

## Supporting Information

### Ring-closing metathesis of unprotected peptides in water

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## 1. General Information

### a. Materials

All reagents and solvents were obtained from Peptide Institute, Inc. (Osaka, Japan), FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Watanabe Chemical Industries, Ltd. (Hiroshima, Japan), Funakoshi Co., Ltd. (Tokyo, Japan), and Sigma-Aldrich Co. LLC. (St. Louis, MO).

### b. HPLC, MS and NMR

Preparative HPLC was carried out on a Shimadzu liquid chromatograph Model LC-8A (Kyoto, Japan) with a YMC-Pack ODS-A (30 x 250 mm) and the following solvent systems: 0.1% TFA in H<sub>2</sub>O and 0.1% TFA in CH<sub>3</sub>CN at a flow rate of 20 mL min<sup>-1</sup> with detection at 220 nm. Analytical HPLC was performed on a Shimadzu liquid chromatograph Model LC-10A (Kyoto, Japan) with a YMC-Pack ODS-A (4.6 x 150 mm) and the following solvent systems: 0.1% TFA in H<sub>2</sub>O and 0.1% TFA in CH<sub>3</sub>CN at a flow rate of 1 mL min<sup>-1</sup> (40 °C) with detection at 220 nm. Low resolution mass spectra (LRMS) were observed with an Agilent G1956B LC/MSD detector using an Agilent 1100 series HPLC system. <sup>1</sup>H NMR spectra were recorded on a JEOL-ECX400 spectrometer (Tokyo, Japan), as solutions in deuterated solvents as specified. Chemical shift values ( $\delta$ ) are given in parts per million (ppm) using residual solvent protons as internal standard.

### c. SPPS

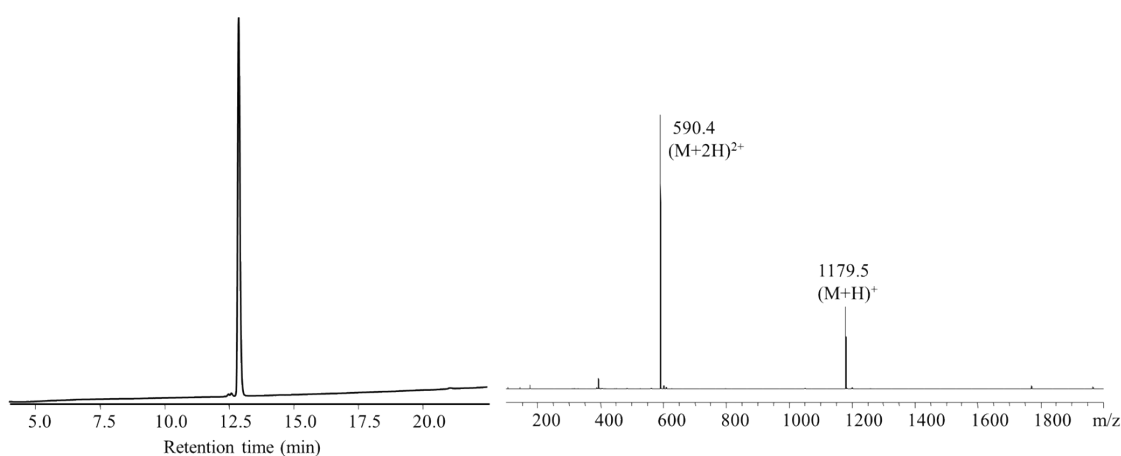
Automated peptide synthesis by Fmoc SPPS was performed on an ABI 433A peptide synthesizer. The peptide chain was elongated using the coupling protocol of Fmoc-amino acid/DIC/OxymaPure.<sup>[1]</sup> The acetyl capping was performed using acetic anhydride/NMP in the presence of DIEA after each coupling step. The following side-chain-protected amino acids were employed: Arg(Pbf), Cys(Trt), Glu(OtBu), His(Trt), Lys(Boc), Ser(*t*Bu), Thr(*t*Bu), Trp(Boc), Tyr(*t*Bu). Fmoc-Pgl (2 equiv) was manually condensed using DIC/OxymaPure (2/2 equiv) in NMP for 14–20 h. After HPLC purification and lyophilization of the target peptide, the identity was confirmed by MS and <sup>1</sup>H NMR (in case of a cyclic peptide), and the purity was determined to be higher than 95% by HPLC unless otherwise noted. RCM fundamentally affords peptide cis-trans mixture, which shows two different peaks in HPLC in all cases of this study. Each isomer, named sequentially as peptide **Xa/Xb** for cyclic peptide **X**, was isolated and analysed separately, while yield of RCM was calculated as the sum of both isomers.

## 2. Experimental Section

### a. Preparation of unprotected peptides for RCM

#### *Ac-Ser-His-Arg-Glu-Gly-Agl-Pro-Tyr-Agl-Val-NH<sub>2</sub> (1)*

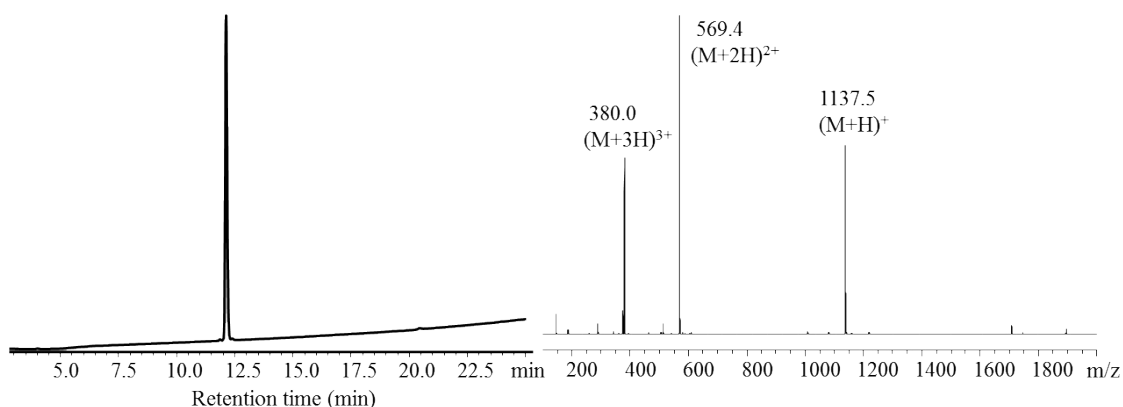
The peptide was assembled on a Rink amide resin (0.125 mmol) using Fmoc SPPS as described in general information (Fmoc-Xaa: 8 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H<sub>2</sub>O (v/v, 95/2.5/2.5) for 1.5 h to give a crude product, which was purified by preparative HPLC to yield the title peptide **1** (65 mg, 44%). Analytical HPLC:  $t_R = 12.9$  min (1–60% CH<sub>3</sub>CN/0.1% TFA for 25 min); LRMS (M+H) calcd for C<sub>53</sub>H<sub>79</sub>N<sub>16</sub>O<sub>15</sub> 1179.6, found 1179.5.



**Figure S1.** Analytical HPLC chromatogram and ESI-MS spectrum of peptide **1**.

#### *Ser-His-Arg-Glu-Gly-Agl-Pro-Tyr-Agl-Val-NH<sub>2</sub> (1')*

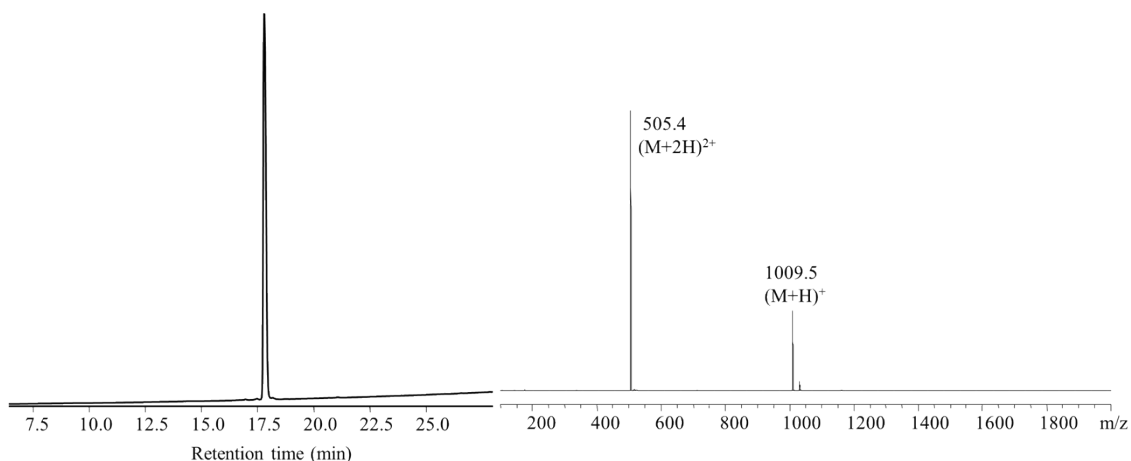
The peptide was assembled on a Rink amide resin (0.125 mmol) using Fmoc SPPS as described in general information (Fmoc-Xaa: 8 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H<sub>2</sub>O (v/v, 95/2.5/2.5) for 1.5 h to give a crude product, which was purified by preparative HPLC to yield the title peptide **1'** (107 mg, 75%). Analytical HPLC:  $t_R = 12.1$  min (1–60% CH<sub>3</sub>CN/0.1% TFA for 25 min); LRMS (M+H) calcd for C<sub>51</sub>H<sub>77</sub>N<sub>16</sub>O<sub>14</sub> 1137.6, found 1137.5.



