Supporting information

Chemical Synthesis of Cys-containing Protein via Chemoselective Deprotection with Different Palladium Complexes

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Experimental

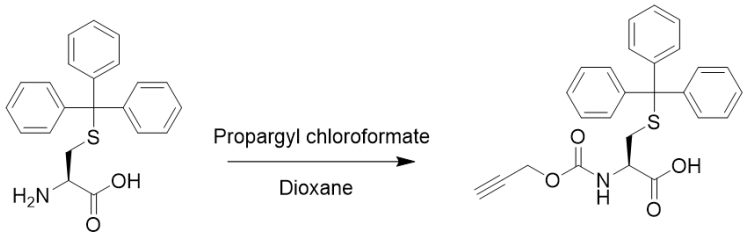
Experimental section

General methods and materials. MALDI-TOF mass spectra were recorded with microflex (BRUKER), using Protein Calibration Standard 2 as an external standard. NMR spectra were recorded with an Avance 600 instrument (Bruker). ESI mass spectra were recoded with. Reversed-phase HPLC was performed on a $5C_{18}$-AR-II and Protein-R column (Nacalai tesque, 4.6 ID and 20 ID × 250 mm for analysis and purification, respectively) with a PU-2080 plus Intelligent HPLC Pump (JASCO) and MD-2018 plus Photodiode Array Detector (JASCO) at 195 to 650 nm. All solvents and reagents were commercially available and used without further purification.

Peptides were prepared by using Fmoc-Gly-Alko-Peg resin (Watanabe Chemical Industries) for peptides **1**, **2**, **3**, and **4**. TentaGel Resin (0.23 mmol/g, 10 μmol scale, HiPep Lab.) for peptide **7**, **8**, and **9**. 2-Cl-(Trt)-Cl resin (Watanabe Chemical Industries) for peptides **10**, **11**, and Fmoc-Ala-Alko-Peg resin (Watanabe Chemical Industries) for peptides **12**. The isolated yields of each peptide were estimated by using the molecular weights of TFA salt at Arg, Lys and His positions.

Fmoc-protected amino acids were purchased from Watanabe Chemical Industries or Novabiochem. 4-mercaprophenylacetic acid (MPAA) was purchased from Sigma-Aldrich. Palladium(II) acetate ($Pd(OAc)_2$), palladium(II) sodium chloride trihydrate ($Na_2PdCl_4 \cdot 3H_2O$), allylpalladium(II) chloride dimer ($[Pd(allyl)Cl]_2$), and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Wako Pure Chemical Industries. 3,3',3"-phosphanetriyltris(benzenesulfonic acid) trisodium salt (TPPTS) and *N*-(hydroxymethyl)acetamide were purchased from Tokyo Chemical Industry Co., Ltd.

Synthesis of *N*-[(2-Propyn-1-yloxy)carbonyl]-S-(triphenylmethyl)-L-cysteine (Proc-Cys(Trt)-OH)



S-trityl-L-cysteine Proc-Cys(Trt)-OH

S-trityl-L-cystine (0.62 g, 1.7 mmol) was dissolved in 1,4-dioxane/2% Na_2CO_3 aq (1/1, 20 ml). The solution was cooled to 4 °C and propargyl chloroformate (0.30g, 2.5 mmol) (prepared by following protocols by S. P. Chakrabarty et al. *Bioorg. Med. Chem*. **2009**, *17*, 8060–8072.) in 1,4-dioxane (3 ml) was added slowly. The mixture was warmed to room temperature, and stirred for 2 h. To the reaction solution 1 N HCl aq (about 1.0 ml) was added to acidify, and the organic solvents were extracted by EtOAc. The collected organic phases were dried over $MgSO_4$, filtered, concentrated in vacuo and purified by column chromatography ($CHCl_3$/MeOH = 30/1) to afford Proc-Cys(Trt)-OH

(0.68 g, 92% yield) as a white solid. **^{1}H NMR** ($CDCl_3$, 600 MHz) d 7.43-7.24 (15 H, m), 5.22 (1 H, d, *J* = 7.6 Hz), 4.68 (2 H, dd, *J* = 15.5 Hz, 12.1 Hz), 4.29 (2 H, m), 2.77-2.71 (2 H, m), 2.52 (1 H, s). **^{13}C NMR** (150 MHz, $CDCl_3$) δ 175.3, 155.2, 144.5, 130.4, 129.9, 129.5, 128.5, 128.1, 75.5, 67.4, 58.4, 34.0. HRMS(ESI): m/z calcd for $C_{26}H_{22}NO_4S$ ($[M-H]^-$): 444.135; found: 444.139.

Peptide synthesis. All peptides were synthesized using Intavis ResPep SL (Intavis). Amino acids protected by 9-fluorenylmethoxycarbonyl (Fmoc) group were coupled with O-(1H-Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as activator and *N*, *N*-diisopropylethylamine (DIEA) as base. For the coupling of Fmoc-His(Trt)-OH, Fmoc-Cys(Trt)-OH, or Alloc-Cys(Trt)-OH, DIC (6 equiv.) and HOAt (6 equiv.) were employed to avoid racemization during coupling reaction.

Peptide cleavage from resin to afford peptides. To each resin was added TFA cocktails (TFA 90%, thioanisole 5%, ethanedithiol 3% and anisole 2%). The mixture was rotated at room temperature for 2 h under argon atmosphere. Then, filtered the mixture to remove resin, and cooled *t*-butyl methyl ether was added. The tube was vortexed well and centrifuged 5,000 × g at room temperature for 1 min. Ether layer was decanted and these operations was repeated three times. The crude precipitate was dried by speed-vac.

Preparation of solution of Pd complexes. 100 mM Pd/TPPTS solution was prepared according to the previous procedure. To the solution of 10 μl $Pd(OAc)_2$ in degassed *N*,*N*-dimethylformamide (200 mM), 8 μl of TPPTS in degassed water (1 M) and 2 μl of degassed water were added and the mixture was vigorously vortexed for 30 s at room temperature under argon atmosphere. The solution can be stored at least for one day at −15 °C under argon atmosphere. Na_2PdCl_4 was dissolved in degassed water, and $[Pd(allyl)Cl]_2$ was dissolved in degassed denaturing buffer (Gn 6 M, NaH_2PO_4 0.2 M at pH 7.0), respectively. To prepare $[Pd(allyl)Cl]_2$/GSH, the mixture of 400 mM $[Pd(allyl)Cl]_2$ (10 μl) and 400 mM GSH (10 μl) in denaturing buffer was vortexed for 30 s.

Deprotection of protecting groups of peptides 1, 2, 3, and 4 with Pd complexes in denaturing buffer. To peptide **1**, **2**, **3**, or **4** solution in denaturing buffer (pH 7.0) was added 100 mM Pd/TPPTS, 200 mM Na_2PdCl_4, $[Pd(allyl)Cl]_2$, or $[Pd(allyl)Cl]_2$/GSH respectively. The final concentration of the reaction mixture was peptide **1** or **2** (1 mM), Pd complex (10 mM, 10 equiv.). The reaction mixture was stirred at room temperature under argon atmosphere. For analysis of each reaction, 2.0 μl from each reaction mixture was treated with 3.0 μl of 500 mM DTT aq and 15 μl of denaturing buffer, followed by stirring for 30 s and injection into analytical HPLC. Only for peptide **3** bearing Thz, the 2.0 μl reaction mixture was treated with 15 μl of denaturing buffer and 3 μl of 500 mM TCEP solu-

tion, followed by stirring for 30 s and injection into analytical HPLC when Na_2PdCl_4 or $[Pd(allyl)Cl]_2$/GSH was employed as Pd complex.

Deprotection of protecting groups of peptides 7, 8, and 9 with Pd complex under NCL conditions.

a) Peptide 7 bearing Alloc and Acm groups. To peptide **7** solution in 6 M Gn· HCl aq containing 0.2 M NaH_2PO_4 at pH 6.0 were added 500 mM TCEP, 500 mM $MgCl_2$, and 500 mM MPAA. Then, to the mixture was added 100 mM Pd/TPPTS solution and the reaction mixture was stirred for 1 h at room temperature under argon atmosphere. The final concentration of the reaction mixture was peptide **7** (2 mM), MPAA (100 mM), TCEP (20 mM), $MgCl_2$ (6 mM), Gn·HCl (6 M), 0.2 M NaH_2PO_4, and Pd/TPPTS (5 mM, 2.5 equiv.). For analysis of the reaction, 2.0 μl from each reaction mixture was treated with 3.0 μl of 500 mM DTT aq and 15 μl of denaturing buffer, followed by stirring for 30 s and injected into analytical HPLC.

b) Peptide 8 bearing Proc and Acm groups. To peptide **8** solution in denaturing buffer were added 500 mM TCEP, and 500 mM MPAA. Then, to the mixture was added 400 mM Na_2PdCl_4 solution and the reaction mixture was stirred for 1 h at room temperature. The final concentration of the reaction mixture was peptide **8** (2 mM), MPAA (40 mM), TCEP (20 mM), and Na_2PdCl_4 (100 mM. 50 equiv.). For analysis of the reaction, 2.0 μl from each reaction mixture was treated with 5.0 μl of 500 mM DTT aq, 15 μl of denaturing buffer, and 3.0 μl of 500 mM TCEP aq to suppress the generation of some precipitation derived from Pd complexes. The mixture was stirred for 30 s, and injected into analytical HPLC.

c) Peptide 9 bearing Thz and Acm groups. To powdered peptide **9** were added 7.2 μl of denaturing buffer, 1.6 μl of 500 mM TCEP, 3.2 μl of 500 mM MPAA, and 16 μl of 500 mM $MgCl_2$ dissolved in denaturing buffer. The mixture was stirred at room temperature for 5 min. Then, 12 μl of 200 mM $[Pd(allyl)Cl]_2$/GSH was added to the mixture. The final concentration of the reaction solution was peptide **9** (2 mM), MPAA (40 mM), TCEP (20 mM), $MgCl_2$ (200 mM. 100 equiv.), and $[Pd(allyl)Cl]_2$/GSH (60 mM, 30 equiv.). For analysis of the reaction, 2.0 μl from each reaction mixture was treated with 5.0 μl of 500 mM DTT aq, 15 μl of denaturing buffer, and 3.0 μl TCEP aq. The mixture was stirred for 30 s, and injected into analytical HPLC.

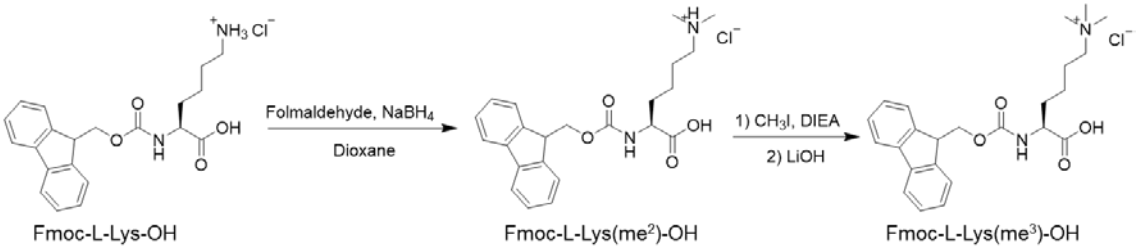
Analysis of coordination of Pd complexes by ^{13}C NMR measurement.

a) Boc-Cys(Acm)-OH with $PdCl_2$. To 33.0 mg of Boc-Cys(Acm)-OH and 20.0 mg of $PdCl_2$ was added 500 μl of DMSO-d_6 and 100 μl of D_2O. The mixture was stirred at room temperature for 1 h. The solution was transferred into a NMR tube and analyzed by NMR measurement.

b) Boc-Cys(Acm)-OH with Pd/TPPTS. 182.0 mg of TPPTS was dissolved in 276 μl of degassed DMSO-d_6, the total volume became about 375 μl. To this solution 18.0 mg of $Pd(OAc)_2$ dissolved in 205 μl of degassed DMSO-d_6 was added, and the mixture was stirred for 1 min under argon atmosphere. This Pd/TPPTS solution was transferred into a NMR tube for NMR analysis. After the measurement, 23.4 mg of Boc-Cys(Acm)-OH dissolved in 100 μl degassed DMSO-d_6 was added to the NMR tube containing Pd/TPPTS solution. The mixture was stirred for 30 min, and the solution was analyzed by NMR measurement.

