Supplementary Information (SI) for RSC Advances.
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Supporting Information

OSMI-4-based PROTACs do not influence O-GlcNAc transferase and O-GlcNAcylation levels in cells

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Supporting Tables, Schemes and Figures

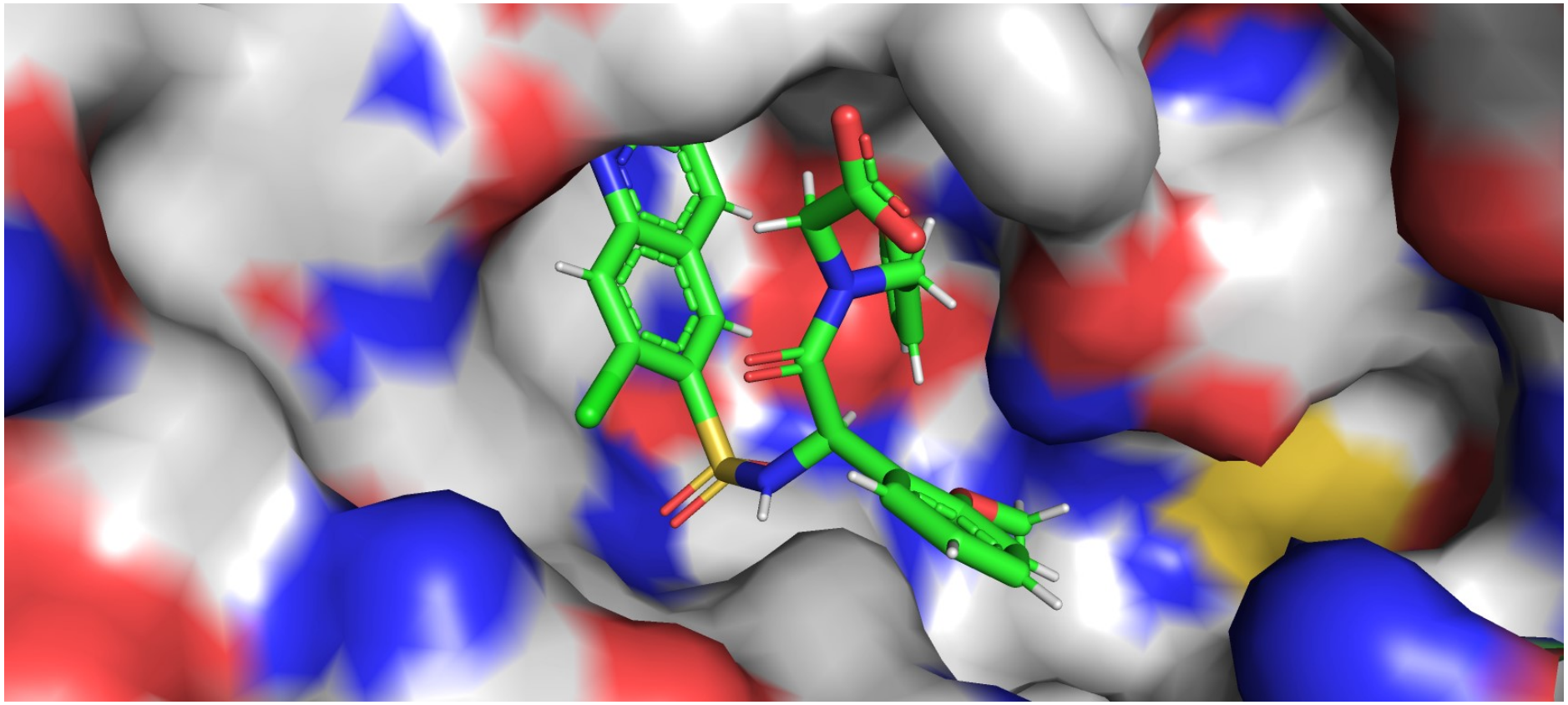


Figure S1: OSMI-4 (in sticks coloured by element: carbon – green, hydrogen – white, oxygen – red, nitrogen – blue, suphur – yellow) in the OGT (presented as surface) UDP-GlcNAc binding site (taken from PDB: 6MA1): the free carboxylate of OSMI-4 points towards the solvent and is a plausible attachment point for a linker coupled with E3 ligase ligand.

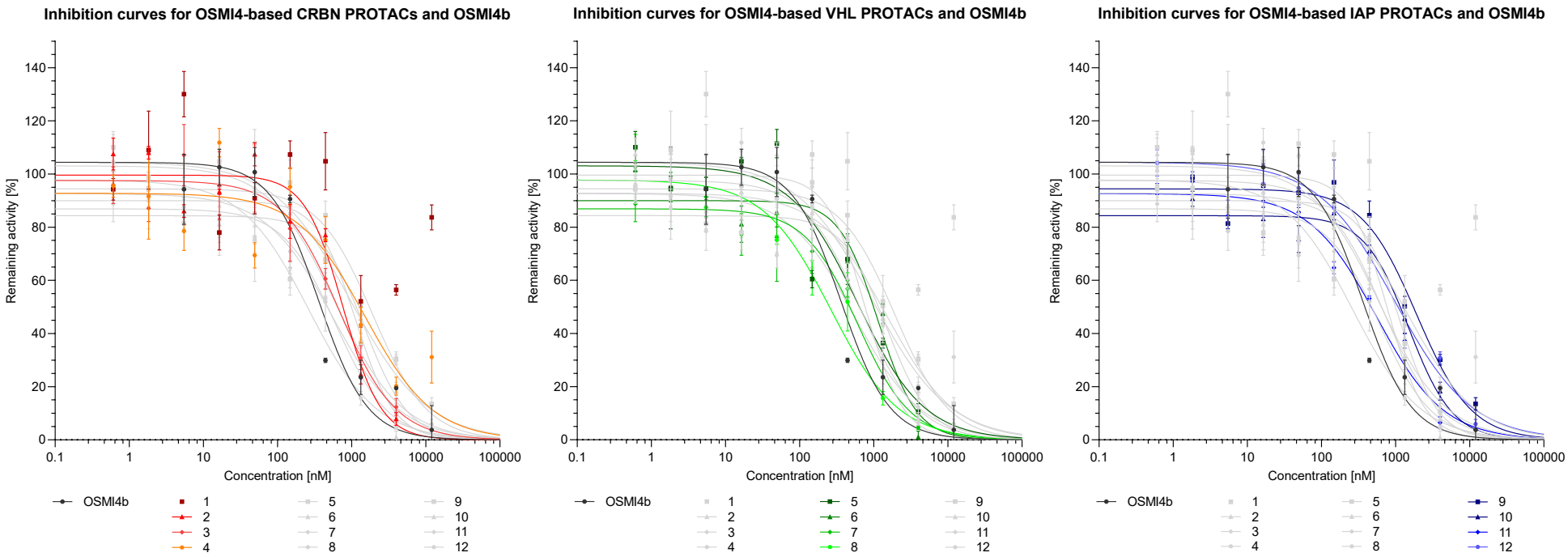


Figure S2: Calculated inhibition curves for compounds from different OSMI4-based compounds and OSMI4b. Graphs correspond to each series a) CRBN PROTACs, b) VHL PROTACs, and c) IAP PROTACs: curves for compounds from the corresponding series are colorized (red, green, blue shades), while inhibition curves for all other compounds are shown in grey. The inhibition curve for OSMI4b is shown in black. Data are given as mean values (n=3) ± SEM

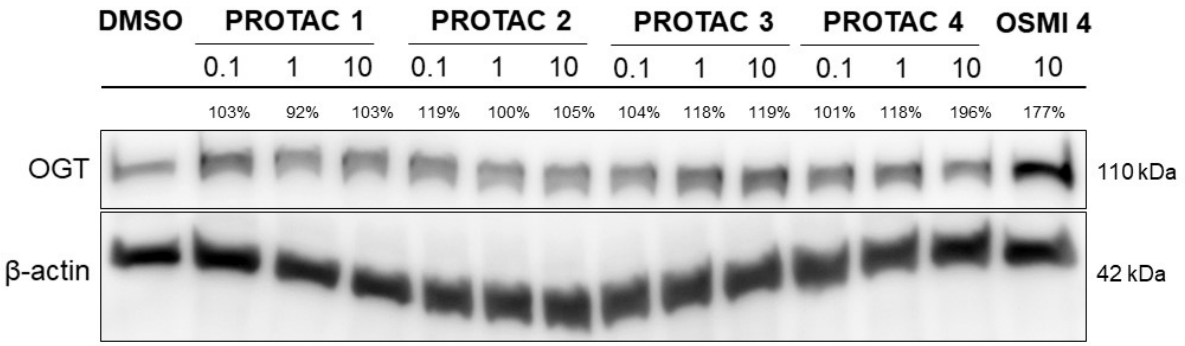


Figure S3: Representative western blot depicting OGT protein levels in MM.1S cells after 16 h treatment with CRBN-series PROTACs **1**-**4** at 0.1, 1, and 10 µM concentration. β-actin was used as loading control. Data represents an average of two independent experiments.

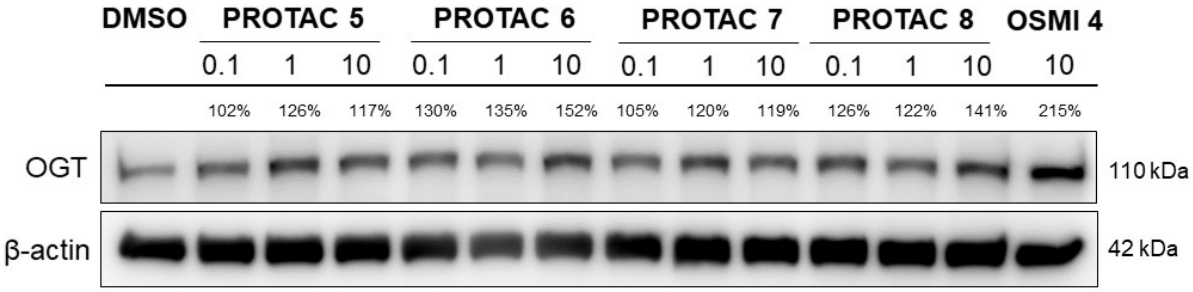


Figure S4: Representative western blot depicting OGT protein levels in MM.1S cells after 16 h treatment with VHL-series PROTACs **5**-**8** at 0.1, 1, and 10 µM concentration. β-actin was used as loading control. Data represents an average of two independent experiments.

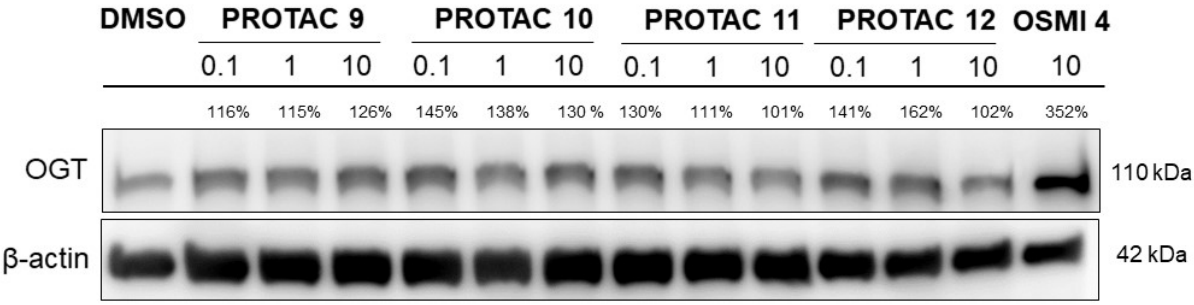


Figure S5: Representative western blot depicting OGT protein levels in MM.1S cells after 16 h treatment with IAP-series PROTACs **9**-**12** at 0.1, 1, and 10 µM concentration. β-actin was used as loading control. Data represents an average of two independent experiments.

E3 ligase ligands

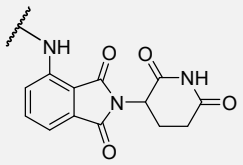
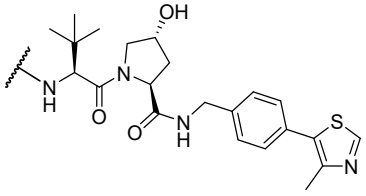
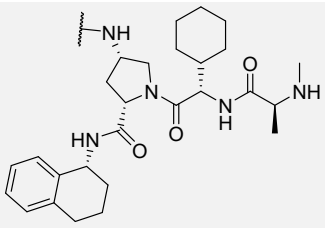
CRBN Series	 CRBN ligand
VHL Series	 VHL ligand
IAP Series	 IAP ligand

Table S1. Overview of different E3 ligase ligands incorporated into final OGT PROTACs.

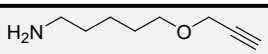
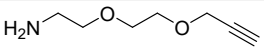
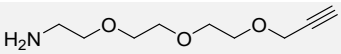
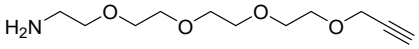
Linker	Pattern	Structure
L1a	5	
L2a	2-2	
L3a	2-2-2	
L4a	2-2-2-2	

Table S2. Structures of linkers used in the CRBN Series PROTACs.