**Figure S2.** Analytical HPLC chromatogram and ESI-MS spectrum of peptide **1'**.

#### *2,7-Agl-octreotide (3)*

The peptide was assembled on an H-Thr(*t*Bu)-ol-Trt(2-Cl)-resin (0.25 mmol) using Fmoc SPPS as described in general information (Fmoc-Xaa: 4 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H<sub>2</sub>O (v/v, 95/2.5/2.5) for 1.5 h to give a crude product, which was purified by preparative HPLC to yield the title peptide **3** (181 mg, 72%). Analytical HPLC:  $t_R$  = 17.8 min (1–60% CH<sub>3</sub>CN/0.1% TFA for 25 min); LRMS (M+H) calcd for C<sub>53</sub>H<sub>73</sub>N<sub>10</sub>O<sub>10</sub> 1009.6, found 1009.5.

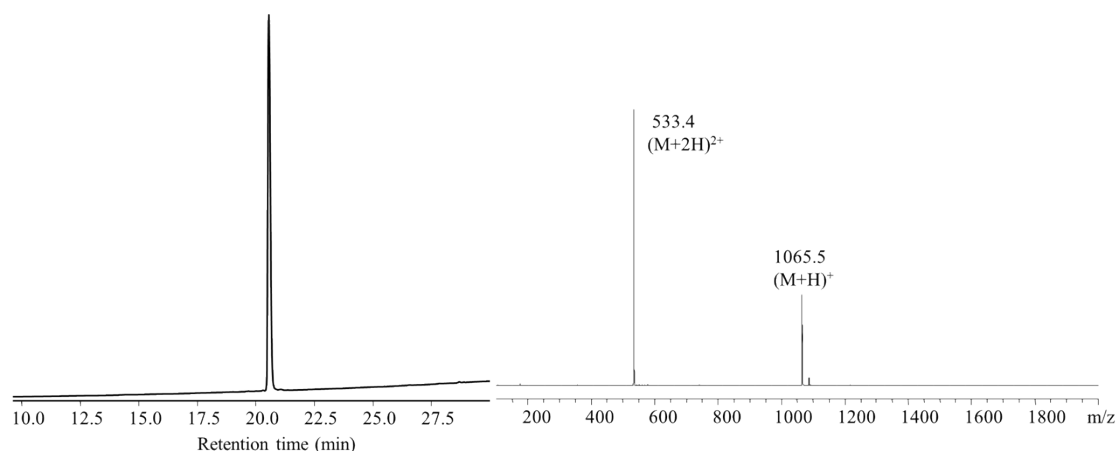


**Figure S3.** Analytical HPLC chromatogram and ESI-MS spectrum of peptide **3**.

#### *2,7-Pgl-octreotide (4)*

The peptide was assembled on an H-Thr(*t*Bu)-ol-Trt(2-Cl)-resin (0.1 mmol) using Fmoc SPPS as described in general information (Fmoc-Xaa: 10 equiv, Fmoc-Pgl: 2 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H<sub>2</sub>O (v/v, 95/5/5) for 1.5 h to give a crude product, which was purified by preparative HPLC to yield the title peptide **4** (62 mg, 58%). Analytical HPLC:  $t_R$  = 20.5 min (1–60% CH<sub>3</sub>CN/0.1% TFA for

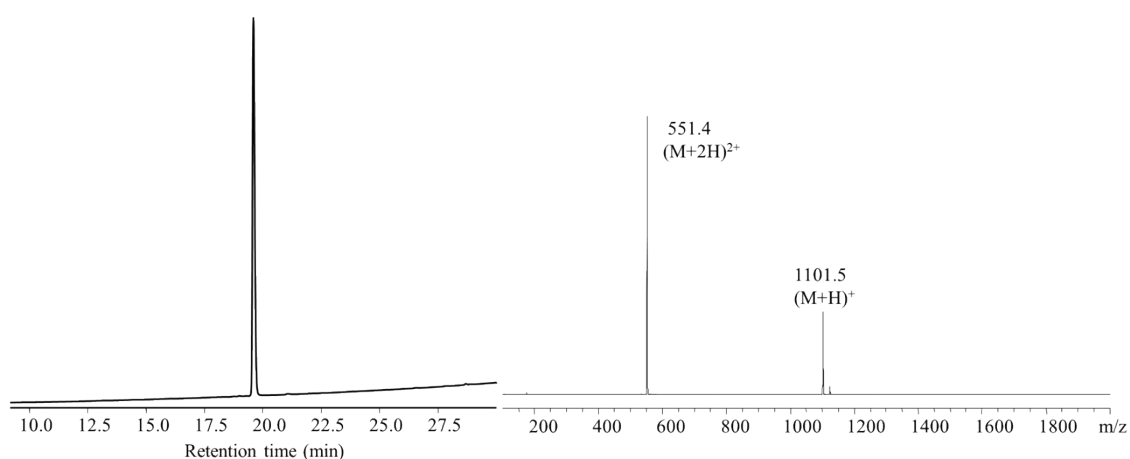
25 min); LRMS (M+H) calcd for C<sub>57</sub>H<sub>81</sub>N<sub>10</sub>O<sub>10</sub> 1065.6, found 1065.5.



**Figure S4.** Analytical HPLC chromatogram and ESI-MS spectrum of peptide **4**.

#### *2,7-Sac-octreotide (5)*

The peptide was assembled on an H-Thr(*t*Bu)-ol-Trt(2-Cl)-resin (0.125 mmol) using Fmoc SPPS as described in general information (Fmoc-Xaa: 8 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H<sub>2</sub>O (v/v, 95/5/5) for 1 h to give a crude product. Then, the obtained peptide dissolved in H<sub>2</sub>O (5 mL) and CH<sub>3</sub>CN (3 mL) was reacted with allyl bromide (21.3 μL, 0.252 mmol) and 1 M NaOH (360 μL, 0.360 mmol) at room temperature. After 10 min, the reaction mixture was quenched with 50 % AcOH aq. (1 mL) and the solution was subjected to preparative HPLC to yield the title peptide **5** (89 mg, 56%). Analytical HPLC:  $t_R = 19.6$  min (1–60% CH<sub>3</sub>CN/0.1% TFA for 25 min); LRMS (M+H) calcd for C<sub>55</sub>H<sub>77</sub>N<sub>10</sub>O<sub>10</sub>S<sub>2</sub> 1101.5, found 1101.5.

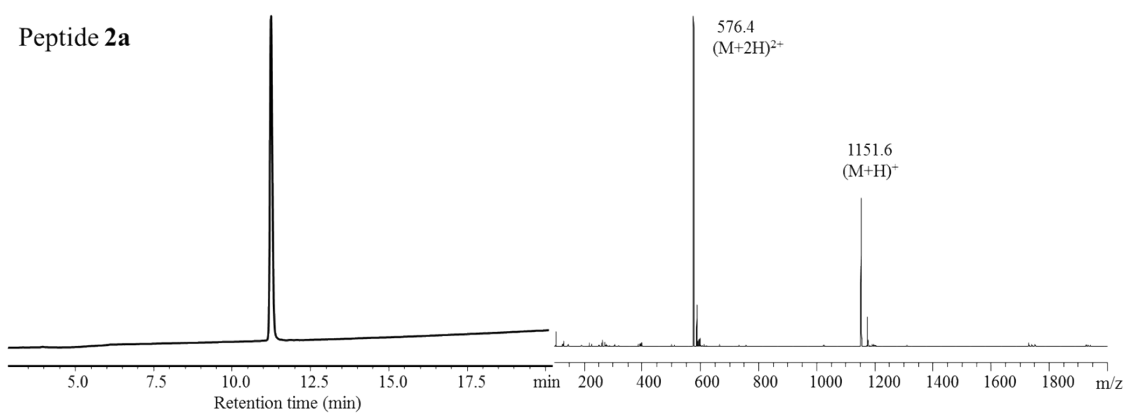


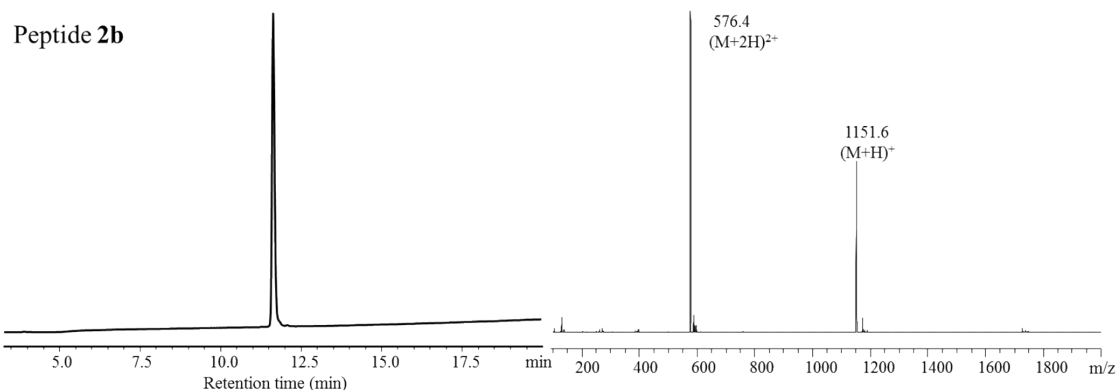
**Figure S5.** Analytical HPLC chromatogram and ESI-MS spectrum of peptide **5**.

