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Electronic Supporting Information

Utilising the Intrinsic Fluorescence of Pomalidomide for Imaging Applications

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General Experimental

All chemical reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. “Dri-Solv” EMD Millipore grade DMF was used. A KD Scientific KDS-210 syringe pump was used for dropwise additions of reagents. Triturations were performed using a VWR model 75T ultrasonic cleaner. Solvents were removed in vacuo using either a Buchi R-300 Rotavapor (equipped with an I-300 Pro Interface, B-300 Base Heating Bath, Welch 2037B-01 DryFast pump, and VWR AD15R-40-V11B Circulating Bath), a Biotage V-10 evaporator, or a Kugelrohr short path distillation apparatus. Reactions were monitored by thin-layer chromatography carried out on Merck glass silica gel plates (60F254) using UV light as a visualizing agent and iodine and/or phosphomolybdic acid stain as developing agents. Manual flash chromatography was performed using Silicycle SiliaFlash F60 silica gel (particle size 0.040−0.063 mm, 230−400 mesh) as well as for automated flash chromatography. Solvents for silica gel chromatography were used as supplied by Sigma-Aldrich. Automated flash chromatography was performed on a Biotage Isolera instrument, equipped with a UV detector and Biotage Dalton mass detector. Chromatograms were recorded at 254 and 280 nm. High-resolution mass spectra (HRMS) and low-resolution mass spectra were obtained using Agilent 6520 Accurate-Mass QTOF LC/MS or Bruker MALDI-TOF Autoflex III and GenTech 5890 series II SSQ 7000 instruments, respectively. Optimization experiments and purity of compounds were assessed (≥95% purity) using an analytical high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity LC equipped with an Agilent 1260 autosampler, an Agilent 1260 multi-wavelength UV detector, and an Agilent 1260 automated fraction collector with a Poroshell 120 EC-C18 4.6 × 50 mm^2 2.7 μm column coupled with a Poroshell 120 EC-C18 4.6 × 5 mm^2 2.7 μm ultra-high performance liquid chromatography guard column. Experiments were run with a flow rate of 1.5 mL/min. Solvents (H_2O, acetonitrile, and isopropanol) containing 0.1% trifluoroacetic acid (TFA) were used. The following gradient was used at 40 °C: 5−95% MeCN in water, 0−20 min. Compound characterisation and purity were analysed by MS and nuclear

magnetic resonance (NMR). ^{1}H NMR (400, 600 MHz) and ^{13}C NMR (100, 151 MHz) spectra were recorded in $CDCl_3$ or DMSO-d_6 at 298K on Bruker Avance III 400 MHz (broadband fluorine observe probe), Bruker DRX 400 MHz (broadband observe (BBO) probe), Bruker Avance 400 MHz (BBO probe), or Bruker Avance III 600 MHz (BBO probe) spectrometers. Chemical shifts (δ) are reported in ppm relative to solvent signals (δ = 7.26 and 77.16 ppm for $CDCl_3$ / 2.50 and 39.52 for DMSO-d_6). Coupling constants (J) are quoted in Hz. Abbreviations used for multiplicity are as follows: s – singlet, d – doublet, t – triplet, q – quartet, br – broad, m – multiplet.

Experimental Procedures and Characterising Data for Compounds

Absorption and Emission Spectra

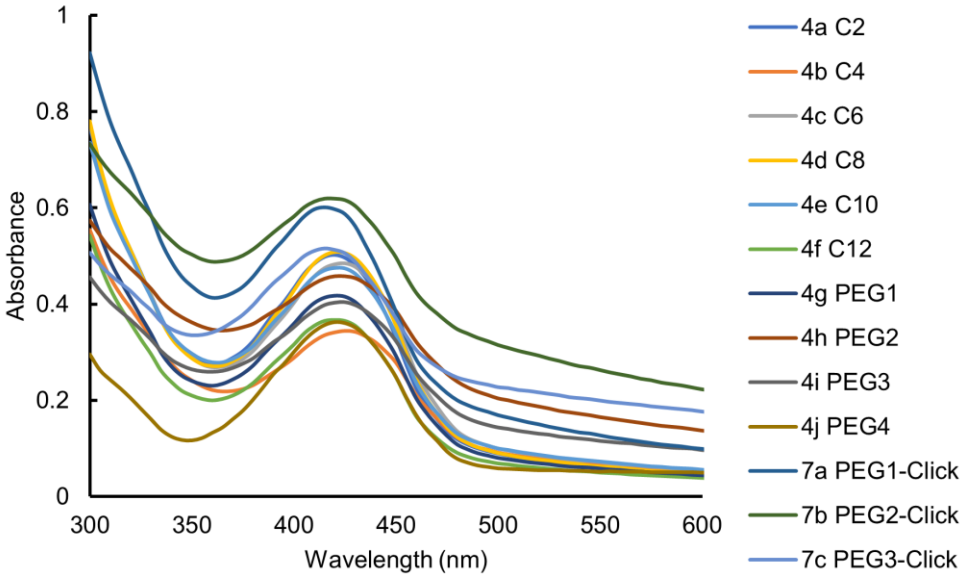


Figure S1. UV-visible light absorption of PROTAC library at 200 μM concentration in PBS solution with 2% DMSO.

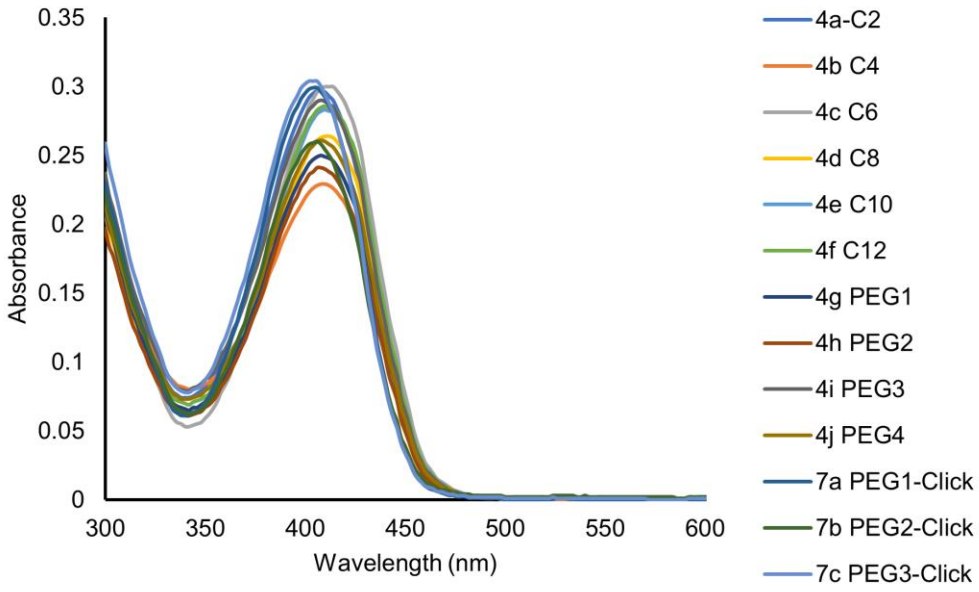


Figure S2. UV-visible light absorption of PROTAC library at 100 µM concentration in octanol.

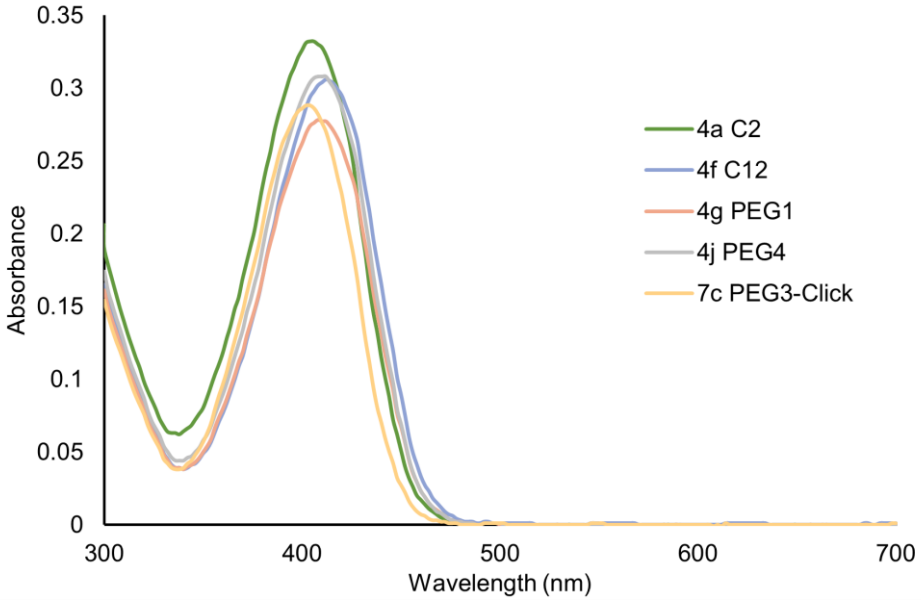


Figure S3. UV-visible light absorption of select examples of PROTAC library at 100 µM concentration in ethanol.

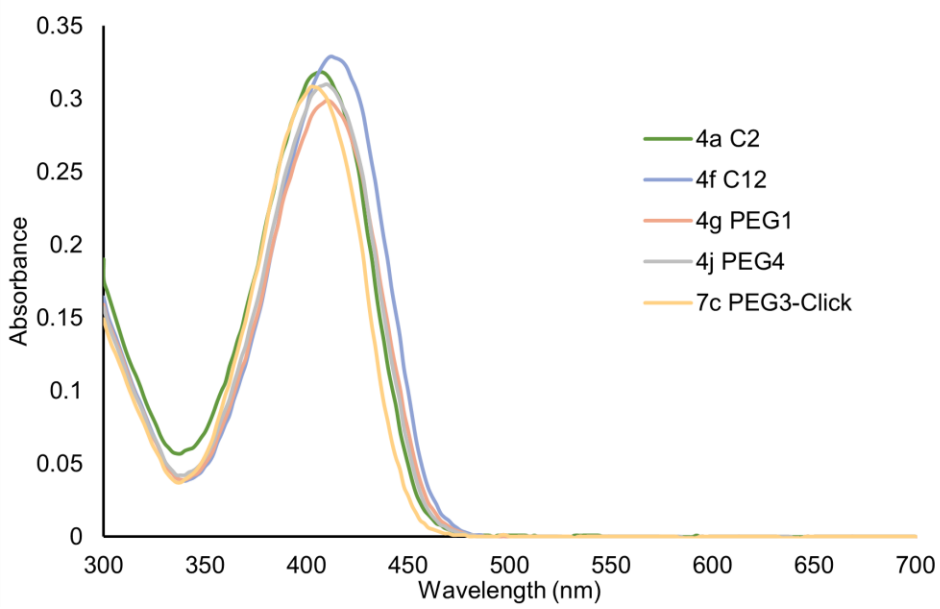


Figure S4. UV-visible light absorption of select examples of PROTAC library at 100 µM concentration in acetonitrile.

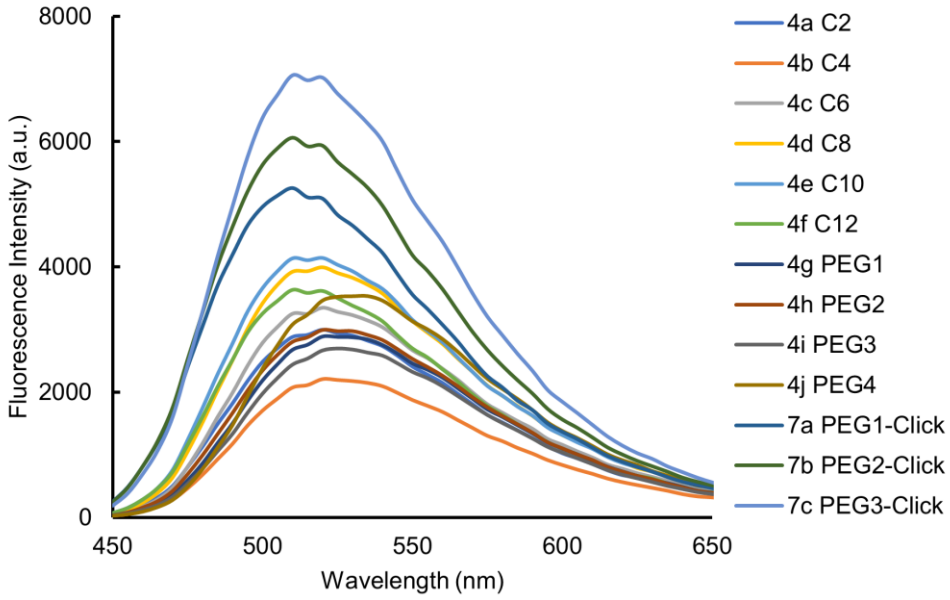


Figure S5. Emission spectra of PROTAC library with excitation at 420 nm. Spectra were recorded at 200 µM concentration in PBS solution with 2% DMSO.

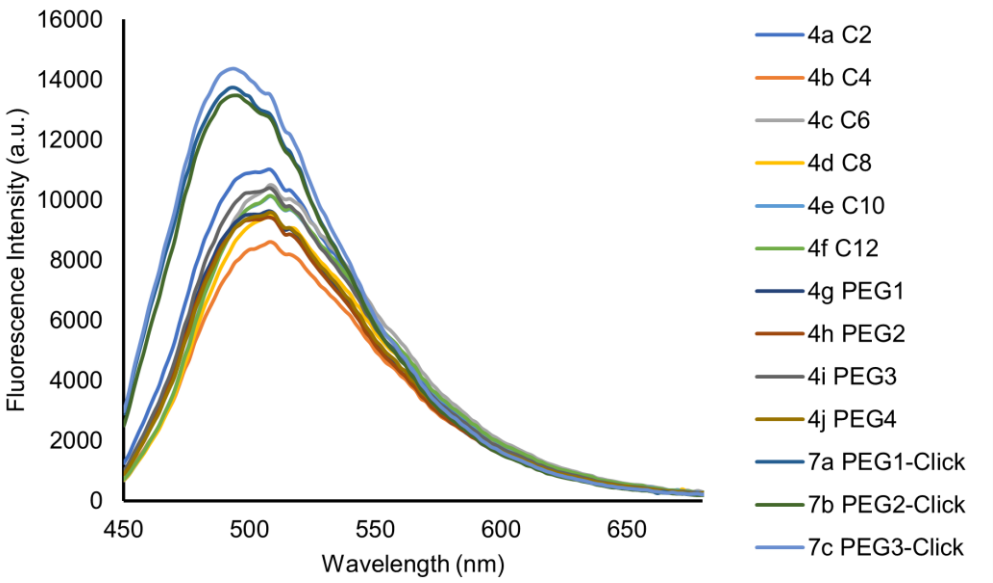


Figure S6. Emission spectra of PROTAC library with excitation at 420 nm at 100 µM concentration in octanol.

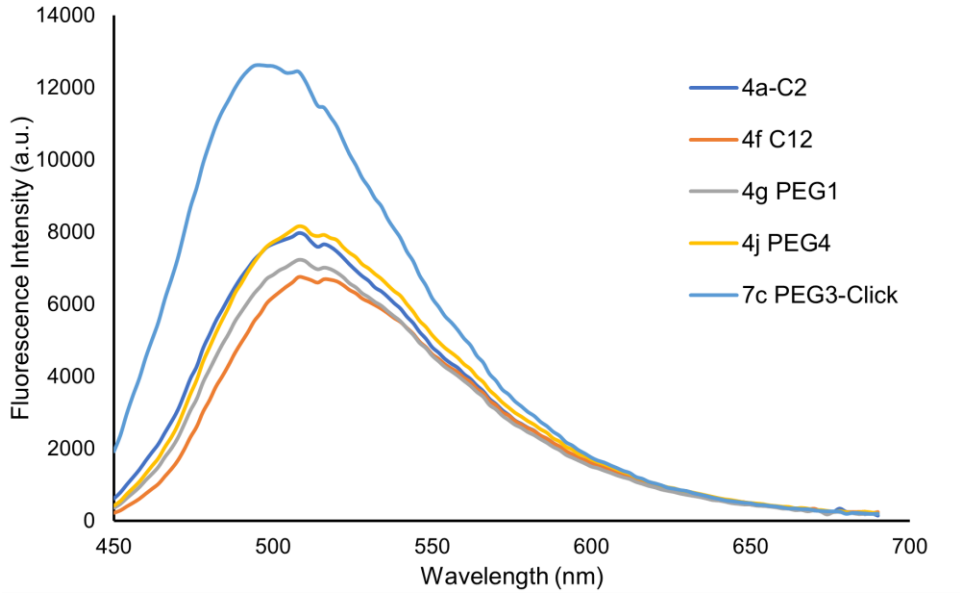


Figure S7. Emission spectra of PROTAC library with excitation at 420 nm at 100 µM concentration in ethanol.

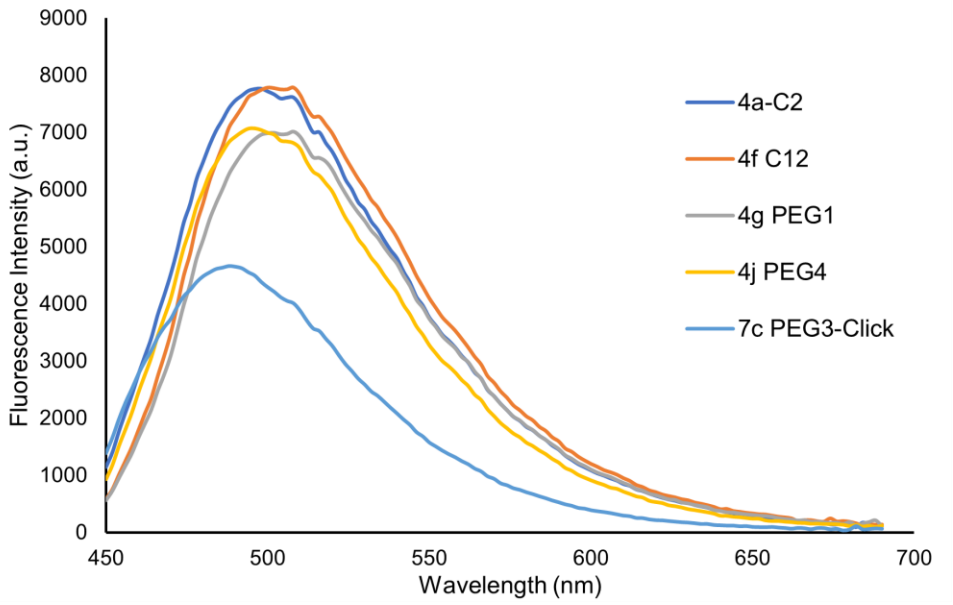


Figure S8. Emission spectra of PROTAC library with excitation at 420 nm at 100 µM concentration in acetonitrile.

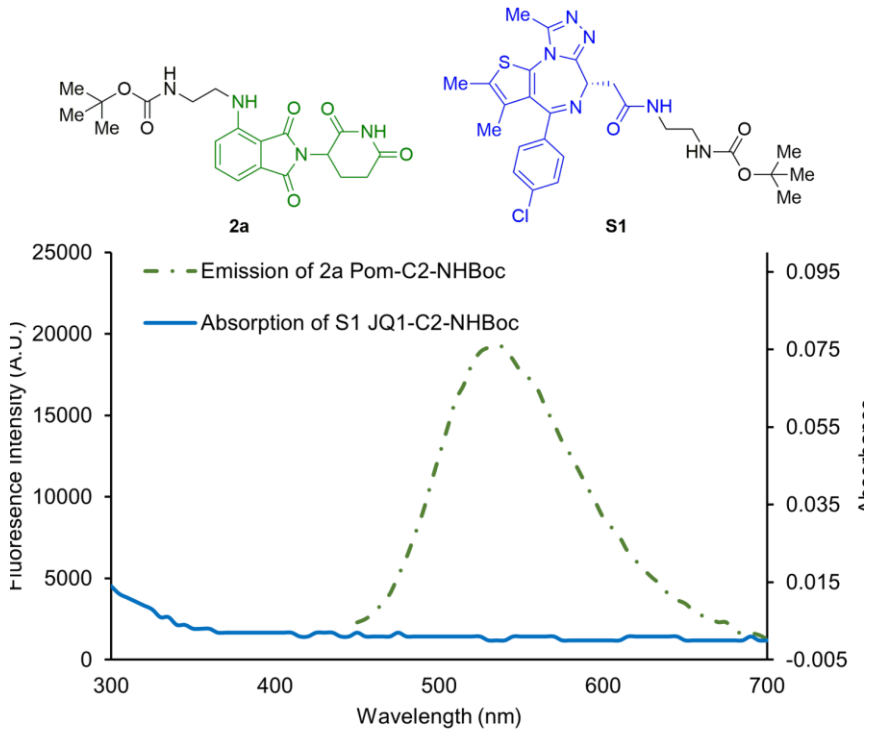


Figure S9. Comparison of spectral overlap of JQ1-linker **S1** absorbance and pomalidomide-linker **2a** emission at 415 nm excitation. Spectra were recorded at 10 µM concentration in PBS solution with 2% DMSO.

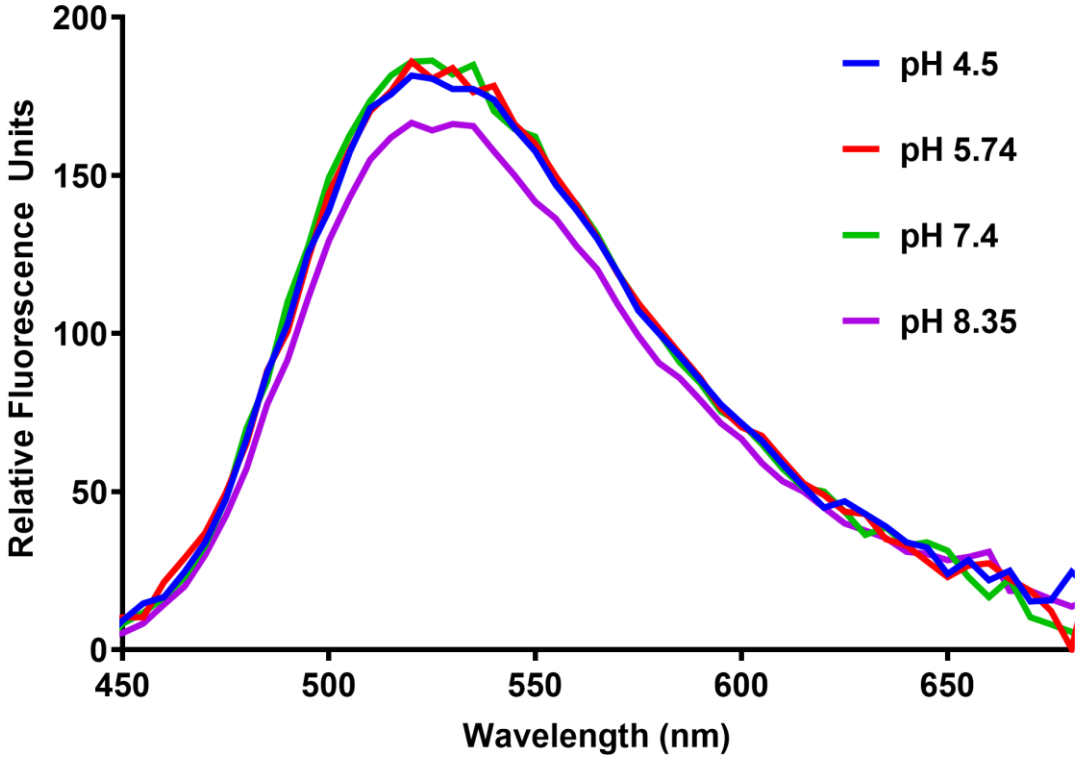


Figure S10. Effect of pH on PROTAC emission spectra. In triplicate, 10 µM of PROTAC **4c** in 2% DMSO with pH 4.5, 5.74, 7.4 and 8.35 buffer. Triplicates were averaged and blank corrected. Blanks were 2% DMSO with pH 4.5, 5.74, 7.4 and 8.35 buffer. Buffers were prepared according to the procedures below and then filtered (0.2 µm).

pH = 8.35, 1L 0.2M Buffer made up using 1.27 g of potassium phosphate monobasic and 50.8 g of sodium phosphate dibasic.

pH = 7.4, 1L 0.2M Buffer made up using 233 mg of sodium phosphate dibasic and 27.5 g of monobasic sodium phosphate.

pH = 5.74, 1L 0.2M Buffer made up using 22.4 g of potassium phosphate monobasic and 3.49 g of sodium phosphate dibasic.

pH = 4.5, 1L 0.2M Buffer made up using 41.580 g of sodium phosphate dibasic and 6.170 g of monobasic sodium phosphate.

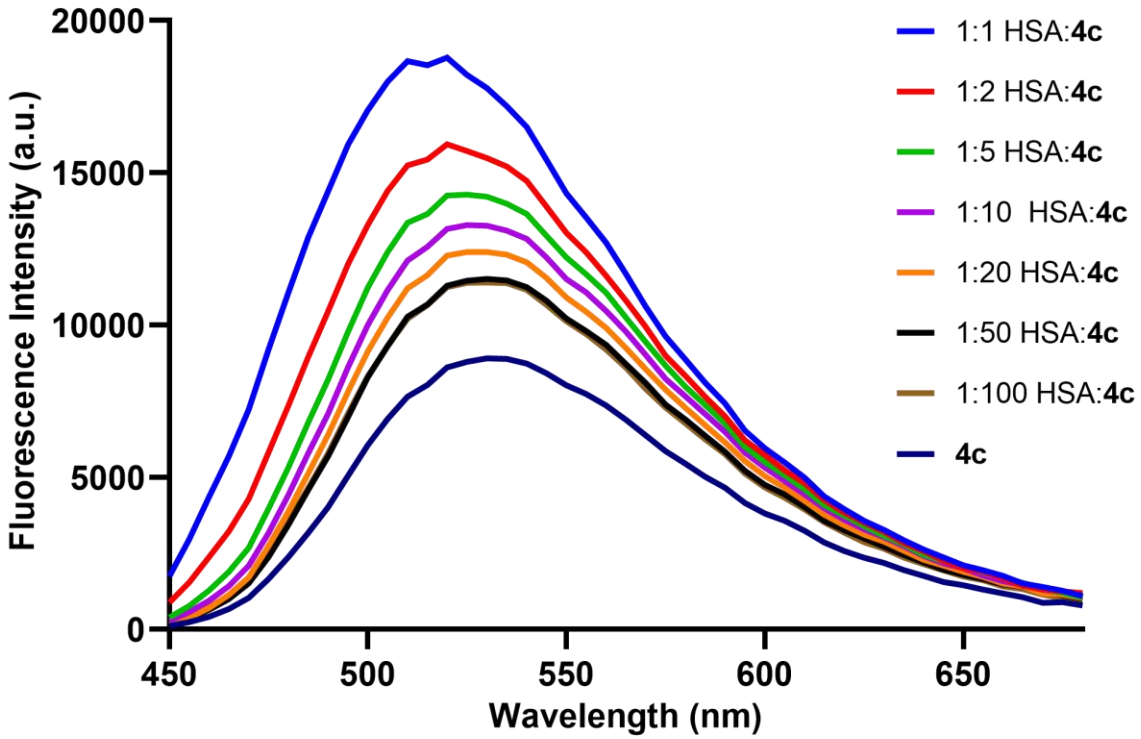


Figure S11. Effect of human serum albumin (HSA) on fluorescence emission of JQ1-pomalidomide PROTAC **4c**. In triplicate, PROTAC **4c** concentration was held constant at 10 µM, with varying ratios to Human Serum Albumin (HSA) [Sigma-Aldrich, Lyophilized powder, ≥96% (agarose gel electrophoresis), A1653] in 2% DMSO with pH 7.4 PBS buffer. Triplicates were averaged and blank corrected to their respective HSA concentration in 2% DMSO with pH 7.4 PBS buffer. Ratios of HSA concentration to PROTAC concentration used were 1: 1 (10 uM: 10

uM), 1: 2 (5 uM, 10 uM), 1: 5 (2 uM: 10 uM), 1: 10 (1 uM: 10 uM), 1: 20 (500 nM: 10 uM), 1: 50 (200 nM: 10 uM), 1: 100 (100 nM: 10 uM).

Photophysical Characterization of PROTAC Library

Compound	Linker	Φ_F (%) 2% DMSO in PBS	Φ_F (%) EtOH	Φ_F (%) MeCN	Φ_F (%) Octanol
4a	$(CH_2)_2$	8	38	37	42
4b	$(CH_2)_4$	7	-	-	42
4c	$(CH_2)_6$	7	-	-	39
4d	$(CH_2)_8$	8	-	-	40
4e	$(CH_2)_{10}$	11	-	-	40
4f	$(CH_2)_{12}$	11	36	37	40
4g	$(CH_2)_2(O(CH_2)_2)_1$	10	41	34	43
4h	$(CH_2)_2(O(CH_2)_2)_2$	7	-	-	43
4i	$(CH_2)_2(O(CH_2)_2)_3$	10	-	-	40
4j	$(CH_2)_2(O(CH_2)_2)_4$	13	42	35	41
7a	$(CH_2)_2(O(CH_2)_2)_1$-(N-N=N-C=C)-CH_2-	12	-	-	49
7b	$(CH_2)_2(O(CH_2)_2)_2$-(N-N=N-C=C)-CH_2-	16	-	-	55

7c	$(CH_2)_2(O(CH_2)_2)_3$-(N-N=N-C=C)-CH_2-	23	67	35	50

Table S1. Relative fluorescence quantum yields (ϕ_F) of PROTACs with 420 nm excitation **4a-j** and **7a-c** in 2% DMSO in PBS solution, EtOH, MeCN and octanol, were determined with Coumarin 153 as a quantum yield standard.[1] Concentrations ranging from 2.5 – 200 µM in 200 µL volumes were used and emission and absorption spectra were recorded using a BioTek™ Cytation™ 5 Cell Imaging Multi-Mode Reader in a 96-well plate.

Compound	**Linker**	**$\varepsilon(M^{-1}cm^{-1})$ 2% DMSO in PBS**	**$\varepsilon(M^{-1}cm^{-1})$ EtOH**	**$\varepsilon(M^{-1}cm^{-1})$ MeCN**	**$\varepsilon(M^{-1}cm^{-1})$ Octanol**
4a	$(CH_2)_2$	5.0×10^3	5.7×10^3	5.4×10^3	5.9×10^3
4b	$(CH_2)_4$	5.7×10^3	-	-	4.4×10^3
4c	$(CH_2)_6$	4.8×10^3	-	-	5.9×10^3
4d	$(CH_2)_8$	5.3×10^3	-	-	5.2×10^3
4e	$(CH_2)_{10}$	4.7×10^3	-	-	5.6×10^3
4f	$(CH_2)_{12}$	3.6×10^3	5.1×10^3	5.6×10^3	5.6×10^3
4g	$(CH_2)_2(O(CH_2)_2)_1$	3.8×10^3	4.6×10^3	5.1×10^3	4.9×10^3
4h	$(CH_2)_2(O(CH_2)_2)_2$	4.8×10^3	-	-	4.7×10^3
4i	$(CH_2)_2(O(CH_2)_2)_3$	4.2×10^3	-	-	5.7×10^3

4j	$(CH_2)_2(O(CH_2)_2)_4$	3.6×10^3	5.2×10^3	5.4×10^3	5.1×10^3
7a	$(CH_2)_2(O(CH_2)_2)_1$-(N-N=N-C=C)-CH_2-	6.1×10^3	-	-	5.7×10^3
7b	$(CH_2)_2(O(CH_2)_2)_2$-(N-N=N-C=C)-CH_2-	6.4×10^3	-	-	4.9×10^3
7c	$(CH_2)_2(O(CH_2)_2)_3$-(N-N=N-C=C)-CH_2-	5.2×10^3	4.2×10^3	5.2×10^3	6.0×10^3

Table S2. Molar absorptivity coefficients of PROTAC library **4a-j** and **7a-c** in 2% DMSO in PBS solution, EtOH, MeCN and octanol. Concentrations ranging from 2.5 – 200 µM in 200 µL volumes were used and absorption spectra were recorded using a BioTek™ Cytation™ 5 Cell Imaging Multi-Mode Reader in a 96-well plate. Pathlength of 0.5 cm was assumed for each well.

Compound	**Linker**	**Brightness ($M^{-1}cm^{-1}$) 2% DMSO in PBS**	**Brightness ($M^{-1}cm^{-1}$) EtOH**	**Brightness ($M^{-1}cm^{-1}$) MeCN**	**Brightness ($M^{-1}cm^{-1}$) Octanol**
4a	$(CH_2)_2$	4.0×10^2	2.2×10^3	2.0×10^3	2.5×10^3
4b	$(CH_2)_4$	4.0×10^2	-	-	1.9×10^3
4c	$(CH_2)_6$	3.4×10^2	-	-	2.3×10^3

4d	$(CH_2)_8$	4.2×10^2	-	-	2.1×10^3
4e	$(CH_2)_{10}$	5.2×10^2	-	-	2.2×10^3
4f	$(CH_2)_{12}$	3.9×10^2	1.8×10^3	2.1×10^3	2.3×10^3
4g	$(CH_2)_2(O(CH_2)_2)_1$	4.2×10^2	1.9×10^3	1.7×10^3	2.19×10^3
4h	$(CH_2)_2(O(CH_2)_2)_2$	3.3×10^2	-	-	2.0×10^3
4i	$(CH_2)_2(O(CH_2)_2)_3$	4.1×10^2	-	-	2.3×10^3
4j	$(CH_2)_2(O(CH_2)_2)_4$	4.7×10^2	2.2×10^3	1.9×10^3	2.1×10^3
7a	$(CH_2)_2(O(CH_2)_2)_1$-(N-N=N-C=C)-CH_2-	7.3×10^2	-	-	2.8×10^3
7b	$(CH_2)_2(O(CH_2)_2)_2$-(N-N=N-C=C)-CH_2-	1.0×10^3	-	-	2.7×10^3
7c	$(CH_2)_2(O(CH_2)_2)_3$-(N-N=N-C=C)-CH_2-	1.2×10^3	2.8×10^3	1.8×10^3	3.0×10^3

Table S3. Brightness values of the PROTAC library in various solvents.

Fluorescence microscopy

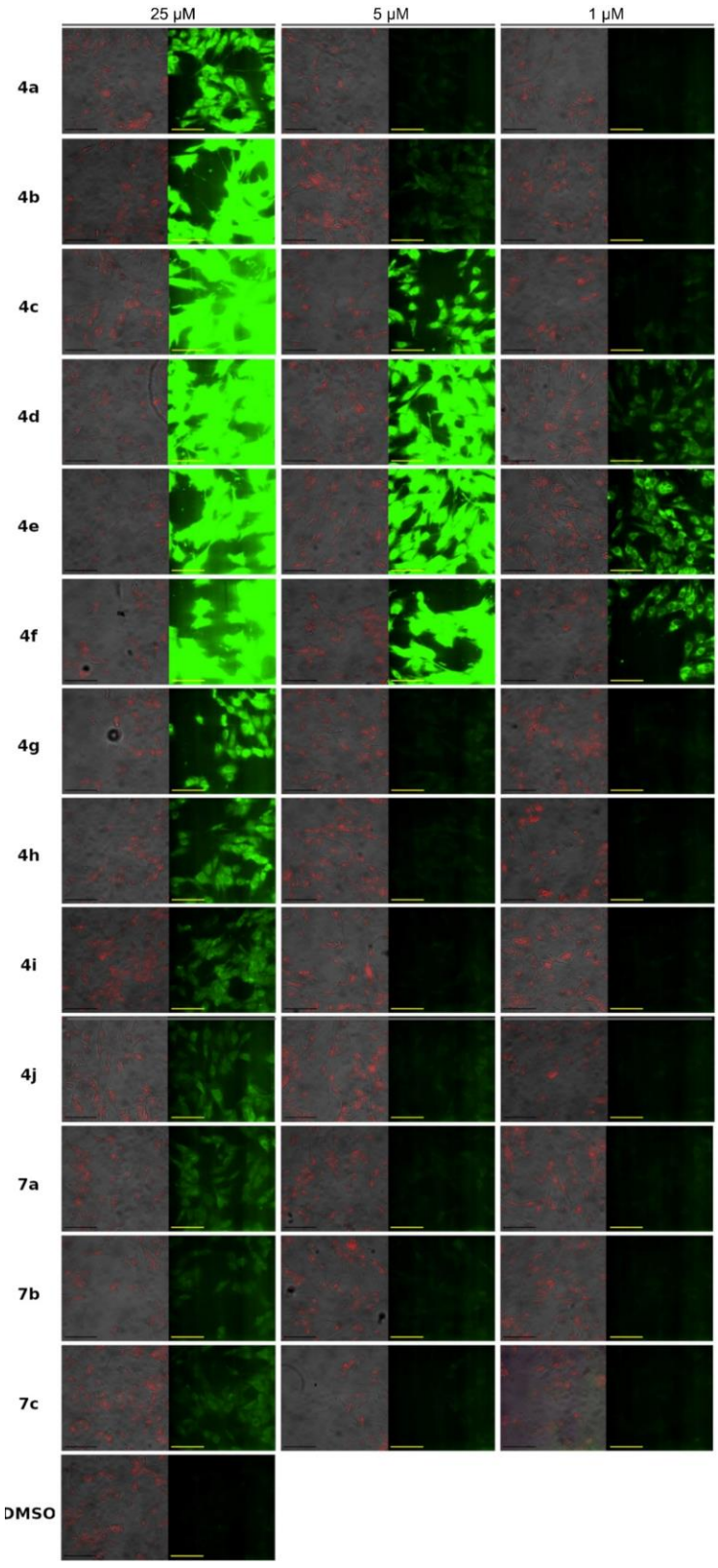


Figure S12. Example images of PROTAC library in MG-63 cells. Scale bars represent 100 µm. MG-63 cells were seeded in 96-well plates at a density of 20,000 cells/well and allowed to grow overnight. Cells were then treated for 30 minutes with a dilution series of candidate PROTACs ranging in concentration from 25 µM to 1 µM, along with NucRed (Thermo) to stain nuclei. Cells were washed then fixed with 1% paraformaldehyde in PBS and imaged at 40x on an automated confocal laser microscope (InCell 6000,GE). Brightfield/Cy5 composite images were collected using a red laser (642 nm) with a Cy5 (706.5/72 nm) emission filter (left column) while PROTACs were imaged with a UV laser (405 nm) and a FITC (525/20 nm) emission filter (right column).

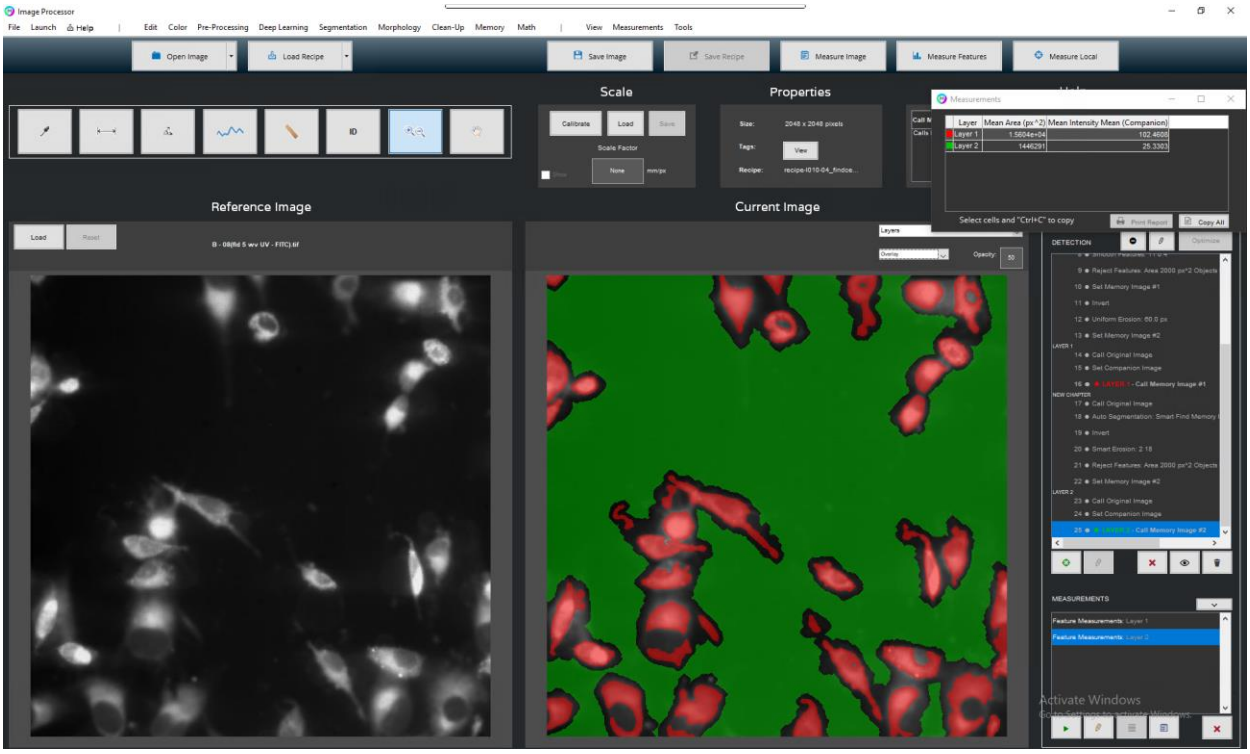


Figure S13. Example image of fluorescence quantitation using MIPAR Image Analysis software suite. Results used in **Fig. 2**, **Fig. S12**, **Fig. S13** and **Fig. S14** are the average of 24 fields per condition (6 fields per well in quadruplicate biological replicate), at 7 different concentrations. Cells are shown in red, and background shown in green.

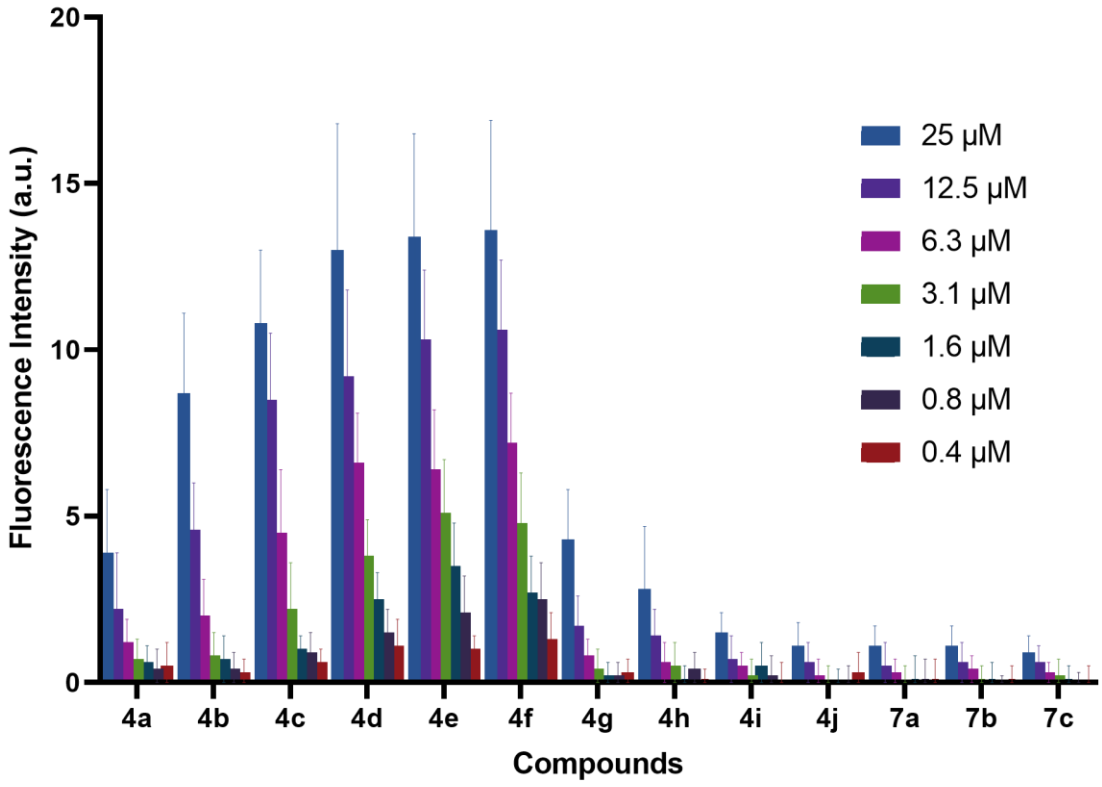


Figure S14. Fluorescence intensity of PROTAC library in MG-63 cells. MG-63 cells were seeded in 96-well plates at a density of 20,000 cells/well in EMEM and allowed to grow overnight. Cells were then treated for 30 minutes with a dilution series of candidate PROTACs ranging in concentration from 25 µM to 0.4 µM. Cells were washed with PBS then fixed with 1% paraformaldehyde in PBS and imaged at 40x on an automated confocal laser microscope (InCell 6000,GE). PROTACs were imaged with a UV laser (405 nm) and a FITC (525/20 nm) emission filter (right column). Intracellular localization of fluorescence was readily determined at 40x magnification in these cells and the mean signal intensity across cells was measured using MIPAR image analysis suite (see **Fig. S13**).

Colocalization imaging

MG-63 cells were seeded in a 96-well plate at 10,000 cells / well and incubated overnight in EMEM media. Cells were then incubated with 25 µM PROTAC **4g** for 30 min, washed with PBS and fixed with

1% paraformaldehyde in PBS for 10 min at room temperature. Following fixation, cells were permeabilized using 0.1% Triton X-100 in PBS for 5 minutes and then blocked in 1% BSA/0.3M glycine/0.1% PBS-Tween 20 for 1 hour. Cells were then incubated with Alexa Fluor 647 conjugated anti-BRD4 antibody (Abcam ab197608) at a 1:100 dilution overnight at 4 °C. After washing with PBS, cells were imaged at 40x Magnification with a UV laser (405 nm) and FITC (525/20 nm) channel or a red laser (642 nm) and Cy5 (706.5/72 nm) channel. Pearson coefficient of colocalized image was determined to be 0.76. Pearson coefficient was calculated in Fiji software (ImageJ v.2.3.0) using colocalization test.

