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Supplementary Information

Accessing three-branched high-affinity cereblon ligands for molecular glue and protein degrader design

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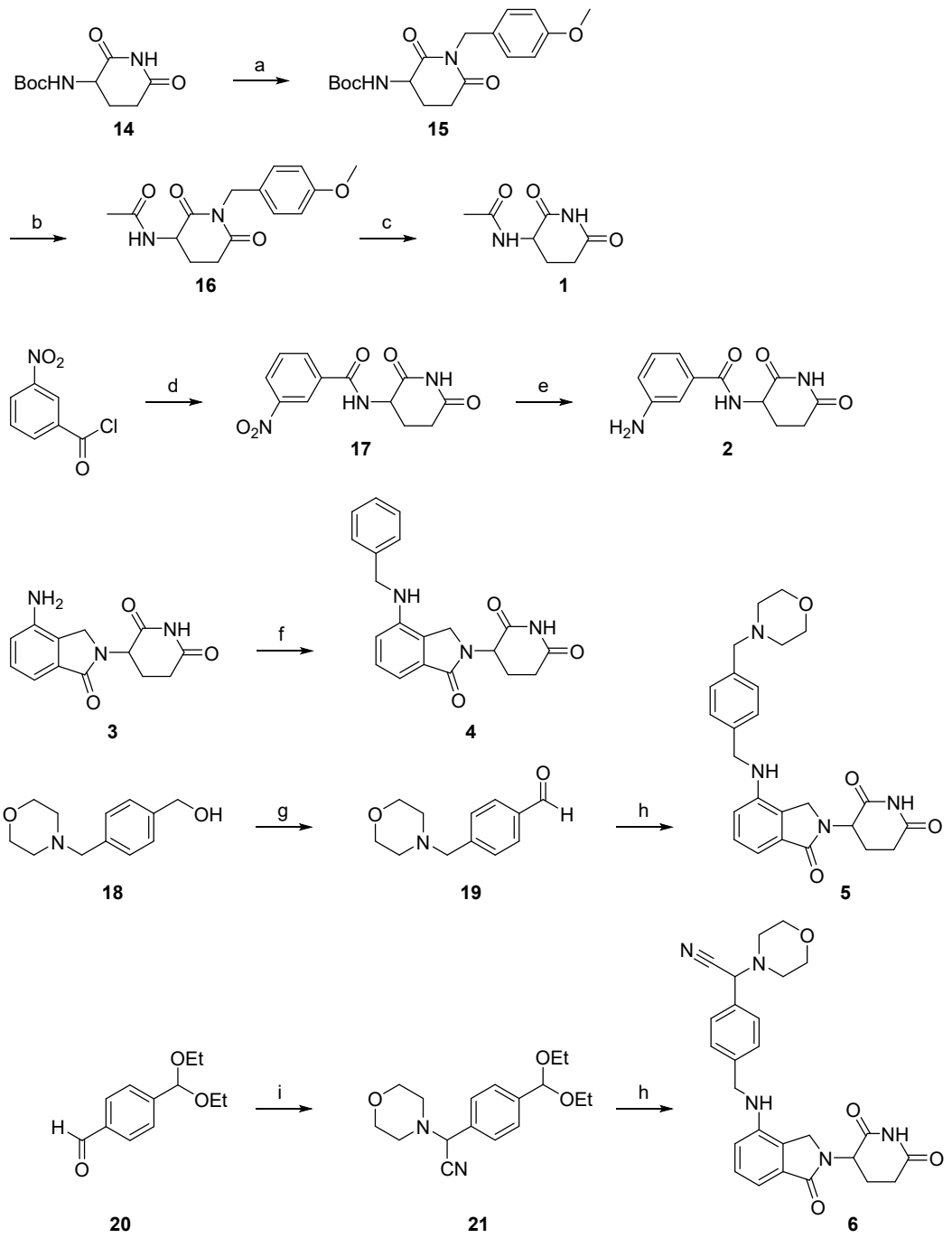
Supplementary Tables, Schemes and Figures

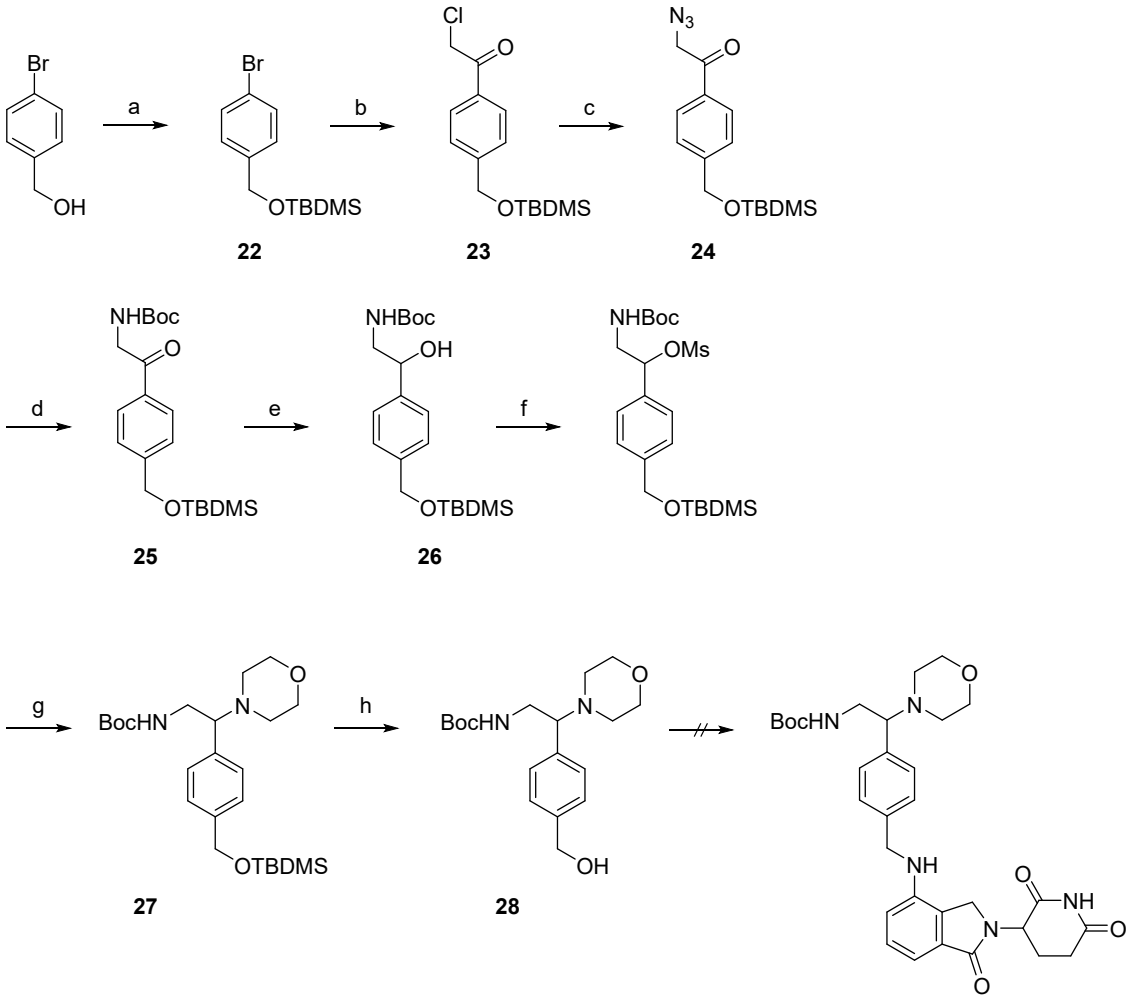
Table S1 Overview on predicted ADME properties.

cmpd	TPSA[a] (Å²)	QPlogS[b]	QPlogHERG[c]	QPPCaco[d] (nm/s)	QPPMDCK[e] (nm/s)
2	101	–2.5	–4.2	81	33
Lenalidomide (3)	93	–2.6	–3.9	100	41
4	79	–4.7	**–5.9**	397	182
5	91	–4.1	**–6.8**	76	34
5[f]	92	–4.2	**–6.8**	72	32
6	115	–5.0	**–7.0**	**20**	**8,0**
9	120	–4.4	**–5.9**	**25**	**15**
Iberdomide	88	–4.1	**–6.9**	106	48
Iberdomide[f]	89	–4.1	**–6.8**	80	36

[a] Topological polar surface area is given in Å². [b] Logarithm of the predicted aqueous solubility, for which S is given in mol × dm^{-3}. [c] Predicted $logIC_{50}$ values for the blockage of HERG K^+ channels. Concern if $logIC_{50}$ is below –5. [d] Predicted apparent Caco-2 cell permeability (non-active transport), a model for the gut-blood absorption. Compounds with values <25 are considered poorly permeable. [e] Predicted apparent MDCK cell permeability, a model for crossing the blood-brain barrier. Compounds with values <25 are considered poorly permeable. [f] Protonated morpholine nitrogen.



Scheme S1: Syntheses of imides **1**–**6**. *Reagents and conditions:* (a) PMB-Cl, K_2CO_3, DMF, rt, ultrasonic, 2 h; (b) Ac_2O, NaOAc, AcOH, 6 h, 100 °C; (c) CAN, MeCN, 2 h, rt; (d) 3-aminopiperidine-2,6-dione hydrochloride, Et_3N, CH_2Cl_2, 0 °C to rt, 18 h; (e) Pd/C, H_2, DMF, rt, 18 h; (f) PhCHO, TES, TFA, CH_2Cl_2, MeCN, rt, 16 h; (g) TEMPO, TBAI, *N*-chlorosuccinimide, 0 °C to rt, 18 h; (h) **3**, TES, TFA, CH_2Cl_2, MeCN, rt, 16 h; (i) KCN, morpholine, H_2O/MeOH, rt, 2 d.



Scheme S2: Linear synthetic approach towards a possible three-branched CRBN ligand. *Reagents and conditions:* (a) TBDMS-Cl, imidazole, DMF, rt, 18 h; (b) 2-chloro-*N*-methoxy-*N*-methylacetamide, *n*-BuLi, –78 °C, 1 h, 40 °C, 1 h; (c) NaN_3, MeCN, 60 °C, 3 h; (d) Pd/C, H_2, $(Boc)_2O$, EtOAc, rt, 2 h; (e) $NaBH_4$, EtOH, 0 °C to rt, 3 h; (f); MsCl, DIPEA, CH_2Cl_2, 0 °C, 30 min; (g); morpholine, DIPEA, CH_2Cl_2, rt, 16 h; (h) TBAF, THF, 0°C to rt, 16 h.

*Supplementary text: For the attachment of **28** to the lenalidomide scaffold, several approaches were tried, for instance, by oxidation of the alcohol to the aldehyde and subsequent reductive amination/alkylation or mesylation and subsequent nucleophilic substitution. In the first attempt, the oxidation system TEMPO/TBAI/NCS in CH_2Cl_2 and an aqueous buffer of 0.5 M $NaHCO_3$ and 0.05 M K_2CO_3 did not provide the aldehyde derived from **28**. Second, the oxidation system BAIB/TEMPO in CH_2Cl_2 failed as well. Lastly, the in situ mesylation and subsequent addition of DIPEA and lenalidomide in CH_2Cl_2 did not yield the final compound either. Due to insufficient amounts of material no further conditions could be employed and the desired product could not be obtained.*

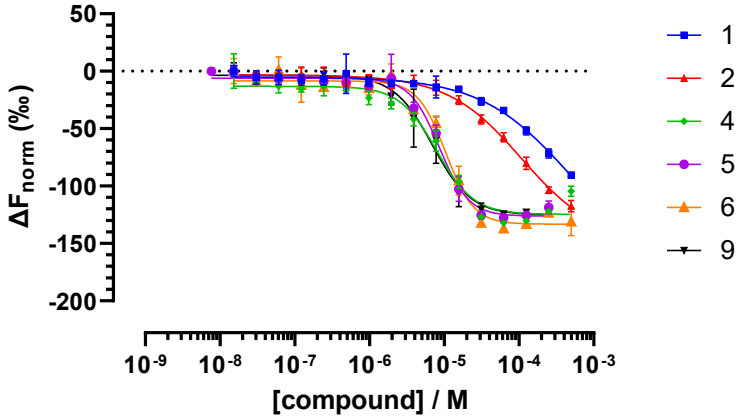


Figure S1: Dose-response curves for compounds **1**-**2**, **4**-**6**, and **9** obtained in competitive MST measurements with BODIPY-uracil and hTBD. Data shown as mean ± s.d. (n=3). See methods for details.

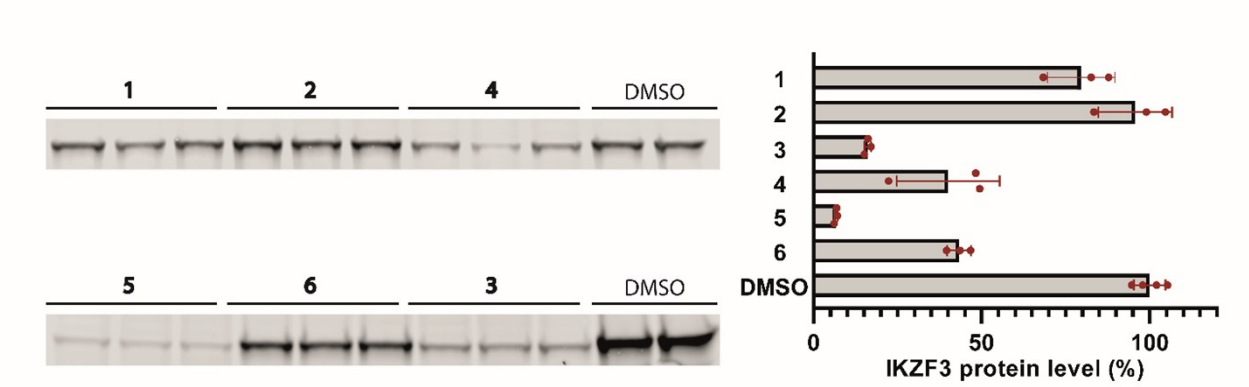


Figure S2: IKZF3 degradation by compounds **1**-**6** in OPM2 cells treated for 24 h at a concentration of 10 µM. Bar graph depicting quantification of remaining IKZF3 protein levels normalized to total protein stain signal and DMSO treated probe. Data is represented as mean ± s.d. (n=3)

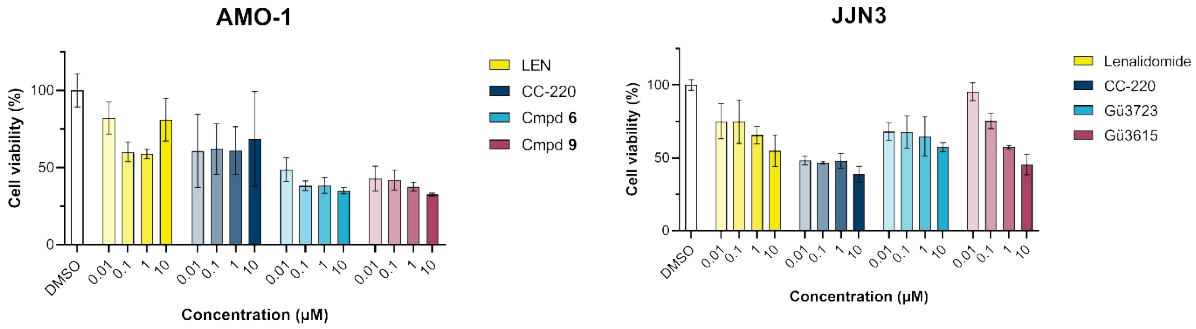


Figure S3: CellTiter-Glo luminescent cell viability assay upon a 96 h treatment with lenalidomide (LEN), CC-220, or three-branched compounds **6** and **9** in the plasmacytoma cell line AMO-1 and the plasma cell leukemia cell line JJN3. Data are shown as mean ± s.d. (n = 3).

