Supporting Information

Table of Contents

General Information

Materials

All Fmoc-protected amino acids were purchased from C S Bio, GL Biochem (Shanghai, China). *N, N'*-diisopropylethylamine (DIPEA), *N, N'*-Diisopropyl-carbodiimide (DIC), Ethyl cyano(hydroxyamino)acetate (Oxyma Pure), 1, 2-bis (bromomethyl)benzene, 1, 3-bis(bromomethyl)benzene, 1, 4-bis(bromomethyl)benzene, 4, 4'-bis(bromomethyl)biphenyl, 2, 6-bis(bromomethyl)pyridine, hexafluorobenzene, decafluorobiphenyl, mercapto-ethylamine (β-MEA), trifluoroacetic acid (TFA), Triisopropylsilane (TIPS), m-cresol, 6-aminohexanoic acid, 1, 2-Phthalaldehyde, 1, 3-Phthalaldehyde, 1, 4-Phthalaldehyde, Sodium cyanoborohydride, 1, 3-dibromoacetone were purchased from Energy Chemical. N, N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), dichloromethane (DCM), diethyl ether (Et_2O), dimethyl sulfoxide (DMSO), acetic anhydride, *2, 6*-Lutidine, sodium hydroxide (NaOH), m-cresol, hydrochloric acid (HCl), sodium nitrate ($NaNO_2$), and hydrazinemonohydrate were ordered from Sinopharm Chemical Reagent. Acetonitrile (HPLC grade) was purchased from J. T. Baker (Phillipsburg, NJ, USA). Rinkamide resin and 2-Chlorotrityl Chloride resin were purchased from Hecheng Technology (Tianjing, China). Phosphate-buffered saline (containing 136.89 mM NaCl, 2.67 mM KCl, 8.1 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) was purchased from Biological Industries Israel Beit Haemek Ltd (Lot: 2129201).

HPLC, Mass Spectrometry (MS)

Reversed phase HPLC experiments were performed on Shimadzu Prominence HPLC systems (SPD-20A). ChromCore™ 120 C18 5μm (4.6 × 250 mm) column was used for peptide analysis, a flow rate of 1.0 mL/min. ChromCore™ 120 C18 5μm (10 × 250 mm) column was used for peptide purification, a flow rate of 3.0 mL/min. **Solvent A**: Water containing 1% acetonitrile and 0.1% TFA; **Solvent B**: acetonitrile containing 1% water and 0.1% TFA. Liquid chromatography mass spectrometer (LC-MS) (Thermo scientific) was used to determine the mass weight of synthesized peptides.

General procedure for Fmoc-based SPPS

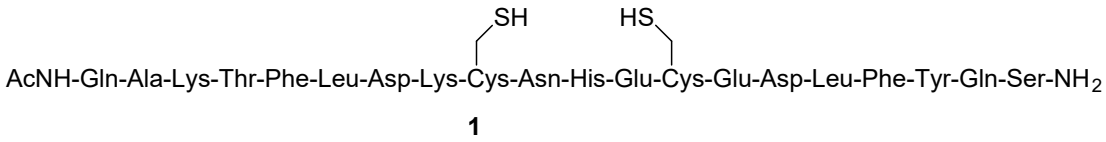
All mentioned peptides were manually synthesized by Fmoc-based procedure. Rinkamide AM resin (~0.43 mmol/g) or hydrazine-2-Cl-Trt-Resin was kept into a syringe equipped with a filter frit. The resin was swollen in DMF for 20 min at RT. Then, 20% piperidine in DMF (2 x 10 min) was transferred to the syringe to remove any Fmoc-group. After washing the resin five times with DMF, the coupling reaction of the first Fmoc-protected amino acid was carried out, using 4.5 eq. Fmoc-protected amino acid (~0.18 M in DMF), 4.5 eq. DIC and 4.5 eq. Oxyma. The coupling was kept at 55 °C for 40 minutes. Then, wash the resin five times with DMF, the capping reagent (DMF/Ac_2O/2, 6-Lutidine: 89/5/6, 2 min x 2) was added to the resin to mask the unreacted amino groups. After washing the resin five times with DMF, the Fmoc-group was removed with 20% piperidine in DMF (2 x 10 min). The coupling process continued until the target peptide was completed. During this process, the amount of the resin-bounded Fmoc group was monitored by UV-320 machine. After the assembly of peptide, the resin was washed thoroughly with DMF and DCM, and dried at room temperature. Then, TFA cleavage cocktail

(TFA/m-cresol/water/TIPS, 88/5/5/2) was added. The cleavage process was kept for two hours at room temperature. The pre-chilled Et_2O (8~10 folds TFA volume) was added to the combined TFA solution to precipitate the crude peptide. After centrifugation, the crude peptide was obtained as a white powder. The crude peptide was dissolved in water containing 0.1% TFA and 1% acetonitrile, and analyzed by HPLC and MS. After preparative HPLC and lyophilization, the purified peptide was obtained as a white powder.

Experimental Section

Synthesis of side-chain cross-linked ACE2 peptide mimetics

1, **1-c1**, **1-c2**, **1-c3**, **1-c4**, **1-c5**, **1-c6** and **1-c7:**



1

1 was prepared by Fmoc-based SPPS using Rink amide AM resin. After preparative HPLC and lyophilization, **1** was obtained as a white powder. Analytical HPLC: t_R = 27.5 min (2-90% B in 40 min); m/z = 2459.6 ($C_{107}H_{158}N_{28}O_{35}S_2$, calcd.: 2460.0 g/mol).

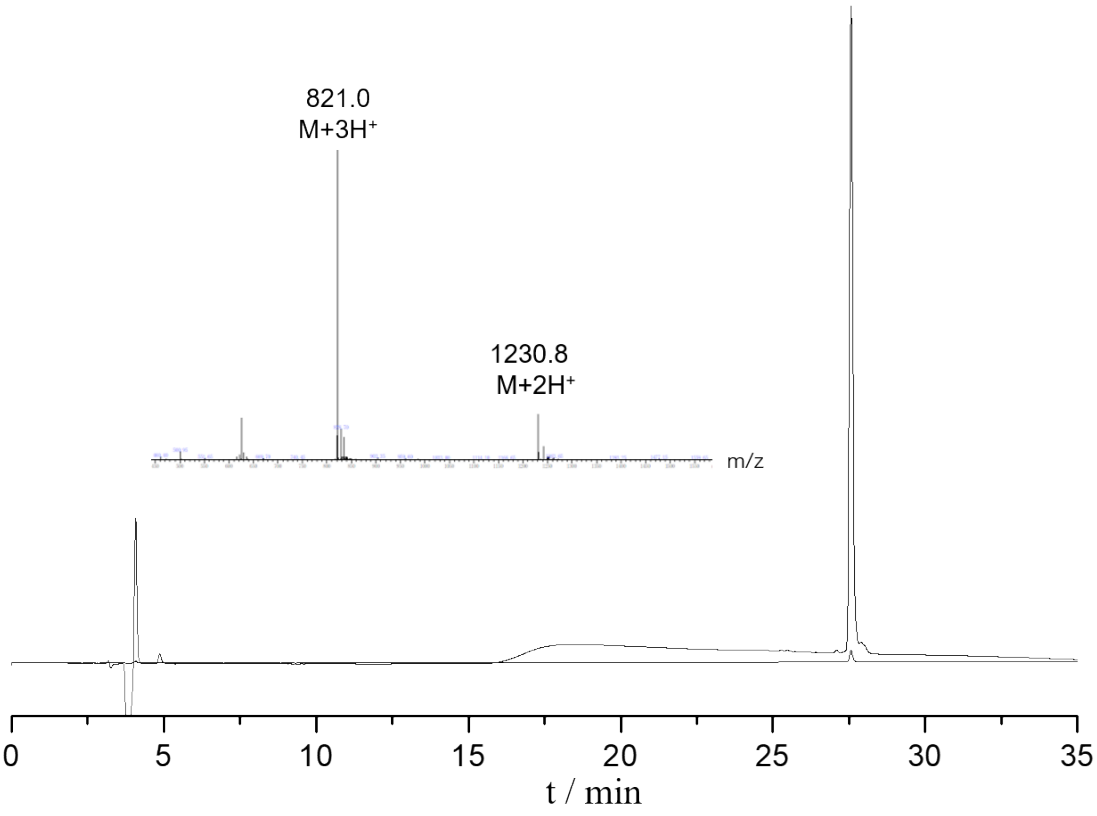
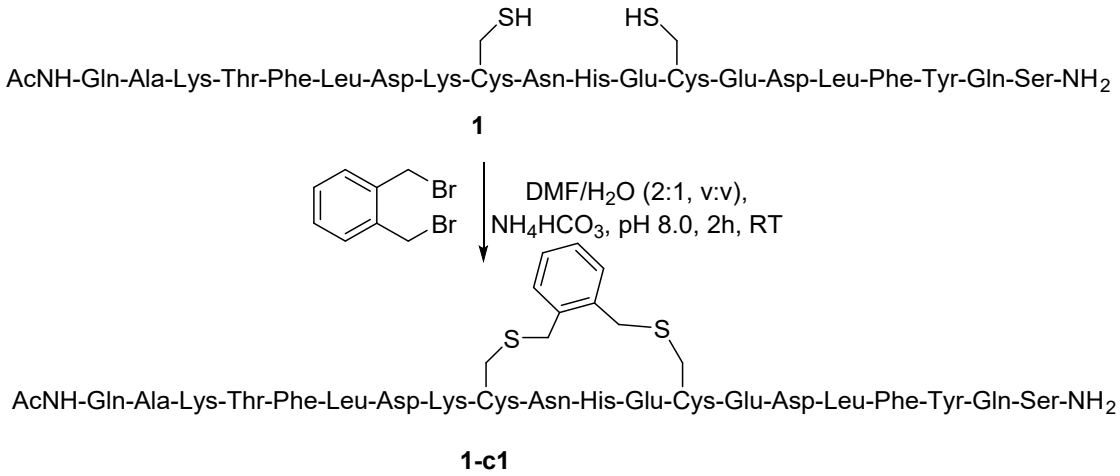


Figure S1. HPLC trace and mass spectrum of the purified **1** (UV: 210nm and 250 nm).



1-c1 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1,2-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c1** was obtained as a white powder (7.0 mg, 35%). Analytical HPLC: t_R = 31.4 min (2-90% B in 40 min); m/z = 2561.8 ($C_{115}H_{164}N_{28}O_{35}S_2$, calcd.: 2562.0 g/mol).

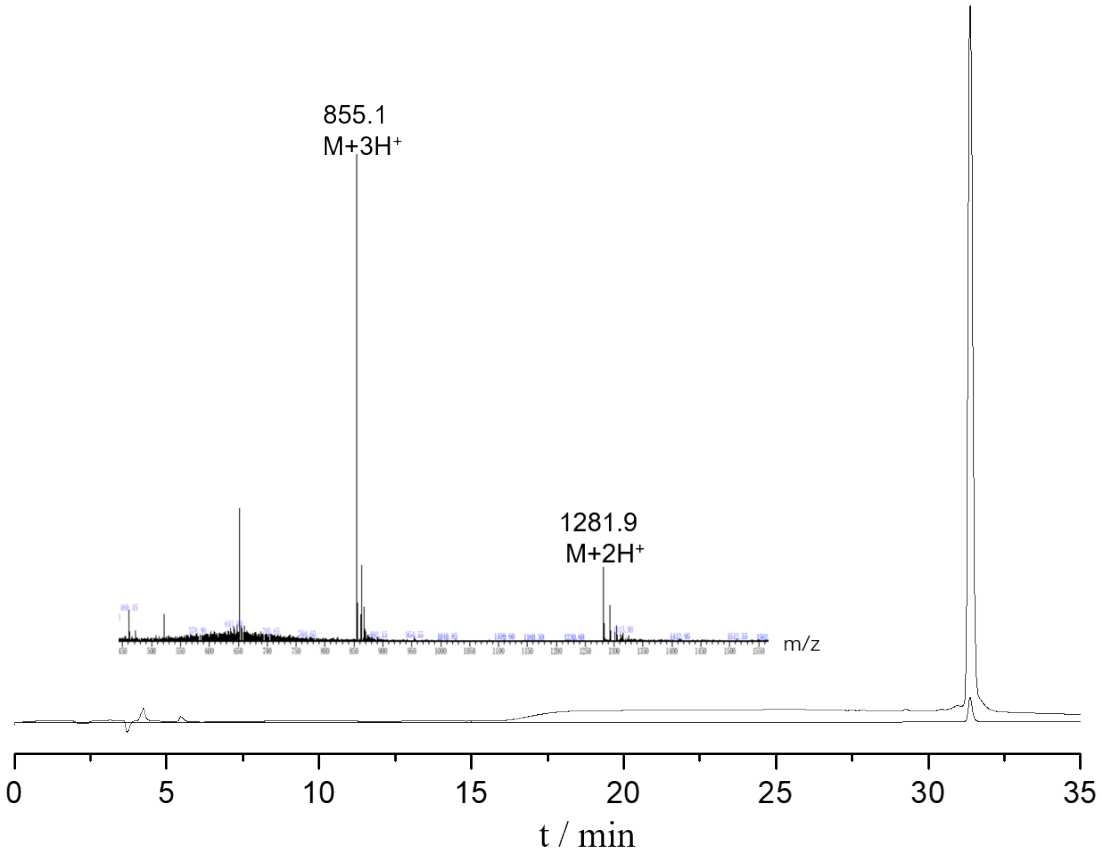
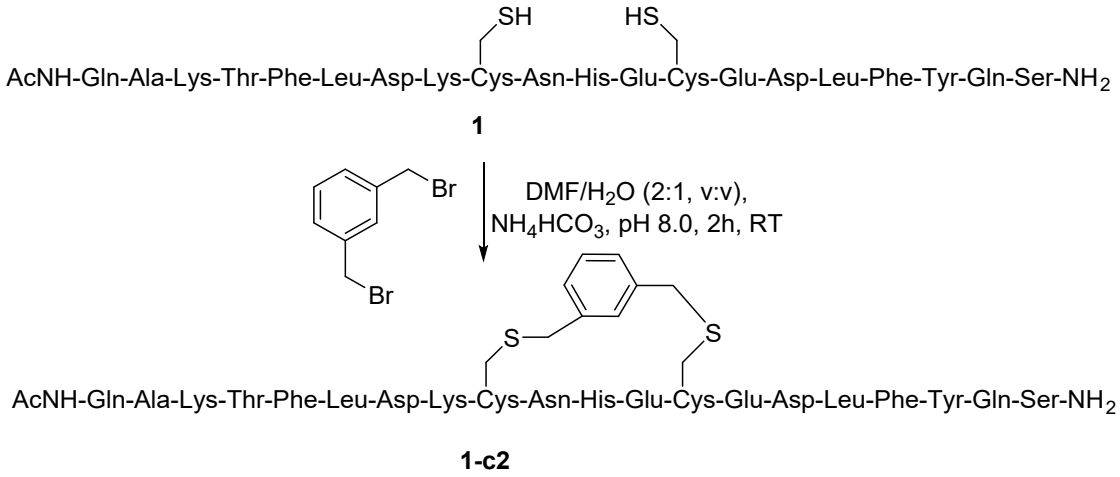


Figure S2. HPLC trace and mass spectrum of the purified **1-c1** (UV: 210nm and 250 nm).



1-c2 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1, 3-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c2** was obtained as a white powder (6.5 mg, 32.5%). Analytical HPLC: t_R = 29.6 min (2-90% B in 40 min); m/z = 2562.4 ($C_{115}H_{164}N_{28}O_{35}S_2$, calcd.: 2562.0 g/mol).

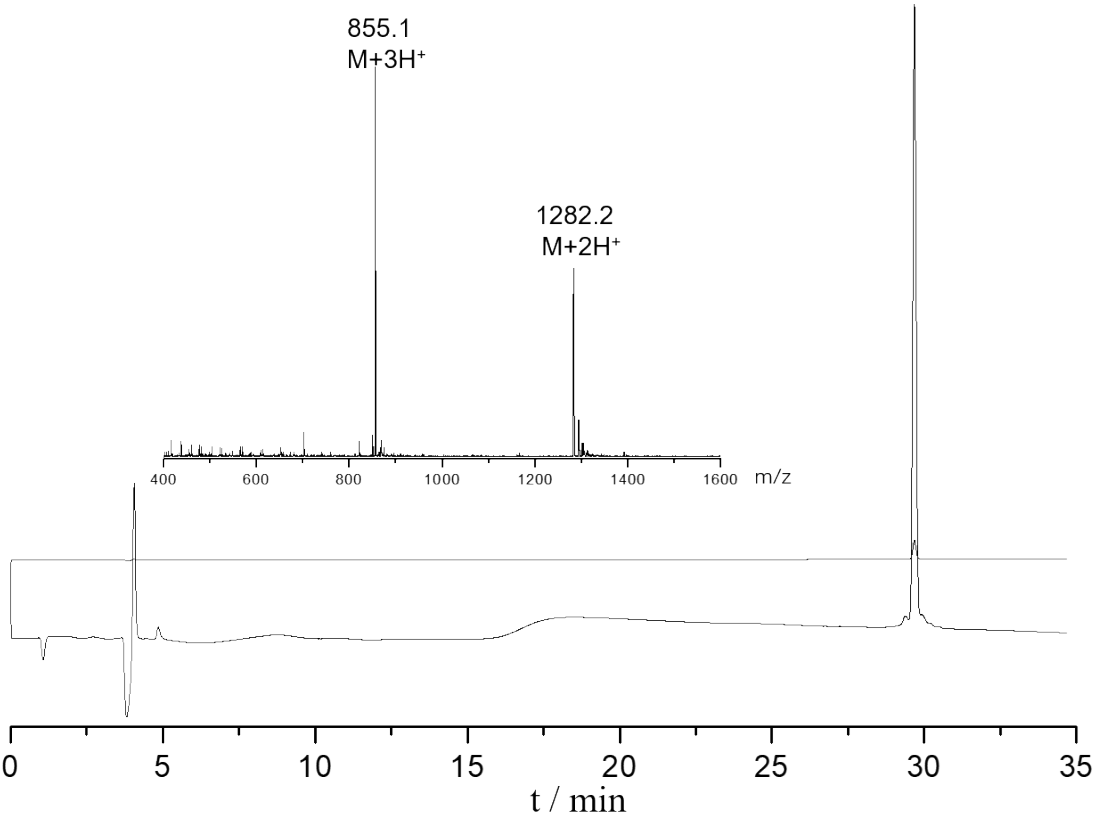
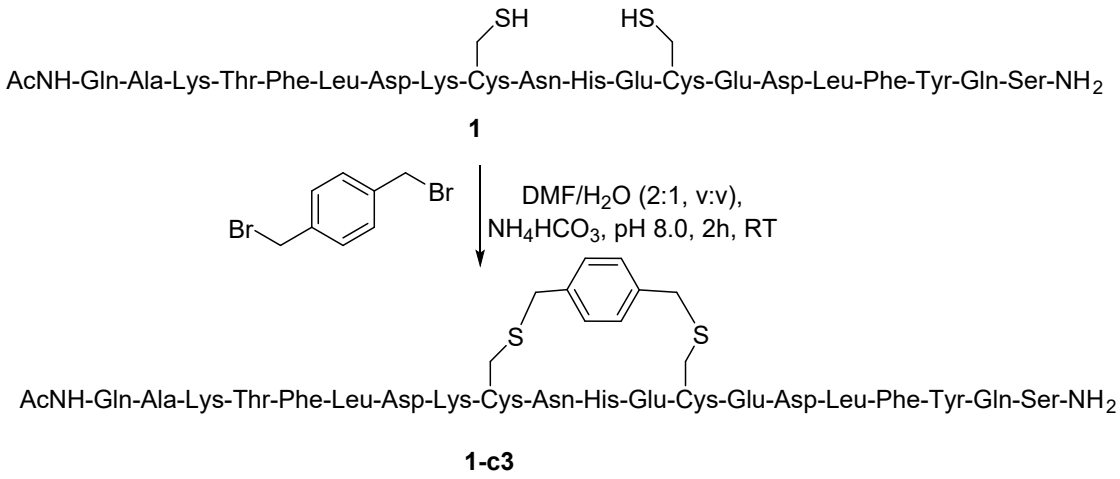


Figure S3. HPLC trace and mass spectrum of the purified **1-c2** (UV: 210nm and 250 nm).



1-c3 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1, 4-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL Acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c3** was obtained as a white powder (6.6 mg, 33%). Analytical HPLC: t_R = 29.3 min (2-90% B in 40 min); m/z = 2562.4 ($C_{115}H_{164}N_{28}O_{35}S_2$, calcd.: 2562.0 g/mol).

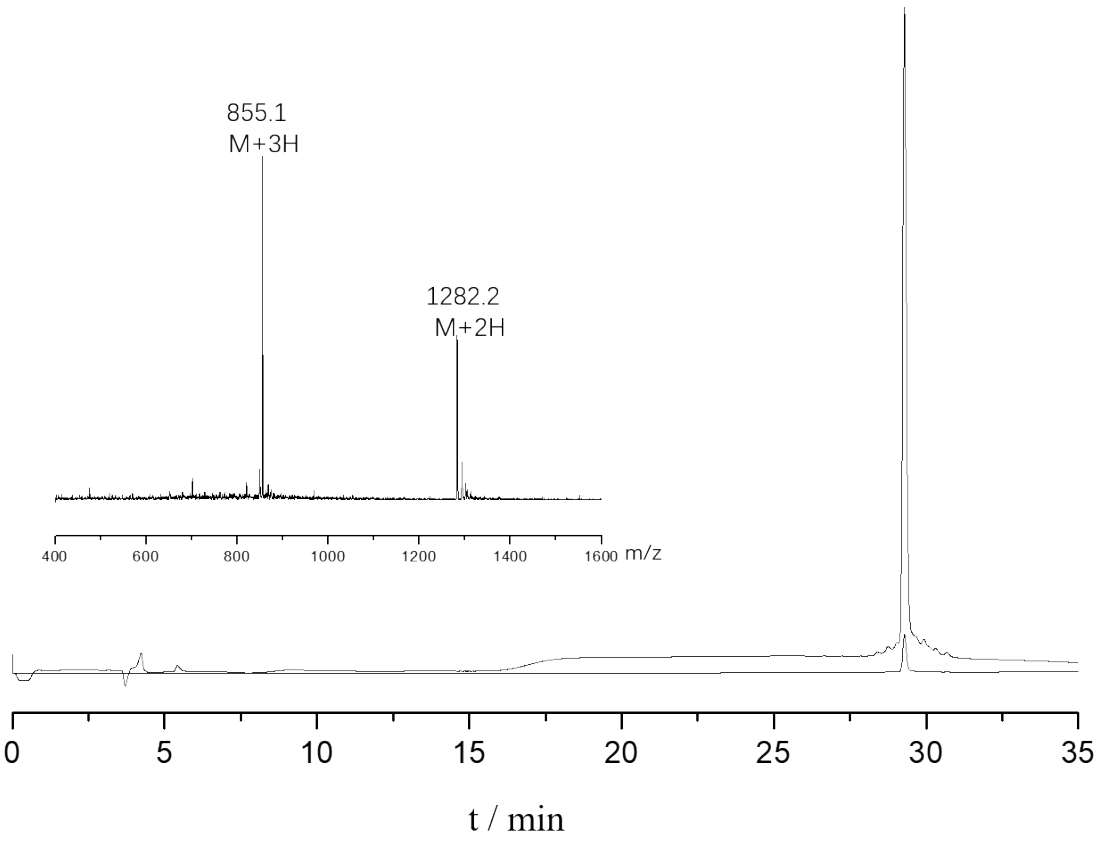
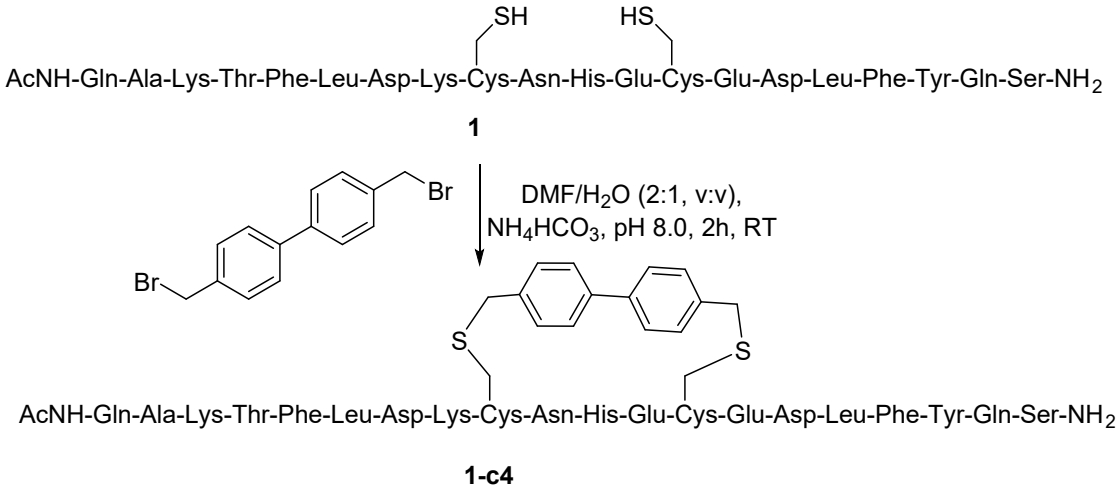


Figure S4. HPLC trace and mass spectrum of the purified **1-c3** (UV: 210nm and 250 nm).



1-c4 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.8 mg of bis(bromomethyl)biphenyl in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c4** was obtained as a white powder (~5 mg, 25%). Analytical HPLC: t_R = 29.1 min (2-90% B in 40 min); m/z = 2637.4 ($C_{121}H_{168}N_{28}O_{35}S_2$, calcd.: 2637.2 g/mol).

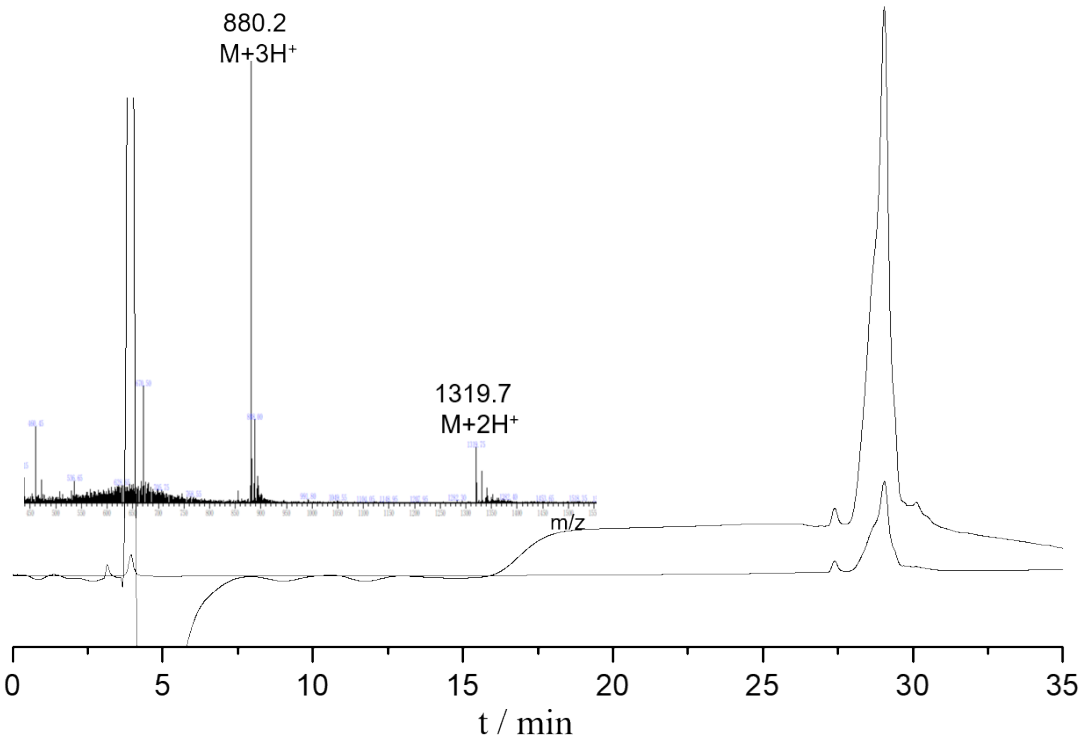
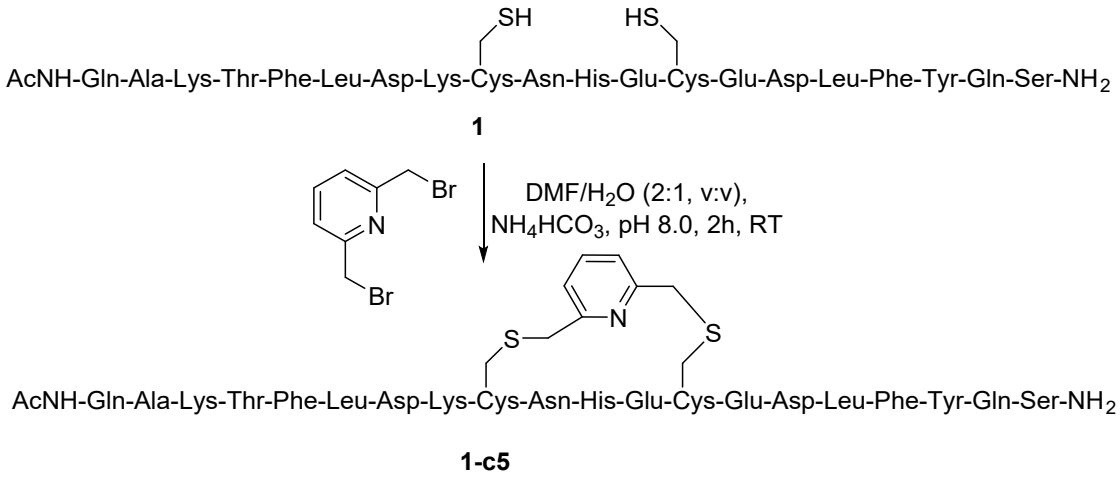


Figure S5. HPLC trace and mass spectrum of the purified **1-c4** (UV: 210nm and 250 nm).



1-c5 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 2,6-bis(bromomethyl)pyridine in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL Acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c5** was obtained as a white powder (~5.8 mg, 29%). Analytical

HPLC: t_R = 26.4 min (2-90% B in 40 min); m/z = 2563.4 ($C_{114}H_{163}N_{29}O_{35}S_2$, calcd.: 2563.2 g/mol).

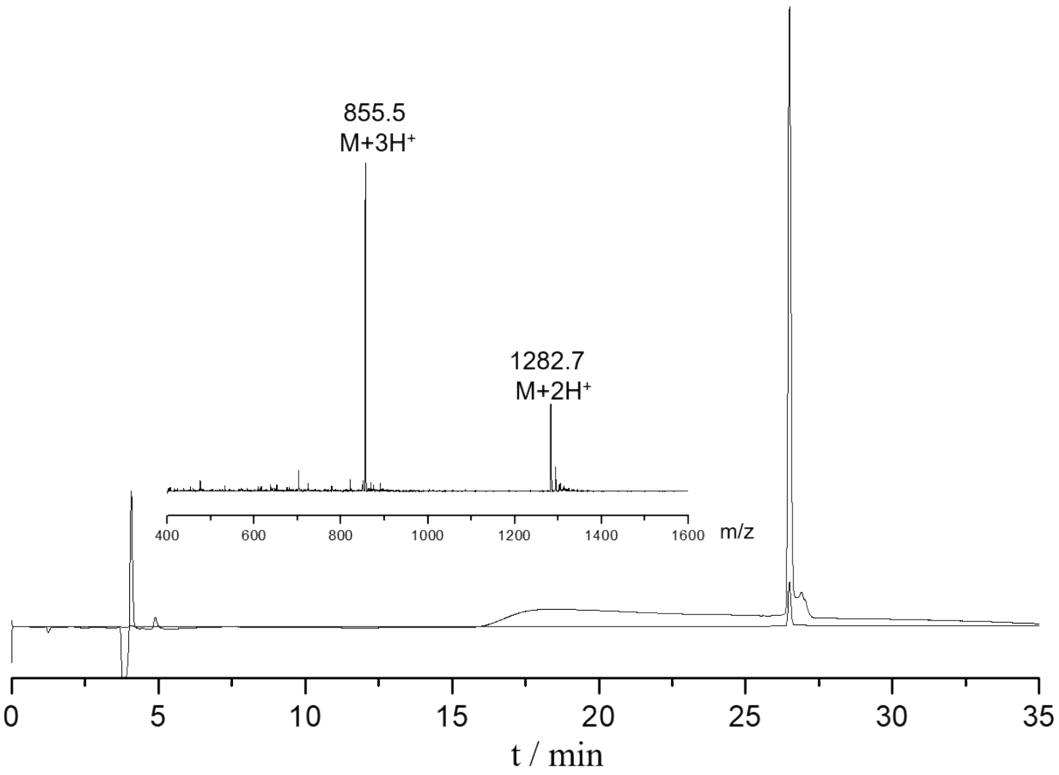
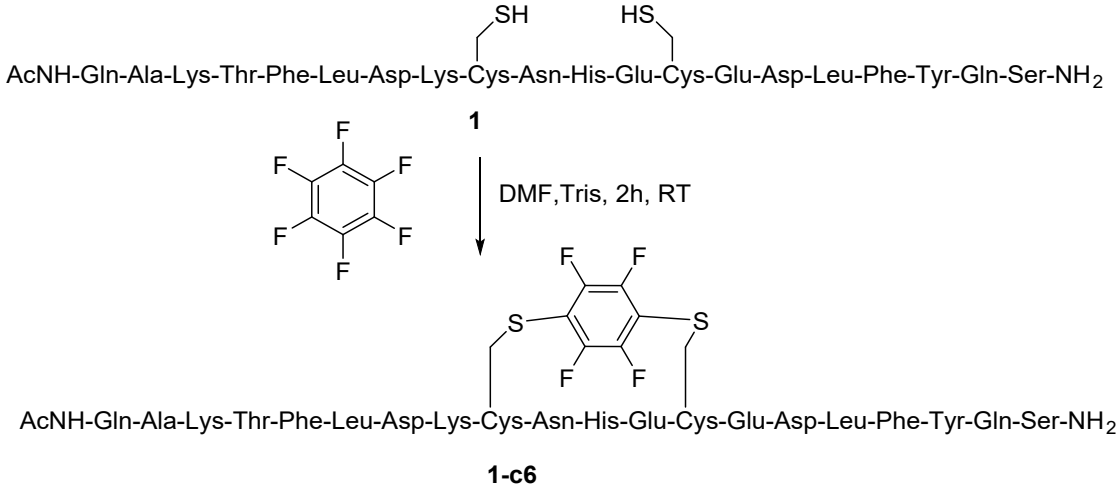


Figure S6. HPLC trace and mass spectrum of the purified **1-c5** (UV: 210nm and 250 nm).



