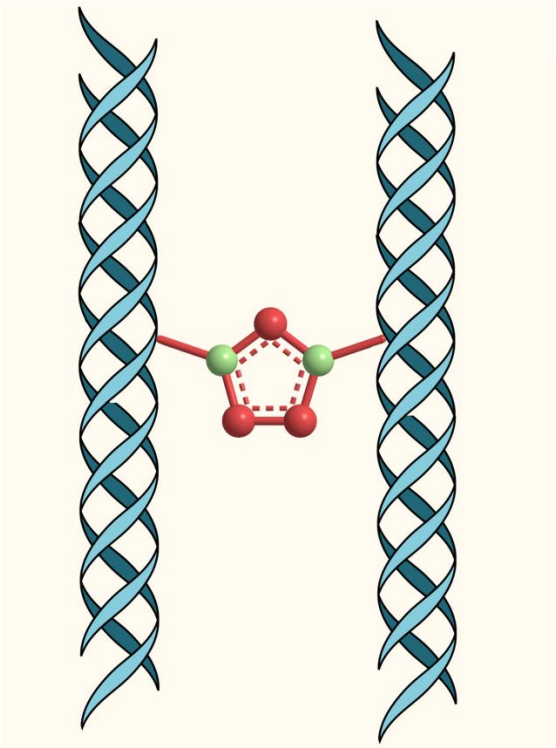
Electronic Supplementary Material (ESI) for ChemComm.



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Site-Specific Cross-linking of Collagen Peptides by Lysyl Advanced Glycation Endproducts

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Supporting Information

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* image adapted from http://commons.wikimedia.org/wiki/File:Tropokollagen_Quervernetzung_Lysyl_Oxidase_%28DE%29.svg

Procedures for Building Block Syntheses

General Remarks

Unless stated, all solvents and reagents were used as supplied from commercial sources. Analytical thin-layer chromatography (TLC) was performed using Kieselgel F254 0.2 mm (Merck) silica plates with visualisation by ultraviolet irradiation (254 nm) followed by staining with potassium permanganate. Flash chromatography was performed using Kieselgel S63-100 µm (Riedel-de-Hahn) silica gel. The solvent compositions reported for all chromatographic separations are on a volume/volume (v/v) basis. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. ^{1}H-NMR spectra were recorded on a 400 MHz Bruker spectrometer and are in parts per million (ppm) on the δ scale relative to d_6-DMSO and ^{13}C-NMR spectra were recorded on 100 MHz Bruker spectrometer and are in parts per million (ppm) on the δ scale relative to d_6-DMSO. The multiplicities of ^{1}H signals are designated by the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad; dd = doublet of doublets; dt = doublet of triplets; dm = doublet of multiplets. All coupling constants J= are reported in hertz. All ^{13}C-NMR spectra were acquired using broadband decoupled mode, and assignments were determined using DEPT sequences. High resolution mass spectra (HRMS) were obtained by electrospray ionisation using a Bruker MicrOTOF-Q II mass spectrometer. Infrared (IR) spectra were recorded as a thin film on a composite zinc selenide and diamond crystal on FT-IR System transform spectrometer.

$Fmoc_2GOLD$ – 2

A solution of formaldehyde in water (37%, 0.660 ml, 8.1 mmol, 2 eq) and a solution of glyoxal in water (40%, 1.2 ml, 8.1 mmol, 2 eq) were added to a round bottomed flask containing Fmoc-Lys-OH (1.5 g, 4.07 mmol, 1 eq) in 5 M AcOH in water/CH_3CN (1:1, 50 ml). The reaction mixture was stirred for 3 h at 50 °C, and partitioned between ethyl acetate (50 ml) and water and the layers separated. The aqueous layer was extracted with ethyl acetate (2 x 40 ml) and the combined extracts washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (0.1% AcOH in EtOAc followed by 5:95 MeOH/CH_2Cl_2 with 0.1% TFA) afforded the title compound as a white solid (1.01g, 1.31 mmol, 65%). TLC R_f = 0.12 (5:95 MeOH/CH_2Cl_2 with 0.1% TFA). $[\alpha]_D^{22}$ = -26.4 ° (c = 2.5 mg/ml in MeOH). M.P. = 75 – 77 °C. IR (ATR) v 3332, 3067, 2947, 1707, 1650, 1522, 1450, 1410, 1333, 1177, 1083, 1043 cm^{-1}. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.18 (s, 1H), 7.89 (d, J = 7.5 Hz, 4H), 7.77 (s, 2H), 7.71 (dd, J = 7.5, 4.7 Hz, 4H), 7.63

(d, J = 8.1 Hz, 2H), 7.42 (t, J = 7.4 Hz, 4H), 7.32 (t, J = 7.4 Hz, 4H), 4.37 – 4.18 (m, 6H), 4.13 (t, J = 7.2 Hz, 4H), 3.94 (td, J = 8.8, 4.7 Hz, 2H), 1.86 – 1.55 (m, 8H), 1.40 – 1.24 (m, 4H). δ. ^{13}C NMR (100 MHz, d_6-DMSO) δ 173.76, 156.15, 143.81, 143.75, 140.71, 135.94, 127.63, 127.04, 125.21, 122.39, 120.11, 65.57, 53.55, 48.58, 46.65, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 30.05, 28.88, 22.27. HRMS (ESI^+) found M^+ 771.3368, $C_{45}H_{47}N_4O_8$ calculated M^+ 771.3388.

$Fmoc_2MOLD$ – 3

A solution of formaldehyde in water (37%, 0.660 ml, 8.1 mmol, 2 eq) and a solution of methylglyoxal in water (40%, 1.6 ml, 8.1 mmol, 2 eq) were added to a round bottomed flask containing Fmoc-Lys-OH (1.5 g, 4.07 mmol, 1 eq) in 5 M AcOH in water/CH_3CN (1:1; 50 ml). The reaction mixture was stirred for 3 h at 50 °C, and partitioned between ethyl acetate (50 ml) and water and the layers separated. The aqueous layer was extracted with ethyl acetate (2 x 40 ml) and the combined extracts washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (0.1% AcOH in EtOAc followed by 5:95 MeOH/CH_2Cl_2 with 0.1% TFA) afforded the title compound as a pale yellow solid (1.19 g, 1.52 mmol, 75%). TLC R_f = 0.12 (5:95 MeOH/CH_2Cl_2 with 0.1% TFA). $[\alpha]_D^{22}$ = -23.3 ° (c = 3.6 mg/ml in MeOH). MP = 75 – 77 °C. IR (ATR) v 3333, 3067, 2948, 1701, 1655, 1522, 1450, 1410, 1333, 1179, 1083, 1043 cm^{-1}. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.09 (d, *J* = 1.7 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 4H), 7.71 (dd, *J* = 7.7, 3.8 Hz, 4H), 7.66 – 7.58 (m, 2H), 7.51 (s, 1H), 7.47 – 7.37 (m, 4H), 7.32 (t, *J* = 7.5 Hz, 4H), 4.37 – 4.16 (m, 6H), 4.13 – 3.87 (m, 6H), 2.25 (s, 3H), 1.87 – 1.50 (m, 8H), 1.45 – 1.21 (m, 4H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 173.76, 156.15, 143.81, 143.75, 140.71, 135.94, 127.63, 127.04, 125.21, 122.39, 120.11, 65.57, 53.55, 48.58, 46.65, 40.15, 39.94, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 30.05, 28.88, 22.27. HRMS (ESI^+) found M^+ 785.3519, $C_{46}H_{49}N_4O_8$ calculated M^+ 785.3545.

