

Supporting Information

**Novel Stapling By Lysine-Tethering Provides Stable and Low Hemolytic
Cationic Antimicrobial Peptide**

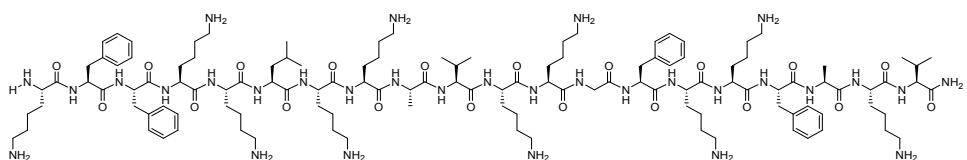
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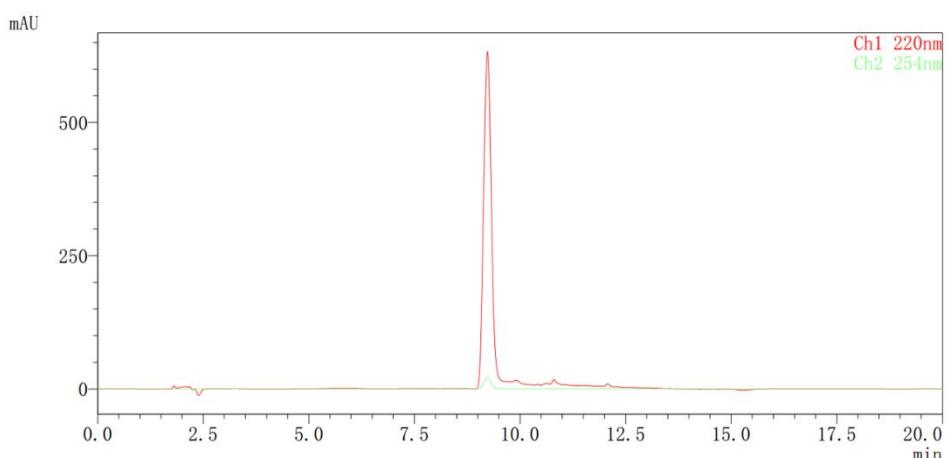
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1. Analytical HPLC chromatograms and HRMS data of purified stapled peptides.

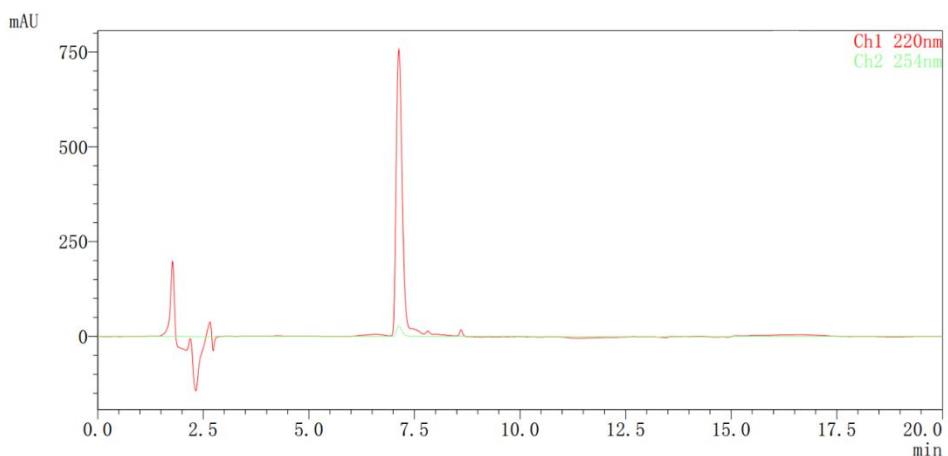


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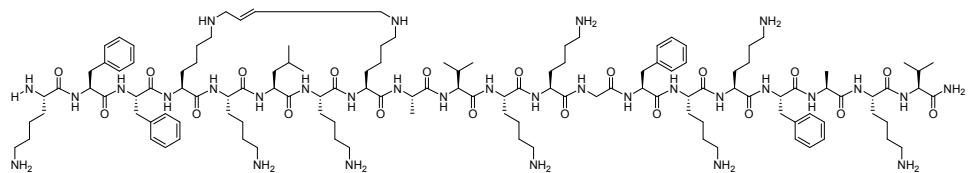
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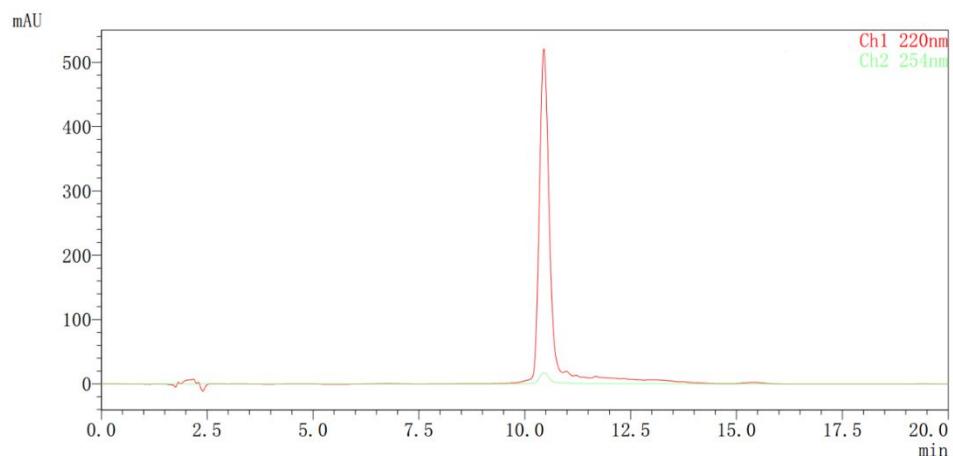


HPLC analysis of linear peptide **1** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 9.258 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 7.131 min; HRMS m/z calcd for C₁₂₀H₂₀₅N₃₁O₂₀ [M+4H]⁴⁺ 600.65279, found 600.65472.

