HaloPROTACS: Use of Small Molecule PROTACs to Induce Degradation of HaloTag Fusion Proteins

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A. Fluorescence Polarization

VHL binding affinity was measured by a fluorescence polarization assay as previously described. In brief, assays were performed in 10% DMSO, 10 mM stocks of the VHL ligands in DMSO were diluted 2 fold 14 times. 9 µL of 1 µM VCB (450 nM final), 2 µL of VHL Ligands in DMSO (10% DMSO final), and 9 µL of 278 nM FAM-DEAL-A-Hyp-YIPD were added to a 384 well plate (Corning 3575). The plate was then shaken for 1 minute, and centrifuged for 1 minute, before reading fluorescence polarization on a Perkin Elmer Envision 2101 Multilabel reader (excitation 486 nM, emission 535 nM). The percent inhibition was determined by normalizing to vehicle alone (DMSO, VCB, and fluorescent peptide) and no protein (DMSO, buffer and fluorescent peptide). IC50s were determined using Prism 5.0 for each independent run, then averaged.

Supplemental Figure 1. Binding of HaloPROTACs and VL285 to VHL/ElonginB/ElonginC complex by fluorescence polarization. Points represent means (n=3), error bars represent SEM.
B. Fluorescence Microscopy

![Fluorescence images of cells treated with different compounds.](image)

**Supplemental Figure 2:** Phase contrast view of cells after treatment with indicated compounds, as in Figure 5. Fluorescence images in Figure 5 are shown again for comparison.

GFP fluorescence of live cells was visually monitored using a fluorescence microscope. HEK 293 FlpIn cells expressing GFP-HaloTag7 were treated with HaloPROTACs for 24 hours in conditions identical to the flow-cytometry assay. Cells were washed with PBS, then imaged. The bright field and fluorescence images depict the same field of view.

C. MTS Assay
Supplemental Figure 3. Toxicity of HaloPROTAC3 was measured using an MTS assay, using a procedure adapted from Hines et al.² HEK 293 Flp-In cells stably expressing GFP-HaloTag7 were seeded in a 96 well plate and treated with the indicated concentration of HaloPROTAC3 for 24 hours (with 5 replicates). 330 µg/ml of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, purchased from Promega) and 25 µM PMS (phenazine methosulfate, purchased from Sigma) were then added and incubated at 37°C for 1.5 hours. Mitochondrial reduction of MTS was monitored by measuring absorbance at 490 nm using a Wallac Victor2 platereader (Perkin-Elmer Life Sciences). Error bars depict the standard error of the mean (SEM).

C. ODD-Luciferase
Supplemental Figure 4. VHL inhibition was measured using an ODD-luciferase assay, which measures the stability of the oxygen dependent degradation (ODD) domain of HIF, a cellular target of VHL. ODD-luc (human neuroblastoma, SH-SY5Y) cells were treated with the indicated concentrations of HaloPROTAC3 or with the positive control, CoCl$_2$ for 24 hours, in triplicate. The cells were then lysed with Passive Lysis Buffer (Promega). Luciferase assay buffer was added, and luminescence was measured using a Wallac Victor2 platereader (Perkin-Elmer Life Sciences). The luminescence was then normalized to protein content as determined by a BCA assay. While CoCl$_2$ increased luminescence 10-fold compared to cells treated with vehicle (0.1% DMSO), no significant difference was observed between HaloPROTAC3 treated cells and the DMSO control.

D. General Chemistry.
All reactions were performed in oven-dried or flame-dried glassware fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. THF, DCM, DMF, toluene and MeCN were obtained from a Pure Solv MD-5 Solvent Purification System from Innovative Technologies. All other solvents were used as obtained from suppliers. Analytical thin layer chromatography (TLC) was performed using glass plates precoated with silica gel (0.25 mm). TLC plates were visualized by exposure to UV light (UV) or KMnO₄. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. ¹H and ¹³C spectra were recorded on 400 or 500 MHz NMR spectrometers. ¹H NMR spectra are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), integration, and coupling constant (J) in Hertz (Hz). ¹H NMR chemical shifts are reported relative to CDCl₃ (7.26 ppm), d₆-DMSO (2.50 ppm) and d₄-CD₃OD (3.31 ppm). ¹³C NMR was recorded relative to the central line of CDCl₃ (77.16 ppm), d₆-DMSO (39.52 ppm) and d₄-CD₃OD (49.00 ppm). In most cases, only peaks of the major rotamer are reported. Mass spectra were obtained using a Perkin-Elmer API 150 EX spectrometer.

E. Synthesis of Chloroalkane PROTACs

(S)-3-methyl-2-(1-oxoisodolin-2-yl)butanoic acid

L-Valine (3.42 g, 29.2 mmol, 1 eq) and phthalaldehyde (4.3 g, 32 mmol, 1.1 eq) were dissolved in MeCN (130 mL). The flask was fitted with a reflux condenser and
heated in a 90 °C oil bath for 3.5 h. The mixture was then cooled to room temperature and then to 4 °C. The mixture was filtered through fluted filter paper and washed with cold MeCN. The resulting light yellow crystals were then dried under vacuum to give the desired product (5.63 g, 24.2 mmol, 83%). $^1$H NMR data matched the data reported in the literature.$^4$

![2-hydroxy-4-(4-methylthiazol-5-yl)benzonitrile](image)

**2-hydroxy-4-(4-methylthiazol-5-yl)benzonitrile**

4-Bromo-2-hydroxybenzonitrile (5 g, 25 mmol, 1 eq), potassium acetate (4.9 g, 50 mmol, 2 eq) and Pd(OAc)$_2$ (56 mg, 0.25 mmol, 1 mol%) were dissolved in DMA (100 mL, 0.25M). 4-methylthiazole (4.6 mL, 50 mmol, 2 eq) was added and the solution was heated to 150 °C under a balloon of argon.$^5$ After 15 h, the mixture was cooled to room temperature and diluted with water and minimal brine. The aqueous layer was extracted 3 times with EtOAc. The combined organic layer was then washed four times with brine. The organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (40 to 67% EtOAc/hexanes with a 100% EtOAc flush) gave a yellow solid (4.13 g, 19.1 mmol, 76%). $^1$H NMR (500 MHz, DMSO) δ 11.34 (s, 1H), 9.07 (s, 1H), 7.70 (d, $J = 8.1$ Hz, 1H), 7.13 (d, $J = 1.5$ Hz, 1H), 7.07 (dd, $J = 8.1, 1.5$ Hz, 1H),
Lithium aluminum hydride (1.68 g, 44.4 mmol, 6 eq) was added to a 3-neck flask under argon and fitted with a vigreux column. The flask was placed in an ice bath and dry THF (45 mL) was added slowly. 2-hydroxy-4-(4-methylthiazol-5-yl)benzonitrile (1.6 g, 7.4 mmol, 1 eq) was dissolved in a separate flask in THF (15 mL) and transferred slowly to the solution of LAH, and then washed once with THF (15 mL). The mixture was then heated to 50 °C for 22 h. The mixture was then cooled to 4 °C and quenched sequentially with water (2 x 3 mL) then 3M sodium hydroxide (2 x 3 mL). The mixture was then filtered through fluted filter paper, then washed three times with THF and 5 times with 20% MeOH/DCM. The organic layer was concentrated and then purified by column chromatography (10 to 30% 0.5N NH₃ (MeOH)/DCM) to give a yellow oil that slowly solidifies upon storage (0.439 g, 1.99 mmol, 27%). \(^1\)H NMR (500 MHz, MeOH) \(\delta\) 8.84 (s, 1H), 7.22 (d, \(J = 7.7\) Hz, 1H), 6.89 (d, \(J = 1.6\) Hz, 1H), 6.85 (dd, \(J = 7.7, 1.7\) Hz, 1H), 3.95 (s, 2H), 2.48 (s, 3H). \(^{13}\)C NMR (126 MHz, MeOH) \(\delta\) 159.18, 152.63, 148.83, 133.62, 133.43, 130.69, 126.57, 120.58, 117.51, 42.65, 15.92. MS (ESI) 221.5 (M+H).

(2S,4R)-tert-butyl 4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate
2-(aminomethyl)-5-(4-methylthiazol-5-yl)phenol (0.613 g, 2.78 mmol, 1 eq) and Boc-Hyp-OH (0.642 g, 2.78 mmol, 1 eq) were dissolved in DMF at 4 °C. DIPEA (1.5 mL, 8.4 mmol, 3 eq) and HATU (1.16 g, 3.06 mmol, 1.10 eq) were added and the mixture was warmed to room temperature after 10 minutes. After 2.5 h, the reaction was diluted with half saturated brine and extracted three times with EtOAc. The organic layer was then washed three times with 10% lithium chloride and once with brine, then dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM gave a foamy yellow oil (0.630 g, 1.45 mmol, 52%); a significant amount of the diacylated aminophenol was also isolated. $^1$H NMR (500 MHz, MeOH) $\delta$ 8.87 (s, 1H), 7.31 (d, $J$ = 7.7 Hz, 1H), 6.99 – 6.83 (m, 2H), 4.52 – 4.28 (m, 4H), 3.67 – 3.47 (m, 2H), 2.51 (d, $J$ = 8.1 Hz, 3H), 2.28 (dd, $J$ = 13.2, 7.6 Hz, 1H), 2.06 (ddd, $J$ = 13.1, 8.6, 4.5 Hz, 1H), 1.51 – 1.27 (m, 9H). $^{13}$C NMR (126 MHz, MeOH) $\delta$ 176.15, 157.09, 156.16, 152.76, 148.92, 133.44, 133.37, 131.70, 126.19, 121.47, 117.50, 81.67, 70.09, 60.68, 55.99, 40.78, 39.73, 28.42, 15.88. MS (ESI) 434.3 (M+H).

