Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

The versatile use of solubilizing trityl tags for difficult peptide/protein synthesis

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Contents

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), Merck KGaA (Darmstadt, Germany) and Sigma-Aldrich Co. LLC. (St. Louis, MO).

b. HPLC and MS

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_2O and 0.1% TFA in CH_3CN at a flow rate of 20 mL min^{-1} with detection at 220 nm. Analytical HPLC was performed on a Shimadzu liquid chromatograph Model LC-10A (Kyoto, Japan) with a DAISO-PAK SP-120-5-ODS-BIO (4.6 x 150 mm) or a YMC-Pack ODS-A (4.6 x 150 mm) and the following solvent systems: 0.1% TFA in H_2O and 0.1% TFA in CH_3CN at a flow rate of 1 mL min^{-1} (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; observed masses (most abundant masses) were derived from the experimental m/z values for each protonation states of a target peptide.

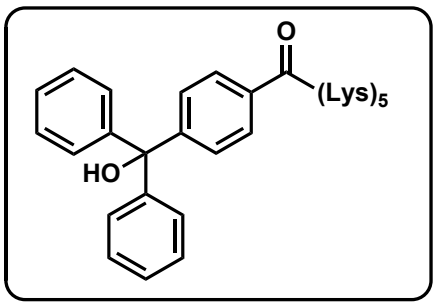
c. SPPS

Automated peptide synthesis by Fmoc SPPS was performed on an ABI 433A peptide synthesizer (Applied Biosystems, USA). The peptide chain was elongated using the coupling protocol of Fmoc-amino acid/DIC/OxymaPure.[1] The following side-chain-protected amino acids were employed: Arg(Pbf), Asn(Trt), Asp(O*t*Bu), Cys(S*t*Bu), Cys(Trt), Glu(O*t*Bu), Gln(Trt), His(Trt), Lys(Boc), Ser(*t*Bu), Thr(*t*Bu), Trp(Boc), Tyr(*t*Bu). Boc-amino acids were selected as N-terminal amino acids, when using MeNbz linker.

2. Experimental Section

a. Synthesis of peptide segments

*Trt(OH)-(Lys)$_5$ (**3**)*



The peptide was assembled on an Fmoc-Lys(Boc)-Wang Resin (0.40 mmol) using automated Fmoc SPPS procedure as described in general information (Fmoc-Lys(Boc) and 4-(diphenylhydroxymethyl)benzoic acid (Sigma-Aldrich): 2.5 equiv). The subsequent deprotection of the resin was carried out by TFA/H_2O (v/v, 95/5) for 40 min to give a crude product, which was purified by preparative HPLC to yield the title compound **3** (395 mg, 65% as 5 TFA base). Analytical HPLC: t_R = 14.7 min (1–60% CH_3CN/0.1% TFA for 25 min); LRMS (M+H) calcd for $C_{50}H_{77}N_{10}O_8$ 945.6, found 945.5.

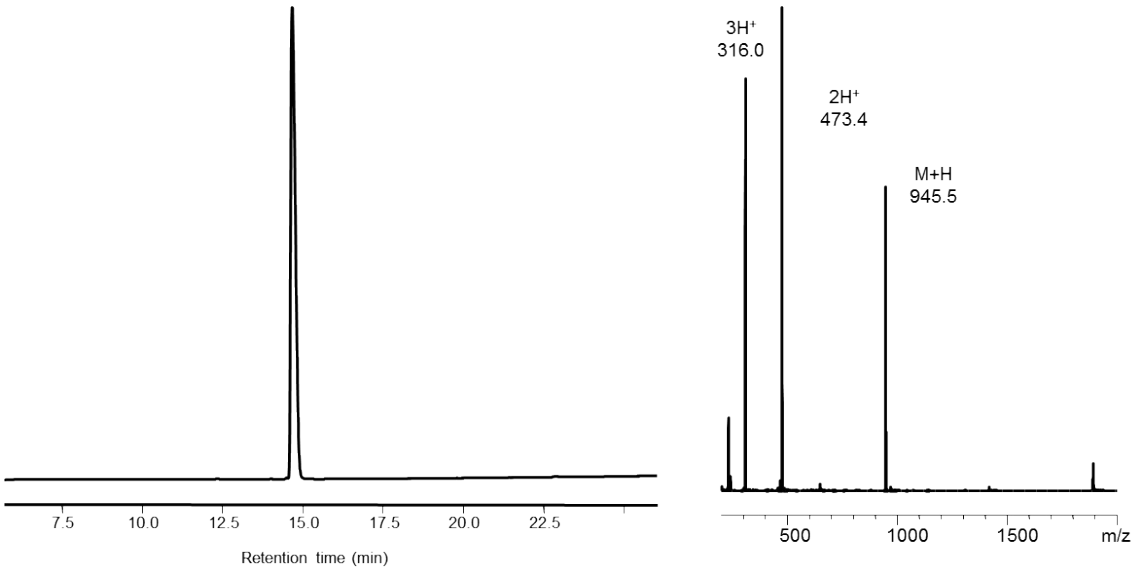


Figure S1. Analytical HPLC chromatogram and ESI-MS spectrum of compound **3**.

