Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Experimental Procedures and Characterization Data

Efficient, Multi-Hundred-Gram Scale Access to E3 Ubiquitin Ligase Ligands for Degrader Development

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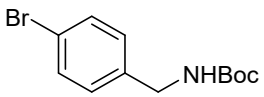
Chemistry General Methods

All reagents and solvents were purchased from commercial sources and used without further purification. Nuclear magnetic resonance spectra were recorded on a Bruker Avance III HD spectrometer operating at 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR and 376 MHz for ^{19}F NMR. ^{1}H NMR and ^{13}C NMR chemical shifts (δ) are reported in parts per million (ppm) and are referenced to residual protium in solvent and to the carbon resonances of the residual solvent peak respectively. DEPT and correlation spectra were run in conjunction to aid assignment. ^{19}F NMR chemical shifts are reported in ppm and are uncorrected. Coupling constants (*J*) are quoted in Hertz (Hz), and the following abbreviations are used to report multiplicity: s= singlet, d= doublet, dd= doublet of doublets, ddd= double doublet of doublets, t= triplet, q= quartet, m= multiplet, br s= broad singlet. Purification by flash column chromatography was carried out using Fisher Scientific silica gel 60Å (35-70 μm), or by using Biotage Selekt, Biotage Isolera, Grace Reveleris or Buchi Pure systems. Analytical thin layer chromatography was performed on glass plates pre-coated with silica gel (Analtech, UNIPLATE™ 250 μm / UV254), with visualization being achieved using UV light (254 nm) and/or by staining with alkaline potassium permanganate dip. Reaction monitoring LC-MS analyses were conducted using Agilent InfinityLab LC/MSD systems. Optical rotations were recorded on a Bellingham & Stanley ADP450 polarimeter. High resolution mass spectral (HRMS) data were collected in the laboratories of the University of Bath Chemistry Department using an Agilent 6545 LC/Q-TOF system. Elemental microanalysis measurements were recorded by MEDAC Ltd. or Elemental Microanalysis Ltd.

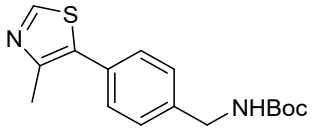
On the largest scales reported, all reactions could be performed either in traditional laboratory glassware (round bottom flasks or on the largest scales, up to 20L flange flasks with Quickfit™ lids, typically with mechanical stirring applied) with conventional means of cooling and heating. Alternatively on the largest scales, jacketed reactor vessels of 10 and 20L capacity were deployed with heating and cooling applied via programmable chiller connected to the vessel jacket.

Synthetic Procedures



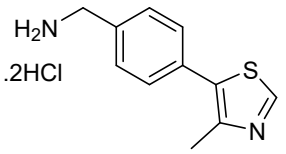
***tert*-Butyl (4-bromobenzyl)carbamate (9)**

To a stirred solution of 4-bromobenzylamine **(8)** (500.00 g, 2.69 mol) in DCM (2.5 L) at ambient temperature was dropwise added a solution of Boc anhydride (586.53 g, 2.69 mol) in DCM (2.5 L) over a period of 3 hours, maintaining the temperature below 25 °C. After stirring for a total of 20 hours, the reaction mixture was concentrated under reduced pressure to a white solid. The residue was triturated with petroleum ether (40:60) (4 L) for 4 hours and left to stand for 72 hours before being filtered, the collected solid being further washed with petroleum ether (40:60) (1.5 L) and then dried under vacuum at 35 °C. This afforded the title compound as a white solid (687.80 g, 86%). ^{1}H NMR ($CDCl_3$) δ: 7.44 (d, *J*= 8.3, 2H), 7.15 (d, *J*= 8.3, 2H), 4.87 (br s, 1H), 4.25 (d *J*= 5.5, 2H), 1.45 (s, 9H). Data consistent with those reported by Young *et al. JACS* (2014), 132, 7899-7906.



***tert*-Butyl (4-(4-methylthiazol-5-yl)benzyl)carbamate (10)**

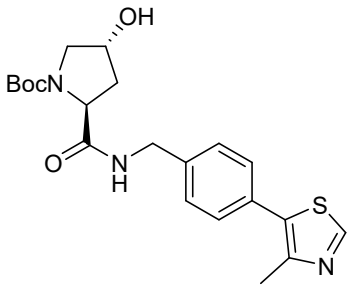
To a stirred solution of *tert*-butyl (4-bromobenzyl)carbamate **(9)** (343.80 g, 1.20 mol) in DMA (1880 mL) at ambient temperature was portion-wise added 4-methylthiazole (238.25 g, 2.40 mol) and potassium acetate (235.82 g, 2.40 mol) with no discernible temperature change. A rapid flow of N_2(g) was bubbled through the stirring reaction mixture for 1 hour before $Pd(OAc)_2$ (2.70 g, 0.01 mol) was added. The reaction mixture was heated to 95 °C and stirring was maintained at this temperature for 18 hours. After cooling to ambient temperature, the reaction mixture was poured into water (3.5 L), and the resulting grey solid was collected by filtration, being further washed with water (1 L). The solid was then triturated with water (2 L) and then filtered, washing with further water (1 L). The damp solid was dissolved in DCM (2 L), and the resulting organic phase was separated, dried over anhydrous magnesium sulfate and concentrated under reduced pressure, affording the title compound as a brown solid (342.90 g, 94%) which was used directly in the next stage without need for further manipulation. ^{1}H NMR ($CDCl_3$) δ: 8.69 (s, 1H), 7.41 (d, *J*= 8.3, 2H), 7.35 (d, *J*= 8.3, 2H), 4.91 (br s, 1H), 4.36 (d, *J*= 5.5, 2H), 2.55 (s, 3H), 1.47 (s, 9H). m/z (ES+): 327.00 $[M+Na^+]^+$. Data consistent with those reported by Steinebach *et al. Synthesis* (2020), 52(17), 2521-2527.



4-(4-Methylthiazol-5-yl)benzylamine dihydrochloride (11)

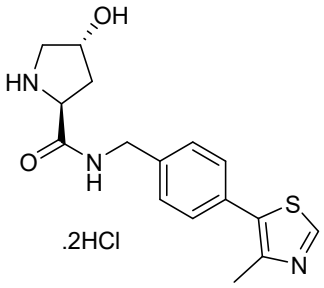
To a stirred solution of *tert*-butyl (4-(4-methylthiazol-5-yl)benzyl)carbamate **(10)** (342.80 g, 1.13 mol) in DCM (4.43 L) at ambient temperature was dropwise added hydrogen chloride (1126.00 mL, 4.50 mol, 4M solution in 1,4-dioxane) over a period of 3 hours in order to keep the temperature below 25 °C. The reaction mixture was stirred for a further 20 hours at ambient temperature, during which time a suspension formed. Diethyl ether (1.25 L) was added to the stirring mixture, and the solid was collected by filtration under nitrogen, washed with DCM (2.5 L) and diethyl ether (1.25 L) and dried under vacuum at 35 °C. This afforded the title compound as a very pale green solid (308.24 g, 99%, 94% w/w accounting for residual solvent). ^{1}H NMR (DMSO-d6) δ: 9.11 (s, 1H), 8.59 (br s, 3H), 7.62 (d, *J*= 8.3, 2H), 7.55 (d, *J*= 8.3, 2H), 6.11 (br s, 1H), 4.09-4.02 (m, 2H), 2.47 (s, 3H). LCMS: >99% purity, m/z (ES+): 205.20 $[M_{(free\ base)}+H^{+}]^{+}$.