^{1}H and ^{13}C NMR Spectra of Building Blocks

$Fmoc_2GOLD$ – 2

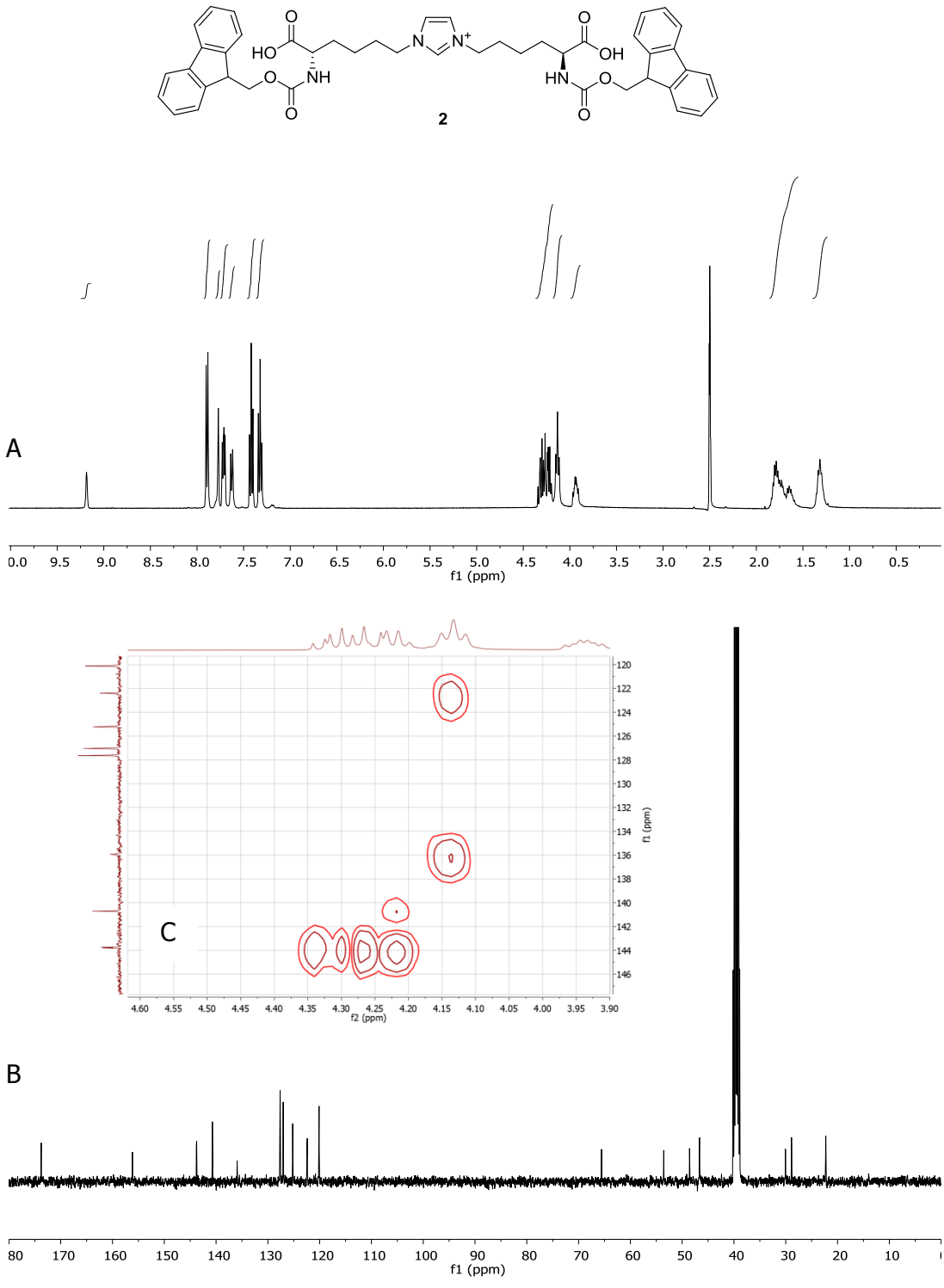

Figure SI-1: ^{1}H (A) and ^{13}C (B) NMR spectra of **2**. Heteronuclear multiple-bound coupling spectrum (C) shows coupling between imidazole carbons at 136 ppm, 122 ppm and the lysyl ε-hydrogens at 3.9 ppm.

