Ag(I)-Promoted fragment coupling of peptide thioamides

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Supplementary Information

Contents

General

All common solvents were purchased from Fisher Scientific. All protected amino acids, coupling reagents and resins were purchased from GL Biochem, Sigma Aldrich or Combi-Blocks and were used without further purification. All reagents were obtained from Sigma-Aldrich. For analytical HPLC, an Alltech Hypersil BDS C18 5 µm 4.6 x 250 mm column was used. A gradient solvent system was used containing acetonitrile and water. High-resolution mass spectrometry was performed on a Thermo Fisher Exactive Plus mass spectrometer using standard electrospray methods.

General procedure: Solid phase peptide synthesis.

Peptides were synthesized using standard Fmoc SPPS coupling methods (peptide C-terminal acids **9** on chlorotrityl resin, peptide thioamide C-terminal amides **8** on Seiber amide resin).[1] The coupling steps were performed using a CEM Liberty Blue microwave peptide synthesizer. All peptides were synthesized on a 0.1 mmol scale using a 4 or 5-fold molar excess of Fmoc-protected amino acid (0.4 or 0.5 mmol for a 0.1mmol scale) that were activated using a 4- or 5-fold excess of HCTU in the presence of DIEA (8–10 equiv). Fmoc deprotection was performed with 20% v/v piperidine in DMF.

Couplings to generate peptide thioamides: The peptide-containing resin **4** (0.10 mmol) was swelled in DMF (4 mL). The required Fmoc-amino acid thiobenzotriazolide **5** (0.08 mmol)[1] and triethylamine (0.011 ml, 0.08 mmol) were added and the reaction mixture was stirred for 2 h. Fmoc deprotection was then performed by treatment with 20% v/v piperidine in DMF to generate peptide thioamide resin **7**.[2]

*Cleavage of peptide thioamide C-terminal amide **8** from resin:* Peptide-containing resin **7** (0.10 mmol) was treated with 1% TFA in CH_2Cl_2 (5 mL) for 15 min. The solvent was drained, and this process was repeated a further two times. The filtrates were combined, and the solvent was evaporated under vacuum to provide the crude peptide thioamide **8**, which was used without purification.

$A^{[S]}S(^tBu)PGY(^tBu)S(^tBu)$-$NH_2$ ***8a***

HPLC retention time 35.5 min. Calculated Mass: $[M+H]^+$ 765.42, Observed *m/z*: 765.42

V[S]S(tBu)PGY(tBu)S(tBu)-NH2 ***8b***

HPLC retention time 39.27 min. Calculated mass: $[M+H]^+$ 806.39, Observed *m/z*: 806.47

L[S]S(tBu)PGY(tBu)S(tBu)-NH2 ***8c***

HPLC retention time 41.89 min. Calculated mass: $[M+H]^+$ 792.05, Observed *m/z*: 792.07

F[S]S(tBu)PGY(tBu)S(tBu)-NH2 ***8d***

HPLC retention time 38.12 min. Calculated mass: $[M+H]^+$ 840.05, Observed *m/z*: 840.04

K[S]S(tBu)PGY(tBu)S(tBu)-NH2 ***8e***

HPLC retention time 37.90 min. Calculated mass: $[M+H]^+$ 921.18, Observed *m/z*: 921.18

Ac-LY(tBu)R(Pbf)AG ***9a***

Linear peptide was synthesized using standard solid phase protocol. HPLC retention time 35.59 min. Calculated Mass: $[M+H]^+$ 929.17, Observed *m/z*: 929.43

Ac-LY(tBu)R(Pbf)AA ***9b***

Linear peptide was synthesized using standard solid phase protocol. HPLC retention time 40.10 min. Calculated Mass: $[M+H]^+$ 943.19, Observed *m/z*: 943.14

Ac-LY(tBu)R(Pbf)AK ***9c***

Linear peptide was synthesized using standard solid phase protocol. HPLC retention time 35.10 min. Calculated mass: $[M+H]^+$ 1100.54, Observed *m/z*: 1100.39.

General procedure: Ag(I)-promoted peptide fragment coupling in solution

The crude peptide thioamide **8** was dissolved in CH_2Cl_2 (2 mL) then peptide **9** and Ag_2CO_3 (0.033 g, 0.12 mmol) were added. The reaction mixture was stirred for 5 h at room temperature. The mixture was centrifuged at 3000 rpm for 1–2 min then the solution decanted to remove the black Ag_2S precipitate. The crude peptide was treated with TFA:TIPS:water (95:2.5:2.5, 10 mL/0.1 mmol of peptide) for 1.5 h to effect deprotection. The solvent was evaporated under a stream of nitrogen, then the residue was treated with ice-cold diethyl ether to precipitate the peptide and the mixture was centrifuged at 3000 rpm for 3–5 min. The solvent was decanted, and the washing was repeated three times by suspending the solid in ice-cold diethyl ether (15 mL) followed by centrifugation and decanting of the diethyl ether solvent. Purification of the peptide was performed using an Agilent RP-HPLC with a C18 Phenomenex 250 x 10 mm, 2µ column. The purity was assessed using an analytical C18 Phenomenex 150 x 4.6mm, 5µ column in a gradient mode with eluent (buffer) A; 0.1% aq. TFA and buffer B; 0.1% TFA in acetonitrile. RP-HPLC was performed using gradient elution with buffer B 0–40% over 40 min, monitoring at a wavelength of 214 nm.

General procedure: Ag(I)-promoted peptide fragment coupling on resin

The resin-bound peptide thioamide **7** (0.1 mmol) was swollen in CH_2Cl_2 (4 mL) then peptide **9** was solubilised in DCM and transferred into the reaction mixture. Ag_2CO_3 (0.095 g, 0.3 mmol) was added, and the reaction mixture was agitated at room temperature for 5–8 h. The resulting black precipitate of Ag_2S was removed by washing the resin with DMF and methanol. Peptide cleavage was performed by treating the resin with a TFA:TIPS:water (95:2.5:2.5, 10 mL/0.1 mmol of peptide) for 1.5 h. The resin was removed by filtration and the cleavage cocktail solution was evaporated under a stream of nitrogen. The residue was treated with ice-cold diethyl ether to precipitate the peptide, and the resulting suspension was centrifuged at 3000 rpm for 3–5 minutes. The diethyl ether supernatant was decanted, and the washing step was repeated three times by resuspending the solid peptide in ice-cold diethyl ether (15 mL), followed by centrifugation and decantation of the solvent. Purification of the peptide was performed using an Agilent RP-HPLC as for the solution-based procedure above.

Ac-LYRAGASPGYS-NH_2 ***10aa***

Peptide **8a** and peptide **9a** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AGAS(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 46.12 min. Calculated Mass: $[M+2H]^{2+}$ 830.53, Observed *m/z*: 830.93.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10aa**. HPLC retention time 11.05 min. Calculated Mass: $[M+H]^+$ 1182.58, $[M+2H]^{2+}$ 591.80, Observed *m/z*: 1182.59, 591.80.

