

Development of Antimicrobial Stapled Peptides Based on Magainin 2 Sequence

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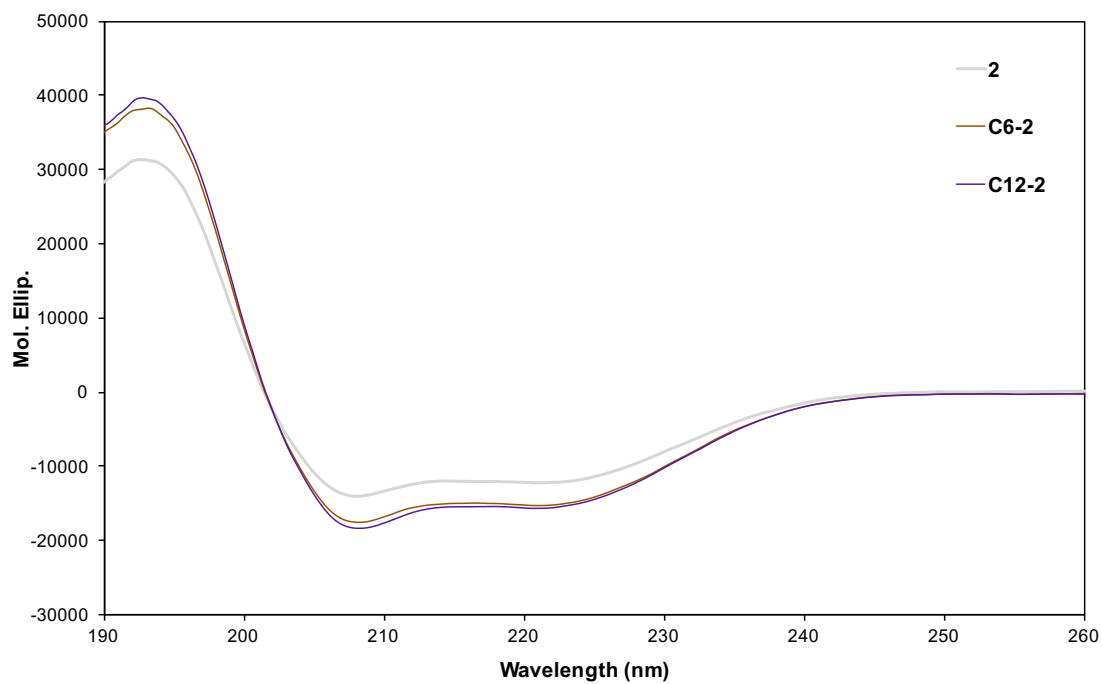


Figure S1. Preferred secondary structure of peptide **2**, **C6-2**, and **C12-2** in pH = 7.4 PBS solution with 1% SDS. Peptide concentration = 100 μ M.

Mag2

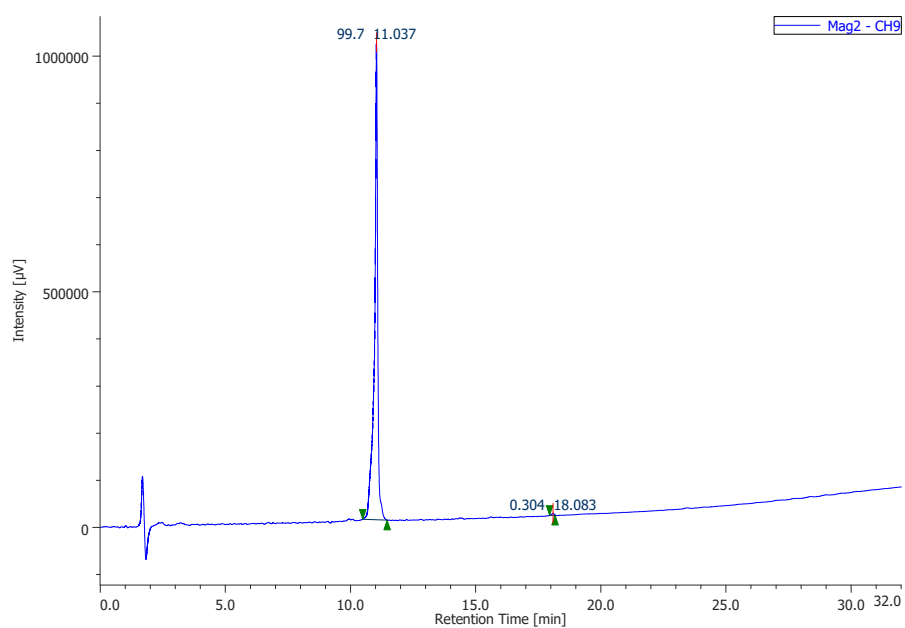
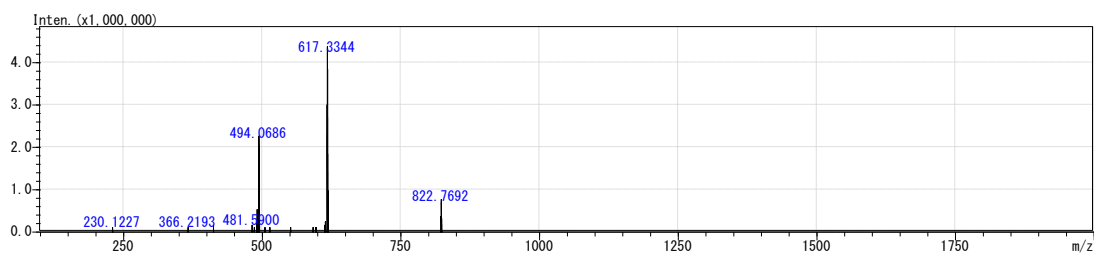
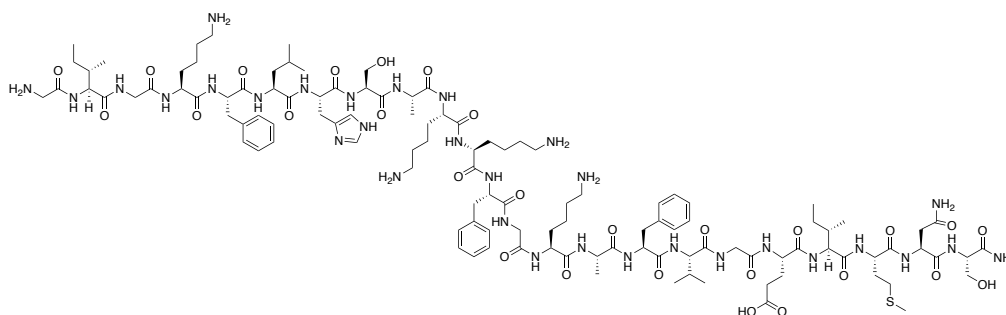
H-Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 99.7%

