Figure S1. Related to Figure 2D.

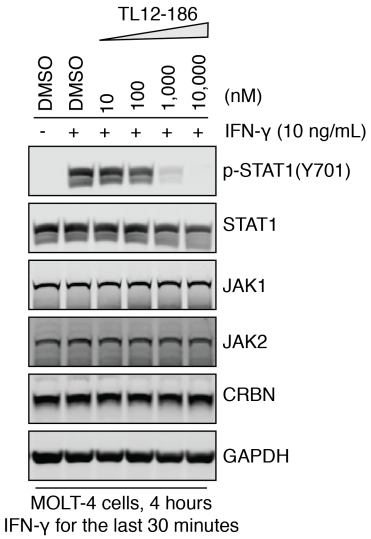


Figure S1. TL12-186 inhibited IFN-γ-stimulated STAT1 phosphorylation without causing degradation of JAK1 or JAK2. Related to Figure 2D.

Immunoblots for phospho-STAT1(Y701), total STAT1, JAK1, JAK2, CRBN and GAPDH in MOLT-4 cells after 4-hour treatment with DMSO or TL12-186 at the indicated concentrations. IFN-γ stimulation (10 ng/mL) occurred 30 minutes before the cells were harvested.

Figure S2. Related to Figure 3.

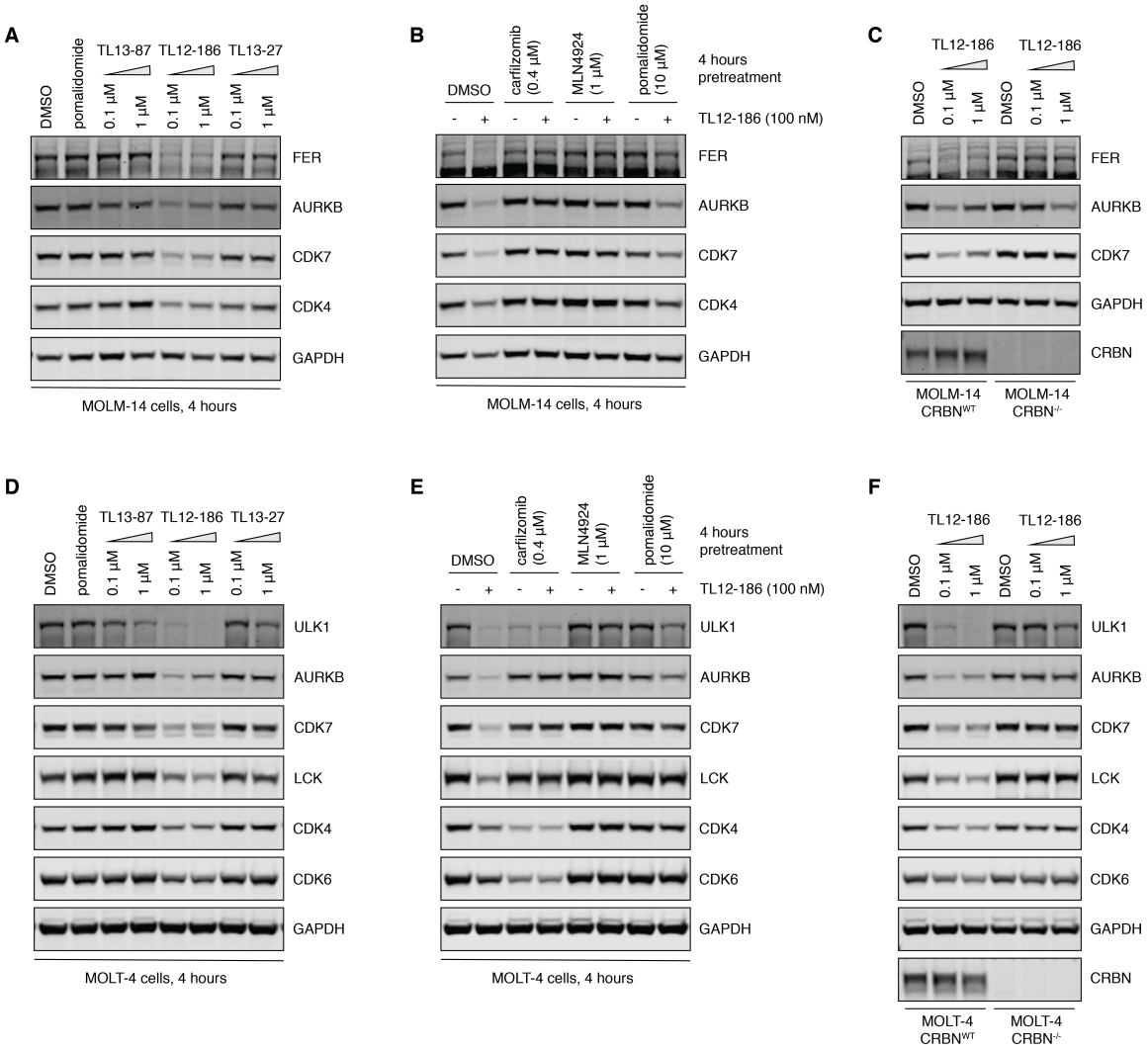


Figure S2. Western blotting validation of multi-kinase degradation induced by TL12-186 as a CRBN-mediated proteasome-dependent process. Related to Figure 3.

(A) Immunoblots for FER, AURKB, CDK7, CDK4 and GAPDH in MOLM-14 cells after 4-hour treatment of DMSO, pomalidomide (10 μM), TL13-87 (0.1 and 1 μM), TL12-186 (0.1 and 1 μM) or TL13-27 (0.1 and 1 μM).

(B) Immunoblots for FER, AURKB, CDK7, CDK4 and GAPDH in MOLM-14 cells pre-treated with DMSO, carfilzomib (0.4 μM), MLN4924 (1 μM) or pomalidomide (10 μM) for 4 hours, followed by 4-hour co-treatment with DMSO, or TL12-186 (0.1 μM).

(C) Immunoblots for FER, AURKB, CDK7, GAPDH and CRBN in WT and CRBN−/− MOLM-14 cells after 4-hour treatment of DMSO or TL12-186 (0.1 and 1 μM).

(D) Immunoblots for ULK1, AURKB, CDK7, LCK, CDK4, CDK6 and GAPDH in MOLT-4 cells after 4-hour treatment of DMSO, pomalidomide (10 μM), TL13-87 (0.1 and 1 μM), TL12-186 (0.1 and 1 μM) or TL13-27 (0.1 and 1 μM).

(E) Immunoblots for ULK1, AURKB, CDK7, LCK, CDK4, CDK6 and GAPDH in MOLT-4 cells pre-treated with DMSO, carfilzomib (0.4 μM), MLN4924 (1 μM) or pomalidomide (10 μM) for 4 hours, followed by 4-hour co-treatment with DMSO, or TL12-186 (0.1 μM).

(F) Immunoblots for ULK1, AURKB, CDK7, LCK, CDK4, CDK6, GAPDH and CRBN in WT and CRBN−/− MOLT-4 cells after 4-hour treatment of DMSO or TL12-186 (0.1 and 1 μM).

Figure S3. Related to Figure 4.