Data consistent with those reported by Yan *et al. ACS Omega* (2022), 7, 26015-26021.



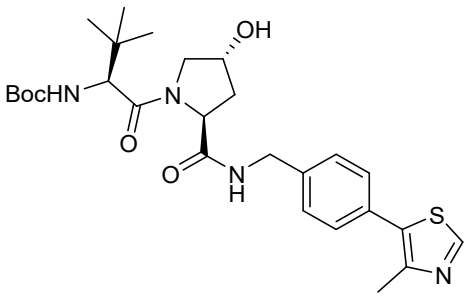
<u>*tert*-Butyl (2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate (12)</u>

To a stirred solution of (2*S*,4*R*)-1-*tert*-butoxycarbonyl-4-hydroxy-pyrrolidine-2-carboxylic acid (Boc-Hyp-OH) (205.64 g, 889.28 mmol, $[\alpha]_D^{23}$= -66.2°, c=1, MeOH) in DMF (2400 mL) at ambient temperature was portion-wise added 4-(4-methylthiazol-5-yl)benzylamine dihydrochloride **(11)** (261.70 g, 889.28 mmol, 94% w/w) followed by the dropwise addition of DIPEA (579.24 mL, 3.38 mol) over 30 minutes. The resulting grey suspension was cooled to 5 °C, after which time HATU (338.13 g, 889.28 mmol) was added in portions over a 40 minute period, maintaining the temperature below 11 °C. The reaction mixture was stirred at ambient temperature overnight and then quenched by addition to water (14 L). For ease of handling, the mixture was split in half. One portion was extracted with ethyl acetate (4 x 1.5 L), with the combined organic extracts being washed with water (4 x 2 L) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The process was duplicated with the remainder. In total, this gave rise to the title compound as a viscous amber-coloured gum (301.53 g, 68%) which was used directly in the next stage without requirement for further manipulation or purification. m/z (ES+): 440.30 $[M+Na^+]^+$. Data consistent with those reported by Cao *et al. JMC* (2020), 63 (19), 11012-11033.



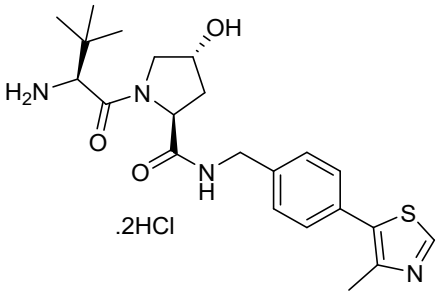
(2*S*,4*R*)-4-Hydroxy-*N*-((4-(4-methylthiazol-5-yl)benzyl) pyrrolidine-2-carboxamide dihydrochloride (13)

To a stirred solution of *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate **(12)** (301.50 g, 722.12 mmol) in ethanol (1580 mL) at ambient temperature was dropwise added hydrogen chloride (720 mL, 4M solution in 1,4-dioxane) at such a rate so as to keep the temperature below 25°C. Upon completion of the addition, the reaction mixture was stirred at ambient temperature overnight, during which time a thick yellow precipitate formed. Diethyl ether (2 L) was added, and the solid was collected by filtration, being further washed with diethyl ether (1L) and then dried under vacuum. This afforded the title compound as a pale yellow solid (245.84 g, 83%). ^{1}H NMR (CD_3OD) δ: 9.92 (s, 1H), 9.02 (t, *J*= 5.8, 1H), 7.60-7.56 (m, 2H), 7.53 (d, *J*= 8.4, 2H), 4.61 (t, *J*= 3.5, 1H), 4.56-4.48 (m, 3H), 3.47-3.40 (m, 1H), 3.34 (br s, 1H), 2.61 (s, 3H), 2.54-2.47 (m, 1H), 2.13-2.05 (m, 1H). m/z (ES+): 340.20 $[M_{(free\ base)}+Na^+]^+$. Data consistent with those reported by Yan *et al. ACS Omega* (2022), 7, 26015-26021.



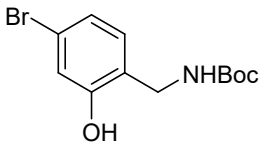
<u>*tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (14)</u>

To a stirred solution of (2*S*,4*R*)-4-hydroxy-*N*-((4-(4-methylthiazol-5-yl)benzyl) pyrrolidine-2-carboxamide dihydrochloride **(13)** (245.80 g, 629.73 mmol) in DMF (2460 mL) under an atmosphere of nitrogen was added (2*S*)-2-(tert-butoxycarbonylamino)-3,3-dimethyl-butanoic acid (Boc-Tle-OH) (138.37 g, 598.24 mmol, $[\alpha]_D^{23}$= -3.2°, c=1, MeOH) portion-wise. The resulting solution was cooled to +6°C before DIPEA (410 mL, 2.39 mol) was added dropwise over 10 minutes with negligible temperature change. HATU (251.41 g, 661.21 mmol) was added portion-wise over 15 minutes maintaining the temperature below 10°C. After 4 hours, the reaction was complete, and the reaction mixture was treated slowly with water (2 L) maintaining the temperature below 30°C. The resulting mixture was extracted with ethyl acetate (3 x 2 L), and the combined organic extracts were washed with water (4 x 1.5 L), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Trituration with diethyl ether followed by drying under vacuum afforded the title compound as a light beige solid (245.06 g, 67%) which was used directly in the next stage without requirement for further manipulation or purification. m/z (ES+): 553.20 $[M+Na^+]^+$.



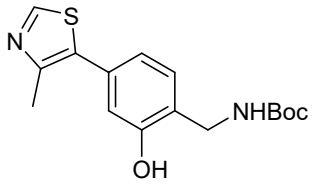
(2*S*,4*R*)-1-((*S*)-2-Amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide dihydrochloride; VH 032, amine (1)

To a stirred solution of *tert*-butyl ((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate **(14)** (245.00 g, 461.67 mmol) in ethanol (1284 mL) at ambient temperature under an atmosphere of nitrogen was dropwise added hydrogen chloride (577 mL, 4M solution in 1,4-dioxane) over 1 hour 45 minutes (during which time the temperature peaked at 26°C), and the resulting amber solution was stirred for 20 hours. Diethyl ether (2 L) was added slowly with stirring for a further 30 minutes. The resulting solid was collected by filtration under nitrogen atmosphere and further washed with diethyl ether before being dried under vacuum, giving a pale yellow solid (211.50 g) containing residual 1,4-dioxane and ethanol. The material was lyophilized from water (3.5 volumes) to afford the title compound as a pale yellow solid (200.65 g, 83%, containing 0.5% w/w residual 1,4-dioxane). ^{1}H NMR (CD_3OD) δ: 9.99 (s, 1H), 7.59-7.50 (m, 4H), 4.71-4.65 (m, 1H), 4.59-4.52 (m, 2H), 4.44-4.38 (m, 1H), 4.07 (s, 1H), 3.88-3.82 (m, 1H), 3.74-3.69 (m, 1H), 2.60 (s, 3H), 2.35-2.27 (m, 1H), 2.12-2.04 (m, 1H), 1.14 (s, 9H). ^{13}C NMR (CD_3OD) δ: 174.1, 168.6, 156.3, 143.0, 142.3, 137.2, 130.5, 129.4, 128.3, 71.2, 61.0, 60.4, 58.1, 43.6, 39.1, 35.8, 26.7, 13.3. LCMS: >98% purity, m/z (ES+): 431.10 $[M_{(free\ base)}+H^+]^+$; Elemental Analysis calculated for $C_{22}H_{30}N_4O_3S.2HCl.1.25H_2O$: C 50.24; H 6.61; N 10.65; found: C 49.92; H 6.82; N 10.37. HRMS (ES+) calculated for $[(C_{22}H_{30}N_4O_3S)+H^+]^+$: 431.2121, found: 431.2120.