HRMS (ESI⁺) calcd for C₁₁₄H₁₈₁N₃₁O₂₈S [M+4H]⁴⁺: 617.0933; found: 617.0840



Peptide 1

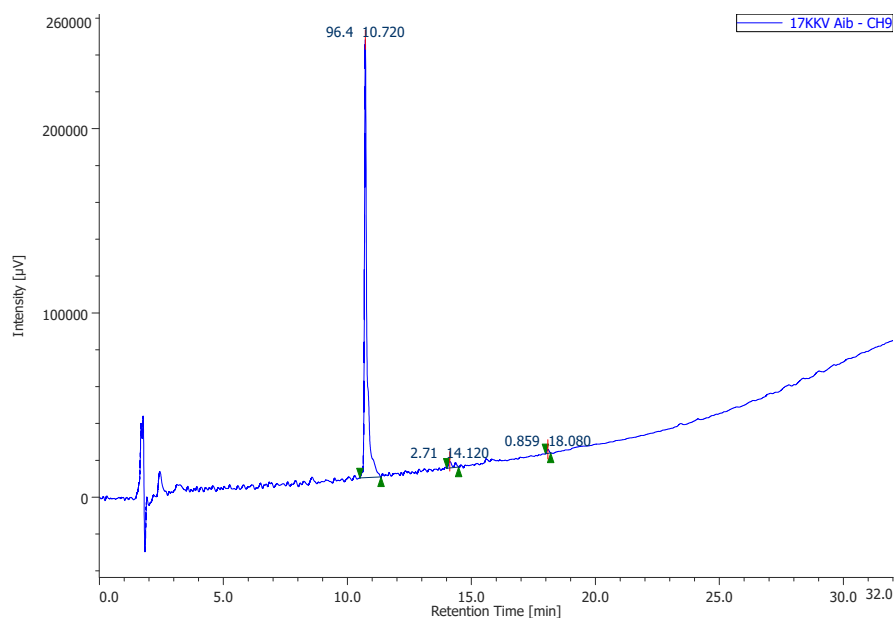
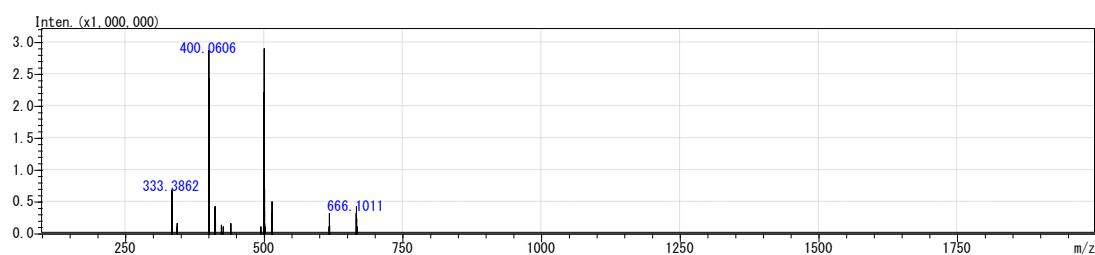
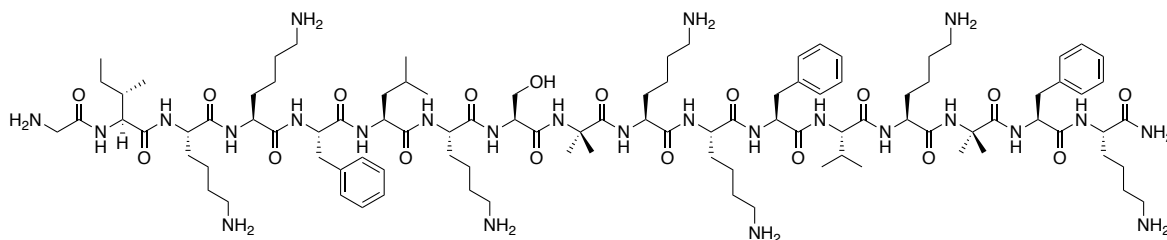
H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Aib-Lys-Lys-Phe-Val-Lys-Aib-Phe-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 96.4%

