Supplementary Information for:

Asparagine-selective Amide Bond Cleavage of Peptides Using Hypervalent Iodine in Neutral Aqueous Solution

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1. General Procedures, Materials, and Preparation of Peptides

General. Analytical HPLC charts were obtained by using a JASCO HPLC system equipped with a UV-2075 spectrometer, PU-2080 pumps, a DG-2080-54 degasser, and an MX-2080-32 mixer, or a HITACHI HPLC system equipped with an L-4200 UV–vis detector, an L-6210 pump or L-6200/L-6000 pumps, and an ERC-3510 or L-5090 degasser. Preparative HPLC were conducted by using a JASCO HPLC system equipped with a UV-2075 spectrometer, PU-2086 pumps, a DG-2080-53 degasser, and an MX-2080-32 mixer. LC/MS/MS (ESI-Q-TOF) analyses were conducted using AB Sciex Triple TOF 4600 equipped with eksigent ekspert microLC 200. MALDI-TOF MS was obtained with a Shimadzu Biotech Axima ToF^2 spectrometer. NMR spectra were recorded on JEOL JNM-ECX500 spectrometer, operating at 500 MHz for ^{1}H NMR and 124.51 MHz for ^{13}C NMR.

Materials. Fmoc-amino acids and 2-chlorotrityl chloride resin were obtained from Peptides Institute, Inc. (Osaka, Japan). 1-Hydroxybenzotriazole (HOBt) was obtained from Watanabe Chemical (Hiroshima, Japan). *N*,*N*-Dimethylformamide (DMF), dichloroethane (DCE), and acetonitrile were purchased from Kanto Chemical (Tokyo, Japan). *N*,*N*-Diisopropylethylamine (DIEA), *N*,*N*'-diisopropylcarbodiimide (DIC), diacetoxyiodobenzene (DIB), and riboflavin were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Piperidine was purchased from nacalai tesque (Kyoto, Japan). Trifluoroacetic acid (TFA) was from Tokyo Chemical Industry (Tokyo, Japan). NovaPEG Rink Amide resin were from Merck (Tokyo, Japan). [Pyr^{11}]Aß11-40 was purchased from Bachem (Bubendorf, Switzerland). Water was purified using a Millipore MilliQ water purification system.

Analytical HPLC. Peptide compositions were evaluated by analytical reverse phase HPLC using a gradient of acetonitrile versus 0.1% TFA in water. Analytical HPLC was carried out as follows: COSMOSIL 5C$_{18}$-MS-II, YMC-Pack ODS-AM, or YMC-Triart-C$_{18}$ (4.6 mm I.D × 150 mm) column using a linear gradient of 0–100% acetonitrile in 0.1% aqueous TFA over 40 min at room temperature with a flow rate of 0.9 mL min^{-1}. The eluent was monitored by absorbance at 301 nm unless otherwise noted.

Analytical LC/MS/MS. Reactions were monitored by LC/MS/MS spectroscopy using a gradient of acetonitrile versus 0.1% formic acid in water. LC was carried out as follows unless otherwise noted: 3C18-CL-120 column (0.5 mm I.D × 100 mm) using linear gradient of 10–70% acetonitrile in 0.1% aqueous formic acid over 18 min at room temperature with a flow rate of 20 μL min^{-1}. The eluent was monitored by on-line ESI-Q-TOF MS.

Preparative HPLC. Peptides were purified by preparative reverse phase HPLC using a gradient of acetonitrile versus 0.1% TFA in water. Preparative HPLC was carried out as follows: YMC-Triart C18 (10 mm I.D × 250 mm) column using a linear gradient of 0-100%

acetonitrile in 0.1% aqueous TFA over 100 min at room temperature with a flow rate of 3.0 mL min^{-1}. The eluent was monitored by absorbance at 230 nm.

General Protocol for Peptide Synthesis. Peptide synthesis was performed manually on a 0.1 mmol scale using chlorotrityl chloride resin or NovaPEG Rink amide resin. Fmoc-protected amino acids (0.25 mmol) were sequentially coupled using a DIC–HOBt method (0.25 mmol each) for 30 min at room temperature after removal of each Fmoc group with 20% piperidine–DMF for 10 min to obtain a peptide-resin. The peptide was cleaved from the resin and simultaneously deprotected by treatment with TFA in the presence of triisopropylsilane and water (95:2.5:2.5) for 60 min at room temperature, concentrated in vacuo, and precipitated with diethyl ether. The product was dissolved with 50% aqueous acetonitrile containing 0.1% TFA and lyophilized. The crude product was purified using preparative HPLC, immediately frozen at –78 °C, and lyophilized to give the desired peptide as a white solid.

Fmoc-Gly-Ser-Asn-Phe-Gly-OH (1a). MS (MALDI-TOF): *m/z* 702.96 (calcd $[M+H]^+$ = 703.27), 725.01 (calcd $[M+Na]^+$ = 725.25). Purity: >95% (HPLC analysis at 230 nm). Retention time: 22.89 min.

Fmoc-Gly-Ser-Asn-Lys-Gly-OH (1b). MS (ESI-TOF): *m/z* 684.29 (calcd $[M+H]^+$ = 684.30). Purity: >95% (HPLC analysis at 230 nm). Retention time: 19.03 min.

Fmoc-Gly-Ser-Asn-Arg-Gly-OH (1c). MS (MALDI-TOF): *m/z* 711.85 (calcd $[M+H]^+$ = 712.31), 733.87 (calcd $[M+Na]^+$ = 734.29). Purity: >95% (HPLC analysis at 230 nm). Retention time: 19.00 min.

Fmoc-Gly-Ser-Asn-Asp-Gly-OH (1d). MS (MALDI-TOF): *m/z* 670.78 (calcd $[M+H]^+$ = 671.23), 692.83 (calcd $[M+Na]^+$ = 693.21). Purity: >95% (HPLC analysis at 230 nm). Retention time: 19.90 min.

Fmoc-Gly-Ser-Asn-His-Gly-OH (1e). MS (MALDI-TOF): *m/z* 692.67 (calcd $[M+H]^+$ = 693.26), 714.68 (calcd $[M+Na]^+$ = 715.25). Purity: >95% (HPLC analysis at 230 nm). Retention time: 18.98 min.

Fmoc-Gly-Ser-Asn-Cys-Gly-OH (1f). MS (MALDI-TOF): *m/z* 658.82 (calcd $[M+H]^+$ = 659.21), 680.87 (calcd $[M+Na]^+$ = 681.20). Purity: >95% (HPLC analysis at 230 nm). Retention time: 20.73 min.

Fmoc-Gly-Ser-Asn-Met-Gly-OH (1g). MS (MALDI-TOF): *m/z* 686.87 (calcd $[M+H]^+$ = 687.24), 708.91 (calcd $[M+Na]^+$ = 709.23). Purity: >95% (HPLC analysis at 230 nm). Retention time: 21.36 min.

Fmoc-Gly-Ser-Asn-Tyr-Gly-OH (1h). MS (MALDI-TOF): *m/z* 718.67 (calcd $[M+H]^+$ = 719.27), 740.72 (calcd $[M+Na]^+$ = 741.25). Purity: >95% (HPLC analysis at 230 nm). Retention time: 21.33 min.

Fmoc-Gly-Ser-Asn-Trp-Gly-OH (1i). MS (MALDI-TOF): *m/z* 742.10 (calcd $[M+H]^+$ = 742.28), 764.13 (calcd $[M+Na]^+$ = 764.27). Purity: >95% (HPLC analysis at 230 nm). Retention time: 23.27 min.

Fmoc-Gly-Ala-Asn-Phe-Gly-OH (5). MS (MALDI-TOF): *m/z* 687.58 (calcd $[M+H]^+$ = 687.28), 709.60 (calcd $[M+Na]^+$ = 709.26). Purity: >95% (HPLC analysis at 230 nm). Retention time: 23.59 min.

Fmoc-Gly-Ser-Asn-Lys-Gly-NH_2 (6). MS (MALDI-TOF): *m/z* 683.71 (calcd $[M+H]^+$ = 683.32), 705.88 (calcd $[M+Na]^+$ = 705.30). Purity: >95% (HPLC analysis at 230 nm). Retention time: 18.57 min.

Pyr-Phe-Ser-Asn-Phe-Gly-OH (7). MS (MALDI-TOF): *m/z* 681.64 (calcd $[M+H]^+$ = 682.28), 703.71 (calcd $[M+Na]^+$ = 704.27). Purity: >95% (HPLC analysis at 230 nm). Retention time: 16.45 min.

For-Phe-Ser-Asn-Phe-Gly-OH (8). MS (MALDI-TOF): *m/z* 598.86 (calcd $[M+H]^+$ = 599.25), 620.82 (calcd $[M+Na]^+$ = 621.23). Purity: >95% (HPLC analysis at 230 nm). Retention time: 16.53 min.

H-Gly-Ser-Asn-Phe-Gly-OH (9). MS (MALDI-TOF): *m/z* 480.74 (calcd $[M+H]^+$ = 480.22), 502.75 (calcd $[M+Na]^+$ = 502.20). Purity: >95% (HPLC analysis at 230 nm). Retention time: 10.74 min.

Fmoc-Gly-Ser-Gln-Phe-Gly-OH (10). MS (MALDI-TOF): *m/z* 716.59 (calcd $[M+H]^+$ = 717.29), 738.61 (calcd $[M+Na]^+$ = 739.27). Purity: >95% (HPLC analysis at 230 nm). Retention time: 22.43 min.

Fmoc-Gly-*D-Ser*-*D-Asn*-*D-Phe*-Gly-OH (11). MS (MALDI-TOF): *m/z* 702.53 (calcd $[M+H]^+$ = 703.27), 724.52 (calcd $[M+Na]^+$ = 725.25). Purity: >95% (HPLC analysis at 230 nm). Retention time: 23.64 min.

Fmoc-Cys-Gly-Arg-Arg-Ala-Cys-Gly-Ser-Asn-Phe-Gly-OH, disulfide bond (12). After the solid phase peptide synthesis of reduced form of **12** ($[M+2H]^{2+}$ obsd: 675.32; $[M+2H]^{2+}$ calcd: 675.28), the resulting product (10 mg) was dissolved in ammonium bicarbonate solution (0.05 M, 50 mL). The mixture was stirred at r.t. under O_2 atmosphere for 18 h. Following oxidation, the mass decreased by 2.1 Da, indicating the formation of a disulfide bond. Then, after lyophilization of the reaction mixture, the desired product was purified by preparative HPLC. MS (ESI-TOF): *m/z* 674.27 (calcd *m/z* for $[M+2H]^{2+}$ = 674.27). Purity: >95% (HPLC analysis at 230 nm). Retention time: 20.93 min.

Pyr-Val-Asp-Pro-Asn-Ile-Gln-OH (13). MS (MALDI-TOF): *m/z* 798.16 (calcd $[M+H]^+$ = 796.38), 818.46 (calcd $[M+Na]^+$ = 818.37). Purity: >95% (HPLC analysis at 230 nm). Retention time: 15.22 min.

Pyr-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg-OH (Fibrinopeptide B, 16). MS (MALDI-TOF): *m/z* 1552.42 (calcd $[M+H]^+$ = 1552.67). Purity: >95% (HPLC analysis at 230 nm). Retention time: 18.18 min.

