Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

A MedChem Toolbox for Cereblon-directed PROTACs

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Supplementary Figures, Schemes, and Tables

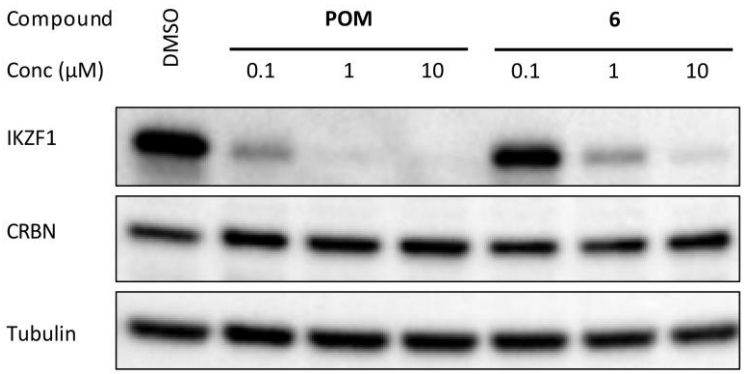


Figure S1: Cell permeabilty and induction of IKZF1 degradation by the biotin labelled pomalidomide analog **6** was shown. The multiple myeloma cell line MM1S was treated either with pomalidomide (**POM**) or compound **6** for 16 h with the indicated concentrations.

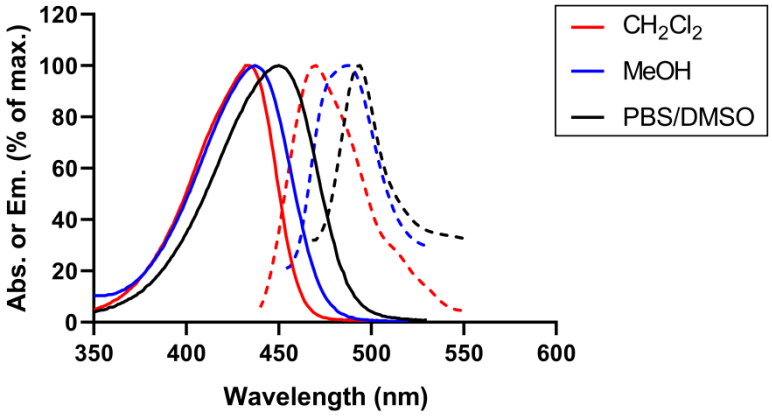


Figure S2: Absorption (Abs., solid lines) and emission (Em., dotted lines) spectra of compound **7** were recorded at concentrations of 10 µM and 1 µM, respectively, in the corresponding solvent containing 1% DMSO. Red lines, CH_2Cl_2, λ_{ex} = 434 nm, λ_{em} = 470 nm, 36 nm Stokes shift; blue lines, MeOH, λ_{ex} = 437 nm, λ_{em} = 488 nm, 51 nm Stokes shift; black lines, PBS/DMSO, λ_{ex} = 450 nm, λ_{em} = 494 nm, 44 nm Stokes shift.

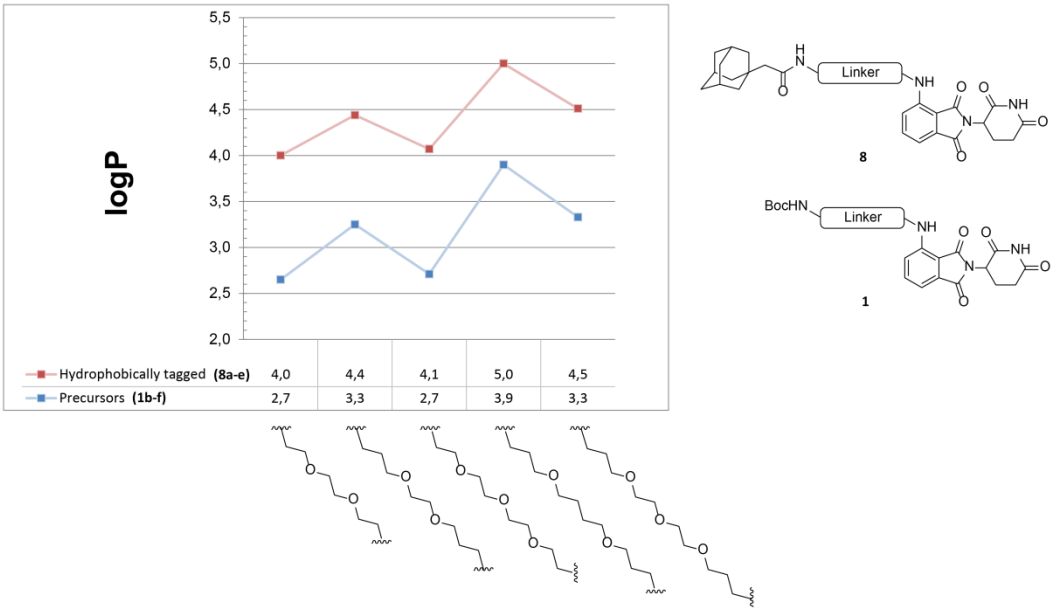


Figure S3: Similar trends can be observed in the logP profiles of synthetic products, chimeras **8a**-**e**, and their precursors, Boc-protected tool compounds **1b**-**f**. The hydrophobicity of the compounds is largely influenced by the linker length and the C/O ratio.

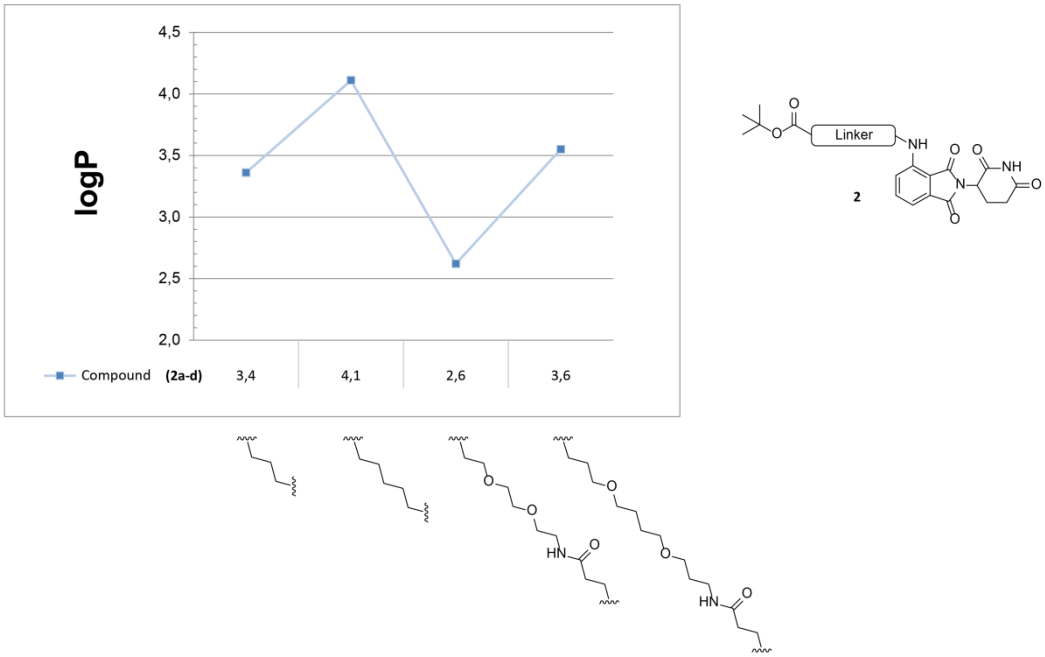


Figure S4: logP values of *tert*-butyl protected carboxy tool compounds **2**. The upper terminus of the linker is connected to the pomalidomide nitrogen.

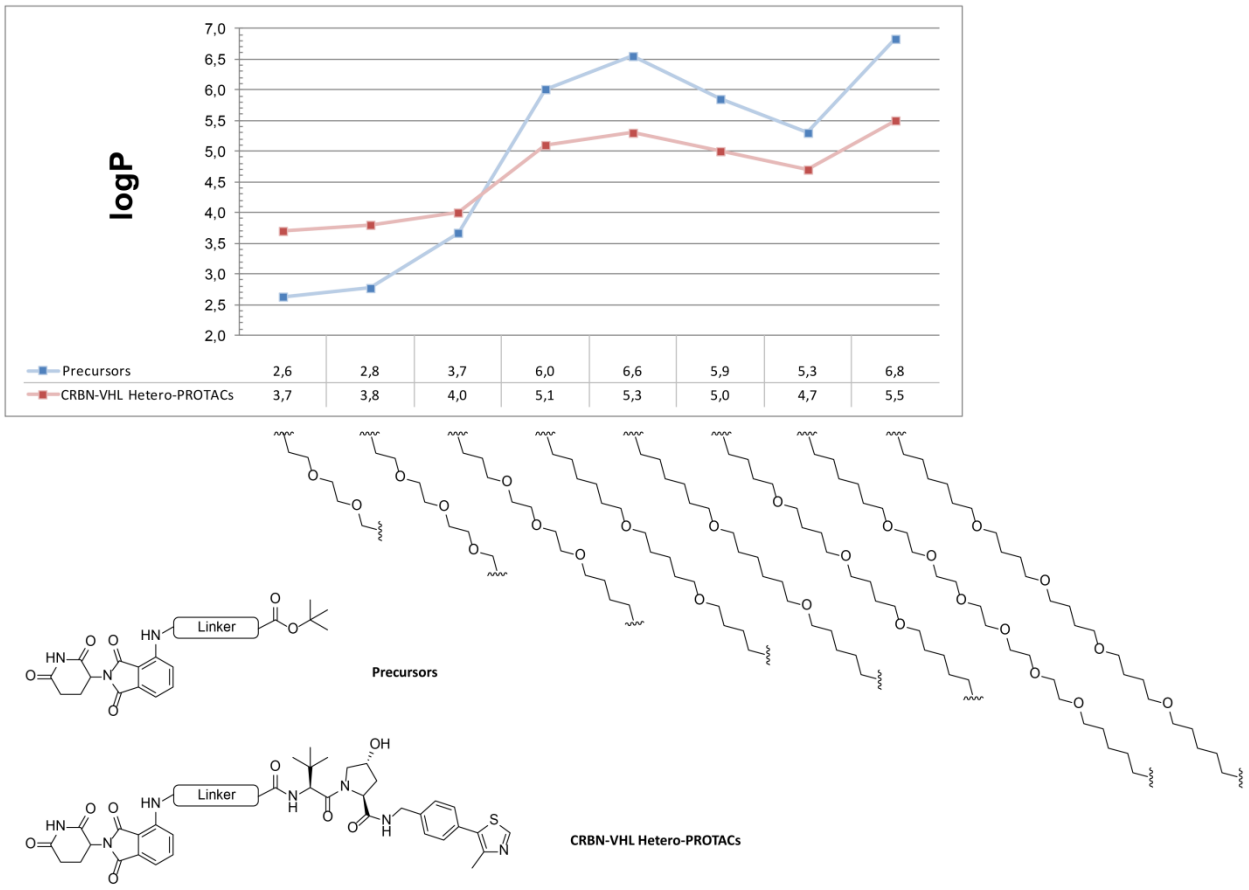


Figure S5: Comparison of logP profiles of our comprehensive pomalidomide-based PROTAC precursor library with recently published CRBN-VHL hetero-PROTACs.[1] The upper terminus of the linker is connected to the pomalidomide nitrogen. The hydrophobicity of the compounds is largely influenced by the linker length and the C/O ratio. The determination of logP values is beneficial to estimate logP values of final PROTAC compounds.[2]

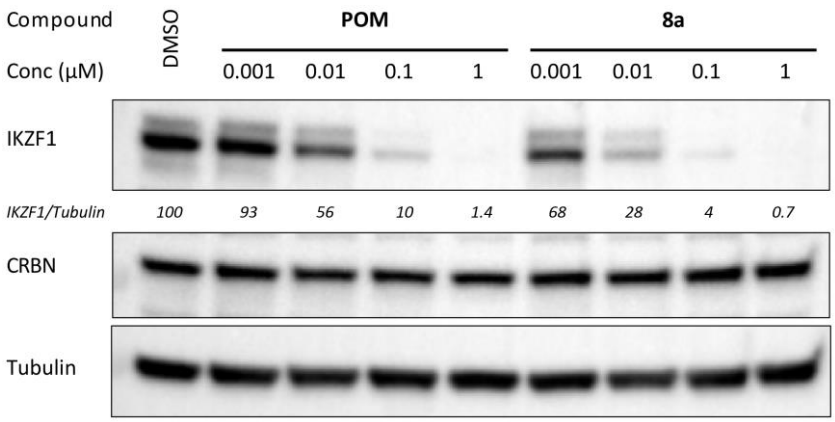


Figure S6: Compound **8a** induces degradation of IKZF1, but does not affect CRBN protein level. The multiple myeloma cell line MM1S was treated either with pomalidomide (**POM**) or compound **8a** for 16 h with the indicated concentrations. The quantification was performed with IKZF1 levels normalized to tubulin, then to DMSO control (100%).

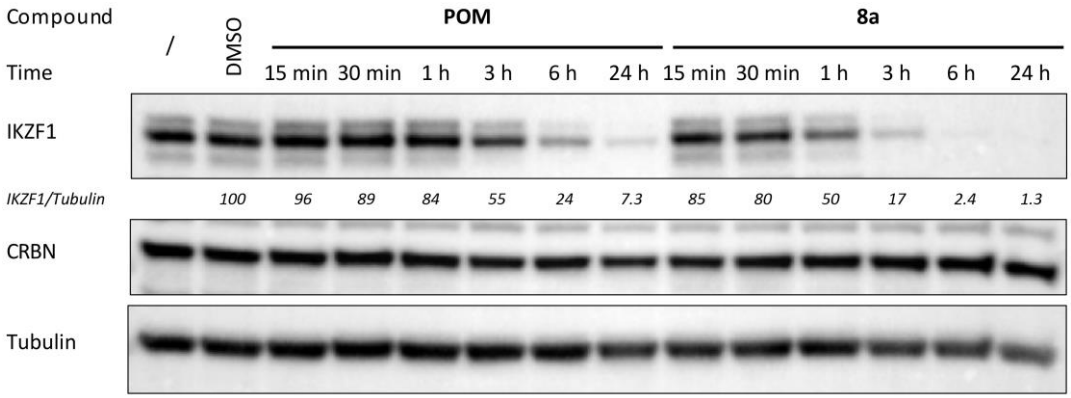


Figure S7: Time course experiment with compound **8a**. The multiple myeloma cell line MM1S was treated with 100 nM pomalidomide (**POM**) or compound **8a** for the indicated time period. The quantification was performed with IKZF1 levels normalized to tubulin, then to DMSO control (100%).

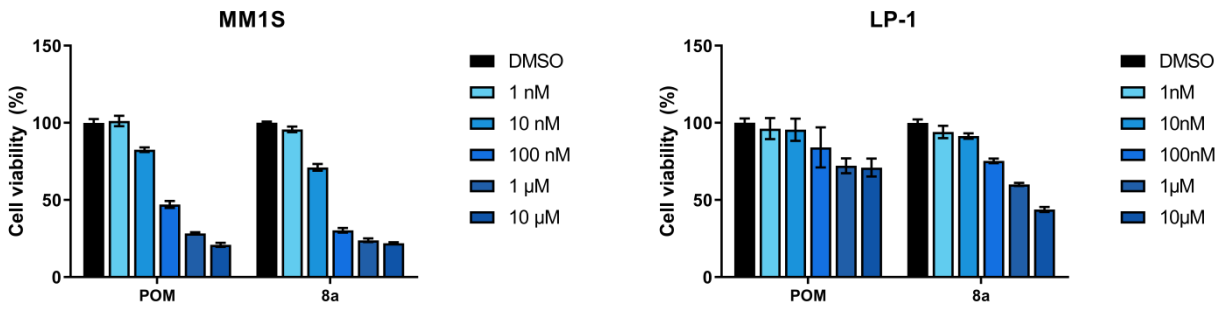


Figure S8: Impact of compound **8a** on cell viability. The multiple myeloma cell lines MM1S and LP-1 were treated with the indicated concentrations of compound **8a** or pomalidomide (**POM**). Cell viability was analyzed after 4 days in triplicate. Error bars express the mean ± SEM from 3 biological replicates.

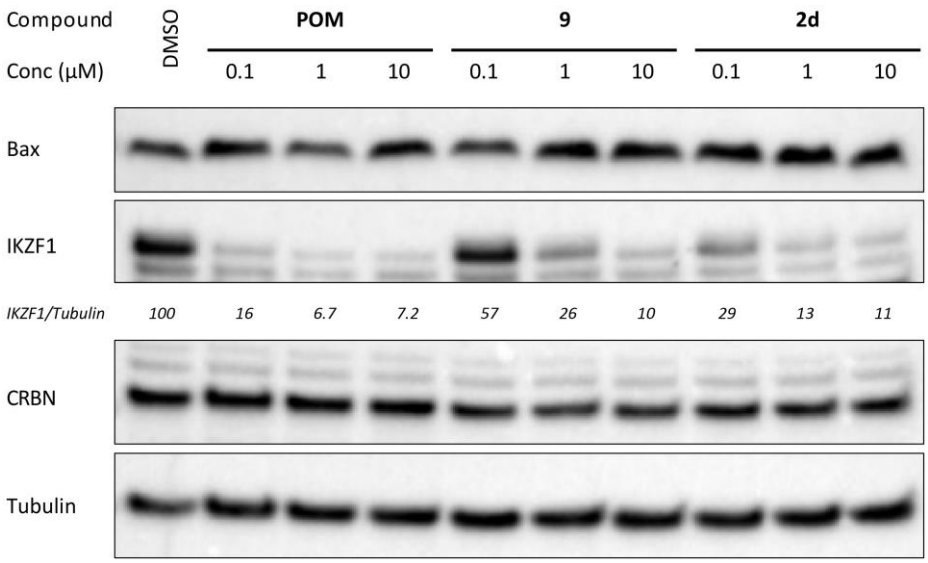


Figure S9: Effect of compound **9** on the cellular protein level of the pro-apoptotic protein Bax, IKZF1, and CRBN. The multiple myeloma cell line MM1S was treated either with pomalidomide (**POM**), compound **9** or **2d** for 16 h with the indicated concentrations. The quantification was performed with IKZF1 levels normalized to tubulin, then to DMSO control (100%).

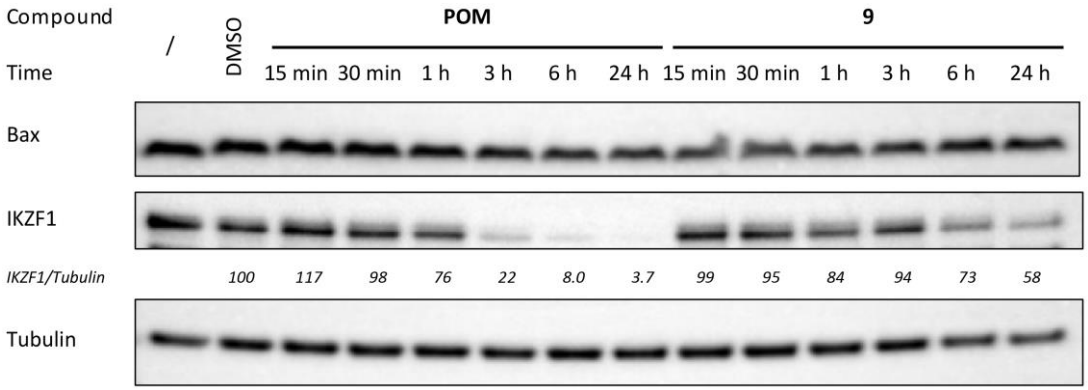
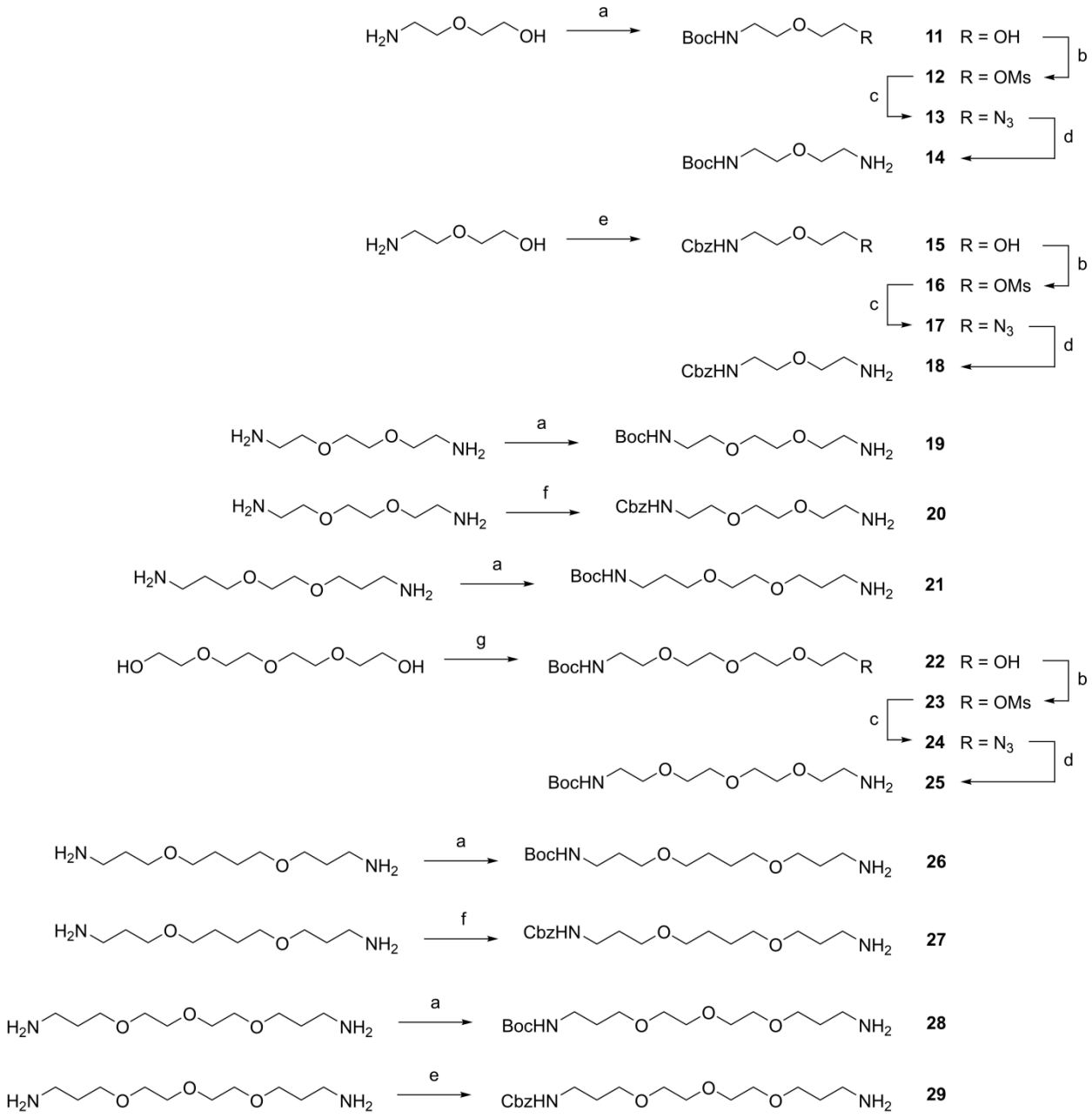
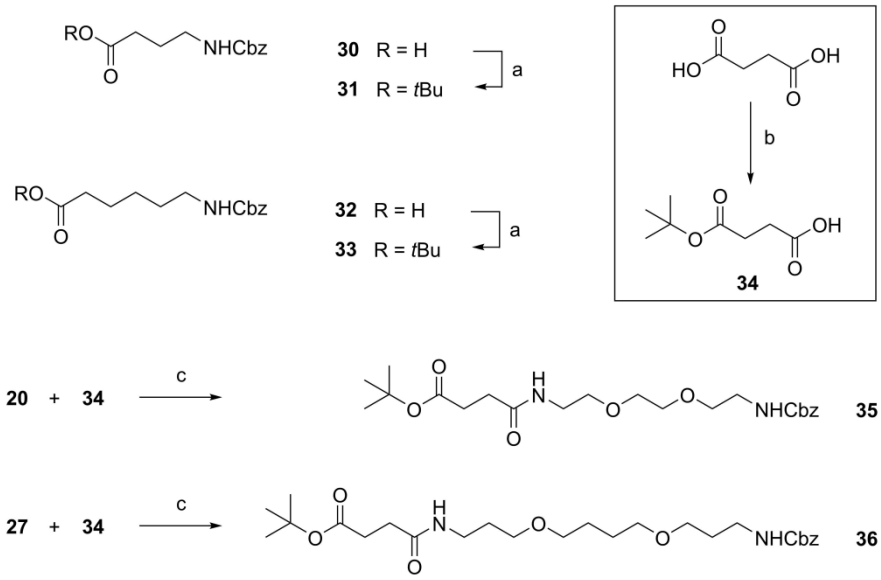


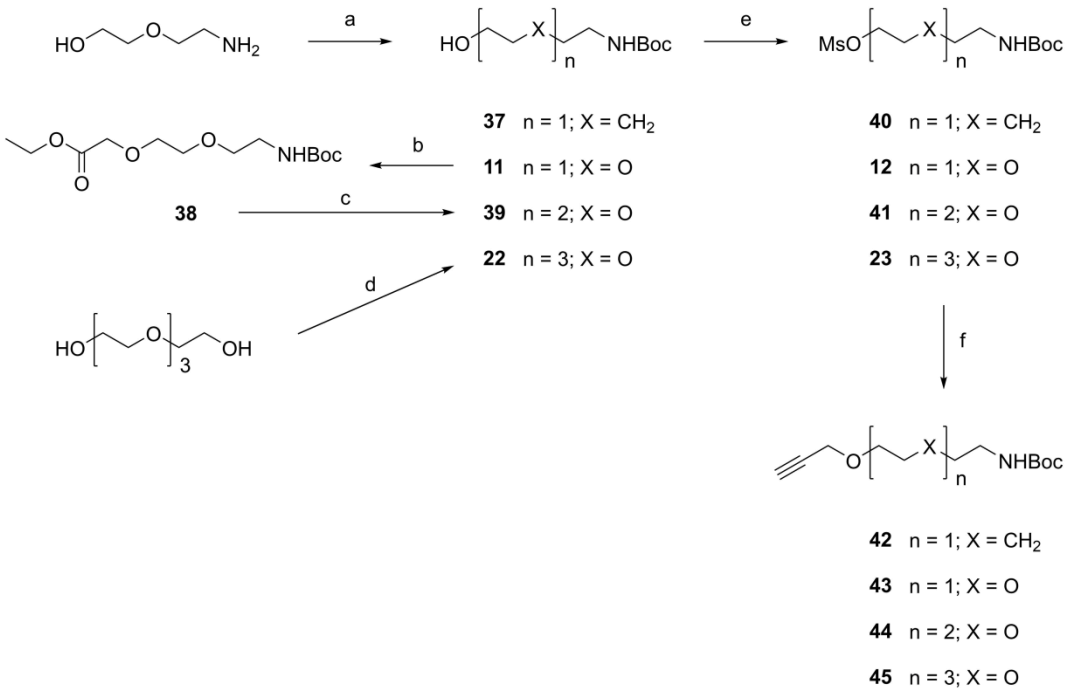
Figure S10: Time course experiment with compound **9**. The multiple myeloma cell line MM1S was treated with 1 µM pomalidomide (**POM**) or compound **9** for the indicated time period. The quantification was performed with IKZF1 levels normalized to tubulin, then to DMSO control (100%).



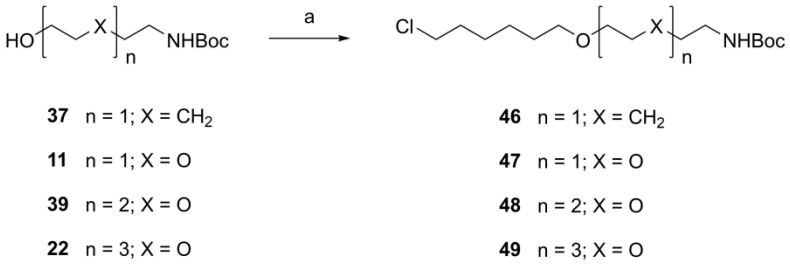
Scheme S1: Synthesis of carbamate-protected amine linkers. *Reagents and conditions:* (a) $(Boc)_2O$, CH_2Cl_2, rt, 18 h; (b) MsCl, Et_3N, CH_2Cl_2, rt, 3 h; (c) NaN_3, DMF, 70 °C, 24 h; (d) PPh_3, H_2O, THF, rt, 24 h; (e) Cbz-Cl, Et_3N, CH_2Cl_2, rt, 24 h; (f) Cbz-Cl, Et_3N, $CHCl_3$, rt, 24 h; (g) (i) MsCl, Et_3N, THF, rt, 18 h; (ii) NaN_3, EtOH, reflux, 6 h; (iii) PPh_3, H_2O, THF, rt, 2 h; (iv) Boc_2O, rt, 18 h.



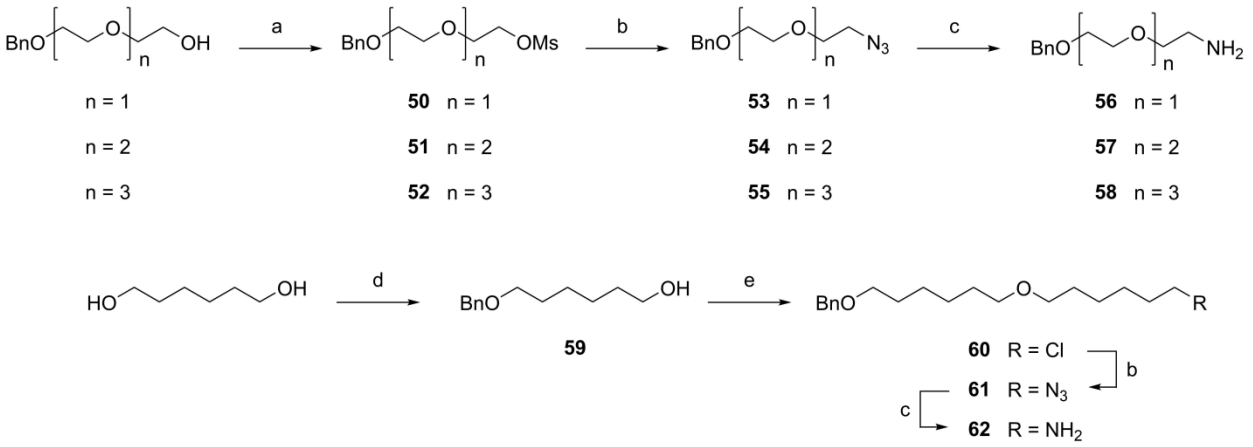
Scheme S2: Synthesis of protected carboxy linkers. *Reagents and conditions:* (a) **30** or **32**, *t*BuOH, DCC, DMAP, CH_2Cl_2, rt, 18 h; (b) NHS, DMAP, toluene, Et_3N, *t*BuOH, reflux, 24 h; (c) (i) **34**, NHS, DCC, DMF, rt, 3 h (ii) **20** or **27**, DIPEA, DMF, rt, 3 h.



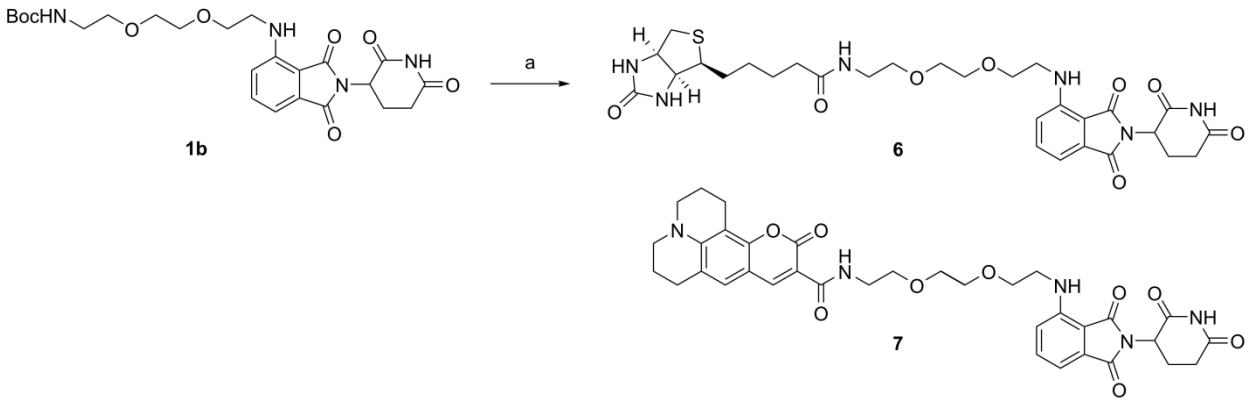
Scheme S3: Synthesis of alkyne linkers. *Reagents and conditions:* (a) $(Boc)_2O$, CH_2Cl_2, rt, 18 h; (b) ethyl bromoacetate, KO*t*Bu, *t*BuOH, rt, 18 h; (c) $NaBH_4$, THF, MeOH, reflux, 2 h; (d) (i) MsCl, Et_3N, THF, rt, 18 h; (ii) NaN_3, EtOH, reflux, 6 h; (iii) PPh_3, H_2O, THF, rt, 2 h; (iv) Boc_2O, rt, 18 h; (e) MsCl, Et_3N, CH_2Cl_2, rt, 3 h; (f) propargyl alcohol, NaH, THF, rt, 18 h.



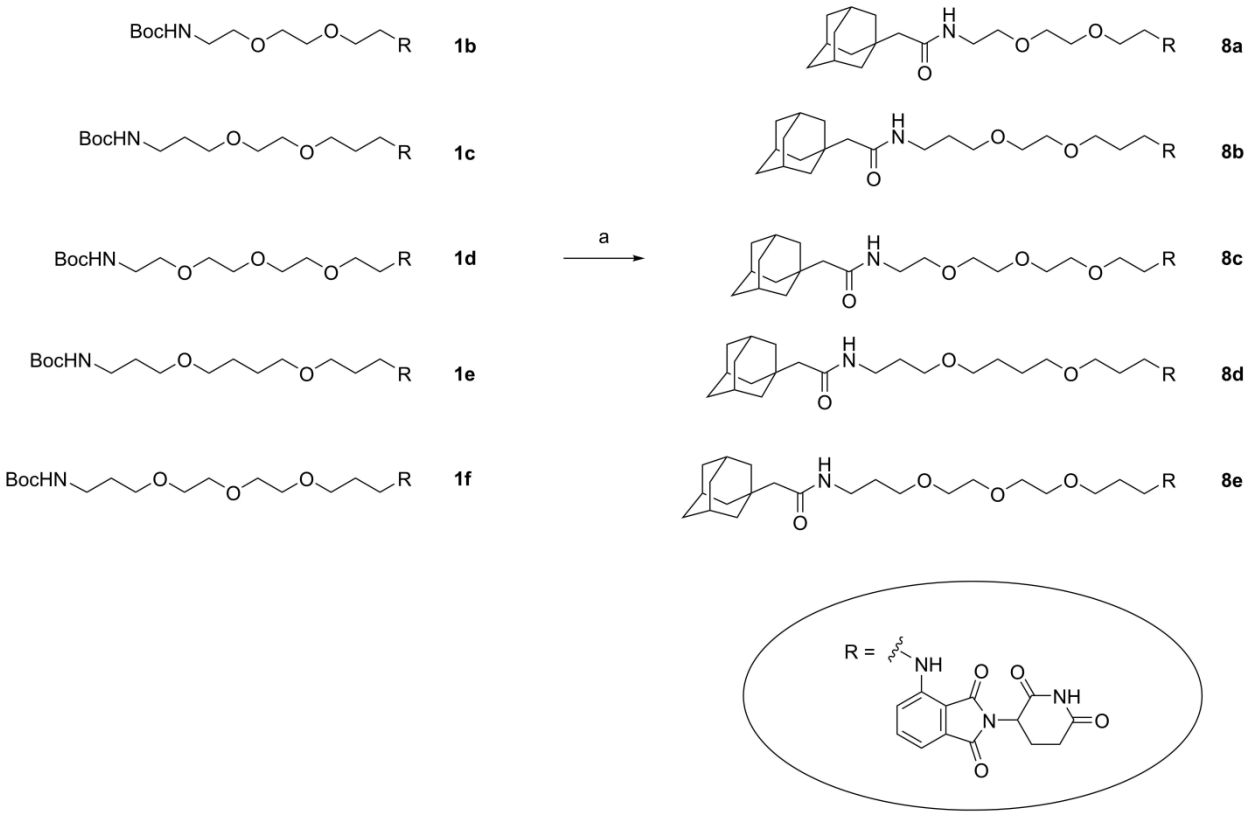
Scheme S4: Synthesis of chloro linkers. *Reagents and conditions:* (a) 1-chloro-6-iodohexane, KO*t*Bu, THF, rt, 18 h.



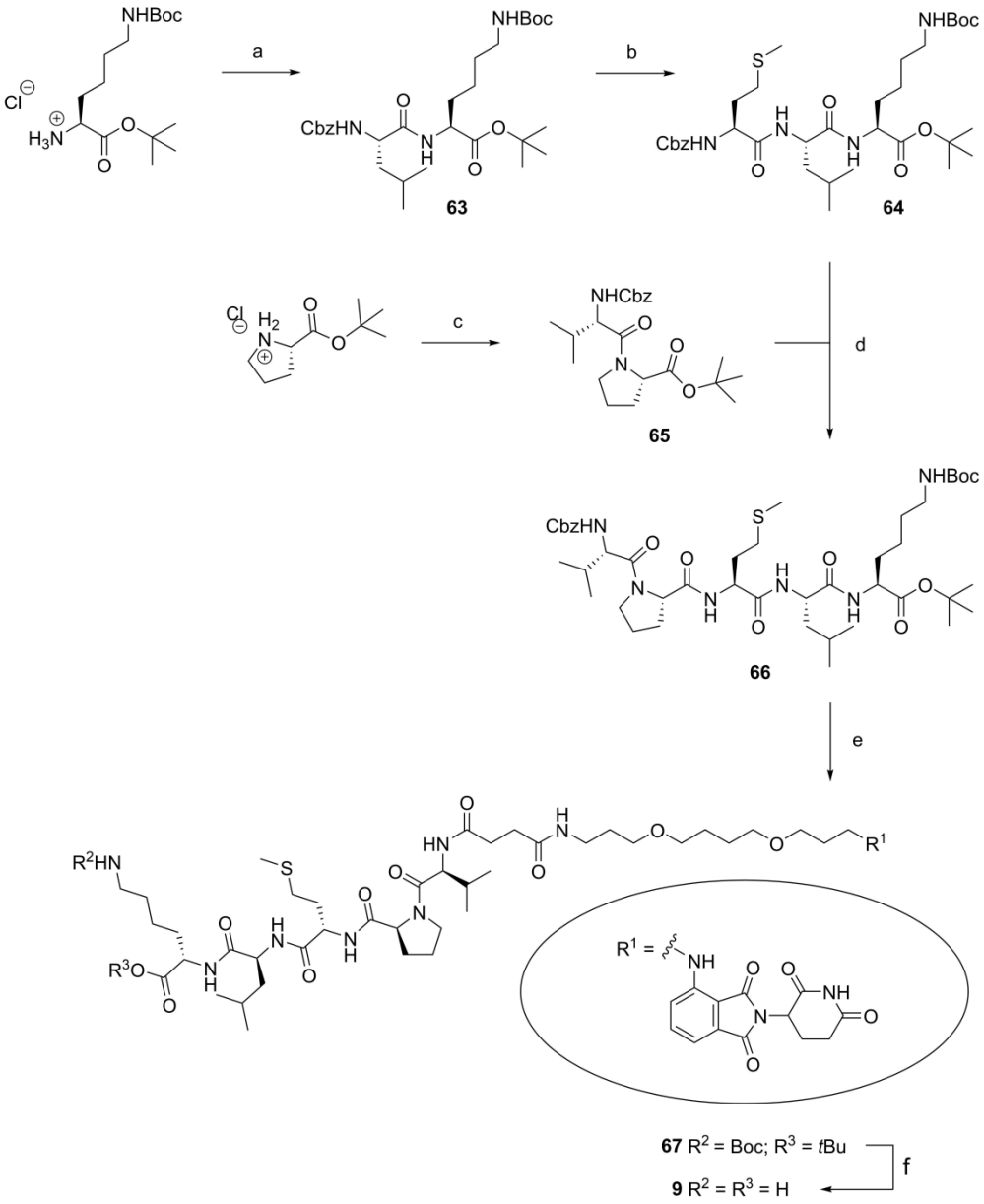
Scheme S5: Synthesis of benzyl-protected hydroxy linkers. *Reagents and conditions:* (a) MsCl, Et_3N, CH_2Cl_2, rt, 3 h; (b) NaN_3, DMF, 70 °C, 24 h; (c) PPh_3, H_2O, THF, rt, 24 h; (d) benzyl bromide, NaH, THF, rt, 24 h; (e) 1-bromo-6-chlorohexane, 50% NaOH, DMSO, rt, 24 h.



