Electronic Supplementary Information

Ligand Amplification in a Dynamic Combinatorial Glycopeptide Library

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S1. Mass Spectrometry

Electrospray ionization-mass spectrometric (ESI-MS) analysis of DCLs was conducted on a Micromass LCT time-of-flight mass spectrometer coupled to a Waters Alliance 2790 HPLC. Samples were loop injected into the MS using the LC autosampler. The ESI capillary and cone voltages were optimized at 2.8 kV and 50 V respectively. DCL samples were quenched by the addition of formic acid (4 %), and diluted 20-fold in MeOH before injection into the MS. ‘Native’ ESI-MS of WGA was performed on a Q-TOFmicro quadrupole-time of flight mass spectrometer. The standard Micromass source was replaced with an Advion BioSciences NanoMate™ chip-based nano-ESI source. Protein samples were sprayed from 10 mM NH₄OAc (pH 7) using a chip nozzle voltage of 1.69 kV, and a cone voltage of 100 V. Collisional cooling of ions was achieved by partially closing a valve on the rotary vacuum pump, leading to an increased pressure in the intermediate vacuum region of the mass spectrometer. CsI was used for calibration.

Denaturing ESI-MS analysis of WGA revealed the presence of two major polypeptide chains with masses of 17086.6 and 17175.8 ± 2 Da, accounting for the multiple signals seen for the dimer in Fig. 3 in the main text.

S2. Experimental

bis-N-Butoxycarbonyl-L-glycinyl-L-cysteine methylester 3
Disopropylamine (1 mL) was added to a solution of bis-L-cysteine methylester (0.39 g, 1.4 mmol), 1,3-dicyclohexylcarbodiimide (0.55 g, 2.8 mmol), hydroxybenzotriazole (0.36 g, 2.8 mmol) and N-Butoxycarbonyl-L-glycine (0.50 g, 2.8 mmol) in DCM (50 mL) and the reaction mixture was stirred at room temperature under Argon. After 24 h, t.l.c. (ethyl acetate:methanol, 5:1) showed the formation of a product (R_f 0.4). The reaction mixture was filtered, diluted with DCM (40 mL) and washed with water (2 x 30 mL). The organic layers was dried (MgSO4), filtered and concentrated in vacuo to yield bis-N-butoxycarbonyl-L-glycinyl-L-cysteine methylester (0.49 g, 60%) as a white amorphous solid; [α]D^21 +35.5 (c, 1.0 in CHCl3); δ_H (400 MHz, CDCl3) 1.46 (18H, s, (CH3)3), 3.20 (4H, m, CH2-Cys), 4.70-4.74 (4H, m, αCH2-Gly), 3.79 (6H, s, OMe), 4.86 (2H, m, αH-Cys), 5.51 (2H, m, NH-Gly), 7.23 (2H, d J_NH,αH-Cys 7.4 Hz, NH-Cys); δ_C (500 MHz, CD3OD) 25.39 (t, CH2(Cys)), 28.15 (q, C(CH3)3), 40.55 (t, CH2(Gly)), 51.78 (q, OCH3), 52.66 (d, α-C-Cys), 80.66 (s, C(CH3)3), 157.87 (s, NC(O)O), 170.49 (s, C(O) Gly), 170.99 (s, C(O) Cys); m/z (ESI+) 605 (M+Na+, 100%); HRMS (ESI+) calcd for C22H39N4O10S2 583.2108. Found 583.2120.

N-Butoxycarbonyl-L-glycinyl-L-cysteine methylester 4

Tributyl phosphine (245 mL, 1.0 mmol) was added to a solution of bis-N-butoxycarbonyl-L-glycinyl-L-cysteine methylester (246 mg, 0.83 mmol) in THF (10 mL) and methanol (2 mL). After 40 min, t.l.c. (1:1, petro:ethyl acetate) indicated formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0.0). The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography to afford N-butoxycarbonyl-L-glycinyl-L-cysteine methylester (496 mg, quantitative) as a foam; [α]D^21 +33.9 (c, 1.1 in CHCl3); ν_max
(thin film) 3320 (br, SH), 1744, 1674 (s, C=O) cm\(^{-1}\); \(\delta_H\) (400 MHz, CDCl\(_3\)) 1.35 (9H, s, (CH\(_3\))\(_3\)), 1.47 (1H, at, \(J\) 8.8 Hz, SH), 2.87-2.91 (2H, m, CH\(_2\)-Cys), 3.68 (3H, s, OMe), 3.76 (2H, d, \(J\) 4.9 Hz, CH\(_2\)-Gly), 4.75-4.79 (1H, m, \(\alpha\)H-Cys), 5.69 (1H, m, NH-Gly), 7.17 (1H, d \(J\) 6.4 Hz, NH-Cys); \(\delta_C\) (100 MHz, CDCl\(_3\)) 26.6 (t, CH\(_2\)-Cys), 28.2 (q, CH\(_3\)), 52.6 (q, OMe), 53.7 (d, \(\alpha\)C), 80.0 (s, C(CH\(_3\))\(_3\)), 156.2, 169.8, 170.4 (3 x s, 3 x C=O); \(m/z\) (ESI\(^+\)) 315 (M+Na\(^{+}\),100), 293 (M+H\(^{+}\), 70 %); HRMS (ESI\(^+\)) calcd. for C\(_{11}\)H\(_{21}\)N\(_2\)O\(_5\)S (M+H\(^{+}\)) 293.1171. Found 293.1174.