1-c6 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF. Then, 2.4 mg of hexafluorobenzene in 0.1 mL DMF and 8 mg of Tris in 1 mL DMF were added. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c6** was obtained as a white powder (~7.5 mg, 37.5%). Analytical HPLC: t_R = 31.0 min (2-90% B in 40 min); m/z = 2606.6

($C_{113}H_{156}F_4N_{28}O_{35}S_2$, calcd.: 2606.0 g/mol).

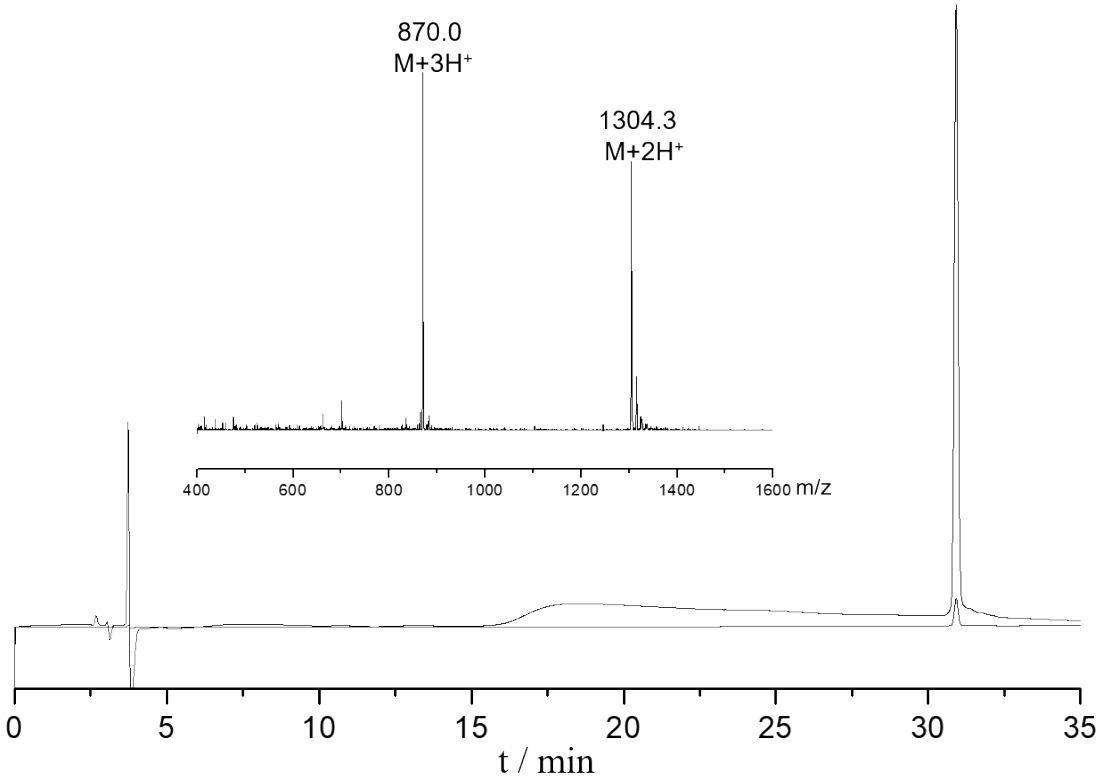
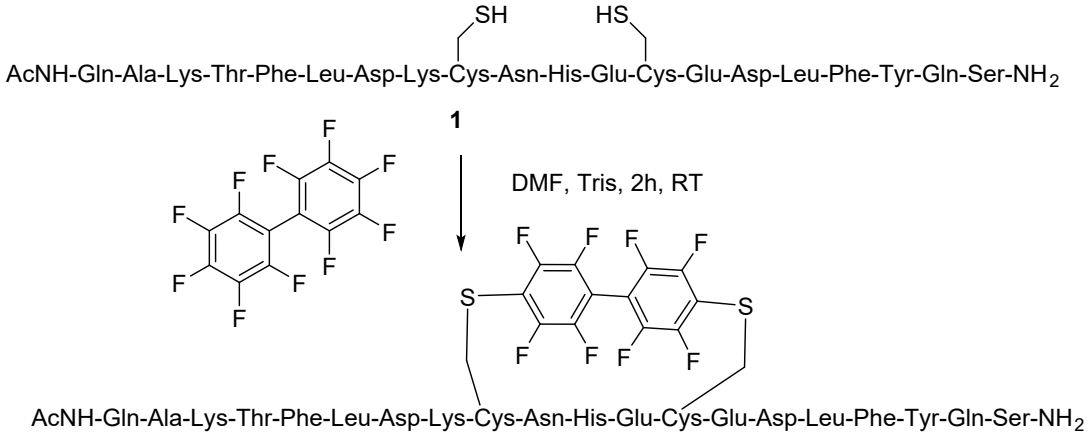


Figure S7. HPLC trace and mass spectrum of the purified **1-c6** (UV: 210nm and 250 nm).



1-c7

1-c7 was prepared by bisalkylation of **1**. 20 mg of crude **1** was dissolved into 2.0 mL DMF. Then, 4.4 mg of decafluorobiphenyl in 0.1 mL DMF and 8 mg of Tris in 1 mL DMF were added. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **1-c7** was obtained as a white powder (~6.8 mg, 34%). Analytical HPLC: t_R = 29.9 min (2-90% B in 40 min); m/z = 2754.2 ($C_{119}H_{156}F_8N_{28}O_{35}S_2$, calcd.: 2754.0 g/mol).

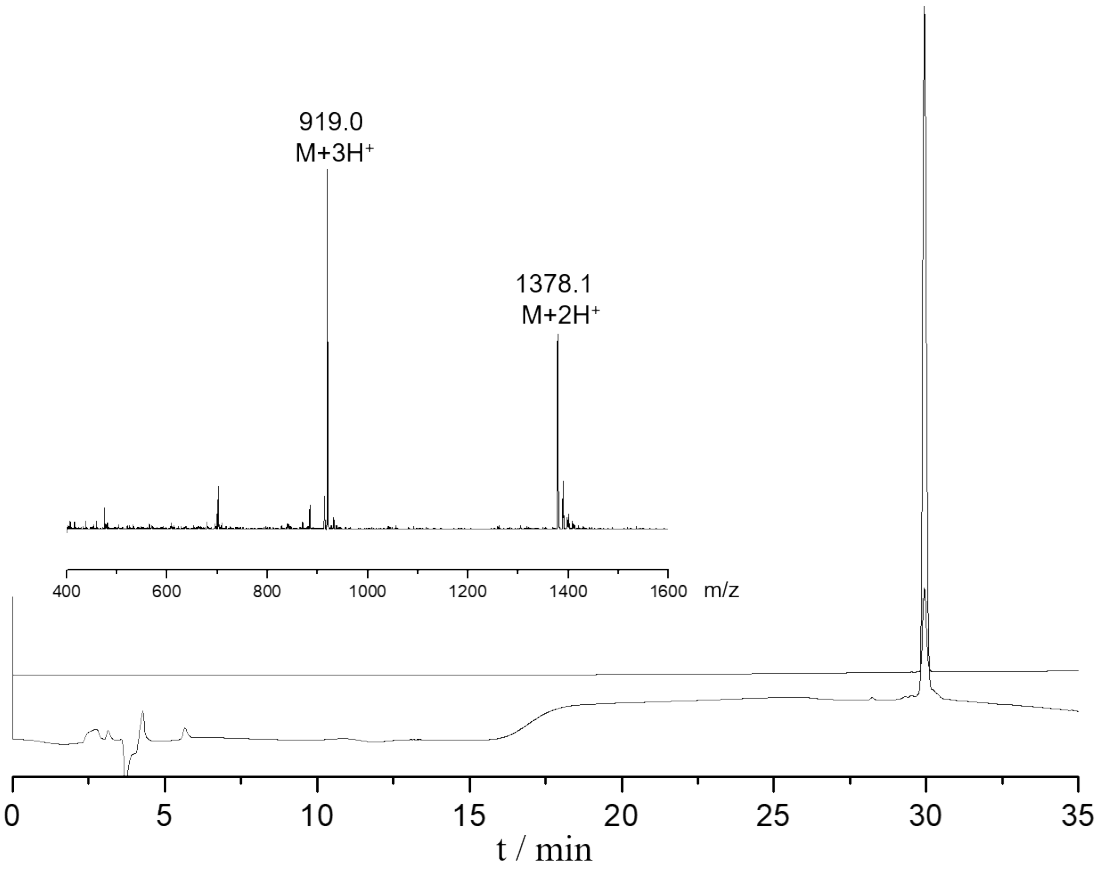
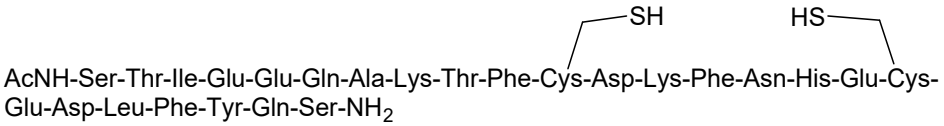


Figure S8. HPLC trace and mass spectrum of the purified **1-c7** (UV: 210nm and 250 nm).

2, **2-c1**, **2-c2**, **2-c3**, **2-c4**, **2-c5**, **2-c6** and **2-c7:**



2

2 was prepared by Fmoc-based SPPS using Rink amide AM resin. After preparative HPLC and lyophilization, **2** was obtained as a white powder. Analytical HPLC: t_R = 31.4 min (2-90% B in 40 min); m/z = 3053.0 ($C_{133}H_{193}N_{33}O_{46}S_2$, calcd.: 3053.3 g/mol).

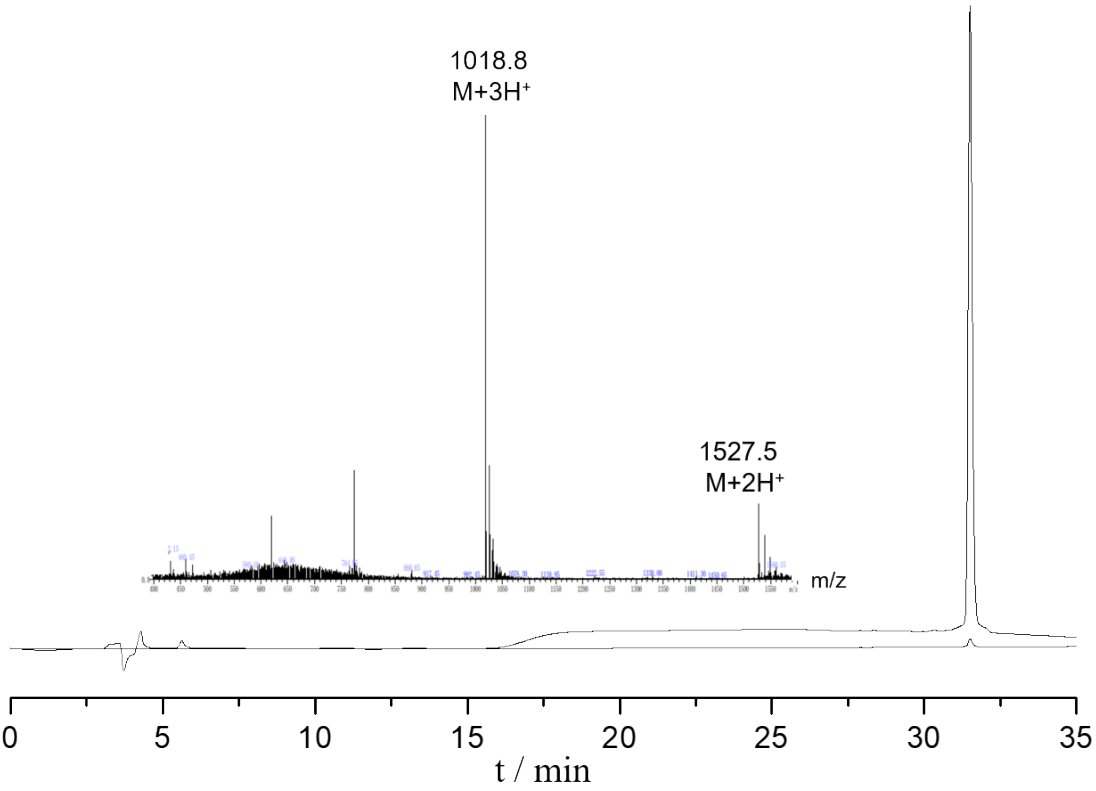
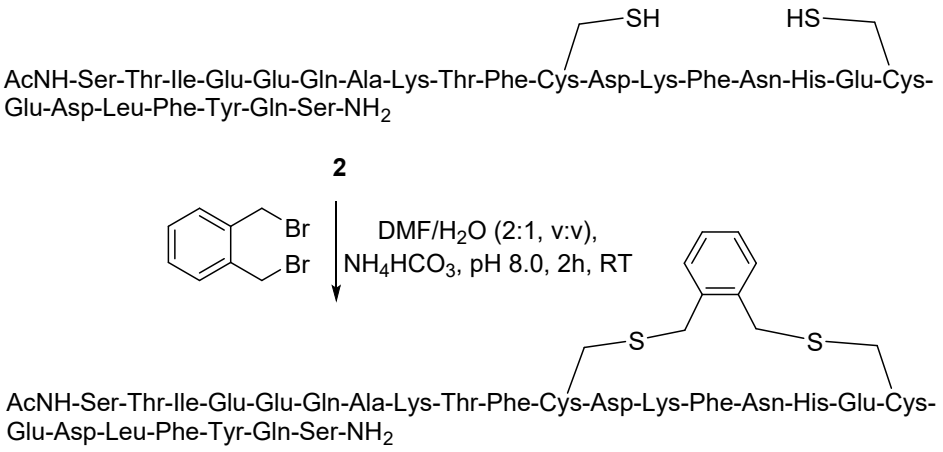


Figure S9. HPLC trace and mass spectrum of the purified **2** (UV: 210nm and 250 nm).



2-c1

2-c1 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1,2-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c1** was obtained as a white powder (7.0 mg, 35%). Analytical HPLC: t_R = 30.3 min (2-90% B in 40 min); m/z = 3155.2 ($C_{141}H_{199}N_{33}O_{46}S_2$, calcd.: 3155.4 g/mol).

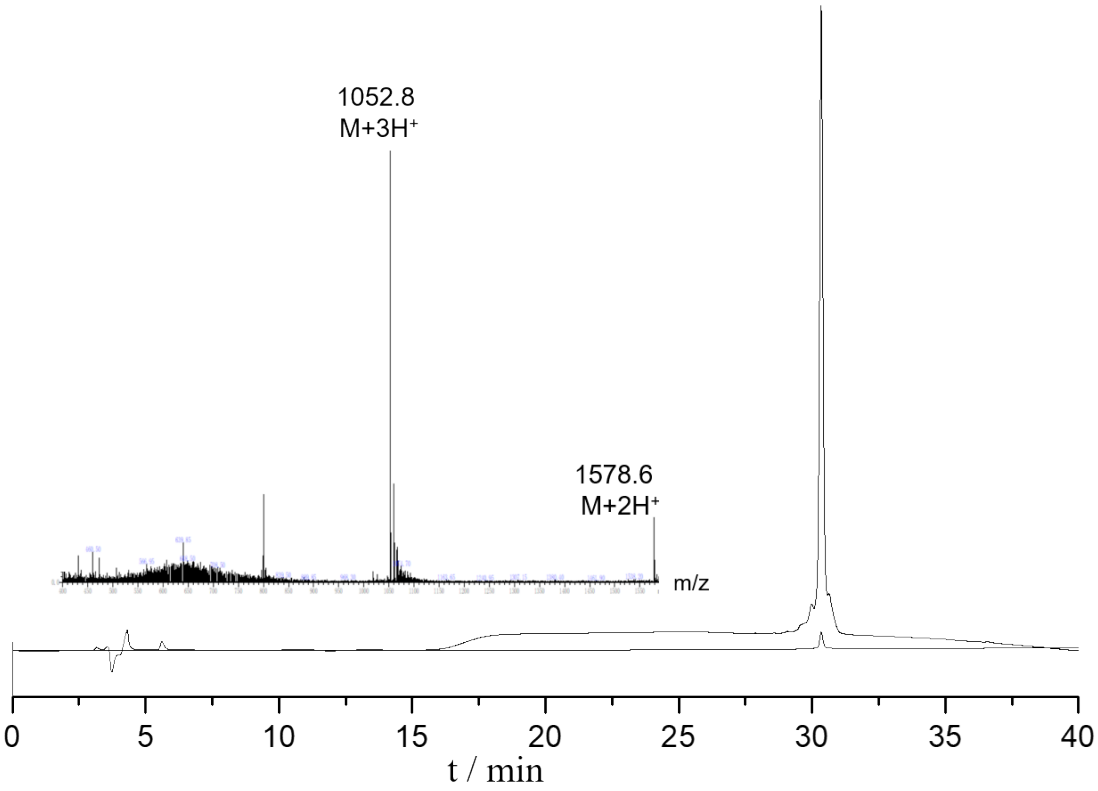
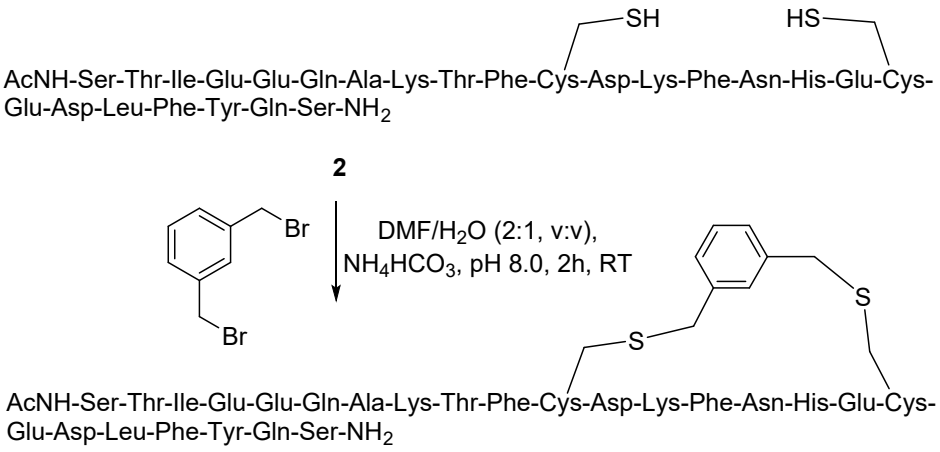


Figure S10. HPLC trace and mass spectrum of the purified **2-c1** (UV: 210nm and 250 nm).



2-c2

2-c2 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1, 3-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c2** was obtained as a white powder (6.5 mg, 32.5%). Analytical HPLC: t_R = 31.5 min (2-90% B in 40 min); m/z = 3155.6 ($C_{141}H_{199}N_{33}O_{46}S_2$, calcd.: 3155.4 g/mol).

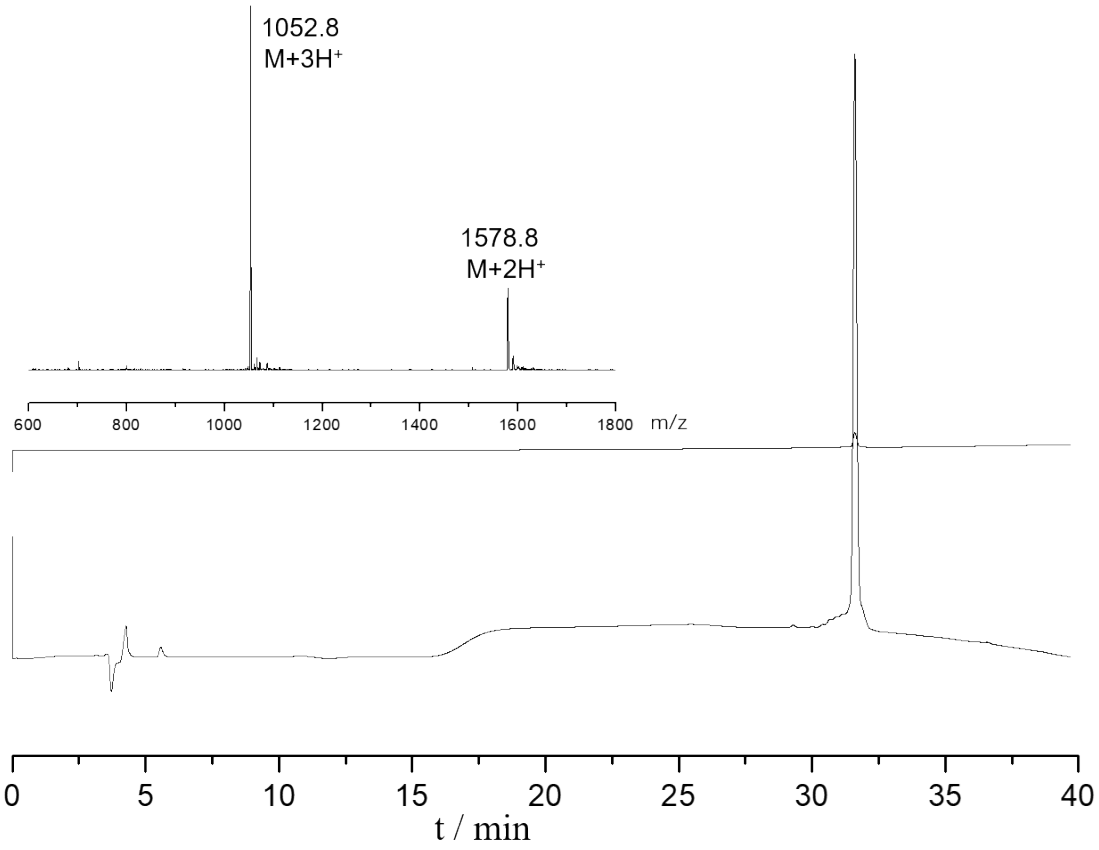
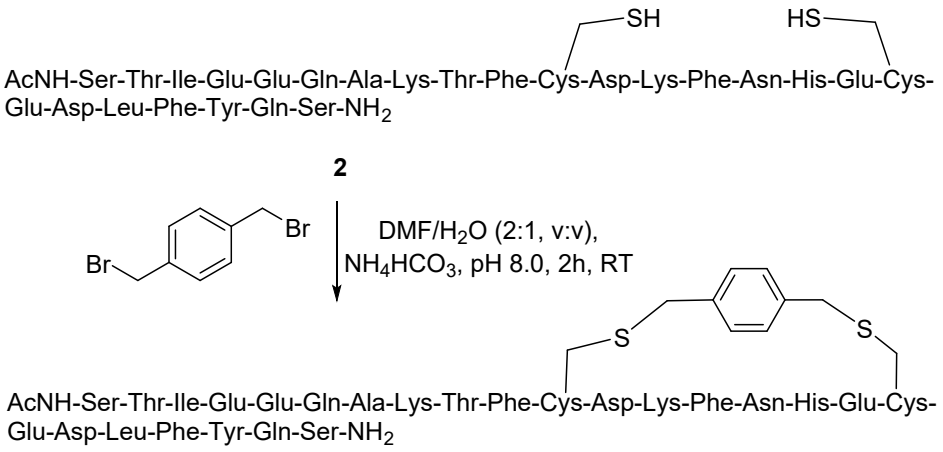


Figure S11. HPLC trace and mass spectrum of the purified **2-c2** (UV: 210nm and 250 nm).



2-c3

2-c3 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1, 4-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c3** was obtained as a white powder (~8 mg, 40%). Analytical HPLC: t_R = 30.1 min (2-90% B in 40 min); m/z = 3155.6 ($C_{141}H_{199}N_{33}O_{46}S_2$, calcd.: 3155.4 g/mol).

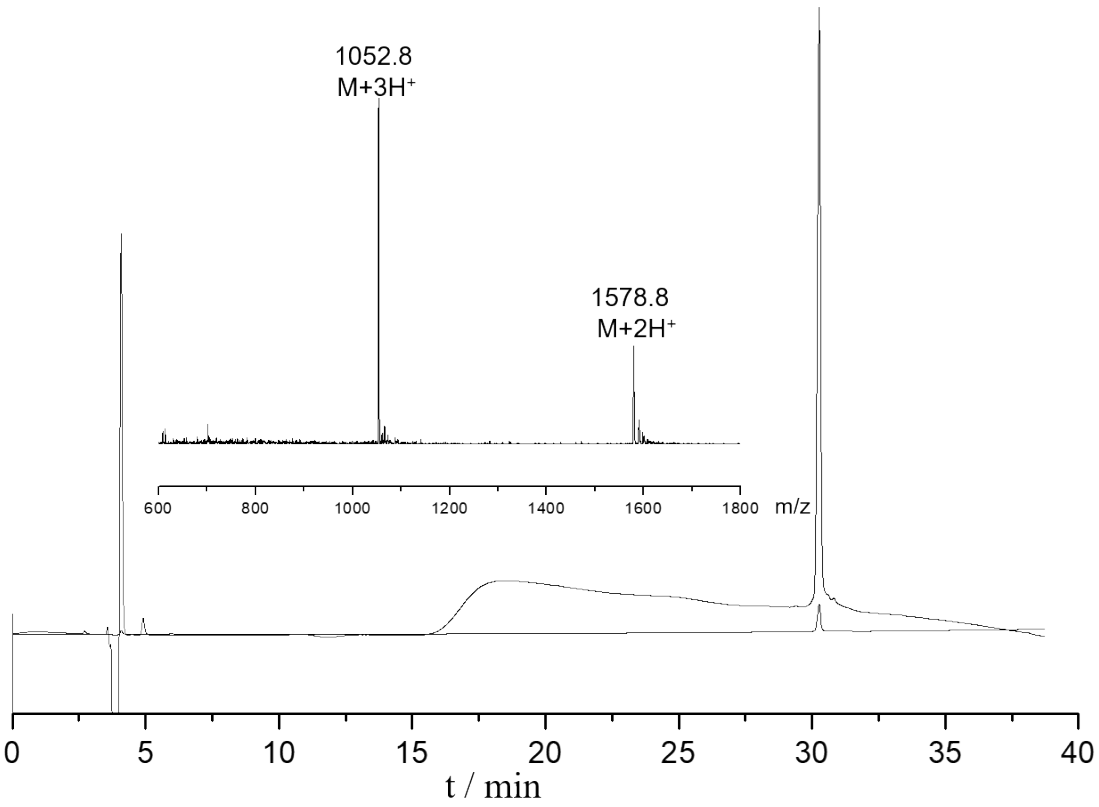
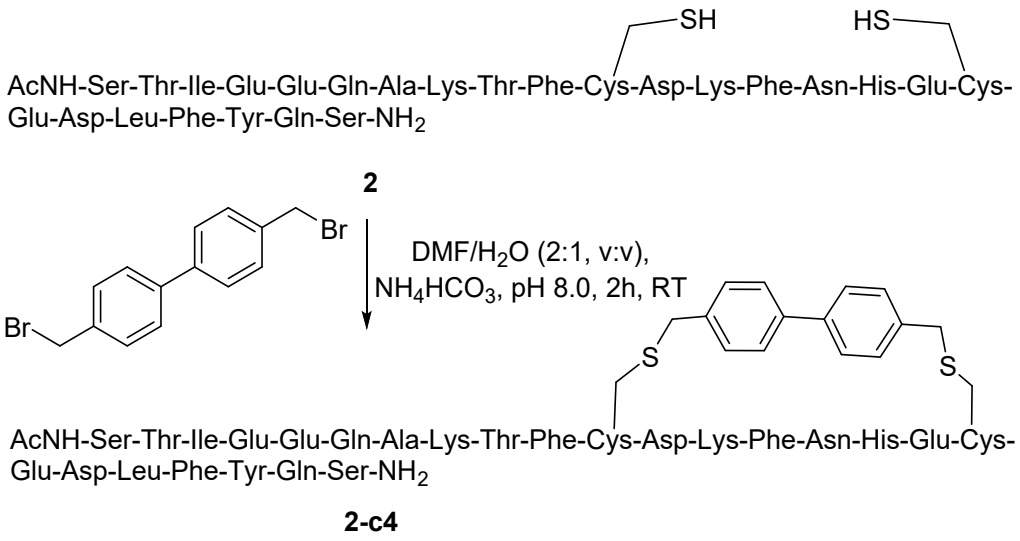


Figure S12. HPLC trace and mass spectrum of the purified **2-c3** (UV: 210nm and 250 nm).



2-c4

2-c4 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.8 mg of bis(bromomethyl)biphenyl in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c4** was obtained as a white powder (~5 mg, 25%). Analytical HPLC: t_R = 34.2 min (2-90% B in 40 min); m/z = 3231.6 ($C_{147}H_{203}N_{33}O_{46}S_2$, calcd.: 3231.4 g/mol).

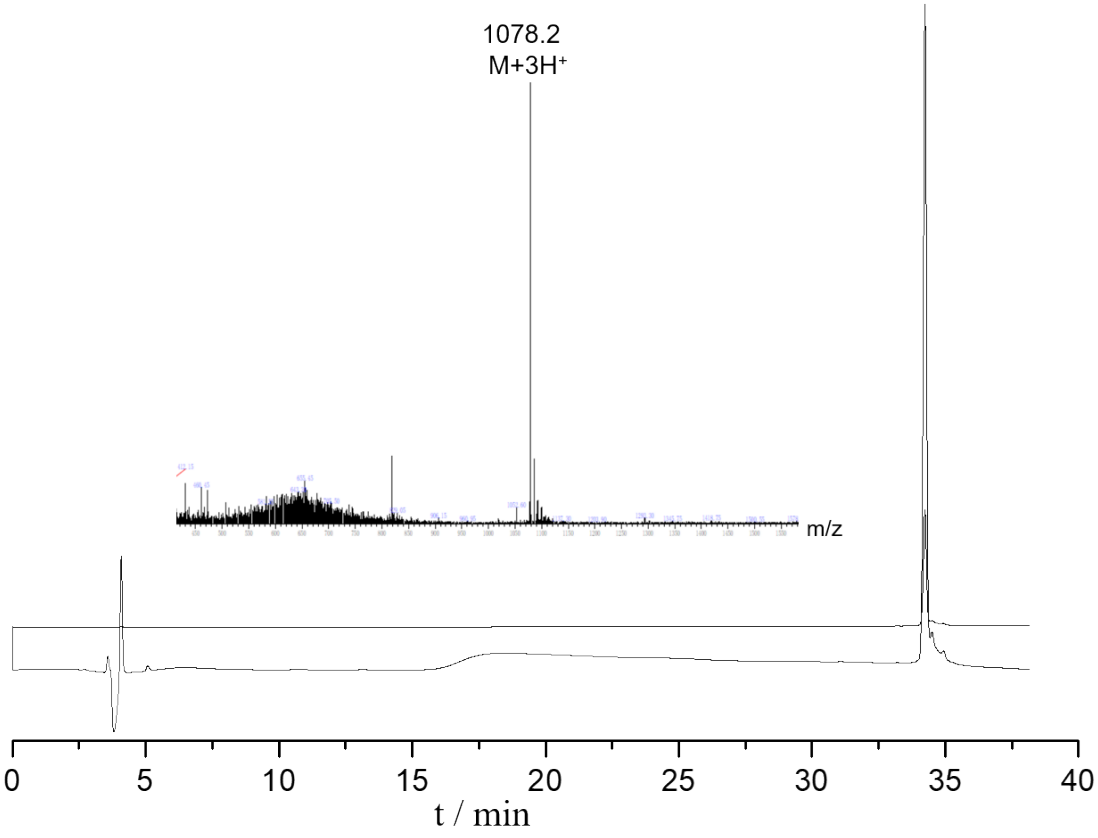
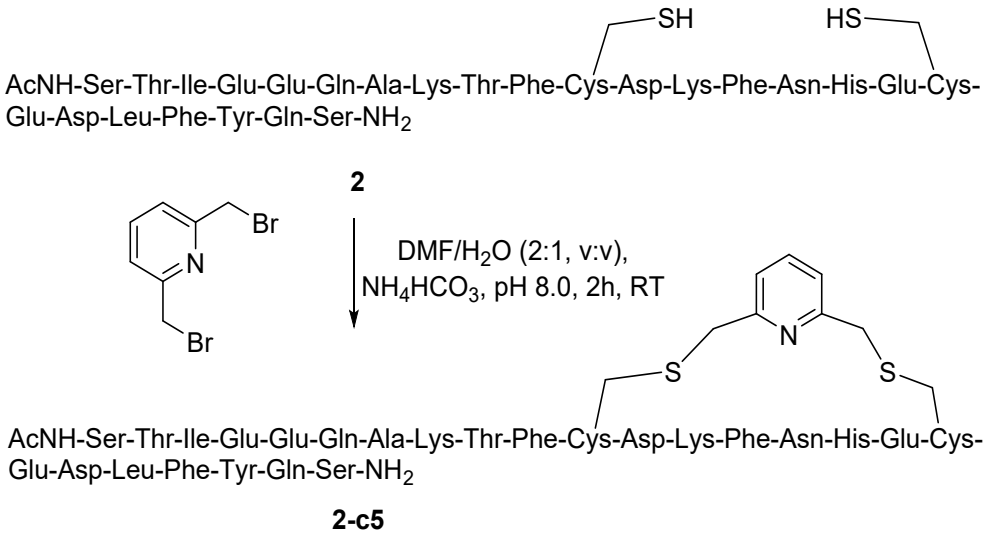


Figure S13. HPLC trace and mass spectrum of the purified **2-c4** (UV: 210nm and 250 nm).



