Supporting Information

Rapid and reversible knockdown of endogenously tagged endosomal proteins via an optimized HaloPROTAC degrader.

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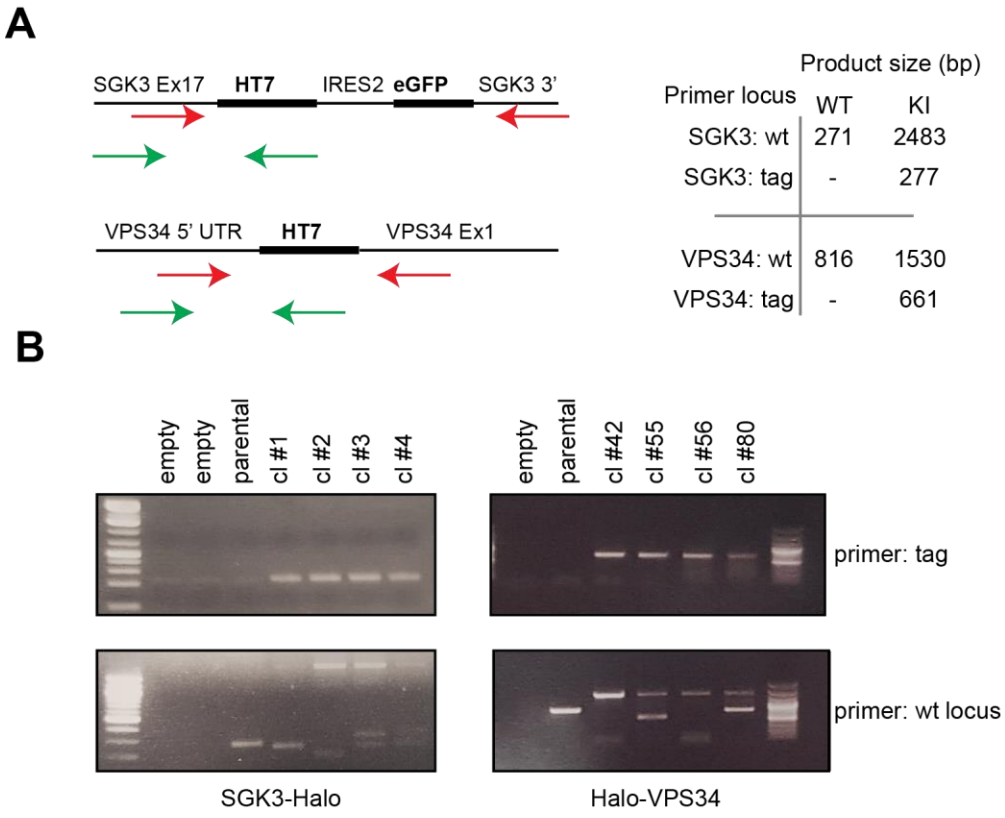
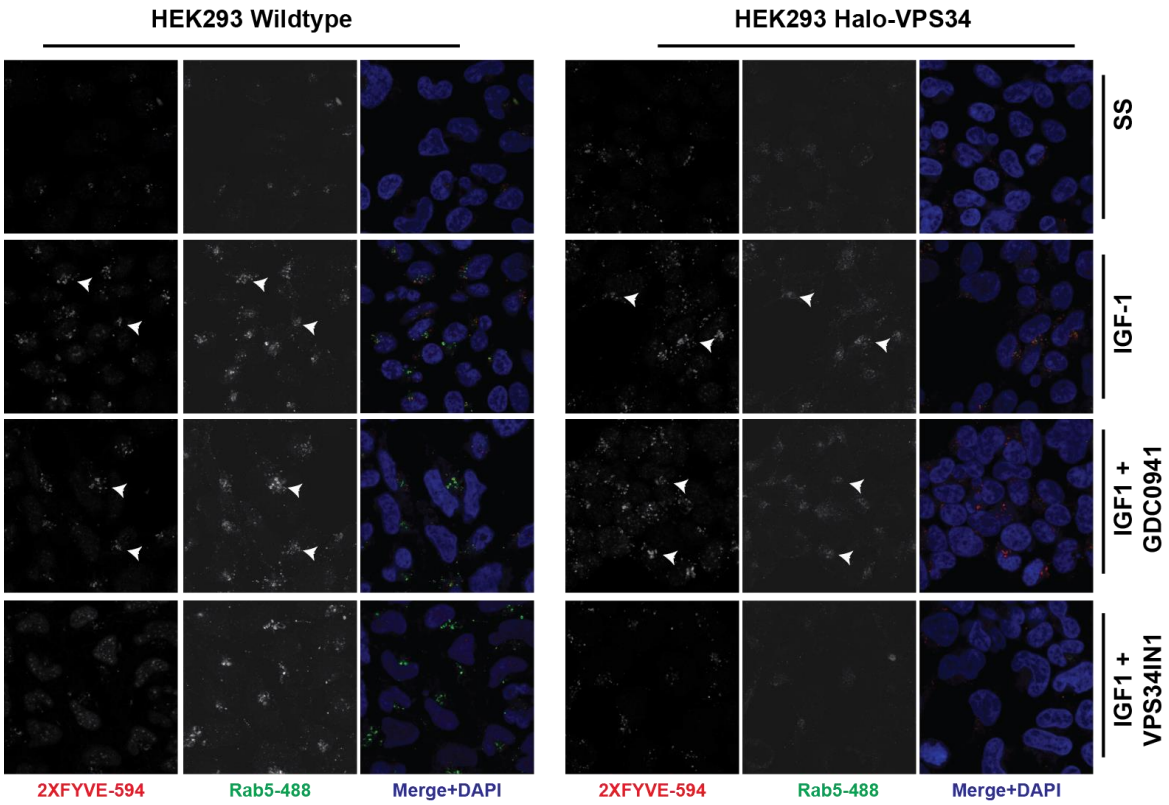
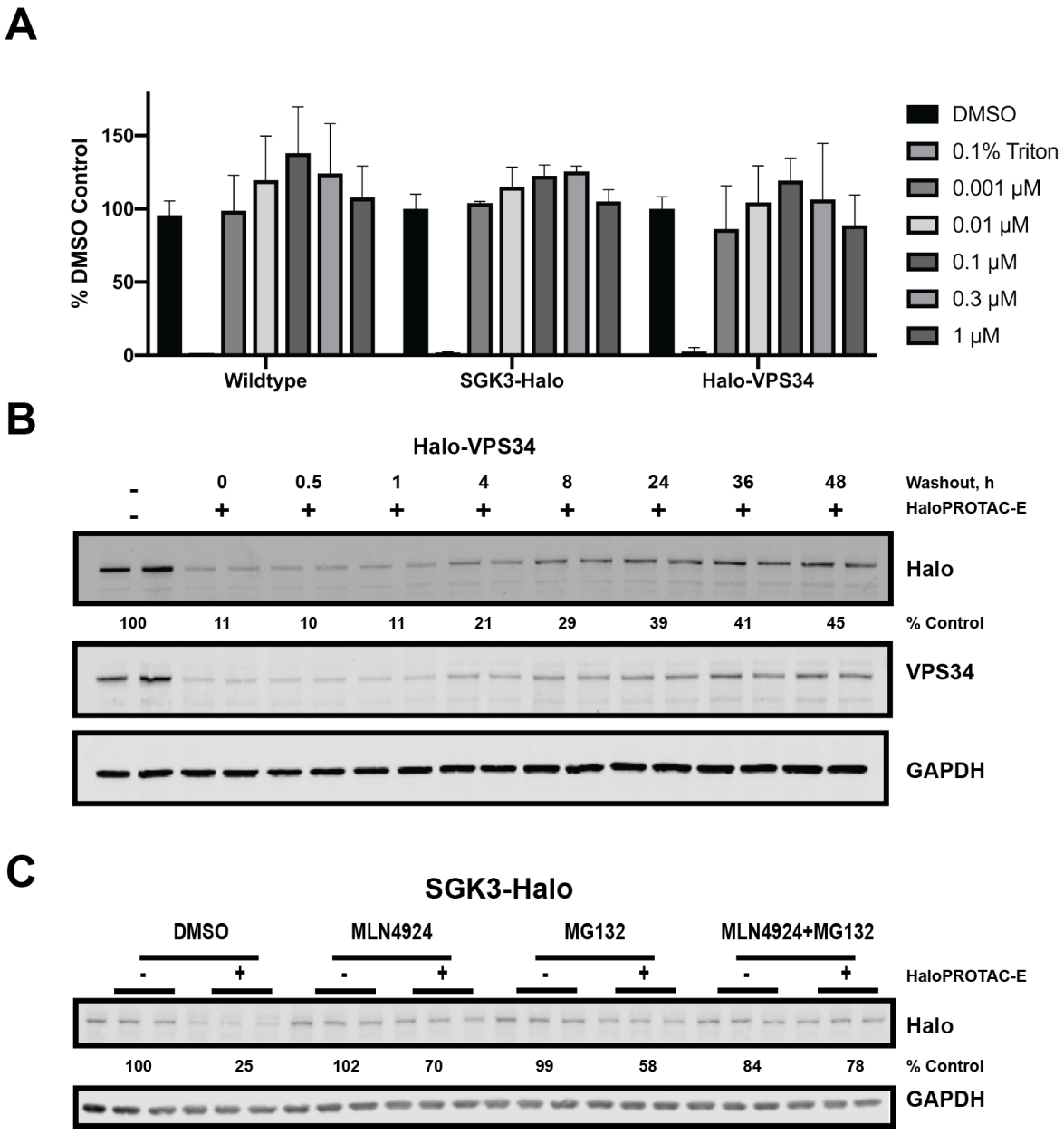
1. SUPPLEMENTARY FIGURES



