Electronic Supplementary Material (ESI) for Chemical Communications.

G4-PROTAC: Targeted degradation of a G-quadruplex binding protein

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Supporting information

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1) General methods:

All chemicals, reagents and solvents were purchased from commercial sources (Sigma Aldrich, Acros, Merck, Alfa Aesar, etc.). DNA phosphoramidite monomers and linker 5'-hexynyl phosphoramidite were purchased from Glen Research. All reagents were used without further purification. Flash column chromatography was performed using Grace Davisil chromatographic silica media (40-63 μm) for the purification of small molecules. Waters preparative RP-HPLC connected to XBridge BEH C18 OBD Prep Column was used to purify some of the small molecules. Chemical reactions for synthesis of small molecules were monitored using Merck TLC silica gel 60 F254 aluminium plates. TLC were visualized by UV light, p-anisaldehyde stain or ninhydrin stain. NMR spectra were recorded on a Bruker Advance 500 or 400 MHz spectrometer. HRMS spectra were recorded on Waters, Xevo G2-XS QTof. Oligomers were synthesized using in-house ABI DNA/RNA synthesizer and Purified by RP-HPLC using XBridge BEH C18 OBD Prep Column, 130Å, 5 μm, 10 mm X 250 mm. MALDI-TOF analysis of synthesized oligomers was carried on Jeol (SpiralTOF JMS-S3000). Circular dichroism (CD) spectra were recorded on JASCO-815 CD spectrometer. PAGE gel was run on Bio-Rad gel apparatus. Western blot images were taken on AI680 and images were processed using image lab software. For Primary antibodies, polyclonal anti-DHX36 (anti-RHAU) (Bethyl Labs) 1:3000, monoclonal anti-GAPDH (Cell Signalling) 1:10000 were used. All primary antibodies used are of rabbit origin. As secondary antibodies, HRP-linked anti-rabbit IgG (Cell Signalling) 1:5000 were used. Antibodies were diluted in 5 % blotting-grade blocker (Bio-rad) in homemade TBST, unless otherwise stated.

2) Circular dichroism (CD)

Circular dichroism (CD) experiments were performed on a JASCO-815 CD spectrometer. Spectra of synthesized oligonucleotide (3 - 5 μM) were annealed and diluted to 500 μL with 20 mM potassium phosphate buffer (Kpi), pH 7 (except for *HP-P* that was diluted with 2 mM Kpi, pH 7). The quartz cuvette with a path length of 1 cm was used to record the spectra at 25 °C. Scans were done from 220-320 nm with 10 accumulations per sample. The values recorded by the instrument were baseline subtracted and each CD spectrum was further corrected at 320 nm.

3) Cell Culture and Transfection

HeLa and K-562 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco) and RPMI-1640 respectively both mediums were supplemented with 10 % fetal bovine serum (FBS) (HyClone), 1× Antibiotic Antimycotic Solution (HyClone) containing 100 units/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B, and 50 μg/mL Gentamicin Sulfate (Gibco) at 37 °C with atmosphere of 5 % CO_2. One day prior to transfection, cells were seeded at 1×10^5 cells/well in 12-well plates and allowed to recover at 37 °C with atmosphere of 5 % CO_2. Treatment samples were prepared using Lipofectamine RNAiMax (Invitrogen) according to manufacturer's instructions. The treatment solutions were added directly to each corresponding well and incubated at 37 °C with 5 % CO_2 atmosphere as specified time duration for each experiment.

4) Proteasome Inhibition

Cells were seeded at 1×10^5 cells/well in 12-well plates and allowed to recover at 37 °C with atmosphere of 5 % CO_2. Two hours prior to treatment, cells were pre-treated with a known proteasomal inhibitor, Bortezomib (SelleckChem), at concentration of 750 nM per well. Treatment samples were prepared as stated above and added to the wells after two-hour incubation with the proteosome inhibitor. The cells were further incubated for 24 hours at 37 °C with 5 % CO_2 atmosphere.

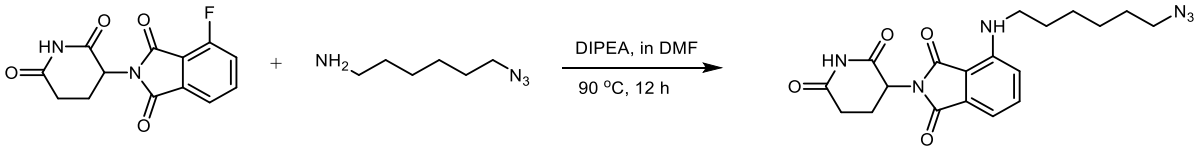
5) Western Blot (WB)

For western blotting, treated HeLa cells were washed twice with ice-cold phosphate-buffered saline (PBS) (Gibco). The cells were scraped with mini cell scrapers, collected, and pelleted at 2.0 × g for 10 minutes at 4 °C. In the case of experiment were K-562 cell (suspension cells) used, cells were collected by centrifugation and washed twice with cold PBS by re-suspension and centrifugation. Cell pellet was lysed at room temperature for 10 minutes with an appropriate volume of Mammalian Protein Extraction Reagent (MPER) (ThermoFisher) supplemented with 1× Pierce Protease Inhibitor tablet (ThermoFisher) pre-dissolved in MPER. The pellet mixture was centrifuged at maximum speed for 30 minutes at 4 °C to settle the cell debris and fragments. The supernatant was collected, and protein concentration was quantified by NanoDrop. All samples were separated by denaturing 10% SDS-PAGE gel with the settings: 80 V for 30 minutes followed by 125 V for 1 hour. Gel transfer onto 0.2 μm polyvinylidene difluoride (PVDF) membrane (Bio-rad) was performed by semi-dry electro blotting using the Bio-rad Trans-Blot Turbo Transfer System (Program: 1 mini gel, High MW setting). All membranes were blocked in 5 % blotting-grade blocker (Bio-rad) in homemade tris-buffered saline with 0.1% Tween20 (TBST) for 1 hour at room temperature before incubation with primary antibody overnight at 4 °C. Membranes were incubated with peroxidase-conjugated secondary antibody for 1 hour at room temperature. Bound antibodies were visualised by enhanced chemiluminescence system (ECL) (Bio-rad) according to manufacturer's instructions using the GE Amersham Imager 680 system. All western blot images were processed using ImageLab software.

6) Chemical synthesis of azide-linked pomalidomide and VH032 derivatives:

A) *Chemical synthesis of azide-linked pomalidomide derivatives:*

i)

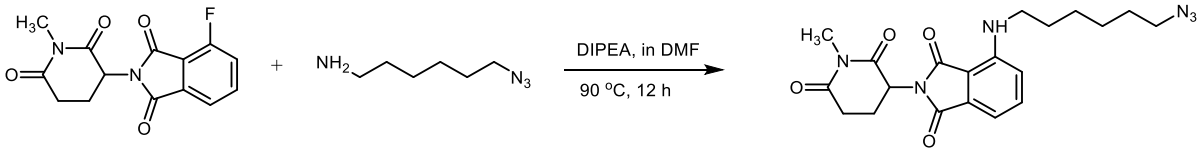


Scheme S1. Synthesis of Pom-N_3

(4-((6-azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione): (Pom-N_3)