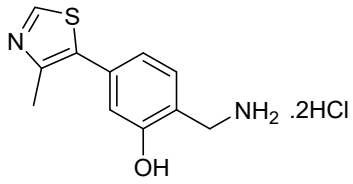
<u>*tert*-Butyl (4-bromo-2-hydroxybenzyl)carbamate (19)</u>

A vessel was charged with 4-bromo-2-hydroxybenzaldehyde **(18)** (200.00 g, 0.99 mol) and *tert*-butyl carbamate (350.00 g, 2.98 mol) and was purged with nitrogen (g) for 1 hour before dissolution with DCM/acetonitrile (1:3, 4 L), the solvent being added over 30 minutes and the resulting stirring pale brown solution having an internal temperature of +14°C. Triethylsilane (480.00 mL, 3.01 mol) was added dropwise *via* dropping funnel over a period of 24 minutes, with no discernible temperature change. The funnel was line-rinsed with acetonitrile before the dropwise addition of TFA (150 mL, 2.02 mol) over a period of 26 minutes, at such a rate that kept the temperature below +25°C. The resulting yellow solution was stirred at ambient temperature overnight. A saturated aqueous solution of sodium hydrogen carbonate (2 L) was added cautiously over 20 minutes, with a small amount of effervescence observed. The organic phase was separated and concentrated under reduced pressure to a yellow solid. The aqueous component was extracted with ethyl acetate (2 x 1L). The obtained yellow solid and the ethyl acetate extracts were combined to solution and washed sequentially with brine (1 L) and 10% citric acid (aq.) (2 x 1L) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Trituration with petroleum ether (40:60) (3 L) afforded a fluffy white solid, containing product and residual *tert*-butyl carbamate. Trituration at ambient temperature for 3 hours with methanol/water (1:3; 3L), followed by a second 4 hour treatment with methanol/water (1:2; 3L), reduced the impurity level to <1mol%. The resulting solid was dissolved in DCM (2 L) and the aqueous phase removed. The organic phase was dried over anhydrous magnesium sulfate and concentrated under reduced pressure, affording the title compound as a white solid (244 g, 81%). ^{1}H NMR ($CDCl_3$) δ: 9.26 (br s, 1H), 7.11 (d, *J*= 1.8, 1H), 6.94 (dd, *J*= 8.0, 1.9, 1H), 6.90 (d, *J*= 8.0, 1H), 5.24 (br s, 1H), 4.17 (d, *J*= 6.8, 2H), 1.44 (s, 9H). m/z (ES-): 302.00 $[M\text{-}H^+]^-$. Data consistent with those reported by Zhou *et al. Eur JMC* (2022), 244, 114830.



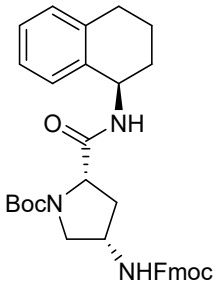
<u>*tert*-Butyl (2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamate (20)</u>

A vessel was charged with *tert*-butyl (4-bromo-2-hydroxybenzyl)carbamate **(19)** (80.00 g, 264.76 mmol), potassium acetate (52.35 g, 533.37 mmol) and Pd(OAc)2 (3.00 g, 13.36 mmol) and the vessel was three-times degassed and back-filled with N_2(g). DMA (250 mL) and 4-methylthiazole (50.00 mL, 549.65 mmol) were sequentially added, followed again by a three-time de-gas and backfill cycle with N_2(g). The reaction mixture was heated to 130°C and stirred for 6 hours before being allowed to cool to ambient temperature with stirring overnight. The reaction mixture was concentrated under reduced pressure, and the residue was taken up in ethyl acetate (200 mL) and filtered through a silica gel plug, washing the pad with further ethyl acetate (4 x 200 mL). After concentration under reduced pressure, the residue was purified by flash column chromatography, eluting with 40%-55% EtOAc/petroleum ether (40:60) to give a yellow oil. Trituration with diethyl ether/petroleum ether (40:60) (1:2; 300 mL) afforded the title compound as a pale-yellow solid (40.70 g, 48%). ^{1}H NMR ($CDCl_3$) δ: 9.21 (br s, 1H), 8.66 (s, 1H), 7.10 (d, *J*= 7.7, 1H), 7.04 (d, *J*= 1.8, 1H), 6.90 (dd, *J*= 7.7, 1.8, 1H), 5.34-5.27 (m, 1H), 4.26 (d, *J*= 6.7, 2H), 2.54 (s, 3H), 1.46 (s, 9H). m/z (ES+): 321.10 $[M+H^+]^+$. Data consistent with those reported by Zhou *et al. Eur JMC* (2022), 244, 114830.



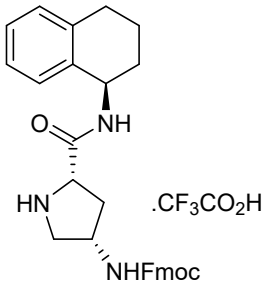
2-(Aminomethyl)-5-(4-methylthiazol-5-yl)phenol dihydrochloride (17)

To a stirred solution of *tert*-butyl (2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamate **(20)** (40.70 g, 127.03 mmol) in 1,4-dioxane (200 mL) and DCM (800 mL) at ambient temperature was dropwise added hydrogen chloride (350.00 mL, 1.40 mol, 4M solution in 1,4-dioxane), and the resulting reaction mixture was stirred at ambient temperature for 3 hours, during which time a precipitate formed. After filtration and washing with further DCM, the solid was suspended in acetonitrile and concentrated under reduced pressure, affording the title compound as a pale yellow solid (37.40 g, quantitative, containing 1%w/w residual solvents), which was used directly in the next stage of onward chemistry without any further manipulation. ^{1}H NMR (DMSO-d6) δ: 10.59 (br s, 2H), 9.09 (s, 1H), 8.31 (br s, 3H), 7.43 (d, *J*= 7.8, 1H), 7.15 (d, *J*= 1.7, 1H), 6.97 (dd, *J*= 7.8, 1.7, 1H), 3.99-3.93 (m, 2H), 2.49 (s, 3H). m/z (ES+): 221.10 $[M_{(free\ base)}+H^{+}]^{+}$.



