ChemBioChem

Supporting Information

Repurposing a DNA-Repair Enzyme for Targeted Protein Degradation

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Experimental

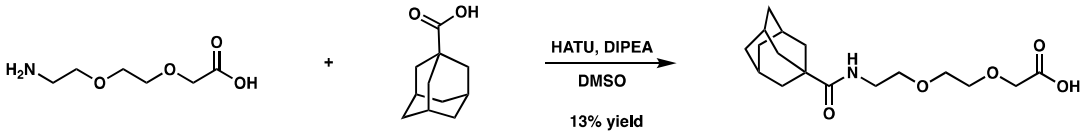
Materials and Methods

All reactions were magnetically stirred. Anhydrous solvents were used where noted. Chemicals were purchased from Combi-blocks, AmBeed, Oakwood, Sigma-Aldrich, Fisher Scientific, or VWR Scientific and used as received. Reactions were monitored by thin-layer chromatography (TLC) on glass-backed silica gel plates (Supelco 1.05175.001, 250 μM). Automated column chromatography was performed with the indicated solvents on a Teledyne ISCO CombiflashRf+ with 20–40 μm spherical silica columns (Redisep Rf gold or Luknova SuperSep HP for regular phase, Redisep Rf gold C18 for reverse phase). Mass spectra were acquired at UC Irvine's Department of Chemistry Mass Spectrometry Facility on a Waters Micromass LCT Premier high resolution mass spectrometer with electrospray ionization (ESI). NMR spectra were taken at ambient temperature at UC Irvine's Department of Pharmaceutical Sciences NMR Facility on a Bruker AvanceNeo Ascend 400. ^{1}H-NMR data was obtained in the specified solvent at 400 MHz. ^{13}C-NMR data was obtained in the specified solvent at 101 MHz. NMR solvents were purchased from Sigma-Aldrich, Acros, or Cambridge Isotope Laboratories. Spectra were calibrated to the residual solvent peak. Chemical shifts are reported in ppm. Coupling constants (*J*) are reported in Hertz (Hz) and rounded to the nearest 0.1 Hz. Multiplicities are defined as: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, td = triplet of doublets, dtd = doublet of triplet of doublets, q = quartet, m = multiplet.

For the purpose of characterization the intermediates were independently synthesized and purified. Since the intermediates were often not fully purified in the syntheses of the final products we elected to

provide methods (and yields) for the final products starting with commercially available materials in addition to the methods used to produce the purified intermediates.

2-(2-(2-((3*r*,5*r*,7*r*)-adamantane-1-carboxamido)ethoxy)ethoxy)acetic acid ((acid)PEG1-Ad)



In a 1 dram vial the adamantane carboxylic acid (0.0818 g, 0.45 mmol, 1 equiv) was combined with DIPEA (0.24 mL, 0.1781 g, 1.38 mmol, 3 equiv) in 1 mL DMSO. The HATU was added in one portion and the reaction mixture stirred at room temperature for 15 min. In a 20 mL scintillation vial the 2-(2-(2-aminoethoxy)ethoxy)acetic acid (0.0746 g, 0.46 mmol, 1 equiv) was combined with DIPEA (0.24 mL, 0.1781 g, 1.38 mmol, 3 equiv) in 1 mL DMSO and sonicated to generate a uniform white suspension. The HATU activated ester was added to this vial, followed by an additional 0.5 mL DMSO. After 1 h half of the reaction mixture was removed for characterization of the title compound and the rest was used to synthesize BG-PEG1-Ad.

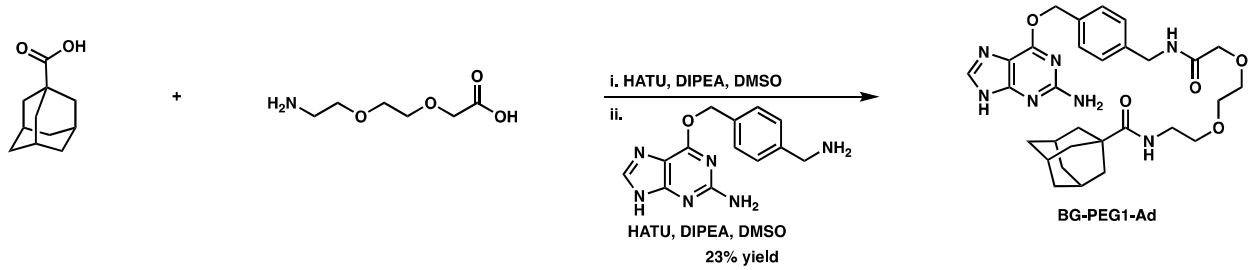
The 1.45 mL aliquot removed was left open to the air and gently heated (40 °C) overnight to drive off the DIPEA. The crude reaction mixture was directly loaded on a 12 g C18 loading cartridge and purified by reverse phase column chromatography on a 50 g C18 modified-silica column with a MeCN/H_2O gradient. 2 CV 0% MeCN, 2 CV 5% MeCN, 10 CV 5→100% MeCN. After lyophilization the product was isolated as a light brown oil (0.0197 g, 13%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.32 (t, *J* = 5.4 Hz, 1H), 4.00 (s, 2H), 3.57 (dd, *J* = 5.7, 3.8 Hz, 2H), 3.51 (dd, *J* = 5.6, 3.9 Hz, 2H), 3.38 (t, *J* = 6.2 Hz, 2H), 3.18 (q, *J* = 6.0 Hz, 2H), 1.94 (s, 3H), 1.74 (d, *J* = 2.3 Hz, 6H), 1.68-1.61 (m, 6H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ 176.98, 171.66, 69.76, 69.55, 68.92, 67.65, 39.79, 38.71, 38.47, 36.16, 27.67.

ESI-HRMS calculated for: $C_{17}H_{27}NO_5Na$ [$(M+Na)^+$]: 348.1787; found 348.1780.

(3*r*,5*r*,7*r*)-*N*-(2-(2-(2-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)amino)-2-



oxoethoxy)ethoxy)ethyl)adamantane-1-carboxamide (BG-PEG1-Ad)

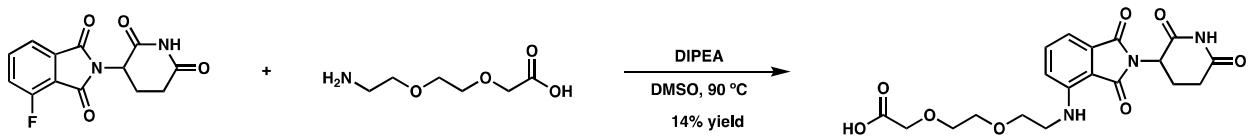
In a 10 mL pear-shaped flask, the adamantane carboxylic acid (0.0456 g, 0.25 mmol, 1 equiv) was dissolved in 0.5 mL DMSO. The Hünig's base (0.17 mL, 0.1261 g, 0.76 mmol, 4 equiv) and the HATU (0.1002 g, 0.26 mol, 1 equiv) were added and the resulting solution stirred for 10 min. A solution of the amine (0.0411 g, 0.25 mmol, 1 equiv) in DMSO (0.75 mL) was then added dropwise to the reaction mixture. After 55 min additional Hünig's base (0.17 mL, 0.1261 g, 0.76 mmol, 4 equiv) was added. At 60 min the second equivalent of HATU (0.0991 g, 0.26 mmol, 1 equiv) was added. After stirring for an additional 5 min the BG-NH_2 was added, and the reaction mixture briefly sonicated to promote dissolution. After stirring for 3 h at room temperature the crude reaction mixture was directly loaded on a 3g C18 loading cartridge and purified by reverse phase chromatography on a 50 g C18-modified silica column with a MeCN/H_2O gradient. 2 CV 0% MeCN, 2 CV 5% MeCN, 10 CV 5→100% MeCN. The product (R_f = 0.28 in 10% MeOH / 90% CH_2Cl_2) was isolated as a white solid (0.0335 g, 23%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.26 (t, *J* = 6.2 Hz, 1H), 7.81 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.35 (t, *J* = 5.6 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 2H), 6.21 (s, 2H), 5.45 (s, 2H), 4.32 (d, *J* = 6.2 Hz, 2H), 3.94 (s, 2H), 3.60-3.53 (m, 4H), 3.38 (t, *J* = 6.3 Hz, 2H), 3.18 (dt, *J* = 9.4, 4.5 Hz, 2H), 1.92 (s, 3H), 1.73 (d, *J* = 2.7 Hz, 6H), 1.66-1.58 (m, 6H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ 177.04, 169.27, 159.48, 159.42, 156.45, 139.30, 138.84, 135.30, 128.46, 127.30, 112.77, 70.27, 70.02, 69.35, 68.93, 66.49, 41.48, 39.78, 38.69, 38.43, 36.13, 27.64.

ESI-HRMS calculated for: $C_{30}H_{40}N_7O_5$ [$(M+H)^+$]: 578.3091; found 578.3099.

2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)acetic acid ((acid)PEG1-Th)



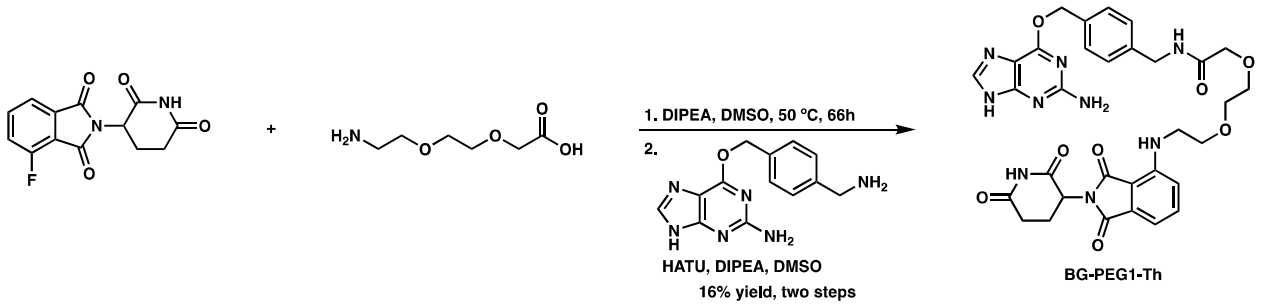
In a 1 dram vial the 2-(2-(2-aminoethoxy)ethoxy)acetic acid (0.0925 g, 0.567 mmol, 1 equiv), the thalidomide fluoride (0.1573 g, 0.57 mmol, 1 equiv), and the DIPEA (0.59 mL, 0.4378 g, 3.39 mmol, 6 equiv) were combined in 1 mL DMSO. The vial was sealed, and the reaction mixture heated to 90 °C for 23 h. The reaction mixture was then left open to the air under moderate heat (50 °C) to drive off the DIPEA. The crude reaction mixture was directly loaded on a 12 g C18 loading cartridge and purified by reverse phase column chromatography on a 50 g C18 modified-silica column with a MeCN/H_2O gradient. 2 CV 0% MeCN, 2 CV 5% MeCN, 10 CV 5→75% MeCN. After lyophilization of the desired fraction the resulting solid was dry loaded on celite and further purified via column chromatography on a 4 g silica column using a gradient of MeOH/CH_2Cl_2. 5 CV 0%, 20 CV 0→20%, 20 CV 20% MeOH. The product was a yellow-brown film (0.0336 g, 14%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.07 (br s, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.60 (t, *J* = 5.6 Hz, 1H), 5.05 (dd, *J* = 12.9, 5.3 Hz, 1H), 3.97 (s, 2H), 3.63-3.59 (m, 4H), 3.47 (q, *J* = 5.4 Hz, 2H), 2.87 (dt, *J* = 17.4, 7.0 Hz, 1H), 2.61-2.47 (m, 2H), 2.06-2.00 (m, 1H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ 172.85, 171.88, 170.12, 168.96, 167.34, 146.44, 136.26, 132.12, 117.47, 110.70, 109.28, 69.83, 69.71, 68.90, 68.00, 48.64, 41.75, 31.03, 22.18.