2-c5

2-c5 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 2,6-bis(bromomethyl)pyridine in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL Acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c5** was obtained as a white powder (~6.7 mg, 33.5%). Analytical HPLC: t_R = 28.8 min (2-90% B in 40 min); m/z = 3156.8 ($C_{140}H_{198}N_{34}O_{46}S_2$, calcd.: 3156.4 g/mol).

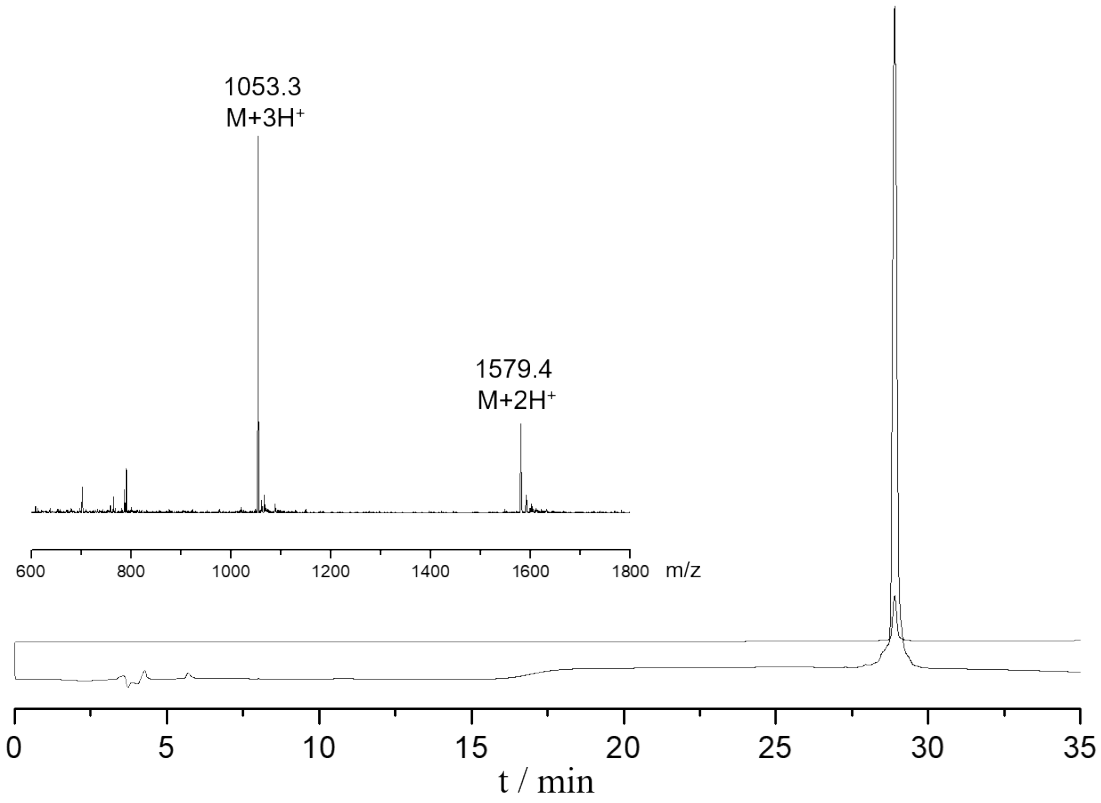
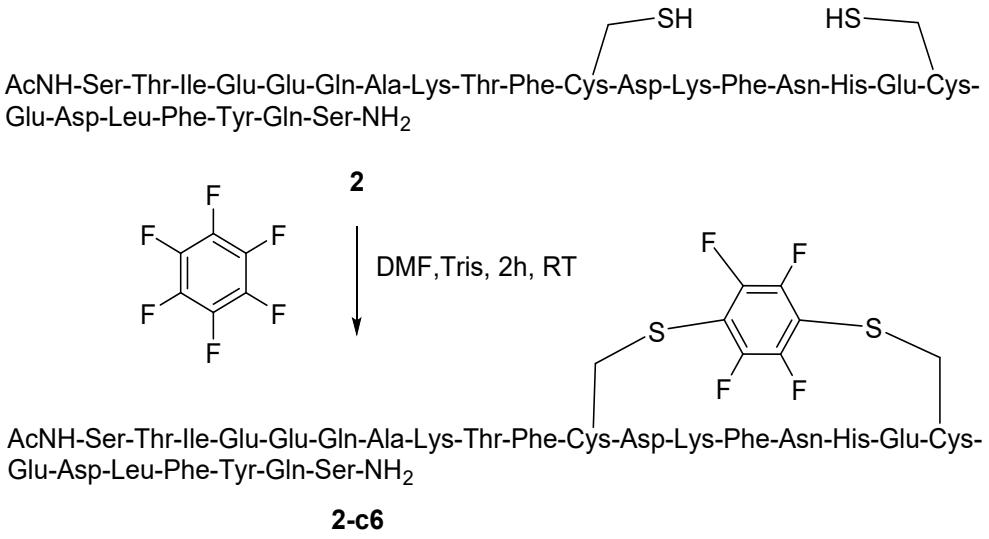


Figure S14. HPLC trace and mass spectrum of the purified **2-c5** (UV: 210nm and 250 nm).



2-c6

2-c6 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF. Then, 2.4 mg of hexafluorobenzene in 0.1 mL DMF and 8 mg of Tris in 1 mL DMF were added. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c6** was obtained as a white powder (~7.2 mg, 36%). Analytical HPLC: t_R = 32.4 min (2-90% B in 40 min); m/z = 3199.8 ($C_{139}H_{191}F_4N_{33}O_{46}S_2$, calcd.: 3199.3 g/mol).

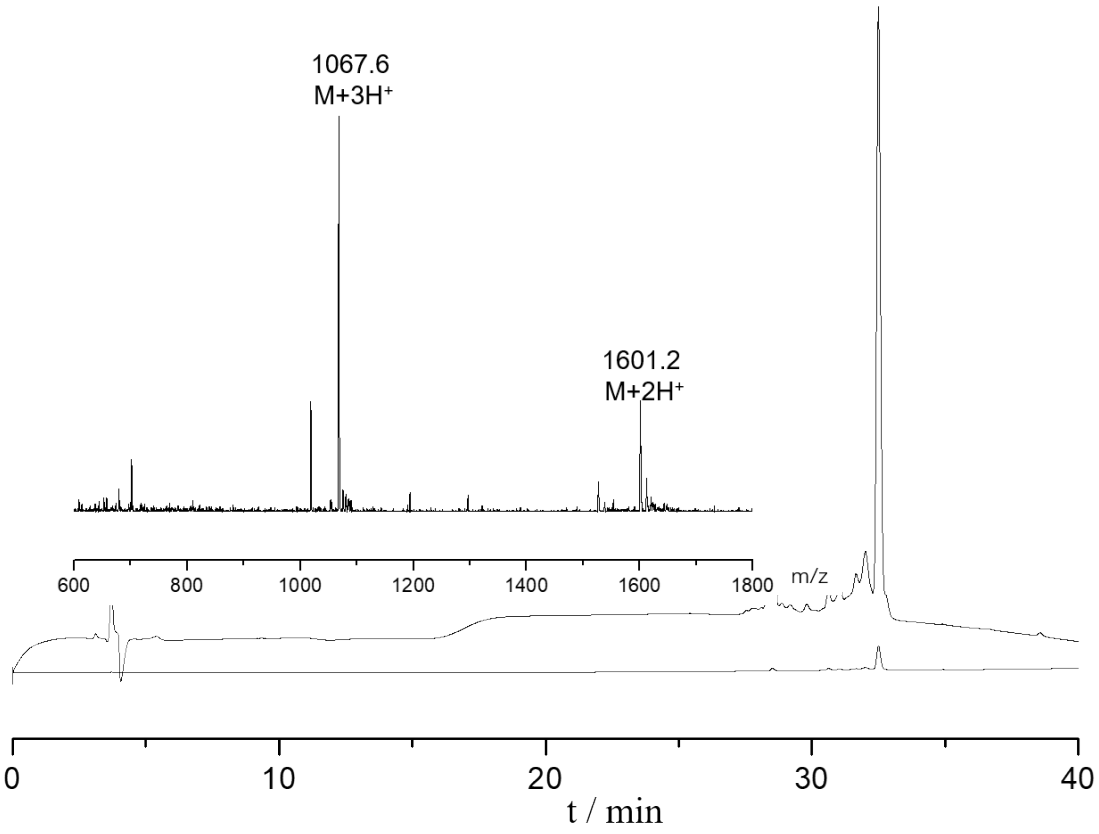
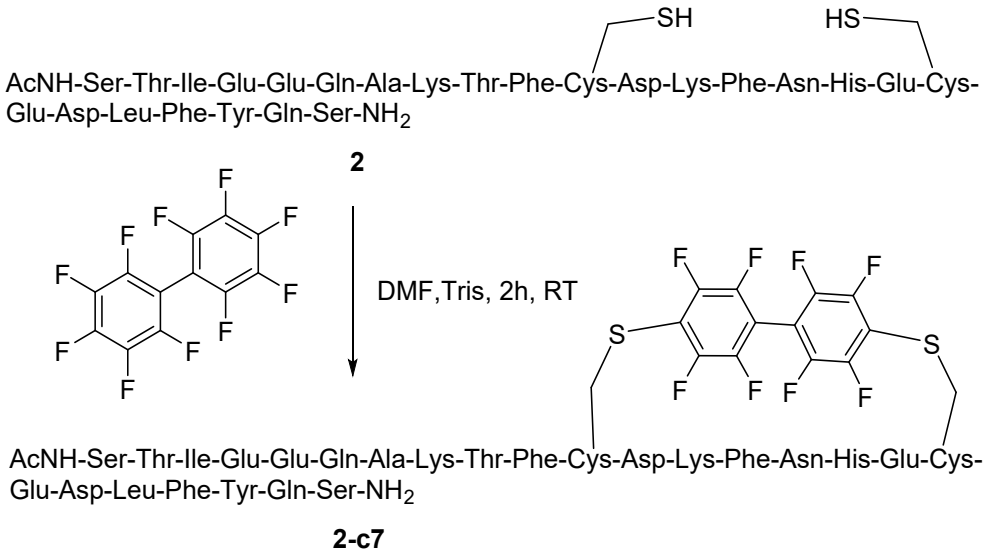


Figure S15. HPLC trace and mass spectrum of the purified **2-c6** (UV: 210nm and 250 nm).



2-c7

2-c7 was prepared by bisalkylation of **2**. 20 mg of crude **2** was dissolved into 2.0 mL DMF. Then, 4.4 mg of decafluorobiphenyl in 0.1 mL DMF and 8 mg of Tris in 1 mL DMF were added. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **2-c7** was obtained as a white powder (~5.8 mg, 29%). Analytical HPLC: t_R = 32.1 min (2-90% B in 40 min); m/z = 3347.6 ($C_{145}H_{191}F_8N_{33}O_{46}S_2$, calcd.: 3347.3 g/mol).

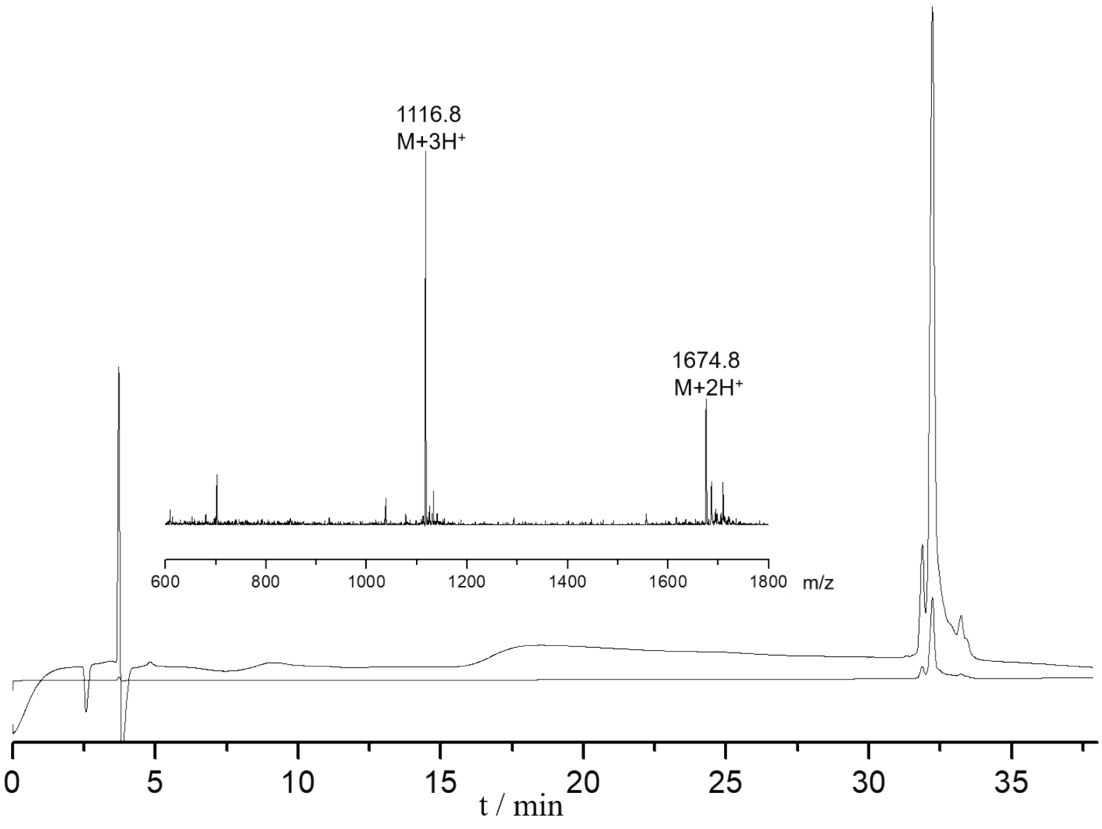
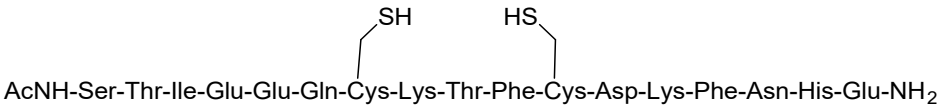


Figure S16. HPLC trace and mass spectrum of the purified **2-c7** (UV: 210nm and 250 nm).

3, **3-c1**, **3-c2**, **3-c3**, **3-c4**, **3-c5**, **3-c6** and **3-c7:**



3

3 was prepared by Fmoc-based SPPS using Rink amide AM resin. After preparative HPLC and lyophilization, **3** was obtained as a white powder. Analytical HPLC: t_R = 28.2 min (2-90% B in 40 min); m/z = 2099.6 ($C_{89}H_{134}N_{24}O_{31}S_2$, calcd.: 2099.0 g/mol).

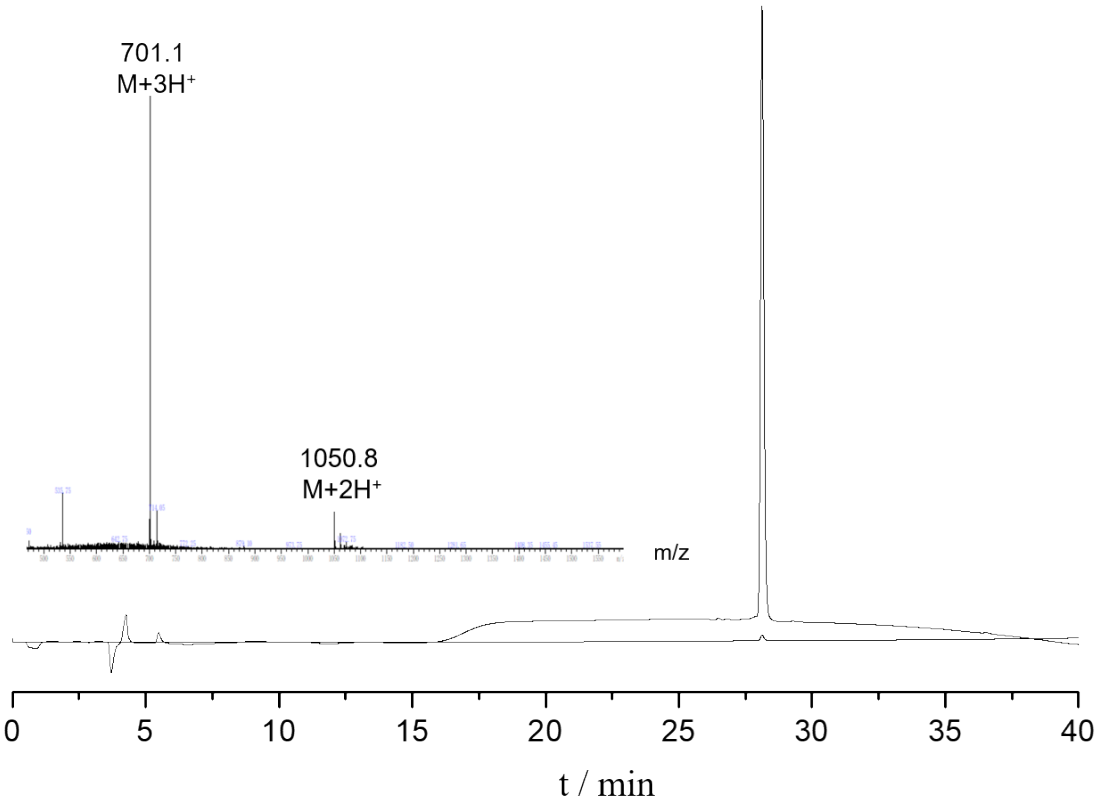
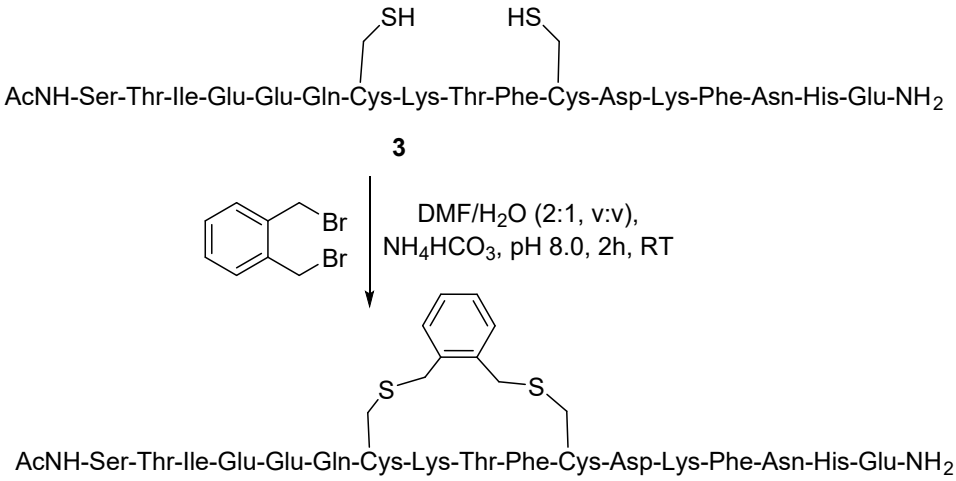


Figure S17. HPLC trace and mass spectrum of the purified **3** (UV: 210nm and 250 nm).



3-c1

3-c1 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1,2-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c1** was obtained as a white powder (7.0 mg, 35%). Analytical HPLC: t_R = 31.2 min (2-90% B in 40 min); m/z = 2201.0 ($C_{97}H_{140}N_{24}O_{31}S_2$, calcd.: 2201.0 g/mol).

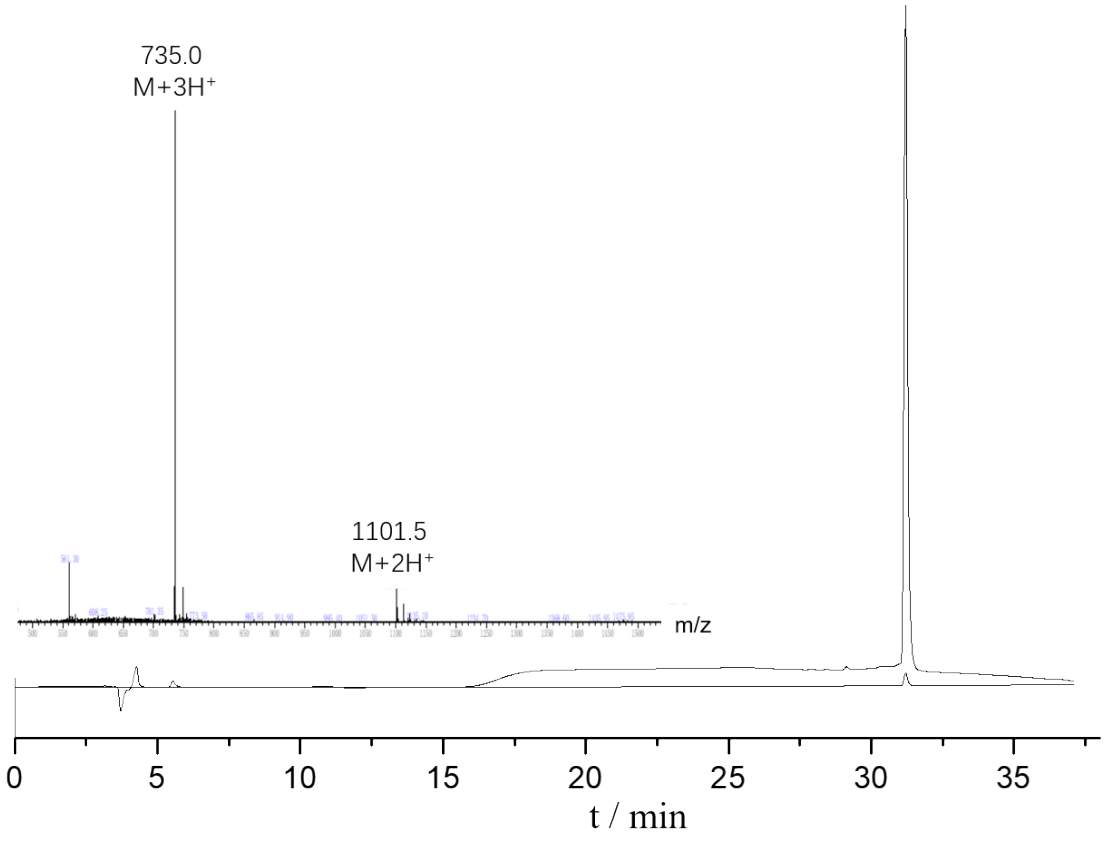
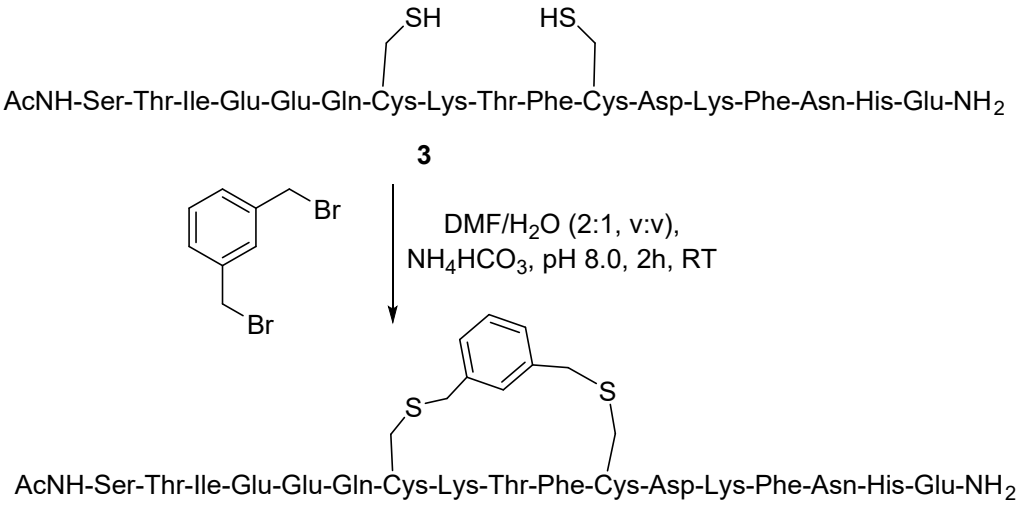


Figure S18. HPLC trace and mass spectrum of the purified **3-c1** (UV: 210nm and 250 nm).



3-c2

3-c2 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 3, 3-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c2** was obtained as a white powder (6.9 mg, 34.5%). Analytical HPLC: t_R = 29.0 min (2-90% B in 40 min); m/z = 2201.0 ($C_{97}H_{140}N_{24}O_{31}S_2$, calcd.: 2201.0 g/mol).

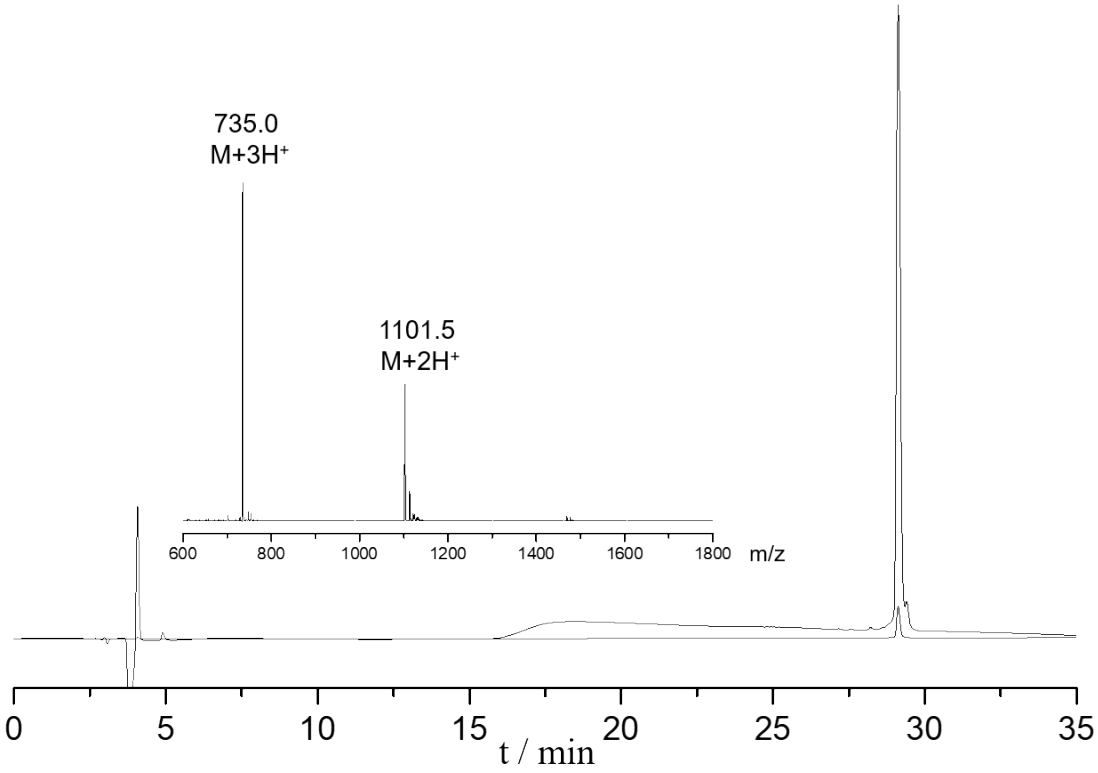
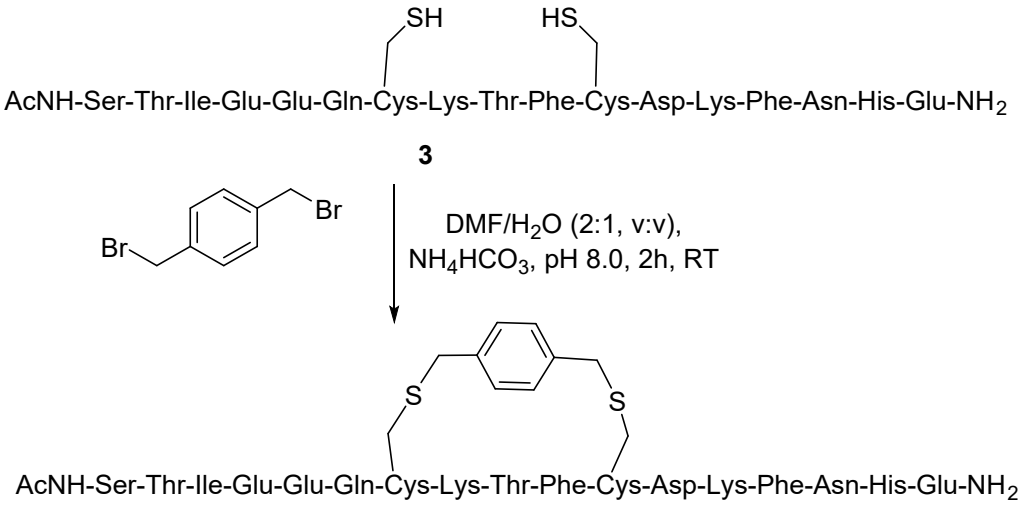


Figure S19. HPLC trace and mass spectrum of the purified **3-c2** (UV: 210nm and 250 nm).



3-c3

3-c3 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 1, 4-bis(bromomethyl)benzene in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c3** was obtained as a white powder (~7.3 mg, 36.5%). Analytical HPLC: t_R = 28.7 min (2-90% B in 40 min); m/z = 2201.0 ($C_{97}H_{140}N_{24}O_{31}S_2$, calcd.: 2201.0 g/mol).

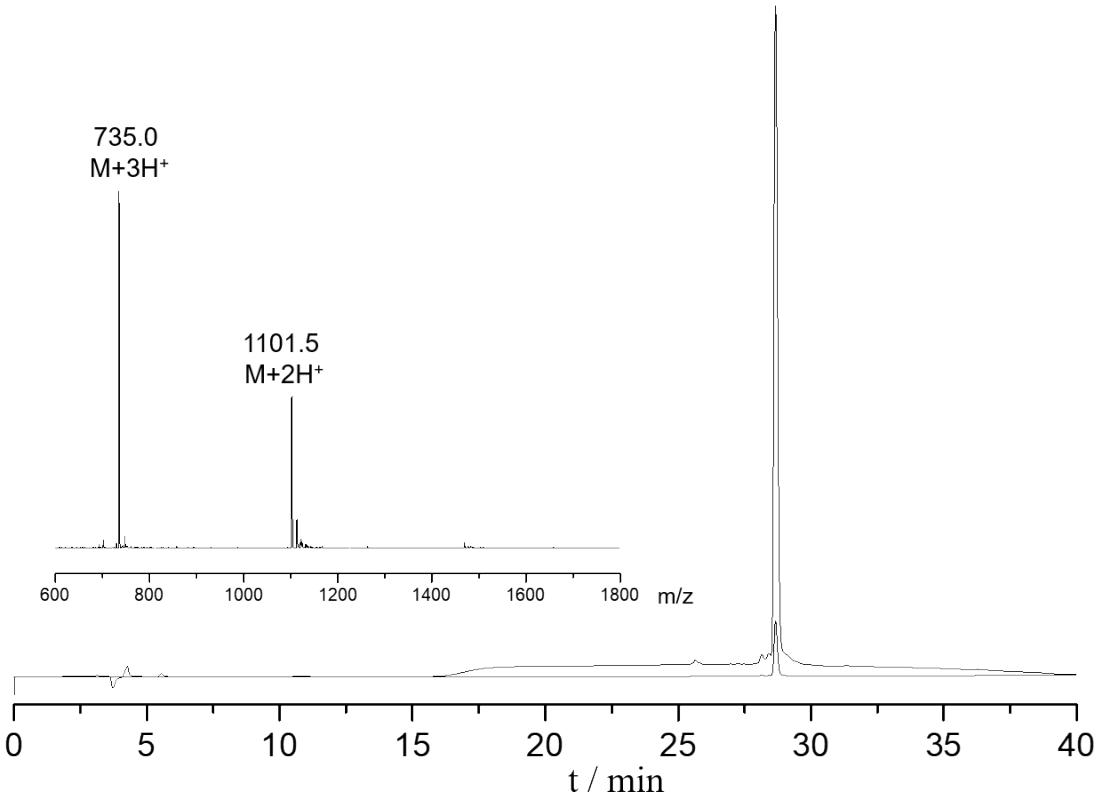
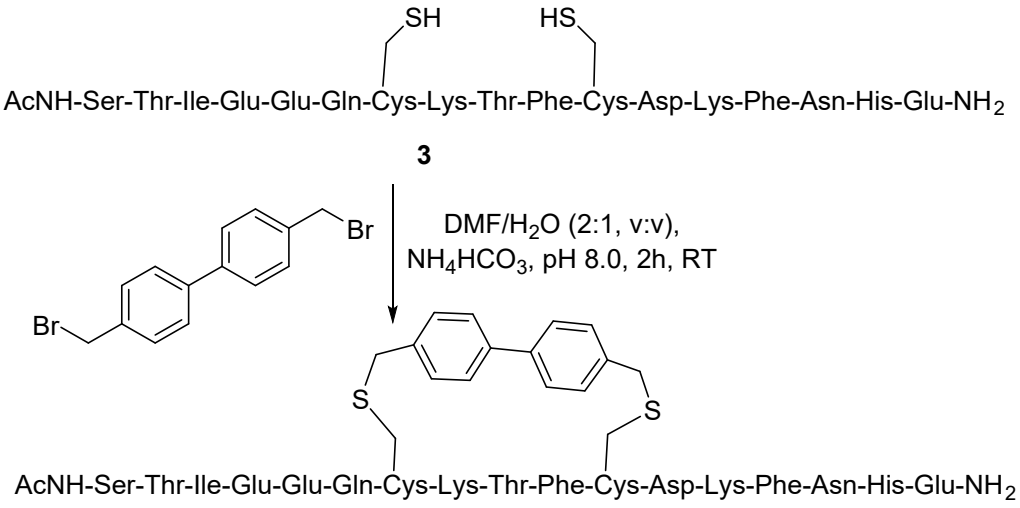


Figure S20. HPLC trace and mass spectrum of the purified **3-c3** (UV: 210nm and 250 nm).