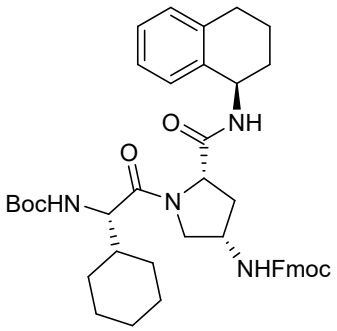
tert-Butyl (2*S*,4*S*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidine-1-carboxylate (22)

To a stirred solution of (2*S*,4*S*)-1-tert-butoxycarbonyl-4-(9*H*-fluoren-9-ylmethoxycarbonylamino)pyrrolidine-2-carboxylic acid **(21)** (104.74 g, 231.47 mmol, $[\alpha]_D^{23}$= -23.6°, c=0.87, MeOH) and (*R*)-(-)-1-aminotetralin (35.48 g, 254.62 mmol, $[\alpha]_D^{23}$= -27.6°, c=1.32, MeOH) in DCM (950 mL) was added DIPEA (200 mL) in a portion-wise fashion before the reaction mixture was cooled to +5°C. HATU (101.21 g, 266.19 mmol) was added in a portion-wise fashion with no appreciable change to the temperature, and the reaction mixture was allowed to warm to ambient temperature and stirred overnight. The resulting orange solution was washed with water (2 x 800 mL) and brine (2 x 800 mL) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was recrystallised from ethanol, collected by filtration and dried under vacuum at 40°C to afford the title compound as a white solid (117.32 g, 87%). ^{1}H NMR ($CDCl_3$) δ: 7.78-7.69 (m, 3H), 7.66-7.62 (m, 2H), 7.42-7.35 (m, 2H), 7.33-7.26 (m, 2H), 7.24-7.04 (m, 5H), 5.23-5.15 (m, 1H), 4.51-4.32 (m, 3H), 4.30-4.23 (m, 2H), 3.63-3.49 (m, 2H), 2.87-2.73 (m, 2H), 2.54-2.45 (m, 1H), 2.28-2.17 (m, 1H), 2.16-2.02 (m, 1H), 1.95-1.77 (m, 3H), 1.40 (s, 9H). m/z (ES+): 482.20 $[(M\text{-}Boc)+H^+]^+$. Data consistent with those reported by Hoffmann-La Roche WO 2014/090709.



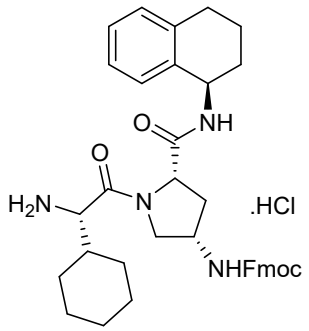
(9*H*-Fluoren-9-yl)methyl ((3*S*,5*S*)-5-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-3-yl)carbamate trifluoroacetate (23)

To a stirred solution of *tert*-butyl (2*S*,4*S*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidine-1-carboxylate **(22)** (117.30 g, 201.65 mmol) in DCM (700 mL) at +4°C was dropwise added TFA (500 mL), and the resulting yellow solution was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was concentrated under reduced pressure, and then reconcentrated from from DCM/heptane (1:1) (6 x 500 mL) to afford the title compound as a beige solid (119.00 g, 99%), which was used directly in the next stage without further manipulation. ^{1}H NMR (DMSO-d6) δ: 9.35 (br s, 1H), 8.84 (d, *J*= 8.3, 1H), 8.76 (br s, 1H), 7.90 (d, *J*= 7.5, 2H), 7.68 (d, *J*= 7.5, 2H), 7.54 (d, *J*= 6.3, 1H), 7.42 (t, *J*= 7.4, 2H), 7.34 (t, *J*= 7.4, 2H), 7.23-7.11 (m, 4H), 4.99 (dd, *J*= 13.5, 5.9, 1H), 4.38 (d, *J*= 6.5, 2H), 4.26-4.12 (m, 3H), 3.48-3.38 (m, 1H), 3.15-3.05 (m, 1H), 2.82-2.65 (m, 2H), 2.62-2.53 (m, 1H), 1.92-1.65 (m, 5H). m/z (ES+): 504.20 [$M_{(free\ base)}$+Na^+]$^+$. Data consistent with those reported by Hoffmann-La Roche WO 2014/090709.



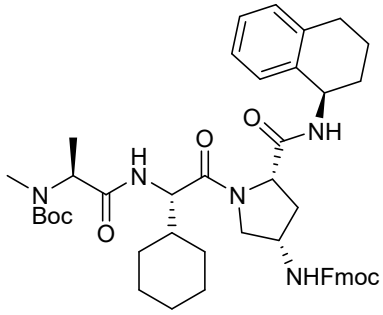
<u>*tert*-Butyl ((*S*)-2-((2*S*,4*S*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)carbamate (24)</u>

To a stirred solution of (9*H*-fluoren-9-yl)methyl ((3*S*,5*S*)-5-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-3-yl)carbamate trifluoroacetate **(23)** (119.00 g, 199.80 mmol) and Boc-Chg-OH (51.47 g, 200.02 mmol, $[\alpha]_D^{24}$= +9.4°, c=1, MeOH) in DMF (350 mL) at ambient temperature was slowly added DIPEA (205 mL), and the reaction mixture was cooled to +4°C. HATU (83.70 g, 220.00 mmol) was added in a portion-wise fashion with no discernible change to the temperature and the reaction mixture was allowed to warm to ambient temperature and stirred overnight. The resulting dark solution was partitioned between water (2 L) and ethyl acetate (750 mL), and the organic phase was separated. The aqueous component was extracted with ethyl acetate (2 x 500 mL), and the combined organic extracts were sequentially washed with water (500 mL), saturated aqueous sodium hydrogen carbonate solution (2 x 500 mL) and brine (2 x 500 mL) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was repeatedly reconcentrated from ethyl acetate/heptane (1:1, 4 x 500 mL) to afford the title compound as a brown foam (142 g, 99% crude yield) which was used directly in the subsequent stage without any further manipulation. m/z (ES+): 621.40 $[(M\text{-Boc})+H^+]^+$. Data consistent with those reported by Hoffmann-La Roche WO 2014/090709.



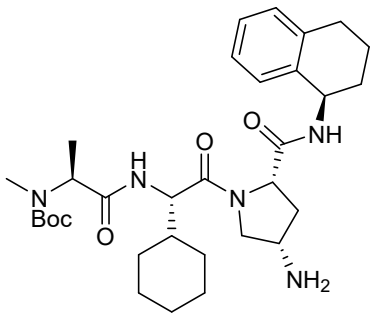
(9*H*-Fluoren-9-yl)methyl ((3*S*,5*S*)-1-((*S*)-2-amino-2-cyclohexylacetyl)-5-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-3-yl)carbamate hydrochloride (25)

To a stirred solution of *tert*-butyl ((*S*)-2-((2*S*,4*S*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)carbamate **(24)** (142 g) in 1,4-dioxane (800 mL) at ambient temperature was dropwise added hydrogen chloride (463 mL, 4M solution in 1,4-dioxane), and the resulting solution was heated to +30°C with stirring maintained at this temperature overnight. After cooling to ambient temperature, the solution was concentrated under reduced pressure and three times reconcentrated from methanol/diethyl ether (1:9). The residue was triturated with diethyl ether (1 L) overnight, collected by filtration, being washed with further diethyl ether (2 x 750 mL) and held under vacuum on the filter cake for 1 hour. Drying for 72 hours at 40°C under vacuum afforded the title compound as an off-white solid (149.46 g, 99%). ^{1}H NMR (DMSO-d6) δ: 8.48 (d, *J*= 8.7, 1H), 8.30-8.10 (m, 3H), 7.90 (d, *J*= 7.5, 2H), 7.72-7.64 (m, 3H), 7.42 (t, *J*= 7.4, 2H), 7.36-7.27 (m, 3H), 7.20-7.07 (m, 3H), 4.98-4.91 (m, 1H), 4.39-4.28 (m, 3H), 4.26-4.20 (m, 1H), 4.14-4.00 (m, 3H), 3.75-3.65 (m, 1H), 3.30-3.20 (m, 1H), 2.81-2.66 (m, 2H). 2.46-2.39 (m, 1H), 1.92-1.58 (m, 12H), 1.22-1.12 (m, 4H). m/z (ES+): 621.30 $[M_{(free\ base)}+H^+]^+$. Data consistent with those reported by Hoffmann-La Roche WO 2014/090709.



