Expanding the scope of sustainable peptide synthesis through post-linear synthesis reactions

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Supporting Information

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General Information and Protocols

Reagents and Instrumentation

All reagents were purchased from commercial sources and used without further purification unless otherwise stated. Standard Fmoc-protected amino acids were purchased from Iris Biotech GmbH, unless specifically stated differently below. Side chain protecting groups of N^{α}-Fmoc amino acids were as follows; Fmoc-Arg(Pbf)-OH (Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl), Fmoc-Cys(Acm)-OH (Acm = acetamidomethyl), Fmoc-Cys(Trt)-OH (Trt = triphenylmethane), Fmoc-Cys(SIT)-OH (SIT = sec-isoamyl mercaptan), Fmoc-Lys(Boc)-OH (Boc = *tert*-butyloxycarbonyl), Fmoc-Lys(Dde)-OH (Dde = 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl) and Fmoc-Tyr(*t*Bu)-OH (*t*Bu = *tert*-butyl).

N-Butylpyrrolidinone (NBP) (TamiSolve®[1] NxG-PS) was purchased from CEM**.** Diethyl ether (Et_2O) was purchased from Rathburn. Triisopropylsilane (TIPS), 2,6-lutidine, iodine and *N*-chlorosuccinimide (NCS) were purchased from Sigma-Aldrich, Merck. *N,N'*-Diisopropylcarbodiimide (DIC), ethyl cyanohydroxyiminoacetate (Oxyma Pure), dithiothreitol (DTT), *N*-bromosuccinimide (NBS), *N*-iodosuccinimide (NIS), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA), *N,N*-diisopropylethylamine (DIPEA), copper (I) iodide (CuI), hydrazine monohydrate, Fmoc-Aha-OH, Fmoc-Pra-OH and sodium ascorbate (NaAsc) were purchased from Fluorochem. Dichloromethane (CH_2Cl_2) and methanol (MeOH) were purchased from VWR. Acetonitrile (MeCN), Trifluoroacetic acid (TFA), piperidine, dimethyl sulfoxide (DMSO) and formic acid (FA) were purchased from Fisher Scientific. Acetic anhydride was purchased from Thermo Scientific. Aminomethyl Rink-Amide resin was purchased from Activotec or Fluorochem.

Analytical reverse-phase high-performance liquid chromatography (RP-HPLC) was performed on a Shimadzu RP-HPLC system with Shimadzu LC-20AT pumps, a Shimadzu SIL20A autosampler and a Shimadzu SPD-20A UV-vis detector using a Phenomenex Aeris Peptide XB-C18 (100Å, 5 µm, 150 × 4.6 mm). Compounds were eluted with linear gradients at column-dependent flow rates (1 mL/min for the Aeris), where buffer A = 0.1% TFA in H_2O and buffer B = 0.1% TFA in MeCN. Data is reported as column retention time (t_R) in minutes (mins).

Liquid chromatography-mass spectrometry (LCMS) was performed on a Thermo Scientific LCQ Fleet Ion Trap Mass Spectrometer using positive mode electrospray ionisation (ESI^+). Where buffer A = 0.1% FA in 95% H_2O/5% MeCN and buffer B = 0.1% FA in 95% MeCN/5% H_2O, a linear gradient of 5% – 95%B over 20 min with a flow rate of 1 mL/min was used with a Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm).

General Protocol 1 for the manual Fmoc-SPPS

Peptides were synthesised batchwise as required on a 0.1 mmol or 0.3 mmol scale and the resin swollen in NBP (5 mL) for 1 hour. The peptides were synthesised Fmoc-rink amide AM resin (0.53 mmol/g loading, 0.566 g). Peptides were elongated in cycles of amino acid coupling followed by Fmoc removal. Fmoc-protected amino acid (3 equiv., 0.1 M in NBP) coupling was achieved by treatment with DIC (3 equiv., 0.1 M in NBP) and Oxyma (3 equiv., 0.1 M in NBP) at r.t. for 2 x 40 mins. Fmoc removal was achieved by treatment with 20% piperidine + 5% formic acid in NBP (8 mL, *v/v/v*) at r.t. for 1 x 5 mins followed by r.t. 1 x 15 mins. The resin was washed with NBP following Fmoc removal (2 x 5 mL), and after coupling NBP (2 x 5 mL), MeOH (2 x 5 mL), and CH_2Cl_2 (2 x 5 mL).

General Protocol 2 for on-resin SIT removal

The on-resin peptide was thoroughly washed with NBP (2 x 1 mL) and swollen in NBP (200 µL) at 50 °C for 20 mins. The resin was cooled to room temperature and treated with a solution of DTT (15 mg, 0.1 mmol, 10 equiv.) dissolved in 4% DIPEA, 4% H_2O in NBP (1 mL, *v/v/v*) for 4 hours. Following DTT treatment, the resin was thoroughly washed with NBP (3 x 1 mL), MeOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and dried under vacuum.

General Protocol 3 for on-resin Acm removal

The on-resin peptide was thoroughly washed with NBP (2 x 1 mL) and swollen in NBP (200 µL) at 50 °C for 20 mins. The resin was cooled to room temperature and treated with a solution of iodine (10 mg, 0.04 mmol, 4 equiv.) dissolved in NBP (400 µL) and $Na_2S_2O_8$ (5 mg, 0.02 mmol, 2 equiv.) dissolved in NBP (400 µL) for 1 hour. Following iodine treatment, the resin was thoroughly washed with NBP (3 x 1 mL), MeOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and dried under vacuum.

General Protocol 4 for on-resin Trt removal

The on-resin peptide was thoroughly washed with NBP (2 x 1 mL) and swollen in NBP (200 µL) at 50 °C for 20 mins. The resin was cooled to room temperature and treated with a solution of NBS (4 mg, 0.022 mmol, 2.2 equiv.) dissolved in 0.5% TFA, 20% H_2O in NBP (800 µL, *v/v/v*) for 30 mins. Following NBS treatment, the resin was thoroughly washed with NBP (3 x 1 mL), MeOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and dried under vacuum.

General Protocol 5 for on-resin Dde removal

The on-resin peptide was thoroughly washed with NBP (2 x 1 mL) and swollen in NBP (200 µL) at 50 °C for 20 mins. The resin was cooled to room temperature and treated with a solution of 4% NH_2NH_2 in NBP (1 mL, *v/v*) for 6 x 5 mins. Following NH_2NH_2treatment, the resin was thoroughly washed with NBP (3 x 1 mL), MeOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and dried under vacuum.

General Protocol 6 for on-resin disulfide formation

The peptidyl resin (1 equiv.) was swollen in NBP (200 µL) at 50 °C for 20 mins. The resin was cooled to room temperature and treated with a solution of NCS (2 equiv.) in NBP (800 µL) for 2 x 15 mins. Upon reaction completion, the resin was thoroughly washed with NBP (3 x 1 mL), MeOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and dried under vacuum.

General Protocol 7 for On-resin 1,4-triazole formation

The peptidyl resin (1 equiv.) was swollen in NBP/DMSO, 9:1, (200 µL, *v/v*) at 50 °C for 20 mins. To this, copper iodide (0.9 equiv.), DIPEA (5 equiv.), 2,6-lutidine (5 equiv.), NaAsc (1.5 equiv.) and TBTA (1.2 equiv.) suspended in NBP (800µL) was added and the reaction heated (60 mins, 55 °C). Upon reaction completion, the resin was washed with NBP (3 x 1 mL) followed by CH_2Cl_2 (3 x 1 mL) and dried under vacuum.

General Protocol 8 for On-resin capping

The peptidyl resin (1 equiv.) was swollen in NBP (200 µL) at 50 °C for 20 mins. The resin was cooled to room temperature. To this, acetic anhydride (3 equiv.) and DIPEA (6 equiv.) dissolved in NBP (800 µL) were added and the reaction was agitated at r.t. for 1 hour. Upon reaction completion, the resin was thoroughly washed with NBP (3 x 1 mL), MeOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and dried under vacuum.

