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Supporting Information

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Supporting Information

for

Thermodynamic and Computational Studies on the Binding of p53-Derived Peptides and Peptidomimetic Inhibitors to HDM2

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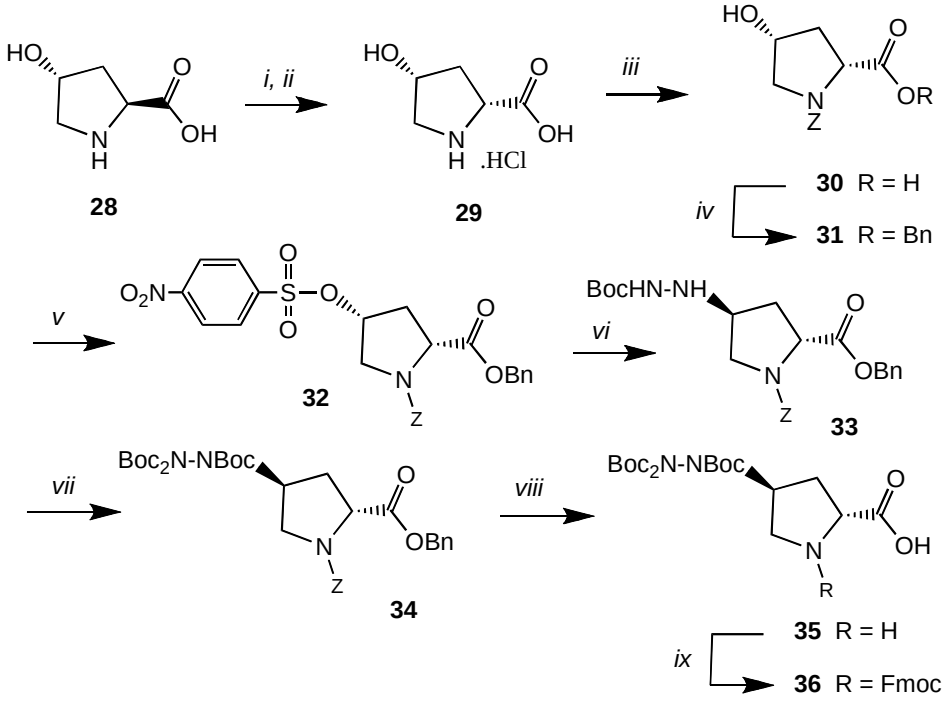
Experimental Section

1. Synthesis of peptides

1.1. Synthesis of Fmoc-4-hydrazino-D-proline



Reagents and conditions: i) Ac_2O, AcOH, reflux ii) HCl (2 N), reflux; iii) Z-Cl, NaOH (1 M), THF, 0 °C $\rightarrow$ RT; iv) BnBr, K_2CO_3, NaI, DMF, RT; v) Nos-Cl, NEt_3, DCM, 0 °C $\rightarrow$ RT; vi) tert-butylcarbazate, dioxane, reflux; vii) di-tert-butyldicarbonate, NEt_3, DMAP, DCM, RT; viii) Pd/C, H_2, MeOH, RT; ix) Fmoc-OSu, Na_2CO_3, H_2O, DMF, RT.

Synthesis of **29**: Freshly distilled acetic anhydride (1.02 kg, 943 mL, 10 mol) was mixed with glacial acetic acid (3.0 L) and heated to 50 °C. 4-L-Hydroxypro-line **28** (247 g, 1.88 mol) was added in one portion and stirred at reflux for 5.5 h. The solvent was removed in vacuum to a thick brown oil. The oil was dissolved in HCl (2 M, 3.5 L) at 50 °C. The mixture was heated to reflux for 3.5 h. Charcoal was added to remove color and the hot mixture was filtered through celite. The solvent was con-centrated in vacuum to 100 mL until white needles started to form. The suspension was left to crystallize overnight at RT and then filtered. The precipitate was washed with ice-cold HCl (2 M, 2 times 50 mL) and dried in vacuum (106.2 g). The mother liquor was left at 4 °C for further crystallization. Filtration and wa shing led to another 25.5 g. Yield: 131.7 g (42 %). White needles. Mp = 144-145 °C. α_D (20 °C, H_2O) = + 23.3 °. IR (KBr) = 3422b, 3238b, 2925s, 1708s, 1376s. ^{1}H NMR (300 MHz, MeOD-d_4): δ = 4.54-4.50 (m, 1 H); 4.47 (d, J = 3.8 Hz, 1 H); 3.39-3.36 (m, 2 H); 2.49 (dd, J = 13.9 Hz, J = 4.6 Hz, 1 H); 2.36 (d,quint, J = 14.1, J = 1.8 Hz, 1 H). ^{13}C NMR (75 MHz, MeOD-d_4): δ = 171.6; 70.0; 59.4; 54.8; 38.2 ppm. CI-MS m/z 166.1 $[M-H]^-$, 168.1 $[M+H]^+$.

Synthesis of **30**: THF (120 mL) and aq. NaOH (1 M, 180 mL) were ad-ded to (2R,4R)-4-hydroxyproline·HCl **29** (10.00 g, 60.4 mmol). The suspension was cooled to 0 °C in an ice/water bath. Simultaneously, a solution of benzyl chloroform-ate (11.4 mL, 76.4 mmol, 1.3 equiv) in THF (60 mL) and NaOH (1 M, 120 mL) were ad-ded dropwise. The milky emulsion was allowed to reach RT and was stirred over-night. The reaction mixture was diluted with H_2O (150 mL) and NaOH (1 M) was ad-ded until pH 9. The mixture was washed with diethyl ether (2 times 50 mL). The aq. layer was acidified with HCl (conc) to pH 3 and extracted with EtOAc (3 x 50 mL). The organic layer was washed with water (2 x 30 mL) and brine (2 x 30 mL) and dried over Na_2SO_4. Solvent was removed in vacuo, which gave a colorless oil. Crys-tallization from $CHCl_3$ resulted in a white solid powder. Yield: 13.15 g (82 %). TLC (Si, EtOAc) R_f = 0.17. Mp = 111-112 °C. α_D (20°C, MeOH) = + 39.3 °. IR (KBr) = 3262m, 1764m, 1686s, 1432.0s. ^{1}H NMR (300 MHz, D_2O): δ = 7.30-7.42 (m, 5 H); 5.12 (s, 1 H); 5.08 (d, J = 6.2 Hz, 1 H); 4.38-4.50 (m, 2 H); 3.63 (td, J = 11.9 Hz, J = 5.2 Hz, 1 H); 3.41 (t, J = 13.4 Hz, 1 H); 2.37-2.52 (m, 1 H); 2.12 ppm (d, J = 15.5 Hz, 1 H).^{13}C NMR (75 MHz, D_2O): δ = 176.5; 156.4; 136.1; 128.7; 128.4; 127.7; 69.2 (C3); 67.7; 58.1 (C1); 54.2; 37.5. CI-MS: 264.1 $[M-H]^-$, 529.2 ppm $[2M-H]^-$. High reso-lution ESI-MS: exact mass calcd for $C_{13}H_{15}N_1O_5Na_1$: 288.0848 ($[M+Na]^+$), m/z found 288.0842.