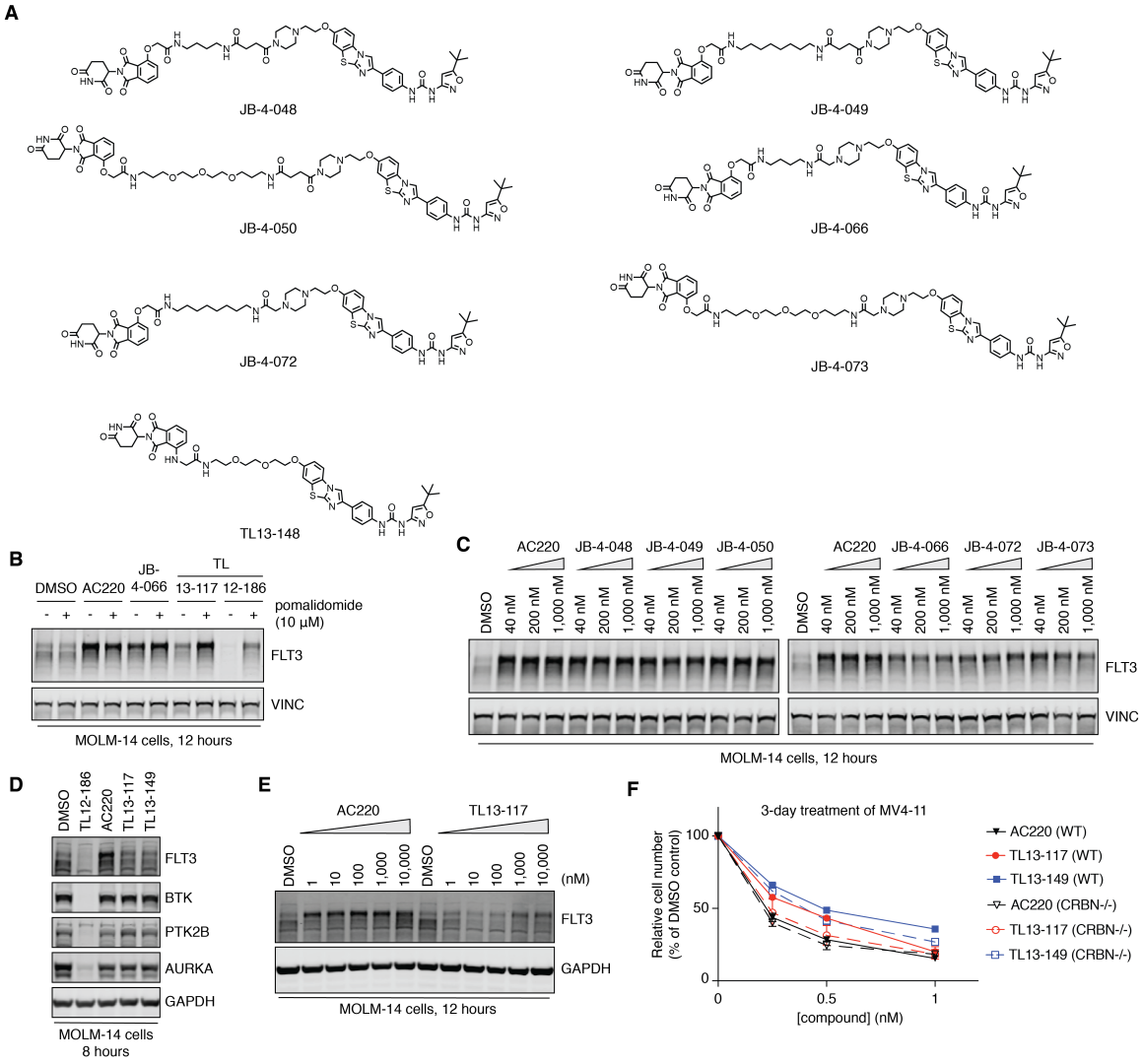


Figure S3. Characterization of AC220-based FLT3 degraders. Related to Figure 4.

(A) Chemical structures of AC220-based FLT3 degraders.

(B) Immunoblots for FLT3 and VINC in MOLM-14 cells pre-treated with pomalidomide (10 μM) for 4 hours, followed by 12-hour co-treatment with DMSO, AC220 (200 nM), JB-04-066 (200 nM), TL13-117 (200 nM) or TL12-186 (200 nM).

(C) Immunoblots for FLT3 and VINC in MOLM-14 cells treated with DMSO, AC220, JB-4-048, -049, -050, -066, -072 or -073 at indicated concentrations for 12 hours.

(D) Immunoblots for FLT3, BTK, PTK2B, AURKA and GAPDH in MOLM-14 cells treated with DMSO, TL12-186 (100 nM), AC220 (100 nM), TL13-117 (100 nM) or TL13-149 (100 nM) for 8 hours.

(E) Immunoblots for FLT3 and GAPDH in MOLM-14 cells treated with DMSO, AC220 or TL13-117 at indicated concentrations for 12 hours.

(F) 3-day proliferation assays of WT and CRBN−/− MV4-11 cells treated with AC220, TL13-117 or TL13-149. Values represent duplicate means ± SD. Error bars shorter than the height of the symbol are not drawn.

Figure S4. Related to Figure 5.

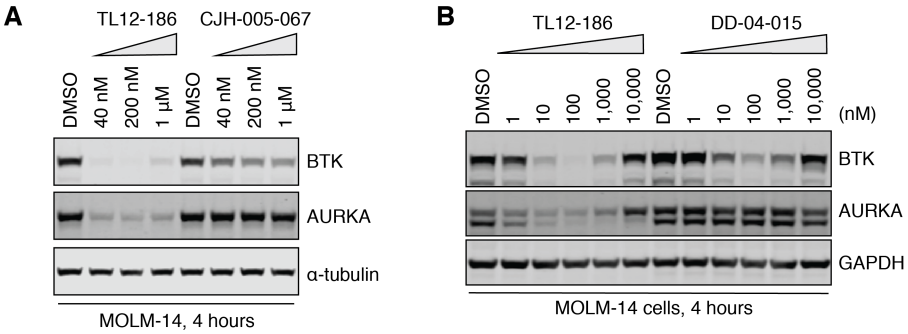


Figure S4. Bosutinib-based degrader CJH-005-067 degrades BTK. Related to Figure 5.
(A) Immunoblots for BTK, AURKA and α-tubulin in MOLM-14 cells treated with DMSO, TL12-186 or CJH-005-067 at indicated concentrations for 4 hours.
(B) Immunoblots for BTK, AURKA and GAPDH in MOLM-14 cells treated with DMSO, TL12-186 or DD-04-015 at indicated concentrations for 4 hours.

Table S6. Related to Figures 2C and 2D.

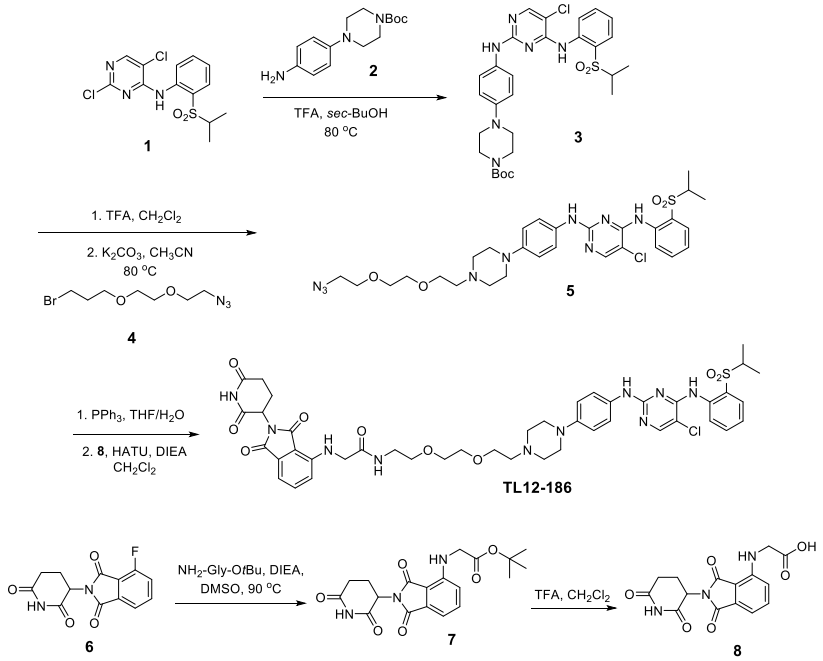
Table S6. IC_{50} values[a] of TL12-186 at inhibiting select kinases. Related to Figures 2C and 2D.

Kinase tested	[ATP] Tested (μM)	IC_{50} (nM)
ABL1 T315I	Km app	4.91
ALK	Km app	14.7
AURKA (Aurora A)	Km app	1.61
BTK	Km app	21.7
CDK2/cyclin A	Km app	73.0
CDK7/cyclin H/MNAT1	Km app	19.1
CDK9/cyclin T1	Km app	55.8
FLT3 ITD		10.3
JAK2 JH1 JH2 V617F	Km app	3.15
JAK2 JH1 JH2	Km app	1.93
JAK3	Km app	3.48
RPS6KA3 (RSK2)	Km app	21.6

[a] The IC_{50} value of FLT3 ITD was obtained from LanthaScreen binding assays. The IC_{50} values of ABL1 T315I, ALK, AURKA, BTK, CDK2, JAK2 V617F, JAK2, JAK3 and RSK2 were obtained from Z'-Lyte activity assays. The IC_{50} values of CDK7 and CDK9 were obtained from Adapta assays. All assays are available from ThermoFisher Scientific (SelectScreen).