Aß19–29 (20). MS (MALDI-TOF): *m/z* 1170.69 (calcd $[M+H]^+$ = 1170.54). Purity: >95% (HPLC analysis at 230 nm). Retention time: 14.24 min.

[Pyr^3]Aß3–16[Asp7Asn] (26). MS (MALDI-TOF): *m/z* 1749.55 (calcd $[M+H]^+$ = 1749.82). Purity: >95% (HPLC analysis at 230 nm). Retention time: 14.36 min.

2. Amide Bond Cleavage of Fmoc-Gly-Ser-Asn-Phe-Gly-OH (1a)

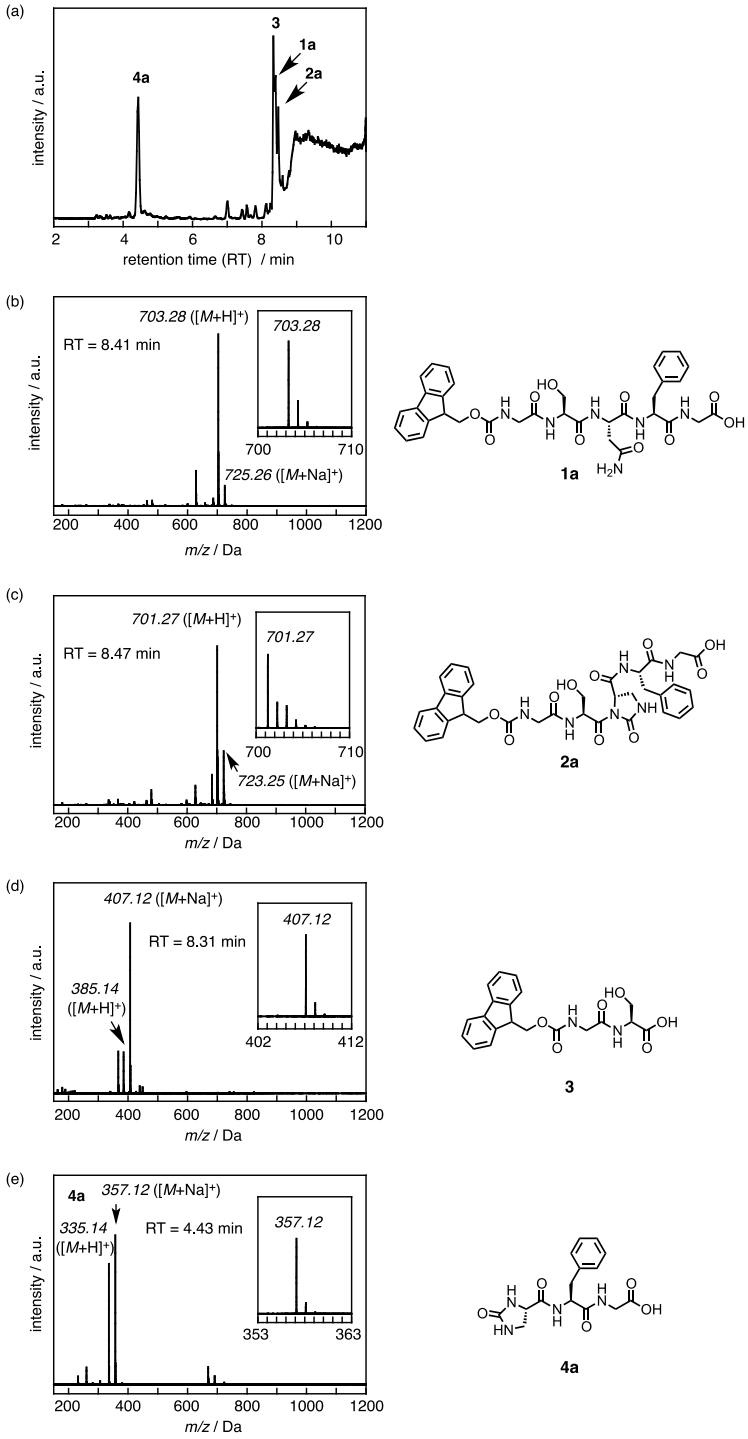

Figure S1. LC/MS charts for the reaction of Fmoc-Gly-Ser-Asn-Phe-Gly-OH (**1a**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after 72 h incubation at 37 ºC. (a) Total ion chromatograph obtained by using linear gradient of 2–35% acetonitrile in 0.1% aqueous

formic acid over 8 min with a flow rate of 20 μL min^{-1}. (b) MS spectrum at a retention time of 8.41 min corresponding to **1a** (calcd. *m/z* for $[M+H]^+$ = 703.27). (c) MS spectrum at a retention time of 8.47 min corresponding to **2a** (calcd. *m/z* for $[M+H]^+$ = 701.26). (d) MS spectrum at a retention time of 8.31 min corresponding to **3** (calcd. *m/z* for $[M+Na]^+$ = 407.12). (e) MS spectrum at a retention time of 4.43 min corresponding to **4a** (calcd. *m/z* for $[M+Na]^+$ = 357.12).

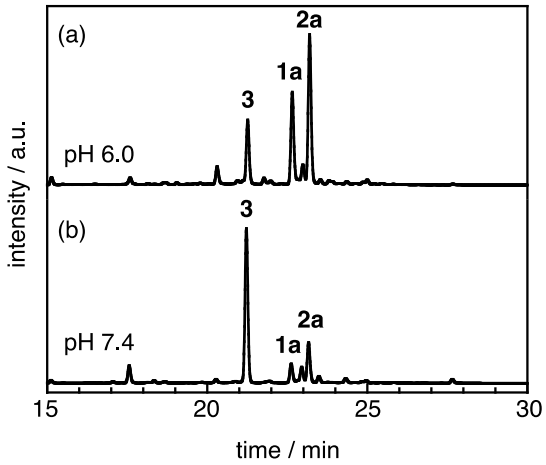

Figure S2. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Phe-Gly-OH (**1a**) with 3 eq. of DIB in 0.2 M phosphate buffer after 72 h incubation at 37 ºC. pH of the reaction mixture was adjusted to (a) 6.0. and (b) 7.4. Chromatographic separations were performed as described in Figure 1a legend.

3. NMR Study for Amide Bond Cleavage of Fmoc-Gly-Asn-OH

Preparation of Dipeptides.

2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-4-amino-4-oxobutanoic acid (Fmoc-Gly-Asn-OH). To a solution of Fmoc-Gly-OH (1.00 mmol, 297 mg) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; 1.05 mmol, 201 mg) in THF (20 mL) was added *N*-hydroxysuccinimide (HOSu; 1.05 mmol, 121 mg) at 0 ºC. After stirred for 18 h at room temperature, the reaction mixture was poured into water. The product was extracted with ethyl acetate (30 mL) three times, and the combined organic layer was washed with sat. $NaHCO_3$ aq., then dried over Na_2SO_4 and filtered. After evaporation of solvent, the product was dried under vacuum to obtain Fmoc-Gly-OSu as a white solid in quantitative yield. This compound was used without further purification. To a solution of Fmoc-Gly-OSu in DMF (20 mL), was added asparagine (1.0 mmol, 150 mg) and stirred for 18 h at room temperature. The reaction mixture was poured into water, and 5N HCl aq. (10 mL) was added. The product was extracted with ethyl acetate (30 mL) three times, the combined organic layer was washed with sat. $NaHCO_3$ aq., then dried over Na_2SO_4 and filtered. After evaporation of solvent, the product was dried under vacuum. The crude product was dissolved in 10 mL of 0.1% TFA aq./CH_3CN (1:1), and purified by preparative

HPLC. MS (ESI-TOF): *m*/*z* 412.15 (calcd $[M+H]^+$ = 412.15). Purity: >95% (HPLC analysis at 230 nm). Retention time: 21.5 min. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.08 (d, 1H, *J* = 8.0 Hz), 7.88 (d, 2H, *J* = 7.5 Hz), 7.71 (d, 2H, *J* = 7.5 Hz), 7.57 (t, 1H, *J* = 6.0 Hz), 7.41 (dd, 2H, *J* = 7.5 Hz, 7.5 Hz), 7.37 (s, 2H), 7.33 (dd, 2H, *J* = 7.5 Hz, 7.5 Hz), 6.90 (s, 1H), 4.55–4.45 (m, 1H), 4.27–4.18 (m, 4H), 3.65–3.57 (m, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 172.69, 171.21, 168.84, 156.45, 143.85, 140.70, 127.63, 127.10, 125.29, 120.10, 65.75, 48.60, 46.61, 43.21, 36.70.

3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetyl)-2-oxoimidazolidine-4-carboxylic acid. Fmoc-Gly-Asn-OH (50 μmol, 20.6 mg) and DIB (50 μmol, 16.1 mg) were dissolved in 50 mL of 0.2 M phosphate buffer (pH 7.4). After stirred for 24 h at 37 ºC, the reaction mixture was freeze-dried. The obtained white solid was dissolved in 10 mL of 0.1% TFA aq., and purified by preparative HPLC. MS (ESI-TOF): *m*/*z* 410.13 (calcd. $[M+H]^+$ = 410.14). Purity: >95% (HPLC analysis at 230 nm). Retention time: 22.36 min. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.88 (d, 2H, *J* = 7.5 Hz), 7.87 (m, 1H), 7.71 (d, 2H, *J* = 7.5 Hz), 7.53 (t, 1H, *J* = 6.0 Hz), 7.40 (dd, 2H, *J* = 7.5 Hz, 7.5 Hz), 7.32 (dd, 2H, *J* = 7.5 Hz, 7.5 Hz), 4.68–4.60 (m, 1H), 4.35–4.15 (m, 4H), 3.69 (t, 1H, *J* = 9.5 Hz), 3.27 (d, 2H, *J* = 9.5 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.23, 169.26, 156.47, 155.35, 143.83, 140.71, 127.62, 127.08, 125.25, 120.11, 65.72, 54.20, 46.59, 44.01, 40.35.

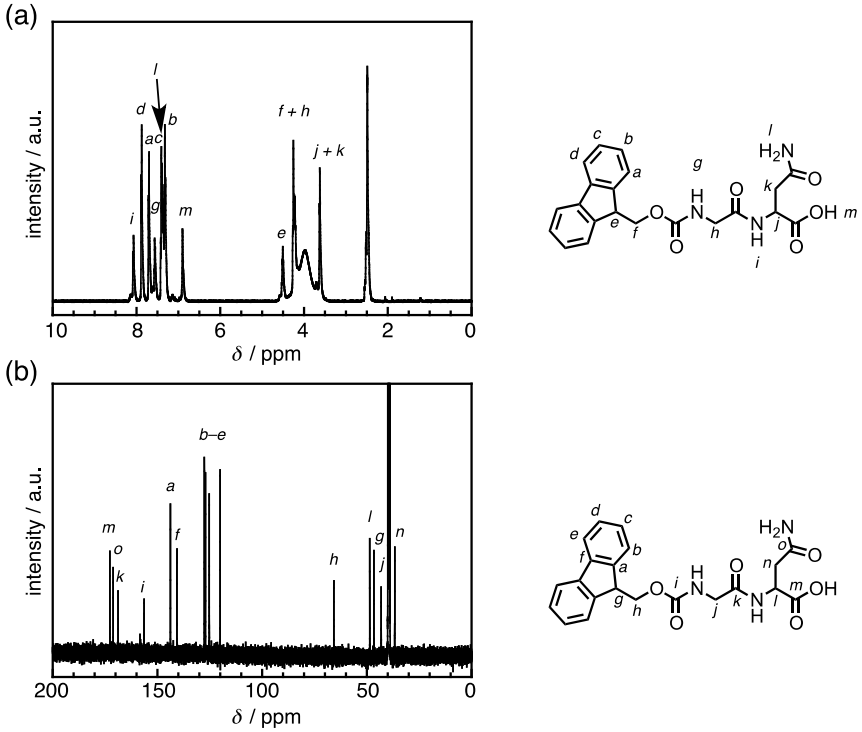

Figure S3. (a) ^{1}H and (b) ^{13}C NMR charts of Fmoc-Gly-Asn-OH measured in DMSO-d_6.