HRMS (ESI⁺) calcd for C₉₉H₁₆₇N₂₅O₁₈ [M+5H]⁵⁺: 399.8662; found: 399.8595



Peptide 2

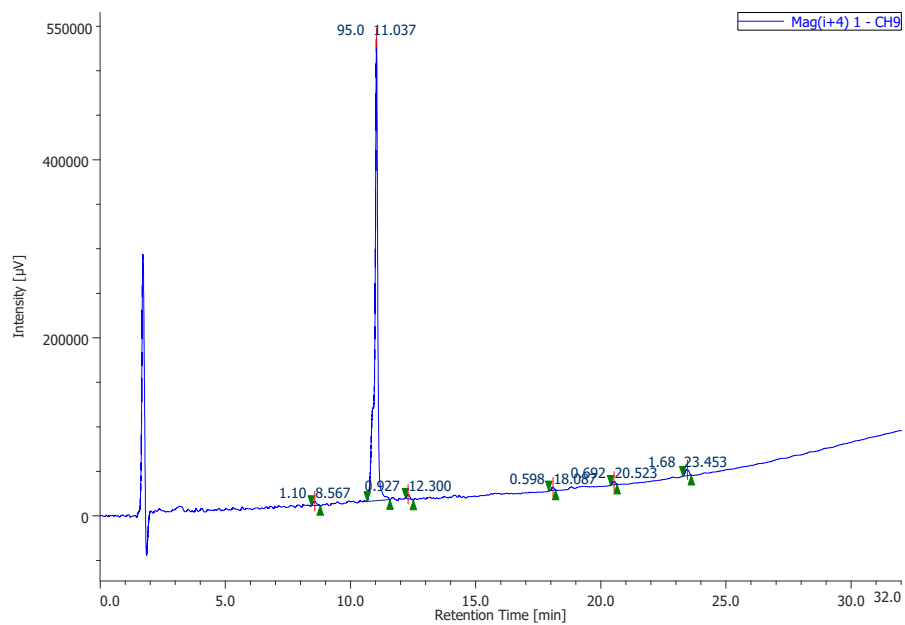
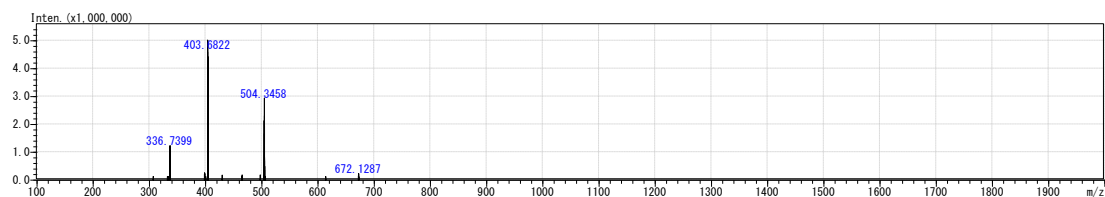
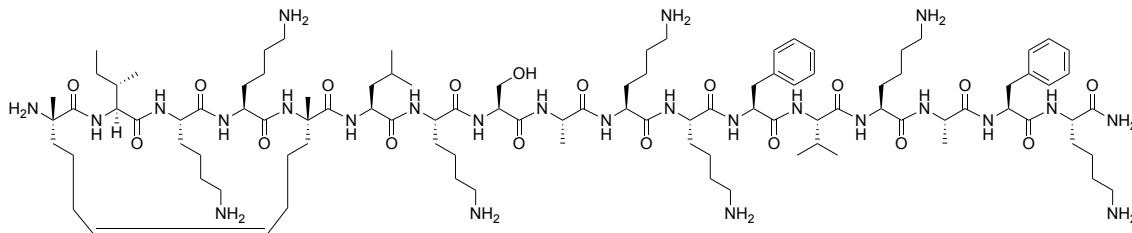
H-S₃-Ile-Lys-Lys-S₃-Leu-Lys-Ser-Ala-Lys-Lys-Phe-Val-Lys-Ala-Phe-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 95.0%

HRMS (ESI⁺) calcd for C₁₀₀H₁₇₃N₂₅O₁₈ [M+5H]⁵⁺: 403.4756; found: 403.4797



Peptide 3

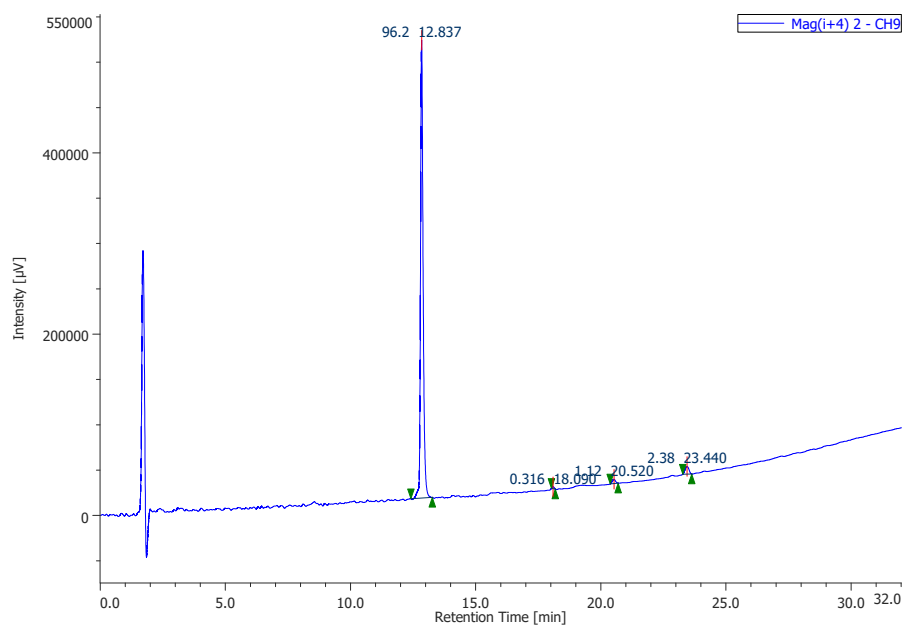
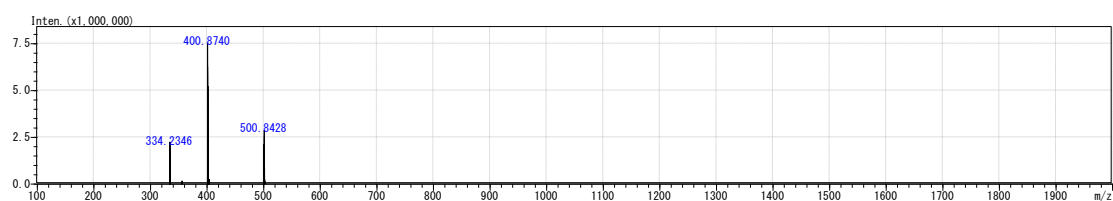
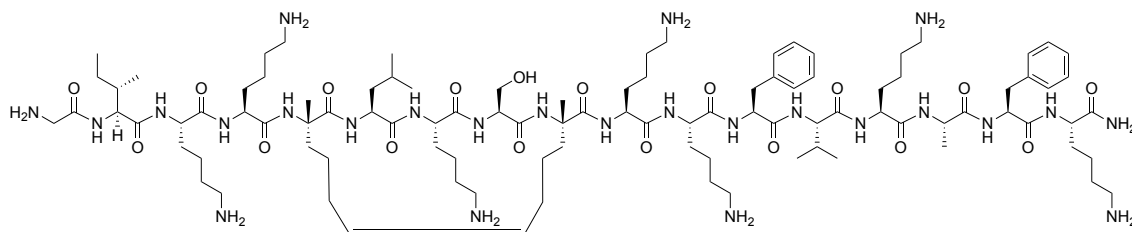
H-Gly-Ile-Lys-Lys-S₅-Leu-Lys-Ser-S₅-Lys-Lys-Phe-Val-Lys-Ala-Phe-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 96.2%

HRMS (ESI⁺) calcd for C₉₉H₁₇₁N₂₅O₁₈ [M+5H]⁵⁺: 400.6725; found: 400.6795



Peptide **4** (E/Z isomers were detected)

