Supplementary Material (ESI) for Chemical Communications

Oligobenzamide proteomimetic inhibitors of the p53-*h*DM2 protein-protein interaction

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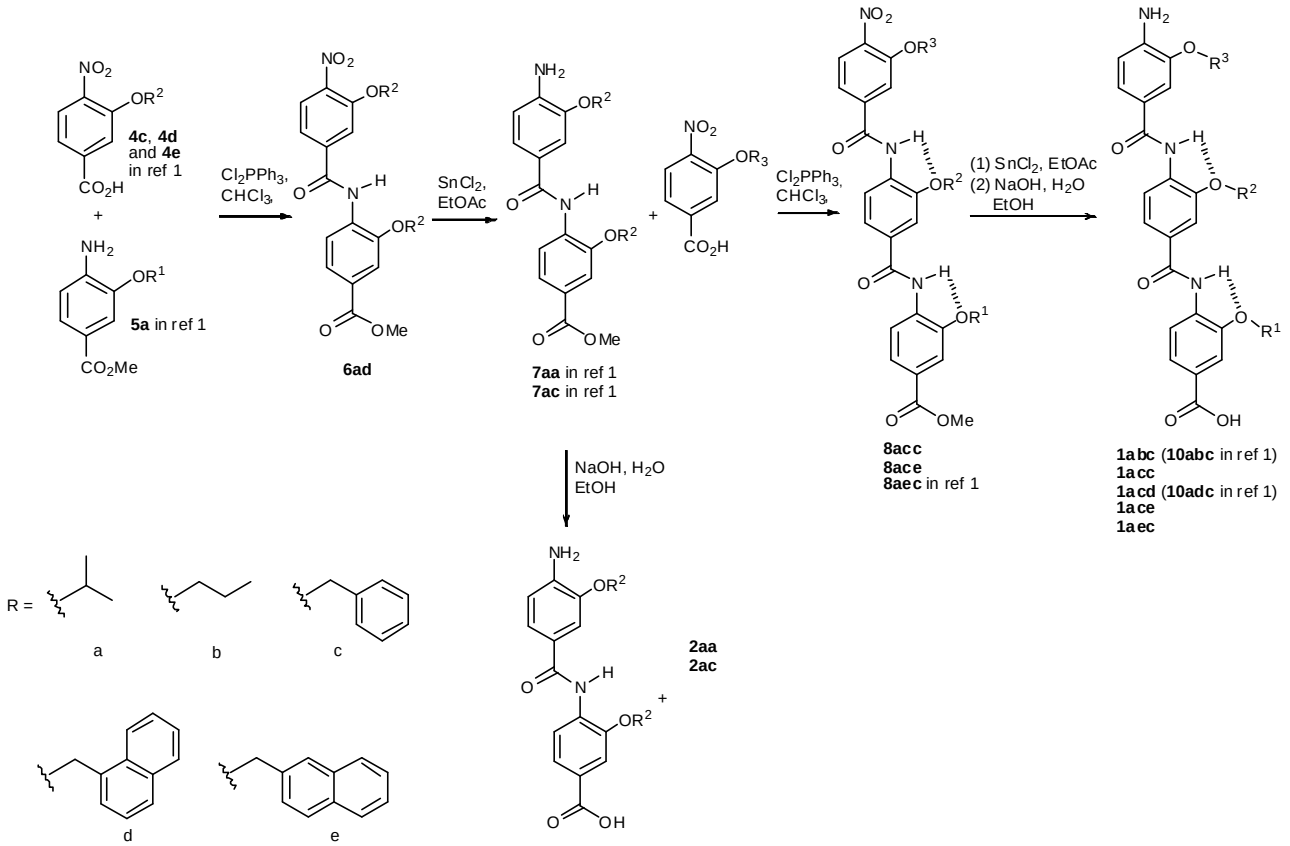
General Experimental Points

All chemicals and solvents were purchased from Aldrich and used without further purification unlseee otherwise stated. Melting points were determined using a Griffin D5 variable temperature apparatus and are uncorrected. ^{1}H Nuclear Magnetic Resonance spectra were recorded using a Bruker Avance DRX500 and Avance300 instruments. ^{1}H spectra are referenced to tetramethylsilane (TMS) and chemical shifts are givin in parts per million downfield from TMS. Coupling constants are reported to the nearest 0.1 Hz. Microanalysis were obtained on a Carlo Erba Elemental Analyser MOD 1106 instrument. Infrared spectra were recorded on a Perkin-Elmer FTIR spectrometer and samples analysed in the solid phase. Mass spectra were obtained on a Bruker microTOF instrument using electro-spray analysis.

The synthesis of compounds was performed in an analogous fashion to those previously described[1] according to scheme ESI1 (the numbering scheme between this paper and our previous synthetic paper[1] is kept the same for clairty). Compounds **1bca** and **1acd** were previously described,[1] as were intermediates **4c**, **4d**, **4e**, **5a**, **7aa**, **7ac**, **8aec**. and **9aaa**.



Scheme ESI 1. Synthesis of new α-helix mimetics

General procedure B for the saponification of esters Oligoamide (1 equiv) was dissolved in THF (25 mL per g of ester) and MeOH (25 mL per g of ester). NaOH (10% aqueous 5 mL per g of ester) was then added and the solution, sealed and stirred overnight. When the reaction was complete as observed by TLC the organic solvent was removed under reduced pressure. The resulting viscous solution was diluted with water (40 mL per g of ester) and acidified to pH of 1 using 10% aqueous HCl. The resulting suspension was then filtered and the solid dried overnight yielding a white solid. Alternatively the acid was dissolved in EtOAc and separated from the spurious water and dried (Na_2SO_4). Removal of the solvents under reduced pressure yielded the acid as a white solid.

General procedure C for the coupling reaction

Dichlorotriphenylphosphorane (4.5 equiv) was added to a stirred solution of amine (1.0 equiv.) and acid (1.4 equiv) in freshly distilled $CHCl_3$ (50 mL per g of amine), under an inert atmosphere and the solution allowed to stir at reflux (80°C) overnight. The solvents were removed under a reduced pressure and the resultant viscous oil subjected to column chromatography (SiO_2, CH_2Cl_2 / Et_2O gradient) to yield the product.

General procedure D for reduction of a nitro group

To a solution of a nitro compound (1 equivalent) in EtOAc (5-10 mL) was added $SnCl_2.2H_2O$ (5 equivalents) and the reaction mixture was heated to 50°C under a drying tube. When TLC indicated complete reduction (typically 24 hours), the reaction mixture was cooled, then poured into NaOH solution (10% 100 mL) and the flask rinsed with ethylacetate (× 2). The combined organics portions were extracted twice with NaOH solution (10% 100 mL) before being washed once with brine (50 mL). The organics were then dried (Na_2SO_4) and the solvent removed under reduced pressure yielding oils that solidify upon standing. Alternatively, the oil was subjected to column chromatography (SiO_2, CH_2Cl_2 /Et_2O gradient) to give a cream white to yellow crystalline product.

