Supporting Information

Rapid Synthesis of Pomalidomide-Conjugates in Minutes *via* Temperature Elevation and Delayed Feeding

Yingying Jiang [a,#], Hang Zhao [a,#], Lunjie Chen [a], Siping Wei [a], Jun Wang[a], Lin Wang [a], Qiang Fu[a,b]*

[a] Green Pharmaceutical Technology Key Laboratory of Luzhou City, Central Nervous System Drug Key Laboratory of Sichuan Province, School of Pharmacy, Southwest Medical University, Luzhou 646000, People's Republic of China. E-mail: fuqiang@swmu.edu.cn;

[b] Ningbo Dayang Technology Co., Ltd, Ningbo 315000, People's Republic of China

[#]The two authors contributed equally to the work

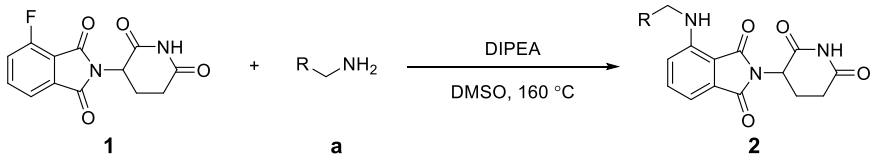
Contents

1.1 General information

All chemical reactions were carried out under an Air atmosphere with Analytical Reagent (AR) solvents, unless otherwise noted. Reagents were purchased from Adamas-beta®, Bide Pharmatech, Energy Chemical, Acmec Biochemical Technology, and Accela as reagent grade and used without further purification, unless otherwise stated. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were obtained with a Bruker AV II-400 spectrometer (^{1}H: 400 MHz, ^{13}C: 101 MHz, ^{19}F: 376 MHz). The chemical shifts (δ) were expressed in ppm and *J* values were given in Hz using tetramethylsilane as the internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets ddt = doublet of doublets of triplets and br = broad. Thin-layer chromatography (TLC) was performed using commercially prepared silica gel plates (GF_{254}), and visualized under UV light 254 nm or iodine stain as developing agents. Flash column chromatography was performed on silica gel (100-200 mesh). Mass analysis data were acquired on a SCIEX UPLC (EXion) – Q-TOF (X500R). Melting points were measured using a Hanon MP470 apparatus.

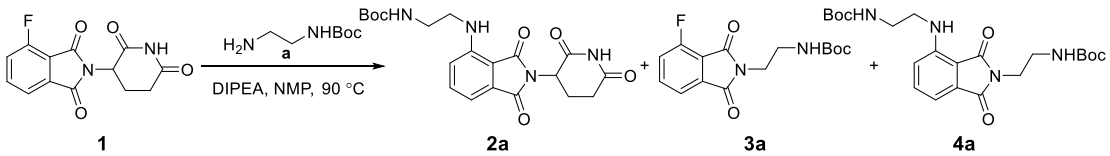
1.2 Experimental procedures

1.2.1 General S_NAr procedures for the synthesis of Pomalidomide-conjugates 2a-2n



4-Fluoro-thalidomide **1** (500 mg, 1.81 mmol, 1.0 equiv.) was dissolved in DMSO (10 mL) and the solution was heated to 160 °C. Subsequently, DIPEA (950 μL, 5.43 mmol, 3.0 equiv.) and the amine **a** (1.90 mmol, 1.05 equiv.) were added sequentially. The reaction mixture was stirred for 5~10 minutes. After the completion of the reaction, the mixture was rapidly cooled to room temperature using cold water, then diluted with 20 mL of ethyl acetate. The mixture was extracted with five volumes of water, and the aqueous phase was further extracted with ethyl acetate three to five times. The ethyl acetate organic phases were combined, and the organic phase was washed with saturated brine. After solvent removal under reduced pressure, the crude residue was then purified by silica gel column chromatography (100-200 mesh, dry loading), eluting with an appropriate organic solvent to obtain the product **2**.

1.2.2 Procedures for the reaction using NMP as the solvent



2-(2,6-Dioxopiperidine-3-yl)-4-fuoroisoindoline-1,3-dione **1** (0.5 g, 1.81 mmol, 1.0 equiv.) was dissolved in N-methy1-2-pyrolidone, mixed with tert-butyl(2-aminoethyl) carbamate (0.31 g, 1.90 mmol, 1.05 equiv.) and *N*,*N*-disopropylethylamine (600 μL, 3.62 mmol, 2.0 equiv.), and then heated at 90 °C for 12 hours. Upon completion of the reaction, the temperature of the reaction mixture was cooled to room temperature, diluted with ethyl acetate, and washed with water and saturated brine. The residue of the organic laver was dried over anhydrous sodium sulfate, filtered and concentrated, and the

residue was purified by silica gel column chromatography (100-200 mesh, dry loading), eluting with EtOAc : hexanes (1:1.5-1:1) to give the compound **1** (0.055g, 11%, white solid), **2a** (0.43 g, 57%, yellow solid), **3a** (0.056 g, 10%, white solid) and **4a** (0.15 g, 18%, yellow solid).

1.3 LC-HRMS analysis of the reaction using NMP as the solvent in scheme 2

A high-performance liquid chromatography (HPLC) system (AB Sciex Exion LC) was coupled to a hybrid quadrupole time-of-flight mass spectrometer (Sciex X500R QTOF). Chromatographic separation was carried out on a C18 column (150.00 mm × 2.10 mm, 2.00 μm) at 35.00°C. The mobile phase consisted of acetonitrile/0.1% formic acid-water (20:80, v/v), and the injection volume was 10.00 μL. The flow rate was set at 0.30 mL/min, and gradient elution was applied. The sample was filtered through a 0.22 μm microporous membrane before injection, and automated injection was used.

The mass spectrometry (MS) analysis was performed using an electrospray ionization (ESI) source in positive ion mode. The ion spray voltage was set to 5000V. The nebulizer gas flow rate was 3 L/min, while the drying gas flow rate was 15 L/min. The source temperature was maintained at 250°C, with an auxiliary gas pressure of 55 psi and a curtain gas pressure of 15 psi. The declustering potential (DP) was set to 37.00V, and the collision energy (CE) was 20.00 eV.

Injection Volume

Injection Volume: 2

Binary Gradient - General

Stop time:	12.00 min
Flow:	0.3000 mL/min
Pressure limits Maximum:	80.0 MPa
Pressure limits Minimum:	0.0 MPa
B.Conc:	20.0 %
B.Curve:	0

Flow program - Table

Time [min]	*Flow [mL/min]*	*B.Conc [%]*	*B.Curve*
1.00	0.3000	20.0	0
6.00	0.3000	40.0	0
8.00	0.3000	40.0	0
10.00	0.3000	55.0	0
11.00	0.3000	55.0	0
11.10	0.3000	20.0	0

Figure 1. Gradient elution conditions for chromatography

1.3.1 Preparation of Standard Solutions

1 Stock Solution: Accurately weigh 3.26 mg of F2 and dissolve in 815 μL of LC-MS grade acetonitrile. Vortex for 2 minutes to obtain a final concentration of 4 mg/mL. Filter through a 0.22 μm PTFE membrane.

2a Stock Solution: Accurately weigh 3.54 mg of P and dissolve in 885 μL of LC-MS grade acetonitrile. Vortex for 2 minutes to obtain a final concentration of 4 mg/mL. Filter through a 0.22 μm PTFE membrane.

3a Stock Solution: Accurately weigh 3.68 mg of F1 and dissolve in 920 μL of LC-MS grade acetonitrile. Vortex for 2 minutes to obtain a final concentration of 4 mg/mL. Filter through a 0.22 μm PTFE membrane.

4a Stock Solution: Accurately weigh 3.62 mg of F4 and dissolve in 3620 μL of LC-MS grade

acetonitrile. Vortex for 2 minutes to obtain a final concentration of 1 mg/mL. Filter through a 0.22 μm PTFE membrane.

Mixed Standard Solution: To prepare a 1 mg/mL mixed standard solution, mix 700 μL of each stock solution (F1, F2, and P) and add 700 μL of LC-MS grade acetonitrile. This solution was serially diluted in LC-MS grade acetonitrile to obtain final concentrations of 0.5, 1, 2, 5, 10, 25, 50, and 100 μg/mL.

Standard Solutions of **4a**: The **4a** stock solution (1 mg/mL) was serially diluted in LC-MS grade acetonitrile to obtain standard solutions with final concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, and 10 μg/mL.

1.3.2 Preparation of sample solutions for the crude product obtained after work-up

(1) Sample Solution for **1**, **2a**, and **3a** Quantification

Accurately weigh 4.48 mg of the sample and dissolve in 4480 μL of LC-MS grade acetonitrile. Vortex for 2 minutes to obtain a 1 mg/mL stock solution, followed by filtration through a 0.22 μm PTFE membrane. The solution was diluted 20-fold with LC-MS grade acetonitrile to prepare a 50 μg/mL working solution for LC-HRMS analysis of **1**, **2a**, and **3a**.

(2) Sample Solution for **4a** Quantification

Accurately weigh 8.92 mg of the sample and dissolve in 8920 μL of LC-MS grade acetonitrile. Vortex for 2 minutes to obtain a 1 mg/mL stock solution, followed by filtration through a 0.22 μm PTFE membrane. The solution was diluted 200-fold with LC-MS grade acetonitrile to prepare a 5 μg/mL working solution for LC-HRMS analysis of **4a**.

1.3.3 Mass spectra, original data of peak area and concentration

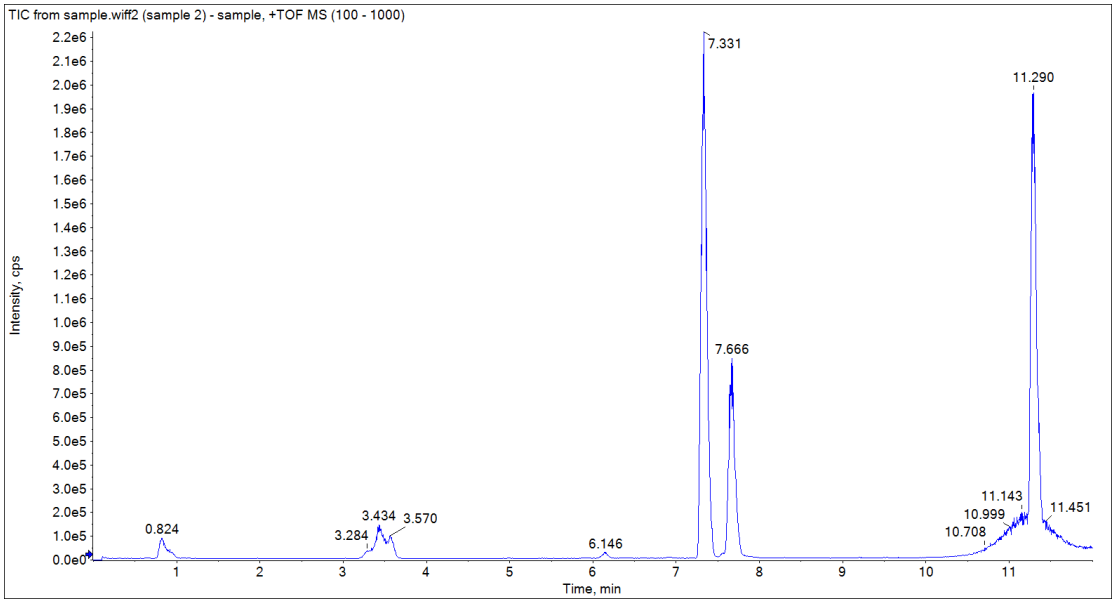


Figure S2. Total mass spectra in positive ion mode of the reaction sample

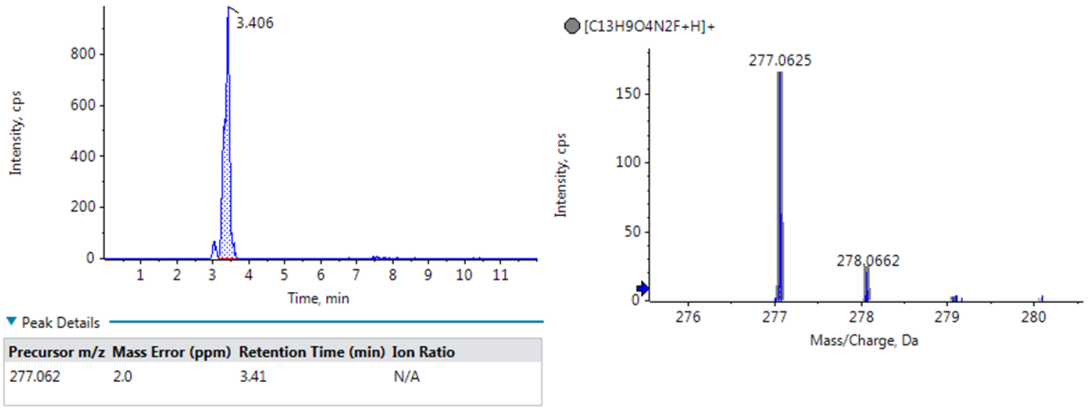


Figure S3. The retention time of substrate **1** and the corresponding mass spectra

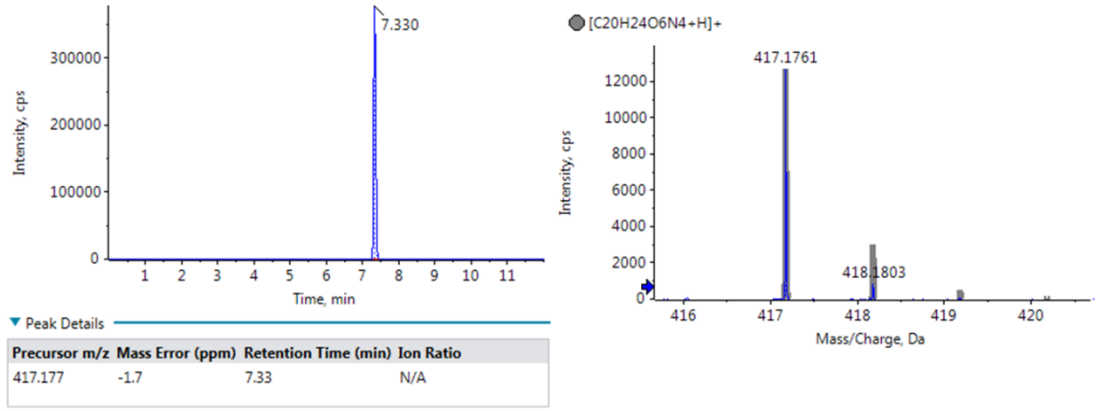


Figure S4. The retention time of product **2a** and the corresponding mass spectra

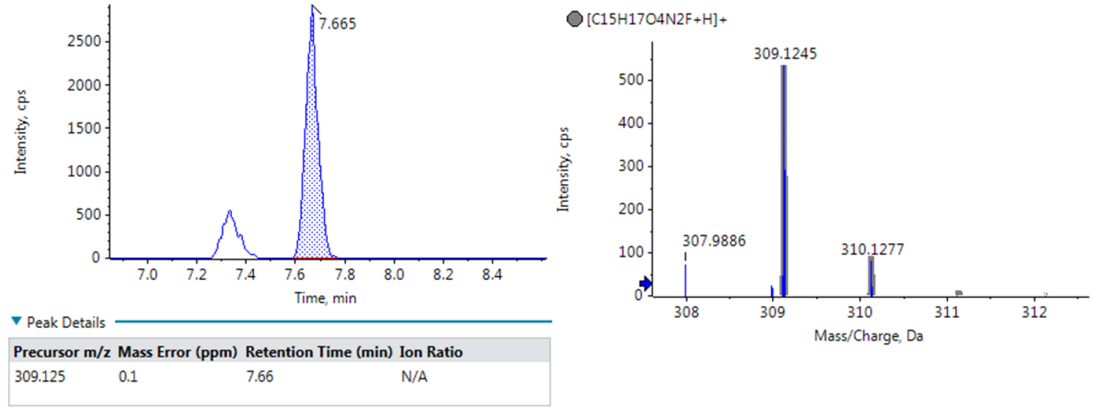


Figure S5. The retention time of byproduct **3a** and the corresponding mass spectra

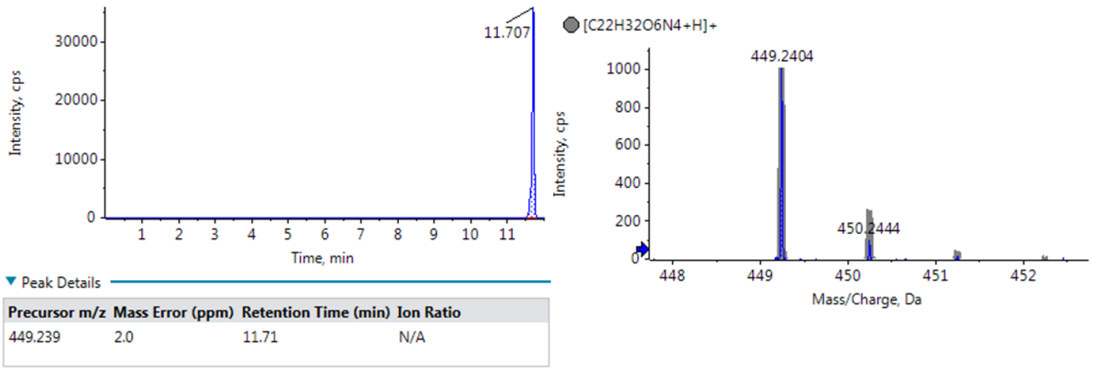


Figure S6. The retention time of byproduct **4a** and the corresponding mass spectra

Table S1. Original data of peak area and concentration relationship for standard solutions

1	Concentration（μg/mL）	2	5	10	25	50	100
	Area	1090	6305	29140	189400	568100	1127000
2a	Concentration（μg/mL）	1	2	5	10	25	100
	Area	1100	6816	71730	327400	1297000	4535000
3a	Concentration（μg/mL）	1	2	5	10	25	50
	Area	1187	3680	15370	37560	85960	162600
4a	Concentration（μg/mL）	0.25	0.5	1	2	5	10
	Area	10490	18080	91240	470000	3427000	7058000

1.3.4 Data processing and results

We utilized GraphPad Prism 10 software for data analysis and generated standard curves for each compound (Figure S7). The results exhibited excellent linearity ($R^2 > 0.9888$). Additionally, LC-MS analysis was conducted to quantify the mass of each compound in the 1 mg/mL sample solution. By integrating the molar mass of each compound with the total sample mass (705.98 mg), we determined the respective yields of each compound (Table S2).