Experimental

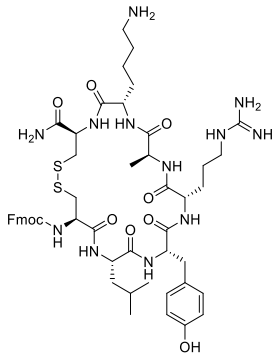
Table of Peptides

Peptide	Sequence	Reaction	Deprotection Conditions	Calculated *m/z*	Observed *m/z*	t_R (mins)
1	*Fmoc-C(SIT)LYRAKC(SIT)-CONH₂*	/	/	1280.59	1281.71	37.8
2	*Fmoc-CLYRAKC-CONH₂*	/	/	1076.49	1077.78	29.6
3	*Fmoc-C(Acm)LYRAK(Dde)C(Acm)-CONH₂*	/	/	1382.65	1384.24	31.9
4	*Fmoc-(Pra)LYRAK(Aha)-CONH₂*	/	/	1091.57	1092.69	29.9
5	*Fmoc-CLYRAKC-CONH₂*	SIT removal	DTT, DIPEA:H_2O:NBP (*v/v/v, 4:4:92*)	1076.49	1077.86	29.8
6	*Fmoc-(CLYRAKC)-CONH₂*	Trt removal, disulfide formation Or oxidation of **5**	NBS, 0.5% TFA Or NCS	1074.48	1075.70	28.8
7	*Fmoc-(CLYRAK(Dde)C)-CONH₂*	ACM removal and disulfide formation	Or I_2, $Na_2S_2O_8$	1238.56	1240.14	33.3
8	*NH_2-C(Acm)LYRAK(Dde)C(Acm)-CONH₂*	Fmoc deprotection	20% piperidine + 5% formic acid	1160.48	1162.18	21.8
9	*NH_2-C(Acm)LYRAKC(Acm)-CONH₂*	DDE removal	NH_2NH_2	996.50	997.51	16.9
10	*Fmoc-(PraLYRAKAha)-CONH₂*	Triazole formation	CuI, DIPEA, 2,6-lutidine, NaAsc, DMSO and TBTA	1091.57	1092.72	28.3

11	*NH_2-(CLYRAKC)-$CONH_2$*	Fmoc deprotection of **6**	20% piperidine + 5% formic acid	853.07	854.54	16.3
12	*Ac-(CLYRAKC)-$CONH_2$*	Acetyl capped	Acetic anhydride and DIPEA	894.42	895.52	18.8

Peptide Synthesis

Synthesis of *Fmoc*-(CLYRAKC), 6–Method 1



C(SIT)LYRAKC(SIT) was synthesised by Fmoc-SPPS as described in **General Protocol 1** (0.3 mmol). Following synthesis of the linear peptide, on-resin SIT removal was achieved following **General Protocol 2.** Disulfide bond formation between residues Cys^7 and Cys^1 were facilitated with NCS employing **General Protocol 6,** to afford peptide **6**.

LCMS: Mass calculated for [$C_{43}H_{67}N_{11}O_{12}S_2$ + H] 1075.32; deconvoluted mass observed: 1074.48 ± 0.56. Charge states; 538.75 $[M+2H]^{2+}$, 1075.70 $[M+H]^{+}$.

RP-HPLC: t_R = 15.3 min. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 3.6%B/min) at 1 mL/min.

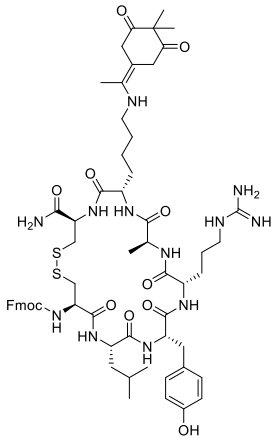
Synthesis of *Fmoc*-(CLYRAKC), 6–Method 2

C(Trt)LYRAKC(Trt) was synthesised by automated Fmoc-SPPS as described in **General Protocol 1** (0.3 mmol). Following synthesis of the linear peptide, on-resin Trt removal and subsequent disulfide formation between residues Cys^7 and Cys^1 was achieved employing **General Protocol 4** to afford peptide **6**.

LCMS: Mass calculated for [$C_{43}H_{67}N_{11}O_{12}S_2$ + H] 1075.32; deconvoluted mass observed: 1074.48 ± 0.56. Charge states; 538.75 $[M+2H]^{2+}$, 1075.70 $[M+H]^{+}$.7

RP-HPLC: t_R = 29.7 min. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min.

Synthesis of *Fmoc*-(CLYRAK(Dde)C), 7

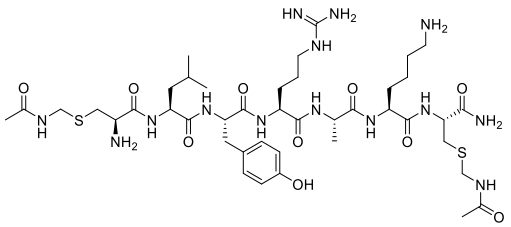


C(Acm)LYRAK(Dde)C(Acm) was synthesised by automated Fmoc-SPPS as described in **General Protocol 1** (0.3 mmol). Following synthesis of the linear peptide, on-resin Acm removal and subsequent disulfide formation between residues Cys^7 and Cys^1 was achieved employing **General Protocol 3** to afford peptide **7**.

LCMS: Mass calculated for [$C_{61}H_{82}N_{12}O_{12}S_2$ + H] 1239.52; deconvoluted mass observed: 1238.56 ± 0.40. Charge states; 620.85 $[M+2H]^{2+}$, 1240.14 $[M+H]^+$.

RP-HPLC: t_R = 33.4 min. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min.

Synthesis of C(Acm)LYRAKC(Acm), 9

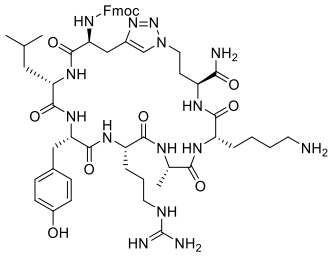


C(Acm)LYRAK(Dde)C(Acm) was synthesised by automated Fmoc-SPPS as described in **General Protocol 1** (0.3 mmol). Following synthesis of the linear peptide and *N*-terminal Fmoc removal, orthogonal on-resin Dde removal was facilitated using hydrazine monohydrate as outlined in **General Protocol 5** to afford peptide **9**.

LCMS: Mass calculated for [$C_{42}H_{72}N_{14}O_{10}S_2$ + H] 997.25 deconvoluted mass observed: 996.5 ± 0.04. Charge states; 997.51 $[M+2H]^{2+}$, 499.23 $[M+H]^+$.

RP-HPLC: t_R = 16.9 min. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min.

Synthesis of *Fmoc*-(PraLYRAKAha), 10

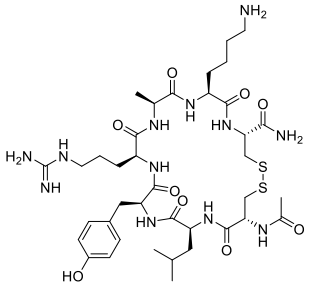


Fmoc-PraLYRAKAha was synthesised by automated Fmoc-SPPS as described in **General Protocol 1** (0.3 mmol). Following synthesis of the linear peptide, on-resin 1,4-triazole formation was facilitated between Pra^7 and Aha^1 according to **General Protocol 7**.

LCMS: Mass calculated for [$C_{54}H_{73}N_{15}O_{10}S_2$ + H] 1092.27; deconvoluted mass observed: 1091.57± 0.38. Charge states; 547.13 $[M+2H]^{2+}$, 1092.72 $[M+H]^+$.

RP-HPLC: t_R = 28.3 min. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min.

Synthesis of *Ac-(*CLYRAKC), 12



Fmoc-(CLYRAKC), was synthesised by following method 1 or 2 outlined above (0.3 mmol). Following synthesis of the cyclic peptide, the resin was treated 20% piperidine + 5% formic acid in NBP (8 mL, *v/v/v*) at r.t. for 1 x 5 mins followed by r.t. 1 x 15 mins affording peptide **11**. The Fmoc deprotected peptide was *N*-terminally capped via the conditions described in **General Protocol 8** to afford peptide, **12**.