Scheme S6: Synthesis of the CRBN-addressing probes **6** and **7** with a biotin and fluorescent tag, respectively. *Reagents and conditions:* (a) (i) TFA, CH_2Cl_2, rt, 2 h; (ii) coumarin 343 or (+)-biotin, HATU, DIPEA, DMF, rt, 18 h.



Scheme S7: Synthesis of the CRBN-addressing hydrophobically tagged compounds **8**. *Reagents and conditions:* (a) (i) TFA, CH_2Cl_2, rt, 2 h; (ii) 1-adamantaneacetic acid, HATU, DIPEA, DMF, rt, 18 h.



Scheme S8: Synthesis of the VPMLK-tagged CRBN ligand **9.** *Reagents and conditions:* (a) Cbz-Leu-OH, isobutyl chloroformate, NMM, Et_3N, rt, 18 h; (b) (i) Pd/C, H_2, MeOH, rt, 24 h; (ii) Cbz-Met-OH, isobutyl chloroformate, NMM, Et_3N, rt, 18 h; (c) Cbz-Val-OH, isobutyl chloroformate, NMM, Et_3N, rt, 18 h; (d) (i) **64**, $Pd(OH)_2/C$, H_2 (60 psi), MeOH, rt, 5 h; (ii) **65**, TFA, CH_2Cl_2, 40 °C, 2 h; (iii) HBTU, DIPEA, DMF, rt, 24 h; (e) (i) **66**, $Pd(OH)_2/C$, H_2, MeOH, rt, 18 h; (ii) **2d**, TFA, CH_2Cl_2, 40 °C, 2 h; (iii) HATU, DIPEA, DMF; rt, 48 h; (f) TFA, CH_2Cl_2, 40 °C, 2 h.

Table S1: Overview on calculated physicochemical properties of toolbox compound **1**–**5**.

Cmpd	SMILES string	TPSA[a]
1a	CC(C)(C)OC(=O)NCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	143
1b	CC(C)(C)OC(=O)NCCOCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	152
1c	CC(C)(C)OC(=O)NCCCOCCOCCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	152
1d	CC(C)(C)OC(=O)NCCOCCOCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	162
1e	CC(C)(C)OC(=O)NCCCOCCCCOCCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	152
1f	CC(C)(C)OC(=O)NCCCOCCOCCOCCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	162
2a	CC(C)(C)OC(=O)CCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	122
2b	CC(C)(C)OC(=O)CCCCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	122
2c	CC(C)(C)OC(=O)CCC(=O)NCCOCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	169
2d	CC(C)(C)OC(=O)CCC(=O)NCCCOCCCCOCCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	169
3a	O=C1CCC(N2C(=O)c3cccc(NCCCCCOCC#C)c3C2=O)C(=O)N1	105
3b	O=C1CCC(N2C(=O)c3cccc(NCCOCCOCC#C)c3C2=O)C(=O)N1	114
3c	O=C1CCC(N2C(=O)c3cccc(NCCOCCOCCOCC#C)c3C2=O)C(=O)N1	123
3d	O=C1CCC(N2C(=O)c3cccc(NCCOCCOCCOCCOCC#C)c3C2=O)C(=O)N1	133
4a	ClCCCCCCOCCCCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	105
4b	ClCCCCCCOCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	114
4c	ClCCCCCCOCCOCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	123
4d	ClCCCCCCOCCOCCOCCOCCNc1cccc2C(=O)N(C3CCC(=O)NC3=O)C(=O)c12	133
5a	O=C1CCC(N2C(=O)c3cccc(NCCOCCOCc4ccccc4)c3C2=O)C(=O)N1	114
5b	O=C1CCC(N2C(=O)c3cccc(NCCOCCOCCOCc4ccccc4)c3C2=O)C(=O)N1	123
5c	O=C1CCC(N2C(=O)c3cccc(NCCOCCOCCOCCOCc4ccccc4)c3C2=O)C(=O)N1	133
5d	O=C1CCC(N2C(=O)c3cccc(NCCCCCCOCCCCCCOCc4ccccc4)c3C2=O)C(=O)N1	114

[a] Topological polar surface area given in Å².

Supplementary Information: Biology

A. Cell Lines

MM1S and LP-1 cell lines were obtained from the American Type Culture Collection (ATCC). 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 harvested and 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), IKZF1 (1:1000, Cell Signaling 14859S, clone: D6N9Y), BAX (1:1000, Cell signaling, clone: D2E11), or α-Tubulin (1:7000, Sigma T5168, clone: B512).

C. Cell Viability Assay

Cells were seeded in biological triplicates in a 96-plate and treated with the indicated concentrations of compounds or pomalidomide for 96 h. 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).

D. Statistical Analysis

Statistical analysis was performed using a two-sided Student *t*-test and Prism (v6.01, GraphPad). Variance of biological replicates is represented as mean ± SEM.

E. Determination of logP values

The determination of the logP values was performed according to the chromatographic method of Donovan and Pescatore (2002).[3] By correlating the retention time with the molecules lipophilicity with the aid of two validated internal standards, it is possible to estimate the logP value. The experiment was performed using a Waters 2695 Separations Module coupled with a Waters 996 Photodiode Array Detector. The column was a 10 × 4 mm LiChrospher 100 RP-18 5 µm EC from Chromatographie Service GmbH (Langerwehe, Germany), which was operated at 25 °C. The aqueous mobile phase consisted of a 0.01 M phosphate buffer solution with a pH of 6.8 to ensure the presence of the unionized compounds. The flow-rate was set to 1 mL/min with a linear gradient from 10 to 100% MeOH in 10 min, followed by a 6 min equilibration time. The detection wavelength was set at 260 nm and 390 nm, respectively. A standard solution of toluene (tol) and triphenylene (triph) as internal standards was prepared as follows: 10 mg triphenylene and 1 mL toluene were added to a volumetric flask and were diluted up to 100 mL of MeOH. Subsequently, approximately 1 mg of the analytes was dissolved in the standard solution and 10 µL were injected. The unknown logP values can be calculated from the retention time with the following equation:

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

F. Molecular Descriptor Calculation

Predicted values for the topological polar surface area (TPSA)[4] were calculated using the KNIME[5] implementation of RDKit.[6]

G. Measurements of Absorption and Emission Spectra

Absorption spectra were recorded on a Varian Cary 50 Bio spectrometer, emission spectra on a Monaco Safas spectrofluorometer flx at ambient temperature.

H. 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 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). 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 the compounds was determined by HPLC-UV obtained on an LC-MS instrument (Applied Biosystems API 2000 LC/MS/MS, HPLC Agilent 1100) or separately on a LC instrument (Acquity UPLC) and mass spectrometer (Thermo Scientific Q Exactive Plus). The purity of all the final compounds was confirmed to be ≥95% purity by LC.

I. Synthesis of the Toolbox Compounds 1-5

General Procedure I: Boc protection of alkanolamines. A solution of di-*tert*-butyl dicarbonate (2.40 g, 11.0 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a stirring solution of the corresponding alkanolamine (10.0 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C and then at rt for 18 h. Subsequently, the solution was diluted with CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$, H_2O and brine (each 50 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

General Procedure II: Mesylation.[7] To a solution of the corresponding alcohol (20 mmol) in dry CH_2Cl_2 (20 mL), Et_3N (3.04 g, 4.18 mL, 30 mmol) was added under argon atmosphere and the mixture was cooled to 0 °C. Subsequently, methanesulfonyl chloride (3.44 g, 2.34 mL, 30 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 3 h. After the reaction was complete (monitored by TLC), MeOH (20 mL) was added to the mixture carefully. The volatiles were then evaporated and the resultant residue was partitioned between EtOAc (100 mL) and H_2O (100 mL). The organic layer was further washed with brine (2 × 100 mL), dried over Na_2SO_4, filtered and evaporated.

General Procedure III: Azidolysis.[7] Sodium azide (1.95 g, 30 mmol) was added to a solution of the corresponding methanesulfonate (10 mmol) in DMF under argon atmosphere at rt. The reaction mixture was stirred at 70 °C for 24 h. Then, DMF was evaporated under reduced pressure and the resultant oily residue was partitioned between EtOAc (100 mL) and H_2O (100 mL). The organic phase was washed with brine (5 × 50 mL), dried over Na_2SO_4, filtered and evaporated.

General Procedure IV: Staudinger reduction.[7] To a solution of the corresponding azide (5 mmol) in THF (15 mL), PPh_3 (2.62 g, 10 mmol) was added under argon atmosphere at rt. After 10 minutes, H_2O (0.14 g, 0.14 mL, 7.5 mmol) was added in a single portion, followed by stirring of the mixture at rt for 24 h. Subsequently, the reaction mixture was evaporated under reduced pressure to yield crude oily products.

General Procedure V: Mono-Cbz protection of bisamines. To a solution of the corresponding bisamine (100 mmol) and Et_3N (1.21 g, 1.67 mL, 12 mmol) in $CHCl_3$ (200 mL), a solution of benzyl chloroformate (1.71 g, 1.43 mL, 10 mmol) in $CHCl_3$ (200 mL) was added dropwise at 0 °C over 3 h period. Subsequently, the reaction mixture was allowed to warm to rt and was stirred for additional 24 h. Then, the mixture was washed with saturated $NaHCO_3$, H_2O and brine (each 200 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure.

General Procedure VI: Mono-Boc protection of bisamines. A solution of di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) in $CHCl_3$ (100 mL) was added dropwise to a stirring solution of the corresponding bisamine (100 mmol) in $CHCl_3$ (100 mL) at 0 °C. The mixture was then stirred at rt 18 h. Subsequently, the solution was washed with H_2O (3 × 200 mL) and brine (200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

General Procedure VII: Etherification of methanesulfonates with propargyl alcohol.[8] To a solution stirred suspension of NaH (60% dispersion in mineral oil, 0.72 g, 18 mmol) in dry THF (10 mL) under an nitrogen atmosphere was added propargyl alcohol (1.68 g, 1.77 mL, 30 mmol) in dry THF (5 mL) at 0 °C and it was stirred for 30 min. Subsequently, a mixture of the corresponding methanesulfonate (10 mmol) in dry THF (5 mL) was added dropwise. The resulting suspension was stirred at rt overnight and was then quenched with saturated NH_4Cl solution (10 mL) and the organic solvent was removed *in vacuo*. The resultant liquid was partitioned between H_2O (50 mL) and EtOAc (100 mL). The organic layer was further washed with saturated NH_4Cl solution, saturated $NaHCO_3$ solution and brine (each 50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*.

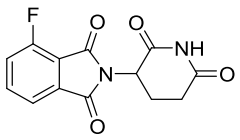
General Procedure VIII: Etherification of Boc-protected alkanolamines with 1-chloro-6-iodohexane. The corresponding boc-protected alkanolamine (5 mmol) was dissolved in dry THF (25 mL) and it was cooled to 0 °C. A mixture of potassium *tert*-butoxide (0.56 g, 5 mmol) in dry THF (10 mL) was added. The yellow mixture was stirred for 30 min at 0 °C. Then, 1-chloro-6-iodohexane (1.48 g, 0.91 mL, 6.0 mmol) was added dropwise at this temperature. After 3 h at 0 °C, stirring was continued at rt 18 h. Subsequently, H_2O (5 mL) was added to the suspension and the organic solvent was evaporated. The remaining residue was partitioned between EtOAc (50 mL) and H_2O (50 mL), and the aqueous layer was extracted again with EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated.

General Procedure IX: Boc-deprotection and nucleophilic aromatic substitution (S_NAr). The Boc-protected amine (1 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and it was treated with trifluoroacetic acid (5 mL). The reaction mixture was stirred for 2 h at rt. The solvent was removed and it was coevaporatd with dry CH_2Cl_2 (3 × 5 mL). The oily residue was further dried in high vacuum. The amine was dissolved in dry DMSO (10 mL) and DIPEA (0.52 g, 0.70 mL, 4 mmol). Finally, 4-fluoro-thalidomide[3] (**10**, 0.28 g, 1 mmol) was added, and the mixture was stirred at 90 °C for 18 h. After cooling to rt, the yellow or green mixture was partitioned between half-saturated brine (100 mL) and EtOAc (3 × 50 mL). The combined organic layers were further washed with saturated NH_4Cl solution, 5% LiCl solution, and brine (each 50 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*.

General Procedure X: Cbz-deprotection and nucleophilic aromatic substitution (S_NAr). The Cbz-protected amine (2 mmol) was dissolved in dry EtOAc (20 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 (20 mL) and DIPEA (0.52 g, 0.70 mL, 4 mmol) as well as 4-fluorothalidomide (**10**, 0.55 g, 2 mmol) were added. The mixture was stirred at 90 °C for 18 h. After cooling, it was poured onto half-saturated brine (200 mL) and it was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with 5% LiCl solution (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated.

General Procedure XI: Boc-deprotection and HATU coupling. The Boc-protected linker-pomalidomide conjugate (0.25 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and it was treated with trifluoroacetic acid (5 mL). The reaction mixture was stirred for 2 h at rt. The solvent was removed and it was coevaporatd with dry CH_2Cl_2 (3 × 5 mL). The oily residue was further dried in high vacuum. 1-Adamantaneacetic acid (49 mg, 0.25 mmol) was dissolved in dry DMF (5 mL) and DIPEA (0.13 g, 0.17 mL, 1.0 mmol) and HATU (105 mg, 0.275 mmol) were added. After stirring for 5 min, the deprotected amine derivative, dissolved in dry DMF (5 mL), was added. The combined mixture was stirred at rt for 18 h. Half-saturated brine (50 mL) was added and it was extracted with EtOAc (3 × 25 mL). The combined organic layers were further washed with saturated NH_4Cl solution, 5% LiCl solution, and brine (each 50 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*.

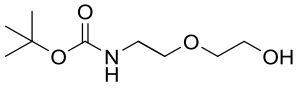
4-Fluorothalidomide (10)



This compound was synthesized as described previously.[9]

1. Carbamate-protected amino linkers:

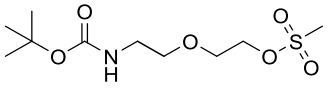
***tert*-Butyl *N*-[2-(2-hydroxyethoxy)ethyl]carbamate (11)**



This compound was prepared using the General Procedure **I** and 2-(2-aminoethoxy)ethan-1-ol (1.05 g, 10 mmol). The crude product was purified by column chromatography (EtOAc/*n*-hexane 1:1) to give a colorless oil.

Yield (1.50 g, 73%); R_f = 0.18 (EtOAc/*n*-hexane 1:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.37 (s, 9H, CH_3), 3.06 (q, *J* = 5.8 Hz, 2H, C$\underline{H}_2$NH), 3.34 – 3.41 (m, 4H, OCH_2), 3.44 – 3.50 (m, 2H, OCH_2), 4.58 (t, *J* = 5.6 Hz, 1H, OH), 6.78 (t, *J* = 5.8 Hz, 1H, NH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 28.30 (C($\underline{C}H_3)_3$), 40.24 (CH_2NH), 61.48 (CH_2OH), 70.20 (OCH_2), 72.17 (OCH_2), 79.24 ($\underline{C}(CH_3)_3$), 156.12 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min), t_R = 0.58 min, *m/z* $[M + H]^+$ calcd for $C_9H_{19}NO_4$, 206.13; found, 206.0; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_9H_{19}NO_4$, 228.1206; found, 228.1198.

2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)ethyl methanesulfonate (12)

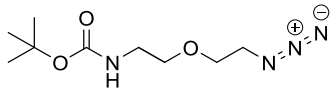


This compound was prepared using the General Procedure **II** and compound **11** (4.12 g, 20 mmol). The crude product (colorless oil) was used in the next step without further purification.

Yield (5.12 g, 90%); R_f = 0.66 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.43 (s, 9H, CH_3), 3.05 (s, 3H, SO_2CH_3), 3.36 (q, *J* = 5.4 Hz, 2H, C$\underline{H}_2$NH), 3.52 – 3.58 (m, 2H, OCH_2), 3.70 – 3.74 (m, 2H, OCH_2), 4.33 – 4.37 (m, 2H,

OCH_2), 4.91 (br s, 1H, NH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 28.25 ($C(\underline{C}H_3)_3$), 37.52 (SO_2CH_3), 40.06 (CH_2NH), 68.51 (OCH_2), 68.78 (OCH_2), 70.21 (OCH_2), 79.22 ($\underline{C}(CH_3)_3$), 155.81 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 194-400 nm), t_R = 6.70 min, 99% purity; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{10}H_{21}NO_6S$, 284.1162; found, 284.1153.

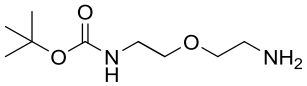
tert-Butyl (2-(2-azidoethoxy)ethyl)carbamate (13)



This compound was prepared using the General Procedure **III** and compound **12** (2.83 g, 10 mmol). The crude product (colorless oil) was used in the next step without further purification.

Yield (1.91 g, 83%); R_f = 0.71 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, CH_3), 3.27 (q, *J* = 5.2 Hz, 2H, $C\underline{H}_2NH$), 3.23 (t, *J* = 5.1 Hz, 2H, N_3CH_2), 3.49 (t, *J* = 5.2 Hz, 2H, OCH_2), 3.60 (t, *J* = 5.1 Hz, 2H, OCH_2), 4.95 (br s, 1H, NH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 28.22 ($C(\underline{C}H_3)_3$), 40.17 (CH_2NH), 50.49 (CH_2N_3), 69.75 (OCH_2), 70.15 (OCH_2), 79.19 ($\underline{C}(CH_3)_3$), 155.84 (CO); **HPLC** (95% H_2O to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 4.87 min, 95% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_9H_{18}N_4O_3$, 253.1271; found, 253.1269.

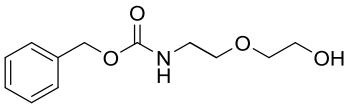
tert-Butyl (2-(2-aminoethoxy)ethyl)carbamate (14)



This compound was prepared using the General Procedure **IV** and compound **13** (1.15 g, 5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 15:1:0.5) to give a colorless oil.

Yield (0.86 g, 84%); R_f = 0.40 (CH_2Cl_2/MeOH/NH_4OH 30:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.42 (br s, 2H, NH_2), 1.43 (s, 9H, CH_3), 2.85 (t, *J* = 5.1 Hz, 2H, $C\underline{H}_2NH_2$), 3.31 (q, *J* = 5.1 Hz, 2H, $C\underline{H}_2NH$), 3.46 (t, *J* = 5.1 Hz, 2H, OCH_2), 3.50 (t, *J* = 5.1 Hz, 2H, OCH_2), 4.95 (br s, 1H, NH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 28.16 ($C(\underline{C}H_3)_3$), 40.11 (CH_2NH), 41.24 (CH_2NH_2), 69.75 (OCH_2), 72.39 (OCH_2), 78.89 ($\underline{C}(CH_3)_3$), 155.87 (CO); **HPLC** (95% H_2O to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 3.19 min, 96% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_9H_{20}N_2O_3$, 205.1547; found, 205.1541.

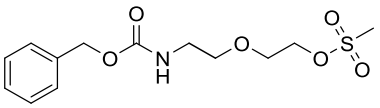
Benzyl (2-(2-hydroxyethoxy)ethyl)carbamate (15)[10]



This compound was synthesized similar to a previously reported procedure.[4] Briefly, to a cooled (0 °C) solution of 2-(2-aminoethoxy)ethan-1-ol (3.15 g, 30 mmol) in CH_2Cl_2 (40 mL), Et_3N (3.04 g, 4.16 mL, 30 mmol) and a solution of benzyl chloroformate (5.18 g, 4.28 mL, 30 mmol) in CH_2Cl_2 (40 mL) were added consecutively. The reaction mixture was first stirred at 0 °C for 2 h, followed by stirring at rt for 24 h. The resulting mixture was then washed with saturated $NaHCO_3$ (30 mL) and this aqueous phase washed again with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The compound was purified by column chromatography (EtOAc) to yield a colorless oil.

Yield (6.30 g, 88%); R_f = 0.38 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.52 (t, *J* = 5.7 Hz, 1H, OH), 3.39 (q, *J* = 5.1 Hz, 2H, C$\underline{H}_2$NH), 3.47 – 3.53 (m, 4H, OCH_2), 3.70 (q, *J* = 5.4 Hz, 2H, $HOCH_2$), 5.09 (s, 2H, CH_2), 5.39 (br s, 1H, NHCO), 7.27 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 40.79 (CH_2NH), 61.63 (CH_2OH), 66.71 (COO$\underline{C}$H$_2$), 70.04 (OCH_2), 72.17 (OCH_2), 128.09 (C-4'), 128.09 (C-2'), 128.47 (C-3'), 136.42 (C-1'), 156.55 (CO); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 3.95 min, 97% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{12}H_{17}NO_4$, 262.1050; found, 262.1040.

2-(2-(((Benzyloxy)carbonyl)amino)ethoxy)ethyl methanesulfonate (16)

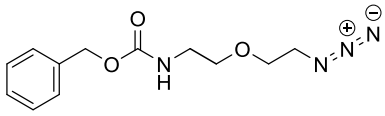


This compound was prepared using the General Procedure **II** and compound **15** (4.78 g, 20 mmol). The crude product (yellow oil) was used in the next step without further purification.

Yield (6.46 g, 99%); R_f = 0.60 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.97 (s, 3H, SO_2CH_3), 3.36 (q, *J* = 5.4 Hz, 2H, C$\underline{H}_2$NH), 3.54 (t, *J* = 5.3 Hz, 2H, OCH_2), 3.64 – 3.70 (m, 2H, OCH_2), 4.27 – 4.34 (m, 2H, OCH_2), 5.07 (s, 2H, CH_2), 5.31 (t, *J* = 5.5 Hz, 1H, NHCO), 7.24 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 37.39 (SO_2CH_3), 40.52 (CH_2NH), 66.48 (COO$\underline{C}$H$_2$), 68.47 (OCH_2), 68.67 (OCH_2), 69.29 (OCH_2), 127.93 (C-4'), 127.95 (C-2'), 128.36 (C-3'), 136.37 (C-1'), 156.28 (CO); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in

10 min, then 95% MeCN for 4 min), t_R = 4.64 min, 94% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{13}H_{19}NO_6S$, 340.0825; found, 340.0812.

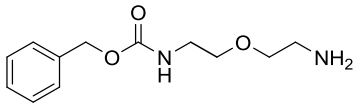
Benzyl (2-(2-azidoethoxy)ethyl)carbamate (17)



This compound was prepared using the General Procedure **III** and compound **16** (3.17 g, 10 mmol). The crude product (yellow oil) was used in the next step without further purification.

Yield (2.45 g, 93%); R_f = 0.76 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.37 (t, *J* = 4.8 Hz, 2H, N_3CH_2), 3.39 (q, *J* = 5.4 Hz, 2H, $C\underline{H}_2NH$), 3.54 (t, *J* = 5.1 Hz, 2H, OCH_2), 3.62 (t, *J* = 5.1 Hz, 2H, OCH_2), 5.09 (s, 2H, CH_2), 5.26 (t, *J* = 5.5 Hz, 1H, NHCO), 7.23 – 7.41 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 40.68 (CH_2NH), 50.44 (CH_2N_3), 66.51 ($COO\underline{C}H_2$), 69.78 (OCH_2), 69.92 (OCH_2), 127.89 (C-4'), 127.92 (C-2'), 128.35 (C-3'), 136.42 (C-1'), 156.31 (CO); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.20 min, 98% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{12}H_{16}N_4O_3$, 287.1115; found, 287.1104.

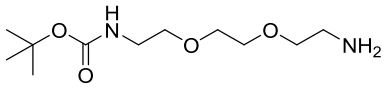
Benzyl (2-(2-aminoethoxy)ethyl)carbamate (18)



This compound was prepared using the General Procedure **VII** and compound **17** (1.32 g, 5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 30:1:0.5) to give a yellow oil.

Yield (1.11 g, 93%); R_f = 0.28 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.47 (br s, 2H, NH_2), 2.81 (t, *J* = 5.1 Hz, 2H, $C\underline{H}_2NH_2$), 3.37 (q, *J* = 5.3 Hz, 2H, $C\underline{H}_2NH$), 3.43 (t, *J* = 5.3 Hz, 2H, OCH_2), 3.50 (t, *J* = 5.1 Hz, 2H, OCH_2), 5.08 (s, 2H, $COOCH_2$), 5.44 (br s, 1H, NHCO), 7.24 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 40.79 (CH_2NH), 41.60 (CH_2NH_2), 66.54 ($COO\underline{C}H_2$), 69.65 (OCH_2), 73.01 (OCH_2), 127.98 (C-2'), 128.00 (C-4'), 128.39 (C-3'), 136.46 (C-1'), 156.38 (CO); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 3.26 min, 98% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{12}H_{18}N_2O_3$, 239.1390; found, 239.1388.

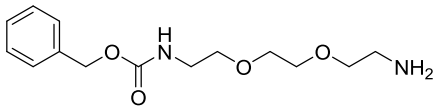
***tert*-Butyl *N*-[2-[2-(2-aminoethoxy)ethoxy]ethyl]carbamate (19)**



This compound was prepared using the General Procedure **VI** and 2,2'-(ethylenedioxy)bis(ethylamine) (14.82 g, 100 mmol) to give a colorless oil.

Yield (1.99 g, 80%); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 2.64 (t, J = 5.7 Hz, 2H, $C\underline{H}_2NH_2$), 3.05 (q, J = 6.0 Hz, 2H, $NHC\underline{H}_2$), 3.30 – 3.41 (m, 4H), 3.44 – 3.53 (m, 4H, OCH_2), 6.72 (br s, 1H, NH). The signals for NH_2 are not visible; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.36 (CH_3), 41.46 (CH_2NH_2), 69.31 (OCH_2), 69.68 (OCH_2), 73.13 (OCH_2), 77.70 ($\underline{C}(CH_3)_3$), 155.72 (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), t_R = 3.26 min, *m/z* $[M + H]^+$ calcd for $C_{11}H_{24}N_2O_4$, 249.18; found, 249.3.

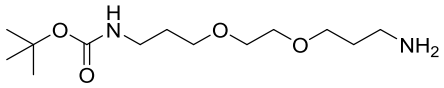
Benzyl *N*-[2-[2-(2-aminoethoxy)ethoxy]ethyl]carbamate (20)



This compound was prepared using the General Procedure **V** and 2,2'-(ethylenedioxy)bis(ethylamine) (14.82 g, 100 mmol) to give a colorless oil.

Yield (2.46 g, 87%); R_f = 0.21 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.65 (t, J = 5.7 Hz, 2H, $C\underline{H}_2NH_2$), 3.14 (q, J = 5.9 Hz, 2H, $NHC\underline{H}_2$), 3.33 – 3.44 (m, 4H), 3.45 – 3.53 (m, 4H, OCH_2), 5.00 (s, 2H, $COOCH_2$), 7.18 – 7.40 (m, 5H, Ar-H); The signals for NH and NH_2 are not visible; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 41.24 (CH_2NH_2), 65.36 ($COO\underline{C}H_2$), 69.26 (OCH_2), 69.69 (2 × OCH_2), 72.63 (OCH_2), 127.85 (C-2'), 127.89 (C-4'), 128.48 (C-3'), 137.36 (C-1), 156.33 (CO). The signal for $NH\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), t_R = 7.10 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{14}H_{22}N_2O_4$, 283.16; found, 282.9.

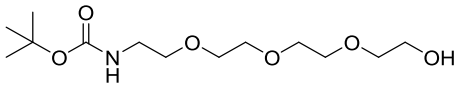
***tert*-Butyl *N*-[3-[2-(3-aminopropoxy)ethoxy]propyl]carbamate (21)**



This compound was prepared using the General Procedure **VI** and ethylene glycol bis(3-aminopropyl) ether (17.63 g, 100 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 19:1:0.5) to give a colorless oil.

Yield (1.74 g, 63%); R_f = 0.18 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 1.49 – 1.64 (m, 4H, CH_2, NH_2), 2.52 – 2.61 (m, 2H, CH_2), 2.89 – 2.99 (m, 4H, CH_2), 3.32 – 3.46 (m, 8H, OCH_2), 6.71 (t, J = 5.9 Hz, 1H, N<u>H</u>CH_2); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.40 (CH_3), 29.87, 33.22 (CH_2), 37.39, 38.86 (NCH_2), 68.24, 68.59, 69.61, 69.68 (OCH_2), 77.52 (<u>C</u>$(CH_3)_3$), 155.72 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 7.78 min, *m/z* $[M + H]^+$ calcd for $C_{13}H_{28}N_2O_4$, 277.21; found, 277.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{13}H_{28}N_2O_4$, 277.2122; found 277.2138.

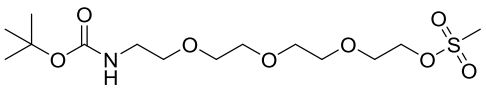
***tert*-Butyl *N*-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]carbamate (22)**[11]



This compound was synthesized similar to a previously reported procedure.[11] The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc) followed by a second column chromatography (gradient of CH_2Cl_2/MeOH 49:1 to 19:1) to yield a light yellow oil.

Yield (40%); R_f = 0.40 (CH_2Cl_2/MeOH 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H CH_3), 3.05 (q, J = 6.0 Hz, 2H, NHC<u>H</u>$_2$), 3.34 – 3.42 (m, 4H), 3.46 – 3.52 (m, 10H, OCH_2), 4.52 (t, J = 5.4 Hz, 1H, OH), 6.68 (br s, 1H, N<u>H</u>CH_2); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.38 (C(<u>C</u>$H_3)_3$), 60.38, 69.32, 69.67, 69.90, 69.92, 69.98, 72.48 (OCH_2), 77.73 (<u>C</u>$(CH_3)_3$), 155.74 (CO). The signal for NH<u>C</u>H_2 is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 8.59 min, *m/z* $[M + H]^+$ calcd for $C_{13}H_{27}NO_6$, 294.19; found, 294.1.

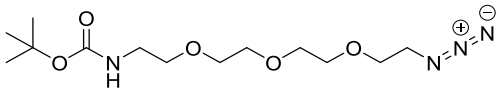
2-[2-[2-[2-(*tert*-Butoxycarbonylamino)ethoxy]ethoxy]ethoxy]ethyl methanesulfonate (23)



This compound was prepared using the General Procedure **II** and compound **22** (5.87 g, 20 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to EtOAc) to yield a colorless oil.

Yield (6.24 g, 84%); R_f = 0.50 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 (s, 9H, $C(CH_3)_3$), 3.05 (q, J = 6.1 Hz, 2H, CH_2), 3.16 (s, 3H, CH_3), 3.36 (t, J = 6.1 Hz, 2H, CH_2), 3.45 – 3.58 (m, 8H), 3.64 – 3.68 (m, 2H), 4.26 – 4.32 (m, 2H, OCH_2), 6.71 (t, J = 5.8 Hz, 1H, $NHCH_2$); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 28.41 ($C(\underline{C}H_3)_3$), 37.01 (CH_3), 68.46, 69.34, 69.68, 69.84, 69.89, 69.93 (OCH_2), 77.79 ($\underline{C}(CH_3)_3$), 155.78 (CO). The signal for $NH\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 9.07 min, *m/z* $[M + H]^+$ calcd for $C_{14}H_{29}NO_8S$, 372.16; found, 372.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{14}H_{29}NO_8S$, 372.1687; found 372.1688.

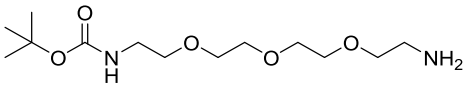
***tert*-Butyl *N*-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]carbamate (24)**



This compound was prepared using the General Procedure **III** and compound **23** (3.71 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to yield a colorless oil.

Yield (1.94 g, 61%); R_f = 0.38 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 3.05 (q, J = 6.0 Hz, 2H, $NHC\underline{H}_2$), 3.33 – 3.41 (m, 4H), 3.46 – 3.56 (m, 8H), 3.57 – 3.62 (m, 2H, OCH_2, N_3CH_2), 6.70 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 28.39 ($C(\underline{C}H_3)_3$), 50.17 (CH_2N_3), 69.33, 69.42, 69.67, 69.87, 69.95, 69.98, 77.74 ($\underline{C}(CH_3)_3$), 155.76 (CO). The signal for $NH\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 10.02 min, *m/z* $[M + H]^+$ calcd for $C_{13}H_{26}N_4O_5$, 319.19; found, 319.0; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{13}H_{26}N_4O_5$, 341.1795; found 341.1796.

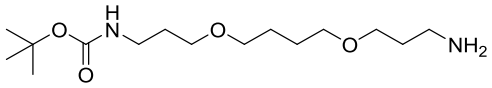
***tert*-Butyl *N*-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl]carbamate (25)**



This compound was prepared using the General Procedure **IV** and compound **24** (1.59 g, 5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 19:1:0.5) to give a colorless oil.

Yield (0.72 g, 49%); R_f = 0.37 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 2.65 (t, J = 5.7 Hz, 2H, C$\underline{H}_2$NH$_2$), 3.05 (q, J = 6.0 Hz, 2H, NHC$\underline{H}_2$), 3.32 – 3.39 (m, 4H), 3.45 – 3.53 (m, 8H, OCH_2), 6.75 (t, J = 5.8 Hz, 1H, N$\underline{H}$CH$_2$). The signals for NH_2 are not visible; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 28.40 (C($\underline{C}$H$_3$)$_3$), 41.33 (CH_2NH_2), 69.34, 69.68, 69.75, 69.92, 69.94, 72.81 (OCH_2), 77.73 ($\underline{C}$(CH$_3$)$_3$), 155.76 (CO). The signal for NH$\underline{C}$H$_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 7.14 min, *m/z* $[M + H]^+$ calcd for $C_{13}H_{28}N_2O_5$, 293.20; found, 293.0.

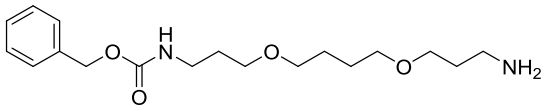
***tert*-Butyl *N*-[3-[4-(3-aminopropoxy)butoxy]propyl]carbamate (26)**



This compound was prepared using the General Procedure **VI** and 4,9-dioxa-1,12-dodecanediamine (20.43 g, 100 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 19:1:0.5) to give a colorless oil.

Yield (2.50 g, 82%); R_f = 0.25 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 1.45 – 1.62 (m, 8H, CH_2), 2.57 (t, J = 6.8 Hz, 2H, C$\underline{H}_2$NH$_2$), 2.94 (q, J = 6.7 Hz, 2H, NHC$\underline{H}_2$), 3.29 – 3.40 (m, 8H, OCH_2), 6.70 (t, J = 5.7 Hz, 1H, N$\underline{H}$CH$_2$). The signals for NH_2 are not visible; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 26.23 (2 × CH_2), 28.41 (C($\underline{C}$H$_3$)$_3$), 29.92, 33.22 (CH_2), 37.46, 38.92 (NCH_2), 67.85, 68.21, 69.97, 70.00 (OCH_2), 77.53 ($\underline{C}$(CH$_3$)$_3$), 155.74 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 8.62 min, *m/z* $[M + H]^+$ calcd for $C_{15}H_{32}N_2O_4$, 305.24; found, 305.0.

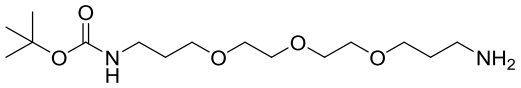
Benzyl *N*-[3-[4-(3-aminopropoxy)butoxy]propyl]carbamate (27)



This compound was prepared using the General Procedure **V** and 4,9-dioxa-1,12-dodecanediamine (20.43 g, 100 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 19:1:0.5) to give a colorless oil.

Yield (3.24 g, 96%); R_f = 0.32 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.43 – 1.65 (m, 9H, CH_2, NHC<u>H</u>H, NH_2), 2.57 (t, *J* = 6.9 Hz, 2H, C<u>H</u>$_2$$NH_2$), 3.03 (q, *J* = 6.6 Hz, 3H, NHCH<u>H</u>, CH_2), 3.31 – 3.40 (m, 8H, OCH_2), 4.99 (s, 2H, OCH_2), 7.20 (t, *J* = 5.9 Hz, 1H, CONH), 7.28 – 7.37 (m, 5H, Ar-H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 26.22, 29.85, 33.14 (CH_2), 37.83, 38.89 (NCH_2), 65.27, 67.67, 68.18, 69.95, 70.00 (OCH_2), 127.86, 127.89 (C-2', C-4'), 128.49 (C-3'), 137.45 (C-1'), 156.24 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 6.73 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{18}H_{30}N_2O_4$, 339.22; found, 339.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{30}N_2O_4$, 339.2274; found, 339.2259.

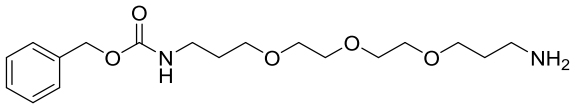
***tert*-Butyl *N*-[3-[2-[2-(3-aminopropoxy)ethoxy]ethoxy]propyl]carbamate (28)**



This compound was prepared using the General Procedure **VI** and diethylene glycol bis(3-aminopropyl) ether (22.03 g, 100 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 19:1:0.5) to give a colorless oil.

Yield (2.60 g, 81%); R_f = 0.18 (CH_2Cl_2/MeOH/Et_3N 19:1:0.5); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 1.44 – 1.71 (m, 4H, CH_2), 2.56 (t, *J* = 6.8 Hz, 2H, C<u>H</u>$_2$$NH_2$), 2.95 (q, *J* = 6.7 Hz, 2H, NHC<u>H</u>$_2$), 3.34 – 3.61 (m, 12H, OCH_2), 6.71 (t, *J* = 5.7 Hz, 1H, N<u>H</u>CH_2). The signals for NH_2 are not visible; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.41 (C(<u>C</u>H_3)$_3$), 29.88, 33.43 (CH_2), 37.40, 38.95 (NCH_2), 68.26, 68.64, 69.67, 69.71, 69.94, 69.96 (OCH_2), 77.54 (<u>C</u>$(CH_3)_3$), 155.75 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min), t_R = 8.00 min, *m/z* $[M + H]^+$ calcd for $C_{15}H_{32}N_2O_5$, 321.23; found, 321.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{15}H_{32}N_2O_5$, 321.2384; found, 321.2400.

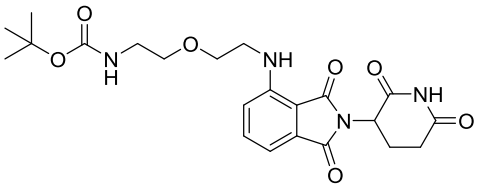
Benzyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (29)



This compound was prepared using the General Procedure **V** and 4,7,10-trioxa-1,13-tridecandiamine (22.03 g, 100 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 15:1:0.5) to give a colorless oil.