ESI-HRMS calculated for: $C_{19}H_{21}N_3O_8Na$ [$(M+Na)^+$]: 442.1226; found 442.1233.

***N*-(4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)-2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)acetamide (BG-PEG1-Th)**



In a 1 dram vial, the amine (0.0149 g, 0.09 mmol, 1.25 equiv) was suspended in 0.5 mL DMSO (anhydrous) under a nitrogen atmosphere. The Hünig's base (0.1 mL, 0.074 g, 0.57 mmol, 6.3 equiv) was added, followed by the thalidomide fluoride (0.0202 g, 0.07 mmol, 1 equiv). The reaction mixture was heated to 50 °C and stirred for 66 h. The reaction mixture was cooled to room temperature and diluted with H_2O for solid phase extraction on a C18 cartridge. The crude material was eluted from the cartridge with 50% MeCN / 50 % H_2O and concentrated *in vacuo* to a green-black oil (0.0230 g, 75% crude yield).

The product of step 1 was dissolved in 0.5 mL of DMSO in a 1 dram vial. The Hünig's base (anhydrous, 0.1 mL, 0.074 g, 0.57 mmol, 10.5 equiv) and the HATU (0.0324 g, 0.09 mol, 1.6 equiv) were added and the resulting solution stirred for 15 min. The BG-NH_2 was added in a single portion and the reaction mixture stirred for 2 h. The reaction mixture was diluted with H_2O for solid phase extraction on a C18 cartridge. The crude material was eluted from the cartridge with 50% MeCN / 50 % H_2O and concentrated *in vacuo* and dry loaded on SiO_2. This material was purified by column chromatography using a 4 g silica column with a MeOH/$CHCl_3$ gradient. 5 CV 0% MeOH, 40 CV 0 → 5% MeOH, 20 CV 5 → 10% MeOH, 20 CV 10 → 20% MeOH, 20 CV 20% MeOH. The product (R_f = 0.528 in 10% MeOH / 90% CH_2Cl_2) was a yellow film (0.0077 g, 21%).

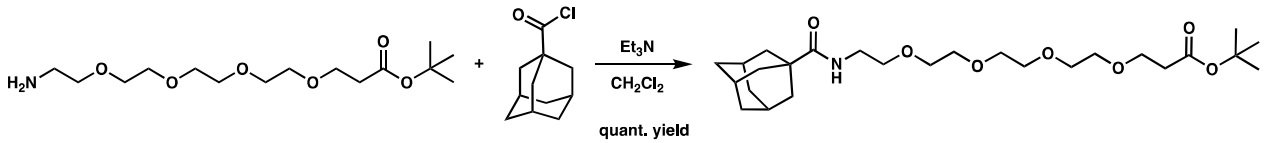
^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H), 11.09 (s, 1H), 8.19 (t, *J* = 6.3 Hz, 1H), 7.79 (s, 1H), 7.56 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.59-6.56 (t, *J* = 5.8 Hz, 1H), 6.27 (s, 2H), 5.43 (s, 2H), 5.04 (dd, *J* =

12.9, 5.4 Hz, 1H), 4.30 (d, *J* = 6.1 Hz, 2H), 3.94 (s, 2H), 3.61-3.58 (m, 6H), 3.45-3.39 (m, 2H), 2.86 (ddd, *J* = 17.3, 14.0, 5.4 Hz, 1H), 2.56 (td, *J* = 13.0, 3.1 Hz, 2H), 2.01 (dtd, *J* = 13.0, 5.3, 2.4 Hz, 1H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ 172.81, 170.09, 169.28, 168.96, 167.29, 159.84, 159.64, 155.18, 146.36, 139.22, 137.79, 136.23, 135.26, 132.07, 128.46, 127.26, 117.42, 113.51, 110.71, 109.25, 70.34, 70.03, 69.46, 68.88, 66.50, 48.57, 41.65, 41.46, 30.99, 22.13.

ESI-HRMS calculated for: $C_{32}H_{34}N_9O_8$ [$(M+H)^+$]: 672.2531; found 672.2541.

***tert*-butyl 1-((3*r*,5*r*,7*r*)-adamantan-1-yl)-1-oxo-5,8,11,14-tetraoxa-2-azaheptadecan-17-oate ((*t*Bu)PEG2-Ad)**



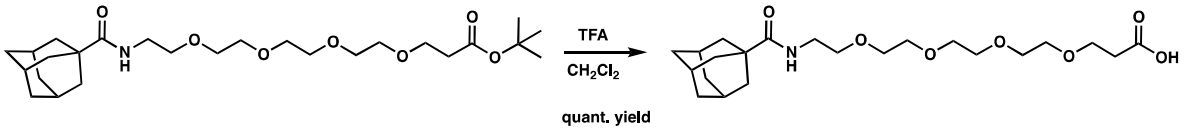
In a 25 mL round bottom flask the amine (0.2626 g, 0.59 mmol, 1 equiv) was dissolved in 1.5 mL CH_2Cl_2. Et_3N (0.25 mL, 0.1815 g, 1.79 mol, 3.1 equiv) was added and the resulting solution stirred at room temperature for 5 min. The acid chloride (0.1860 g, 0.94 mmol, 1.6 equiv) was added in one portion, followed by an additional 2 mL CH_2Cl_2. After stirring at room temperature overnight the reaction mixture was transferred to a separatory funnel with EtOAc. It was washed 1x H_2O, 2x saturated NH_4Cl, 2x saturated $NaHCO_3$, 1x brine, and dried over $MgSO_4$. Filtration and concentration *in vacuo* gave the product as a clear and colorless oil (0.3396 g, 120%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.08 (s, 1H), 3.68 (t, *J* = 6.5 Hz, 2H), 3.63-3.59 (m, 12H), 3.52 (t, *J* = 5.1 Hz, 2H), 3.41 (t, *J* = 7.8 Hz, 2H), 2.47 (t, *J* = 6.6 Hz, 2H), 2.01 (s, 3H), 1.83 (d, *J* = 2.0 Hz, 6H), 1.73-1.65 (m, 6H), 1.42 (s, 9H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 178.04, 170.93, 80.58, 70.69, 70.65, 70.61, 70.58, 70.44, 70.31, 69.99, 66.98, 40.66, 39.27, 38.34, 36.63, 36.33, 28.23, 28.17.

ESI-HRMS calculated for: $C_{26}H_{45}NO_7Na$ [$(M+Na)^+$]: 506.3094; found 506.3087.

1-((3*r*,5*r*,7*r*)-adamantan-1-yl)-1-oxo-5,8,11,14-tetraoxa-2-azaheptadecan-17-oic acid ((acid)PEG2-Ad)



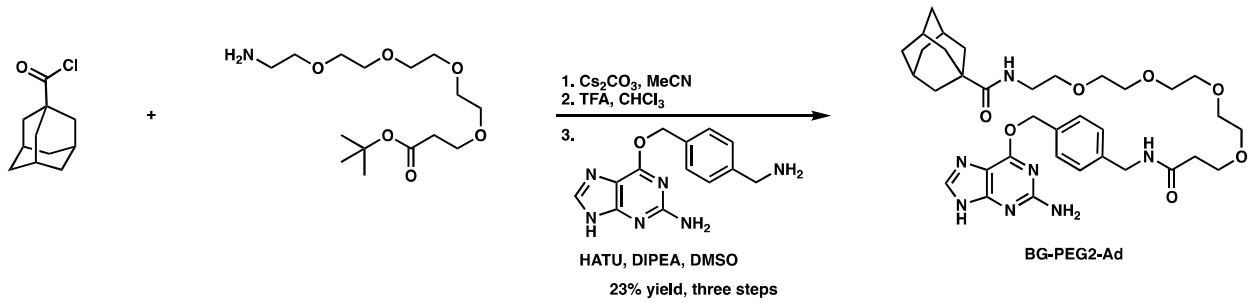
In a 25 mL round bottom flask the ester (0.1001 g, 0.21 mmol, 1 equiv) was dissolved in 3 mL CH_2Cl_2. The TFA (1 mL, 1.49 g, 13.07 mmol, 63.1 equiv) was added slowly and the reaction mixture stirred at room temperature for 6 h. It was concentrated *in vacuo* to afford the TFA salt of the product as a pale-yellow oil (0.1154 g, 103%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.71 (br s, 4H), 6.66 (s, 1H), 3.77 (t, *J* = 6.1 Hz, 2H), 3.65 (d, *J* = 3.4 Hz, 12H), 3.59 (t, *J* = 5.0 Hz, 2H), 3.46 (q, *J* = 5.1 Hz, 2H), 2.63 (t, *J* = 6.0 Hz, 2H), 2.04 (s, 3H), 1.84 (d, *J* = 2.1 Hz, 6H), 1.71 (q, *J* = 11.2 Hz, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 180.28, 175.74, 70.63, 70.50, 70.45, 70.31, 70.29, 70.27, 69.67, 66.53, 40.81, 39.62, 38.89, 36.45, 34.96, 28.09.