Supplementary Figure S1. Sequencing Analysis of SGK3-Halo and Halo-VPS34 KI cell lines. A. Primer design for sequencing. Primers were designed for analysis of the endogenous locus (wt) and internal primers to recognize the incorporated HaloTag (tag). **B** Agarose gel analysis of parental and KI cell lines. In KI cell lines, a shift is observed in the PCR product of the primers design against the endogenous locus. 'Tag' products were sequenced to confirm in-frame incorporation of the donor. 'WT' products were sequenced to detect any knockouts or mutations. **C.** Protein level impact of the truncating mutation in SGK3-Halo Clone #2. Deletion from the 3' end of Exon17 and part of the 3' UTR results in a frameshift and early stop codon 2 residues from the Hydrophobic Motif residue. This will likely destabilize the protein and severely impact on the phosphorylation of this site



Supplementary Figure S2. Colocalization of PtdIns3P to early endosomal structures. HEK293 Parental and Halo–VPS34 cells were treated as described, before staining with the 2XFYVE-594 PtdIns3P binding probe and anti–Rab5 antibody. Colocalization of PtdIns3P with Rab5, detected with anti–Rabbit–AlexaFluor488 secondary antibody was Quantified in Volocity and is shown in Figure 2D. Arrows represent structures colocalizing with both the 2XFYVE and anti-Rab5 stain.



Supplementary Figure S3. MTS assay and mechanistic analysis. **A.** Parental 293, SGK3–Halo and Halo–VPS34 cells seeded in 96–well plates, then treated for 48h with 0.001–1 µM HaloPROTAC–E and subject to MTS analysis. 0.1% Triton was used as a 100% death control. Absorbance readings were normalized to a DMSO control for each cell line. **B.** Halo–VPS34 cells were treated for 24h with 300 nM HaloPROTAC–E. After 24h, cells were washed 3 times with DMEM and replaced with fresh media for the times indicated. Recovery of VPS34–Halo was analyzed by immunoblot. **C.** SGK3–Halo cells were treated for 6h with 300 nM PROTAC E, after pre–inhibition of Cullin neddylation by MLN4924 (3 µM for 3h) or proteasome by MG132 (50 µM for 30 minutes). Cells were lysed and remaining HaloTag7 fusion protein was analyzed by Immunoblot

2. MASS SPECTROMETRY METHODS

Cell culture and proteomic sample preparation

Cell treatments were performed as described in the text. Cells were resuspended in 0.05% Trypsin, resuspended and pelleted at 300g for 5 minutes. After washing with PBS, Cells pellets were lysed with 2 ml of lysis buffer (8 M Urea, 50 mM Ammonium bicarbonate containing protease inhibitor and phosphatase inhibitor). The sample were left on ice for 15 min and were then sonicated for 5 min in ice-cold water bath under high intensity with 30 sec on and off. After sonication, benzonase® endonuclease (Merck Millipore) with the volume ratio of 1: 100 were added into the lysate to degrade DNA and RNA[1]. The lysate were then centrifuged at 12,000 rpm for 15 min at 4 °C, and supernatants were transferred into new eppendorf tubes. The protein concentration were measured with BCA assay (Pierce™ BCA Protein Assay Kit, Thermo). The lysate from each sample was reduced with 5 mM DTT at 45 °C for 30 min. Lysates were briefly centrifuged and were cool down to room temperature before alkylation in the dark with 10 mM iodoacetamide at room temperature. The alkylation was then quenched by the addition of 5 mM DTT. Samples were first digested by Lys-C with the weight ratio of 1:200 (w/w) for 4 h at 30 °c. Then, sample were diluted with 50 mM ammonium bicarbonate to 1.5 M Urea concentration. Samples were then digested with trypsin (Pierce trypsin, Thermo) at an enzyme to protein ratio of 1:50 (w/w) at room temperature with gentle shaking for overnight.

The digest was stopped by the addition of 1% TFA (v/v), centrifuged at 10,000 g for 10 min at room temperature. The supernatant were desalted on 200 mg SepPak tC18 cartridge (Waters). Desalted peptides were dried by vacuum centrifugation using Speedvac (Thermo).

TMT labelling and Basic C18 reverse phase (bRP) chromatography fractionation

Each sample (200 µg of peptides each) was re-suspended in 100 µL of 100 mM TEAB buffer. The TMT labelling reagents were equilibrated to room temperature and 41 µL anhydrous acetonitrile was added to each reagent channel and softly vortexed for 10 min. Peptides were transferred to the corresponding TMT channels and incubated for 1 h at room temperature. The reaction was quenched with 8 µL of 5% hydroxylamine. To ensure complete labelling, 1 µg of labelled samples from each channel were analysed by LC-MS/MS prior to mix. After evaluation, the complete TMT labelled 8 samples were then combined, acidified and dried. Sep-Pak desalting was then performed and the elution was dried to completeness.

Ultimate 3000 high-pressure liquid chromatography (HPLC) system (Dionex) were used for basic C18 reverse phase chromatography fractionation operating at 569 μL/min with two buffers: buffer A (10 mM ammonium formate, pH 10) and buffer B (80% ACN, 10 mM ammonium formate, pH 10). The desalted mixture of TMT-labelled peptides were resuspended in 200 μL of buffer A (10 mM ammonium formate, pH10) and separated on a C18 reverse phase column (4.6 × 250 mm, 3.5 μm, Waters) with a gradient from 3% B to 12.5 % B in 10 min, 12.5% to 40% buffer B in 45 min, 40% B to 60% B in 25 min, 60% B to 80% B in 10 min, 80% B to 100% B in 2.5 min, 100% for 5 min, ramping to 3% B in 2.5 min and then 3% for 10 min. A total of 90 fractions (1 min per fraction) were collected before further concatenation into 30 final fractions. Each fraction was then dried and desalted over a C18 StageTip prior to analysis by mass spectrometry.

LC-MS/MS analysis

The LC separations were performed with a Thermo Dionex Ultimate 3000 RSLC Nano liquid chromatography instrument. Approximately 1 µg of concatenated peptides (Peptides quantitation by Nanodrop) from bRP chromatography were dissolved in 0.1% formic acid and then loaded on C18 trap column with 3 % ACN/0.1%TFA at a flow rate of 5 µL/min. Peptide separations were performed over EASY-Spray column (C18, 2 µm, 75 µm × 50 cm) with an integrated nano electrospray emitter at a flow rate of 300 nL/min. Peptides were separated with a 180 min segmented gradient as follows: the first 10 fractions starting from 7%~35% buffer B in 160 min (Note: the middle 10 fractions starting from 10% and the last 10 fractions starting from 12.5%), 35%~45% buffer B in 40 min, 45%~95% buffer B for 10 min, followed by a 7.5 min 95% B. Eluted peptides were analysed on an Orbitrap Fusion Lumos (Thermo Fisher Scientific, San Jose, CA) mass spectrometer. Spray voltage was set to 2 kV, RF lens

level was set at 30%, and ion transfer tube temperature was set to 275 °C. The Orbitrap Fusion Lumos was operated in positive ion data dependent mode with synchronous precursor selection (SPS)-MS3 analysis for reporter ion quantitation. The mass spectrometer was operated in data-dependent Top speed mode with 3 seconds per cycle. The full scan was performed in the range of 350–1500 m/z at nominal resolution of 120 000 at 200 m/z and AGC set to 4×10^5 with maximal injection time 50 ms, followed by selection of the most intense ions above an intensity threshold of 5000 for collision-induced dissociation (CID)-MS2 fragmentation in the linear ion trap with 35% normalized collision energy. The isolation width was set to 0.7 m/z with no offset. Dynamic exclusion was set to 60 seconds. Monoisotopic precursor selection was set to peptide, maximum injection time was set to 50 msec. Charge states between 2 to 7 were included for MS2 fragmentation. The top 5 fragment ions from each MS2 scan was notched out for MS3. The MS3 scan were performed with an isolation width of 2 m/z in the quadrupole, normalised HCD collision energy of 65% and analysis of fragment ions in the orbitrap using 50 000 resolving power with auto normal range scan from m/z 100 to 500 and AGC target of 5×10^4. The maximal injection time for MS3 scan was set to 86 ms.