Yield (2.16 g, 61%); R_f = 0.39 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.60 – 1.71 (m, 4H, CH_2, NH_2), 1.77 (p, *J* = 6.1 Hz, 2H, CH_2), 2.75 (t, *J* = 6.7 Hz, 2H, $C\underline{H}_2NH_2$), 3.30 (q, *J* = 6.3 Hz, 2H, $C\underline{H}_2NH$), 3.46 – 3.63 (m, 12H, OCH_2), 5.07 (s, 2H, CH_2), 5.62 (br s, 1H, NHCO), 7.26 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 29.29 (CH_2), 33.16 (CH_2), 39.06 (CH_2NH), 39.52 (CH_2NH_2), 66.36 ($COO\underline{C}H_2$), 69.37 (OCH_2), 69.51 (OCH_2), 70.08 (OCH_2), 70.13 (OCH_2), 70.47 (OCH_2), 70.53(OCH_2), 127.93 (C-2'), 128.03 (C-4'), 128.40 (C-3'). 136.76 (C-1'), 156.49 (CO); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 4.13 min, 99% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{30}N_2O_5$, 355.2227; found, 355.2225.

Boc-protected amino tool compound 1a[12]

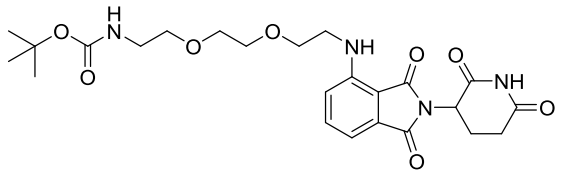


This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.21 g (0.74 mmol) of 4-fluorothalidomide and 0.15 g (0.74 mmol) of compound **14** were used. The crude product was purified by column chromatography (EtOAc/*n*-hexane 2:1) to give a yellow solid.

Yield (0.22 g, 65%); mp 58 – 60 °C; R_f = 0.42 (EtOAc); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 1.99 – 2.06 (m, 1H, 4'-H), 2.46 – 2.62 (m, 2H, 4'-H, 5'-H), 2.83 – 2.94 (m, 1H, 5'-H), 3.08 (q, *J* = 5.9 Hz, 2H, $NHC\underline{H}_2$), 3.39 – 3.50 (m, 4H, OCH_2), 3.59 (t, *J* = 5.4 Hz, 2H, $NHC\underline{H}_2$), 5.05 (dd, *J* = 5.3, 12.7 Hz, 1H, 3'-H), 6.61 (t, *J* = 5.9 Hz, 1H), 6.77 (t, *J* = 5.4 Hz, 1H, $N\underline{H}CH_2$), 7.04 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H, 5-H, 7-H), 7.58 (dd, *J* = 7.0, 8.7 Hz, 1H, 6-H), 11.11 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.08 (C-4'), 28.15 ($C(\underline{C}H_3)_3$), 30.92 (C-5'), 41.61 (Ar-$NH\underline{C}H_2$), 42.03 (CH_2NH), 48.49 (C-3'), 68.54 (OCH_2), 69.02 (OCH_2), 77.57 ($\underline{C}(CH_3)_3$), 109.16 (C-3a), 110.63 (C-7), 117.41 (C-5), 132.02 (C-7a), 136.18 (C-6), 146.34 (C-4), 155.54 (NHCO), 167.24 (C-3), 168.89 (C-1), 170.04 (C-2'), 172.77 (C-6'); **HPLC** (95% H_2O to 95% MeCN in

10 min, then 95% MeCN for 4 min), t_R = 5.24 min, 99% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{22}H_{28}N_4O_7$, 459.1870; found, 459.1879.

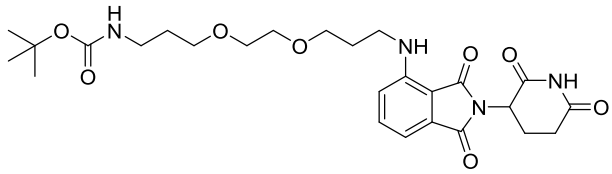
Boc-protected amino tool compound 1b[12]



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.41 g (1.5 mmol) of 4-fluorothalidomide and 0.37 g (1.5 mmol) of compound **19** were used. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a yellow solid.

Yield (0.27 g, 35%); mp 58 – 60 °C; R_f = 0.32 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.35 (s, 9H, CH_3), 1.98 – 2.06 (m, 1H, 4'-H), 2.51 – 2.63 (m, 2H, 4'-H, 5'-H), 2.81 – 2.93 (m, 1H, 5'-H), 3.02 – 3.07 (m, 2H, NHC$\underline{H}_2$), 3.37 (t, *J* = 6.2 Hz, 2H), 3.43 – 3.48 (m, 2H), 3.49 – 3.53 (m, 2H), 3.53 – 3.57 (m, 2H, OCH_2), 3.61 (t, *J* = 5.4 Hz, 2H, NHC$\underline{H}_2$), 5.04 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.59 (t, *J* = 5.8 Hz, 1H, N$\underline{H}CH_2$), 6.63 – 6.72 (m, 1H, CONH), 7.03 (d, *J* = 7.1 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), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.29 (C-4'), 28.36 (C($\underline{C}H_3)_3$), 31.14 (C-5'), 41.88 ($NHCH_2$), 48.73 (C-3'), 69.05, 69.37, 69.67, 69.85 (OCH_2), 77.72 ($\underline{C}(CH_3)_3$), 109.45 (C-3a), 110.82 (C-7), 117.59 (C-5), 132.26 (C-7a), 136.36 (C-6), 146.58 (C-4), 155.73 (CO), 167.43 (C-1), 169.09 (C-3), 170.17 (C-2'), 172.89 (C-6'). One 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 220-400 nm), t_R = 8.07 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{32}N_4O_8$, 505.23; found, 505.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{32}N_4O_8$, 505.2293; found, 505.2294.

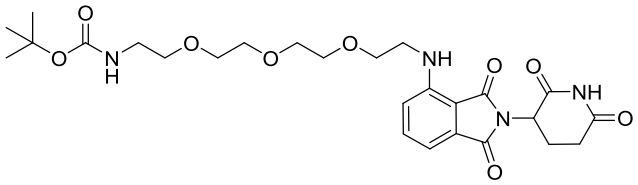
Boc-protected amino tool compound 1c



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.41 g (1.5 mmol) of 4-fluorothalidomide and 0.41 g (1.5 mmol) of compound **21** were used. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a yellow oil.

Yield (0.27 g, 75%); R_f = 0.31 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.35 (s, 9H, CH_3), 1.51 – 1.64 (m, 2H), 1.72 – 1.88 (m, 2H, CH_2), 1.95 – 2.07 (m, 1H, 4'-H), 2.45 – 2.65 (m, 2H, 4'-H, 5'-H), 2.80 – 3.00 (m, 3H, 5'-H, NHC$\underline{H}_2$), 3.31 – 3.43 (m, 4H), 3.43 – 3.59 (m, 6H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.57 – 6.76 (m, 2H, N$\underline{H}CH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.09 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H, 6-H), 11.04 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.31 (C-4'), 28.38 (C($\underline{C}H_3)_3$), 29.04, 29.88 (CH_2), 31.12 (C-5'), 37.40 ($NHCH_2$), 48.69 (C-3'), 68.27, 68.35, 69.62, 69.79 (OCH_2), 77.52 ($\underline{C}(CH_3)_3$), 109.26 (C-3a), 110.51 (C-7), 117.21 (C-5), 132.36 (C-7a), 136.39 (C-6), 146.61 (C-4), 155.70 (CO), 167.46 (C-1), 169.00 (C-3), 170.19 (C-2'), 172.90 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-450 nm), t_R = 10.70 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{26}H_{36}N_4O_8$, 533.26; found, 533.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{26}H_{36}N_4O_8$, 533.2606; found, 533.2588.

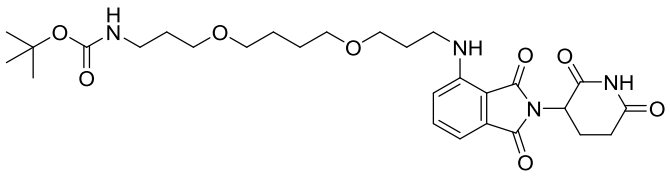
Boc-protected amino tool compound 1d[13]



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.41 g (1.5 mmol) of 4-fluorothalidomide and 0.44 g (1.5 mmol) of compound **25** were used. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to 1:4) to give a yellow solid.

Yield (0.57 g, 69%); mp 50 – 52 °C; R_f = 0.50 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.35 (s, 9H, CH_3), 1.99 – 2.05 (m, 1H, 4'-H), 2.50 – 2.62 (m, 2H, 4'-H, 5'-H), 2.82 – 2.92 (m, 1H, 5'-H), 3.04 (q, *J* = 6.0 Hz, 2H, NHC$\underline{H}_2$), 3.35 (t, *J* = 6.2 Hz, 2H), 3.43 – 3.54 (m, 8H), 3.54 – 3.59 (m, 2H, OCH_2), 3.61 (t, *J* = 5.4 Hz, 2H, NHC$\underline{H}_2$), 5.04 (dd, *J* = 5.5, 12.8 Hz, 1H, 3'-H), 6.59 (t, *J* = 5.8 Hz, 1H), 6.69 (t, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.03 (d, *J* = 7.0 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H, 5-H, 7-H), 7.57 (dd, *J* = 7.0, 8.6 Hz, 1H, 6-H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.31 (C-4'), 28.39 (C($\underline{C}H_3)_3$), 31.14 (C-5'), 41.88 ($NHCH_2$), 48.73 (C-3'), 69.07, 69.32, 69.67, 69.94 (OCH_2), 69.96 (2 × OCH_2), 77.76 ($\underline{C}(CH_3)_3$), 109.44 (C-3a), 110.85 (C-7), 117.63 (C-5), 132.28 (C-7a), 136.40 (C-6), 146.61 (C-4), 155.76 (CO), 167.47 (C-1), 169.12 (C-3), 170.22 (C-2'), 172.95 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 10.29 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{26}H_{36}N_4O_9$, 549.25; found, 549.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{26}H_{36}N_4O_9$, 549.2555; found, 549.2540.

Boc-protected amino tool compound 1e

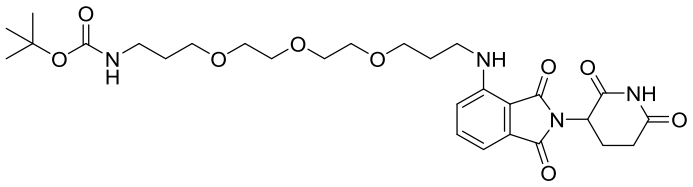


This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.83 g (3.0 mmol) of 4-fluorothalidomide and 0.91 g (3.0 mmol) of compound **26** were used. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:2) to give a yellow oil.

Yield (1.28 g, 76%); R_f = 0.42 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.35 (s, 9H), 1.46 – 1.63 (m, 6H), 1.74 – 1.83 (m, 2H, CH_2), 1.98 – 2.06 (m, 1H, 4'-H), 2.45 – 2.63 (m, 2H, 4'-H, 5'-H), 2.81 – 2.91 (m, 1H, 5'-H), 2.94 (q, *J* = 6.6 Hz, 2H, NHC$\underline{H}_2$), 3.27 – 3.39 (m, 8H, OCH_2), 3.44 (t, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.4, 12.9 Hz, 1H, 3'-H), 6.63 (t, *J* = 5.9 Hz, 1H), 6.70 (t, *J* = 5.8 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* = 7.0, 8.6 Hz, 1H, 6-H), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.32 (C-4'), 26.14, 26.21 (CH_2), 28.41 (C($\underline{C}H_3)_3$), 29.06, 29.92 (CH_2), 31.14 (C-5'), 37.46 ($NHCH_2$), 48.71 (C-3'), 67.84, 68.02, 70.01, 70.22 (OCH_2), 77.54 ($\underline{C}(CH_3)_3$), 109.27 (C-3a), 110.55 (C-7), 117.21 (C-5), 132.39 (C-7a), 136.42 (C-6), 146.61 (C-4), 155.74 (CO), 167.49 (C-1), 169.01 (C-3), 170.24 (C-2'), 172.96 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm),

t_R = 11.21 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{28}H_{40}N_4O_8$, 561.29; found, 561.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{28}H_{40}N_4O_8$, 561.2919; found, 561.2909.

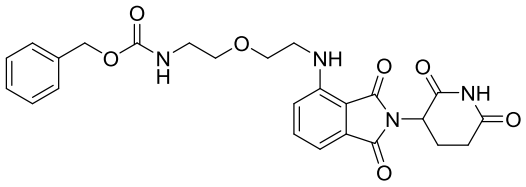
Boc-protected amino tool compound 1f[14]



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.83 g (3.0 mmol) of 4-fluorothalidomide and 0.96 g (3.0 mmol) of compound **28** were used. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:4) to give a yellow oil.

Yield (1.02 g, 59%); R_f = 0.33 (petroleum ether/EtOAc 1:4); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.35 (s, 9H, CH_3), 1.53 – 1.61 (m, 2H), 1.76 – 1.84 (m, 2H, CH_2), 1.98 – 2.05 (m, 1H, 4'-H), 2.46 – 2.61 (m, 2H, 4'-H, 5'-H), 2.83 – 2.91 (m, 1H, 5'-H), 2.94 (q, *J* = 6.6 Hz, 2H, NHC$\underline{H}_2$), 3.33 – 3.38 (m, 4H), 3.42 – 3.46 (m, 2H), 3.46 – 3.56 (m, 8H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H), 6.64 (t, *J* = 5.9 Hz, 1H), 6.70 (t, *J* = 5.2 Hz, 1H, N$\underline{H}CH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.09 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, *J* = 7.0, 8.6 Hz, 1H, 6-H), 11.06 (br s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.34 (C-4'), 28.42 (C($\underline{C}H_3)_3$), 29.07, 29.89 (CH_2), 31.15 (C-5'), 37.42 ($NHCH_2$), 48.72 (C-3'), 68.27, 68.39, 69.72, 69.88, 69.92, 69.97 (OCH_2), 77.57 ($\underline{C}(CH_3)_3$), 109.26 (C-3a), 110.55 (C-7), 117.27 (C-5), 132.39 (C-7a), 136.46 (C-6), 146.65 (C-4), 155.75 (CO), 167.51 (C-1), 169.04 (C-3), 170.25 (C-2'), 172.98 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 10.72 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{28}H_{40}N_4O_9$, 577.28; found, 577.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{28}H_{40}N_4O_9$, 577.2868; found, 577.2877.

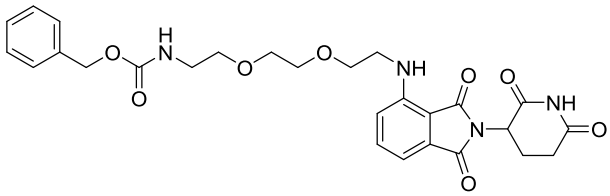
Cbz-protected amino tool compound 1g



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.40 g (1.45 mmol) of 4-fluorothalidomide and 0.35 g (1.45 mmol) of compound **18** were used. The crude product was purified by column chromatography (EtOAc/*n*-hexane 1:1) to give a green solid.

Yield (0.26 g, 36%); mp 51 – 53 °C; R_f = 0.14 (EtOAc/*n*-hexane 1:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.99 – 2.05 (m, 1H, 4'-H), 2.45 – 2.63 (m, 2H, 4'-H, 5'-H), 2.80 – 2.94 (m, 1H, 5'-H), 3.16 (q, *J* = 5.7 Hz, 2H, NHC$\underline{H}_2$), 3.41 – 3.52 (m, 4H, OCH_2), 3.60 (t, *J* = 5.3 Hz, 2H, NHC$\underline{H}_2$), 5.00 (s, 2H, OCH_2), 5.05 (dd, *J* = 5.3, 12.8 Hz, 1H, 3'-H), 6.61 (t, *J* = 5.7 Hz, 1H, $CH_2N\underline{H}$), 7.03 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H, 5-H, 7-H), 7.24 – 7.38 (m, 6H, Ar-H, $CH_2N\underline{H}$), 7.57 (dd, *J* = 7.0, 8.7 Hz, 1H, 6-H), 11.11 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.07 (C-4'), 30.92 (C-5'), 41.60 (NH$\underline{C}H_2$), 48.49 (C-3'), 65.20, 68.60, 68.98 (OCH_2), 109.16 (C-3a), 110.63 (C-7), 117.41 (C-5), 127.66 (C-2''), 127.70 (C-4''), 128.28 (C-3''), 132.02 (C-7a), 136.18 (C-6), 137.12 (C-1''), 146.34 (C-4), 156.13 (NHCO), 167.25 (C-3), 168.88 (C-1), 170.05 (C-2'), 172.77 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.45 min, 98% purity, detection at 210 nm; **HRMS** (ESI) *m/z*$[M - H]^-$ calcd for $C_{22}H_{26}N_4O_7$, 493.1718; found, 493.1723.

Cbz-protected amino tool compound 1h

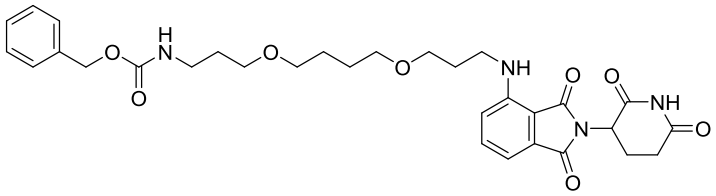


This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.28 g (1.0 mmol) of 4-fluorothalidomide and 0.28 g (1.0 mmol) of compound **20** were used. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc) to give a yellow oil.

Yield (0.29 g, 53%); R_f = 0.42 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.95 – 2.06 (m, 1H, 4'-H), 2.44 – 2.62 (m, 2H, 4'-H, 5'-H), 2.79 – 2.93 (m, 1H, 5'-H), 3.13 (q, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.38 – 3.67 (m, 10H,

OCH_2, $NHC\underline{H}_2$), 4.99 (s, 2H, OCH_2), 5.04 (dd, *J* = 5.4, 12.8 Hz, 1H, 3'-H), 6.58 (t, *J* = 5.8 Hz, 1H), 7.18 (t, *J* = 5.7 Hz, 1H, $CH_2N\underline{H}$), 7.03 (d, *J* = 6.9 Hz, 1H), 7.12 (d, *J* = 8.7 Hz, 1H, 5-H, 7-H), 7.26 – 7.38 (m, 5H, Ar-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 22.29 (C-4'), 31.13 (C-5'), 41.88 ($NHCH_2$), 48.74 (C-3'), 65.36, 69.05, 69.31, 69.73, 69.85 (OCH_2), 109.45 (C-3a), 110.83 (C-7), 117.59 (C-5), 127.85 (C-2''), 127.89 (C-4''), 128.48 (C-3''), 132.26 (C-7a), 136.37 (C-6), 137.35 (C-1''), 146.59 (C-4), 156.32 (CO), 167.45 (C-1), 169.11 (C-3), 170.19 (C-2'), 172.91 (C-6'). One 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 220-450 nm), t_R = 8.24 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{27}H_{30}N_4O_8$, 539.21; found, 539.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{27}H_{30}N_4O_8$, 539.2136; found, 539.2145.

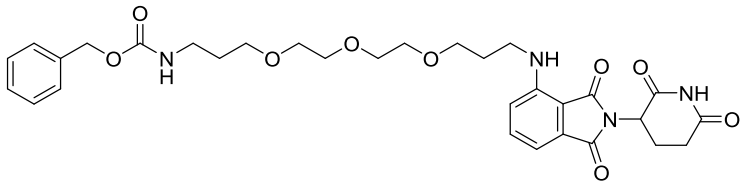
Cbz-protected amino tool compound 1i



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 1.10 g (4.0 mmol) of 4-fluorothalidomide and 1.35 g (4.0 mmol) of compound **27** were used. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 39:1 to 29:1) to give a yellow oil.

Yield (1.09 g, 46%); R_f = 0.38 (CH_2Cl_2/EtOH 29:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.46 – 1.56 (m, 4H), 1.57 – 1.65 (m, 2H), 1.74 – 1.84 (m, 2H, CH_2), 1.92 – 2.12 (m, 1H, 4'-H), 2.46 – 2.63 (m, 2H, 4'-H, 5'-H), 2.81 – 2.93 (m, 1H, 5'-H), 3.03 (q, *J* = 6.6 Hz, 2H), 3.44 (t, *J* = 5.9 Hz, 2H, $NHC\underline{H}_2$), 3.26 – 3.39 (m, 8H, OCH_2), 4.99 (s, 2H, OCH_2), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H, 3'-H), 6.63 (t, *J* = 5.9 Hz, 1H), 7.18 (t, *J* = 5.7 Hz, 1H, $CH_2N\underline{H}$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.26 – 7.37 (m, 5H), 7.56 (dd, *J* = 7.0, 8.6 Hz, 1H, 6-H), 11.06 (br s, 1H, NH). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.32 (C-4'), 26.13, 26.20, 29.05, 29.85 (CH_2), 31.14 (C-5'), 37.84 ($NH\underline{C}H_2$), 48.71 (C-3'), 65.27, 67.68, 68.02, 70.01, 70.20 (OCH_2), 109.27 (C-3a), 110.55 (C-7), 117.20 (C-5), 127.87 (C-2''), 127.90 (C-4''), 128.49 (C-3''), 132.38 (C-7a), 136.41 (C-6), 137.44 (C-1''), 146.60 (C-4), 156.25 (CO), 167.49 (C-1), 169.01 (C-3), 170.24 (C-2'), 172.96 (C-6'). One 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 220-450 nm), t_R = 9.07 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{31}H_{38}N_4O_8$, 595.27; found, 595.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{31}H_{38}N_4O_8$, 595.2762; found, 595.2737.

Cbz-protected amino tool compound 1k

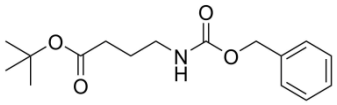


This compound was prepared using the General Procedure IV for the nucleophilic aromatic substitution, where 0.13 g (0.47 mmol) of 4-fluorothalidomide and 0.17 g (0.47 mmol) of compound **29** were used. The crude product was purified by column chromatography (EtOAc/*n*-hexane 4:1) to give a yellow oil.

Yield (0.12 g, 42%); R_f = 0.24 (EtOAc); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.61 (p, *J* = 6.7 H, 2H, CH_2), 1.80 (p, *J* = 6.1 Hz, 2H, CH_2), 1.98 – 2.07 (m, 1H, 4'-H), 2.45 – 2.64 (m, 2H, 4'-H, 5'-H), 2.81 – 2.94 (m, 1H, 5'-H), 3.03 (q, *J* = 6.0 Hz, 2H, Ar-NHC$\underline{H}_2$), 3.34 – 3.40 (m, 4H, OCH_2), 3.44 – 3.57 (m, 10H, OCH_2, C$\underline{H}_2$NH), 4.99 (s, 2H, CH_2), 5.05 (dd, *J* = 5.3, 12.8 Hz, 1H, 3'-H), 6.67 (t, *J* = 6.0 Hz, 1H, Ar-N$\underline{H}CH_2$), 7.02 (d, *J* = 7.0 Hz, 1H), 7.09 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.22 (t, *J* = 5.6 Hz, 1H, CH_2N$\underline{H}$CO), 7.26 – 7.38 (m, 5H, Ar-H), 7.57 (dd, *J* = 7.0, 8.6 Hz, 1H, 6-H), 11.10 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.10 (C-4'), 28.82 (CH_2), 29.57 (CH_2), 30.92 (C-5'), 37.56 (CH_2NH), 39.53 (Ar-NH$\underline{C}H_2$), 48.45 (C-3'), 65.08 (COO$\underline{C}H_2$), 67.86 (OCH_2), 68.15 (OCH_2), 69.48 (OCH_2), 69.63 (OCH_2), 60.67 (OCH_2), 69.72 (OCH_2), 108.97 (C-3a), 110.31 (C-7), 117.03 (C-5), 127.69 (C-2', C-4'), 128.28 (C-3'), 132.13 (C-7a), 136.22 (C-6), 137.19 (C-1'), 146.38 (C-4), 156.01 (NHCO), 167.27 (C-3), 168.78 (C-1), 170.06 (C-2'), 172.78 (C-6'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.86 min, 99% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{31}H_{38}N_4O_9$, 609.2555; found, 609.2556.

2. Protected carboxy linkers:

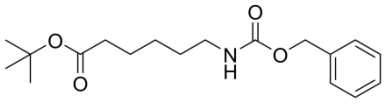
tert-Butyl 4-(benzyloxycarbonylamino)butanoate (31)



Cbz-γ-Abu-OH (**30**, 2.37 g, 10 mmol) was dissolved in dry CH_2Cl_2 and DMAP (0.12 g, 1 mmol) and *t*BuOH (3.71 g, 4.7 mL, 50 mmol) were added. After stirring at 0 °C for 5 min, DCC (2.27 g, 11 mmol) was added. The resulting mixture was stirred for 18 h at rt, after which the precipitate was filtered and it was washed with CH_2Cl_2 (25 mL). The filtrate was washed with H_2O (50 mL) and extracted once again with CH_2Cl_2 (50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a colorless oil which was further purified by column chromatography (CH_2Cl_2).

Yield (1.36 g, 46%); R_f = 0.31 (CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.38 (s, 9H, CH_3), 1.56 – 1.65 (m, 2H), 2.14 – 2.21 (m, 2H, CH_2), 2.99 (q, J = 6.6 Hz, 2H, NHC$\underline{H}_2$), 4.99 (s, 2H, OCH_2), 7.18 – 7.25 (m, 1H, NH), 7.26 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.12 (CH_2), 27.91 (CH_3), 32.24 (CH_2), 65.31 (OCH_2), 79.68 ($\underline{C}(CH_3)_3$), 127.85, 127.89, 128.48 (C-2, C-3, C-4), 137.41 (C-1), 156.28, 172.10 (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 = 11.02 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{16}H_{23}NO_4$, 294.17; found, 294.0.

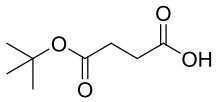
tert-Butyl 6-(benzyloxycarbonylamino)hexanoate (33)



This compound was synthesized in analogy to compound **31**. *N*-Carbobenzoxy-6-aminohexanoic acid (**32**, 2.65 g, 10 mmol) was used as starting material to give a colorless oil.

Yield (1.45 g, 45%); R_f = 0.22 (CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.18 – 1.28 (m, 2H, CH_2), 1.32 – 1.52 (m, 13H, CH_2, CH_3), 2.11 – 2.18 (m, 2H, CH_2), 2.92 – 3.00 (m, 2H, NHC$\underline{H}_2$), 4.99 (s, 2H, OCH_2), 7.18 (t, J = 5.7 Hz, 1H, NH), 7.23 – 7.41 (m, 5H, Ar-H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.77, 27.92 (CH_3), 29.20, 34.87, 40.25, 65.22 (OCH_2), 79.51 ($\underline{C}(CH_3)_3$), 127.82, 127.86, 128.47 (C-2, C-3, C-4), 137.46 (C-1), 156.24, 172.37 (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 = 11.49 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{18}H_{27}NO_4$, 322.20; found, 322.0.

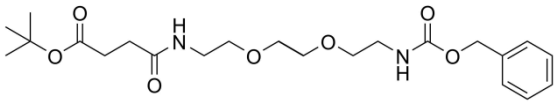
tert-Butyl hemisuccinate (34)[15]



This compound was synthesized similar to a previously reported procedure.[15] In brief, to a suspension of succinic anhydride (10.00 g, 100 mmol), *N*-hydroxysuccinimide (3.45 g, 30 mmol) and DMAP (1.22 g, 10 mmol) in dry toluene (50 mL) was added Et_3N (3.04 g, 4.16 mL, 30 mmol) and *t*BuOH (9.64 g, 12.20 mL, 130 mmol). The mixture was stirred at reflux for 24 h. Subsequently, the dark mixture was diluted with EtOAc (50 mL) and it was washed with 10% citric acid solution (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The brownish oil was triturated with a hot mixture of Et_2O and *n*-hexanes 3:1 (100 mL) and it was filtered. The filtrate was allowed to cool down to rt, filtered again and it was further stored at –18 °C overnight to give colorless crystals.

Yield (7.85 g, 45%); mp 44 – 46 °C, lit. mp 44 – 45 °C; **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.37 (s, 9H, CH_3), 2.35 – 2.43 (m, 4H, CH_2), 12.11 (br s, 1H, COOH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 27.89 (C($\underline{C}H_3)_3$), 28.99, 30.04 (CH_2), 79.85 ($\underline{C}(CH_3)_3$), 171.49, 173.58 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 0.66 min, *m/z* $[M + H]^+$ calcd for $C_8H_{14}O_4$, 175.09; found, 175.1.

tert-Butyl 4-[2-[2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy]ethylamino]-4-oxo-butanoate (35)

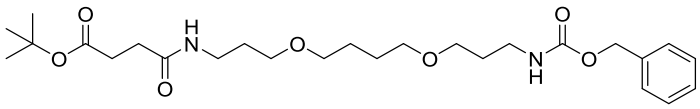


To a solution of **34** (0.52 g, 3 mmol) in dry DMF (3 mL) was added *N*-hydroxysuccinimide (0.33 g, 2.9 mmol) with stirring at rt. Subsequently, DCC (0.60 g, 2.9 mmol) was added to the mixture. After stirring for 3 h at rt, the suspension was filtered and it was washed with dry DMF (5 mL). The filtrate was added to a solution of **20** (0.57 g, 2 mmol) and DIPEA (0.78 g, 1.05 mL, 6 mmol) in dry DMF (5 mL). The resulting

reaction mixture was stirred for another 3 h at rt. Then, it was diluted with EtOAc (50 mL) and the mixture was washed with 10% $KHSO_4$, H_2O, and brine (each 25 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a colorless oil which was further purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc).

Yield (0.53 g, 60%); R_f = 0.37 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 2.28 (t, J = 6.9 Hz, 2H), 2.36 (t, J = 6.6 Hz, 2H, CH_2), 3.10 – 3.20 (m, 4H), 3.36 – 3.42 (m, 4H), 3.46 – 3.51 (m, 4H, OCH_2, $NHC\underline{H}_2$), 5.00 (s, 2H, OCH_2), 7.22 (t, J = 5.6 Hz, 1H, NH), 7.26 – 7.38 (m, 5H, Ar-H), 7.85 (t, J = 5.6 Hz, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 27.93 (CH_3), 30.20, 30.44 (CH_2), 38.77 ($NHCH_2$), 65.41 (OCH_2), 69.30 (2 × OCH_2), 69.68, 69.71 (OCH_2), 79.70 ($\underline{C}(CH_3)_3$), 127.90, 127.94, 128.53 (C-2, C-3, C-4), 137.38 (C-1), 156.37, 171.08, 171.77 (CO). One signal for $NH\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOHN to 20 min, DAD 200-400 nm), t_R = 10.61 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{22}H_{34}N_2O_7$, 439.24; found, 439.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{22}H_{34}N_2O_7$, 439.2439; found, 439.2434.

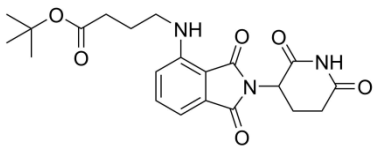
tert-Butyl 4-[3-[4-[3-(benzyloxycarbonylamino)propoxy]butoxy]propylamino]-4-oxo-butanoate (36)



To a solution of **34** (2.61 g, 15 mmol) in dry DMF (15 mL) was added *N*-hydroxysuccinimide (1.67 g, 14.5 mmol) with stirring at rt. Subsequently, DCC (2.99 g, 14.5 mmol) was added to the mixture. After stirring for 3 h at rt, the suspension was filtered and it was washed with dry DMF (5 mL). The filtrate was added to a solution of **27** (3.38 g, 10 mmol) and DIPEA (3.88 g, 5.1 mL, 30 mmol) in dry DMF (10 mL). The resulting reaction mixture was stirred for another 3 h at rt. Then, it was diluted with EtOAc (250 mL) and the mixture was washed with 10% $KHSO_4$, H_2O, and brine (each 125 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a colorless oil which was further purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc).

Yield (3.71 g, 75%); R_f = 0.51 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 1.47 – 1.52 (m, 4H), 1.55 – 1.65 (m, 4H), 2.25 (t, *J* = 6.9 Hz, 2H), 2.37 (t, *J* = 6.9 Hz, 2H, CH_2), 3.00 – 3.08 (m, 4H), 3.31 – 3.35 (m, 8H, OCH_2, $NHC\underline{H}_2$), 4.99 (s, 2H, OCH_2), 7.19 (t, *J* = 5.8 Hz, 1H, NH), 7.26 – 7.38 (m, 5H, Ar-H), 7.76 (t, *J* = 5.6 Hz, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 26.17 (2 × CH_2), 27.87 ($C(\underline{C}H_3)_3$), 29.58, 30.24, 30.44, 30.81 (CH_2), 35.96, 37.79 ($NHCH_2$), 65.24, 67.64, 67.78, 69.95, 69.96 (OCH_2), 79.61 ($\underline{C}(CH_3)_3$), 127.84, 127.87, 128.47 (C-2, C-3, C-4), 137.43 (C-1), 156.22, 170.75, 171.74 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 200-400 nm), t_R = 9.79 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{26}H_{42}N_2O_7$, 495.30; found, 495.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{26}H_{42}N_2O_7$, 495.3065; found, 495.3021.

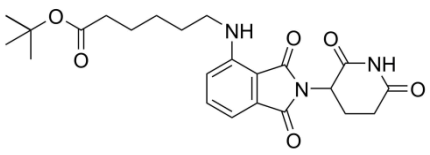
Protected carboxy tool compound 2a



This compound was prepared using the General Procedure **X** and linker **31** (0.59 g, 2 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 1:1) to obtain a green oil.

Yield (0.38 g, 46%); R_f = 0.45 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (600 MHz, DMSO-d_6) 1.38 (s, 9H, CH_3), 1.73 – 1.81 (m, 2H, CH_2), 1.97 – 2.05 (m, 1H, 4'-H), 2.28 (t, *J* = 7.2 Hz, 2H, CH_2), 2.50 – 2.62 (m, 2H, 4'-H, 5'-H), 2.83 – 2.92 (m, 1H, 5'-H), 3.27 – 3.34 (m, 2H, $NHC\underline{H}_2$), 5.04 (dd, *J* = 5.4, 12.8 Hz, 1H, 3'-H), 6.62 (t, *J* = 6.2 Hz, 1H, $N\underline{H}CH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.10 (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'), 24.35 (CH_2), 27.91 ($C(\underline{C}H_3)_3$), 31.13 (C-5'), 32.19 (CH_2), 41.30 ($NHCH_2$), 48.70 (C-3'), 79.87 ($\underline{C}(CH_3)_3$), 109.35 (C-3a), 110.61 (C-7), 117.25 (C-5), 132.41 (C-7a), 136.39 (C-6), 146.47 (C-4), 167.44 (C-1), 168.97 (C-3), 170.20 (C-2'), 172.10, 172.93 (C-6', CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 10.77 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{21}H_{25}N_3O_6$, 416.18; found, 416.0; **HRMS**(ESI) *m/z* $[M + H]^+$ calcd for $C_{21}H_{25}N_3O_6$, 416.1816; found, 416.1814.

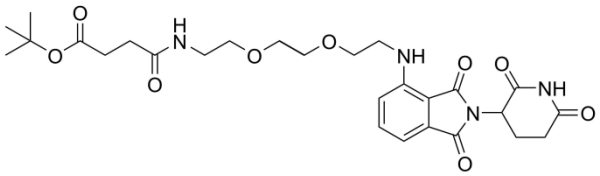
Protected carboxy tool compound 2b



This compound was prepared using the General Procedure **X** and linker **33** (0.64 g, 2 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 1:1) to obtain a yellow oil.

Yield (0.38 g, 43%); R_f = 0.28 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.29 – 1.39 (m, 11H, CH_2, CH_3), 1.46 – 1.60 (m, 4H, CH_2), 1.97 – 2.05 (m, 1H, 4'-H), 2.18 (t, J = 7.3 Hz, 2H, CH_2), 2.45 – 2.62 (m, 2H, 4'-H, 5'-H), 2.82 – 2.92 (m, 1H, 5'-H), 3.28 (q, J = 6.7 Hz, 2H, NHC$\underline{H}_2$), 5.03 (dd, J = 5.5, 12.8 Hz, 1H, 3'-H), 6.51 (t, J = 6.0 Hz, 1H, N$\underline{H}$CH$_2$), 7.01 (d, J = 7.1 Hz, 1H), 7.08 (d, J = 8.6 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 22.33 (C-4'), 24.53, 25.86 (CH_2), 27.91 (C($\underline{C}$H$_3$)$_3$), 28.54 (CH_2), 31.14 (C-5'), 34.85 (CH_2), 41.85 (NHCH$_2$), 48.71 (C-3'), 79.56 ($\underline{C}$(CH$_3$)$_3$), 109.20 (C-3a), 110.54 (C-7), 117.35 (C-5), 132.36 (C-7a), 136.43 (C-6), 146.59 (C-4), 167.47 (C-1), 169.09 (C-3), 170.21 (C-2'), 172.38, 172.95 (C-6', 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 = 9.64 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{29}N_3O_6$, 444.21; found, 444.1; **HRMS**(ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{29}N_3O_6$, 444.2129; found, 444.2123.

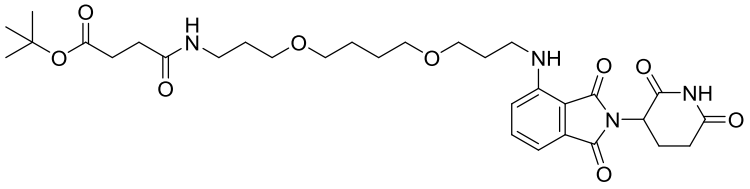
Protected carboxy tool compound 2c



This compound was prepared using the General Procedure **X** and linker **35** (0.44 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to EtOAc) to obtain a yellow oil.

Yield (0.25 g, 45%); R_f = 0.31 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) 1.36 (s, 9H, CH_3), 1.98 – 2.05 (m, 1H, 4'-H), 2.27 (t, *J* = 6.9 Hz, 2H), 2.36 (t, *J* = 7.1 Hz, 2H, CH_2), 2.50 – 2.62 (m, 2H, 4'-H, 5'-H), 2.82 – 2.92 (m, 1H, 5'-H), 3.17 (q, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.38 (t, *J* = 5.9 Hz, 2H), 3.43 – 3.53 (m, 4H), 3.53 – 3.58 (m, 2H, OCH_2), 3.61 (t, *J* = 5.5 Hz, 2H, NHC$\underline{H}_2$), 5.04 (dd, *J* = 5.5, 12.8 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), 7.83 (t, *J* = 5.7 Hz, 1H, N$\underline{H}CH_2$), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.29 (C-4'), 27.89 (C($\underline{C}H_3)_3$), 30.16, 30.40 (CH_2), 31.13 (C-5'), 38.73, 41.87 ($NHCH_2$), 48.72 (C-3'), 69.05, 69.33, 69.74, 69.83 (OCH_2), 79.63 ($\underline{C}(CH_3)_3$), 109.43 (C-3a), 110.83 (C-7), 117.59 (C-5), 132.26 (C-7a), 136.37 (C-6), 146.57 (C-4), 167.43 (C-1), 169.09 (C-3), 170.20 (C-2'), 170.99, 171.73, 172.92 (C-6', 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 = 10.13 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{27}H_{36}N_4O_9$, 561.251; found, 561.2; **HRMS**(ESI) *m/z* $[M + H]^+$ calcd for $C_{27}H_{36}N_4O_9$, 561.2555; found, 561.2549.

Protected carboxy tool compound 2d

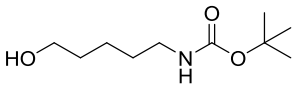


This compound was prepared using the General Procedure **X** and linker **36** (0.99 g, 2 mmol). The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 39:1 to 19:1) to obtain a yellow oil.

