Electronic Supplementary Material (ESI) for ChemComm.

Automated solid-phase concatenation of Aib residues to form long, water-soluble, helical peptides

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Peptide Synthesis

Peptides were synthesised using a microwave-assisted Liberty Blue automated peptide synthesizer (CEM Corporation) on a 0.1 mmol scale on Rink amide MHBA resin (Novabiochem, 0.51 mmol g^{-1}) or phenylalaninol preloaded 2-chlorotrityl chloride resin (Santa Cruz, 0.44 mmol g^{-1}) as stated using Fmoc-coupling chemistry. All Fmoc-protected amino acids were purchased from Carbosynth Ltd (Compton, UK). DMF was purchased from Cambridge Reagents (Hessle, UK). Morpholine was purchased from Merck Millipore (Burlington, USA). All other chemicals were purchased from Sigma Aldrich (Gillingham, UK) or Fisher Scientific (Loughborough, UK) and used without further purification.

Coupling cycle for Pro, Hyp, Aib or any standard amino acid (AA) following Aib (0.1 mmol scale)

Operation	Reagent(s)	Volume (mL)	Time (min)	Temp (°C)
Fmoc deprotection	20% morpholine in DMF	7.0	2	90
Wash (x3)	DMF	4.0, 5.0 & 4.0	-	-
Fmoc deprotection	20% morpholine in DMF	7.0	2	90
Wash (x3)	DMF	4.0, 5.0 & 4.0	-	-
Add amino acid	0.2 M Fmoc-AA-OH in DMF	2.5	-	-
Add DIC	1.0 M DIC in DMF	1.0	-	-
Add Oxyma	0.5 M Oxyma in DMF	1.0	-	-
Coupling	-	-	10	100
Wash (x3)	DMF	2.0, 2.0 & 3.0	-	-

Coupling cycle for any standard amino acid (AA) not following Aib (0.1 mmol scale)

Operation	Reagent(s)	Volume (mL)	Time (min)	Temp (°C)
Fmoc deprotection	20% morpholine in DMF	7.0	2	90
Wash (x3)	DMF	4.0, 5.0 & 4.0	-	-
Fmoc deprotection	20% morpholine in DMF	7.0	2	90
Wash (x3)	DMF	4.0, 5.0 & 4.0	-	-
Add amino acid	0.2 M Fmoc-AA-OH in DMF	2.5	-	-
Add DIC	1.0 M DIC in DMF	1.0	-	-
Add Oxyma	0.5 M Oxyma in DMF	1.0	-	-
Coupling	-	-	3	90
Wash (x3)	DMF	2.0, 2.0 & 3.0	-	-

Final Deprotection cycle (0.1 mmol scale)

Operation	Reagent(s)	Volume (mL)	Time (min)	Temp (°C)
Fmoc deprotection	20% morpholine in DMF	5.0	1	90
Wash (x3)	DMF	5.0, 4.0 & 4.0	-	-
Fmoc deprotection	20% morpholine in DMF	5.0	1	90
Wash (x3)	DMF	5.0, 4.0 & 4.0	-	-

Peptide acetylation and cleavage took place in a fritted syringe with a stopcock tap. *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins at room temperature (rt). Cleavage from the resin was carried out with CH_2Cl_2:TFA:H_2O:TIPS (45:45:5:5 vol%, 15 mL) for peptide **1** and TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) for peptides **2** – **7** with inversion for 1 and 3 h respectively at rt. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins) and the precipitate dissolved in 1:1 MeCN:H_2O solution (5 mL), and lyophilized to give a white solid.

Peptide Purification

Crude peptides were purified by reverse-phase HPLC on a JASCO HPLC system equipped with a Phenomenex Luna C18 column (5 µm particle size; 100 Å pore size; 150 × 10 mm). A gradient of water (0.1 % TFA, buffer A) and acetonitrile (0.1 % TFA, buffer B) between 20 and 80 % or 40 and 100 % buffer B over 30 min at a flow rate of 3 mL min^{-1} with absorbance recorded at 220 and 280 nm was typically used. The fractions collected from HPLC were analysed by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Bruker UltraFlexXtreme II MALDI-TOF mass spectrometer operating in positive or negative-ion reflector mode. Masses reported are the most abundant mass of each species. Peptide purity was confirmed by reverse-phase analytical HPLC on a JASCO chromatography system fitted with a Phenomenex® Kinetex C18 (5 µM particle size; 100 x 4.5 mm) column.

Circular Dichroism Spectroscopy

Circular dichroism (CD) spectra were measured at 5 °C using a JASCO 810 spectropolarimeter fitted with a Peltier temperature controller, a 1 mm pathlength quartz cuvette (Starna), a scanning speed of 100 nm min^{-1}, and a bandwidth of 1 nm. Peptides were prepared at 100 µM concentration (150 µL) in phosphate buffered saline (PBS, 8.2 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.4). CD spectra shown are the averaged results of three replicate spectra.

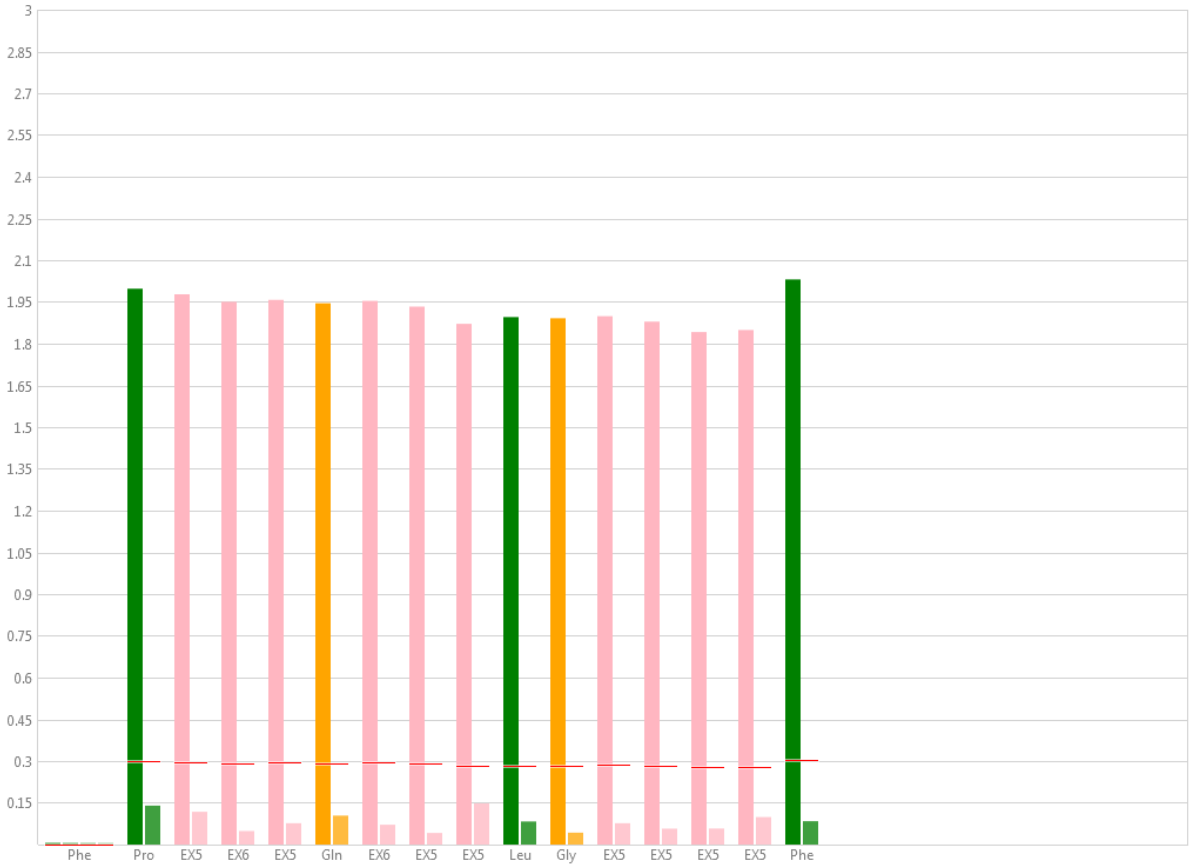
^{1}H NMR Spectra

^{1}H NMR spectra were recorded on a Bruker AVANCE (400 MHz) instrument as an average of 16 scans in CD_3OD.