^{19}F-NMR (376 MHz, $CDCl_3$): δ -75.97.

ESI-HRMS calculated for: $C_{22}H_{38}NO_7$ [$(M+H)^+$]: 428.2648; found 428.2655.



1-((1*s*,3*s*)-adamantane-1-carboxamido)-*N*-(4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)-3,6,9,12-tetraoxapentadecan-15-amide (BG-PEG2-Ad)

In a 10 mL pear-shaped flask, the amine (0.1005 g, 0.31 mmol, 1 equiv) was dissolved in 2.5 mL MeCN (anhydrous). The cesium carbonate (0.5068 g, 1.56 mmol, 5 equiv) was added and the resulting suspension stirred for 3 min. The acid chloride (0.0743 g, 0.37 mmol, 1.2 equiv) was added in a single portion, followed by an additional 0.5 mL MeCN (anhydrous). After stirring for 35 min at room temperature the reaction mixture was quenched with H_2O and transferred to a separatory funnel with CH_2Cl_2. The layers were separated and the aqueous was extracted 3x with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting clear and colorless oil (0.1525 g, quant.) was used without further purification.

The product of step was dissolved in 4 mL $CHCl_3$ in a 10 mL pear-shaped flask, to which 1 mL TFA was added. The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* to give the product as a clear and nearly colorless oil (quant.), which was used without further purification.

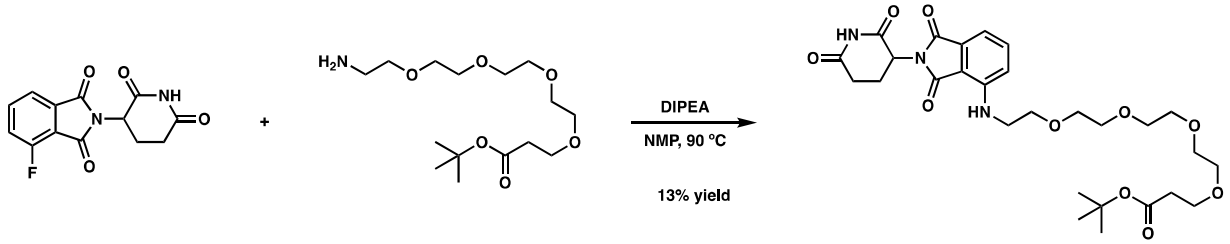
In a 10 mL pear-shaped flask the product of step 2 (0.040 g, 0.09 mmol, 1 equiv) was dissolved in 2.5 mL DMSO (anhydrous) under an atmosphere of N_2. The Hünig's base (0.28 mL, 0.208 g, 1.61 mmol, 17.2 equiv) and the HATU (0.0686 g, 0.18 mol, 1.9 equiv) were both added in two alternating portions over 30 min and the resulting solution stirred for 10 min. The BG-NH_2 was added in a single portion and the reaction mixture stirred for 3 h. This was loaded directly onto a C18 cartridge for solid phase extraction. The crude product was eluted with 95% MeCN / 5% H_2O and concentrated *in vacuo* and dry loaded on celite. Purification by column chromatography on a 4g silica gel cartridge with a MeOH/CH_2Cl_2 gradient. 1 CV 0% MeOH, 15 CV 0 → 5% MeOH, 15 CV 5% MeOH, 5 CV 5 → 10% MeOH, 10 CV 10% MeOH, 5 CV 10 → 20% MeOH, 15 CV 20% MeOH. The product (R_f = 0.19 in 10% MeOH / 90% CH_2Cl_2) was a colorless film (0.0149 g, 23 %).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.42 (s, 1H), 8.35 (t, *J* = 5.9 Hz, 1H), 7.80 (d, *J* = 0.2 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.33 (t, *J* = 5.6 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 2H), 6.27 (s, 2H), 5.45 (s, 2H), 4.27 (d, *J* = 5.9 Hz, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.50-3.47 (m, 12H), 3.37 (m, 2H), 3.17 (q, *J* = 6.0 Hz, 2H), 2.38 (t, *J* = 6.4 Hz, 2H), 1.93 (s, 3H), 1.73 (d, *J* = 2.7 Hz, 6H), 1.59 (s, 6H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ 177.00, 170.13, 159.87, 159.63, 155.20, 139.37, 137.80, 135.21, 128.47, 127.21, 113.52, 69.81, 69.79, 69.73, 69.71, 69.59, 69.55, 68.92, 66.88, 66.53, 41.83, 39.79, 38.72, 38.48, 36.15, 27.66.

ESI-HRMS calculated for: $C_{35}H_{50}N_7O_7$ [$(M+H)^+$]: 680.3772; found 680.3770.

***tert*-butyl 1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxapentadecan-15-oate ((*t*Bu)PEG2-Th)**



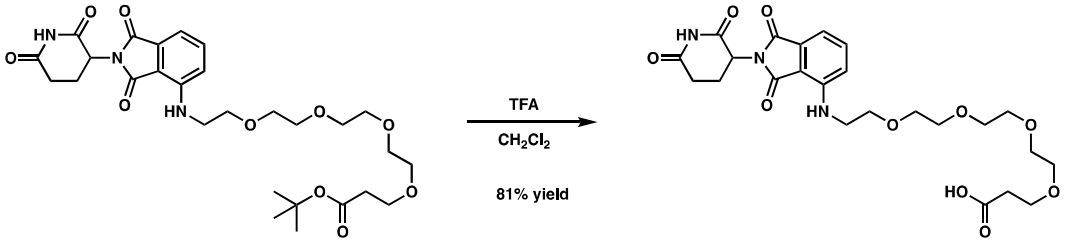
In a 20 mL vial the PEG linker (0.2137 g, 0.48 mmol, 1 equiv) was combined with the thalidomide fluoride (0.1302 g, 0.47 mmol, 1 equiv), and DIPEA (0.5 mL, 0.371 g, 2.87 mmol, 6 equiv) in 1 mL NMP. The vial was sealed and the reaction mixture heated to 90 °C for 3 h. The reaction mixture was then left open to the air overnight under moderate heat (40 °C) to drive off the DIPEA. The reaction mixture was directly loaded on a 12 g C18 cartridge and purified by reverse phase column chromatography on a 50 g C18 modified-silica column with a MeCN/H_2O gradient (+ 0.1% TFA). 2 CV 0% MeCN, 2 CV 5% MeCN, 10 CV 5→75% MeCN. After lyophilization of the desired fractions the resulting solid was dry loaded on florisil and further purified via column chromatography on a 4 g silica column using a gradient of MeOH/CH_2Cl_2 where the MeOH contained 10% $NH_4OH_{(aq)}$. 5 CV 0.1%, 25 CV 0.1→5%, 10 CV 5% MeOH. The product was a bright yellow-green film (0.0364 g, 13%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 6.47 (s, 1H), 4.90 (dd, *J* = 11.7, 5.3 Hz, 1H), 3.72-3.58 (m, 16H), 3.45 (t, *J* = 5.3 Hz, 2H), 2.87-2.67 (m, 3H), 2.48 (t, *J* = 6.5 Hz, 2H), 2.10 (t, *J* = 6.5 Hz, 1H), 1.42 (s, 9H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 171.35, 171.01, 169.34, 168.54, 167.70, 146.92, 136.09, 132.58, 116.87, 111.69, 110.35, 80.61, 70.79, 70.71, 70.67, 70.65, 70.50, 70.44, 69.58, 66.96, 48.95, 42.48, 36.31, 31.50, 28.17, 22.87.

ESI-HRMS calculated for: $C_{28}H_{39}N_3O_{10}Na$ [$(M+Na)^+$]: 600.2533; found 600.2516.

1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxapentadecan-15-oic acid ((acid)PEG2-Th)



In a 20 mL vial the ester (0.0151 g, 0.03 mol, 1 equiv) was dissolved in CH_2Cl_2 (1.5 mL). TFA was added (0.5 mL, 0.745 g, 203 equiv) and the reaction mixture stirred at room temperature for 5 h. It was concentrated *in vacuo* to afford the TFA salt of the product as a yellow film (0.0137 g, 81%).

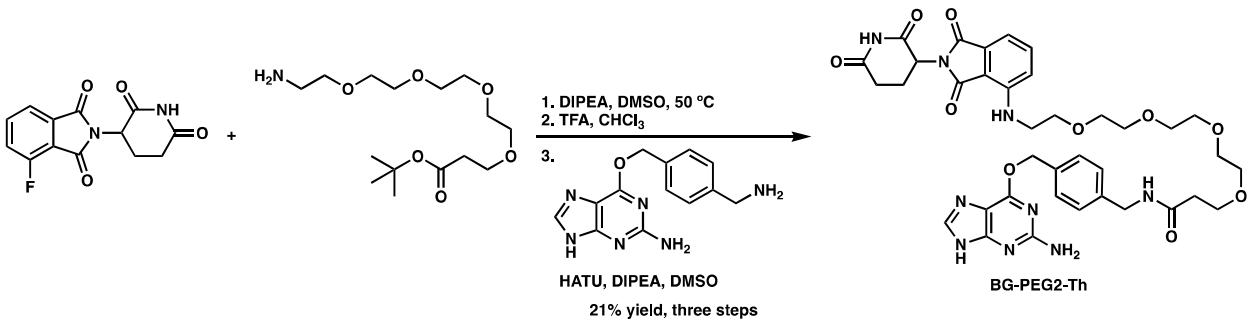
^{1}H-NMR (400 MHz, CD_3OD): δ 7.54 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.09 (d, *J* = 8.6 Hz, 1H), 7.05 (d, *J* = 7.1 Hz, 1H), 5.05 (dd, *J* = 12.6, 5.4 Hz, 1H), 3.71 (q, *J* = 5.6 Hz, 4H), 3.65-3.55 (m, 12H), 3.50 (t, *J* = 5.3 Hz, 2H), 2.91-2.66 (m, 3H), 2.53 (t, *J* = 6.3 Hz, 2H), 2.14-2.08 (m, 1H).

^{13}C-NMR (101 MHz, CD_3OD): δ 175.30, 174.69, 171.56, 170.64, 169.29, 148.21, 137.20, 133.87, 118.27, 112.01, 111.29, 71.64, 71.64, 71.61, 71.59, 71.44, 71.36, 70.63, 67.78, 50.20, 43.26, 35.78, 32.22, 23.82.

^{19}F-NMR (376 MHz, CD_3OD): δ -76.84.