Chemical synthesis of histone H3 bearing K9me3.

a) Synthesis of Fmoc-Lys(me^3)-OH



1st step. Fmoc-L-Lys(me^2)-OH was synthesized by following a previous protocol (R. Wieneke, A. Bernecker, R. Riedel, M. Sumper, C. Steinem and A. Geyer, Org. Biomol. Chem., 2011, 9, 5482–5486.). To a solution of Fmoc-L-Lys-OH (1.0 g, 2.5 mmol), 37% formaldehyde solution (1.0 ml, 12.5 mmol) and acetic acid (3 ml) in 1,4-dioxane (10 ml) were added $NaBH_4$ (0.43 g, 11.3 mmol) in portions at 0 °C. After the addition of the $NaBH_4$, another portion of 37% formaldehyde-solution (1.0 ml, 12.5 mmol) followed by $NaBH_4$ (0.43 g, 11.3 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 1 h. The mixture was diluted with H_2O (10 ml) and the pH was adjusted to around 6 by saturated $NaHCO_3$ aq. The organic solvents were removed in vacuo and the remaining water phase was extracted with $CHCl_3$. The collected organic phases were dried over $MgSO_4$, filtered and concentrated in vacuo to afford Fmoc-L-Lys(me^2)-OH (1.2 g, quant.) as a white solid. The product was used for the next reaction without purification. **^{1}H NMR** (DMSO-d_6, 600 MHz) δ 7.76 (2 H, d, *J* = 3.7 Hz), 7.65 (2 H, t, *J* = 7.8 Hz), 7.40 (2 H, t, *J* = 7.2 Hz), 7.32 (2 H, t, *J* = 7.5 Hz), 7.22 (1 H, t, *J* = 7.5 Hz, NH), 6.14 (1 H, m), 4.34 (3 H, m), 4.22 (1 H, m), 3.00 (2 H, m), 2.78 (6 H, s), 1.96-1.85 (4 H, m), 1.52 (2 H, m).

2nd step. Fmoc-L-Lys(me^3)-OH was synthesized by following an optimized previous protocol (R. Baba, Y. Hori, S. Mizukami, and K. Kikuchi, J. Am. Chem. Soc., 2012, 134, 14310–14313). Fmoc-L-Lys(me^2)-OH (1.4 g, 3.2 mmol) was dissolved in anhydrous MeCN/MeOH (2/1, 40 ml) and

stirred at 0 °C for 10 min. Iodomethane (2.0 ml, 32.3 mmol) and DIEA (1.7 ml, 9.7 mmol) were added. The mixture solvent was stirred at room temperature for 30 min. The solvent was removed *in vacuo* and the residue was dissolved in iPrOH (25 ml) and THF (6 ml). Then, $CaCl_2 \cdot H_2O$ (3.5 g, 23.7 mmol) was added to the solution, and the mixture was cooled to 0 °C. LiOH (140 mg, 5.9 mmol) was dissolved in water (7 ml) and added dropwise to the reaction mixture. The resulting mixture was warmed to room temperature and stirred for 3 h. After 1 h, the mixture was gently neutralized with AcOH and organic solvent was removed in vacuo. The solvent was removed *in vacuo* and purified by column chromatography ($CHCl_3$/MeOH = 2/1) to give Fmoc-L-Lys(me^3)-OH as a white powder (330 mg, 0.095 mmol, 73% yield). **^{1}H NMR** (DMSO-d_6, 600 MHz) δ 7.91 (2 H, d, *J* = 7.7 Hz), 7.71 (2 H, t, *J* = 7.0 Hz), 7.43 (2 H, t, *J* = 7.4 Hz), 7.36 (2 H, m), 4.22-4.28 (3 H, m), 3.77 (1 H, m), 3.29 (2 H, m), 3.07 (9 H, s), 1.64-1.78 (4 H, m), 1.29-1.33 (2 H, m). **^{13}C NMR** (150 MHz, DMSO-d_6) δ 156.5, 144.8, 141.6, 128.5, 128.0, 126.1, 121.0, 66.2, 63.1, 56.1, 52.9, 49.4, 47.6, 43.3, 23.2, 18.0, 10.5. HRMS(ESI): m/z calcd for $C_{24}H_{31}N_2O_4$ ($[M+H]^+$): 411.228; found: 411.272.

b) Synthesis of peptides 10, 11, 12.

Peptide 10. To prepare the C-terminal hydrazide peptide, 2-Cl-(Trt)-Cl resin (10 μmol scale) was used.[1] Briefly, the resin was swelled in 50% DCM/DMF for 30 min. After removing the solvent, 5% hydrazine in DMF (400 μl) was added to the resin and the mixture was agitated for 20 min at r.t. then the solvent was drained and washed by DMF. This operation was conducted again, and the resin was washed by DMF, DCM and DMF. Next, 5% MeOH/DMF (400 μl) was added and stirred for 10 min. After removing the solvent, the resin was washed by DMF, DCM and DMF. Immediately, to the resin were added Fmoc-Pen(Trt)-OH (4 equiv.), HBTU (3.8 equiv.) and DIEA (8 equiv.). The mixture was stirred for 60 min, and then washed by DMF, DCM and DMF three times, respectively. After automated SPPS. To the resin was added cleavage cocktail (90% TFA, 5% thioanisole, 3% EDT and 2% anisole) and the mixture was gently stirred at room temperature for 2 h. Then 10-fold amount of ether was added, vortexed and centrifuged 5,000 × g at room temperature for 1 min. Ether was decanted and washed with ether three times. The crude peptide was dissolved in mixture of water/acetonitrile containing 0.1 % TFA, and purified by RP-HPLC and identified by MALDI-TOF mass spectrometry (25.4 mg, 40% yield calculated from the resin loading).

Peptide 11. The C-terminal hydrazide was prepared in the same procedure as peptide **10**. After ether precipitation, 6 M Gn·HCl and 0.2 M NaH_2PO_4 at pH 3.0 (peptide concentration: 2–3 mM). The solution was cooled to -15 °C and 1 M $NaNO_2$ aq was added (10 equiv.). The mixture was stirred at -15 °C for 15 min, and then 1 M MESNa aq (50 equiv.) was added to the reaction mixture. The pH was adjusted to 6.5–7.0 with 6 N NaOH aq and the solution was stirred at room temperature for 30 min. The peptide solution was diluted by mixture of water/acetonitrile containing 0.1 % TFA and

purified by HPLC (8.4 mg, 11% yield calculated from the resin loading).

Peptide 12. Fmoc-Ala-Alko resin (0.23 mmol/g, 10 μmol scale, Watanabe Chemical Industries.) was used for C-terminal carboxyl peptides. After automated SPPS, the peptide was cleaved by cleavage cocktail (90% TFA, 5% thioanisole, 3% EDT and 2% anisole). The mixture was rotated at room temperature for 2 h under nitrogen atmosphere. To reduce oxidized Met and prevent Acm removals during TFA cleavage, premixed 1% (w/v) of tetrabutylammonium iodide (TBAI) and 10% (v/v) of dimethyl sulfide dissolved in the cleavage cocktail was added to the peptide solution.[2,3] The mixture was stirred for further 10 min and filtered to remove the resin. Then ether was added, vortexed and centrifuged 5,000 × g at room temperature for 1 min. Ether was decanted and washed with ether three times. The crude peptide was dissolved in mixture of water/acetonitrile containing 0.1 % TFA, and purified by RP-HPLC and identified by MALDI-TOF mass spectrometry (9.6 mg, 15% yield calculated from the resin loading).

c) One-pot three-segment ligation. Peptide **11** (0.87 μmol, 5.6 mg) and **12** (0.82 μmol, 5.4 mg) were dissolved in 248 μl NCL buffer (6 M Gn.HCl, 0.2 M NaH_2PO_4, 100 mM MPAA at pH 7.0) (the concentration of peptide was 2.1 mM). The mixture was stirred at 37 °C for 2 h under argon atmosphere (first NCL). Then, 4.9 μl $MgCl_2$ aq (500 mM) (3 equiv.) was added, and pH was reduced to 5.0–6.0 with HCl aq (9 N). The solution was bubbled by argon gas to remove air. To the reaction solution 21 μl Pd/TPPTS solution (100 mM) (2.5 equiv.) was added, and the mixture was stirred for 20 min at 37 °C under argon atmosphere to afford peptide **13**.

In a separated tube, to the solution of peptide **10** (18.0 mg, 2.8 μmol) in 6 M Gn-HCl and 0.2 M NaH_2PO_4 (pH 3.0) was added 7 μl $NaNO_2$ aq (1 M) (2.5 equiv) under -15 °C, and the mixture was stirred for 15 min. To the mixture was added 20 μl MPAA (500 mM) (3.5 equiv versus **10**) in 6 M Gn-HCl and 0.2 M NaH_2PO_4 (pH 6.5) and the value of pH of the mixture was adjusted to pH 6.5-6.7. This peptide solution was transferred into the peptide solution containing peptide 8, and 20 μl TCEP (500 mM) was added to the mixture. The reaction solution was stirred at 37 °C for 4 h under argon atmosphere. For analysis of each reaction, aliquot from each reaction mixture was treated 15 μl MESNa aq (1 M) and 2 μl TCEP (500 mM), followed by stirring for 30 s and injection into analytical HPLC. Finally, 50 μl MESNa aq (1 M) and 20 μl TCEP aq (500 mM) were added to the whole reaction solution and the mixture was stirred at room temperature for 5 min. The peptide solution was diluted by mixture of water/acetonitrile containing 0.1% TFA and purified by HPLC to afford the desired product **9** (5.7 mg, 0.29 μmol) in 39% isolated yield.

d) Desulfurization. To peptide **14** (5.7 mg, 0.29 μmol) were added 82 μl denaturing buffer, 146 μl TCEP solution (500 mM dissolved in denaturing buffer), and 35 μl glutathione solution (1 M). To

the mixture were 29 μl VA-044 solution (200 mM). Final concentration of the mixture was peptide **14** (1 mM), TCEP (250 mM), glutathione (120 mM) and VA-044 (20 mM). The reaction mixture was stirred at 37 °C under argon atmosphere for 2 h. The peptide solution was diluted by mixture of water/acetonitrile containing 0.1 % TFA and purified by HPLC to afford desired product **15** (3.6 mg, 0.19 μmol) with 65% isolated yield.

e) Removal of Acm groups of peptide 15. To peptide **15** (3.6 mg, 0.19 nmol) were added 180 μl denaturing buffer (pH 7.0) and 6 μl Na_2PdCl_4 solution (300 mM). Final concentration of the mixture was peptide **15** (1 mM), Na_2PdCl_4 (10 mM). The reaction solution was stirred at room temperature for 1 h. To the peptide solution 90 μl DTT aq (1M) was added and the mixture was stirred for further 10 min. The precipitation was separated by centrifuge, and the supernatant solution was diluted by mixture of water/acetonitrile containing 0.1 % TFA and purified by HPLC to afford desired product **16** (3.1 mg, 0.16 μmol) with 82% isolated yield.