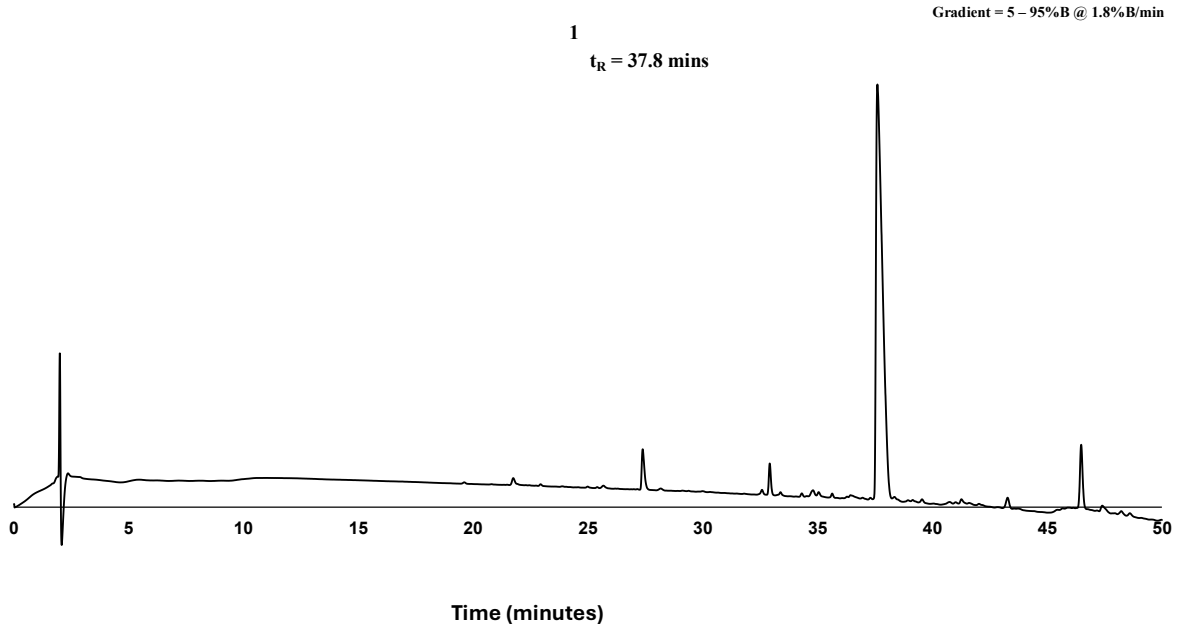
LCMS: Mass calculated for [$C_{38}H_{62}N_{12}O_9S_2$ + H] 895.11; deconvoluted mass observed: 894.42 ± 0.25. Charge states; 448.44 $[M+2H]^{2+}$, 895.52 $[M+H]^+$.

RP-HPLC: t_R = 18.3 min. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min.

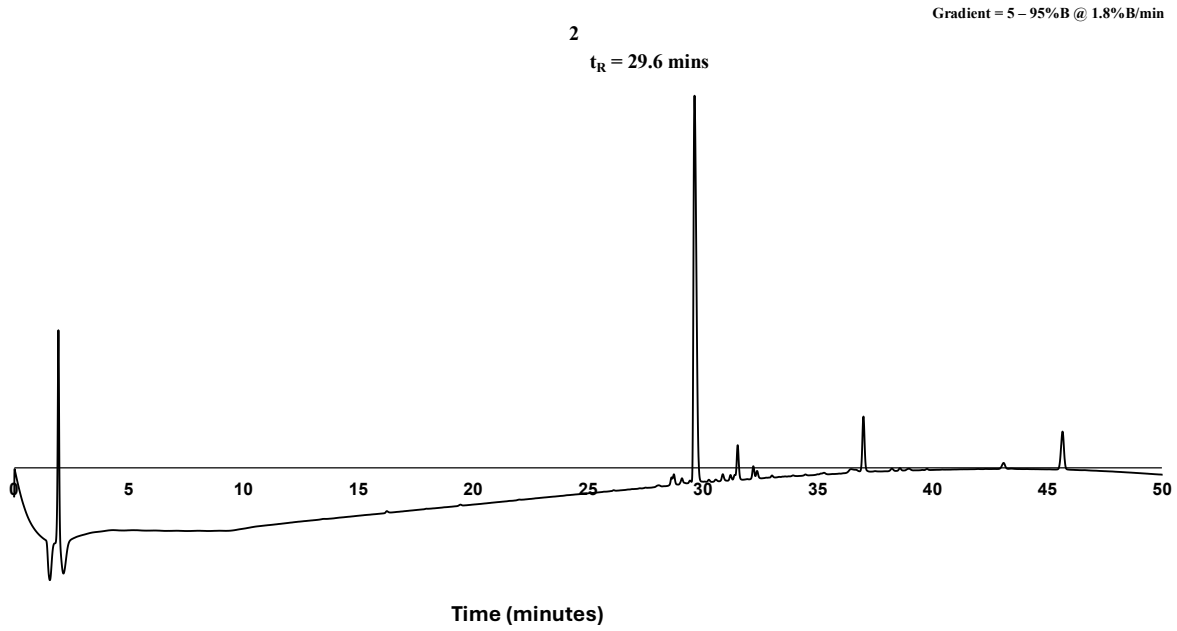
HPLC and LCMS Traces

Reaction progression was monitored by cleavage of a small portion of the peptidyl resin and analysed by RP-HPLC and LCMS. % area determined at 214 nm and rounded to the nearest integer, peaks identified contained an area >5%. Peaks containing <5% total peak area were not included in the calculation. The product in question is indicated by t_R.

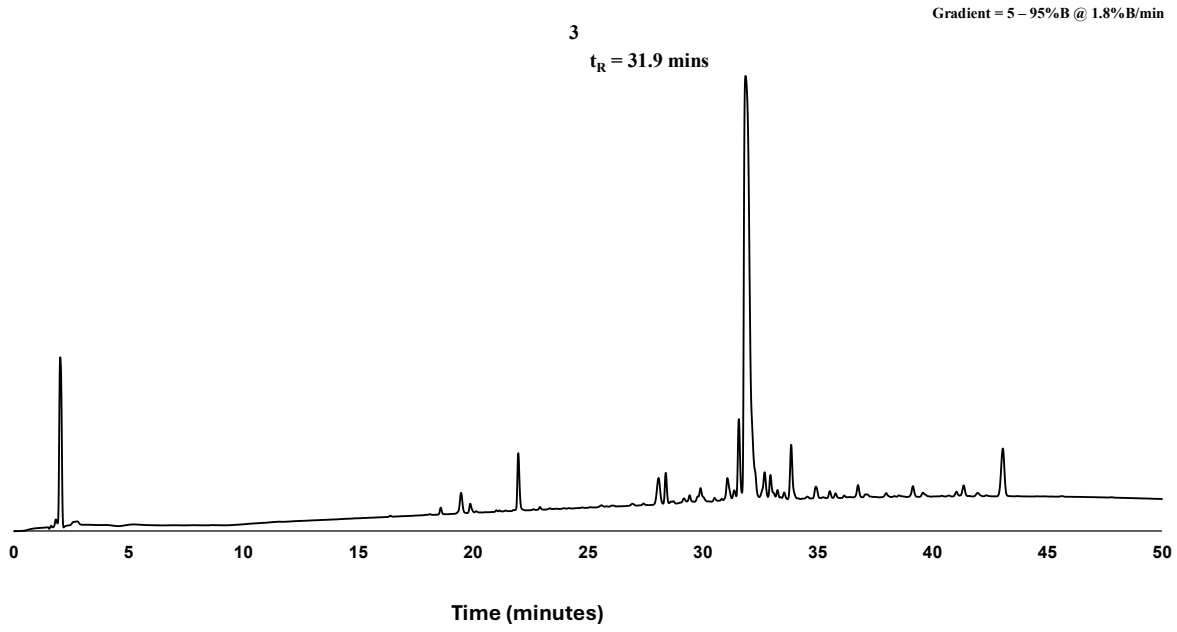
Reverse Phase – High Pressure Liquid Chromatography (RP-HPLC) of crude peptides



