

PROTAC-Mediated Degradation of Class I Histone Deacetylase Enzymes in Corepressor Complexes

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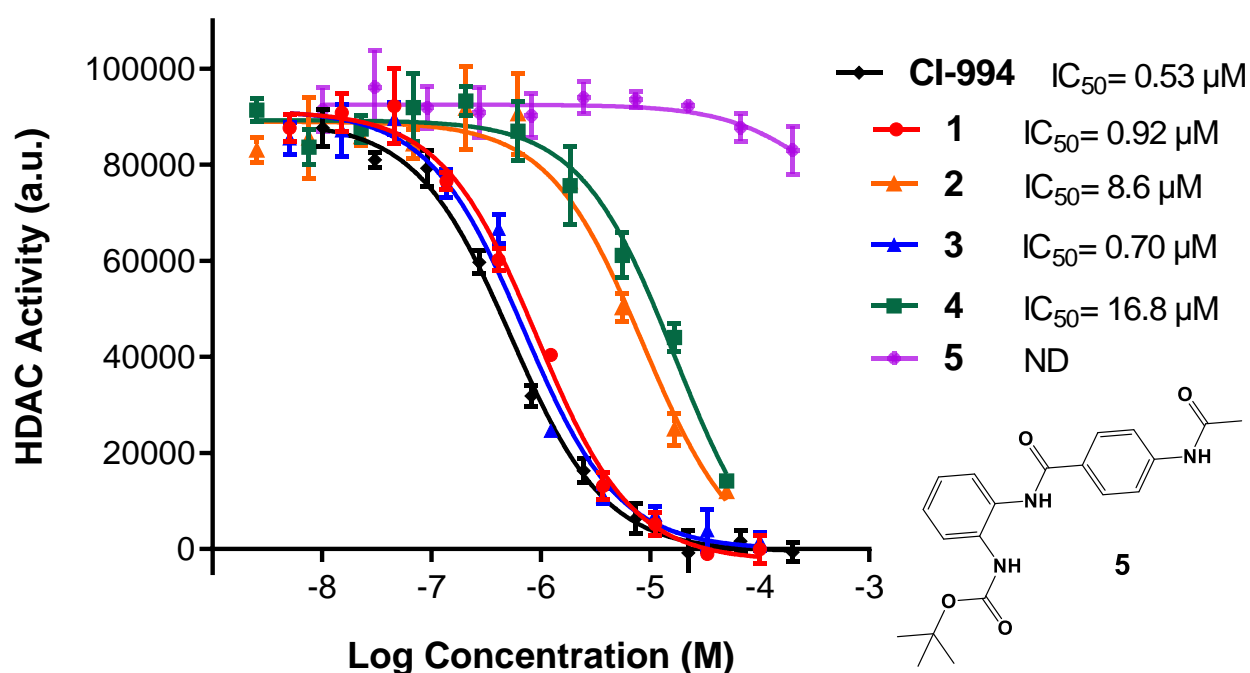
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Supplementary Information: Biology

1. *In vitro* HDAC Assay with CoREST Complex

Inhibition tests against LSD1-HDAC1-CoREST complex were performed using an enzyme fluorescence-based HDAC assay.¹ The inhibitor/PROTACs were dissolved at 50 mM in DMSO, then 1:2 serial dilutions performed using HDAC assay buffer (50 mM Tris pH 7.5, 150 mM NaCl) to afford range of concentrations. 10 μ L of these solutions were added to individual wells, followed by 20 μ L of HDAC complex dissolved in HDAC assay buffer (18 nM) and 20 μ L of the substrate Boc-(Ac)Lys-AMC dissolved in HDAC assay buffer. The assays were performed in black 96-well plates with a reaction volume of 50 μ L per well. All determinations were performed in triplicate. After an incubation of 20 minutes at 37 $^{\circ}$ C and 150 rpm, deacetylation was stopped by the addition of 50 μ L of a developing solution containing trypsin (50 mM Tris pH 7.5, 100 mM NaCl, 10 mg/mL trypsin). Fluorescence intensity was measured with a plate reader (PerkinElmer, 2030 multilabel reader, VICTOR X5, λ_{ex} 335 nm, λ_{em} 460 nm). HDAC Activity was calculated by subtracting the average blank fluorescence from the well fluorescence. Graphpad Prism software was utilised to determine IC₅₀ values.

Figure S1: AMC-Fluorescence Histone Deacetylase inhibition assay with the LSD1-CoREST-HDAC1 complex, including error bars.



2. Cell Lines and Materials

E14 wild type (WT) mouse embryonic stem (mES) cells were maintained on gelatinised plates in standard mES media consisting of Knockout Dulbecco's Modified Eagle Medium (KO DMEM) (GIBCO, 10829-018) supplemented with 15% Fetal Bovine Serum (FBS) (Sigma, F9665), 1X glutamine/penicillin/streptomycin (GIBCO, 10378-016), 100 μ M β -mercaptoethanol (Sigma) and Leukaemia Inhibitory Factor (synthesised in house). HCT116 human colon carcinoma cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO, 41965-039) supplemented with 10% Fetal Bovine Serum (FBS) (Sigma) and 1X glutamine/penicillin/streptomycin (GIBCO, 10378-016). Both cell lines were incubated at 37 °C with 5% CO₂. Cells were treated with PROTACs (0.01-40 μ M) alongside HDAC inhibitors CI-994 (10/40 μ M) and Panobinostat (30 nM) as controls.

3. Western Blotting

HCT116 or mES cells were treated 24 hours after seeding. 24 hours post treatment, cells were harvested, lysed in lysis buffer (50mM Tris-HCl, 150 mM NaCl, 0.5% NP-40, 0.5% Triton X-100) with protease inhibitor (Sigma, P8340), then incubated on ice for 30 minutes, before being centrifuged (18,000 rcf, 15 minutes, 4 °C). The supernatant was collected, and protein concentrations quantified via Bradford Assay using Protein Assay Dye Reagent Concentrate (BIO-RAD). For histone extraction, an equal volume of 0.4 N H₂SO₄ was added to the pellets and the extracts placed at 4 °C overnight. Following overnight incubation, the tubes were centrifuged (18,000 rcf, 15 minutes, 4 °C) and then the supernatant (histone extract) collected.

Western blots were run on NuPAGE™ 4-12% Bis-Tris gels with 20-30 μ g of protein or 10 μ L of acid-extracted histone loaded per lane, using NuPAGE™ LDS Sample Buffer (4X). PageRuler™ Plus Prestained Ladder was used for size standards. After gel electrophoresis at 140V for 75-90 minutes the separated proteins were transferred onto nitrocellulose membrane at 30V for 60 minutes. The membranes were probed with primary antibodies (listed below) for 60-90 minutes. Blots were developed with complimentary IRDye conjugated secondary antibodies and the bands visualised using the Odyssey Infrared Imaging System. Image processing and band intensity quantification was performed using Image Studio Lite.

Antibody Information

Primary Antibodies;

α -tubulin - Sigma, t5168 (1:10,000 dilution)

HDAC1 - Abcam, 109411 (1:2,000 dilution)

HDAC2 - Merck Millipore, 05-814 (1:2,000 dilution)

HDAC3 - Abcam, 32369 (1:2,000 dilution)

H3 - Merck Millipore, 05-499 (1:1,000 dilution)

H3K9Ac - Upstate, 06-942 (1:1,000 dilution)

H3K27Ac - Merck Millipore, 07-360 (1:1,000 dilution)

H3K56Ac - Active Motif, 39281 (1:1,000 dilution)

Secondary Antibodies;

IRDye® 680LT - LI-COR Biosciences, 926-68023 (1:10,000 dilution)

IRDye® 800CW - LI-COR Biosciences, 926-32210 (1:10,000 dilution)

3.1. Western Blots for Histone 3 Lysine 9/27/56 Acetylation (H3KxAc)

Figure S2: B) Histone 3 Lysine 56 Acetylation (H3K56Ac) levels in E14 mouse embryonic stem cells after 24h; CI-994 = 40 μ M, Panobinostat = 30 nM. Fold change in H3K56Ac levels shown.

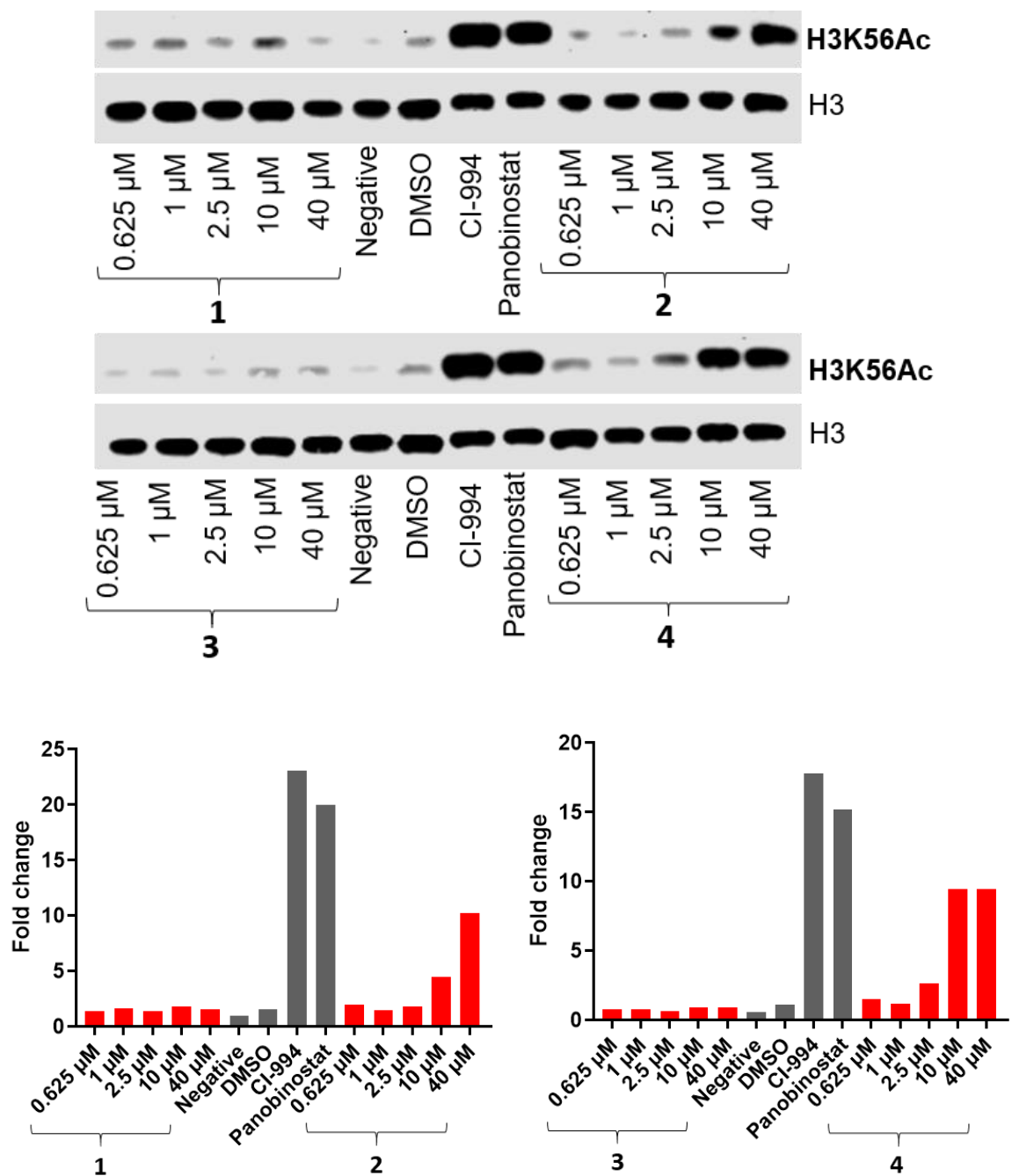
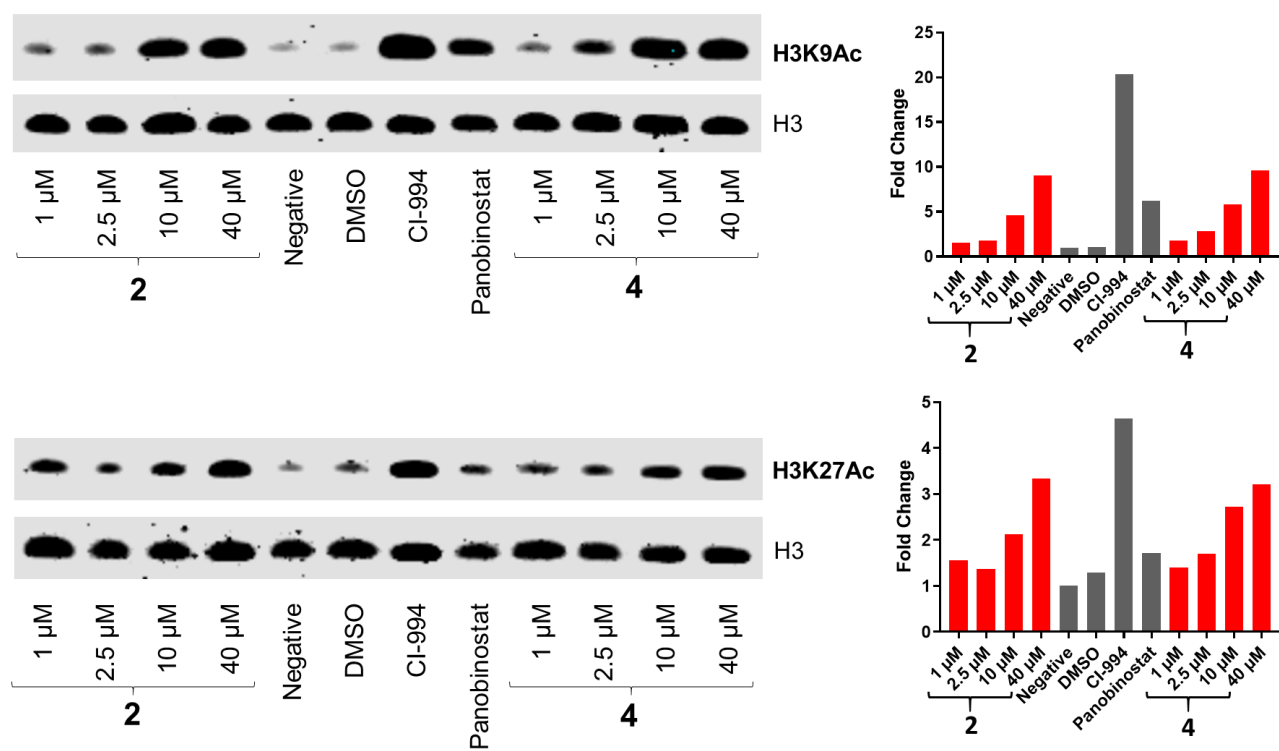


Figure S3: Histone 3 lysine 9 acetylation (H3K9Ac) and histone 3 lysine 27 acetylation (H3K27Ac) levels in HCT116 cells after 24h with **2** and **4**; CI-994 = 40 μ M, Panobinostat = 30 nM. Fold change in H3K9/27Ac levels shown.