Synthesis of **31**: N-Benzyloxycarbonyl-4-hydroxy-D-proline **30** (12.40 g, 46.8 mmol) was dissolved in DMF (85 mL) under N_2. Anhydrous potassium carbon-ate (12.91 g, 93.6 mmol, 3 equiv), sodium iodide (0.71 g, 4.7 mmol) and benzylbromide (16.8 mL, 140.4 mmol, 3 equiv) were added, which led to a yellow suspension. The re-action mixture was left stirring overnight at room temp. The reaction was monitored by TLC (Si, EtOAc/cyclohexane 1:1). H_2O (180 mL) was added and the suspension was extracted with ethyl acetate (3 x 80 mL), which resulted in a colorless aq. solu-tion and an orange organic layer. The combined organic solutions were washed with water (2 x 30 mL), brine (2 x 30 mL) and dried over Na_2SO_4. The solvent was re-moved in vacuo, which gave a brown oil. The product was purified by flash chroma-tography (EtOAc/cyclohexane 1:1). Yield: 14.25 g (85.8 %). Yellow oil. TLC (EtOAc/ cyclohexane 1:1): R_f = 0.29. α_D (20 °C, $CHCl_3$) = + 21.1 °. IR ($CHCl_3$) = 3468bw, 3027m, 3015m, 2959s, 1702bs. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28-7.17 (m, 10 H); 5.21-4.93 (m, 4 H); 4.39 (dd, J = 19.5 Hz, J = 9.6 Hz, 1 H); 4.28 (s, 1H); 3.64 (t, J = 12.0 Hz, 1 H); 3.58-3.49 (m, 1 H)); 2.91 (bs, 1 H); 2.30-2.17 (m, 1 H); 2.05 ppm (t, J = 9.2 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.2; 153.5; 135.3; 134.0; 127.6-126.8; 70.1; 69.1; 66.4; 57.0; 54.8; 37.3 ppm. ESI-MS (MeOH/DCM 3:1 + NaI): m/z 378.2 $[M+Na]^+$. High-resolution ESI-MS: exact mass calcd for $C_{20}H_{21}N_1O_5Na_1$: 378.1317 ($[M+Na]^+$), m/z found 378.1321.

Synthesis of **32**: **31** (10.50 g, 29.6 mmol) was dissolved in DCM (180 mL) and cooled to 0 °C in an ice-water bath. 4-Nitrobenzenesulfony l chloride (8.52 g, 38.5 mmol, 1.3 equiv) and triethylamine (6.2 mL, 44.4 mmol, 1.5 equiv) were added under N_2. The solution turned a dark orange color and was left stirring overnight at RT. The reaction was monitored by TLC (Si, EtOAc/cyclohexane 1:1). The resulting brown suspension was washed with HCl (1 M, 3 x 30 mL) and saturated $NaHCO_3$ solution (3 x 30 mL) and dried over Na_2SO_4. The solvent was removed in vacuo. The product was purified by flash chromatography (EtOAc/cyclohexane 1:2) and obtained as a yellow oil. Yield: 12.52 g (78.3 %). TLC (EtOAc/cyclohexane 1:1): R_f = 0.21. α_D (20 °C, $CHCl_3$) = + 34.5 °. IR ($CHCl_3$) = 3024s, 1709s, 1537s. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (t, J = 8.6 Hz, 2 H); 7.87 (dd, J = 8.7 Hz, J = 3.6 Hz, 2 H); 7.25-7.18 (m, 10 H); 5.21-4.97 (m, 5 H); 4.51 (ddd, J = 26.7 Hz, J = 8.3 Hz, J = 2.7 Hz, 1 H); 3.67 (s, 2 H); 2.47-2.40 ppm (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.0; 135.9; 128.8; 128.5; 128.4; 127.9; 124.4; 80.0; 67.4; 67.1; 57.3; 52.4; 37.1 ppm. ESI-MS (MeOH/DCM 3:1 + NaI): m/z 563.1 $[M+Na]^+$.

Synthesis of **33**: **32** (13.0 g, 24 mmol) was dissolved in dioxane (120 mL) and tert-butylcarbazate (26.3 g, 193 mmol, 8 equiv) was added. The brown solution was heated at reflux overnight. A yellow solid precipitated. The reaction mixture was diluted with H_2O (500 mL) and extracted with DCM (3 x 200 mL). The organic phases were combined, washed with H_2O (2 x 100 mL) and brine (2 x 100 mL) and dried over Na_2SO_4. The solvent was removed in vacuo and the product was purified by flash chromatography on silica gel (AcOEt/cyclohexane 1:3) as a yellow oil. Yield: 10.5 g (93 %). TLC (EtOAc/cyclohexane 1:1): R_f = 0.24. α_D (20 °C, $CHCl_3$) = + 29.0 °. IR ($CHCl_3$) = 3439w, 3024w, 1710bs. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27-7.14 (m, 10 H); 6.71 (bs, 1 H); 6.55 (bs, 1 H); 5.13-4.93 (m, 4 H); 4.49-4.47 (m, 1 H); 3.88-3.58 (m, 3 H); 2.35 (s, 1 H); 2.04 (s, 1H); 1.38 ppm (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.4; 128.4-127.7; 81.3; 67.1; 66.7; 58.1; 57.9; 50.4; 28.1; 26.8. CI-MS: 939.4 $[2M+H]^+$, 883.4, 839.4, 783.3, 487.2 $[M+NH_4]^+$, 431.2 $[M+NH_4\text{-}^t\text{butyl}]^+$, 414.1 $[M\text{-}^t\text{butyl}]^+$, 370.1 ppm $[M\text{-boc}]^+$. High-resolution ESI-MS: exact mass calcd for $C_{25}H_{31}N_3O_6Na_1$: 492.2111 ($[M+Na]^+$), m/z found 492.2107.

Synthesis of **34**: To **33** (11.3 g, 24.1 mmol) and di-tert-butyldicarbonate (15.8 g, 72.3 mmol, 3 equiv) in DCM (150 mL) under N_2 was added dimethylaminopyri-dine (1.47 g, 12.0 mmol, 0.5 equiv) and triethylamine (4.58 mL, 72.3 mmol, 3 equiv). The yellow solution turned orange and was left stirring overnight at RT. TLC showed that reaction was not yet complete yet so further di-tert-butyldicarbonate (15.8 g, 72.3 mmol, 3 equiv) and dimethylaminopyridine (1.47 g, 12.0 mmol, 0.5 equiv) were added and the mixture was left stirring for an additional 5 h at RT. The solvent was removed in vacuo and the product was purified by flash chromatography on silica gel (EtOAc/ cyclohexane 1:3). Yield: 9.50 g (58 %). Yellow oil. TLC (EtOAc/cyclohexane 1:3): R_f = 0.23. α_D (20 °C, EtOAc) = + 22.1 °. IR ($CHCl_3$) = 3693w, 3018s, 2983m, 1799m, 1711bs. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26-7.14 (m, 10 H); 5.18-4.94 (m, 4 H); 4.63 (quint, J = 7.5 Hz, 1 H); 4.39 (qd, J = 7.0 Hz, J = 19.1, J = 4.0 Hz, 1 H); 3.79-3.72 (m, 1 H); 3.57-3.45 (m, 1 H); 2.46-2.30 (m, 1 H); 2.10 (qt, J = 3.2 Hz, J = 7.0 Hz, 1 H); 1.41-1.36 ppm (m, 27 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0; 153.0; 136.3; 128.5; 127.7; 83.8; 81.7; 67.0; 57.7; 56.0; 48.3; 32.5; 27.8. ESI-MS (MeOH/DCM 3:1 + NaI): m/z 692.4 $[M+Na]^+$, 592.3 $[M+Na\text{-boc}]^+$, 591.2 ppm $[M+Na\text{-2boc}]^+$. High-resolution ESI-MS: exact mass calcd for $C_{35}H_{47}N_3O_{10}Na_1$: 692.3159 ($[M+Na]^+$), m/z found 692.3155.

Synthesis of **35**: To **34** (9.5 g, 13.95 mmol) in MeOH (200 mL) under N_2 was added a spatula of Pd/C (10 %). The mixture was stirred vigorously under H_2 overnight. The suspension was filtered over cotton to remove the Pd-charcoal and the solvent was removed in vacuo to give a white solid. No further purification was performed. Yield: 6.27 g (100 %). White powder. TLC (EtOAc/cyclohexane 1:1, 10 % MeOH, 10 % HOAc): R_f = 0.33. Mp = decomposition at 260 °C. IR (KBr) = 3 450bw, 2984m, 1770s, 1746s, 1728s. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.42 (t, J = 7.1 Hz, 1 H); 4.24 (t, J = 7.7 Hz, 1 H); 3.67 (dd, J = 11.9 Hz, J = 7.0 Hz, 1 H); 3.39 (dd, J = 12.7 Hz, J = 6.9 Hz, 1 H); 2.44-2.35 (m, 2 H); 1.45 (d, J = 2.7 Hz, 18 H); 1.35 ppm (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6; 152.8; 150.5; 150.3; 84.0; 81.8; 59.6; 57.4; 46.7; 31.6 (C2); 27.9 ppm. ESI-MS (MeOH + 10 mM NH_4OAc): m/z 446 $[M+H]^+$. High-resolution ESI-MS: exact mass calcd for $C_{20}H_{35}N_3O_8Na_1$: 468.2322 ($[M+Na]^+$), m/z found 468.2326.

