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Supporting Information:

Electrophilic substitution reaction of benzeneselenyl bromide (chloride) for the selective modification of tryptophan side chain in peptides

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Experimental section

Reagents and instruments

Peptides used in this work were synthesized by Fmoc-based solid phase peptide synthesis (SPPS) method and were used without further purification. Somatostatin (HPLC purity 97%) was obtained as gift from professor Tiesheng Shi. Benzeneselenyl bromide (BSB), benzeneselenyl chloride (BSC), trifluoroacetic acid (TFA), and acetonitrile were purchased from Shanghai Aladdin Biochemical Technology (Shanghai, China), and are of analytical grade. The human breast cancer cell line (MCF-7) used in this experiment was purchased from Dalian Meilun Biotechnology Co., Ltd. Peptides were purified on an LC-6AD semi-preparative high performance liquid chromatography system (Prep-HPLC, Shimadzu, Japan) with a 250 mm x 20 mm ODS-C18 column. All the reactions were monitored by an LC-20AB HPLC system (Shimadzu, Japan) with a 250 mm x 4.6 mm C18 column or by an LC-20AD HPLC system with a 250 mm x 4.6 mm C8 column. The UV detector was set at 215 nm for all the HPLC. The flow rate was set as 8.0 mL/min and 1.0 mL/min for semi-preparative HPLC and analytical HPLC, respectively. Two solvent systems consisting of 0.1% TFA in water (referred to as solvents A) and 0.1% TFA in acetonitrile (referred to as solvents B) were used for peptide elution with suitable gradients. Mass spectra were recorded on a Bruker Apex Ultra electrospray mass spectrometer with an ion-trap detector (Bruker Daltonics Inc., Billerica, MA, USA).

Synthesis of VQ7-SePh

11.0 mg of VQ7 (HPLC purity: 92%) was dissolved in HCl solution (2.0 M, 23.0 mL) to give a VQ7 solution (0.5 mM), followed by the addition of BSB (2.0 mM, 23.0 mL; BSB was dissolved in ethanol). The reaction mixture was aged for 40 min at room temperature, and then purified by the Prep-HPLC. 4.3 mg of VQ7-SePh was obtained. The isolated yield of VQ7-SePh was 34%.

Synthesis of FG7-SePh

11.2 mg of FG7 (HPLC purity: 91%) was dissolved in HCl solution (2.0 M, 12.2 mL) to give a VQ7

solution (1.0 mM), followed by the addition of BSB (4.0 mM, 12.2 mL; BSB was dissolved in ethanol). The reaction mixture was aged for 50 min at room temperature, and then purified by the Prep-HPLC. 5.5 mg of FG7-SePh was obtained. The isolated yield of FG7-SePh was 42%.

Synthesis of WN5-SePh

4.9 mg of WN7 (HPLC purity: 95%) was dissolved in HCl solution (2.0 M, 7.0 mL) to give a VQ7 solution (1.0 mM), followed by the addition of BSB (2.7 mM, 10.7 mL; BSB was dissolved in ethanol). The reaction mixture was aged for 40 min at room temperature, and then purified by the Prep-HPLC. 1.7 mg of WN5-SePh was obtained. The isolated yield of FG7-SePh was 28%.

Synthesis of somatostatin-SePh

10.0 mg of somatostatin (HPLC purity: 99%) was dissolved in HCl solution (1.0 M, 6.0 mL) to give a somatostatin solution (1.0 mM), followed by the addition of BSC (1.0 mM, 6.0 mL; BSC was dissolved in ethanol). The reaction was performed at room temperature for 3 h, then BSC (1.8 mM, 6.0 mL; BSC was dissolved in ethanol) and 1.0 M HCl (6.0 mL) were added to the reaction mixture in two portions, and reacted for 3 h, respectively. Finally, the reaction mixture was purified by the Prep-HPLC. 3.1 mg somatostatin-SePh was obtained. The isolated yield of somatostatin-SePh was 28%.

Synthesis of xylyl-somatostatin-SePh

11.4 mg of reduced somatostatin (HPLC purity: 98%) was dissolved in 6.9 mL H_2O to give a 1.0 mM peptide solution, followed by the addition of NH_4HCO_3 solution (2.0 mol/L, 1.0 mL) and *p*-xylyl solution (0.8 mmol/L, 6.9 mL; *p*-xylyl was dissolved in CH_3CN). The reaction mixture was aged for 1 h at room temperature. After that, BSB (4.0 mmol/L, 6.7 mL) and HCl (4.0 mol/L, 6.7 mL) were added to the reaction mixture. The reaction mixture was aged for another 1 h. The product was purified on Prep-HPLC. 4.3 mg of xylyl-somatostatin-SePh was obtained. The isolated yield of xylyl-somatostatin-SePh was 22%. Xylyl-somatostatin was also purified by Pre-HPLC, the isolated yield of xylyl-somatostatin was 53%.

Synthesis of phenylselenyl-tryptophanamide

Tryptophanamide hydrochloride (10 mg) was dissolved in HCl solution (2.0 M, 4.2 mL). The obtained tryptophanamide solution (10 mM, 4.2 mL) was mixed with BSC solution (40 mM, 4.2 mL; BSC dissolved in ethanol) for 1 h at 25 ℃. The pH value of the reaction mixture was adjusted to about 6-7. Then, the solvent was removed using a rotary evaporator, followed by the addition of H_2O (20 mL) to redissolve the generated phenylselenyl-tryptophanamide. After filtration, phenylselenyl-tryptophanamide was purified by Prep-HPLC. Two solvent systems consisting of 0.1% TFA in water (referred to as solvents A) and 0.1% TFA in acetonitrile (referred to as solvents B) were used for the purification. 3.9 mg of phenylselenyl-tryptophanamide (pale yellow solid) was obtained in a yield of 26%.

In vitro cytotoxicity evaluation

In vitro cytotoxicity evaluations were carried out in the Chemical Biology Key Laboratory of Hebei Province using the standard MTT method. The MCF-7 cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution under conditions of 5% CO2 at 37 °C. When the cell density reached 90%, the cells were digested with trypsin for subculture and subsequently used for further experiments. MCF-7 cells were seeded into 96-well plates (5×10^3 cells per well). After 24 hours of incubation, a blank control group and experimental groups were established. The culture medium in the experimental groups was replaced with serum-free medium containing various concentrations of peptides, while the control group received fresh serum-free medium. Following another 24 hours of incubation, 10 μL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 hours. Upon completion of the incubation, the 96-well plates were removed from the incubator, the supernatant was discarded, and 100 μL of DMSO was added to each well. The plates were then shaken on an orbital shaker for 15 minutes. The absorbance (OD value) was measured at 570 nm using a microplate reader. Cell viability was calculated using the following formula:Cell Viability (%) = $(A_s - A_b)/(A_c - A_b) \times 100\%$, where A_s represents the absorbance of the experimental wells, A_c represents the absorbance of the control wells, and A_b represents the absorbance of the blank wells.

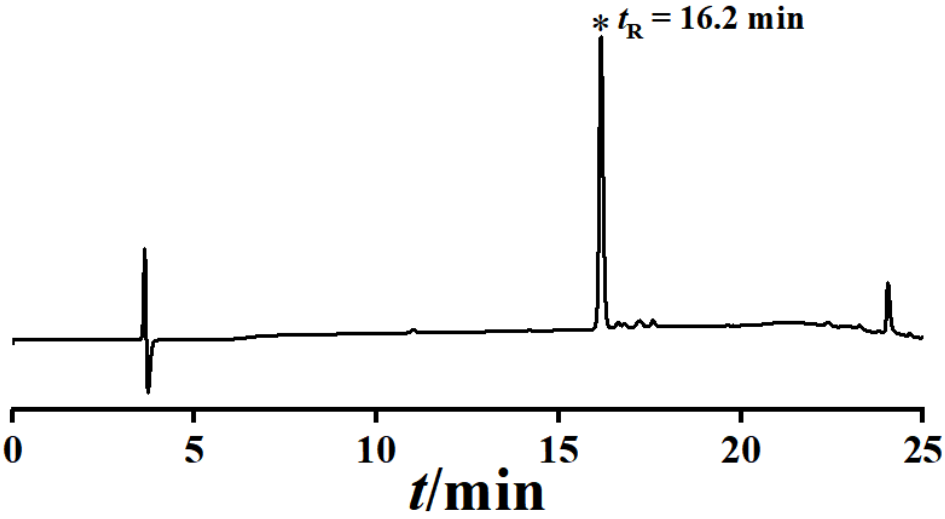

Fig. S1 HPLC chromatogram of the purified phenylselenyl-tryptophanamide; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 45% B over 5 min, and further to 90% over 5 min and then to 10% over 5 min. Retention time = 16.2 min.

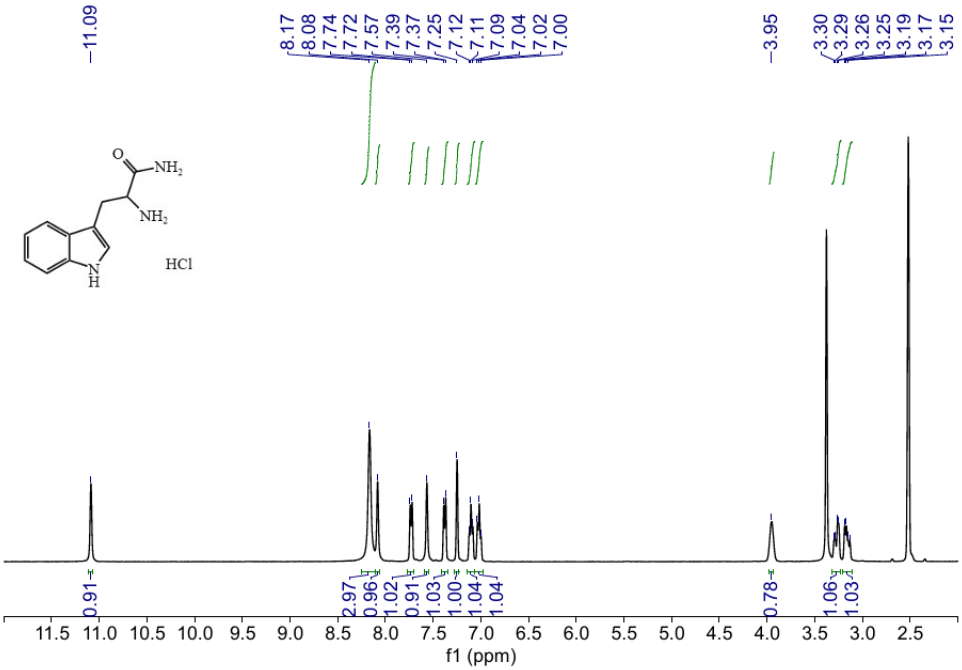

Fig. S2 ^{1}H NMR of tryptophanamide hydrochloride; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.09 (brs, 1H), 8.17 (brs, 3H), 8.08 (s, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.57 (brs, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.11 (t, J = 8.0 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 3.96 (s, 1H), 3.25-3.30 (m, 1H), 3.13-3.19 (m, 1H).