Methyl-3-isopropoxy-4-(3-methyl(-1-napthyl)oxy-4-nitro-benzoylamido)-benzoate 6ad

(Procedure D) **4d** (1.0 g, 3.4 mmol), **5a** (0.50 g, 2.38 mmol), Cl_2PPh_3 (4.20 g, 13.0 mmol), and chloroform (100 mL) afforded the product (0.53 g, 44%) as a yellow solid, after multiple columns to remove a trace impurity; m.p. 162-163 °C; δ_H (300 MHz, $CDCl_3$) 1.41 (6H, d *J* = 6 Hz, iPrCH_3), 3.91 (3H, s, CO_2Me), 4.76 (1H, sep, *J* = 6 Hz, ^{i}PrCH), 5.76 (2H, s, napthyl-CH_2), 7.39 (1H, d, *J* = 8.3 Hz, ArCH), 7.46-7.60 (4H, m, ArCH), 7.70 (1H, d, *J* = 8.4 Hz, ArCH), 7.73 (1H, d, *J* = 8.4 Hz, ArCH), 7.85 (1H, d, *J* = 8.3 Hz, ArCH), 7.89 (1H, d, *J* = 8.3 Hz, ArCH), 7.93 (1H, s, ArCH), 7.95 (1H, d, *J* = 8.3 Hz), 8.05 (1H, d, *J* = 8.5 Hz, ArCH), 8.57 (1H, d, *J* = 8.5 Hz, ArCH), 8.78 (1H, s, NH); δ_C (75 MHz, $CDCl_3$) 22.2, 52.2, 70.2, 71.9, 112.9, 114.9,

117.8, 119.0, 119.5, 123.2, 125.4, 126.1, 126.2, 126.3, 126.8, 128.8, 129.5, 130.2, 131.0, 132.1, 133.7, 139.9, 142.1, 143.3, 145.9, 152.2, 162.9, 166.6; ν_{max}/cm^{-1} (solid state) 3427, 3367, 3109, 2980, 1712, 1602, 1514, 1347, 1110 ,992, 951, 919, 869, 792, 607; ESI-HRMS found *m/z* 515.1801 $[M+H]^+$, $C_{29}H_{27}N_2O_7$ requires 515.1813.

Methyl-3-isopropoxy-4-(3-methyl(-1-napthyl)oxy-4-amino-benzoylamido)-benzoate 7ad

(Procedure C) **6ad** (251.3 mg, 0.50 mmol), $SnCl_2$ (614.3 mg, 2.73 mmol), ethyl acetate (60 mL) afforded the product as a yellow oil that solidified upon standing (161.3 mg, 70%); m.p. 153-154 °C; δ_H (300 MHz, $CDCl_3$) 1.41 (6H, d *J* = 6 Hz, $^iPrCH_3$), 3.89 (3H, s, CO_2Me), 4.20 (2H, br, NH_2), 4.73 (1H, sep, *J* = 6 Hz, iPrCH), 5.57 (2H, s, napthyl-CH_2), 6.74 (1H, d, *J* = 8.0 Hz, ArCH), 7.45-7.60 (5H, m, ArCH), 7.70-7.73 (2H, m, ArCH), 7.86-7.92 (2H, m, ArCH), 8.05 (1H, d, *J* = 8.4 Hz, ArCH), 8.64 (1H, d, *J* = 8.5 Hz, ArCH), 8.77 (1H, s, NH); δ_C (75 MHz, $CDCl_3$) 22.2, 53.5, 69.3, 71.8, 111.4, 113.5, 114.1, 118.5, 120.0, 123.4, 123.7, 124.1, 125.3, 125.9, 126.0, 126.6, 127.1, 127.8, 129.4, 131.3, 131.9, 133.6, 133.8, 140.9, 145.7, 146.0, 165.0, 166.9; ν_{max}/cm^{-1} (solid state) 3442, 3377, 2970, 1720, 1670, 1262, 1006, 963, 889, 746, 608; ESI-HRMS found *m/z* 485.2062 $[M]^+$, $C_{29}H_{28}N_2O_5$ requires 485.2071.

Methyl-3-isopropoxy-4-(3-isopropoxy-4-amino-benzoylamido)-benzoic acid 2aa

(Procedure B) **7aa** (184.3 mg, 0.47 mmol), NaOH (5 mL, 10%), THF (25 mL) and MeOH (25 mL) provided the product as a white powder (170.3 mg, 99 %), m.p. 173-175 °C; δ_H (300 MHz, CD_3OD) 1.39 (6H, d, *J* = 6.0 Hz, $^iPrCH_3$), 1.43 (6H, d, *J* 6.0 Hz, $^iPrCH_3$), 4.83 (2H, m, overlapped ^iPrCH), 6.89 (1H, d, *J* = 8.2 Hz, ArCH), 7.35 (1H, d, *J* = 8.3 Hz, ArCH), 7.41 (1H, s, ArCH), 7.65 (1H, s, ArCH), 7.68 (1H, d, *J* 8.4Hz, ArCH), 8.32 (1H, d, *J* 8.4 Hz, ArCH), 9.02 (1H, br, NH); δ_C (75 MHz, CD_3OD) 22.9, 73.0, 73.9, 114.2, 115.6, 16.3, 121.3, 122.5, 124.6, 124.8, 127.5, 134.7, 143.8, 146.8, 148.6, 168.2, 170.4; ν_{max}/cm^{-1} (solid state) 3442, 3367, 2976, 1692, 1600, 1515, 1486, 1416, 1355, 1276, 1210, 1116, 981, 764; ESI-HRMS found *m/z* 373.1758 $[M+H]^+$, $C_{20}H_{25}N_2O_5$ requires 373.1758.

Methyl-3-isopropoxy-4-(3-benzyloxy-4-amino-benzoylamido)-benzoic acid 2ac

(Procedure B) **7ac** (88.3 mg, 0.20 mmol), NaOH (3 mL, 10%), THF (15 mL) and MeOH (15 mL) provided the product as a white powder (82.7 mg, 99 %), m.p. 178-179 °C; δ_H (300 MHz, $CDCl_3/CD_3OD$) 1.43 (6H, d *J* = 6 Hz, iPrCH_3), 4.81 (1H, sep, *J* = 6 Hz, iPrCH), 5.20 (2H, s, benzyl-CH_2), 6.83 (1H, d, *J* 8.2 Hz, ArCH), 7.31-7.50 (7H, m, ArCH), 7.64 (1H, s, ArCH), 7.67 (1H, d, *J* 8.4 Hz, ArCH), 8.37 (1H, d, *J* 8.4 Hz, ArCH), ; δ_C (75 MHz, $CDCl_3/CD_3OD$) 20.4, 69.5, 71.1, 110.1, 112.6, 118.3, 114.5, 120.1, 121.4, 122.1, 124.8, 126.6, 127.0, 127.5, 132.2, 136.1, 141.5, 144.9, 145.7, 165.4, 167.6; ν_{max}/cm^{-1} (solid state) 3494, 3434, 2976, 2547, 1672,

1598, 1515, 1261, 1111, 1015, 976, 846, 758, 702; ESI-HRMS found *m/z* 421.1752 $[M+H]^+$, $C_{24}H_{25}N_2O_5$ requires 421.1758.

