Electronic Supporting Information

Monosubstituted Alkenyl Amino Acids for Peptide "Stapling"

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General considerations for synthesis

Non-aqueous reactions were carried out in washed and oven-dried glassware. Solvents and reagents were used as supplied from major suppliers without prior purification unless stated. Anhydrous tetrahydrofuran, acetonitrile, dichloromethane and diethyl ether were obtained from the in-house solvent purification system from Innovative Technology Inc. PureSolv®. Anhydrous dimethyl formamide, methanol and chloroform were obtained from major chemical suppliers equipped with a SureSeal (or equivalent). Solvents used for reactions that are not anhydrous were of HPLC quality and provided by Fisher or Sigma-Aldrich. Water in aqueous solutions and quenching was deionised, and water used for buffers and HPLC was ultra-pure 18 mΩ from an ELGA Purelab system. Mixtures of solvents are quoted as ratios and correspond to a volume:volume ratio.

For peptide synthesis, all amino acids and resins were purchased from either Novasyn (Merck) or Sigma-Aldrich. All amino acids were *N*-Fmoc protected and side chains were protected with Boc (Lys, Trp); *O*^{t}Bu (Ser, Thr); Trt (Cys, Asn, Gln). All peptides were synthesised on Rink Amide MBHA resin with a loading capacity of 0.56 mmol g^{-1}.Synthesis of peptides was performed either manually using vaculate reservoirs and draining from a water aspirator or by the use of a microwave assisted automated peptide synthesiser (CEM Liberty). DMF used in peptide synthesis was of ACS grade from Sigma-Aldrich. Peptide identities were confirmed by the LCMS and HRMS machines stated in S.1.1,. Multiple charge states were used to reinforce the assignment of species observed by LCMS, and the monoisotopic mass used in 4 d.p. analysis.

Thin layer chromatography was performed on Merck Kiesegel 60 F_{254} 0.25 mm precoated aluminium plates. Product spots were visualised under UV light (λ_{max} = 254 nm) and/or by staining with potassium permanganate. If any other TLC dip was used, it is stated under the specific experimental procedure of a molecule. Flash chromatography was performed using silica gel 60 (0.043 – 0.063 mm VWR) using pressure by means of head bellows.

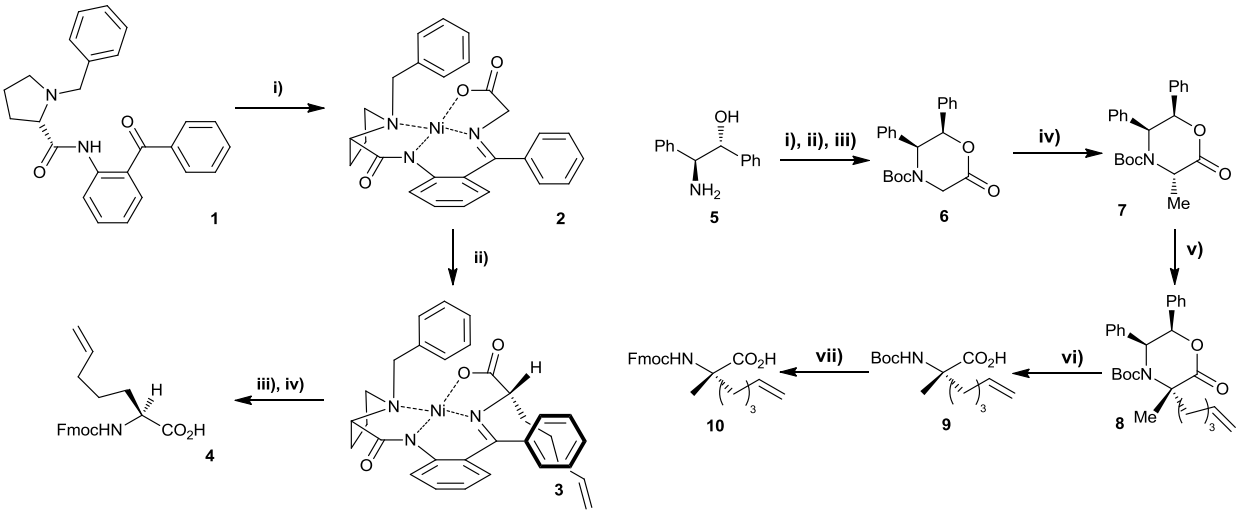
^{1}H NMR spectra were recorded on Bruker DPX 300 (300 MHz) or Avance 500 (500 MHz) spectrometers and referenced to either residual non-deuterated solvent peaks or tetramethylsilane. ^{13}C spectra were recorded on a Bruker Avance 500 (126 MHz) and referenced to the solvent peak. ^{1}H spectra are reported as follows: δ_H (*spectrometer frequency, solvent*): *ppm to two decimal places (number of protons, multiplicity, J coupling constant in Hertz, assignment)*. Chemical shifts are quoted in ppm with signal splitting recorded as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br). Coupling constants, *J*, are measured to the nearest 0.1 Hz. Similarly, ^{13}C spectra are reported as follows: δ_C (*spectrometer frequency, solvent*): *ppm to one decimal place* (*assignment*). Assignments of spectra were assisted by the results of DEPT, COSY, HMQC and HMBC experiments.

Infrared spectra were recorded on a Perkin Elmer Fourier-Transfer spectrometer. Spectra were analysed neat and only structurally important absorptions are quoted. Absorption maxima (ν_{max}) are quoted in wavenumbers (cm^{-1}).

HPLC LC/MS were recorded on a Bruker HCT ultra under the conditions of electrospray ionisation (ESI). HPLC separation was performed on an Agilent 1200 series instrument equipped with a Phenomenex C18 column (50 × 2 mm) using acetonitrile:water as the eluent for positive ion spectra. Values are reported as a ratio of mass to charge. Nominal mass spectra and accurate (4 d.p.) mass spectra were recorded on a Bruker Daltonics micrOTOF Premier Mass Spectrometer, under positive ESI conditions unless otherwise stated.

Optical rotations were recorded on an Optical Activity AA-10 polarimeter using the sodium D line (589 nm). $[\alpha]_D$ are reported in units of 10^{-1} deg dm^2 g^{-1}.

Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. Microanalysis was performed by the School of Chemistry service, utilising a Carlo Erba 1108 Elemental Analyser.



Scheme ESI1 *The synthetic routes of mono and disubstituted alkenyl amino acids. (a) i) $Ni(NO_3)_2$•$6H_2O$, Glycine, NaOMe, MeOH, 96%; ii) 1-iodopent-4-ene 11, NaOH, MeCN, 69%; iii) 1.2M HCl, MeOH, Δ, 95%* ***3a****; iv) Fmoc-OSu, Na_2CO_3, 1,4 dioxane, H_2O, 53%. b) i) Ethyl bromoacetate, Et_3N, THF, 92%* ***5a*** *ii) Boc_2O, toluene, Δ; iii) pTsOH, toluene, 85% both steps; iv) MeI, NaHMDS, THF, –78 °C, 78%; v) 1-Iodopent-4-ene* ***11****, KHMDS, THF, –40 °C, 49%; vi) Li, NH_3 (l), 84%, vii) TFA, DCM then Fmoc-OSu, Na_2CO_3, H_2O:Acetone 1:1, 62%*

Experimental procedures and data for amino acids

(*S*)-Gly-Ni-BPB Schiff Base

(*S*)-2-[*N*-(*N*'-benzylprolyl)amino]-benzophenone **1** (1000 mg, 2.61 mmol), Nickel (II) nitrate hexahydrate (908 mg, 3.13 mmol) and glycine (390 mg, 5.22 mmol) were dissolved in anhydrous methanol (8 mL) and heated to 55 °C. 25% w/v sodium methoxide in methanol (6.26 mL, 22.9 mmol) was added and the reaction diluted to 30 mL by the addition of anhydrous methanol. After 1h, the solution was cooled to rt and acetic acid (1.7 mL, 29.6 mmol) was added, followed by dilution with water (100 mL). The solution was extracted with chloroform (3 × 50 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by silica gel chromatography (Chloroform:Acetone 5:1) afforded Gly-Ni-BPB **2** as a scarlet, glassy solid (1250 mg, 96%): m.p. 213-213 °C (Lit: 208-209 °C)[1]; R_f = 0.2 (Chloroform:Acetone 5:1); δ_H (500 MHz, $CDCl_3$): 2.02-2.10 (1H, m, Pro-γH), 2.12-2.18 (1H, m, Pro-δH), 2.36-2.47 (1H, m, Pro-βH), 2.53-2.63 (1H, m, Pro-βH), 3.29-3.39 (1H, m, Pro-δH), 3.44-3.48 (1H, dd, *J* = 10.7, *J* = 5.6, Pro-αH), 3.64-3.74 (4H, m, Pro-γH, Gly-αH, NC*H*HPh, 4.48 (1H, d, *J* = 12.7, NCH*H*Ph), 6.70 (1H, t, *J* = 7.2, Ar*H*), 6.79 (1H, dd, *J* = 7.9, *J* = 1.6, ArC*H*), 6.96-7.00 (1H, m, ArC*H*), 7.10 (1H, d, *J* = 7.2, ArC*H*), 7.20 (1H, td, *J* = 8.7, *J* = 1.6, ArC*H*), 7.30 (1H, t, *J* = 7.2, ArC*H*), 7.42 (2H, t, *J* = 7.6, ArC*H*), 7.48-7.56 (4H, m, ArC*H*), 8.07 (2H, d, *J* = 7.6, ArC*H*), 8.29 (1H, d, *J* = 8.3, ArC*H*). ESI-HRMS found 520.1159 $[M+Na]^+$, $C_{27}H_{25}N_3NiO_3$ requires 520.1142.

(*S*)-Pent-4-enyl-gly-Ni-BPB 3

Under an atmosphere of N_2, Gly-Ni-BPB **2** (500 mg, 1 mmol) and 1-iodopent-4-ene **11** (151 mg, 0.77 mmol) was suspended in acetonitrile (15 mL) and powdered sodium hydroxide (168 mg, 4.19 mmol) at –22 °C (Methanol–Ice). The reaction was gradually raised to rt and stirred

for 20 h, before the addition of water (30 mL) and the solution extracted with chloroform (3 × 30 mL). The organic fractions were concentrated *in vacuo* and purified by silica gel chromatography (chloroform:acetone 8:1) to afford (*S*)-pent-4-enyl-gly-Ni-BPB **3** (392 mg, 69%) as a viscous, scarlet oil. R_f = 0.3 (chloroform:acetone 5:1); δ_H (500 MHz, $CDCl_3$): 1.57-1.75 (2H, m, alkyl), 1.87-2.28 (6H, m, alkyl, Pro-γH), 2.41-2.59 (1H, m, Pro-βH), 2.69-2.83 (1H, m, Pro-βH), 3.43-3.62 (4H, m, Pro-δH, NC*H*HPh, Gly-αH), 3.91 (1H, dd, *J* = 8.1, *J* = 3.3, Pro-αH), 4.44 (1H, d, *J* = 12.9, NCH*H*Ph), 4.93-5.03 (2H, m, H_2C=C), 5.64-5.79 (1H, m, H_2C=C*H*), 6.61-6.69 (1H, m, ArC*H*), 6.92 (1H, d, *J* = 8.1, ArC*H*), 7.11-7.22 (2H, m, ArC*H*), 7.35 (2H, t, *J* = 7.6, ArC*H*), 8.04 (2H, d, *J* = 7.2, ArC*H*), 8.12 (1H, d, *J* = 8.6, ArC*H*). δ_C (126 MHz, $CDCl_3$): 23.6, 24.6, 30.8, 33.3, 34.8, 57.0, 63.1, 70.3, 70.4, 115.3, 120.8, 123.7, 126.5, 127.2, 127.6, 128.9, 128.9, 129.7, 131.6, 132.1, 133.2, 133.8, 137.8, 142.3, 142.3, 170.4, 179.4, 180.4; ESI-HRMS found *m/z* 566.1926 $[M+H]^+$; $C_{32}H_{34}O_3N_3Ni$ requires 566.1948; $[\alpha]_D^{25}$ ($CHCl_3$, *c* = 0.03) +2533 (Lit: $CHCl_3$ *c* = 0.033, +2560).[1]