Endocytosis inhibition

Fluorescence intensity of selected PROTAC examples in MG-63 cells alongside treatment with hydroxy-Dynasore (HDS) [Sigma-Aldrich, 324413] at 10 μM concentration or Pitstop-2 (PS2) [Sigma-Aldrich, SML1169] at 20 μM concentration. Cells were treated with endocytosis inhibitor for 30 minutes prior to the addition of indicated PROTACs, and then incubated for an addition 30 minutes with PROTAC. Cells were then washed with PBS buffer and then fixed with 1% paraformaldehyde in PBS and then imaged similarly to **Fig. S14**. Image processing was then identical to **Fig. S13**.

Calculated partition coefficient (cLogP)

Compound	cLogP
4a	4.6 ± 0.2
4b	5.3 ± 0.2
4c	6.0 ± 0.2
4d	6.7 ± 0.2
4e	7.4 ± 0.2
4f	8.1 ± 0.2
4g	4.7 ± 0.2
4h	4.9 ± 0.2
4i	5.1 ± 0.3
4j	5.3 ± 0.3
7a	4.7 ± 0.2
7b	5.0 ± 0.2
7c	5.1 ± 0.2

Table S4. PROTACs **4a-j**, **7a-c**, and **10** were uploaded to Maestro (Schrödinger Release 2015–4: MS Jaguar, Schrödinger, LLC, New York, NY, 2015). 3D structures and protonation states at biological pH 7.0 ± 0.5 were generated with Epik and LigPrep (Schrödinger Release 2015-4: LigPrep, Epik, LLC, New York, NY, 2015). From each prepared compound, 100 conformers were generated using ConfGen.[2]

Compounds that cannot be neutralized were removed from this study due to QikProp descriptors calculation limitation. Average values from all conformers were used to determine cLogP using QikProp.[3]

Immunoblotting

MG-63 cells were seeded into 6-well plates at 2-2.5 × 10^5 cells with 2 mL of EMEM supplemented with 10% FBS (v/v) per well and then incubated overnight. MG-63 cell lysates were collected in total lysis buffer (50mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Triton X-100, and 1% SDS) at room temperature. Protein quantitation was performed using the Bio-Rad DC Protein Assay Kit (Bio-Rad, 5000112). Proteins were resolved on mini handcast gels [10 cm X 8 cm X 0.15 cm, (length x width x thickness)] with 8% resolving gel: H2O (4.6 mL), 30% Acrylamide/Bis mix (29:1)[Bio-Rad, 1610156] (2.7 mL), 1.5 M Tris (pH 8.8)[Bio-Rad, 1610798] (2.5 mL), 10% SDS (0.1 mL) [Bio-Rad, 1610416], 10% ammonium persulfate [Bio-Rad, 1610700EDU] (0.1 mL), TEMED [Bio-Rad, 1610800] (0.006 mL) and 5% stacking gel: H2O (1.4 mL), 30% Acrylamide/Bis mix (29:1) [Bio-Rad, 1610156] (0.33 mL), 1.0 M Tris (pH 6.8) [Bio-Rad, 1610799] (0.25 mL), 10% SDS [Bio-Rad, 1610416] (0.02 mL), 10% ammonium persulfate (0.02 mL) TEMED (0.002 mL). This was then followed by transfer to Polyvinylidene Fluoride (PVDF) membranes [Bio-Rad, 1620177] using Bio-Rad Trans-turbo semi-dry transfer apparatus at 25V, 2.5 Amp for 1 hour in transfer buffer consisting of 25 mM Tris, 192 mM glycine, pH 8.3. Membranes were blocked with 1X Tris-Buffered-Saline [Bio-Rad, 1706435] containing 0.1% (v/v) Tween 20 [Sigma-Aldrich, P9416] (TBS-T) and 5% (w/v) skim milk for 30 minutes at room temperature and probed overnight at 4°C for BRD4 using 1:1000 BRD4 rabbit monoclonal antibody [EPR5150(2)] (Abcam, ab128874). 1:10000 GAPDH rabbit polyclonal antibody was used as a loading control (Abcam, ab9485). The following day, membranes were washed with Tris-Buffered-Saline [Bio-Rad, 1706435] containing 0.1% (v/v) Tween 20 [Sigma-Aldrich, P9416] (TBS-T) three times and then probed with goat anti-rabbit horseradish peroxidase-conjugated IgG (Bio-Rad, 1706515) for 1 hour at room temperature. The secondary antibody was diluted 1:6,000 in TBS-T containing 5% (w/v) skim milk. After membranes were washed three times with TBS-T, immunoreactive

proteins were detected using Clarity™ Western ECL Substrate (Bio-Rad, 1705060) and visualized using the Chemidoc-IT Imager (UVP LLC).

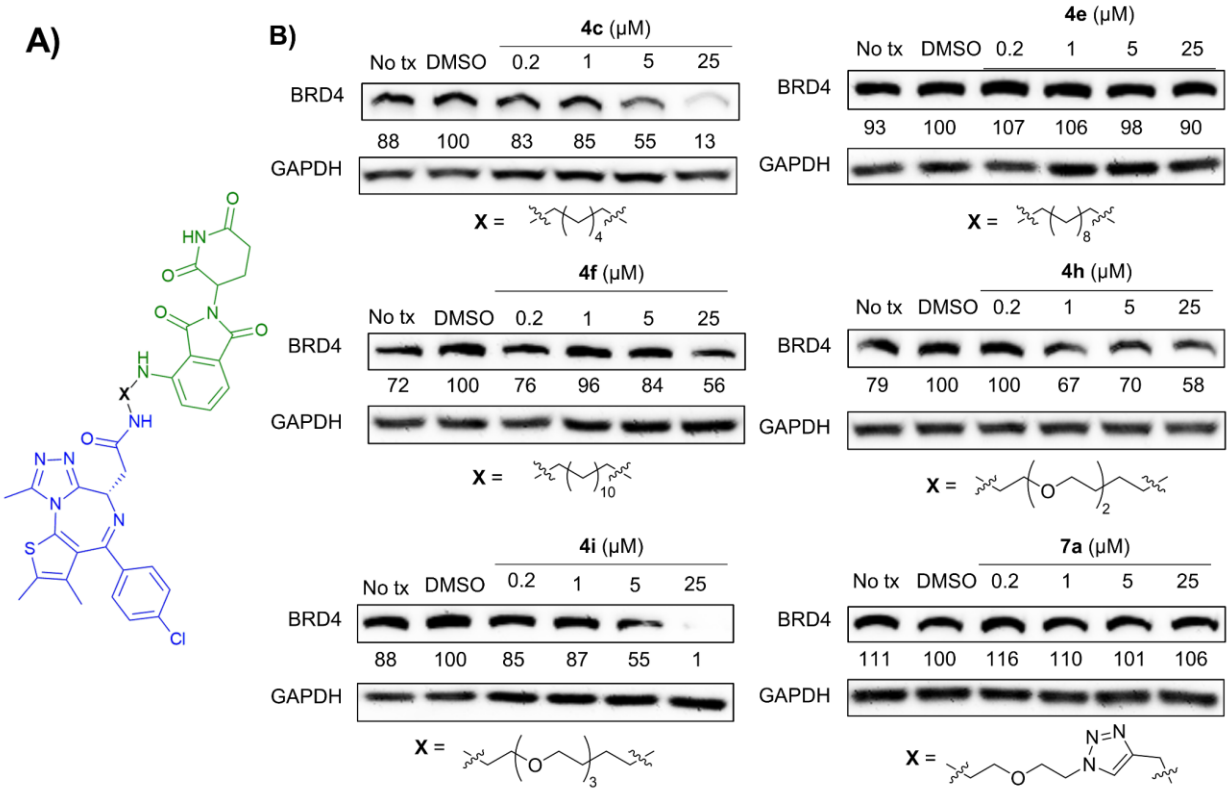


Figure S15. Cellular degradation analysis of BRD4 with selected examples of the prepared fluorescent PROTAC library. A) General chemical structure of fluorescent PROTACs. B) Western blot of BRD4 in MG-63 cells after treatment with specified compound for 24 h.

Cell Culture

MG-63 cells were maintained in EMEM medium supplemented with 10% FBS (v/v) and kept at 37 °C under a 5% CO_2 atmosphere.

Chemical Synthesis

General procedure for preparation of pomalidomide linkers (GP1)

A solution of **1** (1.0 eq.), amine linker (1.1 eq.) and DIPEA (3.0 eq.) in DMSO (0.2 M) was heated to 90 °C for 16 hours. After this time, the solvent was removed by Kugelrohr distillation at 40 °C and 0.1 Torr. The crude residue was then adsorbed to silica gel and purified by flash column chromatography over silica gel, eluting with EtOAc:hexanes (20 – 100 %).

General procedure for the preparation of JQ1-pomalidomide PROTACs (GP2)

To a solution of *N*-Boc protected pomalidomide linker (1.0 eq.) in DCM (0.1 M) was added trifluoroacetic acid (50 eq.) at 0 °C. The mixture was stirred for 10 minutes at 0 °C and was then warmed to room temperature and stirred for an additional hour. Volatiles were then removed *in vacuo* and the deprotected amine was used directly without further purification.

The residue was dissolved in anhydrous DMF (0.1 M) and DIPEA (3 eq.) and **3** (1.2 eq.) were added to the solution. The reaction mixture was then allowed to stir for 16 hours at room temperature. Volatiles were then removed *in vacuo* and the crude residue was adsorbed to silica gel and purified twice, first by silica gel flash column chromatography, eluting with MeOH:DCM (1-12%) and followed by reverse phase C_{18} functionalized silica gel flash column chromatography, eluting H_2O:MeCN (95:5 to 5:95).

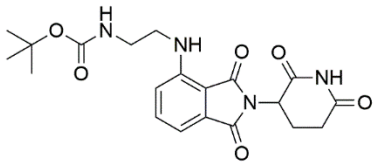
General procedure for the preparation of JQ1-azide linkers (GP3)

To a solution of **3** (1.0 eq.) and DIPEA (3 eq.) in DMF (0.05 M) was added the respective azide-amine PEG linker (1.2 eq., Broadpharm) at room temperature. After one hour, volatiles were removed *in vacuo* and the residue was dissolved in DMF (0.05 M) and potassium carbonate (3 eq.) was added to the mixture. After one hour, the salts were filtered and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography eluting with EtOAc, followed by MeOH in $CHCl_3$ (0 – 10%).

General procedure for the CuAAC prepared JQ1-pomalidomide PROTACs (GP4)

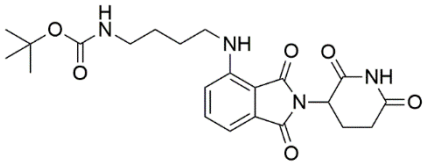
To a solution of **5** (1.0 eq.) in THF (0.1 M) was added the respective JQ1-Azide precursor (1.0 eq.), water (0.2 mL), $CuSO_4 \bullet 5H_2O$ (0.30 eq.) and sodium ascorbate (0.50 eq.) and the reaction mixture was stirred for 16 hours at room temperature. Volatiles were removed *in vacuo* and the crude residue was purified twice, first by silica gel flash column chromatography, eluting with MeOH:$CHCl_3$ (5 – 10%) and then by reverse phase C_{18} functionalized silica gel flash column chromatography, eluting with H_2O:MeCN (95:5 – 5:95).

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



Following **GP1**: Compound **2a** was isolated as a yellow solid (182 mg, 0.44 mmol, 42%): R_f = 0.11 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.24 (s, 1H), 7.50 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.11 (d, *J* = 7.1 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.39 (t, *J* = 6.1 Hz, 1H), 4.95 – 4.85 (m, 2H), 3.44 (q, *J* = 6.1 Hz, 2H), 3.36 (q, *J* = 6.1 Hz, 2H), 2.95 – 2.67 (m, 3H), 2.15 – 2.08 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.2, 169.5, 168.5, 167.7, 156.2, 147.0, 136.4, 132.6, 116.8, 112.0, 110.4, 79.9, 49.0, 42.7, 40.3, 31.6, 28.5, 22.9; HRMS (ESI) *m/z* calculated for $[C_{20}H_{23}N_4O_6\text{-}H]^-$ = 415.1623, found 415.1607.

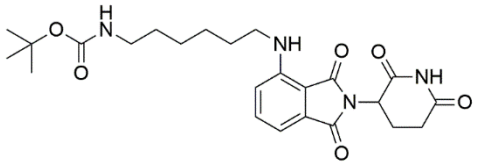
***tert-Butyl (4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)carbamate* (2b)**



Following GP1: Compound **2b** was isolated as a yellow solid (167 mg, 0.38 mmol, 57%): R_f = 0.10 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.83 (s, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.1 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 1H), 6.22 (t, *J* = 5.7 Hz, 1H), 4.90 (dd, *J* = 12.0, 5.4 Hz, 1H), 4.74 (t, *J* = 6.1

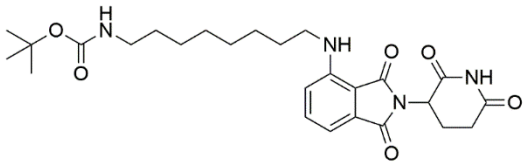
Hz, 1H), 3.25 (q, *J* = 6.6 Hz, 2H), 3.14 (q, *J* = 6.7 Hz, 2H), 2.86 – 2.66 (m, 3H), 2.11 – 2.03 (m, 1H), 1.65 (p, *J* = 7.1 Hz, 2H), 1.56 (p, *J* = 7.1 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.6, 169.6, 168.8, 167.7, 156.1, 146.9, 136.2, 132.5, 116.7, 111.5, 110.0, 79.3, 48.9, 42.3, 40.1, 31.5, 28.5, 27.6, 26.5, 22.8; HRMS (ESI) *m/z* calculated for $[C_{22}H_{28}N_4O_6+H]^+$ = 445.2082, found 445.2087.

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



Following **GP1**: Compound **2c** was isolated as a yellow solid (289 mg, 0.65 mmol, 46%); Rf = 0.30 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 7.57 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 7.0 Hz, 1H), 6.76 (t, *J* = 5.6 Hz, 1H), 6.53 (t, *J* = 6.0 Hz, 1H), 5.05 (dd, *J* = 12.9, 5.4 Hz, 1H), 3.28 (q, *J* = 6.8 Hz, 2H), 2.93 – 2.83 (m, 3H), 2.63 – 2.51 (m, 2H), 2.02 (s, 1H), 1.56 (p, *J* = 7.1 Hz, 2H), 1.36 (s, 15H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.1, 168.9, 167.3, 155.6, 146.4, 136.2, 132.2, 117.1, 110.3, 109.0, 77.3, 48.5, 41.8, 31.0, 29.4, 28.6, 28.3, 26.0, 26.0, 22.1; HRMS (ESI) *m/z* calculated for $[C_{24}H_{31}N_4O_6 - H]^-$ = 471.2249, found 471.2232.

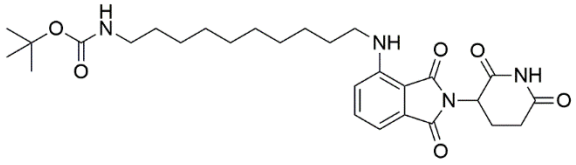
tert-Butyl (8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)carbamate **(2d)**



Following **GP1**: Compound **2d** was isolated as a yellow solid (233 mg, 0.46 mmol, 43%): R_f = 0.43 (50% EtOac in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) 8.19 (br s, 1H), 7.48 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.23 (t, *J* = 5.6 Hz, 1H), 4.91 (dd, *J* = 12.0, 5.3 Hz, 1H), 4.53 (br s, 1H), 3.25 (td, *J* = 6.8, 5.3 Hz, 2H), 3.10 (q, *J* = 6.8 Hz, 2H), 2.91 – 2.65 (m, 3H), 2.18 – 2.07 (m, 1H), 1.72 – 1.58 (m, 3H), 1.50 – 1.22 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.1, 169.7, 168.5, 167.8, 156.1,

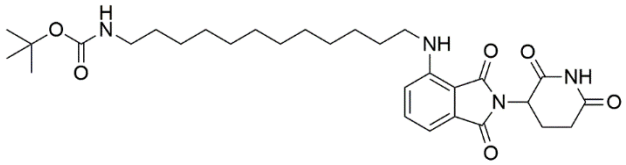
147.2, 136.2, 132.7, 116.8, 111.5, 110.0, 79.2, 49.0, 42.8, 40.8, 31.6, 30.2, 29.3, 29.3, 28.6, 27.0, 26.8, 23.0; HRMS (ESI) *m/z* calculated for $[C_{26}H_{35}N_4O_6 - H]^-$ = 499.2562, found 499.2544.

***tert-Butyl (10-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)decyl)carbamate* (2e)**



Following **GP1**: Compound **2e** was isolated as a yellow solid (128 mg, 0.24 mmol, 24%): R_f = 0.33 (50% EtOac in hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.32 (s, 1H), 7.49 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.23 (t, *J* = 5.6 Hz, 1H), 4.92 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.54 (t, *J* = 5.7 Hz, 1H), 3.32 – 3.20 (m, 2H), 3.10 (q, *J* = 6.8 Hz, 2H), 2.92 – 2.64 (m, 3H), 2.12 (ddt, *J* = 10.4, 5.3, 2.4 Hz, 1H), 1.65 (q, *J* = 7.3 Hz, 2H), 1.50 – 1.37 (m, 13H), 1.35 – 1.26 (m, 10H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 171.2, 169.6, 168.5, 167.8, 156.1, 147.2, 136.2, 132.6, 116.8, 111.4, 110.0, 79.1, 49.0, 42.8, 40.8, 31.6, 30.2, 29.5, 29.3, 28.6, 27.0, 26.9, 22.9; HRMS (ESI) *m/z* calculated for $[C_{28}H_{40}N_4O_6+H]^+$ = 529.3021, found 529.3017.

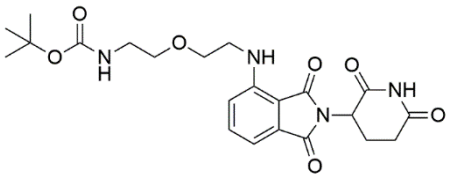
***tert-Butyl (12-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)dodecyl)carbamate* (2f)**



Following **GP1**: Compound **2f** was isolated as a yellow solid (273 mg, 0.49 mmol, 49%): R_f = 0.34 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.09 (br s, 1H), 7.57 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 7.0 Hz, 1H), 6.74 (t, *J* = 5.9 Hz, 1H), 6.52 (t, *J* = 6.0 Hz, 1H), 5.05 (dd, *J* = 12.9, 5.4 Hz, 1H), 3.31 – 3.25 (m, 2H), 2.94 – 2.80 (m, 3H), 2.63 – 2.51 (m, 2H), 2.06 – 1.92 (m, 1H), 1.61 – 1.49 (m, 2H), 1.36 (s, 9H), 1.34 – 1.17 (m, 18H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.8, 170.0, 168.9, 167.3, 155.5, 146.4, 136.2, 132.2, 117.2, 110.3, 109.0, 77.2, 48.5, 41.8, 31.0, 29.4, 29.0, 28.9, 28.9,

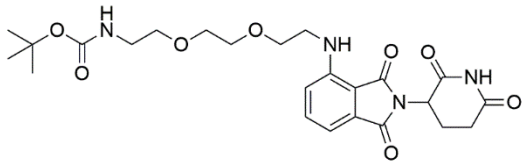
28.7, 28.7, 28.6, 28.3, 26.3, 26.2, 22.1; HRMS (ESI) *m/z* calculated for $[C_{30}H_{44}N_4O_6 + H]^+$ = 557.3334, found 557.3317.

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



Following **GP1**: Compound **2g** was isolated as a yellow solid (255 mg, 0.55 mmol, 55%): R_f = 14 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.20 (br s, 1H), 7.50 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.11 (d, *J* = 7.1 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.51 (t, *J* = 5.8 Hz, 1H), 4.99 (br s, 1H), 4.92 (dd, *J* = 12.5, 5.4 Hz, 1H), 3.68 (t, *J* = 5.4 Hz, 2H), 3.55 (t, *J* = 5.3 Hz, 2H), 3.45 (q, *J* = 5.5 Hz, 2H), 3.33 (q, *J* = 5.5 Hz, 2H), 2.93 – 2.65 (m, 3H), 2.12 (dtd, *J* = 12.5, 4.9, 2.3 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 171.1, 169.5, 168.4, 167.7, 156.1, 147.0, 136.2, 132.6, 116.9, 111.9, 110.6, 79.4, 70.5, 69.4, 49.1, 42.4, 40.6, 31.6, 28.5, 22.9; HRMS (ESI) *m/z* calculated for $[C_{22}H_{28}N_4O_7 + H]^+$ = 461.2031, found 461.2014.

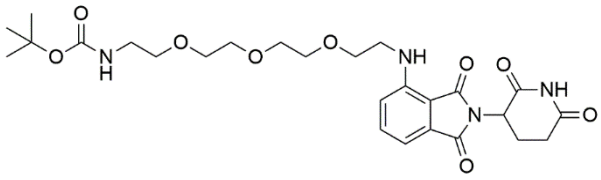
***tert-Butyl (2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)carbamate* (2h)**



Following **GP1**: Compound **2h** was isolated as a yellow film (234 mg, 0.46 mmol, 54%): R_f = 0.45 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.78 (s, 1H), 7.46 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.07 (d, *J* = 7.1 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.49 (t, *J* = 5.3 Hz, 1H), 5.12 – 5.02 (m, 1H), 4.90 (dd, *J* = 12.2, 5.4 Hz, 1H), 3.70 (t, *J* = 5.4 Hz, 2H), 3.66 – 3.59 (m, 4H), 3.54 (t, *J* = 5.3 Hz, 2H), 3.45 (q, *J* = 5.5 Hz, 2H), 3.29 (q, *J* = 5.5 Hz, 2H), 2.89 – 2.64 (m, 3H), 2.12 – 2.05 (m, 1H), 1.40 (s, 9H); ^{13}C NMR (151 MHz,

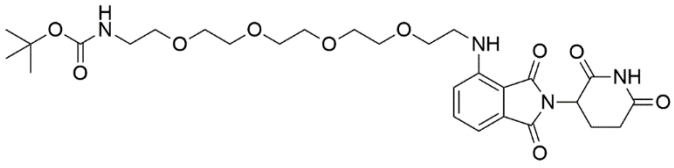
$CDCl_3$) δ 171.5, 169.4, 168.7, 167.7, 156.1, 146.9, 136.1, 132.6, 116.8, 111.7, 110.4, 79.3, 70.8, 70.4, 70.3, 70.2, 69.5, 49.0, 42.4, 40.5, 31.5, 28.5, 22.9; HRMS (ESI) *m/z* calculated for $[C_{24}H_{31}N_4O_8 - H]^-$ = 503.2147, found 503.2137.

tert-Butyl (2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)carbamate **(2i)**



Following **GP1**: Compound **2i** was isolated as a yellow film (236 mg, 0.48 mmol, 48%): R_f = 0.36 (EtOAc);^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 7.58 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 7.04 (d, *J* = 7.0 Hz, 1H), 6.73 (t, *J* = 5.9 Hz, 1H), 6.60 (t, *J* = 5.9 Hz, 1H), 5.05 (dd, *J* = 12.9, 5.4 Hz, 1H), 3.62 (t, *J* = 5.5 Hz, 2H), 3.59 – 3.44 (m, 10H), 3.35 (t, *J* = 6.1 Hz, 2H), 3.04 (q, *J* = 6.0 Hz, 2H), 2.88 (ddd, *J* = 17.1, 13.9, 5.4 Hz, 1H), 2.62 – 2.52 (m, 2H), 2.06 – 1.99 (m, 1H), 1.36 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.0, 168.9, 167.3, 155.5, 146.4, 136.2, 132.1, 117.4, 110.7, 109.2, 77.6, 69.8, 69.5, 69.1, 68.9, 48.5, 41.7, 31.0, 28.2, 22.1; HRMS (ESI) *m/z* calculated for $[C_{26}H_{36}N_4O_9 - H]^-$ = 547.2410, found 547.2395.

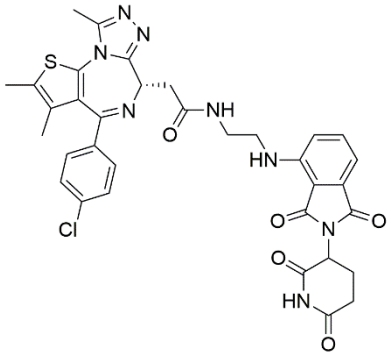
tert-Butyl (14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)carbamate **(2j)**



Following **GP1**: Compound **2j** was isolated as a yellow-green film (296 mg, 0.59 mmol, 59%): R_f = 0.24 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.65 (s, 1H), 7.46 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.07 (d, *J* = 7.1 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.47 (t, *J* = 5.7 Hz, 1H), 5.17 – 4.99 (m, 1H), 4.90 (dd, *J* = 12.3, 5.3 Hz, 1H),

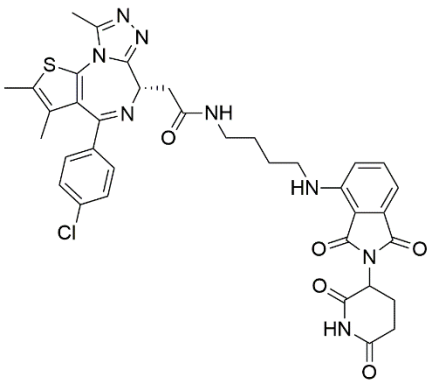
3.70 (t, *J* = 5.4 Hz, 2H), 3.67 – 3.56 (m, 12H), 3.51 (d, *J* = 5.3 Hz, 2H), 3.45 (q, *J* = 5.5 Hz, 2H), 3.28 (q, *J* = 5.3 Hz, 2H), 2.90 – 2.62 (m, 3H), 2.09 (ddd, *J* = 12.2, 6.1, 3.2 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 171.4, 169.3, 168.6, 167.7, 156.1, 146.9, 136.1, 132.6, 116.9, 111.7, 110.4, 79.2, 70.8, 70.7, 70.7, 70.5, 70.3, 69.6, 49.0, 42.5, 40.4, 31.5, 28.5, 22.9; HRMS (ESI) *m/z* calculated for $[C_{28}H_{40}N_4O_{10} + H]^+$ = 593.2817, found 593.2807.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)acetamide (4a)



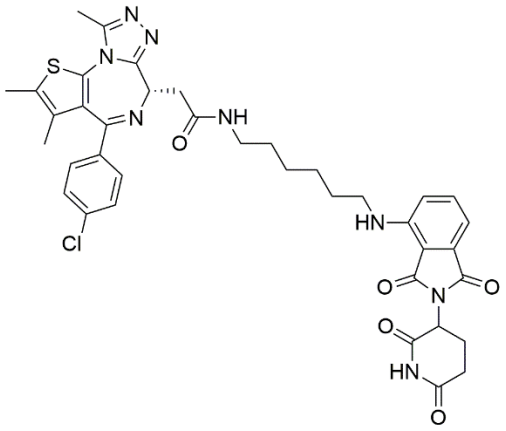
Following **GP2**: Compound **4a** was isolated as a yellow solid (48 mg, 0.07 mmol, 57%): R_f = 0.07 (EtOAc); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.53 – 8.44 (m, 1H), 7.59 (ddd, *J* = 9.0, 7.1, 2.5 Hz, 1H), 7.45 – 7.37 (m, 4H), 7.20 (d, *J* = 8.6 Hz, 1H), 7.06 – 7.01 (m, 1H), 6.78 (t, *J* = 5.9 Hz, 1H), 5.04 (ddd, *J* = 13.6, 8.6, 5.4 Hz, 1H), 4.52 (ddd, *J* = 7.9, 6.3, 2.2 Hz, 1H), 3.41 (dd, *J* = 8.6, 3.9 Hz, 2H), 3.28 – 3.18 (m, 2H), 2.92 – 2.80 (m, 1H), 2.59 (s, 4H), 2.41 (t, *J* = 1.2 Hz, 3H), 1.61 (dd, *J* = 5.6, 0.9 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.2, 170.0, 168.6, 167.3, 163.0, 155.0, 149.8, 146.3, 136.7, 136.2, 135.1, 132.2, 132.2, 130.6, 130.1, 130.1, 129.8, 129.5, 128.4, 117.2, 110.5, 109.3, 53.7, 48.5, 41.8, 38.2, 37.6, 30.9, 22.2, 14.0, 12.7, 11.3; HRMS (ESI) *m/z* calculated for $[C_{34}H_{31}ClN_8O_5S - H]^-$ = 697.1754, found 697.1730.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)acetamide (4b)



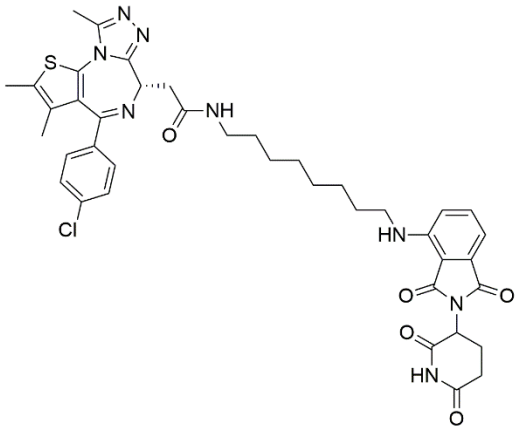
Following **GP2**: Compound **4b** was isolated as a yellow solid (51 mg, 0.070 mmol, 70%): R_f = 0.13 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.08 (s, 1H), 8.23 (t, *J* = 5.8 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.45 (dd, *J* = 8.9, 1.9 Hz, 2H), 7.40 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 7.1 Hz, 1H), 6.53 (d, *J* = 5.5 Hz, 1H), 5.04 (dt, *J* = 12.9, 4.7 Hz, 1H), 4.50 (dd, *J* = 8.2, 5.9 Hz, 1H), 3.35 – 3.05 (m, 6H), 2.92 – 2.81 (m, 1H), 2.63 – 2.51 (m, 5H), 2.40 (s, 3H), 2.05 – 1.96 (m, 1H), 1.64 – 1.56 (m, 5H), 1.56 – 1.48 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.0, 169.4, 168.9, 167.3, 163.0, 155.1, 149.8, 146.4, 136.7, 136.2, 135.2, 132.2, 132.2, 130.6, 130.1, 129.8, 129.5, 128.4, 117.2, 110.4, 109.0, 53.9, 48.5, 41.6, 38.1, 37.7, 31.0, 26.6, 26.1, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{36}H_{35}ClN_8O_5S + H]^+$ = 727.2212, found 727.2225.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)acetamide (4c)



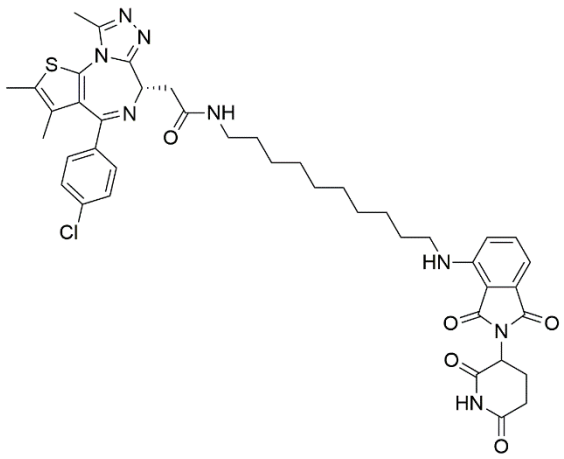
Following **GP2**: Compound **4c** was isolated as a yellow solid (88 mg, 0.11 mmol, 79%): R_f = 0.21 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.10 (br s, 1H), 8.18 (t, *J* = 5.8 Hz, 1H), 7.55 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.44 – 7.38 (m, 2H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.53 (t, *J* = 6.0 Hz, 1H), 5.05 (ddd, *J* = 12.8, 5.4, 1.2 Hz, 1H), 4.52 (dd, *J* = 8.1, 6.0 Hz, 1H), 3.31 – 3.02 (m, 6H), 2.94 – 2.81 (m, 1H), 2.63 – 2.52 (m, 5H), 2.39 (s, 3H), 2.07 – 1.97 (m, 1H), 1.60 (s, 3H), 1.56 (p, *J* = 7.1 Hz, 2H), 1.46 (h, *J* = 6.5 Hz, 2H), 1.42 – 1.29 (m, 4H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.0, 169.3, 168.9, 167.3, 162.9, 155.1, 149.7, 146.4, 136.7, 136.2, 135.2, 132.2, 132.2, 130.7, 130.0, 129.8, 129.5, 128.4, 117.1, 110.3, 109.0, 53.9, 48.5, 41.8, 38.4, 37.7, 31.0, 29.1, 28.7, 26.0, 26.0, 22.2, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{38}H_{39}ClN_8O_5S - H]^-$ = 753.2380, found 753.2369.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)acetamide (4d)



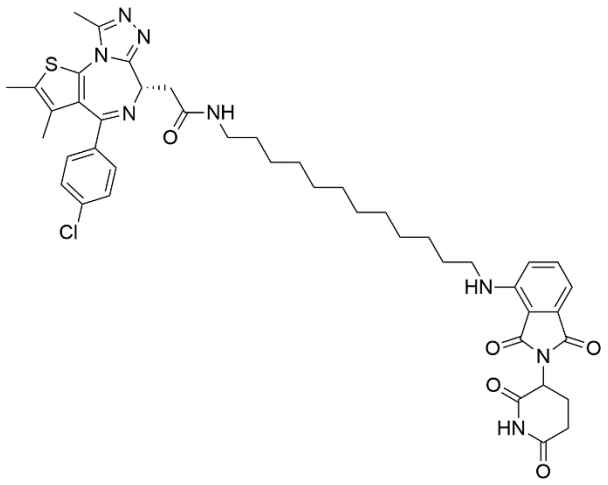
Following **GP2**: Compound **4d** was isolated as a yellow solid (61 mg, 0.078 mmol, 78%): R_f = 0.18 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.16 (t, *J* = 5.7 Hz, 1H), 7.56 (dd, *J* = 8.6, 7.0 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.51 (t, *J* = 5.9 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.50 (dd, *J* = 8.3, 5.8 Hz, 1H), 3.30 – 3.21 (m, 3H), 3.21 – 3.01 (m, 3H), 2.88 (ddd, *J* = 17.0, 13.8, 5.4 Hz, 1H), 2.64 – 2.52 (m, 5H), 2.39 (s, 3H), 2.07 – 1.95 (m, 1H), 1.61 (s, 3H), 1.54 (p, *J* = 7.3 Hz, 2H), 1.43 (q, *J* = 6.7 Hz, 2H), 1.36 – 1.20 (m, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.1, 169.3, 169.0, 167.3, 163.0, 155.1, 149.8, 146.4, 136.7, 136.3, 135.3, 132.3, 132.2, 130.7, 130.1, 129.8, 129.6, 128.4, 117.2, 110.4, 109.0, 53.9, 48.6, 41.8, 38.3, 37.7, 31.0, 29.2, 28.8, 28.8, 28.7, 26.4, 26.3, 22.2, 14.0, 12.7, 11.3; HRMS (ESI) *m/z* calculated for $[C_{40}H_{43}ClN_8O_5S - H]^-$ = 781.2693, found 781.2667.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(10-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)decyl)acetamide (4e)



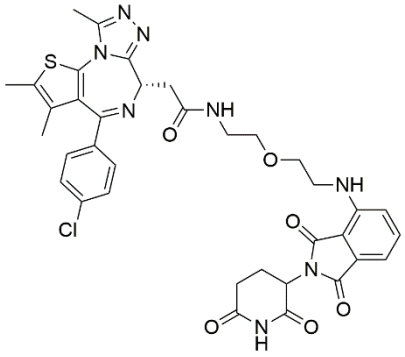
Following **GP2**: Compound **4e** was isolated as a yellow solid (60 mg, 0.074 mmol, 69%): R_f = 0.221 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.15 (t, *J* = 5.7 Hz, 1H), 7.56 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 7.0 Hz, 1H), 6.51 (t, *J* = 6.0 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.3 Hz, 1H), 4.50 (dd, *J* = 8.4, 5.7 Hz, 1H), 3.30 – 3.22 (m, 3H), 3.19 – 3.10 (m, 2H), 3.09 – 3.00 (m, 1H), 2.88 (ddd, *J* = 17.1, 13.9, 5.4 Hz, 1H), 2.63 – 2.51 (m, 5H), 2.40 (s, 3H), 2.02 (ddd, *J* = 12.4, 6.8, 4.2 Hz, 1H), 1.62 (s, 3H), 1.55 (p, *J* = 7.1 Hz, 2H), 1.47 – 1.39 (m, 2H), 1.33 – 1.20 (m, 12H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.0, 169.2, 168.9, 167.3, 162.9, 155.1, 149.7, 146.4, 136.7, 136.2, 135.2, 132.2, 132.2, 130.7, 130.1, 129.8, 129.5, 128.4, 117.1, 110.3, 109.0, 53.9, 48.5, 41.8, 38.3, 37.6, 31.0, 29.3, 29.0, 29.0, 28.8, 28.8, 28.7, 26.4, 26.3, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{42}H_{47}ClN_8O_5S + H]^+$ = 811.3151, found 811.3137. C

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(12-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)dodecyl)acetamide (4f)



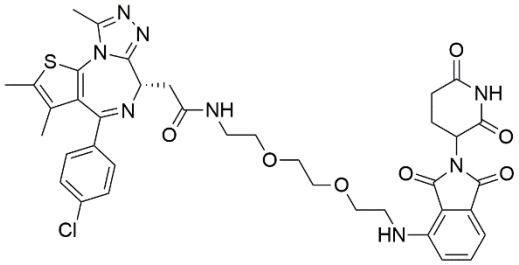
Following **GP2**: Compound **4f** was isolated as a yellow solid (87 mg, 0.10 mmol, 96%): R_f = 0.48 (6% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.10 (br s, 1H), 8.16 (t, *J* = 5.7 Hz, 1H), 7.56 (dd, *J* = 8.6, 7.0 Hz, 1H), 7.49 – 7.44 (m, 2H), 7.44 – 7.38 (m, 2H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 5.04 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.50 (dd, *J* = 8.5, 5.6 Hz, 1H), 3.30 – 3.21 (m, 3H), 3.15 (ddd, *J* = 13.1, 5.9, 2.6 Hz, 2H), 3.07 – 3.00 (m, 1H), 2.88 (ddd, *J* = 17.0, 13.9, 5.4 Hz, 1H), 2.58 (s, 5H), 2.42 – 2.34 (m, 3H), 2.02 (dtd, *J* = 13.0, 5.4, 2.3 Hz, 1H), 1.61 (s, 3H), 1.54 (p, *J* = 7.3 Hz, 2H), 1.42 (p, *J* = 6.7 Hz, 2H), 1.32 – 1.19 (m, 16H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.1, 169.4, 169.0, 167.3, 163.0, 155.2, 149.8, 146.4, 136.7, 136.3, 135.3, 132.3, 132.2, 130.7, 130.1, 129.8, 129.6, 128.4, 117.2, 110.4, 109.0, 54.0, 48.6, 41.9, 38.5, 37.7, 31.0, 29.3, 29.1, 29.0, 29.0, 28.9, 28.8, 28.7, 26.4, 26.3, 22.2, 14.1, 12.7, 11.3; HRMS (ESI) *m/z* calculated for $[C_{44}H_{51}ClN_8O_5S + H]^+$ = 839.3464, found 839.3448.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)acetamide (4g)



Following **GP2**: Compound **4g** was isolated as a yellow solid (42 mg, 0.057 mmol, 50%): R_f = 0.22 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, *J* = 2.7 Hz, 1H), 8.31 – 8.18 (m, 1H), 7.61 – 7.54 (m, 1H), 7.49 – 7.44 (m, 2H), 7.42 (dd, *J* = 8.6, 1.2 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.64 (t, *J* = 5.8 Hz, 1H), 5.05 (ddd, *J* = 12.8, 5.4, 2.1 Hz, 1H), 4.51 (ddd, *J* = 8.3, 5.9, 1.0 Hz, 1H), 3.70 – 3.60 (m, 2H), 3.56 – 3.44 (m, 4H), 3.32 – 3.18 (m, 4H), 2.93 – 2.81 (m, 1H), 2.62 – 2.52 (m, 5H), 2.40 (s, 3H), 2.05 – 1.97 (m, 1H), 1.61 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.0, 169.7, 168.9, 167.2, 162.9, 155.1, 149.8, 146.4, 136.7, 136.2, 135.2, 132.2, 132.1, 130.7, 130.1, 129.8, 129.5, 128.4, 117.4, 110.7, 109.3, 69.0, 68.5, 53.8, 48.5, 41.7, 38.5, 37.5, 30.9, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{36}H_{35}ClN_8O_6S + H]^+$ = 743.2162, found 743.2154.

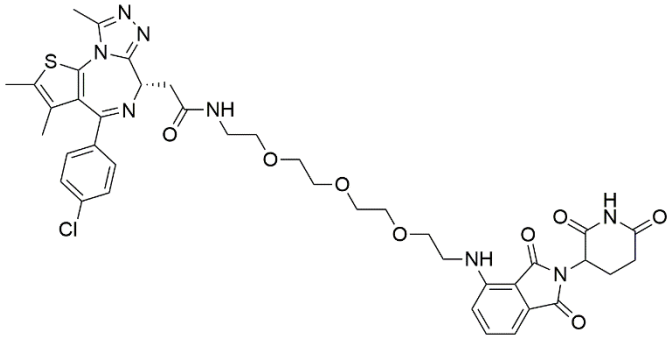
2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)acetamide (4h)



Following **GP2**: Compound **4h** was isolated as a yellow solid (31 mg, 0.039 mmol, 35%): R_f = 0.24 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.26 (t, *J* = 5.6 Hz, 1H), 7.60 – 7.53

(m, 1H), 7.50 – 7.45 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.6 Hz, 1H), 7.02 (dd, *J* = 7.2, 1.0 Hz, 1H), 6.61 (t, *J* = 5.8 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.50 (dd, *J* = 8.1, 6.1 Hz, 1H), 3.63 (t, *J* = 5.5 Hz, 2H), 3.61 – 3.54 (m, 4H), 3.49 – 3.44 (m, 4H), 3.32 – 3.17 (m, 4H), 2.92 – 2.81 (m, 1H), 2.62 – 2.52 (m, 5H), 2.40 (s, 3H), 2.06 – 1.96 (m, 1H), 1.61 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.0, 168.9, 167.2, 162.9, 155.1, 149.7, 146.4, 136.7, 136.2, 135.2, 132.2, 132.1, 130.6, 130.1, 129.8, 129.5, 128.4, 117.4, 110.6, 109.2, 69.7, 69.6, 69.2, 68.9, 53.8, 48.5, 41.7, 38.6, 37.5, 30.9, 22.1, 14.0, 12.6, 11.3. c $[C_{38}H_{39}ClN_8O_7S + H]^+$ = 787.2424, found 787.2414.

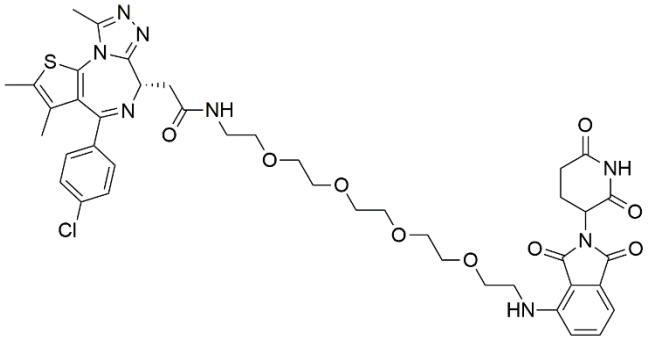
2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)acetamide (4i)



Following **GP2**: Compound **4i** was isolated as a yellow solid (72 mg, 0.087 mmol, 90%): R_f = 0.23 (5% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.27 (t, *J* = 5.7 Hz, 1H), 7.56 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.13 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.60 (t, *J* = 5.9 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.50 (dd, *J* = 8.1, 6.0 Hz, 1H), 3.62 (t, *J* = 5.5 Hz, 2H), 3.58 – 3.50 (m, 9H), 3.48 – 3.42 (m, 4H), 3.32 – 3.16 (m, 4H), 2.88 (ddd, *J* = 17.0, 13.8, 5.5 Hz, 1H), 2.62 – 2.51 (m, 5H), 2.40 (s, 3H), 2.05 – 1.98 (m, 1H), 1.61 (s, 3H); ^{13}C NMR (151 MHz, DMSO) δ = 172.8, 170.0, 169.7, 168.9, 167.3, 163.0, 155.1, 149.8, 146.4, 136.8, 136.2, 135.2, 132.3, 132.1, 130.7, 130.1, 129.8, 129.5, 128.4, 117.4, 110.6, 109.2, 69.8, 69.8, 69.6, 69.2, 68.9, 53.8, 48.6, 41.7, 38.6,

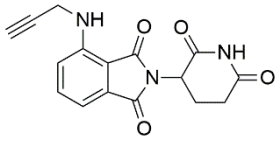
37.5, 31.0, 22.1, 14.0, 12.7, 11.3; HRMS (ESI) *m/z* calculated for $[C_{40}H43ClN_8O_8S + H]^+$ =831.2686, found 831.2672.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)acetamide (4j)



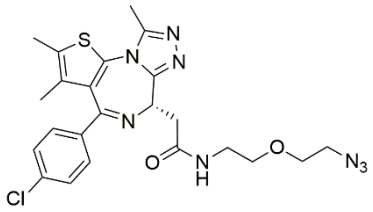
Following **GP2**: Compound **4j** was isolated as a yellow solid (74 mg, 0.084 mmol, 82%): R_f = 0.50 (6% MeOH in DCM); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.10 (br s, 1H), 8.27 (t, *J* = 5.6 Hz, 1H), 7.57 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.50 – 7.46 (m, 2H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.13 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.60 (t, *J* = 5.8 Hz, 1H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.51 (dd, *J* = 8.2, 5.9 Hz, 1H), 3.61 (t, *J* = 5.5 Hz, 2H), 3.58 – 3.48 (m, 13H), 3.48 – 3.42 (m, 4H), 3.32 – 3.17 (m, 4H), 2.93 – 2.82 (m, 1H), 2.62 – 2.51 (m, 5H), 2.40 (s, 3H), 2.07 – 1.97 (m, 1H), 1.61 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.0, 169.6, 168.9, 167.2, 163.0, 155.1, 149.7, 146.4, 136.7, 136.2, 135.2, 132.2, 132.1, 130.6, 130.1, 129.8, 129.5, 128.4, 117.4, 110.6, 69.8, 69.8, 69.8, 69.7, 69.6, 69.2, 68.9, 53.8, 48.5, 41.7, 38.6, 37.5, 31.0, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{42}H_{47}ClN_8O_9S + H]^+$ = 875.2948, found 875.2953.

