Degrading the key component of the inflammasome: Development of an NLRP3 PROTAC

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1. Supplementary Tables, Figures and Schemes

Table S1. Physicochemical and pharmacokinetic properties of NLRP3 PROTACs

Compd	Molecular mass	Linker		*e*log$D_{7.4}$[a]	PPB (%)[b]
		Atoms	Type		
V1	1014.29	6	alkyl	2.3	97
V2	1042.34	8	alkyl	2.5	97
V3	1046.29	8	PEG[c]	2.2	96
V4	1090.34	11	PEG	2.2	96
V5	1134.39	14	PEG	2.3	96
V6	1178.45	17	PEG	2.3	96
C1	828.96	6	alkyl	2.5	97
C2	857.01	8	alkyl	3.0	97
C3	860.96	8	PEG	2.1	97
C4	905.01	11	PEG	2.1	96
C5	949.06	14	PEG	2.2	96
C6	993.12	17	PEG	2.2	96
C7	1181.38	30	PEG[d]	2.6	95
I1	1053.35	6	alkyl	n.d.[e]	n.d.
I4	1129.40	11	PEG	2.3	n.d.
I5	1173.46	14	PEG	2.3	n.d.
I6	1217.51	17	PEG	2.4	n.d.

[a] Experimental distribution coefficient at pH 7.4.
[b] Experimentally determined percentage of compound bound to human serum albumin.
[c] Polyethylene glycol.
[d] Polyethylene glycol linker with a central C6 alkyl part.
[e] not determined.

Table S2. Results of docking NLRP3 and VCB using the ZDOCK server

model	residues to bind	prediction									
		1	2	3	4	5	6	7	8	9	10
		measured distance C25 (MCC950) - C15 (PROTAC JW48) in Å									
1	His360, Asp363, Leu621, Ile623, Asn686, Val707	18.3	25.0	32.7	21.5	16.0	20.4	27.3	69.4	24.6	20.0
2	His360	29.4	26.6	33.6	36.2	24.9	42.2	18.8	55.9	24.3	33.7
3	Asp363	29.4	57.5	36.2	26.6	45.7	42.3	25.6	43.2	33.6	41.5
4	Leu621	24.9	18.8	24.3	15.4	24.8	18.3	18.0	28.4	39.0	65.7
5	Ile623	18.8	18.3	18.0	39.0	65.7	19.9	13.8	25.0	15.5	70.0
6	Asn686	29.4	26.6	36.2	25.6	33.6	40.1	24.9	18.8	43.8	24.3
7	Val707	29.4	36.2	26.6	45.7	36.2	42.3	52.8	25.6	43.2	33.6

Table S3. Results for model 4 prediction 4

mode	affinity	distance from best mode	
	(kcal/mol)	rmsd l.b.[a]	rmsd u.b.[b]
1	-11.1	0.000	0.000
2	-11.1	5.533	11.860
3	-11.1	23.999	29.198
4	-11.0	7.288	16.107
5	-11.0	7.663	16.139
6	-11.0	24.593	29.047
7	-11.0	8.458	15.049
8	-10.9	5.244	7.637
9	-10.9	7.457	13.194
10	-10.9	5.558	6.942
11	-10.9	5.535	12.399
12	-10.8	8.979	15.818
13	-10.8	8.372	14.638
14	-10.8	8.467	18.232
15	-10.8	5.543	7.031
16	-10.8	3.712	6.747
17	-10.7	8.331	14.273
18	-10.7	4.651	8.457
19	-10.7	8.318	14.820
20	-10.7	4.742	10.219

[a] root mean square deviation lower bound
[b] root mean square deviation upper bound

Table S4. Results for model 5 prediction 7

mode	affinity	Distance from best mode	
	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-12.6	0.000	0.000
2	-12.2	6.255	14.070
3	-12.0	5.919	13.493
4	-11.9	7.550	12.800
5	-11.9	3.613	7.038
6	-11.8	13.451	18.146
7	-11.7	8.078	14.138
8	-11.7	6.426	13.065
9	-11.7	6.289	16.121
10	-11.6	7.743	13.021
11	-11.6	12.798	17.596
12	-11.6	11.191	17.105
13	-11.6	11.560	15.791
14	-11.6	5.947	13.552
15	-11.5	9.868	15.375
16	-11.5	15.376	21.153
17	-11.5	7.540	13.017
18	-11.5	13.036	17.559
19	-11.4	9.353	14.725
20	-11.4	5.827	14.616

Table S5. Results for model 5 prediction 9

mode	affinity	dist from best mode	
	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-10.5	0.000	0.000
2	-10.4	11.743	17.593
3	-10.4	0.957	2.020
4	-10.3	10.369	14.547
5	-10.2	11.232	16.958
6	-10.2	9.011	13.439
7	-10.1	11.343	16.550
8	-10.1	11.169	15.384
9	-10.1	11.619	15.994
10	-10.0	7.090	11.631
11	-10.0	8.592	12.751
12	-10.0	6.893	9.828
13	-10.0	1.585	2.769
14	-9.9	7.770	12.503
15	-9.9	10.421	14.427
16	-9.9	10.392	15.819
17	-9.9	7.157	11.803
18	-9.9	9.137	13.634
19	-9.9	9.454	13.926
20	-9.9	11.705	14.992

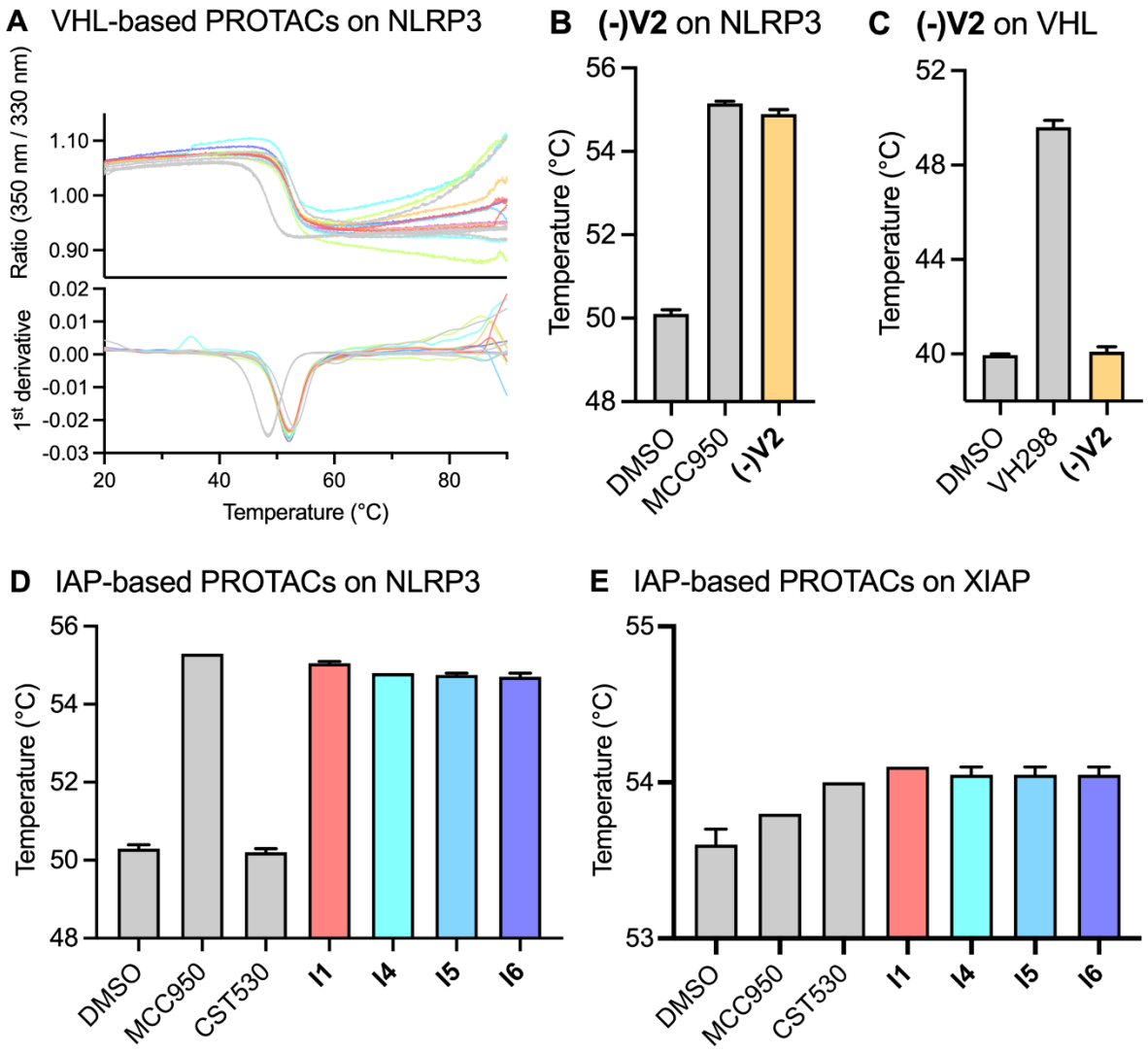


Figure S1. Thermal stability studies of NLRP3 and E3 ligases upon treatment with test compounds. Shown are the ratio between 350 nm and 330 nm and its first derivative for all PROTAC candidates of the **V** series (**A**). The thermal stability of NLRP3 or VHL, respectively, upon treatment with the negative control **(-)V2** (**B**, **C**) as well as of NLRP3 (**D**) and XIAP (X-linked inhibitor of apoptosis, glutathione *S*-transferase-tagged, **E**) treated with IAP-addressing compounds CST530, **I1**, **I4**-**I6** is depicted (**D**, **E**). Graphs show mean + SEM (n = 2). Linker types are color-coded.

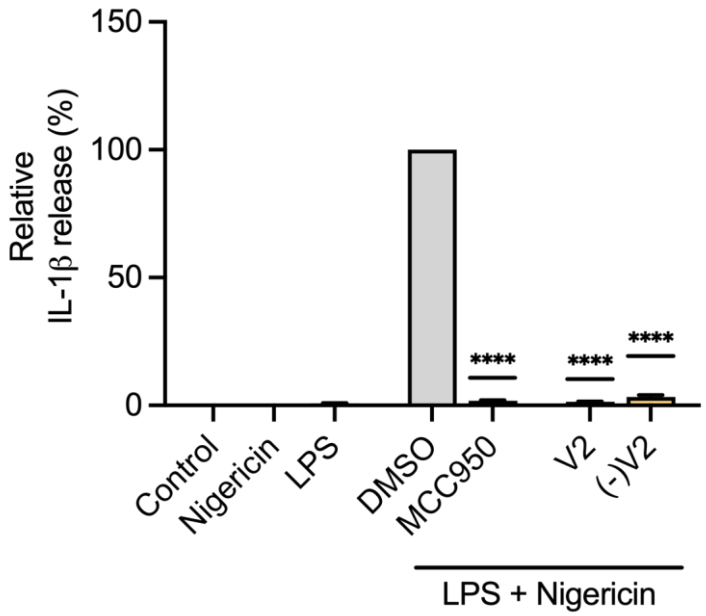


Figure S2. Inhibition of IL-1β release from THP-1 macrophages upon treatment with **V2**, **(-)V2**, and MCC950. THP-1 macrophages were primed with LPS (100 ng/mL) for 3 h. NLRP3 PROTAC **V2** and the chemical negative control **(-)V2** (10 μM) or NLRP3 inhibitor MCC950 (10 μM) were added 1 h before incubation with nigericin (10 μM) for 3 h. DMSO was used as vehicle control. IL-1β release into cell culture supernatants was determined by ELISA. Nigericin-induced IL-1β release of LPS-primed cells was set to 100%, and all other values were calculated accordingly. Bar graphs show mean + SEM (n = 4). One-sample t-test against 100%. ****$P \leq 0.0001$.

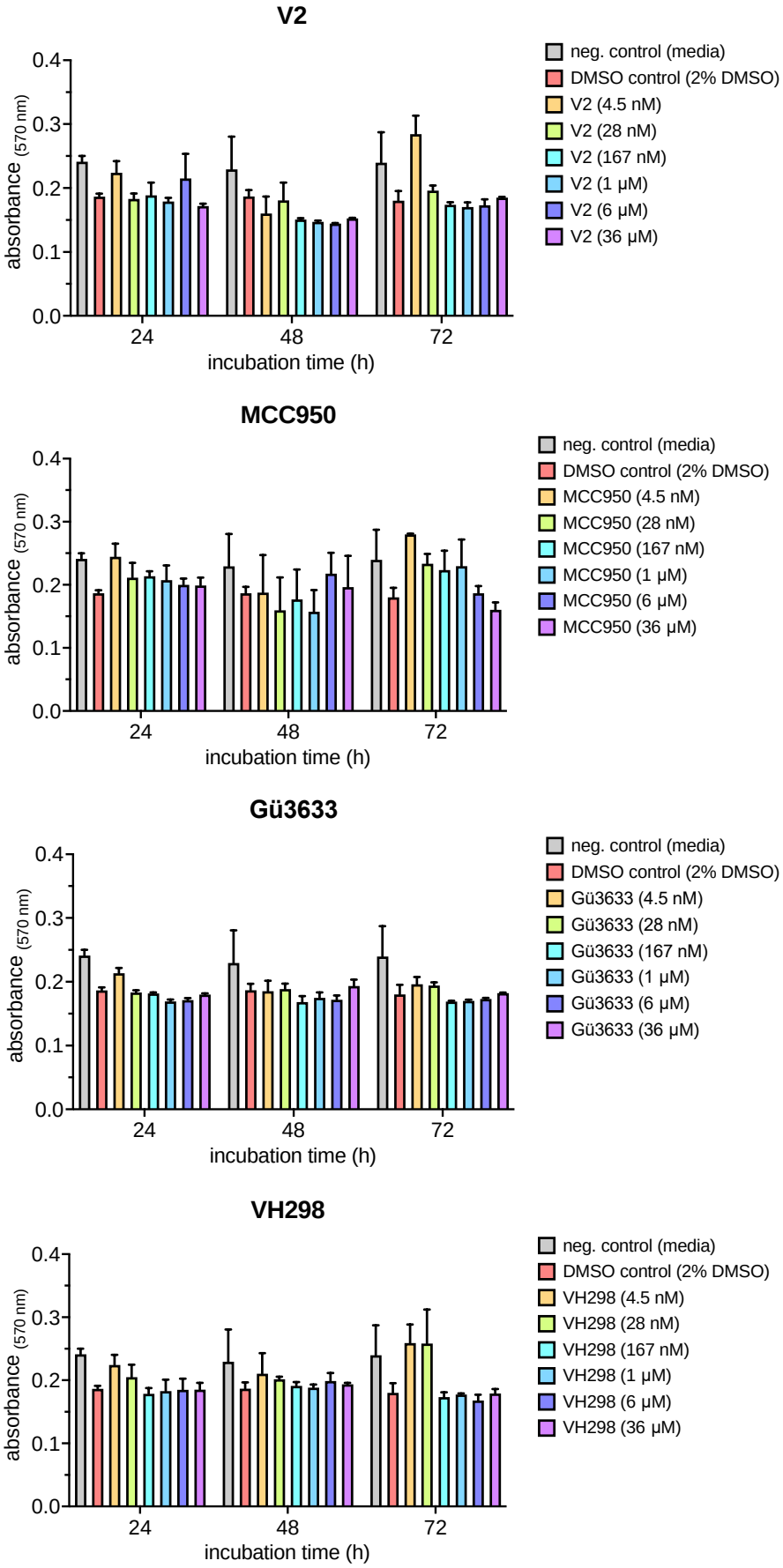


Figure S3. MTT viability assay. THP-1 monocytes were incubated for 24, 48 or 72 hours with increasing concentrations of PROTAC **V2**, NLRP3 ligands MCC950 and Gü3633, as well as VHL ligand VH298. Bar graphs show mean absorbance at 570 nm + SEM (n = 3). Concentrations are color-coded.

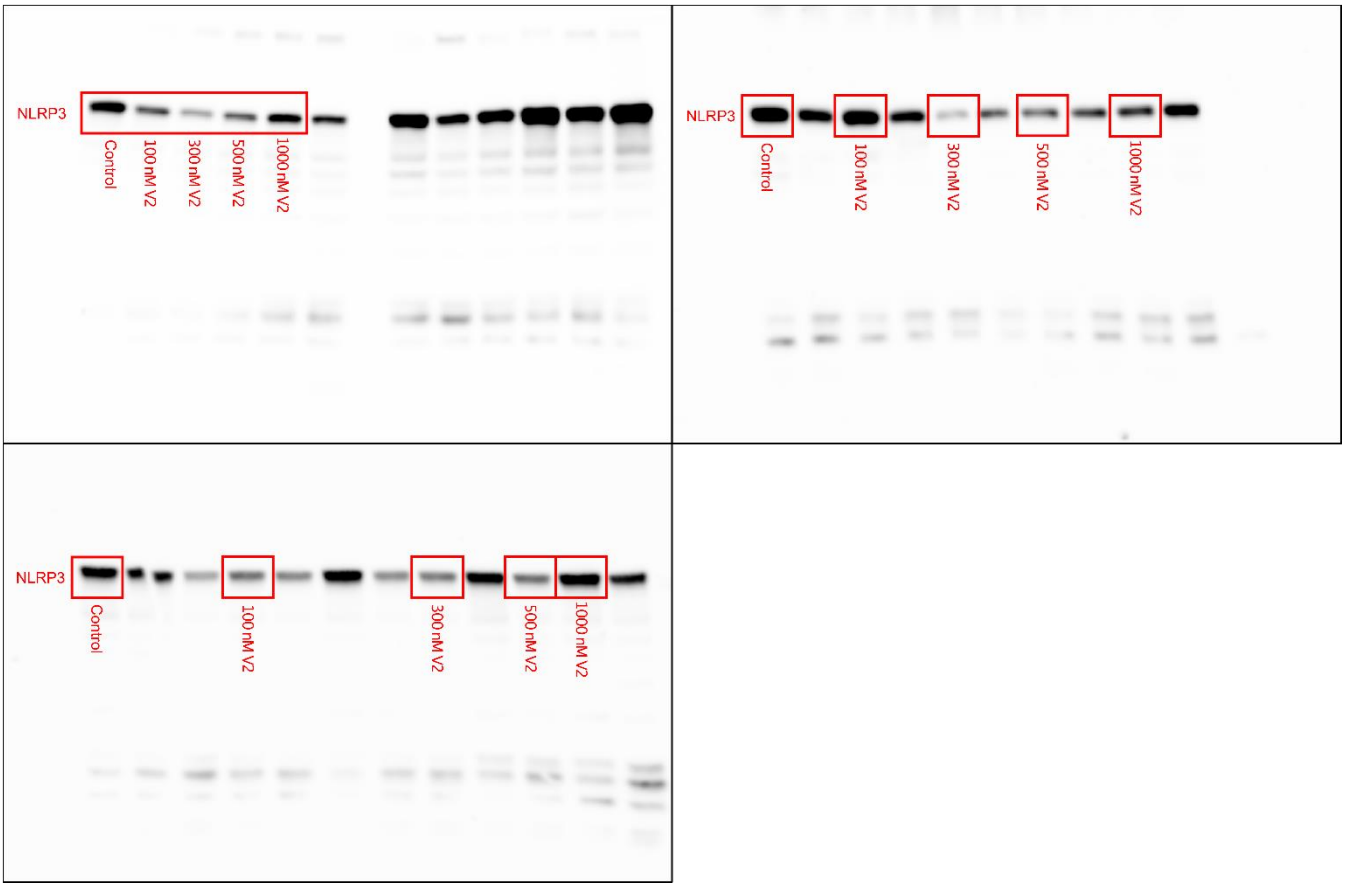


Figure S4. Western blot analyses of the **V2**-induced NLRP3 degradation. Original uncropped images are shown. THP-1 monocytes were incubated with increasing concentrations of **V2** for 18 h. NLRP3 protein level was determined by western blot analysis and normalised to the respective total protein amount.

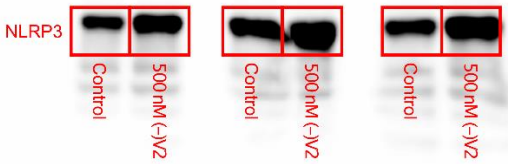


Figure S5. Western blot analyses of NLRP3 after treatment with the negative control **(-)V2**. THP-1 monocytes were incubated with 500 nM **(-)V2** for 18 h. NLRP3 protein level was determined by western blot analysis and normalised to the respective total protein amount. Original uncropped images are shown.

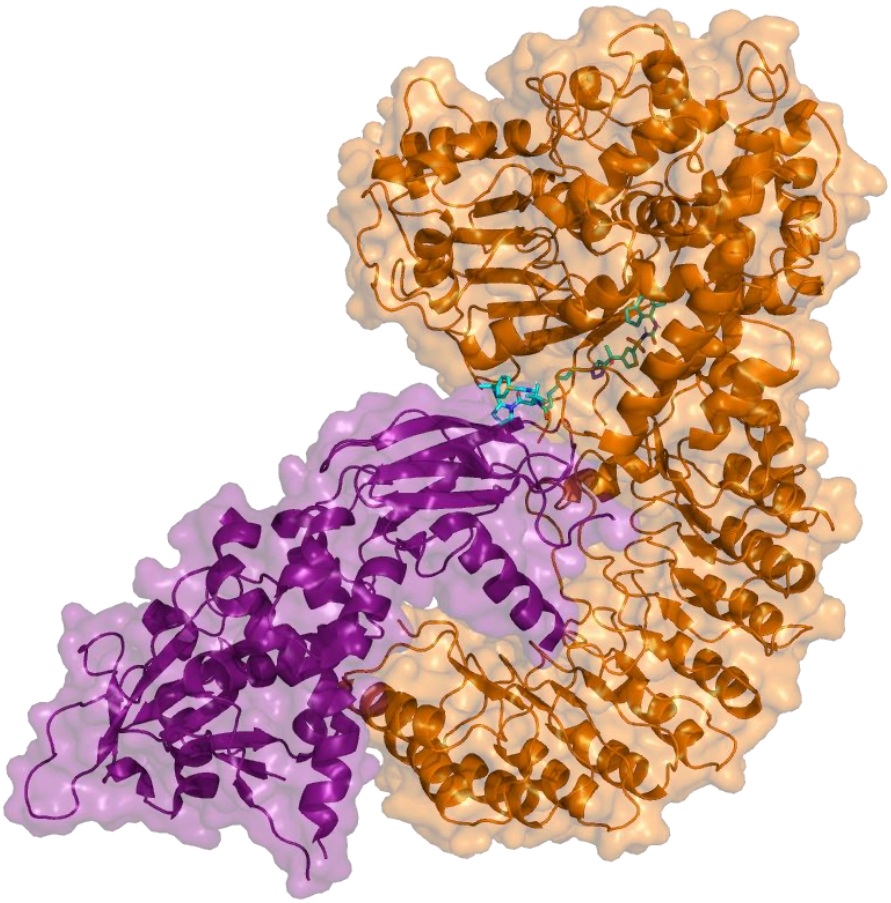


Figure S6. Molecular docking of the ternary complex formed by NLRP3 (orange), PROTAC **V2** (cyan) and VCB (purple). Based on the cryo-EM structure of the NLRP3 decamer binding MCC950 (PDB ID: 7PZC) and the crystal structure of VCB binding a VH298-based PROTAC (PDB ID: 8C13) the ternary NLRP3:**V2**:VCB complex was docked using the ZDOCK server and the AutoDock Vina software.

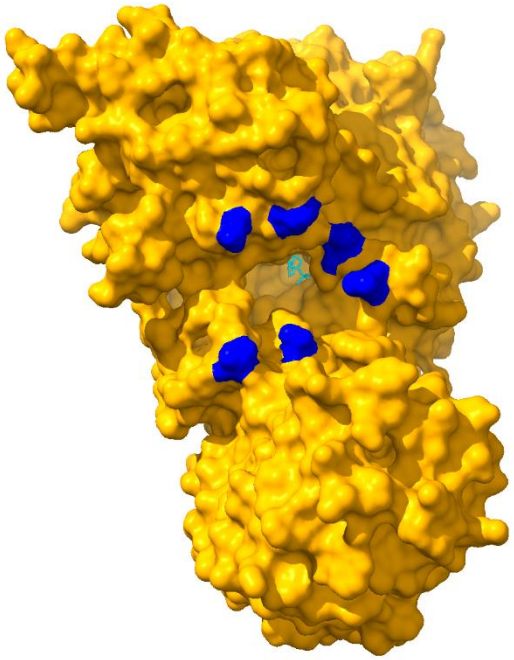


Figure S7. Surface representation of NLRP3 (orange) bound to MCC950 (cyan) (PDB ID: 7PZC). Highlighted in blue are six amino acid residues (His360, Asp363, Leu621, Ile623, Asn686, Val707) that could form part of the binding interface between NLRP3 and VHL.

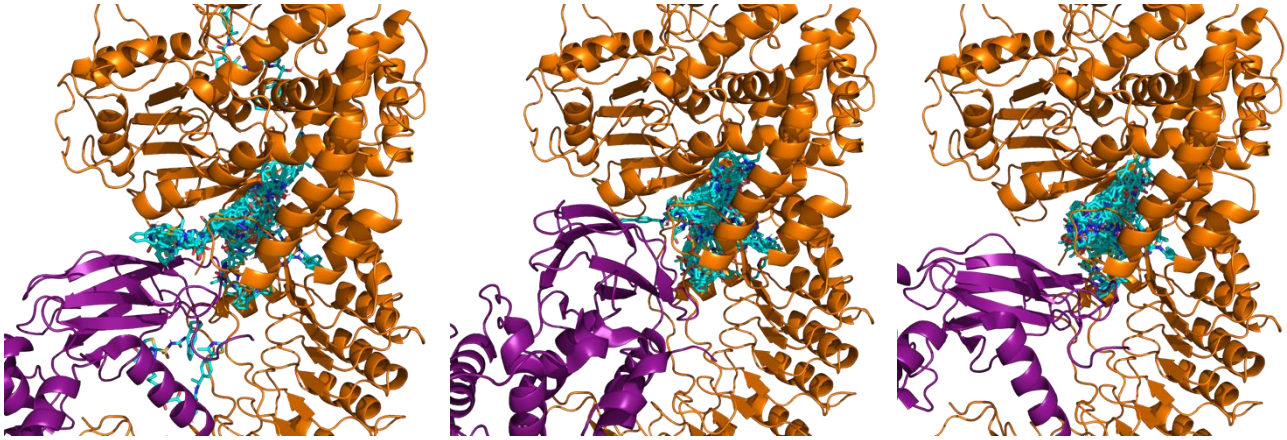
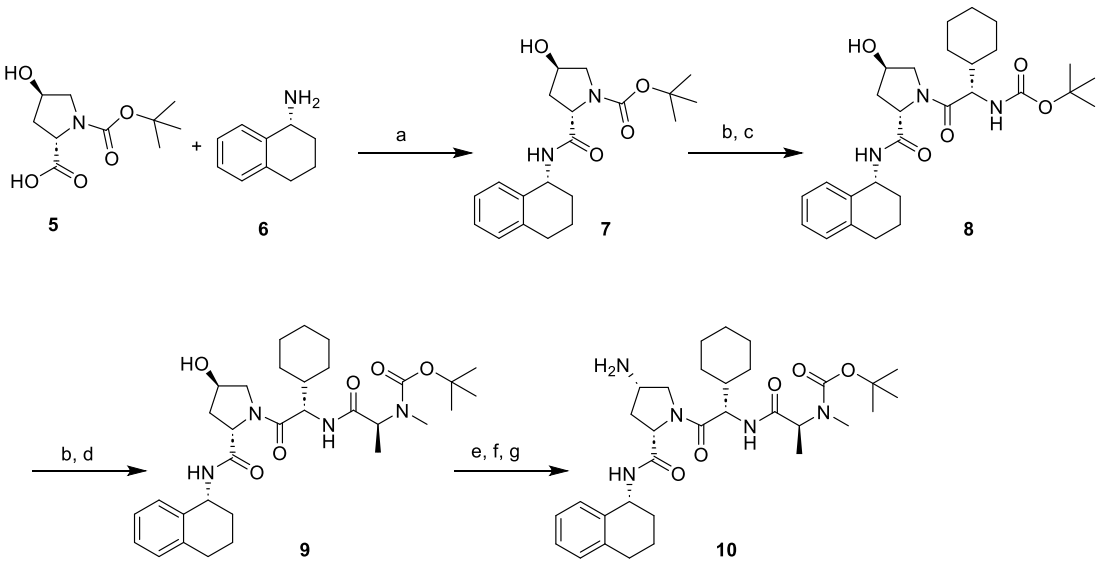
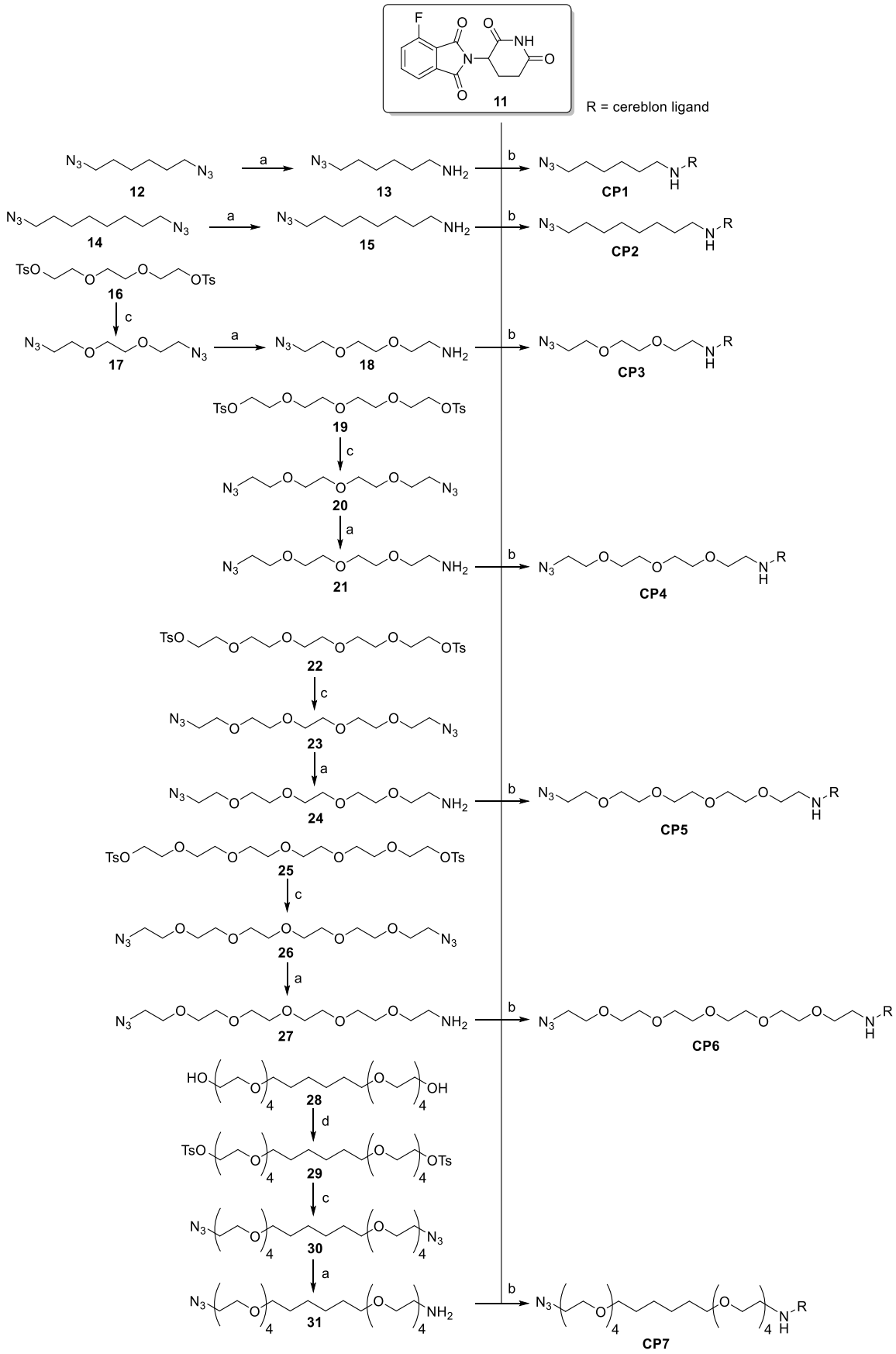


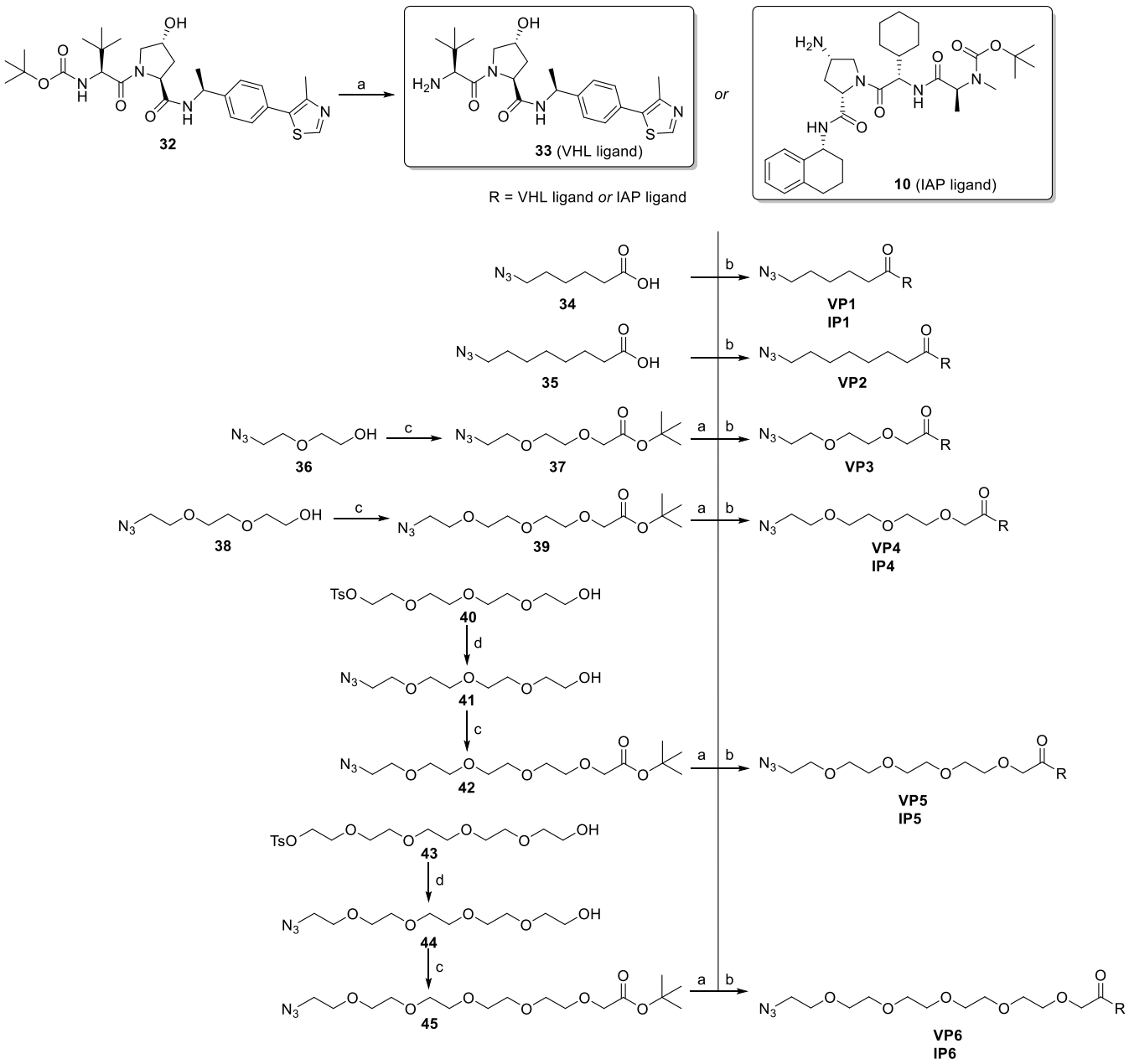
Figure S8. Representation of all calculated modes from AutoDock Vina (cyan) using three different docking results for the protein-protein interaction of NLRP3 (orange) and VCB (purple). Left: model 4 prediction 4, middle: model 5 prediction 7, right: model 5 prediction 9.



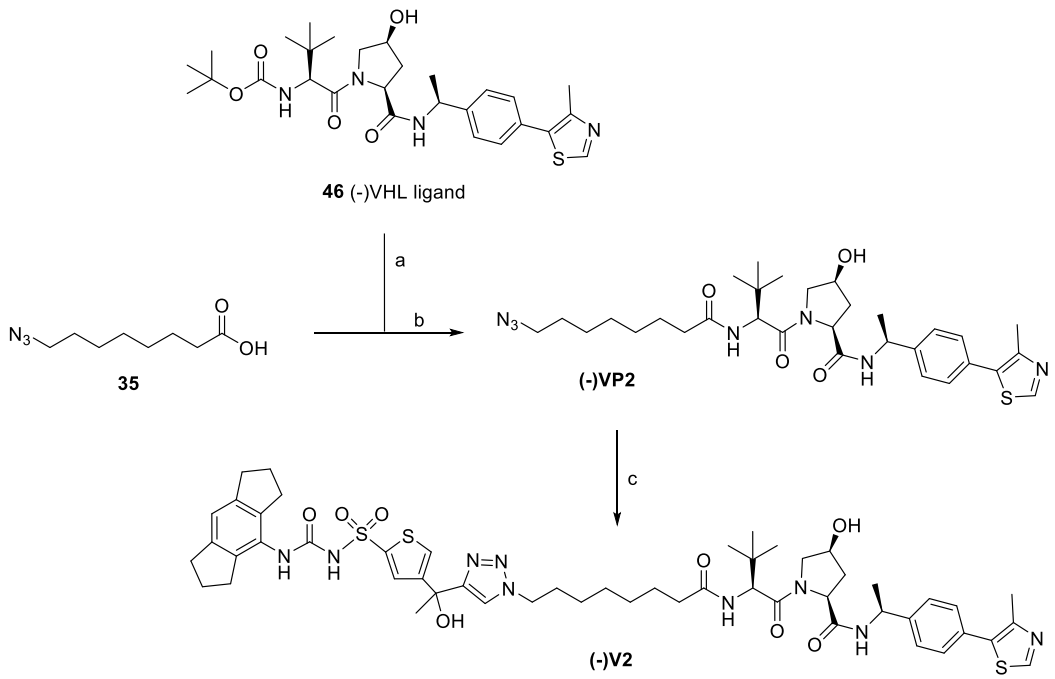
Scheme S1. Synthesis of IAP ligand **10**. Reagents: a) HOBt, TEA, EDC × HCl, CH_2Cl_2; b) 1 N HCl in EtOAc; c) Boc-Chg-OH, HOBt, DIPEA, EDC × HCl, DMF; d) Boc-*N*-Me-L-Ala-OH, HOBt, DIPEA, EDC × HCl, DMF; e) TEA, MsCl, CH_2Cl_2; f) NaN_3, DMF; g) PPh_3, THF, 30% NH_3 in H_2O.



Scheme S2. Synthesis of the precursors **CP1**-**CP7** for the cereblon-addressing compounds **C1**-**C7**. Reagents: a) PPh_3, THF, H_2O; b) DIPEA, DMF; c) NaN_3, DMF; d) TEA, TsCl, CH_2Cl_2.



Scheme S3. Synthesis of the precursors **VP1**-**VP6** and **IP1**, **IP4**-**IP6** for the VHL-addressing compounds **V1**-**V6** and the IAP-addressing compounds **I1, I4-I6**. Reagents: a) TFA, CH_2Cl_2; b) DIPEA, HATU, DMF; c) TBAHS, *tert*-butyl bromoacetate, 9.5 M NaOH (aq), toluene; d) NaN_3, DMF.



Scheme S4. Preparation of the precursor **(-)VP2** for the negative control compound **(-)V2**. Reagents: a) TFA, CH_2Cl_2; b) DIPEA, HATU, DMF; c) NLPR3 ligand **4**, $CuSO_4 \times 5\ H_2O$, sodium L-ascorbate, THF, H_2O.

2. Determination of Physicochemical Properties

2.1. Determination of *e*log$D_{7.4}$ values

The determination of the *e*log$D_{7.4}$ values was performed by a chromatographic method similar to previous reports,[1,2] using a Jasco 2000 Series HPLC. The system was calibrated by plotting the retention times of six different reference substances (atenolol, metoprolol, labetalol, diltiazem, triphenylene, permethrin) *versus* their known distribution coefficient values at pH 7.4 from literature[2,3] to obtain a calibration line ($R^2 \geq 0.98$). Subsequently, the mean retention times (n = 2) of the analytes were taken to calculate their *e*log$D_{7.4}$ values with aid of the calibration line.