## b. Synthesis of cyclic peptides using RCM

### *Cyclised Ac-Ser-His-Arg-Glu-Gly-Agl-Pro-Tyr-Agl-Val-NH<sub>2</sub> (2)*

Peptide **1** (20.0 mg, 17.0  $\mu\text{mol}$ ) and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (1.43 g, 7.04 mmol) were dissolved in 6.0 M  $\text{Gn} \cdot \text{HCl}$ , 0.2 M phosphate (pH 7) (3.5 mL). To the mixture was added a solution of AquaMet (4.3 mg, 5.35  $\mu\text{mol}$ ) in  $\text{H}_2\text{O}$  (0.9 mL). After stirring for 2 h at 60  $^\circ\text{C}$ , the reaction mixture was quenched by TFA, and then subjected to preparative HPLC to obtain **2** (10.9 mg, 56%, **2a:2b** = 40:60 determined by HPLC). Peptide **2a**:  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 8.60 (1H, s), 7.29 (1H, s), 7.14 (2H, d,  $J$  = 8.2 Hz), 6.84 (2H, d,  $J$  = 8.2 Hz), 5.59-5.49 (1H, m), 5.44-5.34 (1H, m), 4.61 (1H, t,  $J$  = 5.0 Hz), 4.39-4.30 (2H, m), 4.25 (1H, dd,  $J$  = 8.2 and 6.0 Hz), 4.16-4.08 (2H, m), 3.92 (2H, AB quartet,  $J$  = 16.9 Hz), 3.80 (2H, d,  $J$  = 5.5 Hz), 3.73-3.64 (1H, m), 3.51-3.41 (1H, m), 3.37-3.26 (2H, m), 3.23-3.11 (3H, m), 2.77 (1H, dd,  $J$  = 14.2 and 11.4 Hz), 2.68-2.52 (2H, m), 2.47-2.26 (4H, m), 2.13-1.93 (7H, m), 1.93-1.67 (4H, m), 1.66-1.54 (2H, m), 1.27-1.17 (1H, m), 0.96 (6H, d,  $J$  = 6.4 Hz); Analytical HPLC:  $t_{\text{R}}$  = 11.2 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min); LRMS (M+H) calcd for  $\text{C}_{51}\text{H}_{75}\text{N}_{16}\text{O}_{15}$  1151.6, found 1151.6. Peptide **2b**:  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 8.61 (1H, d,  $J$  = 1.4 Hz), 7.28 (1H, s), 7.10 (2H, d,  $J$  = 8.2 Hz), 6.85 (2H, d,  $J$  = 8.7 Hz), 5.54-5.43 (1H, m), 5.35-5.24 (1H, m), 4.66 (1H, dd,  $J$  = 10.5 and 4.1 Hz), 4.41-4.29 (3H, m), 4.22 (1H, dd,  $J$  = 8.2 and 6.0 Hz), 4.16 (1H, d,  $J$  = 7.3 Hz), 4.02 (1H, dd,  $J$  = 8.7 and 6.4 Hz), 3.91 (2H, AB quartet,  $J$  = 16.9 Hz), 3.79 (2H, d,  $J$  = 5.5 Hz), 3.72-3.63 (1H, m), 3.57-3.46 (1H, m), 3.36-3.25 (2H, m), 3.22-3.13 (3H, m), 3.04-2.86 (2H, m), 2.82 (1H, dd,  $J$  = 14.2 and 11.0 Hz), 2.42-2.32 (3H, m), 2.15-1.96 (7H, m), 1.96-1.68 (5H, m), 1.65-1.55 (2H, m), 1.49-1.38 (1H, m), 0.97 (6H, d,  $J$  = 6.9 Hz); Analytical HPLC:  $t_{\text{R}}$  = 11.6 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min); LRMS (M+H) calcd for  $\text{C}_{51}\text{H}_{75}\text{N}_{16}\text{O}_{15}$  1151.6, found 1151.6.

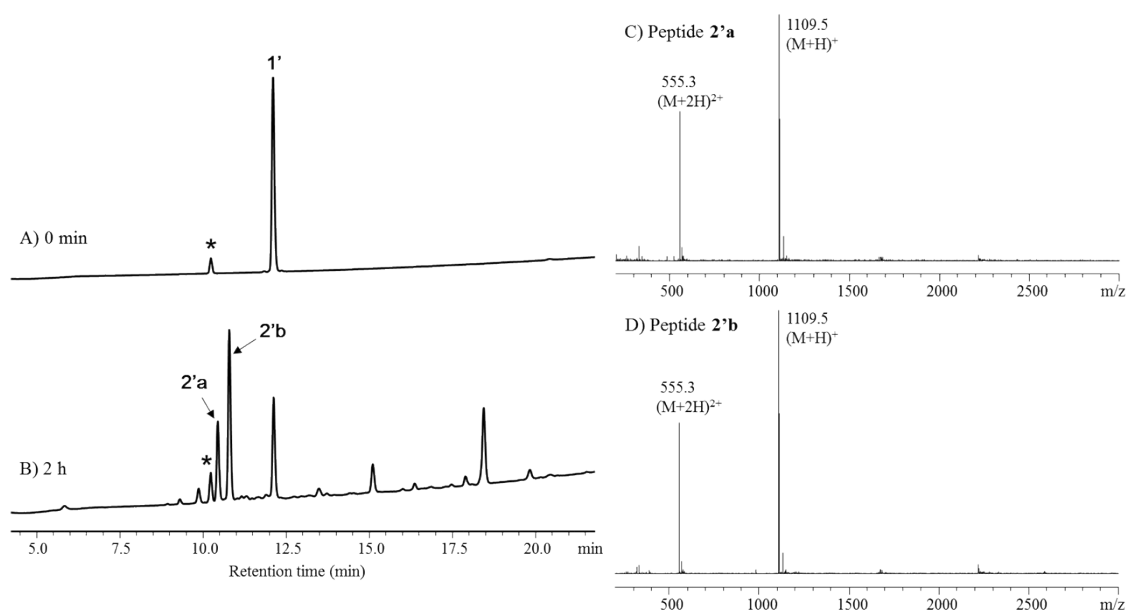




**Figure S6.** Analytical HPLC chromatograms and ESI-MS spectra of peptide **2a** and **2b**.

*Cyclised Ser-His-Arg-Glu-Gly-Agl-Pro-Tyr-Agl-Val-NH<sub>2</sub> (2')*

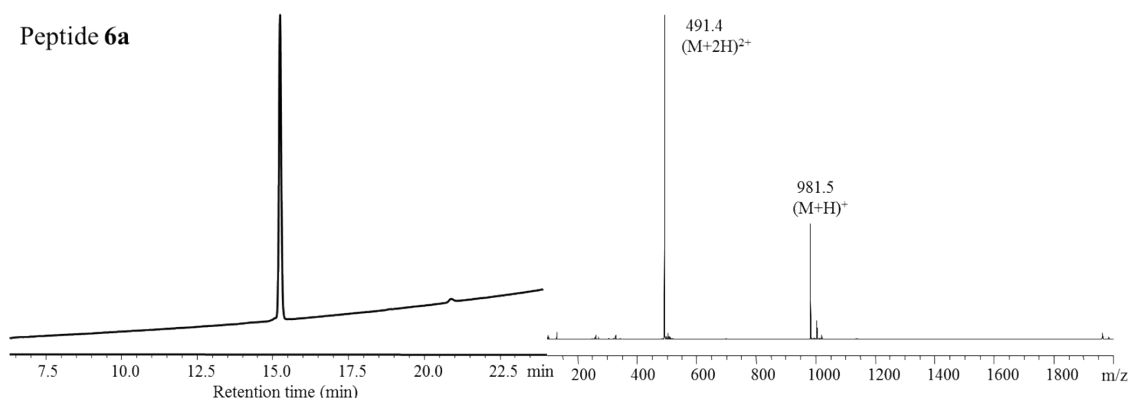
Peptide **1'** (1.1 mg, 1.0  $\mu$ mol) and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (81.3 mg, 400  $\mu$ mol) were dissolved in 0.1 M HCl aq. (0.8 mL). To the mixture was added a solution of AquaMet (0.25 mg, 0.3  $\mu$ mol) in  $\text{H}_2\text{O}$  (0.2 mL). After stirring for 2 h at 60  $^\circ\text{C}$ , the reaction mixture was monitored by analytical HPLC (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min). Peptide **2**; LRMS (M+H) calcd for  $\text{C}_{49}\text{H}_{73}\text{N}_{16}\text{O}_{14}$  1109.6, found 1109.5.



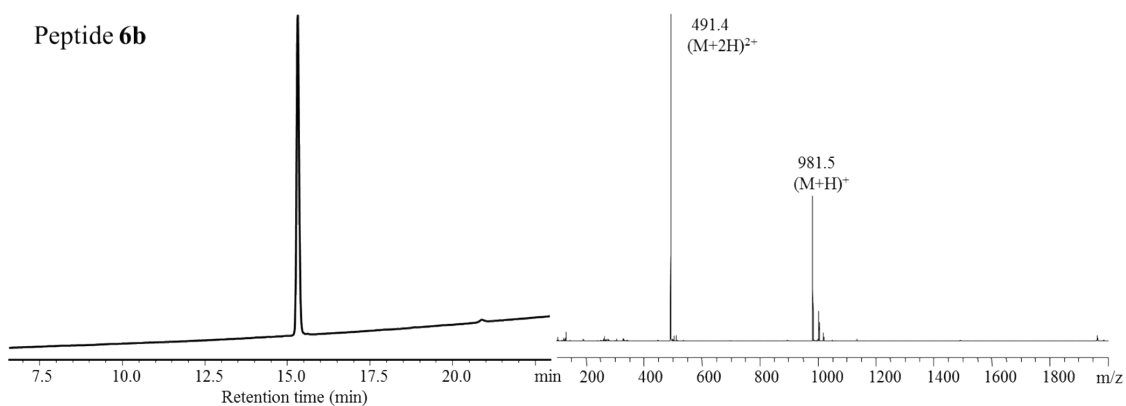
**Figure S7.** Analytical HPLC chromatograms of RCM of model peptide **1'** and ESI-MS spectra of peptide **2'a** and **2'b**: A) reaction mixture ( $t = 0$  h), B) reaction mixture ( $t = 2$  h), C) ESI-MS spectrum of peptide **2'a**, D) ESI-MS spectrum of peptide **2'b**. \* indicates internal standard. [RCM fundamentally affords cis-trans isomers, which are sequentially named as peptide **2'a/2'b** for cyclic peptide **2'**.]