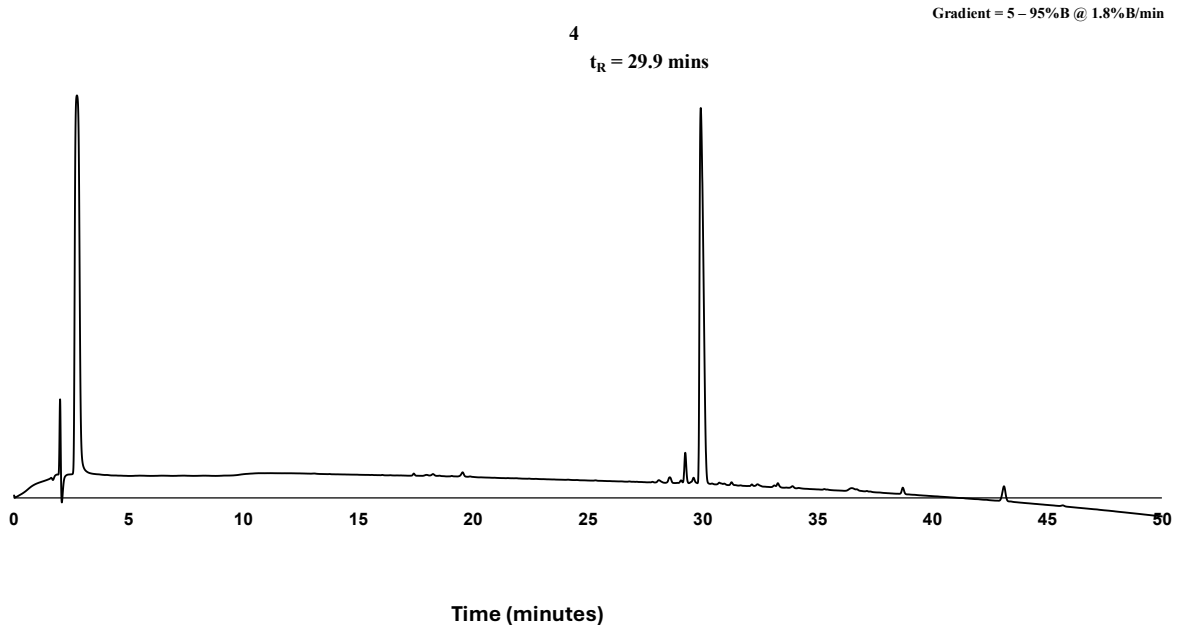
Supporting Information Figure S1. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **1**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 37.8 mins (>90% crude purity).



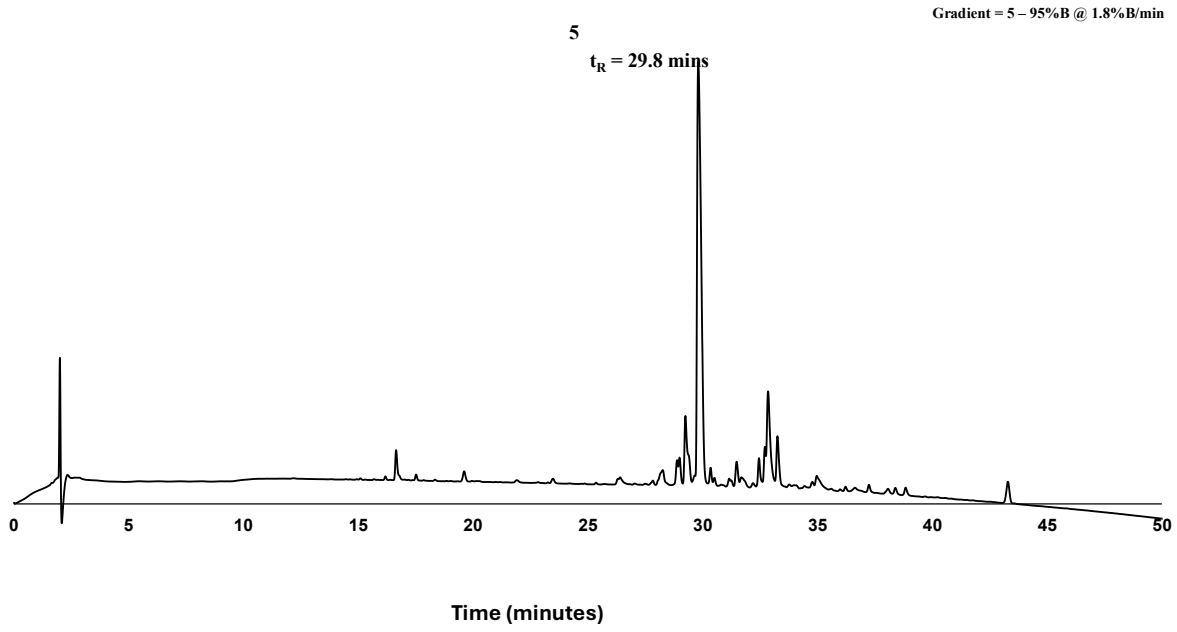
Supporting Information Figure S2. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **2**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 29.6 mins (>90% crude purity).



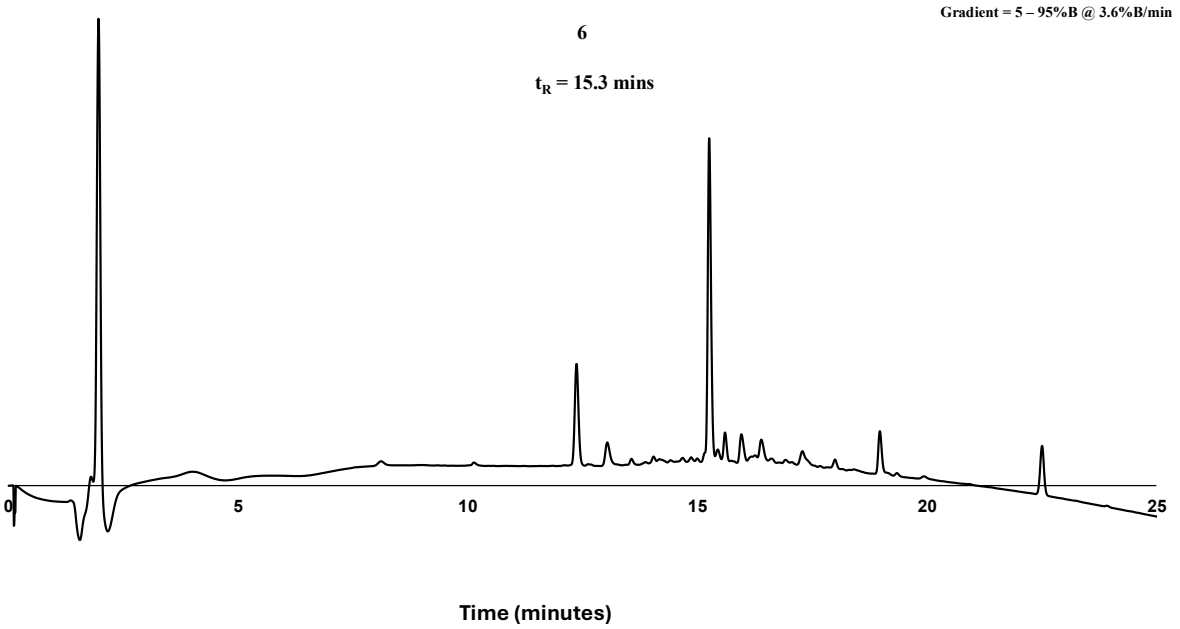
Supporting Information Figure S3. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **3**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 31.9 mins (>90% crude purity).