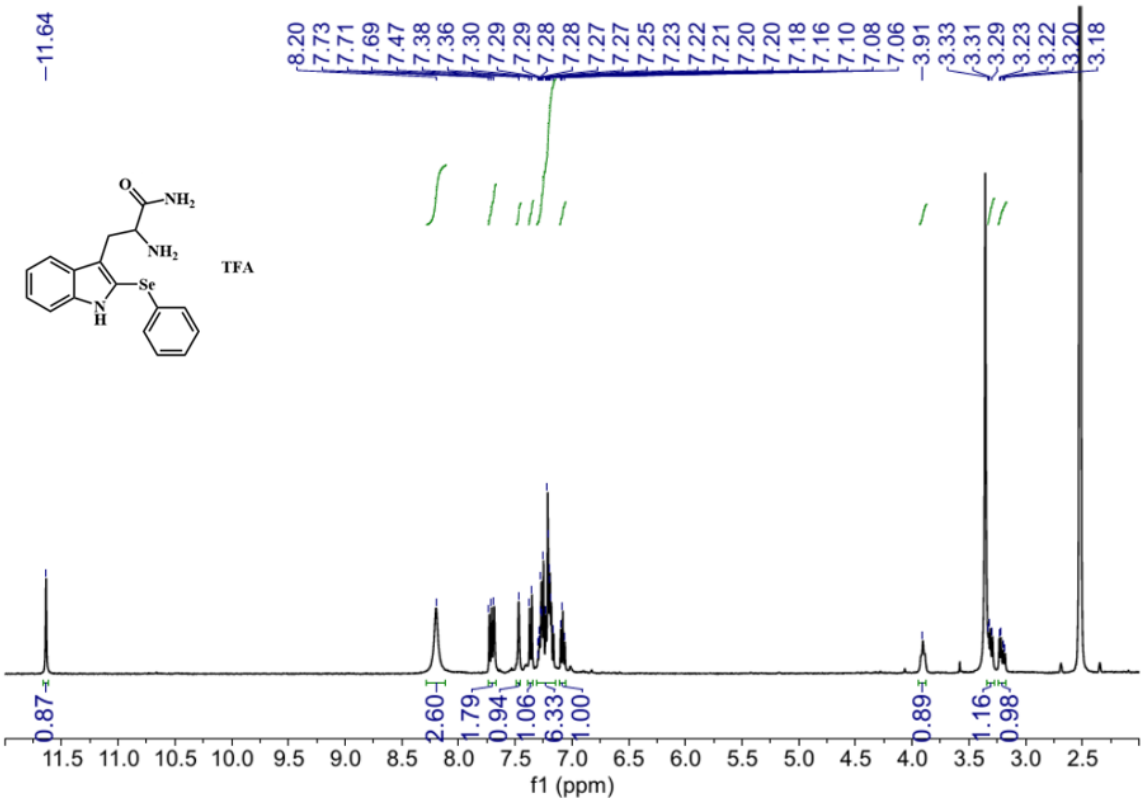

Fig. S3 ^{1}H NMR of phenylselenyl-tryptophanamide; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.64 (brs, 1H), 8.20 (brs, 3H),7.72 (d, J = 8.0 Hz, 1H), 7.69 (brs, 1H), 7.47 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.16-7.30 (m, 6H), 7.08 (t, J = 8.0 Hz, 1H), 3.91 (t, J = 8.0 Hz, 1H), 3.29-3.33 (m, 1H), 3.18-3.23 (m, 1H).

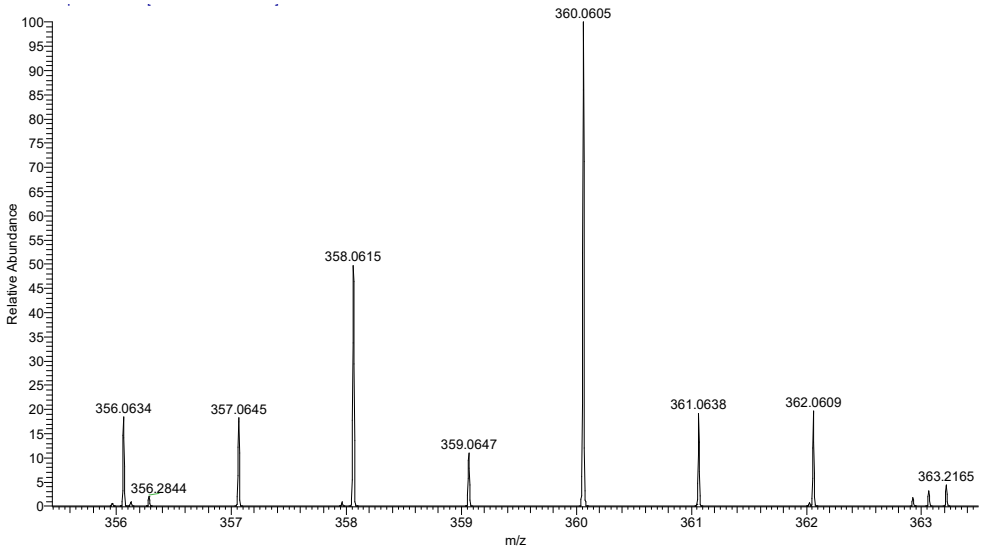

Fig. S4 Mass spectrum of phenylselenyl-tryptophanamide; MS (ESI) m/z: $[M + H]^+$ Calcd. for $C_{17}H_{18}ON_3Se$ 360.0616; found 360.0605.

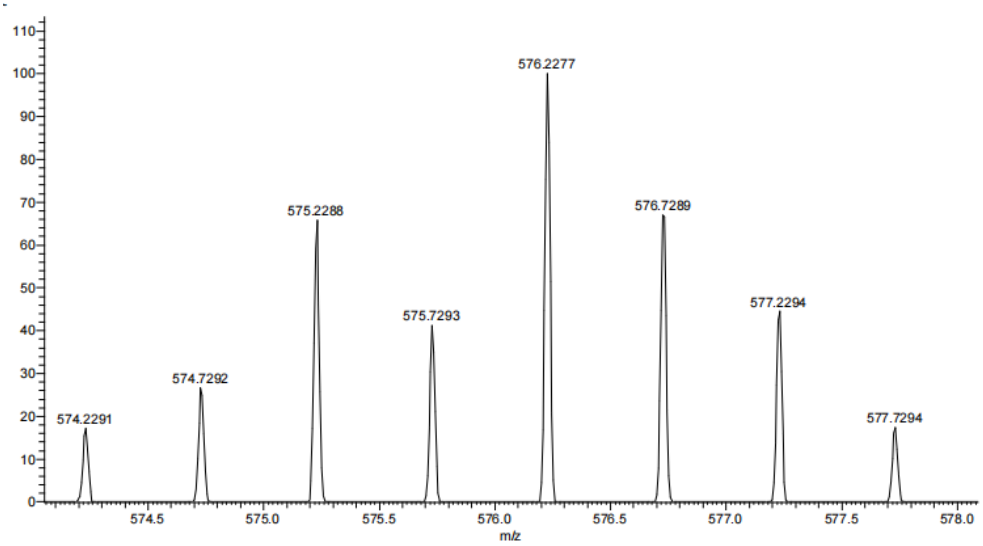

Fig. S5 Mass spectrum of VQ7-SePh; MS (ESI) m/*z*: $[M + 2H]^{2+}$ Calcd. for $C_{51}H_{72}O_{11}N_{10}Se$ 576.2278; found 576.2277.

Kinetic measurements

The kinetics of the reaction of VQ7 with BSC were investigated in a solution mixture of 1.0 M HCl and enthanol (1:1 *v*:*v*) under pseudo first-order reaction conditions ([BSC] ≥ 10[VQ7]) at 25 °C. The kinetic traces were determined by monitoring the consumption of VQ7 using HPLC (Figs. S6-S10). The kinetic traces were fitted well by single exponentials, indicating that the reaction was first-order in [VQ7] and is described in eqn (1).

$$-d[VQ7]/dt = k_{obsd}\,[VQ7] \quad (1)$$

Pseudo first-order rate constants k_{obsd} were derived from the simulations. The values of k_{obsd} reported in this work represent the average of three parallel runs, with standard deviations typically less than 5%. The influence of [BSC] on the reaction rate was also investigated over the range of 2.0 mM ≤ [BSC] ≤ 10.0 mM in the mixture of 1.0 M HCl and ethanol (1:1 *v*:*v*). The k_{obsd}–[BSC] dependence is shown in Fig. S11. The plot is linear without any significant intercepts, indicating that the reaction is pseudo-first-order in [BSC]. The rate equation is described as eqn (2). The pseudo-second-order rate constant *k* was derived from the slope of the linear plot.

$$-d[VQ7]/dt = k_{obsd}\,[VQ7] = k\,[VQ7][BSC] \quad (2)$$

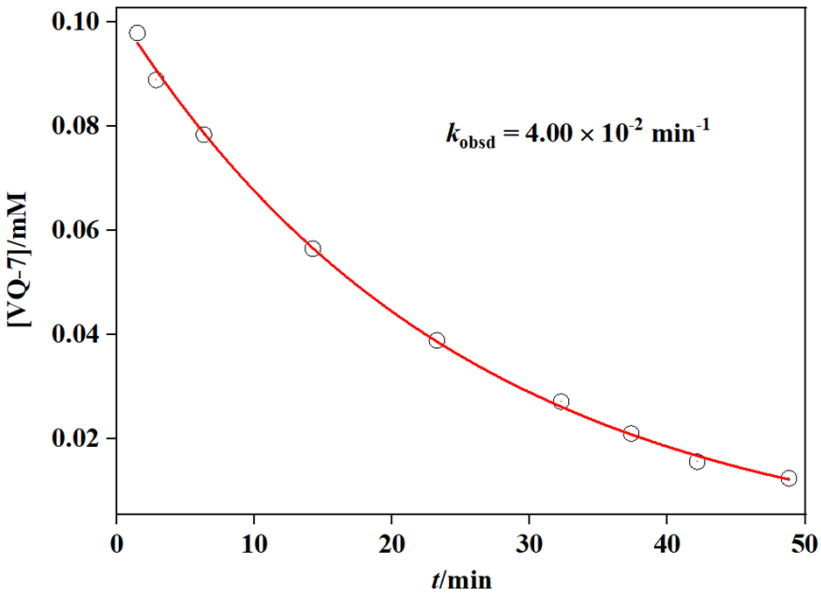

Fig. S6 Kinetic trace for the reaction of VQ7 (0.2 mM) with BSC (2.0 mM); The reaction was stopped by addition of 1.0 equiv. of NaOH (1.0 M), and the data points are from the concentrations of VQ-7 determined by HPLC.

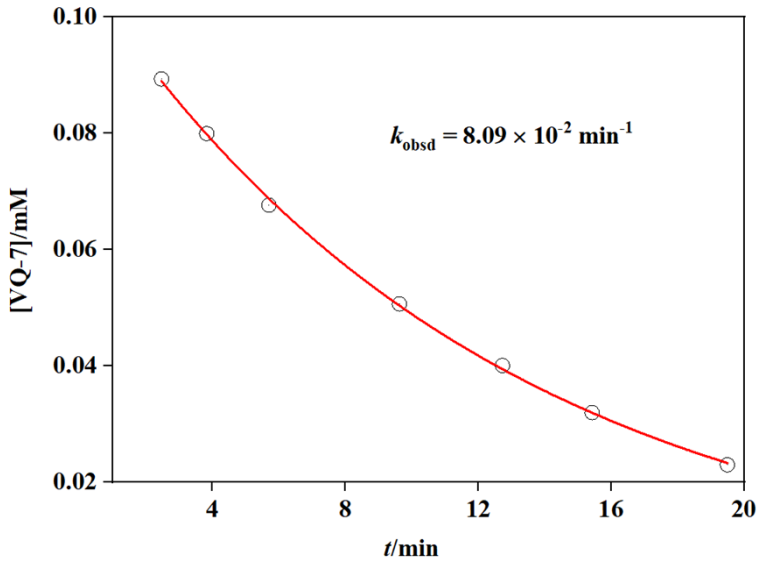

Fig. S7 Kinetic trace for the reaction of VQ7 (0.2 mM) with BSC (4.0 mM); The reaction was stopped by addition of 1.0 equiv. of NaOH (1.0 M), and the data points are from the concentrations of VQ-7 determined by HPLC.