Data Analysis

All the acquired LC-MS data were analysed using Proteome Discoverer software v.2.2 (Thermo Fisher Scientific) with Mascot search engine. A maximum missed cleavages for trypsin digestion was set to 2. Precursor mass tolerance was set to 20 ppm. Fragment ion tolerance was set to 0.6 Da. Carbamidomethylation on cysteine (+57.021 Da) and TMT-10plex tags on N termini as well as lysine (+229.163 Da) were set as static modifications. Variable modifications were set as oxidation on methionine (+15.995 Da) and phosphorylation on serine, threonine, and tyrosine (+79.966 Da). Data were searched against a complete UniProt Human (Reviewed 20,143 entry downloaded at Nov 2018). Peptide spectral match (PSM) error rates with a 1% FDR were determined using the target-decoy strategy coupled to Percolator modelling of true and false matches.

Both unique and razor peptides were used for quantitation. Reporter ion abundances were corrected for isotopic impurities based on the manufacturer's data sheets. Reporter ions were quantified from MS3 scans using an integration tolerance of 20 ppm with the most confident centroid setting. Signal-to-noise (S/N) values were used to represent the reporter ion abundance with a co-isolation threshold of 50% and an average reporter S/N threshold of 10 and above required for quantitation from each MS3 spectra to be used. The S/N value of each reporter ions from each PSM were used to represent the abundance of the identified peptides. The summed abundance of quantified peptides were used for protein quantitation. The total peptide amount was used for the normalisation. Protein ratios were calculated from medians of summed sample abundances of replicate groups. Standard deviation were calculated from all biological replicate values. The standard deviation of all biological replicates lower than 25% were used for further analyses. To determine the significant differences between different treatments, multiple t-tests were used for statistical significance analysis, correcting for false discovery using the original FDR method of Benjamini and Hochberg, with Q=1%.

3. SUPPLEMENTAL BIOLOGY METHODS

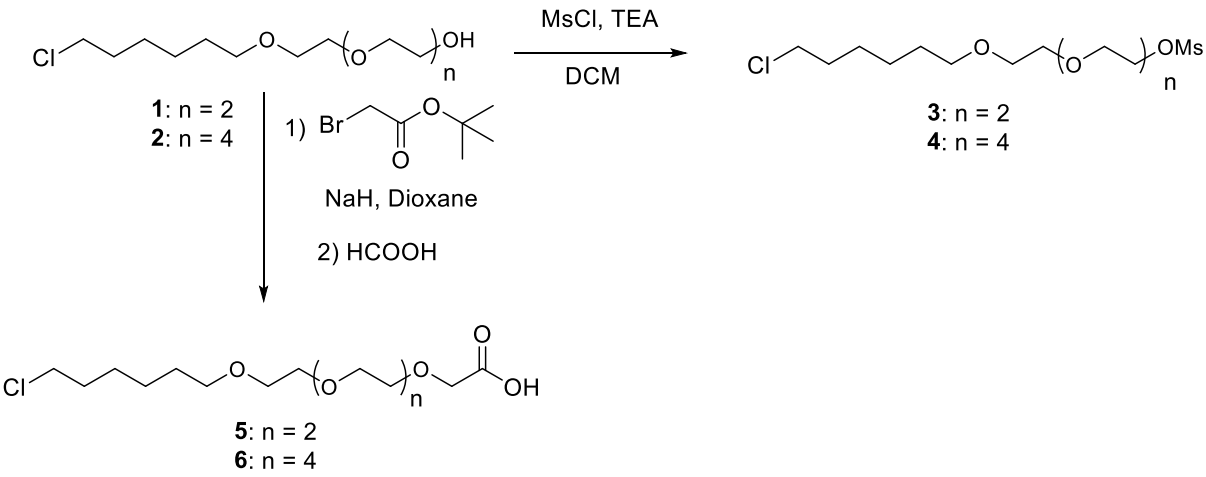
Cell Viability (MTS) Assay

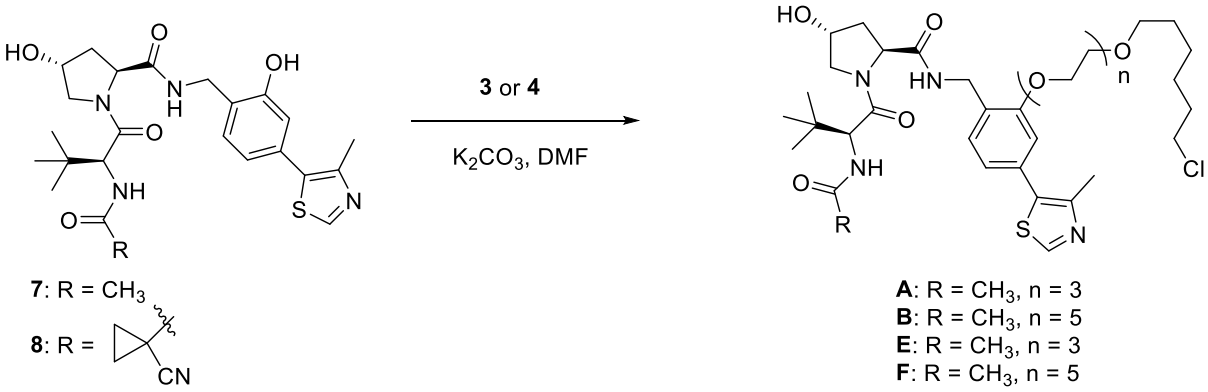
Cells were seeded into 96–well plates at a confluency of 5000 cells/well and allowed to adhere overnight. The next day, cells were treated as described to a final concentration of 0.1% DMSO. After 48h treatment, cell viability was measured using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) kit, which measures the conversion of a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine ethosulfate; PES) to a coloured formazan product. 20µl MTS Reagent was added to cells for 3h at 37°C, before recording absorbance at 490nm. Background absorbance from a no-cell control was subtracted from the readings, before normalizing to a DMSO treatment. 0.1% Triton was used as a 100% cell death control.

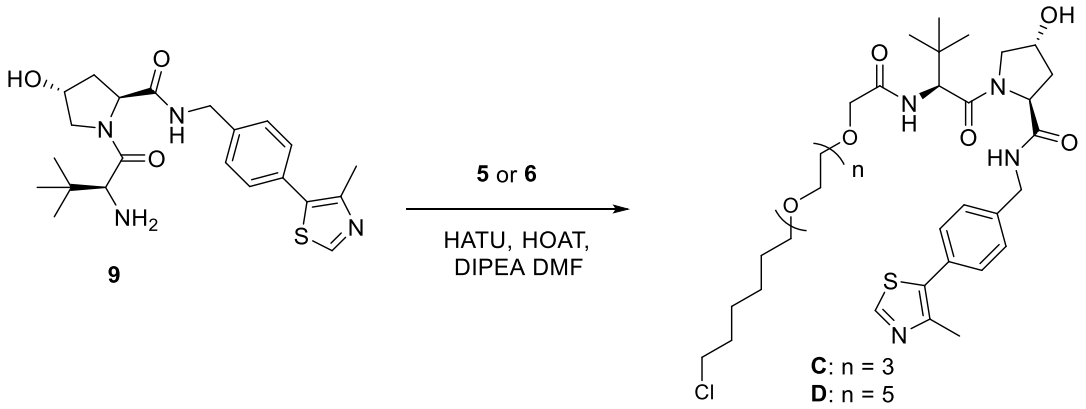
4. CHEMISTRY - GENERAL INFORMATION

All chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**9**), (2S,4R)-1-((S)-2-acetamido-3,3-dimethylbutanoyl)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5- yl)benzyl)pyrrolidine-2-carboxamide (**7**), (2S,4R)-1-((S)-2-(1-cyanocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(2- hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**8**) were prepared as previously reported by us [1,2]. 2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)ethanol (**1**), 21-chloro-3,6,9,12,15-pentaoxahenicosan-1-ol (**2**), 2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)ethyl methanesulfonate (**3**) and 21-chloro-3,6,9,12,15-pentaoxahenicosyl methanesulfonate (**4**) were prepared as previously reported by Buckley et al.[3]. Reactions were magnetically stirred; commercially available anhydrous solvents were used. All reactions requiring anhydrous conditions were carried out under nitrogen atmosphere using oven-dried glassware. Normal phase TLC was carried out on pre-coated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (UV 254/365 nm) and/or basic potassium permanganate solution. Flash column chromatography (FCC) was performed using a Teledyne Isco Combiflash Rf or Rf200i, prepacked columns RediSep Rf Normal Phase. NMR spectra were recorded on a Bruker Ascend 400 or 500 MHz. Chemical shifts are reported in parts per million referenced to residual solvent peaks ($CDCl_3$ = 7.26 ppm, CD_3OD = 3.32 ppm). The following abbreviations were used in reporting spectra, s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets). Only major rotamer NMR spectra are reported. High Resolution Mass Spectra (HRMS) were recorded on a Bruker microTOF. Low resolution MS and analytical HPLC traces were recorded on an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 µm particle size), with a gradient from 5 % to 95% of acetonitrile in water (with 0.1 % of formic acid or aqueous ammonia solution) over 3 or 7 minutes. The flow rate was 0.6 mL/min. Preparative HPLC was performed on a Gilson Preparative HPLC System with a Waters XBridge C18 column (100 mm x 19 mm; 5 µm particle size). Details about the conditions for preparative HPLC are provided in the experimental procedures.