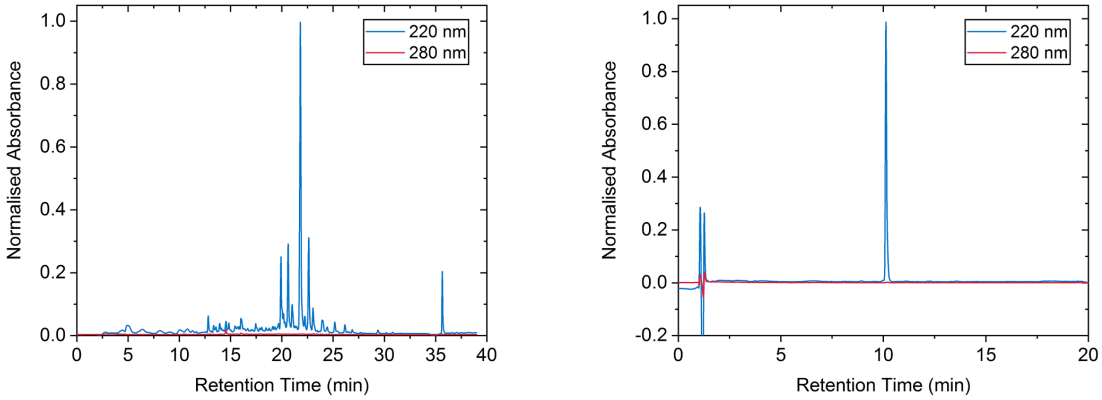
Synthetic Procedures and Analytical Data

Cephaibol D: Ac-Phe-Aib-Aib-Aib-Aib-Gly-Leu-Aib-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Pheol (**1**)

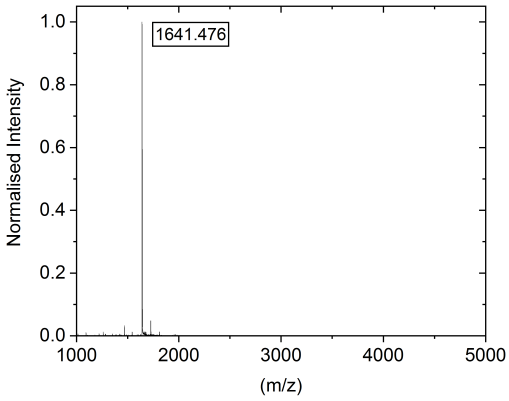
Peptide **1** was synthesised using a phenylalaninol preloaded 2-chlorotrityl chloride resin (0.1 mmol). *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with CH_2Cl_2:TFA:H_2O:TIPS (45:45:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (171.5 mg). 9.0 mg of the crude compound was dissolved in 2 mL 8:2 Buffer A:B and purified by semipreparative HPLC (20-80 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (2.2 mg, 26 %).



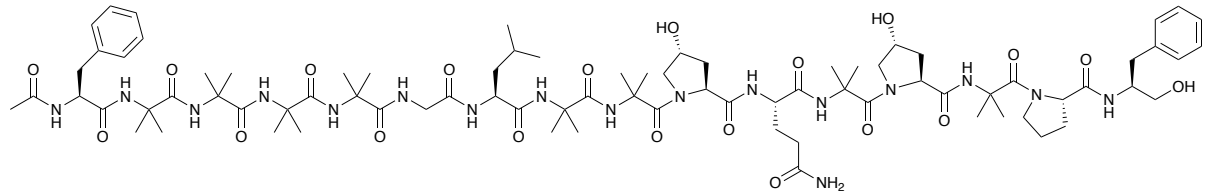
UV Trace from Fmoc removal during synthesis of Cephaibol D (**1**) (EX5 = Aib, EX6 = Hyp).

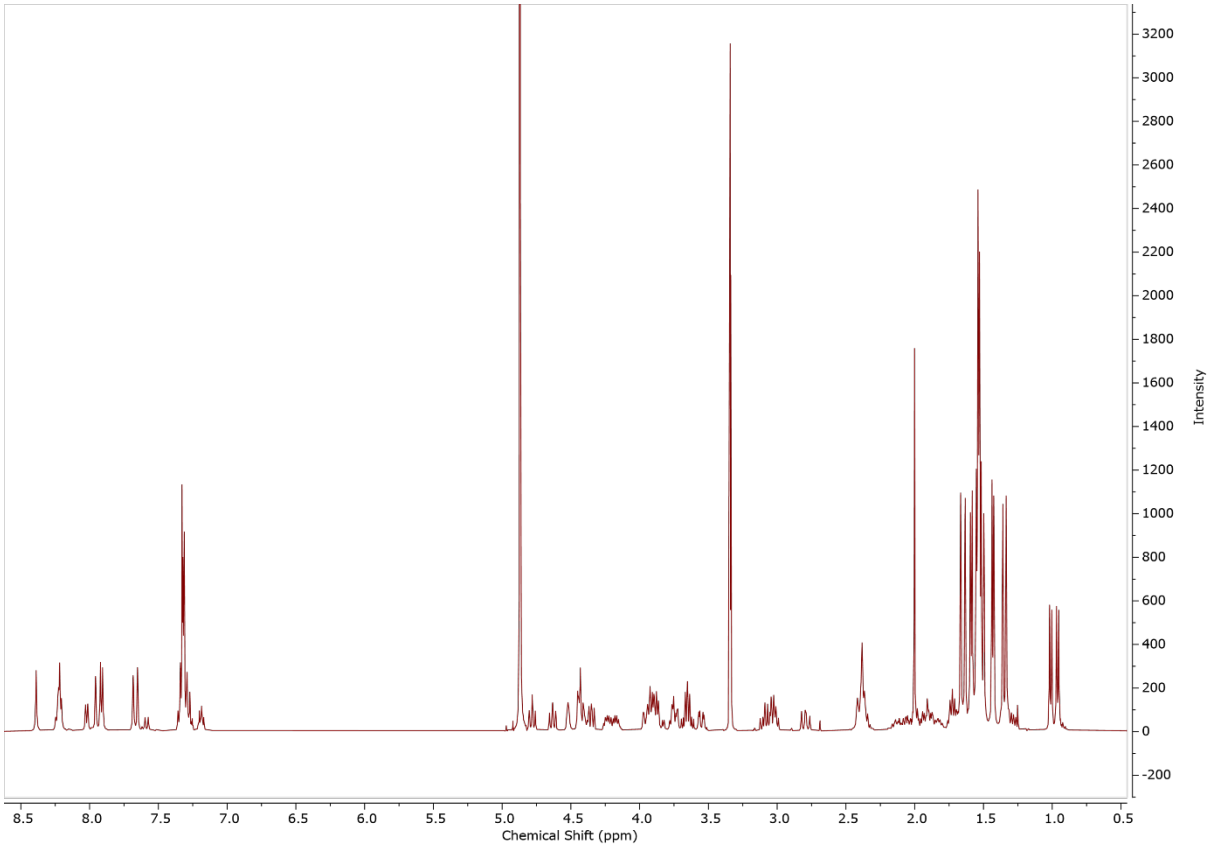


Semiprep HPLC trace of crude peptide(left) and analytical HPLC trace of pure peptide (right).

