Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Physicochemical Studies on the Copper(II) Binding by Glycated Collagen Telopeptides

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Supplementary Information

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Building Block and Peptide Synthesis

All solvents and reagents were used as supplied from commercial sources. Analytical thin-layer chromatography was performed using Kieselgel F254 0.2 mm (Merck) silica plates with visualization by ultraviolet irradiation (254 nm) followed by staining with ninhydrin. Flash chromatography was performed using Kieselgel S63-100 µm (Riedel-de-Hahn) silica gel. All solvent compositions reported are on a volume/volume (v/v) basis. ^{1}H-NMR spectra were recorded on a 400 MHz Bruker spectrometer and are reported in parts per million (ppm) on the δ scale relative to d_6-dimethyl sulfoxide (DMSO). ^{13}C-NMR spectra were recorded on a 100 MHz Bruker spectrometer and are reported in parts 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, *m* = multiplet, *dd* = doublet of doublets. Mass spectra were obtained by electrospray ionization in positive ion mode.

Synthesis of N^{α}-Fmoc-N^{ε}-Ns-L-Lysine

To an ice-cooled solution of Fmoc-Lys-OH (1.0 g, 2.82 mmol, 1 equiv) in 1 M aqueous K_2CO_3 (7 ml) and 1,4-dioxane (10 ml) was added NsCl (0.74 g, 3.36 mmol,1.2 equiv) as a solution in dioxane (5 ml). The reaction was allowed to warm to rt and stirred for 2 h. The reaction mixture was partitioned between ethyl acetate (25 ml) and H_2O (25 ml) and the layers separated. The aqueous layer was extracted with ethyl acetate (2 x 20 ml) and the combined extracts washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (100% ethyl acetate) afforded the title compound as a white solid (1.35 g, 90% yield). Spectroscopic data were in good agreement with those previously reported.[1] ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.08 (t, J = 5.4 Hz, 1H), 7.99-7.93 (m, 2H), 7.90-7.81 (m, 4H), 7.72-7.70 (m, 2H), 7.60-7.57 (m, 1H), 7.42-7.29 (m, 4H), 4.28-4.26 (m, 2H), 4.23-4.19 (m, 1H), 3.90-3.82 (m, 1H), 2.87 (dd, J = 12.8, 6.8 Hz, 2H), 1.66-1.46 (m, 2H), 1.45-1.20 (m, 4H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 174.0, 156.2, 147.8, 143.8, 140.7, 134.0, 132.8, 132.6, 129.4, 127.7, 127.1, 125.3, 124.4, 120.1, 65.6, 53.7, 46.7, 42.5, 30.2, 28.8, 22.7.

Peptide Synthesis

Automated SPPS

Standard SPPS was performed via the Fmoc strategy on aminomethyl resin equipped with a Rink Amide linker using a Biotage Alstra peptide synthesiser (Uppsala, Sweden) on 0.1 mmol scale. The Fmoc group was deprotected with 20% piperidine in *N,N*-dimethylformamide (DMF) for 2 + 3 minutes at 60 °C. The coupling step was performed with 5 equiv of Fmoc-protected amino acid in DMF (0.2 M), 4.5 equiv *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) in DMF (0.45 M) and 10 equivalents of *N,N*-diisopropylethylamine (DIPEA) for 5 min at 75 °C. The final Fmoc-group was removed and the free amine was acetylated by Ac_2O in the presence of DIPEA at room temperature for 10 min.

Peptide Purification

Following completion of syntheses, the peptides were released from resin with concomitant removal of the remaining side-chain protecting groups by treatment with trifluoroacetic acid (TFA)/triisopropyl silane (TIS)/H_2O (38:1:1, 5 ml) 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 acetonitrile/H_2O containing 0.1% TFA and lyophilized. The peptides were analysed for purity by liquid chromatography mass spectrometry (LCMS) (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; 3 x 150 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 acetonitrile). Purification of crude peptides was performed by semipreparative high-performance liquid chromatography (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 acetonitrile). The resulting purified peptides were analysed by the LCMS system used for crude peptide analysis.