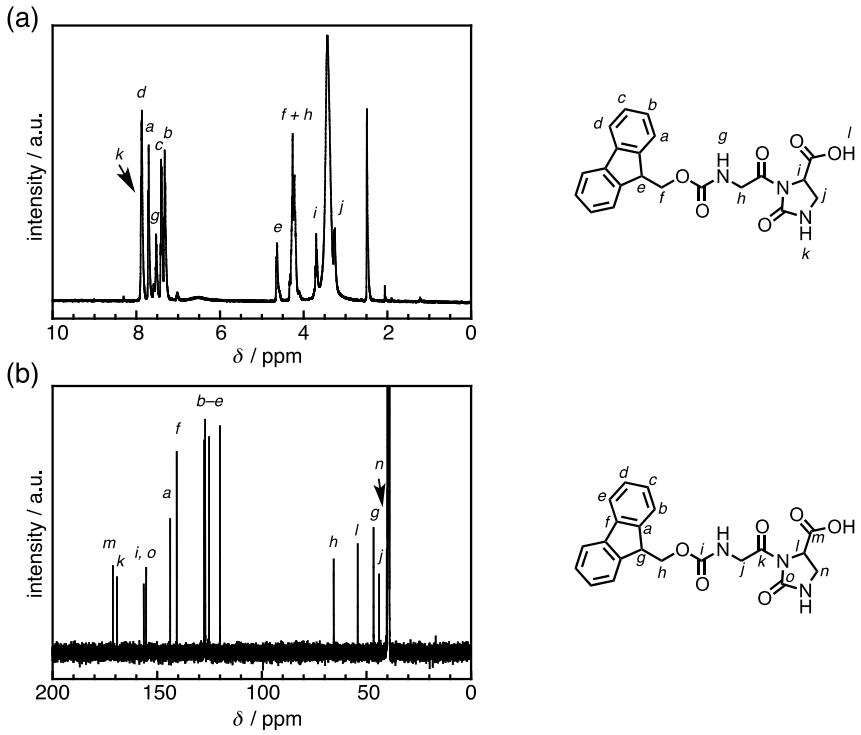

Figure S4. (a) ^{1}H and (b) ^{13}C NMR charts of the intermediate for the reaction of Fmoc-Gly-Asn-OH and DIB measured in DMSO-d_6.

4. HPLC Charts for the Reactions of Amide Bond Cleavage

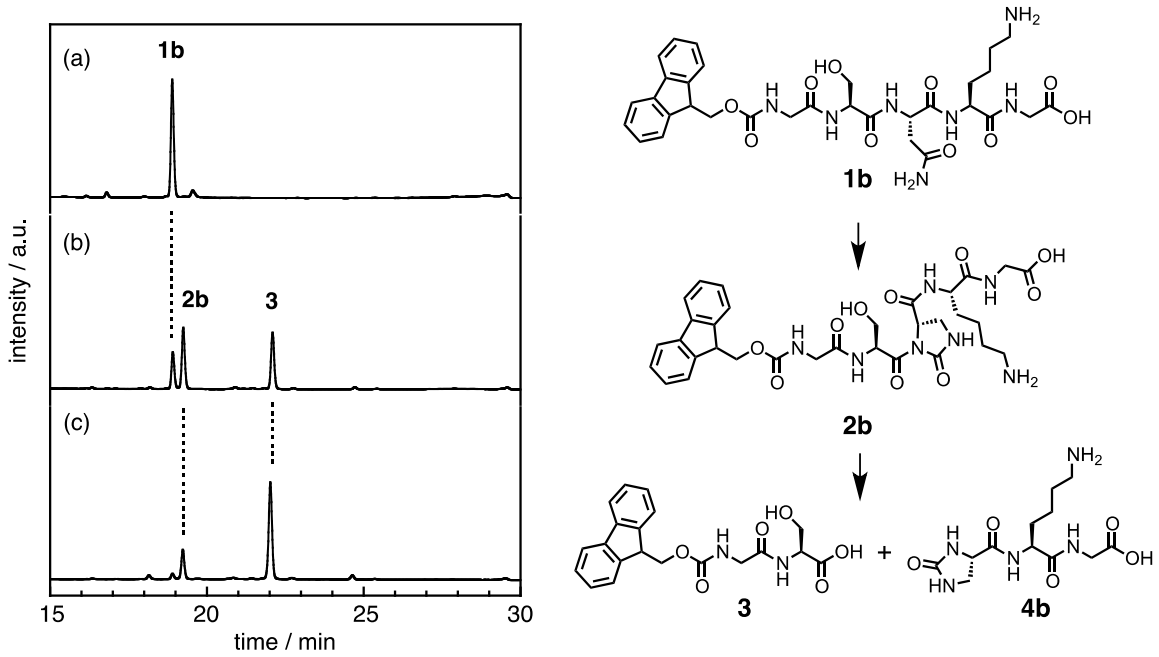

Figure S5. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Lys-Gly-OH (**1b**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

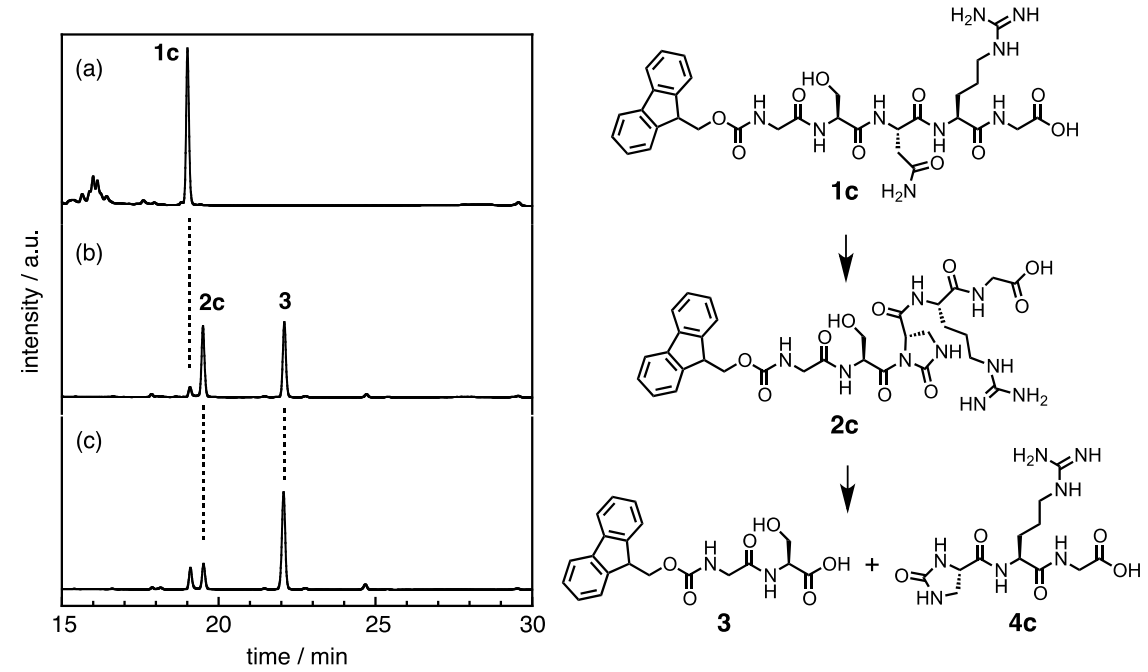

Figure S6. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Arg-Gly-OH (**1c**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

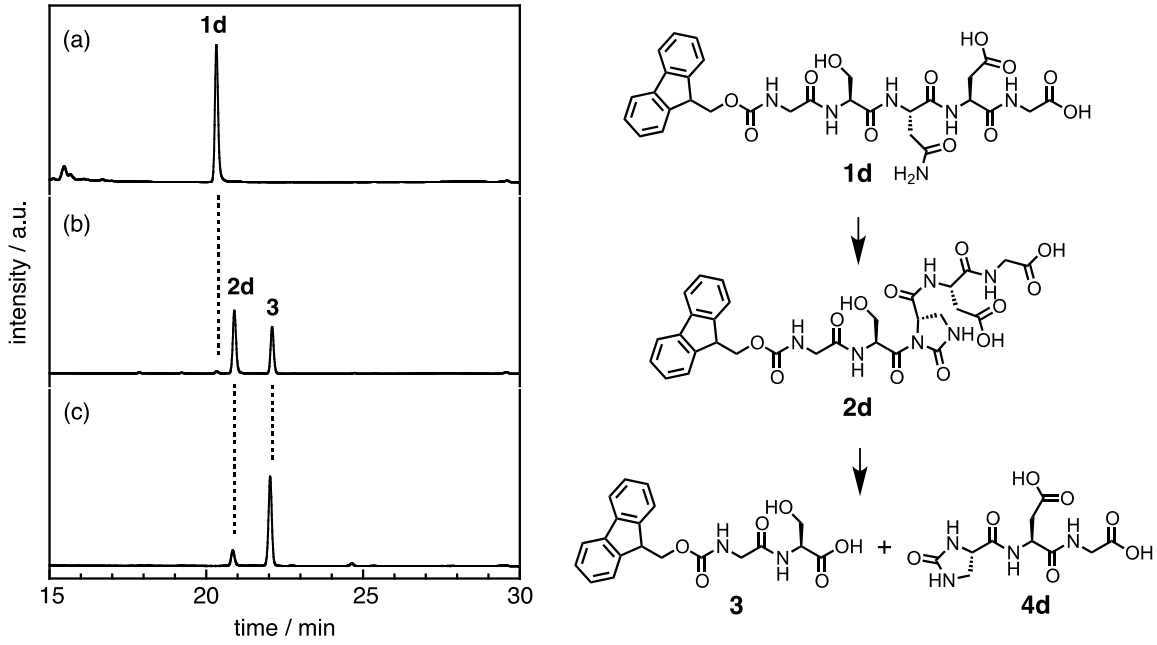

Figure S7. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Asp-Gly-OH (**1d**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