3.2. Western Blots for HDAC1/2&3 Degradation

Figure S4: HDAC 1,2 & 3 degradation occurs in a dose dependent manner with **4**. A) Immunoblot with HDAC 1,2 & 3 antibodies after 24h in HTC116 cell line. Numerical value represents percentage of protein compared to DMSO control = 100%. B) Histone 3 Lysine 9 acetylation levels after 24 hours in HTC116 cell line. C) **6** with the inactive VHL diastereoisomer does not induce degradation. CI-994 = 40 μ M, Panobinostat = 30 nM.

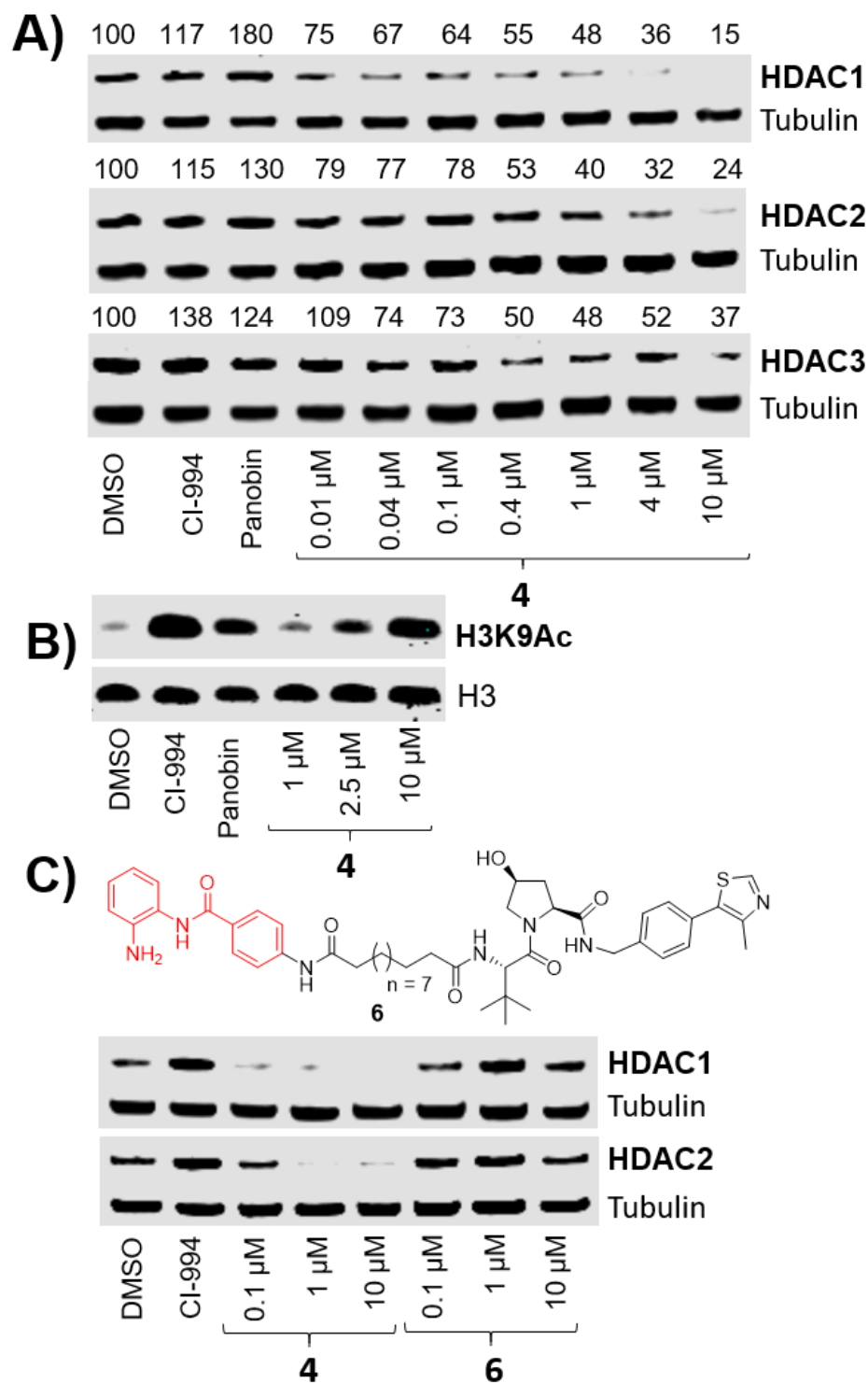
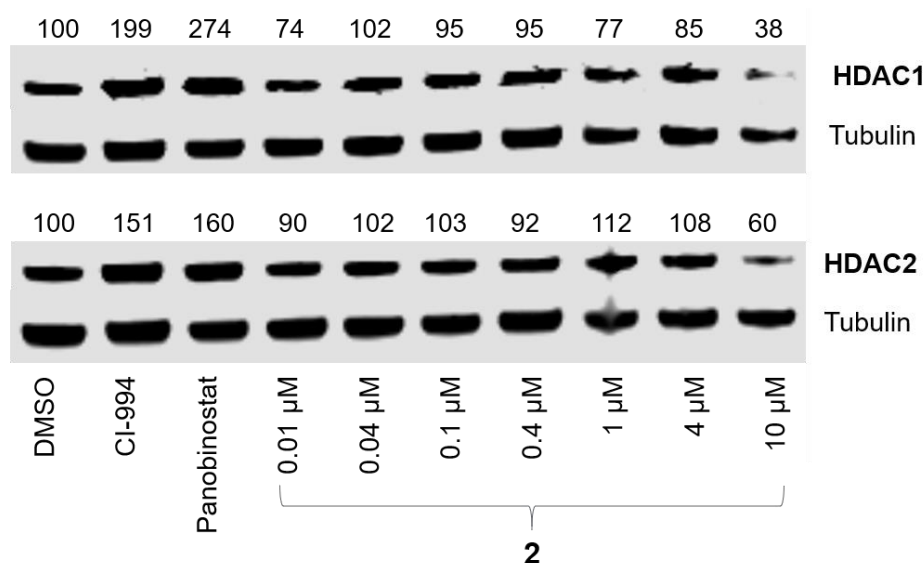


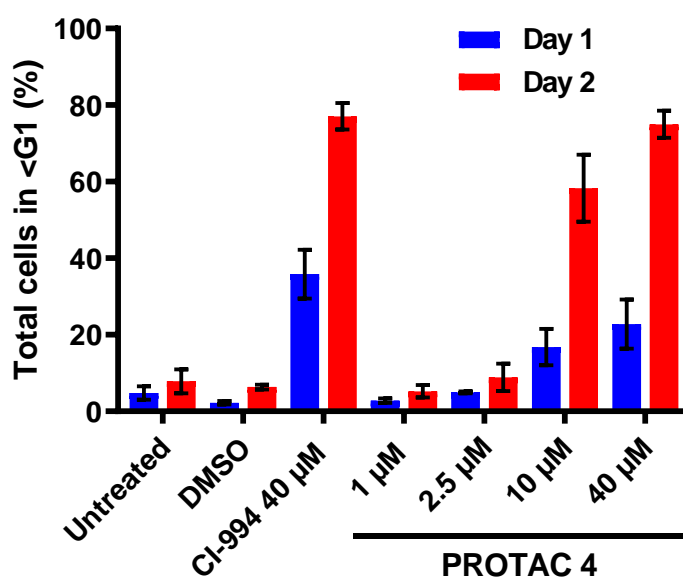
Figure S5: Effect of **2** on the levels of HDAC 1/2 in HCT116 cells following 24h treatment; CI-994 = 40 μ M, Panobinostat = 30 nM.



4. Cell Viability Assay

To analyse cell death, cells were treated with DMSO, CI-994 (40 μ M), or PROTAC **4** (1-40 μ M) 24 hours after seeding. 24 hours post treatment, cells were harvested and fixed with 70% (vol/vol) ethanol at -20°C overnight. Cells were washed in PBS prior to incubation with 50 μ g of propidium iodide and RNase A (10 μ g/mL) for 30 min at room temperature in the dark. Samples were analysed using the BD FACSCanto II flow cytometer (BD Biosciences) in the PE_A channel with BD FACSDiva software.

Figure S5. FACS data of compound **4** and CI-994 in HCT116 colon cancer cell line.



Supplementary Information: Chemistry

5. General Methods

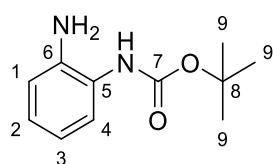
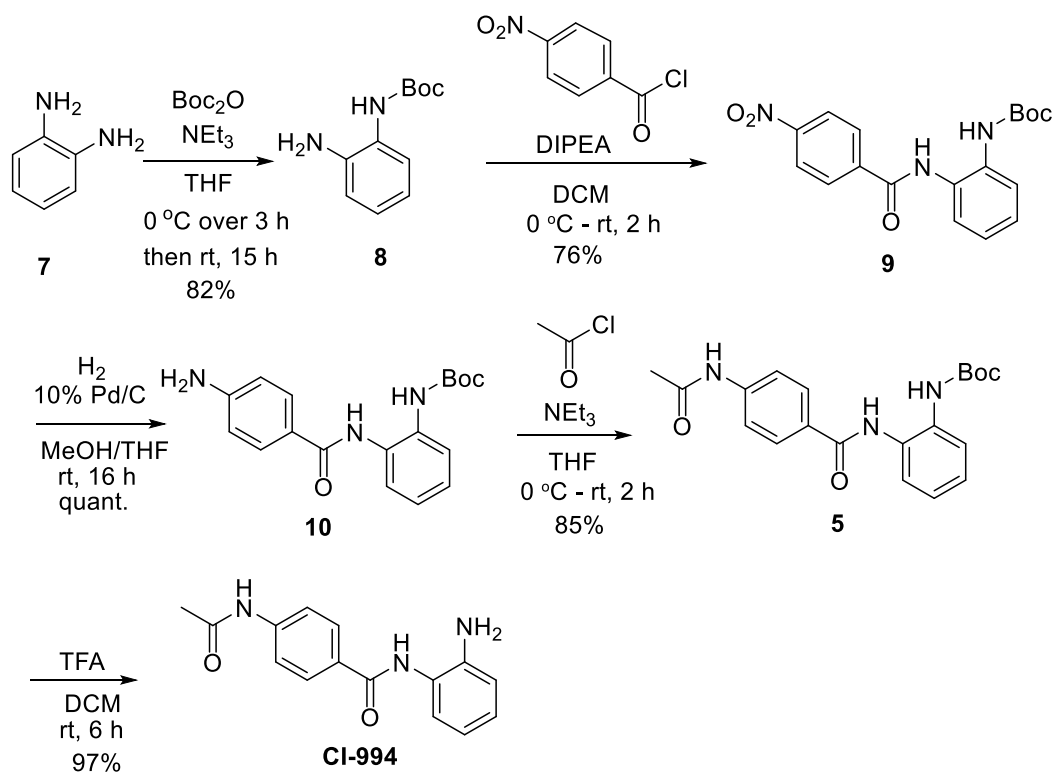
All reagents and solvents were obtained from Sigma Aldrich, Acros Organics, Fisher Scientific and were used as supplied unless stated otherwise. The active VHL ligand (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride and inactive (negative control) VHL ligand (2S,4S)-1-[(2S)-2-Amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide dihydrochloride were purchased from TOCRIS[®]. Biotage[®] Macroporous polystyrene-co-divinylbenzene (MP) carbonate resin (3.02 mmol/g loading capacity) was used for neutralizing amine TFA salts and scavenging excess TFA during tert-butoxycarbonyl deprotection reactions. Glassware was dried in oven at 100 °C for 12 hours for moisture sensitive reactions. Unless otherwise stated reactions were performed under nitrogen using anhydrous solvents. Dried THF and DCM were dried using an Innovative Technology inc. PureSolv solvent purification system. Room temperature refers to ambient temperature. Temperatures of 0 °C were maintained using an ice-water bath. The reactions were monitored by thin-layer chromatography (TLC) on aluminium backed silica gel. Unless otherwise stated Flash column chromatography was carried out with Silica Gel 60 using commercial solvents. All evaporations *in vacuo* were performed under reduced pressure using a Büchi rotary evaporator. All chemical names have been generated using ChemDraw Professional. Preparative column chromatography and flash column chromatography using a Biotage Isolera purification system was performed using silica gel 60 (230-400 mesh).