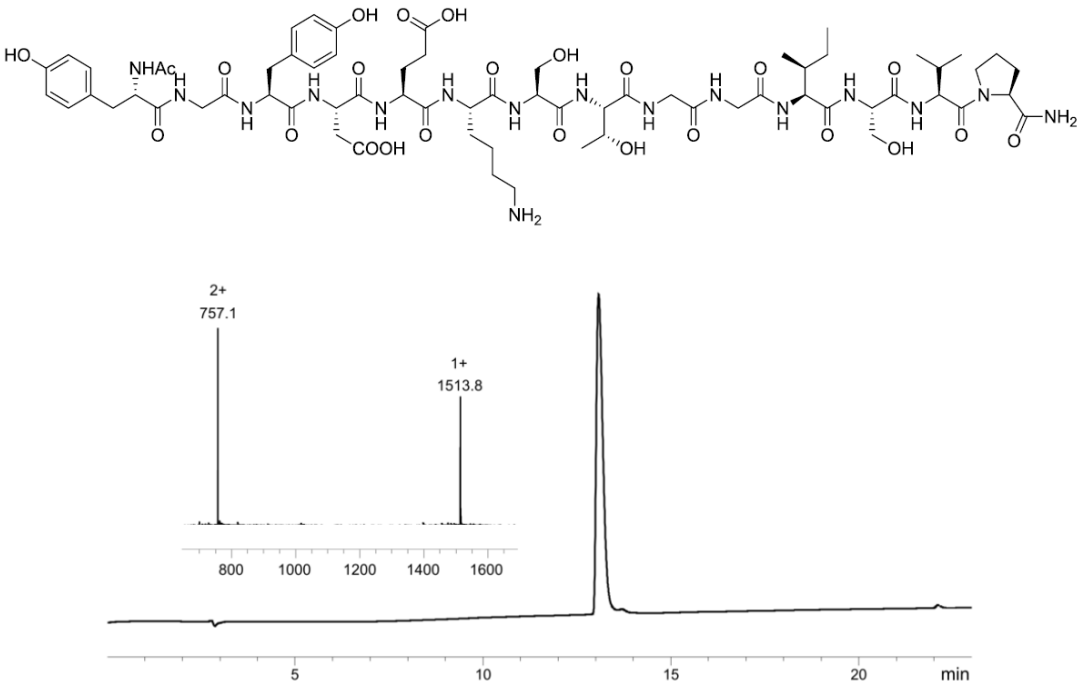


Figure SI-1 Analytical LC-MS of the purified peptide **1**. $[M+H]^+$ calc. 1513.7, obs. 1513.8.

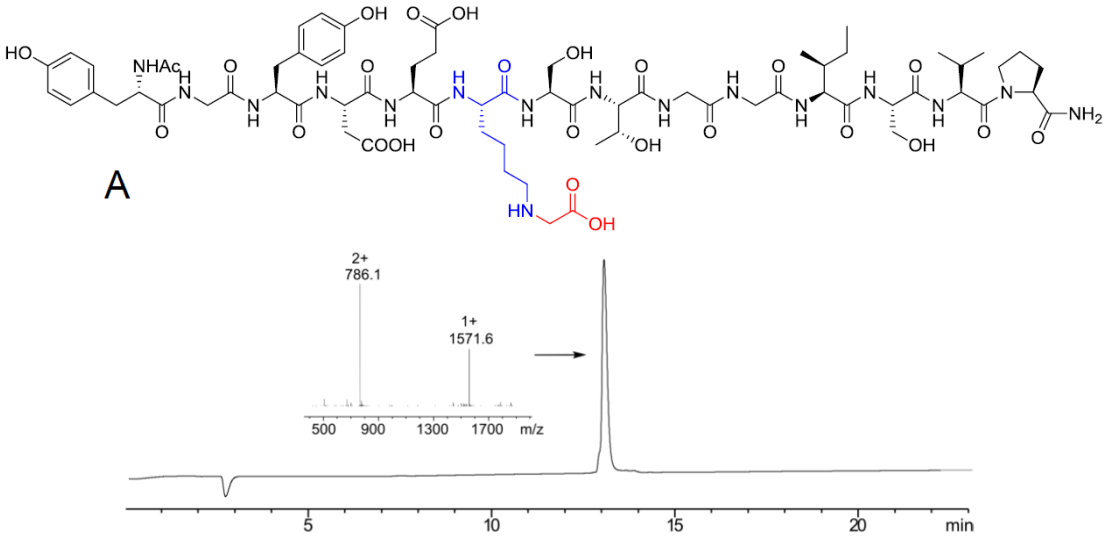
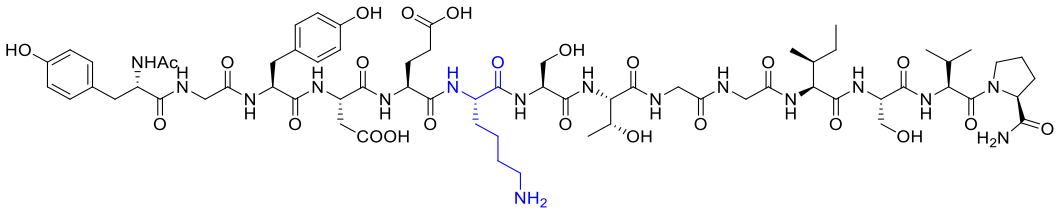
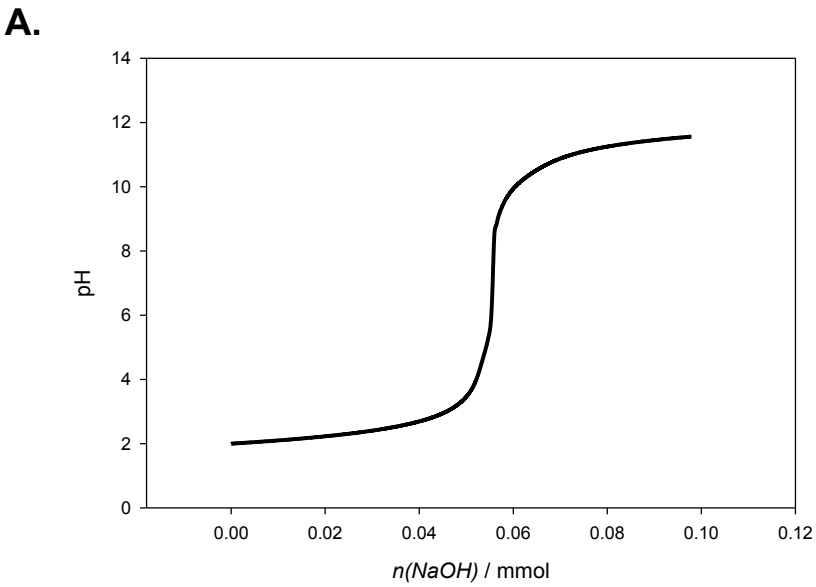