*Ac-Ile-Phe-Cys-Ser-Pro-Cys[Trt-(Lys)$_5$]-Tyr-Ser-NH$_2$ (**1**)*

The peptide was assembled on a Rink Amide Resin (0.25 mmol) using automated Fmoc SPPS procedure as described in general information (Fmoc-Xaa: 4.0 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H_2O (v/v, 95/2.5/2.5) for 1.5 h to give a crude product **2** [Ac-Ile-Phe-Cys(S*t*Bu)-Ser-Pro-Cys-Tyr-Ser-NH_2]. The obtained peptide **2** (21 mg, 20 μmol) and Trt(OH)-$(Lys)_5$ **3** (33 mg, 22 μmol) were dissolved in HFIP (2 mL). After stirring for 2 h, the reaction mixture was concentrated and solidified by diethyl ether. The obtained solid **4** was dissolved in DMSO/H_2O (4/1), and PBu_3 (200 μL, 810 μmol) was added into the solution.[2] After stirring for 6 h at 40 ^oC, the reaction mixture was subjected to preparative HPLC to yield the title peptide **1** (28 mg, 76%). Analytical HPLC: t_R = 16.5 min (10-60% CH_3CN/0.1% TFA for 25 min); LRMS (M+H) calcd for $C_{93}H_{136}N_{19}O_{19}S_2$ 1887.0, found 1888.0.

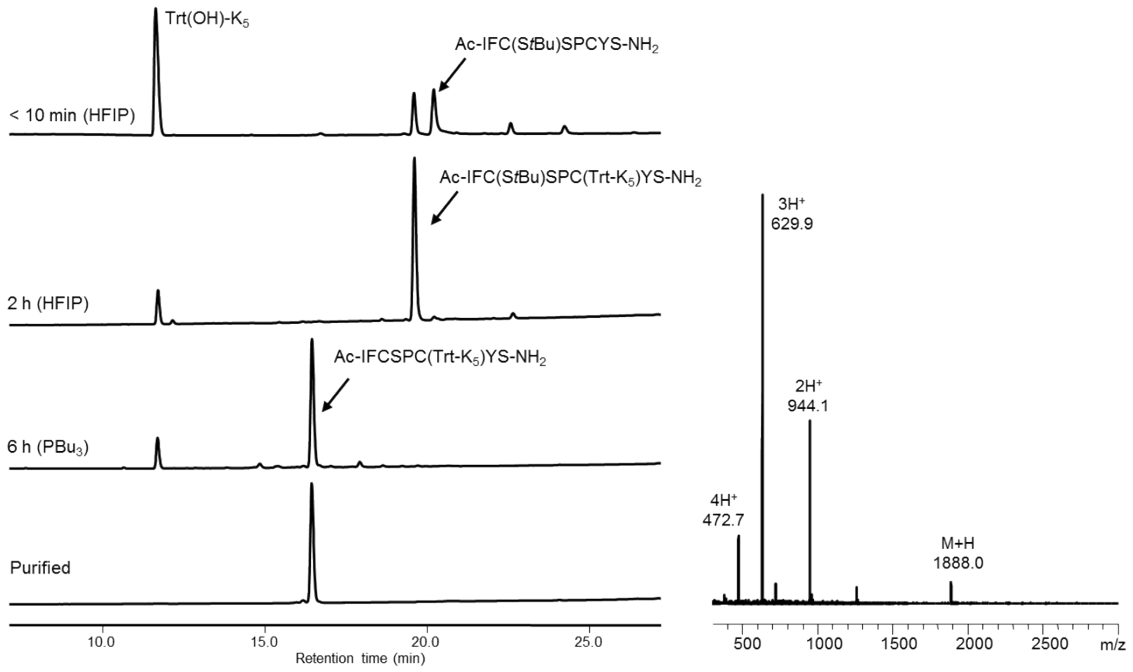
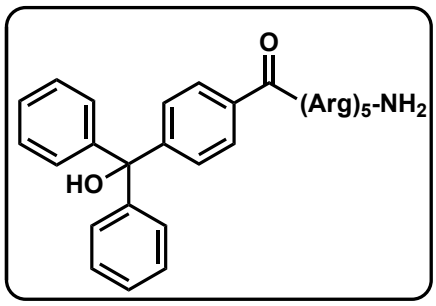


Figure S2. Analytical HPLC chromatograms and ESI-MS spectrum of peptide **1**.

*Trt(OH)-(Arg)$_5$-NH$_2$ (**6**)*



Protect resin of H-(Arg)$_5$-NH$_2$ was assembled on a Rink Amide Resin (0.40 mmol) using automated Fmoc SPPS procedure as described in general information (Fmoc-Arg(Pbf) : 2.5 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H_2O/thiophenol (v/v, 92.5/2.5/2.5/2.5) for 1.5 h to give a crude H-(Arg)$_5$-NH$_2$. 4-(Diphenylhydroxymethyl)benzoic acid (122 mg, 0.40 mmol) and HOSu (51 mg, 0.44 mmol) were dissolved in THF, and then DCC (99 μL, 0.44 mmol) was added to the solution. After stirring for 16 h, the reaction mixture was filtered, and the obtained residue was concentrated. Thus obtained Trt(OH)-OSu was dissolved in DMSO (10 mL), and then crude H-(Arg)$_5$-NH$_2$ in a buffer (6 M Gn.HCl, 0.1 M phosphate, pH 7.2, 10 mL) was added to the DMSO solution. After stirring for 2 h, the reaction mixture was subjected to preparative HPLC to yield the title compound **6** (78 mg, 12% as 5 TFA base). Analytical HPLC: t_R = 15.8 min (1–60% CH_3CN/0.1% TFA for 25 min); LRMS (M+H) calcd for $C_{50}H_{78}N_{21}O_7$ 1084.6, found 1084.6.