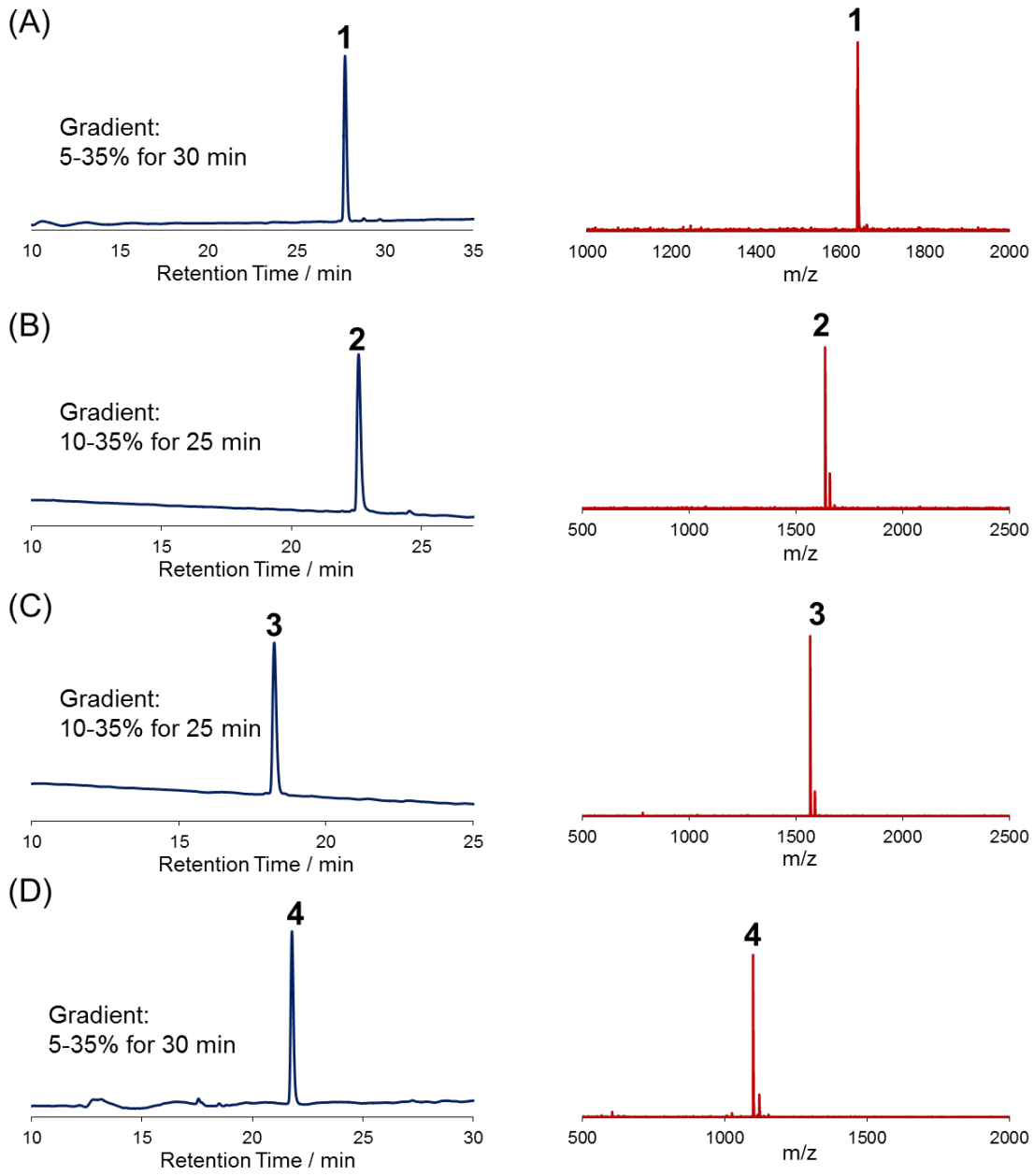


Figure S1. Synthesis of peptides **1**, **2, 3,** and **4**. HPLC profiles and MALDI-TOF mass spectrum of (A) peptide **1**, (B) peptide **2**, (C) peptide **3**, and (D) peptide **4**. HPLC peaks were monitored at 220 nm in the linear gradient of water-acetonitrile containing 0.1% TFA.

Calculated mass of **1** $[M+H]^+$: 1639.8; Mass Found $[M+H]^+$:1640.0.

Calculated mass of **2** $[M+H]^+$: 1637.8; Mass Found $[M+H]^+$:1637.8.

Calculated mass of **3** $[M+H]^+$: 1567.8; Mass Found $[M+H]^+$:1567.9.

Calculated mass of **4** $[M+H]^+$: 1100.3; Mass Found $[M+H]^+$:1100.5.

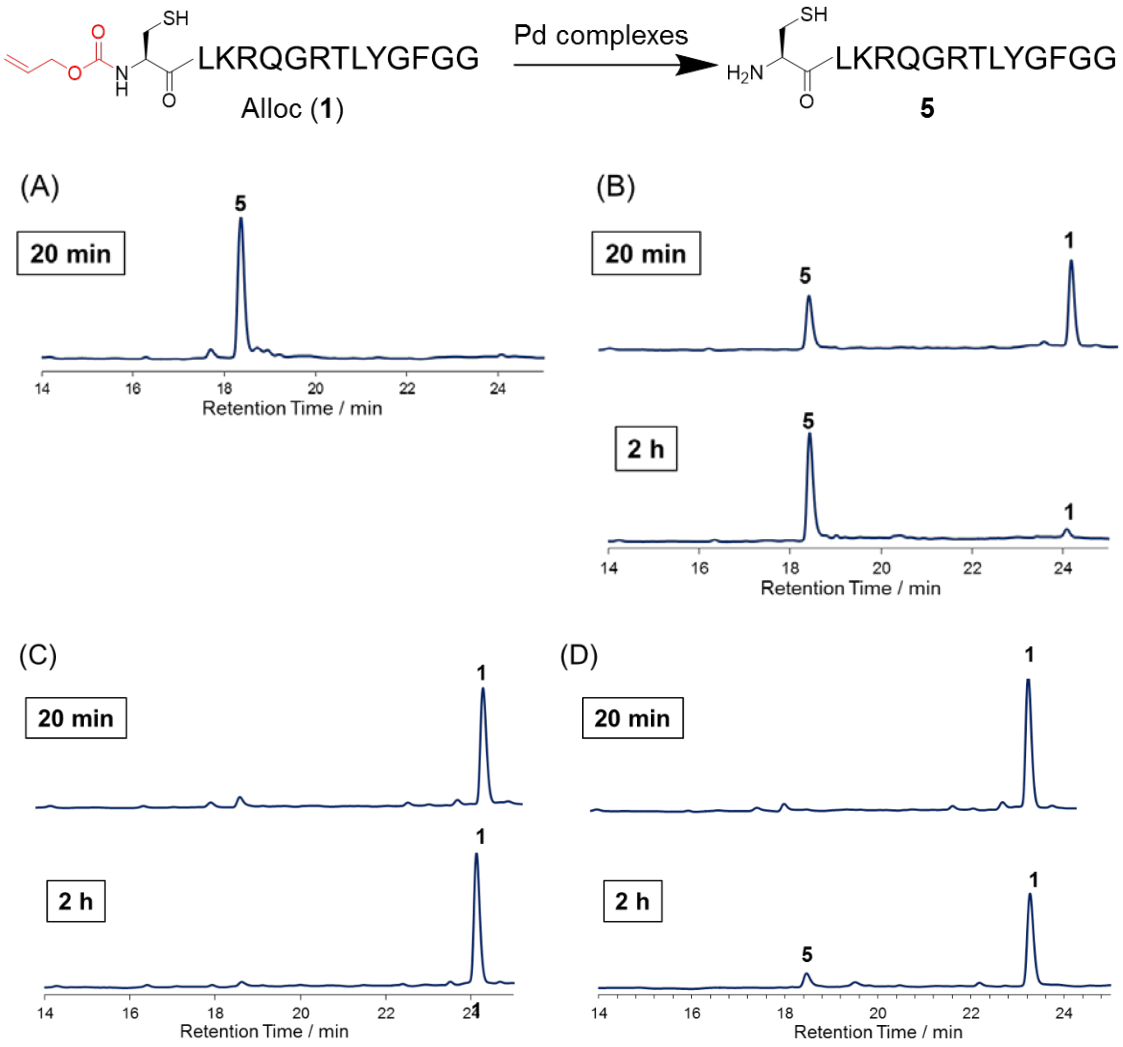


Figure S2. Removal of Alloc groups with Pd complexes. Peptide **1** was reacted with 10 equiv. of (A) Pd/TPPTS complex, (B) Na_2PdCl_4, (C) $[Pd(allyl)Cl]_2$, or (D) $[Pd(allyl)Cl]_2$/GSH. HPLC peaks were monitored at 220 nm in the linear gradient with water/acetonitrile containing 0.1% TFA. Gradient: 10-35% for 25 min.

