Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Scandium(III) triflate-promoted serine/threonine-selective peptide bond cleavage

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1. General Methods

General. Analytical HPLC chart was obtained 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 was conducted 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 (ESI-Q) analysis was performed using Agilent Technologies 6120 Quadrupole LC/MS equipped with 1260 infinity LC. MALDI-TOF MS analysis was carried out with a Shimadzu Biotech Axima ToF2 spectrometer. Column chromatography was performed with silica gel Merck 60 (230-400 mesh ASTM). Reagents were purchased from Aldrich, Tokyo Chemical Industry Co., Ltd. (TCI), Kanto Chemical Co., Inc., Wako Pure Chemical Industries, Ltd., Peptides Institute, Inc., Watanabe Chemical Industries, Ltd., and Nacalai Tesque, Inc. and used without further purification. 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 with a YMC-Triart-C18 column (4.6 mm l.D × 150 mm) using a linear gradient of 0–100% acetonitrile in 0.1% aqueous TFA over 60 min with a flow rate of 1 mL min^{-1} at 40 ^{o}C, detected by absorbance at 254 nm or 230 nm.

Analytical LC/MS.

Reactions were monitored by LC/MS using a gradient of acetonitrile versus 0.1% formic acid in water. LC was carried out with a YMC-Triart-C18 column (4.6 mm l.D × 150 mm) using a linear gradient of 0–100% acetonitrile in 0.1% aqueous formic acid over 50 min with a flow rate of 1 mL min^{-1} at 40 ^{o}C, detected by absorbance at 254 nm or 230 nm and on-line ESI-Q 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 with a YMC-Triart C18 column (10 mm l.D × 250 mm) using a linear gradient of 0-100% acetonitrile in 0.1% aqueous TFA over 100 min with a flow rate of 3.0 mL min^{-1} at 40 ^{o}C, detected 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. Fmoc-protected amino acids were sequentially coupled using a N,N'-diisopropylcarbodiimide and

1-hydroxybenzotriazole after removal of each Fmoc group with 20% piperidine in DMF (10 min) to obtain a peptide-resin. Treating the obtained peptide-resin with TFA–triisopropylsilane (TIS)–water (95:2.5:2.5) for 60 min at room temperature, concentrated in vacuo, and precipitated with diethyl ether gave the crude peptide. The crude peptide was purified using a preparative HPLC with 0.1% aqueous TFA–acetonitrile system and freeze-drying of the collected fraction gave the desired peptide.

2. Experimental Details

Procedure for site-selective cleavage of dipeptide Cbz-Gly-Ser-OMe (1a) and tripeptide Cbz-Phe-Gly-Ser-OMe (1b):

To a test tube with a stirring bar, peptide (0.03 mmol), scandium triflate (7.4 mg, 0.015 mmol), EtOH (0.1 mL), and water (0.1 mL) were added. The tube was sealed and heated to 100 ^{o}C for 18 h. The reaction mixture was analyzed by analytical reverse-phase HPLC and LC/MS spectroscopy, detected by absorbance at 254 nm. HPLC yields = (sum of peak areas of products) / (sum of peak areas of starting material, products, and byproducts).

Representative procedure for site-selective cleavage of bradykinin and its derivatives:

In a sealed tube with a stirring bar, a mixture of peptide (2 mM) and scandium triflate (200 mM) in water was heated to 80 ^{o}C for 40 h. The reaction mixture was analyzed by analytical reverse-phase HPLC and LC/MS spectroscopy, detected by absorbance at 230 nm. HPLC yields = (sum of peak areas of products) / (sum of peak areas of starting material, products, and byproducts).

Enzymatic digestion:

To an Eppendorf tube with phosphate buffer (pH 7.4, 44 μL), peptide substrate **1k** or **1l** (1 μL, 10 mM in water) and endoproteinase Lys-C (5 μL, 20 μg mL^{-1} in water, F. Hoffmann-La Roche Ltd., Basel, Switzerland) was added, incubated at 37 °C for 14 h and analyzed by HPLC or LC/MS.

3. Supporting Data

Analytical data of starting materials.

Cbz-Gly-Ser-OMe (**1a**) MS (ESI): *m/z* 311.1 (calcd $[M+H]^+$ = 311.1). Purity: >95%. Retention time: 17.58 min (HPLC analysis at 254 nm).

Cbz-Phe-Gly-Ser-OMe (**1b**) MS (ESI): *m/z* 458.2 (calcd $[M+H]^+$ = 458.2). Purity: >95%. Retention time: 23.18 min (HPLC analysis at 254 nm).

H-Tyr-Ile-Gly-Ser-Arg-NH_2 (**1c**) MS (ESI): *m/z* 594.4 (calcd $[M+H]^+$ = 594.3). Purity: >95%. Retention time: 11.48 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH (**1d**) MS (ESI): *m/z* 1060.6 (calcd $[M+H]^+$ = 1060.6). Purity: >95%. Retention time: 16.58 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg-OH (**1e**) MS (ESI): *m/z* 1074.6 (calcd $[M+H]^+$ = 1074.6). Purity: >95%. Retention time: 16.58 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Asn-Ser-Pro-Phe-Arg-OH (**1f**) MS (ESI): *m/z* 1027.5 (calcd $[M+H]^+$ = 1027.5). Purity: >95%. Retention time: 13.94 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Met-Ser-Pro-Phe-Arg-OH (**1g**) MS (ESI): *m/z* 1044.5 (calcd $[M+H]^+$ = 1044.5). Purity: >95%. Retention time: 15.41 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-His-Ser-Pro-Phe-Arg-OH (**1h**) MS (ESI): *m/z* 1050.6 (calcd $[M+H]^+$ = 1050.6). Purity: >95%. Retention time: 13.52 min (HPLC analysis at 230 nm).