(*S*)-2-amino-hept-6-enoic acid 3a

(*S*)-pent-4-enyl-gly-Ni-BPB **3** (492 mg, 0.87 mmol) was dissolved in methanol (25 mL), which was added dropwise to a solution of hydrochloric acid (3 M, 7 mL) and heated to 50 °C until a colour change from scarlet to yellow/green was observed (after 15 min) when cooling to rt was followed by the evaporation of the solvent *in vacuo*. The resulting yellow/white residue was extracted with chloroform (3 x 25 mL) to remove the BPB ligand **1** for re-use. The aqueous fraction was loaded on a Dowex 50X2 100 H^+ column (prewashed with H_2O to pH 7), which was rinsed with methanol (50 mL) and water (50 mL) to remove residual nickel, amino acid **3a** was then eluted with 20% ammonium hydroxide:ethanol 1:1, where fractions containing **3a** were identified by ninhydrin staining. Concentration of the fractions *in vacuo* afforded (*S*)-2-amino-hept-6-enoic acid **3a** (118 mg, 95%) as a white, amorphous solid. m.p. 224-225 °C (Lit: 225 °C);[1] δ_H (500 MHz, D_2O): 1.31-1.43 (2H, m, H3), 1.71-1.83 (2H, m, H4), 1.97-2.05 (2H, m, H5), 3.64 (1H, t, *J* = 5.9, H2), 4.94 (1H, d, *J* = 10.3, H7 *cis*), 4.99 (1H, d, *J* = 17.5, H7 *trans*), 5.74-5.84 (1H, m, H6). δ_C (125 MHz, D_2O): 23.5, 29.9, 32.5, 54.7, 115.0, 138.6, 174.9. ESI-HRMS found 144.1044; $C_7H_{14}NO_2$ $[M+H]^+$ requires 144.1019, $[\alpha]_D^{26}$ (H_2O *c* = 0.5) +9.5, Lit (H_2O, *c* = 1.2) +10.0.[1]

(*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)hept-6-enoic acid 4

(*S*)-2-amino-hept-6-eneoic acid **3a** (186 mg, 1.30 mmol) and sodium carbonate (273 mg, 2.59 mmol) were dissolved in water (4 mL) and cooled to 4 °C. 9-fluorenylmethyl succinimidyl carbonate (657 mg, 1.95 mmol) was dissolved in 1,4 dioxane (8 mL) at 4 °C and added dropwise to the amino acid aqueous solution. After 1 h, the reaction was warmed to rt and allowed to continue for a further 14 h. The reaction suspension was diluted with water (15 mL) and extracted with ethyl acetate (2 × 25 mL). The organic layers were back-extracted with saturated sodium hydrogen carbonate solution (20 mL), with the combined aqueous fractions acidified to pH 1 with 3M hydrochloric acid and extracted further with ethyl acetate (3 × 35 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo* to afford a yellow oil. Purification by flash chromatography (Dichloromethane:methanol:acetic acid 95:4:1) afforded (*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)hept-6-enoic acid **4** as a viscous, straw coloured oil (260 mg, 53%). R_f = 0.2 (CH_2Cl_2:MeOH:AcOH 95:4:1, visualised by ninhydrin (violet spot) and permanganate), δ_H (500 MHz, MeOD) 1.47 – 1.58 (2H, m, H3), 1.67 – 1.76 (1H, m, H4), 1.84 – 1.92 (1H, m, H4), 2.09 – 2.15 (2H, m, H5), 4.17 (1H, dd, *J* = 9.1, 4.8, H2), 4.26 (1H, t, *J* = 7.2, H10), 4.38 (2H, d, *J* = 7.2, H9), 4.99 (1H, d, *J* = 9.9, H7 *cis*), 5.05 (1H, d, *J* = 17.1, H7 *trans*), 5.78 – 5.88 (1H, m, H6), 7.33 (2H, t, *J* = 7.6, ArC*H*), 7.41 (2H, t, *J* = 7.6, ArC*H*), 7.65 – 7.72 (2H, m, ArC*H*), 7.82 (2H, d, *J* = 7.6, ArC*H*); δ_C (125 MHz, MeOD): 26.3, 32.3,

34.3, 35.5, 48.3 (*identified by HMQC)*, 55.3, 68.0, 115.6, 121.0, 126.3, 128.2, 128.8, 139.4, 142.6, 145.1, 145.4, 158.7, 175.5; ESI-HRMS found 366.1695 $[M+H]^+$; $C_{22}H_{24}NO_4$ requires 366.1699; $[\alpha]_D^{22.4}$ ($CHCl_3$, *c* = 1) +3.1.

(1*S*, 2*R*)-Ethyl-*N*-(1,2-diphenyl-2-hydroxyethyl)glycinate 5a

(1*R*, 2*S*)-2-amino-1,2-diphenylethanol **5** (1000 mg, 4.76 mmol) was dissolved in anhydrous tetrahydrofuran (25 mL) at rt. Ethyl bromoacetate (760 µL, 7.1 mmol) and triethylamine (1.32 mL, 9.5 mmol) were then added dropwise to the solution. After 18 h, the suspension was passed through a sintered funnel to remove the precipitated triethylammonium bromide salt and the filtrate concentrated *in vacuo* to afford an off-white, amorphous solid. The solid was recrystallised from ethanol (15 mL) to afford (1*S*, 2*R*)-ethyl *N*-(1,2-diphenyl-2-hydroxyethyl)glycinate **5a** (1400 mg, 98%) as white needles; m.p. 125-126 °C (Lit: 126-127 °C)[2]; Found C 71.85%, H 6.95%, N 4.50; $C_{18}H_{21}NO_3$ requires C 72.22%, H 7.07%, N 4.68%; δ_H (500 MHz, $CDCl_3$): 1.20 (3H, t, *J* = 7.2, CH_2CH_3), 1.90 (1H, br s, N*H*), 2.84 (1H, br s, O*H*), 3.15 (1H, d, *J* = 17.5, NHCHH'CO_2Et), 3.28 (1H, d, *J* = 17.5, NHCHH'CO_2Et), 3.95 (1H, d, *J* = 6.0, OHC*H*), 4.11 (2H, q, *J* = 7.2, CH_2CH_3), 4.80 (1H, d, *J* = 6.0, NHC*H*), 7.16-7.22 (4H, m, ArC*H*), 7.23-7.31 (6H, m, ArC*H*); δ_C (126 MHz, $CDCl_3$): 14.2, 48.4, 60.7, 68.3, 126.9, 127.8, 127.9, 128.1, 128.3, 128.4, 128.4, 138.5, 140.2, 172.2; ESI-HRMS found *m/z* 300.1590 $[M+H]^+$; $C_{18}H_{22}NO_3$ requires 300.1594; $[\alpha]_D^{24}$ ($CHCl_3$, *c* = 0.7) +23.7 (Lit: CH_2Cl_2, *c* = 1) +24.2.[2]

(5*S*, 6*R*)-4-(*t*-Butoxycarbonyl)-5,6-diphenylmorpholin-2-one 6

(1*S*, 2*R*)-Ethyl *N*-(1,2-diphenyl-2-hydroxyethyl)glycinate **5a** (1310 mg, 4.38 mmol) was dissolved in toluene (20 mL) and heated to 110 °C. Di-*tert*-butyl dicarbonate (1300 mg, 5.96 mmol) was added slowly as a solution in toluene (10 mL) to the reaction mixture over 1 h. After 12 h, 10 mL of toluene was distilled from the reaction at 130 °C at atmospheric pressure to remove any residual water. Fresh toluene (10 mL) and *p*-toluenesulfonic acid monohydrate (83 mg, 0.438 mmol) were added to the reaction mixture, which was heated at 110 °C for 1 h. 20 mL of toluene was then distilled from the reaction mixture over 2 h, followed by cooling to rt. The resulting solid was filtered and crystallised from hot ethanol to afford two rotamers of (5*S*, 6*R*)-4-(*t*-butoxycarbonyl)-5,6-diphenylmorpholin-2-one **6** (1315 mg, 85%) as colourless, fine needles. m.p. 204-205 °C (Lit: 207 °C)[2]; Found C 71.30%, H 6.55%, N 3.90%; $C_{21}H_{23}NO_4$ requires C 71.37%, H 6.56%, N 3.96%; δ_H (500 MHz, $CDCl_3$) 1.18 (5H, s, ^{t}Bu), 1.44 (4H, s, ^{t}Bu), 4.31 (0.55 H, d, *J* = 18.2, H3), 4.45 (0.45H, d, *J* = 17.9, H3), 4.60 (0.45H, d, *J* = 17.9, H3'), 4.86 (0.55H, d, *J* = 18.2, H3'), 5.03 (0.55H, s, H5), 5.36 (0.45H, s, H5), 5.85 (1H, br s, H6). 6.62-6.80 (2H, m, ArC*H*), 6.96-7.30 (8H, m, ArC*H*); δ_C (75 MHz, $CDCl_3$) 27.9, 28.3, 44.8, 45.7, 58.7, 60.8, 80.4, 81.1, 81.4, 81.8, 126.3, 126.4, 127.7, 127.8, 128.0, 128.2, 128.6, 134.0, 134.3, 135.0, 136.0, 153.2, 153.5, 167.6, 168.0; ν_{max} (neat) 3041, 2974, 2914, 1741, 1687, 1604, 1455, 1432, 1362, 1343, 1273, 1240, 1196; ESI-HRMS found *m/z* 376.1521 $[M+Na]^+$, $C_{21}H_{23}NO_4Na$ requires 376.1519; $[\alpha]_D^{27}$ ($CHCl_3$, *c* = 0.7) -92.0, Lit: (CH_2Cl_2, *c* = 0.2) -87.3.[2]