2-(2,6-Dioxopiperidin-3-yl)-4-(prop-2-yn-1-ylamino)isoindoline-1,3-dione (5)



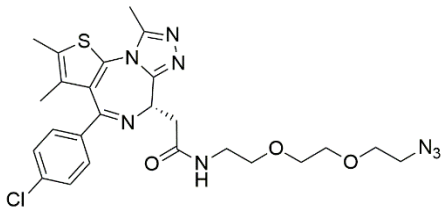
Following **GP1**: Compound **5** was isolated as a yellow solid, (0.117 g, 0.38 mmol, 84%): R_f = 0.37 (50% EtOAc in hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.65 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.93 (t, *J* = 6.2 Hz, 1H), 5.07 (dd, *J* = 12.9, 5.5 Hz, 1H), 4.17 (dd, *J* = 6.2, 2.5 Hz, 2H), 3.17 (t, *J* = 2.4 Hz, 1H), 2.88 (ddd, *J* = 17.1, 14.0, 5.5 Hz, 1H), 2.63 – 2.51 (m, 2H), 2.04 (dtd, *J* = 13.0, 5.4, 2.4 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 172.7, 170.0, 168.5, 167.2, 145.2, 136.0, 132.1, 117.9, 111.3, 110.3, 80.9, 73.7, 48.6, 31.5, 30.9, 22.1; HRMS (ESI) *m/z* calculated for $[C_{16}H_{12}N_3O_4 - H]^-$ = 310.0833, found 310.0827.

(S)-N-(2-(2-Azidoethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide (6a)



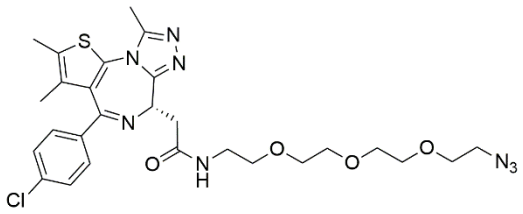
Following **GP3**: Compound **6a** was isolated as a yellow solid (44 mg, 0.086 mmol, 80%): R_f = 0.22 (10% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44 – 7.36 (m, 2H), 7.34 – 7.29 (m, 2H), 6.79 (t, *J* = 5.7 Hz, 1H), 4.62 (t, *J* = 6.9 Hz, 1H), 3.68 – 3.63 (m, 2H), 3.63 – 3.47 (m, 5H), 3.41 – 3.33 (m, 3H), 2.65 (s, 3H), 2.38 (d, *J* = 1.0 Hz, 3H), 1.66 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7, 163.9, 155.8, 149.9, 136.8, 136.7, 132.3, 131.0, 130.8, 130.5, 129.9, 128.8, 70.1, 69.9, 54.5, 50.7, 39.5, 39.3, 14.5, 13.2, 11.9; HRMS (ESI) *m/z* calculated for $[C_{23}H_{25}ClN_8O_2S + H]^+$ = 513.1582, found 513.1583.

(S)-N-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide (6b)



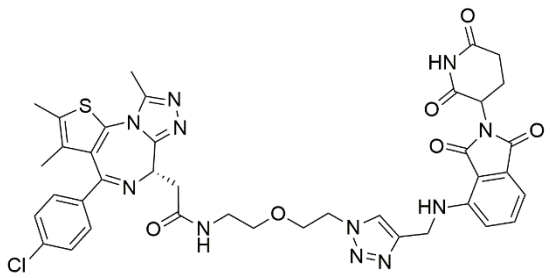
Following **GP3**: Compound **6b** was isolated as a yellow solid (54 mg, 0.097 mmol, 91%): R_f = 0.22 (10% MeOH in $CHCl_3$); ^{1}H NMR (401 MHz, $CDCl_3$) δ = 7.43 – 7.35 (m, 2H), 7.35 – 7.29 (m, 2H), 6.79 (t, *J* = 5.4 Hz, 1H), 4.63 (t, *J* = 7.0 Hz, 1H), 3.72 – 3.63 (m, 6H), 3.62 – 3.44 (m, 5H), 3.42 – 3.34 (m, 3H), 2.65 (s, 3H), 2.38 (s, 1H), 1.65 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 170.7, 164.0, 155.7, 149.9, 136.9, 136.7, 132.2, 131.0, 130.9, 130.6, 129.9, 128.8, 70.7, 70.5, 70.2, 69.9, 54.5, 50.8, 39.5, 39.3, 14.5, 13.2, 11.9; HRMS (ESI) *m/z* calculated for $[C_{25}H_{29}ClN_8O_3S + H]^+$ = 557.1845, found 557.1850.

(S)-N-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide (6c)



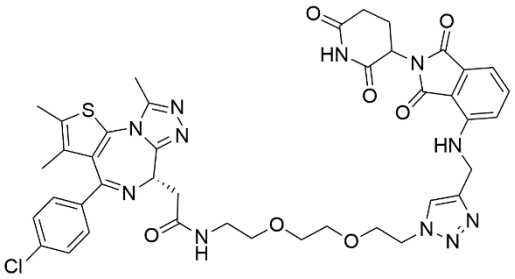
Following **GP3**: Compound **6c** was isolated as a yellow solid (64 mg, 0.11 mmol, 83%): R_f = 0.22 (10% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43 – 7.37 (m, 2H), 7.36 – 7.29 (m, 2H), 6.85 – 6.74 (m, 1H), 4.64 (t, *J* = 7.0 Hz, 1H), 3.71 – 3.64 (m, 10H), 3.63 – 3.43 (m, 5H), 3.42 – 3.32 (m, 3H), 2.66 (s, 3H), 2.39 (s, 2H), 1.66 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.7, 164.0, 155.8, 150.0, 136.9, 136.8, 132.2, 131.0, 130.9, 130.6, 130.0, 128.8, 70.8, 70.8, 70.8, 70.5, 70.2, 69.9, 54.5, 50.8, 39.6, 39.3, 14.5, 13.2, 11.9; HRMS (ESI) *m/z* calculated for $[C_{27}H_{33}ClN_8O_4S + Na]^+$ = 623.1926, found 623.1921.

2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-(4-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethyl)acetamide (7a)



Following **GP4**: Compound **7a** was isolated as a yellow solid (55 mg, 0.067 mmol, 77%): R_f = 0.42 (10% MeOH in $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.23 (t, *J* = 5.6 Hz, 1H), 8.04 (br s, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.3 Hz, 2H), 7.16 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.03 (dd, *J* = 6.9, 4.3 Hz, 2H), 5.05 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.58 (d, *J* = 6.1 Hz, 2H), 4.51 (dd, *J* = 6.6, 4.0 Hz, 3H), 3.80 (t, *J* = 5.5 Hz, 2H), 3.43 (t, *J* = 5.8 Hz, 2H), 3.29 – 3.16 (m, 4H), 2.94 – 2.79 (m, 1H), 2.59 (s, 5H), 2.40 (s, 3H), 2.06 – 1.94 (m, 1H), 1.61 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.0, 169.6, 168.7, 167.2, 163.0, 155.1, 149.8, 145.8, 144.3, 136.7, 136.1, 135.2, 132.2, 132.1, 130.6, 130.1, 129.8, 129.5, 128.4, 123.3, 117.6, 110.9, 109.7, 68.9, 68.5, 53.8, 49.3, 48.5, 38.4, 37.6, 37.5, 30.9, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{39}H_{38}ClN_{11}O_6S + Na]^+$ = 846.2308, found 843.2316.

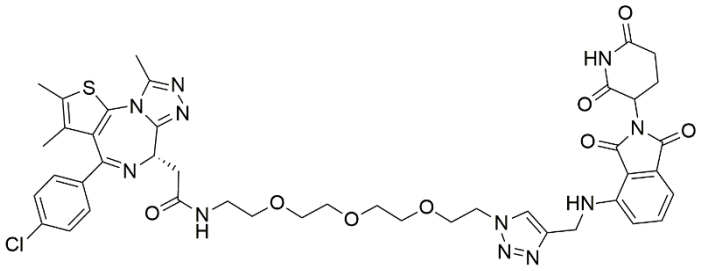
2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-(2-(4-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethyl)acetamide (7b)



Following **GP4**: Compound **7b** was isolated as a yellow solid (70 mg, 0.81 mmol, 81%): R_f = 0.23 (10% MeOH in $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.26 (td, *J* = 5.8, 2.7 Hz, 1H), 7.99

(s, 1H), 7.54 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.50 – 7.45 (m, 2H), 7.45 – 7.37 (m, 2H), 7.19 – 7.13 (m, 1H), 7.07 (t, *J* = 6.1 Hz, 1H), 7.03 (d, *J* = 7.1 Hz, 1H), 5.04 (ddd, *J* = 12.9, 5.5, 2.3 Hz, 1H), 4.58 (d, *J* = 6.1 Hz, 2H), 4.54 – 4.45 (m, 3H), 3.81 – 3.73 (m, 2H), 3.53 – 3.44 (m, 4H), 3.40 (td, *J* = 6.0, 1.5 Hz, 2H), 3.29 – 3.17 (m, 4H), 2.87 (ddd, *J* = 17.0, 13.9, 5.4 Hz, 1H), 2.58 (s, 3H), 2.38 (dd, *J* = 4.1, 0.9 Hz, 3H), 2.06 – 1.95 (m, 1H), 1.59 (dd, *J* = 3.5, 1.0 Hz, 3H).; ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.8, 170.0, 169.7, 168.7, 167.2, 163.0, 155.1, 149.8, 145.8, 144.3, 136.7, 136.1, 135.2, 132.2, 132.1, 130.7, 130.1, 129.8, 129.5, 128.4, 123.3, 117.6, 110.9, 109.6, 69.5, 69.4, 69.1, 68.7, 53.8, 49.4, 48.5, 38.6, 37.6, 37.5, 31.0, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{41}H_{42}ClN_{11}O_7S + H]^+$ = 868.2751, found 868.2788.

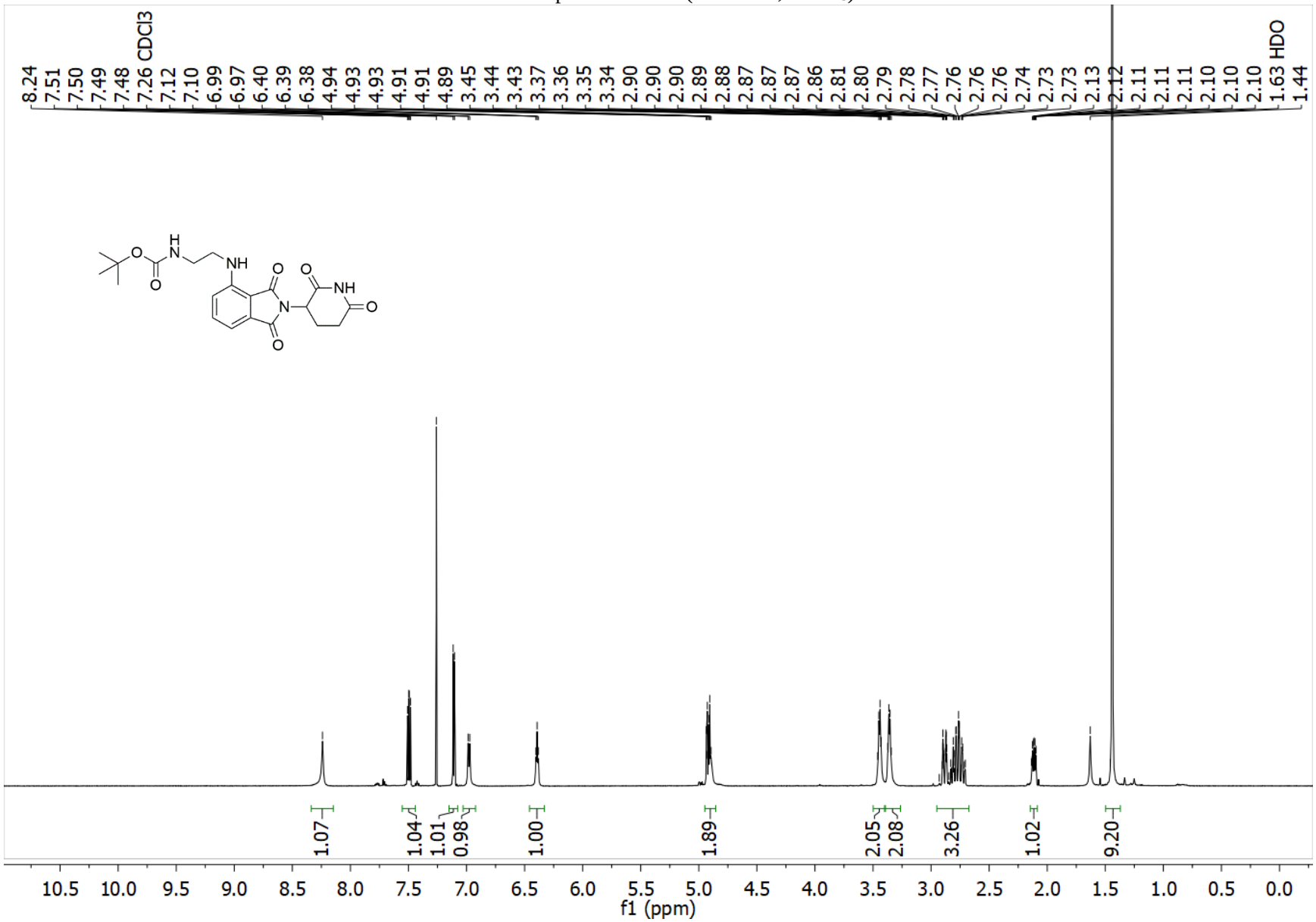
2-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-(2-(2-(4-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)acetamide (7c)



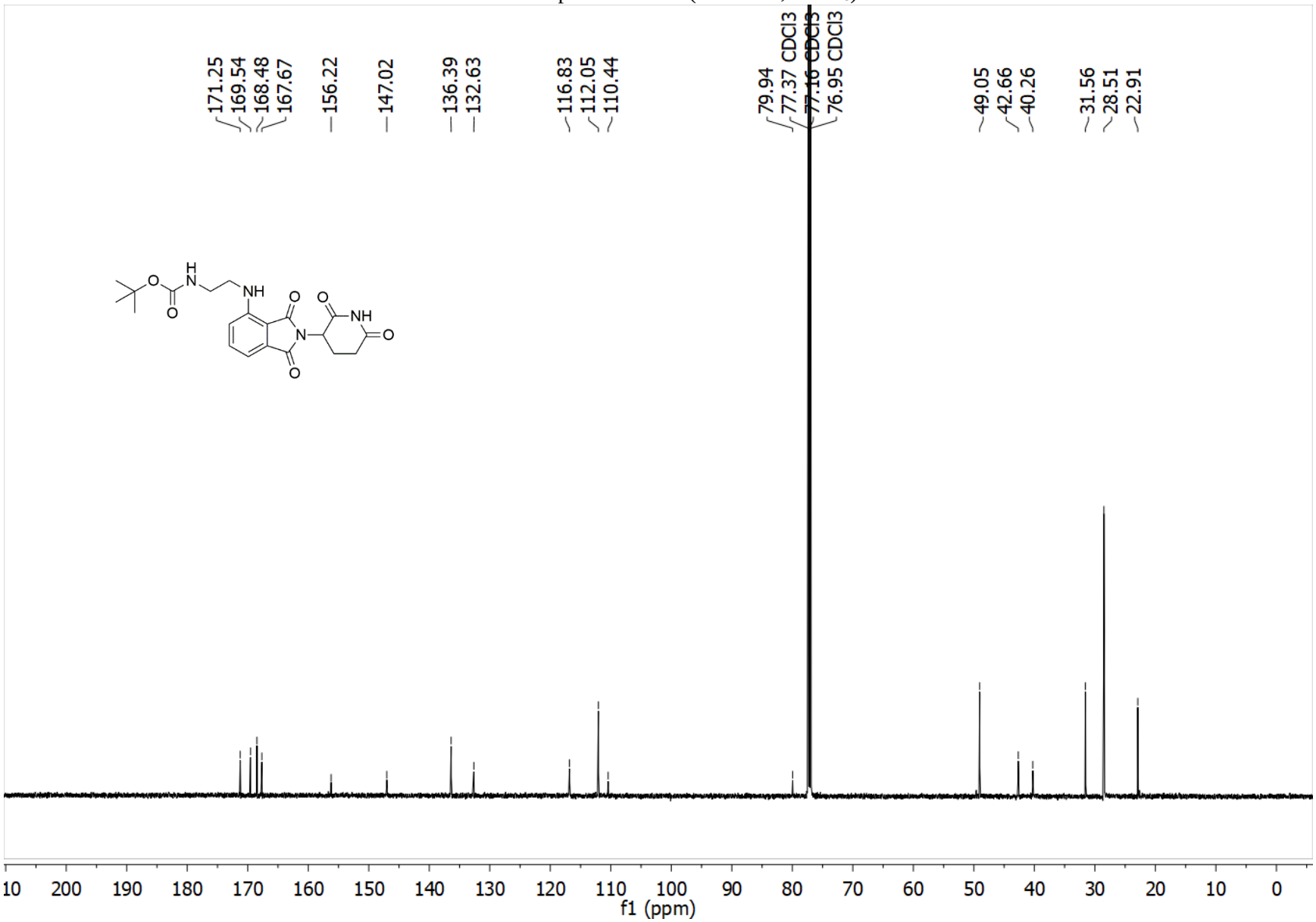
Following **GP4**: Compound **7c** was isolated as a yellow solid (81 mg, 0.089 mmol, 82%): R_f = 0.20 (MeOH in $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.09 (br s, 1H), 8.26 (t, *J* = 5.7 Hz, 1H), 7.99 (br s, 1H), 7.56 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.10 – 7.01 (m, 2H), 5.05 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.58 (d, *J* = 6.1 Hz, 2H), 4.53 – 4.45 (m, 3H), 3.78 (t, *J* = 5.3 Hz, 2H), 3.53 – 3.38 (m, 10H), 3.30 – 3.17 (m, 4H), 2.88 (ddd, *J* = 17.0, 13.8, 5.4 Hz, 1H), 2.63 – 2.51 (m, 5H), 2.40 (s, 3H), 2.01 (ddd, *J* = 13.1, 6.2, 3.2 Hz, 1H), 1.61 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.7, 170.0, 169.6, 168.7, 167.2, 163.0, 155.1, 145.8, 144.3, 136.7, 136.1, 135.2, 132.1, 132.1, 130.6, 130.1, 129.8, 129.5, 128.4, 123.2, 117.6, 110.9, 109.7, 79.1, 69.7, 69.6, 69.6, 69.5, 69.1, 68.7, 53.8,

49.4, 48.5, 38.6, 37.6, 30.9, 22.1, 14.0, 12.6, 11.3; HRMS (ESI) *m/z* calculated for $[C_{43}H_{46}ClN_{11}O_8S + Na]^+$ = 934.2832, found 934.2846.

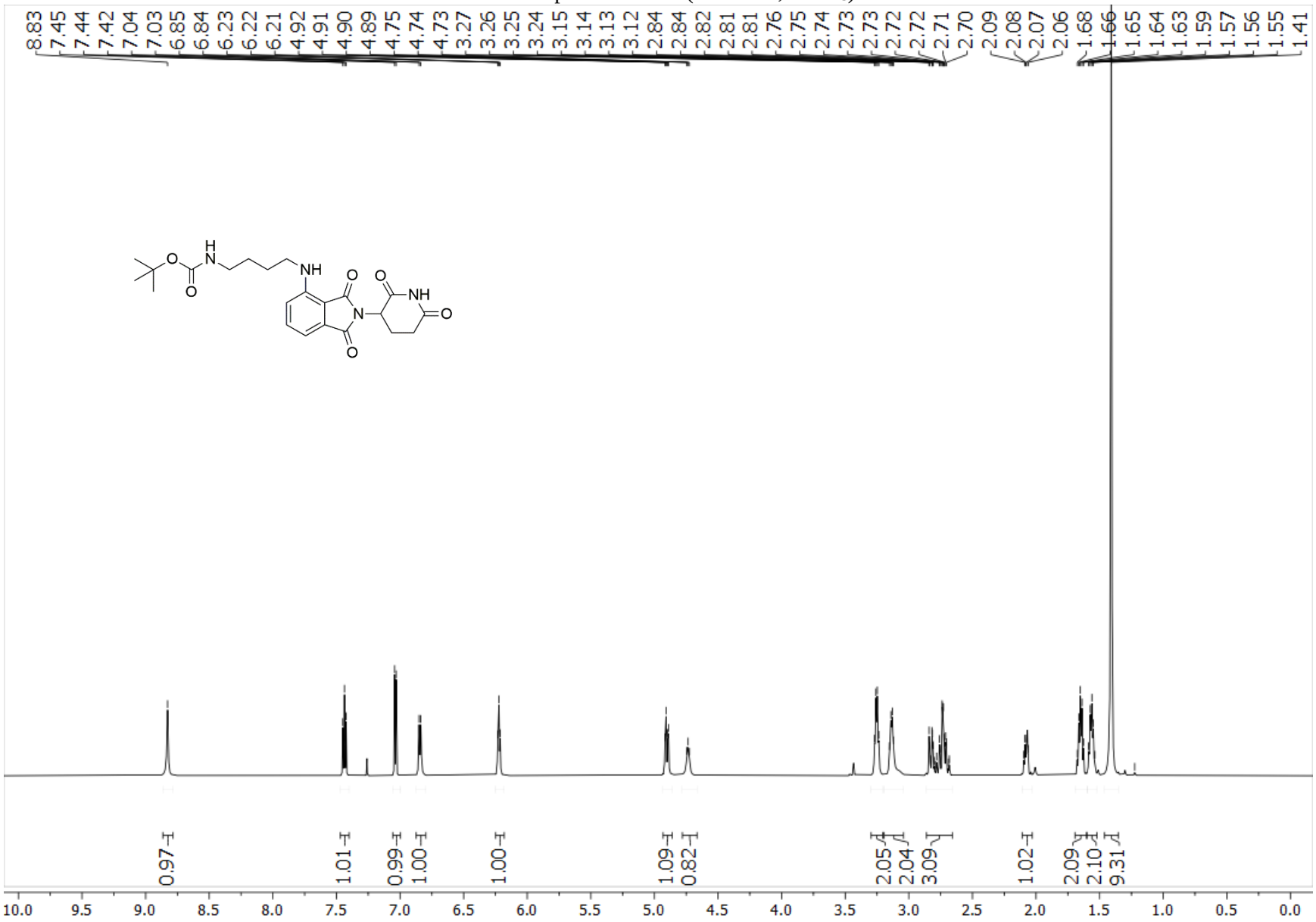
^{1}H NMR spectrum of **2a** (400 MHz, $CDCl_3$)



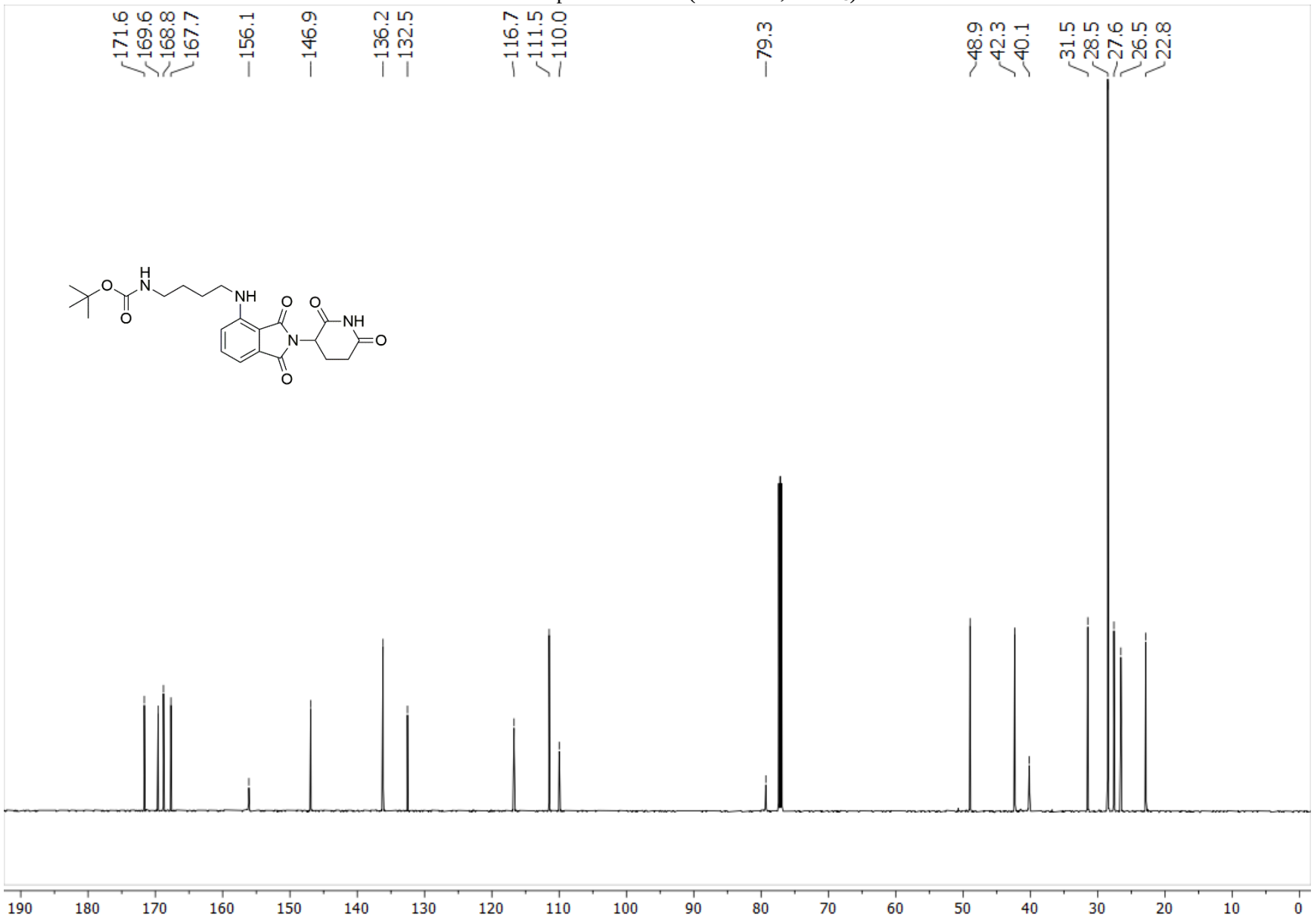
^{13}C NMR spectrum of **2a** (400 MHz, $CDCl_3$)



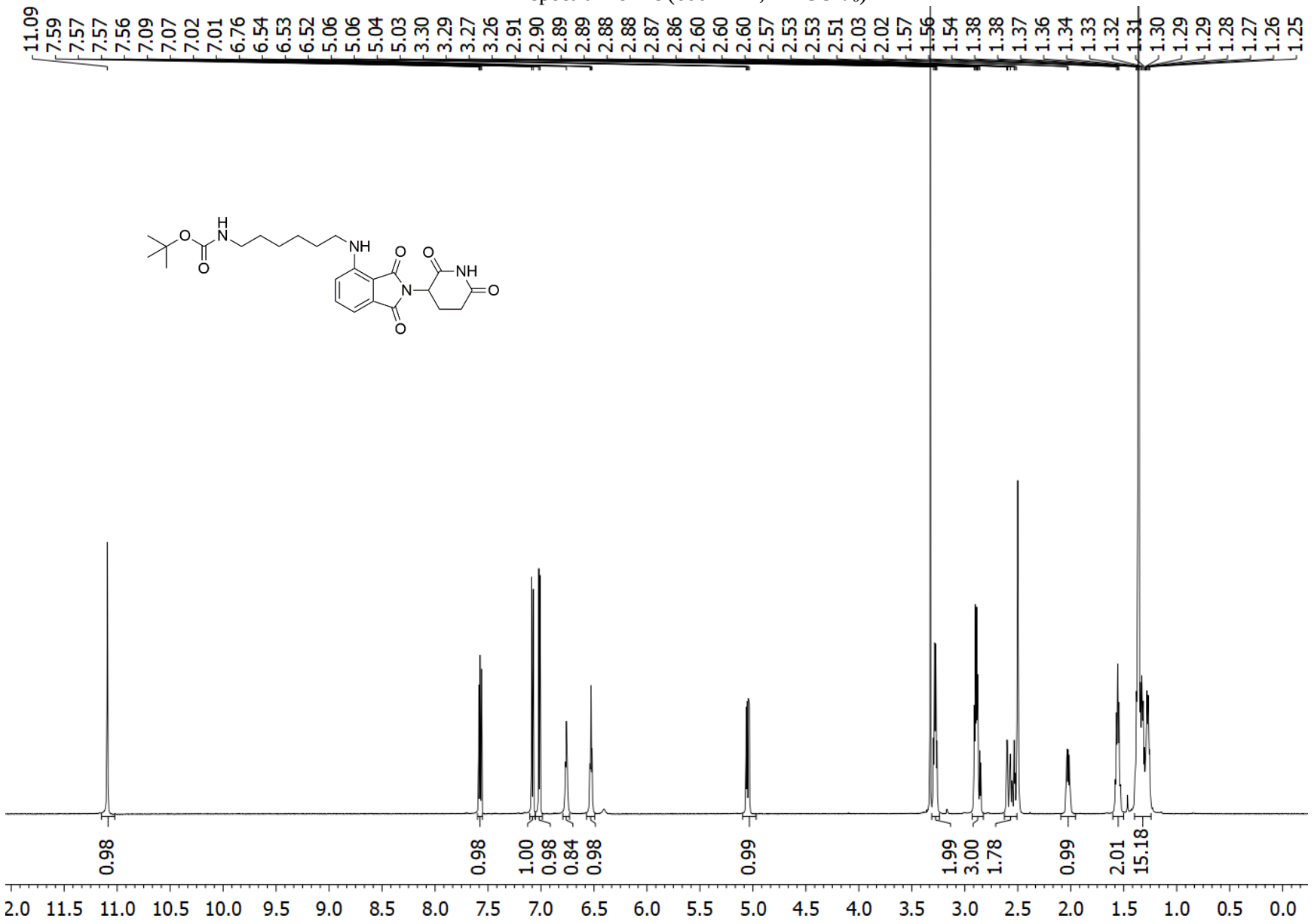
^{1}H NMR spectrum of **2b** (600 MHz, $CDCl_3$)



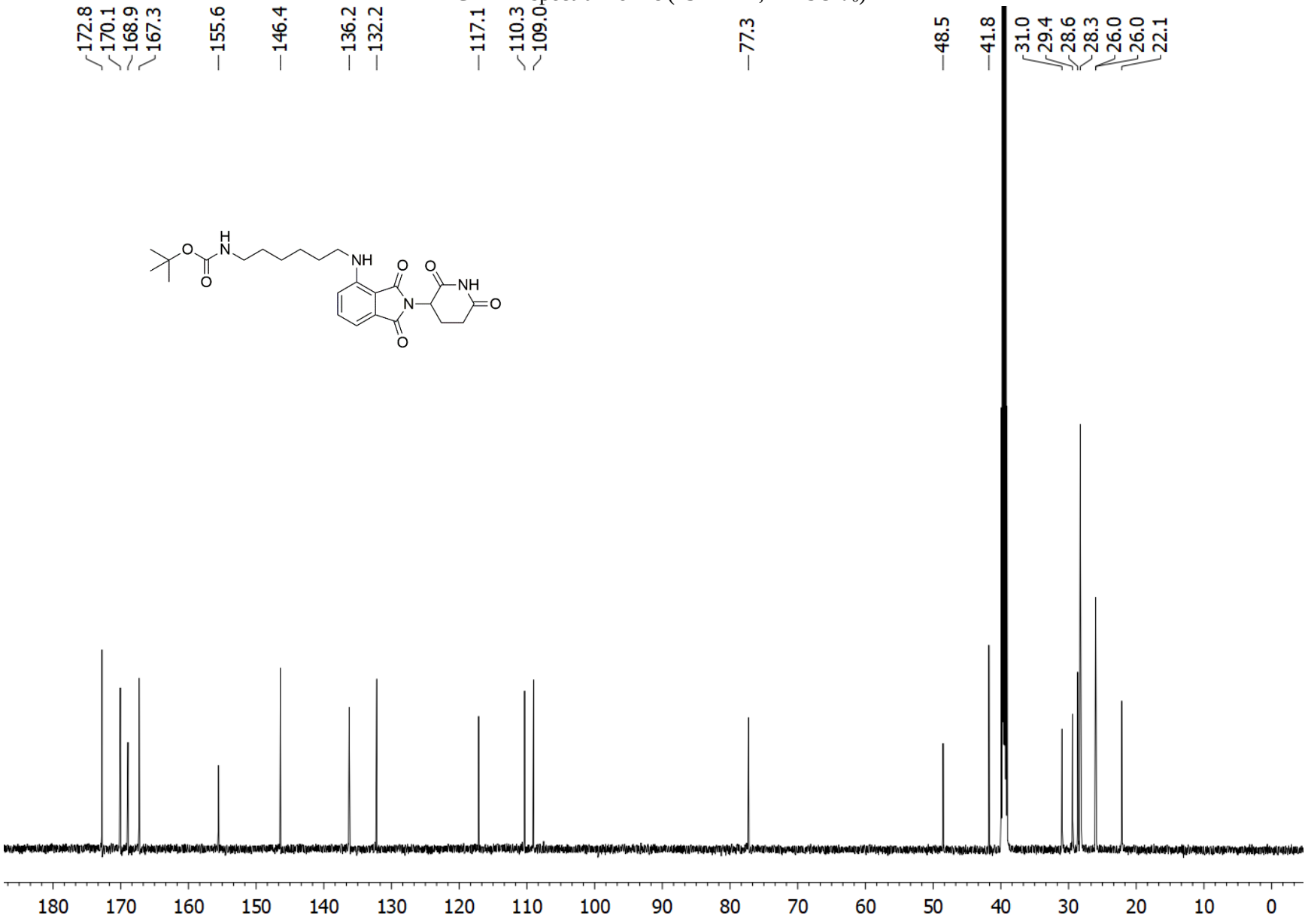
^{13}C NMR spectrum of **2b** (151 MHz, $CDCl_3$)



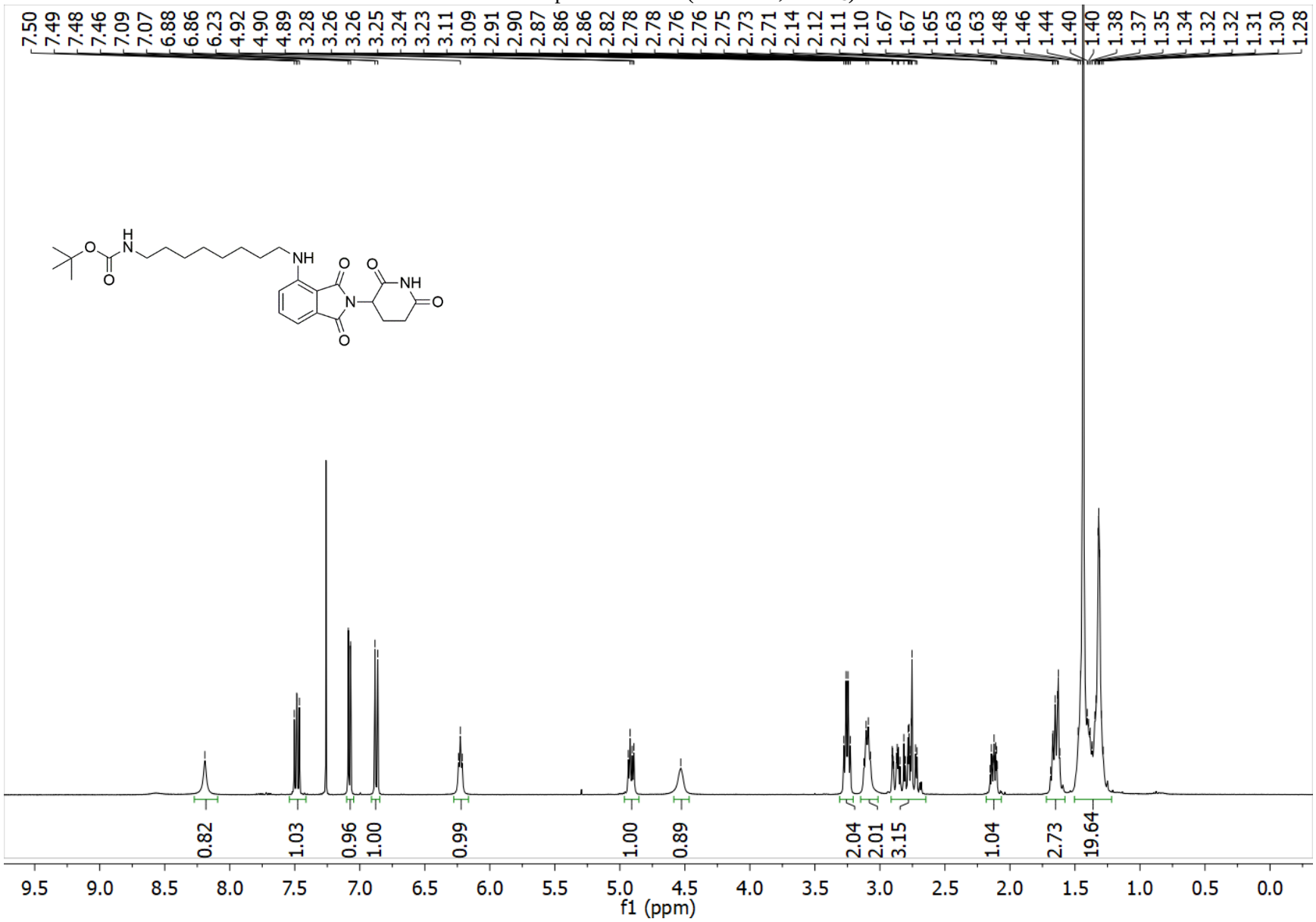
^{1}H NMR spectrum of **2c** (600 MHz, DMSO-d_6)



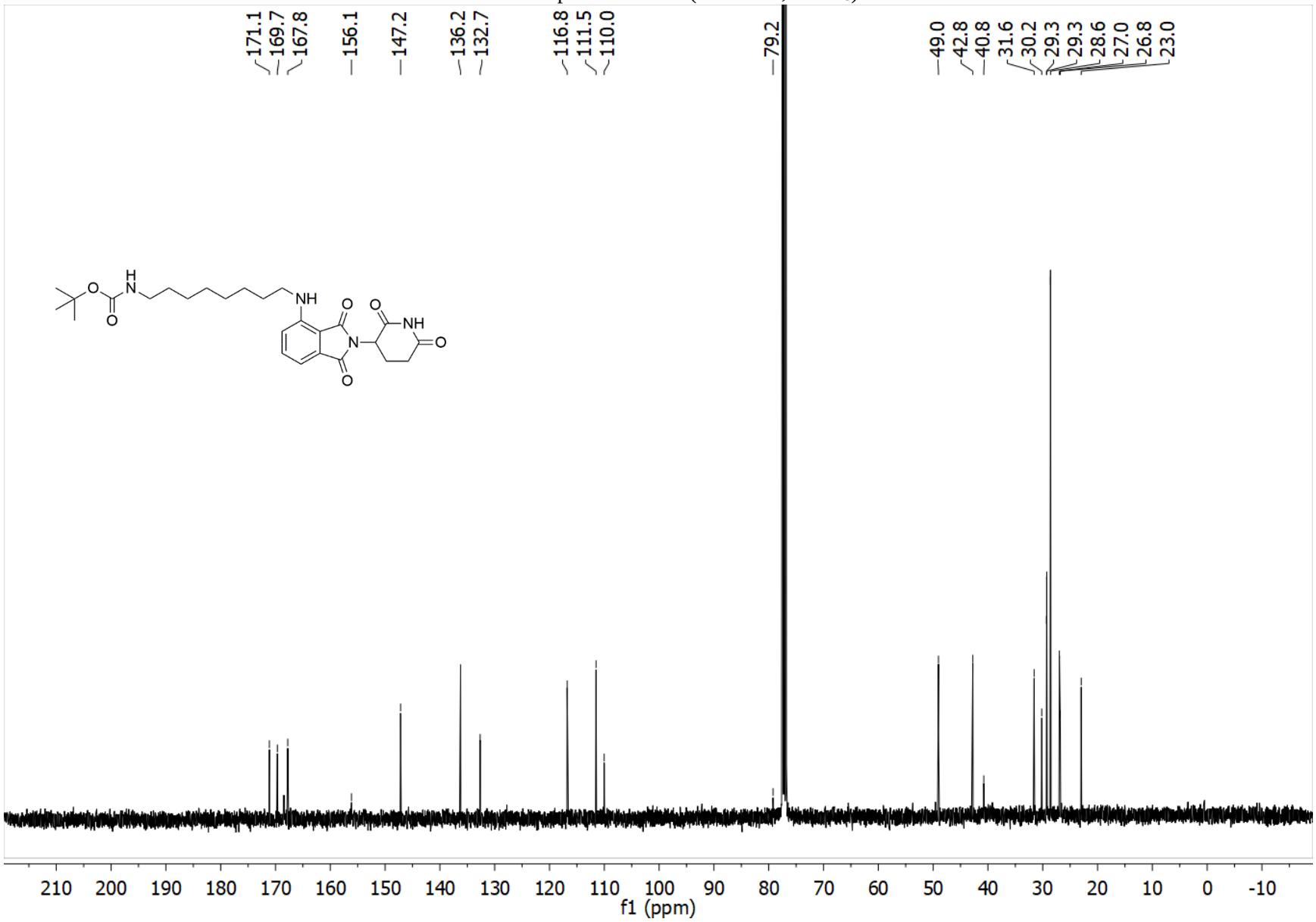
^{13}C NMR spectrum of **2c** (151 MHz, DMSO-d_6)



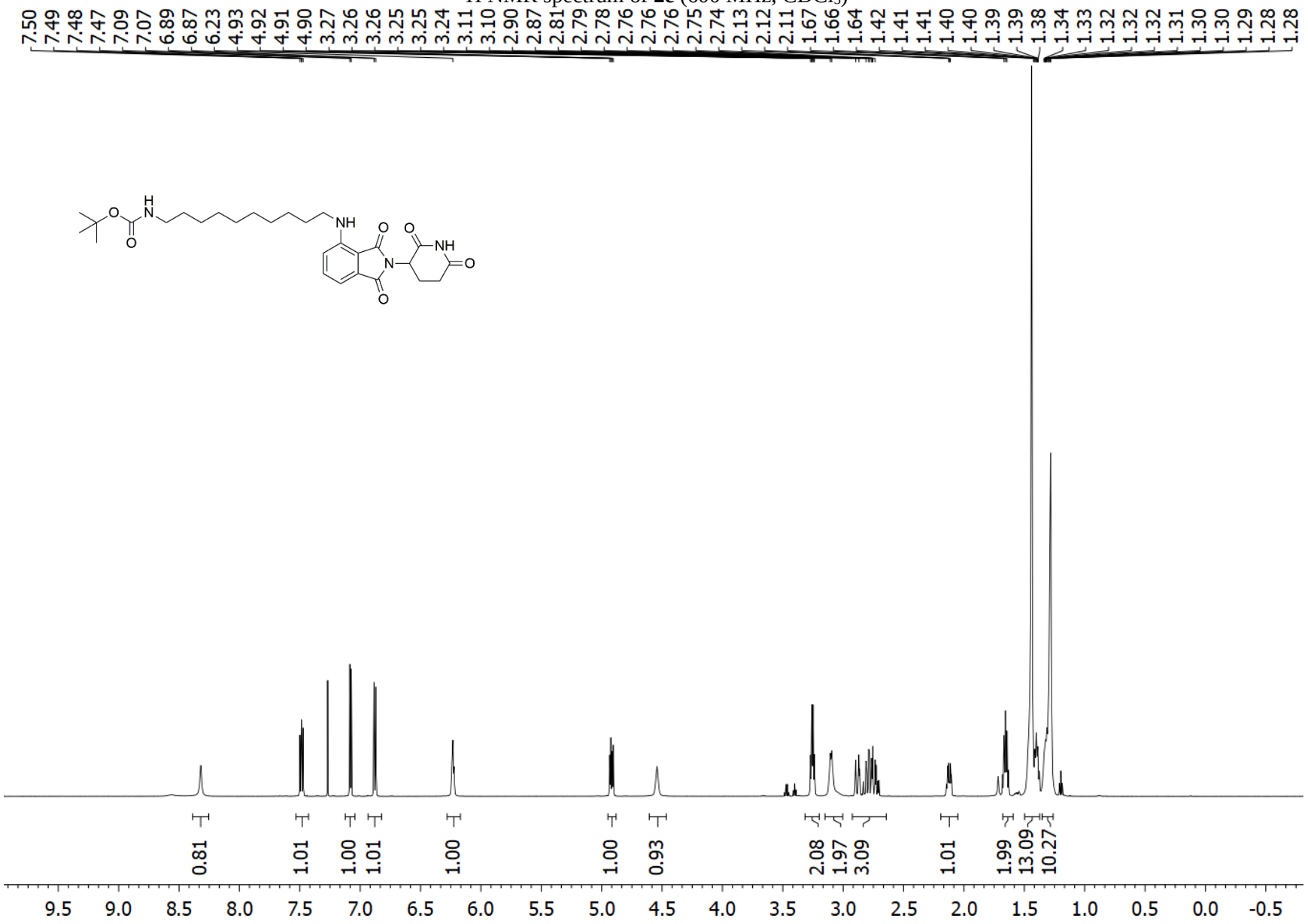
^{1}H NMR spectrum of **2d** (400 MHz, $CDCl_3$)



