Electronic Supplementary Material (ESI) for ChemComm.

On resin synthesis and cross-linking of collagen peptides containing the advanced glycation end-product pyrraline via Maillard condensation

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Supporting Information

Materials and Methods

All solvents and reagents were used as supplied from commercial sources. All solvent compositions reported are on a volume/volume (v/v) basis.

Solid phase synthesis of the peptide backbones

Solid phase peptide synthesis was performed via the Fmoc strategy on Rink Amide resin using a PS3 Synthesiser (Tucson, AZ, USA) on 0.1 mmol scale. The Fmoc group was deprotected with 20% piperidine in *N,N*-dimethylformamide (DMF) for 2 x 5 min. The couplings were performed with Fmoc-AA-OH (5 equiv.) in DMF, *1*-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) (4.5 equiv) and *N*-methylmorpholine (10 equiv.) in DMF for 20 min. The final Fmoc group was removed and the amine was acetylated with Ac_2O in the presence of *N*-methylmorpholine for 10 min. The *N*-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) (Dde) group on the lysyl side-chain was deprotected by a 2 x 3 min treatment with 2% hydrazine hydrate in DMF.

Dihydropyranone coupling optimisation

Resin bound peptide aliquots (0.005 mmol, 1 equiv) were Dde deprotected, swollen in DMF, and filtered. Dihydropyranone **7** (0.015 mmol, 3 equiv) in solvent (0.2 ml) was added to the resin. The reaction mixtures were left standing overnight at rt. The resulting product mixtures were filtered, washed with DMF, washed with DCM, and treated with TFA/TIS (19:1) for 2 h.

Peptide cleavage and purification

The peptides were released from the resin with concomitant removal of the side-chain protecting groups by treatment with either TFA/TIS/H_2O (38:1:1) or TFA/TIS (19:1). Peptides were precipitated with cold diethyl ether, dissolved in 1:1 CH_3CN/H_2O containing 0.1% TFA, lyophilized, and analysed for purity by LC/MS using either a Gemini C18 column (5 µ, 50 x 2 mm;) or a Zorbax C3 column (3.5 µ, 150 x 3mm; Agilent;) at a flow rate of 0.3 ml/min using a linear gradient (5 to 65% B over 21 minutes). The solvent system used was A (0.1% formic acid in H_2O) and B (0.1% formic acid in CH_3CN). Purification of crude peptides was performed by semipreparative HPLC using a Gemini C18 column (10 µ, 10 x 250 mm; Phenomenex) at a flow rate of 5 ml/min using an appropriate shallow gradient. The solvent system used here was A (0.1 % TFA in H_2O) and B (0.1% TFA in CH_3CN). Purified peptides were analysed by LC/MS using a Gemini C18 column (5 µ, 50 x 2 mm; Phenomenex) and subsequently freeze-dried. The purities were extrapolated from integrating the peaks corresponding to **1** (16 min), **2** (12.2 min), **3** (19.1 min).

Synthesis of 3

The Dde deprotected peptide **6** bound to a PS resin via a Rink amide linker (0.01 mmol, 1 equiv) was swollen in DMF, and filtered. A solution of dihydropyranone **7** (0.03 mmol, 3 equiv) in 20% piperidine in DMF (1 ml) was added to the resins. The reaction mixtures were shaken overnight at rt and the resulting resin bound peptides filtered and washed with DMF. The target peptide was cleaved from the resin and purified according to the procedure described above (3.55 ± 0.02 mg, 36%).

Synthesis of 1

The Dde deprotected peptide **10** bound to a PS resin via a Rink amide linker (0.01 mmol, 1 equiv) was swollen in DMF, and filtered. A solution of dihydropyranone **7** (0.03 mmol, 3 equiv) in 20% piperidine in DMF (1 ml) was added to the resins. The reaction mixtures were shaken overnight at rt and the resulting resin bound peptides filtered and washed with DMF. The target peptide was cleaved from the resin and purified according to the procedure described above (5.35 ± 0.02 mg, 33%).