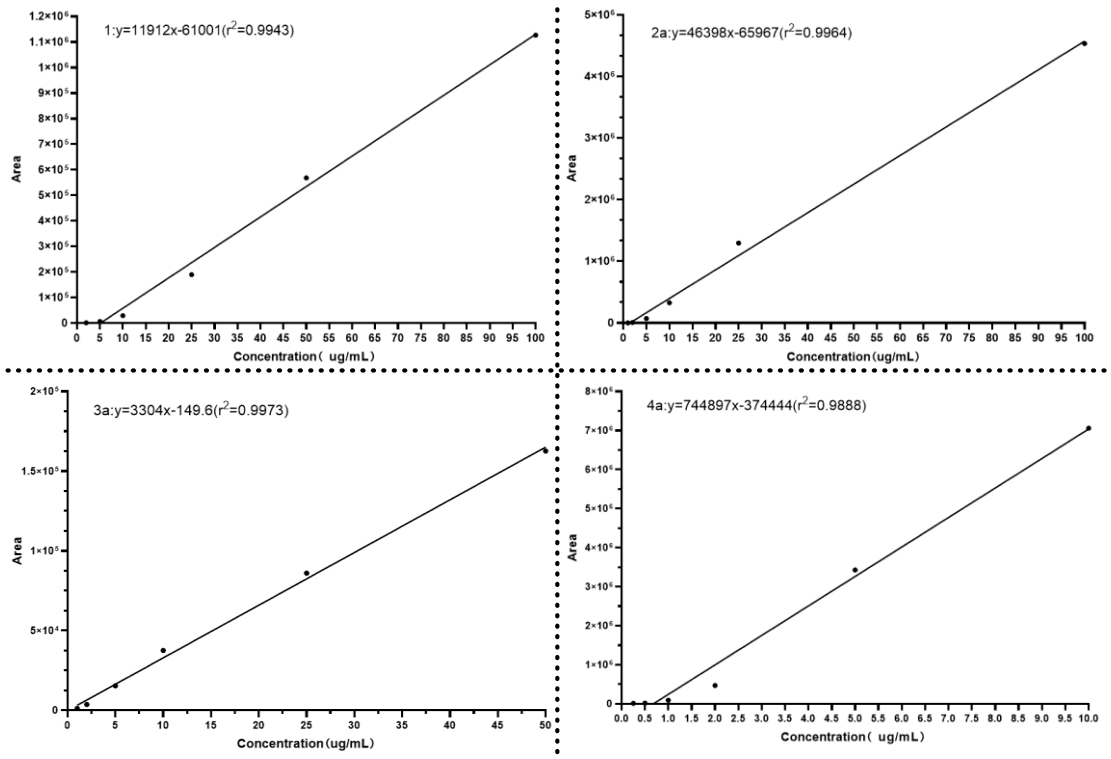


Figure S7. Standard curves of compounds (**1**, **2a**, **3a**, **4a**) based on concentration (μg/mL) and peak area. Note: The standard curves were generated using GraphPad Prism, correlating the concentration of each compound (**1**, **2a**, **3a**, **4a**) with their respective peak areas. The linearity of the calibration curves was validated, demonstrating excellent correlation ($R^2 > 0.988$).

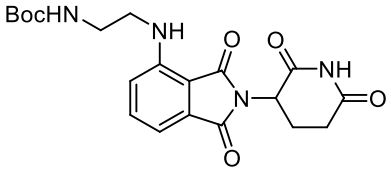
Table S2. LC-HRMS results for the reaction sample

Compounds	Retention time (min)	Peak area	Mass of 1mg/mL (μg/mL)[a]	Molar mass (g/mol)	Mass of Compounds (mg)[b]	Theoretical mass (mg)[c]	Yield (%)[d]
1	3.41	3847	105.89	276.05	74.76	499.65	15
	3.40	284.9					
2a	7.33	1513000	686.2	416.17	484.44	753.27	64
	7.34	1539000					
3a	7.67	8681	57.63	308.12	40.69	557,70	7
	7,67	10060					
4a	11.71	145000	140.03	448.23	98.86	811.30	12
	11.72	149200					

[a] The sample concentration per mL was calculated as follows: the peak areas from two measurements were averaged and substituted into the standard calibration curve to determine the sample concentration, which was then multiplied by the dilution factor; [b] Mass Calculation = Mass in 1 mg/mL sample × 705.98; [c] Theoretical Mass Calculation = Molar Mass × 1.81 mmol; [d] Yield (%) = (Mass of Compound / Theoretical Mass) × 100%

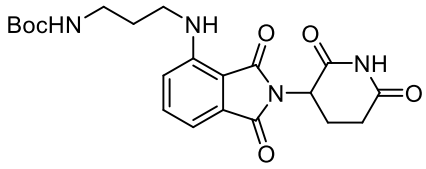
1.4 Experimental details and characterization of the products

tert-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)carbamate (2a)



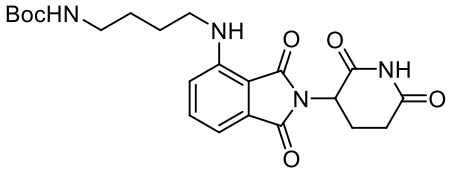
Compound **2a** (0.579 g, 88%) was isolated as a yellow solid. R_f = 0.30 (EtOAc : Dichloromethane : hexanes, 0.3:1:1). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.58 (t, 1H), 7.14 (d, 1H), 7.03 (d, 2H), 6.72 (t, 1H), 5.05 (dd, 1H), 3.37 (d, 2H), 3.12 (q, 2H), 2.96 – 2.83 (m, 1H), 2.57 (td, 2H), 2.06 – 1.96 (m, 1H), 1.36 (s, 7H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.5, 169.2, 167.8, 156.3, 146.8, 136.6, 132.7, 117.5, 110.9, 109.7, 78.2, 55.4, 49.0, 42.0, 31.4, 28.7, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[1])

tert-butyl (3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propyl)carbamate (2b)



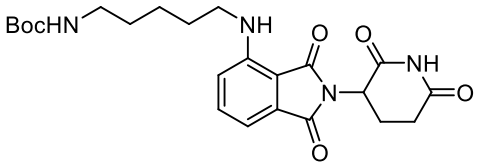
Compound **2b** (0.685 g, 83%) was isolated as a yellow solid. R_f = 0.32 (EtOAc:Dichloromethane, 0.2:1). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 7.58 (dd, 1H), 7.09 (d, 1H), 7.02 (d, 1H), 6.92 (t, 1H), 6.67 (t, 1H), 5.05 (dd, 1H), 3.33 – 3.27 (m, 4H), 3.00 (q, 2H), 2.95 – 2.81 (m, 1H), 2.63 – 2.53 (m, 2H), 2.07 – 1.99 (m, 1H), 1.66 (p, 2H), 1.38 (s, 8H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.6, 167.8, 156.2, 146.7, 136.7, 132.7, 110.8, 78.0, 49.0, 31.5, 28.7, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[2])

tert-butyl (4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)carbamate (2c)



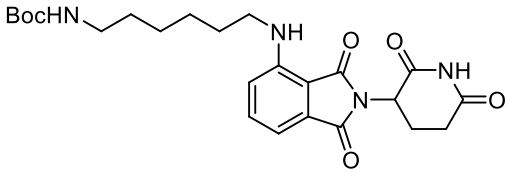
Compound **2c** (0.655 g, 81%) was isolated as a yellow gelatinous substance. R_f = 0.21 (EtOAc : Dichloromethane : hexanes, 1:2:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.48 (s, 1H), 7.43 (t, 1H), 7.03 (d, 1H), 6.85 (d, 1H), 6.27 (t, 1H), 5.09 (t, 1H), 4.97 (q, 1H), 3.26 (q, 2H), 3.15 (q, 2H), 2.78 (p, 2H), 2.14 – 2.06 (m, 1H), 1.71 – 1.62 (m, 2H), 1.62 – 1.53 (m, 2H), 1.43 (s, 10H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.3, 169.4, 169.2, 167.6, 156.1, 146.7, 136.0, 132.3, 116.6, 111.2, 109.7, 79.0, 48.8, 42.1, 40.0, 31.3, 29.6, 28.4, 27.4, 26.4, 22.7. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[3])

tert-butyl (5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentyl)carbamate (2d)



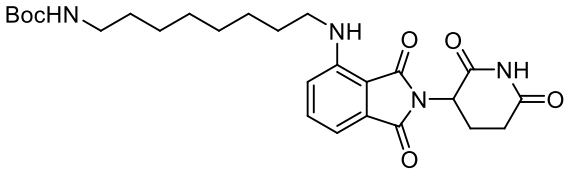
Compound **2d** (0.683 g, 82%) was isolated as a yellow solid. R_f = 0.23 (EtOAc : Dichloromethane : hexanes, 1:2:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.58 (t, 1H), 7.09 (d, 1H), 7.02 (d, 1H), 6.79 (t, 1H), 6.53 (t, 1H), 5.05 (dd, 1H), 3.28 (q, 2H), 2.96 – 2.89 (m, 3H), 2.65 – 2.52 (m, 2H), 2.07 – 1.99 (m, 1H), 1.57 (p,2H), 1.46 – 1.38 (m, 1H), 1.37 (s, 9H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.6, 169.4, 167.8, 156.1, 146.9, 136.7, 132.6, 117.6, 110.8, 109.5, 77.8, 49.0, 42.3, 31.4, 29.7, 28.9, 28.7, 24.1, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[4])

tert-butyl (6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)carbamate (2e)



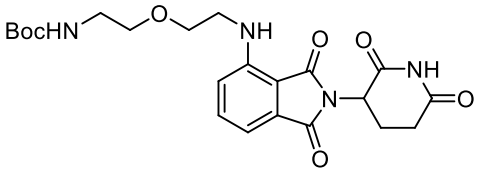
Compound **2e** (0.828 g, 97%) was isolated as a yellow gelatinous substance. R_f = 0.29 (EtOAc : Dichloromethane : hexanes, 1:3.5:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.62 – 7.54 (m, 1H), 7.09 (d, 1H), 7.02 (d,1H), 6.80 (t, 1H), 6.55 (t, 1H), 5.06 (dd,1H), 3.28 (q, 2H), 2.94 – 2.82 (m, 3H), 2.64 – 2.54 (m, 2H), 2.10 – 1.97 (m, 1H), 1.56 (p, 2H), 1.37 (s, 10H), 1.36 – 1.25 (m, 2H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.6, 169.4, 167.8, 156.0, 146.8, 136.7, 132.6, 117.6, 110.8, 109.4, 77.8, 49.0, 42.2, 40.2, 31.4, 29.9, 29.1, 28.7, 26.5, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[4])

tert-butyl (8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)carbamate (2f)



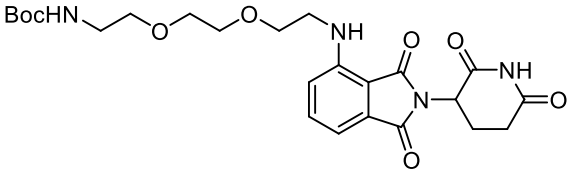
Compound **2f** (0.721 g, 80%) was isolated as a yellow gelatinous substance. R_f = 0.25 (EtOAc : hexanes, 0.4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 7.37 (t, 1H), 6.95 (d,1H), 6.76 (d, 1H), 6.16 (t, 1H), 4.95 – 4.69 (m, 2H), 3.14 (q, 3H), 3.00 (q, 2H), 2.68 (td, 4H), 2.04 – 1.96 (m, 1H), 1.54 (p, 3H), 1.35 (d, 14H), 1.18 (t, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 169.5, 169.1, 167.6, 156.0, 146.8, 136.0, 132.4, 116.6, 111.1, 109.7, 78.9, 48.8, 42.5, 40.5, 31.3, 29.9, 29.6, 29.1, 28.4, 26.8, 22.7. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[5])

tert-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)carbamate (2g)



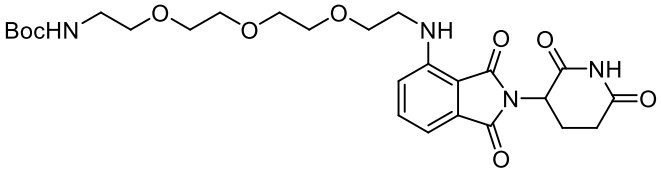
Compound **2g** (0.678 g, 81%) was isolated as a yellow gelatinous substance. R_f = 0.30 (Dichloromethane:Methanol, 60:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 7.46 (t, 1H), 7.06 (d, 1H), 6.90 (d,1H), 6.49 (t, 1H), 5.23 (t, 1H), 5.00 – 4.93 (m, 1H), 3.68 (t, 2H), 3.55 (t, 2H), 3.44 (q, 2H), 3.32 (q, 2H), 2.87 – 2.72 (m, 3H), 2.15 – 2.05 (m, 1H), 1.41 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.1, 169.4, 169.1, 167.6, 156.1, 146.7, 136.0, 132.4, 116.8, 111.6, 110.1, 79.2, 70.1, 69.2, 48.9, 42.1, 40.3, 31.4, 29.6, 28.4, 22.7. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[6])

tert-butyl (2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)carbamate (2h)



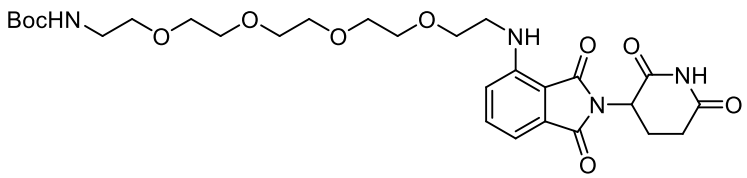
Compound **2h** (0.726 g, 79%) was isolated as a yellow gelatinous substance. R_f = 0.30 (Dichloromethane : Methanol, 50:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.47 (s, 1H), 7.49 – 7.44 (m, 1H), 7.06 (d, 1H), 6.91 (d, 1H), 6.51 (t, 1H), 5.28 (t, 1H), 4.96 (q, 1H), 3.71 (t, 2H), 3.64 (s, 3H), 3.55 (t, 2H), 3.47 (q, 2H), 3.31 (q, 2H), 2.86 – 2.72 (m, 3H), 2.15 – 2.05 (m, 1H), 1.42 (s, 8H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.1, 169.3, 169.1, 167.6, 156.0, 146.7, 135.9, 132.4, 116.7, 111.5, 110.1, 79.1, 70.5, 70.2, 70.0, 69.3, 48.8, 42.2, 40.3, 31.3, 29.6, 28.4, 22.7. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[1])

tert-butyl (2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)carbamate (2h)



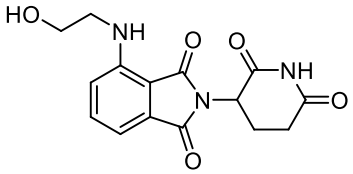
Compound **2h** (0.763 g, 77%) was isolated as a yellow gelatinous substance. R_f = 0.26 (EtOAc : Dichloromethane : hexanes, 1:0.5:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.59 (t, 1H), 7.15 (d, 1H), 7.04 (d, 1H), 6.74 (t, 1H), 6.61 (t, 1H), 5.06 (dd, 1H), 3.62 (t, 2H), 3.59 – 3.55 (m, 1H), 3.55 – 3.52 (m, 1H), 3.35 (t, 6H), 3.05 (q, 2H), 2.95 – 2.83 (m, 1H), 2.65 – 2.53 (m, 2H), 2.09 – 1.99 (m, 1H), 1.36 (s, 9H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.5, 169.4, 167.7, 156.0, 146.9, 136.7, 132.5, 117.9, 111.1, 109.7, 78.0, 70.3, 70.2, 70.0, 69.6, 69.4, 49.0, 42.2, 31.5, 28.7, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[1])

tert-butyl (14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)carbamate (2j)



