Supplementary information

PROTAC-mediated crosstalk between E3 ligases

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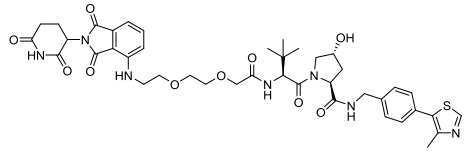
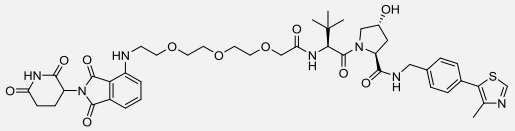
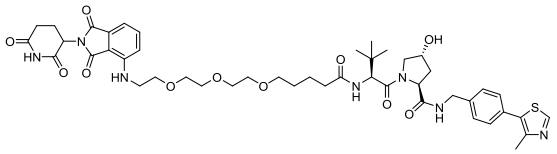
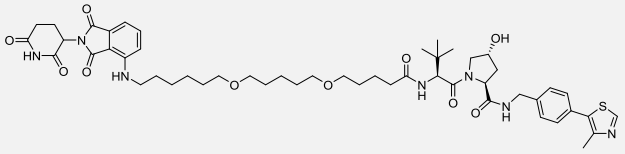
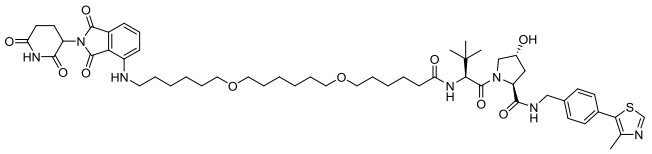
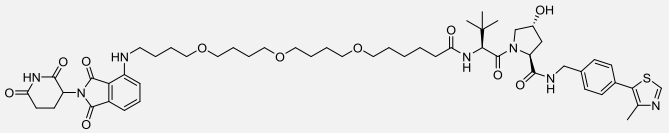
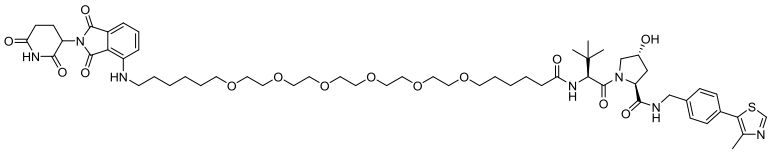
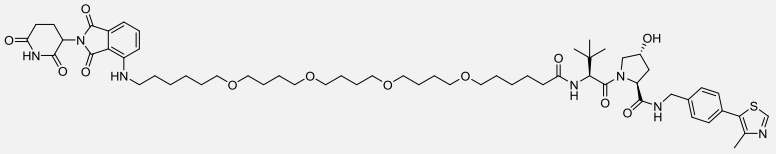
Supplementary Tables and Figures

Table S1 Overview on synthesized CRBN-VHL hetero-PROTACs

Compound	Structure
CRBN-2-2-2-VHL	
CRBN-2-2-2-2-VHL	
CRBN-2-2-2-5-VHL	
CRBN-6-5-5-VHL	
CRBN-6-6-6-VHL	
CRBN-4-4-4-6-VHL	
CRBN-6-(2)$_5$-6-VHL	
CRBN-6-(4)$_3$-6-VHL	

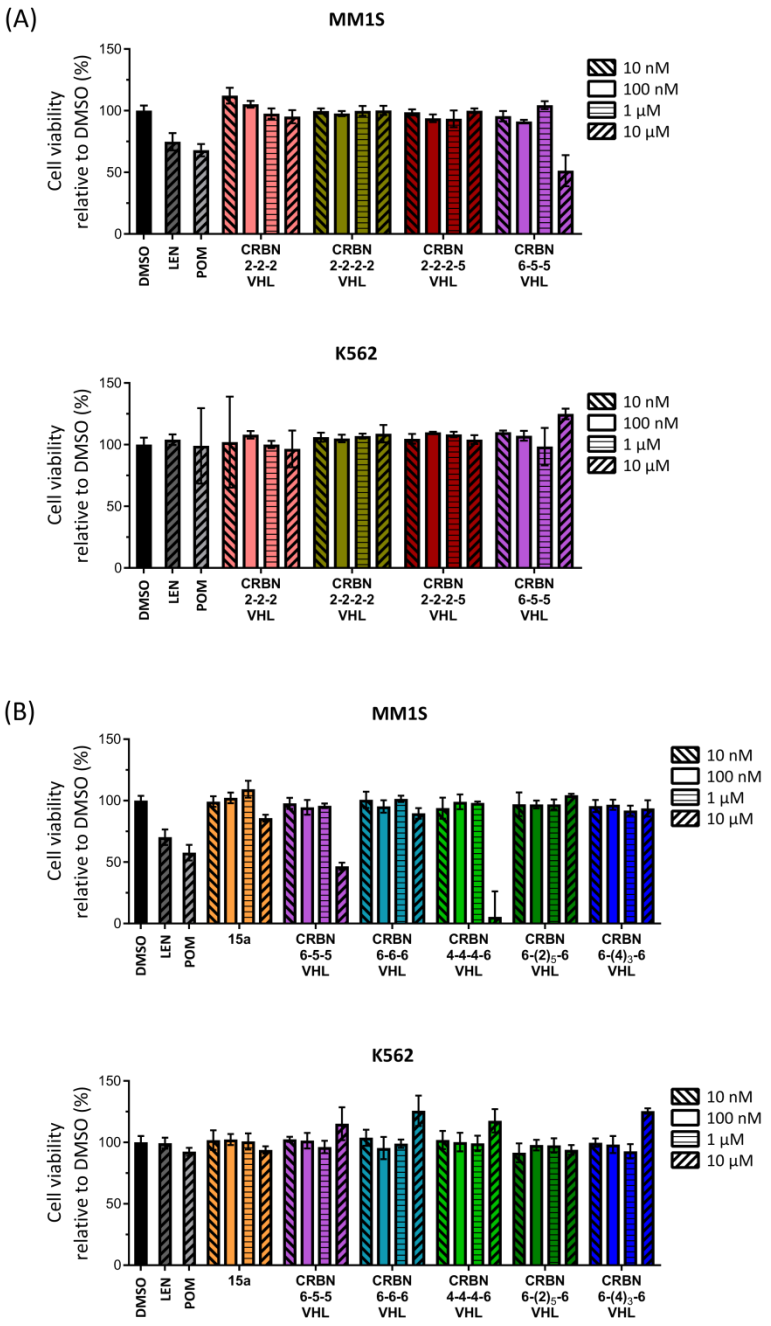


Fig. S1 (A), (B) Impact of hetero-PROTACs, homo-PROTAC **15a**,[1] lenalidomide (LEN) and pomalidomide (POM) on cell viability. IMiD-sensitive multiple myeloma cell line MM1S and pomalidomide-insensitive acute myeloid leukemia cell line K562 were treated with the indicated concentrations of compounds. Cell viability was analyzed after 4 days. Error bars express the standard errors of the mean from three biological replicates.

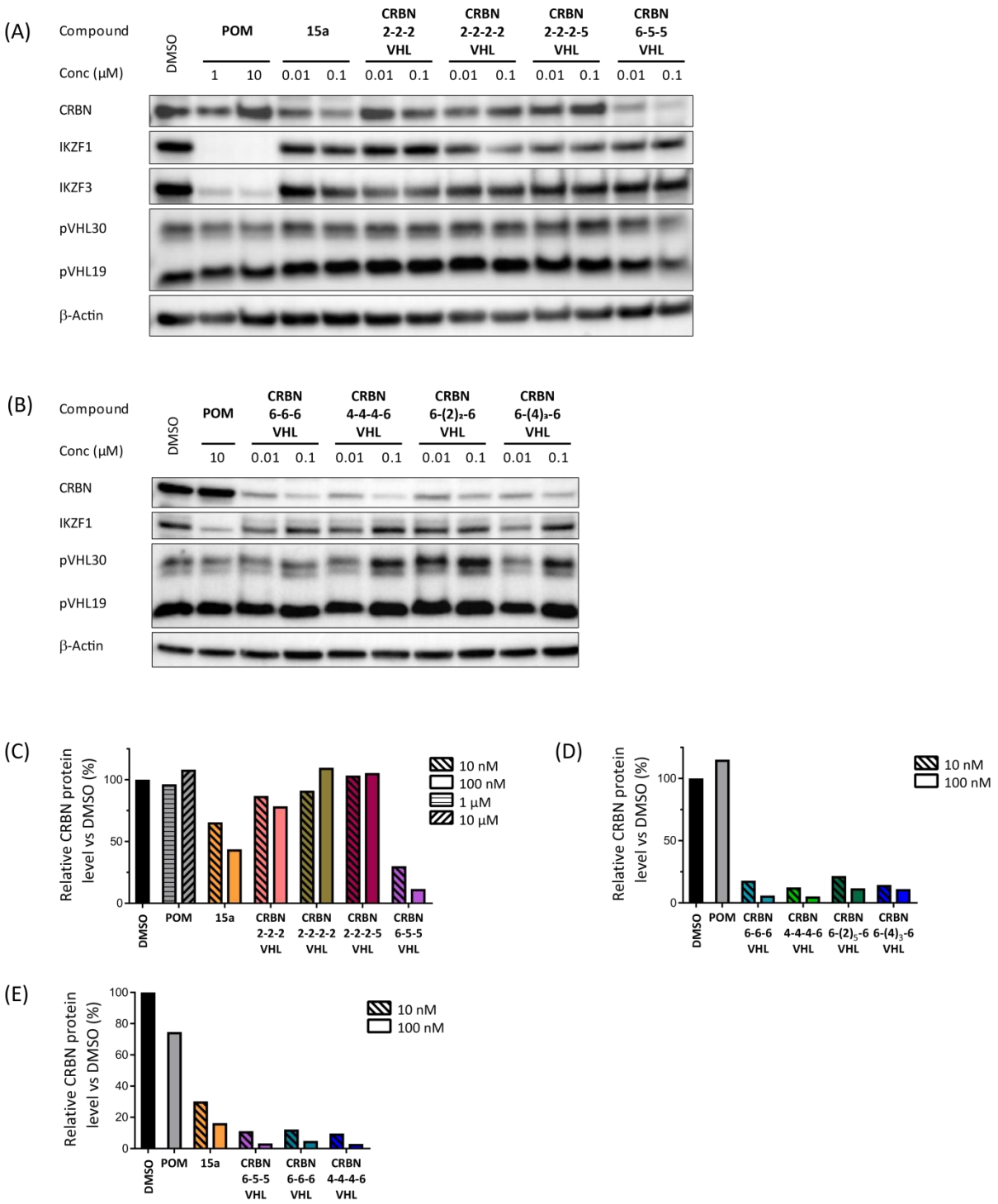


Fig. S2 Pomalidomide- and VH032-based hetero-PROTACs induce degradation of CRBN, but not of VHL. MM1S cells were treated with (A) compounds **CRBN-2-2-2-VHL**, **CRBN-2-2-2-2-VHL**, **CRBN-2-2-2-5-VHL** or **CRBN-6-5-5-VHL** in comparison to pomalidomide-based homo-PROTAC **15a** or (B) compounds **CRBN-6-6-6-VHL**, **CRBN-4-4-4-6-VHL**, **CRBN-6-(2)$_5$-6-VHL** or **CRBN-6-(4)$_3$-6-VHL** for 24 h at the indicated concentrations. (C) Quantification of CRBN protein levels of (A). (D) Quantification of CRBN protein levels of (B). (E) Quantification of CRBN protein levels of **Fig 2**.

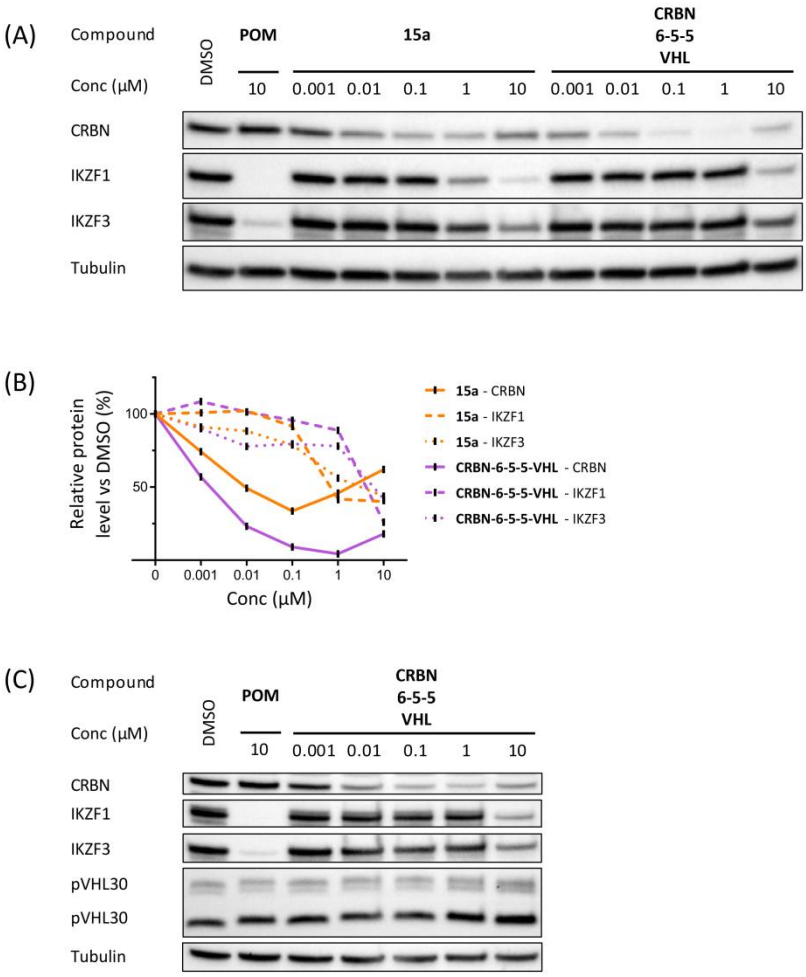


Fig. S3 (A) Homo- and hetero-PROTACs induce degradation of CRBN in a dose-dependent manner. MM1S cells were treated at indicated concentrations for 24 h. The western blot is representative of three independent experiments. (B) Quantification of the concentration series of (A). (C) Effects of elevated concentrations of **CRBN-6-5-5-VHL**. At 10 µM, **CRBN-6-5-5-VHL** did not cause pVHL30 or pVHL19 degradation. A "hook-effect" leads to the degradation of the neo-substrates IKZF1 and IKZF3 at this PROTAC concentration, in accordance with the reduced cell viability of the IMiD-sensitive multiple myeloma cell line MM1S treated with 10 µM **CRBN-6-5-5-VHL** (see Fig. S1)**.**

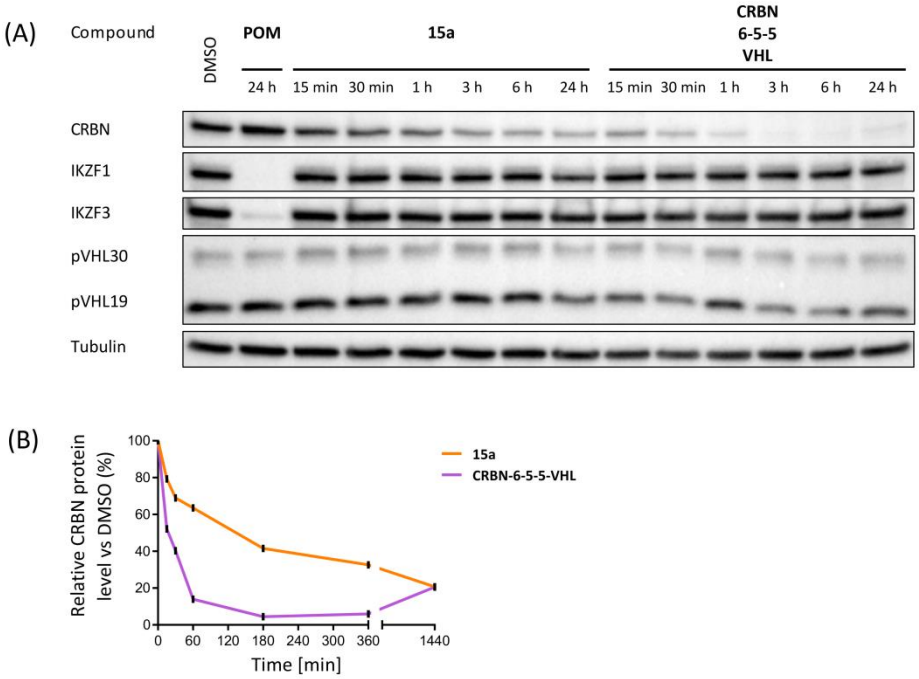
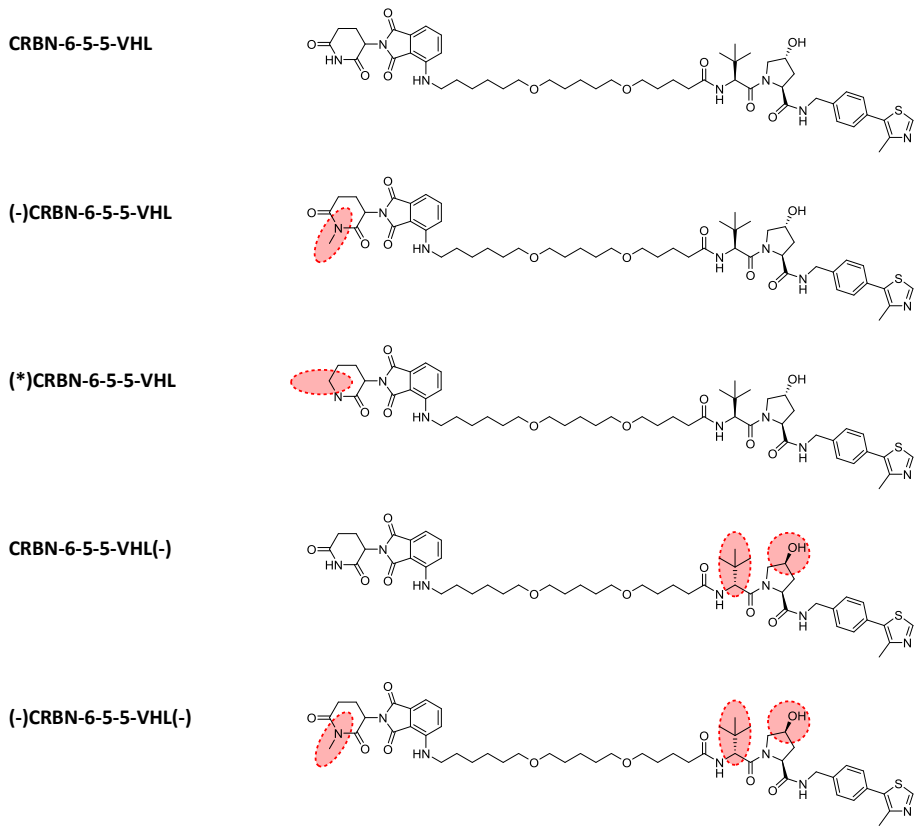


Fig. S4 Homo- and hetero-PROTAC-induced degradation of CRBN. MM1S cells were treated at 100 nM for the indicated time durations. The concentration of pomalidomide (**POM**) was 10 µM. (A) Immunoblotting analysis. The western blot is representative of three independent experiments. (B) Quantification of the time course.

(A)



(B)

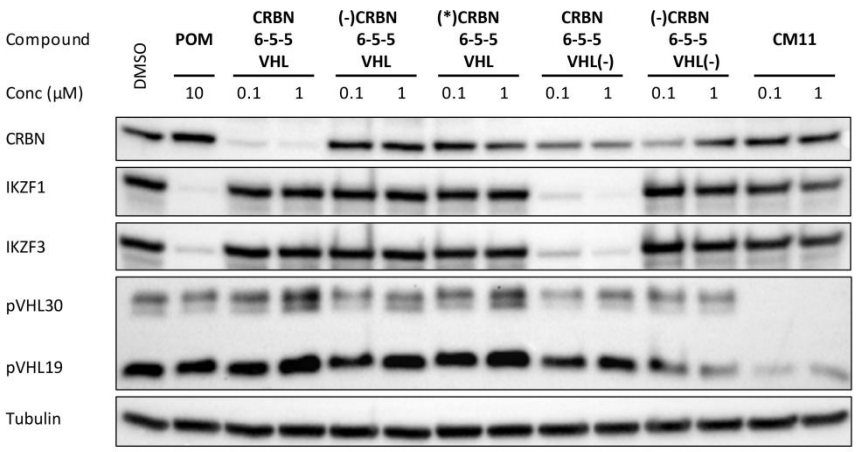


Fig. S5 Negative binding controls. (A) Design of 4 distinct chemical negative controls with different pomalidomide or VH032 modifications that abrogate CRBN degradation. (B) Evaluation of negative controls. MM1S cells were treated at the indicated concentrations with the **CRBN-6-5-5-VHL** hetero-PROTAC, the chemically impaired hetero-PROTACs or the VHL homo-PROTAC **CM11**[2] for 24 h.

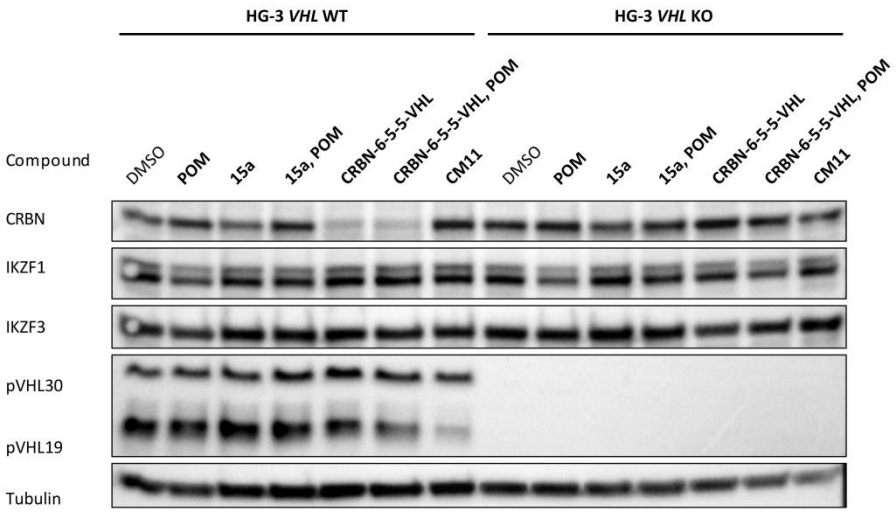


Fig. S6 HG-3 wild-type or HG-3 VHL knockout cells were treated with compounds at 100 nM. The CRBN homo-PROTAC **15a**[1], the VHL homo-PROTAC **CM11**[2] or the hetero-PROTAC **CRBN-6-5-5-VHL** were administered alone for 6 h or cells were pre-treated for 3 h with PROTACs, followed by combined treatment period with PROTAC and pomalidomide (**POM**) for additional 3 h.

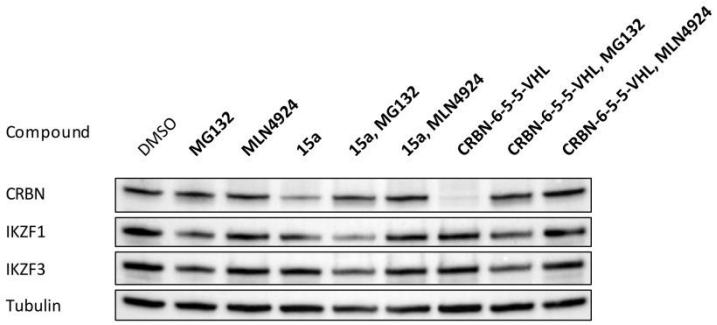


Fig. S7 PROTACs induce ubiquitination and proteasomal degradation of CRBN. MM1S cells were treated with vehicle, 10 µM **MG132**, 10 µM **MLN3924** or 100 nM PROTACs alone for 3 h or cells were pre-treated with inhibitors **MG132** / **MLN4924** for 1 h before addition of PROTACs for additional 3 h.

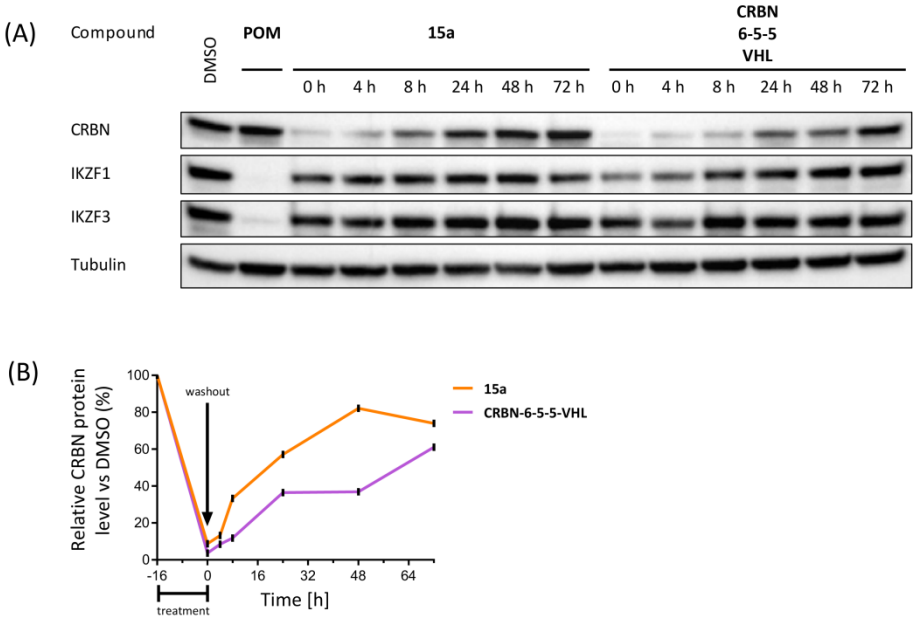


Fig. S8 Persisting effects of PROTAC on CRBN degradation after drug washout. MM1S cells were treated with PROTACs at 100 nM for 16 h before washout with PBS (= 0 h), then kept in plain media until indicated time points. (A) Immunoblotting analysis, and (B) quantification of the time course.

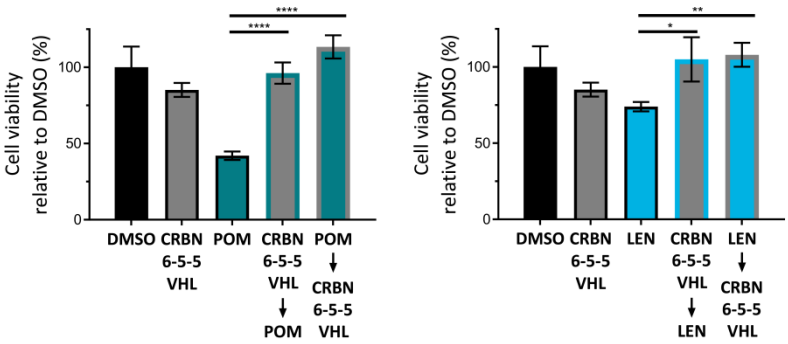


Fig. S9 Impact of **CRBN-6-5-5-VHL**, **POM** or lenalidomide (**LEN**) on cell viability in the IMiD-sensitive cell line MM1S. Cells were treated for 96 h with vehicle (DMSO), **CRBN-6-5-5-VHL** (100 nM)**,** or IMiDs (100 nM) before effects on cell viability were determined. Cell viability readout for DMSO was set to 100%. The second bar shows effects of PROTACs on cell viability. The third bar shows toxic effects of IMiDs. The fourth bar shows rescued cell viability when cells were pre-treated with PROTACs for 3 h, followed by a combined treatment period of PROTAC + IMiD for the next 4 days. The fifth bar shows rescued cell viability of post-treatment with PROTACs. In this, cells were treated with IMiDs for 3 h, followed by combined treatment period of PROTAC + IMiD for the next 4 days. Error bars express the standard errors of the mean from three biological replicates. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$.

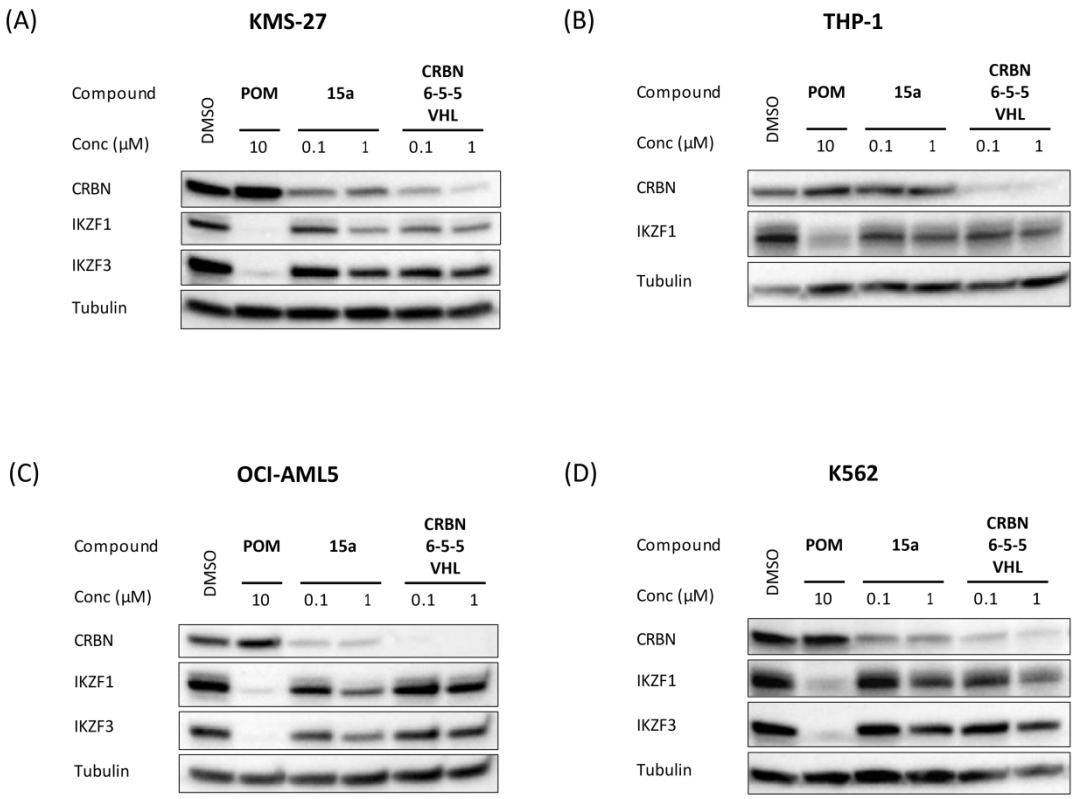


Fig. S10 Homo-PROTAC **15a** and hetero-PROTAC **CRBN-6-5-5-VHL** induce degradation of CRBN in all cell lines tested: (A) Human myeloma cell line KMS-27, (B) human monocytic cell line THP-1, (C) human acute myeloid leukemia cell line OCI-AML5, and the (D) chronic myelogenous leukemia cell line K562. Cells were treated with compounds for 24 h at the indicated concentrations.

Supplementary Information: Biology

A. Cell Lines

Cell lines MM1S and K562 were obtained from the American Type Culture Collection (ATCC) or the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ). Cells were cultured in RPMI-1640 (Biochrom) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biochrom) and 1% penicillin/streptomycin, 1% L-glutamine and kept in a humidified incubator under 5% CO_2. Cells have been authenticated by STR profile analyses and tested for mycoplasma contamination.

B. Immunoblotting

After designated treatment, cells were lysed in IP lysis buffer (Pierce) containing HALT protease and phosphatase inhibitor cocktail (Thermo Scientific). Protein content was determined with a bicinchoninic acid (BCA) assay and equal protein amounts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis at a constant voltage. Protein was transferred onto an Immobilon-P transfer membrane (Millipore). For western blot analyses, the following antibodies were used: CRBN (1:500, Sigma HPA045910), Ikaros (1:1000, Cell Signaling 14859S, clone: D6N9Y), Aiolos (1:1000, Cell Signaling 15103S, clone: D1C1E), VHL (1:1000, Cell Signaling 685475), α-Tubulin (1:7000, Sigma T5168, clone: B512), β-Actin (1:10000, Abcam ab20272, clone: mAbcam8226). Quantification was performed using the software distribution ImageJ + FIJI (v1.51j).[3]

C. Cell Viability Assay

Cells were seeded in a 96- or 384-well plates and treated with the indicated concentrations of test compounds. CellTiter-Glo® Luminescent Cell Viability Assay (Promega) was performed according to the manufacturer's protocol to determine the number of viable cells. Luminescence was readout on a PolarStar plate reader (BMG labtech). Results represent biological triplicates.

D. Statistical Analysis

Statistical analysis was performed using Prism (v6.01, GraphPad). Variance of biological replicates is represented as the standard error of the mean.

Supplementary Information: Chemistry

E. General Remarks

Preparative column chromatography was performed using Merck silica gel 60 (63–200 mesh). Petroleum ether used was a mixture of alkanes boiling between 40 – 60 °C. Melting points were determined on a Büchi 510 oil bath apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 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, version 12.0.4. Chemical shifts are given in parts per million (ppm), coupling constants *J* are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). In case of overlapping extraneous H_2O peaks, multiplet analyses in ^{1}H NMR spectra were performed using qGSD (quantitative Global Spectral Deconvolution). All multiplets related to *J*(C, F) coupling in ^{13}C NMR spectra are centred. HRMS was recorded on a micrOTOF-Q mass spectrometer (Bruker) with ESI-source coupled with an HPLC Dionex Ultimate 3000 (Thermo Scientific). The purity of the compounds was determined by HPLC-UV obtained on an LC-MS instrument (Applied Biosystems API 2000 LC/MS/MS, HPLC Agilent 1100). The purity of all the final compounds was confirmed to be ≥95% purity by LC-MS.

F. Synthesis of the Linkers L1-L8

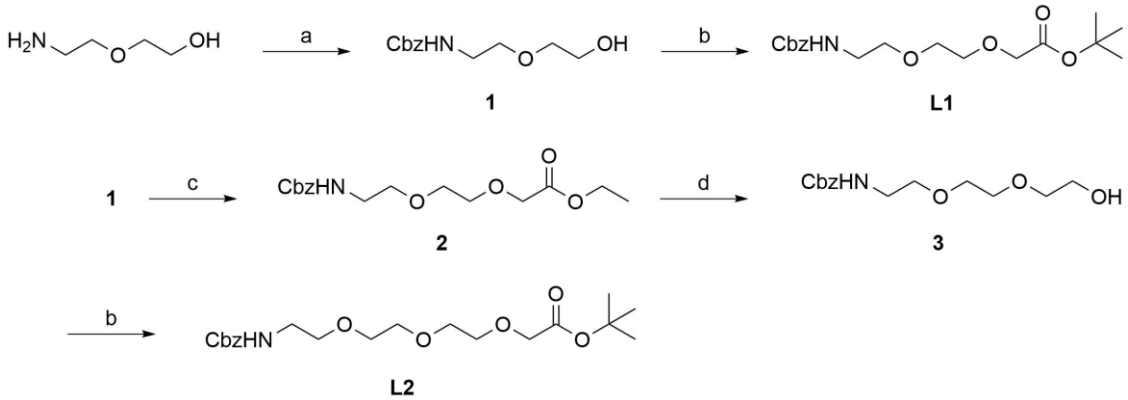
General Procedure I: Monoalkylation of Diols.[4] The corresponding diol (2-5 equiv.) was mixed in DMSO (50 mL) and aqueous NaOH (50%, 2-5 equiv.). After stirring for 10 minutes, 1-bromo-6-chlorohexane (14.96 g, 75 mmol) was added while cooling with a water bath. The resulting suspension was vigorously stirred at rt for 24 h. After the addition of a saturated NH_4Cl solution (150 mL), it was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were washed with H_2O (150 mL) and brine (150 mL), dried over Na_2SO_4, filtered and concentrated.

