

## Supporting Information

### Generation and Biological Evaluation of Degraded Derivatives of the Three *E/Z*-Isomers of Yaku'amide B

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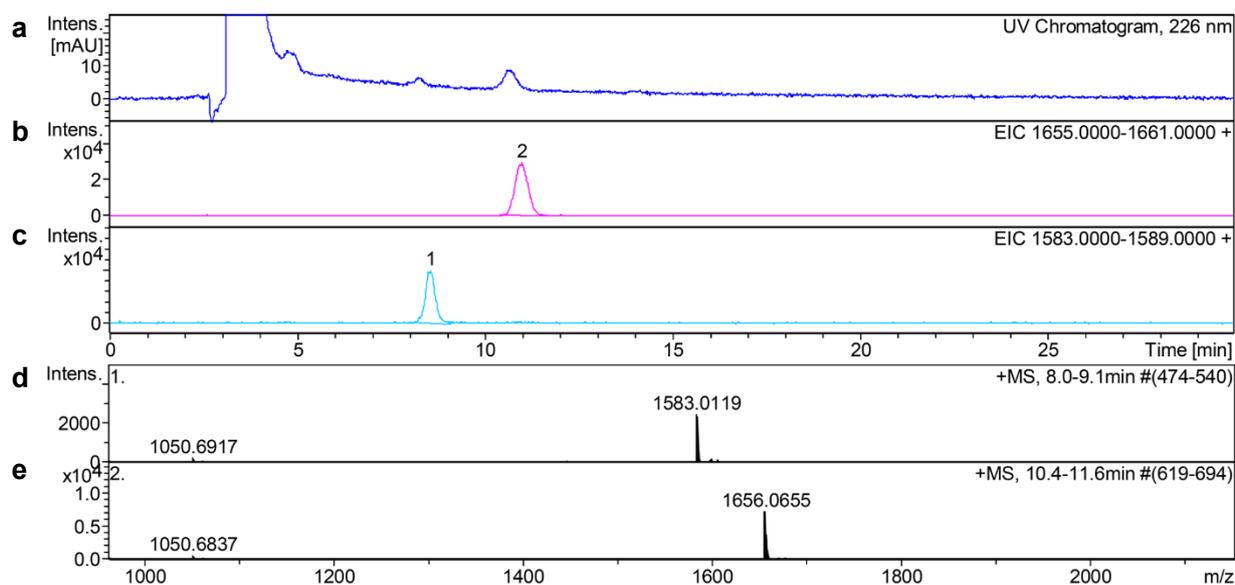
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16 pages

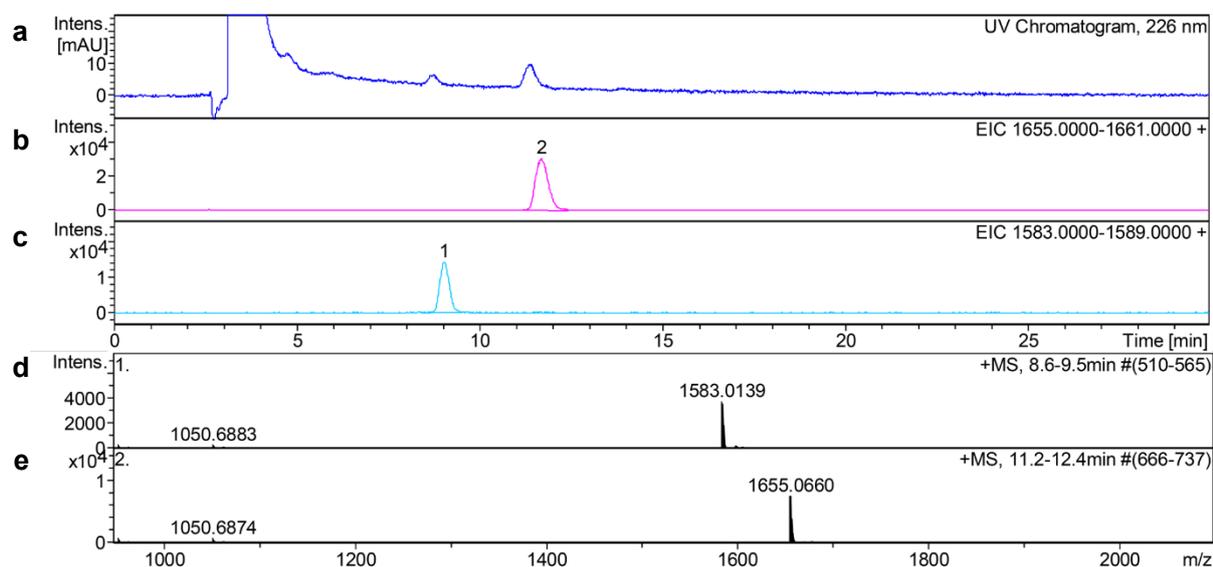
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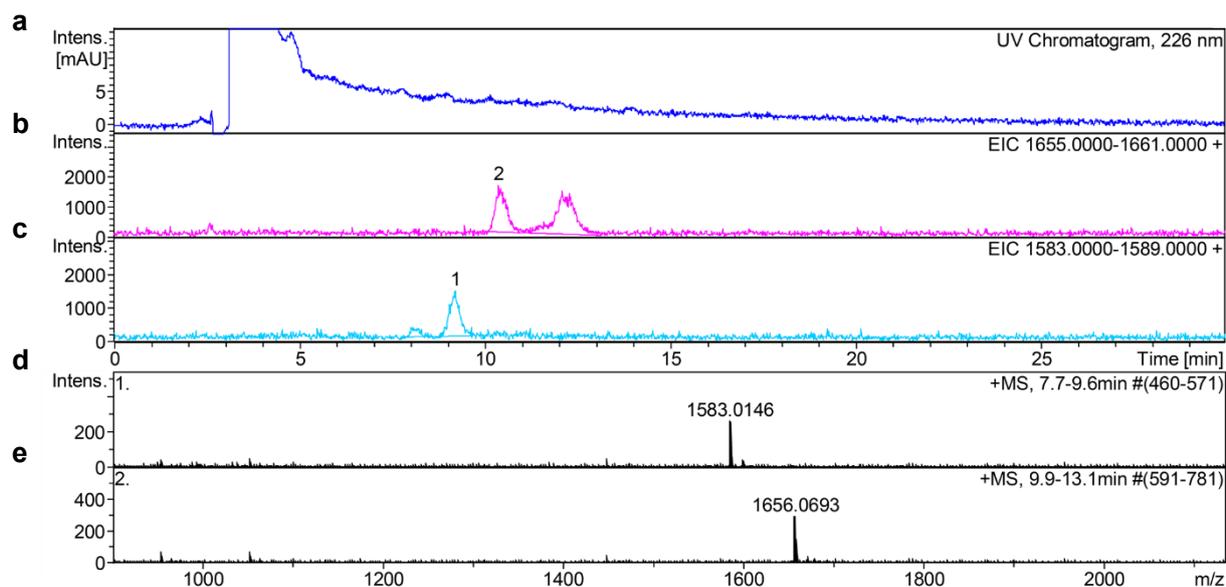
## Supporting Figures