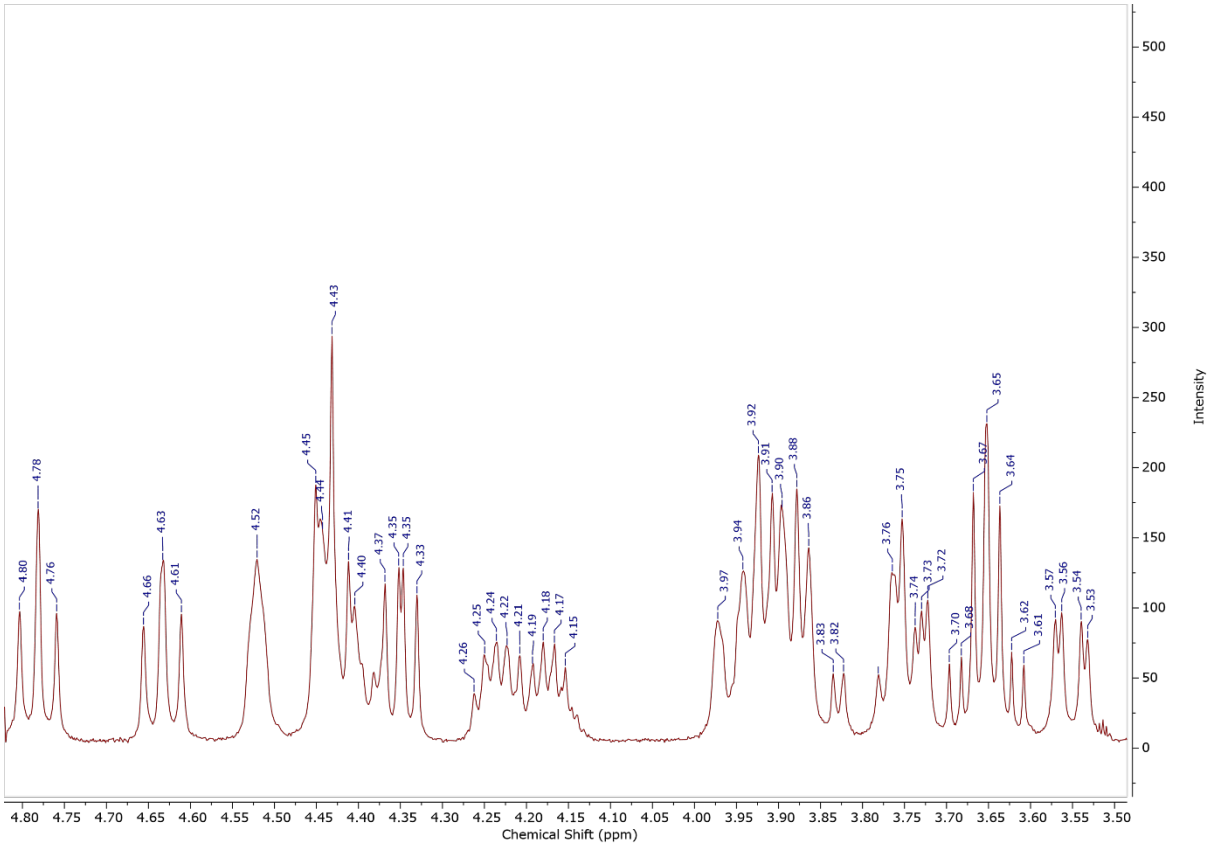


MALDI-TOF MS Trace. Calculated mass = 1641.91 Da $[M-H]^-$, observed mass = 1641.476 $[M-H]^-$.

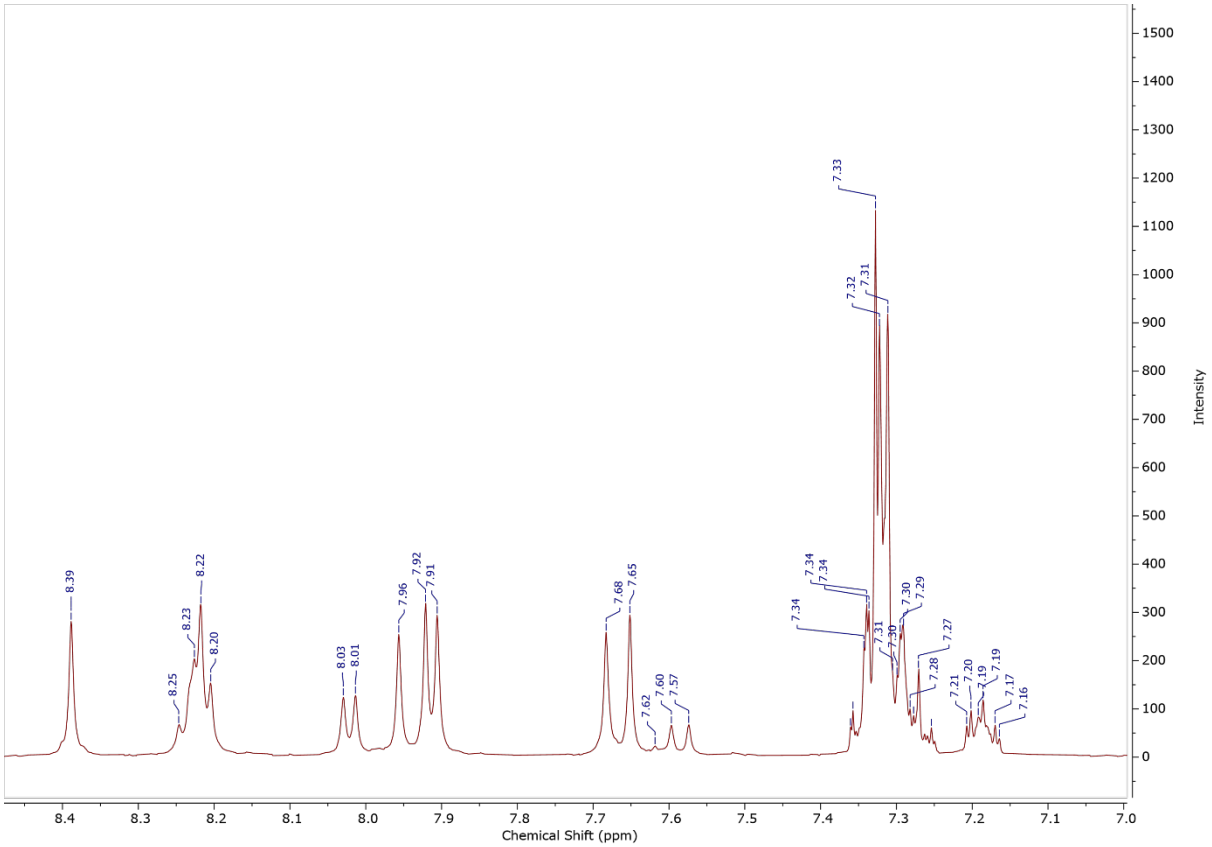




Full ^{1}H NMR spectrum of Cephaibol D (**1**).