2.2. Plasma protein binding studies

Plasma protein binding was estimated by correlating the logarithmic retention times of the analytes on a CHIRALPAK HSA 50 × 3 mm, 5 µm column with the literature known PPB values (converted into logK values) of ten drugs (warfarin, ketoprofen, budesonide, nizatidine, indomethacin, acetylsalicylic acid, carbamazepine, piroxicam, nicardipine, cimetidine).[4] Samples were dissolved in MeCN/DMSO 9:1 to achieve a final concentration of 0.5 mg/mL. The mobile phase A was 50 mM ammonium acetate adjusted to pH 7.4 with aqueous ammonia, while mobile phase B was *i*PrOH. The flow rate was set to 1.0 mL/min, the UV detector was set to 254 nm, and the column temperature was kept at 30 °C. After injecting 2 or 3 µL of the sample, a linear gradient from 100% A to 30% *i*PrOH in 5.4 min was applied. From 5.4 to 18 min, 30% *i*PrOH was kept, followed by switching back to 100% A in 1.0 min and a re-equilibration time of 6 min. With the aid of the calibration line ($R^2 = 0.96$), the logK values of new substances were calculated and converted to their PPB values. At least two independent measurements of each analyte were performed.

3. Biophysical Evaluation of Binary Complexes

3.1. Protein expression and purification

3.1.1. Expression of VCB and XIAP

The recombinant plasmids pET28a-His-tev-VHL (54-213) and pET-DUET-EloB(1-104)-EloC(17-112) or pET28a-GST-tev-XIAP (1-497), respectively, were transformed into *E. coli BL21* cells following a standard heat-shock protocol (incubation with plasmid on ice for 15 min, heat-shock at 42 °C for 45 s and growth in LB-medium at 37 °C for 1 h). A volume of 40 mL of LB-medium (supplemented with kanamycin, 50 µg/mL) was inoculated with the transformed cells and incubated at 37 °C over-night to OD_{600} = 4.0 – 4.4. With this pre-culture, a volume of 1 L LB-medium (supplemented with kanamycin, 50 µg/mL) was inoculated and cultured at 37 °C to OD_{600} = 0.8 – 1.0. Expression was induced by 0.4 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 18 °C for 18 – 20 h. Cells were harvested by centrifugation at 2000×*g* at 4 °C for 20 min (in an Avanti JXN-26 centrifuge, Beckman Coulter, Brea, USA). The pellets were resuspended in 25 mL PBS each, transferred into a 50-mL-Falcon and centrifuged again at 4000×*g* at 4 °C for 10 min (in an Eppendorf centrifuge 5804 R, Eppendorf, Hamburg, Germany). The pellet was snap-frozen in liquid nitrogen and stored at -80 °C until purification.

3.1.2. Purification of VCB

All steps were carried out on ice. The cell pellets were resuspended in 25 mL lysis buffer (20 mM Tris-HCl, 500 mM NaCl, 10 mM imidazole, 1 mM DTT, pH = 8.0) each and to the total volume DNase (1:1000), PMSF (1:100), EDTA free protease inhibitor mix and a spatula tip lysozyme were added. After complete resuspension, the cells were lysed by sonication 4 × 3 min (10 s pulse, 5 s pause) with an amplitude of 40% (Sonopuls mini, BANDELIN electronic, Berlin, Germany). Subsequently, the lysed cells were centrifuged (70 000×*g* at 4 °C for 45 min in an Avanti JXN-26 centrifuge, Beckman Coulter, Brea, USA). After filtration with 0.45 µm syringe filters (Sarstedt, Nümbrecht, Germany), the clear supernatant was applied at a flow rate of 1.5 mL/min on a HisTrap HP 5-mL-column (cytiva, Marlborough, USA) equilibrated in lysis buffer coupled to an ÄKTA start chromatography system (GE Healthcare, Uppsala, Sweden). The column was washed with lysis buffer at a flow rate of 1.5 mL/min (10 column volumes (CV)) and the protein was eluted with a linear gradient starting from 100% lysis buffer and 0% elution buffer (20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, 1 mM DTT, pH = 8.0) up to 0% lysis buffer and 100% elution buffer (≈ 10 mM imidazole to 500 mM) at a flow rate of 2.0 mL/min.

The protein containing elution fractions were pooled and concentrated to a total volume of 1 mL using Amicon Ultra 50 mL centrifugal filters (Merck Millipore, Carrigtwohill, Ireland) with a 3 kDa molecular weight cut-off by centrifuging several times at 3000×*g* at 4 °C for 10 min (in an Eppendorf centrifuge 5804 R, Eppendorf, Hamburg, Germany). To cleave the His-tag from the protein, tobacco etch virus protease (TEV, 3 mg/mL, 1:50) was added

to the protein solution, which was then incubated over night at 4 °C while dialysing it against 2 L of lysis buffer. Next, a reverse Ni^{2+}-affinity chromatography was performed by applying the TEV-digested protein solution at a flow rate of 1.5 mL/min on a HisTrap HP 5-mL-column (cytiva, Marlborough, USA) equilibrated in lysis buffer coupled to an ÄKTA start chromatography system (GE Healthcare, Uppsala, Sweden). The flow-through was collected (2 mL fractions) and the protein containing flow-through fractions were pooled and concentrated to a total volume of 4.5 mL as described before. The concentrated protein solution was loaded at a flow rate of 0.8 mL/min on a HiLoad 16/600 Superdex 75 pg column (cytiva, Marlborough, USA) equilibrated in SEC-buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, pH = 7.0). The SEC (size-exclusion chromatography) was performed at a flow rate of 0.8 mL/min for 1.2 column volumes and fractions of 2 mL were collected. The protein containing fractions were analysed via SDS-PAGE, pooled and concentrated as described above. The concentration of the protein solution was determined using a NanoDrop 2000 (Thermo Scientific, Rockford, USA). The purified protein was aliquoted, snap frozen in liquid nitrogen and stored at -80 °C.

3.1.3. Purification of XIAP

All steps were carried out on ice. The cell pellets were resuspended in 25 mL lysis buffer (25 mM HEPES, 150 mM NaCl, 1 mM TCEP) each and to the total volume DNase (1:1000), PMSF (1:100) and a spatula tip lysozyme were added. After complete resuspension, the cells were lysed by sonication 2 × 5 min (10 s pulse, 5 s pause) with an amplitude of 40% (Sonopuls mini, BANDELIN electronic, Berlin, Germany). Subsequently, the lysed cells were centrifuged as described before. The cleared lysate was applied onto glutathione-agarose (Thermo Scientific, Rockford, USA) equilibrated in lysis buffer. After incubation at 4 °C for 90 min under constant rotation, the protein-bound agarose was packed into a gravity flow column, which was washed two times with 10 CV lysis buffer, before the protein was eluted with 10 CV elution buffer (15 mM glutathione in lysis buffer). The protein containing elution fractions were pooled and concentrated to a total volume of 500 µL as described before (30 kDa molecular weight cut-off). The concentrated protein solution was loaded at a flow rate of 0.5 mL/min on a Superdex 200 Increase 10/300 GL column (cytiva, Marlborough, USA) equilibrated in lysis buffer. The SEC was performed at a flow rate of 0.5 mL/min for 1.2 column volumes and fractions of 500 µL were collected. The protein containing fractions were analysed via SDS-PAGE, pooled, concentrated and stored as described before.

3.1.4. Expression in Sf9 insect cells

His-tagged CRBN (1-442) together with DDB1ΔB (2-395-GNGNSG-706-1140) or MBP-tagged NLRP3 (131-694) were recombinantly expressed in Sf9 insect cells. Therefore, a suspension culture of Sf9 insect cells (1.5×10^6 cells/mL) was infected with 3% (v/v) of baculovirus (V_2) and cultured for 72 h. The cells were harvested by centrifugation at 1,000 × *g* at 4 °C for 20 min (in an Avanti JXN-26 centrifuge, Beckman Coulter GmbH) and washed with cold PBS. Finally, the cell pellets were snap-frozen in liquid nitrogen and stored at -80 °C until purification.

3.1.5. Purification of CRBN/DDB1ΔB

All steps were carried out on ice. The pellets were resuspended in 45 mL lysis buffer (50 mM TRIS-HCl, 200 mM NaCl, 20 mM imidazole, 1 mM DTT, pH 8.0) each and to the total volume DNase (1:1000) and PMSF (1:100) were added. After complete resuspension, the cells were lysed by sonication 4 × 3 min (10 s pulse, 5 s pause) with an amplitude of 40% (Sonopuls mini, BANDELIN electronic GmbH & Co. KG). The lysate was cleared and filtered as described before. The clear supernatant was applied at a flow rate of 3.0 mL/min on a HisTrap FF crude 5-mL-column (cytiva, Marlborough, USA) equilibrated in lysis buffer coupled to an ÄKTA start chromatography system (GE Healthcare, Uppsala, Sweden). The column was washed with 95% lysis buffer and 5% elution buffer (lysis buffer with 1 M imidazole) at a flow rate of 3 mL/min (10 CV) and the protein was eluted with 75% lysis buffer and 25% elution buffer at a flow rate of 3.0 mL/min. The protein containing elution fractions were pooled and concentrated to a total volume of 5 mL as described before (30 kDa molecular weight cut-off).

The concentrated protein solution was diluted in 45 mL AIEX low buffer (50 mM TRIS-HCl, 1 mM DTT, pH 8.0) and applied at a flow rate of 2 mL/min on two HiTrap Q XL 5-mL-columns (Cytiva) equilibrated in AIEX low buffer. The protein was eluted by applying a linear gradient from 0 mM to 700 mM NaCl over a volume of 60 mL at a flow rate of 2 mL/min (AIEX high buffer: 50 mM TRIS-HCl, 1 M NaCl, 1 mM DTT, pH 8.0). The protein containing elution fractions (each 3 mL) were pooled and concentrated to a total volume of 5 ml as described before. For SEC, the concentrated protein solution was loaded at a flow rate of 0.8 mL/min on a HiLoad 16/600 Superdex 200 pg column (Cytiva) equilibrated in SEC-buffer (50 mM HEPES, 200 mM NaCl, 1 mM DTT, pH 7.4). The SEC was performed at a flow rate of 0.8 mL/min for 1.2 column volumes and fractions of 2 mL were collected. The protein containing fractions were analyzed via SDS-PAGE, pooled, concentrated and stored as described before.

3.1.6. Purification of NLRP3

All steps were carried out on ice. The cell pellets were resuspended in 25 mL lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM ADP, 5 mM beta-mercaptoethanol, pH = 7.8) each and to the total volume DNase (1:1000) and PMSF (1:100) were added. After complete resuspension, the cells were lysed by sonication 2 × 5 min (5 s pulse, 10 s pause) with an amplitude of 40% (Sonopuls mini, BANDELIN electronic, Berlin, Germany). Subsequently, the lysate was cleared and filtered as described before. The clear supernatant was applied at a flow rate of 1.5 mL/min on a MBPTrap 5-mL-column (cytiva, Marlborough, USA) equilibrated in lysis buffer coupled to an ÄKTA start chromatography system (GE Healthcare, Uppsala, Sweden). The column was washed with lysis buffer at a flow rate of 1.5 mL/min (10 CV) and the protein was eluted in 1 mL fractions with elution buffer (lysis buffer with 10 mM maltose) at a flow rate of 1.5 mL/min.

The protein containing elution fractions were pooled and concentrated to a total volume of 4.5 mL as described before (50 kDa molecular weight cut-off). The concentrated protein solution was loaded at a flow rate of

0.8 mL/min on a HiLoad 16/600 Superdex 75 pg column (cytiva, Marlborough, USA) equilibrated in SEC-buffer (20 mM HEPES, 150 mM NaCl, 10 mM $MgCl_2$ 1 mM ADP, 1 mM TCEP, pH = 7.8). The SEC was performed at a flow rate of 0.8 mL/min for 1.2 column volumes and fractions of 2 mL were collected. The protein containing fractions were analysed via SDS-PAGE, pooled, concentrated and stored as described above.

3.2. Thermal stability measurements

Thermal stability measurements were performed by nano-differential scanning fluorimetry (nano-DSF) using a Prometheus NT.48 device (NanoTemper Technologies, Munich, Germany). The excitation power was set to 15-20%, depending on the analysed protein. Thermal stability was monitored from 20 to 90 °C at a heating rate of 1.5 °C/min. Each sample contained 10 µM of the respective protein (MBP-NLRP3, VCB, CRBN/DDB1ΔB, GST-XIAP) and 30 µM of the respective test compound (MCC950, VH298, POM, CST530, **V1** – **V6**, **C1** – **C7**, **I1**, **I4** – **I6**, **(-)V2**), the total volume of each sample was 30 µL. The negative controls contained 2% DMSO (Carl Roth, Karlsruhe, Germany) instead of a compound.

4. Cell Biology

4.1. Cell culture

THP-1 cells (ACC 16, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) were cultured in RPMI 1640 medium (11530586, Fisher scientific, Schwerte, Germany) containing 100 U/mL penicillin, 100 µg/mL streptomycin (P4333), 2 mM L-glutamine (G7513, both Sigma-Aldrich, Taufkirchen, Germany) and 10% heat-inactivated fetal bovine serum (FBS; S0615, Sigma-Aldrich, Taufkirchen, Germany) at a density of 2-8 × 10^5 cells/mL. Cells were used from passage 4 to 25 and maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cell lines were regularly tested negative for mycoplasma contamination (VenorGeM Classic Mycoplasma PCR detection kit, 11-8100, Minerva Biolabs, Berlin, Germany). THP-1 derived macrophages for IL-1β release inhibiting experiments were generated by seeding THP-1 monocytes into 24-well plates at a density of 4 × 10^5 cells/mL in growth medium including 25 ng/mL PMA (phorbol 12-myristate 13-acetate; tlrl-pma, Invivogen, Toulouse, France). After 48 h, adherent cells were carefully washed with PBS (phosphate buffered saline; P04-53500, Pan Biotechne, Aidenbach, Germany) and rested in PMA-free medium for 24 h.

4.2. IL-1β release

THP-1 macrophages were primed with LPS (100 ng/mL) for 3 h. NLRP3 PROTACs (10 µM) or NLRP3 inhibitor MCC950 (10 µM) were added 1 h before incubation with nigericin (10 µM) for 3 h. DMSO was used as vehicle control. Cell culture supernatants were collected and analyzed for IL-1β content by using a commercially available ELISA kit (88-7261-88 from Thermofisher Scientific, Darmstadt, Germany).

4.3. Immunoblotting

THP-1 monocytes were incubated with increasing concentrations of **V2** (100 to 1000 nM) or 500 nM of **(-)V2**, respectively, for 18 h. Western blot analysis was performed as described before.[5] Briefly, to determine NLRP3 protein expression, membranes were incubated overnight at 4 °C with anti-NLRP3 rabbit mAb (D2P5E) (1:1000) in blocking buffer. Anti-rabbit HRP conjugated antibody (1:2000) (7074, all from Cell Signaling Technology, Leiden, The Netherlands) was incubated for one hour at room temperature. Blots were developed with ECL reagent (Clarity Western ECL Substrate; 1705060, Bio-Rad, Feldkirchen, Germany) and imaged using ChemiDoc imaging system (Bio-Rad). Values of protein expression were analyzed by densiometry and normalized to total protein levels using Image lab 6.1 (Bio-Rad).

4.4. MTT viability assay

THP-1 cells (kind gift from Marta Lovotti, group of Eicke Latz, Institute of Innate Immunity, University of Bonn, Germany) were cultured in RPMI 1640 medium (gibco, Life Technologies, Thermo Fisher, Paisley, UK) containing 100 U/mL penicillin, 100 µg/mL streptomycin and 10% heat-inactivated FBS (all gibco, Life Technologies, Thermo Fisher, Grand Island, USA) at a density of 5-10 × 10^5 cells/mL. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

To conduct the MTT viability assay, THP-1 cells were preincubated for three hours at a cell-density of 1 × 10^6 cells/mL. In the following, they were seeded at a density 5 × 10^4 cells per well (volume per well: 100 µL, 96-well-plates, VWR, Pennsylvania, USA) with increasing test compound concentrations. After 24 hours incubation, MTT (thiazolyl blue tetrazolium bromide, final concentration: 0.5 mg/mL, Abcam, Amsterdam, Netherlands) was added. Following 4 hours of incubation at 37 °C, media was removed and 100 µL DMSO to solubilize the formazan crystals were added. After complete solubilization the absorbance at 570 nm was measured in a PHERAstar FSX plate reader (BMG Labtech, Ortenberg, Germany).

4.5. Statistical analysis

Data are expressed as means + SEM. For studies of inhibitory effects nigericin-induced IL-1β release was set to 100%. All other values were calculated accordingly. Statistical differences were assessed by one-sample t-test against 100%. Statistical analysis was performed using GraphPad Prism software (Version 9.5.1).

5. Molecular Docking

In a first step, the cryo-EM structure of NLRP3 (PDB ID: 7PZC)[6] was analyzed regarding solvent exposed amino acid residues near the binding site of MCC950, that possibly could form part of the binding interface between NLRP3 and VHL. This resulted in the selection of six amino acid residues (His360, Asp363, Leu621, Ile623, Asn686, Val707) as candidates for direct binding to VHL (Figure S7).

Next, NLRP3 and VCB (PDB ID: 8C13, the VH298-based PROTAC JW48 was removed before docking)[7] were loaded and docked onto the ZDOCK server (version 3.0.2),[8] selecting all 6 binding candidate residues to bind. In parallel, docking of NLRP3 and VCB was performed selecting each candidate residue separately. To evaluate the different NLRP3:VCB models, the available NLRP3 and VCB structures including MCC950 and the VH298-based PROTAC were aligned on the top ten predictions of all 7 docked models and the distance between C25 (MCC950) and C15 (PROTAC JW48)[7] was measured (Table S2).

The minimal distances were obtained for prediction 4 (15.4 Å) of model 4 (Leu621 to bind) and prediction 7 (13.8 Å) and 9 (15.5 Å) of model 5 (Ile623 to bind). In the following, using these predictions as receptor and our PROTAC **V2** as ligand, the ternary complex NLRP3:**V2**:VCB was docked using the AutoDock Vina Software (version 1.1.2).[9] As center the coordinates x = 182.60, y = 215.14, z = 126.34 (center of mass of Ile623) and as search space a box of 80 Å × 80 Å × 80 Å was chosen. The exhaustiveness was set to 50 and the num_modes to 20. In Figure S6, mode 12 of model 4 prediction 4 (calculated binding affinity: -10.8 kcal/mol) is shown, all calculated modes are presented in Figure S8, the results for each calculation (affinities and distances from best mode) are given in Tables S3-S5.

6. Chemistry

6.1. General methods and materials

Commercially available chemicals and starting reagents were purchased from abcr, Acros Organics, BLDpharm, Carl Roth, Fisher Scientific, Fluorochem, Sigma Aldrich/Merck, Tokyo Chemical Industry or VWR Chemicals and were used without further purification. All reactions were conducted using anhydrous solvents. Thin-layer chromatography was carried out on Merck silica gel (60 F_{254}) aluminum sheets. Detection was performed with UV light at 254 and 360 nm or with $AgNO_3$ (10% m/v in H_2O) or ninhydrin (0.2% m/v in EtOH) staining. Spot identification was supported by TLC-MS on an Advion Plate Express plate reader (Ithaca, United States of America) coupled with an Advion expression L compact mass spectrometer with APCI source. Acros Organics silica gel (0.060-0.200 mm, 60 Å) was used for preparative column chromatography. Preparative flash column chromatography was performed on a puriFlash XS520Plus (Interchim, Montluçon, France) with diode-array detection (DAD) from 200 to 400 nm. Uncorrected melting points (mp) were determined on a Büchi (Essen, Germany) 510 or a Büchi M-560 melting point apparatus.

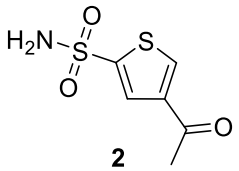
NMR spectra (^1H, ^{13}C) were recorded on a Bruker Avance DRX 500 or on a Bruker Avance III 600 spectrometer. Spectra were processed and analyzed in MestReNova. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (*J*) are given in hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet) or dd (doublet of doublets). Multiplicities are reported as they were measured and might disagree with the expected multiplicity of a signal. LC-ESI-MS analyses were carried out on an API 2000 mass spectrometer (AB Sciex, Darmstadt, Germany) coupled with an Agilent HPLC HP 1100 (Santa Clara, United States of America) using an EC50/2 Nucleodur C18 Gravity 3 µm column (Macherey-Nagel, Düren, Germany) or on an Agilent Infinity Lab LC/MSD-system (Santa Clara, United States of America) coupled with an Agilent HPLC 1260 Infinity II using an EC50/2 Nucleodur C18 Gravity 3 µm column (Macherey-Nagel, Düren, Germany). Samples (1 mg/mL) were dissolved in MeOH, H_2O (each may contain 2 mM NH_4OAc) or MeCN. A volume of either 8 µL or 2 µL were injected into the tempered column at 25 °C or 40 °C, respectively. The flow rate was either 0.3 mL/min or 0.5 mL/min. For LC-MS analyses, the following gradients were applied: (i) 90% H_2O + 2 mM NH_4OAc to 100% MeOH + 2 mM NH_4OAc, then 100% MeOH + 2 mM NH_4OAc to 15 min or 20 min; (ii) 90% H_2O + 2 mM NH_4OAc to 100% MeCN, then 100% MeCN to 15 min or 20 min. Acidic modifiers (HCOOH, CH_3COOH) were added to the mobile phase if stated. The purity of synthesised compounds was determined by LC-DAD. HR-ESI-MS spectra were recorded on a Bruker micrOTOF-Q mass spectrometer coupled with a HPLC Dionex UltiMate 3000 (Thermo Scientific, Braunschweig, Germany) or on a LTQ Orbitrap XL (Thermo Fisher Scientific, Bremen, Germany).

6.2. General procedure for CuAAC

The NLRP3 ligand (**4**, 1 eq), the corresponding azido-precursor (1 eq), (+)-sodium L-ascorbate (0.2 eq) and copper(II) sulfate pentahydrate (0.2 eq) were dissolved in a mixture of THF (2 mL) and water (2 mL). The reaction mixture was stirred for 3 h at room temperature under argon. The mixture was concentrated under high vacuum and purified by flash column chromatography.

6.3. Preparation of compounds

4-Acetylthiophene-2-sulfonamide (**2**)[10]

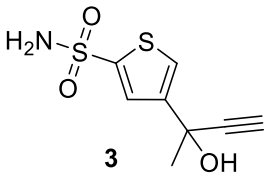


2

Chlorosulfonic acid (10.5 g, 90.0 mmol) was stirred at 0 °C under argon. 1-(Thiophen-3-yl)ethan-1-one (**1**, 1.89 g, 15.0 mmol) was added carefully. The mixture was stirred for 30 min at 0 °C under argon. The reaction mixture was heated to 60 °C for 3 h. The mixture was allowed to cool to room temperature and was carefully poured onto crushed ice. The resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude intermediate was dissolved in MeCN (30 mL) and ammonia gas was added (1 atm, balloon). The reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated *in vacuo*. The precipitate was collected, washed with water (3 × 20 mL) and hexane (3 × 20 mL). The residue was purified by silica gel column chromatography using petroleum ether/EtOAc (1+1) as eluent to yield a white solid (386 mg).

Yield 13%; mp: 124-126 °C , lit.[10] mp: 132-134 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.51 (s, 3H, CH_3), 7.79 (s, 2H, SO_2NH_2), 7.83 (d, J = 1.6 Hz, 1H, $CH_{arom.}$), 8.66 (d, J = 1.6 Hz, 1H, $CH_{arom.}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 27.3 (CH_3), 128.3, 137.4, 141.2, 146.8 ($C_{arom.}$), 191.9 (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 = 4.18 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_6H_7NO_3S_2$ 205.99, found 205.9.

4-(2-Hydroxybut-3-yn-2-yl)thiophene-2-sulfonamide (**3**)



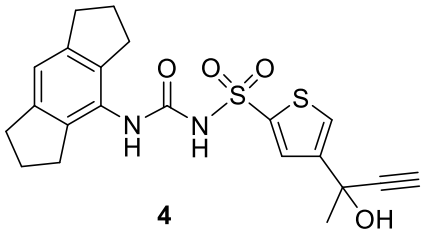
3

Ethynylmagnesium bromide (0.5 M in THF, 45 mL, 22.5 mmol) was stirred at 0 °C under nitrogen. 4-Acetylthiophene-2-sulfonamide (**2**, 924 mg, 4.50 mmol) was dissolved in dry THF (10 mL) and dropwise added at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The reaction was quenched by the addition of

saturated NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over $NaSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 6.5% MeOH) to yield a colourless resin (461 mg).

Yield 44%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.65 (s, 3H, CH_3), 3.53 (s, 1H, CCH), 6.22 (s, 1H, OH), 7.58 (d, J = 1.6 Hz, 1H, $CH_{arom.}$), 7.64 (s, 2H, SO_2NH_2), 7.67 (d, J = 1.7 Hz, 1H, $CH_{arom.}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 32.2 (CH_3), 65.2, 74.0, 87.8 (COH, C<u>C</u>H, <u>C</u>CH), 125.1, 128.4, 145.7, 147.8 ($C_{arom.}$); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 1.58 min, 99% purity, *m/z* $[M - H]^-$ calcd for $C_8H_9NO_3S_2$ 229.99, found 230.0.

N-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)-4-(2-hydroxybut-3-yn-2-yl)thiophene-2-sulfonamide (**4**)



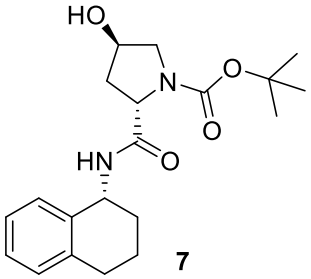
4

Triphosgene (712 mg, 2.40 mmol) was dissolved in dry THF (15 mL). The mixture was stirred for 30 min at 0 °C under nitrogen. TEA (405 mg, 4.00 mmol) was added. 1,2,3,5,6,7-Hexahydro-*s*-indacen-4-amine (416 mg, 2.40 mmol) was dissolved in dry THF (30 mL) and dropwise added over 30 min. The mixture was stirred for additional 30 min at room temperature and then for 30 min at 75 °C. The solvent was evaporated *in vacuo* and the residue was taken up in dry THF (10 mL), filtered and concentrated *in vacuo* to yield the intermediate 4-isocyanato-1,2,3,5,6,7-hexahydro-*s*-indacene. 4-(2-Hydroxybut-3-yn-2-yl)thiophene-2-sulfonamide (**3**, 463 mg, 2.00 mmol) was dissolved in dry THF (7 mL). NaH (60% dispersion in mineral oil, 144 mg, 3.60 mmol) was added and the reaction mixture was stirred at 0 °C under nitrogen for 30 min. The crude intermediate 4-isocyanato-1,2,3,5,6,7-hexahydro-*s*-indacene was dissolved in dry THF (15 mL) and added. The reaction mixture was stirred for 2 h at room temperature under nitrogen. The organic solvent was evaporated *in vacuo*. The residue was dispersed in H_2O (30 mL) and acidified with 2 N HCl. The precipitate was collected and dried under high vacuum. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a beige solid (266 mg).

Yield 31%; mp: decomposition >133 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.64 (s, 3H, CH_3), 1.94 (quint, J = 7.4 Hz, 4H), 2.60 (t, J = 7.4 Hz, 4H), 2.79 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.55 (s, 1H, CCH), 6.28 (s, 1H, OH), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.77 (d, J = 1.7 Hz, 1H), 7.84 (d, J = 1.7 Hz, 1H, $CH_{arom.\ thiophene}$), 8.15 (s, 1H, CONH), 10.64 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 25.0, 30.1, 32.2, 32.4 ($CH_{2\ indacene}$, CH_3), 65.2, 74.1, 87.7 (COH,

C<u>C</u>H, <u>C</u>CH), 118.0, 127.9, 128.6, 131.3, 137.3, 140.7, 143.1, 147.9 ($C_{arom.}$), 149.0 (CO); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 4.11 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{21}H_{22}N_2O_4S_2$ 431.11, found 431.1.

tert-Butyl (2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidine-1-carboxylate (**7**)[11]

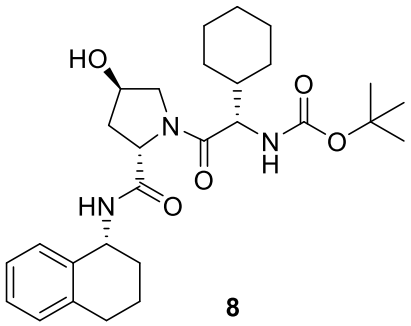


7

Compound **7** was prepared similar to a previously reported procedure.[11] Boc-Hyp-OH (**5**, 11.6 g, 50.0 mmol) was dissolved in dry CH_2Cl_2 (150 mL) at 0 °C under argon. HOBt hydrate (8.42 g, 55.0 mmol), TEA (5.57 g, 55.0 mmol) an EDC × HCl (10.5 g, 55.0 mmol) were added. The mixture was stirred at 0 °C under argon for 15 min. (*R*)-1,2,3,4-Tetrahydronaphthalen-1-amine (**6**, 7.36 g, 50.0 mmol) was added. The reaction mixture was stirred for 18 h at room temperature under argon. The reaction was quenched by the addition of 2 N HCl (100 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (2 × 200 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using a petroleum ether/EtOAc gradient (50% to 100% EtOAc) as eluent to yield a white solid (15.3 g).

Yield 85%; mp: 48-54 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33 – 1.43 (m, 9H), 1.60 – 1.77 (m, 2H), 1.78 – 1.94 (m, 3H), 1.96 – 2.13 (m, 1H), 2.65 – 2.80 (m, 2H), 3.37 – 3.48 (m, 1H), 4.17 – 4.30 (m, 2H), 4.90 – 4.97 (m, 2H), 7.03 – 7.30 (m, 4H), 8.15 – 8.21 (m, 1H), one signal (1H) is obscured by solvent signal; ^{13}C NMR (126 MHz, DMSO-d_6) δ 19.9, 28.0, 28.7, 29.6, 38.5, 46.4, 54.7, 58.6, 67.7, 78.4, 125.5, 126.6, 128.2, 128.6, 137.0, 137.4, 153.6, 171.9; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 5.64 min, 71% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{28}N_2O_4$ 361.21, found 361.2.

tert-Butyl ((*S*)-1-cyclohexyl-2-((2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (**8**)[11]



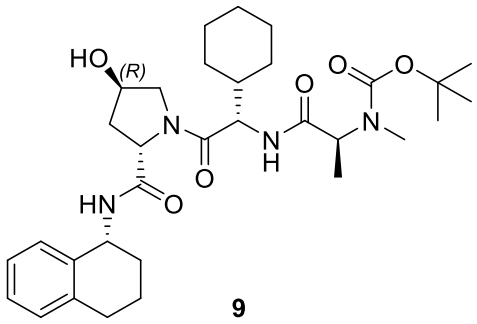
8

Compound **8** was prepared similar to a previously reported procedure.[11] *tert*-Butyl (2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidine-1-carboxylate (**7**, 14.4 g, 40.0 mmol) was stirred in 1 N HCl in EtOAc (100 mL) at room temperature for 18 h. The precipitate was filtered off, washed with dry EtOAc (2 × 15 mL) and dried under high vacuum to yield the intermediate (2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-ium chloride as a white solid (10.6 g, 35.7 mmol). Yield 89%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.66 – 1.79 (m, 2H), 1.80 – 1.93 (m, 3H), 2.25 – 2.33 (m, 1H), 2.65 – 2.82 (m, 2H), 3.07 – 3.15 (m, 1H), 4.29 – 4.37 (m, 1H), 4.40 – 4.46 (m, 1H), 4.92 – 5.03 (m, 1H), 5.55 (s, 1H), 7.07 – 7.25 (m, 4H), 8.68 (s, 1H), 8.98 (d, *J* = 8.4 Hz, 1H), 10.21 (s, 1H), one signal (1H) is obscured by solvent signal; ^{13}C NMR (126 MHz, DMSO-d_6) δ 19.5, 28.6, 29.4, 38.8, 47.0, 53.3, 57.9, 69.0, 125.8, 127.0, 128.4, 128.8, 136.3, 137.1, 167.2; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 4.04 min, 88% purity, *m/z* $[M + H]^+$ calcd for $C_{15}H_{20}N_2O_2$ 261.16, found 261.2.

Boc-Chg-OH (10.8 g, 42.0 mmol) was dissolved in dry DMF (150 mL) at 0 °C under argon. HOBt hydrate (6.43 g, 42.0 mmol), DIPEA (5.43 g, 42.0 mmol) and EDC × HCl (8.05 g, 42.0 mmol) were added. The mixture was stirred at 0 °C under argon for 15 min. The deprotected intermediate (10.4 g, 35.0 mmol) was added. The reaction mixture was stirred for 18 h at room temperature under argon. The mixture was concentrated *in vacuo*. The residue was diluted with H_2O (150 mL) and was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with 1 N HCl (100 mL), saturated $NaHCO_3$ solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using a petroleum ether/EtOAc gradient (50% to 66% EtOAc) as eluent to yield a white solid (14.2 g).

Yield 81%; mp: 80-86 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 – 1.16 (m, 5H), 1.36 (s, 9H), 1.56 – 1.73 (m, 7H), 1.75 – 1.93 (m, 4H), 1.97 – 2.03 (m, 1H), 2.65 – 2.79 (m, 2H), 3.48 – 3.73 (m, 2H), 4.04 – 4.10 (m, 1H), 4.31 – 4.44 (m, 2H), 4.89 – 4.94 (m, 1H), 5.06 (d, *J* = 3.7 Hz, 1H), 6.63 (d, *J* = 8.7 Hz, 1H), 7.04 – 7.16 (m, 3H), 7.25 – 7.32 (m, 1H), 8.17 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 20.3, 25.6, 25.7, 25.9, 27.8, 28.1, 28.7, 28.8, 29.9, 37.8, 46.4, 55.4, 56.6, 58.8, 68.8, 77.9, 125.6, 126.5, 128.2, 128.4, 136.8, 137.6, 155.4, 170.2, 170.9; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 7.34 min, 100% purity, *m/z* $[M + H]^+$ calcd for $C_{28}H_{41}N_3O_5$ 500.31, found 500.5.

tert-Butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-((2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**9**)[11]



9

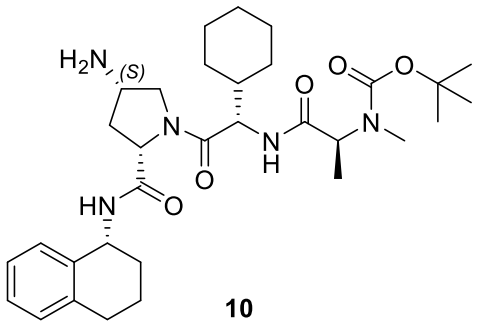
Compound **9** was prepared similar to a previously reported procedure.[11] *tert*-Butyl ((*S*)-1-cyclohexyl-2-((2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (**8**, 14.0 g, 28.0 mmol) was stirred in 1 N HCl in EtOAc (70 mL) at room temperature for 18 h. The precipitate was filtered off, washed with dry EtOAc (2 × 15 mL) and dried under high vacuum to yield the intermediate (*S*)-1-cyclohexyl-2-((2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-2-oxoethan-1-aminium chloride as a white solid (10.6 g, 24.3 mmol). Yield 87%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12 – 1.24 (m, 5H), 1.57 – 1.65 (m, 2H), 1.67 – 1.76 (m, 5H), 1.79 – 1.90 (m, 5H), 2.05 – 2.12 (m, 1H), 2.66 – 2.79 (m, 2H), 3.57 – 3.63 (m, 1H), 3.66 – 3.71 (m, 1H), 3.93 – 3.99 (m, 1H), 4.35 – 4.40 (m, 1H), 4.47 (t, *J* = 8.2 Hz, 1H), 4.90 – 4.96 (m, 1H), 7.06 – 7.16 (m, 3H), 7.29 (d, *J* = 7.4 Hz, 1H), 8.14 – 8.22 (m, 3H), 8.37 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 20.3, 25.4, 25.5, 25.7, 26.9, 27.7, 28.7, 29.9, 38.0, 46.5, 55.2, 55.7, 59.0, 68.9, 125.6, 126.5, 128.1, 128.5, 136.9, 137.6, 166.9, 170.5; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 5.38 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{33}N_3O_3$ 400.26, found 400.3.

Boc-*N*-Me-L-Ala-OH (5.85 g, 28.8 mmol) was dissolved in dry DMF (100 mL) at 0 °C under argon. HOBt hydrate (4.41 g, 28.8 mmol), DIPEA (3.72 g, 28.8 mmol) and EDC × HCl (5.52 g, 28.8 mmol) were added. The mixture was stirred at 0 °C under argon for 15 min. The deprotected intermediate (10.5 g, 24.0 mmol) was added. The reaction mixture was stirred for 18 h at room temperature under argon. The mixture was concentrated *in vacuo*. The residue was diluted with H_2O (100 mL) and was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with 1 N HCl (70 mL), saturated $NaHCO_3$ solution (70 mL) and brine (70 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using EtOAc as eluent to yield a white solid (9.91 g).

Yield 71%; mp: 74-80 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.90 – 1.23 (m, 8H), 1.42 (s, 9H), 1.56 – 1.75 (m, 7H), 1.77 – 1.94 (m, 4H), 1.99 – 2.05 (m, 1H), 2.66 – 2.79 (m, 5H), 3.52 – 3.60 (m, 1H), 3.64 – 3.72 (m, 1H), 4.33 – 4.44 (m, 3H), 4.50 – 4.61 (m, 1H), 4.87 – 4.97 (m, 1H), 5.00 – 5.11 (m, 1H), 7.03 – 7.17 (m, 3H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.43 – 7.78 (m, 1H), 8.21 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.5, 20.4, 25.5, 25.7, 25.8, 27.6, 28.0, 28.7, 28.9, 29.9, 37.8, 46.5, 53.5, 54.5, 55.5, 58.7, 68.8, 79.0, 125.6, 126.5, 128.2, 128.4,

136.8, 137.7, 154.9, 169.6, 170.9, one signal (CO) is missing; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 7.51 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{32}H_{48}N_4O_6$ 585.36, found 585.5.

tert-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-amino-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**10**)[12]

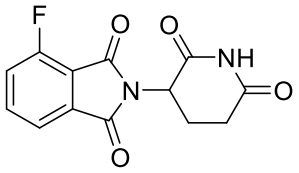


10

tert-Butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-((2*S*,4*R*)-4-hydroxy-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**9**, 9.36 g, 16.0 mmol) was dissolved in dry CH_2Cl_2 (40 mL) and cooled to 0 °C. TEA (2.43 g, 24.0 mmol) and methanesulfonyl chloride (2.75 g, 24.0 mmol) were added. The reaction mixture was allowed to warm to room temperature and was stirred for 24 h. The reaction mixture was concentrated *in vacuo*. The residue was taken up in dry DMF (50 mL) under argon. NaN_3 (3.12 g, 48.0 mmol) was added. The reaction mixture was stirred for 72 h at 70 °C. The mixture was carefully concentrated. The residue was taken up in dry THF (70 mL) under argon. Triphenylphosphine (8.39 g, 32.0 mmol) was added and the reaction mixture was stirred for 1 h. Concentrated aqueous ammonium hydroxide (70 mL) was added and the reaction mixture was stirred for 18 h. The reaction mixture was concentrated *in vacuo* and the aqueous residue was diluted with brine (40 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with water (100 mL), brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9+1) as eluent to yield a white solid (7.11 g).

Yield 76%; mp: 67-73 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 – 1.05 (m, 2H), 1.05 – 1.24 (m, 6H), 1.41 (s, 9H), 1.54 – 1.77 (m, 8H), 1.79 – 1.89 (m, 3H), 2.24 – 2.30 (m, 1H), 2.67 – 2.77 (m, 5H), 3.23 – 3.28 (m, 1H), 3.32 – 3.39 (m, 1H), 3.89 (dd, *J* = 9.7, 6.2 Hz, 1H), 4.20 – 4.27 (m, 1H), 4.35 (t, *J* = 8.0 Hz, 1H), 4.41 – 4.62 (m, 1H), 4.88 – 4.96 (m, 1H), 7.00 – 7.20 (m, 3H), 7.24 – 7.35 (m, 1H), 7.48 – 7.81 (m, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), one signal (2H) is missing due to proton exchange; ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.4, 20.2, 25.5, 25.7, 25.8, 28.0, 28.7, 28.8, 29.8, 30.0, 37.8, 46.5, 51.0, 54.7, 55.4, 59.2, 79.0, 125.6, 126.5, 128.3, 128.5, 136.9, 137.4, 154.8, 169.6, 171.0, two signals are missing; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 7.96 min, 100% purity, *m/z* $[M + H]^+$ calcd for $C_{32}H_{49}N_5O_5$ 584.38, found 584.6.