Methyl-3-isopropoxy-4-(3-benzyloxy-4-(3-methyl(-2-napthyl)oxy-4-nitro-benzoylamido)-benzoylamido)-benzoate 8ace

(Procedure C) **7ac** (0.273 g, 0.63 mmol), **4e** (0.333 g, 1.03 mmol), Cl_2PPh_3 (1.100 g, 3.4 mmol), and chloroform (50 mL) to yield the product (0.389 g, 84%) as a deep yellow powder; m.p. 181–182°C (Found: C, 68.0; H, 5.25; N, 5.45%. $C_{43}H_{37}N_3O_9.H_2O$ requires: C, 68.15; H, 5.19; N, 5.55%); R_f = 0.8 (30% Et_2O in CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 1.47 (6H, d *J* = 6, ^{i}Pr CH_3), 3.93 (3H, s, CO_2Me), 4.78 (1H, sept *J* = 6, ^{i}Pr CH), 5.26 (2H, s, $ArCH_2O$-), 5.37 (2H, s, $ArCH_2O$-), 7.32-7.55 (10H, m, ArCH), 7.62 (1H, s, ArCH), 7.72-7.77 (3H, m, ArCH), 7.86-7.91 (5H, m, ArCH), 8.62 (1H, d *J* 8.5 Hz, ArCH), 8.66 (1H, d *J* 8.4 Hz, ArCH), 8.77 (1H, br, NH), 8.88 (1H, br, NH); δ_C (75 MHz, $CDCl_3$) 22.6, 52.5, 71.9, 72.0, 72.3, 111.9, 113.6, 114.8, 118.7, 119.1, 119.6, 119.8, 123.7, 125.2, 125.6, 126.4, 126.9, 128.2, 128.3, 128.5, 129.1, 129.3, 129.4, 131.1, 131.3, 132.7, 133.3, 133.7, 136.0, 146.2, 148.1, 152.5, 164.5, 165.0, 167.3; ν_{max}/cm^{-1} (solid state) 3430, 3067, 2974, 1700, 1682, 1599, 1516, 1352, 1205, 1128, 1109, 1001, 875, 848, 842, 599. ESI-MS *m/z* 740 $[M+H]^+$, 762 $[M+Na]^+$.

Methyl-3-propyloxy-4-(3-benzyloxy-4-(3-methyl(-2-napthyl)oxy-4-amino-benzoylamido)-benzoylamido)-benzoate 9ace

(Procedure D) **8ace** (70 mg, 0.1 mmol), $SnCl_2.2H_2O$ (126.5 mg, 0.6 mmol) afforded the product (62.3 mg, 94%) as a yellow solid; m.p. 208°C; δ_H (500 MHz, $CDCl_3$) 1.13 (3H, t, *J* = 7.3, CH_3) 1.33 (6H, d, *J* = 5.9, CH_3), 1.94 (2H, tq, J = 7.2 and 6.9, CH_2), 3.92 (3H, s, CO_2Me), 4.14 (2H, t, *J* = 6.3, CH_2), 4.53 (1H, sep, *J* = 6.1, CH), 5.25 (2H, s, benzylic-CH_2), 6.67 (1H, d, *J* = 8.0, Ar CH), 8.86 (1H, s, NH), 7.42-7.20 (4H, m, ArCH), 7.43 (1H, s, ArCH), 7.49-7.46 (5H, m, ArCH), 7.56 (1H, s, NH), 7.74 (2H, d, *J* = 8.4, NH_2), 8.62 (1H, d, *J* = 8.4, ArCH), 8.71 (2H, d, *J* = 5.4, ArCH); δ_C (75 MHz, $CDCl_3$); 9.6, 21.2, 21.5, 51.0, 69.4, 69.6, 70.9, 109.5, 110.3, 112.2, 112.5, 117.6, 118.0, 119.3, 122.4, 123.0 ,123.9, 126.8, 127.3, 127.7, 128.3, 131.0, 132.2, 135.5, 139.8, 144.8, 145.0, 146.6, 163.6, 164.1, 165.8; ν_{max}/cm^{-1} (solid state) 3433, 3378 (NH), 2977, 1701 (CO), 1603, 1520, 1272; ESI-HRMS found *m/z* 634.2524 $[M+Na]^+$, $C_{35}H_{37}N_3O_7.Na$ 634.2529.

3-Isopropoxy-4-(3-isopropoxy-4-(3-isopropoxy-4-amino-benzoylamido)-benzoylamido)-benzoic acid 1aaa

(Procedure B) **9aaa** (112.3 mg, 0.19 mmol), NaOH (3 mL, 10%), THF (15 mL) and MeOH (15 mL) provided the product as a white powder (108.2 mg, 99 %), m.p. 226-227 °C; δ_H (300 MHz, d_4-MeOH) 1.47 (18H, 3 overlapped d, *J* = 6 Hz ^{i}PrCH$_3$), 4.81 (3H, 3 overlapped sept., *J* = 6 Hz, ^{i}PrCH), 7.07 (1H, d *J* = 8.2 Hz, ArCH), 7.43 (1H, d *J* = 8.2 Hz, ArCH), 7.51-7.55 (2H, m, ArCH),

7.63 (1H, s, ArCH), 7.68 (1H, s, ArCH) 7.69 (1H, d *J* = 7.3 Hz, ArCH), 8.36 (1H, d *J* = 8.9 Hz, ArCH) 8.42 (1H, d *J* = 8.4 Hz, ArCH); δ_C (75 MHz, d_4-MeOH) 22.7, 22.8, 72.9, 73.5, 73.6, 113.6, 114.1, 115.4, 118.7, 121.0, 121.8, 121.9, 122.1, 124.3, 128.2, 128.7, 129.2, 131.8, 133.9, 134.3, 148.5, 149.0, 149.4, 167.3, 167.6, 169.8; ν_{max}/cm^{-1} (solid state) 3390, 2978, 1661, 1601, 1419, 1333, 1112, 979; ESI-HRMS found *m/z* 550.2572 $[M+H]^+$, $C_{30}H_{36}N_3O_7$ requires 550.2548.

3-Isopropoxy-4-(3-benzyloxy-4-(3-methyl(-2-napthyl)oxy-4-amino-benzoylamido)-benzoylamido)-benzoic acid 1ace

(Procedure B) **9ace** (76.3 mg, 0.11 mmol) 10% aqueous sodium hydroxide (3 mL), THF (20 mL), and methanol (20 mL), to yield (64.8 mg, 87%) an offwhite powder; m. p. 138–139°C (Found: C, 69.40; H, 5.55; N, 5.50%. $C_{42}H_{47}N_3O_7$ requires: C, 68.93; H, 5.55; N, 5.74%); δ_H (500 MHz, DMSO-d_6) 1.50 (6H, d, *J* = 6.0, ^{i}Pr CH_3), 3.44 (2H, br, NH_2), 4.86 (1H, sept, *J* = 6.0, ^{i}PrCH), 5.44 (2H, s, $ArCH_2O$-), 5.48 (2H, s, $ArCH_2O$-), 6.87 (1H, d, *J* = 8.2, ArCH), 7.42 (1H, t, *J* = 7.4, ArCH), 7.50-7.56 (3H, m, ArCH), 7.62-7.68 (3H, m, ArCH), 7.72-7.75 (6H, m, ArCH), 7.86 (1H, s, ArCH), 8.06-8.10 (3H, m, ArCH), 8.18 (1H, s, ArCH), 8.28 (1H, d, *J* = 8.4, ArCH), 8.35 (1H, d, *J* = 8.4, ArCH), 9.29 (1H, br, NH), 9.46 (1H, br, ArCH); δ_C (75 MHz, d_6-DMSO) 22.1, 70.0, 70.6, 71.8, 111.6, 112.0, 112.9, 114.4, 120.5, 121.1, 121.6, 121.8, 122.1, 122.5, 125.9, 126.3, 126.6, 127.5, 127.8, 128.0, 128.2, 128.4, 128.8, 130.0, 132.0, 132.8, 132.9, 133.2, 135.0, 137.1, 142.9, 144.7, 148.1, 149.0, 164.6, 164.9, 167.3; ν_{max}/cm^{-1} (solid state) 3423, 2931, 1675, 1597, 1514, 1349, 1199, 1118, 1023, 987, 870, 746, 603; ESI-MS *m/z* 696 $[M+H]^+$ 718 $[M+Na]^+$.