Figure SI-2 Analytical LC-MS of the purified peptide **2**. $[M+H]^+$ calc. 1572.4, obs. 1571.6.

Potentiometric Titration Results





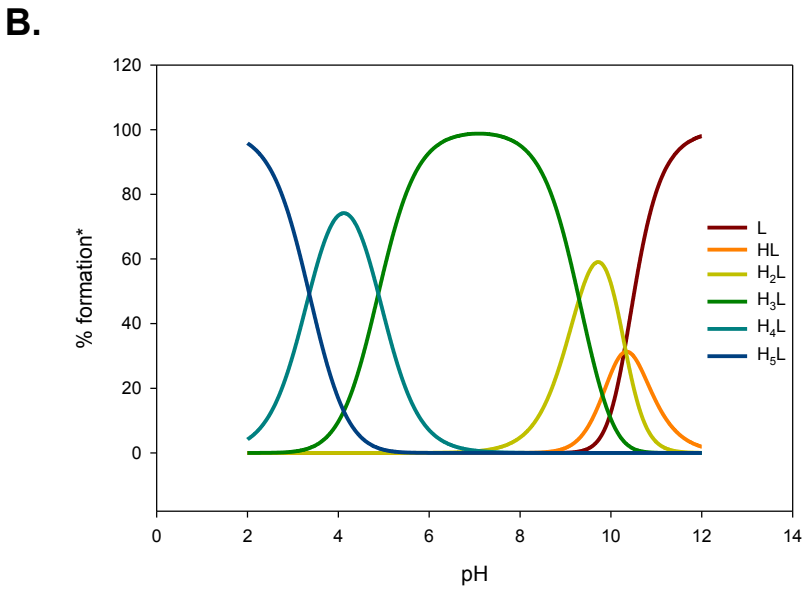
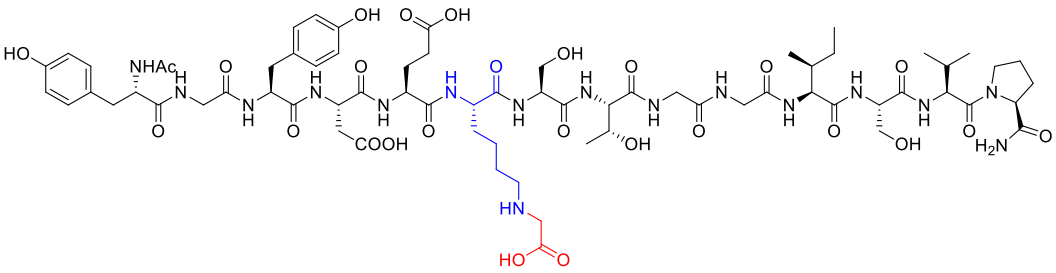
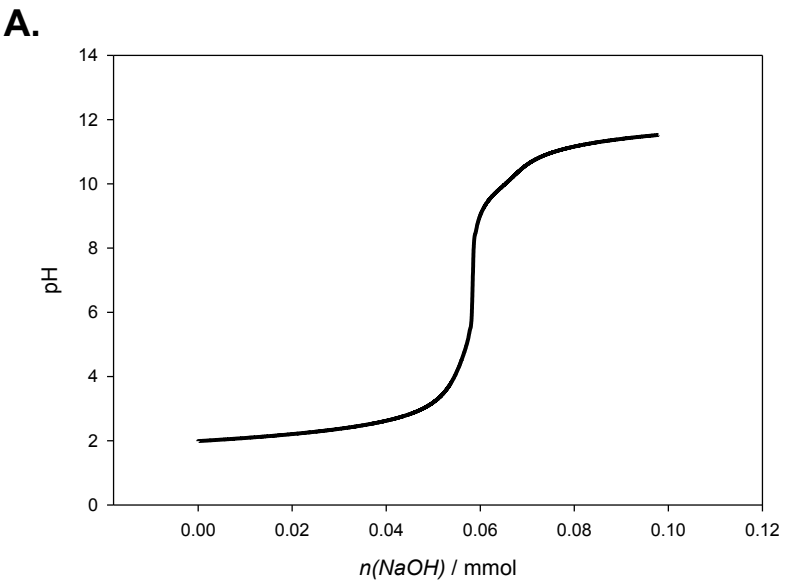


Figure SI-3 Titration curve (A) and the species distribution diagram (B) for titration of **1** with NaOH