(3*S*, 5*S*, 6*R*)-3-Methyl-4-(*t*-butoxycarbonyl)-5,6-diphenylmorpholin-2-one 7

(5*S*, 6*R*)-4-(*t*-Butoxycarbonyl)-5,6-diphenylmorpholin-2-one **7** (2.57 g, 7.28 mmol) was dissolved in tetrahydrofuran (50 mL) and cooled to –78 °C. Sodium *bis*-(trimethylsilyl)amide (7.78 mL, 7.74 mmol, 1M solution in tetrahydrofuran) was added dropwise to the reaction mixture. After 35 min, methyl iodide (4.66 mL, 75 mmol) was added dropwise at –78 °C. After 90 min, the reaction mixture was poured into water (150 mL) and the aqueous layer extracted with ethyl acetate (3 × 120 mL). The combined organic layers were dried over sodium sulphate and concentrated *in vacuo* to afford an orange oil. Purification by flash

chromatography (chloroform:methanol 99:1) afforded two rotamers of (3*S*, 5*S*, 6*R*)-3-methyl-4-(*t*-butoxycarbonyl)-5,6-diphenyl-morpholin-2-one **7** (2.09 g, 78%) as brown, stubby needles (after crystallisation from hot ethanol). R_f = 0.4 (hexane:ethyl acetate 2:1); m.p. 198-200 °C; Found C 71.15%, H 6.85%, N 3.60%; $C_{22}H_{25}NO_4$ requires C 71.91%, H 6.86%, N 3.81%; δ_H (500 MHz, DMSO-d_6, 392 K): 1.23 (9H, br s, ^{t}Bu), 1.72 (3H, d, *J* = 7.0, C*H*$_3$), 4.88 (1H, q, *J* = 7.0, H3), 5.17 (1H, d, *J* = 3.1, H5), 6.15 (2H, d, *J* = 3.1, H6), 6.58 – 6.60 (2H, m, ArC*H*), 7.05 – 7.10 (5H, m, ArC*H*), 7.20 – 7.24 (3H, m, ArC*H*); δ_C (75 MHz, $CDCl_3$): 19.4, 28.7, 52.2, 60.3, 80.1, 125.4, 125.6, 126.3, 126.6, 126.7, 126.8, 127.0, 127.1, 127.5, 127.6, 133.0, 135.3, 152.0, 169.7; ESI-HRMS found *m/z* 390.1689 $[M+Na]^+$; $C_{22}H_{25}NO_4Na$ requires 390.1676;[3] $[\alpha]_D^{26}$ ($CHCl_3$, *c* = 0.7) –69.4 Lit: (CH_2Cl_2, *c* = 0.2) –61.[4]

(3*S*, 5*S*, 6*R*)-*tert*-butyl-3-methyl-2-oxo-3-(pent-4-enyl)-5,6-diphenylmorpholine-4-carboxylate 8

(3*S*, 5*S*, 6*R*)-3-methyl-4-(*t*-butoxycarbonyl)-5,6-diphenylmorpholin-2-one **7** (2100 mg, 5.71 mmol) was dissolved in anhydrous tetrahydrofuran (42 mL) and cooled to –40 °C ($N_{2\,(l)}$:MeCN). 1-Iodopent-4-ene (2800 mg, 14.3 mmol) was added dropwise and the mixture was allowed to stir for 30 min. Potassium *bis*-(trimethylsilyl)amide (7.8 mL, 8.57 mmol, 0.91 M in tetrahydrofuran) was added dropwise and the reaction retained at –40 °C. After 1 h, water (50 mL) was added at –40 °C and the suspension was raised to rt and extracted with ethyl acetate (3 × 100 mL). The organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford an orange, amorphous solid. Purification by flash chromatography (Chloroform:acetone 99:1) afforded (3*S*, 5*S*, 6*R*)-*tert*-butyl-3-methyl-2-oxo-3-(pent-4-enyl)-5,6-diphenylmorpholine-4-carboxylate **8** as a colourless, amorphous solid (970 mg, 49%). R_f = 0.6 (Hexane:ethyl acetate 2:1), δ_H (500 MHz, DMSO-d_6, 392 K): 1.40 (9H, br s, ^{t}Bu), 1.69 (3H, s, C*H*$_3$), 1.45 – 1.57 (2H, m, $CH_2CH_2CH_2$), 2.09 (2H, app q, *J* = 7.3, $CH_2CH{=}CH_2$), 2.21 – 2.28 (1H, m, CH_2CH_2), 2.43 – 2.45 (1H, m, CH_2CH_2), 4.95 – 4.99 (1H, m, CH=C*H*$_2$ *cis*), 5.03 (1H, dq, *J* = 17.2, 2.1, CH=C*H*$_2$ *trans*), 5.57 (1H, d, *J* = 3.6, H5), 5.78 – 5.89 (1H, m, H4), 6.13 (1H, d, *J* = 3.6, H6), 6.94 – 6.97 (2H, d, ArC*H*), 7.12 – 7.14 (3H, m, ArC*H*) 7.19 – 7.24 (5H, m, ArC*H*); δ_c (125 MHz, $CDCl_3$): 24.1, 24.3, 25.2, 28.5, 33.6, 33.7, 57.6, 64.0, 67.3, 80.5, 114.3, 115.4, 125.8, 127.8, 127.9, 128.1, 128.2, 128.3, 129.0, 129.9, 135.4, 135.7, 135.9, 137.9, 172.5, 173.0 $\nu_{(max)}$ 3066, 3034, 2976, 2931, 1747, 1694, 1641, 1498, 1454. ESI-HRMS found 458.2303 $[M+Na]^+$; $C_{27}H_{33}NO_4Na$ requires 458.2302, $[\alpha]_D^{23.3}$ ($CHCl_3$, *c* = 1) +29.9.

(*S*)-2-(*tert*-butoxycarbonyl)amido-2-methylhept-6-enoic acid 9

(3*S*, 5*S*, 6*R*)-*tert*-butyl-3-methyl-2-oxo-3-(pent-4-enyl)-5,6-diphenylmorpholine-4-carboxylate **8** (950 mg, 2.18 mmol) was dissolved in anhydrous tetrahydrofuran (25 mL) and anhydrous ethanol (2 mL) and transferred *via* cannula to liquid ammonia (~50 mL) at –78 °C. Lithium wire (301 mg, 43.7 mmol) was washed in hexane and added to the reaction mixture in several portions. After the blue colour dissipated (15 min), saturated ammonium chloride was added dropwise, and the temperature allowed to rise to rt overnight, to ensure evaporation of ammonia gas. The aqueous solution was extracted with ether (2 × 50 mL), acidified to pH 2 with 1M hydrochloric acid and extracted further with ethyl acetate (3 × 50 mL). The organic layers were combined, dried (sodium sulfate) and concentrated *in vacuo* to afford a colourless solid. Purification by flash chromatography (dichloromethane : methanol 95:1) afforded (*S*)-2-(*tert*-butoxycarbonyl)amido-2-methylhept-6-enoic acid **9** as a colourless, amorphous solid (468 mg, 84%). m.p. 230 °C (decomposes); R_f = 0.2 (CH_2Cl_2:MeOH 95:5, stained with ninhydrin (scarlet spot) and permanganate), δ_H (500 MHz, MeOD) 1.25 – 1.41 (3H, m, H3 & H4), 1.45 & 1.46 (12H, 2 × br s, ^{t}Bu & C*H*$_3$), 1.75 – 1.85

(1H, m, H3), 4.93 (1H, *fine splitting obscured by residual peaks*, H7), 4.98 (1H, d, *J* = 17.0, H7 *trans*), 5.75 –5.95 (1H, m, H6); δ_C (125 MHz, MeOD) 24.3, 25.0, 35.0, 37.3, 61.0, 79.7, 115.0, 139.9, 156.4, 181.2; $\nu_{(max)}$ 3411, 3355, 1667, 1620, 1485, 1454, 1405, 1367, 1170; ESI-HRMS found 280.1507 $[M+Na]^+$; $C_{13}H_{23}NO_4Na$ requires 280.1519, $[\alpha]_D^{24.7}$ (MeOH, *c* = 1) +16.0.

(*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylhept-6-enoic acid 10

(*S*)-2-(*tert*-butoxycarbonyl)amido-2-methylhept-6-enoic acid **9** (468 mg, 1.82 mmol) was suspended in dichloromethane (6 mL) and trifluoroacetic acid (6 mL) was added slowly (2 mL/min) at rt. After 30 min, the solvents were removed *in vacuo* and dried on the high vacuum manifold until less than 2 eq. of trifluoroacetic acid remained by weight. The residue was then dissolved in water:acetone (1:1, 10 mL), cooled to 0 °C and then sodium carbonate (963 mg, 9.10 mmol) and 9-fluorenylmethyl succinimidyl carbonate (644 mg, 1.90 mmol) were added. After 1 h, the suspension was acidified to pH 3 with 1M hydrochloric acid and extracted with ethyl acetate (3 × 120 mL). The combined organic layers were combined, dried (Na_2SO_4) and concentrated *in vacuo* to afford a yellow residue. Purification by flash chromatography (dry loaded, and eluted with 94:5:1 dichloromethane:methanol:acetic acid) afforded (*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylhept-6-enoic acid **10** as a pale yellow oil (430 mg, 62%). R_f = 0.2 (94:5:1 CH_2Cl_2:MeOH:AcOH, stained with ninhydrin (violet)), δ_H (500 MHz, MeOD) 1.29 – 1.42 (5H, m, C*H*$_3$ and H3), 1.81 – 1.93 (2H, m, H4), 2.00 – 2.08 (2H, m, H5), 4.19 (1H, t, *J* = 6.8, H10), 4.28 – 4.35 (2H, m, C*H*$_2$O), 4.93 (1H, d, *J* = 10.7, H7 *cis*), 4.99 (1H, d, *J* = 17.1, H7 *trans*), 5.71 – 5.89 (1H, m, H6), 7.29 (2H, t, *J* = 7.3, ArC*H*), 7.36 (2H, t, *J* = 7.3 ArC*H*), 7.60 – 7.67 (2H, m, ArC*H*), 7.77 (2H, d, *J* = 7.7, ArC*H*); δ_C (125 MHz, MeOD): 24.4, 34.8, 37.6, 49.6 (*identified by HMQC)*, 60.8, 67.5, 115.2, 120.9, 126.2, 126.3, 128.2, 128.8, 139.6, 142.6, 145.3, 158.1, 178.0; ν_{max} 3331 (br), 3029, 2956, 1950, 1913, 1680, 1615, 1535, 1421; ESI-HRMS found 402.1687 $[M+Na]^+$; $C_{23}H_{25}NO_4Na$ requires 402.1676.[5] $[\alpha]_D^{21.1}$ ($CHCl_3$, *c* = 0.3) + 6.5.

1-iodopent-4-ene 11

4-Penten-1-ol (200 mg, 2.32 mmol), triphenyl phosphine (728 mg, 2.78 mmol) and imidazole (237 mg, 3.48 mmol) were dissolved in dichloromethane (5 mL) at 0 ºC. Iodine (703 mg, 2.78 mmol) was added and the solution allowed to stir at 0 ºC for 1 h. The solution was allowed to warm to rt and quenched with saturated NH_4Cl (15 ml) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried (sodium sulfate) and concentrated *in vacuo* to afford a thin, colourless oil. Purification by silica gel chromatography (ethyl acetate:hexane, 1:19) afforded 1-iodopent-4-ene **11** as a thin, volatile oil (270 mg, 60%): R_f = 0.9 (EtOAc:Hexane 1:19); δ_H (500 MHz, $CDCl_3$): 1.92 (2H, quin, *J* 6.8, H2), 2.17 (2H, q, *J* 6.8, H3), 3.20 (2H, t, *J* 6.8, H1), 5.02 (1H, d, *J* 10.3 H5 *cis*), 5.09 (1H, d, *J* 17.1, H5 *trans*), 5.80 – 5.70 (1H, m, H4). δ_C (126 MHz, $CDCl_3$): 6.7, 32.8, 34.7, 116.3, 137.0.

Procedures and methods regarding the solid phase synthesis of peptides[6]

Methods for manual synthesis of peptides

Method A: Resin Swelling

The required quantity of resin was placed in a vaculate reservoir and CH_2Cl_2 (2 ml) was added and the resin was agitated on a blood spinner for 2 h to allow swelling of the resin.