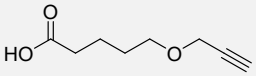
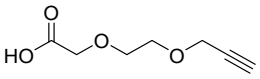
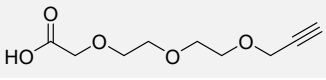
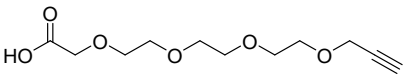
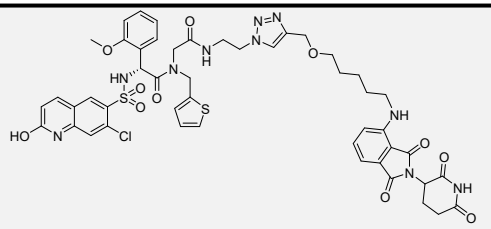
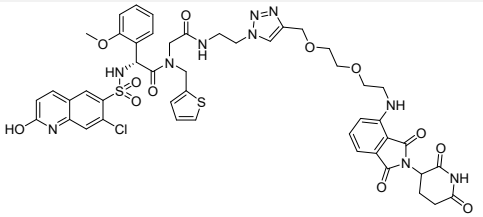
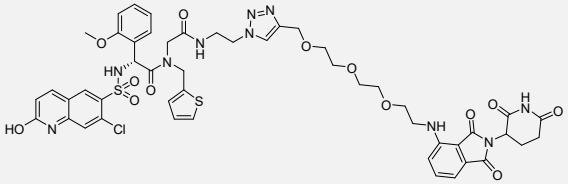
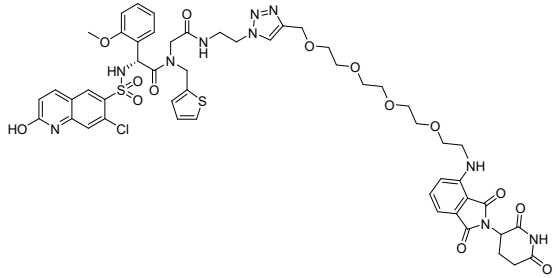
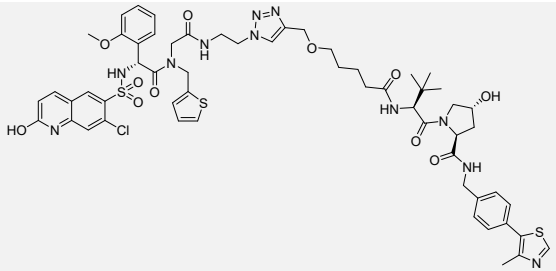
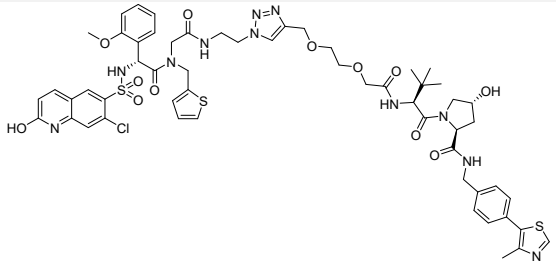
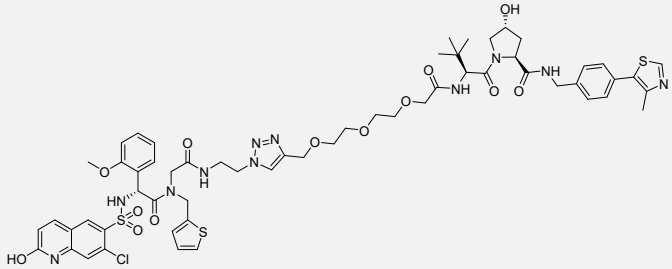
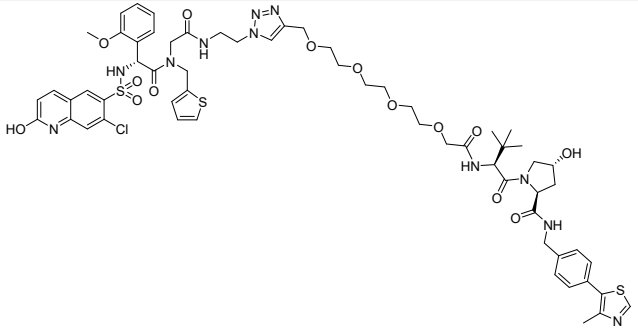
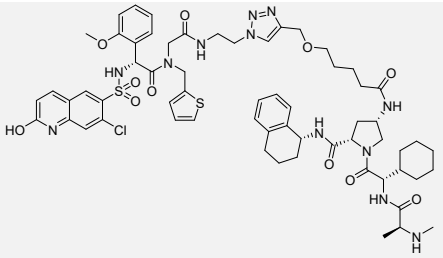
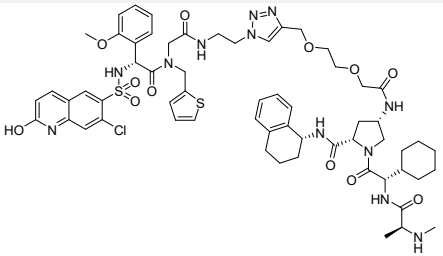
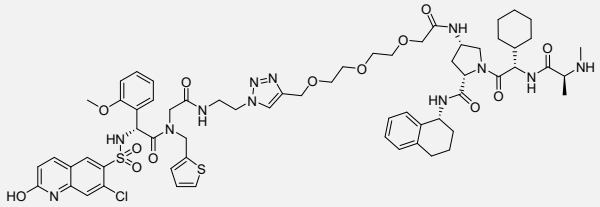
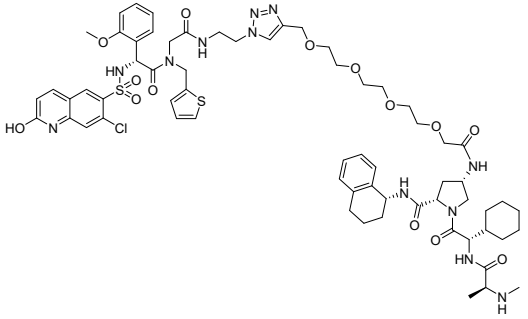
Linker	Pattern	Structure
L1b	5	
L2b	2-2	
L3b	2-2-2	
L4b	2-2-2-2	

Table S3. Structures of linkers used in the IAP and VHL Series PROTACs.

Cmpd	MW (g/mol)	logD[a]	TPSA[b] (Å^2)	NRotB[c]	HBD[d]	HBA[e]	Structure
1	1042	2.8	273	25	5	14	
2	1044	2.4	283	25	5	15	
3	1088	2.5	292	28	5	16	
4	1132	2.5	301	31	5	17	

5	1213	2.4	290	34	6	15	
6	1215	2.3	299	34	6	16	
7	1259	2.3	308	37	6	17	
8	1303	2.3	317	40	6	18	

9	1266	2.6	298	32	7	14	
10	1268	2.4	307	32	7	15	
11	1312	2.5	316	35	7	16	
12	1456	2.5	325	38	7	17	

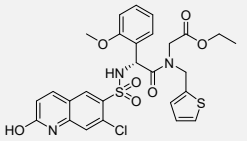
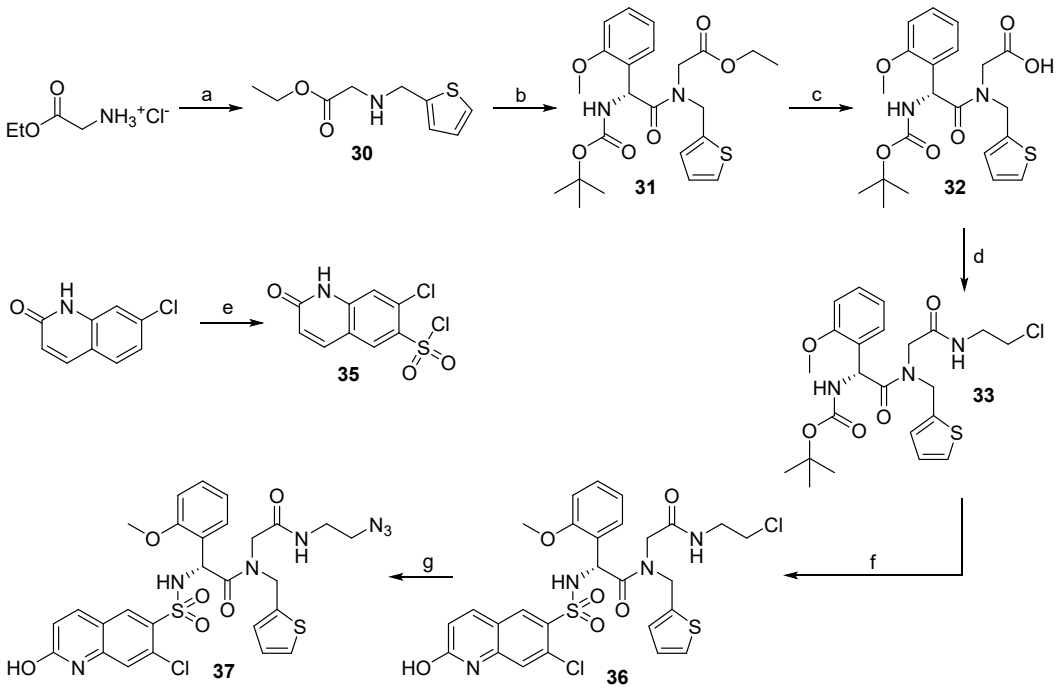
OSMI-4	604	2.8	

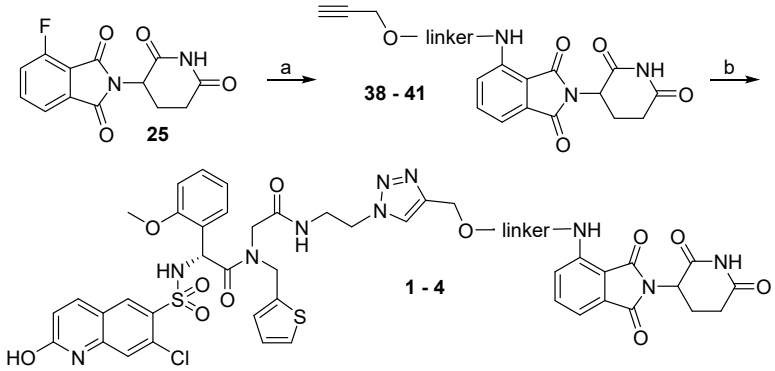
Table S4. Overview on synthesized OGT PROTACs and OSMI-4. [a] Experimental partition coefficient at *p*H 7.4 determined by a fast-gradient HPLC method. [b] Topological polar surface area is given in Å². [c] Number of rotatable bonds. [d] Number of hydrogen bond donors. [e] Number of hydrogen bond acceptors. Molecular descriptor values were obtained using LigandScout 4.4.3.

Scheme S1. Synthesis of OGT ligand **37**[1,2]



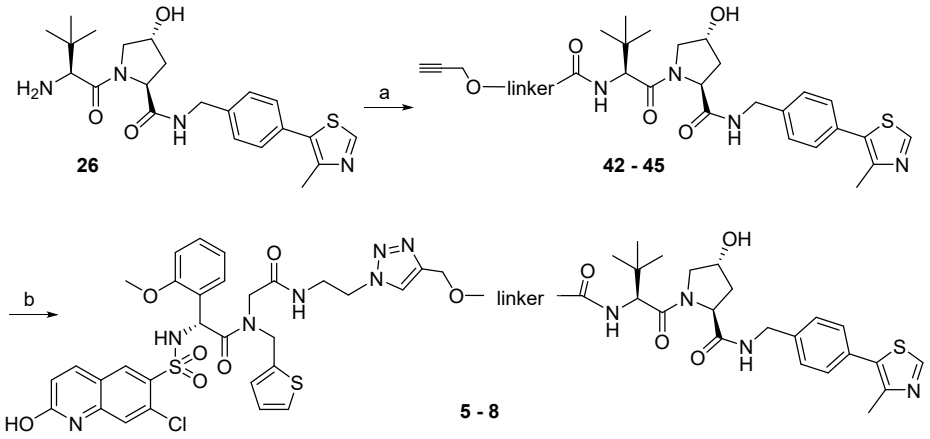
Reagents and conditions: (a) i. thiophene-2-carboxaldehyde, Et_3N, MeOH, rt, 90 min; ii. $NaBH_4$, rt, 30 min; (b) (*R*)-2-((*tert*-butoxycarbonyl)amino)-2-(2-methoxyphenyl)acetic acid, HATU, DIPEA, DMF, rt, 18 h; (c) 1 M LiOH (aq), THF, rt, 5 h; (d) 2-chloroethan-1-amine, HATU, DIPEA, DMF, rt, 18 h; (e) HSO_3Cl, 160 °C, 4 h; (f) i. 4 M HCl in dioxane, CH_2Cl_2, rt, 2 h; ii. **35,** DIPEA, DMF, rt, 18 h; (g) NaN_3, DMF, 80 °C, 4 h.

Scheme S2. Synthesis of OGT-CRBN PROTACs **1**-**4**.[3]



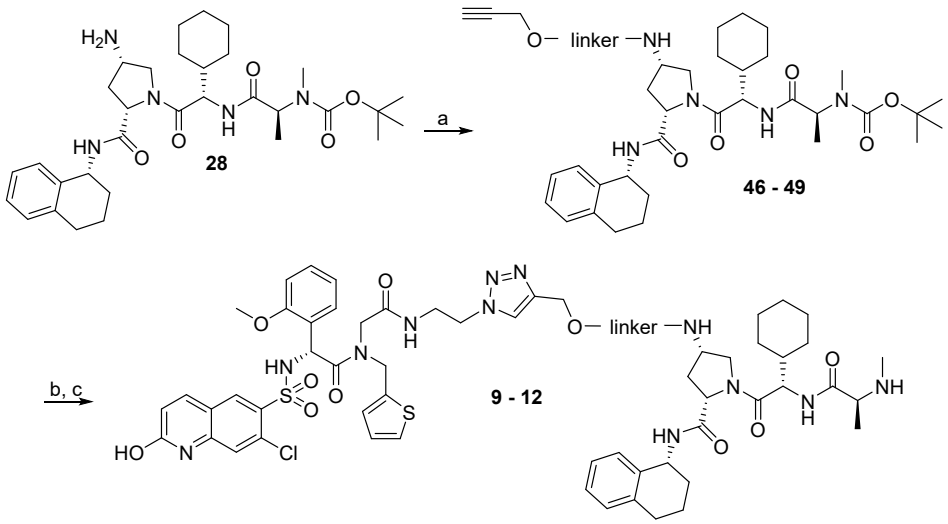
Reagents and conditions: (a) linkers **L1a-L4a** (Table S2), DIPEA, DMSO, 90 °C, 18 h; (b) **37,** $CuSO_4$, sodium ascorbate, THF, H_2O, rt, 18 h.

Scheme S3. Synthesis of the OGT-VHL PROTACs **5**-**8**.



Reagents and conditions: (a) linkers **L1b**-**L1b** (Table S3), HATU, DIPEA, DMF, rt, 18 h; (b) **37,** $CuSO_4$, sodium ascorbate, THF, H_2O, rt, 18 h.

Scheme S4. Synthesis of the OGT-IAP PROTACs **9**-**12**.



Reagents and conditions: (a) linkers **L1b**-**L1b** (Table S3), HATU, DIPEA, CH_2Cl_2, rt, 18 h; (b) **37,** $CuSO_4$, sodium ascorbate, THF, H_2O, rt, 18 h; (c) 4 M HCl in dioxane, CH_2Cl_2, rt, 2 h.

Supporting Information: Biology

IC_{50} determination

OGT reactions were carried out in a 25-µL final volume on Corning® black half-area 96-well plates (Corning, Corning, NY, USA), containing 5.6 µM glycosyl donor Bodipy Fluorescent Labeled-UDP-GlcNAc, 250 nM purified full-length OGT (batch: 18/07/2022, 2nd part of the peak), 9.2 µM glycosyl acceptor HCF-1 Serine (biotinylated substrate) in 1x OGT reaction buffer (1 × PBS pH 7.4, 1 mM DTT, 12.5 mM $MgCl_2$). The inhibitors were preincubated with OGT for 10 min on ice. Reactions were incubated at room temperature for 1 h, in the presence of different concentrations of inhibitor (range of final plate concentrations: 12 µM – 0.61 nM). The reactions were then stopped by the addition of UDP at a final concentration of 2 mM and Nanolink® magnetic streptavidin beads (Vector Laboratories, Burlingame, CA, USA) (3 µL stock solution/well). The plate was centrifuged at 300 RPM for 1 min after addition of each reagent. After incubation at room temperature for another 30 min, the beads were immobilized on a magnetic surface and washed thoroughly with PBS-tween 0.01% (5-times). Finally, the beads were resuspended in PBS-tween 0.01 % for the endpoint fluorescence intensity measurement. Fluorescence was read at Ex/Em 485/535 (bandwidth: 20 nm, 30 flashes, 4x4 reads per well, Z-position: 19700) on TECAN® Spark microplate reader (Tecan Trading AG, Switzerland) after shaking (orbital, 10s, 510 RPM). The data were plotted with GraphPad Prism software (San Diego, CA, USA), version 8, as percentage of remaining maximum activity (measured in the absence of inhibitors). IC_{50} values were calculated using a built in GraphPad Prism analysis with sigmoidal four-point logistic function as fit, constrained at bottom to 0% activity. The experiments were done in triplicate. Adapted from Loi E.M. et al.[2]

Cell Line

MM.1S were provided from prof. Jan Krönke (Charite, Berlin, Germany) and maintained in RPMI-1640 medium (Sigma-Aldrich, Darmstadt, Germany) containing 10% fetal bovine serum (FBS) and supplemented with 1% penicillin/streptomycin and 1% L-glutamine. Cells were maintained at 37 °C with 5% CO_2 in humidified atmosphere.