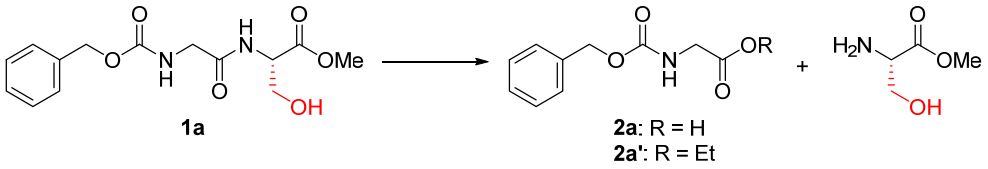
H-Arg-Pro-Pro-Gly-Tyr-Ser-Pro-Phe-Arg-OH (**1i**) MS (ESI): *m/z* 1076.6 (calcd $[M+H]^+$ = 1076.6). Purity: >95%. Retention time: 15.25 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Trp-Ser-Pro-Phe-Arg-OH (**1j**) MS (ESI): *m/z* 1099.6 (calcd $[M+H]^+$ = 1099.6). Purity: >95%. Retention time: 16.81 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Lys-Ser-Pro-Phe-Arg-OH (**1k**) MS (ESI): *m/z* 1041.6 (calcd $[M+H]^+$ = 1041.6). Purity: >95%. Retention time: 13.48 min (HPLC analysis at 230 nm).

H-Arg-Pro-Pro-Gly-Lys(Ac)-Ser-Pro-Phe-Arg-OH (**1l**) MS (ESI): *m/z* 1083.6 (calcd $[M+H]^+$ = 1083.6). Purity: >95%. Retention time: 14.55 min (HPLC analysis at 230 nm).

HPLC Charts.



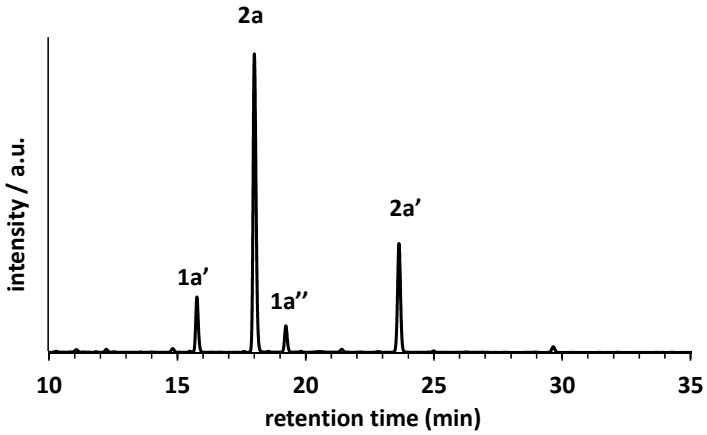


Figure S1. HPLC chart for the reaction of Cbz-Gly-Ser-OMe (**1a**, 150 mM) and $Sc(OTf)_3$ (75 mM) at 100 °C after 18 h. **1a'** = Cbz-Gly-Ser-OH, MS (ESI): *m/z* 297.1 (calcd $[M+H]^+$ = 297.1). **1a''** = Cbz-Gly-Ser-OEt, MS (ESI): *m/z* 325.1 (calcd $[M+H]^+$ = 325.1).

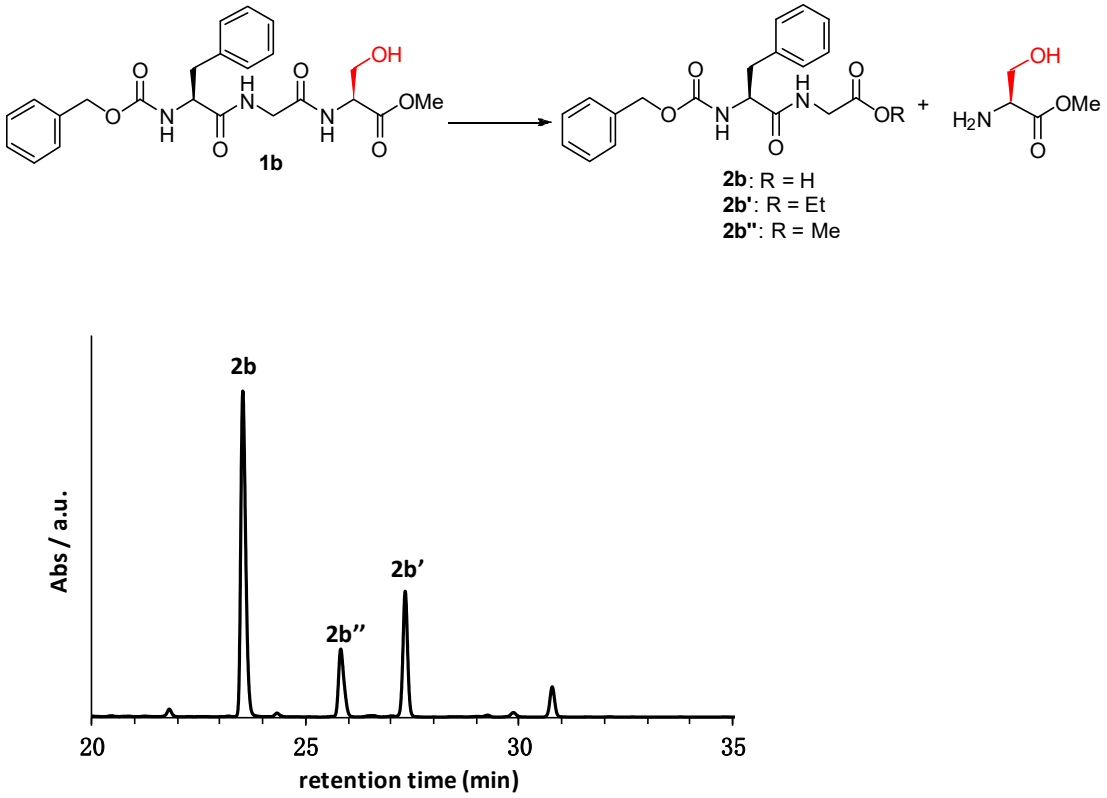
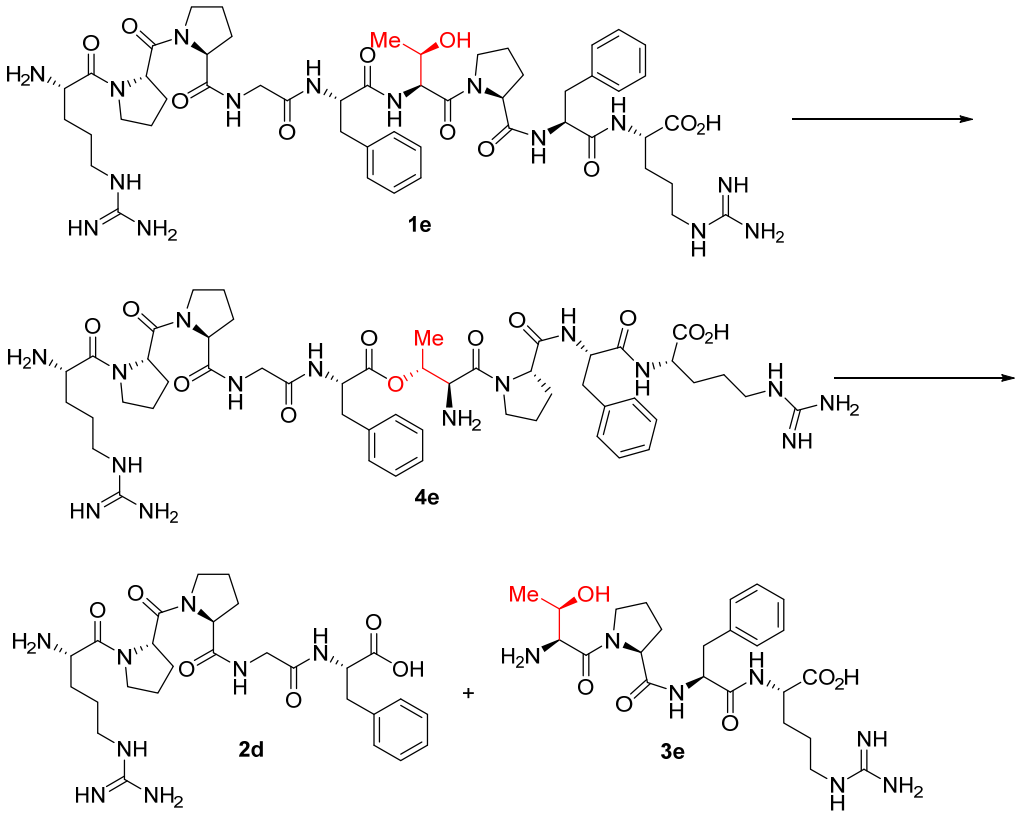