Method B: Deprotection of *N*-Fmoc protecting groups

N-terminal Fmoc protecting groups were removed by the addition of 20% piperidine:DMF (5 × 2 mL × 2 min), followed by rinsing the resin with DMF (5 × 2 mL × 2 min). Successful deprotection was determined by a positive colour test (Methods C & D).

Method C: Kaiser Test[7]

The Kaiser Test was employed for the determination of the successful coupling or deprotection for most of the residues. A small number of resin beads were rinsed in Ethanol and placed in a vial, followed by the addition of two drops of each of the three solutions in the following order:

1) Ninhydrin (5% w/v) in Ethanol, 2) Phenol (80% w/v) in Ethanol, 3) 1 mM $KCN_{(aq.)}$ in pyridine (2% v/v).

The solution was then heated to ca. 150 °C for 1 min. A successful coupling gave no change in the colour of the beads, whereas bright blue beads illustrate a successful deprotection. This colour test was useful for the identification of free primary amines, however inconclusive results are obtained for Asp, Ser, Pro and Asn residues.

Method D: Coupling of Amino Acids with HCTU

The desired amino acid (5 eq.), DIPEA (5 eq.), HOBt (5 eq.) and HCTU (5 eq.) were dissolved in DMF (2 mL) and added to the resin, followed by agitation for 1 h (2 h for unnatural amino acids). For double couplings, this step was repeated. After draining the reagents, the resin was washed with DMF (3 × 2 mL × 2 min) and the success of coupling determined by a negative colour test (Methods C & D). Deprotection of the Fmoc-protected *N*-terminus then followed Method B.

Method E: *N*-terminal acetylation

Acetic anhydride (10 eq.) and DIPEA (10 eq.) were dissolved in DMF (2 mL) and the solution was transferred to the resin. After 2 h, the resin was drained, washed with DMF (3 × 2 mL × 2 min) and successful capping determined by a negative colour test (Methods C & D).

Method F - On-Resin Olefin Metathesis

After the completed peptide elongation and *N*-terminal acetylation, on-resin olefin metathesis was completed by the preparation of a 10 mM solution of Grubbs First Generation Catalyst in degassed dichloroethane (2 mL), which was added to the resin beads and allowed to agitate gently for 2 h, after which time the metathesis was repeated for another 2 h.

Method G: Cleavage and deprotection of Rink Amide MBHA resins

After elongation and *N*-terminal acetylation was complete, the resin was washed with DMF (5 × 2 mL × 2 min), CH_2Cl_2 (5 × 2 mL × 2 min) and then Et_2O (3 × 2 mL × 2 min). Peptides were then simultaneously cleaved and side-chain deprotected with cleavage 'Reagent K' TFA:EDT:Thioanisole:Phenol:H_2O, 82:3:5:5:5 (3 × 2 mL × 1 h). The resin was washed with fresh TFA (2 mL × 2 min) and the solution concentrated *in vacuo*.

The resulting oil was precipitated with ice-cold ether (10 mL) and placed in a centrifuge (3000 rpm × 0.5 min). The supernatants were removed, the precipitate rinsed with ice-cold ether (3 × 10 mL) and dried *in vacuo*.

Method H: **Peptide Purification**

Peptides were purified by preparative scale HPLC using a Jupiter Proteo preparative column (reversed phase) on an increasing gradient of acetonitrile to water (plus 0.1% trifluoroacetic acid v/v) at a flow rate of 20 mL min^{-1}. Crude peptides were suspended in 1:1 acetonitrile:water at an approximate concentration of 15 - 20 mg mL^{-1}. Purification runs injected a maximum of 2.5 mL of crude peptide solution and were allowed to run for 35 min, with acetonitrile increasing from 5 to 95%, and the eluent scanned at 220 nm. Fractions were checked by LCMS, concentrated *in vacuo* and lyophilised. For semi-preparative purification, a Jupiter Proteo Semi-Preparative (reversed phase) column was used, with the flow rate reduced to 5 mL min^{-1}.

The synthesis procedure of peptides followed the order of Methods B, C, D, C. The residue following the unnatural amino acid in the sequence was double-coupled with 10 equivalents of reagents outlined in Method E.

HPLC

Semi-preparative HPLC was performed with a solvent system of water with 0.1% v/v TFA (Solvent A) and acetonitrile with 0.1% v/v TFA (solvent B) and peptides purified using a solvent gradient of 30-95% B over a run time of 35 minutes. The column used was a Phenomenex Jupiter Proteo 90A column, with dimensions 250 mm x 10 mm and 10 micron pore size. Flow rate = 5 ml min^{-1}.

Analytical HPLC for enzymatic degradation and peptide characterisation was performed using a 5-95% B gradient over 5 minutes using an Ascentis Express Peptide ES-C18 column with dimensions 100mm x 2.1 mm and 2.7 micron pore size.

Structures and Mass Spectrometry Data for BID Peptides

BID-WT

Ac-EDIIRNIARHLAQVGDSN$_L$DRSIW-NH$_2$

BID-Aib

BID-DuM

BID-DM

BID-MuM

BID-MM

Ac-EDIIRNIARHLAXVGDXN$_L$DRSIW-NH$_2$

Peptide	$[M+2H]^{2+}$ Obs[d]	$[M+2H]^{2+}$ Exp[d]	$[M+3H]^{2+}$ Obs[d]	$[M+3H]^{3+}$ Exp[d]
BID WT	**1359.7318**	**1359.7253**	**906.8313**	**906.8193**
BID-Aib	**1337.2347**	**1337.2328**	**891.8258**	**891.8243**
BID-DuM	**1391.2777**	**1391.2798**	**927.8572**	**927.8556**
BID-DM	**1377.2529**	**1377.2641**	**918.5114**	**918.5118**
BID-MuM	**1377.2619**	**1377.2641**	**918.5161**	**918.5161**
BID-MM	**1363.2338**	**1363.2485**	**909.1673**	**909.1673**

Masses are in m/z units, with either two or three proton adducts, and the **Obs**erved and **Exp**ected values quoted.