General Procedure II: Williamson Ether Synthesis.[4] The corresponding ω-bromo *tert*-butyl ester (40 mmol) and alcohol (40 mmol) were mixed in toluene (30 mL). While cooling, TBAHS (13.58 g, 40 mmol) and aqueous NaOH (50%, 20 mL) were added. The reaction mixture was vigorously stirred at rt overnight. The yellow mixture was diluted with H_2O (250 mL) and it was extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with H_2O (200 mL) and brine (200 mL), dried over Na_2SO_4, filtered and concentrated.

General Procedure III: Gabriel Synthesis. The corresponding alkyl halide linker (10 mmol) was dissolved in dry DMF (10 mL) and treated with potassium phthalimide (2.00 g, 10.8 mmol). The reaction mixture was heated at 95 °C overnight. The reaction mixture was poured onto half-saturated brine (200 mL) and it was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with 5% aqueous LiCl solution (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated.

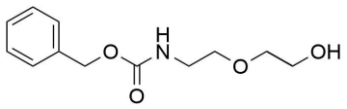
General Procedure IV: Hydrazinolysis and Cbz-Protection. The corresponding phthalimide-protected linker (5 mmol) was dissolved in EtOH (25 mL) and treated hydrazine hydrate (64% hydrazine, 0.50 g, 0.49 mL, 10 mmol). The reaction mixture was heated at 80 °C for 3 h. After evaporation of the volatiles, it was suspended in H_2O (50 mL), ammonium hydroxide solution (25% NH_3 in H_2O, 5 mL) was added and it was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The oily residue was dissolved in dry CH_2Cl_2 (7.5 mL) and Et_3N (5 mmol, 0.51 g, 0.70 mL) and cooled to 0 °C, after which benzyl chloroformate (5 mmol, 0.85 g, 0.70 mL) was added dropwise. After stirring the mixture overnight at rt, saturated $NaHCO_3$ (12.5 mL) was added, and it was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers were washed with H_2O (25 mL), 10% $KHSO_4$ (25 mL), brine (25 mL), dried over Na_2SO_4, filtered and concentrated.

Linker L1 and L2:



Reagents and conditions: (a) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h; (b) *tert*-butyl bromoacetate, KO*t*Bu, dry THF, rt, 18 h; (c) ethyl bromoacetate, KO*t*Bu, *t*BuOH, rt, 18 h; (d) $NaBH_4$, THF, MeOH, reflux, 2 h.

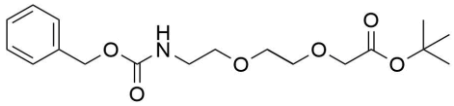
Benzyl *N*-[2-(2-hydroxyethoxy)ethyl]carbamate (1)



This compound was synthesized similar to a previously reported procedure.[5] In brief, 2-(2-aminoethoxy)ethanol (2.63 g, 25 mmol) was dissolved in dry CH_2Cl_2 (25 mL), Et_3N (2.56 g, 3.53 mL, 25 mmol) was added and it was cooled to 0 °C. A solution of benzyl chloroformate (4.26 g, 25 mmol) in dry CH_2Cl_2 (25 mL) was slowly added. The reaction mixture was first stirred for 2 h at 0 °C and then for 18 h at rt. Subsequently, it was washed with a saturated sodium bicarbonate solution (50 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (EtOAc) to obtain the desired product as a colourless oil.

Yield (4.66 g, 78%); R_f = 0.47 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.15 (q, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.37 – 3.43 (m, 4H, OCH_2), 3.47 (q, *J* = 5.2 Hz, 2H, HOC$\underline{H}_2$), 4.53 (t, *J* = 5.5 Hz, 1H, OH), 5.01 (s, 2H, OCH_2Ph), 7.22 (t, *J* = 5.7 Hz, 1H, CONH), 7.27 – 7.40 (m, 5H, 2'-H, 3'-H, 4'-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 60.34 ($HOCH_2$), 65.36 (OCH_2Ph), 69.23, 72.24 (OCH_2), 127.86, 127.88 (C-2', C-4'), 128.47 (C-3'), 137.34 (C-1'), 156.31 (CO). The signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 5.96 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{11}H_{17}NO_4$, 240.12; found, 239.9.

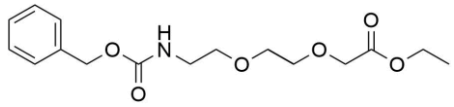
***tert*-Butyl 2-[2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy]acetate (L1)**



This compound was synthesized similar to a previously reported procedure.[5] In brief, benzyl *N*-[2-(2-hydroxyethoxy)ethyl]carbamate (4.79 g, 20 mmol) was dissolved in dry THF (50 mL) and was cooled to 0 °C. A mixture of potassium *tert*-butoxide (2.24 g, 20 mmol) in dry THF (40 mL) was added. The reaction mixture was stirred for 30 min at 0 °C before *tert*-butyl bromoacetate (4.68 g, 3.54 mL, 24 mmol) was added. Stirring was continued for 3 h at 0 °C and for 18 h at rt. Subsequently, H_2O (20 mL) was added and the mixture was evaporated *in vacuo*. The residue was partitioned between EtOAc (100 mL) and water (100 mL) and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (petroleum ether/EtOAc 2:1) to obtain the desired product as a colourrless oil.

Yield (3.18 g, 45%); R_f = 0.38 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.41 (s, 9H, CH_3), 3.14 (q, J = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.41 (t, J = 6.0 Hz, 2H, OCH_2), 3.47 – 3.58 (m, 4H, OCH_2), 3.96 (s, 2H, OCH_2), 5.00 (s, 2H, OCH_2), 7.21 (t, J = 5.1 Hz, 1H, NH), 7.26 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 27.90 (C($\underline{C}H_3)_3$), 65.35, 68.29, 69.23, 69.59, 70.00 (OCH_2), 80.77 ($\underline{C}(CH_3)_3$), 127.83, 127.86, 128.46 (C-2', C-3', C-4'), 137.34 (C-1'), 156.30 (NHCO), 169.49 (CO). The signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 8.72 min, 87% purity, *m/z* $[M + H]^+$ calcd for $C_{18}H_{27}NO_6$, 354.19; found, 354.1.

Ethyl 2-[2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy]acetate (2)

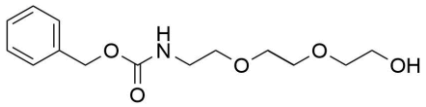


Benzyl *N*-[2-(2-hydroxyethoxy)ethyl]carbamate (19.14 g, 80 mmol) was dissolved in dry toluene (100 mL) and was cooled to 0 °C. A mixture of potassium *tert*-butoxide (13.47 g, 120 mmol) in *t*BuOH (120 mL) was added. The reaction mixture was stirred for 30 min at 0 °C and then ethyl bromoacetate (26.72 g, 17.70 mL, 160 mmol) was added. Stirring of the yellow suspension was continued for 3 h at 0 °C and for 18 h at rt.

Subsequently, 1N HCl (40 mL) was added and the aqueous layer was discarded. The organic layer was further washed with brine (200 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to obtain the desired product as a colourless oil.

Yield (12.2 g, 47%); R_f = 0.38 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.18 (t, J = 7.1 Hz, 3H, CH_3), 3.14 (q, J = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.41 (t, J = 6.0 Hz, 2H, OCH_2), 3.47 – 3.61 (m, 4H, OCH_2), 4.04 – 4.15 (m, 4H, OCH_2), 5.00 (s, 2H, OCH_2), 7.21 (t, J = 5.7 Hz, 1H, NH), 7.26 – 7.40 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 14.19 (CH_3), 60.20 ($\underline{C}H_2CH_3$), 65.35, 67.89, 69.23, 69.62, 70.11 (OCH_2), 127.82, 127.86, 128.45 (C-2', C-3', C-4'), 137.34 (C-1'), 156.30 (NHCO), 170.25 (CO). The signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 7.82 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{16}H_{23}NO_6$, 326.16; found, 326.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{16}H_{23}NO_6$, 326.1598; found, 326.1610; diff., 3.68 ppm.

Benzyl *N*-[2-[2-(2-hydroxyethoxy)ethoxy]ethyl]carbamate (3)

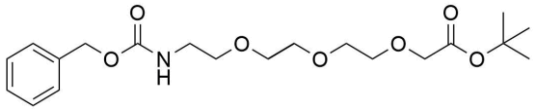


Finely powdered $NaBH_4$ (9.83 g, 0.26 mol) was added portionwise and over a period of 15 min to a stirred solution of **2** (13.0 g, 40 mmol) in THF (200 mL) at reflux. Subsequently, the oil bath was removed and methanol (200 mL) was carefully added dropwise to the stirred suspension over another 30 min. The resulting mixture was left to stir at reflux for a further 60 min. After this time, the colourless solution was allowed to cool to rt before the reaction was quenched with sat. NH_4Cl (200 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with half-saturated brine (100 mL) and the aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude oil afforded was purified by column chromatography (petroleum ether/EtOAc 1:2) to yield a colourless oil.

Yield (7.59 g, 67%); R_f = 0.35 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.14 (q, J = 5.9 Hz, 2H, OCH_2), 3.37 – 3.44 (m, 4H, OCH_2), 3.49 (d, J = 6.9 Hz, 6H, OCH_2), 4.52 (t, J = 5.5 Hz, 1H, OH), 5.00 (s, 2H, OCH_2), 7.22 (t, J = 5.7 Hz, 1H, NH), 7.26 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 60.33, 65.32, 69.22, 69.70, 69.82, 72.45 (OCH_2), 127.80, 127.84, 128.44 (C-2', C.3', C-4'), 137.32 (C-1'), 156.28 (CO). The signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to

100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 6.30 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{14}H_{21}NO_5$, 284.15; found, 284.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{14}H_{21}NO_5$, 284.1492; found, 284.1488; diff., –1.41 ppm.

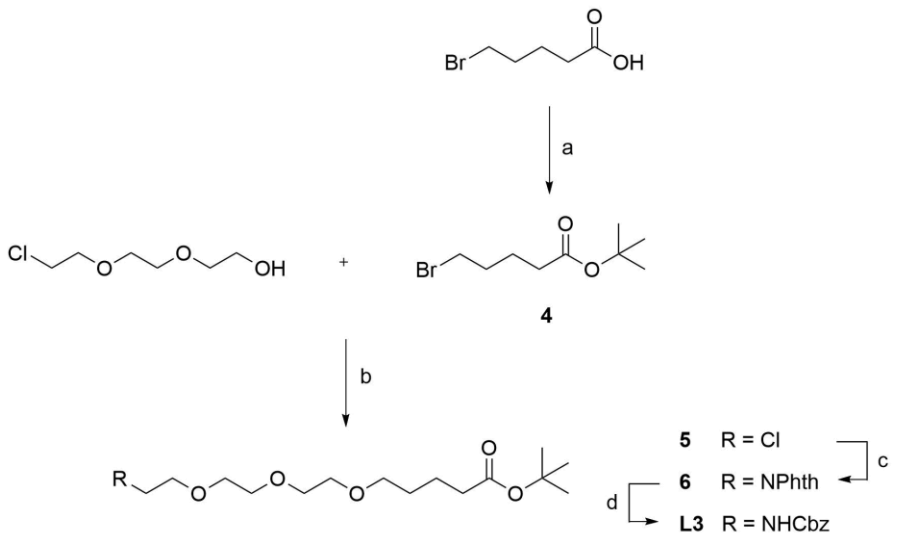
***tert*-Butyl 2-[2-[2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy]ethoxy]acetate (L2)**



Compound **3** (5.67 g, 20 mmol) was dissolved in dry toluene (50 mL) and it was cooled to 0 °C. A mixture of potassium *tert*-butoxide (3.37 g, 30 mmol) in *t*BuOH (30 mL) was added. The reaction mixture was stirred for 30 min at 0 °C and then *tert*-butyl bromoacetate (7.80 g, 5.90 mL, 40 mmol) was added. Stirring of the suspension was continued for 3 h at 0 °C and for 15 h at rt. Subsequently, 1N HCl (10 mL) was added and the aqueous layer was discarded. The organic layer was further washed with brine (50 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (petroleum ether/EtOAc 1:1) to obtain the desired product as a colourless oil.

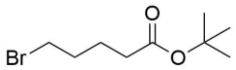
Yield (2.54 g, 32%); R_f = 0.29 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.41 (s, 9H, CH_3), 3.14 (q, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.41 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.47 – 3.53 (m, 6H, OCH_2), 3.54 – 3.57 (m, 2H, OCH_2), 3.96 (s, 2H, OCH_2), 5.00 (s, 2H, OCH_2), 7.20 (t, *J* = 5.2 Hz, 1H, NH), 7.26 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 27.90 (C($\underline{C}H_3)_3$), 65.34, 68.28, 69.24, 69.67, 69.81, 69.83, 70.00 (OCH_2), 80.75 ($\underline{C}(CH_3)_3$), 127.82, 127.86, 128.45 (C-2', C-3', C-4'), 137.34 (C-1'), 156.28 (NHCO), 169.48 (CO). The signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 8.50 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{31}NO_7$, 398.21; found, 398.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{31}NO_7$, 398.2173; found, 398.2189; diff., 4.02 ppm.

Linker L3:



Reagents and conditions: (a) *t*BuOH, DCC, DMAP, CH_2Cl_2, rt, 18 h; (b) toluene, 50% NaOH, TBAHS, rt, 18 h; (c) potassium phthalimide, DMF, 95 °C, 18 h; (d) (i) hydrazine hydrate, EtOH, 80 °C, 3 h; (ii) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h.

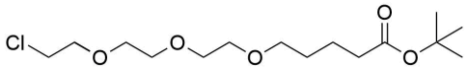
tert-Butyl 5-bromopentanoate (4)



To 5-bromovaleric acid (4.53 g, 25 mmol) in dry CH_2Cl_2 (25 mL) was added *t*BuOH (9.27 g, 11.7 mL, 125 mmol) at 0 °C followed by DMAP (0.31 g, 2.5 mmol). After 5 min, DCC (5.67 g, 27.5 mmol) was added to this solution. The solution was then left to warm to rt and stirred overnight. Subsequently, the mixture was filtered and then partitioned with H_2O (25 mL). The aqueous phase was extracted once more with CH_2Cl_2 (25 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The remaining residue was purified by column chromatography (petroleum ether/CH_2Cl_2 4:1) to give the title product as a colourless oil.

Yield (3.56 g, 60%); R_f = 0.42 (petroleum ether/CH_2Cl_2 3:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.39 (s, 9H, CH_3), 1.55 – 1.63 (m, 2H), 1.75 – 1.83 (m, 2H, CH_2CH_2), 2.22 (t, *J* = 7.4 Hz, 2H, $COCH_2$), 3.52 (t, *J* = 6.6 Hz, 2H, CH_2Br); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 23.41 (C-3), 27.92 (C($\underline{C}H_3)_3$), 30.80 (C-4), 31.66 (C-2), 34.82 (C-5), 79.71 ($\underline{C}(CH_3)_3$), 172.14 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 11.15 min, *m/z* $[M + H]^+$ calcd for $C_9H_{17}{}^{81}BrO_2$, 239.05; found, 239.1.

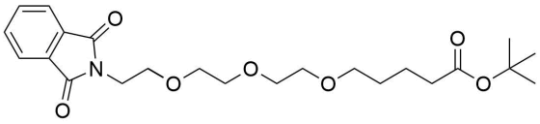
***tert*-Butyl 5-[2-[2-(2-chloroethoxy)ethoxy]ethoxy]pentanoate (5)**



This compound was prepared using the General Procedure **II** and *tert*-butyl 5-bromopentanoate (**4**, 9.49 g, 40 mmol) and 2-(2-(2-chlorethoxy)ethoxy)ethanol (6.74 g, 40 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colourless oil.

Yield (5.72 g, 44%); R_f = 0.47 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.38 (s, 9H, CH_3), 1.42 – 1.55 (m, 4H, CH_2), 2.18 (t, J = 7.0 Hz, 2H, CH_2), 3.36 (t, J = 5.9 Hz, 2H, CH_2Cl), 3.43 – 3.58 (m, 8H, OCH_2), 3.63 – 3.73 (m, 4H, OCH_2); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.58 (CH_2), 27.89 ($C(\underline{C}H_3)_3$), 28.61, 34.63 (CH_2), 43.64 (CH_2Cl), 69.57, 69.82, 69.93, 69.98, 70.68(OCH_2), 79.48 ($\underline{C}(CH_3)_3$), 172.33 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 196-400 nm), t_R = 10.79 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{15}H_{29}ClO_5$, 325.27; found, 325.3; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd for $C_{15}H_{29}ClO_5$, 342.2042; found, 342.2032; diff., –2.92 ppm.

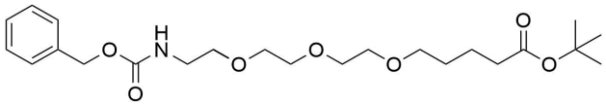
***tert*-Butyl 5-[2-[2-[2-(1,3-dioxoisoindolin-2-yl)ethoxy]ethoxy]ethoxy]pentanoate (6)**



This compound was prepared using the General Procedure **III** and linker precursor **5** (3.25 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to give a colourless oil.

Yield (3.53 g, 81%); R_f = 0.32 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.37 (s, 9H, CH_3), 1.39 – 1.51 (m, 4H, CH_2), 2.15 (t, J = 7.1 Hz, 2H, $COCH_2$), 3.25 – 3.39 (m, 4H), 3.39 – 3.45 (m, 4H), 3.47 – 3.51 (m, 2H), 3.61 (t, J = 5.9 Hz, 2H, OCH_2), 3.74 (t, J = 5.8 Hz, 2H, NCH_2), 7.79 – 7.89 (m, 4H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.59 (CH_2), 27.92 ($C(\underline{C}H_3)_3$), 28.59 (CH_2), 34.65 ($CO\underline{C}H_2$), 37.31 (NCH_2), 67.09, 69.52, 69.67, 69.81, 69.91, 69.96 (OCH_2), 79.52 ($\underline{C}(CH_3)_3$), 123.18 (C-4, C-7), 131.72 (C-3a, C-7a), 134.59 (C-5, C-6), 167.91 (C-1, C-3), 172.37 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 11.14 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{33}NO_7$, 436.23; found, 436.4; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd for $C_{23}H_{33}NO_7$, 453.2595; found, 453.2601; diff., –1.32 ppm.

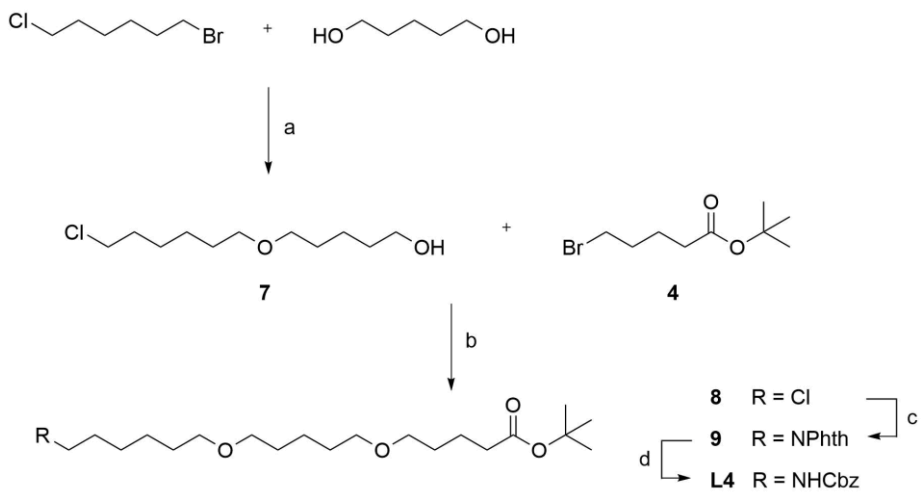
***tert*-Butyl 5-[2-[2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy]ethoxy]pentanoate (L3)**



This compound was prepared using the General Procedure **IV** and linker precursor **6** (2.18 g, 5 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 1:1) to give a colourless oil.

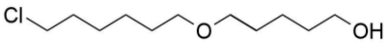
Yield (1.43 g, 65%); R_f = 0.38 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ; 1.38 (s, 9H, CH_3), 1.44 – 1.53 (m, 4H, CH_2), 2.17 (t, J = 7.0 Hz, 2H, CH_2), 3.10 – 3.17 (m, 2H, CH_2), 3.35 (t, J = 6.1 Hz, 2H, CH_2), 3.41 (t, J = 6.0 Hz, 2H, OCH_2, NHC$\underline{H}_2$), 3.43 – 3.46 (m, 2H, OCH_2), 3.47 – 3.51 (m, 6H, OCH_2), 5.00 (s, 2H, $COCH_2$), 7.20 (t, J = 4.9 Hz, 1H, NH), 7.24 – 7.42 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.59 (CH_2), 27.91 (C($\underline{C}H_3$)$_3$), 28.61, 34.64 (CH_2), 65.34, 69.24, 69.58, 69.71, 69.89, 69.93, 70.00 (OCH_2), 79.50 ($\underline{C}(CH_3)_3$), 127.82, 127.86, 128.45 (C-2, C-3, C-4), 137.35 (C-1), 156.29 (CO), 172.35 (CONH); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 9.67 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{37}NO_7$, 440.26; found, 440.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{37}NO_7$, 440.2643; found, 440.2654; diff., 2.50 ppm.

Linker L4:



Reagents and conditions: (a) 50% NaOH, DMSO, rt, 24 h; (b) toluene, 50% NaOH, TBAHS, rt, 18 h; (c) potassium phthalimide, DMF, 95 °C, 18 h; (d) (i) hydrazine hydrate, EtOH, 80 °C, 3 h; (ii) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h.

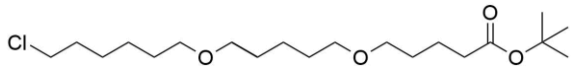
5-(6-Chlorohexoxy)pentane-1-ol (7)



This compound was prepared using the General Procedure **I**, 1,5-pentanediol (5 equiv., 10.05 g, 375 mmol) and aqueous NaOH (50%, 19.3 mL, 375 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to give a colourless oil.

Yield (6.85 g, 41%); ^{1}**H NMR** (600 MHz, DMSO-d_6) δ 1.25 – 1.33 (m, 4H, CH_2), 1.34 – 1.43 (m, 4H, CH_2), 1.43 – 1.50 (m, 4H, CH_2), 1.65 – 1.73 (m, 2H, CH_2), 3.30 – 3.33 (m, 4H, OCH_2), 3.34 – 3.39 (m, 2H, CH_2Cl), 3.61 (t, J = 6.6 Hz, 2H, $C\underline{H}_2OH$), 4.30 (t, J = 5.2 Hz, 1H, OH); ^{13}**C NMR** (151 MHz, DMSO-d_6) δ 22.43, 25.15, 26.25, 29.24, 29.29, 32.18, 32.49 (CH_2), 45.49 (CH_2Cl), 60.82 (CH_2OH), 69.95, 70.15, (OCH_2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 10.85 min, *m/z* $[M + H]^+$ calcd for $C_{11}H_{23}ClO_2$, 223.14; found, 223.1.

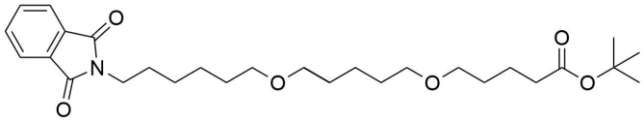
***tert*-Butyl 5-[5-(6-chlorohexoxy)pentoxy]pentanoate (8)**



This compound was prepared using the General Procedure **II** and *tert*-butyl 5-bromopentanoate (**4**, 9.49 g, 40 mmol) and 5-(6-chlorohexoxy)pentane-1-ol (**7**, 8.91 g, 40 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 10:1) to give a colourless oil.

Yield (8.91 g, 31%); R_f = 0.33 (petroleum ether/EtOAc 10:1); ^{1}**H NMR** (500 MHz, DMSO-d_6) δ 1.26 – 1.41 (m, 15H, 3 × CH_2, CH_3), 1.43 – 1.53 (m, 10H, CH_2), 1.65 – 1.74 (m, 2H, CH_2), 2.17 (t, J = 7.0 Hz, 2H, $COCH_2$), 3.31 (t, J = 6.3 Hz, 8H, OCH_2), 3.60 (t, J = 6.6 Hz, 2H, CH_2Cl); ^{13}**C NMR** (126 MHz, DMSO-d_6) δ 21.66, 22.64, 25.11, 26.21 (CH_2), 27.87 ($C(\underline{C}H_3)_3$), 28.64, 29.15, 29.19, 32.15, 34.64 (CH_2), 45.41 (CH_2Cl), 69.62, 69.90, 69.98, 70.01 (OCH_2), 79.43 ($\underline{C}(CH_3)_3$), 172.29 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), t_R = 12.39 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{39}ClO_4$, 379.26; found, 379.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{39}ClO_4$, 379.2610; found, 379.2603; diff., –1.85 ppm.

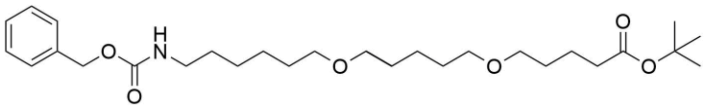
***tert*-Butyl 5-[5-[6-(1,3-dioxoisoindolin-2-yl)hexoxy]pentoxy]pentanoate (9)**



This compound was prepared using the General Procedure **III** and linker precursor **8** (3.79 g, 10 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 4:1) to give a colourless oil.

Yield (2.25 g, 46%); R_f = 0.50 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.23 – 1.32 (m, 6H, CH_2), 1.36 (s, 9H, CH_3), 1.40 – 1.51 (m, 10H, CH_2), 1.53 – 1.61 (m, 2H, CH_2), 2.16 (t, J = 7.1 Hz, 2H, $COCH_2$), 3.25 – 3.32 (m, 8H, OCH_2), 3.54 (t, J = 7.1 Hz, 2H, NCH_2), 7.78 – 7.88 (m, 4H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 21.69, 22.65, 25.46 (CH_2), 26.22 (CH_2), 27.89 ($C(\underline{C}H_3)_3$), 28.03, 28.65, 29.17 (CH_2), 34.66 ($CO\underline{C}H_2$), 37.50 (NCH_2), 69.63, 69.96, 70.00 (OCH_2), 79.46 ($\underline{C}(CH_3)_3$), 123.11 (C-4, C-7), 131.75 (C-3a, C-7a), 134.50 (C-5, C-6), 168.06 (C-1, C-3), 172.33 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 12.88 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{28}H_{43}NO_6$, 490.31; found, 490.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{28}H_{43}NO_6$, 490.3163; found, 490.3145; diff., –3.67 ppm.

***tert*-Butyl 5-[5-[6-(benzyloxycarbonylamino)hexoxy]pentoxy]pentanoate (L4)**

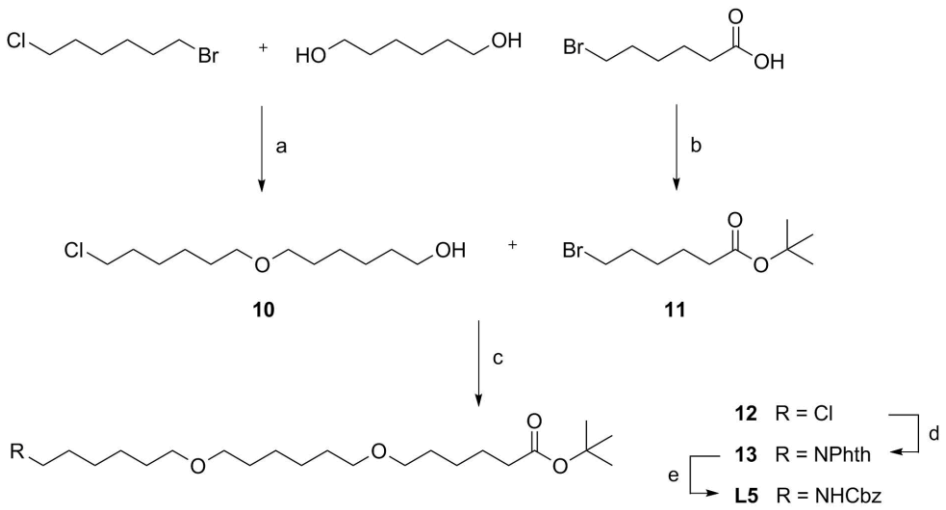


This compound was prepared using the General Procedure **IV** and linker precursor **9** (2.45 g, 5 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 1:1) to give a colourless oil.

Yield (1.56 g, 63%); R_f = 0.16 (petroleum ether/EtOAc 6:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.21 – 1.34 (m, 6H, CH_2), 1.38 (s, 11H, CH_2, CH_3), 1.42 – 1.54 (m, 10H, CH_2), 2.17 (t, J = 7.1 Hz, 2H, CH_2), 2.96 (q, J = 6.7 Hz, 2H, $NHC\underline{H}_2$), 3.27 – 3.34 (m, 8H, OCH_2), 4.99 (s, 2H $COCH_2$), 7.12 – 7.24 (m, 1H, NH), 7.25 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 21.70, 22.66, 25.56, 26.21 (CH_2), 27.91 ($C(\underline{C}H_3)_3$), 28.66, 29.19, 29.33, 29.51, 34.67 (CH_2), 40.38 ($NHCH_2$), 65.20, 69.64, 70.01, 70.02, 70.04 (OCH_2), 79.49 ($\underline{C}(CH_3)_3$), 127.82, 127.85, 128.46 (C-2, C-3, C-4), 137.49 (C-1), 156.22 (CO), 172.36 (CONH); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-

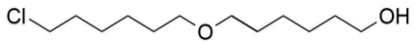
400 nm), t_R = 11.84 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{28}H_{47}NO_6$, 494.34; found, 494.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{28}H_{47}NO_6$, 494.3476; found, 494.3466; diff., –2.02 ppm.

Linker L5:



Reagents and conditions: (a) 50% NaOH, DMSO, rt, 24 h; (b) *t*BuOH, DCC, DMAP, CH_2Cl_2, rt, 18 h; (c) toluene, 50% NaOH, TBAHS, rt, 18 h; (d) potassium phthalimide, DMF, 95 °C, 18 h; (e) (i) hydrazine hydrate, EtOH, 80 °C, 3 h; (ii) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h.

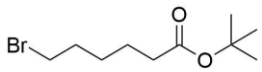
6-(6-Chlorohexoxy)hexane-1-ol (10)



This compound was prepared using the General Procedure **I**, 1,6-hexanediol (10.05 g, 375 mmol) and aqueous NaOH (50%, 19.3 mL, 375 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to give a colourless oil.

Yield (8.52 g, 48%); R_f = 0.39 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.24 – 1.51 (m, 14H, CH_2), 1.62 – 1.76 (m, 2H, CH_2), 3.30 – 3.39 (m, 6H, OCH_2), 3.60 (t, *J* = 6.6 Hz, 2H, CH_2Cl), 4.28 (t, *J* = 5.1 Hz, 1H, OH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.13, 25.50, 25.77, 26.23, 29.22, 29.44, 32.17, 32.66 (CH_2), 45.47 (CH_2Cl), 60.82, 69.93, 70.07 (OCH_2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 11.30 min, *m/z* $[M + H]^+$ calcd for $C_{12}H_{25}ClO_2$, 237.16; found, 237.1.

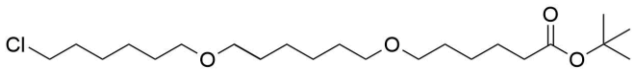
***tert*-Butyl 6-bromohexanoate (11)**



To 6-bromohexanoic acid (4.88 g, 25 mmol) in dry CH_2Cl_2 (25 mL) was added *t*BuOH (9.27 g, 11.7 mL, 125 mmol) at 0 °C followed by DMAP (0.31 g, 2.5 mmol). After 5 min, DCC (5.67 g, 27.5 mmol) was added to this solution. The solution was then left to warm to rt and stirred overnight. Subsequently, the mixture was filtered and then partitioned with H_2O (25 mL). The aqueous phase was extracted once more with CH_2Cl_2 (25 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The remaining residue was purified by column chromatography (petroleum ether/CH_2Cl_2 4:1) to give the title product as a colourless oil.

Yield (4.65 g, 74%); R_f = 0.35 (petroleum ether/CH_2Cl_2 3:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.31 – 1.44 (m, 4H, 4-H, CH_3), 1.44 – 1.55 (m, 2H, 3-H), 1.78 (dt, *J* = 6.8, 14.6 Hz, 2H, 5-H), 2.18 (t, *J* = 7.3 Hz, 2H, 2-H), 3.51 (t, *J* = 6.7 Hz, 2H, 6-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 23.88 (C-3), 27.07 (C-4), 27.90 (C($\underline{C}H_3)_3$), 32.02 (C-5), 34.74, 35.08 (C-1, C-6), 79.55 ($\underline{C}(CH_3)_3$), 172.25 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 11.64 min, *m/z* $[M + H]^+$ calcd for $C_{10}H_{19}{}^{79}BrO_2$, 251.06; found, 251.1.

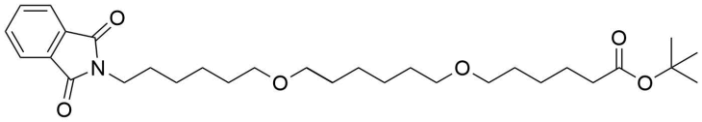
***tert*-Butyl 6-[6-(6-chlorohexoxy)hexoxy]hexanoate (12)**



This compound was prepared using the General Procedure **II** and *tert*-butyl 6-bromohexanoate (**11**, 10.05 g, 40 mmol) and 6-(6-chlorohexoxy)hexane-1-ol (**10**, 9.47 g, 40 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 10:1) to give a colourless oil.

