One-pot synthesis of dual functional peptides by Sortase A-mediated on-resin cleavage and ligation

Xiaozhong Cheng, Tao Zhu, Haofei Hong, Zhifang Zhou, Zhimeng Wu*

Key Laboratory of Carbohydrate Chemistry & Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, China Email: zwu@jiangnan.edu.cn; Tel and Fax: 86-510-85197582

Supporting Information

Contents:

I. General Experimental Methods

Unless otherwise noted, all chemicals were obtained from commercial sources and used without further purification.

RP-HPLC. Analytical scale HPLC were performed with Waters 2695 system and Diamonsil C18 column (250 mm×4. 6 mm, 3.5 μm,100 Å). Semi-preparative purifications were achieved using Waters 1525 and Diamonsil C18 column (250 mm×10 mm, 3.5 μm,100 Å).

Mass Spectrometry. Electrospray ionization-mass spectrometry (ESI-MS) analysis was carried out with a Bruker Amazon SL.

MALDI TOF-MS. Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry was performed on Bruker Ultraflex mass spectrometer.

NMR: Proton chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (TMS). Carbon-13 chemical shifts are reported in ppm (δ) in reference to $CDCl_3$ (δ 77.16) or in reference to the proton signal of the solvent CD_3OD (δ 49.00).

II. Synthesis and characterization of peptides

i. Synthesis of AbzLPETGGK(DNP)S-HMBA, NH_2-BK-Acp-LPETGGS-HMBA and DNS-LPETGGS-HMBA on resin

The resin (PEGA or MBHA resin) was swollen in dry DMF (5ml), then treated with a DMF solution of hydroxymethyl benzoic acid (HMBA) (3 eq) in presence of TBTU (2.85 eq) and DIEA (6 eq). After shaking 4 h at room temperature, the resin was washed with DMF (10×), MeOH (10×), DCM (10×) and dried *in vacuo*. The attachment of first amino acids (Fmoc-Ser(otBu)-OH) was performed using 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT)/methyli-midazole (MeIm) esterification. The resin was swollen in dry DCM, Fmoc-Ser(otBu)-OH) (3 eq), MSNT (3.75 eq) and MeIm (5 eq) was dissolved in DCM and added to the resin. After 1 h, the resin was filtered and washed with DMF (10×), MeOH (10×), DCM (10×). A second coupling was performed with same amount of reagents. The Fmoc group was removed by 20% piperidine/DMF for 20 min. The assembly of the rest Fmoc amino acids or Fmoc amino acid derivatives (Abz,K-Dnp,Acp) was performed using the CEM Liberty automated microwave peptide synthesizer. The coupling reagents was the same as above described. As to DNS (dansyl chloride) coupling, the peptide resin was treated with 5 eq of DNS and 2 eq of DIPEA in 3 ml dry DMF for 4 h at 35℃. After the incorporation of all amino acids, the resin was washed with DMF (10×), MeOH (10×), DCM (10×) and dried. The resin was treated with a solution of TFA (95%), water (2.5%) and Tis (2.5%) for 2 h at room temperature for removing all the side-chain protection groups, then resin was washed with DMF (10×), MeOH (10×), DCM (10×). A few beads were collected and was cleaved with 0.1 M NaOH for 2 h at room temperature. The resin was filtered and the filtrate was neutralized with 0.1 M HCl. The product was analysed by HPLC and MALDI-TOF or ESI.

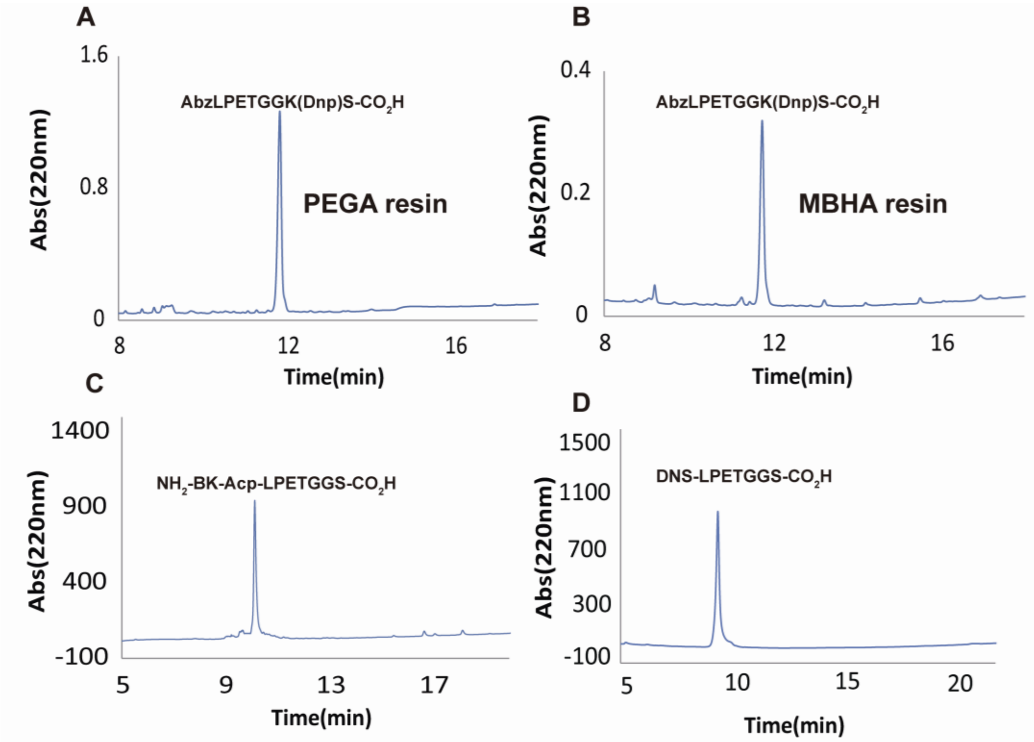
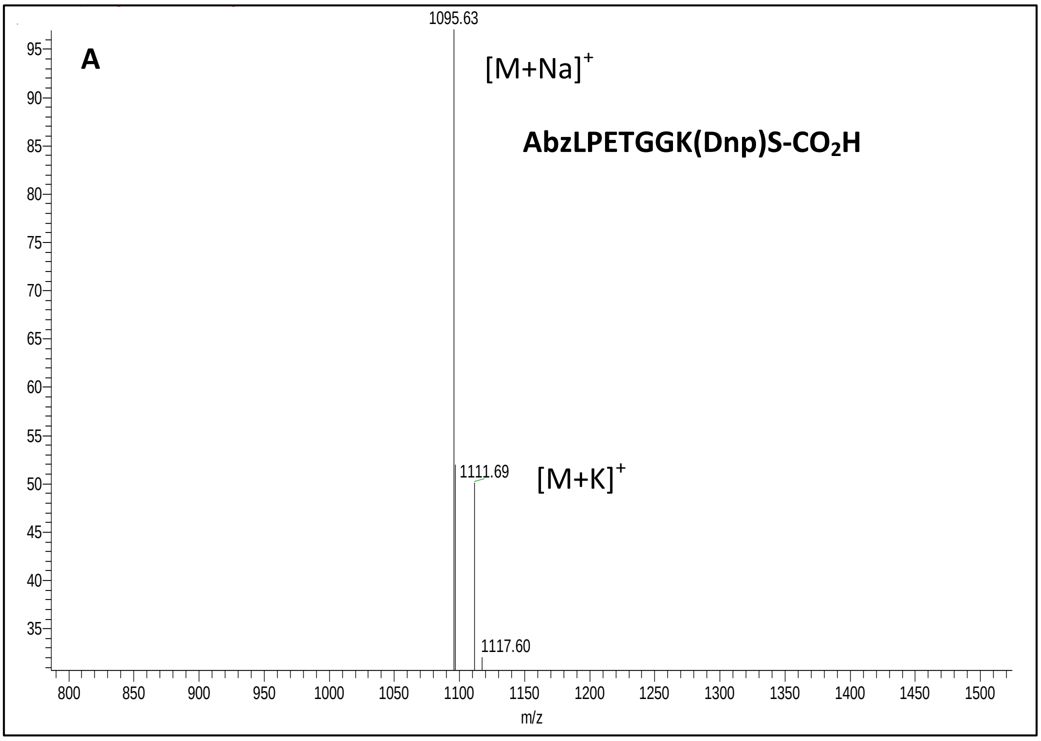
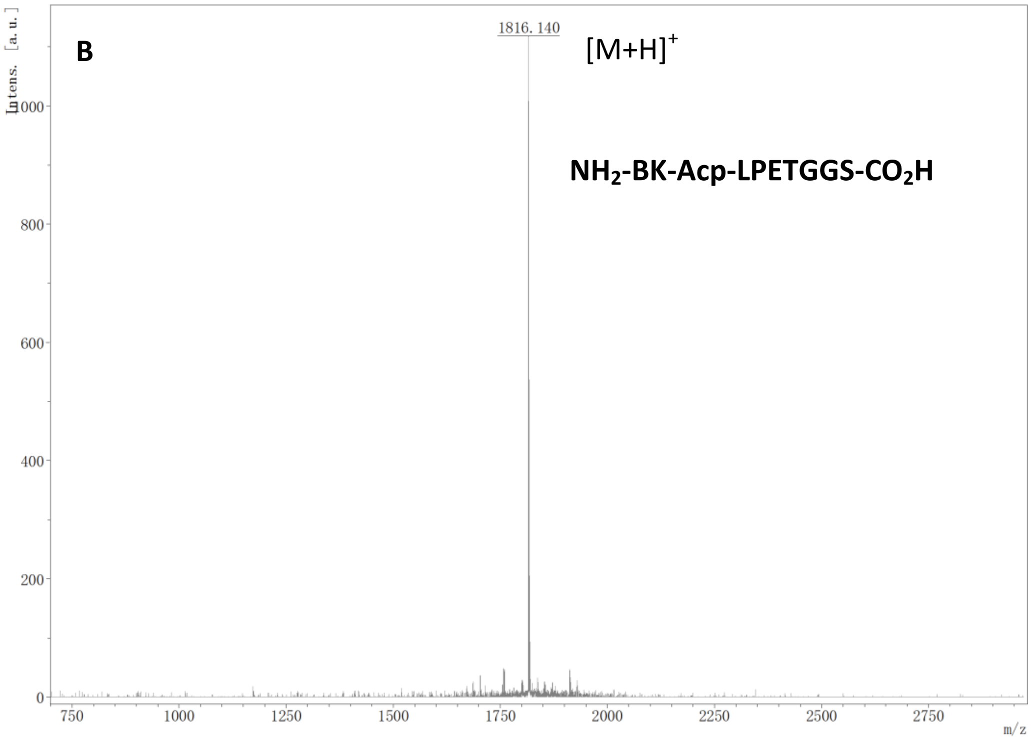


Figure S1. HPLC diagrams of peptide AbzLPETGGK(DNP)S-CO_2H, NH_2-BK-Acp-LPETGGS-CO_2H and DNS-LPETGGS- CO_2H, and monitored with UV at 220 nm. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 90% ACN in 20 min; flow rate: 1 mL/min; R.T.: AbzLPETGGK(DNP)S-CO_2H (11.84 min); NH_2-BK-Acp-LPETGGS-CO_2H (10.13 min); DNS-LPETGGS- CO_2H (9.39 min).





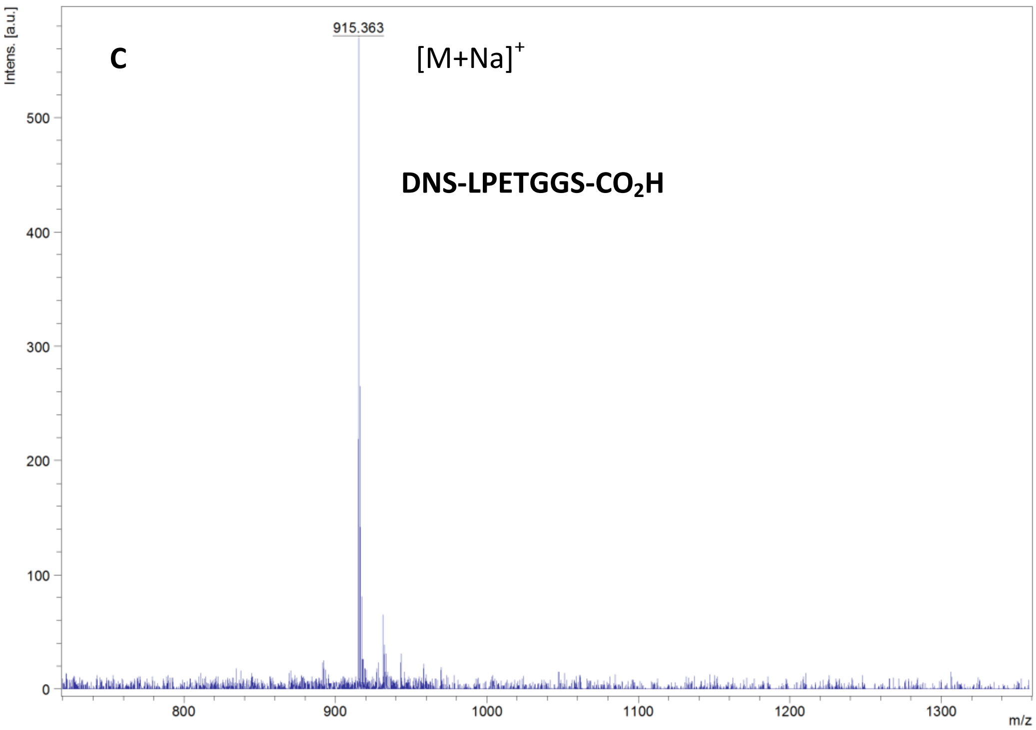


Figure S2. Mass spectrum of peptide AbzLPETGGK(DNP)S-CO_2H, NH_2-BK-Acp-LPETGGS-CO_2H and DNS-LPETGGS- CO_2H. A. ESI-MS of AbzLPETGGK(DNP)S-CO_2H, calcd : 1072.0, observed 1095.6 $[M+Na]^+$, 1111.6 $[M+K]^+$; B. MALDI-TOF-MS of NH_2-BK-Acp-LPETGGS-CO_2H, calcd : 1815.1, observed 1816.1 $[M+H]^+$; C. MALDI-TOF-MS of DNS-LPETGGS-CO_2H, calcd : 892.9, observed 915.4 $[M+Na]^+$.

ii. Synthesis of ACE inhibitory peptides

ACE inhibitory peptides were prepared using the CEM Liberty automated microwave peptide synthesizer. Deprotection was performed with 20% piperazine in DMF. Coupling reactions were performed with 5 fold excess of Fmoc-AA-OH with 1:0.9:2 AA/HBTU/DIEA. After the incorporation of all amino acids, the resin was washed with DMF (10×), MeOH (10×), DCM (10×) and dried. The resin was treated with a solution of TFA (95%), water (2.5%) and Tis (2.5%) for 2h at room temperature for removing all the side-chain protection groups. Following cleavage the peptide was precipitated and washed in cold diethyl ether. The peptide was analyzed by HPLC and MALDI-TOF-MS. Wang resin preloaded with corresponding amino acid was used to synthesize peptide 6-10 and 2-Chlorotrity chloride resin preloaded with Proline residue was used to synthesize peptide 11.