Figure S2. HPLC chart for the reaction of Cbz-Phe-Gly-Ser-OMe (**1b**, 150 mM) and $Sc(OTf)_3$ (75 mM) at 100 °C after 40 h. **2b**, MS (ESI): *m/z* 357.2 (calcd $[M+H]^+ = 357.1$). **2b'**, MS (ESI): *m/z* 385.2 (calcd $[M+H]^+ = 385.2$). **2b''**, MS (ESI): *m/z* 371.1 (calcd $[M+H]^+ = 371.2$).



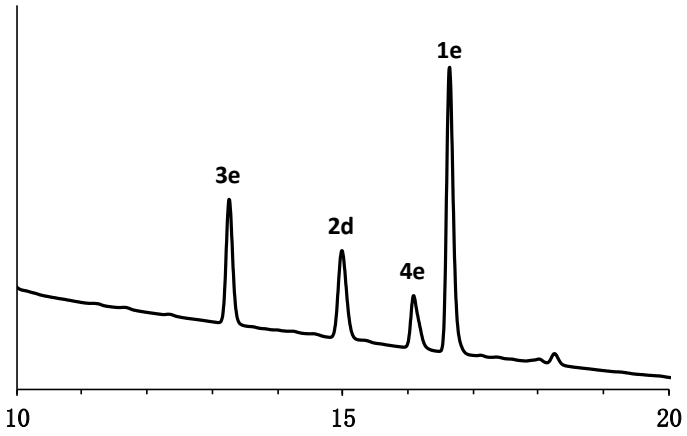


Figure S3. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-Phe-**Thr**-Pro-Phe-Arg-OH (**1e**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **3e**, MS (ESI): m/z 520.3 (calcd $[M+H]^+$ = 520.3). **2d**, MS (ESI): m/z 573.3 (calcd $[M+H]^+$ = 573.3). **4e**, MS (ESI): m/z 1074.6 (calcd $[M+H]^+$ = 1074.6).