**N-Butoxycarbonyl-glycinyl-L-cysteinyl-(S-1-β-D-glucopyranosyl disulfide) methylester G**

A solution of \(N\)-butoxycarbonyl-glycinyl-L-cysteine methylester (45 mg, 0.13 mmol) in methanol (8 mL) was added dropwise to a solution of phenyl 1-selenenylsulfide-β-D-glucopyranoside (100 mg, 0.3 mmol) and triethylamine (15 \(\mu\)L, 0.15 mmol) in methanol (12 mL) and the resulting solution was stirred at room temperature. After 30 min, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a major product (R\(_f\) 0.4). The reaction mixture was concentrated \textit{in vacuo} and the residue was purified by flash column chromatography (ethyl acetate:methanol, 9:1) to yield \(N\)-butoxycarbonyl-glycinyl-L-cysteinyl-(S-1-β-D-glucopyranosyl disulfide) methylester (32 mg, 36 %) as a white amorphous solid; \([\alpha]_D^{21}\) -101.2 (c, 1.2 in MeOH); \(\nu_{\text{max}}\) (thin film) 3346 (br, OH, NH), 1740, 1675 (s, C=O) cm\(^{-1}\); \(\delta_H\) (400 MHz, CD\(_3\)OD) 1.48 (9H, s, C(CH\(_3\))\(_3\)), 3.08 (1H, dd, \(J\)\(_{\text{CH2OH}}\) 8.0 Hz, \(J\)\(_{\text{CH,CIP}}\) 13.9 Hz, CHH\(^-\)-Cys), 3.32-3.38 (5H, m, CHH\(^-\)-Cys, H-4, H-5, CH\(_2\)-Gly), 3.43 (1H, at, \(J\) 8.8 Hz, H-3), 3.53 (1H, at, \(J\) 9.0 Hz, H-2), 3.71 (1H, dd, \(J\)\(_{5,6}\) 5.5 Hz, \(J\)\(_{6,6'}\) 12.0 Hz, H-6), 3.76 (3H, s, OMe), 3.91 (1H, dd, \(J\)\(_{5,6'}\) 1.8 Hz, H-6'), 4.38 (1H, d, \(J\)\(_{1,2}\) 9.4 Hz, H-1), 4.96 (1H, m, \(\alpha\)H); \(\delta_C\) (100
MHz, CD3OD) 27.3 (q, CH3), 4.01 (t, CH2-Cys), 43.1 (t, CH2-Gly), 51.6 (q, OMe), 61.5 (t, C-6), 69.9, 81.1 (2 x d, C-4, C-5), 71.0 (d, C-2), 78.0 (d, C-3), 80.0 (s, C(CH3)3), 90.2 (d, C-1), 171.1 (s, C=O); m/z (ESI+) 509 (M+Na+, 100 %); HRMS calcd. for C17H30N2O10S2Na 509.1240. Found 509.1245.

S3. Library formation

Libraries consisted of 100 µg each of N-butoxycarbonyl-glycinyl-L-cysteinyl-(S-2-acetamino-2-deoxy-1-β-D-glucopyranosyl disulfide) methylester and N-butoxycarbonyl-L-cysteinyl-(S-2-acetamino-2-deoxy-1-β-D-glucopyranosyl disulfide)-L-threonine methylester. Where DTT was used 5 µg was added to the library. Solvents were water, Buffer: a 10mM solution of ammonium acetate adjusted to pH 7.5 by addition of ammonia and a 4% by volume solution of formic acid. 10 µL samples were taken and quenched in 100 µL of 4% formic acid solution before analysis. Wheat Germ Agglutinin (100 µg of a 5 mg/mL solution) was added after 78 h.