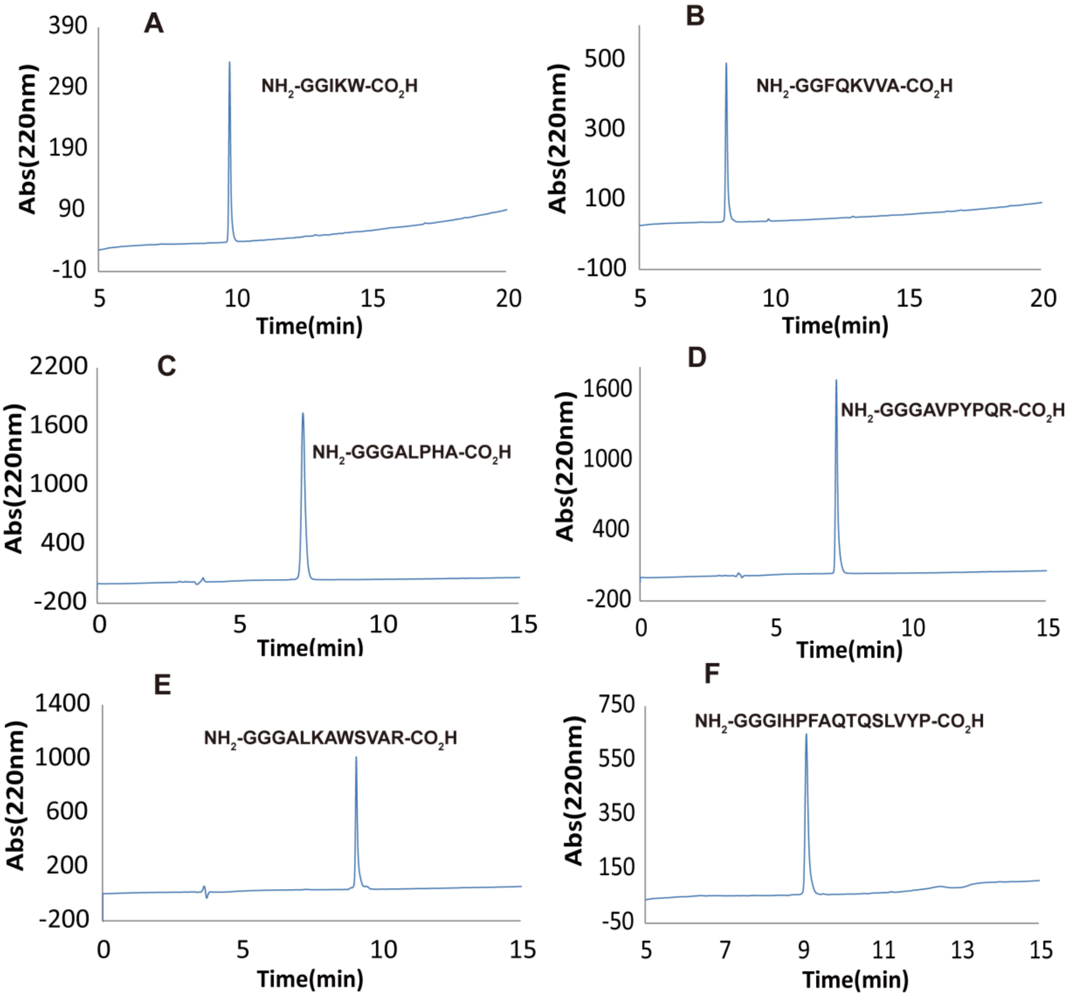
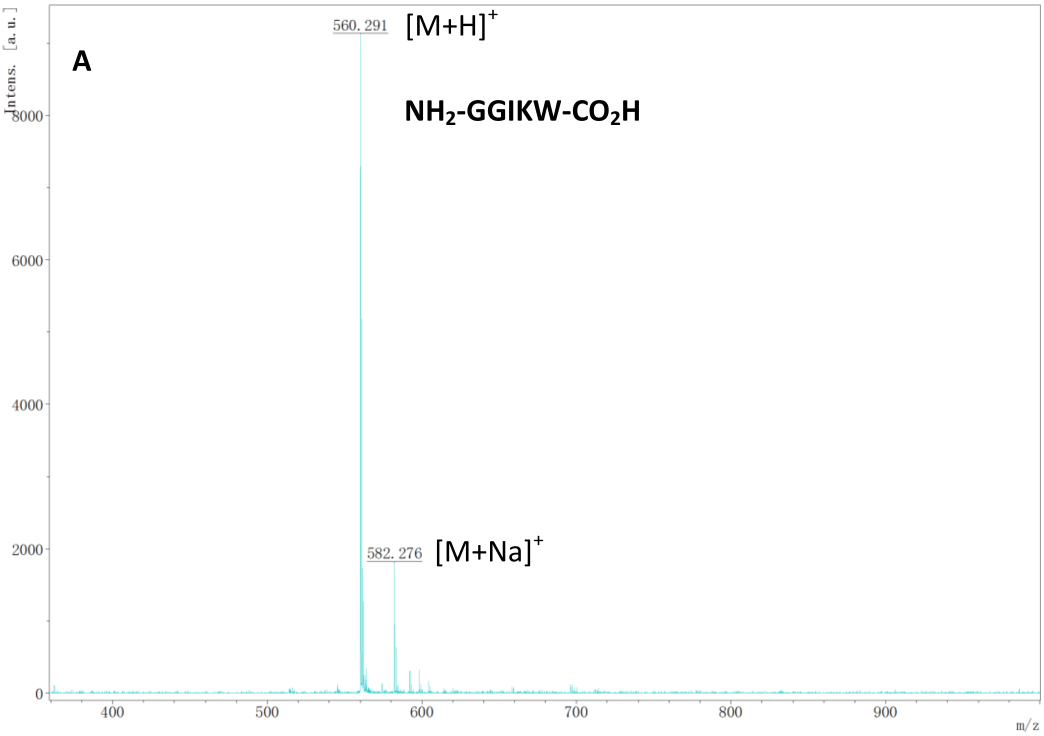
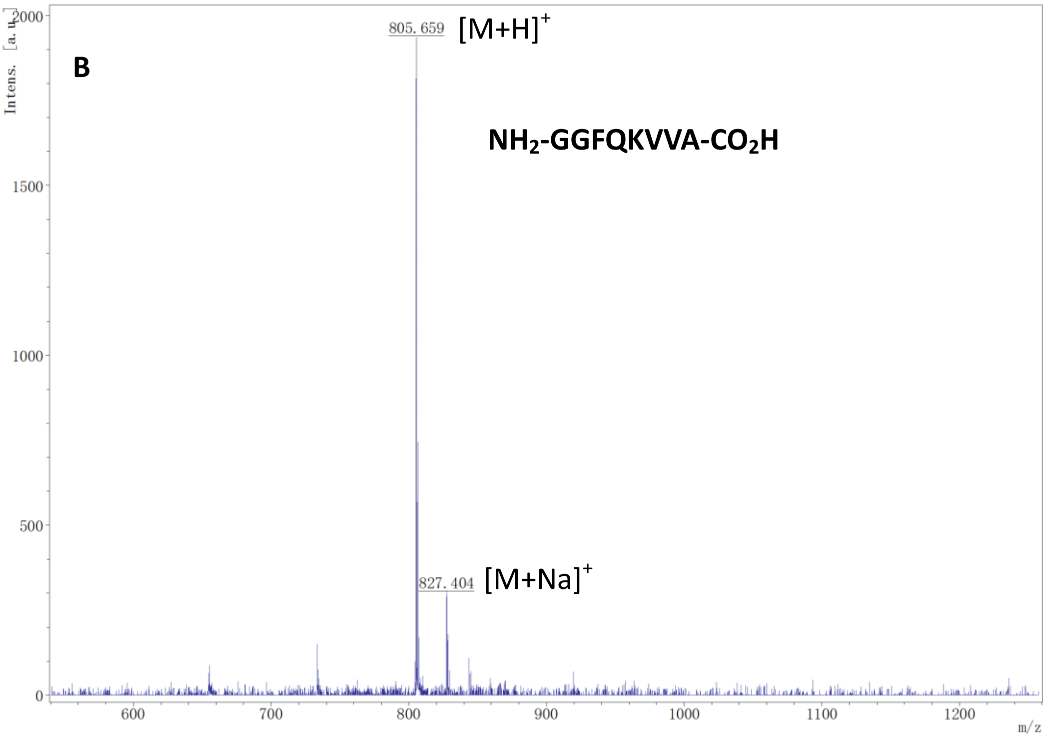


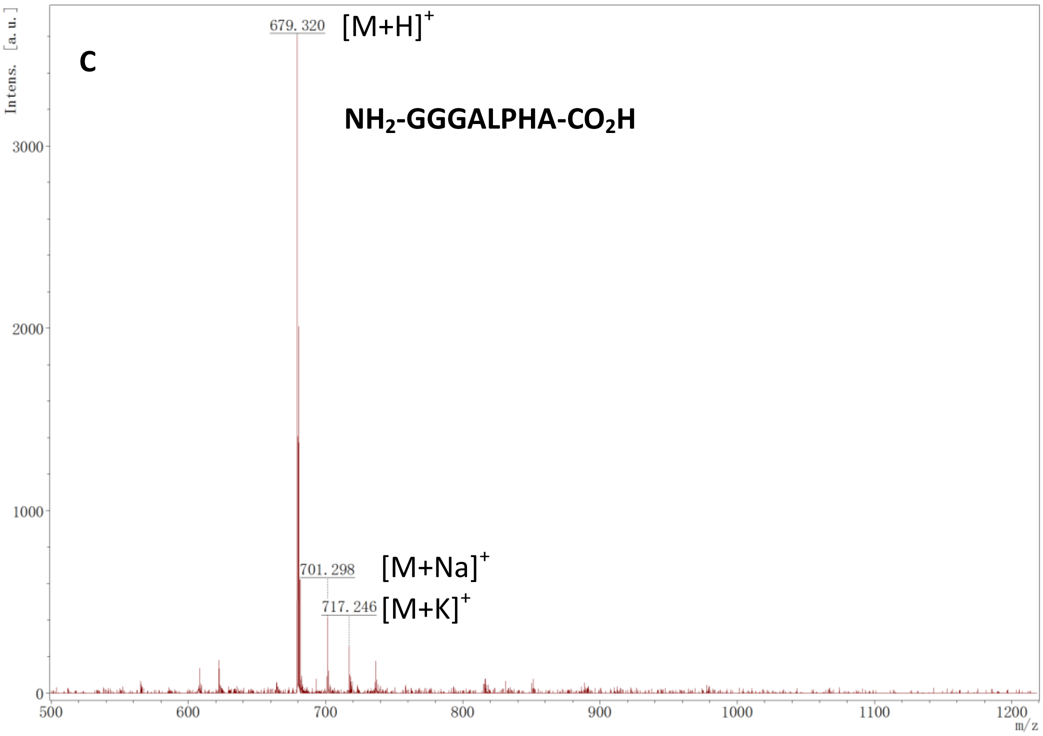
Figure S3. HPLC diagrams of ACE inhibitory peptides monitored with UV at 220 nm. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 90% ACN in H_2O in 20 min; flow rate: 1 mL/min; R.T.: NH_2-GGIKW-CO_2H (9.82 min); NH_2-GGFQKVVA-CO_2H (8.24 min); NH_2-GGGALPHA-CO_2H (7.31 min); NH_2-GGGAVPYPQR-CO_2H (7.25 min); NH_2-GGGALKAWSVAR-CO_2H (9.10 min); NH_2-GGGIHPFAQTQSLVYP-CO_2H (9.09 min).