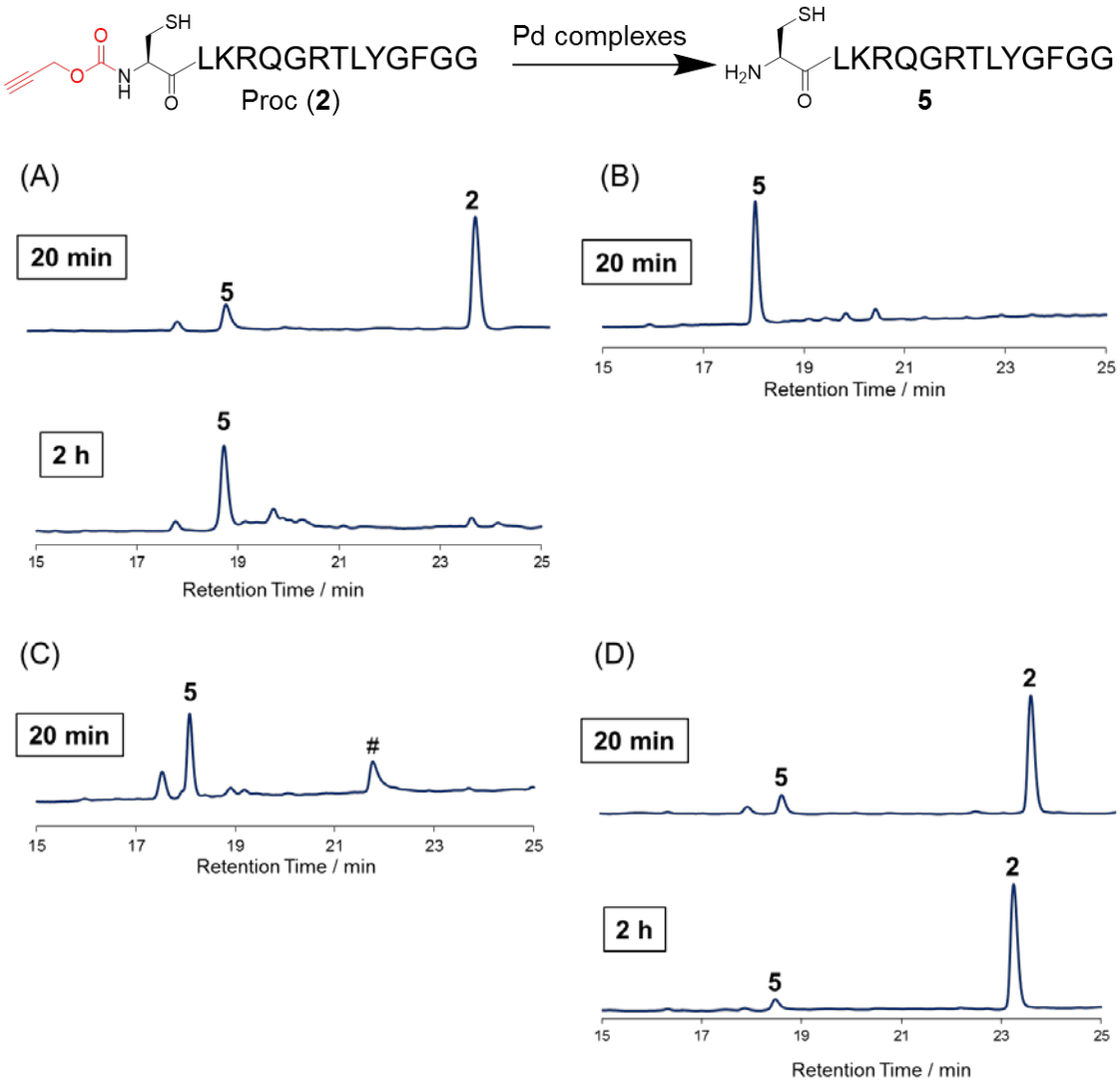


Figure S3. Removal of Proc groups with Pd complexes. Peptide **2** was reacted with 10 equiv. of (A) Pd/TPPTS complex, (B) Na_2PdCl_4, (C) $[Pd(allyl)Cl]_2$, or (D) $[Pd(allyl)Cl]_2$/GSH. HPLC peaks were monitored at 220 nm in the linear gradient with water/acetonitrile containing 0.1% TFA. Gradient: 10-35% for 25 min. #: not derived from peptide.

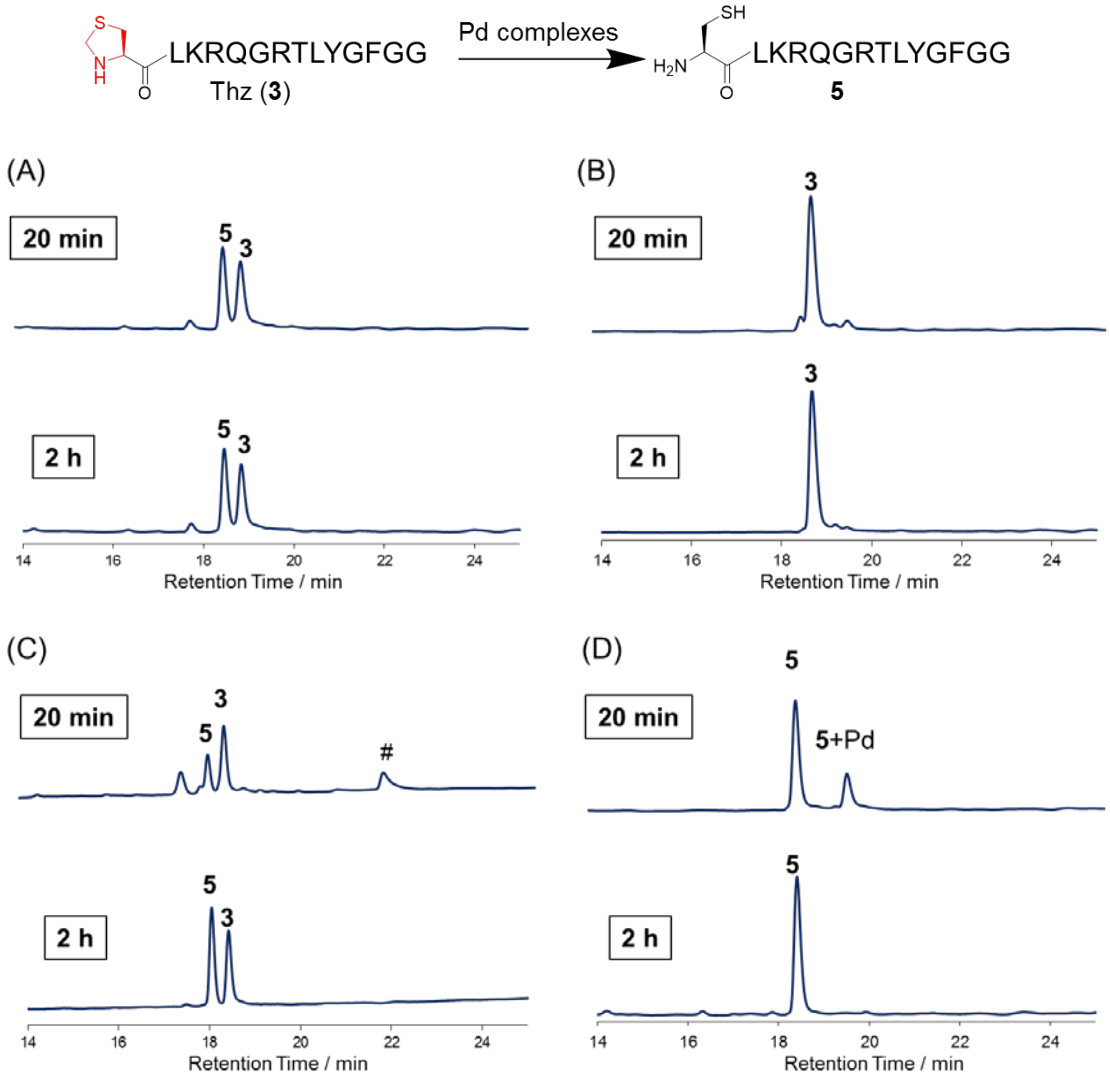


Figure S4. Removal of Thz groups with Pd complexes. Peptide **3** was reacted with 10 equiv. of (A) Pd/TPPTS complex, (B) Na_2PdCl_4, (C) $[Pd(allyl)Cl]_2$, or (D) $[Pd(allyl)Cl]_2$/GSH. HPLC peaks were monitored at 220 nm in the linear gradient with water/acetonitrile containing 0.1% TFA. Gradient: 10-35% for 25 min. #: not derived from peptide.

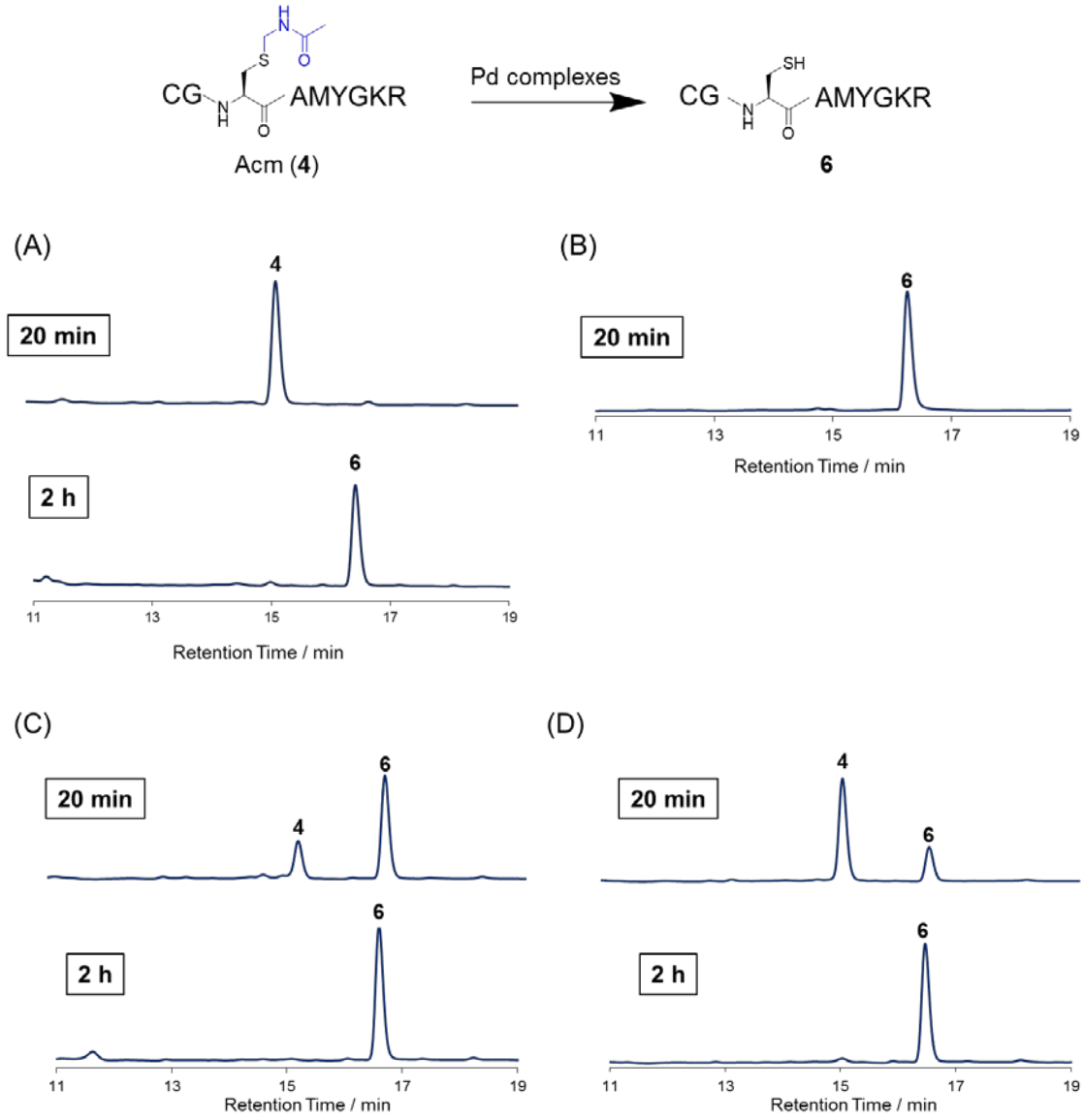
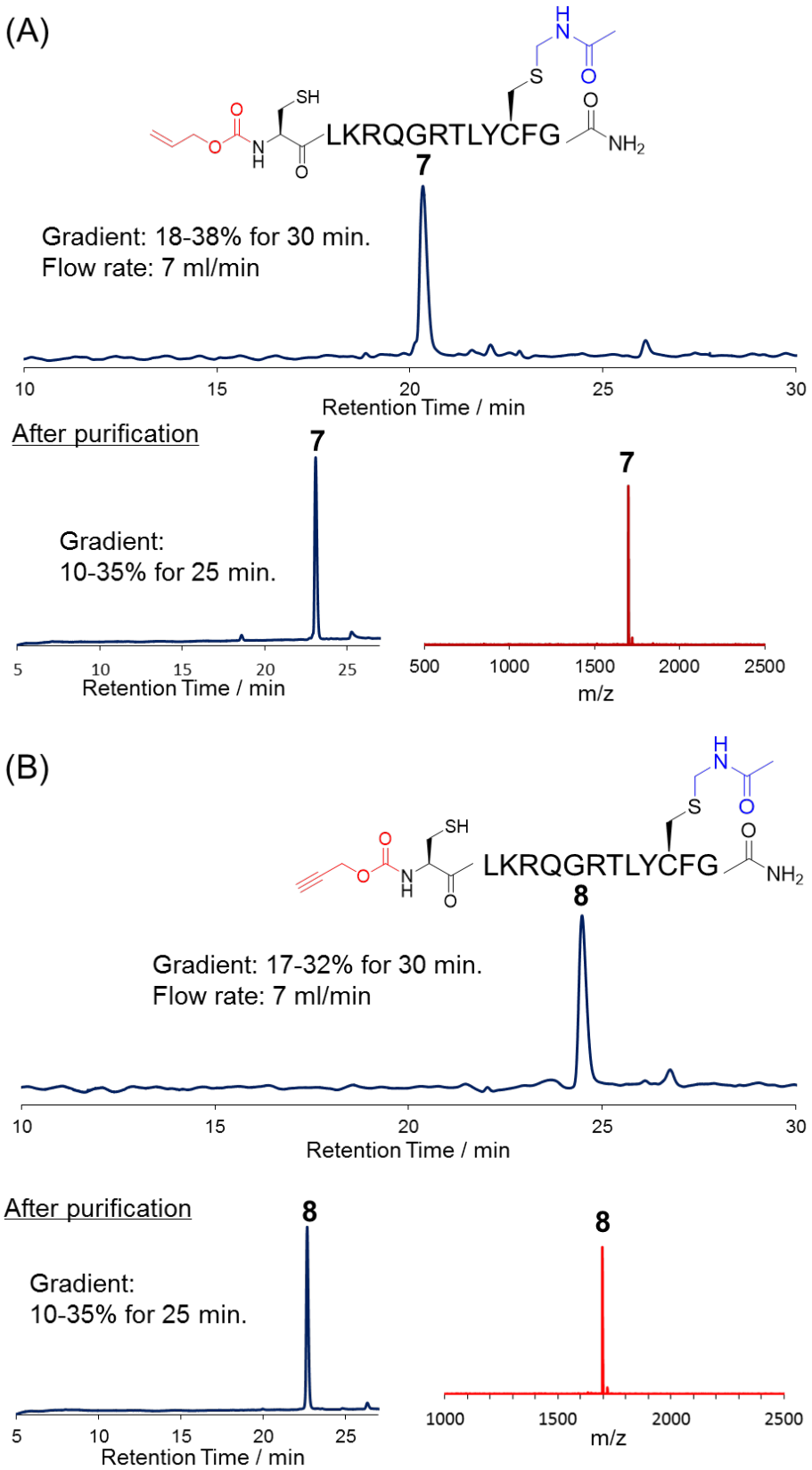