Analytical and semi-preparative HPLC were performed on a ThermoFisher Ultimate 3000 system with Chromeleon software on a Phenomenex Luna C18 column. Method 1, A= H₂O, B= CH₃CN, 5-100% B, 10 mL/min flow, 45 min gradient. Method 2, A= 0.1% TFA in H₂O, B= 0.1% TFA in CH₃CN, 5-100% B, 10 mL/min flow, 45 min gradient. Solutions were either made up in HPLC Grade acetonitrile (MeCN) and deionised water (1:1) or HPLC grade methanol

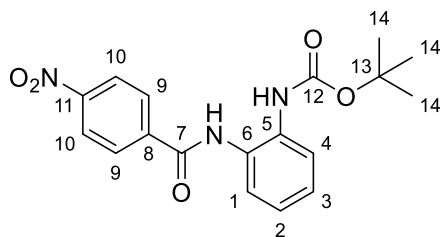
Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker 500 (¹H, 500 MHz; ¹³C 125 MHz) or Bruker 400 (¹H, 400 MHz; ¹³C 100 MHz) instrument at ambient temperature using deuterated solvent as reference - CDCl₃ ($\delta_{\text{H}} = 7.26$ ppm, $\delta_{\text{C}} = 77.00$ ppm), DMSO-*d*₆ ($\delta_{\text{H}} = 2.50$ ppm, $\delta_{\text{C}} = 39.51$ ppm), CD₃OD ($\delta_{\text{H}} = 3.31$ ppm, $\delta_{\text{C}} = 49.15$ ppm), or CD₃CN ($\delta_{\text{H}} = 1.94$ ppm, $\delta_{\text{C}} = 1.39, 118.69$ ppm). ¹H NMR data are reported as: chemical shift, multiplicity [b, broad; s, singlet; d, doublet; t, triplet; q, quartet; quin, m for multiplet; or as a combination (e.g., dd, dt, etc.)], coupling constant(s) and integration. ¹³C NMR spectra were recorded by broadband proton decoupling. ¹³C NMR chemical shifts (δ) are quoted to the nearest 0.1 ppm and referenced to the residual non-deuterated solvent peak. Where ¹H and ¹³C NMR's have been fully assigned, 2D NMR including ¹H-¹H COSY (correlated spectroscopy), ¹H-¹³C HSQC (heteronuclear single quantum coherence) and ¹H-¹³C HMBC (heteronuclear multiple bond coherence) were used to aid assignment. ACDLabs software (Chemsketch and Spectrus Processor) was used for peak picking, integration and calculating coupling

constants. High resolution mass spectra (HRMS) were recorded on a Water Aquity XEVO Q ToF machine and measured in m/z .

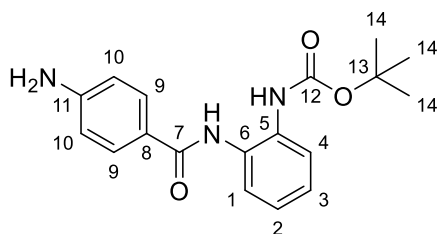
6. Synthesis of CI-994



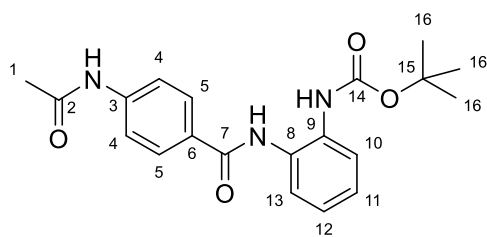
Tert-butyl (2-aminophenyl)carbamate, (8): A solution of Boc_2O (6.05 g, 27.7 mmol) in THF (50 mL) was added dropwise over 3 hours to a solution of **7** (3.00 g, 27.7 mmol) and triethylamine (4.64 mL, 33.3 mmol) in THF (25 mL) at 0 °C, then the mixture was stirred at room temperature for 15 hours. The reaction mixture was concentrated *in vacuo* to afford a grey crystalline solid and then re-dissolved in EtOAc (50 mL). This solution was washed with water (2 x 30 mL) and sat. brine (2 x 30 mL), filtered over Na_2SO_4 , then concentrated *in vacuo* to afford a yellow/grey solid. The crude solid was purified by column chromatography (solid load, 10-25% EtOAc in hexane) to afford **8** (4.72 g, 22.5 mmol, 82% yield) as a yellow/grey solid. ^1H NMR (400 MHz, Chloroform- d) δ_{H} ppm 7.19 (d, $J=7.7$ Hz, 1 H, 1-CH), 6.92 (app. td, $J=7.7, 1.3$ Hz, 1 H, 2-CH), 6.70 (app. td, $J=7.7, 1.3$ Hz, 1 H, 3-CH), 6.68 (dd, $J=7.7, 1.3$ Hz, 1 H, 4-CH), 6.18 (br s, 1 H, NH), 3.64 (br s, 2 H, NH_2), 1.44 (s, 9 H, 9- CH_3). ^{13}C NMR (101 MHz, Chloroform- d) δ_{C} ppm 153.9 (C7), 140.0 (C6), 126.2 (double intensity: C3, C5), 124.8 (C4), 119.6 (C2), 117.6 (C1), 80.5 (C8), 28.4 (C9). HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_2\text{Na}$: 231.1109, found 231.1112.



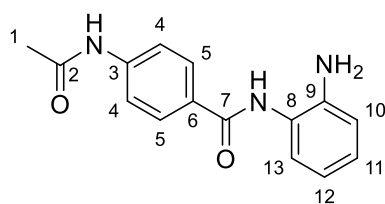
Tert-butyl (2-(4-nitrobenzamido)phenyl)carbamate, (9): DIPEA (5.23 mL, 30.0 mmol) was added to a solution of **8** (4.17 g, 20.0 mmol) in dry DCM (90 mL) at 0 °C, followed by the dropwise addition of 4-nitrobenzoyl chloride (4.09 g, 22.0 mmol) as a solution in dry DCM (10 mL). The mixture was stirred at 0 °C for 30 minutes, then at room temperature overnight. The reaction mixture was diluted with DCM and then washed with sat. NaHCO₄ (100 mL), 1M HCl (100 mL) and sat. brine (100 mL). The organic layer was then dried over Na₂SO₄ and concentrated *in vacuo* to afford a yellow solid (7.64 g). The crude product was triturated in EtOH and then filtered to afford **9** (5.52 g, 15.3 mmol, 76% yield) as a pale yellow solid. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.79 (s, 1 H, NH), 8.30 (d, *J*=8.9 Hz, 2 H, 10-CH), 8.14 (d, *J*=8.9 Hz, 2 H, 9-CH), 7.84 (d, *J*=7.7 Hz, 1 H, 1-CH), 7.20 - 7.26 (m, 1 H, 3-CH), 7.14 - 7.18 (m, 2 H, 2-CH,4-CH), 6.86 (s, 1 H, NH), 1.52 (s, 9 H, 14-CH₃). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 163.3 (C7), 155.0 (C12), 149.8 (C8), 140.0 (C11), 130.5 (C5), 129.6 (C6), 128.6 (C9), 126.3 (C2), 126.2 (C3), 125.9 (C1), 124.4 (C4), 123.7 (C10), 82.0 (C13), 28.3 (C14). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₁₈H₁₉N₃O₅Na: 380.1222, found 380.1223.



Tert-butyl (2-(4-aminobenzamido)phenyl)carbamate, (10): To a solution of **9** (5.52 g, 15.3 mmol) in MeOH/THF (1:1, 100 mL), 10% Pd/C (0.55 g) was added. The reaction flask was filled with nitrogen and evacuated 3 times using a Shlenk line, before a balloon of hydrogen was added and the resultant mixture stirred vigorously for 18 hours. The balloon of hydrogen was removed and the flask was flushed with nitrogen. The reaction mixture was filtered through celite, then the celite was washed with more MeOH (3 x 50 mL) and the filtrate concentrated *in vacuo* to afford **10** (5.23 g, 15.3 mmol, 100% yield) as a fluffy white crystalline solid. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 8.87 (s, 1 H, NH), 7.78 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.64 (dd, *J*=7.7, 1.7 Hz, 1 H, 1-CH), 7.29 (dd, *J*=7.7, 1.7 Hz, 1 H, 4-CH), 7.14 (app. td, *J*=7.7, 1.7 Hz, 1 H, 2-CH), 7.12 (app. td, *J*=7.7, 1.7 Hz, 1 H, 3-CH), 7.06 (s, 1 H, NH), 6.66 (d, *J*=8.7 Hz, 2 H, 10-CH), 4.05 (s, 2 H, 11-NH₂), 1.51 (s, 9 H, 14-CH₃). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 165.7 (C7), 154.6 (C12), 150.1 (C8), 131.0 (C6), 130.3 (C5), 129.3 (C9), 125.7 (C1), 125.6 (C2,C3), 124.5 (C4), 123.5 (C11), 114.1 (C10), 81.0 (C13), 28.3 (C14). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₁₈H₂₁N₃O₃Na: 350.1481, found 350.1486.

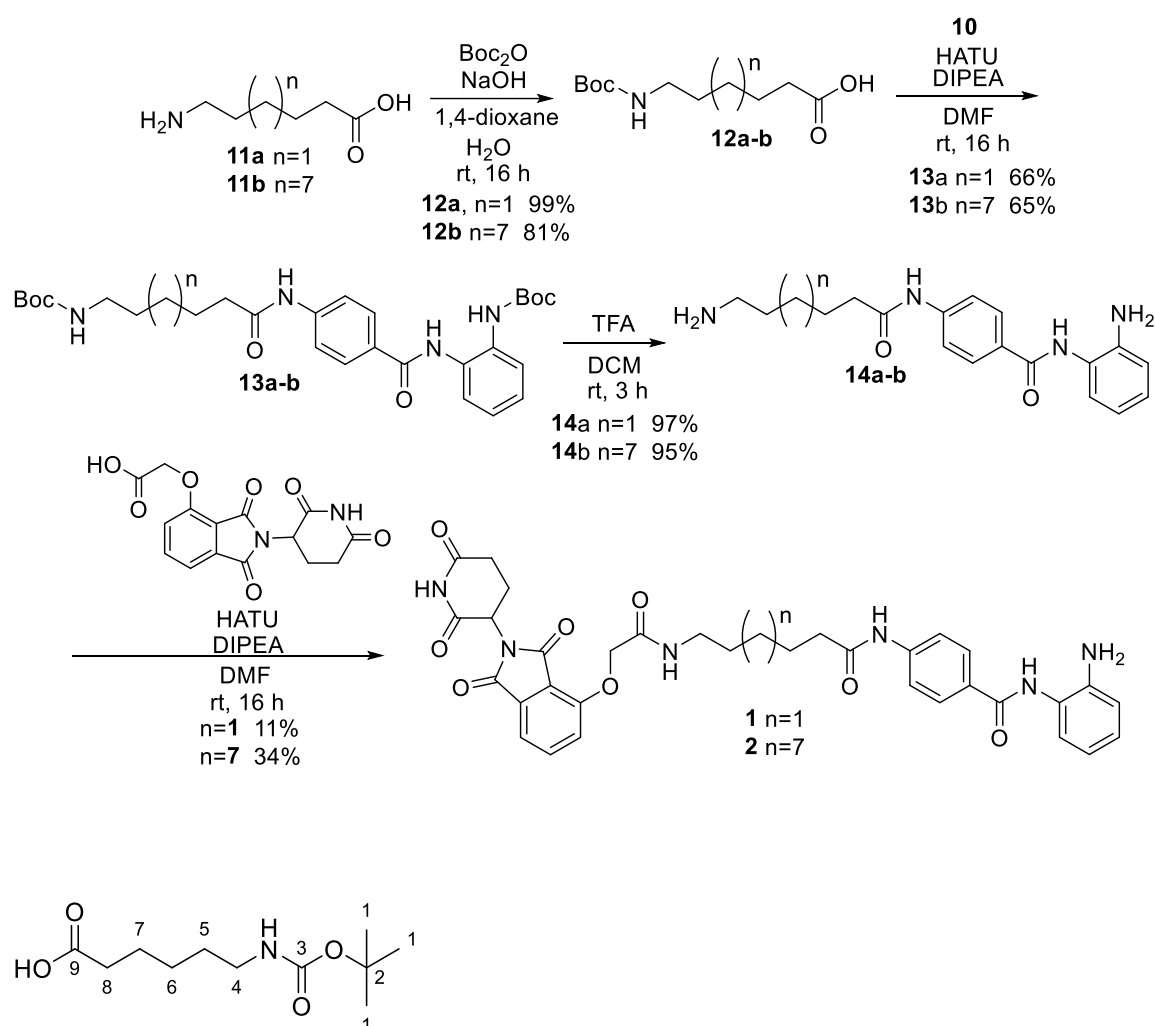


Tert-butyl (2-(4-acetamidobenzamido)phenyl)carbamate, (5): Triethylamine (0.255 mL, 1.833 mmol) was added to a solution of **10** (200 mg, 0.611 mmol) in dry THF (5 mL) at 0 °C, followed by the dropwise addition of acetyl chloride (0.052 mL, 0.733 mmol). The mixture was stirred at 0 °C for 30 minutes, then at room temperature for 2 hours. The reaction mixture was concentrated *in vacuo* to afford a white solid (440 mg). The crude product was purified by column chromatography (dry load, 100% EtOAc) to afford **5** (193 mg, 0.517 mmol, 85% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ_H ppm 10.24 (s, 1 H, NH), 9.74 (s, 1 H, NH), 8.67 (br s, 1 H, NH), 7.91 (d, *J*=8.7 Hz, 2 H, 5-CH), 7.73 (d, *J*=8.7 Hz, 2 H, 4-CH), 7.48 - 7.58 (m, 2 H, 10-CH, 13-CH), 7.11 - 7.22 (m, 2 H, 11-CH, 12-CH), 2.09 (s, 3 H, 1-CH₃), 1.45 (s, 9 H, 16-CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ_C ppm 168.8 (C2), 164.8 (C7), 153.5 (C14), 142.5 (C3), 131.6 (C8), 129.9 (C9), 128.5 (C5), 128.3 (C10), 126.0 (C13), 125.5 (C11), 124.1 (C12), 123.9 (C10), 118.2 (C4), 79.7 (C15), 28.0 (C16), 24.1 (C1). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₂₀H₂₃N₃O₄Na: 392.1586, found 392.1586.