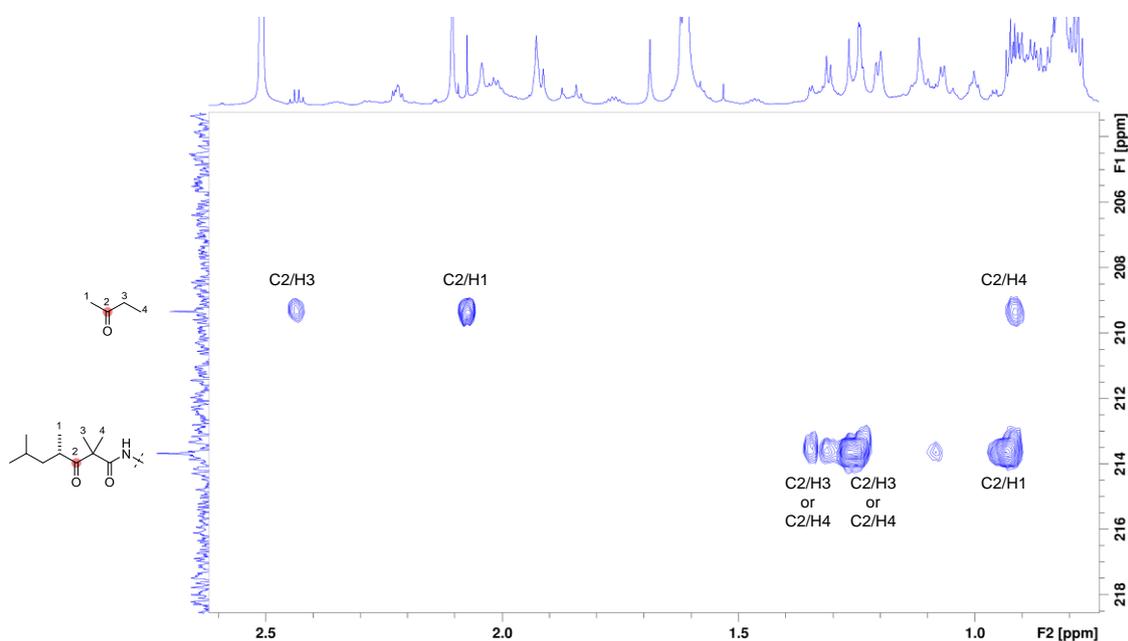
**Figure S1.** LC-MS chart for analysis of **2** after incubation in 0.008% AcOH/DMSO- $d_6$  at room temperature for 7 d. Column: Inertsil C8-3 4.6  $\times$  150 mm, eluent A: MeCN + 0.05% TFA, eluent B: H<sub>2</sub>O + 0.05% TFA, isocratic A/B = 70/30, flow rate: 0.50 mL/min, temperature: 40 °C. (a) UV chromatogram at 226 nm. (b) Extracted ion chromatogram ( $m/z = 1655-1661$ ). (c) Extracted ion chromatogram ( $m/z = 1583-1589$ ). (d) MS (ESI-TOF) signals of peak 1 (**2a**) in Figure S1c. (e) MS (ESI-TOF) signals of peak 2 (**2**) in Figure S1b.



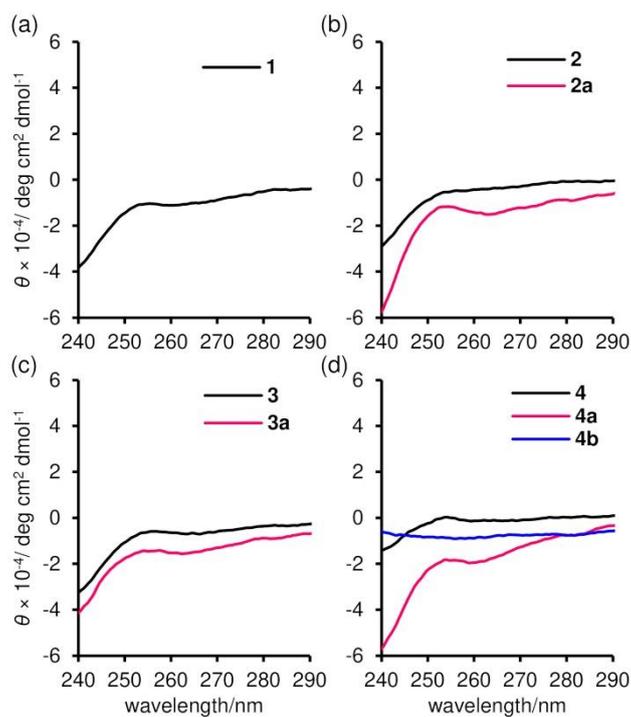
**Figure S2.** LC-MS chart for analysis of **3** after incubation in 0.008% AcOH/DMSO- $d_6$  at room temperature for 7 d. Column: Inertsil C8-3 4.6  $\times$  150 mm, eluent A: MeCN + 0.05% TFA, eluent B: H<sub>2</sub>O + 0.05% TFA, isocratic A/B = 70/30, flow rate: 0.50 mL/min, temperature: 40 °C. (a) UV chromatogram at 226 nm. (b) Extracted ion chromatogram ( $m/z = 1655-1661$ ). (c) Extracted ion chromatogram ( $m/z = 1583-1589$ ). (d) MS (ESI-TOF) signals of peak 1 (**3a**) in Figure S2c. (e) MS (ESI-TOF) signals of peak 2 (**3**) in Figure S2b.