Figure S5. Removal of Acm groups with Pd complexes. Peptide **4** was reacted with 10 equiv. of (A) Pd/TPPTS complex, (B) Na_2PdCl_4, (C) $[Pd(allyl)Cl]_2$, or (D) $[Pd(allyl)Cl]_2$/GSH. HPLC peaks were monitored at 220 nm in the linear gradient with water/acetonitrile containing 0.1% TFA. Gradient: 10-25% for 20 min.


(A)
LKRQGRTLYCFG
7
Gradient: 18-38% for 30 min.
Flow rate: 7 ml/min
Retention Time / min
After purification
Gradient:
10-35% for 25 min.
m/z
(B)
LKRQGRTLYCFG
8
Gradient: 17-32% for 30 min.
Flow rate: 7 ml/min
Retention Time / min
After purification
Gradient:
10-35% for 25 min.
m/z

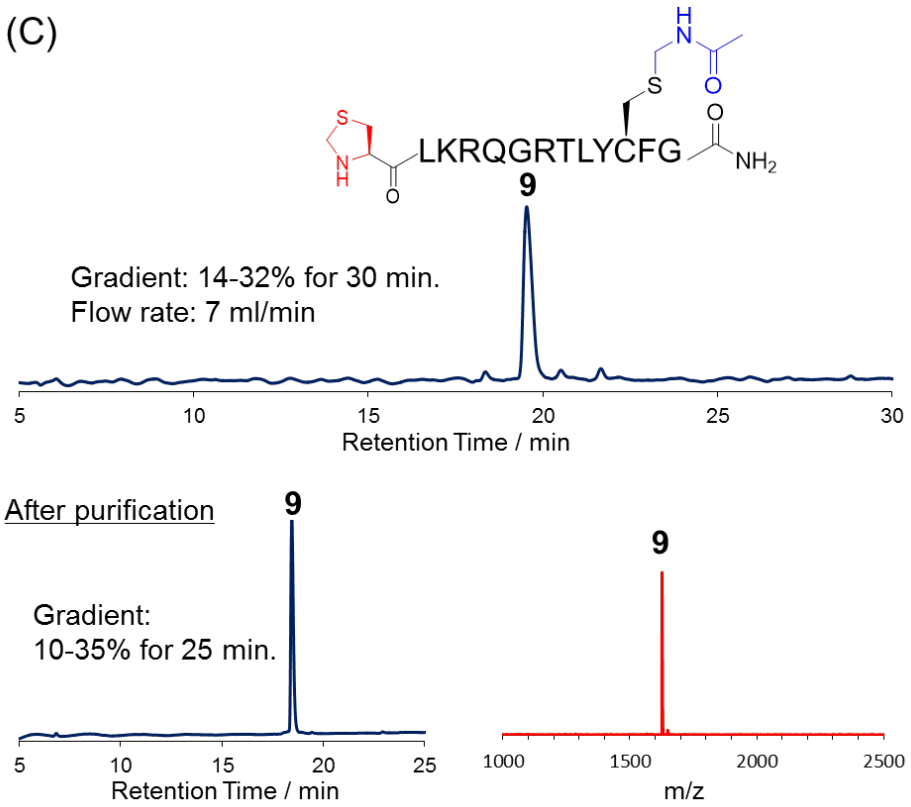


Figure S6. Synthesis of peptides **7**, **8**, and **9**. HPLC charts of crude peptide solutions, purified peptides, and MALDI-TOF (A) of peptide **7,** (B) of peptide **8**, and (C) of peptide **9**.

Calculated mass of **7** $[M+H]^+$: 1698.9; Mass Found $[M+H]^+$:1699.0.

Calculated mass of **8** $[M+H]^+$: 1696.8; Mass Found $[M+H]^+$:1696.5.

Calculated mass of **9** $[M+H]^+$: 1626.8; Mass Found $[M+H]^+$:1626.8.

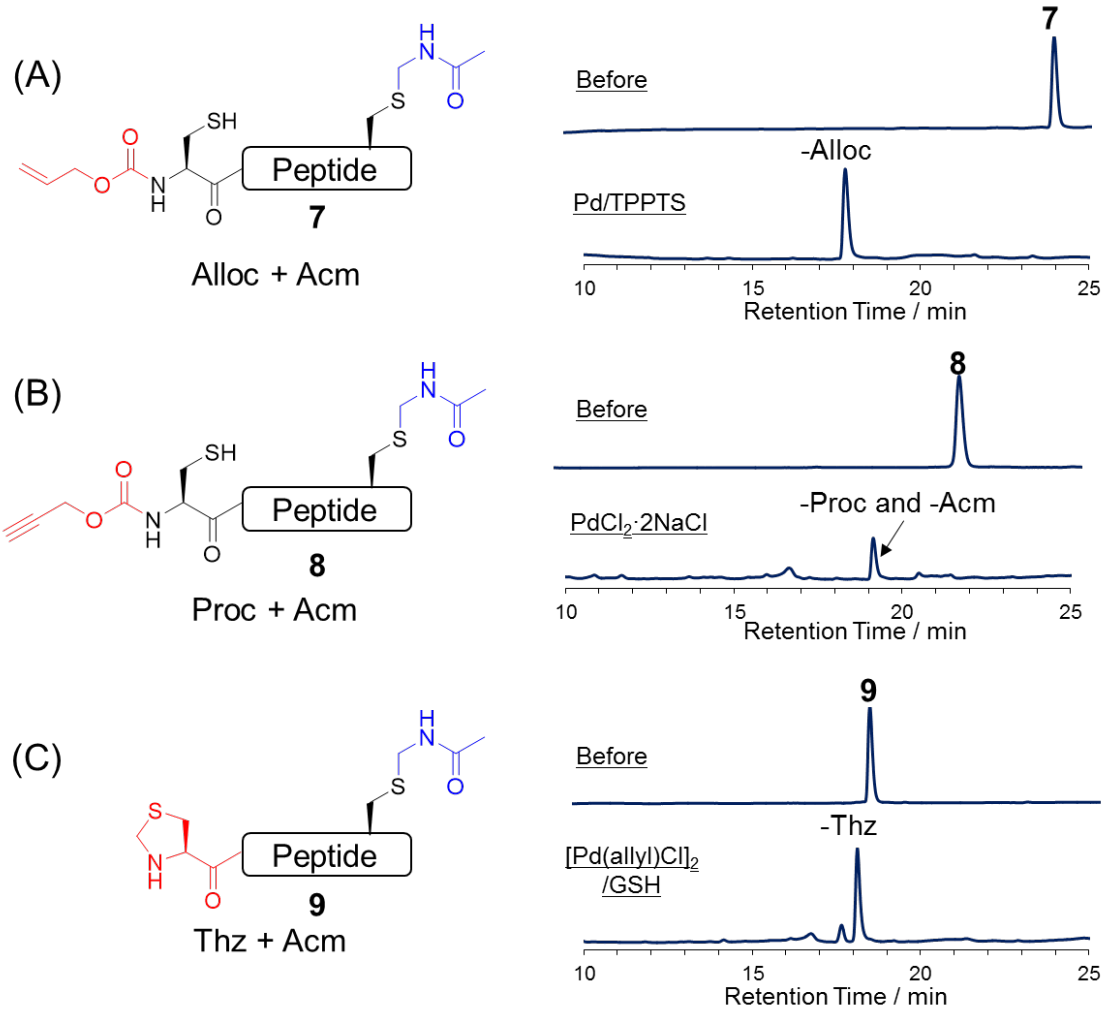


Figure S7. Removal of protecting groups for N-terminal Cys in the presence of Acm groups with Pd complexes under NCL conditions. (A) Alloc groups with Pd/TPPTS complex (2.5 equiv.). Calculated mass of [**7**-Alloc+H]$^+$: 1614.8; Mass Found: 1614.4. (B) Proc groups with Na_2PdCl_4 (50 equiv.). [**8**-Proc-Acm+H]$^+$: 1543.8; Mass Found: 1543.4. (C) Thz groups with $[Pd(allyl)Cl]_2$/GSH (30 equiv.). [**9**-Thz+H]$^+$: 1614.8; Mass Found: 1614.5.