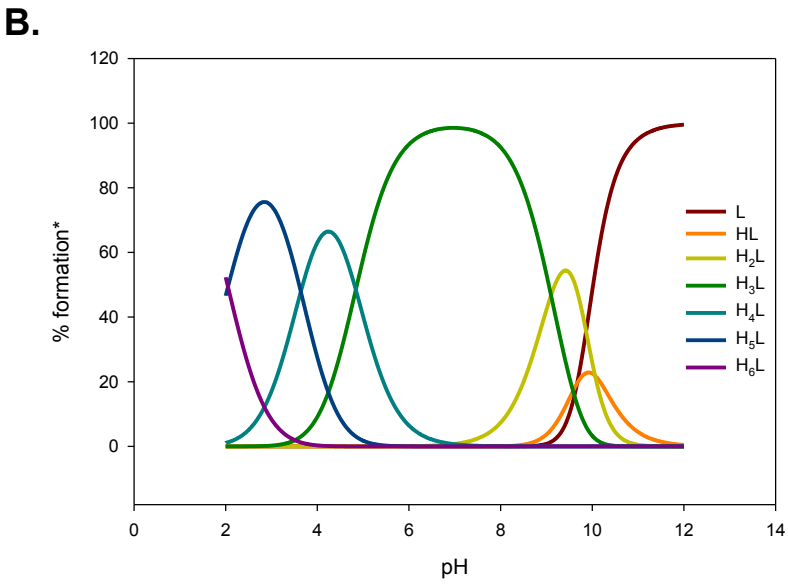
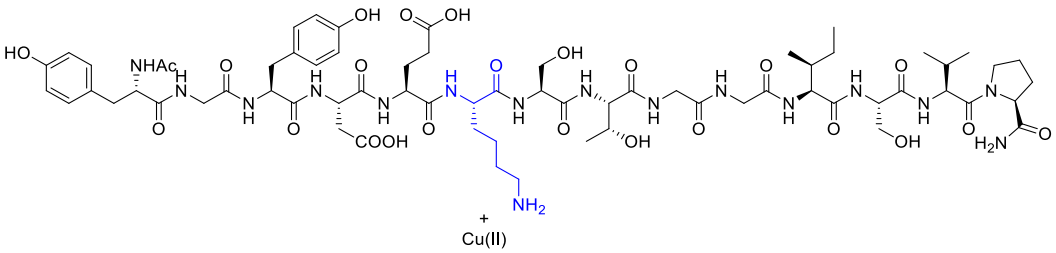
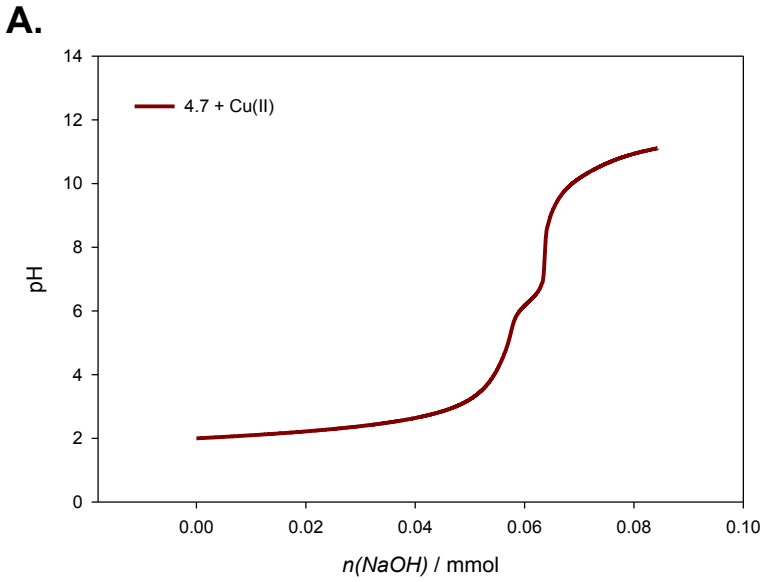


Figure SI-4 Titration curve (A) and the species distribution diagram (B) for titration of **2** with NaOH