5. SYNTHETIC SCHEMES







6. CHEMISTRY - EXPERIMENTAL DATA

General procedure for the synthesis of HaloPROTACs A, B, E and F.

To a solution of compound **9** or **10** (0.061 mmol) in dry DMF (0.5 mL), mesylate derivatives **3** or **4** (0.073 mmol) were added, followed by anhydrous potassium carbonate (21 mg, 0.15 mmol). The reaction mixture was vigorously stirred O/N at 70 °C. The reaction mixture was filtered off to afford the crude product, which was purified by HPLC using a gradient of 5% to 95% v/v of acetonitrile in 0.01% aqueous solution of formic acid over 15 minutes to yield the desired compounds after freeze drying.

General procedure for the synthesis of HaloPROTACs C and D.

To a solution linker **5** or **6** (0.049 mmol) in DMF (0.5 mL) was added HATU (18.6 mg, 0.049 mmol), HOAT (6.7 mg, 0.049 mmol), DIPEA (25 µL, 0.147 mmol) and the solution was stirred at room temperature for 5 min. Compound **13** (22.9 mg, 0.49 mmol) was added. The mixture was stirred at room temperature until no presence of the starting materials was detected by LC-MS. The solvent was evaporated under reduced pressure to give the corresponding crude, which was purified by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.01% aqueous solution of formic acid over 15 minutes to yield the desired compounds after freeze drying.

HaloPROTAC-A:

Obtained 18 mg, 50 % yield, transparent oil. ^{1}H NMR (500 MHz, MeOD) δ: 8.87 (s, 1H), 7.48 (d, *J*=7.8 Hz, 1H), 7.04 (d, *J*=1.2 Hz, 2H), 7.00 (dd, *J*=1.3, 7.9 Hz, 2H), 4.61 - 4.57 (m, 2H), 4.49 - 4.39 (m, 3H), 4.25 - 4.21 (m, 2H), 3.92 - 3.88 (m, 3H), 3.74 (q, *J*=2.9 Hz, 2H), 3.68 - 3.65 (m, 2H), 3.63 - 3.61 (m, 2H), 3.55 - 3.50 (m, 4H), 3.46 - 3.43 (m, 2H), 2.49 (s, 3H), 2.23 - 2.17 (m, 1H), 2.12 - 2.05 (m, 1H), 1.99 (s, 3H), 1.76 - 1.70 (m, 2H), 1.59 - 1.52 (m, 2H), 1.47 - 1.33 (m, 4H), 1.01 (s, 9H). ^{13}C NMR δ: 172.9, 171.6, 170.9, 156.6, 151.4, 147.7, 132.1, 131.4, 128.6, 127.0, 121.4, 112.3, 70.7, 70.4, 70.2, 70.2, 69.8, 69.7, 69.5, 68.0, 59.3, 57.7, 56.5, 44.3, 38.0, 37.4, 35.1, 32.3, 29.1, 26.3, 25.6, 25.1, 20.9, 14.5. HRMS: Calc. for $C_{36}H_{55}ClN_4O_8S$: 738.3429, found: 739.3510 [M+H$^+$].

HaloPROTAC-B

Obtained 19 mg, 47 % yield, transparent oil. ^{1}H NMR (500 MHz, MeOD) δ: 8.87 (s, 1H), 7.48 (d, *J*=7.8 Hz, 1H), 7.05 (d, *J*=0.8 Hz, 1H), 7.01 (d, *J*=0.8 Hz, 1H), 6.99 (d, *J*=1.0 Hz, 1H), 4.62 - 4.56 (m, 2H), 4.50 - 4.38 (m, 3H), 4.26 - 4.21 (m, 2H), 3.93 - 3.88 (m, 2H), 3.76 - 3.72 (m, 2H), 3.69 - 3.65 (m, 2H), 3.64 - 3.58 (m, 11H), 3.56 - 3.52 (m, 4H), 3.47 - 3.43 (m, 2H), 2.49 (s, 3H), 2.24 - 2.18 (m, 1H), 2.12 - 2.05 (m, 1H), 1.99 (s, 3H), 1.78 - 1.71 (m, 2H), 1.60 - 1.52 (m, 2H), 1.48 - 1.34 (m, 4H), 1.02 (s, 9H). ^{13}C NMR δ: 173.0, 171.6, 170.8, 156.6, 151.4, 147.7, 132.1, 131.4, 128.6, 127.1, 121.4, 112.4, 70.7, 70.4, 70.2, 70.2, 70.2, 69.8, 69.7, 69.4, 68.0, 59.3, 57.7, 56.5, 44.3, 38.0, 37.5, 35.1, 32.3, 29.1, 26.3, 25.6, 25.1, 20.9, 14.6. HRMS: Calc. for $C_{40}H_{63}ClN_4O_{10}S$: 826.3953, found: 827.4044 [M+H$^+$].

HaloPROTAC-C

Obtained 26 mg, 73% yield, transparent oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 7.35 - 7.21 (m, 6H), 4.70 - 4.66 (m, 1H), 4.52 - 4.40 (m, 3H), 4.28 (dd, *J*=5.0, 15.0 Hz, 1H), 4.05 - 3.88 (m, 3H), 3.62 - 3.44 (m, 15H), 3.38 (t, *J*=6.6 Hz, 2H), 2.52 - 2.45 (m, 4H), 2.08 - 2.03 (m, 1H), 1.73 - 1.67 (m, 2H), 1.55 - 1.48 (m, 2H), 1.41 - 1.26 (m, 4H), 0.89 (s, 9H). ^{13}C NMR δ: 171.5, 170.6, 150.3, 148.5, 138.1, 131.6, 131.0, 129.5, 128.2, 71.3, 71.2, 70.6, 70.5, 70.4, 70.1, 70.1, 58.3, 57.2, 56.6, 45.1, 43.3, 35.7, 34.8, 32.5, 29.4, 26.7, 26.4, 25.4, 16.1. HRMS: Calc. for $C_{36}H_{55}ClN_4O_8S$: 738.3429, found: 739.3563 [M+H$^+$].

HaloPROTAC-D

Obtained 27 mg, 66% yield, transparent oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 7.35 - 7.22 (m, 5H), 4.67 (t, *J*=8.0 Hz, 1H), 4.52 - 4.41 (m, 3H), 4.28 (dd, *J*=5.1, 14.5 Hz, 1H), 4.04 - 3.89 (m, 3H), 3.63 - 3.49 (m, 22H), 3.48 - 3.45 (m, 2H), 3.40 - 3.37 (m, 2H), 2.51 - 2.45 (m, 3H), 2.08 - 2.03 (m, 1H), 1.73 - 1.66 (m, 2H), 1.55 - 1.49 (m, 2H), 1.41 - 1.26 (m, 4H), 0.89 (s, 9H). ^{13}C NMR δ: 171.5, 170.7, 170.6, 150.3, 148.5, 138.1, 131.6, 131.0, 129.5,

128.2, 71.3, 71.2, 70.6, 70.5, 70.5, 70.4, 70.1, 70.1, 58.4, 57.2, 56.7, 45.1, 43.3, 35.8, 34.9, 32.6, 29.5, 26.7, 26.4, 25.4, 16.1. HRMS: Calc. for $C_{40}H_{63}ClN_4O_{10}S$: 826.3953, found: 827.4048 [M+H$^+$].

HaloPROTAC-E

Obtained 16 mg, 34 % yield, transparent oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 7.38 - 7.33 (m, 2H), 7.04 (d, *J*=8.4 Hz, 1H), 6.99 (dd, *J*=1.6, 7.8 Hz, 1H), 6.93 (d, *J*=1.5 Hz, 1H), 4.67 (t, *J*=7.9 Hz, 1H), 4.56 - 4.51 (m, 4H), 4.50 - 4.46 (m, 4H), 4.30 - 4.18 (m, 2H), 3.97 - 3.57 (m, 9H), 3.56 - 3.51 (m, 2H), 3.46 (t, *J*=6.7 Hz, 2H), 2.54 (s, 3H), 2.45 - 2.37 (m, 1H), 2.17 - 2.10 (m, 1H), 1.82 - 1.73 (m, 2H), 1.72 - 1.34 (m, 11H), 0.95 (s, 9H). ^{13}C NMR δ: 170.5, 170.2, 165.6, 156.9, 150.4, 148.5, 132.3, 131.7, 129.9, 126.8, 122.0, 119.6, 112.9, 71.3, 70.7, 70.5, 70.3, 70.1, 69.6, 68.0, 58.6, 58.4, 56.5, 45.1, 39.3, 36.5, 35.7, 32.5, 29.4, 26.7, 26.3, 25.4, 17.9, 16.1, 13.7. HRMS: Calc. for $C_{39}H_{56}ClN_5O_8S$: 789.3538, found: 790.3620 [M+H$^+$].