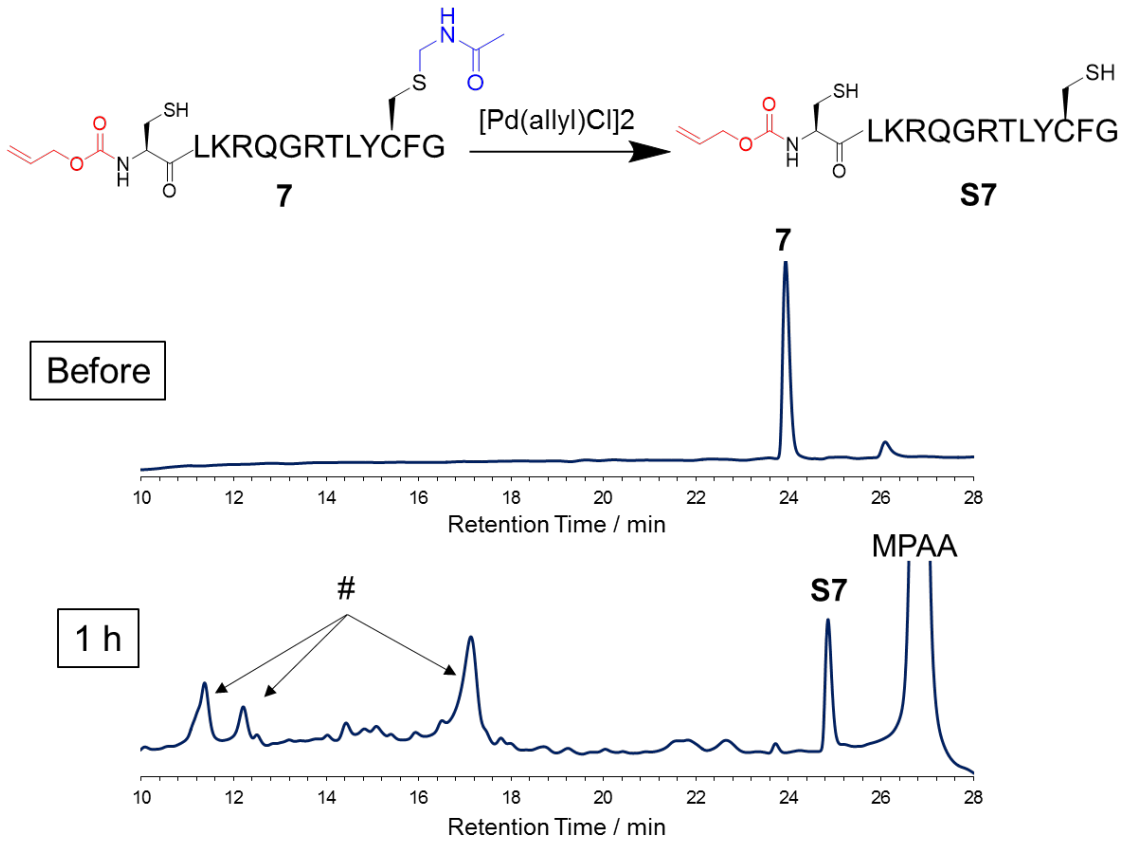


Figure S8. Reaction analysis of peptide **7** with $[Pd(allyl)Cl]_2$. HPLC peaks were monitored at 220 nm (solid line) in a linear gradient of water–acetonitrile containing 0.1% TFA. Gradient: 10-35% for 25 min. #: not derived from peptide.

Calculated mass of **S7** $[M+H]^+$: 1627.8; Mass Found $[M+H]^+$: 1627.7.

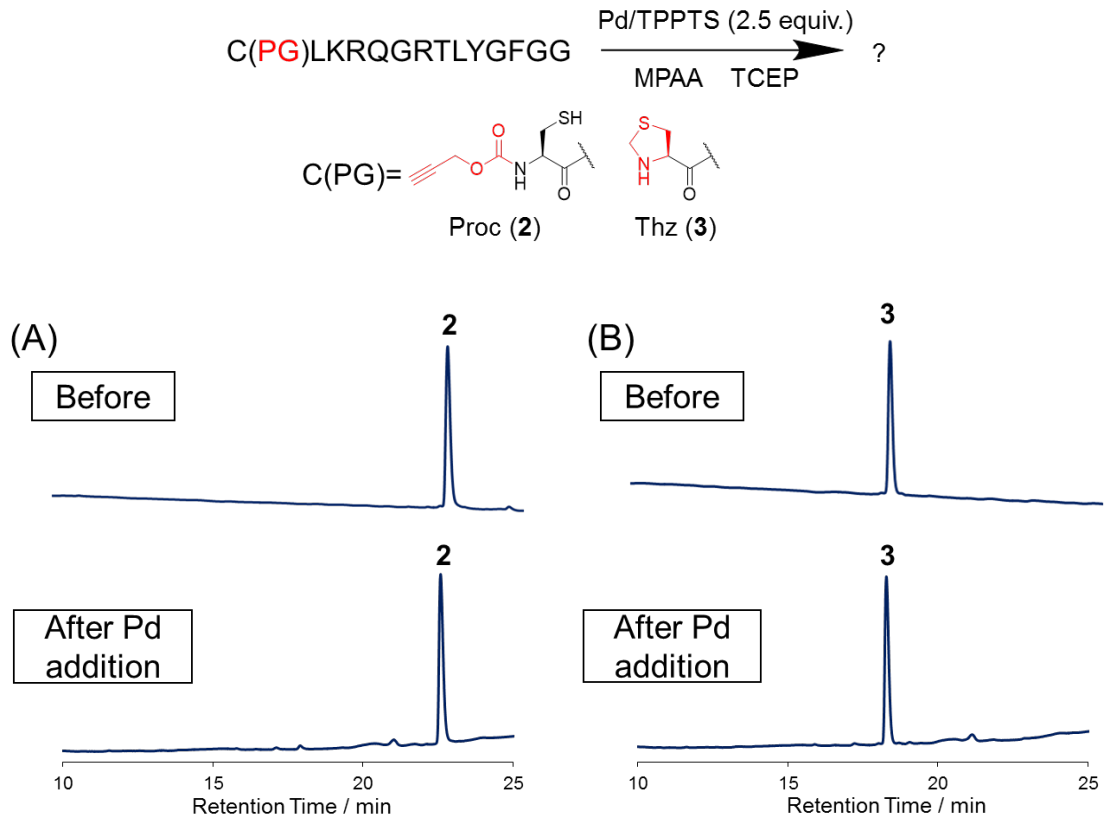
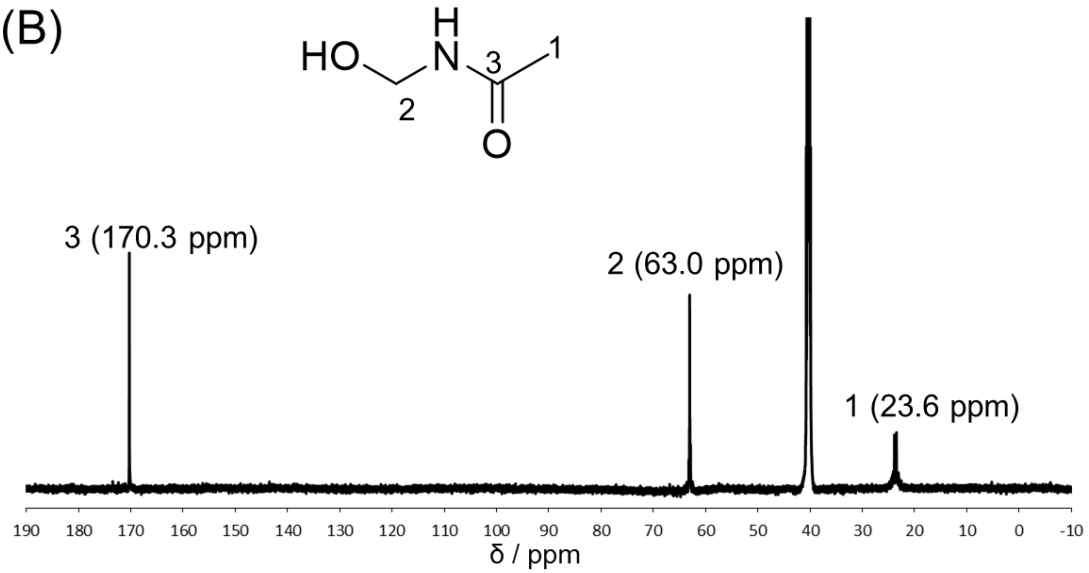


Figure S9. Reaction of peptide **2** or **3** with Pd/TPPTS complex under NCL conditions. (A) Peptide **2** (Proc). (B) Peptide **3** (Thz). Each peptide was treated with 2.5 equiv. of Pd/TPPTS in the presence of MPAA (50 equiv.) and TCEP (20 equiv.). HPLC peaks were monitored at 220 nm (solid line) in a linear gradient of water–acetonitrile containing 0.1% TFA. Gradient: 10-35% for 25 min.

(A)

Pd complexes	^{13}C NMR chemical shift [ppm]								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
No	23.4	29.0	32.7	41.2	54.5	79.1	156.3	170.3	173.4
$PdCl_2$	23.4	28.9	67.9	63.0	55.3	79.3	156.2	171.0	171.6 and 173.0
Pd/TPPTS	22.2	29.1	34.2	41.4	55.3	78.9	156.2	170.7	173.4

(B)



(C)

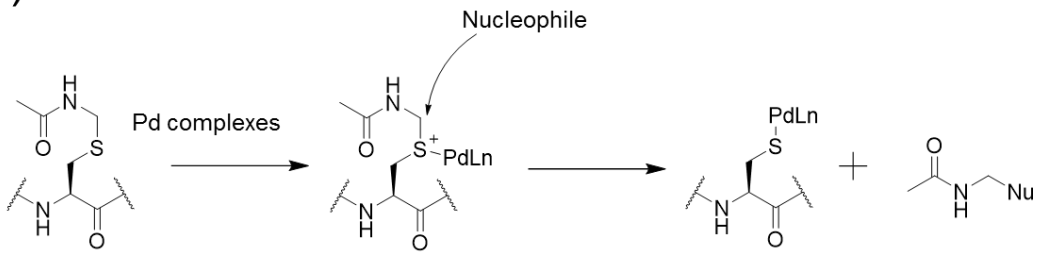
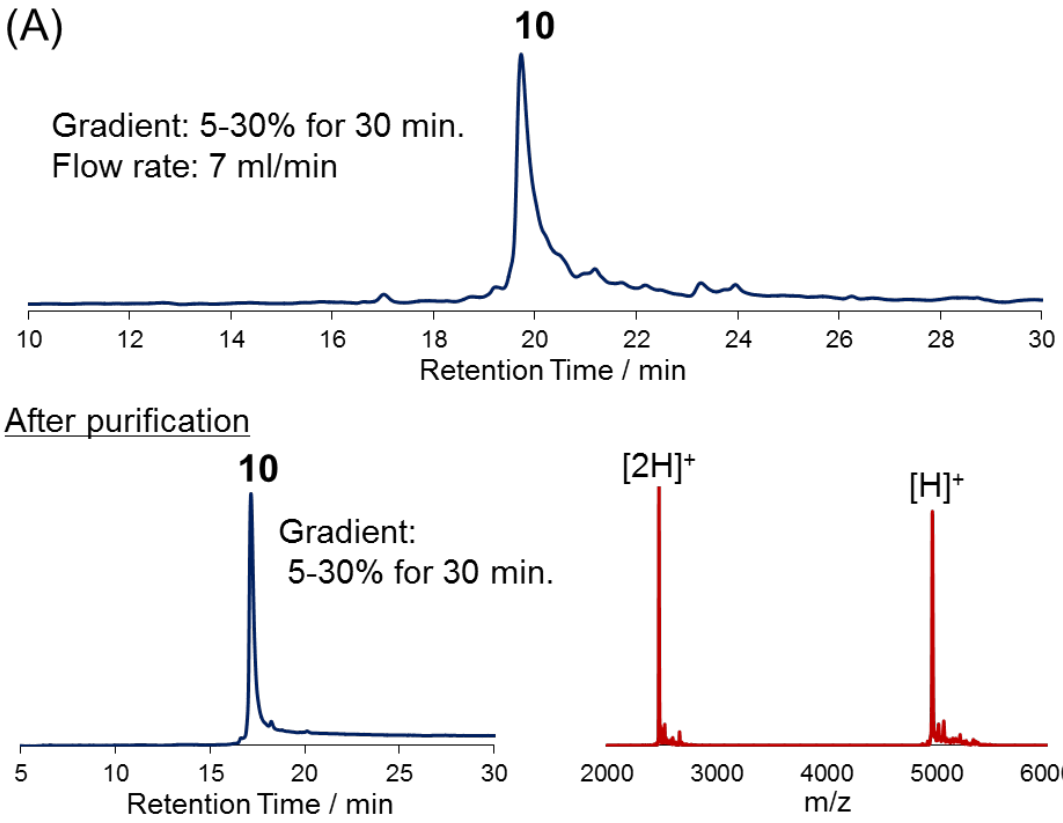
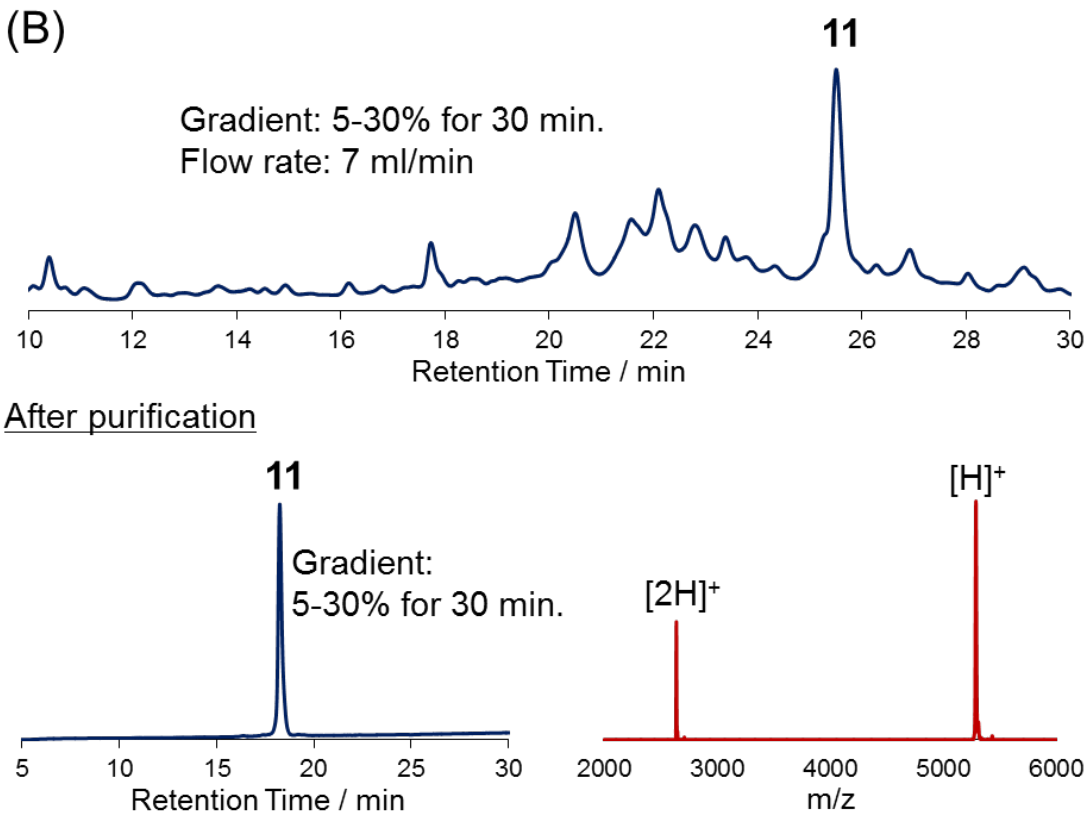