$(2S,4R)$-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride
(2S,4R)-tert-butyl 4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate (0.27 g, 0.62 mmol, 1 eq) was dissolved in DCM (10 mL) and MeOH (0.5 mL) at room temperature. 4M HCl in dioxane (2 mL, 7.9 mmol, 12.5 eq) was added and the mixture was stirred for 16 h. The mixture was then concentrated under a stream of nitrogen, then redissolved in MeOH and DCM and condensed to give a cream colored solid (0.26 g, ~0.7 mmol, quantitative yield) which was deemed sufficiently pure by NMR. \(^1\)H NMR (400 MHz, MeOH) \(\delta\) 9.23 (s, 1H), 8.81 (s, 1H), 7.31 (d, \(J = 8.2\) Hz, 1H), 6.96 (dt, \(J = 3.4, 1.7\) Hz, 2H), 4.60 (s, 1H), 4.55 – 4.38 (m, 3H), 3.75 (dd, \(J = 23.1, 11.1\) Hz, 2H), 2.53 (s, 3H), 2.47 (dd, \(J = 13.5, 7.4\) Hz, 1H), 2.13 – 2.02 (m, 1H). MS (ESI) 334.4 (M+H).

(2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide

(2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (128 mg, 0.346 mmol, 1 eq) and (S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoic acid (89 mg, 0.38 mmol, 1.1 eq) were dissolved in DMF. DIPEA (0.22 mL, 1.2 mmol, 3.5 eq) was added, followed by HATU (138 mg, 0.363 mmol, 1.05 eq). The mixture was stirred for 22 h, then diluted with water and extracted three times with EtOAc. The combined organic layer was washed three times with 10% lithium chloride, then once with saturated ammonium chloride, sodium bicarbonate, water and brine. The
organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) followed by preparative TLC (5% MeOH/DCM) gave a cream colored solid (45.5 mg, 0.083 mmol, 24%). In addition to the product, the diacylated product (MS:764.4), likely resulting from phenol acylation, was recovered (39.1 mg). ¹H NMR (500 MHz, MeOH) δ 8.85 (s, 1H), 7.78 (d, \(J = 7.6\) Hz, 1H), 7.59 (ddd, \(J = 19.0, 12.8, 4.3\) Hz, 2H), 7.49 (t, \(J = 7.4\) Hz, 1H), 7.34 (d, \(J = 8.3\) Hz, 1H), 6.92 (dd, \(J = 5.7, 1.8\) Hz, 2H), 4.84 (s, 2H, obscured by water peak, but detected by HMQC), 4.64 – 4.34 (m, 6H), 4.02 – 3.83 (m, 2H), 2.54 – 2.40 (m, 4H), 2.25 – 2.14 (m, 1H), 2.08 (ddd, \(J = 13.3, 8.9, 4.5\) Hz, 1H), 1.06 (d, \(J = 6.5\) Hz, 3H), 0.83 (d, \(J = 6.7\) Hz, 3H). ¹³C NMR (126 MHz, MeOH) δ 174.40, 170.93, 170.67, 156.74, 152.65, 148.86, 143.80, 133.20, 132.91, 132.48, 130.65, 129.16, 126.13, 124.40, 124.36, 121.42, 116.83, 70.84, 60.62, 60.12, 57.10, 39.54, 39.08, 30.14, 19.67, 18.98, 15.93. MS (ESI) 549.4 (M+H).

(R)-3-methyl-2-(1-oxoisindolin-2-yl)butanoic acid

D-Valine (1.71 g, 14.6 mmol, 1 eq) and phthalaldehyde (2.16 g, 16.1 mmol, 1.1 eq) were dissolved in MeCN (65 mL). The flask was fitted with a reflux condenser and heated in a 90 °C oil bath for 3.5 h. The mixture was then cooled to room temperature and then to
4 °C. The mixture was filtered through fluted filter paper and washed with cold MeCN. The resulting light yellow crystals were then dried under vacuum to give the desired product (2.48 g, 10.6 mmol, 73%). $^1$H NMR data matched the data reported for the enantiomeric (R)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoic acid.$^4$

$^{(2R,4S)}$-tert-butyl 4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate

Boc-D-Hyp-OH (400 mg, 1.7 mmol) was dissolved in 3 mL DMF and charged with HATU (662 mg, 1.7 mmol), 2-(aminomethyl)-5-(4-methylthiazol-5-yl)phenol (300 mg, 1.42 mmol), and DIPEA (0.304 mL, 1.7 mmol). The reaction was left stir for 14 hours upon which time the mixture was diluted with 20 mL NH$_4$Cl, and washed with EtOAc (3 X 20 mL). The organic layers were combined and washed with saturated NaHCO$_3$ (1 X 50 mL), and 10% LiCl (3 X 20 mL), dried with sodium sulfate and concentrated down to yield a crude oil that was dissolved in 5 mL MeOH and charged with 400 mg Na$_2$CO$_3$ and left to stir for 30 minutes upon which time the volatiles were removed by rotovap and the mixture purified by silica gel chromatography (DCM to 10% MeOH in DCM) to yield 130 mg of a pure oil. $^1$H NMR (500 MHz, MeOH) δ 8.81 (s, 1H), 7.27 (d, $J = 7.8$ Hz, 1H), 6.95 – 6.78 (m, 2H), 4.52 – 4.23 (m, 4H), 3.67 – 3.45 (m, 2H), 2.44 (s, 3H), 2.34 – 2.16 (m, 1H), 2.04 (ddd, $J = 13.1, 8.6, 4.5$ Hz, 1H), 1.34 (d, $J = 104.9$ Hz, 9H). $^{13}$C NMR (126 MHz, MeOH) δ 176.06, 156.85, 156.00, 152.66, 148.70, 133.29, 133.20,
MS (ESI) 434.3 (M+H).

(2R,4S)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride

The resultant oil was then dissolved in 3 mL 4 M HCl in dioxanes and let stir for one hour, at which time Nitrogen gas was sparged through until dryness, to yield 130 mg (25% yield over 2 steps) of a light yellow foam after high vacuum. $^1$H NMR (500 MHz, ) δ 9.59 (s, 1H), 8.82 (s, 1H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.07 – 6.95 (m, 2H), 4.60 (s, 1H), 4.56 – 4.42 (m, 3H), 3.80 – 3.63 (m, 1H), 2.57 (s, 3H), 2.48 (dd, $J = 13.6$, 7.4 Hz, 1H), 2.14 – 2.01 (m, 1H). MS (ESI) 334.2 (M -Cl)

(2R,4S)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)-1-((R)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide

(2R,4S)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (210 mg, 0.567 mmol), (R)-3-methyl-2-(1-oxoisindolin-2-yl)butanoic acid (175 mg, 0.737 mmol) were dissolved in 3 mL DMF and charged with
HATU (287 mg, 0.737 mmol) and DIPEA (0.203 mL, 1.12 mmol). Upon 16 h stirring the mixture was diluted with 20 mL NH₄Cl, and washed with EtOAc (3 X 10 mL). The organic layers were combined and washed with saturated NaHCO₃ (1 X 25 mL), and 10% LiCl (3 X 25 mL), dried with sodium sulfate and concentrated down to yield a crude oil that was dissolved in 5 mL MeOH and charged with 400 mg Na₂CO₃ and left to stir for 30 minutes upon which time the volatiles were removed by rotovap and the mixture purified by silica gel chromatography (DCM to 6 % MeOH in DCM by 1 % increments) to yield (130 mg, 42 % yield) of product as a light yellow solid. **¹H NMR** (300 MHz, CD₃OD) δ 8.85 (s, 1H), 7.78 (d, J = 7.6, 1H), 7.65 – 7.45 (m, 4H), 7.34 (d, J = 8.3, 1H), 6.96 – 6.81 (m, 2H), 4.65 – 4.31 (m, 7H), 4.04 – 3.93 (broad m, 1H), 3.92 – 3.83 (m, 1H), 2.48 (s, 3H), 2.46 – 2.38 (m, 1H), 2.24 – 2.14 (m, 1H), 2.13 – 2.04 (m, 1H), 1.06 (d, J = 6.5, 3H), 0.83 (d, J = 6.6, 3H). **MS** (ESI) 549.4 (M+H).

(2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)-1-(2-(3-methylisoxazol-5-yl)acetyl)pyrrolidine-2-carboxamide
3-methyl-5-isoxazolacetic acid (53.2 mg, 0.377 mmol, 1.1 eq) and DIPEA (0.09 mL, 0.52 mmol, 1.5 eq) were dissolved in DMF (1.4 mL) at room temperature. In a separate vial, (2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (126.6 mg, 0.342 mmol, 1 eq) and DIPEA (0.09 mL, 0.52 mmol, 1.5 eq) were dissolved in DMF (1.9 mL). After 10 minutes, the solution of (2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride was added to the solution of 3-methyl-5-isoxazolacetic acid. The mixture was stirred for 15 h, diluted with water (5 mL) and saturated ammonium chloride (10 mL) and then extracted three times with EtOAc. The organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (2 to 20% MeOH/DCM) gave a light brown solid (65.5 mg, 0.143 mmol, 42%). \textsuperscript{1}H NMR (500 MHz, ) δ 8.86 (s, 1H), 8.39 (d, \( J = 8.4 \) Hz, 1H), 7.50 (dd, \( J = 8.3, 4.4 \) Hz, 1H), 7.30 (dd, \( J = 8.0, 5.3 \) Hz, 1H), 6.91 (dd, \( J = 4.1, 2.4 \) Hz, 2H), 6.25 (s, 1H), 4.62 (dd, \( J = 27.4, 19.4 \) Hz, 1H), 4.53 (s, 1H), 4.49 – 4.36 (m, 2H), 3.95 (d, \( J = 2.7 \) Hz, 1H), 3.84 (dt, \( J = 14.3, 7.1 \) Hz, 1H), 3.66 (d, \( J = 11.1 \) Hz, 1H), 2.49 (s, 3H), 2.33 – 2.21 (m, 4H), 2.12 (ddd, \( J = 12.9, 8.1, 4.7 \) Hz, 1H). \textsuperscript{13}C NMR (126 MHz, MeOH) δ 174.40, 170.93, 170.67, 156.74, 152.65, 148.86, 143.80, 133.20, 132.91, 132.48, 130.65, 129.16, 126.13, 124.40, 124.36, 121.42, 116.83, 70.84, 60.62, 60.12, 57.10, 39.54, 39.08, 30.14, 19.67, 18.98, 15.93. MS (ESI) 457.2 (M+H).
(S)-2-(1-oxoisoindolin-2-yl)propanoic acid

L-alanine (1.34 g, 15 mmol, 1 eq) and phthalaldehyde (2.2 g, 16.5 mmol, 1.1 eq) were dissolved in MeCN (50 mL). The flask was fitted with a reflux condenser and heated in a 90 °C oil bath for 3 h. The mixture was then cooled to room temperature and then to 4 °C. The mixture was filtered through fluted filter paper and washed with cold MeCN. The resulting light yellow crystals were then dried under vacuum to give the desired product (2.42 g, 11.8 mmol, 79%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 12.91 (s, 1H), 7.70 (d, $J =$ 7.6 Hz, 1H), 7.66 – 7.59 (m, 2H), 7.55 – 7.47 (m, 1H), 4.84 (q, $J =$ 7.4 Hz, 1H), 4.63 – 4.37 (m, 2H), 1.51 (d, $J =$ 7.5 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO) δ 173.02, 167.53, 142.13, 131.91, 131.54, 127.89, 123.50, 122.83, 49.09, 46.82, 15.33. MS (ESI) 206.2 (M+H).