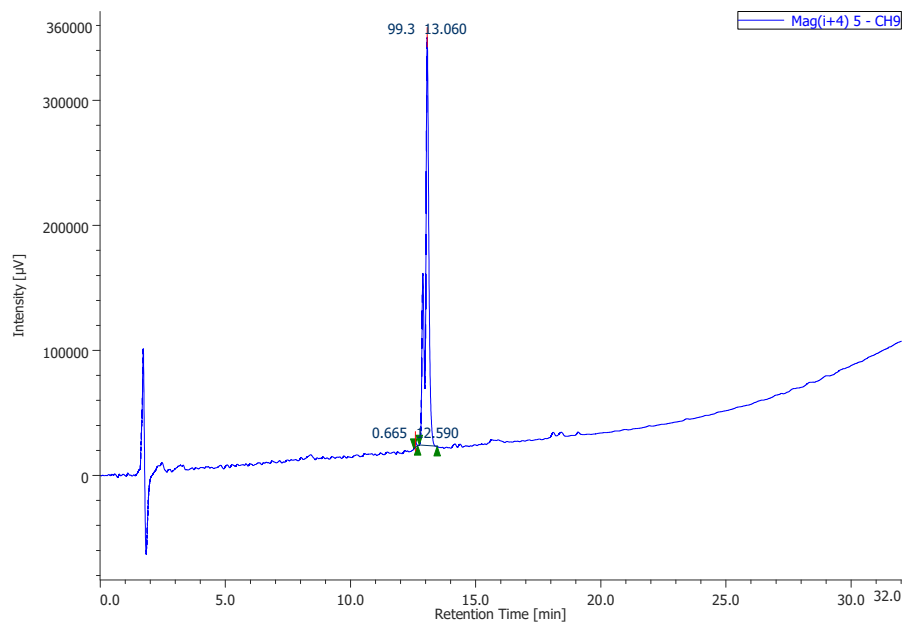
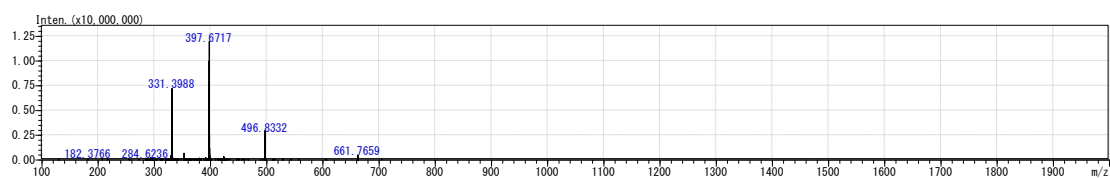
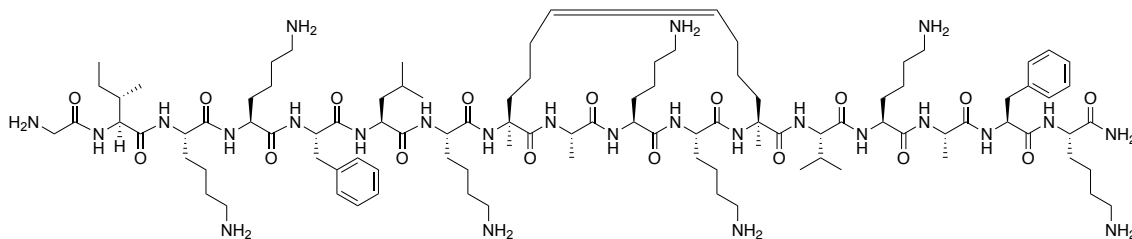
H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-S_ε-Ala-Lys-Lys-S_ε-Val-Lys-Ala-Phe-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 99.3%

HRMS (ESI⁺) calcd for C₉₉H₁₇₁N₂₅O₁₇ [M+5H]⁵⁺: 397.4735; found: 397.4748



Peptide 5

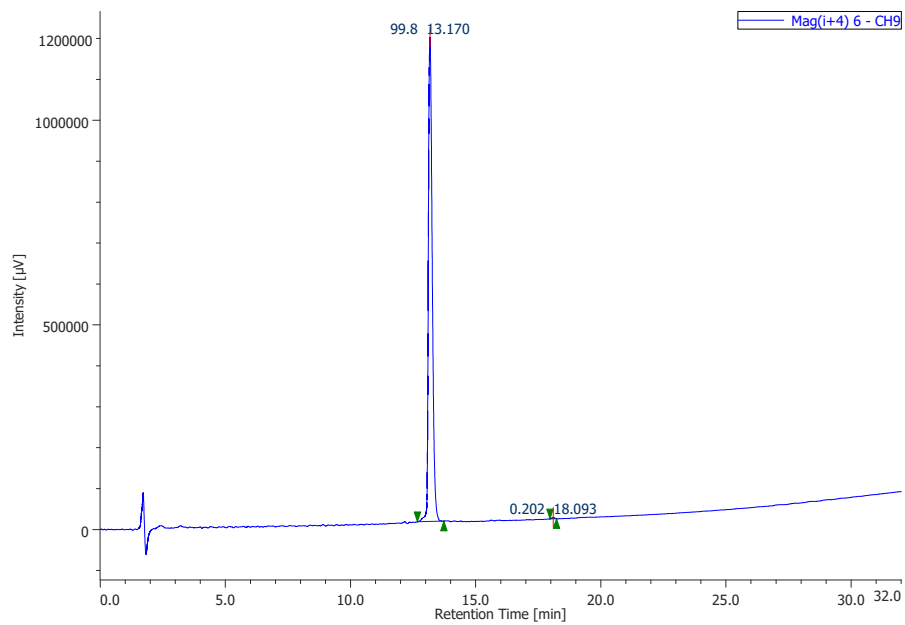
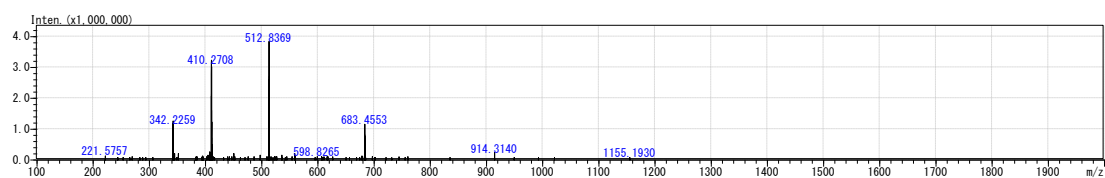
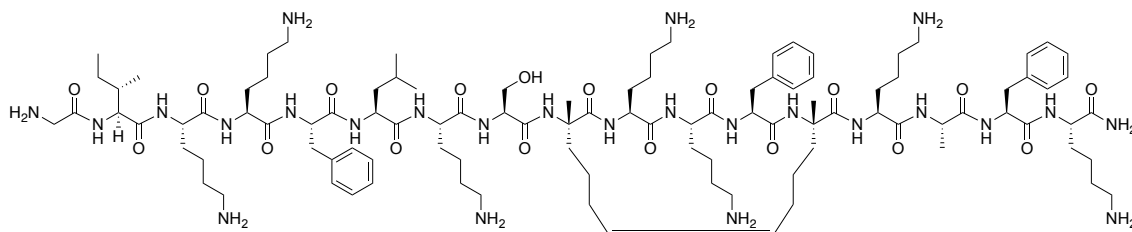
H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-S₃-Lys-Lys-Phe-S₃-Lys-Ala-Phe-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 99.8%

