Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis of Full Length and Truncated Microcin B17 Analogues as DNA Gyrase Poisons

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General Synthetic Experimental

Low-resolution mass spectra were recorded on a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode unless indicated otherwise.

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with a 2996 photodiode array detector and an Alliance series column heater set at 30 °C. A Waters Sunfire 5 μm, 2.1 x 150 mm column (C-18) was used at a flow rate of 0.2 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). Results were analyzed with Waters Empower software.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 230 and 254 nm. Peptides were purified on a Waters Sunfire 5 μm (C-18) preparative column operating at a flow rate of 7 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B).

LC-MS was performed on a Shimadzu LC-MS 2020 instrument consisting of a LC-M20A pump and a SPD-20A UV/Vis detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. Separations were performed on a Waters Sunfire 5 μm, 2.1 x 150 mm column (C18), operating at a flow rate of 0.2 mL min^{-1}. Separations were performed using a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) using gradient elution.

Materials

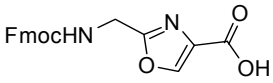
Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254). Flash column chromatography was performed using 230-400 mesh Kieselgel 60 silica eluting with analytical grade solvents as described. Ratios of solvents used for TLC and column chromatography are expressed in v/v as specified. Compounds were visualised by UV light at 254 nm or using vanillin or cerium molybdate stain.

Commercial materials were used as received unless otherwise noted. Dichloromethane was distilled from calcium hydride, and THF was distilled from sodium/benzophenone. Anhydrous methanol, dimethylformamide and diethyl ether were purchased from Sigma Aldrich. Reactions were carried out under an atmosphere of nitrogen or argon unless

otherwise stated. Solid-phase peptide synthesis (SPPS) was performed in Torviq polypropylene fritted syringes. Solid-phase resins were purchased from Novabiochem.

Strains and reagents: Recombinant Microcin B17 was produced from DH5α (*pUC19-mcb*) provided by Christopher T. Walsh (Harvard Medical School, Boston, USA) following the previously reported method.[1] Relaxed pBR322, *E. coli* GyrA and GyrB were purchased from Inspiralis Ltd (Norwich, UK).

Heterocyclic amino acids (11-14)



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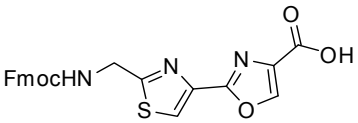

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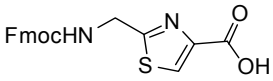

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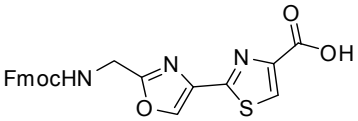
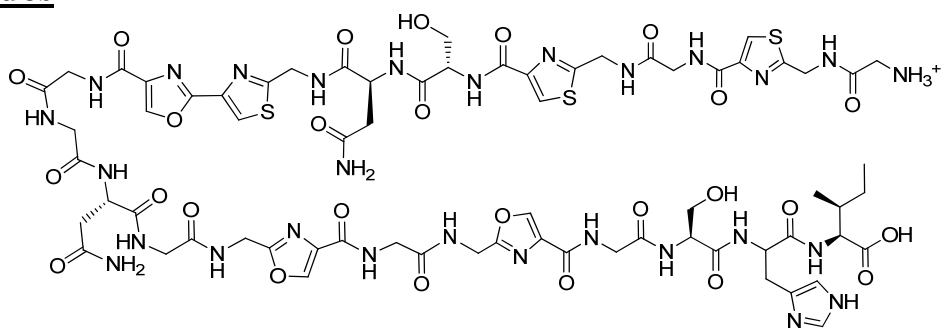


14

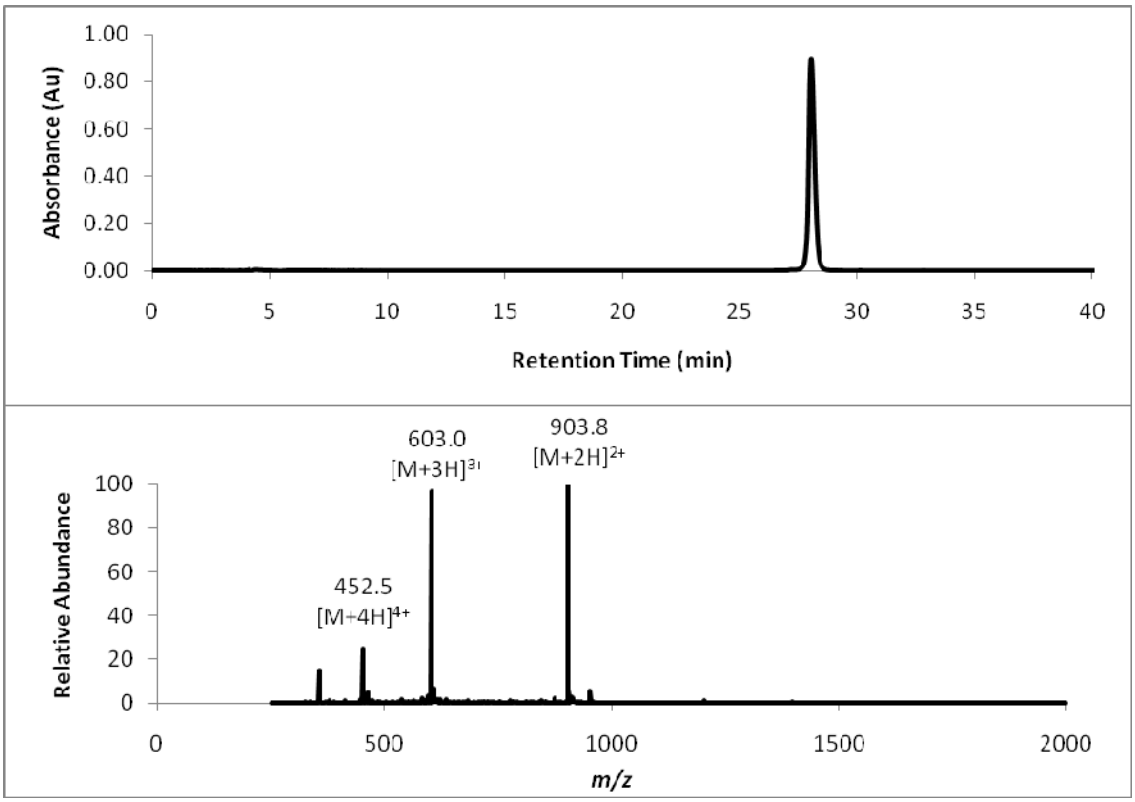
Fmoc-protected amino acids **11-14** were synthesized as previously reported.[2]

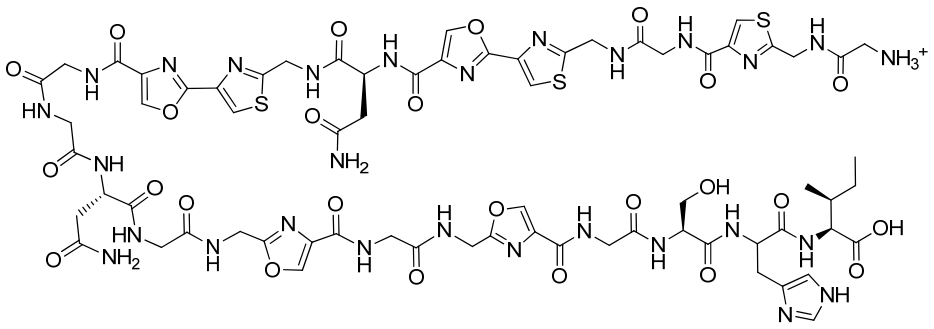
Peptides



10a

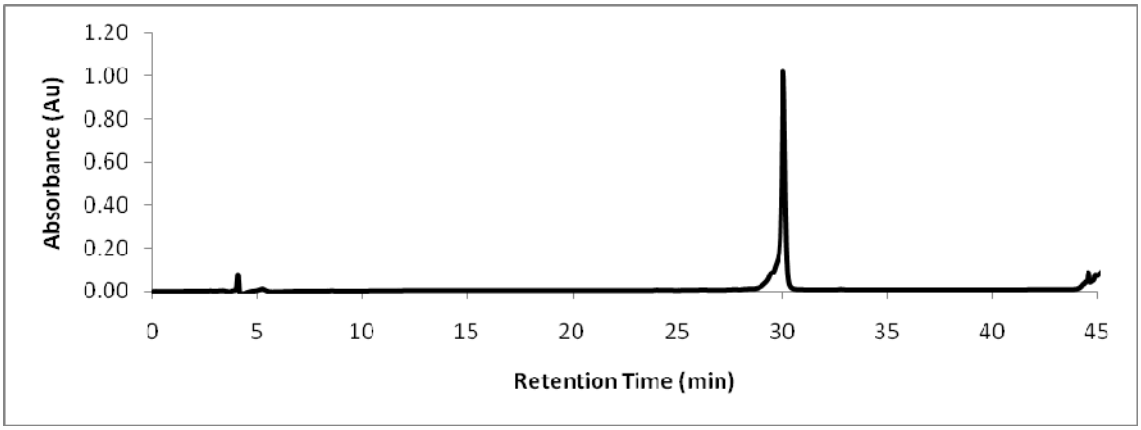
Peptide **10a** was synthesised using the procedures outlined for Fmoc-strategy SPPS on Wang resin preloaded with Fmoc-Ile (25 μmol scale). After the final Fmoc deprotection the peptide was cleaved from the resin and purified using preparative RP-HPLC, affording **10a** as a white solid after lyophilisation (20 mg, 45% yield). Analytical HPLC: R_t 28.0 min (0-30% B over 40 min, 0.1% TFA, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$: 903.3, $[M+3H]^{3+}$: 602.5, $[M+4H]^{4+}$: 452.1,; Mass Found (ESI^+); 903.8 $[M+2H]^{2+}$, 603.0 $[M+3H]^{3+}$, 452.5 $[M+4H]^{4+}$.