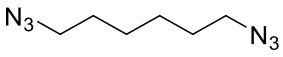
2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**)[13-15]



11

Compound **11** was prepared as described elsewhere.[16]

1,6-Diazidohexane (**12**)[17-19]

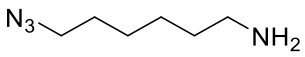


12

1,6-Dibromohexane (1.22 g, 5.00 mmol) was dissolved in dry DMF (10 mL). NaN_3 (1.30 g, 20.0 mmol) was added and the mixture was stirred at 80 °C for 24 h. The reaction mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (30 mL) and was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a slightly yellow liquid (819 mg).

Yield 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 – 1.37 (m, 4H), 1.50 – 1.57 (m, 4H, CH_2), 3.32 (t, J = 6.9 Hz, 4H, CH_2N_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 25.6, 28.0 (CH_2), 50.5 (CH_2N_3).

6-Azidohexan-1-amine (**13**)[17,18]

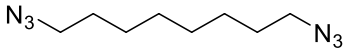


13

1,6-Diazidohexane (**12**, 505 mg, 3.00 mmol) was dissolved in a mixture of THF (10 mL) and H_2O (5 mL). Triphenylphosphine (787 mg, 3.00 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (20 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (30 mL) and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless liquid (192 mg).

Yield 45%; ^{1}H NMR (600 MHz, $CDCl_3$-*d*) δ 1.31 – 1.41 (m, 4H), 1.41 – 1.50 (m, 4H), 1.57 – 1.63 (m, 2H), 2.68 (t, J = 7.0 Hz, 2H, CH_2, NH_2), 3.25 (t, J = 6.9 Hz, 2H, CH_2N_3); ^{13}C NMR (151 MHz, $CDCl_3$-*d*) δ 26.6, 26.7, 28.9, 33.7 (CH_2), 42.2 (CH_2NH_2), 51.5 (CH_2N_3); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 1.99 min, *m/z* $[M + H]^+$ calcd for $C_6H_{14}N_4$ 143.13, found 142.9.

1,8-Diazidooctane (**14**)[20]

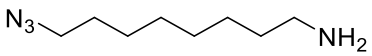


14

1,8-Dibromooctane (2.72 g, 10.0 mmol) was dissolved in dry DMF (10 mL). NaN_3 (2.60 g, 40.0 mmol) was added and the mixture was stirred at 80 °C for 24 h. The reaction mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless liquid (1.60 g).

Yield 82%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.26 – 1.35 (m, 8H), 1.48 – 1.57 (m, 4H, CH_2), 3.31 (t, J = 6.9 Hz, 4H, CH_2N_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 26.0, 28.1, 28.3 (CH_2), 50.6 (CH_2N_3).

8-Azidooctan-1-amine (**15**)[21]

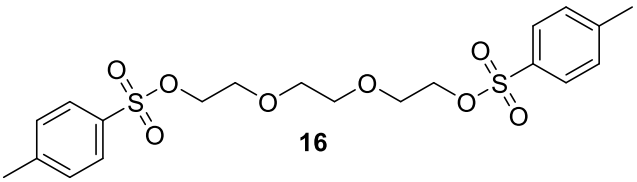


15

1,8-Diazidooctane (**14**, 981 mg, 5.00 mmol) was dissolved in a mixture of THF (10 mL) and H_2O (5 mL). Triphenylphosphine (1.31 g, 5.00 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (20 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (30 mL) and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a slightly yellow liquid (567 mg).

Yield 67%; ^{1}H NMR (600 MHz, $CDCl_3$-*d*) δ 1.27 – 1.39 (m, 10H), 1.40 – 1.44 (m, 2H, CH_2), 1.55 – 1.62 (m, 2H), 2.67 (t, J = 7.0 Hz, 2H, CH_2, NH_2), 3.24 (t, J = 7.0 Hz, 2H, CH_2N_3); ^{13}C NMR (151 MHz, $CDCl_3$-*d*) δ 26.8, 26.9, 28.9, 29.2, 29.5, 33.9 (CH_2), 42.3 (CH_2NH_2), 51.6 (CH_2N_3); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 4.50 min, *m/z* $[M + H]^+$ calcd for $C_8H_{18}N_4$ 171.16, found 171.2.

(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**16**)[22-24]



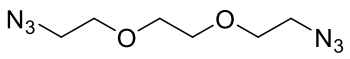
16

Compound **16** was prepared similar to a previously reported procedure.[22] 2,2'-(Ethane-1,2-diylbis(oxy))bis(ethan-1-ol) (6.01 g, 40.0 mmol) was dissolved in dry CH_2Cl_2 (40 mL). TEA (10.1 g, 100 mmol) was added. 4-Methylbenzenesulfonyl chloride (16.8 g, 88.0 mmol) was added and the mixture was cooled with a water bath. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched by the addition of 2 N NaOH

(50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using petroleum ether/EtOAc (1+1) as eluent to yield a white solid (11.6 g).

Yield 63%; mp: 79-81 °C, lit.[23] mp: 82-83 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.41 (s, 6H, CH_3), 3.37 – 3.40 (m, 4H), 3.52 – 3.55 (m, 4H), 4.08 – 4.11 (m, 4H, CH_2), 7.47 (d, J = 8.2 Hz, 4H), 7.75 – 7.80 (m, 4H, $CH_{arom.}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 21.0 (CH_3), 67.8, 69.5, 69.9 (CH_2), 127.5, 130.1, 132.4, 144.8 ($C_{arom.}$); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 7.38 min, 100% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{20}H_{26}O_8S_2$ 476.14, found 476.2.

1,2-Bis(2-azidoethoxy)ethane (**17**)[22,25]

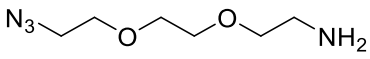


17

Compound **17** was prepared similar to a previously reported procedure.[22] (Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**16**, 11.5 g, 25.0 mmol) was dissolved in dry DMF (30 mL). NaN_3 (6.50 g, 100 mmol) was added. The reaction mixture was stirred at 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a slightly yellow oil (4.36 g).

Yield 87%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.39 (t, J = 4.9 Hz, 4H), 3.57 – 3.59 (m, 4H), 3.60 – 3.63 (m, 4H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 69.2, 69.7 (CH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 4.33 min, *m/z* $[M + NH_4]^+$ calcd for $C_6H_{12}N_6O_2$ 218.14, found 218.1.

2-(2-(2-Azidoethoxy)ethoxy)ethan-1-amine (**18**)[22,26]



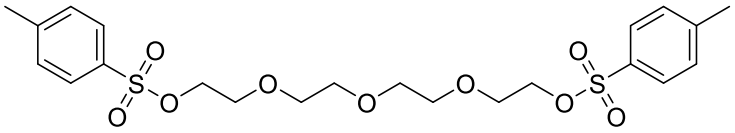
18

Compound **18** was prepared similar to a previously reported procedure.[22] 1,2-Bis(2-azidoethoxy)ethane (**17**, 4.00 g, 20.0 mmol) was dissolved in a mixture of THF (30 mL) and H_2O (10 mL). Triphenylphosphine (5.25 g, 20.0 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (30 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (40 mL) and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a slightly yellow oil (1.74 g).

Yield 50%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.64 (t, J = 5.8 Hz, 2H, C$\underline{H}_2$NH$_2$), 3.34 – 3.41 (m, 4H), 3.50 – 3.54 (m, 2H), 3.55 – 3.59 (m, 2H), 3.59 – 3.62 (m, 2H, CH_2), two protons (NH_2) are missing due to proton exchange;

^{13}C NMR (126 MHz, DMSO-d_6) δ 41.4 (CH_2NH_2), 50.0 (CH_2N_3), 69.2, 69.6, 69.7, 73.1 (CH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 0.75 min, *m/z* $[M + H]^+$ calcd for $C_6H_{14}N_4O_2$ 175.12, found 175.2.

((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**19**)[22,27]

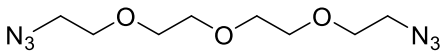


19

Compound **19** was prepared similar to a previously reported procedure.[22] 2,2'-((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethan-1-ol) (7.77 g, 40.0 mmol) was dissolved in dry CH_2Cl_2 (40 mL). TEA (10.1 g, 100 mmol) was added to the solution. 4-Methylbenzenesulfonyl chloride (16.8 g, 88.0 mmol) was added in one portion and the reaction was cooled with a water bath. The reaction mixture was allowed to stir at room temperature for 24 h. The reaction was quenched by the addition of 2 N NaOH (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using petroleum ether/EtOAc (1+1) as eluent to yield a colourless oil (17.1 g).

Yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.42 (s, 6H, CH_3), 3.41 – 3.44 (m, 8H), 3.55 – 3.58 (m, 4H), 4.09 – 4.12 (m, 4H, OCH_2), 7.47 (d, *J* = 8.2 Hz, 4H), 7.78 (d, *J* = 8.1 Hz, 4H, $CH_{arom.}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 21.0 (CH_3), 67.8, 69.6, 69.6, 69.9 (CH_2), 127.5, 130.1, 132.4, 144.8 ($C_{arom.}$); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 7.62 min, 99% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{22}H_{30}O_9S_2$ 520.17, found 520.3.

1-Azido-2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethane (**20**)[22,28]



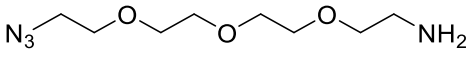
20

Compound **20** was prepared similar to a previously reported procedure.[22] ((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**19**, 16.6 g, 33.0 mmol) was dissolved in dry DMF (30 mL). NaN_3 (8.58 g, 132 mmol) was added and the mixture was heated to 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (7.73 g).

Yield 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.36 – 3.41 (m, 4H), 3.54 – 3.58 (m, 8H), 3.59 – 3.62 (m, 4H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 69.2, 69.7, 69.8 (CH_2); LC-MS (ESI) (90% H_2O to 100% MeCN

in 10 min, then 100% MeCN to 15 min), t_R = 4.41 min, *m/z* $[M + NH_4]^+$ calcd for $C_8H_{16}N_6O_3$ 262.16, found 262.1.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-amine (**21**)[22,28]

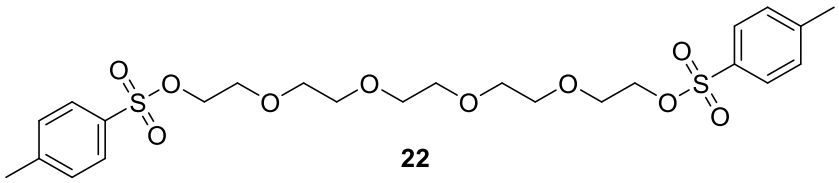


21

Compound **21** was prepared similar to a previously reported procedure.[22] 1-Azido-2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethane (**20**, 7.33 g, 30.0 mmol) was dissolved in a mixture of THF (40 mL) and H_2O (10 mL). Triphenylphosphine (7.87 g, 30.0 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (30 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (40 mL) and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (3.79 g).

Yield 58%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.64 (t, *J* = 5.8 Hz, 2H, C<u>H</u>$_2$NH$_2$), 3.35 (t, *J* = 5.9 Hz, 2H), 3.39 (t, *J* = 4.9 Hz, 2H), 3.47 – 3.57 (m, 8H), 3.60 (t, *J* = 4.9 Hz, 2H, CH_2), two protons (NH_2) are missing due to proton exchange; ^{13}C NMR (126 MHz, DMSO-d_6) δ 41.3 (CH_2NH_2), 50.0 (CH_2N_3), 69.2, 69.5, 69.7, 69.8, 73.1 (CH_2), one signal is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 0.96 min, *m/z* $[M + H]^+$ calcd for $C_8H_{18}N_4O_3$ 219.15, found 219.1.

3,6,9,12-Tetraoxatetradecane-1,14-diyl bis(4-methylbenzenesulfonate) (**22**)[22,29]

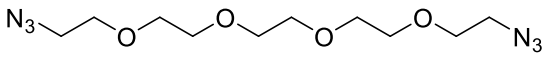


22

Compound **22** was prepared similar to a previously reported procedure.[22] 3,6,9,12-Tetraoxatetradecane-1,14-diol (9.53 g, 40.0 mmol) was dissolved in dry CH_2Cl_2 (40 mL). TEA (10.1 g, 100 mmol) was added to the solution. 4-Methylbenzenesulfonyl chloride (16.8 g, 88.0 mmol) was added in one portion and the reaction was cooled with a water bath. The reaction mixture was allowed to stir at room temperature for 24 h. The reaction was quenched by the addition of 2 N NaOH (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using petroleum ether/EtOAc (1+1) as eluent to yield a colourless oil (15.4 g).

Yield 70%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.42 (s, 6H, CH_3), 3.43 – 3.45 (m, 8H), 3.45 – 3.47 (m, 4H), 3.56 – 3.58 (m, 4H), 4.09 – 4.13 (m, 4H, CH_2), 7.47 (d, *J* = 8.1 Hz, 4H), 7.76 – 7.80 (m, 4H, $CH_{arom.}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 21.0 (CH_3), 67.8, 69.6, 69.7, 69.7, 69.9 (CH_2), 127.5, 130.1, 132.4, 144.8 ($C_{arom.}$); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 7.45 min, 100% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{24}H_{34}O_{10}S_2$ 564.19, found 564.3.

1,14-Diazido-3,6,9,12-tetraoxatetradecane (**23**)[22,30]

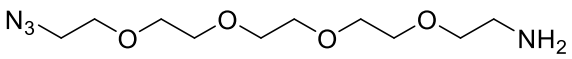


23

Compound **23** was prepared similar to a previously reported procedure.[22] 3,6,9,12-Tetraoxatetradecane-1,14-diyl bis(4-methylbenzenesulfonate) (**22**, 13.7 g, 25.0 mmol) was dissolved in dry DMF (30 mL). NaN_3 (6.50 g, 100 mmol) was added and the mixture was heated to 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (5.90 g).

Yield 82%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.39 (t, J = 4.8 Hz, 4H), 3.53 – 3.57 (m, 12H), 3.59 – 3.61 (m, 4H, CH_2); ^{13}C NMR (151 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 69.2, 69.7, 69.8, 69.8 (CH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 4.84 min, *m/z* $[M + NH_4]^+$ calcd for $C_{10}H_{20}N_6O_4$ 306.19, found 306.3.

14-Azido-3,6,9,12-tetraoxatetradecan-1-amine (**24**)[22,31]

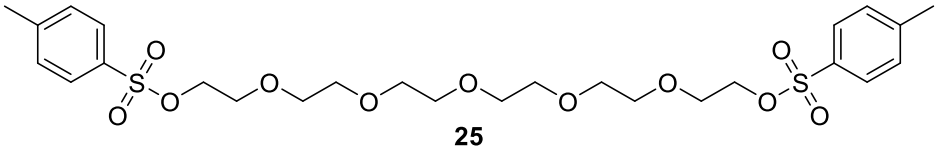


24

Compound **24** was prepared similar to a previously reported procedure.[22] 1,14-Diazido-3,6,9,12-tetraoxatetradecane (**23**, 5.77 g, 20.0 mmol) was dissolved in a mixture of THF (30 mL) and H_2O (10 mL). Triphenylphosphine (5.25 g, 20.0 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (30 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (40 mL) and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (2.16 g).

Yield 41%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.64 (t, J = 5.8 Hz, 2H, C$\underline{H}_2$NH$_2$), 3.33 – 3.37 (m, 2H), 3.37 – 3.41 (m, 2H), 3.48 – 3.57 (m, 12H), 3.58 – 3.62 (m, 2H, CH_2), two protons (NH_2) are missing due to proton exchange; ^{13}C NMR (126 MHz, DMSO-d_6) δ 41.3 (CH_2NH_2), 50.0 (CH_2N_3), 69.2, 69.5, 69.7, 69.8, 69.8, 69.8, 73.1 (CH_2), one signal is missing due to overlapping signals (CH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 1.83 min, *m/z* $[M + H]^+$ calcd for $C_{10}H_{22}N_4O_4$ 263.17, found 263.2.

3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl bis(4-methylbenzenesulfonate) (**25**)[23,32]

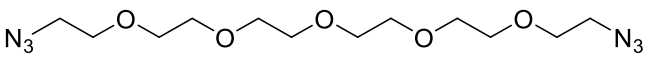


25

3,6,9,12,15-Pentaoxaheptadecane-1,17-diol (4.80 g, 17.0 mmol) was dissolved in dry CH_2Cl_2 (40 mL). TEA (4.30 g, 42.5 mmol) was added to the solution. 4-Methylbenzenesulfonyl chloride (7.13 g, 37.4 mmol) was added in one portion and the reaction was cooled with a water bath. The reaction mixture was allowed to stir at room temperature for 24 h. The reaction was quenched by the addition of 2 N NaOH (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using a gradient petroleum ether/EtOAc gradient (66% to 100% EtOAc) to yield a colourless oil (8.14 g).

Yield 81%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.42 (s, 6H, CH_3), 3.43 – 3.45 (m, 8H), 3.46 – 3.50 (m, 8H), 3.56 – 3.58 (m, 4H), 4.09 – 4.12 (m, 4H, CH_2), 7.46 – 7.50 (m, 4H), 7.76 – 7.81 (m, 4H, $CH_{arom.}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 21.0 (CH_3), 67.8, 69.6, 69.7, 69.7, 69.7, 69.9 (CH_2), 127.6, 130.1, 132.4, 144.8 ($C_{arom.}$); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 7.45 min, 100% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{26}H_{38}O_{11}S_2$ 608.22, found 608.3.

1,17-Diazido-3,6,9,12,15-pentaoxaheptadecane (**26**)[32]

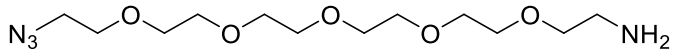


26

3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl bis(4-methylbenzenesulfonate) (**25**, 7.97 g, 13.5 mmol) was dissolved in dry DMF (20 mL). NaN_3 (3.51 g, 54.0 mmol) was added and the mixture was heated to 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (4.02 g).

Yield 90%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.36 – 3.41 (m, 4H), 3.51 – 3.58 (m, 16H), 3.59 – 3.62 (m, 4H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 69.2, 69.7, 69.7, 69.8, 69.8 (CH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 4.99 min, *m/z* $[M + NH_4]^+$ calcd for $C_{12}H_{24}N_6O_5$ 350.21, found 350.3.

17-Azido-3,6,9,12,15-pentaoxaheptadecan-1-amine (**27**)[32]

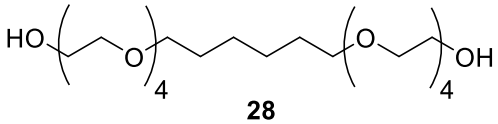


27

1,17-Diazido-3,6,9,12,15-pentaoxaheptadecane (**26**, 3.66 g, 11.0 mmol) was dissolved in a mixture of THF (15 mL) and H_2O (5 mL). Triphenylphosphine (2.89 g, 11.0 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (30 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (40 mL) and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (1.61 g).

Yield 48%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.64 (t, J = 5.8 Hz, 2H, C$\underline{H}_2$NH$_2$), 3.35 (t, J = 5.8 Hz, 2H), 3.37 – 3.40 (m, 2H), 3.47 – 3.52 (m, 2H), 3.50 – 3.53 (m, 10H), 3.51 – 3.57 (m, 4H), 3.59 – 3.62 (m, 2H, CH_2), two protons (NH_2) are missing due to proton exchange; ^{13}C NMR (151 MHz, DMSO-d_6) δ 41.3 (CH_2NH_2), 50.0 (CH_2N_3), 69.2, 69.6, 69.7, 69.8, 69.8, 69.8, 69.8, 73.1 (CH_2), two signals (CH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 2.91 min, *m/z* $[M + H]^+$ calcd for $C_{12}H_{26}N_4O_5$ 307.20, found 307.3.

3,6,9,12,19,22,25,28-Octaoxatriacontane-1,30-diol (**28**)

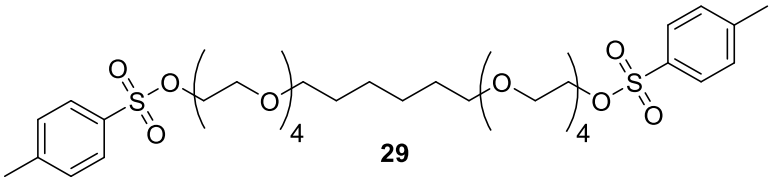


28

2,2'-((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethan-1-ol) (29.1 g, 150 mmol) was stirred at 70 °C. Sodium (1.72 g, 75.0 mmol) was cut into small pieces and was carefully added. After reaction of the sodium under dissolution, the reaction mixture was heated to 100 °C. 1,6-Dibromohexane (7.32 g, 30.0 mmol) was added dropwise and the reaction mixture was stirred for 3 h at 100 °C. The reaction mixture was allowed to cool to room temperature. H_2O (50 mL) was carefully added and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with water (25 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 10% MeOH) as eluent to yield a slightly yellow oil (5.05 g).

Yield 36%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25 – 1.31 (m, 4H), 1.44 – 1.51 (m, 4H, CH_2), 3.37 (t, J = 6.6 Hz, 4H), 3.39 – 3.44 (m, 4H), 3.44 – 3.53 (m, 28H, OCH_2), 4.52 (t, J = 5.5 Hz, 2H, OH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 25.5, 29.1 (CH_2), 60.2 (CH_2OH), 69.4, 69.7, 69.8, 69.8, 70.2, 72.3 (OCH_2), two signals (OCH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 4.16 min, *m/z* $[M + NH_4]^+$ calcd for $C_{22}H_{46}O_{10}$ 488.34, found 488.5.

3,6,9,12,19,22,25,28-Octaoxatriacontane-1,30-diyl bis(4-methylbenzenesulfonate) (**29**)

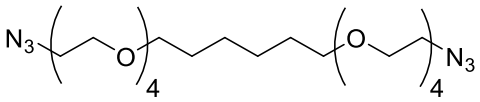


29

3,6,9,12,19,22,25,28-Octaoxatriacontane-1,30-diol (**28**, 2.35 g, 5.00 mmol) was dissolved in dry CH_2Cl_2 (40 mL). TEA (1.26 g, 12.5 mmol) was added to the solution. 4-Methylbenzenesulfonyl chloride (2.10 g, 11.0 mmol) was added in one portion and the reaction was cooled with a water bath. The reaction mixture was allowed to stir at room temperature for 24 h. The reaction was quenched by the addition of 2 N NaOH (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel flash column chromatography using a petroleum ether/EtOAc gradient (0% to 100% EtOAc) to yield a colourless oil (2.24 g).

Yield 58%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.23 – 1.31 (m, 4H), 1.42 – 1.50 (m, 4H, CH_2), 2.42 (s, 6H, CH_3), 3.35 (t, J = 6.6 Hz, 4H), 3.43 – 3.51 (m, 24H), 3.56 – 3.59 (m, 4H), 4.09 – 4.13 (m, 4H, OCH_2), 7.45 – 7.50 (m, 4H), 7.76 – 7.80 (m, 4H, $CH_{arom.}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 21.0 (CH_3), 25.4, 29.1 (CH_2), 67.8, 69.4, 69.6, 69.7, 69.7, 69.9, 70.2 (OCH_2), 127.5, 130.1, 132.4, 144.8 ($C_{arom.}$), two signals (OCH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 8.28 min, 98% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{36}H_{58}O_{14}S_2$ 796.36, found 796.6.

1,30-Diazido-3,6,9,12,19,22,25,28-octaoxatriacontane (**30**)

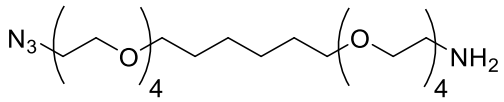


30

3,6,9,12,19,22,25,28-Octaoxatriacontane-1,30-diyl bis(4-methylbenzenesulfonate) (**29**, 1.95 g, 2.50 mmol) was dissolved in dry DMF (10 mL). NaN_3 (650 mg, 10.0 mmol) was added. The mixture was stirred at 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (1.16 g).

Yield 89%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.26 – 1.32 (m, 4H), 1.45 – 1.51 (m, 4H, CH_2), 3.34 – 3.41 (m, 8H), 3.44 – 3.48 (m, 4H), 3.49 – 3.57 (m, 20H), 3.58 – 3.62 (m, 4H, OCH_2, CH_2N_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 25.5, 29.2 (CH_2), 50.0 (CH_2N_3), 69.2, 69.5, 69.7, 69.8, 69.8, 70.2 (OCH_2), two signals (OCH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 6.80 min, *m/z* $[M + NH_4]^+$ calcd for $C_{22}H_{44}N_6O_8$ 538.36, found 538.6.

30-Azido-3,6,9,12,19,22,25,28-octaoxatriacontan-1-amine (**31**)

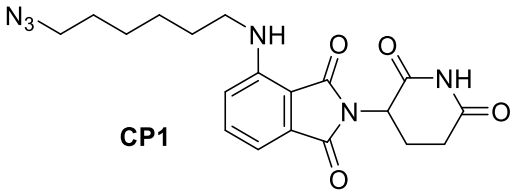


31

1,30-Diazido-3,6,9,12,19,22,25,28-octaoxatriacontane (**30**, 1.04 g, 2.00 mmol) was dissolved in a mixture of THF (10 mL) and H_2O (5 mL). Triphenylphosphine (525 mg, 2.00 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of 2 N HCl (15 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 25 mL). The aqueous phase was adjusted to pH~10 by the addition of 2 N NaOH (20 mL) and was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (641 mg).

Yield 65%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.26 – 1.32 (m, 4H), 1.45 – 1.50 (m, 4H, CH_2), 2.64 (t, J = 5.8 Hz, 2H, C$\underline{H_2}$NH$_2$), 3.32 – 3.41 (m, 8H), 3.44 – 3.48 (m, 4H), 3.48 – 3.57 (m, 20H), 3.58 – 3.63 (m, 2H, OCH_2, CH_2N_3), two protons (NH_2) are missing due to proton exchange; ^{13}C NMR (151 MHz, DMSO-d_6) δ 25.5, 29.2 (CH_2), 41.3 (CH_2NH_2), 50.0 (CH_2N_3), 69.2, 69.5, 69.6, 69.7, 69.8, 69.8, 69.8, 69.8, 70.2, 73.1 (OCH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 5.26 min, *m/z* $[M + H]^+$ calcd for $C_{22}H_{46}N_4O_8$ 495.34, found 495.4.

4-((6-Azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP1**)[33,34]

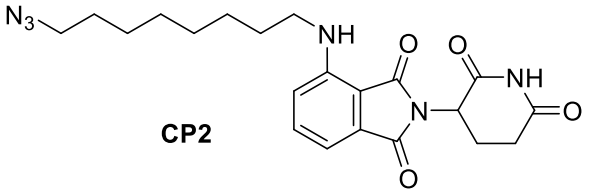


CP1

Compound **CP1** was prepared similar to a previously reported procedure.[33] 6-Azidohexan-1-amine (**13**, 156 mg, 1.10 mmol) was dissolved in dry DMF (8 mL). DIPEA (284 mg, 2.20 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 304 mg, 1.10 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a cyclohexane/EtOAc gradient (0% to 50% EtOAc) as eluent to yield a yellow solid (69 mg).

Yield 16%; mp: 98-100 °C; ^{1}H NMR (600 MHz, $CDCl_3$-*d*) δ 1.42 – 1.48 (m, 4H), 1.60 – 1.65 (m, 2H), 1.66 – 1.71 (m, 2H), 2.10 – 2.16 (m, 1H), 2.69 – 2.84 (m, 2H), 2.86 – 2.92 (m, 1H), 3.25 – 3.30 (m, 4H), 4.91 (dd, J = 12.3, 5.4 Hz, 1H), 6.23 (s, 1H), 6.88 (d, J = 8.5 Hz, 1H), 7.09 (d, J = 7.1 Hz, 1H), 7.49 (dd, J = 8.5, 7.1 Hz, 1H), 8.06 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$-*d*) δ 22.9, 26.6, 26.7, 28.9, 29.3, 31.6, 42.6, 49.0, 51.5, 110.1, 111.6, 116.7, 132.6, 136.3, 147.1, 167.7, 168.4, 169.7, 171.1; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 7.05 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{19}H_{22}N_6O_4$ 399.18, found 399.3.

4-((8-Azidooctyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP2**)[35]

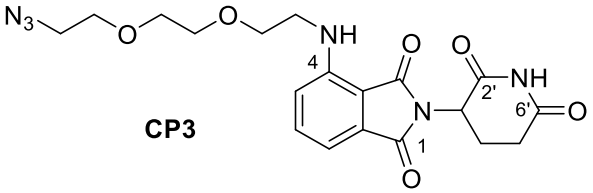


CP2

8-Azidooctan-1-amine (**15**, 341 mg, 2.00 mmol) was dissolved in dry DMF (8 mL). DIPEA (517 mg, 4.00 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 552 mg, 2.00 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a cyclohexane/EtOAc gradient (0% to 50% EtOAc) as eluent to yield a yellow resin (137 mg).

Yield 16%; ^{1}H NMR (600 MHz, $CDCl_3$- *d*) δ 1.32 – 1.44 (m, 8H), 1.57 – 1.63 (m, 2H), 1.63 – 1.69 (m, 2H), 2.10 – 2.15 (m, 1H), 2.69 – 2.84 (m, 2H), 2.86 – 2.92 (m, 1H), 3.22 – 3.29 (m, 4H), 4.91 (dd, *J* = 12.3, 5.4 Hz, 1H), 6.23 (s, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 7.0 Hz, 1H), 7.46 – 7.53 (m, 1H), 8.09 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$-*d*) δ 22.9, 26.8, 27.0, 28.9, 29.2, 29.3, 29.3, 31.5, 42.8, 49.0, 51.6, 110.0, 111.5, 116.8, 132.6, 136.3, 147.1, 167.8, 168.4, 169.6, 171.1, one signal is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 7.98 min, 92% purity, *m/z* $[M + H]^+$ calcd for $C_{21}H_{26}N_6O_4$ 427.21, found 427.3.

4-((2-(2-(2-Azidoethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP3**)[33,34,36]



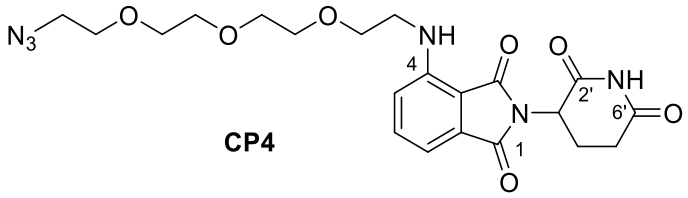
CP3

Compound **CP3** was prepared similar to a previously reported procedure.[36] 2-(2-(2-Azidoethoxy)ethoxy)ethan-1-amine (**18**, 993 mg, 5.70 mmol) was dissolved in dry DMF (10 mL). DIPEA (1.14 g, 8.80 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 1.22 g, 4.40 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient from petroleum ether/EtOAc (1+1) to petroleum ether/EtOAc (1+2) to yield a yellow solid (432 mg).

Yield 23%; mp: 93-95 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.99 – 2.07 (m, 1H, 4'-H), 2.51 – 2.63 (m, 2H, 4'-H, 5'-H), 2.83 – 2.93 (m, 1H, 5'-H), 3.34 – 3.38 (m, 2H), 3.47 (q, *J* = 5.6 Hz, 2H), 3.57 – 3.62 (m, 6H), 3.64 (t, *J* = 5.4 Hz, 2H, CH_2N_3, OCH_2, CH_2N), 5.05 (dd, *J* = 12.7, 5.4 Hz, 1H, 3'-H), 6.60 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 7.04 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.58 (dd, *J* = 8.4, 7.3 Hz, 1H, 6-H), 11.06 (s, 1H, NH $_{imide}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 22.1 (C-4'), 30.9 (C-5'), 41.7 (CH_2NH), 48.5 (C-3'), 50.0 (CH_2N_3), 68.9, 69.3,

69.7, 69.8 (OCH_2), 109.3 (C-3a), 110.6 (C-7), 117.4 (C-5), 132.1 (C-7a), 136.2 (C-6), 146.4 (C-4), 167.2 (C-1), 168.9 (C-3), 170.0 (C-2'), 172.7 (C-6'); LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-800 nm), t_R = 8.98 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{19}H_{22}N_6O_6$ 431.17, found 431.2.

4-((2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP4**)[33,34,36]

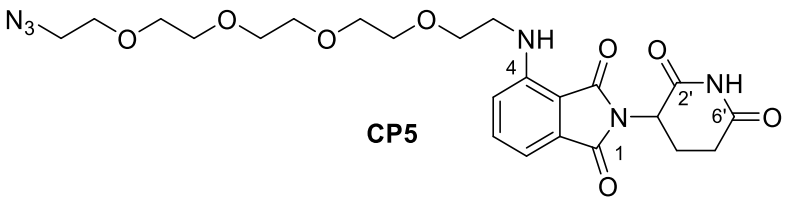


CP4

Compound **CP4** was prepared similar to a previously reported procedure.[36] 2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-amine (**21**, 993 mg, 4.55 mmol) was dissolved in dry DMF (10 mL). DIPEA (905 mg, 7.00 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 967 mg, 3.50 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a petroleum ether/EtOAc gradient (0% to 50% EtOAc) to yield a green resin (397 mg).

Yield 24%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.00 – 2.06 (m, 1H, 4'-H), 2.51 – 2.62 (m, 2H, 4'-H, 5'-H), 2.83 – 2.93 (m, 1H, 5'-H), 3.35 – 3.38 (m, 2H), 3.45 – 3.49 (m, 2H), 3.53 – 3.59 (m, 10H), 3.63 (t, *J* = 5.4 Hz, 2H, CH_2N_3, OCH_2, CH_2N), 5.05 (dd, *J* = 12.7, 5.4 Hz, 1H, 3'-H), 6.59 (t, *J* = 5.9 Hz, 1H, CH_2NH), 7.04 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.58 (dd, *J* = 8.4, 7.2 Hz, 1H, 6-H), 11.06 (s, 1H, NH_{imide}); ^{13}C NMR (126 MHz, DMSO-d_6) δ 22.1 (C-4'), 30.9 (C-5'), 41.7 (CH_2NH), 48.5 (C-3'), 50.0 (CH_2N_3), 68.9, 69.2, 69.7, 69.7, 69.8, 69.8 (OCH_2), 109.2 (C-3a), 110.6 (C-7), 117.4 (C-5), 132.1 (C-7a), 136.2 (C-6), 146.4 (C-4), 167.2 (C-1), 168.9 (C-3), 170.0 (C-2'), 172.7 (C-6'); LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-800 nm), t_R = 9.08 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{21}H_{26}N_6O_7$ 475.19, found 475.3.

4-((14-Azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP5**)[33,34,36]



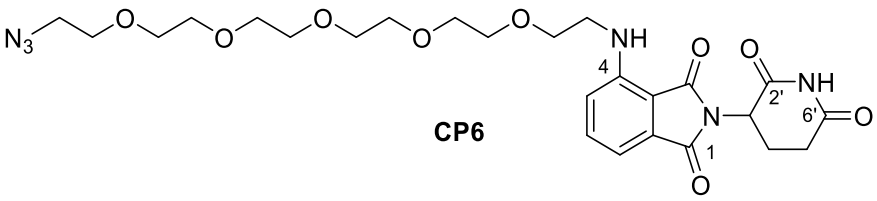
CP5

Compound **CP5** was prepared similar to a previously reported procedure.[36] 14-Azido-3,6,9,12-tetraoxatetradecan-1-amine (**24**, 1.19 g, 4.55 mmol) was dissolved in dry DMF (10 mL). DIPEA (905 mg, 7.00 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 967 mg, 3.50 mmol) were added. The reaction

mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a petroleum ether/EtOAc gradient (0% to 50% EtOAc) as eluent to yield a green resin (288 mg).

Yield 16%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.99 – 2.07 (m, 1H, 4'-H), 2.52 – 2.63 (m, 2H, 4'-H, 5'-H), 2.83 – 2.93 (m, 1H, 5'-H), 3.36 – 3.39 (m, 2H), 3.45 – 3.49 (m, 2H), 3.50 – 3.55 (m, 10H), 3.56 – 3.60 (m, 4H), 3.63 (t, *J* = 5.4 Hz, 2H, CH_2N_3, OCH_2, CH_2N), 5.05 (dd, *J* = 12.7, 5.4 Hz, 1H, 3'-H), 6.59 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 7.04 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.58 (dd, *J* = 8.6, 7.1 Hz, 1H, 6-H), 11.06 (s, 1H, NH $_{imide}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 22.1 (C-4'), 30.9 (C-5'), 41.7 (CH_2NH), 48.5 (C-3'), 50.0 (CH_2N_3), 68.9, 69.2, 69.6, 69.7, 69.8, 69.8, 69.8 (OCH_2), 109.2 (C-3a), 110.6 (C-7), 117.4 (C-5), 132.1 (C-7a), 136.2 (C-6), 146.4 (C-4), 167.2 (C-1), 168.9 (C-3), 170.0 (C-2'), 172.7 (C-6'), one signal (OCH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.59 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{30}N_6O_8$ 519.22, found 519.4.

4-((17-Azido-3,6,9,12,15-pentaoxaheptadecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP6**)[34]

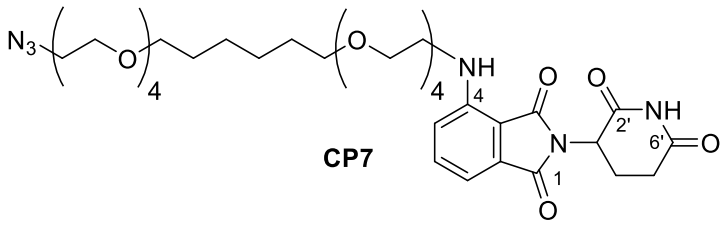


CP6

17-Azido-3,6,9,12,15-pentaoxaheptadecan-1-amine (**27**, 1.39 g, 4.55 mmol) was dissolved in dry DMF (10 mL). DIPEA (905 mg, 7.00 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 967 mg, 3.50 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a cyclohexane/EtOAc gradient (0% to 100% EtOAc) as eluent to yield a green resin (281 mg).

Yield 14%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.97 – 2.07 (m, 1H, 4'-H), 2.51 – 2.65 (m, 2H, 4'-H, 5'-H), 2.83 – 2.96 (m, 1H, 5'-H), 3.36 – 3.40 (m, 2H), 3.45 – 3.60 (m, 20H), 3.63 (t, *J* = 5.4 Hz, 2H, CH_2N_3, OCH_2, CH_2N), 5.05 (dd, *J* = 12.7, 5.5 Hz, 1H, 3'-H), 6.60 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 7.04 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.58 (dd, *J* = 8.4, 7.2 Hz, 1H, 6-H), 11.06 (s, 1H, NH $_{imide}$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 22.1 (C-4'), 30.9 (C-5'), 41.7 (CH_2NH), 48.5 (C-3'), 50.0 (CH_2N_3), 68.9, 69.2, 69.7, 69.7, 69.7, 69.8, 69.8 (OCH_2), 109.2 (C-3a), 110.6 (C-7), 117.4 (C-5), 132.1 (C-7a), 136.2 (C-6), 146.4 (C-4), 167.2 (C-1), 168.9 (C-3), 170.0 (C-2'), 172.7 (C-6'), three signals (OCH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.60 min, 90% purity, *m/z* $[M + H]^+$ calcd for $C_{25}H_{34}N_6O_9$ 563.25, found 563.4.

4-((30-Azido-3,6,9,12,19,22,25,28-octaoxatriacontyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**CP7**)

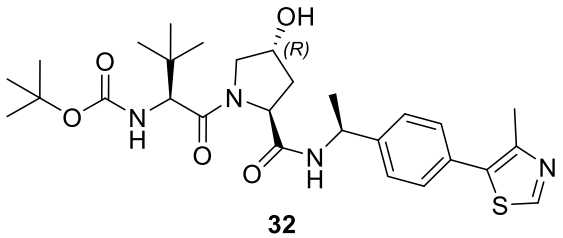


CP7

30-Azido-3,6,9,12,19,22,25,28-octaoxatriacontan-1-amine (**31**, 544 mg, 1.10 mmol) was dissolved in dry DMF (8 mL). DIPEA (284 mg, 2.20 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**11**, 304 mg, 1.10 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) to yield a yellow oil (171 mg).