3-Isopropoxy-4-((3-methyl(-2-napthyl)oxy-4-(3-benzyloxy-4-amino-benzoylamido)-benzoylamido)-benzoic acid 1aec

(Procedure B) **9aec** (148.4 mg, 0.21 mmol), NaOH (3 mL, 10%), THF (15 mL) and MeOH (15 mL) provided the product as a light yellow powder (143.6 mg, 99 %), m.p. 220-221 °C; δ_H (300 MHz, d_6-DMSO) 1.34 (6H, d *J* = 6 Hz, ^{i}PrCH$_3$), 4.73 (1H, sep, *J* = 6 Hz, ^{i}PrCH), 5.10 (2H, s, benzylic-CH_2), 5.52 (2H, s, napthyl-CH_2), 5.54 (2H, br, NH_2), 6.72 (1H, d *J* = 8.2 Hz, ArCH), 7.32-7.44 (6H, m, ArCH), 7.49-7.52 (3H, m, ArCH), 7.58-7.61 (3H, m, ArCH), 7.73 (1H, d, *J* = 8.3 Hz, ArCH), 7.78 (1H, s, ArCH), 7.87-7.89 (2H, m, ArCH), 7.94 (1H, d, *J* = 8.4 Hz, ArCH), 8.10 (1H, s, ArCH), 8.17 (1H, d, *J* = 8.3 Hz, ArCH), 8.23 (1H, d, *J* = 8.3 Hz, ArCH), 9.25 (1H, br, NH), 9.35 (1H, br, NH); δ_C (75 MHz, d_6-DMSO) 22.1, 69.8, 70.7, 71.9, 112.8, 121.1, 121.9, 122.5, 126.3, 127.3, 127.7, 128.2, 128.4, 128.8, 130.1, 132.1, 132.8, 132.9, 133.2, 134.7, 137.4, 142.8, 144.7, 148.1, 149.2, 164.6, 165.0, 167.3; ν_{max}/cm^{-1} (solid state) 3387, 2976, 2550, 1686, 1600, 1509, 1437, 1140, 1017, 874, 747; ESI-HRMS found *m/z* 421.1752 $[M+H]^+$, $C_{24}H_{25}N_2O_5$ requires 421.1758.

Methyl-3-isopropoxy-4-(3-benzyloxy-4-(3-benzyloxy-4-nitro-benzoylamido)-benzoylamido)-benzoate 8acc

(Procedure C) using amine **7ac** (0.730 g, 1.68 mmol), acid **4c** (0.597 g, 2.18 mmol) dichlorotriphenylphosphorane (1.900 g, 6.00 mmol) in chloroform (150 mL) afforded the product as a pale yellow solid after a column (1.100 g, 95%), m.p. 180-181°C; δ_H (300 MHz, $CDCl_3$) 1.46 (6H, d, *J* = 6.0 Hz, $^iPrCH_3$), 3.91 (3H, s, CO_2CH_3) 4.77 (1H, sept *J* = 6.0 Hz, ^iPrCH), 5.19 (2H, s, benzylic-CH_2), 5.26 (2H, s, benzylic-CH_2), 7.29-7.47 (12H, m, ArCH), 7.60 (1H, d, *J* = 1.6 Hz, ArCH), 7.66 (1H, d, *J* = 1.6 Hz, ArCH), 7.72 (1H, dd, *J* = 8.5, 1.6 Hz, ArCH), 7.75 (1H, d, *J* = 1.6 Hz, ArCH), 7.84 (1H, d, *J* = 8.4 Hz, ArCH), 8.61 (1H, d, *J* = 8.5 Hz, ArCH), 8.65 (1H, d, *J* = 8.4 Hz, ArCH), 8.73 (1H, br s, NH), 8.85 (1H, brs, NH); δ_C (75 MHz, $CDCl_3$) 22.6, 52.5, 71.8, 72.0, 72.3, 111.9, 113.6, 114.8, 118.6, 119.1, 119.6, 119.8, 123.7, 125.6, 126.3, 127.7, 128.3, 128.9, 129.2, 129.4, 129.4, 131.1, 131.3, 133.3, 135.3, 136.0, 139.8, 142.5, 146.2, 148.2, 152.5, 153.3, 164.5, 167.1; ν_{max}/cm^{-1} (solid state) 3429, 3035, 1679, 1598, 1529, 1439, 1351, 1205, 1110, 998, 848; ESI-HRMS found *m/z* 690.2451 $[M+H]^+$, $C_{39}H_{36}N_3O_9$ requires 690.2446.

Methyl-3-propyloxy-4-(3-benzyloxy-4-(3-benzyloxy-4-amino-benzoylamido)-benzoylamido)-benzoate 9acc

(Procedure D) using **8acc** (926.4 mg, 1.34 mmol) tin chloride (1.8 g, 7.1 mmol) and ethyl acetate (80 mL) afforded the product after column chrmoatography as a yellow oil that solidified upon standing (753.2 mg 85%); m.p. 84-85°C; δ_H (400 MHz, $CDCl_3$) 1.43 (6H, d *J* = 6 Hz, $^iPrCH_3$), 3.91 (3H, s, CO_2CH_3), 4.23 (2H, brs, $ArCNH_2$), 4.76 (1H, sept, *J* = 6 Hz, ^iPrCH), 5.02 (2H, s, benzylic CH_2), 5.25 (2H, s, benzylic CH_2), 6.68 (1H, d, *J* = 8 Hz, ArCH), 7.23-7.50 (13H, m, ArCH), 7.60 (1H, s, ArCH), 7.68-7.73 (2H, m, ArCH), 8.61 (1H, d, *J* = 8 Hz, ArCH), 8.70 (1H, d, *J* = 8 Hz, ArCH), 8.72 (1H, br s, NH), 8.87 (1H, br s, NH); δ_C (75 MHz, $CDCl_3$) 22.6, 52.5, 70.9, 71.7, 72.3, 111.2,111.6, 113.6, 113.7, 119.0, 119.1,119.9, 121.2, 123.7, 124.1, 125.4, 128.2, 128.3, 128.7, 129.0, 129.3, 129.7, 132.6, 133.5, 136.4, 136.8, 141.2, 146.2, 147.8, 164.9, 165.4, 167.2; ν_{max}/cm^{-1} (solid state) 3432, 3200, 3034, 2868, 1706, 1677, 1537, 1350, 1174, 1107, 997; ESI-HRMS found *m/z* 660.2718 $[M+H]^+$, $C_{39}H_{38}N_3O_7$ requires 660.2704.

3-Isopropoxy-4-((3-benzyloxy-4-(3-benzyloxy-4-amino-benzoylamido)-benzoylamido)-benzoic acid 1acc

(Procedure B) using the ester **9acc** (534.4 mg, 0.81 mmol) 10% NaOH (5 mL) methanol (20 mL) and THF (20 mL) after workup yielded the product as a yellow powder (514.3 mg, 98%). m.p. 159-160°C; δ_H (400 MHz, $CDCl_3/CD_3OD$) 1.46 (6H, d *J* = 6 Hz, $^iPrCH_3$), 4.78 (1H, sep, *J* = 6 Hz, ^iPrCH), 5.01 (2H, s, benzylic CH_2), 5.28 (2H, s, benzylic CH_2), 6.75 (1H, d, *J* 8.2 Hz,

ArCH), 7.36-7.50 (13H, m, ArCH), 7.67-7.61 (3H, m, ArCH), 8.43 (1H, d, *J* 8.4 Hz, ArCH), 8.62 (1H, d, *J* 8.4 Hz, ArCH); ν_{max}/cm^{-1} (solid state) 3353, 3033, 2614, 1675, 1597, 1536, 1490, 1346, 1190, 1108, 984; ESI-HRMS found *m/z* 646.2516 $[M+H]^+$, $C_{38}H_{36}N_3O_7$ requires 646.2548.