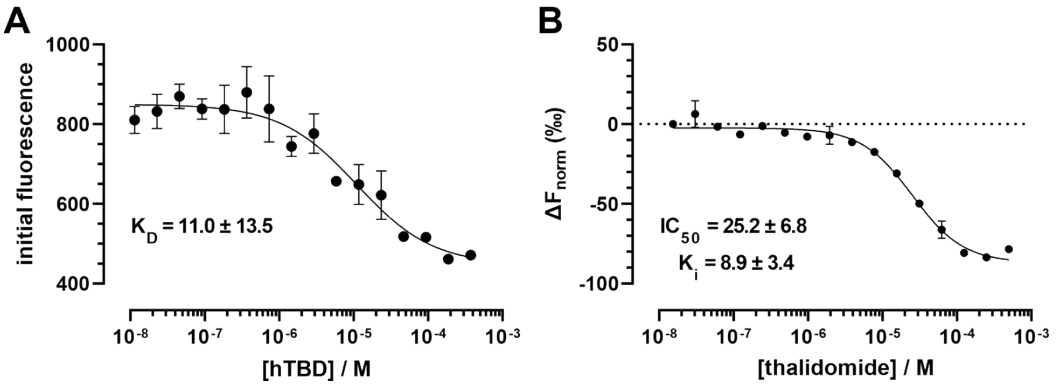


Figure S4: Competitive MST experiments using reporter compound **11**. (A) Dose-response curve and dissociation constant (K_d) for direct binding of **11** to the human thalidomide binding domain (n = 3) evaluated by initial fluorescence. (B) Dose-response curve and affinity values (IC_{50} and K_i) for a competitive MST experiment with thalidomide (n = 2) using the reporter **11**. The K_i of 8.9 µM for thalidomide obtained with this reporter (**11**) is in very good agreement with that previously obtained using the reporter BODIPY-uracil (K_i of 8.5 µM for thalidomide).[1] All values in µM, presented as mean ± 95% CI.

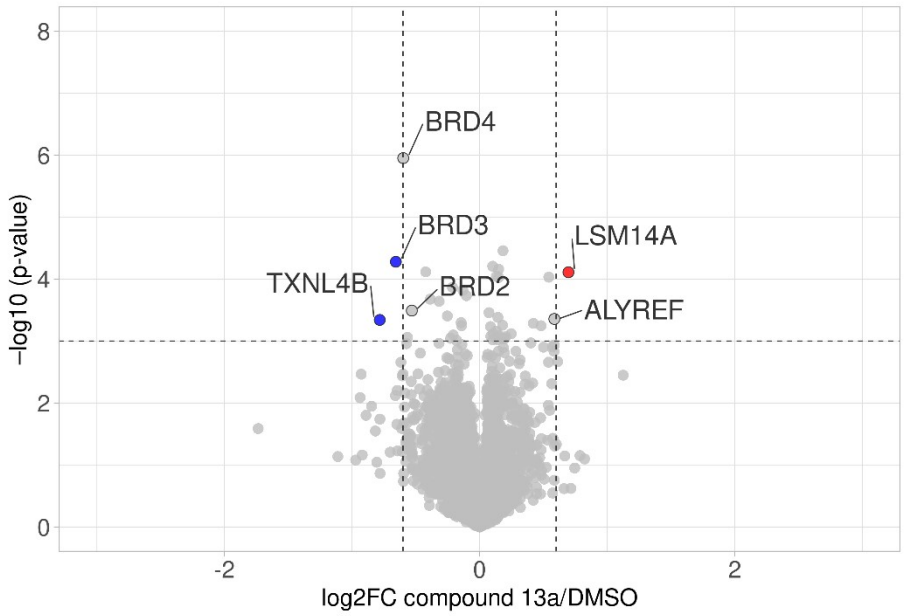


Figure S5: diaPASEF quantitative proteomics for PROTAC **13a**. MOLT4 cells were treated with compound **13a** at 0.01 µM for 8 h. Bioconductor's limma package was used to perform statistical analysis of degrader treatment compared to DMSO vehicle treatment. The identified proteins were plotted as log2 fold change (PROTAC/DMSO) versus −log 10 of p-value. Proteins with –log10 (p-value) >3 (p-value <0.001) and log2 fold change >0.6 or <–0.6 (translating to 1.5-fold up- or down-regulation) were considered to have significantly changed in abundance. Data are mean of biological duplicates.

Supplementary Information: Biochemistry

A. Microscale thermophoresis (MST) assay

MST measurements and data analysis were performed as previously described using the human thalidomide binding domain (hTBD).[1] All compounds were dissolved in DMSO. The alternative reporter **11** was used at a final concentration of 200 nM; 11 µM hTBD were added for the competition experiment with thalidomide (Figure S4). The obtained normalized fluorescence (F_{norm}) values at an MST on-time of 20 s were baseline-corrected to the mean of the normalized fluorescence values for the lowest concentration of peptide ligand (ΔF_{norm}), plotted against the compound concentration and fitted to a nonlinear four-parameter equation using GraphPad Prism 9. For simplicity, only the larger part of the asymmetrical 95% confidence interval for the IC_{50} is given. Conversion of IC_{50} values to K_i values was performed as described by Nikolovska-Coleska and colleagues.[2] K_i error values were calculated as the difference between the K_i and a theoretical K_i calculated from the lower boundary of the IC_{50} 95% confidence interval.

B. Cell lines and treatments

MM.1S, OPM2, AMO-1, JJN3, Namalwa, and RPMI-8226 cell lines were obtained from ATCC or DSMZ and maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and supplemented with 1% penicillin/streptomycin and 1% L-glutamine. Cells were maintained at 37 °C with 5% CO_2 in humidified atmosphere.

OPM2 and Namalwa cells were cultured up to 3×10^5 /mL two days prior to the experiment. At 1×10^6 /mL, the cell suspension was distributed on standard 6-well plates, 4 mL each. Aliquoted cells were treated with 4 µL of the test compound (in DMSO). For MG132 treatment, an additional 4 µL of 5 mM inhibitor (in DMSO) was added. 24 h after treatment, cells were centrifuged at 1000 g. Each pellet was washed with ice-cold PBS, and centrifuged again. The supernatant was discarded, and the pellet was resuspended in 100 µL 1× cell lysis buffer (Cell signaling Technology), supplemented with 125 Units Turbo Nuclease (Jena Bioscience) and 2 mM $MgCl_2$. The cell suspension was kept on ice during lysis for 1 h and vortexed twice. Cell debris was pelleted by centrifugation at 14 000 g for 5 min at 4 °C. Supernatant was collected, and protein concentration was measured and adjusted by BCA assay.

C. Immunoblotting

Samples were mixed with 4x NuPageTM LDS Sample Buffer (ThermoFisher Scientific). Equal amounts were loaded and resolved using NuPageTM 3-8 % Tris-Acetate Mini-Protein-Gel, 1.0 mm (ThermoFisher Scientific) at a constant voltage of 160 V and 80 mA at 4°C. Proteins were transferred onto nitrocellulose membrane using Power Blotter Select Transfer Stacks and Invitrogen Power Blotter XL (ThermoFisher Scientific) using preset programs (10 min for BRD4 and 7 min for IKZF3). The membrane was stained with RevertTM 700 Total Protein Stain and Wash Solution Kit (Licor) following the supplier's protocol for normalization. Signal was detected using Odyssey CLx (Licor) Imager.

The Membrane was blocked for 2 h with 5% milk powder in PBS-T (1x phosphate-buffered saline, 0.2 % Tween20) at 4°C. Primary antibodies were also prepared in 5% milk powder in PBS-T and incubated overnight at 4°C. This was followed by 4 washing steps with PBS-T for 5 min each and secondary antibody incubation for 45 min at room temperature and 4 washing steps again. Chemiluminescence signal was detected using Odyssey Clx (Licor) imager.

The following antibodies were used: Aiolos (D1C1E) Rabbit mAb (Cell Signaling Technology, #15103), BRD4 (E2A7X) Rabbit mAb (Cell Signaling Technology, #13440), IRDye 800CW Goat anti-Rabbit IgG (Licor, NC9401842). Western blot band intensity quantification was performed using Licor Image Studio software version 5.2.5. Signal intensities were normalized using the total protein stain signal and a DMSO control.

D. Cell viability assay

Cells were seeded in 96-well plates at 5000 – 10000 cells per well with respective treatments and concentrations. Plates were incubated at 37 °C for 96 h followed by cell viability readout using CellTiter-Glo® Luminescent Cell Viability Assay. All conditions were normalized to the dimethyl sulfoxide- (DMSO) treated control. Data represents the mean ± s.d. of biological triplicates.

E. Statistical analysis

Statistical analysis of western blots and cell viability were performed with Prism version 9.2.0 (GraphPad Software, San Diego, CA, USA). Variance of biological replicates is represented as standard deviation of mean.

F. Molecular descriptor calculations

Predicted values for the topological polar surface area (TPSA) were calculated using MarvinSketch 17.28.0 (ChemAxon). Calculated ADME descriptors QPlogS, QPlogHERG, QPPCaco, and QPPMDCK were obtained from QikProp (Schrödinger Suite 2020–2, Schrödinger, LLC, New York, NY, 2020).

G. logD Measurements

The determination of the log$D_{7.4}$ values was performed by a chromatographic method as described previously.[3,4] The system was calibrated by plotting the retention times of six different drugs (atenolol, metoprolol, labetalol, diltiazem, triphenylene, permethrin) versus their literature known log$D_{7.4}$ in a calibration line ($R^2 = 0.99$). Subsequently, the mean retention times of the analytes were taken to calculate their log$D_{7.4}$ values with aid of the calibration line. At least two independent measurements of each analyte were performed.

H. Plasma protein binding studies

Plasma protein binding (%PPB) was estimated by correlating the logarithmic retention times of the analytes on a CHIRALPAK HSA 50 × 3 mm, 5 µm column with the literature known %PPB values (converted into logK values) of the following drugs: warfarin, ketoprofen, budesonide, nizatidine, indomethacin, acetylsalicylic acid, carbamazepine, piroxicam, nicardipine, and cimetidine (for details, see Valko *et al.*[5]). Samples were dissolved in MeCN/DMSO 9:1 to achieve a final concentration of 0.5 mg/mL. The mobile phase A was 50 mM ammonium acetate adjusted to *p*H 7.4 with ammonia solution, while mobile phase B was *i*PrOH. The flow rate was set to 1.0 mL/min, the UV detector was set to 254 nm, and the column temperature was kept at 30 °C. After injecting 2 µL of the sample, a linear gradient from 100% A to 30% *i*PrOH in 5.4 min was applied. From 5.4 to 18 min, 30% *i*PrOH was kept, followed by switching back to 100% A in 1.0 min and a re-equilibration time of 6 min. With the aid of the calibration line ($R^2 = 0.94$), the logK values of new substances were calculated and converted to their %PPB values. At least two independent measurements of each analyte were performed.

I. CHI determinations

The experiments were performed as reported previously. In brief, drug-membrane interactions were assessed and characterized by a high-throughput HPLC method on an immobilized artificial membrane (IAM) column that consists of monolayers of phospholipids covalently bound to silica particles.[6] The column was a Regis IAM.PC.DD2 column (100 × 4.6 mm, 10 µm, 300 Å) equipped with a guard cartridge. The column oven was set to 25 °C. Mobile phase A was 50 mM ammonium acetate *p*H 7.4 and mobile phase B was acetonitrile. The retention times were measured with a gradient of 0 to 95% acetonitrile from 0 to 6 min, which was kept at 95% until 6.5 min, then dropped to 0% from 6.5 to 7 min and finally kept at 0% until 9 min. The mobile phase flow rate was 1.5 mL/min. For the conversion of gradient retention times to chromatographic hydrophobicity index values referring to IAM chromatography (CHI_{IAM} values), a calibration was performed by plotting the retention times of an IAM standard solution of paracetamol, acetanilide, acetophenone, propiophenone, butyrophenone, valerophenone and octanophenone against their literature known CHI values.

Importantly, CHI_{IAM} values have been employed for the characeterization of molecular glues and PROTACs.[7,8] While CHI values of over 50 indicate a compound's potential for causing phospholipidosis and for hepatotoxicity, values of less than 10 indicate poor permeability associated with low cellular concentration. For comparison, we determined the CHI_{IAM} values of iberdomide (CHI_{IAM} = 23.0) and the BRD4-targeting PROTAC ARV-825 (CHI_{IAM} = 36.0).