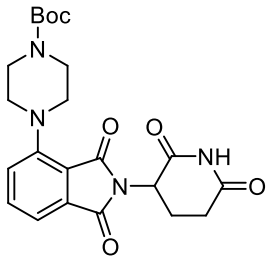
Compound **2j** (0.829 g, 77%) was isolated as a yellow gelatinous substance. R_f = 0.26 (EtOAc : Dichloromethane, 1.5:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 7.36 (t, 1H), 6.96 (d, 1H), 6.82 (d, 1H), 6.39 (t,1H), 5.23 (t, 1H), 4.86 (q, 1H), 3.62 (t, 2H), 3.55 (t, 14H), 3.43 (t, 2H), 3.36 (q, 2H), 3.20 (q,2H), 2.77 – 2.62 (m, 3H), 2.06 – 1.95 (m, 1H), 1.33 (s, 12H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.1, 169.2, 169.0, 167.6, 156.0, 146.7, 135.9, 132.4, 116.7, 111.3, 110.1, 78.9, 70.5, 70.5, 70.4, 70.3, 70.0, 69.4, 48.8, 42.2, 40.2, 31.3, 29.6, 28.4, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[7])

2-(2,6-dioxopiperidin-3-yl)-4-((2-hydroxyethyl)amino)isoindoline-1,3-dione (2k)



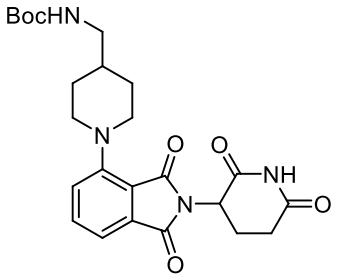
Compound **2k** (0.515 g, 90%) was isolated as a yellow solid. R_f = 0.23 (Dichloromethane : Methanol, 30:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 7.61 – 7.55 (m, 1H), 7.13 (d, 1H), 7.03 (d, 1H), 6.65 (t, 1H), 5.06 (dd, J = 12.9, 5.4 Hz, 1H), 4.93 (t, 1H), 3.60 (q, 2H), 2.94 – 2.83 (m, 0H), 2.64 – 2.54 (m, 2H), 2.07 – 1.99 (m, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.6, 169.4, 167.8, 147.1, 136.7, 132.6, 117.9, 111.0, 109.6, 59.8, 49.0, 44.8, 31.4, 22.6. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[1])

tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate (2l)



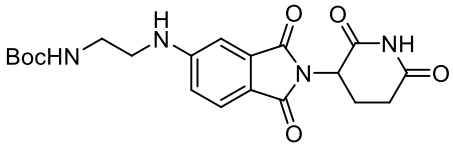
Compound **2i** (0.635 g, 79%) was isolated as a yellow solid. R_f = 0.27 (EtOAc : Dichloromethane, 0.25:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.72 (t, 1H), 5.11 (dd, 1H), 3.51 (t, 4H), 3.26 (d, 4H), 2.94 – 2.83 (m, 1H), 2.65 – 2.53 (m, 1H), 2.08 – 1.98 (m, 1H), 1.43 (s, 8H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.4, 167.5, 166.8, 154.3, 150.0, 136.4, 134.0, 124.4, 117.4, 115.7, 79.5, 50.9, 49.3, 31.4, 28.5, 22.5. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[1])

tert-butyl ((1-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperidin-4-yl)methyl)carbamate (2m)

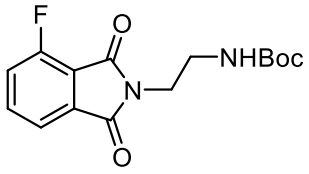


Compound **2m** (0.839 g, 99%) was isolated as a yellow solid. R_f = 0.29 (EtOAc : Dichloromethane, 0.2:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 7.67 (dd, 1H), 7.49 – 7.16 (m, 2H), 6.92 (t, 1H), 5.09 (dd, 1H), 3.67 (d, 2H), 2.90 – 2.78 (m, 5H), 2.63 – 2.52 (m, 2H), 2.07 – 1.98 (m, 1H), 1.75 – 1.66 (m, 2H), 1.59 – 1.49 (m, 1H), 1.38 (s, 9H), 1.34 – 1.28 (m, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.5, 167.6, 166.7, 156.3, 150.6, 136.2, 134.1, 124.4, 116.8, 114.9, 77.9, 51.3, 49.2, 45.9, 36.3, 31.4, 30.1, 28.8, 22.5, 14.4. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[1])

tert-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethyl)carbamate (2n)

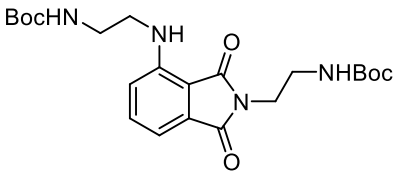


Compound **2n** (0.575 g, 76%) was isolated as a yellow solid. R_f = 0.26 (EtOAc : Dichloromethane, 0.35:1); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 7.57 (d, 1H), 7.14 (t, 1H), 6.98 (d, 1H), 6.93 (t , 1H), 6.86 (dd, 1H), 5.04 (dd, 1H), 3.24 (q, 2H), 3.11 (q, 2H), 2.93 – 2.82 (m, 1H), 2.62 – 2.52 (m, 2H), 2.04 – 1.96 (m, 1H), 1.37 (s, 9H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.3, 170.6, 168.1, 167.6, 156.2, 154.8, 134.7, 125.5, 116.6, 78.3, 55.4, 49.1, 42.6, 31.5, 28.7, 22.7. (The substrate is a known compound. The spectroscopic data is consistent with that previously reported[8])



Compound **3a** (0.045 g, 8%) was isolated as a yellow solid. R_f = 0.26 (EtOAc : Petroleum, 1:8); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.92 – 7.81 (m, 1H), 7.72 (d, J = 7.3 Hz, 1H), 7.66 (t, J = 8.9 Hz, 1H), 6.96 (t, J = 6.3 Hz, 1H), 3.59 (dd, J = 6.4, 4.6 Hz, 2H), 3.17 (q, J = 5.8 Hz, 2H), 1.27 (s, 9H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 167.37 (d, $J_{C\text{-}F}$ = 2.7 Hz), 165.26 , 156.99 (d, $J_{C\text{-}F}$ = 260.9 Hz), 156.23, 137.68 (d, $J_{C\text{-}F}$ = 7.8 Hz), 134.74 (d, $J_{C\text{-}F}$ = 3.9 Hz), 122.69 (d, $J_{C\text{-}F}$ = 19.7 Hz), 119.90 (d, $J_{C\text{-}F}$ = 3.5 Hz), 118.06 (d, $J_{C\text{-}F}$ = 12.5 Hz), 78.14 , 38.65 , 38.33 , 28.50; ^{19}F NMR (376 MHz, DMSO) δ -115.9; HRMS (ESI) *m/z* calculated for $[C_{15}H_{17}FN_2O_4 + H]^+$ = 309.1245, found 309.1261.

tert-butyl (2-((2-(2-((tert-butoxycarbonyl)amino)ethyl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)carbamate 4a)



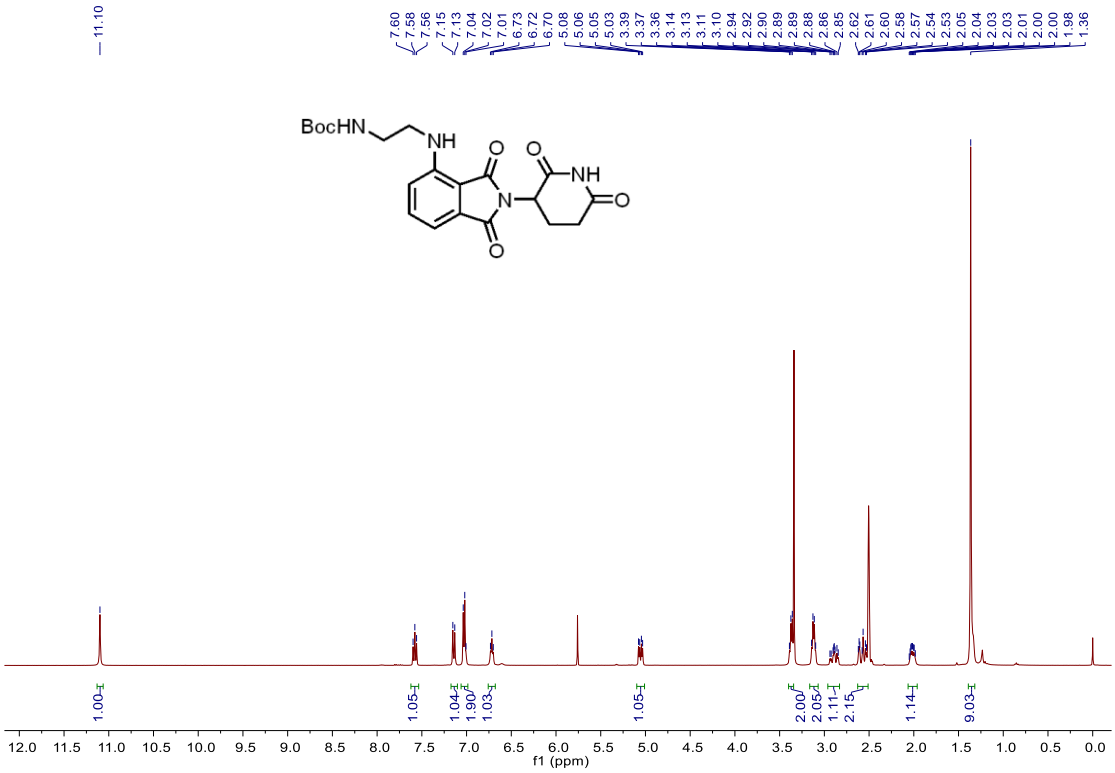
Compound **4a** (0.138 g, 17%) was isolated as a yellow solid. R_f = 0.24 (EtOAc:hexanes, 1:1.5); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.52 (t, 1H), 7.08 (d, 1H), 6.99 (td, 1H), 6.92 (t, *J* = 6.1 Hz, 1H), 6.65 (t,1H), 3.54 (t, 2H), 3.36 (d, 2H), 3.12 (p,4H), 1.37 (s, 8H), 1.30 (s, 9H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.1, 168.5, 156.3, 156.1, 146.4, 136.0, 133.3, 116.8, 110.5, 78.2, 78.1, 42.0, 38.7, 37.9, 28.7, 28.6, 28.3. HRMS (ESI) *m/z* calculated for $[C_{22}H_{32}N_4O_6 + H]^+$ = 449.2395, found 449.2406.

1.5 References

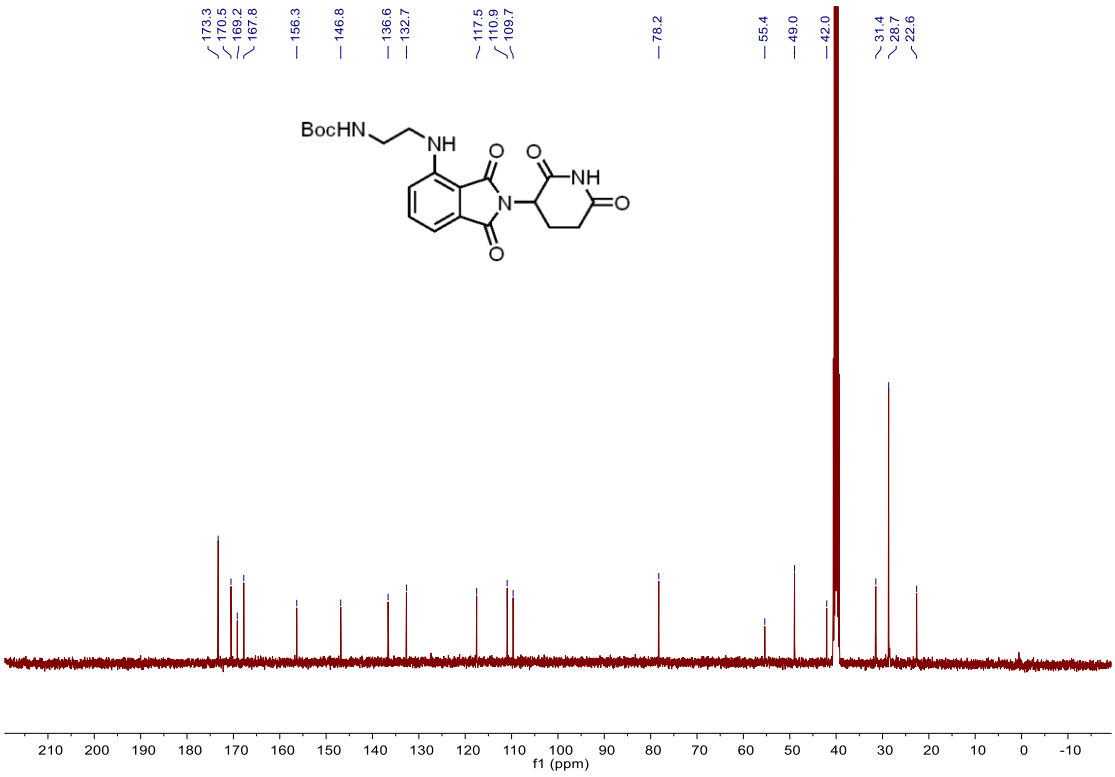
1. Brownsey, D. K.; Rowley, B. C.; Gorobets, E.; Gelfand, B. S.; Derksen, D. J., Rapid Synthesis of Pomalidomide-Conjugates for The Development of Protein Degrader Libraries. *Chem. Sci.* **2021,** *12* (12), 4519-4525.
2. Luo, G. S.; Li, Z. B.; Lin, X.; Li, X. Y.; Chen, Y.; Xi, K.; Xiao, M. X.; Wei, H. L.; Zhu, L. Z.; Xiang, H., Discovery of an orally active VHL-recruiting PROTAC that achieves robust HMGCR degradation and potent hypolipidemic activity in vivo. *Acta Pharm. Sin. B* **2021,** *11* (5), 1300-1314.
3. Xiang, W.; Wang, Q.; Ran, K.; Ren, J.; Shi, Y.; Yu, L., Structure-guided discovery of novel potent and efficacious proteolysis targeting chimera (PROTAC) degrader of BRD4. *Bioor. Chem.* **2021,** *115*.
4. Zhou, B.; Hu, J.; Xu, F.; Chen, Z.; Bai, L.; Fernandez-Salas, E.; Lin, M.; Liu, L.; Yang, C.-Y.; Zhao, Y.; McEachern, D.; Przybranowski, S.; Wen, B.; Sun, D.; Wang, S., Discovery of a Small-Molecule Degrader of Bromodomain and Extra-Terminal (BET) Proteins with Picomolar Cellular Potencies and Capable of Achieving Tumor Regression. *J. Med. Chem.* **2018,** *61* (2), 462-481.
5. Remillard, D.; Buckley, D. L.; Paulk, J.; Brien, G. L.; Sonnett, M.; Seo, H.-S.; Dastjerdi, S.; Wuhr, M.; Dhe-Paganon, S.; Armstrong, S. A.; Bradner, J. E., Degradation of the BAF Complex Factor BRD9 by Heterobifunctional Ligands. *Ange. Chem., Int. Ed.* **2017,** *56* (21), 5738-5743.
6. Hanafi, M.; Chen, X.; Neamati, N., Discovery of a Napabucasin PROTAC as an Effective Degrader of the E3 Ligase ZFP91. *J. Med. Chem.* **2021,** *64* (3), 1626-1648.
7. Brownsey, D. K.; Rowley, B. C.; Gorobets, E.; Mihara, K.; Maity, R.; Papatzimas, J. W.; Gelfand, B. S.; Hollenberg, M. D.; Bahlis, N. J.; Derksen, D. J., Identification of ligand linkage vectors for the development of p300/CBP degraders. *RSC Med. Chem.* **2022,** *13* (6), 726-730.
8. Bricelj, A.; Steinebach, C.; Kuchta, R.; Gütschow, M.; Sosič, I., E3 Ligase Ligands in Successful PROTACs: An Overview of Syntheses and Linker Attachment Points. *Front. Chem.* **2021,** *9*.