The Pom-N_3 compound was synthesized by following literature reported procedure[1], briefly, to an oven dried 25 mL round bottom flask charged with magnetic stirrer bar, 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (300 mg, 1.09 mmol) was dissolved into 3 mL of anhydrous DMF. To that, *N, N*-diisopropylethylamine (351 mg, 2.72 mmol) and 6-azidohexan-1-amine (185 mg, 1.3 mmol) were added. The reaction mixture was stirred at 90 °C for 12 h under inert atmosphere. The crude product was extracted using EtOAc, the organic layer was washed with brine, dried over sodium sulfate. Filtration and evaporation of solvents yielded a crude product. The obtained crude product was re-dissolved into minimum quantity of acetonitrile and purified by preparative RP-HPLC using C18 column. Acetonitrile and water both containing 0.1% TFA were used as solvents for the RP-HPLC. Appropriate fractions of pure product from the HPLC were combined and lyophilized to obtained dry compound (4-((6-azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione) (152 mg, 35 %) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.55 – 7.40 (m, 1H), 7.09 (d, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.23 (t, *J* = 5.3 Hz, 1H), 4.91 (dd, *J* = 12.1, 5.3 Hz, 1H), 3.27 (dd, *J* = 12.8, 6.2 Hz, 4H), 2.94 – 2.63 (m, 3H), 2.19 – 2.06 (m, 1H), 1.75 – 1.56 (m, 4H), 1.53 – 1.34 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.17, 169.73, 168.51, 167.80, 147.17, 136.36, 132.72, 116.80, 111.68, 110.14, 51.54, 49.08, 42.71, 31.62, 29.33, 28.96, 26.72, 26.67, 23.02. HRMS: calculated for C19H23N6O4 $[M + H]^+$, 399.1780; found, 399.1781.

ii)

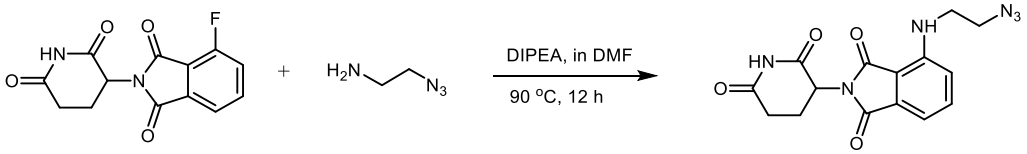


Scheme S2. Synthesis of (*N*-Me)Pom-N_3

4-((6-azidohexyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione: ((*N*-Me)Pom-N_3)

The compound Pom(11)-N_3 has been synthesized and purified by similar procedure as mentioned for Pom-N_3 synthesis except the 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione used in reactants. The pure compound 4-((6-azidohexyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione obtained as yellow solid. (50 mg, 36 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.44 (m, 1H), 7.09 (d, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.96 – 4.86 (m, 1H), 3.28 (m, , 4H), 3.21 (s, 3H), 2.98 (m, 1H), 2.84 – 2.67 (m, 2H), 2.13 – 2.04 (m, 1H), 1.62 (m, 4H), 1.52 – 1.38 (m, 4H). ^{13}C NMR (101 MHz, CDCl3) δ 171.23, 169.71, 169.02, 167.77, 146.93, 136.08, 132.58, 116.39, 111.41, 110.06, 51.34, 49.65, 42.50, 31.93, 29.13, 28.75, 27.25, 26.52, 26.46, 22.15. HRMS: calculated for $C_{20}H_{24}N_6O_4$ $[M + H]^+$, 435.1757; found, 435.1761.

iii)

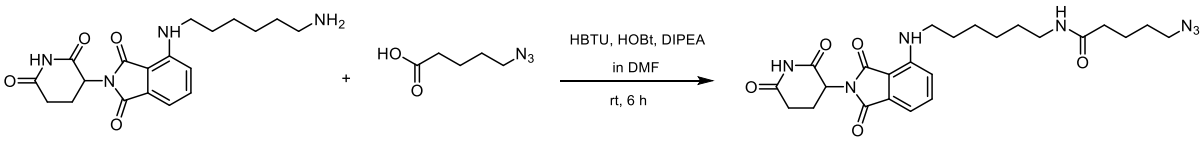


Scheme S3. Synthesis of Pom(L1)-N_3

4-((2-azidoethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione: (Pom(L1)-N_3)

The compound Pom(11)-N_3 has been synthesized and purified by similar procedure as mentioned for Pom-N_3 synthesis except the 2-azidoethan-1-amine used in reactants. The pure compound 4-((2-azidoethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione obtained as yellow solid. (42 mg, 32 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.60 – 7.42 (m, 1H), 7.16 (d, *J* = 7.1 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.44 (s, 1H), 4.99 – 4.83 (m, 1H), 3.54 (dq, *J* = 11.1, 5.6 Hz, 4H), 2.96 – 2.65 (m, 3H), 2.15 (dd, *J* = 12.4, 4.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.82, 169.35, 168.12, 167.39, 146.29, 136.27, 132.61, 116.39, 112.34, 110.89, 50.57, 48.96, 41.85, 31.40, 22.76. HRMS: calculated for $C_{15}H_{15}N_6O_4$ $[M + H]^+$, 343.1155; found, 343.1154.

iv)

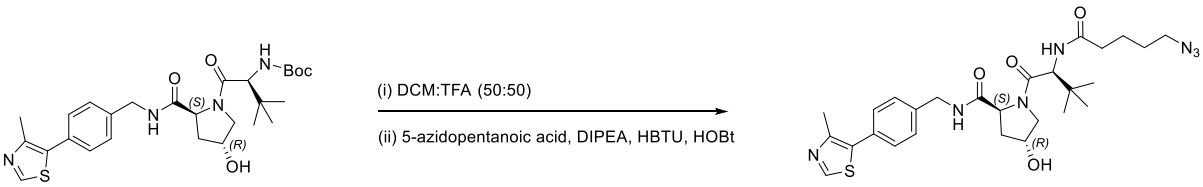


Scheme S4. Synthesis of Pom(L2)-N_3

5-azido-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)pentanamide: (Pom(L2)-N_3)

To an oven dried 10 mL round bottom flask charged with magnetic stirrer bar, 5-azidopentanoic acid (10 mg, 70 µmol) was dissolved into 1 mL of anhydrous DMF. To that, HBTU (26 mg, 70 µmol), HOBt (11 mg, 70 µmol) and *N, N*-diisopropylethylamine (37 µL, 350 µmol) was added under inert atmosphere. Stirred the reaction mix for 10 min. The 4-((6-aminohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione, Trifluoracetate (34 mg, 70 µmol) was dissolved into 1 mL of anhydrous DMF and added to the above reaction mixture under inert atmosphere and stirring was continued for next 6 h. The crude product was extracted using EtOAc, the organic layer was washed with water (5 mL x 2), sat. $NaHCO_3$ (5 mL x 2), brine (5 mL x1) and dried over sodium sulfate. Filtration and evaporation of solvents yielded a crude product. The obtained crude product was re-dissolved into minimum quantity of acetonitrile and purified by preparative RP-HPLC using C18 column. Acetonitrile and water both containing 0.1% TFA were used as solvents for the RP-HPLC. Appropriate fractions of pure product from the HPLC were combined and lyophilized to obtained dry compound 5-azido-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)pentanamide (19 mg, 55 %) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.55 – 7.40 (m, 1H), 7.07 (d, *J* = 7.1 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.22 (m, 1H), 4.91 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.25 (m, 6H), 2.79 (m, 3H), 2.24 – 2.13 (m, 2H), 2.14 – 2.05 (m, 1H), 1.75 – 1.57 (m, 6H), 1.51 (m, 2H), 1.45 – 1.31 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.28, 171.12, 169.50, 168.46, 167.58, 146.93, 136.11, 132.44, 116.63, 111.39, 109.84, 51.13, 48.84, 42.48, 39.33, 35.95, 31.37, 29.65, 29.54, 29.04, 28.34, 26.53, 22.82, 22.76. HRMS: calculated for $C_{24}H_{32}N_7O_5$ $[M + H]^+$, 498.2465; found, 498.2479.

B) *Chemical synthesis of azide-linked VHL032 derivative:*



Scheme S5. Synthesis of VH032-N_3

(2S,4R)-1-((S)-2-(5-azidopentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide: (VH032-N_3)

Step(i): The tert-butyl ((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (100 mg, 0.18 mmol) (synthesized according to literature reported procedure[2]) was added to an oven dried 10 mL round bottom flask charged with magnetic stirrer bar. To that, 4 mL of 50:50 (DCM:TFA) was added and reaction was stirred on ice bath for 30 min. All solvents were evaporated and obtained TFA salt compound. The (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide,
Trifluoracetate (TFA salt) was dried under vacuum and used in the next peptide coupling reaction without further purification.