Yield (5.05 g, 31%); R_f = 0.43 (petroleum ether/EtOAc 10:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.22 – 1.53 (m, 29H, CH_2, CH_3), 1.65 – 1.74 (m, 2H, CH_2), 2.15 (t, *J* = 7.3 Hz, 2H, CH_2), 3.23 – 3.35 (m, 8H, OCH_2), 3.60 (t, *J* = 6.6 Hz, 2H, CH_2Cl); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 24.62 (CH_2), 25.14 (CH_2), 25.36 (CH_2), 25.70 (2 × CH_2), 26.24 (CH_2), 27.90 (C($\underline{C}H_3)_3$), 29.04 (CH_2), 29.22 (CH_2), 29.35 (CH_2), 29.37 (CH_2), 32.18 (CH_2), 34.93 (CH_2), 45.44 (CH_2Cl), 69.89, 69.92, 70.01, 70.03 (OCH_2), 79.44 ($\underline{C}(CH_3)_3$), 172.35 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 12.99 min, *m/z* $[M + H]^+$ calcd for $C_{22}H_{43}ClO_4$, 407.32; found, 407.4; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd for $C_{22}H_{43}ClO_4$, 424.3188; found, 424.3180; diff., –1.89 ppm.

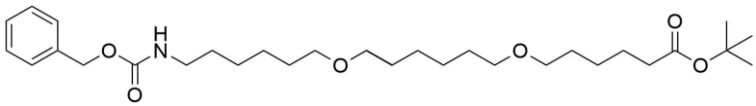
***tert*-Butyl 6-[6-[6-(1,3-dioxoisoindolin-2-yl)hexoxy]hexoxy]hexanoate (13)**



This compound was prepared using the General Procedure **III** and linker precursor **12** (4.07 g, 10 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 4:1) to give a colourless oil.

Yield (3.83 g, 74%); R_f = 0.57 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.20 – 1.34 (m, 10H, CH_2), 1.37 (s, 9H, CH_3), 1.39 – 1.51 (m, 10H, CH_2), 1.52 – 1.62 (m, 2H, CH_2), 2.14 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.24 – 3.32 (m, 8H, OCH_2), 3.55 (t, J = 7.1 Hz, 2H, NCH_2), 7.78 – 7.87 (m, 4H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 24.57, 25.30, 25.41, 25.63, 26.16 (CH_2), 27.84 ($C(\underline{C}H_3)_3$), 27.98, 28.99, 29.12, 29.28, 29.30 (CH_2), 34.87 ($CO\underline{C}H_2$), 37.44 (NCH_2), 69.84, 69.90 (OCH_2), 69.95 (2 × OCH_2), 79.37 ($\underline{C}(CH_3)_3$), 123.05 (C-4, C-7), 131.70 (C-3a, C-7a), 134.44 (C-5, C-6), 167.99 (C-1, C-3), 172.29 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 12.63 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{30}H_{47}NO_6$, 518.34; found, 518.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{30}H_{47}NO_6$, 518.3476; found, 518.3452; diff., –4.63 ppm.

***tert*-Butyl 6-[6-[6-(benzyloxycarbonylamino)hexoxy]hexoxy]hexanoate (L5)**

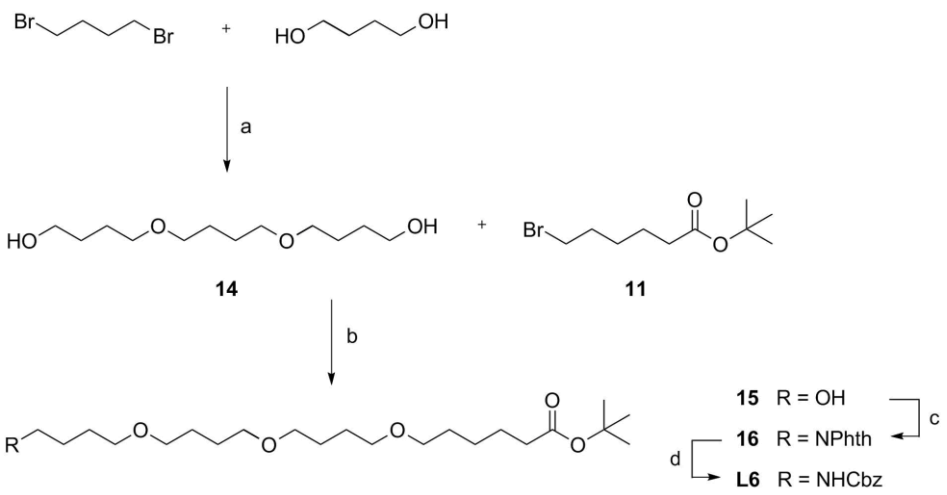


This compound was prepared using the General Procedure **IV** and linker precursor **13** (2.59 g, 5 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 8:1 to 6:1) to give a colourless oil.

Yield (1.10 g, 42%); R_f = 0.39 (petroleum ether/EtOAc 6:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.22 – 1.30 (m, 10H, CH_2), 1.35 – 1.40 (m, 11H, CH_2, CH_3), 1.42 – 1.51 (m, 10H, CH_2), 2.15 (t, J = 7.3 Hz, 2H, $COCH_2$), 2.93 – 2.99 (m, 2H, $NHC\underline{H}_2$), 3.28 – 3.31 (m, 8H, OCH_2), 4.99 (s, 2H, $NHCO_2C\underline{H}_2$), 7.18 (t, J = 5.3 Hz, 1H, NH), 7.26 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.65, 25.38, 25.58, 25.73, 26.23 (CH_2), 27.92 ($C(\underline{C}H_3)_3$), 29.06, 29.35, 29.38, 29.53 (CH_2), 34.94 ($CO\underline{C}H_2$), 65.21 ($NHCO_2\underline{C}H_2$), 69.92, 70.03 (OCH_2), 79.48 ($\underline{C}(CH_3)_3$), 127.83, 127.86, 128.47 (C-2, C-3, C-4), 137.50 (C-1), 156.24 (CONH), 172.40 (CO). A signal for one CH_2-group is missing (overlapping solvent peaks). **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 12.46 min,

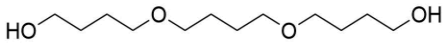
99% purity, *m/z* $[M + H]^+$ calcd for $C_{30}H_{51}NO_6$, 522.38; found, 522.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{30}H_{51}NO_6$, 522.3789; found, 522.3770; diff., –3.64 ppm.

Linker L6:



Reagents and conditions: (a) Sodium, 100 °C, 3 h; (b) toluene, 50% NaOH, TBAHS, rt, 18 h; (c) (i) MsCl, Et_3N, CH_2Cl_2, rt, 2 h; (ii) potassium phthalimide, DMF, 95 °C, 18 h; (d) (i) hydrazine hydrate, EtOH, 80 °C, 3 h; (ii) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h.

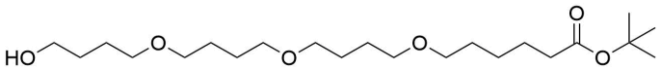
4-[4-(4-Hydroxybutoxy)butoxy]butan-1-ol (14)



This compound was synthesized similar to a previously reported procedure.[6] In brief, sodium (5.75 g, 250 mmol) was cut into small pieces, washed with hexanes (100 mL), and reacted under dissolution in 1,4-butanediol (45.1 g, 44 mL, 500 mmol) at 80 °C while stirring vigorously. The reaction mixture was heated to 100°C and 1,4-dibromobutane (21.6 g, 11.9 mL, 100 mmol) was added carefully and dropwise. The mixture was stirred from 3 h, after which it was cooled to rt. H_2O (50 mL) was carefully added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with half-saturated brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The yellow product was redissolved in Et_2O (200 mL) and recrystallized at –18 °C overnight. The ether was decanted, the product washed with cold Et_2O (50 mL), and redissolved in Et_2O (100 ml). The ether was evaporated *in vacuo* on a rotary evaporator without putting the flask in the water bath. This allowed the product to precipitate during drying and yielded a fine white powder.

Yield (11.95 g, 51%); mp 30 – 32 °C, lit. mp 33 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.37 – 1.54 (m, 12H, CH_2), 3.31 – 3.41 (m, 12H, OCH_2), 4.32 (t, J = 5.2 Hz, 2H, OH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 26.07, 26.26, 29.45 (CH_2), 60.74, 69.88, 70.05 (OCH_2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 195-400 nm), t_R = 8.37 min, *m/z* $[M + H]^+$ calcd for $C_{12}H_{26}O_4$, 235.19; found, 235.2.

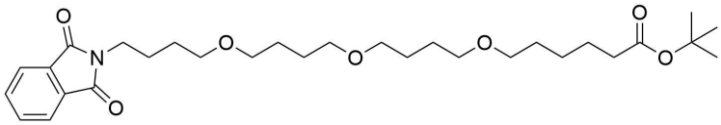
***tert*-Butyl 6-[4-[4-(4-hydroxybutoxy)butoxy]butoxy]hexanoate (15)**



This compound was prepared using the General Procedure **II** and *tert*-butyl 6-bromohexanoate (**11**, 10.05 g, 40 mmol) and 4-[4-(4-hydroxybutoxy)butoxy]butan-1-ol (**14**, 29.43 g, 100 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to give a colourless oil.

Yield (6.80 g, 42%); R_f = 0.29 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.23 – 1.31 (m, 2H, $CO(CH_2)_2CH_2$), 1.38 (s, 9H, CH_3), 1.41 – 1.54 (m, 16H, CH_2), 2.15 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.30 – 3.40 (m, 14H, OCH_2), 4.31 (t, J = 5.1 Hz, 1H, OH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 24.60, 25.33, 26.04, 26.19, 26.21 (CH_2), 27.87 ($C(\underline{C}H_3)_3$), 29.02, 29.41 (CH_2), 34.89 ($COCH_2$), 60.69 (CH_2OH), 69.84, 69.85, 70.01 (OCH_2), 79.42 ($\underline{C}(CH_3)_3$), 172.33 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 11.77 min, *m/z* $[M + H]^+$ calcd for $C_{22}H_{44}O_6$, 405.32; found, 405.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{22}H_{44}O_6$, 405.3211; found, 405.3213; diff., 0.49 ppm.

***tert*-Butyl 6-[4-[4-[4-(1,3-dioxoisoindolin-2-yl)butoxy]butoxy]butoxy]hexanoate (16)**

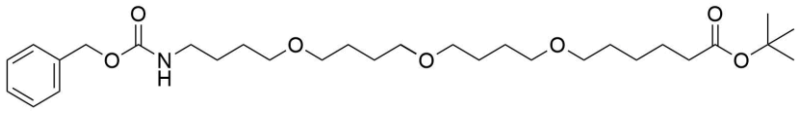


The linker precursor **15** (4.07 g, 10 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and Et_3N (2.02 g, 2.8 mL, 20 mmol) at 0 °C and methanesulfonyl chloride (1.49 g, 1.0 mL, 13 mmol) in dry CH_2Cl_2 (25 mL) was added dropwise. After complete addition, the mixture was stirred at rt for 2 h. The suspension was filtered, the colourless residue was washed with CH_2Cl_2 (25 mL) and the clear filtrate was transferred into a separation funnel. The organic layer was washed with 10% $KHSO_4$ solution

(50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give an orange oil. The crude mesylate intermediate was used without further purification. The envisaged phthalimide was then prepared using the General Procedure **III**. The crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to give a colourless oil.

Yield (3.42 g, 64%): R_f = 0.55 (PE/EtOAc 3:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.24 – 1.30 (m, 2H, CH_2), 1.37 (s, 9H, CH_3), 1.41 – 1.52 (m, 14H, CH_2), 1.58 – 1.66 (m, 2H, CH_2), 2.14 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.24 – 3.37 (m, 12H, OCH_2), 3.57 (t, J = 7.1 Hz, 2H, NCH_2), 7.77 – 7.89 (m, 4H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 24.61, 25.07, 25.34 (CH_2), 26.17 (2 × CH_2), 26.20, 26.21, 26.77 (CH_2), 27.88 ($C(\underline{C}H_3)_3$), 29.02 (CH_2), 34.90 ($COCH_2$), 37.42 (NCH_2), 69.44, 69.81, 69.83 (OCH_2), 69.85 (2 × OCH_2), 69.91 (OCH_2), 79.43 ($\underline{C}(CH_3)_3$), 123.09 (C-4, C-7), 131.75 (C-3a, C-7a), 134.47 (C-5, C-6), 168.05 (C-1, C-3), 172.34 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 12.21 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{30}H_{47}NO_7$, 534.34; found, 534.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{30}H_{47}NO_7$, 534.3425; found, 534.3403; diff., –4.12 ppm.

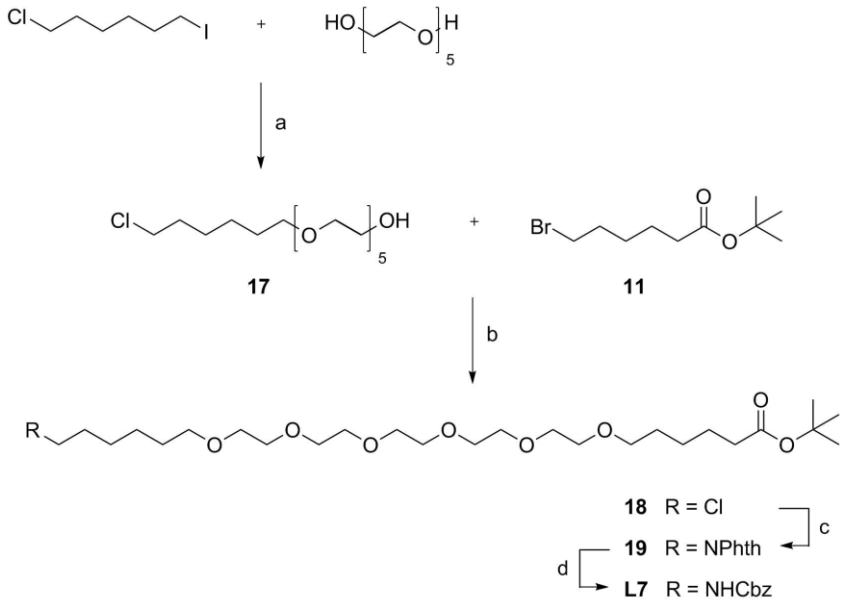
***tert*-Butyl 6-[4-[4-[4-(benzyloxycarbonylamino)butoxy]butoxy]butoxy]hexanoate (L6)**



This compound was prepared using the General Procedure **IV** and linker precursor **16** (2.67 g, 5 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colourless oil.

Yield (1.64 g, 61%); R_f = 0.65 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.23 – 1.31 (m, 2H, CH_2), 1.38 (s, 9H, CH_3), 1.40 – 1.51 (m, 16H, CH_2), 2.15 (t, J = 7.3 Hz, 2H $COCH_2$), 2.98 (q, J = 6.4 Hz, 2H, $NHC\underline{H}_2$), 3.26 – 3.34 (m, 12H, OCH_2), 4.99 (s, 2H, $NHCO_2C\underline{H}_2$), 7.20 (t, J = 5.7 Hz, 1H, NH), 7.26 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.63, 25.36, 26.23, 26.44, 26.72 (CH_2), 27.91 ($C(\underline{C}H_3)_3$), 29.05 (CH_2), 34.92 ($COCH_2$), 40.25 ($NHCH_2$), 65.22 ($NHCO_2\underline{C}H_2$), 69.73, 69.88 (OCH_2), 79.47 ($\underline{C}(CH_3)_3$), 127.83, 127.86, 128.47 (C-2, C-3, C-4), 137.47 (C-1), 156.23 (CONH), 172.39 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 12.14 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{30}H_{51}NO_7$, 538.37; found, 538.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{30}H_{51}NO_7$, 538.3738; found, 538.3710; diff., –5.20 ppm.

Linker L7:



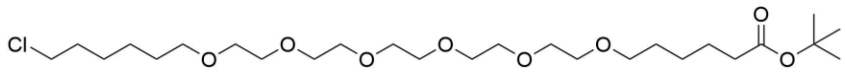
Reagents and conditions: (a) 50% NaOH, DMSO, rt, 24 h; (b) toluene, 50% NaOH, TBAHS, rt, 18 h; (c) potassium phthalimide, DMF, 95 °C, 18 h; (d) (i) hydrazine hydrate, EtOH, 80 °C, 3 h; (ii) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h.

2-[2-[2-[2-[2-(6-Chlorohexoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethanol (17)

This compound was prepared using the General Procedure **I**, pentaethylene glycol (36.98 g, 150 mmol), aqueous NaOH (50%, 7.7 mL, 150 mmol) and 1-chloro-6-iodohexane (18.49 g, 11.4 mL, 75 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 49:1) to give a greenish oil.

Yield (10.71 g, 40%): R_f = 0.26 (CH_2Cl_2/MeOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.25 – 1.41 (m, 4H, CH_2), 1.44 – 1.52 (m, 2H, CH_2), 1.65 – 1.73 (m, 2H, CH_2), 3.34 – 3.42 (m, 4H, OCH_2), 3.43 – 3.52 (m, 18H, OCH_2), 3.61 (t, J = 6.6 Hz, 2H, CH_2Cl), 4.54 (t, J = 5.4 Hz, 1H, OH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 25.09, 26.27, 29.22, 32.19 (CH_2), 45.52 (CH_2Cl), 60.39, 69.65, 69.94, 69.97, 69.98, 70.34, 72.51 (OCH_2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), t_R = 10.38 min, *m/z* $[M + H]^+$ calcd for $C_{16}H_{33}ClO_6$, 357.20; found, 356.8; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{16}H_{33}ClO_6$, 357.2038; found, 357.2028; diff. (ppm): –2.80 ppm.

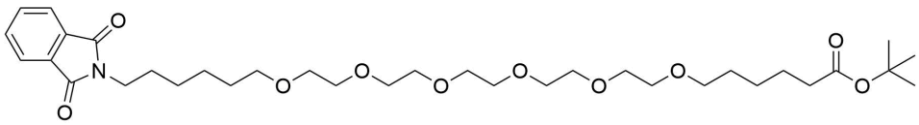
***tert*-Butyl 6-[2-[2-[2-[2-[2-(6-chlorohexoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]hexanoate (18)**



This compound was prepared using the General Procedure **II** and *tert*-butyl 6-bromohexanoate (**11**, 10.05 g, 40 mmol) and 2-[2-[2-[2-[2-(6-chlorohexoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethanol (**17**, 14.28 g, 40 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a colourless oil.

Yield (9.28 g, 44%); R_f = 0.35 (petroleum ether/EtOAc 1:2); **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 1.24-1.33 (m, 4H, CH_2), 1.34-1.40 (m, 2H, CH_2), 1.38 (s, 9H, CH_3), 1.43-1.51 (m, 6H, CH_2), 1.66-1.72 (m, 2H, CH_2), 2.16 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.33-3.39 (m, 4H, OCH_2), 3.43-3.47 (m, 4H, OCH_2), 3.47-3.52 (m, 16H, OCH_2), 3.60 (t, J = 6.6 Hz, 2H, CH_2Cl); **^{13}C-NMR** (151 MHz, DMSO-d_6) δ 24.63, 25.08, 25.28, 26.26 (CH_2), 27.92 (C($\underline{C}H_3$)$_3$), 29.03, 29.21, 32.18 (CH_2), 34.92 ($COCH_2$), 45.49 (CH_2Cl), 69.64, 69.66, 69.97, 69.98, 70.29, 70.33 (OCH_2), 79.48 ($\underline{C}(CH_3)_3$), 172.39 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH, DAD 200-400 nm), t_R = 12.33 min, *m/z* $[M + NH_4]^+$ calcd for $C_{28}H_{51}ClO_8$, 544.36; found 544.2; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{28}H_{51}ClO_8$, 559.3165; found, 549.3137; diff., –5.09 ppm.

***tert*-Butyl 6-[2-[2-[2-[2-[2-[6-(1,3-dioxoisoindolin-2-yl)hexoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]hexanoate (19)**

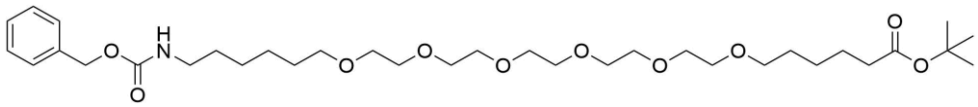


This compound was prepared using the General Procedure **III** and linker precursor **18** (4.07 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a colourless oil.

Yield (4.91 g, 77%); R_f = 0.24 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.22 – 1.32 (m, 6H, CH_2), 1.37 (s, 9H, CH_3), 1.42 – 1.50 (m, 6H, CH_2), 1.54 – 1.60 (m, 2H, CH_2), 2.15 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.34 (t, J = 6.5 Hz, 4H, OCH_2), 3.41 – 3.45 (m, 4H, OCH_2), 3.46 – 3.50 (m, 16H, OCH_2), 3.55 (t, J = 7.1 Hz, 2H, NCH_2) 7.81 – 7.87 (m, 4H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.62, 25.28, 25.39, 26.23 (CH_2), 27.91 (C($\underline{C}H_3$)$_3$), 28.04, 29.03, 29.16 (CH_2), 34.91 ($CO\underline{C}H_2$), 37.51 (NCH_2), 69.61, 69.63, 69.95, 69.97, 70.29, 70.35 (OCH_2), 79.48 ($\underline{C}(CH_3)_3$), 123.14 (C-4, C-7), 131.76 (C-3a, C-7a), 134.52 (C-5, C-6), 168.08 (C-1, C-3), 172.38 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in

10 min, then 100% MeOH, DAD 200-400 nm), t_R = 12.44 min, 99 % purity, *m/z* [M + NH_4]$^+$ calcd for $C_{34}H_{55}NO_{10}$, 655.42; found, 655.1; **HRMS** (ESI) *m/z* [M + NH_4]$^+$ calcd for $C_{34}H_{55}NO_{10}$, 655.4134; found, 655.4164; diff., 4.58 ppm.

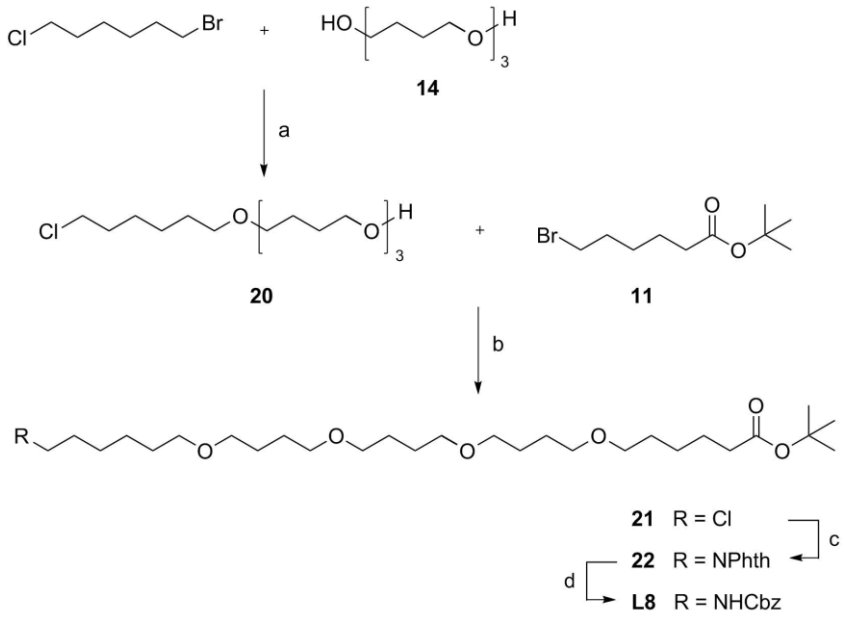
***tert*-Butyl 6-[2-[2-[2-[2-[2-[6-(benzyloxycarbonylamino)hexoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]hexanoate (L7)**



This compound was prepared using the General Procedure **IV** and linker precursor **19** (3.19 g, 5 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 1:2) to give a colourless oil.

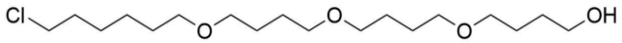
Yield (0.83 g, 26%); R_f = 0.19 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.18 – 1.30 (m, 6H, CH_2), 1.33 – 1.41 (m, 2H, CH_2), 1.38 (s, 9H, CH_3), 1.42 – 1.51 (m, 6H, CH_2), 2.15 (t, *J* = 7.3 Hz, 2H, $COCH_2$), 2.96 (q, *J* = 6.6 Hz, 2H, NHC$\underline{H}_2$), 3.34 (t, *J* = 6.6 Hz, 4H, OCH_2), 3.42 – 3.46 (m, 4H, OCH_2), 3.47 – 3.51 (m, 16H, OCH_2), 4.99 (s, 2H, OCH_2Ph), 7.18 (t, *J* = 5.7 Hz, 1H, CONH), 7.27 – 7.37 (m, 5H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.63, 25.29, 25.51, 26.23 (CH_2), 27.92 (C($\underline{C}H_3$)$_3$), 29.03, 29.31, 29.52 (CH_2), 34.92 (CO$\underline{C}H_2$), 65.21 (OCH_2Ph), 69.64, 69.96, 69.98, 70.30, 70.41 (OCH_2), 79.49 ($\underline{C}$(CH_3)$_3$), 127.84 (C-3'), 128.48 (C-2', C-4'), 137.49 (C-1'), 156.23 (CONH), 172.40 (CO); the signal for NH$\underline{C}H_2$ is missing (overlapping solvent peak); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH, DAD 200-400 nm), t_R = 12.28 min, 99% purity, *m/z* [M + NH_4]$^+$ calcd for $C_{34}H_{59}NO_{10}$, 659.45; found, 659.6; **HRMS** (ESI) *m/z* [M + NH_4]$^+$ calcd for $C_{34}H_{59}NO_{10}$, 659.4459; found, 659.4477; diff., 3.49 ppm.

Linker L8:



Reagents and conditions: (a) 50% NaOH, DMSO, rt, 24 h; (b) toluene, 50% NaOH, TBAHS, rt, 18 h; (c) potassium phthalimide, DMF, 95 °C, 18 h; (d) (i) hydrazine hydrate, EtOH, 80 °C, 3 h; (ii) Z-Cl, Et_3N, CH_2Cl_2, rt, 18 h.

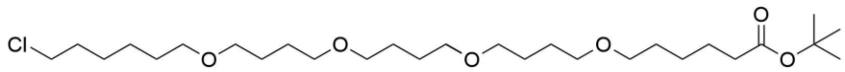
4-[4-[4-(6-Chlorohexoxy)butoxy]butoxy]butan-1-ol (20)



This compound was prepared using the General Procedure **I**, compound **14** (52.73 g, 225 mmol) and aqueous NaOH (50%, 11.6 mL, 225 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 1:1) to give a colourless oil.

Yield (10.59 g, 40%); R_f = 0.40 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.25 – 1.57 (m, 18H, CH_2), 1.65 – 1.74 (m, 2H, CH_2), 3.30 – 3.35 (m, 12H, OCH_2), 3.35 – 3.41 (m, 2H, OCH_2), 3.60 (t, J = 6.6 Hz, 2H, CH_2Cl), 4.30 (t, J = 5.2 Hz, 1H, OH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.13, 26.05 (CH_2), 26.22 (5 × CH_2), 29.21, 29.43, 32.16 (CH_2), 45.44 (CH_2Cl), 60.70 (OCH_2), 69.85 (2 × OCH_2), 69.89, 70.03 (OCH_2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 195-400 nm), t_R = 12.06 min, *m/z* $[M + H]^+$ calcd for $C_{18}H_{37}ClO_4$, 353.24; found, 352.9; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{37}ClO_4$, 353.2453; found, 353.2456; diff., 0.85 ppm.

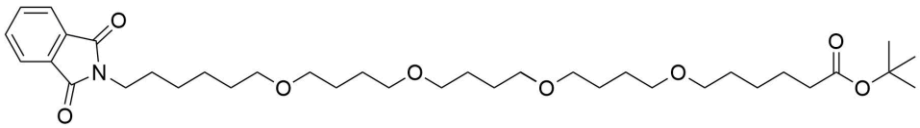
***tert*-Butyl 6-[4-[4-[4-(6-chlorohexoxy)butoxy]butoxy]butoxy]hexanoate (21)**



This compound was prepared using the General Procedure **II** and *tert*-butyl 6-bromohexanoate (**11**, 10.05 g, 40 mmol) and 4-[4-[4-(6-chlorohexoxy)butoxy]butoxy]butan-1-ol (**20**, 14.12 g, 40 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 8:1 to 1:1) to give a colourless oil.

Yield (6.49 g, 31%); R_f = 0.33 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.23 – 1.34 (m, 6H, CH_2), 1.38 (s, 9H, CH_3), 1.41 – 1.52 (m, 18H, CH_2), 1.66 – 1.72 (m, 2H, CH_2), 2.15 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.28 – 3.35 (m, 16H, OCH_2), 3.60 (t, J = 6.6 Hz, 2H, CH_2Cl); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.64, 25.17, 25.37, 26.24 (CH_2), 27.90 ($C(\underline{C}H_3)_3$), 29.07, 29.25, 32.19 (CH_2), 34.93 ($CO\underline{C}H_2$), 45.45 (CH_2Cl), 69.92 (OCH_2), 79.45 ($\underline{C}(CH_3)_3$), 172.36 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH, DAD 200-400 nm), t_R = 13.54 min, *m/z* $[M + H]^+$ calcd for $C_{28}H_{55}ClO_6$, 523.38; found, 523.5; **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{28}H_{55}ClO_6$, 523.3744; found, 523.3760; diff., 3.06 ppm.

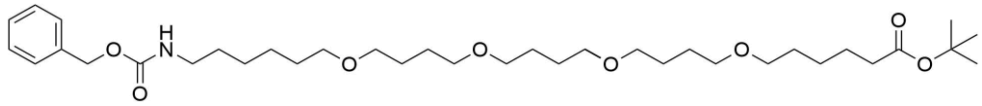
***tert*-Butyl 6-[4-[4-[4-[6-(1,3-dioxoisoindolin-2-yl)hexoxy]butoxy]butoxy]butoxy]hexanoate (22)**



This compound was prepared using the General Procedure **III** and linker precursor **21** (4.07 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colourless oil.

Yield (2.51 g, 48%); R_f = 0.23 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.22 – 1.33 (m, 6H, CH_2), 1.37 (s, 9H, CH_3), 1.41 – 1.51 (m, 18H, CH_2), 1.53 – 1.60 (m, 2H, CH_2), 2.14 (t, J = 7.3 Hz, 2H, $COCH_2$), 3.26 – 3.34 (m, 16H, OCH_2), 3.54 (t, J = 7.1 Hz, 2H, NCH_2), 7.79 – 7.86 (m, 4H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.63, 25.36, 25.46, 26.22, 26.23 (CH_2), 27.88 ($C(\underline{C}H_3)_3$), 28.04, 29.05, 29.18 (CH_2), 34.91 ($CO\underline{C}H_2$), 37.48 (NCH_2), 69.85, 69.88, 69.94 (OCH_2), 79.42 ($\underline{C}(CH_3)_3$), 123.10 (C-4, C-7), 131.74 (C-3a, C-7a), 134.49 (C-5, C-6), 168.03 (C-1, C-3), 172.33 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH, DAD 220-400 nm), t_R = 13.59 min, 94 % purity, *m/z* $[M + H^+]$ calcd for $C_{36}H_{59}NO_8$, 634.43; found, 634.6; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{36}H_{59}NO_8$, 634.4298; found, 634.4313; diff., 3.36 ppm.

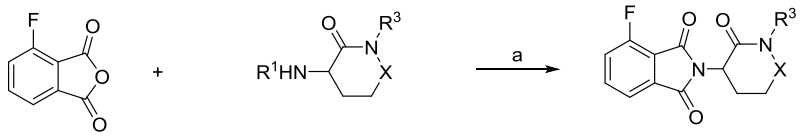
***tert*-Butyl 6-[4-[4-[4-[6-(benzyloxycarbonylamino)hexoxy]butoxy]butoxy]butoxy]hexanoate (L8)**



This compound was prepared using the General Procedure **IV** and linker precursor **22** (3.17 g, 5 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colourless oil.

Yield (0.86 g, 27%); R_f = 0.20 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.20 – 1.31 (m, 6H, CH_2), 1.35 – 1.41 (m, 2H, CH_2), 1.38 (s, 9H, CH_3), 1.42 – 1.47 (m, 4H, CH_2), 1.47 – 1.52 (m, 14H, CH_2), 2.15 (t, *J* = 7.3 Hz, 2H, $COCH_2$), 2.96 (q, *J* = 6.6 Hz, 2H, $NHC\underline{H}_2$), 3.28 – 3.35 (m, 16H, CH_2), 4.99 (s, 2H, OCH_2Ph), 7.18 (t, *J* = 5.7, 1H, CONH), 7.27 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.63, 25.36, 25.57 26.23 (CH_2), 27.90 ($C(\underline{C}H_3)_3$), 29.06, 29.33, 29.51 (CH_2), 34.92 ($CO\underline{C}H_2$), 65.19 (OCH_2Ph), 69.86 (OCH_2), 79.45 ($\underline{C}(CH_3)_3$), 127.82 (C-3'), 128.45 (C-2', C-4'), 137.49 (C-1'), 156.21 (CONH), 172.36 (CO); the signal for $NH\underline{C}H_2$ is missing (overlapping solvent peak); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH, DAD 200-400 nm), t_R = 13.34 min, 96 % purity, *m/z* $[M + H]^+$ calcd for $C_{36}H_{63}NO_8$, 638.46; found, 638.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{36}H_{63}NO_8$, 638.4629; found, 638.4626; diff., –0.47 ppm.

G. Synthesis of the CRBN ligands C1-C3

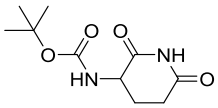


23 R^1 = Boc; X = CO; R^3 = H
24 R^1 = Boc; X = CO; R^3 = CH_3
25 R^1 = H; X = CH_2; R^3 = H

C1 X = CO; R^3 = H
C2 X = CO; R^3 = CH_3
C3 X = CH_2; R^3 = H

Reagents and conditions: (a) NaOAc, AcOH, reflux, 4–6 h.

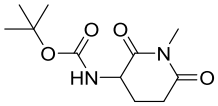
tert-Butyl *N*-(2,6-dioxo-3-piperidyl)carbamate (23)



This compound was synthesized similar to a previously reported procedure.[1] In brief, to a mixture of Boc-Gln-OH (2.46 g, 10 mmol) in THF (50 mL) was added 1,1'-carbonyldiimidazole (1.95 g, 12 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (5 mg). Subsequently, it was heated at reflux for 10 h until a clear solution was formed. After evaporation of the solvent, EtOAc (200 mL) was added to the residue and the mixture was washed with H_2O (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and eluted with EtOAc through a short pad of silica gel. Evaporation of the solvent and drying *in vacuo* gave a colourless solid.

Yield (1.92 g, 84%); R_f = 0.77 (EtOAc).

tert-Butyl *N*-(1-methyl-2,6-dioxo-3-piperidyl)carbamate (24)

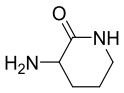


This compound was synthesized similar to a previously reported procedure.[1] In brief, **23** (2.28 g, 10 mmol) was dissolved in DMF (25 mL) and milled potassium carbonate (2.76 g, 20 mmol) and methyl iodide (1.42 g, 0.62 mL, 10 mmol) were added. The reaction mixture was ultrasonicated for 2 h. After dilution with EtOAc (100 mL), the red mixture was washed with 1N NaOH (2 × 25 mL), H_2O (25 mL) and brine (25 mL). The colourless solution was dried (Na_2SO_4), filtrated and evaporated. The

residue was purified by column chromatography (petroleum ether/EtOAc 2:1) to afford a colourless solid.