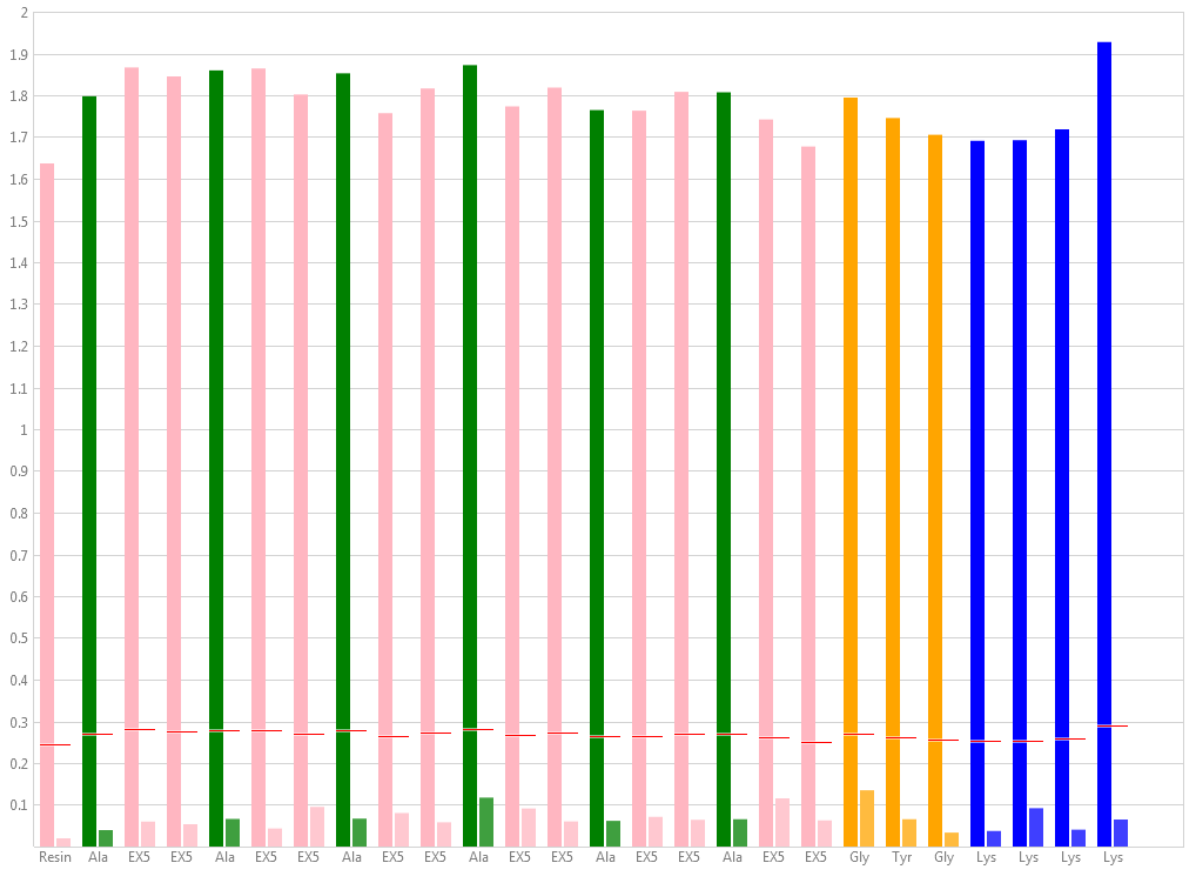
CH-α region



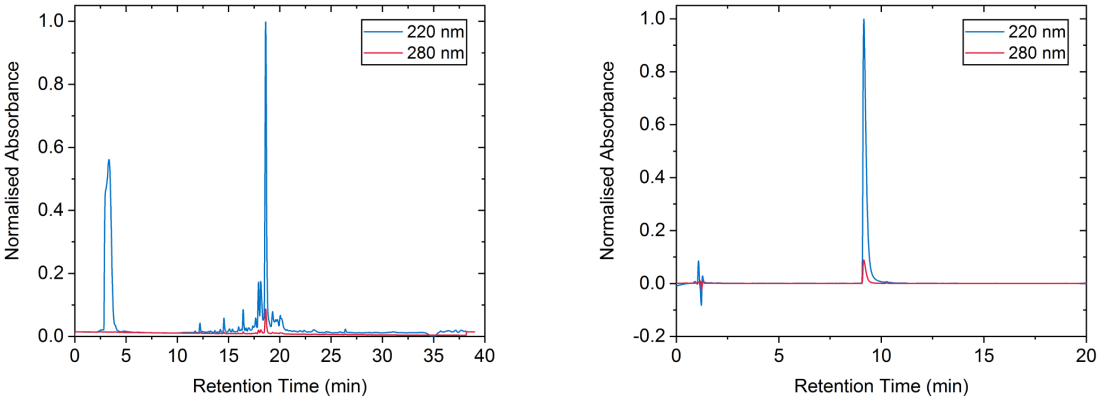
NH and phenylalaninol region

Ac-Lys-Lys-Lys-Lys-Gly-Tyr-Gly-Aib-Aib-Ala-Aib-Aib-Ala-Aib-Aib-Ala-Aib-Aib-Ala-Aib-Aib-Ala-Aib-Aib-Ala-NH_2 (**2**)

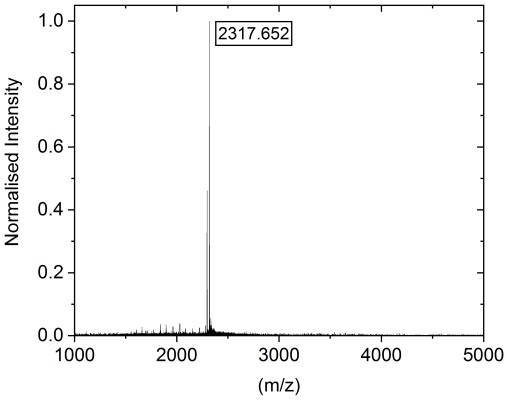
Peptide **2** was synthesised using Rink Amide MHBA resin (0.1 mmol) *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (353.3 mg). 8.5 mg of the crude compound was dissolved in 2 mL 8:2 Buffer A:B and purified by semipreparative HPLC (20-80 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (2.3 mg, 41 %).



UV Trace from Fmoc removal during synthesis of peptide **2** (EX5 = Aib).



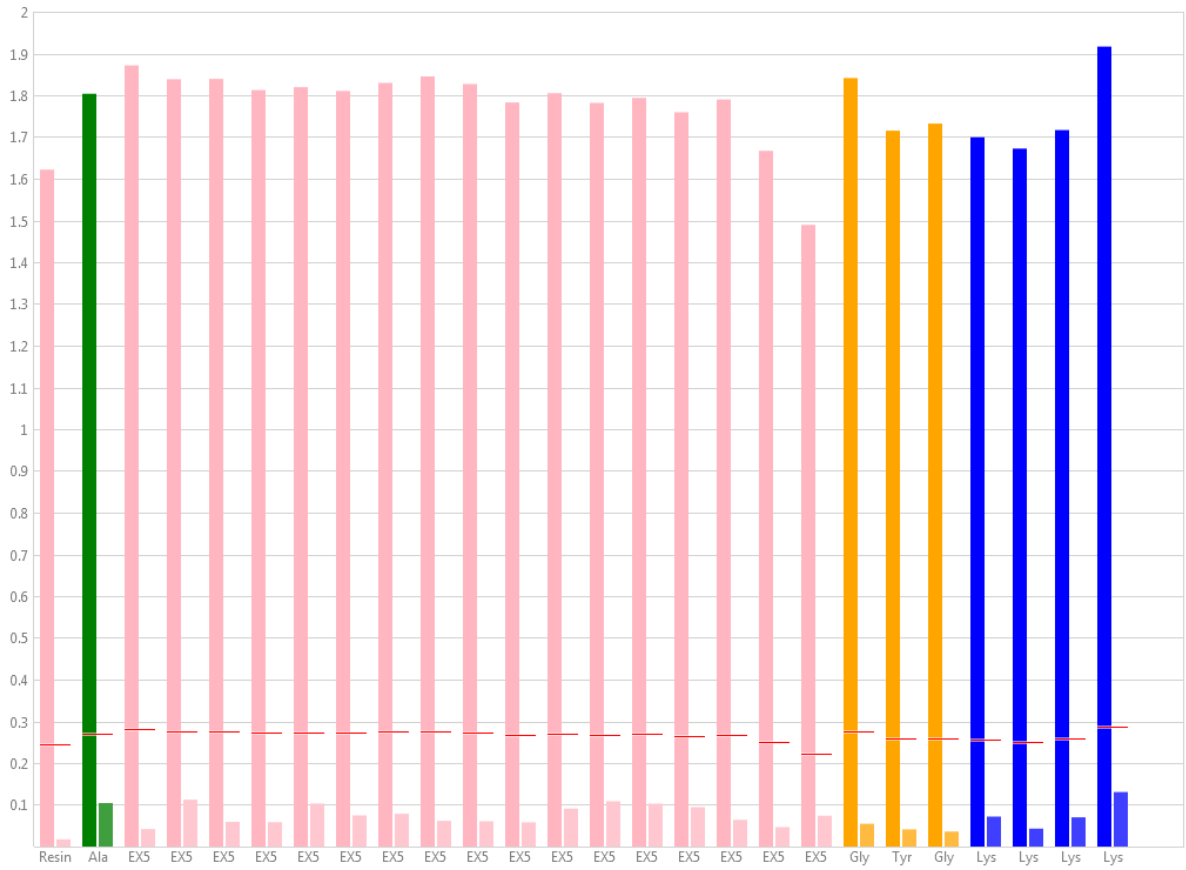
Semiprep HPLC trace of crude peptide (left) and analytical HPLC trace of pure peptide (right).