Immunoblotting

MM.1S were cultured at a density of 1×10^6 cells per mL and treated by the addition of compounds of interest or corresponding vehicle. After 16h cells were harvested, washed with ice-cold PBS and lysed in modified RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5 % Na-deoxycholate, 0.1% SDS, 1 mM EDTA, 1x Halt Phosphatase inhibitor cocktail and 1x Halt Protease inhibitor cocktail (Thermo Scientific)). The lysates were sonicated and centrifuged at 15000 × g at 4 °C for 20 min. Samples containing 20 µg of heat deanturated proteins (96 °C for 5 min) in a sample loading buffer (3% SDS, 10 % glycerol, 62.5 mM Tris–HCl, pH 6.8, 5 % 2-mercaptoethanol and 0.1 % bromphenol blue) were electrophoresed in SDS-polyacrylamide gels and then transferred to PVDF membranes with iBlot2 (Invitrogen). Nonspecific binding sites were blocked for 1 h at room temperature in 5% BSA in TTBS (tris-buffered saline, 0.1% Tween). The membranes were incubated in primary antibodies overnight at 4 °C. Afterwards, membranes were washed and incubated for 1 h at room temperature appropriate secondary antibody conjugated with horseradish peroxidase. Afterwards, SuperSignal West Femto substrate was added and chemiluminescence was detected (Uvitec, Cambridge Alliance). The band intensities were quantified using the Uvitec Imager. To ensure the equal loading of proteins, the membranes were stripped (100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris/HCl, pH = 6.8)

for 45 min at 50 °C and re-probed with appropriate antibodies under the same conditions as those described above. The stripping was performed on membranes depicted in Figure 5A and 5B, where proteins were first detected using O-GlcNAc MultiMab® Rabbit mAb mix antibody and anti-O-linked N-Acetylglucosamine antibody [RL2]. The stripped membranes were then re-probed with antibodies for OGT and β-actin.

Most of the used antibodies were obtained from Cell Signalling Technology and include OGT (#24083), cIAP1 (#7065), IKZF3 (#15103), O-GlcNAc MultiMab® Rabbit mAb mix (#82332), and tubulin (#2148). Anti-*O*-linked *N*-Acetylglucosamine antibody [RL2] was obtained from abcam, while anti-β-Actin (ACTB) Antibody was obtained from Sigma-Aldrich. Secondary antibodies include anti-rabbit IgG HRP (# 7074) and anti-mouse IgG HRP (# 7076), both obtained from Cell Signalling Technology. Quantification of immunoblots was performed using Uvitec Imager (Cambridge).

Cell proliferation and viability

For proliferation analysis, the CellTrace™ Cell Proliferation Kit (Invitrogen Molecular Probes) was utilized following the manufacturer's instructions. Briefly, cells were resuspended in PBS and incubated with CFSE (5 µM) for 10 minutes at 37 °C. Afterward, the cells were washed and resuspended in culture medium, followed by treatment with the compound of interest. Cells were harvested at various time points (24 h, 48 h, 72 h, or 96 h) and analyzed using the Attune NxT flow cytometer (ThermoFisher). The data presented represents the mean ± SD of biological duplicates.

Statistical Analysis

Statistical and graphical analyses of cell viability experiments were performed with Prism version 9.1.0 (GraphPad Software, San Diego, CA, USA).

Supporting Information: Chemistry

G. Molecular descriptor calculations

Predicted values for the topological polar surface area (TPSA), number of rotatable bonds (NRotB), number of hydrogen bond donors (NBD) and acceptors (NBA) were calculated using LigandScout 4.4.3.

H. logD measurements

The determination of the $logD_{7.4}$ values was performed by a chromatographic method as described previously.[4,5] The system was calibrated by plotting the retention times of six different drugs (atenolol, metoprolol, labetalol, diltiazem, triphenylene, permethrin) versus their literature known $logD_{7.4}$ in a calibration line (R^2 = 0.99). Subsequently, the mean retention times of the analytes were taken to calculate their $logD_{7.4}$ values with aid of the calibration line. At least two independent measurements of each analyte were performed.

I. General Remarks

Preparative column chromatography was performed using Merck silica gel 60 (0.063 – 0.200 mm) or using an automated flash chromatography system Biotage Isolera One. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer, Bruker Avance 500 MHz NMR spectrometer, or on a Bruker Avance III 600 MHz NMR spectrometer, respectively. NMR spectra were processed and analyzed in MestReNova. Chemical shifts are given in parts per million (ppm) and are referenced to the deuterated solvent used. Coupling constants *J* are given in Hertz, and the splitting patterns are given as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). *Important note*: The presence of amide rotamers significantly complicated the appearance and validation of the ^{1}H and

^{13}C NMR spectra associated with synthetic intermediates and final PROTACs. Thus, reported resonances and integrals may have limited accuracy. High-resolution mass measurements were recorded on Thermo Scientific Q Exactive Plus mass spectrometer (Thermo Fisher Scientific). The purity of compounds was determined using analytical reversed-phase HPLC on Thermo Scientific Dionex UltiMate 3000 UHPLC modular system (Thermo Fisher Scientific), equipped with a photodiode array detector set to 254 nm. A Waters Acquity UPLC® HSS C18 SB column (1.8 µm, 2.1 mm × 50 mm) was used, thermostated at 40 °C. The mobile phase consisted of 0.1% TFA in H_2O (A) and MeCN (B), employing the following gradient: 95% A to 5% A in 10 min, then 95% B for 4 min, with flow rate of 0.3 mL/min and injection volume of 5 µL. The purities of all test compounds used for the biological evaluations were ≥95%.

J. General procedures

General Procedure I: Etherification of diols with propargyl bromide

To a solution of the corresponding diol (5 mmol) in toluene (5 mL) and 50% NaOH (aq) (5 mL), Bu_4NHSO_4 (0.34 g, 1 mmol) was added and the mixture was cooled to 0 °C. Subsequently, 80% propargyl bromide in toluene (0.12 g, 109 µL, 1 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 18 h. Afterwards, H_2O (50 mL) was added, and the product was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column or flash chromatography.

General procedure II: TEMPO-mediated oxidation of alcohols to carboxylic acids

The corresponding alcohol (5.6 mmol) and BAIB (3.9 g, 12.4 mmol) were dissolved in MeCN/H_2O = 1:1 (20 mL) and then TEMPO (0.22 g, 1.4 mmol) was added. The mixture was stirred at room temperature for 18 h. After evaporation of MeCN, the solution was acidified using 2 M HCl (aq) and the product was extracted with EtOAc (100 mL). The organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column or flash chromatography.

General procedure III: HATU coupling of the VHL ligand and linkers L1b-L4b

The corresponding linker **L1b**-**L4b** (0.38 mmol) was dissolved in dry DMF (5 mL), and DIPEA (0.10 g, 133 µL, 0.77 mmol) was added, followed by the addition of HATU (0.16 g, 0.42 mmol) under argon atmosphere. After stirring for 5 min, VHL ligand **26** (0.18 g, 0.42 mmol) dissolved in dry DMF (5 mL) and DIPEA (0.21 g, 268 µL, 1.54 mmol) were added. The combined mixture was stirred at room temperature for 18 h, after which brine (50 mL) was added, and the product was extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with saturated NH_4Cl solution and brine (each 50 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column or flash chromatography.

General procedure IV: HATU coupling of the IAP ligand and linkers L1b-L4b

The corresponding linker **L1b**-**L4b** (0.34 mmol) was dissolved in dry CH_2Cl_2 (5 mL), and DIPEA (0.14 g, 179 µL, 1.08 mmol) was added, followed by the addition of HATU (0.14 g, 0.38 mmol) under argon atmosphere. After stirring for 5 min, a solution of IAP ligand **28** (0.20 g, 0.34 mmol) in dry CH_2Cl_2 (5 mL) was added. The combined mixture was stirred at room temperature for 18 h, after which the volatiles were evaporated, and the crude product was purified by column or flash chromatography.

General procedure V: Click chemistry azide-alkyne cycloaddition

The corresponding E3 ligase ligand - linker conjugate (**38**-**49**) (0.18 mmol), OGT ligand **37** (113 mg, 0.18 mmol), $CuSO_4$ (6 mg, 35 µmol) and sodium ascorbate (7 mg, 35 µmol) were dissolved in THF (5 mL). Then H_2O (0.5 mL) was added and the mixture was stirred at room temperature for 18 h. After removal of the volatiles, the crude product was purified by column or flash chromatography.

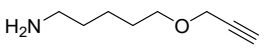
General Procedure VI: Removal of Boc protecting groups.

A solution of the Boc-protected precursor in CH_2Cl_2 (5 mL) was treated with 4 M HCl in dioxane (5 mL) and the mixture was stirred at rt for 2 h. After removal of the volatiles, the crude product was purified by column or flash chromatography.

K. Synthesis of linkers

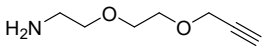
a) Syntheses of L1a-L4a linkers

L1a: 5-(prop-2-yn-1-yloxy)pentan-1-amine (13)



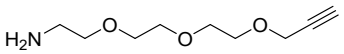
This compound was synthesized as we described previously.[3]

L2a: 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethan-1-amine (14)



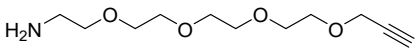
This compound was synthesized as we described previously.[3]

L3a: 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-amine (15)



This compound was synthesized as we described previously.[3]

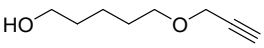
L4a: 3,6,9,12-tetraoxapentadec-14-yn-1-amine (16)



This compound was synthesized as we described previously.[3]

b) Syntheses of L1b-L4b linkers

5-(prop-2-yn-1-yloxy)pentan-1-ol (17)

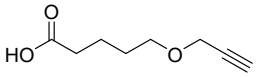


This compound was prepared using the General Procedure **I** and 1,5-pentanediol (5.2 g, 50 mmol). The crude product was purified by column chromatography (EtOAc/*n*-hexanes 4:1) to give an orange oil.

Yield (1.2 g, 84%); R_f = 0.40 (EtOAc/*n*-hexanes 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.41 – 1.47 (m, 2H), 1.56 – 1.66 (m, 4H), 2.41 (t, *J* = 2.4 Hz, 1H), 3.52 (t, *J* = 6.5 Hz, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 4.13 (d, *J* =

2.4 Hz, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 22.32, 29.19, 32.42, 58.03, 62.70, 70.05, 74.18, 79.92; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_8H_{15}O_2$, 143.1067; found, 143.1066.

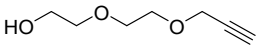
L1b: 5-(prop-2-yn-1-yloxy)pentanoic acid (18)



This compound was prepared using the General Procedure **II** and **17** (1.35 g, 9.5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a colourless oil.

Yield (0.99 g, 67%); R_f = 0.22 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.49 – 1.61 (m, 4H), 2.19 – 2.25 (m, 1H), 3.39 – 3.45 (m, 2H), 3.98 (t, *J* = 6.0 Hz, 2H), 4.09 (d, *J* = 2.4 Hz, 2H), 12.05 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 22.64, 29.61, 32.84, 58.45, 70.47, 74.60, 80.34, 171.73; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_8H_{11}O_3$, 155.0714; found, 155.0705.

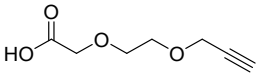
2-(2-(prop-2-yn-1-yloxy)ethoxy)ethan-1-ol (19)



This compound was prepared using the General Procedure **I** and diethylene glycol (5.3 g, 50 mmol). The crude product was purified by column chromatography (EtOAc/*n*-hexanes 4:1) to give an orange oil.

Yield (0.81 g, 56%); R_f = 0.20 (EtOAc/*n*-hexanes 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.44 (t, *J* = 2.4 Hz, 1H), 3.56 – 3.61 (m, 2H), 3.66 – 3.74 (m, 6H), 4.19 (d, *J* = 2.4 Hz, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 58.52, 61.83, 69.20, 70.31, 72.62, 74.83, 79.55; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_7H_{13}O_3$, 145.0859; found, 145.0857.

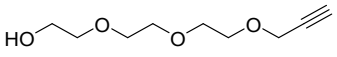
L2b: 2-(2-(prop-2-yn-1-yloxy)ethoxy)acetic acid (20)



This compound was prepared using the General Procedure **II** and **19** (0.53 g, 3.7 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a colourless oil.

Yield (0.5 g, 85%); R_f = 0.22 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.43 (t, *J* = 2.4 Hz, 1H), 3.78 (s, 4H), 4.11 (s, 2H), 4.24 (d, *J* = 2.3 Hz, 2H), 13.59 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 58.58, 68.52, 68.98, 71.02, 75.17, 79.19, 174.12; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_7H_9O_4$, 157.0506; found, 157.0499.

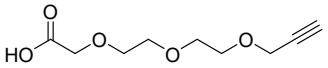
2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-ol (21)



This compound was prepared using the General Procedure **I** and triethylene glycol (15 g, 100 mmol). The crude product was purified by column chromatography (EtOAc/*n*-hexanes 4:1) to give an orange oil.

Yield (1.5 g, 40%); R_f = 0.14 (EtOAc/*n*-hexanes 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.43 (t, *J* = 2.4 Hz, 1H), 2.52 (t, *J* = 6.2 Hz, 1H), 3.58 – 3.62 (m, 2H), 3.64 – 3.74 (m, 10H), 4.19 (d, *J* = 2.4 Hz, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 58.53, 61.87, 69.19, 70.46, 70.50, 70.75, 72.59, 74.71, 79.69; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_9H_{15}O_4$, 187.0979; found, 187.0961.

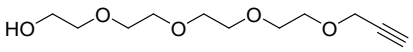
L3b: 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)acetic acid (22)



This compound was prepared using the General Procedure **II** and **21** (0.49 g, 2.6 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a colourless oil.

Yield (0.41 g, 79%); R_f = 0.14 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.44 (t, *J* = 2.4 Hz, 1H), 3.66 – 3.74 (m, 8H), 4.11 (s, 2H), 4.19 (d, *J* = 2.4 Hz, 2H), 10.09 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 58.49, 68.96, 70.28, 70.35, 70.76, 74.91, 79.57, 176.68; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_9H_{13}O_5$, 201.0769; found, 201.0761.

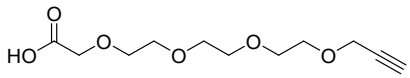
3,6,9,12-tetraoxapentadec-14-yn-1-ol (23)



This compound was prepared using the General Procedure **I** and tetraethylene glycol (0.97 g, 5 mmol). The crude product was purified by column chromatography (EtOAc/*n*-hexanes 2:1) to give an orange oil.

Yield (154 mg, 66%); R_f = 0.16 (EtOAc/n-hexanes 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.57 (d, *J* = 6.0 Hz, 1H), 3.60 – 3.63 (m, 2H), 3.64 – 3.75 (m, 14H), 4.20 (d, *J* = 2.4 Hz, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 58.50, 61.82, 68.95, 70.22, 70.31, 70.55, 70.87, 72.31, 74.84, 79.35; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{11}H_{19}O_5$, 232.1233; found, 232.1242.

L4b: 3,6,9,12-tetraoxapentadec-14-ynoic acid (24)

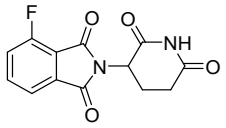


This compound was prepared using the General Procedure **II** and **23** (0.52 g, 2.2 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/AcOH 20:1:0.1) to give a colourless oil.

Yield (0.25 g, 46%); R_f = 0.18 (CH_2Cl_2/MeOH/AcOH 20:1:0.1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 3.42 (t, *J* = 2.4 Hz, 1H), 3.51 (s, 4H), 3.52 – 3.59 (m, 8H), 4.01 (s, 2H), 4.14 (d, *J* = 2.4 Hz, 2H), 12.71 (s, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 57.88, 69.02, 70.27, 70.46, 70.89, 71.03, 72.56, 75.11, 79.56, 178.21; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{19}O_6$, 247.1176; found, 247.1172.