HaloPROTAC-F

Obtained 17 mg, 33 % yield, transparent oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 7.39 - 7.33 (m, 2H), 7.05 (d, *J*=8.4 Hz, 1H), 6.99 (dd, *J*=1.5, 7.8 Hz, 1H), 6.93 (d, *J*=1.7 Hz, 1H), 4.67 (t, *J*=7.9 Hz, 1H), 4.55 - 4.52 (m, 4H), 4.50 - 4.47 (m, 4H), 4.30 - 4.19 (m, 2H), 3.98 - 3.53 (m, 16H), 3.48 - 3.44 (m, 2H), 2.55 (s, 3H), 2.43 - 2.35 (m, 1H), 2.18 - 2.11 (m, 1H), 1.83 - 1.75 (m, 3H), 1.71 - 1.34 (m, 11H), 0.97 (s, 9H). ^{13}C NMR δ: 170.5, 170.2, 165.5, 156.9, 150.3, 148.5, 132.3, 131.7, 130.0, 126.9, 122.0, 119.7, 112.9, 71.2, 70.7, 70.6, 70.5, 70.3, 70.1, 69.6, 68.0, 58.7, 58.4, 56.5, 45.1, 39.3, 36.6, 35.7, 32.5, 29.4, 26.7, 26.3, 25.4, 17.9, 16.2, 13.7. HRMS: $C_{43}H_{64}ClN_5O_{10}S$ 877.4062, found: 878.4152 [M+H$^+$].

General procedure for the synthesis of linkers 5 and 6:

To a solution of alcohol **1** or **2** (1.12 mmol) in anhydrous dioxane (5 mL), a suspension of NaH 60% in mineral oil (54 mg, 1.35 mmol) was added at 0 °C. The resulting mixture was stirred at 0 °C for 1 h. *tert*-butyl bromoacetate (0.33 ml, 2.24 mmol) was added dropwise and the resulting mixture was stirred O/N at r.t. The reaction was quenched with a saturated solution of NH_4Cl and the aqueous phase was extracted with ethyl acetate (×3). The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The resulting oil was purified by column chromatography (from 10 to 90% of ethyl acetate in heptane) to afford a transparent oil, which was treated with formic acid (5 mL) overnight. Volatiles were removed under reduced pressure and the residue was freeze dried in order to remove any traces of formic acid.

18-chloro-3,6,9,12-tetraoxaoctadecanoic acid (5)

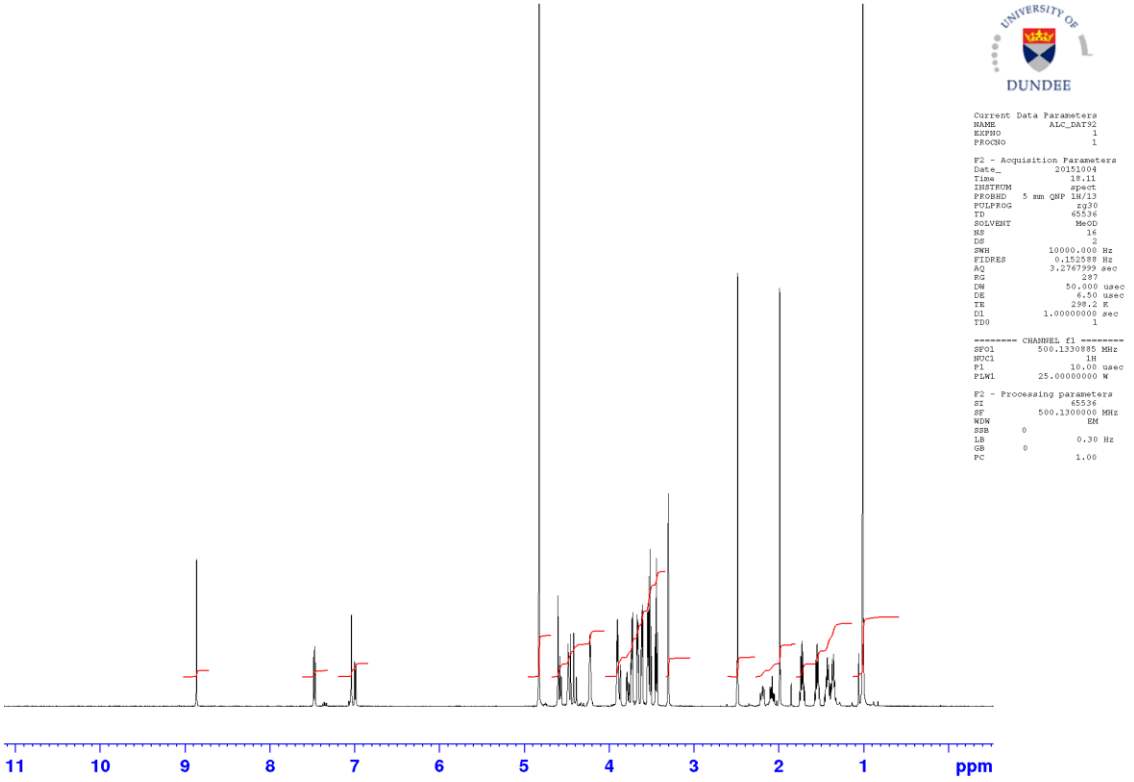
Transparent oil, 212 mg, 58 % yield. Analytical data matches those reported in literature.[4]

24-chloro-3,6,9,12,15,18-hexaoxatetracosanoic acid (6)

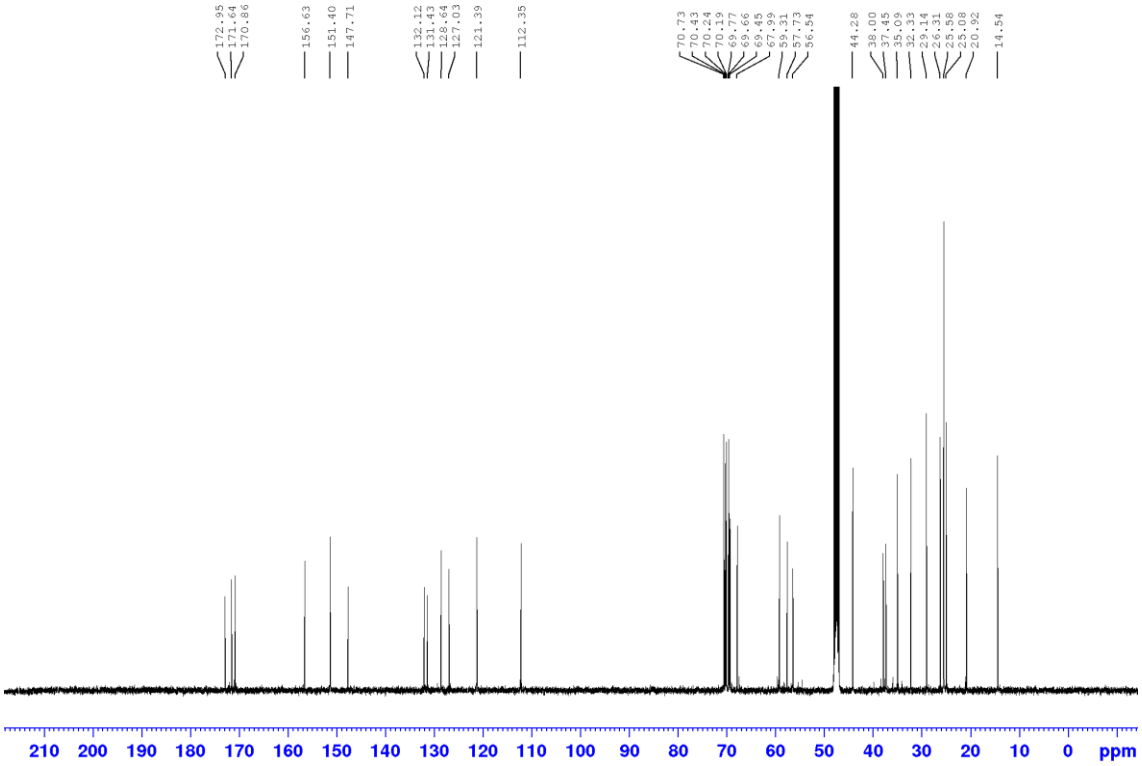
Transparent oil, 240 mg, 51 % yield.^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.50 (s, 1H), 4.10 (s, 2H), 3.71 - 3.37 (m, 20H), 1.75 - 1.66 (m, 2H), 1.57 - 1.47 (m, 2H), 1.43 - 1.24 (m, 4H). MS: calc. for $C_{18}H_{35}ClO_8$ 414.20, found: 413.2 [M-H$^+$].