Method S1. Related to STAR METHODS

Synthesis of TL12-186



***tert*-butyl 4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)piperazine-1-carboxylate (3).**

Intermediate **1** was prepared according to the literature (Galkin et al., 2007), while *tert*-butyl 4-(4-aminophenyl)piperazine-1-carboxylate (**2**) was commercially available. To **1** (693 mg, 2.0 mmol) and **2** (666 mg, 2.4 mmol) in *sec*-butanol (4 mL) was added TFA (185 μL, 2.4 mmol) and the mixture was stirred overnight at 80 °C. The mixture was then concentrated and purified by column chromatography (dichloromethane:methanol = 20:1) to yield 830 mg (71%) of **3** as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 8.53 (d, *J* = 8.4 Hz, 1H), 8.02 (d, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.54 (dd, *J* = 8.8, 8.4 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.30 (dd, *J* = 8.4, 8.4 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 3.60 (m, 4H), 3.22 (m, 1H), 3.11 (m, 4H), 1.49 (m, 9H), 1.31 (d, *J* = 6.8 Hz, 6H). MS (ESI) m/z 587 $(M+H)^+$.

***N*^{2}-(4-(4-(2-(2-(2-azidoethoxy)ethoxy)ethyl)piperazin-1-yl)phenyl)-5-chloro-*N*^{4}-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (5).**

To **3** (590 mg, 1.0 mmol) in dichloromethane (18 mL) was added TFA (1.8 mL), and the mixture was stirred at room temperature (RT) for 2 h, then was concentrated and dried under vacuum. To the obtained crude intermediate in acetonitrile (5 mL) was added commercial available bromide **4** (300 mg, 1.2 mmol) and potassium carbonate (414 mg, 3.0 mmol). The resulted mixture was stirred under 80 °C overnight, then cooled down to RT and diluted with 50 mL of dichloromethane. The precipitation was filtered, and the filtrate was concentrated and purified by column chromatography (dichloromethane:methanol = 10:1) to yield 514 mg (80%) of **5** as a colorless oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 9.32 (s, 1H), 8.65 (br, 1H), 8.23 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.73 (dd, *J* = 8.4, 8.4 Hz, 1H), 7.43 (d, *J* = 9.2 Hz, 2H), 7.36 (dd, *J* = 8.4, 8.0 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 2H), 3.61 (t, *J* = 4.8 Hz, 2H), 3.57 (m, 6H), 3.44 (m, 1H), 3.40 (t, *J* = 5.2 Hz, 2H), 3.06 (m, 4H), 2.58 (m, 4H), 1.16 (d, *J* = 6.8 Hz, 6H). MS (ESI) m/z 644 $(M+H)^+$.

***tert*-butyl (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)glycinate (7).**

Intermediate **6** was prepared according to the literature (Lu et al., 2015). To **6** (550 mg, 2.0 mmol) and glycine *tert*-butyl ester (260 mg, 2.0 mmol) in anhydrous DMSO (20 mL) was added *N*,*N*-diisopropylethylamine (DIEA) (700 μL, 4.0 mmol). The reaction mixture was stirred under 90 °C for 1 day, then cooled down. The mixture was diluted with ethyl acetate (200 mL), washed with water and brine, dried with Na_2SO_4, then filtered and concentrated, purified by column chromatography (dichloromethane:ethyl acetate = 2:1) to yield 530 mg (68%) of **7** as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.51 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 6.76 Hz, 1H), 4.93 (dd, *J* = 12.0, 6.4 Hz, 1H), 3.94 (s, 2H), 2.67-2.92 (m, 2H), 2.12 (m, 1H), 1.93 (m, 1H), 1.50 (s, 9H). MS (ESI) m/z 388 $(M+H)^+$.

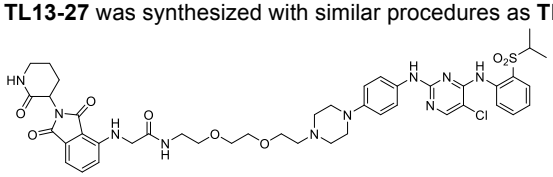
(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)glycine (8).

To **7** (390 mg, 1.0 mmol) in dichloromethane (18 mL) added TFA (1.8 mL). The mixture was stirred at RT overnight, then was concentrated and dried under vacuum to give **8** as a yellow solid, which was used in next step without purification.
MS (ESI) m/z 330 $(M-H)^-$.

***N*-(2-(2-(2-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)acetamide (TL12-186).**
Under a nitrogen atmosphere, to **5** (130 mg, 0.2 mmol) in tetrahydrofuran (18 mL) and water (1.8 mL) was added triphenylphosphine (63 mg, 0.24 mmol). The reaction mixture was stirred overnight, then concentrated and dried under vacuum. To the obtained crude oil in anhydrous dichloromethane (3 mL) was added **8** (73 mg, 0.22 mmol) and (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate) (HATU) and DIEA (110 µL, 0.6 mmol). The reaction mixture was stirred for 2 h, then concentrated and purified by column chromatography (dichloromethane:methanol = 10:1) to yield 136 mg (74%) of **TL12-186** as a yellow oil.
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 9.8 (br, 1H), 9.50 (s, 1H), 9.38 (s, 1H), 8.65 (br, 1H), 8.25 (s, 1H), 8.20 (dd, *J* = 5.5, 5.5 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.75 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.59 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.37 (dd, *J* = 8.5, 8.0 Hz, 1H), 7.08 (d, *J* = 7.0 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 1H), 5.07 (dd, *J* = 13.0, 5.5 Hz, 1H), 3.95 (d, *J* = 5.5 Hz, 2H), 3.80 (m, 2H), 3.74 (m, 2H), 3.59 (m, 6H), 3.46 (m, 3H), 3.18-3.42 (m, 4H), 3.00 (m, 2H), 2.89 (m, 2H), 2.53-2.63 (m, 2H), 2.02 (m, 2H), 1.17 (d, *J* = 6.5 Hz, 6H). MS (ESI) m/z 931 $(M+H)^+$.

Synthesis of TL13-27

TL13-27 was synthesized with similar procedures as **TL12-186**.

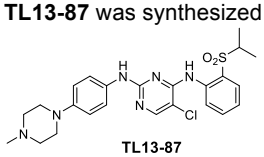


TL13-27

***N*-(2-(2-(2-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-((1,3-dioxo-2-(2-oxopiperidin-3-yl)isoindolin-4-yl)amino)acetamide (TL13-27)**.
^{1}H NMR (400 MHz, DMSO-d_6, TFA salt) δ 9.49 (s, 1H), 9.32 (s, 1H), 8.63 (br, 1H), 8.23 (s, 1H), 8.18 (t, *J* = 5.6 Hz, 1H), 7.83 (m, 2H), 7.73 (dd, *J* = 8.4, 7.6 Hz, 1H), 7.44 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.36 (dd, *J* = 8.0, 7.6 Hz, 1H), 7.03 (dd, *J* = 8.0, 7.6 Hz, 1H), 6.94 (t, *J* = 5.6 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.52 (dd, *J* = 11.6, 6.4 Hz, 1H), 4.11 (dd, *J* = 10.4, 5.2 Hz, 2H), 3.92 (d, *J* = 5.6 Hz, 2H), 3.55 (m, 2H), 3.51 (m, 2H), 3.44 (t, *J* = 6.4 Hz, 2H), 3.28 (dd, *J* = 11.2, 5.2 Hz, 1H), 3.20 (m, 2H), 3.16 (m, 4H), 3.05 (m, 4H), 2.57 (m, 4H), 2.20 (m, 1H), 1.97 (m, 1H), 1.88 (m, 2H), 1.16 (d, *J* = 6.8 Hz, 6H). MS (ESI) m/z 917 $(M+H)^+$.

Synthesis TL13-87

TL13-87 was synthesized with similar procedures as **TL12-186**.

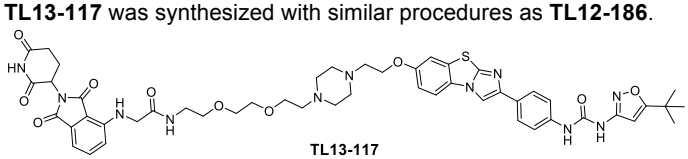


TL13-87

5-chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidine-2,4-diamine (TL13-87).
^{1}H NMR (500 MHz, DMSO-d_6, TFA salt) δ 9.71 (br, 1H), 9.51 (s, 1H), 9.40 (s, 1H), 8.63 (br, 1H), 8.25 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.75 (dd, *J* = 9.0, 8.5 Hz, 1H), 7.49 (d, *J* = 9.0 Hz, 2H), 7.38 (dd, *J* = 8.0, 8.0 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 3.75 (m, 2H), 3.52 (m, 2H), 3.45 (m, 1H), 3.17 (m, 2H), 2.90 (m, 2H), 2.87 (s, 3H), 1.17 (d, *J* = 7.0 Hz, 6H). MS (ESI) m/z 501 $(M+H)^+$.