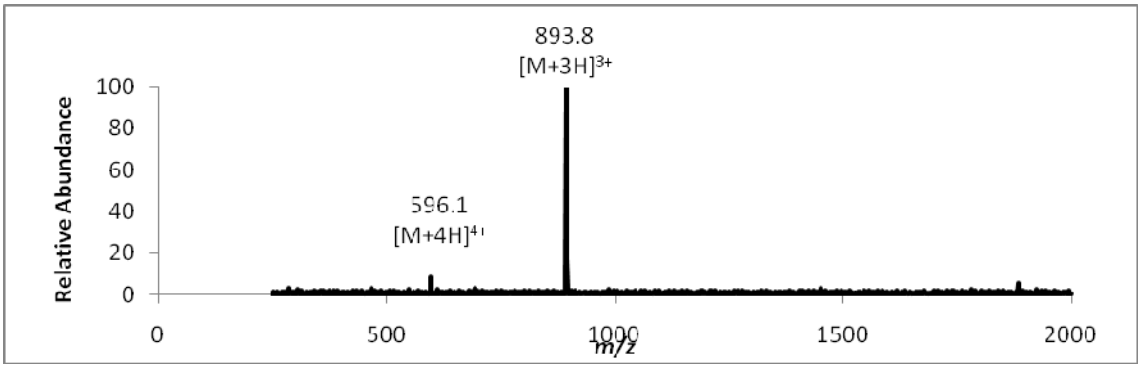


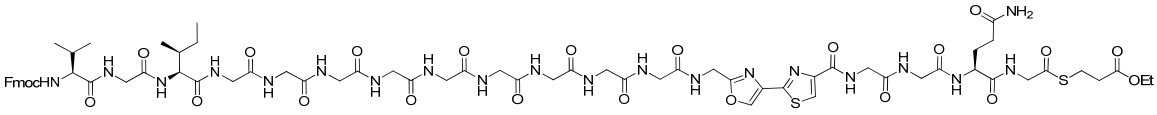


10b

Peptide **10b** was synthesised using the procedures outlined for Fmoc-strategy SPPS on Wang resin preloaded with Fmoc-Ile (25 μmol scale). After the final Fmoc deprotection the peptide was cleaved from the resin and purified using preparative RP-HPLC, affording **10b** as a white solid after lyophilisation (17 mg, 38% yield).Analytical HPLC: R_t 30.0 min (0-30% B over 40 min, 0.1% TFA, λ = 280 nm); Calculated Mass $[M+3H]^{3+}$: 893.3, $[M+4H]^{4+}$: 595.8; Mass Found (ESI^+); 893.8 $[M+3H]^{3+}$, 596.1 $[M+4H]^{4+}$.

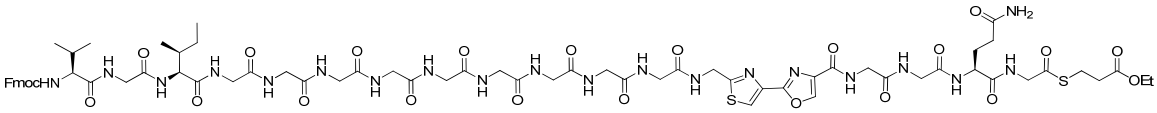






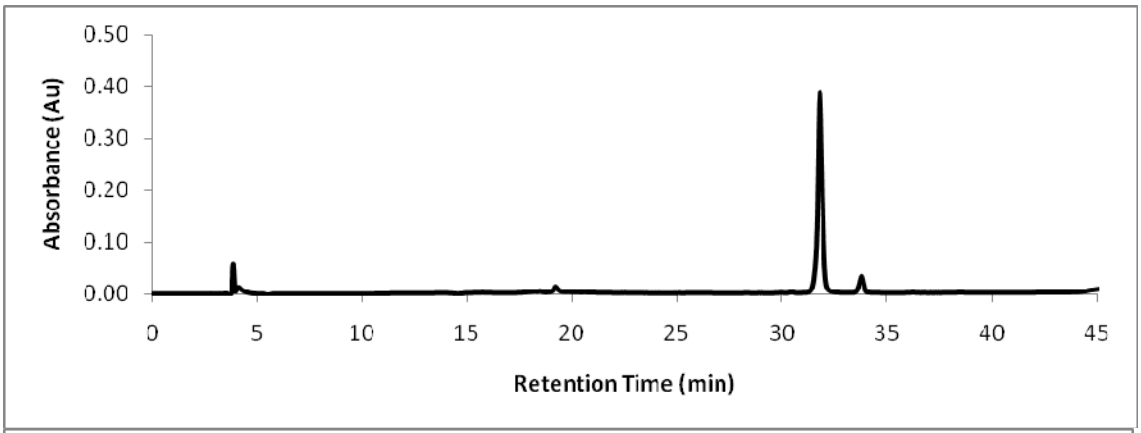
9a

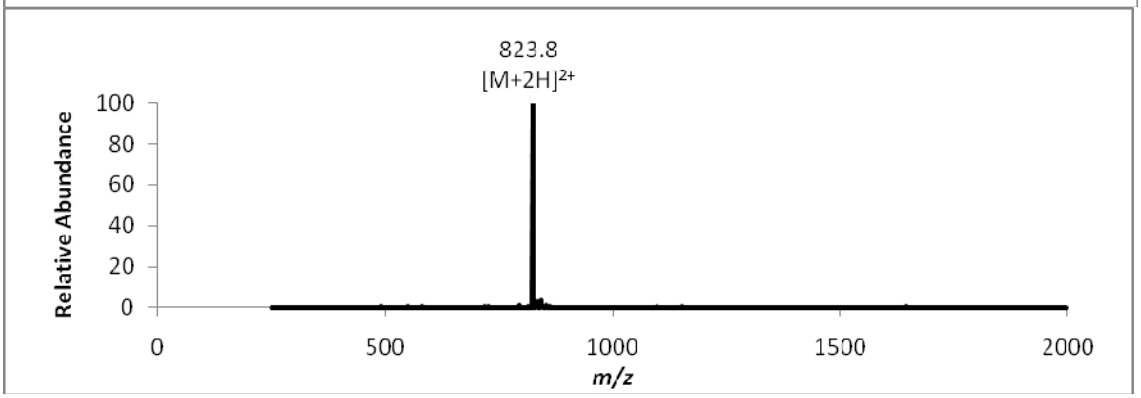
Fmoc-protected thioester **9a** was synthesised on 2-chloro-trityl chloride resin (25 μmol) as previously reported.[2]

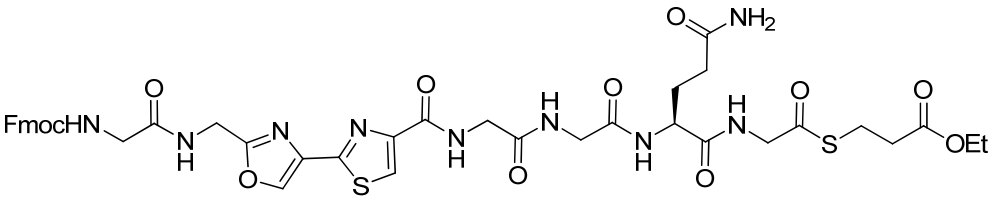


9b

Fmoc-protected thioester **9b** was synthesised on 2-chloro-trityl chloride resin (25 μmol) using the iterative Fmoc-SPPS procedure. Following elongation, the protected peptide was cleaved and thioesterified according to the general procedure affording the title peptide as a fluffy white solid after RP-HPLC purification (9.9 mg, 24 %). Analytical HPLC: R_t 31.8 min (0-70% B over 40 min, 0.1% TFA, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$: 823.3, Mass Found (ESI^+); 823.8 $[M+H]^+$.