### Cyclised 2,7-Agl-octreotide (**6**)

Peptide **3** (10.0 mg, 9.91  $\mu\text{mol}$ ) and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (805 mg, 3.96 mmol) were dissolved in 0.1 M HCl aq. (7.93 mL). To the mixture was added a solution of AquaMet (2.4 mg, 2.97  $\mu\text{mol}$ ) in  $\text{H}_2\text{O}$  (1.98 mL). After stirring for 2 h at 60  $^\circ\text{C}$ , the reaction mixture was subjected to preparative HPLC to obtain **6** (3.5 mg, 36%, **6a:6b** = 63:37 determined by HPLC). Peptide **6a**:  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 7.55-7.34 (8H, m), 7.30-7.23 (5H, m), 7.20 (1H, t,  $J$  = 7.3 Hz), 7.14 (1H, s), 5.37-5.26 (1H, m), 5.14-5.04 (1H, m), 4.62 (1H, dd,  $J$  = 9.2 and 6.4 Hz), 4.42 (1H, dd,  $J$  = 11.4 and 2.7 Hz), 4.27-4.18 (2H, m), 4.12 (1H, dd,  $J$  = 11.0 Hz and 5.5 Hz), 4.10-4.03 (1H, m), 4.01 (1H, d,  $J$  = 4.6 Hz), 4.00-3.92 (2H, m), 3.87-3.80 (1H, m), 3.71 (1H, dd,  $J$  = 11.4 and 5.0 Hz), 3.62 (1H, dd,  $J$  = 11.4 and 7.3 Hz), 3.22-3.08 (2H, m), 3.07-2.89 (3H, m), 2.80 (1H, dd,  $J$  = 13.7 and 5.5 Hz), 2.75-2.55 (3H, m) 2.29-2.01 (3H, m), 1.62-1.50 (1H, m), 1.38-1.25 (5H, m), 1.25-1.07 (4H, m), 0.57-0.43 (1H, m), 0.35-0.22 (1H, m); Analytical HPLC:  $t_{\text{R}}$  = 15.2 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min); LRMS (M+H) calcd for  $\text{C}_{51}\text{H}_{69}\text{N}_{10}\text{O}_{10}$  981.5, found 981.5. Peptide **6b**:  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 7.55 (1H, d,  $J$  = 7.8 Hz), 7.50 (1H, d,  $J$  = 8.2 Hz), 7.45-7.32 (6H, m), 7.31-7.17 (6H, m), 7.14 (1H, s), 5.48-5.37 (1H, m), 5.11-5.02 (1H, m), 4.65 (1H, dd,  $J$  = 8.7 Hz and 6.4 Hz), 4.46 (1H, dd,  $J$  = 10.1 Hz and 4.1 Hz), 4.28-4.20 (2H, m), 4.15 (1H, dd,  $J$  = 8.7 Hz and 5.5 Hz), 4.08-3.98 (3H, m), 3.98-3.90 (1H, m), 3.87-3.80 (1H, m), 3.70 (1H, dd,  $J$  = 11.9 Hz and 5.0 Hz), 3.60 (1H, dd,  $J$  = 11.9 Hz and 7.3 Hz), 3.20-2.87 (5H, m), 2.82 (1H, dd,  $J$  = 14.2 Hz and 6.0 Hz), 2.75-2.57 (2H, m), 2.53-2.42 (1H, m), 2.37-2.17 (3H, m), 1.59-1.46 (1H, m), 1.38-1.23 (5H, m), 1.23-1.07 (4H, m), 0.59-0.45 (1H, m), 0.41-0.27 (1H, m); Analytical HPLC:  $t_{\text{R}}$  = 15.3 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min); LRMS (M+H) calcd for  $\text{C}_{51}\text{H}_{69}\text{N}_{10}\text{O}_{10}$  981.5, found 981.5.



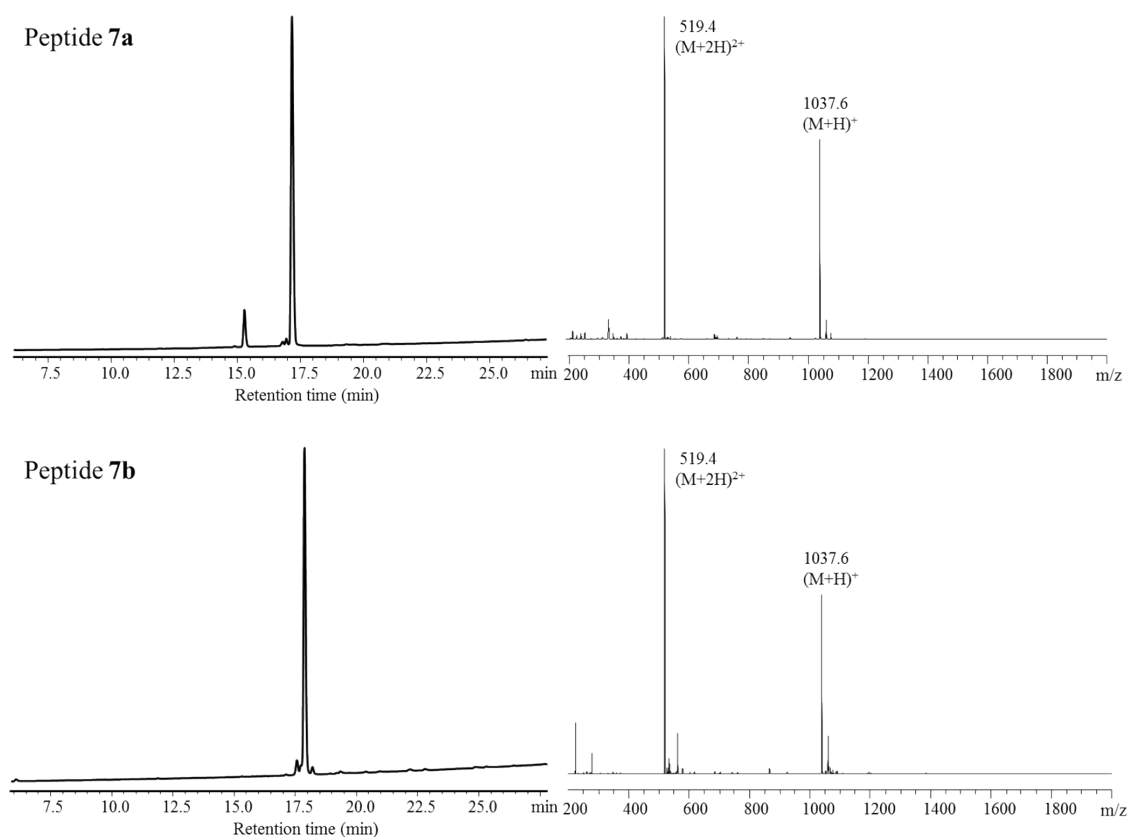




**Figure S8.** Analytical HPLC chromatograms and ESI-MS spectra of peptide **6a** and **6b**.

#### *Cyclised 2,7-Pgl-octreotide (7)*

Peptide **4** (15.0 mg, 14.1  $\mu$ mol) and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (1.15 g, 5.64 mmol) were dissolved in 0.1 M HCl aq. (22.6 mL). To the mixture was added a solution of AquaMet (3.4 mg, 4.23  $\mu$ mol) in  $\text{H}_2\text{O}$  (5.6 mL). After stirring for 30 min at 60  $^\circ\text{C}$ , the reaction mixture was subjected to preparative HPLC to obtain **7** (7.6 mg, 52%, **7a:7b** = 68:32 determined by HPLC). Peptide **7a**:  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 7.58(1H, d,  $J$  = 7.8 Hz), 7.48 (1H, d,  $J$  = 8.2 Hz), 7.43-7.28 (6H, m), 7.28-7.19 (5H, m), 7.16 (1H, t,  $J$  = 7.3 Hz), 7.11 (1H, s), 5.53-5.41 (1H, m), 5.35-5.23 (1H, m), 4.72-4.66 (1H, m), 4.59 (1H, dd,  $J$  = 10.5 Hz and 4.1 Hz), 4.44 (1H, dd,  $J$  = 10.1 Hz and 6.4 Hz), 4.35-4.28 (1H, m), 4.25-4.16 (2H, m), 4.13-3.98 (3H, m), 3.88-3.81 (1H, m), 3.70 (1H, dd,  $J$  = 11.4 Hz and 5.5 Hz), 3.62 (1H, dd,  $J$  = 11.4 Hz and 6.9 Hz), 3.23-3.13 (1H, m), 3.11-2.90 (5H, m), 2.75-2.57 (2H, m), 2.11-1.96(1H, m), 1.94-1.67 (5H, m), 1.61-1.40 (3H, m), 1.40-1.26 (4H, m), 1.26-1.17 (4H, m), 1.14 (3H, d,  $J$  = 6.4 Hz), 1.02-0.90 (2H, m), 0.64-0.50 (1H, m), 0.49-0.35 (1H, m); Analytical HPLC:  $t_{\text{R}}$  = 17.2 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min), purity was 88%; LRMS (M+H) calcd for  $\text{C}_{55}\text{H}_{77}\text{N}_{10}\text{O}_{10}$  1037.6, found 1037.6. Peptide **7b**:  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 7.52 (1H, d,  $J$  = 8.2 Hz), 7.48 (1H, d,  $J$  = 8.2 Hz), 7.43-7.34 (3H, m), 7.34-7.20 (6H, m), 7.19-7.10 (3H, m), 7.04 (1H, s), 5.45-5.35 (1H, m), 5.34-5.23 (1H, m), 4.52 (1H, dd,  $J$  = 8.2 Hz and 6.9 Hz), 4.44-4.38 (1H, m), 4.34 (1H, dd,  $J$  = 10.1 Hz and 4.6 Hz), 4.16-4.03 (5H, m), 4.03-3.95 (1H, m), 3.86-3.78 (1H, m), 3.69 (1H, dd,  $J$  = 11.4 Hz and 5.5 Hz), 3.58 (1H, dd,  $J$  = 11.4 Hz and 7.3 Hz), 3.19 (1H, dd,  $J$  = 13.3 Hz and 6.0 Hz), 3.07-2.92 (3H, m), 2.87 (1H, dd,  $J$  = 13.3 Hz and 8.7 Hz), 2.84-2.74 (3H, m), 2.07-1.66 (6H, m), 1.61-1.50 (1H, m), 1.50-1.26 (7H, m), 1.26-1.16 (3H, m), 1.11 (3H, d,  $J$  = 6.4 Hz), 1.00-0.89 (1H, m), 0.89-0.68 (3H, m); Analytical HPLC:  $t_{\text{R}}$  = 17.9 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min), purity was 91%; LRMS (M+H) calcd for  $\text{C}_{55}\text{H}_{77}\text{N}_{10}\text{O}_{10}$  1037.6, found 1037.6.