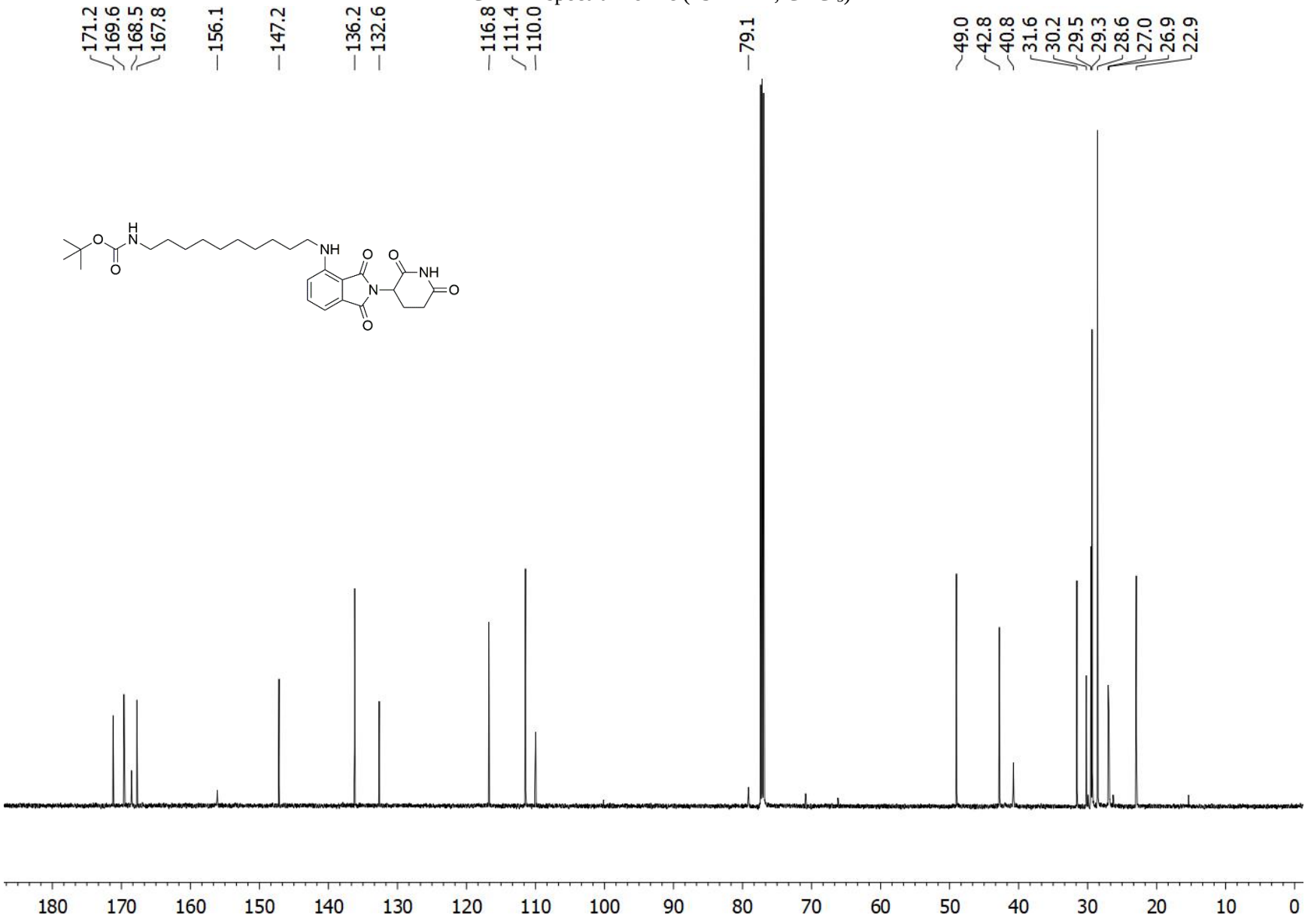
^{13}C NMR spectrum of **2d** (400 MHz, $CDCl_3$)



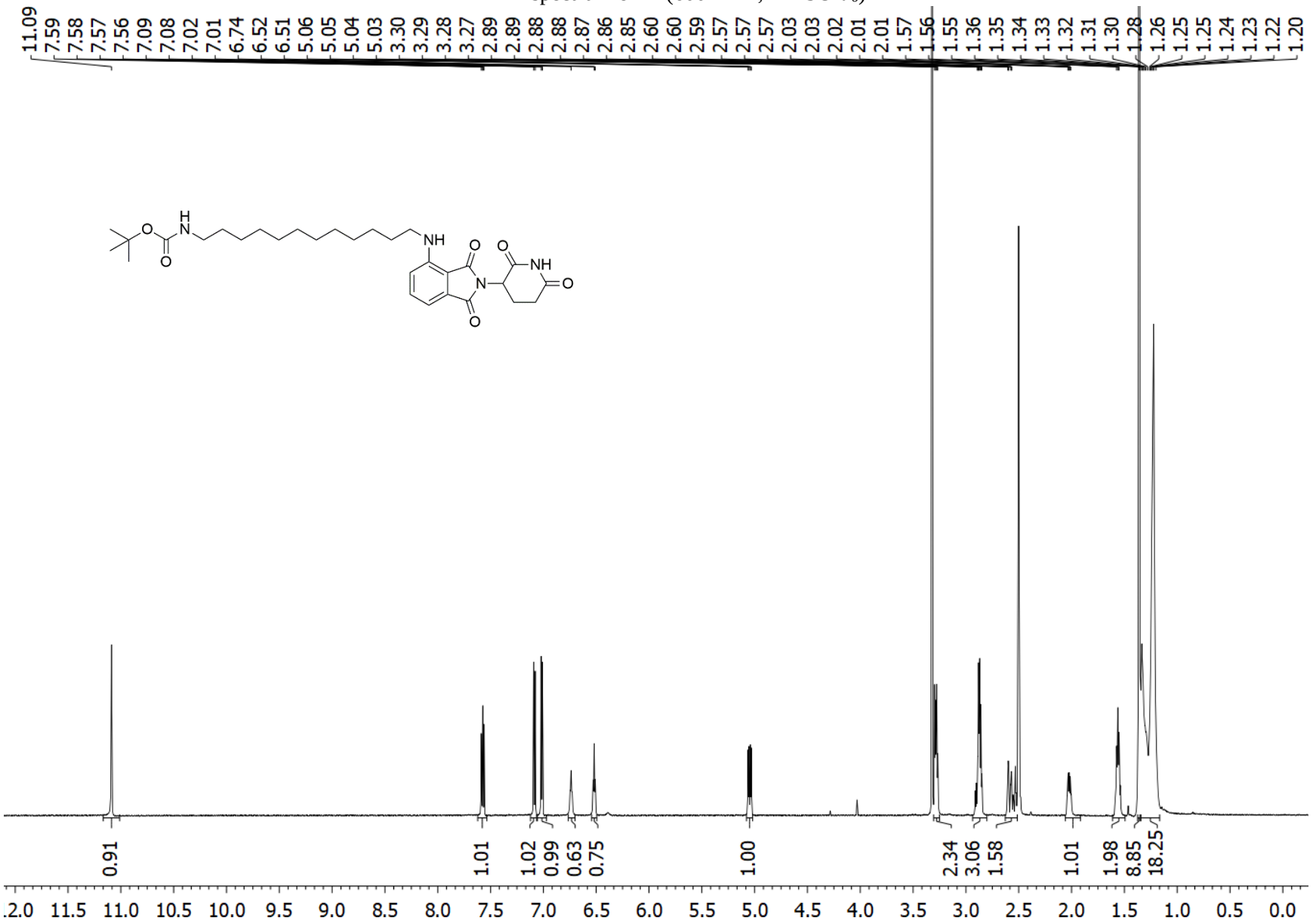
^{1}H NMR spectrum of **2e** (600 MHz, $CDCl_3$)



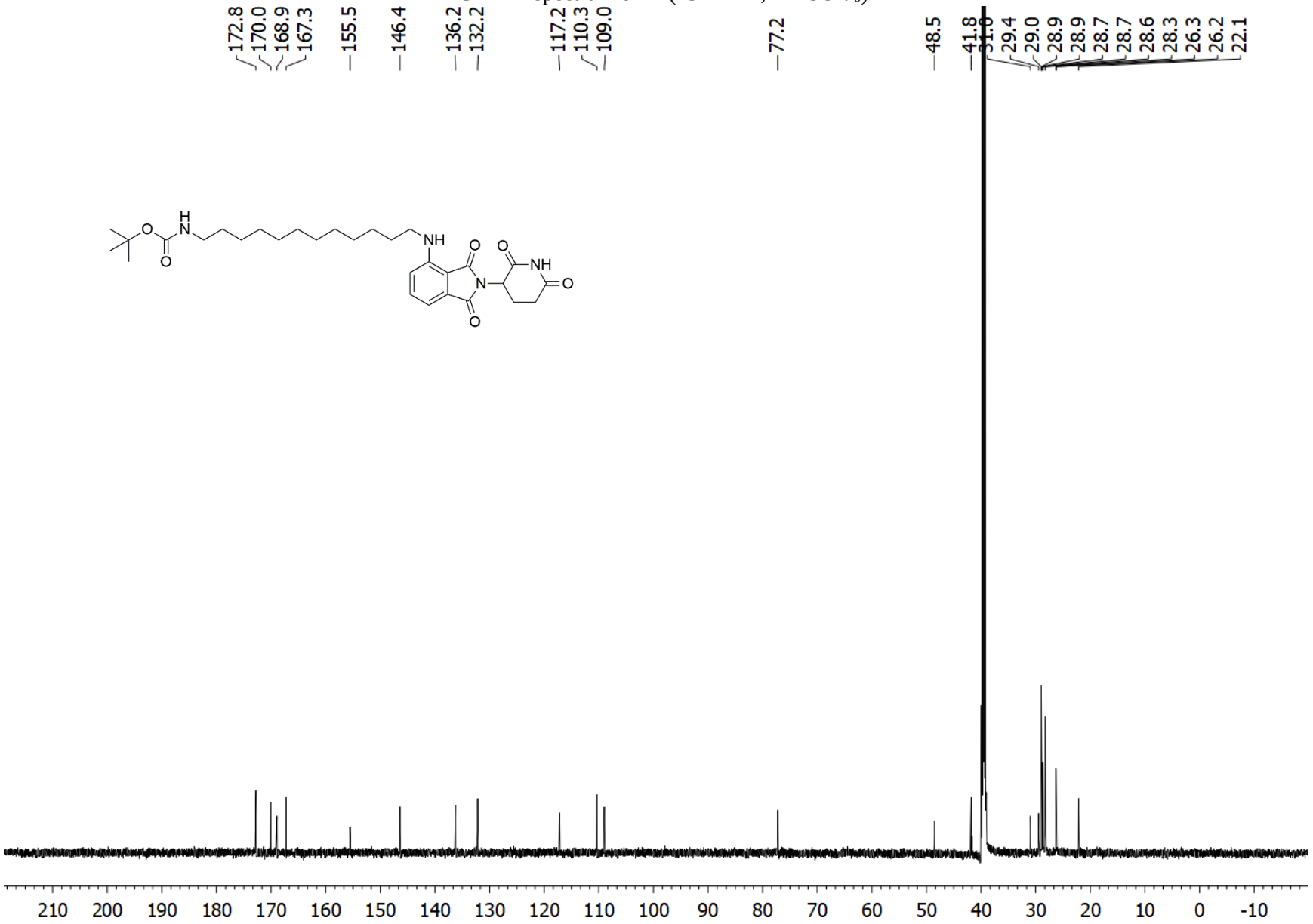
^{13}C NMR spectrum of **2e** (151 MHz, $CDCl_3$)



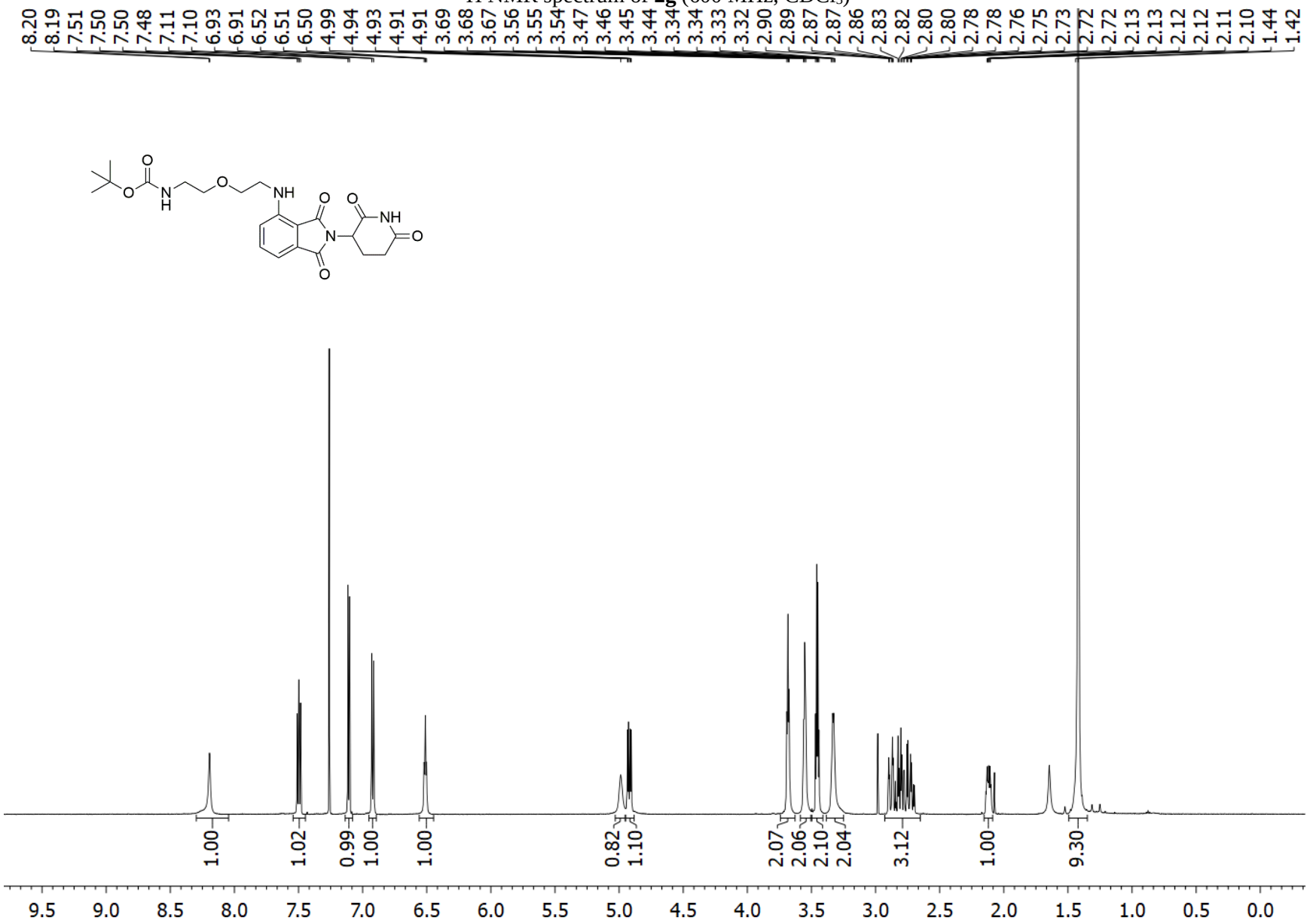
^{1}H NMR spectrum of **2f** (600 MHz, DMSO-d_6)



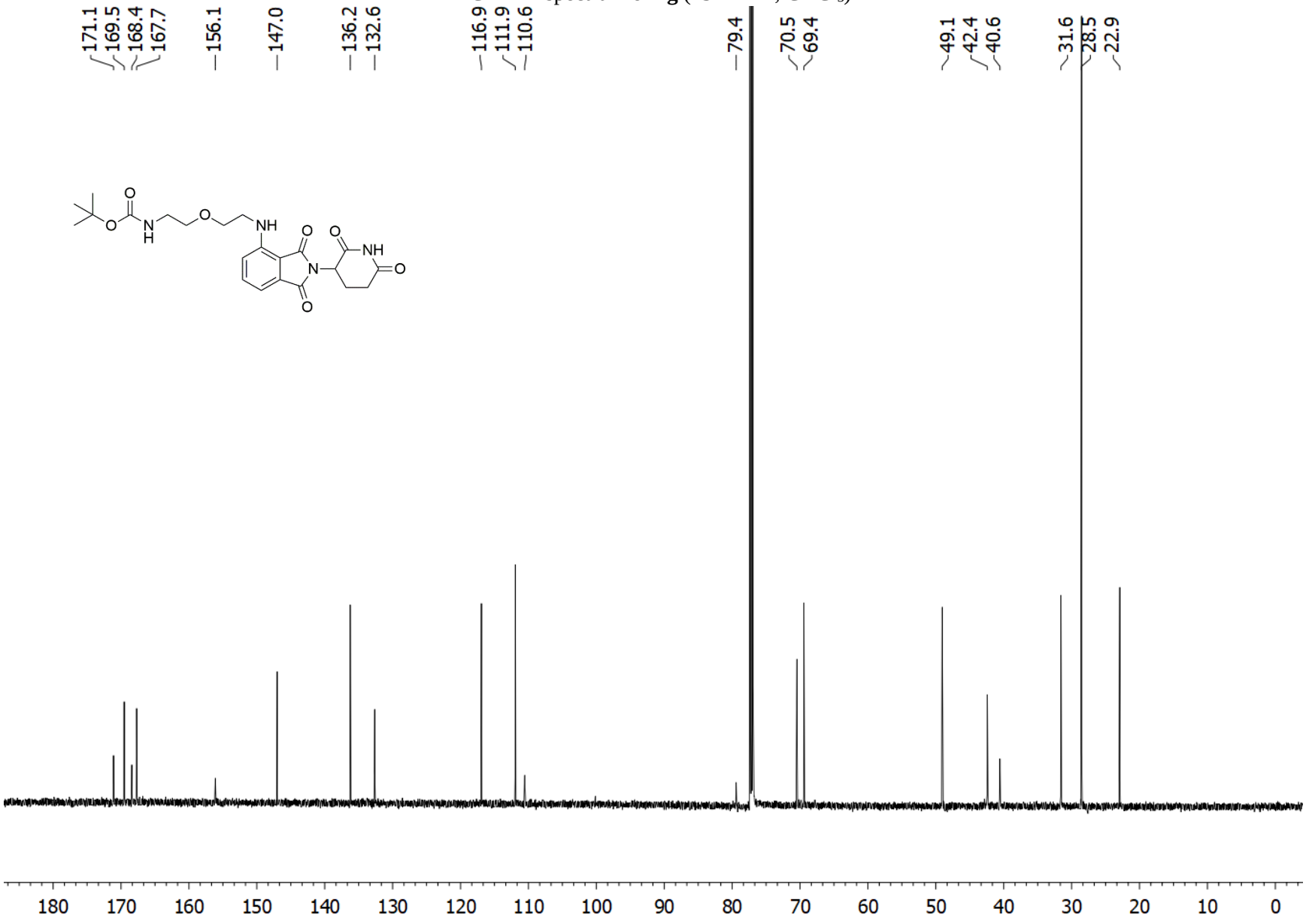
^{13}C NMR spectrum of **2f** (151 MHz, DMSO-d_6)



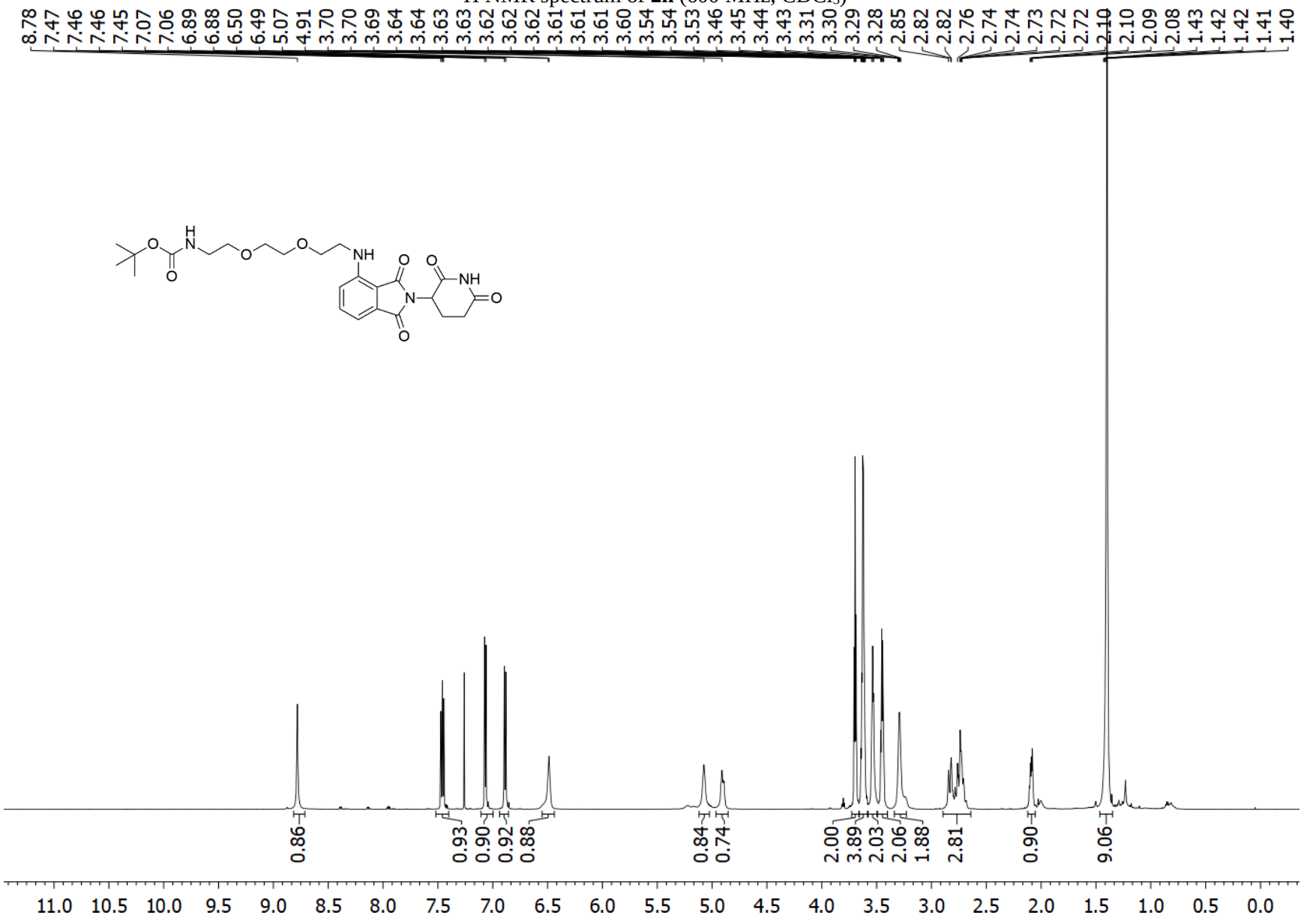
^{1}H NMR spectrum of **2g** (600 MHz, $CDCl_3$)



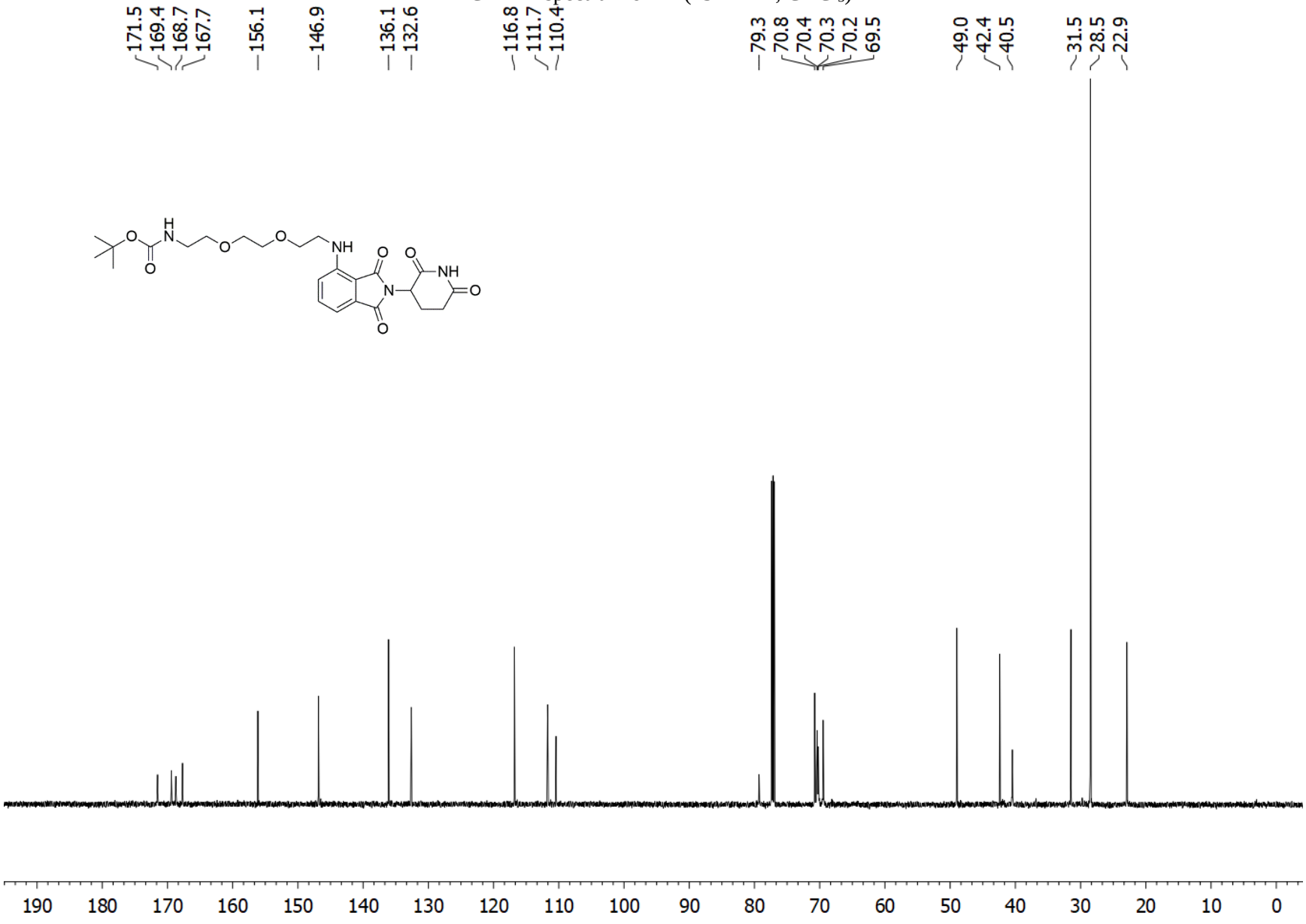
^{13}C NMR spectrum of **2g** (151 MHz, $CDCl_3$)



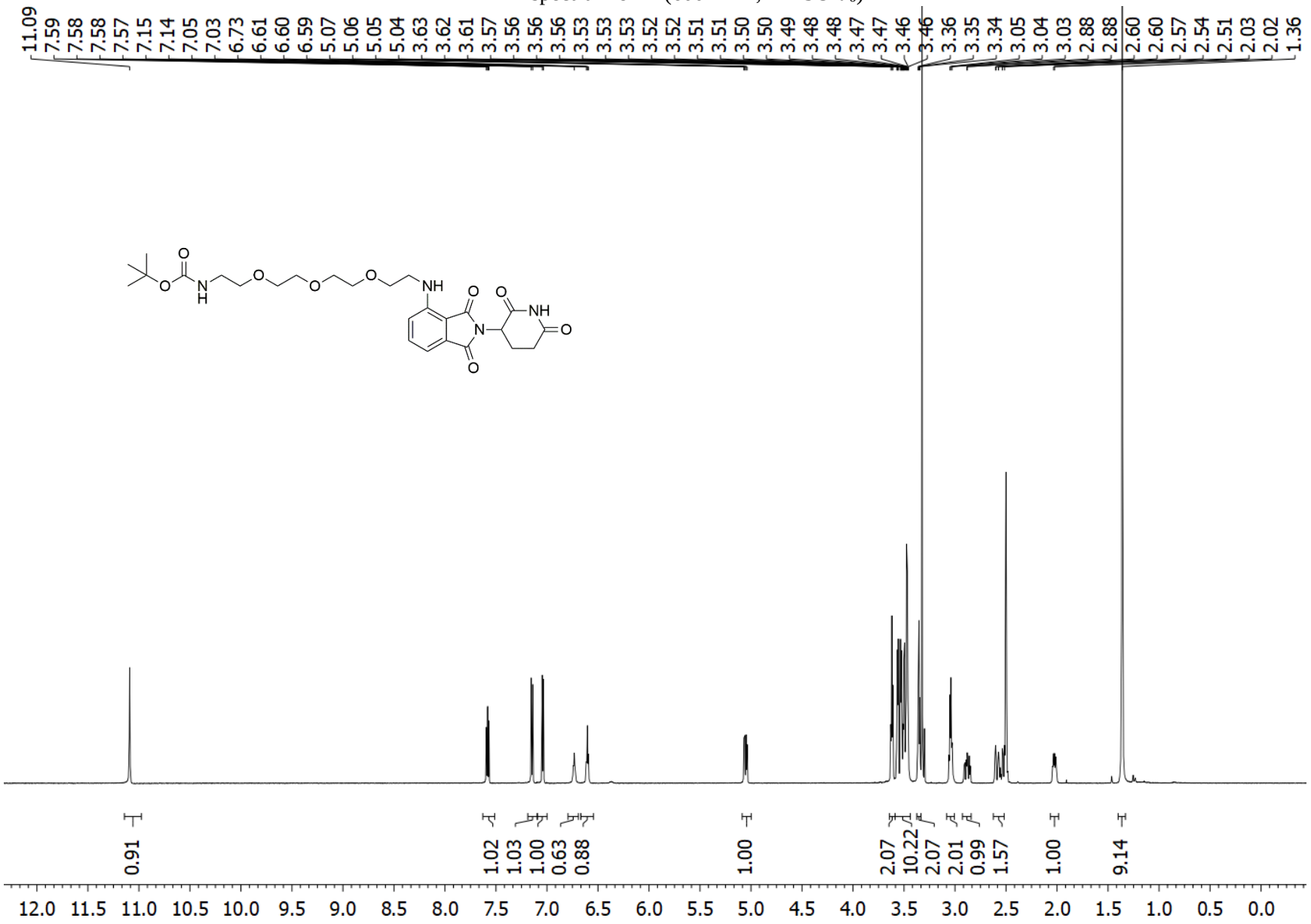
^{1}H NMR spectrum of **2h** (600 MHz, $CDCl_3$)



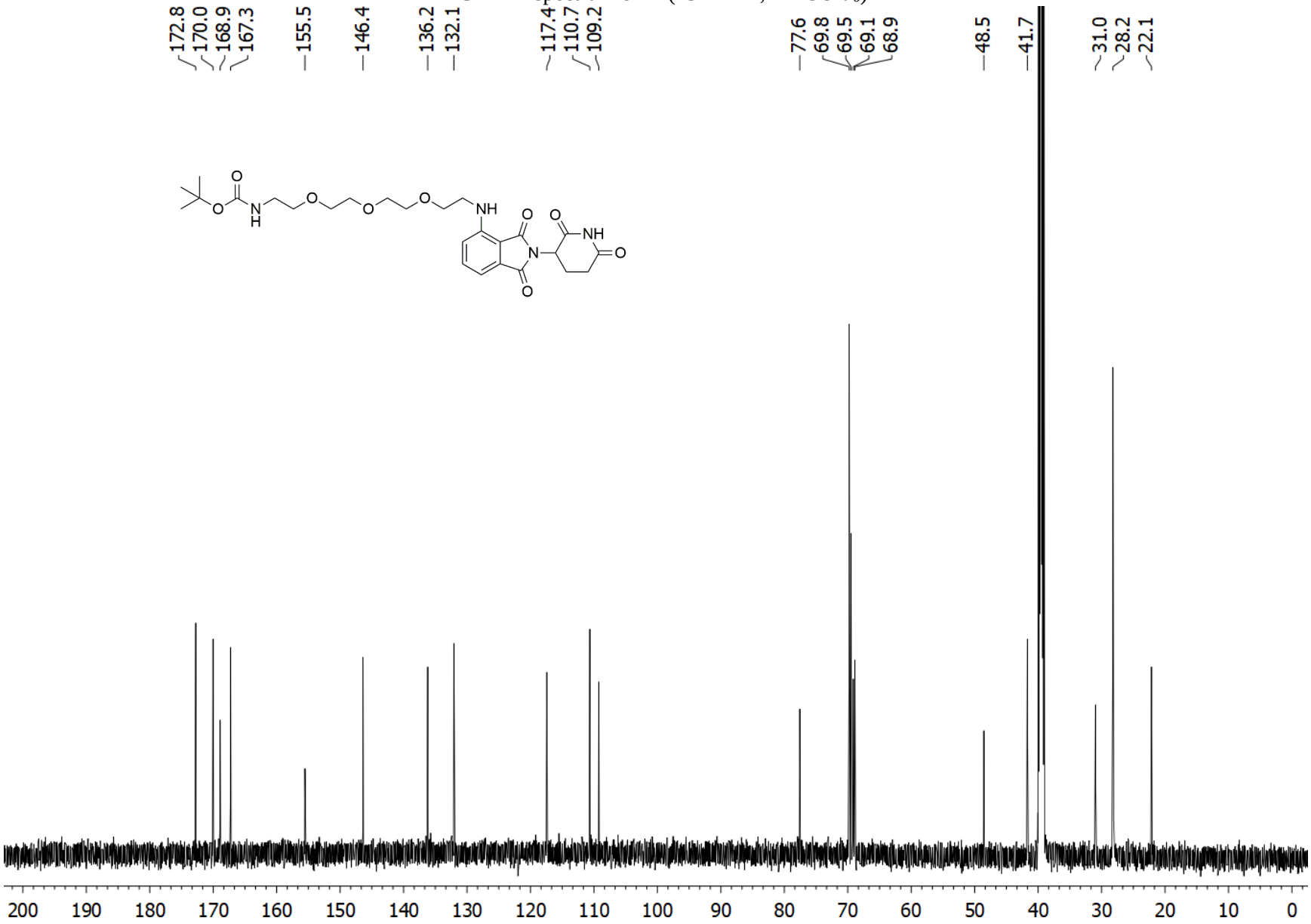
^{13}C NMR spectrum of **2h** (151 MHz, $CDCl_3$)



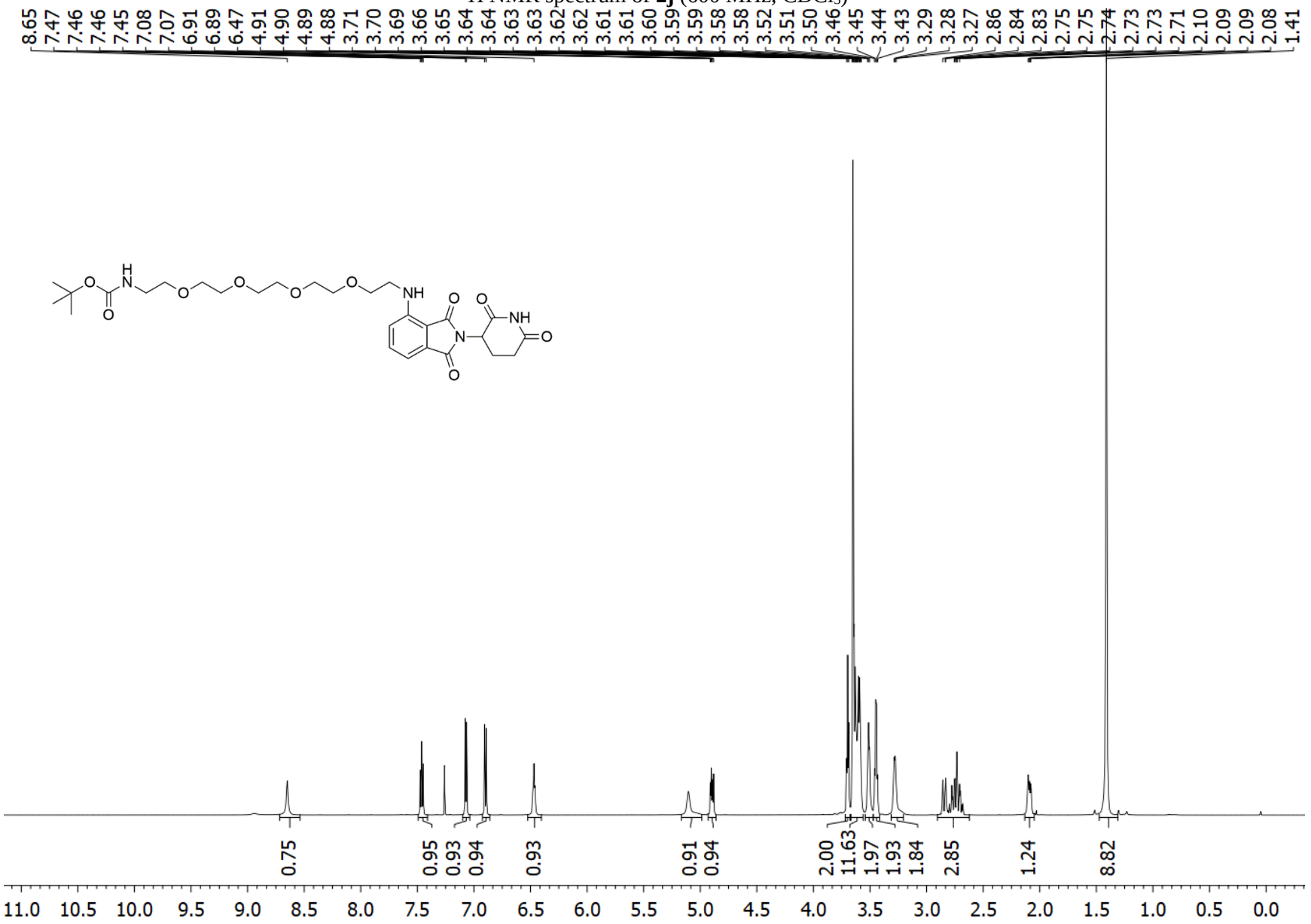
^{1}H NMR spectrum of **2i** (600 MHz, DMSO-d_6)



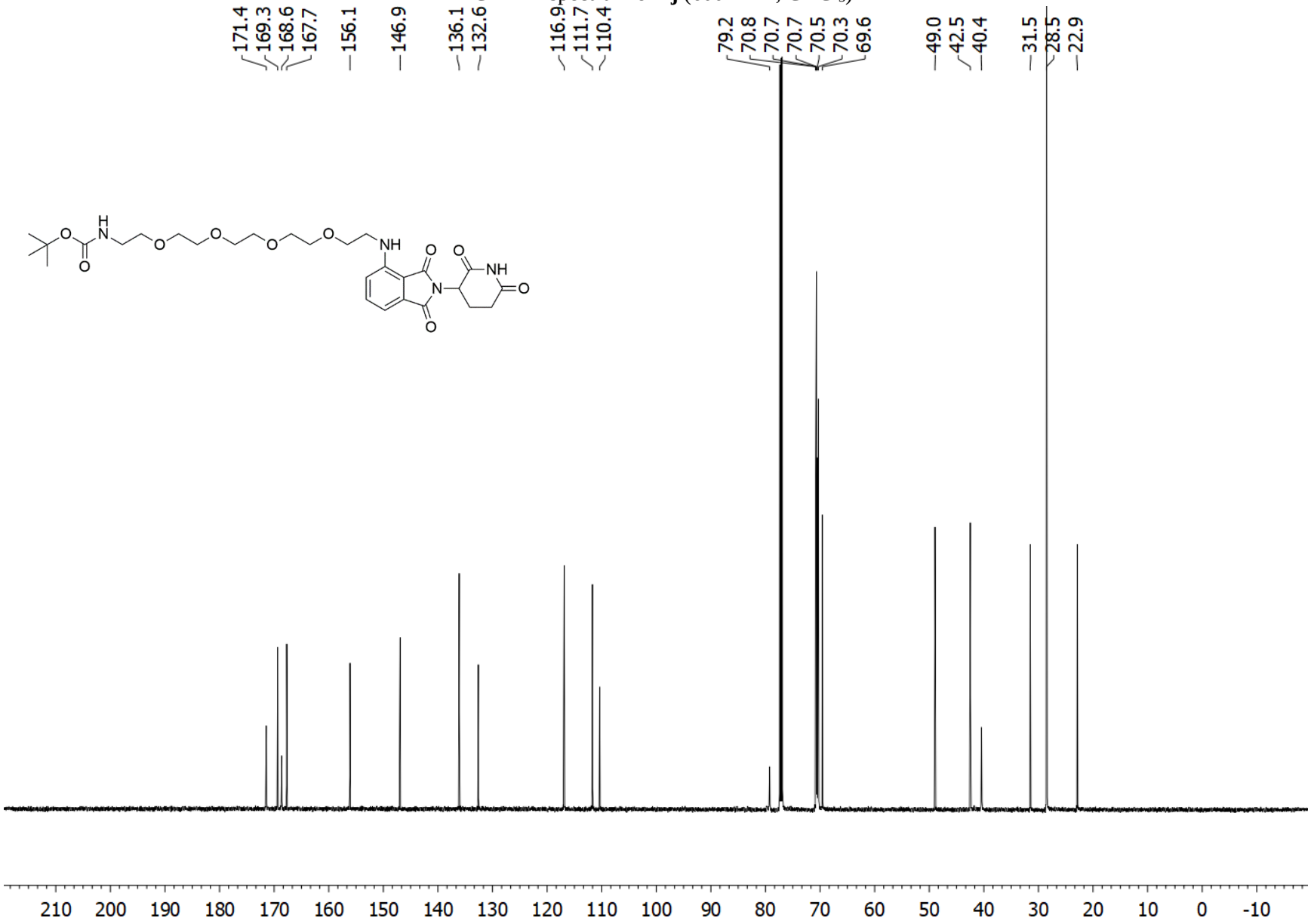
^{13}C NMR spectrum of **2i** (151 MHz, DMSO-d_6)



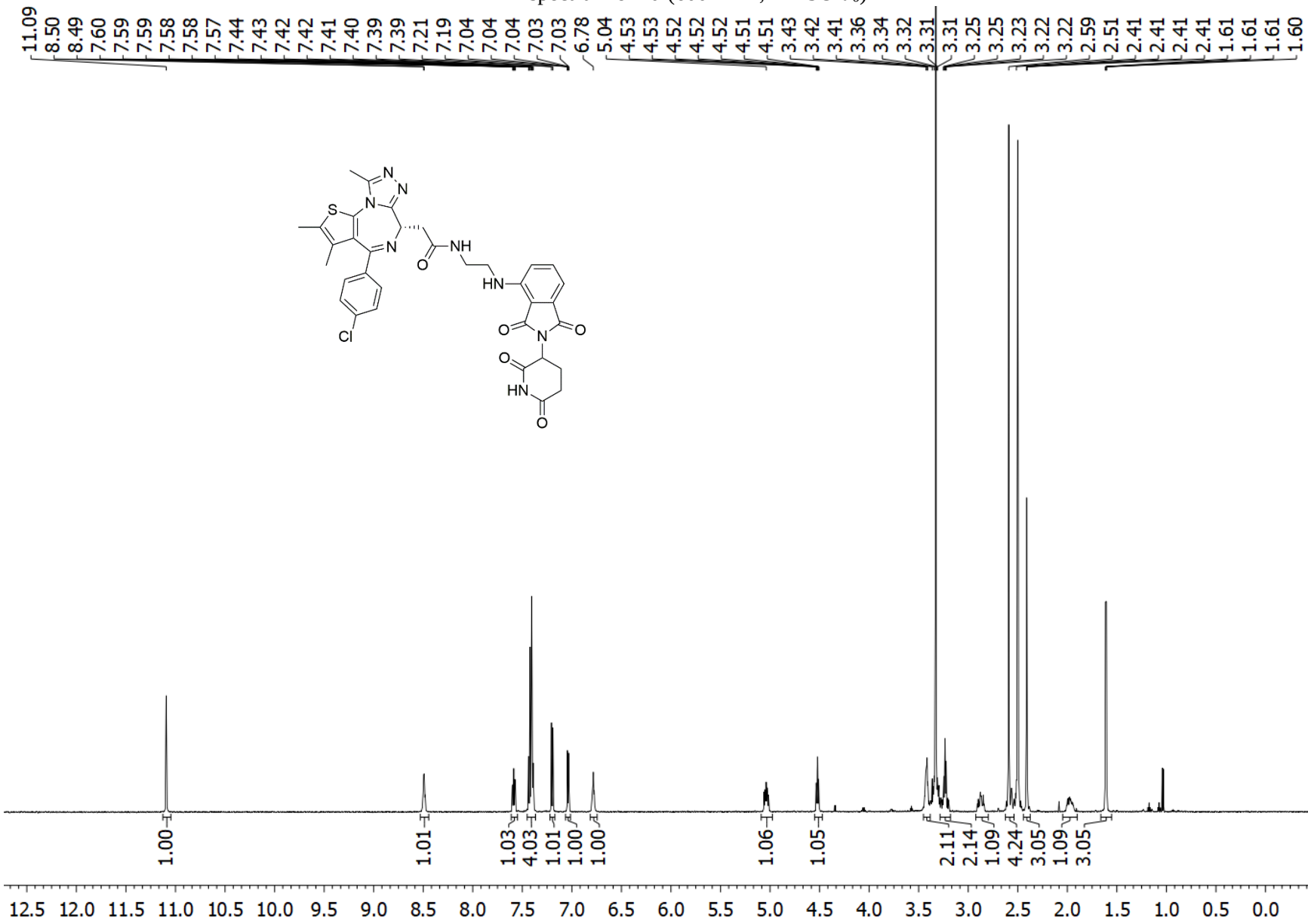
^{1}H NMR spectrum of **2j** (600 MHz, $CDCl_3$)