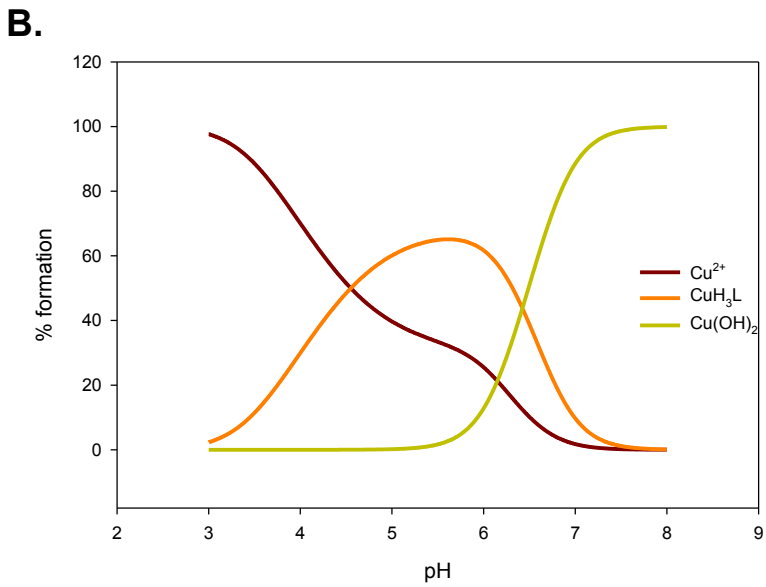
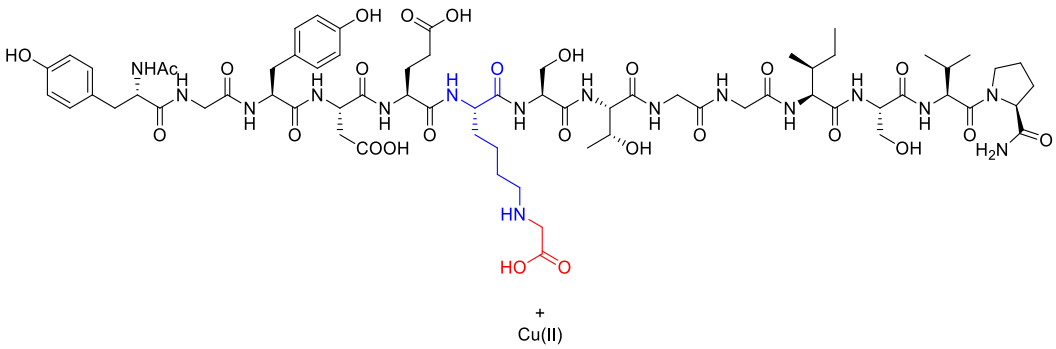
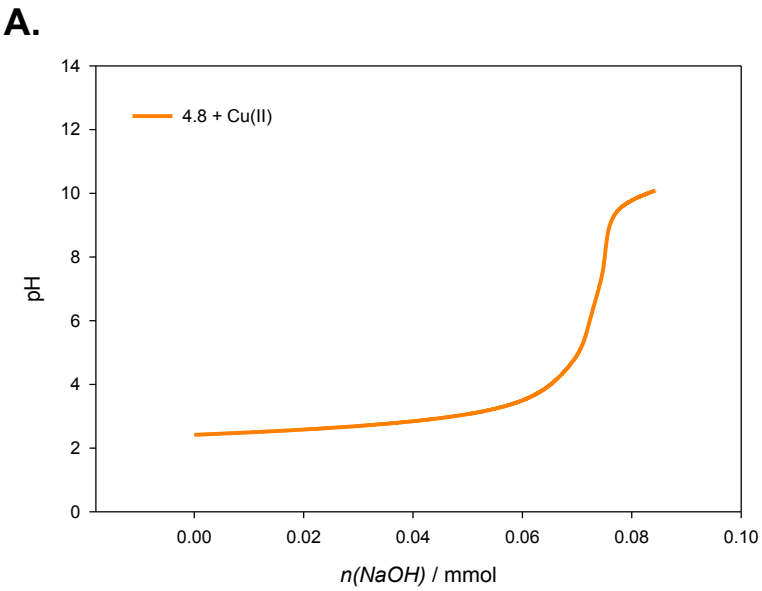


Figure SI-5 Titration curve (A) and the species distribution diagram (B) for titration of **1** with NaOH in presence of Cu(II)