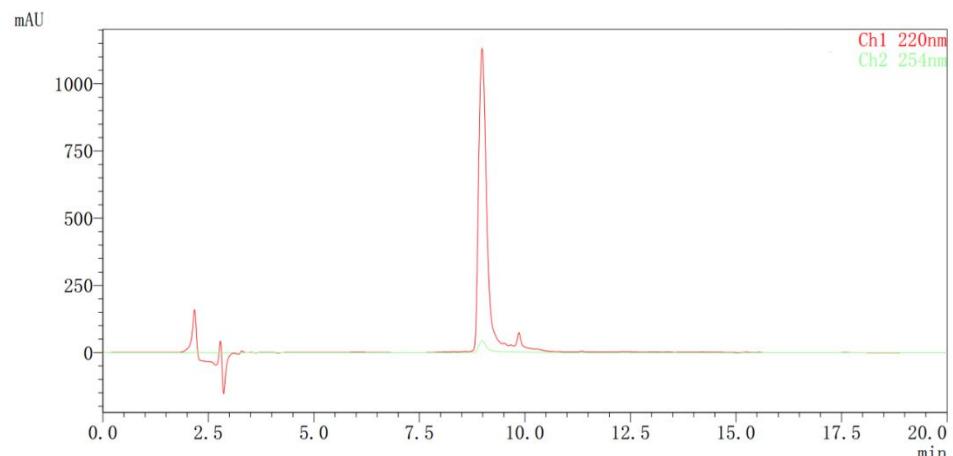


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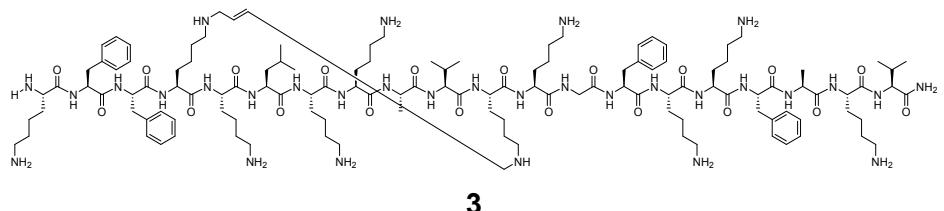
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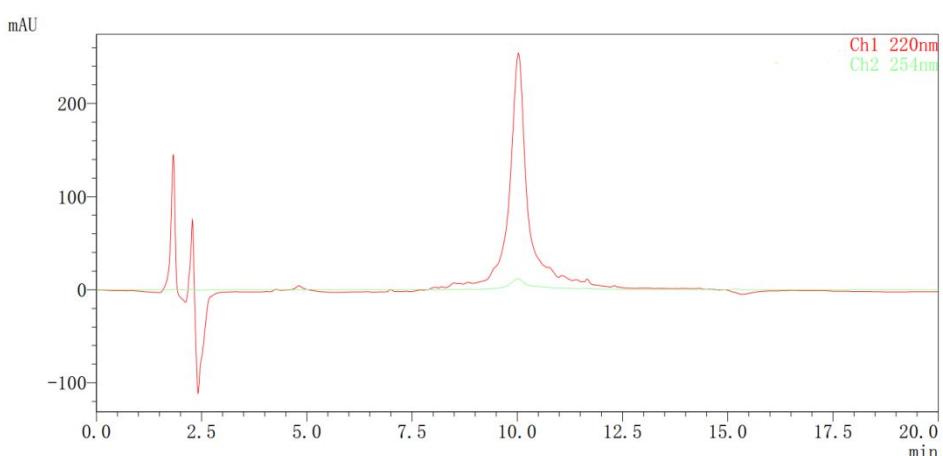
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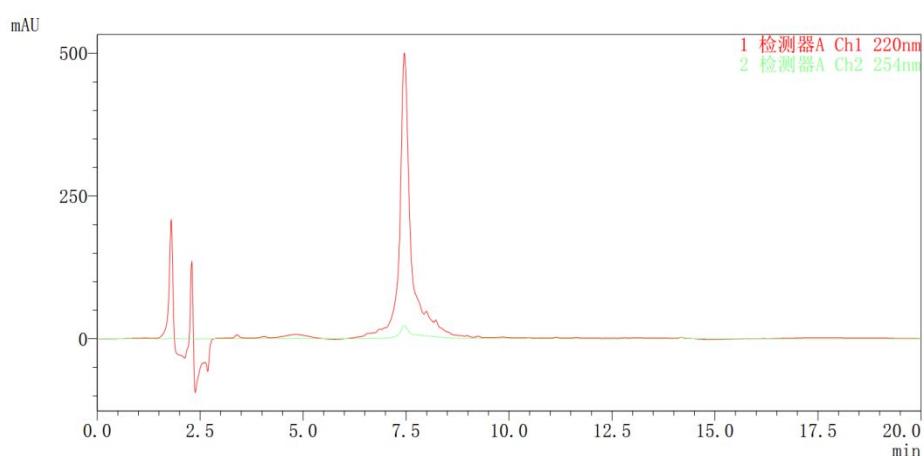
HPLC analysis of stapled peptide **2** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.394 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 9.001 min;
HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40784.



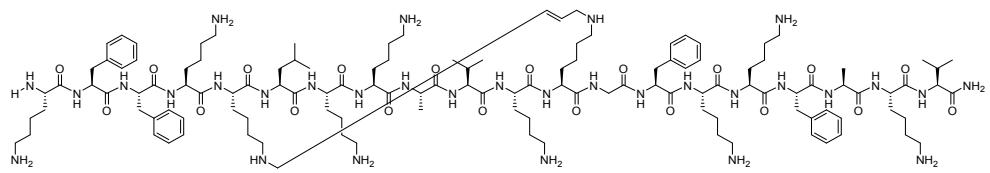
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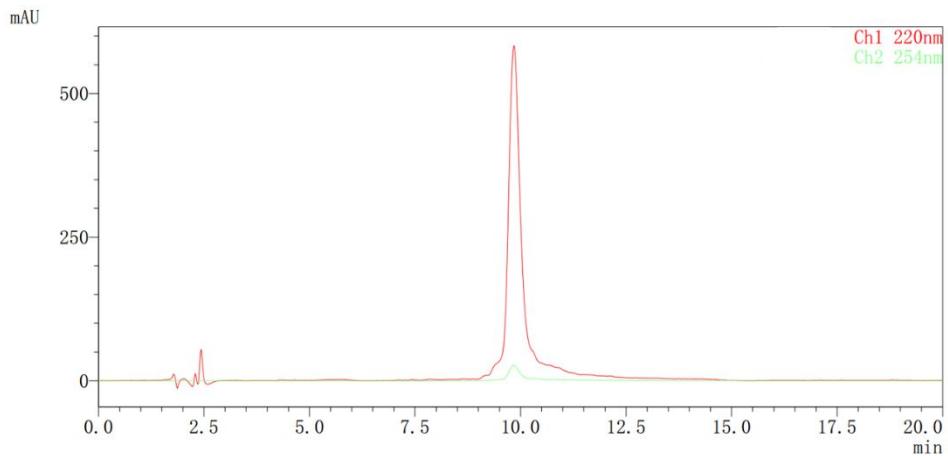


HPLC analysis of stapled peptide **3** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.082 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 7.498 min; HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40879.