Ac-LYRAAASPGYS-NH_2 ***10ba***

Peptide **8a** and peptide **9b** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AAAS(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 44.24 min. Calculated mass: $[M+H]^+$ 1673.22, $[M+2H]^{2+}$ 837.54, Observed *m/z*: 1673.10, 837.52.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10ba**. HPLC retention time 17.16 min. Calculated mass: $[M+H]^+$ 1196.35, $[M+2H]^{2+}$ 599.20, Observed *m/z*: 1196.37, 599.19.

Ac-LYRAKASPGYS-NH_2 ***10ca***

Peptide **9c** and peptide **10a** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AK(Boc)AS(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 43.54. Calculated mass: $[M+H]^+$ 1730.91, $[M+2H]^{2+}$ 866.45, Observed *m/z*: 1730.86, 866.43.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10ca**. HPLC retention time 11.05 min. Calculated mass: $[M+H]^+$ 1253.44, $[M+2H]^{2+}$ 627.63, Observed *m/z*: 1253.40, 627.20.

Ac-LYRAAVSPGYS-NH_2 ***10bb***

Peptide **8b** and peptide **9b** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AAVS(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 46.45 min. Calculated mass: $[M+H]^+$ 1701.16, $[M+2H]^{2+}$ 851.50, Observed *m/z*: 1701.14, 851.47.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10bb**. HPLC retention time 15.96 min. Calculated mass: $[M+H]^+$ 1224.39, $[M+2H]^{2+}$ 612.70, Observed *m/z*: 1224.46, 612.72.

Ac-LYRAALSPGYS-NH_2 ***10bc***

Peptide **9b** and peptide **10c** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AALS(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 41.78 min. Calculated mass: $[M+H]^+$ 1715.20, $[M+2H]^{2+}$ 858.56, Observed *m/z*: 1715.16, 858.53.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10bc**. HPLC retention time 15.10 min. Calculated mass: $[M+H]^+$ 1238.41, $[M+2H]^{2+}$ 619.71, Observed *m/z*: 1238.81, 619.87**.**

Ac-LYRAAFSPGYS-NH_2 ***10bd***

Peptide **9b** and peptide **10d** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AAFS(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 49.91 min. Calculated mass: $[M+H]^+$ 1749.18, $[M+2H]^{2+}$ 875.95, Observed *m/z*: 1749.19, 875.94.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10bd**. HPLC retention time 21.17 min. Calculated mass: $[M+H]^+$ 1272.45, $[M+2H]^{2+}$ 636.78, Observed *m/z*: 1272.45, 636.76.

Ac-LYRAAKSPGYS-NH_2 ***10be***

Peptide **9b** and peptide **10e** were coupled using the general Ag(I)-promoted coupling protocol to generate the protected ligated peptide *Ac-LY(^{t}Bu)R(Pbf)AAK(Boc)S(^{t}Bu)PGY(^{t}Bu)S(^{t}Bu)-NH_2*: HPLC retention time 44.24 min. Calculated mass: $[M+H]^+$ 1730.91, $[M+2H]^{2+}$ 866.45, Observed *m/z*: 1730.88, 866.44.

The protected peptide was deprotected according to the general protocol, then purified by RP-HPLC to provide **10be**. HPLC retention time 9.45 min. Calculated mass: $[M+H]^+$ 1253.44, $[M+2H]^{2+}$ 627.63, Observed *m/z*: 1253.41, 627.61.

INSL-5 peptide; QDLQTLCCTDGCSMTDLSAC-NH_2 13

The crude peptide thioamide **12** was dissolved in CH_2Cl_2 (2 mL) then peptide **11** and Ag_2CO_3 (0.033 g, 0.12 mmol) were added. The reaction mixture was stirred for 8 h at room temperature. The mixture was centrifuged at 3000 rpm for 1–2 min then the solution decanted to remove the black Ag_2S precipitate. The crude peptide was treated with TFA:TIPS:water (95:2.5:2.5, 10 mL/0.1 mmol of peptide) for 1.5 h to effect deprotection. The solvent was evaporated under a stream of nitrogen, then the residue was treated with ice-cold diethyl ether to precipitate the peptide, and the mixture was centrifuged at 3000 rpm for 3–5 min. The solvent was decanted, and the washing was repeated three times by suspending the solid in ice-cold diethyl ether (15 mL) followed by centrifugation and decanting of the diethyl ether solvent. Purification of the peptide was performed using an Agilent RP-HPLC, conditions as above.

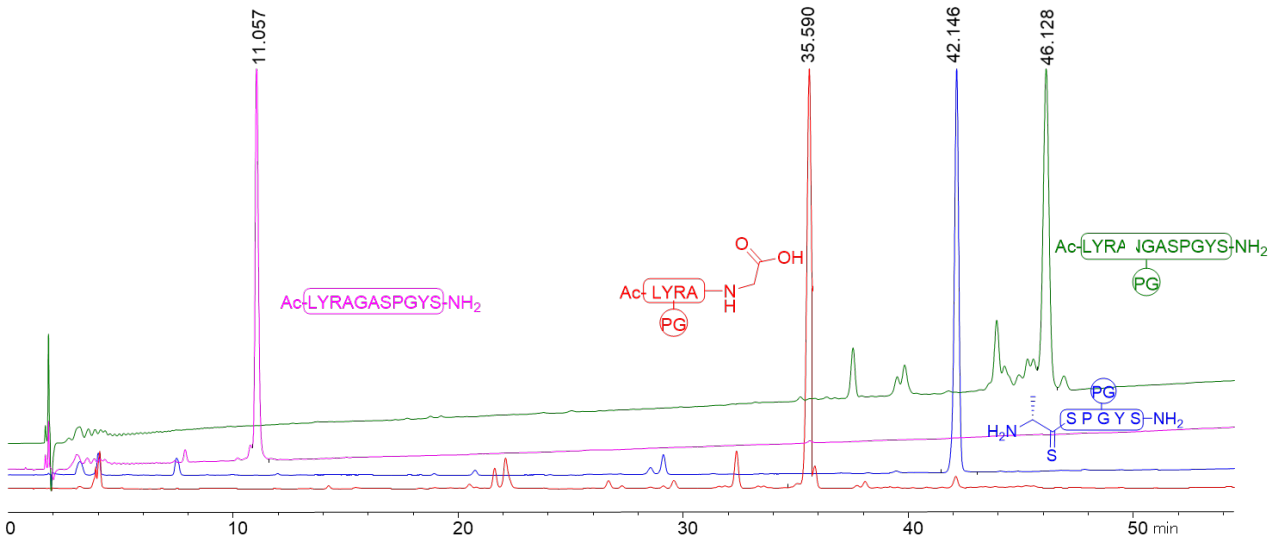

Fig. S11: HPLC traces of crude linear peptide thioamide **8a** (blue), crude linear peptide **9a** (red), crude protected ligated peptide (green) and purified final peptide **10aa** (magenta).

