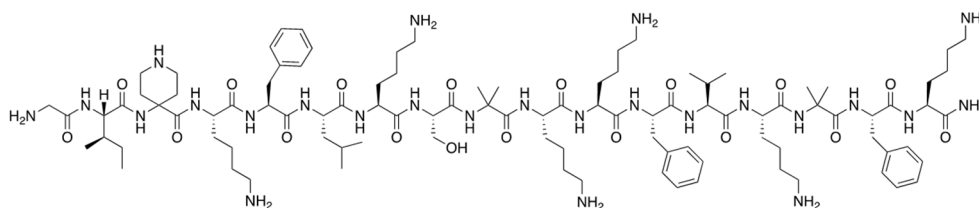
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>167</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.8584, found: 399.8256.



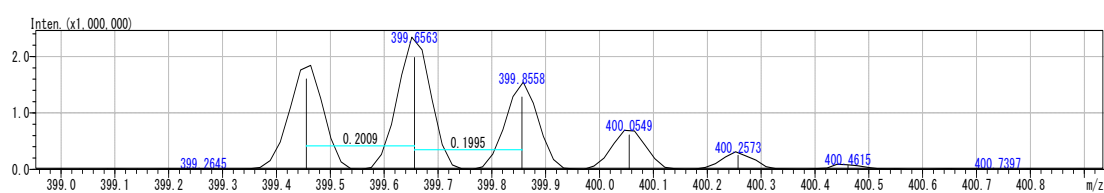
## Pep-2

H-Gly-Ile-Api-Lys-Phe-Leu-Lys-Ser-Aib-Lys-Lys-Phe-Val-Lys-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 97.8%



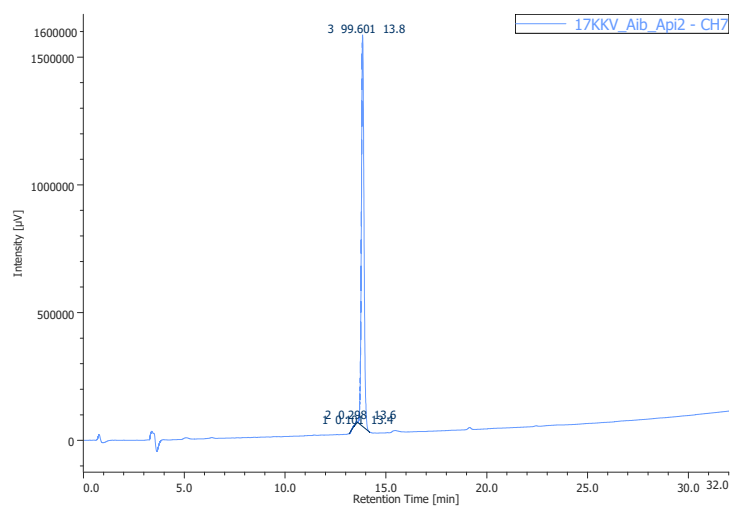
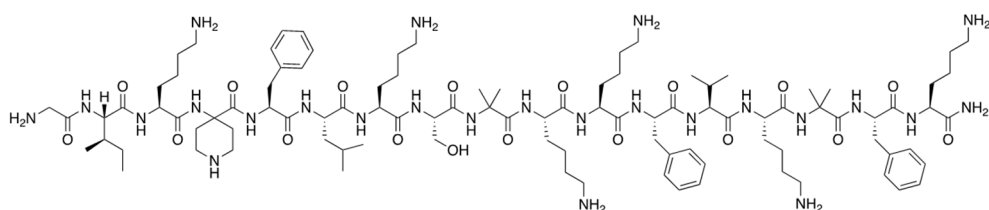
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.4553, found: 399.4554



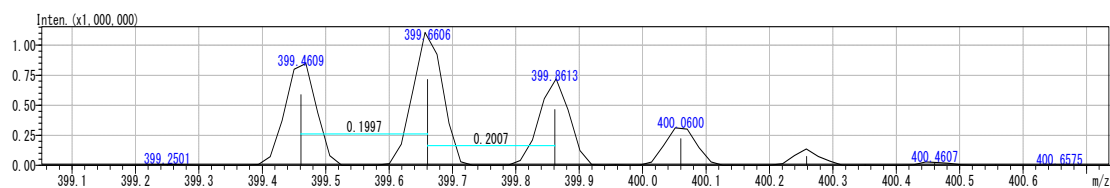
### Pep-3

H-Gly-Ile-Lys-Api-Phe-Leu-Lys-Ser-Aib-Lys-Lys-Phe-Val-Lys-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 99.6%



