Hmb$^{off/on}$ as a switchable thiol protecting group for native chemical ligation

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Supplementary Information

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1. Reagents and materials

2-chlorotrityl resin was purchased from Hecheng Technology (Tianjing, China). Fmoc-amino acids were purchased from GL Biochem (Shanghai, China), CS Bio or Bo Mai Jie Technology (Beijing, China). 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium -hexafluorophosphate (HCTU), 1-[Bis(dimethylamino) methylene]-1H-1,2,3 -triazolo-[4,5-b]pyridinium hexafluorophosphate3-oxide (HATU), 1-Hydroxy-7-azabenzotriazole (HOAt) and 1-Hydroxybenzotriazole (anhydrous) (HOBt) were purchased from GL Biochem (Shanghai, China). *N*,*N'*-Diisopropyl-carbodiimide (DIC), N,N-diisopropylethylamine (DIPEA) and triisopropylsilane (TIPS) were purchased from Ouhe Technology (Beijing, China). 4-mercaptophenylacetic acid (MPAA) was purchased from Alfa Aesar. Acetonitrile (HPLC grade) was purchased from J.T.Baker. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) was purchased from Adamas-beta Co., Ltd. (Shanghai, China). Thioanisole, N,N-Dimethylformamide (DMF), 2-Methyl-2-propanethiol (*t*BuSH) and Trifluoroacetic acid (TFA, HPLC grade) were purchased from J&K Scientific (Beijing, China). Dithiothreitol (DTT) was purchased from Aladdin Reagent (Shanghai, China). Sodium sulphate, Tetrahydrofuran, Pyridine, Piperidine, Phenol (PhOH), Diethyl ether (Et_2O), ethyl alcohol, VA-044, Guanidine hydrochloride (Gn·HCl), Sodium chloride (NaCl), Sodium nitrite ($NaNO_2$), Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4·12H_2O$) and Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4·2H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (AR grade, China). Dichloromethane (DCM), Ethyl Acetate (EtOAc) and Petroleum ether (PE) were purchased from Beijing Chemical Works (Beijing, China).

2. HPLC and mass spectrometry

Analytical and semi-preparative RP-HPLC (Reversed-phase High-Performance Liquid Chromatography) were all performed on Shimadzu Prominence HPLC. For analytical RP-HPLC, Vydac C4 (5 μm, 4.6 × 150 mm) and Vydac C18 (5 μm, 4.6 × 150 mm) columns were used with a flow rate of 1.0 mL/min. For semi-preparative

RP-HPLC, Vydac C4 (10 μm, 10 × 250 mm) and Vydac C18 (10 μm, 10 × 250 mm) columns were used at a flow rate of 3-4 mL/min. Buffers for RP-HPLC: buffer A (0.08-0.1% TFA in CH_3CN) and buffer B (0.1% TFA in water). UV absorption at 214 nm and 254 nm were monitored for the injections. The UV absorption at 214 nm and 254 nm were monitored throughout the injections.

ESI-MS was performed on an Esquire-LC ion trap mass spectrometer or an Agilent 1200 Series HPLC system with LC-MS (Agilent 6340 ion trap mass spectrometer).

3. Synthesis of Fmoc-Cys(Hmb^{off})-OH

To apply the new side-chain protecting group for Fmoc-SPPS, we synthesized the building block Fmoc-Cys(Hmb^{off})-OH **1**. Compound **1** was obtained through three synthetic steps from commercially available **S2** and **S1**. The procedures were detailed below.

Compound **S1** (1.50 g, 6 mmol, 1 equiv.) was dissolved in dry pyridine (6 mL). Then compound **S2** (1.19 g, 7.8 mmol, 1.3 equiv.) was added to the above solution. The reaction mixture was then allowed to stir for 24 h at 25 °C and monitored by TLC (PE/EtOAc, v / v, 1 / 1). Upon completion, the reaction mixture was diluted with EtOAc. Then, the reaction mixture was sequentially washed three times with HCl (1 M) aqueous solution, two times with NaOH (0.2 M) aqueous solution and at last two times with saturated NaCl aqueous solution. The organic extract was then dried with Na_2SO_4. Evaporation of the organic extract afforded a yellow oil product. The crude product was purified by column chromatography on silica gel using a mixture of PE/EtOAc (v / v, 1 / 1) as eluent. Evaporation of the solvent afforded the desired compound **S3** as pale yellow oil (2.04 g, yield 93%). ESI-MS calcd for $C_{18}H_{26}N_2O_6$ 366.2; found ($M+Na^+$) 389.1.

Compound **S3** (1.83 g, 5 mmol, 1 equiv.) was dissolved in 18 mL dry ethyl alcohol and then cooled to 0 °C. $NaBH_4$ (0.11 g, 3 mmol, 0.6 equiv.) was then added to the above solution under 0 °C. The reaction mixture was then stirred under 0-4 °C for about 8 h. The reaction was terminated by the successively addition of 15 mL

water and 15 mL HCl (1 M) aqueous solution under 0-4 °C. The above mixture was extracted with ethyl acetate (40 mL × 3). The combined organic extracts were washed with saturated NaCl solution, and then dried with Na_2SO_4. Evaporation of the organic extract afforded a colorless oil product. The crude product was purified by column chromatography on silica gel using a mixture of PE/EtOAc (v / v, 1 / 1) as eluent. Evaporation of the solvent afforded the desired compound **S4** as colorless oil (1.49 g, yield 81%). ESI-MS calcd for $C_{18}H_{28}N_2O_6$ 368.2; found ($M+Na^+$) 391.2.

Compound **S4** (0.92 g, 2.5 mmol, 1 equiv.) was dissolved in dry CH_2Cl_2 (20 mL). Then compound **S5** (1.2 g, 3.5 mmol, 1.4 equiv.) and TFA (600 uL, 3%) were sequentially added to the above solution. The reaction mixture was then stirred under 25 °C for about 8 h. Upon completion, the reaction mixture was diluted with CH_2Cl_2, washed with saturated NaCl aqueous solution, dried with Na_2SO_4. The organic solvent was removed under reduced pressure and then the crude product was purified by column chromatography on silica gel to afford the target compound **1** as white solid (0.68 g, yield 39%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ. 7.75 – 7.77 (d, J = 8.0 Hz, 2 H), 7.61 – 7.62 (d, J = 4.0 Hz, 2 H), 7.38 – 7.41 (t, J = 6.0 Hz, 2 H), 7.29 – 7.33 (t, J = 8.0 Hz, 2 H), 6.73 – 6.77 (t, J = 8.0 Hz, 1 H), 6.65 (s, 1 H), 5.88 – 5.92 (t, J = 8.0 Hz, 1 H), 4.26 – 4.54 (m, 3 H), 4.20 – 4.24 (t, J = 8.0 Hz, 1 H), 3.41 – 3.83 (m, 9 H), 2.85 – 3.16 (m, 8 H), 1.44 – 1.46 (d, J = 8 Hz, 9 H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ. 173.07, 159.66, 156.17, 154.96, 154.80, 150.27, 143.82, 141.32, 130.93, 127.78, 127.17, 125.31, 121.91, 120.17, 112.13, 109.07, 80.45, 67.28, 55.51, 53.37, 47.13, 35.46, 34.87, 33.64, 33.29, 31.27, 28.50. HRESI-MS calcd for $C_{36}H_{43}N_3O_9SNa^+$ 716.2618; found ($M+Na^+$) 716.2608.

4. Fmoc-based solid-phase peptide synthesis

Peptide synthesis vessels were purchased from Beijing Synthware Glass (Beijing, China). Tbeoc-Thz-OH was prepared according to the previously reported procedure.[1] Peptides were all synthesized manually by Fmoc SPPS. 2-chlorotrityl resin was used for the synthesis of the peptide acid and peptide hydrazide. 2-chlorotrityl resin was

treated with 5% NH_2NH_2 in DMF (twice: 30 min, 30 min) at 30 °C to prepare 2-chlorotrityl-$NHNH_2$ resin.

Proper coupling reactions for each amino acid should be conducted in a constant temperature shaker at 30 °C. A single coupling reaction using HCTU or HATU and a 1.0-1.3 h reaction time is enough for most amino acids. However, a double coupling strategy is recommended for sterically hindered amino acids (e.g., Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Thr(tBu)-OH) and the amino acid right after Proline residue. Moreover, a double coupling strategy is needed when the peptide is assembled beyond 15-20 amino acids. The Fmoc protecting group was removed by 20% piperidine in DMF (twice: 5 min and then about 10 min). After all the coupling reactions were carried out, final cleavage of peptide from the resin was achieved by TFA cocktails for 2-3 h at 25 °C. The TFA cocktails (95% TFA, 2.5% TIPS, 2.5% H_2O) and (85% TFA, 5% thioanisole, 5% EDT, 2.5% phenol and 2.5% H_2O) could be used. The combined mixture was concentrated by blowing with pure nitrogen. The crude peptide was then obtained by precipitation with cold diethyl ether and subsequent centrifugation. Finally, the crude peptide was dissolved in CH_3CN/H_2O and purified by semi-preparative RP-HPLC. The purified peptide was analyzed by ESI-MS.