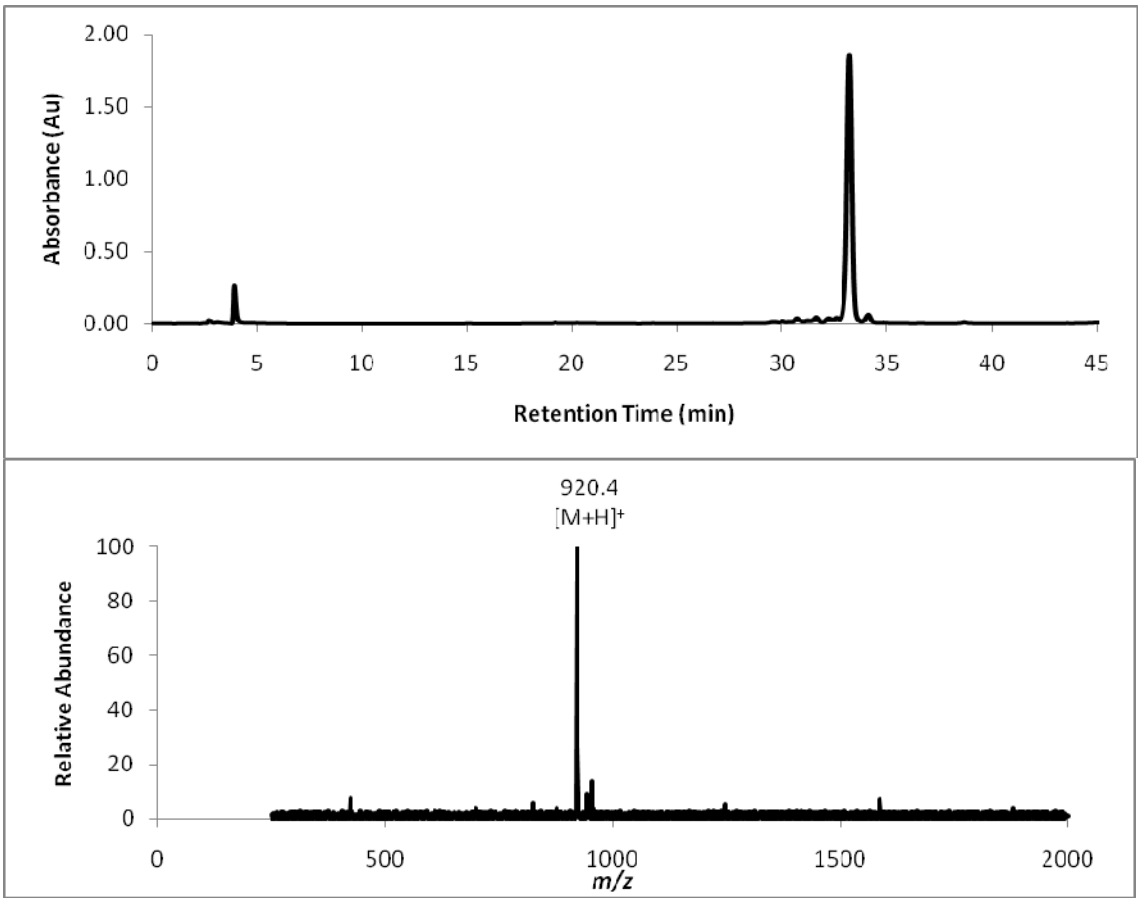


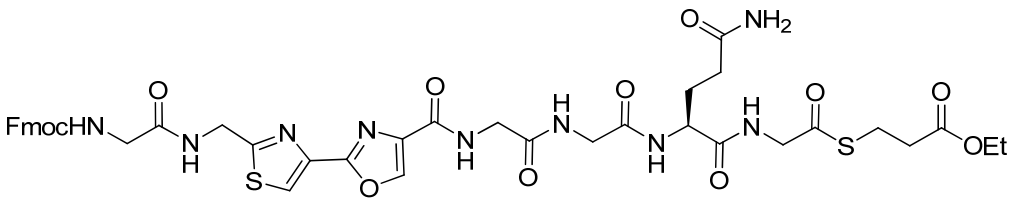




9c

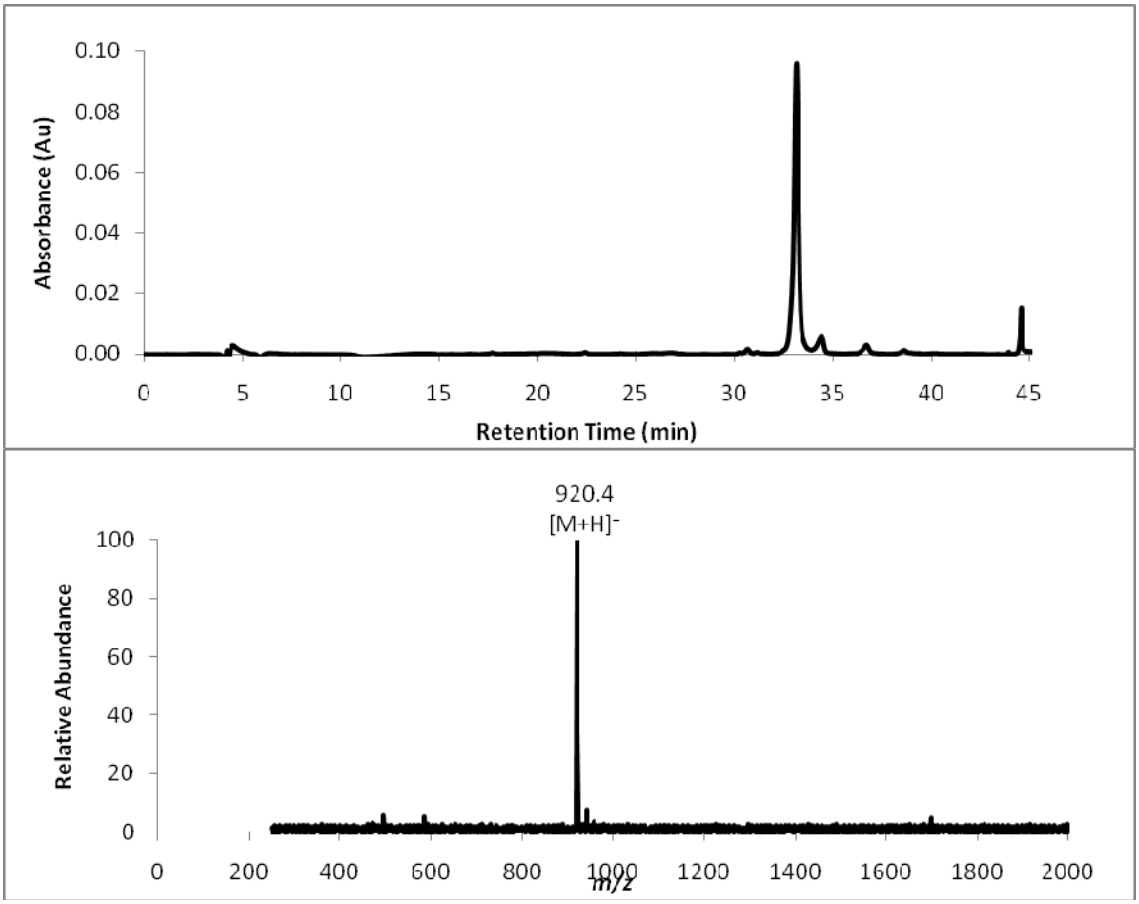
Fmoc-protected thioester **9c** was synthesised on 2-chloro-trityl chloride resin (25 μmol) using the iterative Fmoc-SPPS procedure. Following elongation, the protected peptide was cleaved and thioesterified according to the general procedure affording the title peptide as a fluffy white solid after RP-HPLC purification (17.0 mg, 74%). Analytical HPLC: R_t 33.2 min (0-70% B over 40 min, 0.1% TFA, λ = 280 nm); Calculated Mass $[M+H]^+$: 920.3; Mass Found (ESI^+); 920.4 $[M+H]^+$.