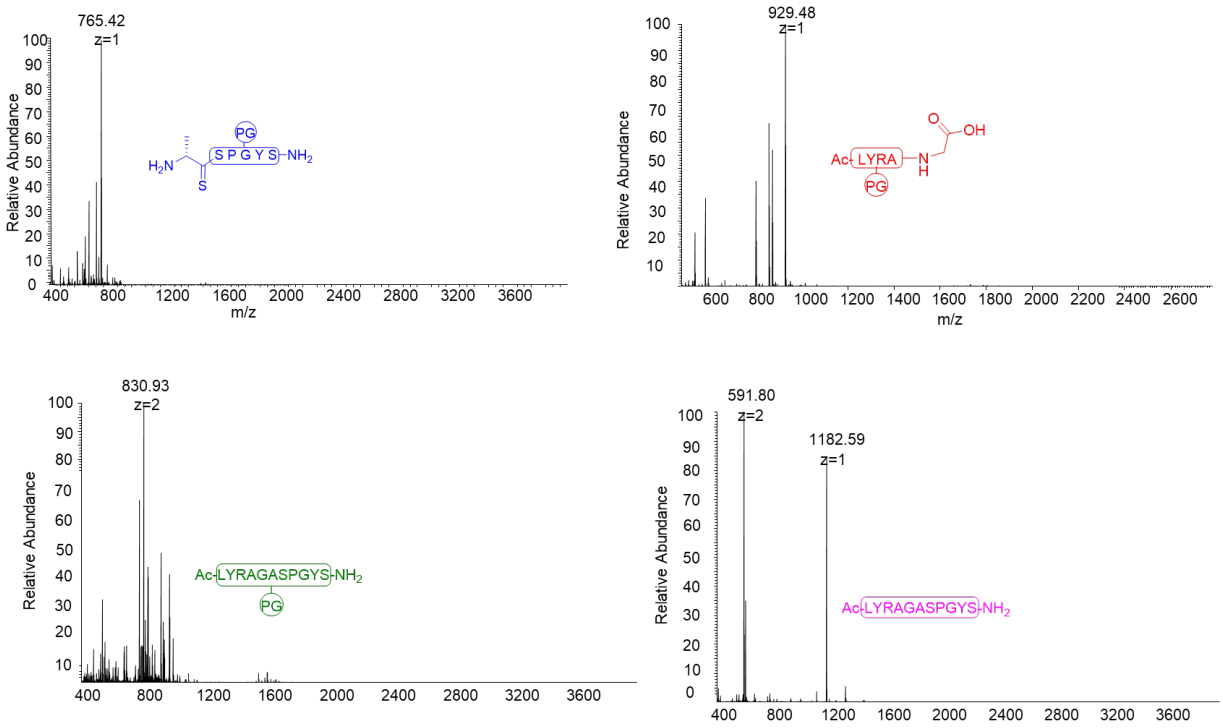

Fig. S12: Mass spectra of crude linear peptide thioamide **8a** (blue), crude linear peptide **9a** (red), crude protected ligated peptide (green) and purified final peptide **10aa** (magenta).

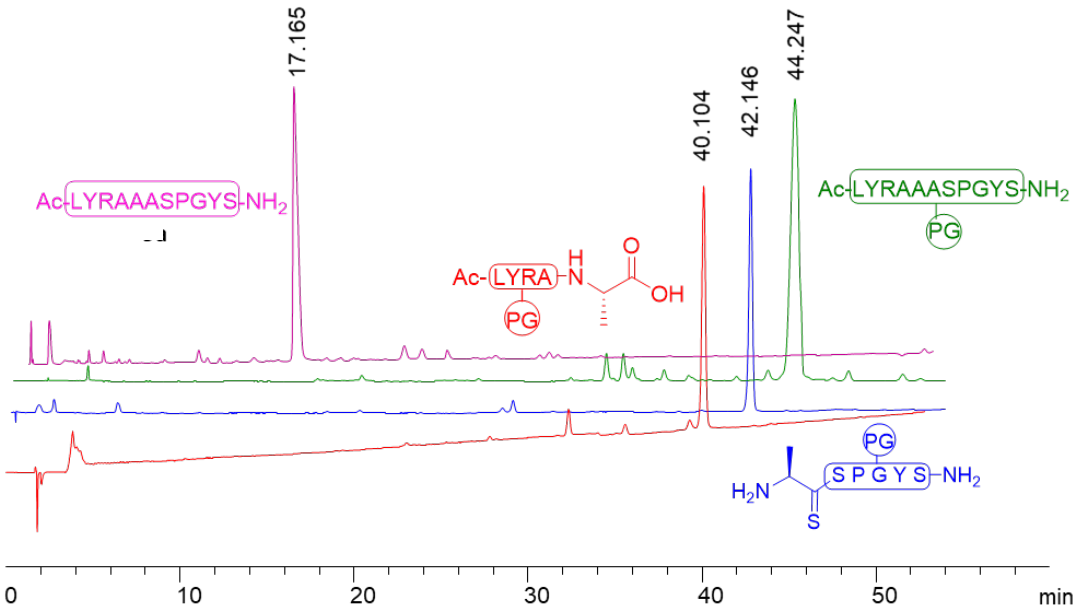

Fig. S13: HPLC traces of crude linear peptide thioamide **8a** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10ba** (magenta).