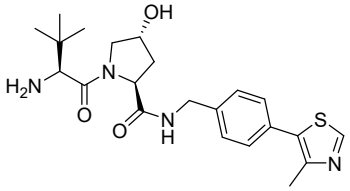
L. Synthesis of E3 ligands

4-Fluorothalidomide (25)



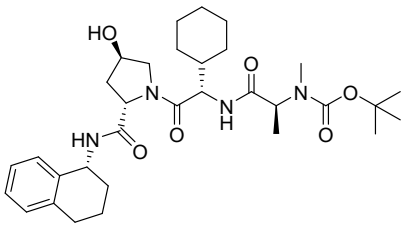
This compound was synthesized as we described previously.[6]

VHL1 ligand (26)



This compound was synthesized as we described previously.[7]

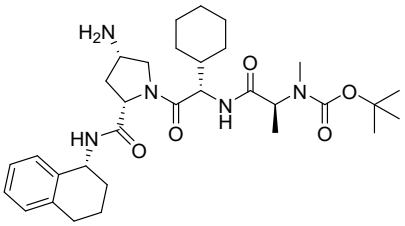
***tert*-Butyl *N*-[(1*S*)-2-[[(1*S*)-1-cyclohexyl-2-[(2*S*,4*R*)-4-hydroxy-2-[[(1*R*)-tetralin-1-yl] carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-1-methyl-2-oxo-ethyl]-*N*-methyl-carbamate (27)**



This compound was synthesized as described previously.[8] A colorless solid was obtained.

Yield (72%); R_f = 0.18 (petroleum ether/EtOAc 1:2); mp 88 – 90 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.83 – 1.29 (m, 8H), 1.39 (s, 9H), 1.52 – 1.93 (m, 11H), 1.96 – 2.04 (m, 1H), 2.65 – 2.75 (m, 2H), 2.73 (s, 3H), 3.49 – 3.64 (m, 1H), 3.64 – 3.79 (m, 1H), 4.28 – 4.45 (m, 3H), 4.45 – 4.69 (m, 1H), 4.88 – 4.96 (m, 1H), 5.07 (d, *J* = 3.6 Hz, 1H), 7.03 – 7.20 (m, 3H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.37 – 7.83 (m, 1H), 8.21 (d, *J* = 8.8 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 15.13, 20.58, 25.75, 25.93, 26.04, 27.82, 28.18, 28.95, 29.14, 30.08, 38.01, 46.69, 53.38, 54.75, 55.71, 58.97, 68.98, 79.24, 125.79, 126.71, 128.41, 128.65, 137.07, 137.86, 155.21, 169.81, 170.51, 171.14; **LC-MS** (ESI) 99% purity, *m/z* $[M + H]^+$ calcd for $C_{32}H_{49}N_4O_6$, 585.36; found, 585.2.

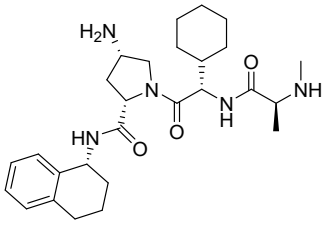
(4*R*)-1-((2S)-2-((*N*-(tert-Butoxycarbonyl)-*N*-methyl-L-alanyl)amino)-2-cyclohexylacetyl)-4-amino-*N*-((1*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)-L-prolinamide (28)



To a solution of **27** (11.12 g, 19 mmol) in dry CH_2Cl_2 (50 mL) under argon, Et_3N (2.88 g, 3.95 mL, 28.5 mmol) was added and the mixture was cooled to 0 °C. Methanesulfonyl chloride (3.27 g, 2.21 mL, 28.5 mmol) was added carefully. The mixture was stirred at room temperature for 3 h. The solvent was then evaporated under reduced pressure. The resulting oil was dissolved in dry DMF (70 mL) under argon and NaN_3 (3.71 g, 57 mmol) was added. The mixture was stirred for 72 h at 70 °C. DMF was then evaporated and the oily residue was dissolved in dry THF (80 mL) under argon and Ph_3P (9.97 g, 38 mmol) was added. After 60 minutes of stirring, 30% NH_4OH(aq) (80 mL) was added and the mixture was stirred for 16 h at room temperature. The organic solvent was evaporated, then brine (50 mL) was added to the aqueous residue, which was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH = 9:1) to give a colorless semisolid.

Yield: (8.99 g, 81%); R_f = 0.29 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.85 – 1.24 (m, 10H), 1.36 – 1.42 (m, 10H), 1.55 – 1.88 (m, 8H), 2.27 – 2.36 (m, 1H), 2.65 – 2.80 (m, 6H), 3.21 – 3.34 (m, 3H), 3.38 (dd, *J* = 5.9, 10.0 Hz, 1H), 3.48 (p, *J* = 6.1 Hz, 1H), 3.91 (dd, *J* = 6.1, 10.1 Hz, 1H), 4.24 – 4.37 (m, 2H), 4.88 – 4.96 (m, 1H), 7.03 – 7.18 (m, 3H), 7.29 (d, *J* = 7.5 Hz, 1H), 8.43 (d, *J* = 8.7 Hz, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 20.36, 25.67, 25.87, 26.01, 28.15, 28.87, 28.95, 29.93, 30.16, 36.69, 46.84, 50.87, 54.53, 54.95, 59.21, 79.13, 125.76, 126.77, 128.47, 128.66, 137.06, 137.45, 169.89, 171.45; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 7.3 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{32}H_{49}N_5O_5$, 584.3767; found, 584.50.

Monomeric IAP ligand (29)



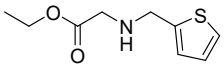
This compound was prepared using the General Procedure **VI** and compound **28** (0.10 g, 0.17 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 15:1:0.1) to give a white solid.

Yield (52 mg, 63%); R_f = 0.18 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.87 – 1.17 (m, 5H), 1.28 (d, *J* = 6.9 Hz, 3H), 1.49 – 1.72 (m, 9H), 1.76 – 1.85 (m, 3H), 1.95 – 2.06 (m, 1H), 2.17 – 2.31 (m, 2H), 2.35 (s, 3H), 2.67 – 2.83 (m, 2H), 3.02 (q, *J* = 6.9 Hz, 1H), 3.37 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.66 – 3.72 (m, 1H), 4.05 (dd, *J* = 10.4, 5.7 Hz, 1H), 4.48 (dd, *J* = 9.1, 7.1 Hz, 1H), 4.55 (dd, *J* = 8.8, 4.0 Hz, 1H), 5.09 – 5.16 (m, 1H), 7.03 – 7.25 (m, 5H), 7.61 (d, *J* = 9.1 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 19.75, 20.16, 25.90, 26.05, 26.19, 28.55, 29.39, 29.87, 30.15, 35.30, 36.19, 40.86, 47.70, 51.30, 54.73,

57.11, 60.42, 60.46, 126.24, 127.23, 128.81, 129.20, 136.72, 137.47, 170.79, 172.35, 175.15; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 4.28 min, 95% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{27}H_{42}N_5O_3$, 484.3282; found, 484.3277.

M. Synthesis of OGT ligand

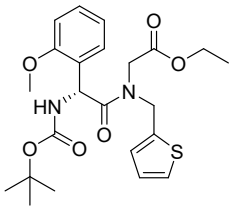
Ethyl (thiophen-2-ylmethyl)glycinate (30)



To a solution of glycine ethyl ester hydrochloride (3.0 g, 21.49 mmol) in dry MeOH (50 mL), Et_3N (2.18 g, 3.0 mL, 21.49 mmol) and thiophene-2-carbaldehyde (2.89 g, 2.37 mL, 25.79 mmol) were added under argon atmosphere. After stirring at room temperature for 1.5 hours, the mixture was cooled to 0 °C. $NaBH_4$ (1.63 g, 42.98 mmol) was slowly added and the mixture was left to stir at room temperature for 30 mins. After removal of the volatiles, H_2O was added (70 mL) and the mixture extracted with EtOAc (2 × 700 mL). The combined organic layers were further washed with brine (150 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/*n*-hexanes 1:2) to give a colorless oil.

Yield (1.71 g, 40 %); R_f = 0.33 (EtOAc/*n*-hexanes 1:2); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.19 (t, *J* = 7.1 Hz, 3H), 2.61 (s, 1H), 3.31 (s, 2H), 3.91 (d, *J* = 0.8 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 6.92 – 6.97 (m, 2H), 7.38 (dd, *J* = 4.7, 1.6 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 14.14, 46.81, 49.05, 59.95, 124.75, 124.86, 126.59, 144.18, 171.93; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_9H_{14}O_2NS$, 200.0740; found, 200.0740.

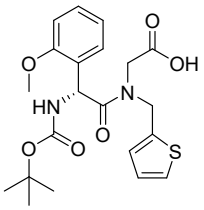
Ethyl (*R*)-*N*-(2-((tert-butoxycarbonyl)amino)-2-(2-methoxyphenyl)acetyl)-*N*-(thiophen-2-ylmethyl) glycinate (31)



(*R*)-2-((*tert*-Butoxycarbonyl)amino)-2-(2-methoxyphenyl)acetic acid (0.85 g, 3.01 mmol) was dissolved in dry DMF (5 mL), and DIPEA (0.43 g, 0.55 mL, 3.31 mmol) was added, followed by the addition of HATU (1.26 g, 3.31 mmol) under argon atmosphere. After stirring for 5 min, a solution of **30** (0.60 g, 3.01 mmol) in dry DMF (5 mL) was added. The reaction mixture was stirred at room temperature for 18 h, after which the solvent was removed. H_2O (40 mL) was then added, and the product was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/*n*-hexanes 1:2) to give a light yellow oil.

Yield (0.87g, 62 %); R_f = 0.30 (EtOAc/*n*-hexanes 1:2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.17 (dt, *J* = 30.3, 7.1 Hz, 3H), 1.41 (d, *J* = 4.3 Hz, 9H), 3.45 – 3.86 (m, 4H), 3.93 – 4.16 (m, 3H), 4.55 – 4.99 (m, 2H), 5.73 – 6.22 (m, 2H), 6.60 – 6.91 (m, 3H), 6.92 – 7.01 (m, 1H), 7.14 – 7.24 (m, 1H), 7.27 – 7.37 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 14.25, 28.52, 45.17, 46.19, 49.40, 58.49, 61.25, 61.52, 79.36, 121.48, 126.05, 126.72, 127.01, 127.39, 128.79, 128.90, 129.68, 129.90, 168.47, 168.95, 172.07; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{31}O_6N_2S$, 463.1897; found, 463.1891.

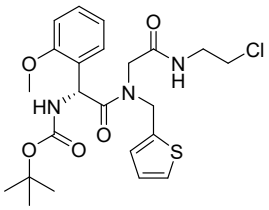
(*R*)-*N*-(2-((*tert*-Butoxycarbonyl)amino)-2-(2-methoxyphenyl)acetyl)-*N*-(thiophen-2-ylmethyl)glycine (32)



Ethyl ester **31** (0.86 g, 1.85 mmol) was dissolved in THF (10 mL) and 1 M LiOH solution (18. 5 mL, 18.5 mmol) was then added. The reaction mixture was stirred for 2 hours at room temperature, after which THF was removed *in vacuo*. The aqueous phase was then acidified by the careful addition of 2 M HCl (10 mL). The mixture was then extracted with EtOAc (100 mL), and organic layer was dried over Na_2SO_4, filtered, and concentrated. The obtained crude white solid was used in the next step without further purification.

Yield (0.75 g, 93%); R_f = 0.55 (CH_2Cl_2/MeOH/AcOH 9:1:0.1); **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{21}H_{27}N_2O_6S$, 435.1584; found, 435.1581.

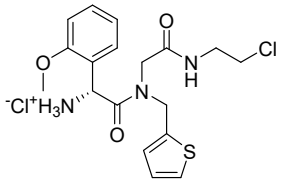
***tert*-Butyl (*R*)-(2-((2-((2-chloroethyl)amino)-2-oxoethyl)(thiophen-2-ylmethyl)amino)-1-(2-methoxyphenyl)-2-oxoethyl)carbamate (33)**



Compound **32** (0.75 g, 1.73 mmol) was dissolved in dry DMF (5 mL), and DIPEA (0.67 g, 0.86 mL, 5.18 mmol) was added, followed by the addition of HATU (0.61 g, 1.90 mmol) under argon atmosphere. After stirring for 5 min, a solution of 2-chloroethan-1-amine (0.22 g, 1.90 mmol) in dry DMF (5 mL) was added. The reaction mixture was stirred at room temperature for 18 h, after which the solvent was removed. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 50:1) to give an off-white oil.

Yield (0.74 g, 92 %); R_f = 0.12 (CH_2Cl_2/MeOH 50:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 3.35 – 3.60 (m, 4H), 3.82 (d, *J* = 8.0 Hz, 4H), 4.14 (dd, *J* = 48.6, 17.1 Hz, 1H), 4.43 – 4.99 (m, 2H), 5.31 – 6.18 (m, 2H), 6.75 (s, 1H), 6.82 – 7.03 (m, 3H), 7.17 (dd, *J* = 17.1, 5.1 Hz, 1H), 7.27 – 7.38 (m, 2H), 7.43 (d, *J* = 8.1 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 28.33, 40.86, 43.36, 55.13, 55.52, 55.89, 56.01, 79.28, 119.75, 120.98, 126.62, 126.95, 127.29, 127.58, 128.50, 131.63, 138.46, 172.37, 177.11, 177.27, 178.04; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{31}N_3O_5ClS$, 496.1668; found, 496.1653.

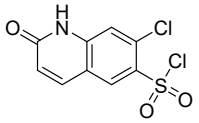
(*R*)-2-((2-((2-Chloroethyl)amino)-2-oxoethyl)(thiophen-2-ylmethyl)amino)-1-(2-methoxyphenyl)-2-oxoethan-1-aminium chloride (34)



This compound was prepared using the General Procedure **VI** and compound **33** (0.74 g, 1.58 mmol). The obtained crude white solid was used in the next step without further purification.

R_f = 0.22 (CH_2Cl_2/MeOH/NH_4OH 15:1:0.1); **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{23}N_3O_3ClS$, 396.1143; found, 396.1133.

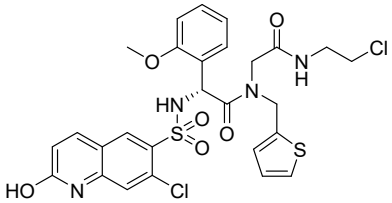
7-chloro-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (35)



To a round bottom flask with 7-chloroquinolin-2-ol (2.5 g, 13.92 mmol) cooled to 0 °C, chlorosulfonic acid (8 mL) was added. The suspension was stirred at 160 °C for 4 h. After cooling down to room temperature, the mixture was poured onto crushed ice, the precipitate was collected by filtration and washed with cooled H_2O and diethyl ether (each 50 mL). The product was crude product was purified by column chromatography (EtOAc/*n*-hexanes 1:1) to give a light brown solid.

Yield (0.97 g, 25%); R_f = 0.60 (EtOAc/*n*-hexanes 4:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 6.49 (dd, *J* = 9.8, 3.8 Hz, 1H), 7.28 (d, *J* = 3.9 Hz, 1H), 7.97 (dd, *J* = 9.5, 3.9 Hz, 1H), 8.15 (d, *J* = 4.1 Hz, 1H), 11.80 (s, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 116.20, 116.88, 122.26, 128.34, 132.79, 138.97, 139.41, 140.20, 161.88; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_9H_4O_3NCl_2S$, 275.9294; found, 275.9295.