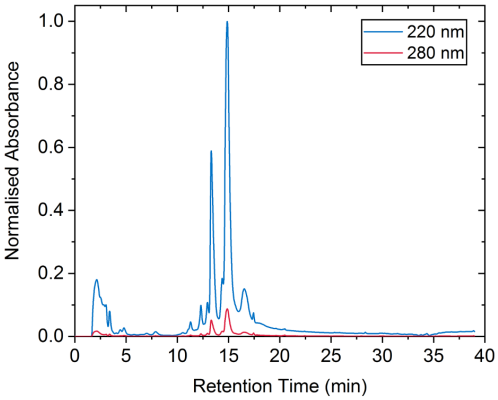
MALDI-TOF MS Trace. Calculated mass = 2317.38 Da $[M+Na]^+$, observed mass = 2317.652 $[M+Na]^+$.

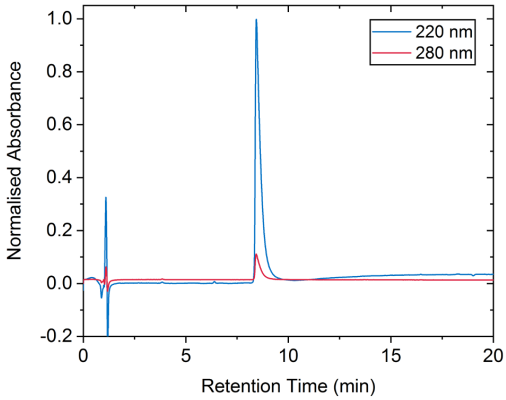
Ac-Lys-Lys-Lys-Lys-Gly-Tyr-Gly-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Ala-NH_2 (**3**)

Peptide **3** was synthesised using Rink Amide MHBA resin (0.1 mmol) *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (258.6 mg). 7.3 mg of the crude compound was dissolved in 2 mL 6:4 Buffer A:B and purified by semipreparative HPLC (40-100 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (1.4 mg, 21 %).

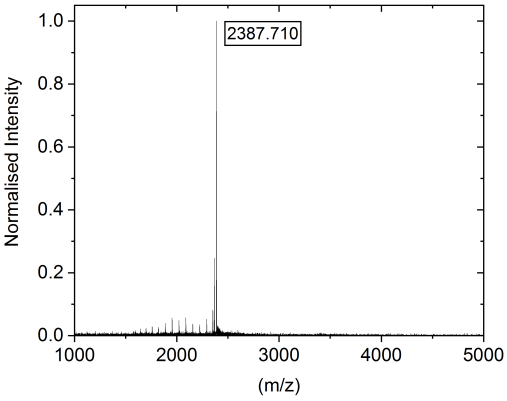


UV Trace from Fmoc removal during synthesis of peptide **3** (EX5 = Aib).





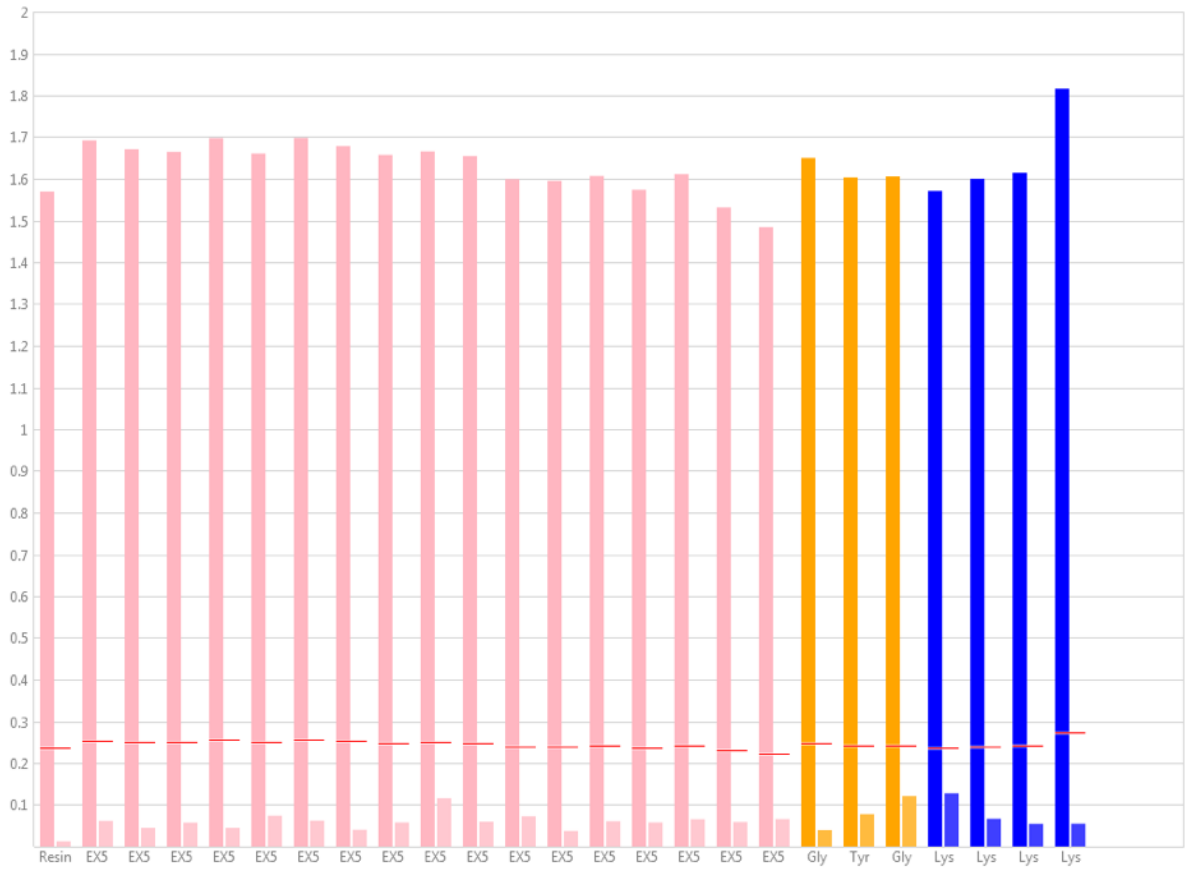
Semiprep HPLC trace of crude peptide (left) The significant peak at 13 mins corresponds to a single Aib deletion, with this same product arising from any one of the 17 coupling steps and analytical HPLC trace of pure peptide (right).