7. COMPOUND NMR SPECTRA

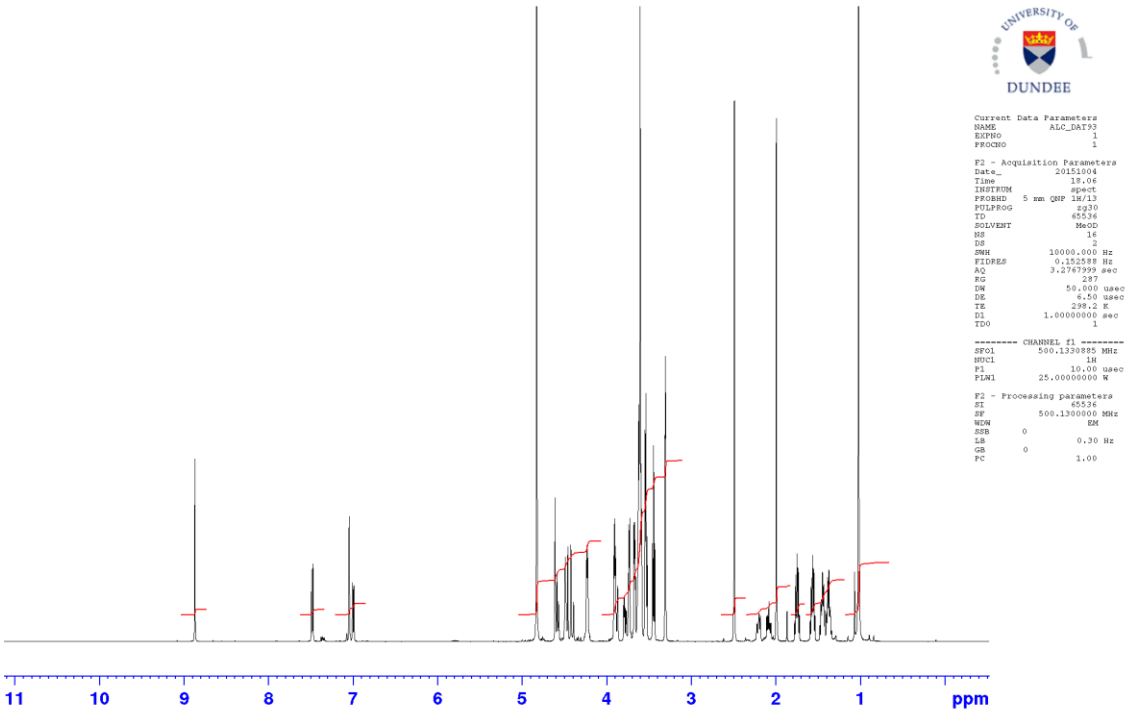
HaloPROTAC-**A**, ^{1}H NMR (CD_3OD)



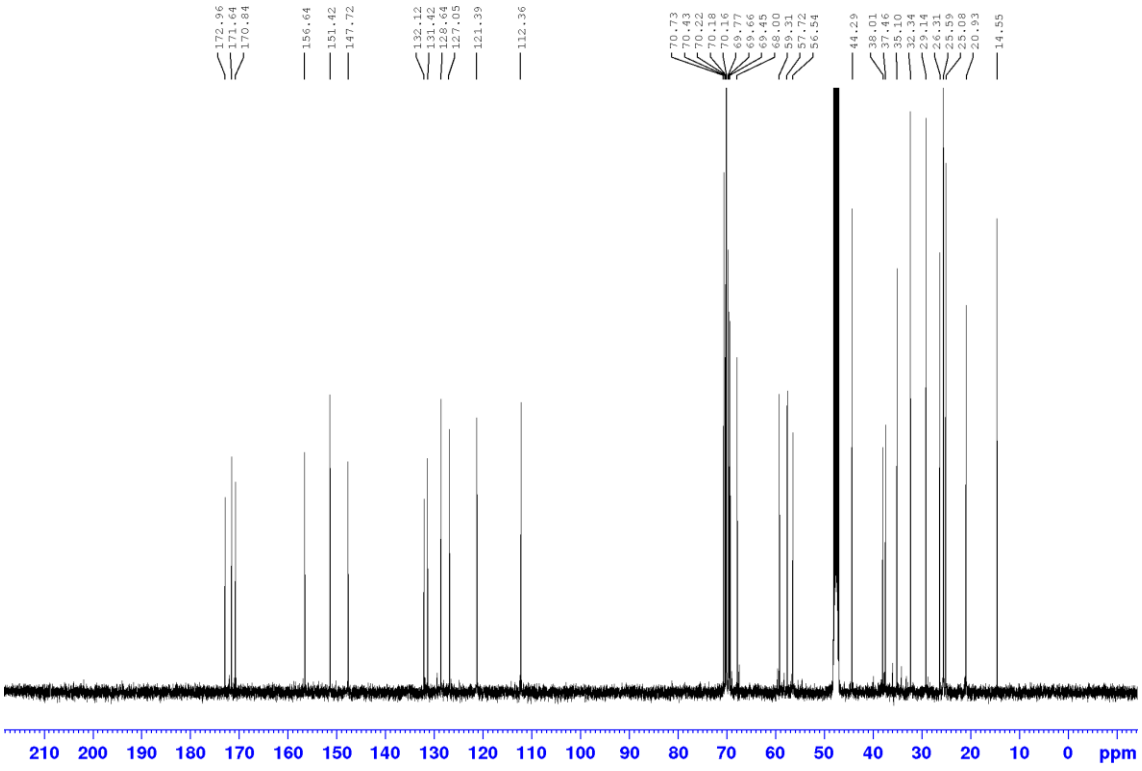
HaloPROTAC-**A**, ^{13}C NMR (CD_3OD)



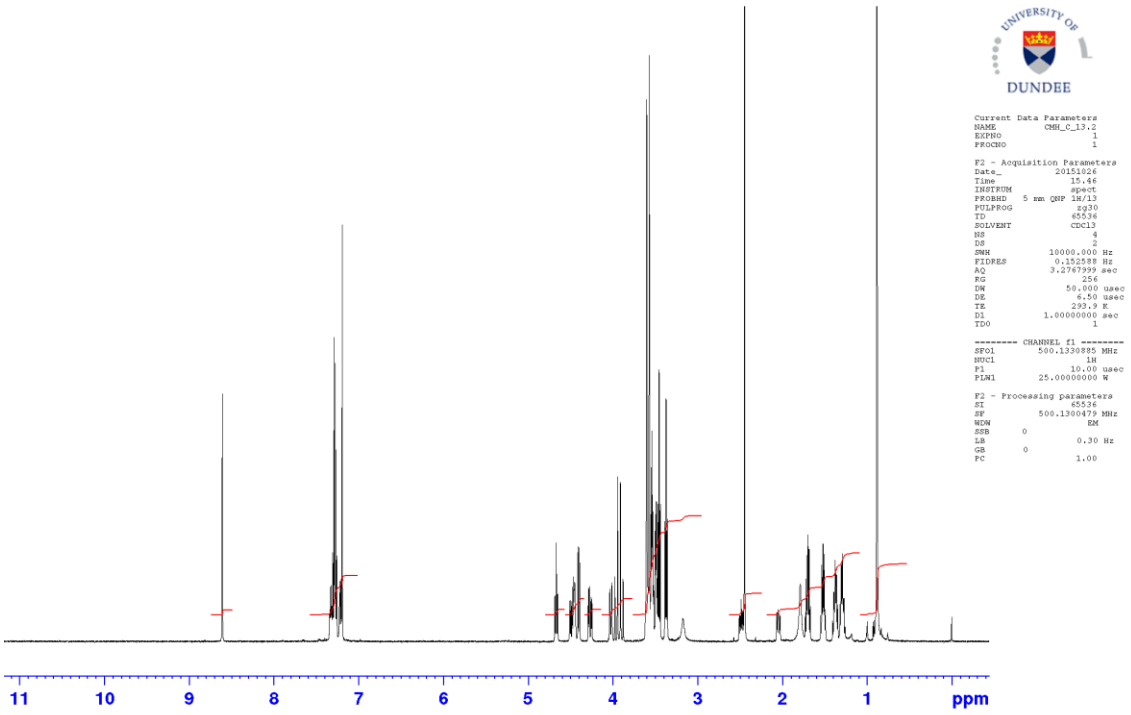
HaloPROTAC-**B**, ^{1}H NMR (CD_3OD)