1.6 Copies of NMR spectra for all the products

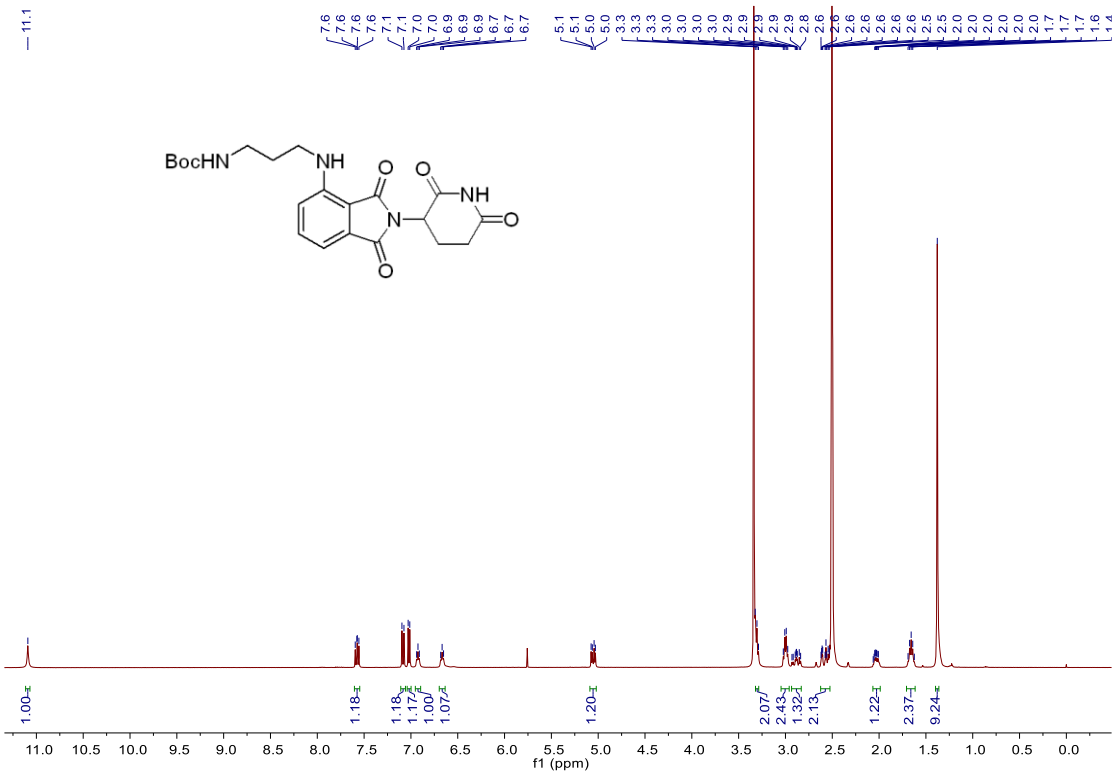
^{1}H NMR spectrum of **2a** (400 MHz, DMSO-d_6)



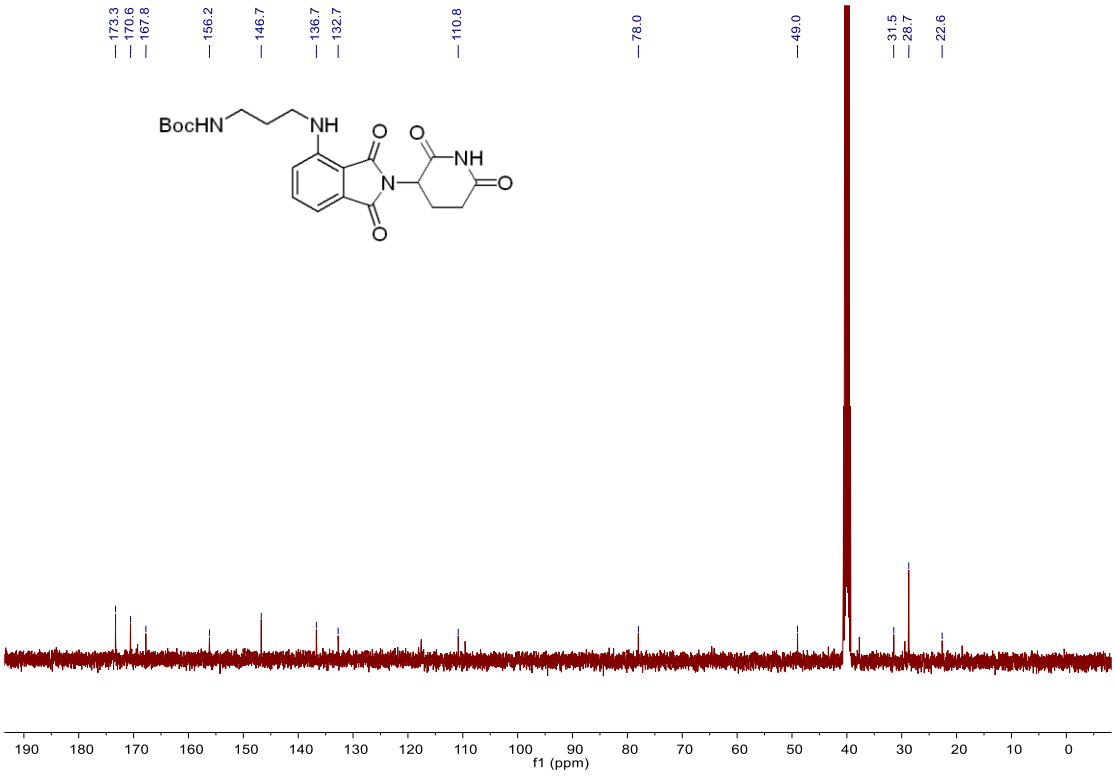
^{13}C NMR spectrum of **2a** (101 MHz, DMSO-d_6)



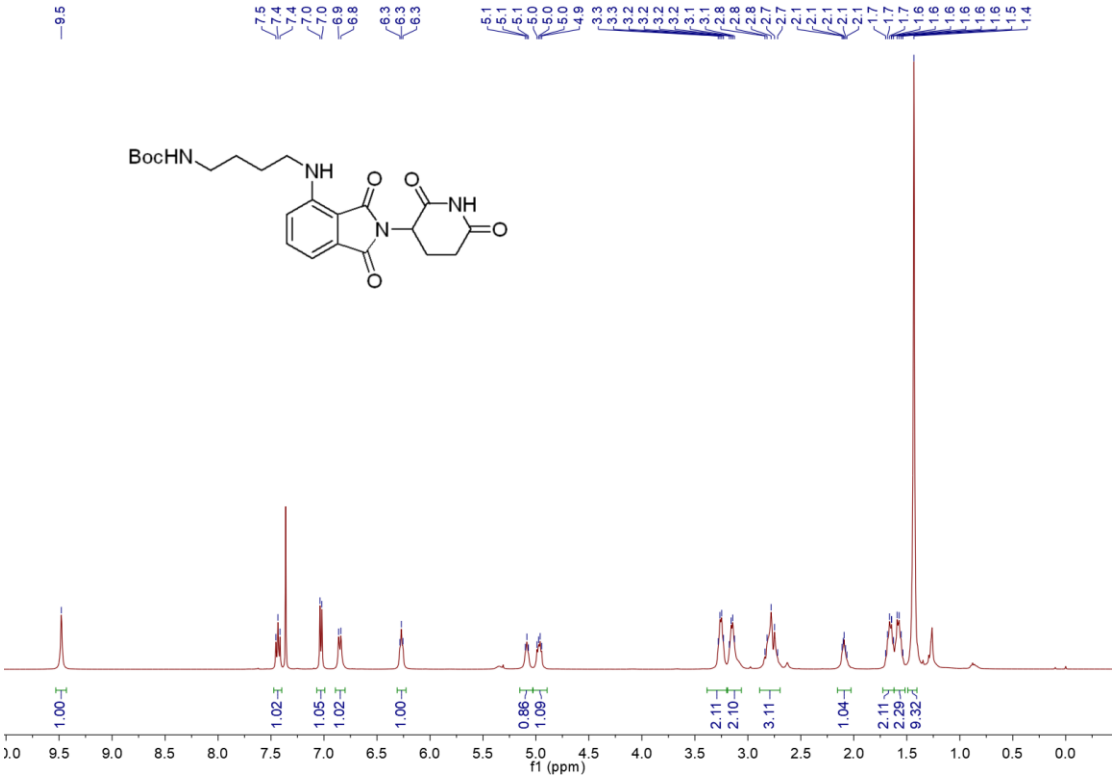
^{1}H NMR spectrum of **2b** (400 MHz, DMSO-d_6)



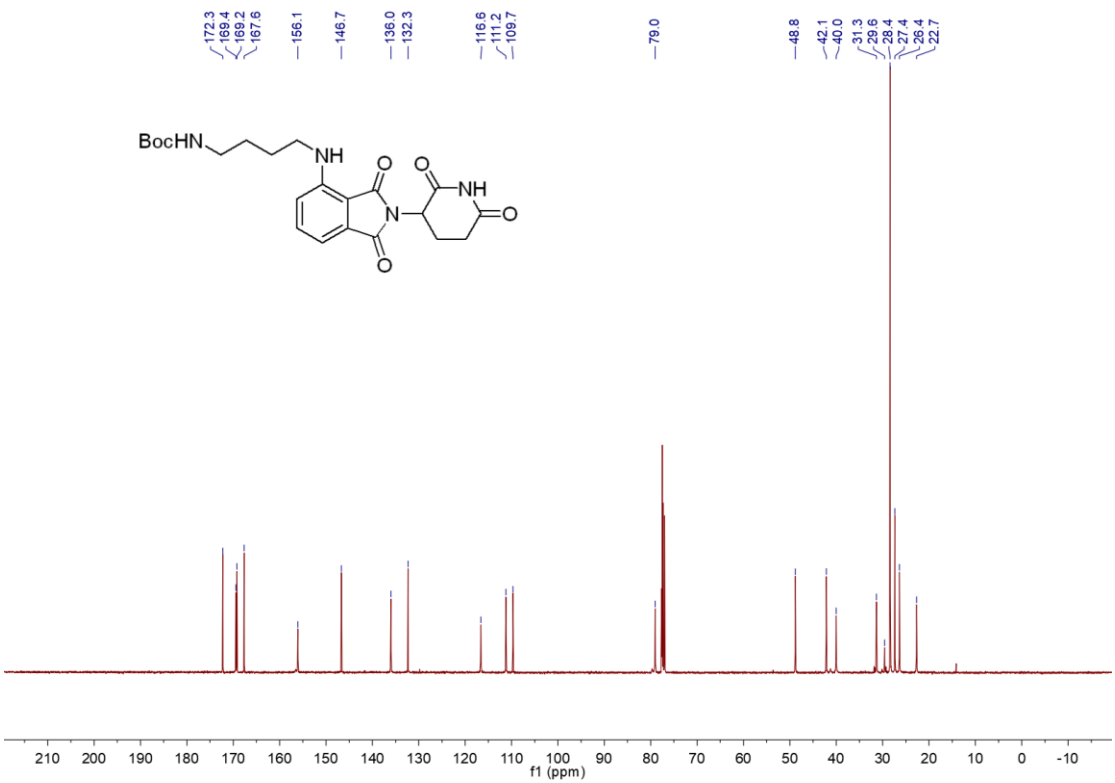
^{13}C NMR spectrum of **2b** (101 MHz, DMSO-d_6)



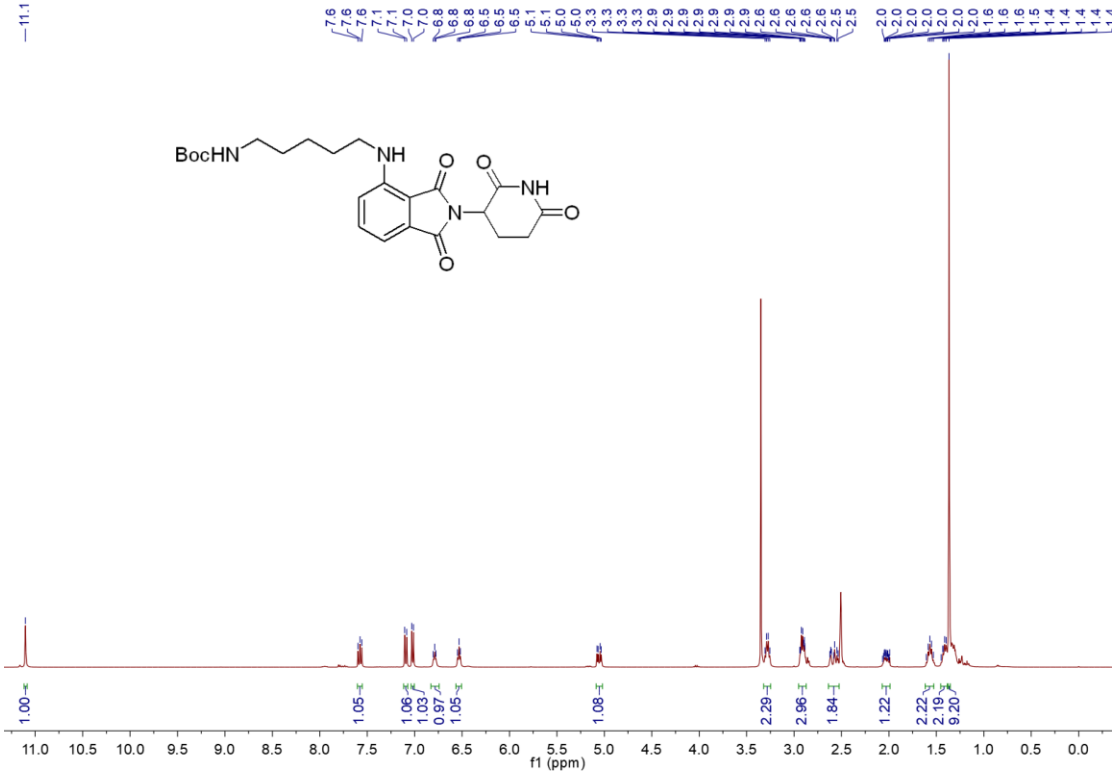
^{1}H NMR spectrum of **2c** (400 MHz, $CDCl_3$)



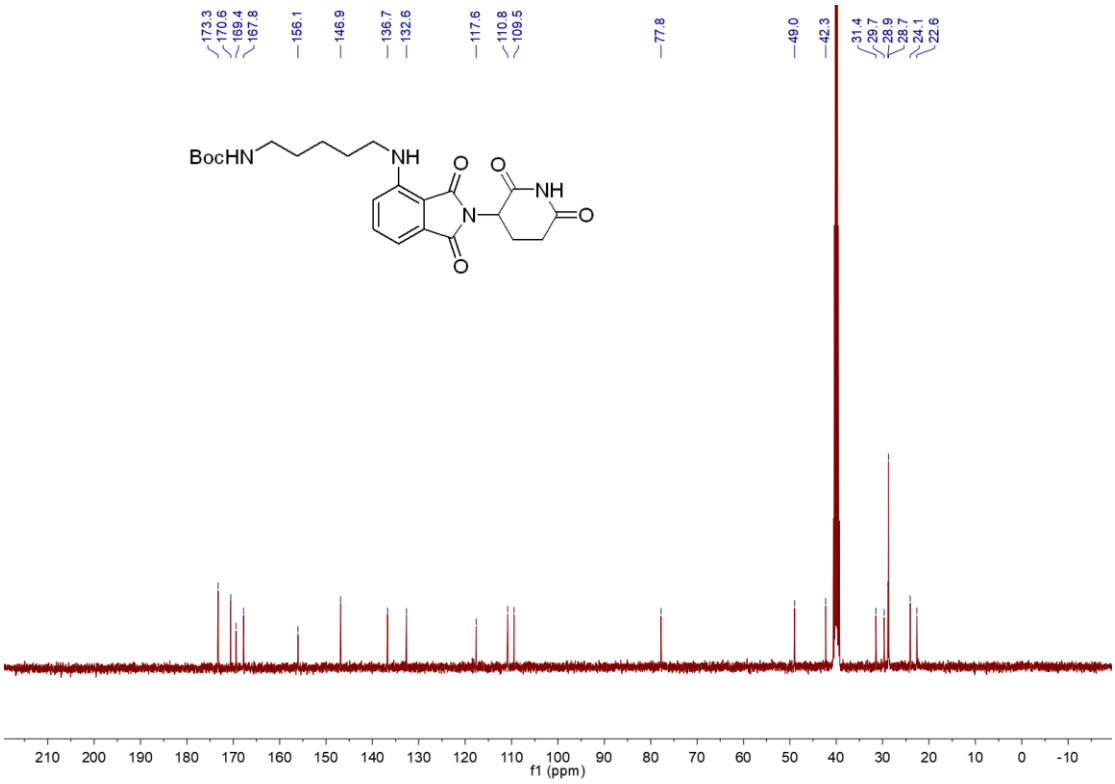
^{13}C NMR spectrum of **2c** (101 MHz, $CDCl_3$)