Yield (0.32 g, 26%); R_f = 0.17 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 1.47 – 1.62 (m, 6H), 1.76 – 1.83 (m, 2H, CH_2), 1.98 – 2.05 (m, 1H, 4'-H), 2.25 (t, *J* = 7.0 Hz, 2H), 2.37 (t, *J* = 7.0 Hz, 2H, CH_2), 2.46 – 2.61 (m, 2H, 4'-H, 5'-H), 2.83 – 2.92 (m, 1H, 5'-H), 3.02 – 3.08 (m, 2H, NHC$\underline{H}_2$), 3.29 – 3.38 (m, 8H, OCH_2), 3.44 (t, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.4, 12.8 Hz, 1H, 3'-H), 6.63 (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* = 7.0, 8.5 Hz, 1H, 6-H), 7.75 (t, *J* = 5.6 Hz, 1H, N$\underline{H}CH_2$), 11.06 (br s, 1H NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.32 (C-4'), 26.14, 26.22 (CH_2), 27.89 (C($\underline{C}H_3)_3$), 29.06, 29.61, 30.27, 30.46 (CH_2), 31.14 (C-5'), 36.00 (CH_2), 48.71 (C-3'), 67.80, 68.02, 70.00, 70.21 (OCH_2), 79.63 ($\underline{C}(CH_3)_3$), 109.28 (C-3a), 110.54 (C-7), 117.20 (C-5), 132.38 (C-7a), 136.41 (C-6), 146.60 (C-4), 167.48 (C-1), 169.01 (C-3), 170.22 (C-2'), 170.78, 171.77, 172.94 (C-6', 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 = 8.54 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{31}H_{44}N_4O_9$, 617.31; found, 617.3; **HRMS**(ESI) *m/z* $[M + H]^+$ calcd for $C_{31}H_{44}N_4O_9$, 617.3181; found, 617.3153.

3. Alkyne linkers:

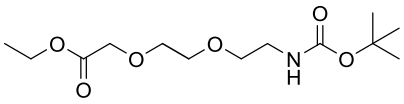
tert-Butyl (5-hydroxypentyl)carbamate (37)



This compound was prepared using the General Procedure **I** and 5-amino-1-pentanol (1.03 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to give a colorless oil.

Yield (1.73 g, 85%); R_f = 0.26 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.18 – 1.28 (m, 2H, CH_2), 1.30 – 1.43 (m, 13H, CH_2, CH_3), 2.84 – 2.92 (m, 2H, CH_2), 3.32 – 3.39 (m, 2H, CH_2), 4.28 (t, J = 5.1 Hz, 1H, NH), 6.60 – 6.77 (m, 1H, OH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.93 (CH_2), 28.40 (CH_3), 29.49, 32.32 (CH_2), 60.77 (OCH_2), 77.38 ($\underline{C}(CH_3)_3$), 155.69 (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), t_R = 6.19 min, *m/z* $[M + H]^+$ calcd for $C_{10}H_{21}NO_3$, 204.16; found, 204.1.

Ethyl 2-[2-[2-(_tert_-butoxycarbonylamino)ethoxy]ethoxy]acetate (38)

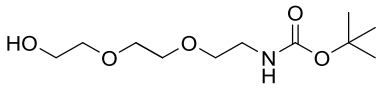


Compound **11** (4.11 g, 20 mmol) was dissolved in dry THF (10 mL) and it was cooled to 0 °C. A mixture of potassium *tert*-butoxide (3.37 g, 30 mmol) in dry THF (60 mL) was added. The reaction mixture was stirred for 30 min at 0 °C, after which ethyl bromoacetate (6.68 g, 4.42 mL, 40 mmol) was added. Stirring of the yellow suspension was continued for 3 h at 0 °C and for 15 h at rt. Subsequently, 10% $KHSO_4$ solution (10 mL) was added and the organic solvent was evaporated. It was further diluted with H_2O (100 mL) and then 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 using petroleum ether/EtOAc 2:1 to obtain a colorless oil.

Yield (2.46 g, 44%); R_f = 0.38 (CH_2Cl_2/acetone 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.19 (t, J = 7.1 Hz, 3H, $CH_2\underline{C}H_3$), 1.36 (s, 9H, $C(CH_3)_3$), 3.02 – 3.08 (m, 2H, $NHCH_2$), 3.35 – 3.39 (m, 2H), 3.49 – 3.53 (m, 2H), 3.56 – 3.59 (m, 2H), 4.07 – 4.14 (m, 4H, OCH_2), 6.70 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 14.21 ($CH_2\underline{C}H_3$), 28.37 ($C(\underline{C}H_3)_3$), 60.22 ($\underline{C}H_2CH_3$), 67.92, 69.32, 69.60, 70.13 (OCH_2), 77.72 ($\underline{C}(CH_3)_3$), 155.73

(CO), 170.26 (CO). The signal for NH<u>C</u>H$_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H$_2$O to 100% MeCN in 10 min, then 100% MeCN to 20 min), t_R = 7.33 min, *m/z* [M + H]$^+$ calcd for $C_{13}H_{25}NO_6$, 292.17; found, 292.1.

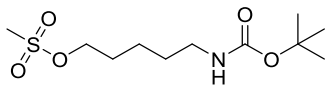
tert-Butyl _N_-[2-[2-(2-hydroxyethoxy)ethoxy]ethyl]carbamate (39)



Finely powdered $NaBH_4$ (1.97 g, 52 mmol) was added portionwise, over a period of 15 min, to a stirred solution of compound **38** (2.33 g, 8.0 mmol) in THF (40 mL) at reflux. Subsequently, the oil bath was removed and methanol (40 mL) was carefully added dropwise to the stirred suspension over another 15 min. The resulting mixture was left to stir at reflux for a further 60 min. After this time, the colorless solution was allowed to cool to rt before the reaction was quenched with half-saturated NH_4Cl solution (40 mL) and the organic layer was evaporated. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), washed with brine (50 mL) and the combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated. The crude oil afforded was purified by column chromatography (petroleum ether/EtOAc 1:2) to yield a colorless oil.

Yield (1.22 g, 61%); R_f = 0.36 (EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 3.01 – 3.08 (m, 2H, OCH_2), 3.35 – 3.42 (m, 4H, OCH_2), 3.45 – 3.51 (m, 6H, OCH_2), 4.52 (t, *J* = 5.4 Hz, 1H, OH), 6.61 – 6.78 (m, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.36 (C(<u>C</u>H$_3$)$_3$), 60.35 (CH_2OH), 69.30, 69.67, 69.85, 72.47 (OCH_2), 77.71 (<u>C</u>(CH$_3$)$_3$), 155.72 (CO). The signal for NH<u>C</u>H$_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H$_2$O to 100 % MeCN in 10 min, then 100 % MeCN to 20 min), t_R = 5.45 min, *m/z* [M + H]$^+$ calcd for $C_{11}H_{23}NO_5$, 250.16; found, 250.3.

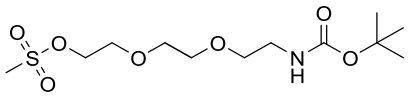
5-(_tert_-Butoxycarbonylamino)pentyl methanesulfonate (40)



This compound was prepared using the General Procedure **II**, where compound **37** was 2.03 g, (10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colorless solid.

Yield (2.17 g, 77%); mp 58 –60 °C; R_f = 0.44 (petroleum ether/EtOAc 2:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.26 – 1.33 (m, 2H, CH_2), 1.34 – 1.42 (m, 11H, CH_2, $C(CH_3)_3$), 1.60 – 1.68 (m, 2H, CH_2), 2.90 (q, J = 6.6 Hz, 2H, CH_2), 3.14 (s, 3H, CH_3), 4.16 (t, J = 6.4 Hz, 2H, OCH_2), 6.76 (s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.37, 28.30, 28.40 ($C(\underline{C}H_3)_3$), 29.01 (CH_2), 36.67 (CH_3), 70.54 (OCH_2), 77.48 ($\underline{C}(CH_3)_3$), 155.74 (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), t_R = 7.75 min, *m/z* $[M + H]^+$ calcd for $C_{11}H_{23}NO_5S$, 282.13; found, 282.1.

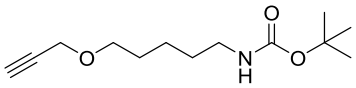
2-[2-[2-(*tert*-Butoxycarbonylamino)ethoxy]ethoxy]ethyl methanesulfonate (41)



This compound was prepared using the General Procedure **II**, where compound **39** was 2.49 g (10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colorless solid.

Yield (2.75 g, 84%); R_f = 0.44 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, $(C(CH_3)_3)$, 3.05 (q, J = 6.0 Hz, 2H, CH_2), 3.16 (s, 3H, CH_3), 3.37 (t, J = 6.1 Hz, 2H), 3.46 – 3.58 (m, 4H), 3.61 – 3.73 (m, 2H), 4.23 – 4.36 (m, 2H, $NHC\underline{H}_2$, OCH_2), 6.70 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.37 ($C(\underline{C}H_3)_3$), 37.00 (CH_3), 68.45, 69.33, 69.59, 69.79, 69.86 (OCH_2), 77.75 ($\underline{C}(CH_3)_3$), 155.74 (CO). The signal for $NH\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 8.84 min, *m/z* $[M + H]^+$ calcd for $C_{12}H_{25}NO_7S$, 328.14; found, 328.0.

***tert*-Butyl *N*-(5-prop-2-ynoxypentyl)carbamate (42)**

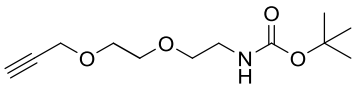


This compound was prepared using the General Procedure **VII** and compound **40** (2.81 g, 10 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 4:1) to give a colorless oil.

Yield (0.94 g, 39%); R_f = 0.32 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.18 – 1.30 (m, 2H, CH_2), 1.31 – 1.41 (m, 11H, CH_2, CH_3), 1.43 – 1.52 (m, 2H, CH_2), 2.76 – 3.00 (m, 2H), 3.32 – 3.43 (m, 3H, $NHC\underline{H}_2$, OCH_2, CH), 4.07 (d, J = 2.5 Hz, 2H, OCH_2), 6.70 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 23.12 (CH_2), 28.45 ($C(\underline{C}H_3)_3$), 28.77, 29.40 (CH_2), 57.43 ($\underline{C}H_2C{\equiv}CH$), 69.28 ($OCH_2$), 76.93 ($C{\equiv}\underline{C}H$), 77.49

(C(CH$_3$)$_3$), 80.73 (C≡CH), 155.78 (CO). The signal for NHCH$_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 10.37 min, *m/z* [M + H]$^+$ calcd for $C_{13}H_{23}NO_3$, 242.17; found, 242.1; **HRMS** (ESI) *m/z* [M + Na]$^+$ calcd for $C_{13}H_{23}NO_3$, 264.1570; found, 264.1548.

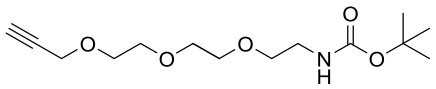
***tert*-Butyl *N*-[2-(2-prop-2-ynoxyethoxy)ethyl]carbamate (43)**[8]



This compound was prepared using the General Procedure **VII** and compound **12** (2.05 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 4:1 to 2:1) to give a colorless oil.

Yield (1.53 g, 63%); R_f = 0.23 (petroleum ether/EtOAc 4:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH$_3$), 3.05 (q, *J* = 6.0 Hz, 2H), 3.32 – 3.40 (m, 3H, OCH$_2$, CH), 3.47 – 3.57 (m, 4H), 4.13 (d, *J* = 2.5 Hz, 2H, OCH$_2$), 6.71 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.36 (C(CH$_3$)$_3$), 57.66 (CH$_2$C≡CH), 68.63, 69.32, 69.38 (OCH$_2$), 77.15 (C≡CH), 77.71 (C(CH$_3$)$_3$), 80.46 (C≡CH), 155.72 (CO). The signal for NHCH$_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H$_2$O to 100% MeCN in 10 min, then 100% MeCN to 20 min), t_R = 7.43 min, *m/z* [M + H]$^+$ calcd for $C_{12}H_{21}NO_4$, 244.15; found, 244.2.

***tert*-Butyl *N*-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethyl]carbamate (44)**

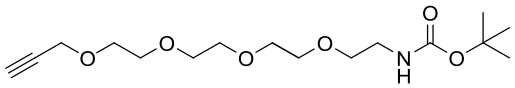


This compound was prepared using the General Procedure **VII** and compound **41** (3.27 g, 10 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to give a colorless oil.

Yield (1.38 g, 48%); R_f = 0.45 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH$_3$), 3.05 (q, *J* = 6.0 Hz, 2H), 3.33 – 3.40 (m, 3H, OCH$_2$, CH), 3.46 – 3.51 (m, 4H), 3.51 – 3.57 (m, 4H), 4.13 (d, *J* = 2.4 Hz, 2H, OCH$_2$), 6.69 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.39 (C(CH$_3$)$_3$), 57.66 (CH$_2$C≡CH), 68.69, 69.33 (OCH$_2$), 69.66 (2 × OCH$_2$), 69.87 (OCH$_2$), 77.19 (C≡CH), 77.76 (C(CH$_3$)$_3$), 80.49 (C≡CH), 155.76 (CO). The signal for NHCH$_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H$_2$O

to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 9.67 min, *m/z* $[M + H]^+$ calcd for $C_{14}H_{25}NO_5$, 288.18; found, 287.8.

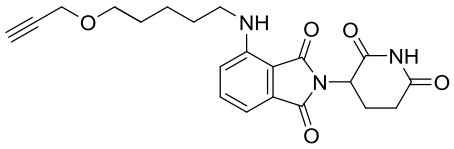
tert-Butyl _N_-[2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethyl]carbamate (45)



This compound was prepared using the General Procedure **VII** and compound **23** (3.71 g, 10 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a colorless oil.

Yield (2.32 g, 70%); R_f = 0.45 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 9H, CH_3), 3.05 (q, *J* = 6.0 Hz, 2H), 3.33 – 3.40 (m, 3H, OCH_2, CH), 3.42 – 3.59 (m, 12H), 4.12 (d, *J* = 2.5 Hz, 2H, OCH_2), 6.68 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.41 ($C(\underline{C}H_3)_3$), 57.67 ($\underline{C}H_2C{\equiv}CH$), 68.71, 69.35, 69.68, 69.92 (OCH_2), 69.96 (2 × OCH_2), 77.18 ($C{\equiv}\underline{C}H$), 77.79 ($\underline{C}(CH_3)_3$), 80.52 ($\underline{C}{\equiv}CH$), 155.79 (CO). The signal for $NH\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 9.71 min, *m/z* $[M + H]^+$ calcd for $C_{16}H_{29}NO_6$, 332.20; found, 332.8; **HRMS** (ESI) *m/z* $[M + K]^+$ calcd for $C_{16}H_{29}NO_6$, 370.1626; found, 370.1612.

Alkyne tool compound 3a

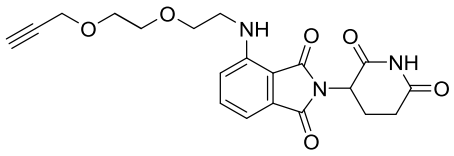


This compound was prepared using the General Procedure **IX** and compound **42** (0.24 g, 1 mmol). The crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to give a yellow oil.

Yield (0.21 g, 52%); R_f = 0.47 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.32 – 1.44 (m, 2H), 1.50 – 1.63 (m, 4H, CH_2), 1.95 – 2.08 (m, 1H, 4'-H), 2.46 – 2.65 (m, 2H, 4'-H, 5'-H), 2.72 – 3.02 (m, 1H, 5'-H), 3.12 – 3.49 (m, 5H, OCH_2, $NHC\underline{H}_2$, CH), 4.09 (d, *J* = 2.5 Hz, 2H, OCH_2), 5.03 (dd, *J* = 5.5, 12.7 Hz, 1H, 3'-H), 6.51 (t, *J* = 6.0 Hz, 1H, $N\underline{H}CH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 8.5 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 22.31 (C-4'), 23.21, 28.62, 28.76 (CH_2), 31.13 (C-5'), 41.95 ($NHCH_2$), 48.72 (C-3'), 57.44 ($\underline{C}H_2C{\equiv}CH$), 69.18 ($OCH_2$), 76.95 ($C{\equiv}\underline{C}H$), 80.72 ($\underline{C}{\equiv}CH$), 109.21 (C-3a), 110.53 (C-7), 117.35 (C-5), 132.36 (C-7a), 136.43 (C-6), 146.61

(C-4), 167.46 (C-1), 169.11 (C-3), 170.21 (C-2'), 172.93 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 10.47 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{21}H_{23}N_3O_5$, 398.17; found, 397.9; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{21}H_{23}N_3O_5$, 398.1710; found, 398.1723.

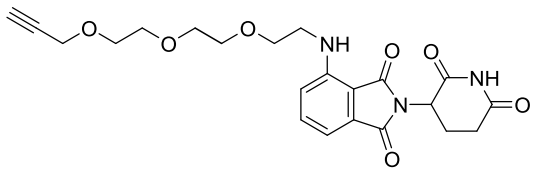
Alkyne tool compound 3b



This compound was prepared using the General Procedure **IX** and compound **43** (0.24 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:2) to give a yellow solid.

Yield (0.22 g, 56%); mp 48 – 50 °C; R_f = 0.53 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.89 – 2.17 (m, 1H, 4'-H), 2.51 – 2.73 (m, 2H, 4'-H, 5'-H), 2.73 – 3.01 (m, 1H, 5'-H), 3.34 – 3.39 (m, 1H), 3.46 (q, *J* = 5.6 Hz, 2H), 3.53 – 3.60 (m, 4H, OCH_2, CH), 3.61 (t, *J* = 5.5 Hz, 2H, $NHC\underline{H}_2$), 4.12 (d, *J* = 2.4 Hz, 2H, OCH_2), 5.04 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.58 (t, *J* = 5.9 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* = 7.0, 8.5 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.33 (C-4'), 31.16 (C-5'), 41.90 ($NHCH_2$), 48.76 (C-3'), 57.73 ($\underline{C}H_2C{\equiv}CH$), 68.72, 69.10, 69.64 ($OCH_2$), 77.21 ($C{\equiv}\underline{C}H$), 80.51 ($\underline{C}{\equiv}CH$), 109.48 (C-3a), 110.88 (C-7), 117.64 (C-5), 132.29 (C-7a), 136.42 (C-6), 146.62 (C-4), 167.49 (C-1), 169.12 (C-3), 170.24 (C-2'), 172.98 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 9.32 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{21}N_3O_6$, 400.15; found, 399.9; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{21}N_3O_6$, 400.1503; found, 400.1518.

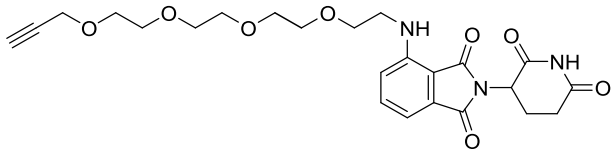
Alkyne tool compound 3c



This compound was prepared using the General Procedure **IX** and compound **44** (0.29 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a yellow oil.

Yield (0.21 g, 47%); R_f = 0.38 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.90 – 2.14 (m, 1H, 4'-H), 2.45 – 2.66 (m, 2H, 4'-H, 5'-H), 2.72 – 3.00 (m, 1H, 5'-H), 3.35 – 3.68 (m, 13H, OCH_2, $NHCH_2$, CH), 4.11 (d, *J* = 2.4 Hz, 2H, OCH_2), 5.03 (dd, *J* = 5.5, 12.7 Hz, 1H, 3'-H), 6.58 (t, *J* = 5.9 Hz, 1H, $NHCH_2$), 7.03 (d, *J* = 7.0 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, *J* = 7.0, 8.5 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.31 (C-4'), 31.14 (C-5'), 41.89 ($NHCH_2$), 48.75 (C-3'), 57.65 ($CH_2C{\equiv}CH$), 68.71, 69.07, 69.73 (OCH_2), 69.94 (2 × OCH_2), 77.14 ($C{\equiv}CH$), 80.51 ($C{\equiv}CH$), 109.44 (C-3a), 110.85 (C-7), 117.63 (C-5), 132.27 (C-7a), 136.40 (C-6), 146.61 (C-4), 167.47 (C-1), 169.11 (C-3), 170.21 (C-2'), 172.94 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 9.51 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{22}H_{25}N_3O_7$, 444.17; found, 443.9; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{22}H_{25}N_3O_7$, 444.1765; found, 444.1776.

Alkyne tool compound 3d



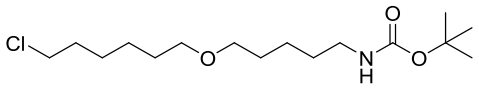
This compound was prepared using the General Procedure **IX** and compound **45** (0.33 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to EtOAc) to give a yellow oil.

Yield (0.18 g, 36%); R_f = 0.25 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.90 – 2.13 (m, 1H, 4'-H), 2.45 – 2.67 (m, 2H, 4'-H, 5'-H), 2.74 – 2.99 (m, 1H, 5'-H), 3.35 – 3.39 (m, 1H, CH), 3.43 – 3.57 (m, 14H, OCH_2), 3.62 (t, *J* = 5.4 Hz, 2H, $NHCH_2$), 4.11 (d, *J* = 2.4 Hz, 2H, OCH_2), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H, 3'-H), 6.58 (t, *J* = 5.8 Hz, 1H, $NHCH_2$), 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, 8.6 Hz, 1H, 6-H), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.33 (C-4'),

31.15 (C-5'), 41.92 ($NHCH_2$), 48.76 (C-3'), 57.66 ($\underline{C}H_2C{\equiv}CH$), 68.70, 69.08, 69.67, 69.95 (OCH_2), 69.97 (2 × OCH_2), 70.00 (OCH_2), 77.17 ($C{\equiv}\underline{C}H$), 80.52 ($\underline{C}{\equiv}CH$), 109.45 (C-3a), 110.87 (C-7), 117.65 (C-5), 132.28 (C-7a), 136.42 (C-6), 146.63 (C-4), 167.49 (C-1), 169.13 (C-3), 170.22 (C-2'), 172.97 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 9.48 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{29}N_3O_8$, 488.20; found, 488.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{29}N_3O_8$, 488.2027; found, 488.2011.

4. Chloro linkers:

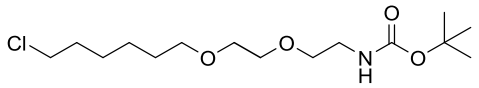
***tert*-Butyl *N*-[5-(6-chlorohexoxy)pentyl]carbamate (46)**



This compound was prepared using the General Procedure **VIII** and compound **37**. The crude product was purified by column chromatography using a gradient of petroleum ether/EtOAc 8:1 to 6:1 to obtain the desired product as a colorless oil.

Yield (0.39 g, 24%); R_f = 0.27 (petroleum ether/EtOAc 8:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.20 – 1.41 (m, 17H, CH_2, CH_3), 1.42 – 1.50 (m, 4H), 1.65 – 1.74 (m, 2H, CH_2), 2.84 – 2.92 (m, 2H, NHC$\underline{H}_2$), 3.14 – 3.45 (m, 4H, OCH_2), 3.61 (t, J = 6.6 Hz, 2H, CH_2Cl), 6.70 (t, J = 5.6 Hz, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 23.16, 25.12, 26.23 (CH_2), 28.40 (C($\underline{C}H_3)_3$), 29.05, 29.22, 29.43, 32.15 (CH_2), 45.46 ($ClCH_2$), 69.92, 70.02 (OCH_2), 77.39 ($\underline{C}(CH_3)_3$), 155.70 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min), t_R = 10.5 min, *m/z* $[M + H]^+$ calcd for $C_{16}H_{32}ClNO_3$, 322.21; found, 322.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{16}H_{32}ClNO_3$, 322.2143; found, 322.2116.

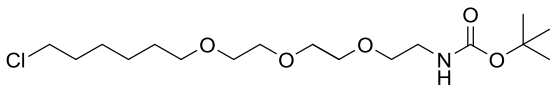
***tert*-butyl *N*-[2-[2-(6-chlorohexoxy)ethoxy]ethyl]carbamate (47)**



This compound was prepared using the General Procedure **VIII** and compound **11**. The crude product was purified by column chromatography using petroleum ether/EtOAc 4:1 to obtain the desired product as a colorless oil.

Yield (0.73 g, 45%); R_f = 0.22 (petroleum ether /EtOAc 4:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.22 – 1.54 (m, 15H, CH_2, CH_3), 1.65 – 1.74 (m, 2H, CH_2), 3.05 (q, J = 6.1 Hz, 2H, NHC$\underline{H}_2$), 3.33 – 3.40 (m, 4H), 3.42 – 3.51 (m, 4H, OCH_2), 3.61 (t, J = 6.7 Hz, 2H, CH_2Cl), 6.68 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.05, 26.24 (CH_2), 28.35 (C($\underline{C}H_3)_3$), 29.18, 32.15 (CH_2), 40.90 ($NHCH_2$), 45.45 (CH_2Cl), 69.29, 69.58, 69.64, 70.31 (OCH_2), 77.68 ($\underline{C}(CH_3)_3$), 155.70 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 11.64 min, *m/z* $[M + H]^+$ calcd for $C_{15}H_{31}ClNO_4$, 324.19; found, 324.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{15}H_{31}ClNO_4$, 324.1936; found, 324.1928.

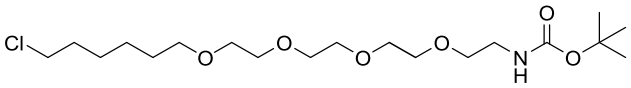
***tert*-Butyl *N*-[2-[2-[2-(6-chlorohexoxy)ethoxy]ethoxy]ethyl]carbamate (48)**



This compound was prepared using the General Procedure **VIII** and compound **39**. The crude product was purified by column chromatography using petroleum ether/EtOAc 1:2 to obtain the desired product as a colorless oil.

Yield (0.40 g, 22%); R_f = 0.42 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.36 (s, 13H, CH_2, CH_3), 1.43 – 1.53 (m, 2H), 1.65 – 1.74 (m, 2H), 3.01 – 3.08 (m, 2H, NHC$\underline{H}_2$), 3.34 – 3.40 (m, 4H), 3.42 – 3.53 (m, 8H, OCH_2), 3.61 (t, J = 6.7 Hz, 2H, CH_2Cl), 6.68 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.05, 26.24 (CH_2), 28.36 (C($\underline{C}H_3)_3$), 29.18, 32.16 (CH_2), 45.47 (CH_2Cl), 69.31, 69.63, 69.67, 69.91, 69.95, 70.32 (OCH_2), 77.71 ($\underline{C}(CH_3)_3$), 155.72 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 11.41 min, *m/z* $[M + H]^+$ calcd for $C_{17}H_{34}ClNO_5$, 368.22; found, 368.2.

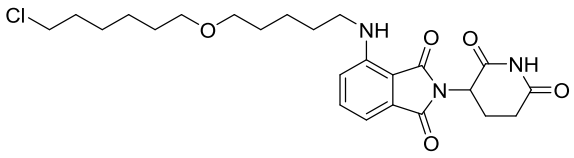
***tert*-Butyl *N*-[2-[2-[2-[2-(6-chlorohexoxy)ethoxy]ethoxy]ethoxy]ethyl]carbamate (49)**



This compound was prepared using the General Procedure **VIII** and compound **22**. The crude product was purified by column chromatography using a gradient of petroleum ether/EtOAc 1:1 to 1:2 to obtain the desired product as a yellow oil.

Yield (0.49 g, 24%); R_f = 0.50 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.25 – 1.42 (m, 13H), 1.44 – 1.53 (m, 2H), 1.65 – 1.75 (m, 2H), 3.05 (q, J = 6.0 Hz, 2H, NHC$\underline{H}_2$), 3.34 – 3.40 (m, 4H), 3.42 – 3.53 (m, 12H, OCH_2), 3.61 (t, J = 6.6 Hz, 2H, CH_2Cl), 6.68 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 25.06, 26.24 (CH_2), 28.36 (C($\underline{C}H_3)_3$), 29.18, 32.16 (CH_2), 45.47 (CH_2Cl), 69.31, 69.63, 69.66, 69.89 (OCH_2), 69.95 (3 × OCH_2), 70.31 (OCH_2), 77.70 ($\underline{C}(CH_3)_3$), 155.72 (CO); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 11.35 min, *m/z* $[M + H]^+$ calcd for $C_{19}H_{38}ClNO_6$, 412.24; found, 412.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{19}H_{38}ClNO_6$, 412.2460; found, 412.2415.

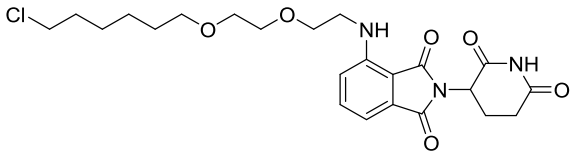
Chloro tool compound 4a



This compound was prepared using the General Procedure **IX** and compound **46** (0.32 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to give a yellow solid.

Yield (86 mg, 18%); mp 36 – 38 °C; R_f = 0.51 (petroleum ether/EtOAc 1:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.24 – 1.41 (m, 6H), 1.42 – 1.61 (m, 6H), 1.64 – 1.71 (m, 2H, CH_2), 1.98 – 2.05 (m, 1H, 4'-H), 2.45 – 2.61 (m, 2H, 4'-H, 5'-H), 2.81 – 2.92 (m, 1H, 5'-H) 3.25 – 3.36 (m, 6H, OCH_2, $NHCH_2$), 3.59 (t, *J* = 6.6 Hz, 2H, CH_2Cl), 5.03 (dd, *J* = 5.4, 12.8 Hz, 1H, 3'-H), 6.51 (t, *J* = 6.0 Hz, 1H, $NHCH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.56 (dd, *J* = 7.0, 8.6 Hz, 1H, 6-H), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.32 (C-4'), 23.31, 25.15, 26.26, 28.69, 29.09, 29.23 (CH_2), 31.14 (C-5'), 32.17 (CH_2), 41.98 ($NHCH_2$), 45.52 (CH_2Cl), 48.71 (C-3'), 69.99 (2 × OCH_2), 109.19 (C-3a), 110.54 (C-7), 117.35 (C-5), 132.37 (C-7a), 136.43 (C-6), 146.62 (C-4), 167.48 (C-1), 169.12 (C-3), 170.23 (C-2'), 172.95 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 10.16 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{32}ClN_3O_5$, 478.21; found, 478.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{32}ClN_3O_5$, 478.2103; found, 478.2100.

Chloro tool compound 4b

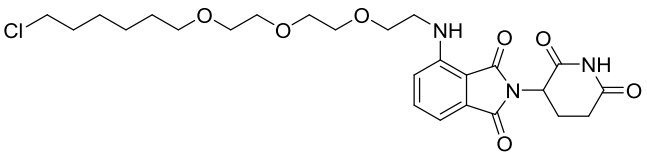


This compound was prepared using the General Procedure **IX** and compound **47** (0.32 g, 1 mmol). The crude product was purified by column chromatography (CH_2Cl_2/EtOH 39:1) to give a yellow oil.

Yield (0.14 g, 30%); R_f = 0.52 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.22 – 1.39 (m, 4H), 1.40 – 1.49 (m, 2H), 1.61 – 1.71 (m, 2H, CH_2), 1.97 – 2.07 (m, 1H, 4'-H), 2.46 – 2.63 (m, 2H, 4'-H, 5'-H), 2.82 – 2.93 (m, 1H, 5'-H), 3.35 (t, *J* = 6.5 Hz, 2H, $NHCH_2$), 3.42 – 3.50 (m, 4H, OCH_2), 3.52 – 3.65 (m, 6H, OCH_2, CH_2Cl), 5.04 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.58 (t, *J* = 5.9 Hz, 1H, $NHCH_2$), 7.03 (d, *J* = 7.1 Hz, 1H), 7.13 (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); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.31 (C-4'), 25.04, 26.24, 29.18 (CH_2), 31.14 (C-5'), 32.15 (CH_2), 41.91 ($NHCH_2$),

45.48 (CH_2Cl), 48.73 (C-3'), 69.09, 69.69, 69.97, 70.39 (OCH_2), 109.44 (C-3a), 110.82 (C-7), 117.60 (C-5), 132.26 (C-7a), 136.35 (C-6), 146.61 (C-4), 167.44 (C-1), 169.09 (C-3), 170.16 (C-2'), 172.90 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 9.26 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{30}ClN_3O_6$, 480.19; found, 480.1; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{30}ClN_3O_6$, 480.1896; found, 480.1896.

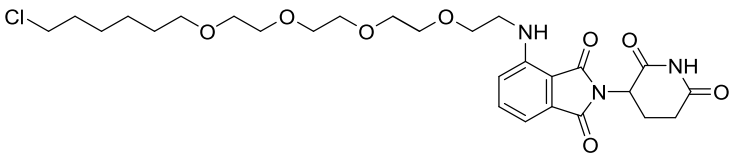
Chloro tool compound 4c



This compound was prepared using the General Procedure **IX** and compound **48** (0.37 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:1 to 1:2) to give a yellow oil.

Yield (0.17 g, 32%); R_f = 0.38 (petroleum ether/EtOAc 1:2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.23 – 1.40 (m, 4H), 1.40 – 1.51 (m, 2H), 1.63 – 1.73 (m, 2H, CH_2), 1.97 – 2.07 (m, 1H, 4'-H), 2.46 – 2.62 (m, 2H, 4'-H, 5'-H), 2.81 – 2.93 (m, 1H, 5'-H), 3.34 (t, *J* = 6.6 Hz, 2H, $NHC\underline{H}_2$), 3.40 – 3.65 (m, 14H, OCH_2, CH_2Cl), 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* = 7.0 Hz, 1H), 7.13 (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); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.31 (C-4'), 25.05, 26.23, 29.18 (CH_2), 31.13 (C-5'), 32.15 (CH_2), 41.90 ($NHCH_2$), 45.48 (CH_2Cl), 48.73 (C-3'), 69.06, 69.63, 69.96, 69.98, 70.02, 70.30 (OCH_2), 109.44 (C-3a), 110.82 (C-7), 117.60 (C-5), 132.26 (C-7a), 136.36 (C-6), 146.60 (C-4), 167.44 (C-1), 169.09 (C-3), 170.16 (C-2'), 172.90 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 9.21 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{25}H_{34}ClN_3O_7$, 524.21; found, 524.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{25}H_{34}ClN_3O_7$, 524.2158; found, 524.2152.

Chloro tool compound 4d

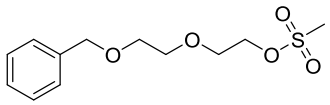


This compound was prepared using the General Procedure **IX** and compound **49** (0.41 g, 1 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to 1:4) to give a yellow oil.

Yield (0.20 g, 35%); R_f = 0.55 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.24 – 1.40 (m, 4H), 1.40 – 1.50 (m, 2H), 1.58 – 1.78 (m, 2H, CH_2), 1.97 – 2.05 (m, 1H, 4'-H), 2.46 – 2.63 (m, 2H, 4'-H, 5'-H), 2.82 – 2.92 (m, 1H, 5'-H), 3.34 (t, *J* = 6.6 Hz, 2H, NHC$\underline{H}_2$), 3.40 – 3.65 (m, 18H, OCH_2, CH_2Cl), 5.04 (dd, *J* = 5.5, 12.8 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), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.32 (C-4'), 25.07, 26.26, 29.20 (CH_2), 31.15 (C-5'), 32.17 (CH_2), 41.89 ($NHCH_2$), 45.51 (CH_2Cl), 48.74 (C-3'), 69.07, 69.64 (OCH_2), 69.96, 69.97 (2 × OCH_2), 70.03, 70.32 (OCH_2), 109.44 (C-3a), 110.84 (C-7), 117.62 (C-5), 132.27 (C-7a), 136.39 (C-6), 146.61 (C-4), 167.46 (C-1), 169.11 (C-3), 170.20 (C-2'), 172.94 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-450 nm), t_R = 9.05 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{27}H_{38}ClN_3O_8$, 568.24; found, 568.4.

5. Benzyl-protected hydroxy linkers:

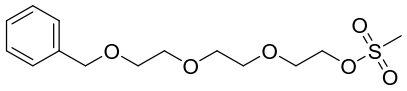
2-(2-(Benzyloxy)ethoxy)ethyl methanesulfonate (50)[7]



This compound was prepared using the General Procedure **II** and 2-(2-(benzyloxy)ethoxy)ethan-1-ol (3.92 g, 20 mmol). The crude product (orange oil) was used in the next step without further purification.

Yield (5.61 g, 99%); R_f = 0.73 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.98 (s, 3H, SO_2CH_3), 3.59 – 3.64 (m, 2H, OCH_2), 3.64 – 3.69 (m, 2H, OCH_2), 3.73 (sym m, 2H, OCH_2), 4.34 (sym m, 2H, OCH_2), 4.53 (s, 2H, CH_2), 7.23 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 37.27 (SO_2CH_3), 68.68 (OCH_2), 69.07 (OCH_2), 69.21 (OCH_2), 70.32 (OCH_2), 72.99 (OCH_2Ph), 127.42 (C-4'), 127.47 (C-2'), 128.13 (C-3'), 137.79 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 4.84 min, 97% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{12}H_{18}O_5S$, 297.0767; found, 297.0757.

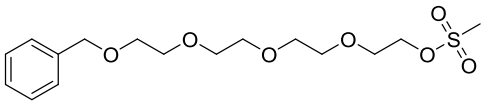
2-(2-(2-(Benzyloxy)ethoxy)ethoxy)ethyl methanesulfonate (51)



This compound was prepared using the General Procedure **II** and 2-(2-(2-(benzyloxy)ethoxy)ethoxy) ethan-1-ol (4.80 g, 20 mmol). The crude product (yellow oil) was used in the next step without further purification.

Yield (6.78 g, 99%); R_f = 0.68 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.01 (s, 3H, SO_2CH_3), 3.58 – 3.69 (m, 8H, OCH_2), 3.73 (sym m, 2H, OCH_2), 4.34 (sym m, 2H, OCH_2), 4.54 (s, 2H, CH_2), 7.24 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 37.30 (SO_2CH_3), 68.68 (OCH_2), 69.13 (OCH_2), 69.18 (OCH_2), 70.23 (OCH_2), 70.29 (2C, OCH_2), 72.89 (OCH_2Ph), 127.36 (C-4'), 127.47 (C-2'), 128.10 (C-3'), 137.92 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 4.95 min, 95% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{14}H_{22}O_6S$, 341.1029; found, 341.1016.

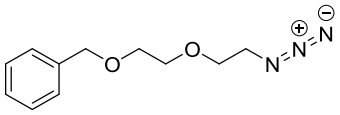
1-Phenyl-2,5,8,11-tetraoxatridecan-13-yl methanesulfonate (52)



This compound was prepared using the General Procedure **II** and 1-phenyl-2,5,8,11-tetraoxatridecan-13-ol (5.68 g, 10 mmol). The crude product (yellow oil) was used in the next step without further purification.

Yield (7.44 g, 99%); R_f = 0.44 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.04 (s, 3H, SO_2CH_3), 3.58 – 3.70 (m, 12H, OCH_2), 3.73 (sym m, 2H, OCH_2), 4.34 (sym m, 2H, OCH_2), 4.55 (s, 2H, CH_2), 7.22 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 37.40 (SO_2CH_3), 68.72 (OCH_2), 69.18 (2C, OCH_2), 70.25 (OCH_2), 70.31 (OCH_2), 70.35 (3C, OCH_2), 72.93 (OCH_2Ph), 127.37 (C-4'), 127.51 (C-2'), 128.13 (C-3'), 138.01 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.02 min, 95% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{16}H_{26}O_7S$, 361.1316; found, 361.1325.

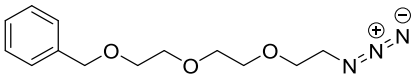
((2-(2-Azidoethoxy)ethoxy)methyl)benzene (53)[7]



This compound was prepared using the General Procedure **III** and compound **50** (2.74 g, 10 mmol). The crude product (orange oil) was used in the next step without further purification.

Yield (2.02 g, 91%); R_f = 0.76 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.34 (t, J = 4.8 Hz, 2H, N_3CH_2), 3.58 – 3.69 (m, 6H, OCH_2), 4.54 (s, 2H, CH_2), 7.21 – 7.37 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 50.34 (CH_2N_3), 69.18 (OCH_2), 69.72 (OCH_2), 70.37 (OCH_2), 72.99 (OCH_2Ph), 127.27 (C-4'), 127.36 (C-2'), 128.04 (C-3'), 137.92 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.59 min, 98% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{11}H_{15}N_3O_2$, 244.1056; found, 244.1048.

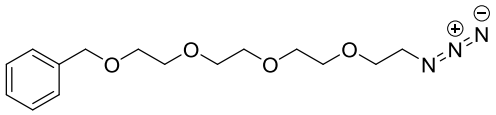
((2-(2-(2-Azidoethoxy)ethoxy)ethoxy)methyl)benzene (54)



This compound was prepared using the General Procedure **III** and compound **51** (3.18 g, 10 mmol). The crude product (yellow oil) was used in the next step without further purification.