Synthesis of TL13-117

TL13-117 was synthesized with similar procedures as **TL12-186**.



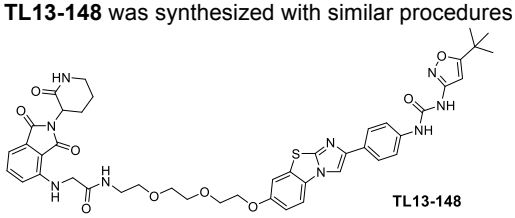
TL13-117

***N*-(2-(2-(2-(4-(2-((2-(4-(3-(5-(tert-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-((1,3-dioxo-2-(2-oxopiperidin-3-yl)isoindolin-4-yl)amino)acetamide (TL13-117).**
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.54 (s, 1H), 8.88 (s, 1H), 8.60 (s, 1H), 8.16 (m, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.77 (d, *J* = 9.0 Hz, 2H), 7.66 (d, *J* = 2.5 Hz, 1H), 7.58 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.14 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.06 (d, *J* = 7.5 Hz, 1H), 6.94 (m, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.52 (s, 1H),

5.07 (dd, *J* = 13.0, 5.5 Hz, 1H), 4.14 (br, 2H), 3.93 (d, *J* = 5.5 Hz, 2H), 3.50 (m, 4H), 3.49 (s, 2H), 3.43 (m, 1H), 3.32 (m, 4H), 3.26 (m, 2H), 2.89 (m, 2H), 2.72 (m, 2H), 2.36-2.62 (m, 8H), 2.03 (m, 1H), 1.30 (s, 9H). MS (ESI) m/z 990 $(M+H)^+$.

Synthesis of TL13-148

TL13-148 was synthesized with similar procedures as **TL12-186**.



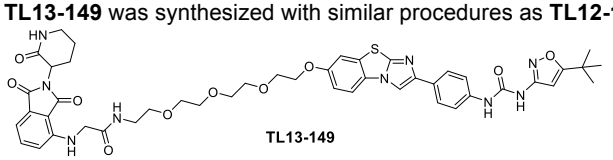
TL13-148

***N*-(2-(2-(3-((2-(4-(3-(5-(tert-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)propoxy)ethoxy)ethyl)-2-((1,3-dioxo-2-(2-oxopiperidin-3-yl)isoindolin-4-yl)amino)acetamide (TL13-148).**

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 9.54 (s, 1H), 8.88 (s, 1H), 8.60 (s, 1H), 8.17 (m, 1H), 7.86 (d, *J* = 9.0 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 2.5 Hz, 1H), 7.58 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.52 (d, *J* = 9.0 Hz, 2H), 7.16 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.05 (d, *J* = 7.0 Hz, 1H), 6.94 (m, 1H), 6.85 (d, *J* = 9.0 Hz, 1H), 6.53 (s, 1H), 5.08 (dd, *J* = 13.0, 5.5 Hz, 1H), 4.17 (m, 2H), 3.94 (d, *J* = 5.5 Hz, 1H), 3.78 (m, 2H), 3.60 (m, 2H), 3.55 (m, 2H), 3.46 (t, *J* = 5.5 Hz, 2H), 3.28 (m, 2H), 2.90 (m, 1H), 2.58 (m, 2H), 2.03 (m, 1H), 1.31 (s, 9H). MS (ESI) m/z 878 $(M+H)^+$.

Synthesis of TL13-149

TL13-149 was synthesized with similar procedures as **TL12-186**.

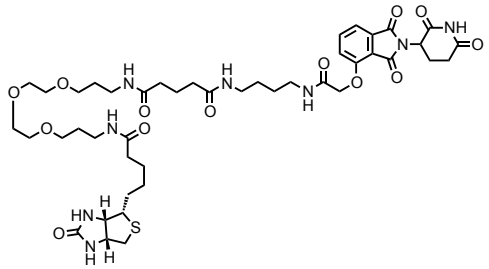


TL13-149

***N*-(2-(2-(2-(2-((2-(4-(3-(5-(tert-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-2-((1,3-dioxo-2-(2-oxopiperidin-3-yl)isoindolin-4-yl)amino)acetamide (TL13-149).**

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.52 (s, 1H), 8.86 (s, 1H), 8.59 (s, 1H), 8.15 (m, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 2.5 Hz, 1H), 7.57 (dd, *J* = 8.5, 7.5 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.15 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.05 (d, *J* = 7.0 Hz, 1H), 6.93 (m, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.52 (s, 1H), 5.06 (dd, *J* = 13.0, 5.5 Hz, 1H), 4.16 (m, 2H), 3.93 (d, *J* = 5.5 Hz, 1H), 3.77 (m, 2H), 3.60 (m, 2H), 3.55 (m, 4H), 3.51 (m, 2H), 3.46 (t, *J* = 5.5 Hz, 2H), 3.28 (m, 2H), 2.90 (m, 1H), 2.58 (m, 2H), 2.03 (m, 1H), 1.30 (s, 9H). MS (ESI) m/z 922 $(M+H)^+$.

Synthesis of biotinylated-thalidomide



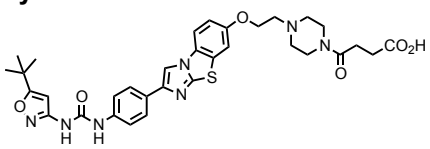
***N*^{1}-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)-*N*^{5}-(15-oxo-19-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)glutaramide (Bio-thal)**

N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (14.3 mg, 0.0277 mmol, 1 eq) (Winter et al., 2015) was added to 5,21-dioxo-25-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-10,13,16-trioxa-6,20-diazapentacosan-1-oic acid N,N-Diisopropylethylamine salt (18.4 mg, 0.0277 mmol, 1 eq) as a 0.1 M solution in DMF (277 microliters). DIPEA (13.9 microliters, 0.0800 mmol, 3 eq) and HATU (10.5 mg, 0.0277 mmol, 1 eq) were added and the mixture was stirred at room temperature. After 19 hours, the mixture was diluted with methanol and purified by preparative phase HPLC to give the desired product as a light yellow oil (18.92 mg, 0.0189 mmol, 68%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.81 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.54 (d, *J* = 6.9 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 5.14 (dd, *J* = 12.5, 5.5 Hz, 1H), 4.76 (s, 2H), 4.48 (dd, *J* = 7.8, 4.3 Hz, 1H), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.63 (dd, *J* = 5.9, 2.9 Hz, 4H), 3.60 – 3.54 (m, 4H), 3.50 (tt, *J* = 5.5, 2.7 Hz, 4H), 3.34 (d, *J* = 5.2 Hz, 1H), 3.27 – 3.17 (m,

6H), 2.95 – 2.62 (m, 6H), 2.18 (q, *J* = 7.7 Hz, 7H), 1.87 (q, *J* = 7.6 Hz, 2H), 1.79 – 1.34 (m, 15H). MS (ESI) m/z 945.45 $(M+H)^+$.

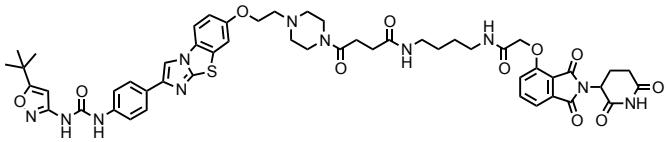
Synthesis of JB-4-048



4-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)-4-oxobutanoic acid

1-(5-(*tert*-butyl)isoxazol-3-yl)-3-(4-(7-(2-(piperazin-1-yl)ethoxy)benzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)phenyl)urea (56.0 mg, 0.10 mmol, 1 eq) was dissolved in DMF (1 mL). Succinic anhydride (15.0 mg, 0.15 mmol, 1.5 eq) and DMAP (12.2 mg, 0.10 mmol, 1 eq) were added and the mixture was stirred for 23 hours. The mixture was then diluted with MeOH and purified by preparative HPLC to give the desired product as a tan solid (68.04 mg).

^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.17 (s, 1H), 7.75 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 2H), 7.44 – 7.36 (m, 3H), 7.13 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.40 (s, 1H), 4.47 – 4.39 (m, 2H), 4.20 – 3.37 (m, 10H), 2.72 – 2.61 (m, 4H), 1.36 (s, 9H). MS (ESI) m/z 660.47 $(M+H)^+$.