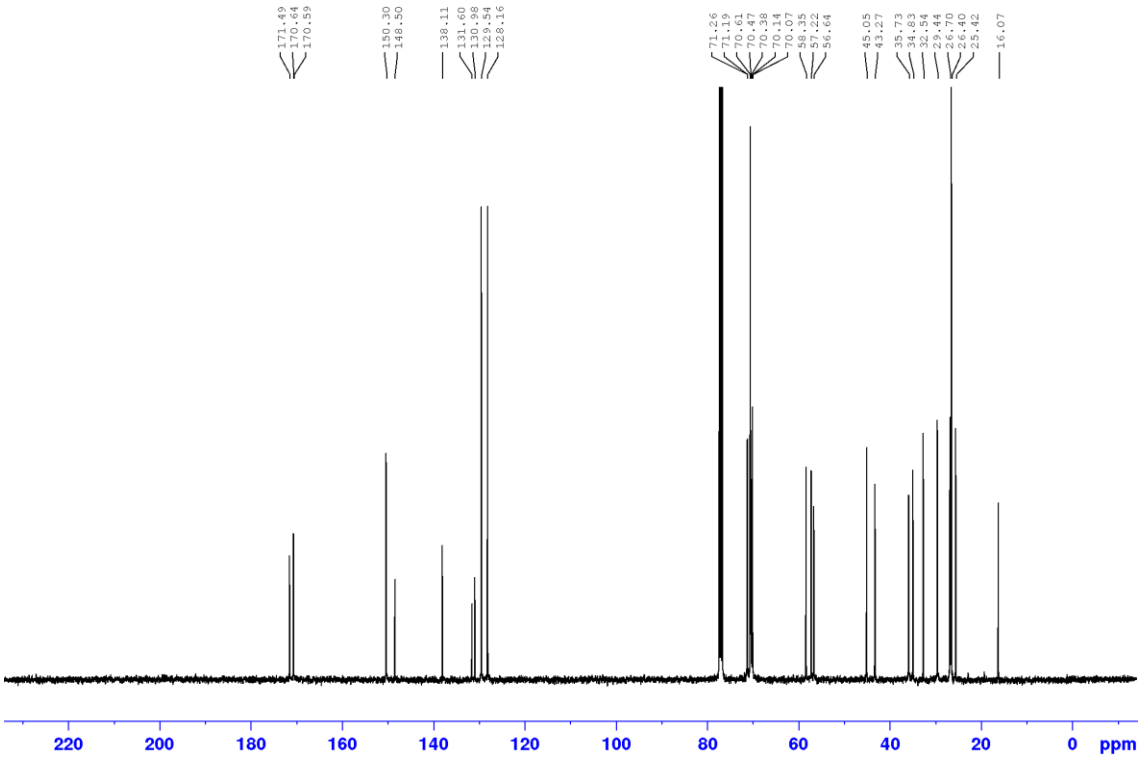
HaloPROTAC-**B**,^{13}C NMR (CD_3OD)



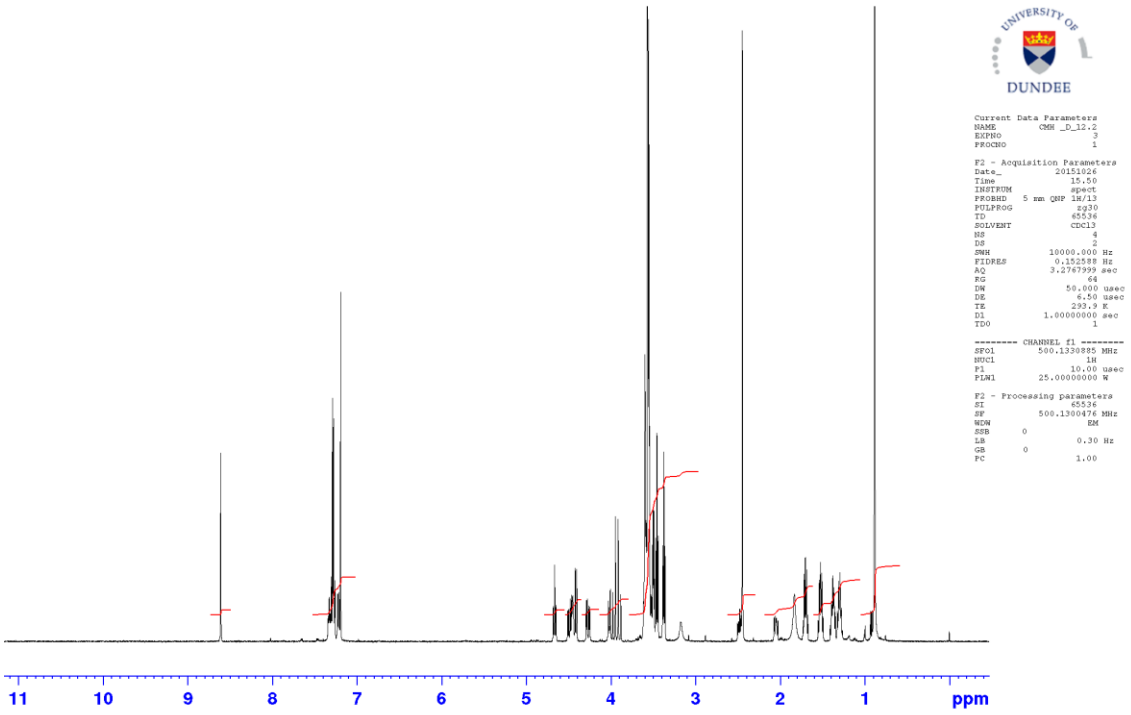
HaloPROTAC-**C**, ^{1}H NMR ($CDCl_3$)



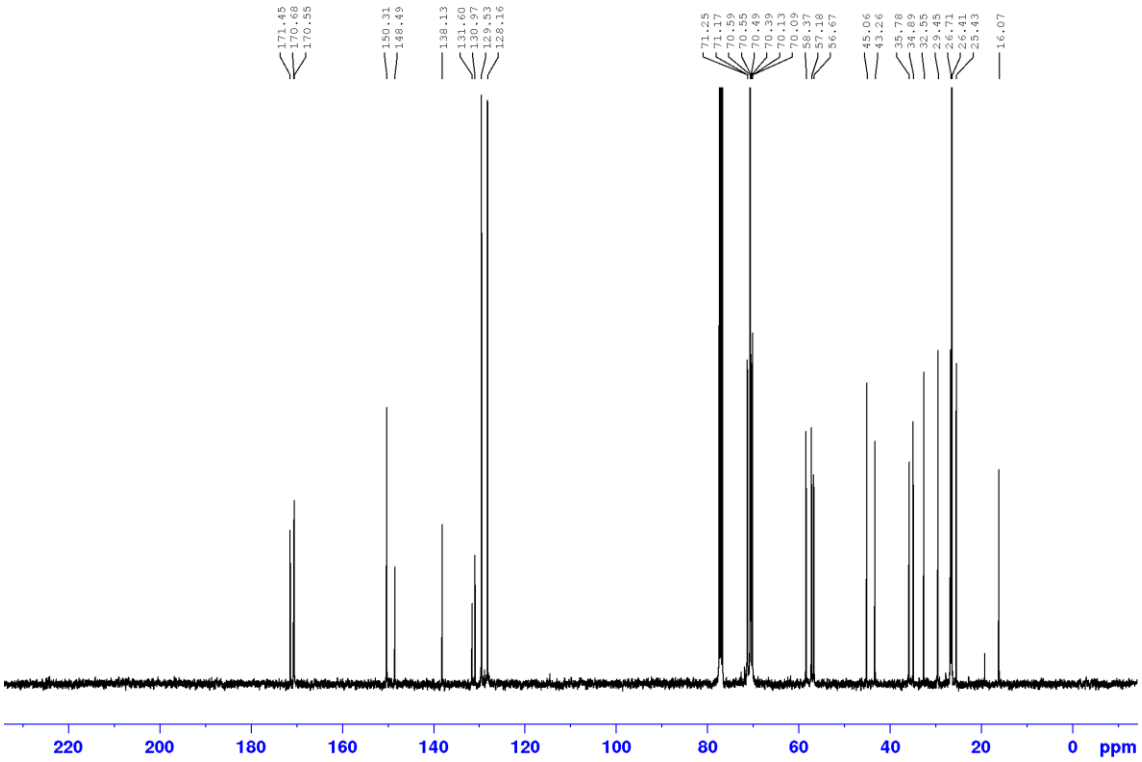
HaloPROTAC-**C**,^{13}C NMR ($CDCl_3$)



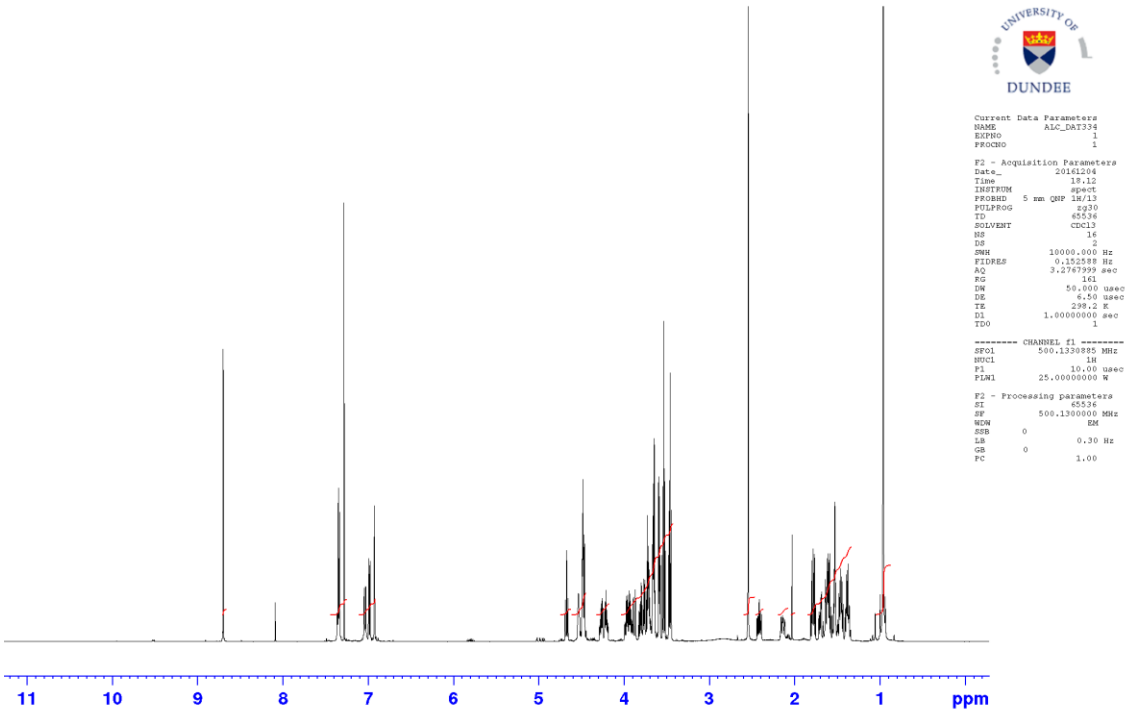
HaloPROTAC-**D**, ^{1}H NMR ($CDCl_3$)