Synthesis of **36**: To **35** (6.20 g, 13.9 mmol) in DCM (150 mL) was ad-ded Fmoc-O-succinimide (6.11 g, 18.1 mmol, 1.3 equiv) and DIEA (5.05 mL, 30.6 mmol, 2.2 equiv). The mixture was stirred 12 h at RT and the reaction was monitored by TLC (Si, EtOAc/cyclohexane 2:1, 1 % HOAc). The solvent was removed in vacuo to leave a yellow oil. The product was purified by flash chromatography (EtOAc/cyclohexane 2:1, 1 % HOAc) to give a clear oil that was crystallized from MeOH/H_2O. Yield: 7.13 g (77 %). White powder. TLC (EtOAc/cyclohexane 2:1, 1 % HOAc): R_f = 0.21. Mp = 102-103 °C. α_D (20°C, MeOH) = + 27.5 °. IR (KBr) = 2927m, 2855m, 1716s. ^{1}H NMR (300 MHz, MeOD-d_4): δ = 7.81 (t, J = 4.6 Hz, 2 H), 7.64 (t, J = 7.0 Hz, 2 H); 7.36 (dt, J = 14.2 Hz, J = 7.3 Hz, 4 H); 4.70-4.66 (m, 1 H); 4.46-4.2 (m, 6 H); 3.79-3.64 (m, 2 H); 2.53-2.49 (m, 1 H); 2.27-2.23 (m, 1 H); 1.54-1.46 ppm (m, 27 H). ^{13}C NMR (500 MHz, MeOD-d_4): δ = 175.8; 156.4; 154.9; 152.6; 145.3; 142.6; 129.9; 128.4; 126.3; 121.0; 85.8; 83.4; 69.5; 69.2; 59.4; 57.4; 50.5; 35.1; 28.5 ppm. ESI-MS (MeOH + NaI): m/z 690 $[M+Na]^+$, 590.2 $[M+Na\text{-}boc]^+$. High-resolution ESI-MS: exact mass calcd for $C_{35}H_{45}N_3O_{10}Na_1$: 690.3003 ($[M+Na]^+$), m/z found 690.2991.

1.2. General protocol for the synthesis of peptide mimetics

Solvents were purchased from *Acros* or *Fisher* in HPLC or peptide synthesis grade. NMP and piperidine were purchased from *Acros Organics* (Geel, Belgium) and used without further purification. DMF was purchased from *Acros Organics* and redistilled under vacuum from ninhydrin, in order to remove amine impurities. DIEA was purchased from *Acros Organics* and redistilled under vacuum first from ninhydrin and subsequently from KOH. DCM was distilled over $CaCl_2$ and over CaH_2 prior to use. High purity water de-ionised water was made using an Elgastat® UHP-UF water purification system (High Wycombe, UK). 2-Chlorotrityl resin (100-200 mesh) with a loading of 1.2 mmol/g and Rink amide resin MBHA (100-200 mesh) were purchased from Novabiochem; Rink amide on ChemMatrix resin was purchased from ChemMatrix. 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 2-[1*H*-benzotriazole-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-hydroxy-9-azabenzotriazole (HOAt), *N*-hydroxybenzotriazole (HOBt) were purchased from *Novabiochem* in peptide synthesis grade. Fmoc protected amino acids and resins were purchased from *Novabiochem* or *Bachem* and the following *N*-α-Fmoc protected L-amino acids were used for routine solid phase peptide synthesis if not stated otherwise: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(^{t}Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-Glu(^{t}Bu)-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Pro-OH, Fmoc-Phe-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Thr(^{t}Bu)-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Trp(Boc)-OH and Fmoc-Val-OH, as well as Fmoc-D-Pro-OH.

The first amino acid was loaded on the resin in general at a loading of 0.3 to 0.5 mmol/g. For this, the calculated amount of *N*-α-Fmoc protected amino acid was dissolved in DCM (10 mL/g resin) and DIEA (4 eq relative to amino acid) was added. The solution was added to the resin and mixed by nitrogen bubbling for 2 h. The resin was washed with DCM/MeOH/DIEA (17:2:1 v/v/v, 3x), DCM (3x), DMF (2x) and DCM (2x), each 20 mL. The resin was dried in vacuum over KOH. For the determination of the amino acid loading, dry resin corresponding to approximately 1 µmol of Fmoc functionality was weighed into two 3 × 10 mm quartz UV cells, and treated with 3 mL of a freshly prepared solution of 20 % piperidine in dry DMF. The cuvettes were agitated occasionally by inversion and the mixture was left at RT until no change in UV_{290} could be detected. The UV absorption at 290 nm was measured on a Cary 3 UV-Vis spectrometer (*Varian*, Palo Alto, US), with 20 % piperidine in DMF as blank.

The amino acid loading was then calculated according to the following equation: loading (mmol/g) = (A_{290}(sample)-A_{290}(blank))/(mg of resin $\times$ 1.75).

Peptides were synthesised on an ABI433A peptide synthesizer (Applied Biosystems) coupled to a Perkin-Elmer 785A UV/VIS detector or a Perkin-Elmer Series 200 UV/VIS detector on a 0.25 mmol scale using the FastMoc™ protocol. Protected amino acids (4.0 eq, relative to resin) were activated with 0.45 M HBTU/HOBt in DMF (3.6 equiv) and 2 M DIEA in NMP (6.0 equiv). After activation for 2 min, the activated amino acid solution was transferred to the resin. Couplings were performed in NMP for 20 (standard) or 40 min (extended) coupling under vortexing. For removal of the N-terminal Fmoc group, 18 % piperidine in NMP was used for 3 min and subsequently 20 % piperidine in NMP for 7 min. Deprotection was monitored by detection of the released *N*-(9-fluorenylmethyl)piperidine by UV absorbance at 301 nm. The deprotection cycle was repeated using 20 % piperidine in DMF for 10 min until the absorption was less than 4 % compared to the previous cycle, up to a maximum of 6 additional deprotection steps. In case of a sluggish deprotection profile, the following amino acid was attached with extended coupling. Capping and N-terminal acetylations were performed using a mixture of 0.5 M Ac_2O, 0.006 M HOBt and 0.136 M DIEA in NMP. Intermittent washing steps during the automated synthesis were carried out with NMP and DCM.

Coupling of non-proteinogenic amino acids was carried out manually by dissolving 1.5 equiv of the *N*-α-Fmoc-protected amino acid in DMF (10 mL/g resin) and addition of 1.45 equiv of HATU and 4 equiv of DIEA. The solution was added to the Fmoc-deprotected resin and agitated for one hour. Completion of the coupling reactions was monitored by the Kaiser test. Upon completion, the resin was transferred back to the ABI synthesizer reaction vessel using DCM and the synthesis was completed after an additional NMP/DCM washing step.

The linear, side-chain protected peptides were cleaved from the 2-chlorotrityl resin after swelling in DCM (10 mL) using 0.8 % TFA in DCM (8 x 2 min with 10 mL) with agitation. The TFA-solution was filtered from the resin and added to a flask containing 1 mL DIEA for neutralization. The filtrate was concentrated in vacuo and the linear protected peptide was precipitated with ice-cold H_2O (50 mL) in the presence of MeOH (5 mL). The precipitate was filtered and washed with H_2O (3x), $NaHCO_3$

(5 %, 2x) H_2O (3x), $NaHSO_4$ (0.05 M, 2x) and H_2O (3x). The peptide was then dried in vacuo.

In the case of Rink amide resins, cleavage of the peptide from the resin side-chain deprotection and was performed in one step.

The linear, side-chain protected peptide was dissolved in DMF (300 mL) and HBTU (2 equiv), HOBt (2 equiv) and DIEA (8 equiv) were added. The bright yellow solution was left stirring for 12 h at RT. The reaction mixture was then evaporated to dryness in vacuo. The cyclic protected peptide was precipitated busing H_2O and dried in vacuo.