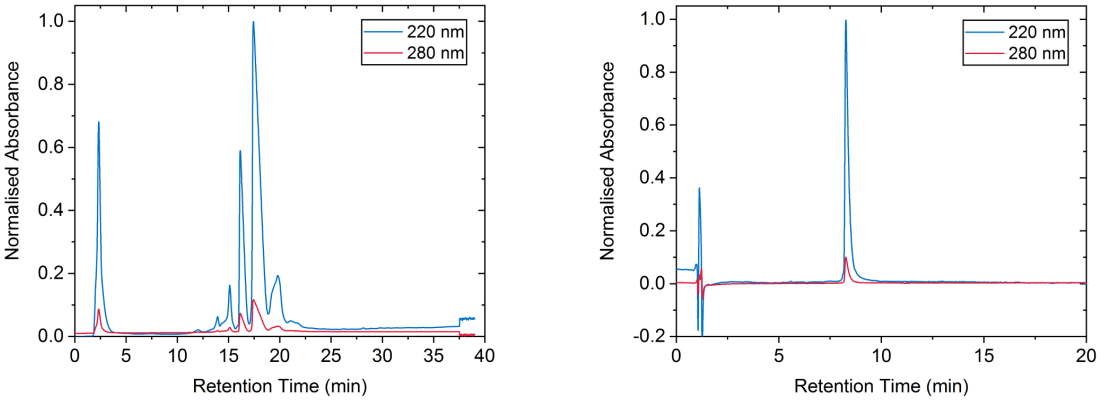
MALDI-TOF MS Trace. Calculated mass = 2387.46 Da $[M+Na]^+$, observed mass = 2387.710 $[M+Na]^+$.

Ac-Lys-Lys-Lys-Lys-Gly-Tyr-Gly-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-NH_2 (**4**)

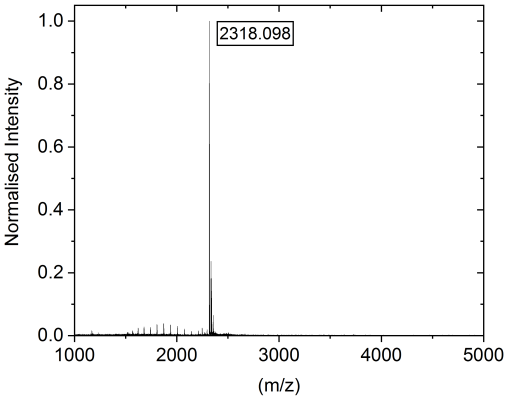
Peptide **4** was synthesised using Rink Amide MHBA resin (0.1 mmol) *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (157.0 mg). 9.9 mg of the crude compound was dissolved in 2 mL 6:4 Buffer A:B and purified by semipreparative HPLC (40-100 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (2.7 mg, 19 %).



UV Trace from Fmoc removal during synthesis of peptide **4** (EX5 = Aib).



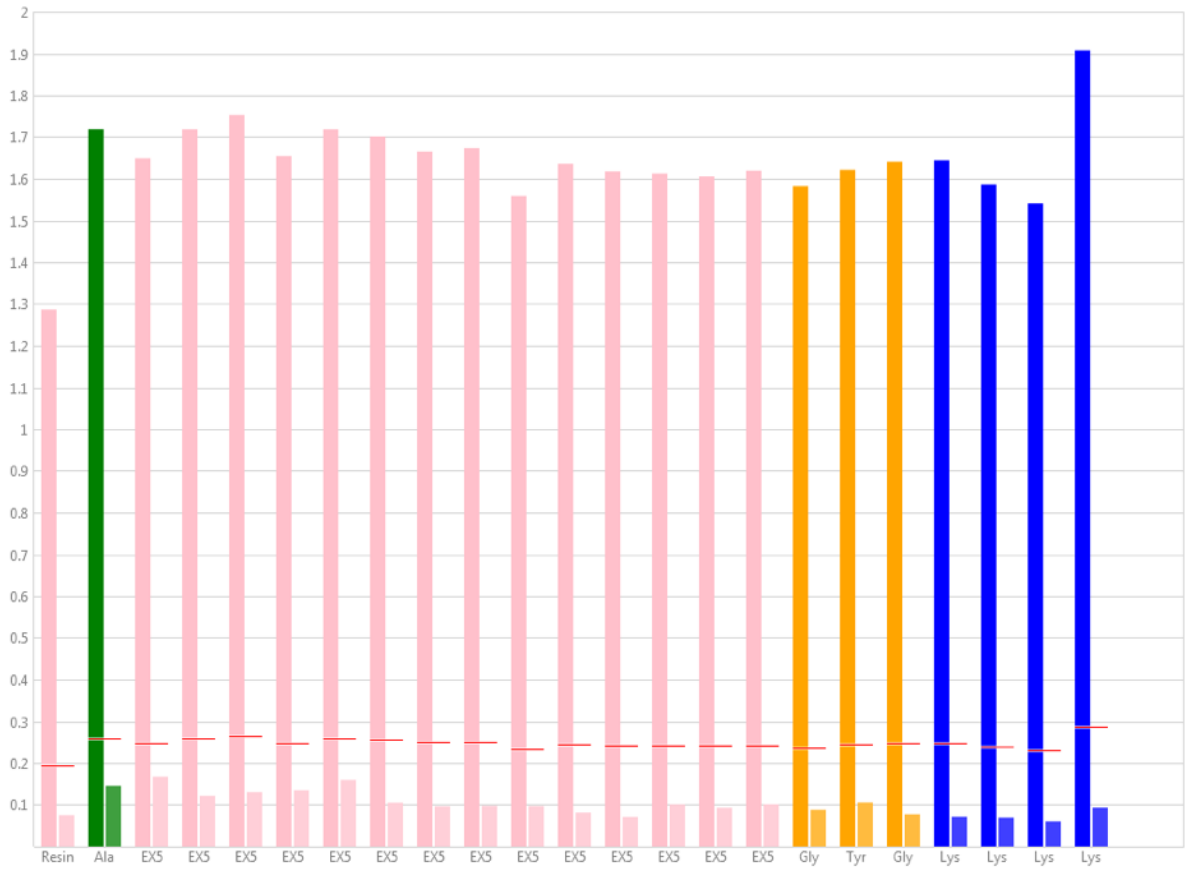
Semiprep HPLC trace of crude peptide (left) and analytical HPLC trace of pure peptide (right).



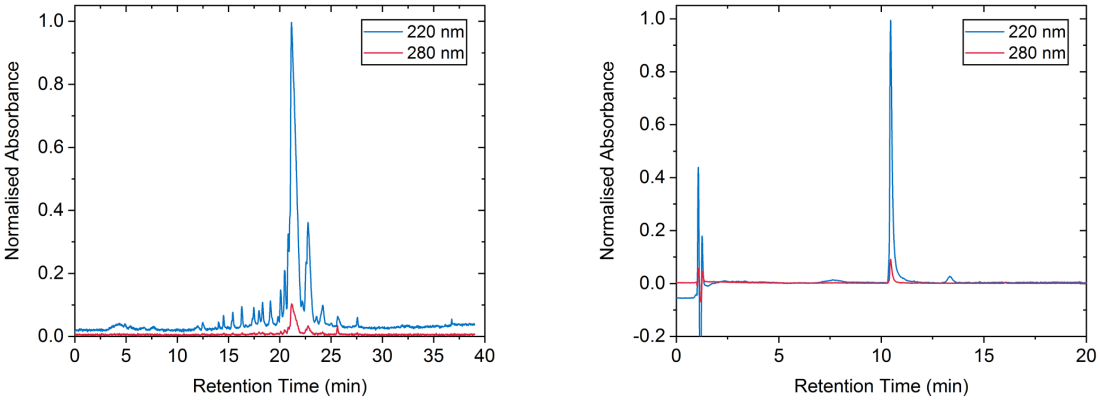
MALDI-TOF MS Trace. Calculated mass = 2317.38 Da $[M+Na]^+$, observed mass = 2318.098 $[M+Na]^+$.