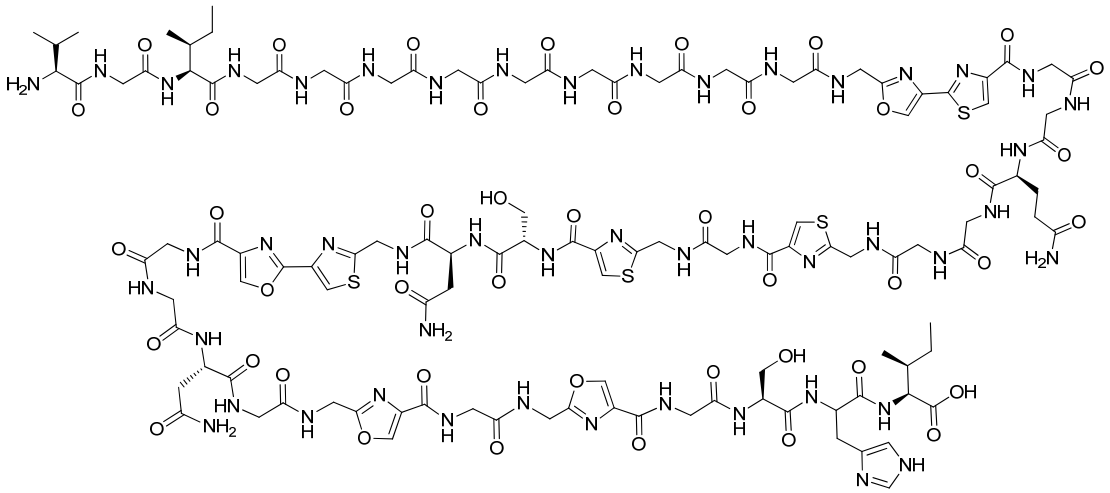




9d

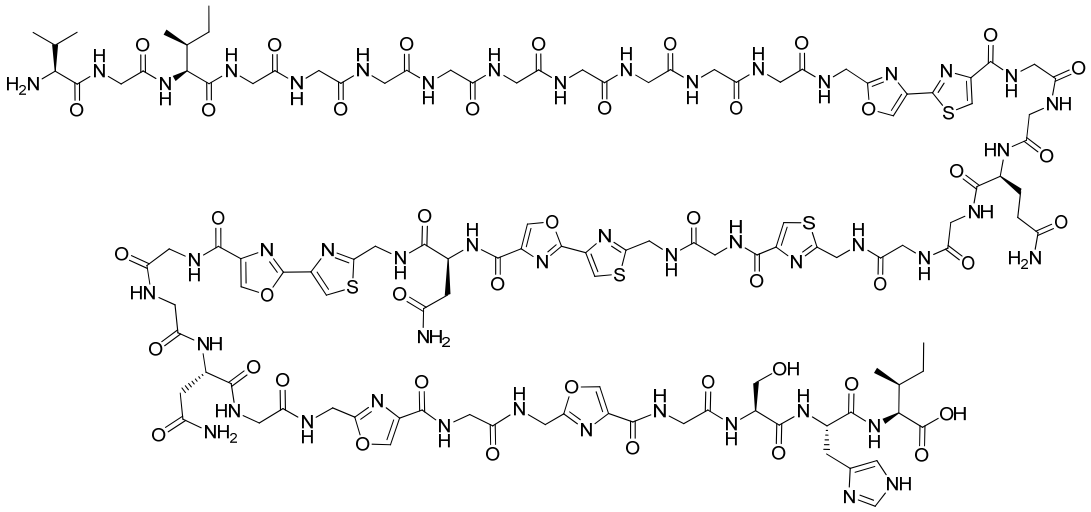
Fmoc-protected thioester **9d** was synthesised on 2-chloro-trityl chloride resin (25 μmol) using the iterative Fmoc-SPPS procedure outlined. Following elongation, the protected peptide was cleaved and thioesterified according to the general procedure affording the title peptide as a fluffy white solid after RP-HPLC purification (15.6 mg, 68%). Analytical HPLC: R_t 33.1 min (0-70% B over 40 min, 0.1% TFA, λ = 280 nm); Calculated Mass $[M+H]^+$: 920.3; Mass Found (ESI^+); 920.4 $[M+H]^+$.





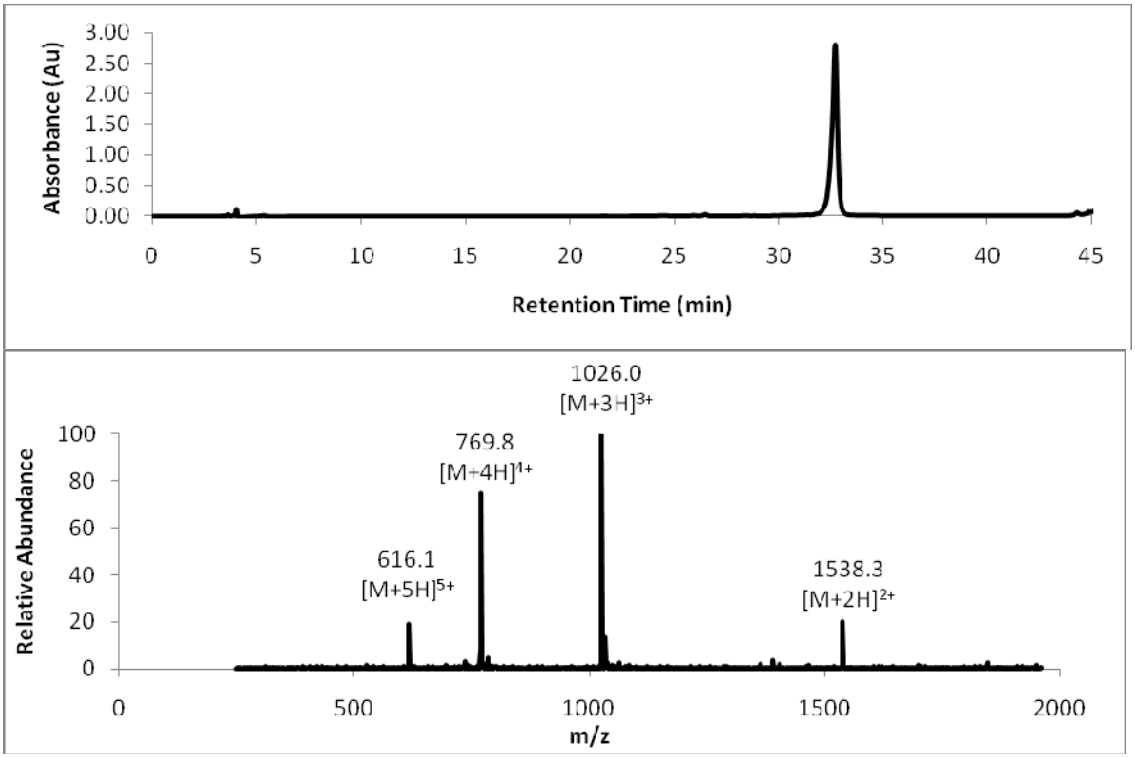
MccB17 (**1**)

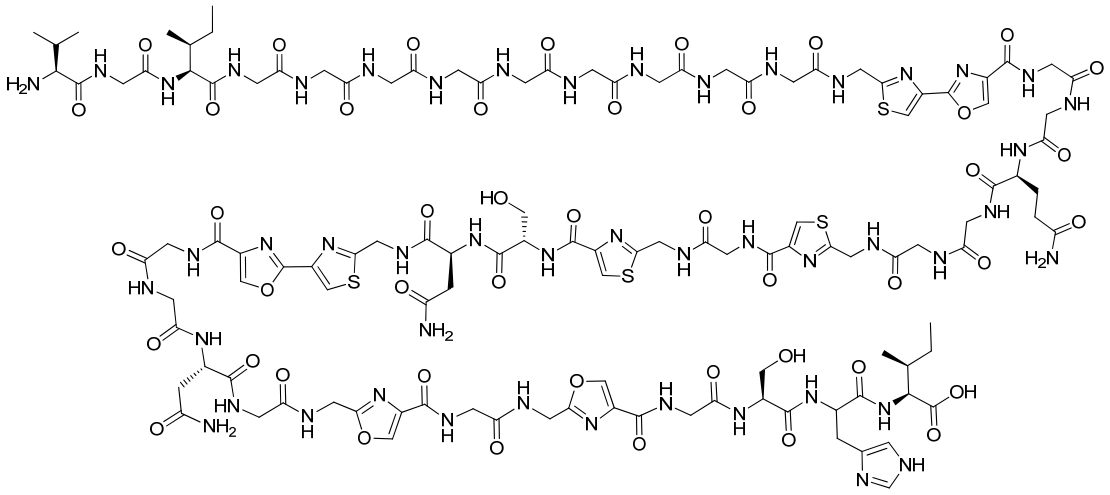
MccB17 (**1**) was synthesised from peptide **10a** (5.2 mg, 2.9 μmol), and thioester **9a** (5.7 mg, 3.5 μmol) as previously reported,[2] affording **1** after RP-HPLC purification (4.1 mg, 46% over 2 steps).