$Fmoc_2MOLD$ – 3

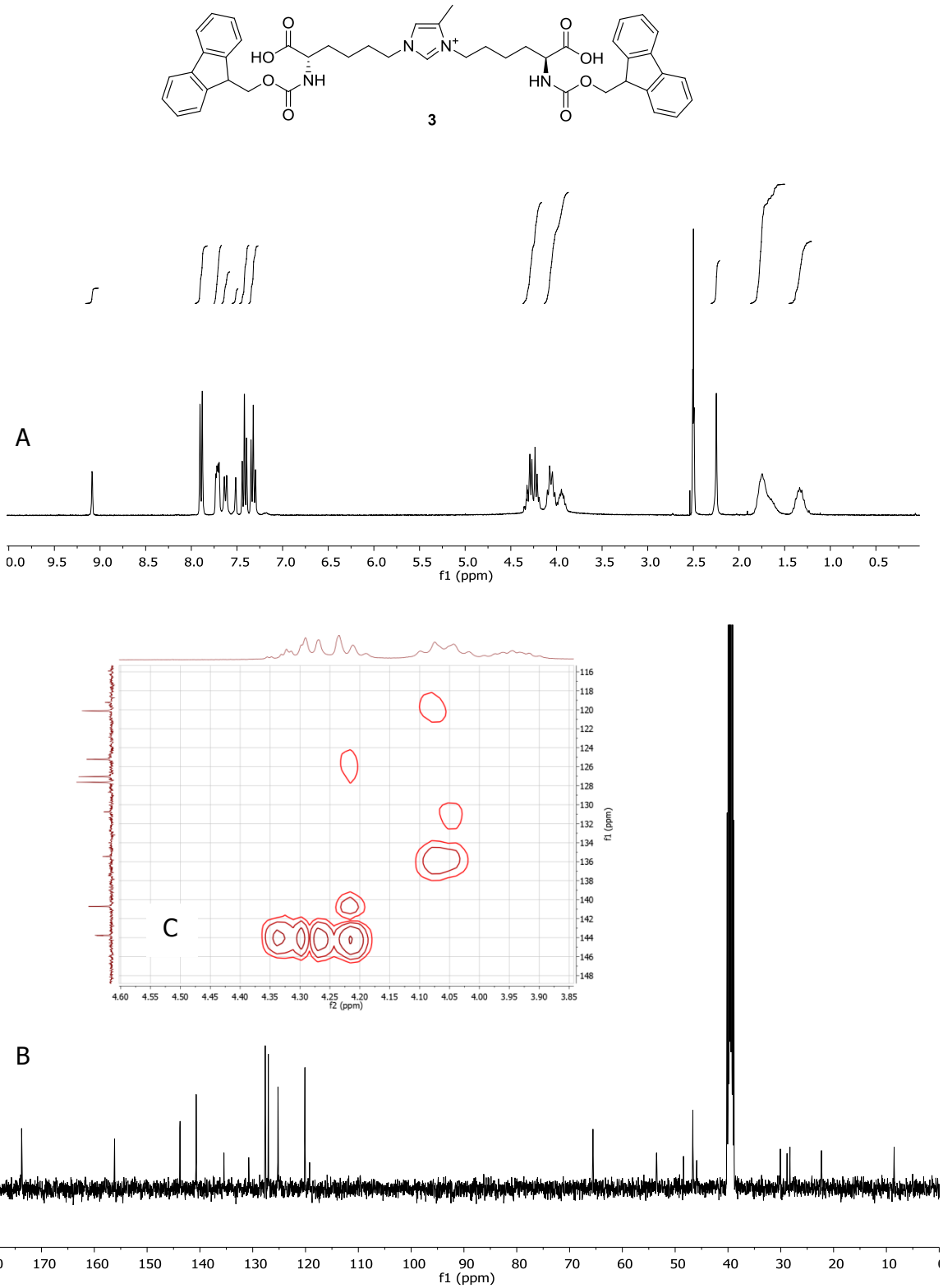

Figure SI-2: ^{1}H (A) and ^{13}C (B) NMR spectra of **3**. Heteronuclear multiple-bound coupling spectrum (C) shows coupling between the methylimidazole carbon at 136 ppm and the lysyl ε-hydrogens at 4.0 ppm.

Procedures for Peptide Syntheses

General Remarks

All solvents and reagents were used as supplied. Standard solid phase synthesis was performed manually using the Fmoc strategy on aminomethyl resin equipped with a Rink Amide linker. The Fmoc group was deprotected with 20% v/v piperidine in DMF for 15 min. The coupling step was performed with 5 eq of Fmoc-protected amino acid in DMF (0.2 M), 4.5 eq HATU in DMF (0.45 M) and 10 equivalents of DIPEA. Unless otherwise stated, couplings were performed for 25 min at rt. Following completion of the sequence, peptides were released from resin with concomitant removal of the side-chain protecting groups by treatment with TFA/TIS/H_2O (38/1/1, v/v/v) at rt for 2 h. The crude peptides were precipitated with cold diethyl ether, isolated by centrifugation, washed in cold diethyl ether, dissolved in 1:1 (v/v) MeCN/H_2O containing 0.1% TFA and lyophilised. The peptides were analysed for purity by LC-MS (Agilent 1120 compact LC system equipped with Agilent 6120 Quadrupole MS and a UV detector at 214 nm) using a Zorbax C3 column (3.5 µ; 250 x 10 mm; Agilent) at 0.3 ml/min using a linear gradient. The solvent system used was A (0.1% formic acid in H_2O) and B (0.1% formic acid in MeCN). The same conditions were employed in the analysis of the purified peptides and the masses confirmed by MS using ESI in the positive mode.

Purification of crude peptides was performed by semipreparative RP-HPLC (Dionex Ultimate 3000 equipped with a 4 channel UV detector) at 210, 230, 254, and 280 nm using a Gemini C18 column (10 µ; 250 x 10 mm; Phenomenex) at 5 ml/min using a shallow linear gradient. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN).

Coupling Optimisation of 2 and 4

Resin-bound peptide **4** (5 µmol, 1 eq) was swollen in CH_2Cl_2 for 30 min and subsequently in DMF for 10 min. Coupling mixtures of building block **2** and the appropriate coupling agents were prepared in 100 µl of DMF, activated with 10 eq of DIPEA, and added to the resin. The reaction mixtures were shaken overnight, filtered, Fmoc-deprotected in 20% piperidine/DMF and coupled with Fmoc-Glu(O^tBu)-OH using standard conditions described in general remarks. The resulting peptide **5** along with all coupling by-products was cleaved, precipitated in ether, washed with ether, lyophilised, and analysed by LC-MS (Figure SI-3).

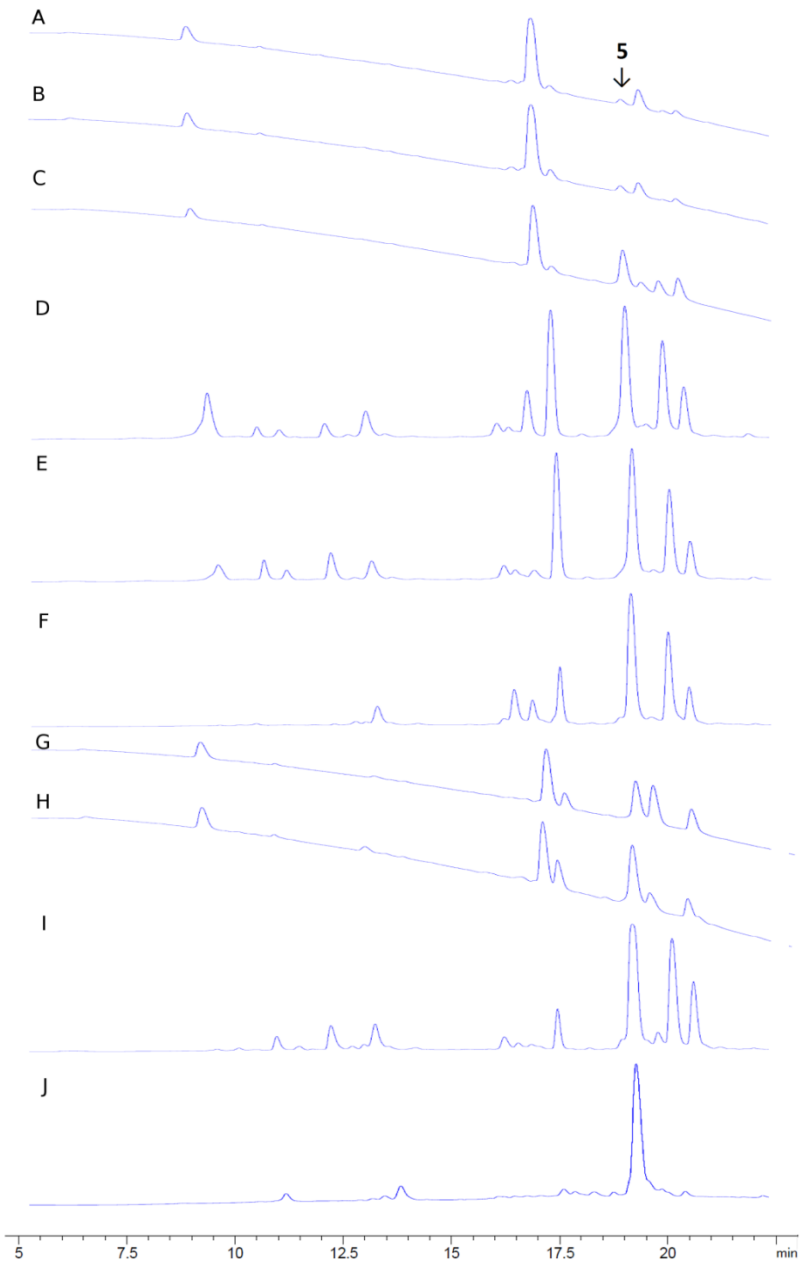

Figure SI-3: HPLC profiles of product mixtures from various couplings of **2** to **4** followed by coupling of Fmoc-Glu(OtBu)-OH. **A:** 2 eq of HOAt, 2 eq of DIC, 1 eq of **2**, 2 eq of **4**, resin loading (rl) = 0.9 mmol/g; **B:** 2 eq of Oxyma, 2 eq of DIC, 1 eq of **2**, 2 eq of **4**, rl = 0.9 mmol/g; **C:** 2 eq of PyBOP, 1 eq of **2**, 2 eq of **4**, rl = 0.9 mmol/g; **D:** 2 eq of PyBOP, 1 eq of **2**, 2 eq of **4**, rl = 0.35 mmol/g; **E:** 2 eq of PyBOP, 1 eq of **2**, 2 eq of **4**, rl = 0.09 mmol/g; **F:** 2 eq of PyBOP, 1 eq of **2**, 1 eq of **4**, rl = 0.09 mmol/g; **G:** 2 eq of HBTU, 1 eq of **2**, 2 eq of **4**, rl = 0.9 mmol/g; **H:** 2 eq of HATU, 1 eq of **2**, 2 eq of **4**, rl = 0.9 mmol/g; **I:** 2 eq of HATU, 1 eq of **2**, 1 eq of **4**, rl = 0.09 mmol/g; **J:** 2.2 eq of HATU, 1 eq of **2**, 2 eq of **4**, rl = 0.09 mmol/g.