Yield 21%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.22 – 1.29 (m, 4H, CH_2), 1.42 – 1.48 (m, 4H, CH_2), 1.98 – 2.04 (m, 1H, 4'-H), 2.50 – 2.61 (m, 2H, 4'-H, 5'-H), 2.84 – 2.91 (m, 1H, 5'-H), 3.31 – 3.35 (m, 4H), 3.36 – 3.39 (m, 2H), 3.41 – 3.57 (m, 26H), 3.57 – 3.60 (m, 2H), 3.61 (t, *J* = 5.5 Hz, 2H, CH_2N_3, OCH_2, CH_2N), 5.04 (dd, *J* = 12.8, 5.4 Hz, 1H, 3'-H), 6.59 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 7.03 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, *J* = 8.4, 7.2 Hz, 1H, 6-H), 11.06 (s, 1H, NH $_{imide}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1 (C-4'), 25.5, 29.2 (CH_2), 30.9 (C-5'), 41.7 (CH_2NH), 48.5 (C-3'), 50.0 (CH_2N_3), 68.9, 69.2, 69.4, 69.4, 69.7, 69.8, 69.8, 69.8, 69.8, 70.2 (OCH_2), 109.2 (C-3a), 110.6 (C-7), 117.4 (C-5), 132.1 (C-7a), 136.2 (C-6), 146.4 (C-4), 167.2 (C-1), 168.9 (C-3), 170.0 (C-2'), 172.7 (C-6'); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 6.79 min, 93% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{35}H_{54}N_6O_{12}$ 768.41, found 768.6.

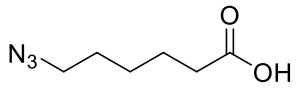
tert-Butyl ((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (**32**)[37-41]



32

Compound **32** was prepared as described elsewhere.[16]

6-Azidohexanoic acid (**34**)[42]

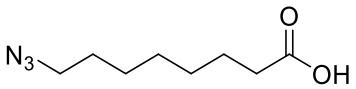


34

Compound **34** was prepared similar to a previously reported procedure.[42] 6-Bromohexanoic acid (975 mg, 5.00 mmol) was dissolved in dry DMF (12 mL). NaN_3 (390 mg, 6.00 mmol) was added. The reaction mixture was stirred for 24 h at 80 °C. The reaction was quenched by the addition of water (30 mL). The aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a colourless oil (724 mg).

Yield 92%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.29 – 1.36 (m, 2H), 1.48 – 1.55 (m, 4H), 2.21 (t, J = 7.3 Hz, 2H, CH_2), 3.31 (t, J = 6.9 Hz, 2H, CH_2N_3), 11.97 (s, 1H, COOH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 24.0, 25.7, 28.0, 33.5 (CH_2), 50.5 (CH_2N_3), 174.3 (COOH); LC-MS (ESI) (90% H_2O + 0.1% HCOOH to 100% MeCN + 0.1% HCOOH in 10 min), t_R = 3.91 min, *m/z* [M - H]$^-$ calcd for $C_6H_{11}N_3O_2$ 156.08, found 156.1.

8-Azidooctanoic acid (**35**)[42]

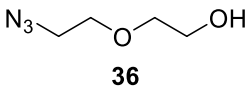


35

Compound **35** was prepared similar to a previously reported procedure.[42] 8-Bromooctanoic acid (1.12 g, 5.00 mmol) was dissolved in dry DMF (12 mL). NaN_3 (390 mg, 6.00 mmol) was added. The reaction mixture was stirred at 80 °C for 24 h. The reaction mixture was allowed to cool to room temperature and was quenched by the addition of water (30 mL). The aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a slightly yellow liquid (833 mg).

Yield 90%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.23 – 1.35 (m, 6H), 1.45 – 1.56 (m, 4H), 2.16 – 2.22 (m, 2H, CH_2), 11.92 (s, 1H, COOH), one signal (2H, CH_2N_3) is obscured by the solvent signal; ^{13}C NMR (126 MHz, DMSO-d_6) δ 24.3, 25.9, 28.1, 28.2, 28.3, 33.6 (CH_2), 50.6 (CH_2N_3), 174.4 (COOH); LC-MS (ESI) (90% H_2O + 0.1% HCOOH to 100% MeCN + 0.1% HCOOH in 10 min, then 100% MeCN + 0.1% HCOOH to 15 min), t_R = 7.00 min, *m/z* [M - H]$^-$ calcd for $C_8H_{15}N_3O_2$ 184.11, 184.0.

2-(2-Azidoethoxy)ethan-1-ol (**36**)[43]



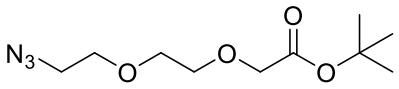
36

NaN_3 (1.15 g, 17.7 mmol) was dissolved in DMF (30 mL). 2-(2-Chloroethoxy)ethan-1-ol (1.42 g, 11.4 mmol) was

added and the reaction mixture was stirred at 100 °C under argon for 48 h. The reaction mixture was diluted with 5% LiCl solution (30 mL) and was extracted with EtOAc (6 × 75 mL). The organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using petroleum ether/EtOAc (1+2) as eluent to yield a colourless oil (1.14 g).

Yield 77%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.38 – 3.40 (m, 2H), 3.45 – 3.47 (m, 2H), 3.49 – 3.52 (m, 2H), 3.59 – 3.61 (m, 2H, CH_2), one signal (1H, OH) is missing due to proton exchange; ^{13}C NMR (151 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 60.2 (CH_2OH), 69.2, 72.1 (OCH_2); LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 1.79 min, *m/z* $[M + H]^+$ calcd for $C_4H_9N_3O_2$ 132.08, found 132.0.

tert-Butyl 2-(2-(2-azidoethoxy)ethoxy)acetate (**37**)[44]

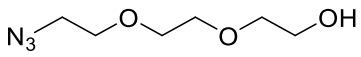


37

2-(2-Azidoethoxy)ethan-1-ol (**36**, 1.13 g, 8.60 mmol) was dissolved in toluene (20 mL) and cooled to 0 °C. Tetrabutylammonium hydrogen sulfate (TBAHS, 1.46 g, 4.30 mmol) and *tert*-butyl bromoacetate (5.03 g, 25.8 mmol) were added. Aqueous 9.5 M NaOH (2.7 mL) was added. The reaction mixture was stirred for 22 h at room temperature. The mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using petroleum ether/EtOAc (3+1) as eluent to yield a colourless oil (907 mg).

Yield 43%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42 (s, 9H, $C(CH_3)_3$), 3.38 – 3.41 (m, 2H), 3.57 – 3.62 (m, 6H, CH_2N_3, OCH_2), 3.99 (s, 2H, CH_2COO); ^{13}C NMR (126 MHz, DMSO-d_6) δ 27.7 ($C(\underline{C}H_3)_3$), 50.0 (CH_2N_3), 68.1, 69.2, 69.6, 69.8 (OCH_2), 80.6 ($\underline{C}(CH_3)_3$), 169.3 (COO); LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 10.12 min, *m/z* $[M + NH_4]^+$ calcd for $C_{10}H_{19}N_3O_4$ 263.17, found 262.9.

2-(2-(2-Azidoethoxy)ethoxy)ethan-1-ol (**38**)[45]

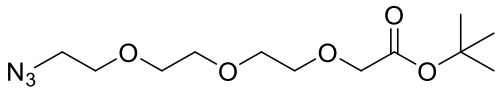


38

Compound **38** was prepared similar to a previously reported procedure.[45] NaN_3 (1.15 g, 17.7 mmol) was dissolved in dry DMF (30 mL). 2-(2-(2-Chloroethoxy)ethoxy)ethan-1-ol (1.92 g, 11.4 mmol) was added and the reaction mixture was stirred at 100 °C under argon for 48 h. The reaction mixture was diluted with 5% LiCl solution (30 mL) and was extracted with EtOAc (6 × 75 mL). The organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using petroleum ether/EtOAc (1+2) as eluent to yield a colourless oil (1.13 g).

Yield 57%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.37 – 3.41 (m, 2H), 3.41 – 3.45 (m, 2H), 3.47 – 3.51 (m, 2H), 3.52 – 3.55 (m, 2H), 3.55 – 3.58 (m, 2H), 3.57 – 3.63 (m, 2H, CH_2N_3, OCH_2), 4.53 (t, J = 5.4 Hz, 1H, OH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 60.2 (CH_2OH), 69.2, 69.7, 69.7, 72.3 (OCH_2); LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 4.19 min, *m/z* $[M + H]^+$ calcd for $C_6H_{13}N_3O_3$ 176.10, found 176.1.

tert-Butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (**39**)[45]

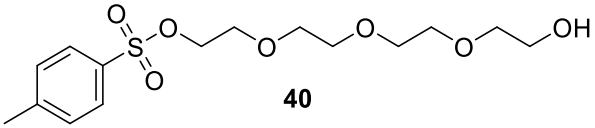


39

Compound **39** was prepared similar to a previously reported procedure.[45] 2-(2-(2-Azidoethoxy)ethoxy)ethan-1-ol (**38**, 1.05 g, 6.00 mmol) was dissolved in toluene (20 mL) and cooled to 0 °C. Tetrabutylammonium hydrogen sulfate (1.02 g, 3.00 mmol) and *tert*-butyl bromoacetate (3.51 g, 18.0 mmol) were added. An aqueous 9.5 M NaOH solution (2 mL) was added. The reaction mixture was stirred at room temperature for 22 h at room temperature. The mixture was diluted with water (100 mL) and extracted with Et_2O (3× 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using petroleum ether/EtOAc (2+1) as eluent to yield a colourless oil (750 mg).

Yield 43%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.42 (s, 9H, $C(CH_3)_3$), 3.37 – 3.40 (m, 2H), 3.53 – 3.58 (m, 8H), 3.59 – 3.61 (m, 2H, CH_2N_3, OCH_2), 3.98 (s, 2H, CH_2COO); ^{13}C NMR (151 MHz, DMSO-d_6) δ 27.7 ($C(\underline{C}H_3)_3$), 50.0 (CH_2N_3), 68.1, 69.2, 69.7, 69.7, 69.8 (OCH_2), 80.6 ($\underline{C}(CH_3)_3$), 169.3 (COO), one signal (OCH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min), t_R = 10.18 min, *m/z* $[M + NH_4]^+$ calcd for $C_{12}H_{23}N_3O_5$ 307.20, found 307.2.

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**40**)[46]



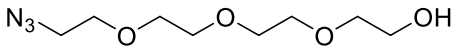
40

2,2'-((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethan-1-ol) (7.77 g, 40.0 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and cooled to 0 °C. TEA (3.04 g, 30.0 mmol) and 4-methylbenzenesulfonyl chloride (3.81 g, 20.0 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of water (50 mL). The organic layer was separated and washed with water (3 × 50 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless oil (2.79 g).

Yield 40%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.39 – 3.42 (m, 2H), 3.45 (s, 4H), 3.46 – 3.50 (m,

6H), 3.56 – 3.59 (m, 2H), 4.10 – 4.13 (m, 2H, OCH_2), 4.54 (t, *J* = 5.5 Hz, 1H, OH), 7.47 – 7.50 (m, 2H), 7.77 – 7.80 (m, 2H, $CH_{arom.}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 21.1 (CH_3), 60.2 (CH_2OH), 67.9, 69.6, 69.7, 69.7, 69.8, 69.9, 72.3 (OCH_2), 127.6, 130.1, 132.4, 144.9 ($C_{arom.}$); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 4.83 min, 99% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{15}H_{24}O_7S$ 366.16, found 366.2.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol (**41**)[47]

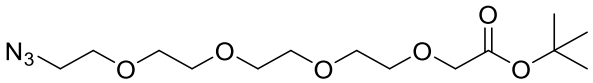


41

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**40**, 1.74 g, 5.00 mmol) was dissolved in dry DMF (10 mL). NaN_3 (650 mg, 10.0 mmol) was added and the mixture was stirred at 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a colourless oil (1.08 g).

Yield 98%; ^1H NMR (600 MHz, DMSO-d_6) δ 3.38 – 3.40 (m, 2H), 3.40 – 3.43 (m, 2H), 3.47 – 3.50 (m, 2H), 3.51 – 3.57 (m, 8H), 3.59 – 3.61 (m, 2H, CH_2N_3, OCH_2), 4.54 (t, *J* = 5.5 Hz, 1H, OH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 50.0 (CH_2N_3), 60.2 (CH_2OH), 69.2, 69.7, 69.7, 69.8, 69.8, 72.3 (OCH_2); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 1.72 min, *m/z* $[M + NH_4]^+$ calcd for $C_8H_{17}N_3O_4$ 237.16, found 237.2.

tert-Butyl 14-azido-3,6,9,12-tetraoxatetradecanoate (**42**)[48]



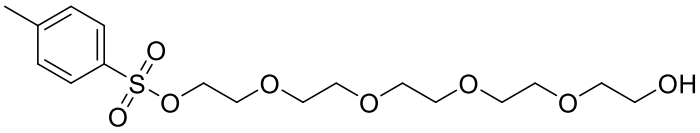
42

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol (**41**, 877 mg, 4.00 mmol) was dissolved in dry toluene (20 mL) and cooled to 0 °C. Tetrabutylammonium hydrogen sulfate (679 mg, 2.00 mmol) and *tert*-butyl bromoacetate (2.34 g, 12.0 mmol) were added. Aqueous 9.5 M NaOH solution (1.26 mL) was added. The reaction mixture was stirred for 22 h at room temperature. The mixture was diluted wit H_2O (100 mL) and was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using CH_2Cl_2/MeOH (29+1) as eluent to yield a colourless oil (338 mg).

Yield 25%; ^1H NMR (600 MHz, DMSO-d_6) δ 1.42 (s, 9H, $C(CH_3)_3$), 3.37 – 3.40 (m, 2H), 3.51 – 3.58 (m, 12H), 3.59 – 3.61 (m, 2H, CH_2N_3, OCH_2), 3.98 (s, 2H, CH_2COO); ^{13}C NMR (151 MHz, DMSO-d_6) δ 27.7 ($C(\underline{C}H_3)_3$),

50.0 (CH_2N_3), 68.1, 69.2, 69.7, 69.7, 69.7, 69.8, 69.8, 69.8 (OCH_2), 80.6 ($\underline{C}(CH_3)_3$), 169.3 (COO); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 5.97 min, *m/z* $[M + NH_4]^+$ calcd for $C_{14}H_{27}N_3O_6$ 351.22, found 351.4.

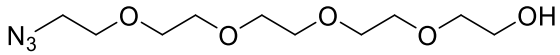
14-Hydroxy-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (**43**)[49]



43

3,6,9,12-Tetraoxatetradecane-1,14-diol (9.53 g, 40.0 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and cooled to 0 °C. TEA (3.04 g, 30.0 mmol) and 4-methylbenzenesulfonyl chloride (3.81 g, 20.0 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of water (50 mL). The organic layer was separated and washed with water (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless oil (2.58 g).
Yield 33%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.41 (t, *J* = 5.3 Hz, 2H), 3.44 – 3.45 (m, 4H), 3.46 – 3.50 (m, 6H), 3.50 – 3.51 (m, 4H), 3.56 – 3.59 (m, 2H), 4.10 – 4.12 (m, 2H, OCH_2), 4.54 (t, *J* = 5.5 Hz, 1H, OH), 7.48 (d, *J* = 8.0 Hz, 2H), 7.77 – 7.80 (m, 2H, $CH_{arom.}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 21.1 (CH_3), 60.2 (CH_2OH), 67.9, 69.6, 69.7, 69.7, 69.8, 69.8, 70.0, 72.3 (OCH_2), 127.6, 130.1, 132.4, 144.9 ($C_{arom.}$), one signal (OCH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 4.95 min, 98% purity, *m/z* $[M + NH_4]^+$ calcd for $C_{17}H_{28}O_8S$ 410.18, found 410.3.

14-Azido-3,6,9,12-tetraoxatetradecan-1-ol (**44**)[30]

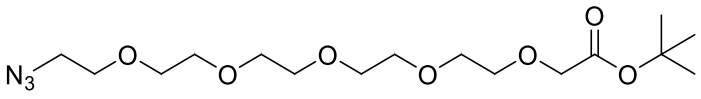


44

14-Hydroxy-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (**43**, 1.96 g, 5.00 mmol) was dissolved in dry DMF (10 mL). NaN_3 (650 mg, 10.0 mmol) was added and the mixture was stirred at 80 °C for 24 h. The mixture was allowed to cool to room temperature and was carefully concentrated. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a colourless oil (1.19 g).
Yield 90%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.36 – 3.41 (m, 2H), 3.40 – 3.43 (m, 2H), 3.47 – 3.50 (m, 2H), 3.50 – 3.53 (m, 8H), 3.53 – 3.57 (m, 4H), 3.59 – 3.61 (m, 2H, CH_2N_3, OCH_2), 4.54 (s, 1H, OH); ^{13}C NMR (151 MHz,

DMSO-d_6) δ 50.0 (CH_2N_3), 60.2 (CH_2OH), 69.2, 69.7, 69.8, 69.8, 69.8, 72.3 (OCH_2), two signals (OCH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 2.66 min, *m/z* $[M + NH_4]^+$ calcd for $C_{10}H_{21}N_3O_5$ 281.18, found 281.3.

tert-Butyl 17-azido-3,6,9,12,15-pentaoxaheptadecanoate (**45**)[50]

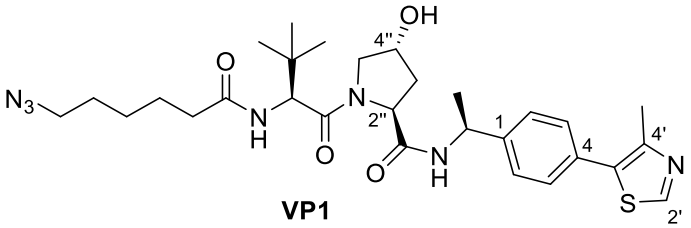


45

14-Azido-3,6,9,12-tetraoxatetradecan-1-ol (**44**, 1.05 g, 4.00 mmol) was dissolved in dry toluene (20 mL) and cooled to 0 °C. Tetrabutylammonium hydrogen sulfate (679 mg, 2.00 mmol) and *tert*-butyl bromoacetate (2.34 g, 12.0 mmol) were added. Aqueous 9.5 M NaOH solution (1.26 mL) was added. The reaction mixture was stirred for 22 h at room temperature. The mixture was diluted wit H_2O (100 mL) and was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by silica gel column chromatography using CH_2Cl_2/MeOH (29+1) as eluent to yield a colourless oil (462 mg).

Yield 31%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.42 (s, 9H, $C(CH_3)_3$), 3.37 – 3.41 (m, 2H), 3.51 – 3.58 (m, 16H), 3.59 – 3.62 (m, 2H, CH_2N_3, OCH_2), 3.98 (s, 2H, CH_2COO); ^{13}C NMR (151 MHz, DMSO-d_6) δ 27.7 ($C(\underline{C}H_3)_3$), 50.0 (CH_2N_3), 68.1, 69.2, 69.7, 69.7, 69.8, 69.8, 69.8, 69.8 (OCH_2), 80.6 ($\underline{C}(CH_3)_3$), 169.3 (COO), two signals (OCH_2) are missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min), t_R = 5.96 min, *m/z* $[M + NH_4]^+$ calcd for $C_{16}H_{31}N_3O_7$ 395.25, found 395.4.

(2*S*,4*R*)-1-((*S*)-2-(6-Azidohexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**VP1**)



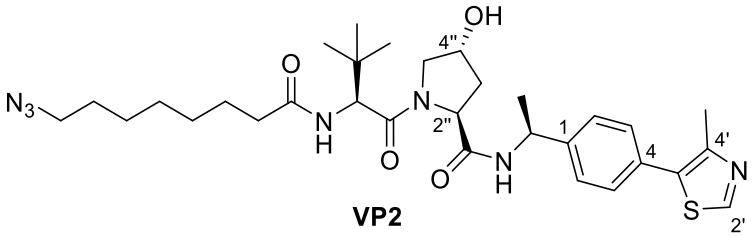
VP1

The Boc-protected VHL ligand **32** (872 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. 6-Azidohexanoic acid (**34**, 251 mg, 1.60 mmol) was dissolved in dry DMF (5 mL). HATU (669 mg, 1.76 mmol) and DIPEA (724 mg, 5.60 mmol) were added under argon. The mixture was allowed to stir for 30 min. The deprotected VHL ligand **33** was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid

compound. The reaction mixture was allowed to stir at room temperature under argon for 4 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless resin (491 mg).

Yield 53%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.94 (s, 9H, C(CH_3)$_3$), 1.28 – 1.33 (m, 2H, CH_2), 1.38 (d, J = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.45 – 1.56 (m, 4H, CH_2), 1.76 – 1.85 (m, 1H), 1.96 – 2.05 (m, 1H, 3''-H), 2.10 – 2.17 (m, 1H), 2.22 – 2.29 (m, 1H, CH_2CON), 2.45 (s, 3H, $CH_{3\ thiazole}$), 3.58 – 3.64 (m, 2H, 5''-H), 4.26 – 4.32 (m, 1H), 4.43 (t, J = 8.0 Hz, 1H), 4.52 (d, J = 9.3 Hz, 1H, 2''-H, 4''-H, NHC<u>H</u>), 4.88 – 4.97 (m, 1H, C<u>H</u>CH$_3$), 5.08 (d, J = 3.6 Hz, 1H, OH), 7.36 – 7.40 (m, 2H), 7.41 – 7.45 (m, 2H, 2-H, 3-H, 5-H, 6-H), 7.78 (d, J = 9.2 Hz, 1H, CONH), 8.34 (d, J = 7.8 Hz, 1H, CONH), 8.97 (s, 1H, 2'-H), one signal (2H, CH_2N_3) is obscured by solvent signal; ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.9 ($CH_{3\ thiazole}$), 22.3 (CH<u>C</u>H$_3$), 24.9, 25.7 (CH_2), 26.4 (C(<u>C</u>H$_3$)$_3$), 27.9(CH_2), 34.7, 35.1 (CH_2, <u>C</u>(CH$_3$)$_3$), 37.7 (C-3''), 47.7 (<u>C</u>HCH$_3$), 50.5 (CH_2N_3), 56.2, 56.3, 58.5 (C-2'', C-5'', NHCH), 68.7 (C-4''), 126.3, 128.8 (C-2, C-3, C-5, C-6), 129.7, 131.1, 144.6 (C-1, C-4, C-5'), 147.7 (C-4'), 151.4 (C-2'), 169.6, 170.6, 171.9 (CO); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 6.16 min, 92% purity, *m/z* $[M + H]^+$ calcd for $C_{29}H_{41}N_7O_4S$ 584.30, found 584.4.

(2*S*,4*R*)-1-((*S*)-2-(8-Azidooctanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**VP2**)



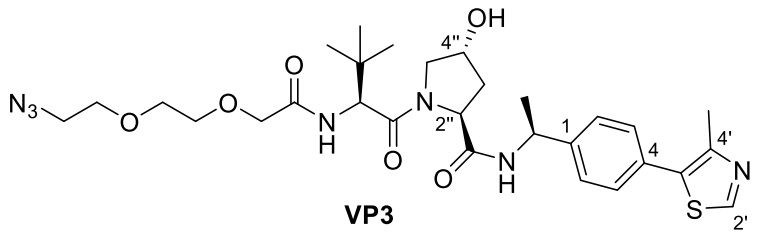
VP2

The Boc-protected VHL ligand **32** (872 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. 8-Azidooctanoic acid (**35**, 296 mg, 1.60 mmol) was dissolved in dry DMF (5 mL). HATU (669 mg, 1.76 mmol) and DIPEA (724 mg, 5.60 mmol) were added under argon. The mixture was allowed to stir for 30 min. The deprotected VHL ligand **33** was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was allowed to stir at room temperature under argon for 4 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a petroleum ether/EtOAc gradient (0% to100% EtOAc) as eluent to yield a colourless resin (436 mg).

Yield 45%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.94 (s, 9H, C(CH_3)$_3$), 1.26 – 1.32 (m, 6H, CH_2), 1.38 (d, J = 7.0 Hz, 3H, CHC<u>H</u>$_3$), 1.41 – 1.55 (m, 4H, CH_2), 1.77 – 1.83 (m, 1H), 1.98 – 2.04 (m, 1H, 3''-H), 2.08 – 2.14 (m, 1H), 2.22 – 2.28 (m, 1H, CH_2CON), 2.45 (s, 3H, $CH_{3\ thiazole}$), 3.59 – 3.63 (m, 2H, 5''-H), 4.25 – 4.31 (m, 1H), 4.42 (t, J = 8.0 Hz, 1H), 4.52 (d, J = 9.3 Hz, 1H, 2''-H, 4''-H, NHC<u>H</u>), 4.85 – 4.96 (m, 1H, C<u>H</u>CH$_3$), 5.08 (d, J = 3.6 Hz,

1H, OH), 7.36 – 7.40 (m, 2H), 7.40 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H), 7.76 (d, *J* = 9.3 Hz, 1H, CONH), 8.35 (d, *J* = 7.8 Hz, 1H, CONH), 8.98 (s, 1H, 2'-H), one signal (2H, CH_2N_3) is obscured by solvent signal; ^{13}C NMR (151 MHz, DMSO-d_6) δ 16.0 ($CH_{3\ thiazole}$), 22.4 (CH<u>C</u>H$_3$), 25.3, 26.0 (CH_2), 26.4 (C(<u>C</u>H$_3$)$_3$), 28.2, 28.2, 28.5 (CH_2), 34.8, 35.2 (CH_2, <u>C</u>(CH$_3$)$_3$), 37.7 (C-3''), 47.7 (<u>C</u>HCH$_3$), 50.6 (CH_2N_3), 56.2, 56.3, 58.5 (C-2'', C-5'', NHCH), 68.7 (C-4''), 126.4, 128.8 (C-2, C-3, C-5, C-6), 129.7, 131.1, 144.6 (C-1, C-4, C-5'), 147.7 (C-4'), 151.4 (C-2'), 169.6, 170.6, 172.0 (CO); LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 6.88 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{31}H_{45}N_7O_4S$ 612.33, found 612.5.

(2*S*,4*R*)-1-((*S*)-2-(2-(2-(2-Azidoethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**VP3**)



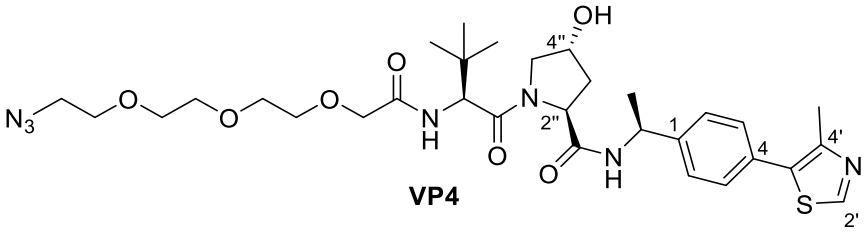
VP3

The Boc-protected VHL ligand **32** (872 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. *tert*-Butyl 2-(2-(2-azidoethoxy)ethoxy)acetate (**37**, 392 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (669 mg, 1.76 mmol) and DIPEA (724 mg, 5.60 mmol) were added under argon. The mixture was allowed to stir for 30 min. The deprotected VHL ligand **33** was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was allowed to stir at room temperature under argon for 4 h. The reaction was quenched by adding H_2O (25 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (75 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a colourless resin (403 mg).

Yield 41%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.95 (s, 9H, C(CH$_3$)$_3$), 1.38 (d, *J* = 7.0 Hz, 3H, CHC<u>H</u>$_3$), 1.75 – 1.82 (m, 1H), 2.01 – 2.08 (m, 1H, 3''-H), 2.46 (s, 3H, $CH_{3\ thiazole}$), 3.41 – 3.45 (m, 2H), 3.55 – 3.66 (m, 8H, 5''-H, CH_2), 3.91 – 4.01 (m, 2H, CH_2CON), 4.26 – 4.31 (m, 1H), 4.45 (t, *J* = 8.2 Hz, 1H), 4.55 (d, *J* = 9.6 Hz, 1H, 2''-H, 4''-H, NHC<u>H</u>), 4.88 – 4.95 (m, 1H, C<u>H</u>CH$_3$), 5.10 (d, *J* = 3.6 Hz, 1H, OH), 7.33 – 7.39 (m, 3H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 8.39 (d, *J* = 7.7 Hz, 1H, CONH), 8.97 (s, 1H, 2'-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.9 ($CH_{3\ thiazole}$), 22.4 (CH<u>C</u>H$_3$), 26.2 (C(<u>C</u>H$_3$)$_3$), 35.7 (<u>C</u>(CH$_3$)$_3$), 37.7 (C-3''), 47.7 (<u>C</u>HCH$_3$), 49.9 (CH_2N_3), 55.7, 56.5, 58.5 (C-2'', C-5'', NHCH), 68.7 (C-4''), 69.3, 69.4, 69.7, 70.4 (OCH_2), 126.3, 128.8 (C-2, C-3, C-5,

C-6), 129.7, 131.1, 144.6 (C-1, C-4, C-5'), 147.7 (C-4'), 151.4 (C-2'), 168.4, 169.0, 170.4 (CO); LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 15 min, DAD 220-400 nm), t_R = 9.13 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{29}H_{41}N_7O_6S$ 616.29, found 616.4.

(2*S*,4*R*)-1-((*S*)-14-Azido-2-(*tert*-butyl)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**VP4**)[41]

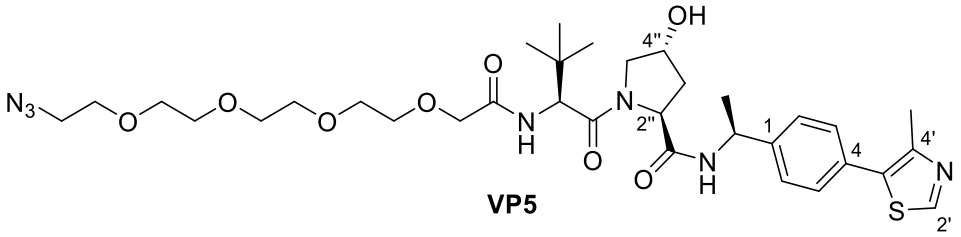


VP4

The Boc-protected VHL ligand **32** (872 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. *tert*-Butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (**39**, 463 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (669 mg, 1.76 mmol) and DIPEA (724 mg, 5.60 mmol) were added under argon. The mixture was allowed to stir for 30 min. The deprotected VHL ligand **33** was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was allowed to stir at room temperature under argon for 4 h. The reaction was quenched by adding H_2O (25 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (75 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a slightly yellow resin (794 mg).

Yield 75%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.95 (s, 9H, $C(CH_3)_3$), 1.38 (d, *J* = 7.0 Hz, 3H, $CHC\underline{H}_3$), 1.74 – 1.82 (m, 1H), 2.01 – 2.08 (m, 1H, 3''-H), 2.45 (s, 3H, $CH_{3\ thiazole}$), 3.38 – 3.41 (m, 2H), 3.57 – 3.63 (m, 12H, 5''-H, CH_2), 3.96 (s, 2H, CH_2CON), 4.26 – 4.31 (m, 1H), 4.45 (t, *J* = 8.3 Hz, 1H), 4.55 (d, *J* = 9.5 Hz, 1H, 2''-H, 4''-H, NHC<u>H</u>), 4.87 – 4.94 (m, 1H, C<u>H</u>CH_3), 5.10 (d, *J* = 3.6 Hz, 1H, OH), 7.33 – 7.39 (m, 3H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 8.40 (d, *J* = 7.7 Hz, 1H, CONH), 8.97 (s, 1H, 2'-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.9 ($CH_{3\ thiazole}$), 22.4 (CH<u>C</u>H_3), 26.2 ($C(\underline{C}H_3)_3$), 35.7 ($\underline{C}(CH_3)_3$), 37.7 (C-3''), 47.7 ($\underline{C}HCH_3$), 50.0 (CH_2N_3), 55.7, 56.5, 58.5 (C-2'', C-5'', NHCH), 68.7 (C-4''), 69.2, 69.6, 69.6, 69.9, 70.4 (OCH_2), 126.3, 128.8 (C-2, C-3, C-5, C-6), 129.7, 131.1, 144.6 (C-1, C-4, C-5'), 147.7 (C-4'), 151.4 (C-2'), 168.5, 169.0, 170.4 (CO), one signal (OCH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 15 min, DAD 220-400 nm), t_R = 9.24 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{31}H_{45}N_7O_7S$ 660.32, found 660.5.

(2*S*,4*R*)-1-((*S*)-17-Azido-2-(*tert*-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**VP5**)

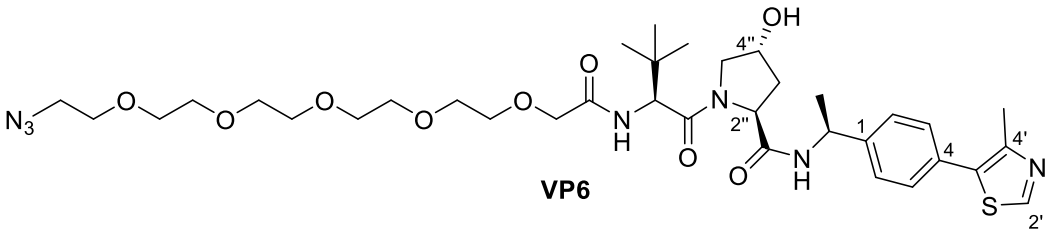


VP5

The Boc-protected VHL ligand **32** (545 mg, 1.00 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. *tert*-Butyl 14-azido-3,6,9,12-tetraoxatetradecanoate (**42**, 333 mg, 1.00 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (418 mg, 1.10 mmol) and DIPEA (452 mg, 3.50 mmol) were added under argon. The deprotected VHL ligand **33** was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was allowed to stir at room temperature under argon for 18 h. The mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a slightly yellow resin (507 mg).

Yield 72%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.95 (s, 9H, C(CH_3)$_3$), 1.38 (d, *J* = 7.0 Hz, 3H, CHC$\underline{H}_3$), 1.74 – 1.82 (m, 1H), 2.01 – 2.08 (m, 1H, 3''-H), 2.45 (s, 3H, $CH_{3\ thiazole}$), 3.37 – 3.40 (m, 2H), 3.53 – 3.63 (m, 16H, 5''-H, CH_2), 3.96 (s, 2H, CH_2CON), 4.26 – 4.30 (m, 1H), 4.45 (t, *J* = 8.1 Hz, 1H), 4.55 (d, *J* = 9.6 Hz, 1H, 2''-H, 4''-H, NHC$\underline{H}$), 4.87 – 4.94 (m, 1H, C$\underline{H}$CH$_3$), 5.11 (d, *J* = 3.5 Hz, 1H, OH), 7.33 – 7.39 (m, 3H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 8.41 (d, *J* = 7.7 Hz, 1H, CONH), 8.98 (s, 1H, 2'-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.9 ($CH_{3\ thiazole}$), 22.4 (CH$\underline{C}$H$_3$), 26.2 (C($\underline{C}$H$_3$)$_3$), 35.7 ($\underline{C}$(CH$_3$)$_3$), 37.7 (C-3''), 47.7 ($\underline{C}$HCH$_3$), 50.0 (CH_2N_3), 55.7, 56.5, 58.5 (C-2'', C-5'', NHCH), 68.7 (C-4''), 69.2, 69.6, 69.6, 69.7, 69.8, 69.8, 70.4 (OCH_2), 126.3, 128.8 (C-2, C-3, C-5, C-6), 129.7, 131.1, 144.7 (C-1, C-4, C-5'), 147.7 (C-4'), 151.4 (C-2'), 168.5, 169.0, 170.4 (CO), one signal (OCH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.99 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{33}H_{49}N_7O_8S$ 704.34, found 704.6.

(2*S*,4*R*)-1-((*S*)-20-Azido-2-(*tert*-butyl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**VP6**)

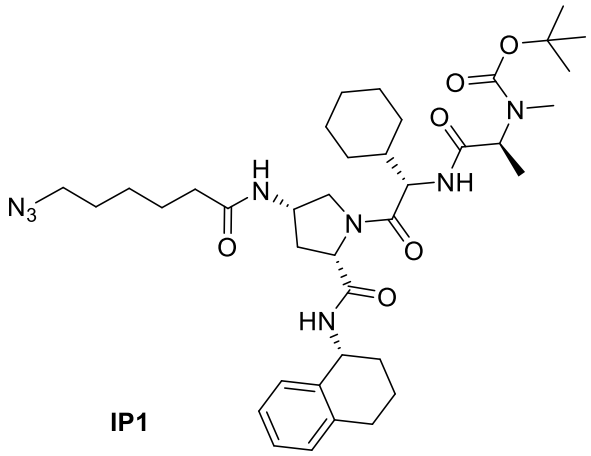


VP6

The Boc-protected VHL ligand **32** (599 mg, 1.10 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. *tert*-Butyl 17-azido-3,6,9,12,15-pentaoxaheptadecanoate (**45**, 415 mg, 1.10 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (460 mg, 1.21 mmol) and DIPEA (498 mg, 3.85 mmol) were added under argon. The deprotected VHL ligand **33** was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was allowed to stir at room temperature under argon for 18 h. The mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19+1) as eluent to yield a slightly yellow resin (598 mg).

Yield 73%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.94 (s, 9H, C(CH_3)$_3$), 1.38 (d, *J* = 7.0 Hz, 3H, CHC$\underline{H}_3$), 1.74 – 1.82 (m, 1H), 2.01 – 2.08 (m, 1H, 3''-H), 2.45 (s, 3H, $CH_{3\ thiazole}$), 3.37 – 3.40 (m, 2H), 3.52 – 3.56 (m, 12H), 3.57 – 3.63 (m, 8H, 5''-H, CH_2), 3.96 (s, 2H, CH_2CON), 4.27 – 4.30 (m, 1H), 4.45 (t, *J* = 8.2 Hz, 1H), 4.55 (d, *J* = 9.5 Hz, 1H, 2''-H, 4''-H, NHC$\underline{H}$), 4.88 – 4.94 (m, 1H, C$\underline{H}$CH$_3$), 5.12 (d, *J* = 3.5 Hz, 1H, OH), 7.33 – 7.40 (m, 3H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 8.41 (d, *J* = 7.7 Hz, 1H, CONH), 8.98 (s, 1H, 2'-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 16.0 ($CH_{3\ thiazole}$), 22.4 (CH$\underline{C}$H$_3$), 26.2 (C($\underline{C}$H$_3$)$_3$), 35.7 ($\underline{C}$(CH$_3$)$_3$), 37.7 (C-3''), 47.7 ($\underline{C}$HCH$_3$), 50.0 (CH_2N_3), 55.7, 56.5, 58.5 (C-2'', C-5'', NHCH), 68.8 (C-4''), 69.2, 69.6, 69.6, 69.7, 69.8, 69.8, 69.8, 69.8, 70.4 (OCH_2), 126.3, 128.8 (C-2, C-3, C-5, C-6), 129.7, 131.1, 144.7 (C-1, C-4, C-5'), 147.7 (C-4'), 151.4 (C-2'), 168.5, 169.0, 170.4 (CO), one signal (OCH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 6.01 min, 100% purity, *m/z* $[M + H]^+$ calcd for $C_{35}H_{53}N_7O_9S$ 748.37, found 748.6.

tert-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-(6-azidohexanamido)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**IP1**)

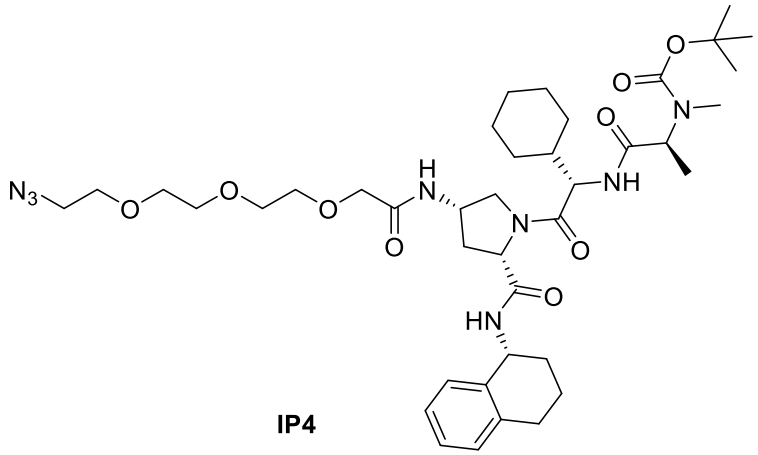


IP1

6-Azidohexanoic acid (**34**, 157 mg, 1.00 mmol) was dissolved in dry DMF (5 mL). HATU (418 mg, 1.10 mmol) and DIPEA (452 mg, 3.5 mmol) were added under argon. The IAP ligand **10** (584 mg, 1.00 mmol) was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was stirred at room temperature under argon for 18 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with saturated NH_4Cl solution (50 mL) and was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was washed brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a white solid (565 mg).