3-c4

3-c4 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.8 mg of bis(bromomethyl)biphenyl in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c4** was obtained as a white powder (~5.3 mg, 26.5%). Analytical HPLC: t_R = 27.4 min (2-90% B in 40 min); m/z = 2277.7 ($C_{103}H_{144}N_{24}O_{31}S_2$, calcd.: 2278.0 g/mol).

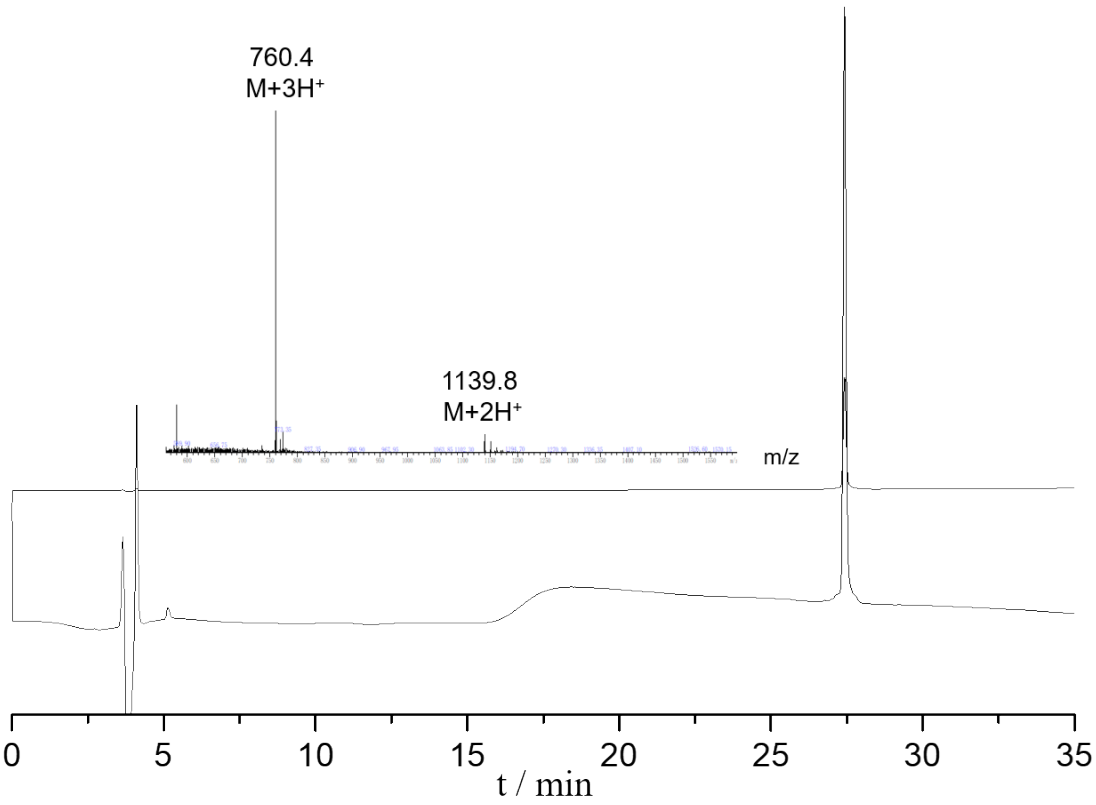
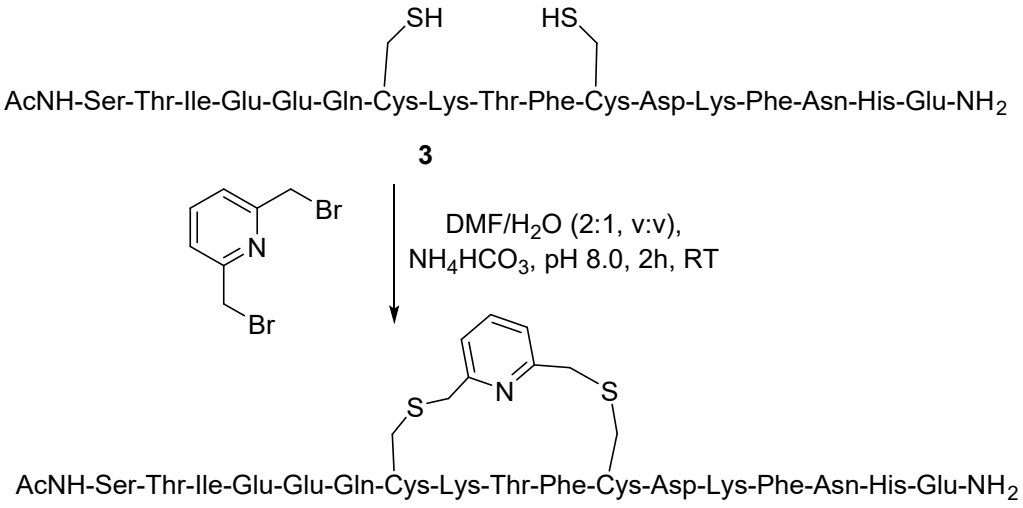


Figure S21. HPLC trace and mass spectrum of the purified **3-c4** (UV: 210nm and 250 nm).



3-c5

3-c5 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of 2,6-bis(bromomethyl)pyridine in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c5** was obtained as a white powder (~6.8 mg, 34%). Analytical HPLC: t_R = 26.3 min (2-90% B in 40 min); m/z = 2203.2 ($C_{96}H_{139}N_{25}O_{31}S_2$, calcd.: 2202.9 g/mol).

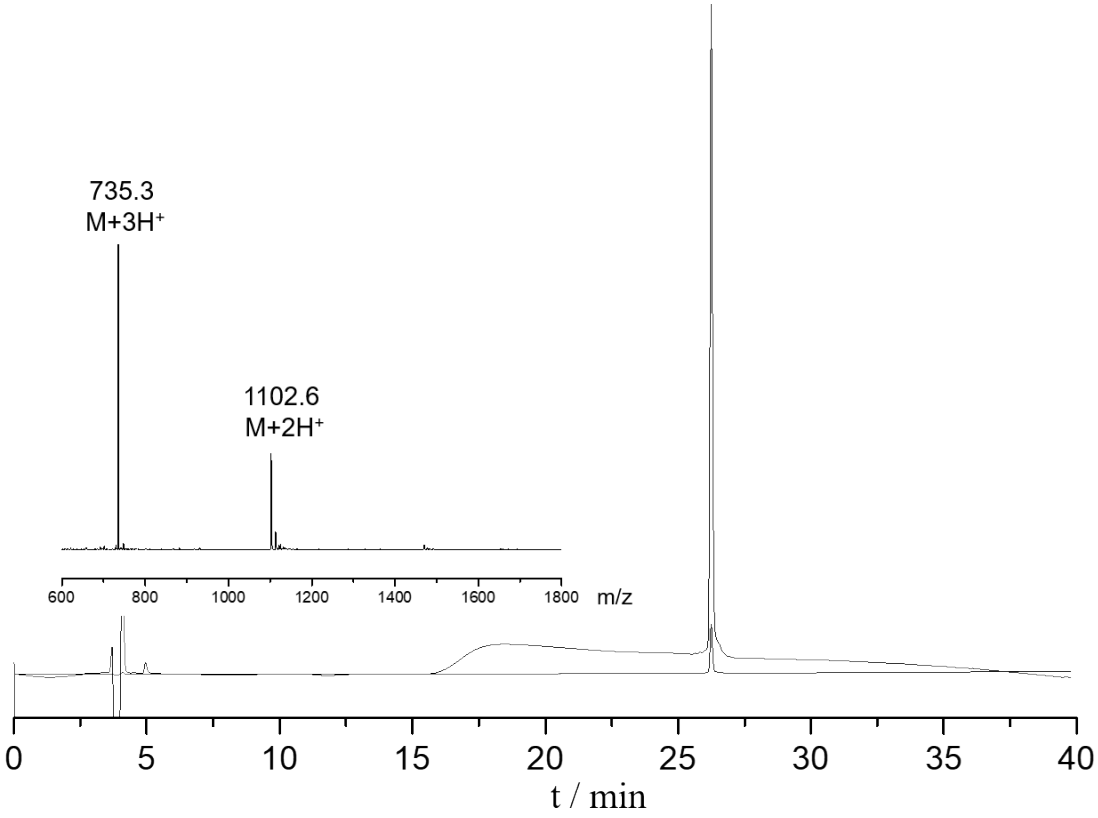
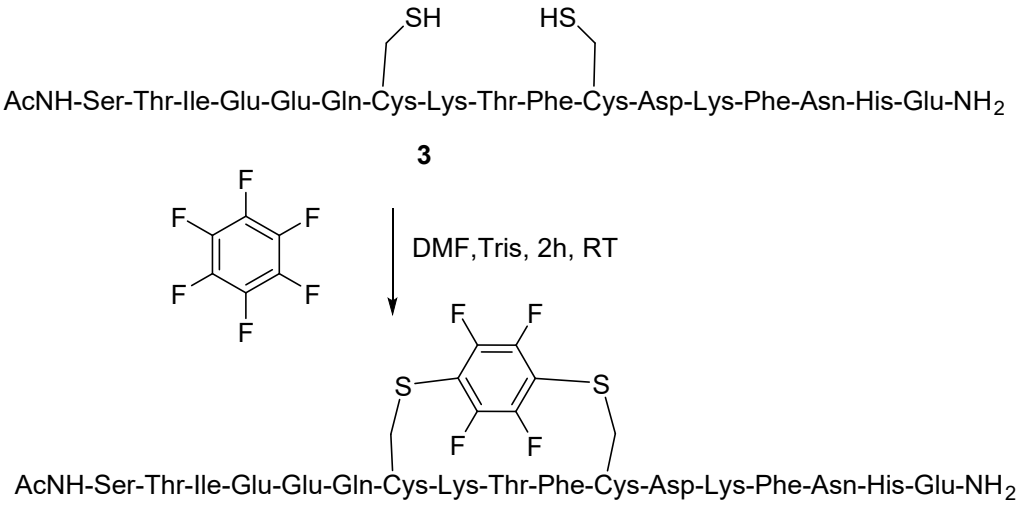


Figure S22. HPLC trace and mass spectrum of the purified **3-c5** (UV: 210nm and 250 nm).



3-c6

3-c6 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF. Then, 2.4 mg of hexafluorobenzene in 0.1 mL DMF and 8 mg of Tris in 1 mL DMF were added. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c6** was obtained as a white powder (~4.7 mg, 23.5%). Analytical HPLC: t_R = 29.6 min (2-90% B in 40 min); m/z = 2245.8 ($C_{95}H_{132}F_4N_{24}O_{31}S_2$, calcd.: 2245.8 g/mol).

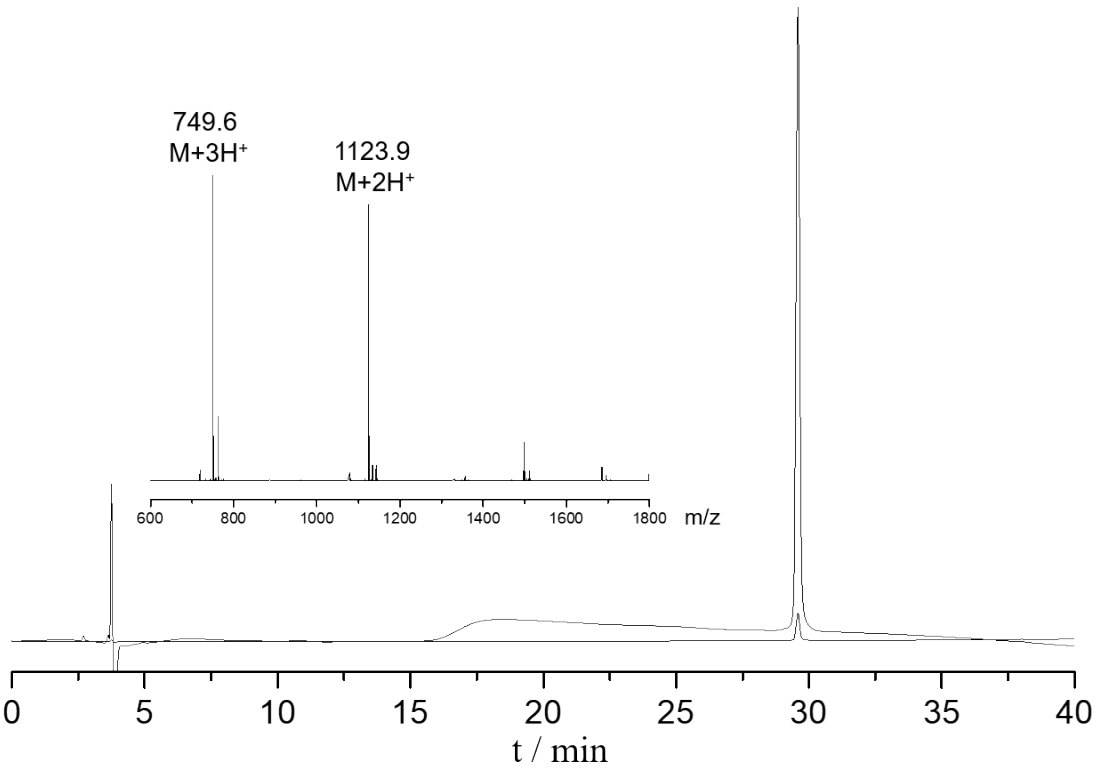
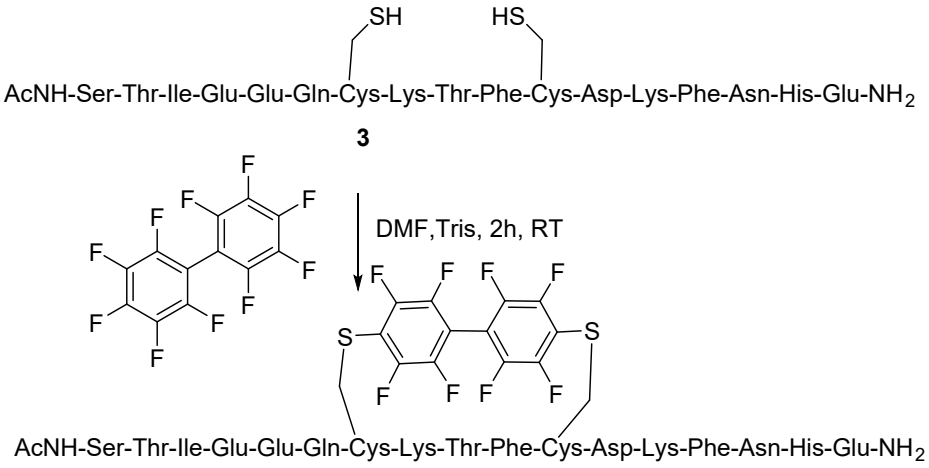


Figure S23. HPLC trace and mass spectrum of the purified **3-c6** (UV: 210nm and 250 nm).



3-c7 was prepared by bisalkylation of **3**. 20 mg of crude **3** was dissolved into 2.0 mL DMF. Then, 4.4 mg of decafluorobiphenyl in 0.1 mL DMF and 8 mg of Tris in 1 mL DMF were added. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **3-c7** was obtained as a white powder (~5.4 mg,27%). Analytical HPLC: t_R = 28.6 min (2-90% B in 40 min); m/z = 2394.4 ($C_{101}H_{132}F_8N_{24}O_{31}S_2$, calcd.: 2393.9 g/mol).

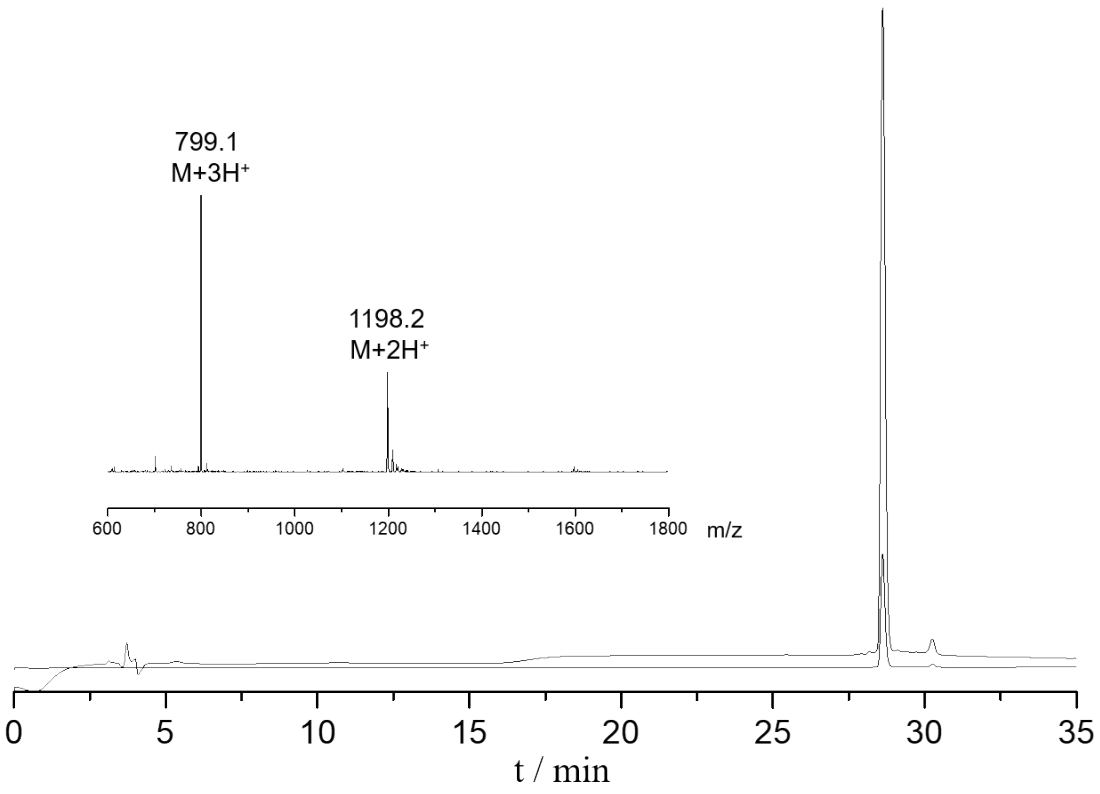
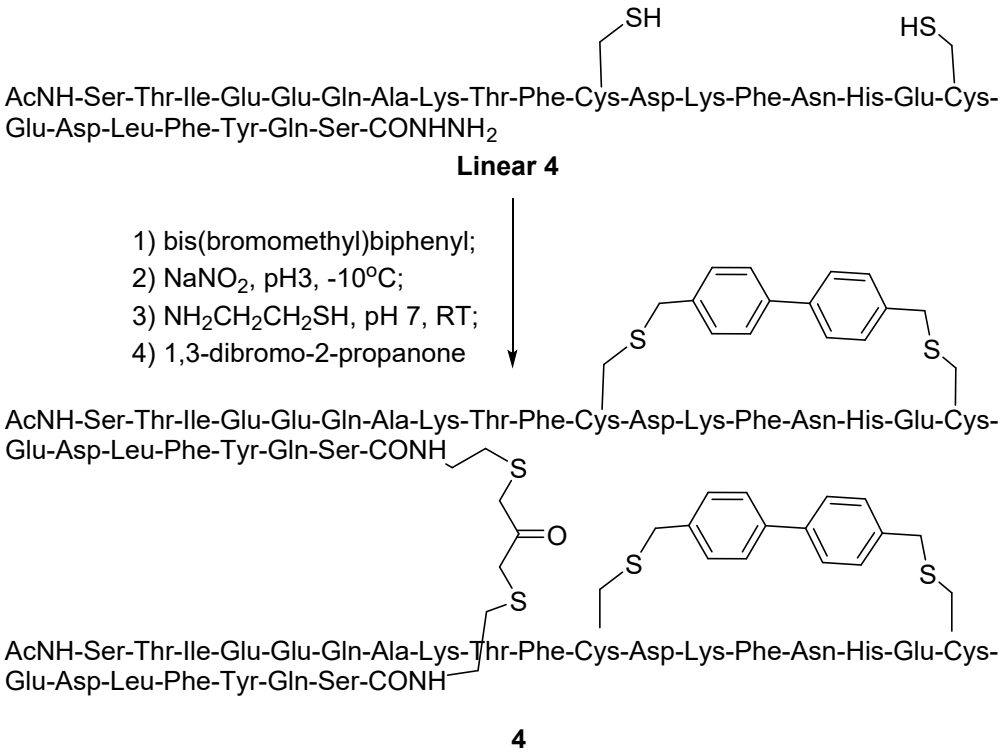


Figure S24. HPLC trace and mass spectrum of the purified **3-c7** (UV: 210nm and 250 nm).

Synthesis of covalent dimeric ACE2 peptide mimetics **4**, **9**, **12**, and **15**:



4

4 was synthesized in several steps, as shown below.

The first step was to prepare hydrazine-Trt(2-Cl) resin. 143 mg of 2-Cl-Trt-Cl resin (80 μmol, loading: 0.56 mmol/g) was transferred to a 10 mL syringe. The resin was swollen in 2 mL of DCM for ~15 minutes. After washing the resin three times with DCM and twice with DMF, 67 μL of hydrazine hydrate in 1.33 mL of DMF (5% v/v) was added to the syringe. Keep the syringe in a shaker at RT. After 30 minutes, the hydrazine mixture was discarded. Repeat this step once again. Then, 267 μL of methanol in 1.33 mL of DMF (20%, v/v) was used to quench the unreacted 2-Cl-Trt-Cl resin. After 20 minutes, the resin was washed five times with DMF. **Linear 4** was prepared by Fmoc-based SPPS using the freshly prepared hydrazine-Trt(2-Cl) resin. After ether precipitation, the crude product of **Linear 4** was obtained as a white powder.

The second step is the reaction between **Linear 4** and bis(bromomethyl)biphenyl. Dissolve 20 mg of crude **Linear 4** in 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.2 mg of bis(bromomethyl)biphenyl in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 with 1.0 M NH_4HCO_3. Keep the reaction in a vortex mixer at room temperature. After 2 hours (Figure S25b), 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. **Cross-linked 4** was obtained by preparative HPLC and lyophilization. The third step is the conversion of **Cross-linked 4** into **Thioester 4**, and the conversion of **Thioester 4** into **C-aminoethanethiol 4**. Dissolve 4.9 mg of **Cross-linked 4** (1.6 μmol) in 1.2 mL of PBS (6.0 M GnHCl, 0.2 M Na_2HPO_4, pH 3.0). Under -10 °C, 80 μL of freshly prepared $NaNO_2$ (200 mM, 10 eq.) was slowly added. After 20 minutes, 1.2 mL of MESNa (200 mM, pH 7) was added. Then, the pH value of the solution was adjusted to 6.8. After 2 hours (Figure S25c), the formed **Thioester 4** was obtained by preparative HPLC. Dissolve 4 mg of **Thioester**

4 in a 2-aminoethanethiol solution (4 mg of 2-aminoethanethiol in 1 mL PBS, pH 7). After 1 hour (Figure S25d), **C-aminoethanethiol 4** was obtained by preparative HPLC and lyophilization. The final step is the dimerization of **C-aminoethanethiol 4** by 1,3-dibromoacetone. 2 mg of **C-aminoethanethiol 4** (~0.6 µmol) in 50 µL of DMF, and ~0.24 µmol of 1,3-dibromoacetone in 50 µL of DMF were added to 2.0 mL of 80 mM sodium borate buffer (20 mg $Na_2B_4O_7$ in 5 mL H_2O, pH 6.5). After 2 hours (Figure S25e), **4** was purified by preparative HPLC. After lyophilization, **4** was obtained as a white powder (1.0 mg, 50%). Analytical HPLC: t_R = 28.6 min (2-90% B in 40 min); m/z = 6637.5 ($C_{301}H_{416}N_{66}O_{93}S_6$, calcd.: 6637.8 g/mol).

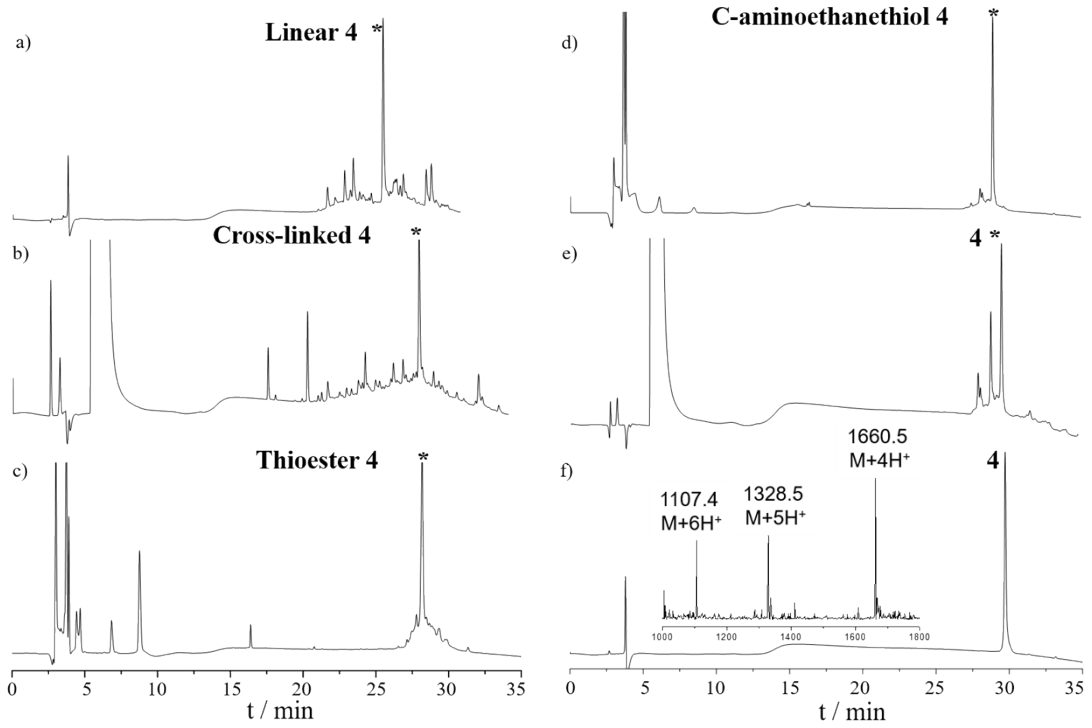
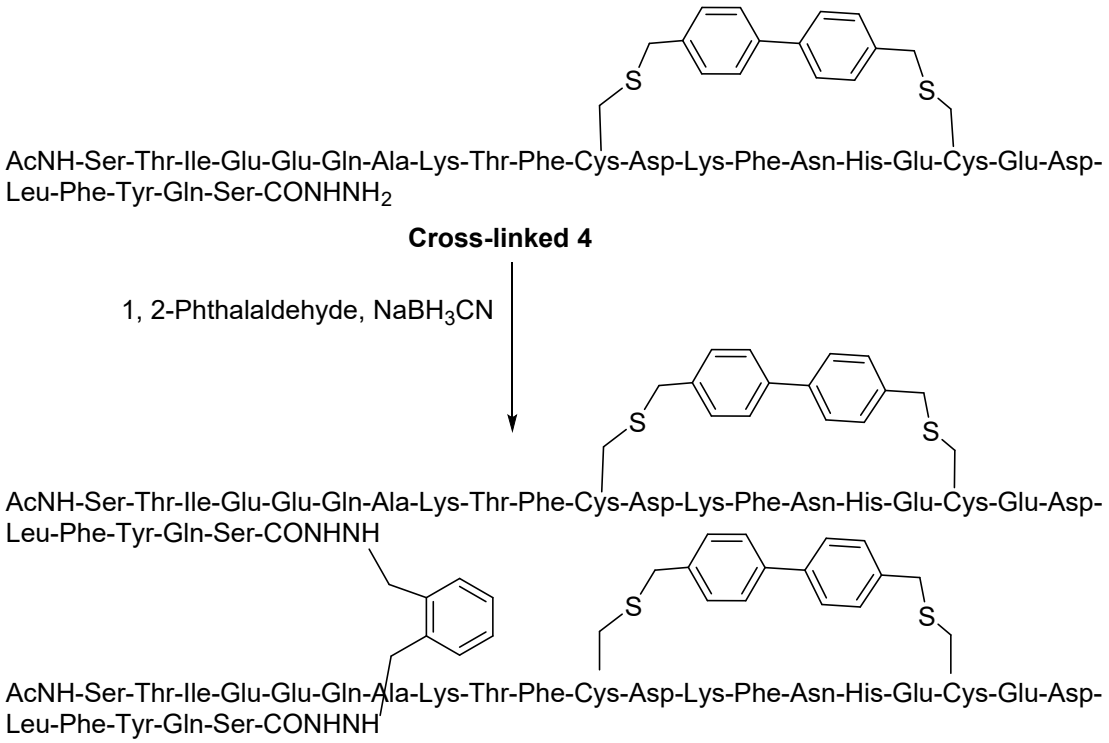


Figure S25. HPLC traces for the preparation of **4** (UV: 210nm). (a) the crude product of **Linear 4**; (b) reaction with bis(bromomethyl)biphenyl; (c) conversion of **Cross-linked 4** into **Thioester 4**; (d) conversion of **Thioester 4** into **C-aminoethanethiol 4**; (e) dimerization of **C-aminoethanethiol 4** by 1,3-dibromoacetone; (f) HPLC trace and mass spectrum of the purified **4**.



9

4 mg of **Cross-linked 4** (~1.23 μmol) was dissolved into 800 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,2-phthalaldehyde was prepared (5.5 mg 1,2-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,2-phthalaldehyde was added to the **Cross-linked 4** solution. Keep the reactor at room temperature for 1 h (Figure S26b). 4.0 mg of $NaBH_3CN$ in 400 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S26c). Then, **9** was purified by preparative HPLC. After lyophilization, **9** was obtained as a white powder (1.5 mg, 37.5%). Analytical HPLC: t_R = 30.8 min (2-90% B in 40 min); m/z = 6595.6 ($C_{302}H_{414}N_{68}O_{92}S_4$, calcd.: 6595.8 g/mol).

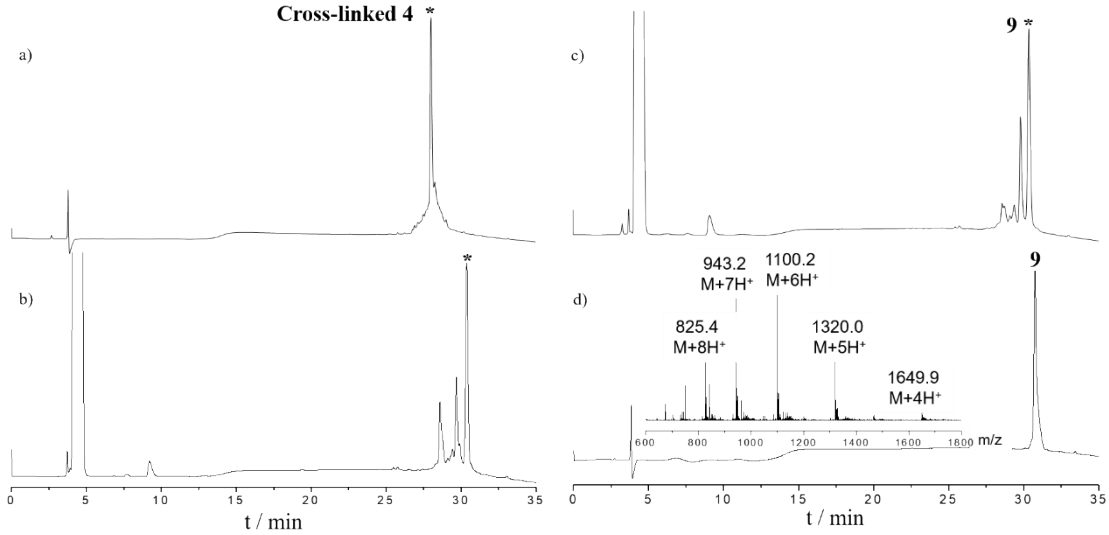
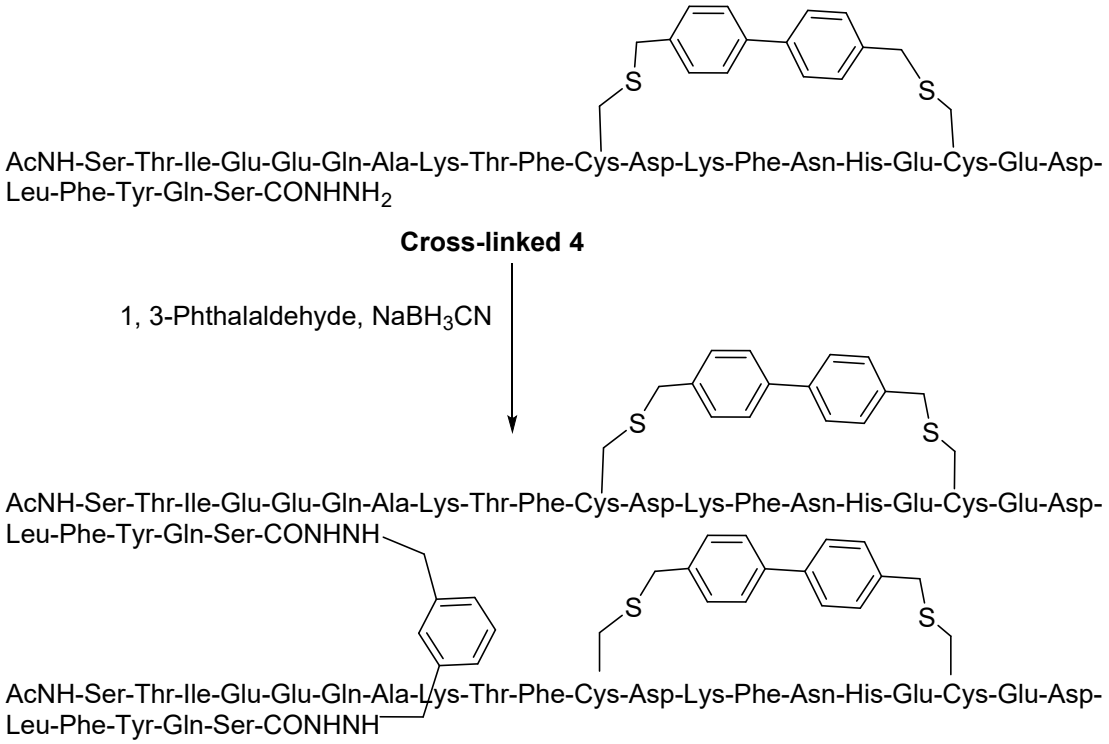


Figure S26. HPLC traces for the preparation of **9** (UV: 210nm). (a) HPLC trace of **Cross-linked 4**; (b) 1 h reaction between **Cross-linked 4** and 1, 2-phthalaldehyde; (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **9**.