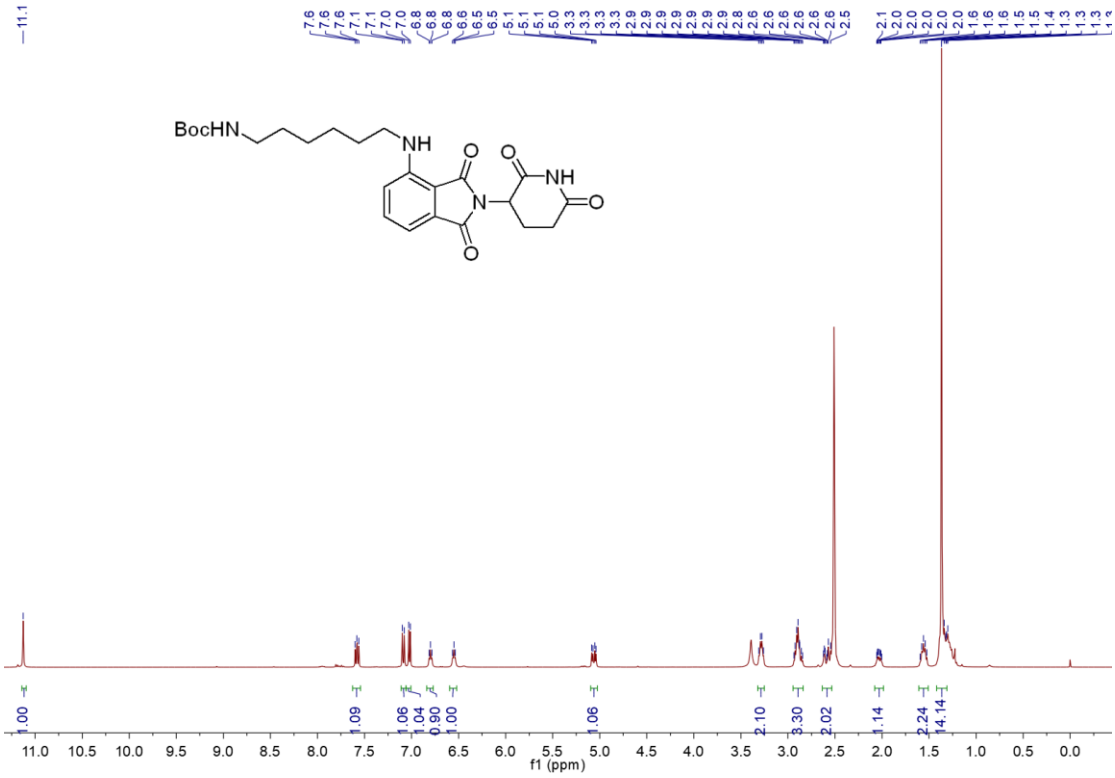
^{1}H NMR spectrum of **2d** (400 MHz, DMSO-d_6)



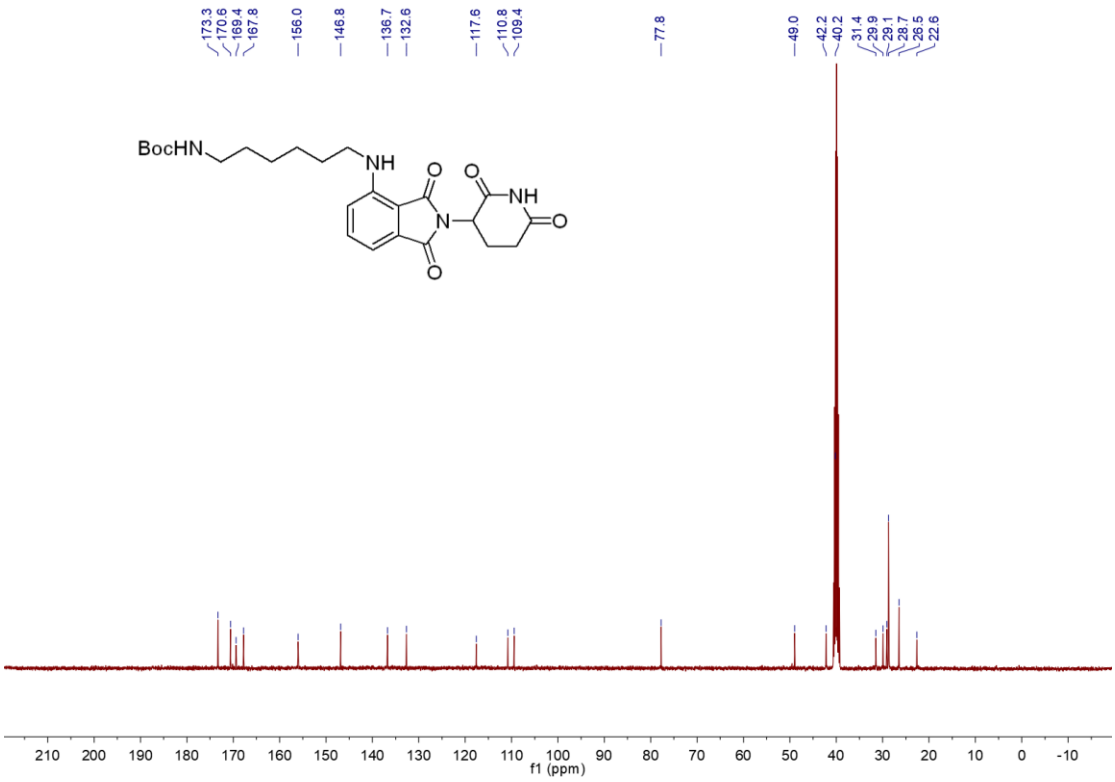
^{13}C NMR spectrum of **2d** (101 MHz, DMSO-d_6)



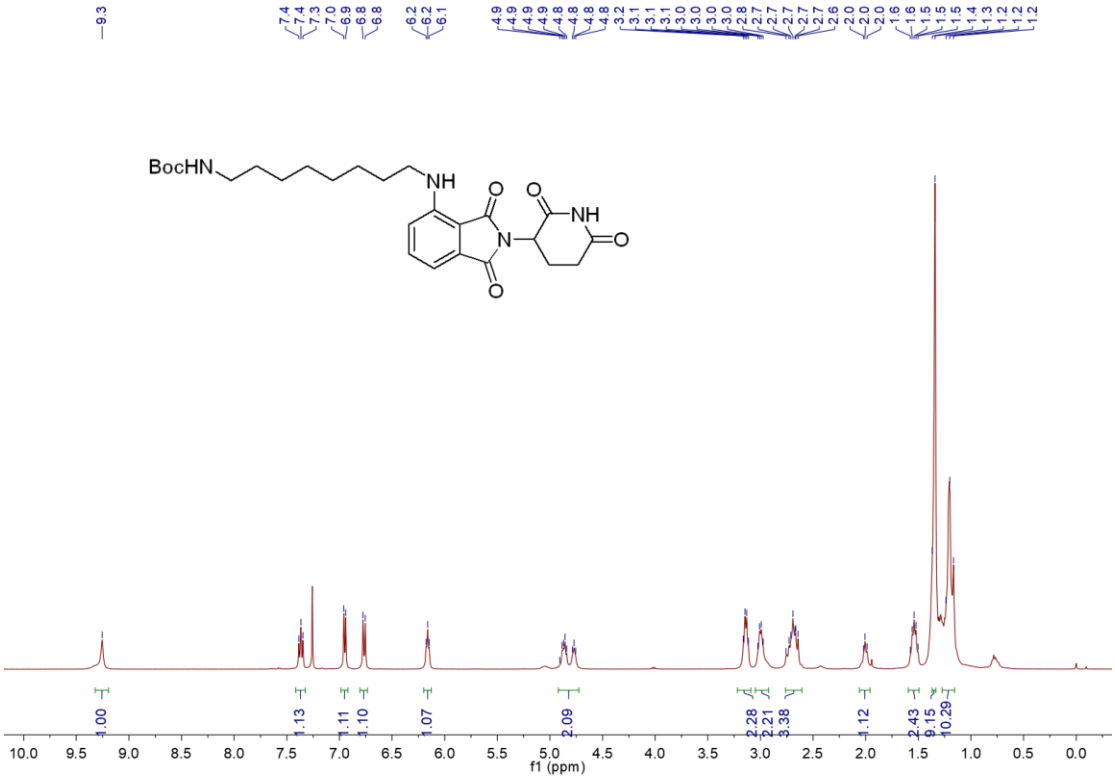
^{1}H NMR spectrum of **2e** (400 MHz, $CDCl_3$)



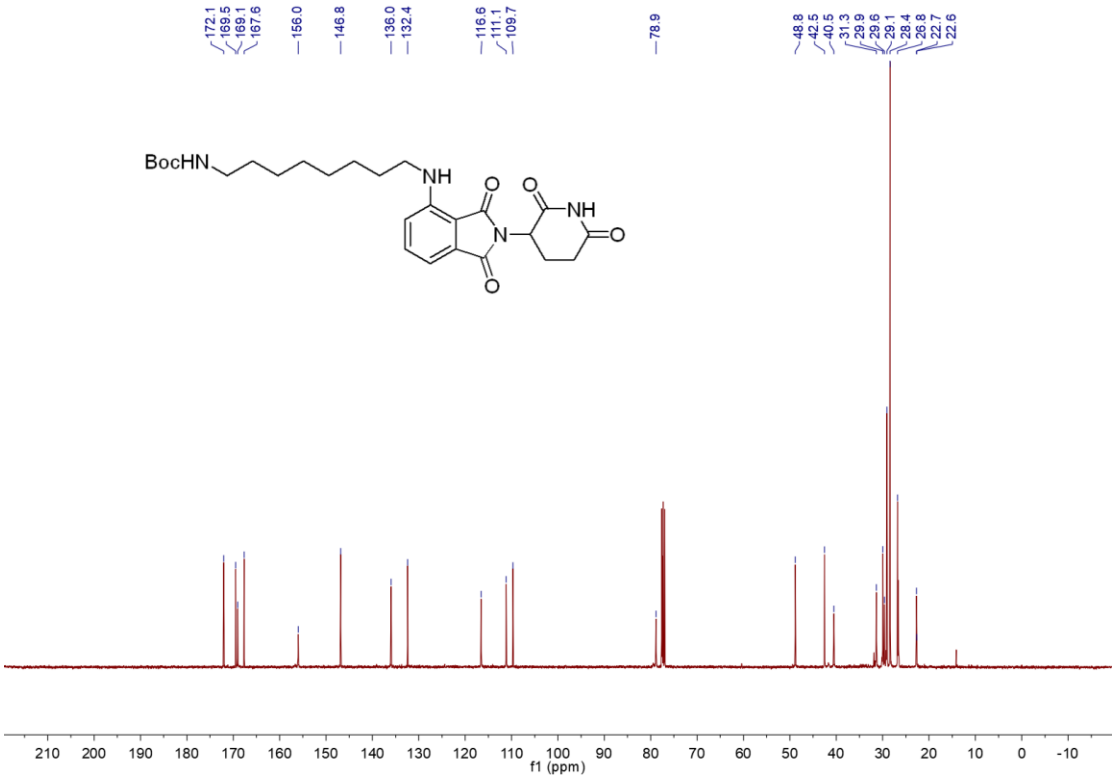
^{13}C NMR spectrum of **2e** (101 MHz,$CDCl_3$)



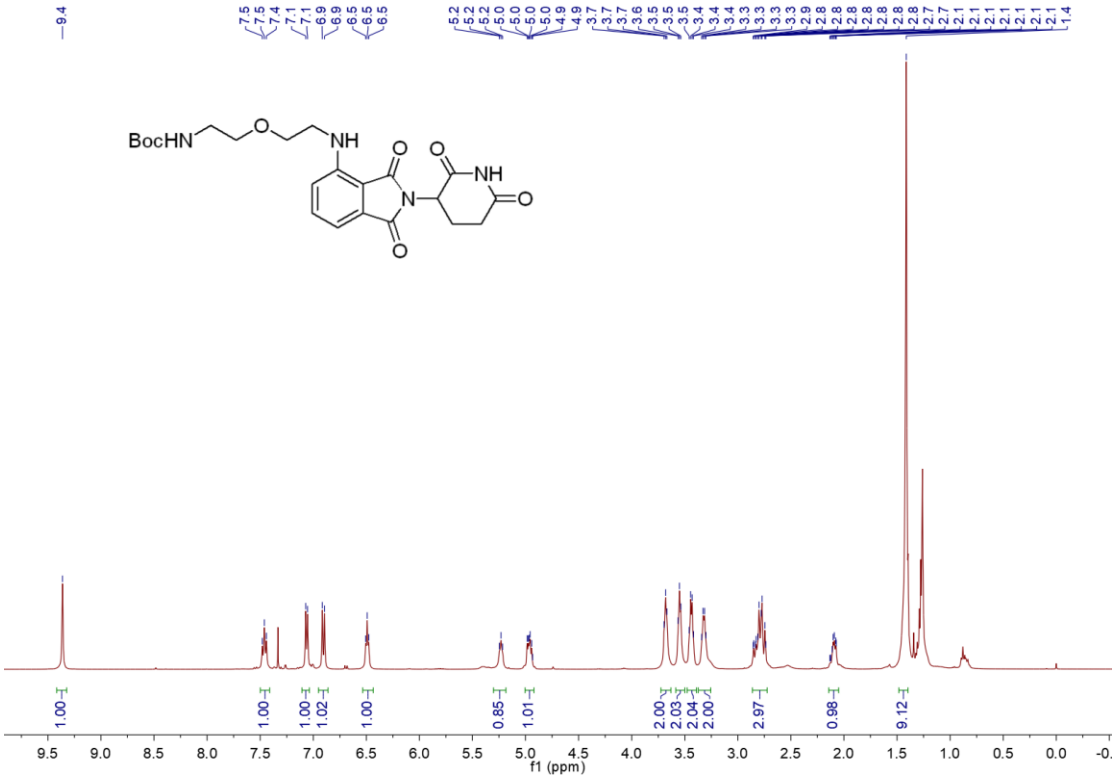
^{1}H NMR spectrum of **2f** (400 MHz, $CDCl_3$)



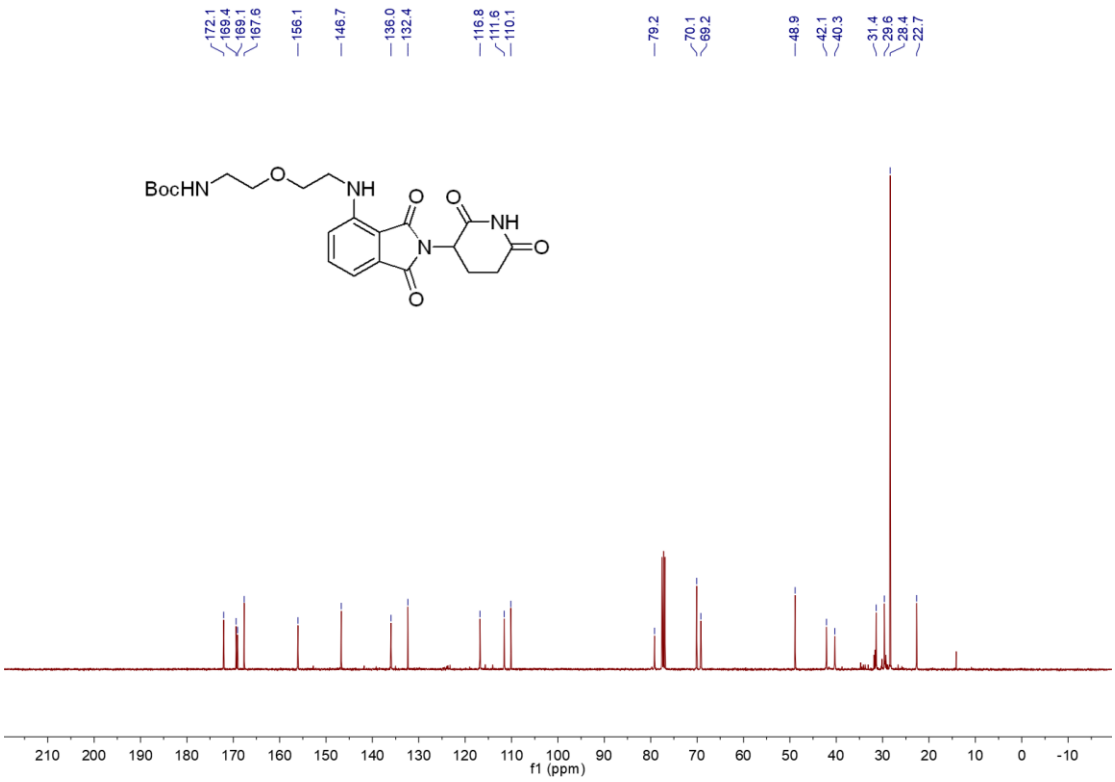
^{13}C NMR spectrum of **2f** (101 MHz, $CDCl_3$)