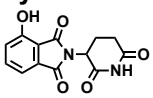


JB-4-048

N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (10.3 mg, 0.020 mmol, 1 eq) (Winter et al., 2015) was added to 4-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)-4-oxobutanoic acid (13.2 mg, 0.020 mmol, 1 eq) as a 0.1M solution in DMF (200 microliters) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) was added, followed by HATU (7.6 mg, 0.020 mmol, 1 eq). After 13 hours, the mixture was diluted with MeOH and purified by preparative HPLC to give the desired product as a yellow residue (14.69 mg, trifluoroacetate salt, 0.0127 mmol, 63%).

^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.16 (s, 1H), 7.79 – 7.72 (m, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.54 – 7.41 (m, 4H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H), 6.38 (s, 1H), 5.04 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.67 (d, *J* = 2.2 Hz, 2H), 4.45 (s, 2H), 3.98 – 3.34 (m, 12H), 3.24 – 3.15 (m, 2H), 2.85 – 2.47 (m, 7H), 2.16 – 2.11 (m, 1H), 1.69 – 1.49 (m, 4H), 1.34 (s, 9H). MS (ESI) m/z 1044.56 $(M+H)^+$.

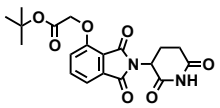
Synthesis of JB-4-049



2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione

3-Hydroxyphthalic anhydride (1.641 g, 10 mmol, 1 eq) and 3-aminopiperidine-2,6-dione hydrochloride (1.646 g, 10 mmol, 1 eq) were dissolved in pyridine (40 mL, 0.25 M) and heated to 110 °C. After 14 hours, the mixture was cooled to room temperature and concentrated under reduced pressure. Purification by column chromatography (ISCO, 24 g silica column, 0-10% MeOH/DCM) gave the desired product as a tan solid (2.424 g, 8.84 mmol, 88%).

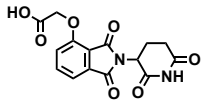
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 2H), 7.65 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.36 – 7.28 (m, 1H), 7.25 (dd, *J* = 8.4, 0.6 Hz, 1H), 5.07 (dd, *J* = 12.8, 5.4 Hz, 1H), 2.88 (ddd, *J* = 17.3, 14.0, 5.4 Hz, 1H), 2.63 – 2.50 (m, 2H), 2.08 – 1.95 (m, 1H).



***tert*-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate**

2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (1.568 g, 5.71 mmol, 1 eq) was dissolved in DMF (57 mL, 0.1 M) at room temperature. Potassium carbonate (1.19 g, 8.58 mmol, 1.5 eq) and tert-butyl bromoacetate (0.843 mL, 5.71 mmol, 1 eq) were then added. After 2 hours, the mixture was diluted with EtOAc and washed once with water then twice with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 24 g silica column, 0-100%EtOAc/hexanes, 21 minute gradient) gave the desired product as a cream colored solid (2.06 g, 5.30 mmol, 93%).

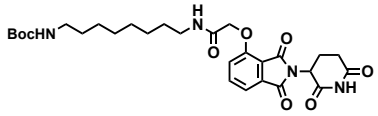
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.94 (s, 1H), 7.67 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 4.97 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.79 (s, 2H), 2.95 – 2.89 (m, 1H), 2.85 – 2.71 (m, 2H), 2.14 (dtd, *J* = 10.2, 5.0, 2.7 Hz, 1H), 1.48 (s, 9H). MS (ESI) m/z 389.33 $(M+H)^+$.



2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid

tert-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (2.06 g, 5.30 mmol, 1 eq) was dissolved in TFA (53 mL, 0.1M) at room temperature. After 4 hours, the solution was diluted with DCM and concentrated under reduced pressure. The resultant cream colored solid (1.484 g, 4.47 mmol, 84%) was deemed sufficiently pure and carried onto the next step without further purification.

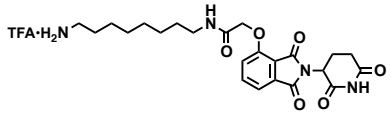
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 7.79 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 1H), 5.10 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.99 (s, 2H), 2.93 – 2.89 (m, 1H), 2.63 – 2.51 (m, 2H), 2.04 (ddd, *J* = 10.5, 5.4, 3.1 Hz, 1H). MS (ESI) m/z 333.25 $(M+H)^+$.



***tert*-butyl (8-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)octyl)carbamate**

Boc-1,8-diaminooctane (2.10 g, 8.59 mmol, 1.1 eq) was dissolved in DMF (86 mL). In a separate flask, 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (2.60 g, 7.81 mmol, 1 eq) was dissolved in DMF (78 mL). The solution of Boc-1,8-diaminooctane in DMF was then added, followed by DIPEA (4.08 mL, 23.4 mmol. 3 eq) and HATU (2.97 g, 7.81 mmol, 1 eq). The mixture was stirred for 19 hours at room temperature, then diluted with EtOAc (600 mL). The organic layer was washed sequentially with 200 mL of half saturated sodium chloride, 200 mL 10% citric acid (aq.), 200 mL of half saturated sodium chloride, 200 mL of saturated sodium bicarbonate (aq.), 200 mL water and twice with 200 mL brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 40 g column, 0-5% MeOH/DCM, 35 minute gradient) gave the desired product as a white solid (3.53 g, 6.32 mmol, 81%).

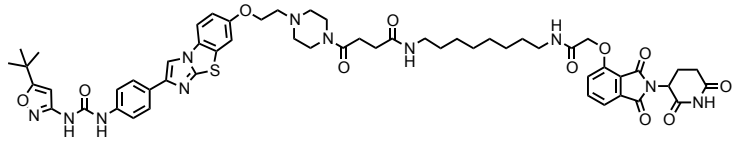
^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.49 (s, 1H), 7.74 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.55 (d, *J* = 7.2 Hz, 1H), 7.39 (t, *J* = 5.3 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 4.97 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.63 (d, *J* = 2.2 Hz, 2H), 4.59 (d, *J* = 10.0 Hz, 1H), 3.36 (q, *J* = 6.9 Hz, 2H), 3.12 – 3.03 (m, 2H), 2.95 – 2.72 (m, 3H), 2.16 (ddt, *J* = 10.3, 5.2, 2.7 Hz, 1H), 1.59 (p, *J* = 7.1 Hz, 2H), 1.37 (d, *J* = 67.6 Hz, 19H). MS (ESI) m/z 559.47 $(M+H)^+$.



***N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate**

tert-butyl (8-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)octyl)carbamate (3.53 g, 6.32 mmol, 1 eq) was dissolved in TFA (63 mL, 0.1M) and heated to 50 °C. After 1 hour, the mixture was cooled to room temperature, diluted with MeOH and concentrated under reduced pressure. The crude material was triturated with diethyl ether and dried under vacuum to give a white solid (2.93 g, 5.12 mmol, 81%).

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.82 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.55 (d, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 5.14 (dd, *J* = 12.5, 5.5 Hz, 1H), 4.76 (s, 2H), 3.33 (dd, *J* = 6.8, 1.8 Hz, 1H), 3.30 (s, 1H), 2.94 – 2.85 (m, 3H), 2.80 – 2.69 (m, 2H), 2.19 – 2.11 (m, 1H), 1.60 (dq, *J* = 24.8, 7.0 Hz, 4H), 1.37 (s, 8H). MS (ESI) m/z 459.45 $(M+H)^+$.

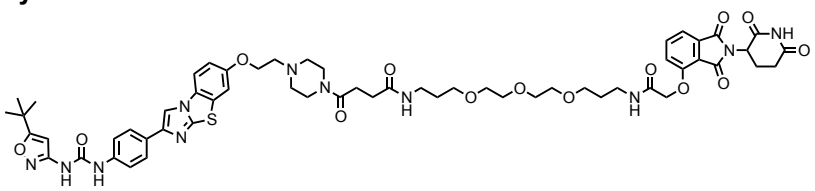


JB-4-049

N-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (11.5 mg, 0.020 mmol, 1 eq) was added to 4-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)-4-oxobutanoic acid (13.2 mg, 0.020 mmol, 1 eq) as a 0.1M solution in DMF (200 microliters) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) was added, followed by HATU (7.6 mg, 0.020 mmol, 1 eq). After 14 hours, the mixture was diluted with MeOH and purified by preparative HPLC to give the desired product as a yellow oil (9.61 mg, trifluoroacetate salt, 0.00791 mmol, 40%).

^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.11 (s, 1H), 7.76 – 7.69 (m, 4H), 7.52 – 7.49 (m, 3H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.12 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.37 (s, 1H), 5.03 – 4.98 (m, 1H), 4.63 (s, 2H), 4.45 – 4.41 (m, 2H), 3.93 – 3.34 (m, 12H), 3.13 (t, *J* = 7.2 Hz, 2H), 2.83 – 2.74 (m, 3H), 2.64 (s, 2H), 2.51 (t, *J* = 6.3 Hz, 2H), 2.16 – 2.11 (m, 1H), 1.60 – 1.53 (m, 2H), 1.45 (d, *J* = 6.8 Hz, 2H), 1.33 (s, 17H). MS (ESI) m/z 1101.55 $(M+H)^+$.