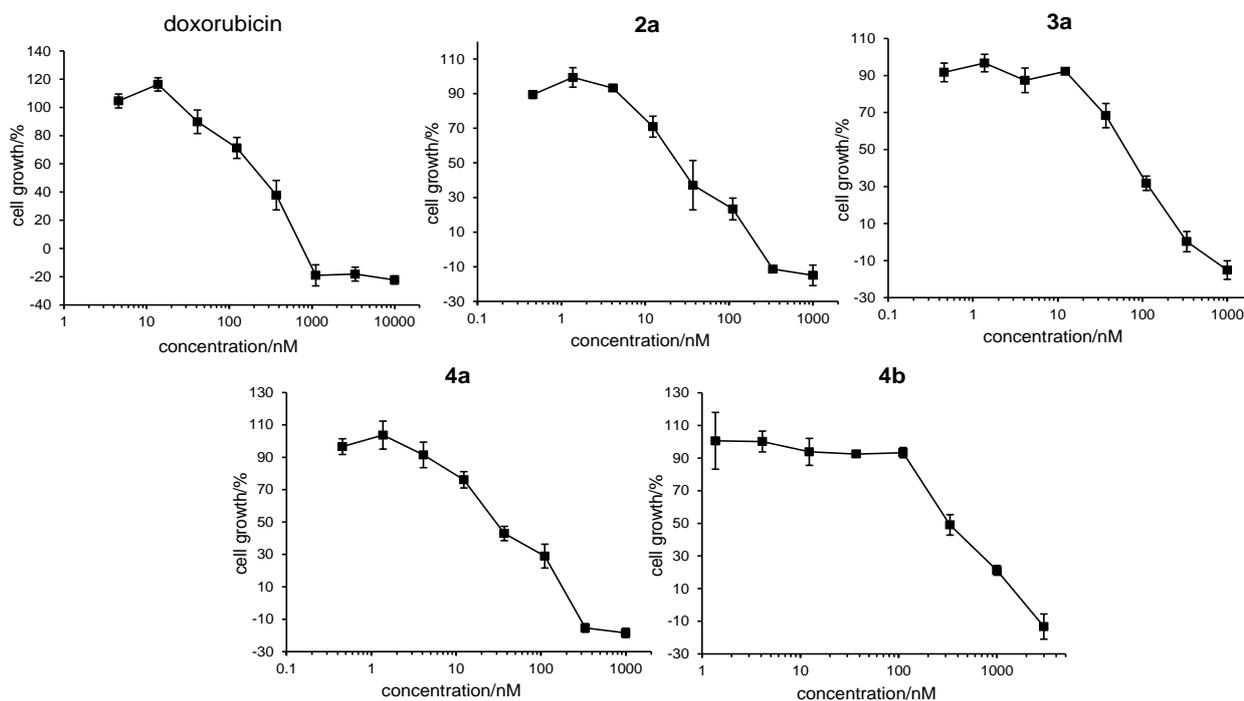
**Figure S3.** LC-MS chart for analysis of **4** after incubation in 0.008% AcOH/DMSO- $d_6$  at room temperature for 7 d. Column: Inertsil C8-3 4.6  $\times$  150 mm, eluent A: MeCN + 0.05% TFA, eluent B: H<sub>2</sub>O + 0.05% TFA, isocratic A/B = 70/30, flow rate: 0.50 mL/min, temperature: 40 °C. (a) UV chromatogram at 226 nm. (b) Extracted ion chromatogram ( $m/z$  = 1655–1661). (c) Extracted ion chromatogram ( $m/z$  = 1583–1589). (d) MS (ESI-TOF) signals of peak 1 (**4a**) in Figure S3c. (e) MS (ESI-TOF) signals of peak 2 (**4**) in Figure S3b.



**Figure S4.**  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **3** after storage at room temperature in DMSO- $d_6$  containing 0.36% AcOH for 7 d. The spectrum was obtained in DMSO- $d_6$  at 27 °C (800 MHz).



**Figure S5.** CD spectra of **1** and its derivatives in  $\text{CHCl}_3$ . The spectra of (a) **1**, (b) **2** and **2a**, (c) **3** and **3a**, (d) **4**, **4a**, and **4b** are displayed. The spectra of **1**, **2**, **3**, and **4** were previously reported (ref. 8).



**Figure S6.** Representative concentration-response curves of **2a**, **3a**, **4a**, and **4b** against MCF-7 cells. Doxorubicin was used as a positive control ( $\text{GI}_{50}$ :  $183.4 \pm 85.2$  nM). The cells were incubated for 2 d in the presence of a peptide, and the cell growth (%) was evaluated. Each plot is displayed as mean  $\pm$  SD of three replicates.