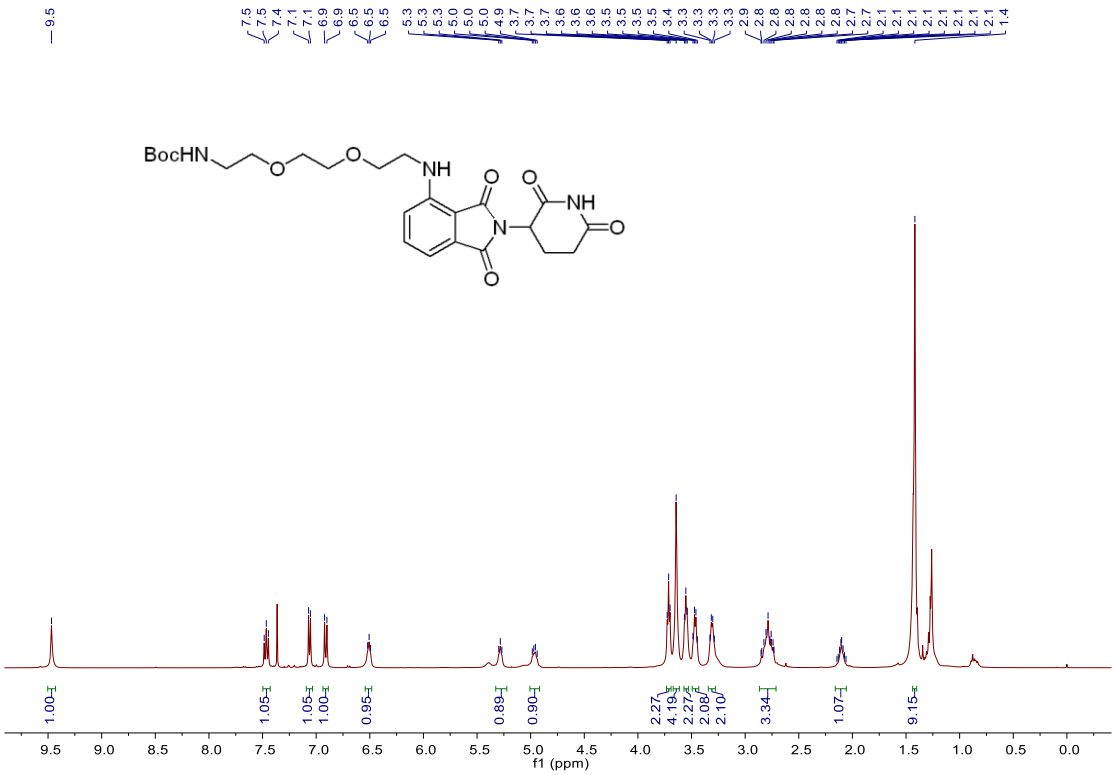
^{1}H NMR spectrum of **2g** (400 MHz, $CDCl_3$)



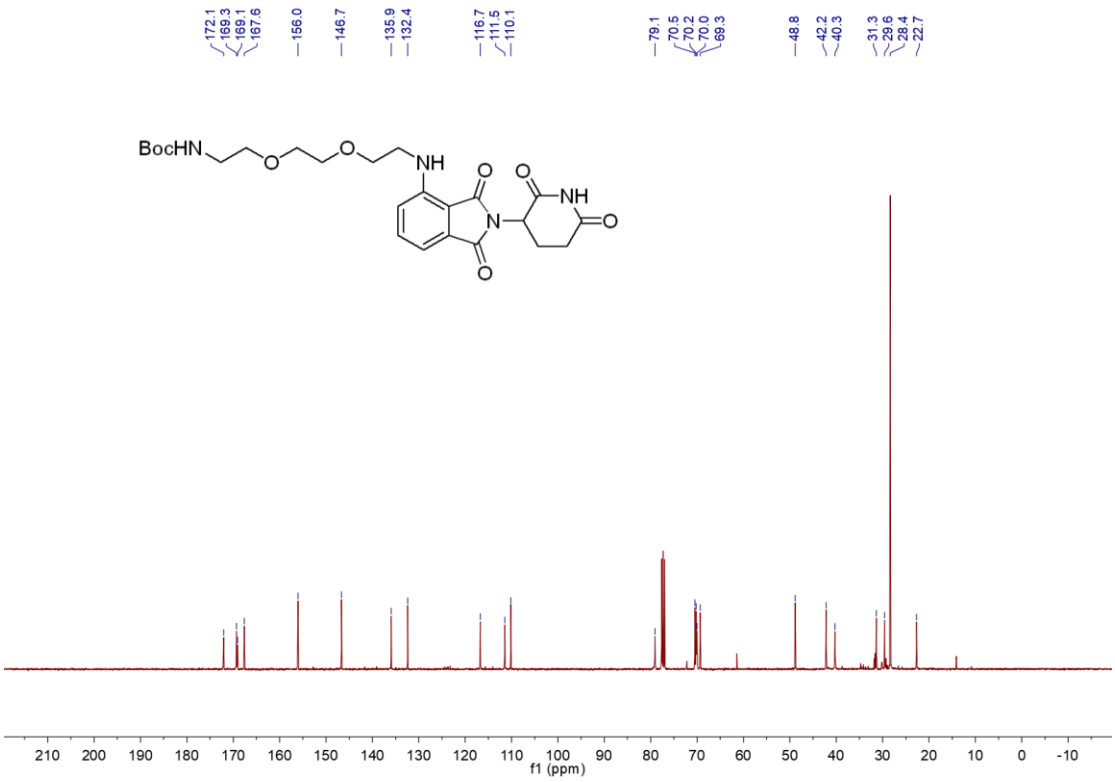
^{13}C NMR spectrum of **2g** (101 MHz, $CDCl_3$)



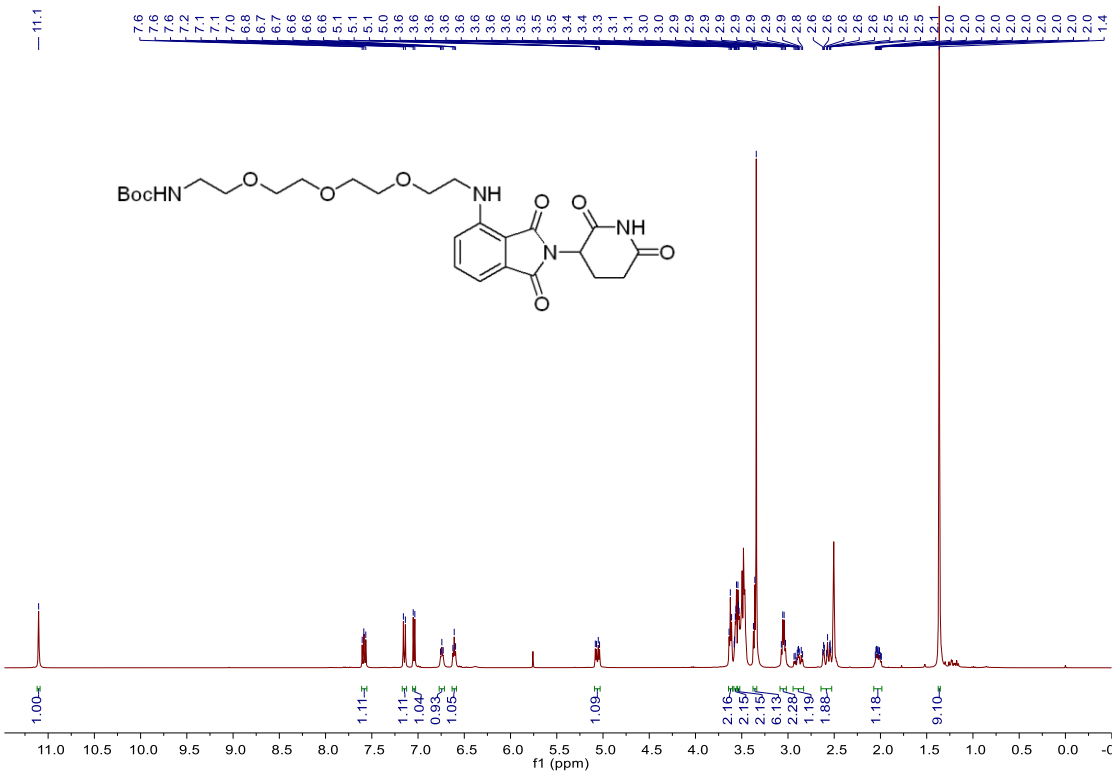
^{1}H NMR spectrum of **2h** (400 MHz, $CDCl_3$)



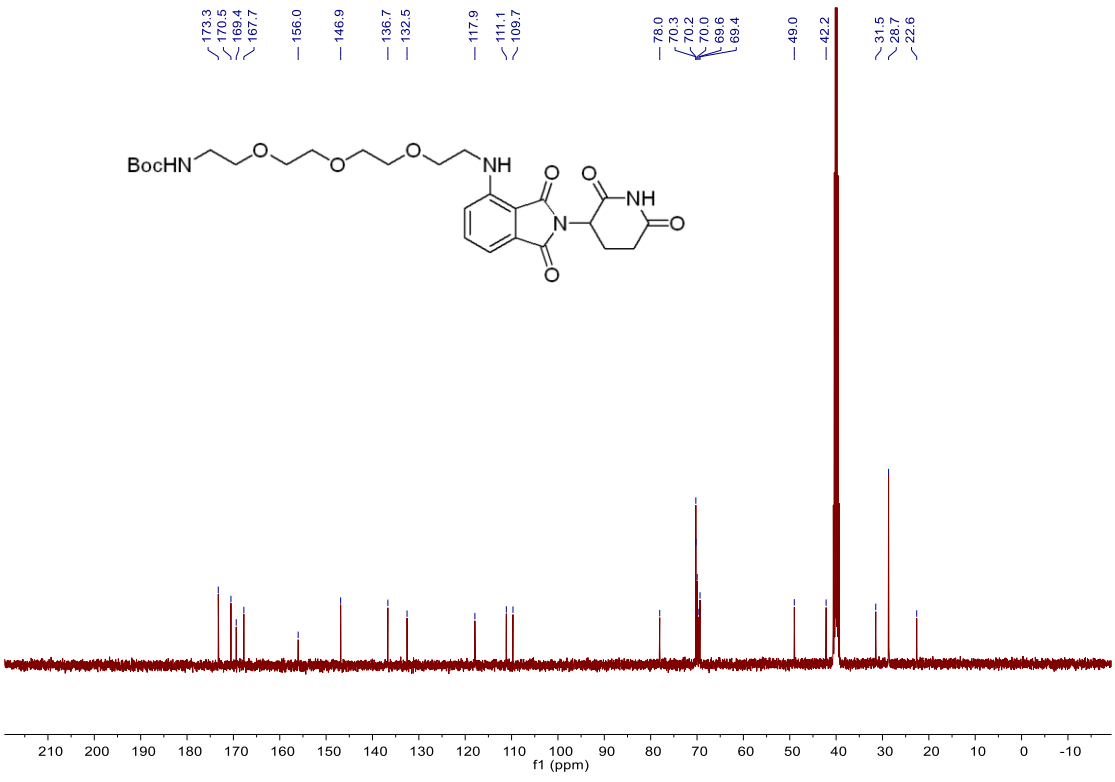
^{13}C NMR spectrum of **2h** (101 MHz, $CDCl_3$)



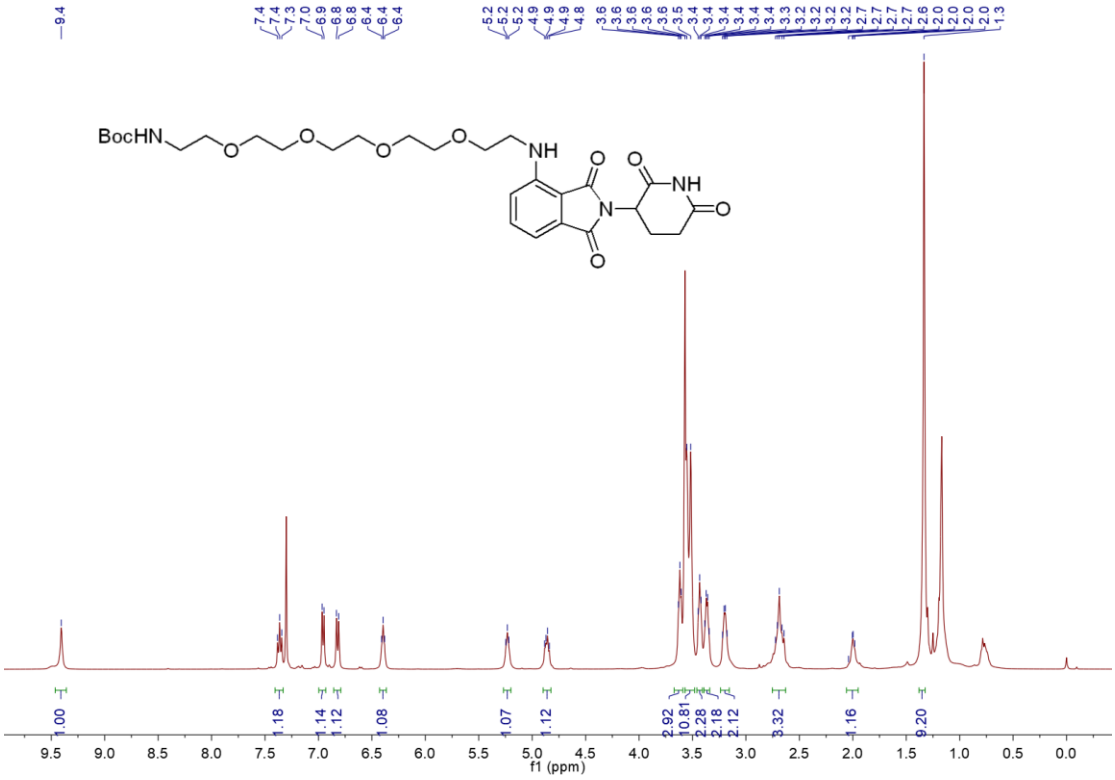
^{1}H NMR spectrum of **2i** (400 MHz, DMSO-d_6)



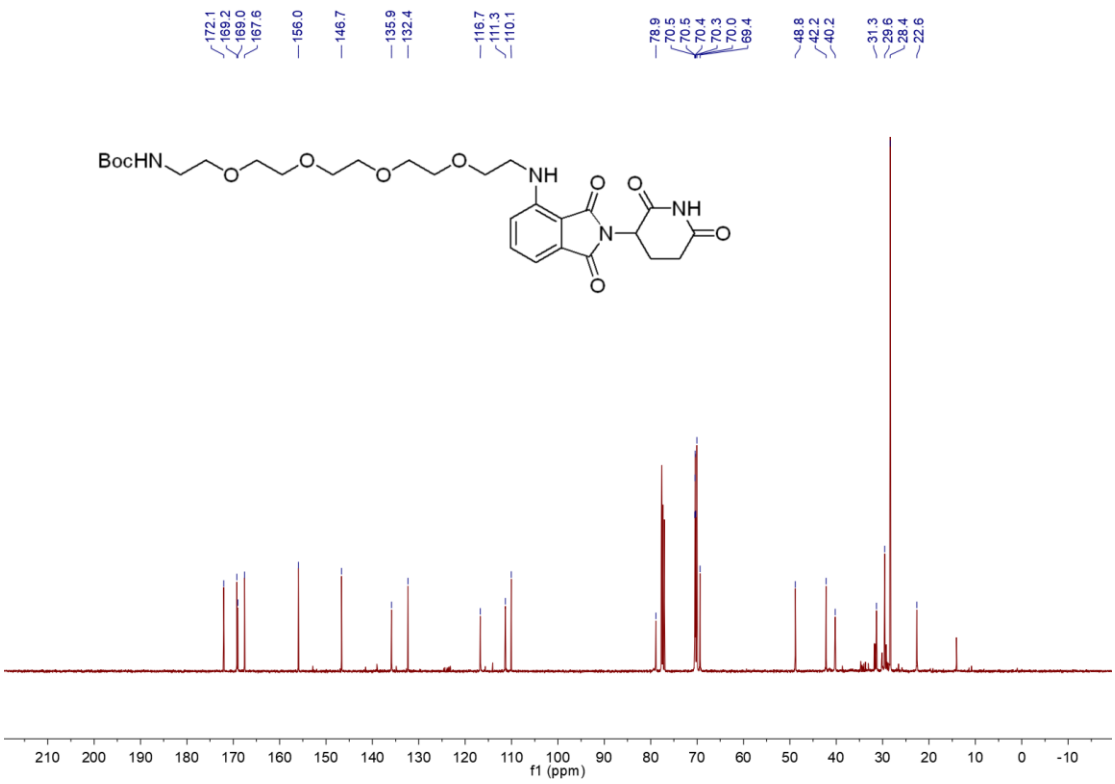
^{13}C NMR spectrum of **2i** (101 MHz, DMSO-d_6)