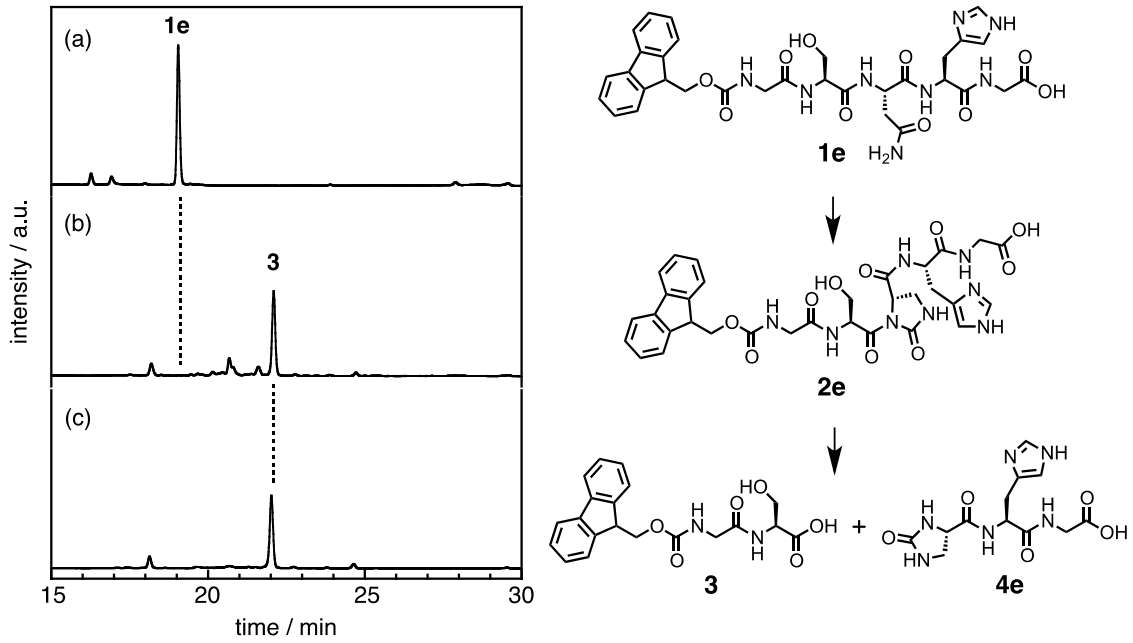

Figure S8. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-His-Gly-OH (**1e**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

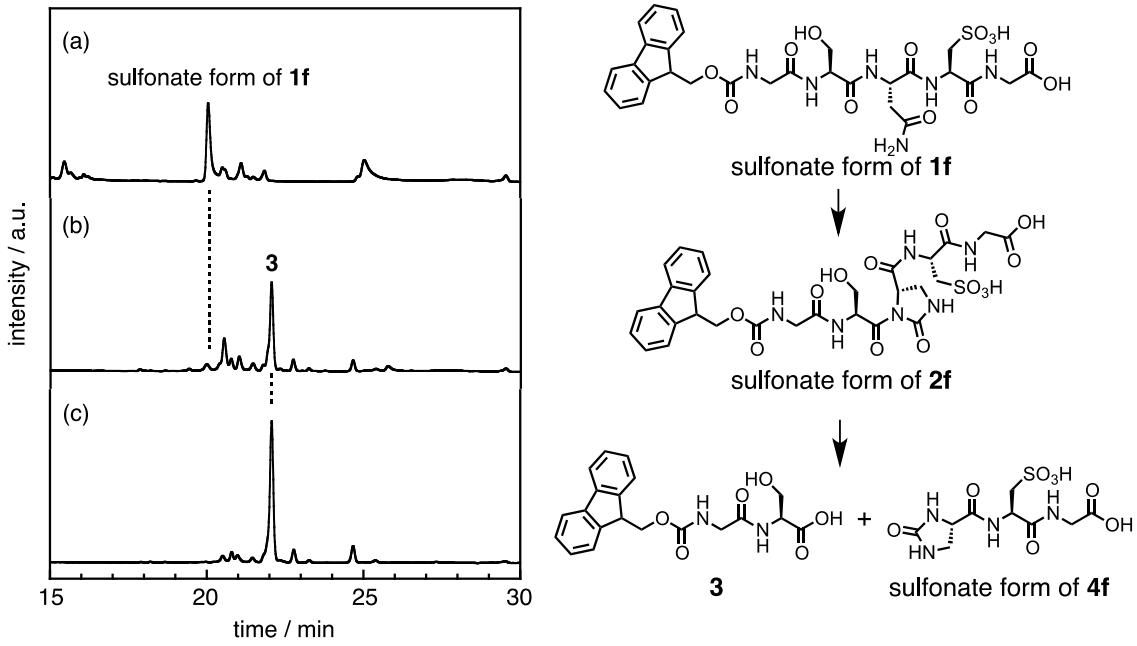

Figure S9. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Cys-Gly-OH (**1f**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

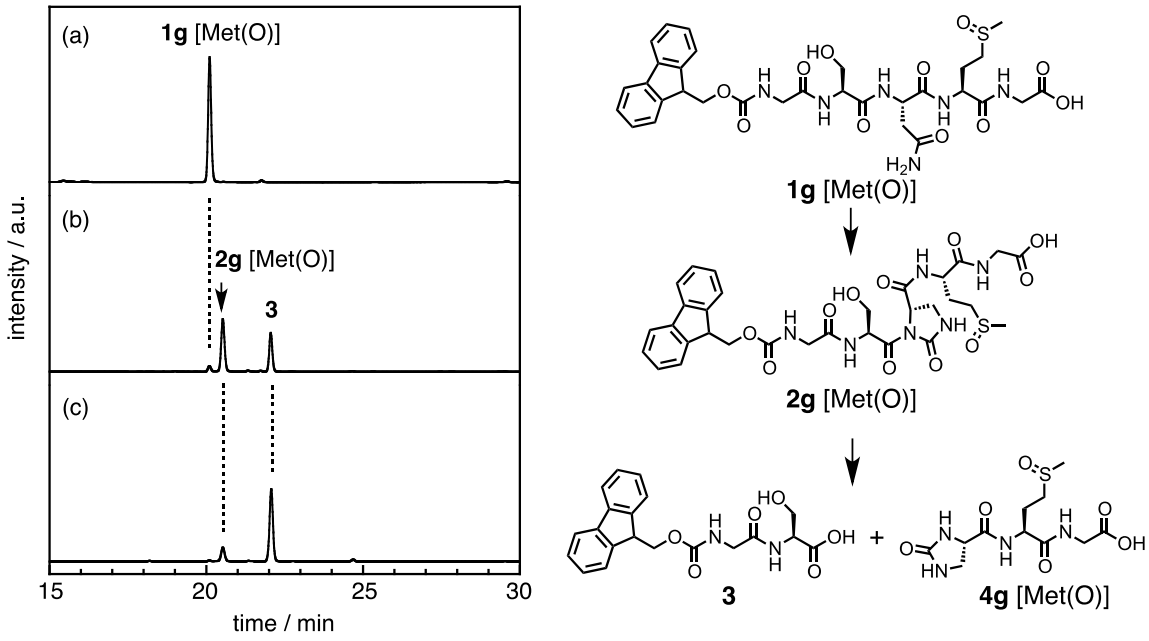

Figure S10. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Met-Gly-OH (**1g**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

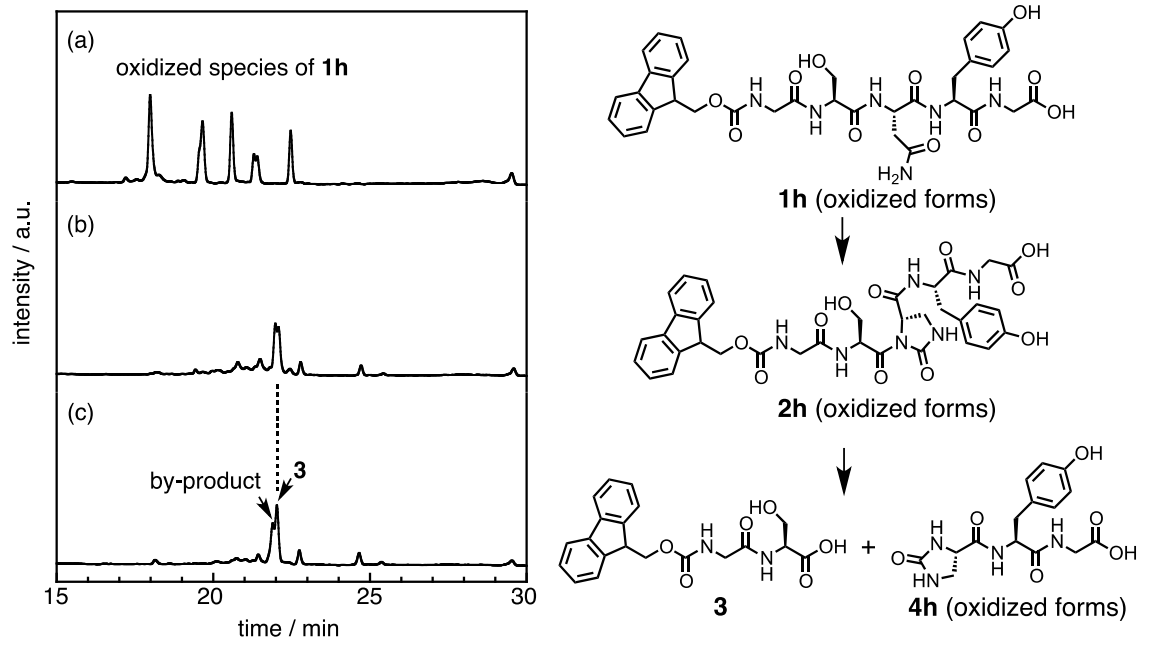

Figure S11. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Tyr-Gly-OH (**1h**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

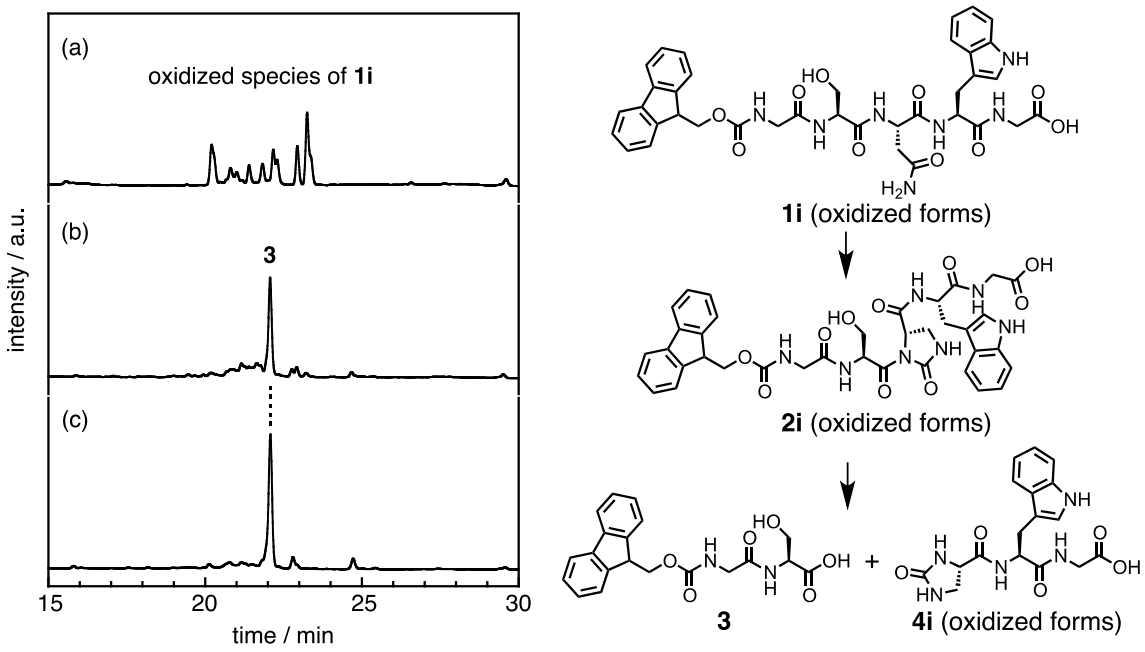

Figure S12. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Trp-Gly-OH (**1i**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC.