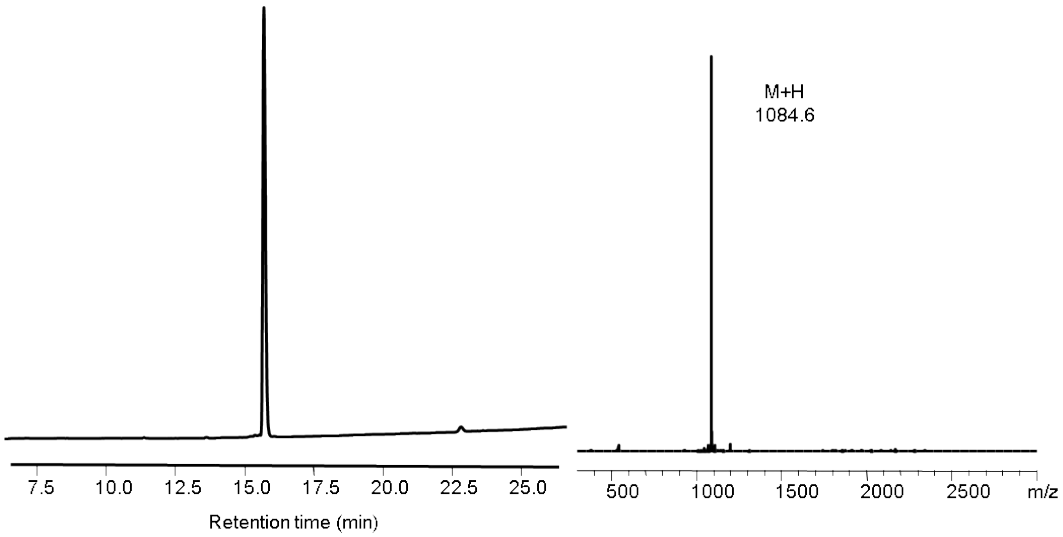


Figure S3. Analytical HPLC chromatogram and ESI-MS spectrum of compound **6**.

*Gly-Tyr-Phe-Cys[Trt-(Arg)$_5$-NH$_2$]-Gly-NH$_2$ (**8**)*

Gly-Tyr-Phe-Cys-Gly-NH$_2$ **7** (11 mg, 20 μmol) and Trt(OH)-(Arg)$_5$-NH$_2$ **6** (37 mg, 22 μmol) were dissolved in HFIP (1 mL). After stirring for 2 h, the reaction mixture was concentrated and solidified by diethyl ether. The obtained solid was dissolved in H_2O with 0.1% TFA and subjected to preparative HPLC to yield the title peptide **8** (30 mg, 66%). Analytical HPLC: t_R = 17.4 min (15-35% CH_3CN/0.1% TFA for 25 min); LRMS (M+H) calcd for $C_{75}H_{108}N_{27}O_{12}S$ 1610.8, found 1610.5.

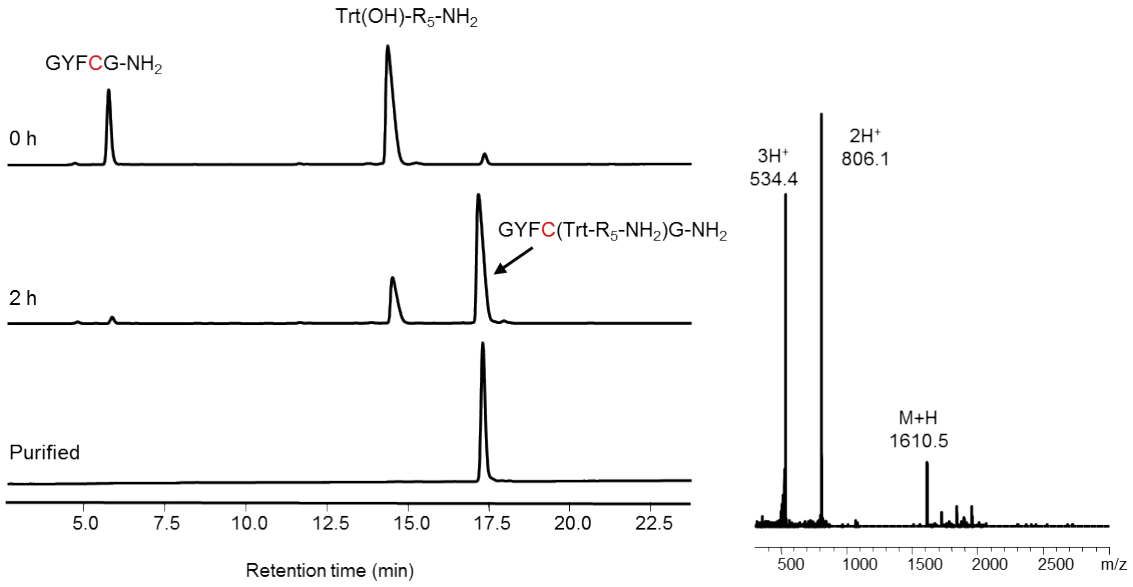


Figure S4. Analytical HPLC chromatograms and ESI-MS spectrum of peptide **8**.

*Ac-Leu-Tyr-Arg-Ala-Asn-Gly-MESNa (**9**)*

The peptide was assembled on a hydrazine-Trt(2-Cl)-Resin (0.40 mmol) using automated Fmoc SPPS procedure as described in general information (Fmoc-Xaa: 2.5 equiv). The subsequent deprotection of the resin was carried out by TFA/TIS/H_2O (v/v, 95/2.5/2.5) for 1.5 h to give a crude peptide (Ac-Leu-Tyr-Arg-Ala-Asn-Gly-N_2H_3). The crude peptide dissolved in a buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 3.0, 8.0 mL) was activated by 1 M $NaNO_2$ (0.8 mL) at -10 °C for 15 min, and then thioesterified with sodium 2-mercaptoethanesulfonate (MESNa) (1.3 g) in a pH 7.0 buffer (8.0 mL). After stirring for 1 h at room temperature, the reaction mixture was subjected to preparative HPLC to yield the title peptide **9** (51 mg, 15%). Analytical HPLC: t_R = 11.9 min (1–60% CH_3CN/0.1% TFA for 25 min); LRMS (M+H) calcd for $C_{34}H_{55}N_{10}O_{12}S_2$ 859.3, found 859.3.