(2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-oxoisoindolin-2-yl)propanoyl)pyrrolidine-2-carboxamide

(2S,4R)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (152.0 mg, 0.411 mmol, 1 eq) and (S)-2-(1-oxoisoindolin-2-yl)propanoic acid (92.8 mg, 0.452 mmol, 1.1 eq) were dissolved in DMF (1.65 mL) at
room temperature. DIPEA (0.214 mL, 1.23 mmol, 3 eq) was added, followed by HATU (164.3 mg, 0.432 mmol, 1.05 eq). After 2.5 hours, the mixture was diluted with saturated sodium bicarbonate and extracted three times with EtOAc. The organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 20% MeOH/DCM) gave a cream colored solid (148.2, 0.285 mmol, 69%). $^1$H NMR (500 MHz, ) δ 8.84 (s, 1H), 7.76 (d, $J = 7.5$ Hz, 1H), 7.64 – 7.51 (m, 2H), 7.48 (d, $J = 7.4$ Hz, 1H), 7.31 (d, $J = 8.3$ Hz, 1H), 6.96 – 6.83 (m, 2H), 5.37 – 5.13 (m, 1H), 4.69 – 4.52 (m, 3H), 4.52 – 4.32 (m, 3H), 3.89 – 3.74 (m, 2H), 2.47 (s, 3H), 2.22 (ddd, $J = 11.1$, 8.0, 1.5 Hz, 1H), 2.06 (ddd, $J = 13.1$, 8.3, 4.7 Hz, 1H), 1.52 (d, $J = 7.1$ Hz, 3H). $^{13}$C NMR (126 MHz, MeOH) δ 174.58, 172.13, 170.49, 156.65, 152.65, 148.83, 143.92, 133.49, 133.12, 132.83, 132.77, 130.47, 129.10, 126.09, 124.37, 124.22, 121.40, 116.70, 70.86, 60.70, 56.48, 50.07, 48.53, 39.43, 38.97, 15.94, 15.49. MS (ESI) 522.3 (M+H), 543.7 (M+Na).

General Procedure for Oligoethylene Glycol Alkylation.
Oligoethylene glycol (5 eq) was added dropwise to a mixture of NaH (95%, dry, 2.5 eq) in DMF (1M) and THF (1M) at 4 °C under dry argon. After 40 minutes, 1-chloro-6-iodohexane (1 eq) was added, and the mixture was warmed to room temperature and stirred overnight. The mixture was then quenched with water, diluted with 1M HCl, and extracted thrice with chloroform. The organic layer was dried over sodium sulfate, filtered, concentrated and purified by column chromatography (25 to 100% EtOAc/hexanes) to give the monoalkylated product.

2-((6-chlorohexyl)oxy)ethanol (n=1)

\[
\text{Cl} - \overset{\text{O}}{\text{C}} - \overset{\text{O}}{\text{C}} - \overset{\text{OH}}{\text{OH}}
\]

2-((6-chlorohexyl)oxy)ethanol, a colorless oil, was synthesized according to the above method in 50% yield. \(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 3.76 – 3.70 (m, 2H), 3.58 – 3.51 (m, 4H), 3.48 (t, \(J = 6.6\) Hz, 2H), 1.89 (s, 1H), 1.84 – 1.73 (m, 2H), 1.66 – 1.56 (m, 2H), 1.52 – 1.43 (m, 2H), 1.43 – 1.33 (m, 2H). \(^1\)C NMR (126 MHz, CDCl\(_3\)) δ 71.88, 71.29, 62.03, 45.17, 32.67, 29.64, 26.84, 25.60. MS (ESI) 202.9 (M+Na).

2-(2-((6-chlorohexyl)oxyethoxy)ethanol (n=2)

\[
\text{Cl} - \overset{\text{O}}{\text{C}} - \overset{\text{O}}{\text{C}} - \overset{\text{OH}}{\text{OH}}
\]

2-(2-((6-chlorohexyl)oxyethoxy)ethanol, a light yellow oil, was synthesized according to the above general method, but under more dilute conditions (using 0.2M THF and 0.2M DMF) to give the monoalkylated product in 85% yield. \(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 3.62 (dd, \(J = 6.1, 3.2\) Hz, 2H), 3.59 – 3.55 (m, 2H), 3.54 – 3.47 (m, 4H), 3.44 (t, \(J = 6.7\) Hz, 2H), 3.38 (t, \(J = 6.7\) Hz, 2H), 3.17 – 2.98 (m, 1H), 1.75 – 1.63 (m,
2H), 1.57 – 1.46 (m, 2H), 1.36 (ddd, \( J = 10.8, 8.8, 7.0 \) Hz, 2H), 1.33 – 1.24 (m, 2H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \( \delta \) 72.49, 71.14, 70.28, 70.05, 61.55, 44.90, 32.41, 29.28, 26.56, 25.27. MS (ESI) 225.2 (M+H).

2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)ethanol (n=3)

\[
\begin{align*}
\text{Cl} & \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{OH} \\
\end{align*}
\]

2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)ethanol, a colorless oil, was synthesized according to the above general method to give the monoalkylated product in 52% yield. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 3.74 – 3.71 (m, 2H), 3.70 – 3.64 (m, 6H), 3.63 – 3.60 (m, 2H), 3.59 (dd, \( J = 5.9, 3.5 \) Hz, 2H), 3.53 (t, \( J = 6.7 \) Hz, 2H), 3.46 (t, \( J = 6.7 \) Hz, 2H), 1.81 – 1.74 (m, 2H), 1.63 – 1.57 (m, 3H), 1.49 – 1.41 (m, 2H), 1.41 – 1.34 (m, 2H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \( \delta \) 72.63, 71.44, 70.80, 70.78, 70.55, 70.23, 61.95, 45.21, 32.70, 29.57, 26.85, 25.56. MS (ESI) 269.2 (M+H).

18-chloro-3,6,9,12-tetraoxaoctadecan-1-ol (n=4)

\[
\begin{align*}
\text{Cl} & \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{OH} \\
\end{align*}
\]

18-chloro-3,6,9,12-tetraoxaoctadecan-1-ol, a colorless oil, was synthesized according to the above general method to give the monoalkylated product in 56% yield. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 3.72 – 3.67 (m, 2H), 3.67 – 3.60 (m, 10H), 3.60 – 3.53 (m, 4H), 3.50 (t, \( J = 6.7 \) Hz, 2H), 3.43 (t, \( J = 6.6 \) Hz, 2H), 2.79 (s, 1H), 1.79 – 1.70 (m, 2H), 1.61 – 1.52 (m, 2H), 1.47 – 1.39 (m, 2H), 1.39 – 1.29 (m, 2H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \( \delta \) 72.62, 71.31, 70.70, 70.69, 70.67, 70.65, 70.43, 70.17, 61.81, 45.12, 32.62, 29.50, 26.77, 25.49. MS (ESI) 313.1 (M+H).
**21-chloro-3,6,9,12,15-pentaoxahenicosan-1-ol (n=5)**

![Chemical Structure]

21-chloro-3,6,9,12,15-pentaoxahenicosan-1-ol, a yellow oil, was synthesized according to the above general method to give the monoalkylated product in 69% yield.

**$^1$H NMR** (500 MHz, CDCl$_3$) $\delta$ 3.74 – 3.70 (m, 2H), 3.68 – 3.64 (m, 12H), 3.64 – 3.56 (m, 6H), 3.53 (t, $J = 6.7$ Hz, 2H), 3.45 (t, $J = 6.7$ Hz, 2H), 1.81 – 1.72 (m, 2H), 1.64 – 1.54 (m, 2H), 1.44 (dt, $J = 8.6$, 7.9 Hz, 2H), 1.40 – 1.31 (m, 2H).

**$^{13}$C NMR** (126 MHz, CDCl$_3$) $\delta$ 72.79, 71.39, 70.76, 70.75, 70.73, 70.70, 70.66, 70.42, 70.20, 61.87, 45.21, 32.70, 29.58, 26.84, 25.56. **MS** (ESI) 357.4 (M+H).

**Mesylation of oligoethylene glycol-hexylchloride**

Oligoethylene glycol-hexylchloride (1 eq) was dissolved in DCM (0.25 M) at room temperature. Triethylamine (3 eq) and methanesulfonyl chloride (1.5 eq) was added and the solution was stirred overnight. The mixture was then diluted with 10% citric acid and extracted thrice with DCM. The combined organic layer was dried over sodium sulfate, filtered and concentrated to give the desired mesylate, which was used without further purification.