Step(ii): To an oven dried 10 mL round bottom flask charged with magnetic stirrer bar, 5-azidopentanoic acid (21 mg, 0.15 mmol) was dissolved into 3 mL of anhydrous DMF under inert gas. To that, HBTU (56 mg, 0.15 mmol), HOBt (23 mg, 0.15 mmol) and *N, N*-diisopropylethylamine (95 mg, 0.75 mmol) were added and reaction mixture was stirred for 10 min at room temperature under inert gas. In another 10 mL round bottom flask dried above mentioned TFA salt was dissolved into 1 mL of anhydrous DMF under inert gas and added to above mentioned reaction mixture. Stirred the obtained reaction mixture at room temperature for next 2 h. Reaction progress was monitored by TLC. Upon completion of reaction it was quenched by addition of 500 uL of water. Volatiles were removed under vacuum and obtained crude was purified flash column chromatography using DCM: MeOH (3-5 % MeOH in DCM) solvents to obtain (2S,4R)-1-((S)-2-(5-azidopentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (42 mg, 40% from two steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.33 (m, 5H), 6.25 (d, *J* = 8.8 Hz, 1H), 4.67 (t, *J* = 7.8 Hz, 1H), 4.53 (m, 3H), 4.32 (dd, *J* = 15.0, 5.3 Hz, 1H), 4.01 (d, *J* = 11.2 Hz, 1H), 3.78 (bs, 1H), 3.62 (dd, *J* = 11.1, 3.7 Hz, 1H), 3.25 (t, *J* = 6.5 Hz, 2H), 2.49 (s, 3H), 2.48 – 2.41 (m, 1H), 2.20 (t, *J* = 7.2 Hz, 2H), 2.07-2.12 (m, 1H), 1.71 – 1.62 (m, 2H), 1.61 – 1.53 (m, 2H), 0.92 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.09, 171.91, 170.98, 150.55, 148.62, 138.25, 131.74, 131.13, 129.68, 128.25, 72.50, 70.17, 58.81, 57.66, 56.88, 51.23, 43.38, 36.23, 35.83, 35.27, 28.44, 26.58, 22.90, 16.21. HRMS: calculated for $C_{27}H_{38}N_7O_4S$ $[M + H]^+$, 556.2706; found, 556.2706

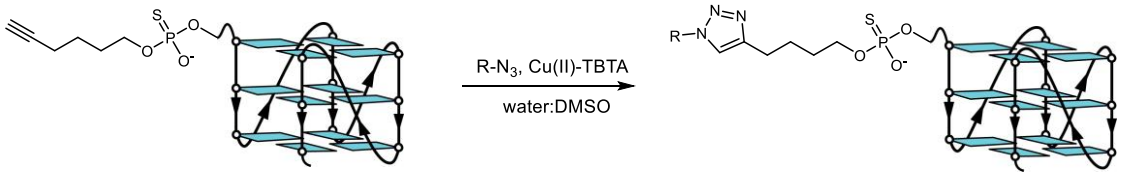
7) Cu(II)-TBTA mediated click reaction between alkyne-linked oligonucleotide and azide-linked small molecules:

The synthesis of conjugates between 5'-alkyne DNA sequences and azide linked-pomalidomide or -VH032 derivative was performed by copper-click reaction. The click reaction protocol was followed from Lumiprobe website (https://www.lumiprobe.com/protocols/click-chemistry-dna-labeling) with slight alterations. Typically, click reaction were carried out on 100-300 uM scale of alkyne linked oligonucleotide.

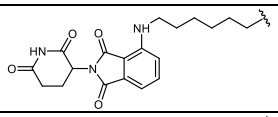
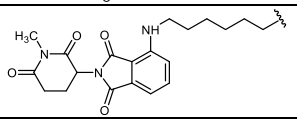
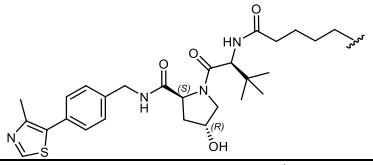
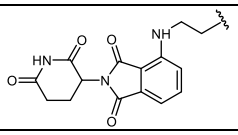
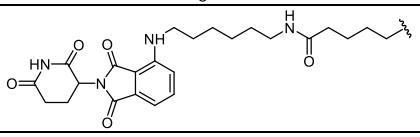
The following click reaction protocol is for 200 uM alkyne-modified oligonucleotide in 400 uL of solvents: In the 1.5 uL plastic reaction tube, 80 uL of oligonucleotide (from 1.0 mM stock in water) was transferred, to that 40 uL of TEAA buffer, pH 7.0 (from 2.0 M stock in water) and 40 uL of DMSO were added and mixture was briefly vortexed. Next, 16 uL of appropriate azide compound (from 20 mM stock prepared in DMSO) was added. 20 uL of sodium ascorbate (from 10 mM stock in water) was added and reaction mixture was vortexed briefly. Degassed the reaction mixture by bubbling the Argon gas approximately for 1 minute and 20 uL of 10 mM Cu-TBTA (stock in 55:45 (DMSO:water)) was added quickly. Appropriate amount of DMSO and water (approximately 50:50 ratios in the final reaction mixture) was added to solubilize all the reagents used in the reaction mixture. Finally, the reaction tube was briefly purged by argon gas and sealed tightly and kept on shaker-heater at 40 ^{o}C (and 1000 rpm) for next 12 h. After completion of reaction, the oligonucleotide from reaction mixture was precipitated by ethanol precipitation protocol. The obtained crude oligonucleotide conjugate was further purified on RP-HPLC by gradient method using 100 mM TEAA and acetonitrile as solvents. The appropriate fractions were collected, lyophilized and the formation of product was confirmed by MALDI-TOF analysis.

Further, the oligonucleotide products were cartridge purified, desalted on commercially available desalting columns, concentration measured using NanoDrop spectrophotometer and were lyophilized. Dried oligonucleotides were re-dissolved into appropriate amount of 20 mM KPi buffer (pH, 7.0), heated into sealed tube at 95 ^{o}C for 5 min and allowed to slow cool until room temperature. The aliquots of synthesized oligonucleotides were stored at -80 ^{o}C until used.

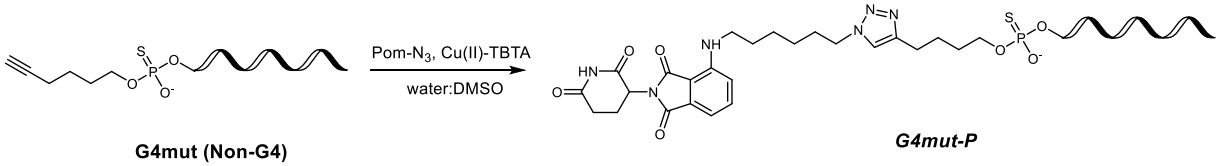
A)



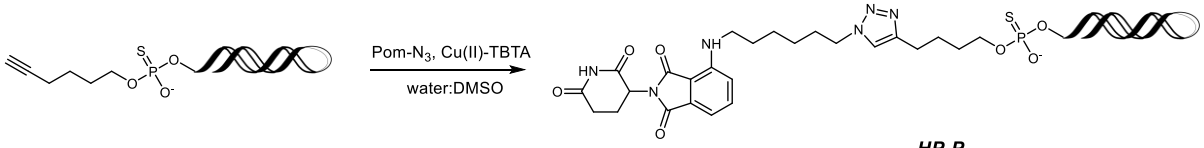
G4-ctrl ***G4-PROTAC***

R	G4-PROTAC
	*G4-P*
	*G4-(N-Me)P*
	*G4-V*
	*G4-P-L1*
	*G4-P-L2*

B)