Yield 78%; mp: 50-56 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 – 1.04 (m, 2H), 1.07 – 1.23 (m, 6H), 1.28 – 1.34 (m, 2H), 1.41 (s, 9H), 1.49 – 1.56 (m, 4H), 1.57 – 1.78 (m, 8H), 1.81 – 1.90 (m, 3H), 2.07 (t, *J* = 7.4 Hz, 2H), 2.33 – 2.42 (m, 1H), 2.70 – 2.79 (m, 5H), 4.01 – 4.09 (m, 1H), 4.23 – 4.38 (m, 3H), 4.42 – 4.62 (m, 1H), 4.90 – 4.97 (m, 1H), 7.06 – 7.12 (m, 2H), 7.12 – 7.17 (m, 1H), 7.31 (d, *J* = 7.4 Hz, 1H), 7.61 – 7.89 (m, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 8.38 (d, *J* = 8.6 Hz, 1H), two overlapping signals (3H) are obscured by solvent signal at 3.30 ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ 20.2, 24.5, 25.5, 25.7, 25.8, 27.9, 28.0, 28.7, 29.7, 29.9, 34.3, 35.2, 46.6, 47.7, 50.5, 52.2, 54.8, 58.4, 78.9, 125.5, 126.5, 128.3, 128.4, 136.9, 137.3, 169.7, 170.8, 171.7; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 9.00 min, 91% purity, *m/z* $[M + H]^+$ calcd for $C_{38}H_{58}N_8O_6$ 723.46, found 723.5.

tert-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetamido)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**IP4**)

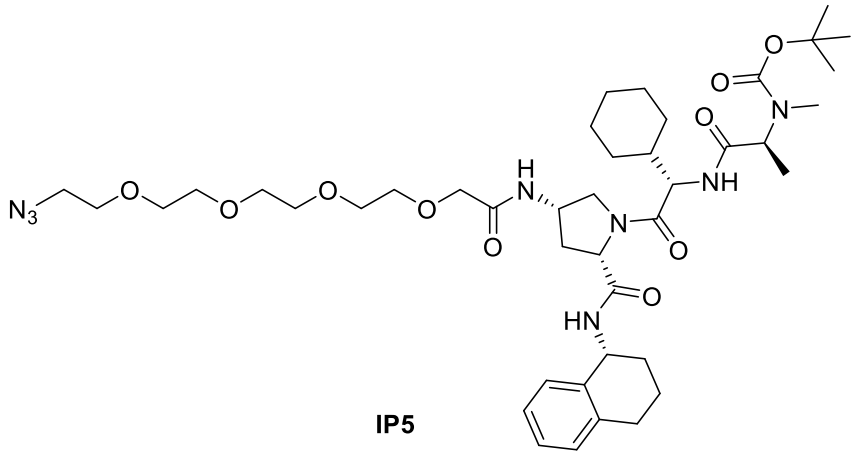


IP4

tert-Butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (**39**, 203 mg, 700 µmol) was dissolved in dry CH_2Cl_2 (5 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (293 mg, 770 µmol) and DIPEA (317 mg, 2.45 mmol) were added under argon. The IAP ligand **10** (409 mg, 700 µmol) was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was stirred at room temperature under argon for 18 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless resin (468 mg).

Yield 84%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.89 – 1.04 (m, 2H), 1.07 – 1.25 (m, 6H), 1.39 (s, 9H), 1.56 – 1.91 (m, 11H), 2.33 – 2.42 (m, 1H), 2.68 – 2.79 (m, 5H), 3.34 – 3.38 (m, 2H), 3.45 – 3.50 (m, 1H), 3.53 – 3.62 (m, 10H), 3.85 – 3.92 (m, 2H), 3.99 (dd, *J* = 10.1, 6.4 Hz, 1H), 4.27 – 4.39 (m, 2H), 4.40 – 4.62 (m, 2H), 4.89 – 4.98 (m, 1H), 7.05 – 7.12 (m, 2H), 7.12 – 7.17 (m, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.62 – 7.89 (m, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 20.2, 25.5, 25.8, 25.8, 28.0, 28.6, 28.7, 29.7, 30.0, 34.2, 46.7, 47.4, 50.0, 52.9, 54.8, 58.5, 69.2, 69.6, 69.8, 70.0, 70.4, 78.9, 125.6, 126.6, 128.3, 128.5, 136.9, 137.3, 169.0, 169.8, 171.1; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 8.22 min, 92% purity, *m/z* $[M + H]^+$ calcd for $C_{40}H_{62}N_8O_9$ 799.47, found 799.8.

tert-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-(14-azido-3,6,9,12-tetraoxatetradecanamido)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**IP5**)

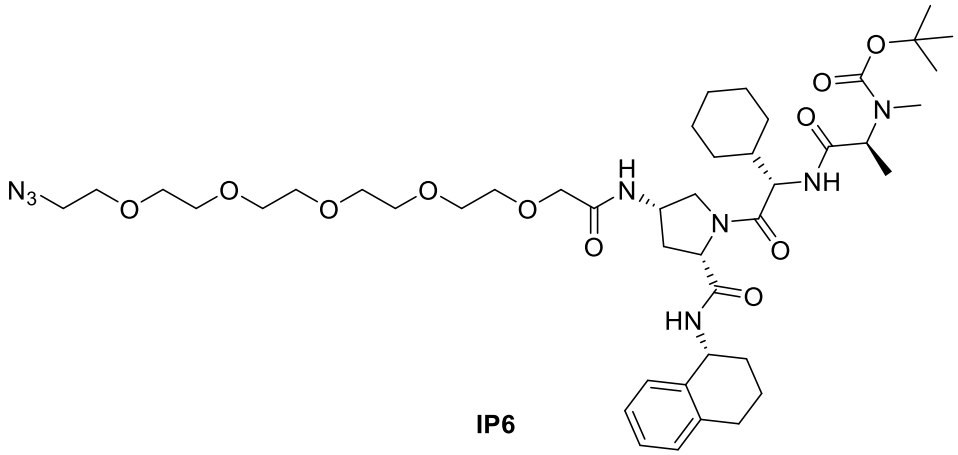


IP5

tert-Butyl 14-azido-3,6,9,12-tetraoxatetradecanoate (**42**, 400 mg, 1.20 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (502 mg, 1.32 mmol) and DIPEA (543 mg, 4.20 mmol) were added under argon. The IAP ligand **10** (701 mg, 1.20 mmol) was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was stirred at room temperature under argon for 18 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless resin (704 mg).

Yield 70%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.89 – 1.04 (m, 2H), 1.07 – 1.25 (m, 6H), 1.41 (s, 9H), 1.57 – 1.90 (m, 11H), 2.33 – 2.42 (m, 1H), 2.68 – 2.79 (m, 5H), 3.35 – 3.39 (m, 2H), 3.45 – 3.49 (m, 1H), 3.49 – 3.63 (m, 14H), 3.84 – 3.93 (m, 2H), 3.99 (dd, *J* = 10.1, 6.4 Hz, 1H), 4.27 – 4.37 (m, 2H), 4.40 – 4.62 (m, 2H), 4.90 – 4.98 (m, 1H), 7.05 – 7.12 (m, 2H), 7.12 – 7.17 (m, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.58 – 7.90 (m, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 20.2, 25.5, 25.8, 25.8, 28.0, 28.6, 28.7, 29.7, 30.0, 34.2, 46.7, 47.4, 50.0, 52.9, 54.8, 58.5, 69.2, 69.6, 69.7, 69.8, 69.8, 69.8, 70.0, 70.4, 78.9, 125.6, 126.6, 128.3, 128.5, 136.9, 137.3, 169.0, 169.8, 171.1; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 8.17 min, 90% purity, *m/z* $[M + H]^+$ calcd for $C_{42}H_{66}N_8O_{10}$ 843.50, found 843.8.

tert-Butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-(17-azido-3,6,9,12,15-pentaoxaheptadecanamido)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**IP6**)

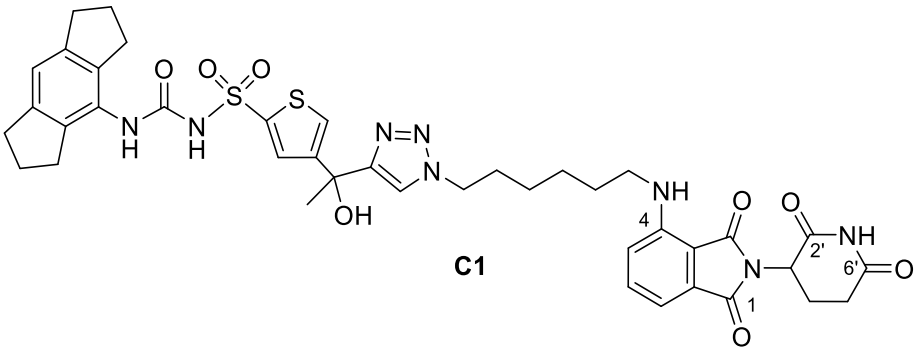


IP6

tert-Butyl 17-azido-3,6,9,12,15-pentaoxaheptadecanoate (**45**, 415 mg, 1.10 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. The deprotected linker was dissolved in dry DMF (5 mL). HATU (460 mg, 1.21 mmol) and DIPEA (498 mg, 3.85 mmol) were added under argon. The IAP ligand **10** (642 mg, 1.10 mmol) was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was stirred at room temperature under argon for 18 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless resin (598 mg).

Yield 61%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.88 – 1.04 (m, 2H), 1.07 – 1.24 (m, 6H), 1.35 – 1.43 (m, 9H), 1.57 – 1.89 (m, 11H), 2.32 – 2.44 (m, 1H), 2.68 – 2.79 (m, 5H), 3.35 – 3.41 (m, 2H), 3.42 – 3.67 (m, 19H), 3.84 – 3.92 (m, 2H), 3.99 (dd, *J* = 10.2, 6.4 Hz, 1H), 4.26 – 4.37 (m, 2H), 4.38 – 4.61 (m, 2H), 4.89 – 4.97 (m, 1H), 7.06 – 7.12 (m, 2H), 7.12 – 7.17 (m, 1H), 7.25 – 7.40 (m, 1H), 7.58 – 7.88 (m, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 20.2, 25.5, 25.8, 25.8, 28.0, 28.6, 28.7, 29.7, 30.0, 34.2, 46.7, 47.4, 50.0, 52.9, 54.8, 58.5, 69.2, 69.6, 69.7, 69.7, 69.7, 69.8, 69.8, 70.0, 70.4, 78.9, 125.6, 126.6, 128.3, 128.5, 136.9, 137.3, 169.0, 169.8, 171.1; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 8.12 min, 93% purity, *m/z* $[M + H]^+$ calcd for $C_{44}H_{70}N_8O_{11}$ 887.52, found 887.8.

4-(1-(1-(6-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C1**)

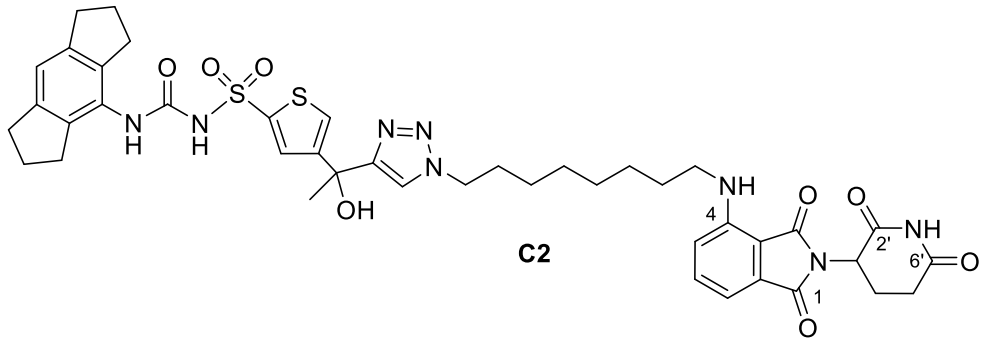


C1

Compound **C1** was synthesised following the general procedure for CuAAC and using azido-precursor **CP1** (150 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 10% MeOH) to yield a yellow solid (63 mg).

Yield 51%; mp: 153-159 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.23 – 1.30 (m, 2H), 1.31 – 1.38 (m, 2H), 1.52 – 1.58 (m, 2H, CH_2), 1.75 – 1.82 (m, 5H, CH_3, CH_2), 1.93 (quint, *J* = 7.5 Hz, 4H, $CH_{2\ indacene}$), 1.99 – 2.05 (m, 1H, 4'-H), 2.52 – 2.63 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.78 (t, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.84 – 2.92 (m, 1H, 5'-H), 3.24 – 3.29 (m, 2H), 4.28 (t, *J* = 7.2 Hz, 2H, CH_2), 5.04 (dd, *J* = 12.8, 5.4 Hz, 1H, 3'-H), 6.07 (s, 1H, OH), 6.51 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 6.93 (s, 1H, $CH_{arom.\ indacene}$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.55 – 7.59 (m, 1H, 6-H), 7.71 (d, *J* = 1.4 Hz, 1H), 7.74 (d, *J* = 1.2 Hz, 1H, $CH_{arom.\ thiophene}$), 7.89 (s, 1H, $CH_{arom.\ triazole}$), 8.10 (s, 1H, NHCO), 10.82 (s, 1H, SO_2NH), 11.07 (s, 1H, $NH_{\ imide}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 25.6, 25.6, 28.4, 29.6, 30.1, 30.6, 31.0, 32.4, 41.7, 48.5, 49.2, 69.5, 109.0, 110.4, 117.1, 118.0, 121.2, 127.5, 128.6, 132.1, 132.2, 136.2, 137.2, 140.1, 143.1, 146.4, 149.0, 149.9, 153.9, 167.3, 168.9, 170.0, 172.8; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.52 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{40}H_{44}N_8O_8S_2$ 829.28, found 829.5; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{40}H_{44}N_8O_8S_2$ 829.2796, found 829.2796.

4-(1-(1-(8-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C2**)

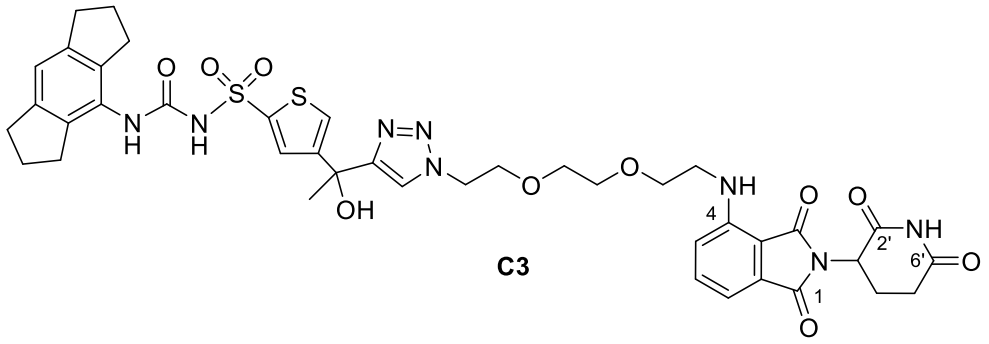


C2

Compound **C2** was synthesised following the general procedure for CuAAC and using azido-precursor **CP2** (150 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a yellow solid (65 mg).

Yield 50%; mp: 137-143 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.21 – 1.34 (m, 8H), 1.51 – 1.59 (m, 2H, CH_2), 1.73 – 1.81 (m, 5H, CH_3, CH_2), 1.92 (quint, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.00 – 2.05 (m, 1H, 4'-H), 2.51 – 2.62 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.78 (t, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.84 – 2.92 (m, 1H, 5'-H), 3.25 – 3.29 (m, 2H), 4.26 (t, *J* = 7.2 Hz, 2H, CH_2), 5.04 (dd, *J* = 12.8, 5.4 Hz, 1H, 3'-H), 6.05 (s, 1H, OH), 6.50 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 6.92 (s, 1H, $CH_{arom.\ indacene}$), 7.01 (d, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H, 5-H, 7-H), 7.55 – 7.60 (m, 1H, 6-H), 7.68 (s, 1H), 7.71 (s, 1H, $CH_{arom.\ thiophene}$), 7.87 (s, 1H, $CH_{arom.\ triazole}$), 8.07 (s, 1H, NHCO), 10.85 (s, 1H, SO_2NH), 11.07 (s, 1H, NH_{imide}); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 25.8, 26.2, 28.3, 28.5, 28.6, 29.6, 30.1, 30.6, 31.0, 32.4, 41.8, 48.5, 49.2, 69.5, 109.0, 110.3, 117.1, 117.8, 121.1, 127.1, 128.9, 131.7, 132.2, 136.2, 137.2, 140.8, 143.0, 146.4, 149.8, 153.9, 167.3, 168.9, 170.0, 172.7; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 6.06 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{42}H_{48}N_8O_8S_2$ 857.31, found 857.5; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{42}H_{48}N_8O_8S_2$ 857.3109, found 857.3104.

4-(1-(1-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C3**)

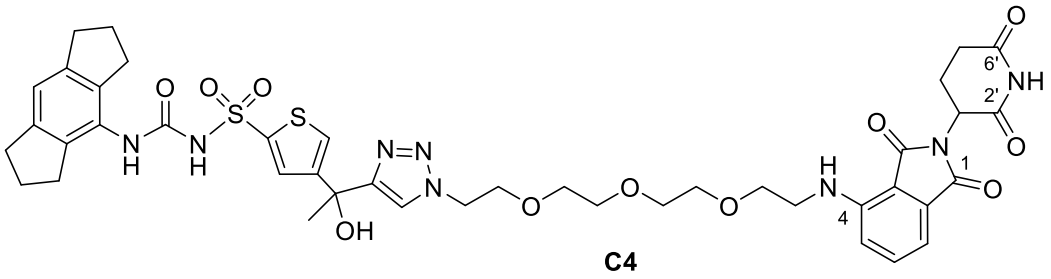


C3

Compound **C3** was synthesised following the general procedure for CuAAC and using azido-precursor **CP3** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a yellow solid (106 mg).

Yield 62%; mp: 142-148 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.78 (s, 3H, CH_3), 1.88 – 1.96 (m, 4H, $CH_{2\ indacene}$), 1.99 – 2.04 (m, 1H, 4'-H), 2.51 – 2.64 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.77 (t, *J* = 7.2 Hz, 4H, $CH_{2\ indacene}$), 2.83 – 2.91 (m, 1H, 5'-H), 3.41 – 3.45 (m, 2H), 3.49 – 3.53 (m, 4H), 3.56 (t, *J* = 5.4 Hz, 2H), 3.79 (t, *J* = 5.1 Hz, 2H), 4.45 (t, *J* = 5.3 Hz, 2H, CH_2), 5.04 (dd, *J* = 12.8, 5.4 Hz, 1H, 3'-H), 6.10 (s, 1H, OH), 6.58 (t, *J* = 5.9 Hz, 1H, $CH_2N\underline{H}$), 6.91 (s, 1H, $CH_{arom.\ indacene}$), 7.03 (d, *J* = 7.0 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H, 5-H, 7-H), 7.54 – 7.59 (m, 1H, 6-H), 7.60 – 7.80 (m, 2H, $CH_{arom.\ thiophene}$), 7.88 (s, 1H, $CH_{arom.\ triazole}$), 10.89 (s, 1H, SO_2NH), 11.07 (s, 1H, NH_{imide}), one signal (1H, NHCO) is missing; ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 30.1, 30.5, 30.9, 32.4, 41.6, 48.5, 49.3, 68.6, 68.8, 69.5, 69.5, 109.2, 110.6, 117.4, 117.7, 121.7, 127.2, 132.0, 136.1, 137.1, 142.9, 146.3, 149.9, 154.0, 167.2, 168.9, 170.0, 172.7; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 4.96 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{40}H_{44}N_8O_{10}S_2$ 861.27, found 861.5; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{40}H_{44}N_8O_{10}S_2$ 861.2695, found 861.2701.

4-(1-(1-(2-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C4**)

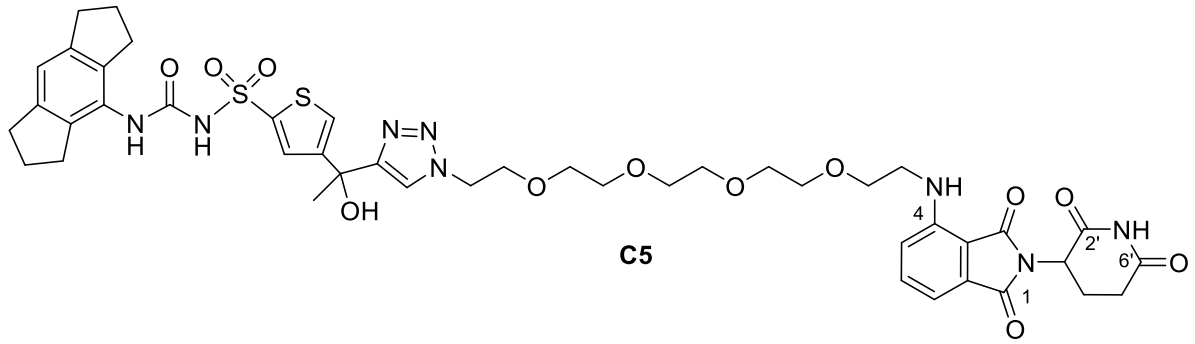


C4

Compound **C4** was synthesised following the general procedure for CuAAC and using azido-precursor **CP4** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a yellow solid (80 mg).

Yield 44%; mp: 145-151 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.79 (s, 3H, CH_3), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 1.99 – 2.05 (m, 1H, 4'-H), 2.52 – 2.62 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.83 – 2.91 (m, 1H, 5'-H), 3.42 – 3.50 (m, 8H), 3.51 – 3.54 (m, 2H), 3.60 (t, J = 5.4 Hz, 2H), 3.77 (t, J = 5.3 Hz, 2H), 4.44 (t, J = 5.0 Hz, 2H, CH_2), 5.04 (dd, J = 12.9, 5.4 Hz, 1H, 3'-H), 6.10 (s, 1H, OH), 6.58 (t, J = 5.8 Hz, 1H, $CH_2N\underline{H}$), 6.93 (s, 1H, $CH_{arom.\ indacene}$), 7.03 (d, J = 7.0 Hz, 1H), 7.12 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.54 – 7.59 (m, 1H, 6-H), 7.71 (s, 1H), 7.74 (s, 1H, $CH_{arom.\ thiophene}$), 7.86 (s, 1H, $CH_{arom.\ triazole}$), 8.10 (s, 1H, NHCO), 10.89 (s, 1H, SO_2NH), 11.08 (s, 1H, $NH_{\ imide}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 30.1, 30.6, 31.0, 32.4, 41.7, 48.6, 49.3, 68.6, 68.9, 69.5, 69.7, 69.7, 109.2, 110.7, 117.4, 118.0, 121.6, 127.6, 128.6, 132.1, 132.1, 136.2, 137.3, 140.1, 143.1, 146.4, 149.0, 149.9, 153.9, 167.3, 168.9, 170.1, 172.8; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.44 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{42}H_{48}N_8O_{11}S_2$ 905.30, found 905.6; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{42}H_{48}N_8O_{11}S_2$ 905.2957, found 905.2976.

4-(1-(1-(14-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C5**)

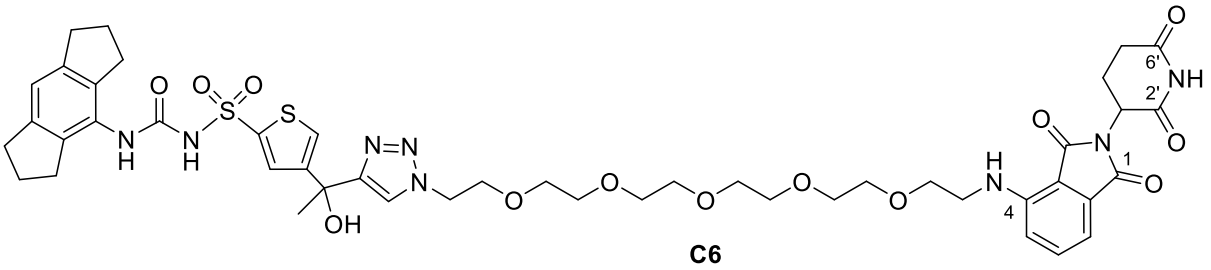


C5

Compound **C5** was synthesised following the general procedure for CuAAC and using azido-precursor **CP5** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a yellow solid (105 mg).

Yield 55%; mp: 104-110 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.79 (s, 3H, CH_3), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 1.99 – 2.05 (m, 1H, 4'-H), 2.51 – 2.62 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.84 – 2.92 (m, 1H, 5'-H), 3.43 – 3.49 (m, 10H), 3.50 – 3.52 (m, 2H), 3.53 – 3.56 (m, 2H), 3.61 (t, J = 5.5 Hz, 2H), 3.77 (t, J = 5.3 Hz, 2H), 4.43 – 4.47 (m, 2H, CH_2), 5.05 (dd, J = 12.8, 5.4 Hz, 1H, 3'-H), 6.10 (s, 1H, OH), 6.59 (t, J = 5.9 Hz, 1H, CH_2N<u>H</u>), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.04 (d, J = 7.0 Hz, 1H), 7.13 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 8.4, 7.2 Hz, 1H, 6-H), 7.72 (d, J = 1.7 Hz, 1H), 7.75 (d, J = 1.7 Hz, 1H, $CH_{arom.\ thiophene}$), 7.87 (s, 1H, $CH_{arom.\ triazole}$), 8.11 (s, 1H, CONH), 10.85 (s, 1H, SO_2NH), 11.07 (s, 1H, NH $_{imide}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 30.1, 30.6, 31.0, 32.4, 41.7, 48.5, 49.3, 68.6, 68.9, 69.5, 69.6, 69.7, 69.7, 69.8, 109.2, 110.6, 117.4, 118.0, 121.6, 127.6, 128.6, 132.1, 132.1, 136.2, 137.2, 140.0, 143.1, 146.4, 148.9, 149.9, 153.9, 167.3, 168.9, 170.0, 172.7; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.13 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{44}H_{52}N_8O_{12}S_2$ 949.32, found 949.6; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{44}H_{52}N_8O_{12}S_2$ 949.3219, found 949.3212.

4-(1-(1-(17-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaheptadecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C6**)

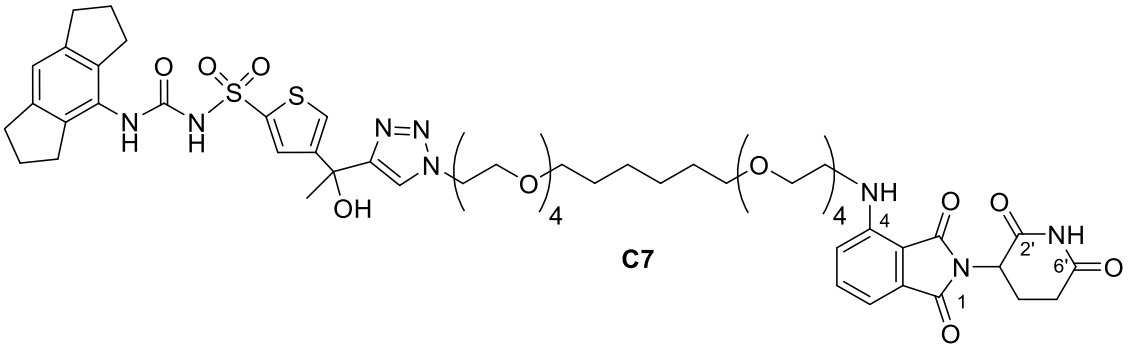


C6

Compound **C6** was synthesised following the general procedure for CuAAC and using azido-precursor **CP6** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a yellow solid (82 mg).

Yield 44%; mp: 100-106 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.79 (s, 3H, CH_3), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.00 – 2.05 (m, 1H, 4'-H), 2.51 – 2.62 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.84 – 2.92 (m, 1H, 5'-H), 3.43 – 3.50 (m, 14H), 3.51 – 3.53 (m, 2H), 3.54 – 3.57 (m, 2H), 3.61 (t, J = 5.5 Hz, 2H), 3.78 (t, J = 5.3 Hz, 2H), 4.46 (t, J = 5.3 Hz, 2H, CH_2), 5.05 (dd, J = 12.8, 5.4 Hz, 1H, 3'-H), 6.09 (s, 1H, OH), 6.59 (t, J = 5.9 Hz, 1H, CH_2N<u>H</u>), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.04 (d, J = 7.0 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 8.3, 7.3 Hz, 1H, 6-H), 7.72 (d, J = 1.6 Hz, 1H), 7.75 (d, J = 1.5 Hz, 1H, $CH_{arom.\ thiophene}$), 7.87 (s, 1H, $CH_{arom.\ triazole}$), 8.10 (s, 1H, CONH), 10.87 (s, 1H, SO_2NH), 11.07 (s, 1H, $NH_{\ imide}$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 30.1, 30.6, 31.0, 32.4, 41.7, 48.5, 49.3, 68.6, 68.9, 69.5, 69.6, 69.7, 69.7, 69.8, 109.2, 110.6, 117.4, 118.0, 121.6, 127.6, 128.6, 132.0, 132.1, 136.2, 137.2, 140.1, 143.1, 146.4, 149.0, 149.9, 153.9, 167.3, 168.9, 170.0, 172.7; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.74 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{46}H_{56}N_8O_{13}S_2$ 993.35, found 993.6; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{46}H_{56}N_8O_{13}S_2$ 993.3481, found 993.3468.

4-(1-(1-(30-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,19,22,25,28-octaoxatriacontyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxyethyl)-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)thiophene-2-sulfonamide (**C7**)

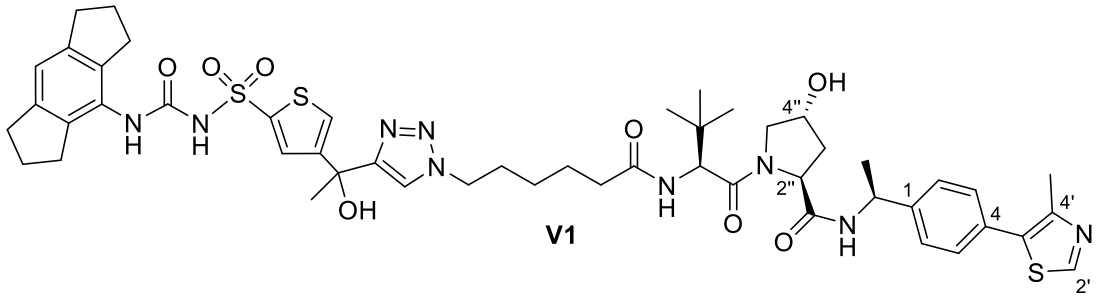


C7

Compound **C7** was synthesised following the general procedure for CuAAC and using azido-precursor **CP7** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a yellow solid (111 mg).

Yield 47%; mp: 88-94 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.24 – 1.27 (m, 4H), 1.41 – 1.49 (m, 4H, CH_2), 1.79 (s, 3H, CH_3), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 1.99 – 2.05 (m, 1H, 4'-H), 2.51 – 2.62 (m, 6H, 4'-H, 5'-H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.84 – 2.92 (m, 1H, 5'-H), 3.32 – 3.35 (m, 4H), 3.41 – 3.52 (m, 22H), 3.52 – 3.54 (m, 2H), 3.55 – 3.58 (m, 2H), 3.62 (t, J = 5.5 Hz, 2H), 3.78 (t, J = 5.3 Hz, 2H), 4.41 – 4.50 (m, 2H, CH_2), 5.05 (dd, J = 12.8, 5.4 Hz, 1H, 3'-H), 6.09 (s, 1H, OH), 6.60 (t, J = 5.8 Hz, 1H, $CH_2N\underline{H}$), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.04 (d, J = 7.0 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.58 (dd, J = 8.4, 7.2 Hz, 1H, 6-H), 7.71 (s, 1H), 7.74 (s, 1H, $CH_{arom.\ thiophene}$), 7.87 (s, 1H, $CH_{arom.\ triazole}$), 8.11 (s, 1H, CONH), 10.87 (s, 1H, SO_2NH), 11.07 (s, 1H, NH_{imide}); ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.1, 25.0, 25.5, 29.2, 30.1, 30.6, 31.0, 32.4, 41.7, 48.5, 49.3, 68.6, 68.9, 69.4, 69.5, 69.6, 69.7, 69.7, 69.8, 69.8, 69.8, 70.2, 109.2, 110.6, 117.4, 118.0, 121.6, 127.5, 128.7, 132.0, 132.1, 136.2, 137.2, 143.1, 146.4, 149.9, 153.9, 167.3, 168.9, 170.0, 172.7; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.93 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{56}H_{76}N_8O_{16}S_2$ 1181.49, found 1181.8; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{56}H_{76}N_8O_{16}S_2$ 1181.4893, found 1181.4895.

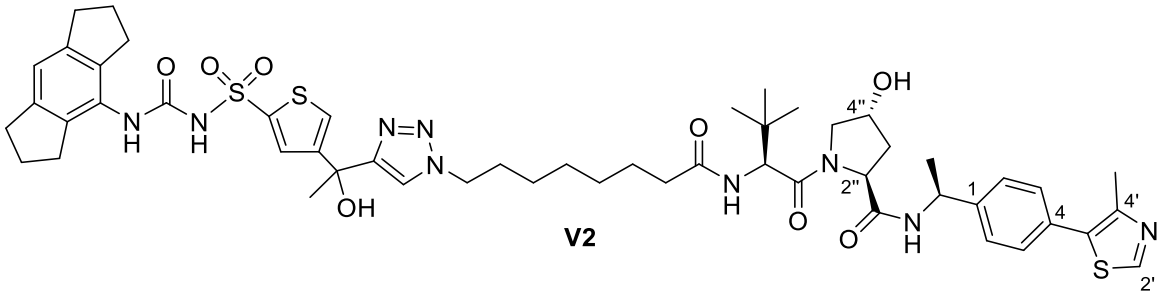
(2*S*,4*R*)-1-((2*S*)-2-(6-(4-(1-(5-(*N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**V1**)



Compound **V1** was synthesised following the general procedure for CuAAC and using azido-precursor **VP1** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (121 mg).

Yield 60%; mp: 135-141 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.93 (s, 9H, $C(CH_3)_3$), 1.19 – 1.24 (m, 2H, CH_2), 1.37 (d, J = 7.0 Hz, 3H, $CHCH_3$), 1.46 – 1.55 (m, 2H, CH_2), 1.74 – 1.83 (m, 6H, CH_2, 3'-H, CH_3CO), 1.93 (quint, J = 7.3 Hz, 4H, $CH_{2\ indacene}$), 1.98 – 2.03 (m, 1H, 3''-H), 2.08 – 2.15 (m, 1H), 2.20 – 2.27 (m, 1H, CH_2CON), 2.45 (s, 3H, $CH_{3\ thiazole}$), 2.58 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.79 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.55 – 3.65 (m, 2H, 5''-H), 4.22 – 4.31 (m, 3H), 4.42 (t, J = 8.0 Hz, 1H), 4.51 (d, J = 9.3 Hz, 1H, 2''-H, 4''-H, NHCH, CH_2), 4.92 (quint, J = 7.2 Hz, 1H, $CHCH_3$), 5.08 (d, J = 2.8 Hz, 1H, CHOH), 6.08 (s, 1H, COH), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.33 – 7.40 (m, 2H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H), 7.70 – 7.73 (m, 1H), 7.73 – 7.76 (m, 1H, $CH_{arom.\ thiophene}$), 7.78 (d, J = 9.3 Hz, 1H, CONH), 7.89 (s, 1H, $CH_{arom.\ triazole}$), 8.11 (s, 1H, NHCON), 8.34 (d, J = 7.8 Hz, 1H, CONH), 8.98 (s, 1H, 2'-H), 10.91 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.9, 22.4, 24.8, 25.0, 25.5, 26.4, 29.4, 30.1, 30.7, 32.4, 34.6, 35.1, 37.7, 47.7, 49.2, 56.2, 56.3, 58.5, 68.7, 69.5, 118.0, 121.1, 126.3, 127.7, 128.5, 128.8, 129.7, 131.1, 132.1, 137.2, 140.0, 143.1, 144.6, 147.7, 148.9, 149.9, 151.4, 153.9, 169.6, 170.6, 171.8; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.60 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{50}H_{63}N_9O_8S_3$ 1014.40, found 1014.7; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{50}H_{63}N_9O_8S_3$ 1014.4034, found 1014.4036.

(2*S*,4*R*)-1-((2*S*)-2-(8-(4-(1-(5-(*N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**V2**)

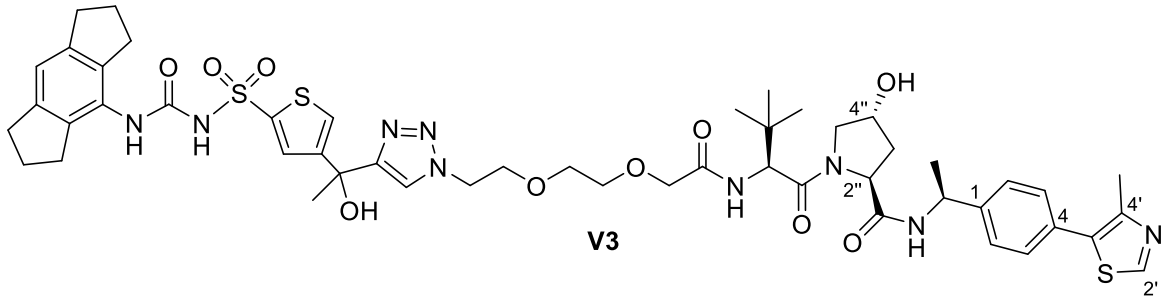


V2

Compound **V2** was synthesised following the general procedure for CuAAC and using azido-precursor **VP2** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (93 mg).

Yield 45%; mp: 126-132 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.93 (s, 9H, $C(CH_3)_3$), 1.14 – 1.25 (m, 6H, CH_2), 1.37 (d, J = 7.0 Hz, 3H, $CHC\underline{H}_3$), 1.41 – 1.52 (m, 2H, CH_2), 1.72 – 1.83 (m, 6H, CH_2, 3’’-H, CH_3CO), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 1.98 – 2.03 (m, 1H, 3’’-H), 2.07 – 2.13 (m, 1H), 2.20 – 2.27 (m, 1H, CH_2CON), 2.45 (s, 3H, $CH_{3\ thiazole}$), 2.58 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.55 – 3.66 (m, 2H, 5’’-H), 4.22 – 4.31 (m, 3H), 4.42 (t, J = 8.0 Hz, 1H), 4.52 (d, J = 9.3 Hz, 1H, 2’’-H, 4’’-H, NHC<u>H</u>, CH_2), 4.89 – 4.95 (m, 1H, C<u>H</u>CH_3), 5.08 (d, J = 3.5 Hz, 1H, CHO<u>H</u>), 6.07 (s, 1H, COH), 6.93 (s, 1H, $CH_{arom.\ indacene}$), 7.38 (d, J = 8.2 Hz, 2H), 7.42 – 7.45 (m, 2H, 2-H, 3-H, 5-H, 6-H), 7.70 (s, 1H), 7.71 – 7.78 (m, 2H, $CH_{arom.\ thiophene}$, CONH), 7.88 (s, 1H, $CH_{arom.\ triazole}$), 8.02 – 8.17 (m, 1H, NHCON), 8.34 (d, J = 7.8 Hz, 1H, CONH), 8.98 (s, 1H, 2’-H), 10.87 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 16.0, 22.4, 25.0, 25.3, 25.8, 26.4, 28.1, 28.4, 29.7, 30.1, 30.6, 32.4, 34.8, 35.2, 37.7, 47.7, 49.2, 56.2, 56.3, 58.5, 68.7, 69.5, 117.9, 121.2, 126.4, 127.4, 128.8, 129.7, 131.1, 131.9, 137.2, 140.4, 143.0, 144.6, 147.7, 149.9, 151.4, 153.9, 169.6, 170.6, 172.0; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.76 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{52}H_{67}N_9O_8S_3$ 1042.43, found 1042.7; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{52}H_{67}N_9O_8S_3$ 1042.4347, found 1042.4346.

(2*S*,4*R*)-1-((2*S*)-2-(2-(2-(2-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**V3**)

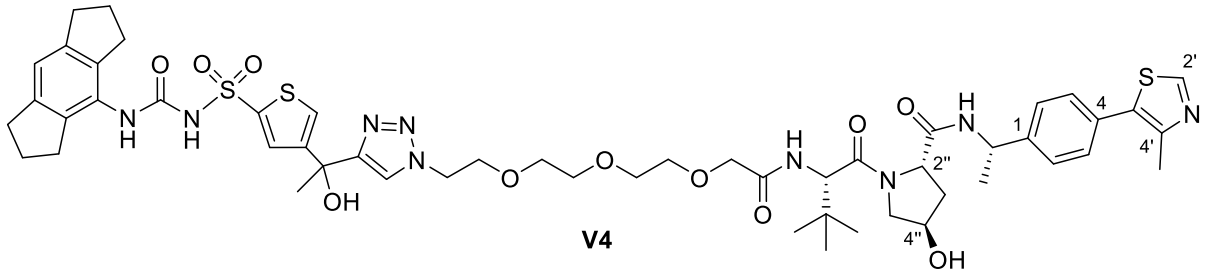


V3

Compound **V3** was synthesised following the general procedure for CuAAC and using azido-precursor **VP3** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (112 mg).