<u>*tert*-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (26)</u>

To a stirred solution of (9*H*-fluoren-9-yl)methyl ((3*S*,5*S*)-1-((*S*)-2-amino-2-cyclohexylacetyl)-5-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-3-yl)carbamate hydrochloride **(25)** (118.30 g, 180.00 mmol) in DMF (650 mL) at ambient temperature was slowly added DIPEA (200 mL) followed by the portion-wise addition of Boc-*N*-Me-Ala-OH (37.31 g, 183.60 mmol, $[\alpha]_D^{21}$= -32.2°, c=1, EtOH). The reaction mixture was cooled to +5°C and HATU (70.50 g, 185.40 mmol) was added in a portion-wise fashion with no discernible change to the temperature, and the reaction mixture was allowed to warm to ambient temperature and stirred for 2 hours. The resulting solution was partitioned between water (2 L) and ethyl acetate (700 mL), and the organic phase was separated. The aqueous component was extracted with ethyl acetate (2 x 500 mL), and the combined organic extracts were sequentially washed with water (1 L), saturated aqueous sodium hydrogen carbonate solution (1 L) and brine (1 L) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was twice reconcentrated from ethyl acetate/heptane (1:1) to give a beige foam. Purification by dry flash chromatography, eluting with 1% 7M methanolic ammonia solution /DCM, afforded the title compound as an off-white solid (141.96 g, 88% crude yield (90%w/w accounting for residual solvents and tetramethyl urea content)) which was used directly in the subsequent stage without any further manipulation. m/z (ES+): 621.30 $[M_{(free\ base)} + H^+]^+$. Data consistent with those reported by Hoffmann-La Roche WO 2014/090709.

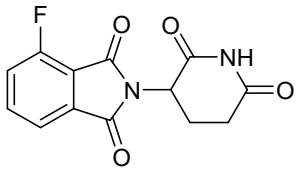


<u>*tert*-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-amino-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate; A 410099.1, amine (3)</u>

To a stirred solution of *tert*-butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate **(26)** (74.02 g, 82.65 mmol, 90%w/w) in DCM (600 mL) at ambient temperature was slowly added piperidine (81.74 mL, 826.53 mmol), and the solution was stirred for 1 hour before being concentrated under reduced pressure and being held under vacuum on the rotary evaporator at 55°C for a further 2 hours after distillation had ceased. Purification by flash column chromatography, eluting with 2.5%-5% 7M methanolic ammonia solution /DCM, afforded the title compound as an off-white solid (45.46 g, 94%). ^{1}H NMR (DMSO-d6) δ: 8.28 (d, *J*= 8.6, 1H), 7.85-7.57 (m, 1H), 7.31 (d, *J*= 7.3, 1H), 7.17-7.05 (m, 3H), 4.95-4.87 (m, 1H), 4.63-4.45 (m, 1H), 4.34 (t, *J*= 7.9, 1H), 4.22 (t, *J*= 7.9, 1H), 3.92-3.86 (m, 1H), 3.36-3.19 (m, 2H), 2.78-2.68 (m, 5H), 2.30-2.21 (m, 1H), 1.90-1.80 (m, 5H), 1.79-1.52 (m, 9H), 1.39 (s, 9H), 1.24-0.90 (m, 7H). LCMS: >98% purity, m/z (ES+): 584.4 $[M+H^+]^+$. Elemental Analysis calculated for $C_{32}H_{49}N_5O_5.H_2O$: C 63.87; H 8.54; N 11.64; found: C 63.74; H 8.44; N 11.71. HRMS (ES+) calculated for $[(C_{32}H_{49}N_5O_5)+H^+]^+$: 584.3820, found: 584.3819. Data consistent with those reported by Hoffmann-La Roche WO 2014/090709.

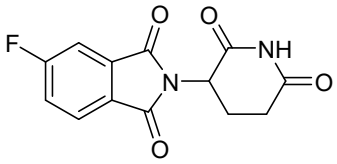
tert-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-amino-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate hydrochloride; A 410099.1, amine hydrochloride

To a stirred solution of A 410099.1, amine **(3)** (5.00 g, 8.57 mmol) in diethyl ether (50 mL) at ambient temperature was added hydrogen chloride (4.28 mL, 8.57 mmol, 2M solution in diethyl ether), and almost immediately a thick white precipitate began to form. Upon completion of the addition, the reaction mixture was concentrated under reduced pressure to an off-white solid. The solid was dissolved in water (100 mL) (pH =5) and freeze dried to afford the title compound as a white solid (5.12 g, 96%). LCMS: 100% purity, m/z (ES+): 606.50 $[M_{(free\ base)}+Na^+]^+$.



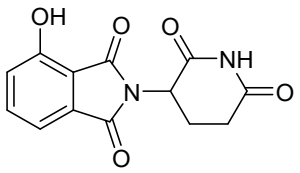
4'-Fluorothalidomide; 2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (4)

To a stirred suspension of 3-fluorophthalic anhydride (198.08 g, 1192.49 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (196.27 g, 1192.48 mmol) in acetic acid (2.4 L) was added sodium acetate (117.39 g, 1431.06 mmol) and the resulting mixture was heated to reflux and stirred for 4.5 hours. After this time, the purple reaction mixture was allowed to cool to ambient temperature with stirring overnight before being poured into water (13 L). The resulting suspended solid was collected by filtration and washed with further water until the washings were running clear in colour (8 L). The product was air-dried under vacuum on the filter for an hour before drying under vacuum at 60°C in the presence of solid desiccants KOH and P_2O_5. This afforded the title compound as a pale grey solid (292.66 g, 89%). ^{1}H NMR (DMSO-d6) δ: 11.14 (s, 1H), 7.98-7.92 (m, 1H), 7.79 (d, *J*= 7.3, 1H), 7.74 (t, *J*= 8.9, 1H), 5.16 (dd, *J*= 12.9, 5.4, 1H), 2.94-2.84 (m, 1H), 2.64-2.56 (m, 1H), 2.54-2.46 (m, 1H), 2.10-2.02 (m, 1H). ^{19}F NMR (DMSO-d6) δ: -114.67. ^{13}C NMR (DMSO-d6) δ:172.72, 169.67, 166.09 (d, *J*= 2.9), 163.96, 156.81 (d, *J*= 262.3), 138.05 (d, *J*= 8.0), 133.45, 123.00 (d, *J*= 19.6), 120.04 (d, *J*= 3.3), 117.03 (d, *J*= 12.6), 49.08, 30.89, 21.83. LCMS: >98% purity, m/z (ES-): 275.10 [M-H^{+}]$^{-}$. Elemental Analysis calculated for $C_{13}H_9FN_2O_4$: C 56.53; H 3.28; N 10.14; found: C 56.52; H 3.26; N 10.14. HRMS (ES-) calculated for [($C_{13}H_9FN_2O_4$)-H^{+}]$^{-}$: 275.0478, found: 275.0478.