FmocGly-1acc

The acid **1acc** (55.3 mg, 0.085 mmol) was added to dry chloroform (20 mL) and heated to reflux. Fmoc-glycine acid chloride (78.3 mg, 0.248 mmol) was dissolved in dry choloroform (10 mL) and added dropwise over an hour and then the solution was allowed to reflux overnight. The solvents were removed under reduced pressure and the residue was triturated with $CHCl_3$ to yield the product as a beige solid (49.6 mg 63%); δ_H (300 MHz, DMSO-d_6) 1.36 (6H, d, *J* = 6.0 Hz, $^{i}PrCH_3$), 3.86 (2H, s, CH_2), 3.91 (2H, d, *J* = 6.2 Hz, CH_2), 4.28 (1H, t, *J* = 6.2 Hz, CH), 4.74 (1H, sept, *J* = 6.0 Hz, $^{i}PrCH$), 5.26 (2H, s, benzylic CH_2), 5.33 (2H, s, benzylic CH_2), 7.27-7.72 (22H, m, ArCH), 7.89 (1H, s, ArCH), 7.91 (1H, s, ArCH), 8.07 (1H, d, *J* = 8.3 Hz, ArCH), 8.18-8.25 (3H, m, ArCH), 9.31 (1H, brs, NH), 9.37 (1H, brs, NH), 9.54 (1H, brs, NH); ν_{max}/cm^{-1} (solid state) 3039, 2858, 1167, 1597, 1532, 1488, 1346, 1189, 1115, 983, 872.

FITC-Gly-1acc

FmocGly-1acc (12.1 mg, 0.013 mmol) was dissolved in DMF (2 mL) and diethylamine (300 µL) was added dropwise. The solution was allowed to stir at room temperature overnight and then the solvents were removed under reduced pressure. The solids were redissolved in DMF (2 mL) and concentrated *in vacuo*. LC-MS revealed the reaction had gone to completion and the material was then used without further purification. The solids were redissolved in DMF and FITC (10.3 mg, 0.026 mmol) was added. The mixture was allowed to stir at room temperature, protected from the light, overnight. The resulting red solution was evaporated to dryness under reduced pressure and the solids were triturated with methanol to afford the product as a red powder (2.3 mg, 10%); δ_H (300 MHz, $CDCl_3/CD_3OD$) 1.41 (6H, d, *J* = 6.0 Hz, $^{i}PrCH_3$), 4.44 (2H, s, CH_2), 4.74 (1H, sept, *J* = 6.0 Hz, $^{i}PrCH$), 5.04 (2H, s, benzylic CH_2), 5.22 (2H, s, benzylic CH_2), 6.47 (1H, d, *J* = 2.4 Hz, FITCArCH), 6.50 (1H, d, *J* = 2.3 Hz, FITCArCH), 6.55-6.65 (4H, m, ArCH), 7.06 (1H, d, *J* = 8.3 Hz, ArCH), 7.21-7.48 (12H, m, ArCH), 7.58 (1H, s, ArCH), 7.66-7.70 (3H, m, ArCH), 7.83 (1H, dd, *J* = 8.2 Hz 2.0 Hz, ArCH), 7.93 (1H, s, ArCH), 8.36 (1H, d, *J* = 8.5 Hz, ArCH), 8.49 (1H, d, *J* = 8.5 Hz, ArCH), 8.58 (1H, d, *J* = Hz, ArCH); ESI-HRMS found 1090.3006 *m/z* $[M-H]^-$, $C_{61}H_{48}N_5O_{13}S$ requires 1090.2975.

Molecular Modelling

Structures were minimized with Macromodel (MacroModel version 9.0, Schrodinger LLC, New York, NY, **2006**) using the Monte Carlo method (5000 structures using the automatic setup) and minimized using the MMFFs forcefield and parameters for water as a solvent. The results were visualized with Maestro (version 7) and the lowest 20 energy structures were compared and found to be similar.

Structural Analysis of Trimers

A complete assigment of the ^{1}H NMR spectrum was not possible for the trimer **1aaa** due to signal overlap of the central protons Hi, H_j, H_o and H_p (Fig. ESI 1). However in polar solvents ^{1}H-^{1}H NOESY analysis indicates cross peaks between protons H_c/ H_d and H_g and H_i/ H_j and H_m suggesting free rotation around each of the Ar-CO bonds (Fig. ESI 1). The absence of cross peaks to H_g and H_m from H_h and H_n indicate intramolecular hydrogen-bonding fixes rotation about the Ar-NH bond (Figure ESI 1). Similarly, The absence of crosspeaks between protons on either of the N or C-terminus confirms an extended linear conformation in polar solvents (Fig. ESI 1).

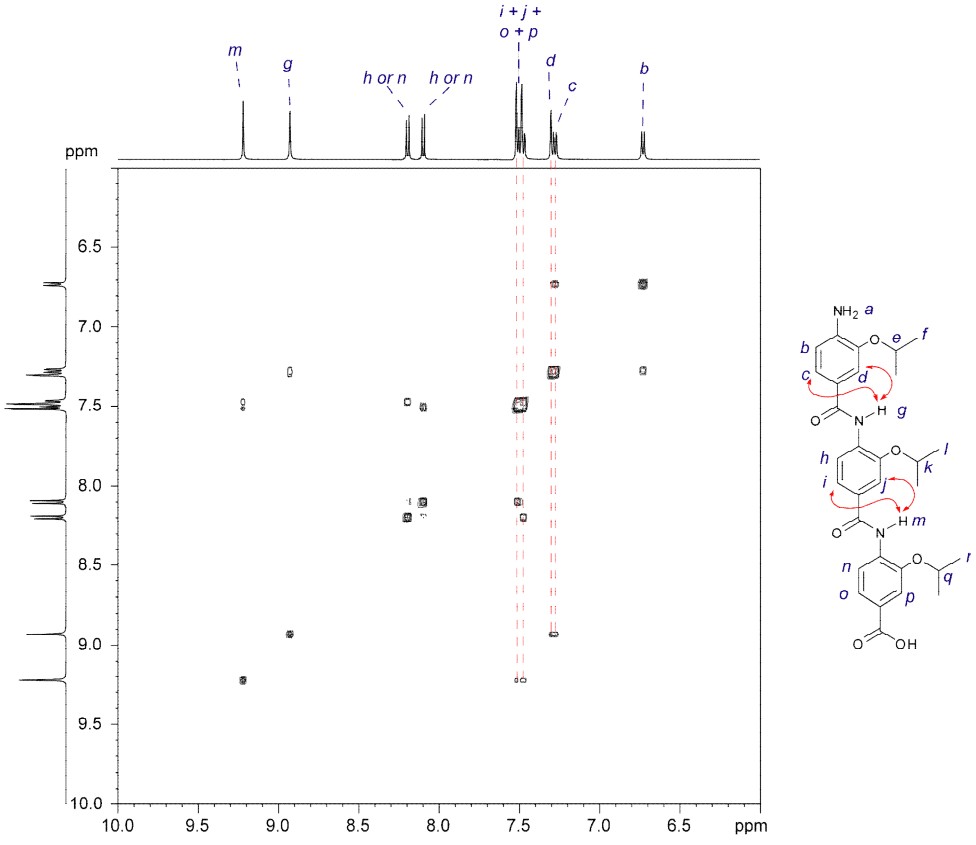


Figure ESI 1. 1H-1H NOESY (500 MHz, DMSO-d_6) of compound **1aaa**.