ESI-HRMS calculated for: $C_{24}H_{31}N_3O_{10}Na$ [$(M+Na)^+$]: 544.1907; found 544.1895.

***N*-(4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)-1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxapentadecan-15-amide (BG-PEG2-Th)**



In a 1 dram vial, the amine (0.0622 g, 0.19 mmol, 1 equiv) was suspended in 0.5 mL DMSO. The thalidomide fluoride (0.0521 g, 0.19 mmol, 1 equiv) was added and the mixture briefly sonicated to promote dissolution. The Hünig's base (0.18 mL, 0.1336 g, 1.03 mmol, 5.3 equiv) was added and the vial sealed with a septum cap, heated to 50 °C and stirred for 3 h. The reaction mixture was cooled to room temperature and transferred to a separatory funnel with EtOAc. The resulting solution was washed 3x H_2O, 1x brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a yellow oil (0.0380 g, 35% crude yield), which was dry loaded on SiO_2. Purification of pooled material was done via column chromatography on a 4 g silica column using a MeOH/CH_2Cl_2 gradient. 2.5 CV 0% MeOH, 20 CV 0 → 5% MeOH, 12.5 CV 5% MeOH, 5 CV 5 → 20% MeOH, 10 CV 20% MeOH. The purified product was a yellow glass.

The product of step 1 was dissolved in 5 mL $CHCl_3$ in a 20 mL scintillation vial. 1 mL of TFA was added and the resulting solution stirred at room temperature for 3 h. The product was isolated as a yellow film by concentrating the reaction mixture *in vacuo* (quant.) and used without further purification.

In a 20 mL scintillation vial, the product of step 2 was dissolved in 1.5 mL DMSO. The Hünig's base (0.1 mL, 0.0742 g, 0.57 mmol, 9.5 equiv) and the HATU (0.0349 g, 0.09 mol, 1.5 equiv) were added and the reaction mixture stirred for 10 min at room temperature. The BG-NH_2 was added as a single portion and stirring continued for 1.5 h. The reaction mixture was then diluted with H_2O for solid phase extraction with a C18 cartridge. The crude product was eluted with MeOH, then MeCN. This solution was concentrated *in vacuo* and dry loaded on celite. Purification by column chromatography on a 4 g silica column was conducted with a MeOH/CH_2Cl_2 gradient. 2.5 CV 0% MeOH, 2.5 CV 5% MeOH, 20 CV 5 → 10% MeOH, 17.5 CV 10% MeOH, 2.5 CV 10 → 20% MeOH, 15 CV 20% MeOH. The product (R_f = 0.28 in 10% MeOH / 90% CH_2Cl_2) was a yellow glass (0.0279 g, 59%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H), 11.09 (s, 1H), 8.33 (t, *J* = 5.8 Hz, 1H), 7.80 (s, 1H), 7.57 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 6.9 Hz, 1H), 6.59 (t, *J* = 5.7 Hz, 1H), 6.27 (s, 2H), 5.45 (s, 2H), 5.05 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.27 (d, *J* = 5.8 Hz, 2H), 4.11 (q, *J* = 5.3 Hz, 1H), 3.61 (q, *J* = 5.6 Hz, 4H), 3.56-3.43 (m, 14H), 3.17 (d, *J* = 5.2 Hz, 3H), 2.92-2.83 (m, 1H), 2.61-2.53 (m, 2H), 2.37 (t, *J* = 6.4 Hz, 2H), 2.02 (dtd, *J* = 11.9, 5.9, 2.9 Hz, 1H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ 172.83, 170.15, 170.09, 168.94, 167.31, 159.87, 159.65, 155.19, 146.41, 139.35, 137.80, 136.24, 135.23, 132.09, 128.48, 127.22, 117.46, 113.53, 110.69, 109.24, 69.85, 69.80, 69.79, 69.77, 69.71, 69.55, 68.88, 66.87, 66.53, 48.63, 41.84, 41.71, 36.16, 31.00, 22.17.

ESI-HRMS calculated for: $C_{37}H_{44}N_9O_{10}$ [$(M+H)^+$]: 774.3211; found 774.3238.

Cell Culture and Cell Line Generation

HEK293T cells (CRL-11268) were purchased from ATCC and cultured in 1x DMEM media with L-Glutamine, 4.5g/L Glucose (10-017-CM; Corning), 10% FBS and 1% Penicillin-Streptomycin (15140122; Gibco). Cells were maintained in 95% humidity with 5% CO^2 and media was replenished every 2-3 days.

Stable transfections were carried out using the standard protocol for the jetPRIME transfection system (Polyplus Transfection) and media was changed 24 hours after transfection. The transfection was carried out with 1ug of plasmid, 0.8ug of Cas9 and 0.2ug of PCR fragmented gRNA in a 6-well plate. Cells were cultured for 10 days before the addition of 1ug/ml puromycin for selection of stable transfected cells.

Fluorescent Imaging

Each transfected HEK293T line was seeded into an 8-well chamber slide with either plain media or 25uM of respective BG and left for 24 hours before imaging. Imaging was done on a Leica Stellaris 8 confocal microscope.

In-Gel Electrophoresis Competition Assay and Analysis

Cells were treated with either vehicle (DMSO) or 20uM of respective BG ligand for 1 hour and then treated with 1uM of TMR-Star (S9105S; New England BioLabs) for 30 minutes before cell lysates were collected. Cells were lysed with RIPA buffer (786-489; G-Biosciences) containing complete proteasome inhibitor (Roche). Protein lysates were run on a 4-20% mini-PROTEAN TGX gel (4561096; BioRad) and then imaged on a ChemiDoc (12003153; BioRad) using the rhodamine filter. Images were processed and analyzed on FIJI.

Immunoblot Assay and Analysis

Cells were treated with either plain media or respective BG ligand 24 hours before cell lysates were collected. Protein lysates were then run on a 4-20% mini-PROTEAN TGX gel (4561096; BioRad) and transferred to a nitrocellulose membrane using the Trans-Blot Turbo Transfer System (1704150; BioRad). The membrane was blocked with 5% nonfat milk in PBS containing 0.1% Tween 20 (PBS-T) and then incubated with primary antibodies overnight. Primary antibodies included mNeonGreen (Cell Signaling, #53061) and GAPDH (AM4300; Invitrogen). The blots were then washed with PBS-T and

then incubated in respective HRP secondary antibody (Cell Signaling) for 2 hours at room temperature. HRP detection was carried out with ECL Western blotting detection reagents (Thermo Scientific).

Flow Cytometry, Protein Degradation Quantification and Analysis

Cells were treated with either plain media, DMSO vehicle or respective BG ligand for 24 hours. The cells were then dissociated with 0.25% Trypsin (15400054; Gibco) and spun down to remove any remaining media or trypsin. The cells were resuspended in PBS with 1% FBS containing 7-AAD viability stain (Biolegend). Each sample set was run on a Flow Cytometer for analysis. Flow cytometry was performed on a NovoSampler Pro (Agilent). Mean Fluorescent Intensity (MFI) was calculated on live (7AAD negative) single cells above 10^4 fluorescent counts with a minimum of 10,000 live cells per well. Non-fluorescing cells were thus gated out. Samples were run in triplicate and errors bars are plotted as standard deviations.

Time Course of Protein Degradation with BG Derivatives

Cells were treated with 25uM of the respective BG derivative and measured at 24, 8, 4, 2, 1, and 0 hours by flow cytometry.

Inhibitor Assay to Demonstrate Proteosome-dependent Degradation

Cells were treated for 24 hours with 10uM BG-PEG1-Ad and either MG-132, Bortezomib or Lenalidomide at varying concentrations, from 10uM to 1nM, or BG-PEG1-Ad alone. After 24 hours, the cells were lifted and analyzed by flow cytometry, using methods outlined above.

Supplementary figures

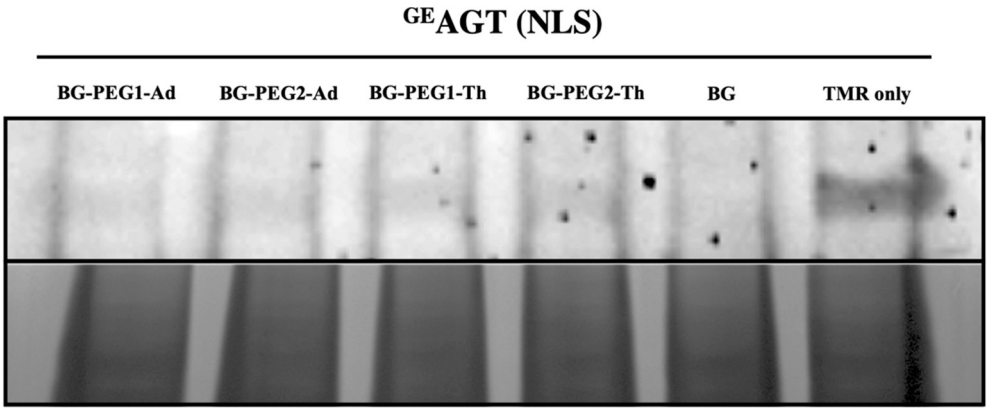


Figure S1. Competition assay with various BG ligands. Cells were treated with 20uM of respective ligand for 1 hr then subsequently treated with 1uM of TMRstar for 30 minutes before protein was collected.

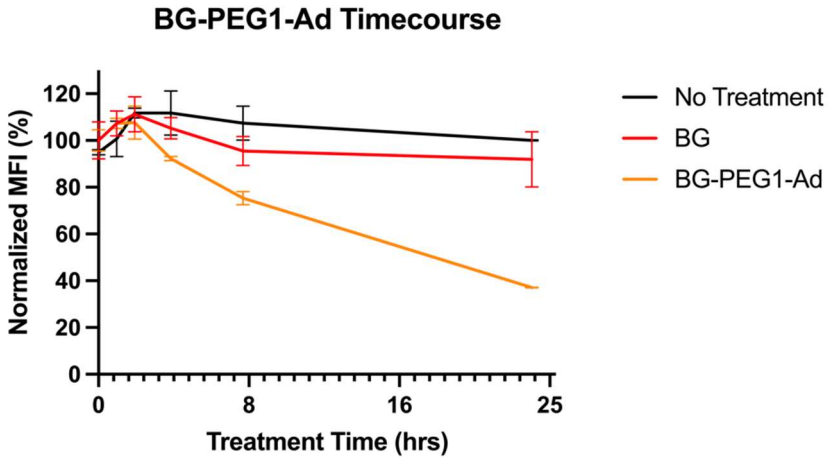


Figure S2. BG-PEG1-Ad timecourse Assay. Cells were treated with 10uM BG-PEG1-Ad, BG, or no treatment and measured at various timepoints over 24 hours before analyzing by flow cytometry.