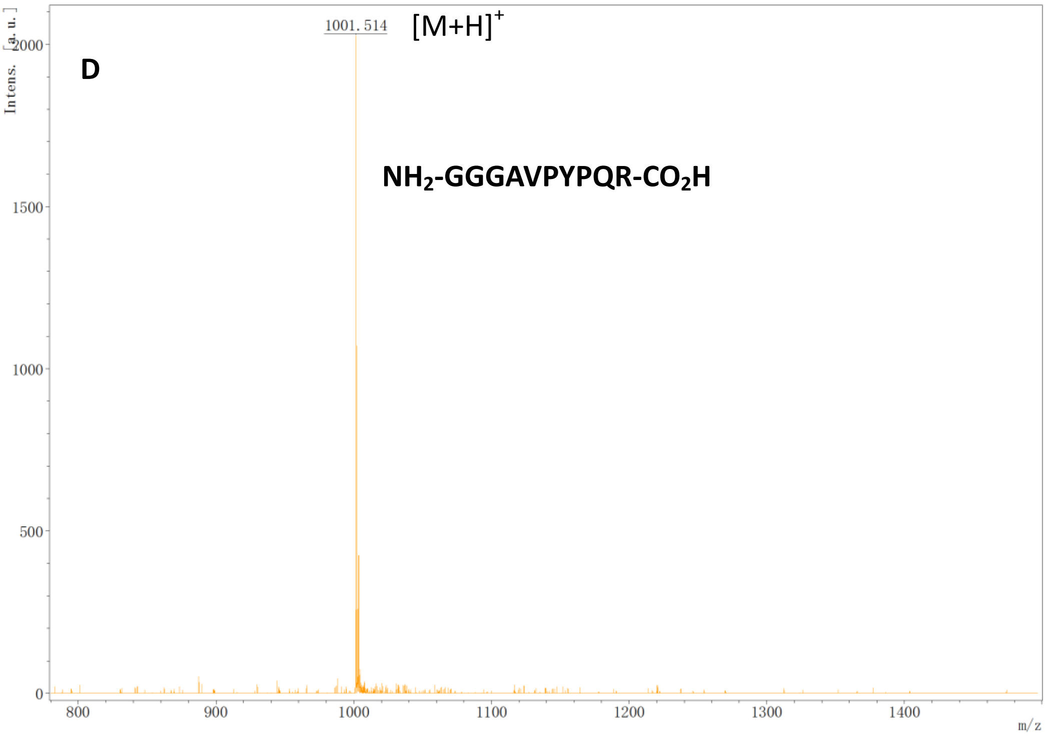


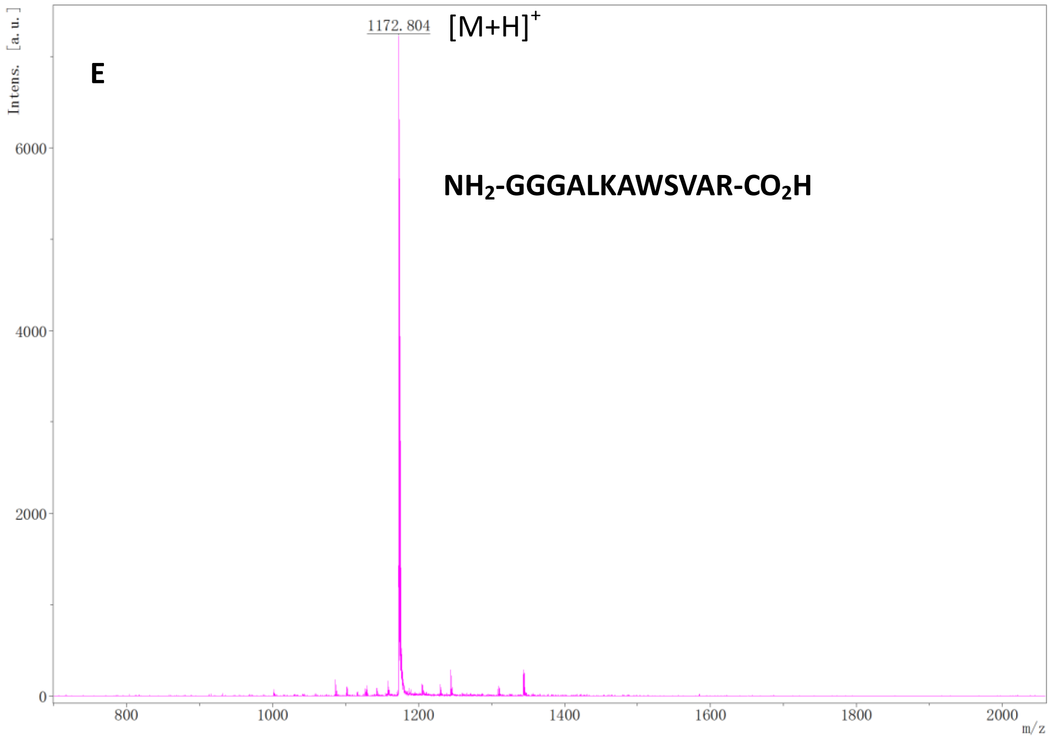
A
560.291 [M+H]+
NH2-GGIKW-CO2H
582.276 [M+Na]+
Intens. [a.u.]
m/z



B
805.659 [M+H]+
NH2-GGFQKVVA-CO2H
827.404 [M+Na]+
Intens. [a.u.]
m/z


C
679.320 [M+H]+
NH2-GGGALPHA-CO2H
701.298 [M+Na]+
717.246 [M+K]+
Intens. [a. u.]
3000
2000
1000
0
500
600
700
800
900
1000
1100
1200
m/z


D
1001.514 [M+H]+
NH2-GGGAVPYPQR-CO2H
Intens. [a. u.]
2000
1500
1000
500
0
800
900
1000
1100
1200
1300
1400
m/z



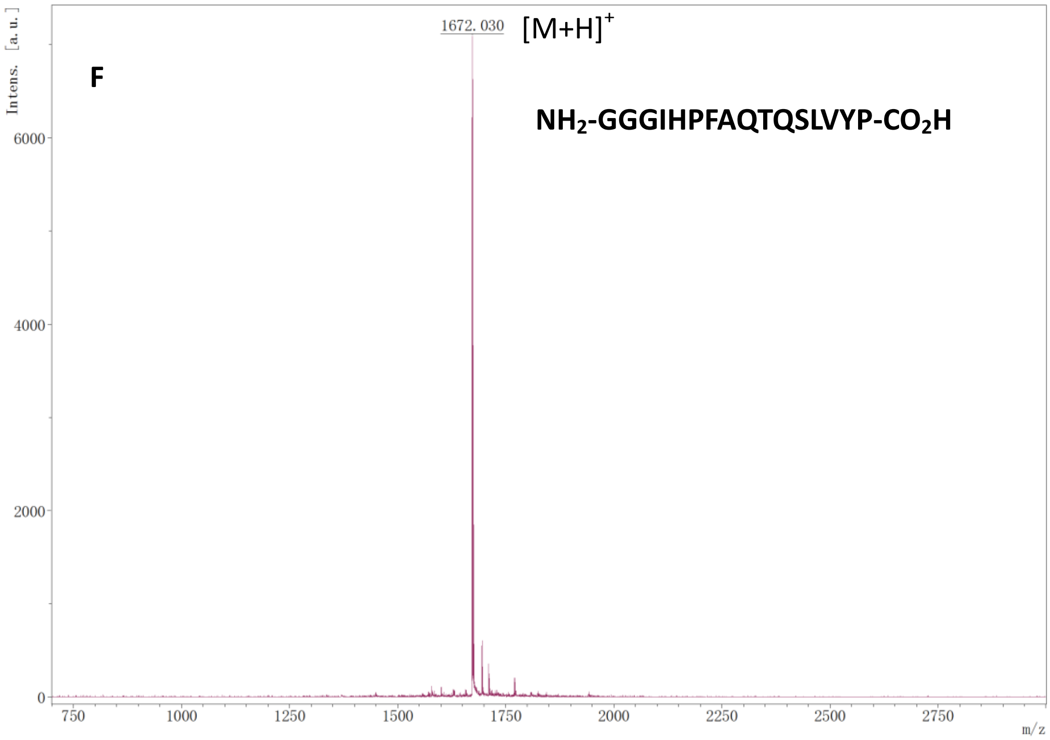
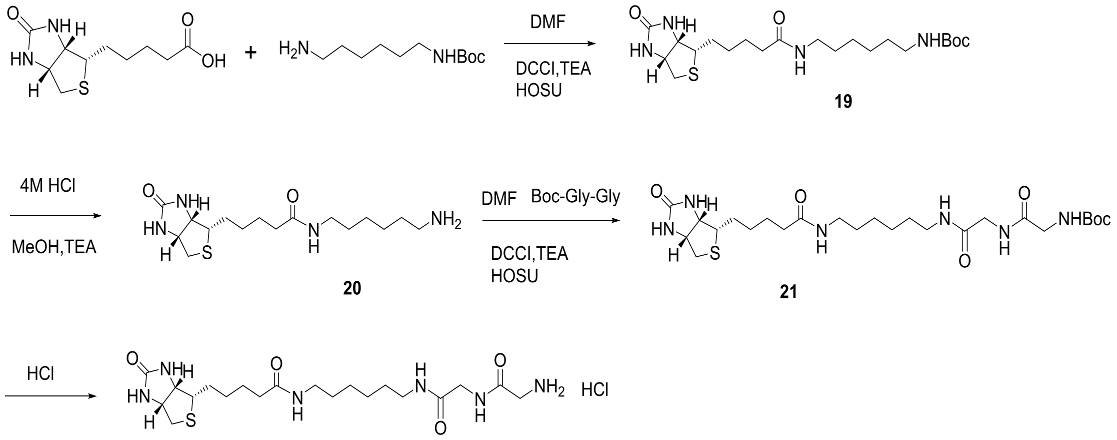


Figure S4. MALDI-TOF MS of ACE inhibitory peptides. A. NH_2-GGIKW-CO_2H, calcd : 559.6, observed 560.2 $[M+H]^+$, 582.2 $[M+Na]^+$; B. NH_2-GGFQKVVA-CO_2H, calcd : 804.9, observed 805.6 $[M+H]^+$, 827.4 $[M+Na]^+$; C. NH_2-GGGALPHA-CO_2H, calcd : 678.7, observed 679.3 $[M+H]^+$, 701.2 $[M+Na]^+$, 717.2 $[M+K]^+$; D. NH_2-GGGAVPYPQR-CO_2H, calcd : 1001.1, observed 1001.5 $[M+H]^+$; E. NH_2-GGGALKAWSVAR-CO_2H, calcd : 1172.3, observed 1172.8 $[M+H]^+$; F. NH_2-GGGIHPFAQTQSLVYP-CO_2H, calcd : 1671.8, observed 1672.0 $[M+H]^+$.

III. Synthesis and characterization of GG-HMDA-Biotin (3), GG-Lipid (12) and GG-PEG2000 (13)

i. Synthesis of GG-HMDA-Biotin **3**



3

19: Biotin (0.2 g, 0.81 mmol) was dissolved in dry dimethylformamide (DMF 4 mL), then N-hydroxysuccinimide (0.102 g, 1.1 eq) and dicyclohexycarbodiimide (0.18 g ,1.1 eq) were added . The reaction was carried out at r.t overnight, then dicyclohexyl urea was removed by filtration and N-(tert-Butoxycarbonyl)-1,6-diaminohexane (0.19 g, 1.1 eq) and TEA (0.112 mL, 1 eq) were added. After 2 h at r.t, the product was precipitated by adding cold water, it was isolated by filtration and rinsed anhydrous diethyl ether and dried in vacuo. yield 82% .

20: Compound **19** was dissolved in 4 M HCl/MeOH. After 30 min, the solvent was evaporated and the product was redissolved in the minimum amount of DCM, and TEA was added to obtain biotinamidohexylamine as the free base. The insoluble product was isolated by filtration, it was rinsed with DCM and cold diethyl ether and dried in vacuo. Yield 65%.

21: Boc-Gly-Gly-OH (0.135 g, 0.583 mmol) was dissolved in dry dimethylformamide (DMF 2 mL), then N-hydroxysuccinimide (0.073 g, 1.1 eq) and dicyclohexycarbodiimide (0.132 g, 1.1 eq) were added . The reaction was carried out at r.t overnight, then dicyclohexyl urea was removed by filtration and compound **20** (0.20 g, 1 eq) and TEA (0.08 mL, 1 eq) were added. After 5 h at r.t, the solvent was removed under reduced pressure, then purified by flash column chromatography (DCM:MeOH=15:1 to 12:1). Yield 40%. ^{1}H NMR (400 MHz, DMSO) δ = 8.06 (t, J = 5.6 Hz, 1H), 7.73 (m, 2H), 7.08 (t, J = 5.8 Hz, 1H), 6.45 (s, 1H), 6.38 (s, 1H), 4.31 (dd, J = 7.5, 5.2 Hz, 1H), 4.18 – 4.08 (m, 1H), 3.65 (d, J = 5.7 Hz, 2H), 3.55 (d, J = 5.9 Hz, 2H), 3.06 (m, 5H), 2.82 (dd, J = 12.4, 5.1 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.04 (t, J = 7.4 Hz, 2H), 1.61 (m, 1H), 1.49 (m, 3H), 1.39 (s, 9H), 1.32 (m, 5H), 1.24 (s, 5H).