**2-((6-chlorohexyl)oxy)ethyl methanesulfonate (n=1)**

![Chemical Structure]

2-((6-chlorohexyl)oxy)ethyl methanesulfonate was synthesized according to the general method, and isolated as a yellow oil in quantitative yield. **$^1$H NMR** (500 MHz, CDCl$_3$) $\delta$ 4.44 – 4.34 (m, 2H), 3.76 – 3.68 (m, 2H), 3.56 (t, $J = 6.7$ Hz, 2H), 3.51 (t, $J =$
6.6 Hz, 2H), 3.08 (s, 3H), 1.87 – 1.76 (m, 2H), 1.67 – 1.58 (m, 2H), 1.55 – 1.45 (m, 2H),
1.45 – 1.36 (m, 2H). 13C NMR (126 MHz, CDCl3) δ 71.48, 69.34, 68.63, 45.14, 37.81,
32.62, 29.52, 26.76, 25.50. MS (ESI) 258.7 (M+H).

2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl methanesulfonate (n=2)

2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl methanesulfonate was synthesized according to the general method and isolated as a yellow oil in quantitative yield. 1H NMR (500 MHz, CDCl3) δ 4.42 – 4.33 (m, 2H), 3.80 – 3.73 (m, 2H), 3.69 – 3.63 (m, 2H),
3.60 – 3.55 (m, 2H), 3.53 (t, J = 6.7 Hz, 2H), 3.45 (t, J = 6.6 Hz, 2H), 3.07 (s, 3H), 1.82 –
1.72 (m, 2H), 1.62 – 1.53 (m, 2H), 1.49 – 1.41 (m, 2H), 1.41 – 1.32 (m, 2H). 13C NMR
(126 MHz, CDCl3) δ 71.41, 70.86, 70.19, 69.40, 69.19, 45.19, 37.85, 32.65, 29.59, 26.81,
25.55. MS (ESI) 303.0 (M+H).

2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)ethyl methanesulfonate (n=3)

2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl methanesulfonate was synthesized according to the general method and isolated as a pale yellow oil in 99% yield. 1H NMR (500 MHz, CDCl3) δ 4.40 – 4.34 (m, 2H), 3.79 – 3.74 (m, 2H), 3.69 –
3.64 (m, 4H), 3.64 – 3.59 (m, 2H), 3.59 – 3.55 (m, 2H), 3.53 (t, J = 6.7 Hz, 2H), 3.44 (t, J
= 6.7 Hz, 2H), 3.08 (d, J = 5.1 Hz, 3H), 1.83 – 1.70 (m, 2H), 1.64 – 1.54 (m, 2H), 1.50 –
1.41 (m, 2H), 1.40 – 1.31 (m, 2H). 13C NMR (126 MHz, CDCl3) δ 71.39, 70.79, 70.77,
18-chloro-3,6,9,12-tetraoxoaodecyl methanesulfonate (n=4)

\[
\text{Cl-}\overset{O}{\begin{array}{c}O\end{array}}\overset{O}{\begin{array}{c}O\end{array}}\overset{O}{\begin{array}{c}O\end{array}}\overset{OMs}{\begin{array}{c}\end{array}}
\]

18-chloro-3,6,9,12-tetraoxaoctadecyl methanesulfonate was synthesized according to the general method and isolated as a yellow oil in 99% yield. \textbf{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}) \(\delta\) 4.42 – 4.31 (m, 2H), 3.81 – 3.71 (m, 2H), 3.69 – 3.59 (m, 10H), 3.59 – 3.54 (m, 2H), 3.52 (t, \(J = 6.7\) Hz, 2H), 3.44 (t, \(J = 6.7\) Hz, 2H), 3.07 (d, \(J = 4.9\) Hz, 3H), 1.80 – 1.72 (m, 2H), 1.63 – 1.54 (m, 2H), 1.49 – 1.40 (m, 2H), 1.40 – 1.32 (m, 2H). \textbf{\textsuperscript{13}C NMR} (126 MHz, CDCl\textsubscript{3}) \(\delta\) 71.36, 70.77, 70.75, 70.72, 70.65, 70.22, 69.43, 69.14, 45.18, 37.87, 32.66, 29.57, 26.81, 25.54. \textbf{MS} (ESI) 390.9 (M+H).

21-chloro-3,6,9,12,15-pentaoxahenicosyl methanesulfonate (n=5)

\[
\text{Cl-}\overset{O}{\begin{array}{c}O\end{array}}\overset{O}{\begin{array}{c}O\end{array}}\overset{O}{\begin{array}{c}O\end{array}}\overset{O}{\begin{array}{c}O\end{array}}\overset{OMs}{\begin{array}{c}\end{array}}
\]

21-chloro-3,6,9,12,15-pentaoxahenicosyl methanesulfonate was synthesized according to the general method and isolated as a yellow oil in quantitative yield. \textbf{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}) \(\delta\) 4.45 – 4.30 (m, 2H), 3.83 – 3.73 (m, 2H), 3.69 – 3.61 (m, 14H), 3.60 – 3.56 (m, 2H), 3.53 (t, \(J = 6.7\) Hz, 2H), 3.45 (t, \(J = 6.7\) Hz, 2H), 3.09 (d, \(J = 7.1\) Hz, 3H), 1.82 – 1.72 (m, 2H), 1.64 – 1.54 (m, 2H), 1.49 – 1.41 (m, 2H), 1.41 – 1.32 (m, 2H). \textbf{\textsuperscript{13}C NMR} (126 MHz, CDCl\textsubscript{3}) \(\delta\) 71.65, 71.06, 71.04, 71.02, 71.00, 70.94, 70.51, 69.72, 69.44, 63.83, 45.49, 38.17, 32.96, 29.87, 27.11, 25.84. \textbf{MS} (ESI) 435.3 (M+H).

**Phenol Alkylation**
The desired phenol (1 eq) and mesylate (1.1-1.2 eq) were dissolved in DMF (0.5 M). Potassium carbonate (2.5 eq) was added and the mixture was heated to 70 °C. The mixture was stirred overnight, then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) gave the desired PROTAC.

**HaloPROTAC5(n=0)**

HaloPROTAC5 was synthesized in an analogous manner to the above general method, substituting 1.2 eq of 1-chloro-6-iodohexane for the mesylate. It was isolated in 46% yield as a colorless oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.61 (s, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.44 (t, J = 7.5 Hz, 1H), 7.34 (dd, J = 7.1, 4.4 Hz, 2H), 7.23 (t, J = 6.9 Hz, 2H), 6.89 (dd, J = 7.7, 1.4 Hz, 1H), 6.80 (d, J = 1.2 Hz, 1H), 4.67 (t, J = 13.8 Hz, 2H), 4.59 (t, J = 7.8 Hz, 1H), 4.49 – 4.29 (m, 5H), 3.96 (dd, J = 7.1, 5.6 Hz, 2H), 3.64 – 3.54 (m, 1H), 3.49 (t, J = 6.6 Hz, 2H), 2.52 – 2.40 (m, 4H), 2.32 (dt, J = 17.7, 6.6 Hz, 1H), 2.03 – 1.90 (m, 1H), 1.82 (d, J = 4.7 Hz, 2H), 1.77 (dd, J = 13.7, 6.9 Hz, 2H), 1.69 – 1.35 (m, 5H), 0.80 (dt, J = 31.3, 15.6 Hz, 6H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.54, 170.49, 169.65, 156.95, 150.41, 148.61, 142.18, 132.35, 131.88, 131.70, 129.43, 128.11, 126.49, 123.93, 122.97, 121.66, 112.12, 70.09, 68.10, 64.00, 58.78, 58.54, 56.04, 47.56, 45.14, 39.00, 35.81, 32.63, 29.23, 28.93, 26.84, 25.67, 19.18, 19.17, 16.26. **MS** (ESI) 667.5 (M+H).
HaloPROTAC6 (n=1)

HaloPROTAC6 was synthesized according to the general method and isolated as a colorless oil in 53% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.67 (s, 1H), 7.75 (d, $J$ = 7.5 Hz, 1H), 7.50 (td, $J$ = 7.5, 1.1 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.35 (t, $J$ = 6.0 Hz, 1H), 7.31 (d, $J$ = 7.7 Hz, 1H), 6.98 (dd, $J$ = 7.7, 1.6 Hz, 1H), 6.91 (d, $J$ = 1.5 Hz, 1H), 4.73 (t, $J$ = 13.9 Hz, 2H), 4.61 (t, $J$ = 7.9 Hz, 1H), 4.53 (dd, $J$ = 14.9, 6.1 Hz, 2H), 4.49 – 4.35 (m, 3H), 4.23 – 4.12 (m, 2H), 3.92 – 3.78 (m, 2H), 3.69 (dd, $J$ = 11.4, 3.5 Hz, 1H), 3.61 – 3.51 (m, 2H), 3.48 (q, $J$ = 6.6 Hz, 2H), 2.52 (s, 3H), 2.47 – 2.34 (m, 2H), 2.06 (dd, $J$ = 13.5, 8.2 Hz, 1H), 1.76 – 1.66 (m, 2H), 1.66 – 1.56 (m, 2H), 1.45 – 1.30 (m, 4H), 0.98 – 0.83 (m, 6H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.72, 170.28, 169.59, 156.95, 150.42, 148.66, 142.20, 132.36, 131.84, 131.74, 129.60, 128.09, 126.98, 123.92, 122.94, 122.12, 112.87, 71.63, 70.14, 69.29, 68.11, 58.77, 58.66, 56.18, 47.54, 45.15, 39.16, 36.25, 32.60, 29.62, 28.95, 26.77, 25.52, 19.19, 16.28. MS (ESI) 711.0 (M+H).