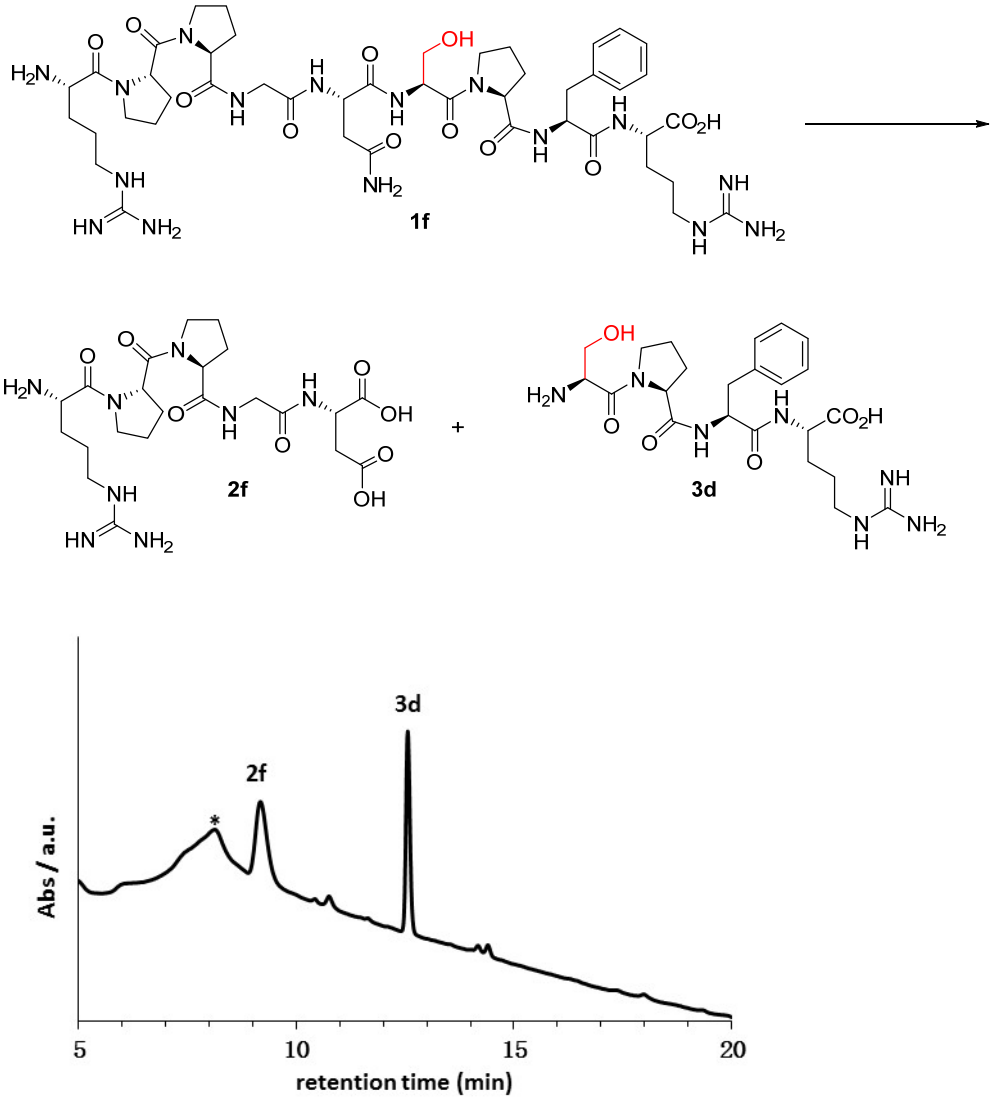


Figure S4. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-Asn-**Ser**-Pro-Phe-Arg-OH (**1f**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **2f**, MS (ESI): m/z 541.3 (calcd $[M+H]^+$ = 541.3). **3d**, MS (ESI): m/z 506.3 (calcd $[M+H]^+$ = 506.3). *The labeled peak is from the HPLC system, but not peptidic compound.

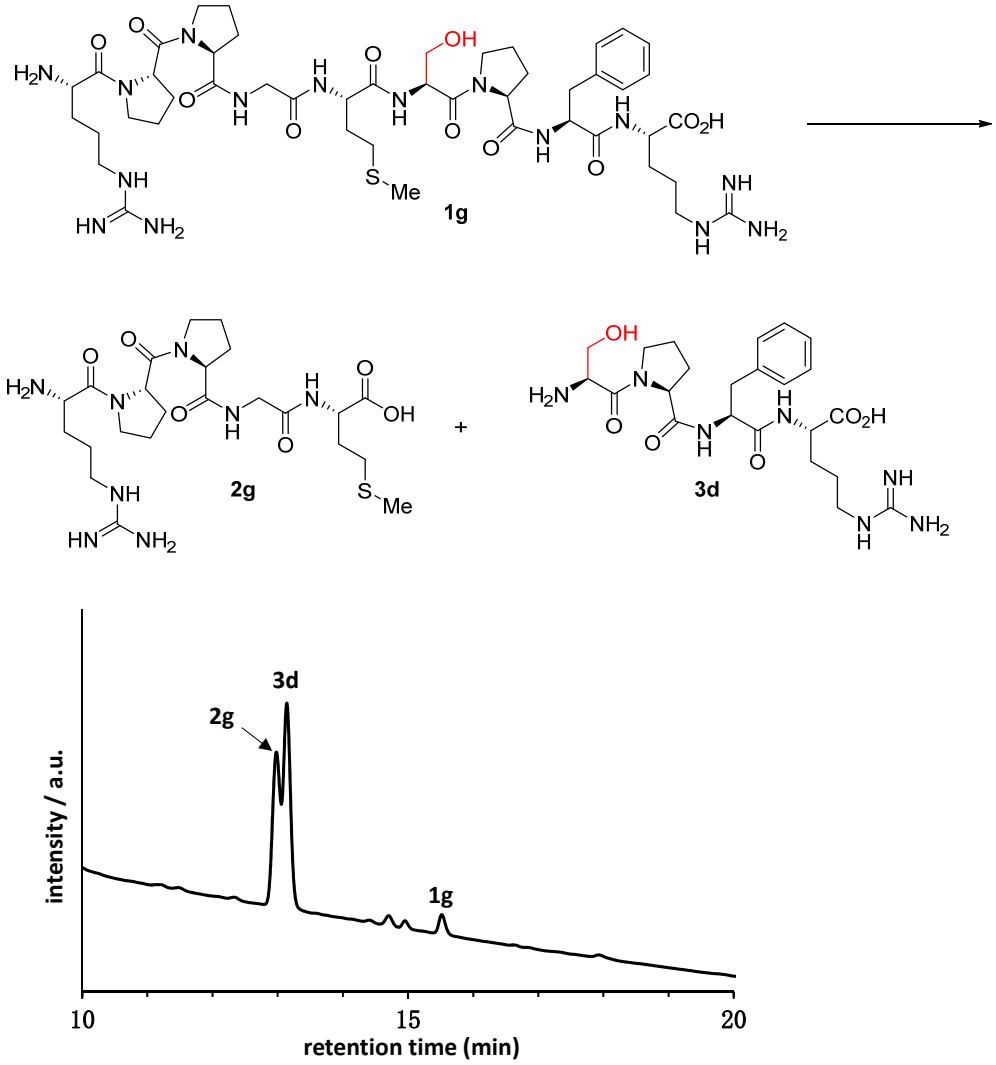


Figure S5. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-Met-**Ser**-Pro-Phe-Arg-OH (**1g**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **2g**, MS (ESI): m/z 557.3 (calcd $[M+H]^+$ = 557.3). **3d**, MS (ESI): m/z 506.3 (calcd $[M+H]^+$ = 506.3).