# MS/MS Spectra

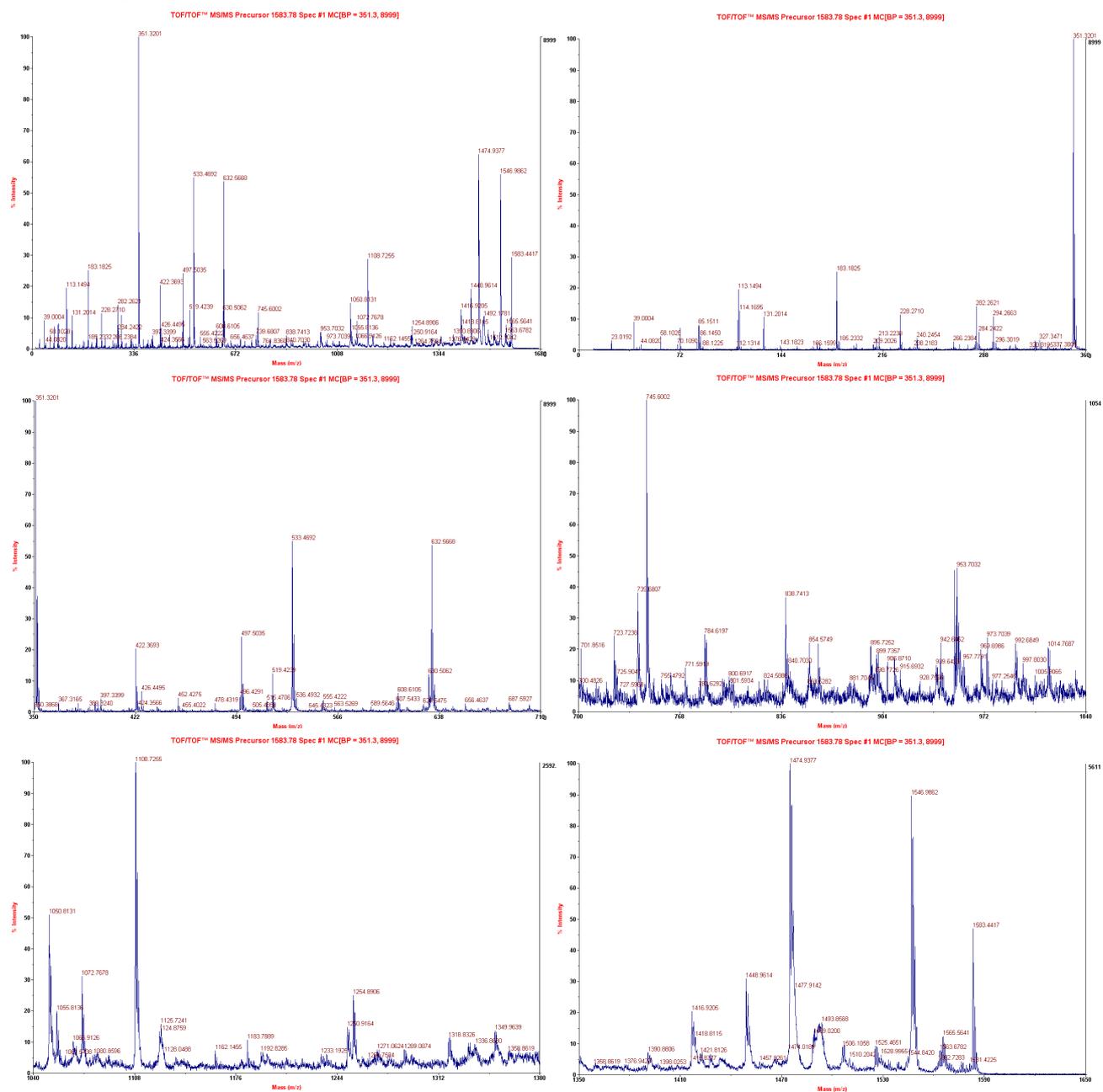


Figure S9. MS/MS spectra of 2a. Mass range:  $m/z$  = 0–1680.

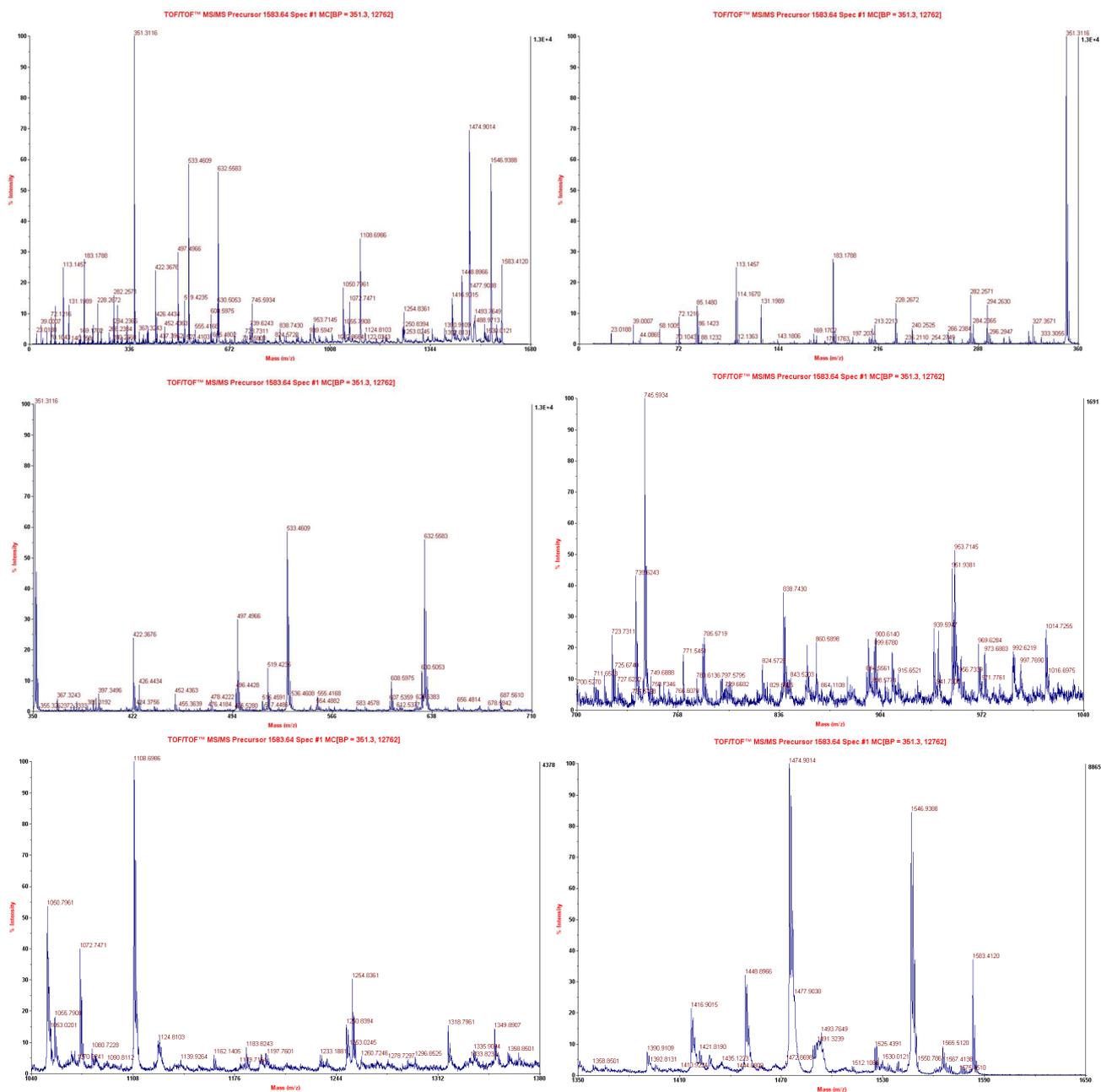


Figure S10. MS/MS spectra of 3a. Mass range:  $m/z = 0-1680$ .