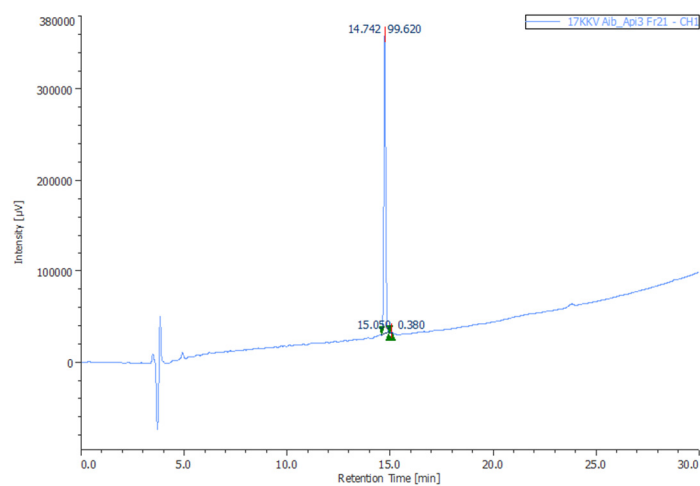
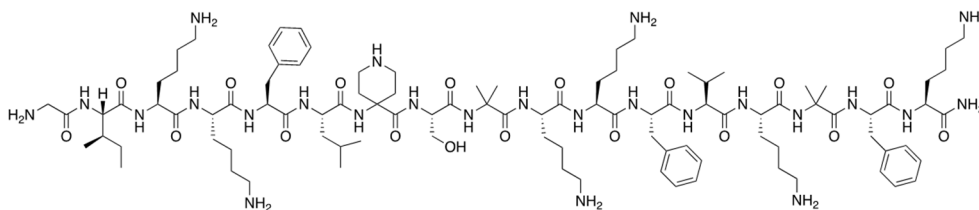
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.4553, found: 399.4609.



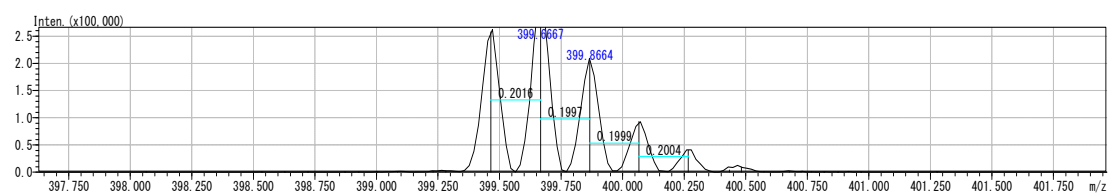
## Pep-4

H-Gly-Ile-Lys-Lys-Phe-Leu-Api-Ser-Aib-Lys-Lys-Phe-Val-Lys-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 99.6%



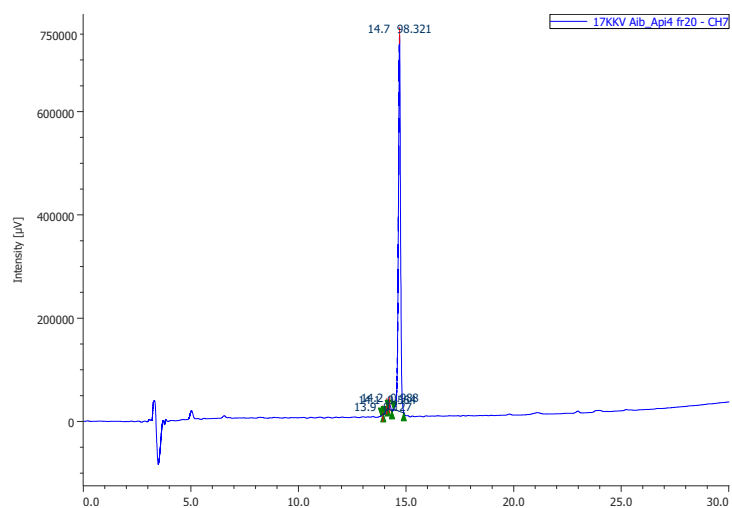
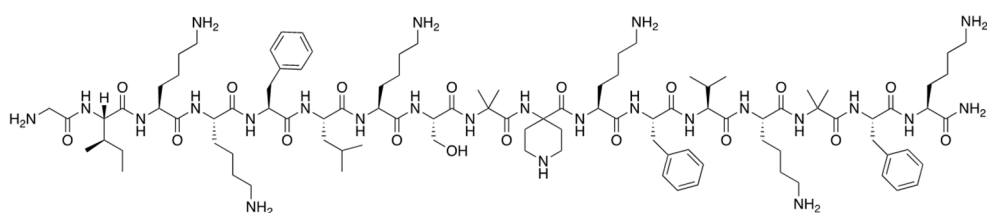
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup>: 399.4553, found: 399.4651.