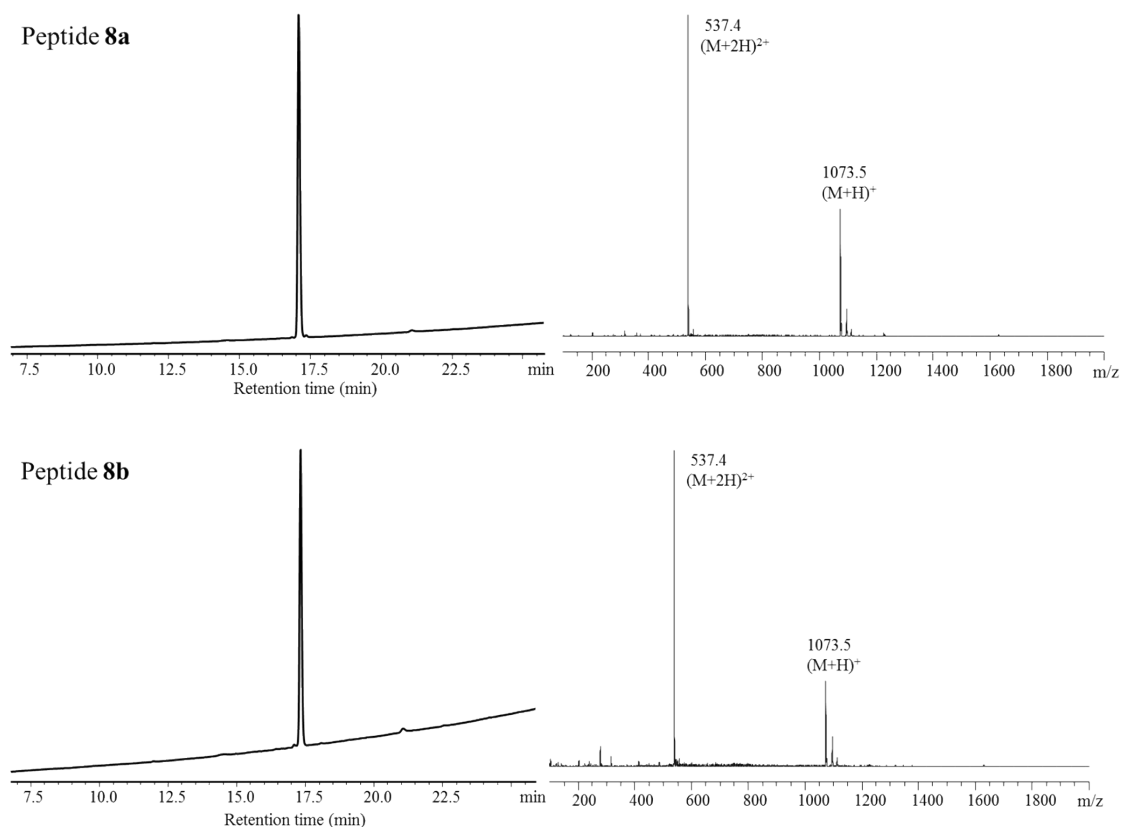


**Figure S9.** Analytical HPLC chromatograms and ESI-MS spectra of peptide **7a** and **7b**.

#### *Cyclised 2,7-Sac-octreotide (8)*

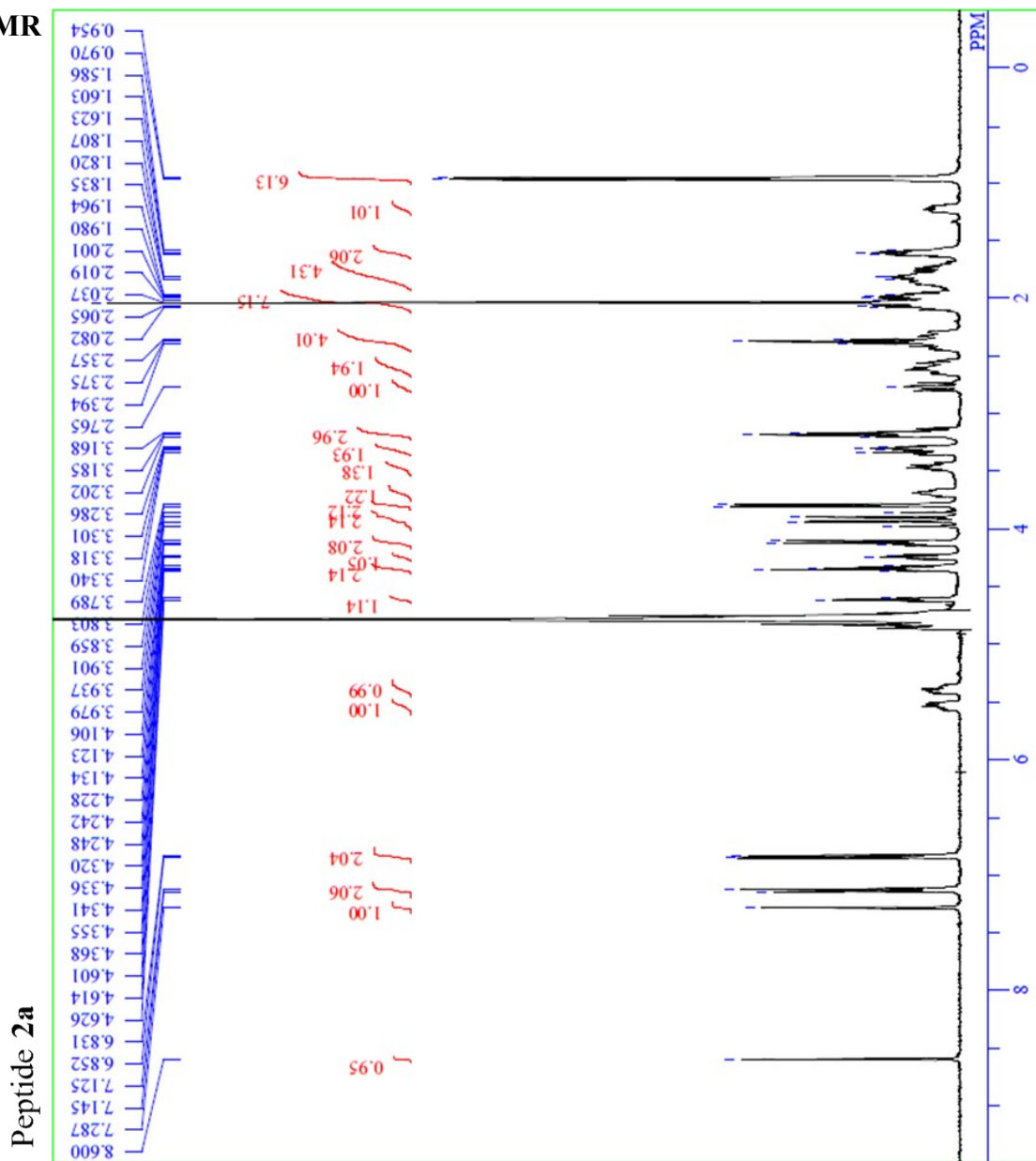
Peptide **5** (10.0 mg, 9.08  $\mu\text{mol}$ ) and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (738 mg, 3.63 mmol) were dissolved in 0.1 M HCl aq. (14.6 mL). To the mixture was added a solution of AquaMet (2.2 mg, 2.72  $\mu\text{mol}$ ) in  $\text{H}_2\text{O}$  (3.6 mL). After stirring for 30 min at 60  $^\circ\text{C}$ , the reaction mixture was subjected to preparative HPLC to obtain **8** (6.1 mg, 63%, **8a:8b** = 77:23 determined by HPLC). Peptide **8a**:  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 7.58 (1H, d,  $J$  = 7.8 Hz), 7.49 (1H, d,  $J$  = 7.8 Hz), 7.42-7.22 (9H, m), 7.20-7.13 (3H, m), 7.10 (1H, s), 5.61-5.49 (2H, m), 4.69-4.60 (2H, m), 4.48 (1H, dd,  $J$  = 9.2 Hz and 6.4 Hz), 4.38 (1H, dd,  $J$  = 8.2 Hz and 6.0 Hz), 4.26-4.15 (2H, m), 4.09-3.98 (2H, m), 3.97-3.89 (1H, m), 3.89-3.82 (1H, m), 3.71 (1H, dd,  $J$  = 11.4 Hz and 5.0 Hz), 3.62 (1H, dd,  $J$  = 11.4 Hz and 6.9 Hz), 3.26-3.12 (2H, m), 3.12-2.91 (9H, m), 2.82-2.70 (3H, m), 2.66 (1H, dd,  $J$  = 13.7 Hz and 6.0 Hz), 2.45 (1H, dd,  $J$  = 13.7 Hz and 8.2 Hz), 1.66-1.54 (1H, m), 1.46-1.27 (3H, m), 1.21 (3H, d,  $J$  = 6.4 Hz), 1.15 (3H, d,  $J$  = 6.4 Hz), 0.81-0.58 (2H, m); Analytical HPLC:  $t_R$  = 17.1 min (1–60%  $\text{CH}_3\text{CN}/0.1\%$  TFA for 25 min); LRMS (M+H) calcd for  $\text{C}_{53}\text{H}_{73}\text{N}_{10}\text{O}_{10}\text{S}_2$  1073.5, found 1073.5. Peptide **8b**:  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  = 7.62 (1H, d,  $J$  = 7.8 Hz), 7.49 (1H, d,  $J$  = 8.2 Hz), 7.40-7.28 (6H, m), 7.27-7.20 (5H, m), 7.20-7.15 (1H, m), 7.14 (1H, s), 5.71-