HRMS (ESI⁺) calcd for C₁₀₃H₁₇₁N₂₅O₁₈ [M+4H]⁴⁺: 512.5887; found: 512.5864



Peptide 6

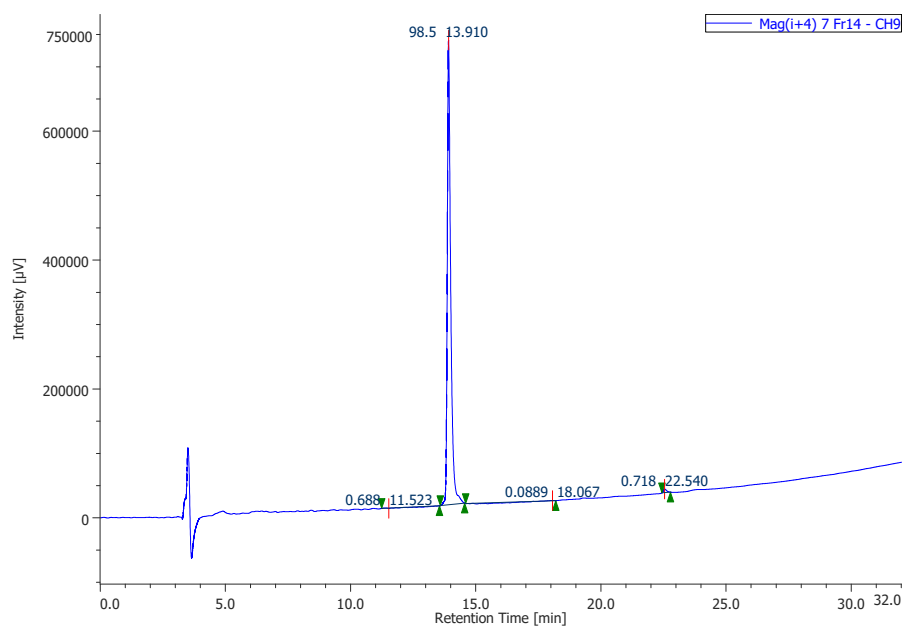
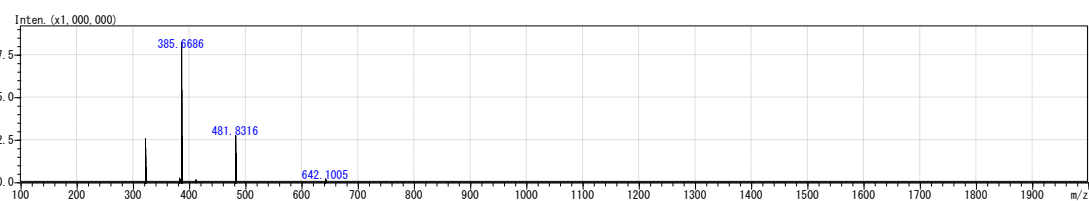
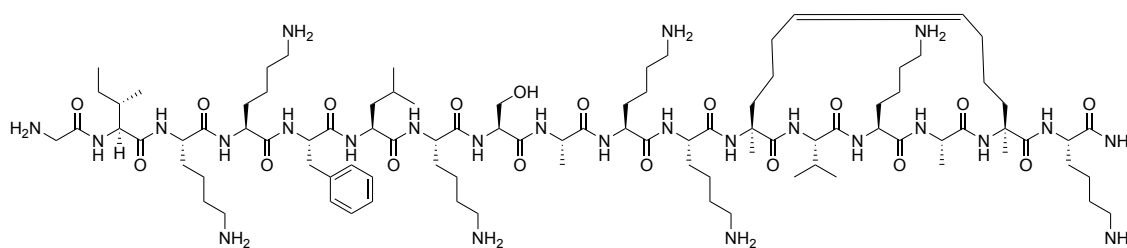
H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-Ala-Lys-Lys-S₅-Val-Lys-Ala-S₅-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 98.5%