HaloPROTAC7 (n=2)

HaloPROTAC7 was synthesized according to the general method and isolated as a colorless oil in 53% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.67 (s, 1H), 7.75 (d, $J$ = 7.5 Hz, 1H), 7.50 (td, $J$ = 7.5, 1.1 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.35 (t, $J$ = 6.0 Hz, 1H), 7.31 (d, $J$ = 7.7 Hz, 1H), 6.98 (dd, $J$ = 7.7, 1.6 Hz, 1H), 6.91 (d, $J$ = 1.5 Hz, 1H), 4.73 (t, $J$ = 13.9 Hz, 2H), 4.61 (t, $J$ = 7.9 Hz, 1H), 4.53 (dd, $J$ = 14.9, 6.1 Hz, 2H), 4.49 – 4.35 (m, 3H), 4.23 – 4.12 (m, 2H), 3.92 – 3.78 (m, 2H), 3.69 (dd, $J$ = 11.4, 3.5 Hz, 1H), 3.61 – 3.51 (m, 2H), 3.48 (q, $J$ = 6.6 Hz, 2H), 2.52 (s, 3H), 2.47 – 2.34 (m, 2H), 2.06 (dd, $J$ = 13.5, 8.2 Hz, 1H), 1.76 – 1.66 (m, 2H), 1.66 – 1.56 (m, 2H), 1.45 – 1.30 (m, 4H), 0.98 – 0.83 (m, 6H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.72, 170.28, 169.59, 156.95, 150.42, 148.66, 142.20, 132.36, 131.84, 131.74, 129.60, 128.09, 126.98, 123.92, 122.94, 122.12, 112.87, 71.63, 70.14, 69.29, 68.11, 58.77, 58.66, 56.18, 47.54, 45.15, 39.16, 36.25, 32.60, 29.62, 28.95, 26.77, 25.52, 19.19, 16.28. MS (ESI) 711.0 (M+H).
HaloPROTAC7 was synthesized according to the general method and isolated as a colorless oil in 37% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.68 (s, 1H), 7.77 (d, $J$ = 7.5 Hz, 1H), 7.51 (t, $J$ = 7.0 Hz, 1H), 7.42 (t, $J$ = 7.6 Hz, 2H), 7.34 (t, $J$ = 5.9 Hz, 1H), 7.31 (d, $J$ = 7.7 Hz, 1H), 6.98 (dd, $J$ = 7.7, 1.5 Hz, 1H), 6.90 (d, $J$ = 1.3 Hz, 1H), 4.75 (dd, $J$ = 14.3, 8.5 Hz, 2H), 4.59 (t, $J$ = 8.0 Hz, 1H), 4.57 – 4.49 (m, 2H), 4.47 – 4.35 (m, 3H), 4.26 – 4.15 (m, 2H), 3.99 – 3.89 (m, 2H), 3.77 – 3.66 (m, 3H), 3.62 – 3.54 (m, 2H), 3.50 (t, $J$ = 6.7 Hz, 2H), 3.41 (t, $J$ = 6.7 Hz, 2H), 2.77 (s, 1H), 2.52 (s, 3H), 2.45 – 2.34 (m, 2H), 2.08 (dd, $J$ = 13.5, 8.2 Hz, 1H), 1.76 – 1.70 (m, 2H), 1.60 – 1.49 (m, 2H), 1.40 (dt, $J$ = 14.5, 7.1 Hz, 2H), 1.31 (dd, $J$ = 15.2, 8.1 Hz, 2H), 0.91 (dd, $J$ = 30.4, 6.6 Hz, 6H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.67, 170.26, 169.56, 156.94, 150.42, 148.67, 142.24, 132.41, 131.83, 131.80, 129.77, 128.09, 126.96, 123.95, 122.97, 122.14, 112.80, 71.40, 70.99, 70.24, 70.14, 69.77, 68.09, 58.76, 58.65, 56.17, 47.55, 45.18, 39.30, 36.35, 32.64, 29.49, 28.94, 26.79, 25.50, 19.22, 16.29. MS (ESI) 755.5 (M+H).

**HaloPROTAC3 (n=3)**

HaloPROTAC3 was synthesized according to the general method and isolated as a light yellow oil in 60% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.67 (s, 1H), 7.76 (d, $J$ = 7.2 Hz, 1H), 7.50 (td, $J$ = 7.2, 1.1 Hz, 1H), 7.41 (t, $J$ = 7.2 Hz, 2H), 7.34 (t, $J$ = 6.1 Hz, 1H), 7.31 (d, $J$ = 7.7 Hz, 1H), 6.97 (dd, $J$ = 7.7, 1.6 Hz, 1H), 6.89 (d, $J$ = 1.5 Hz, 1H), 4.75 (t, $J$ = 13.8 Hz, 2H), 4.58 (t, $J$ = 8.1 Hz, 1H), 4.53 (dd, $J$ = 15.8, 9.5 Hz, 2H), 4.46 – 4.35 (m,
3H), 4.26 – 4.14 (m, 2H), 3.98 – 3.86 (m, 2H), 3.79 – 3.68 (m, 3H), 3.67 – 3.64 (m, 2H),
3.60 – 3.56 (m, 2H), 3.53 – 3.48 (m, 4H), 3.39 (t, J = 6.7 Hz, 2H), 2.52 (s, 3H), 2.44 –
2.29 (m, 2H), 2.08 (dd, J = 13.5, 8.1 Hz, 1H), 1.77 – 1.68 (m, 2H), 1.60 – 1.49 (m, 2H),
1.41 (dt, J = 9.0, 7.1 Hz, 2H), 1.33 (ddd, J = 8.9, 5.4, 3.1 Hz, 2H), 0.95 (d, J = 6.5 Hz,
3H), 0.87 (d, J = 6.6 Hz, 3H). ^13^C NMR (126 MHz, CDCl\textsubscript{3}) δ 170.75, 170.17, 169.50,
156.93, 150.44, 148.63, 142.23, 132.37, 131.80, 131.78, 129.83, 128.06, 126.98, 123.91,
122.96, 122.13, 112.78, 71.33, 70.92, 70.70, 70.67, 70.16, 70.10, 69.72, 68.04, 58.77,

MS (ESI) 800.0 (M+H).

**ent-HaloPROTAC3**

![ent-HaloPROTAC3](image)

*ent*-HaloPROTAC3 was synthesized according to the general method and isolated in 43%
yield. The NMR and MS spectra matched HaloPROTAC3. ^1H NMR (500 MHz, CDCl\textsubscript{3})
δ 8.68 (s, 1H), 7.78 (d, J = 7.3 Hz, 1H), 7.52 (td, J = 7.3, 1.1 Hz, 1H), 7.42 (t, J = 7.0 Hz,
2H), 7.37 – 7.29 (m, 2H), 6.98 (dd, J = 7.7, 1.6 Hz, 1H), 6.90 (d, J = 1.5 Hz, 1H), 4.81 –
4.70 (m, 2H), 4.59 (t, J = 8.0 Hz, 1H), 4.57 – 4.48 (m, 2H), 4.46 – 4.36 (m, 3H), 4.20 (dtt,
J = 12.6, 6.2, 3.6 Hz, 2H), 3.92 (ttdd, J = 11.0, 5.7, 5.3, 3.9 Hz, 2H), 3.79 – 3.68 (m, 3H),
3.67 – 3.62 (m, 2H), 3.58 (td, J = 4.4, 2.4 Hz, 2H), 3.53 – 3.46 (m, 4H), 3.39 (t, J = 6.7
Hz, 2H), 2.52 (s, 3H), 2.44 – 2.32 (m, 2H), 2.08 (dd, J = 13.5, 8.2 Hz, 1H), 1.74 (dd, J =
14.7, 6.9 Hz, 2H), 1.55 (dt, J = 14.5, 6.7 Hz, 2H), 1.46 – 1.37 (m, 2H), 1.33 (ddd, J =
16.1, 9.0, 5.9 Hz, 2H), 0.94 (d, $J = 6.5$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.70, 170.23, 169.53, 156.96, 150.42, 148.67, 142.26, 132.41, 131.84, 131.83, 129.84, 128.09, 126.99, 123.95, 122.99, 122.15, 112.82, 71.34, 70.95, 70.72, 70.70, 70.18, 70.14, 69.74, 68.07, 58.76, 58.67, 56.15, 47.54, 45.18, 39.36, 36.47, 32.66, 29.53, 28.95, 26.81, 25.53, 19.23, 16.29. MS (ESI) 799.6 (M+H).

**HaloPROTAC8 (n=4)**

![HaloPROTAC8](image)

HaloPROTAC8 was synthesized according to the general method and isolated as a colorless oil in 45% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.72 – 8.61 (m, 1H), 7.83 – 7.74 (m, 1H), 7.55 – 7.48 (m, 1H), 7.43 (dd, $J = 7.0$, 5.4 Hz, 2H), 7.34 (t, $J = 6.1$ Hz, 1H), 7.31 (d, $J = 7.7$ Hz, 1H), 6.98 (dd, $J = 7.7$, 1.6 Hz, 1H), 6.90 (d, $J = 1.4$ Hz, 1H), 4.81 – 4.70 (m, 2H), 4.61 – 4.48 (m, 3H), 4.46 – 4.35 (m, 3H), 4.24 – 4.13 (m, 2H), 3.98 – 3.84 (m, 2H), 3.78 – 3.61 (m, 6H), 3.58 (tdd, $J = 9.1$, 5.7, 3.2 Hz, 6H), 3.53 (t, $J = 2.3$ Hz, 1H), 3.51 (t, $J = 5.4$ Hz, 2H), 3.42 (t, $J = 6.7$ Hz, 2H), 2.53 (s, 3H), 2.38 (ddt, $J = 12.8$, 7.9, 5.7 Hz, 2H), 2.12 – 2.02 (m, 1H), 1.77 – 1.72 (m, 2H), 1.60 – 1.53 (m, 2H), 1.43 (dt, $J = 8.8$, 7.0 Hz, 2H), 1.38 – 1.30 (m, 2H), 0.95 (d, $J = 6.5$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.72, 170.21, 169.50, 156.98, 150.42, 148.68, 142.27, 132.42, 131.82, 129.87, 128.09, 127.02, 123.95, 123.00, 122.16, 112.84, 71.35, 70.94, 70.67, 70.18, 70.13, 69.74, 68.08, 58.76, 58.71, 56.15, 47.54, 45.18, 39.38, 36.53, 32.68, 29.56, 28.96, 26.83, 25.54, 19.23, 19.21, 16.30. MS (ESI) 843.2 (M+H).
ent-HaloPROTAC8 was synthesized according to the general method and isolated as a colorless oil in 35% yield. $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 8.68 (s, 1H), 7.78 (d, $J$ = 8.1 Hz, 1H), 7.52 (td, $J$ = 7.6, 1.1 Hz, 1H), 7.45 – 7.40 (m, 2H), 7.36 (t, $J$ = 6.1 Hz, 1H), 7.32 (d, $J$ = 7.7 Hz, 1H), 6.98 (dd, $J$ = 7.7, 1.6 Hz, 1H), 6.90 (d, $J$ = 1.5 Hz, 1H), 4.81 – 4.69 (m, 2H), 4.60 – 4.46 (m, 3H), 4.46 – 4.34 (m, 3H), 4.27 – 4.11 (m, 2H), 3.98 – 3.84 (m, 2H), 3.76 – 3.62 (m, 6H), 3.61 – 3.54 (m, 6H), 3.53 – 3.52 (m, 1H), 3.52 – 3.49 (m, 2H), 3.45 – 3.35 (m, 2H), 2.44 – 2.27 (m, 2H), 2.09 (dd, $J$ = 13.5, 8.1 Hz, 1H), 1.78 – 1.72 (m, 2H), 1.60 – 1.52 (m, 2H), 1.42 (dd, $J$ = 11.9, 10.2, 7.1 Hz, 2H), 1.34 (ddd, $J$ = 8.9, 5.4, 3.2 Hz, 2H), 0.96 (t, $J$ = 8.0 Hz, 3H), 0.91 – 0.82 (m, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.79, 170.18, 169.52, 156.98, 150.44, 148.67, 142.29, 132.43, 131.84, 129.94, 128.10, 127.03, 123.93, 123.01, 122.18, 112.87, 71.36, 70.89, 70.62, 70.14, 70.12, 69.74, 68.06, 58.76, 56.20, 47.57, 45.18, 39.39, 36.62, 32.67, 29.53, 28.98, 26.81, 25.52, 19.22, 16.28.