UV Chromatograms and LC Traces of Peptides

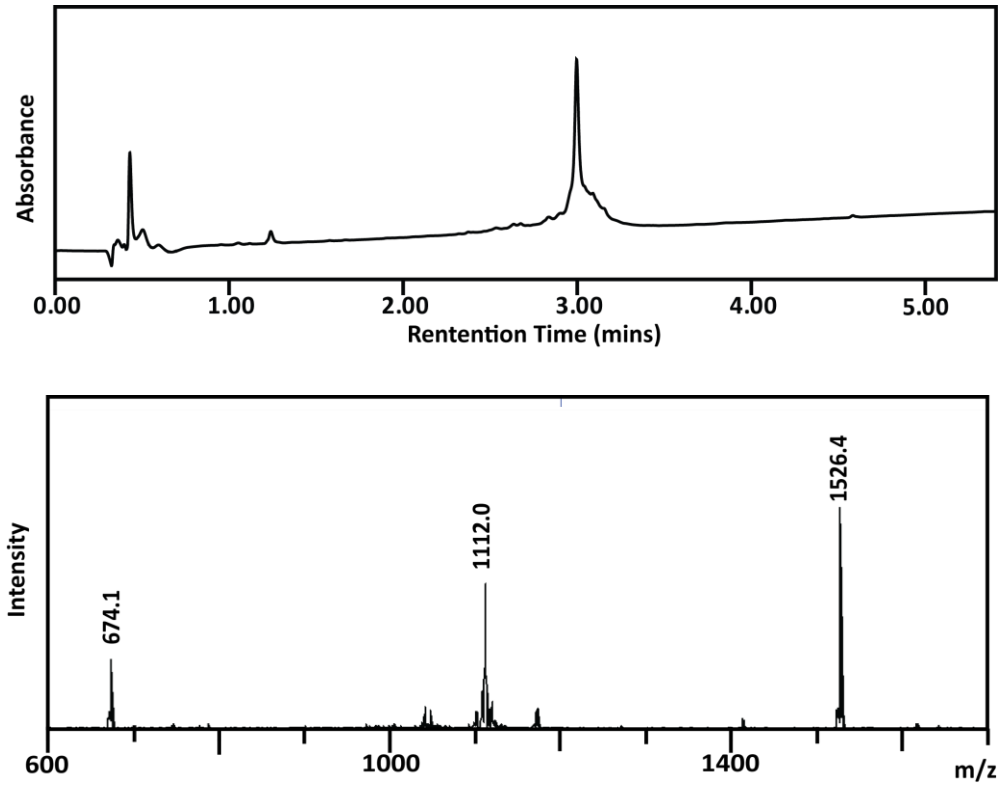


Figure ESI-1. LC and MS traces for p53 peptide

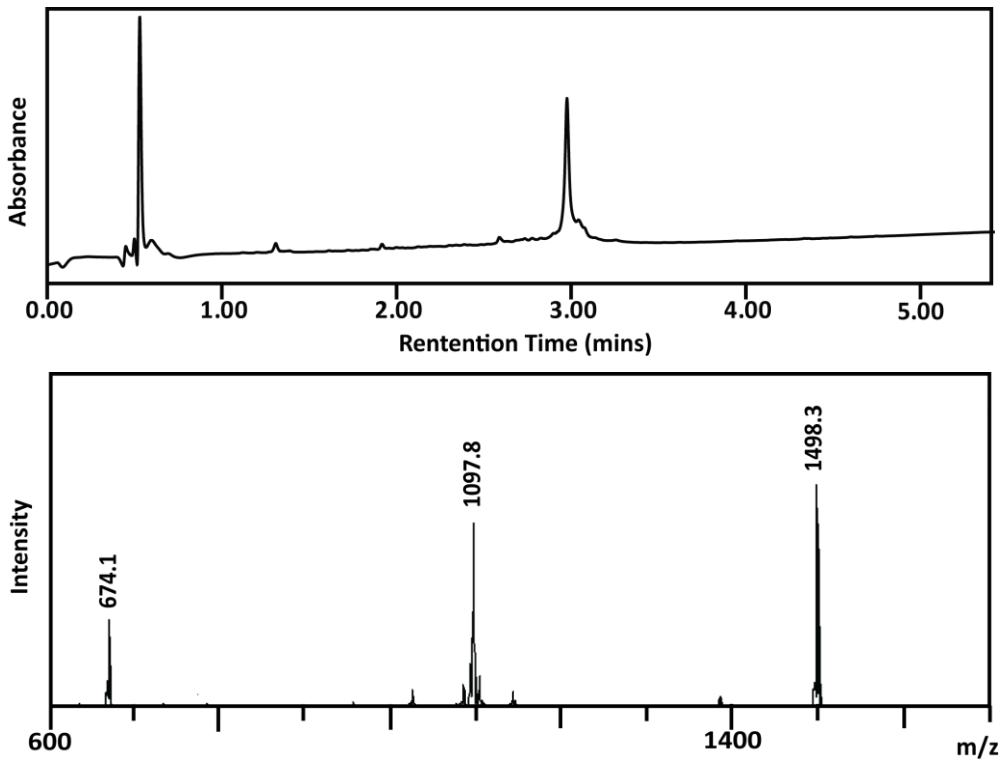


Figure ESI-2. LC and MS traces for "stapled" p53 peptide

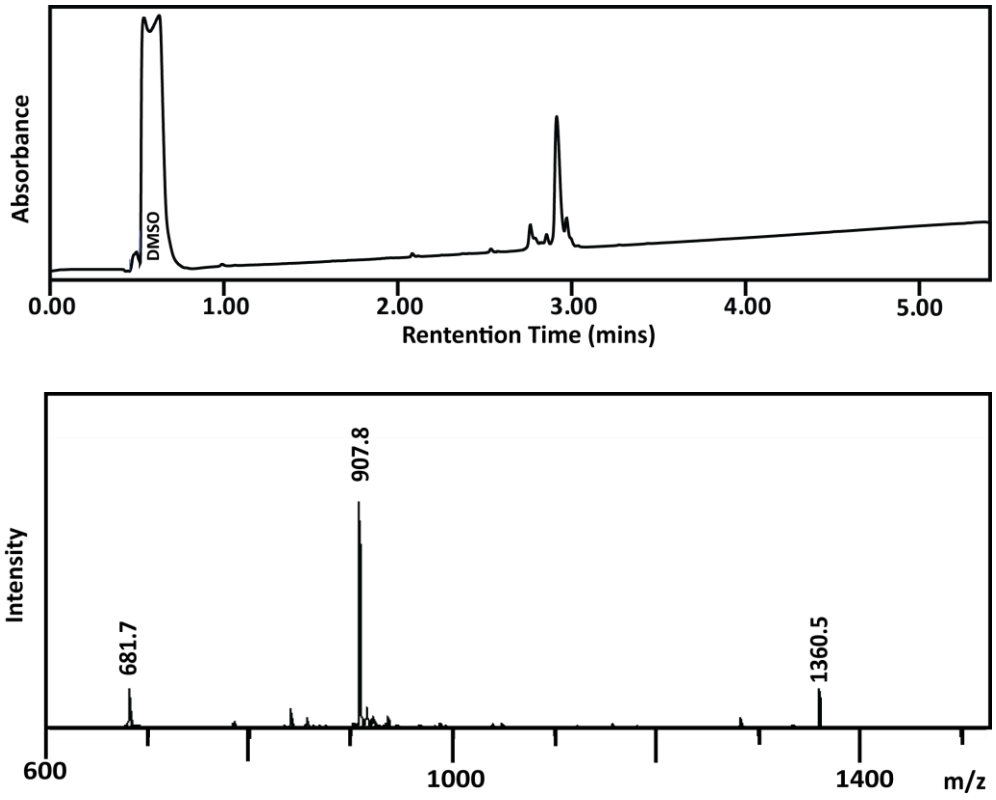


Figure ESI-3. LC and MS traces for BID-WT

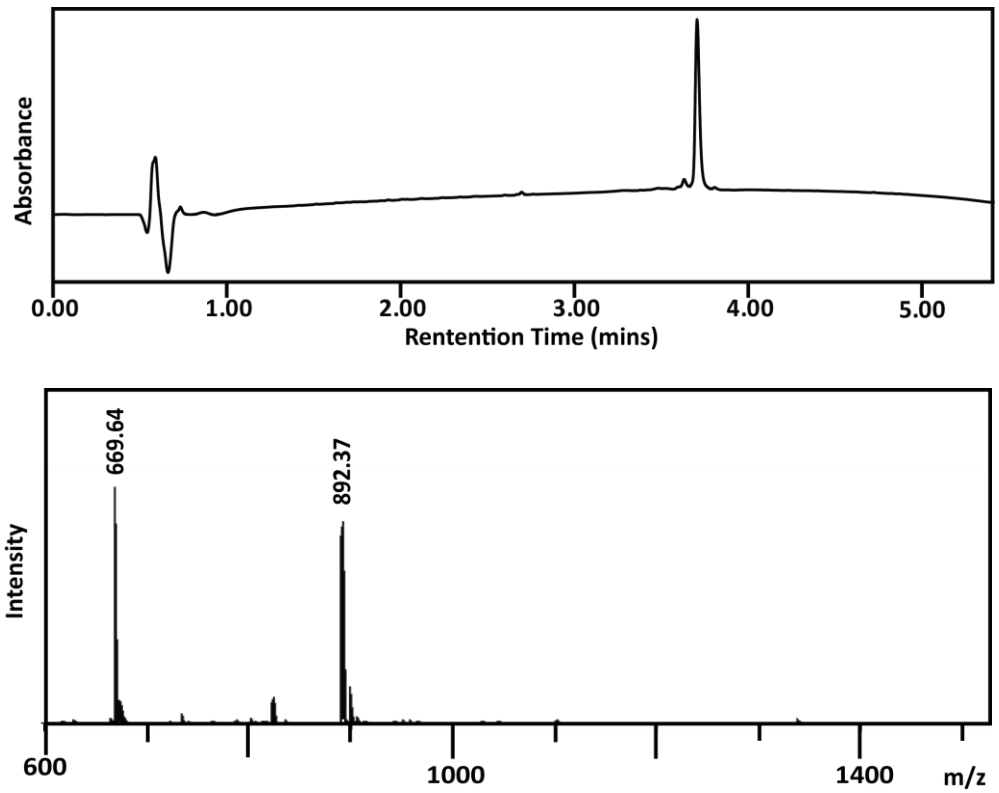


Figure ESI-4. LC and MS traces for BID-AIB peptide

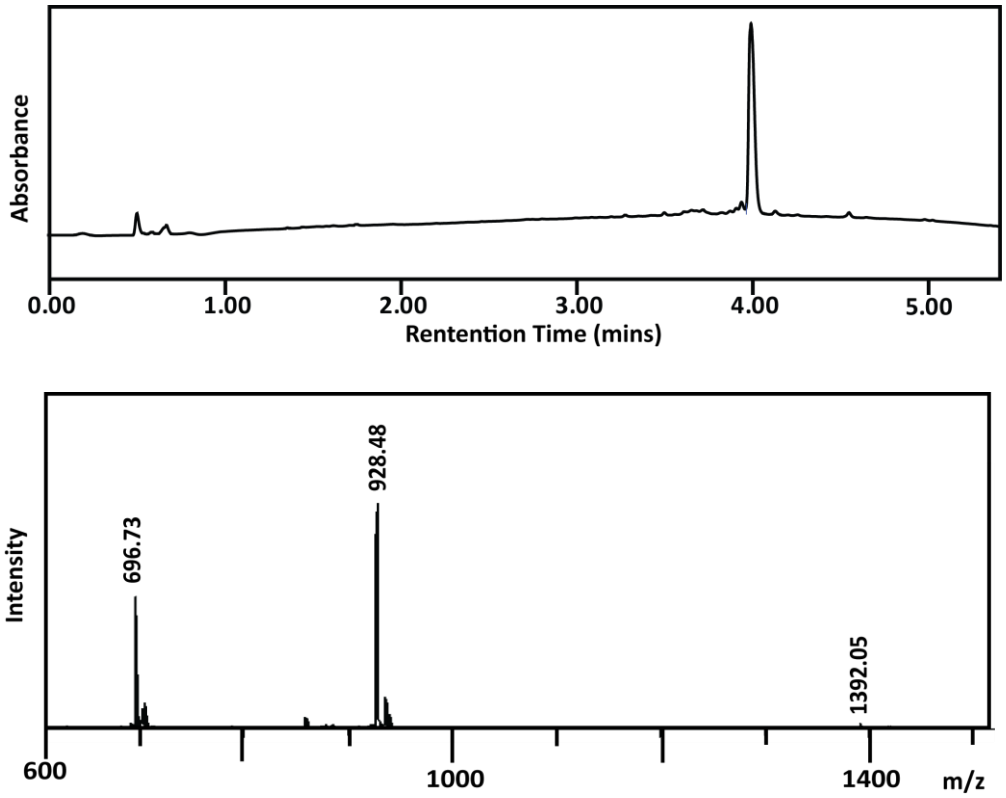


Figure ESI-5. LC and MS traces for BID-DuM

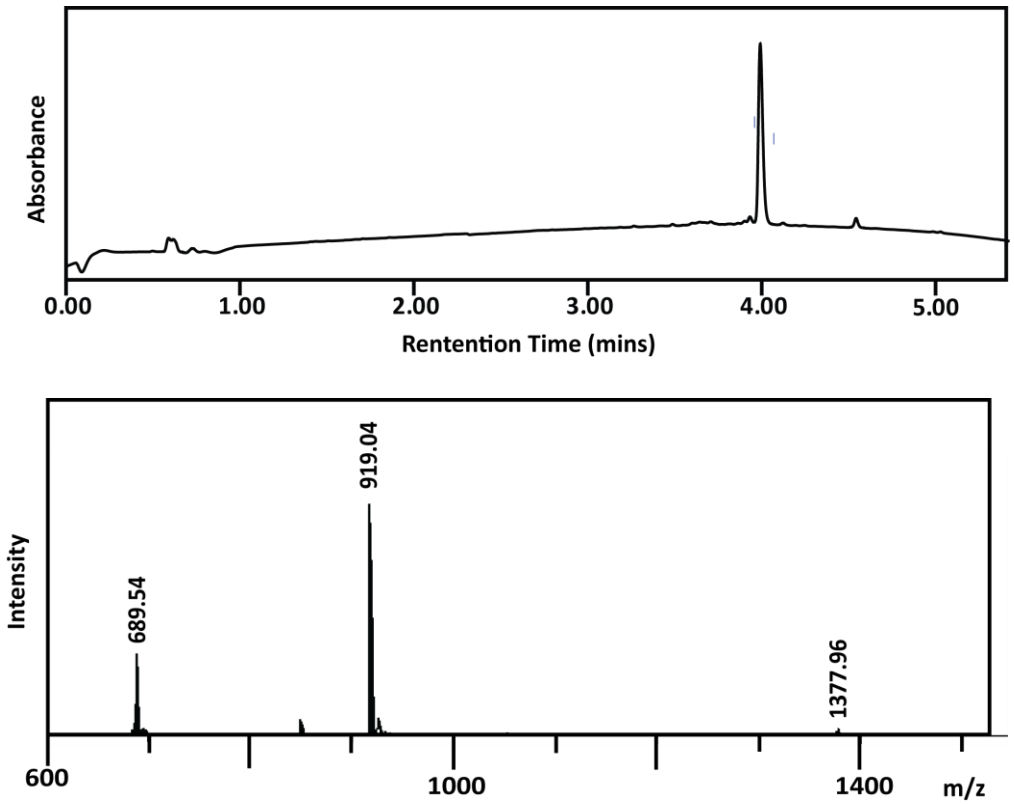


Figure ESI-6. LC and MS traces for BID-DM

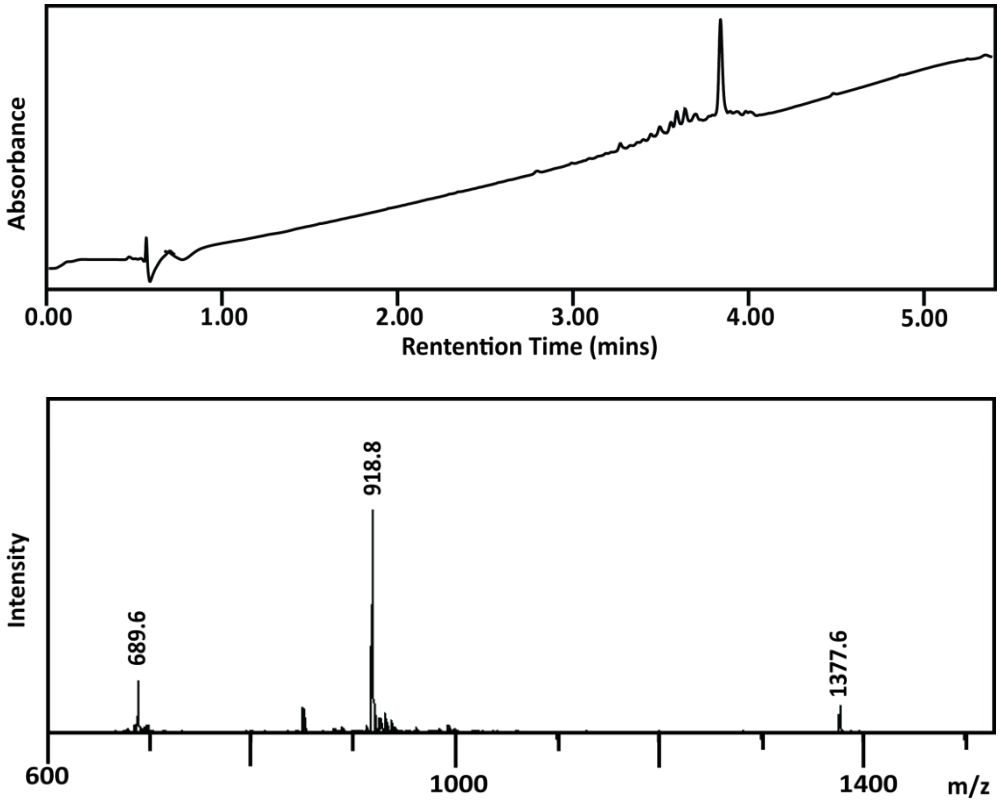


Figure ESI-7. LC and MS traces for BID-MuM

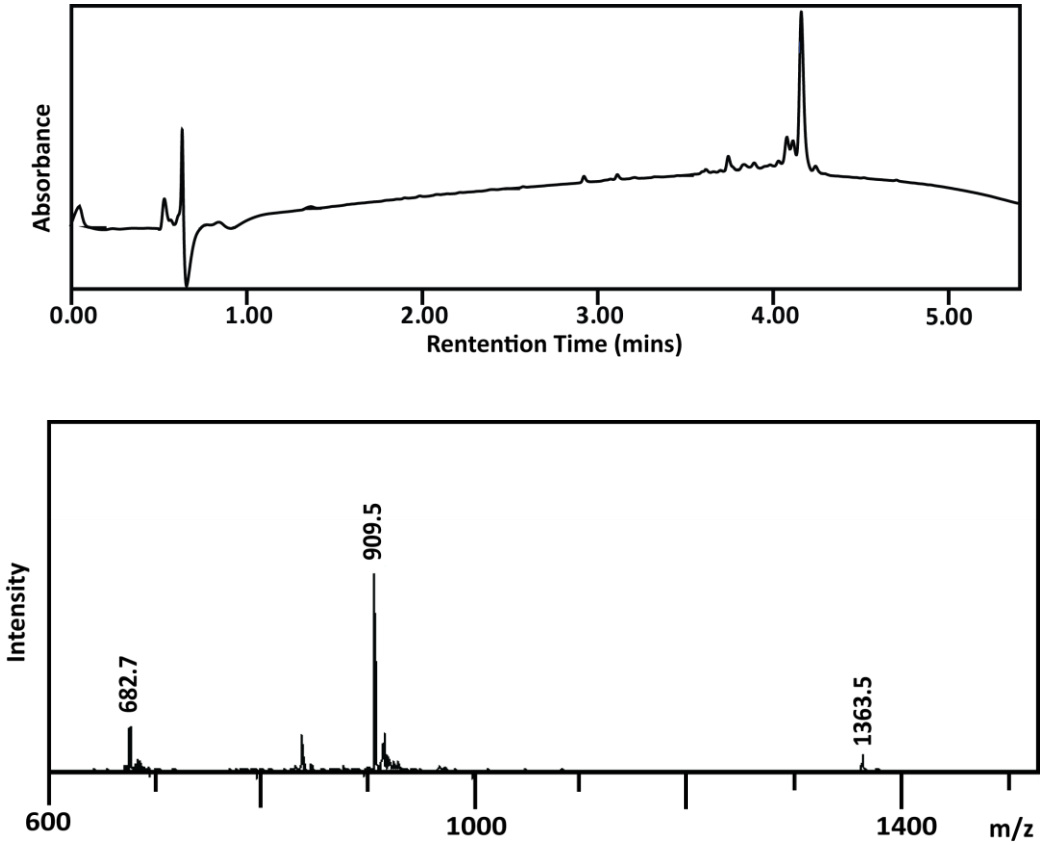


Figure ESI-8. LC and MS traces for BID-MM

Protein expression and purification

Bcl-x_L 'no loop'

The pGEX Bcl-x_L 'no loop' construct[8] was kindly provided by Prof W. D. Fairlie (The Walter and Eliza Hall Institute for Medical Research, Victoria). The pGEX Bcl-x_L 'no loop' construct was over-expressed in the *E.coli* strain Rosetta 2. 15 ml of overnight starter culture was used to inoculate 1 L 2 xYT containing 100 μg/ml Ampicillin. Cultures were grown at 37 °C plus shaking until OD_{600} ~ 0.6 – 0.8, the temperature was then switched to 18 °C and protein expression induced by the addition of 0.8mM IPTG. Induced cultures were grown at 18 °C plus shaking overnight before harvesting by centrifugation. Cells were resuspended in 20 mM Sodium Phosphate, 140 mM NaCl, pH 7.5 containing 0.1% Triton X-100 and lysed by sonication in the presence of 10 μL of 1 $U.ml^{-1}$ DNase I per litre of over-expression culture and 5mM $MgCl_2$. DTT was then added to the lysed cells to give a final concentration of 1 mM. The cell lysate was centrifuged (Beckman JA25.50 rotor, 17,000 rpm, 30 min, 4 °C) and the supernatant filtered (0.22 μM syringe filter) before mixing with 5ml of Glutathione Superflow (SuperGlu) resin (Generon) at 4 °C for 1 hour. The resin had previously been equilibrated with 5 CV of 20 mM Sodium Phosphate pH 7.5, 140 mM NaCl, 1 mM DTT (low salt buffer). The cleared cell lysate was then allowed to flow through the SuperGlu resin under gravity flow. The resin was then washed with 5 CV of low salt buffer followed by 7 CV of 20 mM Sodium Phosphate pH 7.5, 1 M NaCl, 1 mM DTT (high salt buffer) and a further 7 CV of low salt buffer.

The GST-Bcl-x_L fusion protein was cleaved on the resin. 10 ml of low salt buffer was added to the resin along with 3 x 100 μL aliquots of PreScission protease (prepared by A. Ariza, protease concentration unknown). The resin was mixed gently at 4 °C overnight. To remove any cleaved Bcl-x_L 'no loop' protein the column was washed with 6 CV followed by 5 CV of low salt buffer. The resin was then washed with 6 CV of 20 mM Sodium Phosphate pH 7.5, 140 mM NaCl, 20 mM reduced glutathione to remove the GST. The eluent from each step was collected and samples run on a 15% SDS-PAGE gel.