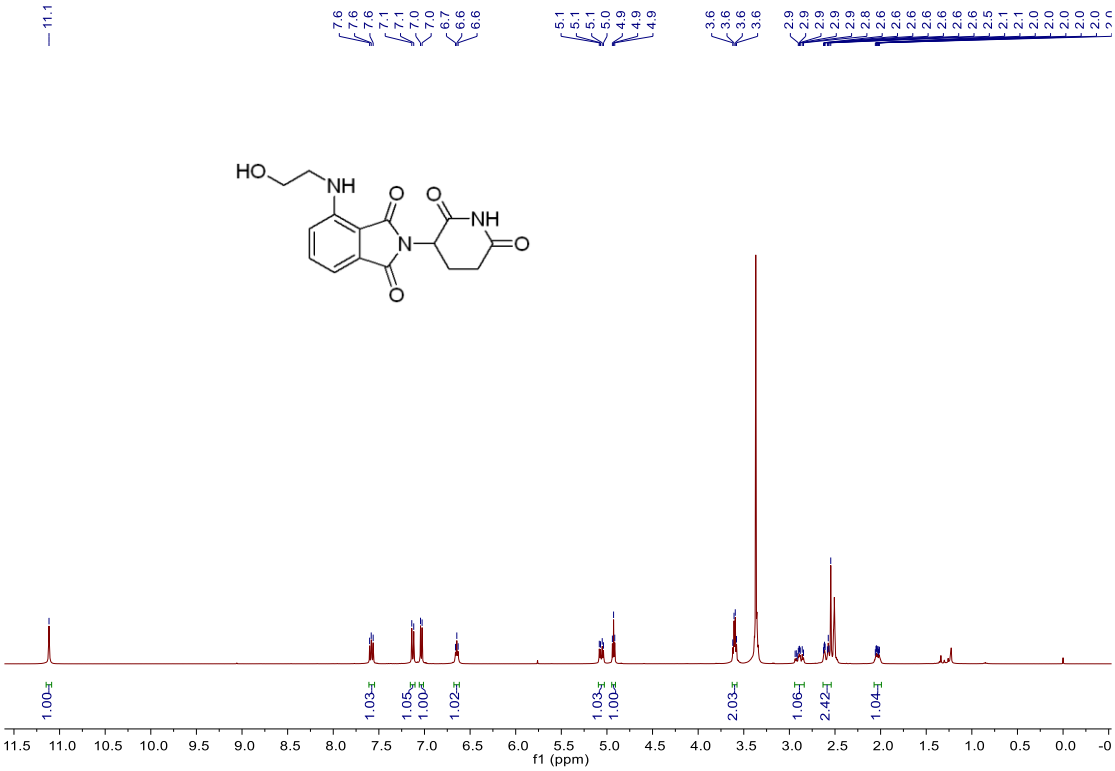
^{1}H NMR spectrum of **2j** (400 MHz, $CDCl_3$)



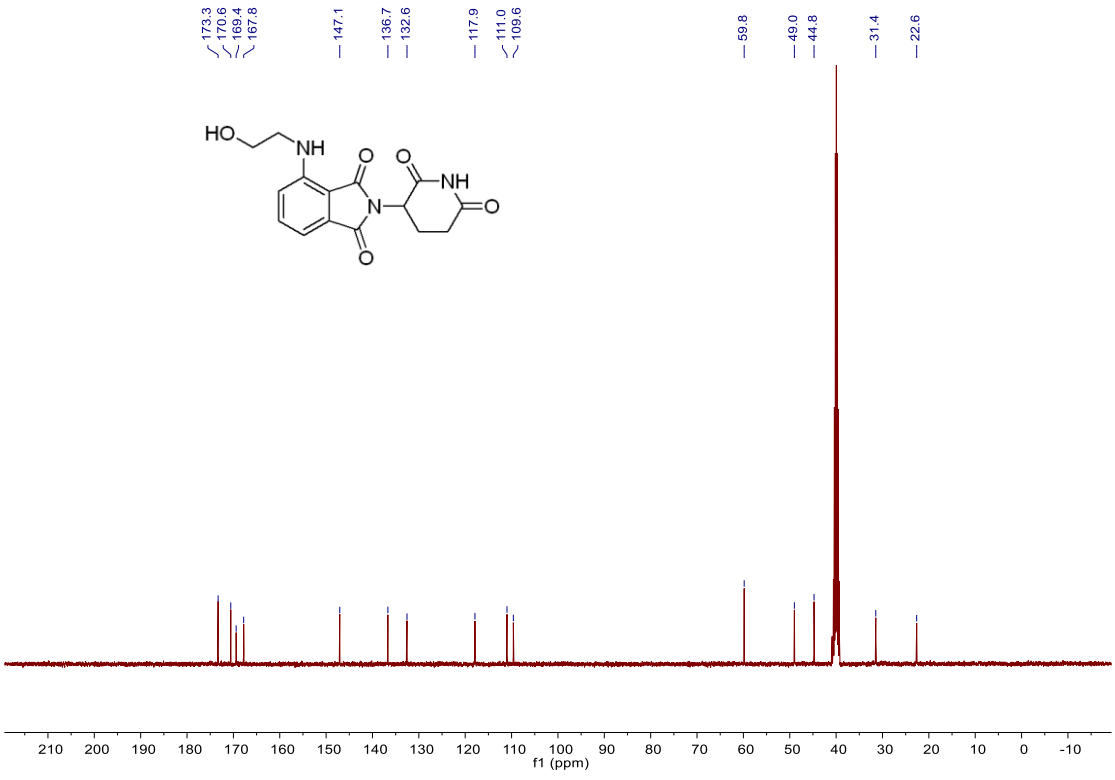
^{13}C NMR spectrum of **2j** (101 MHz, $CDCl_3$)



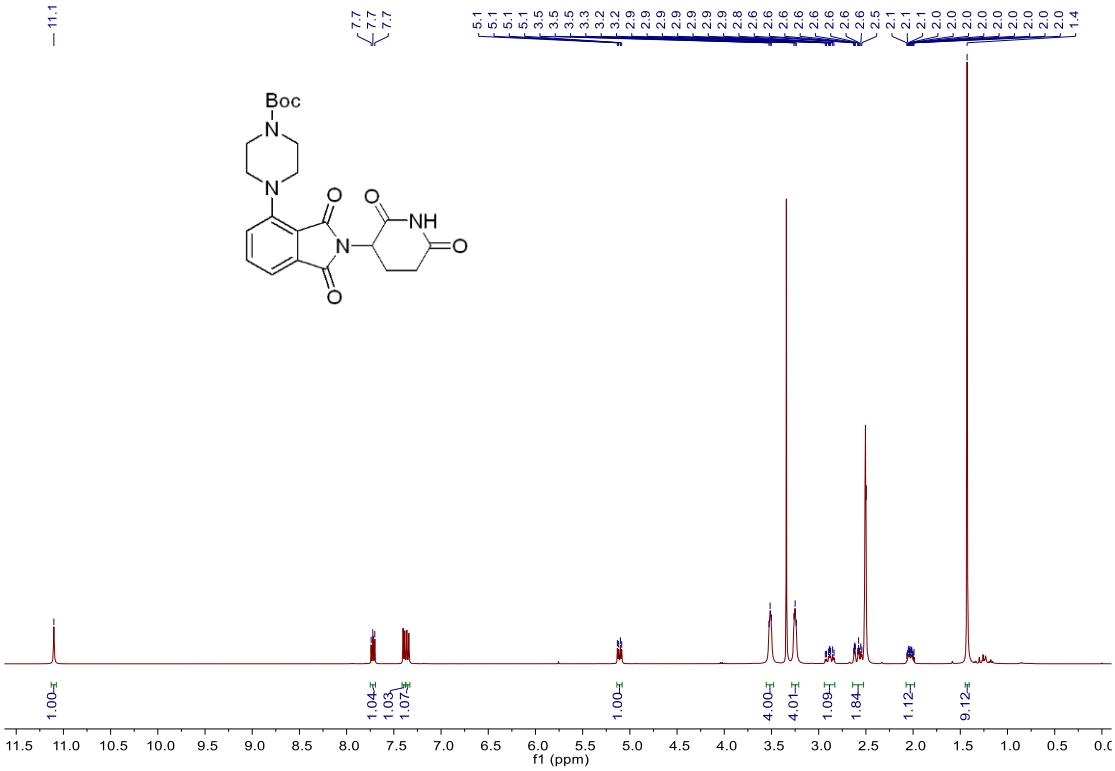
^{1}H NMR spectrum of **2k** (400 MHz, DMSO-d_6)



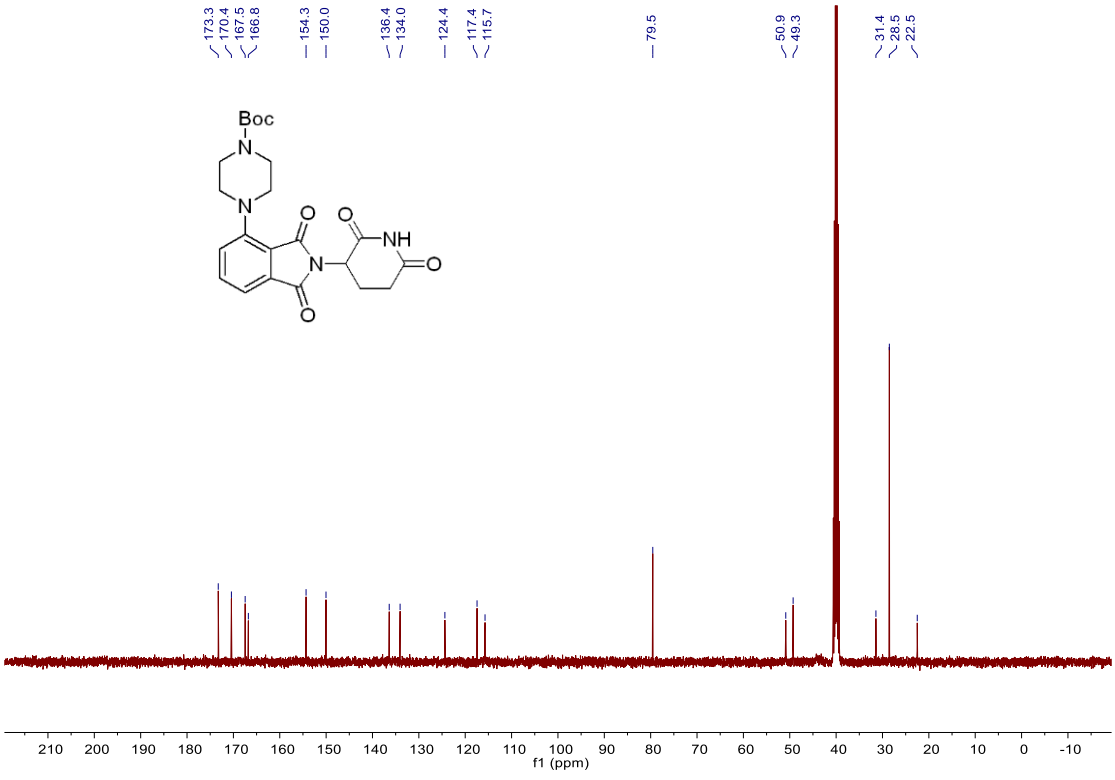
^{13}C NMR spectrum of **2k** (101 MHz, DMSO-d_6)



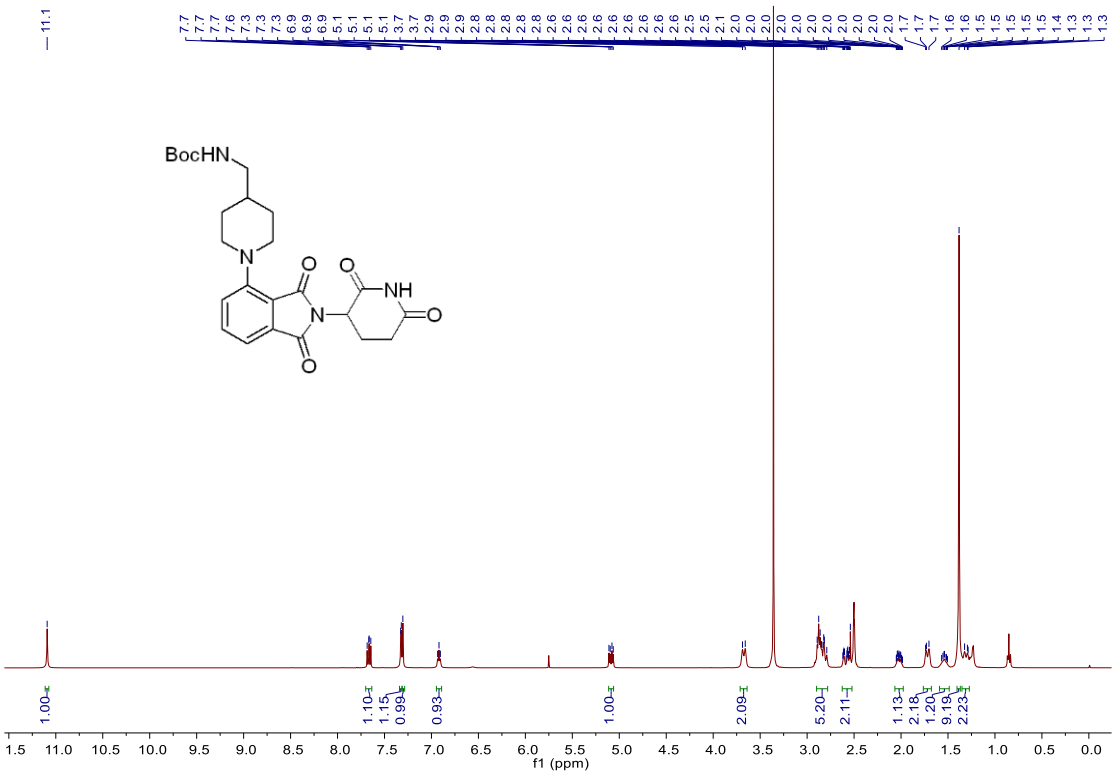
^{1}H NMR spectrum of **2l** (400 MHz, DMSO-d_6)



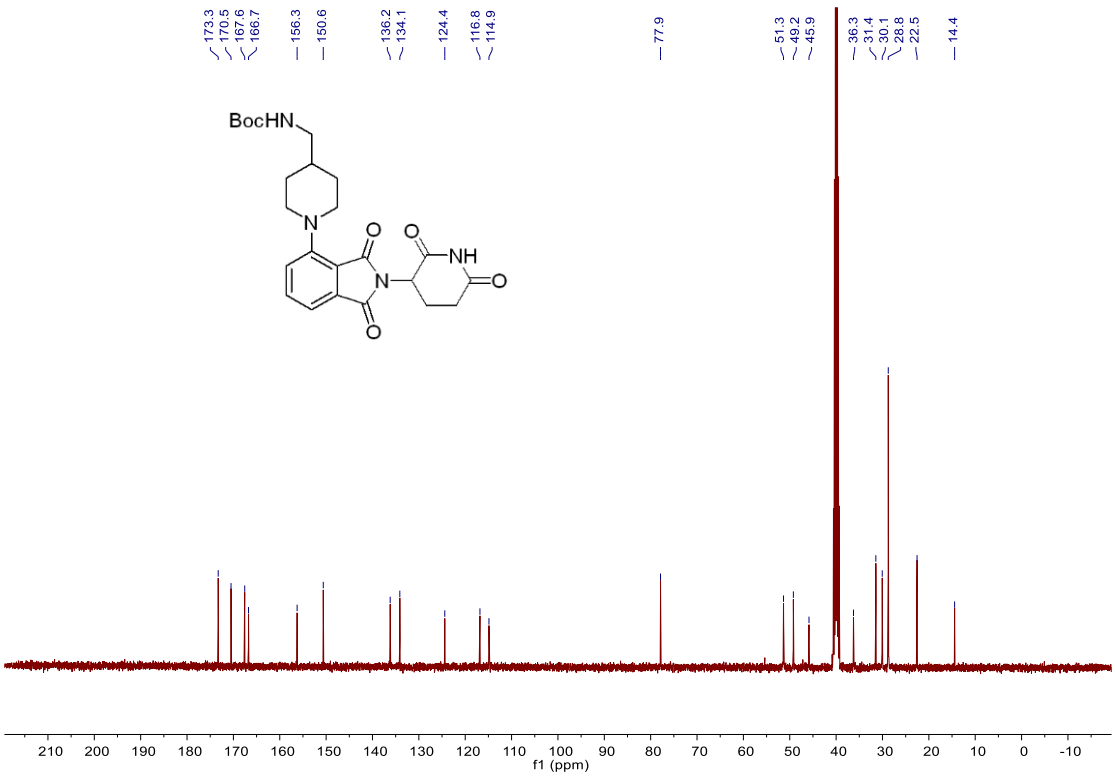
^{13}C NMR spectrum of **2l** (101 MHz, DMSO-d_6)