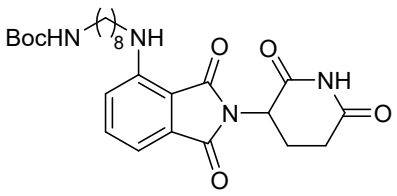
5'-Fluorothalidomide; 2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (5)

To a stirred suspension of 4-fluorophthalic anhydride (201.00 g, 1210.07 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (199.17 g, 12.10.07 mmol) in acetic acid (2.5 L) was added sodium acetate (119.12 g, 1452.08 mmol) and the resulting mixture was heated to reflux and stirred for 1 hour. After this time, the purple reaction mixture was allowed to cool to ambient temperature with stirring overnight before being poured into water (13 L). The resulting suspended solid was collected by filtration, washed with water (2 L) and air-dried under vacuum on the filter for 30 minutes. The resulting solid was suspended in water (3 L) and stirred for 30 minutes before being collected by filtration and further washed with water (2 L). The product was air-dried under vacuum on the filter for an hour before drying under vacuum at 50°C in the presence of KOH(s), replacing desiccant as required and drying to constant weight. This afforded the title compound as a pale grey solid (307.00 g, 92%). ^{1}H NMR (DMSO-d6) δ: 11.14 (s, 1H), 8.01 (dd, *J*= 8.3, 4.5, 1H), 7.85 (dd, *J*= 7.5, 2.3, 1H), 7.72 (ddd, *J*= 9.4, 8.3, 2.3, 1H), 5.16 (dd, *J*= 12.8, 5.4, 1H), 2.95-2.84 (m, 1H), 2.65-2.47 (m, 2H), 2.11-2.02 (m, 1H). ^{19}F NMR (DMSO-d6) δ: -102.35. ^{13}C NMR (DMSO-d6) δ: 172.73,169.74, 166.18, 165.99 (d, *J*= 252.5), 165.89 (d, *J*= 2.8), 134.20 (d, *J*= 9.9), 127.43 (d, *J*= 2.6), 126.27 (d, *J*= 9.9), 121.75 (d, *J*= 23.8), 111.43 (d, *J*= 25.3), 49.17, 30.91, 21.93. LCMS: 100% purity, m/z (ES+): 299.00 $[M+Na^{+}]^{+}$. Elemental Analysis calculated for $C_{13}H_9FN_2O_4$: C 56.53; H 3.28; N 10.14; found: C 56.42; H 3.25; N 10.11. HRMS (ES-) calculated for $[(C_{13}H_9FN_2O_4)-H^{+}]^{-}$: 275.0479, found: 275.0478.



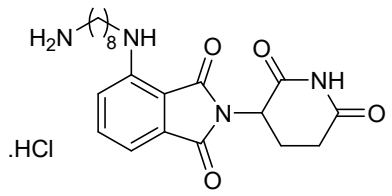
4'-Hydroxythalidomide; 2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (6)

To a stirred suspension of 3-hydroxyphthalic anhydride (108.10 g, 658.81 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (108.40 g, 658.81 mmol) in acetic acid (1.3 L) was added sodium acetate (120.22 g, 1465.62 mmol) and the resulting mixture was heated to reflux and stirred for 4 hours. After this time, the reaction mixture was allowed to cool to ambient temperature with stirring overnight before being concentrated under reduced pressure, and three-times re-concentrated from heptane (1L). The resulting grey solid was slurried in water (1.5 L) for 2 hours before being collected by filtration, the filter cake being washed with water (1L) and air-dried under vacuum for 20 minutes. The solid was triturated with methanol (500 mL), the suspension cooled in an ice bath, and then collected by filtration washing with methanol (250 mL) and diethyl ether (500 mL). Drying under vacuum at 50°C afforded the title compound as a pale beige solid (161.30 g, 89%). ^{1}H NMR (DMSO-d6) δ: 11.22 (s, 1H), 11.09 (s, 1H), 7.64 (dt, *J*= 11.7, 5.9, 1H), 7.33-7.30 (m, 1H), 7.27 (dd, *J*= 8.4, 0.5, 1H), 5.07 (dd, *J*= 12.9, 5.4, 1H), 2.94-2.83 (m, 1H), 2.6-2.46 (m, 2H), 2.07-1.98 (m, 1H). ^{13}C NMR (DMSO-d6) δ: 172.8, 170.0, 167.0, 165.8, 155.5, 136.4, 133.2, 123.6, 114.4, 114.3, 48.6, 31.0, 22.0. LCMS: 100% purity, m/z (ES-): 273.10 $[M-H^{+}]^{-}$. Elemental Analysis calculated for $C_{13}H_{10}N_2O_5.0.25H_2O$: C 56.02; H 3.80; N 10.05; found: C 56.19; H 3.64; N 10.14. HRMS (ES-) calculated for $[(C_{13}H_{10}N_2O_5)-H^{+}]^{-}$: 273.0521, found: 273.0520.



<u>*tert*-Butyl (8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)carbamate (27)</u>

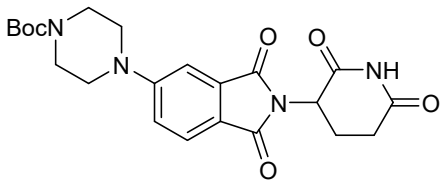
To a stirred solution of 4'-fluorothalidomide **(4)** (15.00 g, 54.30 mmol) in NMP (350 mL) at ambient temperature was added *tert*-butyl *N*-(8-aminooctyl)carbamate (14.60 g, 59.74 mmol) followed by DIPEA (19.20 mL, 110.23 mmol), and the resulting dark solution was heated to 85°C and stirred at this temperature overnight. After cooling to ambient temperature, the reaction mixture was partitioned between water (500 mL) and ethyl acetate (500 mL) and the organic phase was separated. The aqueous component was extracted with ethyl acetate (500 mL), and the combined organic extracts were sequentially washed with water (2 x 500mL) and brine (2 x 500 mL) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Purification by flash column chromatography, eluting with 50% ethyl acetate/petroleum ether (40:60), afforded the title compound as a yellow oil (13.78 g, 50%). ^{1}H NMR ($CDCl_3$) δ: 8.53 (br s, 1H), 7.46 (dd, *J*= 8.5, 7.2, 1H), 7.06 (d, *J*= 7.2, 1H), 6.85 (d, *J*= 8.5, 1H), 6.22 (t, *J*= 5.5, 1H), 4.91 (dd, *J*= 11.8, 5.4, 1H), 4.57 (br s, 1H), 3.27-3.20 (m, 2H), 3.12-3.03 (m, 2H), 2.89-2.67 (m, 3H), 2.14-2.07 (m, 1H), 1.68-1.57 (m, 2H), 1.49-1.26 (m, 19H). m/z (ES+): 401.30 $[(M\text{-}Boc)+H^+]^+$; 523.30 $[M+Na^+]^+$. Data consistent with those reported by Ishoey *et al. ACS Chemical Biology* (2018), 13 (3), 553-560.



4-((8-Aminooctyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione hydrochloride (28)

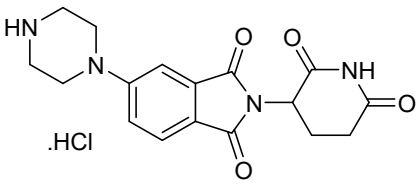
To a stirred solution of *tert*-Butyl (8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)carbamate **(27)** (13.78 g, 27.53 mmol) in 1,4-dioxane (150 mL) at ambient temperature was dropwise added hydrogen chloride (68.82 mL, 275.27 mmol, 4M solution in 1,4-dioxane), and the resulting solution was heated to +35°C with stirring maintained at this temperature overnight. After cooling to ambient temperature, the reaction mixture was cooled to ambient temperature and concentrated under reduced pressure, followed by three times reconcentration from methanol. The residue was triturated with diethyl ether/methanol (15:1) for 2 hours, and the resulting yellow solid was collected by filtration, dissolved in MeCN/H_2O (1:1) and freeze dried to give the title compound as a pale-yellow solid (11.12 g, 92%).