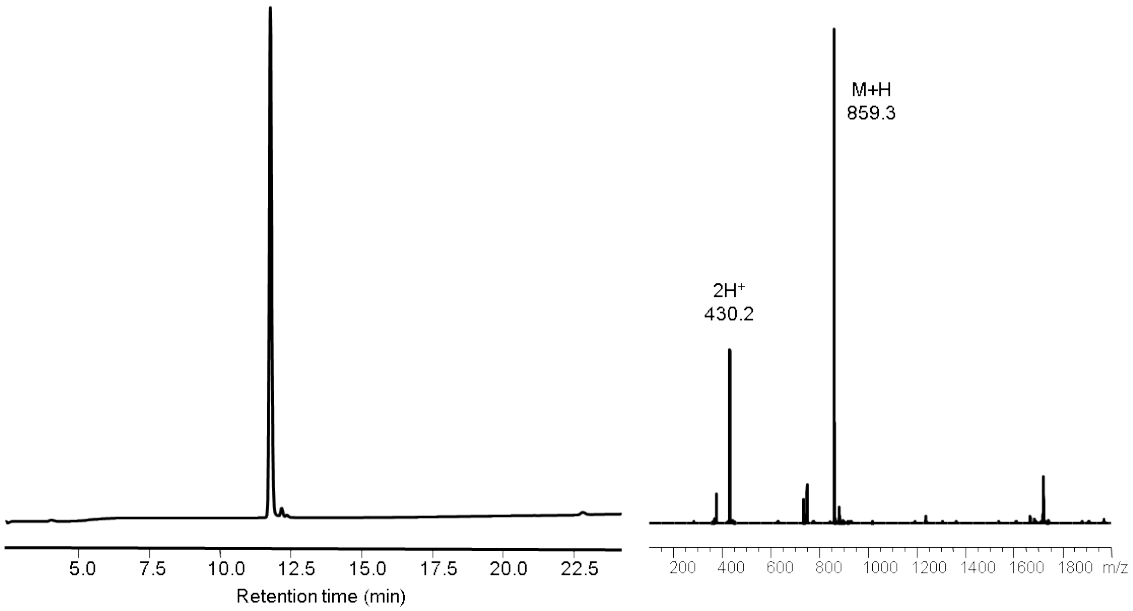


Figure S5. Analytical HPLC chromatogram and ESI-MS spectrum of peptide **9**.

*MLEPFQILSIC(Trt-K_5)SFILSALHFL-MeNbz-K_3-NH_2 (**13**)*

The peptide was assembled on an Fmoc-Leu-MeDbz-[Lys(Boc)$_3$]-TentaGel S RAM Resin (0.1 mmol) using Fmoc SPPS as described in general information (Fmoc-Xaa: 10 equiv, Boc-Met as N-terminal Xaa). The obtained peptide resin was activated by the method reported by Dawson *et al*.[3] The final deprotection was carried out by TFA/TIS/H_2O/thiophenol (v/v, 92.5/2.5/2.5/2.5) for 1.5 h to give a crude product **12**. A part of the obtained crude peptide (80 mg, 27 μmol) and Trt(OH)-(Lys)$_5$ **3** (61 mg, 40 μmol) were dissolved in HFIP (1 mL). After stirring for 6 h, the reaction mixture was concentrated and solidified by diethyl ether. The obtained solid was dissolved in 50% AcOH with H_2O and subjected to preparative HPLC to yield the title peptide **13** (26 mg, 25%). Analytical HPLC: t_R = 15.2 min (20-98% CH_3CN/0.1% TFA for 25 min); LRMS (ESI) calcd for $C_{193}H_{298}N_{44}O_{38}S_2$ 3906.2, found 3907.1.

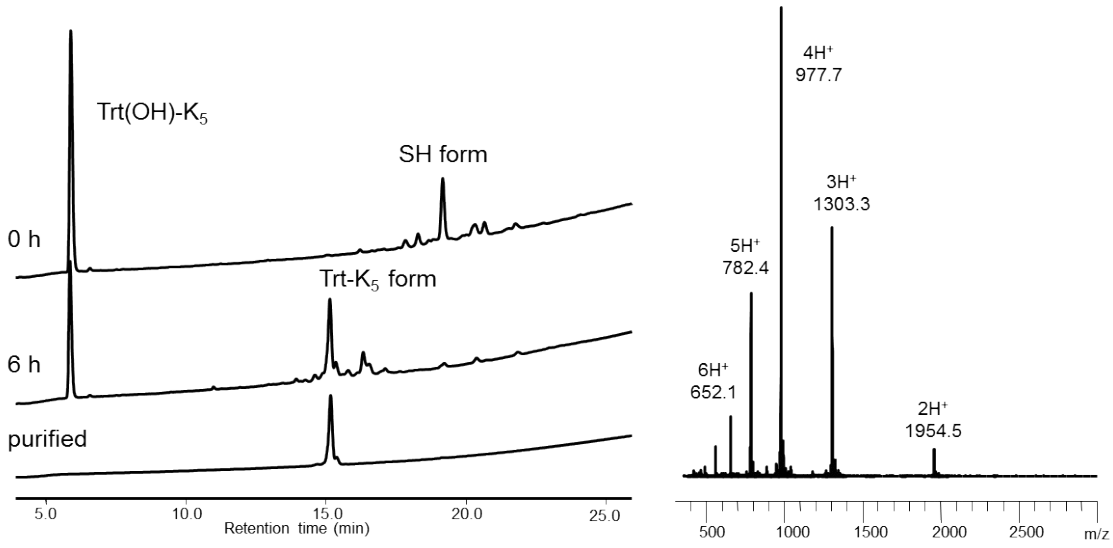


Figure S6. Analytical HPLC chromatograms and ESI-MS spectrum of peptide **13**.