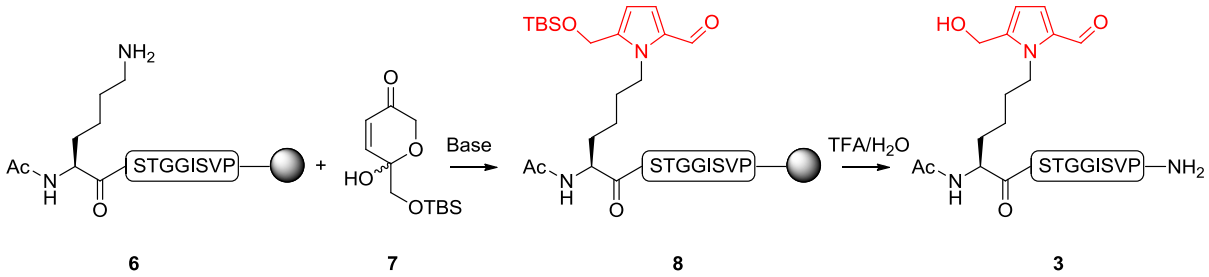
Synthesis of 2

The Dde deprotected intermediate peptide (POGPOGPOGPKGPOGPOGPOG, where O denotes *4R*-hydroxyproline) bound to a PS resin via a Rink amide linker (0.01 mmol, 1 equiv) was swollen in DMF, and filtered. A solution of dihydropyranone **7** (0.03 mmol, 3 equiv) in 20% piperidine in DMF (1 ml) was added to the resins. The reaction mixtures were shaken overnight at rt and the resulting resin bound peptides filtered and washed with DMF. The peptide was cleaved from the resin and purified according to the procedure described above (4.30 ± 0.02 mg, 21%).

Synthesis of 13

The Dde deprotected peptide **6** bound to a Tentagel resin via a Rink amide linker (0.02 mmol, 1 equiv) was swollen in DMF, and filtered. A solution of dihydropyranone **7** (0.06 mmol, 3 equiv) in 20% piperidine in DMF (1 ml) was added to the resin. The reaction mixture was left standing overnight at rt and the resulting product mixture filtered and washed with DMF. The target peptide was cleaved from the resin and purified according to the procedure described above (3.41 0.02 mg, 18%).

Table SI-1 On-resin Maillard-type condensation



6 7 8 3

Entry	Resin (loading)	Base and solvent	% Yield 3
1	Tentagel (0.37 mmol/g)	10% pyridine in DMF	3
2	Tentagel (0.37 mmol/g)	10% DIPEA in NMP	7
3	Tentagel (0.37 mmol/g)	10% piperidine in DMF	18
4	Tentagel (0.37 mmol/g)	20% piperidine in DMF	25
5	Tentagel (0.37 mmol/g)	30% piperidine in DMF	23
6	PS (0.92 mmol/g)	10% pyridine in DMF	7
7	PS (0.92 mmol/g)	10% DIPEA in NMP	15
8	PS (0.92 mmol/g)	20% piperidine in DMF	36

Trypsin Stability Assay

Bovine trypsin (0.3 mg, type XI, 9090 units/mg, Sigma) was dissolved in H_2O (1ml) and 3.3 µl (9 units) of this solution further diluted to 1 ml using Tris buffer (pH 8.0) and incubated at 37 °C for 30 min. Substrate (0.2 µmol) was added in a single portion and 50 µl aliquots were 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 a flow rate of 0.3 ml/min using a linear gradient of 5 to 65% B over 21 minutes. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in CH_3CN).