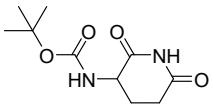
J. Synthesis: general remarks

Preparative column chromatography was performed using Merck silica gel 60 (0.063 – 0.200 mm) or using an automated flash chromatography system puriFlash XS 520Plus. Melting points were determined on a Büchi 510 oil bath apparatus or on a Reichelt hot-stage apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer, Bruker Avance 500 MHz NMR spectrometer or on a Bruker Avance III 600 MHz NMR spectrometer, respectively. NMR spectra were processed and analyzed in MestReNova. Chemical shifts are given in parts per million (ppm), 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 solvent peaks, multiplet analyses in ^{1}H NMR spectra were performed using qGSD (quantitative Global Spectral Deconvolution). Resonance assignments were made based on one- and two-dimensional NMR techniques which include ^{1}H, ^{13}C, DEPT, HSQC, and HMBC experiments. HRMS was recorded on a micrOTOF-Q mass spectrometer (Bruker) with

ESI-source coupled with an HPLC Dionex UltiMate 3000 (Thermo Scientific). The purity and identity of compounds were determined on an Infinity Lab LC/MSD-system (Agilent) with ESI-source coupled with a HPLC 1260 Infinity II (Agilent) using a EC50/2 Nucleodur C18 Gravity 3 µm column (Macherey-Nagel). The column temperature was 40 °C. HPLC conditions started with 90% H_2O containing 2 mM NH_4Ac. The gradient ramped up to 100% MeCN in 10 min, followed by further flushing with 100% MeCN for 5 min. The flow rate was 0.5 mL/min. The samples were dissolved in H_2O, MeOH or MeCN (approx. 1 mg/mL), and 2 µL sample solution was injected. Positive total ion scans were observed from 100–1000 *m*/*z* (or more if necessary) and UV absorption was detected from 190–600 nm using a diode array detector (DAD). The purity was determined at 220–600 nm.

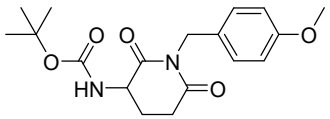
K. Synthesis: procedures

tert-Butyl _N_-(2,6-dioxo-3-piperidyl)carbamate (14)



This compound was synthesized as described previously.[9]

tert-Butyl _N_-[1-[(4-methoxyphenyl)methyl]-2,6-dioxo-3-piperidyl]carbamate (15)

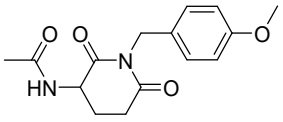


Compound **14** (2.28 g, 10 mmol) was dissolved in DMF (25 mL). 4-Methoxybenzyl chloride (1.57 g, 1.37 mL, 10 mmol) and potassium carbonate (2.80 g, 20 mmol) were added, and the solution was sonicated for two hours at rt. The solution was diluted with EtOAc (100 mL) and washed with 1 M NaOH (2 × 50 mL), 5% LiCl solution (100 mL) and brine (100 mL). Evaporation of the organic solvent and column chromatography on silica gel with *n*-hexanes/EtOAc = 2:1 yielded the title compound as a colourless solid.

Yield (1.67 g, 48%); R_f = 0.45 (*n*-hexanes/EtOAc 2:1); mp 109 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.30 (s, 1H), 1.40 (s, 9H), 1.88 – 1.99 (m, 2H), 2.64 – 2.71 (m, 1H), 2.84 – 2.93 (m, 1H), 3.71 (s, 3H), 4.34 – 4.41 (m,

1H), 4.73 (s, 1H), 6.81 – 6.86 (m, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 7.23 (d, *J* = 8.9 Hz, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 23.43, 28.13, 31.22, 40.04, 42.01, 50.98, 55.00, 78.14, 113.54, 128.94, 129.36, 155.43, 158.24, 171.82, 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 = 6.25 min, 93% purity, *m/z* $[M + H]^+$ calcd for $C_{18}H_{25}N_2O_5$, 349.17; found, 293.10 $[M - C_4H_9]$; *m/z* $[M - H]^-$ calcd for $C_{18}H_{23}N_2O_5$, 347.16; found, 347.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{23}N_2O_5$, 349.1758; found, 349.1750.

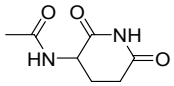
***N*-[1-[(4-Methoxyphenyl)methyl]-2,6-dioxo-3-piperidyl]acetamide (16)**



Compound **15** (348 mg, 1 mmol) was dissolved in acetic acid (5 mL). Acetic anhydride (306 mg, 283 µL, 3 mmol) and sodium acetate (98 mg, 1.2 mmol) was added, and the solution was stirred for 6 h at 120 °C. After quenching with ice and sat. aqueous $NaHCO_3$ the neutral solution was extracted with EtOAc (3 × 80 mL). The combined organic phases were washed with brine and dried over Na_2SO_4. Evaporation of the solvent and column chromatography on silica gel with CH_2Cl_2/MeOH = 19:1 yielded the title compound as a colourless solid.

Yield (176 mg, 61%); R_f = 0.38 (CH_2Cl_2/MeOH 19:1); mp 141°C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.87 (s, 3H), 1.88 – 1.98 (m, 2H, 4-H), 2.68 (dt, *J* = 17.5, 3.8 Hz, 1H), 2.82 – 2.95 (m, 1H), 3.71 (s, 3H), 4.61 – 4.70 (m, 1H), 4.73 (s, 2H), 6.72 – 6.95 (m, 2H), 7.05 – 7.21 (m, 2H), 8.27 (d, *J* = 8.3 Hz, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.62, 23.60, 31.35, 42.19, 49.77, 55.19, 113.75, 129.17, 129.55, 158.44, 169.30, 171.89, 171.96; **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), t_R = 3.79 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{15}H_{19}N_2O_4$, 291.13; found, 291.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{15}H_{19}N_2O_4$, 291.1339 found 291.1333.

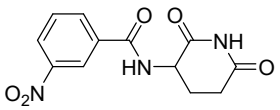
***N*-(2,6-Dioxo-3-piperidyl)acetamide (1)**



The starting material (88 mg, 0.3 mmol) was dissolved in MeCN (3 mL), mixed with ceric ammonium nitrate (666 mg, 1.2 mmol) in H_2O (660 µL) and stirred for 2 h at rt. After addition of $NaHSO_3$, the organic phase was separated, mixed with silica, and evaporated to dryness. Column chromatography on silica with CH_2Cl_2/MeOH = 19:1 to 9:1 yielded the title compound as a beige solid.

Yield (35 mg, 69%); R_f = 0.43 (CH_2Cl_2/MeOH 9:1); mp 160°C (dec); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.86 (s, 3H), 1.87 – 1.93 (m, 2H), 2.46 (t, *J* = 3.8 Hz, 1H), 2.64 – 2.76 (m, 1H), 4.47 – 4.56 (m, 1H), 8.17 (d, *J* = 8.3 Hz, 1H), 10.75 (s, 1H). One signal for 5'-H is missing (overlapping solvent peaks); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.65, 24.50, 31.03, 49.16, 169.30, 172.38, 173.04.

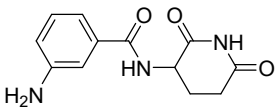
***N*-(2,6-Dioxo-3-piperidyl)-3-nitro-benzamide (17)**[10]



3-Aminopiperidine-2,6-dione hydrochloride (0.33 g, 2.0 mmol) was suspended in dry CH_2Cl_2 (20 mL), and it was cooled to 0 °C. Subsequently, Et_3N (0.40 g, 0.56 mL, 4.0 mmol) and 3-nitrobenzoyl chloride (0.37 g, 2.0 mmol) were added. After stirring the mixture for 18 h at rt, it was quenched by the addition of half-saturated NH_4Cl solution (100 mL), and it was extracted with 10% MeOH in EtOAc (2 × 100 mL). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc) to give a colourless solid.

Yield (0.34 g, 61%); R_f = 0.54 (EtOAc); mp 206 – 210 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.98 – 2.05 (m, 1H), 2.08 – 2.19 (m, 1H), 2.52 – 2.60 (m, 1H), 2.77 – 2.86 (m, 1H), 4.80 – 4.87 (m, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 8.29 – 8.33 (m, 1H), 8.38 – 8.43 (m, 1H), 8.71 (t, *J* = 2.0 Hz, 1H), 9.17 (d, *J* = 8.3 Hz, 1H), 10.89 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.22, 31.12, 49.92, 122.13, 126.34, 130.45, 133.98, 135.42, 148.00, 164.25, 172.11, 173.15; **LC-MS** (ESI) t_R = 6.84 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{12}H_{12}N_3O_5$, 278.08; found, 278.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{12}H_{12}N_3O_5$, 278.0767; found, 278.0772.

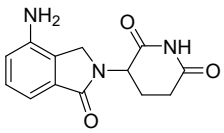
3-Amino-*N*-(2,6-dioxo-3-piperidyl)benzamide (2)



To a Schlenk tube was added a solution of compound **17** (0.25 g, 0.90 mmol) in dry DMF (9 mL) and Pd/C 10% w/w (25 mg, 10% w/w). The vessel was closed, evacuated, and refilled with nitrogen gas (3 ×), followed by hydrogen gas. The black mixture was stirred for 18 h at rt. The hydrogen gas was removed, and the flask was refilled with nitrogen gas, the vessel was opened, and it was filtrated through a pad of celite, and washed with EtOAc (3 × 20 mL). After evaporation of the solvents, the crude material was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to EtOAc to EtOAc/EtOH 9:1) to obtain a colourless solid.

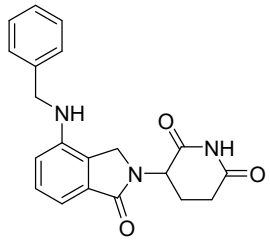
Yield (0.19 g, 87%); R_f = 0.49 (EtOAc/EtOH 9:1); mp 196 – 198 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.90 – 2.01 (m, 1H), 2.01 – 2.14 (m, 1H), 2.49 – 2.59 (m, 1H), 2.65 – 2.87 (m, 1H), 4.73 (ddd, *J* = 5.3, 8.3, 12.4 Hz, 1H), 5.23 (s, 2H), 6.63 – 6.76 (m, 1H), 6.90 – 7.00 (m, 1H), 7.04 (t, *J* = 2.0 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 10.80 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 24.39, 31.16, 49.56, 113.02, 114.54, 116.82, 128.83, 135.05, 148.88, 167.01, 172.48, 173.21; **LC-MS** (ESI) t_R = 3.02 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{14}N_3O_3$, 248.10; found, 247.9; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{14}N_3O$, 248.1030; found, 248.1022.

Lenalidomide (3)



This compound was used as commercially supplied (ABCR, Germany).

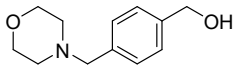
3-[4-(Benzylamino)-1-oxo-isoindolin-2-yl]piperidine-2,6-dione (4)



Lenalidomide (104 mg, 0.4 mmol) in MeCN (3 mL) and benzaldehyde (85 mg, 0.8 mmol) in dry CH_2Cl_2 (1 mL) were mixed and treated with trifluoroacetic acid (91 mg, 61 µL, 0.8 mmol) and triethylsilane (140 mg, 191 µL, 1.2 mmol). After stirring at rt for 16 h, the colourless precipitate was filtered and washed with EtOAc to yield the title compound as a colourless solid.

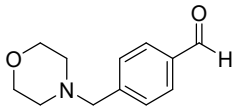
Yield (113 mg, 81%); R_f = 0.69 (EtOAc); mp 248 – 250 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.02 – 2.07 (m, 1H), 2.23 – 2.37 (m, 1H), 2.59 – 2.64 (m, 1H), 2.88 – 2.95 (m, 1H), 4.20 (d, *J* = 17.1 Hz, 1H), 4.31 (d, *J* = 17.1 Hz, 1H), 4.39 (d, *J* = 5.9 Hz, 2H), 5.04 – 5.18 (m, 1H), 6.32 (t, *J* = 6.0 Hz, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 6.87 – 6.94 (m, 1H), 7.13 – 7.26 (m, 2H), 7.27 – 7.35 (m, 2H), 7.35 – 7.43 (m, 2H), 10.97 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.76, 31.21, 40.04, 45.74, 46.06, 51.51, 110.26, 112.28, 126.67, 127.02, 128.26, 129.01, 132.05, 139.68, 143.29, 168.75, 171.18, 172.84; **LC-MS** (ESI) t_R = 7.70 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{20}N_3O_3$, 350.15; found, 350.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{20}N_3O_3$, 350.1499; found, 350.1497.

[4-(Morpholinomethyl)phenyl]methanol (18)



This compound was used as commercially supplied (TCI, Germany).

4-(Morpholinomethyl)benzaldehyde (19)

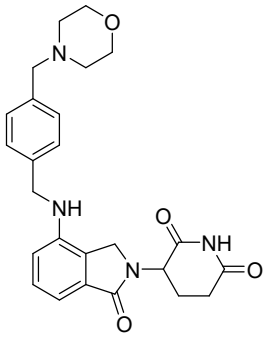


An aqueous buffer solution (*p*H = 8) of $NaHCO_3$ (1.260 g, 0.5 M) and K_2CO_3 (207 mg, 0.05 M) in 30 mL water was prepared. Compound **18** (0.60 g, 2.9 mmol) was portioned between CH_2Cl_2 (30 mL) and the buffer solution. At 0 °C, *N*-chlorosuccinimide (774 mg, 5.8 mmol), tetrabutylammonium iodide (107 mg, 0.29 mmol) and 2,2,6,6-tetramethylpiperidinyloxyl (45 mg, 0.29 mmol) were added, and it was stirred for 2.5 h. The phases were separated, and the aqueous phase was extracted CH_2Cl_2 (2 × 10 mL). The combined organic phases were washed with brine (50 mL) and evaporated. Column chromatography on silica with EtOAc yielded the corresponding aldehyde as a yellow oil.

Yield (0.42 g, 70%); R_f = 0.50 (EtOAc); **LC-MS** (ESI) t_R = 3.80 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{12}H_{16}NO_2$, 206.12; found, 206.1.

Due to its instability the aldehyde was used immediately without further analysis.

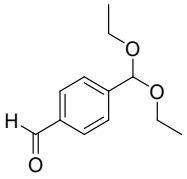
3-[4-[[4-(Morpholinomethyl)phenyl]methylamino]-1-oxo-isoindolin-2-yl]piperidine-2,6-dione (5)



Lenalidomide (492 mg, 1.9 mmol) in MeCN (11 mL) and compound **19** (388 mg, 1.9 mmol) in dry CH_2Cl_2 (3.8 mL) were mixed and treated with trifluoroacetic acid (433 mg, 289 µL, 3.8 mmol) and triethylsilane (661 mg, 906 µL, 5.7 mmol). After stirring at rt for 16 h all volatiles were evaporated. Column chromatography on silica with EtOAc/MeOH = 9:1 yielded the title compound as an off-white solid.