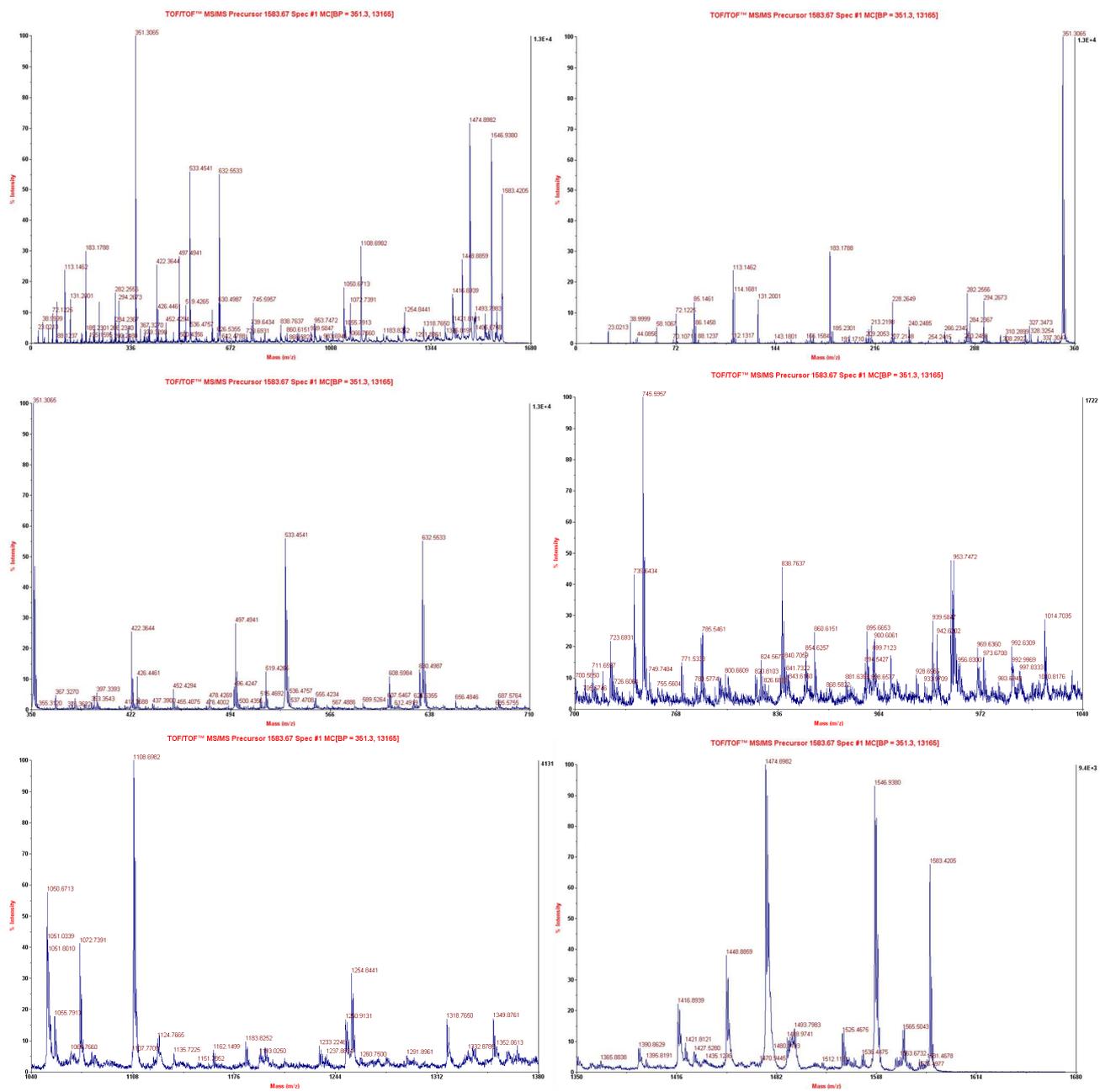
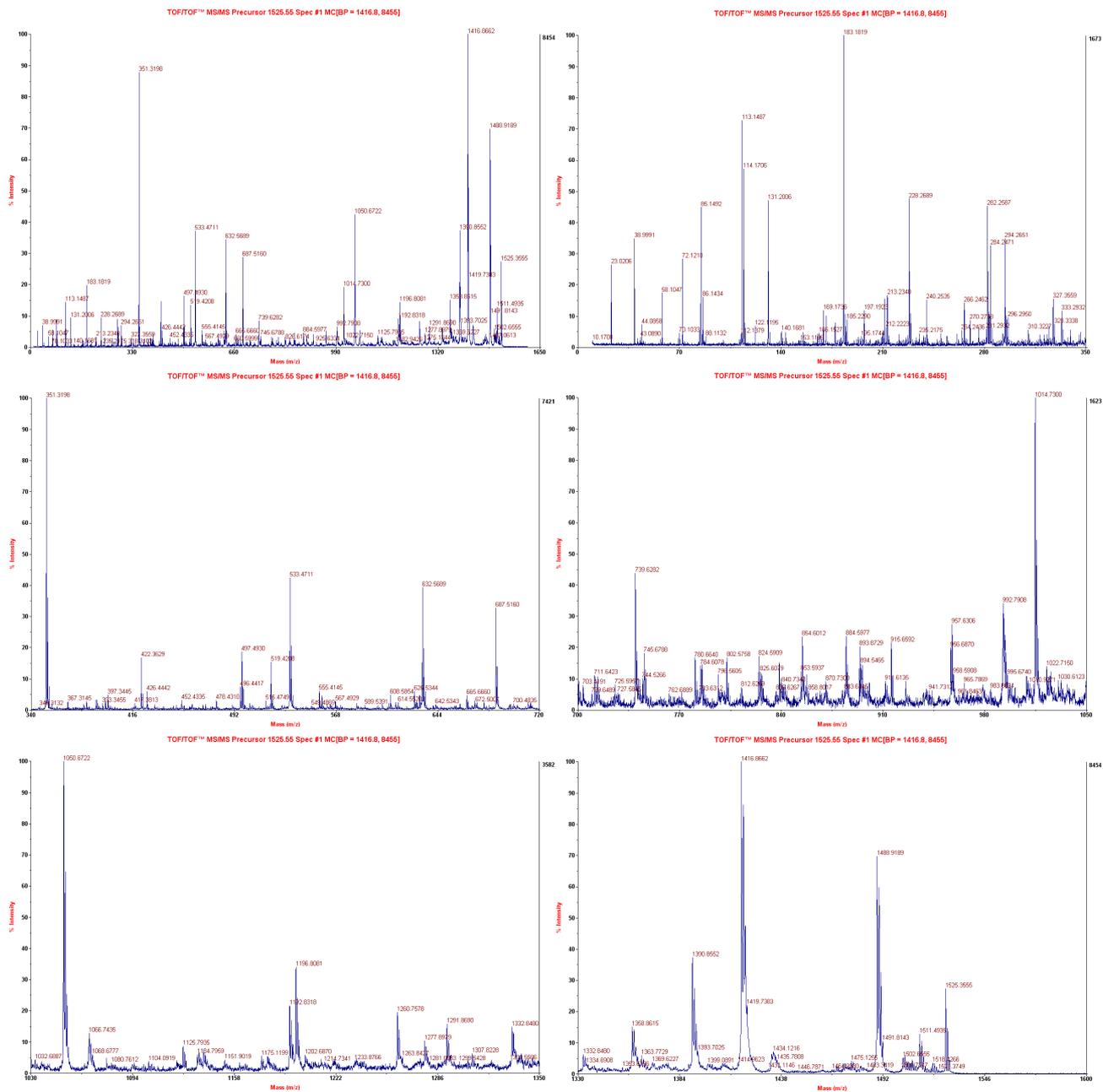
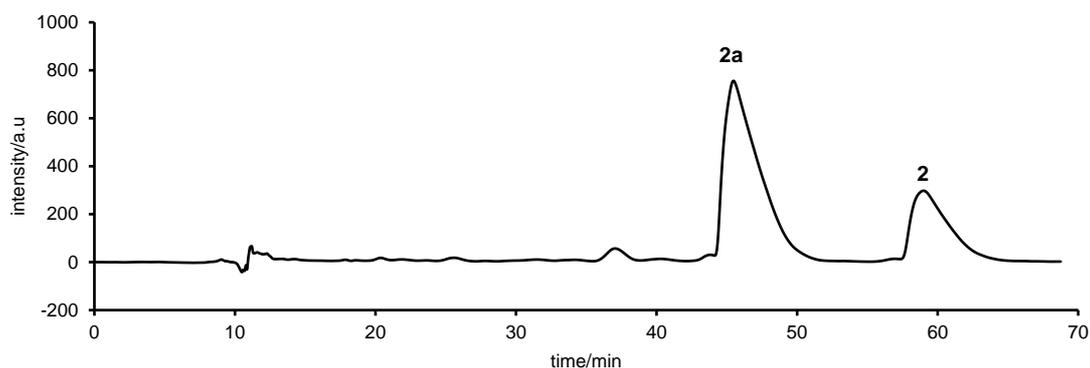