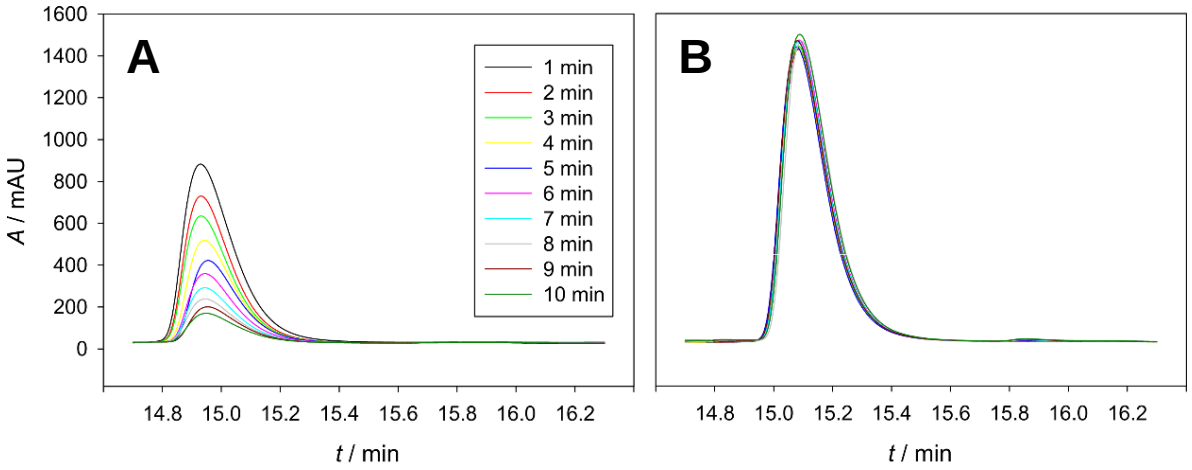
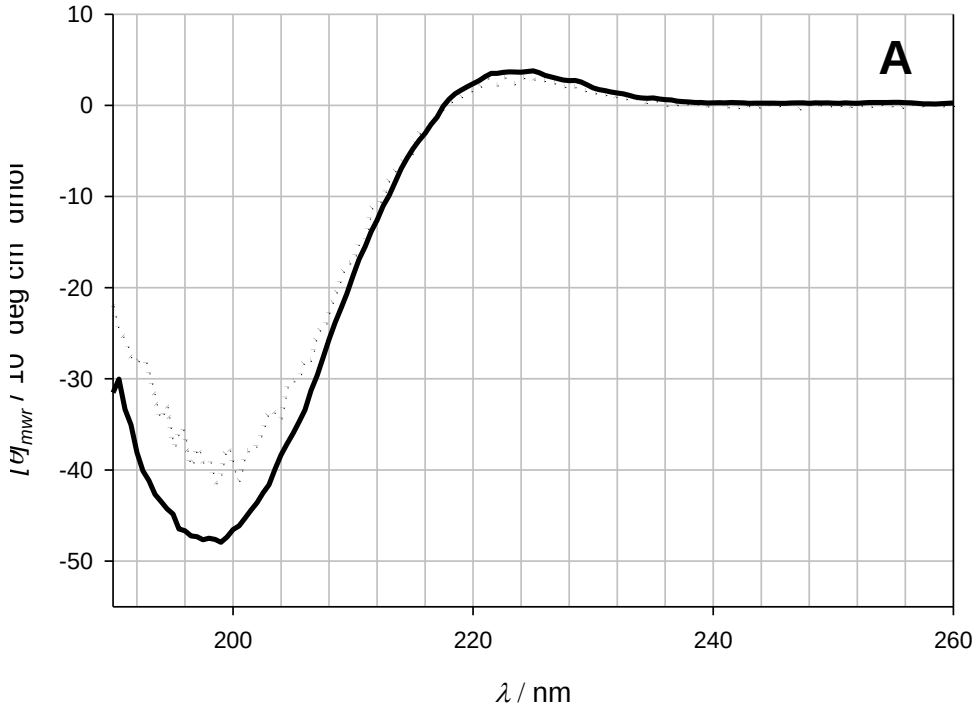


Figure SI-1 Trypsin digestion of collagen telopeptides. Relevant peaks in HPLC elution profiles during the incubation of peptides **11** (A) and **1** (B) at various time (coloured lines) with trypsin.

CD analysis

CD spectra were recorded on peptide solutions (0.05 mM in 20 mM AcOH) that had been incubated at 5 °C for a minimum of 24 h. Spectra were recorded in 1 nm increments with 3 s scans at 20 °C and averaged over 10 scans. Thermal ramp experiments were recorded on peptide solutions (0.5 mM in 20 mM AcOH) that had also 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 logged at each temperate interval with 25 s scans and averaged over 5 scans.



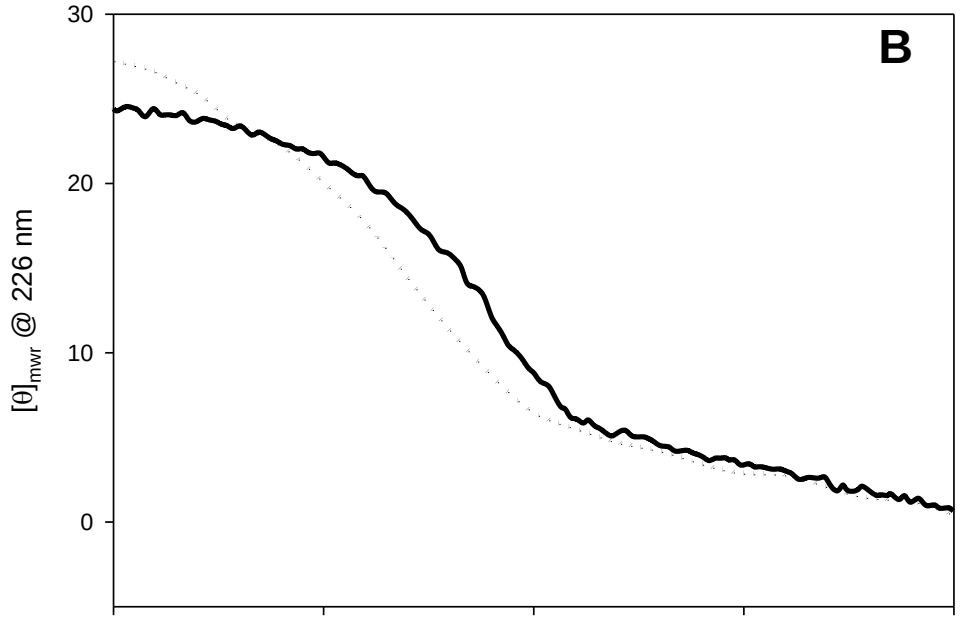


Figure SI-2. Circular Dichroism spectrum (A) and thermal denaturation curve (B) of peptide **12** (solid) and **2** (dotted)