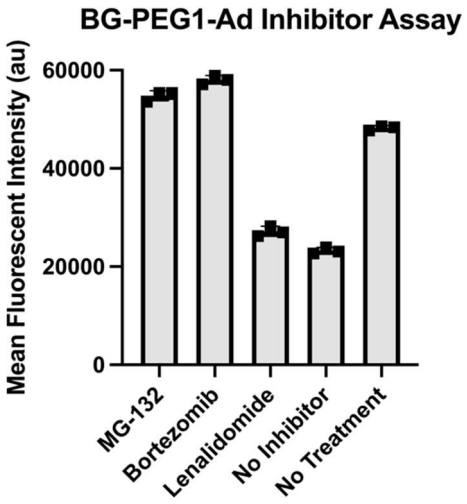


Figure S3. BG-PEG1-Ad inhibitor assay. Cells were treated with 5uM BG-PEG1-Ad and either 10nM MG-132, 10nM Bortezomib, or 1uM Lenalidomide for 24 hours before analyzing by flow cytometry.

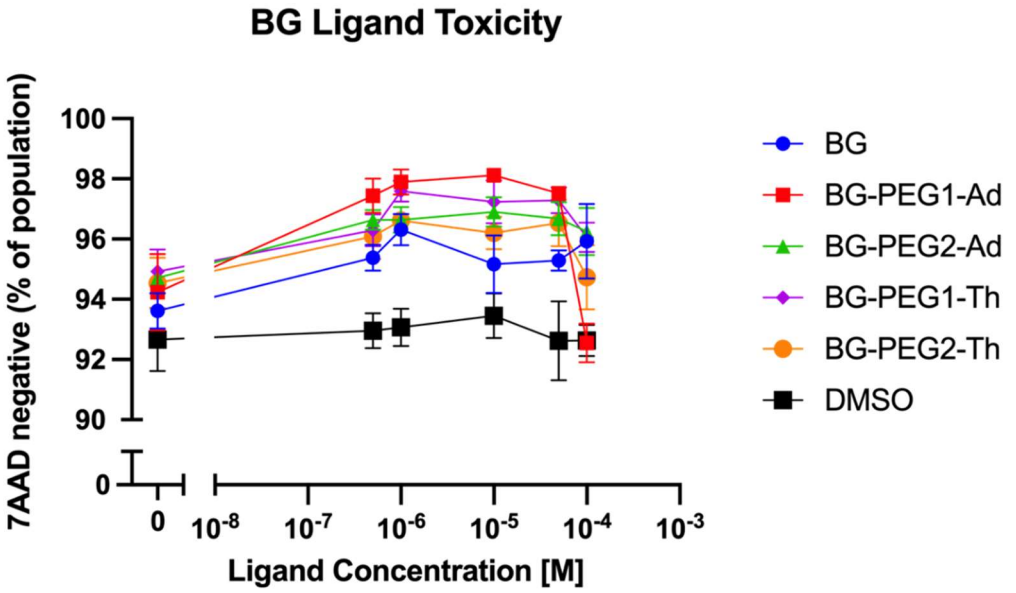


Figure S4. BG ligand toxicity (7AAD as live-dead stain). Cells were treated with ligand concentrations ranging from 0.5uM to 100mM then subsequently treated with 7AAD for 15 minutes before quantification by flow cytometry.

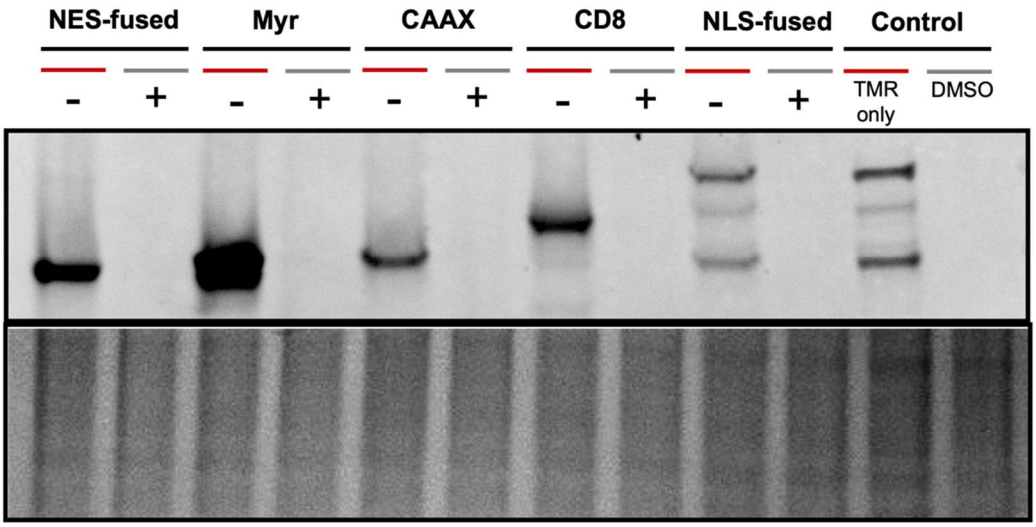


Figure S5. Competition assay in various localizations. Cells were treated with 20uM of BG-PEG1-Ad for 1 hr then subsequently treated with 1uM of TMRstar for 30 minutes before protein was collected.

Table S1: Annotated plasmid map of all relevant constructs

Plasmid Number	Plasmid Description
pJZ0387	CAG-BP_NLS-SpCas9-BP_NLS-WPRE-BGH_polyA
pJZ0338	U6_T7_AASV1gRNA_T2

pJZ1654	AAVS1-HITI-PGK-Puro-P2A-BP_NLS-mNeonGreen-GE_AGT
pJZ1682	AAVS1-HITI-PGK-Puro-GSG-P2A-NES-mNeonGreen-GE_AGT
pJZ1683	AAVS1-HITI-PGK-Myris-mNeonGreen-GE_AGT-GSG-P2A-Puro
pJZ1684	AAVS1-HITI-PGK- Puro-GSG-P2A-CD8_SS-mNeonGreen-CD8_TM- GE_AGT
pJZ1685	AAVS1-HITI-PGK- Puro-GSG-P2A-mNeonGreen-CAAX

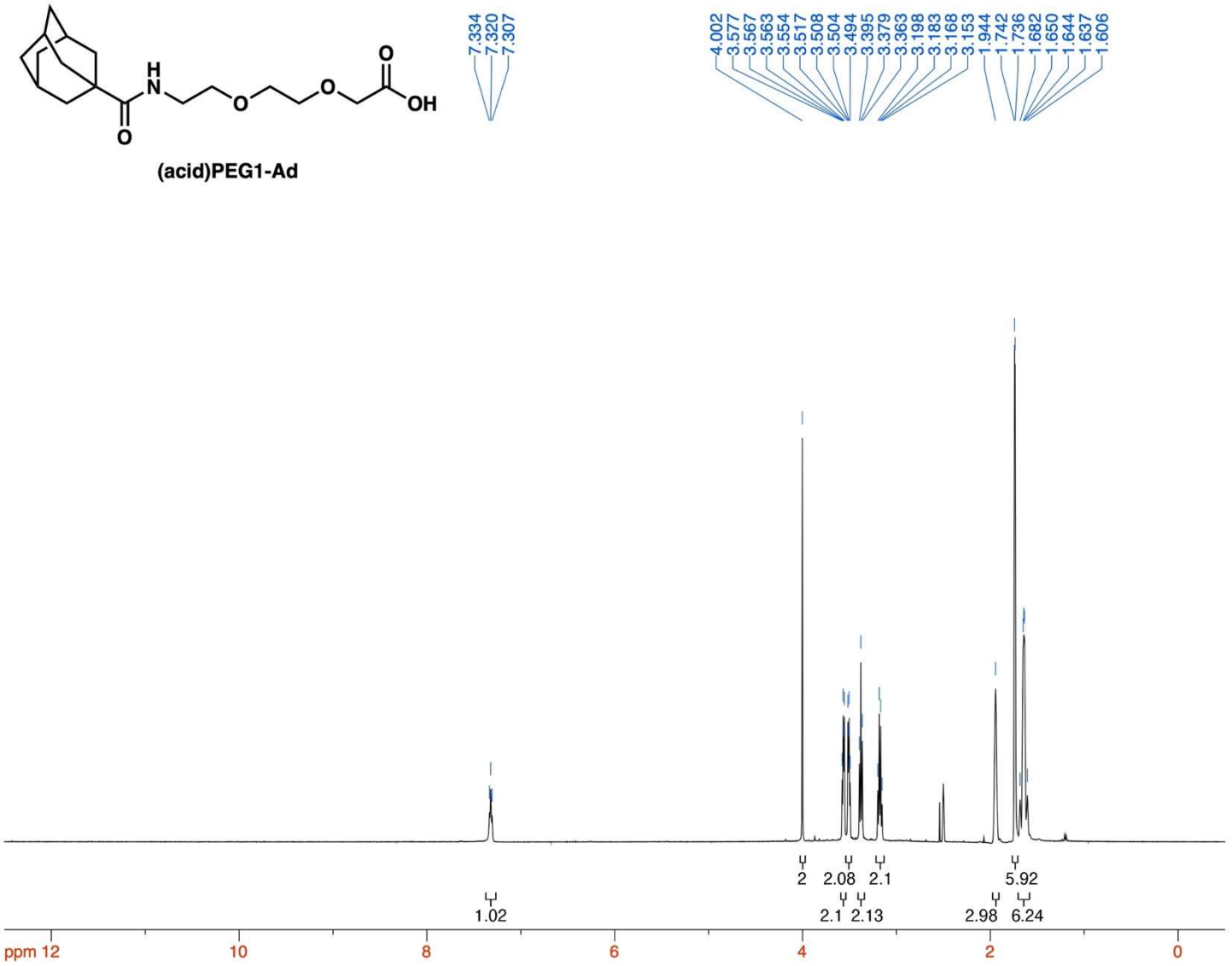


Figure S6. ^{1}H-NMR spectrum of (acid)PEG1-Ad (400 MHz; DMSO-d_6).