The eluent fraction containing cleaved Bcl-x_L 'no loop' was concentrated (Amicon Ultra centrifugal filter, MWCO 3,500) to approximately 5 ml. The sample was then filtered before being loaded onto a Superdex 75 column (GE healthcare) equilibrated in 40 mM Sodium Phosphate, 140 mM NaCl, 5% glycerol, 1 mM DTT, pH 7.5. The purified Bcl-x_L 'no loop' was concentrated to ~ 0.5 mg/ml and stored at – 80 °C. the mass of the purified protein confirmed by mass spectrometry.

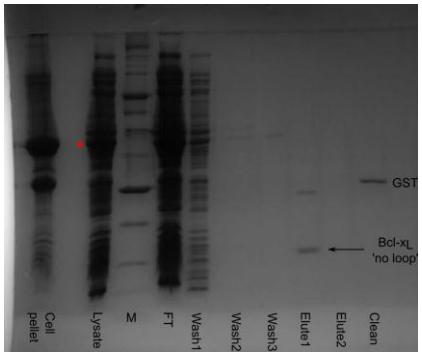


Figure ESI-9 Purification of Bcl-x_L 'no loop' using Glutathione Superflow resin. The band corresponding to the GST-Bcl-x_L 'no loop' fusion is marked with a*. The lane labelled FT contains the flow through from the column when the cleared cell lysate was first loaded. Washes 1 and 3 were with low NaCl buffer (20 mM Sodium Phosphate pH 7.5, 140 mM NaCl, 1 mM DTT), wash 2 was with high NaCl buffer (20 mM Sodium Phosphate pH 7.5, 1 M NaCl, 1 mM DTT). Washes elute 1 and 2 were with low NaCl buffer to remove cleaved Bcl-x_L 'no loop', the clean wash contained 20 mM reduced Glutathione to remove the GST.

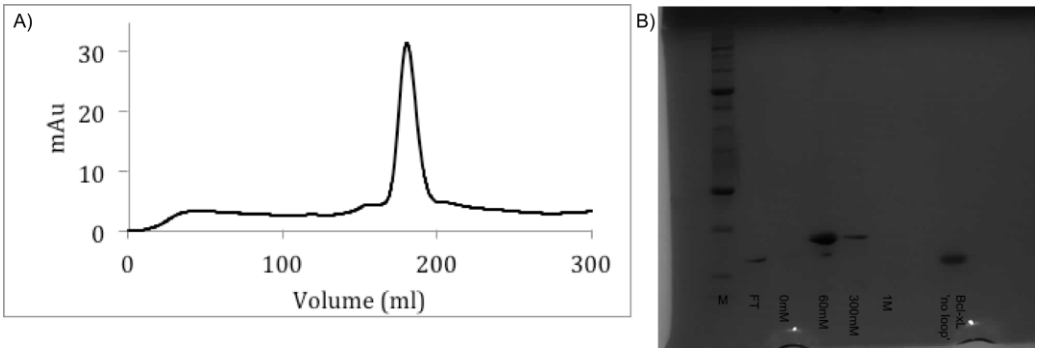


Figure ESI-10 Final purification of Bcl-x_L 'no loop' using the Superdex75 SEC column (GE Healthcare). A) Absorbance profile from the gel filtration column. B). SDS-PAGE gel showing the concentrated Bcl-x_L 'no loop' sample, individual fractions from the SEC column were not concentrated enough to be visible on the SDS-PAGE gel. The other lanes on the gel contain samples from another prep. The marker lane contains a broad range (2-212 kDa) protein marker (New England Biolabs).

Circular Dichroism

Circular Dichroism was performed on an Applied Photophysics ChiraScan Apparatus and Software. For each scan, the following parameters were used: 180-260nm range; point time 1s; 1 nm per point; step = 1; bandwidth 5 nm; path length 10mm; temperature 20°C. Scans were done in triplicate. Samples were dissolved in 1:4 acetonitrile: 50 mM sodium phosphate buffer pH 7.50 to concentrations between 5 - 20 µM. The raw circular dichroism data obtained for the peptides was processed by the subtraction of the solvent signal and converted into a mean residue elipticity:

$$[\theta] = \frac{\theta}{10 \times c \times l}$$

$$[\theta]_{MRE} = \frac{[\theta]}{(R\ -\ 1)}$$

Where θ = circular dichroism at a given wavelength, c = molar concentration, l = path length in cm, R = number of residues in the peptide sequence.