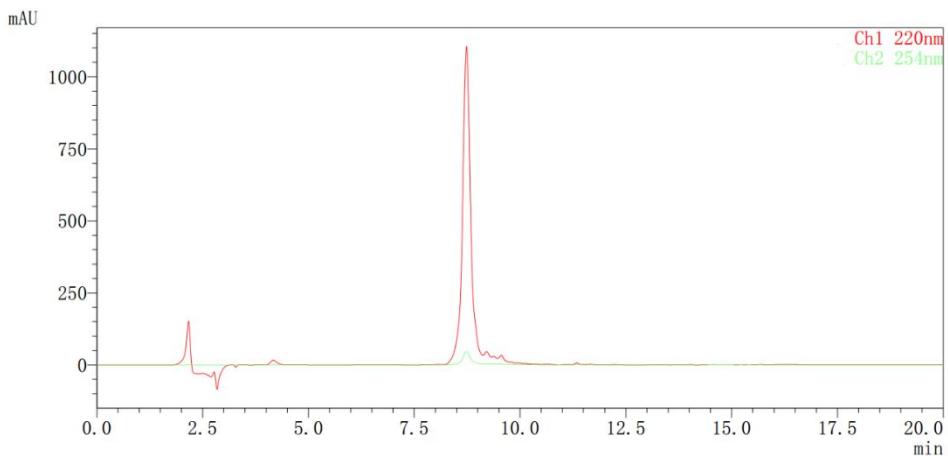


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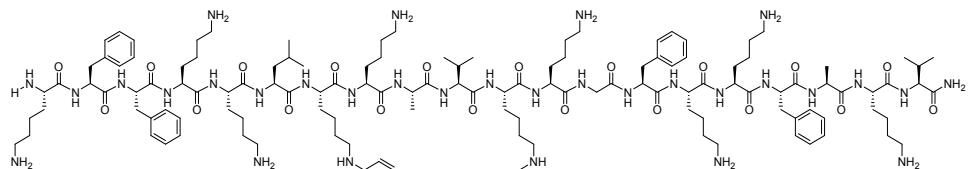
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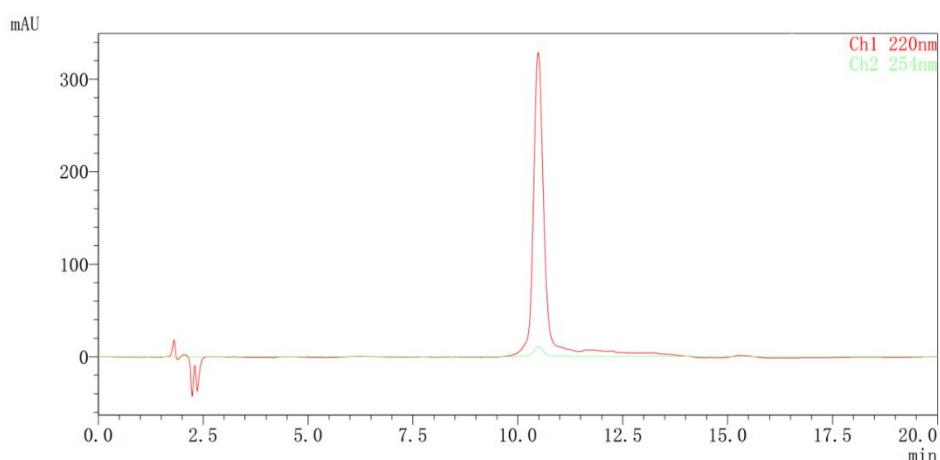


HPLC analysis of stapled peptide **4** was performed using a linear gradient of a) 5- 60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 9.863 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.781 min;
HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40850.

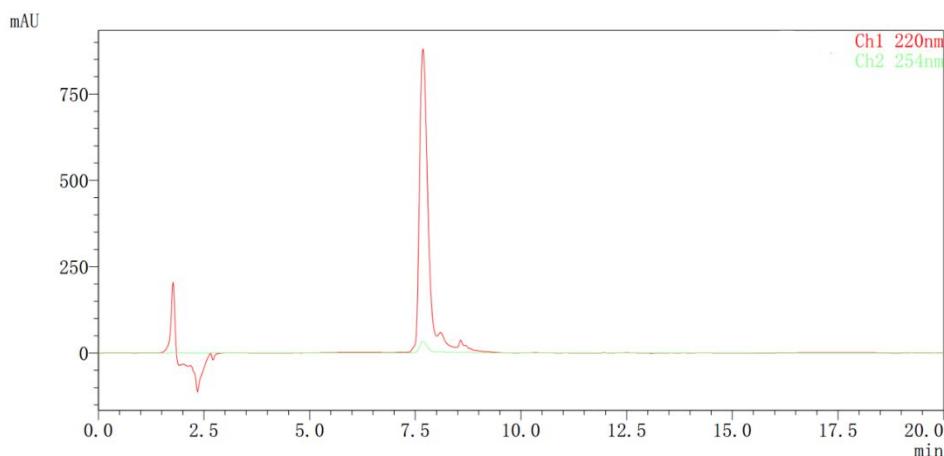


5

a)

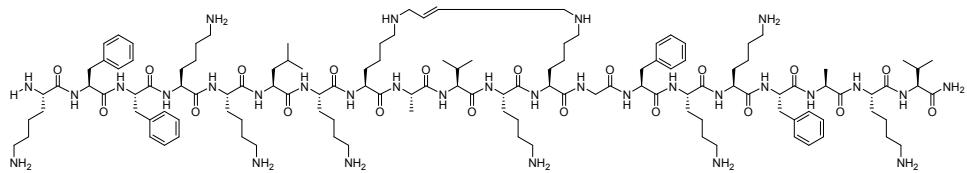


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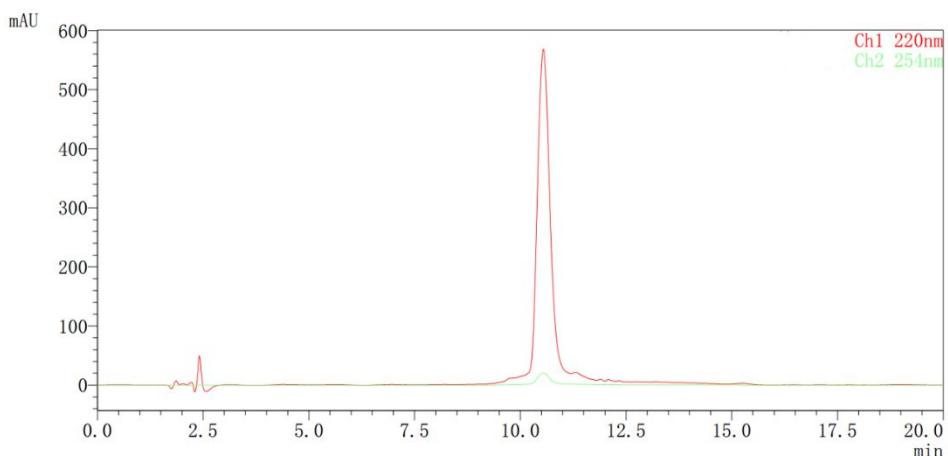
HPLC analysis of stapled peptide **5** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.504 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 7.699 min;

HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40965.