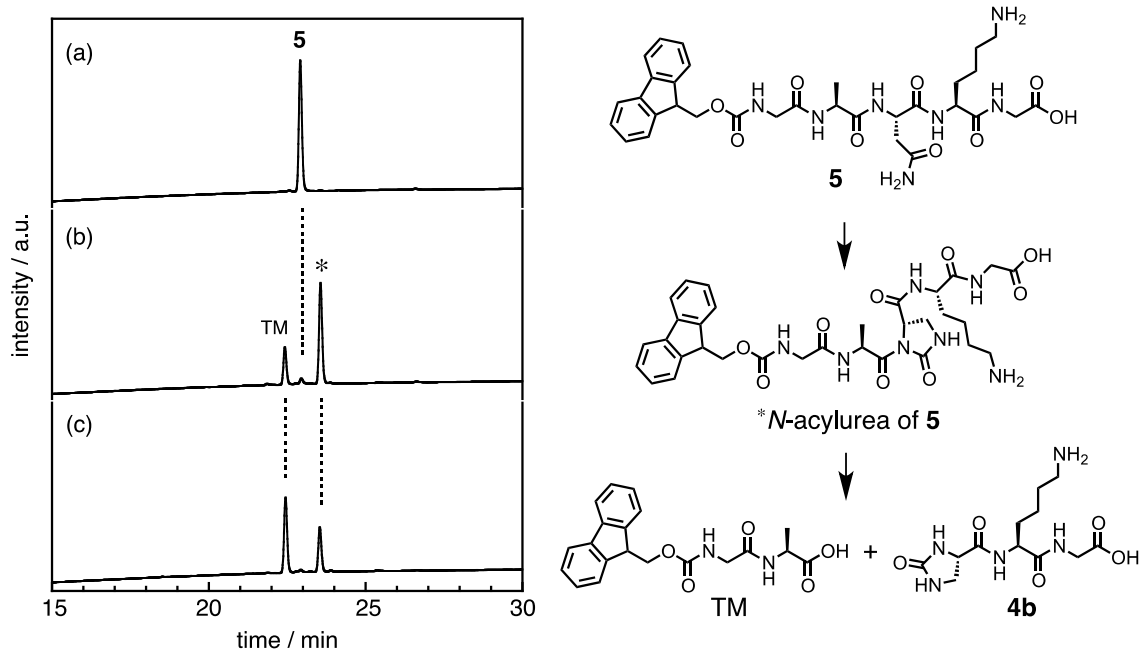

Figure S13. HPLC charts for the reaction of Fmoc-Gly-Ala-Asn-Lys-Gly-OH (**5**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC. TM: Fmoc-Gly-Ala-OH. **N*-acylurea form.

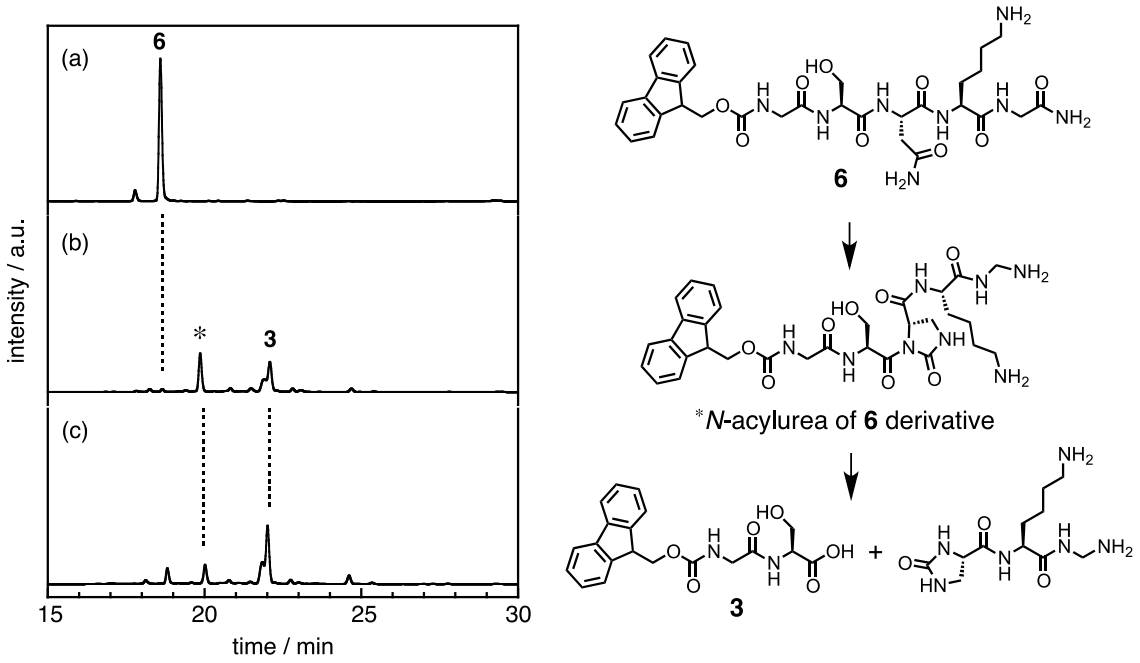

Figure S14. HPLC charts for the reaction of Fmoc-Gly-Ser-Asn-Lys-Gly-NH_2 (**6**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC. **N*-acylurea form.

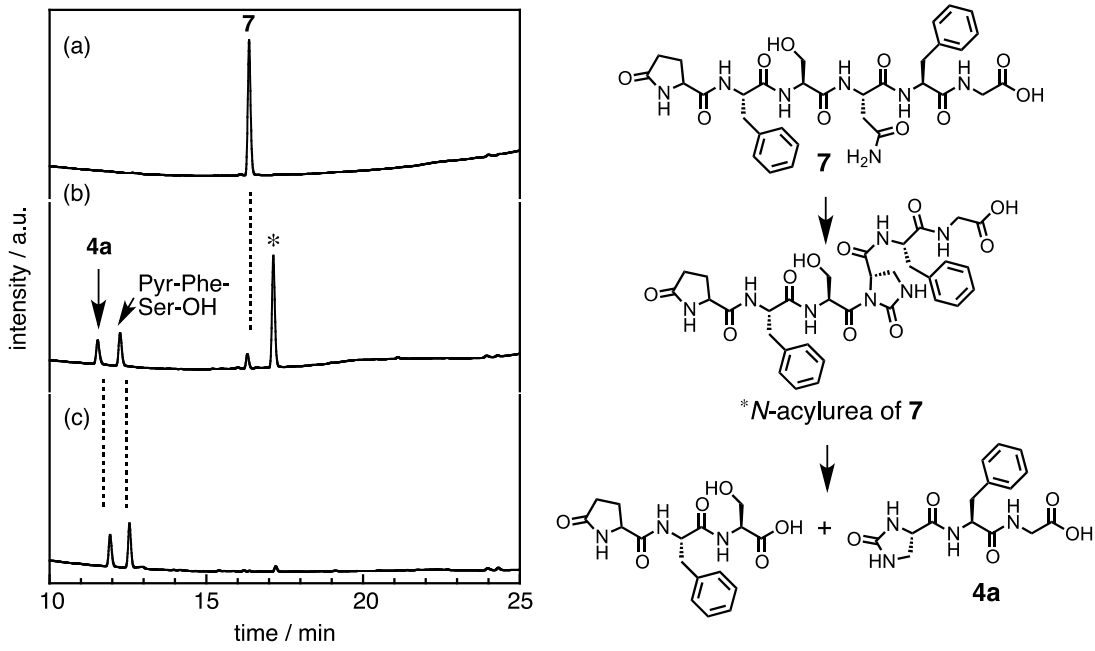

Figure S15. HPLC charts for the reaction of Pyr-Phe-Ser-Asn-Phe-Gly-OH (**7**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC. Monitoring wavelength is 230 nm. **N*-acylurea form. Each product was identified using ESI-TOF MS.

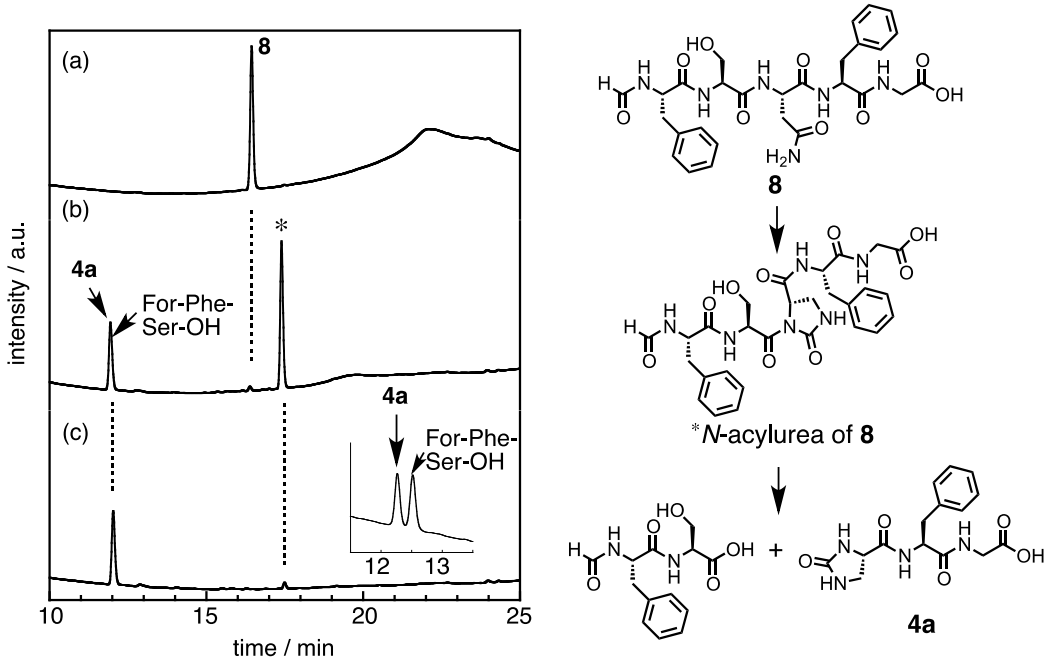

Figure S16. HPLC charts for the reaction of For-Phe-Ser-Asn-Phe-Gly-OH (**8**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC. Monitoring wavelength is 230 nm. **N*-acylurea form. Two peaks of target materials were overlapped into one peak by using cosmosil-packed ODS column. Inset shows another HPLC chart obtained using YMC-packed ODS column. Each product was identified using ESI-TOF MS.