Figure S11. MS/MS spectra of 4a. Mass range:  $m/z = 0-1680$ .

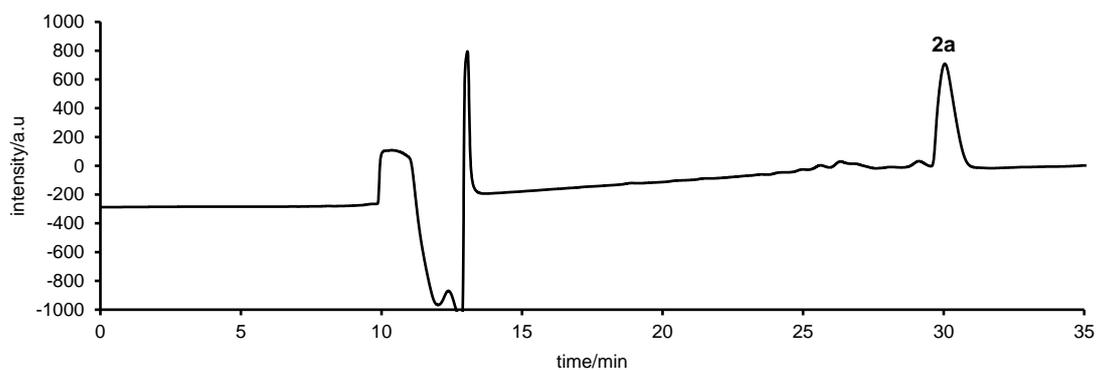


**Figure S12.** MS/MS spectra of **4b**. Mass range:  $m/z = 0-1650$ .

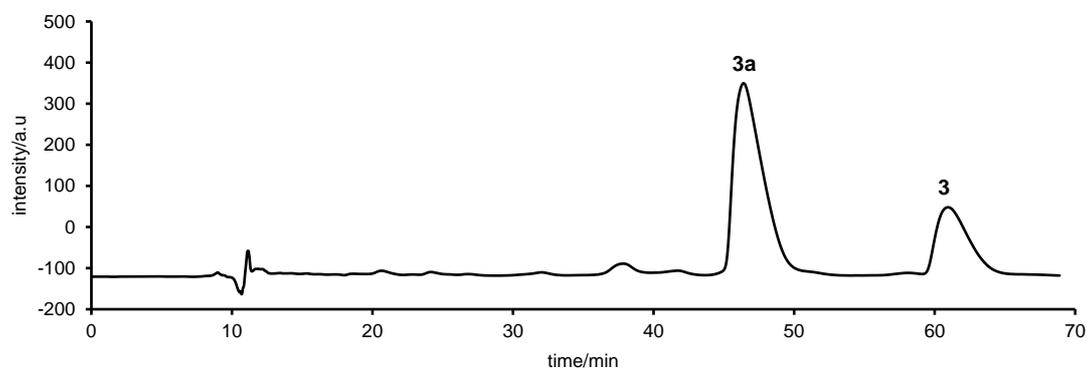
## HPLC Charts for Purification of Peptides



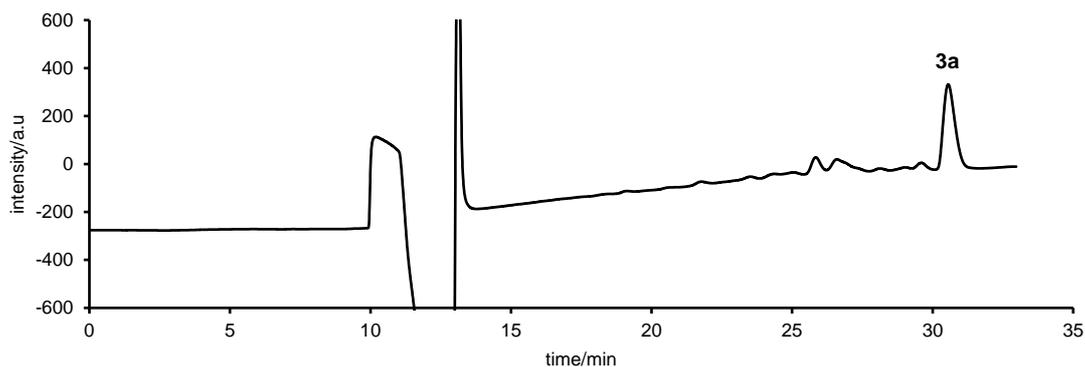
**Figure S13.** HPLC chart for 1st HPLC purification of **2a**. Column: Inertsil C8-3 10 × 250 mm connected with Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN/THF (33/66/1) + 0.05% TFA, eluent B: H<sub>2</sub>O + 0.05% TFA, isocratic A/B = 75/25, flow rate: 3.0 mL/min, detection: UV 226 nm.



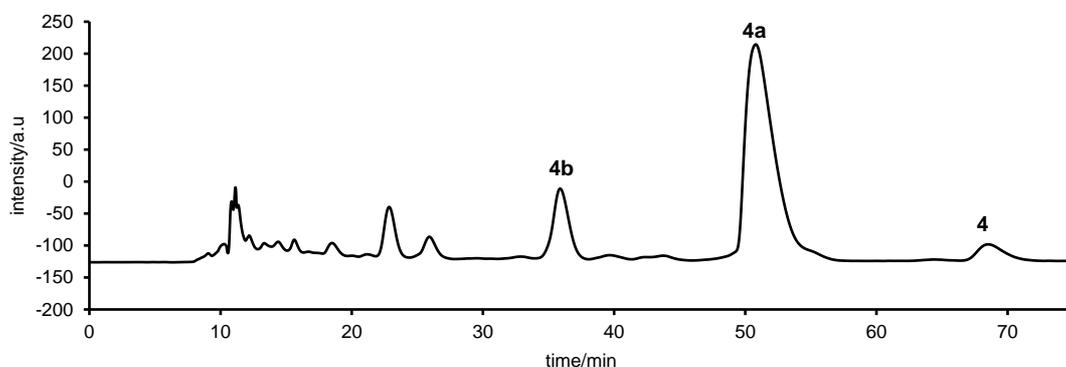
**Figure S14.** HPLC chart for 2nd HPLC purification of **2a**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, linear gradient A/B = 30/70 to 100/0 over 60 min, flow rate: 1.5 mL/min, detection: UV 226 nm.