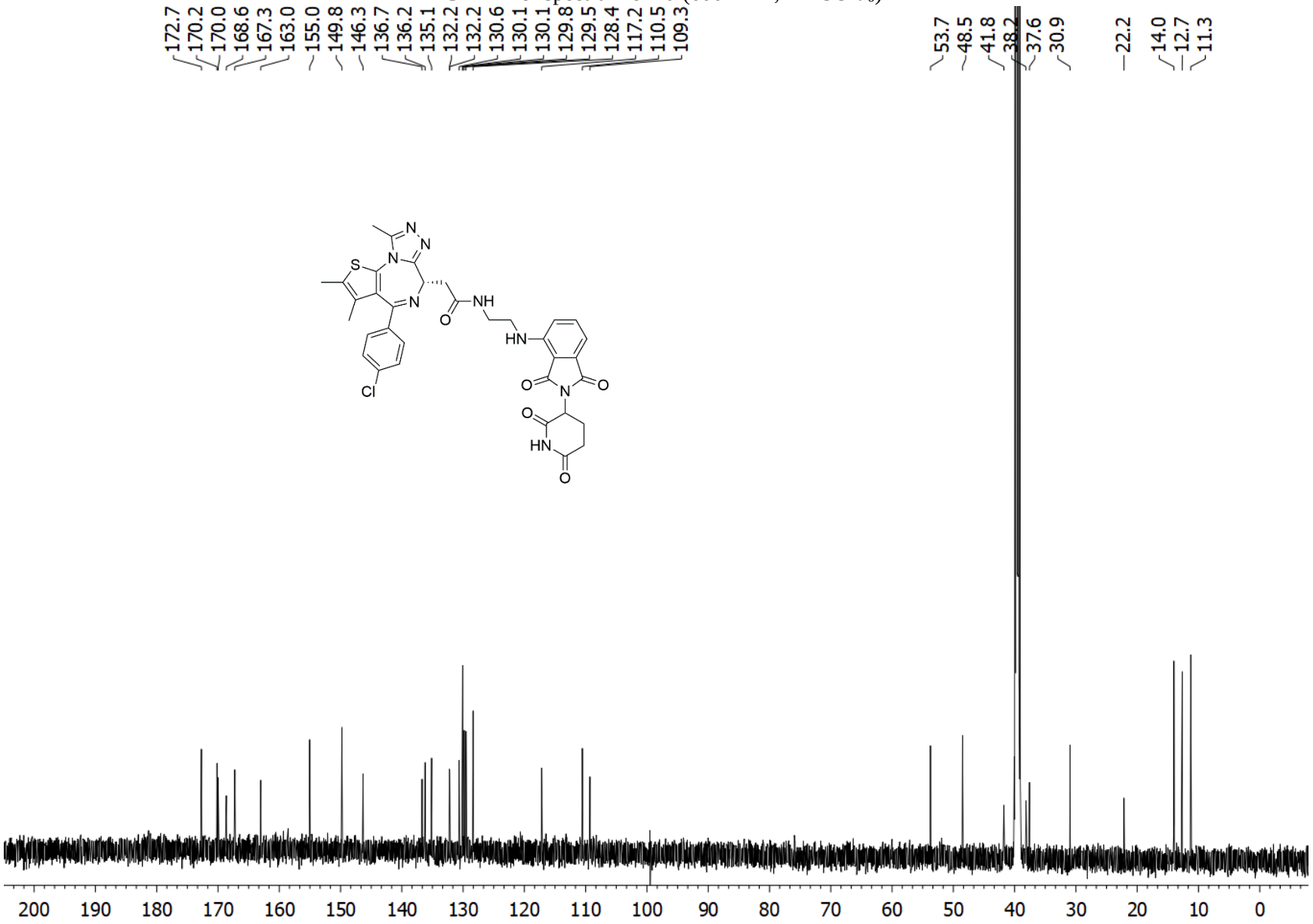
^{13}C NMR spectrum of **2j** (600 MHz, $CDCl_3$)



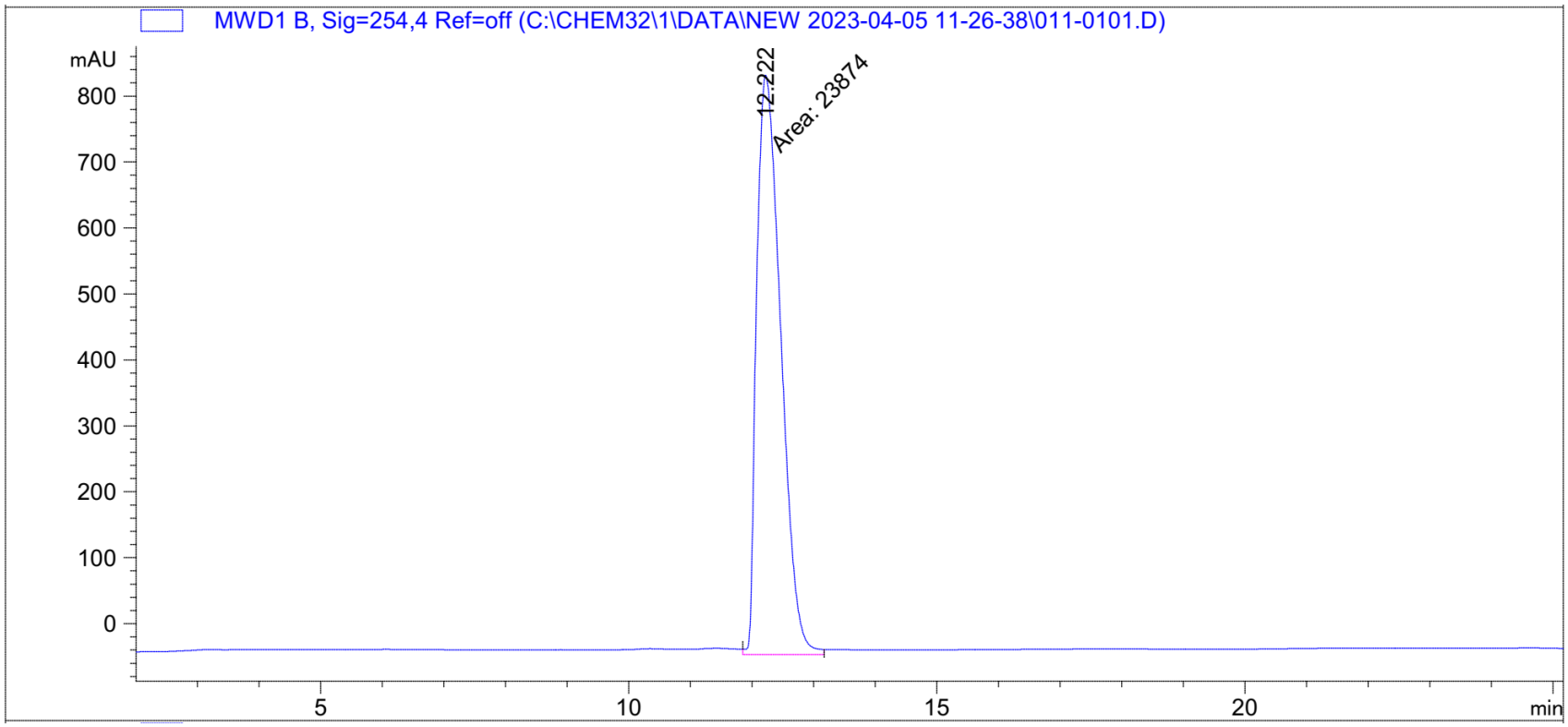
^{1}H NMR spectrum of **4a** (600 MHz, DMSO-d_6)



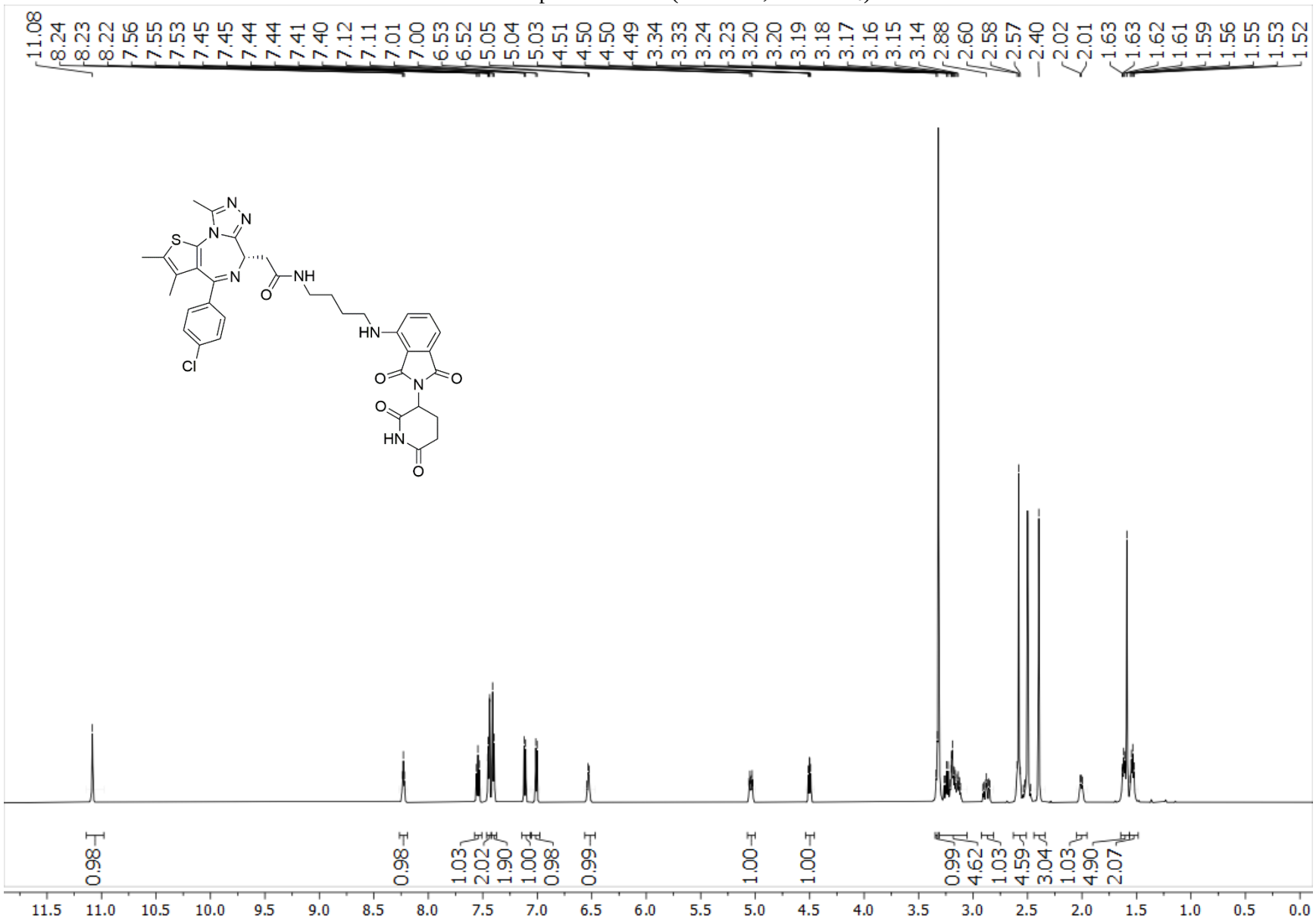
^{13}C NMR of spectrum of **4a** (600 MHz, DMSO-d_6)



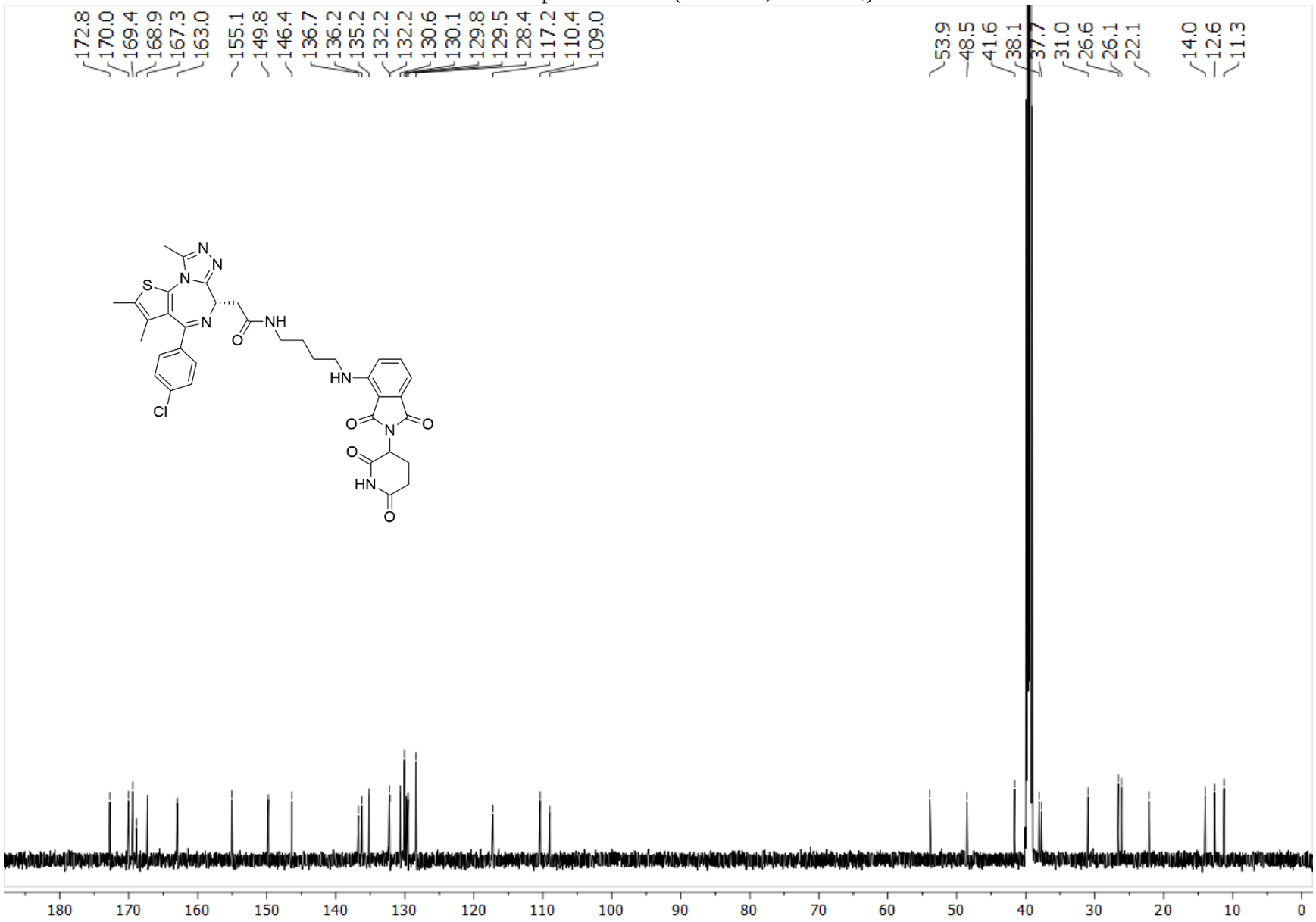
HPLC trace of **4a** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



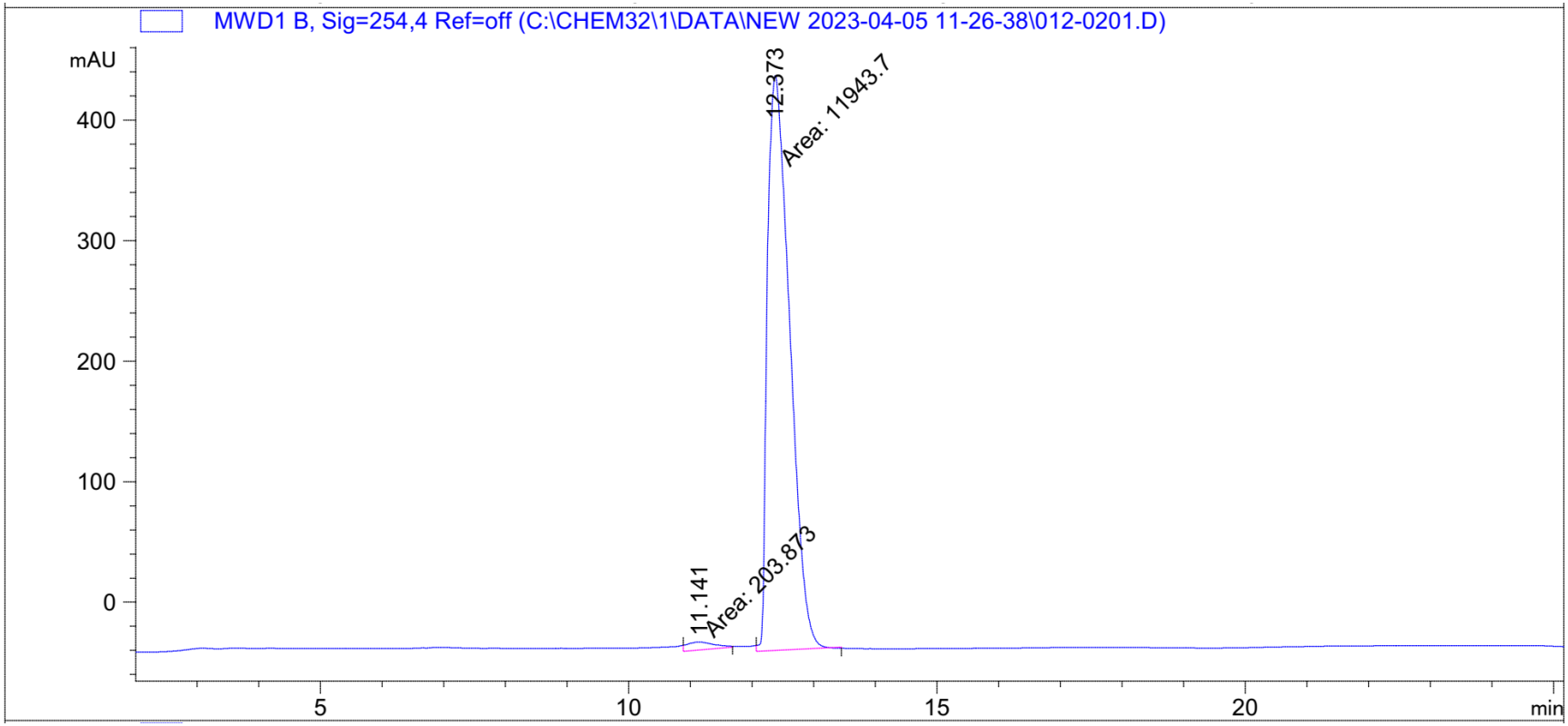
^{1}H NMR spectrum of **4b** (600 MHz, DMSO-d_6)



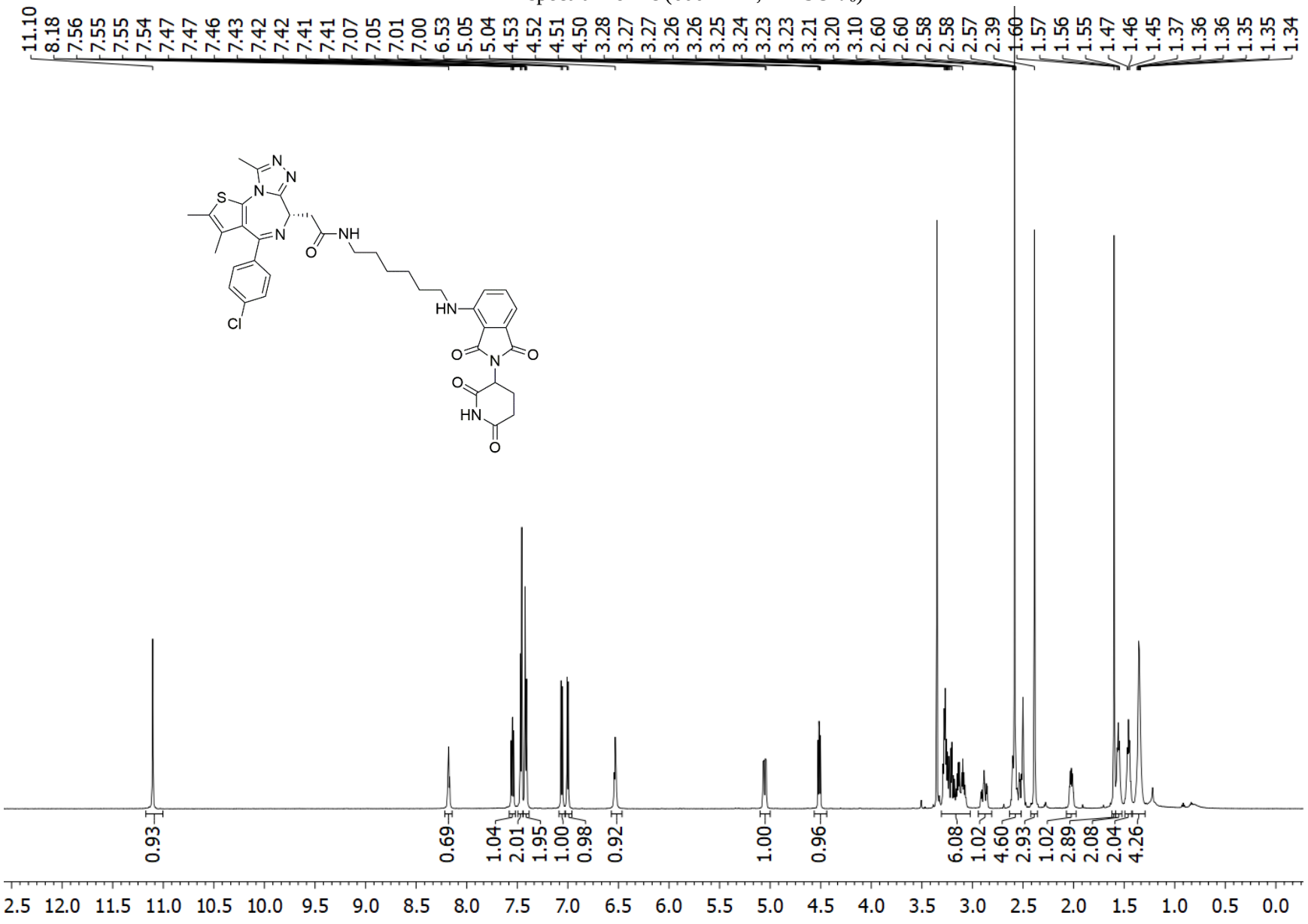
^{13}C NMR spectrum of **4b** (151 MHz, DMSO-d_6)



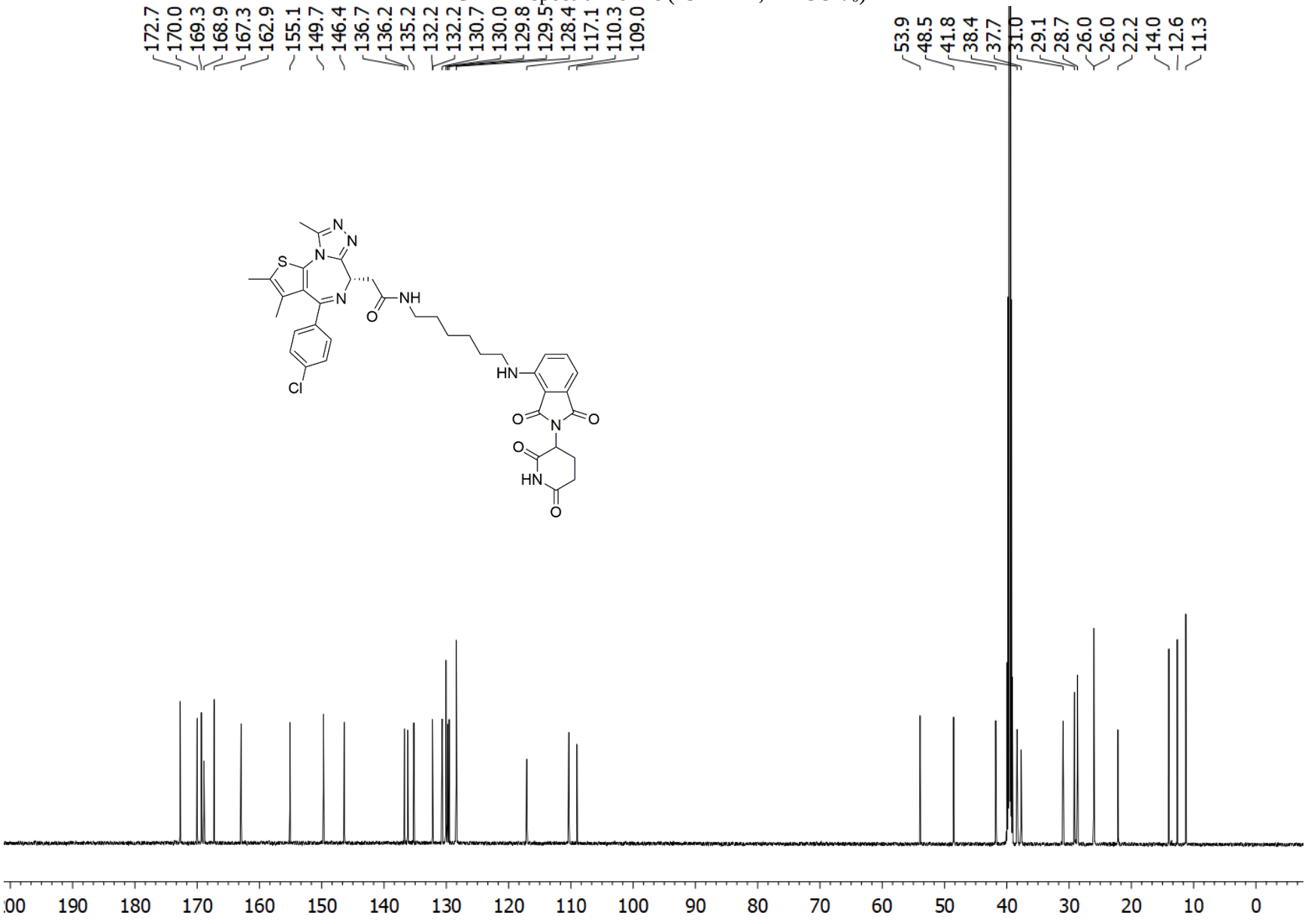
HPLC trace of **4b** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



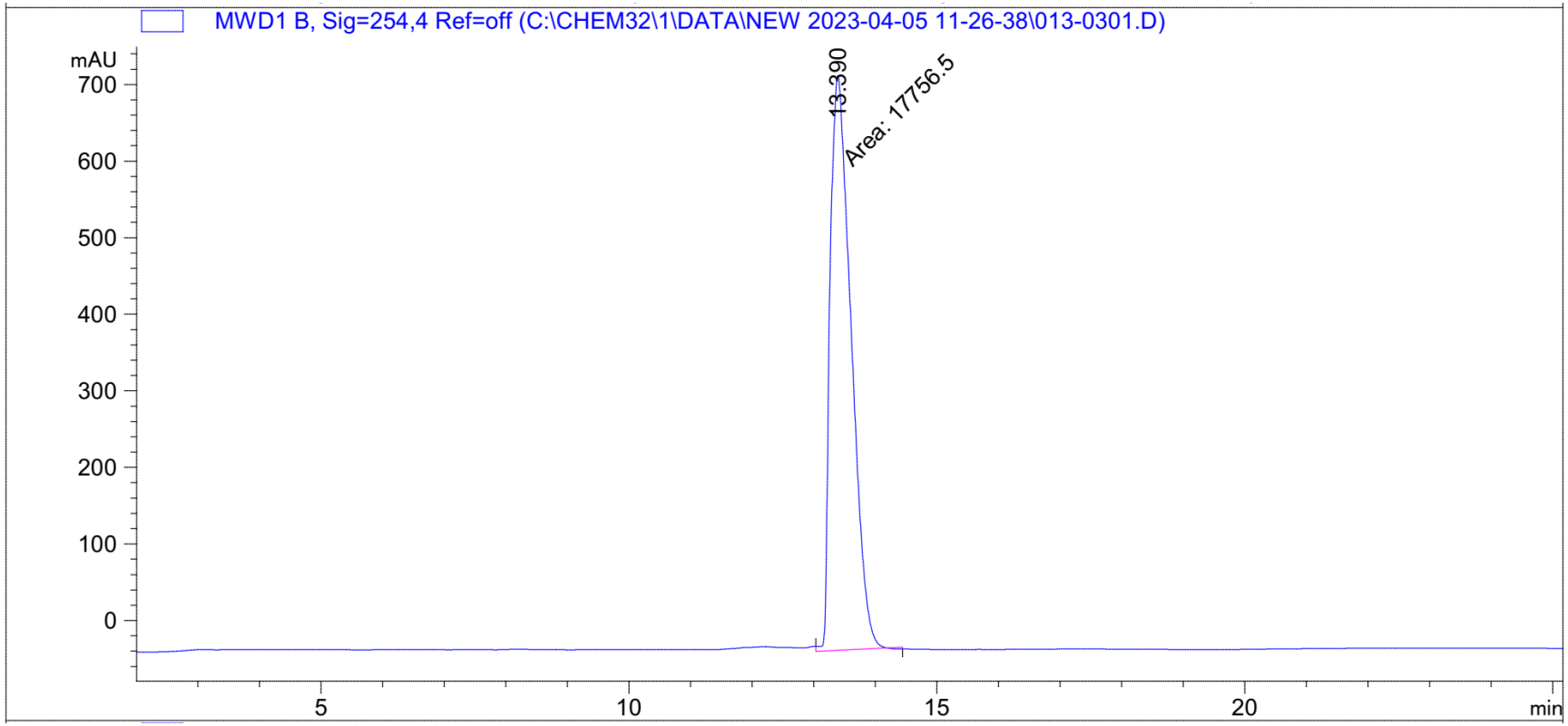
^{1}H NMR spectrum of **4c** (600 MHz, DMSO-d_6)



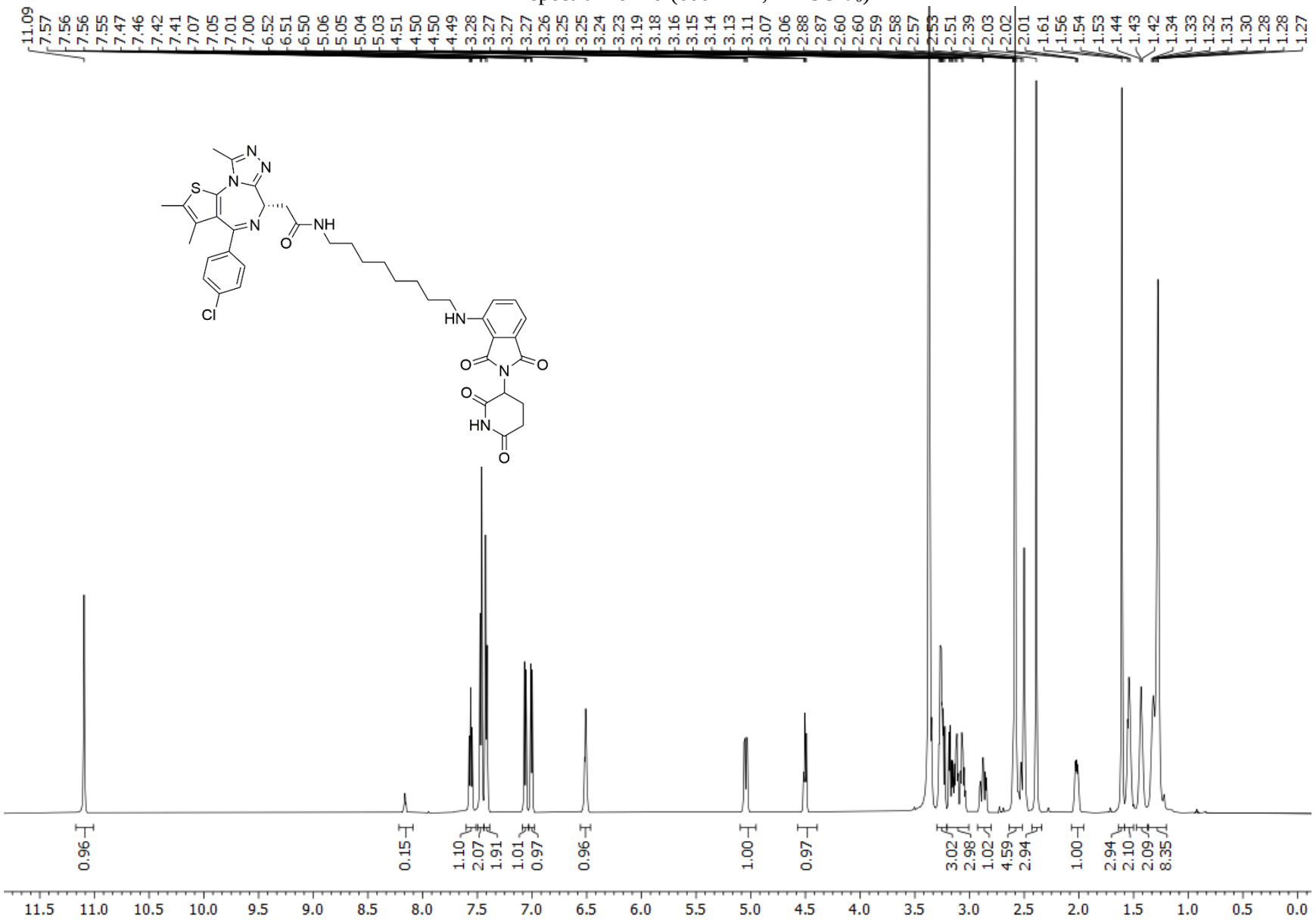
^{13}C NMR spectrum of **4c** (151 MHz, DMSO-d_6)



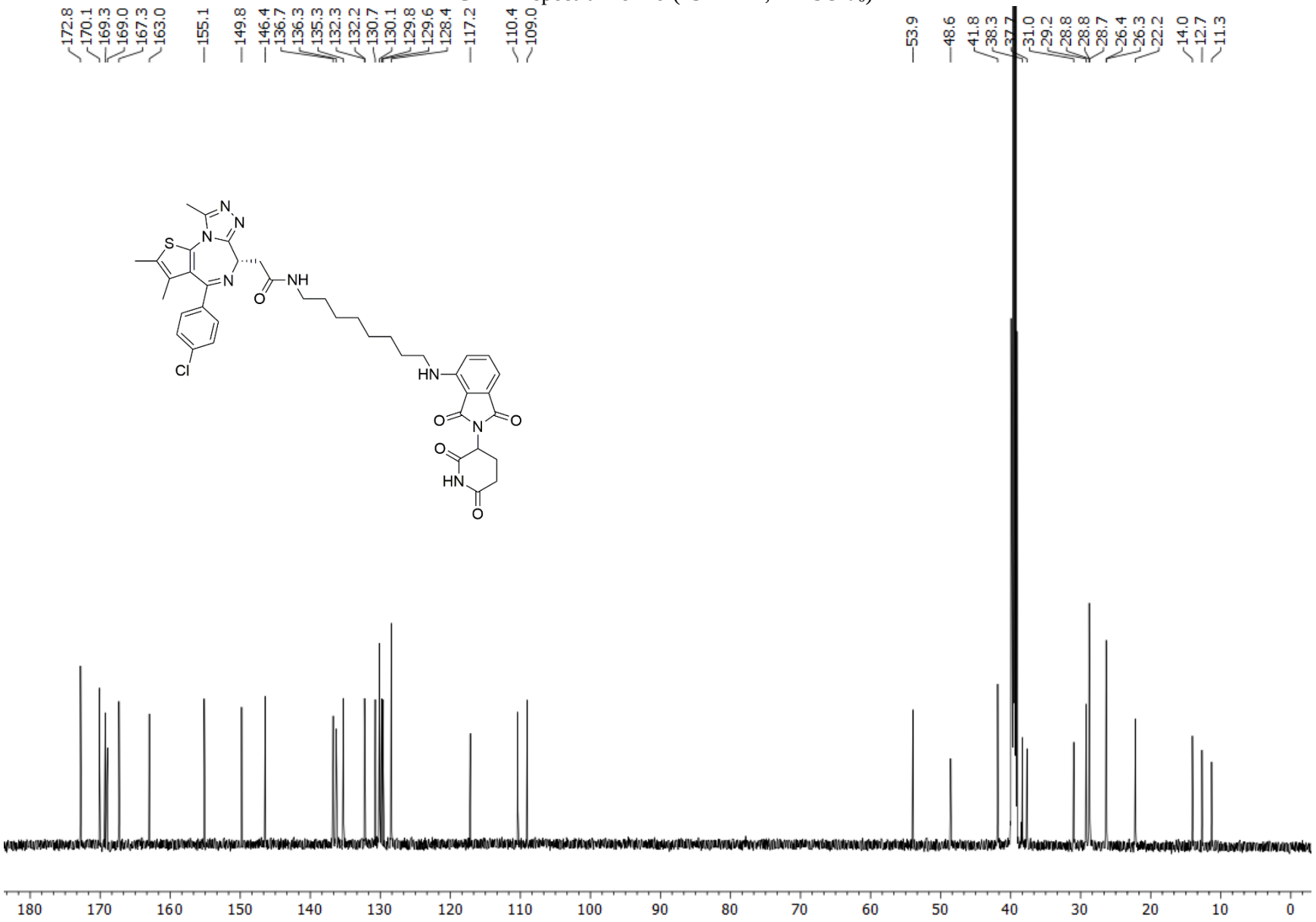
HPLC trace of **4c** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



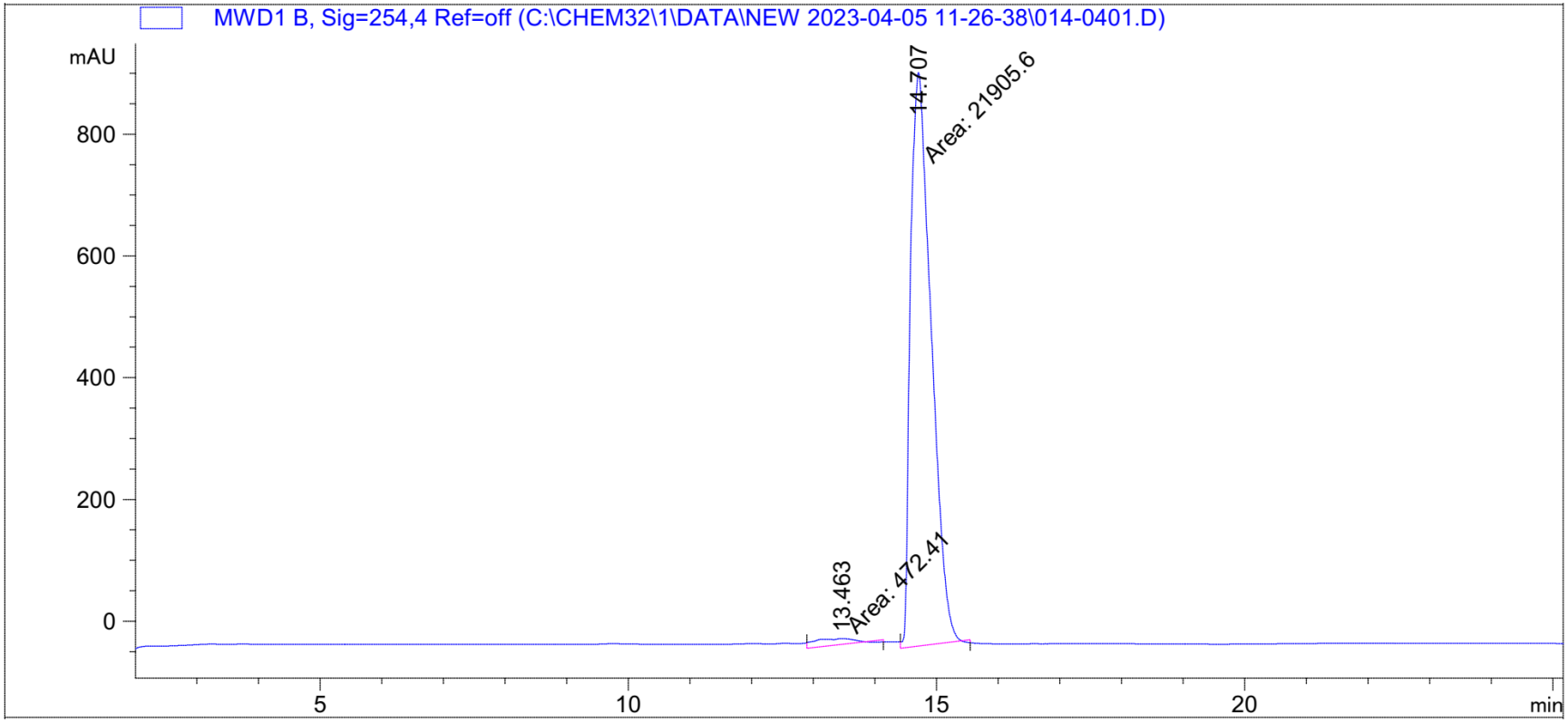
^{1}H NMR spectrum of **4d** (600 MHz, DMSO-d_6)



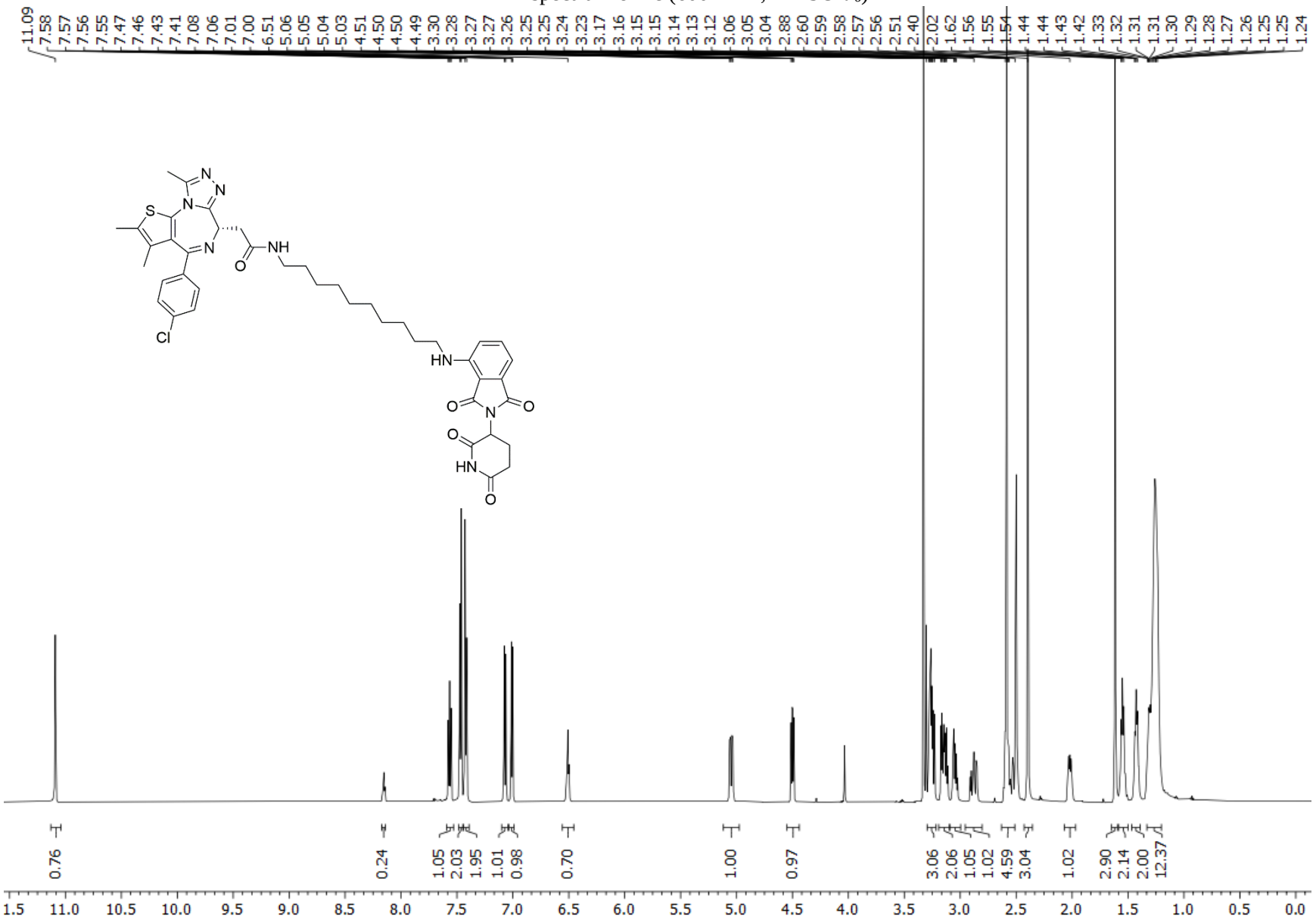
^{13}C NMR spectrum of **4d** (151 MHz, DMSO-d_6)