Fmoc-Cys(Hmb^{off})-OH **1** could be coupled onto the resin via standard Fmoc SPPS coupling. Peptide hydrazide EPO[Cys^{98}-Ala^{127}]-$NHNH_2$ was synthesized on 2-chlorotrityl-$NHNH_2$ resin following the standard Fmoc SPPS procedures described above. Model peptide **2**, **10** and **11** were synthesized on 2-chlorotrityl resin following the standard Fmoc-SPPS procedures described above.

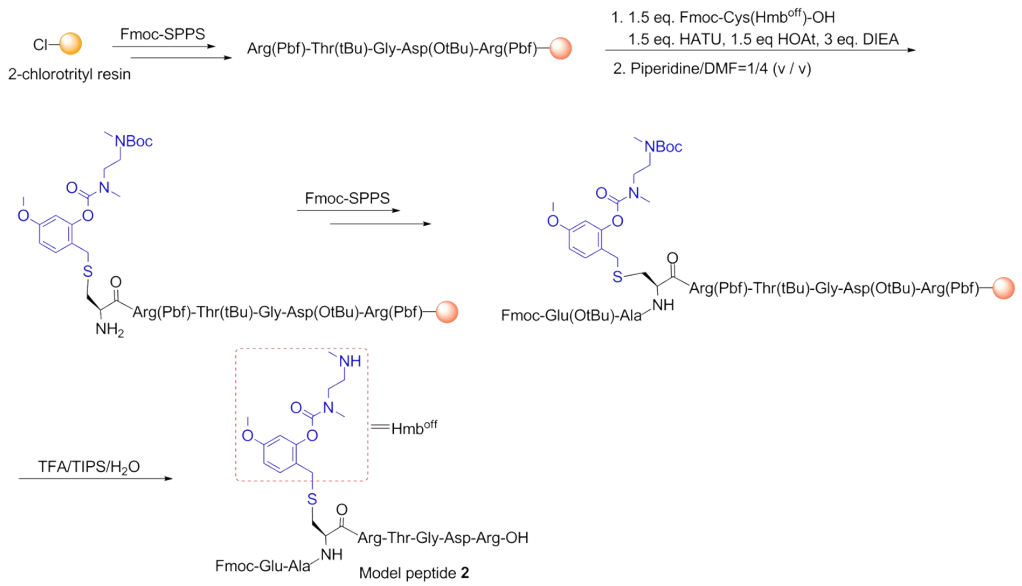

Fig. S1 The procedure for Fmoc SPPS synthesis of model peptide **2** with removable Hmb^{off} group.

5. Tests of model peptide 2

5.1 Stability test of model peptide 2 in TFA cleavage cocktails

To test the stability of Cys(Hmb^{off})-containing peptide **2** in TFA cocktails, the stability test was conducted during the Fmoc SPPS. After all the coupling reactions were carried out, 6 mL TFA cocktails (TFA/TIPS/H_2O = 95/2.5/2.5) (2-3 mL per 100 mg of resin) was added to the dried peptide-containing resin. The mixture was then incubated in a constant temperature shaker at 25 °C. Analytic RP-HPLC and ESI-MS were used to monitor the reaction mixture. No degradation of peptide **2** was detected in 3 h.

The reaction was monitored by analytic RP-HPLC chromatogram (λ = 214 nm) (Fig. 1B in main text). Time points: 1 h and 3 h after the treatment of peptide-containing resin in TFA cocktails. HPLC conditions: a linear gradient of 5-95% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-95% for 30 min) on a Vydac C4 (4.6 × 150 mm) column.

5.2. The intramolecular cyclization-elimination reaction on Cys(Hmb^{off}) residue

In 1990, Saari and co-workers investigated the intramolecular cyclization-elimination reaction for a series of basic carbamates of 4-hydroxyanisole at pH 7.4, 37 ^{o}C. The model peptide **2**, Fmoc-EAC(Hmb^{off})RTGDR-OH, was selected to study the intramolecular cyclization-elimination reaction under native chemical ligation buffer (0.2 M Na_2HPO_4 solution containing 6 M Gn·HCl, pH 7.4). Model peptide **2** (4.14 mg, 3 μmol) was dissolved in 1.5 mL of ligation buffer. The pH of the reaction mixture was adjusted to pH 7.4 and then stirred at 25 °C. Analytic RP-HPLC and ESI-MS were used to monitor the reaction mixture. Intramolecular cyclization reaction occurred soon after the dissolution of peptide **2** in ligation buffer. The intramolecular cyclization reaction was completed up to more than 95% within 5 h. Moreover, peptide **3** was totally stable up to 24 h under the above buffer. Finally, the reaction mixture was purified by semi-preparative RP-HPLC, affording pure peptide **3** in 43% isolated yield (1.63 mg, 1.29 μmol).

The reaction was monitored by analytical RP-HPLC chromatograms (λ = 214 nm) (Fig. 1C in main text). Time points: 5 min and 5 h after the treatment of peptide **2** in pH 7.4 neutral aqueous solution at 25 °C. HPLC conditions: a linear gradient of 5-95% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-95% for 30 min) on a Vydac C4 (4.6 × 150 mm) column.

5.3. The Deprotection of Hmb^{on} group by TFA cocktails

According to the study of Johnson *et al*, the reversible protection group *N*-(2-hydroxy-4-methoxybenzyl) (Hmb) group which was attached to the nitrogen atom of peptide bond could be cleaved by TFA cocktails. We then studied whether Hmb^{on} attached to the sulfur atom of cysteine could be cleaved by TFA cocktails. To this end, the purified peptide **3** (4 mg, 3.16 μmol) was dissolved in 2 mL of TFA cocktails (TFA/TIPS/H_2O, 95/2.5/2.5, v/v/v). The reaction mixture was then incubated at 25 ºC. According to the RP-HPLC analysis and mass spectra data, Hmb^{on} residue was completely cleaved from peptide **3** within 2 h, affording the native cysteine residue in peptide **4**. Subsequently, the reaction mixture was concentrated by blowing with pure nitrogen. The concentrated mixture was precipitated with cold Et_2O and then

centrifuged. Finally, the crude peptide was dissolved in H_2O/CH_3CN and purified by semi-preparative RP-HPLC, affording pure peptide **4** in 63% isolated yield (2.26 mg, 2.0 μmol).

The reaction was monitored by analytical RP-HPLC chromatograms (λ = 214 nm) (Fig. 1D in main text). Time points: 1 min and 2 h after the treatment of peptide **3** in TFA cocktails. HPLC conditions: a linear gradient of 5-95% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-95% for 30 min) on a Vydac C4 (4.6 × 150 mm) column.

6. Ligation of EPO[Cys^{98}-Ala^{127}]-NHNH$_2$ with EPO[Cys^{128}-Arg^{166}]-OH

EPO[Cys^{98}-Ala^{127}]-NHNH$_2$ **5** (6.43 mg, 2 μmol, 1 equiv.) was dissolved in 1 mL aqueous buffer (containing 6 M Gn·HCl and 0.2 M NaH_2PO_4, pH = 3.0-3.1) in a 2-ml Eppendorf reaction tube. The reaction solution was then cooled to approximately -15 °C in an ice-salt bath and constantly stirred by magnetic stirring. $NaNO_2$ (10-15 equiv, 0.2 M) dissolved in aqueous buffer (6 M Gn·HCl and 0.2 M NaH_2PO_4, pH = 3.0) was then added dropwise into the above solution. The reaction solution was then stirred for 20 min at -15 °C to oxidize the peptide hydrazide to the peptide azide. Then, 0.5 mL of MPAA (about 50 equiv., 200 mM) dissolved in aqueous buffer (6 M Gn·HCl and 0.2 M Na_2HPO_4, pH 6.6) and peptide EPO[Cys^{128}-Arg^{166}]-OH **6** (10.6 mg, 2.2 μmol, 1.1 equiv.) were sequentially added into the reaction solution. The reaction was then taken out of the ice-salt bath, stirred and warmed to room temperature. The pH value of the reaction mixture was then adjusted to 6.6 slowly with aqueous NaOH solution (2 M) to initiate the NCL process. The reaction mixture was then gently stirred at 25 °C. Analytical RP-HPLC and ESI-MS were used to monitor the reaction process. The ligation between **5** and peptide **6** was completed within 10 h. To remove the Tbeoc and *N*-methyl-*N*-[2-(methylamino)ethyl]carbamoyl groups, 0.3 mL of 0.2 M TCEP aqueous solution (containing 6 M Gn·HCl, pH = 7.0) was added into the reaction solution. Subsequently, the pH value of reaction mixture was raised to 7.4 to accelerate the intramolecular cyclization-elimination reactions. The intramolecular cyclization-elimination reactions were completed within 6 h, generating peptide **8**.