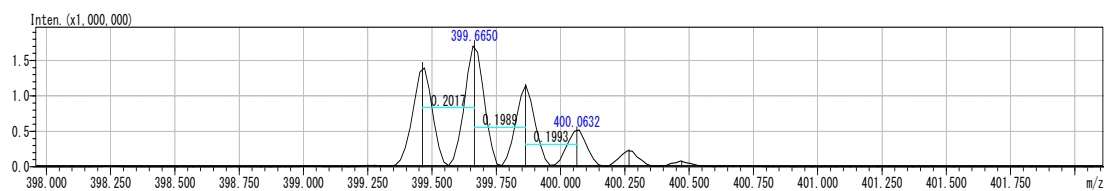
## Pep-5

H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Aib-Api-Lys-Phe-Val-Lys-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 98.3 %



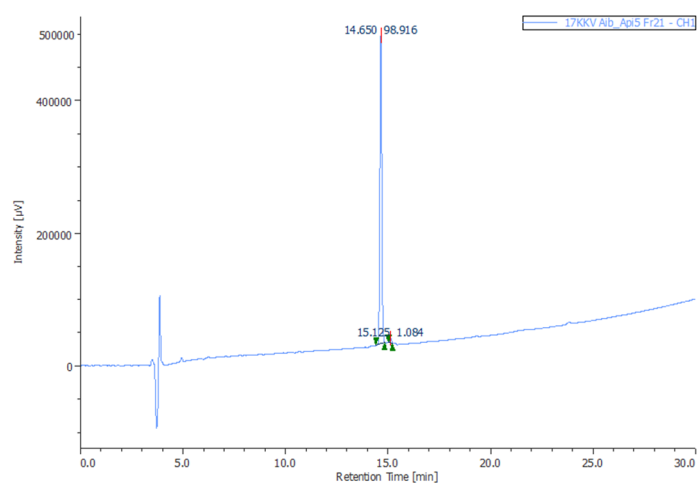
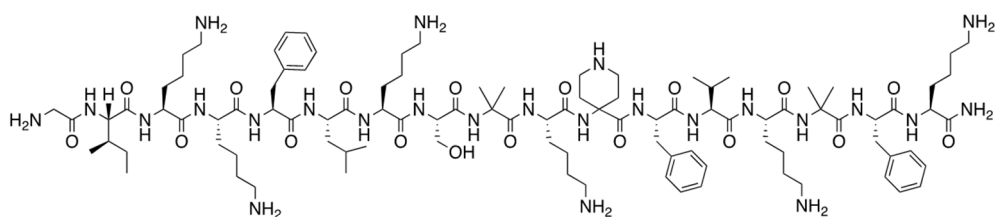
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.4553, found: 399.4633.



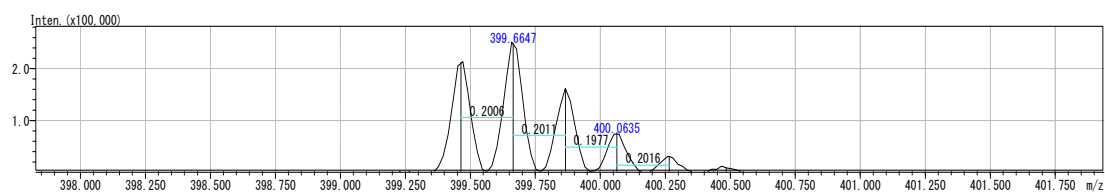
## Pep-6

H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Aib-Lys-Api-Phe-Val-Lys-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 98.9%