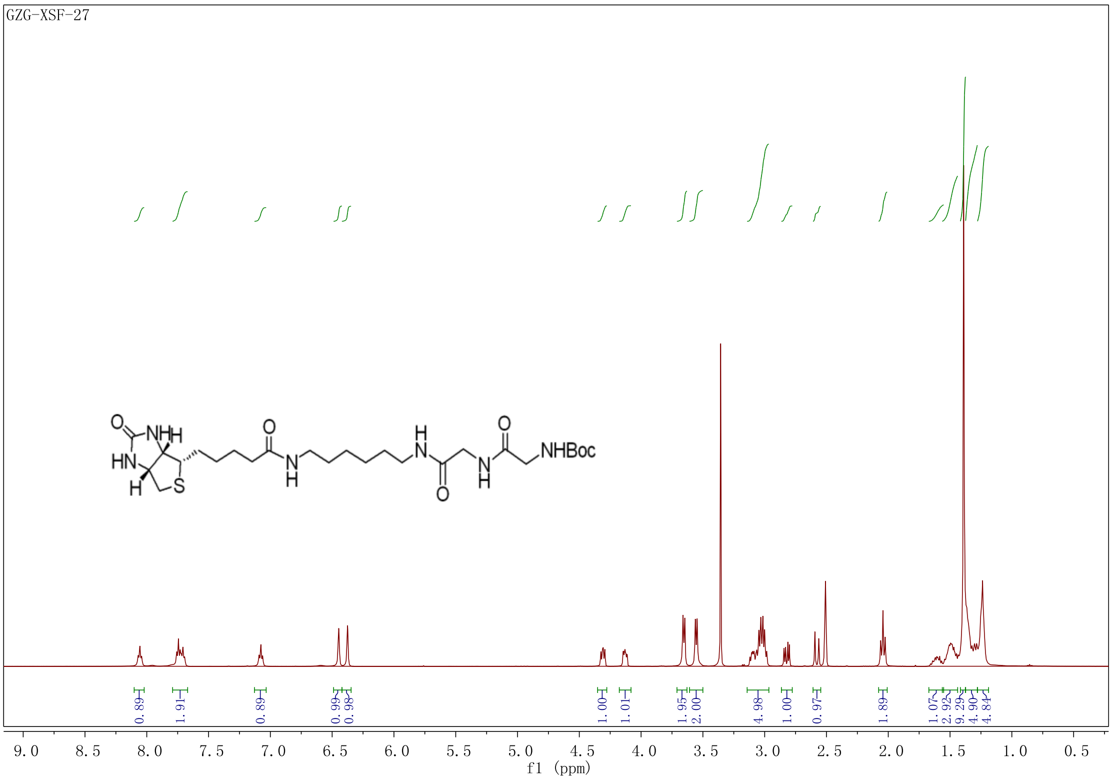


Figure S5. ^{1}H-NMR of **21** at 400 MHz in DMSO.

3: Compound **21** (100 mg) was dissolved in the DCM (2m) at 0 ^oC, then 1 mL HCl in 1 mL dioxane was added and stirred for 30 min at r.t, then the solvents were removed with toluene in vacuo to give **3** in quantitive yield. ^{1}H NMR (400 MHz, D_2O) δ = 4.57 – 4.51 (t, 1H), 4.35 (m, 1H), 3.88 (s, 1H), 3.82 (s, 1H), 3.29– 3.24 (m, 1H), 3.08 – 3.18 (m, 4H), 2.93 (dd, J = 13.0, 4.9 Hz, 1H), 2.71 (d, J = 13.1 Hz, 1H), 2.18 (t, J = 7.0 Hz, 2H), 1.66 – 1.53 (m, 4H), 1.46 – 1.42 (m, 4H), 1.37-1.32 (m, 2H), 1.25 (s, 4H). ^{13}C NMR (100 MHz, D_2O) δ = 176.6, 170.7, 167.7, 165.4, 62.2, 60.3, 55.4, 42.5, 40.6, 39.8, 39.4 , 39.3,35.6, 28.3, 27.9 , 27.7,25.7, 25.3.

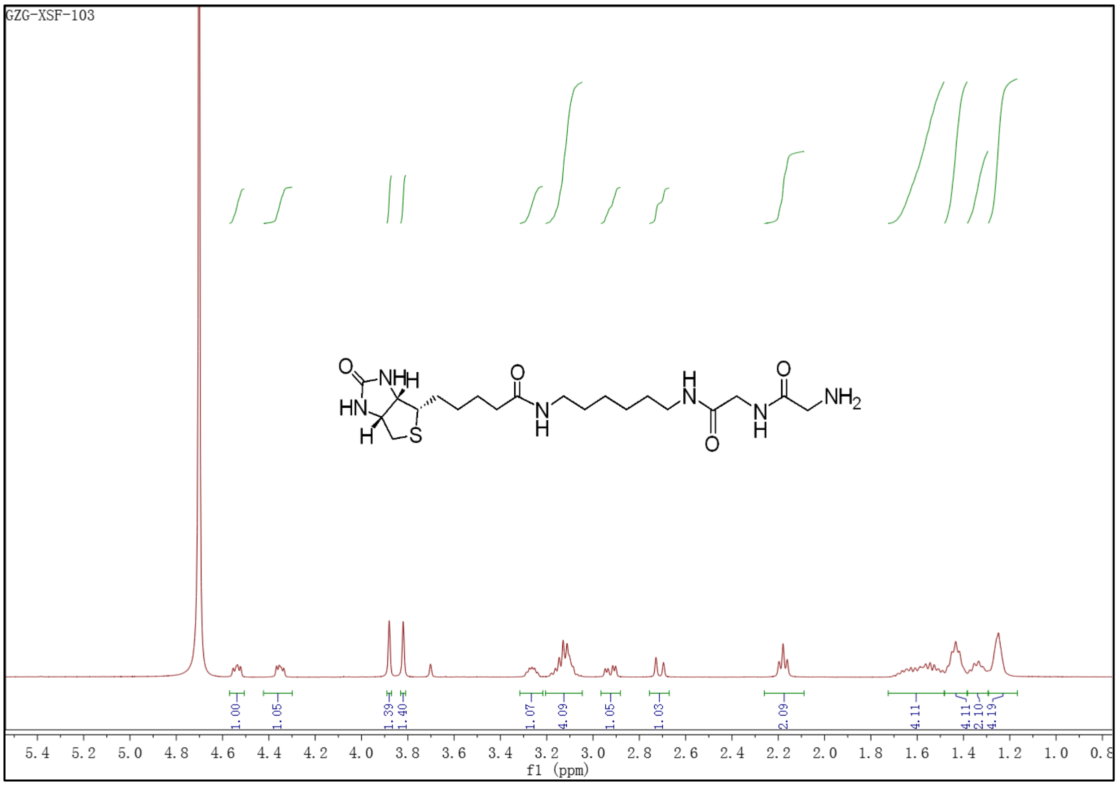


Figure S6. ^{1}H-NMR of **3** at 400 MHz in D_2O.

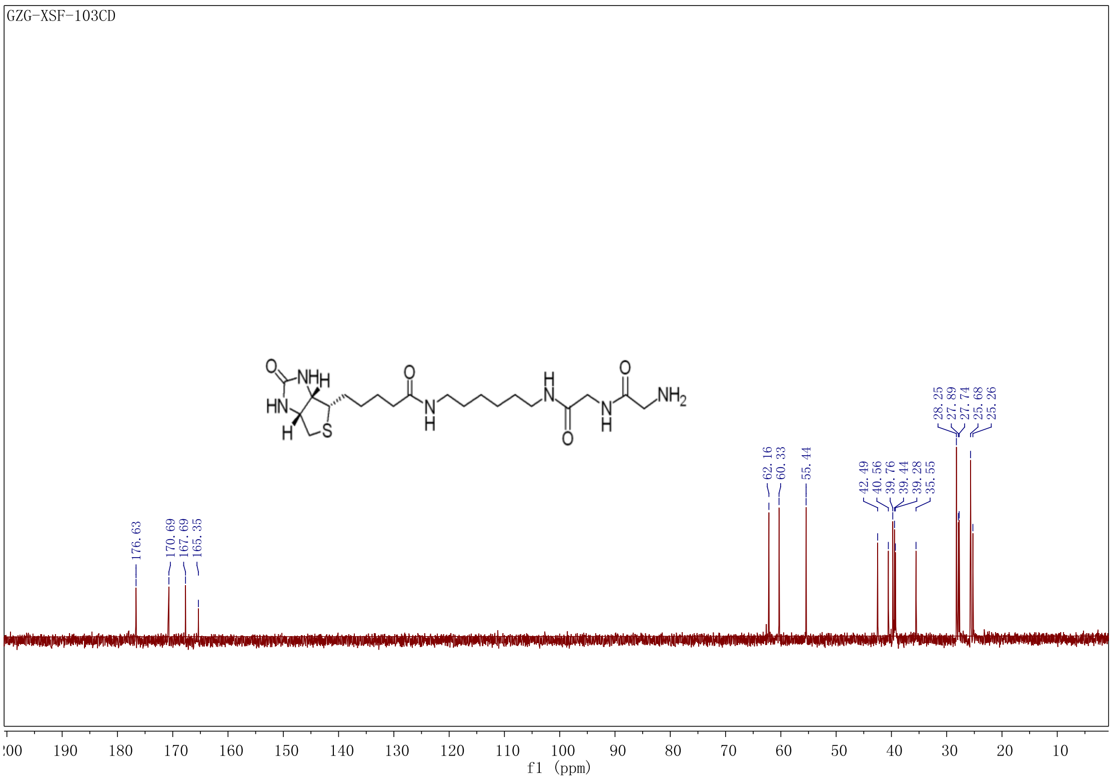
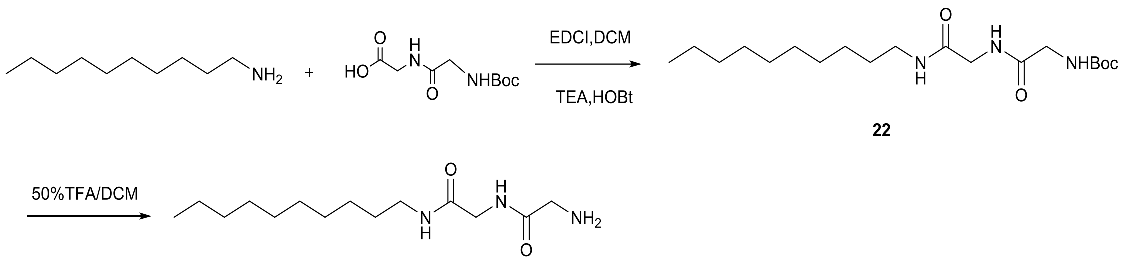


Figure S7.^{13}C-NMR of **3** at 100 MHz in D_2O.

ii. Synthesis of GG-Lipid **12**



22: Boc-Gly-Gly-OH (1.2 eq) was dissolved in DCM, then added EDCI (1.2 eq), HOBt (1.2 eq), and stirred at 0 °C for 30 min. Then TEA (2 eq) and alkylamine were added and reaction mixture was stirring at r.t for 34 h. The reaction mixture was washed with water and the organic was dried over Na_2SO_4. The solvent was removed in vacuo, then purified by flash column chromatography (DCM:MeOH =50:1 to 40:1). Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.95 (s, 1H), 6.40 (s, 1H), 5.28 (t, J = 5.7 Hz, 1H), 3.86 (d, J = 5.6 Hz, 2H), 3.75 (d, J = 5.7 Hz, 2H), 3.16 (dd, J = 13.3, 7.0 Hz, 2H), 1.45 – 1.41 (m, 2H), 1.38 (s, 9H), 1.19 (s, 14H), 0.81 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.0, 167.5, 155.3, 79.6, 43.4, 42.1, 38.7, 30.9, 28.5(2C), 28.4, 28.3 (2C), 27.3, 25.9, 21.7, 13.1.

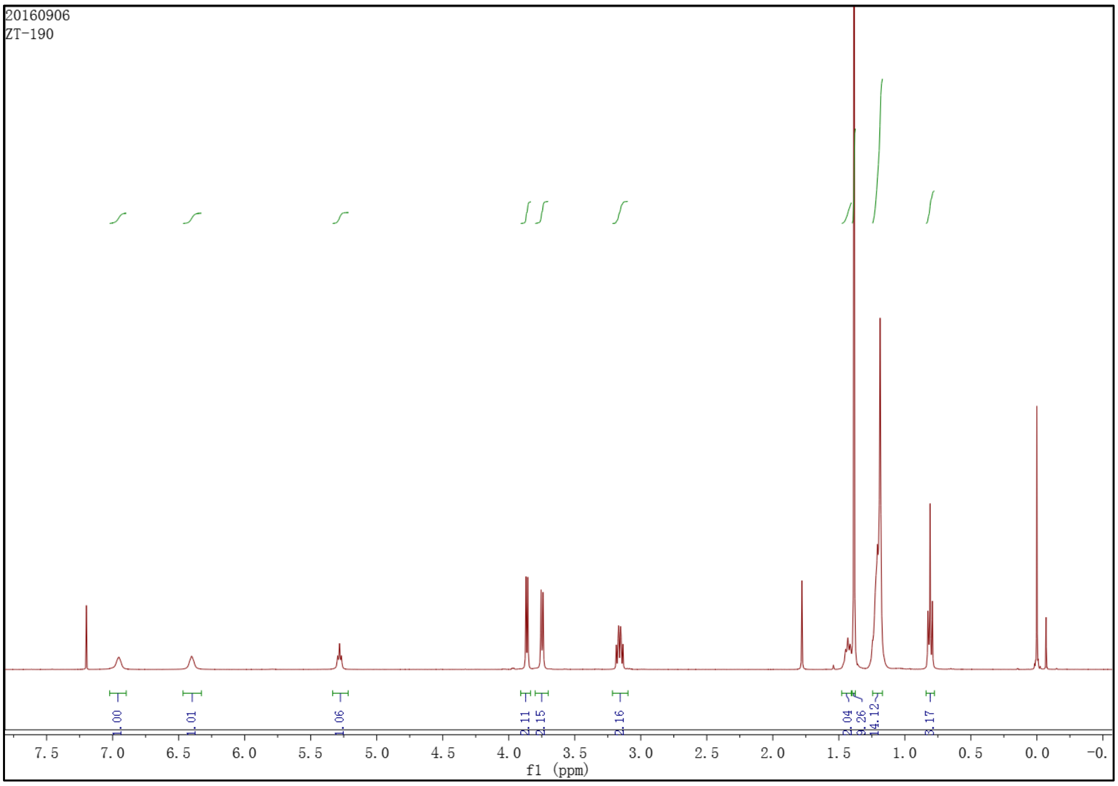


Figure S8. ^{1}H-NMR of **22** at 400 MHz in $CDCl_3$.