HRMS (ESI⁺) calcd for C₉₃H₁₆₇N₂₅O₁₈ [M+5H]⁵⁺: 385.4662; found: 385.4703



Peptide 8

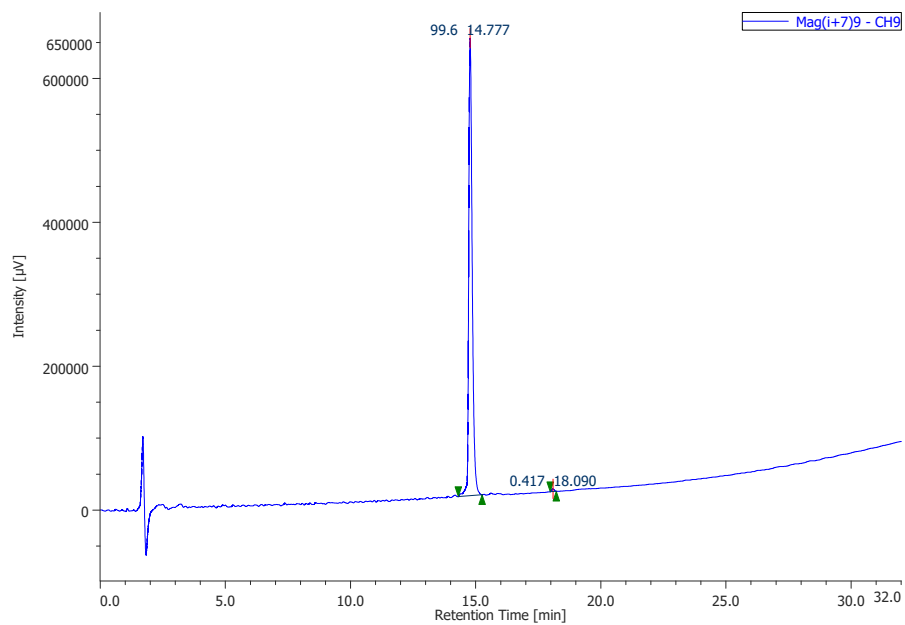
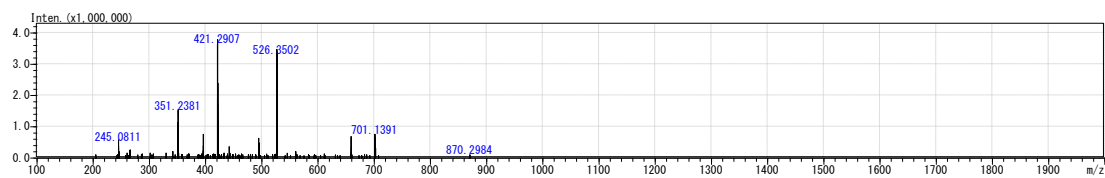
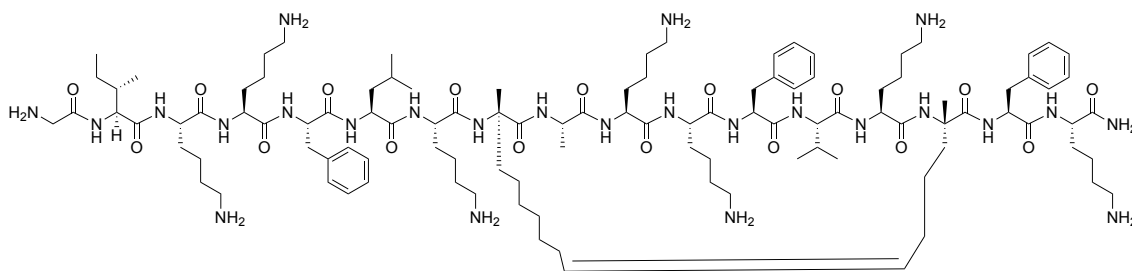
H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-R_s-Ala-Lys-Lys-Phe-Val-Lys-S₅-Phe-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 99.6%

HRMS (ESI⁺) calcd for C₁₀₈H₁₈₁N₂₅O₁₇ [M+5H]⁵⁺: 421.0892; found: 421.0873



Peptide 9

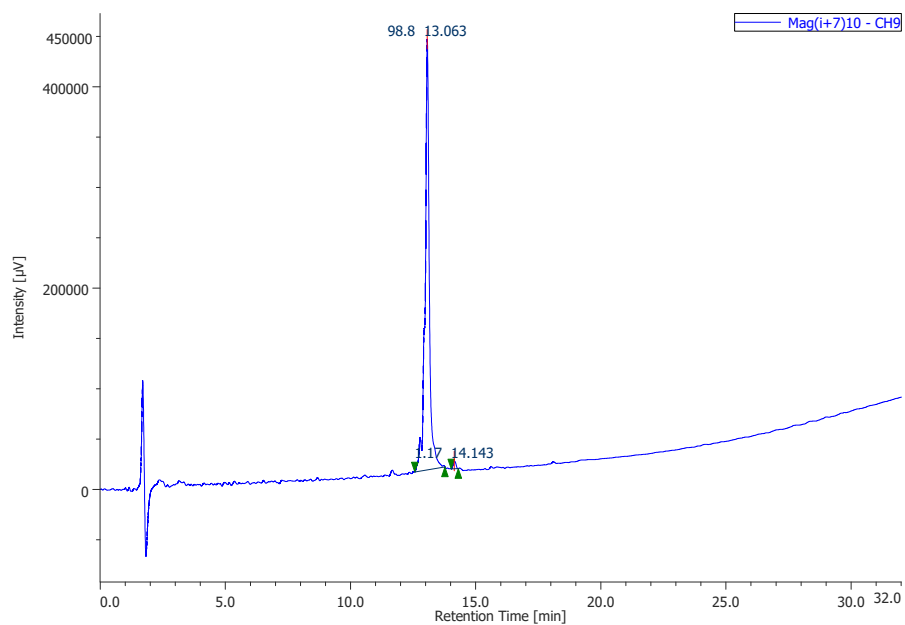
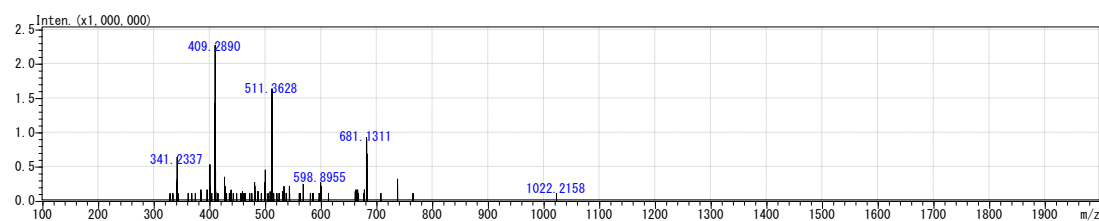
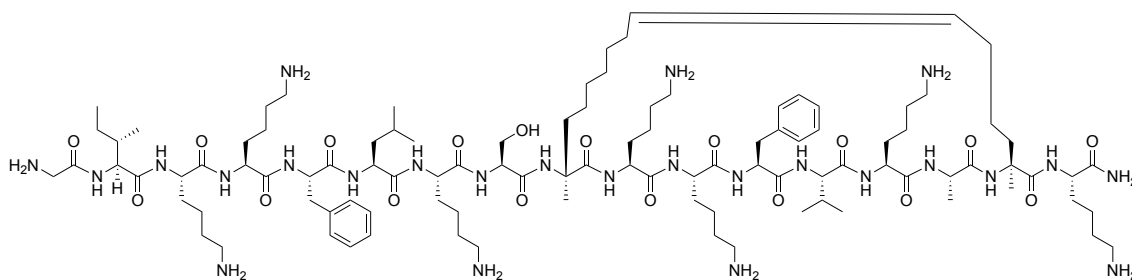
H-Gly-Ile-Lys-Lys-Phe-Leu-Lys-Ser-R_s-Lys-Lys-Phe-Val-Lys-Ala-S₅-Lys-NH₂

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/CH₃CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 98.8%

HRMS (ESI⁺) calcd for C₁₀₂H₁₇₇N₂₅O₁₈ [M+3H]³⁺: 681.1313; found: 681.1311



C6-2 (E/Z isomers were detected)