Yield (2.41 g, 91%); R_f = 0.74 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.39 (t, *J* = 4.9 Hz, 2H, N_3CH_2), 3.59 – 3.72 (m, 10H, OCH_2), 4.55 (s, 2H, CH_2), 7.22 – 7.39 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 50.34 (CH_2N_3), 69.16 (OCH_2), 69.74 (OCH_2), 70.39 (2 × OCH_2), 70.41 (OCH_2), 72.89 (OCH_2Ph), 127.28 (C-4'), 127.42 (C-2'), 128.05 (C-3'), 138.01 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.64 min, 96% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{13}H_{19}N_3O_3$, 266.1499; found, 266.1490.

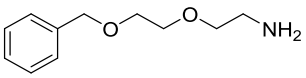
13-Azido-1-phenyl-2,5,8,11-tetraoxatridecane (55)



This compound was prepared using the General Procedure **III** and compound **52** (3.62 g, 10 mmol). The crude product (yellow oil) was used in the next step without further purification.

Yield (2.77 g, 89%); R_f = 0.58 (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.34 (t, *J* = 4.9 Hz, 2H, N_3CH_2), 3.55 – 3.74 (m, 14H, OCH_2), 4.55 (s, 2H, CH_2), 7.22 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 50.36 (CH_2N_3), 69.16 (OCH_2), 69.74 (OCH_2), 70.36 (3 × OCH_2), 70.39 (2 × OCH_2), 72.91 (OCH_2Ph), 127.30 (C-4'), 127.45 (C-2'), 128.07 (C-3'), 138.02 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.66 min, 96% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{15}H_{23}N_3O_4$, 332.1581; found, 332.1568.

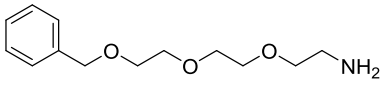
2-(2-(Benzyloxy)ethoxy)ethan-1-amine (56)[7]



This compound was prepared using the General Procedure **IV** and compound **53** (1.11 g, 5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 15:1:0.5) to give a colorless oil.

Yield (0.91 g, 93%); R_f = 0.42 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.41 (br s, 2H, NH_2), 2.86 (t, *J* = 5.1 Hz, 2H, C$\underline{H_2}NH_2$), 3.50 (t, *J* = 5.4 Hz, 2H, OCH_2), 3.59 – 3.67 (m, 4H, OCH_2), 4.57 (s, 2H, CH_2), 7.23 – 7.37 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 41.77 (CH_2NH_2), 69.28 (OCH_2), 70.29 (OCH_2), 73.17, 73.45, 127.55 (C-4'), 127.66 (C-2'), 128.30 (C-3'), 138.13 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 3.18 min, 96% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{17}NO_2$, 196.1332; found, 196.1328.

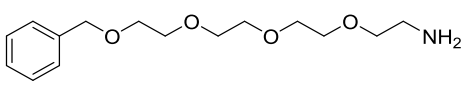
2-(2-(2-(Benzyloxy)ethoxy)ethoxy)ethan-1-amine (57)



This compound was prepared using the General Procedure **IV** and compound **54** (1.33 g, 5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 15:1:0.5) to give a yellow oil.

Yield (1.05 g, 88%); R_f = 0.37 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.52 (br s, 2H, NH_2), 2.84 (t, *J* = 5.3 Hz, 2H, C$\underline{H_2}NH_2$), 3.49 (t, *J* = 5.4 Hz, 2H, OCH_2), 3.59 – 3.70 (m, 8H, OCH_2), 4.56 (s, 2H, CH_2), 7.23 – 7.37 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 41.05 (CH_2NH_2), 69.08 (OCH_2), 69.91 (OCH_2), 70.24 (OCH_2), 70.27 (OCH_2), 72.18 (OCH_2), 72.88 (OCH_2Ph), 127.30 (C-4'), 127.45 (C-2'), 128.05 (C-3'), 137.89 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 3.53 min, 94% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{13}H_{21}NO_3$, 262.1414; found, 262.1403.

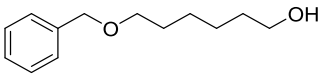
1-Phenyl-2,5,8,11-tetraoxatridecan-13-amine (58)



This compound was prepared using the General Procedure **IV** and compound **55** (1.55 g, 5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 20:1:0.5) to give a yellow oil.

Yield (1.33 g, 94%); R_f = 0.37 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.20 (br s, 2H, NH_2), 2.82 (t, *J* = 5.1 Hz, 2H, C<u>H</u>$_2$$NH_2$), 3.48 (t, *J* = 5.3 Hz, 2H, OCH_2), 3.56 – 3.70 (m, 12H, OCH_2), 4.55 (s, 2H, CH_2), 7.22 – 7.38 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 41.24 (CH_2NH_2), 69.02 (OCH_2), 69.86 (OCH_2), 70.15 (OCH_2), 70.18 (OCH_2), 70.22 (2 × OCH_2), 72.74 (OCH_2), 72.78 (OCH_2Ph), 127.18 (C-4'), 127.32 (C-2'), 127.94 (C-3'), 137.83 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 3.82 min, 94% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{15}H_{25}NO_4$, 284.1856; found, 284.1845.

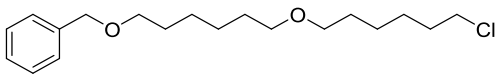
6-(Benzyloxy)hexan-1-ol (59)[16]



This compound was synthesized similar to a previously reported procedure.[16] In brief, to a cooled (0 °C) suspension of NaH (60% dispersion in mineral oil, 0.56 g, 14.0 mmol) in dry THF (10 mL), a solution of 1,6-hexanediol (1.50 g, 12.7 mmol) in dry THF (10 mL) was slowly added. After 1 h of stirring at 0 °C, benzyl bromide (1.95 g, 11.4 mmol) was added and the reaction mixture stirred at rt for 24 h. Subsequently, saturated NH_4Cl solution (10 mL) was added and the majority of THF evaporated. The aqueous phase was extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (EtOAc/*n*-hexane 1:2) to obtain a colorless oil.

Yield (1.23 g, 46%); R_f = 0.62 (EtOAc/*n*-hexane 1:2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.34 – 1.45 (m, 5H, CH_2, OH), 1.54 – 1.68 (m, 4H, CH_2), 3.47 (t, *J* = 6.6 Hz, 2H, CH_2O), 3.63 (t, *J* = 6.6 Hz, 2H, HOC<u>H</u>$_2$), 4.50 (s, 2H, CH_2), 7.25 – 7.37 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 25.48 (CH_2), 25.89 (CH_2), 29.59 (CH_2), 32.56 (CH_2), 62.67 (OCH_2), 70.23 (OCH_2), 72.77 (OCH_2Ph), 127.36 (C-4'), 127.47 (C-2'), 128.10 (C-3'), 137.92 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.53 min, 94% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{13}H_{20}O_2$, 231.1356; found, 231.1347.

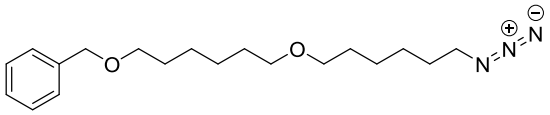
(((6-((6-Chlorohexyl)oxy)hexyl)oxy)methyl)benzene (60)



To a solution of **59** (1.20 g, 5.75 mmol) in DMSO (10 mL), NaOH (50%, 2 mL) and 1-bromo-6-chlorohexane (1.04 g, 5.23 mmol) were added and the mixture stirred at rt for 24 h. Then, saturated NH_4Cl (100 mL) was added and the mixture extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (EtOAc/*n*-hexane 1:6) to obtain a colorless oil.

Yield (0.79 g, 42%); R_f = 0.65 (EtOAc/*n*-hexane 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.32 – 1.48 (m, 8H, CH_2), 1.54 – 1.68 (m, 6H, CH_2), 1.74 – 1.82 (m, 2H, CH_2), 3.35 – 3.42 (m, 4H, CH_2), 3.46 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.52 (t, *J* = 6.7 Hz, 2H, OCH_2), 4.49 (s, 2H, CH_2), 7.23 – 7.36 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 25.50 (CH_2), 26.04 (2 × CH_2), 26.69 (CH_2), 29.56 (CH_2), 29.67 (CH_2), 29.70 (CH_2), 32.53 (CH_2), 45.05 (CH_2Cl), 70.37 (OCH_2), 70.64 (OCH_2), 70.86 (OCH_2), 72.82 (OCH_2Ph), 127.44 (C-4'), 127.58 (C-2'), 128.31 (C-3'), 138.63 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 8.36 min, 97% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{19}H_{31}O_2Cl$, 349.1905; found, 349.1891.

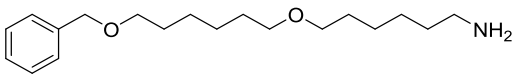
(((6-((6-Azidohexyl)oxy)hexyl)oxy)methyl)benzene (61)



This compound was prepared using the General Procedure **III** on a smaller scale, where compound **60** was 0.80 g (2.44 mmol). The crude product (colorless oil) was used in the next step without further purification.

Yield (0.82 g, 99%); R_f = 0.47 (EtOAc/*n*-hexane 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.30 – 1.44 (m, 8H, CH_2), 1.52 – 1.68 (m, 8H, CH_2), 3.26 (t, *J* = 7.0 Hz, 2H, N_3CH_2), 3.36 – 3.44 (m, 4H, OCH_2), 3.47 (t, *J* = 6.6 Hz, 2H, OCH_2), 4.50 (s, 2H, CH_2), 7.24 – 7.36 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 25.82 (CH_2), 26.09 (CH_2), 26.10 (CH_2), 26.60 (CH_2), 28.82 (CH_2), 29.63 (CH_2), 29.73 (CH_2), 29.75 (CH_2), 51.41 (CH_2N_3), 70.42 (OCH_2), 70.68 (OCH_2), 70.91 (OCH_2), 72.87 (OCH_2Ph), 127.49 (C-4'), 127.63 (C-2'), 128.36 (C-3'), 138.68 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 8.30 min, 93% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{19}H_{31}N_3O_2$, 356.2308; found, 356.2295.

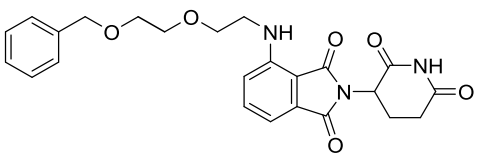
6-((6-(Benzyloxy)hexyl)oxy)hexan-1-amine (62)



This compound was prepared using the General Procedure **IV** on a smaller scale where compound **61** was 0.80 g (2.40 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 15:1:0.5) to give a colorless oil.

Yield (0.44 g, 59%); R_f = 0.43 (CH_2Cl_2/MeOH/NH_4OH 9:1:0.5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.28 – 1.48 (m, 13H, CH_2, NH_2), 1.50 – 1.64 (m, 5H, CH_2), 2.67 (t, *J* = 7.1 Hz, 2H, $C\underline{H}_2NH_2$), 3.38 (t, *J* = 6.6 Hz, 4H, OCH_2), 3.46 (t, *J* = 6.7 Hz, 2H, OCH_2), 4.49 (s, 2H, CH_2), 7.24 – 7.36 (m, 5H, Ar-H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 25.96 (2 × CH_2), 25.98 (CH_2), 26.64 (CH_2), 29.64 (3 × CH_2), 32.90 (CH_2), 41.66 (CH_2NH_2), 70.29 (OCH_2), 70.71 (OCH_2), 70.76 (OCH_2), 72.74 (OCH_2Ph), 127.36 (C-4'), 127.49 (C-2'), 128.22 (C-3'), 138.55 (C-1'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.48 min, 97% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd for $C_{19}H_{33}NO_2$, 330.2404; found, 330.2391.

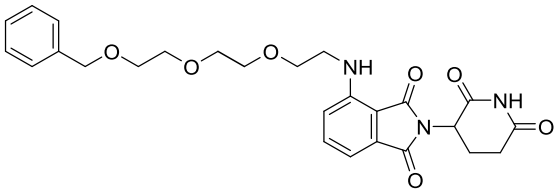
Benzyl-protected hydroxy tool compound 5a



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.40 g (1.45 mmol) of 4-fluorothalidomide and 0.28 g (1.45 mmol) of compound **56** were used. The crude product was purified by column chromatography (EtOAc/*n*-hexane 1:1) to give a green oil.

Yield (0.26 g, 40%); R_f = 0.24 (EtOAc/*n*-hexane 1:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.96 – 2.04 (m, 1H, 4'-H), 2.44 – 2.62 (m, 2H, 4'-H, 5'-H), 2.81 – 2.94 (m, 1H, 5'-H), 3.48 (q, *J* = 5.6 Hz, 2H, Ar-NHC$\underline{H}_2$), 3.54 – 3.64 (m, 6H, OCH_2), 4.48 (s, 2H, CH_2), 5.05 (dd, *J* = 5.3, 12.8 Hz, 1H, 3'-H), 6.63 (t, *J* = 5.6 Hz, 1H, Ar-N$\underline{H}CH_2$), 7.03 (d, *J* = 6.8 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H, 5-H, 7-H), 7.22 – 7.34 (m, 5H, Ar-H), 7.57 (dd, *J* = 6.8, 8.7 Hz, 1H, 6-H), 11.11 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.07 (C-4'), 30.93 (C-5'), 41.65 (Ar-NH$\underline{C}H_2$), 48.49 (C-3'), 68.91 (OCH_2), 69.13 (OCH_2), 69.75 (OCH_2), 72.03 (OCH_2Ph), 109.17 (C-3a), 110.61 (C-7), 117.38 (C-5), 127.27 (C-4'), 127.37 (C-2'), 128.12 (C-3'), 132.02 (C-7a), 136.14 (C-6), 138.40 (C-1'), 146.35 (C-4), 167.24 (C-3), 168.88 (C-1), 170.04 (C-2'), 172.77 (C-6'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.71 min, 96% purity, detection at 210 nm; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{24}H_{25}N_3O_6$, 450.1660; found, 450.1665.

Benzyl-protected hydroxy tool compound 5b

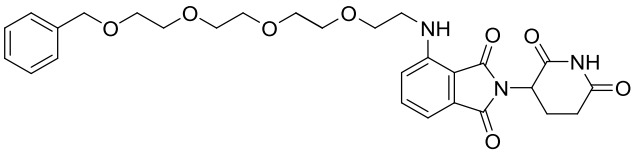


This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.40 g (1.45 mmol) of 4-fluorothalidomide and 0.35 g (1.45 mmol) of compound **57** were used. The crude product was purified by column chromatography (EtOAc/*n*-hexane 4:1) to give a green oil.

Yield (0.31 mg, 44%); R_f = 0.16 (EtOAc/*n*-hexane 1:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.94 – 2.05 (m, 1H, 4'-H), 2.44 – 2.62 (m, 2H, 4'-H, 5'-H), 2.81 – 2.94 (m, 1H, 5'-H), 3.40 – 3.58 (m, 10H, OCH_2, Ar-NHC$\underline{H}_2$), 3.62 (t, *J* = 5.3 Hz, 2H, OCH_2), 4.46 (s, 2H, CH_2), 5.05 (dd, *J* = 5.3, 12.8 Hz, 1H, 3'-H), 6.61 (t, *J* = 5.7 Hz, 1H, Ar-N$\underline{H}CH_2$), 7.03 (d, *J* = 6.7 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.22 – 7.36 (m, 5H, Ar-H), 7.57 (dd, *J* = 6.7, 8.6 Hz, 1H, 6-H), 11.11 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.09 (C-4'), 30.93 (C-5'),

41.63 (Ar-NH<u>C</u>H$_2$), 48.49 (C-3'), 68.84 (OCH$_2$), 69.10 (OCH$_2$), 69.75 (OCH$_2$), 69.79 (OCH$_2$), 69.80 (OCH$_2$), 71.96 (OCH$_2$Ph), 109.17 (C-3a), 110.61 (C-7), 117.36 (C-5), 127.28 (C-4'), 127.40 (C-2'), 128.13 (C-3'), 132.02 (C-7a), 136.41 (C-6), 138.41 (C-1'), 146.33 (C-4), 167.24 (C-3), 168.88 (C-1), 170.03 (C-2'), 172.76 (C-6'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.74 min, 97% purity, detection at 210 nm; **HRMS** (ESI) *m/z* [M – H]$^-$ calcd for $C_{26}H_{29}N_3O_7$, 494.1922; found, 494.1927.

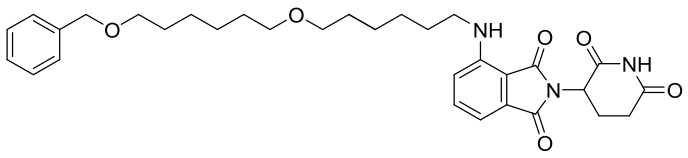
Benzyl-protected hydroxy tool compound 5c



This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.40 g (1.45 mmol) of 4-fluorothalidomide and 0.41 g (1.45 mmol) of compound **58** were used. The crude product was purified by column chromatography (EtOAc) to give a green oil.

Yield (0.33 g, 43%); R_f = 0.18 (EtOAc/*n*-hexane 1:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.96 – 2.05 (m, 1H, 4'-H), 2.44 – 2.62 (m, 2H, 4'-H, 5'-H), 2.81 – 2.94 (m, 1H, 5'-H), 3.45 (q, *J* = 5.7 Hz, 2H, Ar-NHC<u>H</u>$_2$), 3.48 – 3.58 (m, 12H, OCH$_2$), 3.61 (t, *J* = 5.4 Hz, 2H, OCH$_2$), 4.47 (s, 2H, CH$_2$), 5.05 (dd, *J* = 5.3, 12.8 Hz, 1H, 3'-H), 6.60 (t, *J* = 5.7 Hz, 1H, Ar-N<u>H</u>CH$_2$), 7.04 (d, *J* = 6.7 Hz, 1H), 7.13 (d, *J* = 8.7 Hz, 1H, 5-H, 7-H), 7.23 – 7.36 (m, 5H, Ar-H), 7.57 (dd, *J* = 6.7, 8.7 Hz, 1H, 6-H), 11.11 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.09 (C-4'), 30.92 (C-5'), 41.62 (Ar-NH<u>C</u>H$_2$), 48.49 (C-3'), 68.82 (OCH$_2$), 69.06 (OCH$_2$), 69.70 (OCH$_2$), 69.73 (OCH$_2$), 69.75 (OCH$_2$), 69.79 (OCH$_2$), 69.79 (OCH$_2$), 71.95 (OCH$_2$Ph), 109.16 (C-3a), 110.62 (C-7), 117.40 (C-5), 127.31 (C-4'), 127.43 (C-2'), 128.16 (C-3'), 132.03 (C-7a), 136.18 (C-6), 138.41 (C-1'), 146.34 (C-4), 167.25 (C-3), 168.88 (C-1), 170.04 (C-2'), 172.78 (C-6'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 5.76 min, 98% purity, detection at 210 nm; **HRMS** (ESI) *m/z* [M – H]$^-$ calcd for $C_{28}H_{33}N_3O_8$, 538.2184; found, 538.2184.

Benzyl-protected hydroxy tool compound 5d

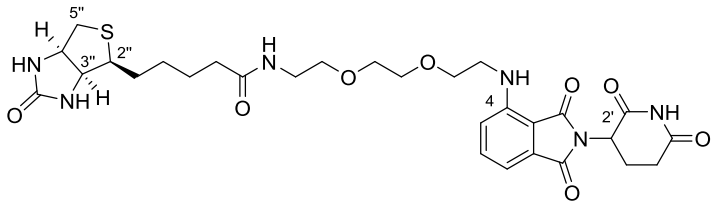


This compound was prepared using the General Procedure **IX** for the nucleophilic aromatic substitution, where 0.13 g (0.47 mmol) of 4-fluorothalidomide and 0.15 g (0.47 mmol) of compound **62** were used. The crude product was purified by column chromatography (EtOAc/*n*-hexane 1:1) to give a yellow oil.

Yield (75 mg, 28%); R_f = 0.28 (EtOAc/*n*-hexane 1:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.20 – 1.38 (m, 8H, CH_2), 1.40 – 1.58 (m, 8H, CH_2), 1.97 – 2.06 (m, 1H, 4'-H), 2.45 – 2.64 (m, 2H, 4'-H, 5'-H), 2.82 – 2.94 (m, 1H, 5'-H), 3.24 – 3.34 (m, 6H, OCH_2, Ar-NHC$\underline{H}_2$), 3.39 (t, *J* = 6.4 Hz, 2H, OCH_2), 4.42 (s, 2H, CH_2), 5.05 (dd, *J* = 5.3, 12.8 Hz, 1H, 3'-H), 6.52 (t, *J* = 5.7 Hz, 1H, Ar-N$\underline{H}$CH_2), 7.01 (d, *J* = 7.1 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.22 – 7.37 (m, 5H, Ar-H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H, 6-H), 11.11 (br s, 1H, NH); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 22.63 (C-4'), 25.95 (CH_2), 26.04 (2C, CH_2), 26.61 (CH_2), 29.12 (CH_2), 29.64 (CH_2), 29.67 (2C, CH_2), 31.45 (C-5'), 42.25 (Ar-NH$\underline{C}$H$_2$), 48.99 (C-3'), 70.02 (OCH_2), 70.30 (OCH_2), 70.35 (OCH_2), 72.24 (OCH_2Ph), 109.46 (C-3a), 110.83 (C-7), 117.60 (C-5), 127.75 (C-4'), 127.81 (C-2'), 128.66 (C-3'), 132.65 (C-7a), 136.73 (C-6), 139.19 (C-1'), 146.87 (C-4), 167.77 (C-3), 169.42 (C-1), 170.56 (C-2'), 173.29 (C-6'); **HPLC** (95% H_2O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), t_R = 7.77 min, 96% purity, detection at 210 nm; **HRMS** (ESI) *m/z* [M – H]$^-$ calcd for $C_{32}H_{41}N_3O_6$, 562.2912; found, 562.2913.

J. Synthesis of the Chemical Probes 6 and 7

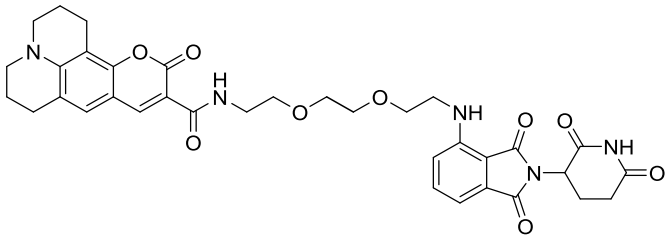
Biotin-tagged CRBN ligand 6



Compound **1b** (0.76 g, 1.5 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and trifluoroacetic acid (5 mL) was added. The mixture was stirred for 2 h at rt. The solvent was removed and it was coevaporatd with dry CH_2Cl_2 (3 × 5 mL). The oily residue was further dried in high vacuum. Subsequently, the residue was redissolved in dry DMF (10 mL) and DIPEA (0.58 g, 0.78 mL, 4.5 mmol). In a separate flask, (+)-Biotin (0.24 g, 1.0 mmol) was dissolved in dry DMF/dry CH_2Cl_2 1:1 (40 mL) and HATU (0.42 g, 1.1 mmol) as well as DIPEA (0.13 g, 0.17 mL, 1.0 mmol) were added. The mixture was stirred for 10 min at rt, before the first solution containing the deprotected amine was added. The resulting yellow mixture was stirred for 18 h at rt. Subsequently, the solvents were removed and the residue was dissolved in CH_2Cl_2 (100 mL) and it was wasehed with 10% $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O and brine (each 25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 19:1 to 9:1) to yield a yellow solid.

Yield (0.48 g, 76%); mp 92 – 94 °C; R_f = 0.32 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.19 – 1.37 (m, 2H), 1.37 – 1.54 (m, 3H), 1.54 – 1.67 (m, 1H, CH_2), 1.90 – 2.16 (m, 3H, $COCH_2$, 4'-H), 2.52 – 2.61 (m, 2H, 4'-H, 5'-H), 2.76 – 2.94 (m, 2H, 5'-H, 5''-H), 3.03 – 3.12 (m, 1H, 5''-H), 3.17 (q, *J* = 5.8 Hz, 2H, CONHC$\underline{H}_2$), 3.36 – 3.49 (m, 5H, OCH2, 2''-H), 3.49 – 3.58 (m, 4H, OCH_2), 3.61 (t, *J* = 5.5 Hz, 2H, NHC$\underline{H}_2$), 4.06 – 4.15 (m, 1H), 4.25 – 4.31 (m, 1H, 3''-H, 4''-H), 5.04 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.31 (br s, 1H), 6.36 (br s, 1H, NHCONH), 6.59 (t, *J* = 5.8 Hz, 1H, N$\underline{H}$CH$_2$), 7.03 (d, *J* = 7.1 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.58 (dd, *J* = 7.1, 8.6 Hz, 1H, 6-H), 7.75 (t, *J* = 5.7 Hz, 1H, CONH), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.30 (C-4'), 25.39, 28.18, 28.32 (CH_2), 31.14 (C-5'), 35.25 (CH_2CO), 38.60 (C-5''), 41.90 ($NHCH_2$), 48.74 (C-3'), 55.55 (C-2''), 59.36 (C-4''), 61.20 (C-3''), 69.06, 69.36, 69.73, 69.85 (OCH_2), 109.45 (C-3a), 110.85 (C-7), 117.60 (C-5), 132.27 (C-7a), 136.40 (C-6), 146.59 (C-4), 162.86 (CO), 167.45 (C-1), 169.11 (C-3), 170.21 (C-2'), 172.29 (CO), 172.93 (C-6'). One 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 220-500 nm), t_R = 6.47 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{29}H_{38}N_6O_8S$, 631.25; found, 631.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{29}H_{38}N_6O_8S$, 631.2545; found, 631.2536.

Coumarin-labelled CRBN ligand 7

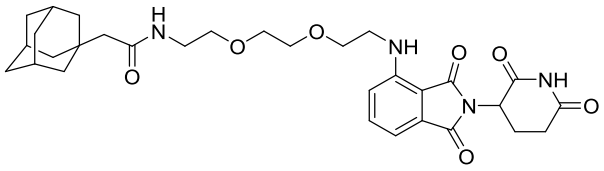


Compound **1b** (0.18 g, 0.35 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and trifluoroacetic acid (2.5 mL) was added. The mixture was stirred for 2 h at rt. The solvent was removed and it was coevaporatd with dry CH_2Cl_2 (3 × 5 mL). The oily residue was further dried in high vacuum. Subsequently, the residue was redissolved in dry DMF (5 mL) and DIPEA (0.18 g, 0.24 mL, 1.4 mmol). In a separate flask, coumarin 343[4] (71 mg, 0.25 mmol) was dissolved in dry DMF/CH_2Cl_2 1:1 (10 mL) and HATU (105 mg, 0.275 mmol) as well as DIPEA (32 mg, 44 µL, 0.25 mmol) were added. The mixture was stirred for 10 min at rt, before the first solution containing the deprotected amine was added. The resulting yellow mixture was stirred for 18 h at rt. Subsequently, the solvents were removed and the residue was dissolved in CH_2Cl_2 (50 mL) and it was wasehed with 10% $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O and brine (each 12.5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 29:1 to 19:1) to yield an orange solid.

Yield (128 mg, 76%); mp 214 – 216 °C; R_f = 0.44 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.76 – 1.94 (m, 4H, CH_2), 1.97 – 2.07 (m, 1H, 4'-H), 2.45 – 2.63 (m, 2H, 4'-H, 5'-H), 2.63 – 2.71 (m, 4H, CH_2), 2.80 – 2.92 (m, 1H, 5'-H), 3.21 – 3.76 (m, 16H, OCH_2, NCH_2, $NHCH_2$), 5.02 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.53 (t, *J* = 5.8 Hz, 1H, $NHCH_2$), 6.93 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.16 (s, 1H), 7.50 (dd, *J* = 7.1, 8.6 Hz, 1H, 6-H), 8.43 (s, 1H), 8.76 (t, *J* = 5.5 Hz, 1H), 11.04 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 19.71 (2 × CH_2), 20.68, 22.28 (C-4'), 26.92, 31.14 (C-5'), 38.95, 41.91 ($NHCH_2$), 48.71 (C-3'), 49.13, 49.67, 69.05, 69.27, 69.88, 70.01 (OCH_2), 104.73, 107.49, 107.99, 109.36 (C-3a), 110.64 (C-7), 117.37 (C-5), 119.51, 127.22, 132.13 (C-7a), 136.18 (C-6), 146.47 (C-4), 147.52, 148.09, 152.17, 161.98, 162.57 (CO), 167.40 (C-1), 169.00 (C-3), 170.17 (C-2'), 172.89 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 8.41 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{35}H_{37}N_5O_9$, 672.26; found, 672.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{35}H_{37}N_5O_9$, 672.2664; found, 672.2644.

K. Synthesis of the hydrophobically tagged CRBN Ligands 8

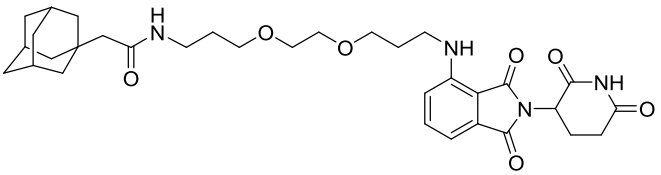
Hydrophobically tagged CRBN ligand 8a



This compound was prepared using the General Procedure **XI** and compound **1b** (126 mg, 0.25 mmol). The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc) to give a yellow solid.

Yield (23 mg, 16%); mp 42 – 44 °C; R_f = 0.33 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.50 – 1.63 (m, 12H, CH_2), 1.79 (s, 2H, $COCH_2$), 1.83 – 1.90 (m, 3H, CH), 1.99 – 2.05 (m, 1H, 4'-H), 2.46 – 2.64 (m, 2H, 4'-H, 5'-H), 2.75 – 3.00 (m, 1H, 5'-H), 3.15 (q, *J* = 5.8 Hz, 2H), 3.39 (t, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.43 – 3.63 (m, 8H, OCH_2), 5.04 (dd, *J* = 5.4, 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, 8.6 Hz, 1H, 6-H), 7.62 (t, *J* = 5.7 Hz, 1H, N$\underline{H}CH_2$), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.32(C-4'), 28.22 (3 × CH), 31.15 (C-5'), 32.28 (C), 36.63 (3 × CH_2), 38.49, 41.89 ($NHCH_2$), 42.23 (3 × CH_2), 48.74 (C-3'), 50.14 (CO$\underline{C}H_2$), 69.06, 69.42, 69.72, 69.89 (OCH_2), 109.47 (C-3a), 110.87 (C-7), 117.60 (C-5), 132.28 (C-7a), 136.41 (C-6), 146.59 (C-4), 167.46 (C-1), 169.13 (C-3), 170.14 (CO), 170.22 (C-2'), 172.94 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), t_R = 8.68 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{31}H_{40}N_4O_7$, 581.29; found, 581.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{31}H_{40}N_4O_7$, 581.2970; found, 581.2984.

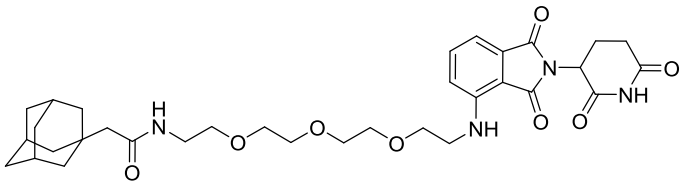
Hydrophobically tagged CRBN ligand 8b



This compound was prepared using the General Procedure **XI** and compound **1c** (133 mg, 0.25 mmol). The crude product was purified by column chromatography (gradient of CH_2Cl_2 to CH_2Cl_2/EtOH 19:1) to give a yellow solid.

Yield (87 mg, 57%); mp 80 – 82 °C; R_f = 0.28 (CH_2Cl_2/EtOH 29:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.47 – 1.67 (m, 12H, CH_2), 1.75 – 1.85 (m, 4H, CH_2), 1.78 (s, 2H, $COCH_2$), 1.85 – 1.90 (m, 3H, CH), 1.97 – 2.05 (m, 1H, 4'-H), 2.45 – 2.63 (m, 2H, 4'-H, 5'-H), 2.80 – 2.94 (m, 1H, 5'-H), 3.00 – 3.08 (m, 2H, NHC$\underline{H}_2$), 3.26 – 3.55 (m, 10H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.5, 12.7 Hz, 1H, 3'-H), 6.64 (t, *J* = 6.0 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), 7.46 – 7.67 (m, 2H, 6-H, N$\underline{H}CH_2$), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.34 (C-4'), 28.22 (3 × CH), 29.07, 29.67 (CH_2), 31.14 (C-5'), 32.30 (C), 35.79 ($NHCH_2$), 36.63 (3 × CH_2), 42.30 (3 × CH_2), 48.72 (C-3'), 50.25 ($CO\underline{C}H_2$), 68.38 (2 × OCH_2), 69.68, 69.82 (OCH_2), 109.28 (C-3a), 110.55 (C-7), 117.24 (C-5), 132.38 (C-7a), 136.43 (C-6), 146.64 (C-4), 167.49 (C-1), 169.02 (C-3), 169.96 (CO), 170.21 (C-2'), 172.94 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-450 nm), t_R = 11.66 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{33}H_{44}N_4O_7$, 609.32; found, 609.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{33}H_{44}N_4O_7$, 609.3283; found, 609.3281.

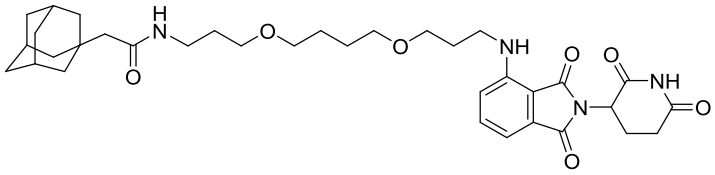
Hydrophobically tagged CRBN ligand 8c



This compound was prepared using the General Procedure **XI** and compound **1d** (137 mg, 0.25 mmol). The crude product was purified by column chromatography (gradient of CH_2Cl_2 to CH_2Cl_2/EtOH 19:1) to give a yellow solid.

Yield (155 mg, 99%); mp 70 – 72 °C; R_f = 0.36 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.47 – 1.68 (m, 12H, CH_2), 1.80 (s, 2H, $COCH_2$), 1.83 – 1.92 (m, 3H, CH), 1.97 – 2.06 (m, 1H, 4'-H), 2.46 – 2.63 (m, 2H, 4'-H, 5'-H), 2.75 – 3.00 (m, 1H, 5'-H), 3.15 (q, *J* = 5.8 Hz, 2H), 3.37 (t, *J* = 5.9 Hz, 2H), 3.42 – 3.58 (m, 10H, OCH_2, NHC$\underline{H}_2$), 3.61 (t, *J* = 5.5 Hz, 2H, NHC$\underline{H}_2$), 5.04 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.58 (t, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.03 (d, *J* = 7.0 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H, 5-H, 7-H), 7.57 (dd, *J* = 7.1, 8.6 Hz, 1H, 6-H), 7.63 (t, *J* = 5.8 Hz, 1H, N$\underline{H}CH_2$), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.29 (C-4'), 28.20 (3 × CH), 31.12 (C-5'), 32.26 (C), 36.60 (3 × CH_2), 38.47, 41.88 ($NHCH_2$), 42.22 (3 × CH_2), 48.72 (C-3'), 50.11 ($CO\underline{C}H_2$), 69.06, 69.33, 69.69, 69.92, 69.95, 69.96 (OCH_2), 109.43 (C-3a), 110.82 (C-7), 117.58 (C-5), 132.25 (C-7a), 136.35 (C-6), 146.58 (C-4), 167.42 (C-1), 169.08 (C-3), 170.10 (CO), 170.16 (C-2'), 172.89 (C-6'); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 11.47 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{33}H_{44}N_4O_8$, 625.32; found, 625.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{33}H_{44}N_4O_8$, 625.3232; found, 625.3241.

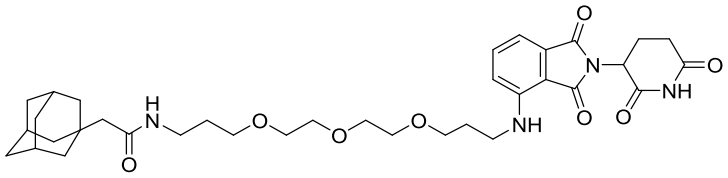
Hydrophobically tagged CRBN ligand 8d



This compound was prepared using the General Procedure **XI** and compound **1e** (140 mg, 0.25 mmol). The crude product was purified by column chromatography (gradient of CH_2Cl_2 to CH_2Cl_2/EtOH 29:1) to give a yellow solid.

Yield (91 mg, 57%); mp 68 – 70 °C; R_f = 0.30 (CH_2Cl_2/EtOH 29:1); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.47 – 1.68 (m, 18H,), 1.74 – 1.84 (m, 4H, CH_2, $COCH_2$), 1.85 – 1.93 (m, 3H, CH), 1.97 – 2.06 (m, 1H, 4'-H), 2.45 – 2.63 (m, 2H, 4'-H, 5'-H), 2.74 – 2.97 (m, 1H, 5'-H), 2.97 – 3.15 (m, 2H, NHC$\underline{H}_2$), 3.30 – 3.40 (m, 8H, OCH_2), 3.44 (t, *J* = 6.0 Hz, 2H, NHC$\underline{H}_2$), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H, 3'-H), 6.63 (t, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.45 – 7.68 (m, 2H, 6-H, N$\underline{H}CH_2$), 11.05 (br s, 1H, NH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.31 (C-4'), 26.14, 26.19 (CH_2), 28.20 (3 × CH), 29.05, 29.66 (CH_2), 31.12 (C-5'), 32.28 (C), 35.79 ($NHCH_2$), 36.61 (3 × CH_2), 42.28 (3 × CH_2), 48.70 (C-3'), 50.23 ($CO\underline{C}H_2$), 67.88, 68.00, 70.02, 70.19 (OCH_2), 109.27 (C-3a), 110.52 (C-7), 117.18 (C-5), 132.37 (C-7a), 136.38 (C-6), 146.60 (C-4), 167.46 (C-1), 168.99 (C-3), 169.90 (CO), 170.18 (C-2'), 172.90 (C-6'). One signal for NH$\underline{C}H_2$ is missing (overlapping solvent peaks); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-500 nm), t_R = 12.07 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{35}H_{48}N_4O_7$, 637.36; found, 637.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{35}H_{48}N_4O_7$, 637.3596; found, 637.3608.

Hydrophobically tagged CRBN ligand 8e

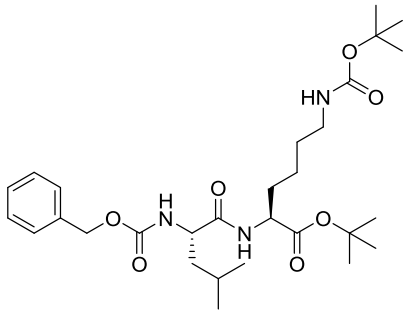


This compound was prepared using the General Procedure **XI** and compound **1f** (144 mg, 0.25 mmol). The crude product was purified by column chromatography (gradient of CH_2Cl_2 to CH_2Cl_2/EtOH 19:1) to give a yellow solid.

Yield (122 mg, 75%); mp 64 – 66 °C; R_f = 0.25 (CH_2Cl_2/EtOH 19:1); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.48 – 1.66 (m, 14H, CH_2), 1.75 – 1.83 (m, 4H, CH_2, $COCH_2$), 1.84 – 1.92 (m, 3H, CH), 1.97 – 2.05 (m, 1H, 4'-H), 2.45 – 2.62 (m, 2H, 4'-H, 5'-H), 2.81 – 2.92 (m, 1H, 5'-H), 3.03 (q, J = 6.6 Hz, 2H, NHC$\underline{H}_2$), 3.29 – 3.63 (m, 14H, OCH_2, NHC$\underline{H}_2$), 5.03 (dd, J = 5.5, 12.9 Hz, 1H, 3'-H), 6.64 (t, J = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.00 (d, J = 7.1 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.51 – 7.67 (m, 2H, 6-H, N$\underline{H}CH_2$), 11.06 (br s, 1H, NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.37 (C-4'), 28.24 (3 × CH), 29.09, 29.67 (CH_2), 31.17 (C-5'), 32.34 (C), 35.82 ($NHCH_2$), 36.65 (3 × CH_2), 42.33 (3 × CH), 48.74 (C-3'), 50.28 (CO$\underline{C}H_2$), 68.37, 68.42, 69.77, 69.90, 69.94, 69.99 (OCH_2), 109.27 (C-3a), 110.59 (C-7), 117.30 (C-5), 132.40 (C-7a), 136.50 (C-6), 146.68 (C-4), 167.54 (C-1), 169.06 (C-3), 170.04 (CO), 170.28 (C-2'), 173.02 (C-6'). One signal for NH$\underline{C}H_2$ 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 = 11.70 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{35}H_{48}N_4O_8$, 653.35; found, 653.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{35}H_{48}N_4O_8$, 653.3545; found, 653.3544.