Expression and purification of *h*DM2 17-126 L33E

*h*DM2 (17-126) construct was kindly provided by John Robinson at University of Zürich. The pET14b plasmid (Novagen) containing cDNA encoding *h*DM2 residues Ser17 to Asn126, with a single mutation L33E was introduced into *E.coli* BL21 (DE3) Codon plus.[2] Protein production in 2xYT medium with ampicilin (100 μg/ml) was induced with IPTG (0.8 mM) when the optical density of the cell suspension reached OD_{600} = 0.8. Induced cells were grown at 18°C for 12 h and harvested by centrifugation at 6000 RPM for 10 min. Cells were resuspended in buffer A (20 mM Tris, 500 mM NaCl, pH 7.9) with 0.1% Triton X-100, sonicated in batched until completely lysed (5 seconds on, 10 seconds off 5 cycles) in presence of DNaseI, 1U/ml (EPICENTRE Biotechnologies) and 5 mM $MgCl_2$. Cell lysate was centrifuged at 17000 RPM for 30 min. Supernatant was loaded onto a Ni^{2+}-nitriloacetic acid (NTA) column equilibrated with buffer A. His_6-tagged *h*DM2 recombinant fragment was washed with 50 ml of the buffer A and the buffer A with 60 mM imidazole. The protein was eluted with 120 mM and 300 mM imidazole and the column was washed with 1 M imidazole in buffer A. Fractions containing protein were detected by 16% SDS PAGE. Pooled fractions containing *h*DM2 fragment were loaded on Superdex™ 75, washed with buffer B (25 mM Tris, 150 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM DTT, pH 7.3). The purified protein was concentrated to the final concentration 83 μM and stored at -70°C until use.

Fluorescence polarization assays

$p53_{15\text{-}31}$ transactivation domain peptide (Ac-SQETFSDLWKLLPENNVC-NH_2) ($p53_{15\text{-}31}$) and its fluorescein-labeled analogue (Ac-SQETFSDLWKLLPENNVC(Flu)-NH_2) ($p53_{15\text{-}31Flu}$) were purchased from Peptide Protein Research Ltd. Fluorescence polarization assays were performed in 96 well plates (160 μL per well). All experiments were performed in 40 mM Sodium Phosphate buffer pH 7.54 containing 200 mM NaCl and 0.02 mg/ml of Bovine Serum Albumin (Buffer C). The standard dilution profile would be: (using 10000 μM as the most concentrated point) 10000, 5000, 2000, 1000, 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2. All solutions were diluted, then loaded into the well after pipette mixing.

Determination of the binding of $p53_{15\text{-}31Flu}$ to hDM2

*h*DM2 was serially diluted through buffer C containing 54.5 nM $p53_{15\text{-}31Flu}$, over such a range that equilibrium was reached for both the fully bound and the fully unbound. Each dilution was performed in triplicate 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}) as illustrated in Fig. ESI 2.

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

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

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

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

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

Using equation 3, the data for the anisotropy was converted to fraction bound and multiplied by the $p53_{15\text{-}31Flu}$ concentration then fitted in origin 7 (Eq. 4) to give the dissociation constant (K_d) for the interaction as illustrated in Fig. ESI 2.

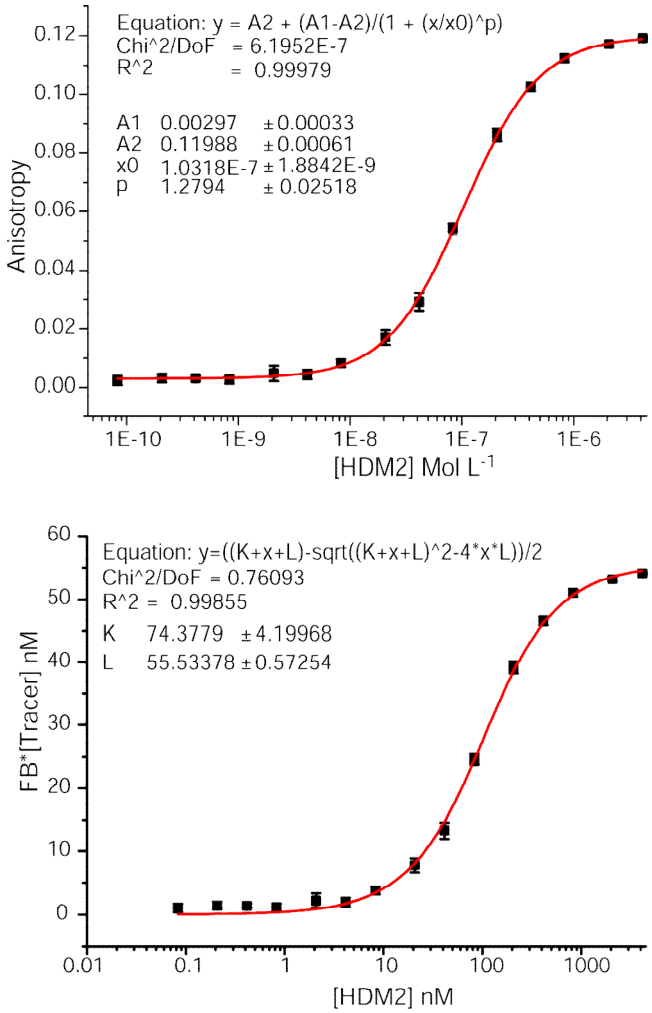


Figure ESI 2. Fluorescence polarization titration for binding of *h*DM2 to $p53_{15\text{-}31Flu}$ (54.5 nM) in buffer C

A reverse titration was also performed whereby the protein concentration was held constant and $p53_{15\text{-}31Flu}$ was serially diluted through buffer C. 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 $p53_{15\text{-}31Flu}$ concentration at each point and fit (Eq. 4) in origin 7 (Fig. ESI 3), where y is the fraction bound times the tracer concentration and x is the concentration of added tracer. Although the two values are similar, the data for 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 all the competition experiments

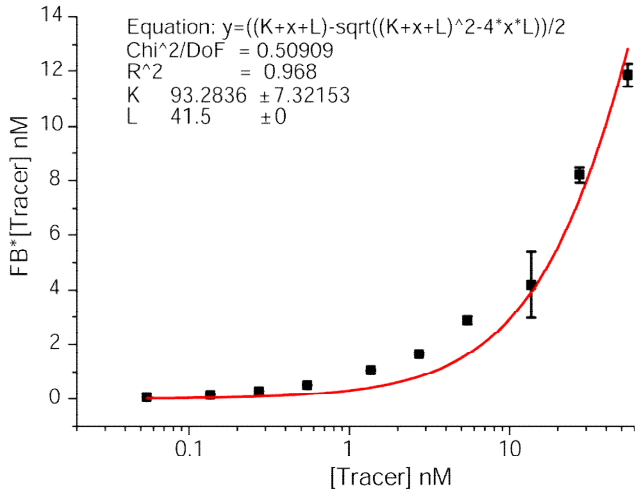


Figure ESI 3. Fluorescence polarization titration for binding of $p53_{15\text{-}31Flu}$ to *h*DM2 (41.5 nM) in buffer C

Displacement assay between mimetics and $p53_{15\text{-}31Flu}$ - hDM2

A stock solution of the mimetic in DMSO (all 10 mM) was diluted through buffer C containing 41.5 nM *h*DM2 and 54.5 nM $p53_{15\text{-}31Flu}$. The compounds that were found to have poor solubility had less of the stock added to the first point. The plates were allowed to incubate for 30 minutes at room temperature and then read. The intensity and anisotropy was calculated for each point, converted into a fraction bound and then multiplied by the $p53_{15\text{-}31Flu}$ concentration and plotted vs. the concentration of added mimetic in origin 7. A fit to a logistic model available in Origin 7 afforded IC_{50} values (Fig. ESI 4). Compound **1acc** is shown separately as the concentration of *h*DM2 in this experiment was slightly different. As the anisotropy was observed to drop to a level lower than r_{min} as determined in the $p53_{15\text{-}31Flu}$ -*h*DM2 titration, it was not possible to use the data to calculate K_i.