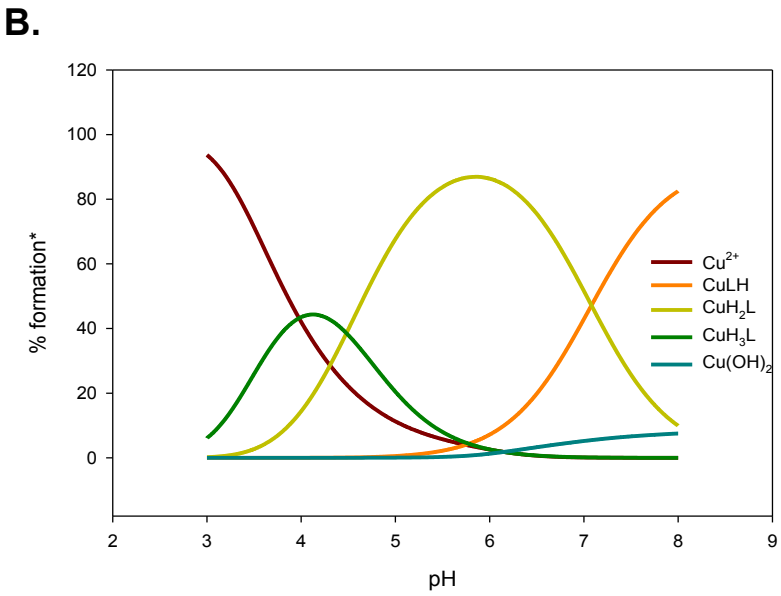
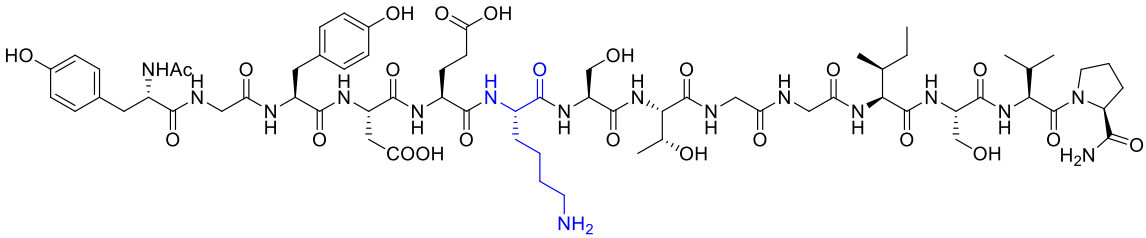
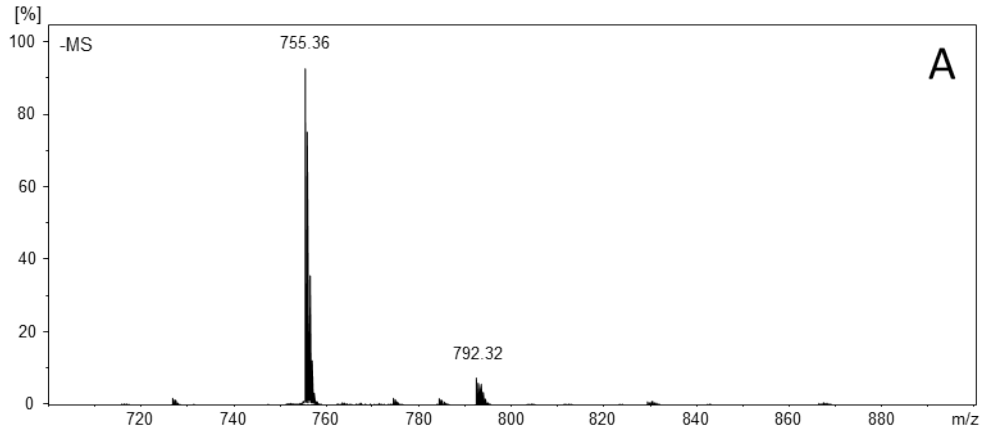
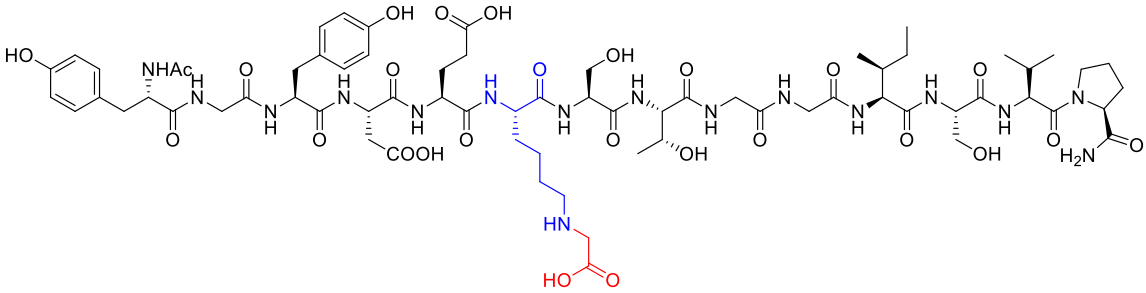


Figure SI-6 Titration curve (A) and the species distribution diagram (B) for titration of **2** with NaOH in presence of Cu(II)

Mass Spectrometry Results







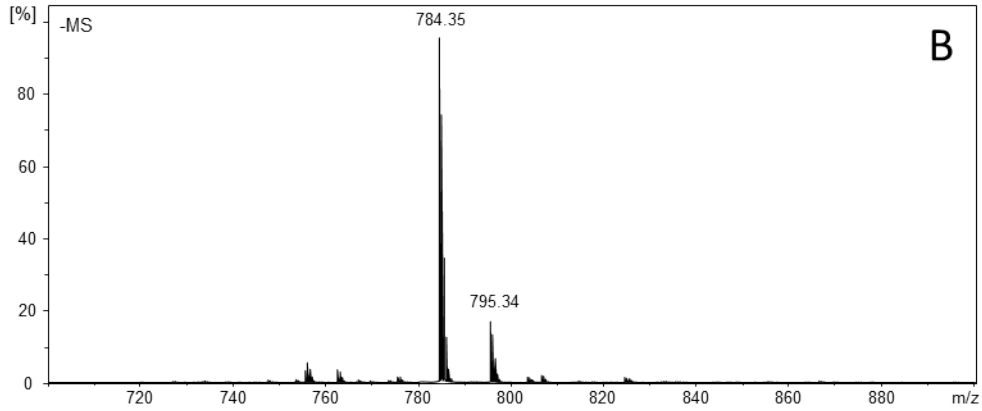
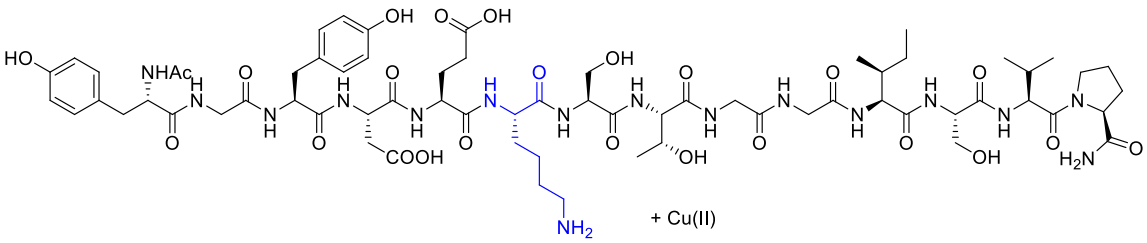