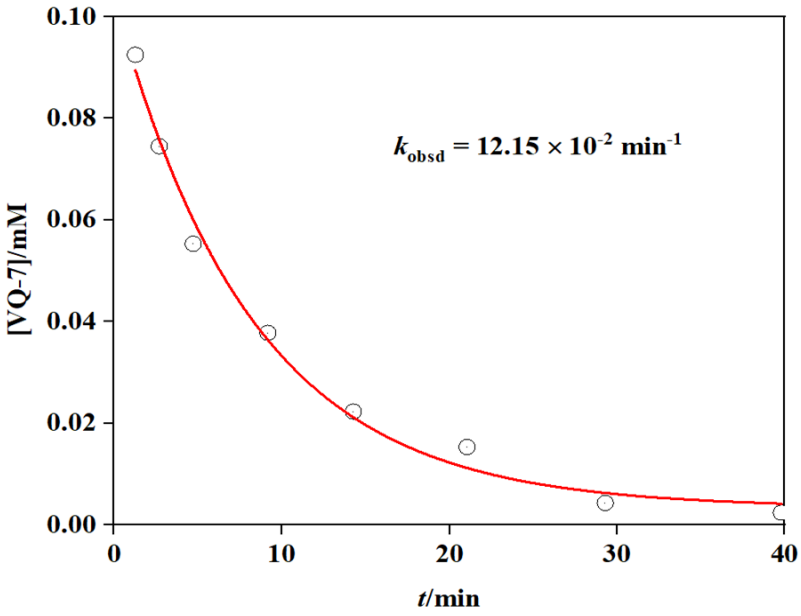

Fig. S8 Kinetic trace for the reaction of VQ7 (0.2 mM) with BSC (6.0 mM); The reaction was stopped by addition of 1.0 equiv. of NaOH (1.0 M), and the data points are from the concentrations of VQ-7 determined by HPLC.

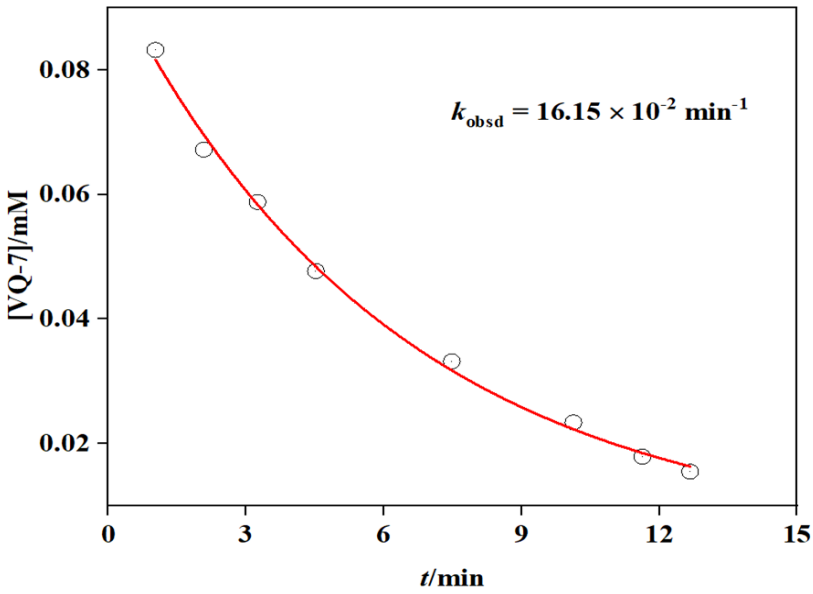

Fig. S9 Kinetic trace for the reaction of VQ7 (0.2 mM) with BSC (8.0 mM); The reaction was stopped by addition of 1.0 equiv. of NaOH (1.0 M), and the data points are from the concentrations of VQ-7 determined by HPLC.

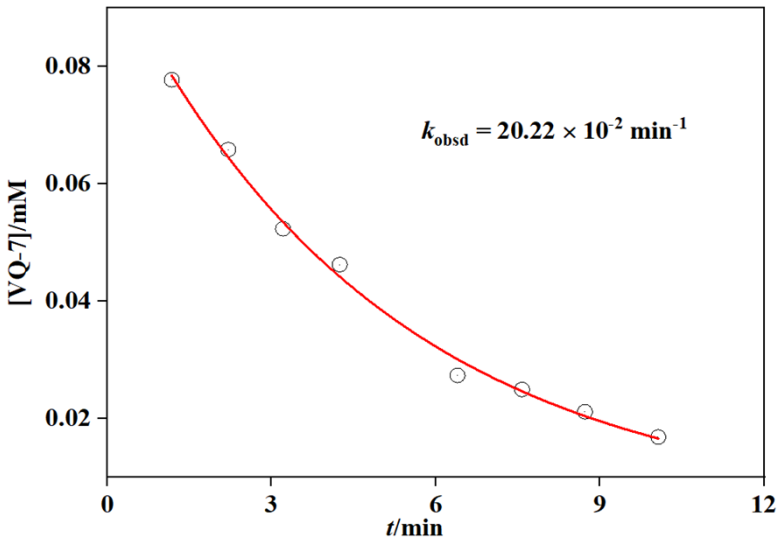

Fig. S10 Kinetic trace for the reaction of VQ7 (0.2 mM) with BSC (10.0 mM); The reaction was stopped by addition of 1.0 equiv. of NaOH (1.0 M), and the data points are from the concentrations of VQ-7 determined by HPLC.

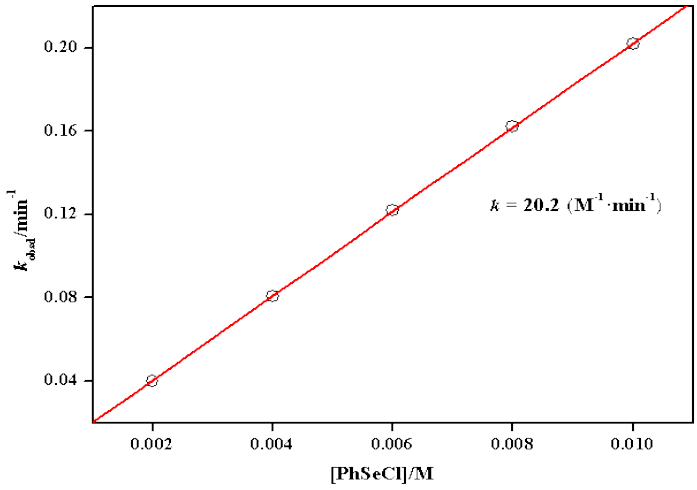

Fig. S11 Plot of k_{obsd} versus [BSC].

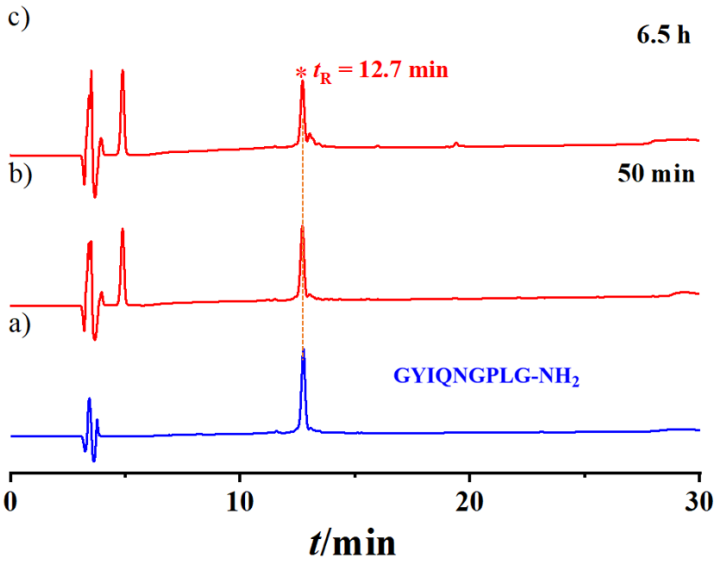

Fig. S12 HPLC chromatograms of a) tyrosine-containing peptide (GYIQNGPLG-NH_2); b, c) the reaction of tyrosine-containing peptide (0.5 mM) with 4.0 equiv. of BSC in the solution mixture containing 2.0 M HCl and ethanol (1:1 *v*:*v*) at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-50% B over 25 min, and further to 90% B over 10 min. Retention time = 12.7 min.

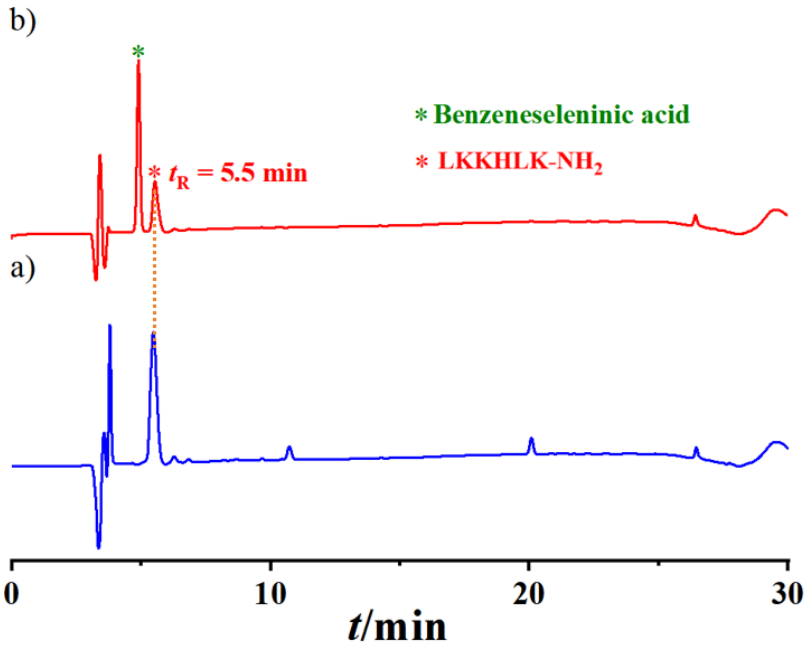

Fig. S13 HPLC chromatograms of a) the His-containing peptide (LKKHLK-NH_2); b) the reaction of His-containing peptide (1.0mM) with 4.0 equiv. of BSC in the mixing solution containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 5 h at room temperature. After the reaction, the solvent was evaporated by

rotary evaporation and redissolved in pure water; the peptide concentration is 0.33 mM; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 3 min. Retention time = 5.5 min.