L. Synthesis of the VPMLK-tagged CRBN Ligand 9

(5*S*,8*S*)-*tert*-Butyl 5-isobutyl-16,16-dimethyl-3,6,14-trioxo-1-phenyl-2,15-dioxa-4,7,13-tri azahepta-decane-8-carboxylate (63)

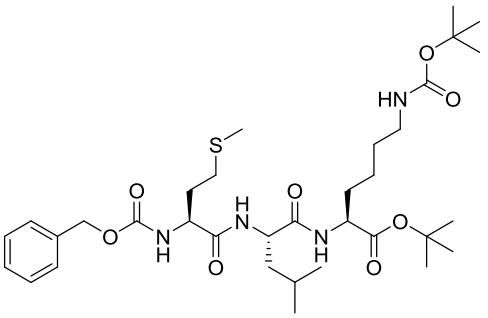


The acid component Cbz-Leu-OH (4.38 g, 16.5 mmol) was dissolved in dry THF (3.7 mL) and cooled at –25 °C. To the stirred solution, NMM (1.67 g, 1.82 mL, 16.5 mmol) and isobutyl chloroformate (2.25 g, 2.16 mL, 16.5 mmol) were added consecutively. The amine component *N*-ε-Boc-L-lysine *tert*-butyl ester hydrochloride (5.08 g, 15 mmol) was dissolved in dry THF (12 mL) and Et_3N (1.52 g, 2.08 mL, 15 mmol) was added. This was given to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. The mixture was allowed to warm up to rt within 30 min and was stirred overnight. After evaporation of the solvent, the residue was dissolved in ethyl acetate (50 mL) and washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), saturated $NaHCO_3$ (2 × 30 mL), H_2O (30 mL) and brine (30-50 mL). The solvent was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography using CH_2Cl_2/MeOH 29:1 to obtain the desired product as a colorless oil.

Yield (7.52 g, 91%); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.85, 0.87 (each d, J = 7.2 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 1.23 – 1.30 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2NH$), 1.32 – 1.35 (m, 2H, C$\underline{H}_2CH_2CH_2CH_2NH$), 1.36, 137 (each s, each 9H, CHCOOC(C$\underline{H}_3$)$_3$, NHCOOC(C$\underline{H}_3$)$_3$), 1.40 – 1.45 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2NH$), 1.52 – 1.59 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 1.59 – 1.69 (m, 2H, C$\underline{H}_2$CH(CH$_3$)$_2$), 2.88 (q, J = 6.5 Hz, 2H, $CH_2CH_2CH_2C\underline{H}_2NH$), 4.01 – 4.10 (m, 2H, C$\underline{H}CH_2$CH(CH$_3$)$_2$, C$\underline{H}CH_2CH_2CH_2CH_2NH$), 5.01 (s, 2H, NHCOOC$\underline{H}_2$Ph), 6.70 (t, J = 5.3 Hz, 1H, $CH_2CH_2CH_2CH_2N\underline{H}$), 7.27 – 7.37 (m, 6H, 2-H – 6-H, N$\underline{H}$COOCH$_2$Ph), 8.02 (d, J = 7.4 Hz, 1H, CHCON$\underline{H}$CH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.63 ($CH_2\underline{C}H_2CH_2CH_2NH$), 22.73 (CH(C$\underline{H}_3$)$_2$), 23.15 (CH(C$\underline{H}_3$)$_2$), 24.28 (C$\underline{H}$(CH$_3$)$_2$), 27.74, 28.40 (NHCOOC(C$\underline{H}_3$)$_3$, CHCOOC(C$\underline{H}_3$)$_3$), 29.19 ($CH_2CH_2\underline{C}H_2CH_2NH$), 30.76 ($\underline{C}H_2CH_2CH_2CH_2NH$), 40.29 ($CH_2CH_2CH_2\underline{C}H_2NH$) 40.97 ($\underline{C}H_2CH(CH_3)_2$), 52.81, 52.96 ($\underline{C}HCH_2CH_2CH_2CH_2NH$, $\underline{C}HCH_2CH(CH_3)_2$), 65.44 (NHCOO$\underline{C}H_2$Ph), 77.45, 80.48 (NHCOO$\underline{C}$(CH$_3$)$_3$, CHCOO$\underline{C}$(CH$_3$)$_3$), 127.75 (C-2, C-6), 127.87 (C-4), 128.43(C-3, C-5), 137.22 (C-1), 155.69, 155.99 (NH$\underline{C}$OOC(CH$_3$)$_3$, NH$\underline{C}$OOCH$_2$Ph), 171.23 (CHC$\underline{O}$NHCH), 172.50 (CH$\underline{C}$OOC(CH$_3$)$_3$); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100%

MeCN for 10 min, DAD 220-400 nm), t_R = 10.30 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{29}H_{47}N_3O_7$, 550.34; found, 550.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{29}H_{47}N_3O_7$, 550.3487; found, 550.3456.

(5*S*,8*S*,11*S*)-*tert*-Butyl 8-isobutyl-19,19-dimethyl-5-(2-(methylthio)ethyl)-3,6,9,17-tetra oxo-1-phenyl-2,18-dioxa-4,7,10,16-tetraazaicosane-11-carboxylate (64)

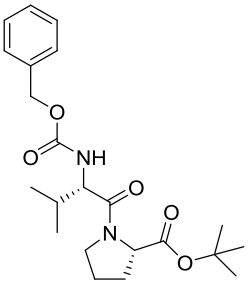


Compound **63** (8.25 g, 15 mmol) was dissolved in ethyl acetate (17 mL) and deprotected by hydrogenation with H_2 over Pd/C (10%, 0.83 mg) at rt over 24 h. Filtration of Pd/C over a celite pad and evaporation of the solvent yielded the derivative H-Leu-Lys(Boc)-O*t*Bu that was used as amine component as described below. The acid component Cbz-Met-OH (4.25 g, 15 mmol) was dissolved in dry THF (17 mL) and cooled to –25 °C. To the stirred solution, NMM (2.23 g, 2.42 mL, 22 mmol) and isobutyl chloroformate (3.00 g, 2.86 mL, 22 mmol) were added consecutively. The amine component H-Leu-Lys(Boc)-O*t*Bu was dissolved in dry THF (5 mL) and added to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. The mixture was allowed to warm up to rt within 30 min and was stirred overnight. After evaporation of the solvent, the residue was dissolved in ethyl acetate (56 mL) and washed with 10% $KHSO_4$ solution (33 mL), H_2O (33 mL), saturated $NaHCO_3$ solution (2 × 33 mL), H_2O (33 mL) and brine (50 mL). The solvent was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to obtain the desired product as a colorless solid.

Yield (7.05 g, 69%); mp 110 – 114 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.84, 0.88 (each d, *J* = 6.5 Hz, 6H, $CH_2CH(C\underline{H}_3)_2$), 1.21 – 1.30 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2NH$), 1.31 – 1.35 (m, 2H, $C\underline{H}_2CH_2CH_2CH_2NH$), 1.35, 1.37 (each s, each 9H, $CHCOOC(C\underline{H}_3)_3$, $NHCOOC(C\underline{H}_3)_3$), 1.41 – 1.49 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2NH$), 1.51 – 1.57 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.57 – 1.67 (m, 2H, $C\underline{H}_2CH(CH_3)_2$), 1.74 – 1.82 (m, 1H, $C\underline{H}_2CH_2SCH_3$), 1.82 – 1.90 (m, 1H, $C\underline{H}_2CH_2SCH_3$), 2.01 (s, 3H, $CH_2CH_2SC\underline{H}_3$), 2.41 – 2.46 (m, 2H, $CH_2C\underline{H}_2SCH_3$), 2.88 (q, *J* = 6.6 Hz, 2H, $CH_2CH_2CH_2C\underline{H}_2NH$), 4.02 (q, *J* = 7.1 Hz, 1H, $C\underline{H}CH_2CH_2CH_2CH_2NH$), 4.09 (dt, *J* = 8.5 Hz, *J* = 5.0 Hz, 1H, $C\underline{H}CH_2CH(CH_3)_2$), 4.34 (dt, *J* = 8.8 Hz, *J* = 5.7 Hz, 1H, $C\underline{H}CH_2CH_2SCH_3$), 5.02 (s, 2H, $NHCOOC\underline{H}_2Ph$), 6.70 (t, *J* = 5.5 Hz, 1H, $N\underline{H}COOC(CH_3)_3$), 7.27 – 7.37 (m, 5H, 2-H – 6-H), 7.43 (d, *J* = 8.1 Hz, 1H, $N\underline{H}COOCH_2Ph$),

7.86 (d, *J* = 8.3 Hz, 1H, CHCONHCHCONH), 8.05 (d, *J* = 7.3 Hz, 1H, CHCONHCHCONH); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 14.73 (SCH_3, $CH_2CH_2SCH_3$), 21.81 ($CH_2CH_2CH_2CH_2NH$), 22.72 ($CH_2CH(CH_3)_2$), 23.17 ($CH_2CH(CH_3)_2$), 24.22 ($CH_2CH(CH_3)_2$), 27.73, 28.39 ($CHCOOC(CH_3)_3$, $NHCOOC(CH_3)_3$), 29.21 ($CH_2CH_2SCH_3$), 29.75 ($CH_2CH_2CH_2CH_2NH$), 30.71 ($CH_2CH_2CH_2CH_2NH$), 40.29 ($CH_2CH_2CH_2CH_2NH$) 41.20 ($CH_2CH(CH_3)_2$), 50.79, 52.87, 54.04 ($CHCH_2CH(CH_3)_2$), $CHCH_2CH_2CH_2CH_2NH$, $CHCH_2CH_2SCH_3$), 65.53 ($NHCOOCH_2Ph$), 77.44, 80.46 ($NHCOOC(CH_3)_3$, $CHCOOC(CH_3)_3$), 127.75 (C-2, C-6), 127.87 (C-4), 128.43 (C-3, C-5), 137.13 (C-1), 155.67, 156.03 ($NHCOOC(CH_3)_3$, $NHCOOCH_2Ph$), 171.12, 171.20, 172.06 ($CHCONHCHCO$, $CHCOOC(CH_3)_3$); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220-400 nm), t_R = 11.92 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{34}H_{56}N_4O_8S$, 681.39; found, 681.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{34}H_{56}N_4O_8S$, 681.3892; found, 681.3877.

(*S*)-*tert*-Butyl 1-((*S*)-2-(benzyloxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (65)

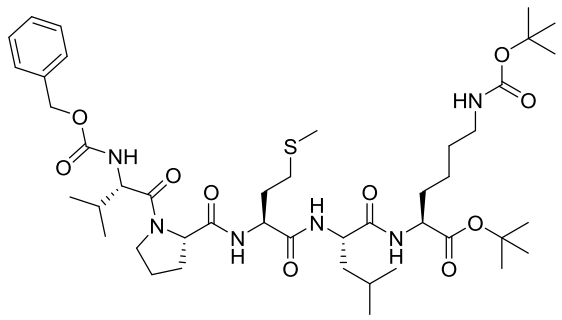


Cbz-Val-OH (1.26 g, 5 mmol) was dissolved in dry THF (7.5 mL) and cooled to –25 °C. To the stirred solution, NMM (0.51 g, 0.55 mL, 5 mmol) and isobutyl chloroformate (0.68 g, 0.65 mL, 5 mmol) were added consecutively. H-Pro-O*t*Bu hydrochloride (1.14 g, 5.5 mmol) was dissolved in dry THF (12 mL) and Et_3N (0.56 g, 0.76 mL, 5.5 mmol) was added. This was added to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. The mixture was allowed to warm up to rt within 30 min and was stirred overnight. After evaporation of the solvent, the residue was dissolved in EtOAc (50 mL) and washed with 10% $KHSO_4$ solution (30 mL), H_2O (30 mL), saturated $NaHCO_3$ solution (2 × 30 mL), H_2O (30 mL) and brine (30-50 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 2:1 to 1:1) to obtain the desired product as a colorless oil.

Yield (0.75 g, 38%); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.88 (d, *J* = 5.6 Hz, 3H, $CHCH(CH_3)_2$), 0.93 (d, *J* = 5.7 Hz, 3H, $CHCH((CH_3)_2$), 1.37 (s, 9H, $(CH_3)_3$), 1.73 – 1.81 (m, 1H, proline CH_2), 1.81 – 1.99 (m, 3H, proline CH_2), 2.08 – 2.18 (m, 1H, $CH(CH_3)_2$), 3.52 – 3.59 (m, 1H, proline NCH_2), 3.70 –3.78 (m, 1H, proline NCH_2), 4.06 (t, *J* = 6.9 Hz, 1H, proline CH), 4.17 (dd, *J* = 4.3, 7.2 Hz, Hz, 1H, $CHCH(CH_3)_2$), 4.98 and 5.03 (each d, *J* = 12.7 Hz and *J* = 12.7 Hz, 2H, $NHCOOCH_2Ph$), 7.27 – 7.38 (m, 5H, 2-H – 6-H), 7.41 (d, *J* = 7.1 Hz, 1H,

NH); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 18.49 ($(CH_3)_2$), 19.11 ($(CH_3)_2$), 24.69 (proline CH_2), 27.75 ($(CH_3)_3$), 28.87 (proline CH_2), 29.93 ($\underline{C}H(CH_3)_2$), 46.97 (proline NCH_2), 57.88 ($\underline{C}HCH(CH_3)_2$), 59.58 (proline CH), 65.57 ($NHCOO\underline{C}H_2Ph$), 80.45 ($\underline{C}(CH_3)_3$), 127.81 (C-2, C-6), 127.93 (C-4) 128.49 (C-3, C-5), 137.22 (C-1), 156.35 ($NH\underline{C}OOCH_2$), 170.28 ($\underline{C}OOC(CH_3)_3$), 171.15 ($CHNH\underline{C}OCH$); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220-400 nm), t_R = 11.28 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{22}H_{32}N_2O_5$, 405.23; found, 405.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{22}H_{32}N_2O_5$, 405.2384; found, 405.2362.

(*S*)-*tert*-Butyl 2-((*S*)-2-((*S*)-2-((*S*)-1-((*S*)-2-(benzyloxycarbonylamino)-3-methylbutanoyl) pyrrolidine-2-carboxamido)-4-(methylthio)butanamido)-4-methylpentanamido)-6-(*tert*-butoxycarbonylamino) hexanoate (66)



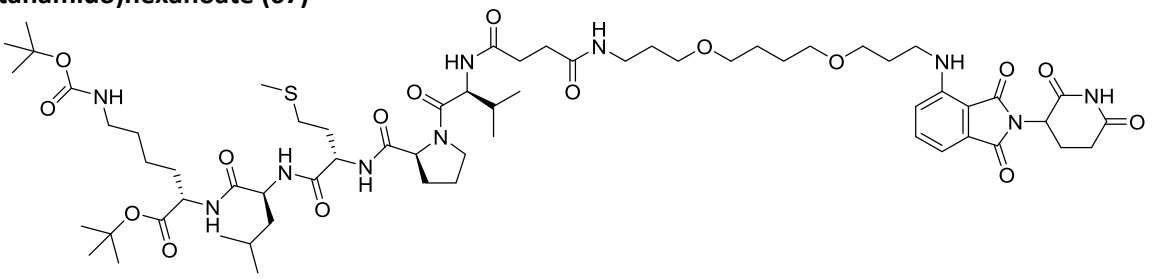
Cbz-Met-Leu-Lys(Boc)-O*t*Bu (**64**, 0.81 g, 1.2 mmol) was dissolved in MeOH (10 mL) and deprotected by hydrogenation over $Pd(OH)_2/C$ (20%, 167 mg) at rt for 5 h at 60 psi. Filtration of $Pd(OH)_2/C$ over a celite pad and evaporation of the solvent yielded the derivative H-Met-Leu-Lys(Boc)-O*t*Bu that was used as amine component as described below.

To deprotect the dipeptide Cbz-Val-Pro-O*t*Bu (**65**, 0.98 g, 2.4 mmol), it was dissolved in dry CH_2Cl_2/ trifluoroacetic acid 1:1 (10 mL) and stirred at 40 °C for 2 h. Removal of the solvent, coevaporation with CH_2Cl_2 (4 × 5 mL) and further drying *in vacuo* yielded the crude acid derivative, which was redissolved in dry MeCN (10 mL). After stirring of this light yellow solution together with HBTU (1.14 g, 3.0 mmol) and DIPEA (0.93 g, 1.25 mL, 7.2 mmol) for 10 min at rt, the amine component H-Met-Leu-Lys(Boc)-O*t*Bu in dry MeCN (10 mL) and DIPEA (0.31 g, 0.42 mL, 2.4 mmol) was added. The combined mixture was stirred at rt for 12 h. The yellow solution was then diluted with EtOAc (200 mL) and washed with 10% $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O and brine (each 25 mL). The yellow organic layer was dried over Na_2SO_4, filtrated and concentrated. The crude material was twice purified by column chromatography (gradient of CH_2Cl_2/MeOH 39:1 to 29:1) to obtain a colorless oil, which was subjected

to a further purification by reversed-phase flash chromatography (20% H_2O, 80% MeCN) to obtain a colorless solid.

Yield (0.23 g, 28%); mp 86 – 90 °C; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.77 – 0.92 (m, 12H, $CH_2CH(C\underline{H}_3)_2$, $CHCH(C\underline{H}_3)_2$), 1.22 – 1.28 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2NH$), 1.29 –1.34 (m, 2H, $C\underline{H}_2CH_2CH_2CH_2NH$), 1.36 (each s, each 9H, $CHCOOC(C\underline{H}_3)_3$, $NHCOOC(C\underline{H}_3)_3$), 1.39 – 1.46 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2NH$), 1.46 – 1.71 (m, 4H, $CH_2C\underline{H}(CH_3)_2$, $C\underline{H}_2CH(CH_3)_2$, proline CH_2), 1.71 – 1.99 (m, 6H, $C\underline{H}_2CH_2SCH_3$, $CHC\underline{H}(CH_3)_2$, proline CH_2), 2.01 (s, 3H, $CH_2CH_2SC\underline{H}_3$), 2.37 – 2.44 (m, 1H, $CH_2C\underline{H}_2SCH_3$), 2.44 – 2.48 (m, 1H, $CH_2C\underline{H}_2SCH_3$), 2.88 (q, *J* = 6.6 Hz, 2H, $CH_2CH_2CH_2C\underline{H}_2NH$), 3.50 - 3.61 (m, 1H, proline NCH_2), 3.66 – 3.76 (m, 1H, proline NCH_2), 3.97 – 4.07 (m, 2H, $C\underline{H}CH_2CH_2CH_2CH_2NH$, $C\underline{H}CH_2CH(CH_3)_2$), 4.24 – 4.37 (m, 3H, $C\underline{H}CH_2CH_2SCH_3$, proline CH, $C\underline{H}CH(CH_3)_2$), 5.00 (d, *J* = 13.6 Hz, 1H, $NHCOOC\underline{H}_2Ph$), 5.06 (d, *J* = 13.6 Hz, 1H, $NHCOOC\underline{H}_2Ph$), 6.70 (t, *J* = 5.3 Hz, 1H, $CH_2CH_2CH_2CH_2N\underline{H}$), 7.27 – 7.41 (m, 6H, 2-H - 6-H, $CHN\underline{H}CO$), 7.77 (d, *J* = 8.3 Hz, 1H, $CHN\underline{H}CO$), 8.00 (t, *J* = 7.8 Hz, 2H, $CHN\underline{H}CO$); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 14.80 ($CH_2CH_2S\underline{C}H_3$), 18.66, 19.12 ($CHCH(\underline{C}H_3)_2$), 21.78 ($CH_2\underline{C}H_2CH_2CH_2NH$), 22.73, 23.15 ($CH_2CH(\underline{C}H_3)_2$), 24.23 (proline CH_2), 24.67 ($CH_2\underline{C}H(CH_3)_2$), 27.74, 28.41 ($NHCOOC(\underline{C}H_3)_3$, $CHCOOC(\underline{C}H_3)_3$), 29.10 ($CH_2\underline{C}H_2SCH_3$), 29.23 (proline CH_2), 29.58 ($CH_2CH_2\underline{C}H_2CH_2NH$), 29.97 ($\underline{C}H_2CH_2CH_2CH_2NH$), 30.74 ($CH\underline{C}H(CH_3)_2$), 32.15 ($\underline{C}H_2CH_2SCH_3$), 40.29 ($CH_2CH_2CH_2\underline{C}H_2NH$), 41.19 ($\underline{C}H_2CH(CH_3)_2$), 47.28 (proline NCH_2), 50.82 ($\underline{C}HCH_2CH(CH_3)_2$), 52.06 ($\underline{C}HCH_2CH_2CH_2CH_2NH$), 52.83 ($\underline{C}HCH_2CH_2SCH_3$), 58.11, 59.51 ($\underline{C}HCH(CH_3)_2$, proline CH), 65.55 ($NCOO\underline{C}H_2Ph$), 77.47, 80.51 ($NHCOO\underline{C}(CH_3)_3$, $CHCOO\underline{C}(CH_3)_3$), 127.77 (C-2, C-6), 127.90 (C-4), 128.47 (C-3, C-5), 137.22 (C-1), 155.69 ($NH\underline{C}OOC(CH_3)_3$), 156.36 ($N\underline{C}OOCH_2Ph$), 170.44, 170.81, 171.12, 171.74, 171.99 ($CH\underline{C}OOC(CH_3)_3$, $CHNH\underline{C}OCH$, $CHN\underline{C}OCH$); **LC-MS** (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220-400 nm), t_R = 11.24 min, 94% purity, *m/z* $[M + H]^+$ calcd for $C_{44}H_{72}N_6O_{10}S$, 877.51; found, 877.6; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{44}H_{72}N_6O_{10}S$, 877.5103; found, 877.5119.

(2*S*)-*tert*-Butyl 6-(*tert*-butoxycarbonylamino)-2-((2*S*)-2-((2*S*)-2-((2*S*)-1-((2*S*)-20-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)-2-isopropyl-4,7-dioxo-12,17-dioxa-3,8-diazaicosan-1-oyl)pyrrolidine-2-carboxamido)-4-(methylthio)butanamido)-4-methyl pentanamido)hexanoate (67)

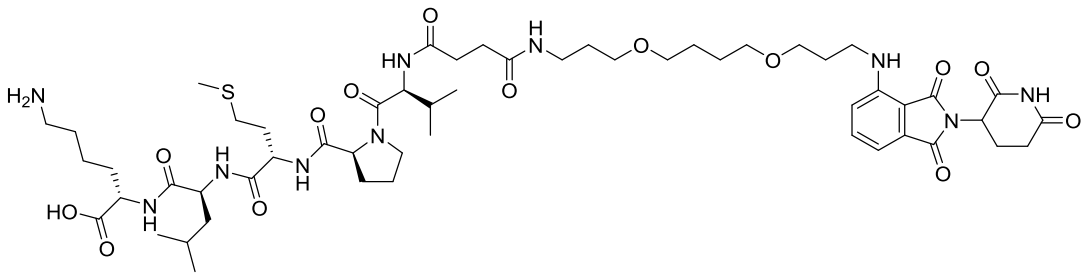


Pentapeptid **66** (0.20 g, 0.27 mmol) was stirred in HCl/dioxane (4N, 10 mL) for 4 h at rt. The solvent was evaporated and it was coevaporated with dry CH_2Cl_2 (4 × 5 mL) and further dried *in vacuo*.

In a separate flask, compound **2d** (0.20 g, 0.32 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and trifluoroacetic acid (5 mL) was added. The mixture was stirred for 2 h at 40 °C. The solvent was evaporated and it was coevaporated with dry CH_2Cl_2 (4 × 5 mL) and further dried *in vacuum*. The crude acid was dissolved in dry DMF (10 mL) and DIPEA (124 mg, 167 µL, 0.96 mmol) and HATU (134 mg, 0.352 mmol) were added. After stirring this solution for 10 min, the deprotected pentapeptid in dry DMF (5 mL) and DIPEA (35 mg, 47 µL, 0.267 mmol) was added. The combined mixture was stirred for 48 h at rt. The yellow mixture was diluted with CH_2Cl_2 (200 mL) and it was washed with 10% $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O and brine (each 25 mL). The organic layer was dried over Na_2SO_4, filtered, and concentraced *in vacuo*. The crude product was purified by column chromatography (gradient of CH_2Cl_2/EtOH 25:1 to 9:1).

Yield (207 mg, 60%); mp 96 – 98 °C; R_f = 0.25 (CH_2Cl_2/EtOH 19:1); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN for 10 min, DAD 220-450 nm), t_R = 9.58 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{63}H_{100}N_{10}O_{16}S$, 1285.71; found, 1285.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{63}H_{100}N_{10}O_{16}S$, 1285.7112; found, 1285.6843.

VPMLK-tagged CRBN ligand 9

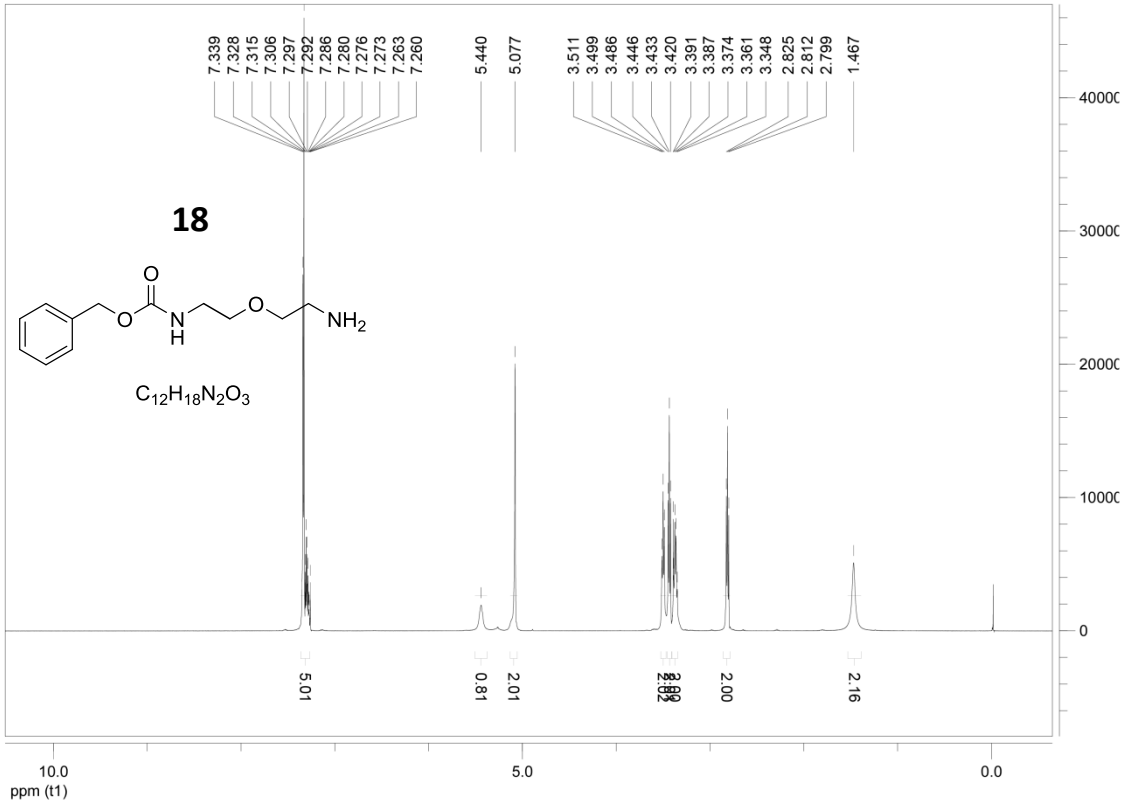


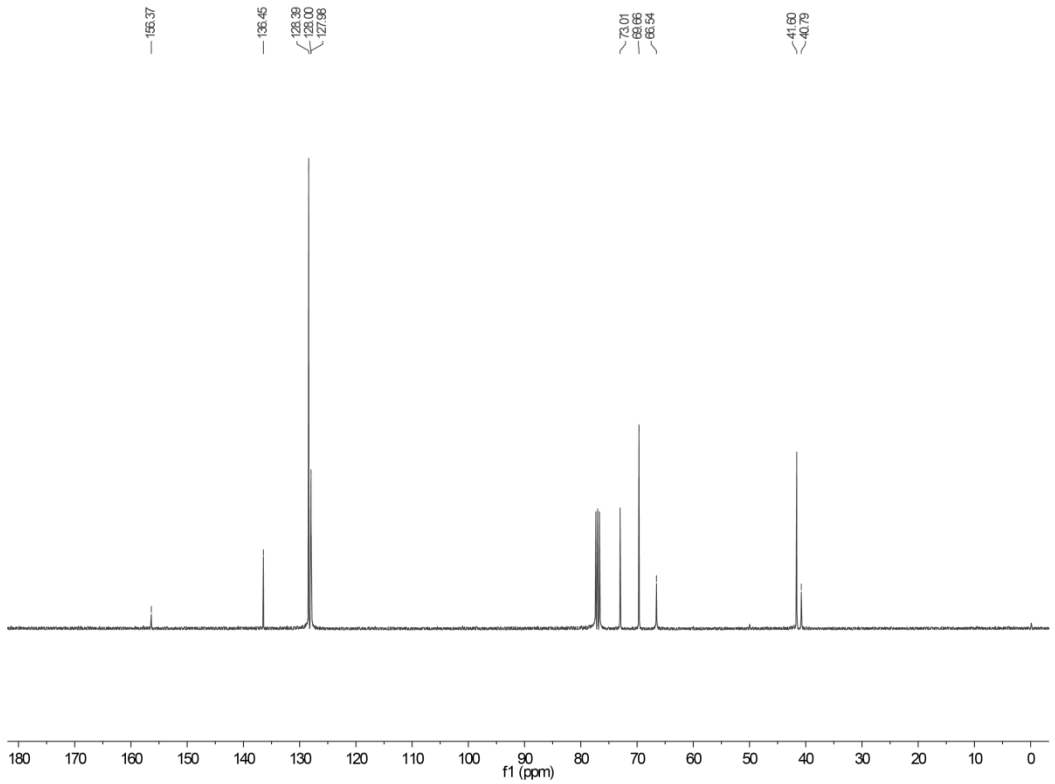
Compound **67** (30.4 mg, 23.6 µmol) was deprotected through stirring with dry CH_2Cl_2/trifluoroacetic acid 1:1 (10 mL) for 2 h at 40 °C. Subsequently, the solvent was removed and it was coevaporated with dry CH_2Cl_2 (4 × 5 mL). The crude product was further dried *in vacuo* and gave a yellow resin.

Yield (quant.); **LC-MS** (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN for 10 min, DAD 220-450 nm), t_R = 6.92 min, 94% purity, *m/z* $[M + H]^+$ calcd for $C_{54}H_{84}N_{10}O_{14}S$, 1129.59; found, 1130.0; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{54}H_{84}N_{10}O_{14}S$, 1129.5962; found, 1129.5924.

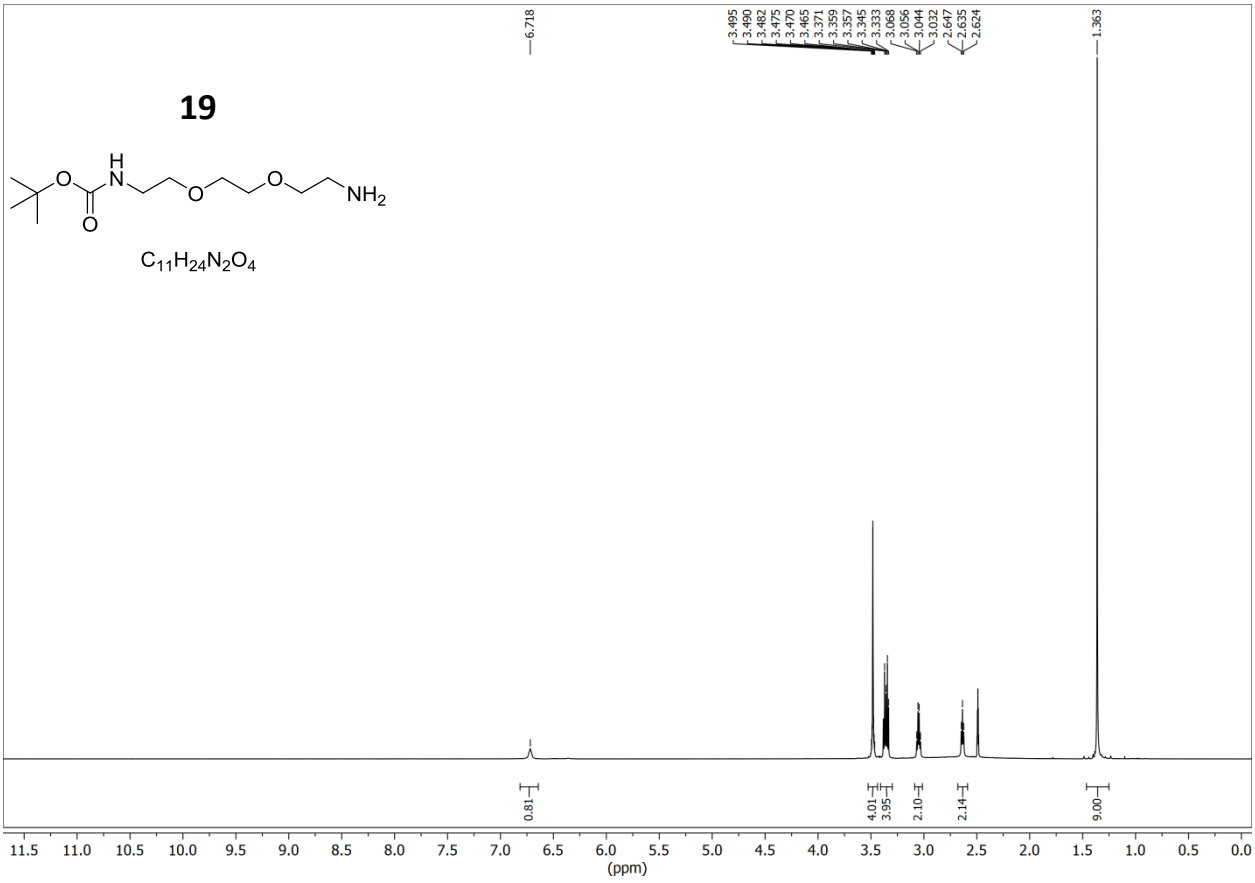
M. Selected ^{1}H and ^{13}C NMR Spectra

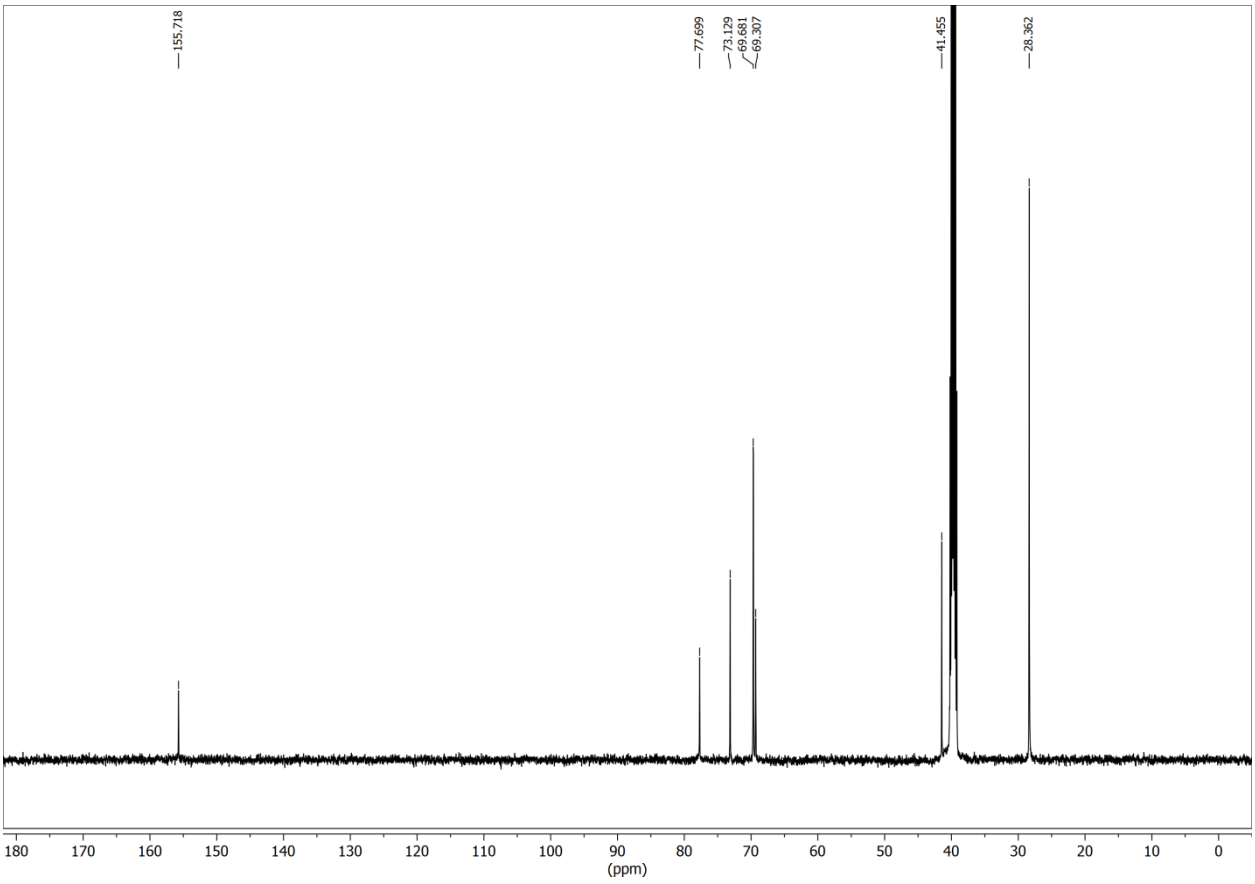
^{1}H and ^{13}C NMR spectrum of compound **18**.