Then, methoxyamine hydrochloride (67 mg, 0.8 mmol) was added, and then the pH value of the reaction mixture was adjusted to 4.0. Finally, the reaction solution was stirred for about 8 h at 25 °C. The target product **9** with deprotected Cys^{98} and $Cys(Hmb^{on})^{161}$ was obtained with an overall isolated yield of 43% through semi-preparative RP-HPLC.

The reaction was monitored by analytical RP-HPLC chromatograms (λ = 214 nm) (Fig. 2C in main text). Before RP-HPLC analysis and purification, the reaction mixture was reduced by aqueous TCEP (50 mM, containing 6 M Gn·HCl, pH 6.6). The peak marked with **5'** corresponds to the peptide thioester generated from peptide hydrazide **5**. HPLC conditions for Fig. 2C: a linear gradient of 20-55% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 35 min (5% for 2 min, then 20%-55% for 35 min) on a Vydac C4 (4.6 × 150 mm) column. HPLC conditions for Fig. 2D: a linear gradient of 20-60% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 20%-60% for 30 min) on a Vydac C18 (4.6 × 150 mm) column.

7. Stability test of Hmb protecting group in free-radical-based desulfurization

7.1. The intramolecular cyclization-elimination reaction on $Cys(Hmb^{off})$ residue

Model peptide **10,** H-CAC(Hmb^{off})RTGDR-OH, (3.39 mg, 3 μmol, crude product) was dissolved in 1.5 mL of native chemical ligation buffer (0.2 M Na_2HPO_4 solution containing 6 M Gn·HCl, pH 7.4). The pH of the reaction mixture was adjusted to pH 7.4 and then stirred at 25 °C. Analytic RP-HPLC and ESI-MS were used to monitor the reaction mixture. The intramolecular cyclization reaction was completed up to more than 95% within 10 h, generating peptide **12** H-CAC(Hmb^{on})RTGDR-OH. Moreover, peptide **12** was totally stable up to 48 h with continuous magnetic stirring under the above buffer at 25 °C. Finally, the reaction mixture was purified by semi-preparative RP-HPLC, affording pure peptide **12** in 41% isolated yield (1.25 mg, 1.23 μmol).

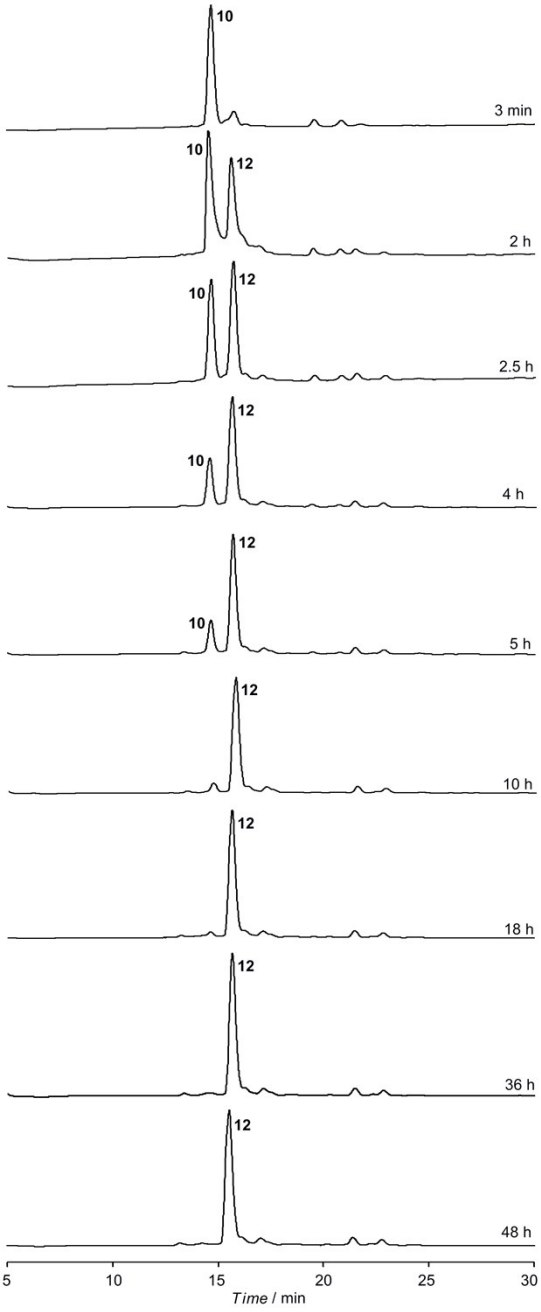

Fig. S2 Analytical RP-HPLC traces for the intramolecular cyclization-elimination of **10**. The reaction was monitored by analytical RP-HPLC chromatograms (λ = 214 nm). Time points: 3 min, 2h, 2.5 h, 4 h, 5 h, 10 h, 18 h, 36 h and 48 h after the treatment of peptide **10** in pH 7.4 neutral aqueous solution at 25 °C. HPLC conditions: a linear gradient of 5-35% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-35% for 30 min) on a Vydac C18 (4.6 × 150 mm) column. Before RP-HPLC analysis and purification, the reaction mixture was reduced by aqueous TCEP (**10**0 mM, containing 6 M Gn·HCl, pH 6.6).

Model peptide **11,** H-AAC(Hmb^{off})RTGDR-OH, (3.30 mg, 3 μmol, crude product) was dissolved in 1.5 mL of native chemical ligation buffer (0.2 M Na_2HPO_4 solution containing 6 M Gn·HCl, pH 7.4). The pH of the reaction mixture was adjusted to pH 7.4 and then stirred at 25 °C. Analytic RP-HPLC and ESI-MS were used to monitor the reaction mixture. The intramolecular cyclization reaction was completed up to more than 95% within 12 h, generating peptide **13** H-AAC(Hmb^{on})RTGDR-OH. Moreover, peptide **13** was totally stable up to 48 h with continuous magnetic stirring under the above buffer at 25 °C. Finally, the reaction mixture was purified by semi-preparative RP-HPLC, affording pure peptide **13** in 38% isolated yield (1.12 mg, 1.14 μmol).

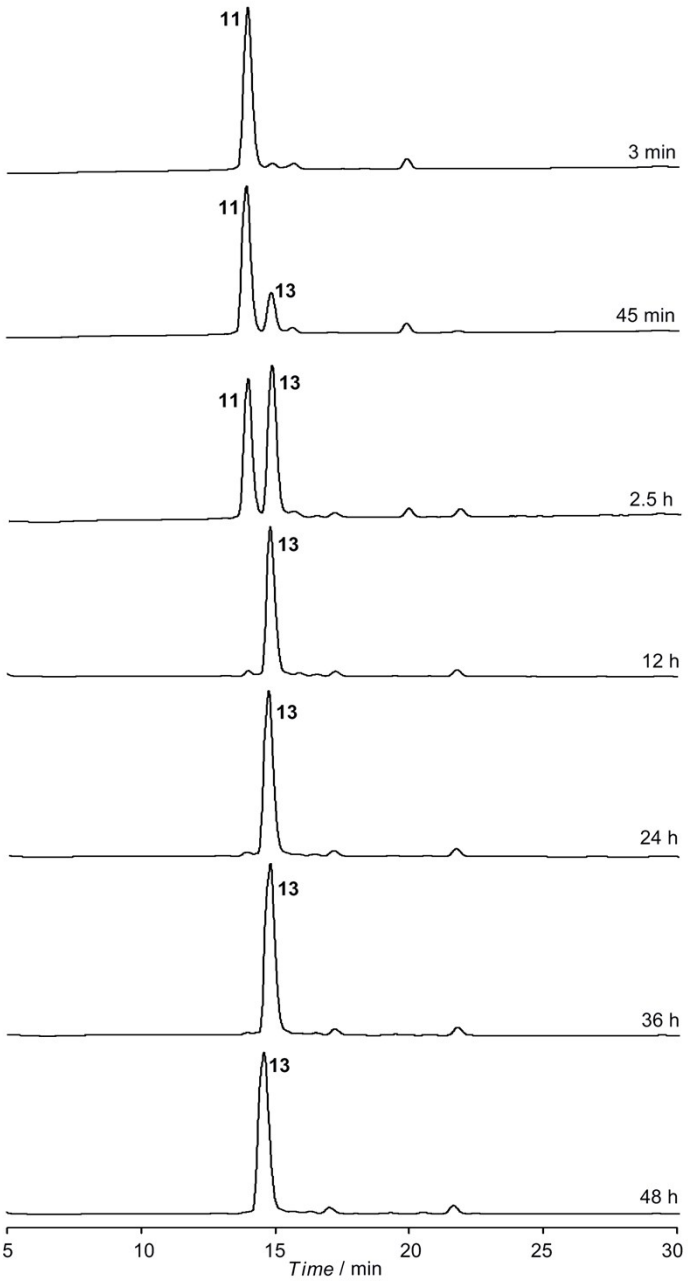

Fig. S3 Analytical RP-HPLC traces for the intramolecular cyclization-elimination of **10**. The reaction was monitored by analytical RP-HPLC chromatograms (λ = 214 nm). Time points: 3 min, 45 min, 2.5 h, 12 h, 24 h, 36 h and 48 h after the treatment of peptide **11** in pH 7.4 neutral aqueous solution at 25 °C. HPLC conditions: a linear gradient of 5-35% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-35% for 30 min) on a Vydac C18 (4.6 × 150 mm) column.