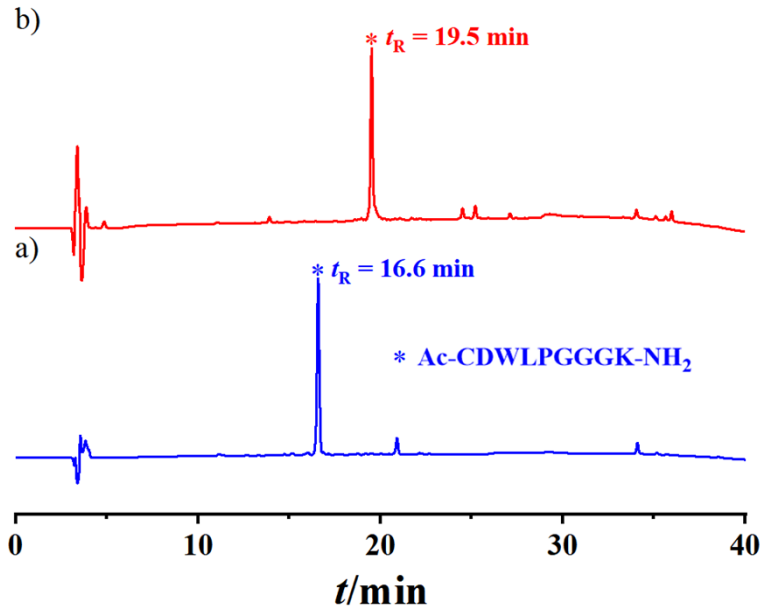

Fig. S14 HPLC chromatograms of a) CK9 (Ac-CDWLPGGGK-NH_2); b) CK9 reaction with 4.0 equiv. of BSB in the mixing solution containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 70 min at room temperature, followed by the addition of NaOH (1.0 M) until pH = 7.0. The elution protocol for analytical HPLC (C8) was set as follows: 10-50% B over 25 min, and then to 90% over 10 min. Retention time = 19.5 min.

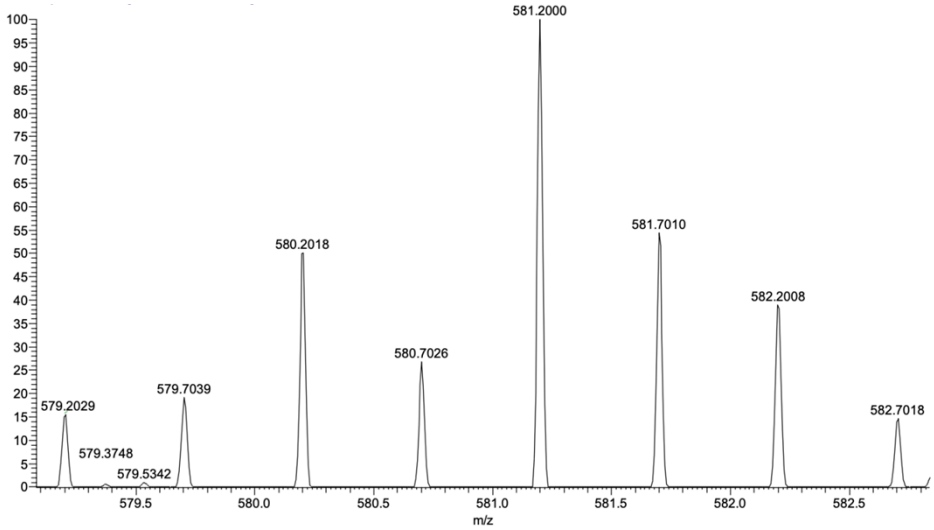

Fig. S15 Mass spectrum of the compound (t_R = 19.5 min) as shown in **Fig. S14b**; MS (ESI) m/*z*: [M + 2H]$^{2+}$ Calcd. for $C_{49}H_{70}O_{14}N_{12}SSe$ 581.2013; found 581.2000; the compound is characterized as

phenylselenyl CK9 with incorporation of two oxygen atoms according to the mass spectrometry.

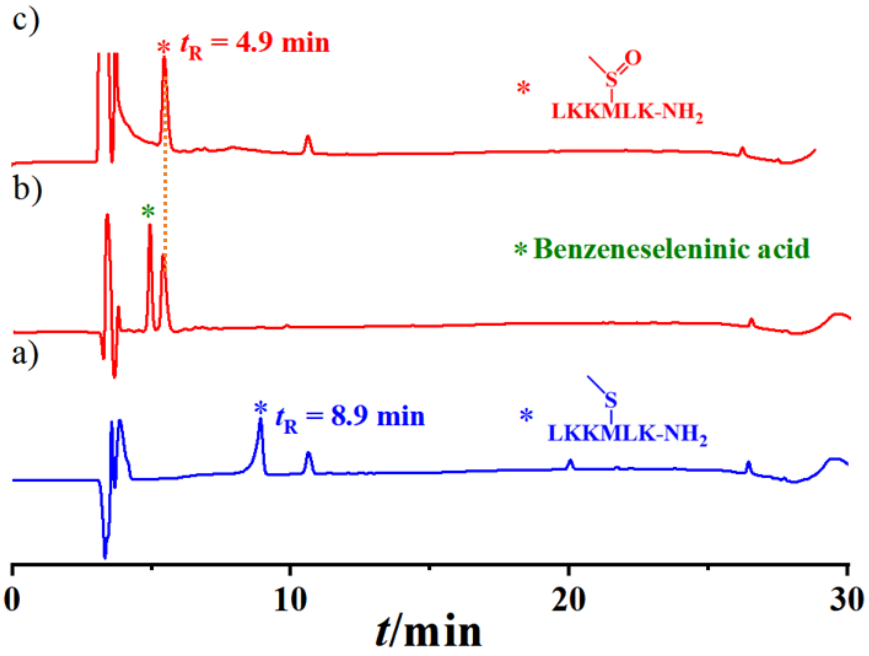

Fig. S16 HPLC chromatograms of a) Peptide LK6 (LKKMLK-NH_2); b) the reaction of LK6 (2.0 mM) with 4.0 equiv. of BSB in the mixing solution containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 1 h at room temperature. After the reaction, the solvent was evaporated by rotary evaporation, and redissolved in pure water (1.0 mM); c) the reaction of LK6 (2.0 mM) with 1.0 equiv. of $K_2[PtBr_2(CN)_4]$ in 0.1 M NaBr for 30 min at room temperature; $K_2[PtBr_2(CN)_4]$ can oxidize methionine residue to its sulfoxide derivate in peptide [1]. The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 3 min. Retention time = 4.9 min.

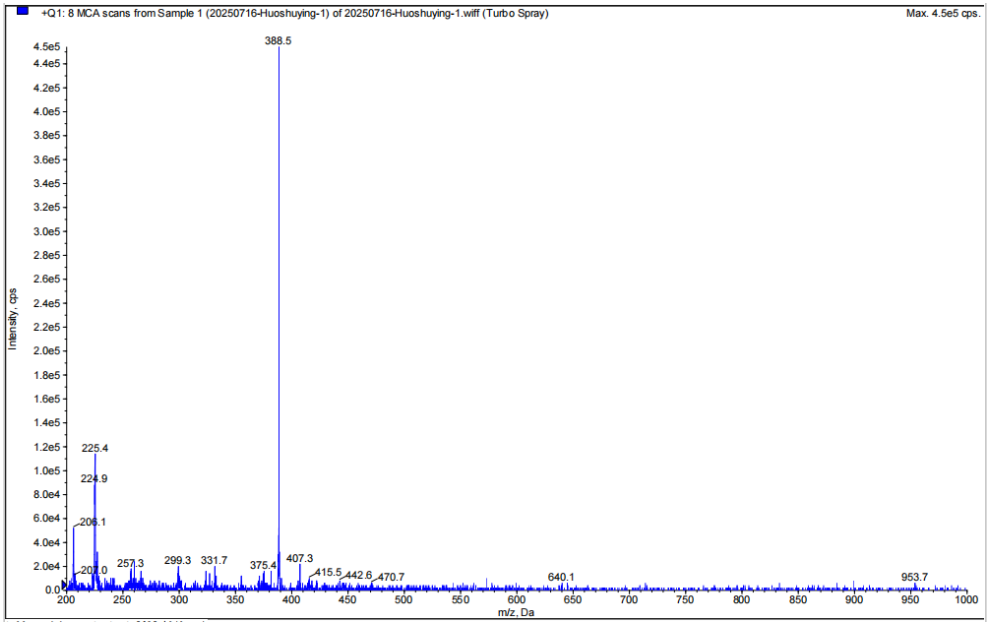

Fig. S17 Mass spectrum of LK(O)6; MS (ESI) m/*z*: $[M + 2H]^{2+}$ Calcd. for $C_{35}H_{72}N_{10}O_7S$ 388.5; found 388.5.

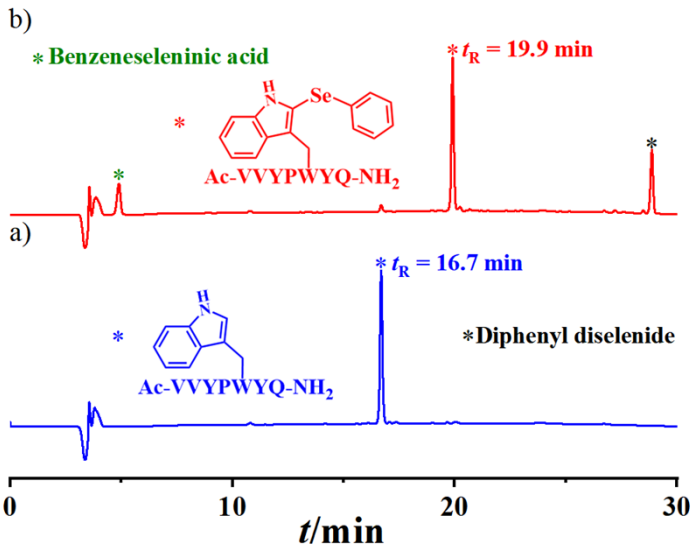

Fig. S18 HPLC chromatograms of a) peptide VQ7 (Ac-VVYPWYQ-NH_2; HPLC purity, 92%); b) the reaction of VQ7 (0.25mM) with 4.0 equiv. of BSC in the solution mixture containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 40 min at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 16.7 min.

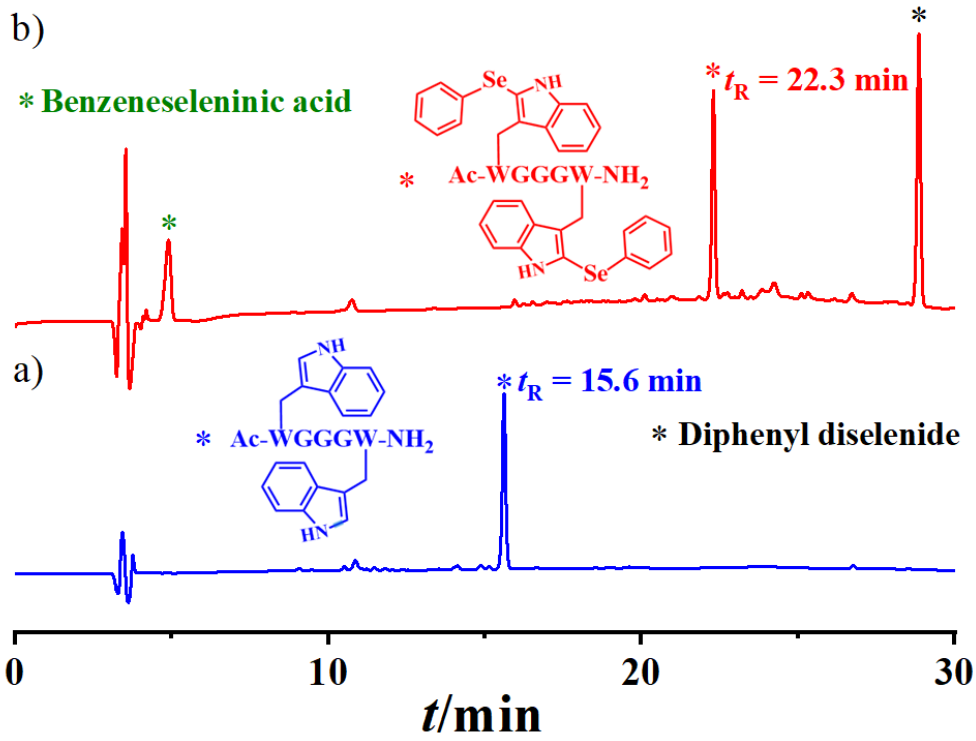

Fig. S19 HPLC chromatograms of a) peptide WW5 (Ac-WGGGW-NH_2; HPLC purity, 82%); b) the reaction of WW5 (0.17 mM) reaction with 4.0 equiv. of BSB in the solution mixture containing 1.5 M HCl and ethanol (1:1, *v*:*v*) for 3 h at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 22.3 min.