1H NMR (DMSO-d6) δ: 11.09 (br s, 1H), 7.96 (br s, 2H), 7.58 (dd, *J*= 8.5, 7.1, 1H), 7.09 (d, *J*= 8.5, 1H), 7.02 (d, *J*= 7.1, 1H), 6.52 (t, *J*= 5.9, 1H), 5.05 (dd, *J*= 12.8, 5.4, 1H), 3.34-3.25(m, 2H), 2.94-2.83 (m, 1H), 2.77-2.73 (m, 2H), 2.63-2.45 (m, 2H), 2.07-1.98 (m, 1H), 1.62-1.48 (m, 4H), 1.38-1.26 (m, 8H). ^{13}C NMR (DMSO-d6) δ: 172.8, 170.1, 169.0, 167.3, 146.4, 136.3, 132.2, 117.2, 110.4, 109.0, 48.5, 41.8, 38.7, 31.0, 28.6, 28.5, 28.4, 26.9, 26.2, 25.8, 22.2. LCMS: >98% purity, m/z (ES+): 401.30 $[M_{(free\ base)}+H^+]^+$. Elemental Analysis calculated for $C_{21}H_{28}N_4O_4$.HCl.0.75 H_2O: C 56.00; H 6.82; N 12.44; found: C 55.87; H 6.72; N 12.35. HRMS (ES+) calculated for $[(C_{21}H_{28}N_4O_4)+H^+]^+$: 401.2194, found: 401.2194.



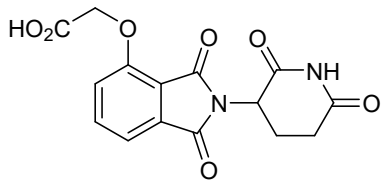
<u>*tert*-Butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperazine-1-carboxylate (29)</u>

To a stirred solution of 5'-fluorothalidomide **(5)** (15.70 g, 56.84 mmol) in NMP (225 mL) at ambient temperature was added *N*-Boc piperazine (11.64 g, 62.52 mmol) followed by DIPEA (29.70 mL, 170.54 mmol), and the resulting dark solution was heated to 90°C and stirred at this temperature overnight. After cooling to ambient temperature, the reaction mixture was partitioned between water (500 mL) and ethyl acetate (500 mL) and the organic phase was separated. The aqueous component was extracted with ethyl acetate (500 mL), and the combined organic extracts were sequentially washed with water (500mL) and brine (2 x 500 mL) before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Purification by flash column chromatography, eluting with 20-30% ethyl acetate/DCM, afforded the title compound as a yellow solid (14.04 g, 56%). ^{1}H NMR ($CDCl_3$) δ: 8.16 (br s, 1H), 7.70 (d, *J*= 8.5, 1H), 7.27 (d, *J*= 2.3, 1H), 7.05 (dd, *J*= 8.5, 2.3, 1H), 4.94 (dd, *J*= 12.4, 5.3, 1H), 3.62-3.58 (m, 4H), 3.43-3.39 (m, 4H), 2.92-2.68 (m, 3H), 2.16-2.09 (m, 1H), 1.49 (s, 9H). m/z (ES+): 443.30 $[M+H^+]^+$ Data consistent with those reported by Zhang *et al. JMC* (2022), 65 (13), 9096-9125.



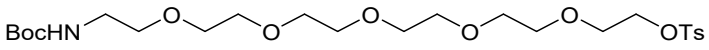
2-(2,6-Dioxopiperidin-3-yl)-5-(piperazin-1-yl)isoindoline-1,3-dione hydrochloride (30)

To a stirred solution of *tert*-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperazine-1-carboxylate **(29)** (14.00 g, 31.73 mmol) in DCM (100 mL) at ambient temperature was dropwise added hydrogen chloride (79.66 mL, 318.64 mmol, 4M solution in 1,4-dioxane), and the resulting yellow suspension was stirred at ambient temperature overnight. The reaction mixture was partially concentrated under reduced pressure to approximately 50% of the initial volume, and diethyl ether was then added. After stirring at ambient temperature for 1 hour, the resulting solid was collected by filtration, dissolved in MeCN (30 mL) and H_2O (150 mL) and freeze dried to give the title compound as a pale-yellow solid (10.81 g, 90%). ^{1}H NMR (DMSO-d6) δ: 11.09 (s, 1H), 9.46 (br s, 2H), 7.74 (d, *J*= 8.5, 1H), 7.44 (d, *J*= 2.2, 1H), 7.32 (dd, *J*= 8.5, 2.2, 1H), 5.09 (dd, *J*= 12.9, 5.4, 1H), 3.73-3.68 (m, 4H), 3.23-3.17 (m, 4H), 2.94-2.84 (m, 1H), 2.63-2.48 (m, 2H), 2.07-1.98 (m, 1H). ^{13}C NMR (DMSO-d6) δ: 172.8, 170.0, 167.4, 166.9, 154.4, 133.8, 125.0, 119.6, 118.6, 108.9, 48.8, 44.1, 42.1, 31.0, 22.1. LCMS: 100% purity, m/z (ES+): 343.20 $[M_{(free\ base)}+H^+]^+$. Elemental Analysis calculated for $C_{17}H_{18}N_4O_4.HCl.1.5H_2O$: C 50.31; H 5.46; N 13.81; found: C 50.47; H 5.17; N 13.66. HRMS (ES+) calculated for $[(C_{17}H_{18}N_4O_4)+H^+]^+$: 343.1393, found: 343.1411.



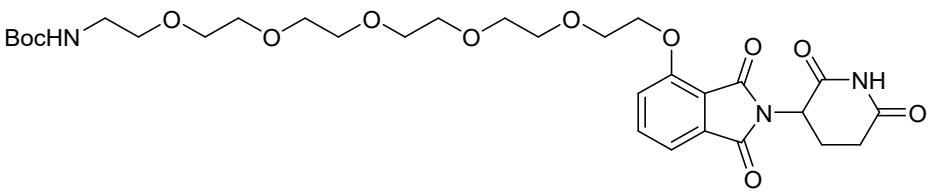
2-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]oxyacetic acid (7)