G4mut (Non-G4) ***G4mut-P***



Hairpin DNA (Non-G4) ***HP-P***

Scheme S6. Schematic for the synthesis of representative G4-PROTACs and derivatives. G4 forming sequence containing PROTACs *G4-P*, *G4-V*, *G4-P-L1* and *G4-P-L2*, G4-forming sequence containing non-PROTAC *G4-(N-Me)P* (Panel A), Non-G4 forming sequences containing PROTAC *G4mut-P* and *HP-P* (Panel B)

Table S1: List of all synthesized oligomers for the study and their MALDI-TOF analysis

Label	DNA sequence[a]	Cal. MW (Da)	Obs.MW (Da)
G4-ctrl	linker-*TT GGG T GGG T GGG T GGG T	5889.7	5888.7
G4-P	Pom-linker-*TT GGG T GGG T GGG T GGG T	6287.7	6289.1
G4-(N-Me)P	(*N*-Me)Pom-linker-*TT GGG T GGG T GGG T GGG T	6301.7	6303.7
G4-V	VHL-linker-*TT GGG T GGG T GGG T GGG T	6444.7	6447.0
G4mut-P	Pom-linker-*TT GIG T GIG T GIG T GIG T	6187.8	6186.0
HP-P	Pom-linker-*ATC TGA GAA TCA GAT	5174.2	5170.9
G4(htelo)-P	Pom-linker-*TAG GGT TAG GGT TAG GGT TAG GGT T	8453.21	8454.6
G4(parp1)-P	Pom-linker-*TGG GGT CCG AGG CGG GGC TTG GG	7807 .8	7806.8
G4-P-L1	Pom-linker1-*TT GGG T GGG T GGG T GGG T	6231.8	6235.6
G4-P-L2	Pom-linker2-*TT GGG T GGG T GGG T GGG T	6386.9	6389.9

[a] 'linker-', 'linker1-' and 'linker2-' as drawn in Scheme S6 (ESI†), 'Pom-' represents pomalidomide and 'VH032-' represents VH032 ligand, '*' represents a phosphorothioate linkage.

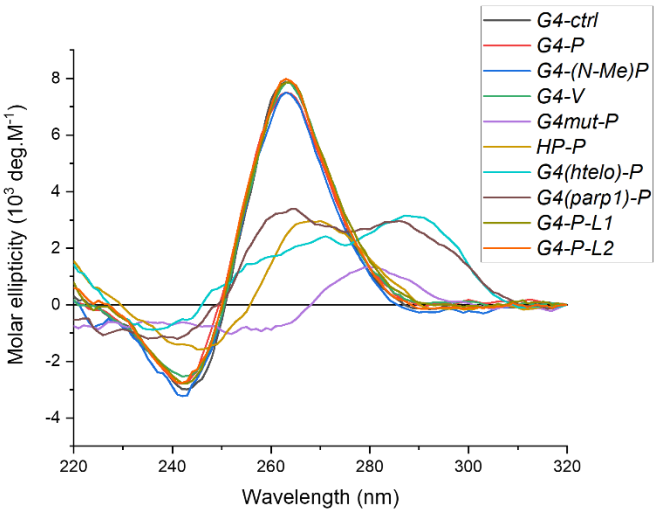


Figure S1. CD spectra of synthesized oligomers used in this study.

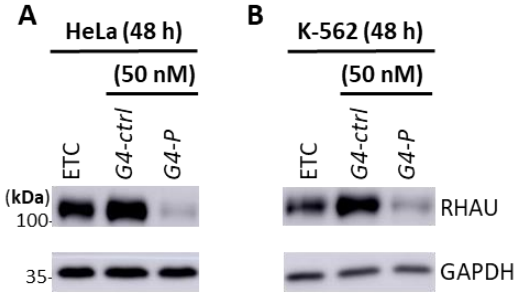


Figure S2. Degradation of RHAU in different cell lines. Cells were transfected with ETC, *G4-ctrl* (50 nM) and *G4P* (50 nM), harvested after 48 h treatment and analyzed by Western blot; the results for HeLa and K-562 cells are shown in panel A and B respectively.

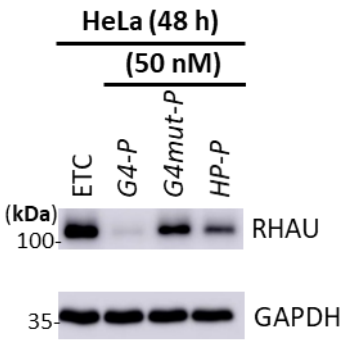


Figure S3. Effect of G4-PROTAC and (non-G4)-PROTAC on RHAU degradation. Cells were transfected with ETC, *G4-P* (50 nM), *G4mut-P* (50 nM) and *HP-P* (50 nM), harvested after 48 h treatment and analysed by Western blot.

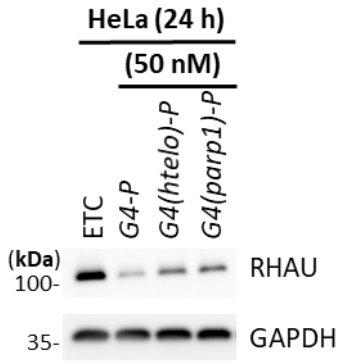


Figure S4. Effect of G4-topology in G4-PROTAC on RHAU degradation. Cells were transfected with ETC*, G4-P* (50 nM), *G4(htelo)-P* (50 nM) and *G4(parp1)-P* (50 nM), harvested after 24 h treatment and analyzed by Western blot.

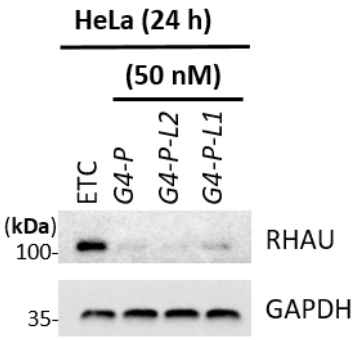


Figure S5. Effect of linker length in G4-PROTAC on RHAU degradation. Cells were transfected with ETC and *G4-P* (50 nM), *G4-P-L1* (50 nM) and *G4-P-L2* (50 nM), harvested after 24 h of treatment and analyzed by Western blot.