Ac-Lys-Lys-Lys-Lys-Gly-Tyr-Gly-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Ala-NH_2 (**5**)

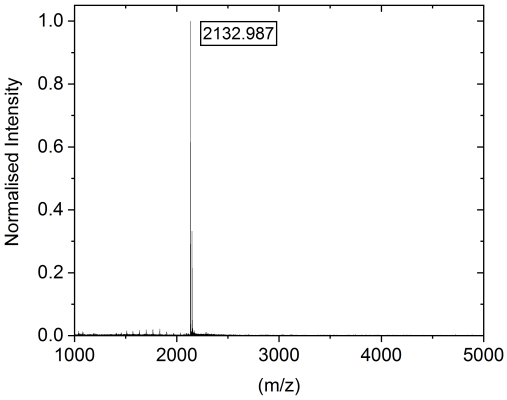
Peptide **5** was synthesised using Rink Amide MHBA resin (0.1 mmol) *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (202.2 mg). 9.0 mg of the crude compound was dissolved in 2 mL 8:2 Buffer A:B and purified by semipreparative HPLC (20-80 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (3.1 mg, 33 %).



UV Trace from Fmoc removal during synthesis of peptide **5** (EX5 = Aib).



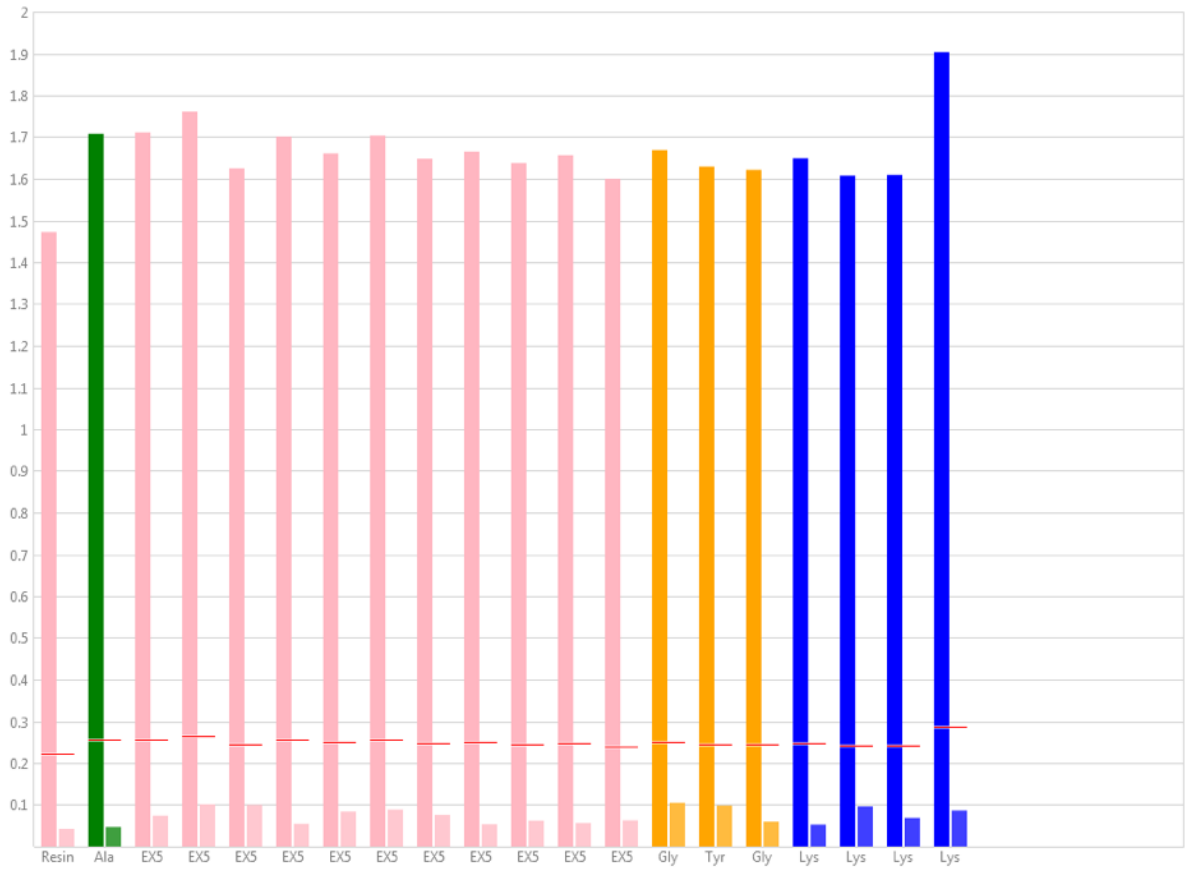
Semiprep HPLC trace of crude peptide (left) and analytical HPLC trace of pure peptide (right).



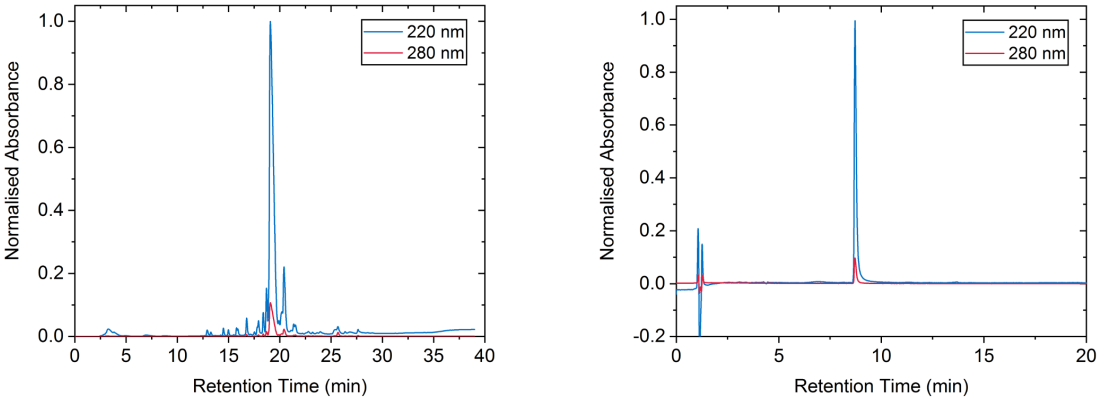
MALDI-TOF MS Trace. Calculated mass = 2133.30 Da $[M+Na]^+$, observed mass = 2132.987 $[M+Na]^+$.

Ac-Lys-Lys-Lys-Lys-Gly-Tyr-Gly-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Ala-NH_2 (**6**)

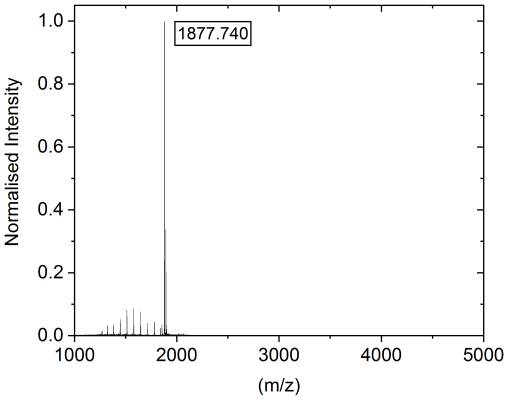
Peptide **6** was synthesised using Rink Amide MHBA resin (0.1 mmol) *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (209.8 mg). 9.1 mg of the crude compound was dissolved in 2 mL 8:2 Buffer A:B and purified by semipreparative HPLC (20-80 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (3.8 mg, 55 %).



UV Trace from Fmoc removal during synthesis of peptide **6** (EX5 = Aib).