Yield (0.70 g, 29%); R_f = 0.34 (petroleum ether/EtOAc 2:1).

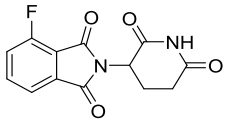
3-Aminopiperidin-2-one (25)



This compound was synthesized as reported previously.[7] In brief, L-ornithine hydrochloride (10 g, 59 mmol) was stirred together with a solution of NaOH pellets (2.38 g, 59 mmol) in H_2O (10 mL) for 10 min. Aluminium oxide was suspended in toluene (100 mL) and the aqueous solution was added via syringe. After refluxing the combined mixture for 3 h and collecting the water with a Dean-Stark apparatus, the mixture was cooled to rt, the alumina was filtered and it was washed with 10% MeOH in CH_2Cl_2 (30 mL). The filtrate was evaporated *in vacuo* to give a yellow oil.

Yield (3.87 g, 57%); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 – 1.47 (m, 1H, 4-H), 1.57 – 1.68 (m, 1H, 5-H), 1.71 – 1.79 (m, 1H, 5-H), 1.90 – 1.98 (m, 1H, 4-H), 3.03 – 3.10 (m, 3H, 3-H, 6-H), 7.34 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 21.14 (C-5), 29.77 (C-4), 41.62 (C-6), 51.22 (C-3), 173.87 (C-2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 198-400 nm), t_R = 0.71 min, *m/z* $[M + H]^+$ calcd for $C_5H_{10}N_2O$, 115.08; found, 114.8; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_5H_{10}N_2O$, 115.0866; found, 115.0869; diff., 2.61 ppm.

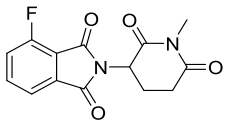
4-Fluoro-thalidomide (C1)



This compound was synthesized similar to a previously reported procedure.[1] In brief, a mixture of 3-fluorophthalic anhydride (1.25 g, 7.5 mmol) and **23** (1.14 g, 5 mmol) and a solution of sodium acetate (0.50 g, 6.0 mmol) in glacial acetic acid (20 mL) was refluxed for 6 h. After cooling, it was poured onto H_2O (100 mL) and the solid formed was collected by filtration, washed with H_2O (3 × 5 mL) and petroleum ether (3 × 5 mL). The purple solid was further dried *in vacuo*.

Yield (1.23 g, 89%); R_f = 0.67 (petroleum ether/EtOAc 1:2).

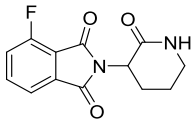
4-Fluoro-2-(1-methyl-2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (C2)



This compound was synthesized similar to a previously reported procedure.[1] In brief, A mixture of 3-fluorophthalic anhydride (1.25 g, 7.5 mmol) and **24** (1.21 g, 5 mmol) and a solution of sodium acetate (0.50 g, 6.0 mmol) in glacial acetic acid (20 mL) was refluxed for 6 h. After cooling, it was poured onto H_2O (100 mL) and the solid formed was collected by filtration, washed with H_2O (3 × 5 mL) and petroleum ether (3 × 5 mL). The purple solid was further dried *in vacuo*.

Yield (1.19 g, 82%); R_f = 0.55 (petroleum ether/EtOAc 1:1).

4-Fluoro-2-(2-oxo-3-piperidyl)isoindoline-1,3-dione (C3)



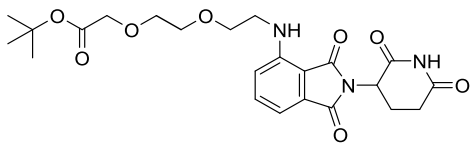
3-fluorophthalic anhydride (1.25 g, 7.5 mmol) and **25** (0.57 g, 5 mmol) and a solution of sodium acetate (0.50 g, 6.0 mmol) in glacial acetic acid (20 mL) was refluxed for 4 h. After cooling, it was poured onto H_2O (100 mL) and the solid formed was collected by filtration, washed with H_2O (3 × 5 mL) and petroleum ether (3 × 5 mL). The colourless solid was further dried *in vacuo*.

Yield (0.85 g, 65%); mp >250 °C; R_f = 0.16 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.82 – 1.95 (m, 2H, 4'-H, 5'-H), 1.96 – 2.04 (m, 1H, 5'-H), 2.15 – 2.25 (m, 1H, 4'-H), 3.16 – 3.27 (m, 2H, 6'-H), 4.59 (dd, *J* = 6.4, 11.9 Hz, 1H, 3'-H), 7.65 – 7.76 (m, 2H), 7.83 – 7.94 (m, 2H, Ar-H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 21.78 (C-5'), 25.71 (C-4'), 41.55 (C-6'), 49.35 (C-3'), 117.28 (d, ^2J (C, F) = 12.7 Hz), 122.98 (d, ^2J (C, F) = 19.6 Hz, C-3a, C-5), 120.00 (d, ^4J (C, F) = 2.7 Hz, C-7), 133.83, 138.02 (d, ^3J (C, F) = 7.8 Hz, C-6, C-7a), 156.93 (d, ^1J (C, F) = 262 Hz, C-4), 164.40 (C-1), 166.53 (d, ^3J (C, F) = 2.2 Hz, C-3), 166.91 (C-2'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 210-400 nm), t_R = 5.73 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{13}H_{11}FN_2O_3$, 263.08; found, 262.8; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{13}H_{11}FN_2O_3$, 285.0646; found, 285.0642; diff., –1.40 ppm.

H. Synthesis of the Linker-conjugated CRBN ligands CL1-CL10

General Procedure V: Nucleophilic aromatic substitution reaction. The corresponding orthogonal protected linker **5** (1 mmol) was dissolved in dry EtOAc (10 mL) and treated with 10% Pd/C (10% m/m). The reaction mixture was stirred under H_2 (1 atm, balloon) overnight. The mixture was filtered through celite and the filtrate was concentrated. The oily residue was redissolved in dry DMSO (10 mL) and DIPEA (2 mmol, 0.34 mL) and the corresponding CRBN Ligand **C** (1 mmol) were added. The mixture was stirred at 90 °C for 18 h. After cooling, it was poured onto half-saturated brine (100 mL) and it was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with 5% aqueous LiCl solution (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated.

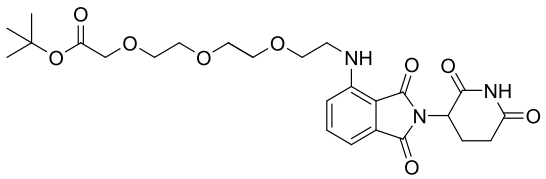
***tert*-Butyl 2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy] acetate (CL1)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L1**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a yellow solid.

Yield (0.14 g, 30%); mp 66 – 68 °C; R_f = 0.23 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.40 (s, 9H, CH_3), 1.98 – 2.06 (m, 1H, 4'-H), 2.45 – 2.63 (m, 2H, 4'-H, 5'-H), 2.80 – 2.95 (m, 1H, 5'-H), 3.46 (q, J = 5.6 Hz, 2H, OCH_2), 3.58 (s, 4H, OCH_2), 3.62 (t, J = 5.5 Hz, 2H, NHC$\underline{H}_2$), 3.96 (s, 2H, OCH_2), 5.04 (dd, J = 5.5, 12.7 Hz, 1H, 3'-H), 6.59 (t, J = 5.8 Hz, 1H, N$\underline{H}CH_2$), 7.03 (d, J = 7.0 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 7.1, 8.6 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.27 (C-4'), 27.88 (C($\underline{C}H_3$)$_3$), 31.11 (C-5'), 41.87 ($NHCH_2$), 48.71 (C-3'), 68.33, 69.04, 69.76, 70.06 (OCH_2), 80.75 ($\underline{C}(CH_3)_3$), 109.41 (C-3a), 110.79 (C-7), 117.56 (C-5), 132.23 (C-7a), 136.33 (C-6), 146.56 (C-4), 167.41 (C-1), 169.05 (C-3), 169.46 (C-2'), 170.13 (C-6'), 172.86 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 8.24 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{29}N_3O_8$, 476.20; found, 476.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{29}N_3O_8$, 476.2027; found, 476.2009; diff., –3.78 ppm.

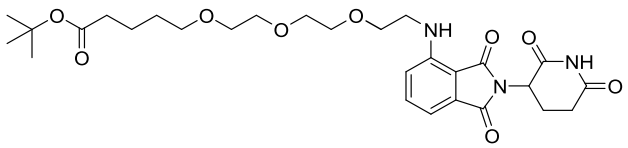
***tert*-Butyl 2-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy] ethoxy]ethoxy]acetate (CL2)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L2**. The crude product was purified by column chromatography (EtOAc) to give a yellow oil.

Yield (0.14 g, 27%); R_f = 0.73 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.39 (s, 9H, CH_3), 1.98 – 2.05 (m, 1H, 4'-H), 2.46 – 2.62 (m, 2H, 4'-H, 5'-H), 2.82 – 2.92 (m, 1H, 5'-H), 3.46 (q, J = 5.6 Hz, 2H, OCH_2), 3.50 – 3.57 (m, 8H, OCH_2), 3.61 (t, J = 5.5 Hz, 2H, NHC$\underline{H}_2$), 3.95 (s, 2H, OCH_2), 5.04 (dd, J = 5.5, 12.9 Hz, 1H, 3'-H), 6.59 (t, J = 5.8 Hz, 1H, N$\underline{H}CH_2$), 7.03 (d, J = 7.0 Hz, 1H), 7.13 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 7.1, 8.5 Hz, 1H, 6-H), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.31 (C-4'), 27.92 (C($\underline{C}H_3$)$_3$), 31.15 (C-5'), 41.88 ($NHCH_2$), 48.74 (C-3'), 68.30, 69.07, 69.92, 70.04 (OCH_2), 80.78 ($\underline{C}(CH_3)_3$), 109.44 (C-3a), 110.83 (C-7), 117.61 (C-5), 132.27 (C-7a), 136.38 (C-6), 146.59 (C-4), 167.45 (C-1), 169.10 (C-3), 169.52 (C-2'), 170.20 (C-6'), 172.94 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-450 nm), t_R = 8.27 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{25}H_{33}N_3O_9$, 520.23; found, 520.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{25}H_{33}N_3O_9$, 520.2290; found, 520.2303; diff., 2.50 ppm.

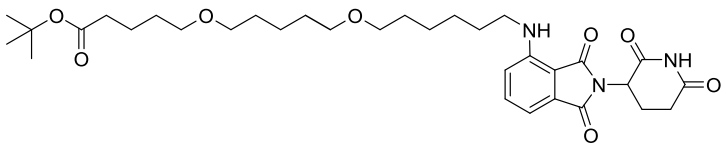
***tert*-Butyl 5-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy] ethoxy]pentanoate (CL3)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L3**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a yellow oil.

Yield (0.35 g, 62%); R_f = 0.32 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.37 (s, 9H, CH_3), 1.40 – 1.54 (m, 4H, CH_2), 1.96 – 2.06 (m, 1H, 4'-H), 2.13 – 2.19 (m, 2H, CH_2), 2.45 – 2.62 (m, 2H, 4'-H, 5'-H), 2.79 – 2.93 (m, 1H, 5'-H), 3.25 – 3.38 (m, 2H), 3.40 – 3.59 (m, 10H, OCH_2), 3.62 (t, J = 5.4 Hz, 2H, NHC$\underline{H}_2$), 5.04 (dd, J = 5.4, 12.7 Hz, 1H, 3'-H), 6.58 (t, J = 5.8 Hz, 1H, N$\underline{H}$CH$_2$), 7.03 (d, J = 7.1 Hz, 1H), 7.13 (d, J = 8.7 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 7.1 Hz, 8.6, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.61 (CH_2), 22.32 (C-4'), 27.93 (C($\underline{C}H_3$)$_3$), 28.63 (CH_2), 31.14 (C-5'), 34.66 (CH_2), 41.91 ($NHCH_2$), 48.75 (C-3'), 69.08, 69.61, 69.96 (OCH_2), 70.00 (3 × OCH_2), 79.53 ($\underline{C}(CH_3)_3$), 109.45 (C-3a), 110.83 (C-7), 117.61 (C-5), 132.26 (C-7a), 136.37 (C-6), 146.61 (C-4), 167.45 (C-1), 169.09 (C-3), 170.17 (C-2'), 172.39 (C-6'), 172.91 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 9.00 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{28}H_{39}N_3O_9$, 562.27; found, 562.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{28}H_{39}N_3O_9$, 562.2759; found, 562.2742; diff., –3.02 ppm.

***tert*-Butyl 5-[5-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy] pentoxy]pentanoate (CL4)**

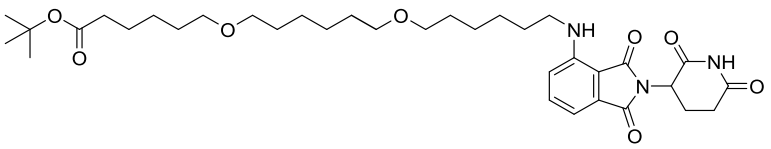


This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L4**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 1:1) to give a yellow oil.

Yield (0.31 g, 51%); R_f = 0.50 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.27 – 1.35 (m, 6H, CH_2), 1.37 (s, 9H, CH_3), 1.41 – 1.53 (m, 10H, CH_2), 1.53 – 1.61 (m, 2H, CH_2), 1.95 – 2.06

(m, 1H, 4'-H), 2.16 (t, J = 7.1 Hz, 2H, CH_2), 2.45 – 2.62 (m, 2H, 4'-H, 5'-H), 2.80 – 2.94 (m, 1H, 5'-H), 3.19 – 3.43 (m, 10H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, J = 5.4, 12.7 Hz, 1H, 3'-H), 6.49 (t, J = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.00 (d, J = 7.0 Hz, 1H), 7.07 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.56 (dd, J = 7.0, 8.6 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO) δ 21.71 (CH_2), 22.33 (C-4'), 22.67, 25.62, 26.29 (CH_2), 27.92 (C($\underline{C}H_3$)$_3$), 28.67, 28.83 (CH_2), 29.19 (2 × CH_2), 29.32 (CH_2), 31.14 (C-5'), 34.69 (CH_2), 41.99 ($NHCH_2$), 48.73 (C-3'), 69.65, 70.00, 70.02, 70.06 (OCH_2), 79.51 ($\underline{C}(CH_3)_3$), 109.22 (C-3a), 110.55 (C-7), 117.32 C-5), 132.36 (C-7a), 136.43 (C-6), 146.63 (C-4), 167.46 (C-1), 169.13 (C-3), 170.19 (C-2'), 172.38 (C-6'), 172.93 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 11.51 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{33}H_{49}N_3O_8$, 616.36; found, 616.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{33}H_{49}N_3O_8$, 616.3592; found, 616.3588; diff., –0.65 ppm.

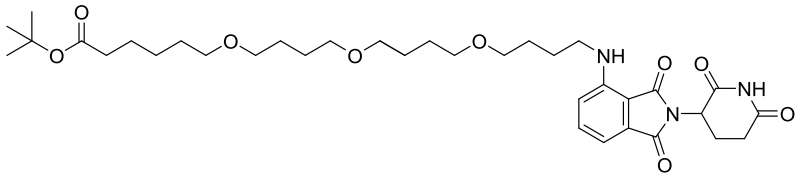
***tert*-Butyl 6-[6-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy]hexoxy] hexanoate (CL5)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L5**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to give a yellow oil.

Yield (0.30 g, 47%); R_f = 0.51 (CH_2Cl_2/EtOH 29:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.24 – 1.35 (m, 10H, CH_2), 1.37 (s, 9H, CH_3), 1.39 – 1.52 (m, 10H, CH_2), 1.52 – 1.61 (m, 2H, CH_2), 1.98 – 2.07 (m, 1H, 4'-H), 2.14 (t, J = 7.3 Hz, 2H, CH_2), 2.44 – 2.62 (m, 2H, 4'-H, 5'-H), 2.81 – 2.94 (m, 1H, 5'-H), 3.23 – 3.40 (m, 10H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, J = 5.4, 12.7 Hz, 1H), 6.49 (t, J = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.00 (d, J = 7.0 Hz, 1H), 7.07 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.56 (dd, J = 7.1, 8.6 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO) δ 22.32 (C-4'), 24.63, 25.36, 25.61 (CH_2), 25.70 (2 × CH_2), 26.28 (CH_2), 27.91 (C($\underline{C}H_3$)$_3$), 28.82, 29.04, 29.31 (CH_2), 29.36 (2 × CH_2), 31.13 (C-5'), 34.93 (CO$\underline{C}H_2$), 41.97 ($NHCH_2$), 48.72 (C-3'), 69.90, 69.98, 70.01, 70.03 (OCH_2), 79.46 ($\underline{C}(CH_3)_3$), 109.21 (C-3a), 110.53 (C-7), 117.30 (C-5), 132.35 (C-7a), 136.41 (C-6), 146.61 (C-4), 167.44 (C-1), 169.11 (C-3), 170.17 (C-2'), 172.38 (C-6'), 172.90 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 12.25 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{35}H_{53}N_3O_8$, 644.39; found, 644.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{35}H_{53}N_3O_8$, 644.3905; found, 644.3917; diff., 1.86 ppm.

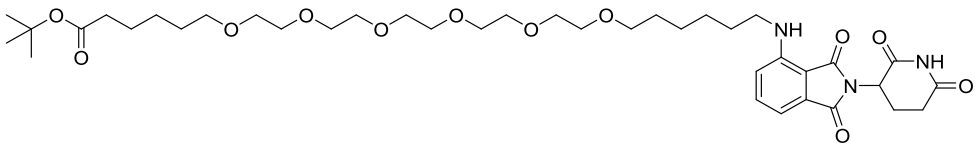
***tert*-Butyl 6-[4-[4-[4-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]butoxy]butoxy] butoxy]hexanoate (CL6)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L6**. The crude product was purified by column chromatography (CH_2Cl_2/EtOH 29:1) to give a yellow oil.

Yield (0.22 g, 34%); R_f = 0.42 (CH_2Cl_2/EtOH 29:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.23 – 1.30 (m, 2H, CH_2), 1.37 (s, 9H, CH_3), 1.41 – 1.52 (m, 12H, CH_2), 1.53 – 1.64 (m, 4H, CH_2), 1.97 – 2.05 (m, 1H, 4'-H), 2.12 – 2.18 (m, 2H, CH_2), 2.50 – 2.61 (m, 2H, 4'-H, 5'-H), 2.82 – 2.92 (m, 1H, 5'-H), 3.27 – 3.30 (m, 4H), 3.31 – 3.40 (m, 10H, OCH_2, $NHC\underline{H}_2$), 5.03 (dd, J = 5.4, 12.8 Hz, 1H, 3'-H), 6.54 (t, J = 6.0 Hz, 1H, $N\underline{H}CH_2$), 7.00 (d, J = 7.0 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H, 5-H, 7-H), 7.56 (dd, J = 7.1, 8.5 Hz, 1H, 6-H), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.30 (C-4'), 24.62, 25.34, 25.75, 26.23, 26.65 (CH_2), 27.89 ($C(\underline{C}H_3)_3$), 29.03 (CH_2), 31.12 (C-5'), 34.90 (CH_2), 41.77 ($NHCH_2$), 48.69 (C-3'), 69.69, 69.85, 69.86, 69.94 (OCH_2), 79.45 ($\underline{C}(CH_3)_3$), 109.21 (C-3a), 110.50 (C-7), 117.30 (C-5), 132.36 (C-7a), 136.36 (C-6), 146.55 (C-4), 167.43 (C-1), 169.06 (C-3), 170.18 (C-2'), 172.38 (C-6'), 172.90 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 10.97 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{35}H_{53}N_3O_9$, 660.38; found, 660.6; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{35}H_{53}N_3O_9$, 660.3855; found, 660.3849; diff., –0.91 ppm.

***tert*-Butyl 6-[2-[2-[2-[2-[2-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]hexanoate (CL7)**

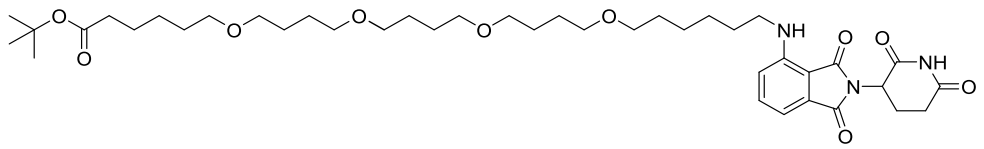


This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L7**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to EtOAc) to give a yellow oil.

Yield (0.33 g, 43%); R_f = 0.67 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.24 – 1.36 (m, 6H, CH_2), 1.37 (s, 9H, CH_3), 1.41 – 1.62 (m, 8H, CH_2), 1.98 – 2.05 (m, 1H, 4'-H), 2.15 (t, J = 7.3 Hz, 2H, CH_2), 2.43 –

2.65 (m, 2H, 4‘-H, 5‘-H), 2.77 – 2.98 (m, 1H, 5‘-H), 3.23 – 3.32 (m, 2H), 3.32 – 3.40 (m, 4H), 3.42 – 3.46 (m, 4H), 3.47 – 3.52 (m, 16H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H, 3‘-H), 6.50 (t, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, *J* = 7.1, 8.6 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO) δ 22.30 (C-4‘), 24.59, 25.25, 25.53, 26.27 (CH_2), 27.90 (C($\underline{C}H_3)_3$), 28.80, 29.00, 29.26 (CH_2), 31.11 (C-5‘), 34.89 (CH_2), 41.96 ($NHCH_2$), 48.70 (C-3‘), 69.61 (2 × OCH_2), 69.93 (6 × OCH_2), 70.26, 70.36 (OCH_2), 79.46 ($\underline{C}(CH_3)_3$), 109.19 (C-3a), 110.51 (C-7), 117.30 (C-5), 132.34 (C-7a), 136.40 (C-6), 146.59 (C-4), 167.43 (C-1), 169.09 (C-3), 170.17 (C-2’), 172.36 (C-6’), 172.89 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 10.45 min, 99% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{39}H_{61}N_3O_{12}$, 781.46; found, 781.4; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd for $C_{39}H_{61}N_3O_{12}$, 781.4593; found, 781.4560; diff., –4.22 ppm.

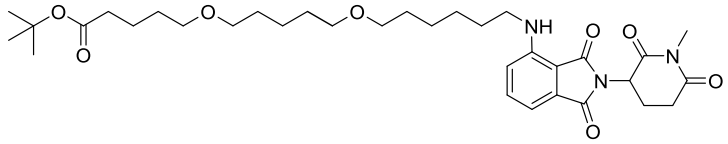
***tert*-Butyl 6-[4-[4-[4-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy]butoxy] butoxy]butoxy]hexanoate (CL8)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C1** (0.28 g) and linker **L8**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 1:1) to give a yellow oil.

Yield (0.41 g, 54%); R_f = 0.61 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.22 – 1.35 (m, 6H, CH_2), 1.37 (s, 9H, CH_3), 1.42 – 1.51 (m, 18H), 1.52 – 1.60 (m, 2H, CH_2), 1.97 – 2.05 (m, 1H, 4‘-H), 2.15 (t, *J* = 7.3 Hz, 2H, CH_2), 2.50 – 2.61 (m, 2H, 4‘-H, 5‘-H), 2.82 – 2.92 (m, 1H, 5‘-H), 3.23 – 3.37 (m, 18H, OCH_2), 5.03 (dd, *J* = 5.4, 12.8 Hz, 1H, 3‘-H), 6.50 (t, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.53 – 7.59 (m, 1H, 6-H), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO) δ 22.64 (C-4‘), 24.96, 25.68, 25.95, 26.56 (6 × CH_2), 26.61, 28.22 (C($\underline{C}H_3)_3$), 29.14, 29.38, 29.64, 31.45 (C-5‘), 35.24 (CH_2), 42.29 ($NHCH_2$), 49.03 (C-3‘), 70.18, 70.20, 70.22 (7 × OCH_2), 70.29 (OCH_2), 79.78 ($\underline{C}(CH_3)_3$), 109.52 (C-3a), 110.84 (C-7), 117.61 (C-5), 132.66 (C-7a), 136.72 (C-6), 146.92 (C-4), 167.76 (C-1), 169.42 (C-3), 170.50 (C-2’), 172.69 (C-6’), 173.23 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 12.79 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{41}H_{65}N_3O_{10}$, 760.47; found, 760.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{41}H_{65}N_3O_{10}$, 760.4743; found, 760.4728; diff., –1.97 ppm.

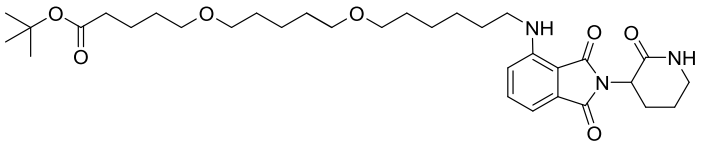
***tert*-Butyl 5-[5-[6-[[2-(1-methyl-2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy] pentoxy]pentanoate (CL9)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C2** (0.29 g) and linker **L5**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a yellow oil.

Yield (0.32 g, 50%); R_f = 0.36 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.25 – 1.34 (m, 6H, CH_2), 1.37 (s, 9H, $C(CH_3)_3$), 1.41 – 1.53 (m, 10H, CH_2), 1.53 – 1.60 (m, 2H, CH_2), 2.00 – 2.06 (m, 1H, 4'-H), 2.16 (t, J = 7.1 Hz, 2H, CH_2), 2.50 – 2.57 (m, 1H, 4'-H), 2.71 – 2.77 (m, 1H, 5'-H), 2.89 – 2.97 (m, 1H, 5'-H), 3.00 (s, 3H, NCH_3), 3.20 – 3.40 (m, 10H, OCH_2, $NHC\underline{H}_2$), 5.10 (dd, J = 5.4, 13.0 Hz, 1H, 3'-H), 6.50 (t, J = 5.9 Hz, 1H, $N\underline{H}CH_2$), 7.01 (d, J = 7.0 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 7.1, 8.6 Hz, 1H, 6-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 21.56 (C-4'), 21.71, 22.68, 25.62, 26.29 (CH_2), 26.75 (CH_3), 27.92 ($C(\underline{C}H_3)_3$), 28.67, 28.82 (CH_2), 29.19 (2 × CH2), 29.32 (CH_2), 31.28 (C-5'), 34.68 ($COCH_2$), 41.98 ($NHCH_2$), 49.28 (C-3'), 69.66, 69.99, 70.01, 70.06 (OCH_2), 79.52 ($\underline{C}(CH_3)_3$), 109.16 (C-3a), 110.57 (C-7), 117.36 (C-5), 132.35 (C-7a), 136.47 (C-6), 146.65 (C-4), 167.44 (C-1), 169.10 (C-3), 169.97 (C-2'), 171.96 (C-6'), 172.39 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 12.38 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{34}H_{51}N_3O_8$, 630.37; found, 630.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{34}H_{51}N_3O_8$, 630.3749; found, 630.3738; diff., –1.74 ppm.

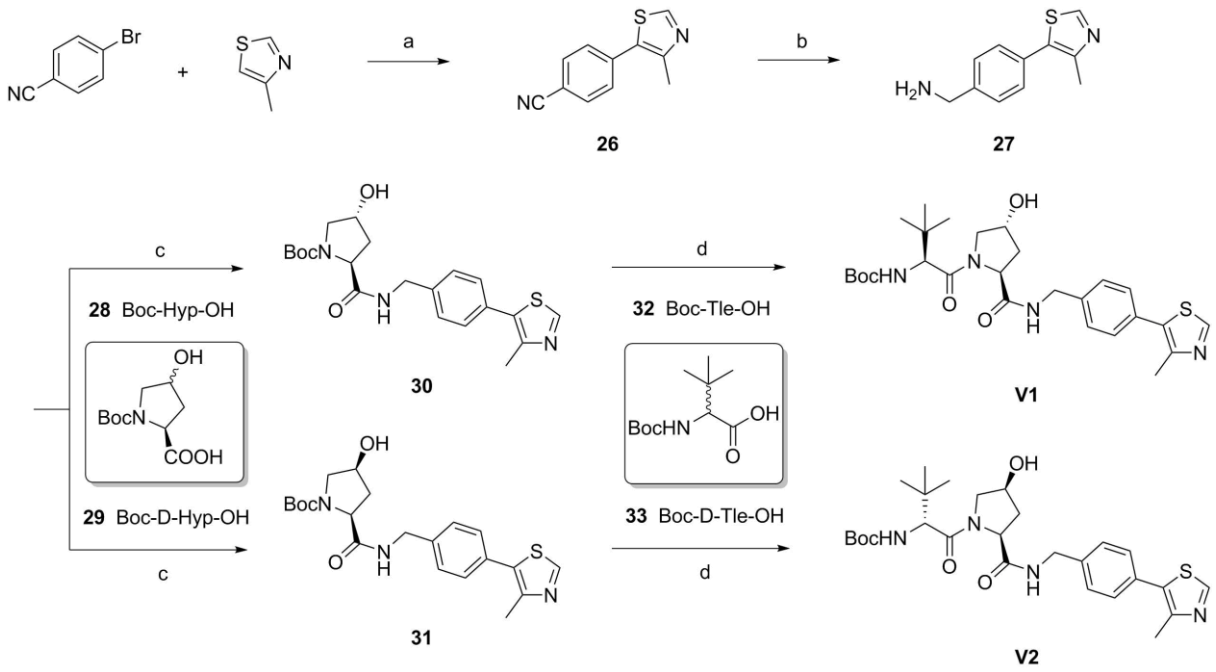
***tert*-Butyl 5-[5-[6-[[1,3-dioxo-2-(2-oxo-3-piperidyl)isoindolin-4-yl]amino]hexoxy]pentoxy] pentanoate (CL10)**



This compound was prepared using the General Procedure **V**, CRBN ligand **C3** (0.26 g) and linker **L6**. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc) to give a yellow oil.

Yield (0.30 g, 50%); R_f = 0.67 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.20 – 1.37 (m, 6H, CH_2), 1.37 (s, 9H, CH_3), 1.39 – 1.61 (m, 12H, CH_2), 1.81 – 2.00 (m, 3H, 4'-H, 5'-H), 2.13 – 2.25 (m, 3H, $COCH_2$, 4'-H), 3.13 – 3.40 (m, 12H, OCH_2, NHC$\underline{H}_2$, 6'-H), 4.49 (dd, *J* = 6.4, 11.9 Hz, 1H, 3'-H), 6.46 (t, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 6.97 (d, *J* = 7.1 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H, 5-H, 7-H), 7.54 (dd, *J* = 7.1, 8.5 Hz, 1H, 6-H), 7.76 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.67 (C-5'), 21.90, 22.64, 25.59, 26.04, 26.27 (CH_2), 27.89 (C($\underline{C}H_3$)$_3$), 28.64, 28.81 (CH_2), 29.16 (2 × CH_2), 29.29, 34.66 (CH_2), 41.55 (C-6'), 41.95 ($NHCH_2$), 48.72 (C-3'), 69.62, 69.97, 69.99, 70.03 (OCH2), 79.47 ($\underline{C}(CH_3)_3$), 109.48 (C-3a), 110.29 (C-7), 117.02 (C-5), 132.52 (C-7a), 136.17 (C-6), 146.46 (C-4), 167.27, 167.67 (C-1, C-3), 169.48 (C-2'), 172.34 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 11.51 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{33}H_{51}N_3O_7$, 602.38; found, 602.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{33}H_{51}N_3O_7$, 602.3800; found, 602.3773; diff., –4.48 ppm.

J. Synthesis of the VHL Ligands

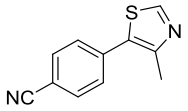


Reagents and conditions: (a) $Pd(OAc)_2$, KOAc, NMP, 140 °C, 24 h; (b) $NaBH_4$, $CoCl_2$, MeOH, 0 °C, 2 h; (c) HATU, DIPEA, DMF, rt, 1 h; (d) (i) CH_2Cl_2, TFA, rt, 2 h; (ii) HATU, DIPEA, DMF, rt, 1 h.

General Procedure VI: Amide bond formation. The corresponding amine (1 mmol) was dissolved in dry DMF (5 mL) and the appropriate acid (1 mmol) was added. While stirring the solution, DIPEA (0.70 mL, 4 mmol) was added, followed by the addition of HATU (1.1 mmol) after 5 minutes. The mixture was stirred at room temperature for 1 h, after which H_2O (50 mL) was added, and it was extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*.

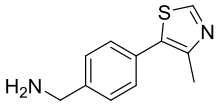
General Procedure VII: Removal of Boc protecting groups. The corresponding Boc-protected amine (1 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and TFA (5 mL) was added. The mixture was stirred at room temperature for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum.

4-(4-Methylthiazol-5-yl)benzonitrile (26)[8]



A solution of 4-bromobenzonitrile (1.46 g, 8 mmol) and $Pd(OAc)_2$ (2 mg, 0.08 mmol, 0.1 mol %) in NMP (8 mL) were added KOAc (1.57 g, 16 mmol) and 4-methyl thiazole (1.59 g, 1.46 mL, 16 mmol). The resulting mixture was heated to 140 °C and stirred for 24 h. The mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtrated and evaporated. The crude cyano intermediate was used in the next step without further purification.

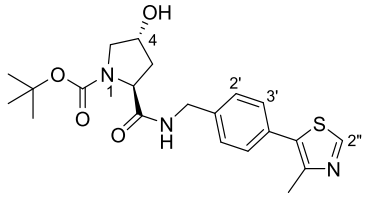
(4-(4-Methylthiazol-5-yl)phenyl)methanamine (27)[8]



Compound **26** was dissolved in MeOH (90 mL) and the dark solution was cooled to 0 °C. Anhydrous $CoCl_2$ (1.56 g, 12 mmol) was added, followed by portionwise addition of $NaBH_4$ (1.51 g, 40 mmol). The resulting mixture was stirred for 2 h, after which the black suspension was quenched with 1N NaOH (50 mL) and the mixture was transferred to centrifuge tubes. After centrifugation (10.000 × g, 10 min), the supernatant was decanted and the sediment was washed with more of the same solvent. The combined supernatants were extracted with $CHCl_3$ (3 × 50 mL), washed with brine (50 mL), dried over Na_2SO_4, filtrated and concentrated. The crude material was further purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 19:1:0.5) to obtain a yellow oil.

Yield (0.44 g, 27%); R_f = 0.36 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 7.37 – 7.47 (m, 4H, Ar-H), 8.96 (s, 1H, 2-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 16.07 (CH_3), 45.41 (CH_2), 127.71, 128.82 (C-2', C-3'), 129.43, 131.45 (C-5, C-1'), 144.31 (C-4'), 147.79 (C-4), 151.41 (C-2); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 6.33 min, 93% purity, *m/z* $[M + H]^+$ calcd for $C_{11}H_{12}N_2S$, 205.08; found, 204.6.