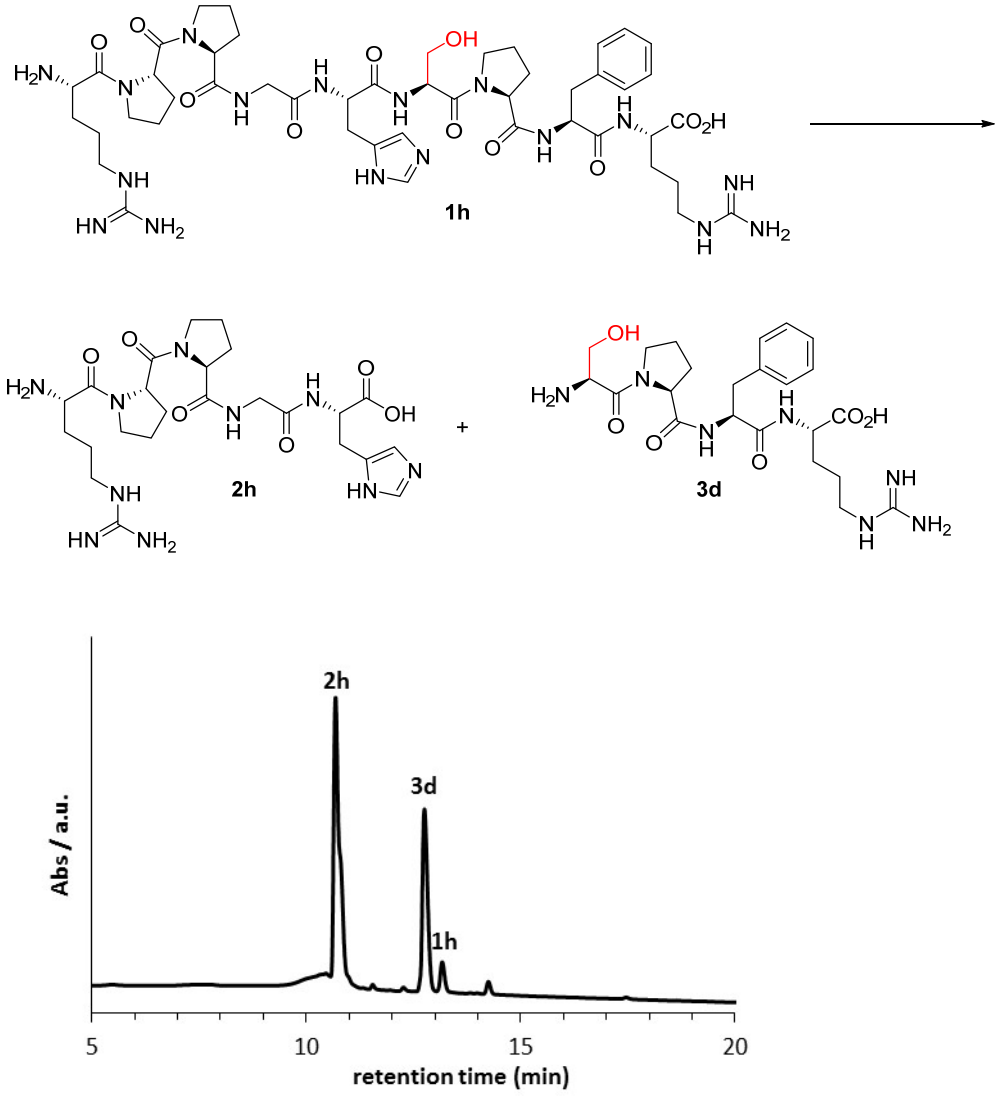


Figure S6. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-His-**Ser**-Pro-Phe-Arg-OH (**1h**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **2h**, MS (ESI): m/z 563.4 (calcd $[M+H]^+$ = 563.3). **3d**, MS (ESI): m/z 506.3 (calcd $[M+H]^+$ = 506.3).