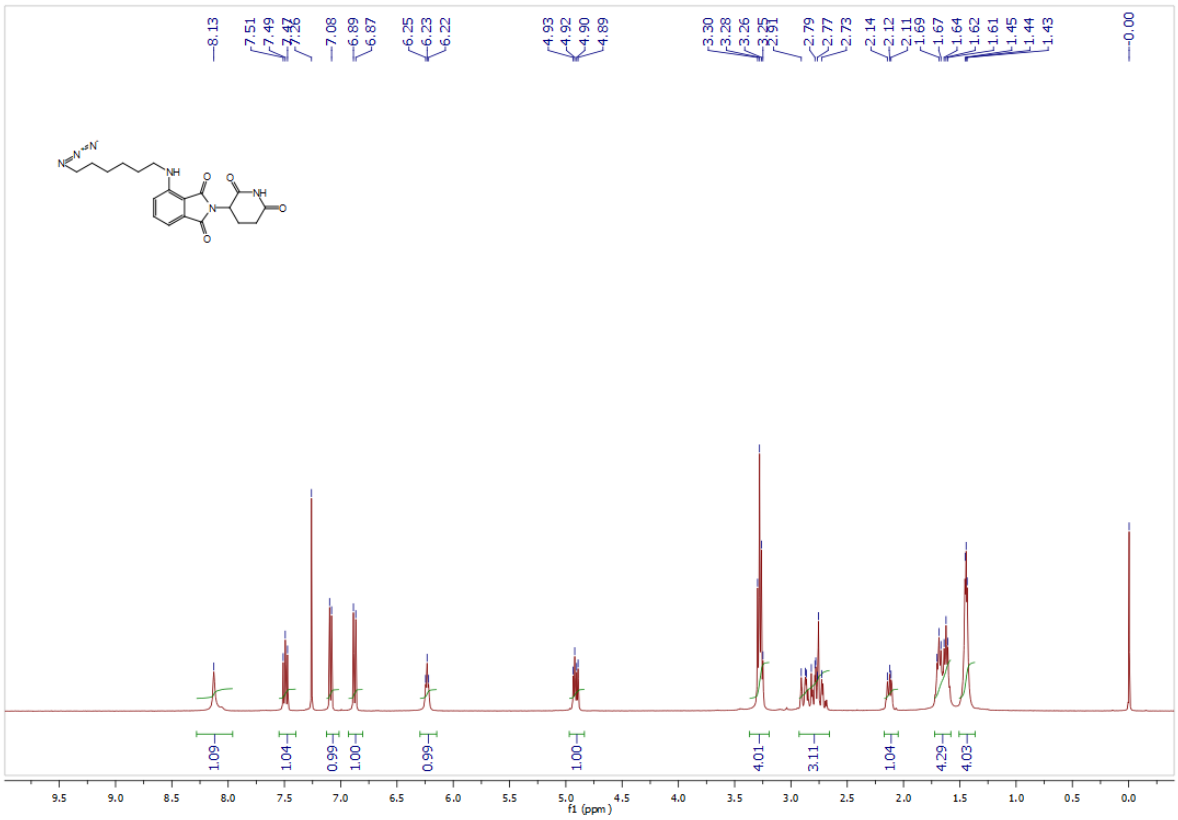


Figure S6. ^{1}H NMR spectrum of Pom-N_3

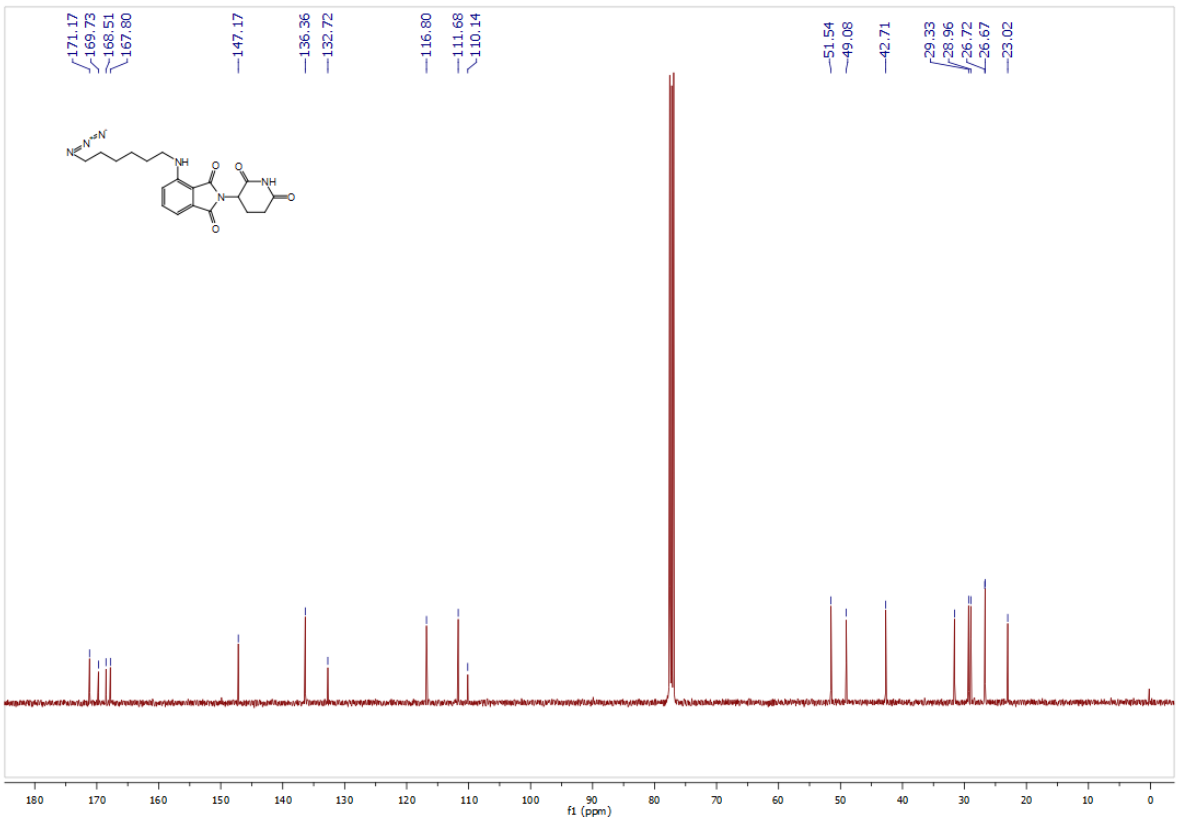


Figure S7. ^{13}C NMR spectrum of Pom-N_3

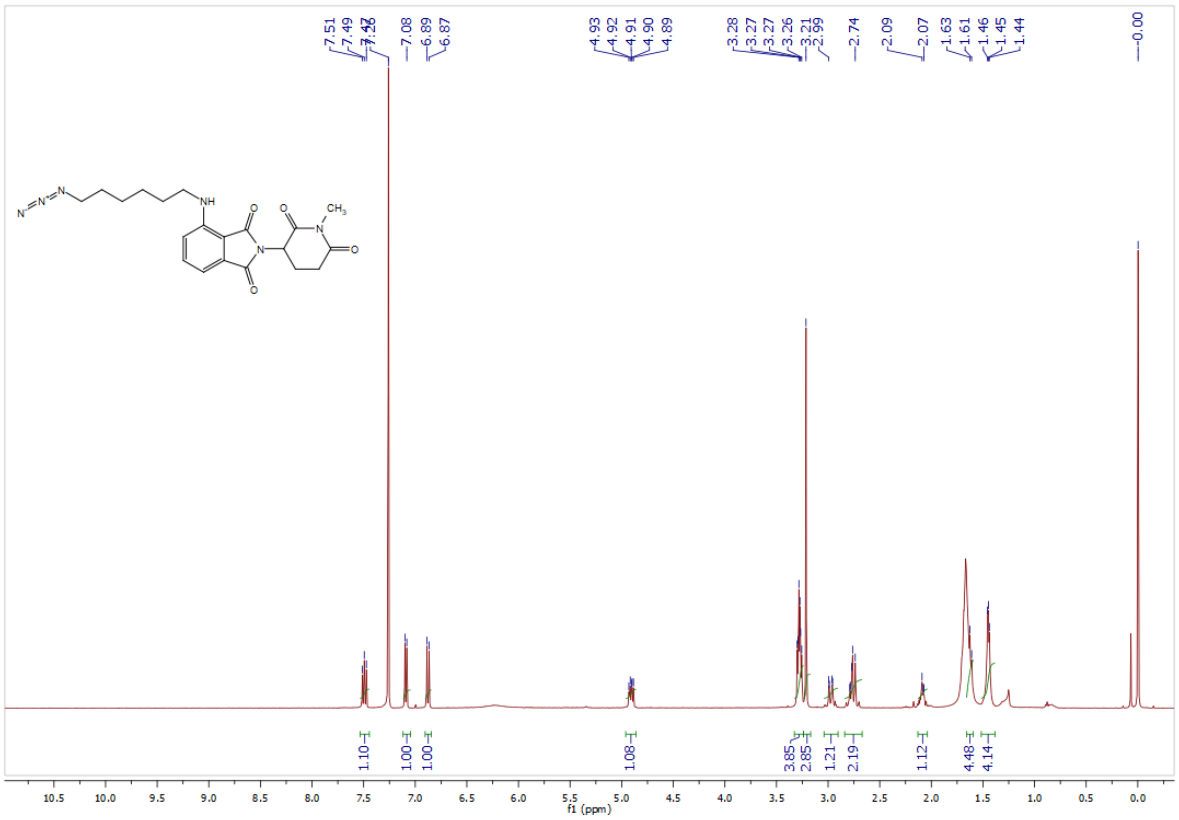


Figure S8. ^{1}H NMR spectrum of (*N*-Me)Pom-N_3

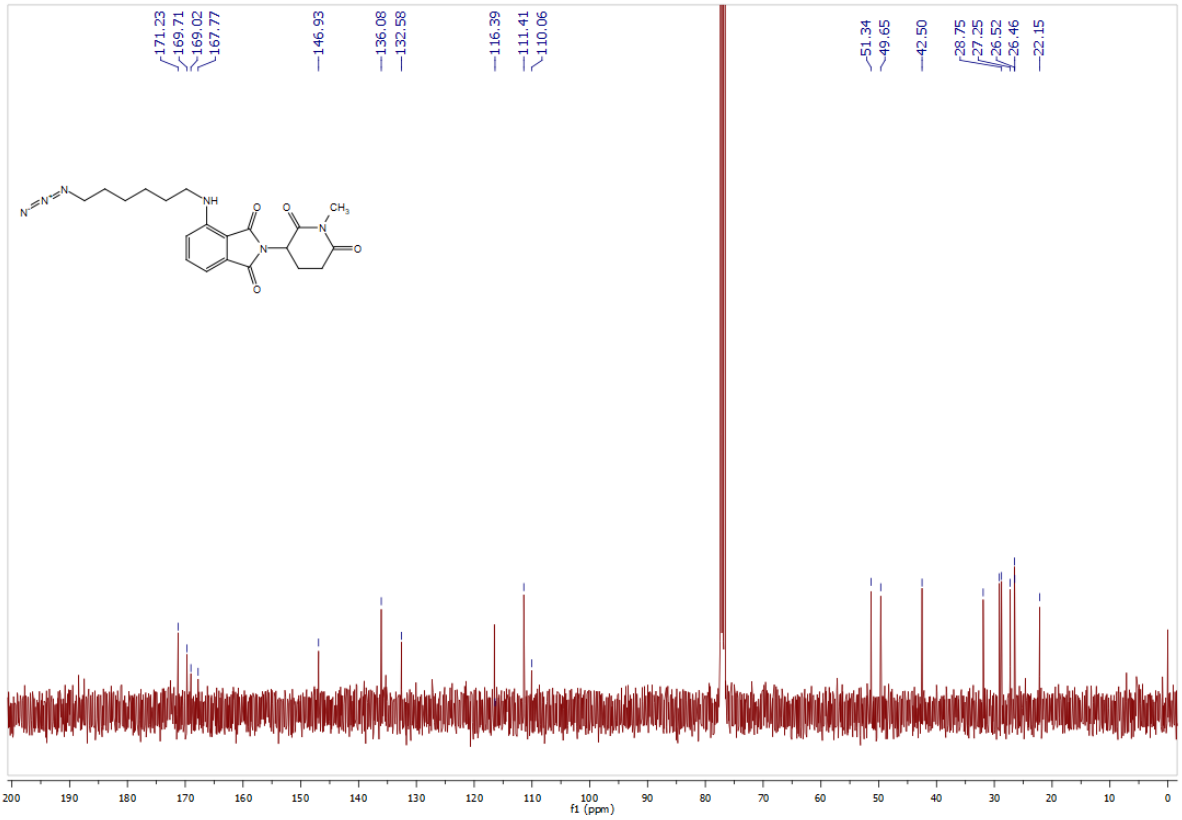