Optimised conditions for coupling GOLD and MOLD:

Building block (~ 43 mg, 0.055 mmol, 1.1 eq) and HATU (42 mg, 0.11 mmol, 2.2 eq) were dissolved in a minimal amount of DMF, and the mixture was added to the resin-bound peptide (125 mg, 0.1 mmol, 2 eq, 0.09 mmol/g loading). DIPEA (175 µL, 1 mmol, 20 eq) was then added in one portion and the reaction mixture shaken for 3 h. Completion of the coupling was checked by the Kaiser test and the resulting peptide was subjected to further SPPS using standard conditions.

Peptide Chromatograms and Mass Spectra

Native CTP

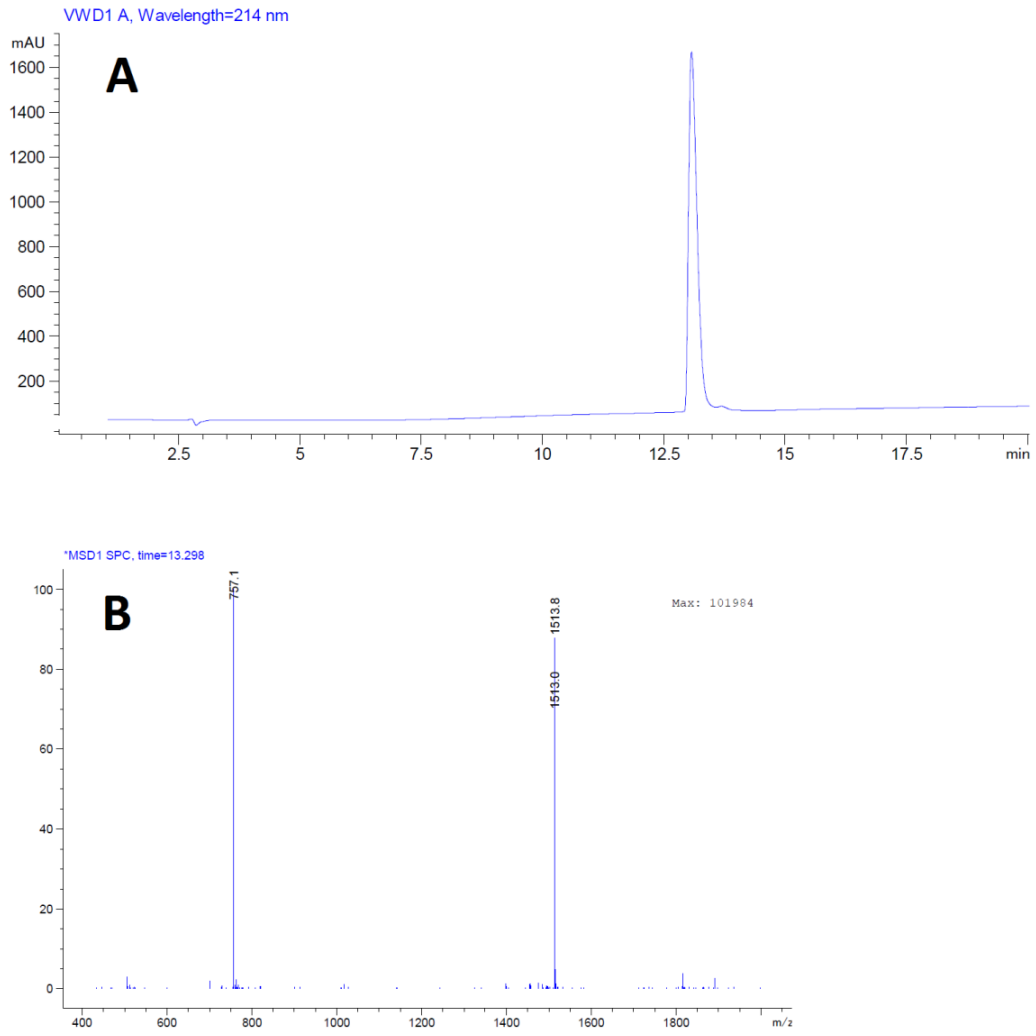

Figure SI-4: HPLC chromatogram of the purified native CTP (A), ESI MS of the major peak (B) ($C_{67}H_{100}N_{16}O_{24}$ $[M+H]^+$ calc. 1513.7, obs. 1513.8).