2

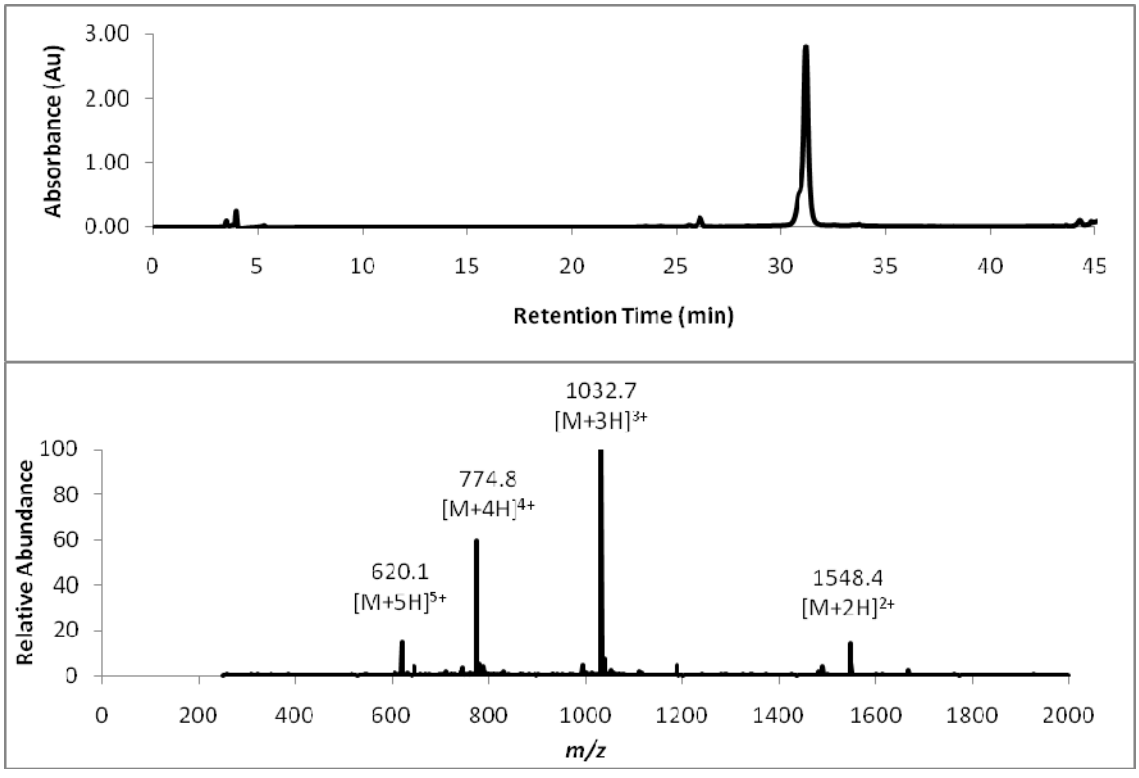
Peptide **2** was synthesised from peptide **10b** (3.7 mg, 2.0 µmol), and thioester **9a** (4.4 mg, 2.7 µmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **2** after RP-HPLC purification (2.4 mg, 38% over 2 steps). Analytical HPLC: R_t 32.7 min (0-30% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1538.0, $[M+3H]^{3+}$: 1025.7, $[M+4H]^{4+}$: 769.5, $[M+5H]^{5+}$: 615.8; Mass Found (ESI^+); 1538.3 $[M+2H]^{2+}$, 1026.0 $[M+3H]^{3+}$, 769.8 $[M+4H]^{4+}$, 616.1 $[M+5H]^{5+}$.

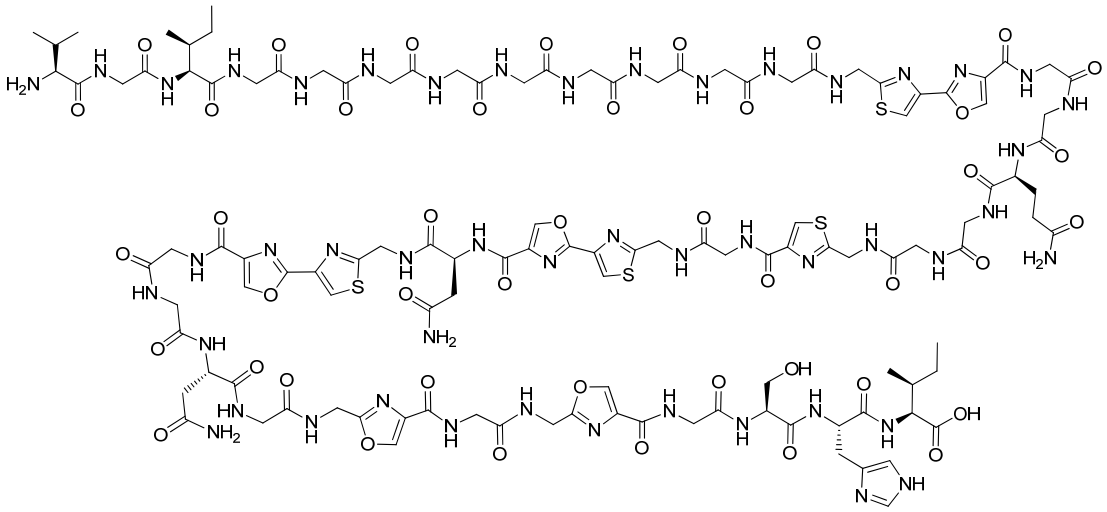




3

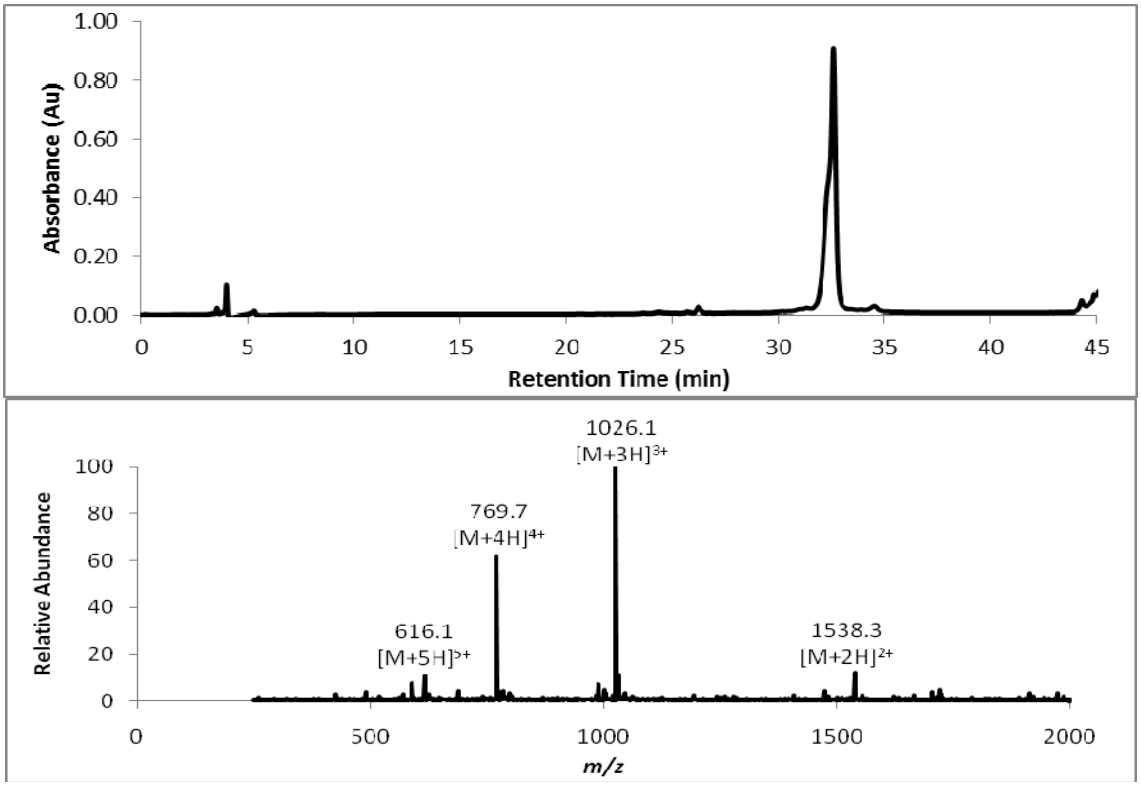
Peptide **3** was synthesised from peptide **10a** (3.6 mg, 2.0 µmol), and thioester **9b** (4.3 mg, 2.6 µmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **3** after RP-HPLC purification (3.2 mg, 52% over 2 steps). Analytical HPLC: R_t 31.2 min (0-30% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1548.0, $[M+3H]^{3+}$: 1032.4, $[M+4H]^{4+}$: 774.5, $[M+5H]^{5+}$: 619.8; Mass Found (ESI^+); 1548.4 $[M+2H]^{2+}$, 1032.7 $[M+3H]^{3+}$, 774.8 $[M+4H]^{4+}$, 620.1 $[M+5H]^{5+}$.