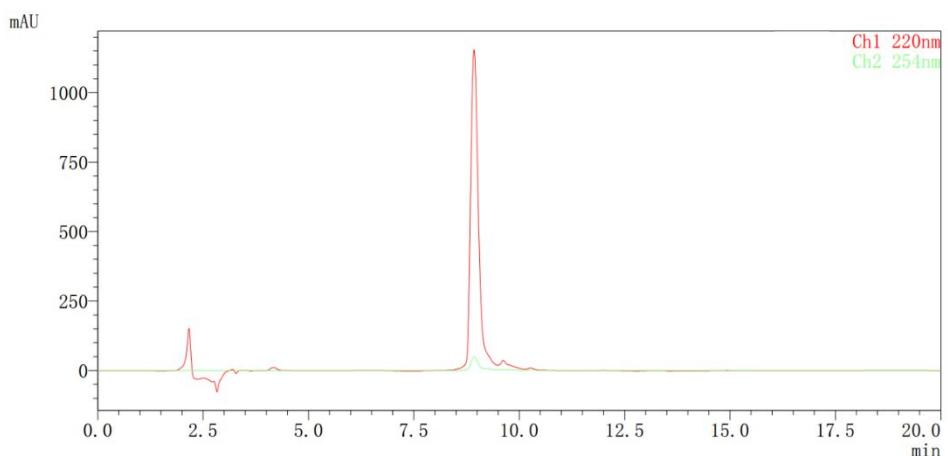


6

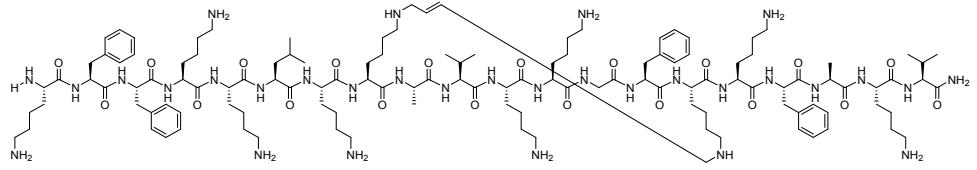
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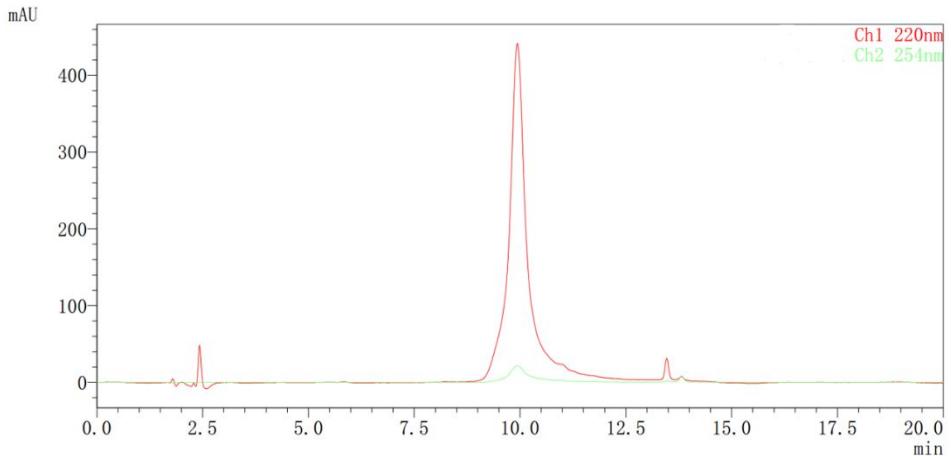


HPLC analysis of stapled peptide **6** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.559 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.928 min; HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40743.

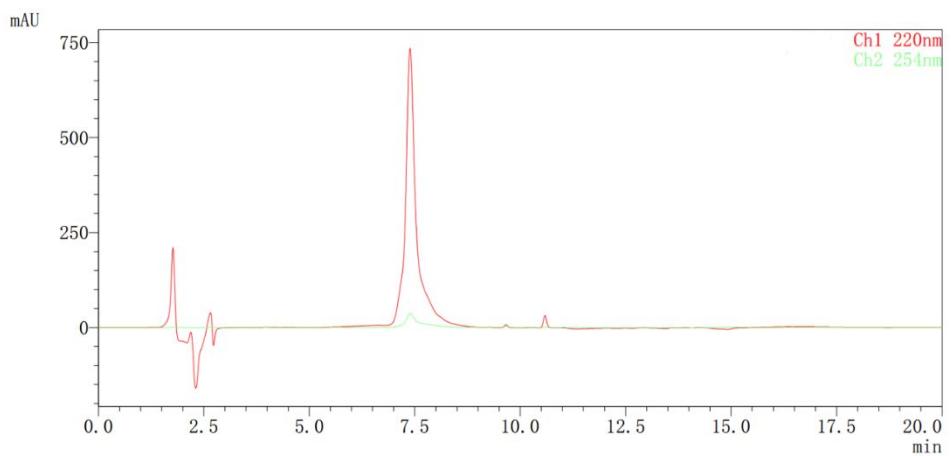


7

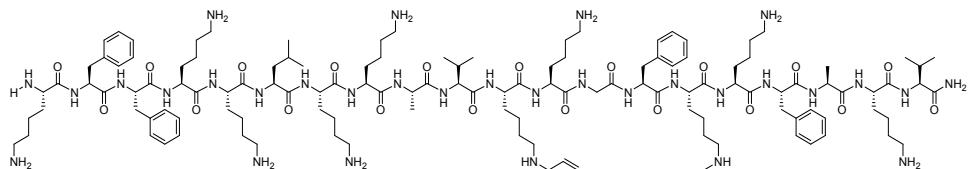
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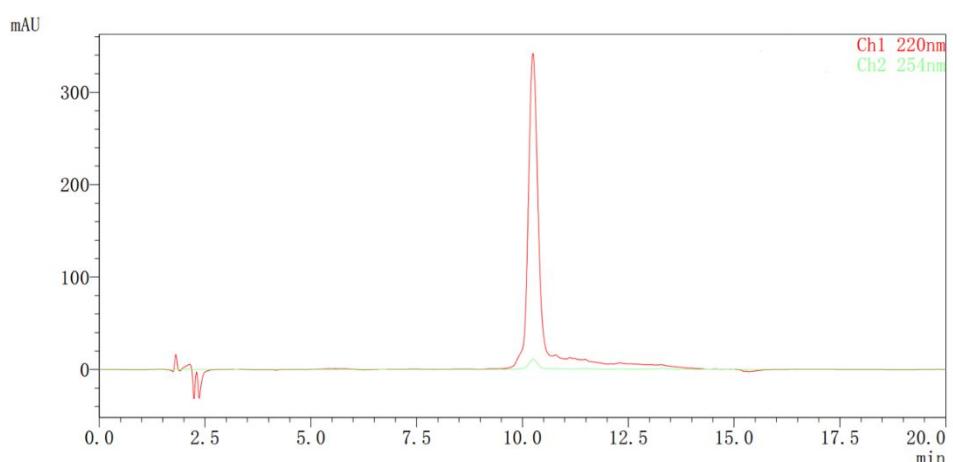
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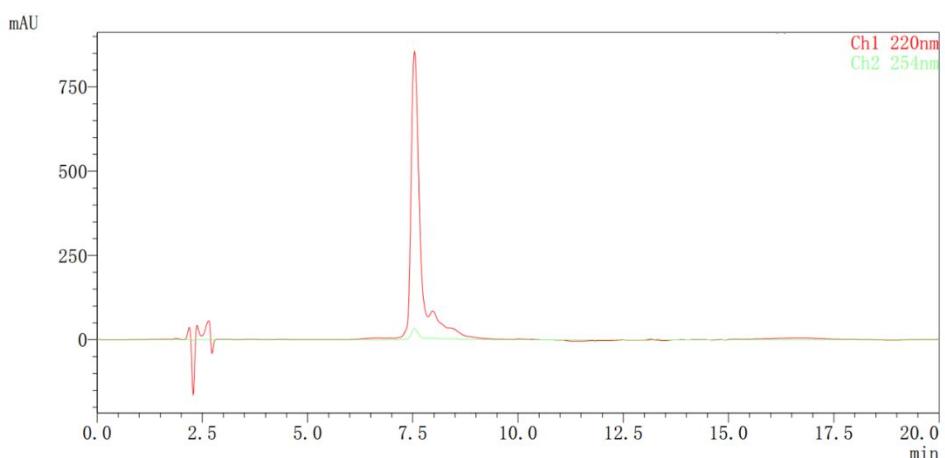
HPLC analysis of stapled peptide **7** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 9.954 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 7.406 min; HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40996.