Yield 54%; mp: 127-133 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.93 (s, 9H, C(CH_3)$_3$), 1.31 – 1.38 (m, 3H, CHC<u>H</u>$_3$), 1.75 – 1.81 (m, 4H, 3''-H, CH_3CO), 1.93 (quint, *J* = 7.3 Hz, 4H, $CH_{2\ indacene}$), 2.02 – 2.08 (m, 1H, 3''-H), 2.45 (s, 3H, $CH_{3\ thiazole}$), 2.59 (t, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.78 (t, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.52 – 3.63 (m, 6H, 5''-H, CH_2), 3.79 – 3.86 (m, 2H, CH_2), 3.87 – 3.97 (m, 2H, CH_2CON), 4.27 – 4.31 (m, 1H), 4.42 – 4.47 (m, 1H), 4.48 – 4.53 (m, 2H), 4.55 (d, *J* = 9.5 Hz, 1H, 2''-H, 4''-H, NHC<u>H</u>, CH_2), 4.87 – 4.93 (m, 1H, C<u>H</u>CH_3), 5.12 (d, *J* = 2.8 Hz, 1H, CHO<u>H</u>), 6.10 (s, 1H, COH), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.33 – 7.45 (m, 5H, 2-H, 3-H, 5-H, 6-H, CONH), 7.70 – 7.74 (m, 1H), 7.74 – 7.77 (m, 1H, $CH_{arom.\ thiophene}$), 7.89 – 7.92 (m, 1H, $CH_{arom.\ triazole}$), 8.11 (s, 1H, NHCON), 8.36 – 8.45 (m, 1H, CONH), 8.98 (s, 1H, 2'-H), 10.87 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 16.0, 22.4, 25.0, 26.2, 30.1, 30.6, 32.4, 35.8, 37.7, 47.7, 49.2, 55.7, 56.5, 58.6, 68.8, 68.8, 69.4, 69.5, 69.6, 70.3, 118.0, 121.7, 126.3, 127.6, 128.6, 128.8, 129.7, 131.1, 132.0, 137.2, 140.1, 143.1, 144.6, 147.7, 149.9, 151.4, 153.9, 168.5, 169.0, 170.4; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.50 min, 96% purity, *m/z* [M + H]$^+$ calcd for $C_{50}H_{63}N_9O_{10}S_3$ 1046.39, found 1046.7; HRMS (ESI) *m/z* [M + H]$^+$ calcd for $C_{50}H_{63}N_9O_{10}S_3$ 1046.3933, found 1046.3933.

(2*S*,4*R*)-1-((2*S*)-2-(*tert*-Butyl)-14-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**V4**)

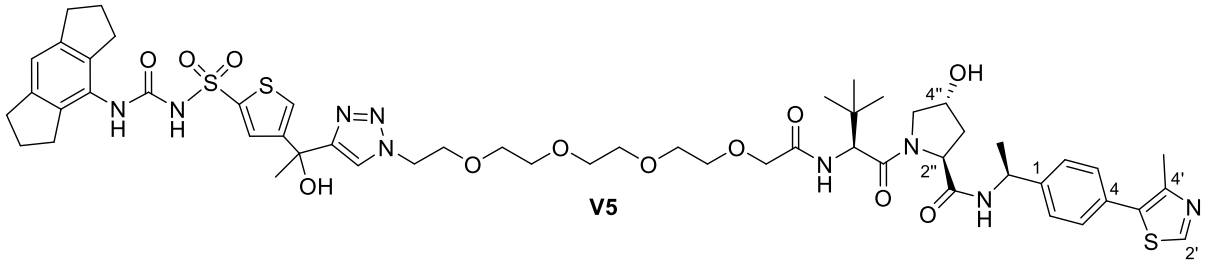


V4

Compound **V4** was synthesised following the general procedure for CuAAC and using azido-precursor **VP4** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a slightly beige solid (63 mg).

Yield 29%; mp: 113-119 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.94 (s, 9H, C(CH_3)$_3$), 1.36 (d, *J* = 7.0 Hz, 3H, CHC<u>H</u>$_3$), 1.74 – 1.81 (m, 4H, 3’’-H, CH_3CO), 1.93 (quint, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.02 – 2.08 (m, 1H, 3’’-H), 2.45 (s, 3H, $CH_{3\ thiazole}$), 2.59 (t, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.78 (t, *J* = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.49 – 3.55 (m, 6H), 3.55 – 3.62 (m, 4H, 5’’-H, CH_2), 3.80 (t, *J* = 5.4 Hz, 2H, CH_2), 3.95 (s, 2H, CH_2CON), 4.26 – 4.31 (m, 1H), 4.43 – 4.49 (m, 3H), 4.55 (d, *J* = 9.5 Hz, 1H, 2’’-H, 4’’-H, NHC<u>H</u>, CH_2), 4.87 – 4.93 (m, 1H, C<u>H</u>CH_3), 5.11 (d, *J* = 3.4 Hz, 1H, CHO<u>H</u>), 6.10 (s, 1H, COH), 6.93 (s, 1H, $CH_{arom.\ indacene}$), 7.33 – 7.39 (m, 3H), 7.41 – 7.45 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 7.71 (s, 1H), 7.74 (s, 1H, $CH_{arom.\ thiophene}$), 7.88 (s, 1H, $CH_{arom.\ triazole}$), 8.10 (s, 1H, NHCON), 8.41 (d, *J* = 7.7 Hz, 1H, CONH), 8.98 (s, 1H, 2’-H), 10.86 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 16.0, 22.4, 25.0, 26.2, 30.1, 30.6, 32.4, 35.7, 37.7, 47.7, 49.3, 55.7, 56.5, 58.5, 68.7, 68.7, 69.5, 69.6, 69.6, 69.7, 70.4, 117.9, 121.6, 126.3, 127.4, 128.8, 129.7, 131.1, 131.9, 137.2, 143.0, 144.7, 147.7, 149.9, 151.5, 153.9, 168.5, 169.0, 170.4; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.64 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{52}H_{67}N_9O_{11}S_3$ 1090.42, found 1090.7; HRMS (ESI) *m/z* $[M + Na]^+$ calcd for $C_{52}H_{67}N_9O_{11}S_3$ 1112.4014, found 1112.4067.

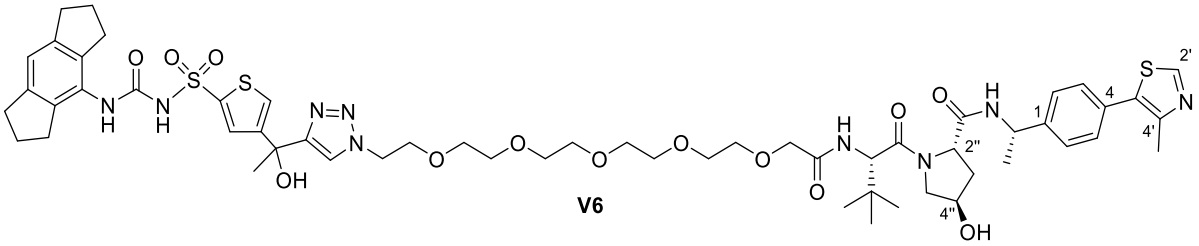
(2*S*,4*R*)-1-((2*S*)-2-(*tert*-Butyl)-17-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**V5**)



Compound **V5** was synthesised following the general procedure for CuAAC and using azido-precursor **VP5** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (106 mg).

Yield 47%; mp: 107-113 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.94 (s, 9H, $C(CH_3)_3$), 1.37 (d, J = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.73 – 1.84 (m, 4H, 3''-H, CH_3CO), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.02 – 2.08 (m, 1H, 3''), 2.45 (s, 3H, $CH_{3\ thiazole}$), 2.58 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.45 – 3.63 (m, 14H, 5''-H, CH_2), 3.79 (t, J = 5.3 Hz, 2H, CH_2), 3.92 – 3.99 (m, 2H, CH_2CON), 4.25 – 4.31 (m, 1H), 4.41 – 4.50 (m, 3H), 4.55 (d, J = 9.6 Hz, 1H, 2''-H, 4''-H, NHC<u>H</u>, CH_2), 4.87 – 4.94 (m, 1H, C<u>H</u>CH_3), 5.11 (d, J = 3.0 Hz, 1H, CHO<u>H</u>), 6.10 (s, 1H, COH), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.33 – 7.40 (m, 3H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 7.70 – 7.73 (m, 1H), 7.73 – 7.77 (m, 1H, $CH_{arom.\ thiophene}$), 7.88 (s, 1H, $CH_{arom.\ triazole}$), 8.12 (s, 1H, NHCON), 8.41 (d, J = 7.7 Hz, 1H, CONH), 8.98 (s, 1H, 2'-H), 10.85 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 16.0, 22.4, 25.0, 26.2, 30.1, 30.6, 32.4, 35.7, 37.7, 47.7, 49.3, 55.7, 56.5, 58.5, 68.6, 68.7, 69.5, 69.6, 69.6, 69.6, 69.7, 69.8, 70.4, 118.0, 121.6, 126.3, 127.6, 128.6, 128.8, 129.7, 131.1, 132.0, 137.2, 143.1, 144.7, 147.8, 149.9, 151.5, 153.9, 168.5, 169.0, 170.4; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.49 min, 95% purity, m/z $[M + H]^+$ calcd for $C_{54}H_{71}N_9O_{12}S_3$ 1134.45, found 1134.7; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{54}H_{71}N_9O_{12}S_3$ 1134.4457, found 1134.4444.

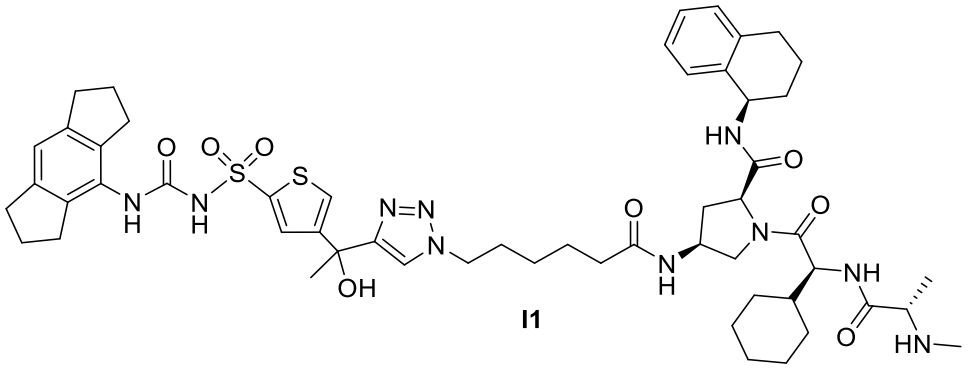
(2*S*,4*R*)-1-((2*S*)-2-(*tert*-Butyl)-20-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**V6**)



Compound **V6** was synthesised following the general procedure for CuAAC and using azido-precursor **VP6** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (100 mg).

Yield 42%; mp: 92-98 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.94 (s, 9H, $C(CH_3)_3$), 1.37 (d, J = 7.0 Hz, 3H, CHC<u>H</u>$_3$), 1.75 – 1.81 (m, 4H, 3’’-H, CH_3CO), 1.93 (quint, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.02 – 2.07 (m, 1H, 3’’-H), 2.45 (s, 3H, $CH_{3\ thiazole}$), 2.58 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 2.78 (t, J = 7.4 Hz, 4H, $CH_{2\ indacene}$), 3.45 – 3.54 (m, 12H), 3.55 – 3.63 (m, 6H, 5’’-H, CH_2), 3.79 (t, J = 5.3 Hz, 2H, CH_2), 3.96 (s, 2H, CH_2CON), 4.26 – 4.31 (m, 1H), 4.42 – 4.48 (m, 3H), 4.54 (d, J = 9.5 Hz, 1H, 2’’-H, 4’’-H, NHC<u>H</u>, CH_2), 4.91 (quint, J = 7.1 Hz, 1H, C<u>H</u>CH_3), 5.11 (d, J = 2.9 Hz, 1H, CHO<u>H</u>), 6.10 (s, 1H, COH), 6.94 (s, 1H, $CH_{arom.\ indacene}$), 7.32 – 7.40 (m, 3H), 7.41 – 7.46 (m, 2H, 2-H, 3-H, 5-H, 6-H, CONH), 7.70 – 7.73 (m, 1H), 7.73 – 7.76 (m, 1H, $CH_{arom.\ thiophene}$), 7.88 (s, 1H, $CH_{arom.\ triazole}$), 8.11 (s, 1H, NHCON), 8.41 (d, J = 7.7 Hz, 1H, CONH), 8.98 (s, 1H, 2’-H), 10.91 (s, 1H, SO_2NH); ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.9, 22.4, 25.0, 26.2, 30.1, 30.6, 32.4, 35.7, 37.7, 47.7, 49.3, 55.7, 56.5, 58.5, 68.6, 68.7, 69.5, 69.6, 69.6, 69.7, 69.8, 70.4, 118.0, 121.6, 126.3, 127.6, 128.6, 128.8, 129.7, 131.1, 132.1, 137.2, 140.0, 143.1, 144.7, 147.7, 148.9, 149.9, 151.4, 153.9, 168.5, 169.0, 170.4; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 200-600 nm), t_R = 5.48 min, 98% purity, m/z $[M + H]^+$ calcd for $C_{56}H_{75}N_9O_{13}S_3$ 1178.47, found 1178.8; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{56}H_{75}N_9O_{13}S_3$ 1178.4719, found 1178.4706.

(2*S*,4*S*)-1-((*S*)-2-Cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-4-(6-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)hexanamido)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)pyrrolidine-2-carboxamide (**I1**)

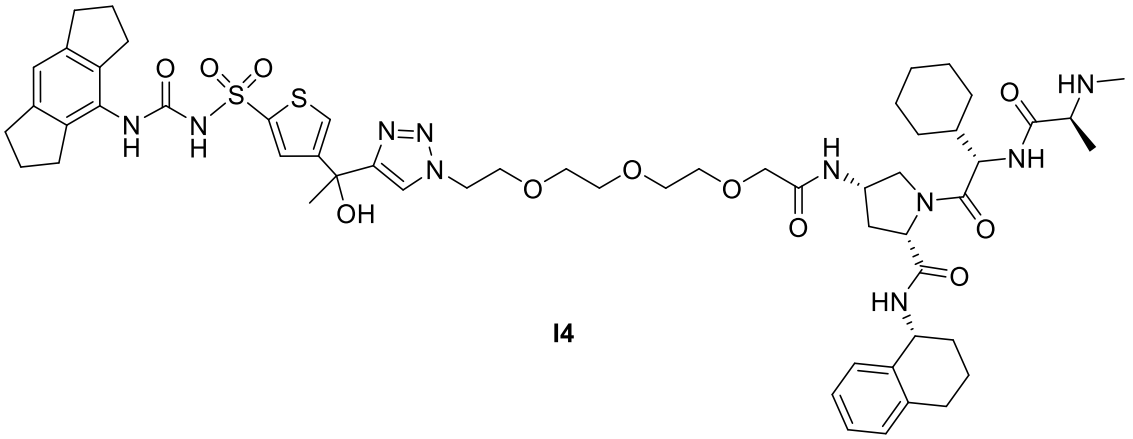


I1

Compound **IP1** (145 mg, 200 µmol) was dissolved in dry CH_2Cl_2 (2 mL), treated with TFA (2 mL) and stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. Compound **I1** was then synthesised following the general procedure for CuAAC and using the deprotected azido-precursor of **IP1**. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (23 mg).

Yield 11%; mp: 145-151 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.94 – 1.30 (m, 8H), 1.31 (d, *J* = 6.9 Hz, 3H), 1.47 – 1.54 (m, 2H), 1.59 – 1.81 (m, 13H), 1.81 – 1.97 (m, 7H), 2.01 – 2.09 (m, 2H), 2.33 – 2.39 (m, 1H), 2.48 (s, 3H), 2.61 (t, *J* = 7.5 Hz, 4H), 2.66 – 2.75 (m, 2H), 2.77 (t, *J* = 7.4 Hz, 4H), 3.79 – 3.84 (m, 1H), 4.08 – 4.14 (m, 1H), 4.19 – 4.33 (m, 4H), 4.37 – 4.41 (m, 1H), 4.90 – 4.97 (m, 1H), 5.95 (s, 1H), 6.86 (s, 1H), 7.05 – 7.12 (m, 2H), 7.12 – 7.17 (m, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.48 (s, 1H), 7.57 (s, 1H), 7.85 (s, 1H), 7.87 – 7.95 (m, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 8.35 – 8.43 (m, 1H), 8.72 (d, *J* = 8.0 Hz, 1H), two signals (2H) are missing due to proton exchange; ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.7, 20.1, 24.4, 25.0, 25.5, 25.7, 25.8, 28.0, 28.1, 28.5, 28.7, 29.4, 29.8, 30.3, 30.5, 30.8, 32.5, 34.3, 35.1, 46.6, 47.6, 49.1, 52.2, 55.4, 55.9, 58.4, 69.5, 116.9, 121.1, 125.6, 126.6, 128.3, 128.5, 136.9, 137.0, 137.3, 142.6, 149.3, 154.1, 168.8, 169.2, 170.6, 171.9; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 6.39 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{54}H_{72}N_{10}O_8S_2$ 1053.50, found 1053.8; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{54}H_{72}N_{10}O_8S_2$ 1053.5049, found 1053.5052.

(2*S*,4*S*)-1-((*S*)-2-Cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-4-(2-(2-(2-(2-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)acetamido)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)pyrrolidine-2-carboxamide (**I4**)

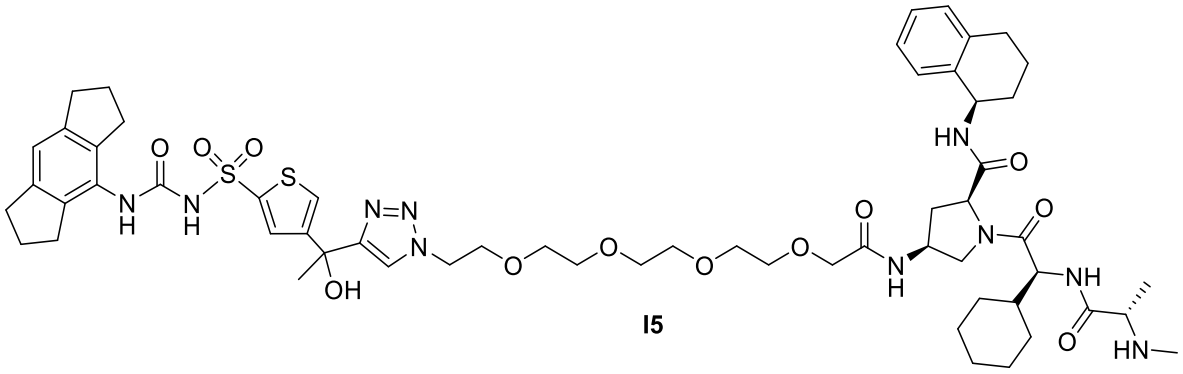


I4

Compound **IP4** (160 mg, 200 µmol) was dissolved in dry CH_2Cl_2 (2 mL), treated with TFA (2 mL) and stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. Compound **I4** was then synthesised following the general procedure for CuAAC and using the deprotected azido-precursor of **IP4**. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (32 mg).

Yield 14%; mp: 122-128 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.98 – 1.07 (m, 2H), 1.09 – 1.21 (m, 3H), 1.31 (d, *J* = 7.0 Hz, 3H), 1.56 – 1.75 (m, 7H), 1.78 (s, 3H), 1.80 – 1.99 (m, 8H), 2.33 – 2.41 (m, 1H), 2.53 – 2.66 (m, 4H), 2.66 – 2.83 (m, 6H), 3.47 – 3.61 (m, 10H), 3.75 – 3.93 (m, 5H), 4.00 – 4.07 (m, 1H), 4.28 – 4.50 (m, 5H), 4.92 – 4.98 (m, 1H), 6.00 (s, 1H), 6.88 (s, 1H), 7.07 – 7.11 (m, 2H), 7.13 – 7.18 (m, 1H), 7.29 (d, *J* = 6.9 Hz, 1H), 7.52 (s, 1H), 7.56 – 7.67 (m, 1H), 7.84 (s, 1H), 7.90 – 8.15 (m, 1H), 8.26 – 8.34 (m, 1H), 8.39 – 8.46 (m, 1H), 8.73 (d, *J* = 7.5 Hz, 1H), two signals (2H) are missing due to proton exchange, one signal (2H) is obscured by solvent signal; ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.7, 20.2, 25.0, 25.5, 25.7, 25.8, 28.1, 28.4, 28.7, 29.7, 30.2, 30.5, 30.8, 32.5, 34.2, 46.7, 47.3, 49.2, 52.7, 55.4, 55.9, 58.5, 68.6, 69.2, 69.5, 69.6, 69.8, 69.9, 70.3, 117.1, 121.5, 125.6, 126.6, 128.3, 128.5, 137.0, 137.0, 137.3, 142.7, 149.4, 154.1, 168.7, 169.1, 169.3, 170.8; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 6.20 min, 93% purity, *m/z* $[M + H]^+$ calcd for $C_{56}H_{76}N_{10}O_{11}S_2$ 1129.52, found 1129.9; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{56}H_{76}N_{10}O_{11}S_2$ 1129.5209, found 1129.5211.

(2*S*,4*S*)-1-((*S*)-2-Cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-4-(14-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-3,6,9,12-tetraoxatetradecanamido)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)pyrrolidine-2-carboxamide (**I5**)

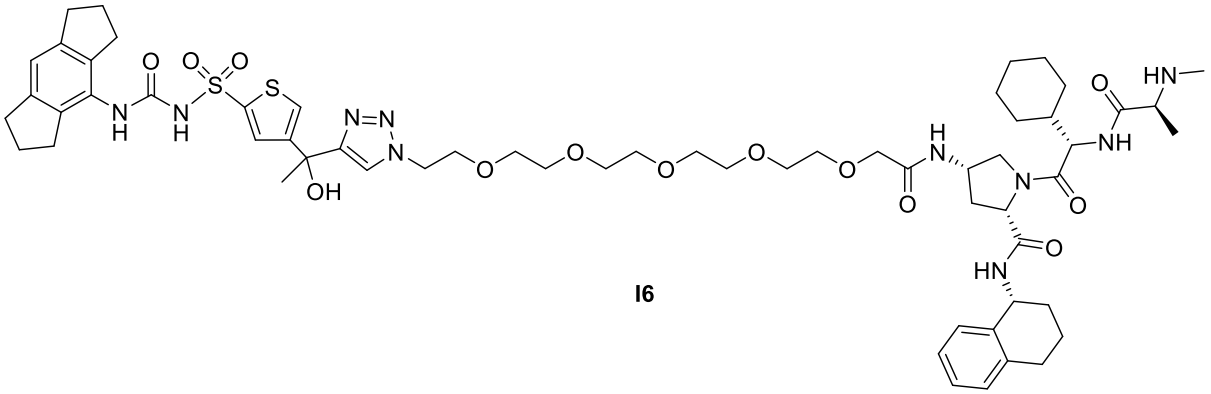


I5

Compound **IP5** (169 mg, 200 µmol) was dissolved in dry CH_2Cl_2 (2 mL), treated with TFA (2 mL) and stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. Compound **I5** was then synthesised following the general procedure for CuAAC and using the deprotected azido-precursor of **IP5**. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (61 mg).

Yield 26%; mp: 117-123 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.98 – 1.07 (m, 2H), 1.11 – 1.19 (m, 3H), 1.31 (d, *J* = 6.7 Hz, 3H), 1.59 – 1.74 (m, 7H), 1.78 (s, 3H), 1.81 – 1.95 (m, 8H), 2.34 – 2.39 (m, 1H), 2.61 (t, *J* = 6.9 Hz, 4H), 2.67 – 2.82 (m, 6H), 3.37 – 3.65 (m, 14H), 3.77 (t, *J* = 5.3 Hz, 2H), 3.81 – 3.86 (m, 1H), 3.86 – 3.95 (m, 2H), 3.95 – 4.10 (m, 1H), 4.29 – 4.52 (m, 5H), 4.89 – 4.98 (m, 1H), 6.01 (s, 1H), 6.88 (s, 1H), 7.06 – 7.12 (m, 2H), 7.12 – 7.18 (m, 1H), 7.27 – 7.31 (m, 1H), 7.54 (s, 1H), 7.62 (s, 1H), 7.84 (s, 1H), 7.95 – 8.20 (m, 1H), 8.27 – 8.38 (m, 1H), 8.43 (d, *J* = 8.7 Hz, 1H), 8.71 – 8.77 (m, 1H), two signals (2H) are missing due to proton exchange, one signal (2H) is obscured by solvent signal; ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.7, 20.2, 25.0, 25.5, 25.7, 25.8, 28.2, 28.4, 28.7, 29.7, 30.2, 30.5, 30.8, 32.5, 34.2, 46.7, 47.3, 49.2, 52.8, 55.4, 55.9, 58.5, 68.7, 69.5, 69.6, 69.6, 69.7, 69.8, 69.9, 70.4, 117.2, 121.6, 125.6, 126.6, 128.3, 128.5, 137.0, 137.0, 137.3, 142.8, 149.5, 154.1, 168.7, 169.1, 169.3, 170.9; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 6.11 min, 96% purity, *m/z* $[M + H]^+$ calcd for $C_{58}H_{80}N_{10}O_{12}S_2$ 1173.55, found 1173.9; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{58}H_{80}N_{10}O_{12}S_2$ 1173.5471, found 1173.5452.

(2*S*,4*S*)-1-((*S*)-2-Cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-4-(17-(4-(1-(5-(*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-3,6,9,12,15-pentaoxaheptadecanamido)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)pyrrolidine-2-carboxamide (**I6**)

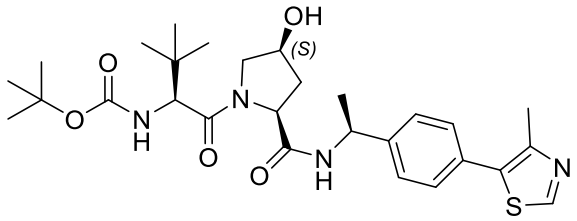


I6

Compound **IP6** (177 mg, 200 µmol) was dissolved in dry CH_2Cl_2 (2 mL), treated with TFA (2 mL) and stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. Compound **I6** was then synthesised following the general procedure for CuAAC and using the deprotected azido-precursor of **IP6**. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 25% MeOH) to yield a white solid (44 mg).

Yield 18%; mp: 118-124 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.97 – 1.09 (m, 2H), 1.09 – 1.23 (m, 3H), 1.29 – 1.37 (m, 3H), 1.57 – 1.77 (m, 7H), 1.79 (s, 3H), 1.80 – 1.96 (m, 8H), 2.33 – 2.40 (m, 1H), 2.59 (t, *J* = 7.4 Hz, 4H), 2.68 – 2.76 (m, 2H), 2.78 (t, *J* = 7.4 Hz, 4H), 3.41 – 3.63 (m, 18H), 3.78 (t, *J* = 5.4 Hz, 2H), 3.82 – 3.91 (m, 3H), 3.99 – 4.08 (m, 1H), 4.32 – 4.49 (m, 5H), 4.91 – 4.98 (m, 1H), 6.08 (s, 1H), 6.93 (s, 1H), 7.05 – 7.12 (m, 2H), 7.12 – 7.19 (m, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.69 (s, 1H), 7.73 (s, 1H), 7.87 (s, 1H), 8.25 (s, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 8.7 Hz, 1H), 8.75 (d, *J* = 8.0 Hz, 1H), two signals (2H) are missing due to proton exchange, one signal (2H) is obscured by solvent signal; ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.6, 20.2, 25.0, 25.5, 25.7, 25.8, 28.1, 28.4, 28.7, 29.7, 30.1, 30.6, 30.9, 32.4, 34.2, 46.7, 47.3, 49.2, 52.8, 55.4, 55.8, 58.5, 68.6, 69.5, 69.6, 69.6, 69.7, 69.8, 69.9, 70.4, 117.9, 121.6, 125.6, 126.6, 127.4, 128.3, 128.5, 131.9, 136.9, 137.2, 137.3, 143.0, 149.9, 153.9, 168.6, 169.1, 169.3, 170.8; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 6.23 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{60}H_{84}N_{10}O_{13}S_2$ 1217.57, found 1217.9; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{60}H_{84}N_{10}O_{13}S_2$ 1217.5734, found 1217.5731.

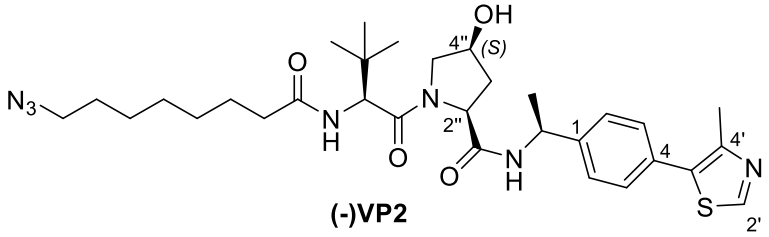
tert-Butyl ((*S*)-1-((2*S*,4*S*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (**46**)



46 (-)VHL ligand

Compound **46** was prepared as described elsewhere.[16]

(2*S*,4*S*)-1-((*S*)-2-(8-Azidooctanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**(-)VP2**)



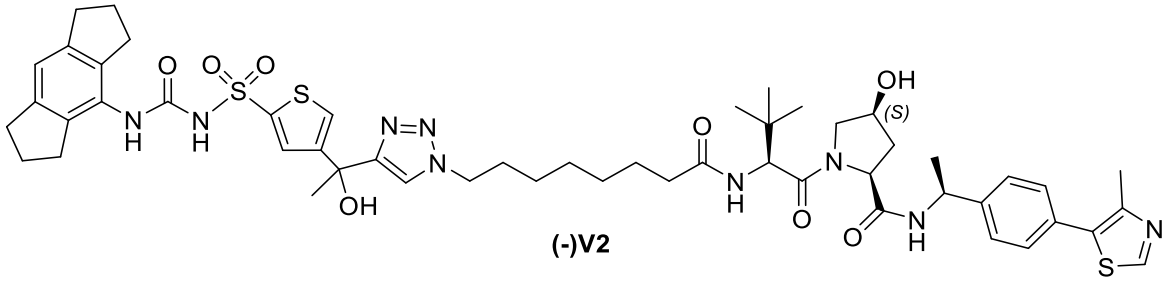
(-)VP2

The Boc-protected (-)VHL ligand **46** (436 mg, 800 µmol) was dissolved in dry CH_2Cl_2 (5 mL) and was treated with TFA (5 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo*. 8-Azidooctanoic acid (**35**, 148 mg, 800 µmol) was dissolved in dry DMF (5 mL). HATU (335 mg, 880 µmol) and DIPEA (362 mg, 2.80 mmol) were added under argon. The deprotected (-)VHL ligand was dissolved in dry DMF (5 mL) and was added to the mixture, containing the activated acid compound. The reaction mixture was allowed to stir at room temperature under argon for 18 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using a CH_2Cl_2/MeOH gradient (0% to 5% MeOH) as eluent to yield a colourless resin (265 mg).

Yield 54%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.95 (s, 9H, $C(CH_3)_3$), 1.17 – 1.33 (m, 6H, CH_2), 1.38 (d, J = 6.9 Hz, 3H, CHC$\underline{H}_3$), 1.42 – 1.55 (m, 4H, CH_2), 1.63 – 1.69 (m, 1H), 2.06 – 2.14 (m, 1H), 2.20 – 2.27 (m, 1H), 2.27 – 2.34 (m, 1H, 3''-H, CH_2CON), 2.46 (s, 3H, $CH_{3\ thiazole}$), 3.36 – 3.41 (m, 1H), 3.86 – 3.94 (m, 1H, 5''-H), 4.16 – 4.23 (m, 1H), 4.33 (dd, J = 8.7, 6.1 Hz, 1H), 4.45 (d, J = 8.8 Hz, 1H, 2''-H, 4''-H, NHC$\underline{H}$), 4.89 – 4.96 (m, 1H, C$\underline{H}CH_3$), 5.31 (d, J = 6.5 Hz, 1H, OH), 7.35 – 7.41 (m, 2H), 7.42 – 7.50 (m, 2H, 2-H, 3-H, 5-H, 6-H), 7.78 (d, J = 8.7 Hz, 1H, CONH), 8.29 (d, J = 7.7 Hz, 1H, CONH), 8.98 (s, 1H, 2'-H), one signal (2H, CH_2N_3) is obscured by solvent signal; ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.9 ($CH_{3\ thiazole}$), 22.1 (CH$\underline{C}H_3$), 25.2, 26.0 (CH_2), 26.4 ($C(\underline{C}H_3)_3$), 28.1, 28.4 (CH_2), 34.6, 34.7, 36.7 (CH_2, $C(CH_3)_3$, C-3''), 47.7 ($\underline{C}HCH_3$), 50.6 (CH_2N_3), 55.5, 56.6, 58.5 (C-2'', C-5'', NHCH), 68.9 (C-4''), 126.4, 128.8 (C-2, C-3, C-5, C-6), 129.7, 131.0, 144.2 (C-1, C-4, C-5'),

147.7 (C-4'), 151.4 (C-2'), 169.9, 171.0, 172.3 (CO), one signal (CH_2) is missing due to overlapping signals; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 7.12 min, 95% purity, *m/z* $[M + H]^+$ calcd for $C_{31}H_{45}N_7O_4S$ 612.33, found 612.5.

(2*S*,4*S*)-1-((2*S*)-2-(8-(4-(1-(5-(*N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)thiophen-3-yl)-1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**(-)V2**)



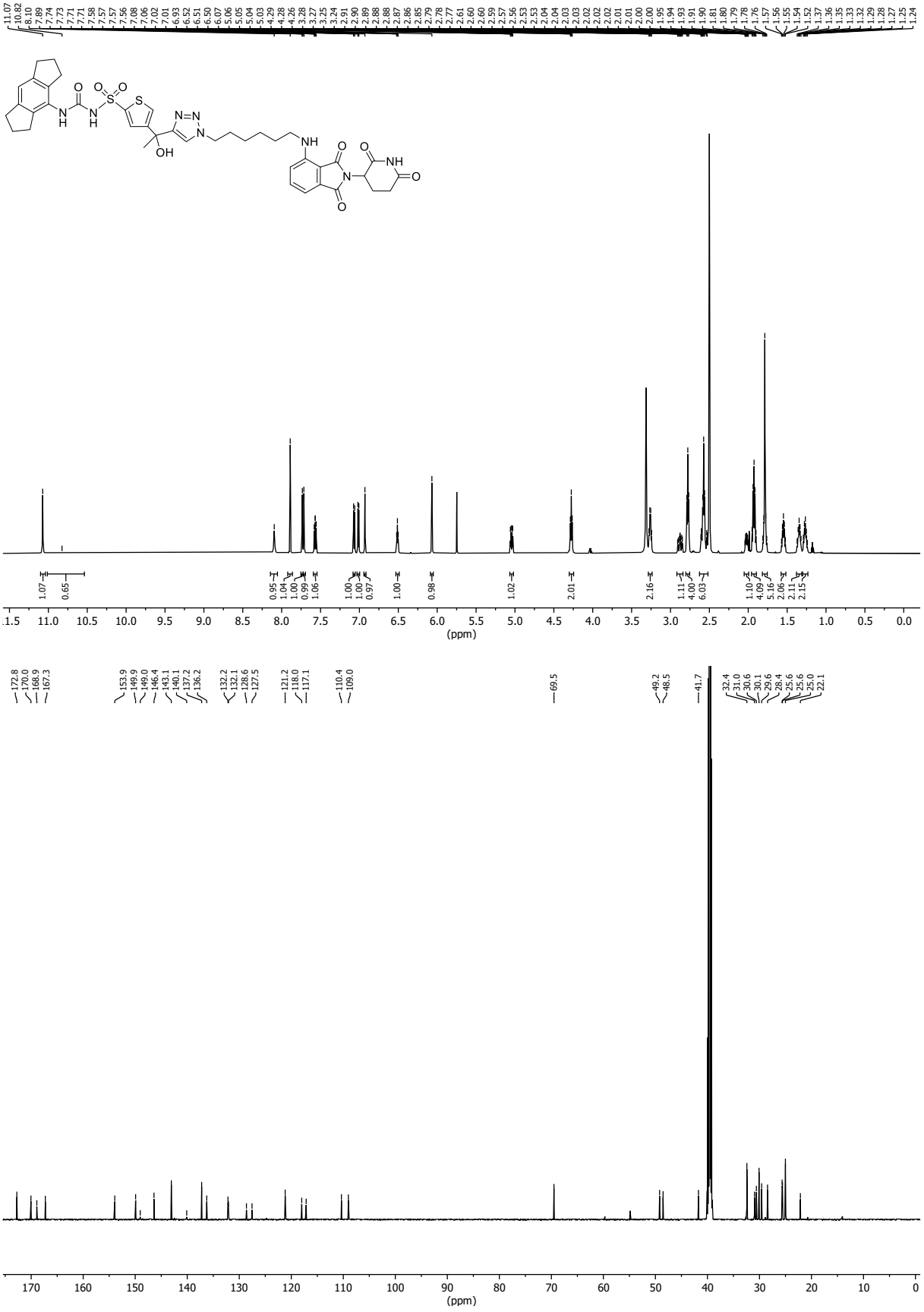
(-)V2

Compound **(-)V2** was synthesised following the general procedure for CuAAC and using azido-precursor **(-)VP2** (200 µmol). The crude product was purified by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (0% to 20% MeOH) to yield a white solid (118 mg).