If not stated otherwise, the crude cyclized peptides were deprotected using the cocktail TFA/TIS/H_2O (95:2.5:2.5 *v/v/v*, 10 mL) with stirring for 1 h. The solution was concentrated in vacuo and then precipitated by slow addition of diethyl ether or di-isopropyl ether (20 mL) cooled to -20 °C, and the p recipitate was washed with the ice-cold ether (3 x 20 mL).

In the case of Rink amide resin, if not stated otherwise, the above-mentioned cleavage cocktail (10 mL/g resin) was added to the resin in a glass vessel and agitated for 1 h at RT. The cleavage solution was filtered off and the resin washed with cleavage cocktail (2 x 2 mL). Solvent evaporation and peptide precipitation were performed as described above.

Preparative scale reversed phase HPLC separations of peptides were performed using a Waters XBridge™ (C18, 50 × 19 mm, 5 µm, 135 Å) or a Agilent Zorbax Eclipse XDB-C18 (C18, 250 x 21.2 mm, 5 µm, 300 Å) column. Preparative-scale HPLC separations of lipopeptides were performed using a Laubscher Labs Interchrom UP10WC4/25M (C4, 250 x 21.2 mm, 5 µm, 300 Å) column. Purifications were done at ÄKTApurifier 100 system (GE Healthcare). Flow rates were generally between 10 and 20 mL/min and detection was by UV at wavelengths 226, 254 and 278 nm. Typical yields for cyclic peptidomimetics were in the range of 30 to 50 %.

Semi-preparative scale reversed phase HPLC separations of peptides were performed on a ÄKTApurifier 10 system (*GE Healthcare*) using a Agilent Zorbax Eclipse XDB-C18 (C18, 250 × 9.4 mm, 5 µm, 300 Å), and a Laubscher Labs Interchrom UP5WC4.25M (C4, 250 × 10.0 mm, 5 µm, 300 Å) at flow rates of 4 or 5 mL/min. Detection was by UV at wavelengths 226, 254 and 278 nm.

Analytical reversed phase HPLC of crude and pure peptides was performed on a ÄKTApurifier 10 system (*GE Healthcare*) using a Laubscher Labs Interchrom 218QTP54 (C18, 250 × 4.6 mm, 5 µm, 300 Å) or a Agilent Zorbax Eclipse XDB-C18 (C18, 250 × 4.6 mm, 5 µm, 300 Å) column, and for lipopeptides a Laubscher Labs Interchrom UP5WC4-25QS (C4, 250 × 4.6 mm, 5 µm, 300 Å) column. Flow rates were 1 mL/min and detection was by UV at wavelengths 226, 254 and 278 nm. After purification, all peptides were analyzed by HPLC, MS and NMR.

1.2.1 Alanine scanning library (cyclic peptides **20**-**27**)

These peptides were prepared using the methods described above, and were ≥95% pure by RP-HPLC. Retention times on analytical RP-HPLC (Interchrom C18) with a gradient from 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.7 min at a flow rate of 1 mL/min, and ESI-MS (MeOH/MeCN 1:1 + HCOOH 0.1 %) data are provided in the table below.

	$MS_{theoret}$ [g/mol]	MS_{exp} *m/z*	Retention time (min)
20	1305.9	1305.6	10.2
21	1323.9	1323.6	12.1
22	1232.4	1232.6	10.0
23	1339.9	1339.5	10.7
24	1337.9	1337.6	12.2
25	1266.8	1266.5	10.3
26	1323.9	1323.5	11.6
27	1305.9	1305.5	10.0

1.2.2. Peptidomimetics **6**, **7** and **8**

For peptide **6**, retention time on analytical RP-HPLC (Zorbax C18): 11.2 min with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min at a flow rate of 1 mL/min. ESI-MS (MeCN): *m/z* 1347.7 $[M+H]^+$, 1370.6 $[M+Na]^+$, 1385.6 $[M+2Na]^+$.

Syntheses of **7** and **8** used the racemic amino acids Fmoc-6-chloro-D/L-Trp-OH and Fmoc-6-methyl-D/L-Trp-OH, respectively. The resulting diastereomeric peptides could be separated by preparative RP-HPLC on a Zorbax XDB, C18 column using a gradi-

ent from 30-65 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 3.5 column volumes at a flow rate of 15 mL/min. Retention times on analytical RP-HPLC (Zorbax C18) column with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min at a flow rate of 1 mL/min were (the **B** isomers contain D-X-Trp, where X=Cl or Me) **7** 15.3 min, **7B** 10.9 min; **8** 12.7 min, **8B** 10.8 min. ESI-MS (MeCN) of mimetic **7**: *m/z* 1381.6 $[M+H]^+$, 1403.7 $[M+Na]^+$, 1419.5 $[M+Na+H_2O]^+$; ESI-MS (MeCN) of mimetic **7B**: *m/z* 1403.7 $[M+Na]^+$. ESI-MS (MeOH/MeCN 1:1 + HCOOH 0.1 %) of mimetic **8**: *m/z* 1361.6 $[M+H]^+$, 1383.6 $[M+Na]^+$; ESI-MS (MeOH/MeCN 1:1 + HCO_2H 0.1 %) of mimetic **8B**: *m/z* 1361.7 $[M+H]^+$, 1383.6 $[M+Na]^+$. ^1H NMR assignments for **7** and a crystal structure in complex with HDM2 were reported earlier.[1, 2] ^1H NMR assignments for **8** are given below:

^1H NMR (600 MHz, 300 K, in $[D_6]$DMSO) chemical shift assignment for **8**.

Residue	NH	H-C(α)	H-C(β)	Others
Phe1	7.50	4.83	2.97, 3.08	Aromat. 7.24, 7.30, 7.86
Glu2	8.86	5.04	1.77, 1.90	CH_2(γ) 2.24, 2.24
6-Me-Trp3	8.60	4.68	2.97, 3.22	Aromat. 6.74, 6.98, 7.52, 7.85, 8.23; NH 10.62
Leu4	8.70	3.47	1.43, 1.66	CH(γ) 1.13, CH_3(δ) 0.72
Asp5	8.23	4.27	2.71, 2.79	
Trp6	7.86	4.77	2.93, 3.19	Aromat. 6.96, 7.14, 7.28, 7.67, 8.23, 8.69; NH 10.80
Glu7	8.60	4.83	1.84	CH_2(γ) 2.25, 2.25
Phe8	8.58	4.89	2.80, 3.06	Aromat. 7.24, 7.50
D-Pro9	-	4.48	1.77	CH(γ) 2.04, 2.04; CH_2(δ) 3.40, 3.40
Pro10	-	4.43	1.54, 1.76	CH_2(γ) 0.76, 0.76; CH_2(δ) 3.45, 3.71

1.2.3. Synthesis peptididomimetics **9**-**12**

Peptidomimetics **9** & **10**. The cyclic peptide containing D-hydrazinoproline was prepared using the procedure described above. The peptide was isolated by preparative RP-HPLC (*Zorbax XDB C18*) with a gradient of 30-80 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 3 column volumes at a flow rate of 15 mL/min. Analytical HPLC (Zorbax C18): product eluted after 8.7 min with a gradient of 5-30 % acetonitrile

(0.1 % TFA) in H_2O (0.1 % TFA) in 16.7 min at a flow rate of 1 mL/min. ESI-MS (MeOH/MeCN 1:1 + HCOOH 0.1 %): *m/z* 1377.7 $[M+H]^+$, 1399.7 $[M+Na]^+$. This cyclic peptide (1.58 mg, 1.15 µmol) was dissolved in pyridine (1 mL) and cooled to 0 °C in ice/water bath. Acetic anhydride (1 mL of 1.15 mM in pyridine, 1.0 equiv) was added with stirring. After 20 min H_2O (0.5 mL) was added and the product **9** was directly purified by semi-preparative RP-HPLC (Zorbax C18) with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 4 column volumes at a flow rate of 5 mL/min. Retention time of **9** on analytical RP-HPLC (Interchrom C18) was 9.5 min, with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 12.5 min at a flow rate of 1 mL/min. ESI-MS (MeCN): *m/z* 1441.8 $[M+Na]^+$.