To a stirred mixture of 4'-hydroxythalidomide **(6)** (40.00 g, 145.86 mmol) and potassium carbonate (30.24 g, 218.80 mmol) in DMF (400 mL) at ambient temperature was portion-wise added *tert*-butyl bromoacetate (21.47 mL, 145.43 mmol). The resulting reaction mixture was stirred at ambient temperature overnight before being poured into water (1200 mL) and extracted with ethyl acetate. The combined organic extracts were sequentially washed with saturated aqueous sodium hydrogen carbonate solution (2 x 500 mL), water (500 mL) and brine (500 mL) and were then dried over anhydrous magnesium sulfate and concentrated under reduced pressure, being held on the rotary evaporator for a further 1 hour at 45°C under vacuum after distillation had ceased. Trituration of the residue with diethyl ether (750 mL) afforded a white solid (containing 2%w/w of a dialkylated adduct). The solid was treated with TFA (500 mL) and stirred at ambient temperature for 1 hour before being concentrated under reduced pressure and subsequently three times re-concentrated from acetonitrile. The residue was suspended in acetonitrile (300 mL) and THF (30 mL) was added. The suspension was heated to reflux, and immediately allowed to cool to ambient temperature with stirring for 18 hours. The resulting suspended solid was collected by filtration and dried under vacuum to afford the title compound as a white solid (35.41 g, 73%). ^{1}H NMR (DMSO-d6) δ: 13.27 (br s, 1H), 11.11 (br s, 1H), 7.79 (dd, *J*= 8.5, 7.2, 1H), 7.48 (d, *J*= 7.2, 1H), 7.39 (d, *J*= 8.5, 1H), 5.10 (dd, *J*= 12.8, 5.4, 1H), 4.99 (s, 2H), 2.95-2.84 (m, 1H), 2.64-2.47 (m, 2H), 2.08-2.00 (m, 1H). ^{13}C NMR (DMSO-d6) δ: 172.8, 169.9, 169.5, 166.7, 165.2, 155.1, 136.8, 133.3, 119.9, 116.3, 115.8, 65.0, 48.8, 31.0, 21.0. LCMS: 100% purity, m/z (ES+): 355.00 $[M+Na^{+}]^{+}$. Elemental Analysis calculated for $C_{15}H_{12}N_2O_7$: C 54.22; H 3.64; N 8.43; found: C 54.20; H 3.65; N 8.60. HRMS (ES+) calculated for $[(C_{15}H_{12}N_2O_7)+Na^{+}]^{+}$: 355.0543, found: 355.0543.



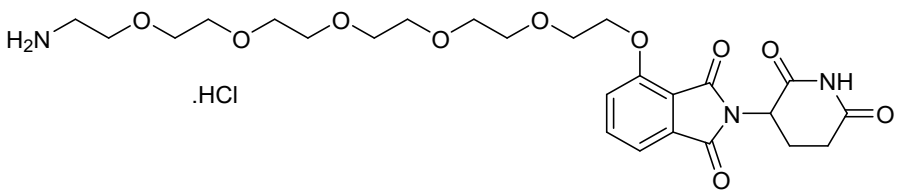
***N*-Boc-PEG6-OTs (34)**

To a stirred solution of *N*-Boc-PEG6-alcohol **(33)** (14.96 g, 39.22 mmol) in DCM (230 mL) at 0-5°C was sequentially added TsCl (9.72 g, 50.98 mmol), TEA (8.20 mL, 58.83 mmol) and DMAP (0.24 g, 1.96 mmol), and the resulting solution was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was partitioned between water and DCM and the organic phase was separated. The aqueous component was twice extracted with DCM, and the combined organics were washed with brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to a pale oil. Purification by flash column chromatography, eluting with 50-100% ethyl acetate/petroleum ether (40:60) afforded the title compound as a colourless oil (20.52 g, 93%). ^{1}H NMR ($CDCl_3$) δ: 7.79 (d, *J*= 8.1, 2H), 7.34 (d, *J*= 8.1, 2H), 5.07 (br s, 1H), 4.17-4.13 (m, 2H), 3.70-3.49 (m, 20H), 3.33-3.26 (m, 2H), 2.44 (s, 3H), 1.43 (s, 9H). m/z (ES+): 558.40 $[M+Na^+]^+$. Data consistent with those reported by Mueller *et al. Chemical Communications* (2011), 47 (1), 310-312.



<u>*tert*-Butyl (17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-3,6,9,12,15-pentaoxaheptadecyl)carbamate; Thalidomide 4'ether-PEG5-amine, *N*-Boc protected (31)</u>

To a stirred solution of 4-hydroxythalidomide **(6)** (9.98 g, 36.39 mmol) and *N*-Boc-PEG6-OTs **(34)** (20.52 g, 36.39 mmol, 95%w/w) in DMF (100 mL) at ambient temperature was added potassium hydrogen carbonate (5.47 g, 54.59 mmol) and potassium iodide (0.60 g, 3.64 mmol), and the resulting mixture was stirred for 72 hours. The reaction mixture was poured into water (500 mL) and three times extracted with ethyl acetate. The organic extracts were washed with water, twice with 1M potassium carbonate solution (aq.) and twice with brine, before being dried over anhydrous magnesium sulfate and concentrated under reduced pressure to a pale oil. Purification by flash column chromatography, eluting with 1-3% 7M methanolic ammonia/DCM afforded the crude product as a viscous colourless oil. A second chromatographic purification, eluting with 3-5% MeOH/EtOAc, afforded the product as a very viscous colourless oil (12.46 g, 51%, containing 5%w/w residual EtOAc). ^{1}H NMR ($CDCl_3$) δ: 8.38 (br s, 1H, 7.66 (dd, *J*= 8.4, 7.2, 1H), 7.46 (dd, *J*= 7.2, 0.4, 1H), 7.29-7.25 (m, 1H), 5.10 (br s, 1H), 4.94 (dd, *J*= 12.4, 5.3, 1H), 4.34-4.32 (m, 2H), 3.95-3.91 (m, 2H), 3.78-3.75 (m, 2H), 3.67-3.58 (m, 14H), 3.54-3.50 (m, 2H), 3.32-3.26 (m, 2H), 2.91-2.67 (m, 3H), 2.16-2.08 (m, 1H), 1.43 (s, 9H). m/z (ES+): 538.30 $[(M-_{Boc})+H^+]^+$; 660.30 $[M+Na^+]^+$. Data consistent with those reported by Merck WO 2020/152067..



Thalidomide 4'ether-PEG5-amine hydrochloride (32)

To a stirred solution of **(31)** (12.45 g, 18.55 mmol, 95%w/w) in 1,4-dioxane (170 mL) at ambient temperature was dropwise added hydrogen chloride (46.37 mL, 185.48 mmol, 4M solution in 1,4-dioxane), and the resulting solution was heated to +35°C with stirring maintained at this temperature overnight. A further portion of hydrogen chloride solution (20.00 mL) was added and the temperature was increased to +45°C with stirring for a further 4 hours to complete the reaction. After cooling to ambient temperature, the reaction mixture was cooled to ambient temperature and concentrated under reduced pressure to a sticky colourless foam which was dissolved in water (200 mL) and freeze dried. The residue was dissolved in ethanol and concentrated under reduced pressure to a white foam, which was dissolved in water and freeze dried to afford the title compound as a colourless solid (10.65 g, 100%). ^{1}H NMR (CD_3OD) δ: 7.83 (dd, *J*= 8.5, 7.3, 1H), 7.53-7.50 (m, 2H), 5.14 (dd, *J*= 12.6, 5.5, 1H), 4.45-4.41 (m, 2H), 4.00-3.96 (m, 2H), 3.84-3.81 (m, 2H), 3.75-3.61 (m, 16H), 3.34-3.31 (m, 3H), 3.22-3.17 (m, 2H), 2.96-2.68 (m, 3H), 2.20-2.12 (m, 1H). ^{13}C NMR (CD_3OD) δ: 174.56, 171.42, 168.41, 167.85, 157.55, 138.20, 135.11, 120.83, 118.33, 117.00, 71.47, 71.37, 71.32, 71.25, 71.20, 71.08, 71.00, 70.63, 70.34, 70.16, 67.87, 50.50, 40.72, 32.17, 23.64. LCMS: >99% purity, m/z (ES+): 538.30 $[M+H^+]^+$. Elemental Analysis calculated for $C_{25}H_{35}N_3O_{10}.HCl.1H_2O$: C 50.72; H 6.47; N 7.10; found: C 50.48; H 6.49; N 6.75. HRMS (ES+) calculated for $[(C_{25}H_{35}N_3O_{10})+H^+]^+$: 538.2430, found: 538.2405.