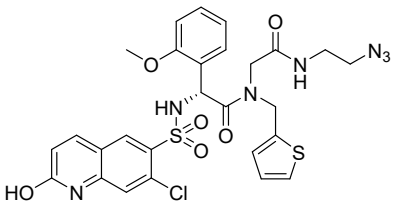
(*R*)-2-((7-Chloro-2-hydroxyquinoline)-6-sulfonamido)-*N*-(2-((2-chloroethyl)amino)-2-oxoethyl)-2-(2-methoxyphenyl)-*N*-(thiophen-2-ylmethyl)acetamide (36)



Compound **34** (1.19 g, 2.75 mmol) was dissolved in dry DMF (10 mL), and DIPEA (1.07 g, 1.41 mL, 8.25 mmol) was added. Afterwards, a solution of **35** (0.77 g, 2.75 mmol) in dry DMF (10 mL) was added. The reaction mixture was stirred at room temperature for 18 h, after which the solvent was removed. The crude product was purified by column chromatography (EtOAc/*n*-hexanes 4:1) to give a white solid.

Yield (1.17 g, 67%); R_f = 0.25 (EtOAc/*n*-hexanes 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.35 – 3.60 (m, 4H), 3.70 – 4.15 (m, 5H), 4.43 – 4.82 (m, 2H), 5.75 – 6.31 (m, 2H), 6.61 – 7.04 (m, 6H), 7.09 – 7.33 (m, 4H), 7.63 (d, *J* = 9.5 Hz, 1H), 7.93 – 8.05 (m, 1H), 11.96 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 45.59, 46.64, 49.71, 50.40, 50.69, 56.01, 111.47, 117.25, 118.04, 121.77, 122.96, 126.55, 127.26, 127.76, 128.08, 128.66, 130.83, 133.53, 137.00, 137.72, 140.21, 140.85, 162.80, 163.89, 167.31, 168.13; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{27}H_{27}N_4O_6Cl_2S_2$, 637.0744; found, 637.0725.

(*R*)-*N*-(2-((2-Azidoethyl)amino)-2-oxoethyl)-2-((7-chloro-2-hydroxyquinoline)-6-sulfonamido)-2-(2-methoxyphenyl)-*N*-(thiophen-2-ylmethyl)acetamide (37)

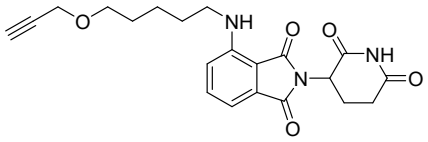


Compound **36** (1.17 g, 1.84 mmol) was dissolved in dry DMF (15 mL), followed by the addition of NaN_3 (0.24 g, 3.67 mmol) under argon atmosphere. The reaction mixture was stirred at 80 °C for 4 h, after which the solvent was removed. H_2O (100 mL) was then added, and the product was extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The obtained crude white solid was used in the next step without further purification.

Yield (1.07 g, 91%); R_f = 0.14 (CH_2Cl_2/MeOH 20:1); **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{27}H_{27}N_7O_6ClS_2$, 644.1147; found, 644.1126.

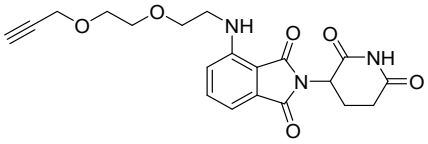
N. Synthesis of E3-linker conjugates

CRBN ligand-L1 linker conjugate (38)



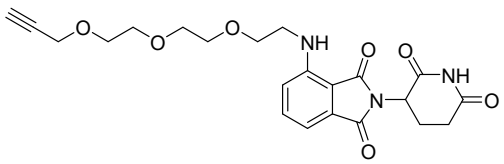
This compound was synthesized as we described previously.[3]

CRBN ligand-L2 linker conjugate (39)



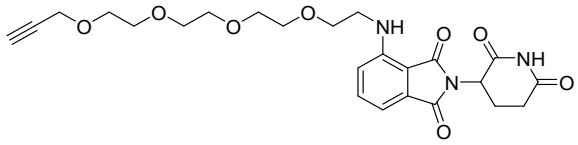
This compound was synthesized as we described previously.[3]

CRBN ligand-L3 linker conjugate (40)



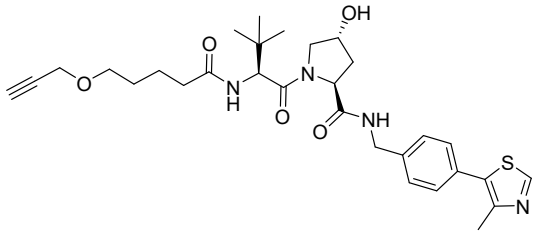
This compound was synthesized as we described previously.[3]

CRBN ligand-L4 linker conjugate (41)



This compound was synthesized as we described previously.[3]

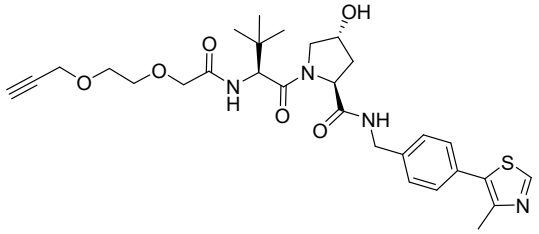
VHL ligand-L1b linker conjugate (42)



This compound was prepared using the General Procedure **III** and linker **L1b** (60 mg, 0.38 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a colorless waxy solid.

Yield (115 mg, 45%); R_f = 0.40 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.92 (s, 9H), 2.03 (s, 2H), 2.08 – 2.20 (m, 1H), 2.19 – 2.30 (m, 2H), 2.51 (s, 3H), 2.52 – 2.56 (m, 1H), 3.40 (s, 1H), 3.48 (s, 4H), 3.52 (t, *J* = 6.0 Hz, 1H), 3.59 (dt, *J* = 11.4, 4.5 Hz, 1H), 3.71 (p, *J* = 6.7 Hz, 1H), 4.00 – 4.15 (m, 3H), 4.33 (dd, *J* = 15.0, 5.2 Hz, 1H), 4.46 – 4.62 (m, 3H), 4.71 (td, *J* = 8.0, 2.4 Hz, 1H), 7.30 – 7.41 (m, 5H), 8.67 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 12.98, 16.52, 21.48, 26.85, 35.28, 36.15, 43.72, 44.23, 56.30, 57.10, 57.66, 58.86, 69.55, 70.93, 71.03, 75.09, 128.62, 129.99, 131.44, 134.32, 138.55, 150.75, 152.67, 171.08, 171.55, 171.94; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{30}H_{41}N_4O_5S$, 569.2792; found, 569.2782.

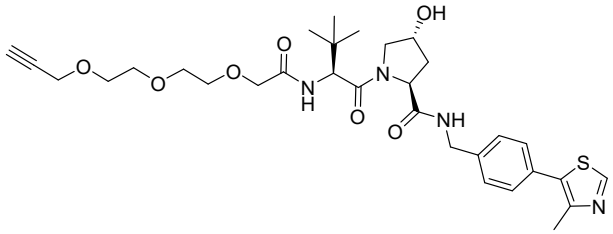
VHL ligand-L2b linker conjugate (43)



This compound was prepared using the General Procedure **III** and linker **L2b** (46 mg, 0.29 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a colorless waxy solid.

Yield (150 mg, 90%); R_f = 0.60 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 2.07 – 2.15 (m, 1H), 2.43 (t, *J* = 2.4 Hz, 1H), 2.50 (s, 3H), 3.62 (dd, *J* = 11.3, 3.8 Hz, 1H), 3.67 – 3.75 (m, 6H), 3.94 – 4.00 (m, 2H), 4.04 – 4.09 (m, 1H), 4.13 – 4.26 (m, 2H), 4.32 (dd, *J* = 15.0, 5.3 Hz, 1H), 4.45 – 4.58 (m, 3H), 4.72 (t, *J* = 7.9 Hz, 1H), 7.31 – 7.44 (m, 6H), 8.67 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 12.70, 26.50, 35.00, 36.05, 43.34, 43.80, 55.86, 56.78, 57.47, 58.50, 58.61, 68.78, 70.26, 71.15, 75.08, 79.40, 128.21, 129.62, 131.03, 131.74, 138.24, 148.58, 150.43, 170.71, 170.92, 171.47; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{29}H_{39}N_4O_6S$, 571.2585; found, 571.2602.

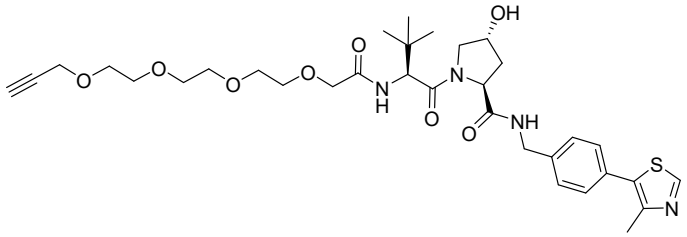
VHL ligand-L3b linker conjugate (44)



This compound was prepared using the General Procedure **III** and linker **L3b** (47 mg, 0.23 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a colorless waxy solid.

Yield (137 mg, 95%); R_f = 0.55 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 2.07 – 2.15 (m, 1H), 2.42 (t, *J* = 2.4 Hz, 1H), 2.52 (s, 3H), 2.53 – 2.61 (m, 1H), 3.07 (d, *J* = 4.4 Hz, 1H), 3.18 (q, *J* = 7.4 Hz, 1H), 3.60 (dd, *J* = 11.4, 3.7 Hz, 1H), 3.66 – 3.71 (m, 8H), 4.00 (d, *J* = 8.4 Hz, 2H), 4.10 (d, *J* = 11.6 Hz, 1H), 4.17 (d, *J* = 2.4 Hz, 2H), 4.34 (dd, *J* = 14.9, 5.3 Hz, 1H), 4.46 (d, *J* = 8.4 Hz, 1H), 4.56 (dd, *J* = 15.0, 6.7 Hz, 2H), 4.74 (t, *J* = 7.9 Hz, 1H), 7.32 – 7.40 (m, 5H), 8.68 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 13.02, 26.82, 35.31, 36.37, 43.66, 44.11, 56.18, 57.10, 57.78, 58.82, 58.93, 69.09, 70.57, 71.46, 74.45, 75.40, 79.72, 128.53, 129.94, 131.35, 132.06, 138.56, 148.90, 150.75, 171.43, 171.24, 171.79; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{31}H_{43}N_4O_7S$, 615.2847; found, 615.2835.

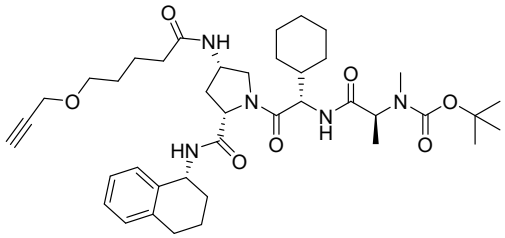
VHL ligand-L4b linker conjugate (45)



This compound was prepared using the General Procedure **III** and linker **L4b** (77 mg, 0.31 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a colorless waxy solid.

Yield (222 mg, 95%); R_f = 0.48 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 2.43 (t, *J* = 2.4 Hz, 1H), 2.52 (s, 3H), 2.53 – 2.60 (m, 1H), 3.13 (d, *J* = 4.5 Hz, 1H), 3.18 (q, *J* = 7.4 Hz, 1H), 3.60 (dd, *J* = 11.4, 3.7 Hz, 1H), 3.63 – 3.75 (m, 13H), 3.93 – 4.07 (m, 2H), 4.10 (d, *J* = 11.5 Hz, 1H), 4.19 (d, *J* = 2.4 Hz, 2H), 4.34 (dd, *J* = 14.9, 5.3 Hz, 1H), 4.48 (d, *J* = 8.5 Hz, 1H), 4.52 – 4.59 (m, 2H), 4.74 (t, *J* = 7.9 Hz, 1H), 7.32 – 7.40 (m, 5H), 8.68 (s, 1H); **^{13}C NMR** 101 MHz, $CDCl_3$) δ 12.66, 26.53, 34.96, 35.83, 43.40, 43.91, 55.98, 56.78, 57.35, 58.47, 58.54, 69.24, 70.29, 70.51, 70.61, 70.71, 71.31, 74.77, 128.30, 129.67, 131.12, 131.57, 138.23, 148.50, 150.43, 170.76, 171.62; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{33}H_{47}N_4O_8S$, 659.3109; found, 659.3090.

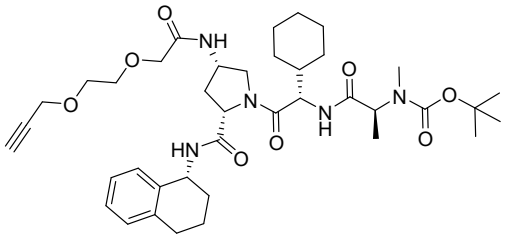
IAP ligand-L1b linker conjugate (46)



This compound was prepared using the General Procedure **IV** and linker **L1b** (54 mg, 0.34 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a beige waxy solid.

Yield (230 mg, 93%); R_f = 0.55 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.78 – 1.16 (m, 5H), 1.27 (d, *J* = 7.2 Hz, 3H), 1.63 – 1.78 (m, 4H), 1.79 – 1.90 (m, 3H), 2.02 (s, 3H), 2.12 – 2.28 (m, 3H), 2.37 – 2.43 (m, 1H), 2.75 (s, 3H), 3.46 – 3.56 (m, 1H), 3.66 (d, *J* = 10.7 Hz, 1H), 4.01 (dd, *J* = 10.8, 5.0 Hz, 1H), 4.07 (t, *J* = 6.2 Hz, 1H), 4.31 (t, *J* = 7.9 Hz, 1H), 4.58 (q, *J* = 6.0 Hz, 1H), 4.73 (d, *J* = 8.8 Hz, 1H), 5.06 – 5.14 (m, 1H), 6.62 (s, 1H), 7.03 – 7.18 (m, 4H), 7.79 (s, 1H), 8.01 (dd, *J* = 21.1, 6.6 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 20.08, 21.08, 22.26, 22.44, 25.91, 26.06, 28.31, 28.47, 28.63, 29.14, 29.20, 29.35, 30.01, 31.26, 36.34, 36.49, 38.74, 40.64, 48.10, 49.58, 55.38, 55.94, 58.16, 60.31, 64.20, 69.73, 74.33, 126.17, 127.52, 128.27, 129.40, 136.13, 137.37, 171.29, 171.62, 172.54, 172.86, 173.11; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{40}H_{60}N_5O_7$, 722.4487; found, 722.4491.

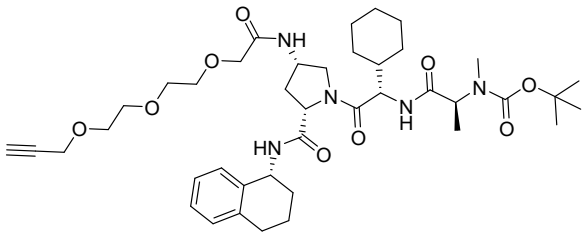
IAP ligand-L2b linker conjugate (47)



This compound was prepared using the General Procedure **IV** and linker **L2b** (47 mg, 0.30 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a beige waxy solid.