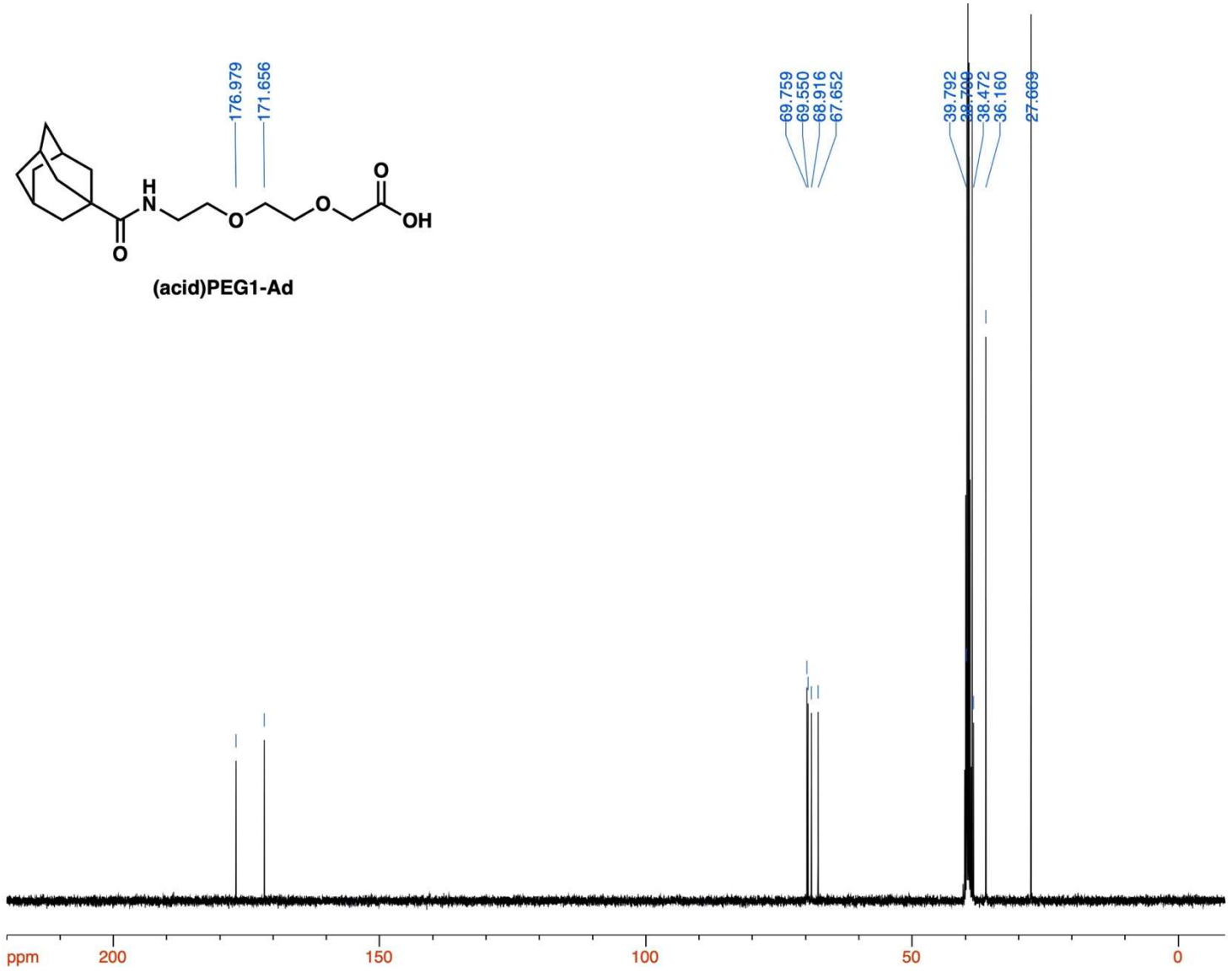


Figure S7. ^{13}C-NMR spectrum of (acid)PEG1-Ad (101 MHz; DMSO-d_6).

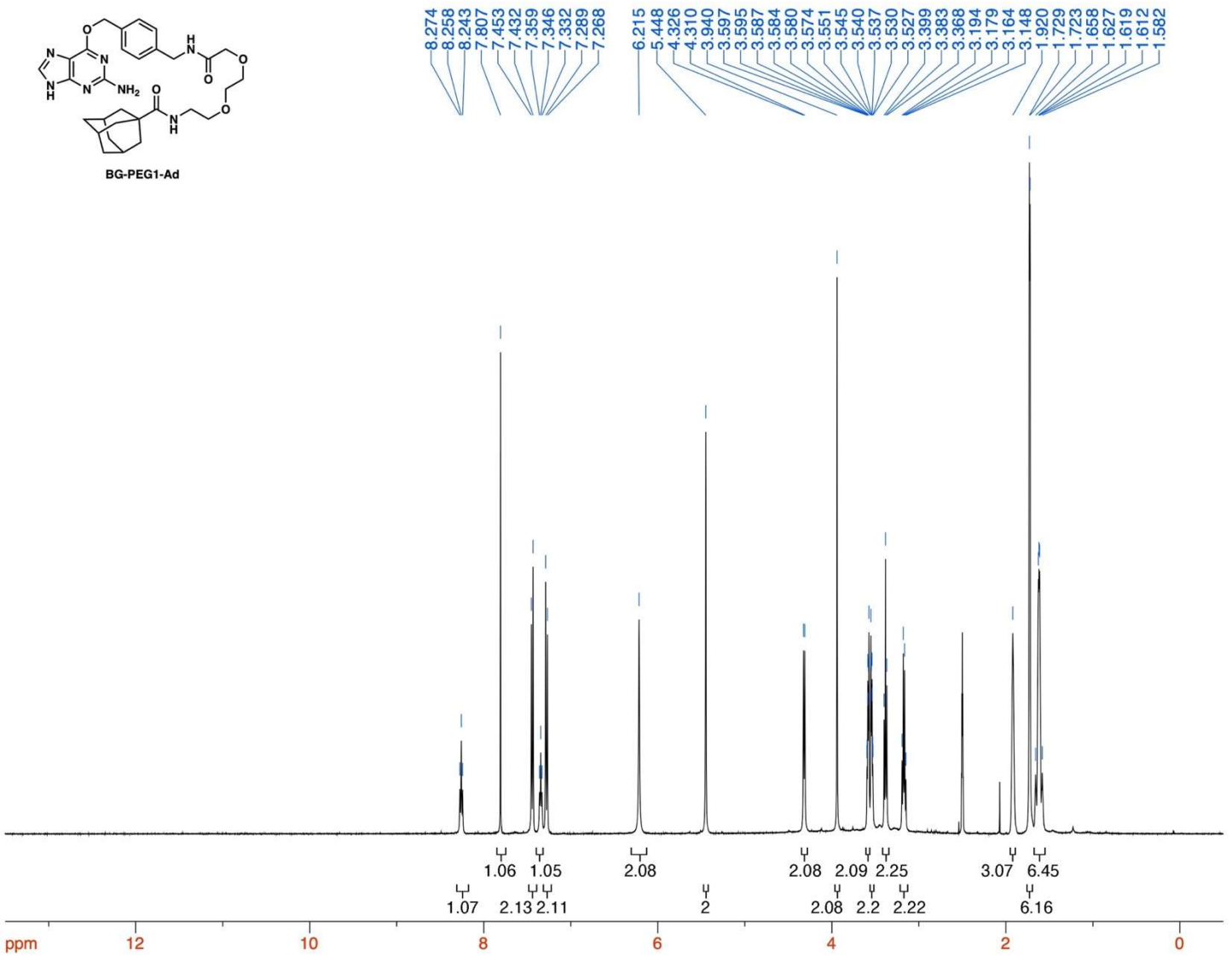


Figure S8. ^{1}H-NMR spectrum of BG-PEG1-Ad (400 MHz; DMSO-d_6).

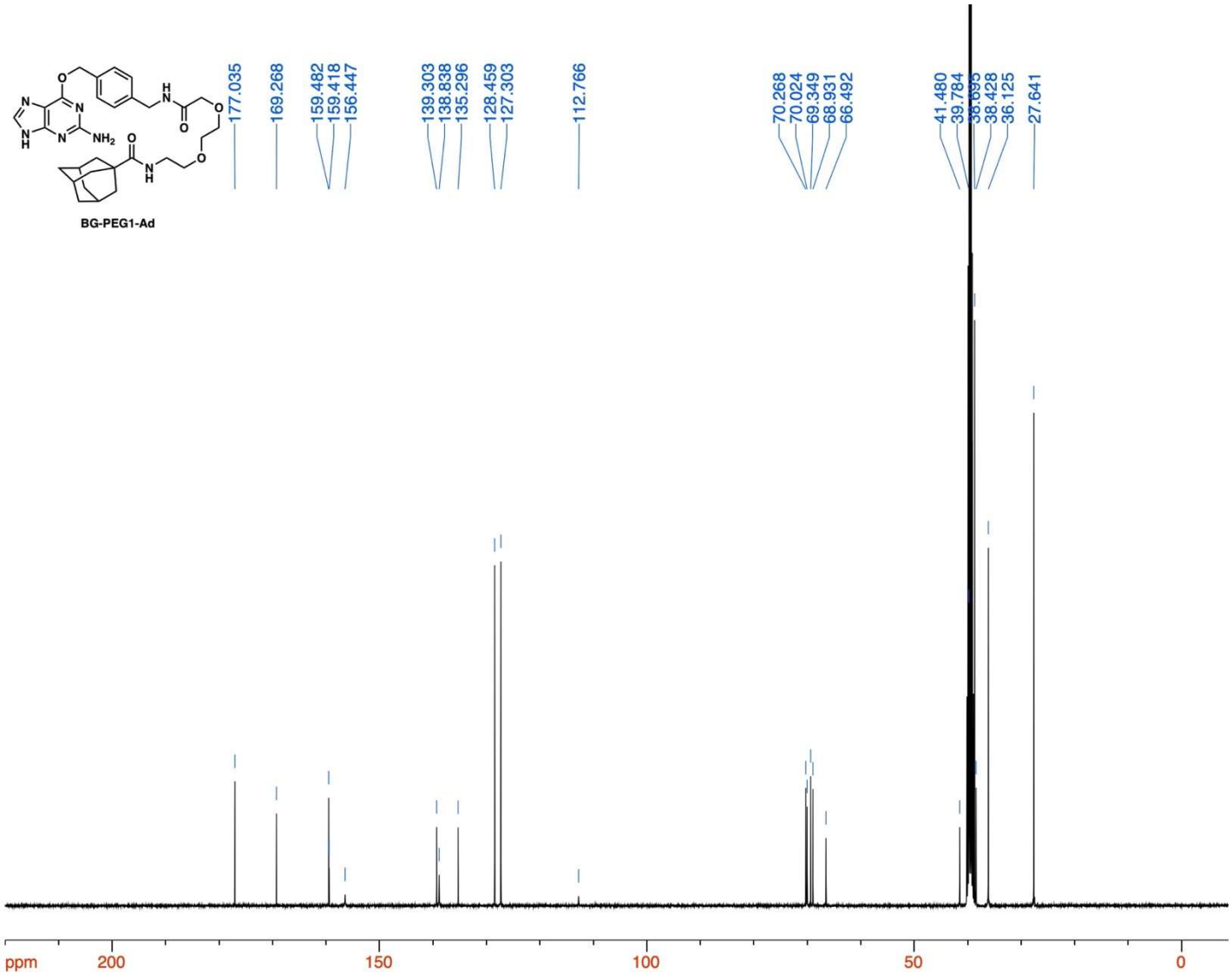


Figure S9. ^{13}C-NMR spectrum of BG-PEG1-Ad (101 MHz; DMSO-d_6).