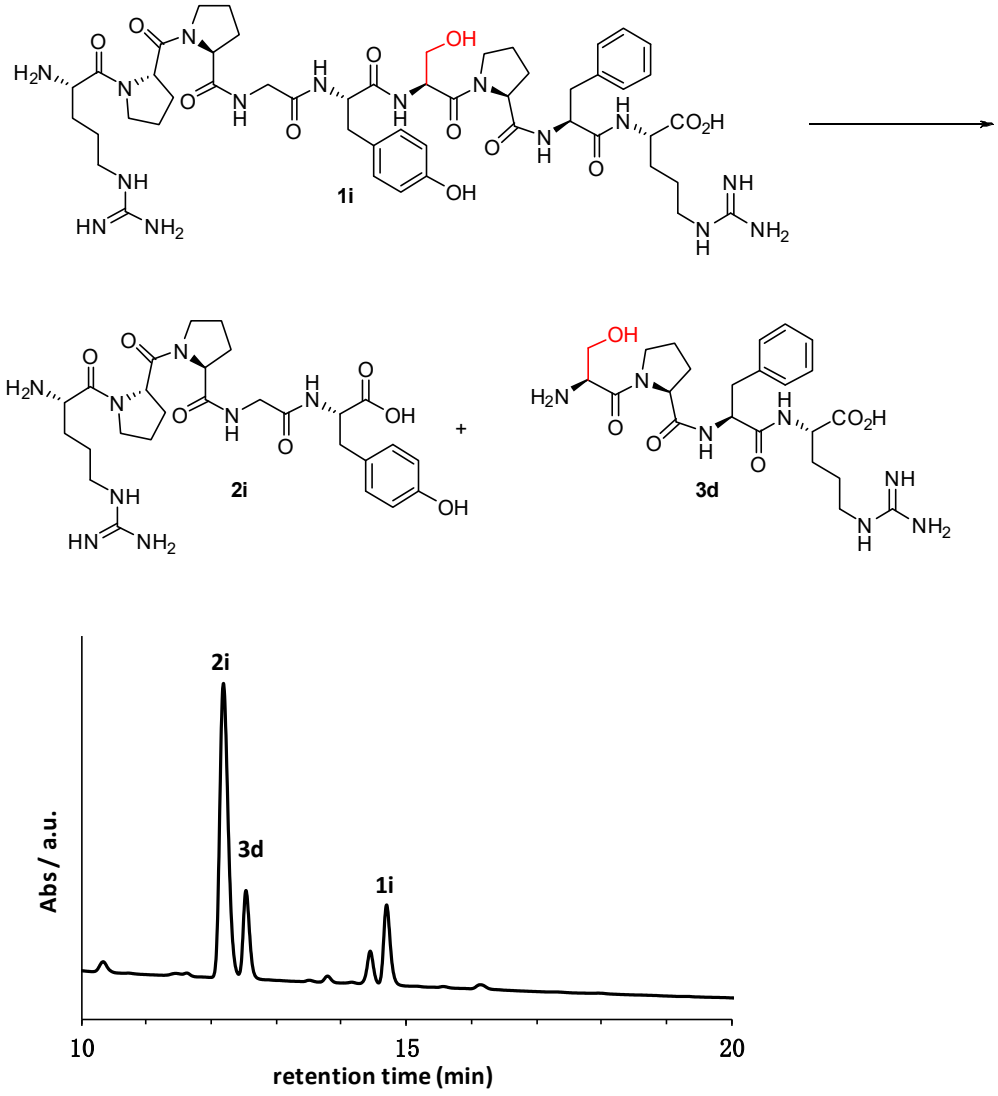


Figure S7. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-Tyr-**Ser**-Pro-Phe-Arg-OH (**1i**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **2i**, MS (ESI): m/z 589.3 (calcd $[M+H]^+$ = 589.3). **3d**, MS (ESI): m/z 506.3 (calcd $[M+H]^+$ = 506.3).

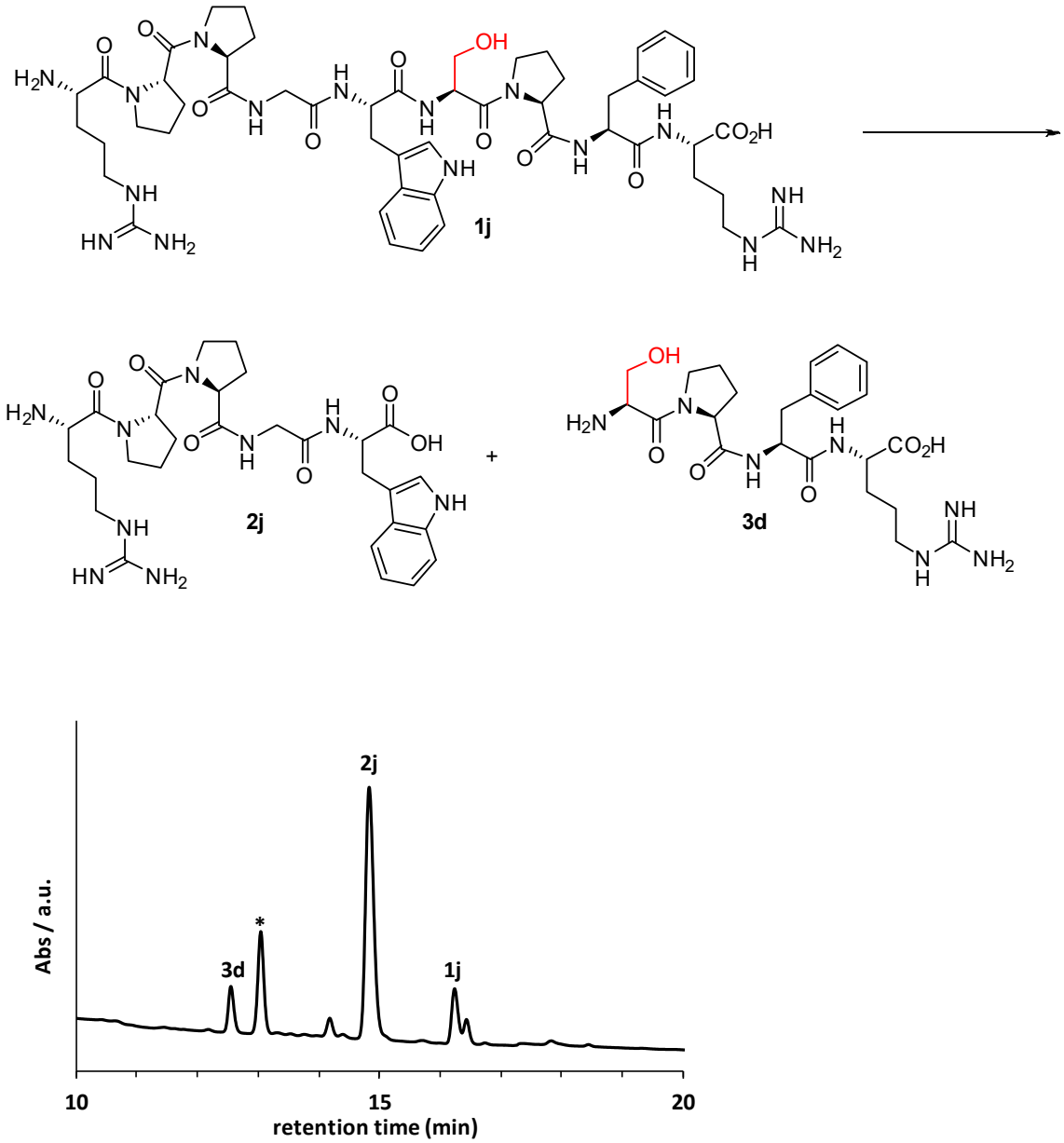


Figure S8. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-Trp-**Ser**-Pro-Phe-Arg-OH (**1j**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **3d**, MS (ESI): m/z 506.3 (calcd $[M+H]^+$ = 506.3). **2j**, MS (ESI): m/z 612.3 (calcd $[M+H]^+$ = 612.3). *MS of the labeled peak (m/z 628.4) corresponds to oxidized **2j**.