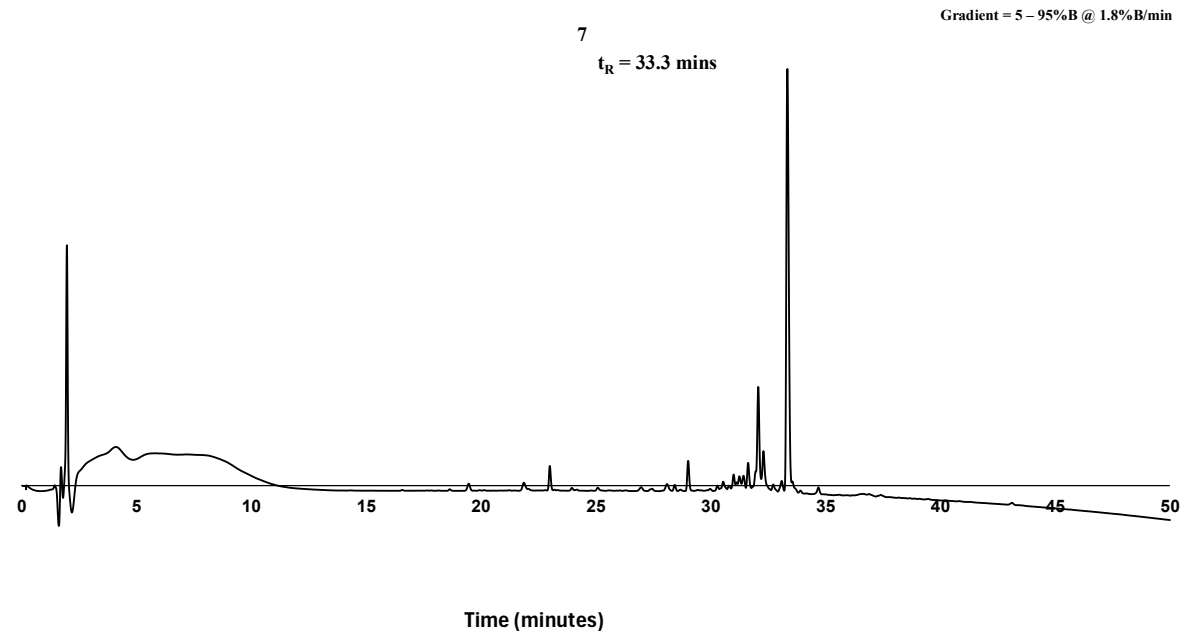
Supporting Information Figure S4. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **4**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 29.9 mins (>90% crude purity).



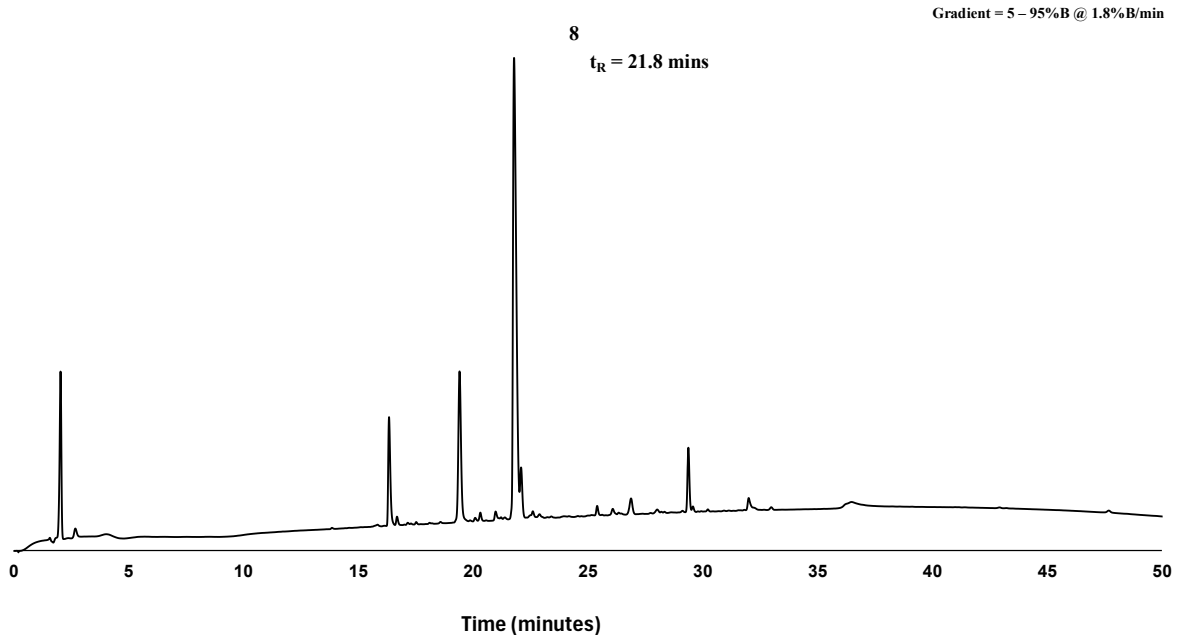
Supporting Information Figure S5. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **5**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 29.8 mins (92% reaction conversion).



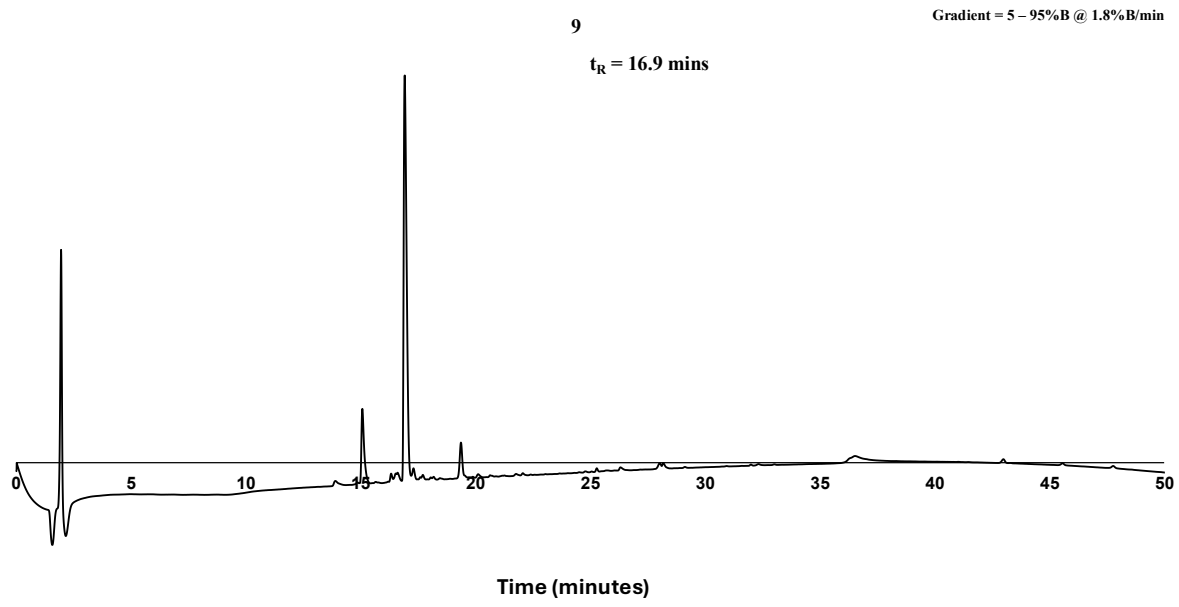
Supporting Information Figure S6. Analytical RP-HPLC chromatogram (214 nm) of peptide, **6** (method 1). Phenomenex Aeris Peptide XB-C18 (100 Å, 5 μm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 25 min (*ca.* 3.6%B/min) at 1 mL/min. t_R = mins (85% reaction conversion).