4 mg of **Cross-linked 4** (~1.23 μmol) was dissolved into 800 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,3-phthalaldehyde was prepared (5.5 mg 1,3-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,3-phthalaldehyde was added to the **Cross-linked 4** solution. Keep the reactor at room temperature for 1 h (Figure S27b). 4.0 mg of $NaBH_3CN$ in 400 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S27c). Then, **12** was purified by preparative HPLC. After lyophilization, **12** was obtained as a white powder (1.4 mg, 35%). Analytical HPLC: t_R = 29.8 min (2-90% B in 40 min); m/z = 6595.6 ($C_{302}H_{414}N_{68}O_{92}S_4$, calcd.: 6595.8 g/mol).

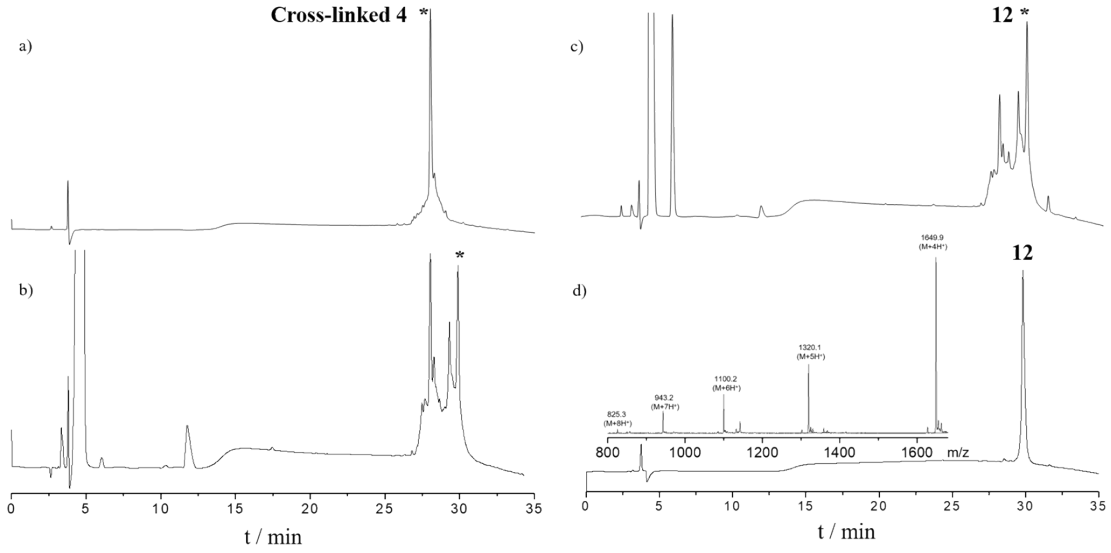
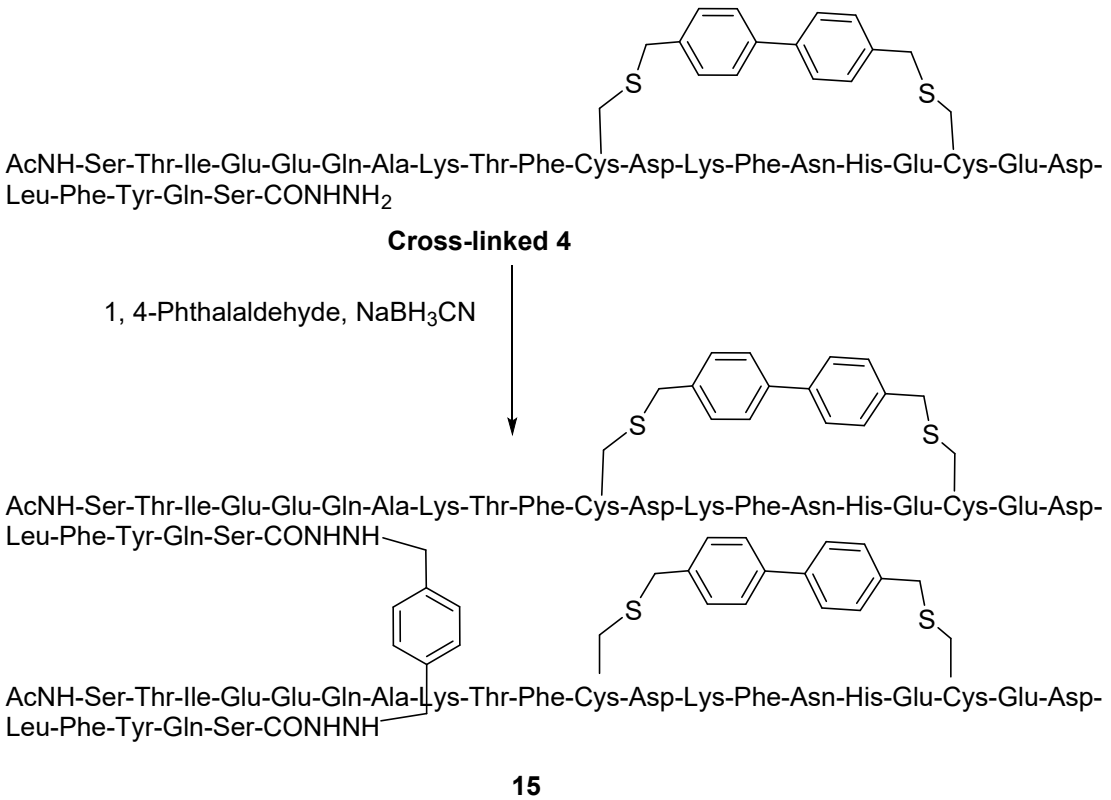


Figure S27. HPLC traces for the preparation of **12** (UV: 210nm). (a) HPLC trace of **Cross-linked 4**; (b) 1 h reaction between **Cross-linked 4** and 1, 3-phthalaldehyde; (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **12**.



15

4 mg of **Cross-linked 4** (~1.23 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,4-phthalaldehyde was prepared (5.5 mg 1,4-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,4-phthalaldehyde was added to the **Cross-linked 4** solution. Keep the reactor at room temperature

for 1 h (Figure S28b). 4.0 mg of $NaBH_3CN$ in 400 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S28c). Then, **15** was purified by preparative HPLC. After lyophilization, **15** was obtained as a white powder (1.4 mg, 35%). Analytical HPLC: t_R = 29.6 min (2-90% B in 40 min); m/z = 6595.6 ($C_{302}H_{414}N_{68}O_{92}S_4$, calcd.: 6595.8 g/mol).

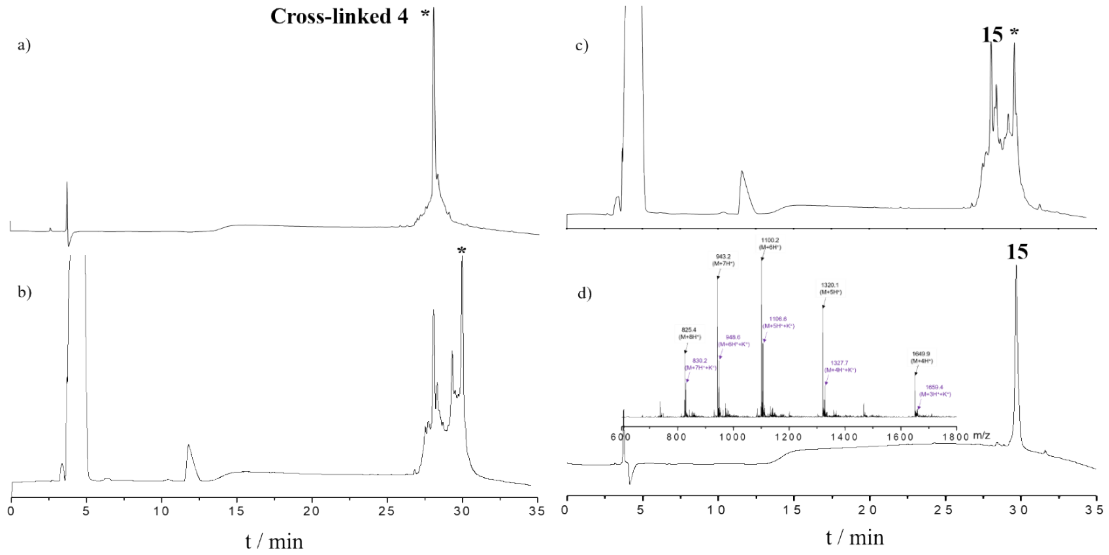
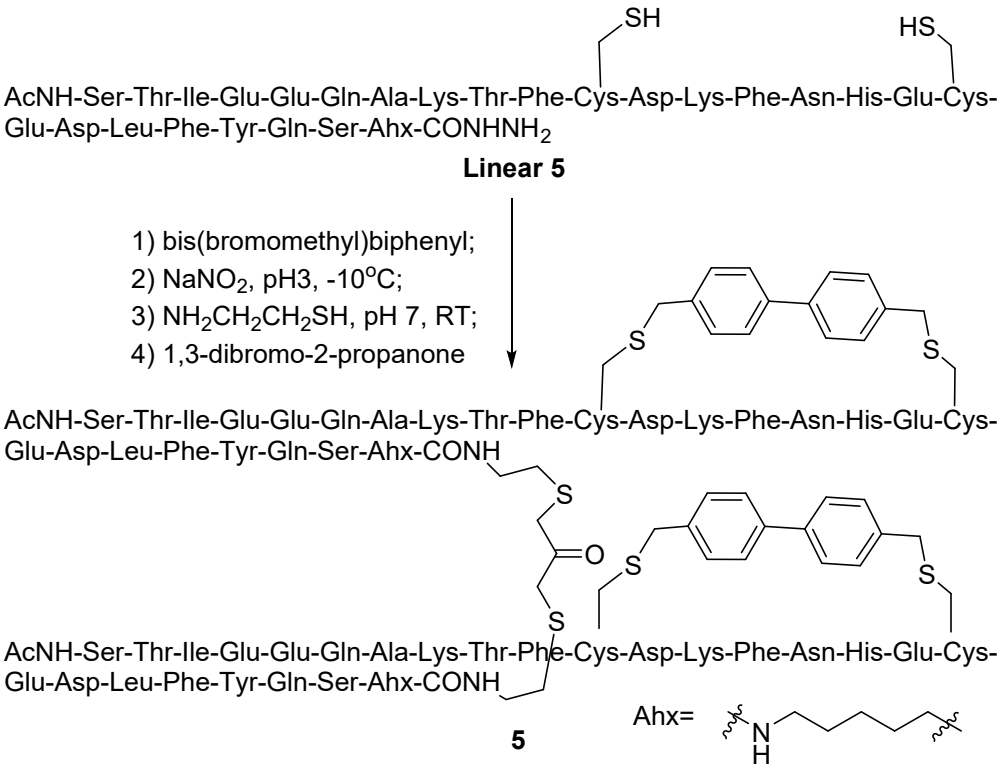


Figure S28. HPLC traces for the preparation of **15** (UV: 210nm). (a) HPLC trace of **Cross-linked 4**; (b) 1 h reaction between **Cross-linked 4** and 1, 4-phthalaldehyde; (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **15**.

5, **10**, **13**, and **16**:



5 was synthesized in several steps, as shown below.

The first step was to prepare hydrazine-Trt(2-Cl) resin. 143 mg of 2-Cl-Trt-Cl resin (80 μmol, loading: 0.56 mmol/g) was transferred to a 10 mL syringe. The resin was swollen in 2 mL of DCM for ~15 minutes. After washing the resin three times with DCM and twice with DMF, 67 μL of hydrazine hydrate in 1.33 mL of DMF (5% v/v) was added to the syringe. Keep the syringe in a shaker at RT. After 30 minutes, the hydrazine mixture was discarded. Repeat this step once again. Then, 267 μL of methanol in 1.33 mL of DMF (20%, v/v) was used to quench the unreacted 2-Cl-Trt-Cl resin. After 20 minutes, the resin was washed five times with DMF. **Linear 5** was prepared by Fmoc-based SPPS using the freshly prepared hydrazine-Trt(2-Cl) resin. After ether precipitation, the crude **Linear 5** was obtained as a white powder.

The second step is the reaction between **Linear 5** and bis(bromomethyl)biphenyl. Dissolve 20 mg of crude **Linear 5** in 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.1 mg of bis(bromomethyl)biphenyl in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 with 1.0 M NH_4HCO_3. Keep the reaction in a vortex mixer at room temperature. After 2 hours (Figure S29b), 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. **Cross-linked 5** was obtained by preparative HPLC and lyophilization. The third step is the conversion of **Cross-linked 5** into **Thioester 5**, and the conversion of **Thioester 5** into **C-aminoethanethiol 5**. Dissolve 5.0 mg of **Cross-linked 5** (1.6 μmol) in 1.2 mL of PBS (6.0 M GnHCl, 0.2 M Na_2HPO_4, pH 3.0). Under -10 °C, 80 μL of freshly prepared $NaNO_2$ (200 mM, 10 eq.) was slowly added. After 20 minutes, 1.2 mL of MESNa (200 mM, pH 7) was added. Then, the pH value of the solution was adjusted to 6.8. After 2 hours (Figure S29c), the formed **Thioester 5** was obtained by preparative HPLC. Dissolve 4 mg of **Thioester 5** in a 2-aminoethanethiol solution (4 mg of 2-aminoethanethiol in 1 mL PBS, pH 7). After 1 hour (Figure S29d), **C-aminoethanethiol 5** was obtained by preparative HPLC and lyophilization. The final step is the dimerization of **C-aminoethanethiol 5** by 1,3-dibromoacetone. 2 mg of **C-aminoethanethiol 5** (~0.6 μmol) in 50 μL of DMF, and ~0.24 μmol of 1,3-dibromoacetone in 50 μL of DMF were added to 2.0 mL of 80 mM sodium borate buffer (20 mg $Na_2B_4O_7$ in 5 mL H_2O, pH 6.5). After 2 hours (Figure S29e), **5** was purified by preparative HPLC. After lyophilization, **5** was obtained as a white powder (1.0 mg, 50%). Analytical HPLC: t_R = 29.6 min (2-90% B in 40 min); m/z = 6863.6 ($C_{313}H_{438}N_{68}O_{95}S_6$, calcd.: 6864.0 g/mol).

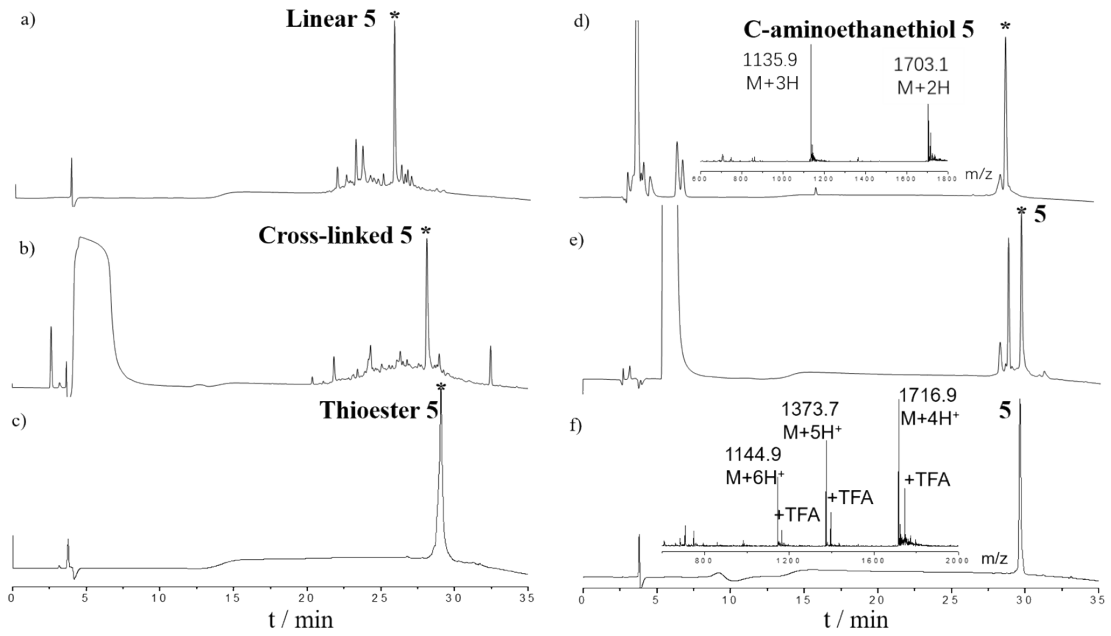
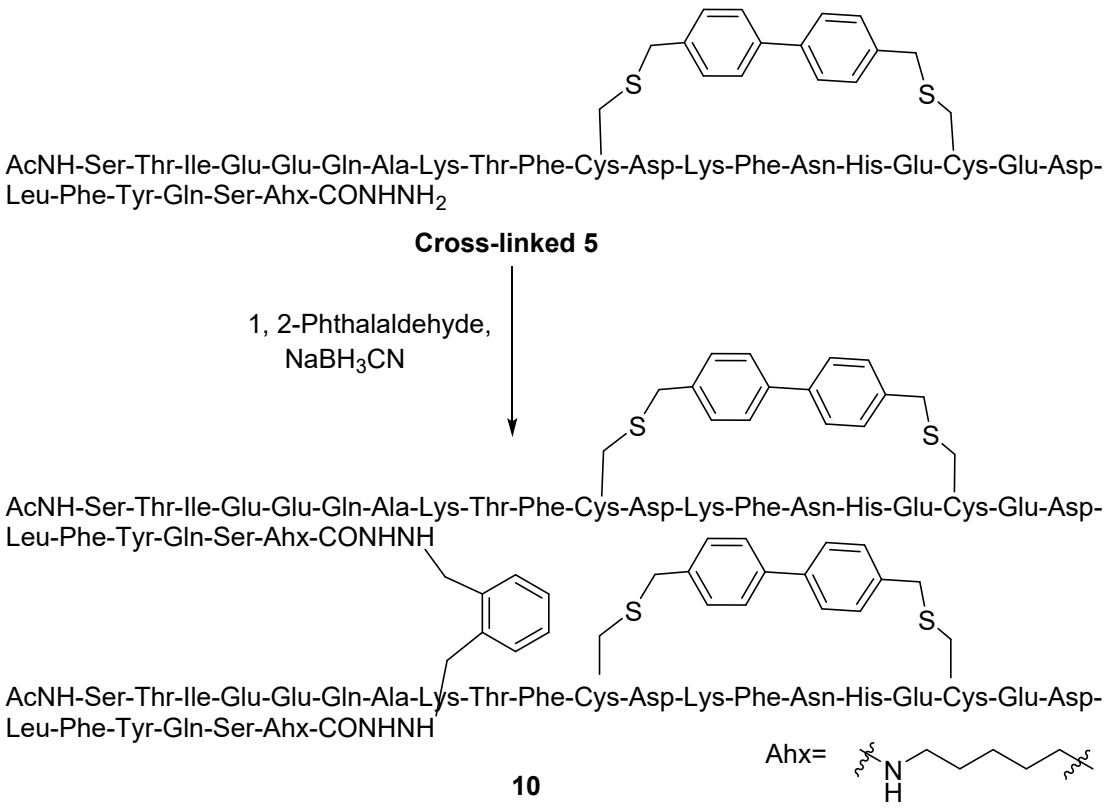


Figure S29. HPLC traces for the preparation of **5** (UV: 210nm). (a) the crude product of **Linear 5**; (b) reaction with bis(bromomethyl)biphenyl; (c) conversion of **Cross-linked 5** into **Thioester 5**; (d) conversion of **Thioester 5** into **C-aminoethanethiol 5**; (e) dimerization of **C-aminoethanethiol 5** by 1,3-dibromoacetone; (f) HPLC trace and mass spectrum of the purified **5**.



3 mg of **Cross-linked 5** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture

(1:1, v:v). Then, a soluton of 1,2-phthalaldehyde was prepared 4.1 mg 1,2-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,2-phthalaldehyde was added to the **Cross-linked 5** solution. Keep the reactor at room temperature for 1 h (Figure S30b). 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S30c). Then, **10** was purified by preparative HPLC. After lyophilization, **10** was obtained as a white powder (1.2 mg, 40%). Analytical HPLC: t_R = 29.7 min (2-90% B in 40 min); m/z = 6819.6 ($C_{314}H_{436}N_{70}O_{94}S_4$, calcd.: 6819.1 g/mol).

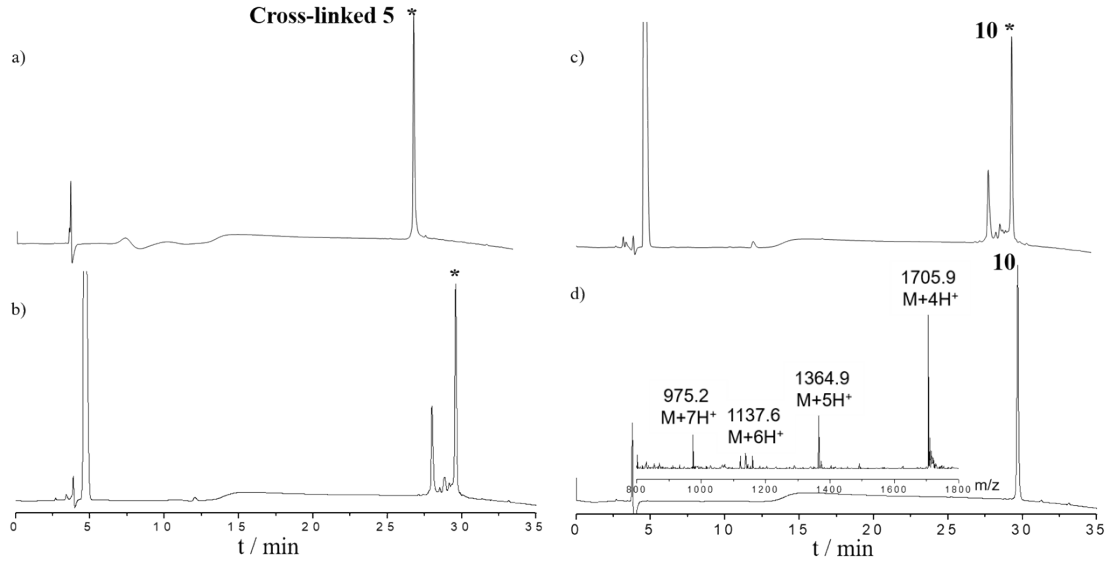
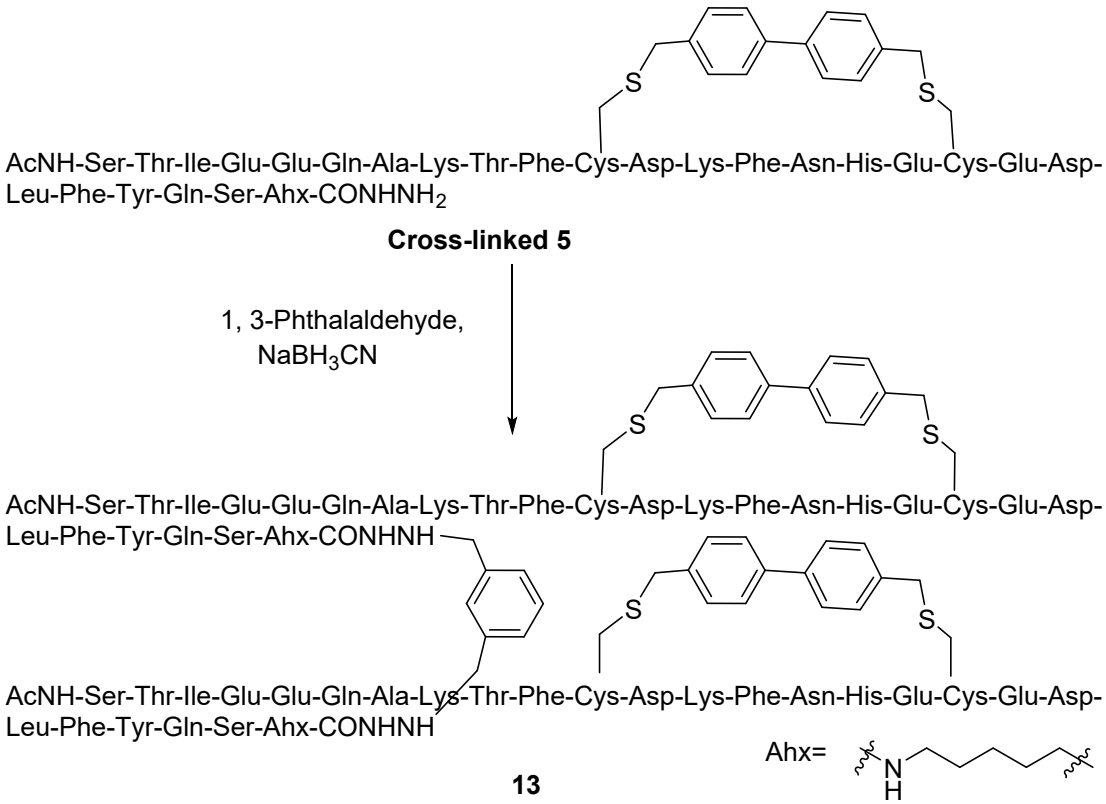


Figure S30. HPLC traces for the preparation of **10** (UV: 210nm). (a) HPLC trace of **Cross-linked 5**; (b) 1 h reaction between **Cross-linked 5** and 1, 2-phthalaldehyde; (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **10**.



3 mg of **Cross-linked 5** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,3-phthalaldehyde was prepared (4.1 mg 1,3-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,3-phthalaldehyde was added to the **Cross-linked 5** solution. Keep the reactor at room temperature for 1 h (Figure S31b). 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S31c). Then, **13** was purified by preparative HPLC. After lyophilization, **13** was obtained as a white powder (0.8 mg, 27%). Analytical HPLC: t_R = 29.4 min (2-90% B in 40 min); m/z = 6820.4 ($C_{302}H_{414}N_{68}O_{92}S_4$, calcd.: 6819.1 g/mol).

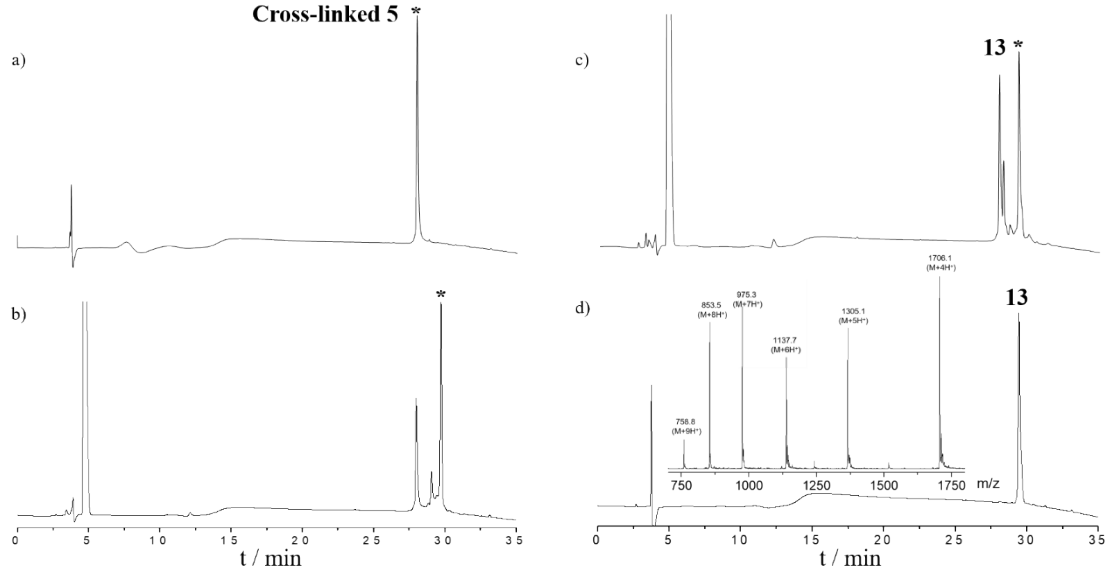
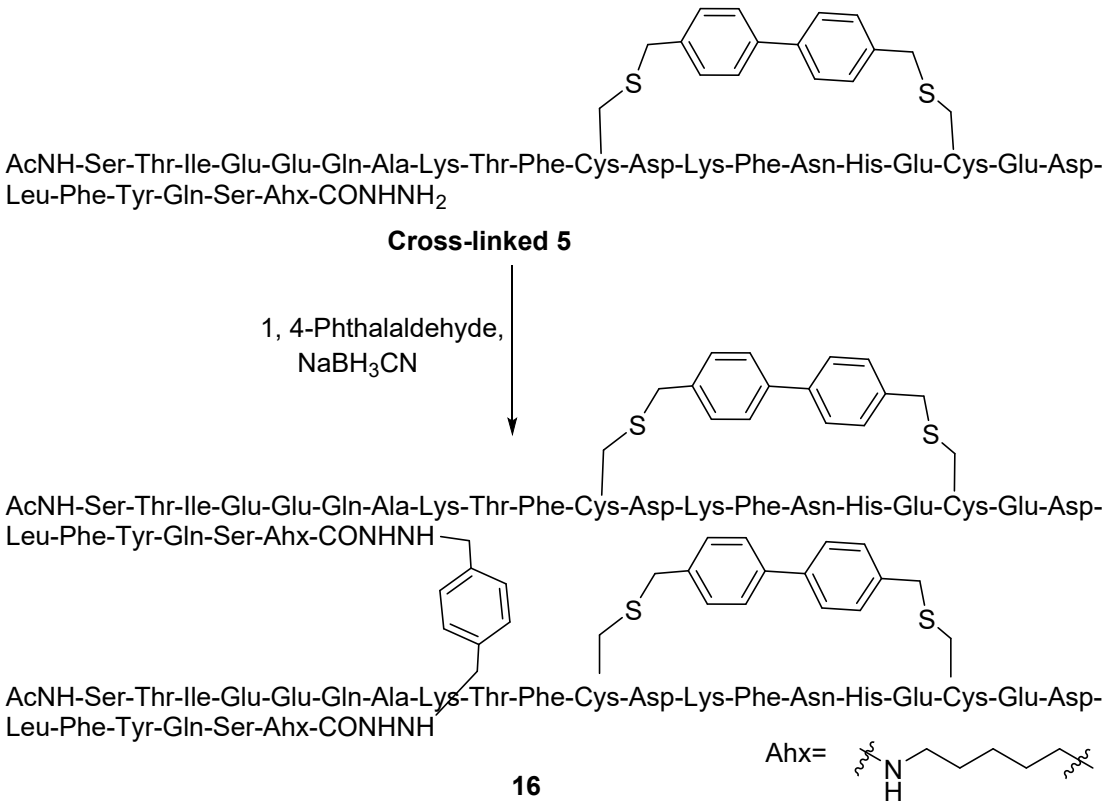


Figure S31. HPLC traces for the preparation of **13** (UV: 210nm). (a) HPLC trace of **Cross-linked 5**; (b) 1 h reaction between **Cross-linked 5** and 1, 3-phthalaldehyde; (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **13**.



3 mg of **Cross-linked 5** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,4-phthalaldehyde was prepared (4.1 mg 1,4-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,4-phthalaldehyde was added to the **Cross-linked 5** solution. Keep the reactor at room temperature for 1 h (Figure

S32b). 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S32c). Then, **16** was purified by preparative HPLC. After lyophilization, **16** was obtained as a white powder (0.8mg, 27%). Analytical HPLC: t_R = 29.5 min (2-90% B in 40 min); m/z = 6820.0 ($C_{302}H_{414}N_{68}O_{92}S_4$, calcd.: 6819.1 g/mol).