5.62 (1H, m), 5.61-5.52 (1H, m), 5.10 (1H, dd,  $J = 8.7$  Hz and  $6.9$  Hz), 4.54 (1H, dd,  $J = 10.5$  Hz and  $6.0$  Hz), 4.33 (1H, d,  $J = 6.9$  Hz), 4.29-4.21 (1H, m), 4.10-4.02 (2H, m), 3.94-3.88 (1H, m), 3.85-3.73 (2H, m), 3.69 (1H, dd,  $J = 11.4$  Hz and  $6.9$  Hz), 3.28-3.11 (3H, m), 3.11-2.90 (6H, m), 2.88-2.74 (2H, m), 2.73-2.54 (3H, m), 2.47 (1H, dd,  $J = 14.2$  Hz and  $9.6$  Hz), 2.22 (1H, dd,  $J = 14.2$  Hz and  $6.4$  Hz), 1.70-1.58 (1H, m), 1.35-1.19 (7H, m), 1.19-1.10 (4H, m), 0.60-0.47 (1H, m), 0.43-0.30 (1H, m); Analytical HPLC:  $t_R = 17.3$  min (1–60% CH<sub>3</sub>CN/0.1% TFA for 25 min); LRMS (M+H) calcd for C<sub>53</sub>H<sub>73</sub>N<sub>10</sub>O<sub>10</sub>S<sub>2</sub> 1073.5, found 1073.5.