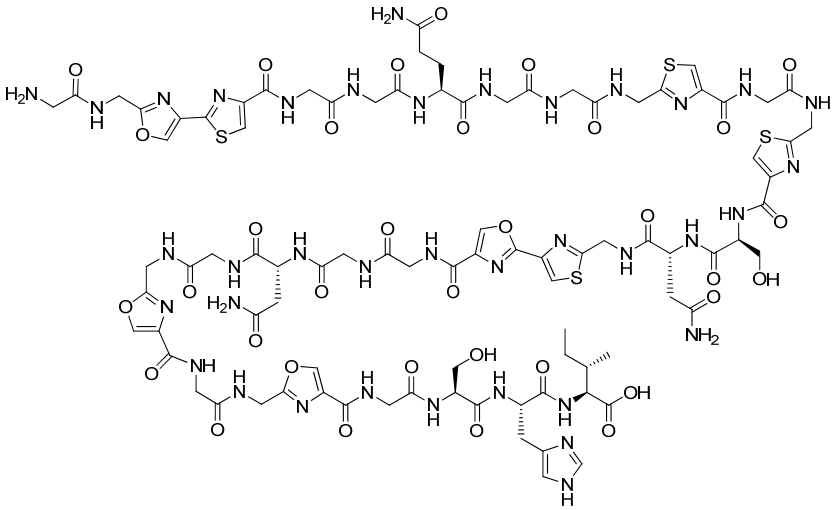




4

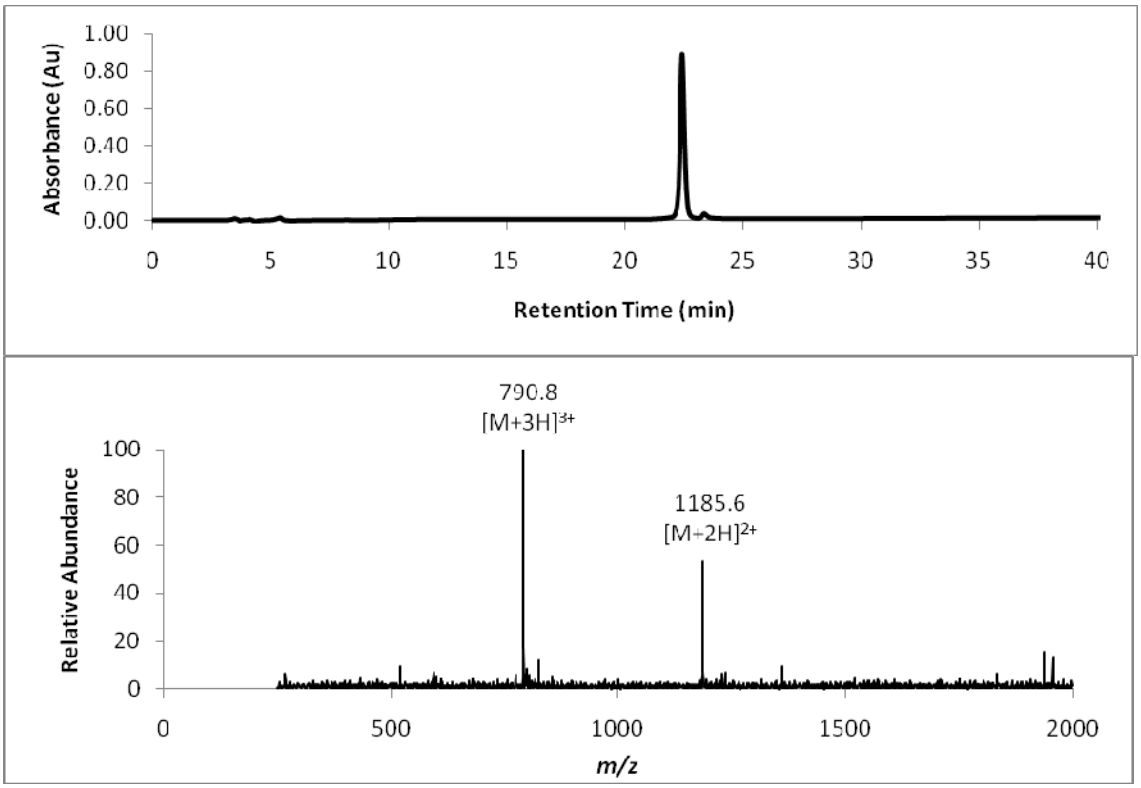
Peptide **4** was synthesised from peptide **10b** (3.6 mg, 2.0 μmol), and thioester **9b** (4.4 mg, 2.7 μmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **4** after RP-HPLC purification (2.2 mg, 35% over 2 steps. Analytical HPLC: R_t 32.6 min (0-50% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1538.0, $[M+3H]^{3+}$: 1025.7, $[M+4H]^{4+}$: 769.5, $[M+5H]^{5+}$: 615.8; Mass Found (ESI^+); 1538.3 $[M+2H]^{2+}$, 1026.1 $[M+3H]^{3+}$, 769.7 $[M+4H]^{4+}$, 616.1 $[M+5H]^{5+}$.

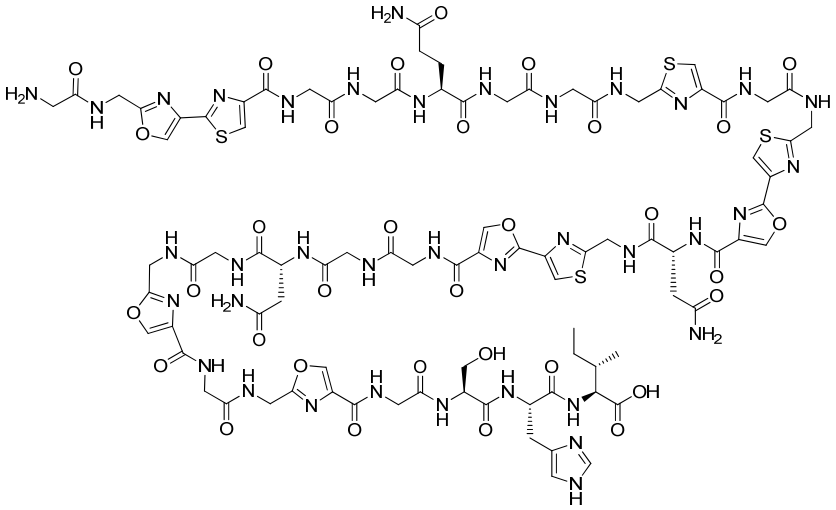




5

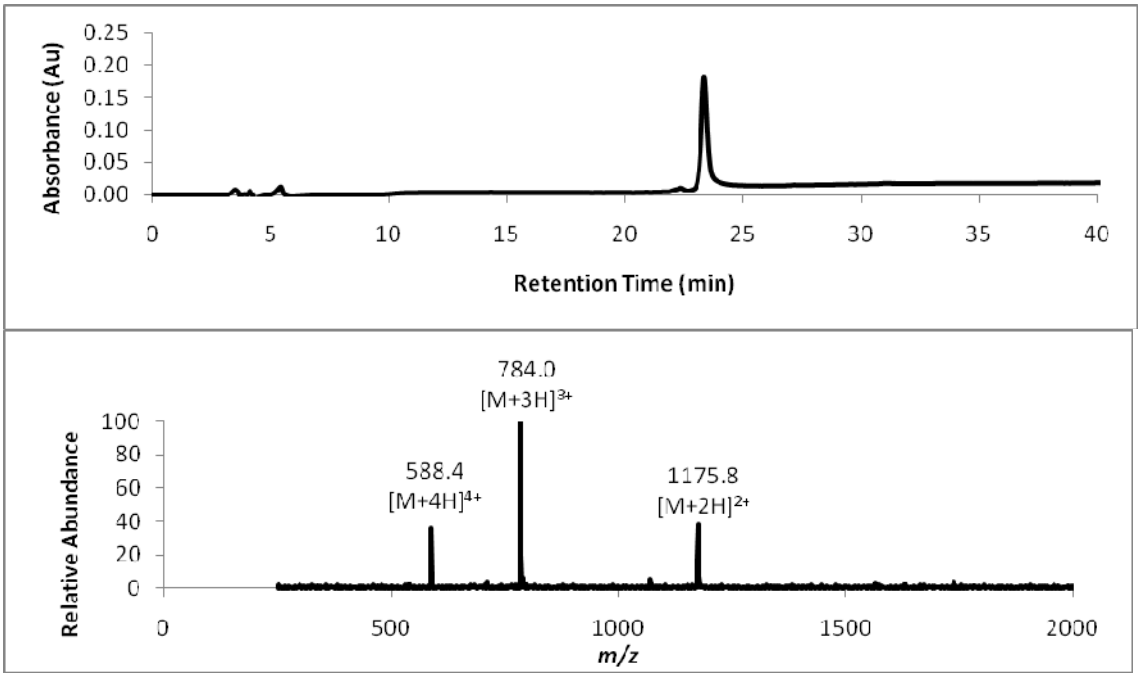
Peptide **5** was synthesised from peptide **10a** (2.0 mg, 1.1 μmol), and thioester **9c** (1.5 mg, 1.6 μmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **5** after RP-HPLC purification (1.7 mg, 63% over 2 steps). Analytical HPLC: R_t 22.4 min (0-50% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1184.8, $[M+3H]^{3+}$: 790.2; Mass Found (ESI^+); 1185.6 $[M+2H]^{2+}$, 790.8 $[M+3H]^{3+}$..