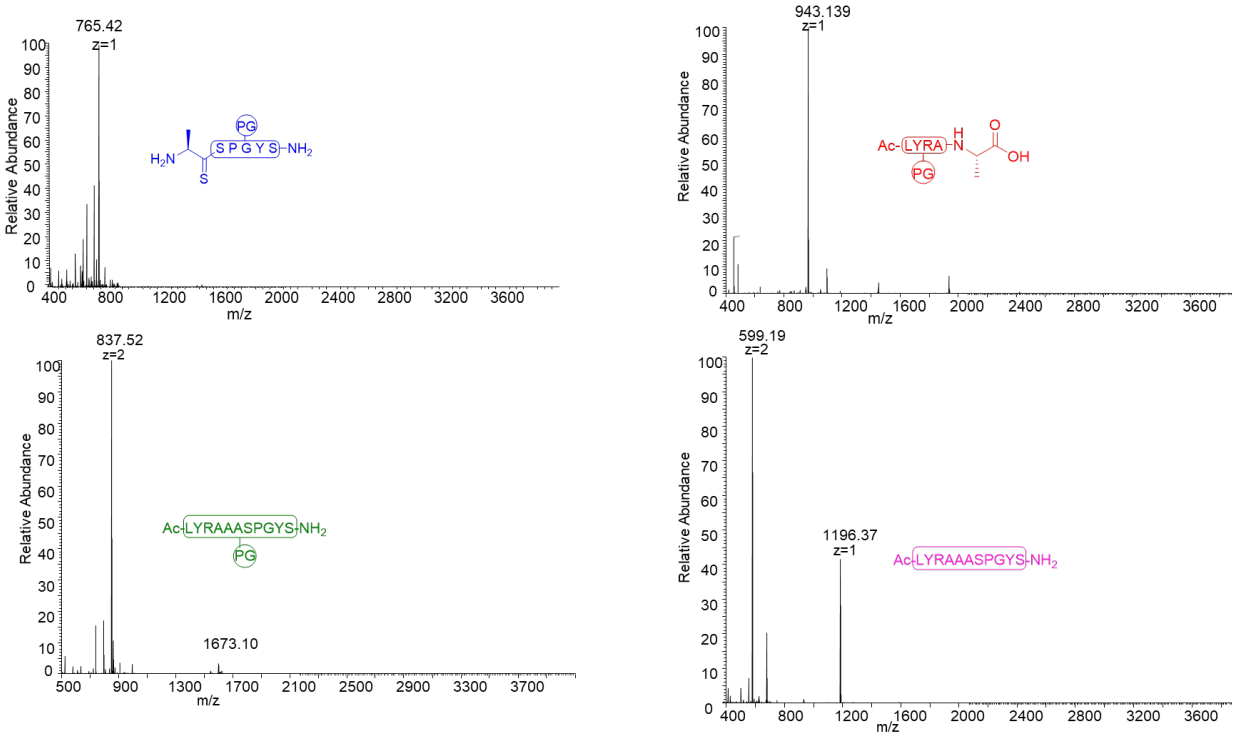

Fig. S14: Mass spectra of crude linear peptide thioamide **8a** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10ba** (magenta).

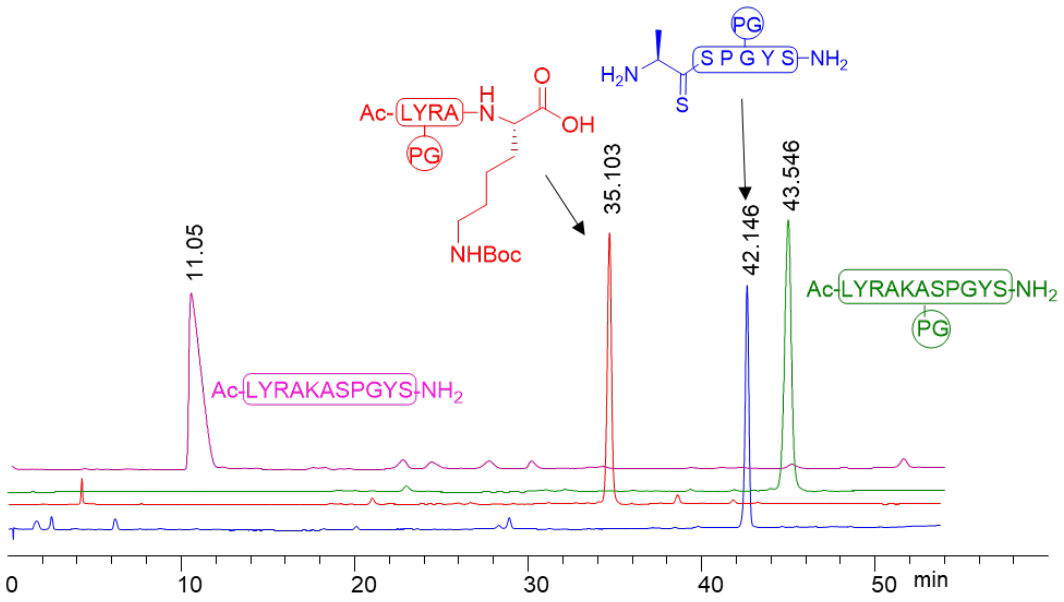

Fig. S25: HPLC traces of crude linear peptide thioamide **8a** (blue), crude linear peptide **9c** (red), crude protected ligated peptide (green) and purified final peptide **10ca** (magenta).

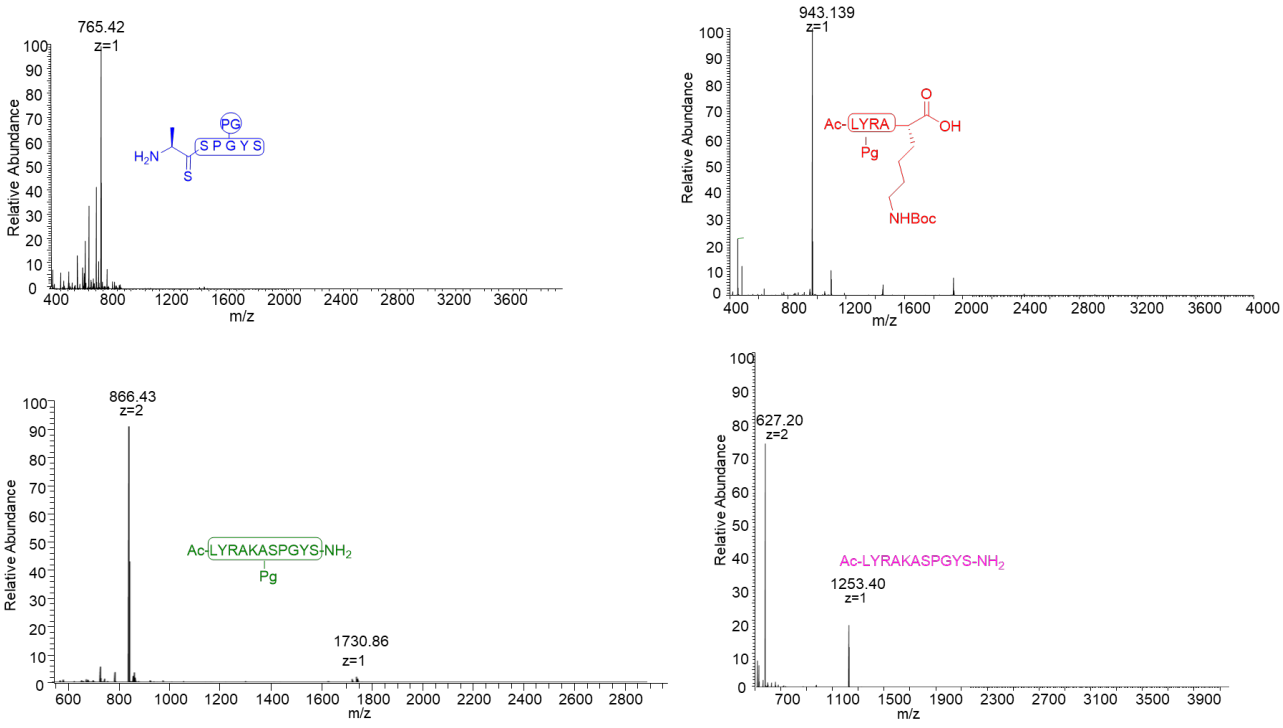

Fig. S26: Mass spectra of crude linear peptide thioamide **8a** (blue), crude linear peptide **9c** (red), crude protected ligated peptide (green) and purified final peptide **10ca** (magenta).