HaloPROTAC4 (n=5)
HaloPROTAC4 was synthesized according to the general method and isolated as a colorless oil in 47% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.67 (s, 1H), 7.77 (d, $J = 7.3$ Hz, 1H), 7.56 – 7.47 (m, 1H), 7.41 (dd, $J = 7.0$, 5.2 Hz, 2H), 7.33 (dd, $J = 15.6$, 6.8 Hz, 2H), 6.97 (dd, $J = 7.7$, 1.5 Hz, 1H), 6.89 (d, $J = 1.4$ Hz, 1H), 4.75 (dd, $J = 14.2$, 11.6 Hz, 2H), 4.54 (dt, $J = 14.4$, 8.0 Hz, 3H), 4.46 – 4.32 (m, 3H), 4.25 – 4.12 (m, 2H), 3.98 – 3.84 (m, 2H), 3.73 (dd, $J = 9.4$, 5.0 Hz, 2H), 3.70 – 3.53 (m, 15H), 3.51 (t, $J = 6.7$ Hz, 2H), 3.43 (t, $J = 6.7$ Hz, 2H), 2.52 (s, 3H), 2.44 – 2.27 (m, 2H), 2.15 – 2.02 (m, 1H), 1.81 – 1.70 (m, 2H), 1.63 – 1.52 (m, 2H), 1.47 – 1.28 (m, 4H), 0.95 (d, $J = 6.5$ Hz, 3H), 0.87 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.78, 170.14, 169.45, 156.95, 150.44, 148.63, 142.24, 132.37, 131.84, 131.82, 131.80, 129.86, 128.06, 127.01, 123.91, 122.98, 122.14, 112.81, 71.35, 70.90, 70.67, 70.65, 70.61, 70.60, 70.59, 70.18, 70.08, 69.72, 68.05, 58.75, 58.72, 56.17, 47.53, 45.19, 39.36, 36.61, 32.66, 29.55, 28.99, 26.82, 25.53, 19.21, 19.18, 16.28. MS (ESI) 887.52.

$^*$

$^{ent}$-HaloPROTAC4

$^{ent}$-HaloPROTAC4 was synthesized according to the general method and isolated as a colorless oil in 39% yield. The NMR and MS spectra matched HaloPROTAC4. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.67 (s, 1H), 7.82 – 7.75 (m, 1H), 7.57 – 7.49 (m, 1H), 7.47 – 7.39 (m, 2H), 7.38 – 7.28 (m, 2H), 6.98 (dd, $J = 7.7$, 1.5 Hz, 1H), 6.90 (d, $J = 1.5$ Hz, 1H), 4.76 (dd, $J = 14.3$, 7.5 Hz, 2H), 4.55 (dt, $J = 13.8$, 7.8 Hz, 3H), 4.41 (ddd, $J = 12.9$, 10.7,
5.9 Hz, 3H), 4.28 – 4.12 (m, 2H), 3.97 – 3.82 (m, 2H), 3.73 (dt, $J = 7.8, 3.6$ Hz, 2H), 3.69 – 3.54 (m, 15H), 3.52 (t, $J = 6.7$ Hz, 2H), 3.43 (t, $J = 6.7$ Hz, 2H), 2.52 (s, 3H), 2.46 – 2.30 (m, 2H), 2.09 (dd, $J = 13.6, 8.2$ Hz, 1H), 1.80 – 1.70 (m, 2H), 1.63 – 1.52 (m, 2H), 1.48 – 1.30 (m, 4H), 0.95 (d, $J = 6.5$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.74, 170.19, 169.49, 156.98, 150.42, 148.68, 142.27, 132.41, 131.87, 131.83, 129.88, 128.09, 127.03, 123.95, 123.00, 122.16, 112.84, 77.41, 77.16, 76.91, 72.67, 71.37, 70.93, 70.67, 70.63, 70.20, 70.12, 69.74, 68.08, 58.75, 58.72, 56.15, 47.54, 45.19, 39.38, 36.57, 32.68, 29.57, 28.96, 26.83, 25.55, 19.21, 16.29. MS (ESI) 887.6 (M+H).

**HaloPROTAC9**

HaloPROTAC9 was synthesized according to the general method and was isolated as a yellow oil in 21% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.67 (s, 1H), 7.29 (d, $J = 7.7$ Hz, 1H), 6.99 (dd, $J = 7.6, 1.5$ Hz, 1H), 6.90 (d, $J = 1.5$ Hz, 1H), 6.11 (s, 1H), 4.64 – 4.35 (m, 4H), 4.24 – 4.11 (m, 2H), 3.95 – 3.85 (m, 2H), 3.83 – 3.77 (m, 2H), 3.77 – 3.67 (m, 4H), 3.66 – 3.60 (m, 3H), 3.59 – 3.54 (m, 3H), 3.51 (t, $J = 6.7$ Hz, 2H), 3.44 (t, $J = 6.8$ Hz, 2H), 2.52 (s, 3H), 2.39 – 2.31 (m, 1H), 2.24 (s, 3H), 2.16 – 2.06 (m, 1H), 1.79 – 1.72 (m, 2H), 1.62 – 1.53 (m, 2H), 1.47 – 1.38 (m, 2H), 1.38 – 1.31 (m, 2H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.83, 166.74, 165.63, 160.27, 157.08, 150.45, 148.74, 132.57, 131.76, 130.05, 126.85, 122.26, 113.05, 104.20, 71.42, 70.64, 70.52, 70.37, 70.11, 69.70, 68.05, 59.28,
HaloPROTAC10 was synthesized according to the general method and isolated as a colorless oil in 54% yield. ¹H NMR (500 MHz, Chloroform-d) δ 8.66 (s, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.50 (td, J = 7.6, 1.1 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.33 (t, J = 6.0 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 6.96 (dd, J = 7.7, 1.6 Hz, 1H), 6.89 (d, J = 1.5 Hz, 1H), 5.24 (q, J = 7.0 Hz, 1H), 4.72 (d, J = 17.2 Hz, 1H), 4.59 (t, J = 7.9 Hz, 1H), 4.52 – 4.45 (m, 2H), 4.44 – 4.41 (m, 1H), 4.20 – 4.13 (m, 2H), 3.92 – 3.84 (m, 2H), 3.74 – 3.67 (m, 3H), 3.66 – 3.63 (m, 2H), 3.60 – 3.57 (m, 2H), 3.56 (d, J = 2.7 Hz, 1H), 3.53 – 3.48 (m, 4H), 3.40 (t, J = 6.7 Hz, 2H), 2.51 (s, 3H), 2.34 – 2.24 (m, 1H), 2.13 – 2.07 (m, 2H), 1.80 – 1.67 (m, 2H), 1.55 (dd, J = 14.4, 7.3 Hz, 2H), 1.50 (d, J = 7.1 Hz, 3H), 1.44 – 1.38 (m, 2H), 1.32 (ddd, J = 11.8, 5.4, 3.2 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 171.12, 171.07, 168.93, 156.86, 150.41, 148.64, 142.19, 132.30, 132.00, 131.78, 129.49, 128.09, 127.06, 123.79, 122.97, 122.12, 112.84, 71.32, 70.89, 70.68, 70.67, 70.25, 70.15, 69.74, 68.08, 58.88, 55.70, 48.19, 47.50, 45.16, 39.20, 36.76, 32.64, 29.50, 26.78, 25.49, 16.28, 15.74. MS (ESI) 771.8 (M+H).

Synthesis of VHL binding fragment for N-terminal linker PROTAC
tert-butyl \((\langle S\rangle -1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate

Boc-tert-Leu-OH (0.160 g, 0.505 mmol, 1 eq) was dissolved in DMF (1.6 mL, 0.5M) at room temperature. HATU (0.332 g, 0.872 mmol, 1.10 eq) and DIPEA (0.43 mL, 2.4 mmol, 3 eq) were added and the solution was stirred for 30 minutes. \((2S,4R)\)-4-hydroxy-\(N\)-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide\(^1\) was then added as a solution in 0.8 mL DMF. The mixture was stirred for 17 h, then diluted with half saturated NaCl and extracted thrice with EtOAc. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) gave a yellow foamy oil (0.135 g, 0.565 mmol, 65%).