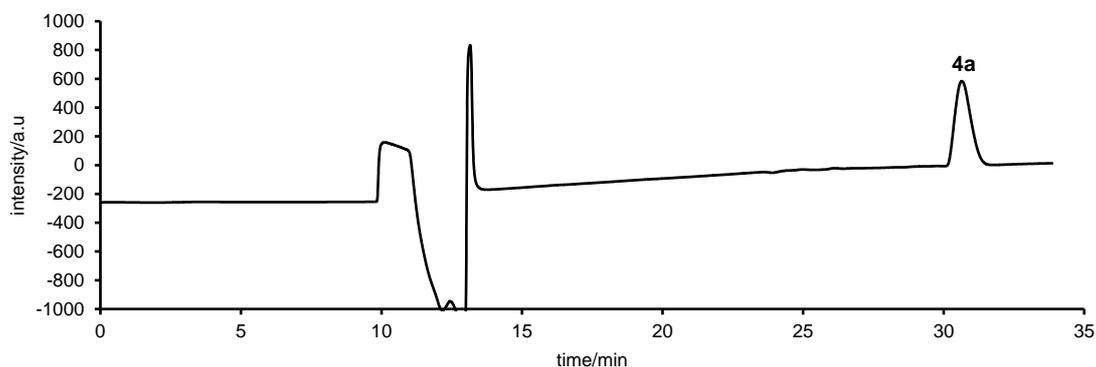
**Figure S15.** HPLC chart for 1st HPLC purification of **3a**. Column: Inertsil C8-3 10 × 250 mm connected with Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN/THF (33/66/1) + 0.05% TFA, eluent B: H<sub>2</sub>O + 0.05% TFA, isocratic A/B = 75/25, flow rate: 3.0 mL/min, detection: UV 226 nm.



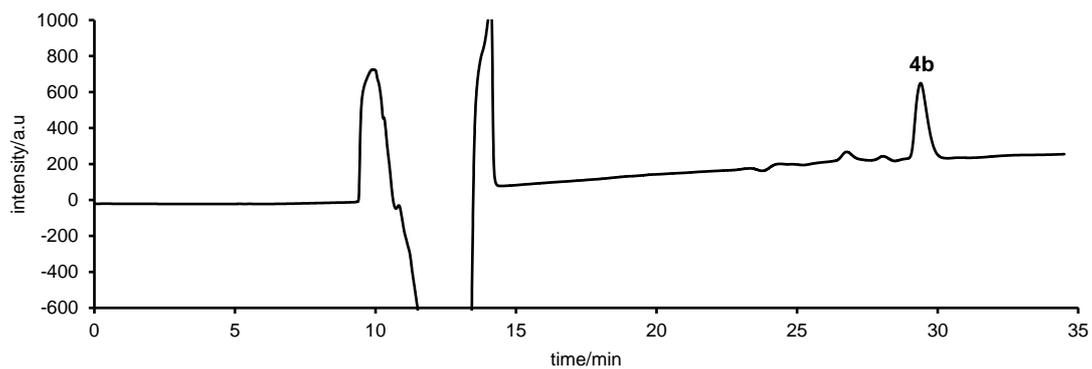
**Figure S16.** HPLC chart for 2nd HPLC purification of **3a**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, linear gradient A/B = 30/70 to 100/0 over 60 min, flow rate: 1.5 mL/min, detection: UV 226 nm.



**Figure S17.** HPLC chart for 1st HPLC purification of **4a** and **4b**. Column: Inertsil C8-3 10 × 250 mm connected with Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN/THF (33/66/1) + 0.05% TFA, eluent B: H<sub>2</sub>O + 0.05% TFA, isocratic A/B = 75/25, flow rate: 3.0 mL/min, detection: UV 226 nm.

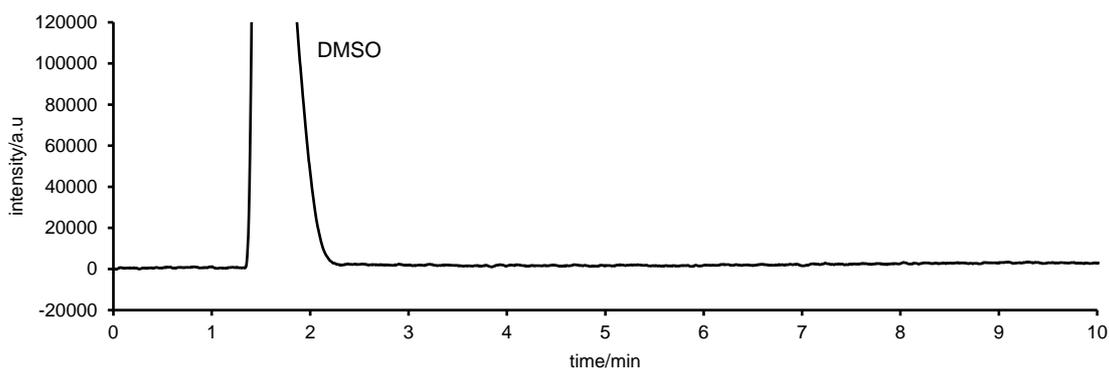


**Figure S18.** HPLC chart for 2nd HPLC purification of **4a**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, linear gradient A/B = 30/70 to 100/0 over 60 min, flow rate: 1.5 mL/min, detection: UV 226 nm.