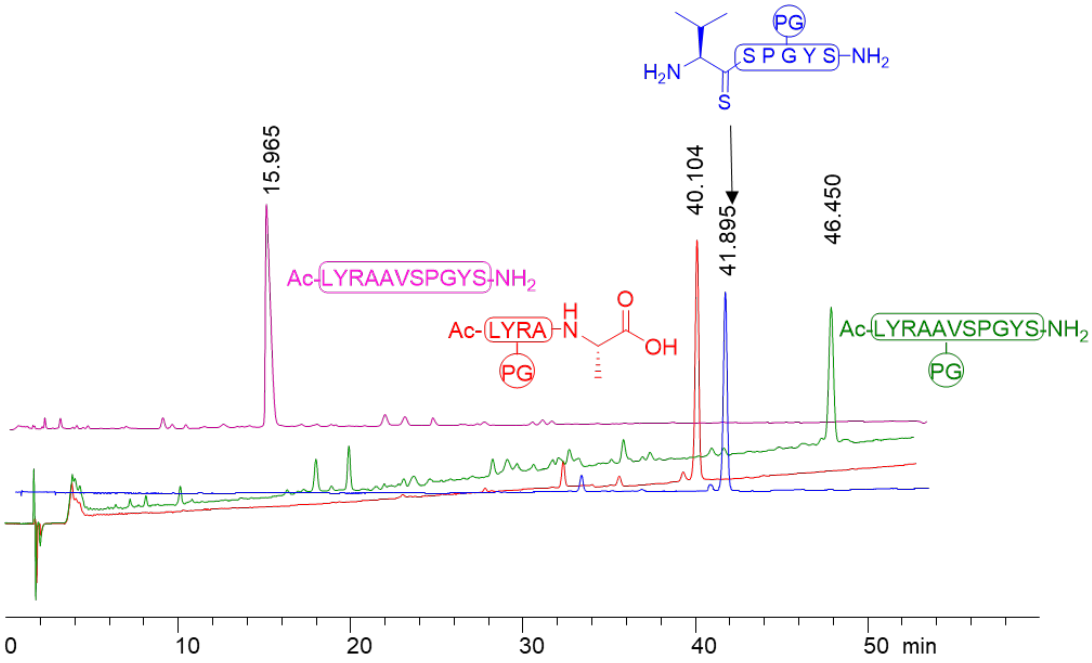

Fig. S17: HPLC traces of crude linear peptide thioamide **8b** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10bb** (magenta).

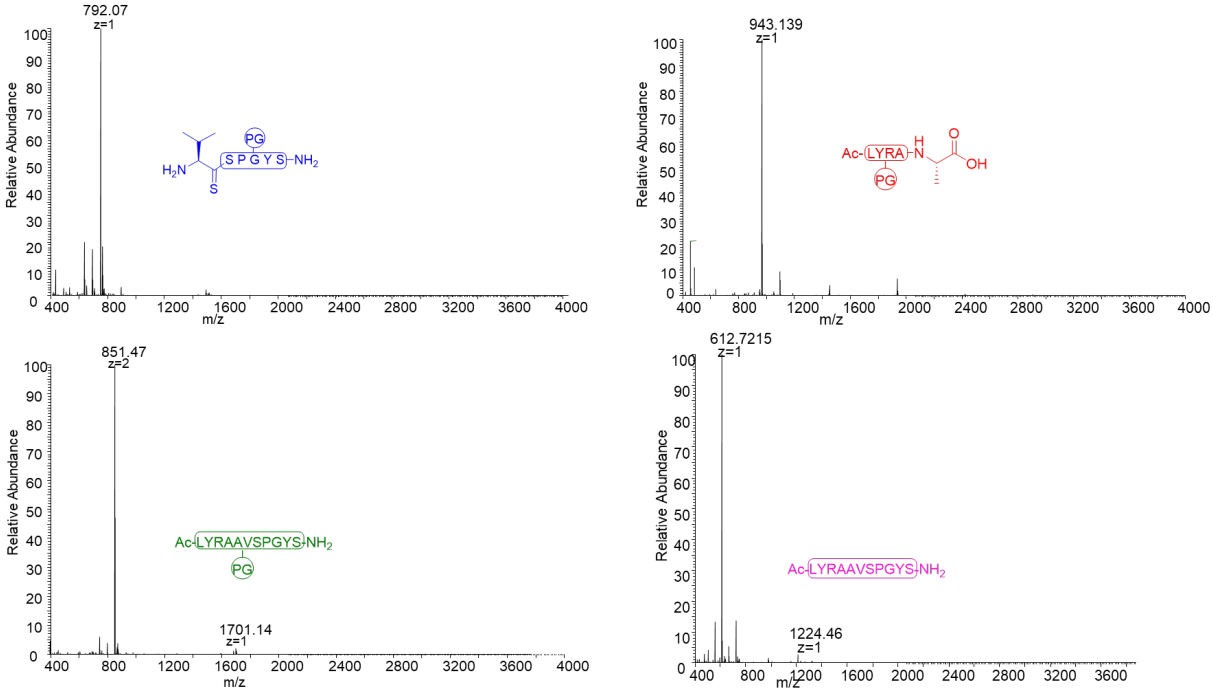

Fig. S18: Mass spectra of crude linear peptide thioamide **8b** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10bb** (magenta).