The cyclic peptide from above (3.5 mg, 2.54 nmol) was dissolved in ice-cold $NaHCO_3$ buffer (50 mM, pH 8.6, 4 mL). Biotin-$(PEO)_4$-NHS-propionate (Molecular Biosciences) (2.2 mg, 3.81 nmol, 1.5 equiv) was added and the reaction mixture was stirred on ice for 45 min. The product **10** was directly purified by semi-preparative RP-HPLC (Zorbax XDB C18) using a gradient from 30-60 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 4 column volumes at a flow rate of 5 mL. Retention time of **10** on analytical RP-HPLC (Zorbax C18) with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min at a flow rate of 1 mL/min was 8.5 min. ESI-MS of peptide **10** (MeCN): *m/z* 948.5 $[M+Na]^{2+}$, 1874.0 $[M+Na]^+$. High-resolution ESI-MS of **10**: exact mass calcd for $C_{91}H_{119}N_{17}O_{23}S$: 1849.8385 ($[M]^+$), *m/z* found 1849.8368. The ^1H NMR spectrum of the cyclic peptide backbone was also assigned in $[D_6]$DMSO:

^1H NMR (600 MHz, 300 K, $[D_6]$DMSO) chemical shift assignments for the peptide portion of **10**.

Residue	NH	H-C(α)	H-C(β)	Others
Phe1	7.52	4.88	3.04, 3.04	aromat 7.06, 7.22, 7.28, 7.40
Glu2	8.89	5.09	1.78, 1.92	CH(γ) 2.24, 2.24
Trp3	8.63	4.84	2.96, 3.22	aromat 7.06, 7.26, 7.63, 7.72; NH 10.81
Leu4	8.76	3.41	1.41, 1.66	CH(γ) 1.03, CH_3(δ) 0.67
Asp5	8.20	4.23	2.70, 2.78	-
Trp6	7.92	4.76	2.90, 3.23	aromat 6.97, 7.16, 7.28, 7.67, 7.92; NH 10.80

Glu7	8.63	4.74	1.82, 1.82	$CH_2(\gamma)$ 2.24, 2.24
Phe8	8.57	4.94	2.78, 3.05	aromat 7.06, 7.22, 7.27, 7.41, 7.65
D-Pro9	-	4.30	2.57, 2.81	$CH(\gamma)$ 3.08; $CH_2(\delta)$ 4.12, 4.12; NHNH 6.40, 6.35
Pro10	-	4.22	1.53, 1.77	$CH_2(\gamma)$ 0.79, 0.79; $CH_2(\delta)$ 3.42, 3.75

Peptidomimetic **11**. The cyclic precursor was prepared using **36** and Fmoc-6-chloro-D/L-Trp-OH, using the method described above. The diastereomeric peptides (**A** and **B**) could be isolated after purification by preparative RP-HPLC (Zorbax C18) with a gradient of 30-60 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 4 column volumes. Both peptides were isolated after lyophilisation as white powders. Retention times on analytical RP-HPLC (Interchrom C18) with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 10.0 min: **A** 6.9 min. **B** 7.5 min. ESI-MS for **A** (MeOH/ MeCN 1:1 + HCOOH 0.1 %): *m/z* 1411.6 $[M+H]^+$, 1427.5 $[M+H_2O]^+$. ESI-MS for **B** (MeOH/MeCN 1:1 + HCOOH 0.1 %): *m/z* 1411.6 $[M+H]^+$, 1427.6 $[M+H_2O]^+$.

The isomer **A** was biotinylated in the same way as for **10** above. Retention time for **11** on analytical RP-HPLC with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min at a flow rate of 1 mL/min (Interchrom C18) was 8.8 min. ESI-MS (MeCN): *m/z* 1907.8 $[M+Na]^+$. High-resolution ESI-MS, exact mass calcd for $C_{91}H_{118}ClN_{17}O_{23}SNa_2$: 1929.7791 ($[M+2Na]^+$), *m/z* found 1929.7809. The ^1H NMR spectrum of the cyclic peptide backbone was also assigned in[D_6]DMSO:

^1H NMR (600 MHz, 300 K, [D_6]DMSO) chemical shift assignments for the peptide portion of **11**.

Residue	NH	H-C(α)	H-C(β)	Others
Phe1	7.51	4.86	3.02, 3.02	aromat 7.29, 7.41
Glu2	8.87	5.07	1.77, 1.90	$CH(\gamma)$ 2.23, 2.23
Trp3	8.63	4.67	2.95, 3.21	aromat 6.89, 7.10, 7.24, 7.59; NH 10.96
Leu4	8.76	3.43	1.42, 1.63	$CH(\gamma)$ 0.10, $CH_3(\delta)$ 0.70
Asp5	8.24	4.23	2.72, 2.79	-
Trp6	7.89	4.75	2.88, 3.22	aromat 6.97, 7.03, 7.14, 7.29, 7.66; NH 10.80

Glu7	8.63	4.75	1.82, 1.82	$CH_2(\gamma)$ 2.23, 2.23
Phe8	8.58	4.94	2.79, 3.08	aromat 6.42, 7.07, 7.21, 7.27
D-Pro9	-	4.61	1.84, 2.10	$CH(\gamma)$ 3.64; $CH_2(\delta)$ 3.38, 3.38;
Pro10	-	4.21	1.52, 1.76	$CH_2(\gamma)$ 0.77, 0.77; $CH_2(\delta)$ 3.42, 3.74

Peptidomimetic **12**. The cyclic precursor was prepared using **36** and Fmoc-6-methyl-D/L-Trp-OH, using the method described above. The diastereomeric peptides (**A** and **B**) could be isolated after purification by preparative RP-HPLC (Zorbax C18) with a gradient of 30-60 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 4 column volumes. Both peptides were isolated after lyophilisation as white powders. Retention times on analytical RP-HPLC (Interchrom C18) with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 10.0 min: **A** 7.4 min. **B** 6.8 min. (the **A** isomer contains L-Me-Trp) ESI-MS for peptide **A** (MeOH/MeCN 1:1 + HCOOH 0.1 %): *m/z* 1391.7 $[M+H]^+$.

The isomer **A** was biotinylated in the same way as for **10** above. Retention time for **12** on analytical RP-HPLC with a gradient of 30-100 % acetonitrile (Interchrom C18) was 9.0 min. ESI-MS of peptide **12** (MeCN/H_2O 1:1 + TFA 0.1 %): *m/z* 1865.8 $[M+H]^+$, 1886.8 $[M+Na]^+$. High-resolution ESI-MS of **31**: exact mass calcd for $C_{92}H_{122}N_{17}O_{23}$-NaS: 1887.8518 ($[M+H+Na]^{2+}$), *m/z* found 1887.8487. The ^1H NMR spectrum of the cyclic peptide backbone was also assigned in [D_6]DMSO:

^1H NMR (600 MHz, 300 K, [D_6]DMSO) chemical shift assignments for the peptide portion of **12**.

Residue	NH	H-C(α)	H-C(β)	Others
Phe1	7.51	4.87	3.02, 3.02	aromat 6.74, 7.02, 7.51
Glu2	8.88	5.08	1.78, 1.91	$CH(\gamma)$ 2.25, 2.25
Trp3	8.61	4.68	2.95, 3.21	aromat 6.97, 7.30, 7.66; NH 10.63
Leu4	8.72	3.43	1.43, 1.66	$CH(\gamma)$ 1.08, $CH_3(\delta)$ 0.70
Asp5	8.22	4.23	2.71, 2.79	-
Trp6	7.90	4.75	2.89, 3.22	aromat 7.15; NH 10.80
Glu7	8.62	4.75	1.83, 1.83	$CH_2(\gamma)$ 2.24, 2.24
Phe8	8.57	4.94	2.79, 3.07	aromat 7.06, 7.22, 7.27
D-Pro9	-	4.61	1.85, 2.10	$CH(\gamma)$ 3.64; $CH_2(\delta)$ 3.39, 3.39;

				NHNH 9.36
Pro10	-	4.22	1.53, 1.77	$CH_2(\gamma)$ 0.79, 0.79; $CH_2(\delta)$ 3.43, 3.76

1.2.4. Synthesis of linear peptides

Linear peptide **19**: The peptide was synthesized on Rink amide MBHA resin (0.35 g resin, loading: 0.72 mmol/g) using the standard protocol, and the N terminus was acetylated using acetic anhydride. After cleavage from the resin and deprotection, the peptide was purified by preparative RP-HPLC (Zorbax C18) with a gradient of 30-55 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 3.5 cv. Analytical RP-HPLC (Interchrom C18): t_R = 7.5 min with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min. ESI-MS (MeOH/MeCN 1:1 + HCOOH 0.1 %): *m/z* 1212.5 $[M+H]^+$.