CMP-K

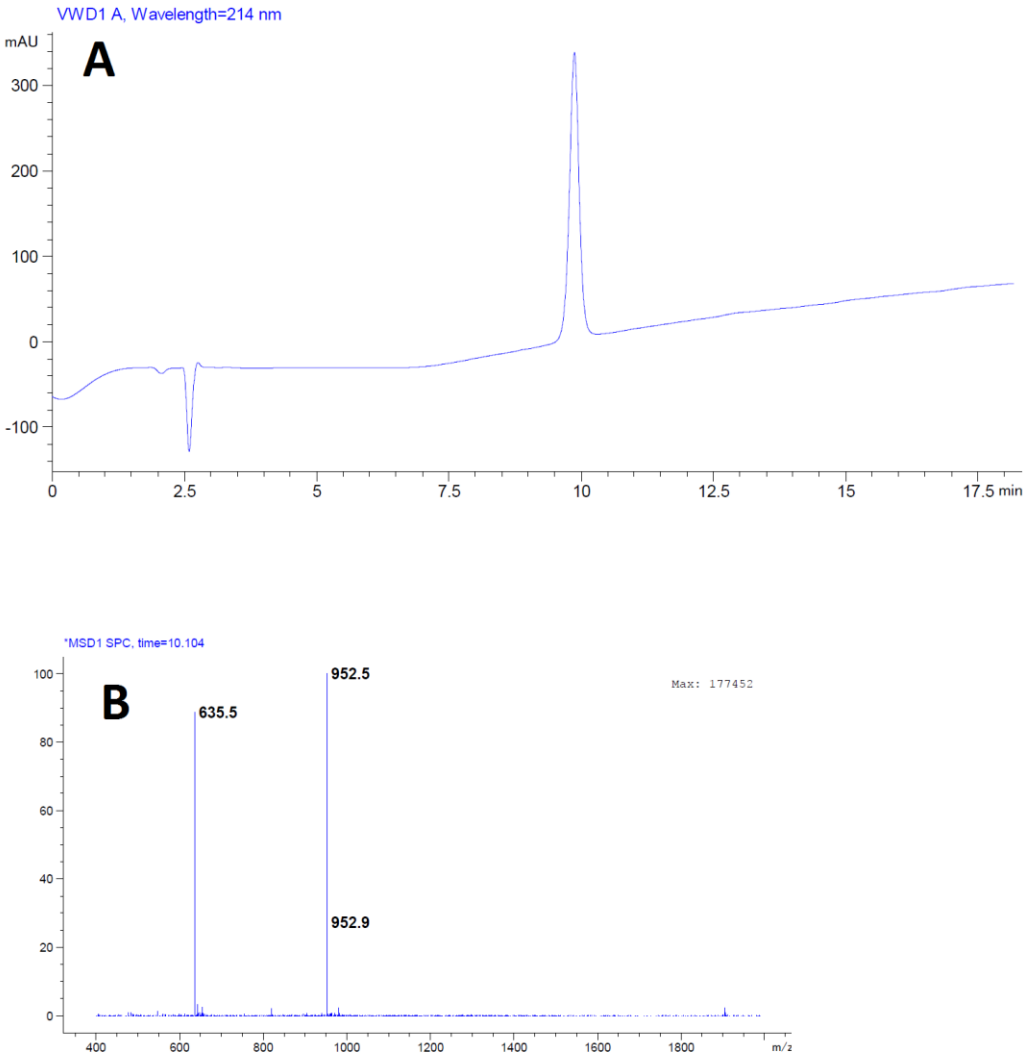

Figure SI-5: HPLC chromatograms of the purified CMP-K (A), ESI MS of the major peak (B) ($C_{85}H_{126}N_{22}O_{28}^{+}$ $[M+2H]^{2+}$ calc. 952.5, obs. 952.5).

CTP-GOLD – 7

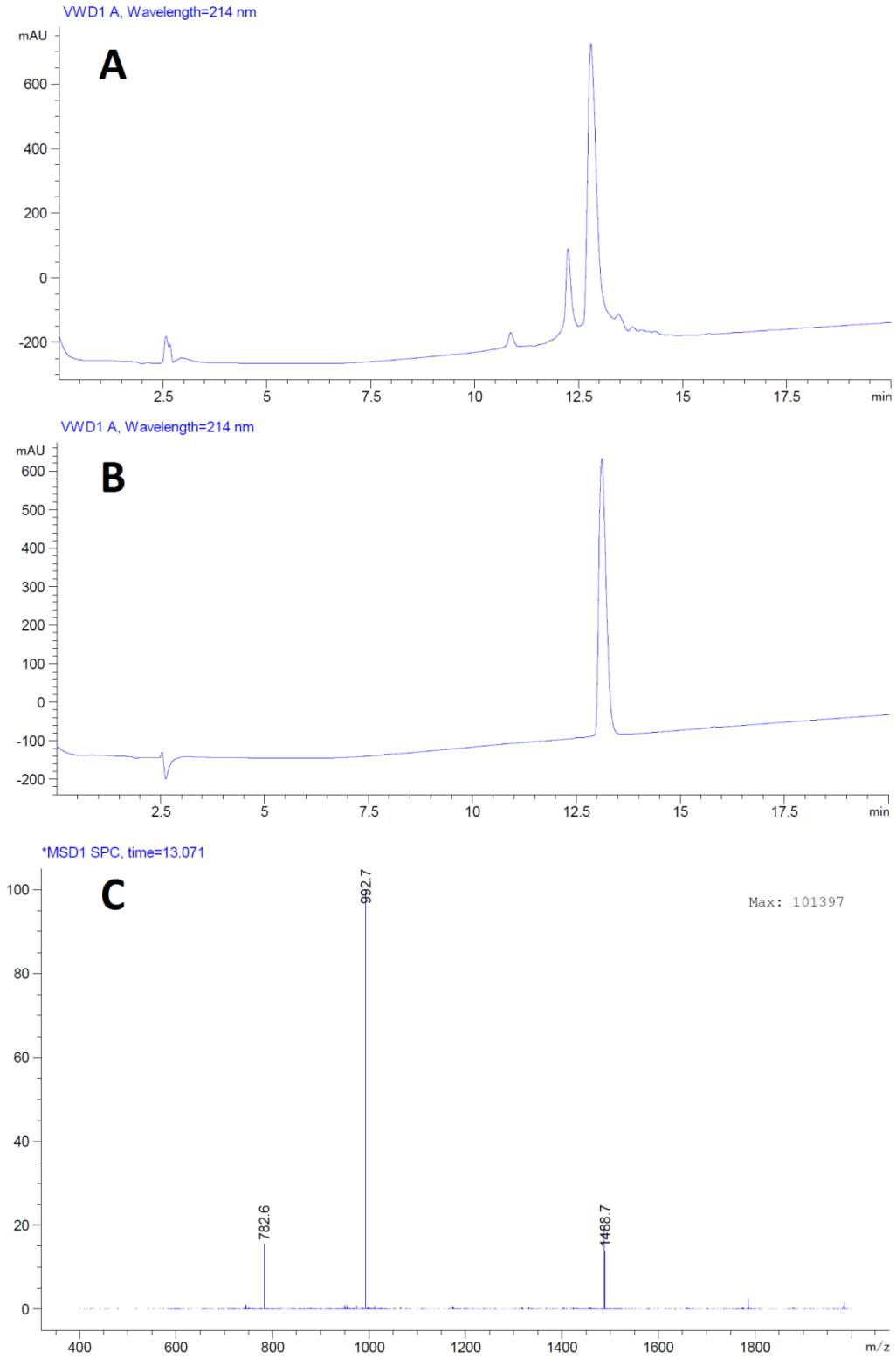

Figure SI-6: HPLC chromatograms of crude (A) and purified (B) peptide **7**, ESI MS of the major peak of **7** (C) ($C_{133}H_{195}N_{32}O_{46}^{+}$ $[M+H]^{2+}$ calc. 1488.7, obs. 1489.0).