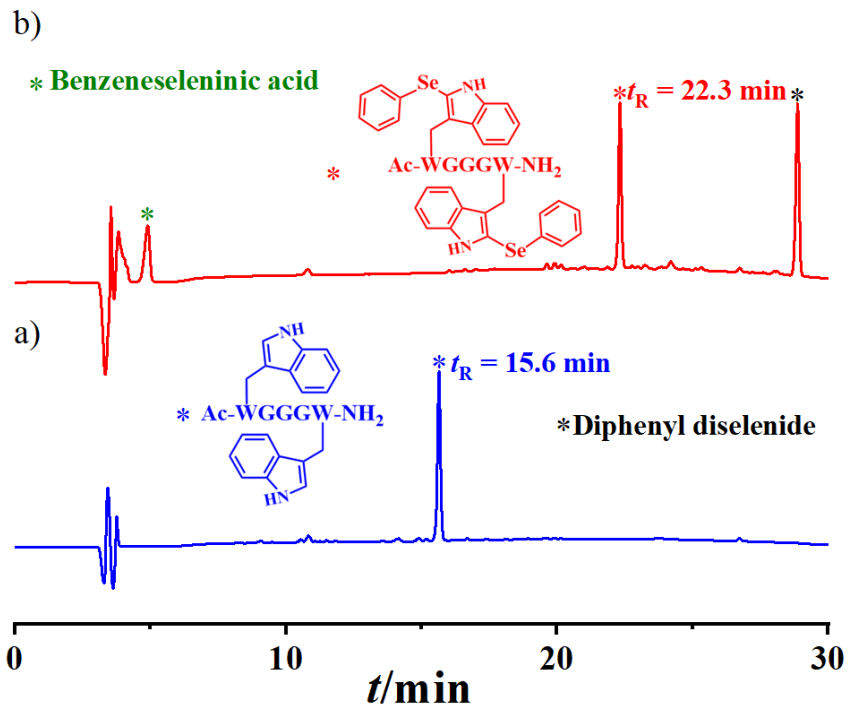

Fig. S20 HPLC chromatograms of a) peptide WW5 (Ac-WGGGW-NH_2; HPLC purity, 82%); b) the reaction of WW5 (0.17 mM) with 4.0 equiv. of BSC in the solution mixture containing 1.5 M HCl and ethanol (1:1, *v*:*v*) for 3 h at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 22.3 min.

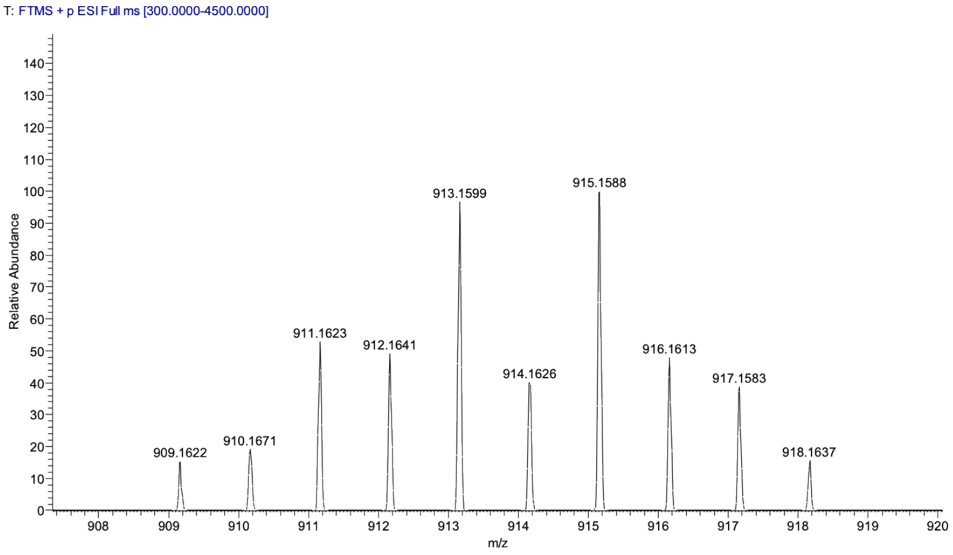

Fig. S21 Mass spectrum of WW5-SePh; MS (ESI) m/*z*: $[M + H]^+$ Calcd. for C42H43O6N8Se2 915.1647; found 915.1588.

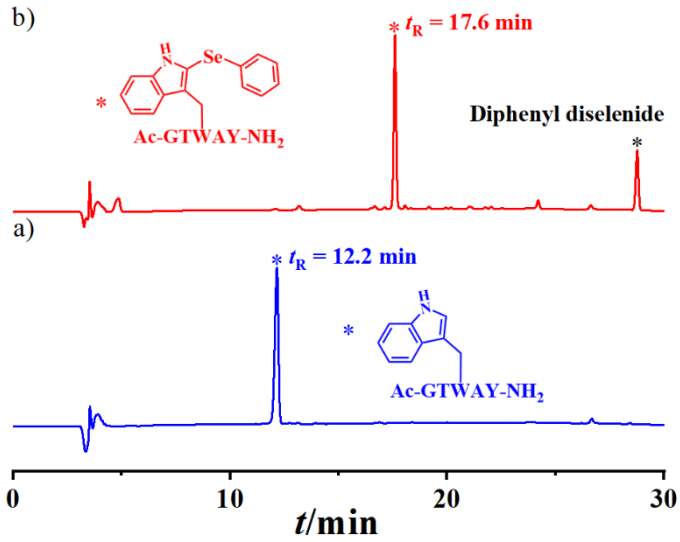

Fig. S22 HPLC chromatograms of a) peptide GY5 (Ac-GTWAY-NH_2; HPLC purity, 96%); b) the reaction of GY5 (0.25mM) with 4.0 equiv. of BSB in the solution mixture containing 2.0 M HCl and

ethanol (1:3, *v*:*v*) for 3 h at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 17.6 min.

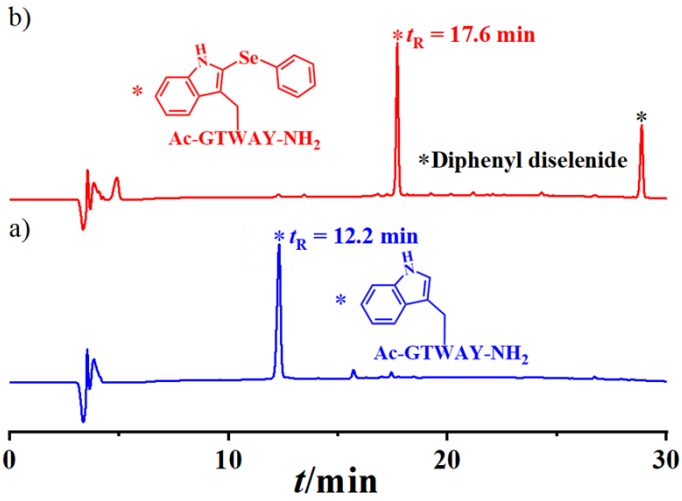

Fig. S23 HPLC chromatograms of a) peptide GY5 (Ac-GTWAY-NH_2; HPLC purity, 96%); b) the reaction of GY5 (0.25mM) with 4.0 equiv. of BSC in the solution mixture containing 2.0 M HCl and ethanol (1:3, *v*:*v*) for 3 h at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 17.6 min.

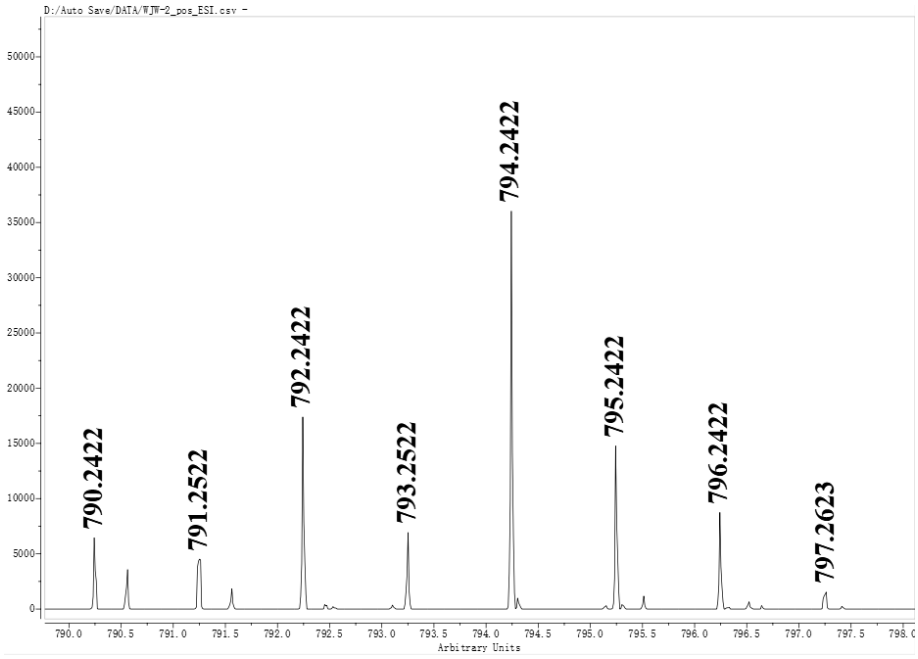

Fig. S24 Mass spectrum of GY5-SePh; MS (ESI) m/*z*: $[M + H]^+$ Calcd. for $C_{37}H_{44}O_8N_7Se$ 794.242; found 794.242.

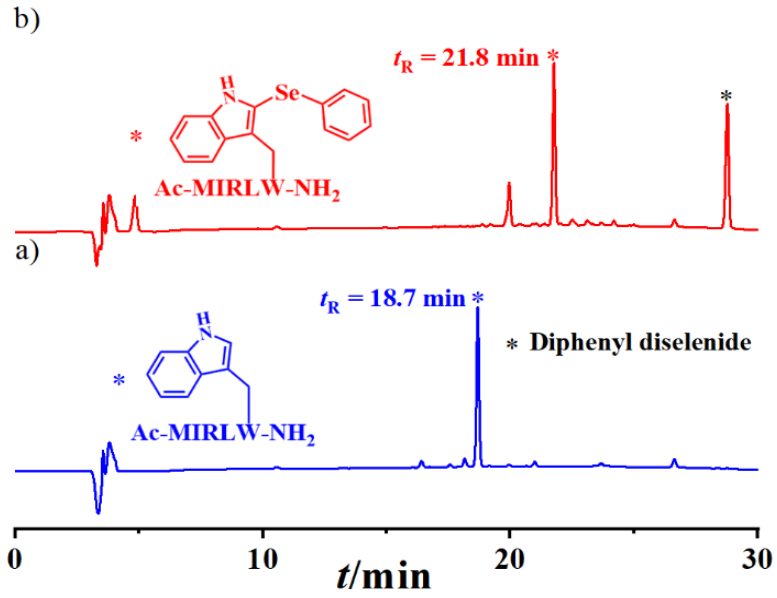

Fig. S25 HPLC chromatograms of a) peptide MW5 (Ac-MIRLW-NH_2; HPLC purity, 86%); b) the reaction of MW5 (0.25mM) with 4.0 equiv. of BSB in the solution mixture containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 40 min at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 21.8 min.