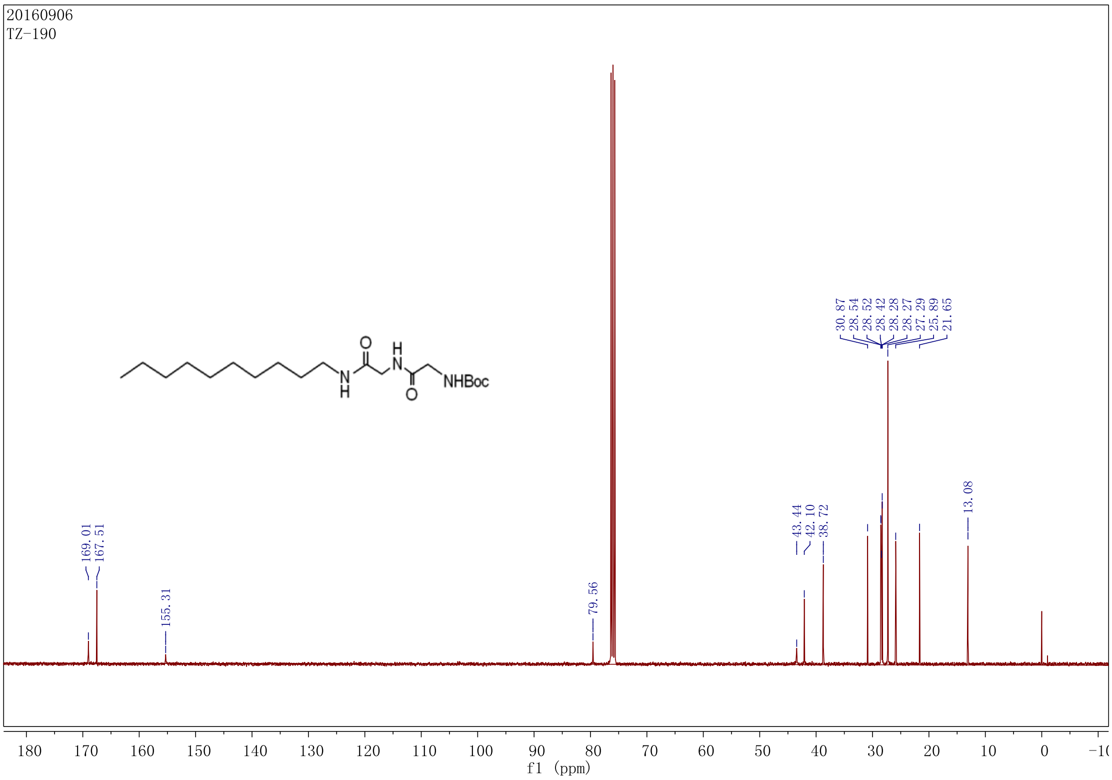


Figure S9.^{13}C-NMR of **22** at 100 MHz in $CDCl_3$.

12: Compound **22** was dissolved in the 50% TFA/DCM at 0 °C, stirred 30 min at r.t, then removed the solvents with toluene in vacuo. ^{1}H NMR (400 MHz, MeOD) δ = 3.91 (s, 2H), 3.75 (s, 2H), 3.20 (t, J = 7.1 Hz, 2H), 1.52-1.49 (t, J =7.2 Hz, 2H), 1.30 (s, 14H), 0.91 (t, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ = 169.5, 166.5, 41.8, 40.1, 39.1, 31.6, 29.3, 29.0, 26.6, 22.3, 13.0.

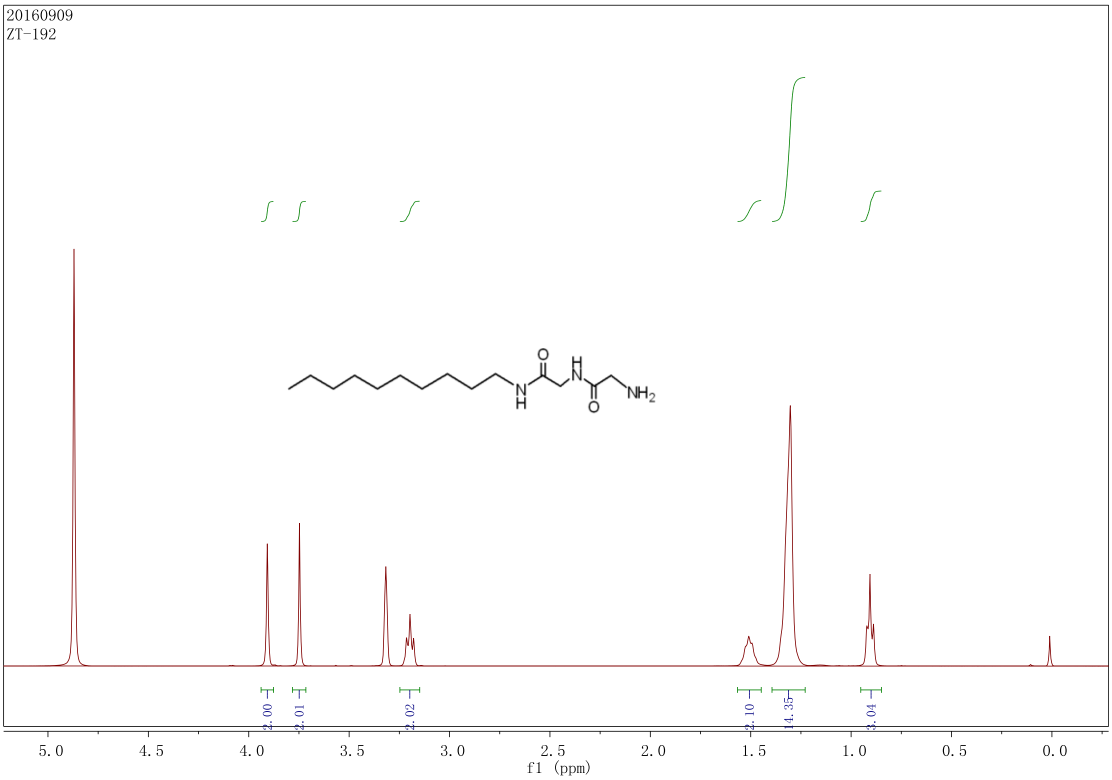


Figure S10. ^{1}H-NMR of **12** at 400 MHz in MeOD.

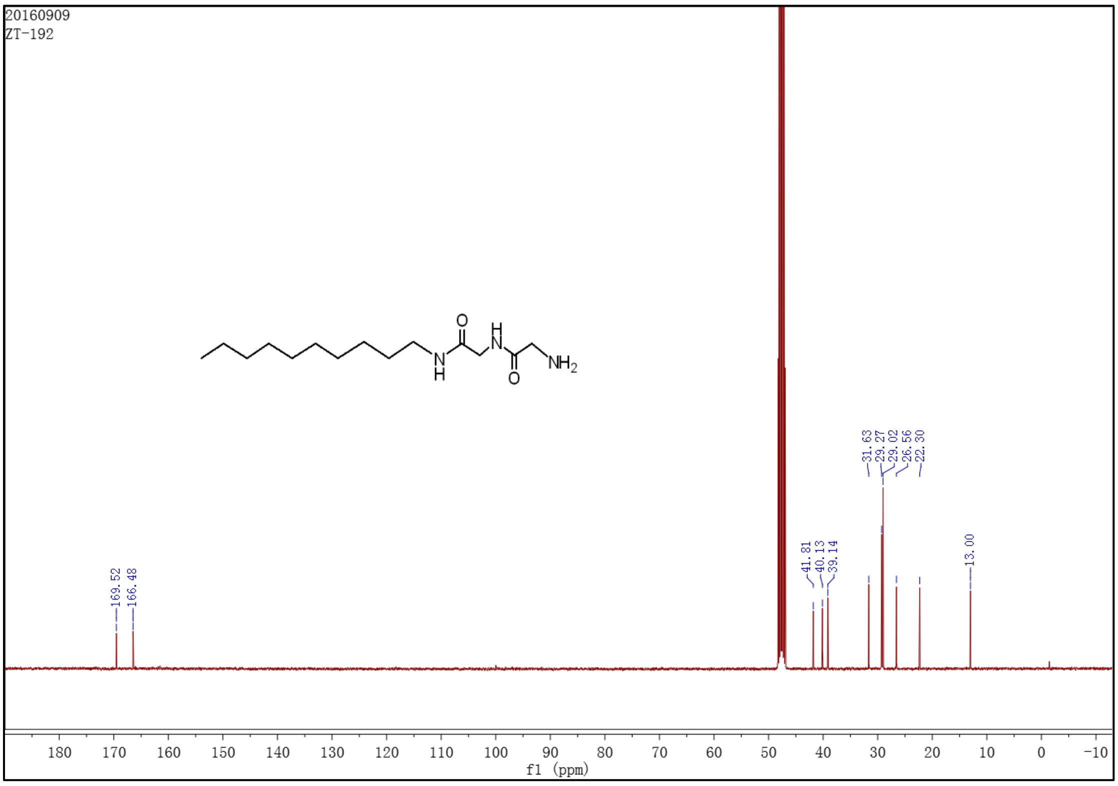


Figure S11.[13]C-NMR of **12** at 100 MHz in MeOD.

iii. Synthesis of GG-PEG2000 **13**

23: Boc-Gly-Gly-OH (1 g, 4.3 mmol), N-hydroxysuccinimide (0.5 g, 1 eq) and dicyclohexycarbodiimide (0.887 g, 1 eq) were added at 0 °C. Then the reaction mixture was stirred for 2 h at 0 °C and for 20 h at r.t. After the precipitated dicyclohexylured was filtered and wash with DCM, organic phase was evaporated under reduced pressure. The residue was recrystallized from hot isopropyl alcohol. Yield 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.95 (s, 1H), 5.30 (s, 1H), 4.41 (d, J = 5.6 Hz, 2H), 3.86 (d, J = 5.1 Hz, 2H), 2.84 (s, 4H), 1.44 (s, 9H).

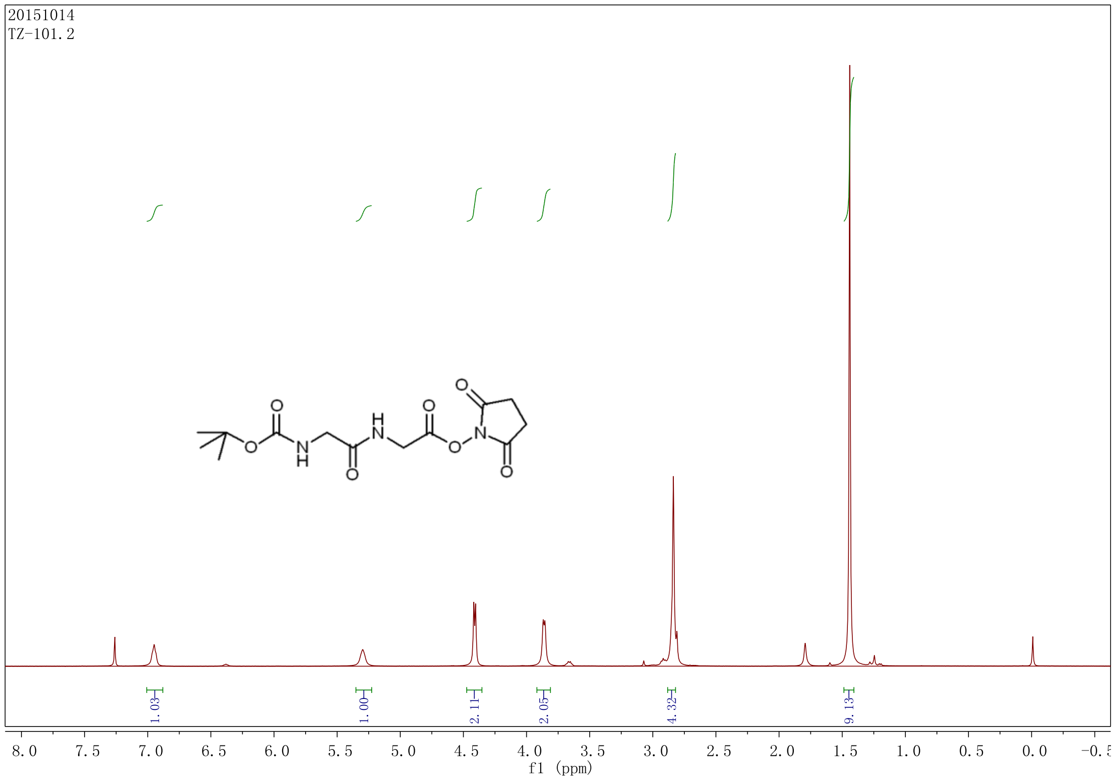


Figure S12. ^{1}H-NMR of **23** at 400 MHz in $CDCl_3$.

24: After PEG2000-NH_2 (0.1 g, 0.05 mmol) was dissolved in DCM, compound **23** (0.033 g, 2 eq) and TEA (14 µL, 2 eq) were added to the solution. The reaction was stirred at r.t overnight. The solution was concentrated under reduced pressure, purified by flash column chromatography to give 22. (DCM: MeOH =30:1, 25:1, 20:1). Yield 85%. ^{1}H NMR (400 MHz, MeOD) δ = 3.89 (s, 2H), 3.77 (s, 2H), 3.65(s, PEG), 3.38 (s, 3H), 1.48 (s, 9H).