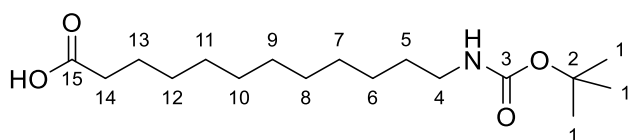


(4-acetamido-N-(2-aminophenyl)benzamide) CI-994: TFA (0.2 mL) was added to a stirring solution of **5** (47.5 mg, 0.129 mmol) in DCM (1 mL) and the resulting reaction mixture stirred at room temperature for 6 hours. The reaction mixture was concentrated *in vacuo* to afford a brown oil (51.7 mg). The crude oil was dissolved in MeOH (1 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 150 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **CI-994** (35.6 mg, 0.126 mmol, 97 % yield) as a yellow/white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ_H ppm 10.19 (s, 1 H, NH), 9.54 (s, 1 H, NH), 7.93 (d, *J*=8.7 Hz, 2 H, 5-CH), 7.69 (d, *J*=8.7 Hz, 2 H, 4-CH), 7.15 (dd, *J*=7.7, 1.4 Hz, 1 H, 13-CH), 6.96 (app. td, *J*=7.7, 1.4 Hz, 1 H, 11-CH), 6.77 (dd, *J*=7.7, 1.4 Hz, 1 H, 10-CH), 6.59 (app. td, *J*=7.7, 1.4 Hz, 1 H, 12-CH), 4.87 (s, 2 H, 9-NH₂), 2.08 (s, 3 H, 1-CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ_C ppm 168.7 (C2), 164.7 (C7), 143.1 (C9), 142.1 (C3), 128.8 (C6), 128.7 (C5), 126.7 (C13), 126.4 (C11), 123.5 (C8), 118.0 (C4), 116.3 (C10), 116.1 (C12), 24.1 (C1). HRMS (ESI) *m/z*: [M+H]⁺ calculated for C₁₅H₁₆N₃O₂: 270.1243, found 270.1247. Matches literature data.²

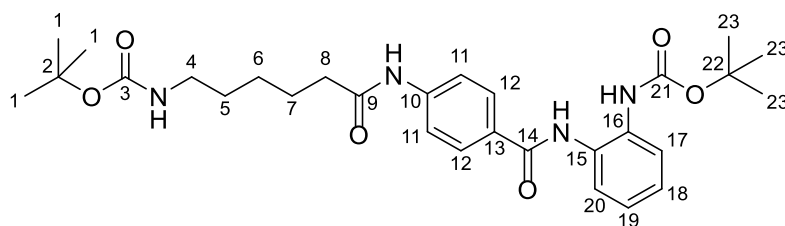
7. Synthesis of HDACi-linker Conjugates with Thalidomide Derivative



6-((Tert-butoxycarbonyl)amino)hexanoic acid, (12a): A solution of Boc₂O (3.66 g, 16.8 mmol) in 1,4-dioxane/water (2:1, 10 mL) was added slowly to a solution of **11a** (2.00 g, 15.2 mmol) and NaOH (0.61 g, 15.2 mmol) in 1,4-dioxane/water (2:1, 50 mL) at 0 °C, and then the mixture was stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo*, then the basic residue redissolved in water (100 mL) and washed with EtOAc (2 x 50 mL). The aqueous phase was then acidified with 1 M HCl to pH 1 and extracted with EtOAc (3 x 100 mL). The organic phases were combined, dried over Na₂SO₄, filtered and concentrated *in vacuo* to afford **12a** (3.54 g, 15.0 mmol, 98% yield) as a colourless oil, which slowly crystallised to a white solid. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 10.95 (br s, 1 H, 9-CO₂H), 4.57 (br s, 1 H, NH), 3.03 - 3.17 (m, 2 H, 4-CH₂), 2.35 (t, *J*=7.5 Hz, 2 H, 8-CH₂), 1.65 (quin, *J*=7.5 Hz, 2 H, 7-CH₂), 1.46 - 1.54 (m, 2 H, 5-CH₂), 1.44 (s, 9 H, 1-CH₃), 1.33 - 1.41 (m, 2 H, 6-CH₂). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 179.0 (C9), 156.0 (C3), 79.2 (C2), 40.4 (C4), 33.9 (C8), 29.7 (C5), 28.4 (C1), 26.2 (C6), 24.3 (C7). MS (ESI) *m/z* 254 [M+Na]⁺, 203 [M-H]⁻.

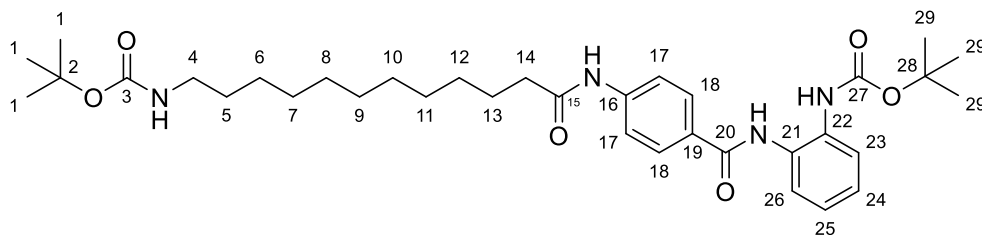


12-((Tert-butoxycarbonyl)amino)dodecanoic acid, (12b): A solution of Boc₂O (2.23 g, 10.22 mmol) in 1,4-dioxane/water (2:1, 10 mL) was added slowly to a solution of **11b** (2.00 g, 9.29 mmol) and NaOH (0.37 g, 9.29 mmol) in 1,4-dioxane/water (2:1, 50 mL) at 0 °C, and then the mixture was stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo*, then the basic residue redissolved in water (100 mL) and washed with EtOAc (2 x 50 mL). The aqueous phase was then acidified with 1 M HCl (ca. 15 mL) to pH 1 and extracted with EtOAc (3 x 100 mL). The organic phases were combined, dried over Na₂SO₄, filtered and concentrated *in vacuo* to afford **12b** (2.41 g, 7.56 mmol, 81% yield) as a fluffy white solid. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 10.95 (br s, 1 H, CO₂H), 4.54 (br s, 1 H, NH), 3.02 - 3.15 (m, 2 H, 4-CH₂), 2.34 (t, *J*=7.4 Hz, 2 H, 14-CH₂), 1.63 (quin, *J*=7.4 Hz, 2 H, 13-CH₂), 1.41 - 1.50 (m, 11 H, 1-CH₃, 5-CH₂), 1.24 - 1.36 (m, 14 H, (6-12)-CH₂). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 179.2 (C15), 156.0 (C3), 79.1 (C2), 40.6 (C4), 34.0 (C14), 30.0 (C5), 29.5 (alkyl CH₂), 29.4 (alkyl CH₂), 29.3 (alkyl CH₂), 29.2 (alkyl CH₂), 29.1 (alkyl CH₂), 29.0 (alkyl CH₂), 28.4 (C1), 26.8 (alkyl CH₂), 24.7 (C13). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₁₇H₃₃NO₄Na: 338.2307, found 338.2307.



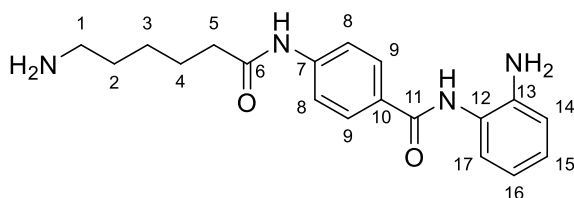
Tert-butyl (2-(4-(6-((tert-butoxycarbonyl)amino)hexanamido)benzamido)phenyl)carbamate, (13a): To a solution of **12a** (233 mg, 1.01 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.48 mL, 2.75 mmol) and HATU (453 mg, 1.19 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (300 mg, 0.92 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. NaHCO₃ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a crude brown oil (816 mg). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **13a** (328 mg, 0.60 mmol, 66% yield) as a yellow crystalline solid. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.35 (br s, 1 H, NH), 8.53 (br s, 1 H, NH), 7.88 (d, *J*=8.7 Hz, 2 H, 12-CH), 7.66 - 7.70 (m, 1 H, 20-CH), 7.63 (d, *J*=8.7 Hz, 2 H, 11-CH), 7.32 - 7.36 (m, 1 H, 17-CH), 7.29 (br s, 1 H, NH), 7.10 - 7.18 (m, 2 H, 18-CH, 19-CH), 4.73 (br s, 1 H, NH), 3.06 (q, *J*=6.8 Hz, 2 H, 4-CH₂), 2.30 (br t, *J*=7.3 Hz, 2 H, 8-CH₂), 1.60 - 1.71 (m, 2 H, 7-CH₂), 1.49 (s, 9 H, 23-CH₃), 1.44 (s, 9 H, 1-CH₃), 1.39 - 1.47 (m, 2 H, 5-CH₂), 1.23-1.30 (m, 2 H, 6-CH₂). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 172.1 (C9), 165.5 (C14), 156.2 (C3), 154.6 (C21), 141.8 (C10), 130.6 (C15), 130.5 (C16), 129.0 (C13), 128.4 (C12), 126.0 (C18/19), 125.8 (C20), 125.6 (C18/19), 124.4 (C17), 119.1 (C11), 81.2 (C22), 79.2 (C2), 40.3 (C4), 37.3 (C8), 29.7 (C5), 28.4

(C1), 28.3 (C23), 26.2 (C6), 25.0 (C7). HRMS (ESI) m/z : $[M+Na]^+$ calculated for $C_{29}H_{40}N_4O_6Na$: 563.2846, found 563.2840.



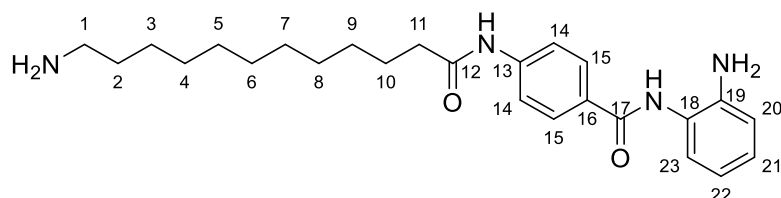
Tert-butyl (2-(4-(12-((tert-butoxycarbonyl)amino)dodecanamido)benzamido)phenyl)carbamate, (13b):

To a solution of **12b** (318 mg, 1.01 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.48 mL, 2.75 mmol) and HATU (454 mg, 1.19 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (300 mg, 0.92 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude brown oil (0.92 g). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **13b** (374 mg, 0.59 mmol, 65% yield) as a pale yellow crystalline solid. 1H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.21 (br s, 1 H, NH), 7.94 (s, 1 H, NH), 7.90 (d, $J=8.7$ Hz, 2 H, 18-CH), 7.73 (dd, $J=7.7, 1.7$ Hz, 1 H, 26-CH), 7.62 (d, $J=8.7$ Hz, 2 H, 17-CH), 7.29 (dd, $J=7.7, 1.7$ Hz, 1 H, 23-CH), 7.11 - 7.21 (m, 2 H, 24-CH, 25-CH), 7.03 (s, 1 H, NH), 4.56 (br s, 1 H, NH), 3.09 (q, $J=6.6$ Hz, 2 H, 4- CH_2), 2.36 (t, $J=7.5$ Hz, 2 H, 14- CH_2), 1.67 - 1.75 (m, 2 H, 13- CH_2), 1.51 (s, 9 H, 29- CH_3), 1.41 - 1.48 (m, 11 H, 1- $CH_3, 5-CH_2$), 1.23 - 1.34 (m, 14 H, (6-12)- CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 172.0 (C15), 165.2 (C20), 156.1 (C3), 154.6 (C27), 141.6 (C16), 130.8 (C21), 130.1 (C22), 129.2 (C19), 128.4 (C18), 125.9 (C24/25), 125.8 (C24/25), 125.7 (C26), 124.4 (C23), 119.1 (C17), 81.3 (C28), 79.1 (C2), 40.6 (C4), 37.7 (C14), 30.0 (alkyl CH_2), 29.4 (2x alkyl CH_2), 29.3 (alkyl CH_2), 29.2 (alkyl CH_2), 29.1 (2x alkyl CH_2), 28.4 (C1), 28.3 (C29), 26.7 (alkyl CH_2), 25.4 (C13). HRMS (ESI) m/z : $[M+Na]^+$ calculated for $C_{35}H_{52}N_4O_6Na$: 647.3785, found 647.3792.

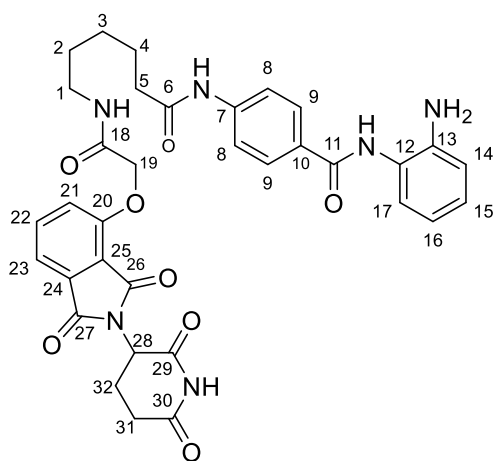


4-(6-Aminohexanamido)-N-(2-aminophenyl)benzamide, (14a): TFA (0.52 mL, 6.82 mmol) was added to a stirring solution of **13a** (185 mg, 0.341 mmol) in DCM (10 mL) and the resulting reaction mixture stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (280 mg). The crude oil was dissolved in MeOH (10 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 0.677 g) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **14a** (114 mg, 0.334 mmol, 97% yield) as a yellow/brown solid. 1H NMR (400 MHz, Methanol-*d_4*) δ_H ppm 7.95 (d, $J=8.7$ Hz, 2 H, 9-CH), 7.73 (d, $J=8.7$ Hz, 2 H, 8-CH), 7.18 (dd, $J=8.0, 1.3$ Hz, 1 H, 17-CH), 7.07 (app. td, $J=8.0, 1.3$

Hz, 1 H, 15-CH), 6.90 (dd, $J=8.0, 1.3$ Hz, 1 H, 14-CH), 6.76 (app. td, $J=8.0, 1.3$ Hz, 1 H, 16-CH), 2.79 (t, $J=7.2$ Hz, 2 H, 1-CH₂), 2.43 (t, $J=7.2$ Hz, 2 H, 5-CH₂), 1.73 (quin, $J=7.2$ Hz, 2 H, 4-CH₂), 1.61 (quin, $J=7.2$ Hz, 2 H, 2-CH₂), 1.44 (quin, $J=7.2$ Hz, 2 H, 3-CH₂). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_c ppm 174.8 (C6), 168.4 (C11), 144.0 (C13), 143.6 (C7), 130.5 (C10), 129.9 (C9), 128.7 (C17), 127.9 (C15), 125.4 (C12), 120.4 (C8), 119.8 (C16), 118.8 (C14), 41.5 (C1), 37.9 (C5), 30.7 (C2), 27.4 (C3), 26.4 (C4). HRMS (ESI) m/z : [M+H]⁺ calculated for C₁₉H₂₅N₄O₂: 341.1978, found 341.1977.