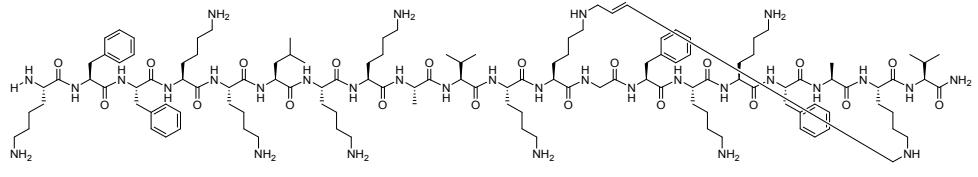
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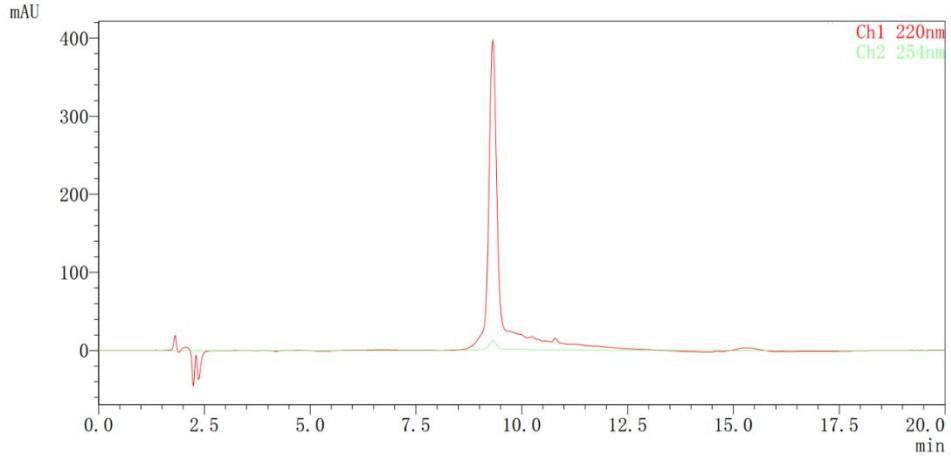


HPLC analysis of stapled peptide **8** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.266 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 7.571 min; HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40845.

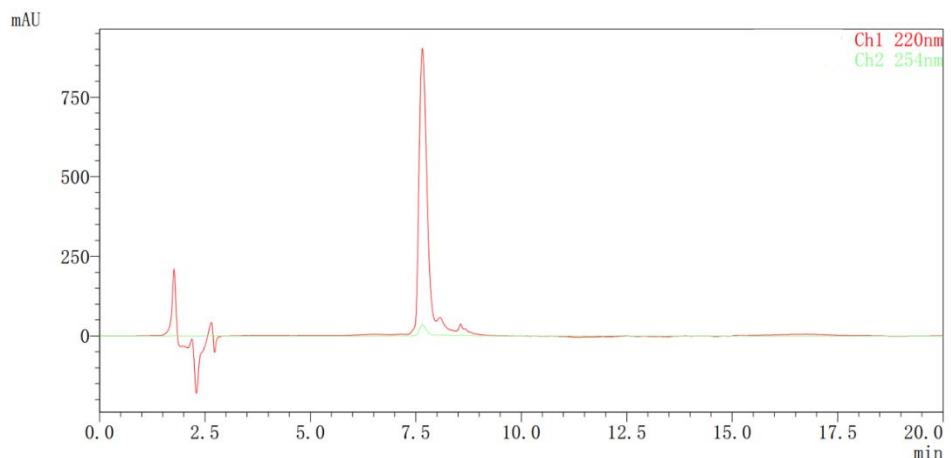


9

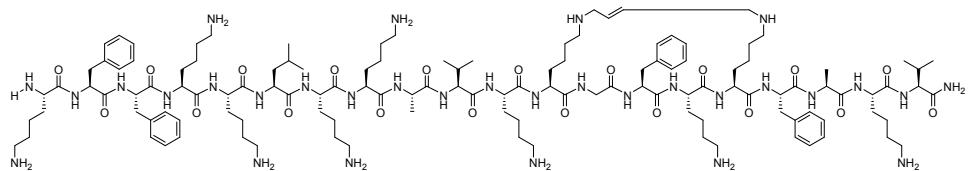
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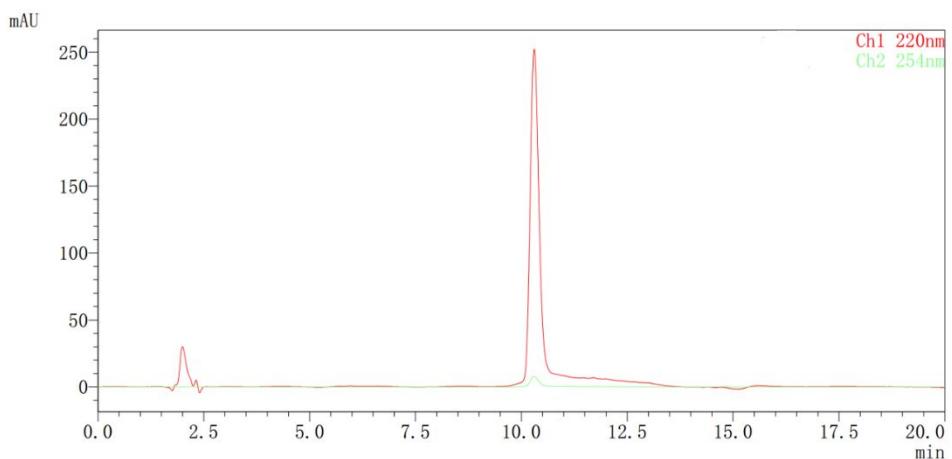


HPLC analysis of stapled peptide **9** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 9.331 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 7.663 min; HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40736.