7.2. The desulfurization reaction

H-CAC(Hmb^{on})RTGDR-OH **12** (1.2 mg, 1.2 μmol) was dissolved in 1 mL aqueous solution (containing 0.2 M Na_2HPO_4, 6 M Gn·HCl and 500 mM TCEP, pH 6.8). Then 50 μl of *t*BuSH and 500 μl of 0.1 M VA-044 dissolved in aqueous solution (containing 0.2 M Na_2HPO_4, pH 6.6) were sequentially added into the above solution. The reaction mixture was adjusted to pH 6.8 and then stirred on a magnetic stirrer at 37 ^{o}C. Analytical RP-HPLC and ESI-MS were used to monitor the reaction mixture. It was found that most peptide **12** was converted to peptide **14** within 3 h.

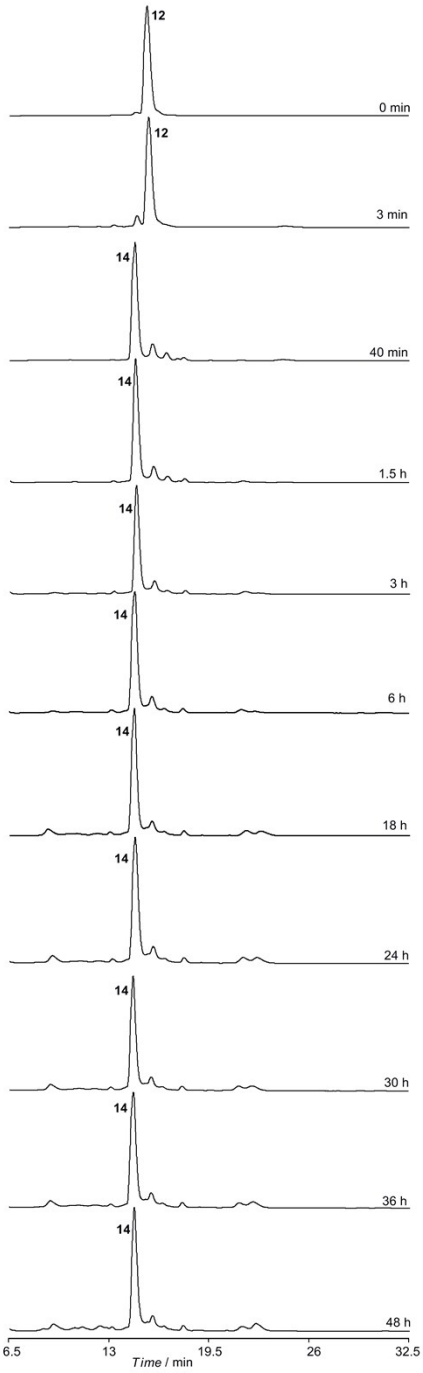

Fig. S4 The desulfurization reaction of peptide **12** in free-radical-based desulfurization system. The reaction was monitored by analytical RP-HPLC chromatograms (λ = 214 nm). Time points: 0 min, 3 min, 40 min, 1.5 h, 3 h, 6 h, 18 h, 24 h, 30 h, 36 h and 48 h after the treatment of peptide **12** in desulfurization system with continuous stirring under 37 ^{o}C. HPLC conditions: a linear gradient of 5-35% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-35% for 30 min) on a Vydac C18 (4.6 × 150 mm) column.

8. The ESI-MS spectra

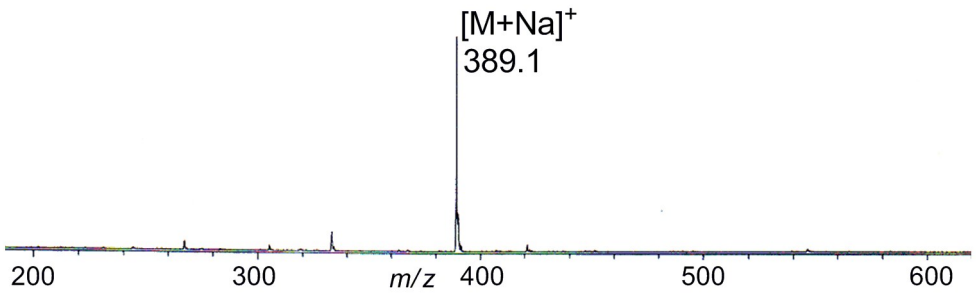

Fig. S5 The ESI-MS spectrum of compound **S3.** ESI-MS calcd for $C_{18}H_{26}N_2O_6$ 366.2; found ($M+Na^+$) 389.1.

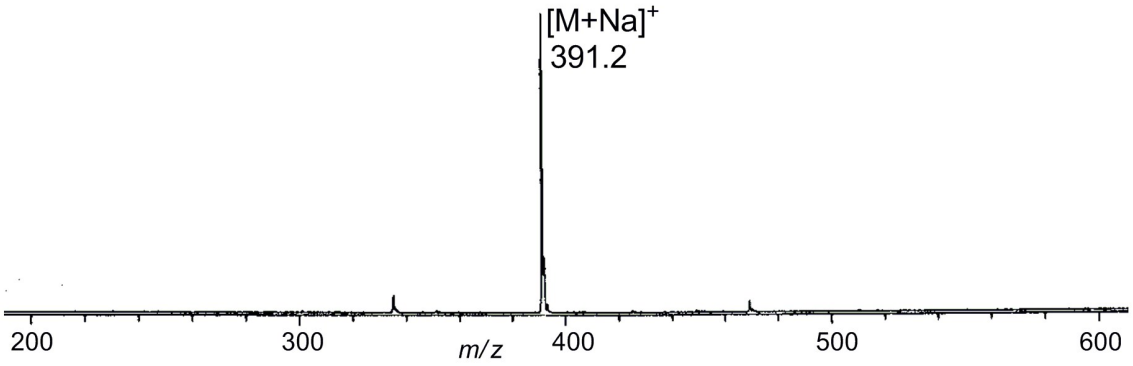

Fig. S6 The ESI-MS spectrum of compound **S4**. ESI-MS calcd for $C_{18}H_{28}N_2O_6$ 368.2; found ($M+Na^+$) 391.2.

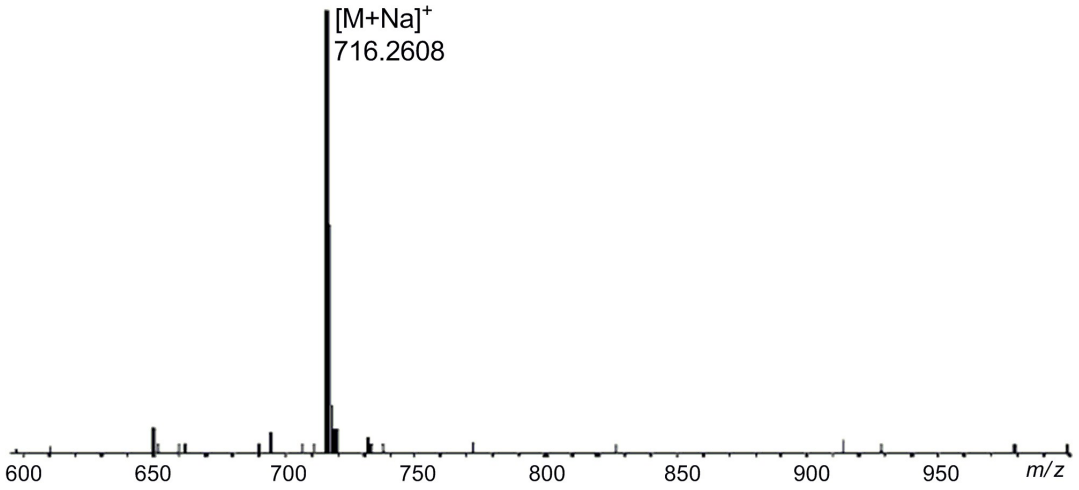

Fig. S7 The HRESI-MS spectrum of compound **1**. HRESI-MS calcd for $C_{36}H_{43}N_3O_9SNa^+$ 716.2618; found ($M+Na^+$) 716.2608.