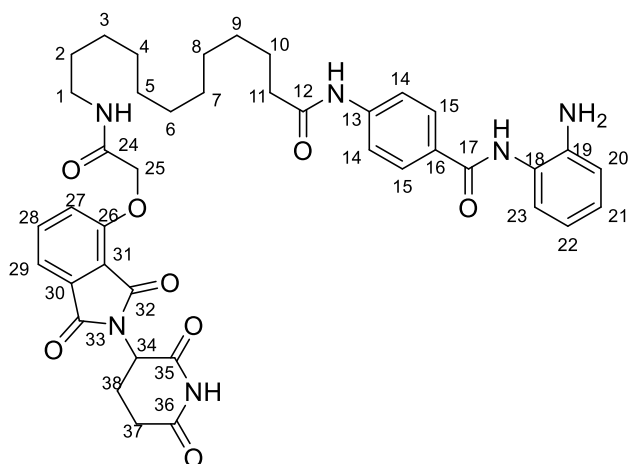


4-(12-Aminododecanamido)-N-(2-aminophenyl)benzamide, (14b): TFA (0.39 mL, 5.10 mmol) was added to a stirring solution of **13b** (159 mg, 0.255 mmol) in DCM (10 mL) and the resulting reaction mixture stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (322 mg). The crude oil was dissolved in MeOH (10 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 0.80 g) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **14b** (104 mg, 0.242 mmol, 95% yield) as a pale yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ_H ppm 10.15 (s, 1 H, NH), 9.55 (br s, 1 H, NH), 7.94 (d, $J=8.7$ Hz, 2 H, 15-CH), 7.71 (m, $J=8.7$ Hz, 2 H, 14-CH), 7.16 (dd, $J=7.7, 1.4$ Hz, 1 H, 23-CH), 6.96 (app. td, $J=7.7, 1.4$ Hz, 1 H, 21-CH), 6.78 (dd, $J=7.7, 1.4$ Hz, 1 H, 20-CH), 6.59 (app. td, $J=7.7, 1.4$ Hz, 1 H, 22-CH), 4.87 (br s, 2 H, NH₂), 3.78 (br s, 2 H, NH₂), 2.58 (t, $J=7.2$ Hz, 2 H, 1-CH₂), 2.34 (t, $J=7.2$ Hz, 2 H, 11-CH₂), 1.60 (br t, $J=7.2$ Hz, 2 H, 10-CH₂), 1.33 - 1.42 (m, 2 H, 2-CH₂), 1.25 (br s, 14 H, (3-9)-CH₂). ¹³C NMR (101 MHz, DMSO-*d*₆) δ_c ppm 171.7 (C12), 164.7 (C17), 143.1 (C19), 142.1 (C13), 128.7 (C16), 128.6 (C15), 126.6 (C23), 126.3 (C21), 123.5 (C18), 118.1 (C14), 116.3 (C22), 116.1 (C20), 40.8 (C1), 36.5 (C11), 31.3 (C2), 29.0 (alkyl CH₂), 28.95 (alkyl CH₂), 28.9 (alkyl CH₂), 28.85 (alkyl CH₂), 28.8 (alkyl CH₂), 28.7 (alkyl CH₂), 26.2 (alkyl CH₂), 25.0 (C10). HRMS (ESI) m/z : [M+H]⁺ calculated for C₂₅H₃₇N₄O₂: 425.2917, found 425.2918.



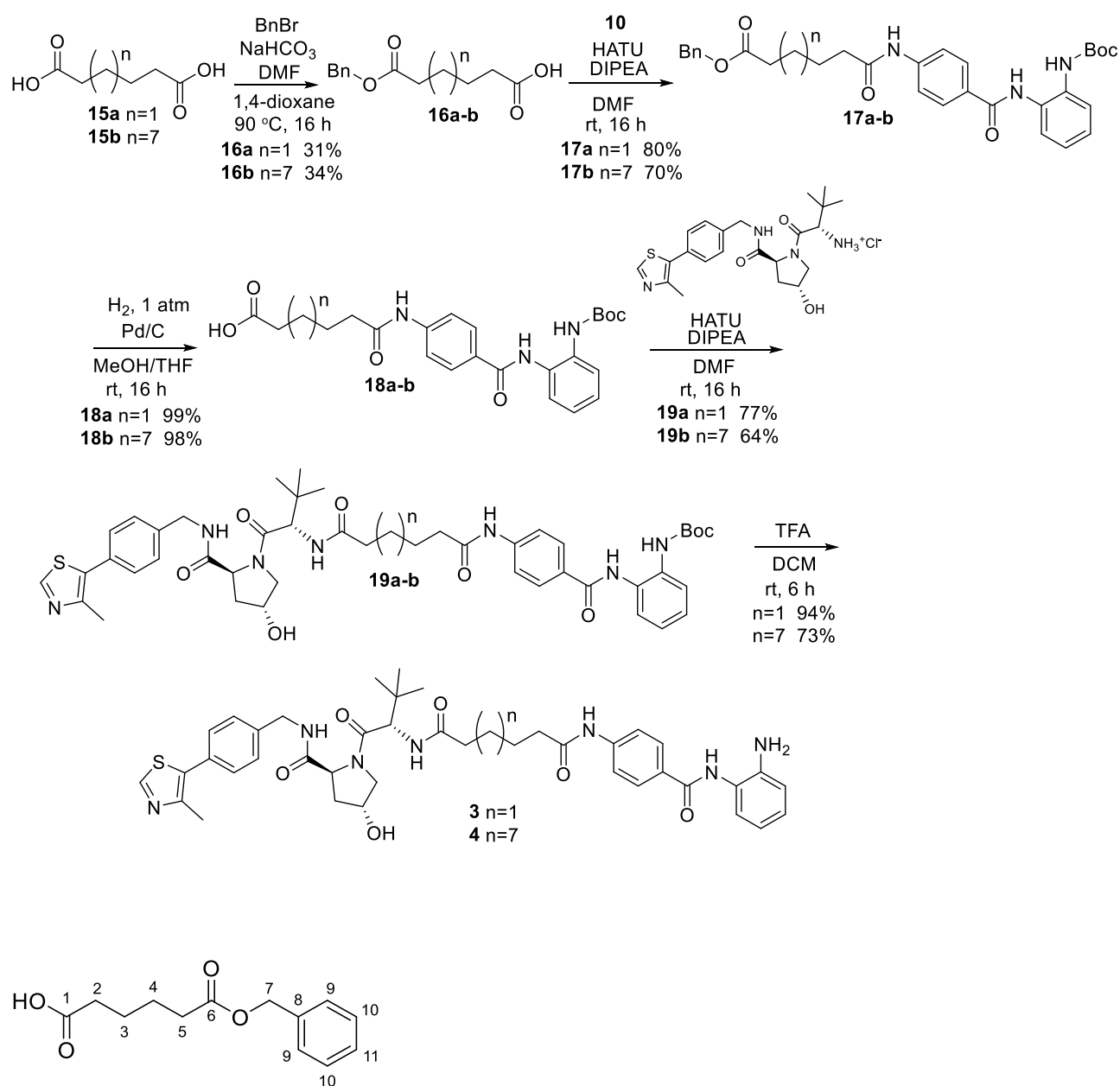
***N*-(2-aminophenyl)-4-(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetamido)**

hexanamido)benzamide, (1): The acetic acid functionalised thalidomide was prepared as previously reported in the literature.³ To a solution of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetic acid (41.5 mg, 0.125 mmol) in dry DMF (2 mL) at 0 °C, DIPEA (0.065 mL, 0.375 mmol) and HATU (57.0 mg, 0.150 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **14a** (42.5 mg, 0.125 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. NaHCO₃ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a pale yellow solid (45.8 mg). The crude product was purified by column chromatography (5% MeOH in DCM) to afford **1** (9.7 mg, 0.014 mmol, 11% yield) as a pale yellow solid. ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 7.89 (d, *J*=8.8 Hz, 2 H, 9-CH), 7.77 (dd, *J*=8.4, 7.3 Hz, 1 H, 22-CH), 7.67 (d, *J*=8.8 Hz, 2 H, 8-CH), 7.50 (dd, *J*=7.3, 0.5 Hz, 1 H, 23-CH), 7.39 (dd, *J*=8.4, 0.5 Hz, 1 H, 21-CH), 7.19 (dd, *J*=7.7, 1.5 Hz, 1 H, 17-CH), 7.07 (app. td, *J*=7.7, 1.5 Hz, 1 H, 15-CH), 6.90 (dd, *J*=7.7, 1.5 Hz, 1 H, 14-CH), 6.77 (td, *J*=7.7, 1.5 Hz, 1 H, 16-CH), 5.12 (dd, *J*=12.5, 5.5 Hz, 1 H, 28-CH), 4.72 (s, 2 H, 19-CH₂), 3.32 - 3.37 (m, 2 H, 1-CH₂), 2.80 - 2.92 (m, 1 H, 31-CH), 2.65 - 2.78 (m, 2 H, 31-CH,32-CH), 2.41 (t, *J*=7.4 Hz, 2 H, 5-CH₂), 2.08 - 2.18 (m, 1 H, 32-CH), 1.74 (quin, *J*=7.7 Hz, 2 H, 4-CH₂), 1.60 - 1.69 (m, 2 H, 2-CH₂), 1.41 - 1.52 (m, 2 H, 3-CH₂). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_C ppm 174.8 (C6), 174.7 (C30), 171.6 (C29), 170.0 (C18), 168.4 (C27), 168.3 (C11), 167.9 (C26), 156.3 (C20), 144.0 (C13), 143.6 (C7), 138.4 (C22), 135.0 (C24), 130.4 (C10), 129.9 (C9), 128.7 (C15), 127.9 (C17), 125.5 (C12), 121.8 (C21), 120.4 (C8), 119.8 (C16), 119.4 (C25), 118.9 (C14), 118.2 (C23), 69.5 (C19), 50.7 (C28), 40.1 (C1), 38.0 (C5), 32.3 (C31), 30.0 (C2), 27.5 (C3), 26.4 (C4), 23.8 (C32). HRMS (ESI) *m/z*: [M+H]⁺ calculated for C₃₄H₃₅N₆O₈: 655.2516, found 655.2515.



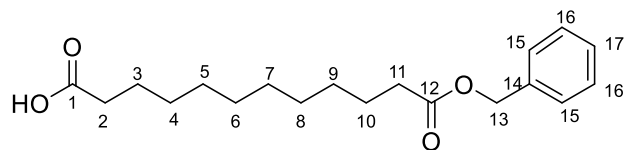
N-(2-aminophenyl)-4-(12-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetamido)dodecanamido)benzamide, (2): To a solution of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetic acid (31.6 mg, 0.095 mmol) in dry DMF (2 mL) at 0 °C, DIPEA (0.050 mL, 0.285 mmol) and HATU (43.4 mg, 0.114 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **14b** (40.4 mg, 0.095 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 18 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. NaHCO₃ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a brown oil (74.2 mg). The crude product was purified by column chromatography (5% MeOH in DCM) to afford **2** (25.4 mg, 0.033 mmol, 34% yield) as a pale yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ_H ppm 11.11 (br s, 1 H, NH), 10.11 (s, 1 H, NH), 9.54 (s, 1 H, NH), 7.93 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.91 (s, 1H, NH), 7.81 (dd, *J*=8.4, 7.2 Hz, 1 H, 28-CH), 7.70 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.50 (d, *J*=7.2 Hz, 1 H, 29-CH), 7.39 (d, *J*=8.4 Hz, 1 H, 27-CH), 7.15 (dd, *J*=7.7, 1.4 Hz, 1 H, 23-CH), 6.96 (app. td, *J*=7.7, 1.4 Hz, 1 H, 21-CH), 6.78 (dd, *J*=7.7, 1.4 Hz, 1 H, 20-CH), 6.59 (app. td, *J*=7.7, 1.4 Hz, 1 H, 22-CH), 5.12 (dd, *J*=12.9, 5.4 Hz, 1 H, 34-CH), 4.87 (s, 2 H, NH₂), 4.76 (s, 2 H, 25-CH₂), 3.13 (q, *J*=6.5 Hz, 2 H, 1-CH₂), 2.85 - 2.95 (m, 1 H, 37-CH), 2.53 - 2.63 (m, 2 H, 37-CH,38-CH), 2.33 (t, *J*=7.4 Hz, 2 H, 11-CH₂), 2.00 - 2.07 (m, 1 H, 38-CH), 1.59 (quin, *J*=7.0 Hz, 2 H, 10-CH₂), 1.39 - 1.47 (m, 2 H, 2-CH₂), 1.23 - 1.31 (m, 14 H, (3-9)-CH₂). ¹³C NMR (101 MHz, DMSO-*d*₆) δ_C ppm 172.8 (C36), 171.7 (C12), 169.9 (C35), 166.7 (C24), 166.6 (C33), 165.5 (C32), 164.7 (C17), 155.0 (C26), 143.1 (C19), 142.1 (C13), 136.9 (C28), 133.0 (C30), 128.7 (C16), 128.6 (C15), 126.6 (C23), 126.3 (C21), 123.5 (C18), 120.4 (C27), 118.1 (C14), 116.8 (C31), 116.3 (C22), 116.1 (C20), 116.0 (C29), 67.7 (C25), 48.8 (34), 38.3 (C1), 36.5 (C11), 31.0 (C37), 29.0 (alkyl CH₂), 28.9 (3x alkyl CH₂), 28.8 (alkyl CH₂), 28.7 (alkyl CH₂), 28.6 (alkyl CH₂), 26.3 (alkyl CH₂), 25.0 (C10), 22.0 (C38). HRMS (ESI) *m/z*: [M+H]⁺ calculated for C₄₀H₄₇N₆O₈: 739.3455, found 739.3455.

8. Synthesis of HDACi-linker Conjugates with VHL Ligand

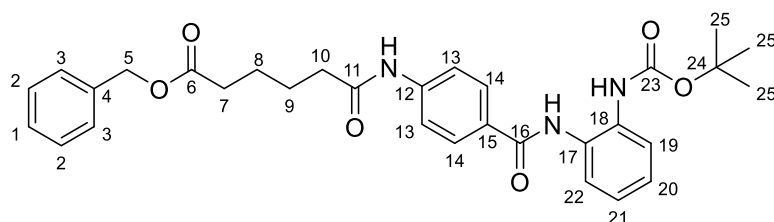


6-(benzyloxy)-6-oxohexanoic acid, (16a): To a solution of **15a** (1.00 g, 6.84 mmol) in 1,4-dioxane/DMF (1:1, 40 mL), was added BnBr (0.81 mL, 6.84 mmol), followed by the addition of NaHCO₃ (0.59 g, 7.02 mmol). The resulting suspension was heated at 90 °C for 16 hours. The reaction mixture was left to cool to room temperature and then concentrated *in vacuo* to afford an off-white oil. The crude residue was then suspended in EtOAc (50 mL) and washed with sat. NaCl (50 mL) and water (50 mL). The organic phase was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a cloudy white oil (0.965 g). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **16a** (0.499 g, 2.09 mmol, 31% yield) as a colourless oil. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 11.04 (br s, 1 H, CO₂H), 7.30 - 7.41 (m, 5 H, (9-11)-CH), 5.13 (s, 2 H, 7-CH₂), 2.34 - 2.42 (m, 4 H, 2-CH₂,5-CH₂), 1.64 - 1.77 (m, 4 H, 3-CH₂,4-CH₂).