Yield (200 mg, 92%); R_f = 0.30 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.80 – 1.11 (m, 5H), 1.23 – 1.31 (m, 3H), 1.45 (s, 9H), 1.50 – 1.68 (m, 8H), 1.76 – 1.89 (m, 3H), 1.97 – 2.06 (m, 2H), 2.16 – 2.28 (m, 1H), 2.36 (t, *J* = 2.4 Hz, 1H), 2.48 (d, *J* = 13.8 Hz, 1H), 2.75 (s, 4H), 3.57 – 3.68 (m, 1H), 3.71 – 3.81 (m, 3H), 4.04 – 4.06 (m, 2H), 4.09 (dd, *J* = 10.9, 5.6 Hz, 1H), 4.19 (d, *J* = 2.4 Hz, 2H), 4.29 – 4.40 (m, 1H), 4.64 (q, *J* = 6.4 Hz, 1H), 4.72 (d, *J* = 8.5 Hz, 1H), 5.12 (q, *J* = 6.7 Hz, 1H), 6.63 (s, 1H), 7.02 – 7.18 (m, 4H), 7.51 – 7.80 (m, 1H), 8.60 (d, *J* = 7.0 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 20.17, 25.89, 26.06, 28.48, 29.29, 29.51, 30.10, 31.32, 38.76, 40.59, 47.88, 48.97, 55.34, 55.53, 58.69, 60.26, 69.24, 70.94, 71.25, 74.73, 79.71, 126.24, 127.41, 128.47, 129.29, 136.41, 137.34, 170.18, 170.60, 171.58, 172.87; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{39}H_{58}N_5O_8$, 724.4280; found, 724.4264.

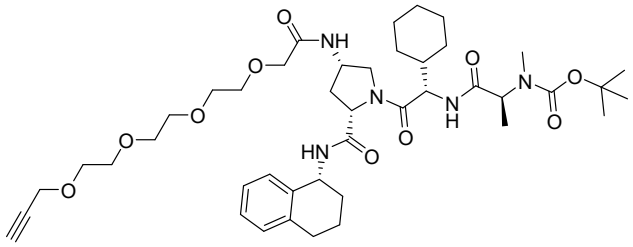
IAP ligand-L3b linker conjugate (48)



This compound was prepared using the General Procedure **IV** and linker **L3b** (61 mg, 0.30 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a beige waxy solid.

Yield (213 mg, 92%); R_f = 0.48 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.80 – 1.13 (m, 5H), 1.28 (d, *J* = 7.2 Hz, 3H), 1.45 (s, 9H), 1.48 – 1.65 (m, 8H), 1.75 – 1.89 (m, 3H), 1.94 – 2.06 (m, 1H), 2.20 (m, 1H), 2.39 (t, *J* = 2.4 Hz, 1H), 2.44 – 2.51 (m, 1H), 2.74 (s, 4H), 3.46 – 3.63 (m, 1H), 3.63 – 3.77 (m, 8H), 4.05 (s, 2H), 4.07 – 4.11 (m, 1H), 4.14 (d, *J* = 2.4 Hz, 2H), 4.34 (t, *J* = 7.7 Hz, 1H), 4.64 (t, *J* = 6.3 Hz, 1H), 4.73 (d, *J* = 8.7 Hz, 1H), 5.12 (t, *J* = 7.4 Hz, 1H), 6.63 (s, 1H), 7.00 – 7.18 (m, 4H), 7.56 – 7.81 (m, 1H), 8.58 (d, *J* = 7.0 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 25.43, 25.88, 26.05, 28.48, 29.30, 29.49, 30.11, 31.25, 38.76, 40.53, 47.84, 49.01, 55.35, 55.56, 58.50, 60.27, 69.21, 70.66, 71.00, 71.46, 74.67, 79.77, 126.24, 127.41, 128.44, 129.30, 136.45, 137.33, 170.18, 170.35, 171.61, 172.92, 173.12; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{41}H_{62}N_5O_9$, 768.4542; found, 768.4528.

IAP ligand-L4b linker conjugate (49)

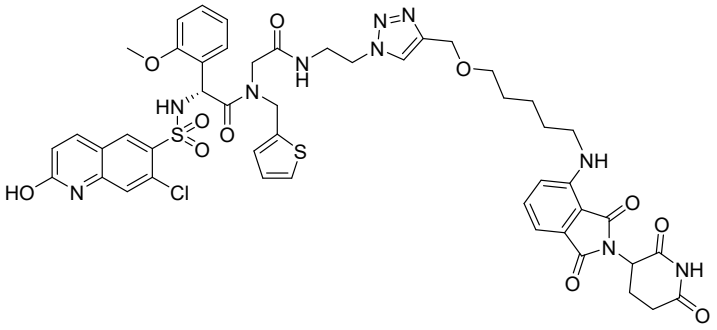


This compound was prepared using the General Procedure **IV** and linker **L4b** (84 mg, 0.34 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) to give a beige waxy solid.

Yield (244 mg, 88%); R_f = 0.48 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.77 – 1.13 (m, 5H), 1.22 – 1.33 (m, 3H), 1.45 (s, 9H), 1.49 – 1.65 (m, 7H), 1.78 – 1.89 (m, 3H), 1.96 – 2.06 (m, 1H), 2.16 – 2.25 (m, 1H), 2.42 (t, *J* = 2.4 Hz, 1H), 2.48 (d, *J* = 14.3 Hz, 1H), 2.75 (s, 4H), 3.48 (d, *J* = 4.4 Hz, 2H), 3.58 – 3.68 (m, 8H), 3.69 – 3.78 (m, 4H), 4.05 (s, 2H), 4.09 (dd, *J* = 11.1, 5.7 Hz, 1H), 4.17 (d, *J* = 2.4 Hz, 2H), 4.33 (t, *J* = 7.5 Hz, 1H), 4.63 (q, *J* = 6.4 Hz, 1H), 4.73 (d, *J* = 8.6 Hz, 1H), 5.12 (t, *J* = 7.3 Hz, 1H), 6.62 (s, 1H), 7.05 (d, *J* = 7.7 Hz, 2H), 7.09 – 7.16 (m, 2H), 7.63 (s, 1H), 8.57 (d, *J* = 7.1 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 20.19, 25.88, 26.05, 28.48, 29.30, 29.48, 30.11, 31.22, 38.76, 40.57, 47.82, 49.01, 55.34, 55.56, 58.52, 60.26, 69.22, 70.51, 70.64, 70.69, 70.81, 71.00, 71.43, 74.66, 79.80, 126.23, 127.40, 128.44, 129.29, 136.45, 137.31, 170.35, 170.60, 171.58, 172.92; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{43}H_{66}N_5O_{10}$, 812.4804; found, 812.4780.

O. Synthesis of Boc-protected and final PROTACs

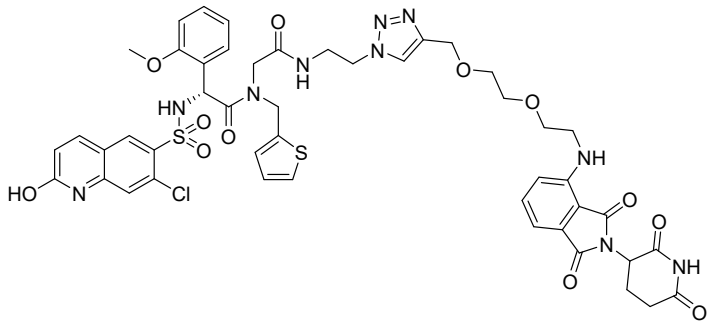
CRBN Series PROTAC 1



This compound was prepared using the General Procedure **V** and CRBN ligand-linker conjugate **38** (37 mg, 93 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 50:1) to give a yellow solid.

Yield (39 mg, 40%); R_f = 0.22 (CH_2Cl_2/MeOH 30:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.36 – 1.73 (m, 8H), 2.12 – 2.20 (m, 1H), 2.70 – 2.94 (m, 3H), 3.17 – 3.44 (m, 2H), 3.48 – 3.95 (m, 8H), 4.29 – 4.47 (m, 2H), 4.54 – 4.69 (m, 4H), 4.92 – 5.03 (m, 1H), 5.66 – 6.41 (m, 3H), 6.52 – 6.63 (m, 1H), 6.64 – 6.94 (m, 6H), 7.02 – 7.22 (m, 4H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 19.5 Hz, 1H), 7.68 (t, *J* = 10.6 Hz, 1H), 7.98 (dd, *J* = 10.2, 4.9 Hz, 1H), 9.07 – 9.59 (m, 1H), 11.34 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 23.03, 23.67, 29.00, 29.28, 31.60, 39.34, 39.64, 42.61, 46.75, 49.06, 49.27, 55.84, 64.35, 70.52, 109.87, 111.21, 111.60, 116.93, 117.38, 117.90, 121.57, 123.12, 123.55, 123.81, 126.45, 126.80, 127.13, 127.58, 128.66, 130.72, 132.61, 133.64, 136.35, 137.25, 140.26, 140.90, 145.55, 145.74, 147.13, 155.92, 163.69, 165.20, 167.82, 168.54, 169.78, 171.65, 171.75; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.34 min, 97% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{48}H_{50}N_{10}O_{11}ClS_2$, 1041.2785; found, 1041.2764.

CRBN Series PROTAC 2

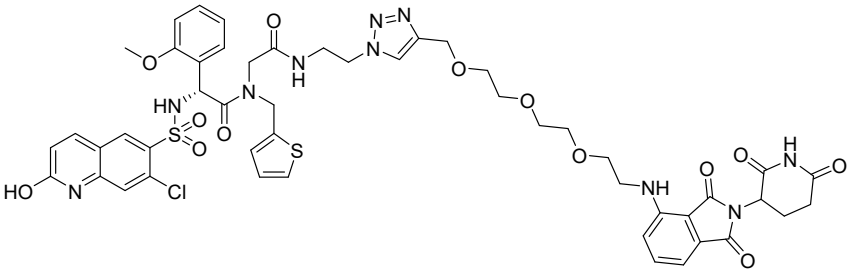


This compound was prepared using the General Procedure **V** and CRBN ligand-linker conjugate **39** (31 mg, 78 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 50:1) to give a yellow solid.

Yield (18 mg, 22%); R_f = 0.33 (CH_2Cl_2/MeOH 20:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.14 – 1.46 (m, 2H), 1.52 – 1.73 (m, 3H), 2.04 – 2.20 (m, 1H), 2.65 (d, *J* = 9.3 Hz, 2H), 2.71 – 2.93 (m, 3H), 3.34 – 3.49 (m, 2H), 3.52 – 3.81 (m, 10H), 4.24 – 4.45 (m, 2H), 4.51 – 4.76 (m, 4H), 4.89 – 6.38 (m, 2H), 6.43 – 6.58 (m, 1H),

6.58 – 6.96 (m, 5H), 6.98 – 7.25 (m, 4H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.58 – 7.73 (m, 2H), 7.93 – 8.05 (m, 1H), 9.35 – 9.82 (m, 1H), 11.45 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 22.97, 27.13, 31.61, 37.02, 39.54, 42.49, 46.72, 49.07, 49.19, 55.86, 64.76, 69.52, 69.96, 70.76, 110.29, 111.26, 111.78, 117.08, 117.37, 117.98, 121.59, 123.07, 123.98, 126.41, 127.12, 127.60, 128.03, 128.67, 130.71, 131.04, 132.62, 133.65, 136.26, 137.27, 137.79, 140.29, 141.01, 145.30, 146.98, 155.93, 163.63, 167.86, 168.54, 169.51, 170.02, 171.97, 172.14; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.89 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{47}H_{48}N_{10}O_{12}ClS_2$, 1043.2578; found, 1043.2565.

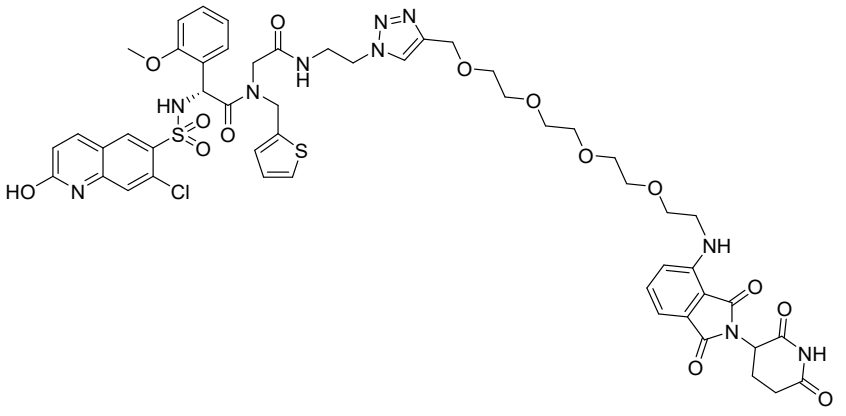
CRBN Series PROTAC 3



This compound was prepared using the General Procedure **V** and CRBN ligand-linker conjugate **40** (45 mg, 0.10 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 50:1) to give a yellow solid.

Yield (55 mg, 50%); R_f = 0.28 (CH_2Cl_2/MeOH 20:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.66 – 1.75 (m, 2H), 2.08 – 2.17 (m, 1H), 2.69 – 2.90 (m, 3H), 3.43 (q, *J* = 5.4 Hz, 2H), 3.51 – 3.91 (m, 15H), 4.25 – 4.42 (m, 2H), 4.54 – 4.73 (m, 4H), 4.93 – 5.04 (m, 1H), 5.68 – 6.39 (m, 2H), 6.46 – 6.73 (m, 5H), 6.75 – 6.85 (m, 2H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.99 – 7.21 (m, 5H), 7.47 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.57 – 7.69 (m, 2H), 7.93 – 7.99 (m, 1H), 9.55 (dd, *J* = 63.4, 24.6 Hz, 1H), 11.51 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 22.99, 31.58, 39.62, 42.47, 46.68, 49.03, 49.31, 52.00, 53.57, 55.86, 64.46, 69.45, 69.88, 70.62, 70.76, 70.93, 110.37, 111.23, 111.72, 111.76, 116.96, 117.31, 118.02, 121.56, 123.06, 124.01, 126.38, 126.78, 127.12, 127.58, 127.97, 128.69, 130.99, 132.63, 133.62, 136.21, 137.34, 137.85, 140.24, 141.00, 145.27, 146.91, 155.74, 155.93, 163.66, 167.85, 168.58, 169.39, 170.32, 172.19; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.98 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{49}H_{52}N_{10}O_{13}ClS_2$, 1087.2840; found,1087.2816.

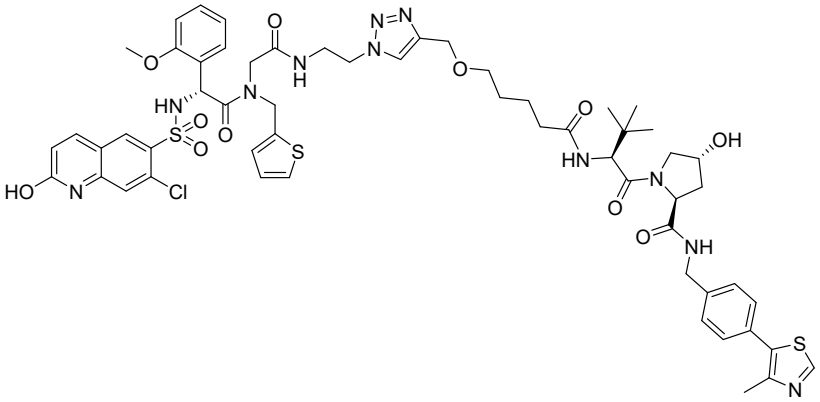
CRBN Series PROTAC 4



This compound was prepared using the General Procedure **V** and CRBN ligand-linker conjugate **41** (38 mg, 78 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 50:1) to give a yellow solid.