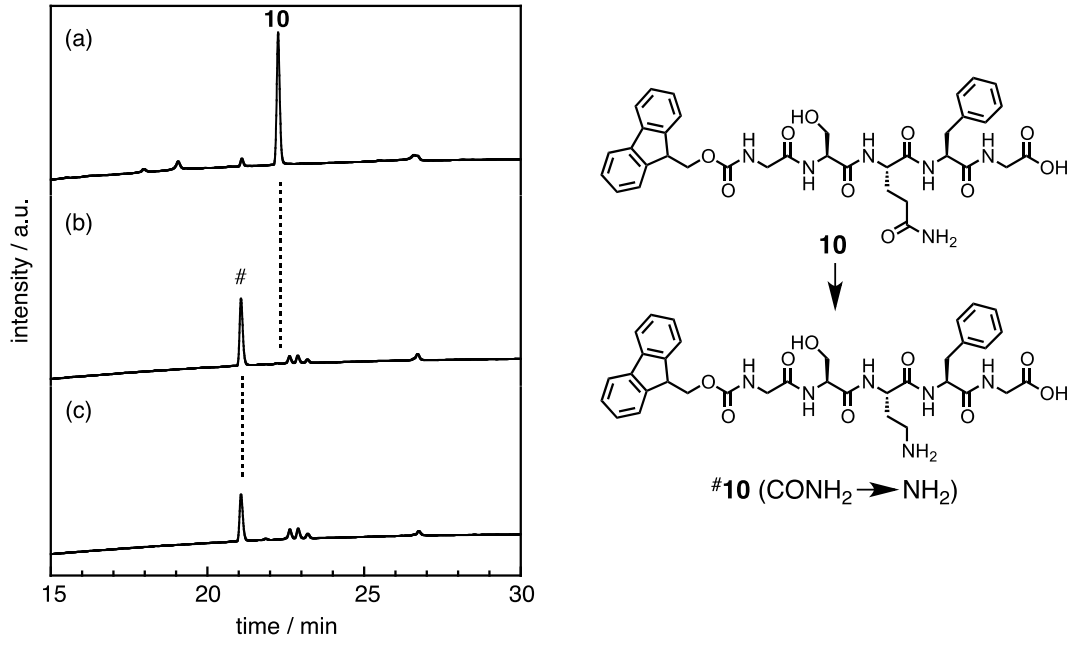

Figure S17. HPLC charts for the reaction of Fmoc-Gly-Ser-Gln-Phe-Gly-OH (**10**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC. #Fmoc-Gly-Ser-Gln($CONH_2$→NH_2)-Phe-Gly-OH.

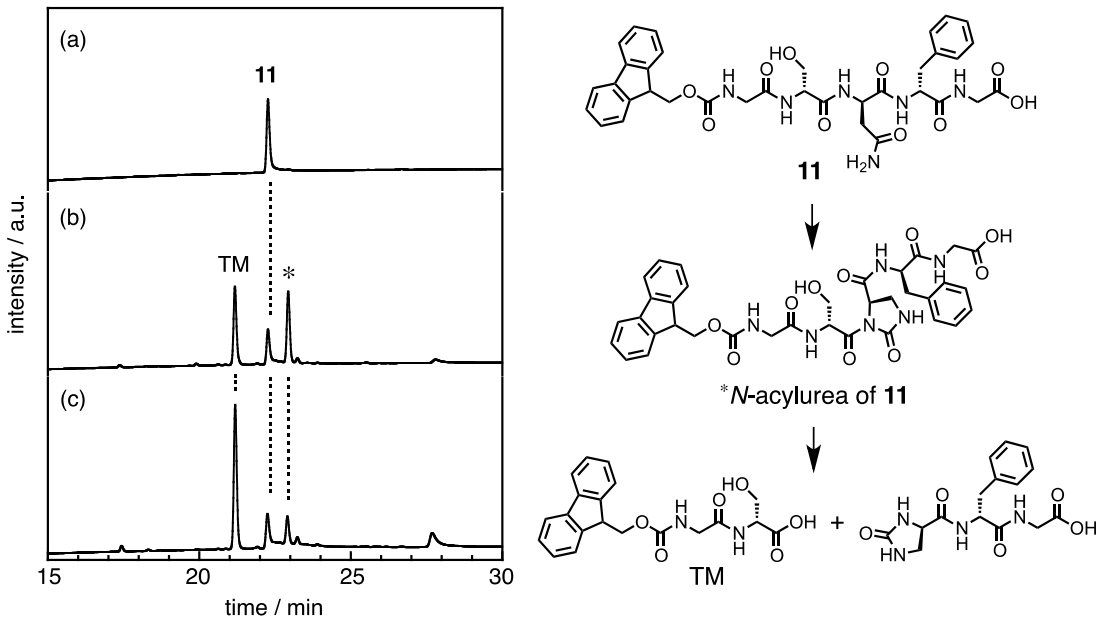

Figure S18. HPLC charts for the reaction of Fmoc-Gly-*D-Ser-D-Asn-D-Phe*-Gly-OH (**11**) with 3 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 72 h incubation at 37 ºC. TM = Fmoc-Gly-*D-Ser*-OH. **N*-acylurea form.

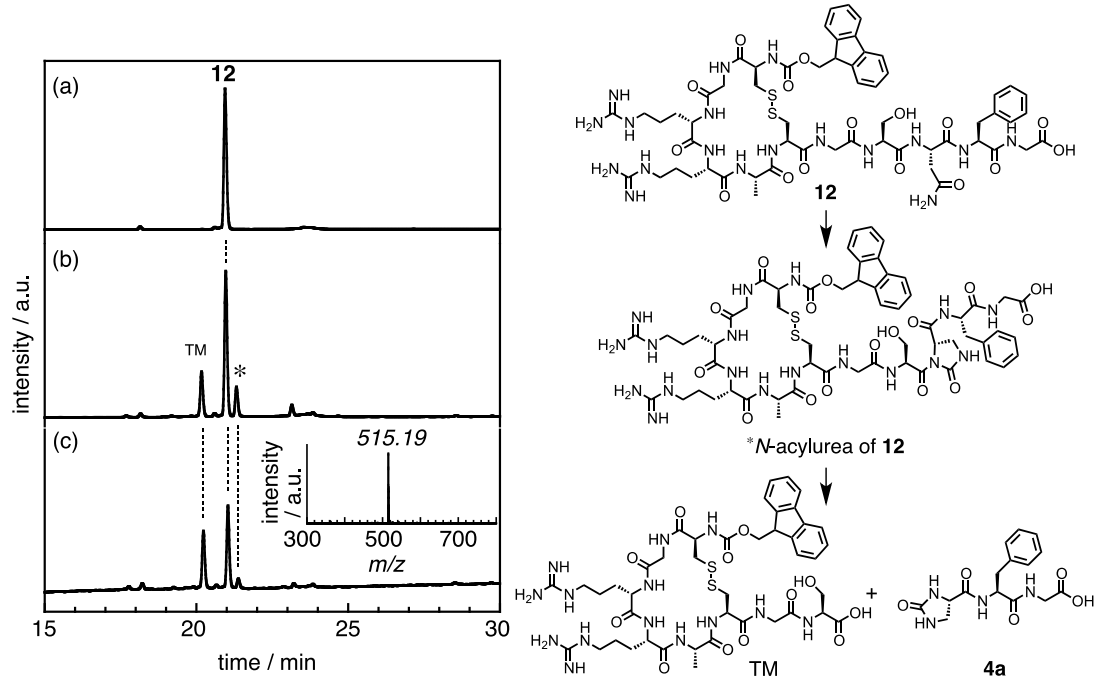

Figure S19. HPLC charts for the reaction of Fmoc-Cys-Gly-Arg-Arg-Ala-Cys-Gly-Ser-Asn-Phe-Gly-OH, disulfide bond (**12**) with 1 eq. of DIB in 0.1 M phosphate buffer (pH 7.4) after (a) 0 h, (b) 24 h, and (c) 96 h incubation at 37 ºC. Inset shows a mass spectrum of TM (calcd. *m/z* for $[M+2H]^{2+}$ = 515.21). TM = Fmoc-Cys-Gly-Arg-Arg-Ala-Cys-Gly-Ser-OH (disulfide bond). Cyclourea-Phe-Gly-OH was also detected by LC/MS analysis with *m/z* of 335.12 (calcd. *m/z* for $[M+H]^+$ = 335.14). **N*-acylurea form.

5. LC/MS Charts for the Reactions with Biologically Active Peptides

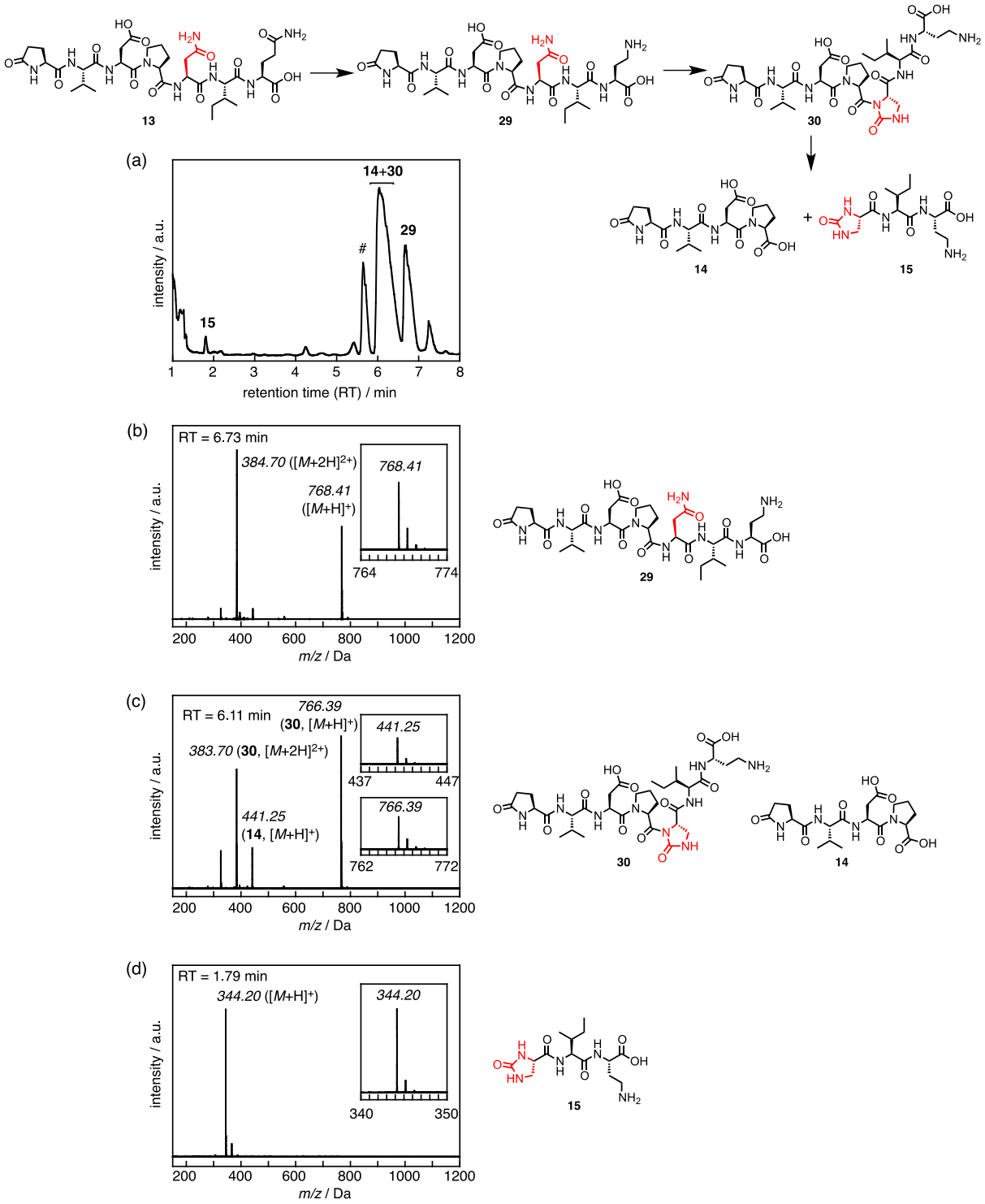

Figure S20. Reaction of Pyr-Val-Asp-Pro-Asn-Ile-Gln-OH (**13**) with 3 eq. of DIB after 4 days incubation at 37 ºC: (a) Total ion chromatogram obtained by using linear gradient of 5–20% acetonitrile in 0.1% aqueous formic acid over 11 min; (b) MS spectrum at a retention time of 6.73 min, corresponding to **29** (calcd. *m/z* for $[M+H]^+$ = 768.39); (c) MS spectrum at a retention time of 6.11 min, corresponding to **30** (calcd. *m/z* for $[M+H]^+$ = 766.37) and **14** (calcd. *m/z* for $[M+H]^+$ = 441.20); (d) MS spectrum at a retention time of 1.79 min, corresponding to **15** (calcd. *m/z* for $[M+H]^+$ = 344.19). #Pyr-Val-Asp-Pro-

Asn($CONH_2 \rightarrow NH_2$)-Ile-Gln($CONH_2 \rightarrow NH_2$)-OH, generated via the Hofmann rearrangement of **13** followed by hydrolysis of isocyanate at both Asn and Gln residues.