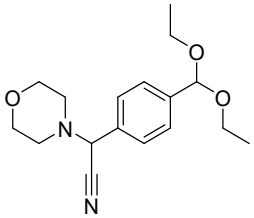
Yield (203 mg, 24%); R_f = 0.25 (EtOAc/MeOH 9:1); mp 235 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 2.00 – 2.10 (m, 1H), 2.22 – 2.40 (m, 5H), 2.58 – 2.68 (m, 1H), 2.85 – 2.98 (m, 1H), 3.41 (s, 2H), 3.55 (t, *J* = 4.7 Hz, 4H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.37 (d, *J* = 5.9 Hz, 2H), 5.11 (dd, *J* = 5.1, 13.3 Hz, 1H), 6.32 (t, *J* = 6.0 Hz, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.92 (d, *J* = 7.4 Hz, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.7 Hz, 2H), 7.33 (d, *J* = 7.7 Hz, 2H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 23.28, 31.73, 46.27, 46.39, 52.03, 53.62, 62.66, 66.64, 110.76, 112.76, 127.18, 127.45, 129.42, 129.55, 132.57, 143.85, 169.27, 171.70, 173.36; **LC-MS** (ESI) t_R = 4.84 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{25}H_{29}N_4O_4$, 449.22; found, 449.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{25}H_{29}N_4O_4$, 449.2183; found 449.2178.

Terephthalaldehyde monodiethylacetal (20)



This compound was used as commercially supplied (TCI, Germany).

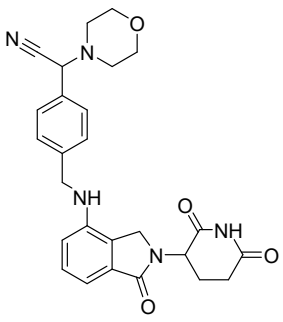
2-[4-(Diethoxymethyl)phenyl]-2-morpholino-acetonitrile (21)



A solution of 4-(diethoxymethyl)benzaldehyde (625 mg, 601 µL, 3 mmol) in MeOH (5 mL) was cooled to 0 °C and morpholine (1.045 g, 1.045 mL, 12 mmol) in H_2O (5 mL) was added. To the colourless suspension was added 2 M hydrochloric acid (1.8 mL, 3.6 mmol). The *p*H was checked to still be basic (*p*H = 8 – 9). Potassium cyanide (391 mg, 6 mmol) was added, and the suspension stirred at rt for 40 h. The off-white suspension was cooled to 0 °C, the precipitate filtered, washed with cold water and dried under vacuum to yield a colourless solid.

Yield (755 mg, 83%); R_f = 0.53 (EtOAc/*n*-hexanes 1:2); mp 58 – 59 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.15 (t, *J* = 7.0 Hz, 6H), 2.35 – 2.42 (m, 2H), 2.52 (s, 2H), 3.44 – 3.65 (m, 8H), 5.38 (s, 1H), 5.50 (s, 1H), 7.38 – 7.54 (m, 4H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 15.08, 49.50, 60.73, 60.84, 60.86, 65.85, 100.54, 115.73, 126.81, 127.60, 132.76, 139.90; **LC-MS** (ESI) t_R = 6.88 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{17}H_{25}N_2O_3$, 305.19; found, 305.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{17}H_{25}N_2O_3$, 305.1860; found, 305.1854

2-[4-[[[2-(2,6-Dioxo-3-piperidyl)-1-oxo-isoindolin-4-yl]amino]methyl]phenyl]-2-morpholino-acetonitrile (6)

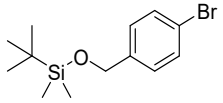


Compound **21** (243 mg, 0.8 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with lenalidomide (207 mg, 0.8 mmol) and trifluoroacetic acid (273 mg, 183 µL, 2.4 mmol) in MeCN (15 mL). After 10 min, triethylsilane (278 mg, 381 µL, 2.4 mmol) was added and the solution was stirred for 16 h at rt.

Evaporation of all volatile compounds and column chromatography on silica with 5% CH_2Cl_2 in MeOH yielded the title compound as a colourless solid.

Yield (214 mg, 56%); R_f = 0.35 (EtOAc); mp 183 – 185 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.01 – 2.10 (m, 1H), 2.26 – 2.38 (m, 1H), 2.35 – 2.42 (m, 2H), 2.49 – 2.56 (m, 1H), 2.59 – 2.67 (m, 1H), 2.87 – 2.98 (m, 1H), 3.53 – 3.65 (m, 4H), 4.21 (d, *J* = 17.1 Hz, 1H), 4.32 (d, *J* = 17.1 Hz, 1H), 4.42 (d, *J* = 5.9 Hz, 2H), 5.12 (dd, *J* = 5.1, 13.2 Hz, 1H), 5.33 (s, 1H), 6.36 (t, *J* = 6.0 Hz, 1H), 6.64 (d, *J* = 8.1 Hz, 1H), 6.91 – 6.96 (m, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 10.99 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.95, 31.39, 45.92, 49.67, 51.71, 60.87, 66.01, 110.57, 112.41, 115.99, 126.90, 127.63, 128.05, 129.23, 131.49, 132.28, 140.76, 143.40, 168.90, 171.34, 172.99; **LC-MS** (ESI) t_R = 5.21 min, 97% purity, *m/z* [M + H]$^+$ calcd for $C_{26}H_{27}N_5O_4$, 474.21; found, 474.3; **HRMS** (ESI) *m/z* [M + Na]$^+$ calcd for $C_{26}H_{27}N_5NaO_4$, 496.1955; found, 496.1968.

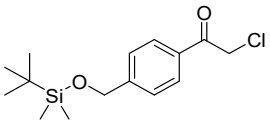
(4-Bromophenyl)methoxy-tert-butyl-dimethyl-silane (22)



4-Bromobenzyl alcohol (22.44 g, 120 mmol) was dissolved in dry CH_2Cl_2 (500 mL) and Et_3N (33 mL, 240 mL) and TBDMSCl (27.13 g, 180 mmol) was added while cooling with a water bath. The mixture was stirred at rt for 18 h. The purple solution was washed with NH_4Cl solution (500 mL) and the aqueous phase was extracted with CH_2Cl_2 (300 mL). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4, filtered, and evaporated. Column chromatography on silica (gradient of 0 to 5% EtOAc in cyclohexane) gave the title compound as a colourless liquid.

Yield (11.80 g, 33%); R_f = 0.33 (Cyclohexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.06 (s, 6H), 0.89 (s, 9H), 4.67 (s, 2H), 7.23 – 7.29 (m, 2H), 7.47 – 7.55 (m, 2H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ –5.19, 18.09, 25.91, 63.71, 119.91, 128.26, 131.16, 140.76; **LC-MS** (ESI) t_R = 11.41 min, 99% purity, *m/z* [M + H]$^+$ calcd for $C_{13}H_{22}BrOSi$, 301.06; mass not found.

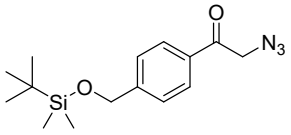
1-[4-[[*tert*-Butyl(dimethyl)silyl]oxymethyl]phenyl]-2-chloro-ethanone (23)[11]



To a cooled (–78 °C) and argon-purged solution of **22** (10.0 g, 33.2 mmol) in freshly distilled THF (120 mL), *n*-BuLi (2.5 M in hexanes, 15.9 mL, 39.8 mmol) was added slowly and the resulting mixture was stirred at –78 °C for 1 h. A solution of 2-chloro-*N*-methoxy-*N*-methylacetamide (5.5 g, 39.8 mmol) in freshly distilled THF (100 mL) was subsequently added dropwise, followed by heating the reaction mixture to –40 °C, where it was stirred for additional 1 h. To quench the reaction, saturated aqueous solution of NH_4Cl (200 mL) was added, the mixture heated to rt, and extracted with EtOAc (3 × 200 mL). The combined organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Column chromatography on silica with EtOAc/*n*-hexane = 1:7 yielded the title compound as a colourless solid.

Yield (8.29 g, 84%); R_f = 0.54 (EtOAc/*n*-hexanes 1:4); mp 29 – 30 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.95 (s, 9H), 4.71 (s, 2H), 4.81 (s, 2H), 7.42 – 7.47 (m, 2H), 7.91 – 7.96 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ –5.33, 18.38, 25.88, 46.00, 64.35, 126.06, 128.61, 132.93, 148.18, 190.75; **LC-MS** (ESI) t_R = 9.66 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{15}H_{24}ClO_2Si$, 299.12; found, 299.2.

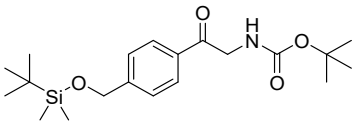
2-Azido-1-[4-[[*tert*-butyl(dimethyl)silyl]oxymethyl]phenyl]ethanone (24)



Compound **23** (8.0 g, 26.8 mmol) and NaN_3 (1.9 g, 29.4 mmol) were dissolved in MeCN (120 mL) and stirred at 60 °C for 3 h. Subsequently, MeCN was evaporated under reduced pressure, followed by the addition of EtOAc (200 mL) and H_2O (200 mL) to the crude residue. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give an off-white solid.

Yield (7.32 g, 89%); R_f = 0.49 (EtOAc/*n*-hexanes 1:4); mp 56 – 57 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.95 (s, 9H), 4.55 (s, 2H), 4.80 (s, 2H), 7.43 – 7.47 (m, 2H), 7.85 – 7.92 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ –5.35, 18.36, 25.86, 54.81, 64.33, 126.12, 127.98, 133.06, 148.30, 192.83; **LC-MS** (ESI) t_R = 9.67 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{15}H_{24}O_2N_3Si$, 306.16; found, 306.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{15}H_{24}O_2N_3Si$, 306.1632; found, 306.1630.

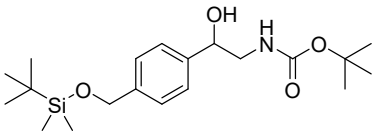
***tert*-Butyl *N*-[2-[4-[[*tert*-butyl(dimethyl)silyl]oxymethyl]phenyl]-2-oxo-ethyl]carbamate (25)**



To a solution of **24** (3.77 g, 12.34 mmol) and $(Boc)_2O$ (5.39 g, 24.68 mmol) in EtOAc (80 mL), Pd/C (10% 377 mg) was added. The reaction mixture was stirred under H_2 (1 atm, balloon) at rt for 4 h. The mixture was then filtered through a pad of celite, the filtrate evaporated, and the crude residue purified by column chromatography on silica (EtOAc/*n*-hexanes 1:7) to obtain title compound as a colourless solid.

Yield (4.12 g, 88%); R_f = 0.28 (EtOAc/*n*-hexanes 1:7); mp 58 – 60 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.95 (s, 9H), 1.48 (s, 9H), 4.65 (d, *J* = 4.4 Hz, 2H), 4.80 (s, 2H), 5.55 (br s, 1H), 7.41 – 7.46 (m, 2H), 7.91 – 7.94 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ –5.34, 18.36, 25.87, 28.35, 47.44, 64.38, 79.75, 126.04, 127.87, 133.24, 148.02, 155.75, 194.07; **LC-MS** (ESI) t_R = 9.95 min, 99% purity, *m/z* $[M - H]^-$ calcd for $C_{20}H_{34}NO_4Si$, 378.21; found, 378.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{34}NO_4Si$, 380.2252; found, 380.2247.

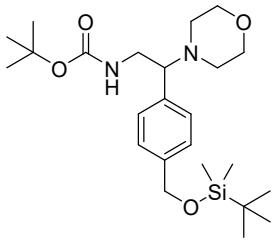
***tert*-Butyl *N*-[2-[4-[[*tert*-butyl(dimethyl)silyl]oxymethyl]phenyl]-2-hydroxy-ethyl]carbamate (26)**



Compound **25** (3.94 g, 10.37 mmol) was weighed into a round-bottom flask and thoroughly purged with argon. EtOH (50 mL) was added, and the solution cooled to 0 °C, followed by portionwise addition of $NaBH_4$ (471 mg, 12.44 mmol). It was stirred at rt for 3 h and then quenched by addition of H_2O (5 mL). The volatiles were evaporated and EtOAc (200 mL) and H_2O (200 mL) were added to the residue. The phases were separated, and the aqueous layer was washed with EtOAc (2 × 100 mL). The organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield a colourless solid.

Yield (3.59 g, 91%); R_f = 0.39 (EtOAc/*n*-hexane 1:2); mp 73 – 74 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.94 (s, 9H), 1.45 (s, 9H), 2.91 – 3.00 (m, 1H), 3.20 – 3.31 (m, 1H), 3.47 – 3.54 (m, 1H), 4.73 (s, 2H), 4.78 – 4.86 (m, 1H), 4.90 (br s, 1H), 7.28 – 7.37 (m, 4H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ –5.27, 18.41, 25.93, 28.34, 48.32, 64.71, 73.83, 79.80, 125.77, 126.20, 140.43, 141.04, 156.96; **LC-MS** (ESI) t_R = 9.22 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{36}NO_4Si$, 382.24; found, 382.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{36}NO_4Si$, 382.2408; found, 382.2403.

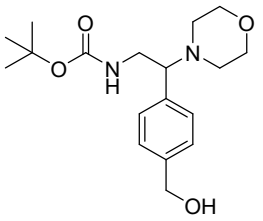
***tert*-Butyl *N*-[2-[4-[[*tert*-butyl(dimethyl)silyl]oxymethyl]phenyl]-2-morpholino-ethyl]carbamate (27)**



Compound **26** (763 mg, 2 mmol) in dry CH_2Cl_2 (20 mL) was treated with DIPEA (517 mg, 699 µL, 4 mmol) and mesyl chloride (458 mg, 310 µL, 4 mmol) at 0 °C. After 30 min DIPEA (517 mg, 699 µL, 4 mmol) and morpholine (348 mg, 348 µL, 4 mmol) were added and stirred for 16 h at rt. Water (20 mL) was added, the layers separated and the aqueous layer extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. Column chromatography with MeOH in CH_2Cl_2 yielded the title compound as a colourless oil.

Yield (241 mg, 27%); R_f = 0.50 (CH_2Cl_2/MeOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.07 (s, 6H), 0.90 (s, 8H), 1.30 (s, 1H), 1.32 (s, 9H), 2.27 (s, 2H), 2.30 – 2.36 (m, 2H), 3.15 – 3.22 (m, 1H), 3.46 – 3.55 (m, 6H), 4.69 (s, 2H), 6.47 (t, *J* = 5.6 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 2H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ -4.80, 18.49, 26.31, 28.69, 41.60, 50.48, 64.60, 66.92, 68.50, 77.97, 126.06, 129.00, 136.56, 140.48, 155.95; **LC-MS** (ESI) t_R = 9.86 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{43}N_2O_4Si$, 451.3; found, 451.4.