*CWTIGHLNQIKRGINMKIRIKGPNKETINR **(14)***

The peptide was assembled on a Wang-PEG Resin (0.125 mmol) using automated Fmoc SPPS as described in general information (Fmoc-Xaa: 8 equiv). The final deprotection was carried out by TFA/TIS/H_2O (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 **14** (48 mg, 11%). Analytical HPLC: t_R = 13.4 min (10–60% CH_3CN/0.1% TFA for 25 min); LRMS (ESI) calcd for $C_{154}H_{263}N_{51}O_{40}S_2$ 3532.0, found 3532.7

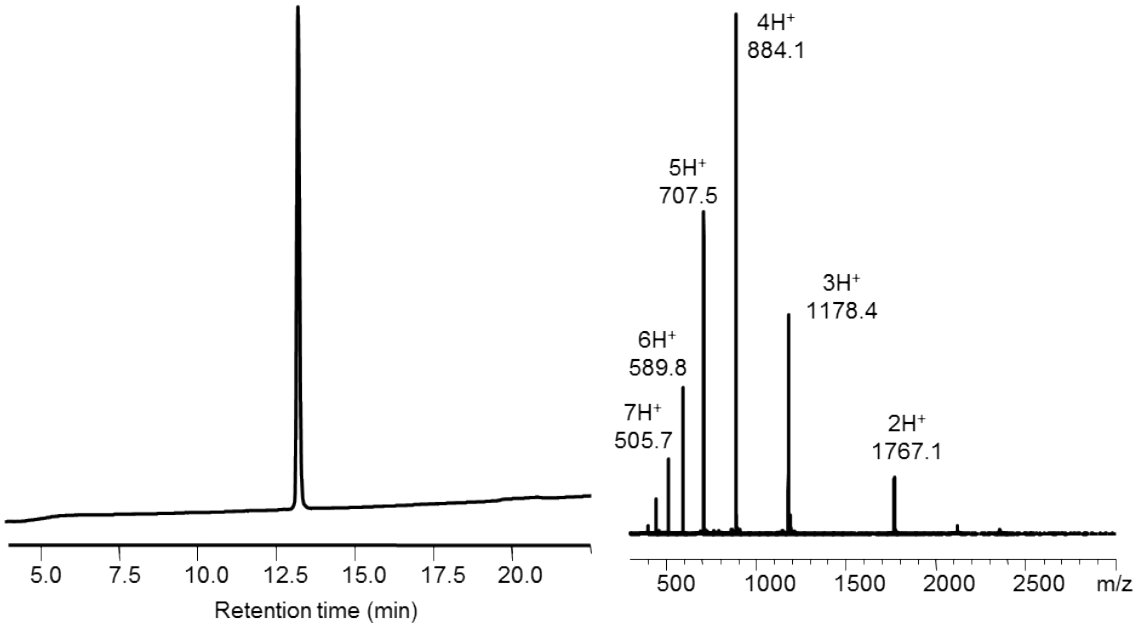


Figure S7. Analytical HPLC chromatogram and ESI-MS spectrum of peptide **14**.

b. Investigation using Trt-tagged peptides under various ligation conditions

Desulfurization of a Trt-K_5-tagged model peptide

Ac-IFCSPC(Trt-K_5)YS-NH_2 **1** (0.34 mg) was dissolved in 0.18 mL of a buffer (6 M Gn·HCl, 100 mM Na_2HPO_4, 400 mM TCEP, pH 6.5), and the solution was incubated under the condition in the presence of VA-044 (2.8 mg, 50 equiv) and GSH (2.7 mg, 50 equiv) at 40 °C. Then, the solution was monitored by analytical HPLC (1–60% CH_3CN/0.1% TFA for 25 min). Ac-IFASPC(Trt-K_5)YS-NH_2 **5**; LRMS (M+H) calcd for $C_{93}H_{136}N_{19}O_{19}S$ 1855.0, found 1855.9.