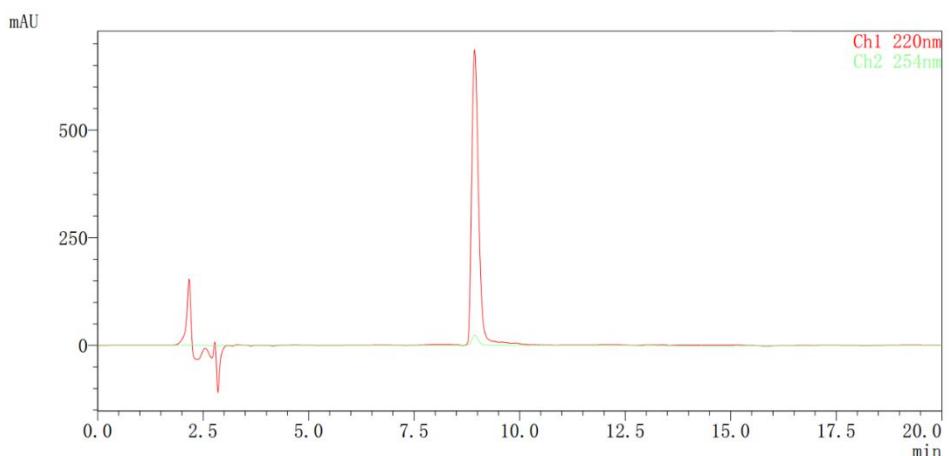


10

a)

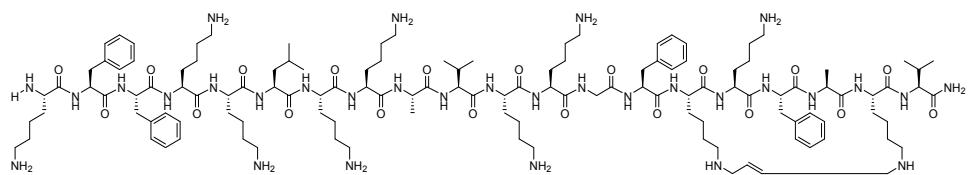


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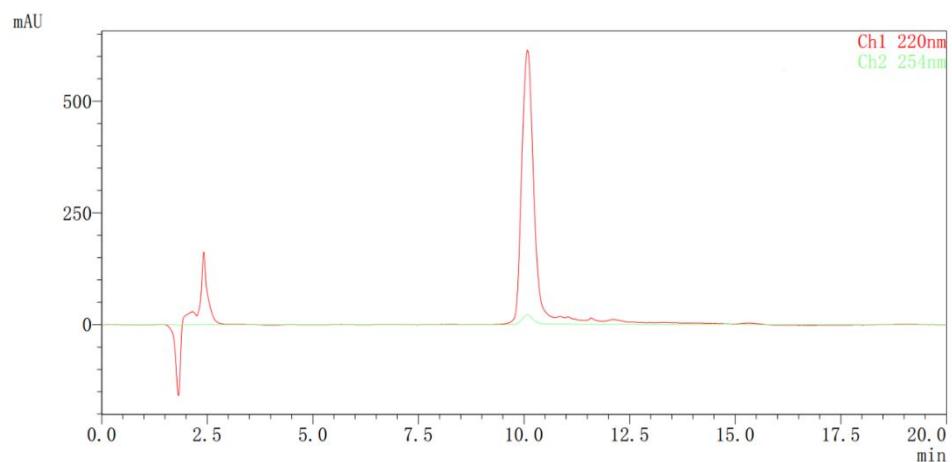
HPLC analysis of stapled peptide **10** was performed using a linear gradient of a) 5- 60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.302 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.946 min;

HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40963.

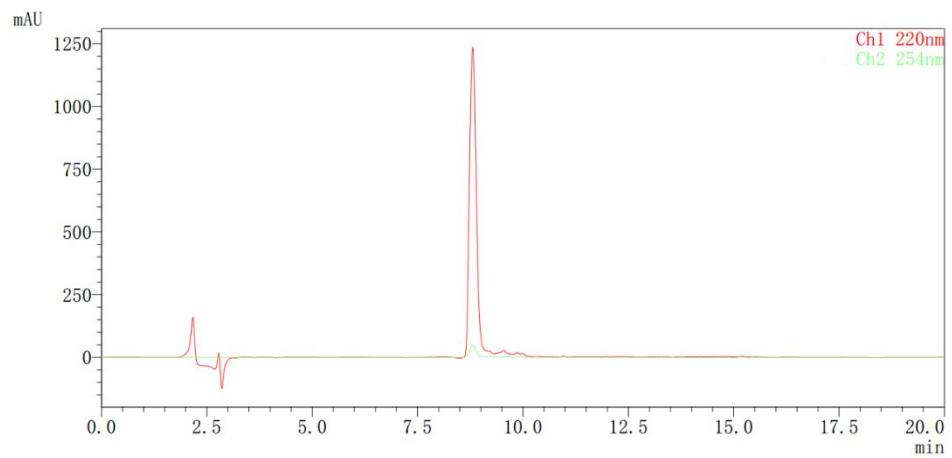


11

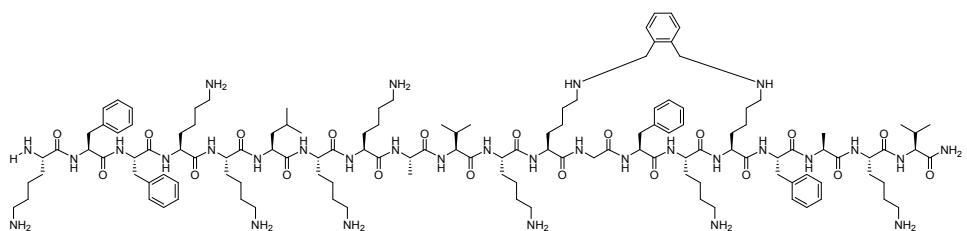
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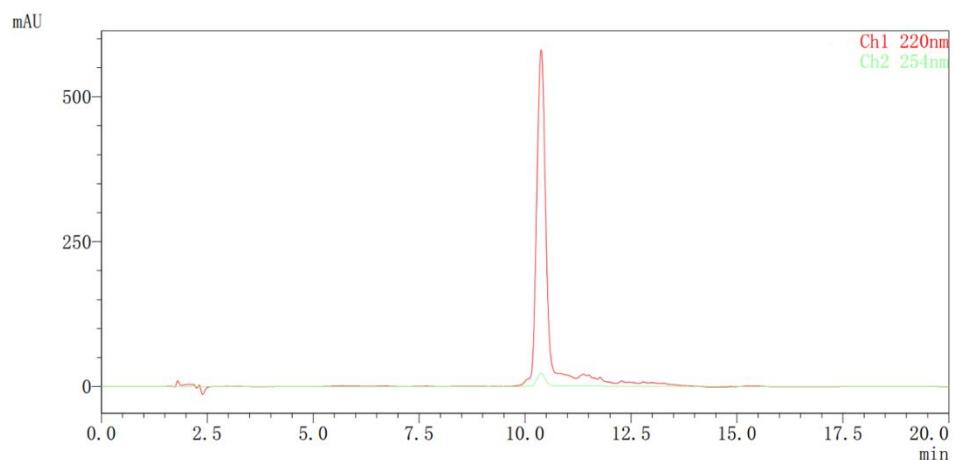