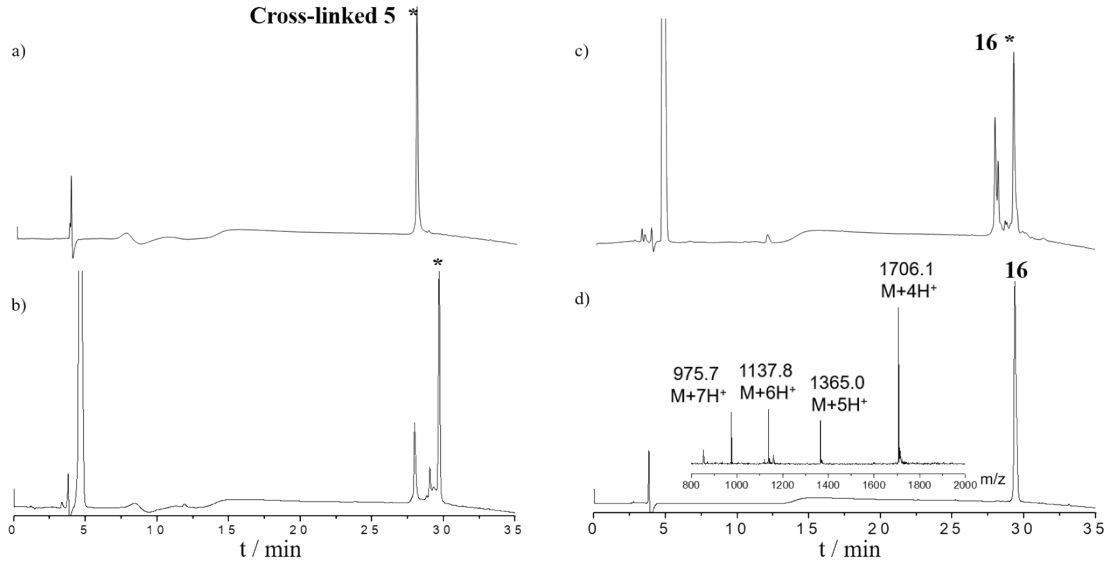
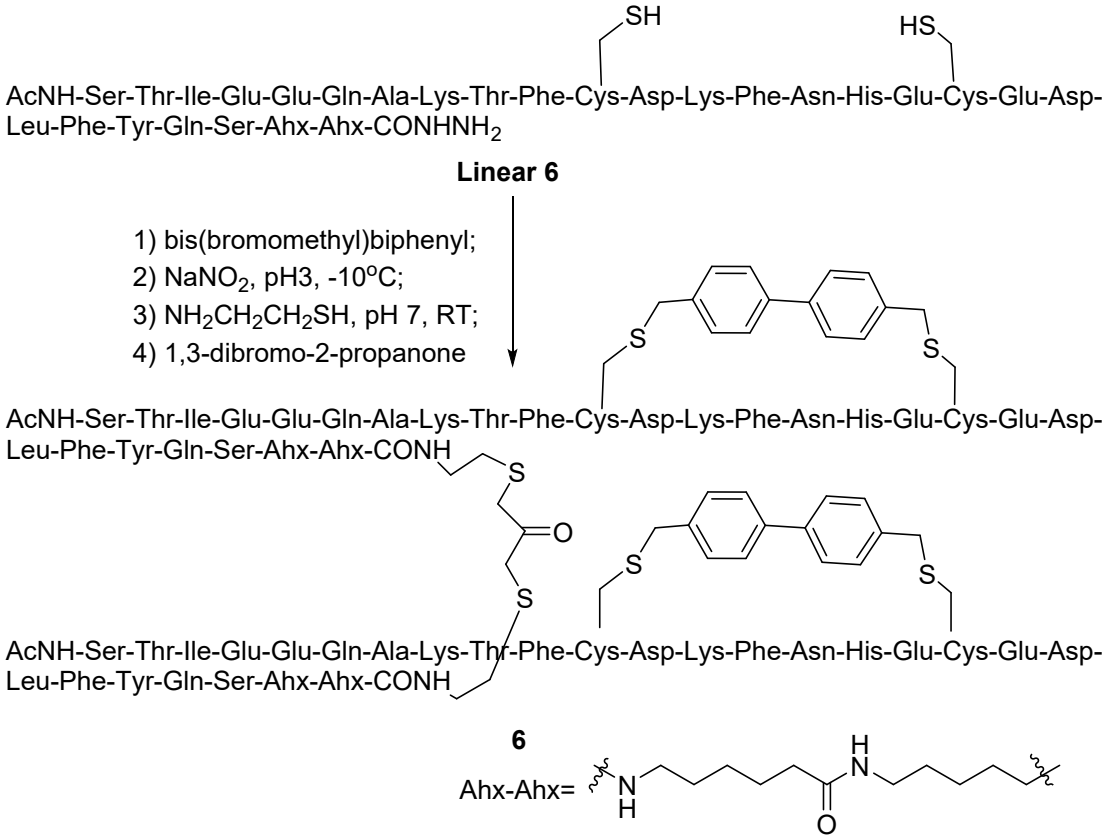


Figure S32. HPLC traces for the preparation of **16** (UV: 210nm). (a) HPLC trace of **Cross-linked 5**; (b) 1 h reaction between **Cross-linked 5** and 1, 4-phthalaldehyde; (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **16**.

6, **11**, **14**, and **17**:



6 was synthesized in several steps, as shown below.

The first step was to prepare hydrazine-Trt(2-Cl) resin. 143 mg of 2-Cl-Trt-Cl resin (80 μmol, loading: 0.56 mmol/g) was transferred to a 10 mL syringe. The resin was swollen in 2 mL of DCM for ~15 minutes. After washing the resin three times with DCM and twice with DMF, 67 μL of hydrazine hydrate in 1.33 mL of DMF (5% v/v) was added to the syringe. Keep the syringe in a shaker at RT. After 30 minutes, the hydrazine mixture was discarded. Repeat this step once again. Then, 267 μL of methanol in 1.33 mL of DMF (20%, v/v) was used to quench the unreacted 2-Cl-Trt-Cl resin. After 20 minutes, the resin was washed five times with DMF. **Linear 6** was prepared by Fmoc-based SPPS using the freshly prepared hydrazine-Trt(2-Cl) resin. After ether precipitation, the crude product of **Linear 6** was obtained as a white powder.

The second step is the reaction between **Linear 6** and bis(bromomethyl)biphenyl. Dissolve 20 mg of crude **Linear 6** in 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 2.1 mg of bis(bromomethyl)biphenyl in 0.2 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 with 1.0 M NH_4HCO_3. Keep the reaction in a vortex mixer at room temperature. After 2 hours (Figure S33b), 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. **Cross-linked 6** was obtained by preparative HPLC and lyophilization. The third step is the conversion of **Cross-linked 6** into **Thioester 6**, and the conversion of **Thioester 6** into **C-aminoethanethiol 6**. Dissolve 5.0 mg of **Cross-linked 6** (1.4 μmol) in 1.2 mL of PBS (6.0 M GnHCl, 0.2 M Na_2HPO_4, pH 3.0). Under -10 °C, 80 μL of freshly prepared $NaNO_2$ (200 mM, 10 eq.) was slowly added. After 20 minutes, 1.2 mL of MESNa (200 mM, pH 7) was added. Then, the pH value of the solution was adjusted to 6.8. After 2 hours (Figure

S33c), the formed **Thioester 6** was obtained by preparative HPLC. Dissolve 4 mg of **Thioester 6** in a 2-aminoethanethiol solution (4 mg of 2-aminoethanethiol in 1 mL PBS, pH 7). After 1 hour (Figure S33d), **C-aminoethanethiol 6** was obtained by preparative HPLC and lyophilization. The final step is the dimerization of **C-aminoethanethiol 6** by 1,3-dibromoacetone. 2 mg of **C-aminoethanethiol 6** (~0.6 µmol) in 50 µL of DMF, and ~0.24 µmol of 1,3-dibromoacetone in 50 µL of DMF were added to 2.0 mL of 80 mM sodium borate buffer (20 mg $Na_2B_4O_7$ in 5 mL H_2O, pH 6.5). After 2 hours (Figure S33e), **6** was purified by preparative HPLC. After lyophilization, **6** was obtained as a white powder (1.0 mg, 50%). Analytical HPLC: t_R = 29.6 min (2-90% B in 40 min); m/z = 7090.5 ($C_{325}H_{460}N_{70}O_{97}S_6$, calcd.: 7090.1 g/mol).

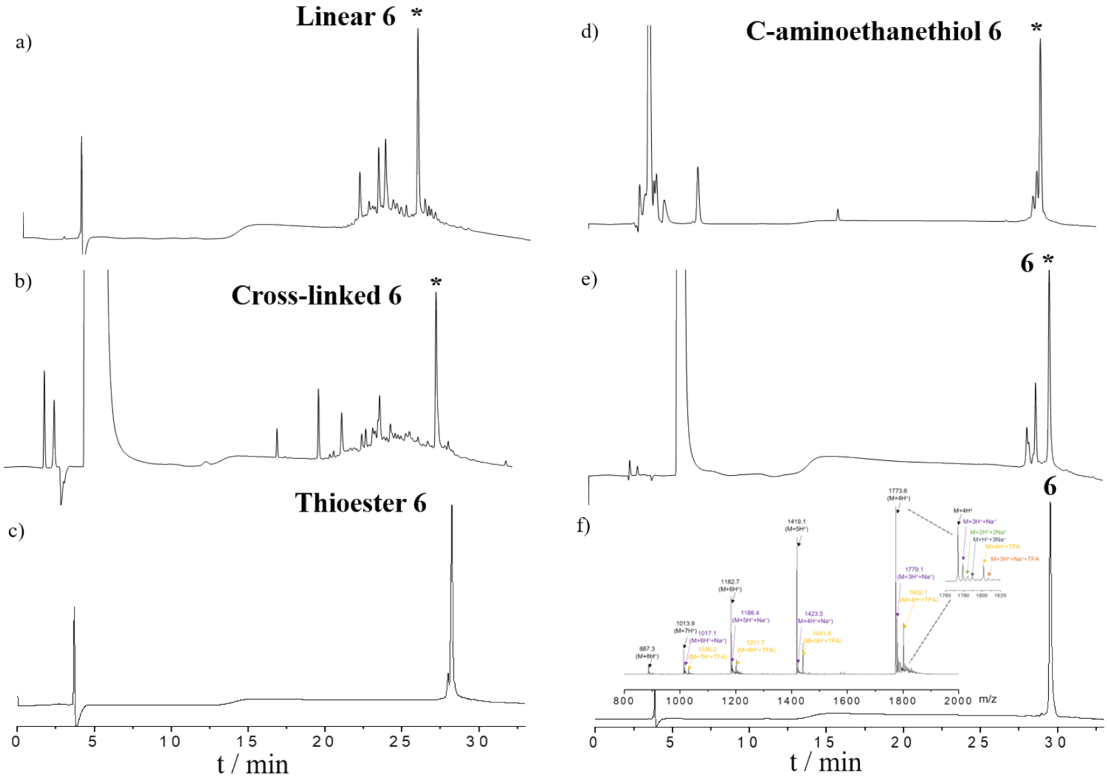
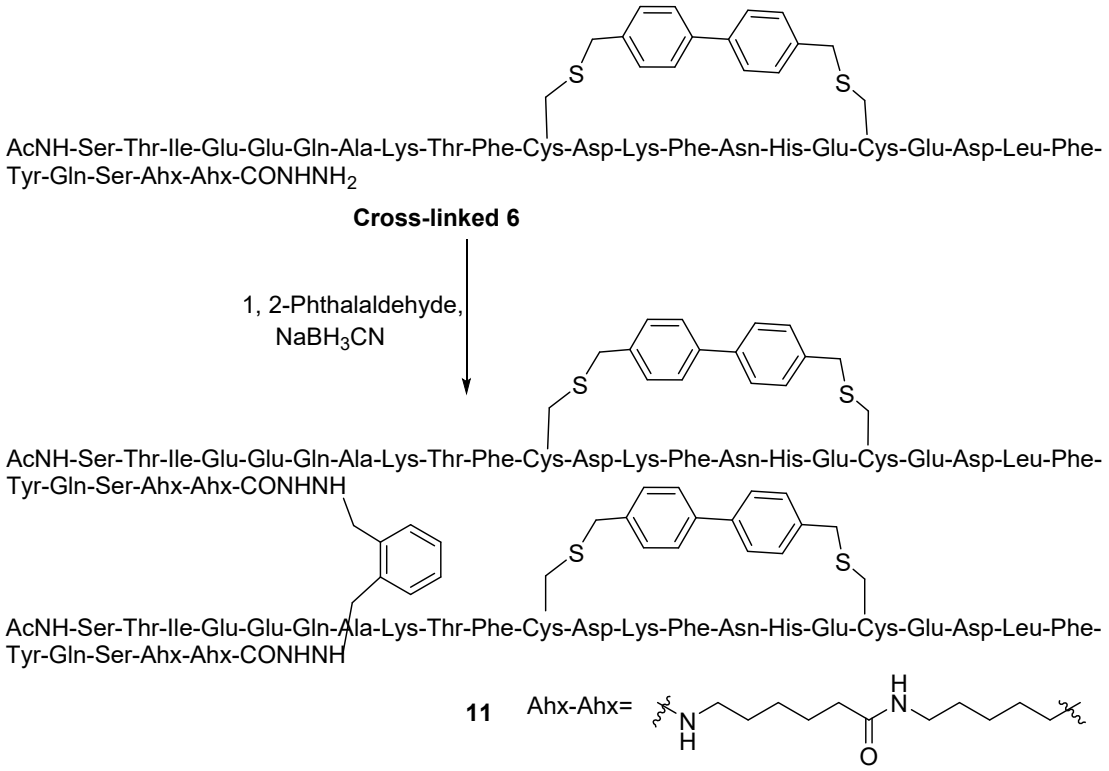


Figure S33. HPLC traces for the preparation of **6** (UV: 210nm). (a) the crude product of **Linear 6**; (b) reaction with bis(bromomethyl)biphenyl; (c) conversion of **Cross-linked 6** into **Thioester 6**; (d) conversion of **Thioester 6** into **C-aminoethanethiol 6**; (e) dimerization of **C-aminoethanethiol 6** by 1,3-dibromoacetone; (f) HPLC trace and mass spectrum of the purified **6**.



3 mg of **Cross-linked 6** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,2-phthalaldehyde was prepared (3.9 mg 1,2-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,2-phthalaldehyde was added to the **Cross-linked 6** solution. Keep the reactor at room temperature for 1 h. 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S34a). Then, **11** was purified by preparative HPLC. After lyophilization, **11** was obtained as a white powder (1.1 mg, 37%). Analytical HPLC: t_R = 29.8 min (2-90% B in 40 min); m/z = 7047.6 ($C_{326}H_{458}N_{72}O_{96}S_4$, calcd.: 7048.2 g/mol).

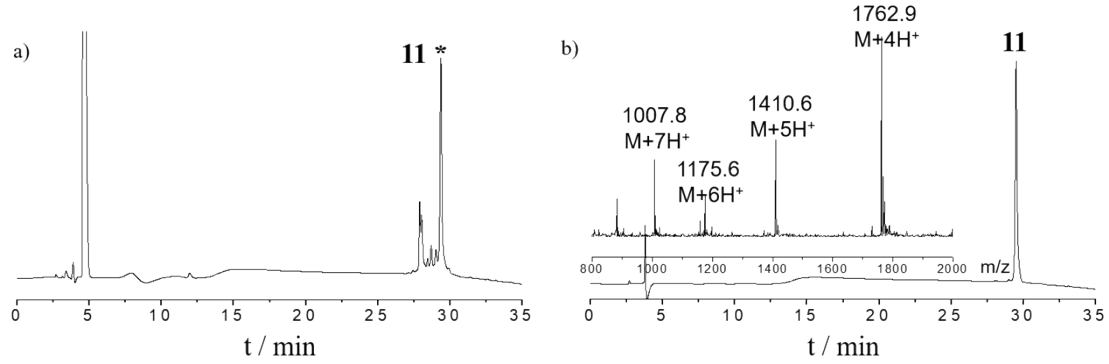
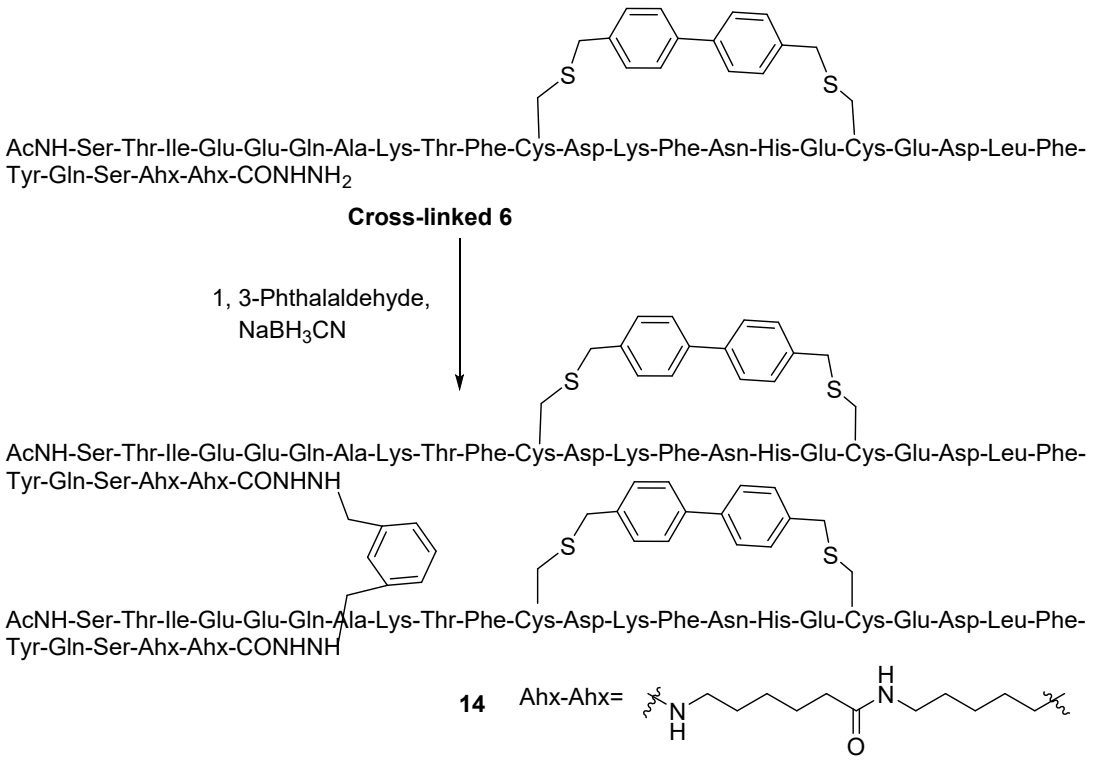


Figure S34. HPLC traces for the preparation of **11** (UV: 210nm). (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **11**.



3 mg of **Cross-linked 6** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,3-phthalaldehyde was prepared (3.9 mg 1,3-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,3-phthalaldehyde was added to the **Cross-linked 6** solution. Keep the reactor at room temperature for 1 h. 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S35a). Then, **14** was purified by preparative HPLC. After lyophilization, **14** was obtained as a white powder (1.5 mg, 50%). Analytical HPLC: t_R = 29.3 min (2-90% B in 40 min); m/z = 7048.0 ($C_{326}H_{458}N_{72}O_{96}S_4$, calcd.: 7048.2 g/mol).

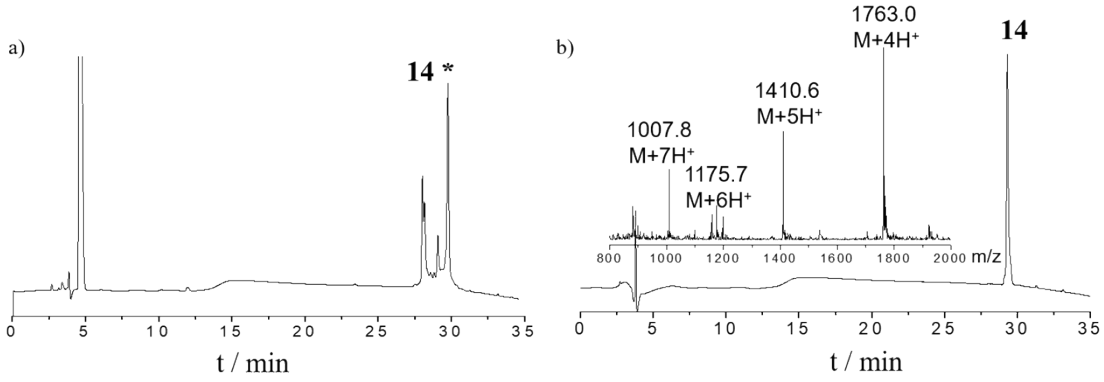
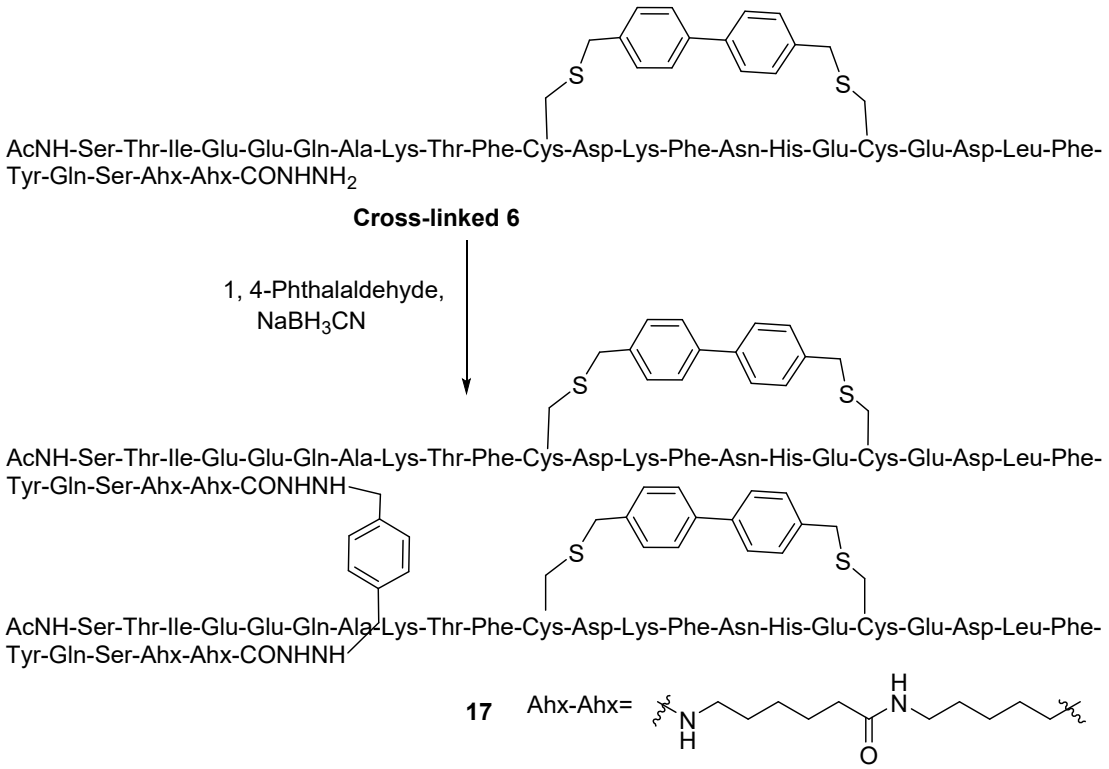


Figure S35. HPLC traces for the preparation of **14** (UV: 210nm). (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **14**.



3 mg of **Cross-linked 6** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,4-phthalaldehyde was prepared (3.9 mg 1,4-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,4-phthalaldehyde was added to the **Cross-linked 6** solution. Keep the reactor at room temperature for 1 h. 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S36a). Then, **17** was purified by preparative HPLC. After lyophilization, **17** was obtained as a white powder (1.0 mg, 33%). Analytical HPLC: t_R = 29.5 min (2-90% B in 40 min); m/z = 7048.4 ($C_{326}H_{458}N_{72}O_{96}S_4$, calcd.: 7048.2 g/mol).

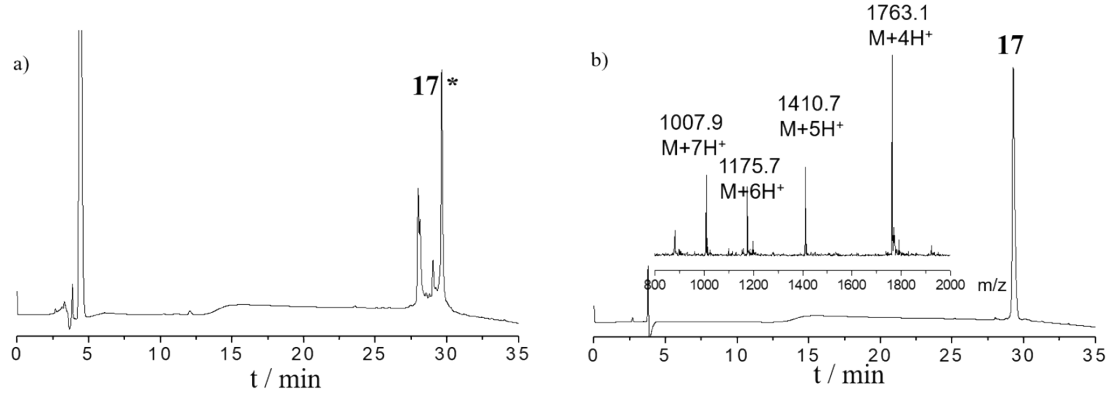
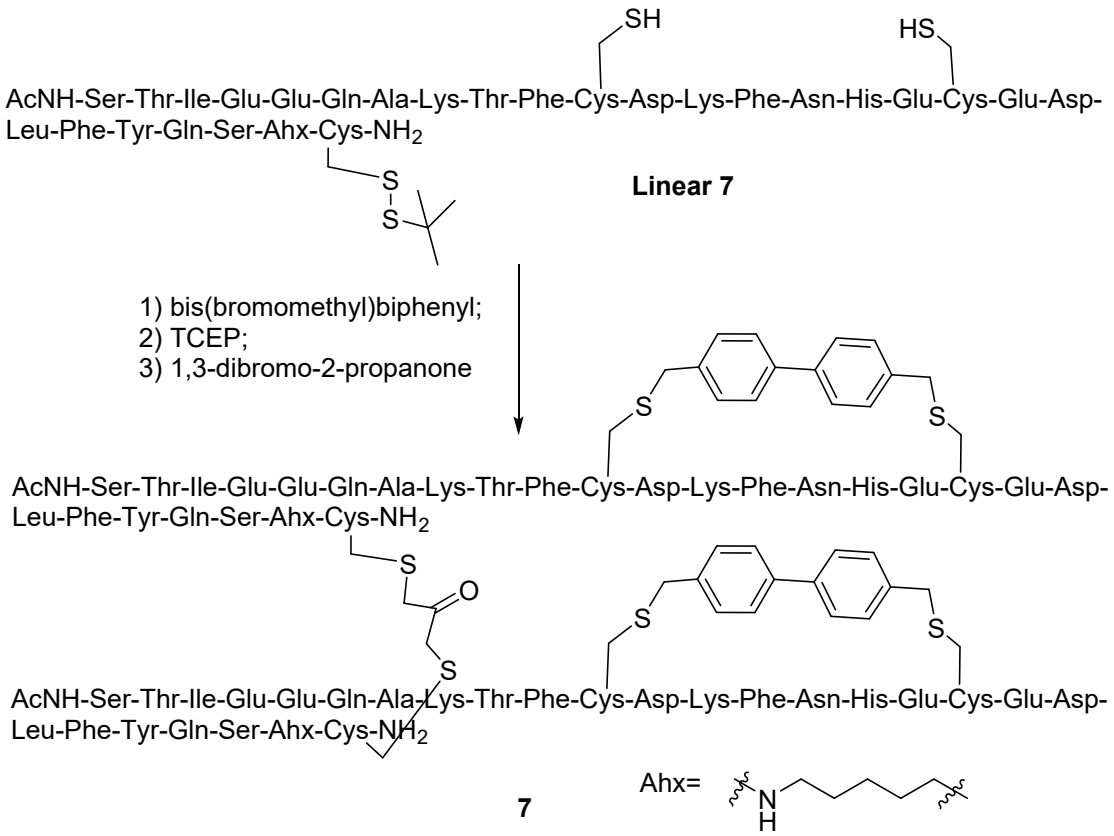


Figure S36. HPLC traces for the preparation of **17** (UV: 210nm). (c) 1 h later after addition of $NaBH_3CN$; (d) HPLC trace and mass spectrum of the purified **17**.

7 and **8**:



7 was synthesized in several steps, as shown below.

First, **Linear 7** was prepared by Fmoc-based SPPS using Rink amide AM resin. After preparative HPLC and lyophilization, **Linear 7** was obtained as a white powder.

The second step is the reaction between **Linear 7** and bis(bromomethyl)biphenyl. Dissolve 10 mg of crude **Linear 7** in 1.3 mL DMF. Then, 1.0 equ. of bis(bromomethyl)biphenyl in 0.2 mL DMF was added, followed by adding 10 μL DIPEA. Keep the reaction in a vortex mixer at room temperature. After 2 hours, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction (Figure 37b). The **Cross-linked 7** was directly purified by preparative HPLC and subsequent lyophilization. Then, 2 mg of **Linear 7** was dissolved into 1 mL of PBS (6 M GnHCl, 0.2 M Na_2HPO_4, pH=7), and 10 equiv. of freshly prepared TCEP solution in 0.5 mL water was added. The pH value of the mixture was adjusted to 8. After 30 minutes, the **Cross-linked 7'** was purified by preparative HPLC (Figure S37d). The final step is the dimerization of **Cross-linked 7'** by 1,3-dibromoacetone. 2 mg of **Cross-linked 7'** (~0.6 μmol) in 50 μL of DMF, and ~0.24 μmol of 1,3-dibromoacetone in 50 μL of DMF were added to 2.0 mL of 80 mM sodium borate buffer (20 mg $Na_2B_4O_7$ in 5 mL H_2O, pH 6.5). After 2 hours (Figure S37e), **7** was purified by preparative HPLC. After lyophilization, **7** was obtained as a white powder (0.7 mg, 35%). Analytical HPLC: t_R = 29.4 min (2-90% B in 40 min); m/z = 6950.0 ($C_{315}H_{440}N_{70}O_{97}S_6$, calcd.: 6950.0 g/mol).

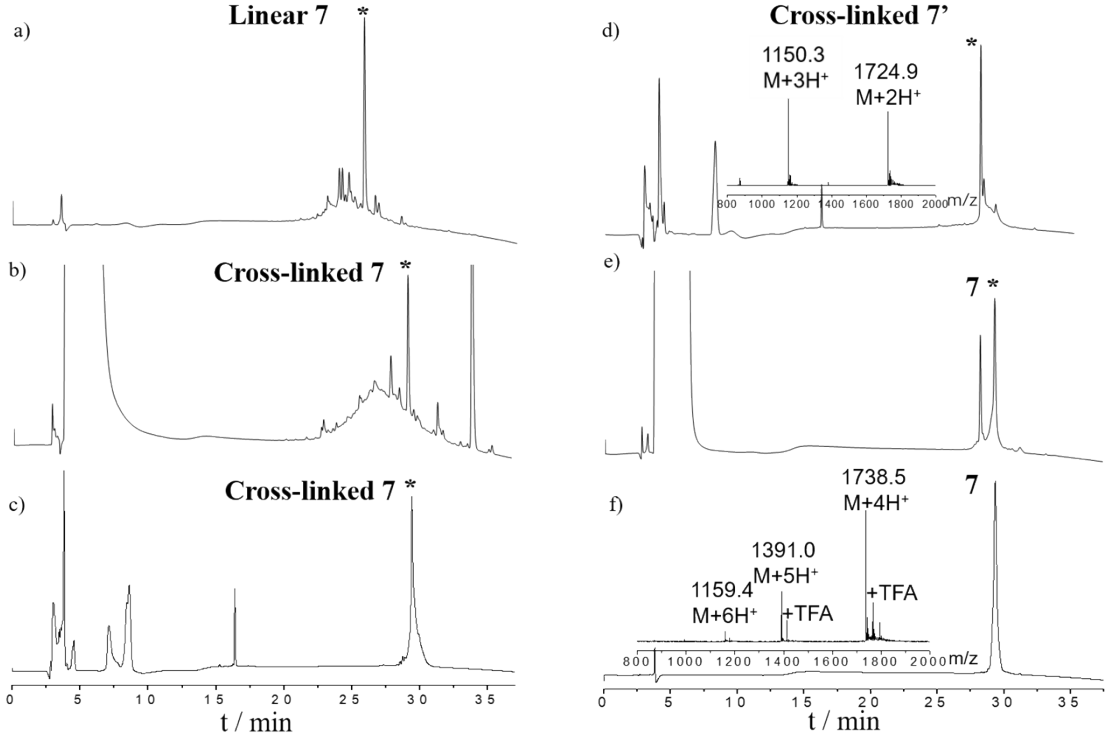
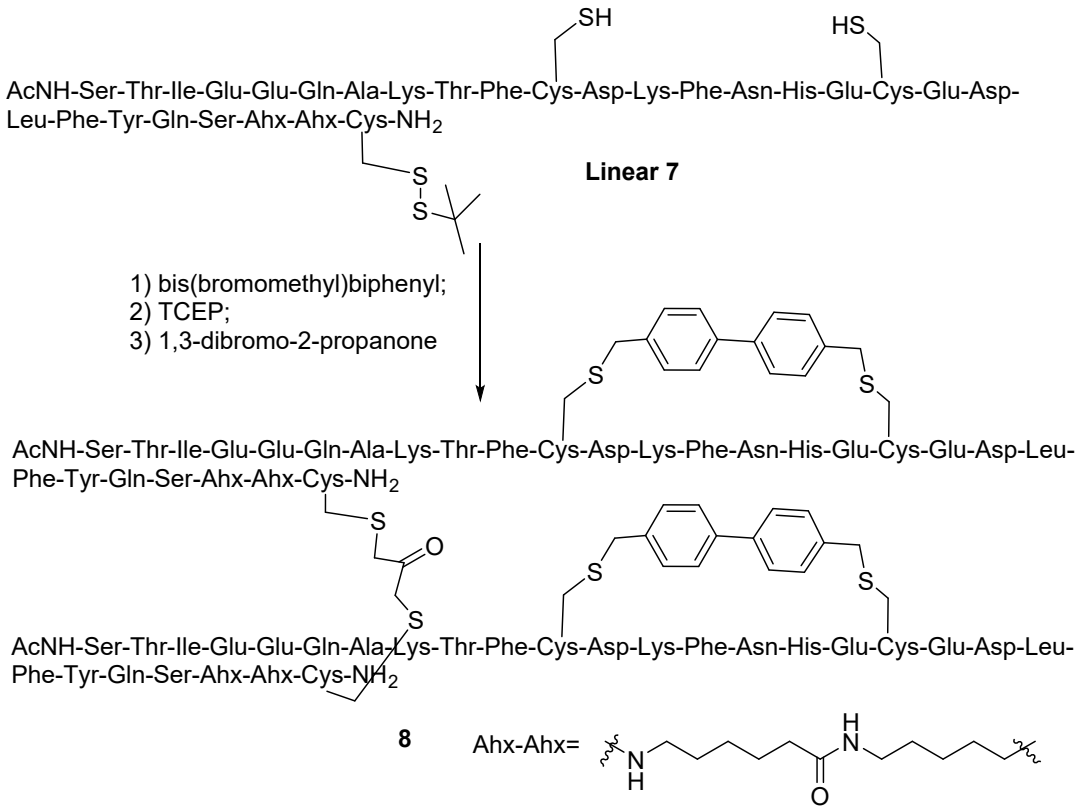


Figure S37. HPLC traces for the preparation of **7** (UV: 210nm). (a) the crude product of **Linear 7**; (b) reaction with bis(bromomethyl)biphenyl; (c) the purified **Cross-linked 7**; (d) after TCEP treatment; (e) dimerization of **Cross-linked 7'** by 1,3-dibromoacetone; (f) HPLC trace and mass spectrum of the purified **7**.