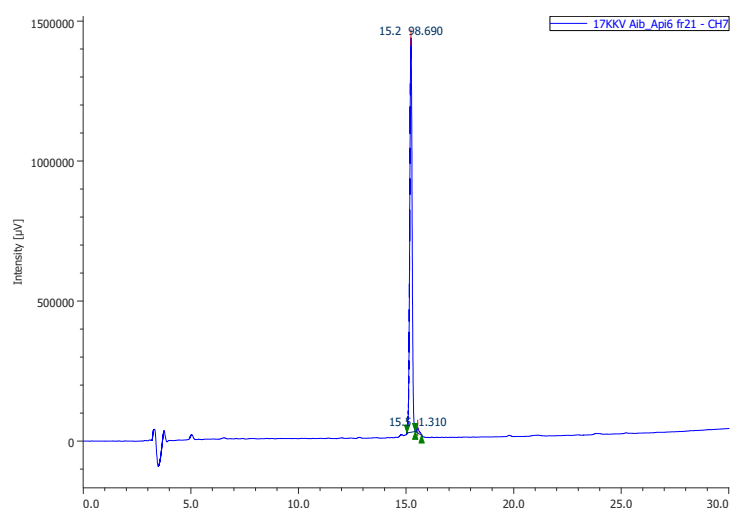
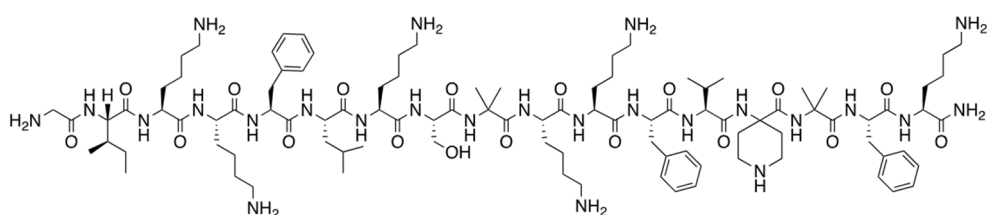
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.4553, found: 399.4641.



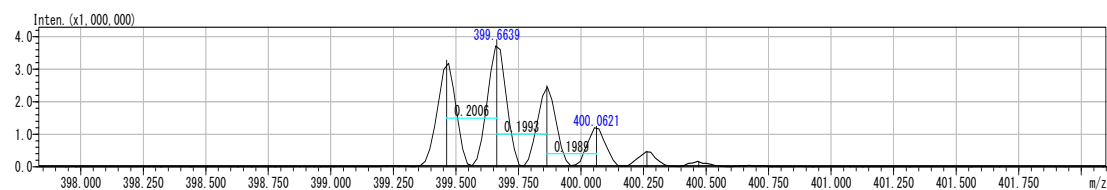
# Pep-7

H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Aib-Lys-Lys-Phe-Val-Api-Aib-Phe-Lys-NH<sub>2</sub>

Purity : 98.7%



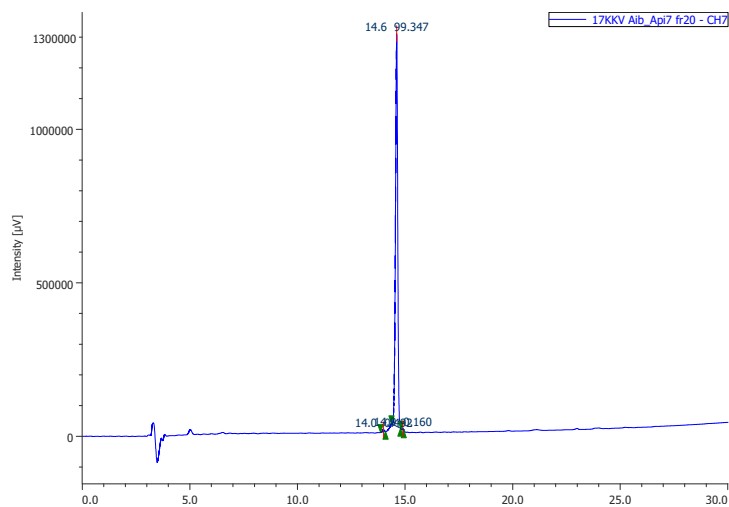
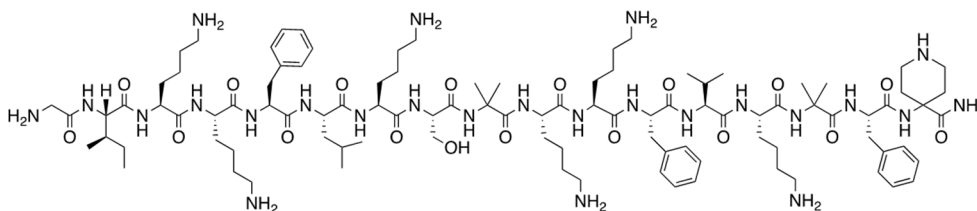
LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.45528, found: 399.4633.



# Pep-8

H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Aib-Lys-Lys-Phe-Val-Lys-Aib-Phe-Api-NH<sub>2</sub>

Purity : 99.3%



LC-MS (ESI<sup>+</sup>) calcd for C<sub>99</sub>H<sub>165</sub>N<sub>25</sub>O<sub>18</sub>[M+5H]<sup>5+</sup> : 399.45528, found: 399.4633.