Peptide chromatograms and mass spectra

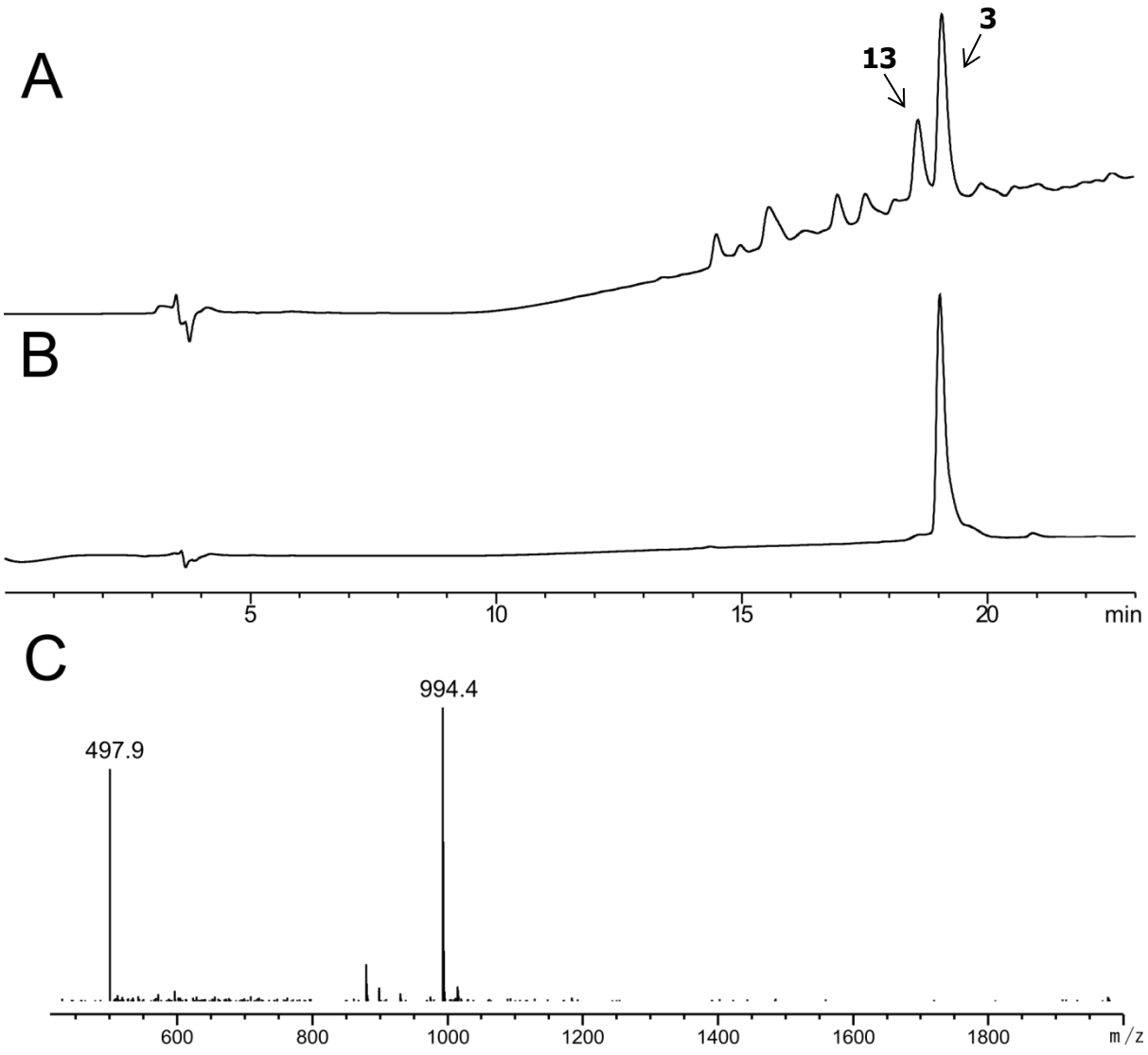


Figure SI-3. LC/MS chromatograms of crude peptide **3** (A, Gemini C18, 44% by LC/MS), of purified peptide **3** (B, Gemini C18), and ESI-MS of the major peak (C) (>95% purity, $[M+H]^+$ calc. 994.5, obs. 994.4).