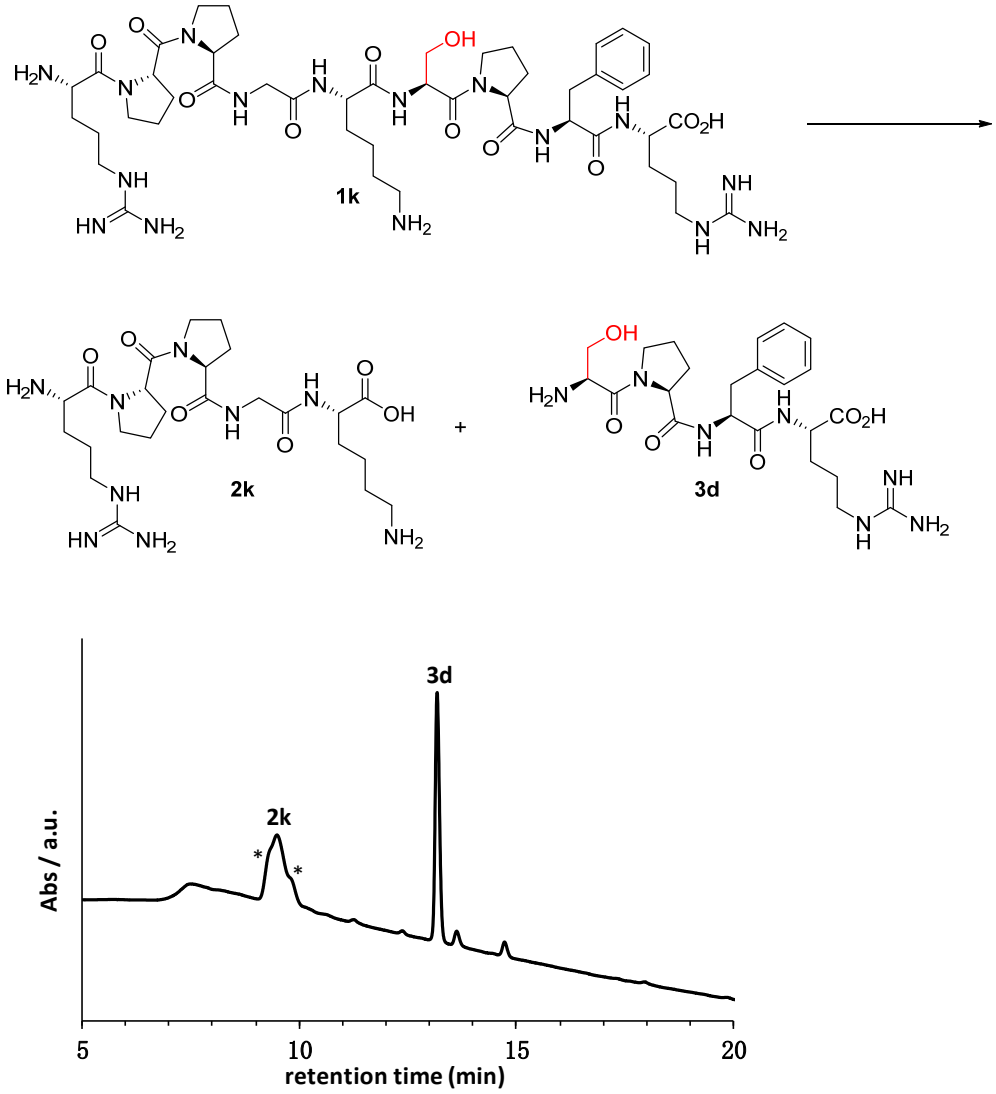
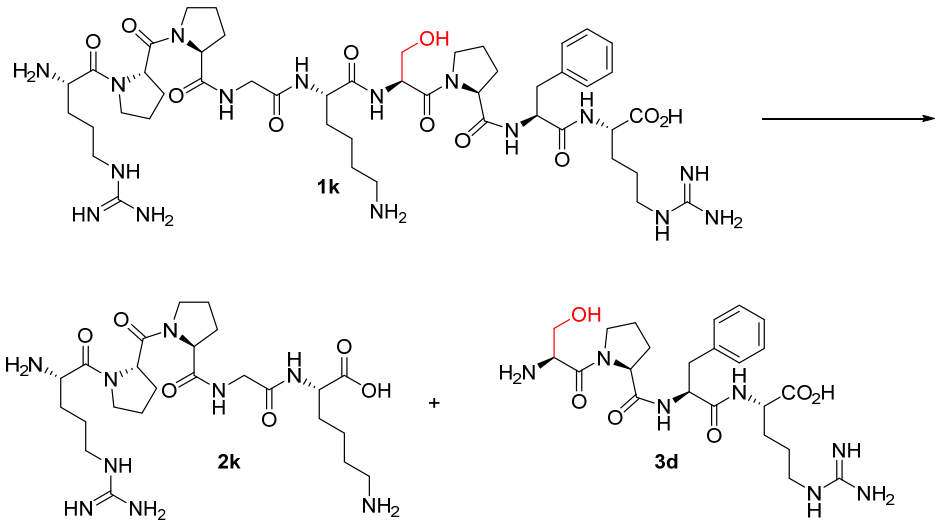
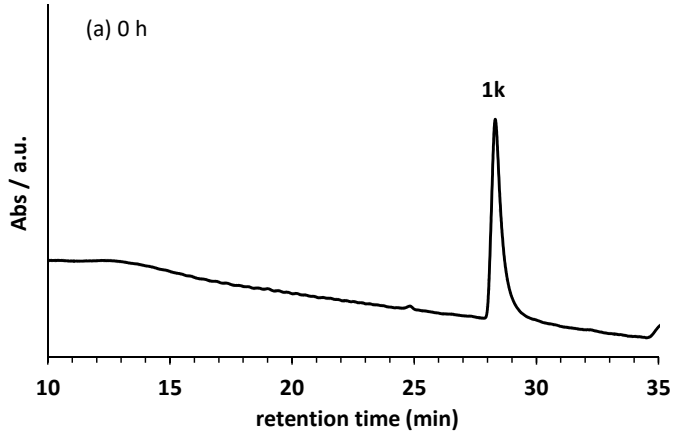