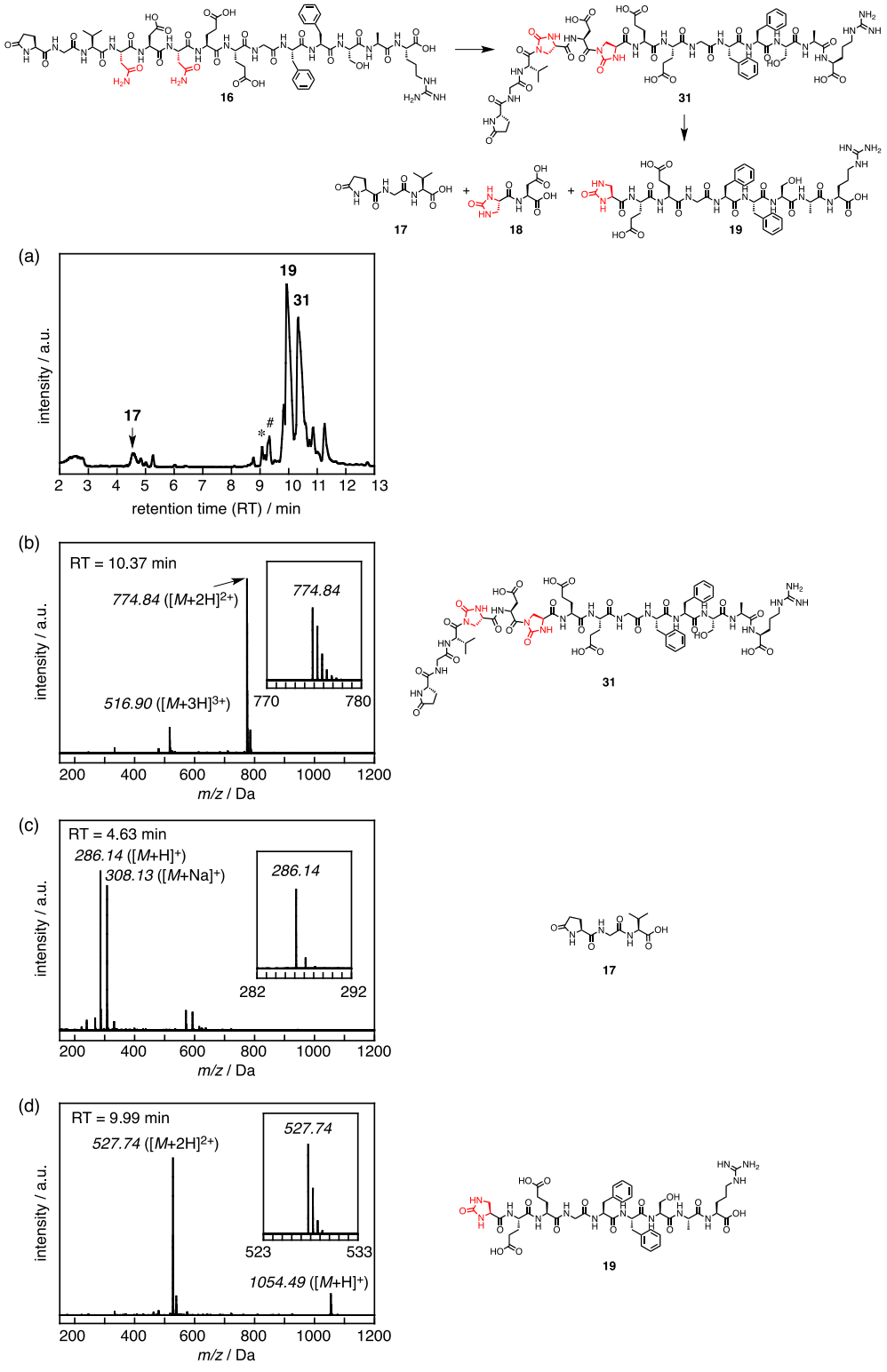

Figure S21. Reaction of fibrinopeptide B (**16**) with 3 eq. of DIB after 3 days incubation at 37 ºC: (a) Total ion chromatogram obtained by using linear gradient of 2–25% acetonitrile in 0.1% aqueous formic acid over 11 min; (b) MS spectrum at a retention time of 10.37 min, corresponding to **31** (calcd. *m/z* for $[M+2H]^{2+}$ = 774.82); (c) MS spectrum at a retention time of 4.63 min, corresponding to **17** (calcd. *m/z* for $[M+H]^{+}$ = 286.14); (d) MS spectrum at a

retention time of 9.99 min, corresponding to **19** (calcd. *m/z* for $[M+2H]^{2+}$ = 527.73). $^{*,\#}N$-acylurea at one Asn residue and $CONH_2 \rightarrow NH_2$ conversion at side chain of the other Asn.

6. Reactions with a Free *N*-terminal Peptide

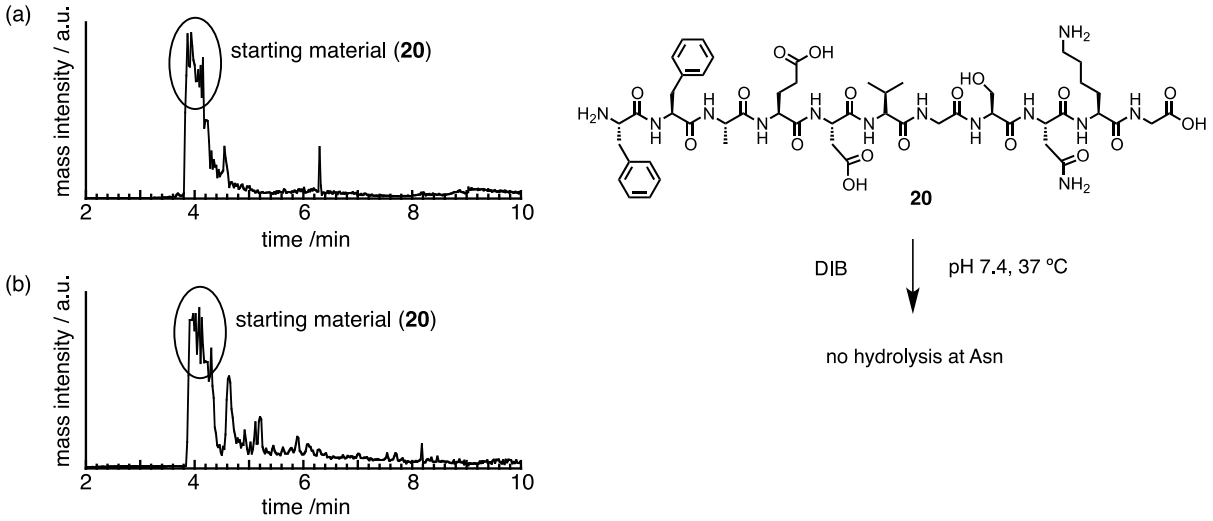

Figure S22. Total ion chromatogram of LC/MS analyses for the reaction using Aß19–29 (**20**): (a) starting material (**20**); (b) after reaction with DIB for 72 h. The right scheme indicates the reaction of **20** with DIB.

Acetylation and Cleavage of Aß19–29 (20). To a 45 µL of aqueous solution of **20** (0.13 mg, 0.1 µmol), acetic anhydride (1.0 µL, 10.0 µmol) and 5 µL of aqueous NaOH (0.5 mg, 12.5 µmol) were added, and the reaction mixture was incubated at 37 ºC for 4 h. Then, a solution of DIB (0.096 mg, 0.3 µmol) in 0.2 M phosphate buffer (pH 7.4) was added into the reaction mixture, and the reaction mixture was incubated at 37 ºC for 72 h.