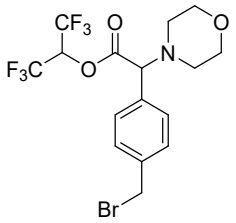
***tert*-Butyl *N*-[2-[4-(hydroxymethyl)phenyl]-2-morpholino-ethyl]carbamate (28)**



Compound **27** (128 mg, 0.28 mmol) in dry THF (3 mL) was treated dropwise with TBAF solution (1 M in THF, 0.70 mL, 4 mmol) at 0 °C and stirred for 16 h at rt. The reaction mixture was diluted with saturated. NH_4Cl solution and brine (20 mL each), the layers were separated, and the organic layer washed with saturated NH_4Cl solution and brine (3 × 20 mL), dried over Na_2SO_4, filtered and evaporated to dryness. Column chromatography (gradient of 0 to 4% MeOH in CH_2Cl_2) yielded the title compound as a colourless oil.

Yield (40 mg, 27%); R_f = 0.42 (CH_2Cl_2/MeOH 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.33 (s, 9H), 2.27 (s, 2H), 2.33 (s, 2H), 3.16 (d, *J* = 7.8 Hz, 1H), 3.51 (d, *J* = 4.6 Hz, 6H), 4.47 (d, *J* = 5.5 Hz, 2H), 5.10 (t, *J* = 5.7 Hz, 1H), 6.44 (s, 1H), 7.15 (d, *J* = 7.7 Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 2H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.19, 41.11, 45.71, 49.91, 62.67, 66.38, 67.93, 77.50, 126.00, 128.37, 141.41, 155.46; **LC-MS** (ESI) t_R = 4.63 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{18}H_{29}N_2O_4$, 337.2; found, 337.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{29}N_2O_4$, 337.2122; found, 337.2108.

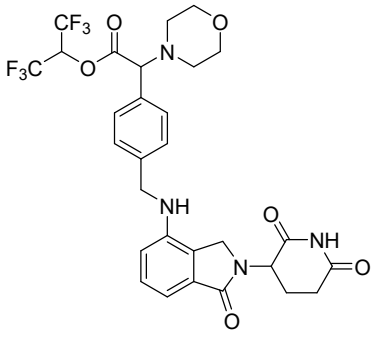
[2,2,2-Trifluoro-1-(trifluoromethyl)ethyl] 2-[4-(bromomethyl)phenyl]-2-morpholino-acetate (7)



4-(Bromomethyl)phenylboronic acid (2.15 g, 10 mmol) and glyoxylic acid hydrate (921 mg, 10 mmol) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (20 mL) and 4-(dimethylamino)pyridine (122 mg, 1 mmol) and morpholine (871 mg, 871 µL, 10 mmol) were added. The mixture was stirred for 5 h at rt. Subsequently, EDC × HCl (2.11 g, 11 mmol) was added, and the solution was further stirred for three days at rt. Silica was added and the solvent was evaporated. Column chromatography on silica with hexanes/EtOAc = 4:1 yielded the title compound as a colourless solid.

Yield (2.97 g, 64%); R_f = 0.52 (EtOAc/hexanes = 1:4); mp 182 – 183 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.33 – 2.47 (m, 4H), 3.52 – 3.63 (m, 4H), 4.57 (d, *J* = 4.1 Hz, 1H), 4.69 (s, 1H), 4.75 (s, 1H), 6.85 (p, *J* = 6.2 Hz, 1H), 7.33 – 7.43 (m, 2H), 7.43 – 7.51 (m, 2H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 33.82, 45.66, 50.40, 66.17, 71.35, 119.54, 121.77, 128.99, 129.27, 129.74, 133.81, 138.42, 138.83, 167.83, 167.85; **LC-MS** (ESI) t_R = 8.35 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{16}H_{17}BrF_6NO_3$, 464.0; found, 464.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{16}H_{17}BrF_6NO_3$, 464.0291; found, 464.0287.

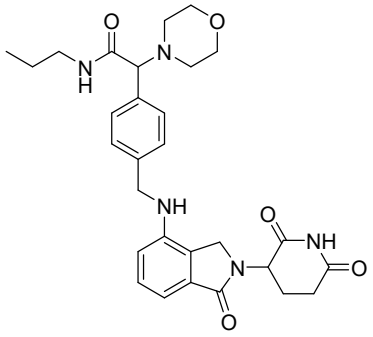
[2,2,2-Trifluoro-1-(trifluoromethyl)ethyl] 2-[4-[[[2-(2,6-dioxo-3-piperidyl)-1-oxo-isoindolin-4-yl]amino]methyl]phenyl]-2-morpholino-acetate (8)



Lenalidomide (892 mg, 3.4 mmol) and DIPEA (1.13 g, 1.52 mL, 8.7 mmol) in DMF (5 mL) was added to compound **7** (1.35 g, 2.9 mmol) in MeCN (20 mL). Sodium iodide (174 mg, 1.16 mmol) was added, and the solution was stirred at 100 °C for 16 h. The solvent was concentrated, and the resulting solution was added to H_2O (100 mL). The precipitate was filtered and washed with H_2O (2 × 25 mL). Column chromatography on silica with MeOH in CH_2Cl_2 yielded the title compound as a colourless solid.

Yield (206 mg, 11%); R_f = 0.50 (EtOAc); mp 170 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.98 – 2.10 (m, 1H), 2.23 – 2.45 (m, 5H), 2.58 – 2.65 (m, 1H), 2.86 – 2.97 (m, 1H), 3.56 (dt, *J* = 3.0, 5.9 Hz, 4H), 4.20 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.39 (d, *J* = 5.9 Hz, 2H), 4.50 (d, *J* = 1.6 Hz, 1H), 5.05 – 5.17 (m, 1H), 6.32 (t, *J* = 6.0 Hz, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 6.81 (h, *J* = 6.4 Hz, 1H), 6.92 (d, *J* = 7.4 Hz, 1H), 7.17 (t, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 10.97 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.76, 31.21, 45.72, 45.78, 50.25, 51.52, 65.99, 71.38, 110.37, 112.19, 119.38, 121.54, 126.72, 127.35, 128.52, 128.96, 131.99, 132.08, 140.55, 143.26, 167.84, 168.72, 171.15, 172.81; **LC-MS** (ESI) t_R = 7.13 min, 96% purity, *m/z* [M + H]$^+$ calcd for $C_{29}H_{29}F_6N_4O_6$, 643.2; found, 643.3; **HRMS** (ESI) *m/z* [M + H]$^+$ calcd for $C_{29}H_{29}F_6N_4O_6$, 643.1947; found, 643.1976.

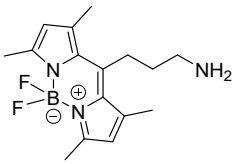
2-[4-[[[2-(2,6-Dioxo-3-piperidyl)-1-oxo-isoindolin-4-yl]amino]methyl]phenyl]-2-morpholino-*N*-propyl-acetamide (9)



Compound **8** (208 mg, 0.32 mmol) was dissolved in anhydrous MeCN (5 mL), treated with *n*-propylamine (57 mg, 80 µL, 0.97 mmol) and stirred at rt for 16 h. The solvent and residual *n*-propylamine were evaporated. Column chromatography on silica with (gradient of 3 – 10% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

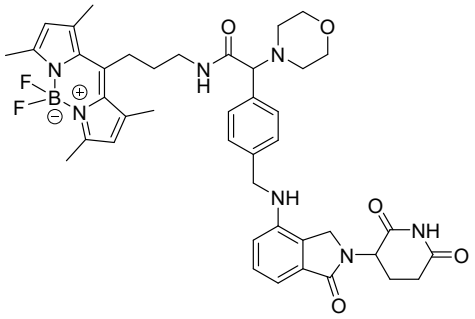
Yield (112 mg, 66%); R_f = 0.25 (7% MeOH in CH_2Cl_2); mp 168 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.76 (t, *J* = 7.4 Hz, 3H), 1.37 (h, *J* = 7.2 Hz, 2H), 2.01 – 2.10 (m, 1H), 2.22 – 2.36 (m, 5H), 2.59 – 2.66 (m, 1H), 2.87 – 3.06 (m, 3H), 3.57 (t, *J* = 4.7 Hz, 4H), 3.70 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.36 (d, *J* = 5.9 Hz, 2H), 5.06 – 5.15 (m, 1H), 6.25 – 6.33 (m, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.90 – 6.96 (m, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.29 – 7.40 (m, 4H), 8.05 (t, *J* = 5.8 Hz, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 11.78, 22.77, 23.28, 31.73, 40.61, 46.26, 46.39, 51.95, 52.02, 66.55, 75.35, 110.81, 112.70, 127.19, 127.42, 129.04, 129.58, 132.58, 136.23, 139.63, 143.85, 169.28, 170.31, 171.69, 173.37; **LC-MS** (ESI) t_R = 4.99 min, 96% purity, *m*/*z* $[M + H]^+$ calcd for $C_{29}H_{36}N_5O_5$, 534.3; found, 534.3; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{29}H_{36}N_5O_5$, 534.2672; found, 534.2707.

8-(3-Aminopropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (10)[12]



This compound was synthesized as described previously.[12]

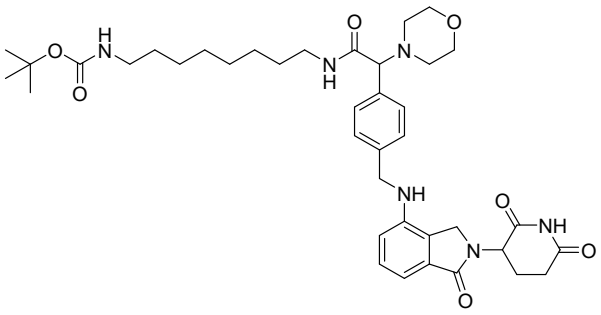
BODIPY-labeled CRBN ligand 11



Compound **10** (27 mg, 90 µmol), compound **8** (57 mg, 90 µmol) and cesium carbonate (53 mg, 160 µmol) were stirred in anhydrous MeCN (3 mL) at rt for 16 h. The mixture was diluted with EtOAc (20 mL), washed with H_2O and brine (20 mL each), dried over Na_2SO_4, filtered, and evaporated to dryness. Column chromatography on silica (gradient of 0 – 4% MeOH in CH_2Cl_2) yielded the title compound as a red solid.

Yield (17 mg, 24%); R_f = 0.38 (5% MeOH in CH_2Cl_2); mp 189 – 194 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.60 – 1.69 (m, 2H), 2.04 (d, *J* = 12.8 Hz, 1H), 2.18 – 2.44 (m, 17H), 2.62 (d, *J* = 17.4 Hz, 1H), 2.81 – 2.98 (m, 3H), 3.19 – 3.25 (m, 2H), 3.53 – 3.59 (m, 4H), 3.69 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.26 – 4.33 (m, 1H), 4.36 (d, *J* = 6.0 Hz, 2H), 5.07 – 5.15 (m, 1H), 6.16 – 6.20 (m, 2H), 6.25 – 6.32 (m, 1H), 6.63 (d, *J* = 8.1 Hz, 1H), 6.90 (d, *J* = 7.4 Hz, 1H), 7.11 – 7.18 (m, 1H), 7.34 (s, 4H), 8.28 (t, *J* = 6.0 Hz, 1H), 10.98 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 14.18, 15.92, 22.95, 25.50, 31.40, 38.91, 45.91, 46.00, 51.68, 66.20, 75.28, 110.45, 112.29, 121.82, 126.83, 127.13, 128.77, 129.19, 130.72, 132.25, 135.59, 139.46, 140.93, 143.49, 146.29, 153.28, 168.93, 170.40, 171.33, 173.00; **LC-MS** (ESI) t_R = 6.74 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{42}H_{49}BF_2N_7O_5$, 780.4; found, 780.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{42}H_{49}BF_2N_7O_5$, 780.3851; found, 780.3822.

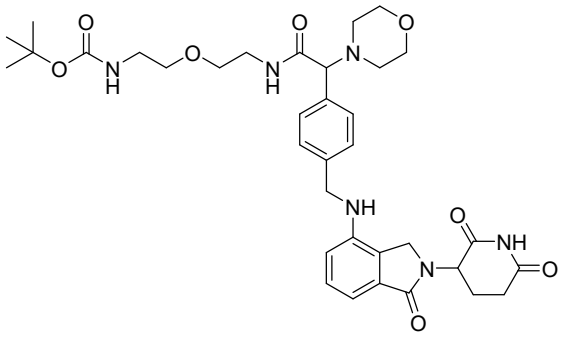
Linker-connected CRBN ligand 12a



Compound **8** (329 mg, 0.50 mmol) and *tert*-butyl *N*-(8-aminooctyl)carbamate (122 mg, 0.50 mmol) were stirred in MeCN (6 mL) at rt for 3 days. The precipitated product was filtered off. Washing with *n*-hexanes (10 mL) and drying yielded the title compound as a colourless solid.

Yield (270 mg, 75%); R_f = 0.29 (5% MeOH in CH_2Cl_2); mp 66 – 68 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.18 (s, 9H), 1.36 (s, 13H), 2.00 – 2.09 (m, 1H), 2.19 – 2.37 (m, 5H), 2.59 – 2.66 (m, 1H), 2.82 – 3.06 (m, 5H), 3.56 (t, *J* = 4.7 Hz, 4H), 3.70 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.36 (d, *J* = 5.9 Hz, 2H), 5.08 – 5.14 (m, 1H), 6.26 – 6.32 (m, 1H), 6.63 – 6.67 (m, 1H), 6.72 (t, *J* = 5.7 Hz, 1H), 6.89 – 6.94 (m, 1H), 7.20 (t, *J* = 7.8 Hz, 1H), 7.30 – 7.37 (m, 4H), 8.03 (t, *J* = 5.8 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.77, 26.13, 26.19, 28.23, 28.54, 28.60, 28.89, 29.40, 31.21, 38.25, 45.72, 45.74, 45.88, 51.43, 51.50, 66.03, 74.81, 74.82, 77.23, 110.29, 112.15, 126.68, 126.88, 128.52, 129.05, 132.06, 135.69, 139.10, 143.34, 155.53, 168.75, 169.70, 171.17, 172.84; **LC-MS** (ESI) t_R = 6.63 min, 93% purity, *m*/*z* $[M + H]^+$ calcd for $C_{39}H_{55}N_6O_7$, 719.41; found, 719.70; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{39}H_{55}N_6O_7$, 719.4127; found, 719.4155.