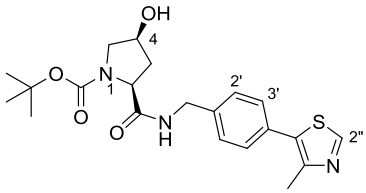
***tert*-Butyl (2*S*,4*R*)-4-hydroxy-2-[[4-(4-methylthiazol-5-yl)phenyl]methylcarbamoyl]pyrrolidine-1-carboxylate (30)**[8]



This compound was prepared using the General Procedure **VI**, precursor **26** (0.20 g) and Boc-Hyp-OH (**28**, 0.23 g). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 19:1) to give a yellow solid.

Yield (0.44 g, 75%); mp 78 – 80 °C; R_f = 0.42 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.25 and 1.40 (each s, 9H, major and minor rotamer, $C(CH_3)_3$), 1.81 – 1.91 (m, 1H, 3-H), 2.00 – 2.12 (m, 1H, 3-H), 2.43 (s, 3H, CH_3), 3.23 – 3.33 (m, 1H), 3.35 – 3.48 (m, 1H), 4.13 – 4.29 (m, 3H), 4.31 – 4.41 (m, 1H), 4.97 (d, *J* = 3.5, 1H, major and minor rotamer, OH), 7.33 – 7.45 (m, 4H, 2'-H, 3'-H), 8.38 – 8.48 (m, 1H, NH), 8.97 (s, 1H, 2''-H); **^{13}C NMR** (126 MHz, DMSO-d_6, the major rotamer) δ 15.99 (CH_3), 28.04 ($C(\underline{C}H_3)_3$), 41.96 ($NHCH_2$), 54.90, 59.09 (C-2, C-5), 67.97 (C-4), 78.63 ($\underline{C}(CH_3)_3$), 128.25, 128.96 (C-2', C-3'), 130.11, 131.21 (C-5'', C-1'), 139.60 (C-4'), 147.95 (C-4''), 151.60 (C-2''), 153.69 (NCO), 172.66 (CONH). A signal for one CH_2-group is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 9.91 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{11}H_{12}N_2S$, 205.08; found, 204.6.

***tert*-Butyl (2*S*,4*S*)-4-hydroxy-2-[[4-(4-methylthiazol-5-yl)phenyl]methylcarbamoyl]pyrrolidine-1-carboxylate (31)**[9]

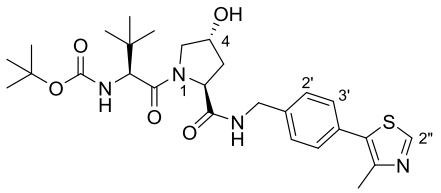


This compound was prepared using the General Procedure **VI**, precursor **26** (0.20 g) and Boc-D-Hyp-OH (**29**, 0.23 g). The crude product was purified by column chromatography (gradient of CH_2Cl_2/MeOH 29:1 to 19:1) to give a brown oil.

Yield (0.12 g, 28%); R_f = 0.23 (CH_2Cl_2/MeOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.25 and 1.40 (each s, 9H, major and minor rotamer, $C(CH_3)_3$), 1.80 – 1.90 (m, 1H, 3-H), 2.01 – 2.11 (m, 1H, 3-H),

2.43 (s, 3H, CH_3), 3.22 – 3.48 (m, 2H), 4.13 – 4.29 (m, 3H), 4.31 – 4.40 (m, 1H), 4.98 and 5.00 (d, *J* = 3.7 Hz, 1H, major and minor rotamer, OH), 7.34 – 7.44 (m, 4H, 2'-H, 3'-H), 8.40 – 4.50 (m, 1H, NH), 8.97 (s, 1H, 2''-H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.08 (CH_3), 28.07 (C($\underline{C}H_3$)$_3$), 41.99 ($NHCH_2$), 54.94, 59.12 (C-2, C-5), 68.00 (C-4), 78.87 ($\underline{C}(CH_3)_3$), 127.64, 128.30 (C-2', C-3'), 129.00, 131.25 (C-5'', C-1'), 139.64 (C-4'), 147.99 (C-4''), 151.61 (C-2''), 153.73 (NCO), 172.74 (CONH); the C-3 signal is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 10.65 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{21}H_{27}N_3O_4S$, 418.18; found, 418.0.

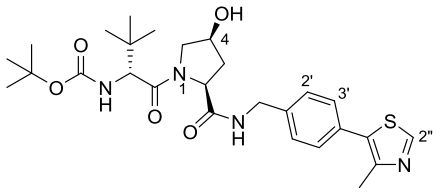
***tert*-Butyl *N*-[(1*S*)-1-[(2*S*,4*R*)-4-hydroxy-2-[[4-(4-methylthiazol-5-yl)phenyl]methylcarbamoyl] pyrrolidine-1-carbonyl]-2,2-dimethyl-propyl]carbamate (V1)**[8]



This compound was prepared using the General Procedure **VII** and precursor **30** (0.42 g). After deprotection, the crude material was subjected to amide bond formation (General Procedure **VI**) while using Boc-Tle-OH (**32**, 0.23 g). The crude product was purified by column chromatography (EtOAc) to give a colourless oil.

Yield (0.36 g, 67%); R_f = 0.44 (CH_2Cl_2/MeOH 9:1); **^{1}H NMR** (500 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H, C(CH_3)$_3$), 1.38 (s, 9H, OC(CH_3)$_3$), 1.85 – 1.93 (m, 1H, 3-H), 2.00 – 2.06 (m, 1H, 3-H), 2.43 (s, 3H, CH_3), 3.59 (d, *J* = 10.8 Hz, 1H), 3.65 (dd, *J* = 4.1, 10.5 Hz, 1H), 4.14 (d, *J* = 9.3 Hz, 1H), 4.23 (dd, *J* = 5.6, 15.8 Hz, 1H), 4.33 – 4.47 (m, 3H, 2-H, 4-H, 5-H, NHC$\underline{H}$, NHC$\underline{H}_2$), 5.08 (br s, 1H, OH), 6.39 (d, *J* = 9.3 Hz, 1H, BocNH), 7.36 – 7.41 (m, 4H, 2'-H, 3'-H), 8.53 (t, *J* = 6.1 Hz, 1H, NH), 8.96 (s, 1H, 2''-H). **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.04 (CH_3), 26.40 (C($\underline{C}H_3$)$_3$), 28.30 (OC($\underline{C}H_3$)$_3$), 35.49 ($\underline{C}(CH_3)_3$), 38.01 (C-3), 41.79 ($NHCH_2$), 56.45, 58.51, 58.87 (C-2, C-5, NHCH), 69.03 (C-4), 78.22 (O$\underline{C}(CH_3)_3$), 127.59, 128.79 (C-2', C-3'), 129.80, 131.28 (C-5", C-1'), 139.58 (C-4'), 147.86 (C-4''), 151.52 (C-2''), 155.46 (NCO), 169.96, 172.01 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 10.97 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{27}H_{38}N_4O_5S$, 531.26; found, 531.5.

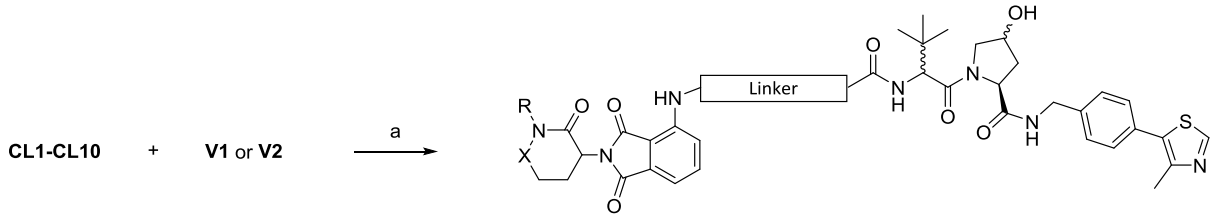
***tert*-Butyl *N*-[(1*R*)-1-[(2*S*,4*S*)-4-hydroxy-2-[[4-(4-methylthiazol-5-yl)phenyl]methylcarbamoyl] pyrrolidine-1-carbonyl]-2,2-dimethyl-propyl]carbamate (V2)**



This compound was prepared using the General Procedure **VII** and precursor **31** (0.42 g). After deprotection, the crude material was subjected to amide bond formation (General Procedure **VI**) while using Boc-D-Tle-OH (**33**, 0.23 g). The crude product was purified by column chromatography (gradient of CH_2Cl_2/MeOH 39:1 to 19:1) to give a yellow oil.

Yield (0.48 g, 91%); R_f = 0.26 (CH_2Cl_2/MeOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H, $C(CH_3)_3$), 1.37 (s, 9H, $OC(CH_3)_3$), 1.85 – 1.93 (m, 1H, 3-H), 2.00 – 2.06 (m, 1H, 3-H), 2.43 (s, 3H, CH_3), 3.56 – 3.62 (m, 1H), 3.62 – 3.68 (m, 1H), 4.14 (d, *J* = 9.3 Hz, 1H), 4.22 (dd, *J* = 5.6, 15.8 Hz, 1H), 4.31 – 4.47 (m, 3H, 2-H, 4-H, 5-H, NHC<u>H</u>, NHC<u>H_2</u>), 5.12 (br s, 1H, OH), 6.41 (d, *J* = 9.4 Hz, 1H, BocNH), 7.35 – 7.43 (m, 4H, 2'-H, 3'-H), 8.54 (t, *J* = 5.8 Hz, 1H, NH), 8.96 (s, 1H, 2''-H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.11 (CH_3), 26.46 ($C(\underline{C}H_3)_3$), 28.37 ($OC(\underline{C}H_3)_3$), 35.56 ($\underline{C}(CH_3)_3$), 38.06 (C-3), 41.84 ($NHCH_2$), 56.52, 58.57, 58.93 (C-2, C-5, NHCH), 69.09 (C-4), 78.29 ($O\underline{C}(CH_3)_3$), 127.64, 128.86 (C-2', C-3'), 129.85, 131.34 (C-5'', C-1'), 139.64 (C-4'), 147.92 (C-4''), 151.61 (C-2'), 155.52 (NCO), 170.02, 172.09 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), t_R = 11.18 min, 92% purity, *m/z* $[M + H]^+$ calcd for $C_{27}H_{38}N_4O_5S$, 531.26; found, 531.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{27}H_{38}N_4O_5S$, 531.2636; found, 531.2638; diff., 0.38 ppm.

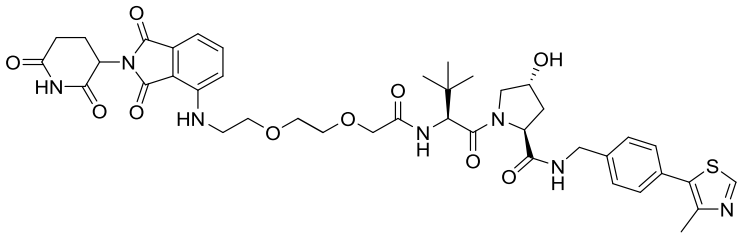
K. Synthesis of the Final CRBN-VHL Hetero-PROTACs



Reagents and conditions: (a) (i) CH_2Cl_2, TFA, 2 h; (ii) HATU, DIPEA, DMF, rt, 18 h.

General Procedure VIII: Synthesis of the CRBN-VHL Hetero-PROTACs. The corresponding linker-conjugated CRBN ligand **CL** (0.25 mmol) was dissolved in dry CH_2Cl_2 (2.5 mL) and TFA (2.5 mL) was added. The mixture was stirred at 40 °C for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. In a second flask, the corresponding Boc-protected VHL ligand **V** (0.132 g, 0.25 mmol) was dissolved in dry CH_2Cl_2 (2.5 mL) and TFA (2.5 mL) was added. The mixture was stirred at room temperature for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. Both deprotected intermediates were dissolved in dry DMF (each 4 mL) and DIPEA (each 0.75 mmol, 97 mg, 0.13 mL). HATU (0.275 mmol, 0.105 g) was added to the first flask and it was stirred for 5 minutes, before the second solution was transferred to the first flask. The combined mixture was stirred at rt overnight. The yellow mixture was quenched with half-saturated brine (50 mL), and it was extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with saturated NH_4Cl solution (50 mL), 5% aqueous LiCl solution (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*.

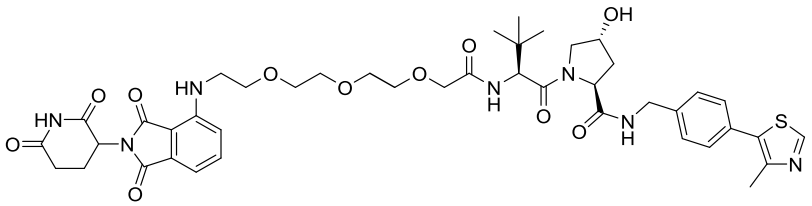
CRBN-2-2-2-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL1** (0.119 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 9:1) to give a yellow solid.

Yield (0.187 g, 90%); mp 96 – 98 °C; R_f = 0.47 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.86 – 1.94 (m, 1H), 1.98 – 2.09 (m, 2H), 2.41 (s, 3H), 2.51 – 2.61 (m, 2H), 2.82 – 2.91 (m, 1H), 3.46 – 3.52 (m, 2H), 3.56 – 3.69 (m, 8H), 3.96 (s, 2H), 4.20 – 4.27 (m, 1H), 4.33 – 4.40 (m, 2H), 4.44 (t, *J* = 8.4 Hz, 1H), 4.56 (d, *J* = 9.6 Hz, 1H), 5.04 (dd, *J* = 5.4, 12.9 Hz, 1H), 5.13 (d, *J* = 3.5 Hz, 1H), 6.60 (tt, *J* = 3.0, 6.0 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.11 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.33 – 7.45 (m, 5H), 7.51 – 7.58 (m, 1H), 8.54 (q, *J* = 5.9 Hz, 1H), 8.94 (s, 1H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.37, 22.62, 26.64, 31.46, 36.20, 38.39, 42.13, 42.17, 49.03, 56.18, 57.05, 59.21, 69.35, 69.56, 70.01, 70.12, 70.97, 109.75, 111.14, 117.85, 127.92, 129.14, 130.17, 131.58, 132.56, 136.67, 139.87, 146.86, 148.20, 151.85, 167.76, 169.05, 169.38, 169.65, 170.52, 172.21, 173.24; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 7.56 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{41}H_{49}N_7O_{10}S$, 832.33; found, 832.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{41}H_{49}N_7O_{10}S$, 832.3334; found, 832.3346; diff., 1.44 ppm.

CRBN-2-2-2-2-VHL

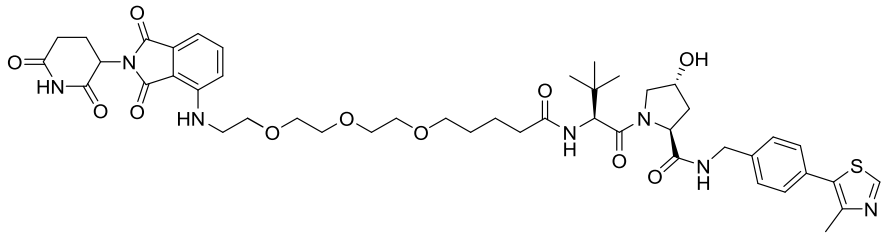


This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL2** (0.130 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 9:1) to give a yellow solid.

Yield (0.187 g, 33%); mp 98 – 100 °C; R_f = 0.62 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.86 – 1.93 (m, 1H), 1.97 – 2.08 (m, 2H), 2.42 (s, 3H), 2.51 – 2.61 (m, 2H), 2.82 – 2.91 (m, 1H), 3.39 – 3.46 (m, 2H), 3.53 – 3.68 (m, 12H), 3.94 (s, 2H), 4.20 – 4.29 (m, 1H), 4.31 – 4.46 (m, 3H), 4.55 (d, *J* = 9.6 Hz, 1H), 5.03 (dd, *J* = 5.4, 12.9 Hz, 1H), 5.13 (d, *J* = 3.5 Hz, 1H), 6.57 (t, *J* = 5.8 Hz, 1H), 7.02 (d, *J* = 7.0 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 7.35 – 7.45 (m, 5H), 7.55 (dd, *J* = 7.1, 8.6 Hz, 1H), 8.55 (t, *J* = 6.1 Hz, 1H), 8.95 (s, 1H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.07, 22.31, 26.33, 31.14, 35.87, 38.07, 41.85, 48.73, 55.85, 56.72, 58.91, 69.04, 69.07, 69.77, 69.84, 69.99, 70.04, 70.67, 109.42, 110.83, 117.59, 127.63, 128.84, 129.86, 131.30, 132.24, 136.37, 139.59, 146.58, 147.91, 151.58, 167.45, 168.78, 169.09, 169.31, 170.21, 171.92, 172.94; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500

nm), t_R = 10.74 min, 99% purity, *m/z* [M + H]$^+$ calcd for $C_{43}H_{53}N_7O_{11}S$, 876.36; found, 876.8; **HRMS** (ESI) *m/z* [M + H]$^+$ calcd for $C_{43}H_{53}N_7O_{11}S$, 876.3597; found, 876.3585; diff., –1.37 ppm.

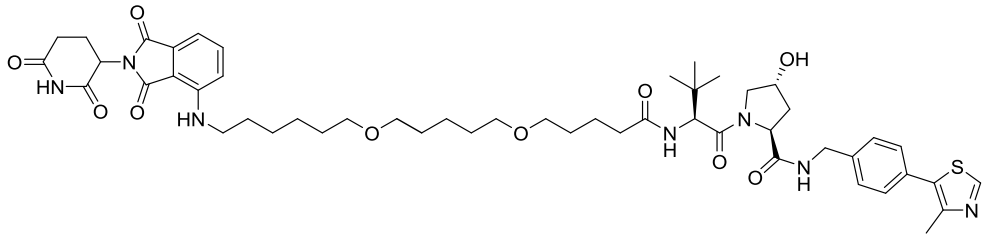
CRBN-2-2-2-5-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL3** (0.140 g) and VHL ligand **V1**. The crude product was purified by column chromatography (CH_2Cl_2/EtOH 19:1) to give a yellow solid.

Yield (0.103 g, 45%); mp 94 – 96 °C; R_f = 0.23 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.20 – 1.28 (m, 2H), 1.39 – 1.55 (m, 4H), 1.86 – 1.93 (m, 1H), 1.97 – 2.06 (m, 2H), 2.07 – 2.15 (m, 1H), 2.21 – 2.29 (m, 1H), 2.43 (s, 3H), 2.50 – 2.62 (m, 2H), 2.82 – 2.92 (m, 1H), 3.41 – 3.57 (m, 10H), 3.58 – 3.69 (m, 4H), 4.21 (dd, *J* = 5.6, 15.8 Hz, 1H), 4.30 – 4.37 (m, 1H), 4.38 – 4.45 (m, 2H), 4.53 (d, *J* = 9.3 Hz, 1H), 5.04 (dd, *J* = 5.4, 12.9 Hz, 1H), 5.09 (d, *J* = 3.6 Hz, 1H), 6.59 (t, *J* = 5.8 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 7.35 – 7.45 (m, 4H), 7.57 (dd, *J* = 7.0, 8.5 Hz, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.07 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.10, 22.32, 22.38, 26.54, 28.95, 31.15, 34.79, 35.37, 38.10, 41.83, 41.89, 48.74, 56.48, 56.51, 58.86, 69.03, 69.07, 69.63, 69.95, 69.98, 70.18, 109.43, 110.84, 117.62, 127.60, 128.80, 129.81, 131.33, 132.26, 136.38, 139.67, 146.60, 147.88, 151.59, 167.46, 169.10, 169.88, 170.20, 172.11, 172.16, 172.94; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-450 nm), t_R = 7.82 min, 99% purity, *m/z* [M + H]$^+$ calcd for $C_{46}H_{59}N_7O_{11}S$, 918.40; found, 918.7; **HRMS** (ESI) *m/z* [M + H]$^+$ calcd for $C_{46}H_{59}N_7O_{11}S$, 918.4066; found, 918.4060; diff., –0.65 ppm.

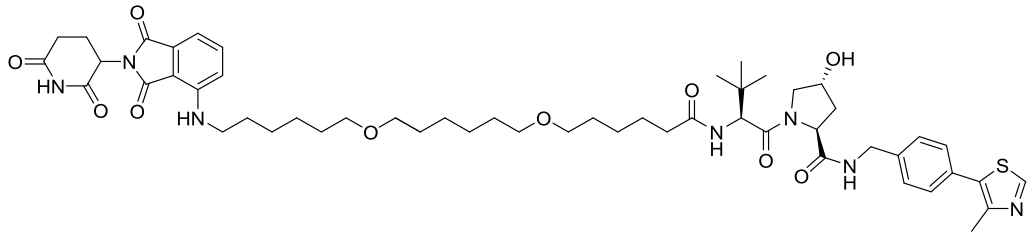
CRBN-6-5-5-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL4** (0.154 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 9:1) to give a yellow solid.

Yield (0.185 g, 76%); mp 88 – 92 °C; R_f = 0.57 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.21 – 1.36 (m, 8H), 1.39 – 1.60 (m, 12H), 1.86 – 1.93 (m, 1H), 1.97 – 2.05 (m, 2H), 2.07 – 2.15 (m, 1H), 2.21 – 2.30 (m, 1H), 2.43 (s, 3H), 2.50 – 2.61 (m, 2H), 2.82 – 2.92 (m, 1H), 3.23 – 3.34 (m, 8H), 3.58 – 3.69 (m, 2H), 4.21 (dd, *J* = 5.5, 15.8 Hz, 1H), 4.31 – 4.37 (m, 1H), 4.38 – 4.47 (m, 2H), 4.53 (d, *J* = 9.4 Hz, 1H), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H), 5.09 (d, *J* = 3.6 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.7 Hz, 1H), 7.35 – 7.43 (m, 4H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.08, 22.31, 22.42, 22.66, 25.60, 26.28, 26.53, 28.81, 28.97, 29.20, 29.31, 31.13, 34.78, 35.36, 38.09, 41.81, 41.96, 48.70, 56.44, 56.48, 58.84, 69.02, 69.79, 69.99, 70.05, 109.19, 110.52, 117.31, 127.58, 128.78, 129.80, 131.31, 132.35, 136.41, 139.65, 146.60, 147.87, 151.56, 167.45, 169.10, 169.86, 170.21, 172.08, 172.13, 172.92. **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-450 nm), t_R = 9.26 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{69}N_7O_{10}S$, 972.49; found, 972.8; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{69}N_7O_{10}S$, 972.4899; found, 972.4911; diff., 1.23 ppm.

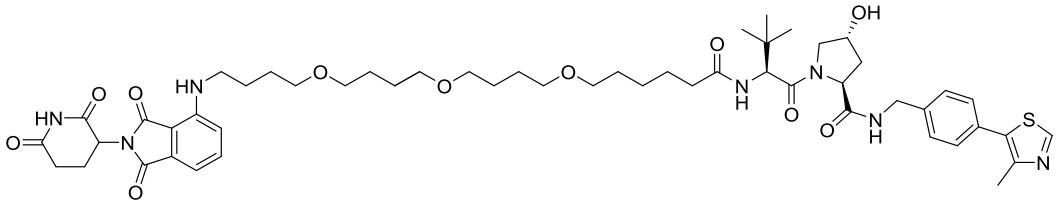
CRBN-6-6-6-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL5** (0.161 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 12:1) to give a yellow solid.

Yield (0.133 g, 53%); mp 84 – 86 °C; R_f = 0.78 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.21 – 1.28 (m, 6H), 1.29 – 1.36 (m, 4H), 1.40 – 1.51 (m, 10H), 1.52 – 1.59 (m, 2H), 1.85 – 1.93 (m, 1H), 1.97 – 2.05 (m, 2H), 2.06 – 2.13 (m, 1H), 2.20 – 2.28 (m, 1H), 2.43 (s, 3H), 2.50 – 2.61 (m, 2H), 2.82 – 2.91 (m, 1H), 3.26 – 3.32 (m, 10H), 3.60 – 3.69 (m, 2H), 4.17 – 4.23 (m, 1H), 4.31 – 4.36 (m, 1H), 4.38 – 4.45 (m, 2H), 4.52 (d, *J* = 9.4 Hz, 1H), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.49 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.34 – 7.43 (m, 4H), 7.53 – 7.59 (m, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.11, 22.36, 25.50, 25.59, 25.65, 25.75, 25.77, 26.31, 26.57, 28.85, 29.18, 29.35, 29.40, 29.43, 31.17, 35.06, 35.39, 38.12, 41.85, 42.00, 48.74, 56.50, 58.89, 69.06, 70.02, 70.07, 70.08, 109.21, 110.58, 117.36, 127.62, 128.83, 129.83, 131.36, 132.38, 136.47, 139.68, 146.64, 147.91, 151.62, 167.50, 169.15, 169.92, 170.26, 172.15, 172.28, 172.99; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 10.04 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{53}H_{73}N_7O_{10}S$, 1000.52; found, 1000.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{53}H_{73}N_7O_{10}S$, 1000.5212; found, 1000.5114; diff., –9.79 ppm.

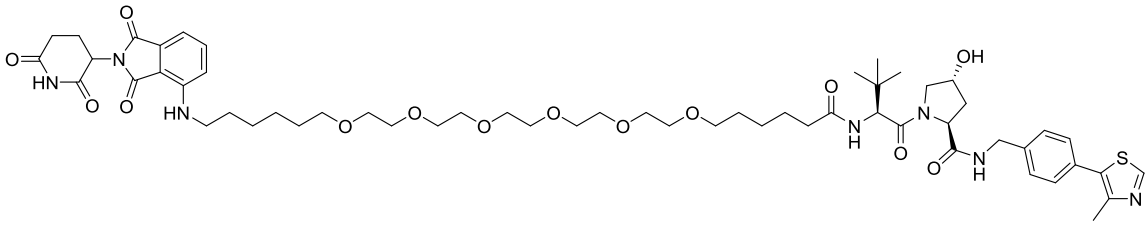
CRBN-4-4-4-6-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL6** (0.165 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 19:1) to give a yellow solid.

Yield (89 mg, 35%); mp 84 – 86 °C; R_f = 0.70 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.22 – 1.26 (m, 2H), 1.43 – 1.51 (m, 12H), 1.53 – 1.61 (m, 4H), 1.86 – 1.95 (m, 1H), 1.97 – 2.05 (m, 2H), 2.05 – 2.14 (m, 1H), 2.20 – 2.29 (m, 1H), 2.43 (s, 3H), 2.46 – 2.64 (m, 2H), 2.80 – 2.93 (m, 1H), 3.24 – 3.39 (m, 14H), 3.58 – 3.71 (m, 2H), 4.20 (dd, *J* = 5.5, 15.8 Hz, 1H), 4.30 – 4.37 (m, 1H), 4.37 – 4.46 (m, 2H), 4.52 (d, *J* = 9.4 Hz, 1H), 5.03 (dd, *J* = 5.6, 12.8 Hz, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.54 (t, *J* = 6.0 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.35 – 7.41 (m, 4H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.11, 22.35, 25.50, 25.59, 25.79, 26.26, 26.28, 26.56, 26.69, 29.18, 31.16, 35.06, 35.39, 38.12, 41.82, 41.85, 48.74, 56.50, 58.89, 69.06, 69.74, 69.91, 69.92, 69.99, 70.05, 109.24, 110.57, 117.37, 127.63, 128.83, 129.83, 131.36, 132.40, 136.44, 139.68, 146.61, 147.91, 151.62, 167.50, 169.12, 169.92, 170.25, 172.15, 172.29, 172.99; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 9.19 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{53}H_{73}N_7O_{11}S$, 1016.51; found, 1016.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{53}H_{73}N_7O_{11}S$, 1016.5162; found, 1016.5075; diff., –8.56 ppm.

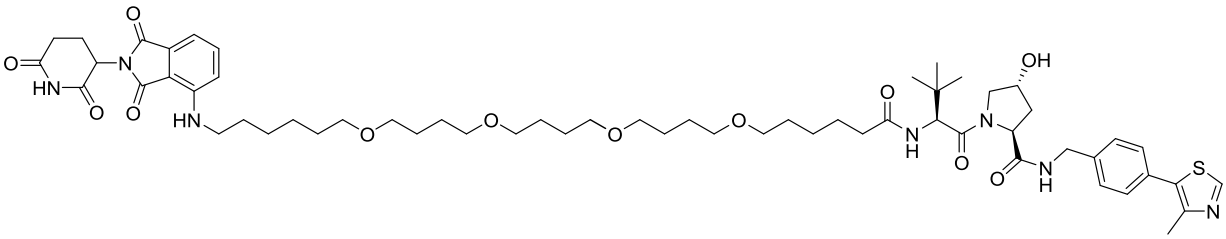
CRBN-6-(2)$_5$-6-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL7** (0.191 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 29:1 to 12:1) to give a yellow solid.

Yield (0.202 g, 72%); mp 76 – 78 °C; R_f = 0.65 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (500 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.22 – 1.27 (m, 2H), 1.31 – 1.36 (m, 4H), 1.43 – 1.52 (m, 6H), 1.53 – 1.61 (m, 2H), 1.85 – 1.94 (m, 1H), 1.97 – 2.05 (m, 2H), 2.06 – 2.14 (m, 1H), 2.19 – 2.29 (m, 1H), 2.43 (s, 3H), 2.52 – 2.61 (m, 2H), 2.81 – 2.93 (m, 1H), 3.34 (dd, *J* = 6.6, 12.0 Hz, 4H), 3.43 – 3.46 (m, 4H), 3.46 – 3.52 (m, 18H), 3.60 – 3.69 (m, 2H), 4.21 (dd, *J* = 5.5, 15.8 Hz, 1H), 4.31 – 4.37 (m, 1H), 4.38 – 4.46 (m, 2H), 4.53 (d, *J* = 9.4 Hz, 1H), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H), 5.08 (d, *J* = 3.6 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.1 Hz, 1H), 7.07 (d, *J* = 8.7 Hz, 1H), 7.35 – 7.42 (m, 4H), 7.56 (dd, *J* = 7.1, 8.5 Hz, 1H), 7.79 (d, *J* = 9.3 Hz, 1H), 8.51 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.05 (br s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6, the major rotamer) δ 16.07, 22.31, 25.44, 25.47, 25.54, 26.28, 26.53, 28.81, 29.08, 29.27, 31.13, 35.01, 35.33, 38.08, 41.82, 41.97, 48.71, 56.45, 58.84, 69.01, 69.60, 69.62, 69.94, 70.37, 70.40, 109.20, 110.53, 117.31, 127.58, 128.78, 129.80, 131.31, 132.34, 136.42, 139.65, 146.60, 147.87, 151.54, 167.44, 169.10, 169.88, 170.18, 172.07, 172.19, 172.91; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 8.48 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{57}H_{81}N_7O_{14}S$, 1120.56; found, 1120.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{57}H_{81}N_7O_{14}S$, 1120.5635; found, 1120.5525; diff., –9.82 ppm.

CRBN-6-(4)$_3$-6-VHL

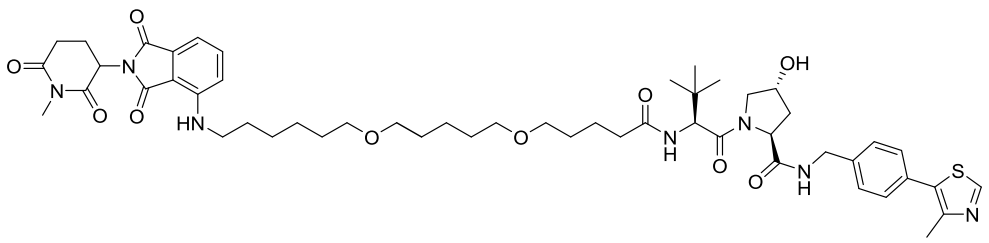


This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL8** (0.190 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 29:1 to 9:1) to give a yellow solid.

Yield (0.170 g, 61%); mp 72 – 74 °C; R_f = 0.76 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.20 – 1.29 (m, 2H), 1.29 – 1.36 (m, 4H), 1.41 – 1.52 (m, 18H), 1.53 – 1.60 (m, 2H), 1.86 – 1.94 (m, 1H), 1.97 – 2.05 (m, 2H), 2.06 – 2.13 (m, 1H), 2.21 – 2.28 (m, 1H), 2.43 (s, 3H), 2.51 – 2.61 (m, 2H), 2.82 – 2.91 (m, 1H), 3.23 – 3.34 (m, 18H), 3.58 – 3.71 (m, 2H), 4.20 (dd, *J* = 5.5, 15.8 Hz, 1H), 4.31 – 4.37 (m, 1H), 4.38 – 4.46 (m, 2H), 4.53 (d, *J* = 9.4 Hz, 1H), 5.03 (dd, *J* = 5.5, 12.9 Hz, 1H), 5.10 (d, *J* = 3.5 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.34 – 7.43 (m, 4H), 7.56 (dd, *J* = 7.1, 8.5 Hz, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.11, 22.35, 25.49,

25.59, 25.65, 26.25, 26.31, 26.56, 28.84, 29.17, 29.34, 31.16, 35.05, 35.38, 38.11, 41.84, 41.99, 48.74, 56.48, 56.50, 58.88, 69.05, 69.89, 69.92, 69.99, 70.05, 109.21, 110.56, 117.34, 127.62, 128.81, 129.83, 131.35, 132.37, 136.45, 139.68, 146.63, 147.90, 151.60, 167.49, 169.14, 169.91, 170.24, 172.13, 172.25, 172.97; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 10.36 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{59}H_{85}N_7O_{12}S$, 1116.60; found, 1116.9; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{59}H_{85}N_7O_{12}S$, 1116.6050; found, 1116.5940; diff., –9.85 ppm.

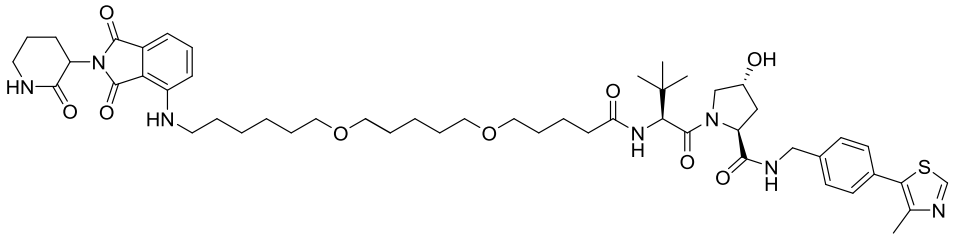
(-)CRBN-6-5-5-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL9** (0.157 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 19:1) to give a yellow solid.