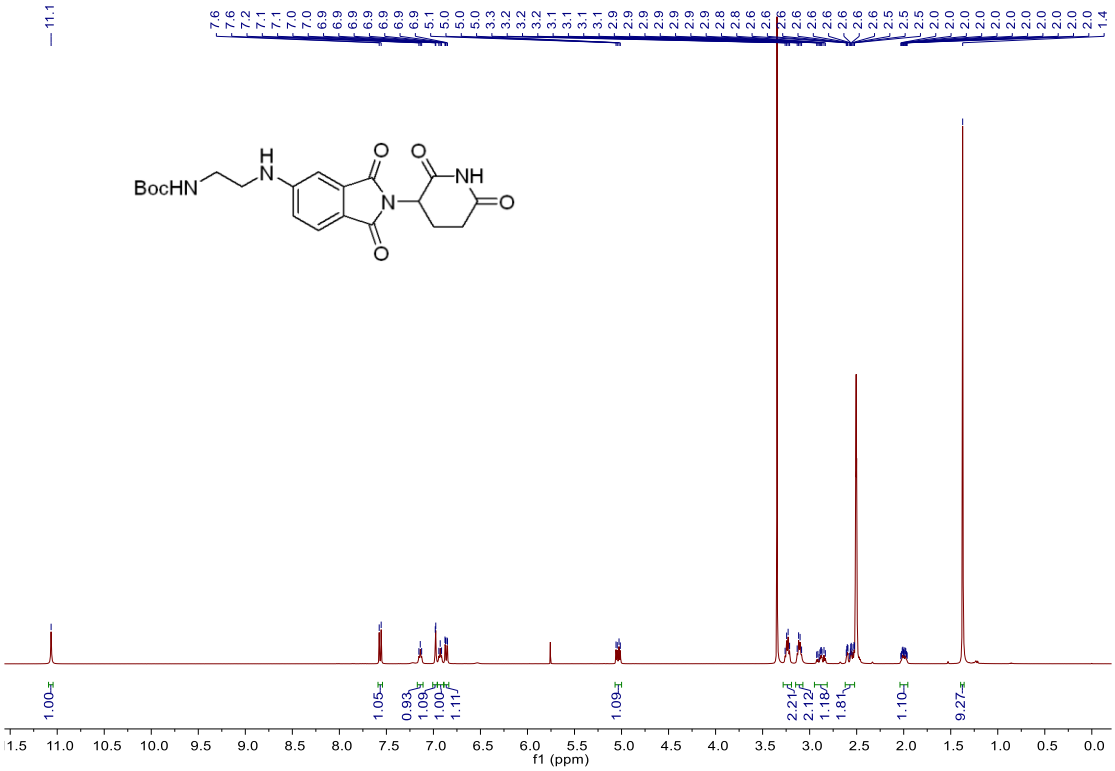
^{1}H NMR spectrum of **2m** (400 MHz, DMSO-d_6)



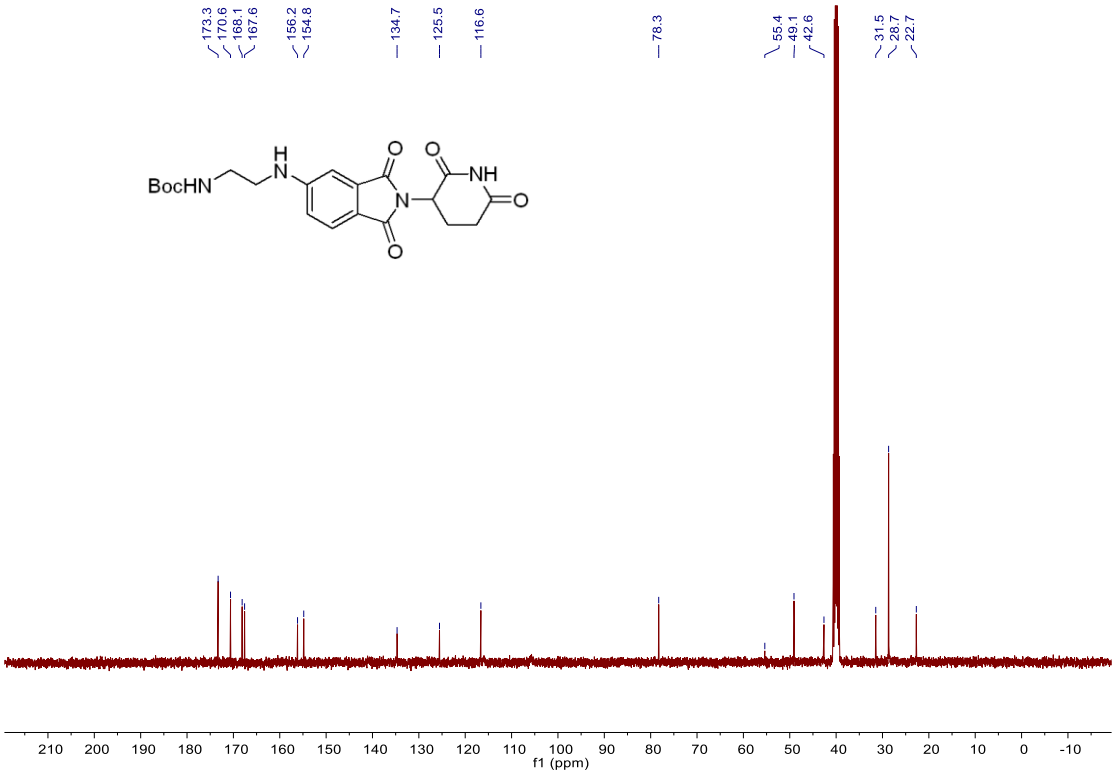
^{13}C NMR spectrum of **2m** (101 MHz, DMSO-d_6)



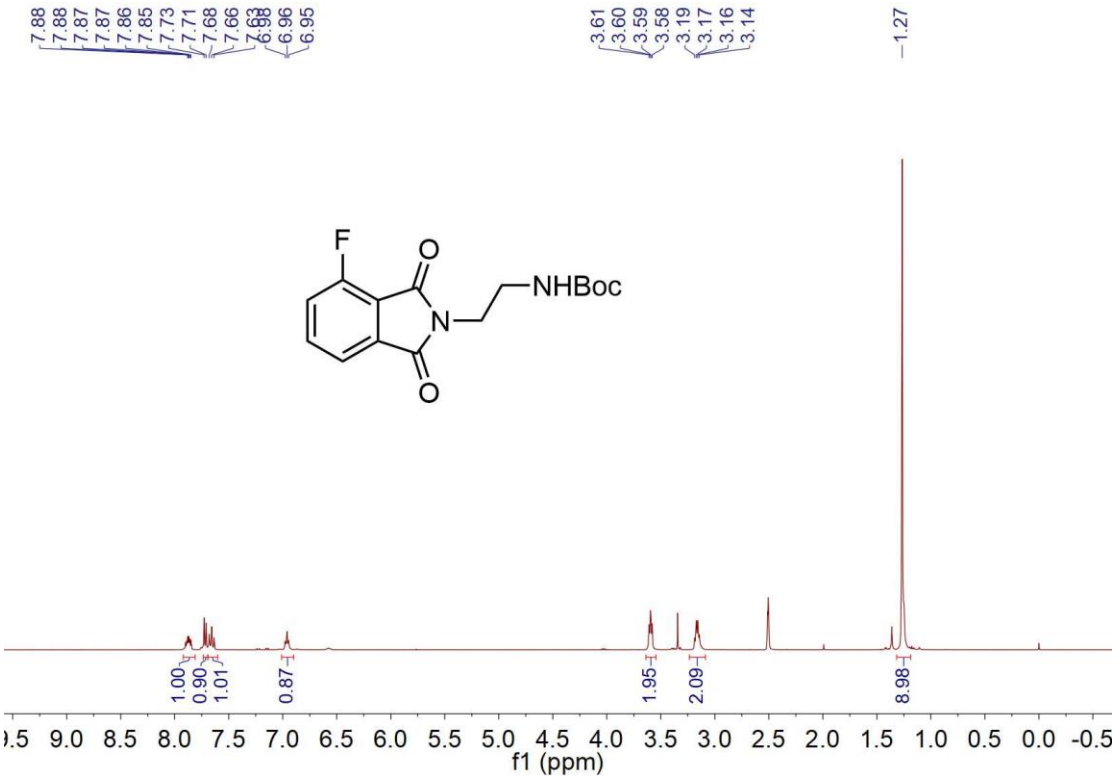
^{1}H NMR spectrum of **2n** (400 MHz, DMSO-d_6)



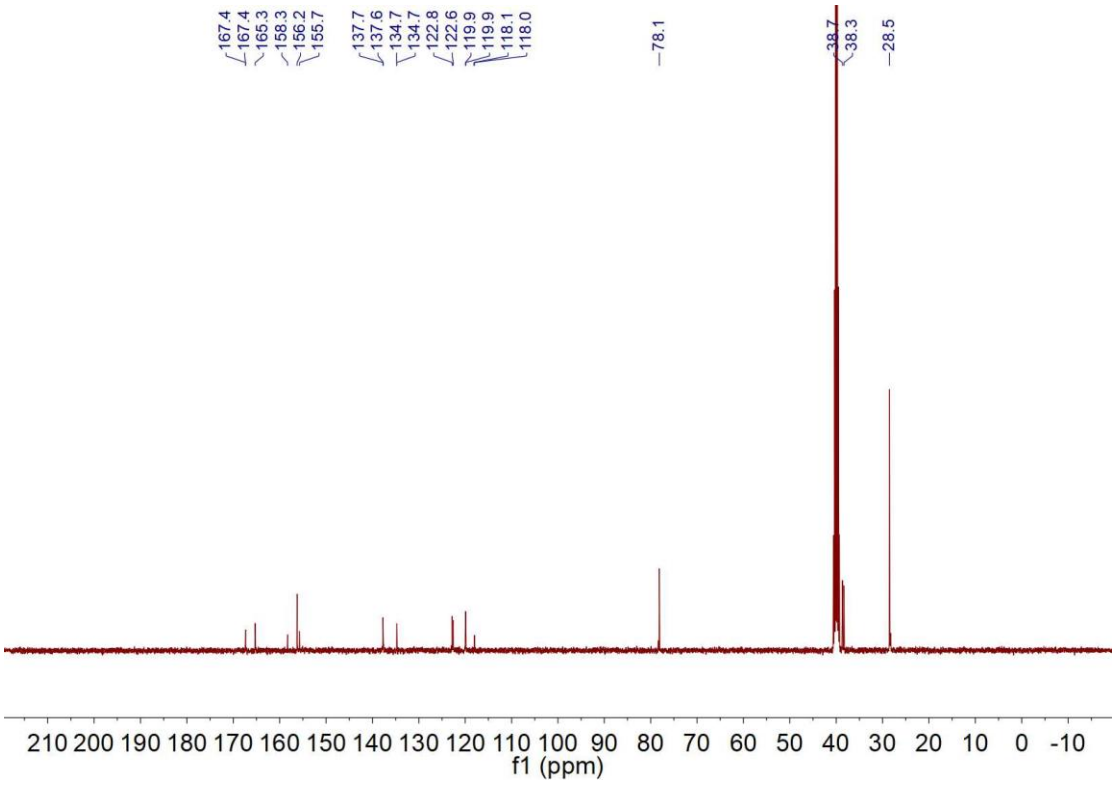
^{13}C NMR spectrum of **2n** (101 MHz, DMSO-d_6)



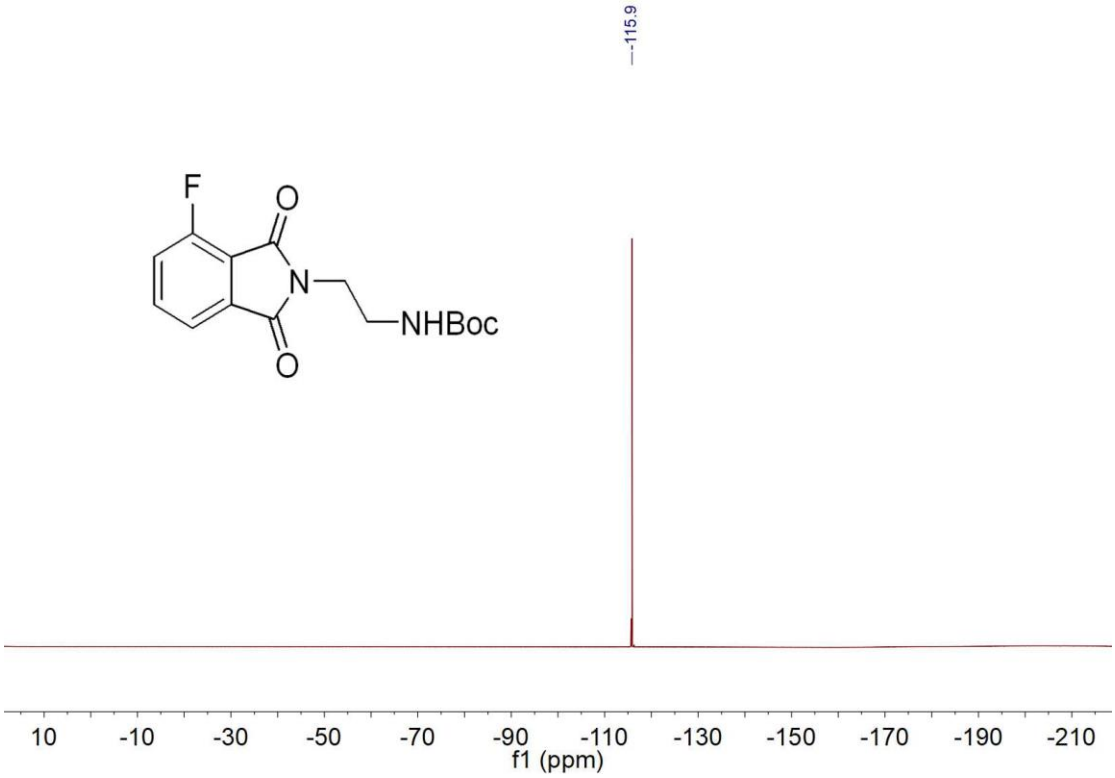
^{1}H NMR spectrum of **3a** (400 MHz, DMSO-d_6)



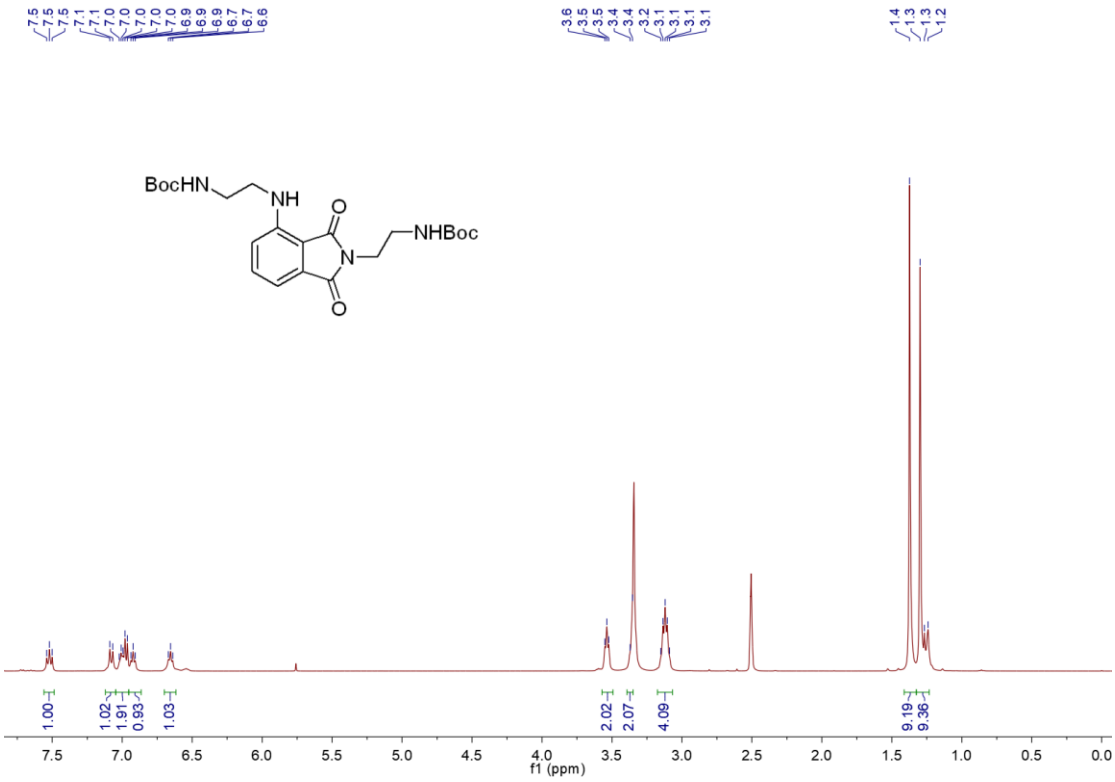
^{13}C NMR spectrum of **3a** (101 MHz, DMSO-d_6)



^{19}F NMR spectrum of **3a** (376 MHz, DMSO-d_6)



^{1}H NMR spectrum of **4a** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of **4a** (101 MHz, DMSO-d_6)