HPLC analysis of stapled peptide **11** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.082 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.779 min; HRMS m/z calcd for C₁₂₄H₂₀₉N₃₁O₂₀ [M+4H]⁴⁺ 613.40866, found 613.40985.

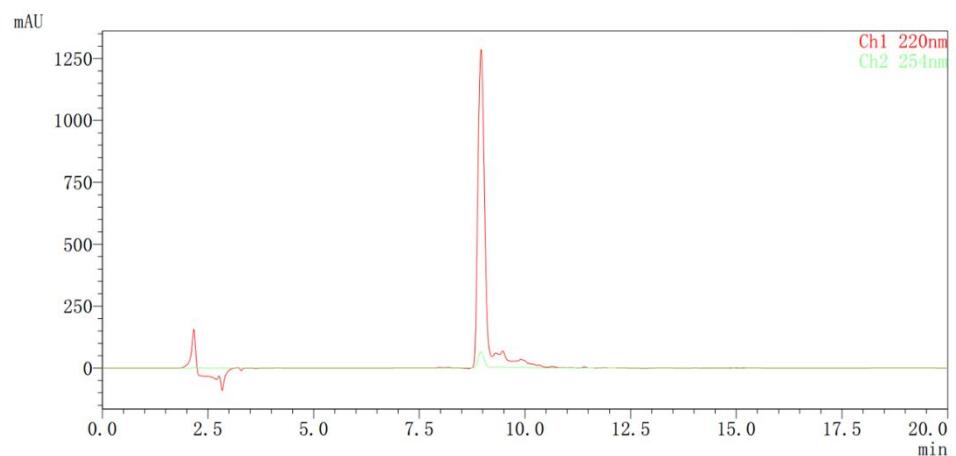


12

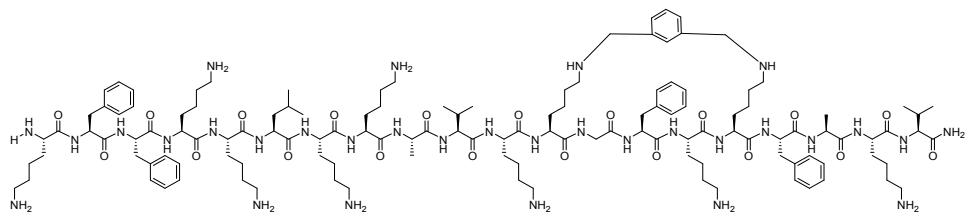
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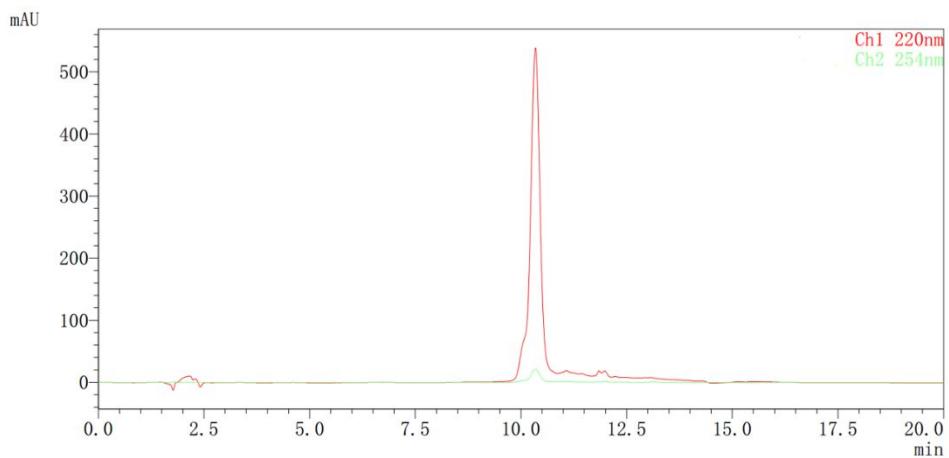


HPLC analysis of stapled peptide **12** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.376 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.983 min; HRMS m/z calcd for C₁₂₈H₂₁₁N₃₁O₂₀ [M+4H]⁴⁺ 626.16453, found 626.16497.

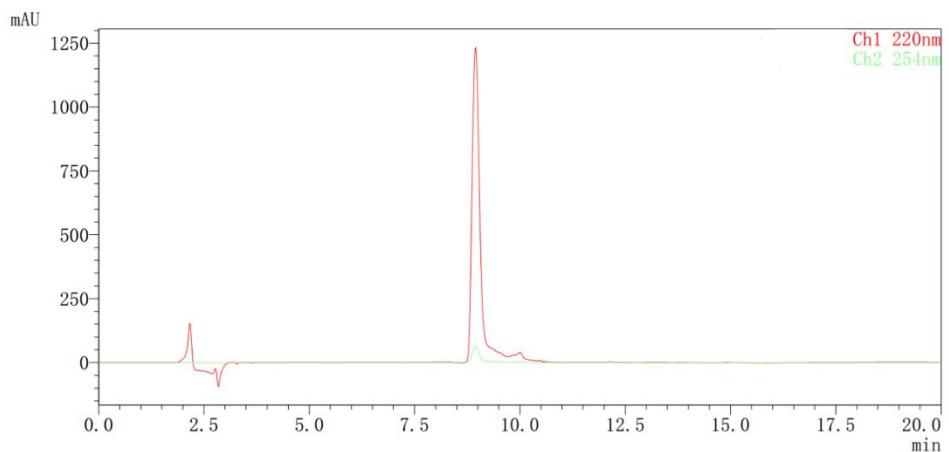


13

a)