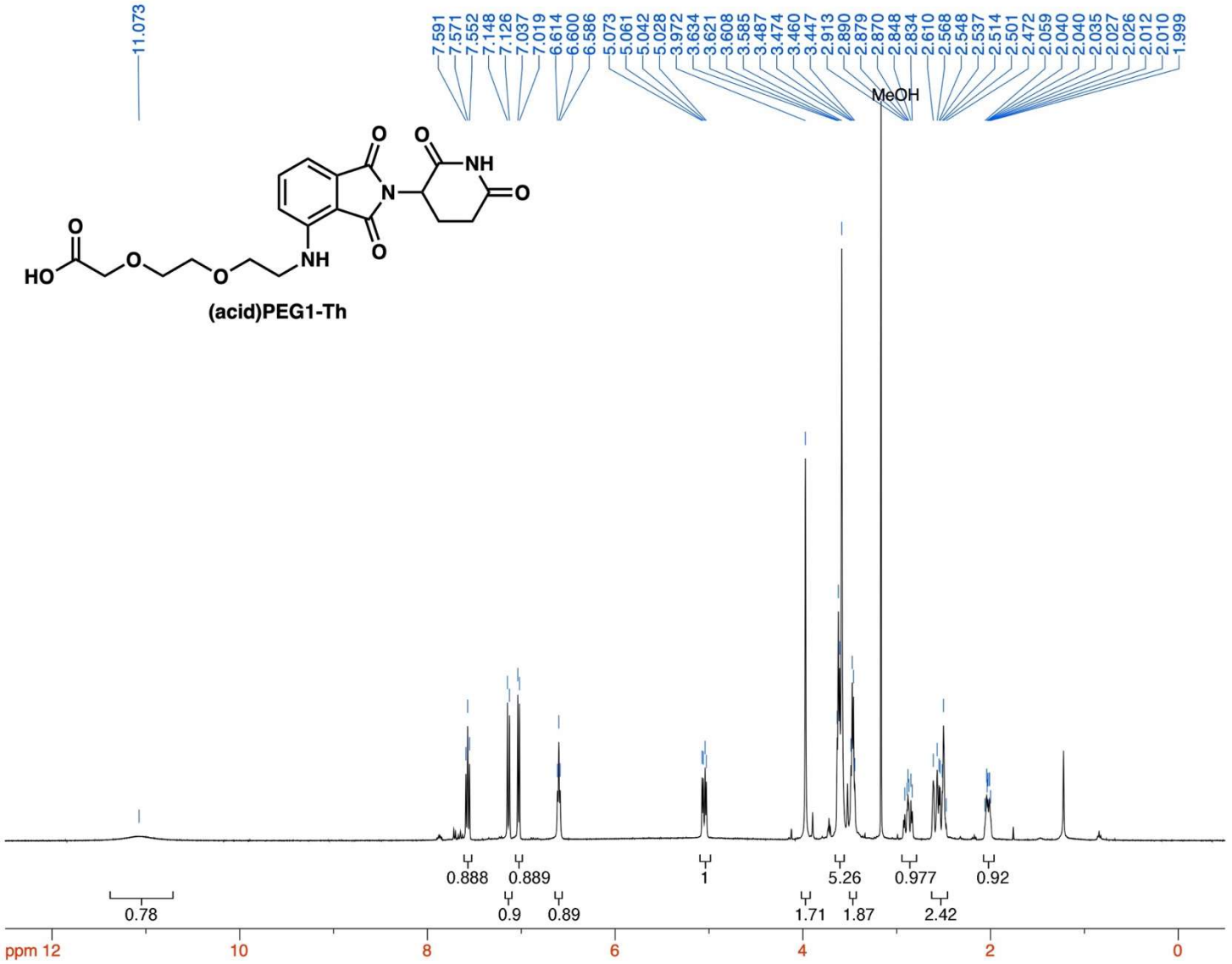


Figure S10. ^{1}H-NMR spectrum of (acid)PEG1-Th (400 MHz; DMSO-d_6).

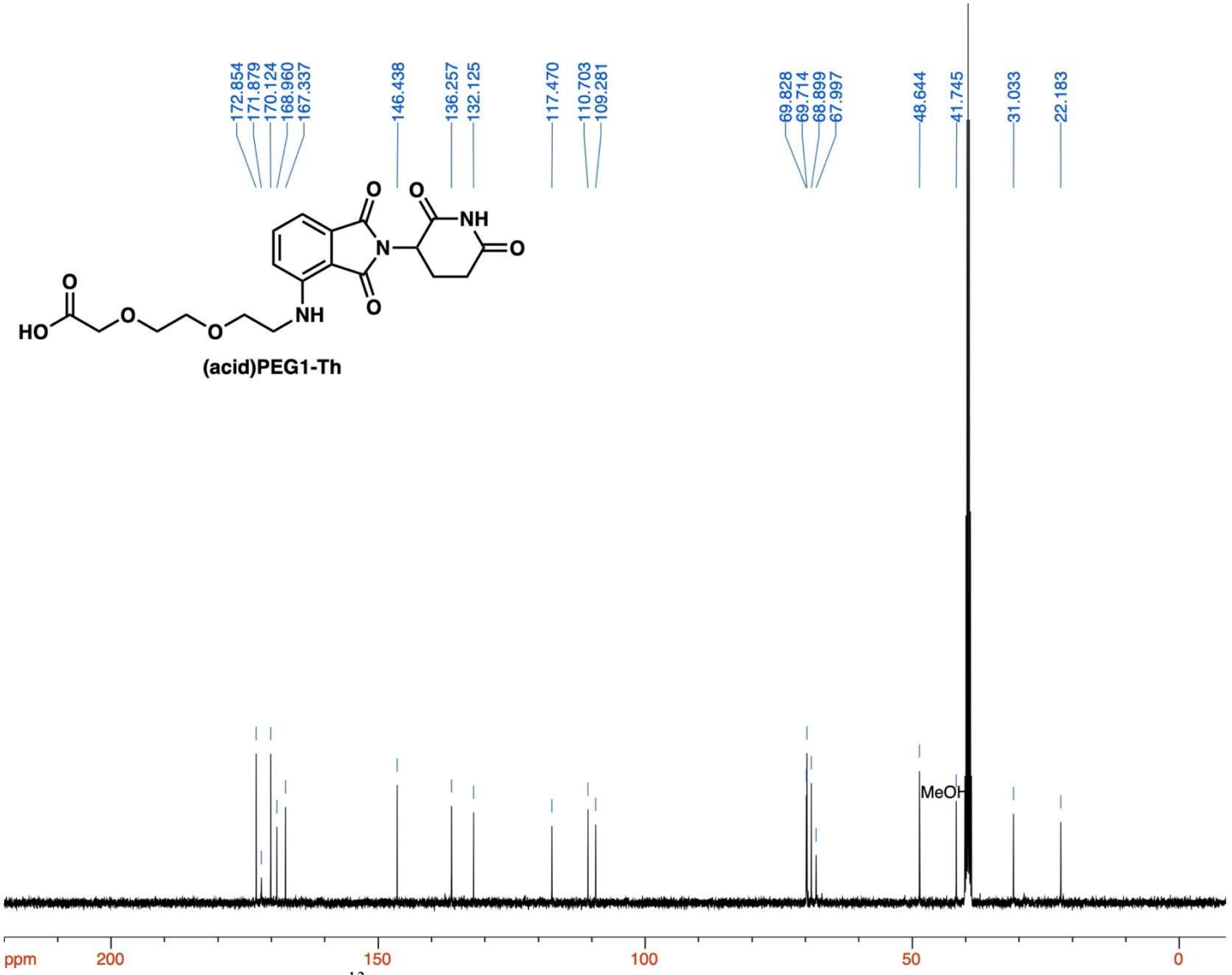


Figure S11. ^{13}C-NMR spectrum of (acid)PEG1-Th (101 MHz; DMSO-d_6).