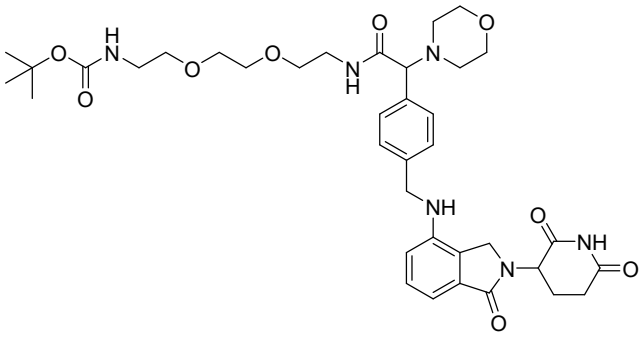
Linker-connected CRBN ligand 12b



Compound **8** (200 mg, 0.31 mmol) and *tert*-butyl *N*-[2-(2-aminoethoxy)ethyl]carbamate (76 mg, 0.37 mmol) were stirred in MeCN (3 mL) at 60°C for 4 h. The solvent was evaporated. Column chromatography on silica (gradient of 0 – 6% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (178 mg, 85%); R_f = 0.51 (10% MeOH in CH_2Cl_2); mp 124 – 129 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.37 (s, 9H), 2.01 – 2.08 (m, 1H), 2.20 – 2.36 (m, 4H), 2.59 – 2.66 (m, 1H), 2.88 – 2.97 (m, 1H), 3.04 (q, *J* = 6.0 Hz, 2H), 3.14 – 3.23 (m, 2H), 3.30 – 3.39 (m, 5H), 3.57 (t, *J* = 4.7 Hz, 4H), 3.74 (s, 1H), 4.19 (d, *J* = 17.0 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.36 (d, *J* = 5.9 Hz, 2H), 5.11 (dd, *J* = 5.1, 13.3 Hz, 1H), 6.26 – 6.31 (m, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.72 (t, *J* = 5.6 Hz, 1H), 6.90 – 6.94 (m, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.34 (s, 4H), 8.05 – 8.10 (m, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.52, 27.92, 30.96, 38.04, 45.47, 45.61, 51.15, 51.24, 65.80, 68.33, 68.65, 74.47, 77.34, 110.04, 111.91, 126.42, 126.69, 128.34, 128.81, 131.81, 135.12, 138.93, 143.08, 155.29, 168.49, 169.81, 170.91, 172.58; **LC-MS** (ESI) t_R = 5.50 min, 99% purity, *m*/*z* [M + H]$^+$ calcd for $C_{35}H_{47}N_6O_8$, 679.4; found, 679.6; **HRMS** (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{35}H_{47}N_6O_8$, 679.3450; found, 679.3437.

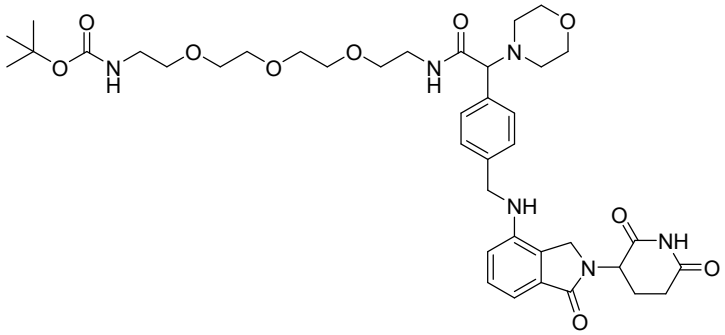
Linker-connected CRBN ligand 12c



Compound **8** (200 mg, 0.31 mmol) and *tert*-Butyl *N*-[2-[2-(2-aminoethoxy)ethoxy]ethyl]carbamate (92 mg, 0.37 mmol) were stirred in MeCN (3 mL) at 60 °C for 4 h. The solvent was evaporated. Column chromatography on silica (gradient of 0 – 6% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (210 mg, 94%); R_f = 0.51 (10% MeOH in CH_2Cl_2); mp 104-108 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.37 (s, 9H), 2.01 – 2.08 (m, 1H), 2.21 – 2.37 (m, 5H), 2.59 – 2.66 (m, 1H), 2.88 – 2.97 (m, 1H), 3.03 – 3.09 (m, 2H), 3.15 – 3.24 (m, 2H), 3.34 – 3.41 (m, 4H), 3.45 (s, 4H), 3.52 – 3.61 (m, 4H), 3.74 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.36 (d, *J* = 6.0 Hz, 2H), 5.11 (dd, *J* = 5.1, 13.3 Hz, 1H), 6.26 – 6.32 (m, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.73 (t, *J* = 5.7 Hz, 1H), 6.92 (dd, *J* = 0.7, 7.5 Hz, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.34 (s, 4H), 8.04 – 8.09 (m, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.78, 28.19, 31.22, 38.30, 45.74, 45.87, 51.41, 51.51, 66.07, 68.81, 69.14, 69.44, 69.45, 74.73, 77.57, 110.31, 112.18, 126.68, 126.95, 128.63, 129.08, 132.08, 135.36, 139.20, 143.34, 155.56, 168.76, 170.07, 171.18, 172.85; **LC-MS** (ESI) t_R = 5.50 min, 97% purity, *m*/*z* $[M + H]^+$ calcd for $C_{37}H_{51}N_6O_9$, 723.4; found, 723.4; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{37}H_{51}N_6O_9$, 723.3712; found, 723.3697.

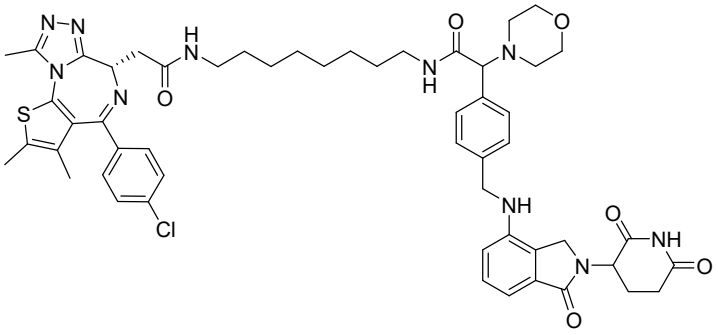
Linker-connected CRBN ligand 12d



Compound **8** (200 mg, 310 µmol) and *tert*-butyl *N*-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl] carbamate (108 mg, 370 µmol) were stirred in MeCN (3 mL) at 60°C for 4 h. The solvent was evaporated. Column chromatography on silica (gradient of 0 – 6% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (206 mg, 87%); R_f = 0.51 (10% MeOH in CH_2Cl_2); mp 83 – 87 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.37 (s, 9H), 2.01 – 2.08 (m, 1H), 2.21 – 2.27 (m, 2H), 2.27 – 2.36 (m, 3H), 2.59 – 2.66 (m, 1H), 2.88 – 2.97 (m, 1H), 3.03 – 3.09 (m, 2H), 3.15 – 3.24 (m, 2H), 3.34 – 3.41 (m, 4H), 3.42 – 3.49 (m, 8H), 3.54 – 3.59 (m, 1H), 3.57 (s, 3H), 3.74 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.36 (d, *J* = 6.0 Hz, 2H), 5.08 – 5.14 (m, 1H), 6.26 – 6.32 (m, 1H), 6.66 (d, *J* = 8.1 Hz, 1H), 6.72 (t, *J* = 5.8 Hz, 1H), 6.90 – 6.95 (m, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.34 (s, 4H), 8.07 (t, *J* = 5.8 Hz, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.77, 28.18, 31.21, 38.29, 45.73, 45.86, 51.40, 51.50, 66.06, 68.80, 69.14, 69.46, 69.69, 69.73, 74.72, 77.54, 110.30, 112.16, 126.67, 126.93, 128.62, 129.07, 132.06, 135.34, 139.19, 143.33, 155.53, 168.75, 170.05, 171.17, 172.84; **LC-MS** (ESI) t_R = 5.54 min, 98% purity, *m*/*z* $[M + H]^+$ calcd for $C_{39}H_{55}N_6O_{10}$, 767.4; found, 767.5; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{39}H_{55}N_6O_{10}$, 767.3974; found, 767.3959.

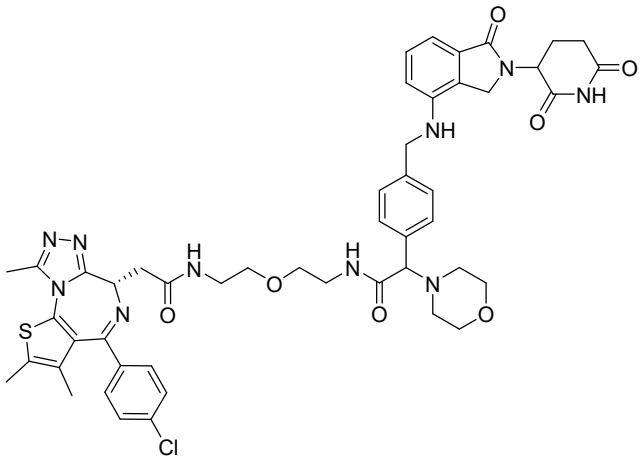
BRD4-targeting PROTAC 13a



Compound **12a** (59 mg, 82 µmol) was dissolved in dry CH_2Cl_2 (3 mL) and was treated with trifluoroacetic acid (3 mL). The reaction mixture was stirred for 2 h at 40 °C. The solvent was removed and coevaporatd with dry CH_2Cl_2 (2 × 5 mL). The residue was further dried under high vacuum. The deprotected amine derivative in dry DMF (4 mL) was treated with DIPEA (42 mg, 57 µL, 328 µmol), JQ1 carboxylic acid (33 mg, 82 µmol) and HATU (34 mg, 90 µmol). The combined mixture was stirred at rt for 16 h. Half-saturated brine (50 mL) was added, and it was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated NH_4Cl solution, 5% LiCl solution, and brine (each 30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography on silica (gradient of 0 – 10% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (33 mg, 40%); R_f = 0.35 (5% MeOH in CH_2Cl_2); mp 174 – 179 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.12 – 1.29 (m, 6H), 1.34 (p, *J* = 7.0 Hz, 2H), 1.42 (p, *J* = 6.9 Hz, 2H), 1.60 – 1.63 (m, 3H), 2.00 – 2.07 (m, 1H), 2.18 – 2.36 (m, 6H), 2.39 – 2.42 (m, 3H), 2.59 (s, 3H), 2.58 – 2.65 (m, 1H), 2.88 – 3.15 (m, 6H), 3.18 (dd, *J* = 6.0, 15.0 Hz, 1H), 3.24 (dd, *J* = 8.2, 15.0 Hz, 1H), 3.56 (t, *J* = 4.7 Hz, 4H), 3.69 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (dd, *J* = 1.6, 17.1 Hz, 1H), 4.35 (d, *J* = 5.9 Hz, 2H), 4.50 (dd, *J* = 6.0, 8.1 Hz, 1H), 5.08 – 5.14 (m, 1H), 6.26 – 6.31 (m, 1H), 6.62 – 6.67 (m, 1H), 6.92 (dd, *J* = 0.8, 7.5 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 7.30 – 7.37 (m, 4H), 7.39 – 7.45 (m, 2H), 7.45 – 7.49 (m, 2H), 8.03 (t, *J* = 5.8 Hz, 1H), 8.13 (t, *J* = 5.6 Hz, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) *δ* 11.76, 13.15, 14.50, 23.29, 26.75, 26.79, 29.10, 29.20, 29.44, 29.70, 31.73, 38.16, 38.76, 38.95, 46.25, 46.39, 51.94, 52.02, 54.41, 66.55, 75.33, 110.80, 112.66, 127.19, 127.40, 128.90, 129.04, 129.56, 130.05, 130.28, 130.58, 131.18, 132.58, 132.74, 135.70, 136.21, 137.22, 139.62, 143.85, 150.25, 155.61, 163.43, 169.26, 169.78, 170.21, 171.68, 173.35; **LC-MS** (ESI) t_R = 6.86 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{53}H_{62}ClN_{10}O_6S$, 1001.43; found, 1001.70; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{53}H_{62}ClN_{10}O_6S$, 1001.4258; found, 1001.4239.

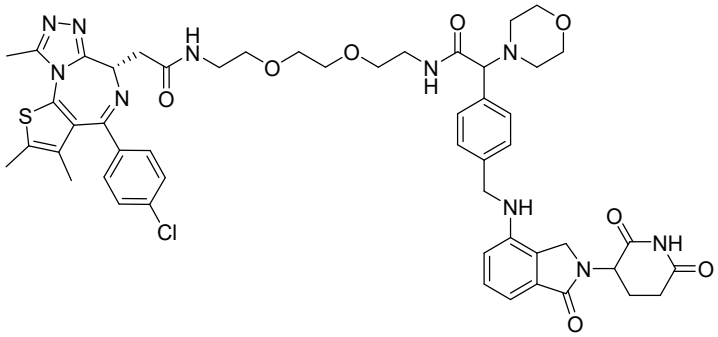
BRD4-targeting PROTAC 13b



Compound **12b** (75 mg, 0.11 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and was treated with trifluoroacetic acid (3 mL). The reaction mixture was stirred for 2 h at 40 °C. The solvent was removed and coevaporated with dry CH_2Cl_2 (2 × 5 mL). The residue was further dried in high vacuum. The deprotected amine derivative in dry DMF (4 mL) was treated with DIPEA (52 mg, 70 µL, 0.40 mmol), JQ1 carboxylic acid (40 mg, 0.10 mmol) and HATU (42 mg, 0.11 µmol). The combined mixture was stirred at rt for 16 h. Half-saturated brine (50 mL) was added, and it was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated NH_4Cl solution, 5% LiCl solution, and brine (each 30 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Column chromatography on silica (gradient of 0 – 10% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (76 mg, 79%); R_f = 0.44 (10% MeOH in CH_2Cl_2); mp 272 – 277 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.61 (s, 3H), 2.00 – 2.08 (m, 1H), 2.17 – 2.36 (m, 5H), 2.40 (s, 3H), 2.59 (s, 3H), 2.59 – 2.65 (m, 2H), 2.88 – 2.97 (m, 1H), 3.17 (s, 1H), 3.18 – 3.30 (m, 3H), 3.36 – 3.45 (m, 5H), 3.56 (s, 4H), 3.75 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (dd, *J* = 1.9, 17.1 Hz, 1H), 4.36 (s, 2H), 4.51 (dd, *J* = 6.3, 7.8 Hz, 1H), 5.11 (dd, *J* = 5.1, 13.3 Hz, 1H), 6.28 – 6.31 (m, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 7.4 Hz, 1H), 7.16 – 7.22 (m, 1H), 7.35 (s, 4H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 2H), 8.10 (s, 1H), 8.22 (s, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 11.26, 12.64, 13.99, 18.52, 22.78, 31.22, 37.53, 38.50, 45.74, 45.84, 51.52, 53.83, 54.87, 55.99, 66.06, 68.60, 68.84, 110.33, 112.16, 126.69, 127.00, 128.43, 128.65, 129.07, 129.52, 129.79, 130.10, 130.67, 132.09, 132.24, 135.20, 136.73, 143.31, 149.79, 155.07, 163.01, 168.75, 169.67, 171.18, 172.85; **LC-MS** (ESI) t_R = 6.29 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{49}H_{54}ClN_{10}O_7S$, 961.4; found, 961.6; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{49}H_{54}ClN_{10}O_7S$, 961.3581; found, 3961.3562.