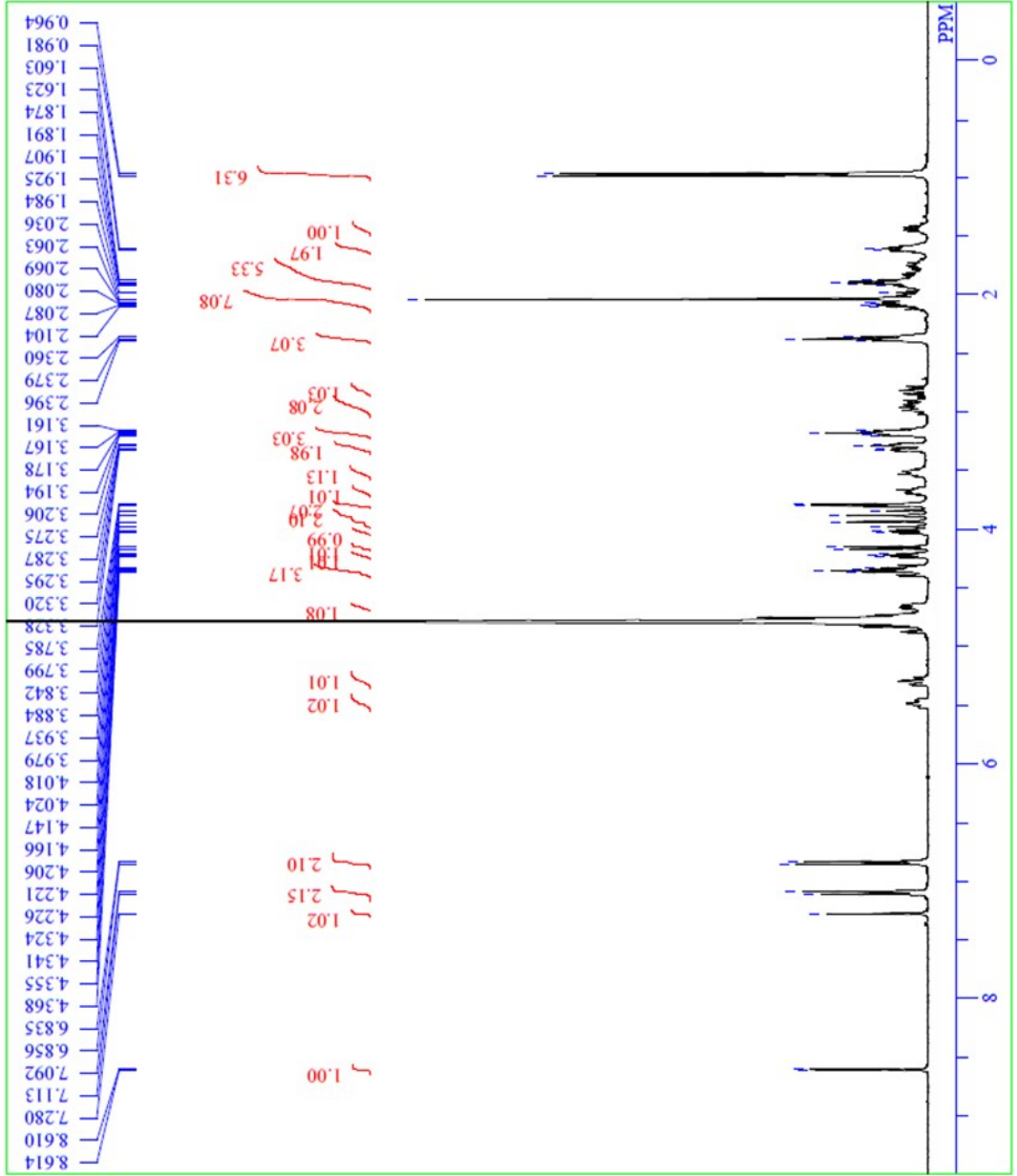


**Figure S10.** Analytical HPLC chromatograms and ESI-MS spectra of peptide **8a** and **8b**.

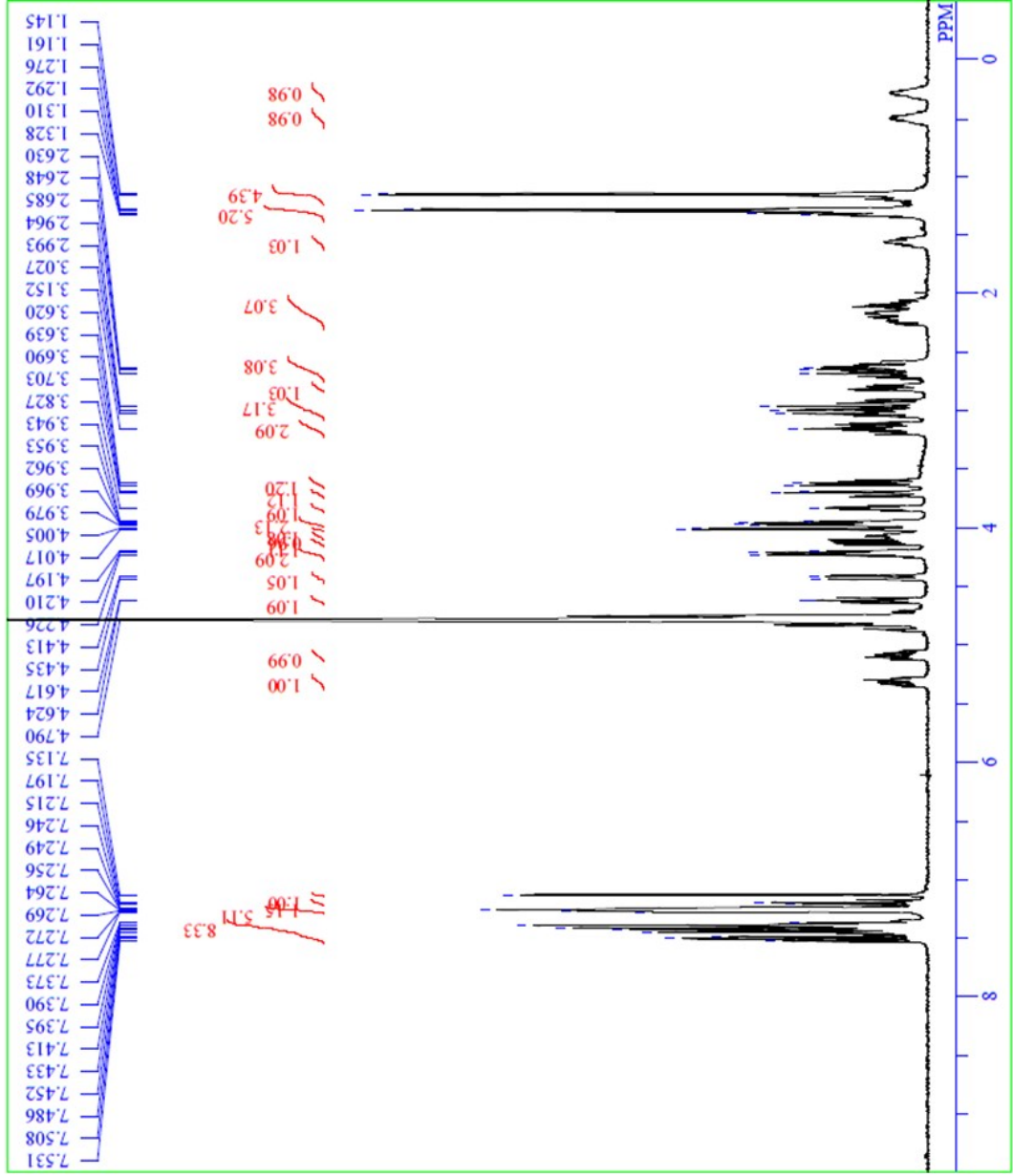
### 3. NMR



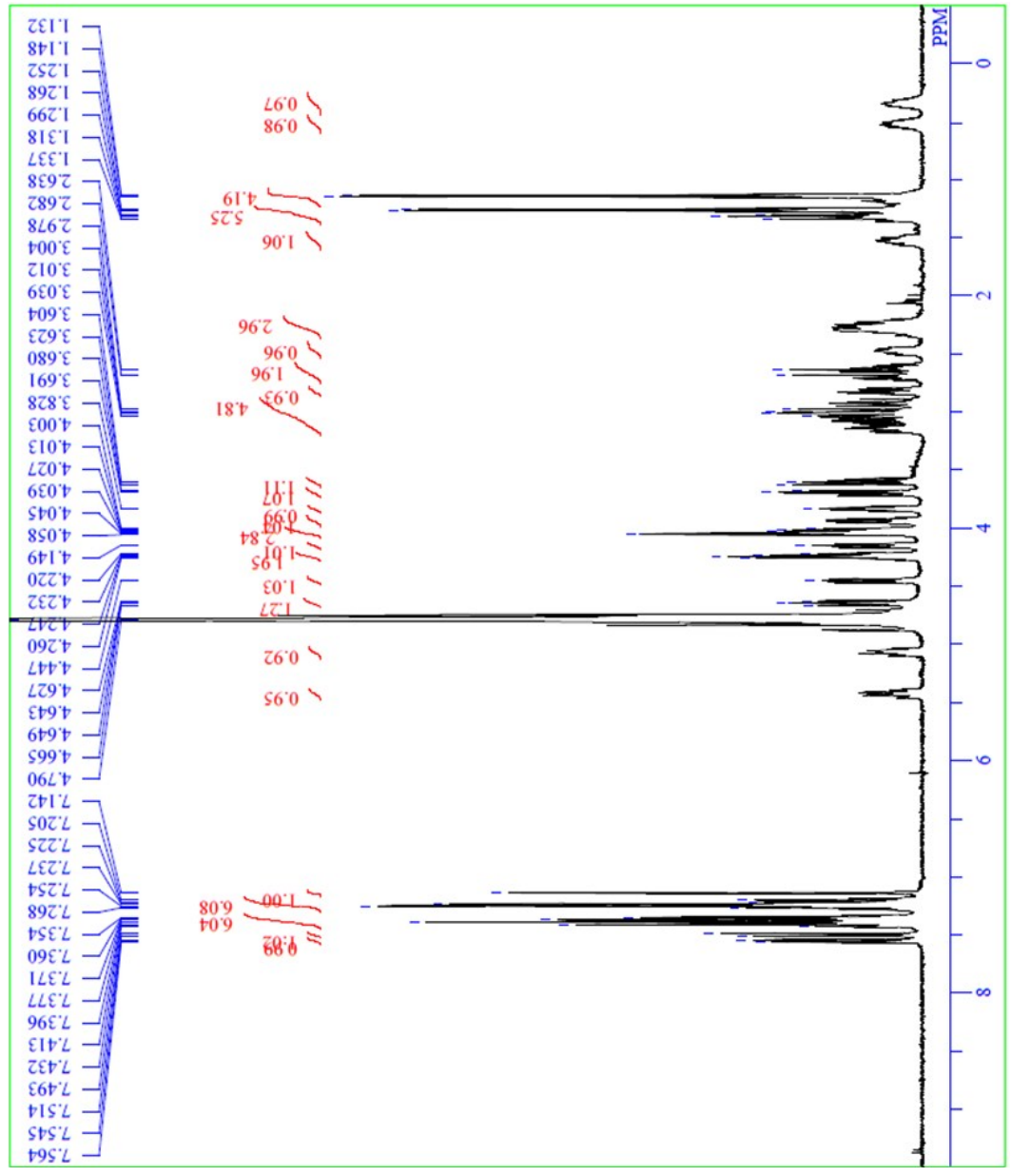
Peptide 2b



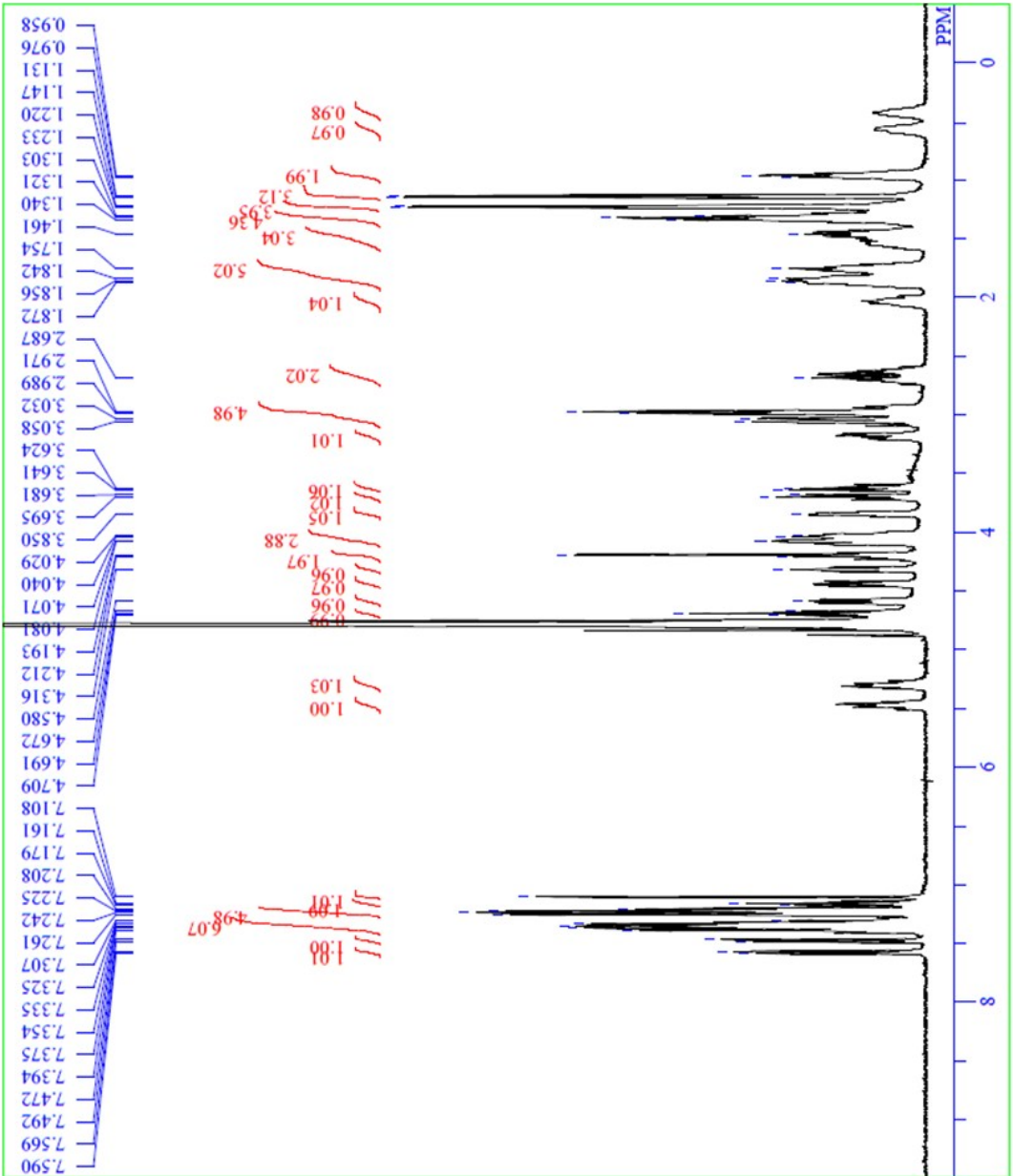
Peptide 6a