CTP-MOLD – 8

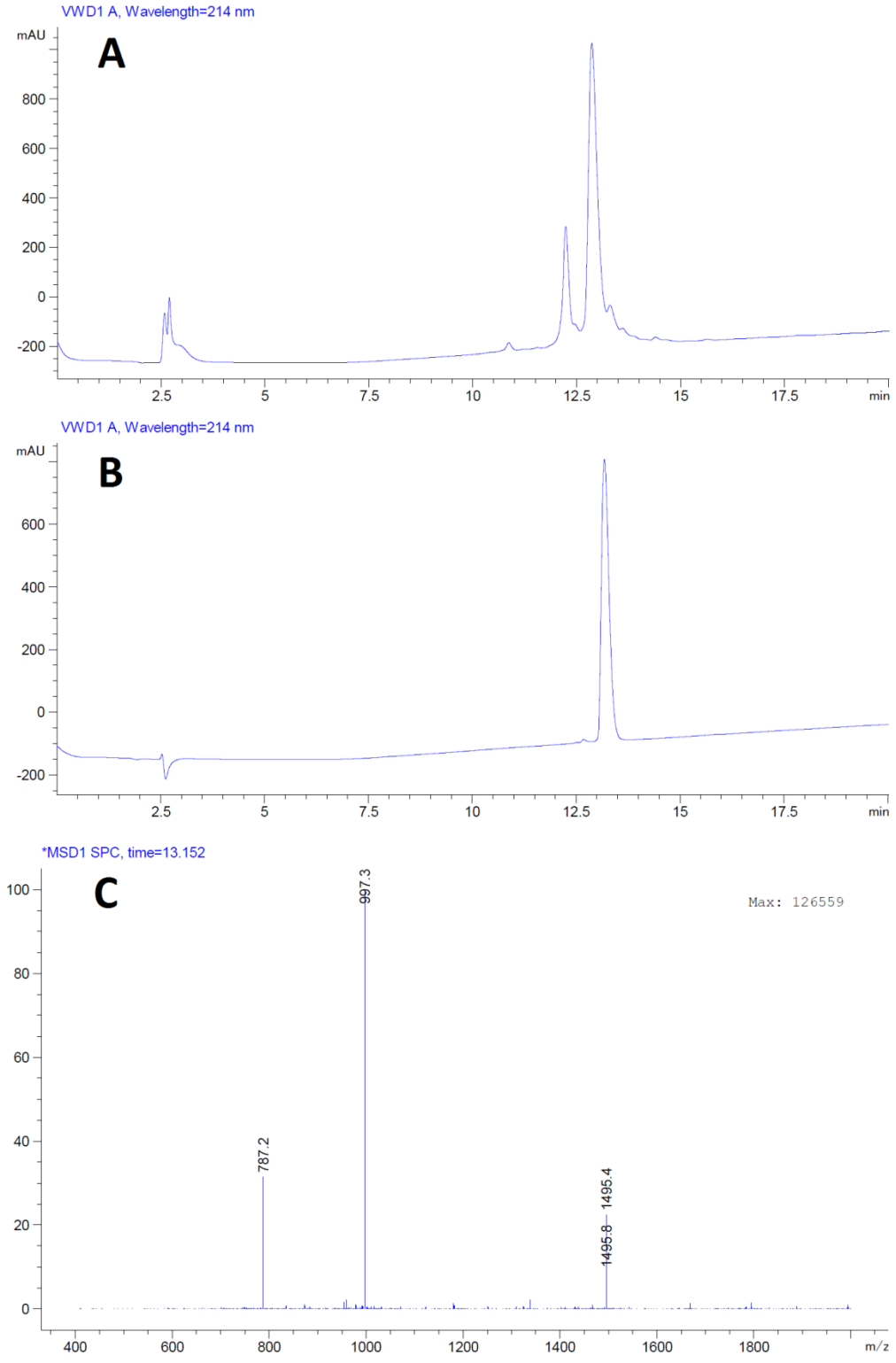

Figure SI-7: HPLC chromatograms of crude (A) and purified (B) peptide **8**, ESI MS of the major peak of **8** (C) ($C_{134}H_{197}N_{32}O_{46}^{+}$ $[M+H]^{2+}$ calc. 1495.7, obs. 1495.7).

CMP-GOLD – 9

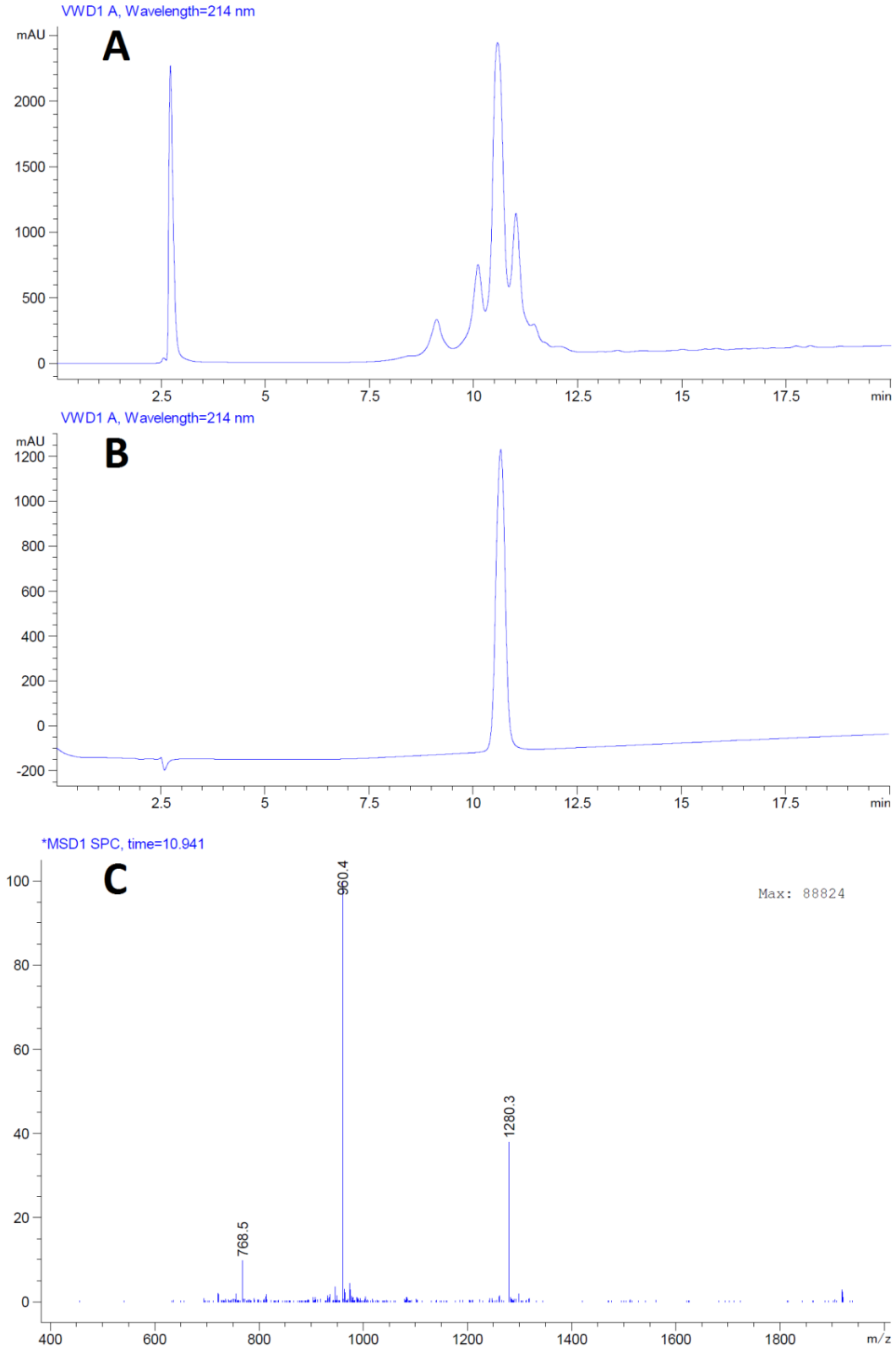

Figure SI-8: HPLC chromatograms of crude (A) and purified (B) peptide **9**, ESI MS of the major peak of **9** (C) ($C_{173}H_{253}N_{46}O_{54}^{+}$ $[M+H]^{2+}$ calc. 1919.9, obs. 1919.8).