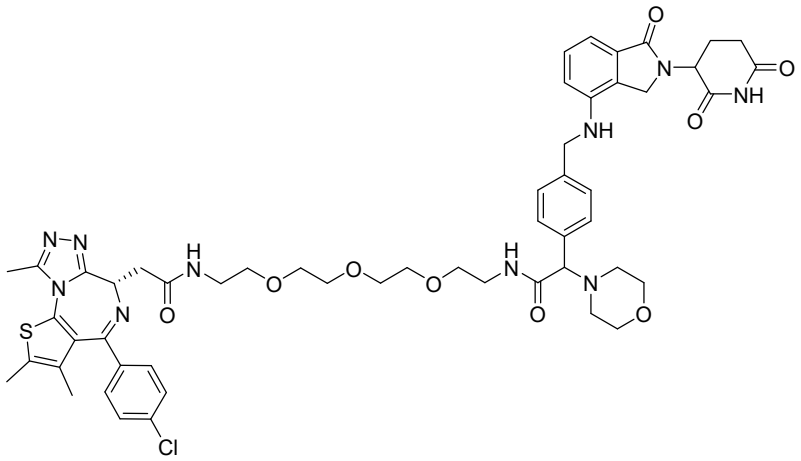
BRD4-targeting PROTAC 13c



Compound **12c** (80 mg, 0.11 µmmol) was dissolved in dry CH_2Cl_2 (3 mL) and was treated with trifluoroacetic acid (3 mL). The reaction mixture was stirred for 2 h at 40 °C. The solvent was removed and coevaporated with dry CH_2Cl_2 (2 × 5 mL). The residue was further dried in high vacuum. The deprotected amine derivative in dry DMF (4 mL) was treated with DIPEA (52 mg, 70 µL, 0.40 mmol), JQ1 carboxylic acid (40 mg, 0.10 mmol) and HATU (42 mg, 0.11 mmol). The combined mixture was stirred at rt for 16 h. Half-saturated brine (50 mL) was added, and it was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated NH_4Cl solution, 5% LiCl solution, and brine (each 30 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Column chromatography on silica (gradient of 0 – 10% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (52 mg, 52%); R_f = 0.44 (10% MeOH in CH_2Cl_2); mp 151 – 155 °C (dec.); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.61 (s, 3H), 2.00 – 2.08 (m, 1H), 2.18 – 2.36 (m, 5H), 2.40 (s, 3H), 2.51 – 2.56 (m, 1H), 2.59 (s, 4H), 2.87 – 2.97 (m, 1H), 3.16 – 3.31 (m, 4H), 3.36 – 3.62 (m, 13H), 3.72 – 3.76 (m, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (dd, *J* = 1.6, 17.2 Hz, 1H), 4.35 (d, *J* = 5.8 Hz, 2H), 4.51 (dd, *J* = 6.2, 7.9 Hz, 1H), 5.11 (dd, *J* = 5.1, 13.2 Hz, 1H), 6.29 (s, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.92 (d, *J* = 7.4 Hz, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.34 (s, 4H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 8.5 Hz, 2H), 8.08 (s, 1H), 8.25 (t, *J* = 5.7 Hz, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 9.07, 11.76, 13.15, 14.43, 14.51, 22.53, 23.29, 31.42, 31.73, 34.81, 38.01, 38.84, 39.08, 46.17, 46.26, 46.36, 51.92, 52.02, 54.32, 66.57, 69.32, 69.65, 69.96, 70.08, 75.22, 110.82, 112.67, 127.19, 127.46, 128.92, 129.16, 129.58, 130.03, 130.30, 130.62, 131.17, 132.58, 132.74, 135.69, 137.25, 143.83, 150.28, 155.58, 163.48, 169.26, 170.17, 171.68, 173.35; **LC-MS** (ESI) t_R = 6.26 min, 96% purity, *m*/*z* $[M + H]^+$ calcd for $C_{51}H_{58}ClN_{10}O_8S$, 1005.4; found, 1005.7; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{51}H_{58}ClN_{10}O_8S$, 1005.3843; found, 1005.3825.

BRD4-targeting PROTAC 13d

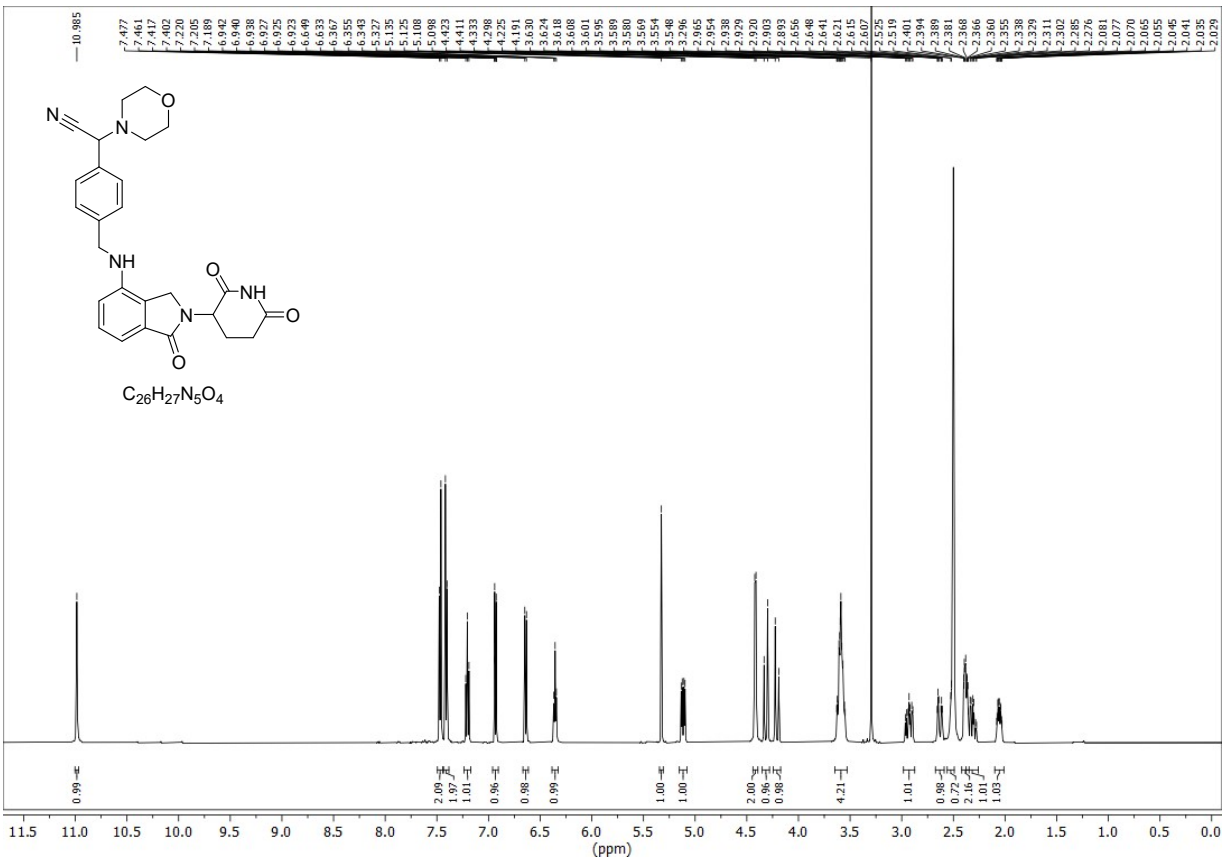


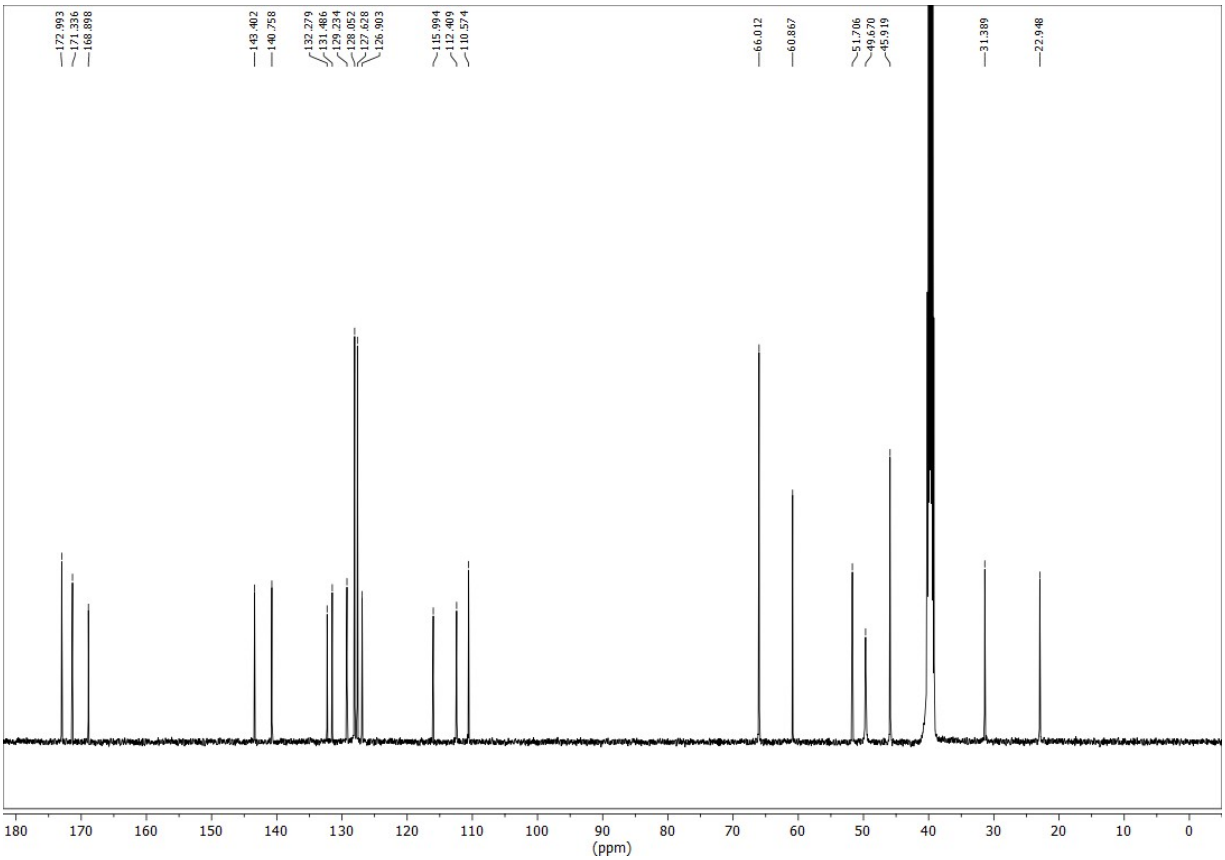
Compound **12d** (84 mg, 0.11 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and was treated with trifluoroacetic acid (3 mL). The reaction mixture was stirred for 2 h at 40 °C. The solvent was removed and coevaporated with dry CH_2Cl_2 (2 × 5 mL). The residue was further dried in high vacuum. The deprotected amine derivative in dry DMF (4 mL) was treated with DIPEA (52 mg, 70 µL, 0.40 mmol), JQ1 carboxylic acid (40 mg, 0.10 mmol) and HATU (42 mg, 0.11 mmol). The combined mixture was stirred at rt for 16 h. Half-saturated brine (50 mL) was added, and it was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated NH_4Cl solution, 5% LiCl solution, and brine (each 30 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Column chromatography on silica (gradient of 0 – 10% MeOH in CH_2Cl_2) yielded the title compound as a colourless solid.

Yield (83 mg, 79%); R_f = 0.44 (10% MeOH in CH_2Cl_2); mp 233 – 238 °C (dec.); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.60 – 1.63 (m, 3H), 2.01 – 2.08 (m, 1H), 2.19 – 2.36 (m, 5H), 2.39 – 2.42 (m, 3H), 2.59 (s, 3H), 2.59 – 2.65 (m, 1H), 2.88 – 2.97 (m, 1H), 3.15 – 3.31 (m, 6H), 3.39 (t, *J* = 5.8 Hz, 2H), 3.41 – 3.60 (m, 15H), 3.73 (s, 1H), 4.19 (d, *J* = 17.1 Hz, 1H), 4.30 (d, *J* = 17.1 Hz, 1H), 4.35 (d, *J* = 5.9 Hz, 2H), 4.50 (dd, *J* = 6.0, 8.1 Hz, 1H), 5.11 (dd, *J* = 5.1, 13.3 Hz, 1H), 6.26 – 6.31 (m, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.90 – 6.94 (m, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.33 (s, 3H), 7.39 – 7.45 (m, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 8.07 (t, *J* = 6.0 Hz, 1H), 8.25 (t, *J* = 5.7 Hz, 1H), 10.99 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 11.26, 12.64, 14.01, 22.02, 22.79, 30.91, 31.22, 37.50, 38.31, 38.60, 45.75, 45.88, 51.41, 51.52, 53.81, 66.07, 68.82, 69.18, 69.49, 69.59, 69.73, 69.76, 74.72, 110.31, 112.17, 126.68, 126.94, 128.42, 128.64, 129.08, 129.53, 129.79, 130.11, 130.66, 132.08, 132.24, 135.18, 135.35, 136.74, 139.20, 143.34, 149.76, 155.08, 162.97, 168.76, 169.64, 170.07, 171.18, 172.85; **LC-MS** (ESI) t_R = 6.30 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{53}H_{62}ClN_{10}O_9S$, 1049.4; found, 1049.8; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{53}H_{62}ClN_{10}O_9S$, 1049.41050; found, 1049.4087.