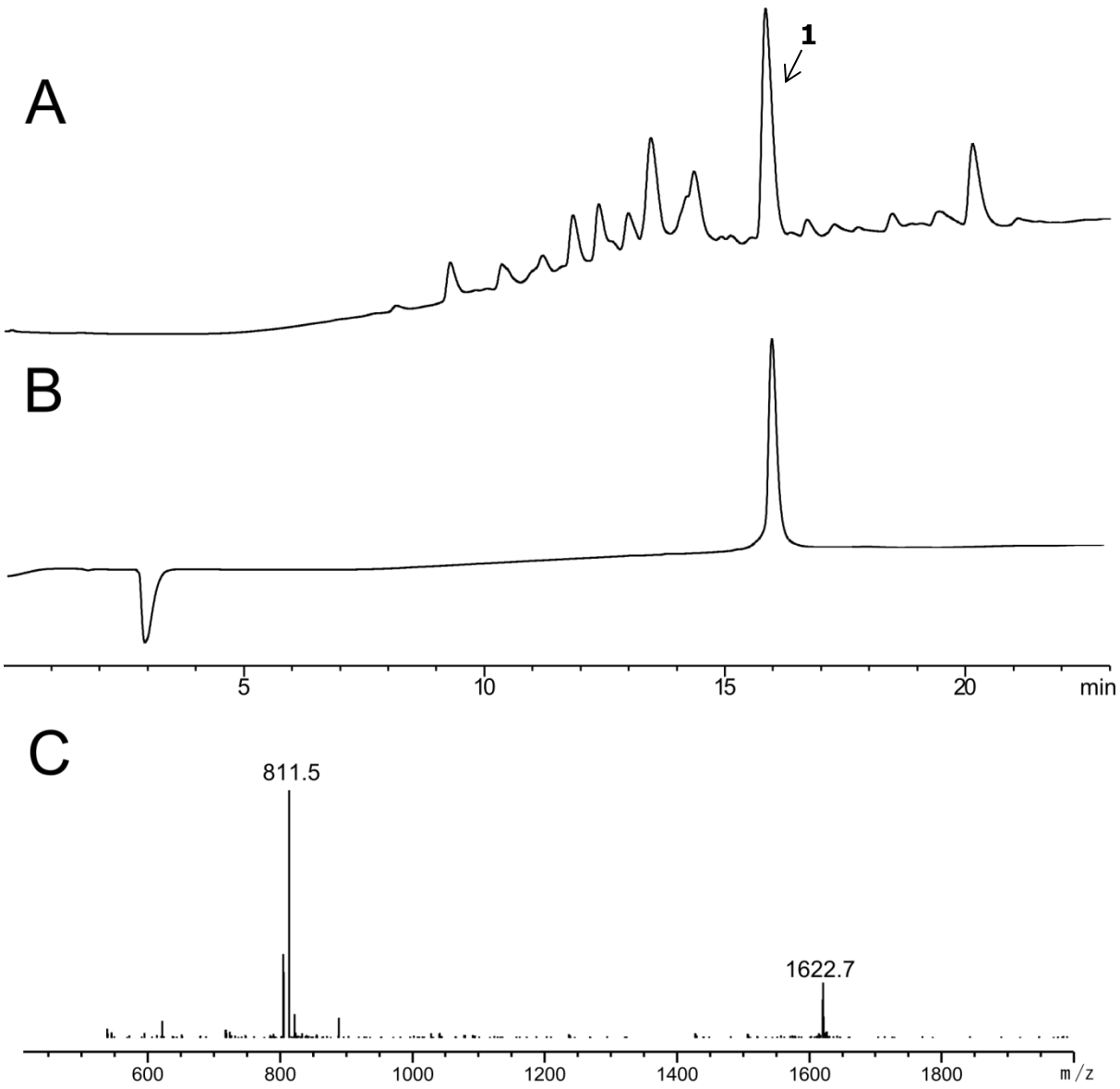


Figure SI-4. LC/MS chromatograms of crude peptide **1** (A, Gemini C18, 38 % by LC/MS), of purified peptide **1** (B, Gemini C18), and ESI-MS of the major peak (C) (>95% purity, $[M+H]^+$ calc. 1622.7, obs. 1622.7).