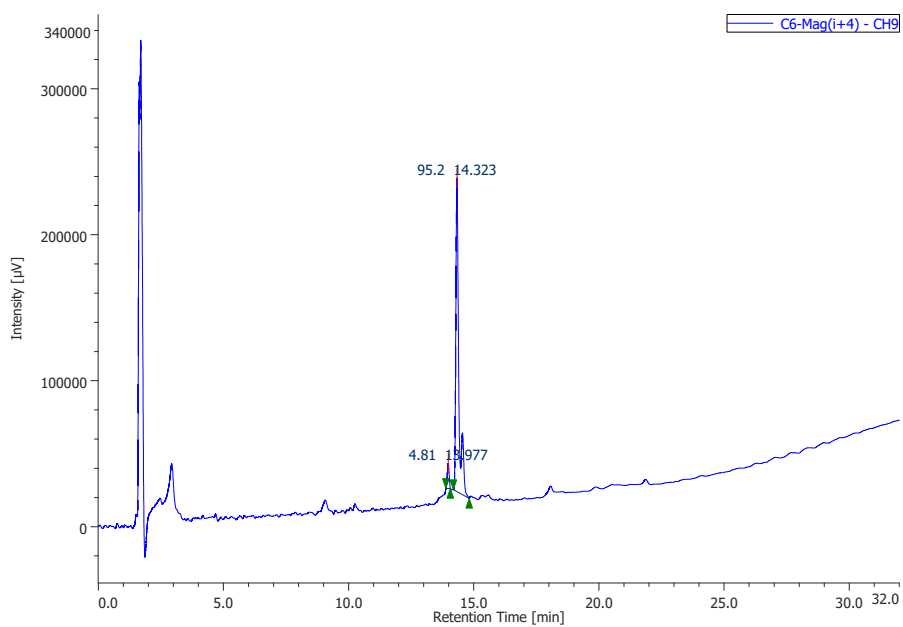
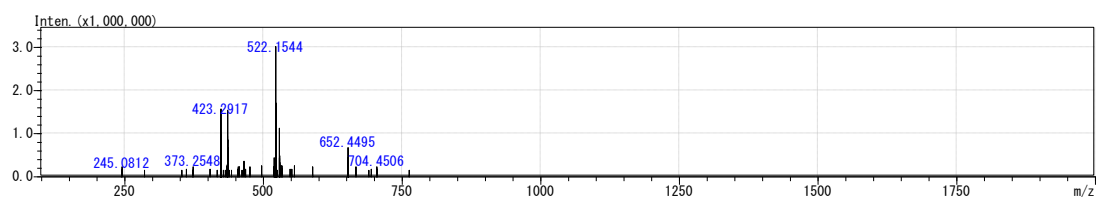
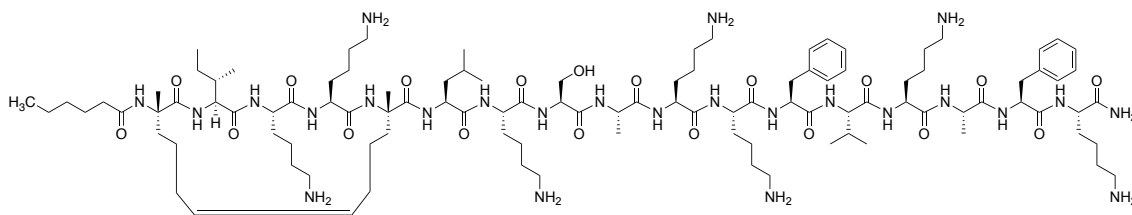
$\text{CH}_3(\text{CH}_2)_4\text{CO-S}_5\text{-Ile-Lys-Lys-S}_5\text{-Leu-Lys-Ser-Ala-Lys-Lys-Phe-Val-Lys-Ala-Phe-Lys-NH}_2$

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/ CH_3CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 95.2%

HRMS (ESI⁺) calcd for $\text{C}_{106}\text{H}_{183}\text{N}_{25}\text{O}_{19}$ $[\text{M}+5\text{H}]^{5+}$: 423.0902; found: 423.0912



C12-2

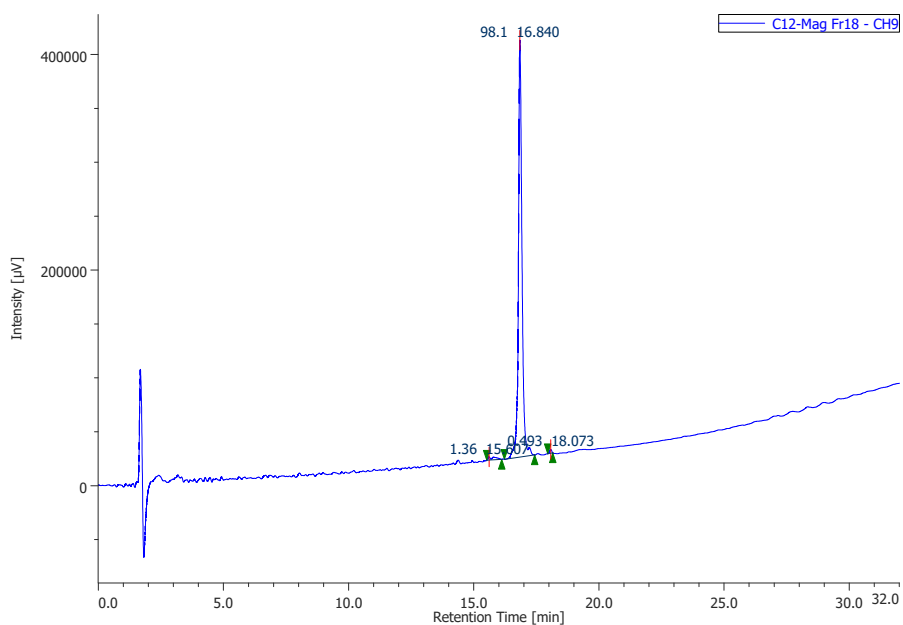
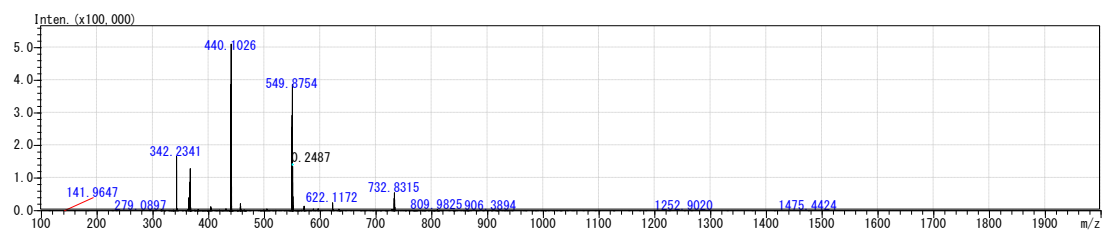
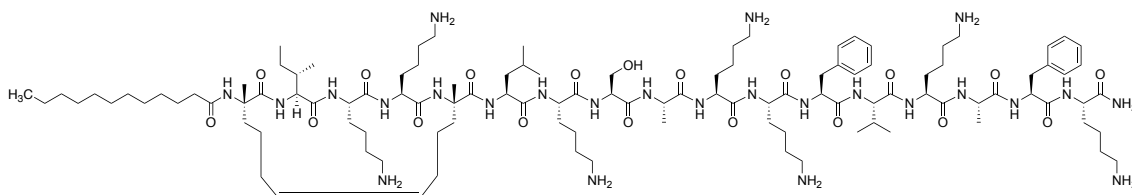
$\text{CH}_3(\text{CH}_2)_{10}\text{CO-S}_5\text{-Ile-Lys-Lys-S}_5\text{-Leu-Lys-Ser-Ala-Lys-Lys-Phe-Val-Lys-Ala-Phe-Lys-NH}_2$