CMP-MOLD – 10

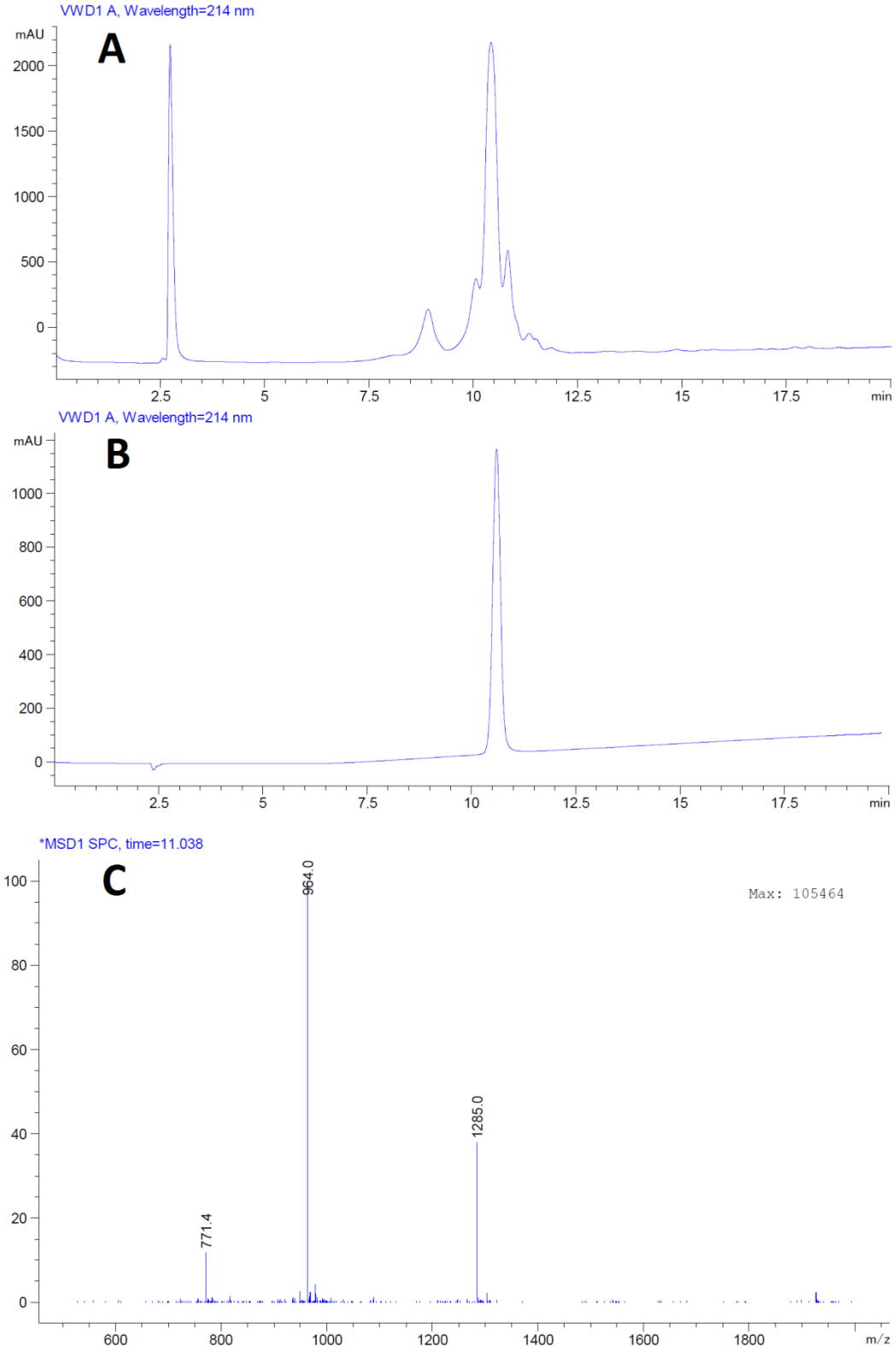

Figure SI-9: HPLC chromatograms of crude (A) and purified (B) peptide **10**, ESI MS of the major peak of **10** (C) ($C_{174}H_{255}N_{46}O_{54}^{+}$ $[M+H]^{2+}$ calc. 1926.9, obs. 1926.8).

Procedure for Trypsin Digest

Bovine trypsin (0.3 mg, type XI, 9090 units/mg, Sigma) was dissolved in H_2O (1 ml), 3.3 µl (9 units) of this solution diluted to 1 ml using Tris buffer (pH 8.0) and incubated at 37 °C for 30 min. Substrate (0.21 µmol) was added in one portion and 50 µl aliquots removed every minute, quenched with 1 M HCl (50 µl), and analysed by analytical RP-HPLC (Dionex Ultimate 3000 equipped with a 4 channel UV detector) at 210 nm using a Luna C18(2) column (3µ; 150 x 3 mm; Phenomenex) at 0.3 ml/min on a linear gradient (Figure SI-10).

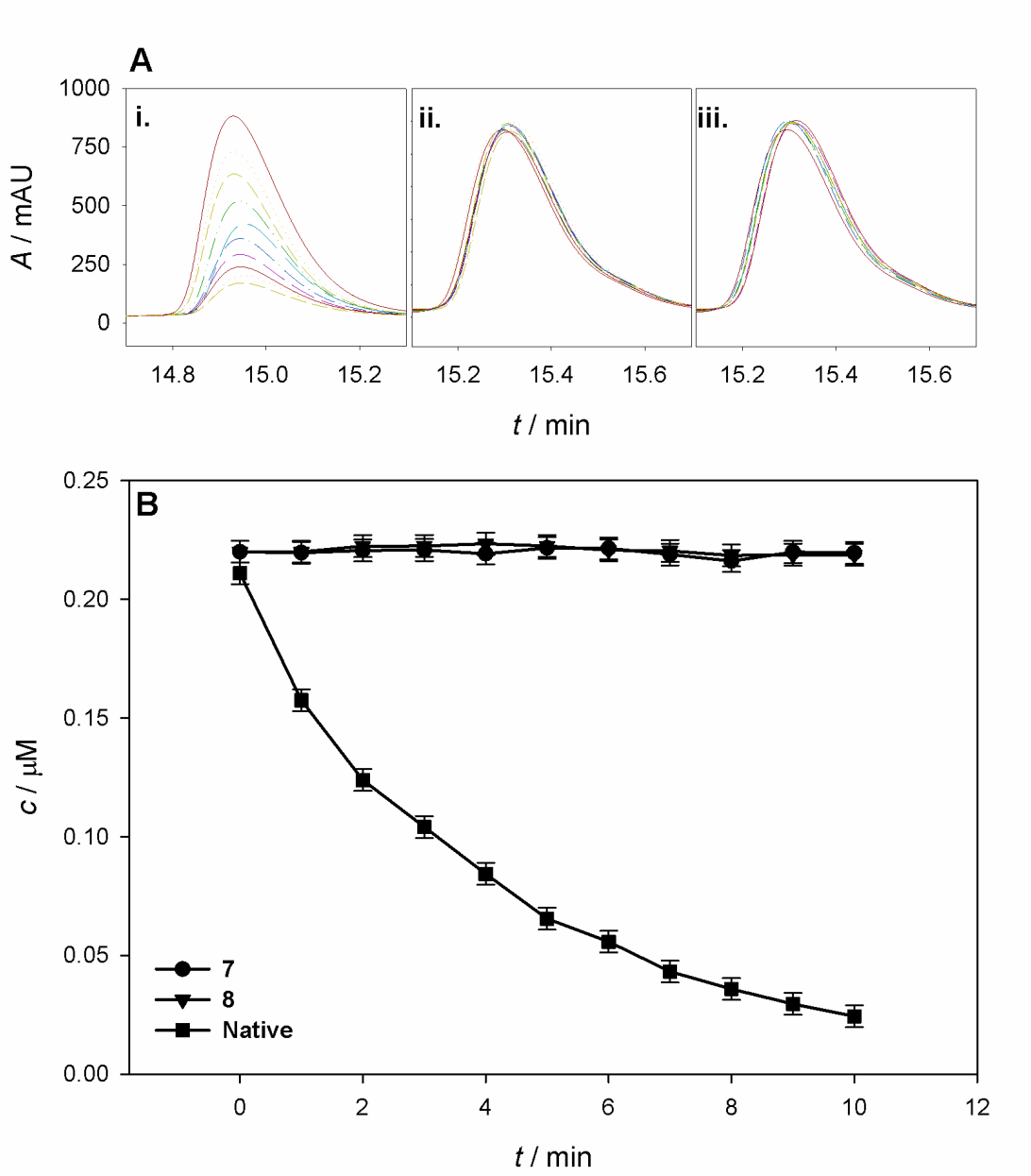

Figure SI-10: Trypsin digestion of CTP peptides. A: HPLC elution profiles of the incubation pf native CTP peptide (i.), **7** (ii.), and **8** (iii.) at 210 nm at various time points (coloured lines) with trypsin; B: relative peptide concentrations over time extrapolated from HPLC.