Yield (0.150 g, 61%); mp 80 – 82 °C; R_f = 0.87 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.24 – 1.36 (m, 6H), 1.39 – 1.52 (m, 10H), 1.53 – 1.60 (m, 2H), 1.85 – 1.93 (m, 1H), 1.98 – 2.06 (m, 2H), 2.06 – 2.14 (m, 1H), 2.22 – 2.29 (m, 1H), 2.43 (s, 3H), 2.50 – 2.56 (m, 1H), 2.70 – 2.78 (m, 1H), 2.89 – 2.97 (m, 1H), 3.00 (s, 3H), 3.24 – 3.31 (m, 10H), 3.60 – 3.69 (m, 2H), 4.20 (dd, *J* = 5.5, 15.8 Hz, 1H), 4.31 – 4.36 (m, 1H), 4.38 – 4.45 (m, 2H), 4.53 (d, *J* = 9.3 Hz, 1H), 5.07 – 5.13 (m, 2H), 6.50 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.1 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.34 – 7.43 (m, 4H), 7.56 (dd, *J* = 7.0, 8.6 Hz, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.10, 21.57, 22.45, 22.68, 25.63, 26.31, 26.55, 26.77, 28.83, 29.00, 29.23, 29.33, 31.30, 34.82, 35.38, 38.11, 41.84, 41.99, 49.29, 56.48, 56.51, 58.87, 69.05, 69.82, 70.02, 70.07, 109.17, 110.58, 117.38, 127.61, 128.81, 129.82, 131.34, 132.35, 136.48, 139.67, 146.65, 147.89, 151.60, 167.45, 169.11, 169.89, 169.99, 171.97, 172.12, 172.19; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 9.94 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{52}H_{71}N_7O_{10}S$, 986.50; found, 986.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{52}H_{71}N_7O_{10}S$, 986.5056; found, 986.5046; diff., –1.01 ppm.

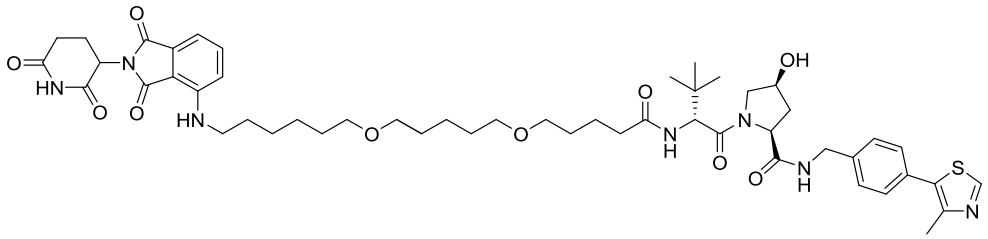
(*)CRBN-6-5-5-VHL



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL10** (0.150 g) and VHL ligand **V1**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 12:1) to give a yellow solid.

Yield (96 mg, 40%); mp 98 – 100 °C; R_f = 0.59 (CH_2Cl_2/EtOH 9:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.27 – 1.35 (m, 6H), 1.42 – 1.51 (m, 10H), 1.52 – 1.57 (m, 2H), 1.80 – 1.92 (m, 3H), 1.92 – 2.04 (m, 2H), 2.06 – 2.14 (m, 1H), 2.15 – 2.29 (m, 2H), 2.43 (s, 3H), 3.17 – 3.22 (m, 2H), 3.24 – 3.28 (m, 2H), 3.28 – 3.31 (m, 8H), 3.60 – 3.69 (m, 2H), 4.20 (dd, *J* = 5.5, 15.9 Hz, 1H), 4.31 – 4.37 (m, 1H), 4.38 – 4.45 (m, 2H), 4.45 – 4.55 (m, 2H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.46 (t, *J* = 5.9 Hz, 1H), 6.97 (d, *J* = 7.0 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 7.35 – 7.42 (m, 4H), 7.54 (dd, *J* = 7.0, 8.5 Hz, 1H), 7.76 – 7.83 (m, 2H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6, the major rotamer) δ 16.10, 21.93, 22.45, 22.69, 25.63, 26.07, 26.31, 26.55, 28.85, 29.00, 29.23, 29.33, 34.82, 35.38, 38.11, 41.60, 41.84, 41.98, 48.75, 56.48, 56.51, 58.88, 69.05, 69.82, 70.02, 70.08, 109.50, 110.34, 117.08, 127.61, 128.81, 129.82, 131.34, 132.56, 136.24, 139.67, 146.49, 147.89, 151.60, 167.34, 167.72, 169.52, 169.90, 172.12, 172.19; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 9.16 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{71}N_7O_9S$, 958.51; found, 958.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{71}N_7O_9S$, 958.5107; found, 958.5044; diff., –6.57 ppm.

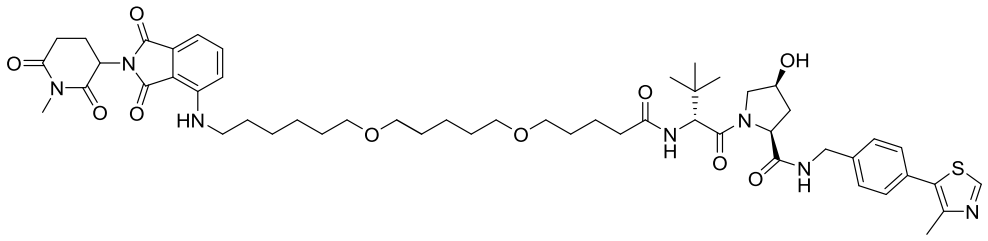
CRBN-6-5-5-VHL(-)



This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL4** (0.154 g) and VHL ligand **V2**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 29:1 to 9:1) to give a yellow solid.

Yield (87 mg, 36%); mp 94 – 96 °C; R_f = 0.27 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.24 – 1.37 (m, 6H), 1.40 – 1.52 (m, 10H), 1.53 – 1.61 (m, 2H), 1.85 – 1.94 (m, 1H), 1.97 – 2.06 (m, 2H), 2.07 – 2.15 (m, 1H), 2.21 – 2.29 (m, 1H), 2.43 (s, 3H), 2.50 – 2.62 (m, 2H), 2.81 – 2.92 (m, 1H), 3.23 – 3.33 (m, 10H), 3.58 – 3.73 (m, 2H), 4.21 (dd, *J* = 5.5, 15.9 Hz, 1H), 4.30 – 4.38 (m, 1H), 4.38 – 4.46 (m, 2H), 4.53 (d, *J* = 9.4 Hz, 1H), 5.03 (dd, *J* = 5.5, 12.7 Hz, 1H), 5.09 (d, *J* = 3.6 Hz, 1H), 6.49 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.34 – 7.43 (m, 4H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.79 (d, *J* = 9.3 Hz, 1H), 8.51 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H), 11.05 (br s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6, the major rotamer) δ 16.09, 22.34, 22.44, 22.67, 25.62, 26.29, 26.55, 28.83, 28.99, 29.22, 29.32, 31.15, 34.82, 35.37, 38.09, 41.85, 41.99, 48.74, 56.49, 58.87, 69.04, 69.81, 70.01, 70.07, 109.21, 110.56, 117.34, 127.61, 128.80, 129.82, 131.34, 132.36, 136.44, 139.66, 146.63, 147.89, 151.58, 167.48, 169.13, 169.90, 170.21, 172.11, 172.19, 172.94; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 9.30 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{69}N_7O_{10}S$, 972.49; found, 972.9; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{69}N_7O_{10}S$, 972.4899; found, 972.4881; diff., –1.85 ppm.

(-)CRBN-6-5-5-VHL(-)



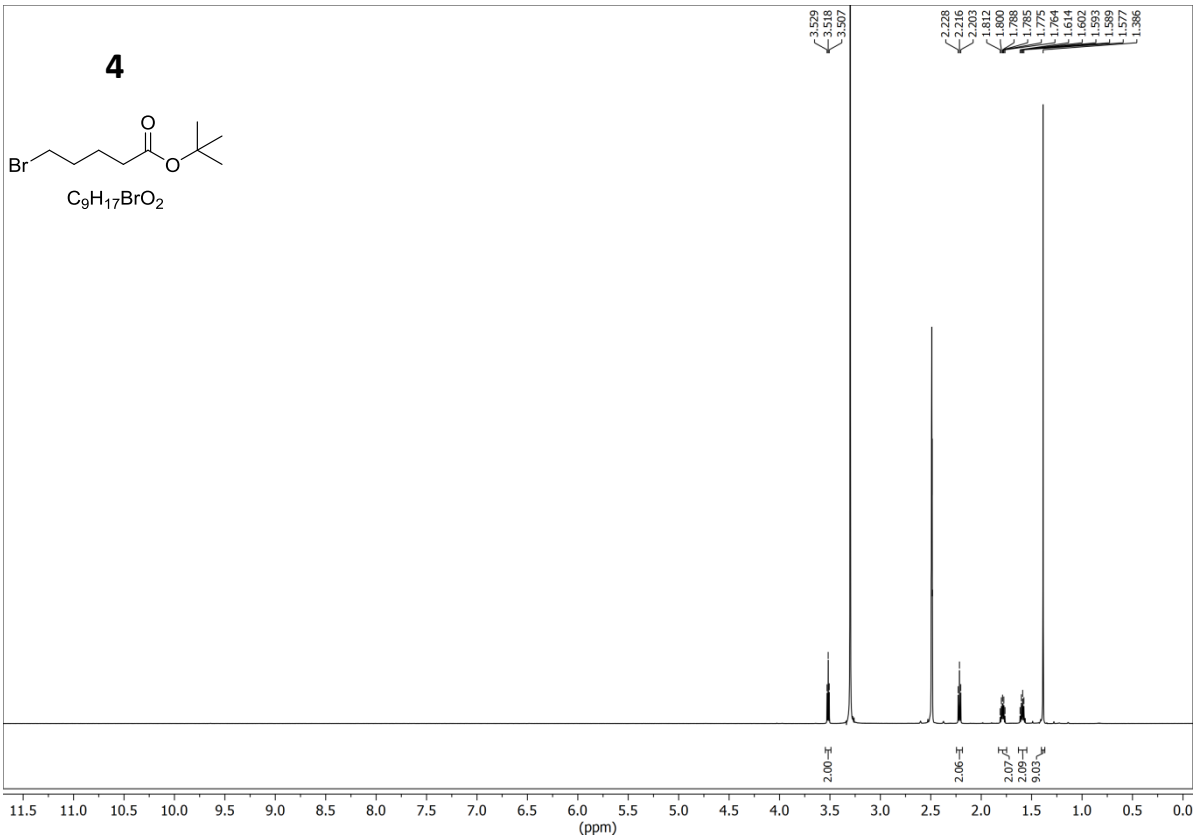
This compound was prepared using the General Procedure **VIII**, the linker-conjugated CRBN ligand **CL9** (0.157 g) and VHL ligand **V2**. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 49:1 to 19:1) to give a yellow solid.

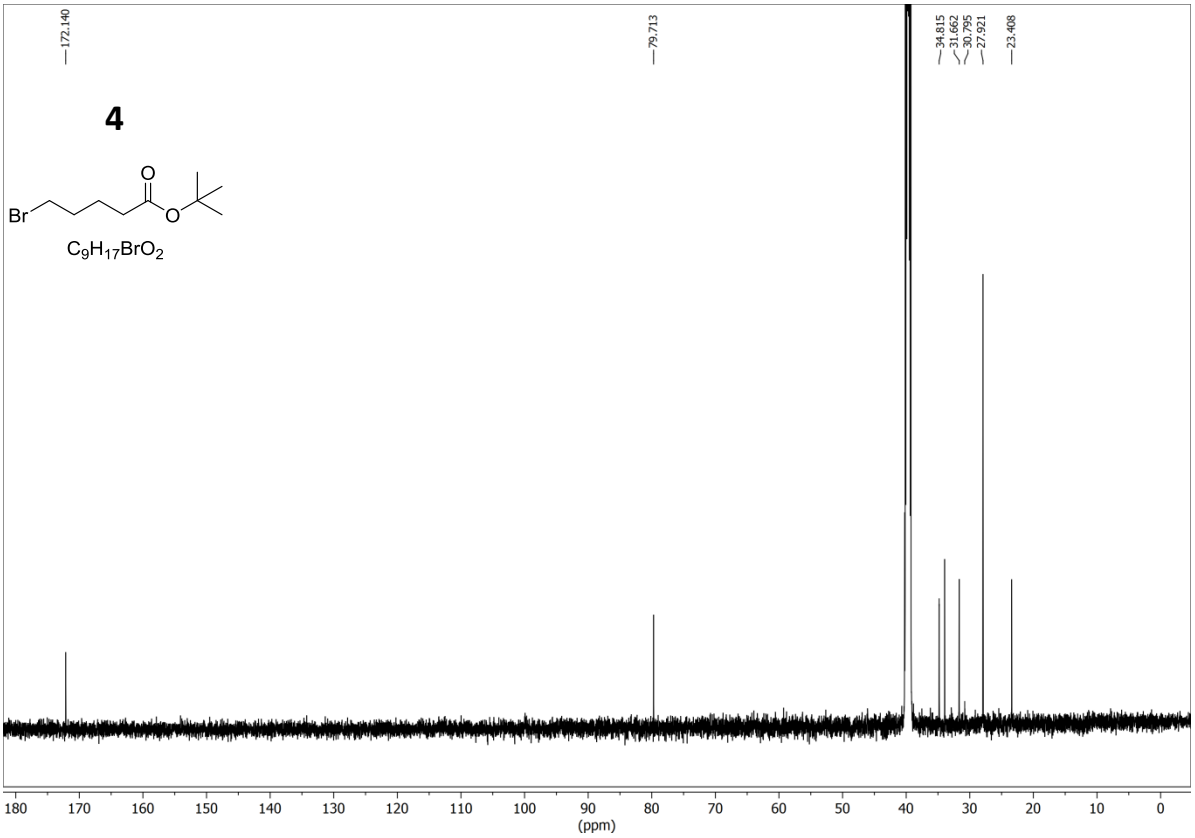
Yield (88 mg, 36%); mp 80 – 82 °C; R_f = 0.37 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6, the major rotamer) δ 0.92 (s, 9H), 1.24 – 1.35 (m, 6H), 1.42 – 1.50 (m, 10H), 1.52 – 1.58 (m, 2H), 1.84 – 1.95 (m, 1H), 1.98 – 2.06 (m, 2H), 2.08 – 2.15 (m, 1H), 2.19 – 2.29 (m, 1H), 2.43 (s, 3H), 2.50 – 2.57 (m, 1H), 2.70 – 2.82 (m, 1H), 2.87 – 2.98 (m, 1H), 3.00 (s, 3H), 3.21 – 3.31 (m, 9H), 3.59 – 3.70 (m, 2H), 4.21 (dd, *J* = 5.6, 15.8 Hz, 1H), 4.30 – 4.37 (m, 1H), 4.37 – 4.46 (m, 2H), 4.53 (d, *J* = 9.3 Hz, 1H), 5.06 – 5.14 (m, 2H), 6.50 (t, *J* = 6.0 Hz, 1H), 7.01 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.35 – 7.42 (m, 4H), 7.57 (dd, *J* = 7.0, 8.5 Hz, 1H), 7.78 (d, *J* = 9.5 Hz, 1H), 8.51 (t, *J* = 6.1 Hz, 1H), 8.96 (s, 1H).; **^{13}C NMR** (126 MHz, DMSO-d_6, the major rotamer) δ 16.05, 21.52, 22.40, 22.63, 25.58, 26.25, 26.51, 26.72, 28.78, 28.95, 29.18, 29.28, 31.25, 34.78, 35.33, 38.05, 41.80, 41.95, 49.25, 56.44, 58.83, 69.00, 69.77,

69.97, 70.03, 109.14, 110.53, 117.33, 127.57, 128.76, 129.78, 131.29, 132.31, 136.43, 139.62, 146.61, 147.85, 151.53, 167.40, 169.06, 169.85, 169.93, 171.91, 172.06, 172.13; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 9.67 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{52}H_{71}N_7O_{10}S$, 986.50; found, 986.7; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{52}H_{71}N_7O_{10}S$, 986.5056; found, 986.4996; diff., –6.08 ppm.

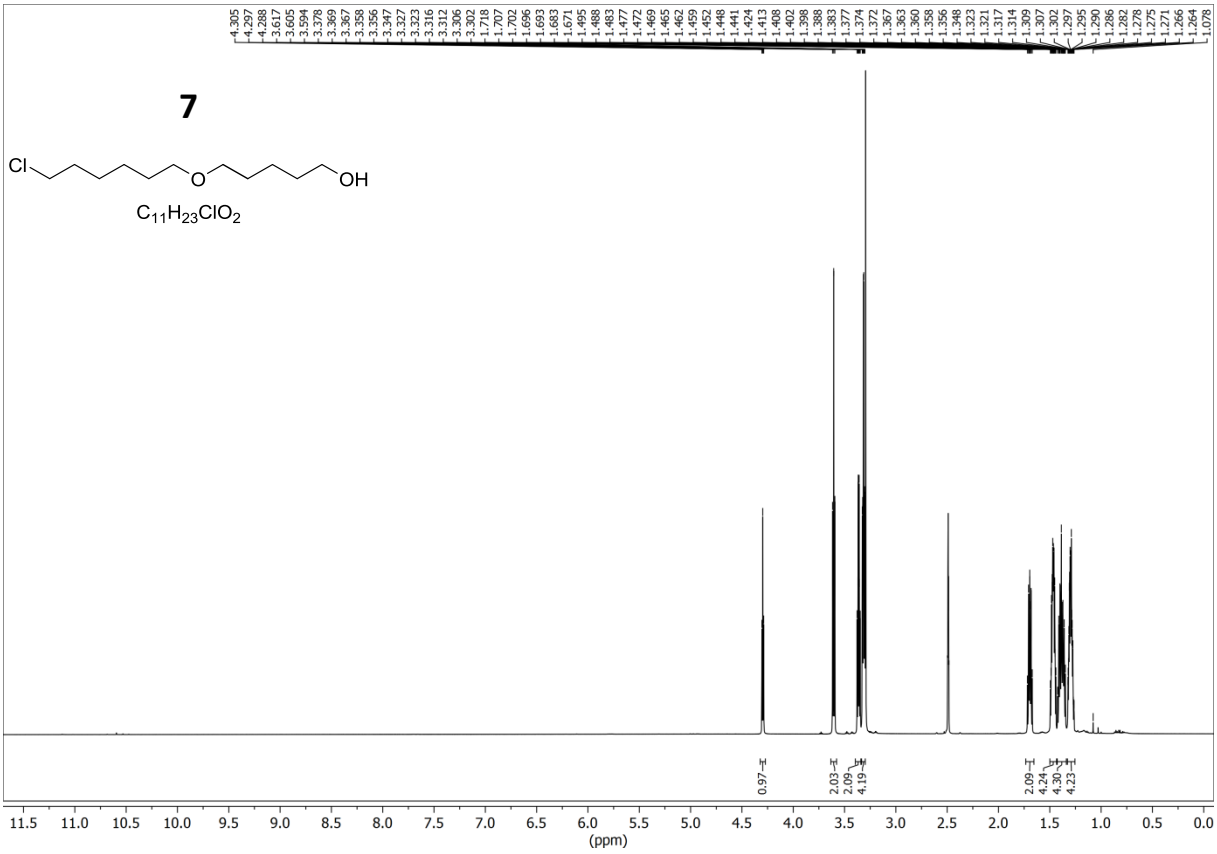
L. Selected ^{1}H and ^{13}C NMR Spectra

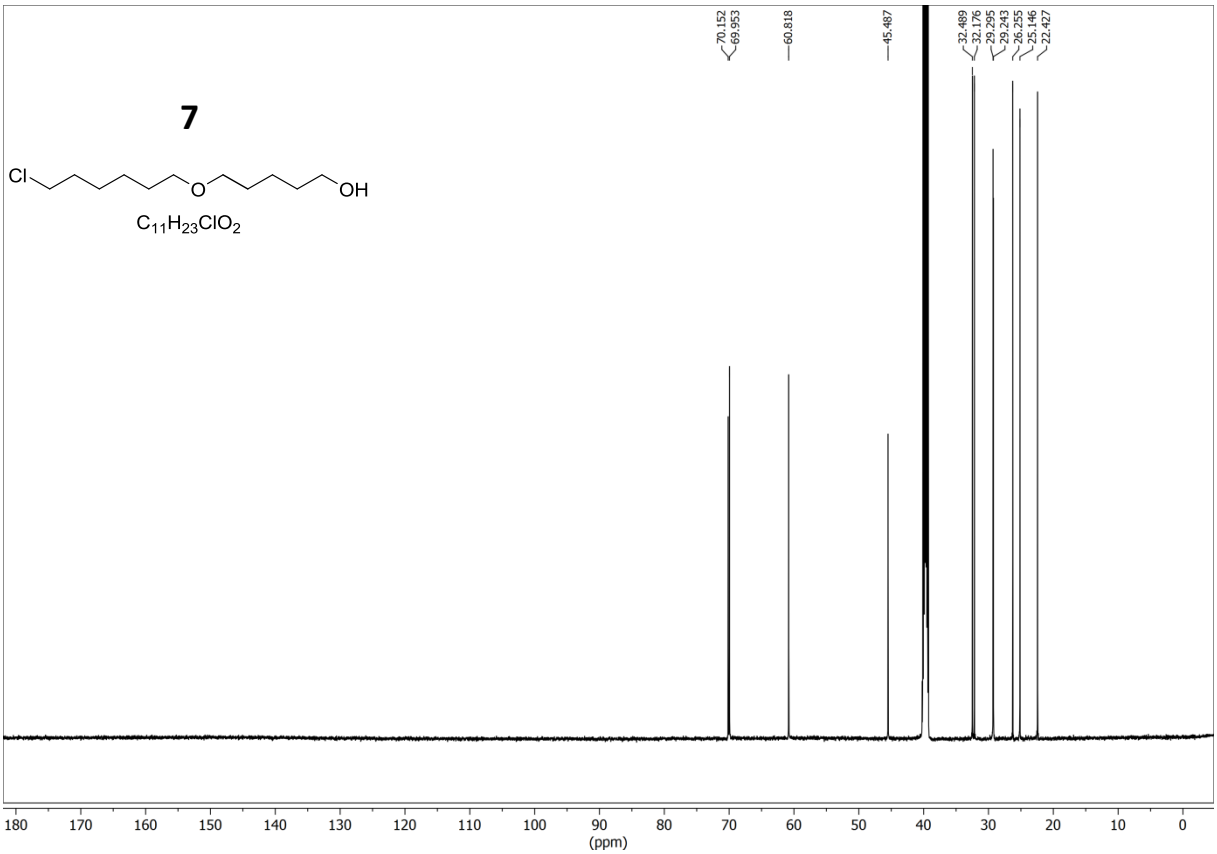
^{1}H and ^{13}C NMR spectrum of compound **4**.



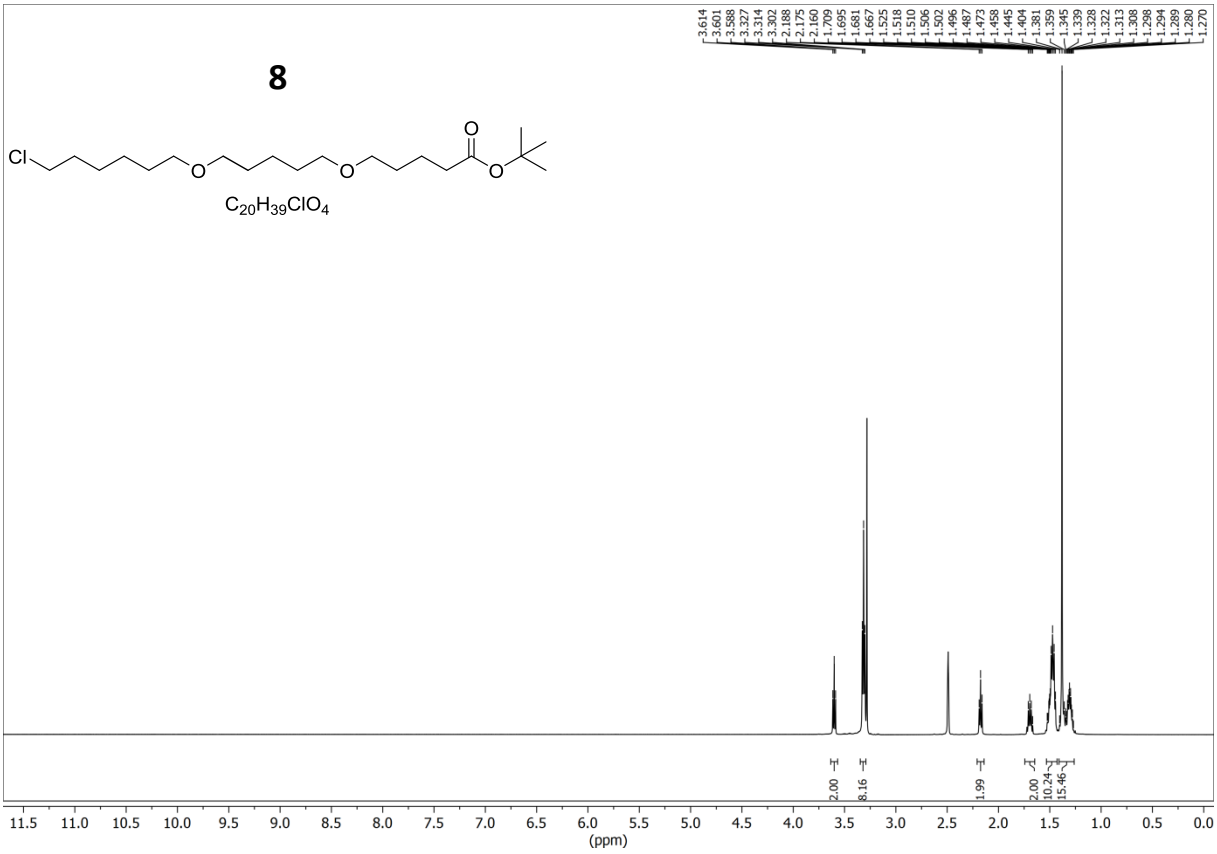


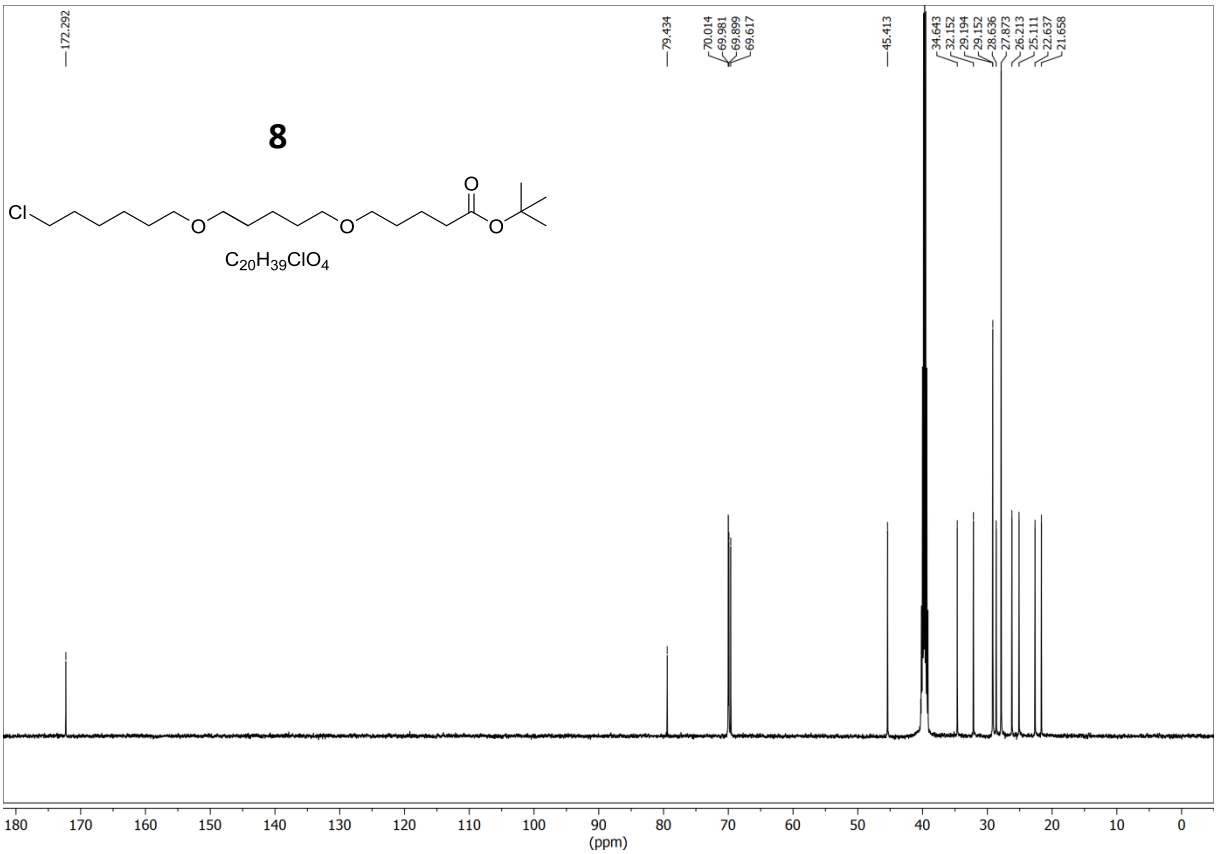
^{1}H and ^{13}C NMR spectrum of compound **7**.



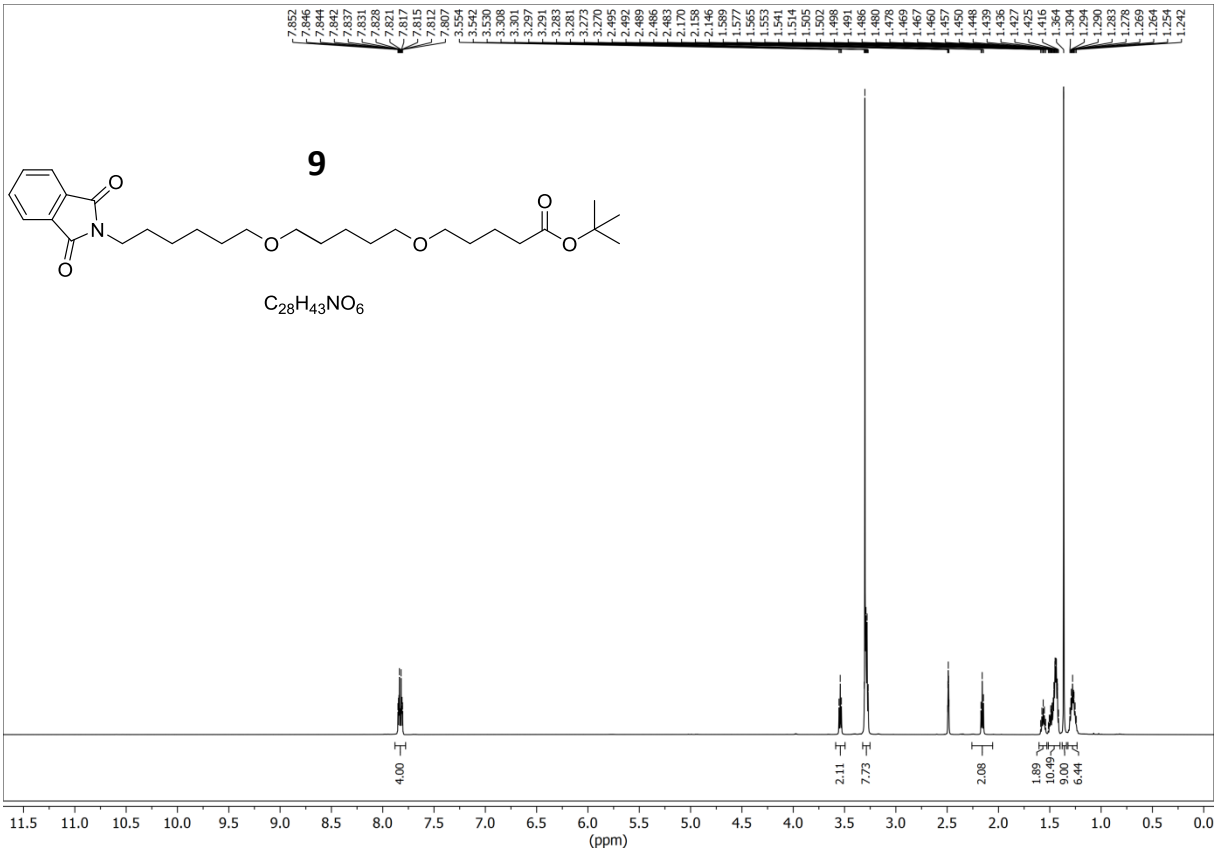


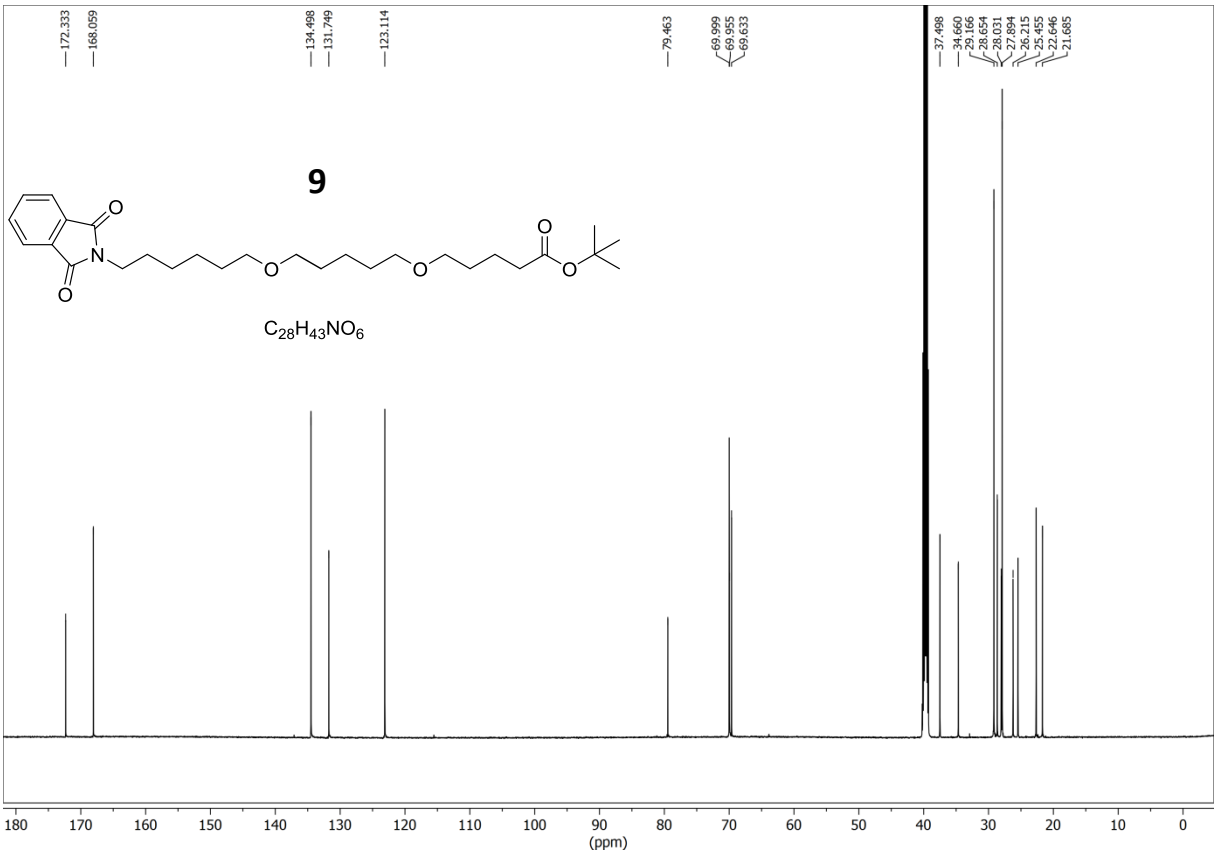
^{1}H and ^{13}C NMR spectrum of compound **8**.