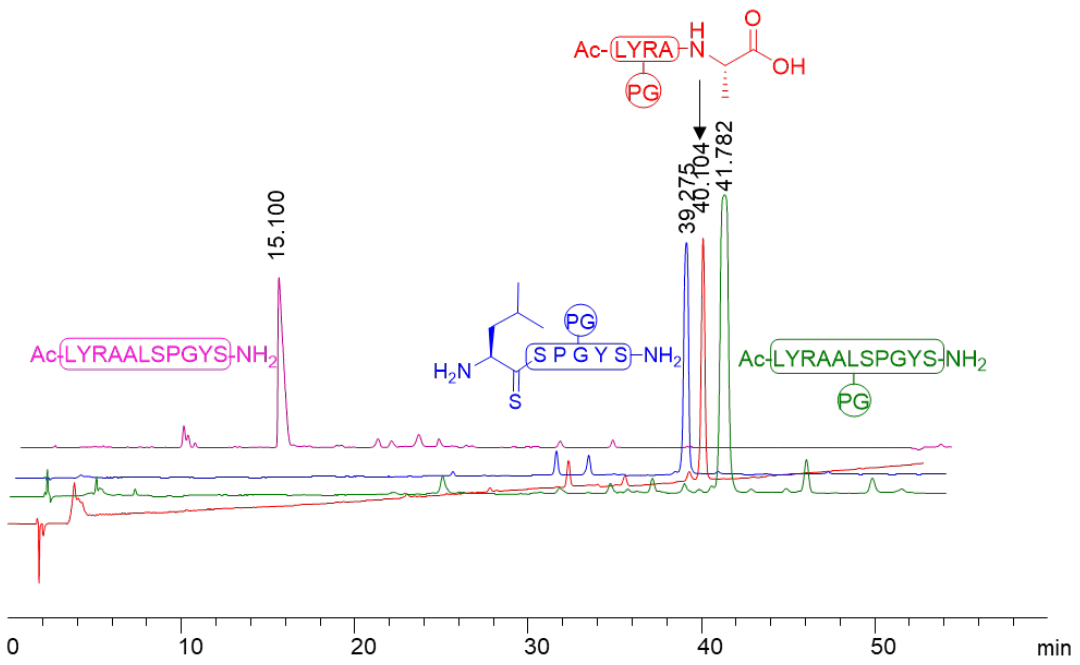

Fig. S19: HPLC traces of crude linear peptide thioamide **8c** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10bc** (magenta).

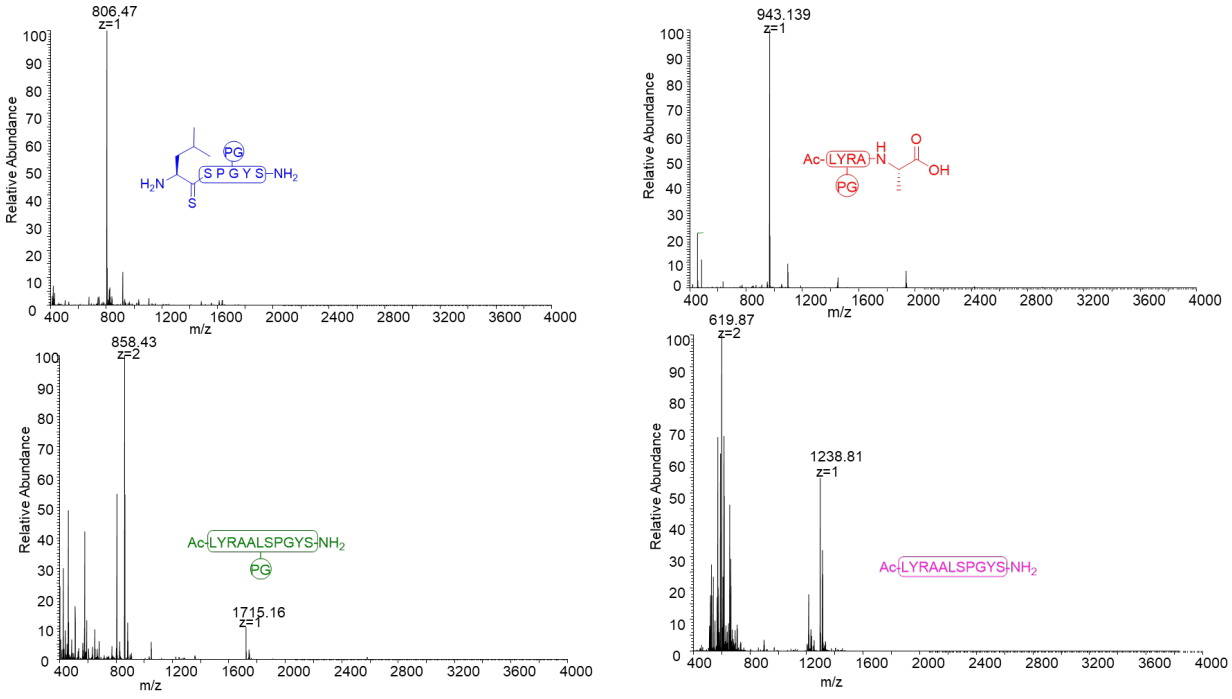

Fig. S20: Mass spectra of crude linear peptide thioamide **8c** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10bc** (magenta).

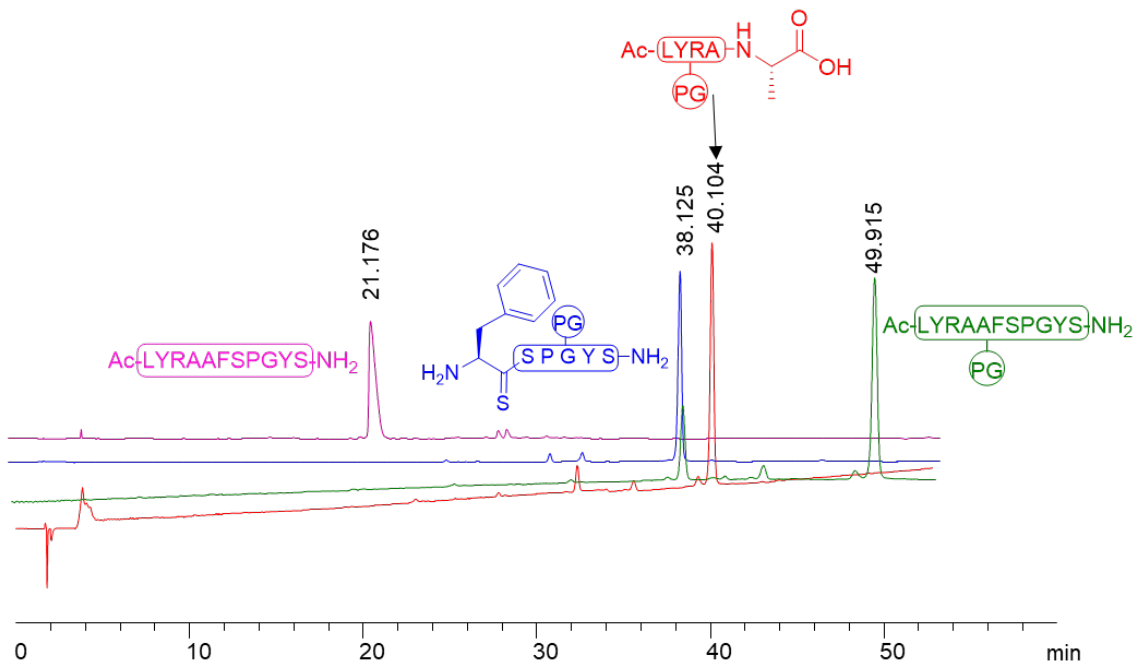

Fig. S21: HPLC traces of crude linear peptide thioamide **8d** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10bd** (magenta).