Figure S9. ^{13}C NMR spectrum of (*N*-Me)Pom-N_3

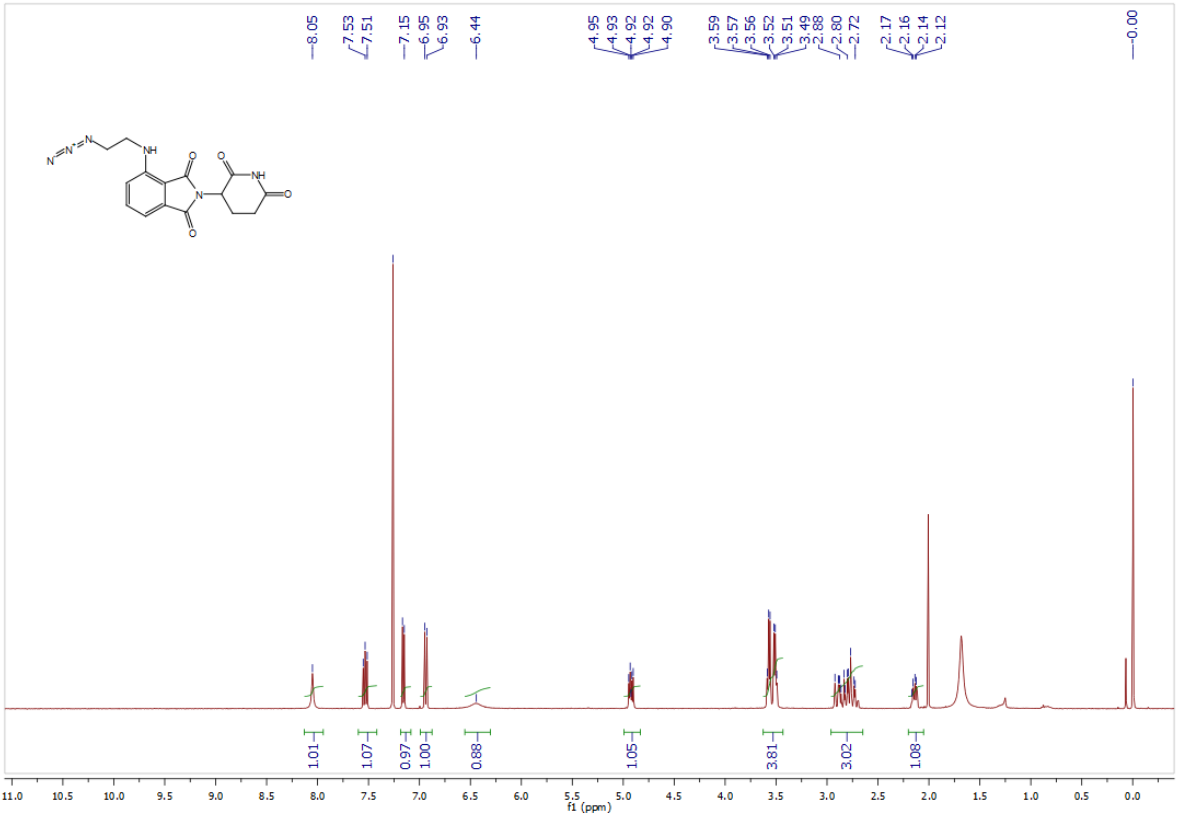


Figure S10. ^{1}H NMR spectrum of Pom(L1)-N_3

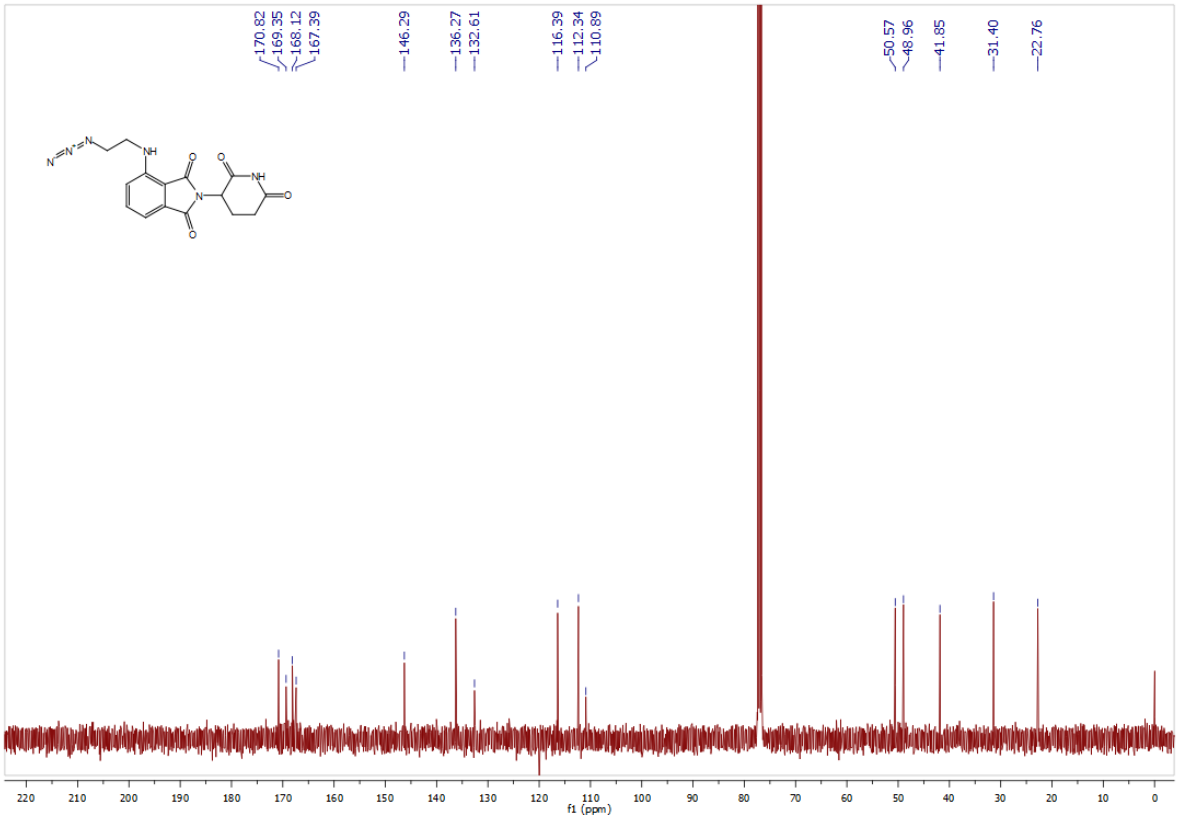


Figure S11. ^{13}C NMR spectrum of Pom(L1)-N_3

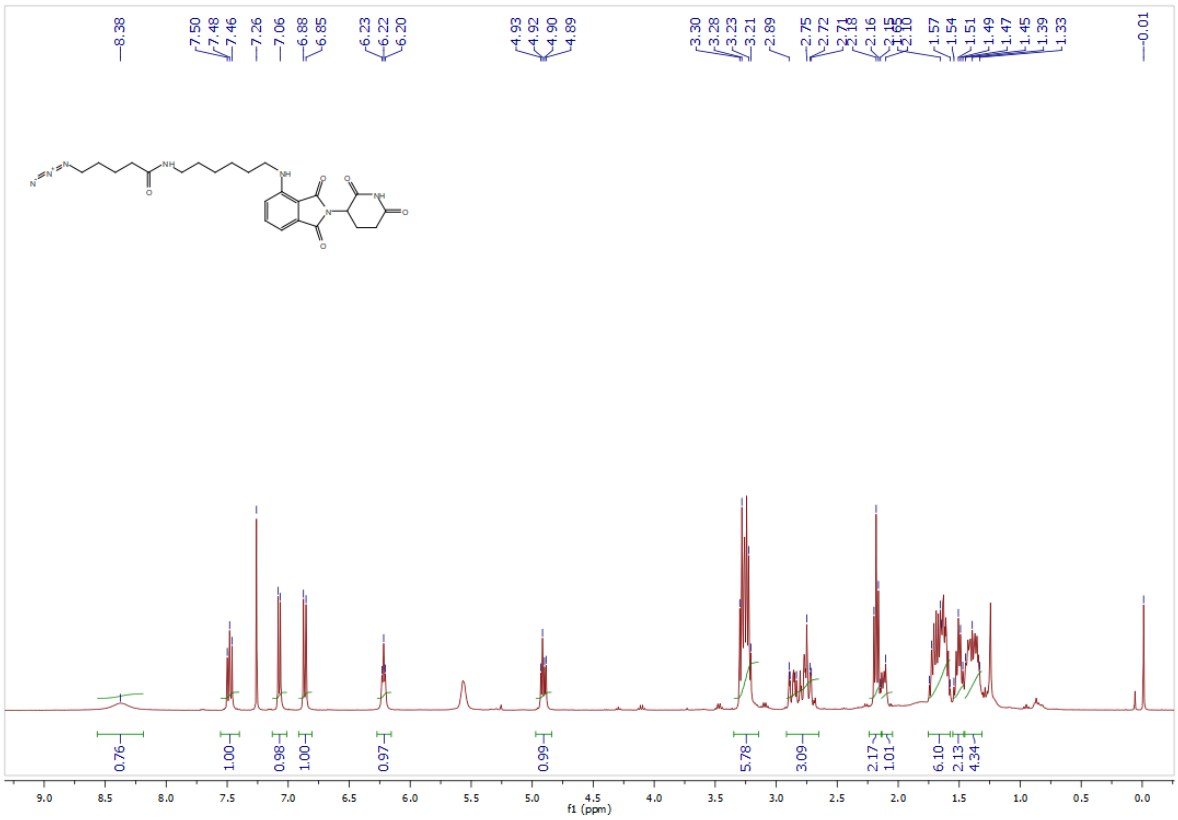


Figure S12. ^{1}H NMR spectrum of Pom(L2)-N_3