Linear peptide **13**: The peptide was synthesized on Rink amide MBHA resin (0.42 g, loading: 0.66 mmol/g) using the standard protocol, and the N terminus was acetylated using acetic anhydride. After cleavage from the resin and deprotection, the peptide was purified by preparative RP-HPLC (*Zorbax XDB C18*) with a gradient from 25-40 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 3.5 column volumes. Retention time on analytical HPLC (Interchrom C18) t_R 5.71 min with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min. ESI-MS (MeOH/H_2O 1:1 + HCOOH 0.1 %): *m/z* 936.1 $[M+2H]^{2+}$, 1849.0 $[M+H]^+$. ^{1}H NMR assignments are given below.

Peptide **13**: ^{1}H NMR (500 MHz, 300 K, H_2O/D_2O 9:1, pH 5.3) chemical shift assignments.

Residue	NH	H-C(α)	H-C(β)	Others
Ser1	8.33	4.43	3.87, 3.87	acetyl 2.07
Gln2	8.58	4.34	1.98, 2.13	$CH_2(\gamma)$ 2.36, 2.36
Glu3	8.46	4.30	1.91, 1.91	$CH_2(\gamma)$ 2.25, 2.25
Thr4	8.14	4.26	4.13	$CH_3(\gamma)$ 1.11
Phe5	8.24	4.61	3.04, 3.16	aromat 7.22, 8.13
Ser6	8.11	4.33	3.74, 3.87	-
Asp7	8.29	4.56	2.61, 2.61	-
Leu8	7.96	4.10	1.41, 1.52	CH(γ) 0.87, $CH_3(\delta)$ 0.80

Trp9	7.85	4.55	3.31, 3.31	aromat 7.28, 7.58, 7.97, 8.28; NH 10.17
Lys10	7.58	4.07	1.70, 1.70	$CH_2(\gamma)$ 1.13, 1.13; $CH_2(\delta)$ 1.60, 1.60, $CH_2(\varepsilon)$ 2.93, 2.93
Leu11	7.85	4.29	1.63, 1.63	$CH(\gamma)$ 0.93, $CH_3(\delta)$ 0.86
Leu12	7.96	4.62	1.67, 1.67	$CH(\gamma)$ 1.58, $CH_3(\delta)$ 0.92
Pro13	-	4.38	2.31, 2.31	$CH_2(\gamma)$ 1.94, 2.04; $CH_2(\delta)$ 3.69, 3.83
Glu14	8.72	4.22	1.98, 2.05	$CH_2(\gamma)$ 2.29, 2.29
Asn15	8.33	4.70	2.77, 2.77	-

Synthesis of linear peptides **14** and **15**: The synthesis was performed on rink amide MBHA resin (0.42 g, loading: 0.66 mmol/g) using Fmoc-6-chloro-D/L-Trp-OH (**for 14**) and Fmoc-6-methyl-D/L-Trp-OH (**for 15**), and the procedures described above. After the addition of the 15th residue (Ser) each batch of resin was divided into two portions. One portion was used to acetylate the N-terminus, to give **14** and **15**, and the other portion was used for the synthesis of **17** and **18** (see below). After cleavage from the resin and deprotection, the peptides **14** and **15** were isolated by preparative RP-HPLC (Zorbax C18) with a gradient of 25-40 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 3.5 column volumes. Retention times on analytical RP-HPLC (Interchrom C18) with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min. (The **A** isomers contains L-Cl/Me-Trp): **14A**: 5.8 min. **14B**: 5.4 min. **15A**: 5.8 min. **15B**: 5.0 min. ESI-MS of mimetic **14A** (MeOH/H_2O 1:1 + HCOOH 0.1 %): *m/z* 942.1 $[M+2H]^{2+}$, 1883.1 $[M+H]^{+}$. ESI-MS of mimetic **14B** (MeOH/H_2O 1:1 + HCO_2H 0.1 %): *m/z* 942.1 $[M+2H]^{2+}$, 1883.0 $[M+H]^{+}$. ESI-MS of mimetic **15A** (MeOH/H_2O 1:1 + HCOOH 0.1 %): *m/z* 932.1 $[M+2H]^{2+}$, 1863.0 $[M+H]^{+}$. ESI-MS of mimetic **15B** (MeOH/H_2O 1:1 + HCOOH 0.1 %): *m/z* 932.1 $[M+2H]^{2+}$, 1863.0 $[M+H]^{+}$.

Linear peptides **16**-**18**: The synthesis of peptide **16** was described earlier.[2] For the synthesis of **17** and **18**, the chain assembly was completed, as described above for **14** and **15**, and finally biotin was coupled at the N-terminus. For this, biotin (44 mg, 0.188 mmol, 1.5 equiv) was dissolved in DMF/DCM (1:1, 8 mL) and HATU (61 mg, 0.16 mmol, 1.45 equiv), HOAT (22 mg, 0.16 mmol, 1.45 equiv) and DIEA (160 µL, 0.9 mmol, 8 equiv) were added to the resin with agitation for 90 min. Cleavage, deprotection and precipitation were performed using the standard protocol, and pep-

tides were purified by preparative RP-HPLC (*C18*) with a gradient of 20-50 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 3.5 column volumes. Retention times on analytical RP-HPLC with a gradient of 30-100 % acetonitrile (0.1 % TFA) in H_2O (0.1 % TFA) in 16.6 min at a flow rate of 1 mL/min. Retention times: **17** (Zorbax C18): 5.4 min. **18** (Interchrom C18): 5.4 min. ESI-MS of peptide **17** (MeCN/H_2O 1:1 + HCO_2H 0.1 %): *m/z* 1178.6 $[M+H]^{2+}$. ESI-MS of peptide **18** (MeCN/H_2O 1:1 + HCO_2H 0.1 %): *m/z* 1168.1 $[M+H]^{2+}$.

2. Production of HDM2

Recombinant HDM2, comprising residues 17 to 125 of human double minute protein 2, was produced in *E. coli* BL21(DE3)LysS and purified as described earlier.[1] The concentration of purified $HDM2^{17-125}$ was determined spectrophotometrically at 280 nm using an extinction coefficient of 11400 $cm^{-1}\,M^{-1}$, as determined by amino acid analysis. The protein was stored at -20 °C.

Prior to ITC measurements, the HDM2 protein was purified further by affinity chromatography. The affinity column was prepared as follows. An NHS-activated HiTrap™ HP column (1 mL, *Amersham Biosciences*) was washed with ice-cold HCl (1 mM, 6 mL) at a flow rate of 1 mL/min. A linear p53-derived peptide (**13a**) comprising $p53^{15-29}$ (2 mg/mL) was coupled in buffer (0.2 M $NaHCO_3$, 0.5 M NaCl, pH 7.3) at RT for 30 min. The column was then washed alternately with deactivation buffer (0.5 M ethanolamine, 0.5 M NaCl, pH 8.3), washing buffer (0.1 M NaOAc, 0.5 M NaCl, pH 4.0) and again deactivation buffer (each 6 mL). After repeating the washing steps, the column was washed with storage buffer (0.05 M Na_2HPO_4, 0.1 % NaN_3, pH 7.0) and stored at 4 °C.

HDM2 purification by affinity chromatography. The affinity column was first equilibrated in phosphate buffer (50 mM, pH 6.9) (10 mL) at a flow rate of 1 mL/min. Purified HDM2 was loaded on the column at a flow rate of 1 mL/min in phosphate buffer (50 mM, pH 6.9) in the concentrations obtained from ion exchange chromatography (max. 5 mg protein per run). The column was washed with phosphate buffer (5 mL) and the active protein was eluted with eluting buffer (10 mM glycine-HCl, pH 3.4) at a flow rate of 1 mL/min. The solution was immediately neutralized by addition of neutralization buffer (1 M Tris-HCl, pH 9.0, ca. 10 µL). The protein was dialyzed into ITC buffer (50 mM phosphate, 150 mM NaCl, 1 mM DDT, pH 7.4) overnight, prior to ITC measurements.