^{13}C NMR (101 MHz, Chloroform-*d*) δ_{C} ppm 179.4 (C1), 173.2 (C6), 136.0 (C8), 128.6 (C10), 128.3 (C11), 128.2 (C9), 66.3 (C7), 33.9 (C5), 33.6 (C2), 24.3 (C3/4), 24.1 (C3/4). HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{13}\text{H}_{16}\text{O}_4\text{Na}$: 259.0946, found 259.0946.

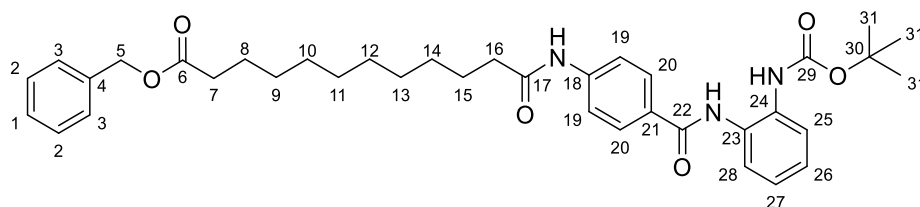


12-(benzyloxy)-12-oxododecanoic acid, (16b): To a solution of **15b** (2.00 g, 8.68 mmol) in 1,4-dioxane/DMF (1:1, 40 mL), was added BnBr (1.03 mL, 8.68 mmol), followed by the addition of NaHCO_3 (0.73 g, 8.68 mmol). The resulting suspension was heated at 90 °C for 16 hours. The reaction mixture was left to cool to room temperature and then concentrated *in vacuo* to afford a cloudy oil. The crude residue was then suspended in EtOAc (50 mL) and washed with sat. NaCl (50 mL) and water (50 mL). The organic phase was dried over MgSO_4 , filtered and concentrated *in vacuo* to afford an off-white solid (2.39 g). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **16b** (0.998g, 2.99 mmol, 34% yield). ^1H NMR (400 MHz, Chloroform-*d*) δ_{H} ppm 11.07 (br s, 1 H, 1-CO₂H), 7.32 - 7.40 (m, 5 H, (15-17)-CH), 5.13 (s, 2 H, 13-CH₂), 2.30 - 2.42 (m, 4 H, 2-CH₂, 11-CH₂), 1.57 - 1.71 (m, 4 H, 3-CH₂, 10-CH₂), 1.23 - 1.37 (m, 12 H, (4-9)-CH₂). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_{C} ppm 179.9 (C1), 173.7 (C12), 136.1 (C14), 128.5 (C16), 128.2 (C15), 128.1 (C17), 66.1 (C13), 34.3 (C11), 34.0 (C2), 29.4 (alkyl CH₂), 29.3 (alkyl CH₂), 29.2 (2x alkyl CH₂), 29.1 (alkyl CH₂), 29.0 (alkyl CH₂), 24.9 (C10), 24.6 (C3). HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{19}\text{H}_{28}\text{O}_4\text{Na}$: 343.1885, found 343.1887.

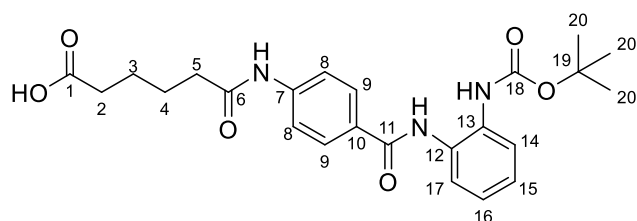


Benzyl 6-((4-((2-((tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-6-oxohexanoate, (17a) : To a solution of **16a** (164 mg, 0.693 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.28 mL, 1.60 mmol) and HATU (304 mg, 0.799 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (174 mg, 0.533 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 64 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. NaHCO_3 (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over MgSO_4 , filtered and concentrated *in vacuo* to afford a crude brown oil (510 mg). The crude product was purified by column chromatography (50% EtOAc in hexane), to give **17a** (234 mg, 0.424 mmol, 80% yield) as a pale yellow crystalline solid. ^1H NMR (400 MHz, Chloroform-*d*) δ_{H} ppm 9.24 (br s, 1 H, NH), 8.09 (s, 1 H, NH), 7.89 (d, $J=8.8$ Hz, 2 H, 14-CH), 7.71 (dd, $J=7.7, 1.7$ Hz, 1 H, 22-CH), 7.63 (d, $J=8.8$ Hz, 2 H, 13-CH), 7.32 - 7.38 (m, 5 H, (1-3)-CH), 7.27 (dd, $J=7.7, 1.7$ Hz, 1 H, 19-CH), 7.11 - 7.20 (m, 2 H, 20-CH, 21-CH), 7.10 (s, 1 H, NH), 5.14 (s, 2 H, 5-CH₂), 2.40 (t, $J=6.9$ Hz, 2 H, 7-CH₂), 2.32 (t, $J=6.9$ Hz, 2 H, 10-CH₂), 1.65 - 1.75 (m, 4 H, 8-

CH₂,9-CH₂), 1.51 (s, 9 H, 25-CH₃). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 173.6 (C6), 171.3 (C11), 165.3 (C16), 154.6 (C23), 141.5 (C12), 135.8 (C4), 130.7 (C17), 130.2 (C18), 129.1 (C15), 128.6 (C2), 128.4 (C1), 128.3 (C14), 128.2 (C3), 126.0 (C21), 125.8 (C22), 125.7 (C20), 124.5 (C19), 119.1 (C13), 81.3 (C24), 66.4 (C5), 37.0 (C10), 33.8 (C7), 28.3 (C25), 24.6 (C9), 24.2 (C8). HRMS (ESI) m/z: [M+Na]⁺ calculated for C₃₁H₃₅N₃O₆Na: 568.2424, found 568.2422.

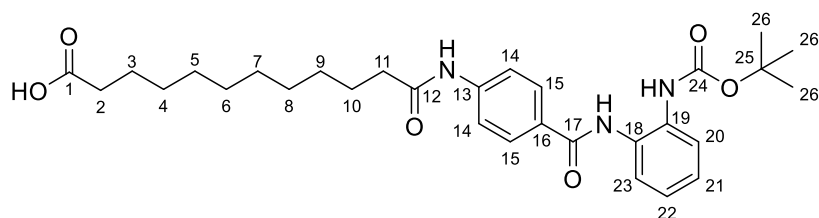


Benzyl 12-((4-((2-((tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-12-oxododecanoate, (17b): To a solution of **16b** (257 mg, 0.802 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.32 mL, 1.833 mmol) and HATU (356 mg, 0.936 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (200 mg, 0.611 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 18 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. NaHCO₃ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a crude brown oil (632 mg). The crude product was purified by column chromatography (50% EtOAc in hexane) to give **17b** (274 mg, 0.430 mmol, 70%) as a pale yellow solid. ¹H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.20 (br s, 1 H, NH), 7.89 (d, *J*=8.7 Hz, 2 H, 20-CH), 7.78 (br s, 1 H, NH), 7.73 (dd, *J*=7.7, 1.6 Hz, 1 H, 28-CH), 7.60 (d, *J*=8.7 Hz, 2 H, 19-CH), 7.31 - 7.40 (m, 5 H, 1,2,3-CH), 7.28 (dd, *J*=7.7, 1.6 Hz, 1 H, 25-CH), 7.11 - 7.22 (m, 2 H, 26-CH,27-CH), 7.01 (s, 1 H, NH), 5.12 (s, 2 H, 5-CH₂), 2.30 - 2.40 (m, 4 H, 7-CH₂,16-CH₂), 1.68 - 1.74 (m, 2 H, 15-CH₂), 1.60 - 1.68 (m, 2 H, 8-CH₂), 1.51 (s, 9 H, 31-CH₃), 1.24 - 1.37 (m, 12 H, (9-14)-CH₂). ¹³C NMR (101 MHz, Chloroform-*d*) δ_C ppm 173.8 (C6), 171.9 (C17), 165.2 (C22), 154.6 (C29), 141.5 (C18), 136.0 (C4), 130.8 (C23), 130.1 (C24), 129.2 (C21), 128.5 (C2), 128.4 (C20), 128.2 (C1), 128.1 (C3), 125.9 (C27), 125.8 (C26), 125.7 (C28), 124.5 (C25), 119.1 (C19), 81.3 (C30), 66.1 (C5), 37.7 (C16), 34.3 (C7), 29.3 (2x alkyl CH₂), 29.25 (alkyl CH₂), 29.2 (alkyl CH₂), 29.1 (alkyl CH₂), 29.0 (alkyl CH₂), 28.3 (C31), 25.4 (C15), 24.9 (C8). HRMS (ESI) m/z: [M+Na]⁺ calculated for C₃₇H₄₇N₃O₆Na: 652.3363, found 652.3364.

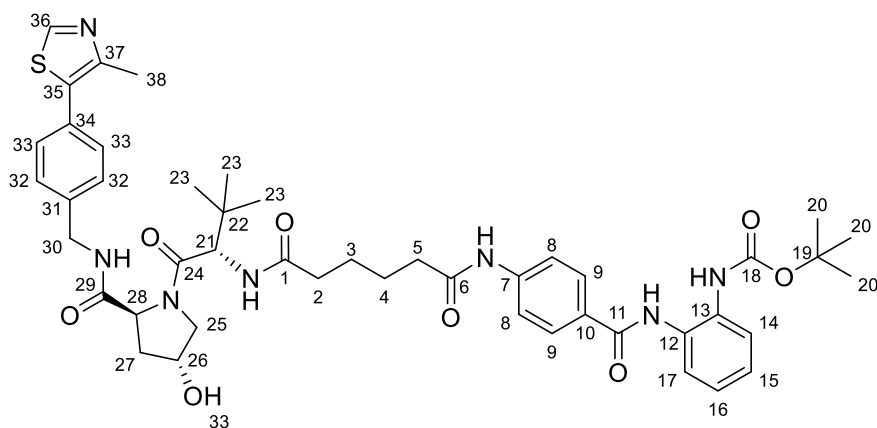


6-((4-((2-((Tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-6-oxohexanoic acid, (18a): To a solution of **17a** (190 mg, 0.348 mmol) in MeOH (20 mL), 10% Pd/C (20.0 mg) was added. The reaction flask was filled with nitrogen and evacuated 3 times using a Shlenk line, before a balloon of hydrogen was added and the resultant mixture stirred vigorously for 18 hours, after which the solid had crashed out as a white

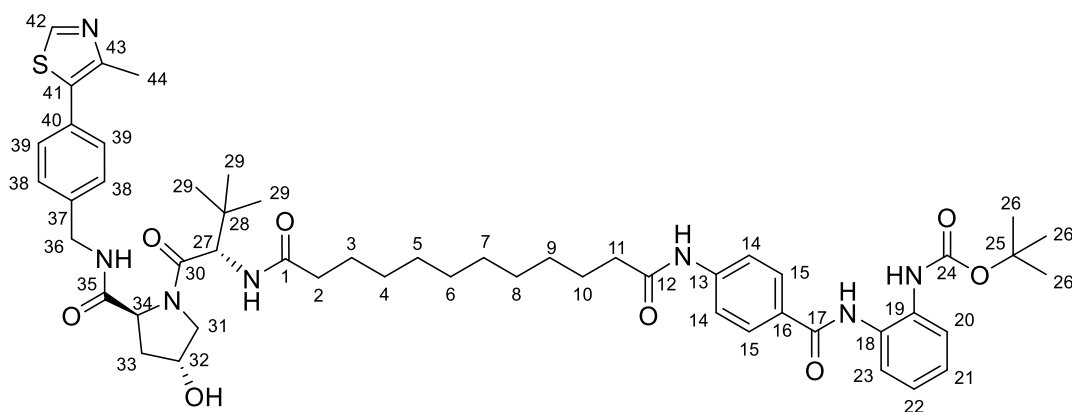
precipitate. The balloon of hydrogen was removed and the flask was flushed with nitrogen. The reaction mixture was diluted in more MeOH (20 mL) and THF (5 mL) to dissolve the precipitate, filtered through a glass microfiber filter paper, and the filtrate concentrated *in vacuo* to afford **18a** (159 mg, 0.345 mmol, 99% yield) as a white crystalline solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ_H ppm 11.97 (br s, 1 H, CO₂H), 10.21 (s, 1 H, NH), 9.75 (s, 1 H, NH), 8.68 (s, 1 H, NH), 7.91 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.74 (d, *J*=8.7 Hz, 2 H, 8-CH), 7.48 – 7.58 (m, 2 H, 14-CH,17-CH), 7.11 - 7.22 (m, 2 H, 15-CH,16-CH), 2.36 (t, *J*=7.1 Hz, 2 H, 5-CH₂), 2.25 (t, *J*=7.1 Hz, 2 H, 2-CH₂), 1.52 - 1.68 (m, 4 H, 3-CH₂,4-CH₂), 1.45 (s, 9 H, 20-CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ_C ppm 174.4 (C1), 171.6 (C6), 164.7 (C11), 153.5 (C18), 142.5 (C7), 131.6 (C13), 130.0 (C12), 128.5 (C9), 128.3 (C10), 126.0 (C17), 125.5 (C16), 124.2 (C15), 123.9 (C14), 118.2 (C8), 79.7 (C19), 36.2 (C5), 33.5 (C2), 28.0 (C20), 24.5 (C4), 24.2 (C3). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₂₄H₂₉N₃O₆Na: 478.1954; found 478.1955.