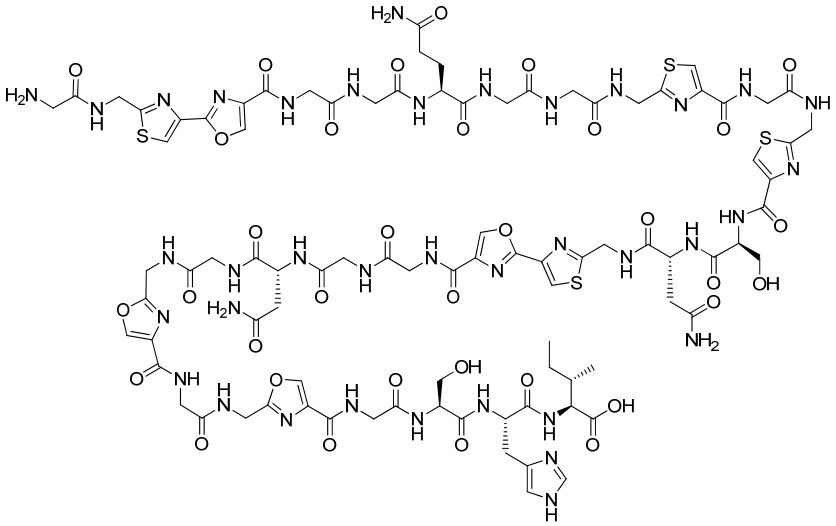




6

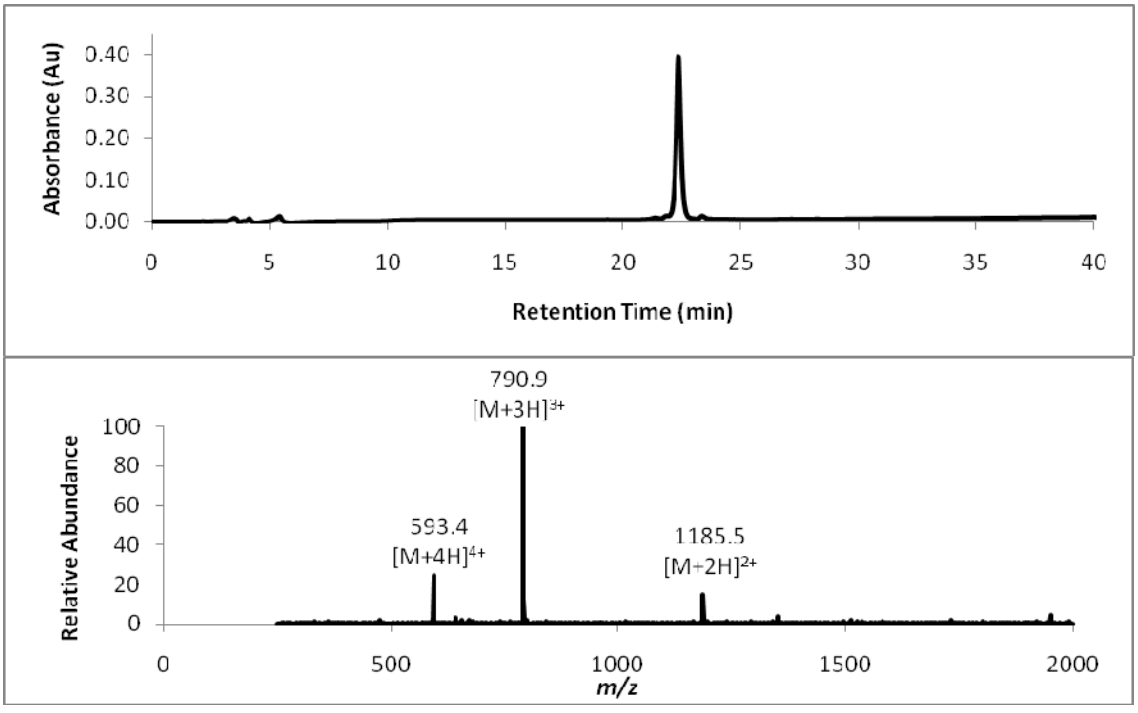
Peptide **6** was synthesised from peptide **10b** (2.0 mg, 1.1 μmol), and thioester **9c** (1.5 mg, 1.6 μmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **6** after RP-HPLC purification (1.4 mg, 54% over 2 steps). Analytical HPLC: R_t 23.3 min (0-50% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1174.8, $[M+3H]^{3+}$: 783.6, $[M+4H]^{4+}$: 587.9; Mass Found (ESI^+); 1175.8 $[M+2H]^{2+}$, 784.0 $[M+3H]^{3+}$, 588.4 $[M+4H]^{4+}$.

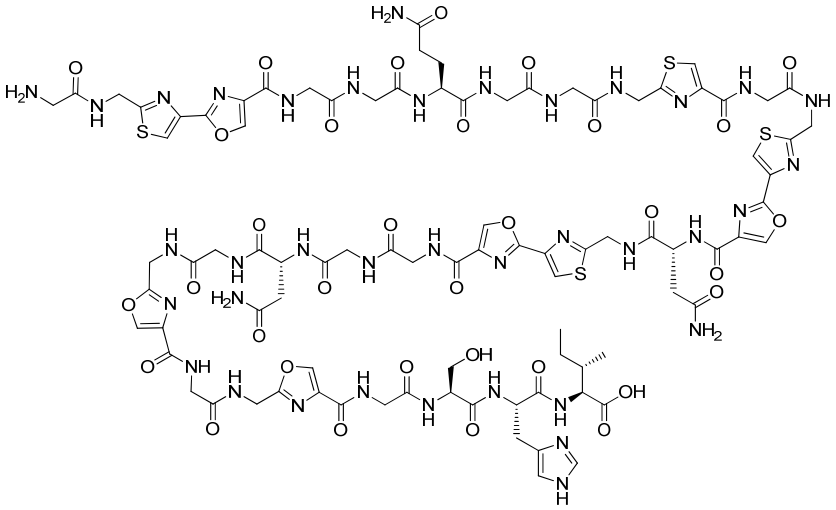




7

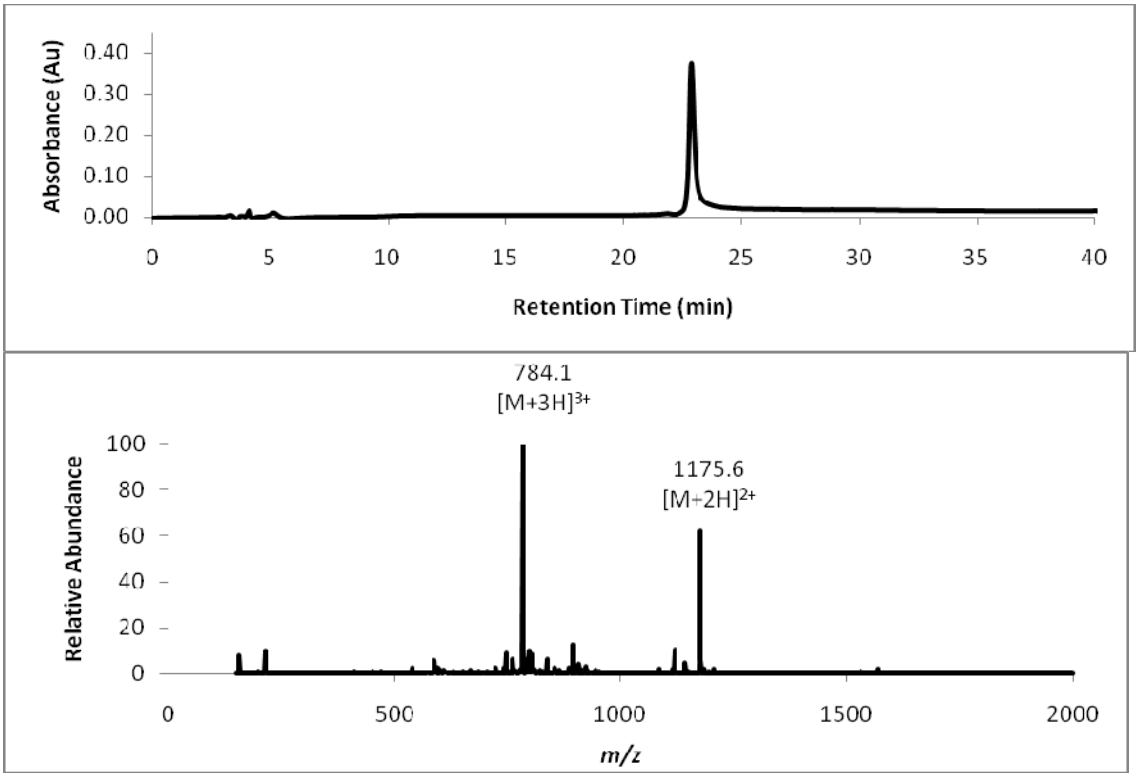
Peptide **7** was synthesised from peptide **10a** (2.0 mg, 1.1 µmol), and thioester **9d** (1.5 mg, 1.6 µmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **7** after RP-HPLC purification (1.9 mg, 72% over 2 steps). Analytical HPLC: R_t 22.3 min (0-50% B over 40 min, 0.1% TFA, λ = 230 nm); Analytical HPLC: R_t 22.4 min (0-50% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1184.8, $[M+3H]^{3+}$: 790.2; Mass Found (ESI^+); 1185.6 $[M+2H]^{2+}$, 790.8 $[M+3H]^{3+}$.