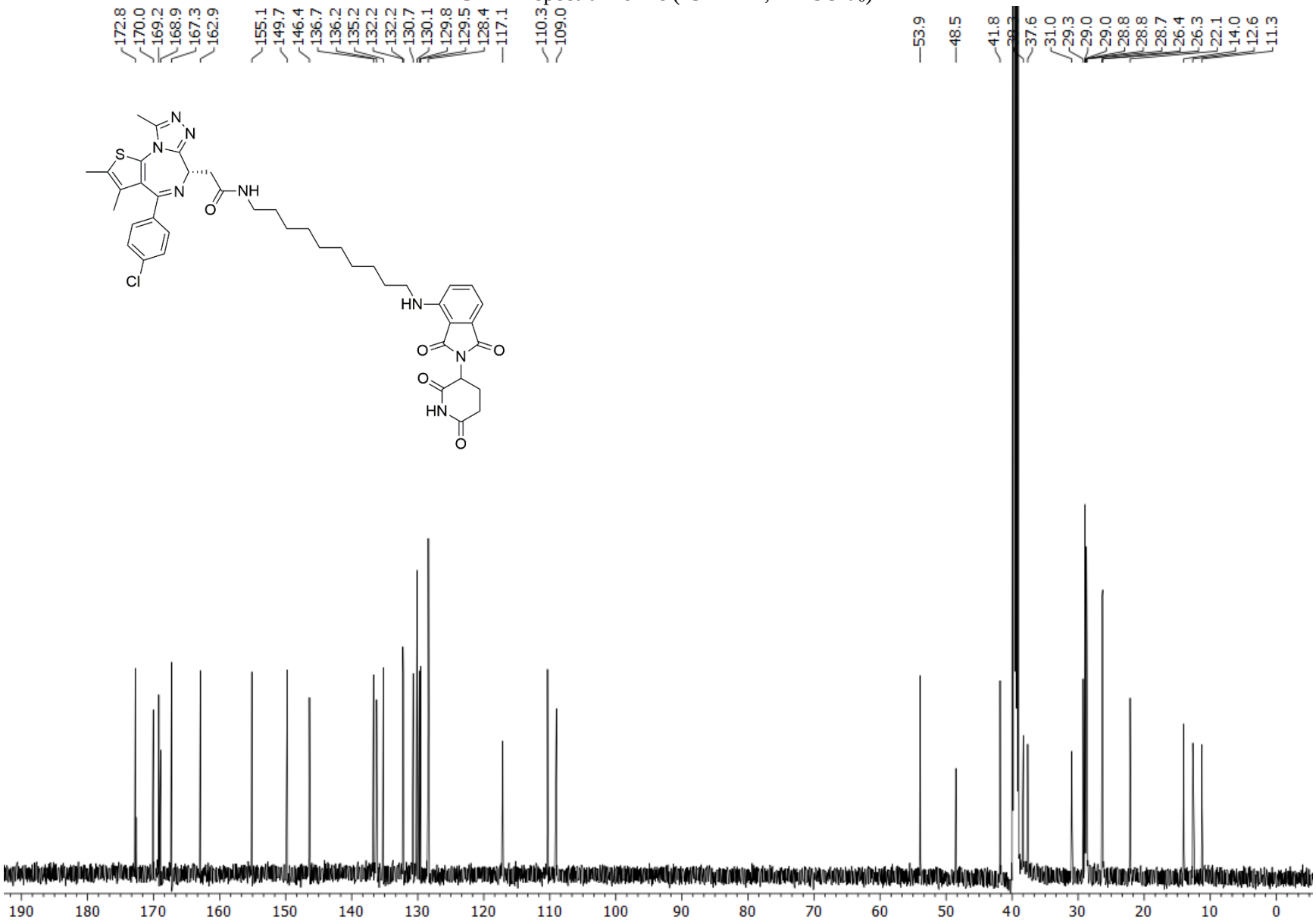
HPLC trace of **4d** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



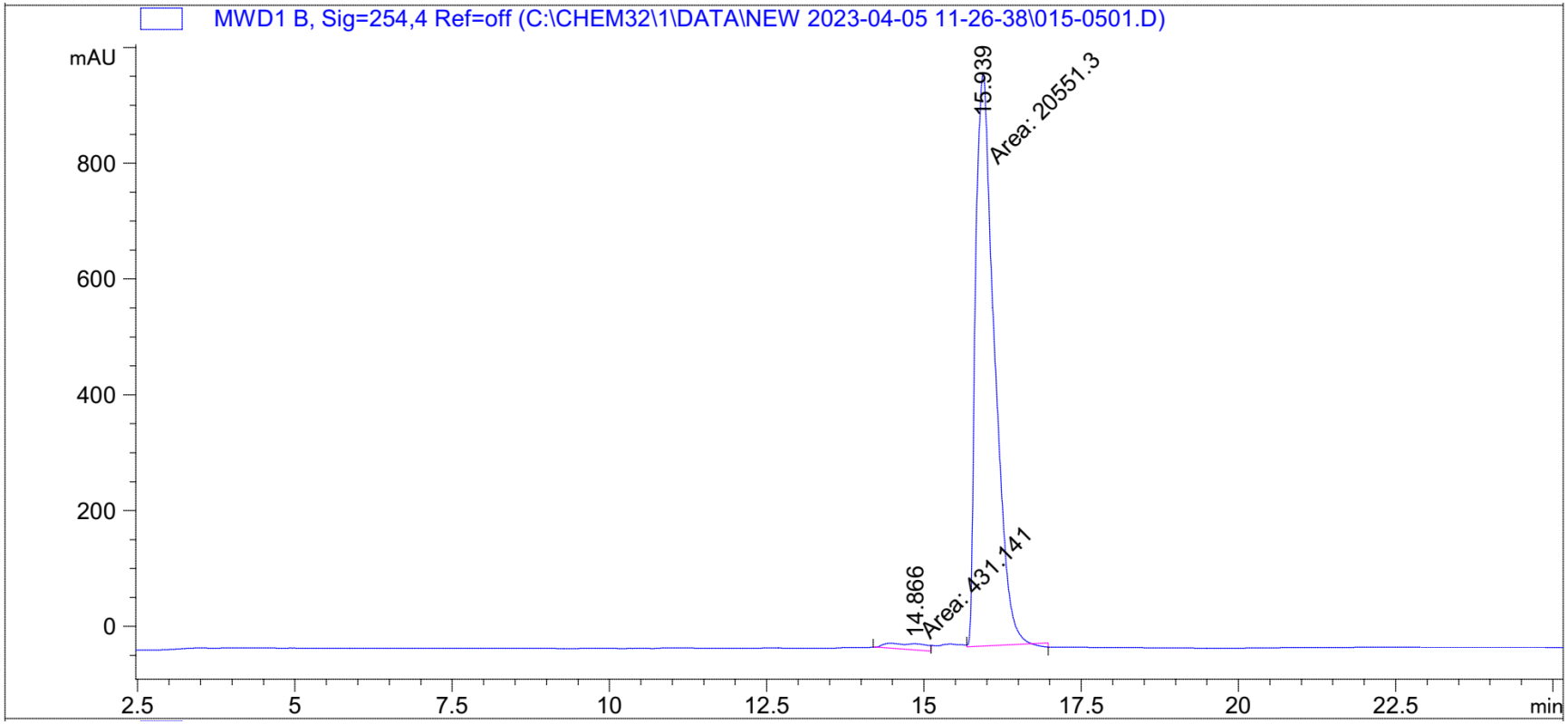
^{1}H NMR spectrum of **4e** (600 MHz, DMSO-d_6)



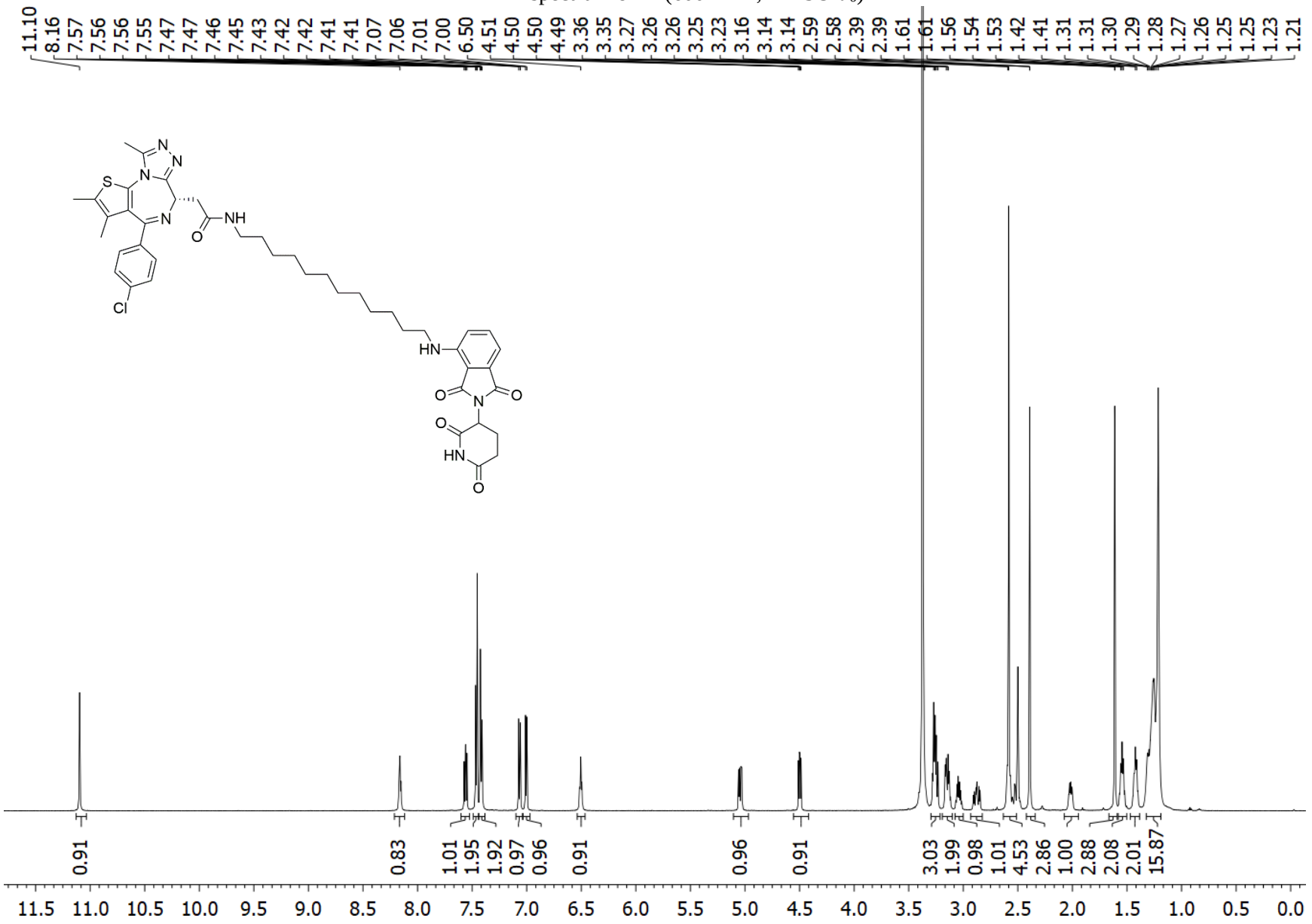
^{13}C NMR spectrum of **4e** (151 MHz, DMSO-d_6)



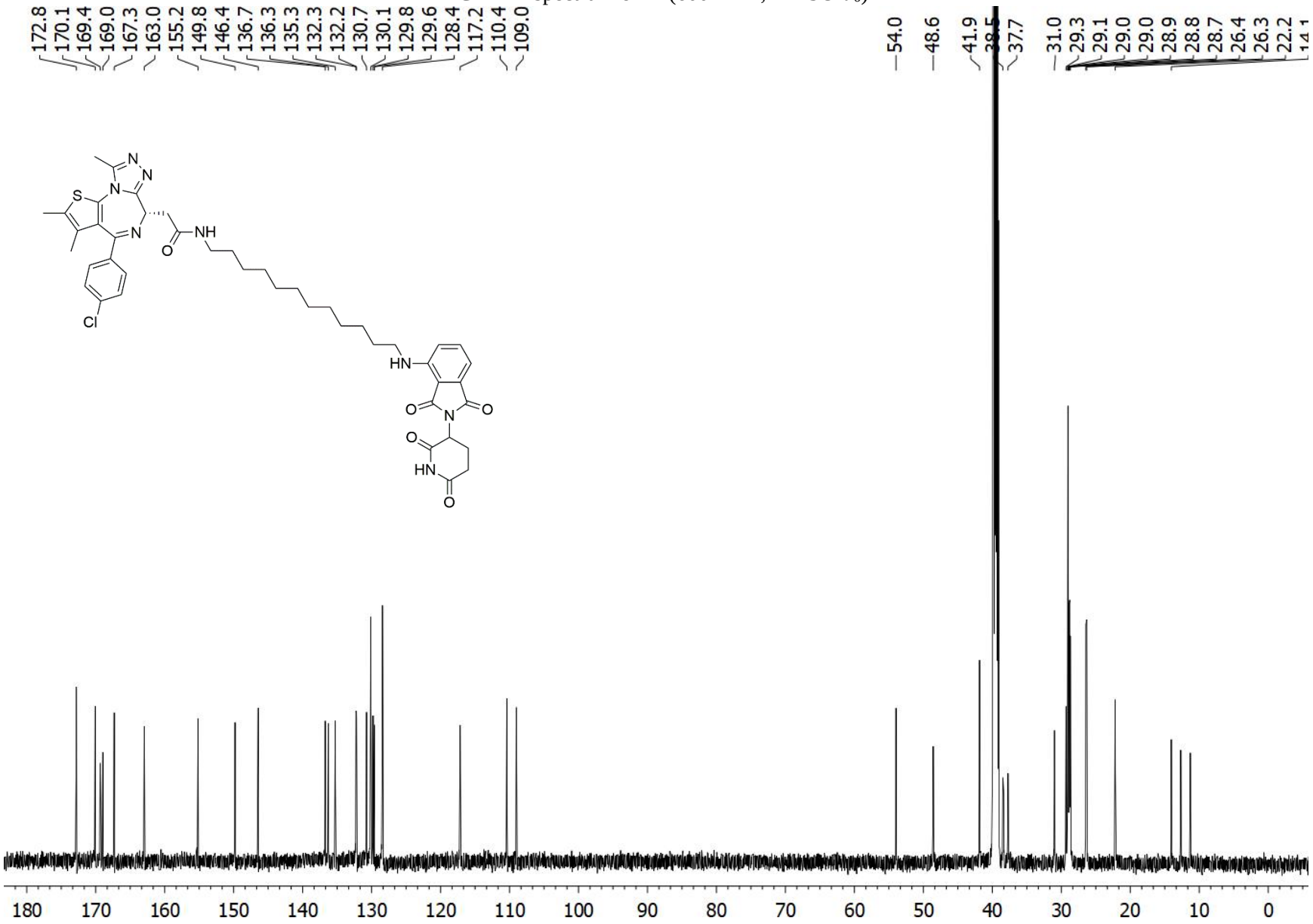
HPLC trace of **4e** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



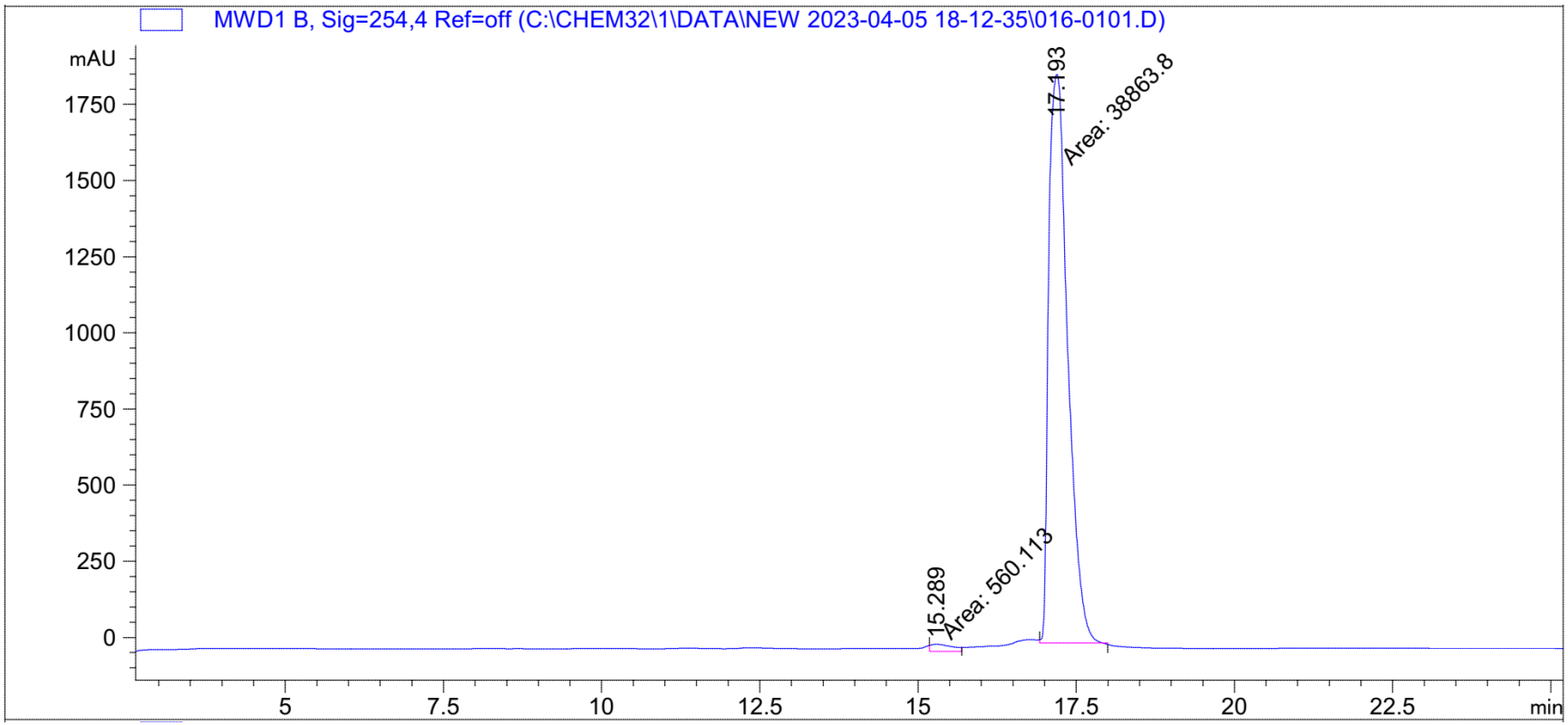
^{1}H NMR spectrum of **4f** (600 MHz, DMSO-d_6)



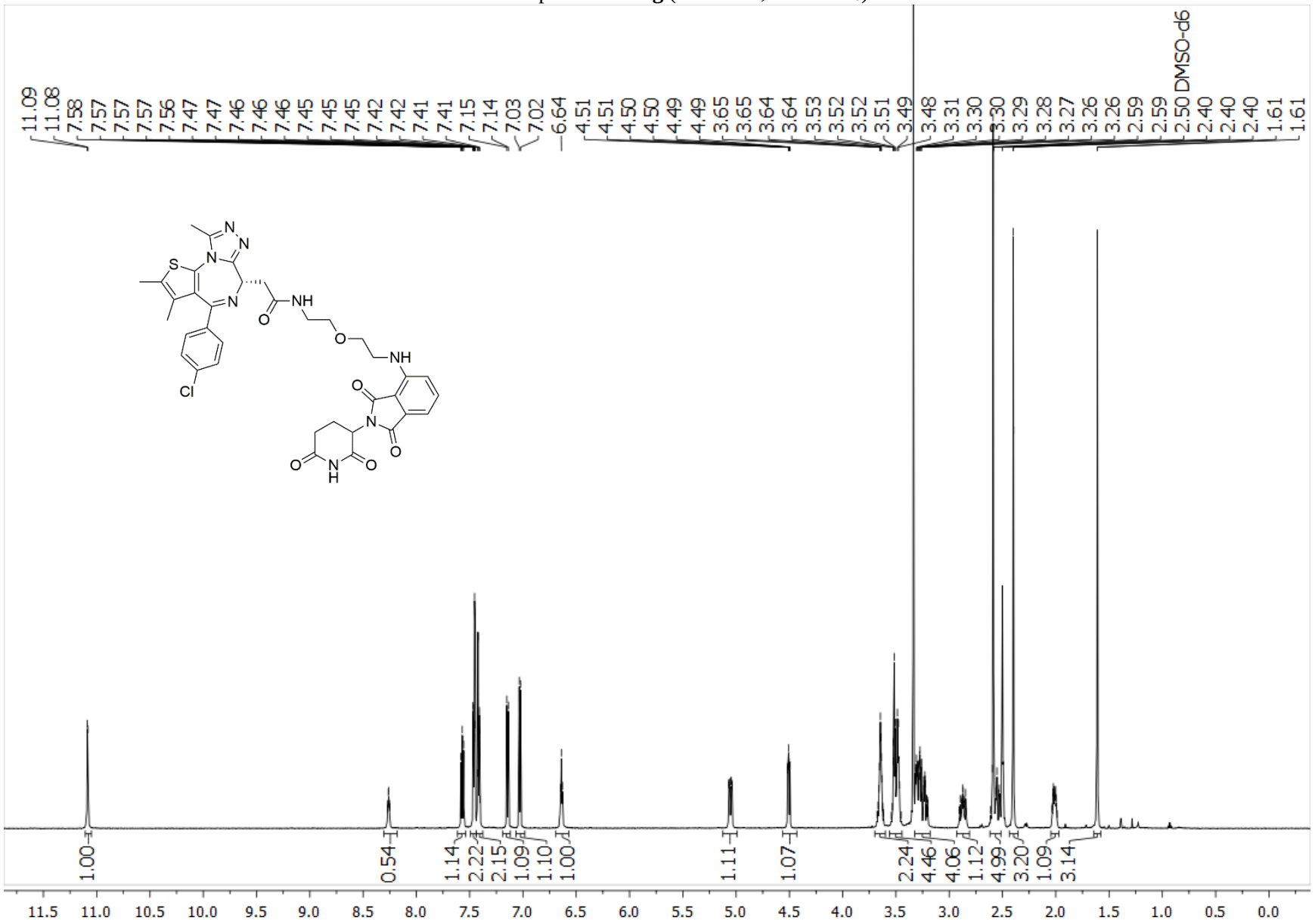
^{13}C NMR spectrum of **4f** (600 MHz, DMSO-d_6)



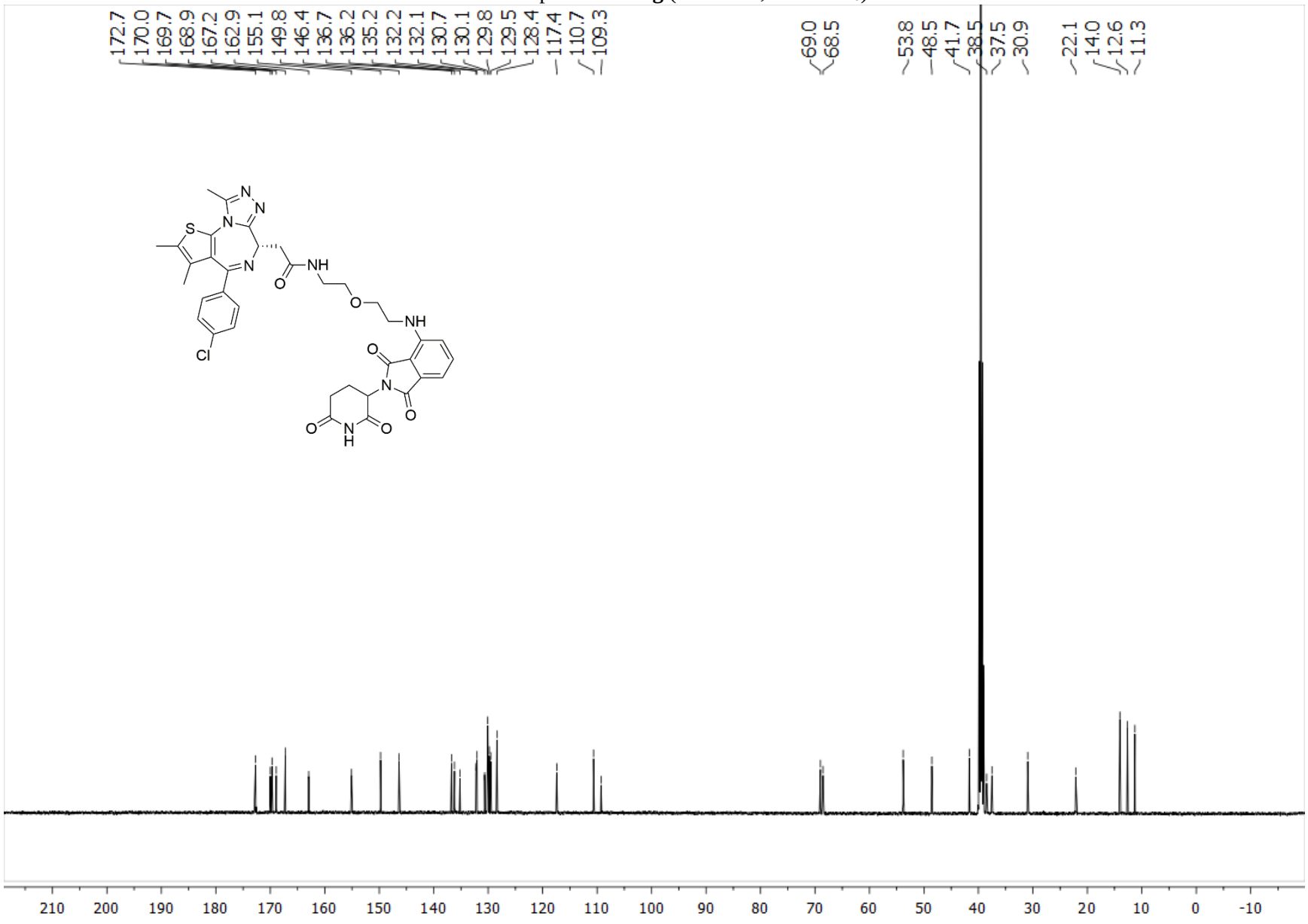
HPLC trace of **4f** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



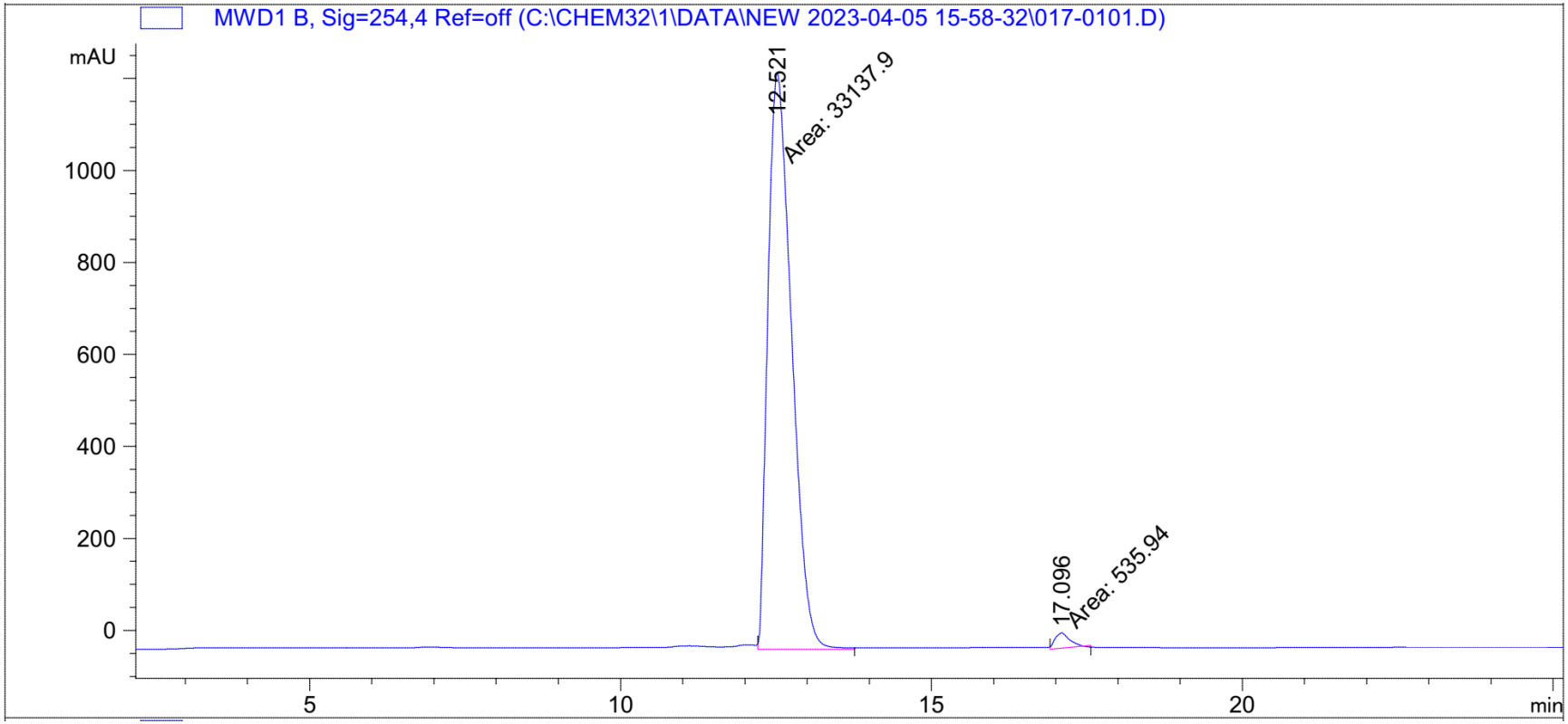
^{1}H NMR spectrum of **4g** (600 MHz, DMSO-d_6)



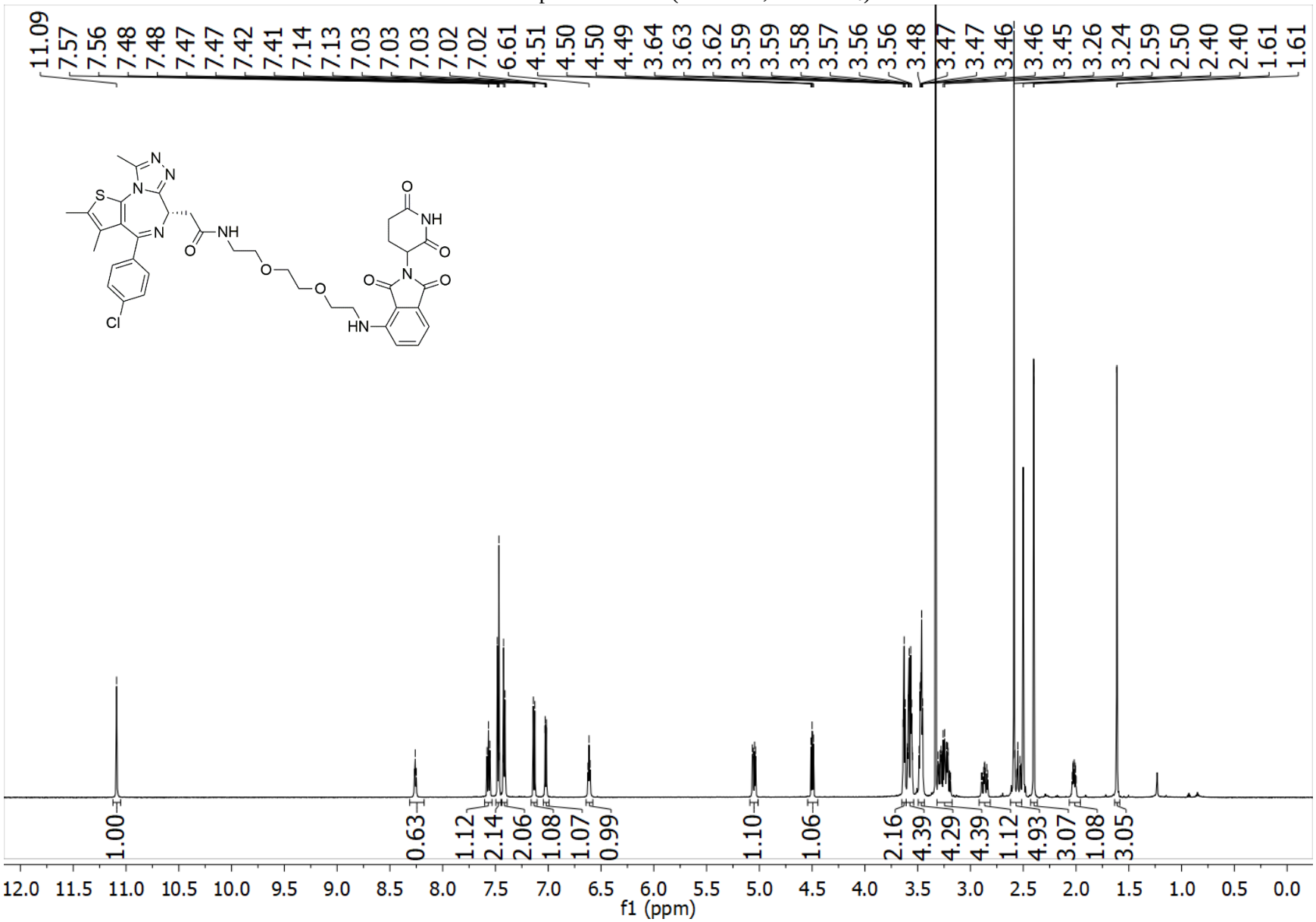
^{13}C NMR spectrum of **4g** (151 MHz, DMSO-d_6)



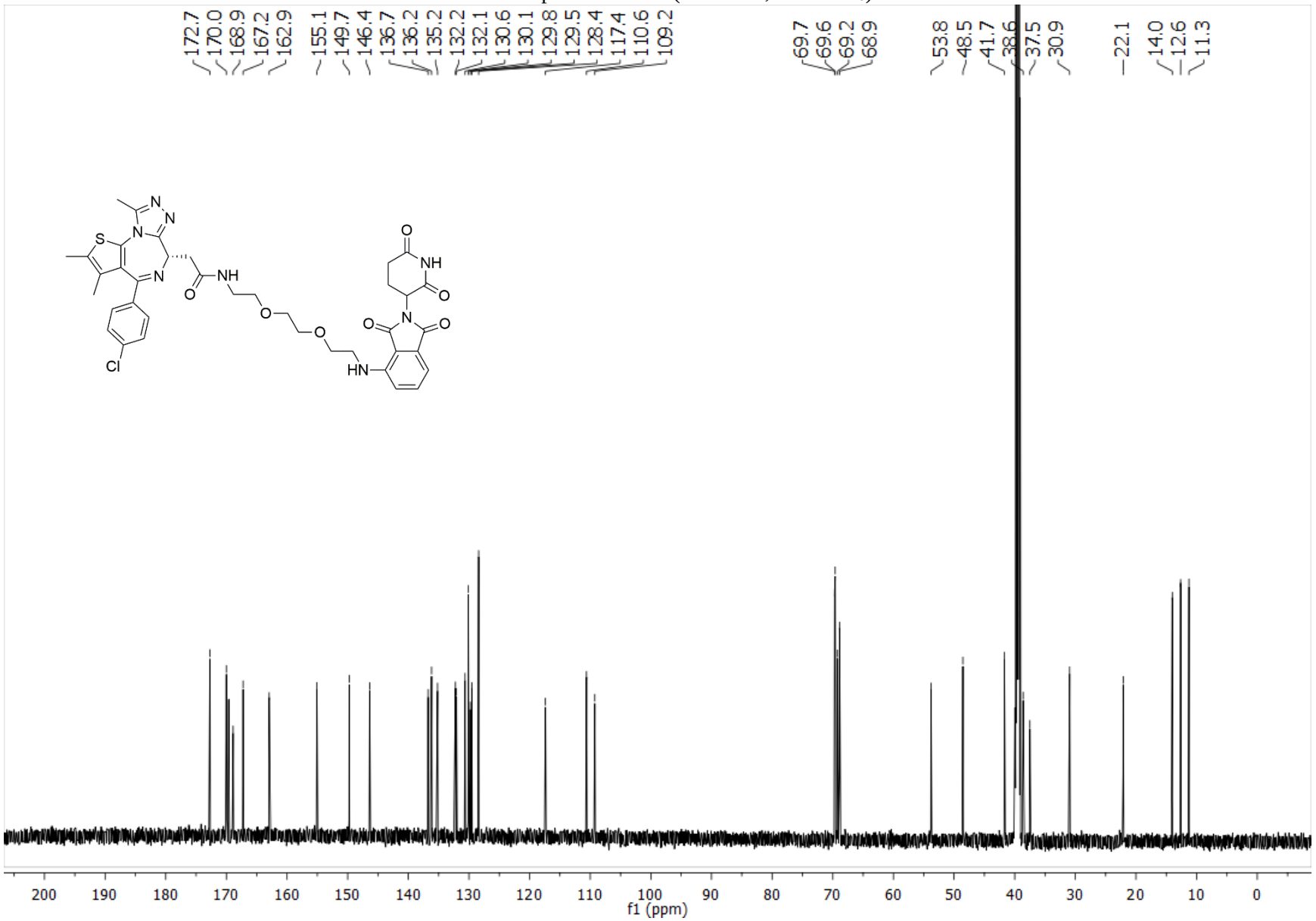
HPLC trace of **4g** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



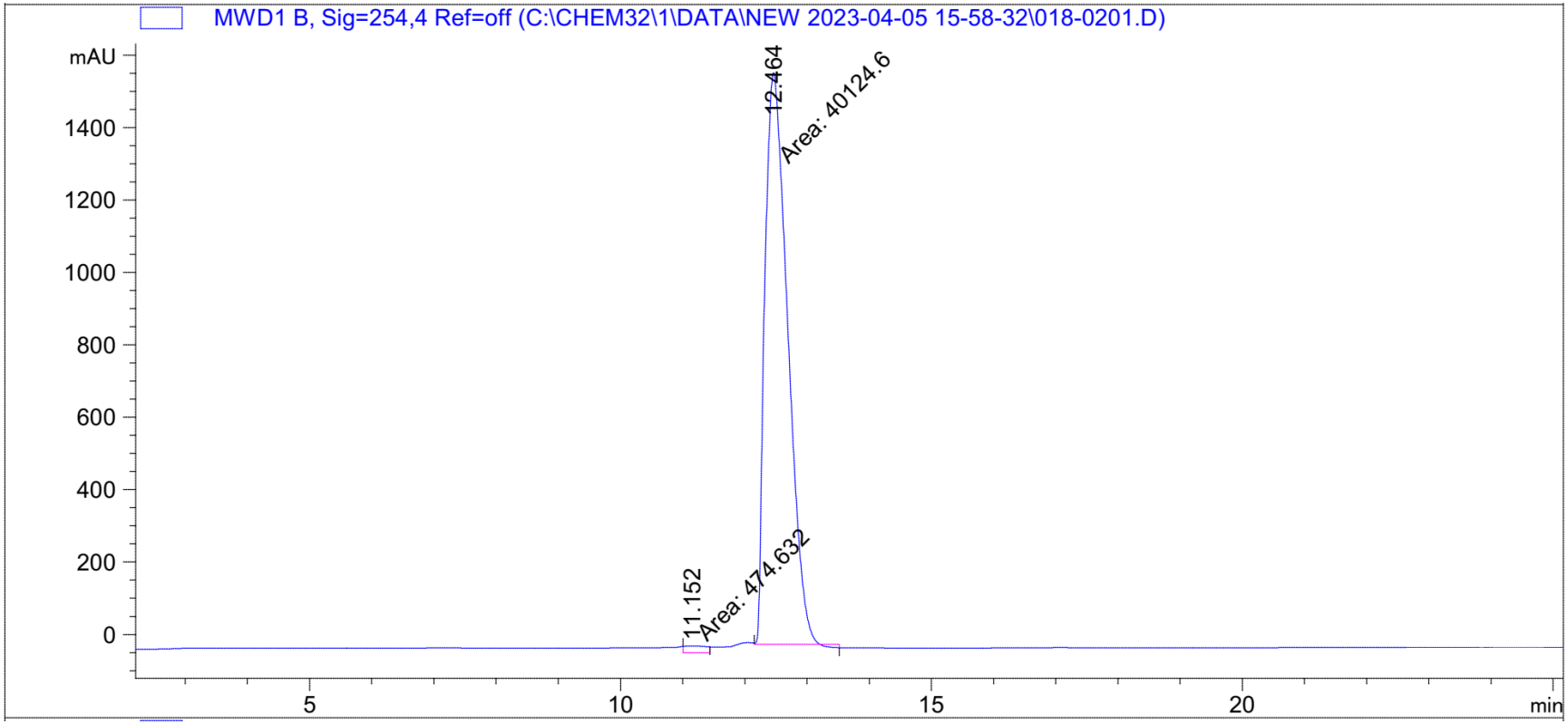
^{1}H NMR spectrum of **4h** (600 MHz, DMSO-d_6)



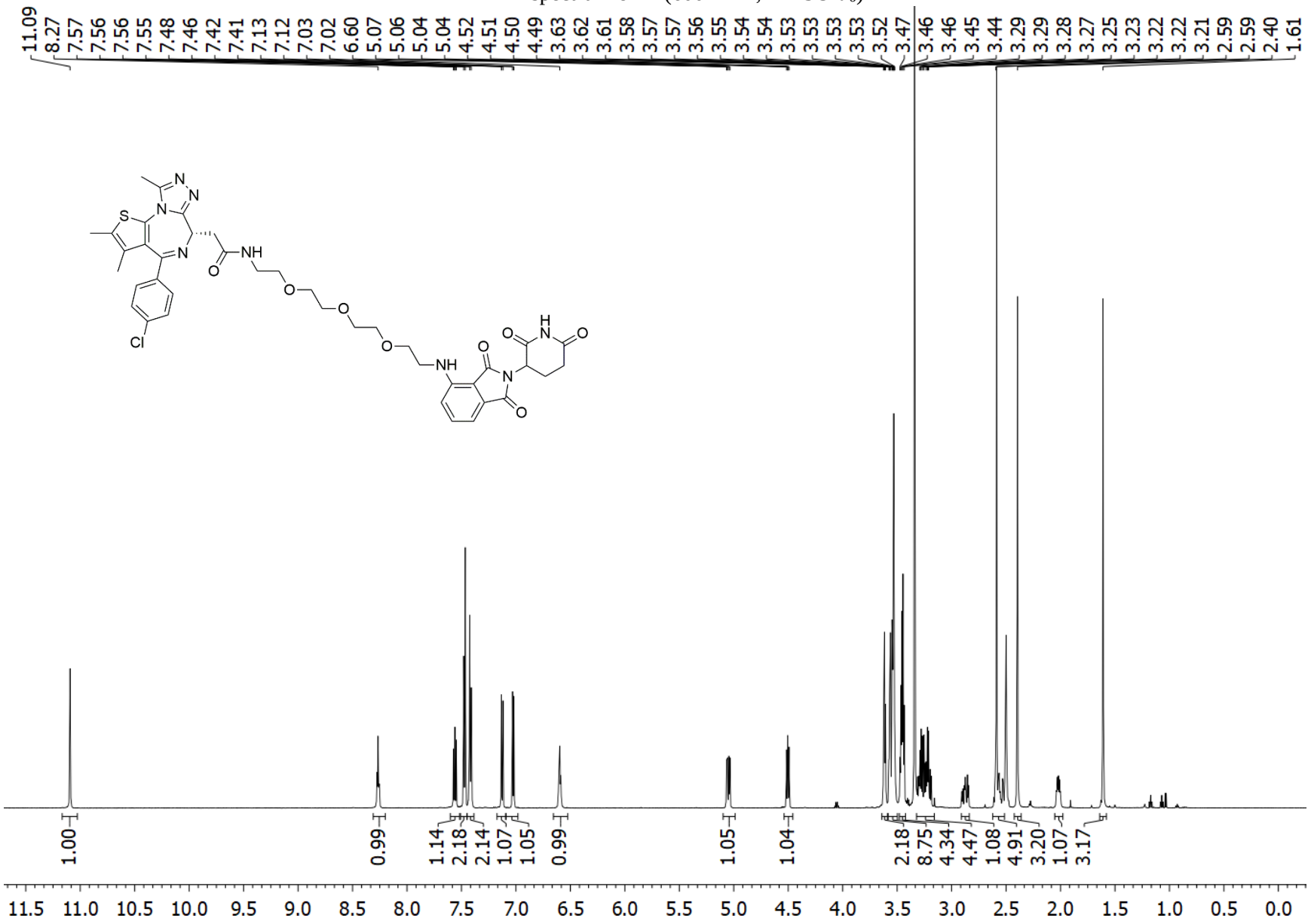
^{13}C NMR spectrum of **4h** (151 MHz, DMSO-d_6)