Peptide 6b

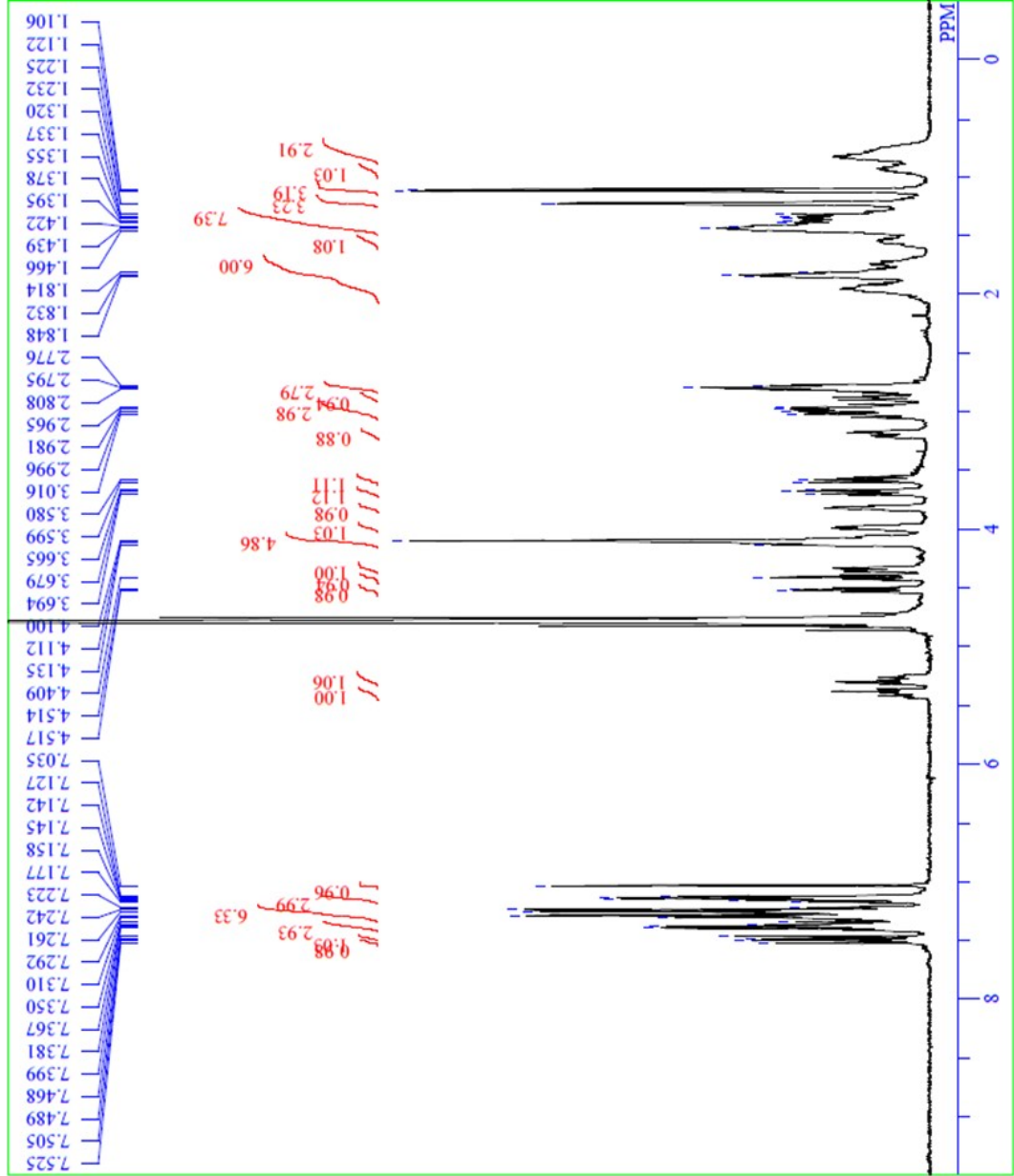


Peptide 7a

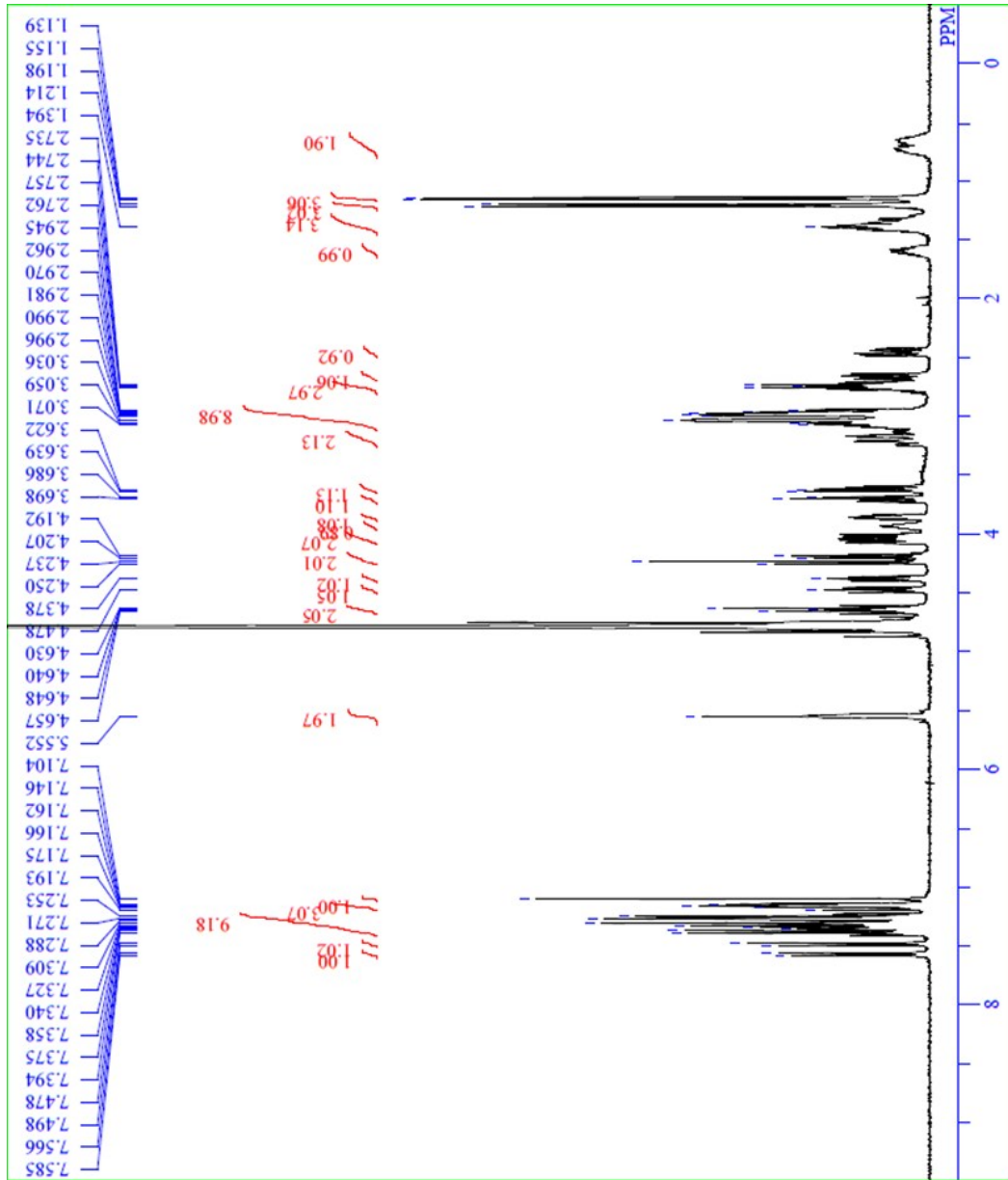


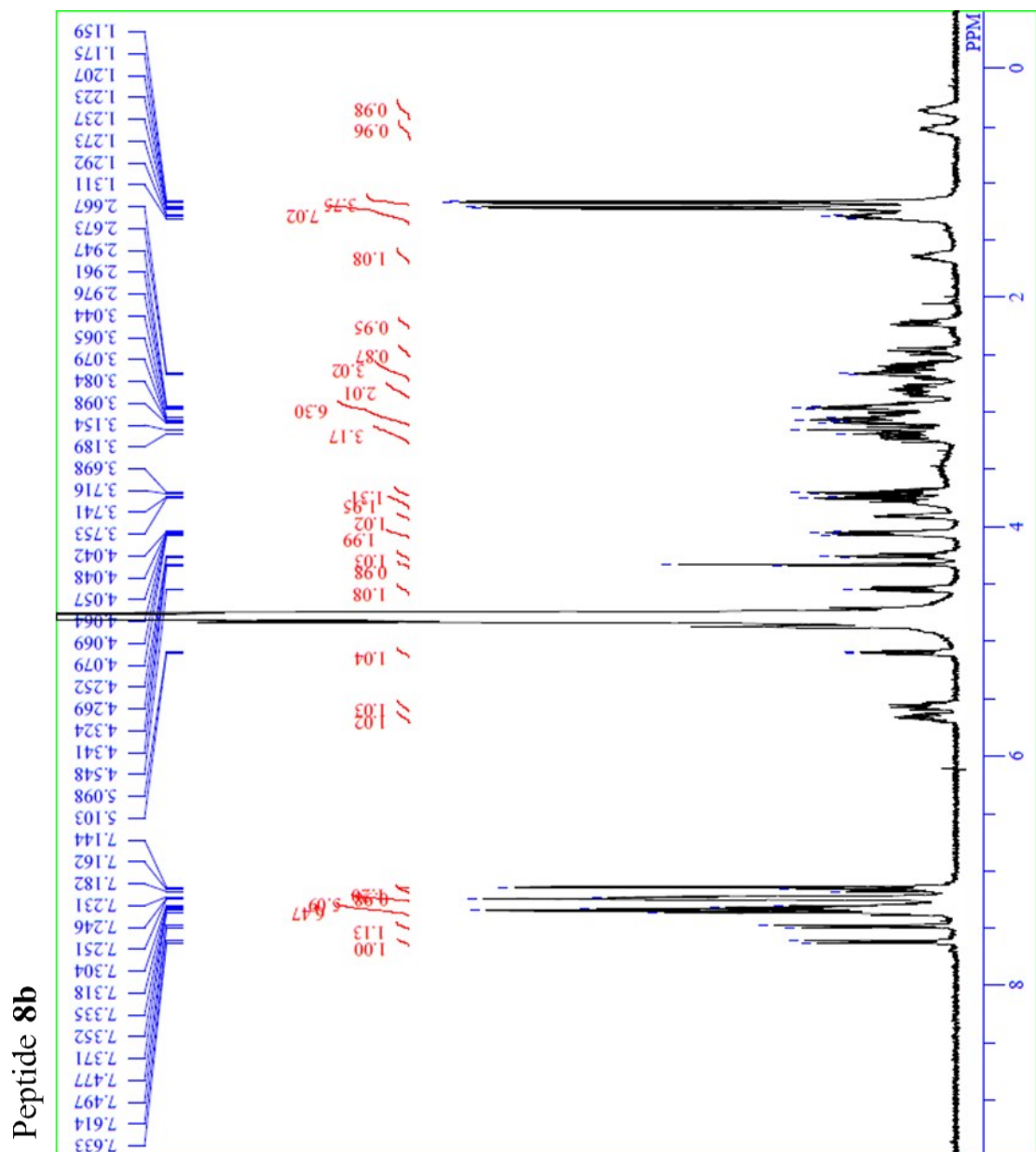


Peptide 7b



Peptide 8a





#### 4. Reference

- [1] Subiros-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chem. Eur. J.* **2009**, *15*, 9394–9403.