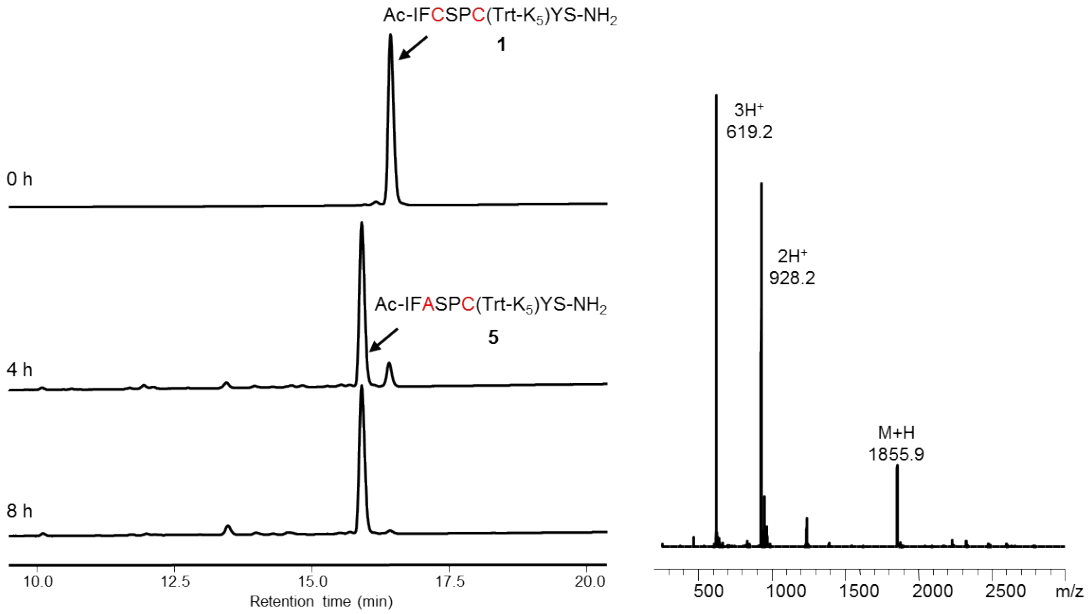


Figure S8. Analytical HPLC chromatograms of desulfurization of **1** and ESI-MS spectrum of **5**

Ag-mediated thioester method of a Trt-R_5-tagged model peptide

Ac-LYRANG-MESNa **9** (2.2 mg, 2.5 μmol), GYFC(Trt-R_5-NH_2)G-NH_2 **8** (4.0mg, 2.5 μmol) and HOOBt (25 mg) were dissolved in DMSO (250 μL). Then, a tiny portion of AgCl and DIEA (50 μL) were added to the mixture. After stirring for 4 h at 40 °C, the solution was quenched by 0.1% TFA with H_2O, and subjected to preparative HPLC to yield the target peptide **10** (3.8 mg, 66%). Analytical HPLC: t_R = 17.3 min (1-60% CH_3CN/0.1% TFA for 25 min); LRMS (ESI) calcd for $C_{107}H_{155}N_{37}O_{21}S$ 2327.2, found 2327.3.

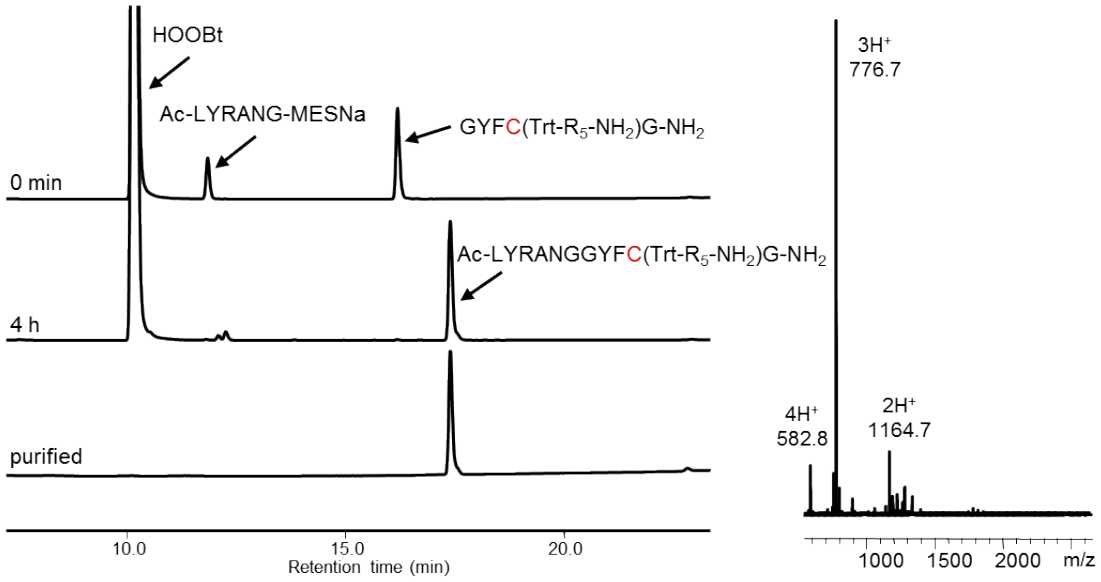


Figure S9. Analytical HPLC chromatograms of thioester method reaction between **8** and **9** and ESI-MS spectrum of **10**.

Investigation of deprotection of a Trt-R_5-tag

Ac-LYRANGGYFC(Trt-R_5-NH_2)G-NH_2 **10** (0.40 mg) was treated with TFA/TIS (95 μL/5 μL). After 30 min, the solution was concentrated. Then, the residue was dissolved in CH_3CN/H_2O (1/1) and monitored by analytical HPLC (1–60% CH_3CN/0.1% TFA for 25 min); LRMS (M+H) calcd for $C_{57}H_{81}N_{16}O_{15}S$ 1261.6, found 1261.5.