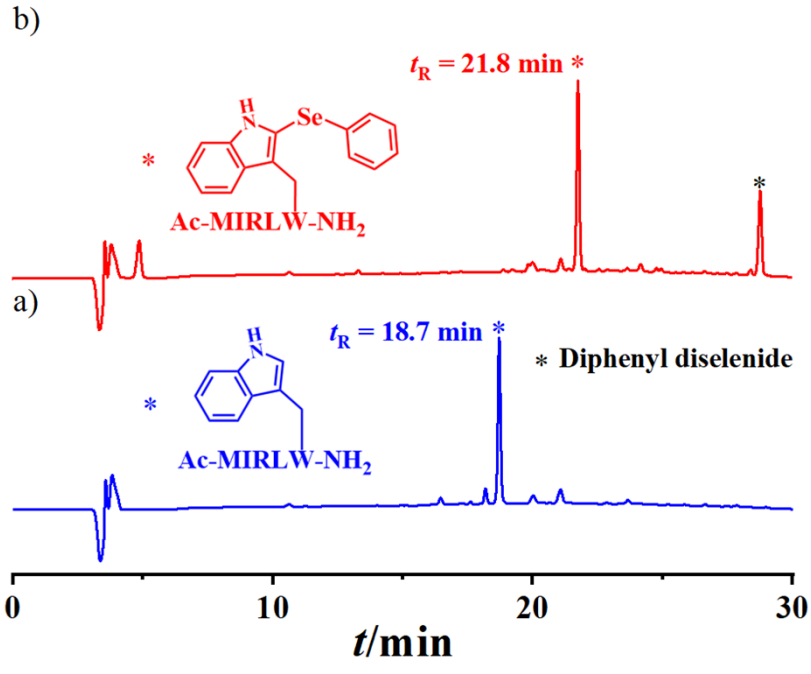

Fig. S26 HPLC chromatograms of a) peptide MW5 (Ac-MIRLW-NH_2; HPLC purity, 86%); b) the reaction of MW5 (0.25mM) with 4.0 equiv. of BSC in the solution mixture containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 40 min at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 21.8 min.

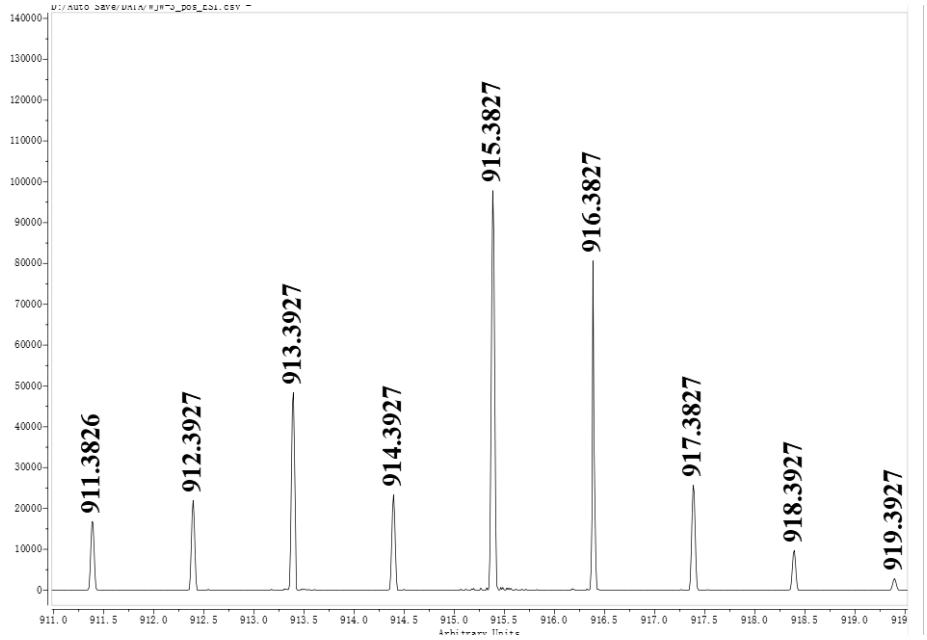

Fig. S27 Mass spectrum of MW5-SePh; MS (ESI) m/*z*: $[M + H]^+$ Calcd. for $C_{42}H_{63}O_6N_{10}SSe$ 915.382; found 915.382.

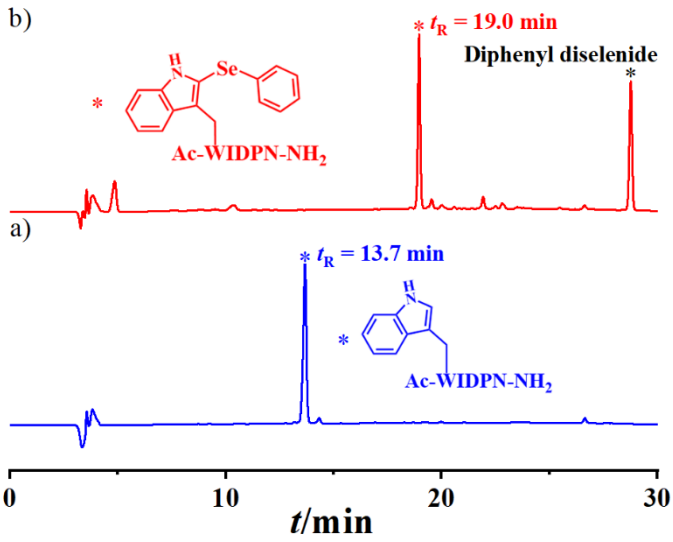

Fig. S28 HPLC chromatograms of a) peptide WN5 (Ac-WIDPN-NH_2; HPLC purity, 95%); b) the reaction of WN5 (0.4 mM) with 4.0 equiv. of BSB in the solution mixture containing 2.0 M HCl and

ethanol (2:3, *v*:*v*) for 40 min at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 19.0 min.

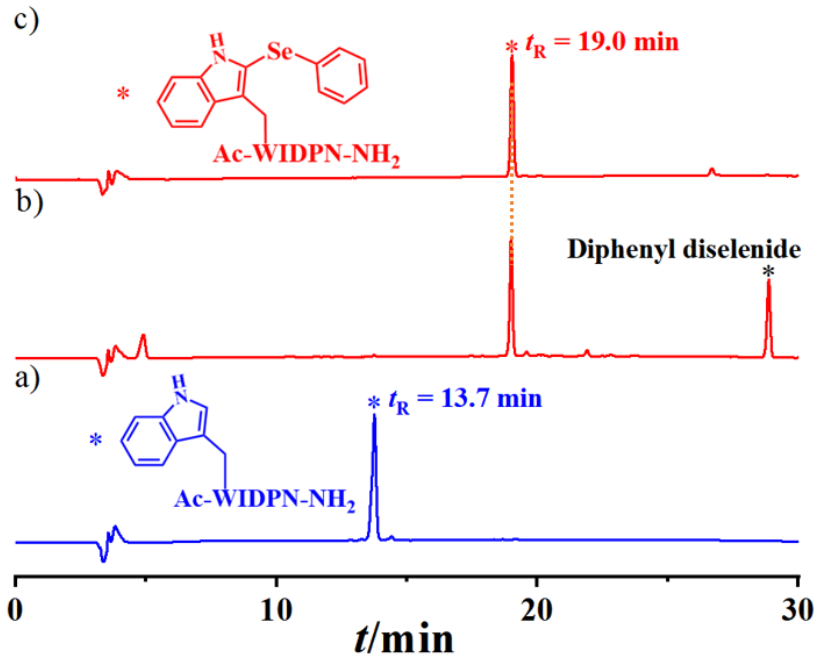

Fig. S29 HPLC chromatograms of a) peptide WN5 (Ac-WIDPN-NH_2; HPLC purity, 95%); b) the reaction of WN5 (0.4 mM) with 4.0 equiv. of BSC in the solution mixture containing 2.0 M HCl and ethanol (2:3, *v*:*v*) for 40 min at room temperature; c) the purified WN5-SePh The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 19.0 min.

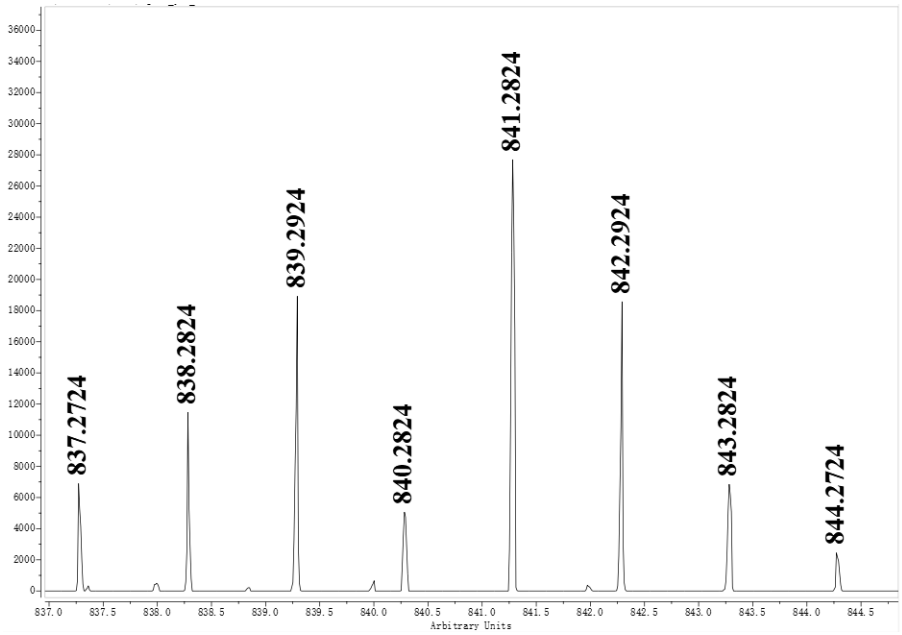

Fig. S30 Mass spectrum of WN5-SePh; MS (ESI) m/*z*: $[M + H]^+$ Calcd. for $C_{38}H_{49}O_9N_8Se$ 841.2792; found 841.2824.