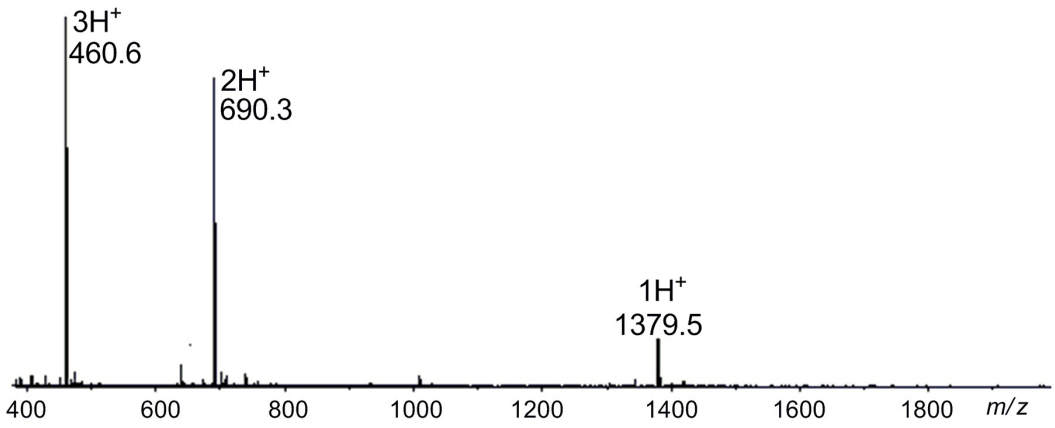

Fig. S8 ESI-MS spectrum of purified peptide **2.** The spectrum gave an observed mass of 1378.6 Da (calculated 1379.5 Da, average isotopes).

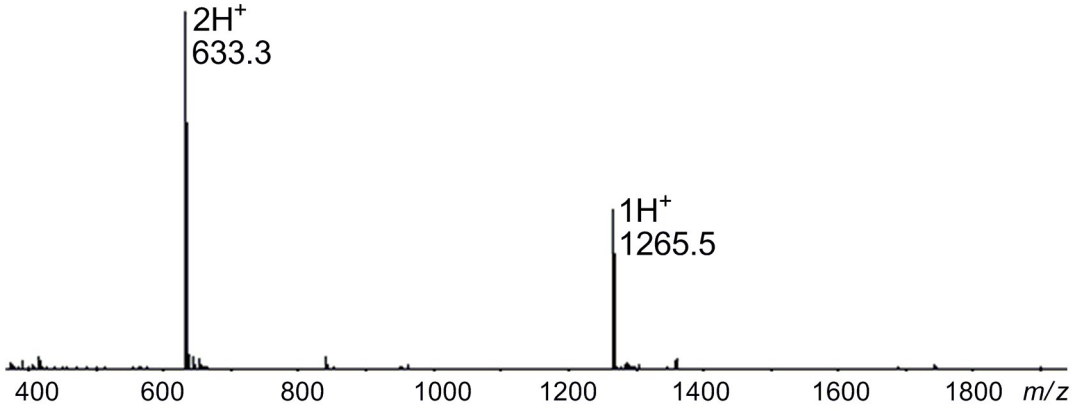

Fig. S9 ESI-MS spectrum of purified peptide **3.** The spectrum gave an observed mass of 1264.6 Da (calculated 1265.4 Da, average isotopes).

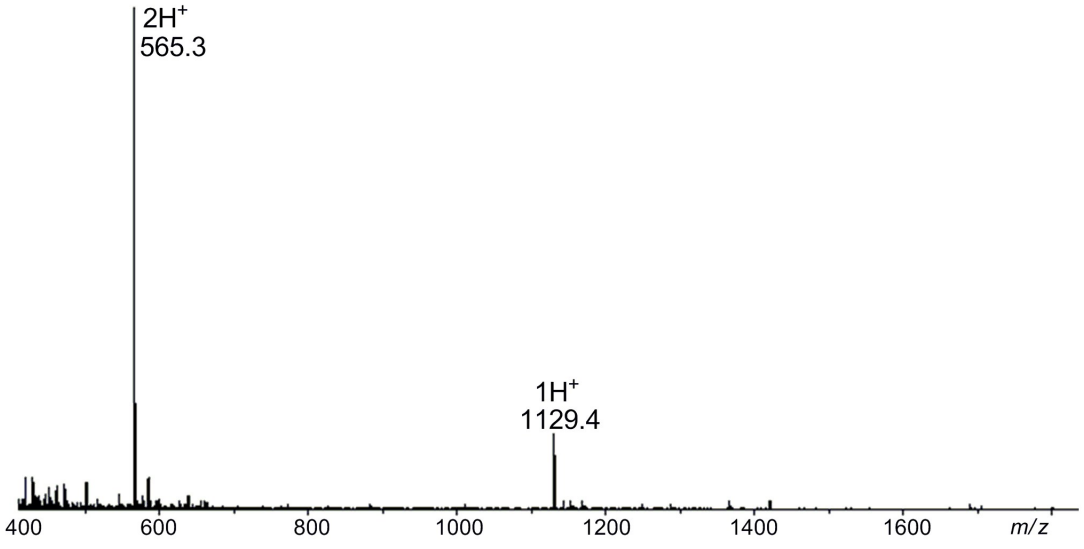

Fig. S10 ESI-MS spectrum of purified peptide **4.** The spectrum gave an observed mass of 1128.5 Da (calculated 1129.2 Da, average isotopes).

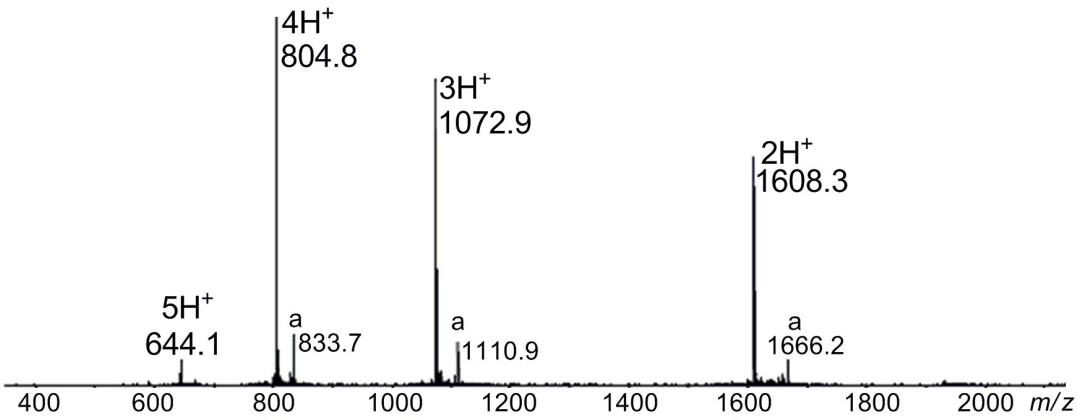

Fig. S11 ESI-MS spectrum of purified peptide **5.** The spectrum gave an observed mass of 3215.3 Da (calculated 3215.8 Da, average isotopes). Peaks marked by **a** corresponded to compound with the mass of approximately M+115. The ratio of these peaks to the main peaks varied from injections to injections. These peaks were thought to be associated with the arginine residues.[2]

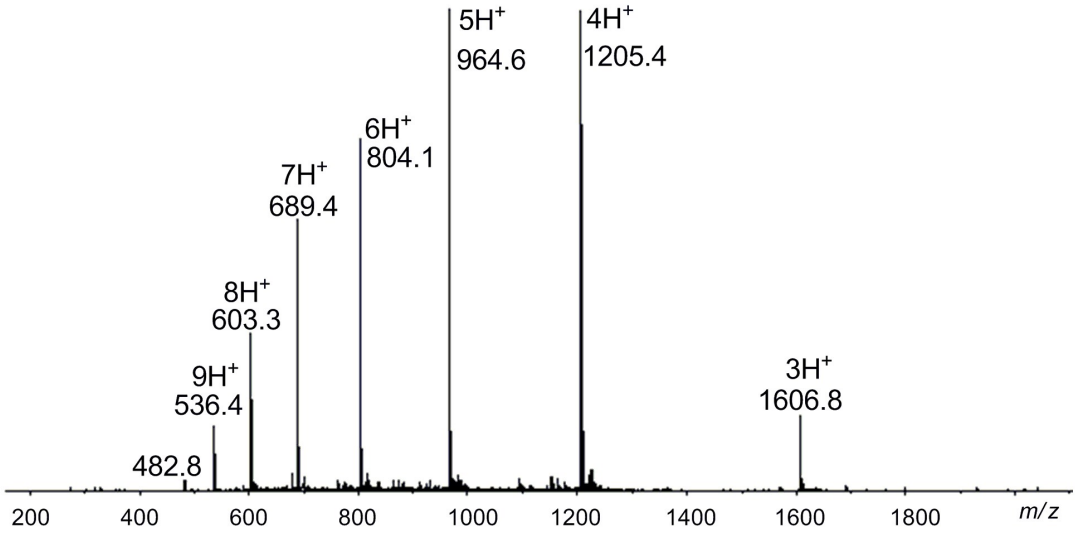

Fig. S12 ESI-MS spectrum of purified peptide **6.** The spectrum gave an observed mass of 4818.2 Da (calculated 4818.6 Da, average isotopes).