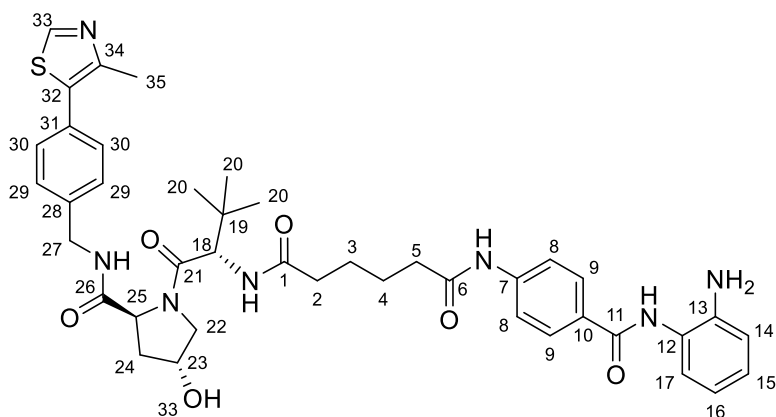
12-((4-((2-((Tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-12-oxododecanoic acid, (18b) : To a solution of **17b** (171 mg, 0.271 mmol) in MeOH (20 mL), 10% Pd/C (20.0 mg) was added. The reaction flask was filled with nitrogen and evacuated 3 times using a Shlenk line, before a balloon of hydrogen was added and the resultant mixture stirred vigorously for 18 hours, after which the solid had crashed out as a white precipitate. The balloon of hydrogen was removed and the flask was flushed with nitrogen. The reaction mixture was diluted in more MeOH (20 mL) and THF (5 mL) to dissolve the precipitate, filtered through a glass microfiber filter paper, and the filtrate concentrated *in vacuo* to afford **18b** (146 mg, 0.266 mmol, 98% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ_H ppm 11.96 (br s, 1 H, CO₂H), 10.17 (s, 1 H, NH), 9.74 (s, 1 H, NH), 8.68 (br s, 1 H, NH), 7.91 (d, *J*=8.5 Hz, 2 H, 15-CH), 7.74 (d, *J*=8.5 Hz, 2 H, 14-CH), 7.48 - 7.59 (m, 2 H, 20-CH,23-CH), 7.09 - 7.24 (m, 2 H, 21-CH,22-CH), 2.34 (t, *J*=7.3 Hz, 2 H, 11-CH₂), 2.18 (t, *J*=7.3 Hz, 2 H, 2-CH₂), 1.55 - 1.65 (m, 2 H, 10-CH₂), 1.46 - 1.51 (m, 2 H, 3-CH₂), 1.45 (s, 9 H, 26-CH₃), 1.23 - 1.30 (m, 12 H, (4-9)-CH₂). ¹³C NMR (101 MHz, DMSO-*d*₆) δ_C ppm 174.5 (C1), 171.8 (C12), 164.7 (C17), 153.5 (C24), 142.6 (C13), 131.6 (C19), 130.0 (C18), 128.5 (C15), 128.2 (C16), 126.0 (C23), 125.4 (C22), 124.1 (C21), 123.9 (C20), 118.2 (C14), 79.7 (C25), 36.5 (C11), 33.7 (C2), 28.9 (2x alkyl CH₂), 28.8 (alkyl CH₂), 28.7 (alkyl CH₂), 28.6 (alkyl CH₂), 28.5 (alkyl CH₂), 28.0 (C26), 25.0 (C10), 24.5 (C3). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₃₀H₄₁N₃O₆Na: 562.2893; found 562.2886.



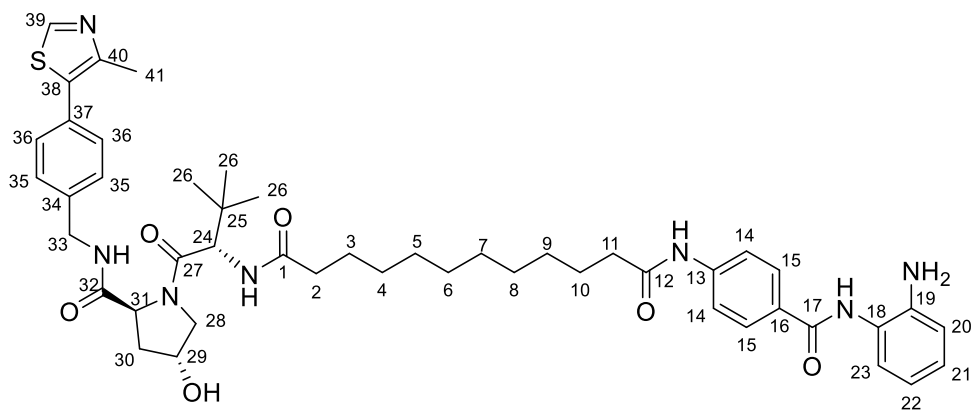
Tert-butyl(2-(4-(6-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanamido)benzamido)phenyl)carbamate, (19a): To a solution of **18a** (54.3 mg, 0.119 mmol) in dry DMF (1 mL) at 0 °C, DIPEA (0.05 mL, 0.298 mmol) and HATU (45.4 mg, 0.119 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride (50.0 mg, 0.099 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. NaHCO₃ (2 x 5 mL) and sat. brine (2 x 5 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a pale-yellow tar (125 mg). The crude product was purified by column chromatography (0-5% MeOH in DCM) to afford **19a** (70.0 mg, 0.077 mmol, 77% yield) as a white solid. ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 8.86 (s, 1 H, 36-CH), 7.92 (d, *J*=8.9 Hz, 2 H, 9-CH), 7.73 (d, *J*=8.9 Hz, 2 H, 8-CH), 7.56 - 7.62 (m, 1 H, 17-CH), 7.43 - 7.46 (m, 2 H, 33-CH), 7.41 - 7.43 (m, 1 H, 14-CH), 7.38 - 7.41 (m, 2 H, 32-CH), 7.16 - 7.26 (m, 2 H, 15-CH,16-CH), 4.61 - 4.66 (m, 1 H, 21-CH), 4.54 - 4.60 (m, 1 H, 28-CH), 4.47 - 4.54 (m, 2 H, 26-CH,30-CH), 4.32 - 4.38 (m, 1 H, 30-CH), 3.86 - 3.97 (m, 1 H, 25-CH), 3.77 - 3.84 (m, 1 H, 25-CH), 2.46 (s, 3 H, 38-CH₃), 2.42 (t, *J*=7.1 Hz, 2 H, 5-CH₂), 2.30 - 2.37 (m, 2 H, 2-CH₂), 2.17 - 2.26 (m, 1 H, 27-CH), 2.04 - 2.12 (m, 1 H, 27-CH), 1.66 - 1.77 (m, 4 H, 3-CH₂,4-CH₂), 1.48 (s, 9 H, 20-CH₃), 1.04 (s, 9 H, 23-CH₃). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_C ppm 175.8 (C1), 174.6 (C6), 174.5 (C29), 172.4 (C24), 167.9 (C11), 156.4 (C18), 153.0 (C36), 149.2 (C37), 143.9 (C7), 140.4 (C31), 133.6 (C35), 133.2 (C13), 131.8 (C12), 131.6 (C34), 130.6 (C10), 130.5 (C32), 129.7 (C9), 129.1 (C33), 127.5 (C15/16), 127.3 (C17), 126.4 (C15/16), 125.7 (C14), 120.5 (C8), 81.9 (C19), 71.2 (C26), 61.0 (C28), 59.2 (C21), 58.2 (C25), 43.8 (C30), 39.1 (C27), 37.9 (C5), 36.7 (C2), 36.5 (C22), 28.8 (C20), 27.2 (C23), 26.7 (C3), 26.5 (C4), 16.0 (C38). HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₄₆H₅₇N₇O₈SNa: 890.3887, found 890.3884.



Tert-butyl(2-(4-(12-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecanamido)benzamido)phenyl)carbamate, (19b): To a solution of **18b** (65.5 mg, 0.121 mmol) in dry DMF (1 mL) at 0 °C, DIPEA (0.05 mL, 0.298 mmol) and HATU (48.0 mg, 0.126 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride (50.0 mg, 0.099 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. NaHCO₃ (2 x 5 mL) and sat. brine (2 x 5 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a dark yellow tar (147 mg). The crude product was purified by column chromatography (0-5% MeOH in DCM) to afford **19b** (61.9 mg, 0.064 mmol, 64% yield) as a white solid. ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 8.86 (s, 1 H, 42-CH), 7.93 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.73 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.56 - 7.63 (m, 1 H, 23-CH), 7.44 - 7.47 (m, 2 H, 39-CH), 7.42 (m, 1 H, 20-CH), 7.38 - 7.41 (m, 2 H, 38-CH₂), 7.15 - 7.27 (m, 2 H, 21-CH,22-CH), 4.61 - 4.66 (m, 1 H, 27-CH), 4.55 - 4.60 (m, 1 H, 34-CH), 4.50 - 4.55 (m, 1 H, 36-CH), 4.47 - 4.50 (m, 1 H, 32-CH), 4.30 - 4.39 (m, 1 H, 36-CH), 3.84 - 3.94 (m, 1 H, 31-CH), 3.76 - 3.82 (m, 1 H, 31-CH), 2.46 (s, 3 H, 44-CH₃), 2.39 (t, *J*=7.5 Hz, 2 H, 11-CH₂), 2.17 - 2.32 (m, 3 H, 2-CH₂,33-CH), 2.03 - 2.11 (m, 1 H, 33-CH), 1.65 - 1.75 (m, 2 H, 3-CH₂), 1.59 (m, 2 H, 10-CH₂), 1.49 (s, 9 H, 26-CH₃), 1.29 - 1.37 (m, 12 H, (4-9)-CH₂), 1.03 (s, 9 H, 29-CH₃). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_C ppm 176.2 (C1), 175.1 (C12), 174.6 (C35), 172.5 (C30), 167.9 (C17), 156.4 (C24), 153.0 (C42), 149.1 (C43), 143.9 (C13), 140.4 (C37), 133.6 (C41), 133.2 (C19), 131.8 (C18), 131.6 (C40), 130.6 (C16), 130.5 (C38), 129.7 (C15), 129.1 (C39), 127.5 (C21/22), 127.3 (C23), 126.4 (C21/22), 125.7 (C20), 120.4 (C14), 81.9 (C25), 71.2 (C32), 61.0 (C34), 59.1 (C27), 58.2 (C31), 43.8 (C36), 39.0 (C33), 38.2 (C11), 36.8 (C2), 36.7 (C28), 30.7 (alkyl CH₂), 30.6 (alkyl CH₂), 30.55 (alkyl CH₂), 30.5 (alkyl CH₂), 30.4 (2x alkyl CH₂), 28.8 (C26), 27.2 (C29), 27.1 (C10), 26.9 (C3), 16.0 (C44). HRMS (ESI) *m/z*: [M+H]⁺ calculated for C₅₂H₇₀N₇O₈S: 952.5007, found 952.5009.