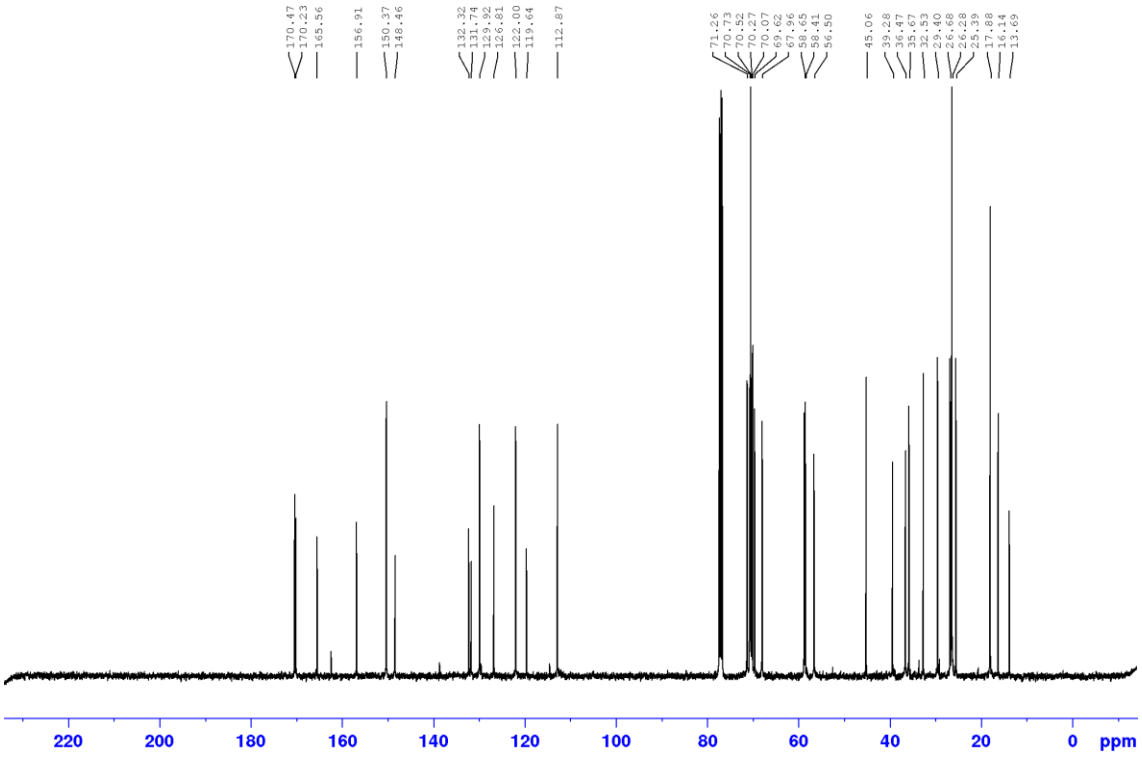
HaloPROTAC-**D**,^{13}C NMR ($CDCl_3$)



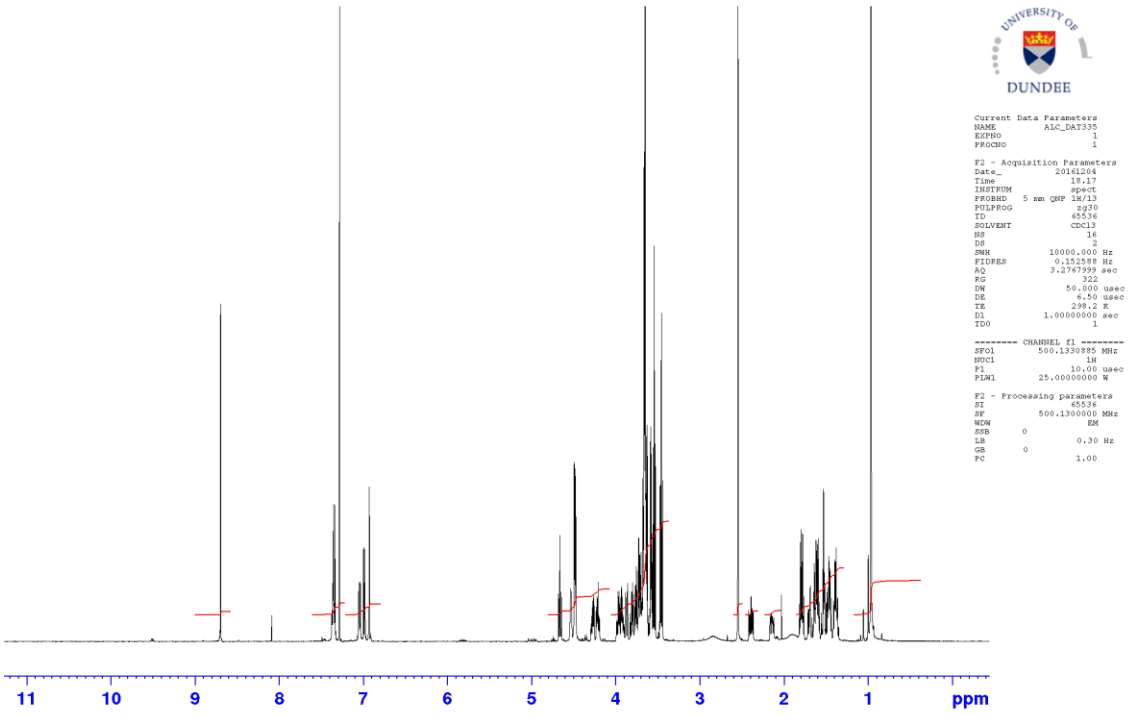
HaloPROTAC-**E**, ^{1}H NMR ($CDCl_3$)



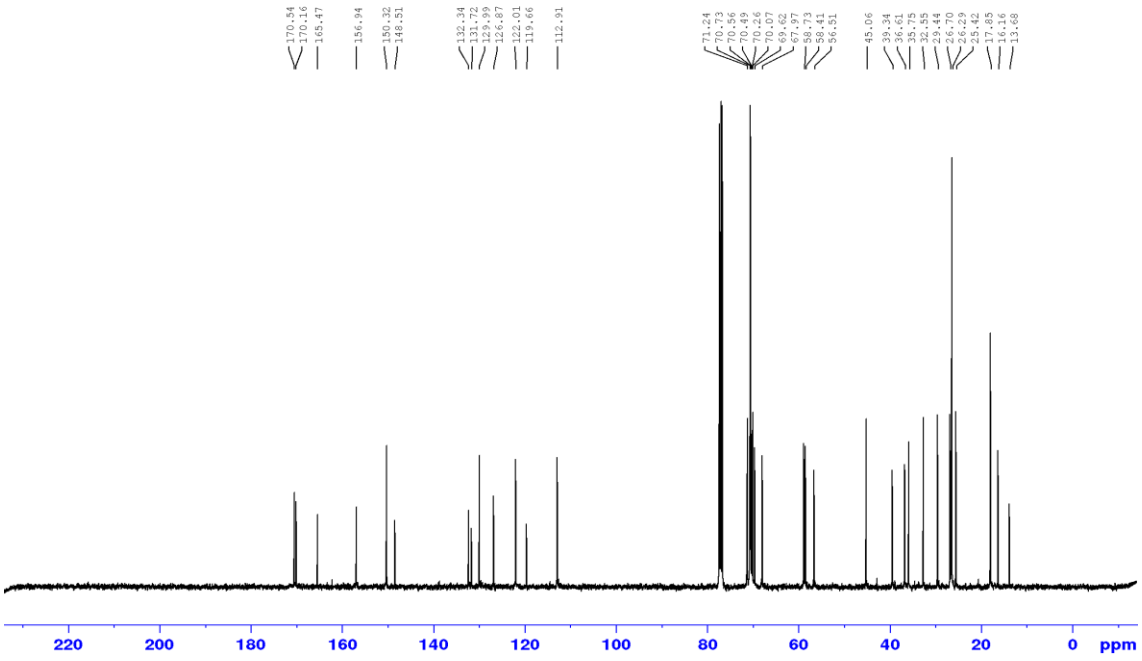
HaloPROTAC-**E**,^{13}C NMR ($CDCl_3$)



HaloPROTAC-**F**, ^{1}H NMR ($CDCl_3$)



HaloPROTAC-**F**,^{13}C NMR ($CDCl_3$)



8. REFERENCES

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