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/ CH_3CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 98.1%

HRMS (ESI⁺) calcd for $\text{C}_{112}\text{H}_{195}\text{N}_{25}\text{O}_{19}$ $[\text{M}+4\text{H}]^{4+}$: 549.6343; found: 549.6267



C18-2 (E/Z isomers were detected)

$\text{CH}_3(\text{CH}_2)_{16}\text{CO-S}_5\text{-Ile-Lys-Lys-S}_5\text{-Leu-Lys-Ser-Ala-Lys-Lys-Phe-Val-Lys-Ala-Phe-Lys-NH}_2$

HPLC: Conditions = solvent A 0.1% TFA/water, solvent B 0.1% TFA/ CH_3CN

Gradient = 10-90% gradient of solvent B over 30 min

Purity: 96.2%

HRMS (ESI⁺) calcd for $\text{C}_{118}\text{H}_{207}\text{N}_{25}\text{O}_{19}$ $[\text{M}+5\text{H}]^{5+}$: 456.7278; found: 456.7152

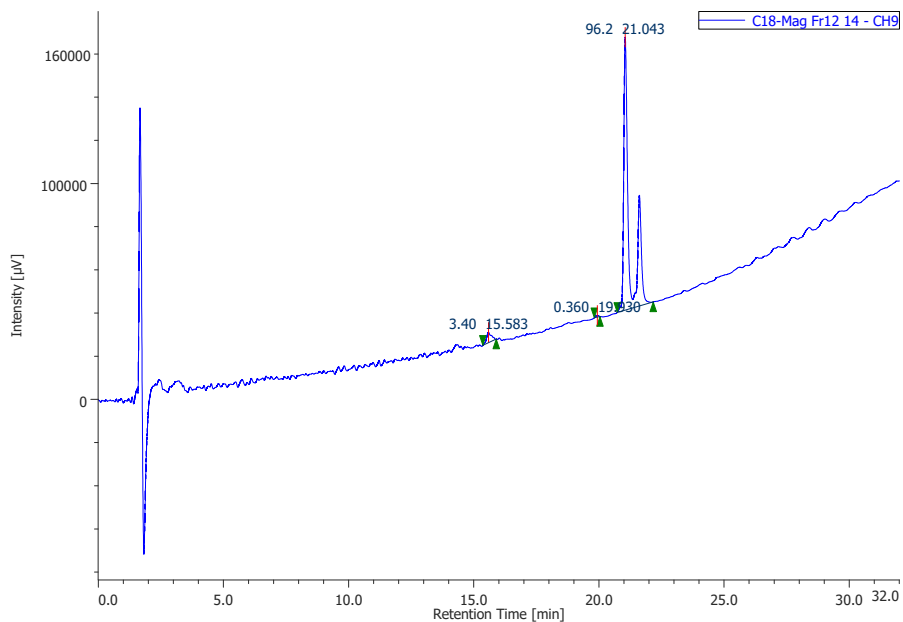
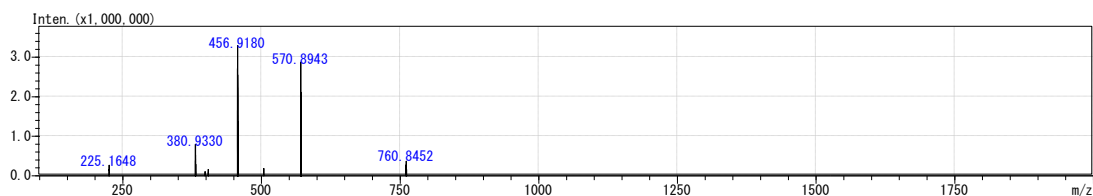
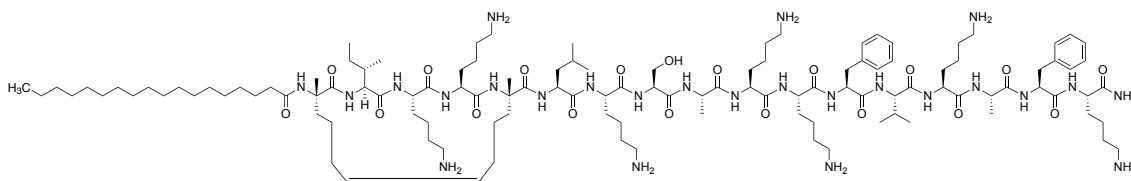


Table S1 The pore forming score for antimicrobial activities of peptides 1, 2, and 8 in DOPE/DOPG membrane.

Peptide	1	2	8
Pore stability (%)	48	49	38
Pore diameter (nm)	1.1 ± 0.01	1.2 ± 0.6	0.5 ± 0.01
Charge flux (pC sec ⁻¹)	32.5 ± 10.3	68.1 ± 13.6	42.9 ± 8.6
Pore forming activity	2.5	1.1	0.4

Table S2 The pore forming score for antimicrobial activities of peptides 1, 2, and 8 in DOPC membrane.

Peptide	1	2	8
Pore stability (%)	47	53	60
Pore diameter (nm)	N/A	1.2 ± 0.3	1.7 ± 0.4
Charge flux (pC sec ⁻¹)	25.3 ± 8.5	21.0 ± 8.4	92.1 ± 16.0
Pore forming activity	0.4	1.1	0.9