Figure SI-7 Mass spectra of peptides **1** (A, pH 7.3) and **2** (B, pH 6.9) in the range *m/z* 700 – 900 featuring double negatively charged anions.



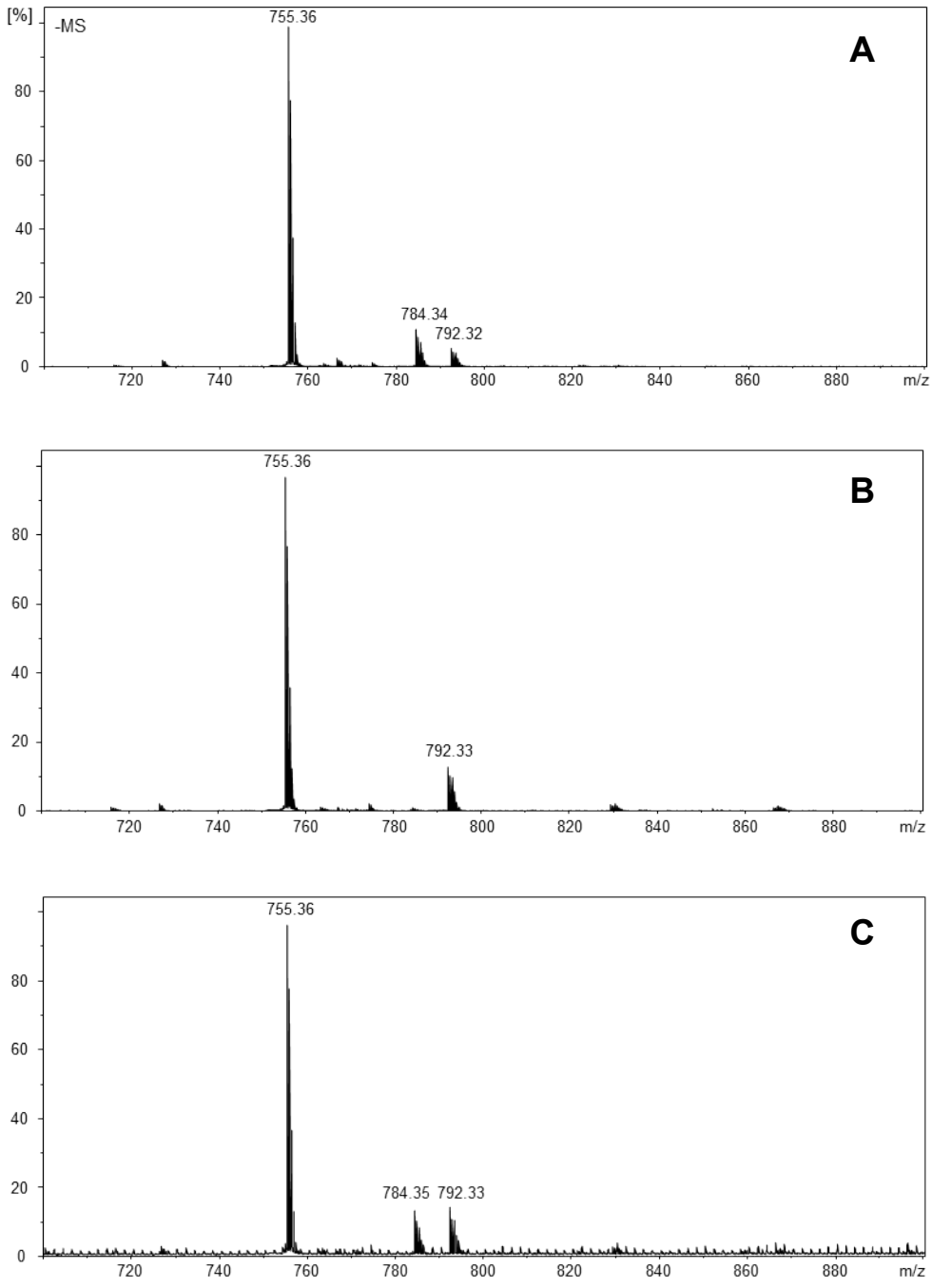
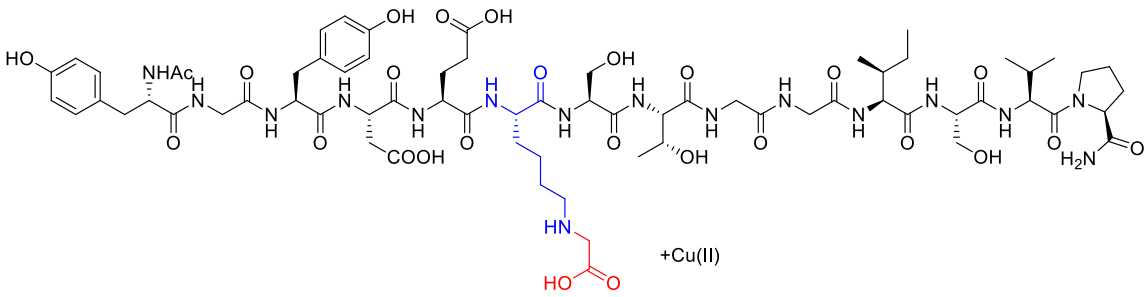