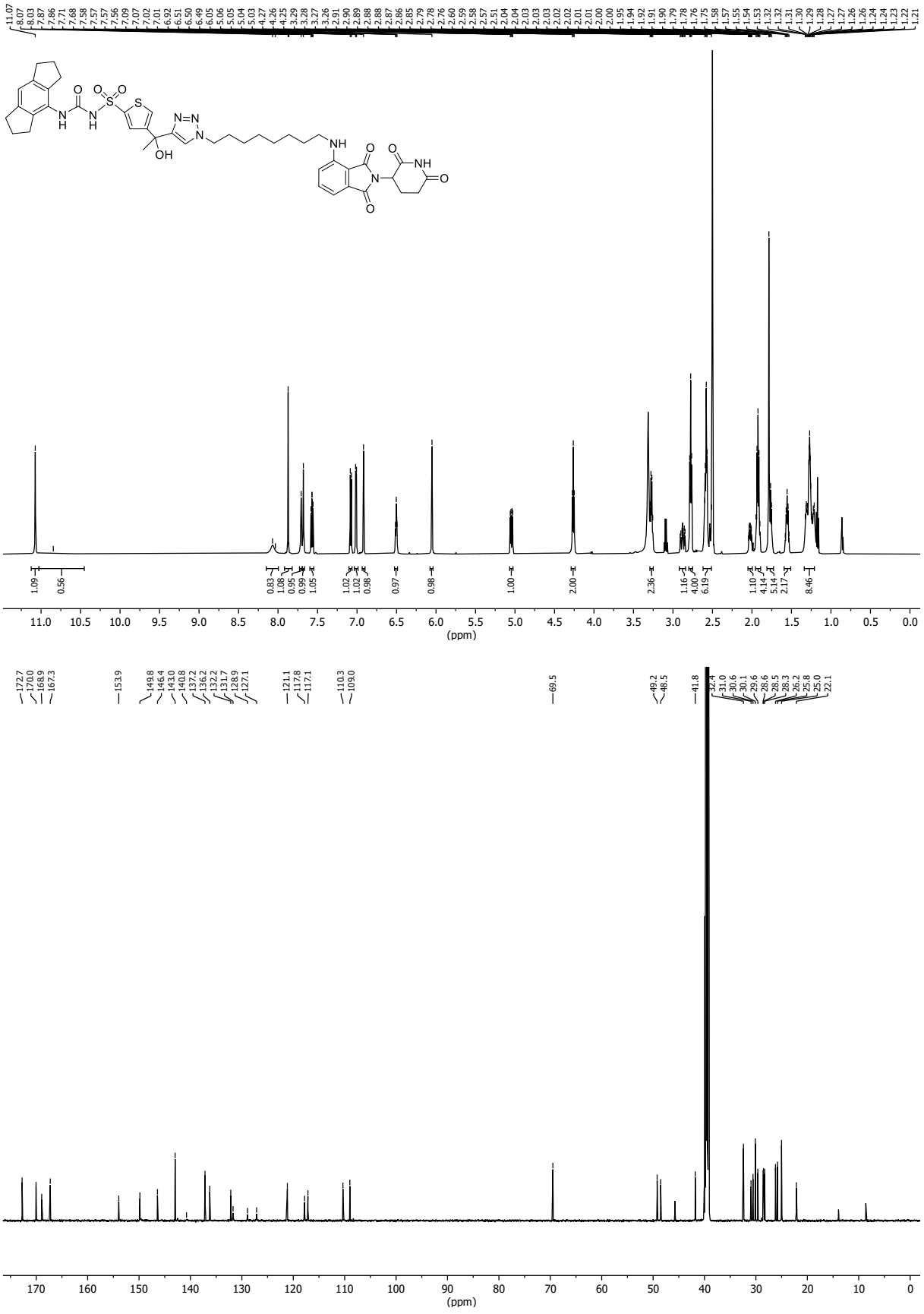
Yield 57%; mp: 133-139 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.95 (s, 9H), 1.17 – 1.24 (m, 6H), 1.38 (d, *J* = 7.0 Hz, 3H), 1.40 – 1.51 (m, 2H), 1.63 – 1.69 (m, 1H), 1.73 – 1.81 (m, 5H), 1.93 (quint, *J* = 7.4 Hz, 4H), 2.06 – 2.12 (m, 1H), 2.19 – 2.25 (m, 1H), 2.27 – 2.33 (m, 1H), 2.46 (s, 3H), 2.58 (t, *J* = 7.4 Hz, 4H), 2.78 (t, *J* = 7.4 Hz, 4H), 3.39 (dd, *J* = 10.1, 5.2 Hz, 1H), 3.90 (dd, *J* = 10.2, 5.6 Hz, 1H), 4.16 – 4.22 (m, 1H), 4.27 (t, *J* = 7.2 Hz, 2H), 4.33 (dd, *J* = 8.7, 6.1 Hz, 1H), 4.44 (d, *J* = 8.8 Hz, 1H), 4.89 – 4.96 (m, 1H), 5.31 (d, *J* = 6.5 Hz, 1H), 6.07 (s, 1H), 6.94 (s, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 1.4 Hz, 1H), 7.74 (d, *J* = 1.4 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.88 (s, 1H), 8.11 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.98 (s, 1H), 10.88 (s, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 15.9, 22.2, 25.0, 25.2, 25.8, 26.4, 28.0, 28.4, 29.6, 30.1, 30.6, 32.4, 34.6, 34.7, 36.8, 47.7, 49.2, 55.5, 56.6, 58.5, 69.0, 69.5, 118.0, 121.2, 126.4, 127.6, 128.6, 128.8, 129.8, 131.1, 132.1, 137.2, 140.0, 143.1, 144.2, 147.8, 148.9, 149.9, 151.4, 153.9, 169.9, 171.0, 172.3; LC-MS (ESI) (90% H_2O to 100% MeCN in 10 min, then 100% MeCN to 15 min, DAD 220-600 nm), t_R = 5.96 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{52}H_{67}N_9O_8S_3$ 1042.43, found 1042.7; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{52}H_{67}N_9O_8S_3$ 1042.4347, found 1042.4347.

7. NMR Spectra

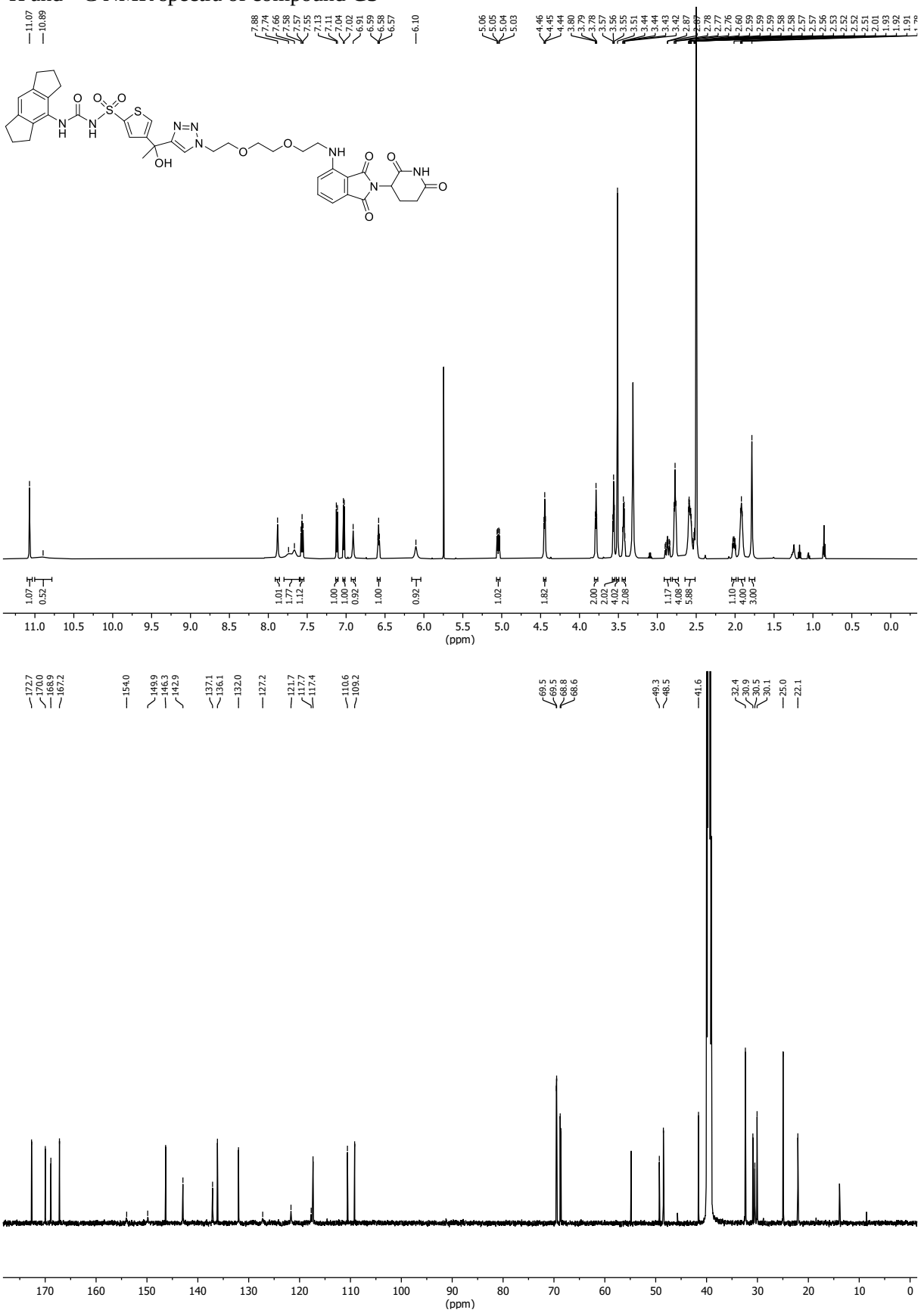
^{1}H and ^{13}C NMR spectra of compound **C1**



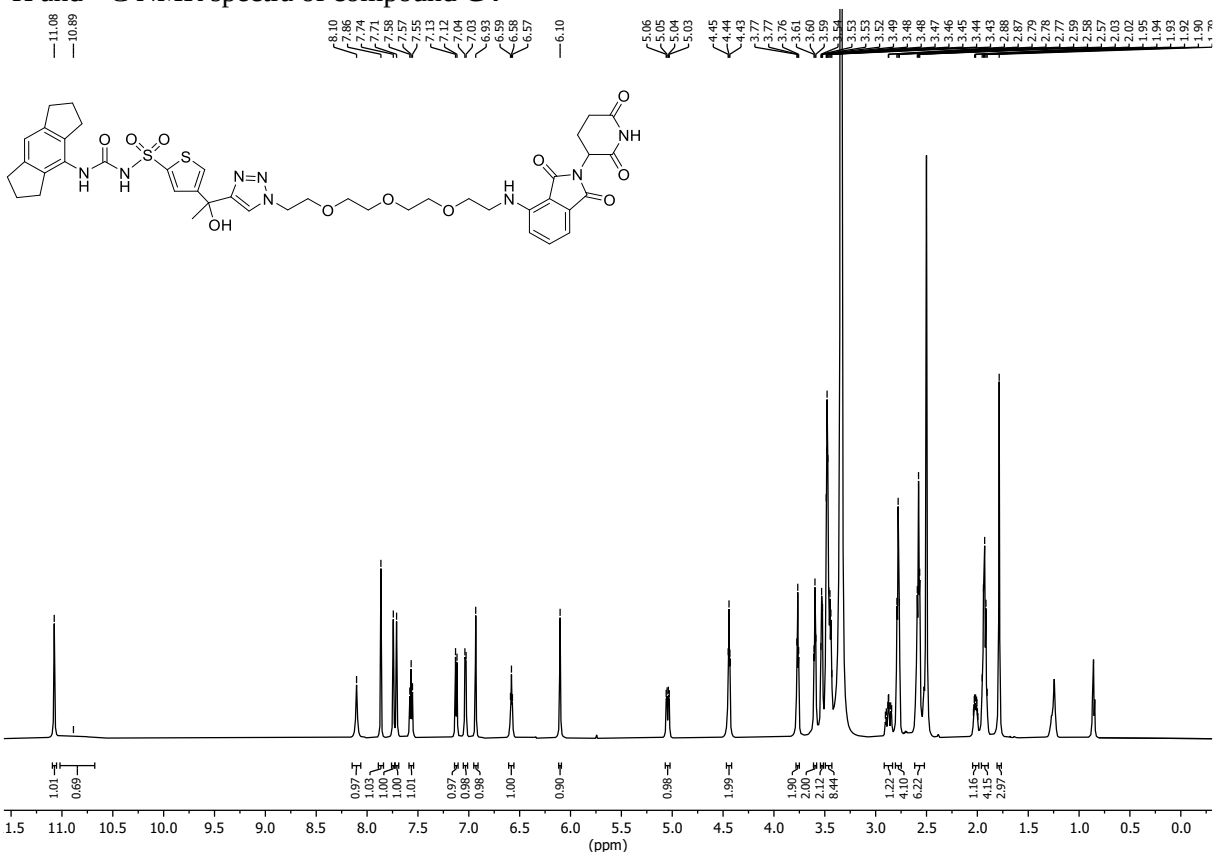
^{1}H and ^{13}C NMR spectra of compound **C2**

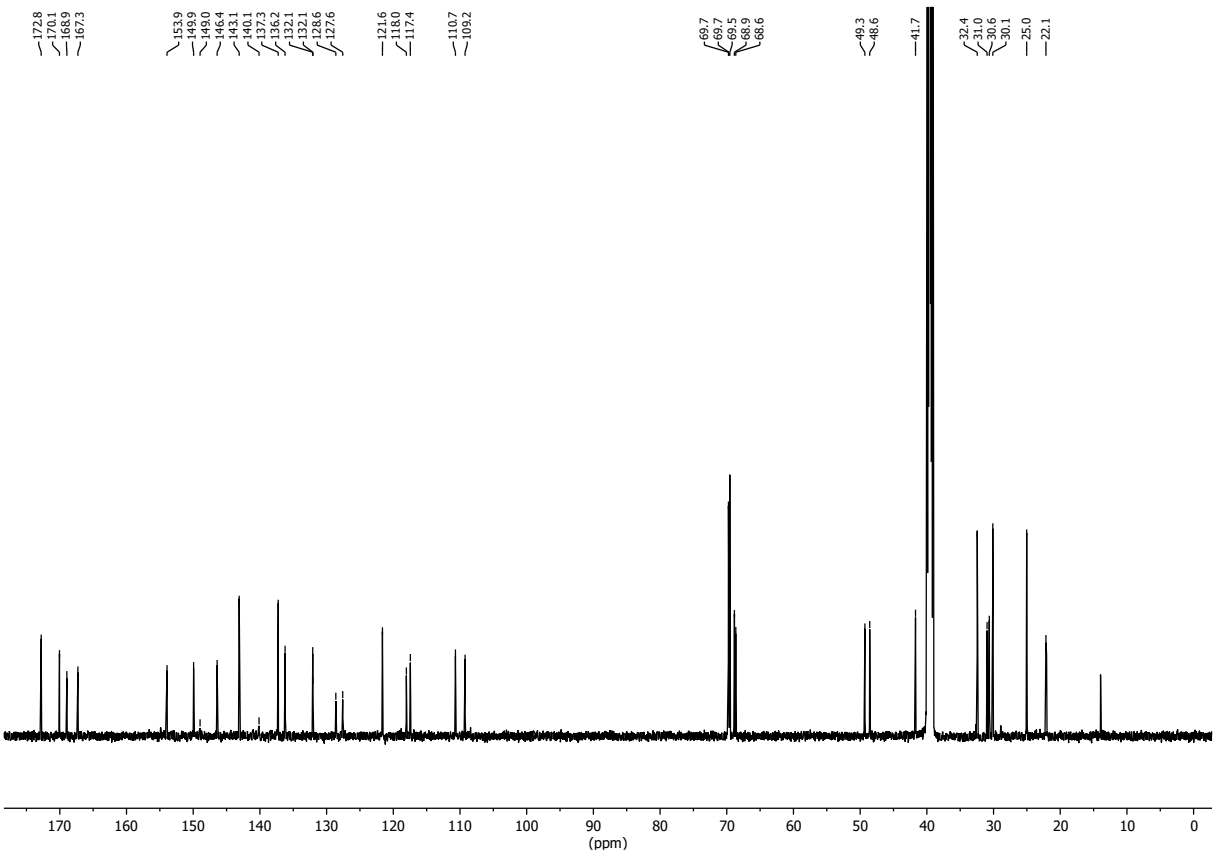


^{1}H and ^{13}C NMR spectra of compound **C3**

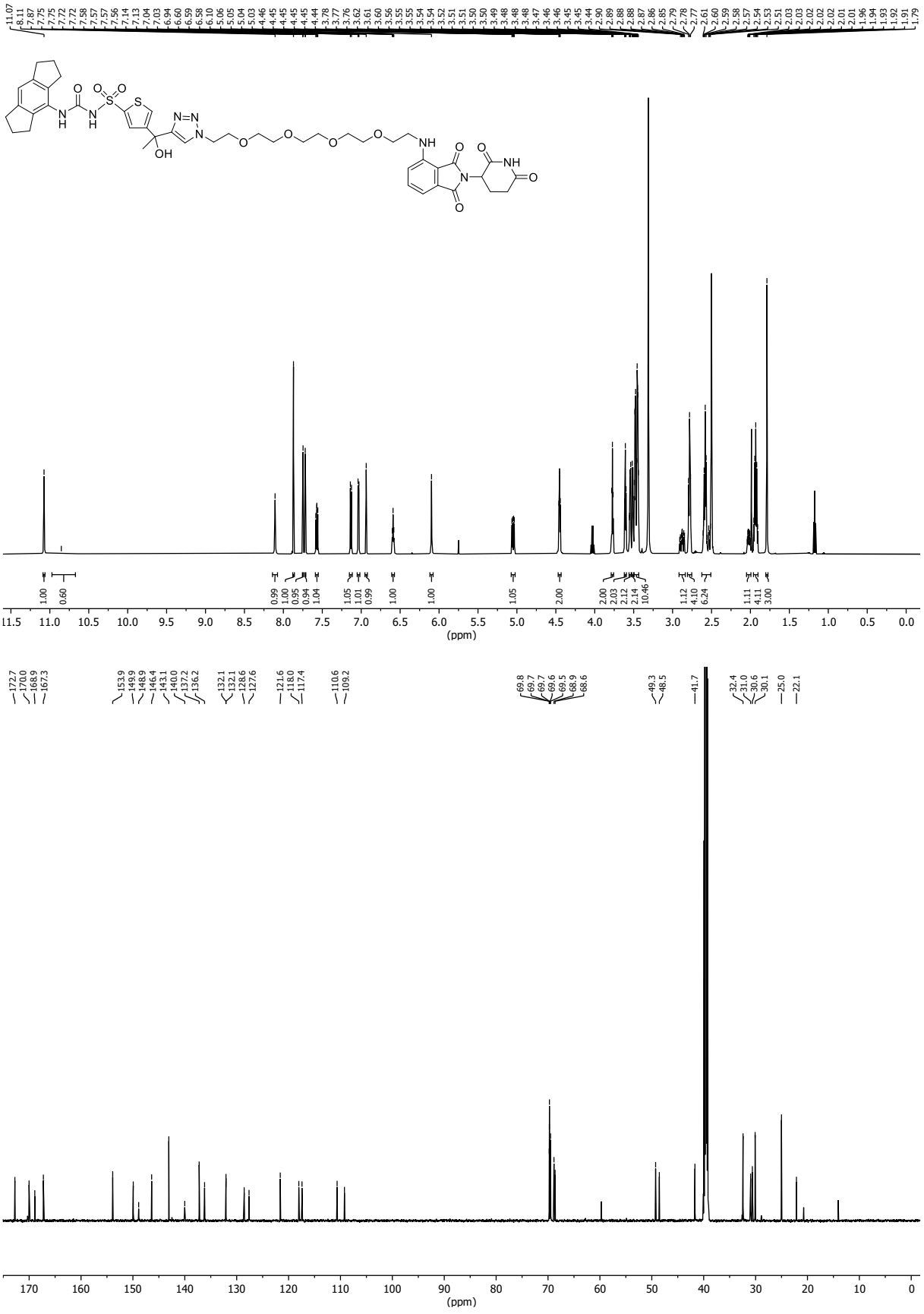


^{1}H and ^{13}C NMR spectra of compound **C4**

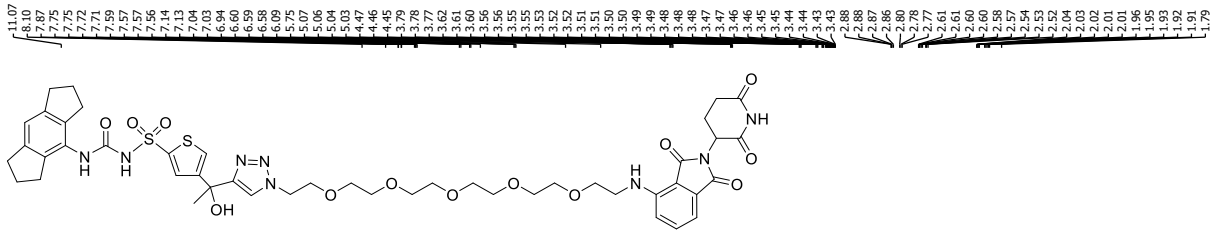


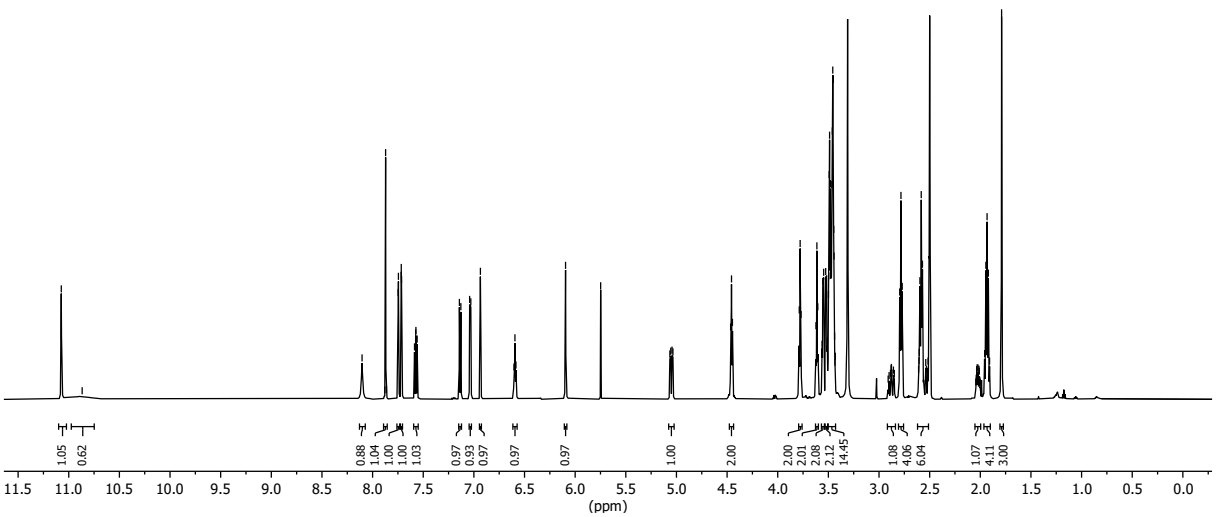


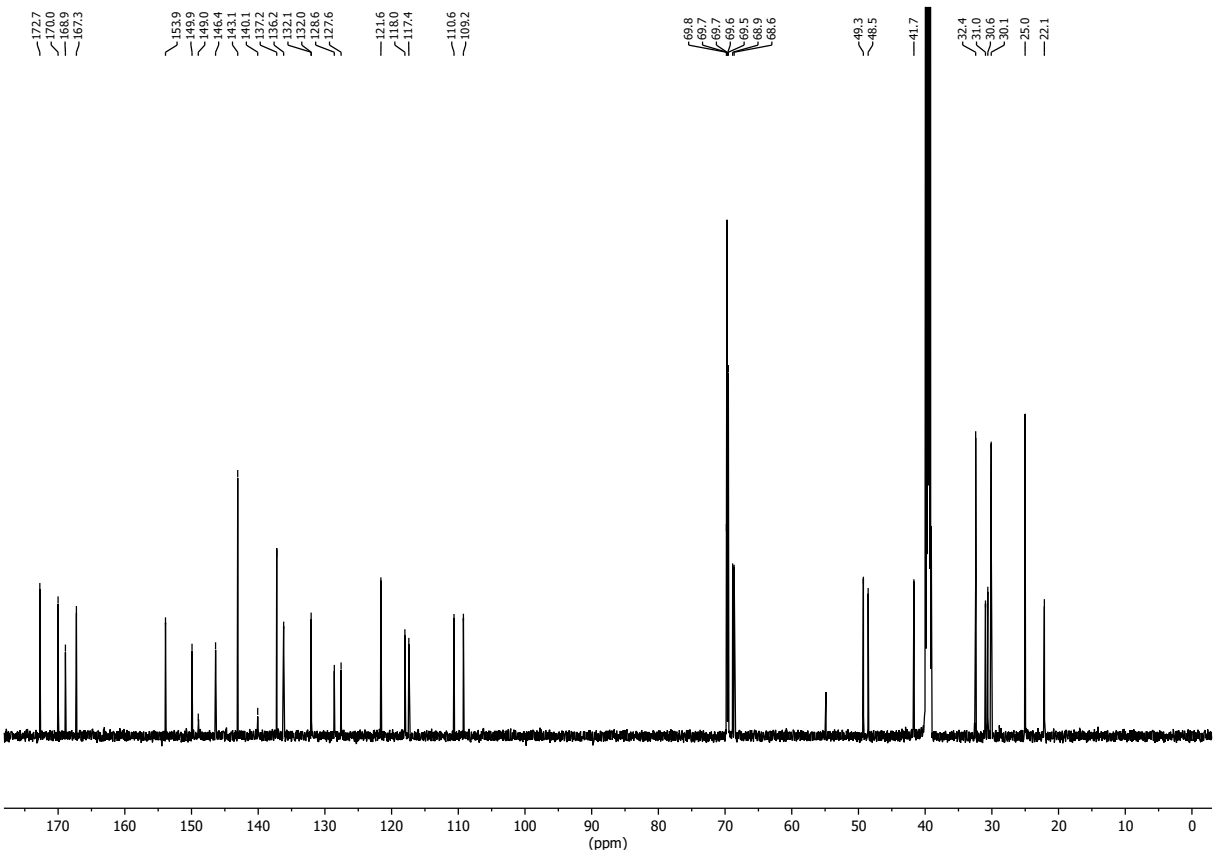
^{1}H and ^{13}C NMR spectra of compound **C5**



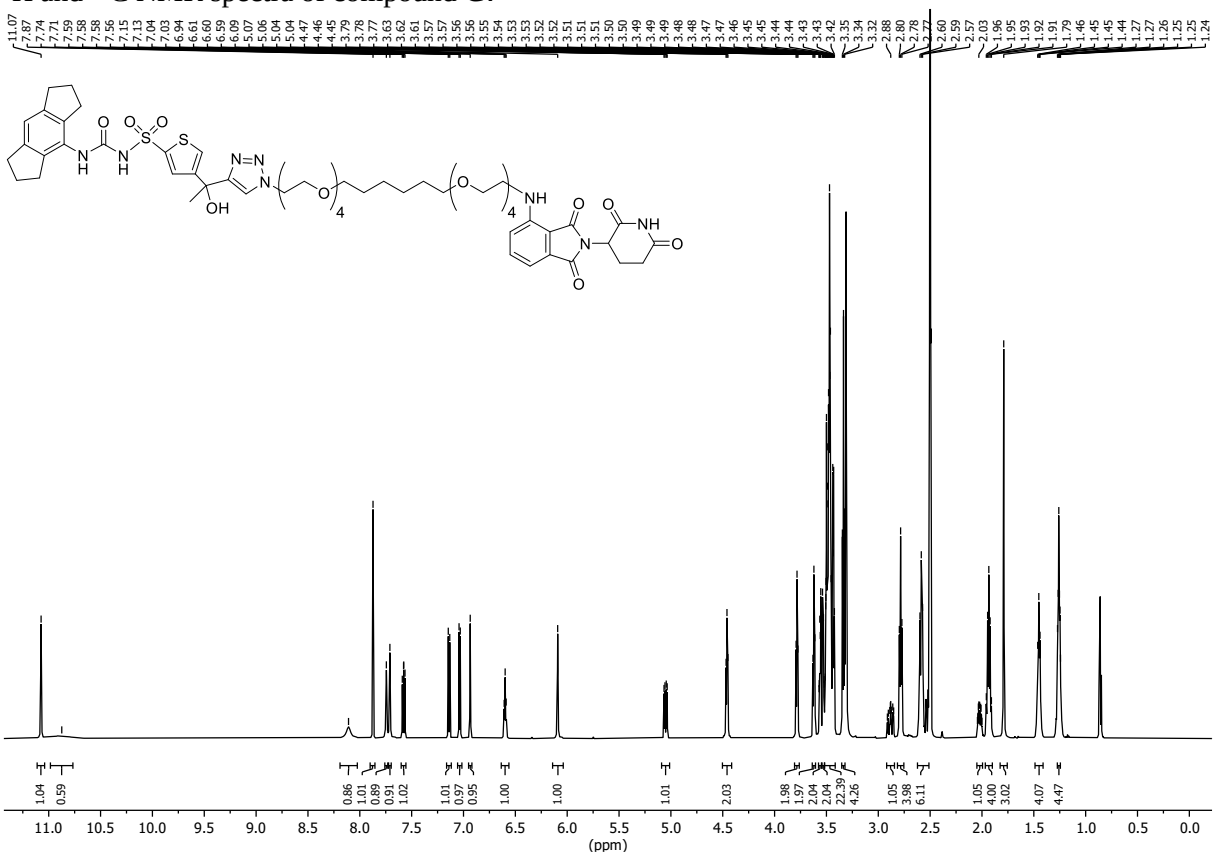
^{1}H and ^{13}C NMR spectra of compound **C6**

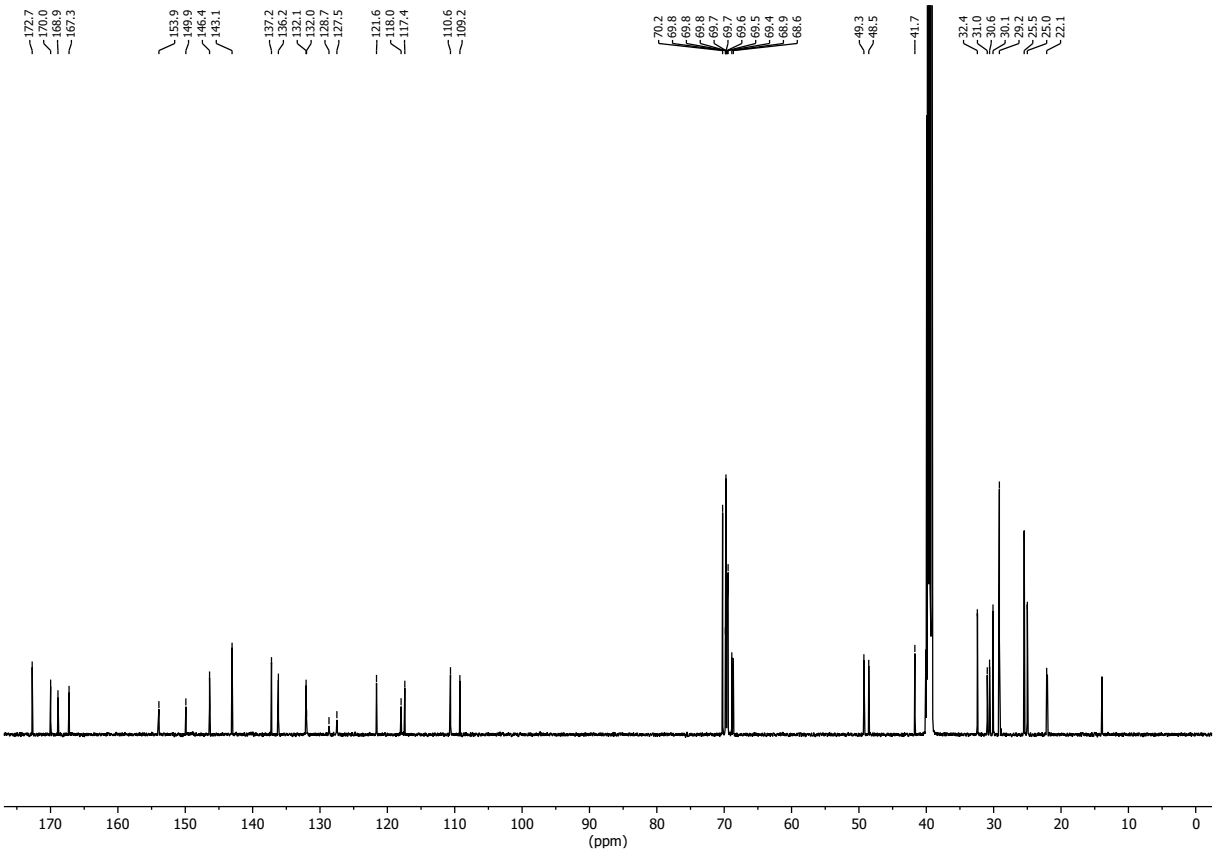




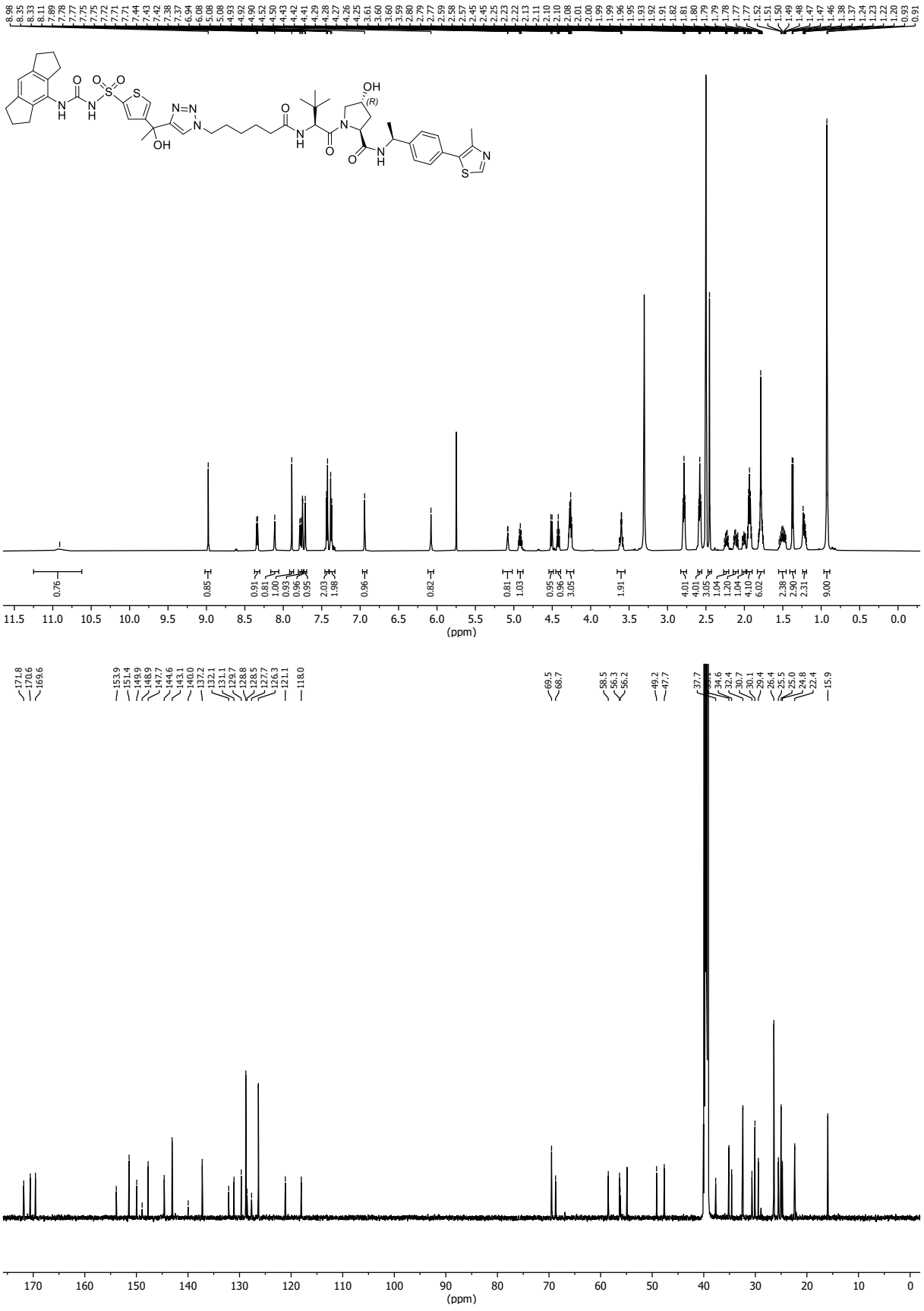


^{1}H and ^{13}C NMR spectra of compound **C7**

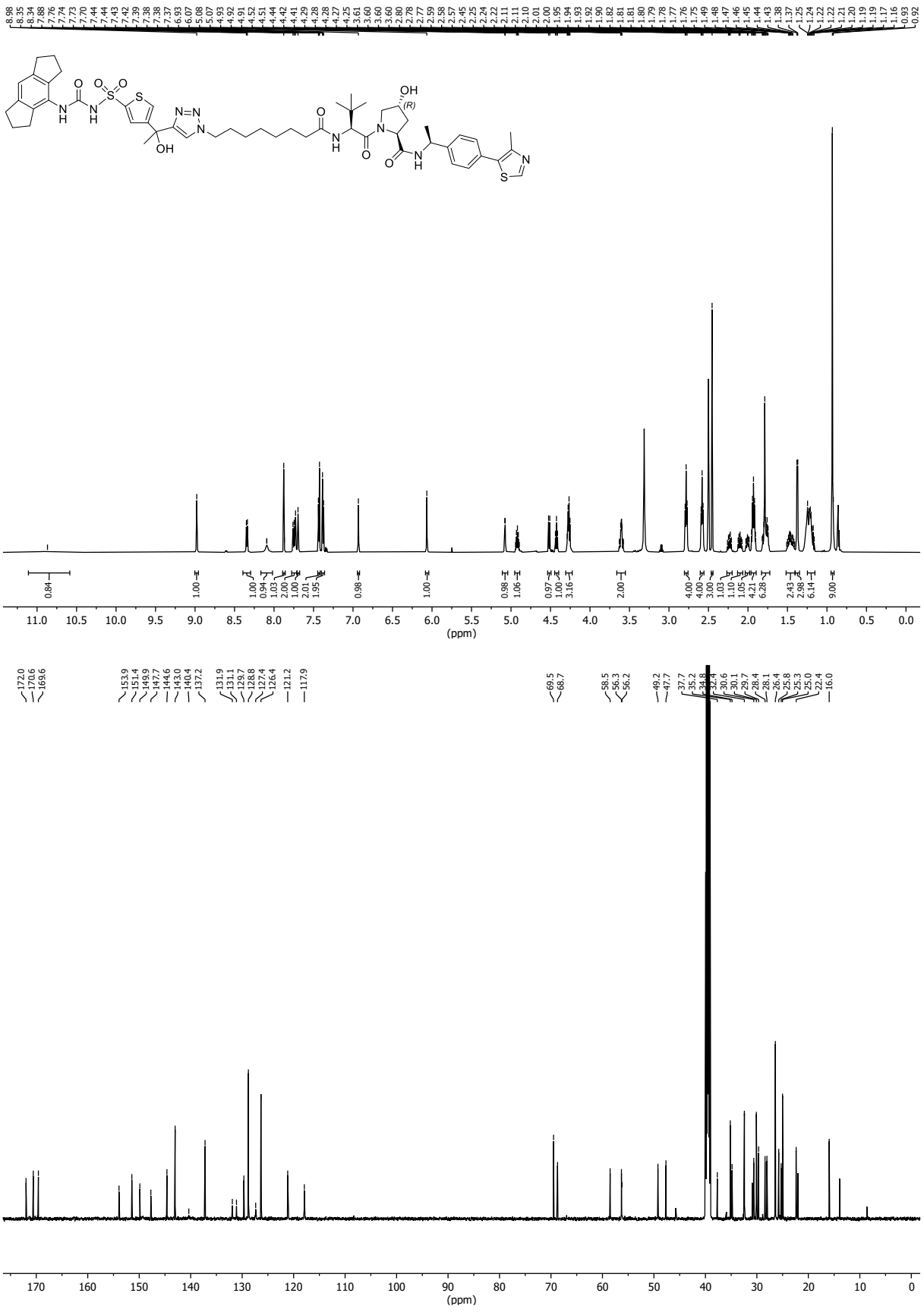




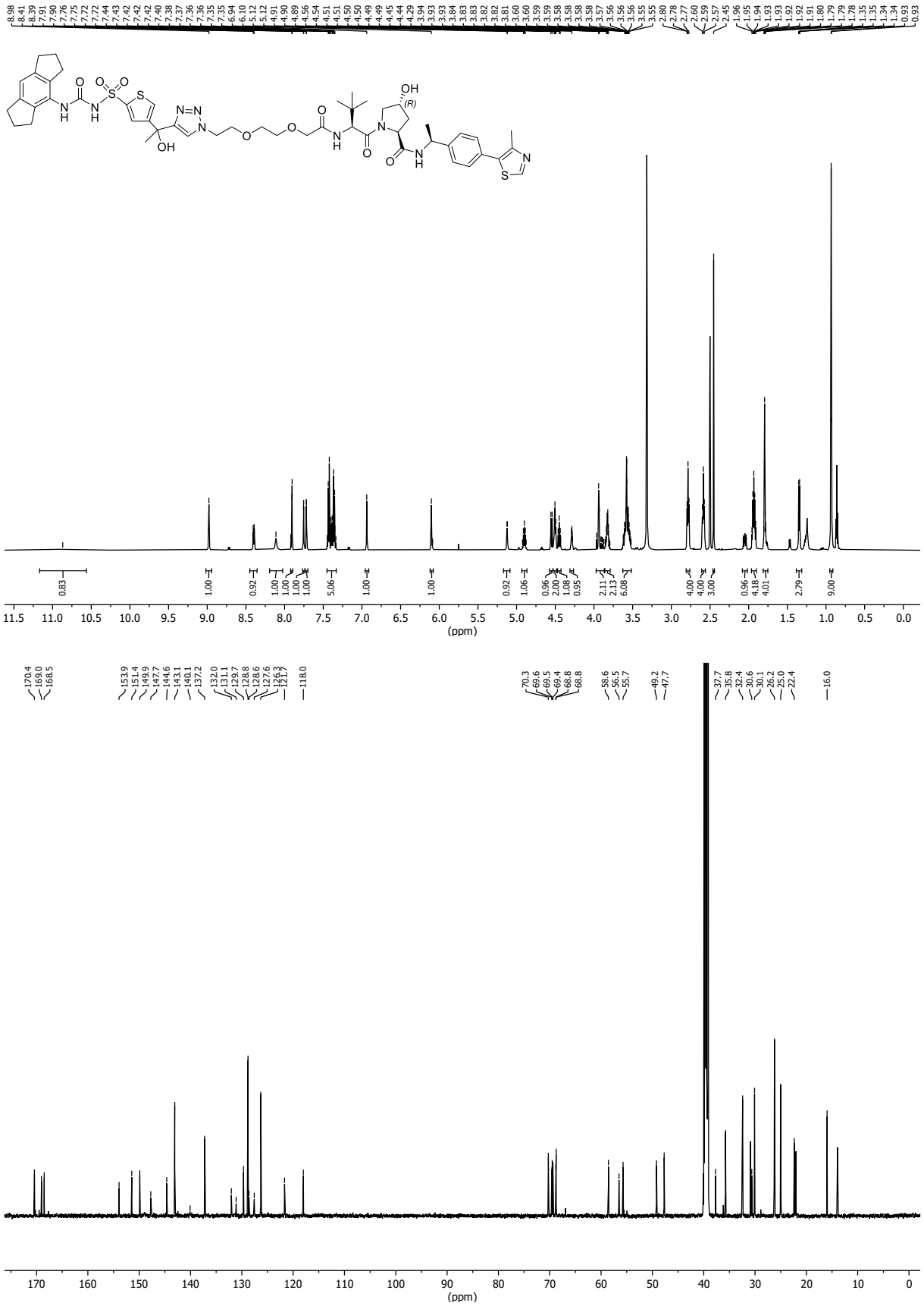
^{1}H and ^{13}C NMR spectra of compound **V1**