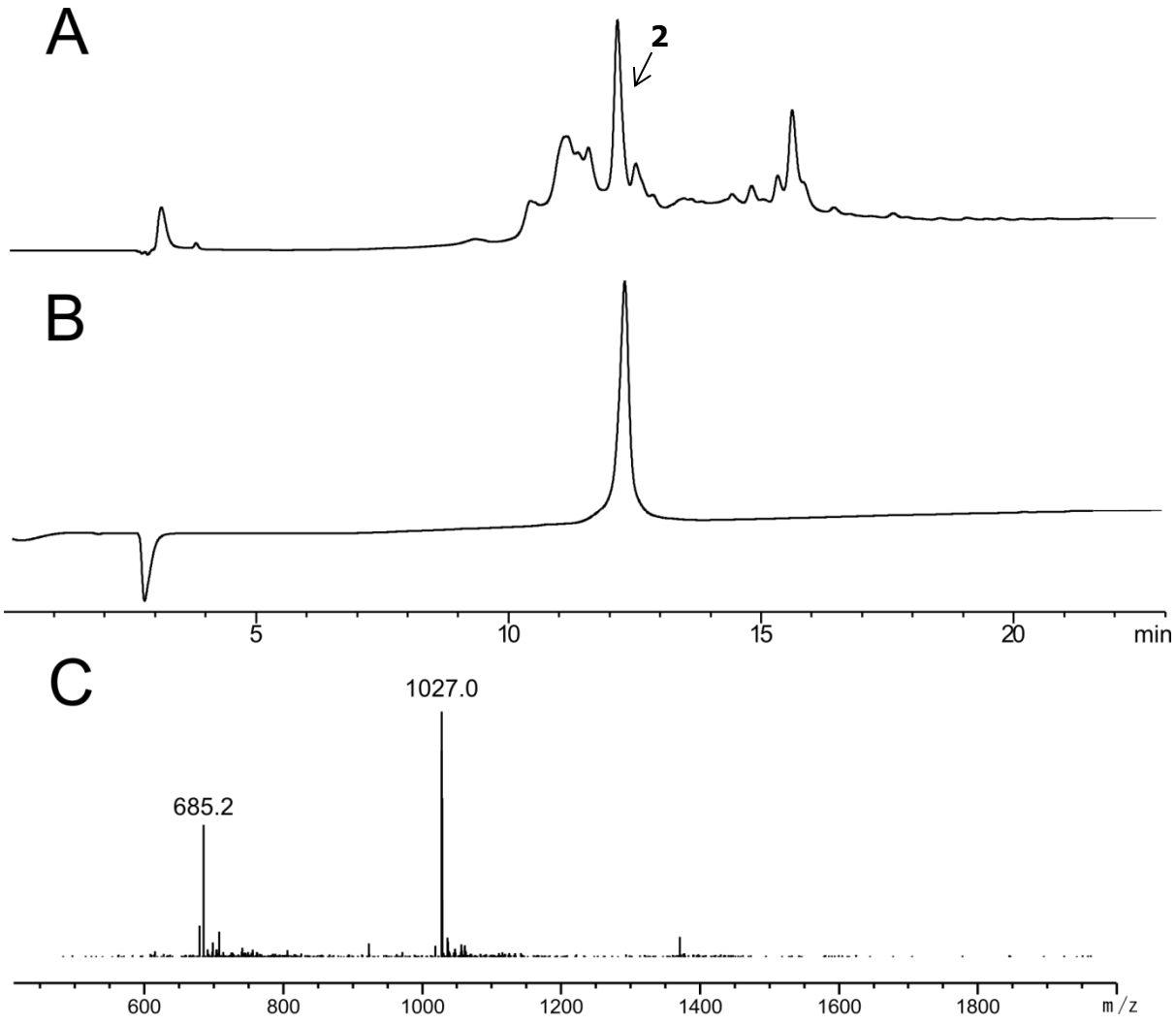


Figure SI-5. LC/MS chromatograms of crude peptide **2** (A, Gemini C18, 29% by LC/MS), of purified peptide **2** (B, Gemini C18), and ESI-MS of the major peak (C) (>95% purity, $[M+2H]^{2+}$ calc. 1027.5, obs. 1027.0).