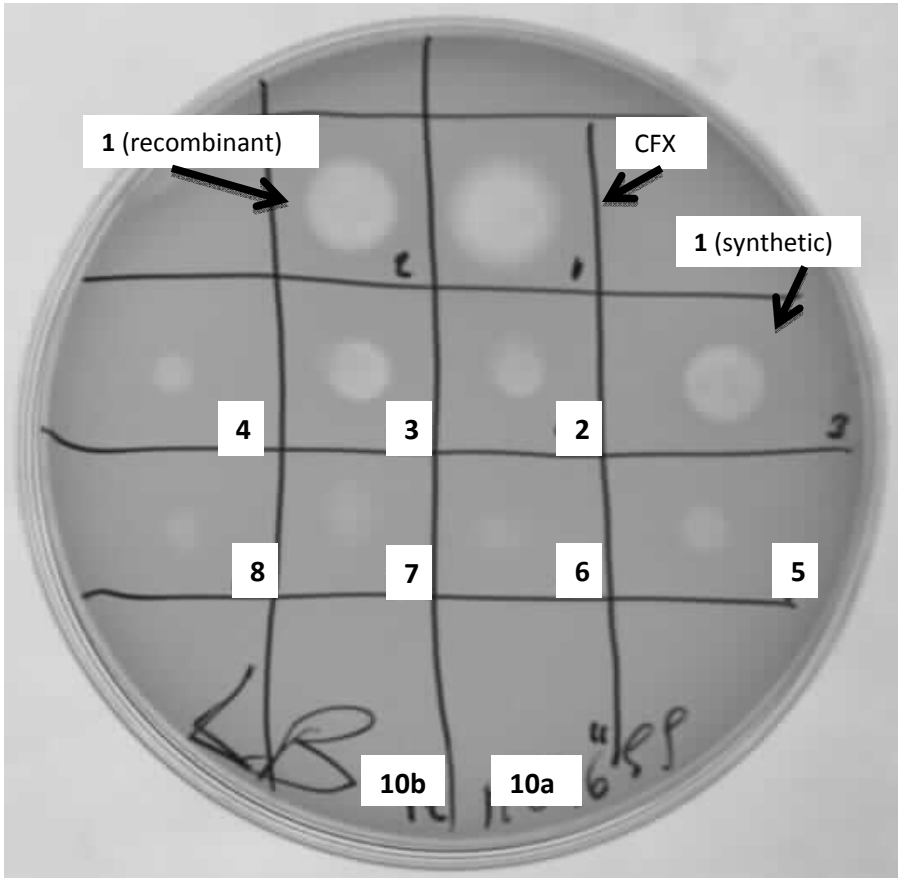


8

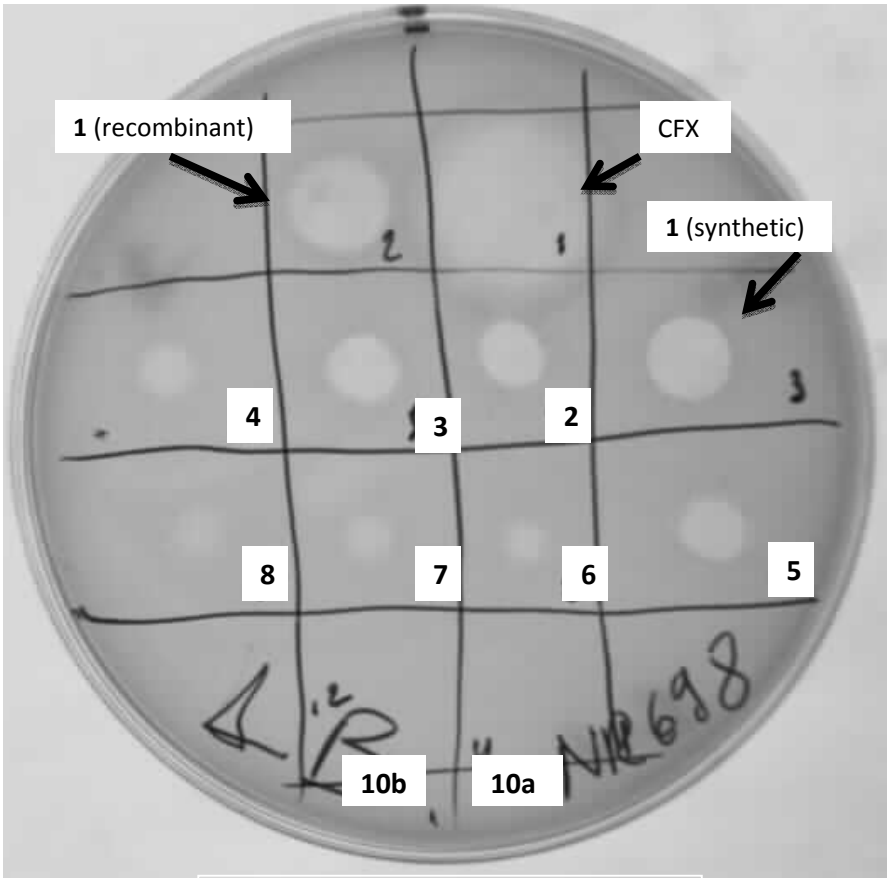
Peptide **8** was synthesised from peptide **10b** (2.0 mg, 1.1 μmol), and thioester **9d** (1.5 mg, 1.6 μmol) as outlined in the silver(I)-promoted fragment condensation general procedure, affording **8** after RP-HPLC purification (1.6 mg, 60% over 2 steps). Analytical HPLC: R_t 22.8 min (0-50% B over 40 min, 0.1% TFA, λ = 230 nm); Calculated Mass $[M+2H]^{2+}$: 1174.8, $[M+3H]^{3+}$: 783.6; Mass Found (ESI^+); 1175.6 $[M+2H]^{2+}$, 784.1 $[M+3H]^{3+}$.



Halo Assay Results



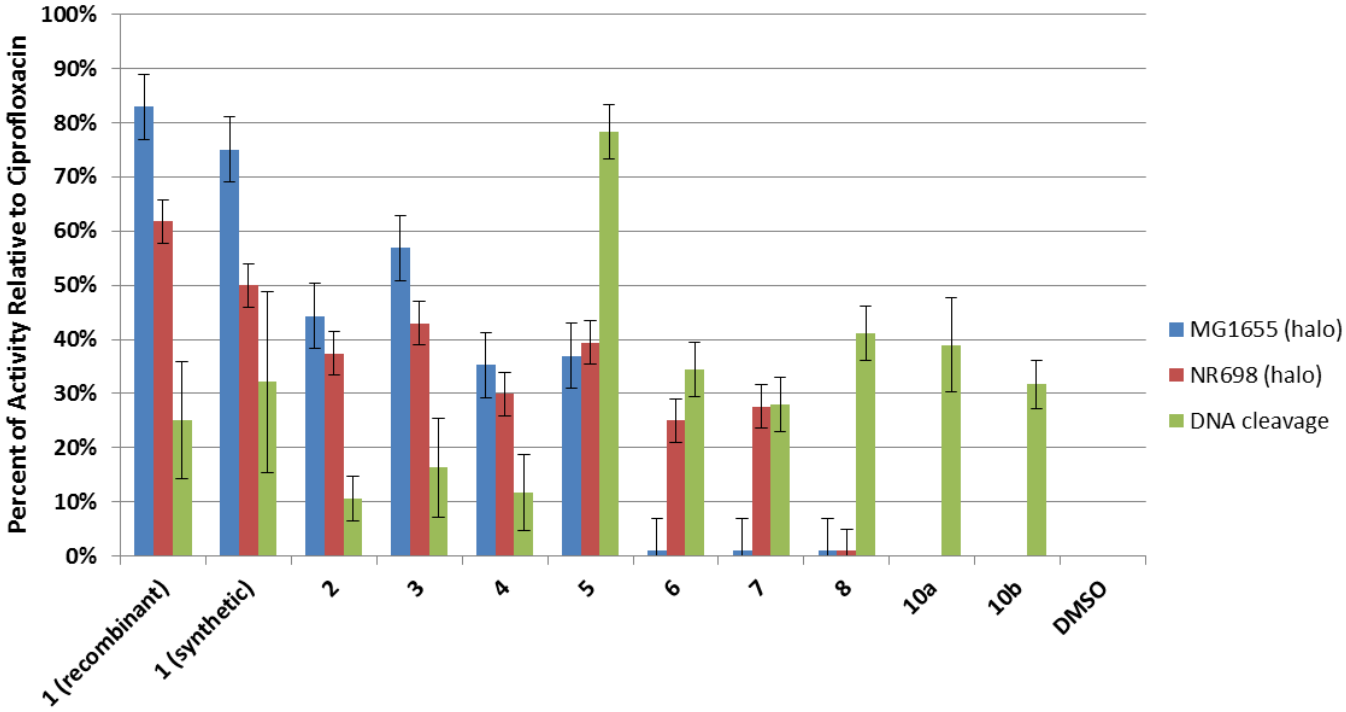
***E. coli* standard strain (MG1655)**



***E. coli* "permeable" strain (NR698)**

Bacteria were grown on LB agar plates; 2 µl of DMSO solution containing MccB17 analogues or ciprofloxacin (CFX) were added to the plates: CFX at 120 µM, compounds **1-8**, **10a** and **10b** at 1.5 mM.

Summary of Biological Activities



Summary of the biological activities for compounds **1**-**8**, **10a** and **10b** relative to Ciprofloxacin as described in the manuscript. Data include halo assays against *E. coli* strains MG1655 (red) and NR698 (blue), and gyrase/DNA cleavage assays (green).

References

[1] R. S. Roy, N. L. Kelleher, J. C. Milne, C. T. Walsh, *Chem Biol* **1999**, *6*, 305-318.
[2] R. E. Thompson, K. A. Jolliffe, R. J. Payne, *Org Lett* **2011**, *13*, 680-683.