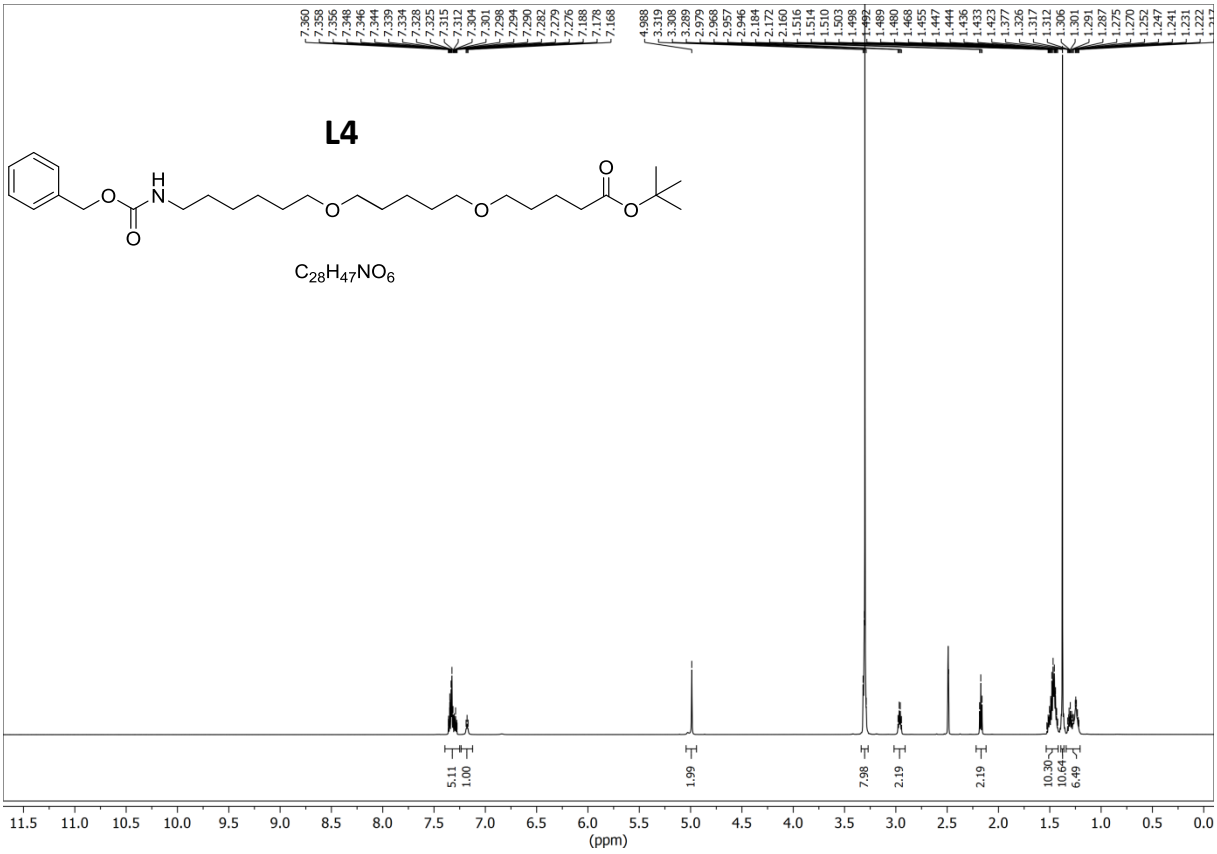


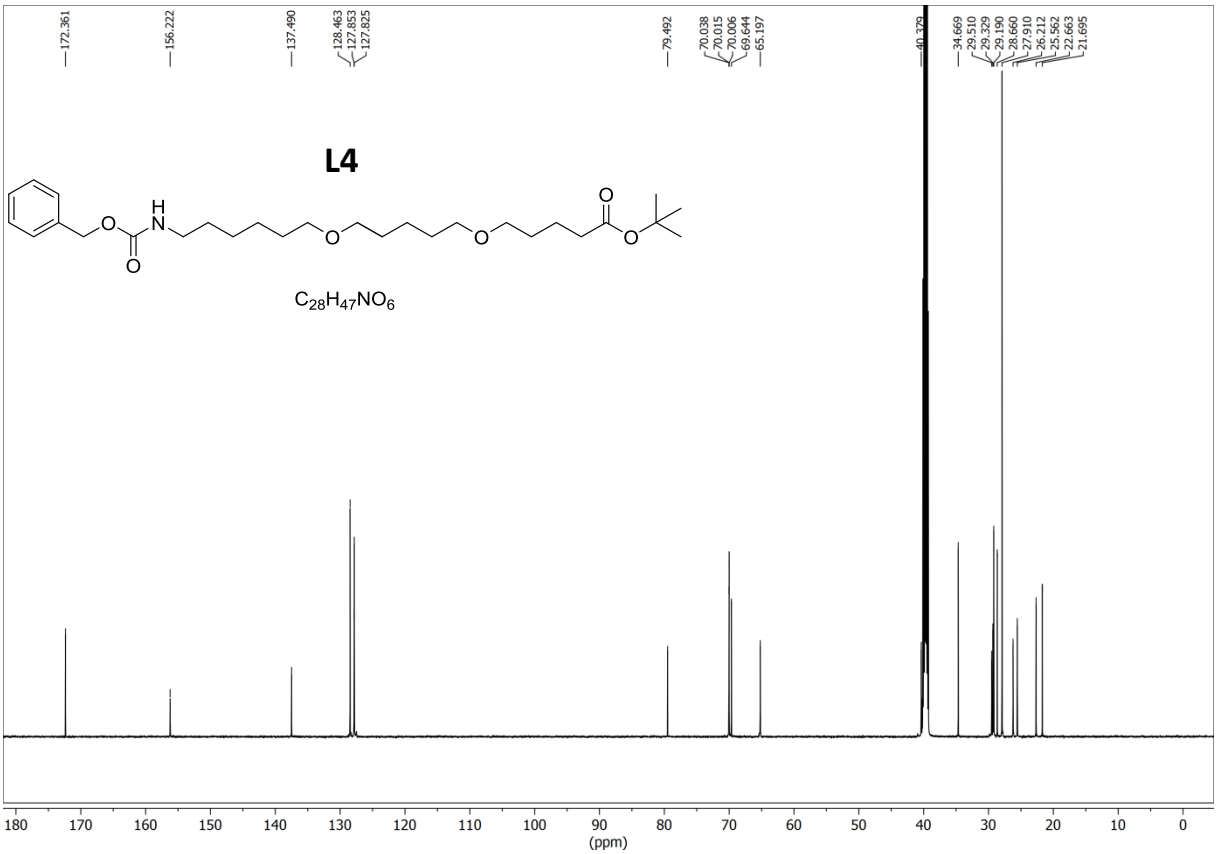
^{1}H and ^{13}C NMR spectrum of compound **9**.



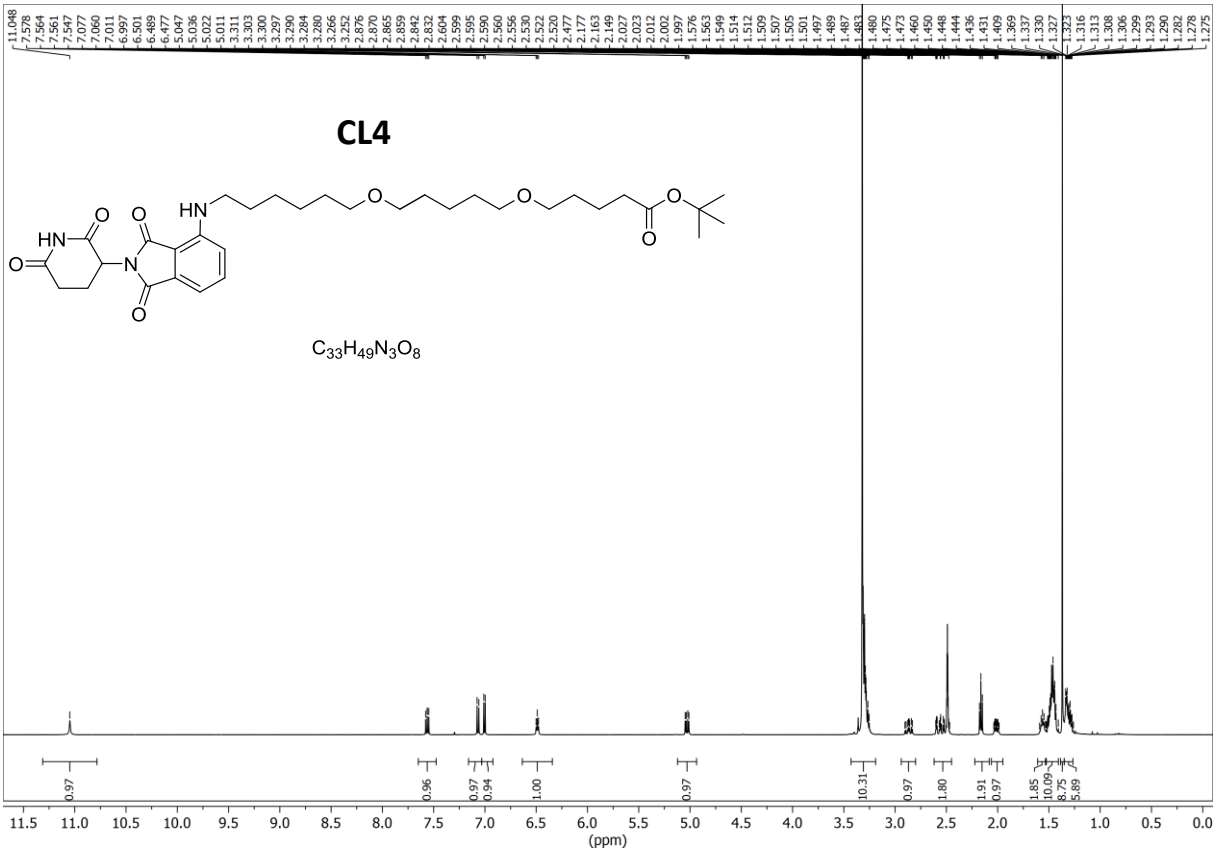


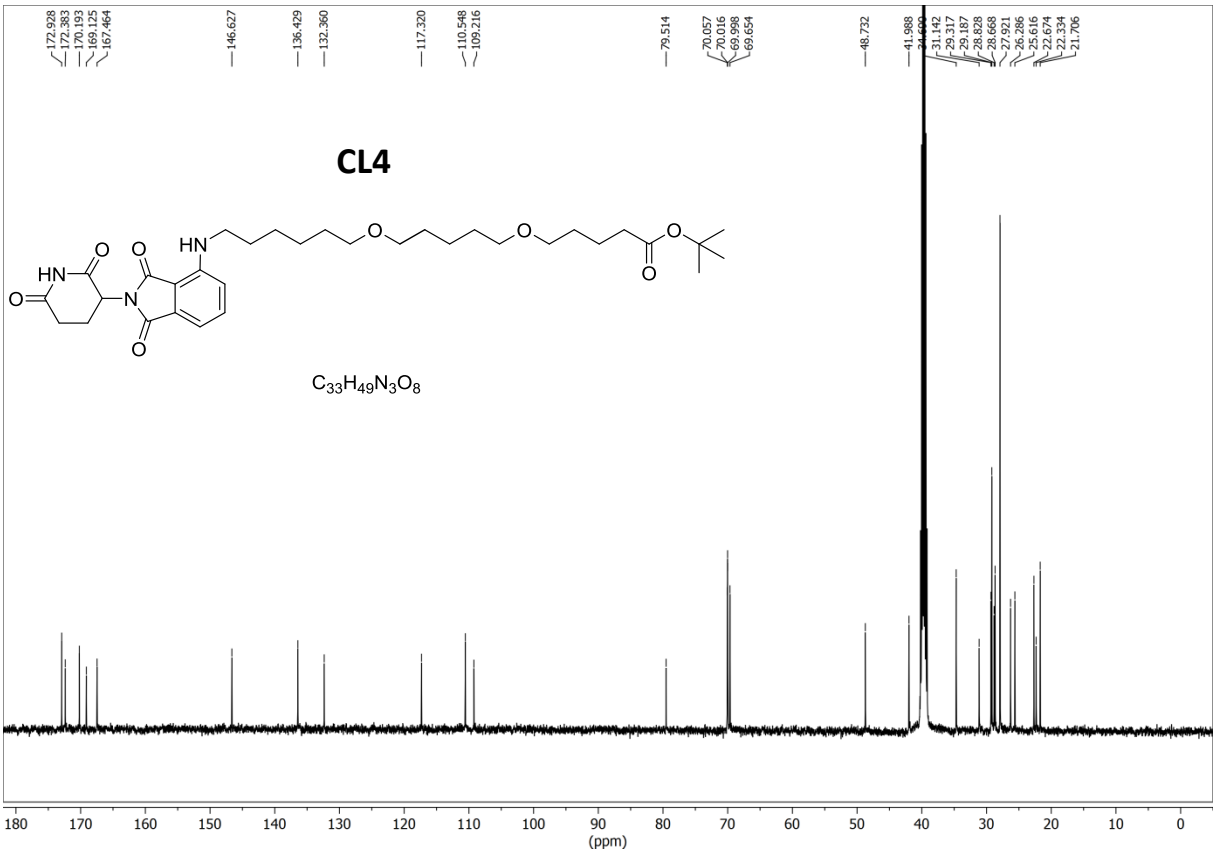
^{1}H and ^{13}C NMR spectrum of linker **L4**.



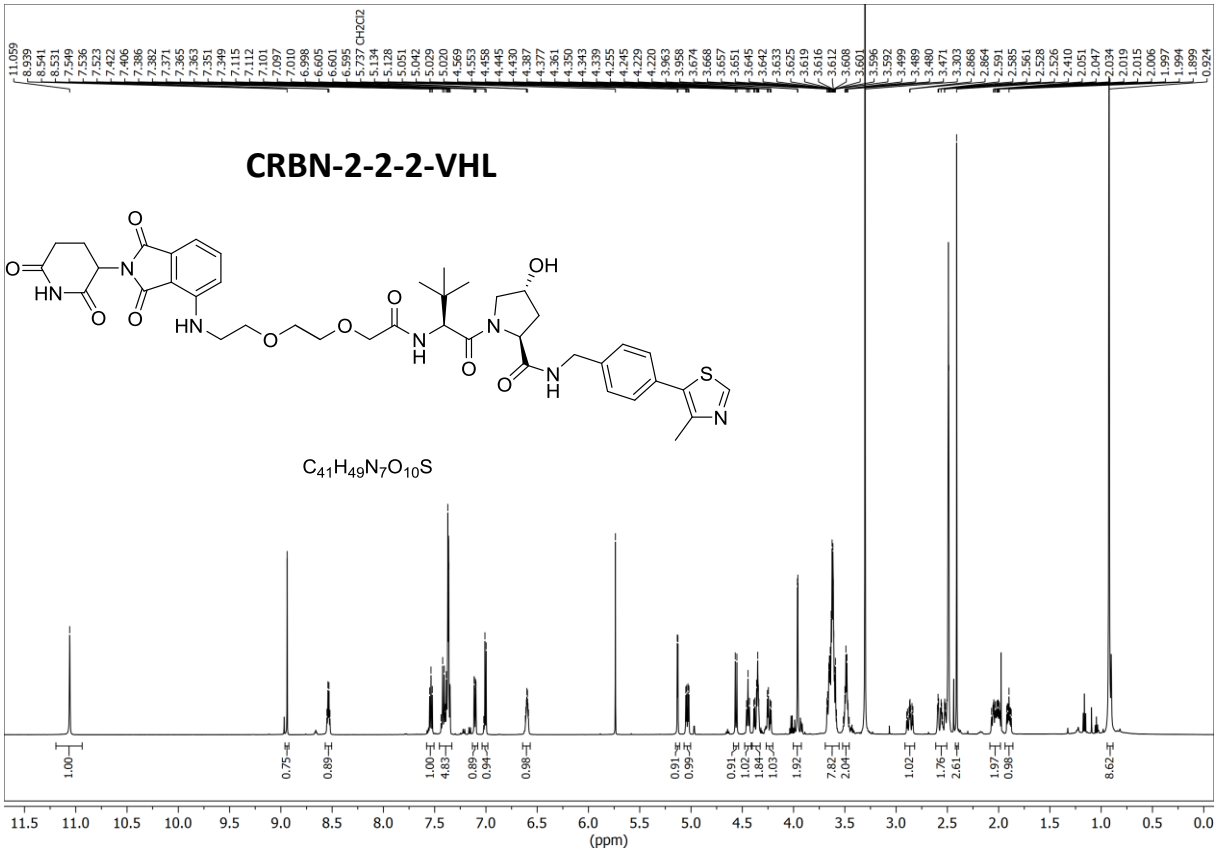


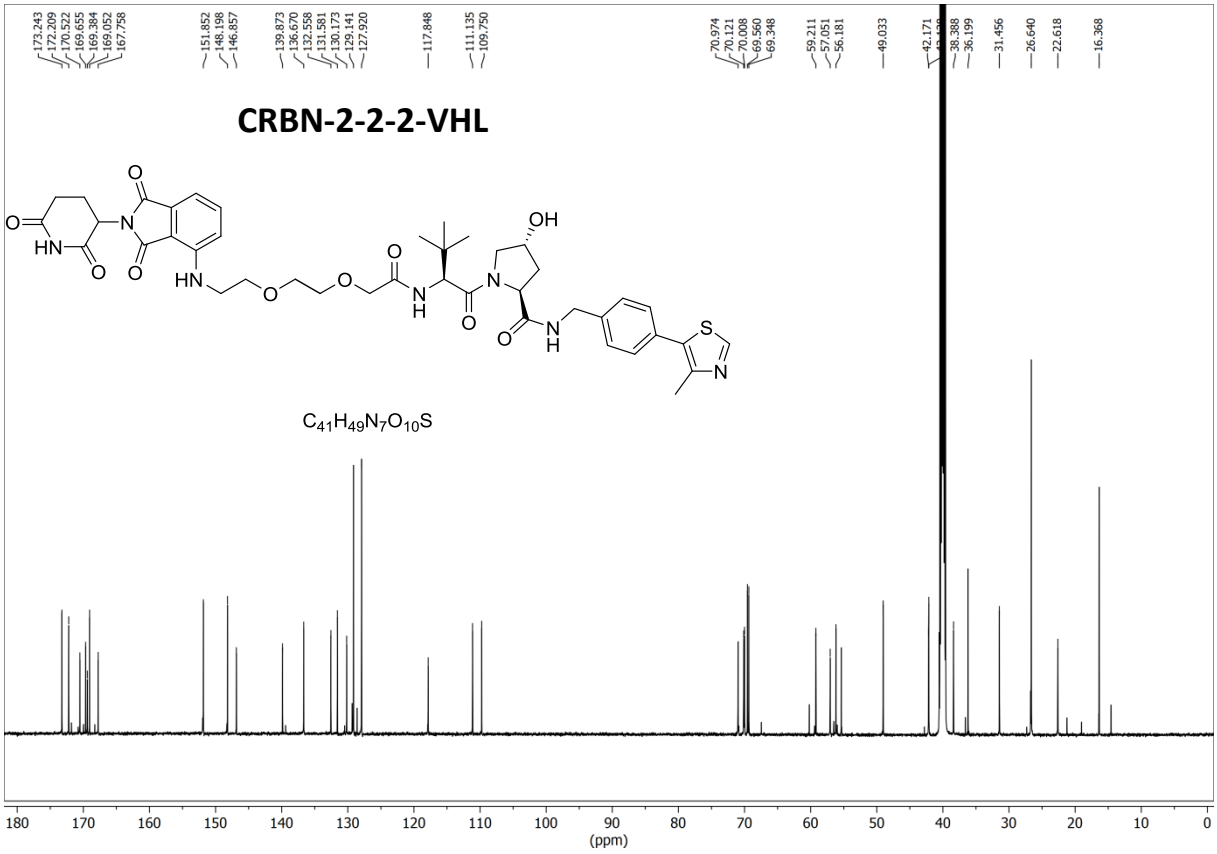
^{1}H and ^{13}C NMR spectrum of linker **CL4**.



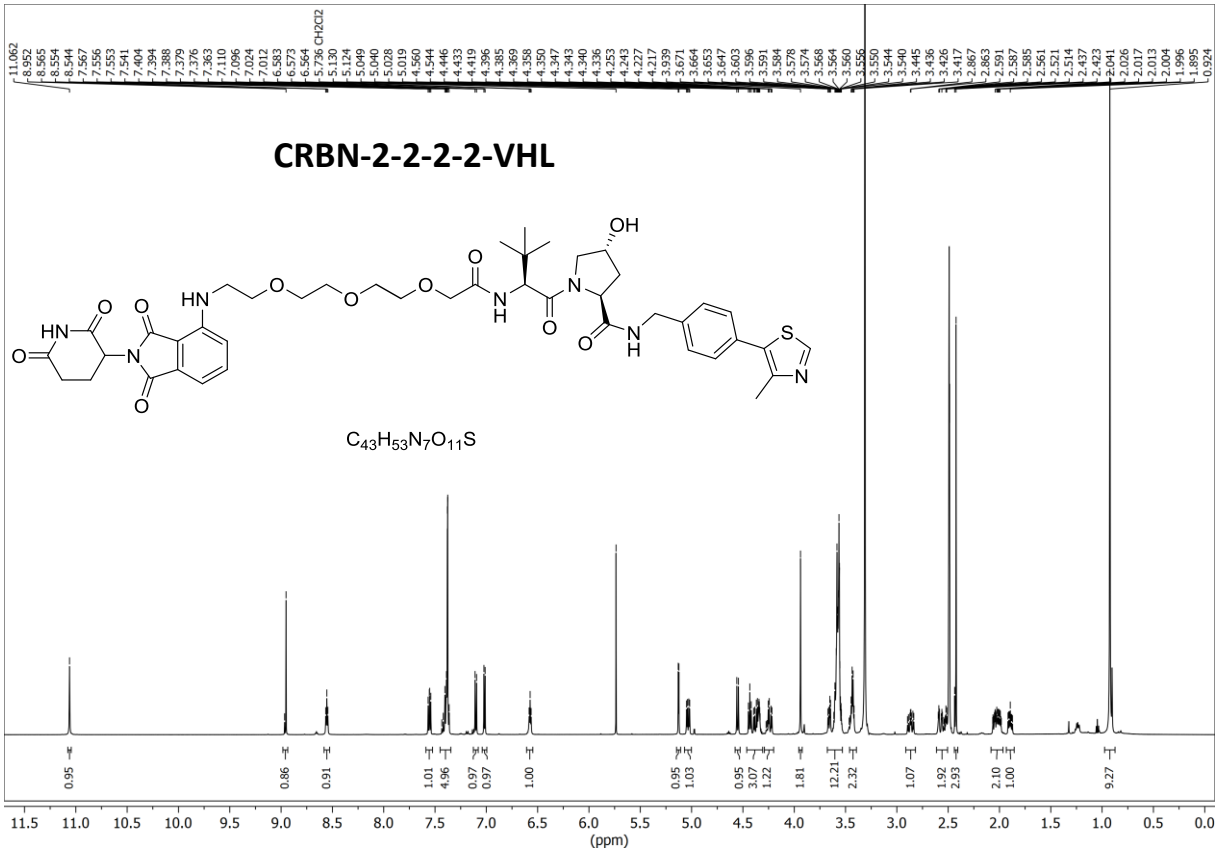


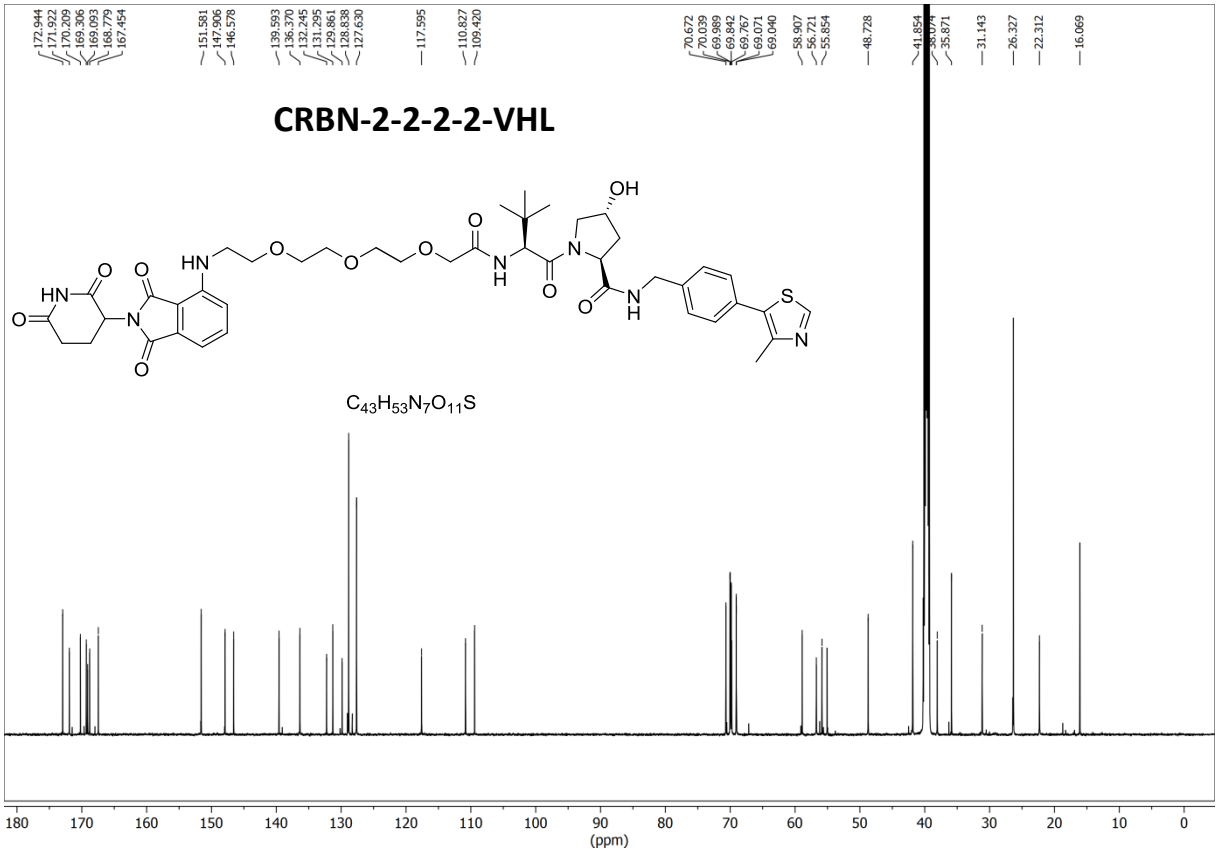
^{1}H and ^{13}C NMR spectrum of linker **CRBN-2-2-2-VHL**.



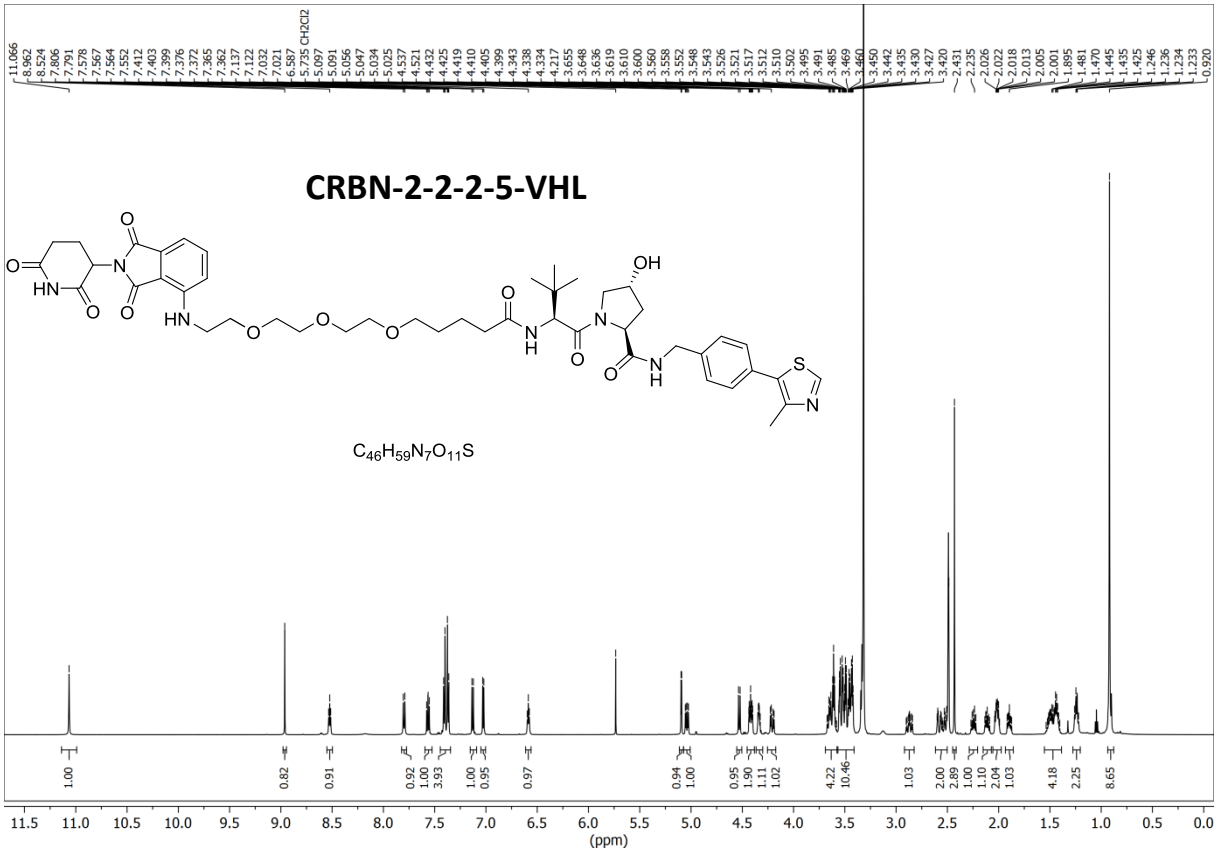


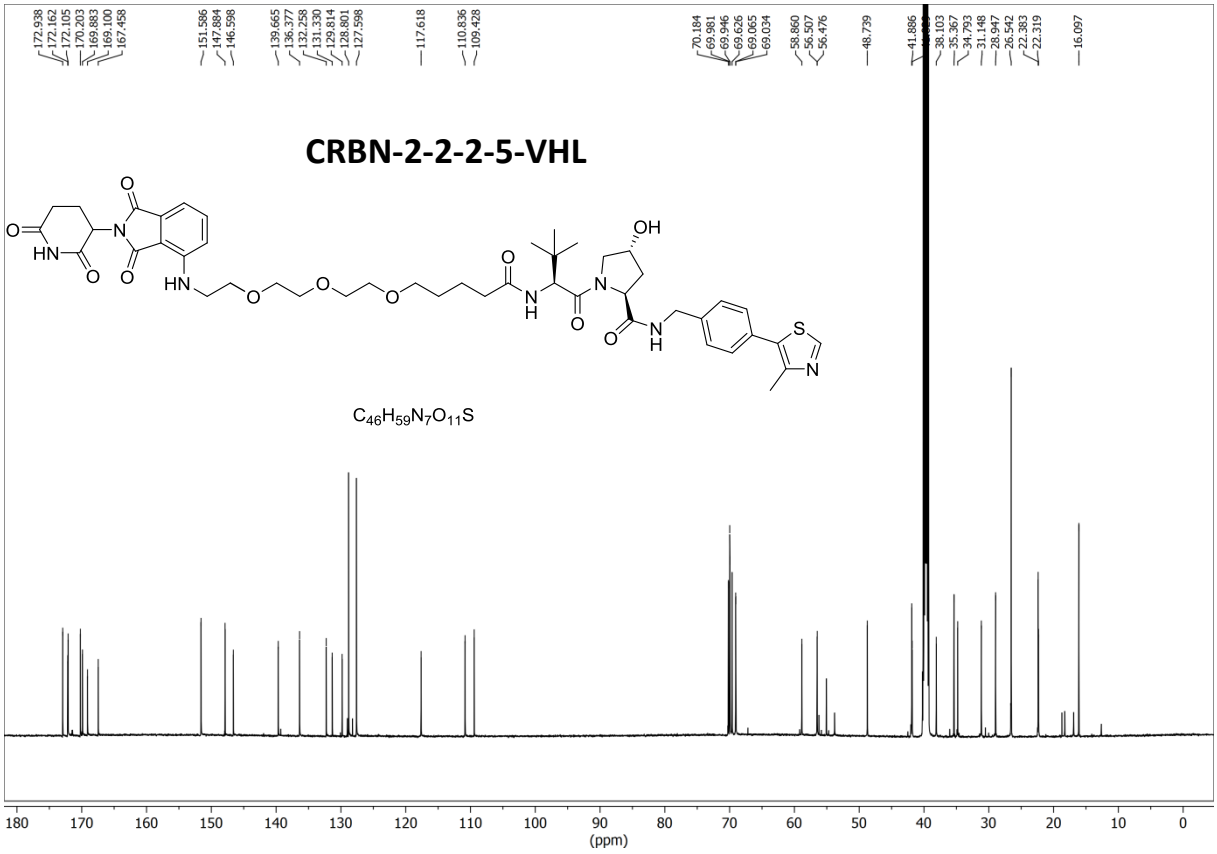
^{1}H and ^{13}C NMR spectrum of linker **CRBN-2-2-2-2-VHL**.