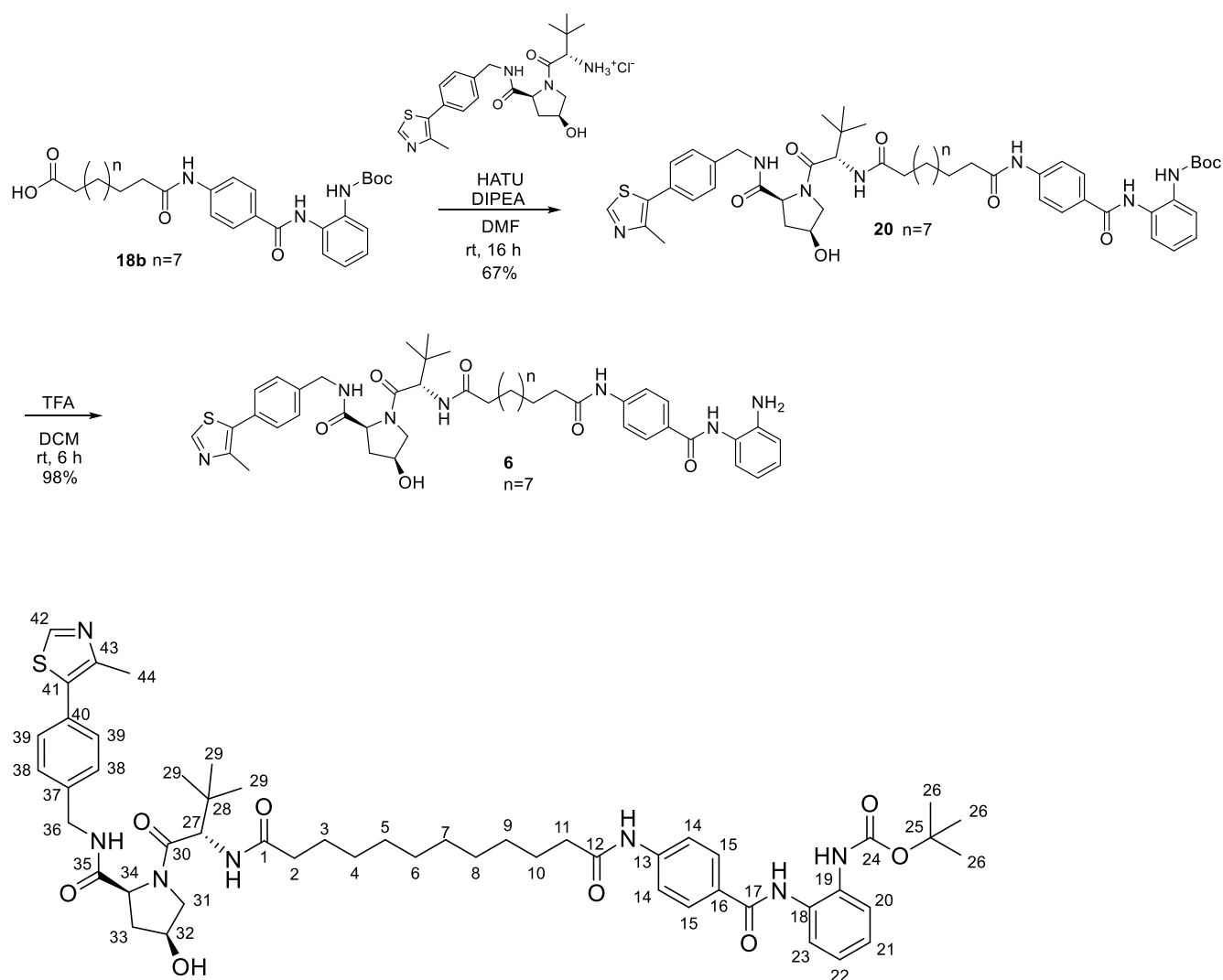


N-1-(4-((2-aminophenyl)carbamoyl)phenyl)-N6-((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)adipamide, (3): TFA (0.2 mL) was added to a stirring solution of **19a** (25.3 mg, 0.029 mmol) in DCM (1 mL) and the resulting reaction mixture stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* to afford an orange oil. The crude oil was dissolved in MeOH (2 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 200 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **3** (21.0 mg, 0.027 mmol, 94% yield) as a pale yellow solid. Prior to biological evaluation the product was further purified by semi-preparative HPLC (5-95% MeCN in H₂O, 260 nm, 45 min gradient). ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 8.87 (s, 1 H, 33-CH), 7.95 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.73 (d, *J*=8.7 Hz, 2 H, 8-CH), 7.43 - 7.48 (m, 2 H, 30-CH), 7.39 - 7.43 (m, 2 H, 29-CH), 7.18 (dd, *J*=8.0, 1.5 Hz, 1 H, 17-CH), 7.08 (app. td, *J*=8.0, 1.5 Hz, 1 H, 15-CH), 6.90 (dd, *J*=8.0, 1.4 Hz, 1 H, 14-CH), 6.77 (app. td, *J*=8.0, 1.4 Hz, 1 H, 16-CH), 4.63 (s, 1 H, 18-CH), 4.53 - 4.59 (m, 1 H, 25-CH), 4.43 - 4.52 (m, 2 H, 23-CH, 27-CH), 4.33 - 4.39 (m, 1 H, 27-CH), 3.89 - 3.95 (m, 1 H, 22-CH), 3.77 - 3.84 (m, 1 H, 22-CH), 2.47 (s, 3 H, 35-CH₃), 2.43 (br t, *J*=6.9 Hz, 2 H, 5-CH₂), 2.30 - 2.37 (m, 2 H, 2-CH₂), 2.18 - 2.25 (m, 1 H, 24-CH), 2.05 - 2.12 (m, 1 H, 24-CH), 1.67 - 1.77 (m, 4 H, 3-CH₂, 4-CH₂), 1.04 (s, 9 H, 20-CH₃). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_C ppm 175.8 (C1), 174.7 (C6), 174.6 (C26), 172.5 (C21), 168.4 (C11), 153.0 (C33), 149.2 (C34), 143.9 (C13), 143.6 (C7), 140.4 (C28), 133.6 (C32), 131.7 (C31), 130.6 (C10), 130.5 (C29), 129.9 (C9), 129.1 (C30), 128.6 (C15), 127.8 (C17), 125.6 (C12), 120.5 (C8), 119.8 (C16), 118.9 (C14), 71.2 (C23), 61.0 (C25), 59.2 (C18), 58.2 (C22), 43.9 (C27), 39.1 (C24), 37.9 (C5), 36.7 (C19), 36.5 (C2), 27.2 (C20), 26.7 (C3), 26.5 (C4), 16.0 (C35). HRMS (ESI) *m/z*: [M+H]⁺ calculated for C₄₁H₅₀N₇O₆S: 768.3543, found 768.3543.



N1-(4-((2-aminophenyl)carbamoyl)phenyl)-N12-((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)dodecanediamide, (4): TFA (0.2 mL) was added to a stirring solution of **19b** (37.6 mg, 0.0395 mmol) in DCM (2 mL) and the resulting reaction mixture stirred at room temperature for 7 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (54 mg). The crude oil was dissolved in MeOH (2 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 210 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **4** (25.8 mg, 0.0288 mmol, 73% yield) as a pale yellow solid. Prior to biological evaluation the product was further purified by semi-preparative HPLC (5-95% MeCN in H₂O, 260 nm, 45 min gradient). ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 8.86 (s, 1 H, 39-CH), 7.95 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.72 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.43 - 7.48 (m, 2 H, 36-CH), 7.38 - 7.42 (m, 2 H, 35-CH), 7.18 (dd, *J*=7.8, 1.3 Hz, 1 H, 23-CH), 7.07 (app. td, *J*=7.8, 1.3 Hz, 1 H, 21-CH), 6.90 (dd, *J*=7.8, 1.3 Hz, 1 H, 20-CH), 6.76 (app. td, *J*=7.8, 1.3 Hz, 1 H, 22-CH), 4.60 - 4.66 (m, 1 H, 24-CH), 4.55 - 4.60 (m, 1 H, 31-CH), 4.50 - 4.55 (m, 1 H, 33-CH), 4.47 - 4.50 (m, 1 H, 29-CH), 4.31 - 4.39 (m, 1 H, 33-CH), 3.86 - 3.93 (m, 1 H, 28-CH), 3.76 - 3.83 (m, 1 H, 28-CH), 2.47 (s, 3 H, 41-CH₃), 2.40 (t, *J*=7.5 Hz, 2 H, 11-CH₂), 2.18 - 2.33 (m, 3 H, 2-CH₂,30-CH), 2.03 - 2.12 (m, 1 H, 30-CH), 1.70 (quin, *J*=7.2 Hz, 2 H, 3-CH₂), 1.53 - 1.64 (m, 2 H, 10-CH₂), 1.28 - 1.41 (m, 12 H, (4-9)-CH₂), 1.03 (s, 9 H, 26-CH₃). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_C ppm 176.2 (C1), 175.1 (C12), 174.6 (C32), 172.5 (C27), 168.4 (C17), 153.0 (C39), 149.2 (C40), 144.0 (C19), 143.7 (C13), 140.4 (C34), 133.6 (C38), 131.7 (C37), 130.6 (C16), 130.5 (C35), 129.9 (C15), 129.1 (C36), 128.6 (C21), 127.8 (C23), 125.6 (C18), 120.4 (C14), 119.8 (C22), 118.9 (C20), 71.2 (C29), 61.0 (C31), 59.1 (C24), 58.2 (C28), 43.8 (C33), 39.1 (C30), 38.2 (C11), 36.8 (C25), 36.7 (C2), 30.7 (alkyl CH₂), 30.6 (alkyl CH₂), 30.55 (alkyl CH₂), 30.5 (alkyl CH₂), 30.4 (2x alkyl CH₂), 27.2 (C26), 27.1 (C10), 26.9 (C3), 16.0 (C41). HRMS (ESI) *m/z*: [M+H]⁺ calculated for C₄₇H₆₂N₇O₆S: 852.4476, found 852.4482.

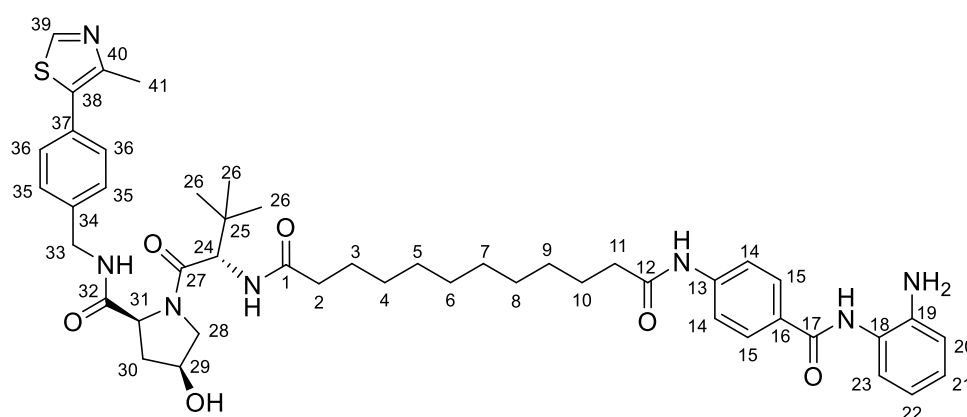
9. Synthesis of HDACi-linker Conjugate with Inactive Isomer of VHL Ligand



Tert-butyl (2-(4-(12-(((S)-1-((2S,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecanamido)benzamido)phenyl)carbamate,

(20): To a solution of **18b** (51.4 mg, 0.095 mmol) in dry DMF (1 mL) at 0 °C, DIPEA (0.04 mL, 0.238 mmol) and HATU (39.3 mg, 0.103 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of (2S,4S)-1-[(2S)-2-Amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide dihydrochloride (40.0 mg, 0.079 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. NaHCO₃ (2 x 5 mL) and sat. brine (2 x 5 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to afford a dark yellow tar (104 mg). The crude product was purified by column chromatography (0-5% MeOH in DCM) to afford **20** (50.8 mg, 0.053 mmol, 67% yield) as a white solid. ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 8.86 (s, 1 H, 42-CH), 7.93 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.83 (d, *J*=8.3 Hz, 1 H, NH), 7.73 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.56 - 7.63 (m, 1 H, 23-CH), 7.41 - 7.47 (m, 3 H, 20-CH, 39-CH₂), 7.38 - 7.41 (m, 2 H, 38-CH), 7.17 - 7.26 (m, 2 H, 21-CH, 22-CH), 4.47 - 4.56 (m, 3 H, 27-CH, 34-CH, 36-CH), 4.32 - 4.41 (m, 2 H, 32-CH, 36-CH), 4.03 (dd, *J*=10.6, 5.1 Hz, 1

H, 31-CH), 3.66 - 3.75 (m, 1 H, 31-CH), 2.46 (s, 3 H, 44-CH₃), 2.42 - 2.45 (m, 1 H, 33-CH), 2.39 (t, $J=7.5$ Hz, 2 H, 11-CH₂), 2.18 - 2.33 (m, 2 H, 2-CH₂), 1.93 - 2.02 (m, 1 H, 33-CH), 1.70 (quin, $J=7.3$ Hz, 2 H, 3-CH₂), 1.54 - 1.63 (m, 2 H, 10-CH₂), 1.49 (s, 9 H, 26-CH₃), 1.34 - 1.38 (m, 4 H, 4-CH₂,9-CH₂), 1.27 - 1.33 (m, 8 H, (5-8)-CH₂), 1.03 (s, 9 H, 29-CH₃). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_c ppm 176.6 (C1), 175.1 (C12), 175.0 (C35), 172.8 (C30), 167.9 (C17), 156.4 (C24), 153.0 (C42), 149.2 (C43), 143.9 (C13), 140.1 (C37), 133.5 (C41), 133.2 (C19), 131.8 (C18), 131.7 (C40), 130.5 (C38), 130.3 (C16), 129.7 (C15), 129.2 (C39), 127.5 (C21/22), 127.3 (C23), 126.4 (C21/22), 125.7 (C20), 120.4 (C14), 81.9 (C25), 71.6 (C32), 61.1 (C34), 59.4 (C27), 57.8 (C31), 44.0 (C36), 38.2 (C11), 38.0 (C33), 36.6 (C2), 36.1 (C28), 30.7 (alkyl CH₂), 30.6 (alkyl CH₂), 30.55 (alkyl CH₂), 30.5 (alkyl CH₂), 30.4 (2x alkyl CH₂), 28.8 (C26), 27.2 (C29), 27.1 (C10), 26.9 (C3), 16.0 (C44). HRMS (ESI) m/z : [M+H]⁺ calculated for C₅₂H₇₀N₇O₈S: 952.5007, found 952.4999.



N1-(4-((2-aminophenyl)carbamoyl)phenyl)-N12-((S)-1-((2S,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)dodecanediamide, (6): TFA (0.4 mL) was added to a stirring solution of **20** (29.0 mg, 0.030 mmol) in DCM (2 mL) and the resulting reaction mixture stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (33 mg). The crude oil was dissolved in MeOH (2 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 100 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **6** (25.6 mg, 0.029 mmol, 98% yield) as a pale yellow solid. Prior to biological evaluation the product was further purified by semi-preparative HPLC (5-95% MeCN in H₂O, 260 nm, 45 min gradient). ¹H NMR (400 MHz, Methanol-*d*₄) δ_H ppm 8.86 (s, 1 H, 39-CH) 7.95 (d, $J=8.7$ Hz, 2 H, 15-CH) 7.72 (d, $J=8.7$ Hz, 2 H, 14-CH) 7.44 (d, $J=8.5$ Hz, 2 H, 36-CH) 7.40 (d, $J=8.5$ Hz, 2 H, 35-CH) 7.18 (dd, $J=7.8, 1.3$ Hz, 1 H, 23-CH) 7.07 (app. td, $J=7.8, 1.3$ Hz, 1 H, 21-CH) 6.90 (dd, $J=7.8, 1.3$ Hz, 1 H, 20-CH) 6.76 (app. td, $J=7.8, 1.3$ Hz, 1 H, 22-CH), 4.47 - 4.58 (m, 3 H, 24-CH,31-CH,33-CH) 4.32 - 4.40 (m, 2 H, 29-CH,33-CH) 4.03 (dd, $J=10.5, 5.1$ Hz, 1 H, 28-CH) 3.69 (dd, $J=10.5, 3.5$ Hz, 1 H, 28-CH) 2.47 (s, 3 H, 41-CH₃) 2.42 - 2.45 (m, 1 H, 30-CH) 2.40 (t, $J=7.4$ Hz, 2 H, 11-CH₂) 2.18 - 2.32 (m, 2 H, 2-CH₂) 1.97 (dt, $J=13.3, 4.3$ Hz, 1 H, 30-CH) 1.70 (quin, $J=7.3$ Hz, 2 H, 3-CH₂) 1.53 - 1.63 (m, 2 H, 10-CH₂) 1.33 - 1.39 (m, 4 H, 4-CH₂,9-CH₂) 1.29 - 1.33 (m, 8 H, (5-8)-CH₂) 1.03 (s, 9 H, 26-CH₃). ¹³C NMR (101 MHz, Methanol-*d*₄) δ_c ppm 176.5 (C1), 175.1 (C12), 175.0 (C32), 172.8 (C27), 168.4 (C17), 153.0 (C39), 149.2 (C40), 143.9 (C19), 143.7 (C13), 140.2 (C34), 133.5 (C38), 131.7 (C37), 130.6 (C16), 130.5 (C35), 129.9 (C15), 129.2 (C36), 128.6 (C21), 127.8 (C23), 125.6 (C18), 120.4 (C14), 119.8 (C22), 118.9 (C20), 71.6 (C29), 61.1 (C31), 59.4 (C24), 57.8 (C28), 44.0 (C33),

38.2 (C11), 38.0 (C30), 36.6 (C2), 36.1 (C25), 30.7 (alkyl CH₂), 30.6 (alkyl CH₂), 30.55 (alkyl CH₂), 30.5 (alkyl CH₂), 30.4 (2x alkyl CH₂), 27.2 (C26), 27.1 (C10), 26.9 (C3), 16.0 (C41). HRMS (ESI) m/z: [M+H]⁺ calculated for C₄₇H₆₂N₇O₆S: 852.4482, found 852.4483.

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Appendix: ^1H NMR and ^{13}C NMR of Novel Compounds