Circular Dichroism Measurements

CD spectra were recorded on peptide solutions at 0.2 mM (by weight) in 20 mM AcOH that had been incubated at 5 °C for a minimum of 24 h in 1 nm increments with 3 s scans at 20 °C and averaged over 10 scans (Figure SI-11). Thermal ramp experiments were recorded on peptide solutions at 0.5 mM (by weight) in 50 mM aqueous AcOH that had been incubated at 5 °C for a minimum of 24 h. The solutions were heated from 5 to 50 °C in 1 °C steps. The ellipticity at 224 nm was monitored at each temperature with 25 s scans and averaged over 5 scans (Figure SI-12).

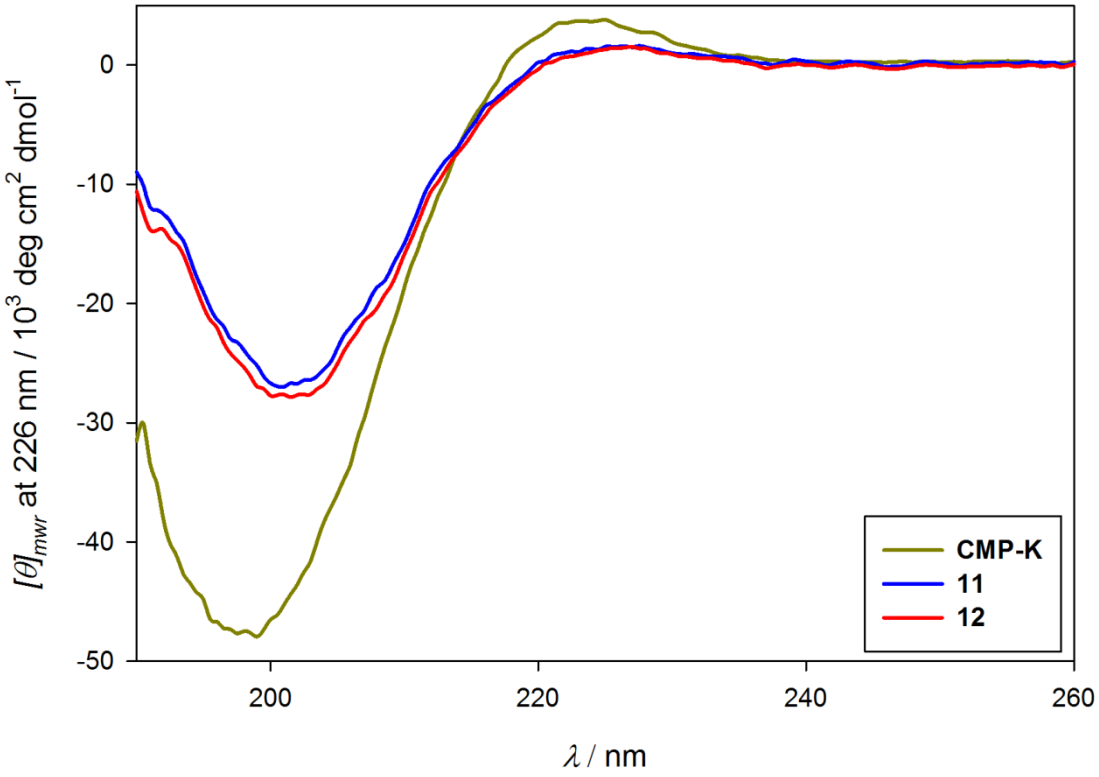

Figure SI-11: Circular dichroism of CMP-K, **9**, and **10**

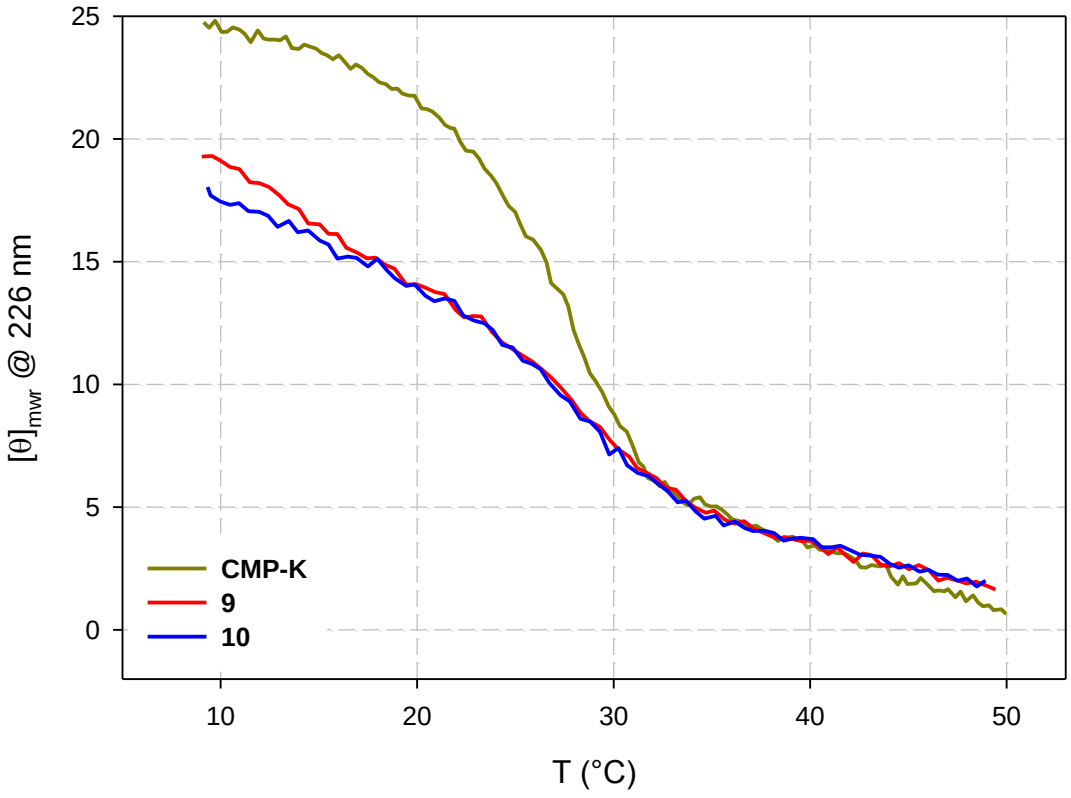

Figure SI-12: Thermal ramp CD spectra of 9, 10, and CMP-K