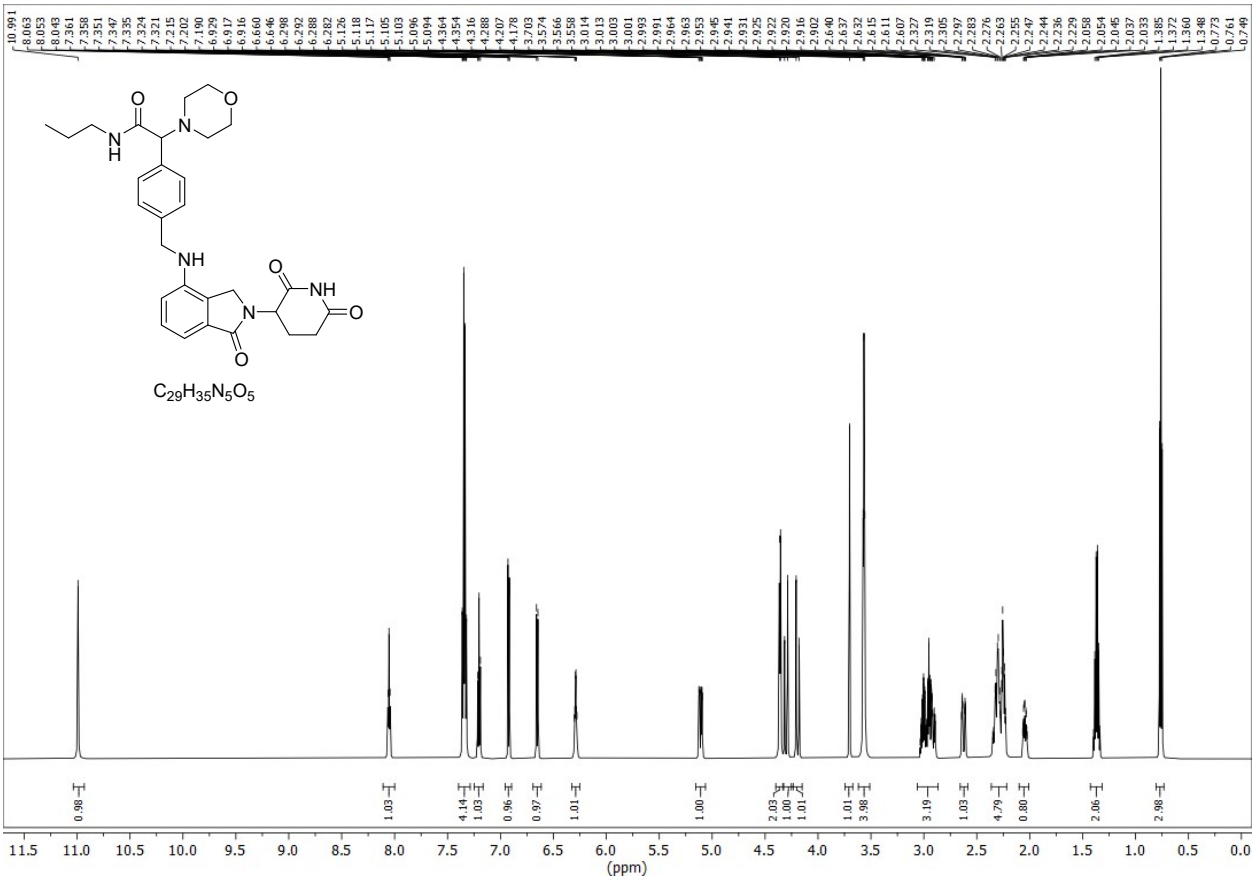
L. Selected ^{1}H and ^{13}C NMR spectra

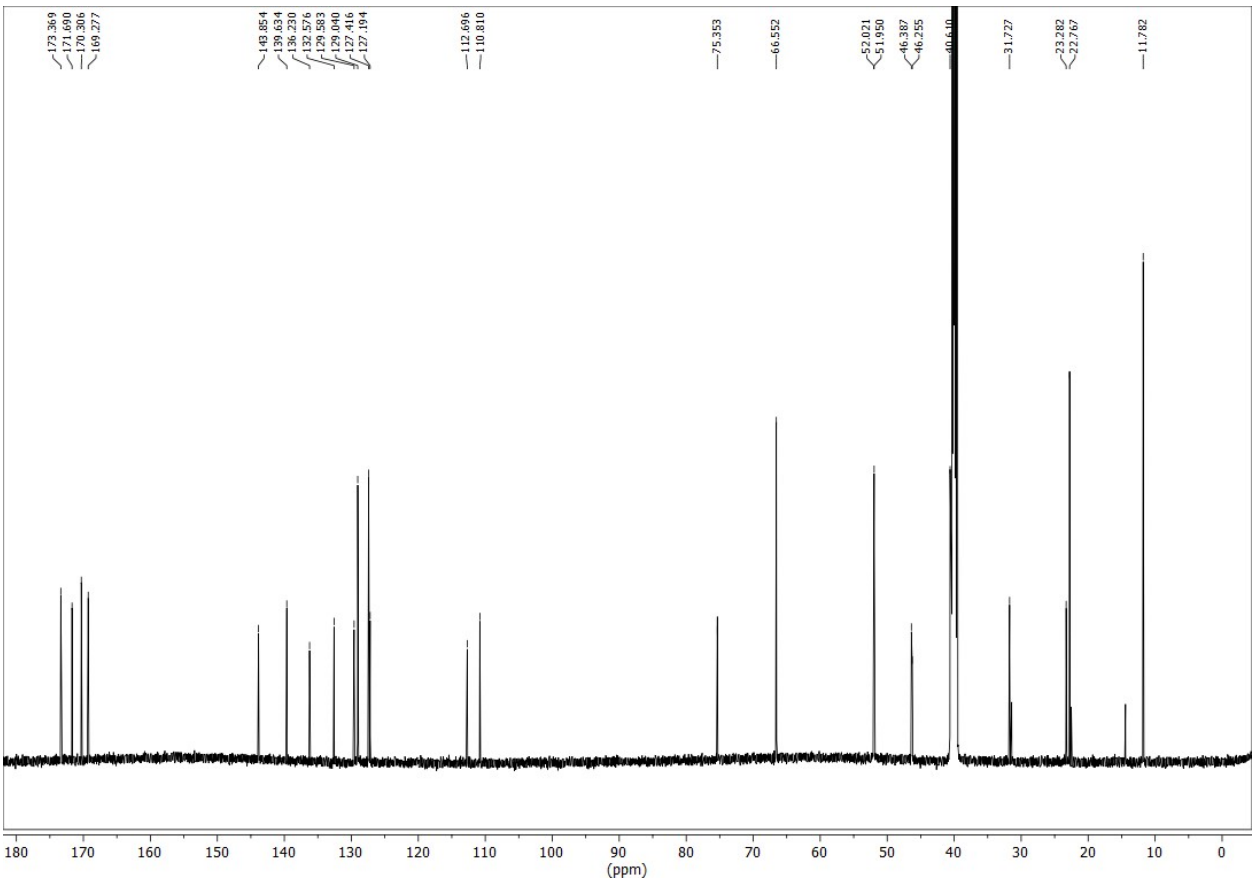
^{1}H and ^{13}C NMR spectrum of compound **6**.



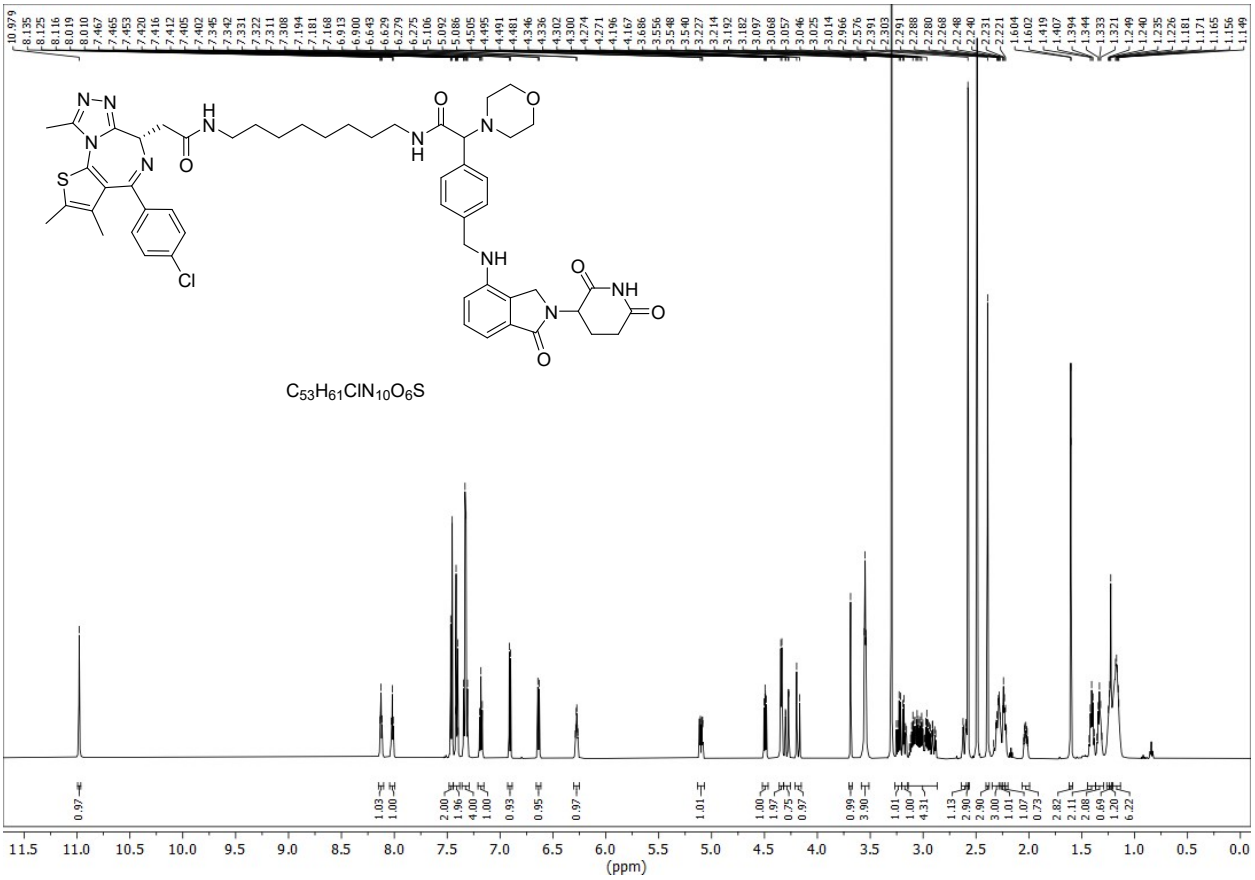


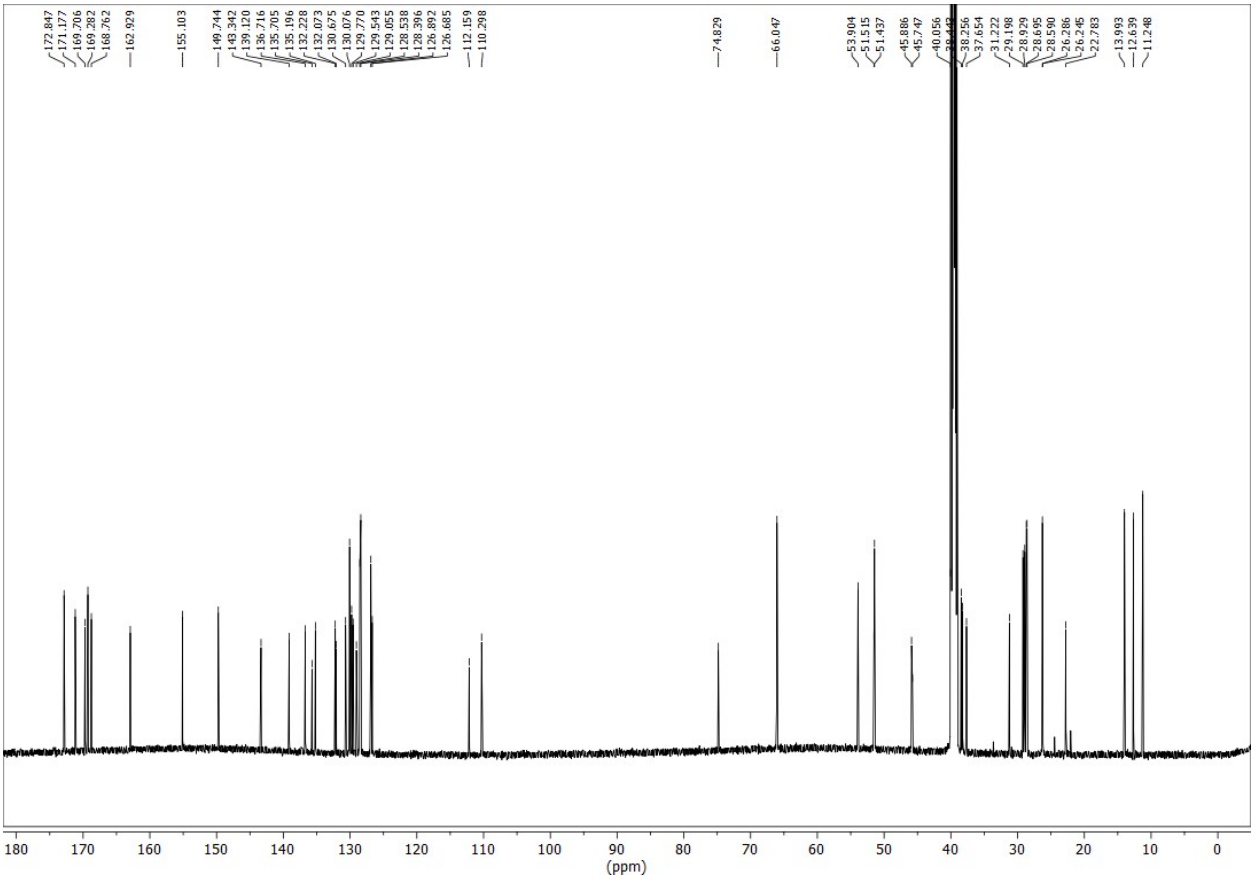
^{1}H and ^{13}C NMR spectrum of compound **9**.





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





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