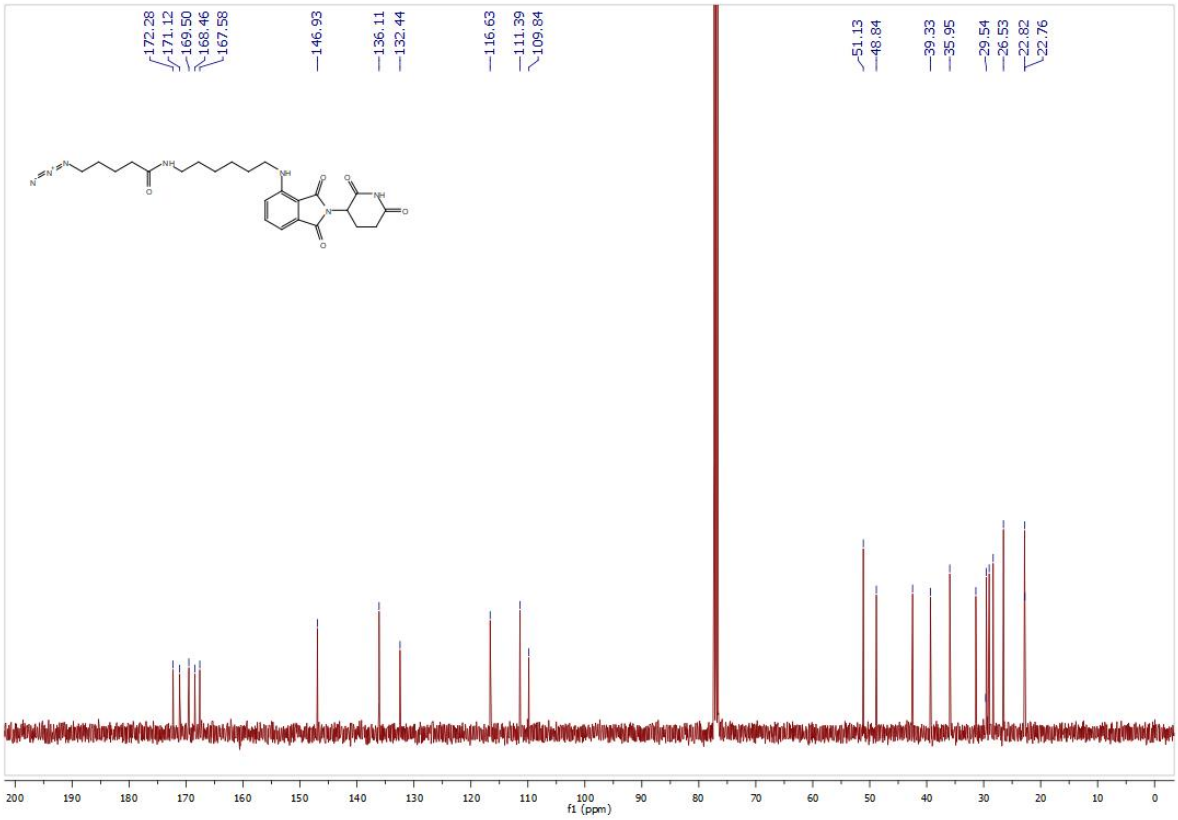


Figure S13. ^{13}C NMR spectrum of Pom(L2)-N_3

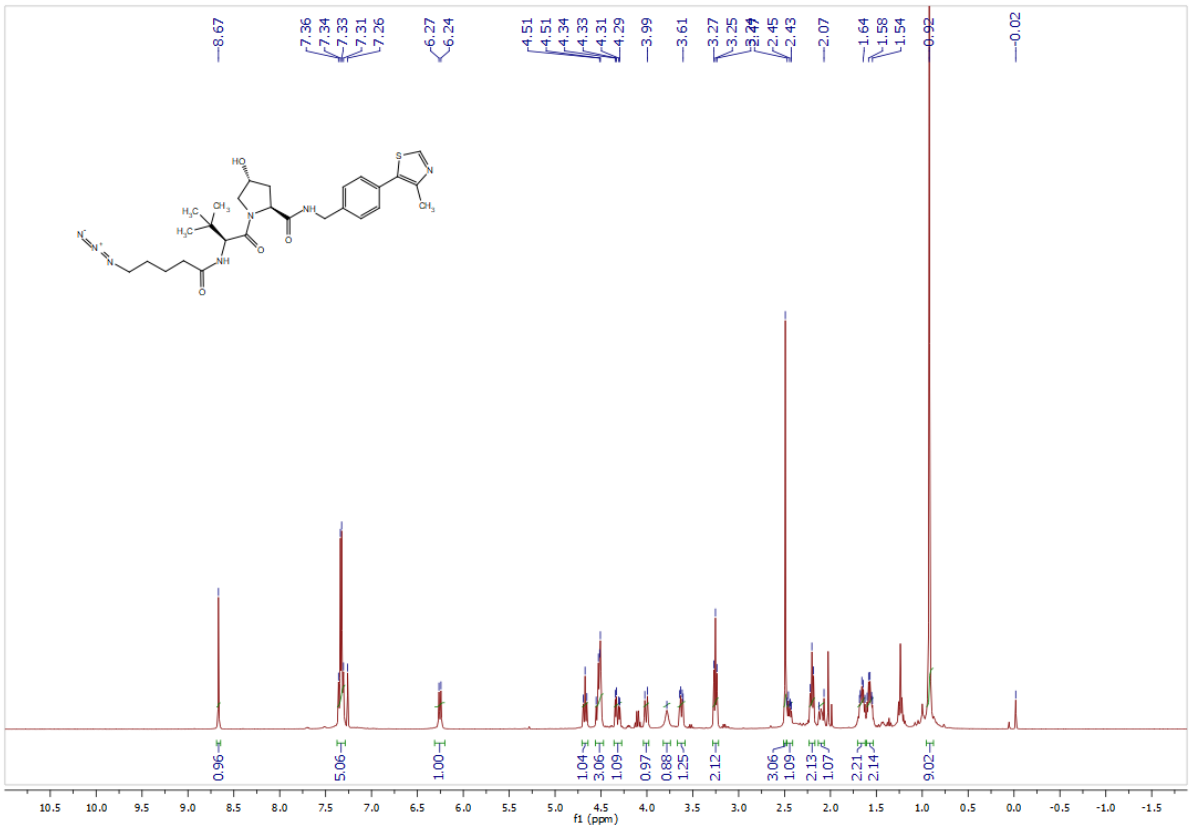


Figure S14. ^{1}H NMR spectrum of VH032-N_3

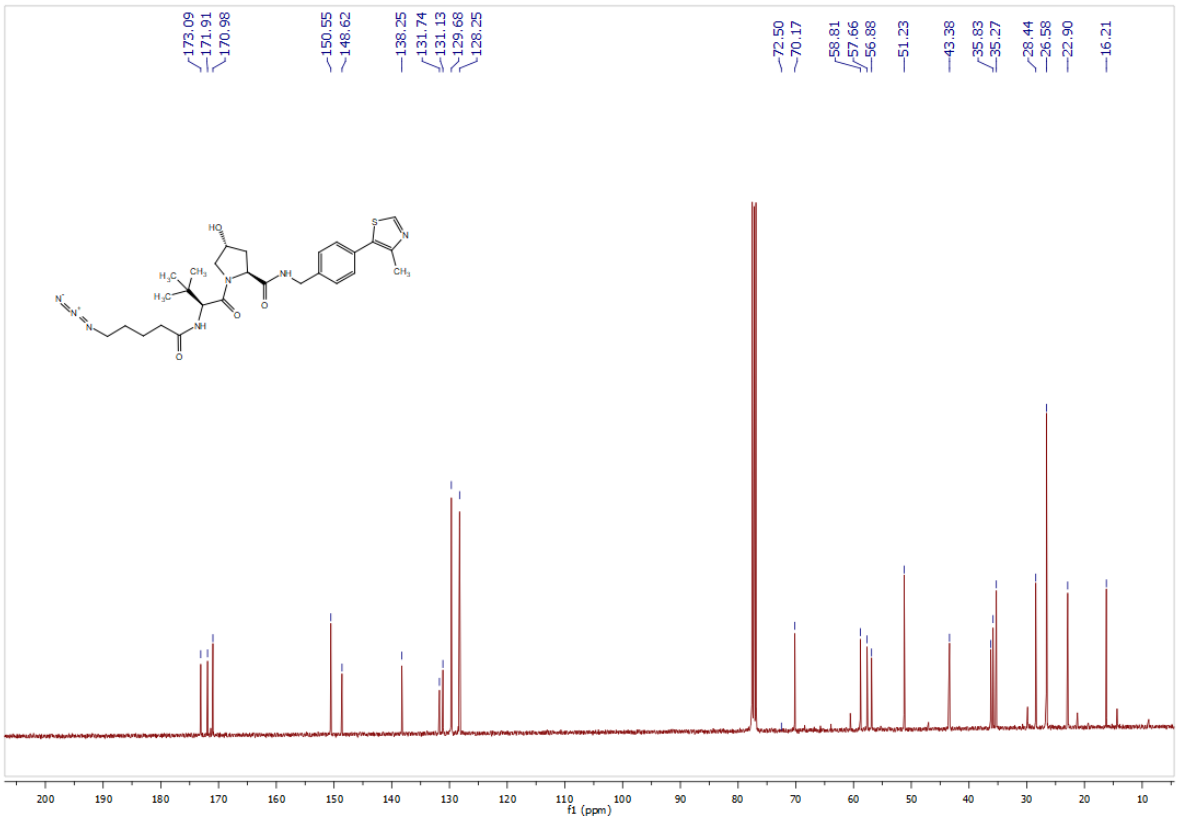


Figure S15. ^{13}C NMR spectrum of VH032-N_3

8) References.

[1] M.-X. Li, Y. Yang, Q. Zhao, Y. Wu, L. Song, H. Yang, M. He, H. Gao, B.-L. Song, J. Luo, Y. Rao, *Journal of Medicinal Chemistry* **2020**, *63*, 4908-4928.

[2] C. Steinebach, H. Kehm, S. Lindner, L. P. Vu, S. Köpff, Á. López Mármol, C. Weiler, K. G. Wagner, M. Reichenzeller, J. Krönke, M. Gütschow, *Chemical Communications* **2019**, *55*, 1821-1824.