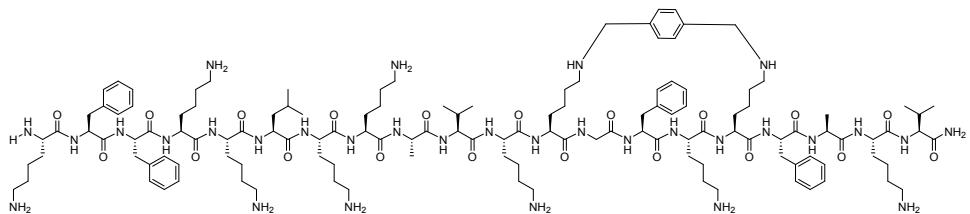


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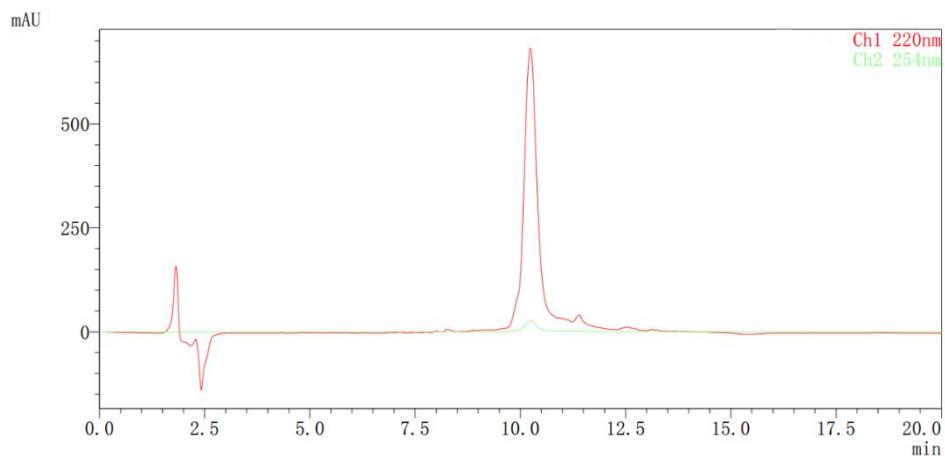
HPLC analysis of stapled peptide **13** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.339 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.964 min;

HRMS m/z calcd for C₁₂₈H₂₁₁N₃₁O₂₀ [M+4H]⁴⁺ 626.16453, found 626.16419.

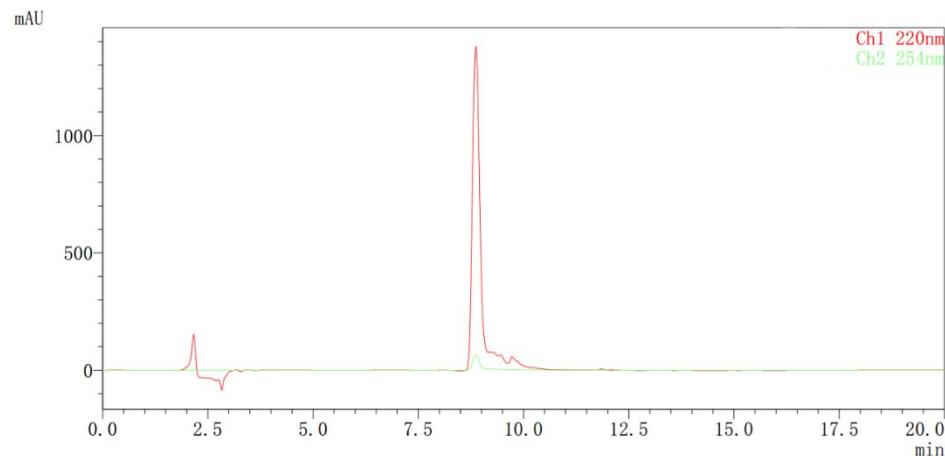


14

a)



b)



HPLC analysis of stapled peptide **14** was performed using a linear gradient of a) 5-60% of MeOH containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeOH for 8 min, R.T. = 10.264 min; b) 5-60% of MeCN containing 0.1% FA in H₂O containing 0.1% FA over 12 min, then at 5% MeCN for 8 min, R.T. = 8.861 min;

HRMS m/z calcd for C₁₂₈H₂₁₁N₃₁O₂₀ [M+4H]⁴⁺ 625.91091, found 625.91257.

2. Circular Dichroism Specturm

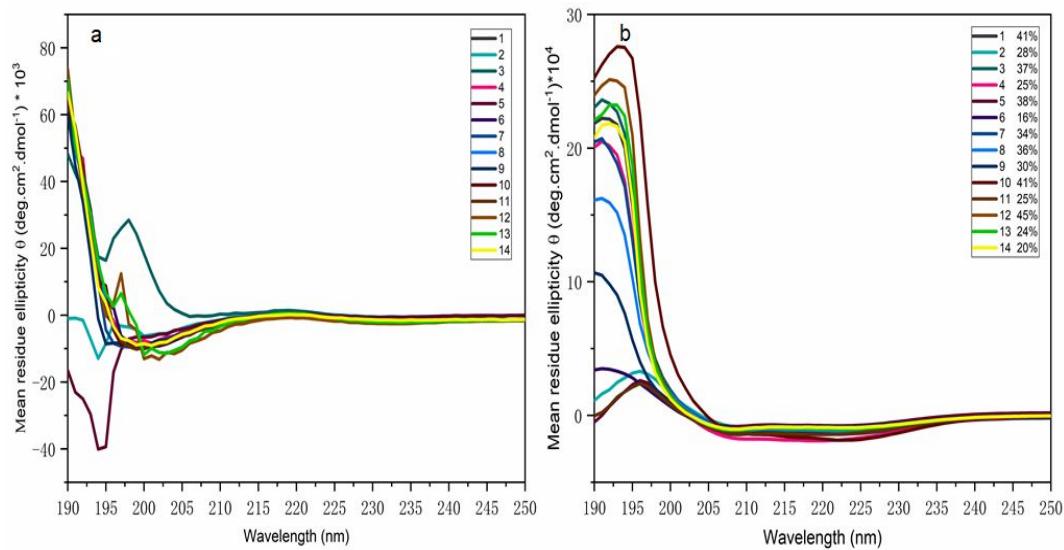


Figure S1. Circular Dichroism Spectra (CD). a) CD spectra of peptide **1-14** in PBS; b) CD spectra of peptide **1-14** in 30 mM SDS.

3. Standard curve of Serum Digestion experiment.

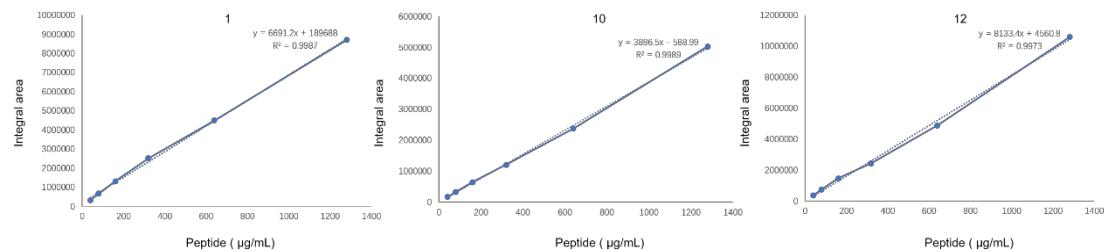


Figure S2. Standard curve of the integral area versus peptide concentration.