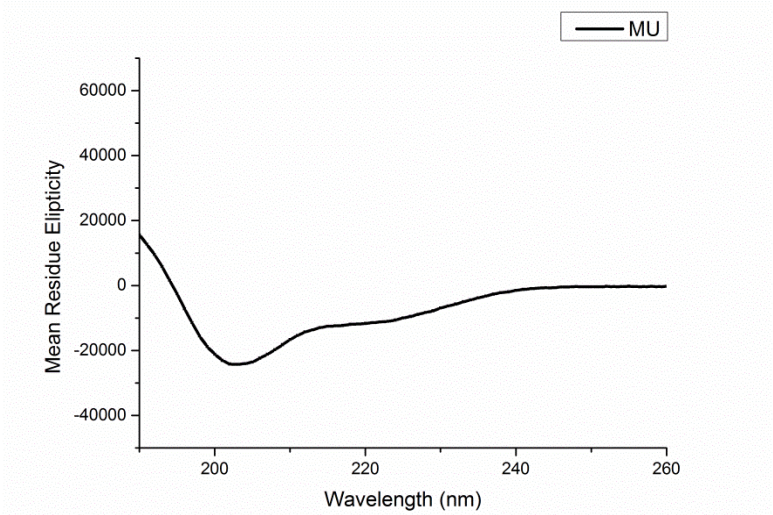


Figure ESI-11. Circular dichroism spectrum of BID-monounstaple. The % helicity is calculated as 17% versus 73 % for BID-monostaple

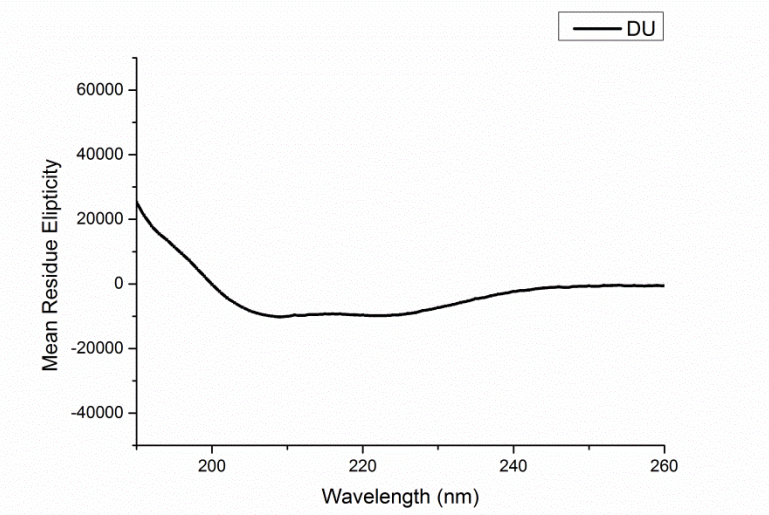


Figure ESI-12. Circular dichroism spectrum of BID-diunstaple. The % helicity is calculated as 20% versus 80% for BID-distaple

Fluorescence Anisotropy Competition Assay

Determination of the binding of BODIPY-BAK to Bcl-x_L

The procedure followed was adapted from our previous paper on *O*-alkylated aromatic oligoamides.[9] Briefly: Bcl-x_L was serially diluted (685 pM – 0.3 µM) into a solution of BODIPY-BAK (100 nM) (40 mM Sodium phosphate buffer pH 7.54, 200 mM NaCl, 0.02 mg/ml of bovine serum albumin) – the total volume of each well was 100 µL, the plates were allowed to incubate at room temperature for 6 hours. Each experiment was run in triplicate and the fluorescence anisotropy measured using a Perkin Elmer EnVision™ 2103 MultiLabel plate reader, with excitation at 531 nm and emission at 595 nm (5 nM bandwidth) and the intensity (Eq. 1) was calculated for each point. This was used to calculate anisotropy (Eq. 2) and plotted to a sigmoidal fit in origin 7 to determine the minimum and maximum anisotropies (r_{min} and r_{max}). Using equation 3, the data for the anisotropy was converted to fraction bound and multiplied by the BODIPY-BAK concentration then fitted in origin 7 (Eq.

4) to give the dissociation constant K_d = 4.2 ± 3.7 nM. The intensity (Eq. 1) was plotted against concentration of protein **(ESI-13a)** and fitted to a sigmoidal fit in order to calculate λ from the upper and lower asymptotes, which represent I_{bound} and $I_{unbound}$ respectively.

$$I = (2PG) + S \qquad \textit{(Eq. 1)}$$

$$r = \frac{S - PG}{I} \qquad \textit{(Eq. 2)}$$

$$L_b = \frac{(r - r_{\min})}{(\lambda(r_{\max} - r) + r - r_{\min})} \qquad \textit{(Eq. 3)}$$

$$y = \frac{\{(k_1 + x + [FL]) - \sqrt{\{(k_1 + x + [FL])^2 - 4x[FL]\}}}{2} \qquad \textit{(Eq. 4)}$$

r = anisotropy, I = total intensity, P = perpendicular intensity, S = parallel intensity, L_b = fraction ligand bound, λ = $I_{bound}/I_{unbound}$ = 4.603, [FL] = concentration of fluorescent ligand (i.e. $p53_{15\text{-}31Flu)}$, $k_1 = K_d$, y = L_b* BODIPY-BAK and x = [added titrant], G is an instrument factor set to 1.

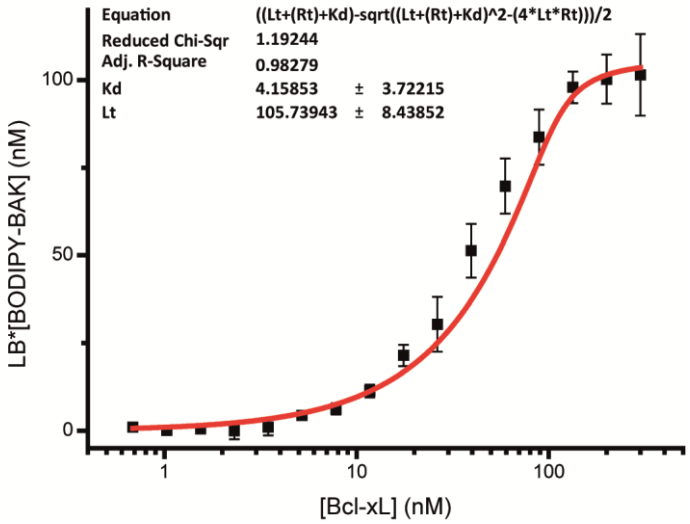


Figure ESI-13. Fluorescence anisotropy titration assays for the addition of Bcl-x_L to a constant concentration of BODIPY-BAK;

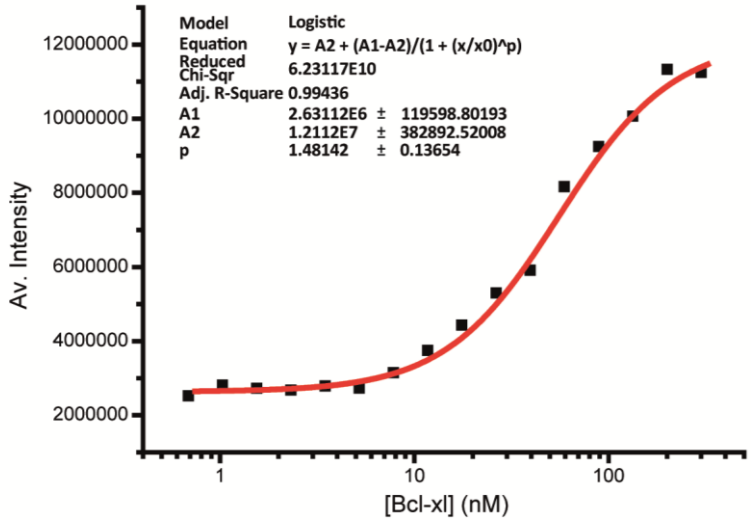


Figure ESI-14. Change of intensity during addition of Bcl-$x_L$in order to calculate λ.

A reverse titration was also performed whereby the protein concentration was held constant and BODIPY-BAK was serially diluted. The intensity and anisotropy was calculated for each point as before and converted to a fraction bound using the same value of r_{min} and r_{max}. As saturation approaches, the anisotropy begins to drop, whilst the intensity increases, so the asymptote is not reached in this experiment. The fraction bound was then multiplied by the total BODIPY-BAK concentration at each point and fit (Eq. 4) in origin 7, where y is the fraction bound times the tracer concentration and x is the concentration of added tracer. The protein titration fit the model better and did not have the added complication of saturation of the detector so that value was used for subsequent competition experiments.

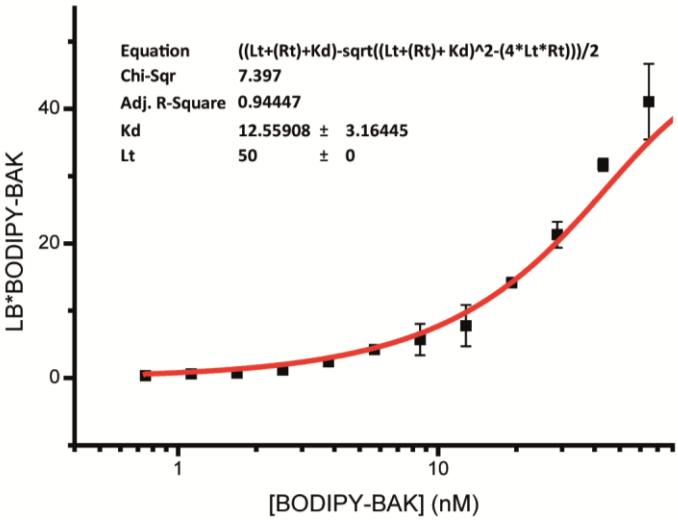


Figure ESI-15. Fluorescence anisotropy titration assays for the addition of BODIPY-BAK to a constant concentration of Bcl-x_L K_d = 12.6 ± 3.2 nM

Competition assay

For fluorescence anisotropy competition assays, BODIPY-labelled BAK (BODIPYTMRX-GQVGRQLAIIGDDINR-NH_2) was used as the tracer and binding assays were performed in 96 or 384 well plates. After a protein titration confirmed the K_d of BODIPY-BAK to be 4.15 ± 3.7 nM, the inhibiting peptide was dissolved in Buffer A (40 mM sodium phosphate pH 7.50, 200 mM sodium chloride, 0.02 mg mL^{-1} of bovine serum albumin) to concentrations between 45 - 300 μM. The competing peptides were then serially diluted across the plate and then BODIPY-BAK (43 nM) and Bcl-x_L (131 nM) was added for the total volume of each well to be 150 μL. A series of control experiments were also run where the competing peptides were serially diluted across the plate and Bcl-x_L (131 nM) and Buffer A (50 μL) were added. All experiments were recorded in triplicate using a Perkin Elmer EnVision 2103 MultiLabel plate reader at 25.0 °C with excitation at 531 nm and emission at 595 nm (5 nM bandwidth) and the intensity plotted for each point (Equation 1). This was used to calculate anisotropy (equation 2) and using the maximum and minimum anisotropies (r_{max} and r_{min}) from the tracer titration fitted against peptide concentration in a dose-response model to obtain an IC_{50} value (Equation 5). Replicating the competition assays with the tracer concentration doubled did not have an impact on the IC_{50} values calculated.