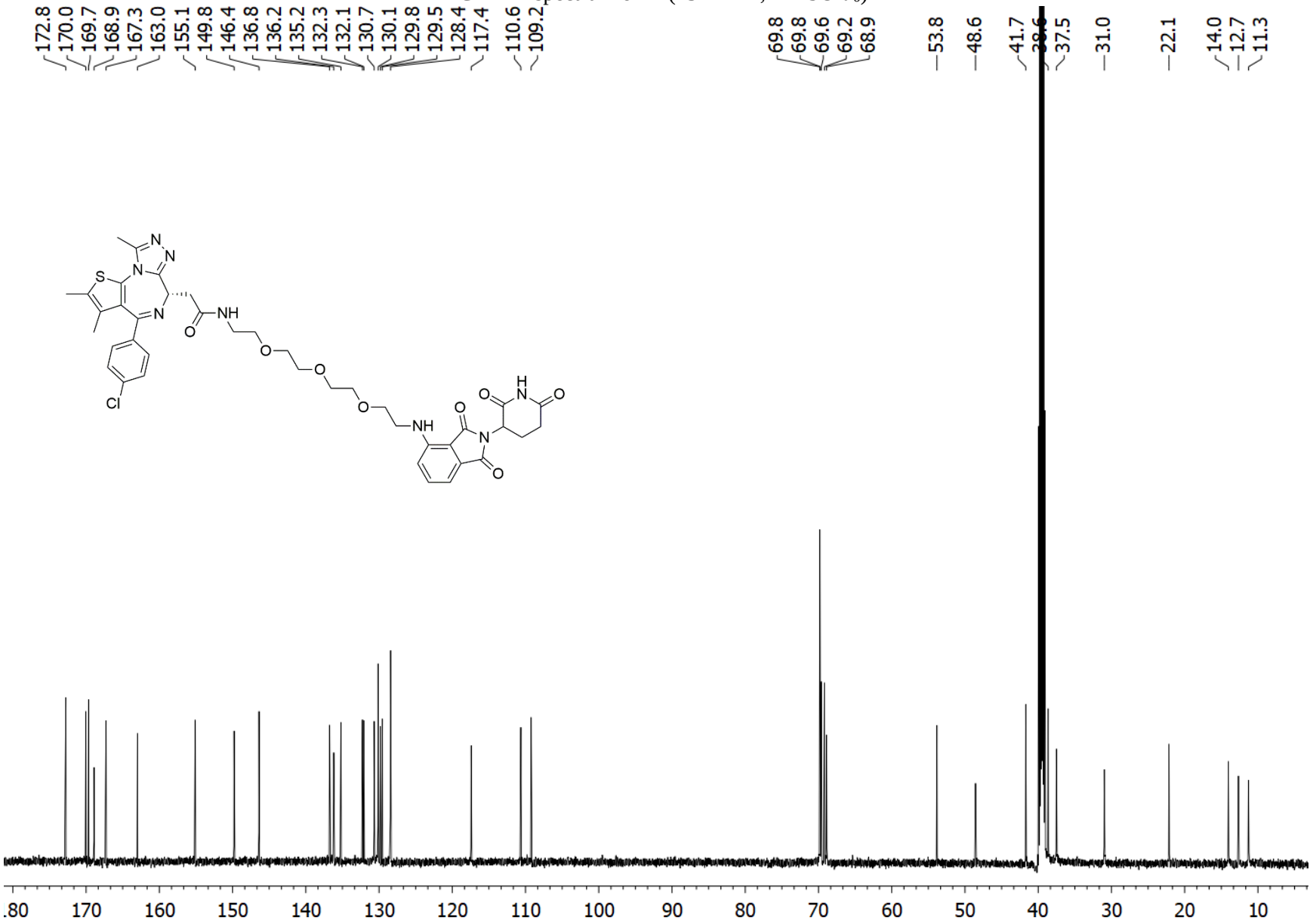
HPLC trace of **4h** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



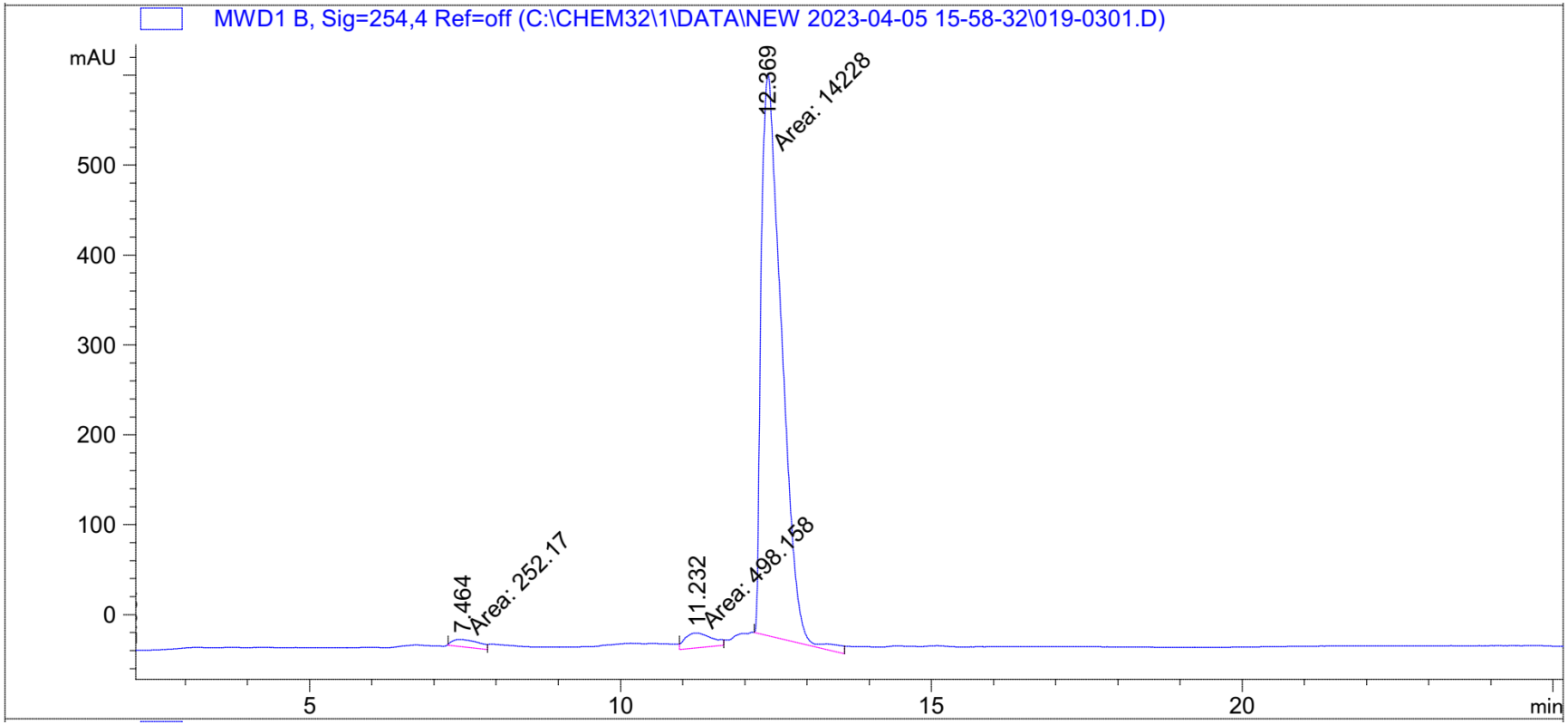
^{1}H NMR spectrum of **4i** (600 MHz, DMSO-d_6)



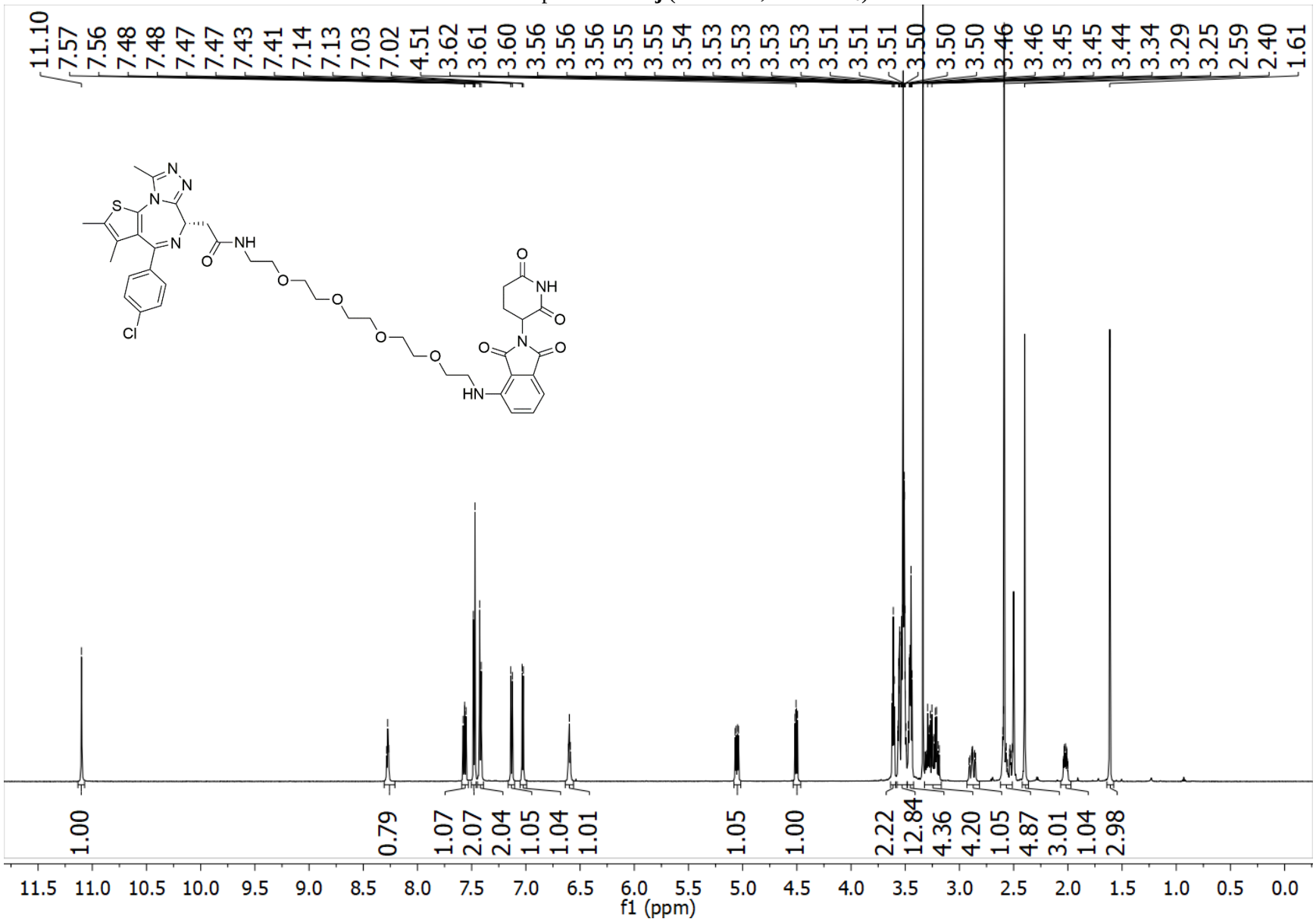
^{13}C NMR spectrum of **4i** (151 MHz, DMSO-d_6)



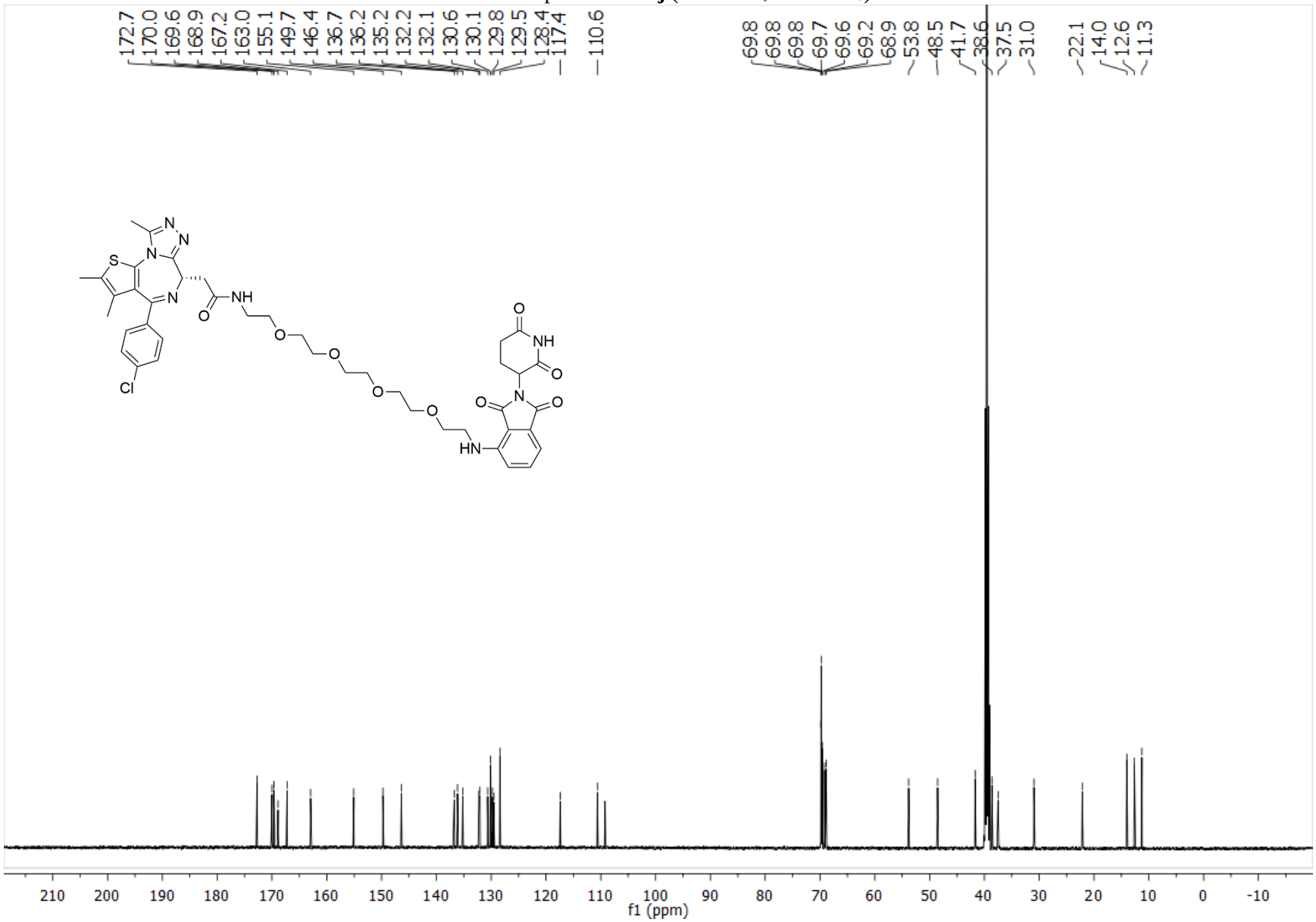
HPLC trace of **4i** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



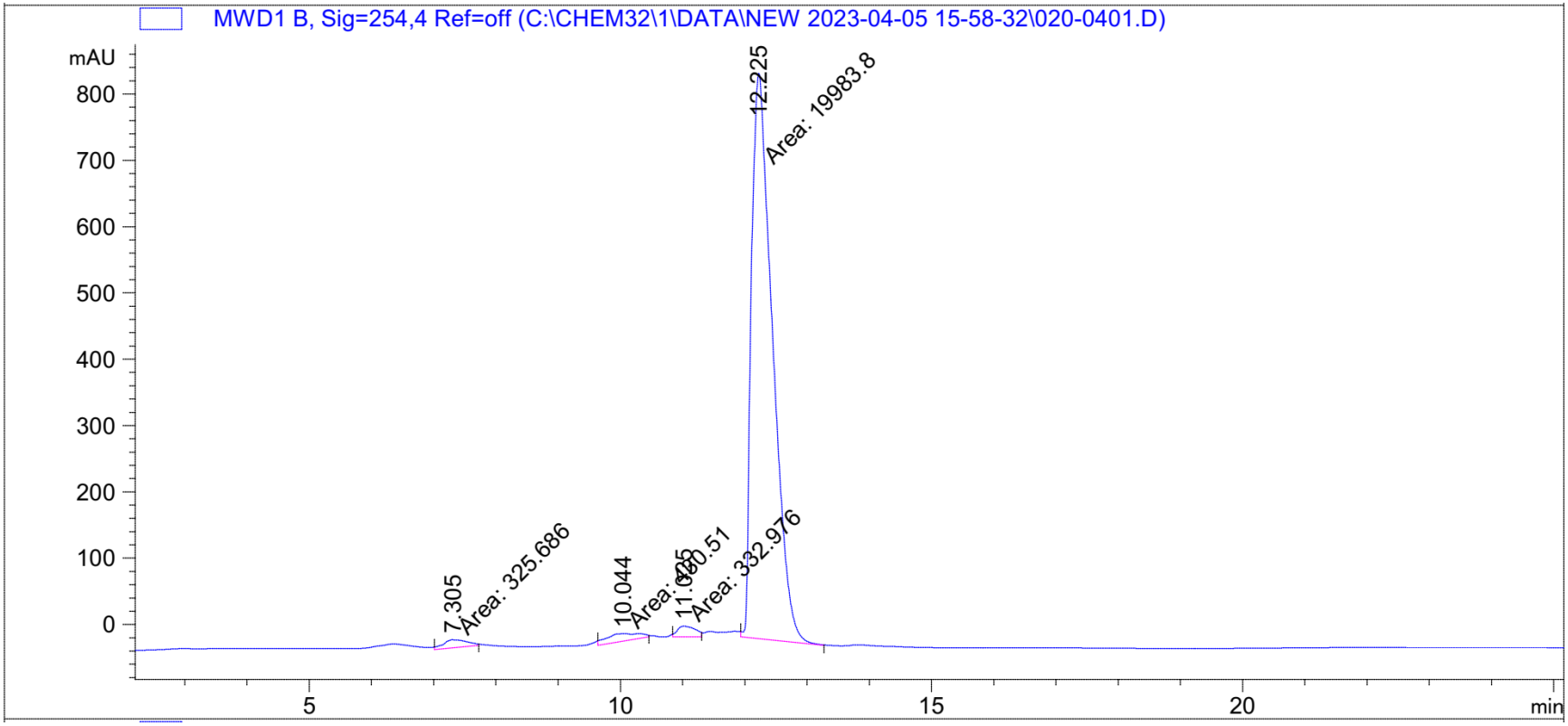
^{1}H NMR spectrum of **4j** (600 MHz, DMSO-d_6)



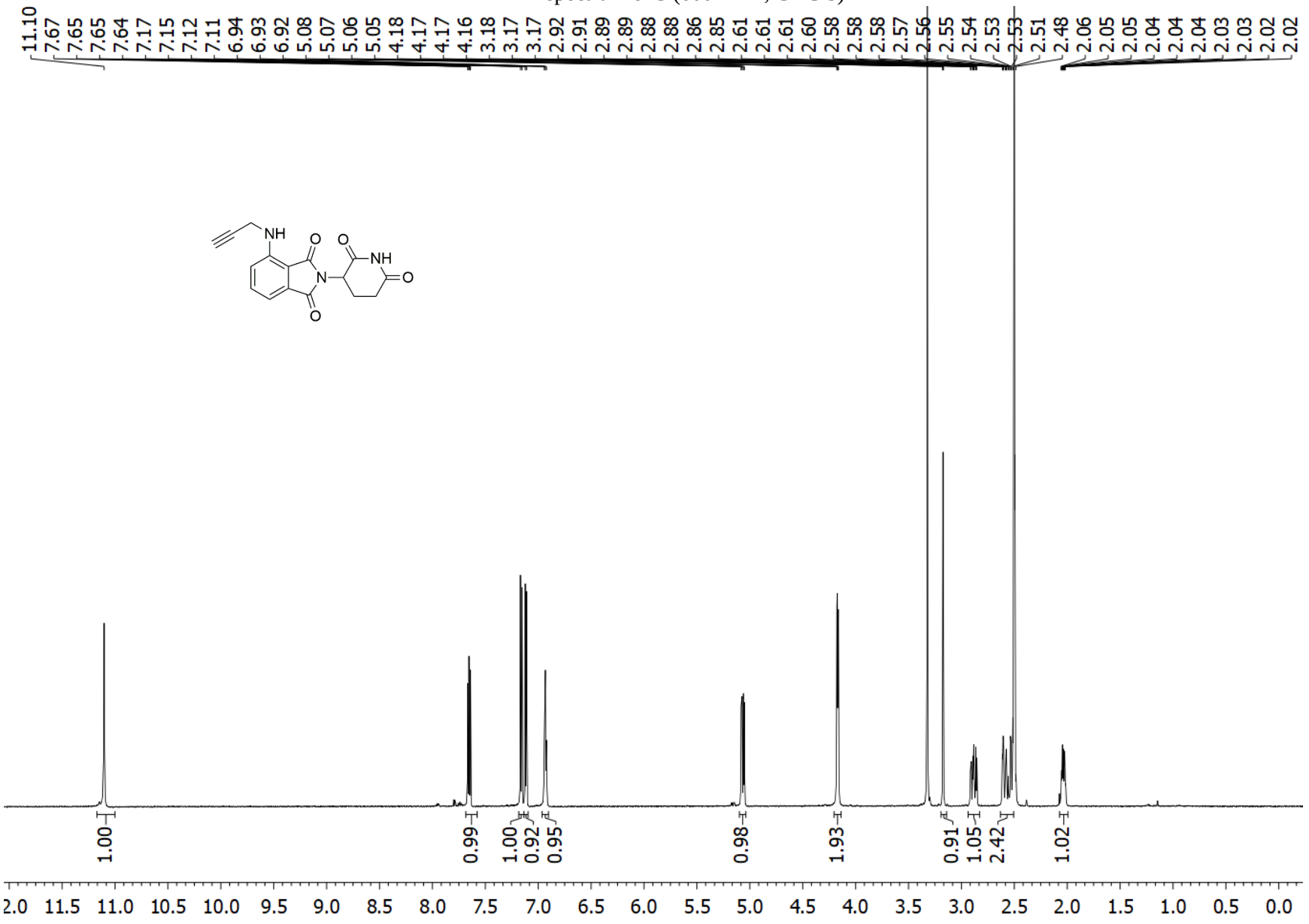
^{13}C NMR spectrum of **4j** (151 MHz, DMSO-d_6)



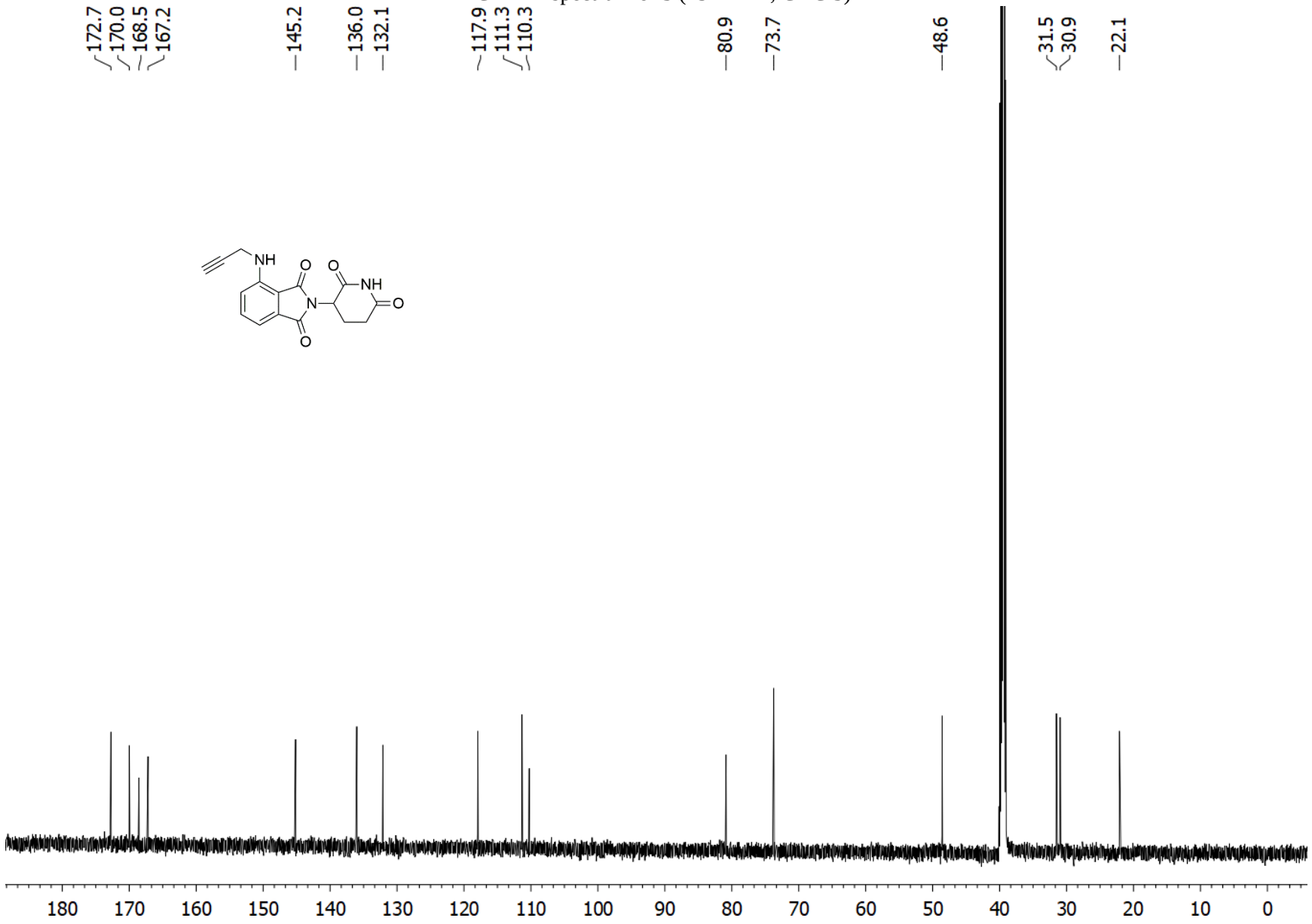
HPLC trace of **4j** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



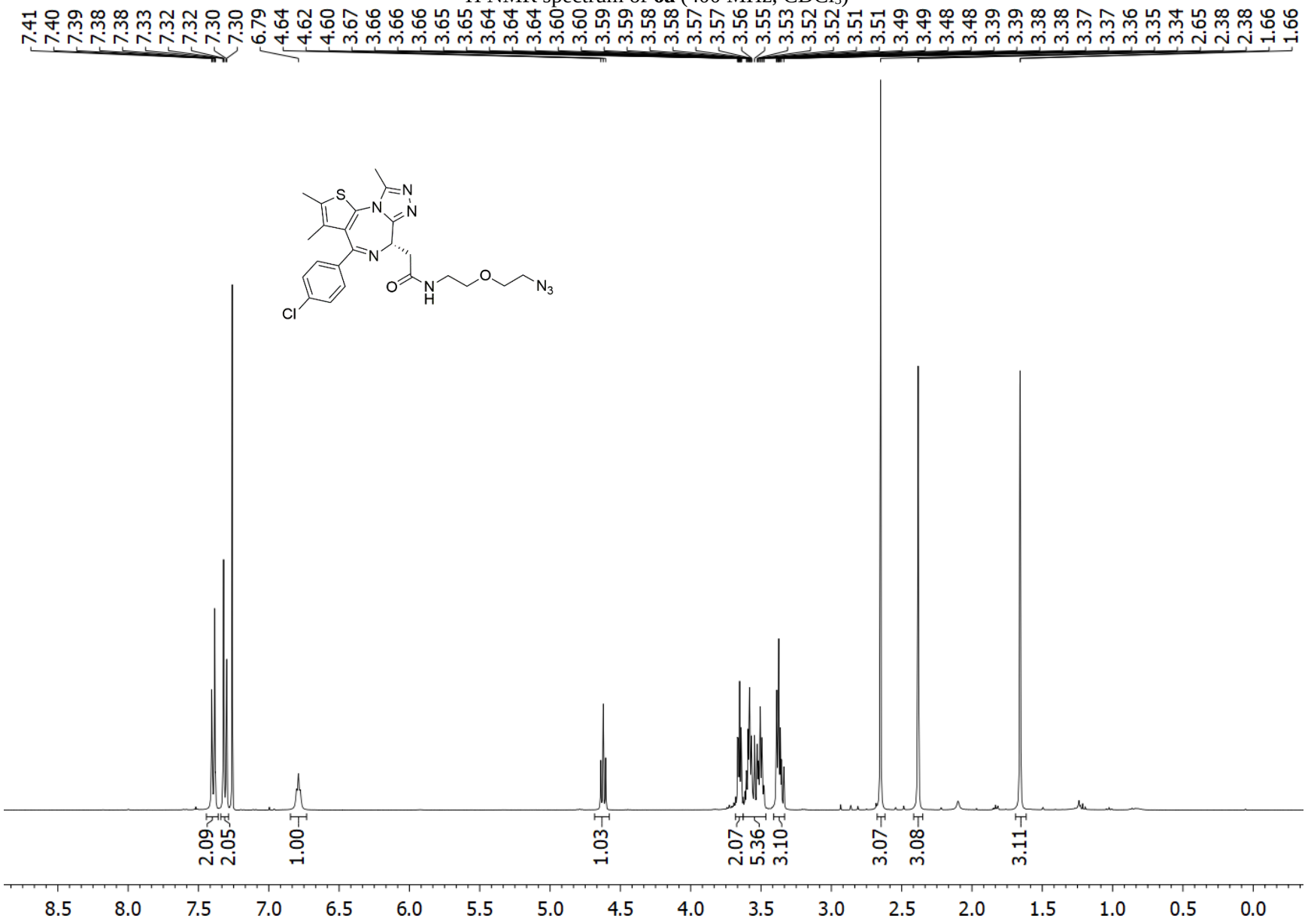
[1]H NMR spectrum of **5** (600 MHz, CDCl3)



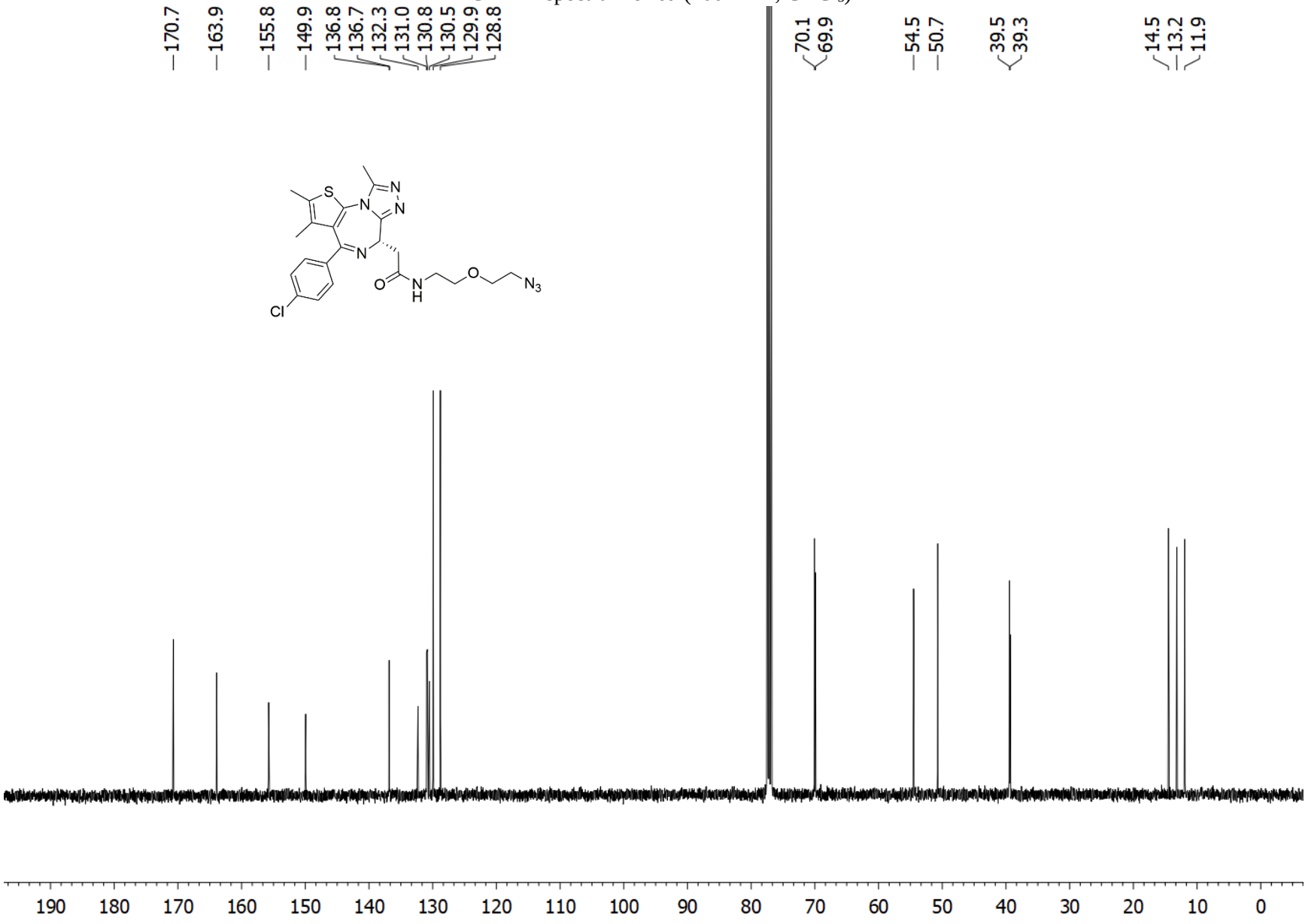
^{13}C NMR spectrum of **5** (151 MHz, CDCl3)



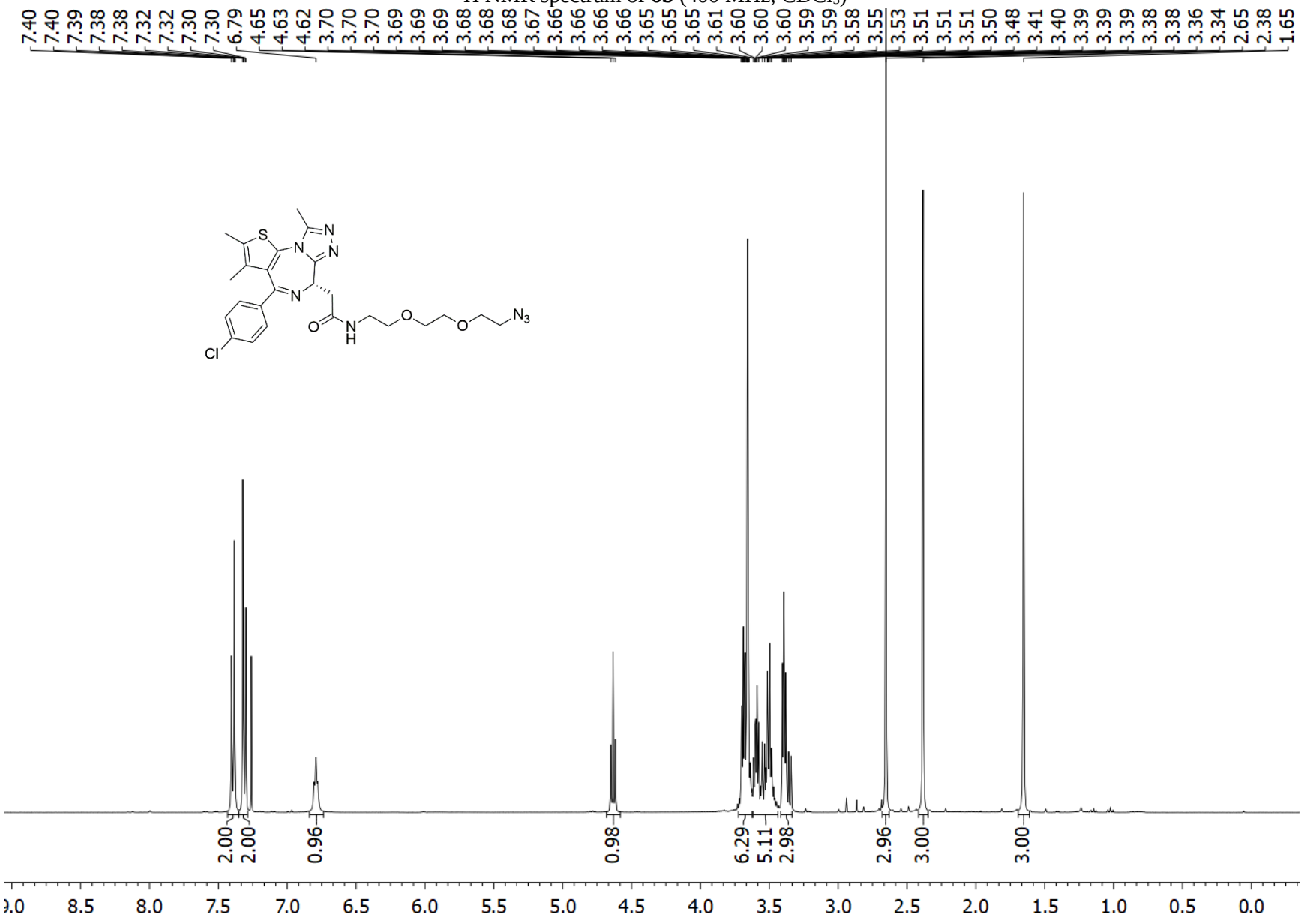
^{1}H NMR spectrum of **6a** (400 MHz, $CDCl_3$)



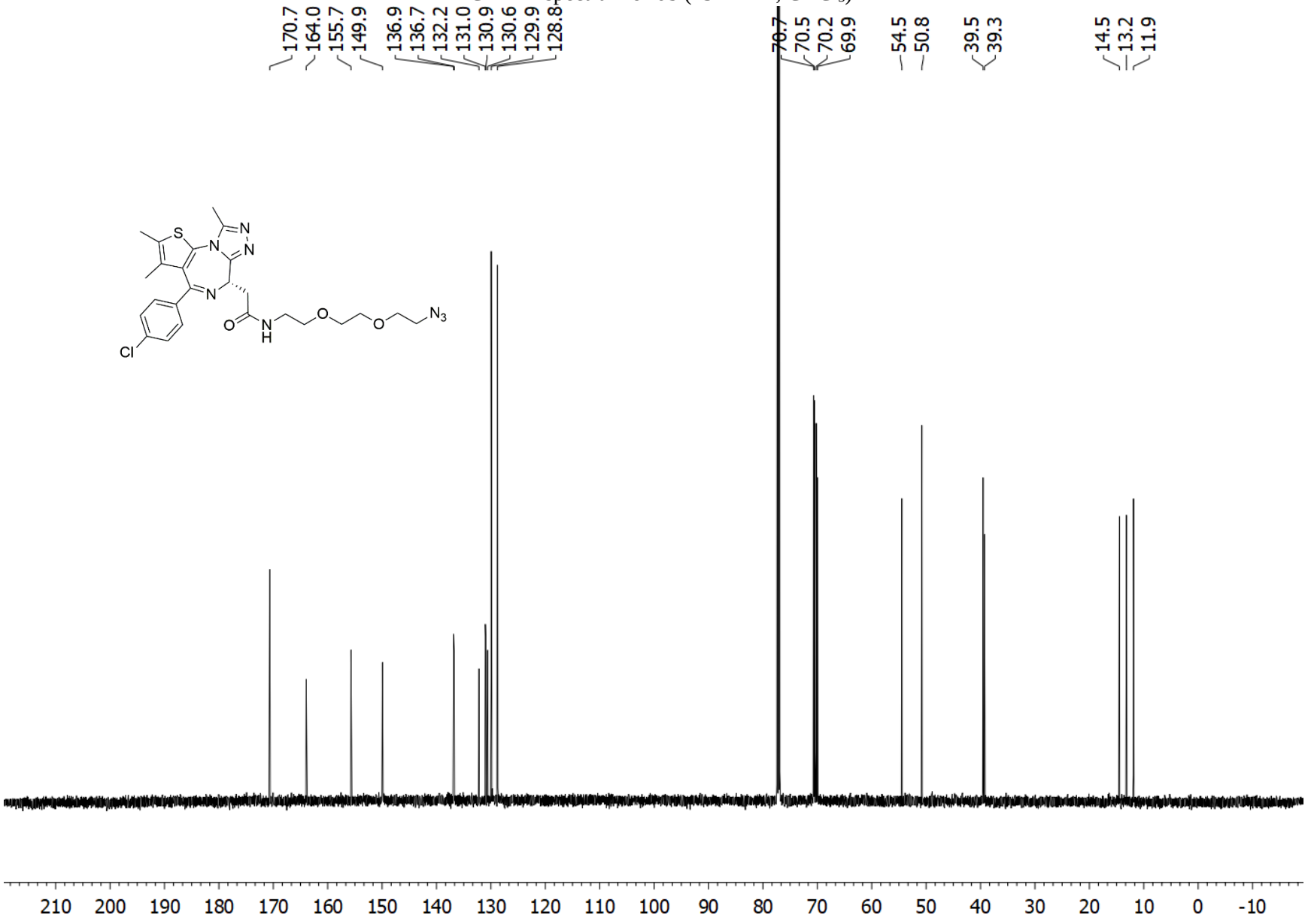
^{13}C NMR spectrum of **6a** (100 MHz, $CDCl_3$)



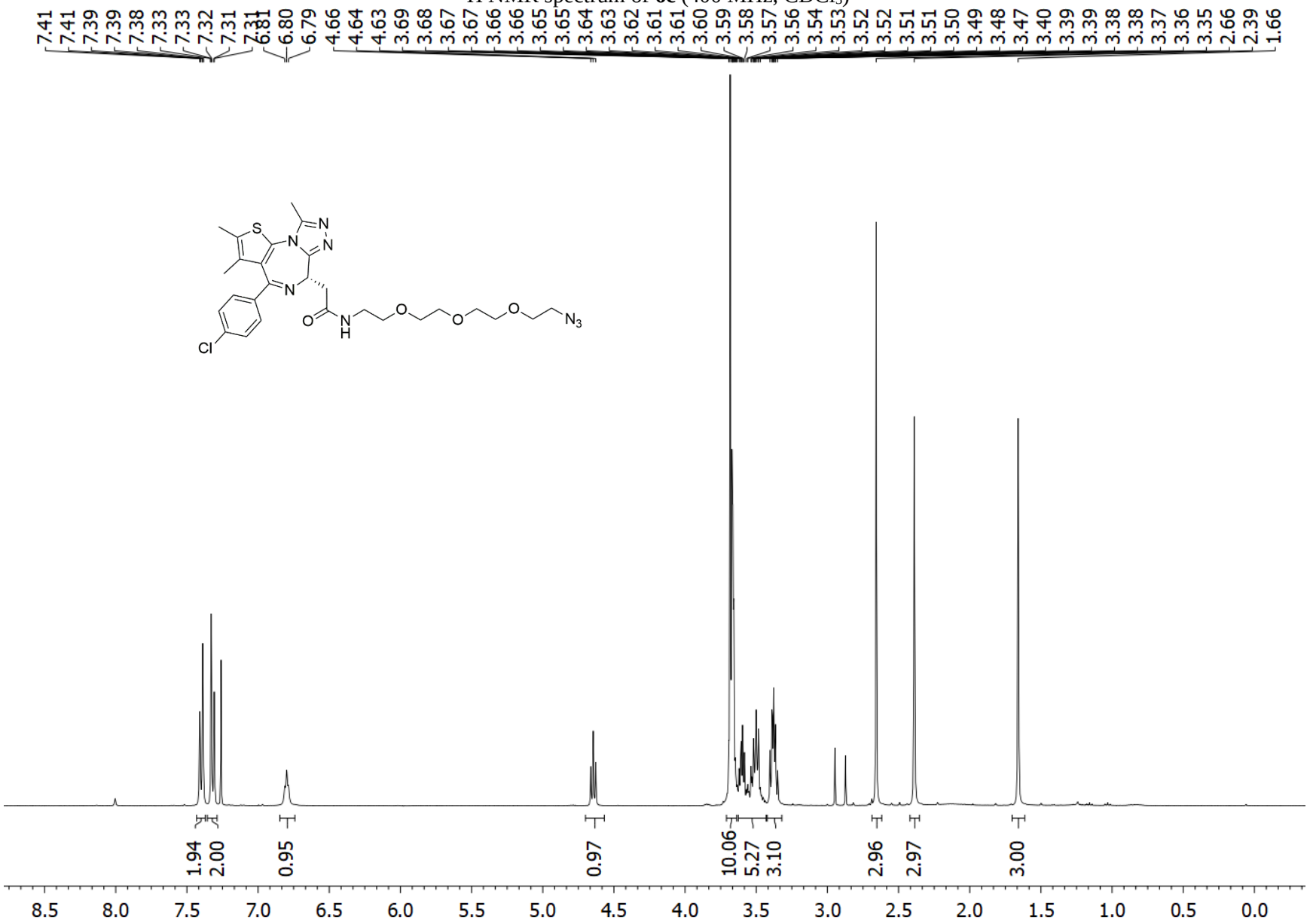
^{1}H NMR spectrum of **6b** (400 MHz, $CDCl_3$)



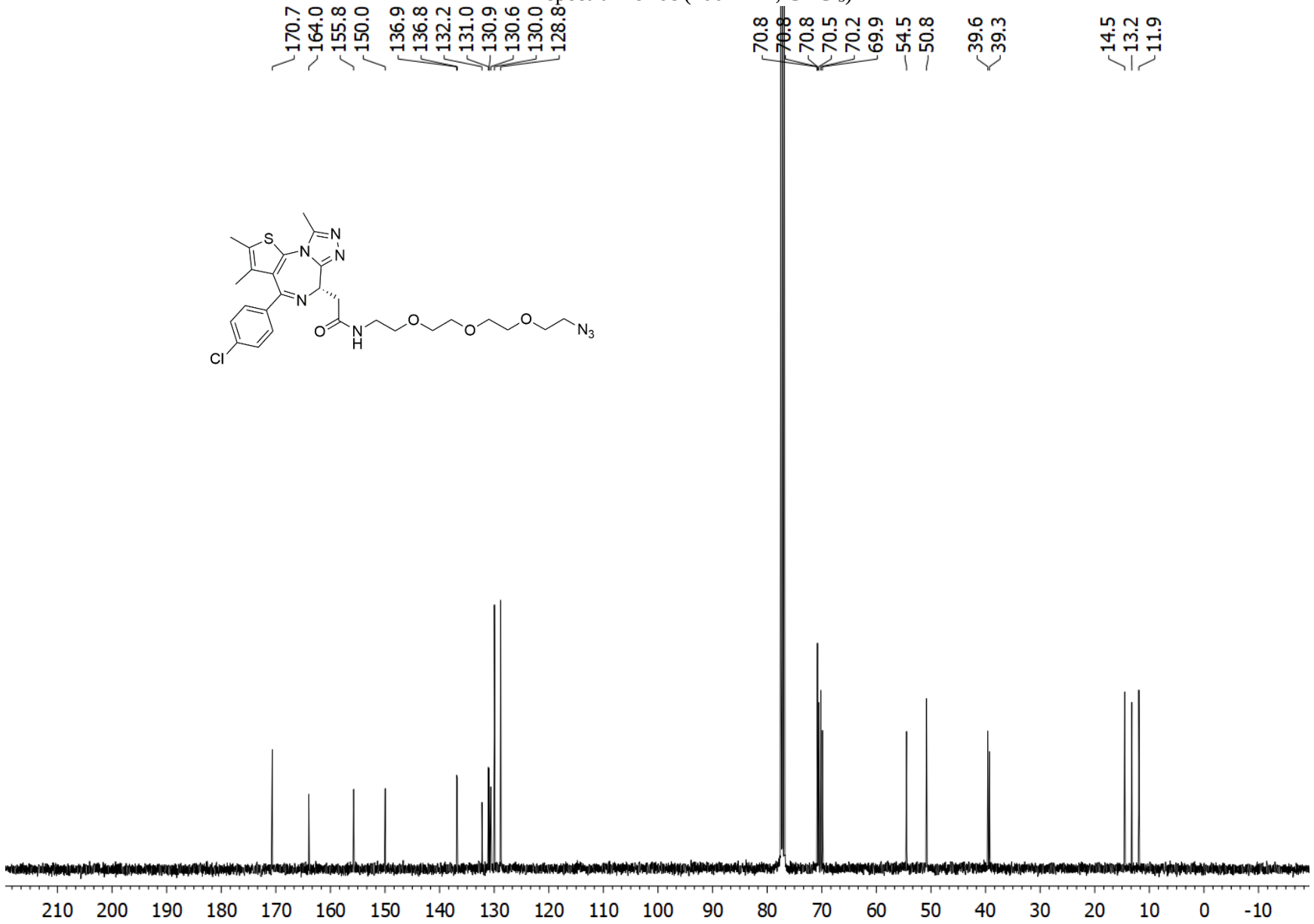
^{13}C NMR spectrum of **6b** (151 MHz, $CDCl_3$)