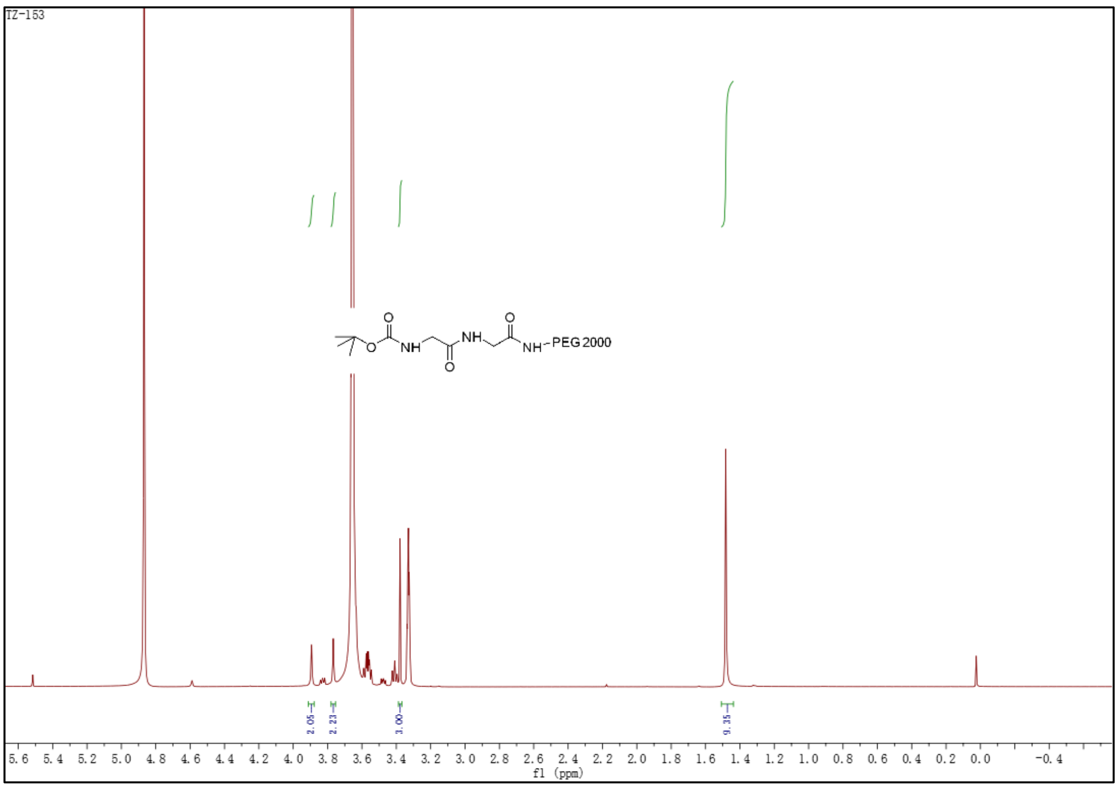


Figure S13. ^{1}H-NMR of **24** at 400 MHz in MeOD.

13: Compound **24** was dissolved in the 50% TFA/DCM at 0 °C, stirred 30min at r.t, then removed the solvents with toluene in vacuo to give 13. ^{1}H NMR (400 MHz, D_2O) δ = 3.91 (s, 2H), 3.82 (s, 2H), 3.63(s, PEG), 3.31 (s, 3H).

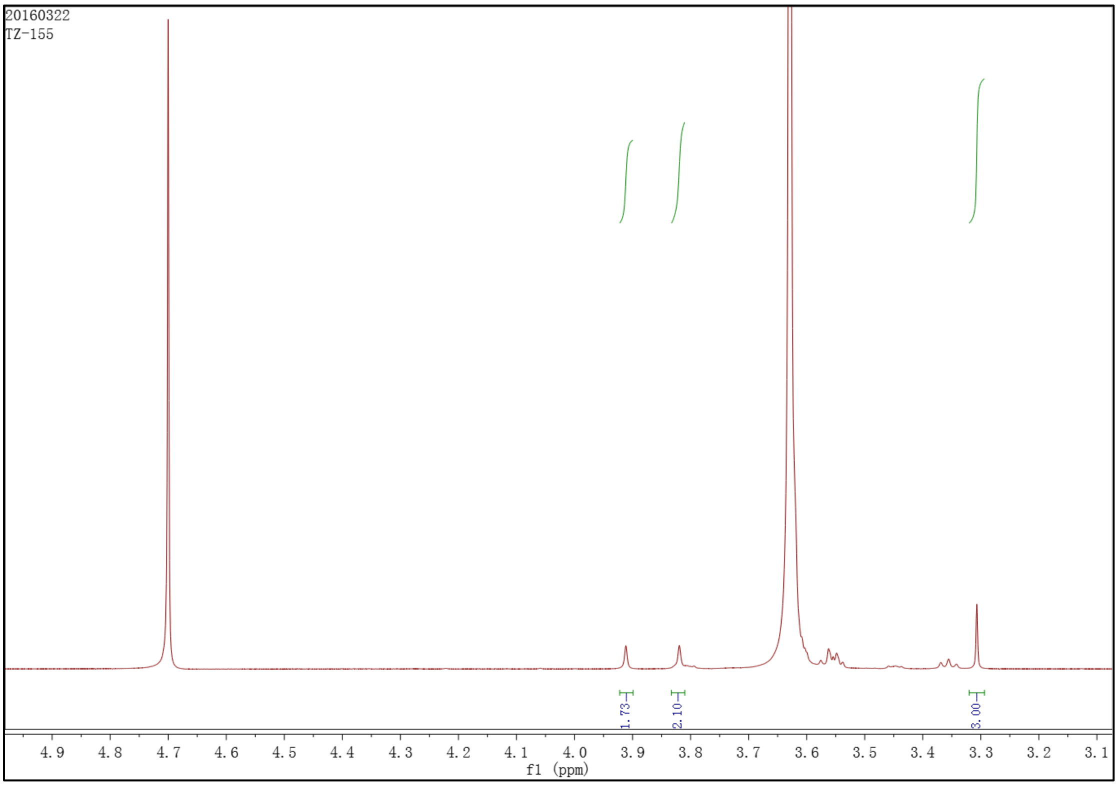


Figure S14. ^{1}H-NMR of **13** at 400 MHz in D_2O.

IV. Sortase-mediated ligation studies

i. Sortase-mediated cleavage of **1**, **5** and **17**

Dried peptide resin (0.6 µmol) was swollen in 100µl reaction buffer (Tris-HCl 0.3 M pH 7.5, 150 mM NaCl, 5 Mm $CaCl_2$, and 0.5 mM mercaptoethanol) for 30 min. Then 20 µL SrtA (100 µM dissolved in buffer) and 80 µL buffer was added to reach total 200 µL. After the reaction was kept at 37 °C for 20 h, 20 µL of the reaction mixture was withdrawn and quenched with the same volume of 0.1% of TFA. The aliquots were then analyzed by HPLC using a RP C18 column and the fractions were analyzed by MALDI-TOF MS.

In large scale reaction: 10 mg of dry peptide PEGA resin **1**, **5** and **17** was treated with SrtA for 20 h. After filtration and isolation by HPLC. The yield of product **2**, NH_2-BK-Acp-LPET-CO_2H and DNS-LPET-CO_2H was 87.5% (1.1 mg), 73.2% (2.3 mg) and 80% (1.2 mg), respectively.

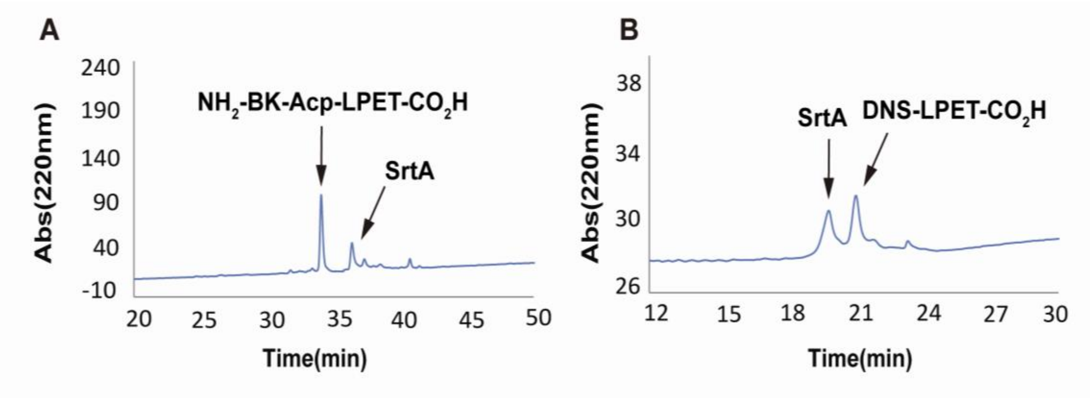
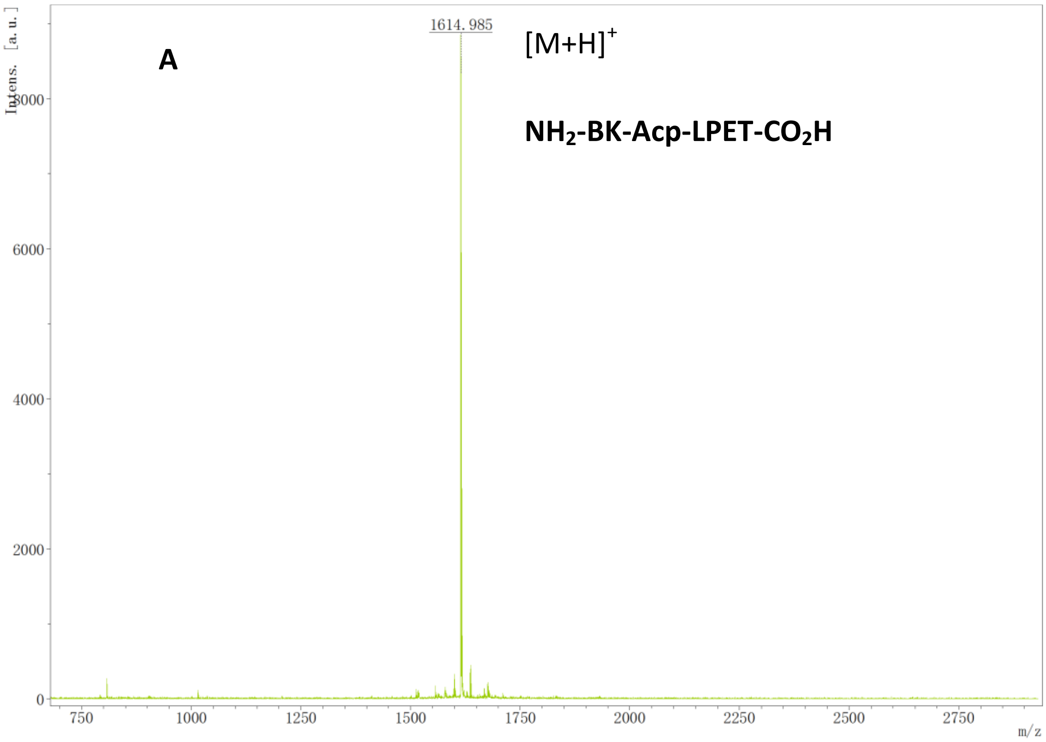


Figure S15. HPLC profile of NH_2-BK-Acp-LPET-CO_2H and DNS-LPET-CO_2H cleaved from PEGA resin by SrtA, monitored with UV at 220 nm. A. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 40% ACN in H_2O in 50 min; flow rate: 1 mL/min; R.T.: NH_2-BK-Acp-LPET-CO_2H (34.03 min); SrtA (36.41 min). B. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 50% ACN in H_2O in 30 min; flow rate: 1 mL/min; R.T.: DNS-LPET-CO_2H (21.05 min); SrtA (19.92 min).



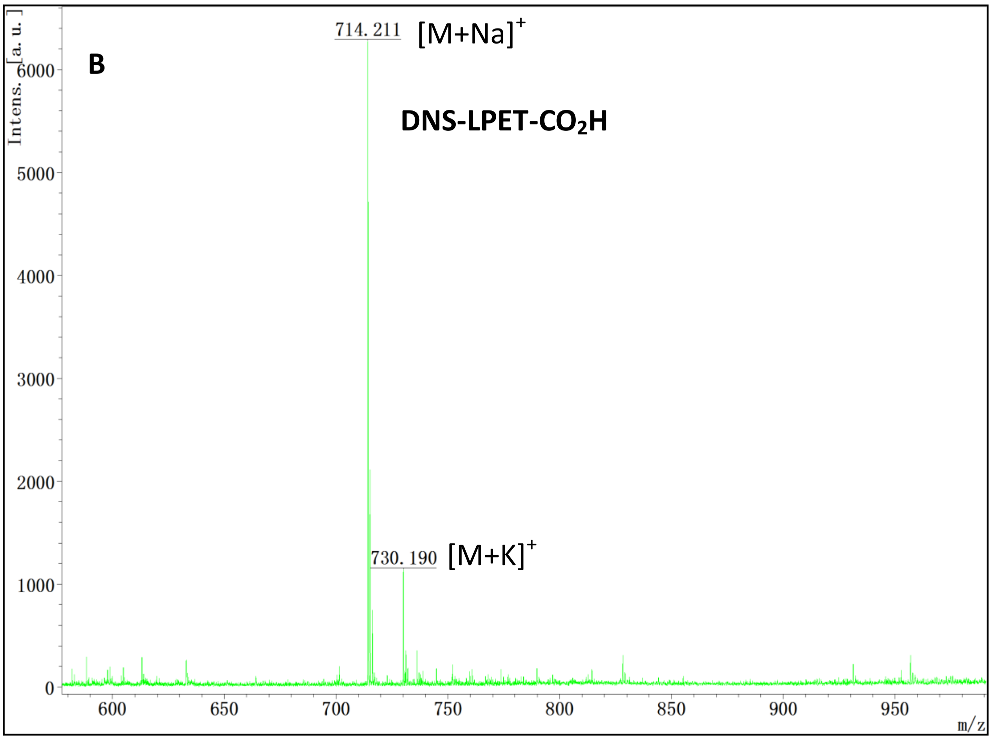


Figure S16. MALDI-TOF MS of NH_2-BK-Acp-LPET-CO_2H and DNS-LPET-CO_2H. A. NH_2-BK-Acp-LPET-CO_2H, calcd : 1613.8, observed 1614.9 $[M+H]^+$. B. DNS-LPET-CO_2H, calcd : 691.8, observed 714.2 $[M+Na]^+$, 730.2 $[M+K]^+$.

ii. Sortase-mediated ligation of **1** and **5** with polyglycine nucleophiles in one-pot

Dried peptide resin (0.6 µmol) was swollen in 100 µL reaction buffer (Tris-HCl 0.3 M pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, and 0.5 mM mercaptoethanol) for 30min. Then 20 µL SrtA (100 µM dissolved in buffer) and 5 eq polyglycine nucleophiles were added. The final volume was 200ul. After the reaction was kept at 37 ^oC for 20 h, 20 µL of the reaction mixture was withdrawn and quenched with the same volume of 0.1% of TFA. The aliquots were then analyzed by HPLC using a RP C18 column and the fractions were analyzed by MALDI-TOF MS.