$$y = r_{min} + \frac{r_{max} - r_{min}}{1 + 10^{(x - \log x_o)}} \quad (Eq.\ 5)$$

A direct comparison of our IC_{50} values with previous IC_{50} values determined by Chen *et al.*[10] is difficult because the prior work employed surface plasmon resonance (SPR) and used a different ligand to be displaced (BAK in this work, BIM for the SPR). Moreover, because an IC_{50} value depends on the upon the affinity of the ligand being displaced and the concentrations of the protein and ligand being displaced, hence such a comparison would not be valid. What can be indicated is that the ligand being displaced in our assay (BODIPY-BAK) binds with a dissociation constant of ~ 1 nM which is consistent with the literature.[11]

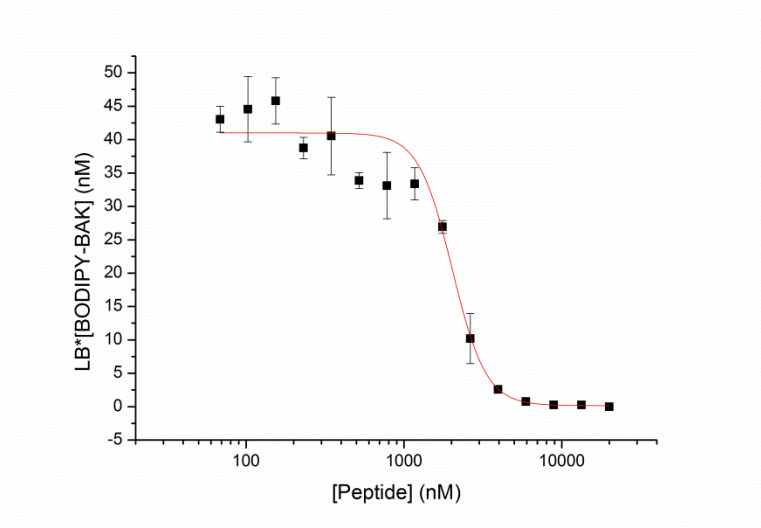


Figure ESI-16. Fluorescence anisotropy competition titration assays for BID-DuM titrating against BODIPY-BAK/Bcl-x_L – an IC_{50} of 2.02 µM ± 0.08 µM was measured

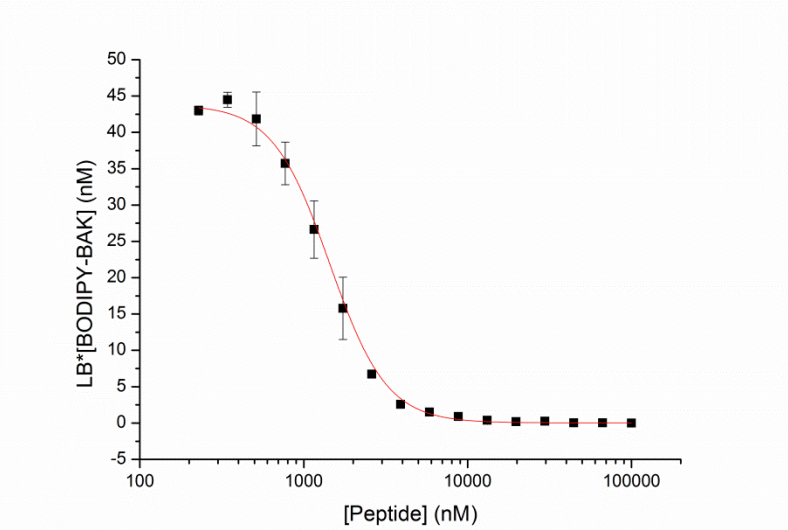


Figure ESI-17. Fluorescence anisotropy competition titration assays for BID-MuM titrating against BODIPY-BAK/Bcl-x_L – an IC_{50} of 1.44 µM ± 0.10 µM was measured

Enzymatic Degradation

Peptides were dissolved in PBS Buffer (0.01M, pH 7.47) at concentrations of 200 µM. Trypsin (Promega, Sequencing Grade) was made into a 0.01 nM stock. 60 µL of each stock were added and mixed and the digestion monitored by HPLC at 25°C, with aliquots removed after 0, 5, 12, 21, 45 and 90 minutes and the digestion quantified by the integration of the peak at 220 nm corresponding to undigested peptide. The HPLC used an Ascentis Analytical Peptide Column (20 µL injection, 0.5 mL min^{-1} flow rate, 5-95% acetonitrile:water gradient, 5.5 min run time). Experiments were done in duplicate, and assume first order kinetics. The HPLC time points were converted into a percentage of the original substrate (S), and plotted as a natural logarithm (lnS) against time (t) in minutes. Half lives ($t_{1/2}$) were calculated by dividing –ln(2) by the slope of the graph, plotted in Origin.

Half Lives were as follows

BID WT	20 min
BID-Aib	30 min
BID-DuM	25.6 min
BID-DM	46.5 min
BID-MuM	25.1 min
BID-MM	38.3 min

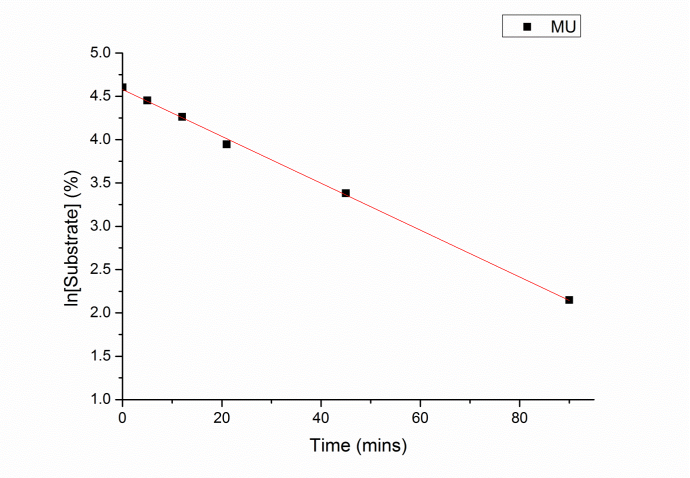


Figure ESI-18. Enzymatic degredation assays for BID-DuM using trypsin

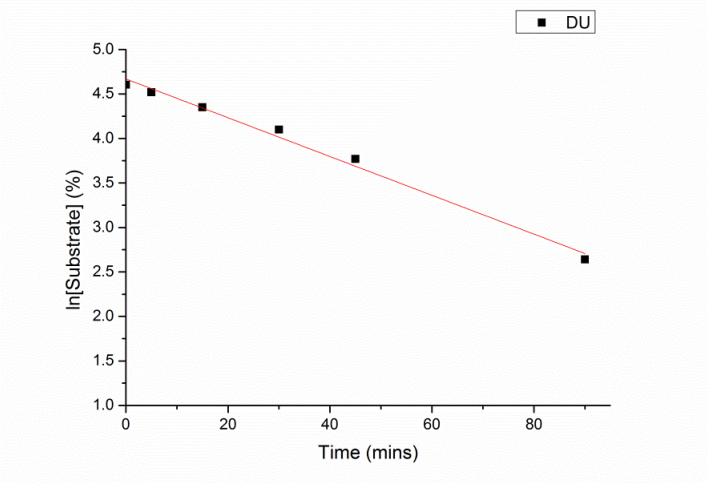


Figure ESI-19. Enzymatic degredation assays for BID-MuM using trypsin

p53/hDM2 Initial Investigation

The stapled p53 peptides used were based upon the following sequence: Ac-S^{15}QE*T*FSD*L*WKLLPENNVC-NH_2 with the italicised residues replaced with the monosubstituted amino acids. Peptides were synthesised and purified as outlined above. The positioning of the unnatural amino acids was rationalised to insert in positions that did not interfere with those of the key binding residues (F^{19}, W^{23}, L^{26}) and that the hydrocarbon crosslink would not interfere with the tertiary structure of *h*DM2 surrounding the binding pocket.

The circular dichroism data showed stabilisation of a helical conformation in solution, between the stapled and unstapled peptides, but the binding affinity of the mutated p53 peptides was weaker than that of the wild type peptide. The anisotropy assay was based on our previous work in this area.[12]

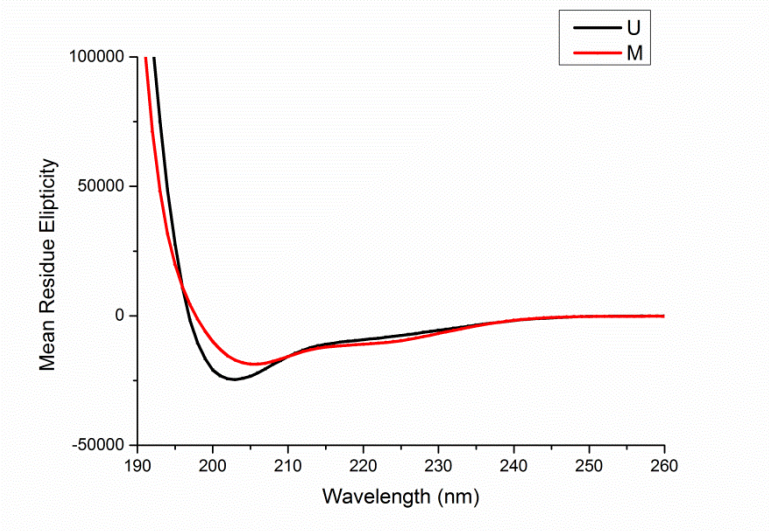


Figure ESI-20 Circular dichroism spectrum of p53 unstapled (black) and stapled (red).

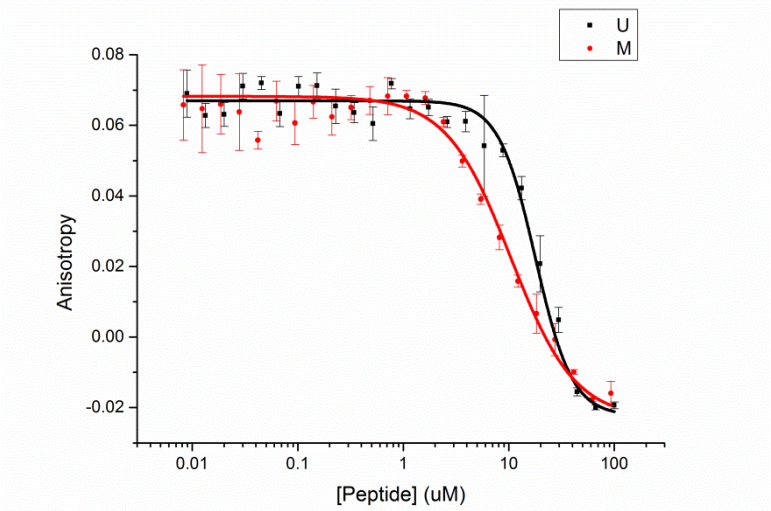


Figure ESI-21. Fluorescence anisotropy competition titration assays for (a) p53-monounstaple and (b) p53-monostaple titrating against FITC-p53/*h*DM2 – an IC_{50} of 12.4 µM ± 0.64 µM and an IC_{50} of 17.8 µM ± 1.28 µM were measured respectively.

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