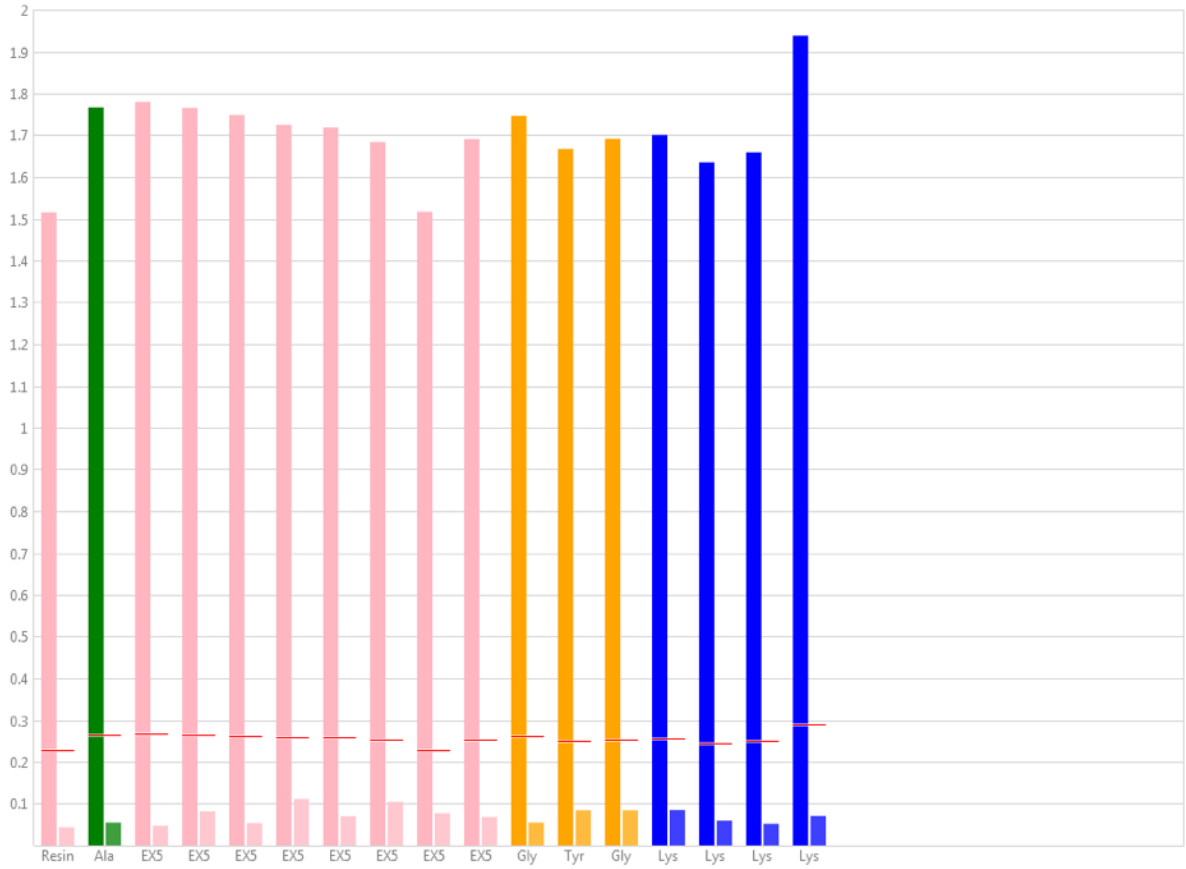
Semiprep HPLC trace of crude peptide (left) and analytical HPLC trace of pure peptide (right).



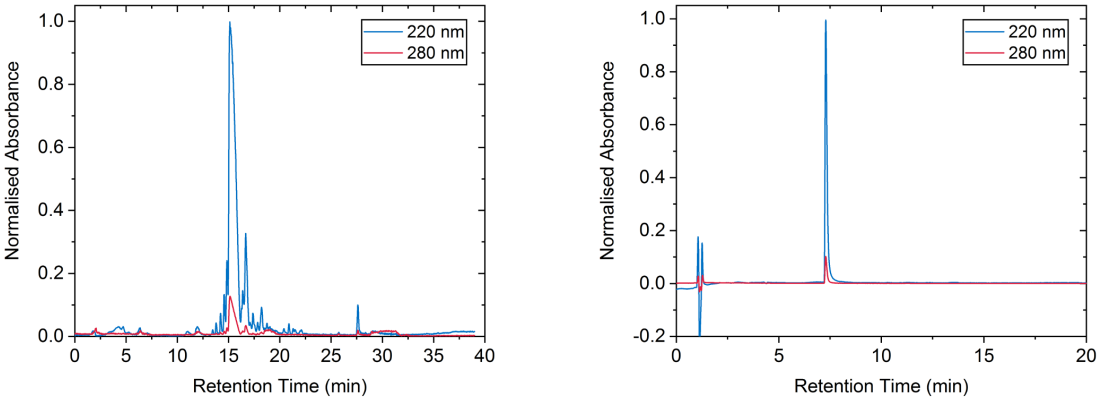
MALDI-TOF MS Trace. Calculated mass = 1878.14 Da $[M+Na]^+$, observed mass = 1877.740 $[M+Na]^+$.

Ac-Lys-Lys-Lys-Lys-Gly-Tyr-Gly-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Aib-Ala-NH_2 (**7**)

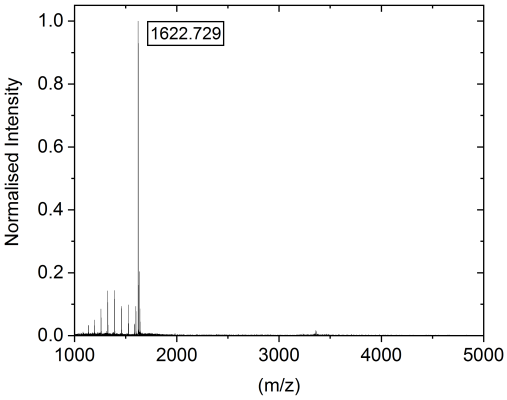
Peptide **7** was synthesised using Rink Amide MHBA resin (0.1 mmol) *N*-acetylation was carried out using excess Ac_2O (1 mL) and DIPEA (2 mL) in DMF (7 mL) with inversion for 30 mins. Cleavage from the resin was carried out with TFA:H_2O:TIPS (90:5:5 vol%, 15 mL) with inversion for 3 h. Following cleavage, the TFA solution was reduced to ~5 mL under a flow of nitrogen. The crude peptides were precipitated by cold Et_2O (~30 mL) and isolated by refrigerated centrifugation (3000 rpm, 10 mins). The precipitate dissolved in 1:1 MeCN:H_2O solution, 5 mL and lyophilized to give a white solid (185.2 mg). 10.0 mg of the crude compound was dissolved in 2 mL 8:2 Buffer A:B and purified by semipreparative HPLC (20-80 % buffer B in A). The pure fractions were combined and lyophilized to give the pure peptide (5.2 mg, 52 %).



UV Trace from Fmoc removal during synthesis of peptide **7** (EX5 = Aib).



Semiprep HPLC trace of crude peptide (left) and analytical HPLC trace of pure peptide (right).



MALDI-TOF MS Trace. Calculated mass = 1622.98 Da $[M+Na]^+$, observed mass = 1622.729 $[M+Na]^+$.

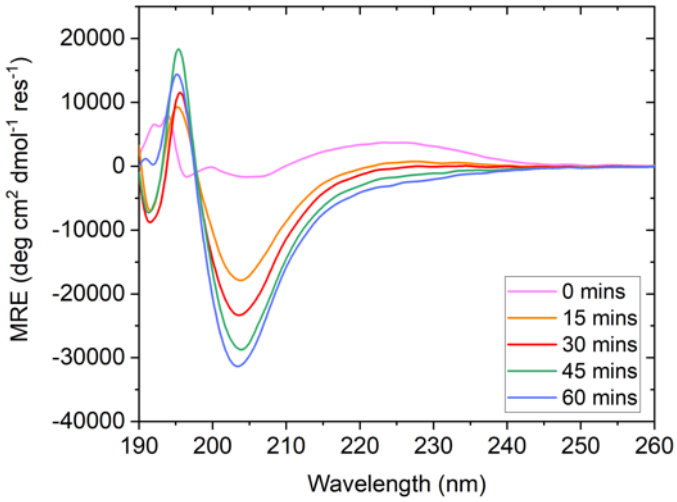


Figure S1: Time dependent CD spectrum of peptide **5**. The legend denotes time sample was kept at room temperature until spectra run at 5 °C (100 µM peptide, 1 mm pathlength, PBS, pH 7.4).