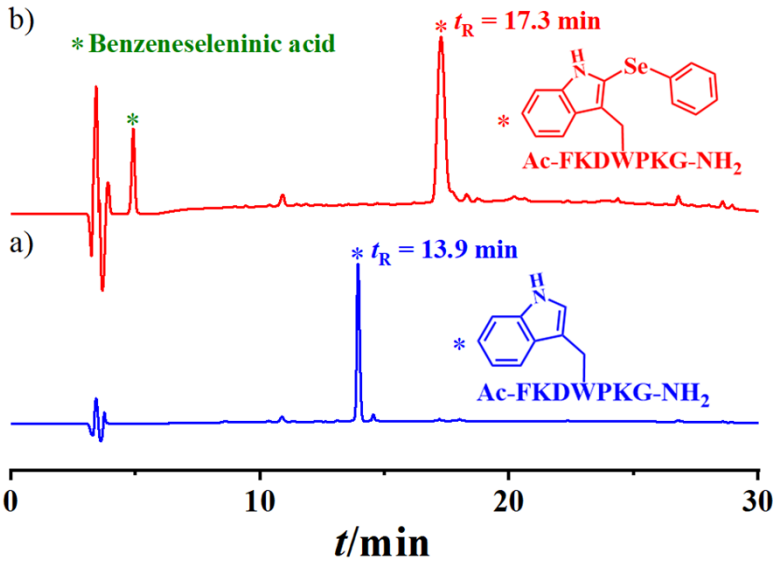

Fig. S31 HPLC chromatograms of a) peptide FG7 (Ac-FKDWPKG-NH_2; HPLC purity, 91%); b) the reaction of FG7 (0.5 mM) with 4.0 equiv. of BSB in the solution mixture containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 40 min at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 17.3 min.

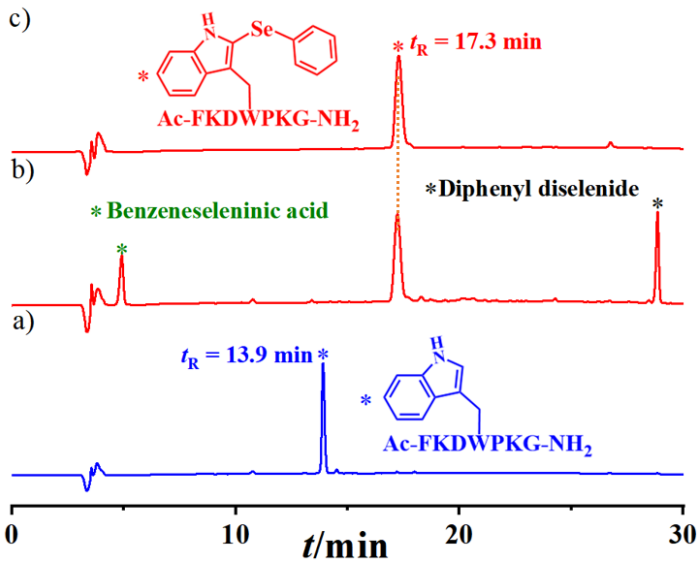

Fig. S32 HPLC chromatograms of a) peptide FG7 (Ac-FKDWPKG-NH_2; HPLC purity, 91%); b) the reaction of FG7 (0.5 mM) with 4.0 equiv. of BSC in the solution mixture containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 40 min; c) the purified FG7-SePh; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min at room temperature, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 17.3 min.

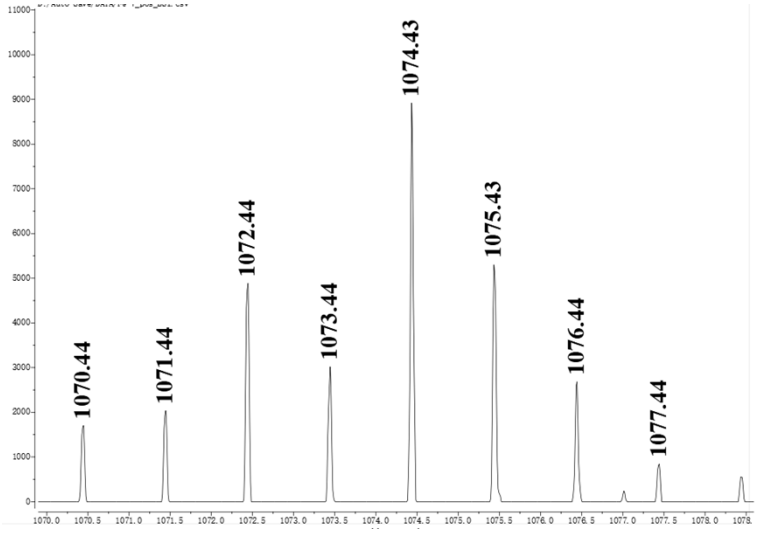

Fig. S33 Mass spectrum of FG7-SePh. MS (ESI) m/z: $[M + H]^+$ Calcd. for $C_{51}H_{68}O_{10}N_{11}Se$ 1074.43; found 1074.43.

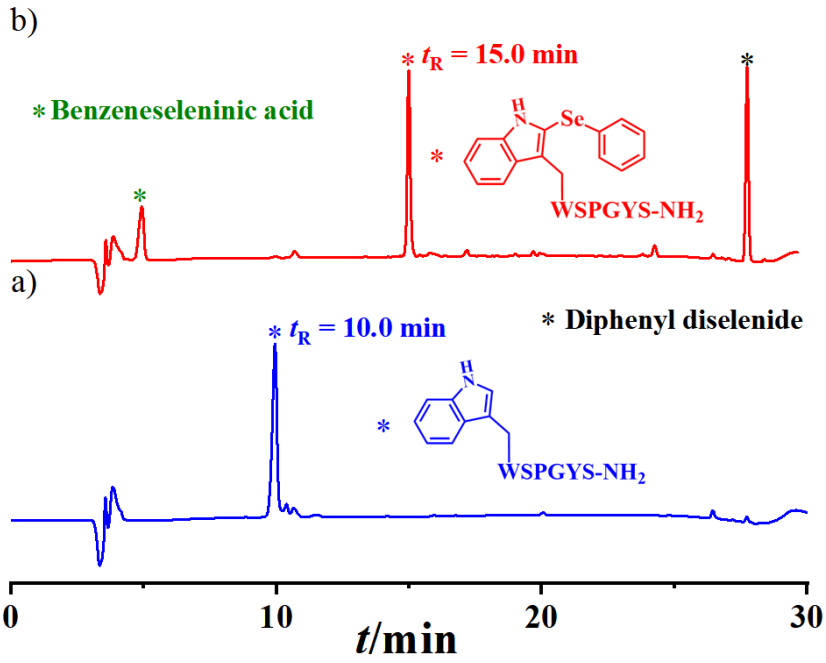

Fig. S34 HPLC chromatograms of a) peptide WS6 (WSPGYS-NH_2 HPLC purity, 93%); b) the reaction of WS6 with 4.0 equiv. BSC in the mixing solution containing 2.0 M HCl and ethanol (1:1, *v*:*v*) for 1.5 h at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 3 min. Retention time = 15.0 min.

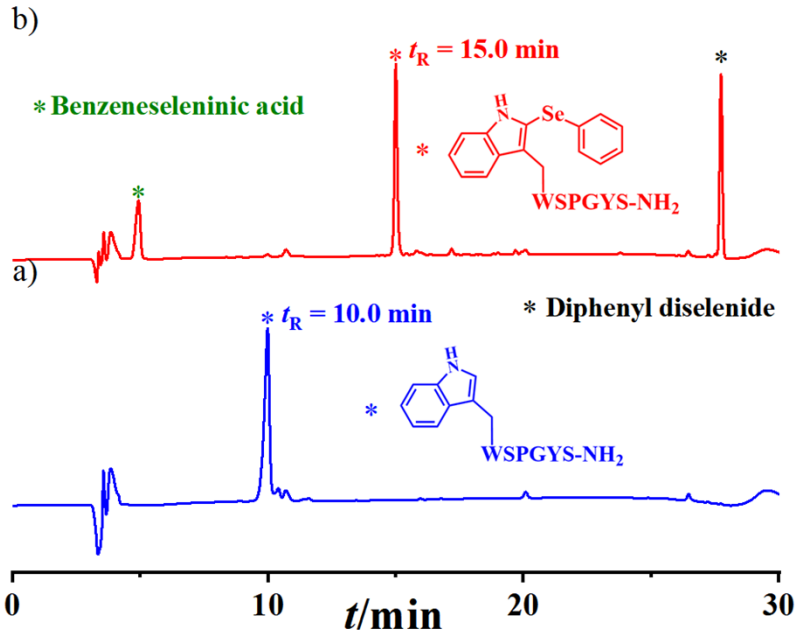

Fig. S35 HPLC chromatograms of a) peptide WS6 (WSPGYS-NH_2 HPLC purity, 93%); b) the reaction of WS6 with 4.0 equiv. BSB in the mixing solution containing 2.0 M HCl and ethanol (1:1,

v:*v*) for 1.5 h at room temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 3 min. Retention time = 15.0 min.

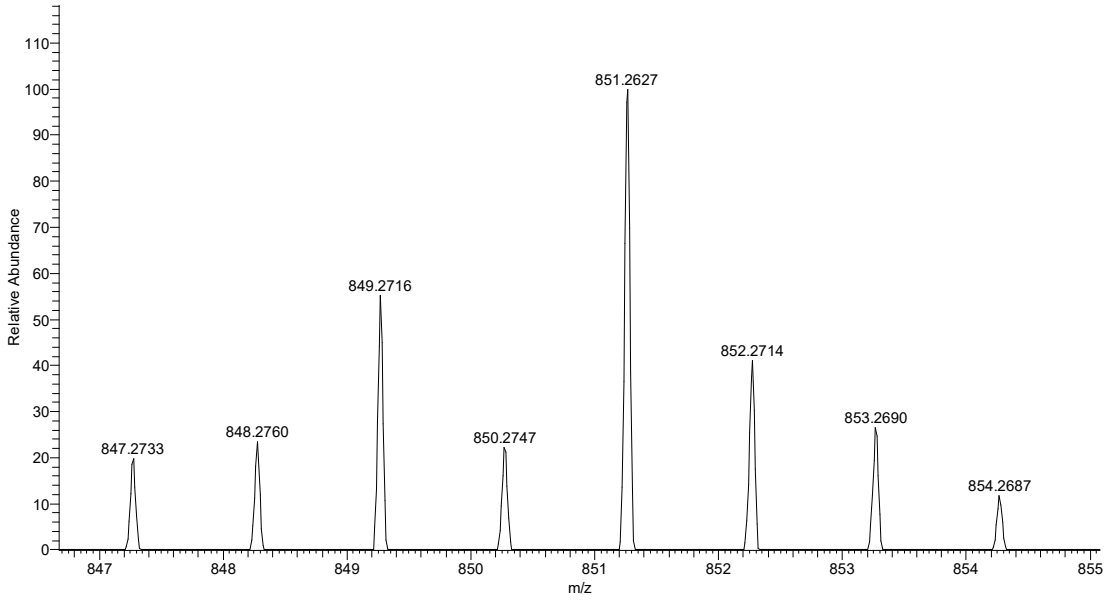

Fig. S36 Mass spectrum of WS6-SePh; MS (ESI) m/*z*: $[M + H]^+$ Calcd. for $C_{39}H_{47}N_8O_9Se$ 851.2636; found 851.2627.