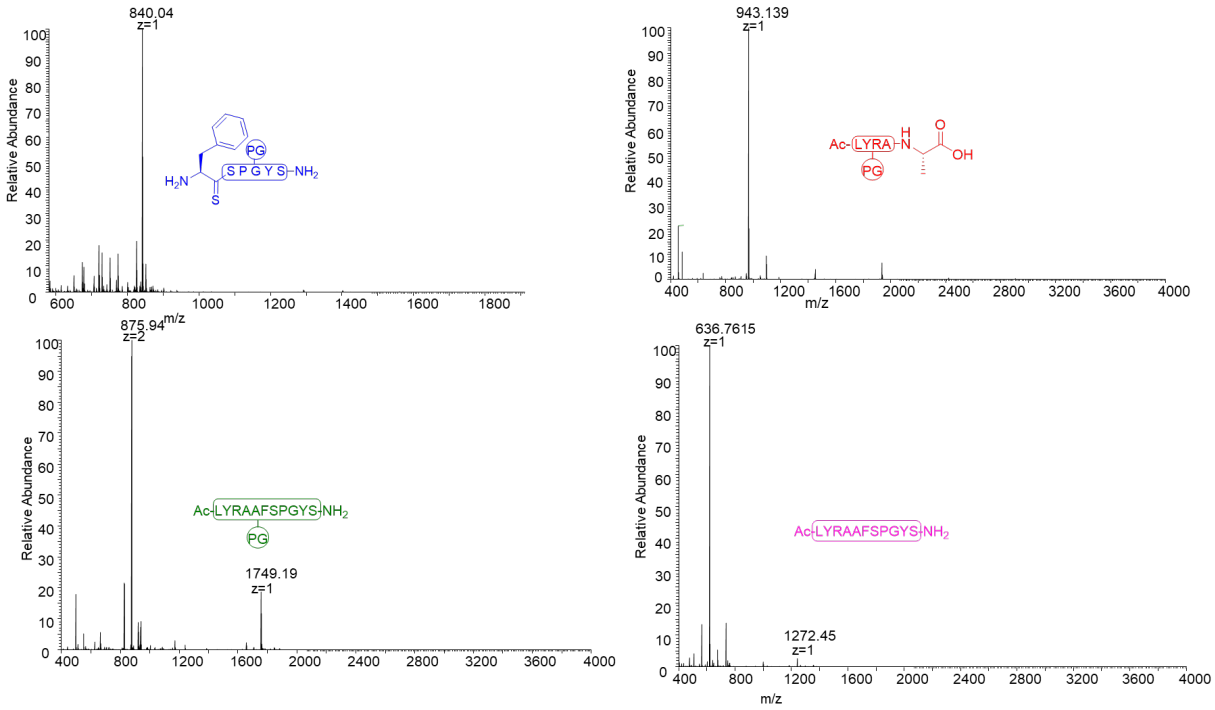

Fig. S22: Mass spectra of crude linear peptide thioamide **8d** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10bd** (magenta).

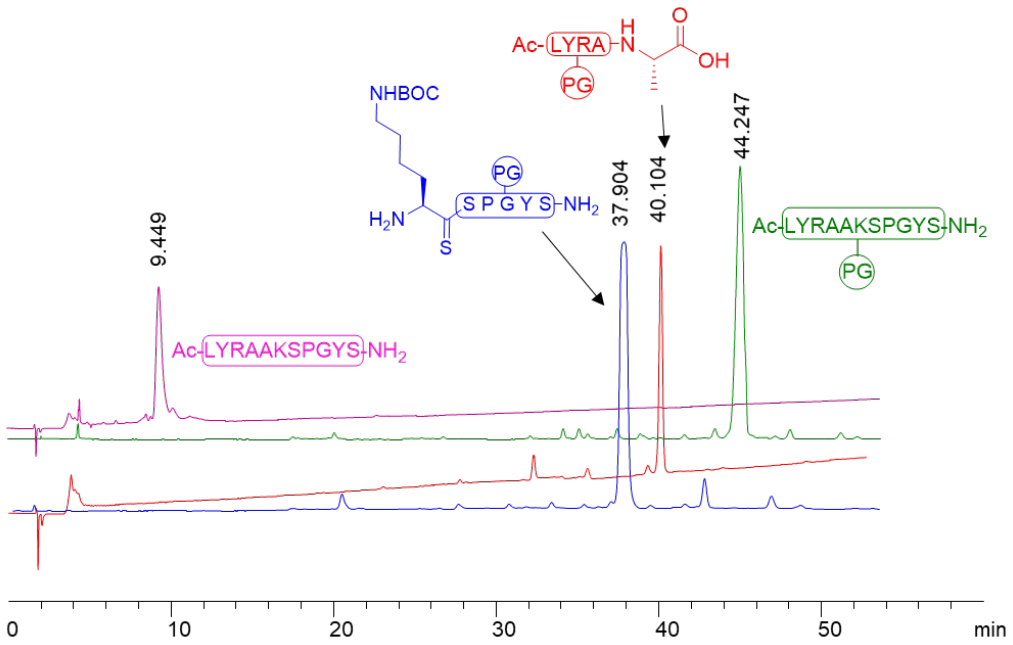

Fig. S23: HPLC traces of crude linear peptide thioamide **8e** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10be** (magenta).