Figure SI-8 Mass spectra of peptide **1** in presence of Cu(II) at pH 6.7 (A), pH 5.9 (B), and pH 2.9 (C) in the range *m/z* 700 – 900 featuring double negatively charged anions.



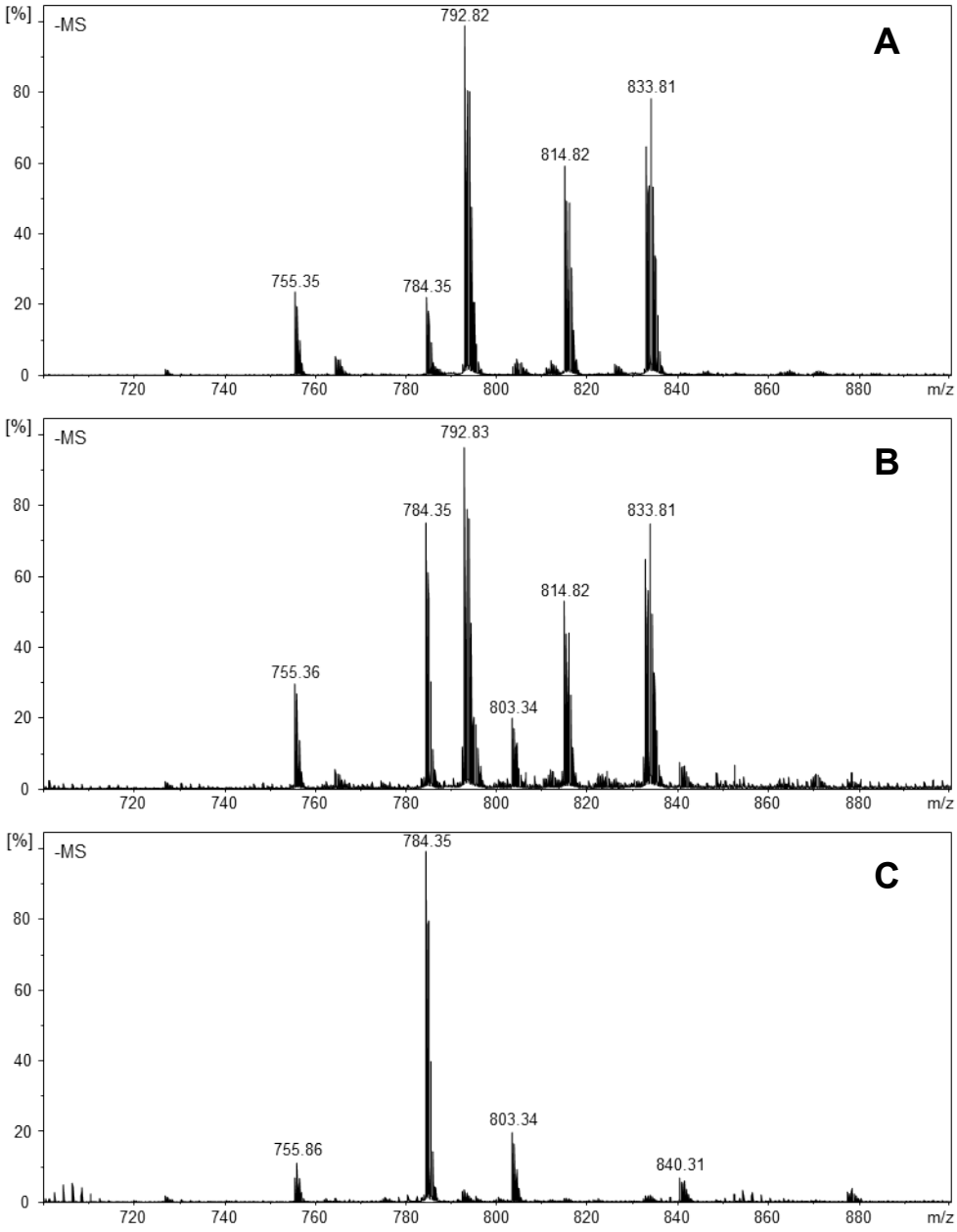


Figure SI-9 Mass spectra of peptide **2** in presence of Cu(II) at pH 7.0 (A), pH 5.4 (B), and pH 3.2 (C) in the range *m/z* 700 – 900 featuring double negatively charged anions.