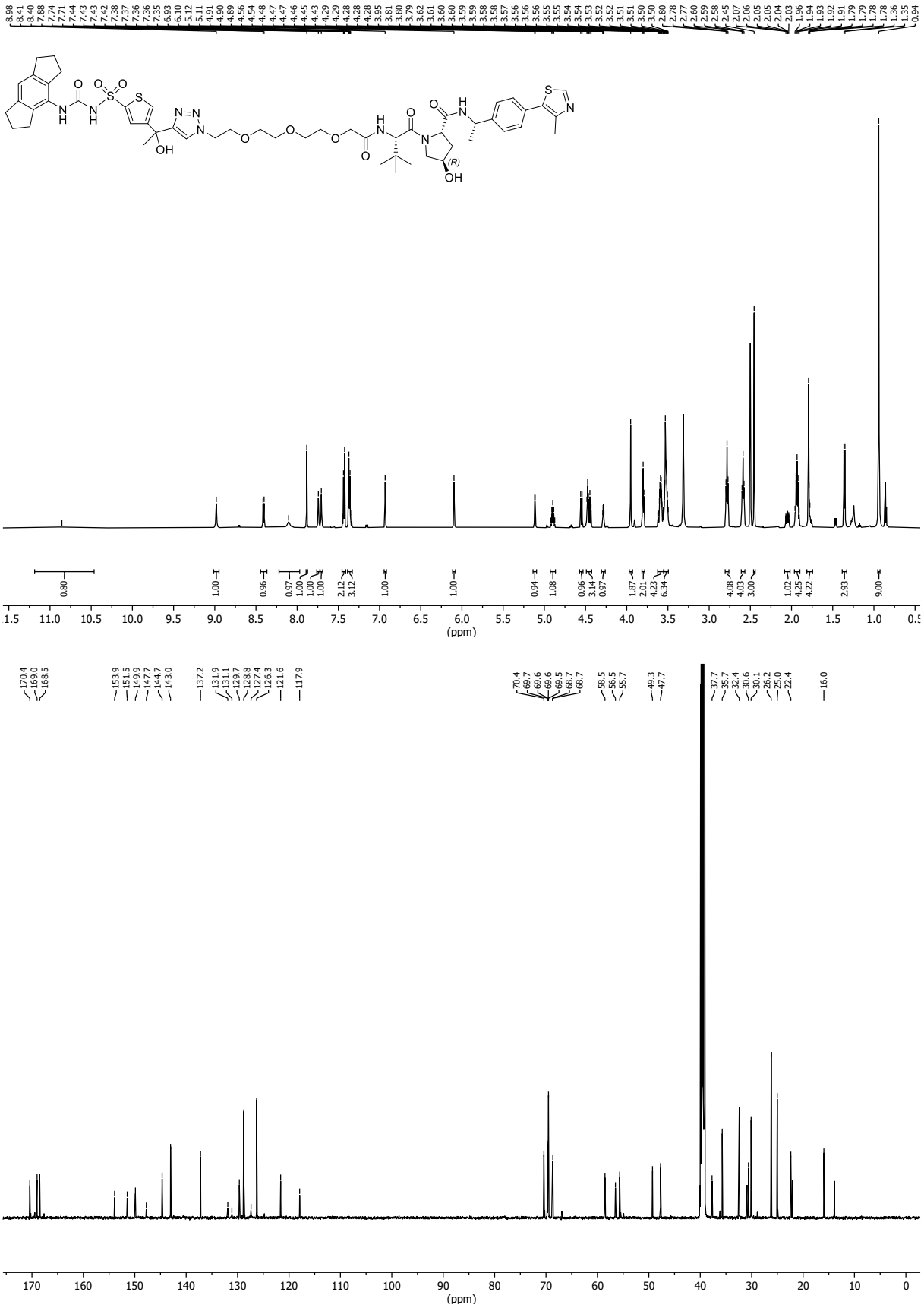
^{1}H and ^{13}C NMR spectra of compound **V2**



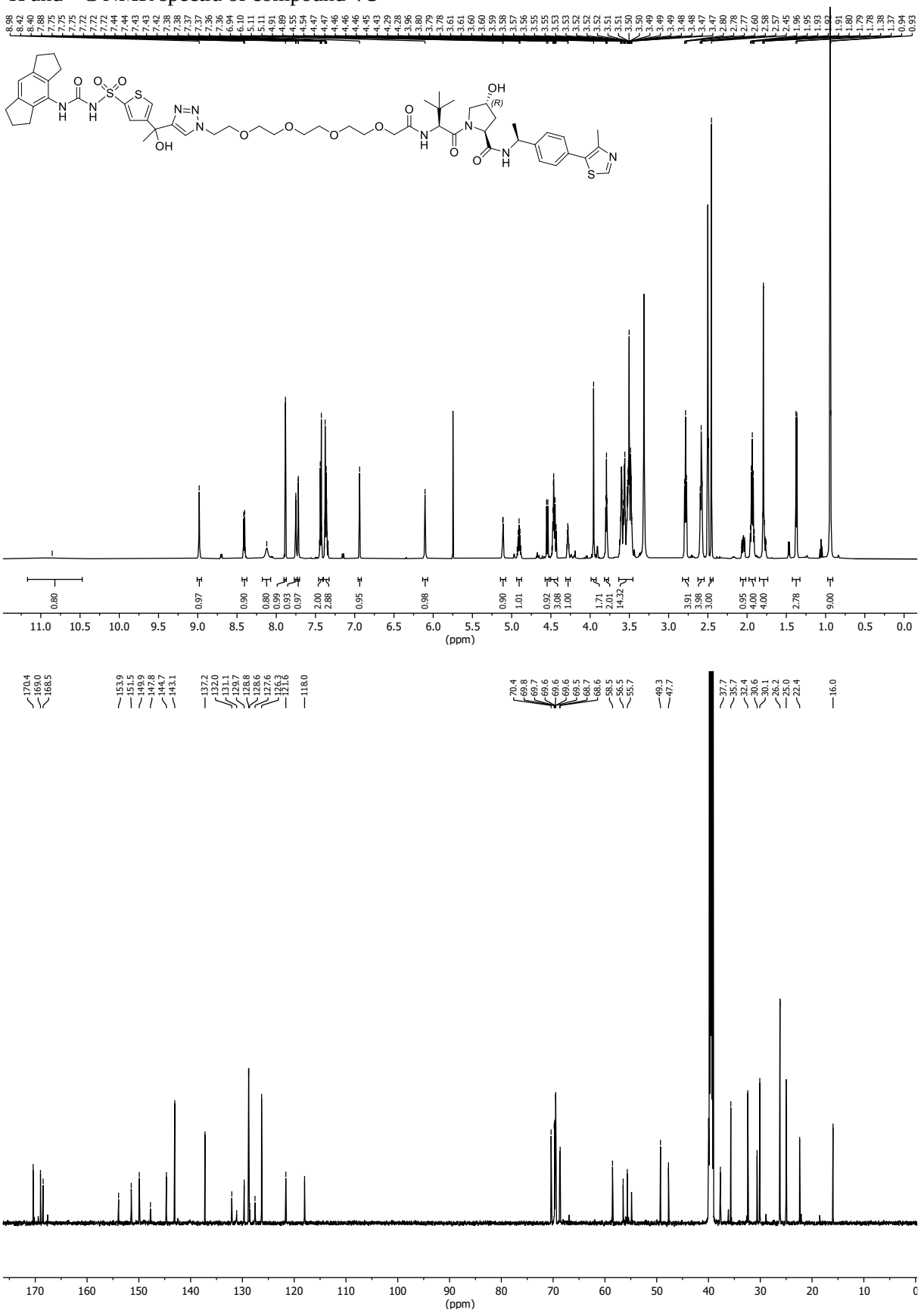
^{1}H and ^{13}C NMR spectra of compound **V3**



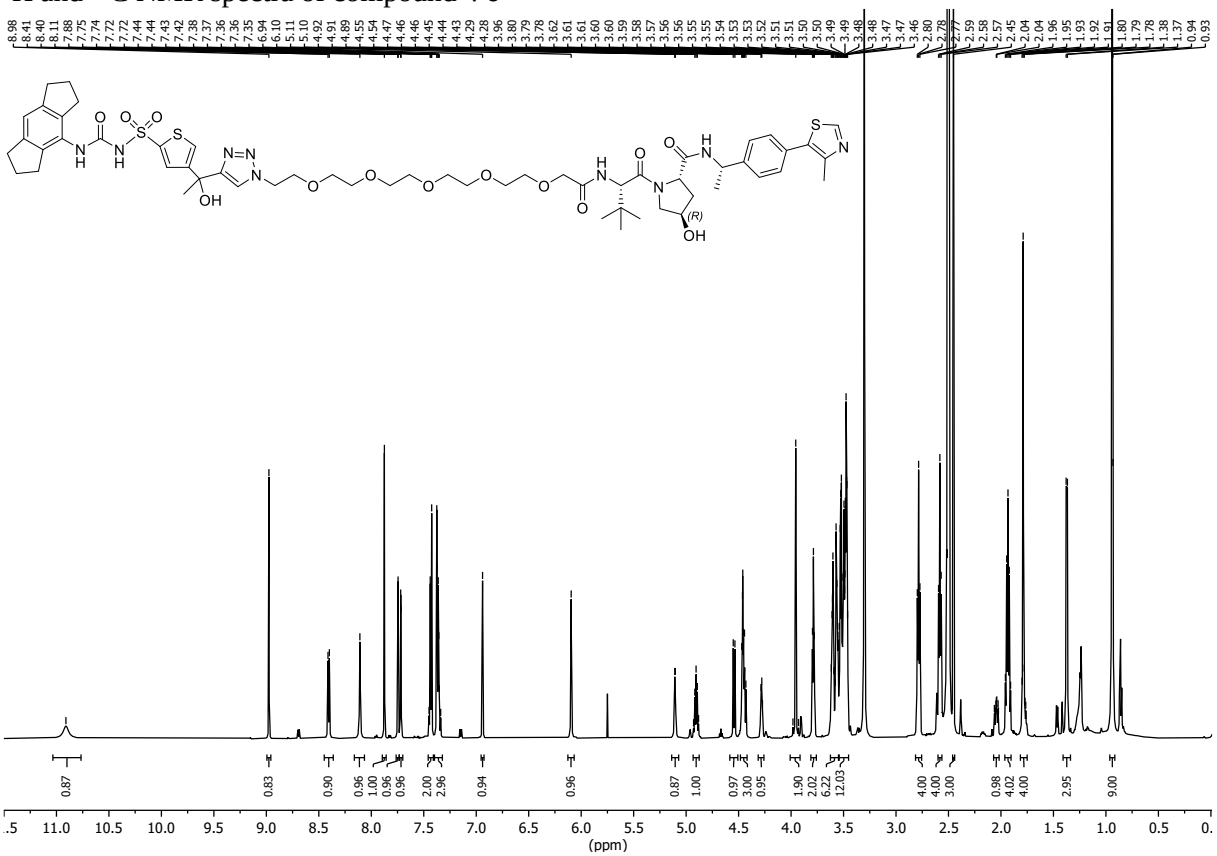
^{1}H and ^{13}C NMR spectra of compound **V4**

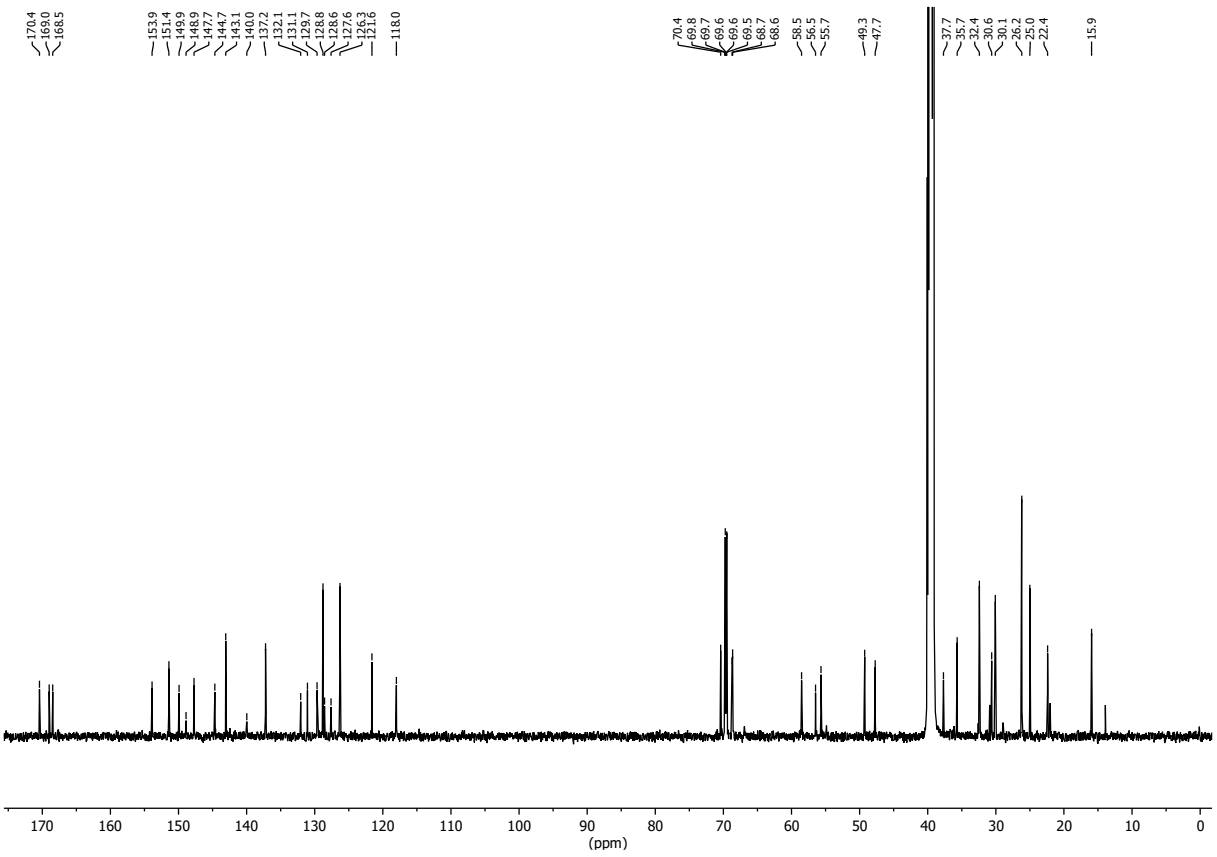


^{1}H and ^{13}C NMR spectra of compound **V5**

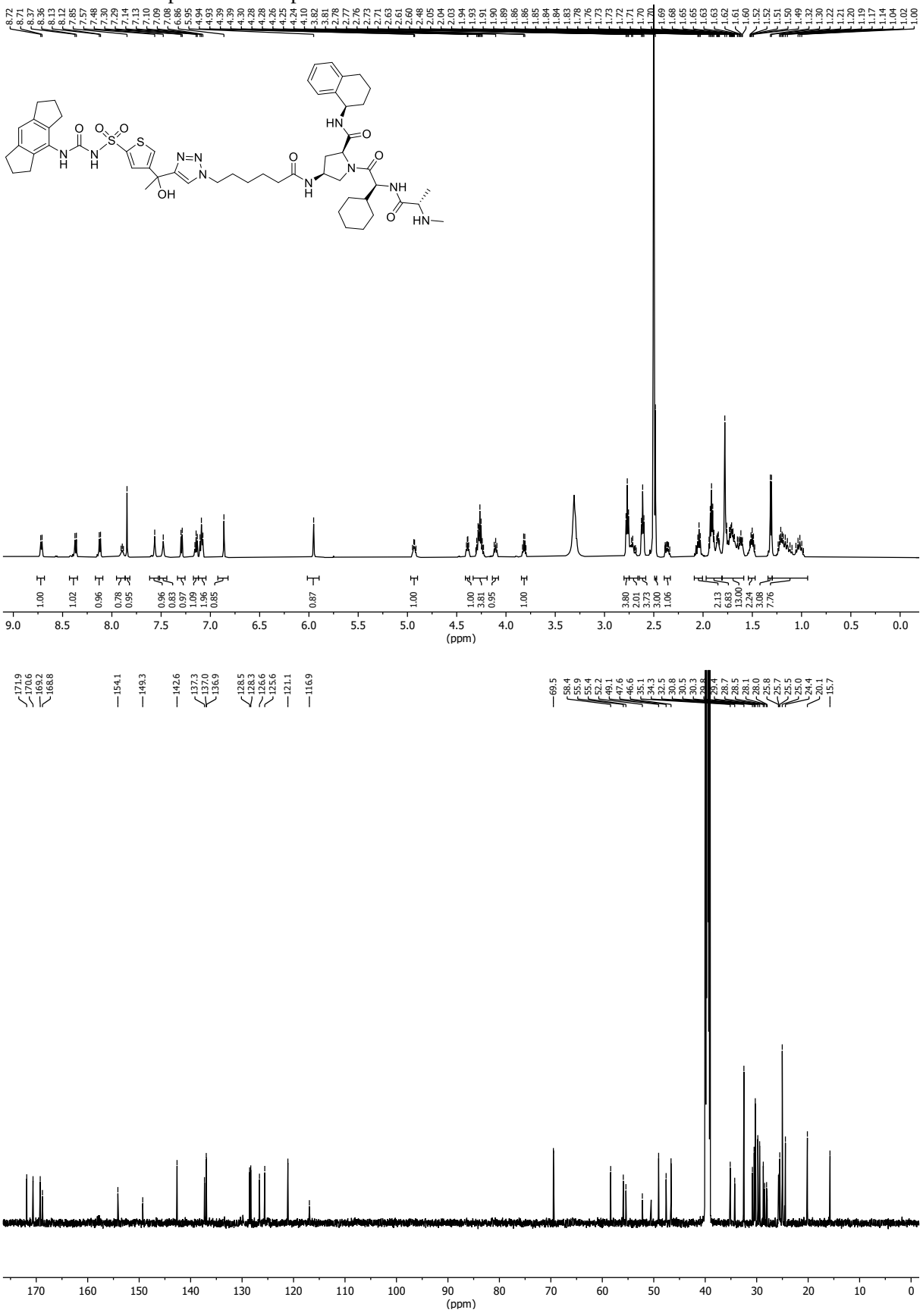


^{1}H and ^{13}C NMR spectra of compound **V6**

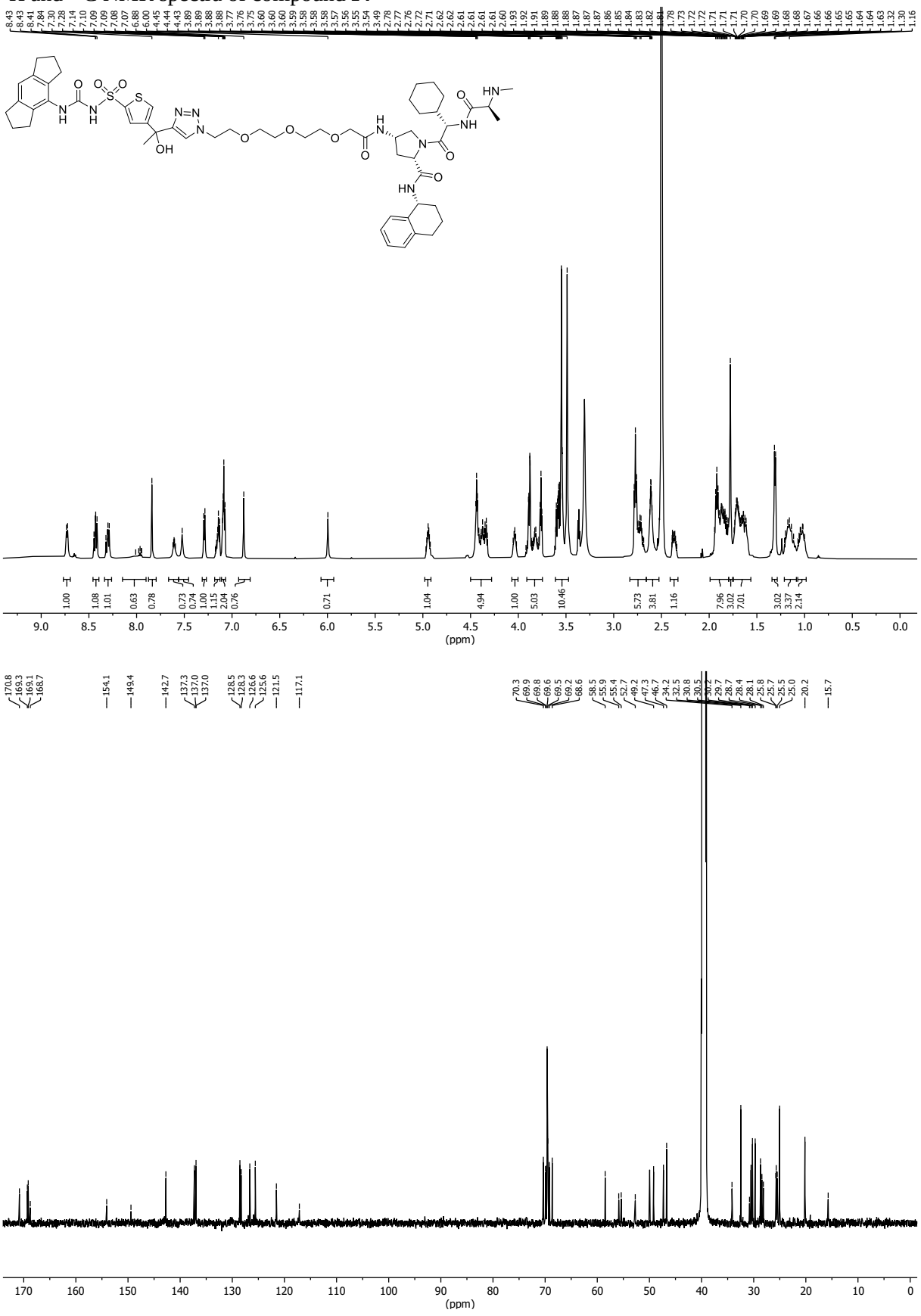




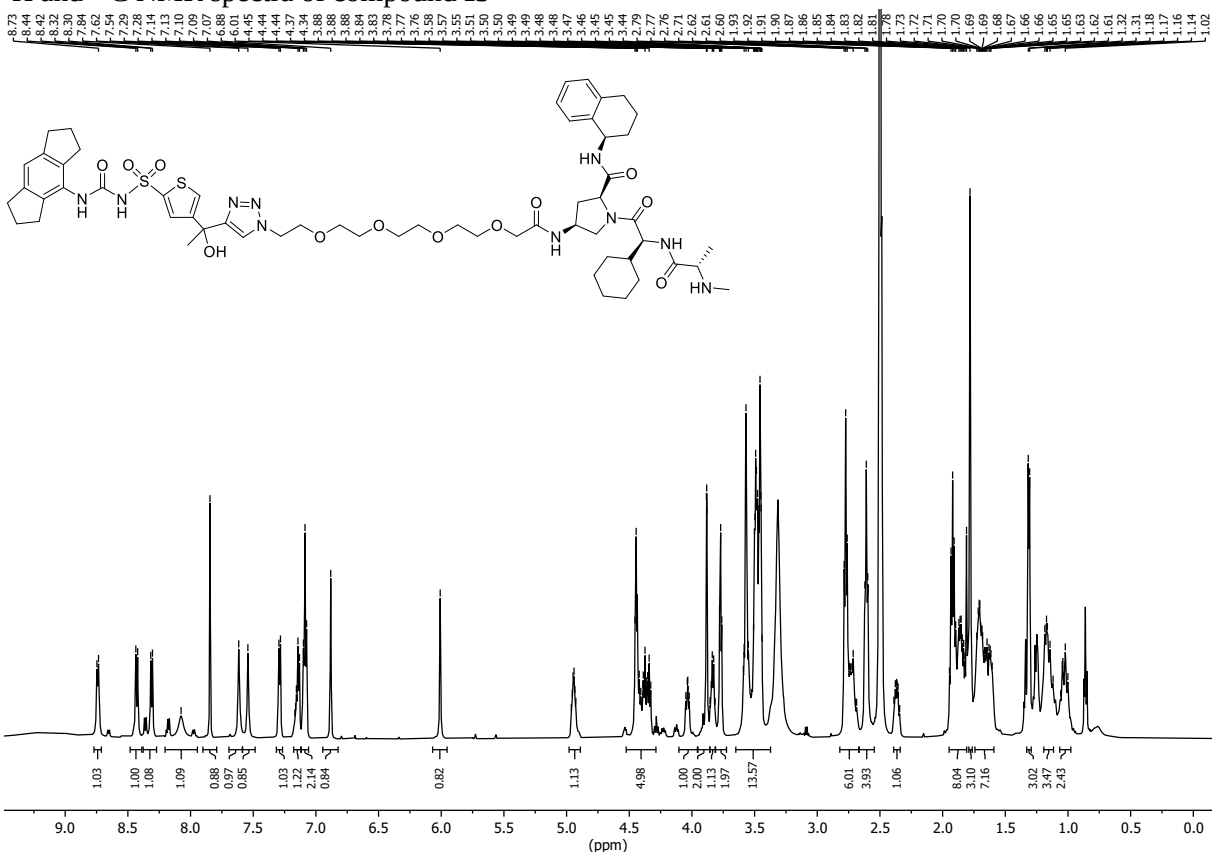
^{1}H and ^{13}C NMR spectra of compound **I1**

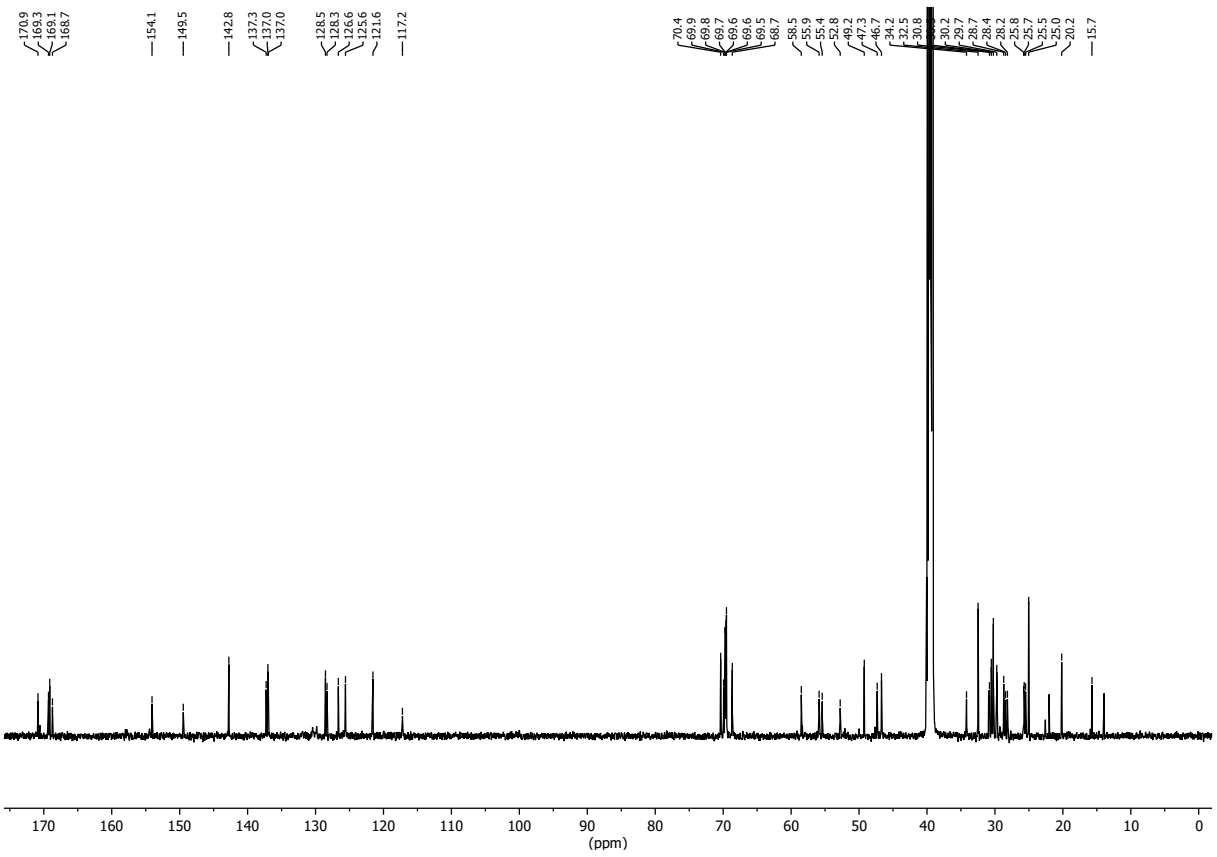


^{1}H and ^{13}C NMR spectra of compound **I4**

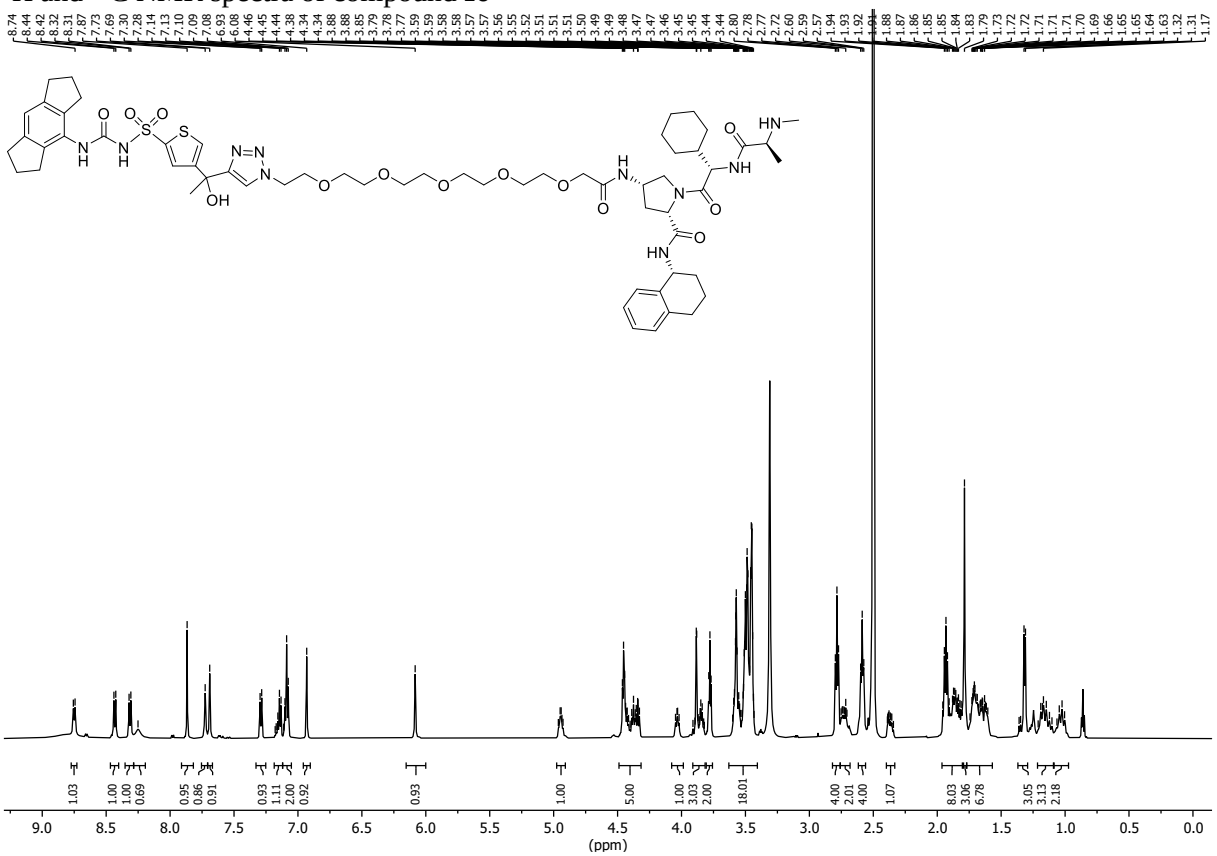


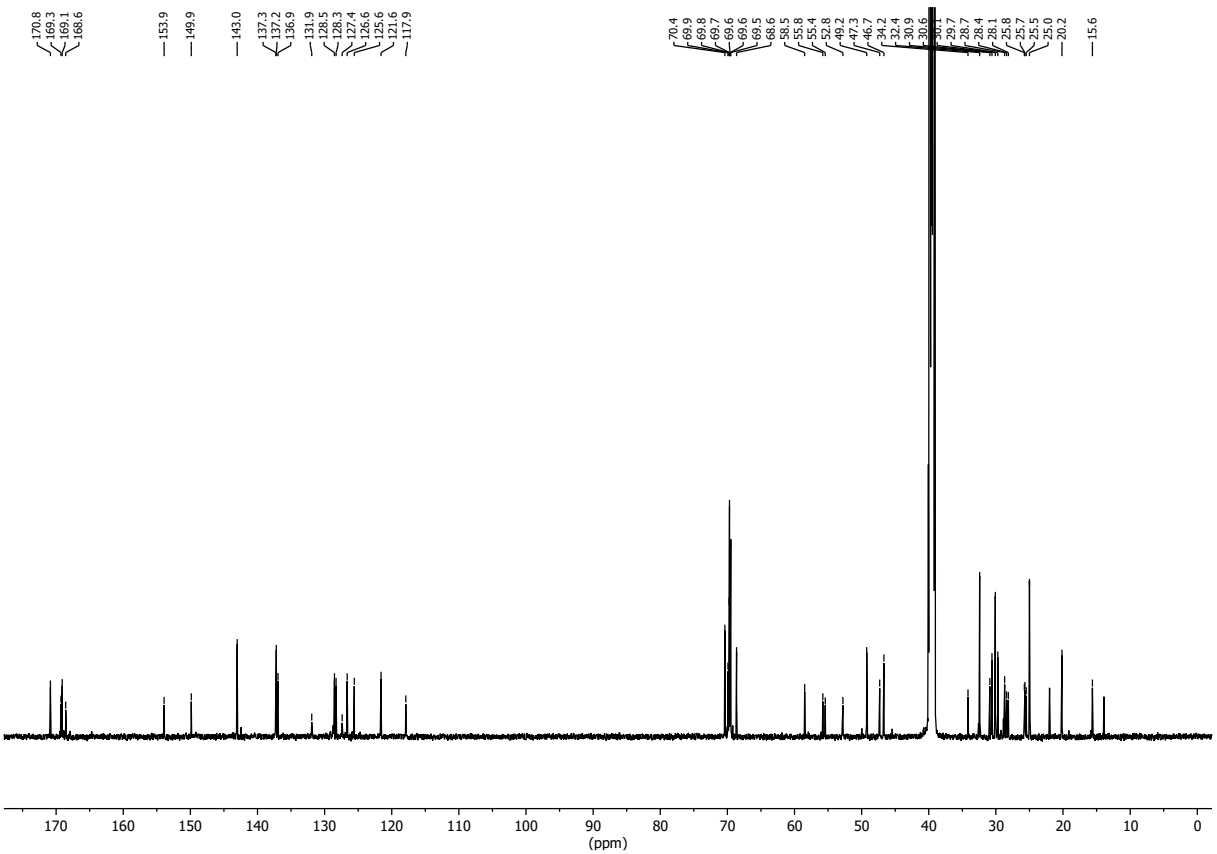
^{1}H and ^{13}C NMR spectra of compound **I5**



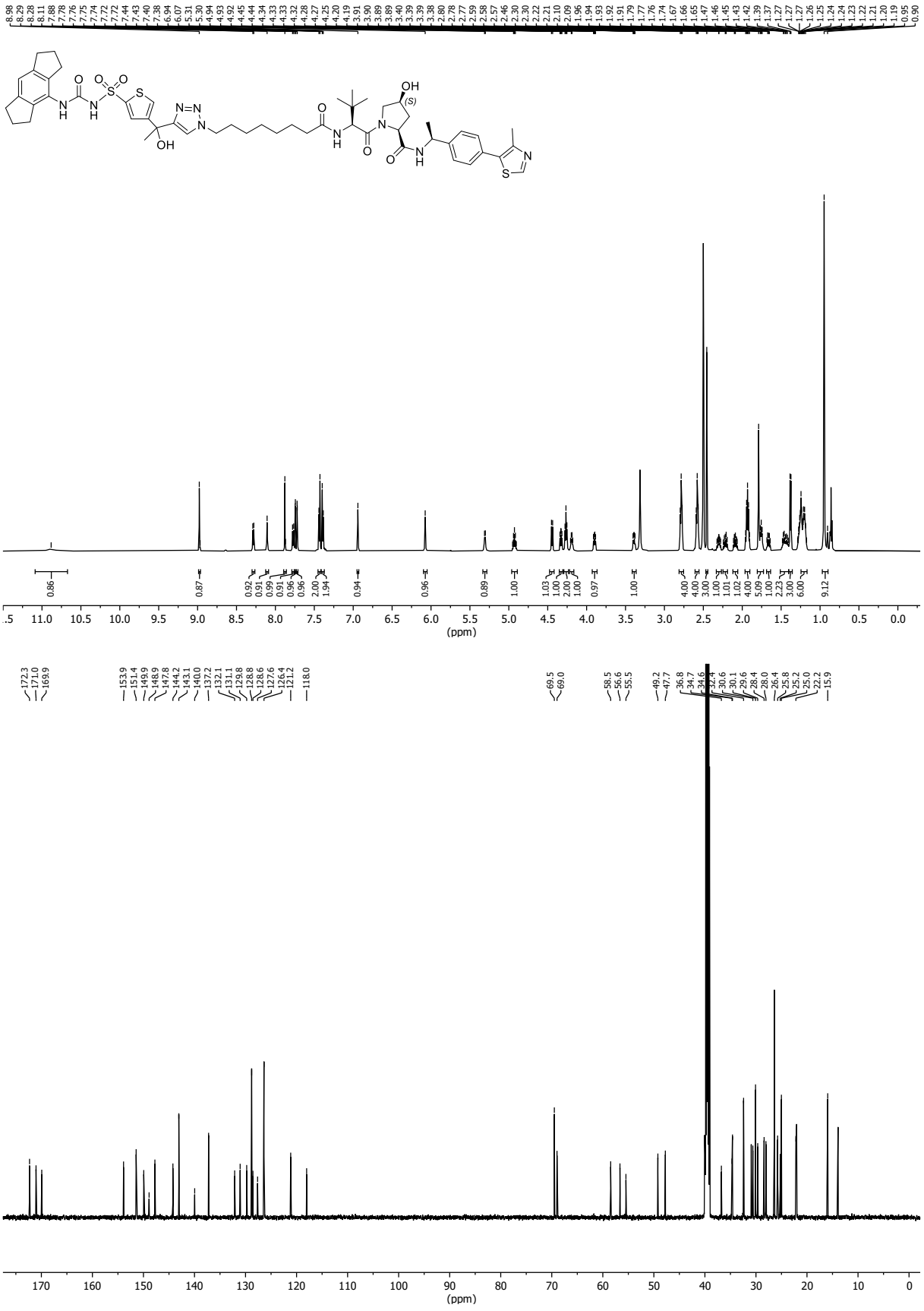


^{1}H and ^{13}C NMR spectra of compound **I6**





^{1}H and ^{13}C NMR spectra of compound **(-)V2**



8. References

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