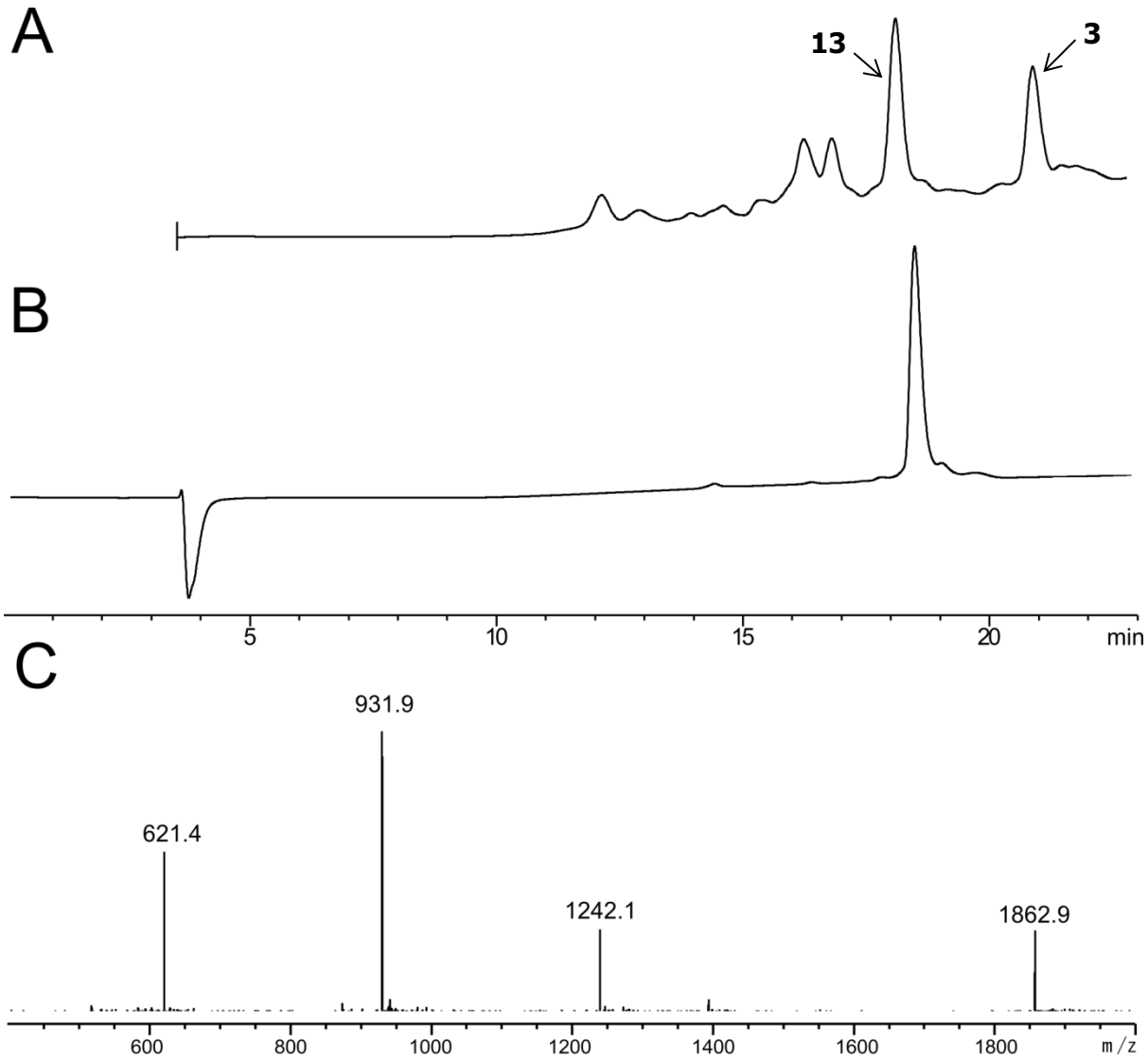


Figure SI-6. LC/MS chromatograms of crude peptide **13** (A, Zorbax C3), of purified peptide **13** (B, Gemini C18), and ESI-MS of the major peak (C) ([M+H]$^+$ calc. 1863.1, obs. 1862.9)