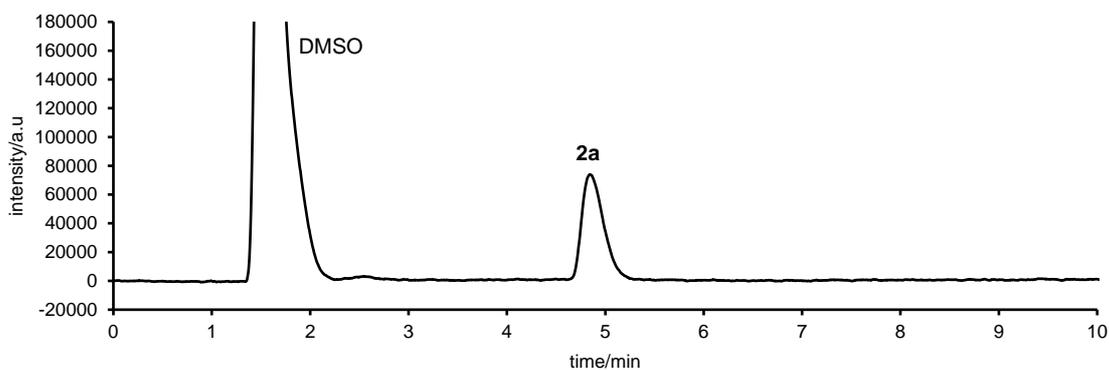


**Figure S19.** HPLC chart for 2nd HPLC purification of **4b**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, linear gradient A/B = 30/70 to 100/0 over 60 min, flow rate: 1.5 mL/min, detection: UV 226 nm.

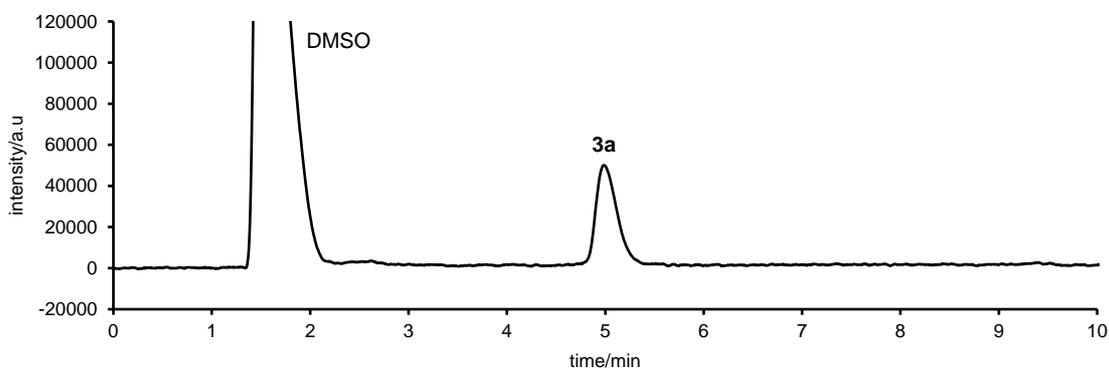
## UHPLC Charts for Analysis of Purified Peptides



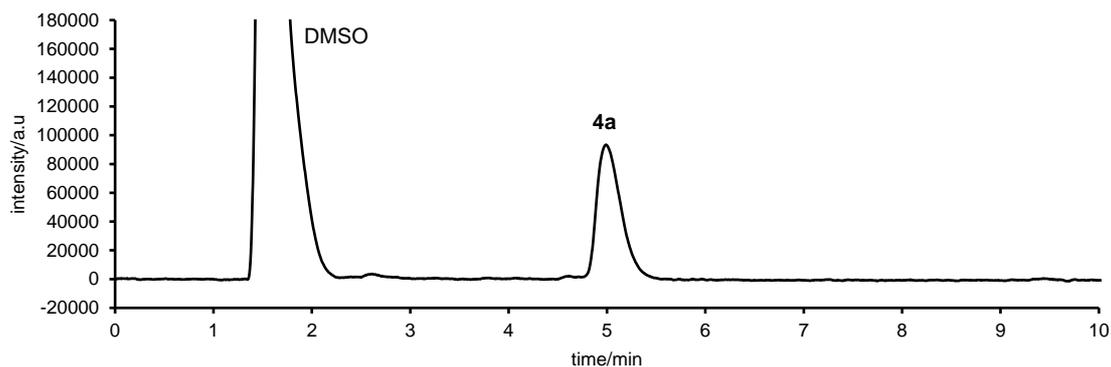
**Figure S20.** UHPLC chart of the injection solvent (DMSO). Column: Accucore C18 2.1 × 150 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, isocratic A/B = 35/65, flow rate: 0.20 mL/min, temperature: 40 °C, detection: photodiode array detector (UV 226 nm).



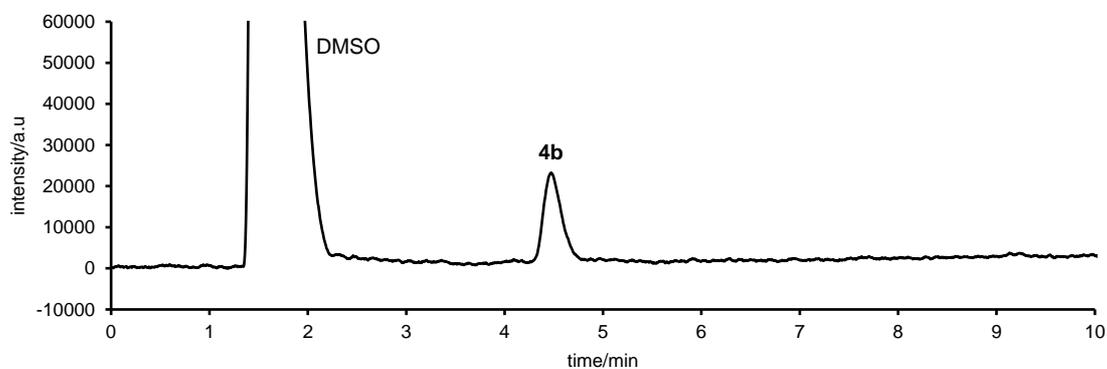
**Figure S21.** UHPLC chart of purified **2a**. Column: Accucore C18 2.1 × 150 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, isocratic A/B = 35/65, flow rate: 0.20 mL/min, temperature: 40 °C, detection: photodiode array detector (UV 226 nm).



**Figure S22.** UHPLC chart of purified **3a**. Column: Accucore C18 2.1 × 150 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, isocratic A/B = 35/65, flow rate: 0.20 mL/min, temperature: 40 °C, detection: photodiode array detector (UV 226 nm).



**Figure S23.** UHPLC chart of purified **4a**. Column: Accucore C18 2.1 × 150 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, isocratic A/B = 35/65, flow rate: 0.20 mL/min, temperature: 40 °C, detection: photodiode array detector (UV 226 nm).



**Figure S24.** UHPLC chart of purified **4b**. Column: Accucore C18 2.1 × 150 mm, eluent A: *n*-PrOH + 1% AcOH, eluent B: H<sub>2</sub>O + 1% AcOH, isocratic A/B = 35/65, flow rate: 0.20 mL/min, temperature: 40 °C, detection: photodiode array detector (UV 226 nm).