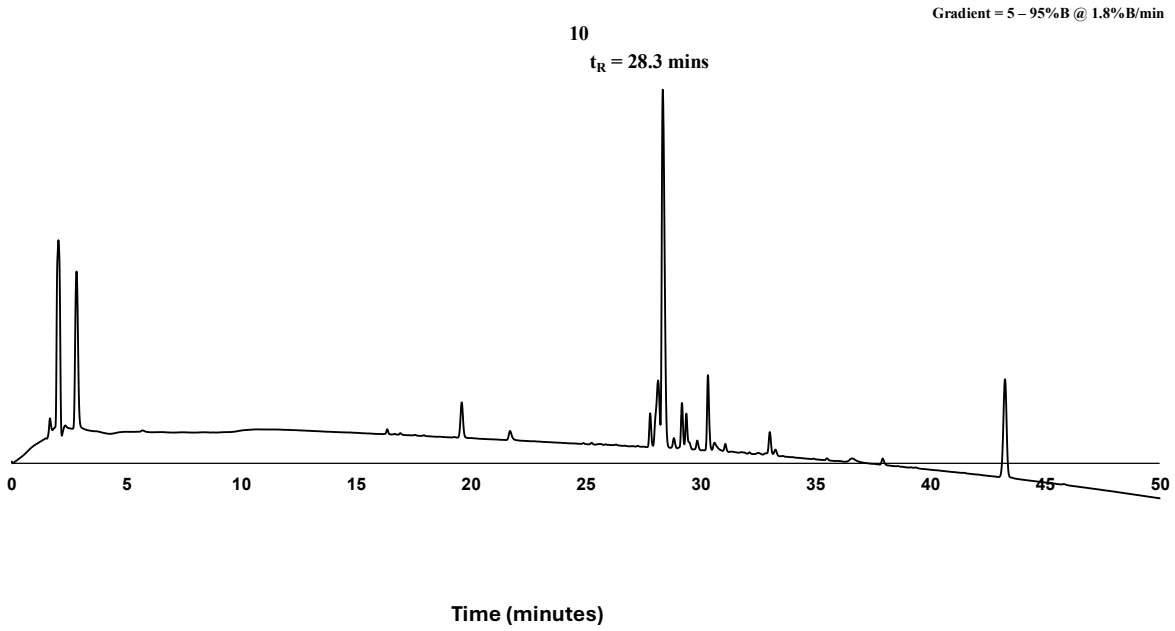
Supporting Information Figure S7. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **7**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 μm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 33.3 mins (83% reaction conversion).



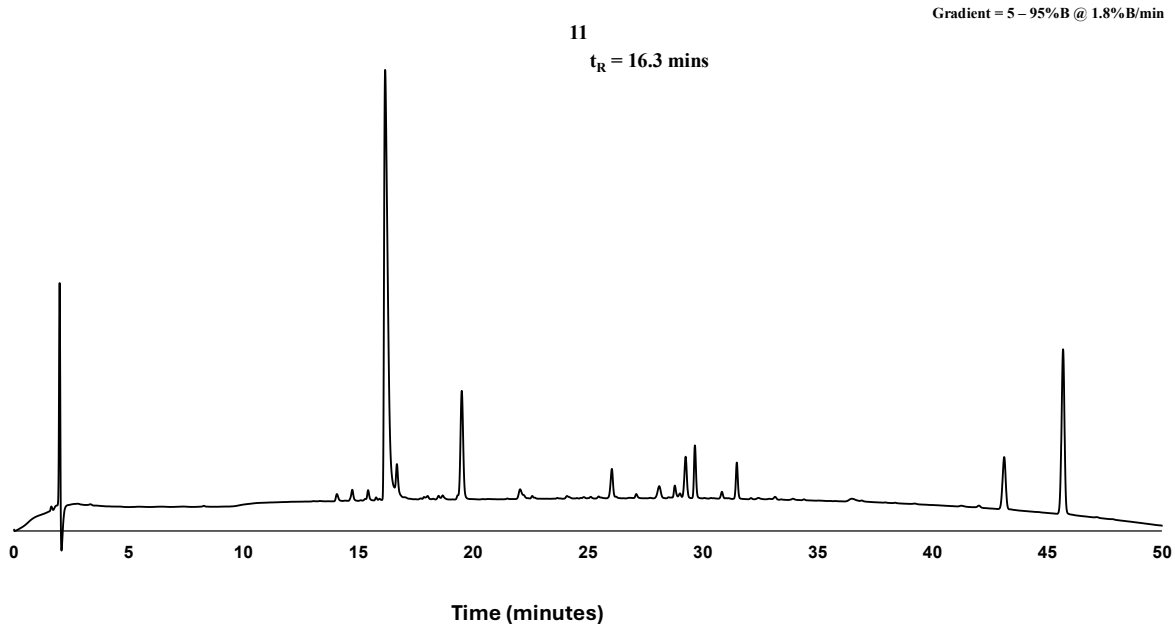
Supporting Information Figure S8. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **8**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 21.8 mins.



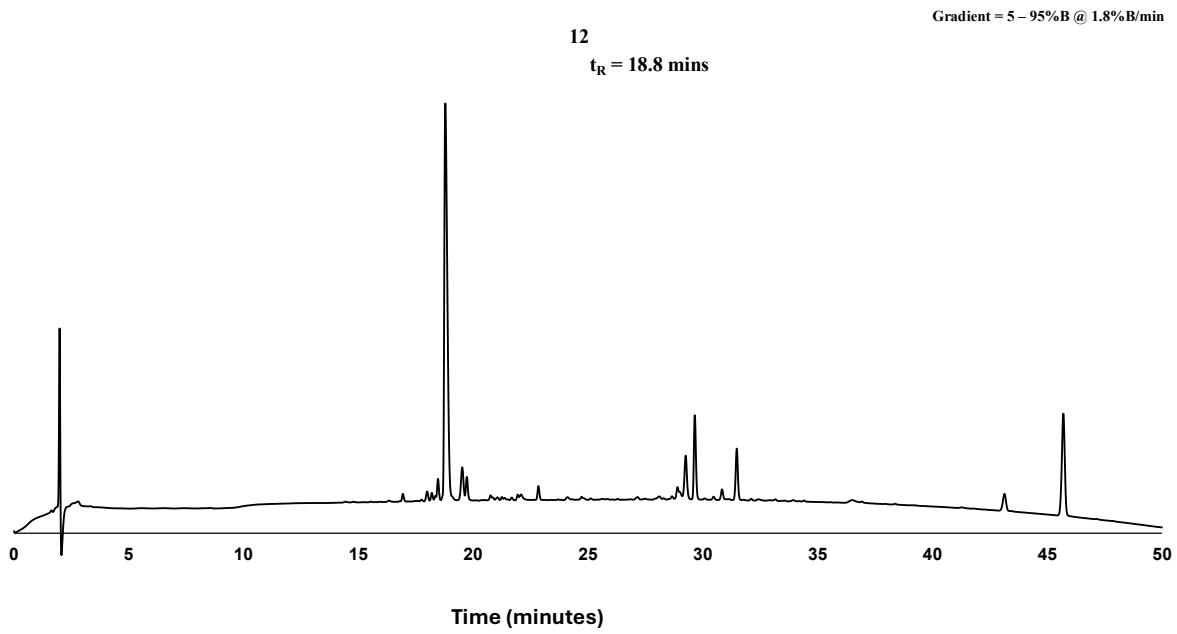
Supporting Information Figure S9. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **9**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 16.9 mins (>99% reaction conversion).



Supporting Information Figure S10. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, 10. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (ca. 1.8%B/min) at 1 mL/min. tR = 28.3 mins (>99% reaction conversion).

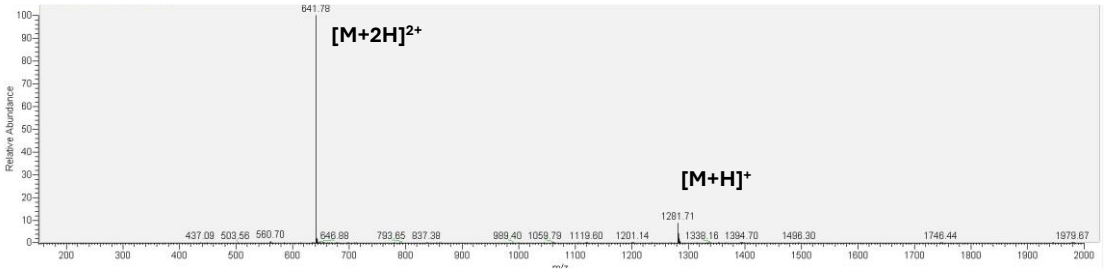


Supporting Information Figure S11. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **11**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 µm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 16.3 mins (>90% crude purity).