3. Surface plasmon resonance

If not stated otherwise, all measurements were carried out at 25 ℃ in HBS buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005 % P20, pH 7.4) as running buffer on a BIAcore 1000 (Biacore, Uppsala) or a BIAcore 3000 (Biacore) instrument. All solutions were filtered through a 0.2 µm filter.

Direct binding assay: Surface plasmon resonance experiments were performed on a BIAcore 1000 instrumnent. Streptavidin coated (SA) sensor chips were preconditioned by three one-minute injections of NaCl (1 M) in NaOH (50 mM). The biotinylated linear peptides **16**, **17** and **18** (40 nM in HBS buffer) were immobilized at a flow rate of 5 µL/min to an extent of 300 to 400 RU. HDM2 was diluted to concentrations ranging from 9 nM to 2.5 µM and injected over the sample and empty reference cells (120 to 180 µL). Experiments were performed at different flow rates (10 µL/min, 20 µL/min, 30 µL/min, 40 µL/min and 60 µL/min). The surface was regenerated at the end of the injection with a 30 s pulse of HCl (10 mM).

Biosensor data were elaborated with *BIAevaluation* software. The response from the reference surface was subtracted from the binding responses collected on the sample flow cell to correct for bulk refractive index changes. The response from the buffer injection was also subtracted. The interactions reached steady-state binding during sample injection, and binding isotherms were obtained by plotting the equilibrium response against the peptide concentration (Figure-1S). Equilibrium dissociation constants (K_d) were determined by nonlinear curve fitting analysis of the binding isotherms. Each binding analysis was further analyzed in a Scatchard plot by plotting R_{eq}/HDM2 concentration versus the R_{eq} value. The negative reciprocal value of the slope is equal to the K_d of the interaction. Reported mean values and standard deviations derive from at least three independent experiments.

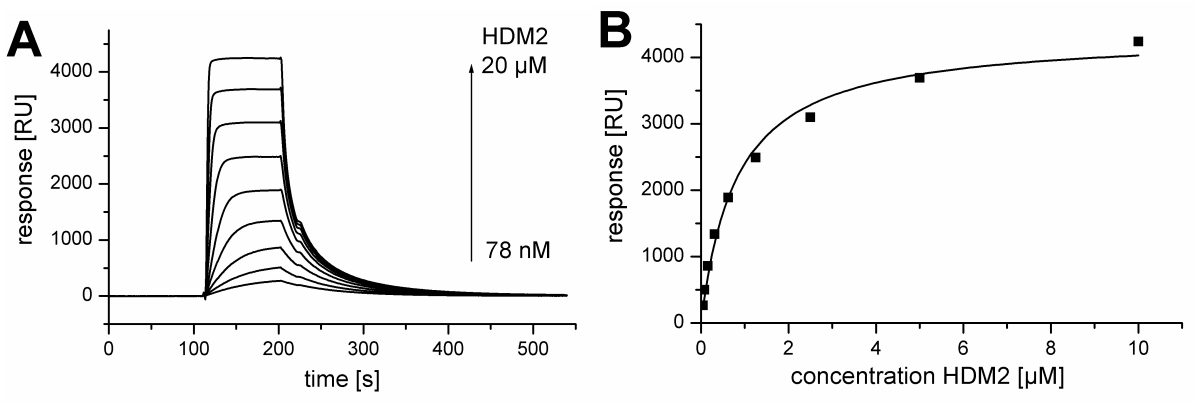


Figure S1. A) SPR sensorgrams showing direct binding of HDM2 to immobilized peptide 16. B) concentration dependence of the steady state response.

Biotinylated cyclic peptidomimetics (**10**-**12**) were immobilized on carboxylated BIAcore sensor chip (CM5) to which streptavidin had been coupled by random amine coupling. Sample and reference flow cells were activated by a 7 min injection (5 µL/ min) of NHS (0.05 M)/EDC (0.2 M). Streptavidin (100 µg/mL in acetate 10 mM, pH 4.5) was immobilized by a 7 min injection at a flow rate of 5 µL/min to a level of about 300 RU. Unreacted binding sites were deactivated by a 7 min injection of ethanolamine (1 M, pH 8.5). The biotin-PEG-peptidomimetics **10**-**12** (20 nM in HBS) were immobilized on the sample flow cell to 30 RU at a flow rate of 5 µL/min. HDM2 was injected in concentrations from 30 nM to 1 µM at varying flow rates (20 µL/min, 40 µL/min, 60 µL/min, 80 µL/min) in HBS buffer and 120 to 180 µL HDM2 solution was injected.

From the binding curves were subtracting the buffer signal and the signal from the reference flow cell. The referenced binding data were then analyzed by nonlinear global curve fitting to a simple reversible bimolecular interaction model using the BIA-evaluation software (Figure S2). Having determined the rate constants (k_{on} and k_{off}), equilibrium dissociation constants (K_d) were calculated from the quotient k_{off}/k_{on}. The good quality of the best-fit parameters was indicated by the small and randomly distributed residuals (on average, $\pm$ 2 RU) and the small standard errors (on average, < 2 %) in the parameter estimates (R_{max}, k_{on}, k_{off}). For the interactions that reached steady-state binding during sample injection, binding isotherms were obtained by plotting the equilibrium response against the peptide concentration. Equilibrium dissociation constants (K_d) were determined by nonlinear curve fitting analysis of the binding isotherms as described above. The reliability of the kinetic rates was also

supported by the good agreement between the kinetically derived K_d values and the equilibrium dissociation constants determined by steady-state analysis.

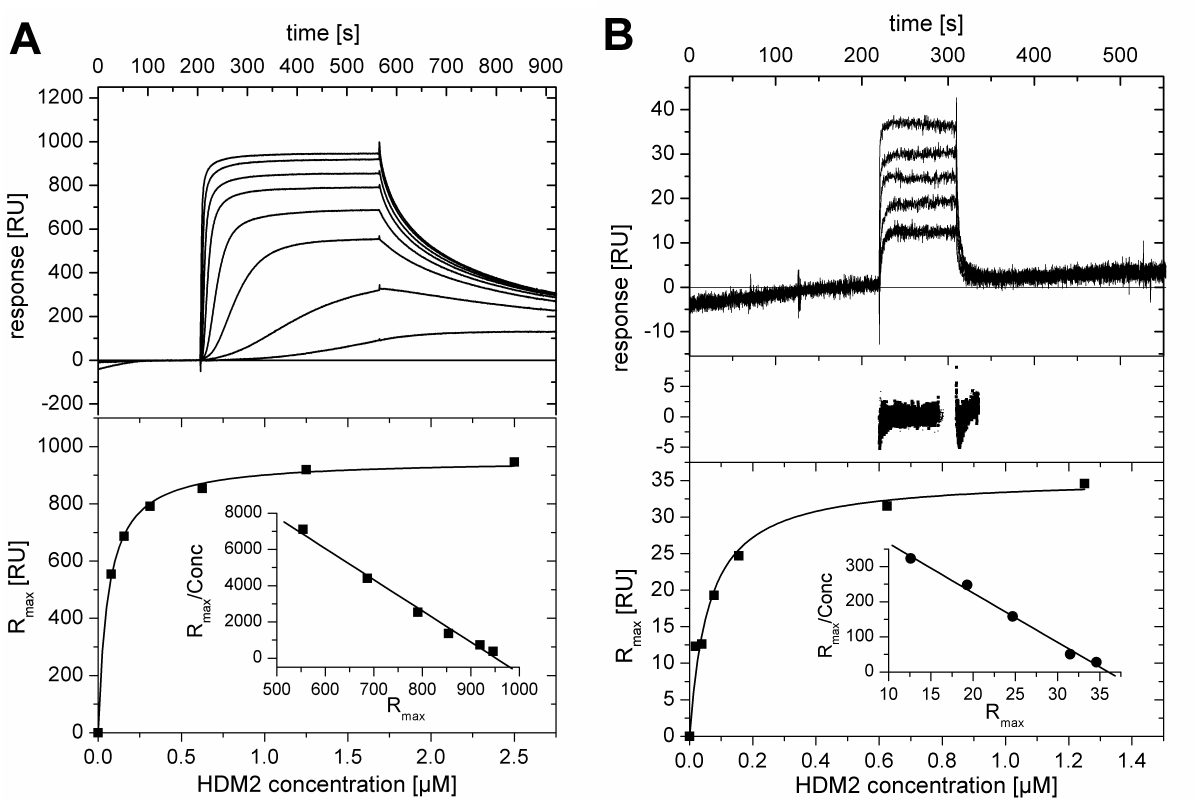


Figure S2. Top panels SPR sensorgrams of HDM2 binding to A) immobilized peptides **17**, and B) immobilized peptide **11**. The lower panels show the concentration dependence of the steady state response and a Scatchard plot. In (B), middle, the residuals from fitting the kinetic data.