During the course of the titration the quantity of DMSO decreases from a maximal amount of 2.5% DMSO over 5 log units. A blank titration of DMSO into a solution of 41.5 nM *h*DM2 and 54.5 nM $p53_{15\text{-}31Flu}$ reveals no change in fluorescence signal within this concentration range.

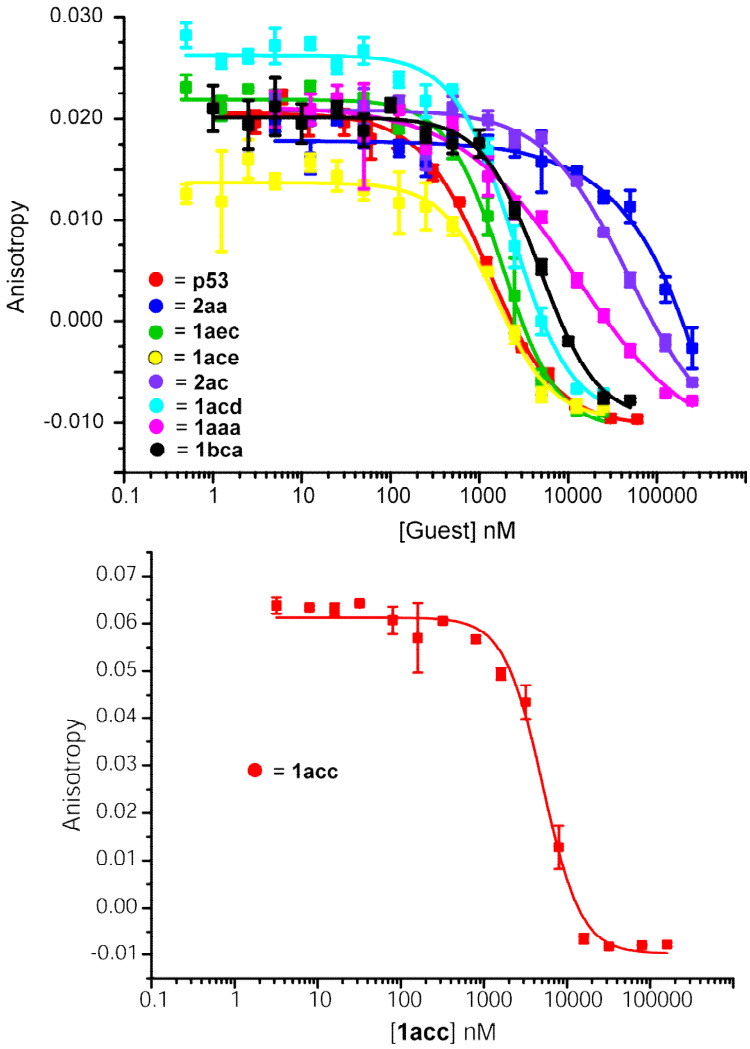


Figure ESI 4. Raw anisotropies and IC_{50} titration curves for each compound.

Derivation of three state equation

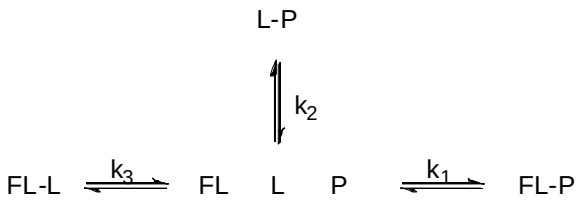


Figure ESI 5: Multiple binding equilibrium diagram

Assuming that $p53_{15\text{-}31Flu}$ interacts with the competitor, the equilibrium for the competition experiment can be illustrated as shown in Fig. ESI 5 where P is *h*DM2, L is the competitor and FL

is $p53_{15\text{-}31Flu}$. The model for such a system was derived using steady state approximations i.e. ignoring any self-association of $p53_{15\text{-}31Flu}$ (FL) and assuming that it FL does not contribute to the anisotropy

The total anisotropy is given by equation 4.

$$r = \frac{\sum_i f_i Q_i r_i}{\sum_i f_i Q_i} \quad \textbf{(Eq. 4)}$$

If one substitutes for all species outlined in Fig. ESI 4 then the total anisotropy is given by:

$$r = \frac{f_{FL.P}Q_{FL.P}r_{FL.P} + f_{FL.L}Q_{FL.L}r_{FL.L} + f_{FL}Q_{FL}r_{FL}}{f_{FL.P}Q_{FL.P} + f_{FL.L}Q_{FL.L} + f_{FL}Q_{FL}} \quad \textbf{(Eq. 5)}$$

For each species, anisotropies are given by *r*, quantum yields are given by *Q* and mol fractions are given by *f*

As one substitutes the concentrations in the place of the mole fractions it is possible to divide through by a common denominator (the sum of all of the concentrations in the sample) to simplify equation 6 into equation 7.

$$r = \frac{\left(\frac{[FL.P]}{[FL.P]+[FL.L]+[FL]}\right)Q_{FL.P}r_{FL.P} + \left(\frac{[FL.L]}{[FL.P]+[FL.L]+[FL]}\right)Q_{FL.L}r_{FL.L} + \left(\frac{[FL]}{[FL.P]+[FL.L]+[FL]}\right)Q_{FL}r_{FL}}{\left(\frac{[FL.P]}{[FL.P]+[FL.L]+[FL]}\right)Q_{FL.P} + \left(\frac{[FL.L]}{[FL.P]+[FL.L]+[FL]}\right)Q_{FL.L} + \left(\frac{[FL]}{[FL.P]+[FL.L]+[FL]}\right)Q_{FL}} \quad \textbf{(Eq. 6)}$$

$$r = \frac{[FL.P]Q_{FL.P}r_{FL.P} + [FL.L]Q_{FL.L}r_{FL.L} + [FL]Q_{FL}r_{FL}}{[FL.P]Q_{FL.P} + [FL.L]Q_{FL.L} + [FL]Q_{FL}} \quad \textbf{(Eq. 7)}$$

This leaves three different concentrations to determine in addition to six different constants that we can measure. The individual quantum yields (Q) and anisotropies (r) can be obtained from the endpoint in carefully selected titrations (the Q's from the intensity values and the r's from the anisotropy endpoints). Returning to the equilibrium (Fig. ESI 5) we can write out the following equations that are then substituted into equation 7 to yield equation 8.

$$[FL.P] = k_1[P][FL] \qquad [FL.L] = k_3[L][FL]$$

$$r = \frac{k_1[P]Q_{FL.P}r_{FL.P} + k_3[L]Q_{FL.L}r_{FL.L} + Q_{FL}r_{FL}}{k_1[P]Q_{FL.P} + k_3[L]Q_{FL.L} + Q_{FL}} \quad \textbf{(Eq. 8)}$$

The concentration of free L we assume is close to the total L concentration as it is higher in concentration than either species and is the eventual x variable in the choosen fitting program. We know the Q's and r's and calculate k_1 previously from the fit of the fluorescent ligand to the protein (k_3 can be determined as described below). We wish to find the value for k_2 so require the free protein concentration ([P] where $[P_{TOT}]$ is the total protein concentration) which is determined by equation 9.

$$[P] = \frac{[P_{TOT}]}{k_2[L] + k_1[FL] + 1} \quad \textbf{(Eq. 9)}$$

Unfortunately the free protein concentration is dependant on the free FL concentration i.e. $[p53_{15\text{-}31Flu\ free}]$. So combining Eq. 9 with Eq. 10 we obtain Eq. 11 in which we can solve for [FL] where the total concentration of $p53_{15\text{-}31Flu}$ is $[FL_{TOT}]$

$$[FL] = \frac{[FL_{TOT}]}{1 + k_1[P] + k_3[L]} \quad \textbf{(Eq. 10)}$$

$$[FL_{TOT}] = [FL] + \frac{k_1[P_{TOT}][FL] + k_3[L][FL]}{k_2[L] + k_1[FL] + 1} \quad \textbf{(Eq. 11)}$$