In large scale reaction: 10 mg of dry peptide PEGA resin **1** was treated with SrtA and **3** (5 eq) for 20 h. 1.5 mg of product **4** (yield 68%) was afforded after isolation by semi preparative HPLC. For reaction of peptide PEGA resin **5** with **3**, **12** and **13**, 10 mg of dry peptide PEGA resin **5** was treated with SrtA and **3** (5 eq)**, 12** (5 eq) and **13** (5 eq) for 20 h respectively. After filtration and isolation by HPLC, the yield of product **14**, **15** and **16** was 71% (2.9 mg) , 61% (2.5 mg) and 65% (4.8 mg) respectively. For reaction of peptide PEGA resin **5** with ACE inhibitory peptides, ACE inhibitory peptides (5 eq) and SrtA were added to 10 mg peptide PEGA resin **5** and reacted for 20 h. The product was isolated by HPLC. The yield of produc of **6**, **7**, **8**, **9**, **10** and **11** was 57% (2.4 mg) , 53% (2.5 mg) , 52% (2.3 mg), 63% (3.2 mg), 64% (3.5 mg) and 68% (4.3 mg) respectively.

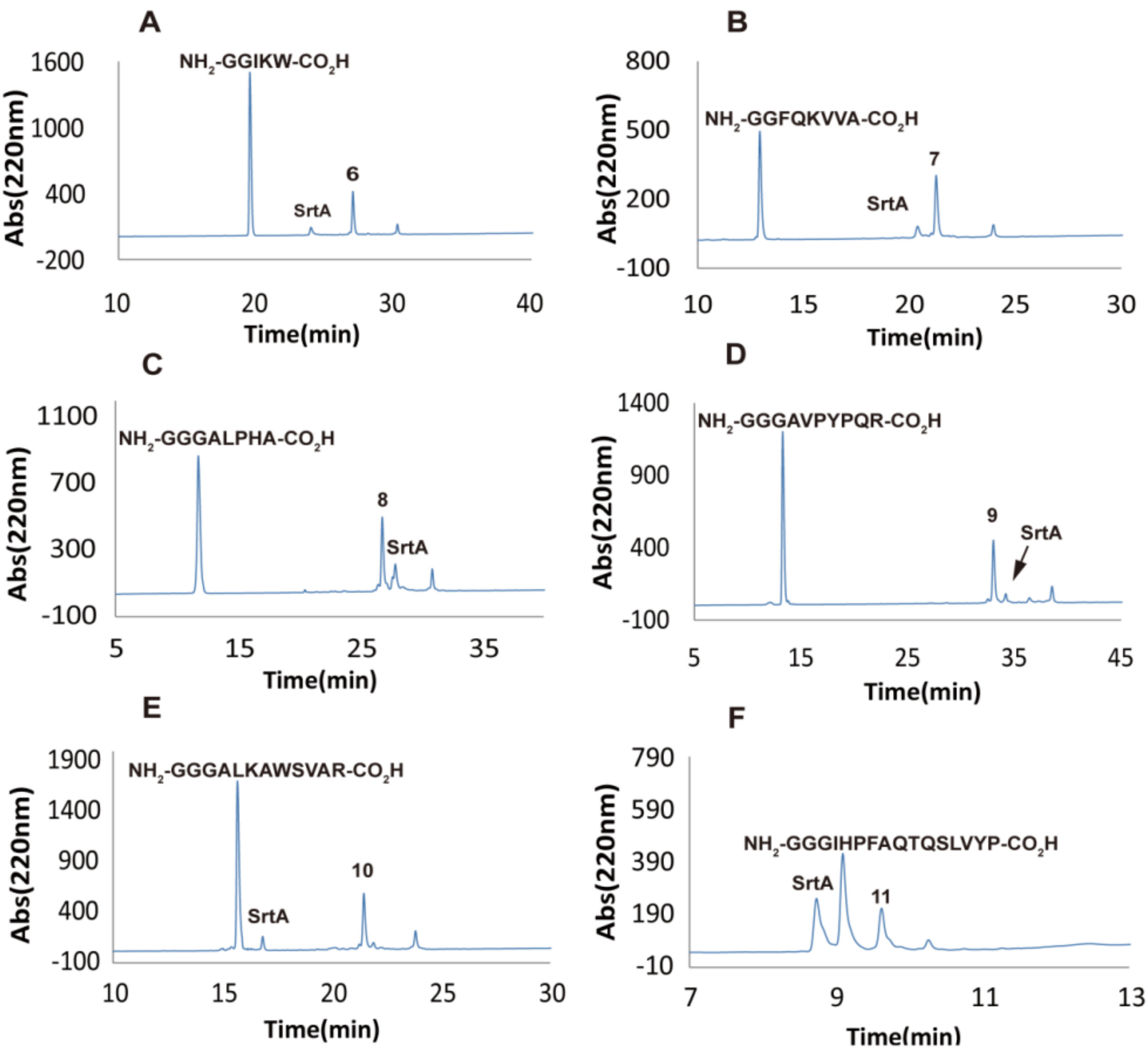
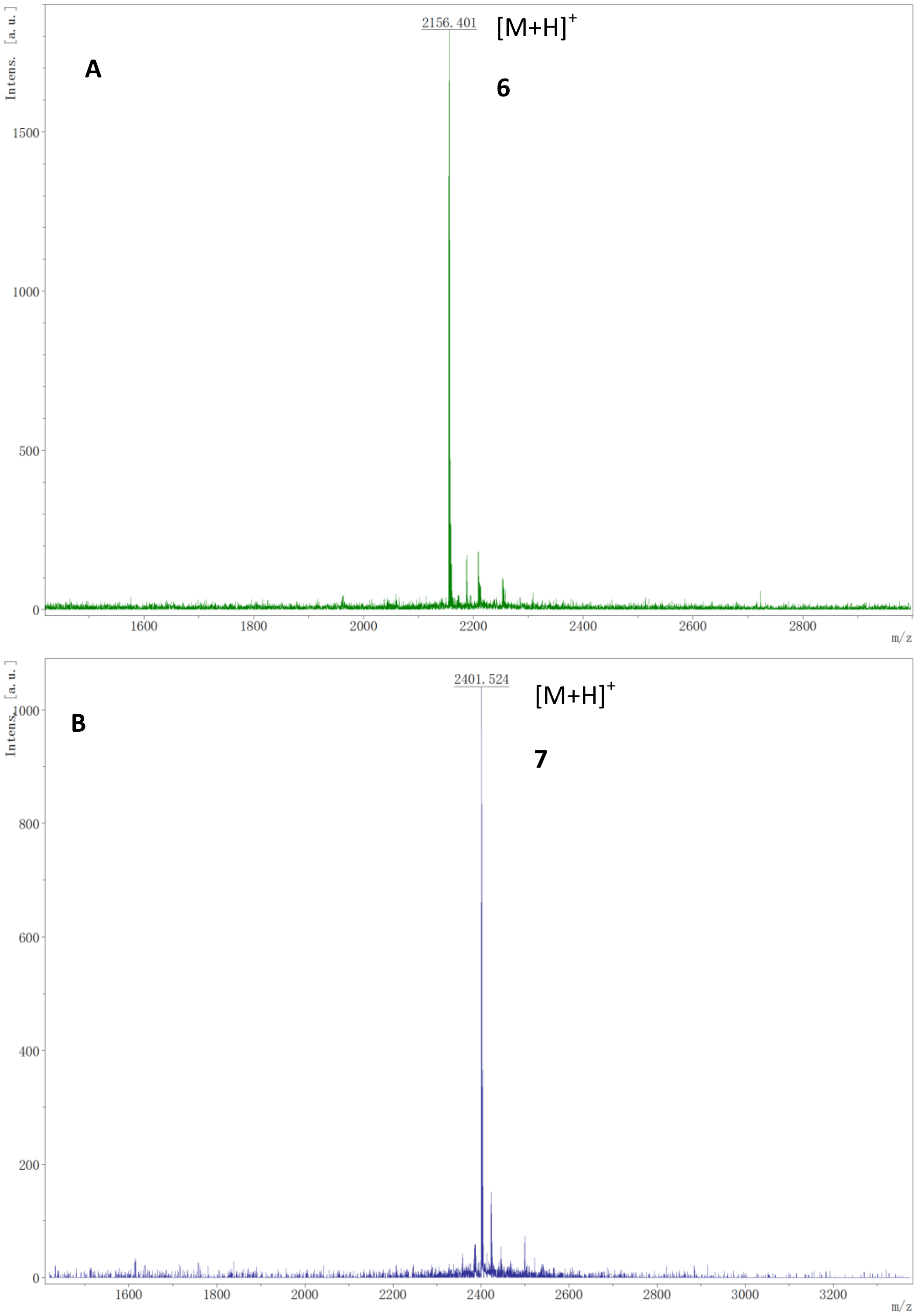
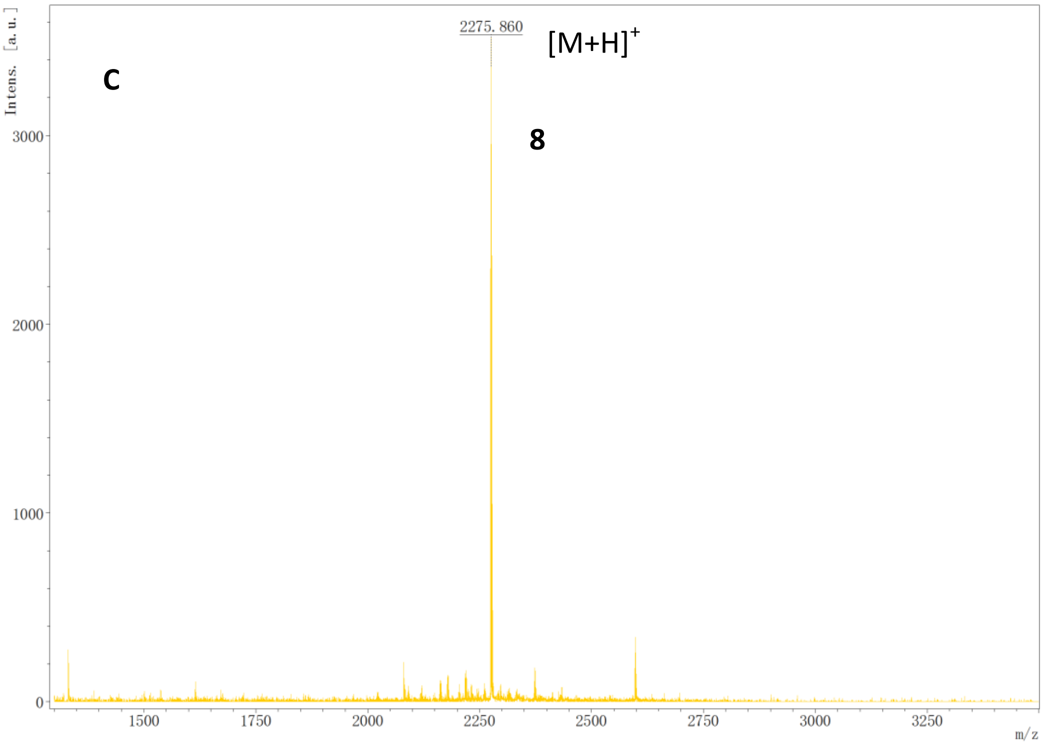


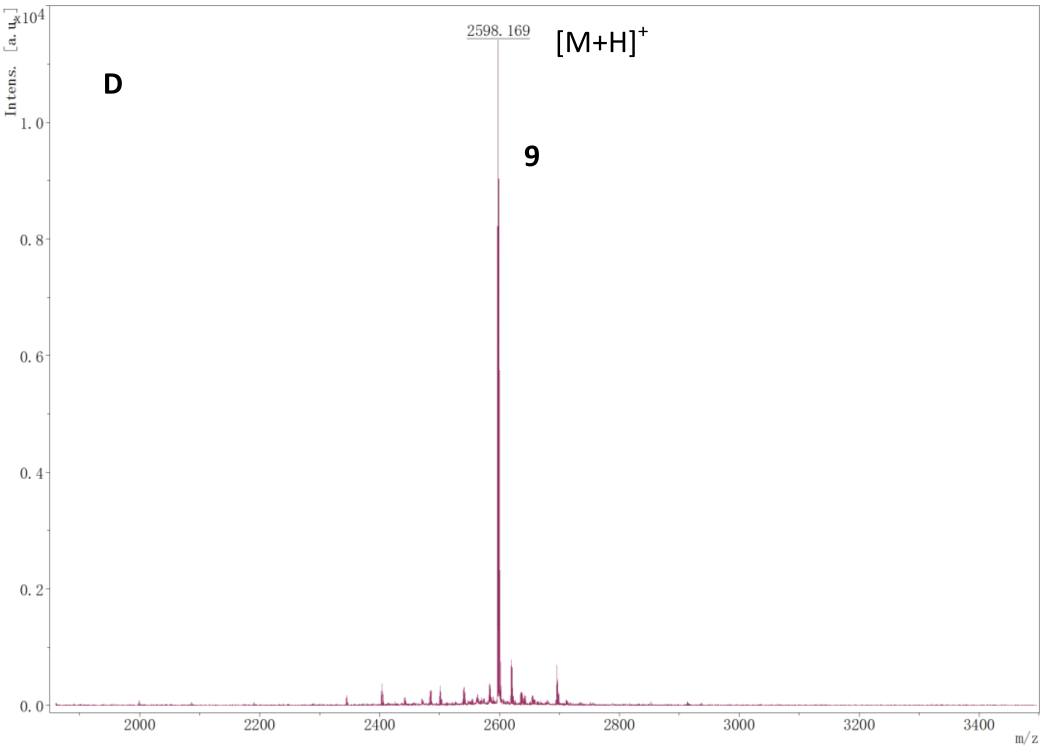
Figure S17. HPLC profile of ligation of BK peptide with ACE inhibitory peptides in one-pot on PEGA resin, monitored with UV at 220 nm. A. Ligation of BK peptide with NH_2-GGIKW-CO_2H. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 60% ACN in H_2O in 50 min; flow rate: 1 mL/min; R.T.: **6** (27.01 min); B. Ligation of BK peptide with NH_2-GGFQKVVA-CO_2H. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 60% ACN in H_2O in 40 min; flow rate: 1 mL/min; R.T.: **7** (21.24 min); C. Ligation of BK peptide with NH_2-GGGALPHA-CO_2H. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 60% ACN in H_2O in 60 min; flow rate: 1 mL/min; R.T.: **8** (26.78 min); D. Ligation of BK peptide with NH_2-GGGAVPYPQR-CO_2H. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 40% ACN in H_2O in 50 min; flow rate: 1 mL/min; R.T.: **9** (33.06 min); E. Ligation of BK peptide with NH_2-GGGALKAWSVAR-CO_2H. HPLC conditions: 10% ACN in H_2O (both containing 0.1% TFA) to 60% ACN in H_2O in 60 min; flow rate: 1 mL/min; R.T.: **10**(29.04 min); F. Ligation of BK peptide with NH_2-GGGIHPFAQTQSLVYP-CO_2H. HPLC conditions: 5% ACN in H_2O (both containing 0.1% TFA) to 95% ACN in H_2O in 10 min; flow rate: 1 mL/min; R.T.: **11** (9.64 min).