Figure S10. NMR analysis of deprotection of Acm groups. (A) ^{13}C NMR chemical shift assignments (ppm) for Boc-Cys(Acm)-OH in DMSO-d_6. NMR spectrum is shown in Figure 2. (B) ^{13}C NMR spectra of *N*-(hydroxymethyl)acetamide in DMSO-d_6. (C) Proposed mechanism of removal of Acm group with Pd complexes. (Maity, S. K.; Jbara, M.; Laps, S.; Brik, A. *Angew. Chem. Int. Ed*. **2016**, *55*, 8108–8112.)


(A)
10
Gradient: 5-30% for 30 min.
Flow rate: 7 ml/min
10 12 14 16 18 20 22 24 26 28 30
Retention Time / min
After purification
10
Gradient:
5-30% for 30 min.
5 10 15 20 25 30
Retention Time / min
[2H]+
[H]+
2000 3000 4000 5000 6000
m/z


(B)
11
Gradient: 5-30% for 30 min.
Flow rate: 7 ml/min
10 12 14 16 18 20 22 24 26 28 30
Retention Time / min
After purification
11
Gradient:
5-30% for 30 min.
5 10 15 20 25 30
Retention Time / min
[H]+
[2H]+
2000 3000 4000 5000 6000
m/z

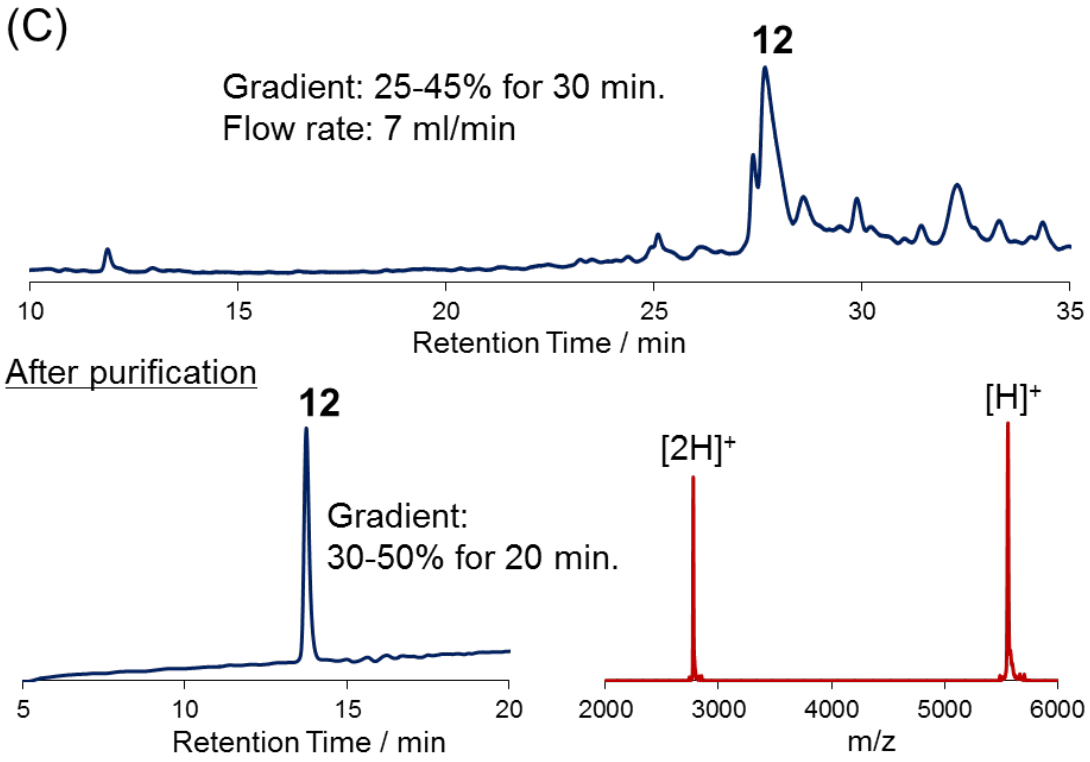


Figure S11. Synthesis of peptides **10**, **11**, and **12**. HPLC charts of crude peptide solutions, purified peptides, and MALDI-TOF (A) of peptide **10,** (B) of peptide **11**, and (C) of peptide **12**.

Calculated mass of **10** $[M]^+$: 4960.8; Mass Found $[M+H]^+$:4961.0.

Calculated mass of **11** $[M+H]^+$: 5292.2; Mass Found $[M+H]^+$:5291.7.

Calculated mass of **12** $[M+H]^+$: 5561.6; Mass Found $[M+H]^+$:5561.1.

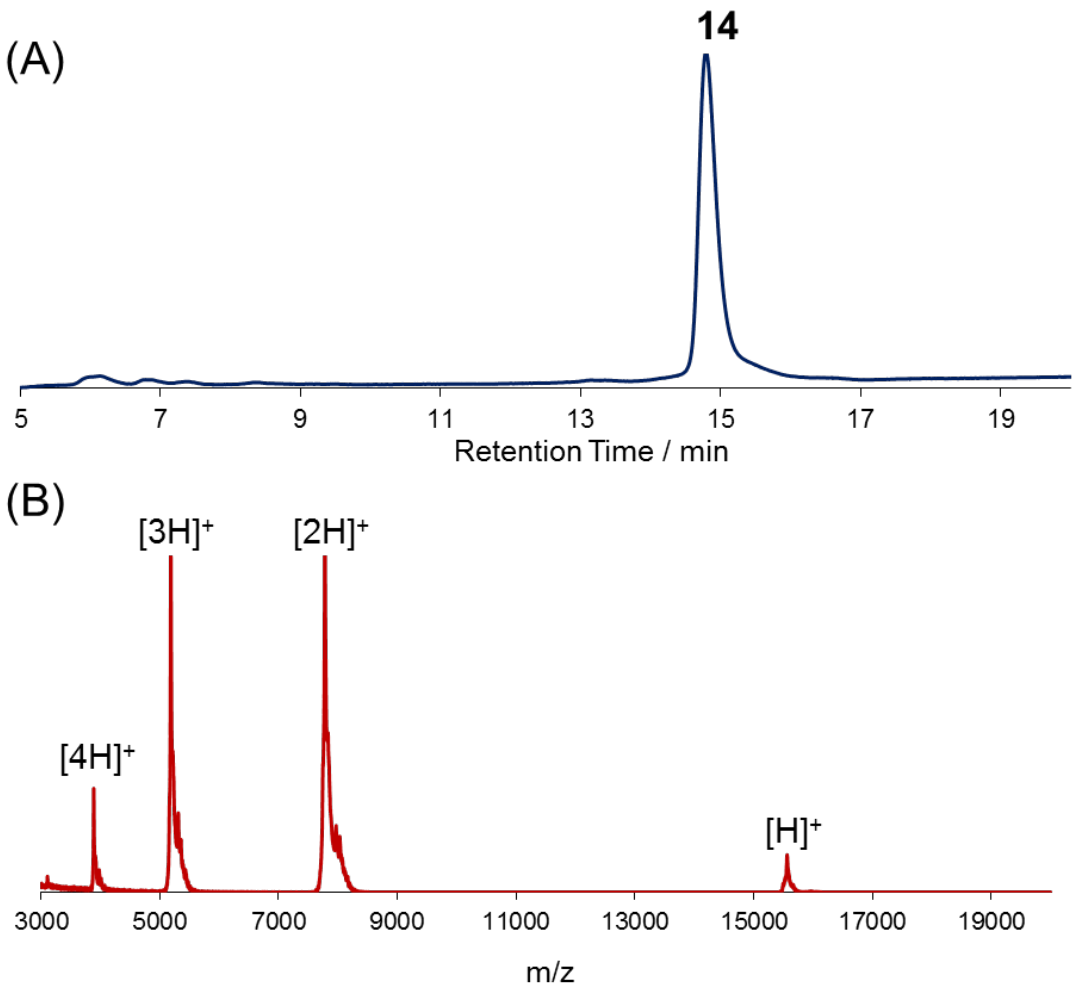


Figure S12. Identification of peptide **14**. HPLC peaks were monitored at 220 nm in the linear gradient with water/acetonitrile containing 0.1% TFA. (A) HPLC chart of purified peptide **9**. Gradient: 38-58% for 20 min. (B) MALDI-TOF mass spectrum of peptide **9**. Calculated mass of **5** $[M]^+$: 15554.4; Mass Found $[M]^+$:15555.4