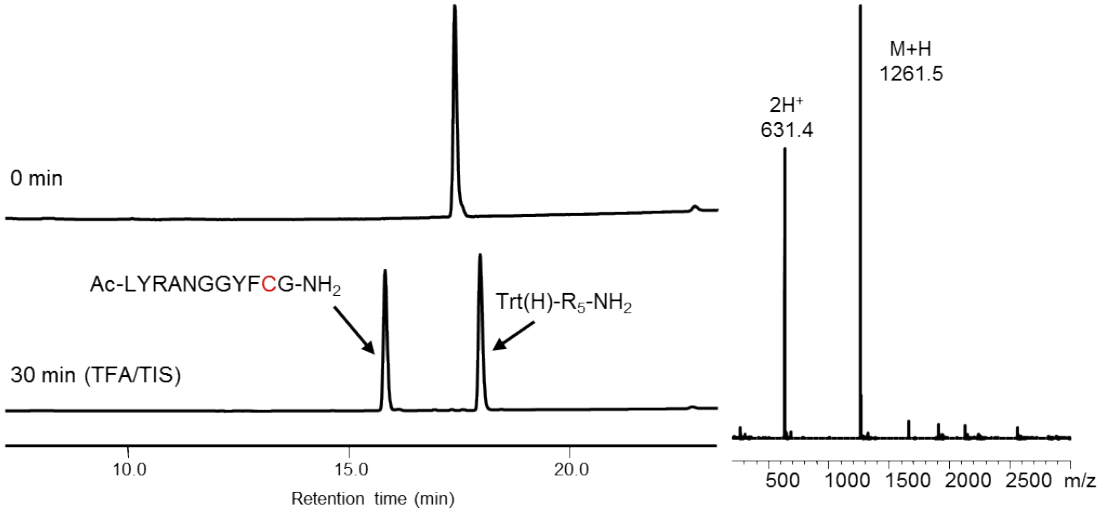


Figure S10. Analytical HPLC chromatograms of TFA cleavage and ESI-MS spectrum of Ac-LYRANGGYFCG-NH_2.

Synthesis of [Cys^{11}(Trt-K$_5$), Leu^{21}, Cys^{22}]BM2(1-51) by NCL

*MLEPFQILSIC(Trt-K$_5$)SFILSALHFLCWTIGHLNQIKRGINMKIRIKGPNKETINR (**15**)*

MLEPFQILSIC(Trt-K$_5$)SFILSALHFL-MeNbz-K$_3$-NH$_2$ **13** (11.7 mg, 3.0 μmol) was reacted with CWTIGHLNQIKRGINMKIRIKGPNKETINR **14** (14.8 mg, 4.2 μmol) in 1.5 mL of a ligation buffer (6 M Gn·HCl, 100 mM Na_2HPO_4, 200 mM MPAA, 50 mM TCEP, pH 7.0) at 40 °C. After stirring for 8 h, the solution was quenched by 50% AcOH with H_2O, and directly subjected to preparative HPLC to yield the title peptide **15** (9.3 mg, 45%). Analytical HPLC: t_R = 19.7 min (10-98% CH_3CN/0.1% TFA for 25 min); LRMS (ESI) calcd for $C_{320}H_{516}N_{86}O_{73}S_4$ 6862.8, found 6864.4.

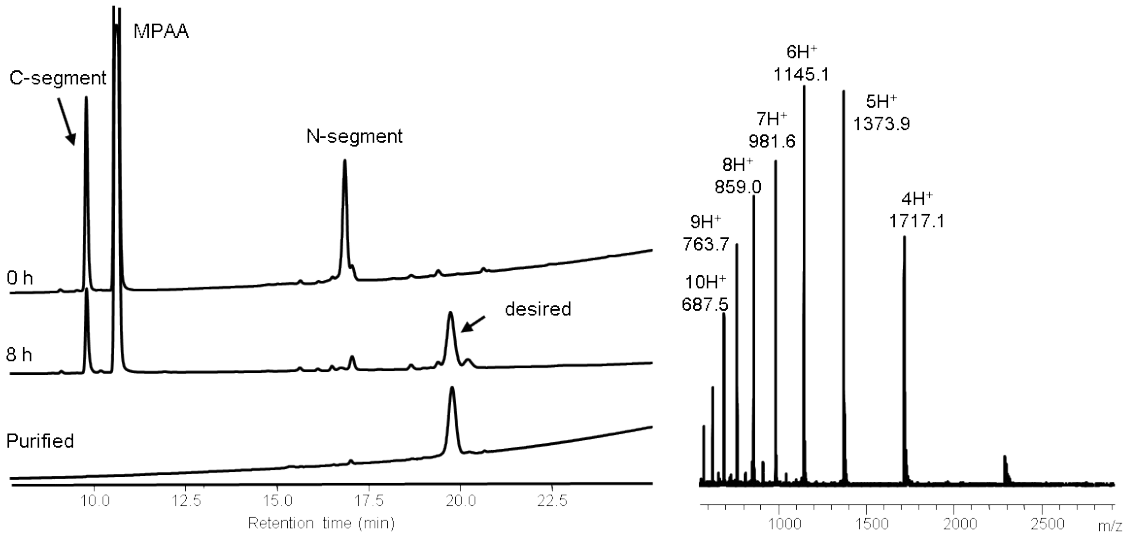


Figure S11. Analytical HPLC chromatograms of NCL and ESI-MS spectrum of **15**.

Synthesis of [Cys^{11}(Trt-K$_5$), Leu^{21}, Ala^{22}]BM2(1-51) by desulfurization

*MLEPFQILSIC(Trt-K$_5$)SFILSALHFLAWTIGHLNQIKRGINMKIRIKGPNKETINR (**16**)*

[Cys(Trt-K$_5$)^{11}, Leu^{21}, Cys^{22}]BM2(1-51) **15** (8.4 mg, 1.22 μmol) was dissolved in 1.4 mL of a buffer (6 M Gn·HCl, 100 mM Na_2HPO_4, 400 mM TCEP, pH 6.4), and the solution was incubated under the condition in the presence of VA-044 (17.2 mg, 54.9 μmol) and MESNa (9.0 mg, 54.9 μmol) at 42 °C. After stirring for 24 h, the solution was quenched by 50% AcOH with H_2O, and subjected to preparative HPLC to yield the title peptide **16** (3.8 mg, 45%). Analytical HPLC: t_R = 19.5 min (10-98% CH_3CN/0.1% TFA for 25 min); LRMS (ESI) calcd for $C_{320}H_{516}N_{86}O_{73}S_3$ 6830.9, found 6832.3.