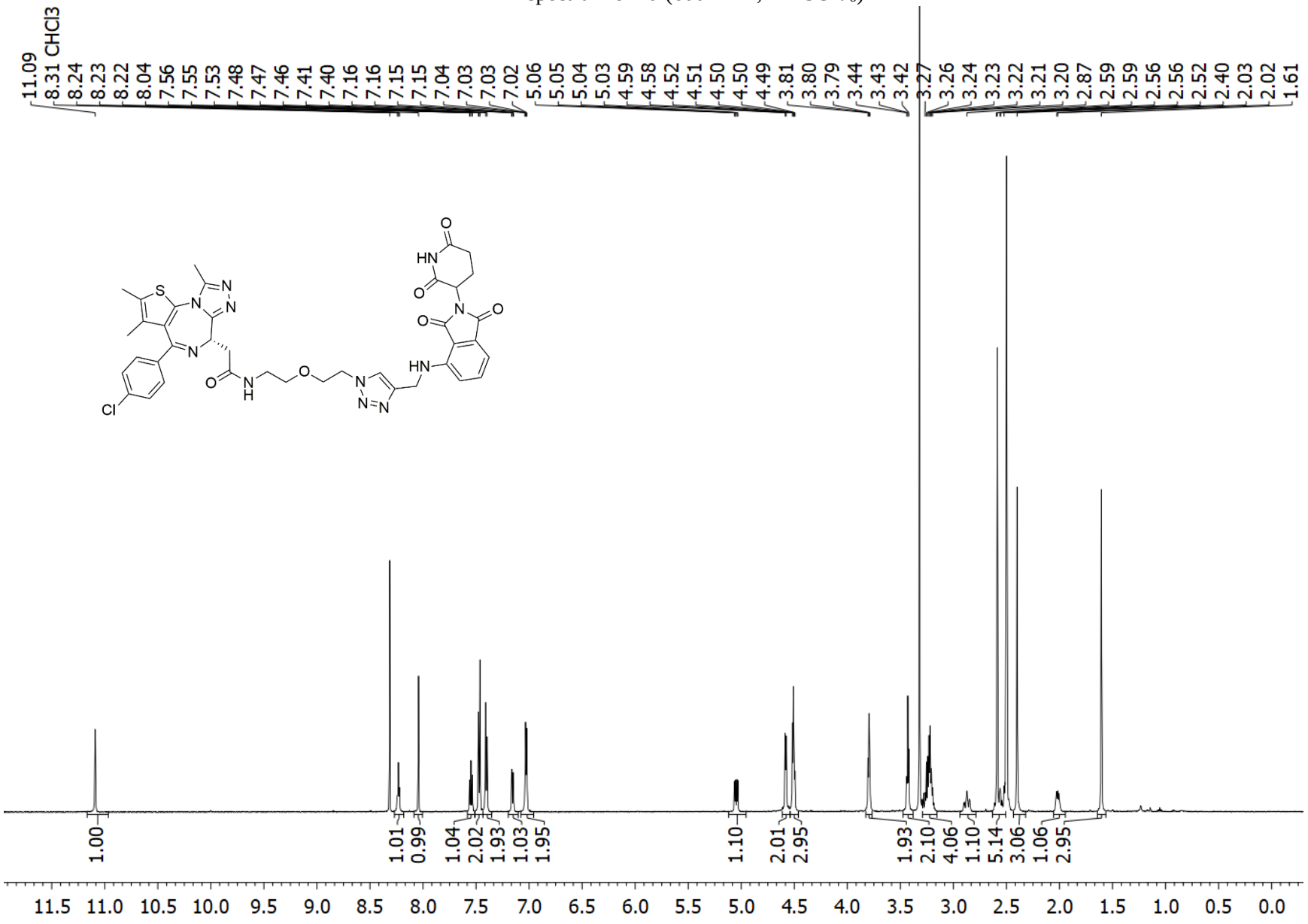
^{1}H NMR spectrum of **6c** (400 MHz, $CDCl_3$)



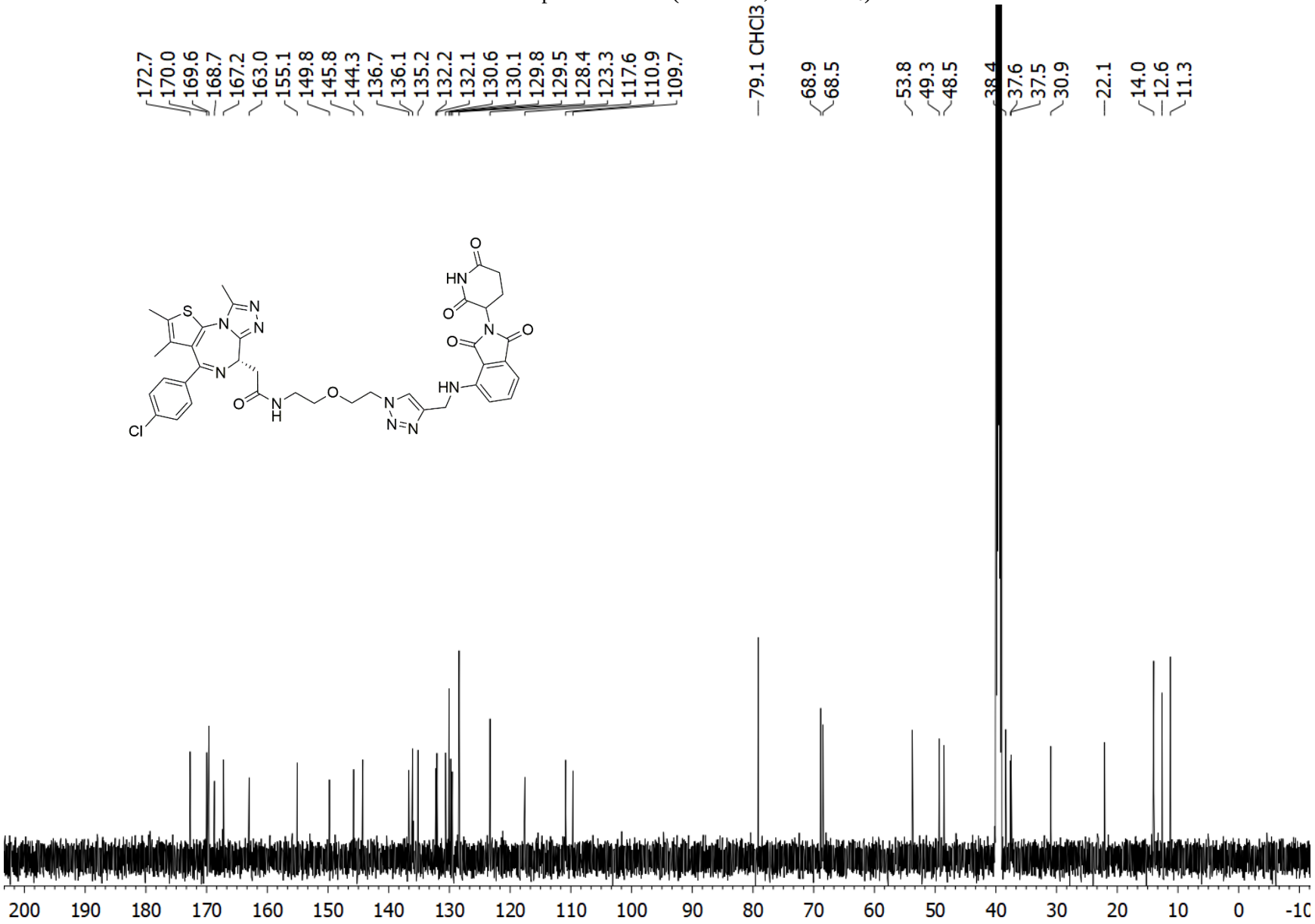
^{1}H NMR spectrum of **6c** (100 MHz, $CDCl_3$)



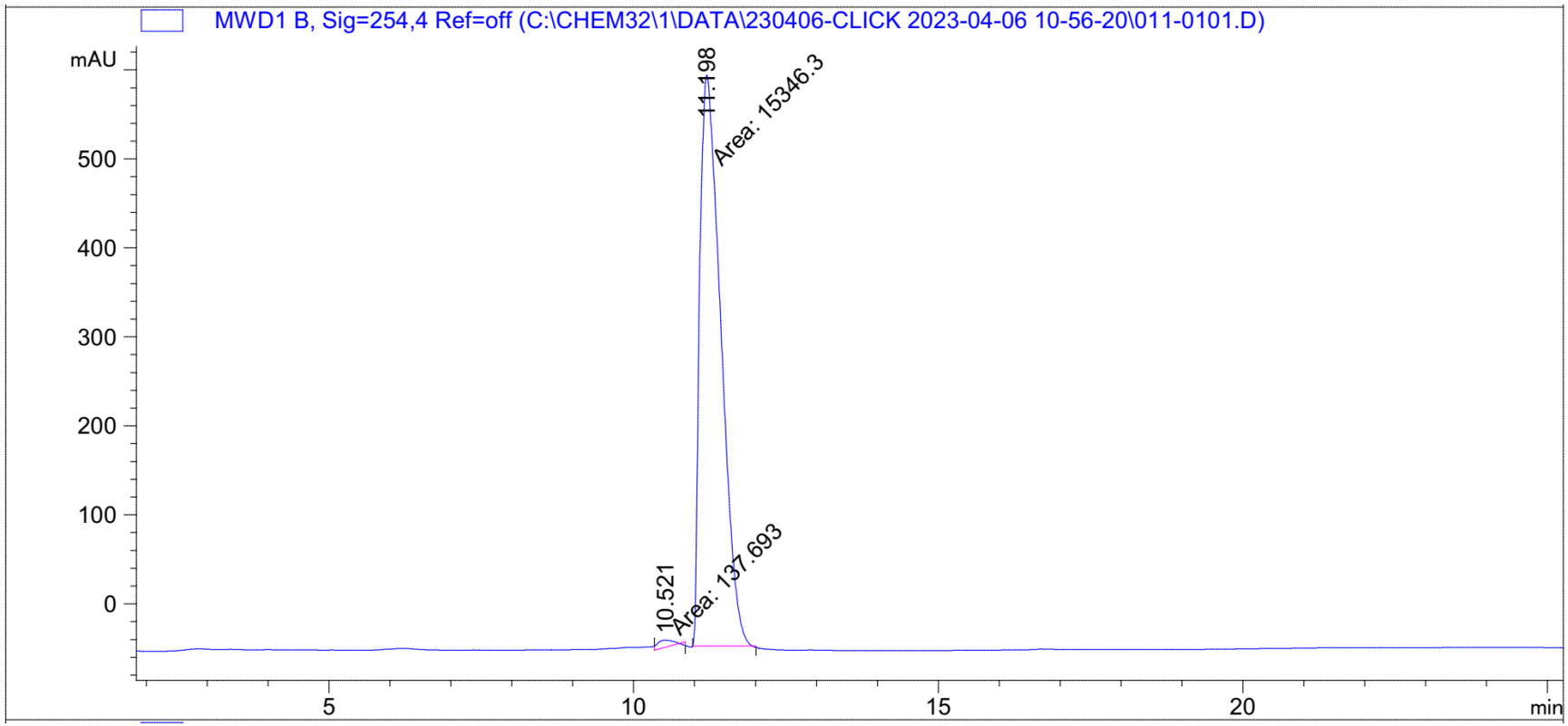
^{1}H NMR spectrum of **7a** (600 MHz, DMSO-d_6)



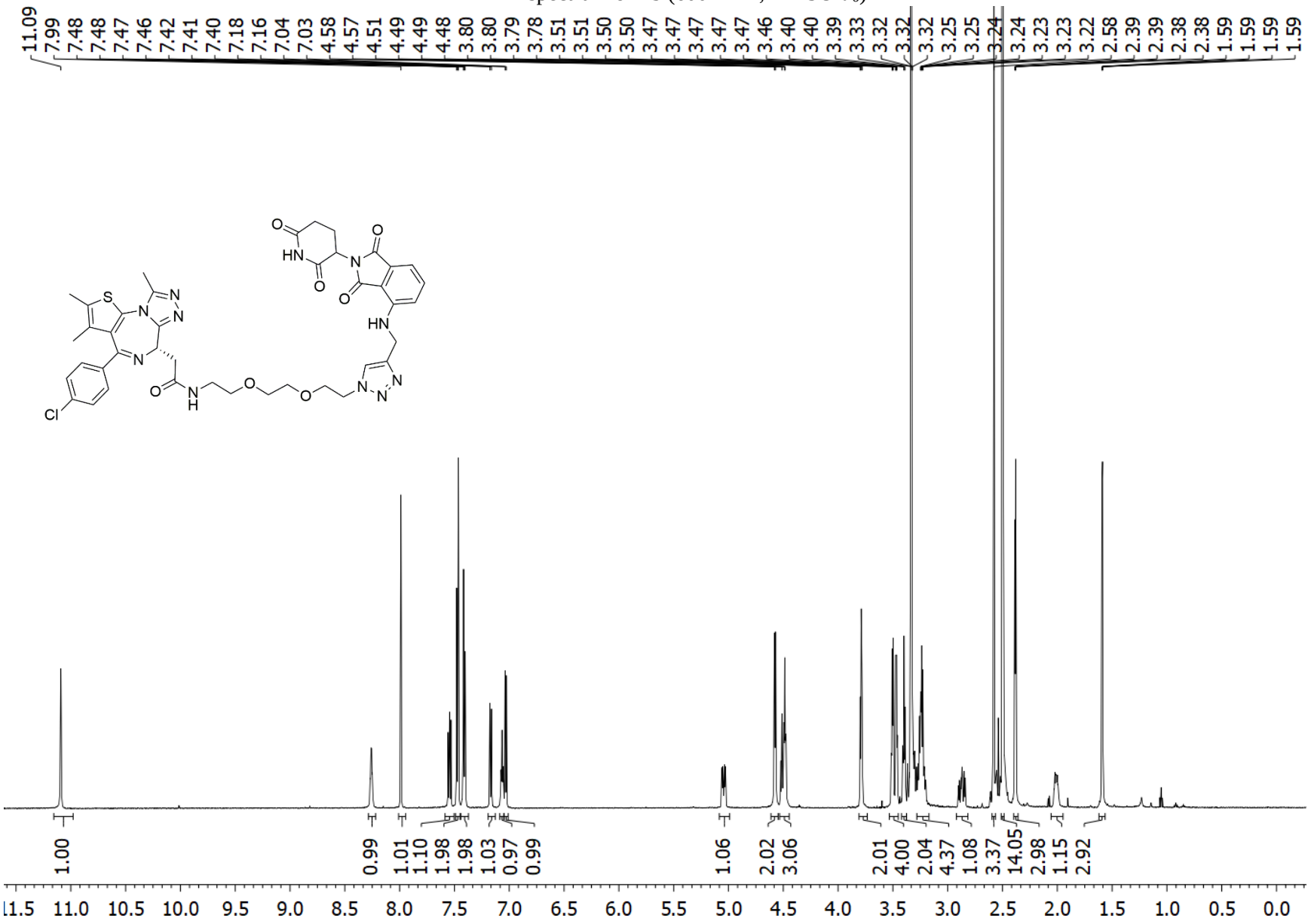
^{13}C NMR spectrum of **7a** (151 MHz, DMSO-d_6)



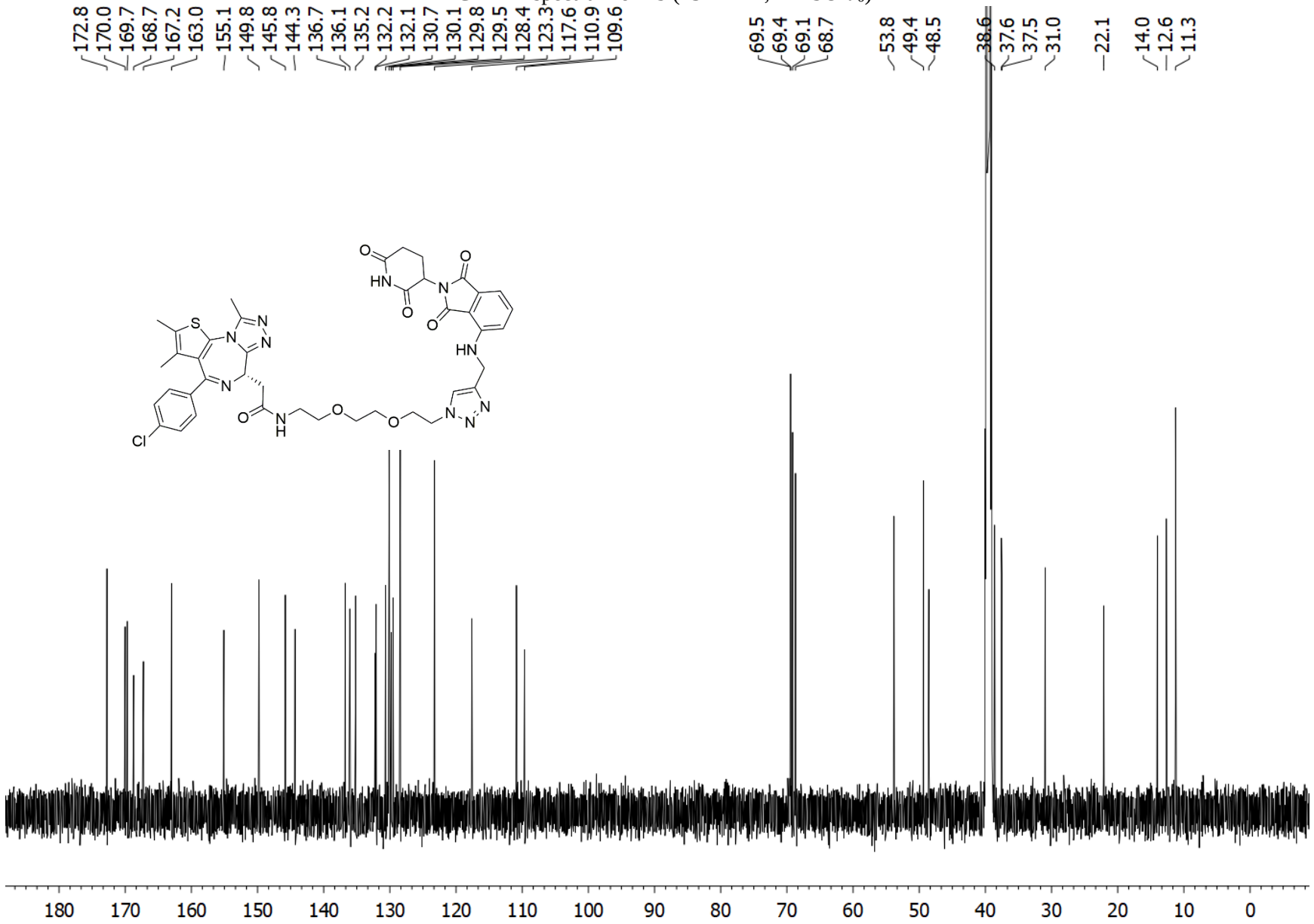
HPLC trace of **7a** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



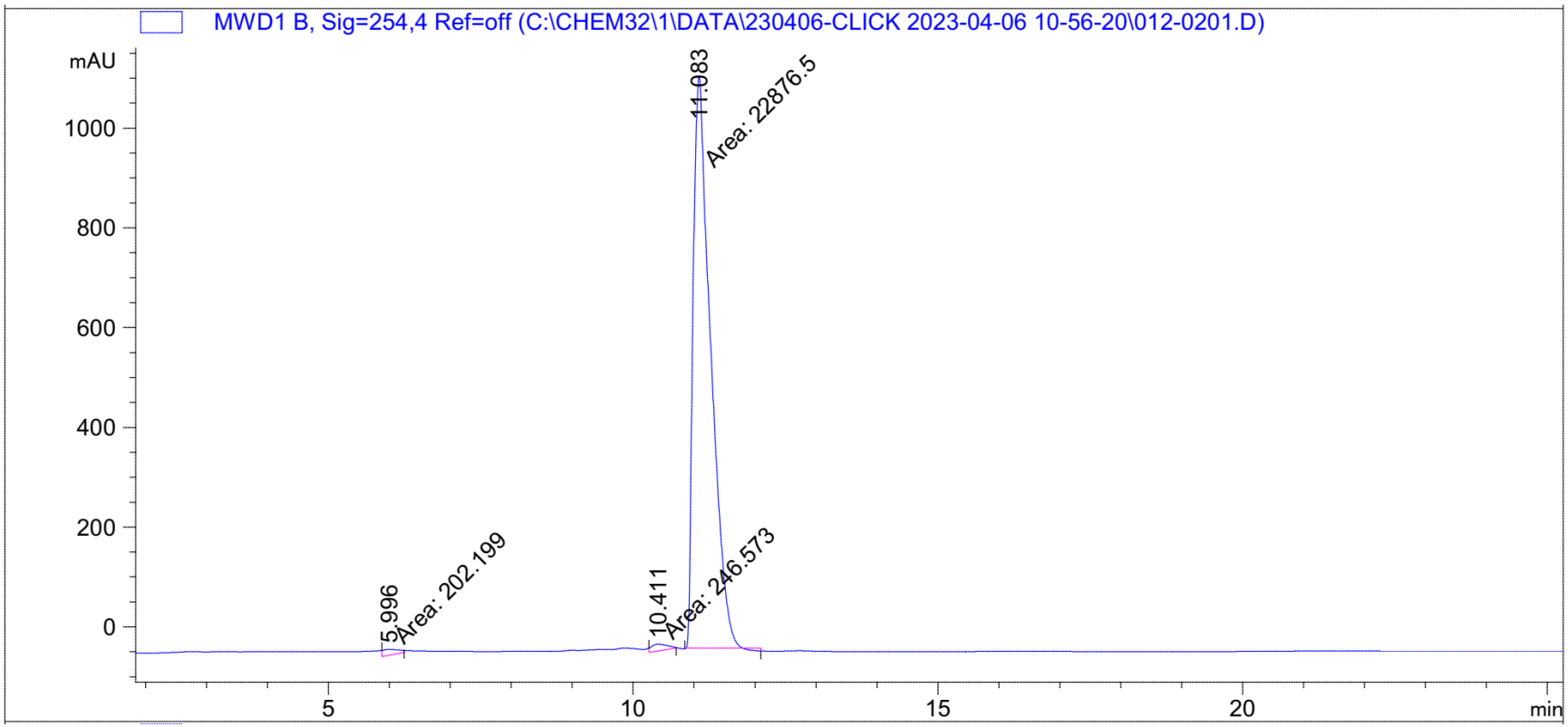
^{1}H NMR spectrum of **7b** (600 MHz, DMSO-d_6)



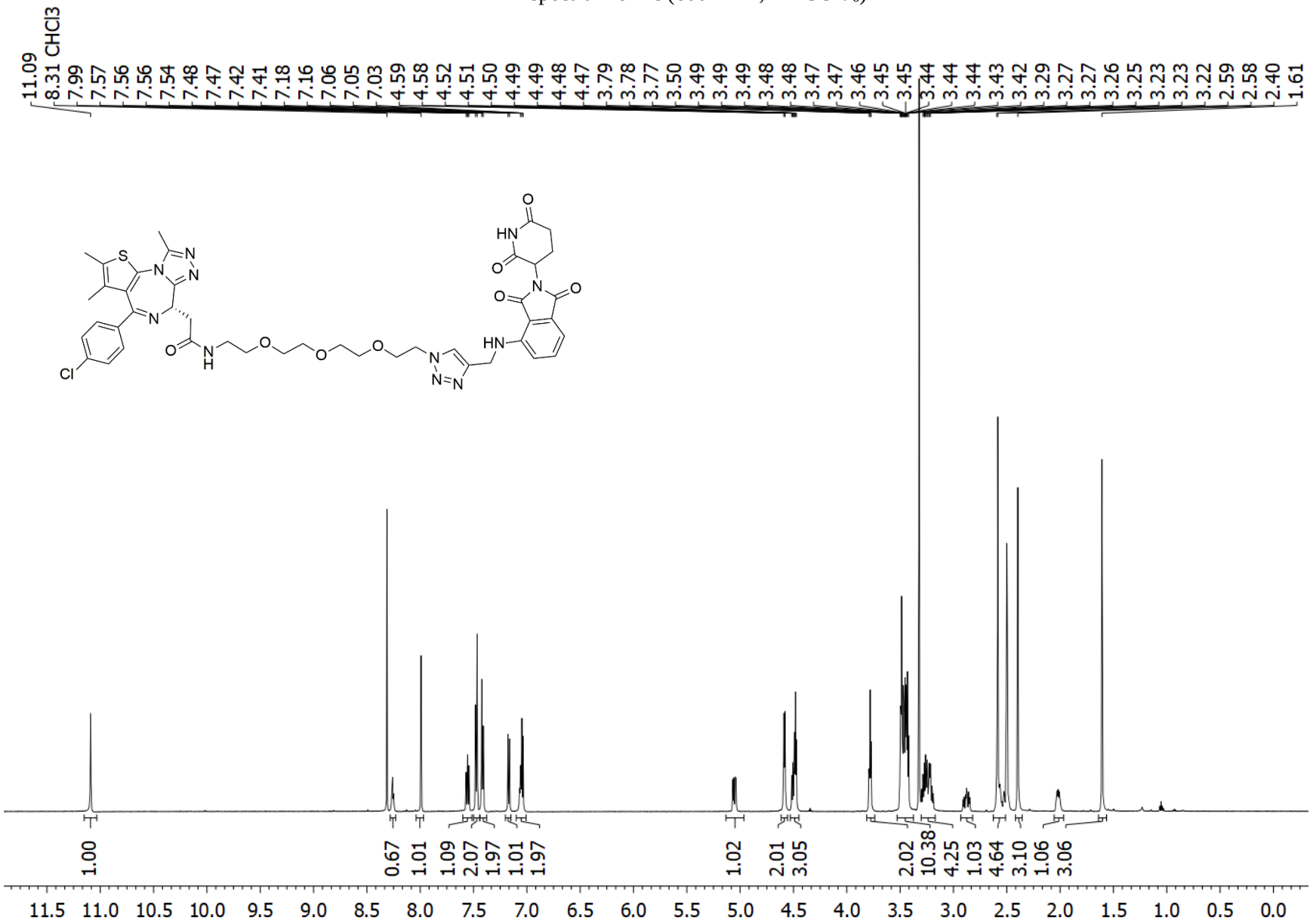
^{13}C NMR spectrum of **7b** (151 MHz, DMSO-d_6)



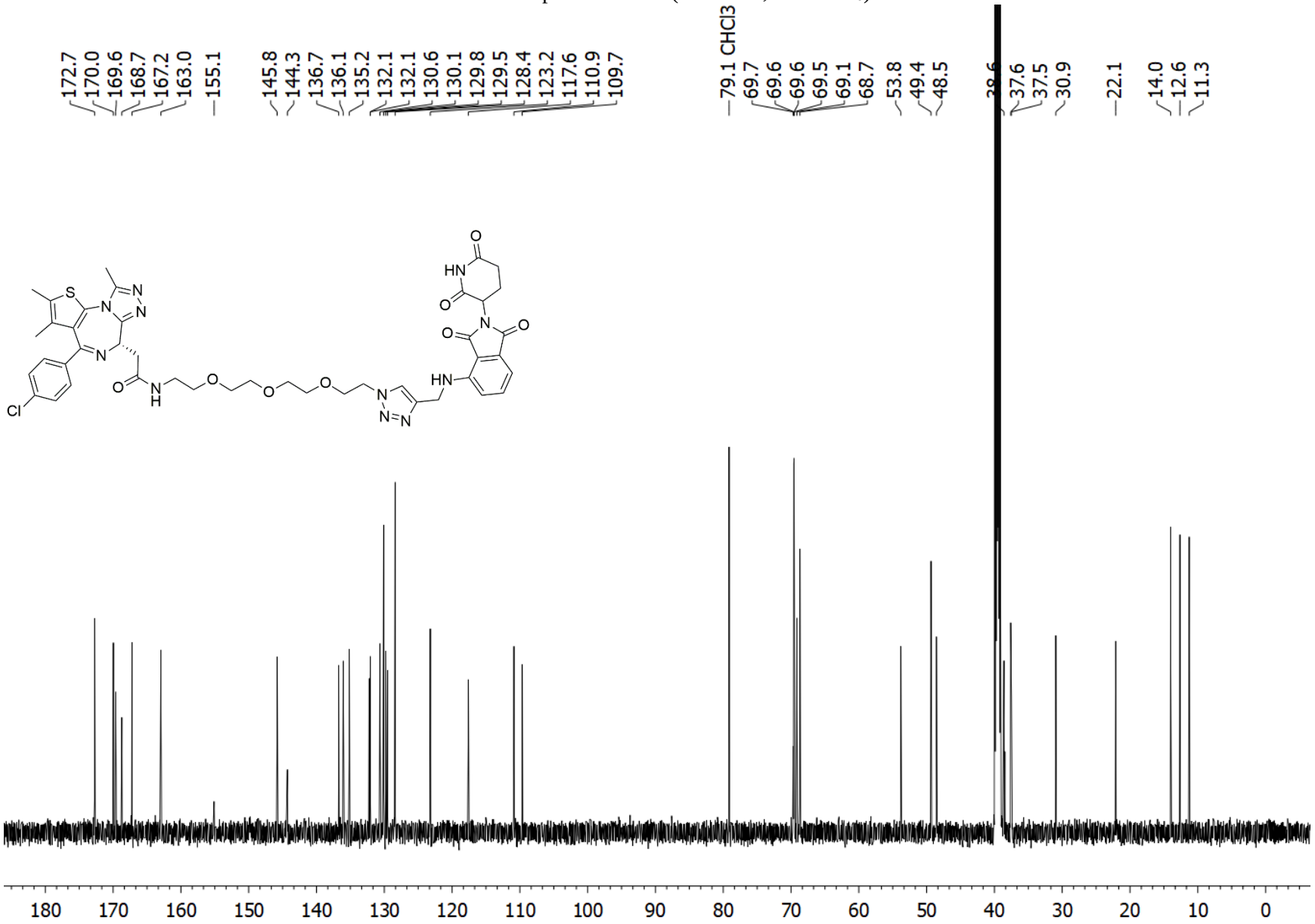
HPLC trace of **7b** (5:95 – 95:5 MeCN:H_2O over 20 minutes)



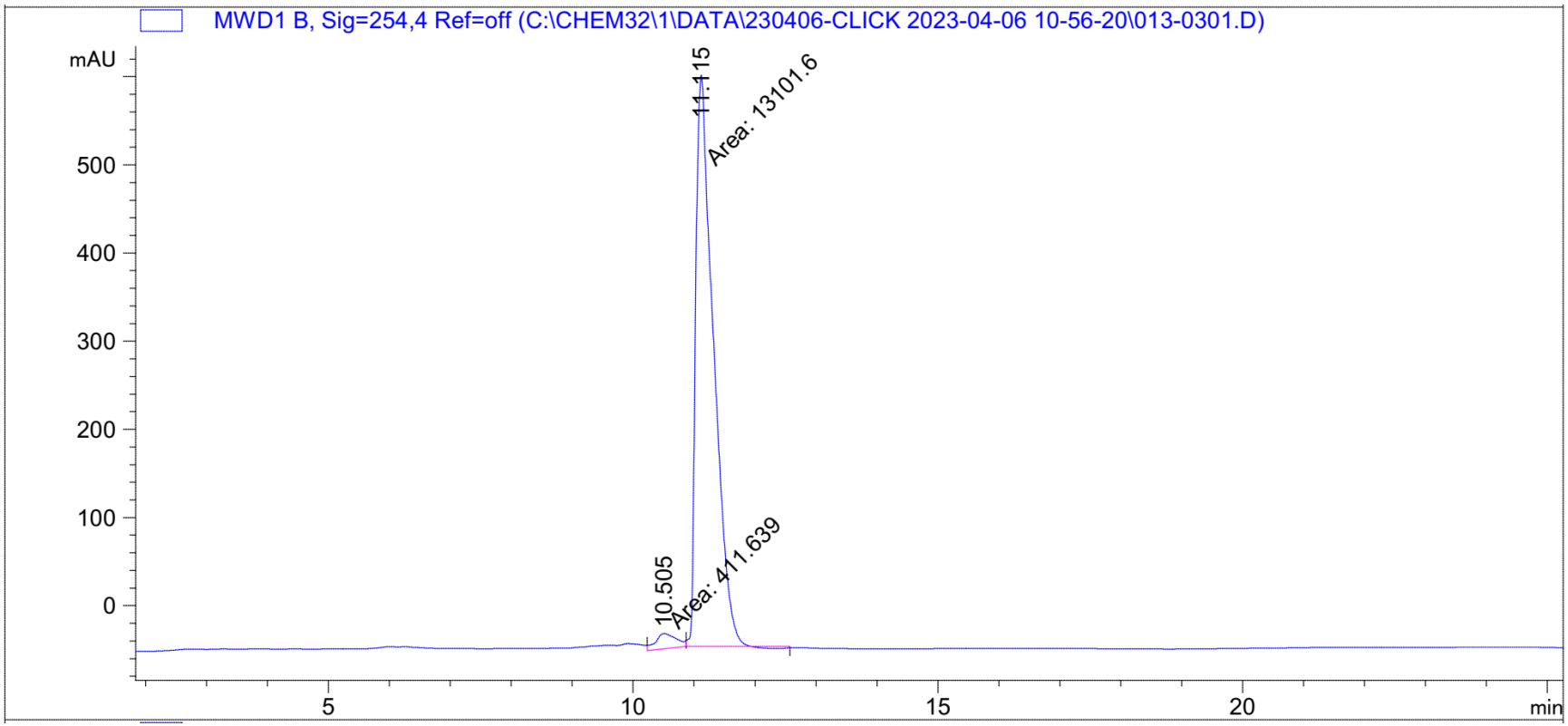
^{1}H NMR spectrum of **7c** (600 MHz, DMSO-d_6)



^{13}C NMR spectrum of **7c** (151 MHz, DMSO-d_6)



HPLC trace of **7c** (5:95 – 95:5 MeCN:H_2O over 20 minutes)

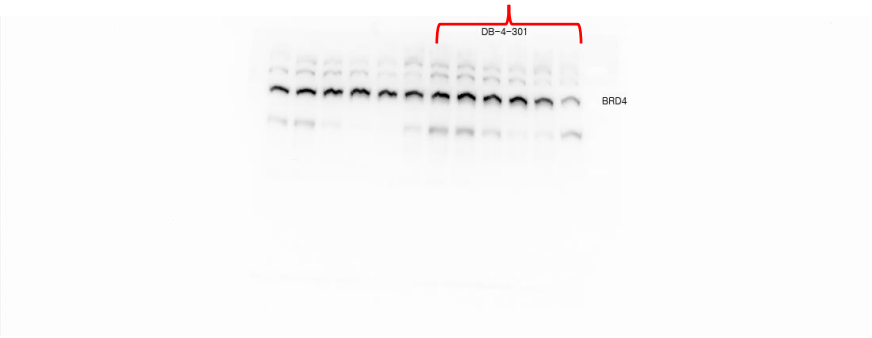


References:

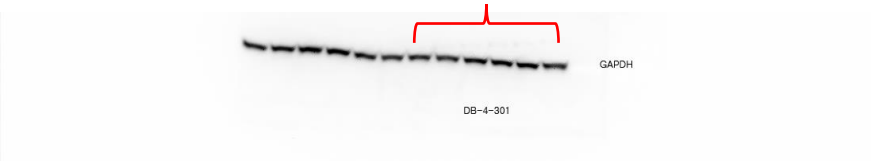
1. C. Würth, M. Grabolle, J. Pauli, M. Spieles and U. Resch-Genger, *Nat. Protoc.*, 2013, **8**, 1535-1550.
2. K. S. Watts, P. Dalal, R. B. Murphy, W. Sherman, R. A. Friesner and J. C. Shelley, *J. Chem Inf. Model.*, 2010, **50**, 534-546.
3. W. L. Jorgensen and E. M. Duffy, *Adv. Drug Deliv. Rev.*, 2002, **54**, 355-366.

Copies of immunoblots:

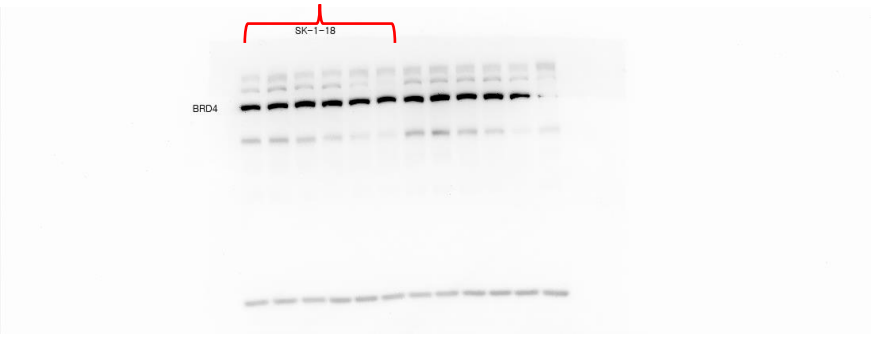
Immunoblot for BRD4 after treatment with **4c** (DB-4-301)



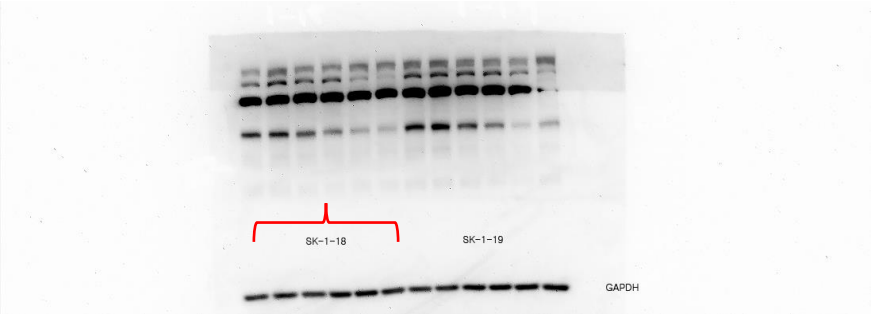
Immunoblot for GAPDH (loading control) after treatment with **4c** (DB-4-301)



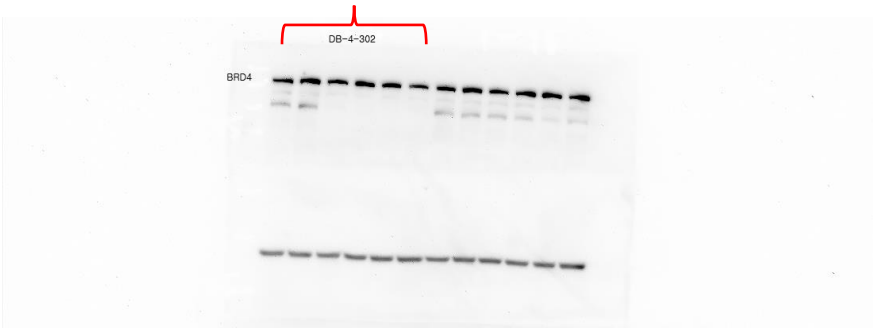
Immunoblot for BRD4 after treatment with **4e** (SK-1-18)



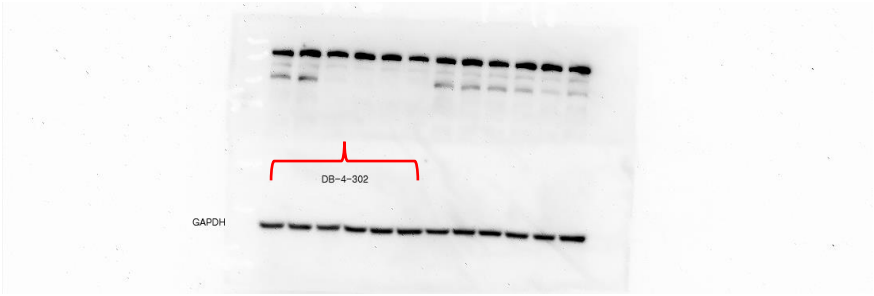
Immunoblot for GAPDH (loading control) after treatment with **4e** (SK-1-18)



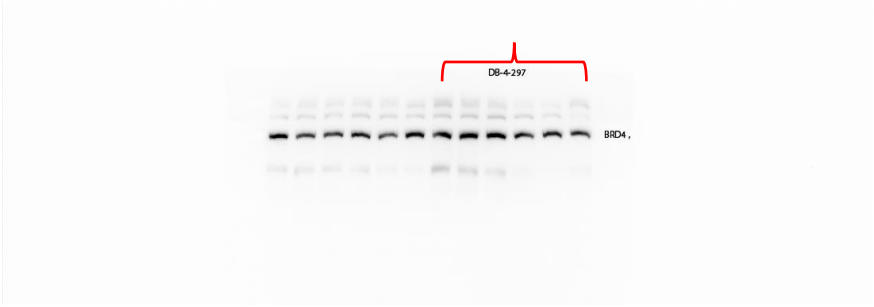
Immunoblot for BRD4 after treatment with **4f** (DB-4-302)



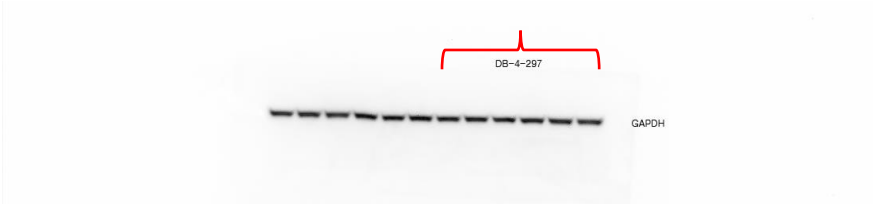
Immunoblot for GAPDH (loading control) after treatment with **4f** (DB-4-302)



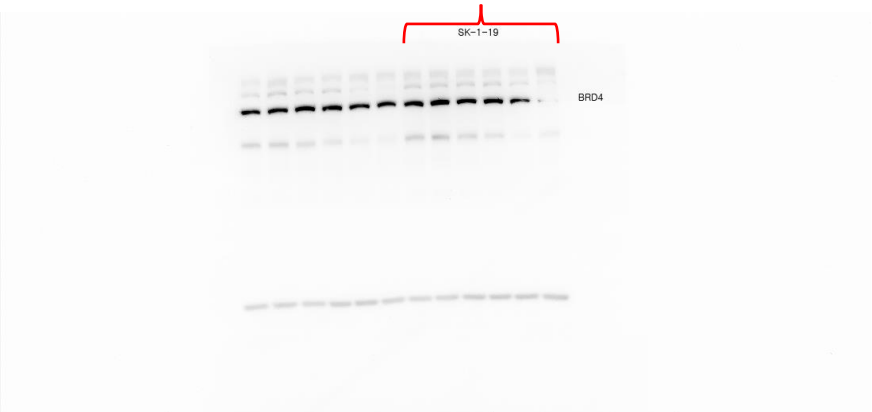
Immunoblot for BRD4 after treatment with **4h** (DB-4-297)



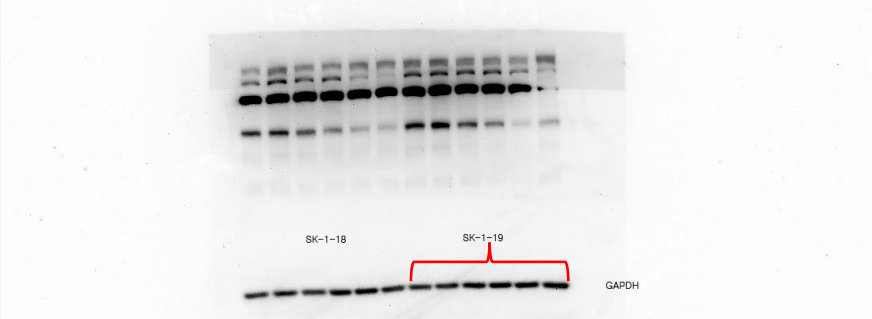
Immunoblot for GAPDH (loading control) after treatment with **4h** (DB-4-297)



Immunoblot for BRD4 after treatment with **4i** (SK-1-19)



Immunoblot for GAPDH (loading control) after treatment with **4i** (SK-1-19)



Immunoblot for BRD4 after treatment with **7a** (PROTAC2)

Immunoblot for GAPDH (loading control) after treatment with **7a** (PROTAC2)