Figure S9. HPLC chart for the reaction of H-Arg-Pro-Pro-Gly-Lys-**Ser**-Pro-Phe-Arg-OH (**1k**, 2 mM) and $Sc(OTf)_3$ (200 mM) at 80 °C after 40 h. **2k**, MS (ESI): m/z 554.3 (calcd $[M+H]^+$ = 554.3). **3d**, MS (ESI): m/z 506.3 (calcd $[M+H]^+$ = 506.3). *The labeled peaks are from the HPLC system, but not peptidic compounds.





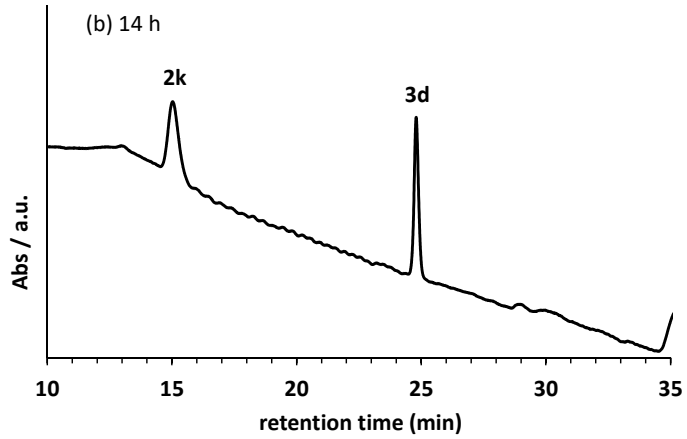


Figure S10. HPLC charts for the reaction of H-Arg-Pro-Pro-Gly-Lys-**Ser**-Pro-Phe-Arg-OH (**1k**, 10 mM) and endoproteinase Lys-C (2 μg mL^{-1}) at 37 °C after 0 h (a) and 14 h (b). The eluent condition was modified: a linear gradient of 2–32% acetonitrile in 0.1% aqueous TFA over 30 min. **2k**, MS (ESI): *m/z* 554.4 (calcd $[M+H]^+$ = 554.3). **3d**, MS (ESI): *m/z* 506.3 (calcd $[M+H]^+$ = 506.3).

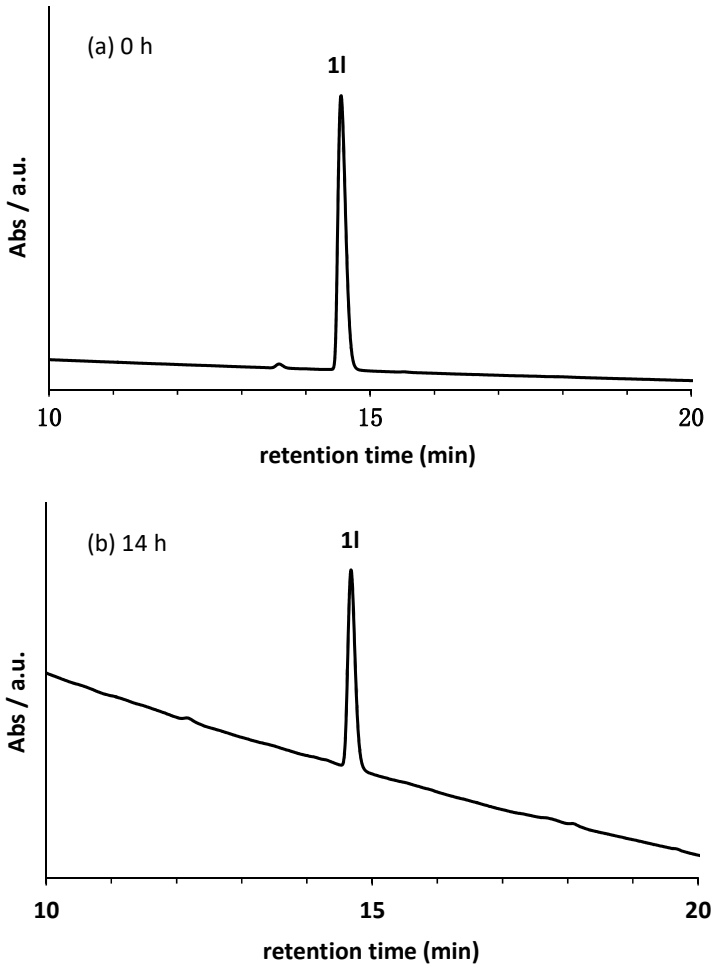


Figure S11. HPLC charts for the reaction of H-Arg-Pro-Pro-Gly-Lys(Ac)-**Ser**-Pro-Phe-Arg-OH (**1l**, 10 mM) and endoproteinase Lys-C (2 μg mL^{-1}) at 37 °C after 0 h (a) and 14 h (b).