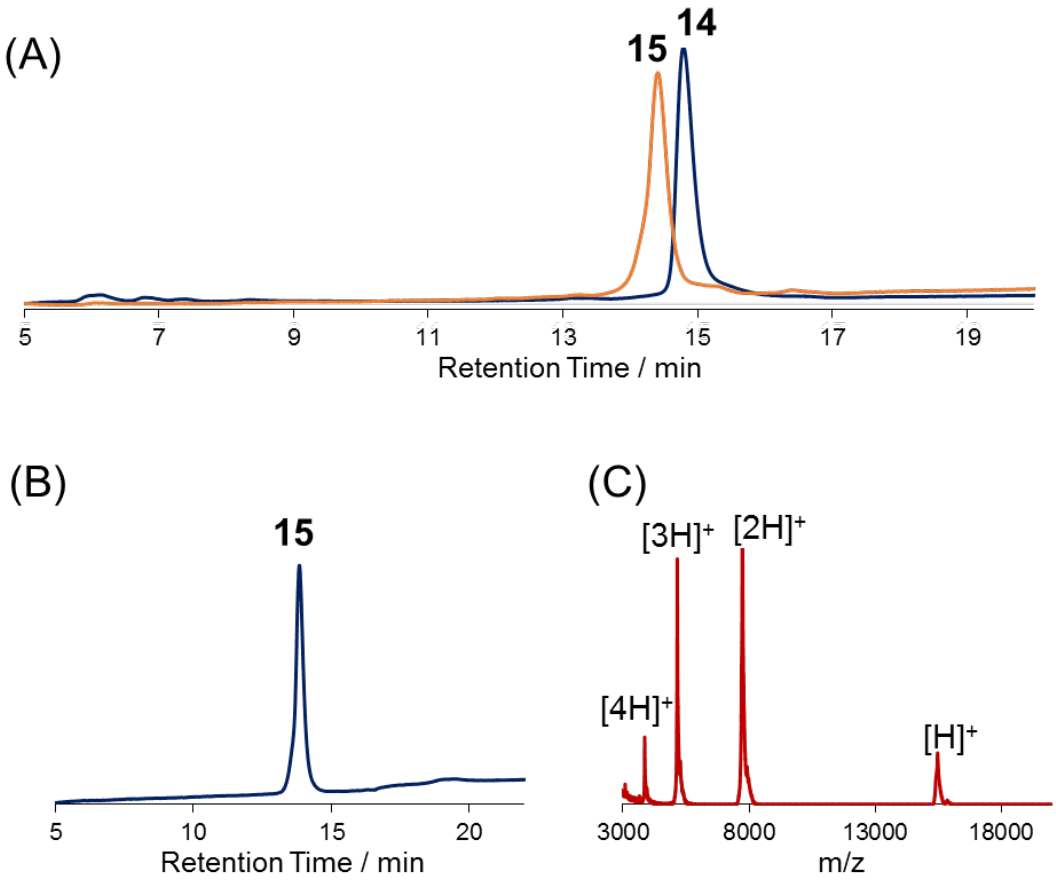
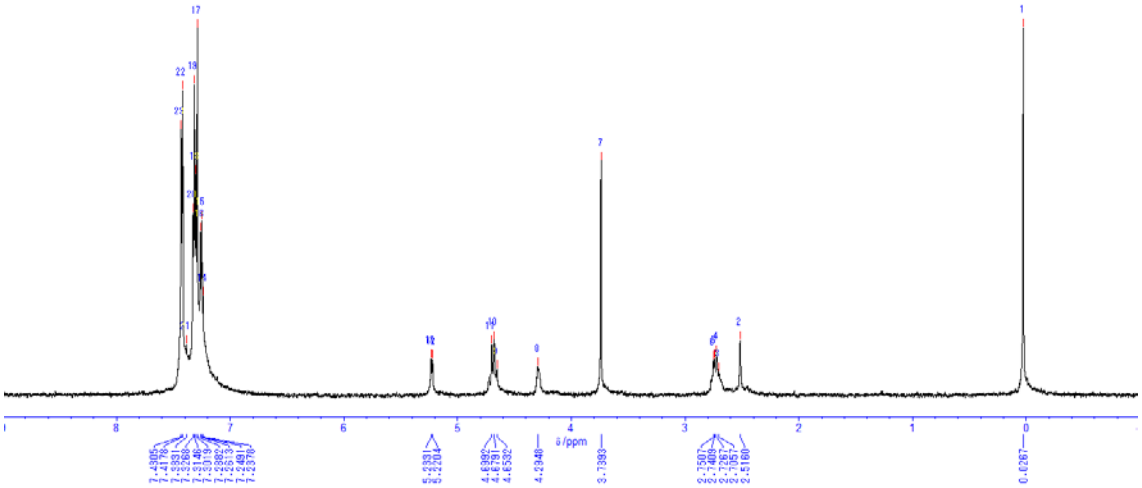
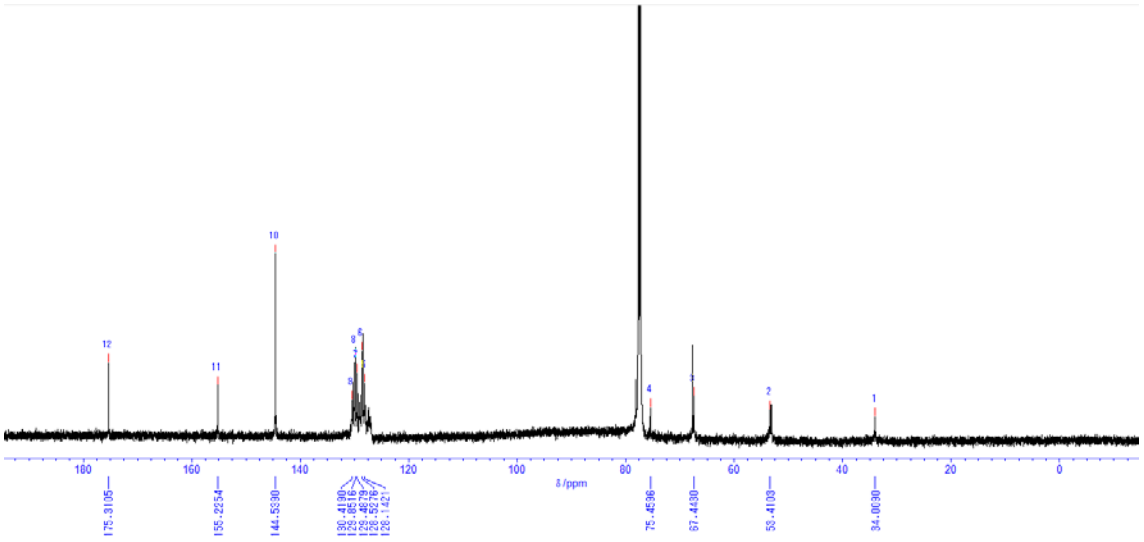


Figure S13. Desulfurization of peptide **14** and identification of peptide **15**. HPLC peaks were monitored at 220 nm in the linear gradient with water/acetonitrile containing 0.1% TFA. (A) HPLC analysis of desulfurization reaction. Peptide **14** (before reaction) and **15** (after 4 h reaction) were shown in blue and orange line, respectively. Gradient: 38-58% for 20 min. (B) HPLC profile of purified peptide **10**. Gradient: 38-58% for 20 min. (C) MALDI-TOF mass spectrum of peptide **10**. Calculated mass of **5** $[M]^+$: 15458.2; Mass Found $[M]^+$: 15459.4

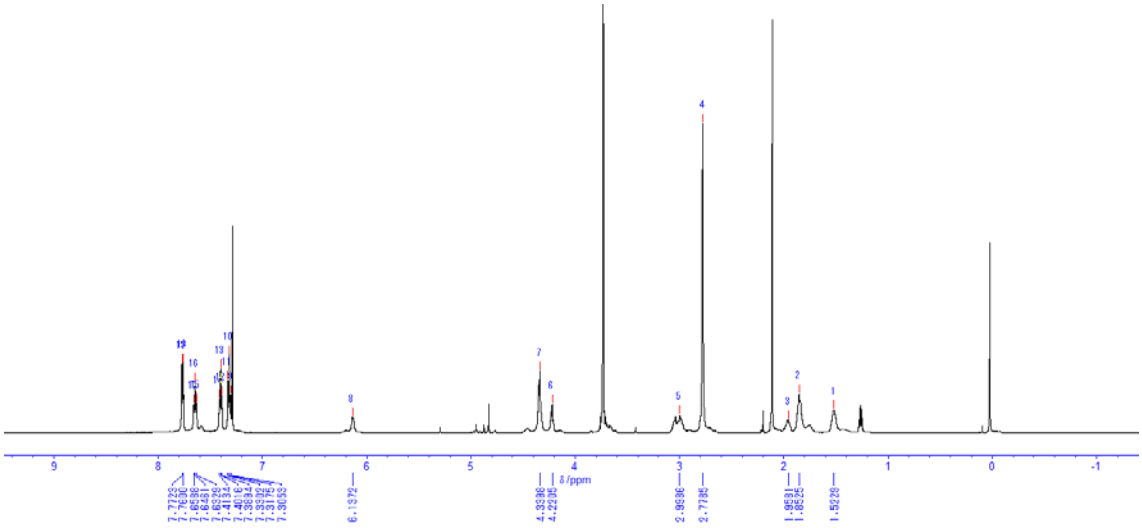
Proc-Cys(Trt)-OH (^{1}H NMR, $CDCl_3$, 600 MHz)



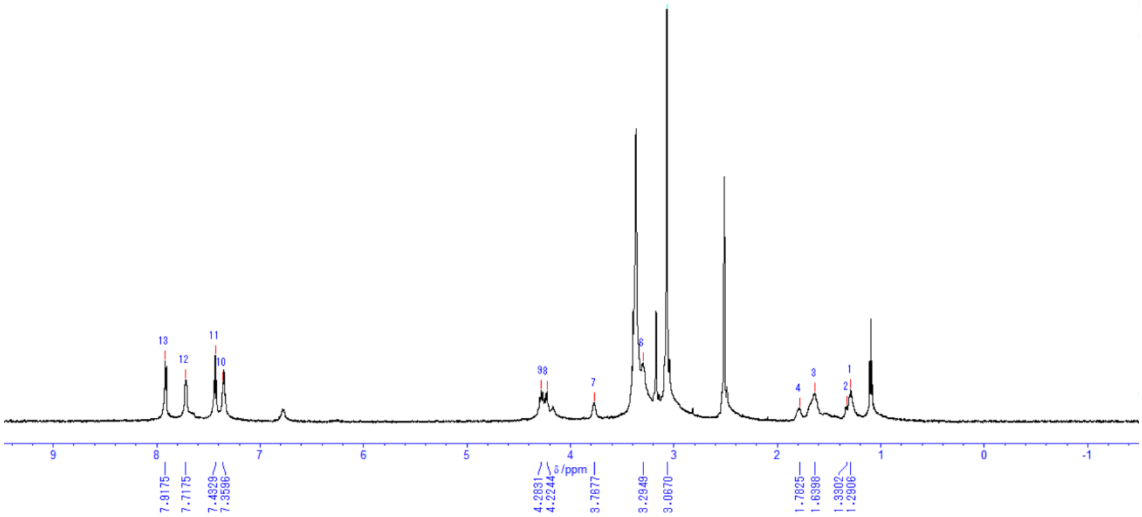
Proc-Cys(Trt)-OH (^{13}C NMR, $CDCl_3$, 150 MHz)



Fmoc-Lys(me^2)-OH (^{1}H NMR, $CDCl_3$, 600 MHz)



Fmoc-Lys(me^3)-OH (^{1}H NMR, $CDCl_3$, 600 MHz)



Fmoc-Lys(me^3)-OH (^{13}C NMR, $CDCl_3$, 150 MHz)