Yield (20 mg, 23%); R_f = 0.30(CH_2Cl_2/MeOH 20:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.25 (s, 2H), 1.78 (s, 2H), 2.05 – 2.17 (m, 1H), 2.69 – 2.87 (m, 3H), 3.43 (q, *J* = 5.5 Hz, 2H), 3.53 – 3.91 (m, 20H), 4.35 (dd, *J* = 26.2, 5.7 Hz, 2H), 4.52 – 4.77 (m, 3H), 4.88 – 5.01 (m, 1H), 5.68 – 6.41 (m, 2H), 6.46 – 6.74 (m, 4H), 6.74 – 6.94 (m, 3H), 6.99 – 7.23 (m, 4H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.60 – 7.70 (m, 2H), 7.91 – 8.02 (m, 1H), 9.19 – 9.56 (m, 1H), 11.52 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 22.97, 29.83, 31.56, 39.69, 42.49, 46.68, 49.03, 49.30, 52.00, 53.57, 55.85, 64.54, 69.50, 69.91, 70.53, 70.62, 70.74, 70.85, 110.36, 111.20, 111.76, 116.99, 117.31, 118.05, 121.54, 123.07, 124.08, 126.36, 126.78, 127.12, 127.56, 127.96, 128.71, 130.68, 131.01, 132.07, 132.63, 136.22, 137.38, 140.22, 141.03, 145.17, 146.93, 155.94, 163.66, 167.84, 168.57, 169.40, 169.51, 171.98, 172.10; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.03 min, 95% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{56}N_{10}O_{14}ClS_2$, 1131.3102; found, 1131.3084.

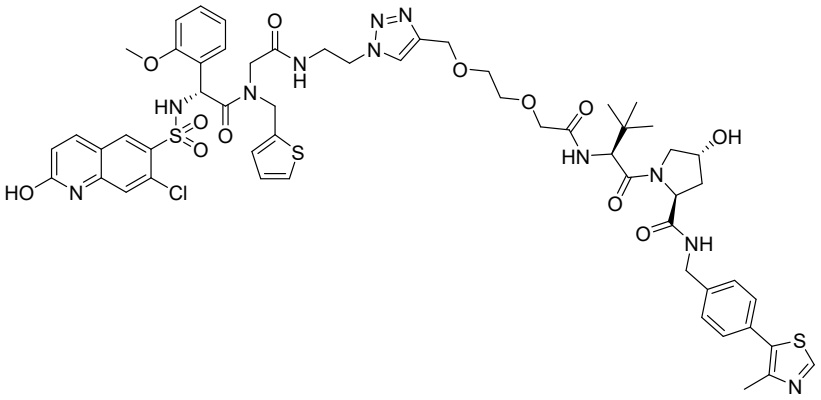
VHL Series PROTAC 5



This compound was prepared using the General Procedure **V** and VHL ligand-linker conjugate **42** (80 mg, 0.12 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a off-white solid.

Yield (35 mg, 24%); R_f = 0.18 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.04 (s, 9H), 2.16 – 2.39 (m, 9H), 2.42 (d, *J* = 4.8 Hz, 3H), 3.41 – 3.69 (m, 10H), 4.17 – 4.44 (m, 4H), 4.48 – 4.79 (m, 8H), 5.32 – 6.33 (m, 1H), 6.51 – 6.86 (m, 6H), 7.10 – 7.25 (m, 4H), 7.27 – 7.46 (m, 5H), 7.47 – 7.71 (m, 4H), 7.91 (s, 1H), 8.64 (s, 1H), 11.31 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 16.21, 22.67, 22.82, 26.55, 26.64, 28.75, 29.84, 35.48, 35.93, 37.26, 39.79, 43.25, 46.74, 48.84, 49.29, 50.71, 51.80, 55.34, 58.06, 59.40, 63.92, 70.09, 70.19, 70.37, 111.30, 117.34, 118.27, 121.46, 123.10, 124.44, 126.27, 126.76, 127.13, 127.36, 128.23, 128.50, 130.72, 130.84, 131.66, 133.24, 134.52, 137.51, 138.43, 140.14, 141.28, 145.07, 148.46, 150.44, 153.60, 156.06, 163.13, 167.77, 168.66, 170.05, 171.55, 171.99; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.08 min, 97% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{57}H_{65}N_{11}O_{11}ClS_3$, 1210.3721; found, 1210.3714.

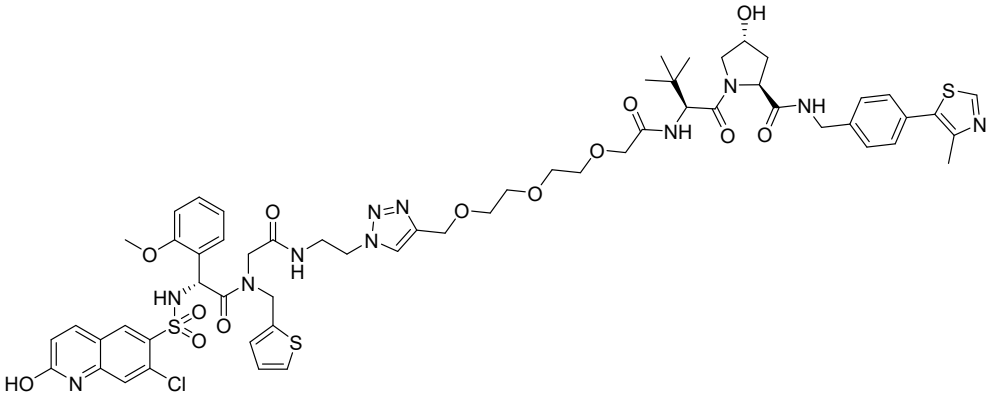
VHL Series PROTAC 6



This compound was prepared using the General Procedure **V** and VHL ligand-linker conjugate **43** (100 mg, 0.18 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a off-white solid.

Yield (75 mg, 35%); R_f = 0.18 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.03 (d, *J* = 4.0 Hz, 12H), 2.24 – 2.35 (m, 2H), 2.40 (d, *J* = 1.8 Hz, 3H), 3.50 – 4.03 (m, 12H), 4.11 – 4.51 (m, 4H), 4.54 – 4.80 (m, 8H), 5.61 – 6.31 (m, 1H), 6.53 – 6.85 (m, 7H), 7.00 – 7.25 (m, 5H), 7.27 – 7.45 (m, 4H), 7.51 – 7.95 (m, 4H), 8.64 (d, *J* = 14.6 Hz, 1H), 11.25 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 12.65, 16.18, 26.58, 35.78, 37.55, 39.83, 43.16, 43.68, 46.85, 49.22, 55.70, 55.81, 57.34, 59.33, 64.55, 69.67, 70.41, 70.56, 71.28, 111.12, 117.32, 118.13, 121.39, 122.72, 123.02, 124.17, 124.72, 126.34, 126.80, 127.10, 128.19, 128.63, 129.29, 129.35, 130.69, 130.79, 131.65, 133.27, 137.42, 138.47, 140.13, 141.14, 144.85, 145.14, 148.38, 150.53, 156.03, 163.29, 167.95, 168.84, 170.60, 171.09, 171.77; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.95 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{56}H_{65}N_{11}O_{12}ClS_3$, 1214.3659; found, 1214.3649.

VHL Series PROTAC 7

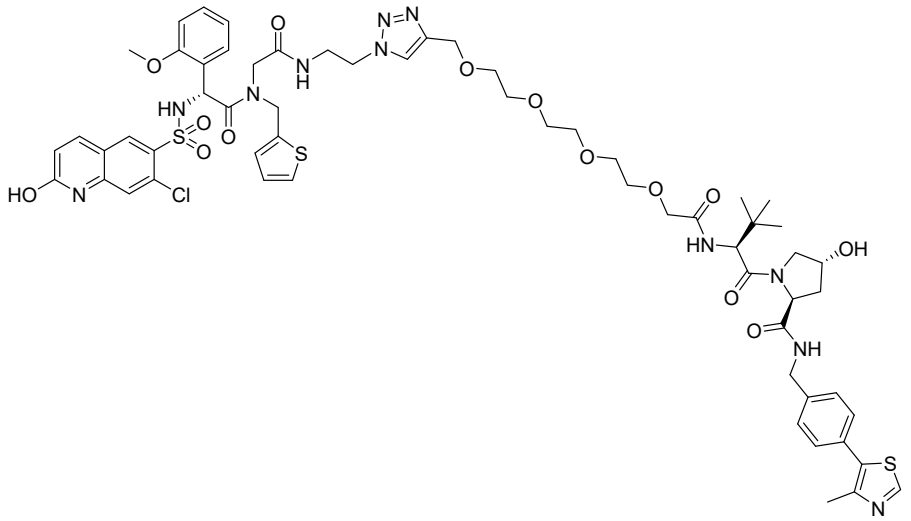


This compound was prepared using the General Procedure **V** and VHL ligand-linker conjugate **44** (95 mg, 0.16 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to give a off-white solid.

Yield (58 mg, 30%); R_f = 0.12 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.00 (d, *J* = 9.2 Hz, 9H), 1.98 – 2.16 (m, 6H), 2.19 – 2.39 (m, 2H), 2.45 (d, *J* = 4.8 Hz, 3H), 3.52 – 3.75 (m, 9H), 3.78 – 4.17 (m, 4H), 4.24 – 4.45 (m, 4H), 4.47 – 4.80 (m, 8H), 5.62 – 6.04 (m, 1H), 6.53 – 6.85 (m, 6H), 6.97 – 7.20 (m,

4H), 7.26 – 7.48 (m, 6H), 7.55 – 7.95 (m, 4H), 8.64 (d, *J* = 2.6 Hz, 1H), 11.61 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 16.19, 26.58, 35.70, 35.82, 37.19, 39.80, 43.21, 46.80, 48.88, 49.40, 51.84, 55.82, 57.34, 59.20, 64.52, 69.91, 70.32, 70.36, 70.55, 70.82, 71.11, 111.11, 117.30, 118.16, 121.45, 123.06, 124.40, 126.27, 126.78, 127.10, 127.35, 127.79, 128.19, 128.68, 129.36, 130.64, 130.80, 131.72, 133.44, 137.56, 137.99, 138.50, 140.08, 141.21, 145.01, 145.28, 148.42, 150.49, 155.93, 163.41, 167.90, 168.63, 170.21, 170.54, 171.08, 171.67; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.02 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{58}H_{69}N_{11}O_{13}ClS_3$, 1258.3922; found, 1258.3902.

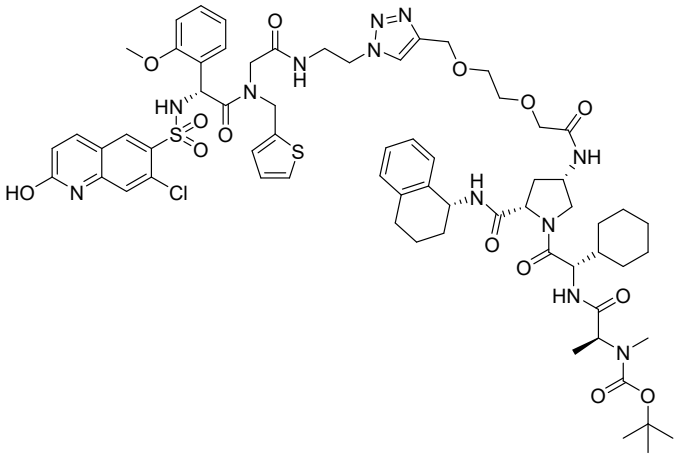
VHL Series PROTAC 8



This compound was prepared using the General Procedure **V** and VHL ligand-linker conjugate **45** (210 mg, 0.32 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 15:1) to give a off-white solid.

Yield (145 mg, 35%); R_f = 0.25 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.00 (s, 9H), 2.30 (s, 8H), 2.45 (d, *J* = 3.0 Hz, 3H), 3.48 – 3.74 (m, 14H), 3.79 – 4.14 (m, 4H), 4.28 – 4.47 (m, 4H), 4.49 – 4.78 (m, 7H), 5.63 – 6.05 (m, 1H), 6.50 – 6.70 (m, 3H), 6.73 – 6.94 (m, 3H), 6.97 – 7.21 (m, 4H), 7.27 – 7.49 (m, 6H), 7.59 – 7.98 (m, 4H), 8.64 (s, 1H), 11.65 (d, *J* = 30.1 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 16.18, 26.56, 35.74, 37.19, 39.71, 43.19, 45.86, 46.74, 48.61, 49.37, 51.84, 55.81, 55.86, 57.25, 57.39, 59.16, 64.44, 69.75, 69.88, 70.34, 70.38, 70.43, 70.52, 70.60, 71.05, 111.16, 117.37, 118.19, 121.45, 123.00, 123.17, 124.44, 126.25, 127.05, 127.36, 128.14, 128.63, 129.39, 130.64, 130.76, 130.86, 131.84, 133.50, 137.56, 138.01, 138.54, 140.20, 141.24, 144.84, 148.42, 150.49, 155.92, 163.43, 167.94, 168.61, 170.13, 170.50, 171.04, 171.60, 171.71; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.08 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{60}H_{73}N_{11}O_{14}ClS_3$, 1302.4184; found, 1302.4158.

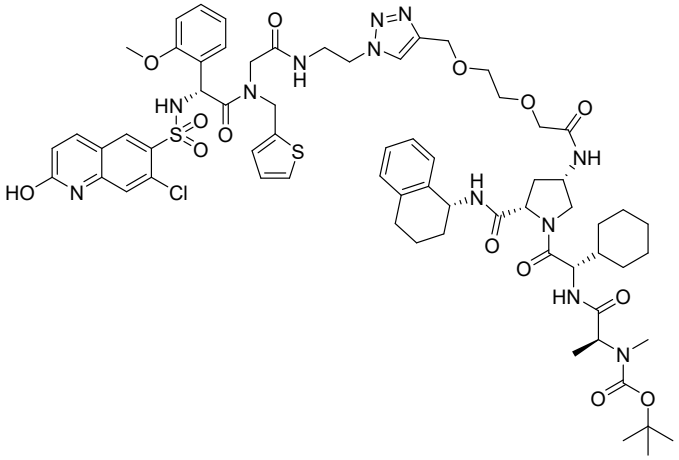
L1b-based Boc-protected IAP Series PROTAC (50)



This compound was prepared using the General Procedure **V** and IAP ligand-linker conjugate **46** (225 mg, 0.31 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 15:1) to give a off-white solid.

Yield (67 mg, 16%); R_f = 0.25 (CH_2Cl_2/MeOH 15:1). Due to the presence of *N*-Boc protecting group resulting in additional set of rotamers, NMR data is only provided for the deprotected final PROTAC. **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{67}H_{86}N_{12}O_{13}ClS_2$, 1365.5562; found, 1365.5558.

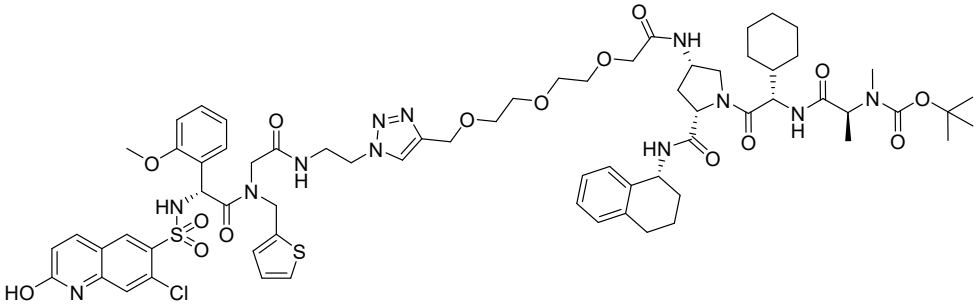
L2b-based Boc-protected IAP Series PROTAC (51)



This compound was prepared using the General Procedure **V** and IAP ligand-linker conjugate **47** (193 mg, 0.30 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 15:1) to give a off-white solid.