8 was synthesized in several steps, as shown below.

First, **Linear 7** was prepared by Fmoc-based SPPS using Rink amide AM resin. After preparative HPLC and lyophilization, **Linear 7** was obtained as a white powder.

The second step is the reaction between **Linear 7** and bis(bromomethyl)biphenyl. Dissolve 10 mg of crude **Linear 7** in 1.3 mL DMF. Then, 1.0 equ. of bis(bromomethyl)biphenyl in 0.2 mL DMF was added, followed by adding 10 μL DIPEA. Keep the reaction in a vortex mixer at room temperature. After 2 hours, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction (Figure 38b). **Cross-linked 7** was directly purified by preparative HPLC and subsequent lyophilization. Then, 2 mg of **Cross-linked 7** was dissolved into 1 mL of PBS (6 M GnHCl, 0.2 M Na_2HPO_4, pH=7), and 10 equiv. of freshly prepared TCEP solution in 0.5 mL water was added. The pH value of the mixture was adjusted to 8. After 30 minutes, **Cross-linked 7'** was purified by preparative HPLC (Figure S38d). The final step is the dimerization of **Cross-linked 7'** by 1,3-dibromoacetone. 2 mg of **Cross-linked 7'** (~0.6 μmol) in 50 μL of DMF, and ~0.24 μmol of 1,3-dibromoacetone in 50 μL of DMF were added to 2.0 mL of 80 mM sodium borate buffer (20 mg $Na_2B_4O_7$ in 5 mL H_2O, pH 6.5). After 2 hours (Figure S38e), **8** was purified by preparative HPLC. After lyophilization, **8** was obtained as a white powder (0.9 mg, 45%). Analytical HPLC: t_R = 29.8 min (2-90% B in 40 min); m/z = 7176.4 ($C_{327}H_{462}N_{72}O_{99}S_6$, calcd.: 7176.2 g/mol).

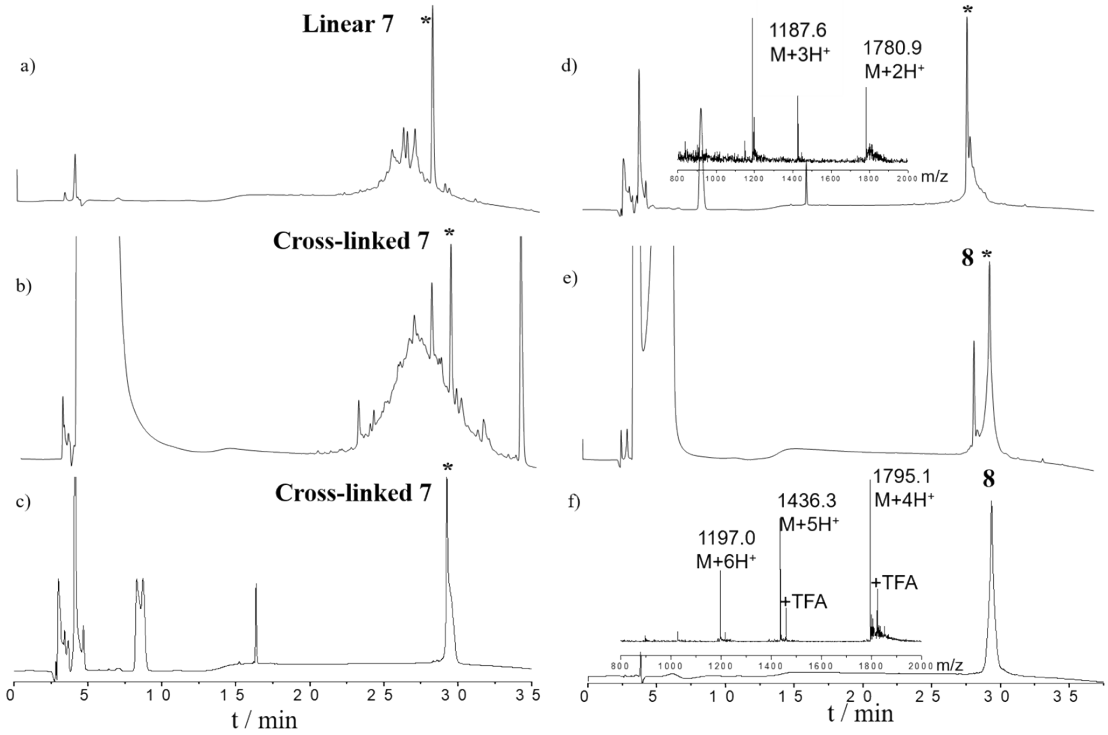
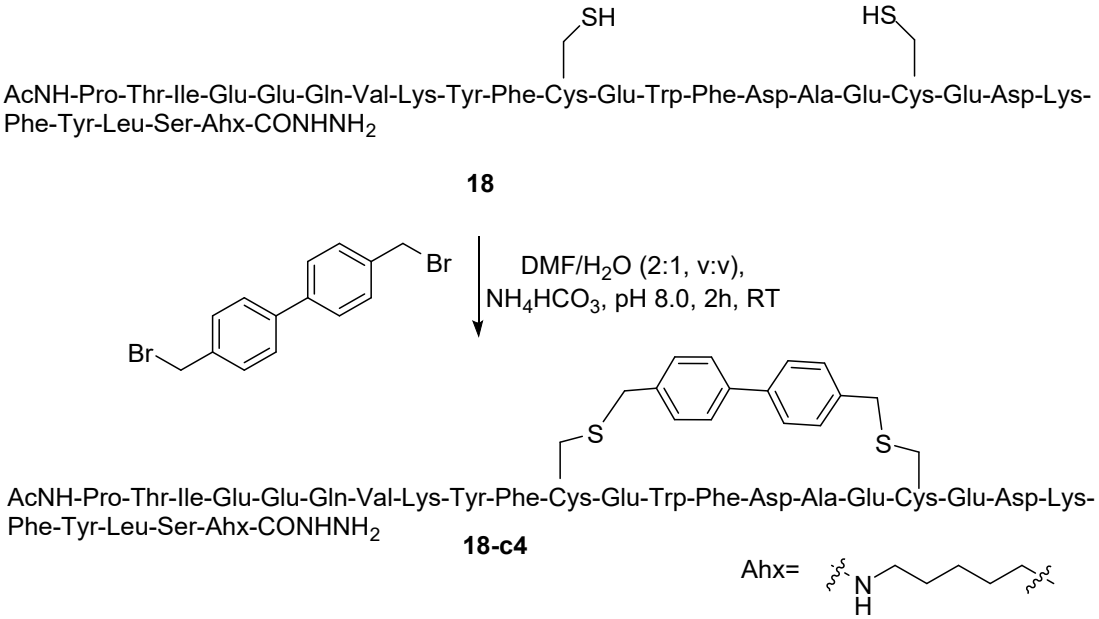


Figure S38. HPLC traces for the preparation of **8** (UV: 210nm). (a) the crude product of **Linear 7**; (b) reaction with bis(bromomethyl)biphenyl; (c) the purified **Cross-linked 7**; (d) after TCEP treatment; (e) dimerization of the **Cross-linked 7'** by 1,3-dibromoacetone; (f) HPLC trace and mass spectrum of the purified **8**.

Synthesis of **18**, **18-c4**, **19**, **19-c4**, **20**, **21** and **22:**



18-c4 was prepared by bisalkylation of **18**. 10 mg of crude **18** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 1.4 mg of bis(bromomethyl)biphenyl in 0.1 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, the desired **18** was obtained as a white powder (~3.5 mg, 35%). Analytical HPLC: t_R = 30.1 min (2-90% B in 40 min); m/z = 3467.0 ($C_{166}H_{224}N_{32}O_{46}S_2$, calcd.: 3466.6 g/mol).

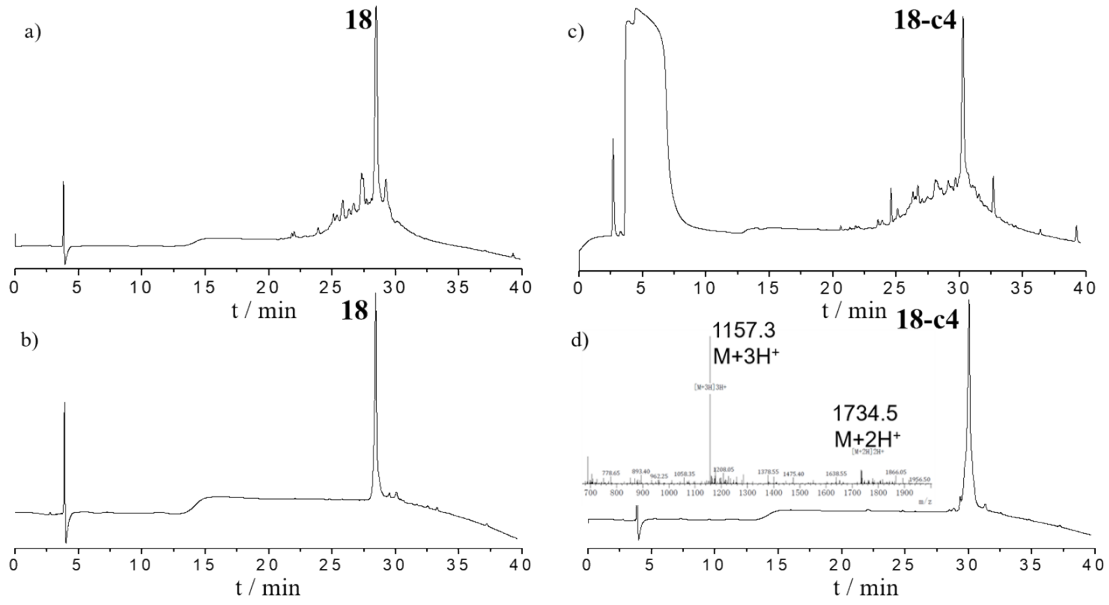
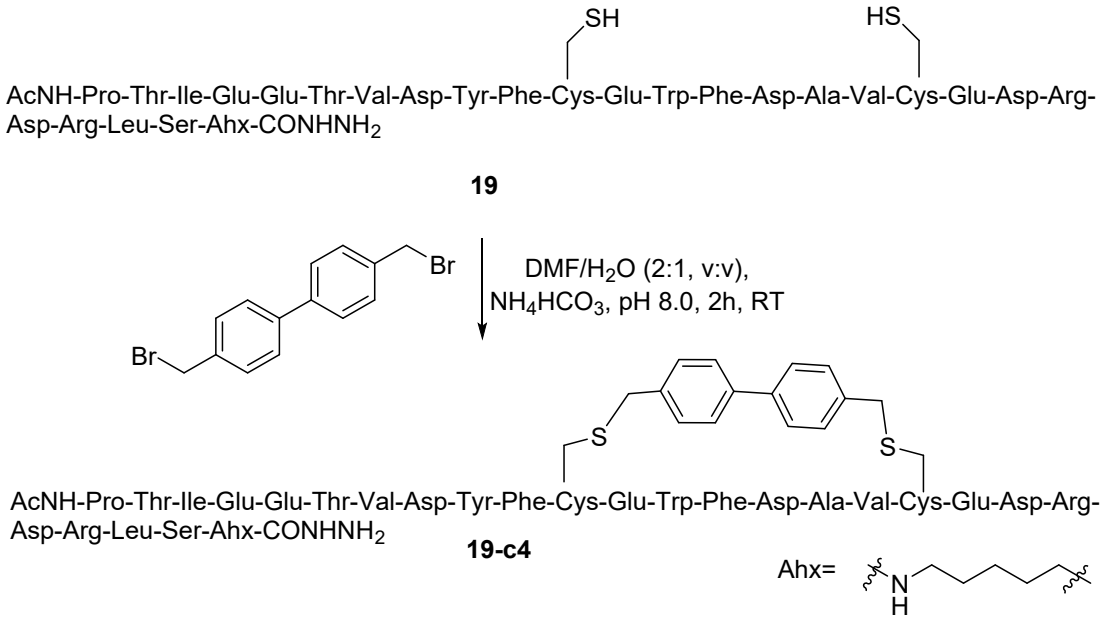


Figure S39. HPLC traces for the preparation of **18-c4** (UV: 210nm). (a) the crude product of **18**; (c) the purified **18**; (c) after reaction with bis(bromomethyl)biphenyl; (d) HPLC trace and mass spectrum of the purified **18-c4**.



19-c4 was prepared by bisalkylation of **19**. 10 mg of crude **19** was dissolved into 2.0 mL DMF-H_2O mixture (2:1, v:v). Then, 1.4 mg of bis(bromomethyl)biphenyl in 0.1 mL DMF was added. The pH of the reaction mixture was adjusted to 8.0 by 1.0 M NH_4HCO_3. The reaction was hold into vortex mixer at room temperature. 2 hours later, 4.0 mL acetonitrile-H_2O mixture (1:1, v:v, 0.1% TFA) was added to quench the reaction. After preparative HPLC and lyophilization, **19-c4** was obtained as a white powder (~3 mg, 30%). Analytical HPLC: t_R = 32.9 min (2-90% B in 40 min); m/z = 3404.2 ($C_{159}H_{219}N_{35}O_{45}S_2$, calcd.: 3403.5 g/mol).

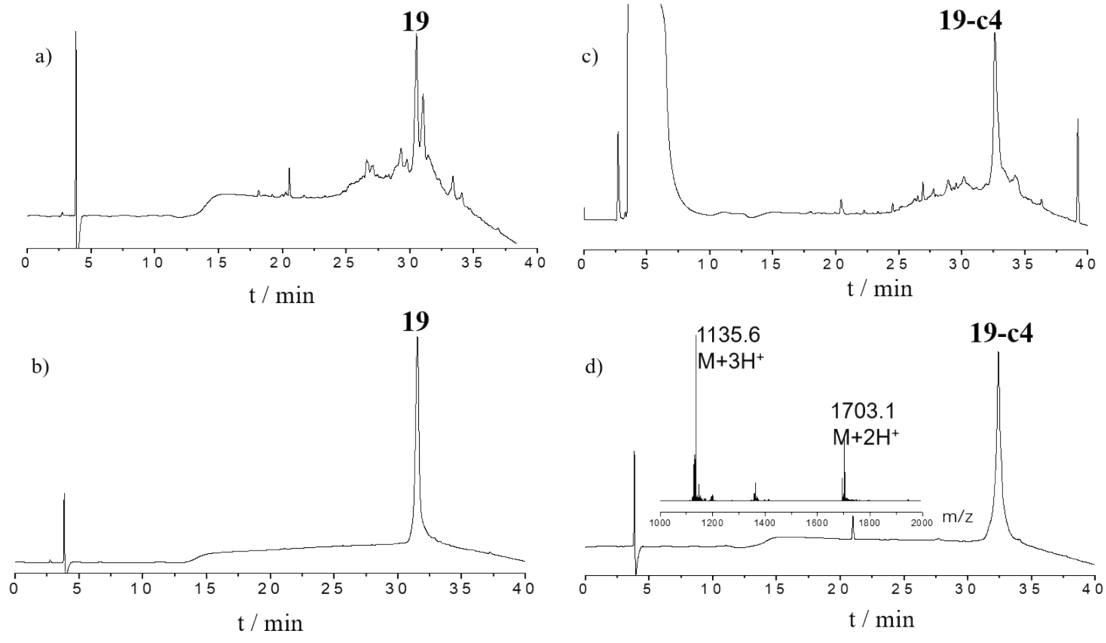
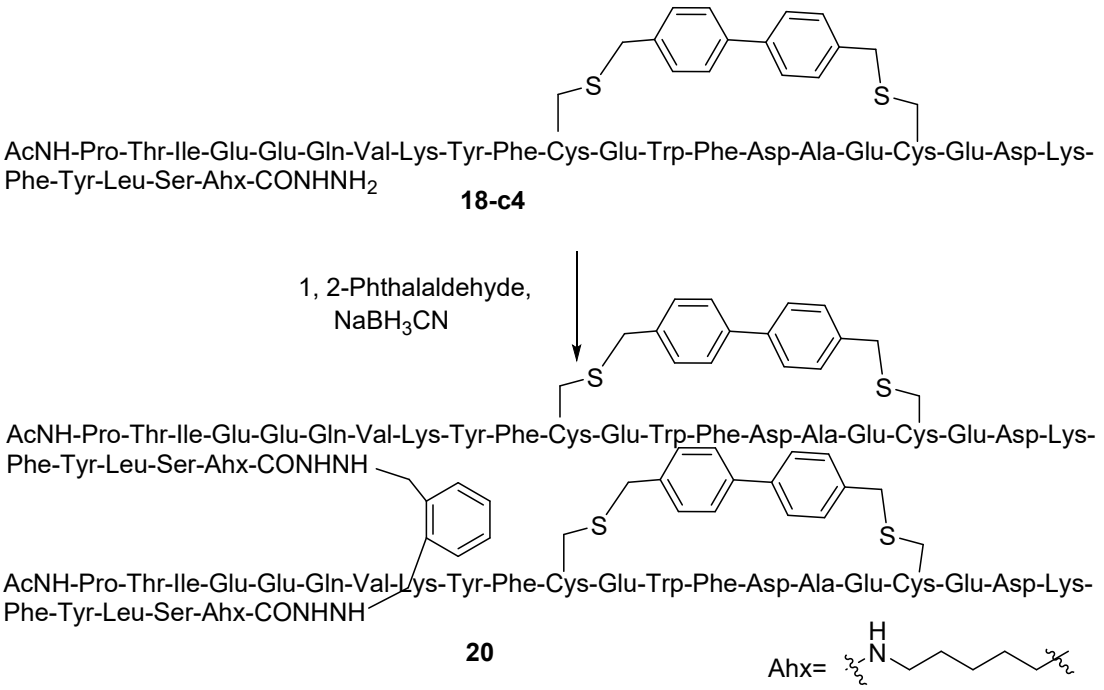


Figure S40. HPLC traces for the preparation of **19-c4** (UV: 210nm). (a) the crude product of **19**; (c) the purified **19**; (c) after reaction with bis(bromomethyl)biphenyl; (d) HPLC trace and mass spectrum of the purified **19-c4**.



3 mg of **18-c4** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,2-phthalaldehyde was prepared (4.0 mg 1,2-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,2-phthalaldehyde was added to the **18-c4** solution. Keep the reactor at room temperature for 1 h. 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S41a). Then, **20** was purified by preparative HPLC. After lyophilization, **20** was obtained as a white powder (0.8 mg, 27%). Analytical HPLC: t_R = 32.1 min (2-90% B in 40 min); m/z = 7034.4 ($C_{340}H_{454}N_{64}O_{92}S_4$, calcd.: 7035.0 g/mol).

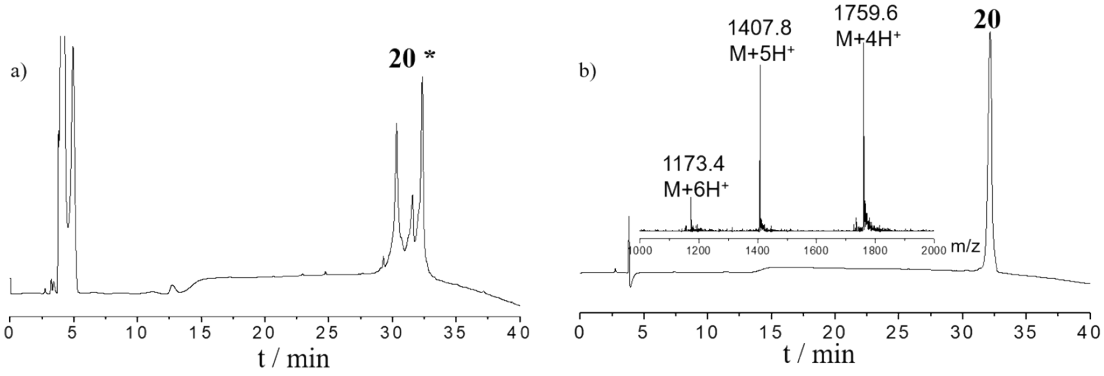
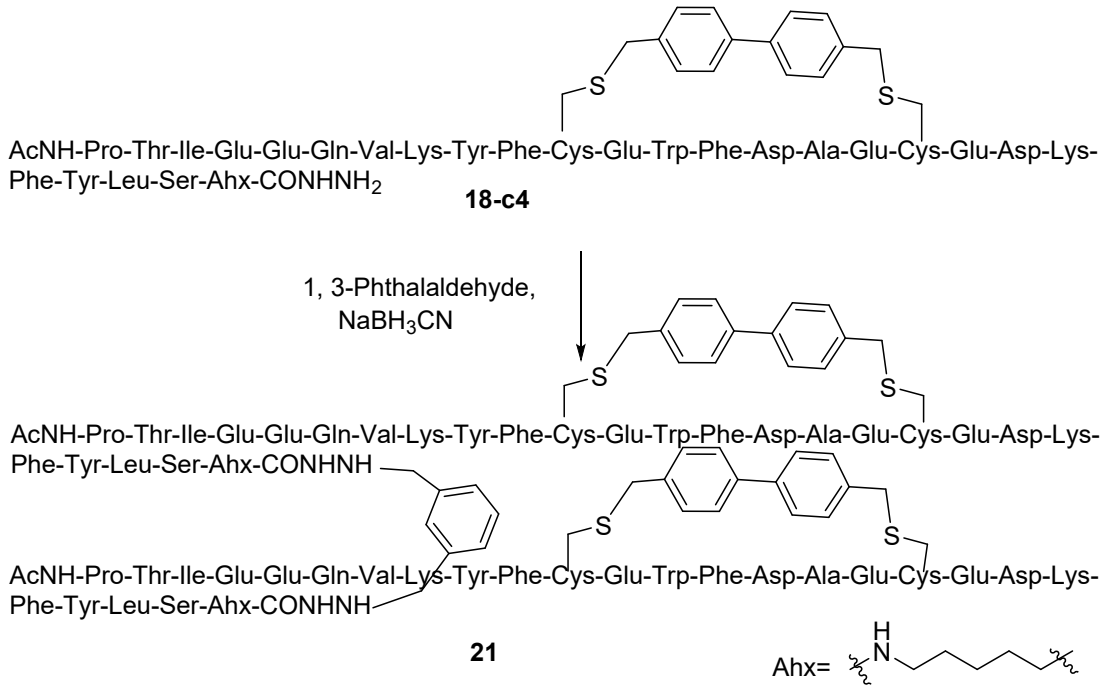


Figure S41. HPLC traces for the preparation of **20** (UV: 210nm). (a) 1 h later after addition of $NaBH_3CN$; (b) HPLC trace and mass spectrum of the purified **20**.



3 mg of **18-c4** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,3-phthalaldehyde was prepared (4.0 mg 1,3-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,3-phthalaldehyde was added to the **18-c4** solution. Keep the reactor at room temperature for 1 h. 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S42a). Then, **21** was purified by preparative HPLC. After lyophilization, **21** was obtained as a white powder (0.9 mg, 30%). Analytical HPLC: t_R = 31.8 min (2-90% B in 40 min); m/z = 7036.0 ($C_{340}H_{454}N_{64}O_{92}S_4$, calcd.: 7035.7 g/mol).

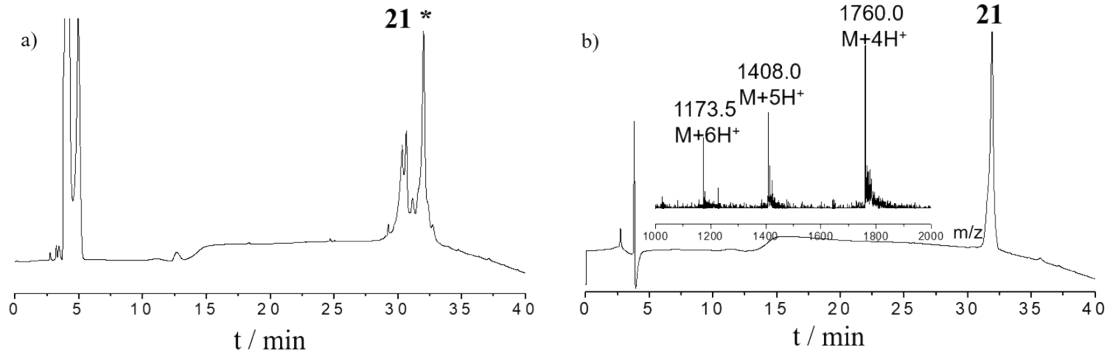
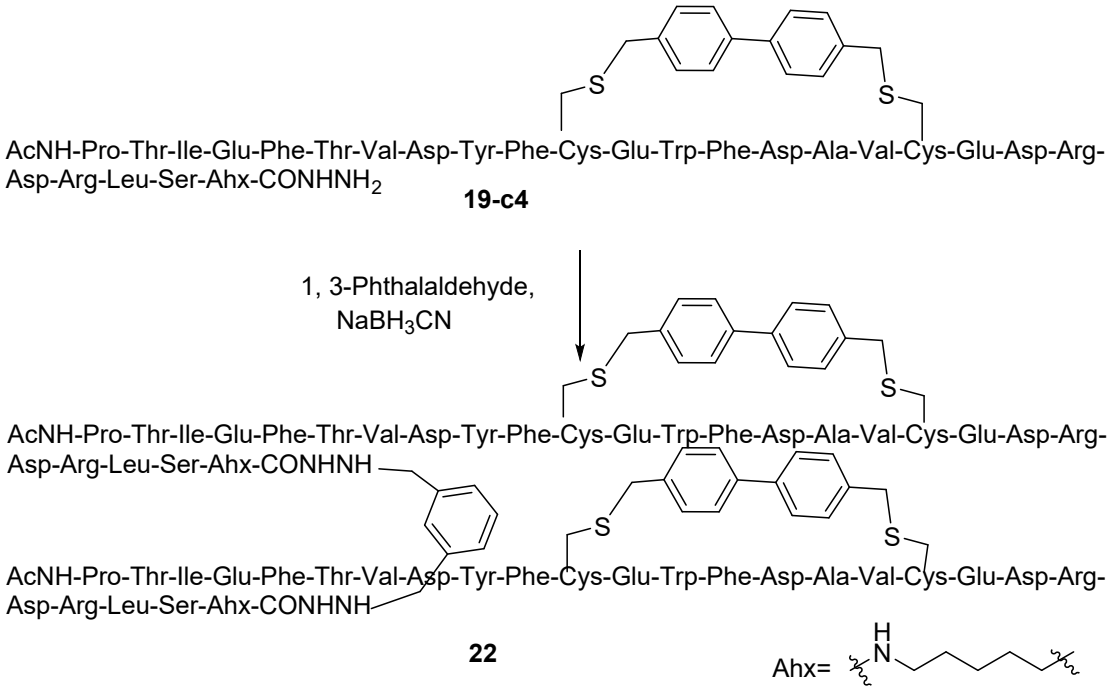


Figure S42. HPLC traces for the preparation of **21** (UV: 210nm). (a) 1 h later after addition of $NaBH_3CN$; (b) HPLC trace and mass spectrum of the purified **21**.



3 mg of **19-c4** (~0.9 μmol) was dissolved into 600 μL of acetic acid-methanol mixture (1:1, v:v). Then, a soluton of 1,3-phthalaldehyde was prepared (4.1 mg 1,3-phthalaldehyde in 1.0 mL of acetic acid-methanol mixture). 10 μL of the freshly prepared 1,3-phthalaldehyde was added to the **19-c4** solution. Keep the reactor at room temperature for 1 h. 3.0 mg of $NaBH_3CN$ in 300 μL H_2O was added to the reaction mixture. Keep the reactor at room temperature for another 1 hour (Figure S43a). Then, **22** was purified by preparative HPLC. After lyophilization, **22** was obtained as a white powder (0.6 mg, 20%). Analytical HPLC: t_R = 35.7 min (2-90% B in 40 min); m/z = 6912.0 ($C_{326}H_{444}N_{70}O_{90}S_4$, calcd.: 6911.6 g/mol).

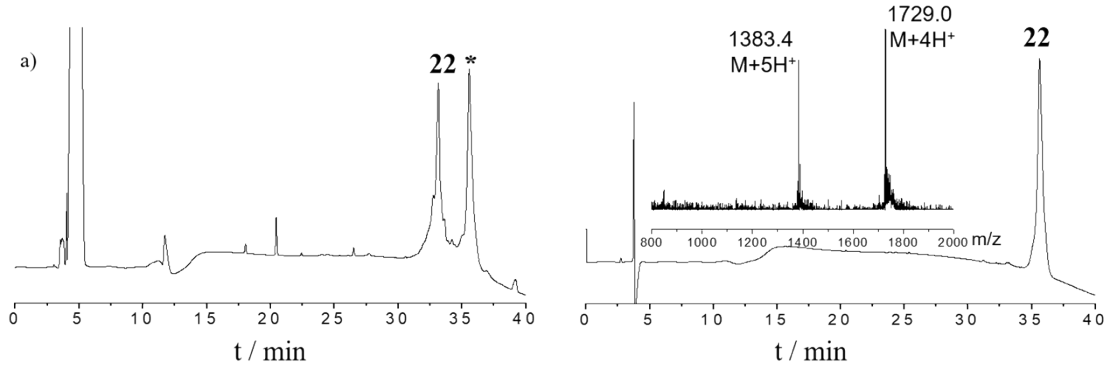
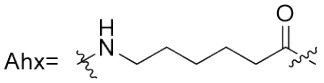


Figure S43. HPLC traces for the preparation of **22** (UV: 210nm). (a) 1 h later after addition of $NaBH_3CN$; (b) HPLC trace and mass spectrum of the purified **22**.

Biotin-Ahx-Ile-Glu-Glu-Gln-Ala-Lys-Thr-Phe-Leu-Asp-Lys-Phe-Asn
-His-Glu-Ala-Glu-Asp-Leu-Phe-Tyr-Gln-Ser-NH_2

Biotin-**SBP1**



Biotin-**SBP1** was prepared by Fmoc-based SPPS using Rink amide AM resin. After preparative HPLC and lyophilization, Biotin-**SBP1** was obtained as a white powder. Analytical HPLC: t_R = 26.0 min (2-90% B in 40 min); m/z = 3140.2 ($C_{143}H_{210}N_{34}O_{44}S$, calcd.: 3140.5 g/mol).

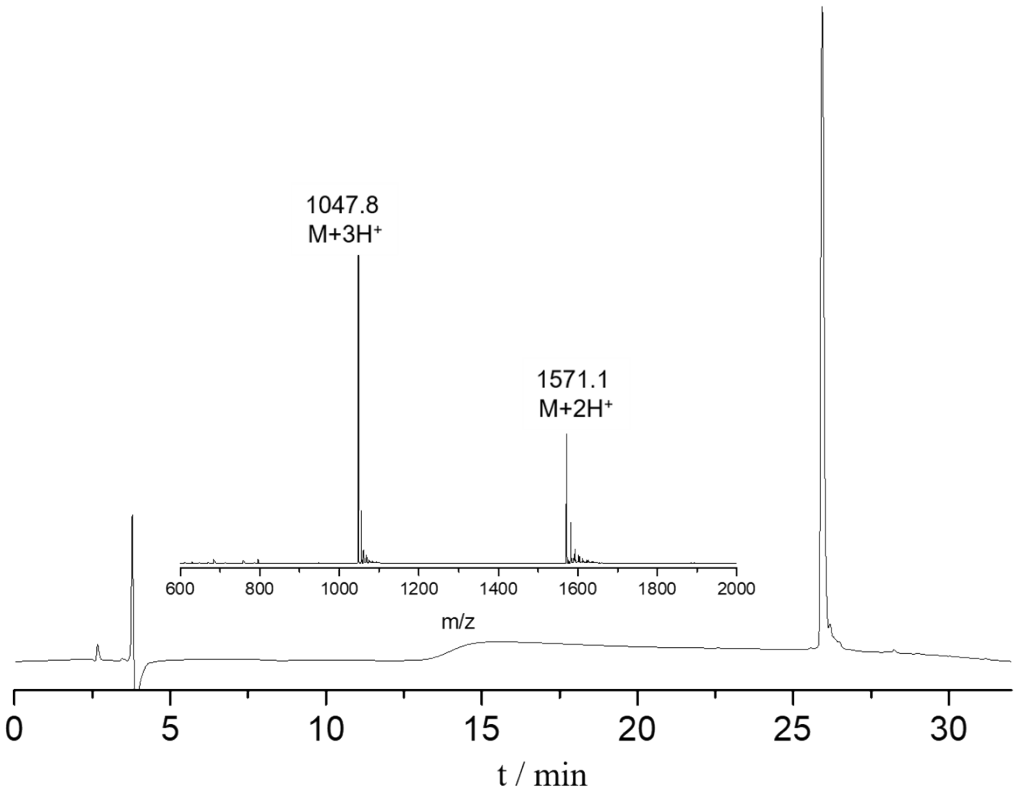


Figure S44. HPLC trace and mass spectrum of the purified Biotin-**SBP1** (UV: 210nm).