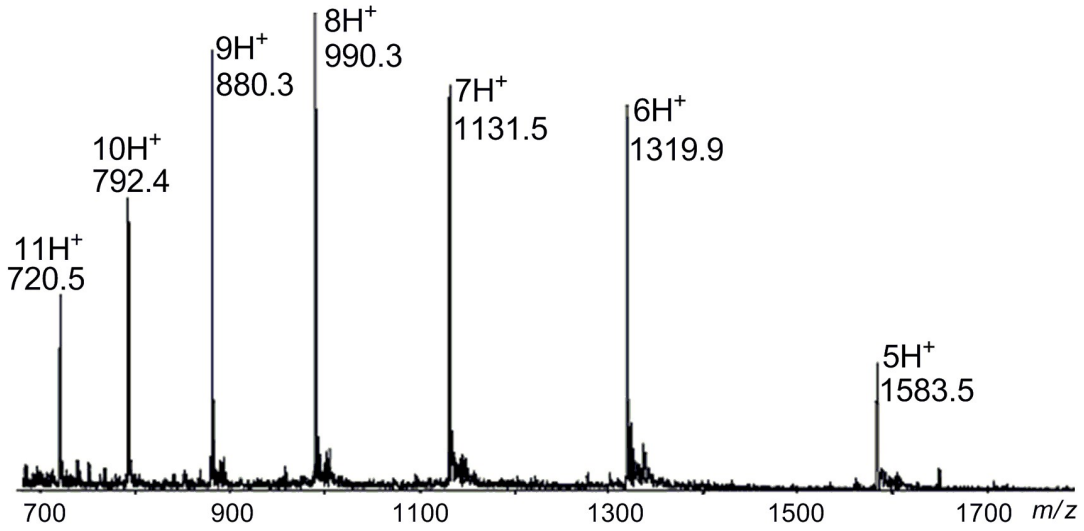

Fig. S13 ESI-MS spectrum of purified peptide **7.** The spectrum gave an observed mass of 7913.7 Da (calculated 7914.2 Da, average isotopes).

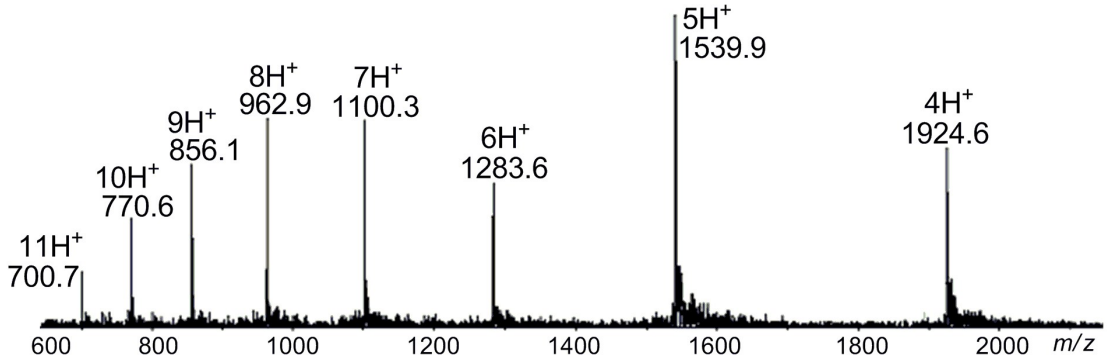

Fig. S14 ESI-MS spectrum of purified peptide **8.** The spectrum gave an observed mass of 7695.4 Da (calculated 7696.0 Da, average isotopes).

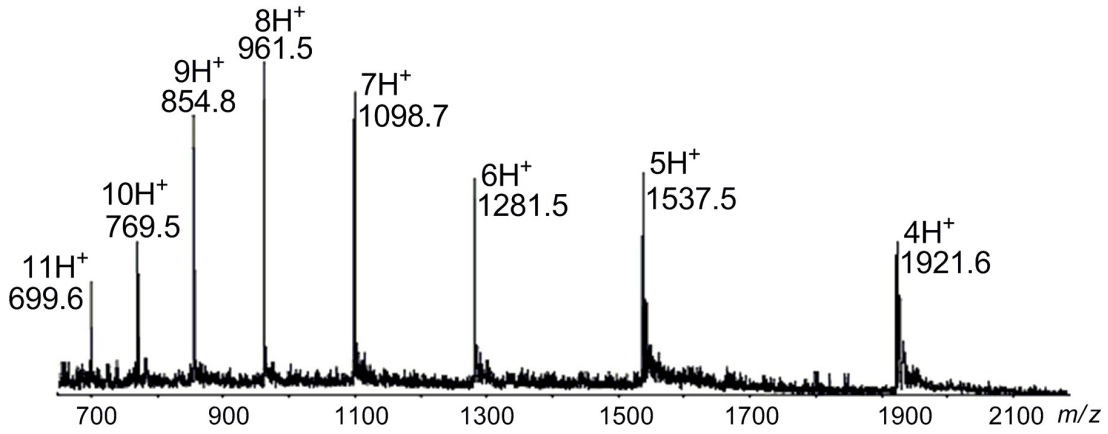

Fig. S15 ESI-MS spectrum of purified peptide **9.** The spectrum gave an observed mass of 7683.7 Da (calculated 7683.9 Da, average isotopes).

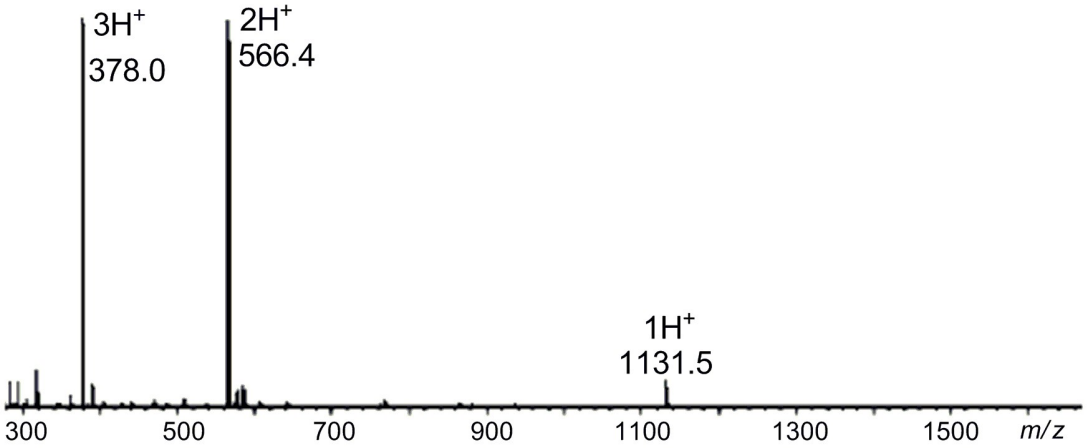

Fig. S16 ESI-MS spectrum of purified peptide **10.** The spectrum gave an observed mass of 1130.8 Da (calculated 1131.3 Da, average isotopes).

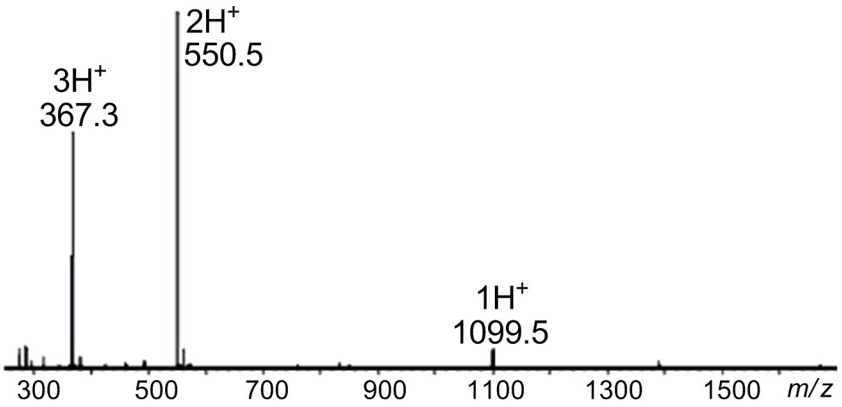

Fig. S17 ESI-MS spectrum of purified peptide **11.** The spectrum gave an observed mass of 1098.8 Da (calculated 1099.2 Da, average isotopes).