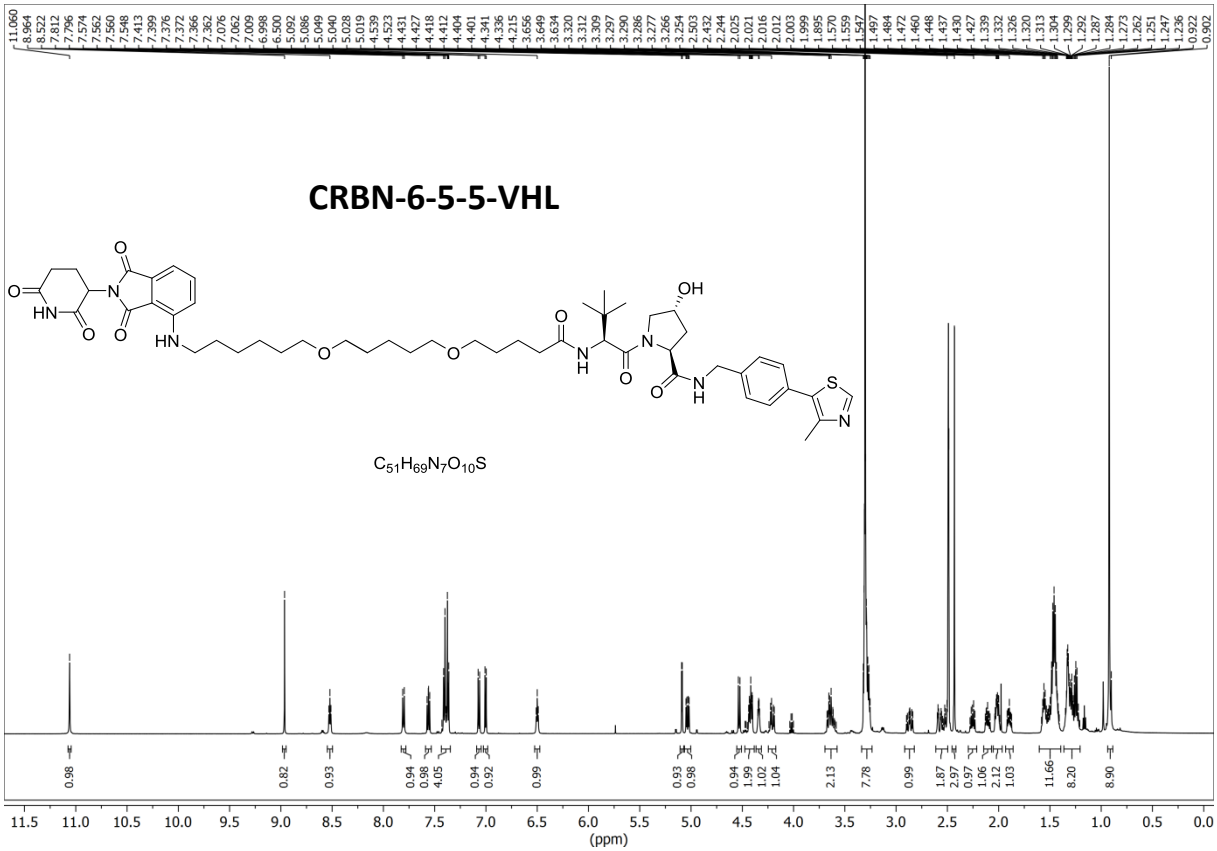


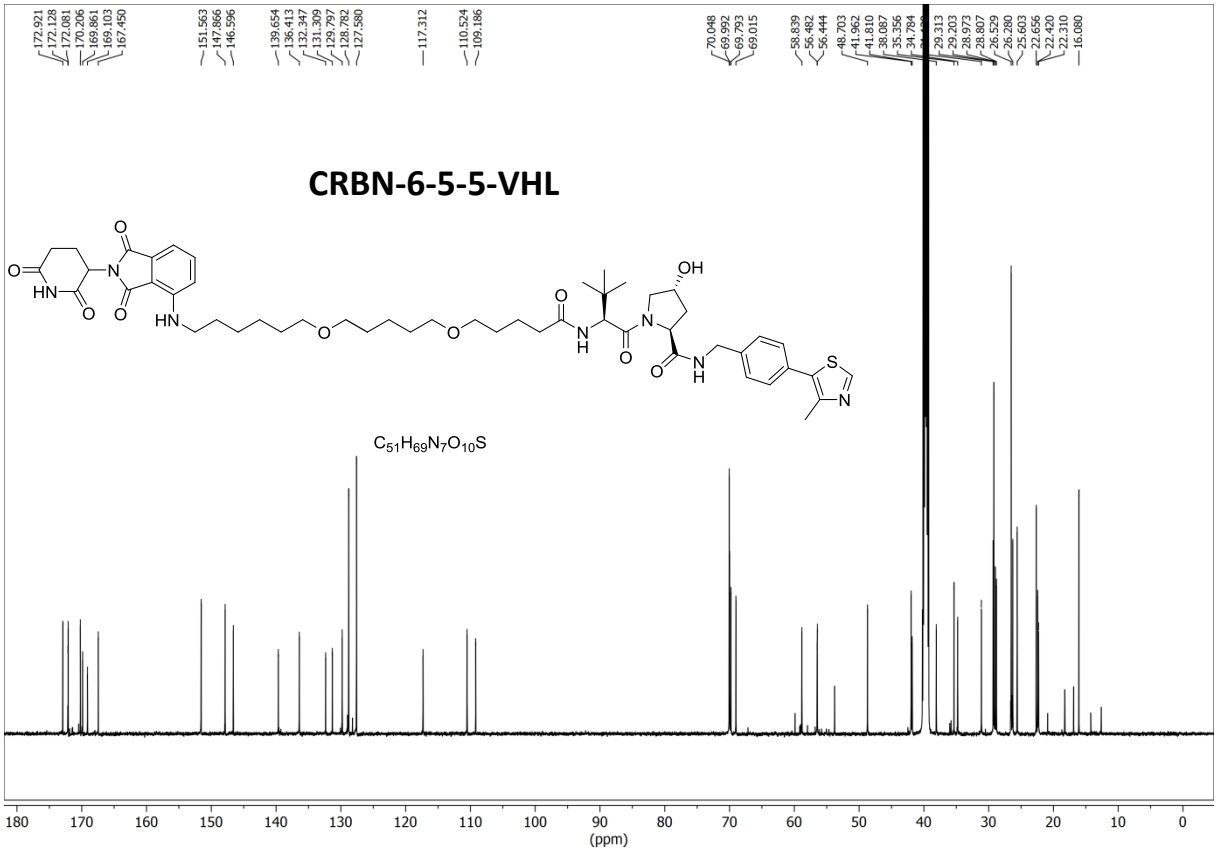
^{1}H and ^{13}C NMR spectrum of linker **CRBN-2-2-2-5-VHL**.



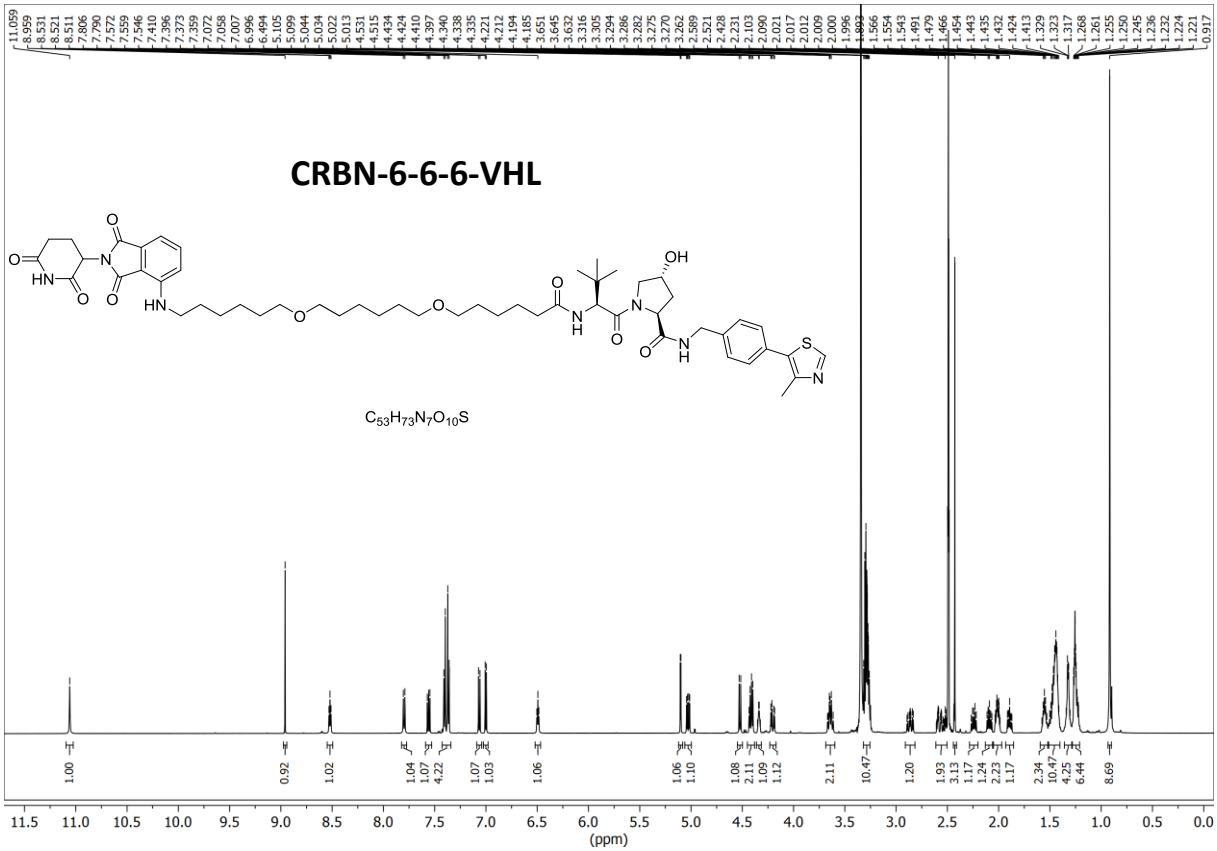


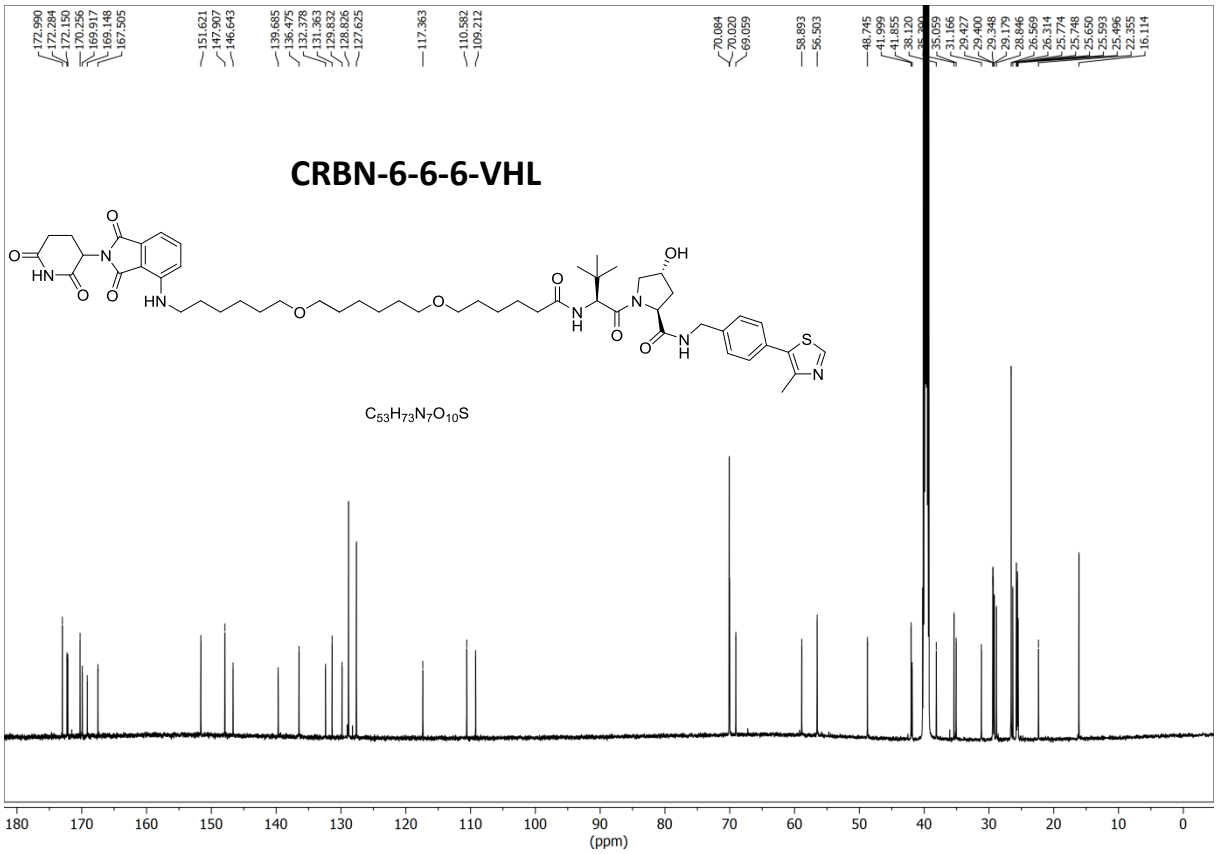
^{1}H and ^{13}C NMR spectrum of linker **CRBN-6-5-5-VHL**



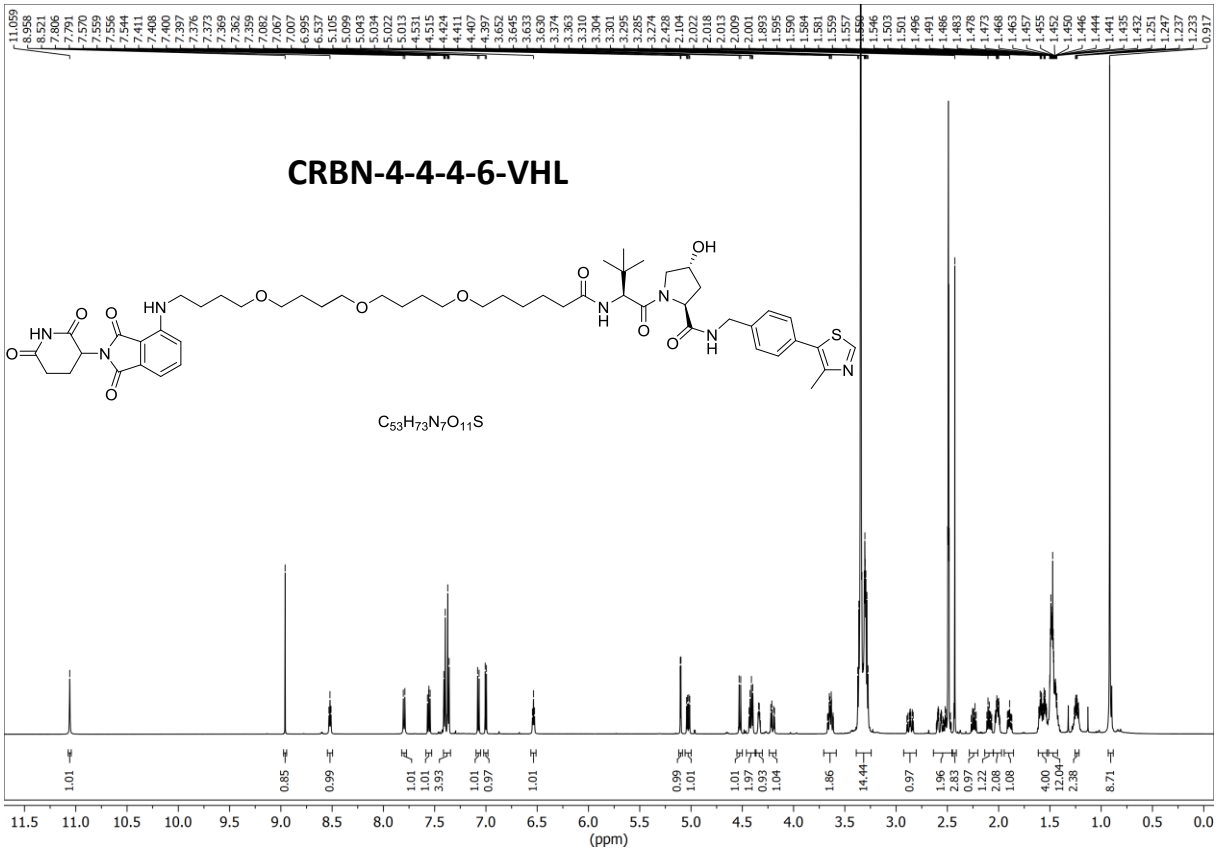


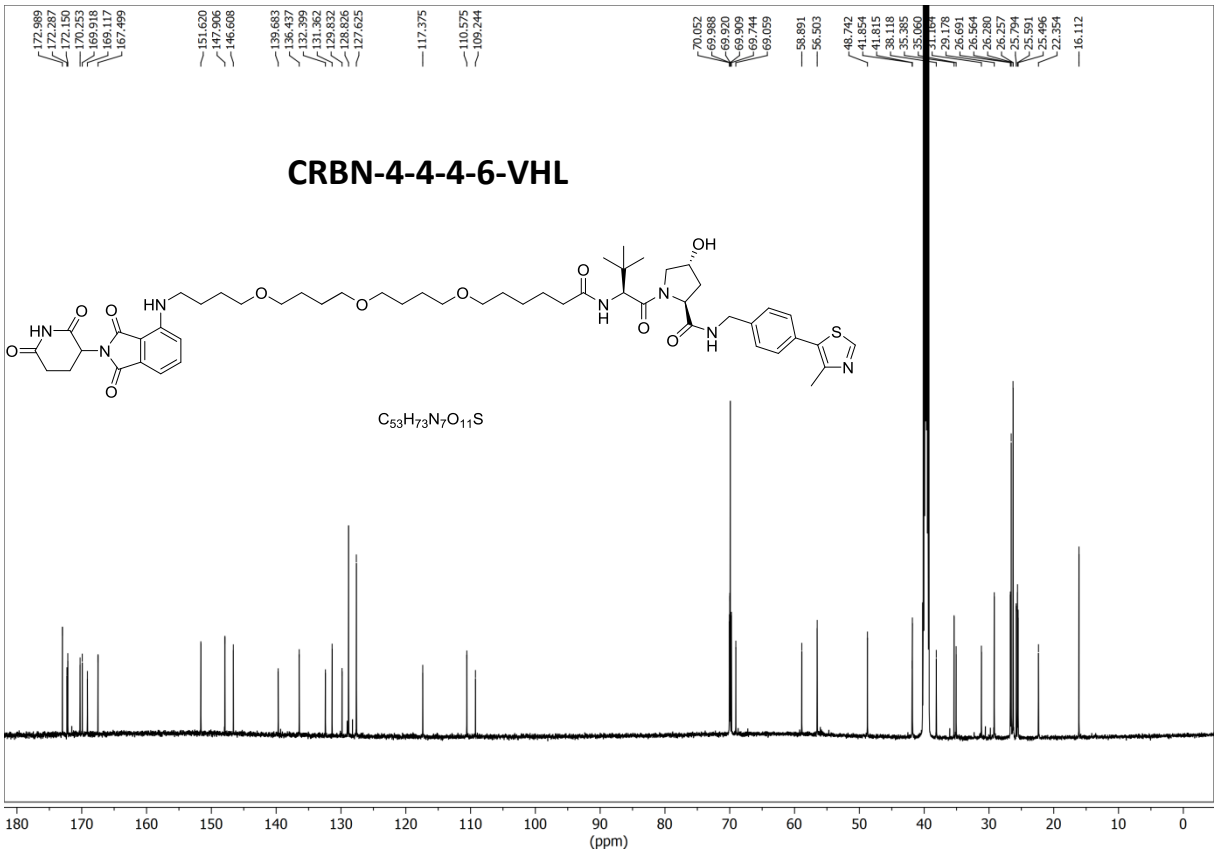
^{1}H and ^{13}C NMR spectrum of linker **CRBN-6-6-6-VHL**.



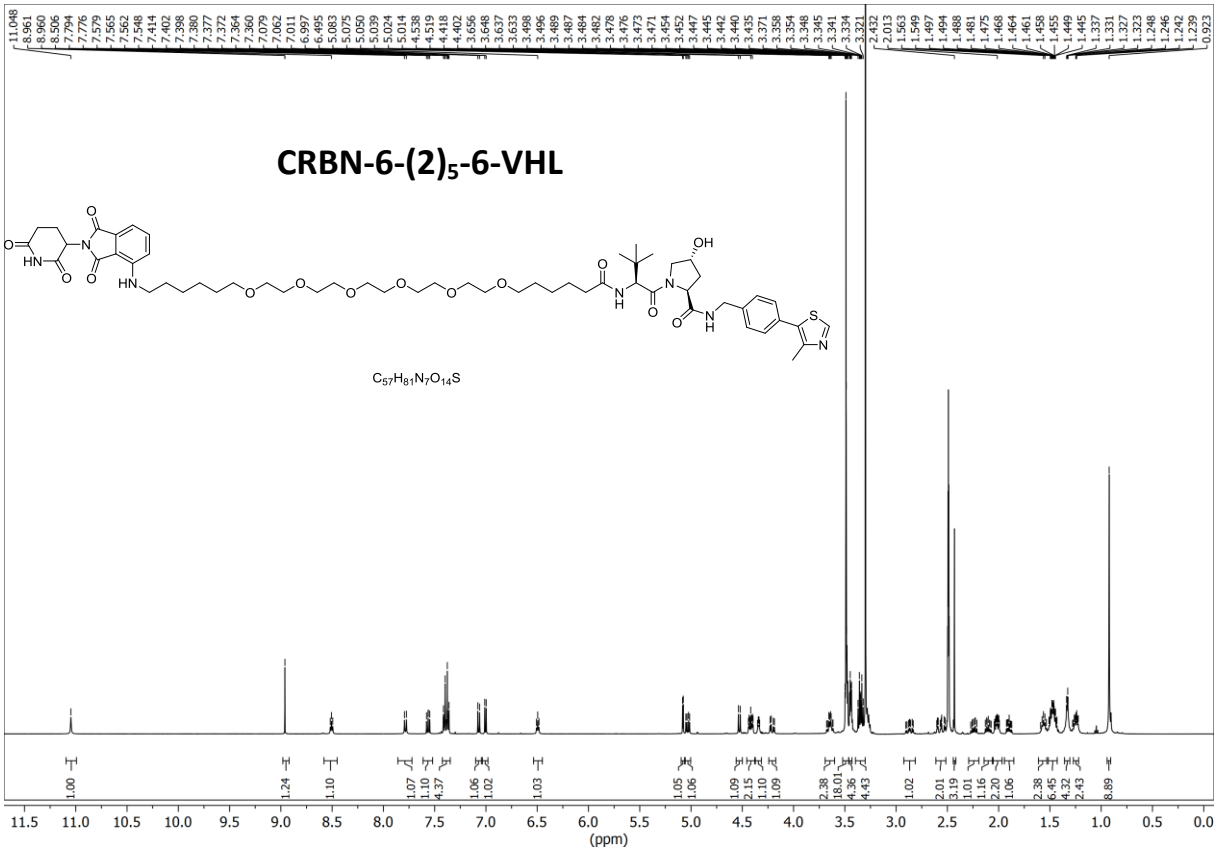


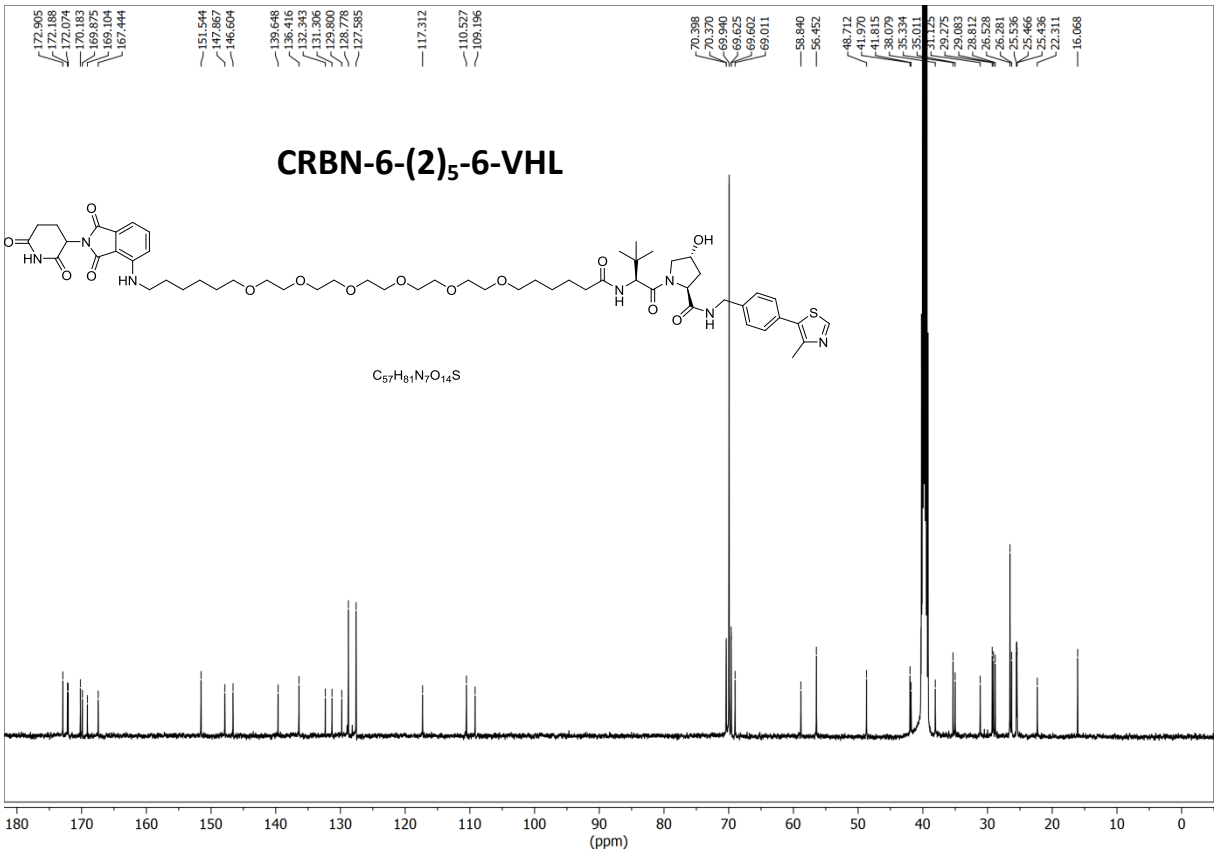
^{1}H and ^{13}C NMR spectrum of linker **CRBN-4-4-4-6-VHL**.



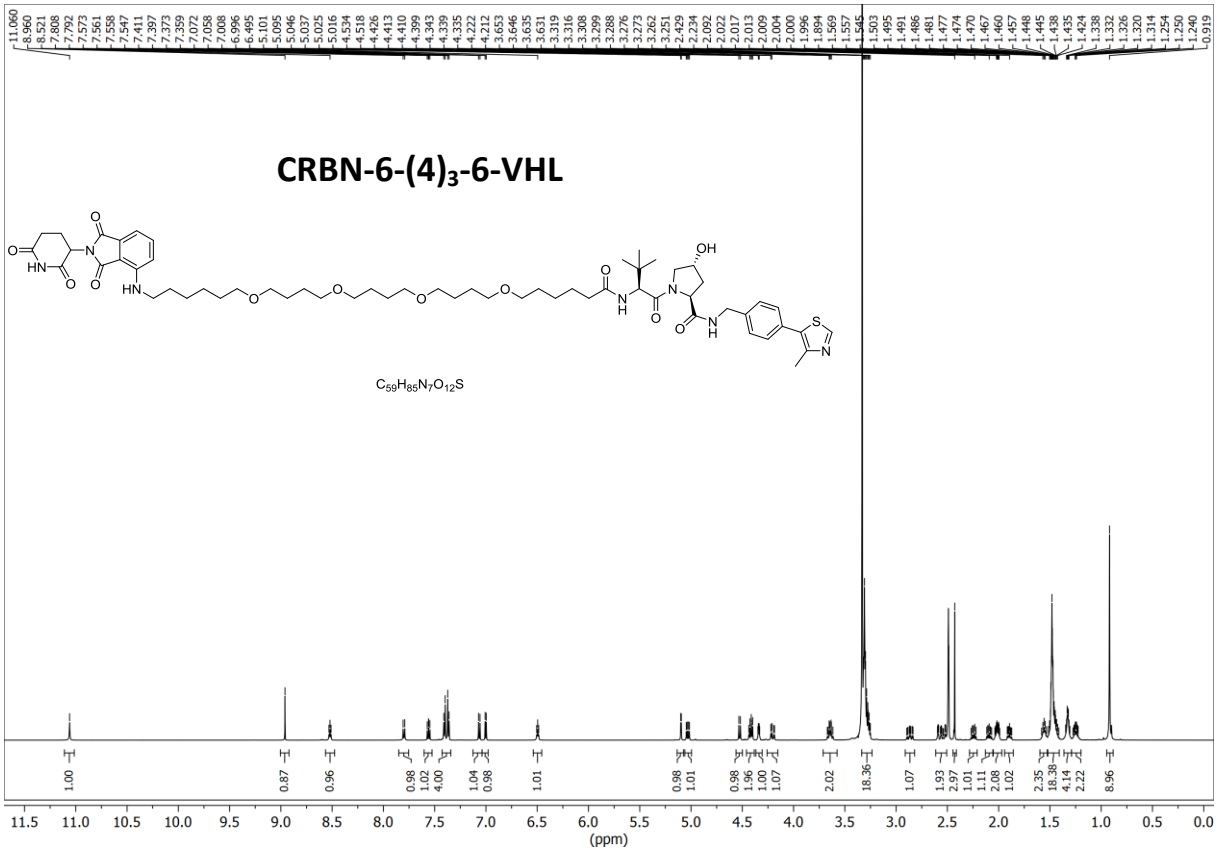


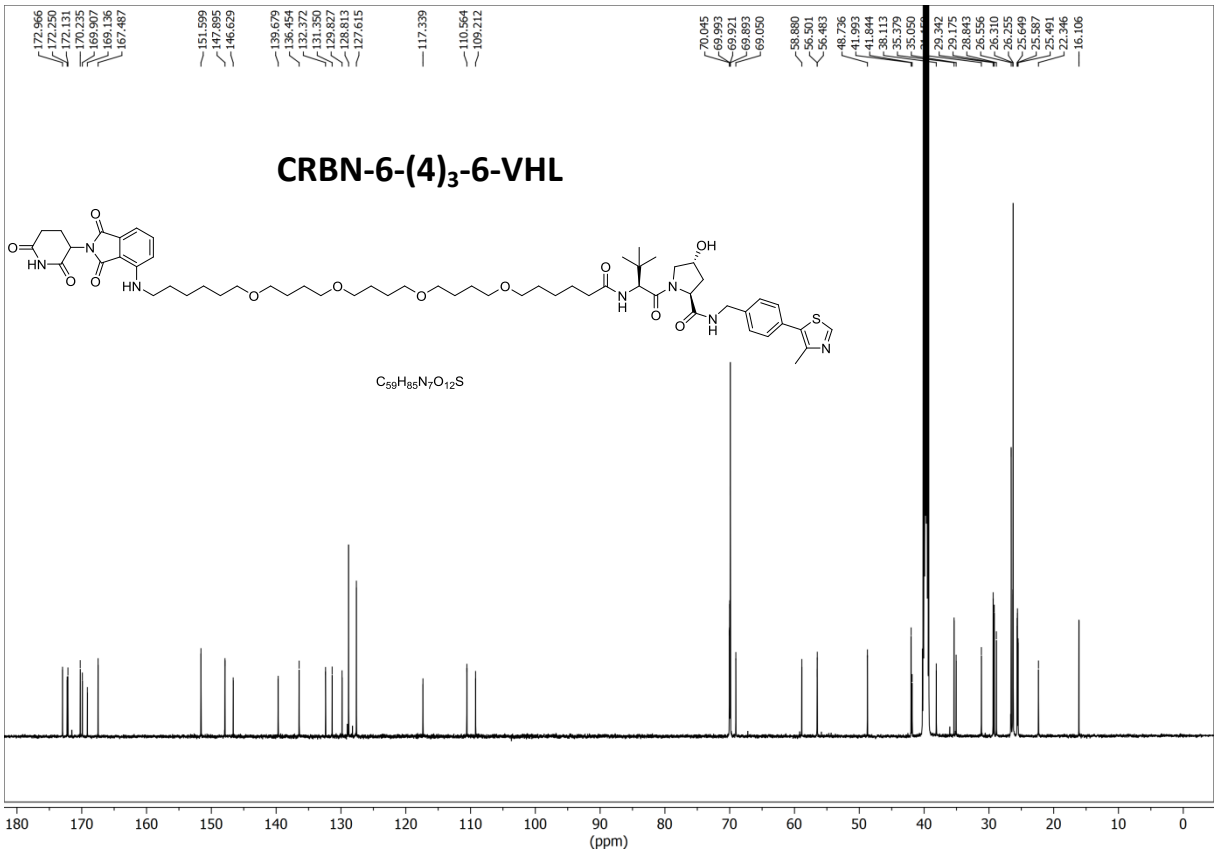
^{1}H and ^{13}C NMR spectrum of linker **CRBN-6-(2)$_5$-6-VHL**.





^{1}H and ^{13}C NMR spectrum of linker **CRBN-6-(4)$_3$-6-VHL**.





M. Molecular Descriptor Calculation

Predicted values for the topological polar surface area (TPSA)[10] were calculated using the KNIME[11] implementation of RDKit[12].

N. Physicochemical Measurements

The eLogP values of the PROTAC molecules were determined following the chromatographic method described in 2002 by Donovan and Pescatore.[13] Briefly, the molecules lipophilicity was estimated from their retention times under a rapid methanol-water gradient in a 10 × 4 mm Lichrospher 100 RP-18 5 µm EC precolumn (Chromatographie Service GmbH, Langerwehe, Germany). The chromatographic system was composed by a Waters 2695 Separations Module coupled with a Waters 996 Photodiode Array Detector. The column was operated at 25 °C and the buffer was prepared from a 0.01 M sodium phosphate solution adjusted to pH 5.0 with ortophosphoric acid to ensure the presence of neutral compounds during the determinations. Experimental conditions were set to a 1.50 mL/min flow-rate and a linear gradient from 10 to 100% MeOH in 10 min, followed by 6 min equilibration time between runs. The 269.5 nm output from the diode array UV detector was measured. With the purpose of determining the eLogP values of the analytes, the elution times from two internal standards, toluene (tol) and triphenylene (triph), were taken as a reference. An initial solution was prepared by adding 10 mg triphenylene to 1 mL of toluene and these were diluted up to 100 mL of methanol. Approximately 1 mg of the analytes was dissolved in 1 mL of the initial solution and 10 µL of the solution were injected. Under the previously described chromatographic conditions, toluene and triphenylene eluted at 4.670 ± 0.118 and 8.290 ± 0.050 min, respectively. Knowing the retention times of the standards and analytes, as well as the logP values of the two standards, the elogP of the analytes can be easily calculated by means of the following equation:

$$logP_{analyte} = \frac{\left(logP_{tol} - logP_{triph}\right) t_{analyte} + t_{tol}\, logP_{triph} - t_{triph}\, logP_{tol}}{t_{tol} - t_{triph}}$$

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