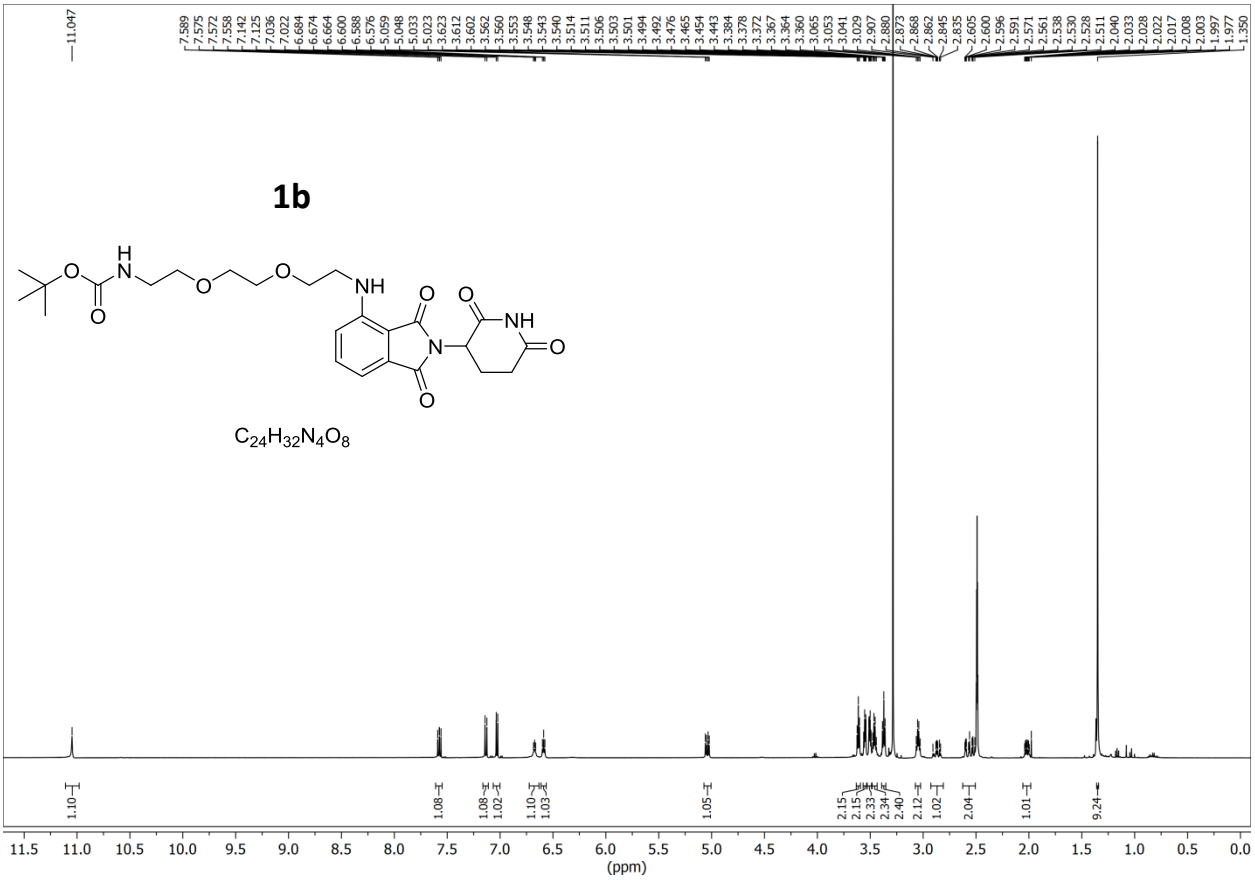


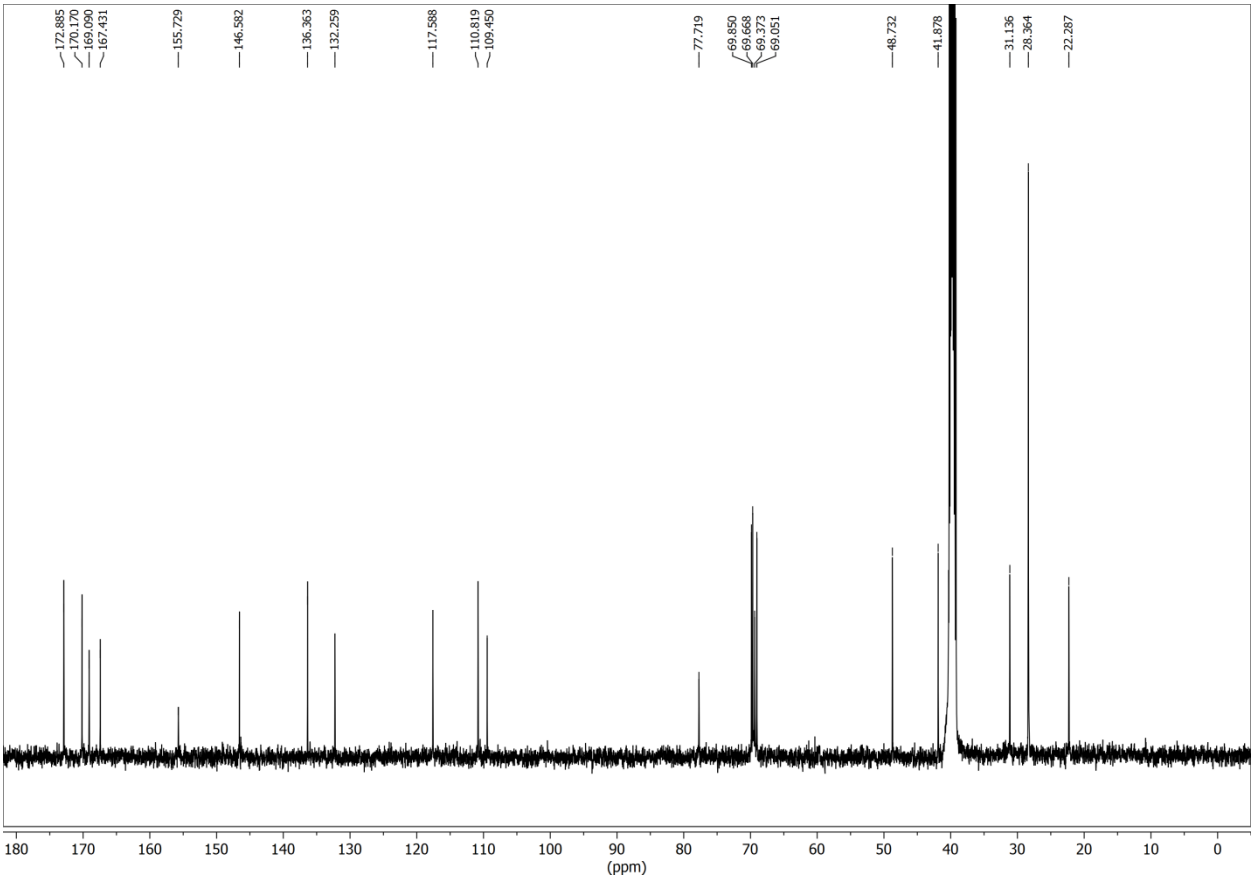
^{1}H and ^{13}C NMR spectrum of compound **19**.



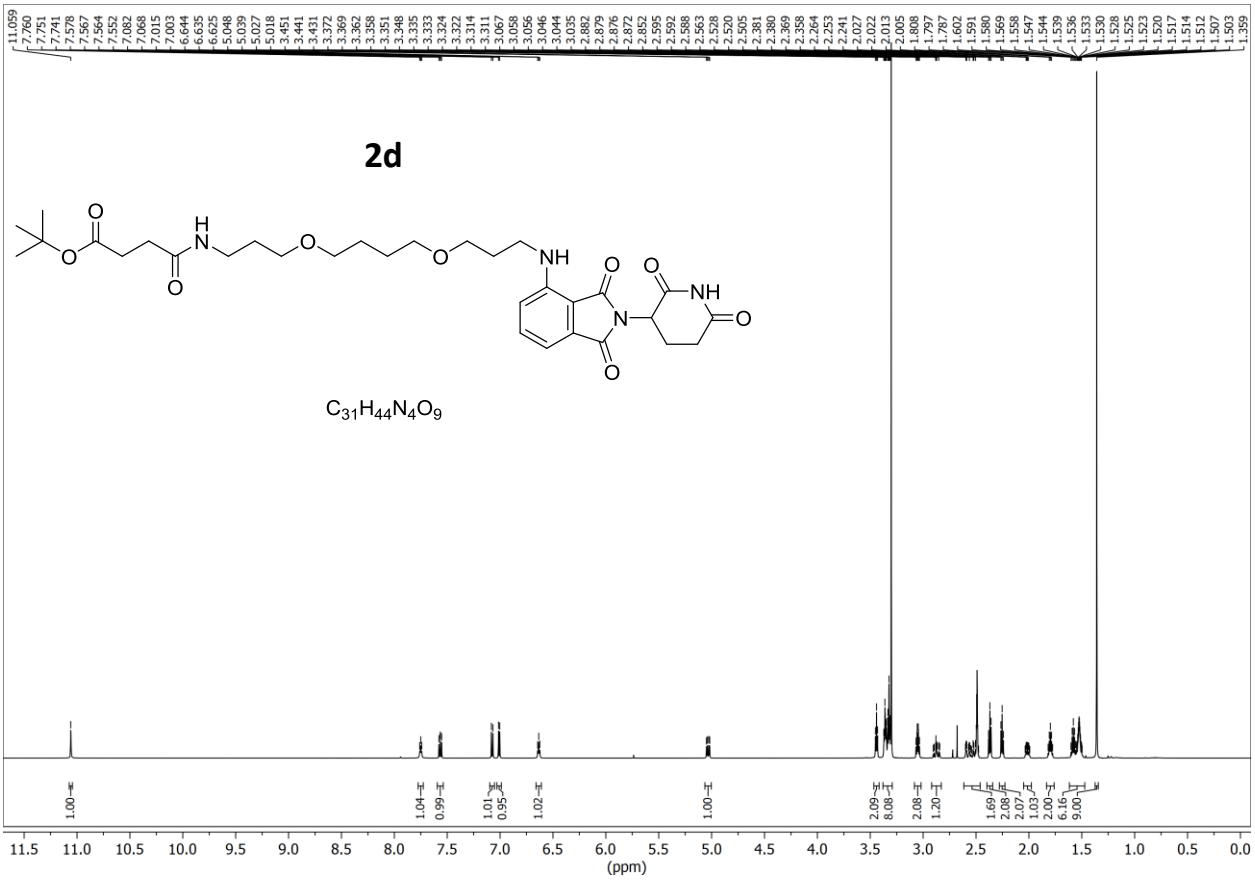


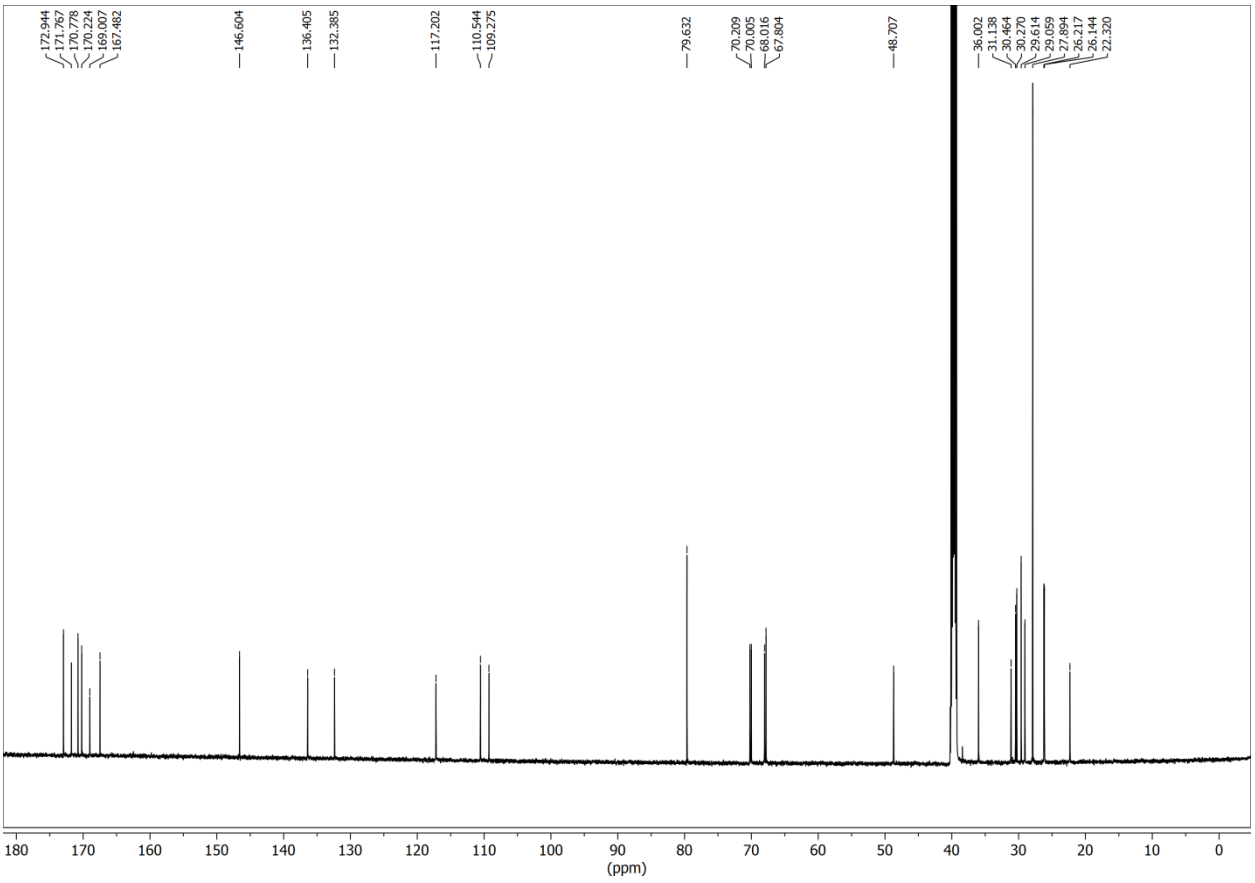
^{1}H and ^{13}C NMR spectrum of compound **1b**.



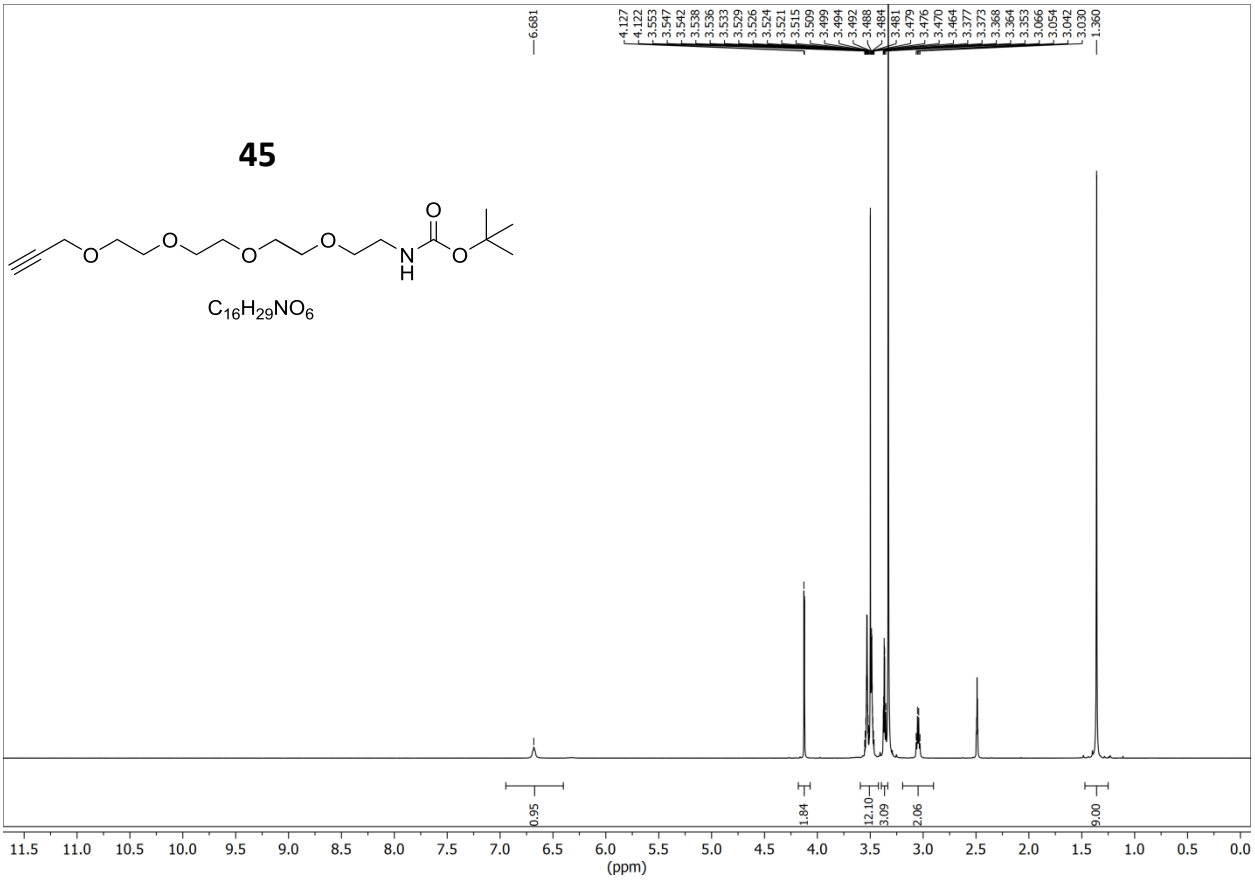


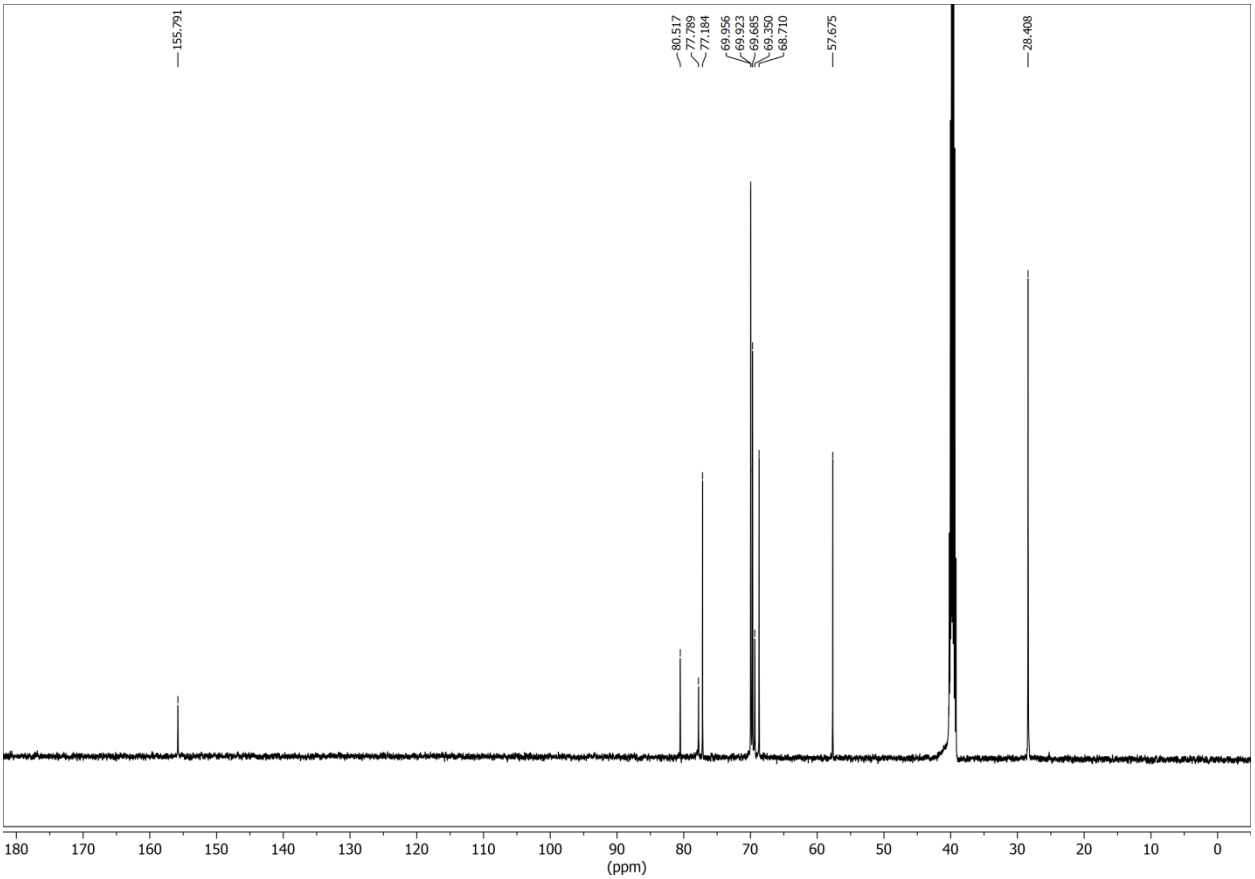
^{1}H and ^{13}C NMR spectrum of compound **2d**.



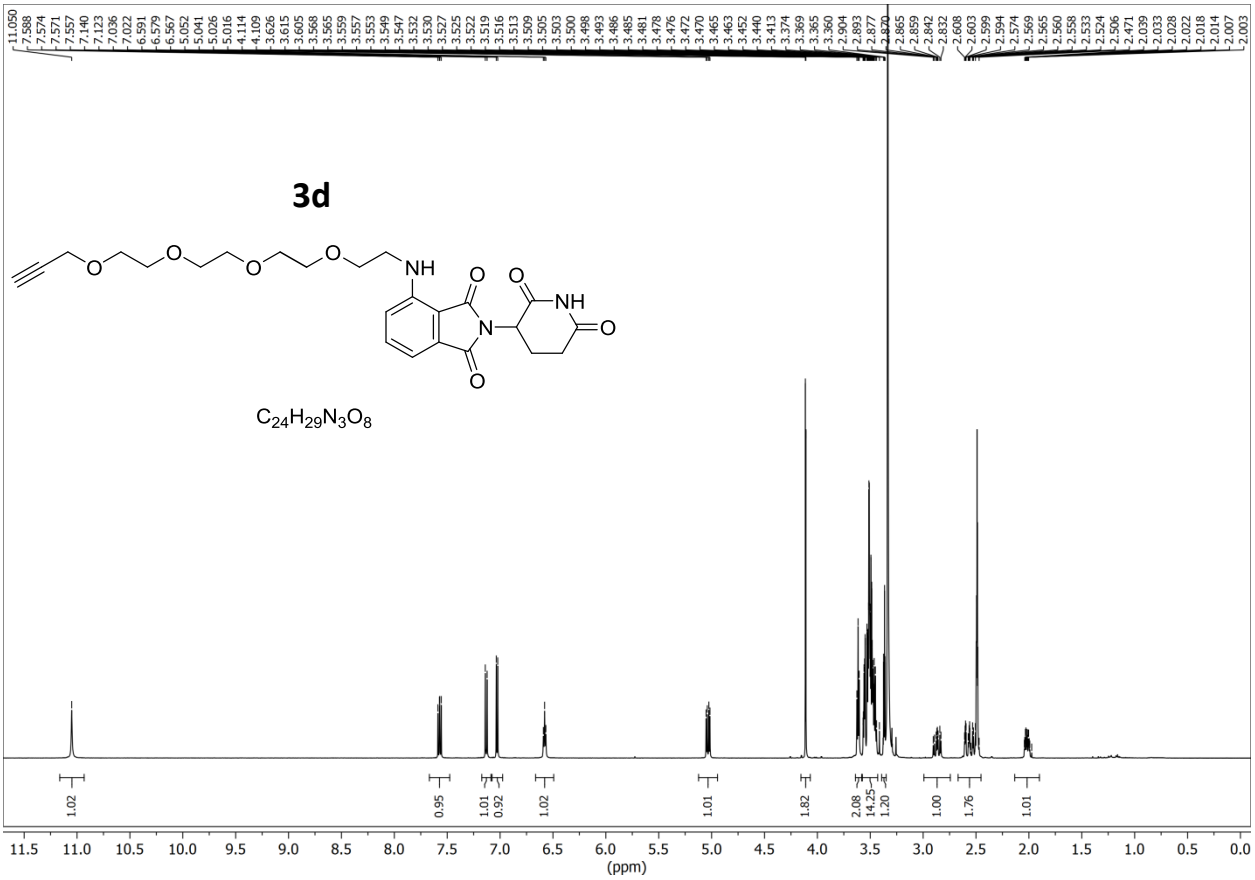


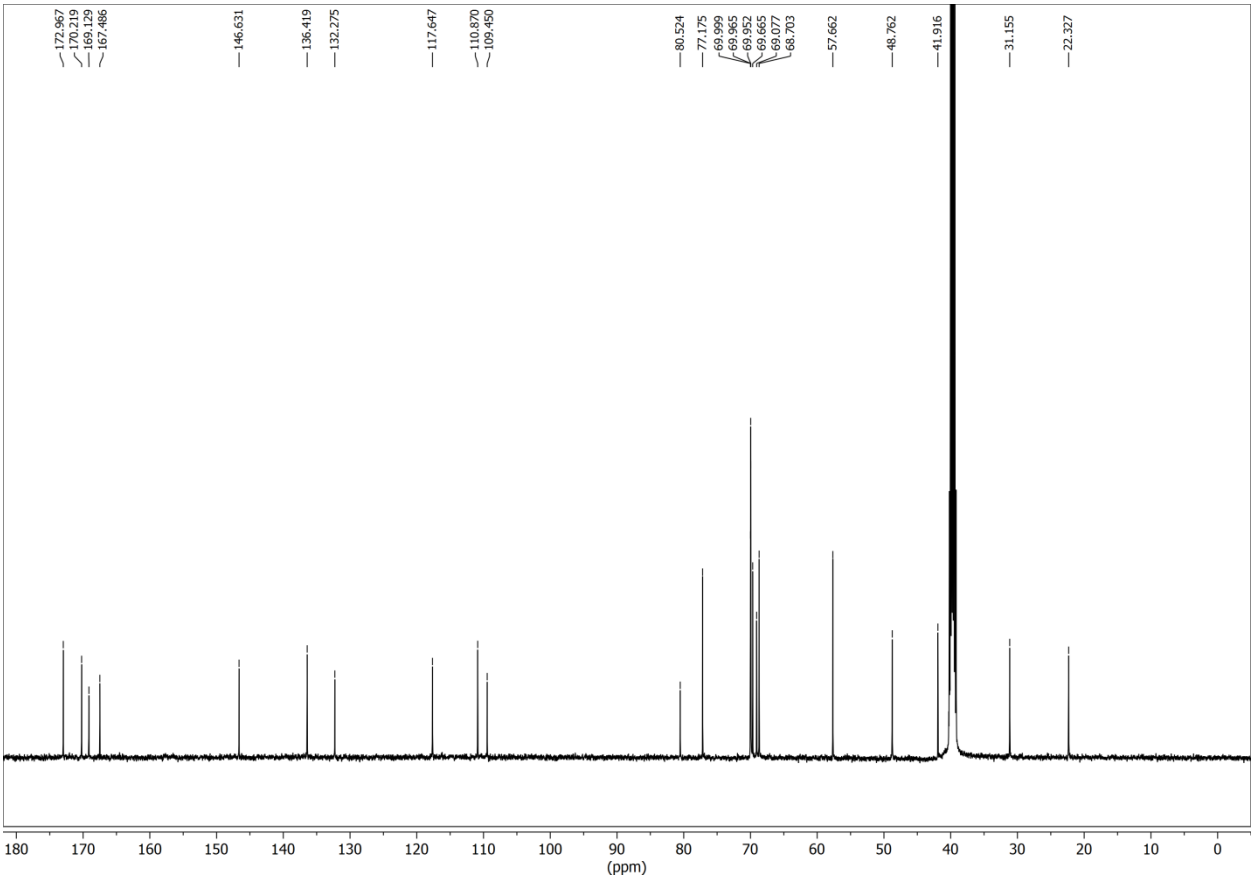
^{1}H and ^{13}C NMR spectrum of compound **45**.



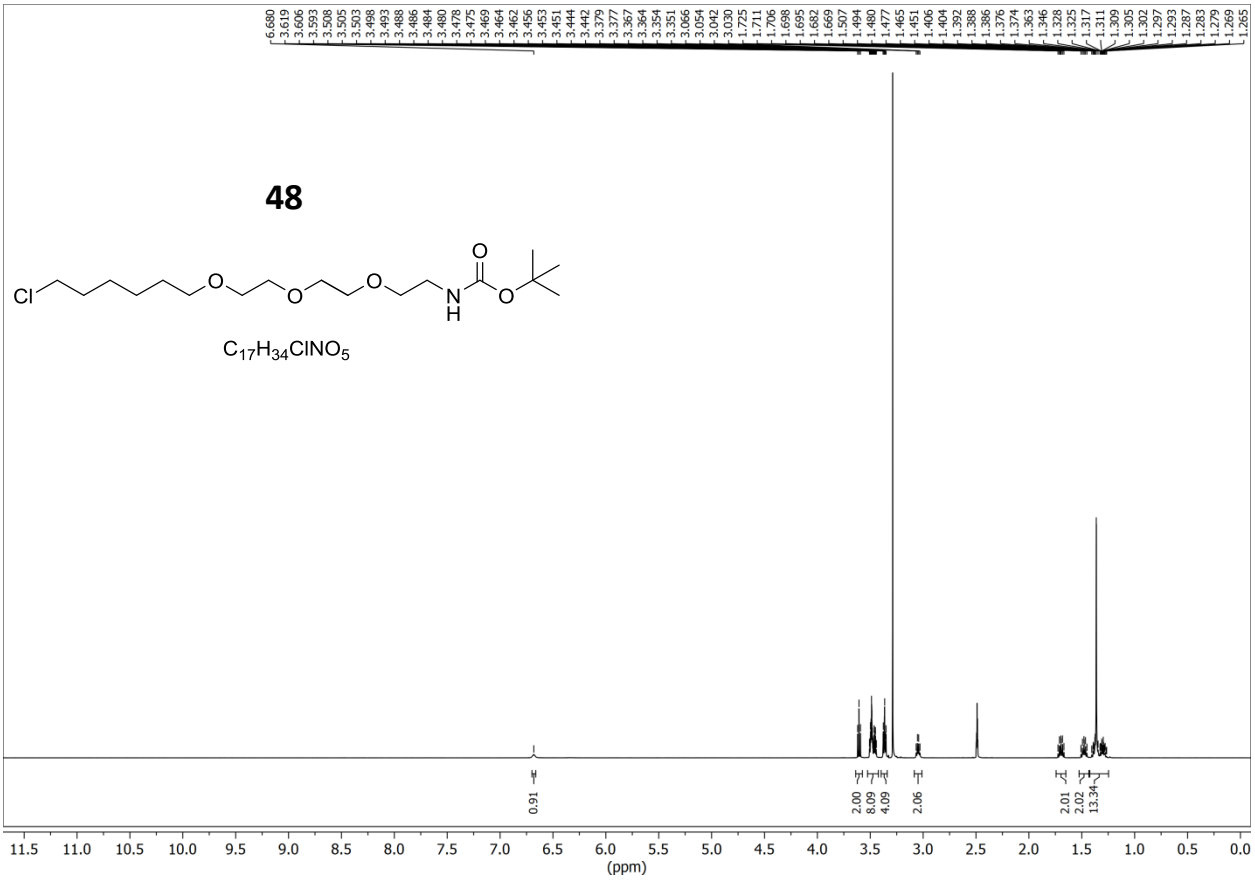


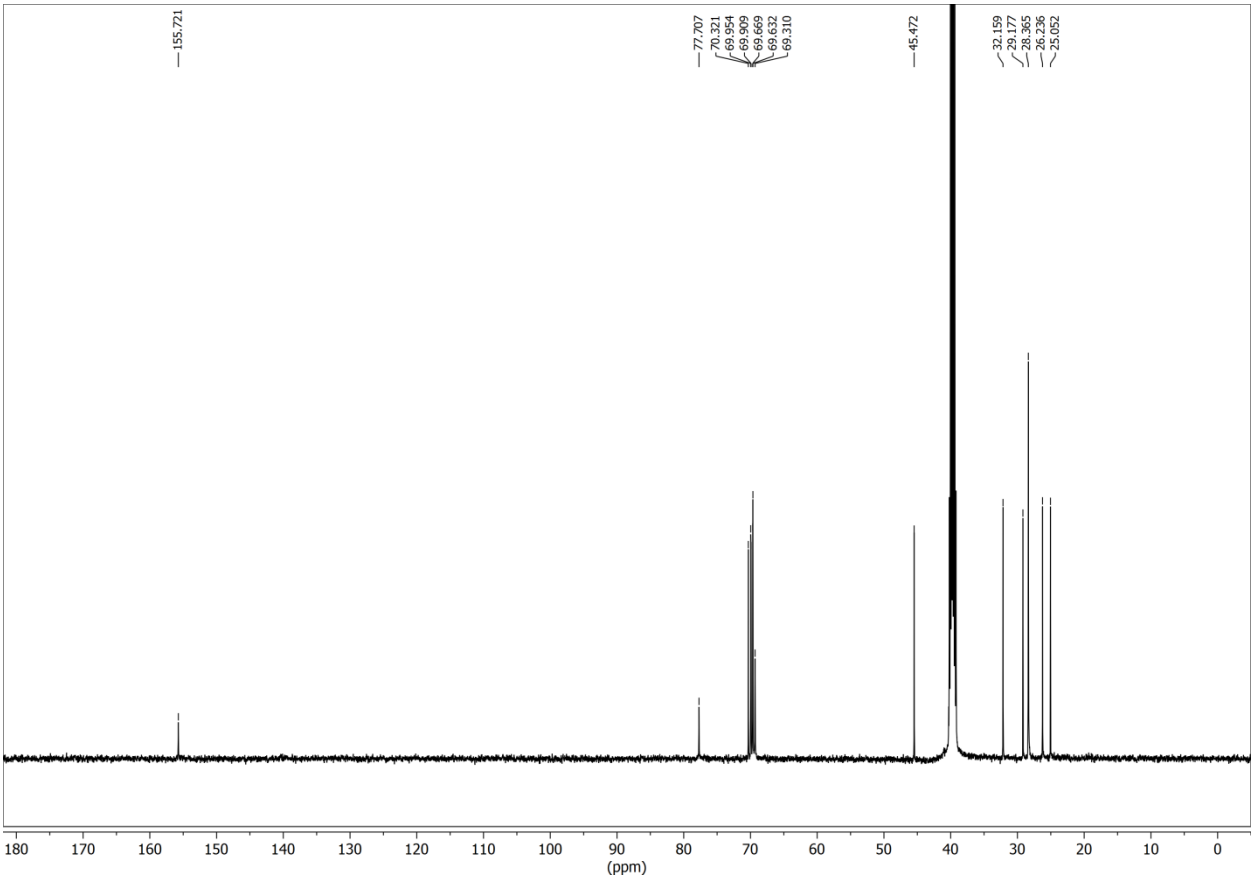
^{1}H and ^{13}C NMR spectrum of compound **3d**.



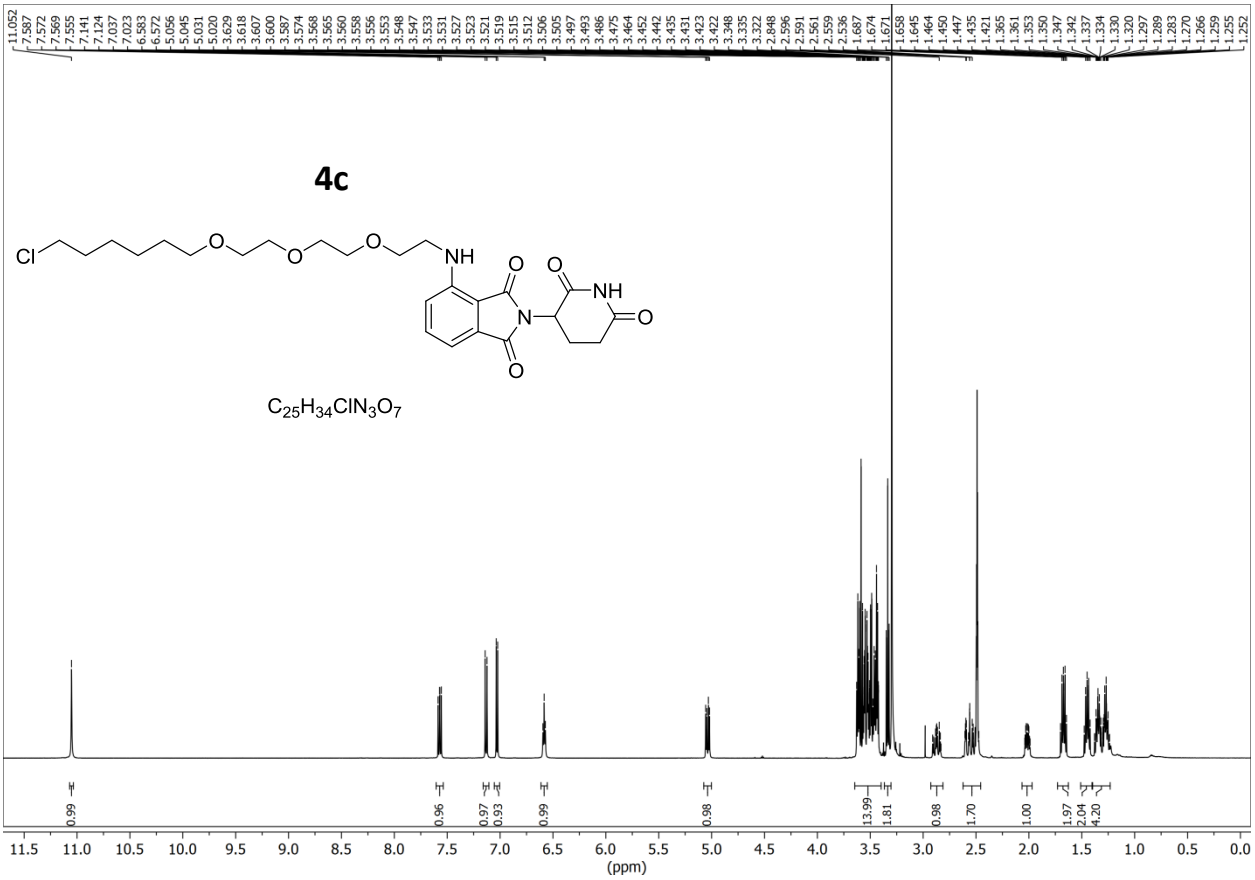


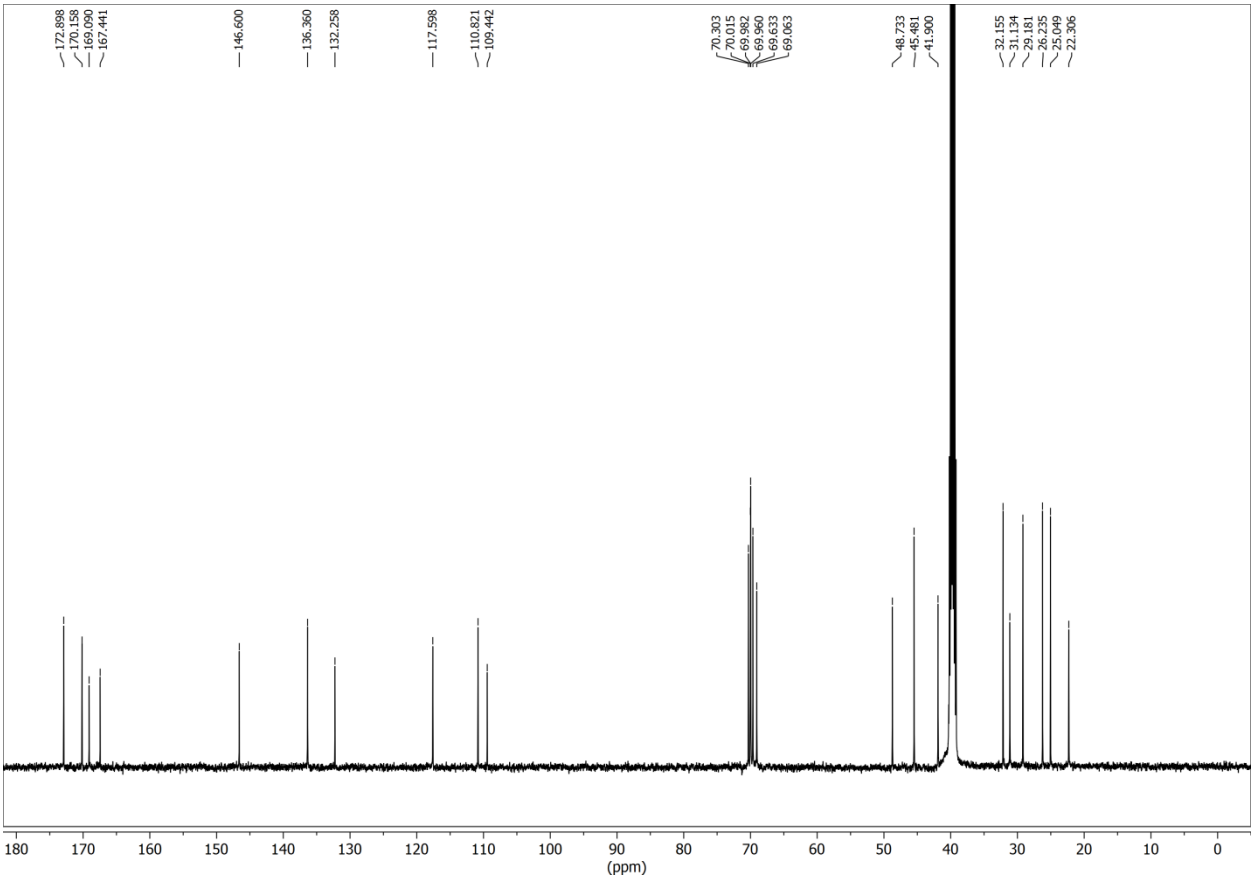
^{1}H and ^{13}C NMR spectrum of compound **48**.