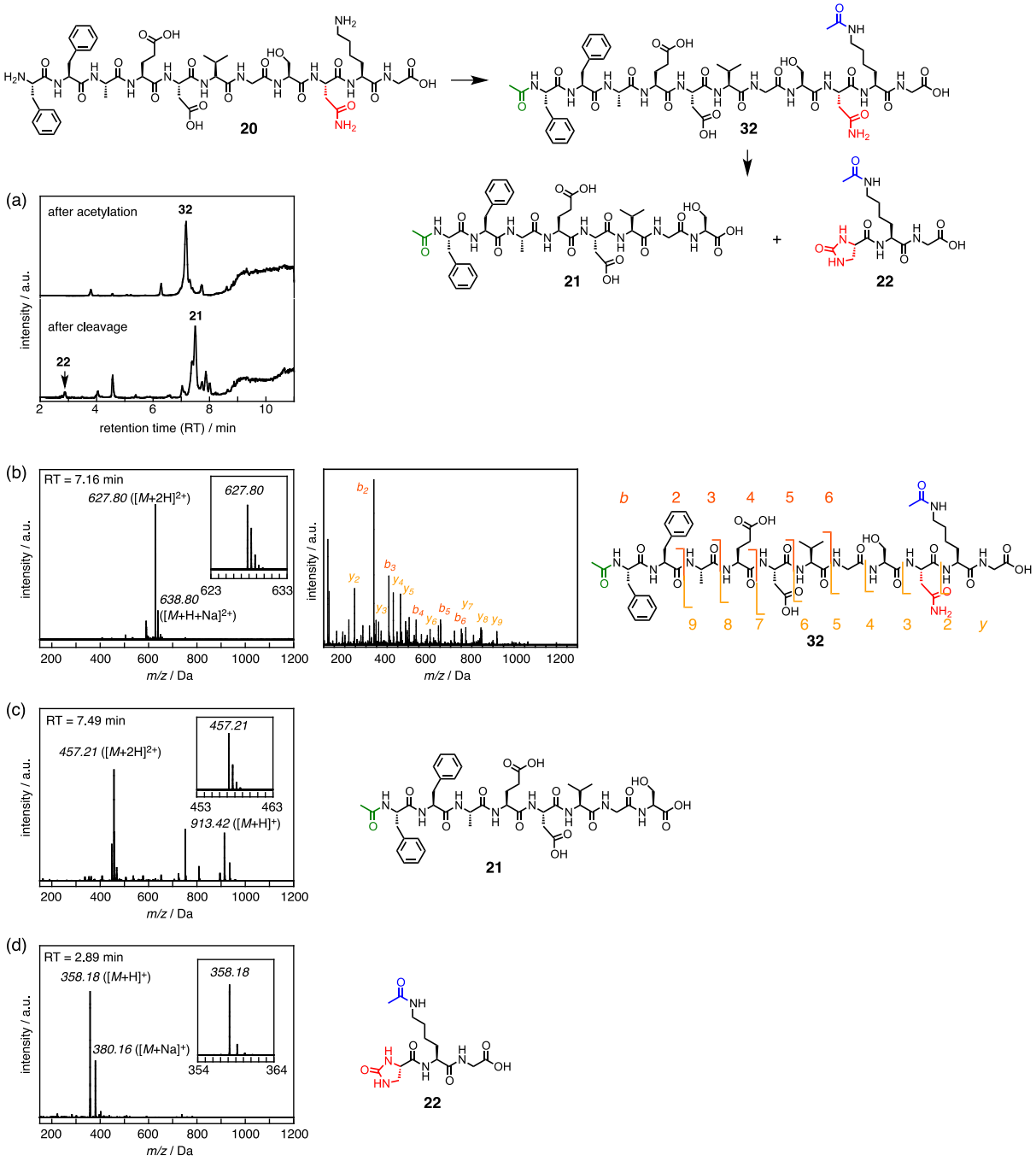

Figure S23. One-pot acetylation (100 eq. Ac_2O, 125 eq. NaOH, 37 ºC, 30 min) plus DIB treatment (3 eq. DIB, 37 ºC, 72 h) of Aß19–29 (**20**): (a) Total ion chromatogram at an linear gradient of 2–35% acetonitrile in 0.1% aqueous formic acid over 6 min with a flow rate of 20 µL min^{-1}; (b) MS spectrum (left) and MS/MS spectrum (right) at a retention time of 7.16 min (upper chromatograph of a), corresponding to **32** (calcd. *m/z* for $[M+2H]^{2+}$ = 627.79); (c) MS spectrum at a retention time of 7.49 min (lower chromatograph of a), corresponding to **21** (calcd. *m/z* for $[M+H]^{+}$ = 457.20); (d) MS spectrum at a retention time of 2.89 min (lower chromatograph of a), corresponding to **22** (calcd. *m/z* for $[M+H]^{+}$ = 358.17).

7. Photo-Oxidation and Enzymatic Digestion of [Pyr^3]Aß3–16[Asp7Asn]

Photo-Oxidation of [Pyr^3]Aß3–16[Asp7Asn] (26) using Riboflavin. 0.1% aq. TFA solution of **26** (1 mM, 20 μL) was diluted with an equal volume of 0.2 M aq. ammonium bicarbonate, and additional 0.1 M aq. ammonium bicarbonate (60 μL) was added to obtain 200 μM of the peptide solution. To the solution, 5 μL of riboflavin (200 μM) in water-ethanol (1 : 1) was added to obtain a 10 μM (5 mol%) final concentration of riboflavin. The mixture was irradiated with a fluorescent lamp (24 W, natural white, approximately 3 cm distance) at room temperature for 4 h to afford **27**. A reaction mixture without light irradiation was also prepared as a control sample (unoxidized **26**).

Enzymatic Digestion. To 30 μL of the reaction mixture containing oxidized **27** or unoxidized **26**, 2 μL of endoproteinase Glu-C or chymotrypsin (0.1 mg mL^{-1}; F. Hoffmann-La Roche Ltd., Basel, Switzerland) dissolved in water was added, and incubated at room temperature for 18 h.

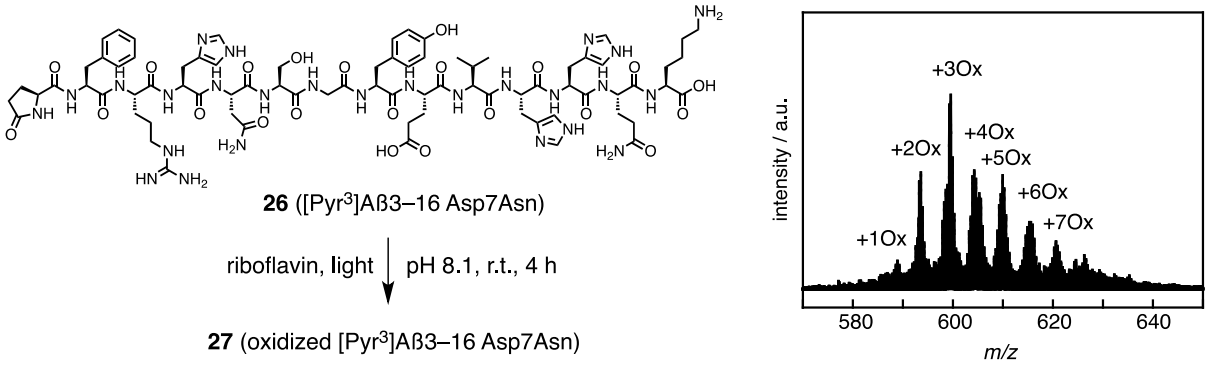

Figure S24. Scheme of oxidation of [Pyr^3]Aß3–16[Asp7Asn] (**26**) and ESI-TOF MS spectrum of oxidized [Pyr^3]Aß3–16[Asp7Asn] (**27**).

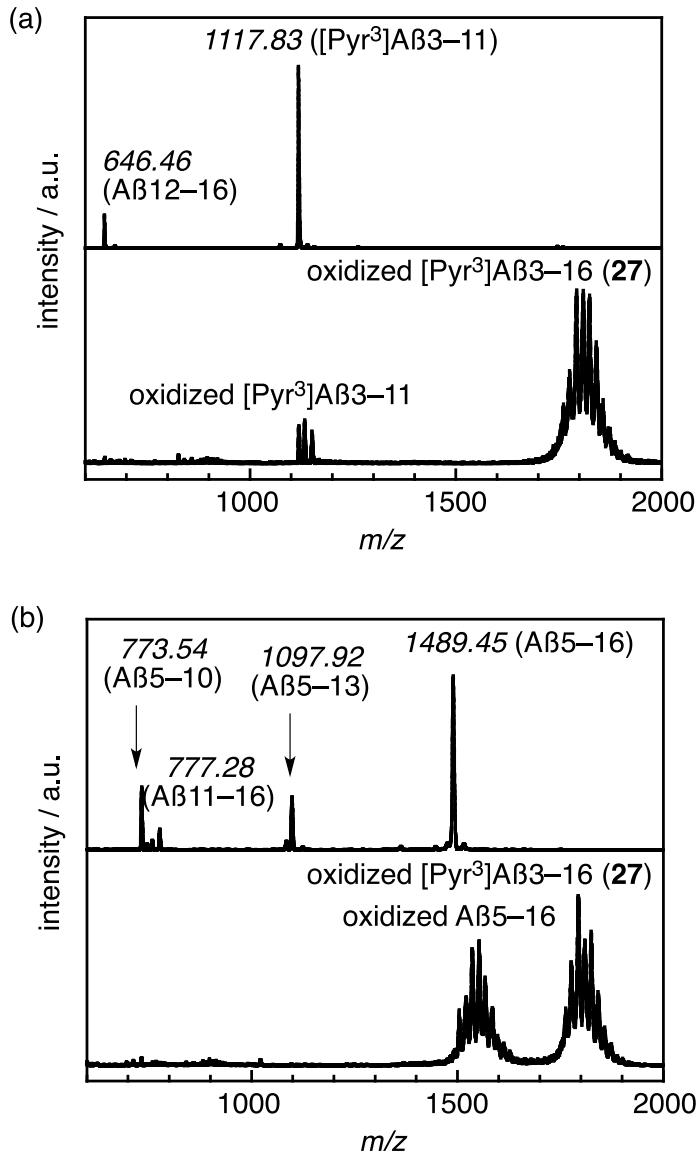

Figure S25. MALDI-TOF MS spectra of **26** and **27** after treatment with (a) Glu-C and (b) chymotrypsin. For both charts, the upper spectra indicate MS spectra of **26 (**unoxidized), and the bottom spectra indicate those of **27** (oxidized). (a) Peaks of oxidized [Pyr^3]Aβ3–11 include 1133.8 and 1150.8, and peaks of oxidized [Pyr^3]Aβ3–16 (**27**) include 1776.3, 1793.0, 1809.5, 1825.6, and 1840.1; (b) peaks of oxidized Aβ5-16 include 1504.2, 1520.2, 1535.6, 1552.8, 1567.2, and 1583.9, and peaks of oxidized [Pyr^3]Aβ3–16 (**27**) include 1776.7, 1793.6, 1809.2, 1824.9, and 1840.8.

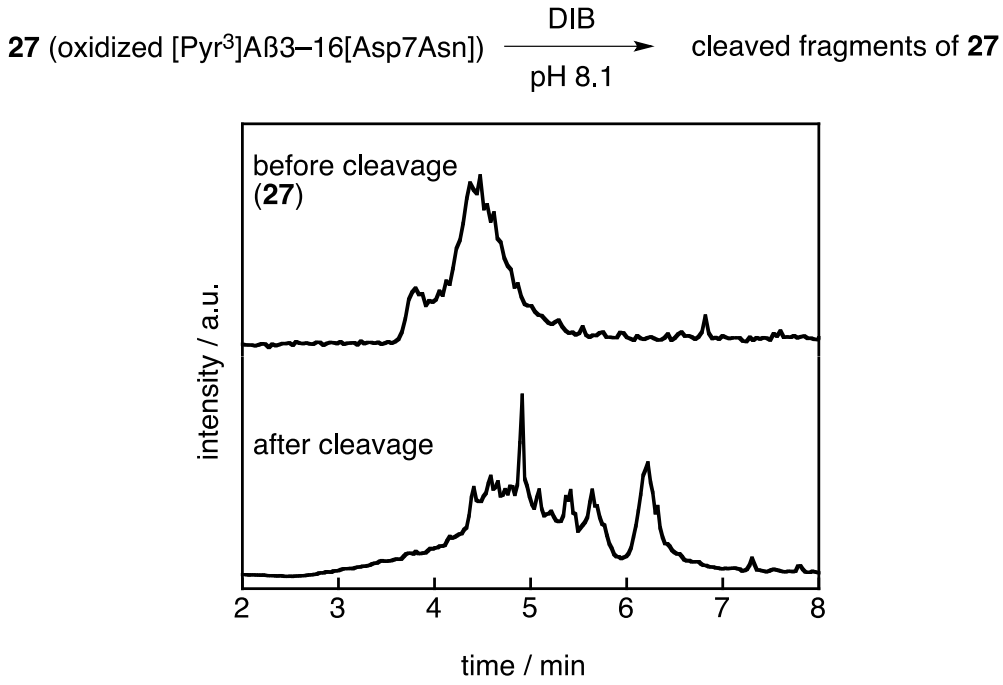

Figure S26. Total ion chromatographs of **27**, before (upper) and after (lower) cleavage by DIB, obtained at an linear gradient of 2–35% acetonitrile in 0.1% aqueous formic acid over 6 min with a flow rate of 20 μL min^{-1}