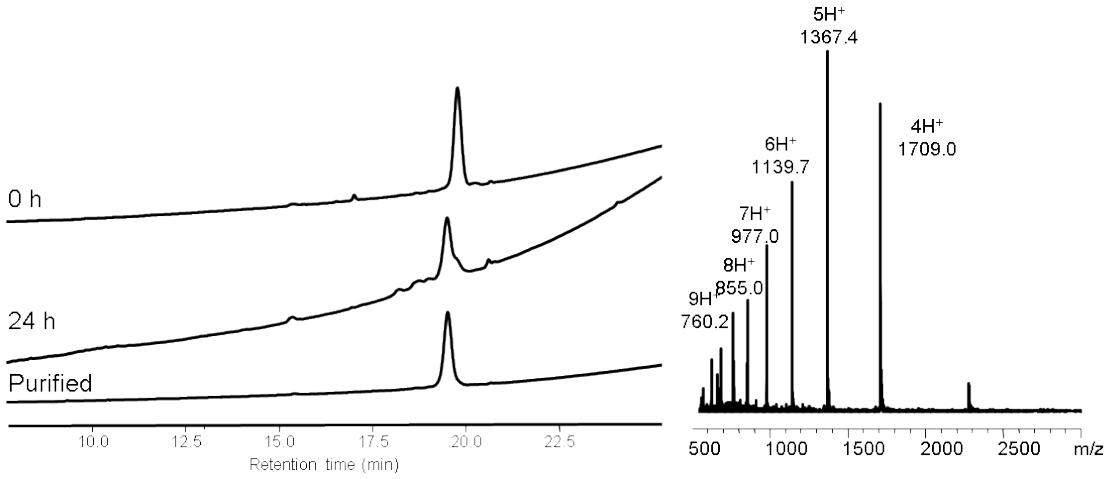


Figure S12. Analytical HPLC chromatograms of desulfurization and ESI-MS spectrum of **16**.

Synthesis of [Leu^{21}]BM2(1-51)

*MLEPFQILSICSFILSALHFLAWTIGHLNQIKRGINMKIRIKGPNKETINR (**11**)*

[Cys(Trt-K_5)^{11}, Leu^{21}, Ala^{22}]BM2(1-51) **16** (3.0 mg, 0.44 μmol) was treated with TFA/TIS (95 μL/5 μL). After 15 min, the solution was concentrated. The residue was dissolved in 3 M Gn·HCl in 50% AcOH/H_2O, and subjected to preparative HPLC to yield the title peptide **11** (1.5 mg, 58%). Analytical HPLC: t_R = 23.9 min (10-98% CH_3CN/0.1% TFA for 25 min); LRMS (ESI) calcd for $C_{270}H_{441}N_{75}O_{67}S_3$ 5904.3, found 5905.1.

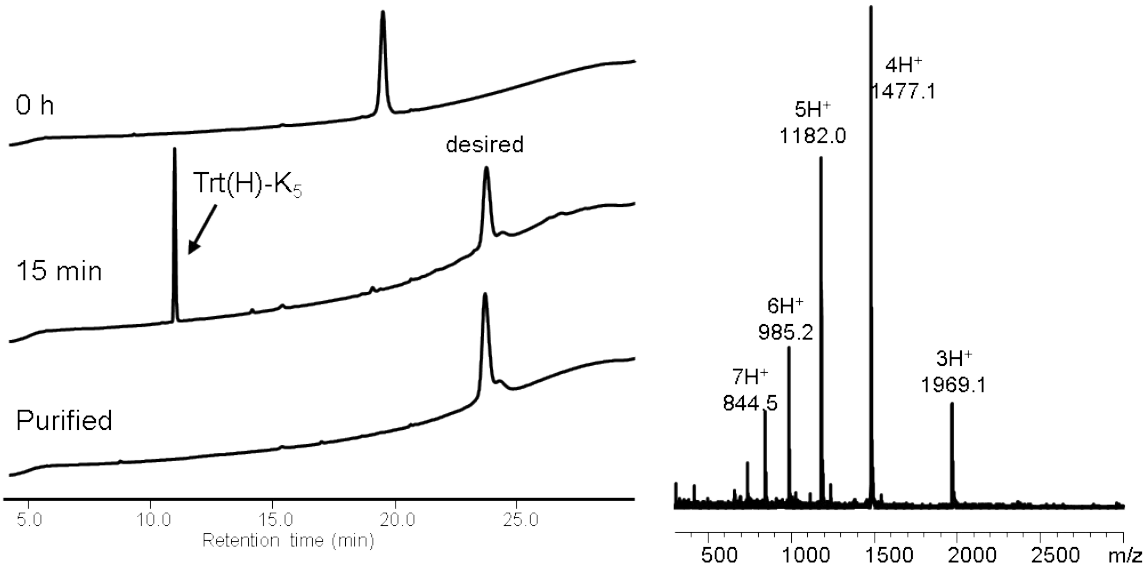


Figure S13. Analytical HPLC chromatograms of TFA cleavage and ESI-MS spectrum of **11**.

3. References

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

[2] M. Mochizuki, S. Tsuda, K. Tanimura and Y. Nishiuchi, *Org. Lett.,* 2015, **17**, 2202–2205.

[3] J. B. Blanco-Canosa, B. Nardone, F. Albericio and P. E. Dawson, *J. Am. Chem. Soc.,* 2015, **137**, 7197–7209.