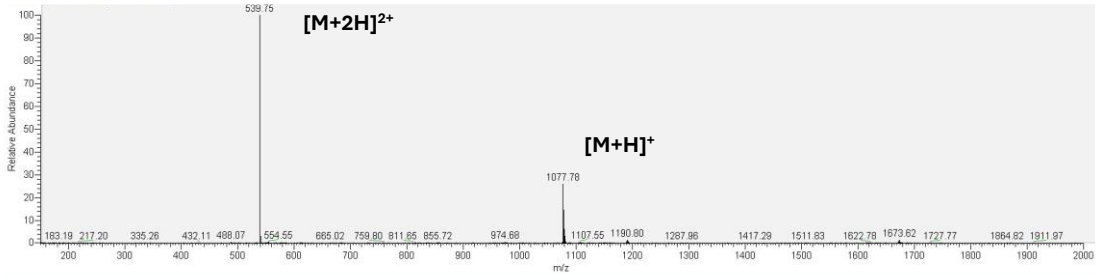


Supporting Information Figure S12. Analytical RP-HPLC chromatogram (214 nm) of crude peptide, **12**. Phenomenex Aeris Peptide XB-C18 (100 Å, 5 μm, 150 mm x 4.6 mm), linear gradient 5% – 95%B over 50 min (*ca.* 1.8%B/min) at 1 mL/min. t_R = 18.8 mins. (>99% reaction conversion).

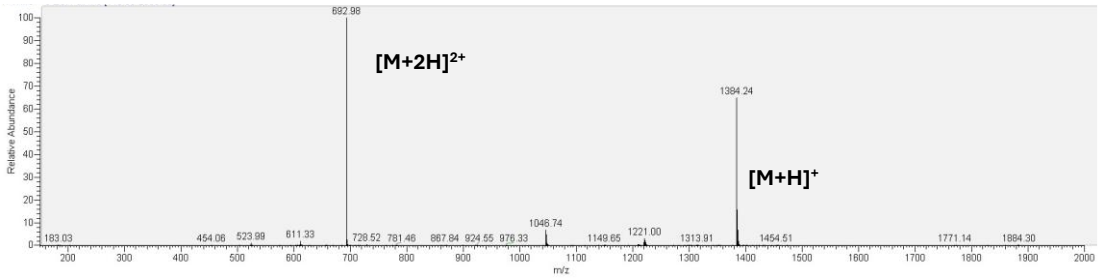
Liquid Chromatography Mass Spectrometry (LCMS)



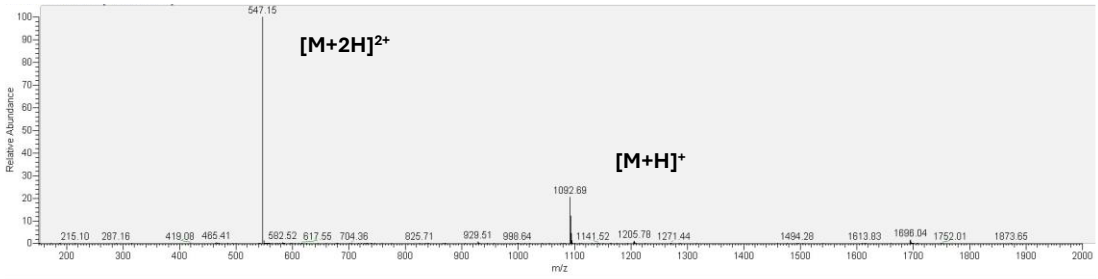
Supporting Information Figure S13. LCMS of peptide, **1**, mass calculated for $[C_{61}H_{92}N_{12}O_{10}S_4 + H]$ 1281.72; deconvoluted mass observed: 1280.59 ± 0.60. Charge states; 641.78 $[M+2H]^{2+}$, 1281.71 $[M+H]^{+}$.



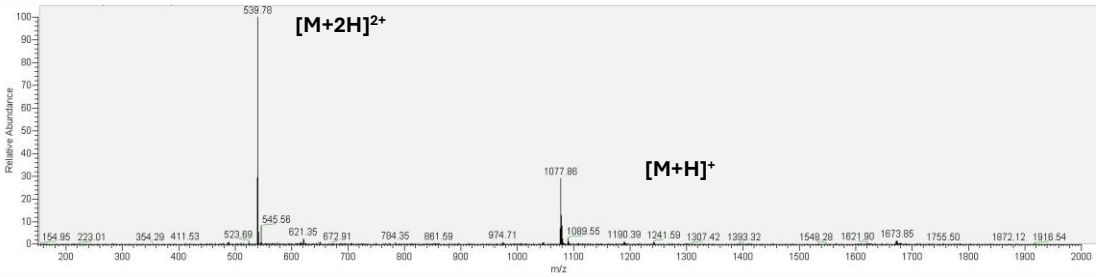
Supporting Information Figure S14. LCMS of peptide, **2**, mass calculated for $[C_{51}H_{72}N_{12}O_{10}S_2 + H]$ 1077.33; deconvoluted mass observed: 1076.49 ± 0.51. Charge states; 539.75 $[M+2H]^{2+}$, 1077.78 $[M+H]^{+}$.



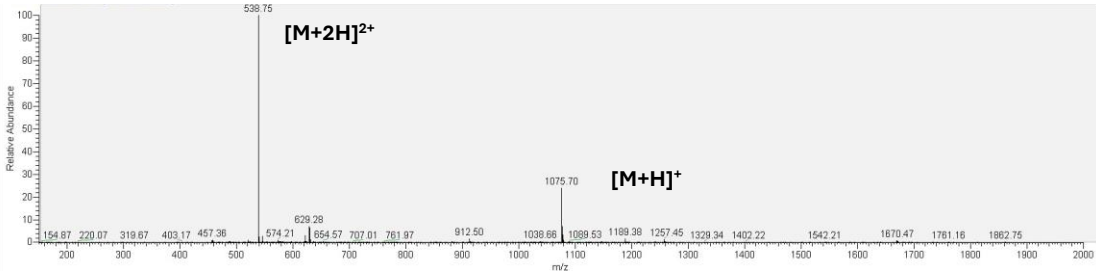
Supporting Information Figure S15. LCMS of peptide, **3**, mass calculated for $[C_{67}H_{94}N_{14}O_{14}S_2 + H]$ 1383.69; deconvoluted mass observed: 1382.65 ± 0.51. Charge states; 692.98 $[M+2H]^{2+}$, 1384.24 $[M+H]^{+}$.



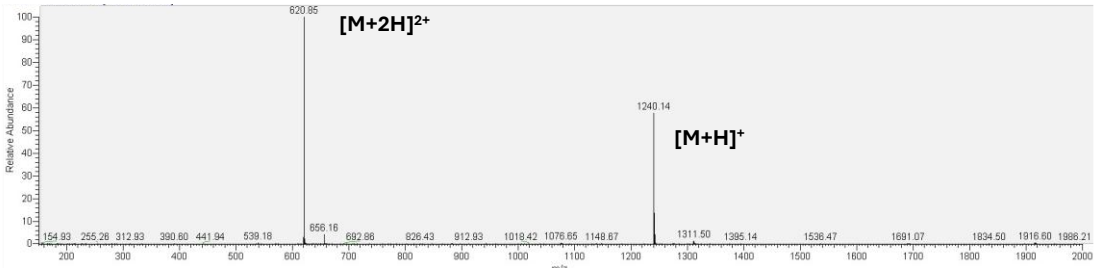
Supporting Information Figure S16. LCMS of peptide, **4**, mass calculated for [$C_{54}H_{73}N_{15}O_{10}$ + H] 1092.27; deconvoluted mass observed: 1091.57 ± 0.43. Charge states; 547.15 $[M+2H]^{2+}$, 1092.69 $[M+H]^{+}$.