Synthesis of JB-4-050

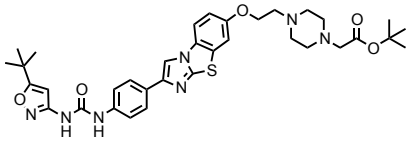


JB-4-050

N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (13.0 mg, 0.020 mmol, 1 eq) (Winter et al., 2015) was added to 4-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)-4-oxobutanoic acid (13.2 mg, 0.020 mmol, 1 eq) as a 0.1M solution in DMF (200 microliters) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) was added, followed by HATU (7.6 mg, 0.020 mmol, 1 eq). After 21 hours, the mixture was diluted with MeOH/DMF and purified by preparative HPLC to give the desired product as a dark yellow oil (16.88 mg, trifluoroacetate salt, 0.0131 mmol, 65%).

^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.18 (s, 1H), 7.75 (q, *J* = 7.0 Hz, 2H), 7.67 (d, *J* = 6.2 Hz, 2H), 7.50 (d, *J* = 7.2 Hz, 3H), 7.44 (d, *J* = 2.3 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.14 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.39 (s, 1H), 5.03 (dd, *J* = 12.4, 5.5 Hz, 1H), 4.45 (s, 2H), 4.05 – 3.36 (m, 24H), 3.24 (t, *J* = 6.7 Hz, 2H), 2.80 (q, *J* = 6.0, 5.6 Hz, 2H), 2.75 – 2.61 (m, 3H), 2.52 (t, *J* = 6.4 Hz, 2H), 2.13 (dt, *J* = 12.7, 5.0 Hz, 1H), 1.84 (p, *J* = 6.5 Hz, 2H), 1.74 (p, *J* = 6.4 Hz, 2H), 1.34 (s, 9H). MS (ESI) m/z 1177.62 $(M+H)^+$.

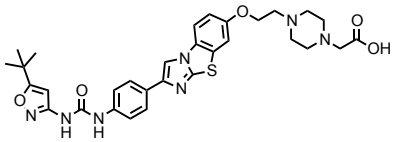
Synthesis of JB-4-066



***tert*-butyl 2-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)acetate**

1-(5-(*tert*-butyl)isoxazol-3-yl)-3-(4-(7-(2-(piperazin-1-yl)ethoxy)benzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)phenyl)urea (56.0 mg, 0.10 mmol, 1 eq) was dissolved in THF (2 mL) at room temperature. Triethylamine (41.8 microliters, 0.30 mmol, 3 eq) was added, followed by *tert*-butyl bromoacetate (22.1 microliters, 0.15 mmol, 1.5 eq). After 26 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography (ISCO, 0-15%MeOH/DCM, 15 minute gradient) to give the desired product as a cream colored solid (0.05g).

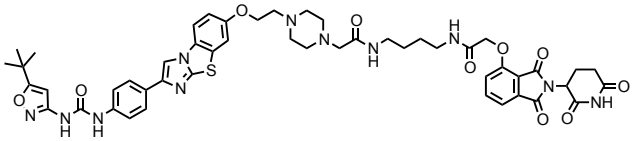
^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.04 (s, 1H), 7.75 – 7.70 (m, 2H), 7.62 (d, *J* = 8.9 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.31 (d, *J* = 2.4 Hz, 1H), 7.05 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.39 (s, 1H), 4.16 (t, *J* = 5.4 Hz, 2H), 3.11 (s, 2H), 2.85 (t, *J* = 5.4 Hz, 2H), 2.66 (d, *J* = 17.2 Hz, 8H), 1.45 (s, 9H), 1.33 (s, 9H). MS (ESI) m/z 674.65 $(M+H)^+$.



2-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)acetic acid

tert-butyl 2-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)acetate (0.05 g, 0.10 mmol) was dissolved in DCM (10 mL) at room temperature. TFA (2 mL) was added and the mixture was stirred for 21 hours. The reaction mixture was concentrated under reduced pressure, triturated with diethyl ether, then dried under vacuum to give a cream colored solid (67 mg, quantitative yield), which was used without further purification.

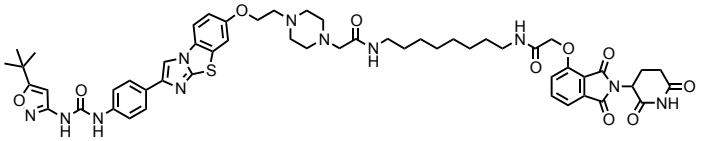
^{1}H NMR (500 MHz, Methanol-d_4) δ 8.41 (s, 1H), 7.90 (d, *J* = 8.9 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.25 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.40 (s, 1H), 4.45 – 4.40 (m, 2H), 3.58 – 3.50 (m, 4H), 3.45 – 3.34 (m, 4H), 3.13 (d, *J* = 34.1 Hz, 4H), 1.36 (s, 9H). MS (ESI) m/z 618.48 $(M+H)^+$.



JB-4-066

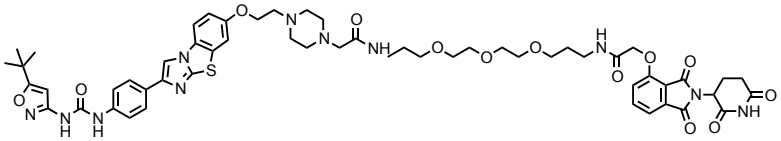
N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (10.3 mg, 0.020 mmol, 1 eq) (Winter et al., 2015) was added to 2-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)acetic acid (12.4 mg, 0.020 mmol, 1 eq) as a 0.1M solution in DMF (200 microliters) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) was added, followed by HATU (7.6 mg, 0.020 mmol, 1 eq). After 20 hours, the mixture was diluted with MeOH/DMF and purified by preparative HPLC to give the desired product as a light brown oil (10.84 mg mg, trifluoroacetate salt, 0.00971 mmol, 49%).
^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.24 (s, 1H), 7.75 (ddd, *J* = 17.8, 11.9, 8.7 Hz, 4H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 7.3 Hz, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.15 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.38 (s, 1H), 5.05 (dd, *J* = 12.5, 5.5 Hz, 1H), 4.67 (s, 2H), 4.40 (s, 2H), 3.52 (s, 2H), 3.38 (d, *J* = 26.9 Hz, 5H), 3.29 – 3.16 (m, 5H), 2.98 (d, *J* = 8.7 Hz, 4H), 2.85 – 2.70 (m, 3H), 2.17 – 2.12 (m, 1H), 1.59 (d, *J* = 5.6 Hz, 4H), 1.34 (s, 9H). MS (ESI) m/z 1002.56 $(M+H)^+$.