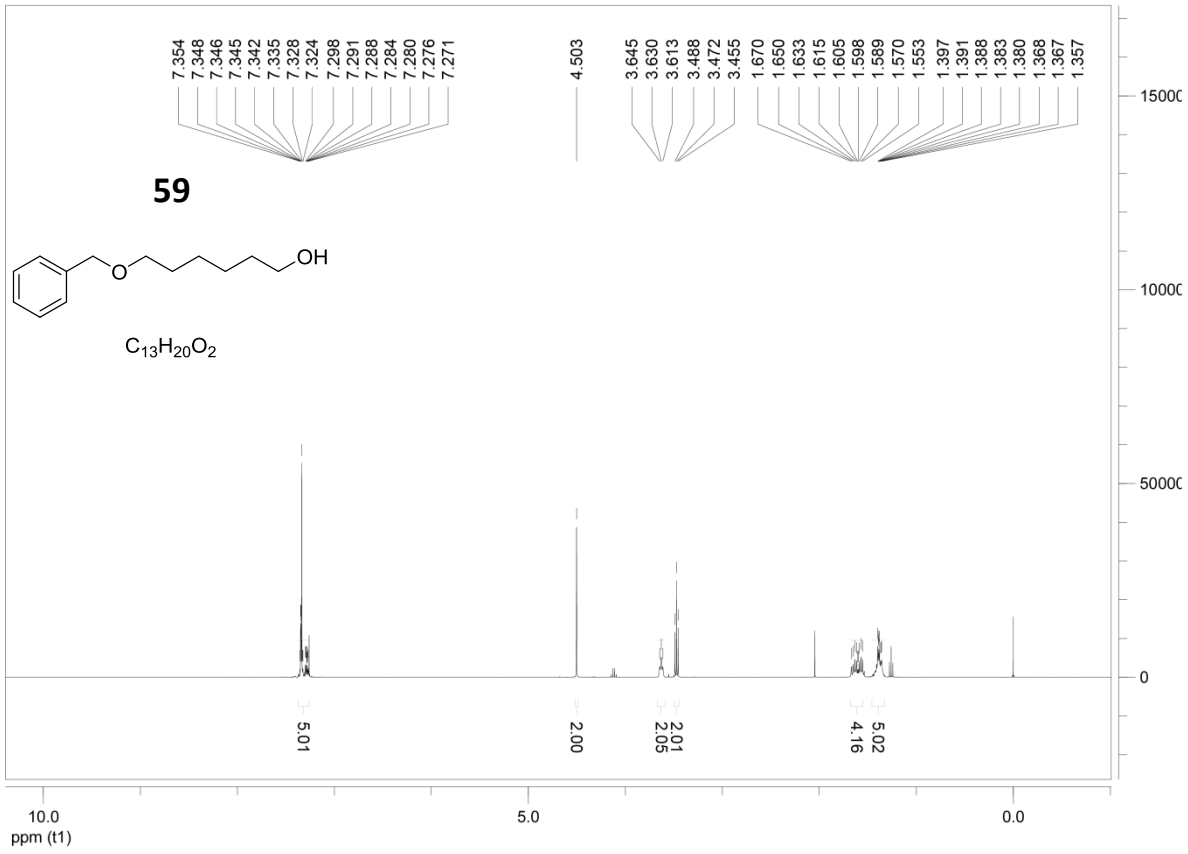


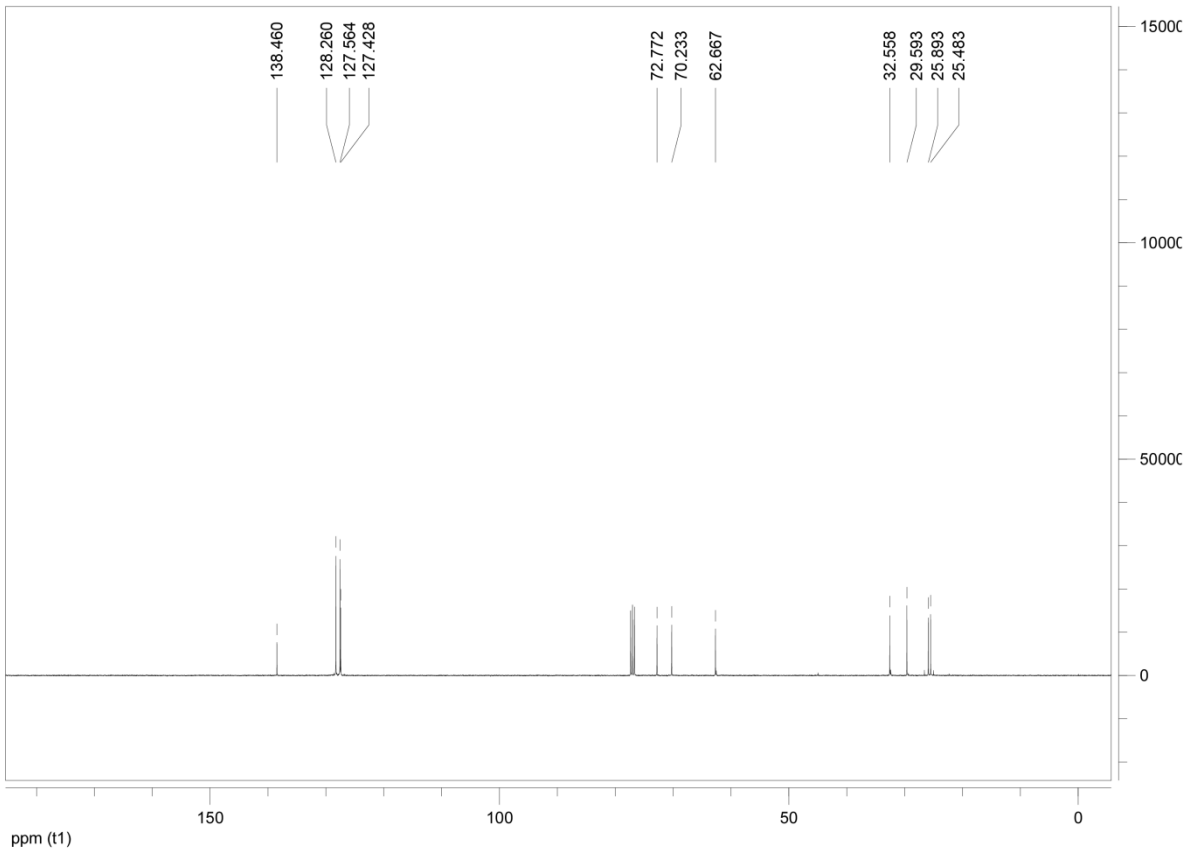
^{1}H and ^{13}C NMR spectrum of compound **4c**.



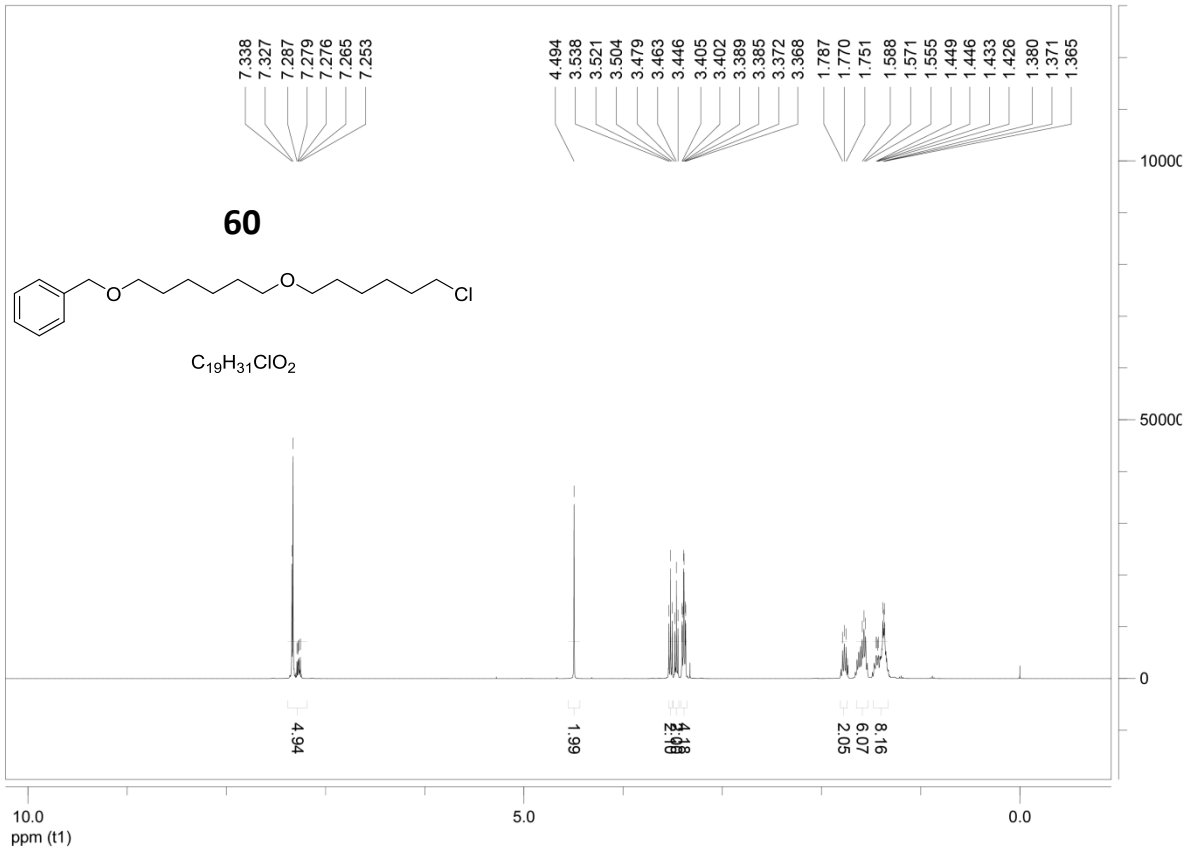


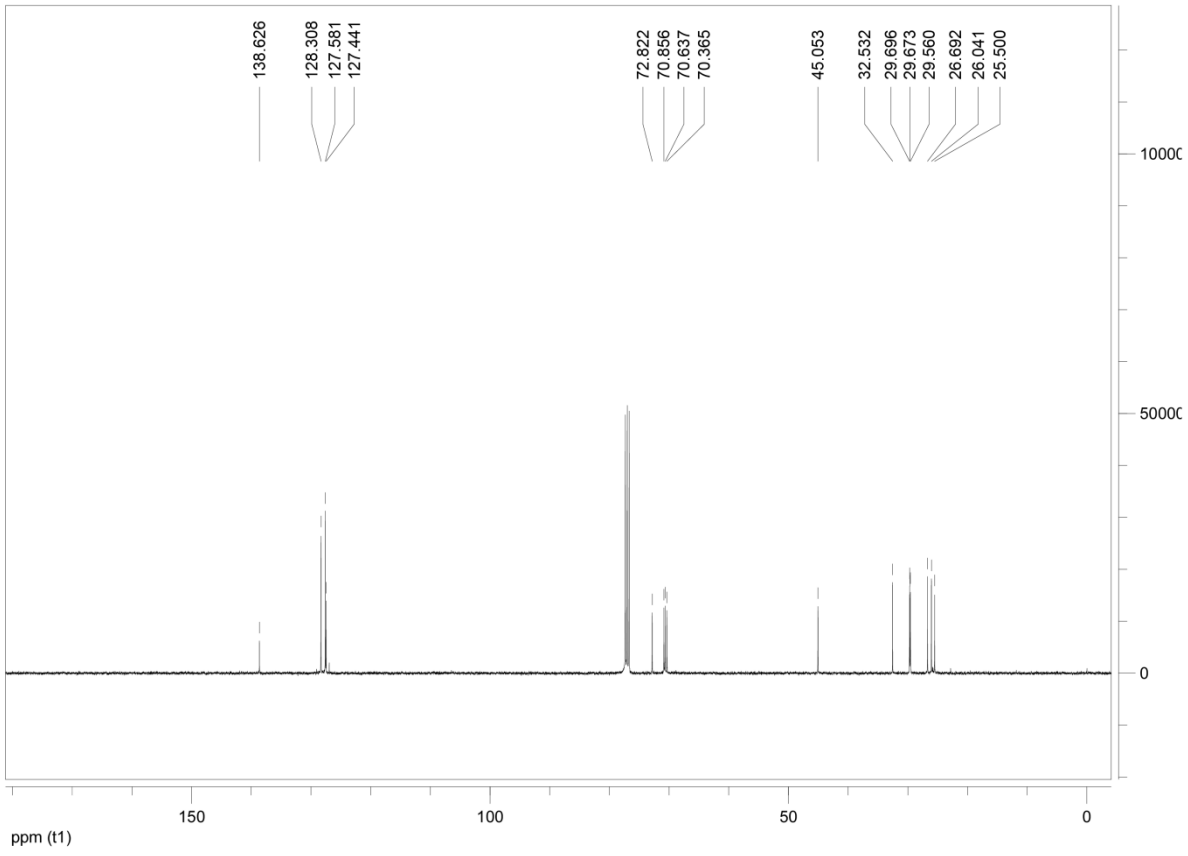
^{1}H and ^{13}C NMR spectrum of compound **59**.



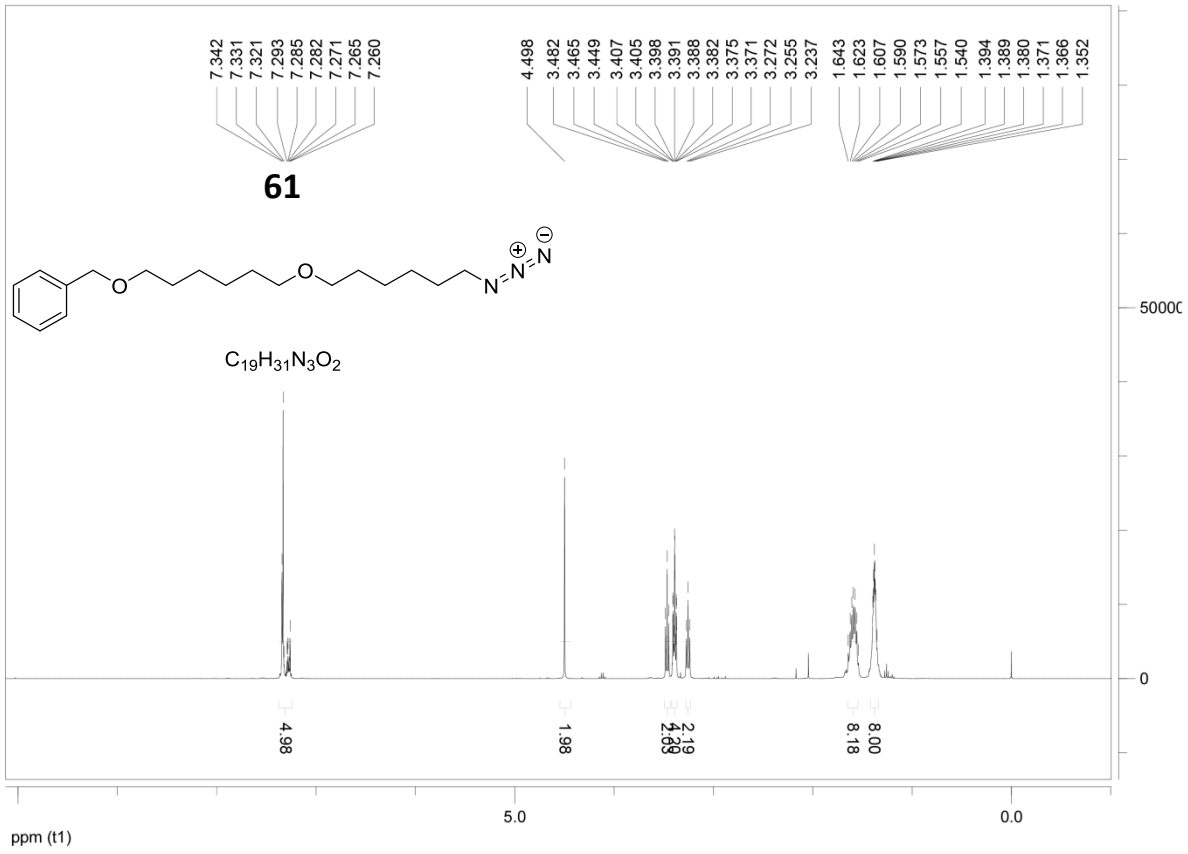


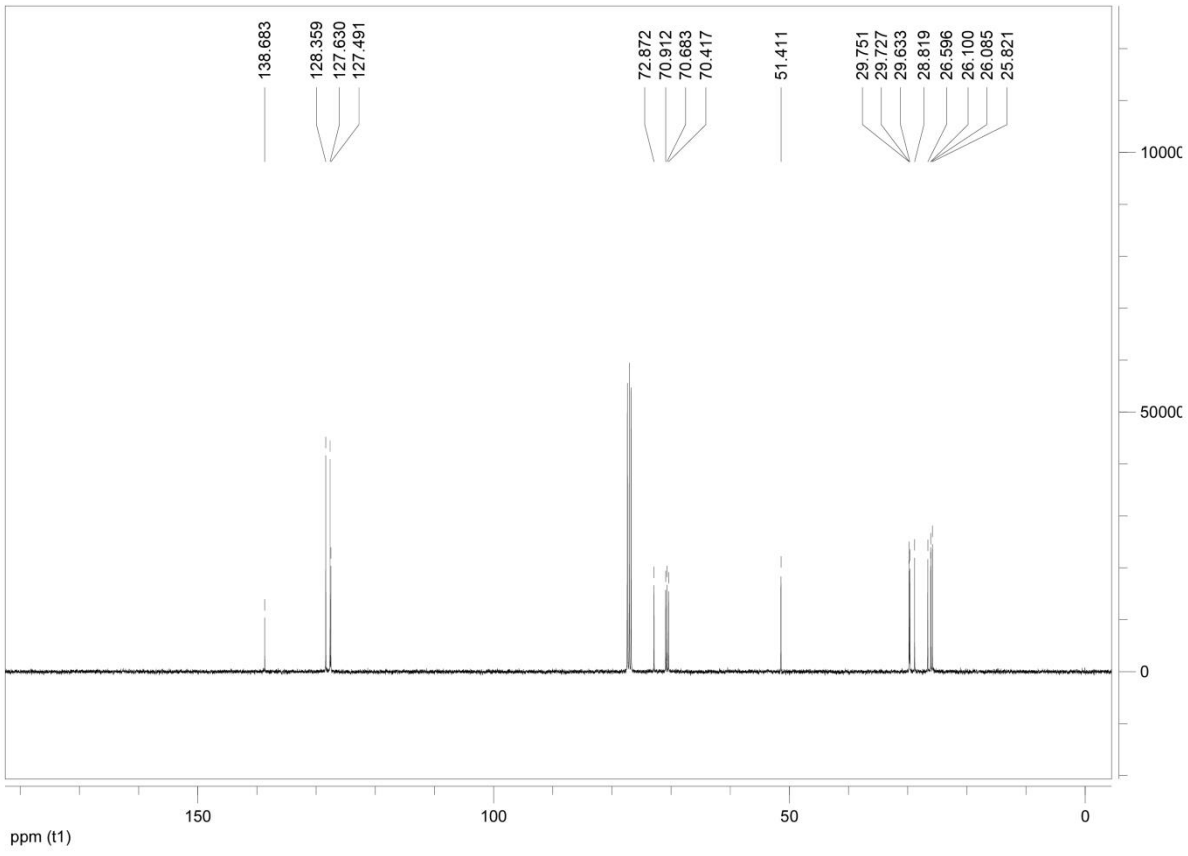
^{1}H and ^{13}C NMR spectrum of compound **60**.



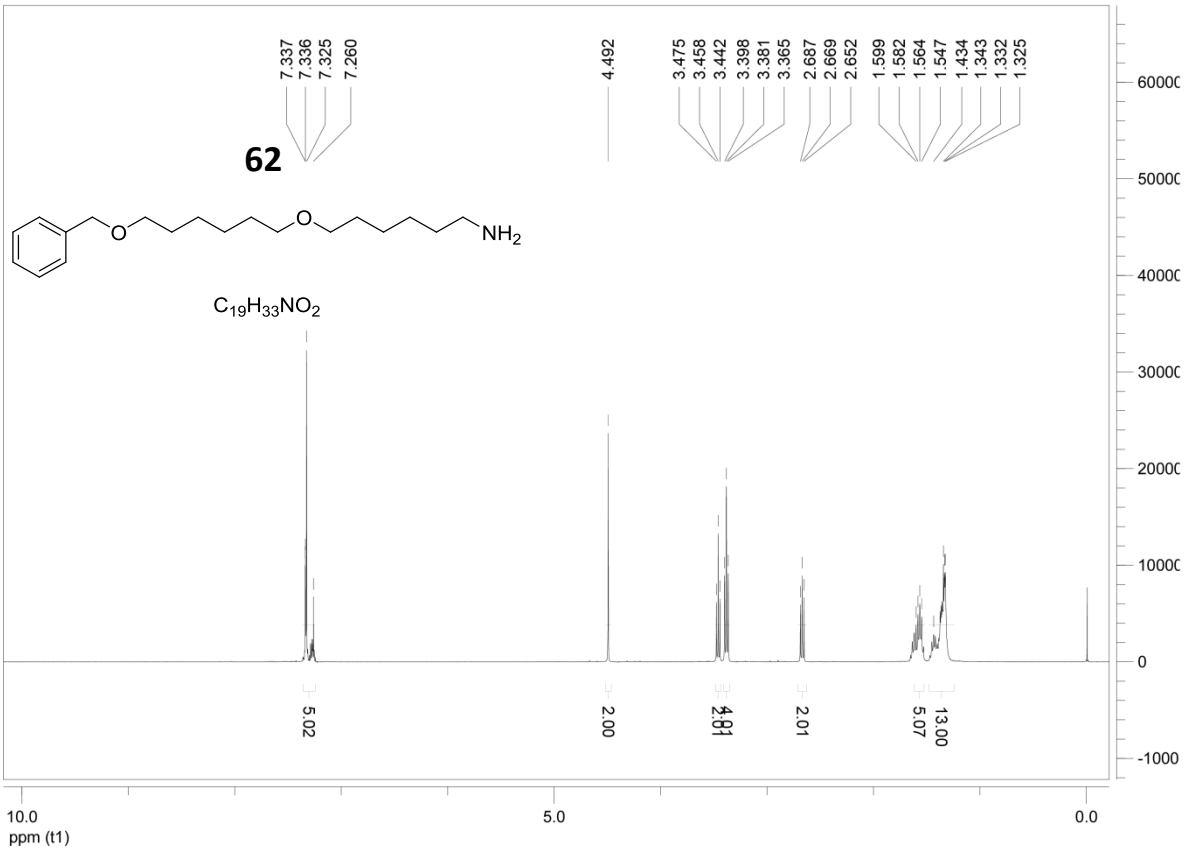


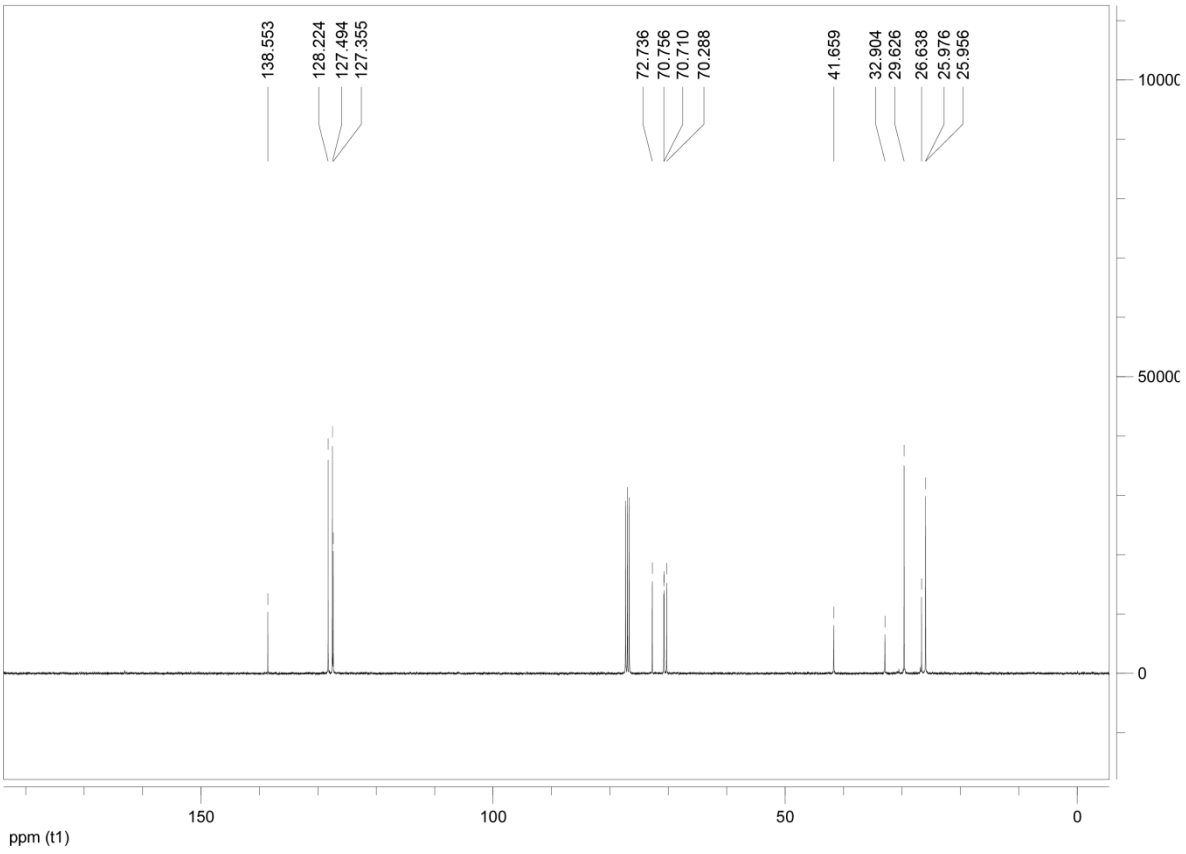
^{1}H and ^{13}C NMR spectrum of compound **61**.



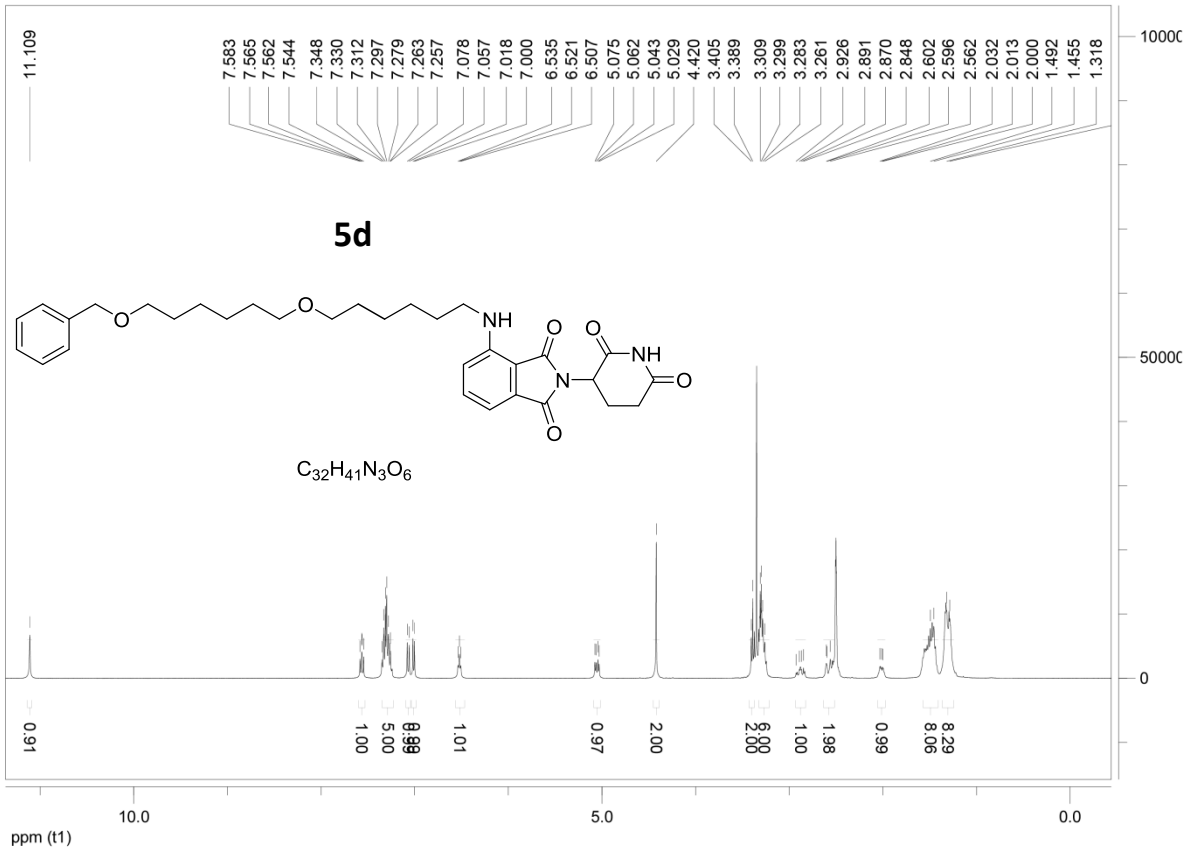


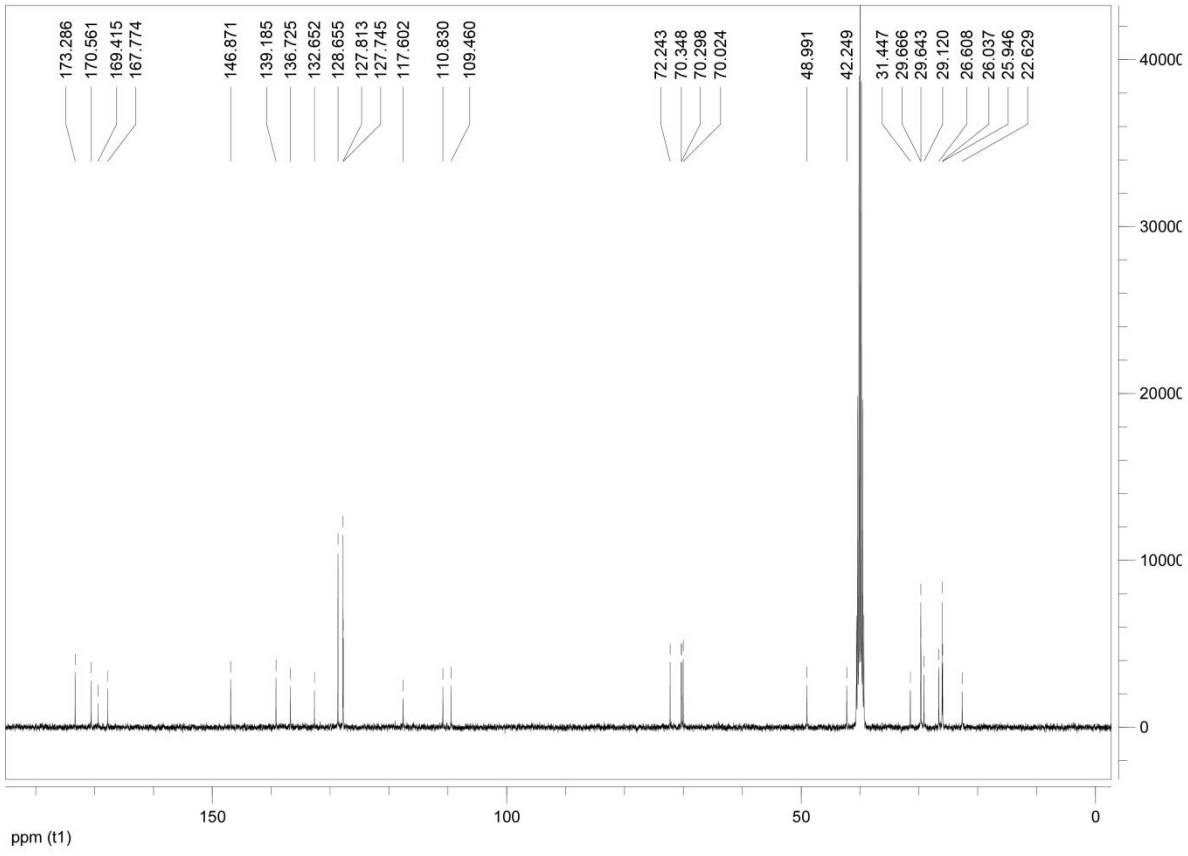
^{1}H and ^{13}C NMR spectrum of compound **62**.



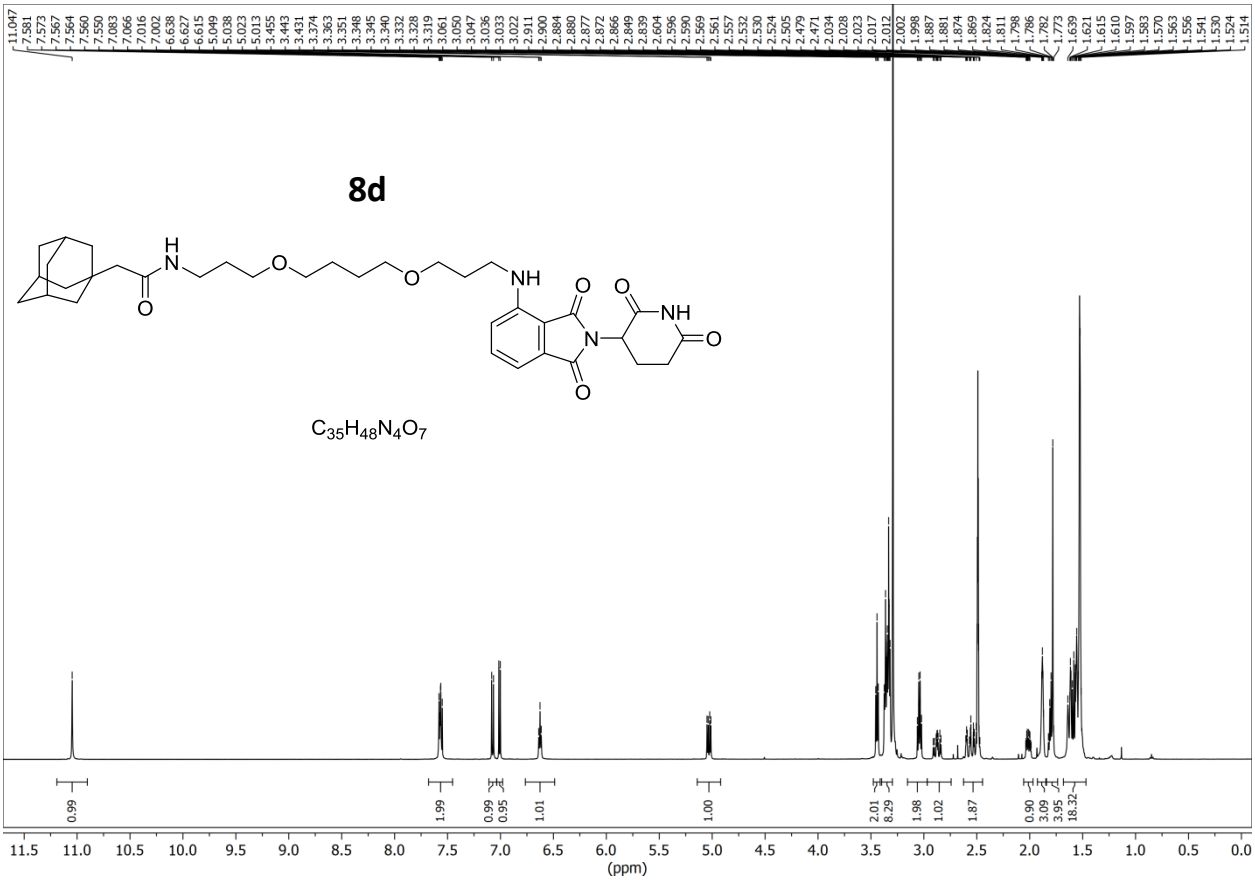


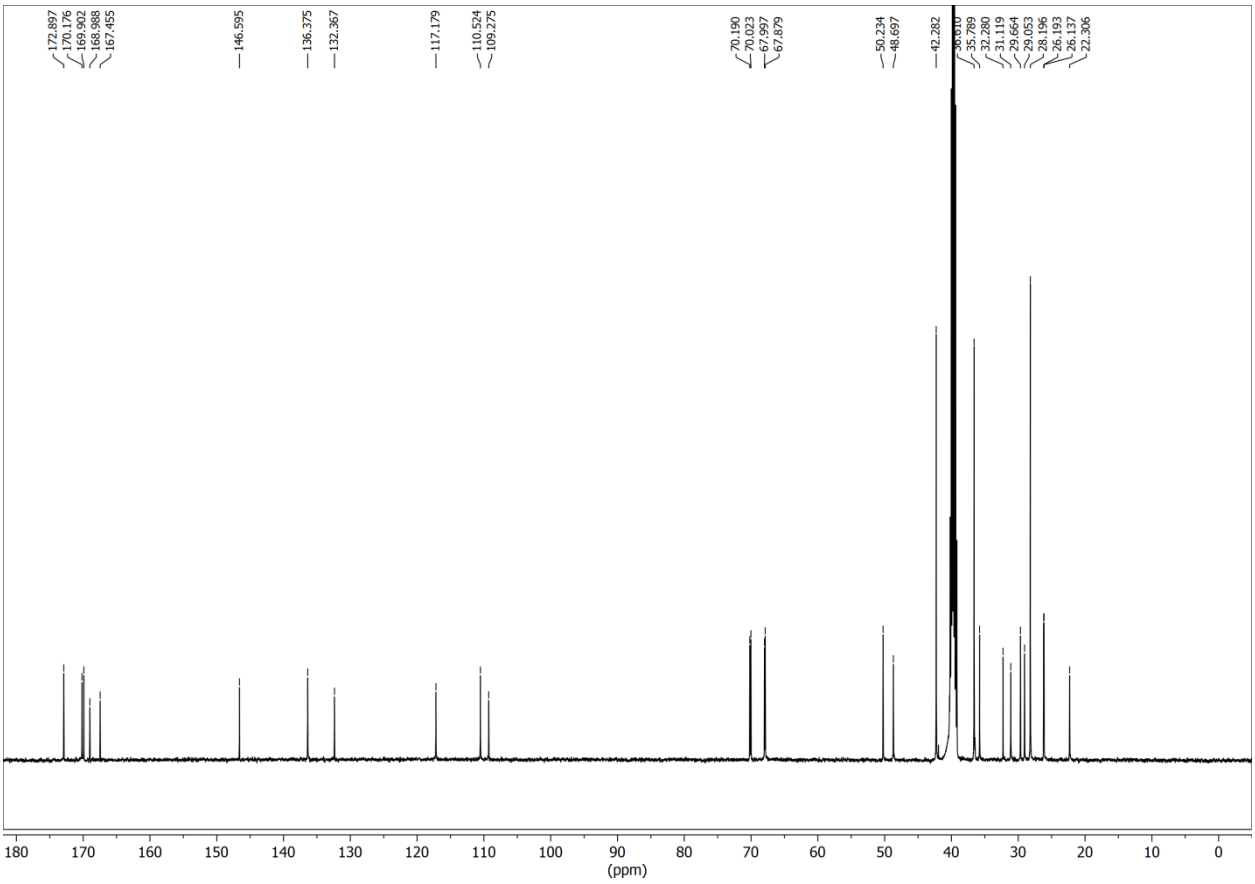
^{1}H and ^{13}C NMR spectrum of compound **5d**.





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





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