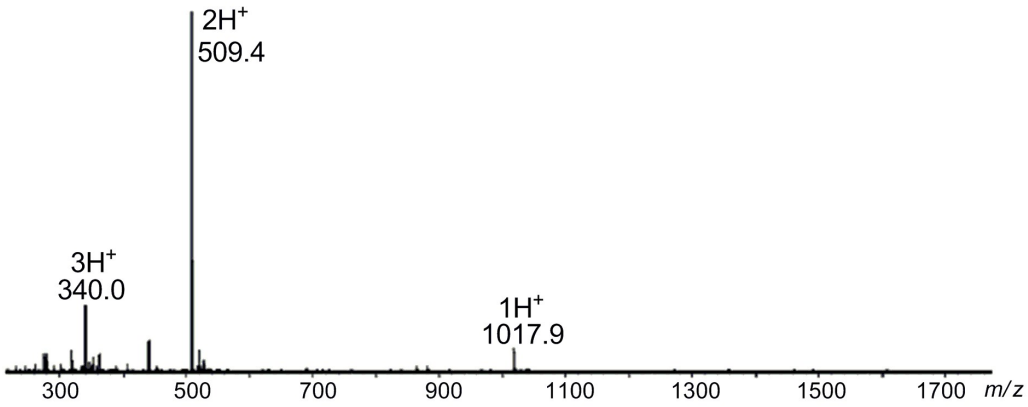

Fig. S18 ESI-MS spectrum of purified peptide **12.** The spectrum gave an observed mass of 1016.9 Da (calculated 1017.2 Da, average isotopes).

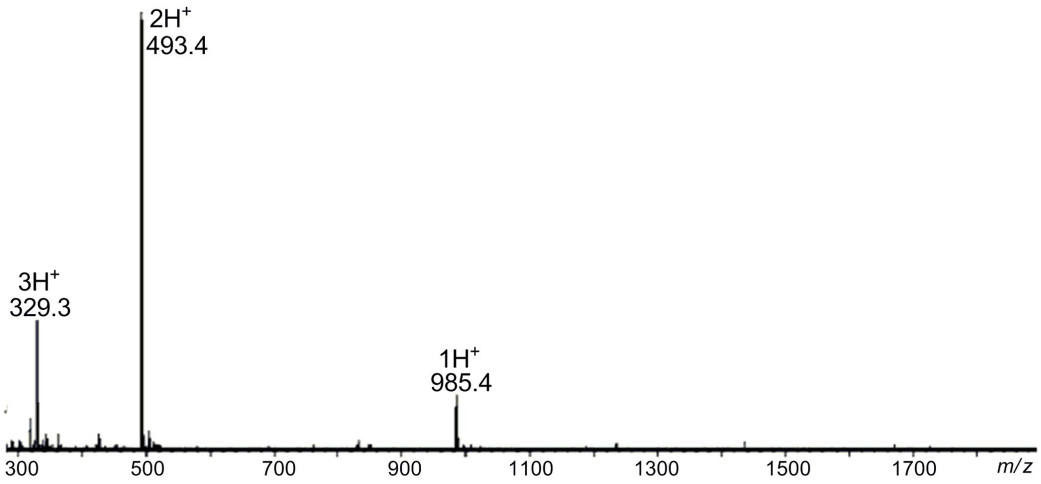

Fig. S19 ESI-MS spectrum of purified peptide **13.** The spectrum gave an observed mass of 984.7 Da (calculated 985.1 Da, average isotopes).

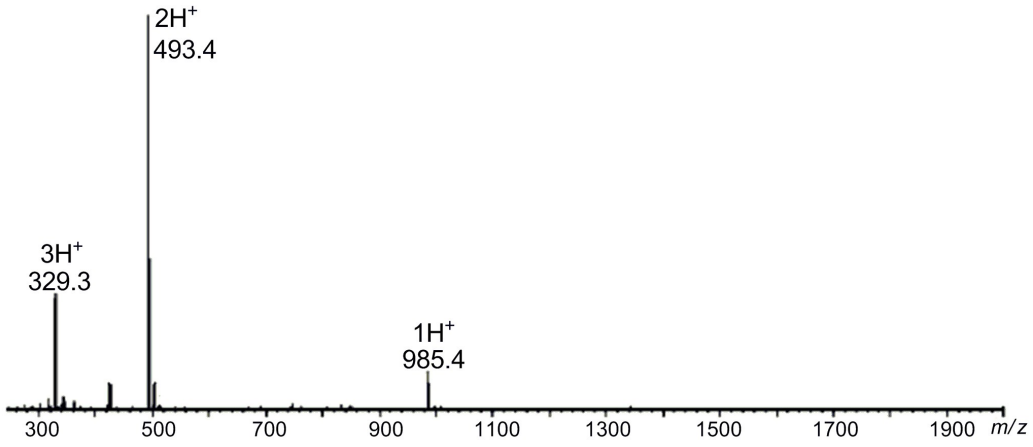

Fig. S20 ESI-MS spectrum of purified peptide **14.** The spectrum gave an observed mass of 984.7 Da (calculated 985.1 Da, average isotopes).

9. The NMR spectra

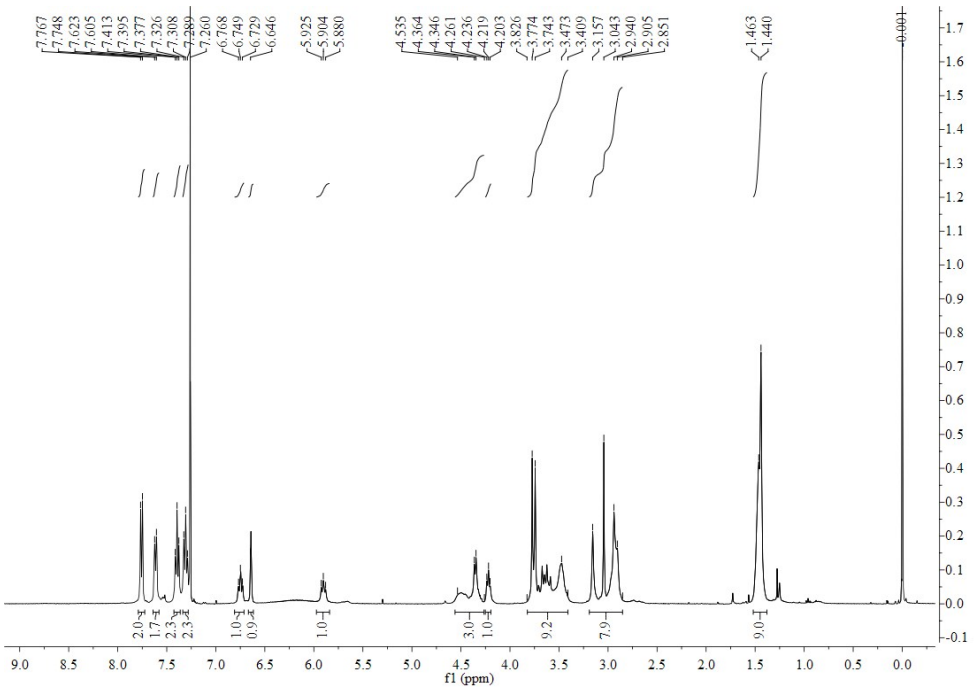

Fig. S21 The ^{1}H-NMR spectrum of compound **1**

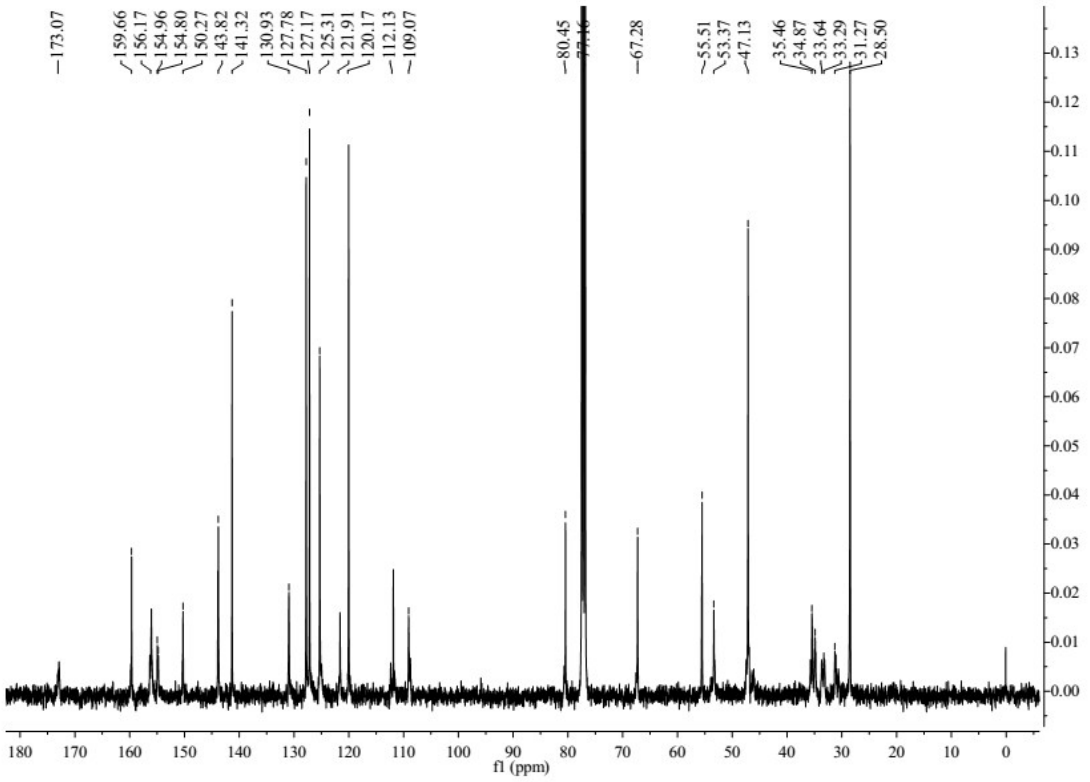

Fig. S22 The ^{13}C-NMR spectrum of compound **1**.