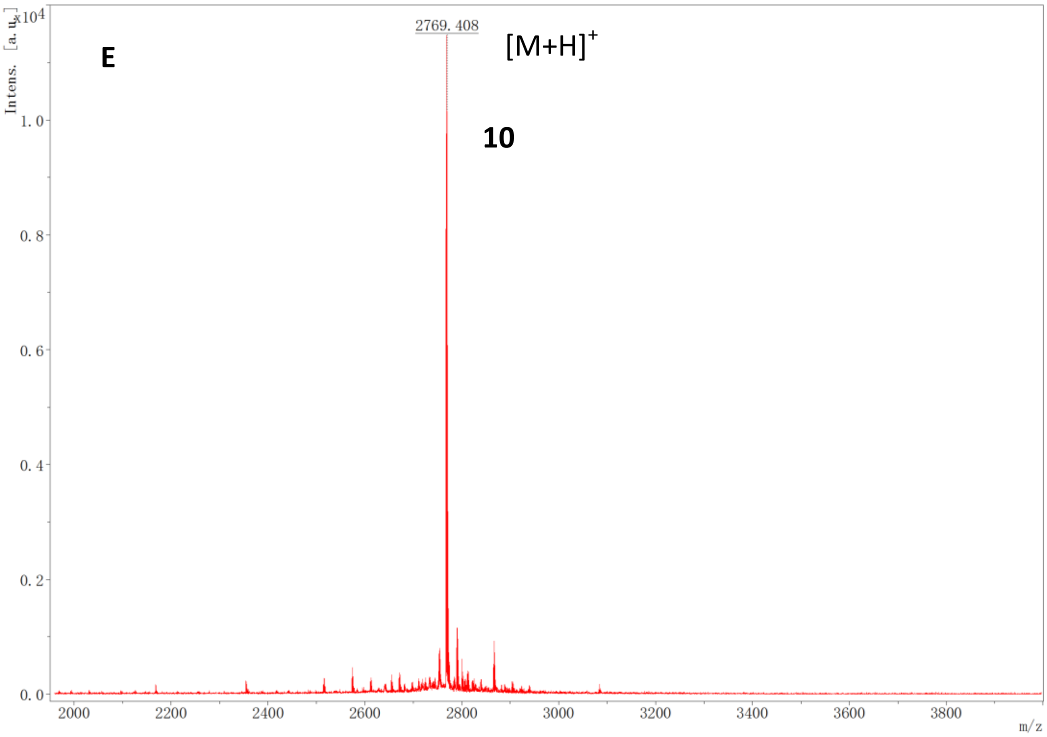
A
2156.401
[M+H]+
6
Intens. [a. u.]
1500
1000
500
0
1600
1800
2000
2200
2400
2600
2800
m/z
B
2401.524
[M+H]+
7
Intens. [a. u.]
1000
800
600
400
200
0
1600
1800
2000
2200
2400
2600
2800
3000
3200
m/z



Intens. [a. u.]
C
2275. 860
[M+H]+
8
3000
2000
1000
0
1500
1750
2000
2250
2500
2750
3000
3250
m/z



Intens. [a. u.]
x10^4
D
2598. 169
[M+H]+
9
1. 0
0. 8
0. 6
0. 4
0. 2
0. 0
2000
2200
2400
2600
2800
3000
3200
3400
m/z



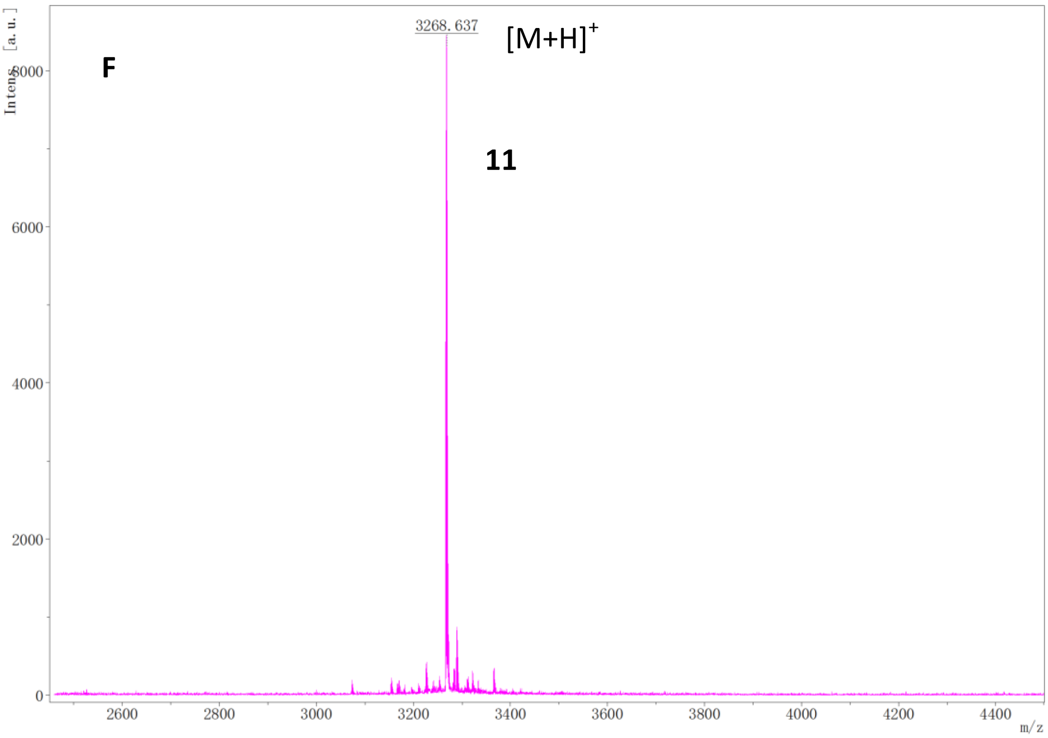


Figure S18. MALDI-TOF MS of ligation product of ACE inhibitory peptides. A. **6**, calcd : 2155.5, observed 2156.4 $[M+H]^+$; B. **7**, calcd : 2400.8, observed 2401.5 $[M+H]^+$; C. **8**, calcd : 2274.6, observed 2275.8 $[M+H]^+$; D. **9**, calcd : 2596.9, observed 2598.1 $[M+H]^+$; E. **10**, calcd : 2768.2, observed 2769.4 $[M+H]^+$; F. **11**, calcd : 3267.7, observed 3268.6 $[M+H]^+$.

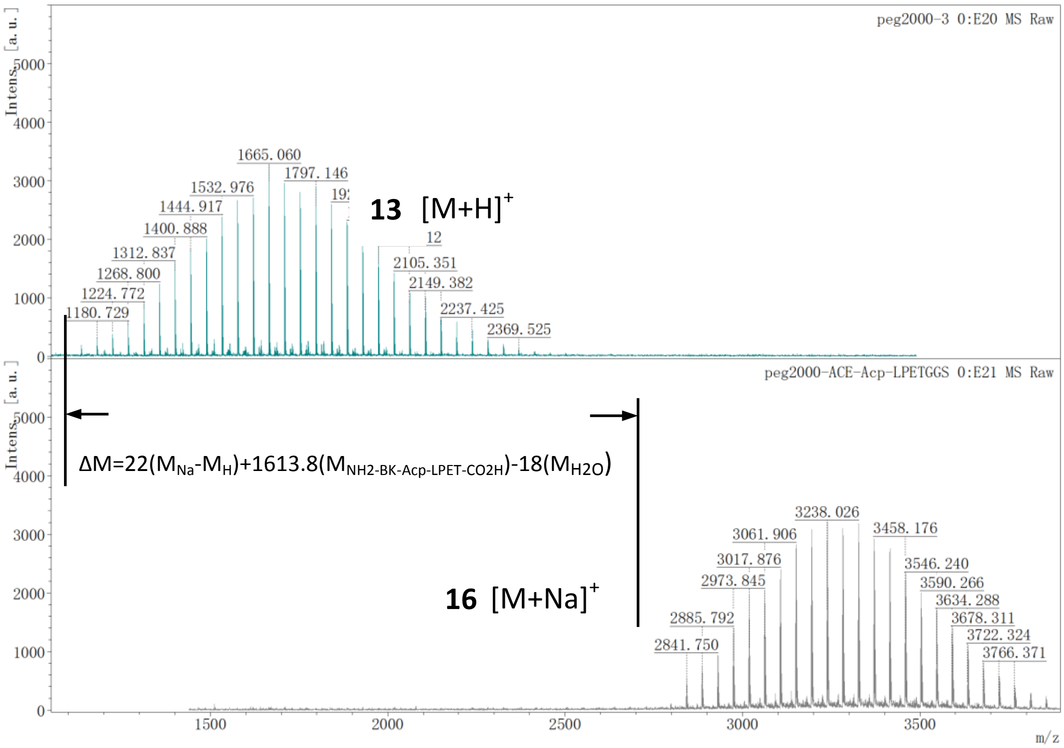


Figure S19. MALDI-TOF MS of peptide **16**. **13** (upper); ligation product **16** (bottom).

iii. SrtA-mediated ligation of **17** with protein in one-pot

Dried peptide resin **17** (0.6 µmol) was swollen in 100 µL reaction buffer (Tris-HCl 0.3 M pH 7.5, 150 mM NaCl, 5 mM CaCl2, and 0.5 mM mercaptoethanol) for 30min. Then 20 µL SrtA (100 µM dissolved in buffer) and 5 eq insulin were added. The final volume was 200ul. After the reaction was kept at 37 ^{o}C for 20 h, 20 µL of the reaction mixture was withdrawn and quenched with the same volume of 0.1% of TFA. The aliquots were then analyzed by HPLC using a RP C18 column and the fractions were analyzed by MALDI-TOF MS.

In large scale reaction: 10 mg of dry peptide PEGA resin **17** was treated with SrtA and insulin (5 eq) for 20 h. 3.1 mg of product **18** (yield 71%) was afforded after isolation by semi preparative HPLC.

In another procedure, 10 equiv. of dried peptide resin **17** (1.8 µmol) was swollen in 100 µL reaction buffer (Tris-HCl 0.3 M pH 7.5, 150 mM NaCl, 5 mM CaCl2, and 0.5 mM mercaptoethanol) for 30min. Then 20 µL SrtA (100 µM dissolved in buffer) and 0.18 µmol insulin were added. The final volume was 200ul. After the reaction was kept at 37 ^{o}C for 20 h, 20 µL of the reaction mixture was withdrawn and quenched with the same volume of 0.1% of TFA. The aliquots were then analyzed by HPLC using a RP C18 column and the fractions were analyzed by MALDI-TOF MS. The convertion yield is 46% based on the HPLC analysis.

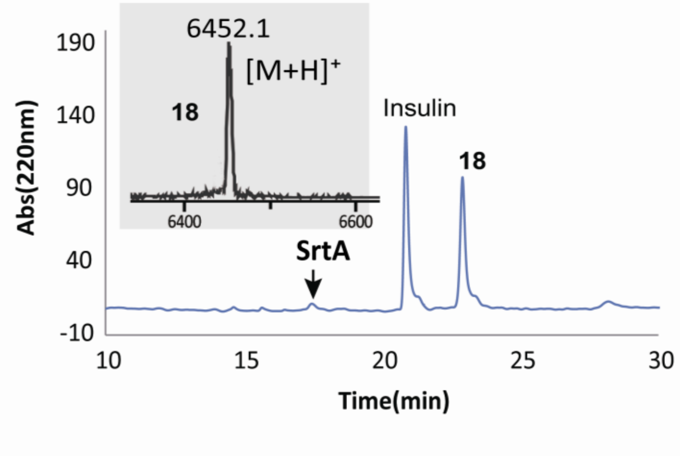


Figure S20. SrtA-mediated *in situ* N-terminal modification of insulin using PEGA resin 17 in one-pot. Reaction condition: dried peptide resin 17 (1.8 μmol), 10 uM SrtA, 0.3 M Tris buffer (pH 7.5) containing 150 mM NaCl, 5 mM $CaCl_2$, and 0.5 mM mercapehtnaol and 1 equiv. of insulin.