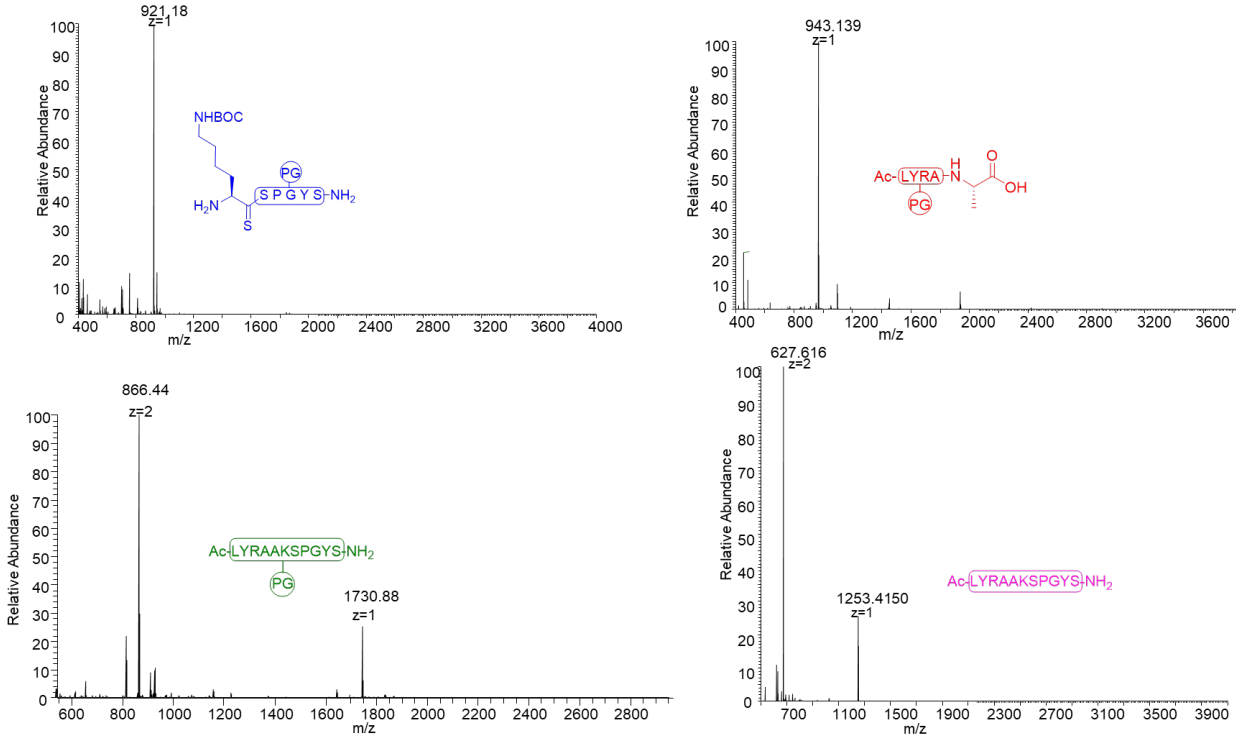

Fig. S24: Mass spectra of crude linear peptide thioamide **8e** (blue), crude linear peptide **9b** (red), crude protected ligated peptide (green) and purified final peptide **10be** (magenta).

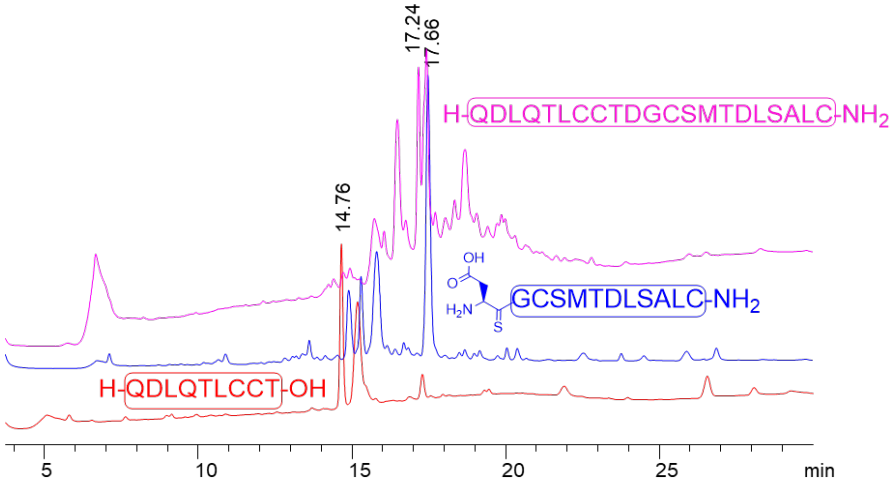

Fig. S25: HPLC traces of crude linear peptide thioamide **12** (blue), crude linear peptide **11** (red), and crude final peptide **13** (magenta).

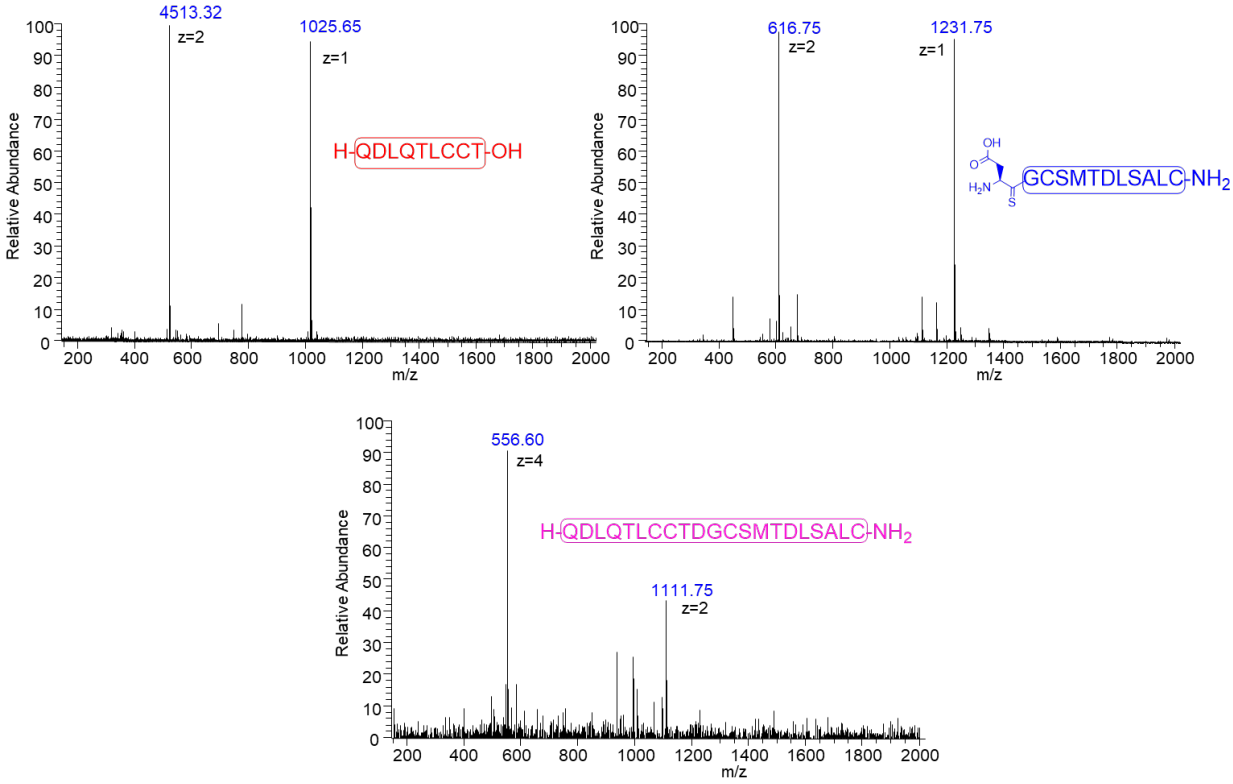

Fig. S26: Mass spectra of crude linear peptide **11** (red), crude linear peptide thioamide **12** (blue), and purified final peptide **13** (magenta).

References

[1] a) J. Shang, V. J. Thombare, C. L. Charron, U. Wille, C. A. Hutton, *Chem. Eur. J.* **2021**, *27*, 1620-1625;
b) V. J. Thombare, C. A. Hutton, *Angew. Chem. Int. Ed.* **2019**, *58*, 4998-5002.

[2] a) Y. J. Wang, D. M. Szantai-Kis, E. J. Petersson, *Org. Biol. Chem.* **2016**, *14*, 6262-6269;
b) S. Batjargal, Y. J. Wang, J. M. Goldberg, R. F. Wissner, E. J. Petersson, *J. Am. Chem. Soc.* **2012**, *134*, 9172-9182.