10. The RP-HPLC spectra

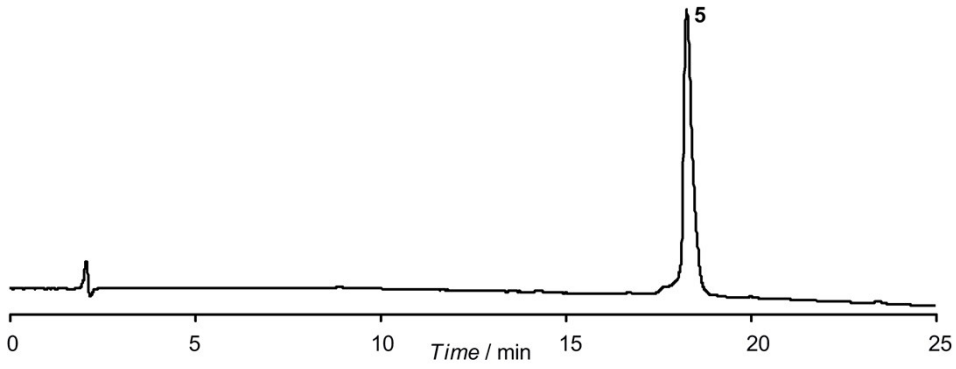

Fig. S23 Analytical RP-HPLC chromatogram of purified peptide **5**. HPLC conditions: a linear gradient of 25-65% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 20 min (25% for 2 min, then 25%-65% for 20 min) on a Vydac C4 (4.6 × 150 mm) column.

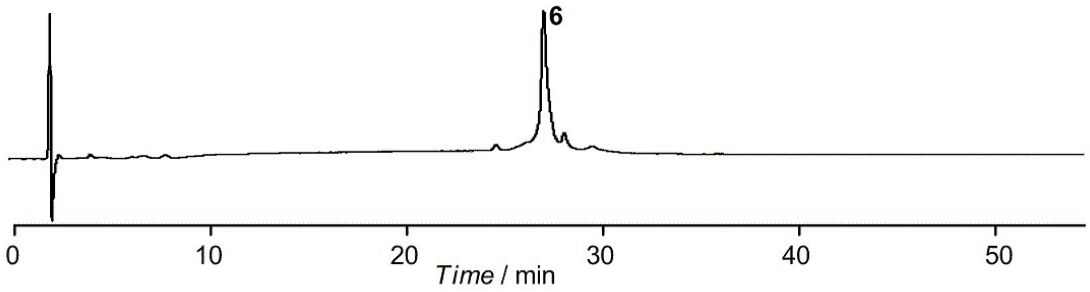

Fig. S24 Analytical RP-HPLC chromatogram of purified peptide **6**. HPLC conditions: a linear gradient of 20-50% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (20% for 2 min, then 20%-50% for 30 min) on a Vydac C4 (4.6 × 150 mm) column.

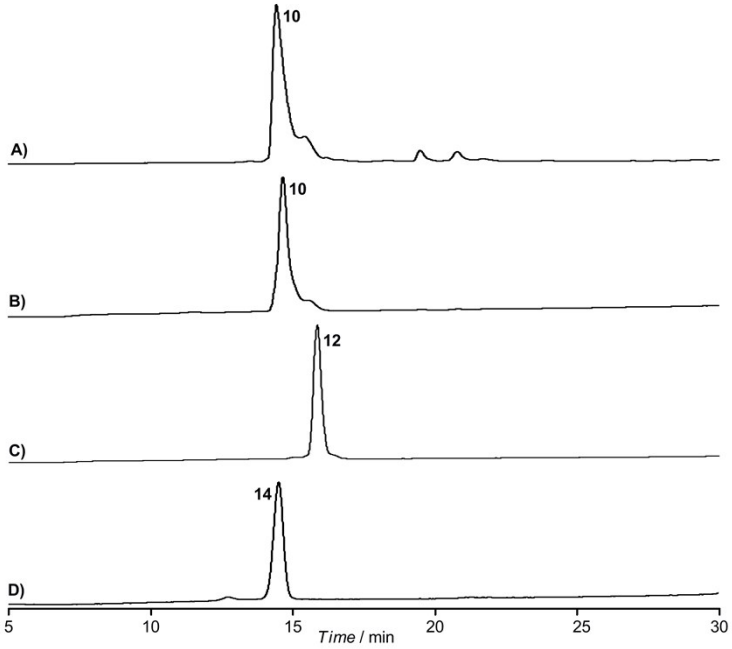

Fig. S25 Analytical RP-HPLC chromatogram of A) crude peptide **10**, B) purified peptide **10**, C) purified peptide **12** and D) purified peptide **14**. HPLC conditions: a linear gradient of 5-35% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-35% for 30 min) on a Vydac C18 (4.6 × 150 mm) column.

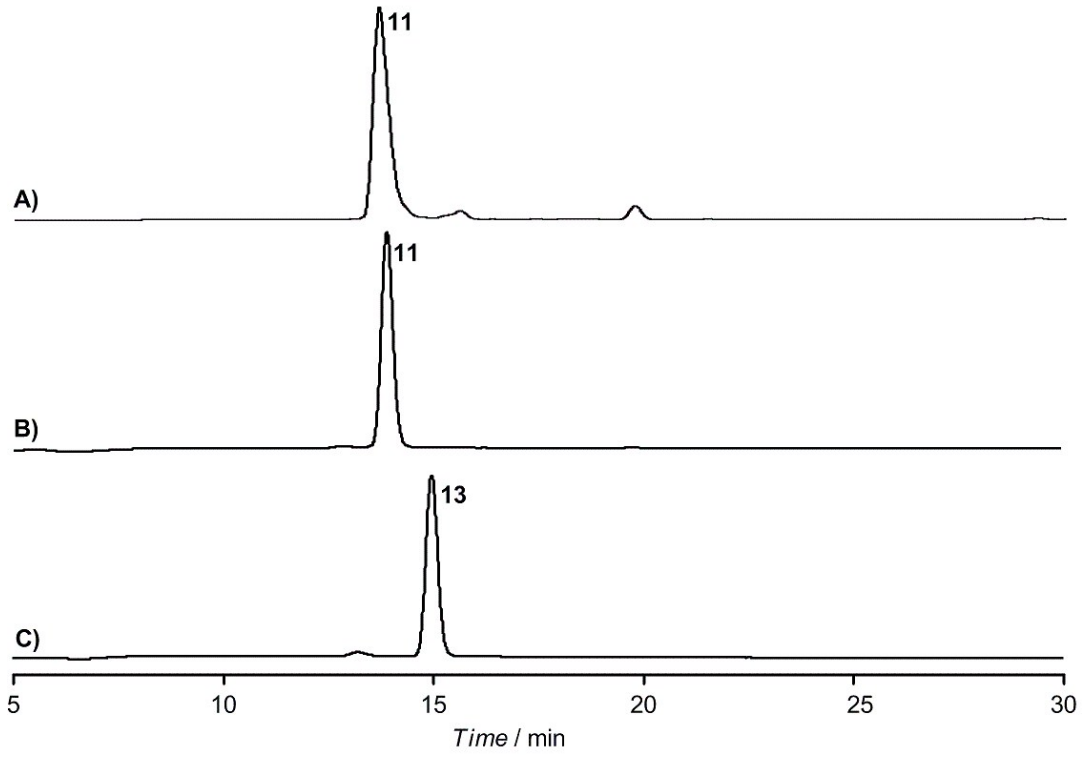

Fig. S26 Analytical RP-HPLC chromatogram of A) crude peptide **11**, B) purified peptide **11** and C) purified peptide **13**. HPLC conditions: a linear gradient of 5-35% acetonitrile (with 0.08-0.1% TFA) in water (with 0.1% TFA) over 30 min (5% for 2 min, then 5%-35% for 30 min) on a Vydac C18 (4.6 × 150 mm) column.

11. References

1. G. M. Fang, J. X. Wang and L. Liu, *Angew. Chem., Int. Ed.*, 2012, **51**, 10347.

2. J. X. Wang, G. M. Fang, Y. He, D. L. Qu, M. Yu, Z. Y. Hong and L. Liu, *Angew. Chem., Int. Ed.*, 2015, **54**, 2194.