Competition assay: Competition experiments were performed on a BIAcore 1000 instrument. A streptavidin coated sensor chip SA (*Biacore*) was preconditioned by three consecutive 1 min injections of NaCl (1 M) in NaOH (50 mM). The biotinylated p53-derived peptide **16** (80 μM in HBS) was captured on the sensor chip at a density of 570-600 RU by injecting at a flow rate of 10 μL/min for 1 min. An untreated flow cell was used as reference surface to correct for bulk refractive index changes. A solution of $HDM2^{17-125}$ at a concentration of 0.250 μM in HBS was incubated with a given concentration of inhibitor and 20 μL of this solution was injected over the specific and reference surfaces at a flow rate of 10 μL/min. An injection with buffer alone was also included. Remaining surface-bound protein was completely removed with a 30 s pulse of HCl (10 mM). Specific binding curves for each concentration of inhibitor were obtained by subtracting the response in the reference surface from the response in

the p53-peptide-coated surface. Equilibrium response values ($R_{equiv)}$ at steady state were calculated by averaging the last 10 s of each sample injection. For each inhibitor, eight different concentrations were used and each experiment was repeated twice. Inhibition curves were obtained by plotting the decrease in binding response against the logarithmic increase of the inhibitor concentration. IC_{50} values were determined by sigmoidal curve fit using Origin software. The reported mean values and standard deviations derive from at least three independent experiments. A control standard (peptide **13**) was included in each set of experiments to verify the reproducibility of the assay.

4. Isothermal titration calorimetry

All measurements were carried out on a VP-ITC microcalorimeter (Microcal). The protein was purified by affinity chromatography prior to titration experiments and dialyzed against ITC buffer (50 mM sodium phosphate, 150 mM NaCl, 1 mM DTT, pH 7.4) and diluted to concentrations of 20 to 40 µM. Peptides were prepared in identical buffer at concentrations of 250 to 400 µM. All measurements were performed at 25 °C with a reference power of 10 µCal/s. The sample cell was filled with 1.4 mL of HDM2 protein solution (20-40 µM) and a syringe was filled with peptide ligand (250-400 µM). The system was equilibrated for ca. 30 min until the baseline had stabilized. Peptide solution was added in steps of 10 µL (2 µL for the first step) during 10 s (2 s for the first step). Spacings of 300 s between the single titration steps were applied. 30 injections were completed for each compound. The heat changes for injections 2 to 30 were plotted and integrated using *Origin* software. The resulting ΔH values were plotted in kcal/mol of injectant vs molar ratio of peptide. The binding data were analyzed by sigmoidal global curve fitting to a 1:1 binding model to obtain the stoichiometry of the titration, entropy ΔS and K_d-value. At least two independent measurements were performed for each ligand. The homogeneity of HDM2 could be verified by the stoichiometry of binding, which was in the range of 0.95 to 1.1 for all measurements.

5. Computational studies

To characterize the electronic nature of the interactions between the three linear p53-derived peptides (**13**-**15**), and three cyclic ß-hairpin peptidomimetics (**6**-**8**) and HDM2, computational quantum chemistry and hybrid quantum/classical electrostatic methods were applied. The 3D structure of HDM2 bound to the β-hairpin peptide **7** was

obtained from PDB entry 2AXI,[1] and the complex with 15-mer linear p53 peptide **13a** was from PDB entry 1YCR.[Kussie, 1996 #5] PDB entry 2GV2 contains the HDM2 protein bound to the octamer peptide **3**.[3] The binding energy of each ligand in the respective complexes was analyzed with the Adaptive Poisson–Boltzmann Solver (APBS) package,[4] to solve numerically the linearized Poisson–Boltzmann Equation. The preparation of the receptor was carried out in several steps. Partial atomic charges and radii required to solve the PBE were assigned with the program PDB2PQR[5] according to the PARSE[6] force field. Protonation and optimization of the hydrogen bond network of HDM2 was carried out using the program PROPKA.[7] The ligand was treated using density functional techniques with the program B3LYP,[8] together with the double-ζ valence DZV(2d,p) basis set of Dunning, as specified in the GAMESS software.[9] Constrained as well as unconstrained geometry optimizations were carried out on a variety of ligand structures. Constrained optimizations were used together with the receptor in the APBS computations, and the unconstrained optimizations were investigated for comparison purposes. Ligand charges for use in APBS were determined using the CHELPG scheme.[10] The VMD software[11] was used for visualization of complexes.

The value of the protein dielectric constant, which depends on polarization, flexibility, and protonation state of the side chains, is an active subject of discussion in the scientific community.[12-17] The typical value for the protein environment is between 2-4, according to experimental as well as theoretical results, although it has been determined that the value will fluctuate between 1 to 20.[14] The choice of the dielectric depends also on the continuum model used to study a particular macromolecular system.[15] Models based on Poisson-Boltzmann typically incorporate a dielectric constant between 4-8, with 4 the more consistent value. The present work involves the use of the adaptive Poisson-Boltzmann equation for the entire ligand-HDM2 complexes using APBS, incorporating the accurate QM structural and atomic charge data for the peptides, carried out in low dielectric ($\varepsilon = 4$), to describe the protein interior environment, and high dielectric ($\varepsilon = 78.54$) for the surrounding solvent. The ionic strength was assigned as 0.150 M with an ion exclusion radius of 2 Å. Boundary conditions were applied using the single Debye Hückel method. A grid dimension of 225 x 193 x 193 was used, and the Poisson–Boltzmann equation solved to a 0.4 Å resolution. The nonelectrostatic energy was calculated following the methods described by Wagoner and Baker.[18]

Calculated electrostatic properties were used to derive total binding energies ($\Delta E_{\text{tot binding}}$) for linear and cyclic peptides bound to HDM2. The calculated total binding energy ($\Delta E_{\text{tot binding}}$) was calculated using the Adaptive Poisson-Boltzmann Solver (APBS) method[19], which includes both electrostatic interactions ($\Delta E_{\text{total elect}}$) arising from both coulombic (ΔE_{elect}) and polar solvation ($\Delta E_{\text{solvation}}$) contributions, as well as non-polar solvation effects ($\Delta E_{\text{total non-elect}}$) arising from cavitation and dispersion terms, i.e.:

$$\Delta E_{\text{tot binding}} = \Delta E_{\text{total elect}} + \Delta E_{\text{total non-elect}} \quad (1)$$

$$\text{where, } \Delta E_{\text{total elect}} = \Delta E_{\text{elect}} + \Delta E_{\text{solvation}} \quad (2)$$

Each of these energies are evaluated for the complex (E_{complex}) and for the separated protein (E_{protein}) and the ligand (E_{ligand}), i.e.:

$$\Delta E = E_{\text{complex}} - (E_{\text{protein}} + E_{\text{ligand}}) \quad (3)$$

Unconstrained quantum chemical calculations were performed on the ligand, and ligand plus surrounding residues to better understand the electronic nature of these interactions. In particular, larger fragments included Trp23 plus Leu54, Trp23 plus the Phe86 and Phe91 side chains, to better understand the hydrogen bond interaction and the vdW interactions between those residues, respectively. These computations were performed in vacuum and low dielectric environment using both the B3LYP as well as the more modern and accurate M06-2X[20] functional, both incorporating the DZV(2d,p) basis set.

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