Yield (147 mg, 36%); R_f = 0.44 (CH_2Cl_2/MeOH 9:1). Due to the presence of *N*-Boc protecting group resulting in additional set of rotamers, NMR data is only provided for the deprotected final PROTAC. **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{66}H_{84}N_{12}O_{14}ClS_2$, 1367.5354; found, 1367.5346.

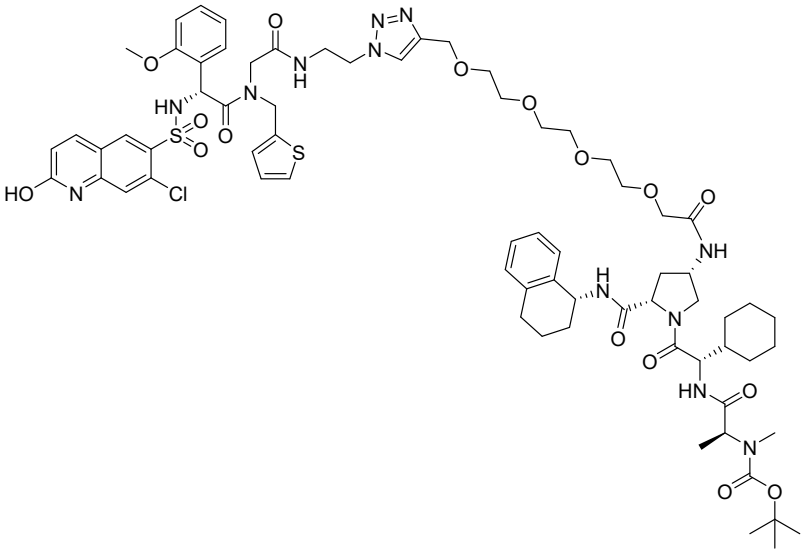
L3b-based Boc-protected IAP Series PROTAC (52)



This compound was prepared using the General Procedure **V** and IAP ligand-linker conjugate **48** (167 mg, 0.26 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 15:1) to give a off-white solid.

Yield (108 mg, 29%); R_f = 0.38 (CH_2Cl_2/MeOH 9:1). Due to the presence of *N*-Boc protecting group resulting in additional set of rotamers, NMR data is only provided for the deprotected final PROTAC. **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{68}H_{88}N_{12}O_{15}ClS_2$, 1411.5617; found, 1411.5591.

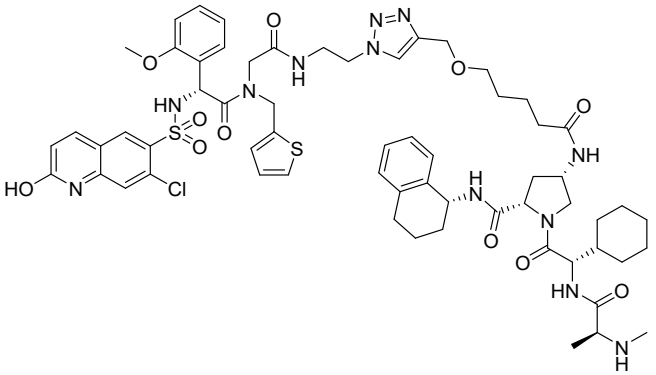
L4b-based Boc-protected IAP Series PROTAC (53)



This compound was prepared using the General Procedure **V** and IAP ligand-linker conjugate **49** (167 mg, 0.26 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 15:1) to give a off-white solid.

Yield (104 mg, 25%); R_f = 0.28 (CH_2Cl_2/MeOH 9:1). Due to the presence of *N*-Boc protecting group resulting in additional set of rotamers, NMR data is only provided for the deprotected final PROTAC. **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{70}H_{92}N_{12}O_{16}ClS_2$, 1455.5879; found, 1455.5869.

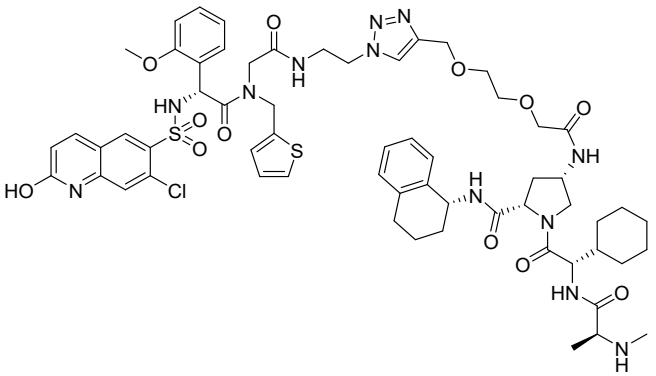
IAP Series PROTAC 9



This compound was prepared using the General Procedure **VI** and PROTAC precursor **50** (53 mg, 39 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1) to give a colorless solid.

Yield (42 mg, 85%); R_f = 0.40 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.81 – 1.18 (m, 6H), 1.26 (d, *J* = 7.2 Hz, 6H), 1.55 – 1.73 (m, 8H), 1.77 – 2.07 (m, 5H), 2.15 – 2.34 (m, 7H), 2.64 – 2.84 (m, 2H), 2.97 – 3.40 (m, 1H), 3.51 – 3.62 (m, 4H), 3.63 – 3.69 (m, 4H), 3.76 – 3.87 (m, 2H), 4.07 (dd, *J* = 11.0, 5.3 Hz, 1H), 4.30 – 4.44 (m, 3H), 4.56 – 4.77 (m, 6H), 5.04 – 5.15 (m, 1H), 5.71 – 6.02 (m, 1H), 6.46 – 6.89 (m, 6H), 6.95 – 7.22 (m, 7H), 7.32 (d, *J* = 28.3 Hz, 1H), 7.56 – 7.72 (m, 3H), 7.84 (s, 1H), 7.94 (d, *J* = 16.7 Hz, 1H), 8.11 (d, *J* = 7.2 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 19.45, 20.17, 22.46, 25.96, 26.12, 28.70, 29.17, 29.22, 29.39, 29.81, 30.03, 31.90, 35.01, 36.38, 39.51, 39.74, 40.78, 45.84, 46.65, 48.04, 48.95, 49.32, 49.39, 51.80, 53.56, 54.90, 55.81, 55.89, 60.18, 64.33, 70.30, 111.13, 117.30, 118.14, 121.48, 123.16, 123.42, 123.76, 126.11, 126.33, 127.10, 127.37, 128.36, 129.30, 130.90, 133.31, 136.16, 137.39, 137.90, 140.00, 141.30, 145.50, 155.87, 163.47, 167.66, 168.60, 170.16, 171.45, 173.07; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.36 min, 95% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{62}H_{78}N_{12}O_{11}ClS_2$, 1265.5038; found, 1265.5021.

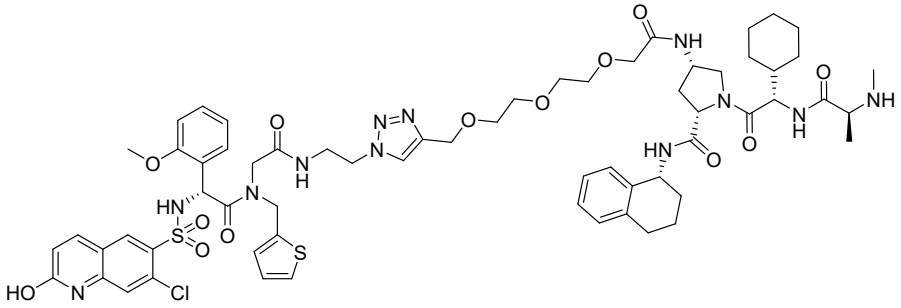
IAP Series PROTAC 10



This compound was prepared using the General Procedure **VI** and PROTAC precursor **51** (130 mg, 95 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1) to give a colorless solid.

Yield (77 mg, 64%); R_f = 0.20 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.86 – 1.19 (m, 6H), 1.27 (d, *J* = 6.8 Hz, 3H), 1.51 – 1.72 (m, 2H), 1.73 – 1.89 (m, 5H), 1.90 – 2.03 (m, 2H), 2.19 – 2.28 (m, 1H), 2.31 (d, *J* = 8.2 Hz, 3H), 2.34 – 2.45 (m, 1H), 2.63 – 2.82 (m, 2H), 3.51 – 3.66 (m, 4H), 3.68 – 3.87 (m, 10H), 3.97 – 4.07 (m, 2H), 4.09 – 4.19 (m, 1H), 4.28 – 4.44 (m, 3H), 4.52 – 4.77 (m, 6H), 5.09 (s, 1H), 5.73 – 6.03 (m, 1H), 6.45 – 6.72 (m, 4H), 6.77 – 6.87 (m, 3H), 6.95 – 7.08 (m, 4H), 7.09 – 7.24 (m, 4H), 7.36 (s, 1H), 7.58 – 7.70 (m, 4H), 7.97 (d, *J* = 24.5 Hz, 1H), 8.55 (d, *J* = 7.4 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 19.62, 20.24, 25.92, 26.08, 28.63, 29.28, 29.48, 30.10, 31.94, 35.23, 39.82, 40.72, 46.67, 47.83, 48.73, 49.23, 51.92, 54.88, 55.09, 55.86, 60.14, 60.33, 64.77, 64.93, 70.05, 70.90, 71.36, 111.24, 117.35, 118.15, 121.51, 123.21, 123.40, 123.59, 123.93, 126.16, 126.33, 127.11, 127.29, 127.47, 128.46, 128.70, 129.25, 130.66, 130.90, 133.41, 136.36, 137.35, 137.50, 139.99, 141.26, 145.18, 155.93, 163.42, 167.70, 168.68, 170.40, 170.97, 172.95, 175.27; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.19 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{61}H_{76}N_{12}O_{12}ClS_2$, 1267.4830; found, 1267.4816.

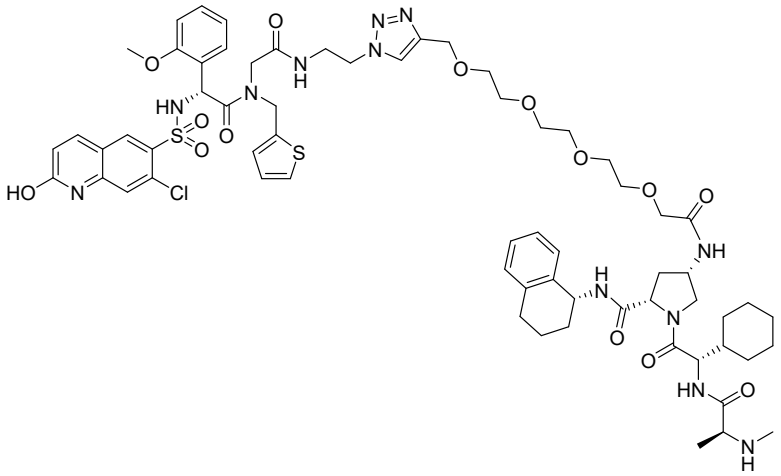
IAP Series PROTAC 11



This compound was prepared using the General Procedure **VI** and PROTAC precursor **52** (110 mg, 78 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1) to give a colorless solid.

Yield (87 mg, 85%); R_f = 0.28 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.87 – 1.19 (m, 6H), 1.27 (d, *J* = 6.9 Hz, 3H), 1.50 – 1.73 (m, 7H), 1.73 – 2.02 (m, 8H), 2.29 (d, *J* = 12.1 Hz, 2H), 2.36 (d, *J* = 10.6 Hz, 1H), 2.63 – 2.82 (m, 2H), 2.99 – 3.46 (m, 1H), 3.51 – 3.88 (m, 16H), 4.14 – 4.21 (m, 1H), 4.33 – 4.46 (m, 3H), 4.55 – 4.68 (m, 5H), 4.74 (d, *J* = 8.7 Hz, 1H), 5.12 (d, *J* = 7.7 Hz, 1H), 5.68 – 6.04 (m, 1H), 6.50 – 6.88 (m, 6H), 6.92 – 7.09 (m, 4H), 7.09 – 7.23 (m, 4H), 7.34 (s, 1H), 7.59 – 7.78 (m, 4H), 7.97 (d, *J* = 15.5 Hz, 1H), 8.59 (dd, *J* = 17.7, 7.5 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 19.52, 20.20, 25.94, 26.12, 28.62, 29.29, 29.48, 30.09, 35.05, 39.63, 40.70, 45.83, 46.61, 47.71, 48.79, 49.35, 51.96, 53.57, 54.90, 55.39, 55.86, 60.15, 64.67, 69.90, 70.64, 70.89, 71.00, 71.19, 111.24, 117.34, 118.19, 121.52, 123.15, 123.32, 123.88, 126.12, 126.33, 126.51, 126.77, 127.10, 127.46, 127.92, 128.53, 129.23, 130.65, 130.86, 131.91, 133.30, 136.49, 137.34, 137.93, 140.01, 141.27, 145.20, 155.94, 163.39, 167.68, 168.49, 170.14, 170.44, 170.96, 172.89; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.24 min, 99% purity, detection at 254 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{63}H_{80}N_{12}O_{13}ClS_2$, 1311.5092; found, 1311.5078.

IAP Series PROTAC 12



This compound was prepared using the General Procedure **VI** and PROTAC precursor **53** (110 mg, 76 µmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1) to give a colorless solid.

Yield (64 mg, 63%); R_f = 0.38 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.86 – 1.19 (m, 6H), 1.27 (d, *J* = 6.9 Hz, 3H), 1.54 – 1.73 (m, 7H), 1.75 – 1.91 (m, 5H), 1.94 – 2.05 (m, 2H), 2.29 (d, *J* = 9.0 Hz, 3H), 2.35 – 2.43 (m, 1H), 2.66 – 2.81 (m, 2H), 3.07 (s, 1H), 3.53 – 3.88 (m, 19H), 4.02 (s, 2H), 4.17 (dd, *J* = 11.0, 5.7 Hz, 1H), 4.30 – 4.46 (m, 3H), 4.54 – 4.78 (m, 6H), 5.12 (s, 1H), 5.70 – 6.44 (m, 1H), 6.55 – 6.74 (m, 3H), 6.74 – 6.88 (m, 2H), 6.92 – 7.23 (m, 7H), 7.34 (s, 1H), 7.64 (dd, *J* = 15.2, 5.6 Hz, 3H), 7.97 (d, *J* = 13.7 Hz, 1H), 8.59 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 19.52, 20.24, 25.95, 26.14, 28.62, 29.30, 29.49, 30.10, 35.07, 39.63, 40.70, 46.64, 47.73, 48.81, 48.95, 49.33, 51.91, 54.92, 55.35, 55.88, 60.16, 64.65, 69.90, 70.00, 70.53, 70.62, 70.91, 71.34, 111.28, 117.32, 118.18, 121.54, 123.19, 123.94, 126.12, 126.35, 126.55, 126.79, 127.13, 127.24, 127.50, 127.94, 128.54, 128.68, 129.22, 130.68, 130.89, 133.41, 136.51, 137.32, 137.47, 137.91, 139.99, 141.30, 145.22, 155.95, 163.33, 167.66, 168.49, 170.15, 170.39, 170.92, 172.95; **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 6.30 min, 98% purity, detection at 254 nm; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{65}H_{84}N_{12}O_{14}ClS_2$, 1355.5354; found, 1355.5338.

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