Synthesis of JB-4-072



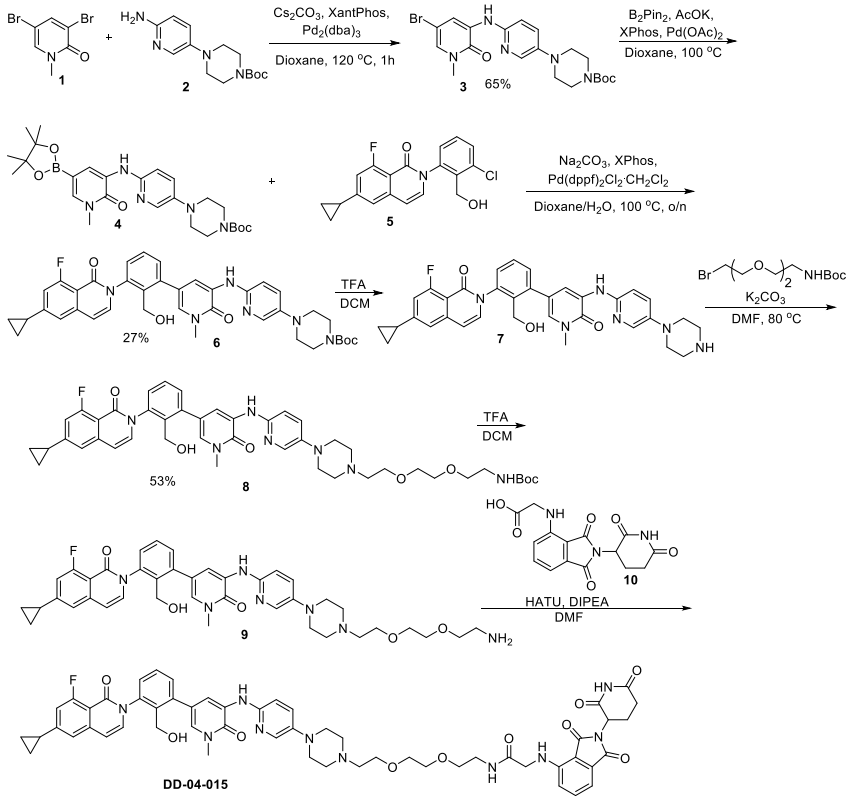
JB-4-072
N-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (11.5 mg, 0.020 mmol, 1 eq) was added to 2-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)acetic acid (12.4 mg, 0.020 mmol, 1 eq) as a 0.1M solution in DMF (200 microliters) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) was added, followed by HATU (7.6 mg, 0.020 mmol, 1 eq). After 18 hours, the mixture was diluted with MeOH and purified by preparative HPLC to give the desired product as a light brown oil (11.00 mg, trifluoroacetate salt, 0.00938 mmol, 47%).
^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 1H), 7.81 – 7.70 (m, 4H), 7.55 – 7.46 (m, 4H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.15 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.38 (s, 1H), 5.05 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.65 (s, 2H), 4.40 (s, 2H), 3.52 (s, 2H), 3.46 – 3.32 (m, 4H), 3.30 – 3.18 (m, 6H), 2.99 (d, *J* = 21.2 Hz, 4H), 2.84 – 2.72 (m, 3H), 2.17 – 2.11 (m, 1H), 1.60 – 1.54 (m, 2H), 1.52 – 1.47 (m, 2H), 1.34 (s, 17H). MS (ESI) m/z 1058.59 $(M+H)^+$.

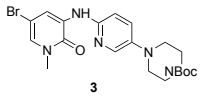
Synthesis of JB-4-073



JB-4-073
N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt (13.0 mg, 0.020 mmol, 1 eq) (Winter et al., 2015) was added to 2-(4-(2-((2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido)phenyl)benzo[*d*]imidazo[2,1-*b*]thiazol-7-yl)oxy)ethyl)piperazin-1-yl)acetic acid (12.4 mg, 0.020 mmol, 1 eq) as a 0.1M solution in DMF (200 microliters) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) was added, followed by HATU (7.6 mg, 0.020 mmol, 1 eq). After 20 hours, the mixture was diluted with MeOH/DMF and purified by preparative HPLC to give the desired product as a yellow oil (14.77 mg, trifluoroacetate salt, 0.0118 mmol, 59%).
^{1}H NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) δ 8.19 (s, 1H), 7.78 – 7.69 (m, 4H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.49 – 7.43 (m, 2H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.14 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.38 (s, 1H), 5.03 (dd, *J* = 12.4, 5.6 Hz, 1H), 4.62 (s, 2H), 4.40 (s, 2H), 3.66 – 3.48 (m, 14H), 3.45 – 3.33 (m, 6H), 3.24 (s, 4H), 2.97 (s, 4H), 2.83 – 2.71 (m, 3H), 2.14 (dt, *J* = 8.2, 4.8 Hz, 1H), 1.84 (p, *J* = 6.6 Hz, 2H), 1.77 (p, *J* = 6.3 Hz, 2H), 1.34 (s, 9H). MS (ESI) m/z 1134.60 $(M+H)^+$.

Synthesis of DD-04-015

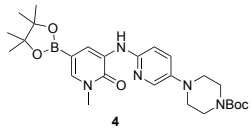




3

***tert*-butyl 4-(6-((5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)amino)pyridin-3-yl)piperazine-1-carboxylate (3).**

Compounds **1** and **2** were commercially available. Compound **1** (800 mg, 3.0 mmol, 1 equiv.), compound **2** (1.0 g, 3.6 mmol, 1.2 equiv.), Cs_2CO_3 (2.1 g, 6.4 mmol, 2.1 equiv.), and XantPhos (175 mg, 0.3 mmol, 0.1 equiv.) were dissolved in anhydrous 1,4-dioxane (15 mL) and the mixture was degassed by bubbling N_2 (g) through it for 10 min. Then $Pd_2(dba)_3$ (275 mg, 0.3 mmol, 0.1 equiv.) was added, the vial was sealed, then stirred at 120 °C for 1h. The reaction was cooled to r.t. and filtered through Celite. The filtrate was concentrated then purified via silica gel column chromatography to obtain the product as brown oil (900 mg, 1.94 mmol, 65% yield). MS (ESI) m/z 465 $(M+H)^+$.

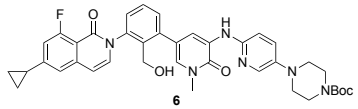


4

tert-butyl 4-(6-((1-methyl-2-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-3-yl)amino)pyridin-3-yl)piperazine-1-carboxylate (4).

Compound **3** (145 mg, 0.31 mmol, 1.0 equiv.), B_2Pin_2 (159 mg, 0.63 mmol, 2.0 equiv.), and KOAc (92 mg, 0.94 mmol, 3.0 equiv.), and $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (31 mg, 0.04 mmol, 0.1 equiv.) were stirred in anhydrous 1,4-dioxane (3 mL) at 90 °C for 2h. The reaction was cooled to r.t. and filtered through Celite. The filtrate was concentrated then used in the next step without further purification.

MS (ESI) m/z 511 $(M+H)^+$.

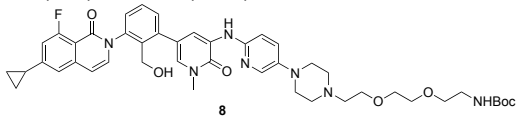


6

***tert*-butyl 4-(6-((5-(3-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)phenyl)-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)amino)pyridin-3-yl)piperazine-1-carboxylate (6).**

Compound **5** was prepared according to the literature (Lou et al., 2015). Compound **5** (487 mg, 1.4 mmol, 1.0 equiv.), compound **4** (1.45 g, 2.8 mmol, 2.0 equiv.), Na_2CO_3 (376 mg, 3.5 mmol, 2.5 equiv.), and XPhos (135 mg, 0.28 mmol, 0.2 equiv.) were dissolved in 1,4-dioxane (20 mL) and water (2.7 mL) and the mixture was degassed by bubbling N_2 (g) through it for 10 min. Then $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (116 mg, 0.14 mmol, 0.1 equiv.) was added, the vial was sealed, then stirred at 100 °C overnight. The reaction was cooled to r.t. and filtered through Celite. The filtrate was concentrated then purified via silica gel column chromatography to obtain the product as an oil (259 mg, 0.374 mmol, 26% yield).

MS (ESI) m/z 693 $(M+H)^+$.

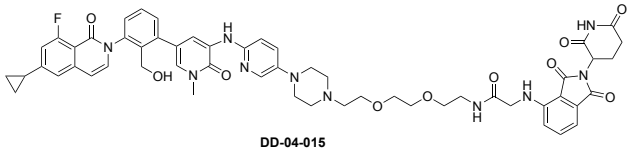


8

***tert*-butyl (2-(2-(2-(4-(6-((5-(3-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)phenyl)-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)amino)pyridin-3-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)carbamate (8).**

To a solution of compound **6** (259 mg, 0.374 mmol, 1.0 equiv.) in DCM (2 mL) was added trifluoroacetic acid (2 mL) then stirred for 30 min. The solution was concentrated then redissolved in DMF (1 mL). *Tert*-butyl (2-(2-(2-bromoethoxy)ethoxy)ethyl)carbamate (117 mg, 0.3 mmol, 1 equiv.) and K_2CO_3 (92 mg, 0.67 mmol, 1.8 equiv.) were added and stirred in a sealed vial at 80 °C for 3h. The reaction was cooled to r.t. then purified using an HPLC with a reverse phase C18 column to afford the product as an oil (131 mg, 0.16 mmol, 53% yield over two steps).

MS (ESI) m/z 825 $(M+H)^+$.