\(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)) \(\delta 8.69 \text{ (s, 1H)}, 7.64 \text{ (t, } J = 5.9 \text{ Hz, 1H)}, 7.37 - 7.28 \text{ (m, 4H)}, 5.34 \text{ (d, } J = 9.2 \text{ Hz, 1H)}, 4.72 \text{ (t, } J = 7.9 \text{ Hz, 1H)}, 4.53 \text{ (dd, } J = 14.8, 6.7 \text{ Hz, 2H)}, 4.33 \text{ (dd, } J = 15.1, 5.3 \text{ Hz, 1H)}, 4.23 \text{ (d, } J = 9.3 \text{ Hz, 1H)}, 3.94 \text{ (d, } J = 11.1 \text{ Hz, 1H)}, 3.71 - 3.63 \text{ (m, 1H)}, 2.56 - 2.45 \text{ (m, 3H)}, 2.39 \text{ (ddd, } J = 12.7, 7.8, 4.6 \text{ Hz, 1H)}, 2.17 - 2.05 \text{ (m, 1H)}, 1.41 \text{ (s, 9H)}, 0.94 \text{ (s, 9H)}\).

\(^{13}\text{C NMR}\) (126 MHz, CDCl\(_3\)) \(\delta 172.06, 171.22, 156.05, 150.45, 148.22, 138.23, 131.68, 130.64, 129.36, 127.89, 80.08, 69.94, 58.81, 56.57, 55.44, 43.04, 36.48, 35.43, 28.31, 26.28, 15.96\). MS (ESI) 531.0 (M+H).
(2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

tert-butyl

((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (0.163 g, 0.307 mmol, 1 eq) was dissolved in DCM and cooled to 4 °C. 4M HCl in dioxane (1.5 mL, 6.1 mmol, 20 eq) was added and the mixture was warmed to room temperature. After 70 minutes, the mixture was concentrated under a stream of nitrogen, then diluted with 10 mL 3M NaOH (aq) and extracted twice with 10 mL chloroform, once with 10 mL DCM and once with 5 mL EtOAc. The combined organic layer was dried over sodium sulfate, filtered and concentrated to give a light yellow foam (97 mg, 0.23 mmol, 73%), which was deemed sufficiently pure and carried forward without further purification.

\(^1\)H NMR (500 MHz, MeOH) \(\delta\) 8.87 (s, 1H), 7.43 (dd, \(J = 22.5, 8.3\) Hz, 4H), 4.64 – 4.32 (m, 4H), 3.81 – 3.70 (m, 2H), 3.41 (s, 1H), 2.47 (s, 3H), 2.29 – 2.18 (m, 1H), 2.09 (ddd, \(J = 13.2, 8.9, 4.5\) Hz, 1H), 1.01 (s, 9H). \(^13\)C NMR (126 MHz, MeOH) \(\delta\) 175.28, 174.55, 152.80, 149.01, 140.24, 133.38, 131.48, 130.36, 128.94, 71.07, 61.21, 60.69, 57.69, 43.68, 38.91, 36.53, 26.77, 15.82. MS (ESI) 430.8 (M+H).
Oxidation of oligoethylene glycol-hexylchloride

Oligoethylene glycol-hexylchloride (1 eq) was dissolved in DMF (0.25 M). PDC (6 eq) was added and the mixture was stirred overnight. The mixture was then diluted with water, then 2M HCl (aq), and extracted repeatedly with diethyl ether. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 20% MeOH/DCM) gave the desired carboxylic acid fragment.

2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)acetic acid (n=2)

2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)acetic acid was synthesized from 2-(2-((6-chlorohexyl)oxy)ethoxy)ethanol in an analogous method to the general method, using only 4 eq of PDC. It was isolated as a green oil in 29% yield. \( ^1H \text{NMR} \) (500 MHz, CDCl\(_3\)) \( \delta \) 4.16 (s, 2H), 3.77 (dd, \( J = 5.5, 3.1 \) Hz, 2H), 3.75 – 3.63 (m, 4H), 3.60 (dd, \( J = 5.6, 3.6 \) Hz, 2H), 3.53 (t, \( J = 6.7 \) Hz, 2H), 3.47 (t, \( J = 6.6 \) Hz, 2H), 1.82 – 1.73 (m, 2H), 1.63 – 1.56 (m, 2H), 1.45 (dt, \( J = 8.7, 6.8 \) Hz, 2H), 1.41 – 1.31 (m, 2H). \( ^{13}C \)
NMR (126 MHz, CDCl$_3$) $\delta$ 171.77, 71.77, 71.49, 70.96, 70.16, 69.96, 69.09, 45.22, 32.67, 29.51, 26.81, 25.53. MS (ESI) 283.0 (M+H).

21-chloro-3,6,9,12,15-pentaoxahenicosan-1-oic acid (n=4)

\[
\text{Cl} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{OH}
\]

21-chloro-3,6,9,12,15-pentaoxahenicosan-1-oic acid was synthesized from 21-chloro-3,6,9,12,15-pentaoxahenicosan-1-ol according to the general method and isolated as a green oil in 56% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.14 (s, 2H), 3.74 (s, 2H), 3.64 (s, 12H), 3.58 (s, 2H), 3.52 (t, $J$ = 6.5 Hz, 2H), 3.45 (d, $J$ = 4.1 Hz, 2H), 1.79 – 1.73 (m, 2H), 1.58 (d, $J$ = 6.3 Hz, 2H), 1.43 (d, $J$ = 6.3 Hz, 2H), 1.36 (d, $J$ = 6.2 Hz, 2H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 171.99, 71.33, 70.72, 70.65, 70.63, 70.50, 70.44, 70.37, 70.16, 70.02, 69.23, 45.14, 32.62, 29.43, 26.76, 25.45. MS (ESI) 393.1 (M+Na).

General Procedure for Amide Coupling

The desired carboxylic acid fragment (1.2 eq) was dissolved in DCM (0.5 M) along with EDC (1.1 eq) and HOBT (1.1 eq). The mixture was stirred for 15 minutes, then DIPEA (1 eq) was added. After 5 minutes, (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (1 eq) was added as a 100 mg/mL solution in DCM. The mixture was stirred over night, then diluted with half saturated sodium chloride and extracted thrice with EtOAc. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) gave the desired PROTAC.
HaloPROTAC1 (n=2)

HaloPROTAC1 was synthesized according to the general method and isolated as a colorless oil in 20% yield. $^1$H NMR (500 MHz, MeOH) $\delta$ 8.88 (s, 1H), 7.45 (dd, $J = 22.3, 8.1$ Hz, 4H), 4.70 (d, $J = 7.0$ Hz, 1H), 4.64 – 4.47 (m, 3H), 4.35 (d, $J = 15.5$ Hz, 1H), 4.15 – 3.96 (m, 2H), 3.94 – 3.76 (m, 2H), 3.74 – 3.61 (m, 6H), 3.58 (t, $J = 4.6$ Hz, 2H), 3.54 (t, $J = 6.6$ Hz, 2H), 3.44 (t, $J = 6.5$ Hz, 2H), 2.48 (s, 3H), 2.21 (d, $J = 7.5$ Hz, 1H), 2.13 – 2.04 (m, 1H), 1.81 – 1.69 (m, 2H), 1.56 (dd, $J = 14.2, 7.1$ Hz, 2H), 1.47 – 1.40 (m, 2H), 1.37 (dd, $J = 12.7, 5.9$ Hz, 2H), 1.05 (s, 9H). $^{13}$C NMR (126 MHz, MeOH) $\delta$ 174.41, 172.13, 171.69, 154.92, 149.05, 140.27, 133.43, 131.50, 130.40, 128.94, 72.34, 72.17, 71.69, 71.54, 71.27, 71.11, 71.07, 60.81, 58.15, 58.10, 45.70, 43.71, 38.95, 37.10, 33.75, 30.56, 27.73, 26.97, 26.51, 15.83. MS (ESI) 695.4 (M+H).

HaloPROTAC2 (n=4)

HaloPROTAC2 was synthesized according to the general method and isolated as a pale green oil in 36% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.67 (s, 1H), 7.35 (q, $J = 8.4$ Hz, 4H), 4.73 (s, 1H), 4.59 – 4.46 (m, 3H), 4.34 (dd, $J = 14.8, 4.9$ Hz, 1H), 4.03 (dt, $J = 42.6, 13.4$ Hz, 3H), 3.71 – 3.60 (m, 14H), 3.59 – 3.54 (m, 3H), 3.52 (t, $J = 6.7$ Hz, 2H),
3.44 (t, *J* = 6.6 Hz, 2H), 2.57 – 2.49 (m, 4H), 2.12 (s, 1H), 1.80 – 1.72 (m, 2H), 1.63 – 1.54 (m, 2H), 1.44 (dt, *J* = 11.1, 6.6 Hz, 2H), 1.39 – 1.31 (m, 2H), 0.93 (d, *J* = 8.1 Hz, 9H). ¹³C NMR (126 MHz, cdcl₃) δ 171.73, 170.74, 170.71, 150.34, 148.73, 138.32, 131.74, 131.23, 129.72, 129.70, 128.33, 71.39, 70.82, 70.81, 70.78, 70.65, 70.63, 70.35, 70.29, 58.52, 57.40, 56.78, 45.10, 43.47, 35.91, 34.96, 32.73, 29.64, 26.87, 26.58, 25.60, 16.18. MS (ESI) 783.6 (M+H).