Expansion of eq. 11 leaves a quadratic equation for [FL].

$$[FL]^2(k_1 + k_1k_3[L]) + [FL](k_2[L] + 1 + k_1[P_{TOT}] + k_2k_3[L]^2 + k_3[L] - k_1[FL_{TOT}][L] - [FL_{TOT}] = 0 \quad \textbf{(Eq. 12)}$$

Solving the quadratic using the quadratic gives a final equation 13 for [FL].

$$[FL] = \frac{-(k_2[L]+1)(k_3[L]+1) - k_1([P_{TOT}]-[FL_{TOT}]) + \sqrt{[(k_2[L]+1)(k_3[L]+1)]^2 + 2k_1([P_{TOT}]-[FL_{TOT}])(k_2[L]+1)(k_3[L]+1) + k_1^2([P_{TOT}]-[FL_{TOT}])^2 + 4k_1(k_3[L]+1)(k_2[L]+1)([FL_{TOT}])}}{2k_1(k_3[L]+1)}$$

(Eq. 13)

In summary we can fit:

$$r = \frac{k_1[P]Q_{FL.P}r_{FL.P} + k_3[L]Q_{FL.L}r_{FL.L} + Q_{FL}r_{FL}}{k_1[P]Q_{FL.P} + k_3[L]Q_{FL.L} + Q_{FL}}$$

where:

$$[P] = \frac{[P_{TOT}]}{k_2[L] + k_1[FL] + 1}$$

and

$$[FL] = \frac{-\{k_1([P_{TOT}]-[FL_{TOT}]) + (k_2[L]+1)(k_3[L]+1)\} + \sqrt{\{(k_2[L]+1)(k_3[L]+1) + k_1([P_{TOT}]+[FL_{TOT}]\}^2 - 4k_1{}^2[FL_{TOT}][P_{TOT}]}}{2k_1(k_3[L]+1)}$$

Using *r* as the *y* variable and [L] as the *x* variable.

Using the equation:

The equation was inserted into origin 7 script and the following values were used for Q,r, and k:

$Q_{FL.P}$ = 90, $r_{FL.P}$ = 0.12 (from variable protein – $p53_{15\text{-}31Flu}$ titration)

Q_{FL} = 140, r_{FL} = 0.0123 (from variable protein – $p53_{15\text{-}31Flu}$ titration)

k_1 = 0.01351 (from variable protein – $p53_{15\text{-}31Flu}$ titration)

$Q_{FL.L}$ = 118 (this value tends not to change between different compounds), k_3 = 0.00074 (from $p53_{15\text{-}31}$ – $p53_{15\text{-}31Flu}$ titration)

$r_{FL.L}$ and k_2 were fit from the data.

Unfortunately for a majority of the compounds the error involved for the k_2 fit was approximately the same as the value for k_2 itself. This means that while the equation works, it does not give an acceptable fit for the data and so it is impossible to determine with any degree of certainty the value for k_2. Simulation reveals this is due to the fact that the binding constant between P and L has only a subtle effect on the shape of the curve and any effect that could be seen is within the error for each point.

Determination of the affinity (k_3) between the mimetics and $p53_{15\text{-}31Flu}$

A stock solution of the mimetic in DMSO (all 10 mM) was diluted through buffer C containing 54.5 nM $p53_{15\text{-}31Flu}$. The plates were allowed to incubate at room temperature for 30 minutes and then read. It was noticed that the anisotropy values changed from 0 to -0.15 which fits as the minimum of the displacement titration. A new r_{min} and r_{max} were found from a fit to the titration

with $p53_{15\text{-}31}$ against $p53_{15\text{-}31Flu}$ and the calculated anisotropies were converted to fraction bound, multiplied by $p53_{15\text{-}31Flu}$ concentration and fit to a 1:1 binding model described (Eq. 4) (Fig. ESI 6). Because the signal change is small, only those compounds that gave useful data are shown.

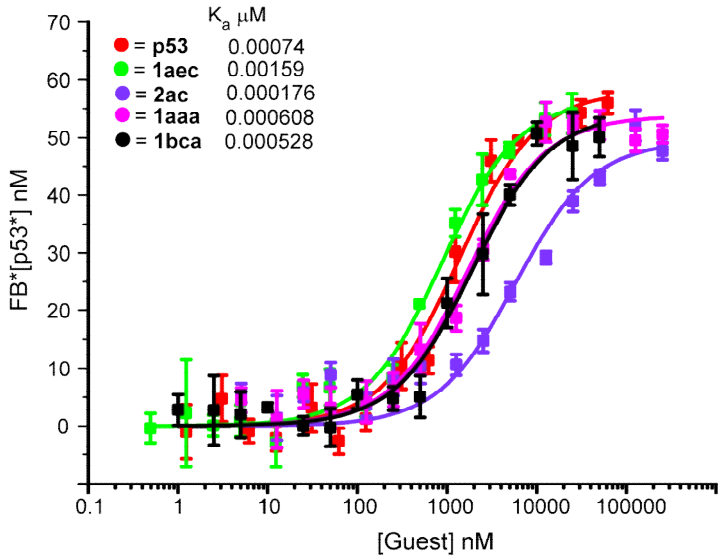


Figure ESI 6. Fluorescence polarization titration for binding of $p53_{15\text{-}31Flu}$ to ligands **1** and **2**

Direct titration of FITC labelled mimetic ***FITC-Gly-1acc***

*h*DM2 (30 µL 83 µM) was diluted through a standard dilution series consisting of 102 nM FITC labelled mimetic **FITC-Gly-1acc** in buffer C. This was plotted to a logistic fit in origin 7 to get a value for r_{min} and r_{max}. Those values were used along with equation 3 to determine a fraction bound, that was then multiplies by the tracer concentration. The resulting curve (Fig. ESI 7) was fit to equation 5 to determine the binding affinity.

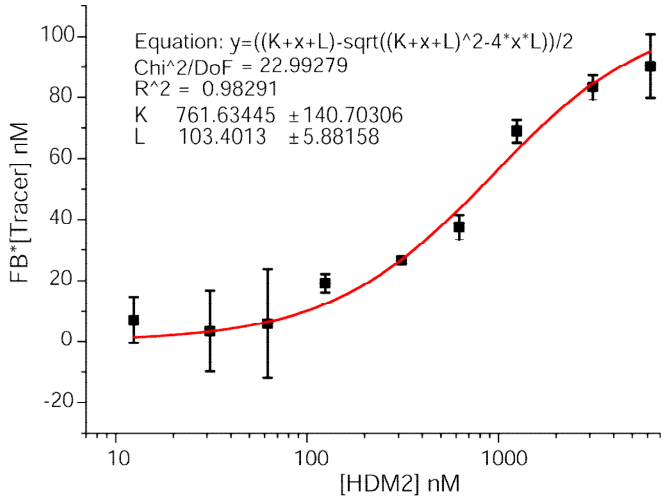


Figure ESI 7. Fluorescence polarization titration for binding of *h*DM2 to **FITC-Gly-1acc** in buffer C

(1) J. Plante, F. Campbell, B. Malkova, C. Kilner, S. L. Warriner, and A. J. Wilson, *Org. Biomol. Chem.*, 2009, **6**, 138-146.
(2) L. T. Vassilev, B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, and E. A. Liu, *Science*, 2004, **303**, 844.