Pseudovirus neutralization assay

The plasmid pCDNA3.1(+)-HnCoV-S with codon-optimized SARS-CoV-2 S glycoprotein DNA sequence was purchased from Shanghai Generay Biotech, and the plasmid pCDNA3.1(+)-3xFlag-hACE2(NM_021804) encoding human ACE2 protein was purchased from FengHui Biotech (Hunan, China). The plasmid pCDNA3.1(+)-SARS-S encoding SARS-CoV S glycoprotein and the HIV-backbone plasmid PNL4-3R$^-$E$^-$ containing the firefly luciferase reporter gene was generously gifted by Linqi Zhang, Tsinghua University. The 293 T and Huh7 cells were kindly given by Prof. Linqi Zhang (School of Medicine, Tsinghua University).

Pseudovirus neutralization assay was performed as previously described with some modifications[1]. The plasmids of pCDNA3.1(+)-HnCoV-S or pCDNA3.1(+)-SARS-S and HIV-backbone plasmid PNL4-3R$^-$E$^-$ were co-transfected into the HEK 293T cells by Lipofectamine 3000 transfection reagent (Invitrogen) to produce SARS-CoV-2 and SARS-CoV pseudovirus, at room temperature. After 48 h, the supernatant was centrifuged to remove cell debris and used as the viral stocks. 239T-ACE2 or Huh7-hACE2 cells expressing recombinant human ACE2 were established by transfection of pCDNA3.1(+)-3xFlag-hACE2 through lipofectamine 6000 transfection reagent (Biyuntian Biotech). 239T-hACE2 or Huh7-hACE2 cells were seeded at a density of 2 x10^4 cells per well in 96-well plates at 37℃ overnight. The diluted compounds were incubated for 1 h with a certain titer of pseudovirus (relative luminescence units (RLU) ranging from 20,000 to 40,000 per well in 96-well plates) and then added to 239T-hACE2 or Huh7-hACE2 cells. After cultured for 2 days at 37℃, the cells were harvested by cell lysis buffer containing the luciferase detection reagent and detected as manufacturer's protocol (Bright-Glo™ Luciferase Assay System, Promega). The read time of the luciferase detection in the experiment is 1 s.

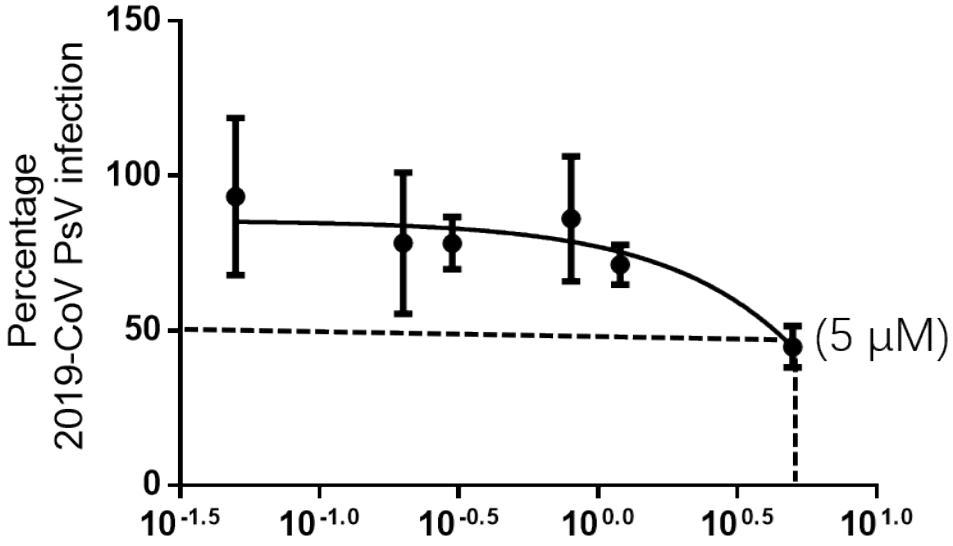


Figure S45. The dose-dependent test of **2-c4** on inhibition of SARS-CoV-2 PsV in Huh7-hACE2 cell lines (IC_{50}: 5.0 μM). All results of antiviral activity are shown as the mean ± s.d. for triplicate samples.

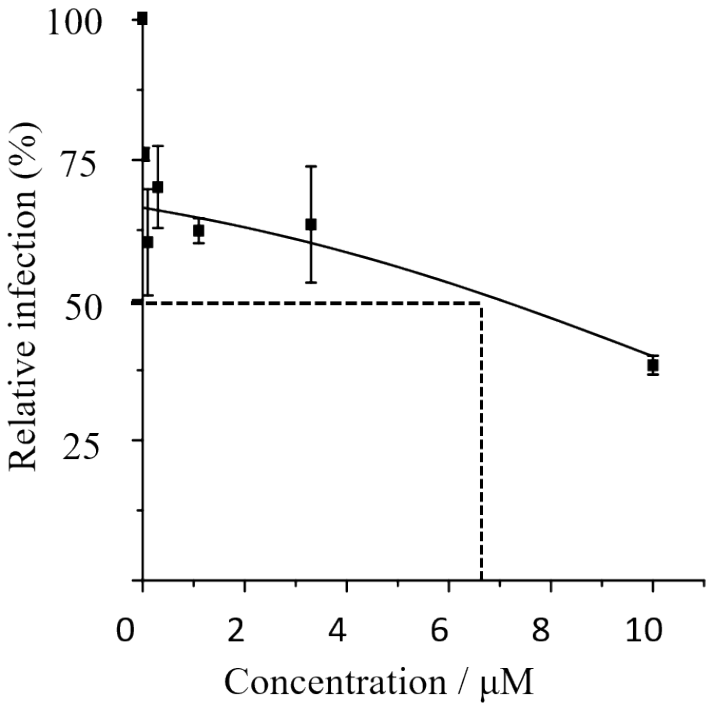


Figure S46. The dose-dependent test of **EK1** on inhibition of SARS-CoV-2 PsV in Huh7-hACE2 cell lines (IC_{50}: 7.0 μM). The point of **EK1** concentration: 0 μM, 0.03 μM, 0.1 μM, 0.3 μM, 1.1 μM, 3.3 μM and 10 μM. All results of antiviral activity are shown as the mean ± s.d. for triplicate samples.

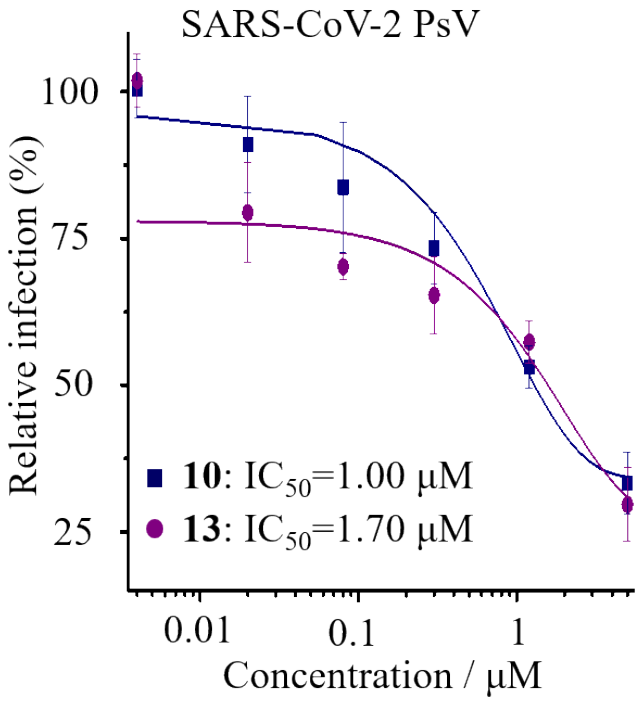


Figure S47. the dose-dependent test of **10** and **13** on inhibition of SARS-CoV-2 PsV infection in Huh7-hACE2 cell lines. All results of antiviral activity are shown as the mean ± s.d. for triplicate samples.

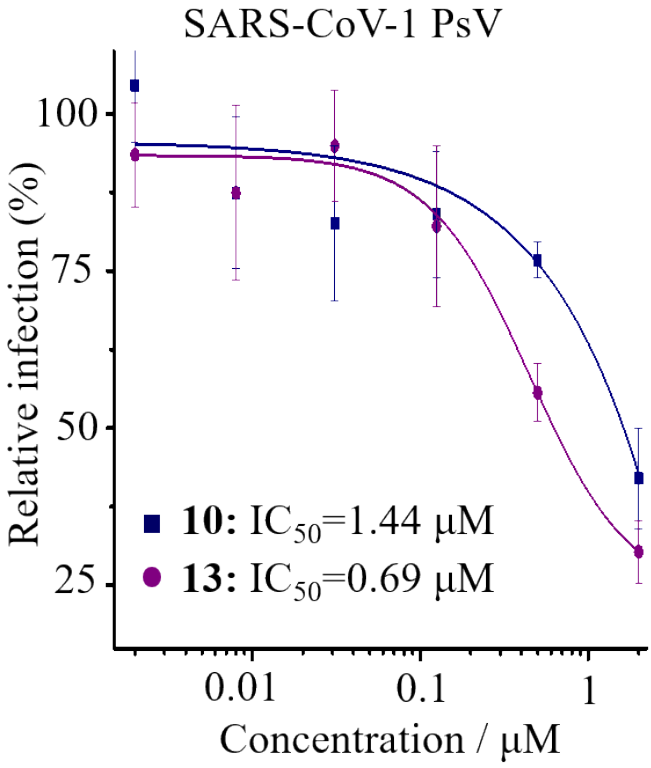


Figure S48. the dose-dependent test of **10** and **13** on inhibition of SARS-CoV-1 PsV infection in Huh7-hACE2 cell lines. All results of antiviral activity are shown as the mean ± s.d. for triplicate samples.

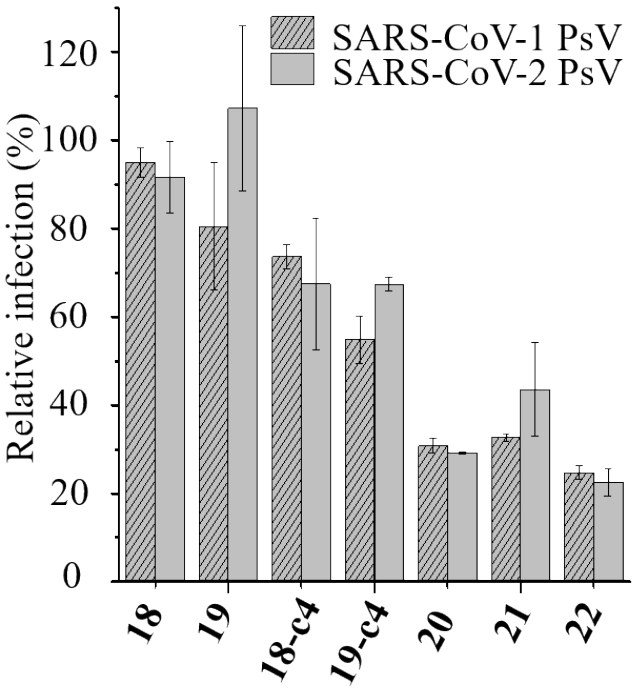


Figure S49. Inhibitory activity of 2.0 μM of ACE2 peptide mimetics **18**, **19**, **18-c4**, **19-c4**, **20**, **21**, **22** on SARS-CoV-2 pseudovirus (PsV) and SARS-CoV-1 pseudovirus (PsV).

Authentic SARS-CoV-2 virus neutralization assay

Authentic SARS-CoV-2 experiment was performed in a biosafety level 3 facility at Fudan University. Briefly, Caco-2 cells were seeded into a 96-well plate at a density of $3x10^4$ per cell and cultured at 37 °C for 12 hours. 50 μL of SARS-CoV-2 (GenBank accession number: MT121215) were premixed with 50 μL of culture medium with or without peptide mimetics for 60 minutes. Then, the mixture was added to Caco-2 cells (MOI = 0.01) and incubated at 37 °C for 1 hour. After discarding the mixture, incubate the Caco-2 cells with fresh DMEM containing 2% FBS. After culture for 48-hour incubation at 37 °C, the supernatant was collected for the RT-qPCR assay. According to the manufacturer's manual, we used Trizol LS reagent (Invitrogen, USA) to extract the total RNA from the supernatant. The qPCR was carried out by One-Step PrimeScrip RT-PCR Kit (Takara, Japan). The program for qPCR reaction: 95 °C for 10 seconds, 42 oC for 5 minutes; 40 cycles of 95 °C for 5 seconds, 50 °C for 30 seconds, 72 °C for 30 seconds on Bio-Rad CFX96. Viral loads were quantified by using a standard curve based on a plasmid containing SARS-CoV-2 nucleocapsid protein (N) gene (purchased from BGI, China).

The primers were as follows:

SARS-CoV-2-N-F: 5'-GGGGAACTTCTCCTGCTAGAAT-3';

SARS-CoV-2-N-R: 5'-CAGACATTTTGCTCTCAAGCTG-3';

SARS-CoV-2-N-probe: 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'.

Circular dichroism spectroscopy

CD spectra were obtained using circular dichrosim spectrometer.

*CD spectra of **2**, **2-c1**, **2-c2**, **2-c3**, **2-c4**, **2-c5**, **2-c6** and **2-c7***

0.2 mg of the peptide was dissolved into 1.0 mL phosphate-buffered saline (containing 136.89 mM NaCl, 2.67 mM KCl, 8.1 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4)) to afford a final concentration of 0.2 mg/mL. 100 μL of the solution was transferred to a 0.1 mm path length cell. CD data were recorded at room temperature using an Applied Photophysics Chirascan Spectrometer, with wavelength scanning from 190 nm to 250 nm, an interval of 1 nm and a bandwidth of 1nm. Take the average value after repeating the measurement three times for each sample. The content of helicity was calculated by the following equation: Helicity % = $[\theta]_{222}/[\theta]_{max}$. Of note, $[\theta]_{222} = (mdeg * 10^6)/(0.1\ mm * c * n)$; mdeg: the measured value at 222 nm; c: the concentration of the peptide, unit of μM; n is the number of amino acid residues of the measured peptide. $[\theta]_{max} = (-44000+250T)*(1-k/n)$, T = 20 °C, k = 4, n: the number of amino acid residues of the studied peptide.

*CD spectra of **Linear 5**, **10**, **13** and **22***

0.2 mg of the peptide was dissolved into 1.5 mL phosphate-buffered saline containing 2% trifluoroethanol (v/v) to afford a final concentration of 0.13 mg/mL. 100 μL of the solution was transferred to a 0.1 mm path length cell. The CD spectra were obtained according to the above mentioned procedure.

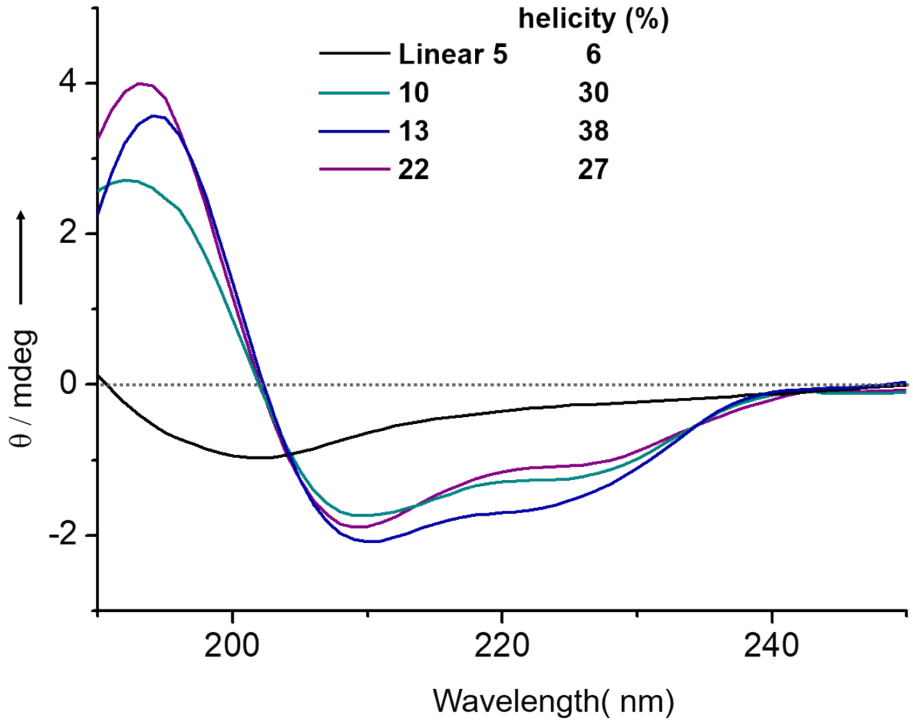


Figure S50. CD spectra of **Linear 5**, **10**, **13** and **22** (0.13 mg/mL) measured in 10 mM phosphate buffer pH 7.4: TFE (98:2) at room temperature. **Linear 5** is the corresponding monomeric un-crosslinked peptide of **10** and **13**. TFE was added to avoid precipitation of peptides.

Binding affinity measurement

The kinetic binding assay was performed on a ForteBio Octet RED96 Bio-Layer Interferometry system.

The first step was the biotinylation of SARS-CoV-2 spike RBD. After receiving ~100 μg of SARS-CoV-2 spike RBD-His (MW.: 26.54KD) from Sino Biological, we added 0.4 mL ddH_2O to the sample bottle to afford a concentration of 0.36 mg/mL (or 13.5 μM, determined by onedrop machine). To 200 μL of SARS-CoV-2 spike RBD-His (1.0 equ.) was added 0.8 μL of NHS-PEG12-Biotin (10 mM, 3.0 equ.). The remaining SARS-CoV-2 spike RBD-His was stocked in -80 °C. The reaction of biotinylation was kept at 4 °C for 1 hour. Then, the sample was desalted by using a spin desalting column (2 mL, Thermo, Nr. 89889), at room temperature. After washing five times with ddH_2O, the desalting column was equilibrated with HEPES buffer (20 mM HEPES, 100 mM NaCl, 0.02% Tween 20, pH 7.5) five times (2 mL x 5). Transfer the biotinylated RBD sample to the desalting column, at room temperature. When the solution of the RBD sample flows out, add 300 μL of HEPES buffer (20 mM HEPES, 100 mM NaCl, 0.02% Tween 20, pH 7.5) to the desalting column. The eluted HEPES buffer was collected into a labeled tube. The HEPES washing step was repeated five times. By one-drop measurement, we found the desired protein in the second and third eluted HEPES buffers, with a concentration of 0.1 mg/mL and total volume of 600 μL.

The second step was to load the SARS-CoV-2 spike RBD-His onto streptavidin-based SA sensor. Add 100 μL of HEPES buffer (20 mM HEPES, 100 mM NaCl, 0.02% Tween 20, pH 7.5) and 2 μL of DMSO (0.5%, v/v) to 300 μL of biotinylated SARS-CoV-2 spike RBD-His.

The concentration of SARS-CoV-2 spike RBD-His in the loading buffer is 75 μg/mL (for sensor loading, the concentration of loading protein is usually set to be ~100 μg/mL). The 400 μL of SARS-CoV-2 spike RBD-His solution was equally divided into two chambers for the loading of six SA sensors, with temperature set at 37 °C. The equilibration time for SA sensor was set to 5 minutes, and the loading time was set to 10 minutes. After SARS-CoV-2 spike RBD-His loading, we observed that the RBD loading signal in each SA sensor is almost at the same level.

Then, we measured the binding of **22** to SARS-CoV-2 spike RBD by ForteBio Octet RED96. Double reference-based method was used to perform the experiments, with temperature set at 37 °C. The aforementioned six SARS-CoV-2 spike RBD modified SA probes are used as ligand sensors, and six untreated SA probes are used as reference sensors. The concentration of the tested ACE2 peptide mimetic 22 was set to 0 nM, 125 nM, 250 nM, 500 nM, 1000 nM and 2000 nM, respectively. The buffer we used consists of 20 mM HEPES, 100 mM NaCl, 0.02% Tween 20, pH 7.5, and 0.5% DMSO. Each sensor will first equilibrate in the buffer for 5 minutes (Baseline), then transfer into the sample for 7 minutes (Association), and finally dissociate in the buffer for 7 minutes (Dissociation). The data of each concentration was obtained by subtracting the background signal from both 0 nM sample and the untreated SA sensor. Note that: by using the ForteBio Octet RED96 Bio-Layer Interferometry, we did not observe the binding of SARS-CoV-2 spike RBD to Biotin-**SBP1**, the previously reported linear ACE2 peptide mimetic.

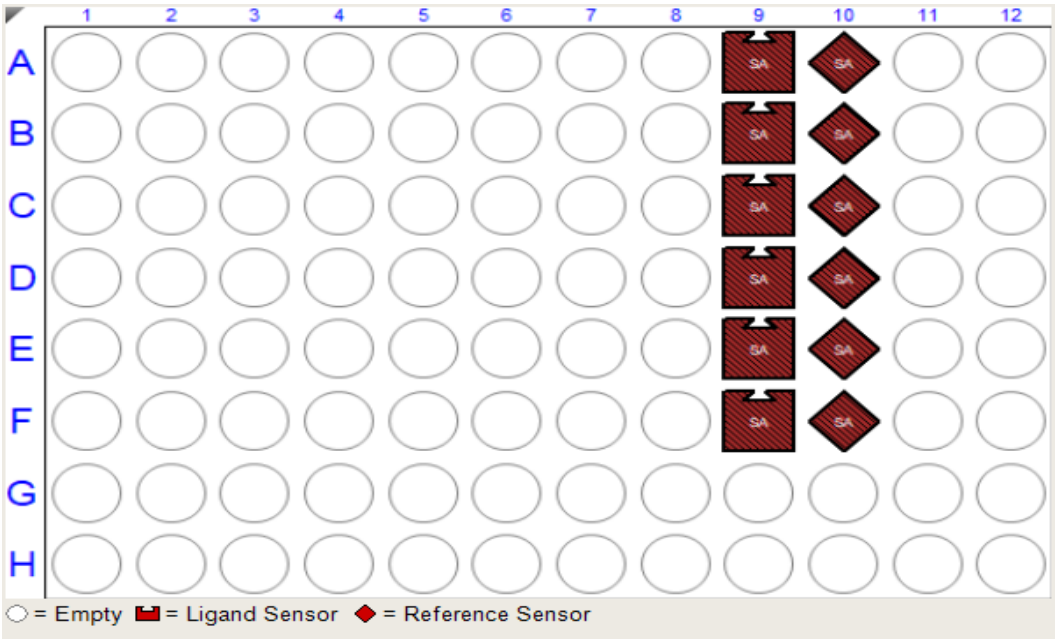


Figure S51. SA sensor arrangement used in our ForteBio Octet system.

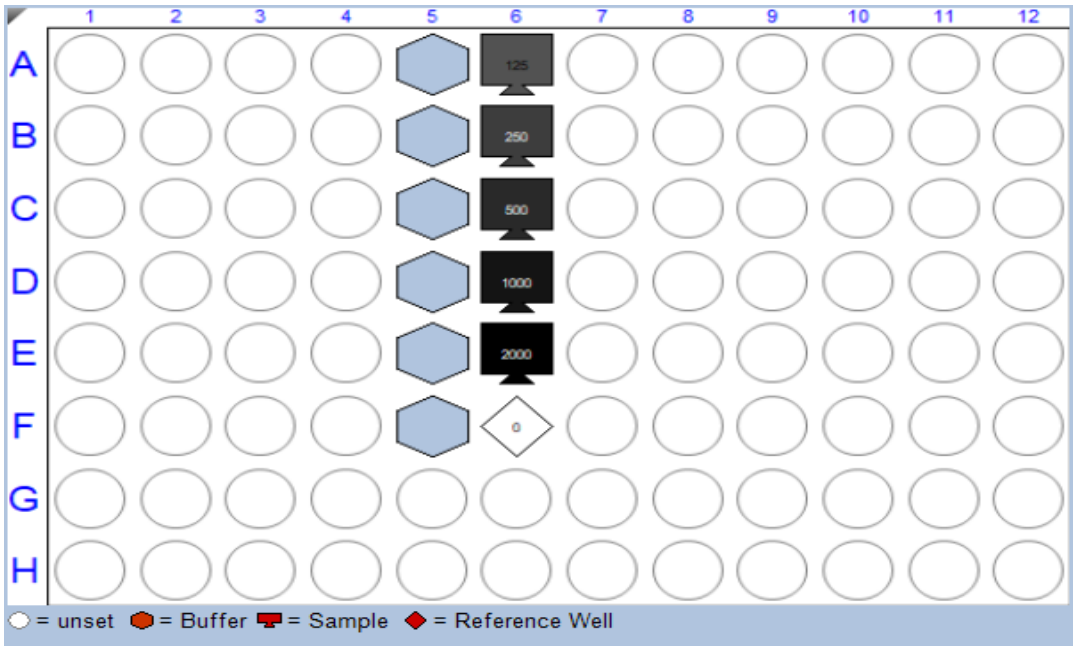


Figure S52. Sample concentration used in our ForteBio Octet system.

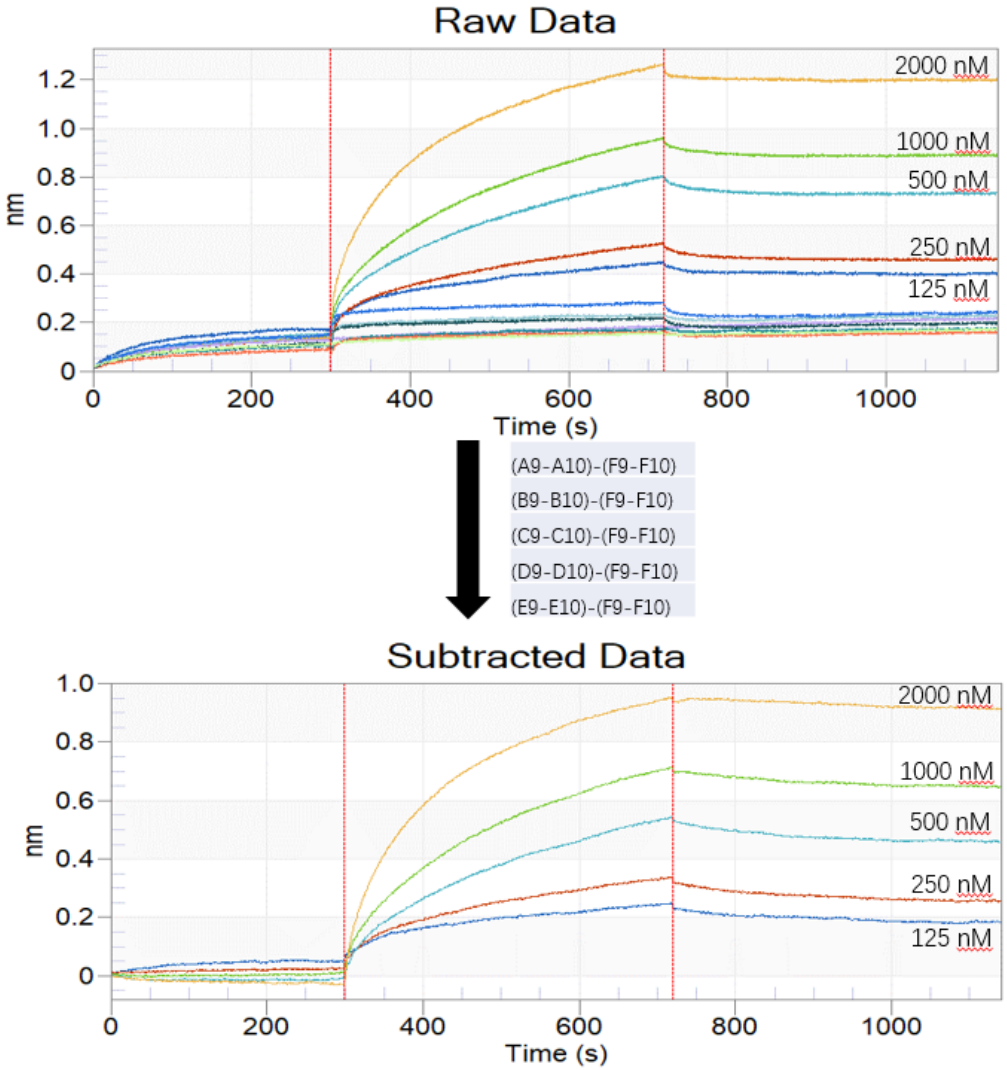


Figure S53. Signal before and after background subtraction.

[1] a) B. Ju, Q. Zhang, J. Ge, R. Wang, J. Sun, X. Ge, J. Yu, S. Shan, B. Zhou, S. Song, X. Tang, J. Yu, J. Lan, J. Yuan, H. Wang, J. Zhao, S. Zhang, Y. Wang, X. Shi, L. Liu, J. Zhao, X. Wang, Z. Zhang, L. Zhang, *Nature* **2020**, *584*, 115-119; b) X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Z. Xiang, Z. Mu, X. Chen, J. Chen, K. Hu, Q. Jin, J. Wang, Z. Qian, *Nature communications* **2020**, *11*, 1620.

Cell viability assay

Caco2 or Huh7-hACE2 Cells were seeded in 96-well plates at a density of 1 × 104 cells per well, cultured overnight at 37 °C, and incubated with the serially diluted peptides. After 48 hours, the cell viability was determined by using MTT Cell Proliferation and Cytotoxicity Assay Kit (Solarbio Life Science, M1020). The absorbance (OD490) was measured to determine the cytotoxicity of the peptides according to the manufacturer's protocol.

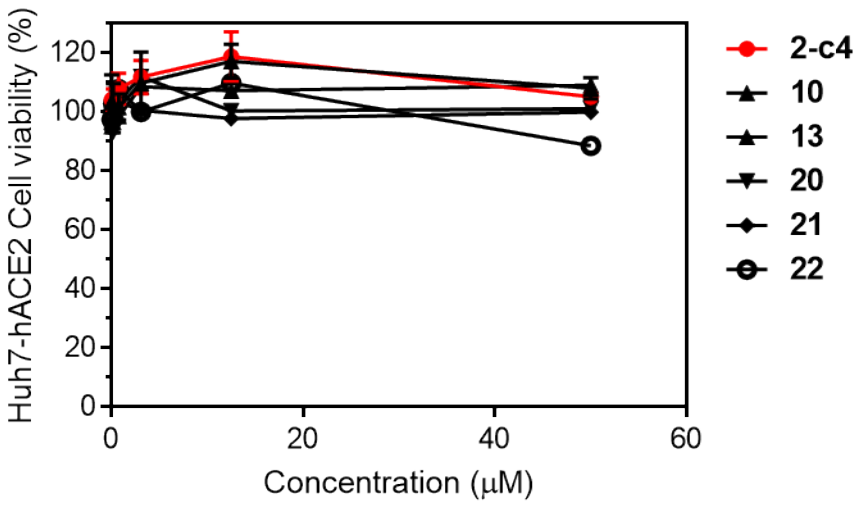


Figure S54. Cytotoxicity of **2-c4**, **10**, **13**, **20**, **21** and **22** to Huh7-hACE2 cells. All results are shown as the mean ± s.d. for triplicate samples.

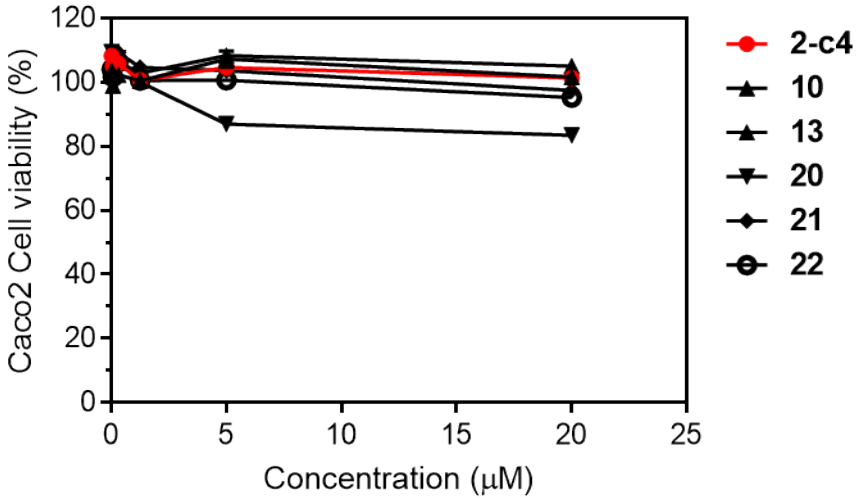


Figure S55. Cytotoxicity of **2-c4**, **10**, **13**, **20**, **21** and **22** to Caco2 cells. All results are shown as the mean ± s.d. for triplicate samples.