![Chemical structure](image)

**tert-butyl**

**((R)-1-((2R,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate**

Boc-**D-tert**-leucine (0.155, 0.67 mmol, 1 eq) was dissolved in DMF (1.34 mL, 0.5 M) at room temperature. HATU (0.28 g, 0.736 mmol, 1.1 eq) and DIPEA (0.35 mL, 2.0 mmol, 3 eq) were added and the mixture was stirred for 30 minutes at which point **(2R,4S)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide** (0.2337 g, 0.736 mmol, 1.1 eq) was added as a solution in DMF (0.75 mL). The mixture was stirred for 15 hours, before being diluted with half saturated NaCl (aq) and extracted 3 times with EtOAc. The organic layer was washed 5 times with 10% LiCl (aq) and once with saturated ammonium chloride, sodium bicarbonate, water, and brine. The organic layer was dried over sodium sulfate, filtered and concentrated. Purification by column...
chromatography (1 to 10% MeOH/DCM) gave a light yellow solid (0.30 g, 0.565 mmol, 77%). \textbf{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}) \textit{δ} 8.65 (s, 1H), 7.52 (t, \textit{J} = 5.8 Hz, 1H), 7.33 – 7.27 (m, 4H), 5.24 (d, \textit{J} = 9.2 Hz, 1H), 4.69 (t, \textit{J} = 7.8 Hz, 1H), 4.51 (dd, \textit{J} = 15.2, 6.5 Hz, 2H), 4.27 (dd, \textit{J} = 15.1, 5.2 Hz, 1H), 4.17 (d, \textit{J} = 9.3 Hz, 1H), 3.93 (d, \textit{J} = 10.6 Hz, 1H), 3.61 (dd, \textit{J} = 11.1, 3.7 Hz, 1H), 2.47 (d, \textit{J} = 4.2 Hz, 3H), 2.44 – 2.36 (m, 1H), 2.12 – 2.02 (m, 1H), 1.38 (s, 9H), 0.90 (s, 9H). \textbf{\textsuperscript{13}C NMR} (126 MHz, CDCl\textsubscript{3}) \textit{δ} 172.36, 171.03, 156.22, 150.45, 148.40, 138.22, 131.71, 130.83, 129.49, 128.02, 80.26, 70.06, 58.89, 58.67, 56.58, 43.19, 36.25, 35.30, 28.38, 26.36, 16.07. \textbf{MS} (ESI) 531.6 (M+H).

(\textit{2R,4S})-1-((\textit{R})-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

\begin{align*}
\text{tert-butyl} & \quad (\textit{R})-1-((\textit{2R,4S})-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate \quad (0.30 \text{ g, 0.565 mmol, 1 eq}) \text{ was dissolved in DCM (19 mL) at room temperature. TFA (1 mL, 5\% by volume) was added and the mixture was stirred for 14 hours then condensed. The resultant mixture was then diluted with water, basified with 3M NaOH and extracted three times with chloroform. The resultant off-white foam (0.22 g, 0.511 mmol, 90\%) was found to be sufficiently pure by NMR.} \textbf{\textsuperscript{1}H NMR} (500 MHz, MeOD) \textit{δ} 8.86 (s, 1H), 7.49 – 7.37 (m, 4H), 4.64 – 4.58 (m, 1H), 4.52 (d, \textit{J} = 15.6 Hz, 2H), 4.41 – 4.32 (m, 1H), 3.81 – 3.69 (m, 2H), 3.40 (s, 1H), 2.46 (s, 3H), 2.27 – 2.18 (m, 1H), 2.09 (ddd, \textit{J} = 13.2, 8.9, 4.5 Hz, 1H), 1.01 (s, 9H). & \quad \textbf{\textsuperscript{13}C NMR} (126 MHz, MeOD) \textit{δ} 175.28, 174.52, 152.78, 150.45, 148.40, 138.22, 131.71, 130.83, 129.49, 128.02, 80.26, 70.06, 58.89, 58.67, 56.58, 43.19, 36.25, 35.30, 28.38, 26.36, 16.07. \textbf{MS} (ESI) 531.6 (M+H).}
\end{align*}
ent-HaloPROTAC1

2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)acetic acid (22.2 mg, 0.0785 mmol, 1 eq) was dissolved in DMF (0.31 mL, 0.25M). HATU (32.9 mg, 0.0864 mmol, 1.1 eq) and DIPEA (41 µL, 0.236 mmol, 3 eq) were added. After 35 minutes, (2R,4S)-1-((R)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (33.8 mg, 0.0785 mmol, 1 eq) was added. After 20 hours, the mixture was diluted with half saturated NaCl, then extracted three times with EtOAC. The organic layer was then washed once with saturated ammonium chloride, three times with 10% LiCl, and once with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) gave a yellow oil (18.2 mg, 0.0262 mmol, 33%). \(^1\)H NMR (500 MHz, MeOD) δ 8.88 (s, 1H), 7.52 – 7.36 (m, 4H), 4.70 (s, 1H), 4.61 – 4.46 (m, 3H), 4.35 (d, \(J = 15.5\) Hz, 1H), 4.10 – 3.99 (m, 2H), 3.90 – 3.78 (m, 2H), 3.74 – 3.60 (m, 6H), 3.58 (t, \(J = 4.6\) Hz, 2H), 3.54 (t, \(J = 6.6\) Hz, 2H), 3.45 (dd, \(J = 16.8, 10.3\) Hz, 2H), 2.48 (s, 3H), 2.23 (dd, \(J = 13.1, 7.6\) Hz, 1H), 2.09 (ddd, \(J = 13.3, 9.3, 4.4\) Hz, 1H), 1.77 – 1.70 (m, 2H), 1.59 – 1.50 (m, 2H), 1.47 – 1.40 (m, 2H), 1.40 – 1.31
(m, 2H), 1.05 (s, 9H). \(^{13}\text{C}\) NMR (126 MHz, MeOD) \(\delta\) 174.39, 172.12, 171.68, 152.85, 149.04, 140.27, 133.43, 131.49, 130.39, 130.39, 128.93, 72.33, 72.17, 71.68, 71.53, 71.27, 71.10, 71.06, 60.80, 58.14, 58.10, 45.71, 43.71, 38.94, 37.10, 33.75, 30.56, 27.72, 26.97, 26.50, 15.84. MS (ESI) 695.2 (M+H).

**ent-Halo PROTAC2**

21-chloro-3,6,9,12,15-pentaoxahenicosan-1-oic acid (23.6 mg, 0.0636 mmol, 1 eq) was dissolved in DMF (0.26 mL). HATU (26.6 mg, 0.0636 mmol, 1.1 eq) and DIPEA (33.2 µL, 0.191 mmol, 3 eq) were added. After 35 minutes, (2R,4S)-1-((R)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (27.4 mg, 0.0636 mmol, 1 eq) was added. After 22 hours, the mixture was diluted with half saturated NaCl, then extracted three times with EtOAC. The organic layer was then washed once with saturated ammonium chloride, three times with 10% LiCl, and once with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) gave a yellow-green oil (8.24 mg, 0.0105 mmol, 17%). \(^{1}\text{H}\) NMR (501 MHz, CDCl\(_3\)) \(\delta\) 8.68 (s, 1H), 7.36 (q, \(J = 8.1\) Hz, 4H), 4.75 (t, \(J = 8.0\) Hz, 1H), 4.61 – 4.50 (m, 2H), 4.47 (d, \(J = 8.4\) Hz, 1H), 4.34 (dd, \(J = 14.8, 5.2\) Hz, 1H), 4.12 (d, \(J = 11.2\) Hz, 1H), 4.00 (dd, \(J = 32.8, 15.6\) Hz, 2H), 3.69 – 3.60 (m, 14H), 3.56 (dd, \(J = 6.1, 3.7\) Hz, 3H), 3.52 (t, \(J = 6.7\) Hz, 2H), 3.44 (t, \(J = 6.7\) Hz, 2H),
2.63 – 2.54 (m, 1H), 2.52 (s, 3H), 2.16 – 2.07 (m, 1H), 1.80 – 1.73 (m, 4H), 1.63 – 1.54 (m, 3H), 1.44 (dt, $J = 14.2, 6.9$ Hz, 2H), 1.36 (dd, $J = 15.2, 8.1$ Hz, 2H), 0.95 (s, 9H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 171.79, 170.77, 170.67, 150.36, 148.73, 138.29, 131.74, 131.24, 129.72, 128.35, 71.41, 71.38, 70.91, 70.80, 70.77, 70.66, 70.61, 70.37, 70.29, 58.42, 57.44, 56.75, 45.14, 43.49, 35.80, 34.86, 32.73, 29.64, 26.87, 26.58, 25.61, 16.21.

MS (ESI) 783.2 (M+H).

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(2S,4R)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (150.6 mg, 0.474 mmol, 1 eq) and (S)-3-methyl-2-(1-oxoisooindolin-2-yl)butanoic acid (121.8 mg, 0.522 mmol, 1.1 eq) were dissolved in DMF (1.6 mL, 0.3 M) at room temperature. DIPEA (0.25 mL, 1.42 mmol, 3 eq) was added, followed by HATU (198.5 mg, 0.522 mmol, 1.1 eq). After 16 hours, the mixture was diluted with half saturated NaCl and extracted three times with EtOAc. The organic layer was then washed three times with 10% LiCl, and washed one time with saturated ammonium chloride, saturated sodium bicarbonate, water and brine. The organic layer was then washed over sodium sulfate, filtered and condensed. Purification by column chromatography (1 to 10% MeOH/DCM) and preparative TLC (5% MeOH/DCM) gave a white solid (127 mg, 0.238 mmol, 50%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.67 (s, 1H), 7.74 (d, $J = 7.5$ Hz, 1H), 7.49 (td, $J = 7.5, 1.1$ Hz, 1H), 7.43 – 7.31 (m, 7H), 4.80 – 4.63 (m, 3H), 4.52 (s, 1H), 4.50 – 4.33 (m, 4H), 3.67
(dd, $J = 11.4$, 3.6 Hz, 1H), 2.52 (s, 4H), 2.41 (dt, $J = 10.9$, 6.6 Hz, 1H), 2.07 – 2.01 (m, 1H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.87 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.79, 170.68, 169.64, 150.42, 148.66, 142.16, 138.25, 131.89, 131.71, 131.64, 131.15, 129.66, 128.15, 128.12, 123.92, 122.97, 70.03, 58.80, 58.67, 56.15, 47.57, 43.42, 35.94, 28.91, 19.22, 19.14, 16.20. MS (ESI) 533.8 (M+H)