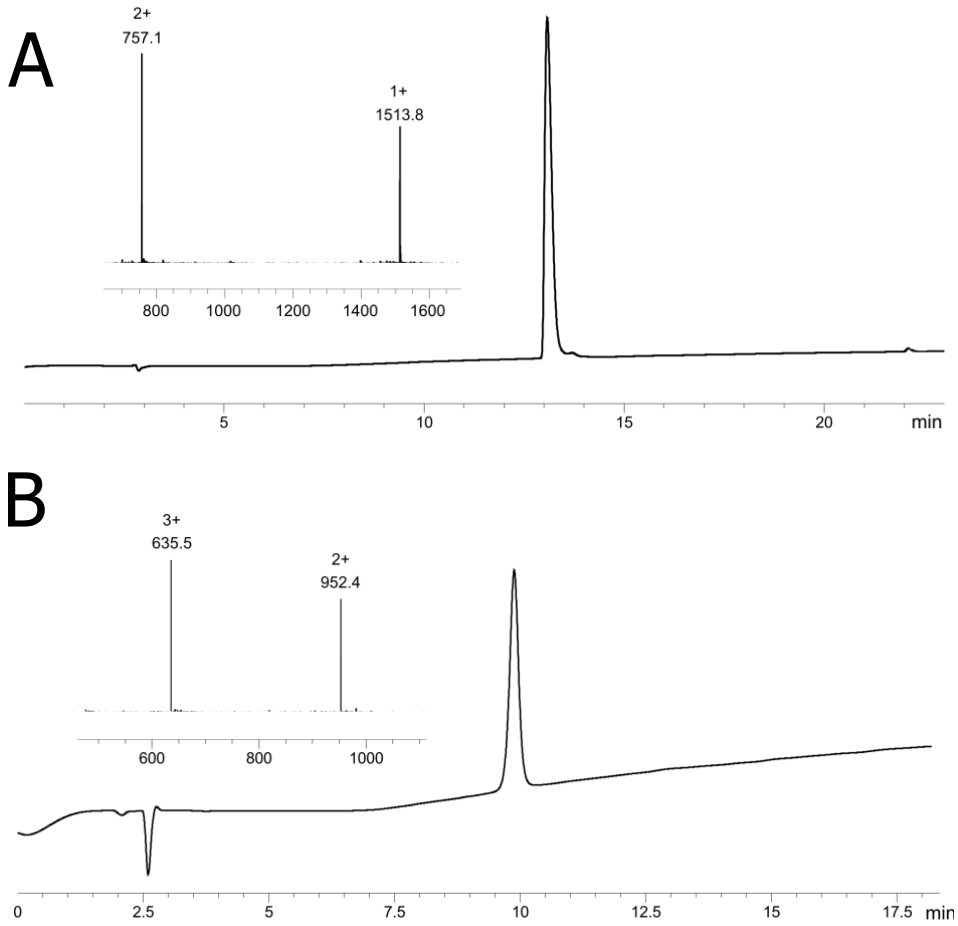


Figure SI-7 LCMS chromatograms of purified peptides **11** (A, Zorbax C3) and **12** (B, Zorbax C3) (**11**: >95% pure, $[M+H]^+$ calc. 1513.7, obs. 1513.8; **12**: >95% pure, $[M+2H]^{2+}$ calc. 952.5, obs. 952.4).

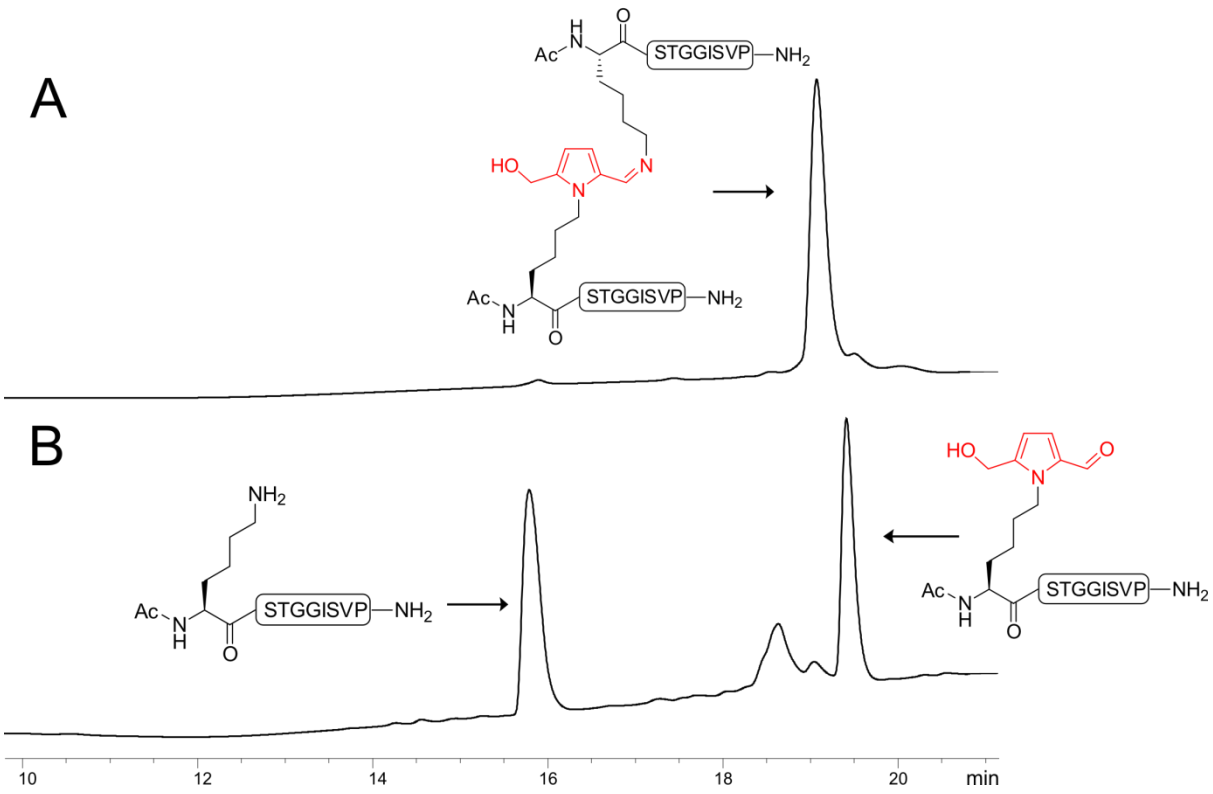


Figure SI-8 Monitoring degradation of peptide **13** by LC/MS following 24 h incubation at pH 6 (A) and 24 h incubation at pH 8 (B)