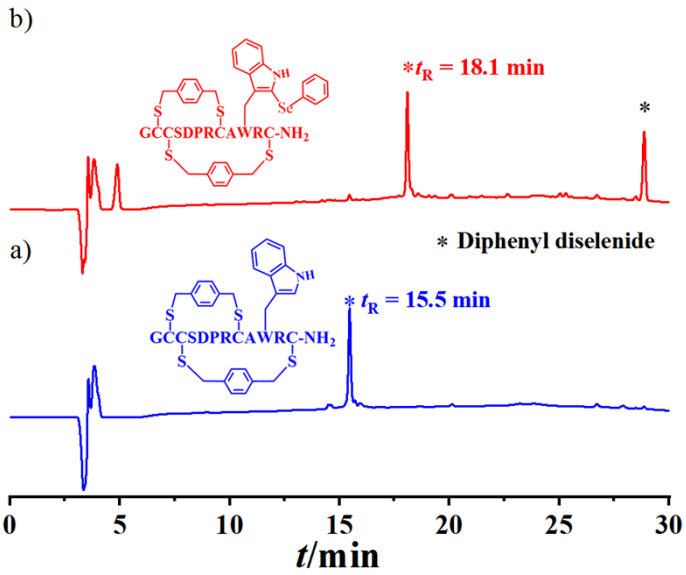

Fig. S37 HPLC chromatograms of a) the xylxyl bridged bicycle *a*-conotoxin IMI (bixylyl-conontoxin IMI, HPLC purity, 85%); b) the reaction of bixylyl-conotoxin IMI (0.125mM) with 4.0 equiv. of BSB (0.5 mM) in the solution mixture containing 2.0 M HCl and ethanol (2:3, *v*:*v*) for 40 min at room

temperature; The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 18.1 min.

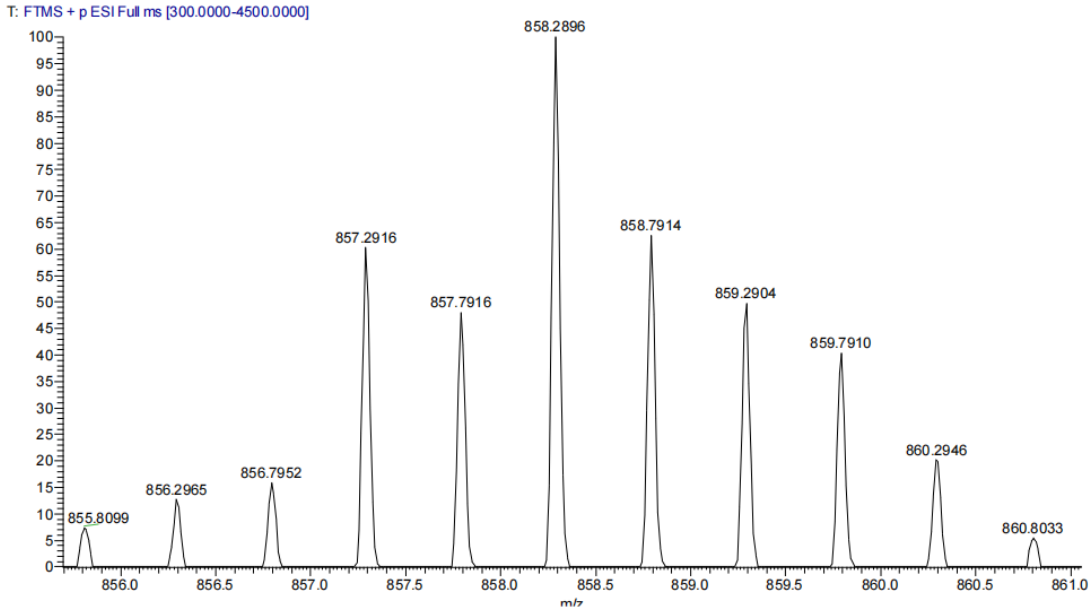

Fig. S38 Mass Spectrum of bixylyl-conotoxin IMI-SePh; MS (ESI) m/*z*: $[M + 2H]^{2+}$ Calcd. for $C_{74}H_{100}N_{20}O_{15}S_4Se$ 858.2868; found 858.2896.

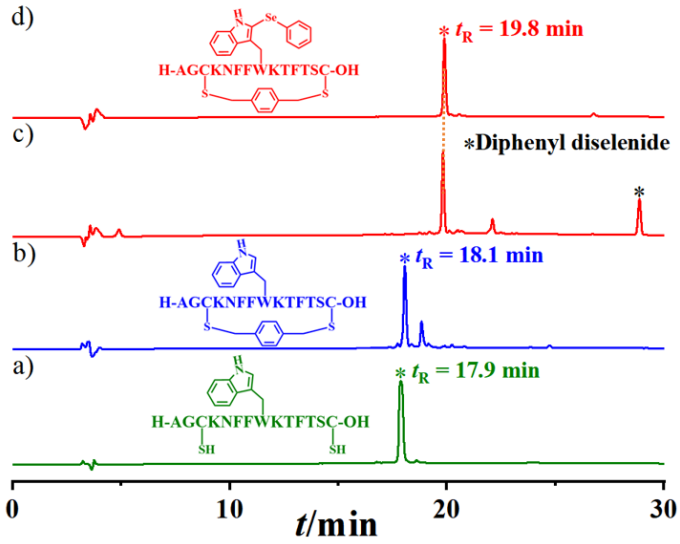

Fig. S39 HPLC chromatograms of a) reduced somatostatin (HPLC purity, 98%) b) the reaction of reduced somatostatin (0.5mM) with 0.8 equiv. of xylyl (0.4mM) for 40 min in a 75 mM NH_4HCO_3 solution containing equal volume of ethanol at room temperature; c) the reaction mixture aided by the addition of HCl, followed by addition of 2.0 equiv. of BSB to modified Trp residue. The solution

mixture containing 2.0 M HCL and ethanol (1:1, *v*:*v*); d) the purified xylyl-somatostatin-SePh. The elution protocol for analytical HPLC (C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 19.8 min.

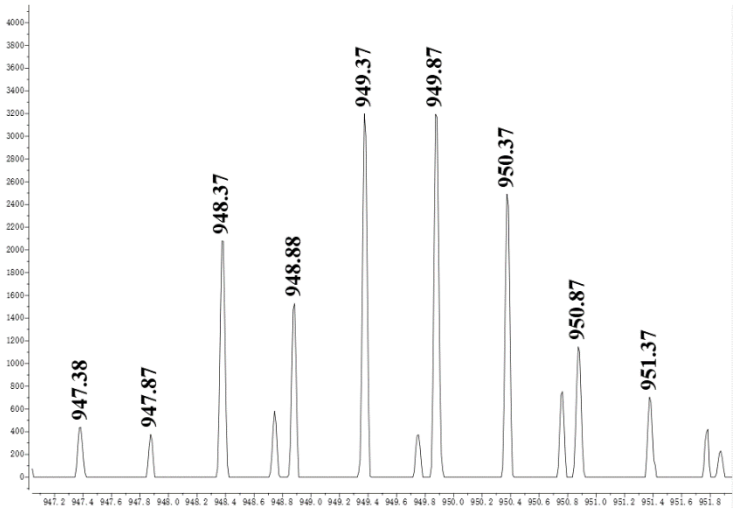

Fig. S40 Mass spectrum of xylyl-somatostatin-SePh; MS(ESI) m/*z*: $[M + 2H]^{2+}$ Calcd. for $C_{90}H_{118}N_{18}O_{19}S_2Se$ 949.37; found 949.37.

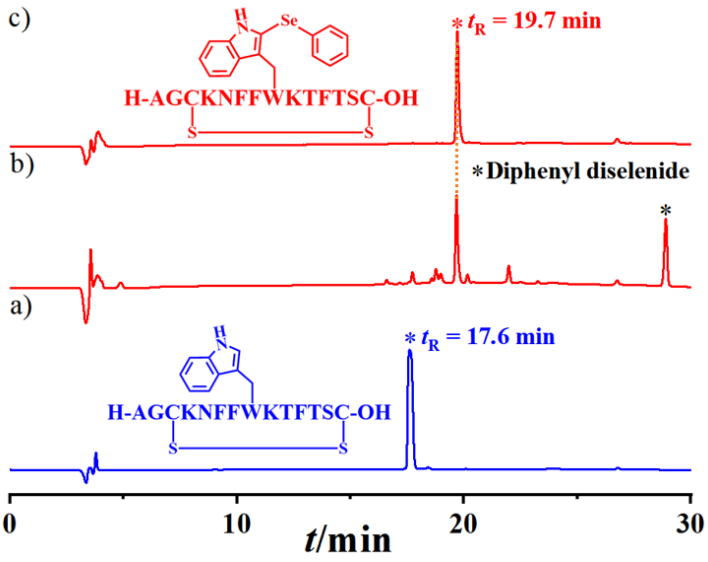

Fig. S41 HPLC chromatograms of a) somatostatin (AGCKNFFWKTFTSC-OH Cys3/Cys14; HPLC purity, 99%); b) the reaction of somatostatin (0.5mM) with 2.0 equiv. of BSB (1.0mM) in the mixing solution containing 1.0 M HCL and ethanol (1:1, *v*:*v*) for 3 h. Then, equal volume the mixture solution containing 2.0 equiv. 0f BSB was added to the above reaction mixture, and aged for another 3 h, affording the product; c) the purified somatostatin-SePh. The elution protocol for analytical HPLC

(C8) was set as follows: 10-30% B over 10 min, and further to 60% B over 10 min, and then to 90% over 10 min. Retention time = 19.7 min.

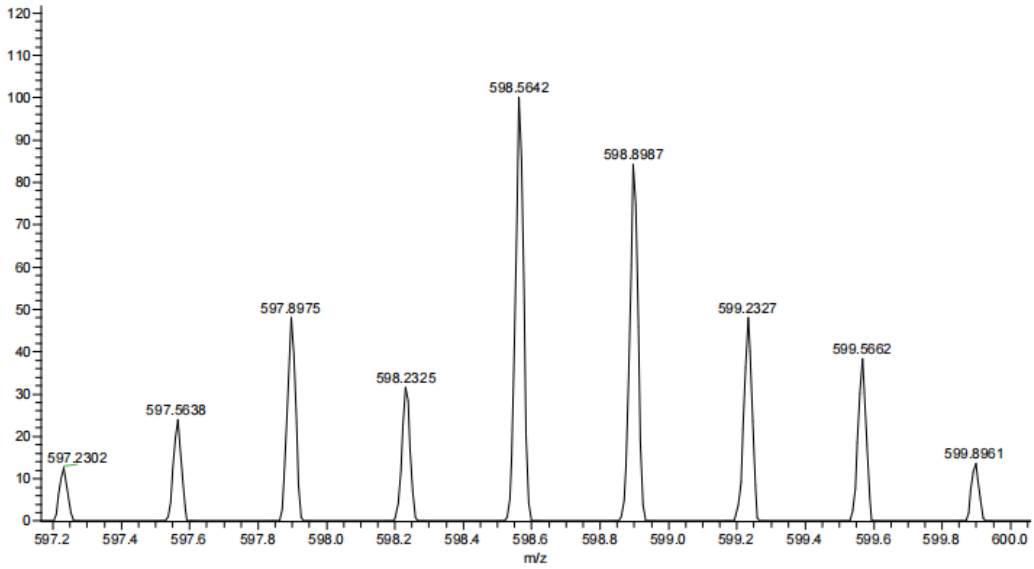

Fig. S42 Mass spectrum of somatostatin-SePh; MS (ESI) m/*z*: $[M + 3H]^{3+}$ Calcd. for $C_{82}H_{111}N_{18}O_{19}S_2Se$ 598.5632; found 598.5642.

References

1. D. Ma, J. Sun, S. Shen, H. Chen, W. Xu, Y. Wang, C. Song, T. Shi and S. Huo. *J. Org. Chem.*, 2022, **87**, 1470−1476.