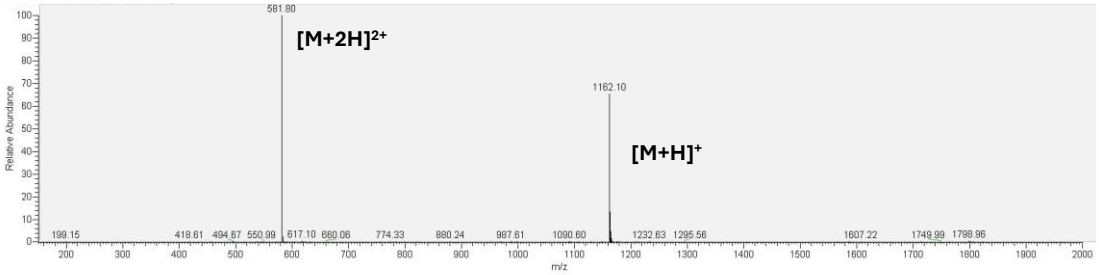
Supporting Information Figure S17. LCMS of peptide, **5**, mass calculated for [$C_{51}H_{72}N_{12}O_{10}S_2$ + H] 1077.33; deconvoluted mass observed: 1076.49 ± 0.50. Charge states; 539.78 $[M+2H]^{2+}$, 1077.86 $[M+H]^{+}$.



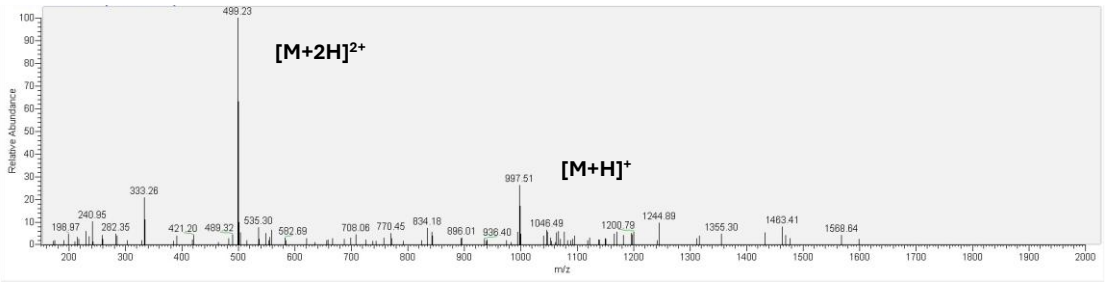
Supporting Information Figure S18. LCMS of peptide, **6**, mass calculated for [$C_{51}H_{70}N_{12}O_{10}S_2$ + H] 1075.32; deconvoluted mass observed: 1074.48 ± 0.57. Charge states; 538.75 $[M+2H]^{2+}$, 1075.70 $[M+H]^{+}$.



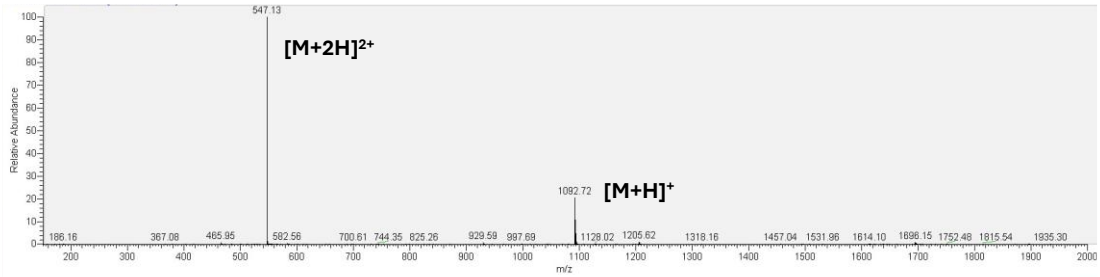
Supporting Information Figure S19. LCMS of peptide, **7**, mass calculated for [$C_{61}H_{82}N_{12}O_{12}S_2$ + H] 1239.52; deconvoluted mass observed: 1238.56 ± 0.40. Charge states; 620.85 $[M+2H]^{2+}$, 1240.14 $[M+H]^{+}$.



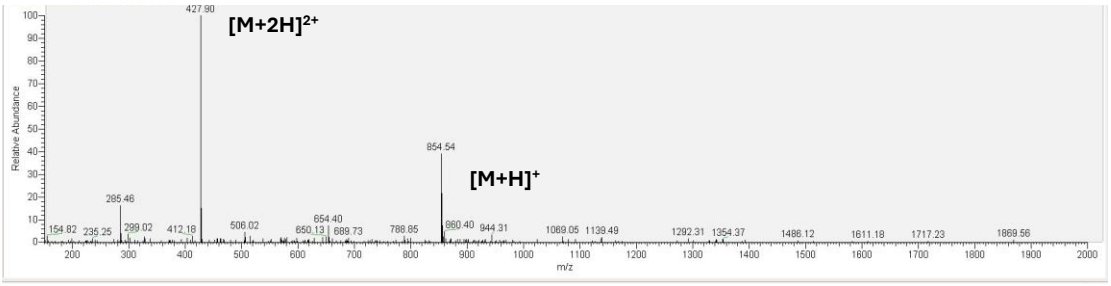
Supporting Information Figure S20. LCMS of peptide, **8**, mass calculated for [$C_{52}H_{84}N_{14}O_{12}S_2$ + H] 1161.45; deconvoluted mass observed: 1160.48 ± 0.35. Charge states; 581.80 $[M+2H]^{2+}$, 1162.10 $[M+H]^{+}$.



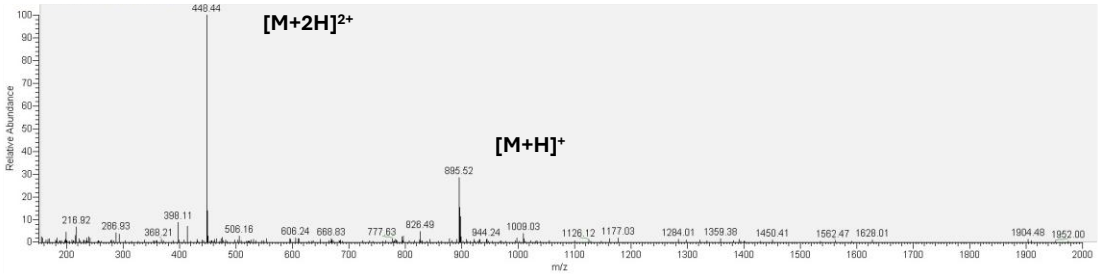
Supporting Information Figure S21. LCMS of peptide, **9**, mass calculated for [$C_{42}H_{72}N_{14}O_{10}S_2$ + H] 997.25; deconvoluted mass observed: 996.50 ± 0.04. Charge states; 499.23 $[M+2H]^{2+}$, 997.51 $[M+H]^{+}$.



Supporting Information Figure S24. LCMS of peptide, **10**, mass calculated for [$C_{54}H_{73}N_{15}O_{10}$ + H] 1092.27; deconvoluted mass observed: 1091.57 ± 0.38. Charge states; 547.13 $[M+2H]^{2+}$, 1092.72 $[M+H]^{+}$.



Supporting Information Figure S22. LCMS of peptide, **11**, mass calculated for $[C_{36}H_{60}N_{12}O_8S_2 + H]$ 853.07; deconvoluted mass observed: 852.41 ± 0.18. Charge states; 427.90 $[M+2H]^{2+}$, 854.54 $[M+H]^{+}$.



Supporting Information Figure S23. LCMS of peptide, **12**, mass calculated for $[C_{38}H_{62}N_{12}O_9S_2 + H]$ 895.11; deconvoluted mass observed: 894.42 ± 0.25. Charge states; 448.44 $[M+2H]^{2+}$, 895.52 $[M+H]^{+}$.