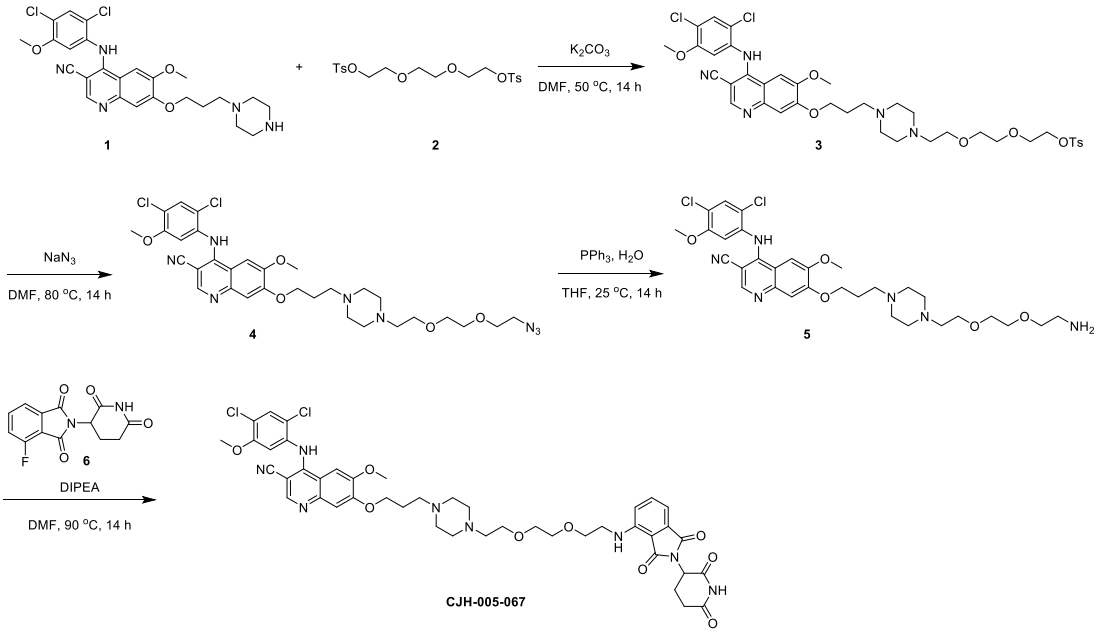
DD-04-015

N-(2-(2-(2-(4-(6-((5-(3-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)phenyl)-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)amino)pyridin-3-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)acetamide (DD-04-015)

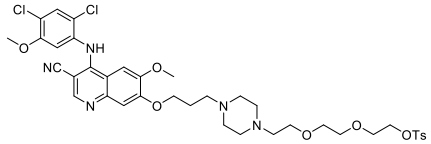
To a solution of compound **8** (58 mg, 0.07 mmol, 1.0 equiv.) in DCM (1 mL) was added trifluoroacetic acid (1 mL) then stirred for 30min. The solution was concentrated then redissolved in DMF (1 mL). HATU (35 mg, 0.09 mmol, 1.3 equiv.), DIPEA (37 µL, 0.2 mmol, 3.0 equiv.), and compound **10** (28 mg, 0.08 mmol, 1.2 equiv.) were added and the solution stirred for 1h, then purified using an HPLC with a reverse phase C18 column to afford the product as a white solid (10 mg, 0.009 mmol, 15% yield over two steps).

^{1}H NMR (500 MHz, DMSO) δ 11.10 (s, 1H), 8.56 (d, *J* = 2.2 Hz, 1H), 8.36 (s, 1H), 8.16 (t, *J* = 5.6 Hz, 1H), 7.85 (d, *J* = 2.5 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.46 – 7.40 (m, 1H), 7.37 – 7.29 (m, 4H), 7.26 (d, *J* = 1.3 Hz, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 7.05 (t, *J* = 11.8 Hz, 1H), 7.02 – 6.90 (m, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 6.58 (dt, *J* = 17.1, 8.5 Hz, 1H), 5.07 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.76 (t, *J* = 4.4 Hz, 1H), 4.31 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.26 – 4.15 (m, 1H), 3.93 (d, *J* = 5.6 Hz, 2H), 3.66 – 3.47 (m, 10H), 3.44 (dd, *J* = 12.4, 6.4 Hz, 2H), 3.26 (dd, *J* = 11.5, 5.8 Hz, 2H), 2.99 (d, *J* = 30.9 Hz, 4H), 2.94 – 2.82 (m, 1H), 2.14 – 1.94 (m, 2H), 1.16 – 1.03 (m, 2H), 0.93 – 0.78 (m, 2H). MS (ESI) m/z 1038 $(M+H)^+$.

Synthesis of CJH-005-067

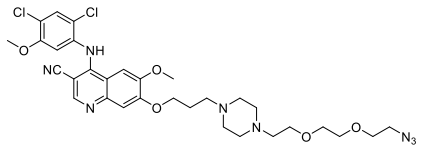


Preparation of **1** was previously described (Boschelli et al., 2001).
Preparation of **6** was previously described (Lu et al., 2015).



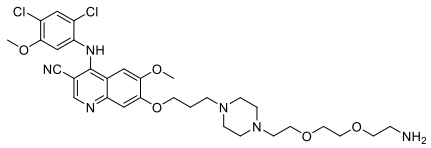
2-(2-(2-(4-(3-((3-cyano-4-((2,4-dichloro-5-methoxyphenyl)amino)-6-methoxyquinolin-7-yl)oxy)propyl)piperazin-1-yl)ethoxy)ethoxy)ethyl-4-methylbenzenesulfonate (3)

To a solution of **1** (1.0 eq) and **2** (1.0 eq) in DMF (0.2 M) was added K_2CO_3 (4.0 eq) at 25 °C. The mixture was heated to 50 °C and then stirred for 14 h. After the reaction, the mixture was cooled to room temperature, poured into water, and extracted with EtOAc (x3). The combined organic layer was washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2 to 1:10 MeOH:CH_2Cl_2) to afford **3**.
MS (ESI) m/z 802 $(M+H)^+$.



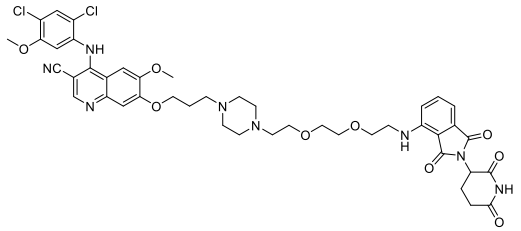
7-(3-(4-(2-(2-(2-azidoethoxy)ethoxy)ethyl)piperazin-1-yl)propoxy)-4-((2,4-dichloro-5-methoxyphenyl)amino)-6-methoxyquinoline-3-carbonitrile (4)

To a solution of **3** (1.0 eq) in DMF (0.1 M) was added NaN_3 (2.0 eq) at 25 °C. The mixture was heated to 80 °C and then stirred for 14 h. After the reaction, the mixture was cooled to room temperature, poured into water, and extracted with EtOAc (x3). The combined organic layer was washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The resulting residue (**4**) was used to next step without further purification.
MS (ESI) m/z 673 $(M+H)^+$.



7-(3-(4-(2-(2-(2-aminoethoxy)ethoxy)ethyl)piperazin-1-yl)propoxy)-4-((2,4-dichloro-5-methoxyphenyl)amino)-6-methoxyquinoline-3-carbonitrile (5)

To a solution of **4** (1.0 eq) in THF (0.1 M) was added PPh_3 (1.5 eq) and water (1.5 eq) at 25 °C. The mixture was stirred for 14 h, and then the solvent was removed. The crude residue was purified by flash chromatography (CH_2Cl_2 to 1:10 MeOH (5% NH_4OH):CH_2Cl_2) to afford **5**.
MS (ESI) m/z 647 $(M+H)^+$.



4-((2,4-dichloro-5-methoxyphenyl)amino)-7-(3-(4-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)piperazin-1-yl)propoxy)-6-methoxyquinoline-3-carbonitrile (CJH-005-067)
To a solution of **5** (1.0 eq) and **6** (1.0 eq) in DMF (0.1 M) was added DIPEA (2.0 eq) at 25 °C. The mixture was heated to 90 °C, and then stirred for 14 h. After the reaction, the mixture was cooled to room temperature, poured into water, and extracted with EtOAc (x3). The combined organic layer was washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The resulting residue was purified by preparative HPLC to afford the desired compound. Yield: 12 %, yellow oil.
^{1}H NMR (400 MHz, d_6-DMSO) d 11.13 (s, 1H), 8.74 (s, 1H), 7.97 (s, 1H), 7.82 (s, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.06 (d, *J* = 7.0 Hz, 1H), 6.60 (br, 1H), 5.07 (m, 1H), 4.42 (t, *J* = 5.4 Hz, 2H), 4.12 (br, 8H), 3.95 (s, 3H), 3.89 (m, 3H), 3.87 (s, 3H), 3.64 (m, 4H), 3.48 (m, 2H), 3.16 (br, 4H), 2.84-3.01 (m, 4H), 2.17 (m, 2H), 2.04 (m, 2H). MS (ESI) m/z 903 $(M+H)^+$. HPLC (R_t = 4.84 min).