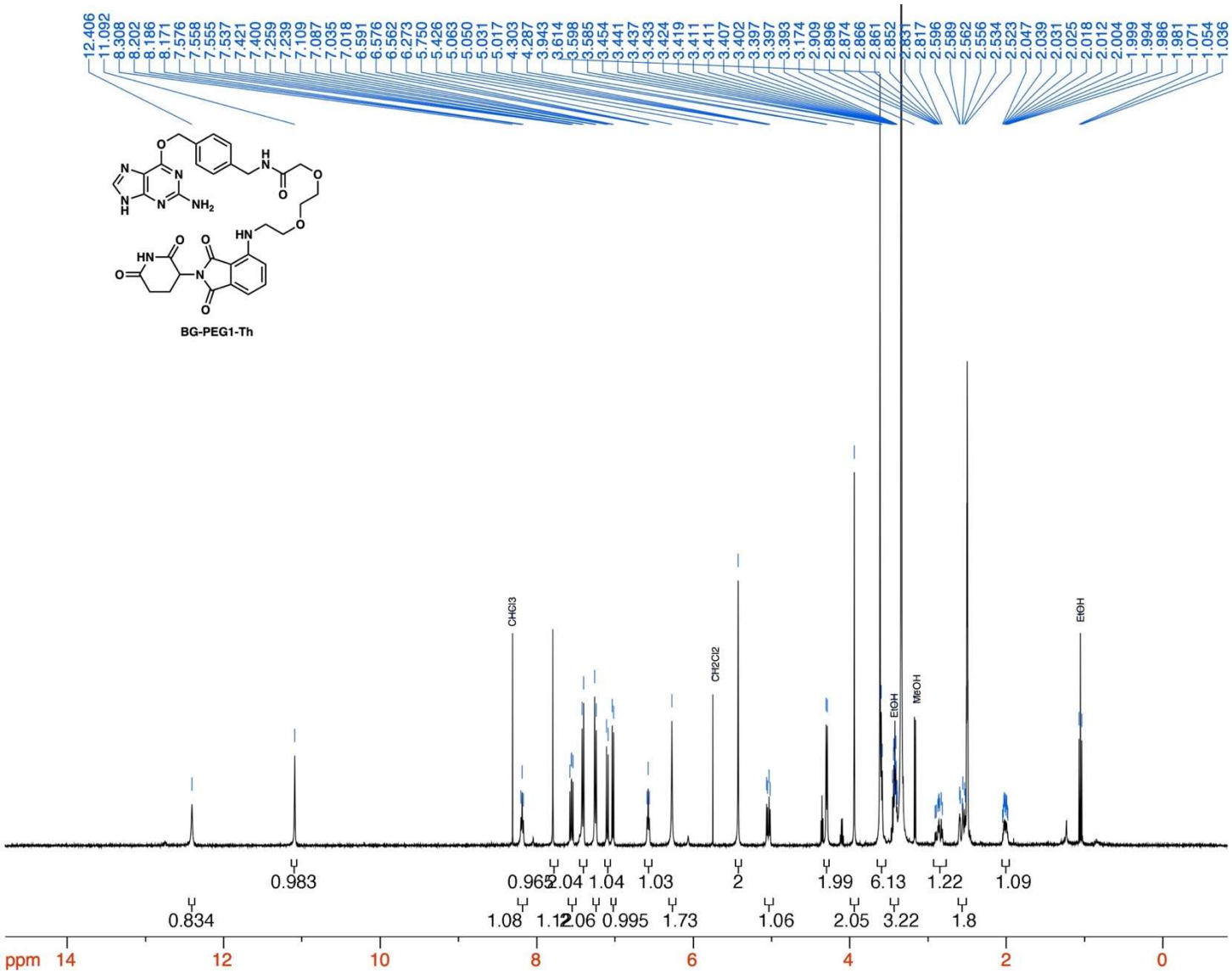


Figure S12. ^{1}H-NMR spectrum of BG-PEG1-Th (400 MHz; DMSO-d_6).

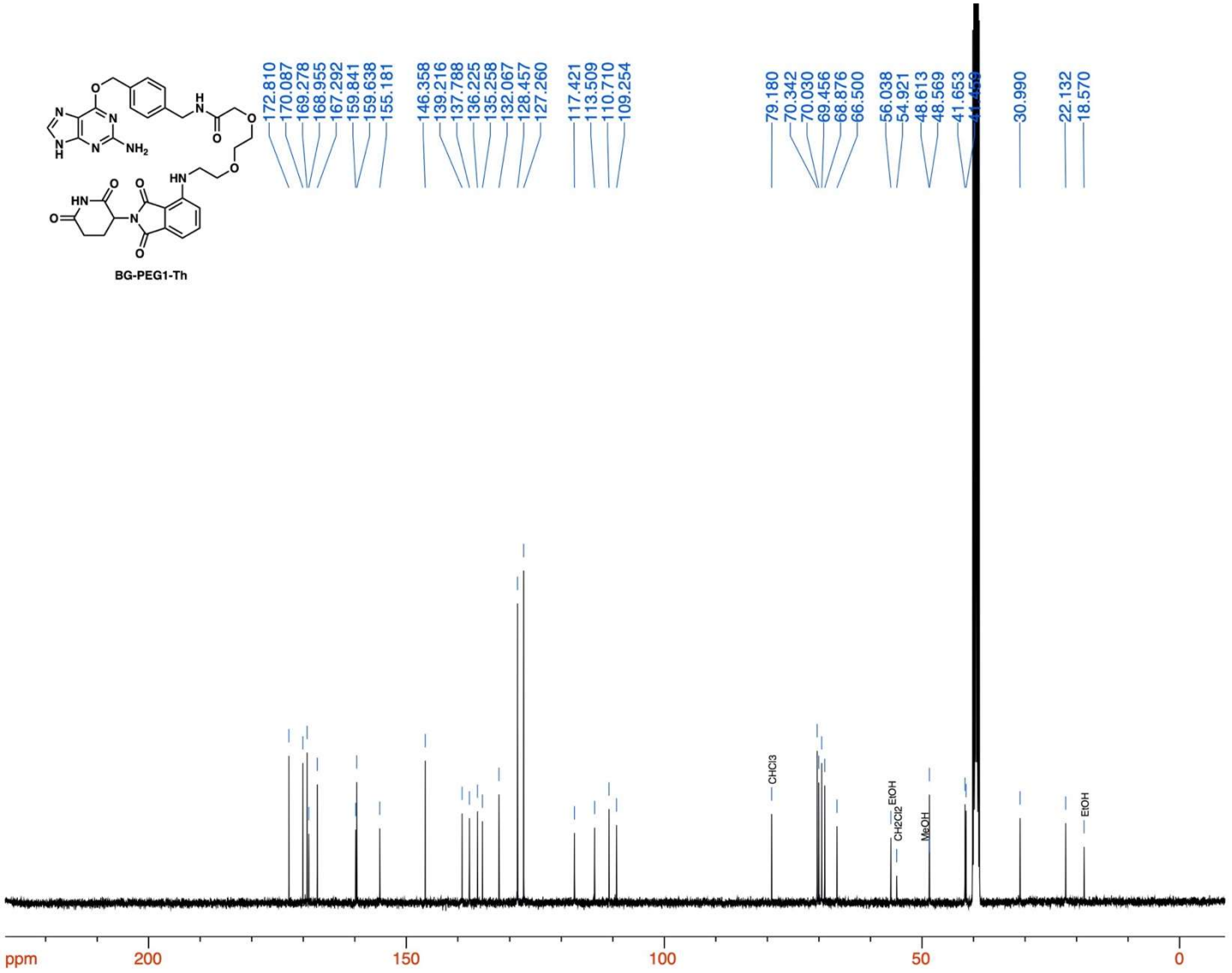


Figure S13. ^{13}C-NMR spectrum of BG-PEG1-Th (101 MHz; DMSO-d_6).

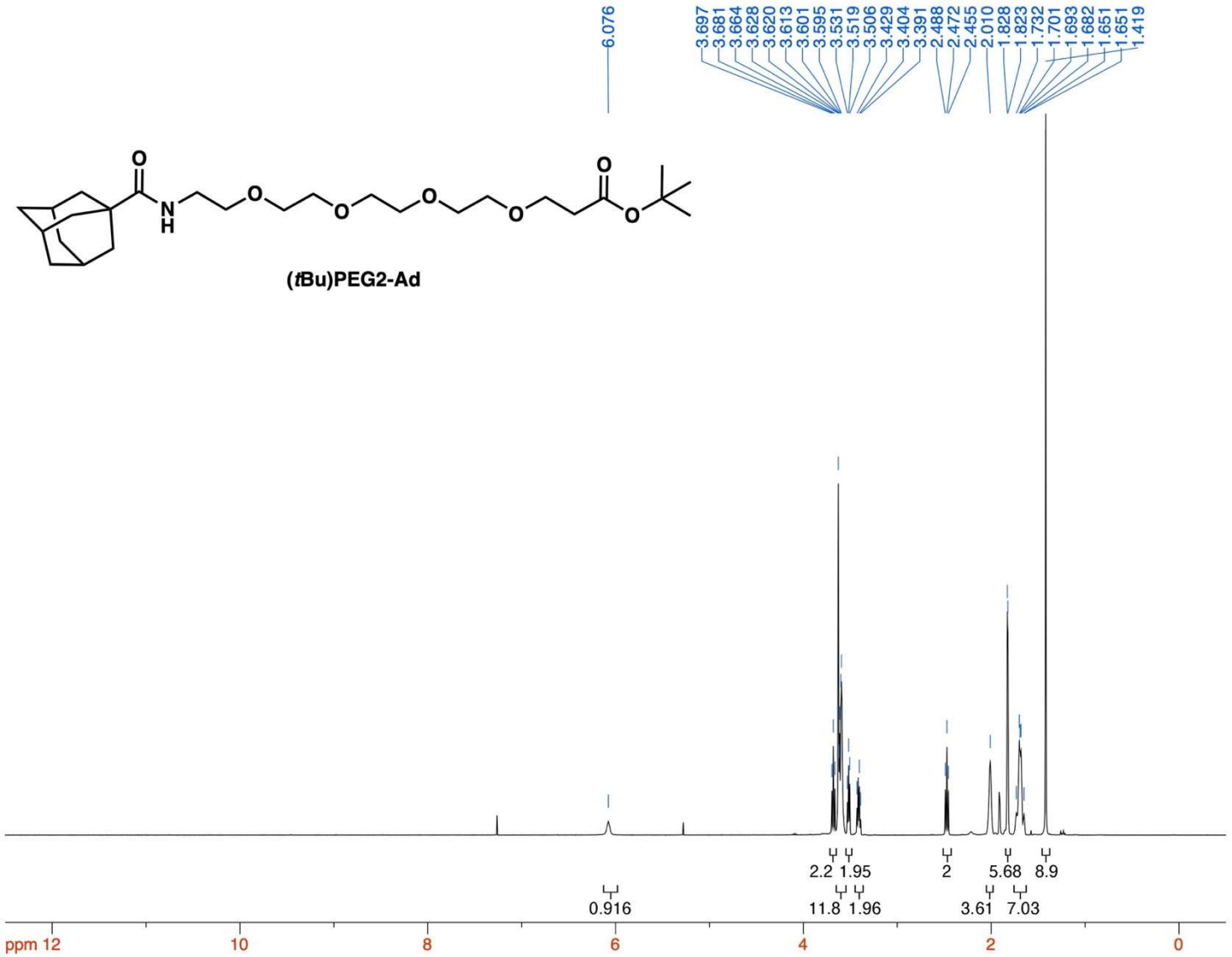


Figure S14. ^{1}H-NMR spectrum of (*t*Bu)PEG2-Ad (400 MHz; $CDCl_3$).

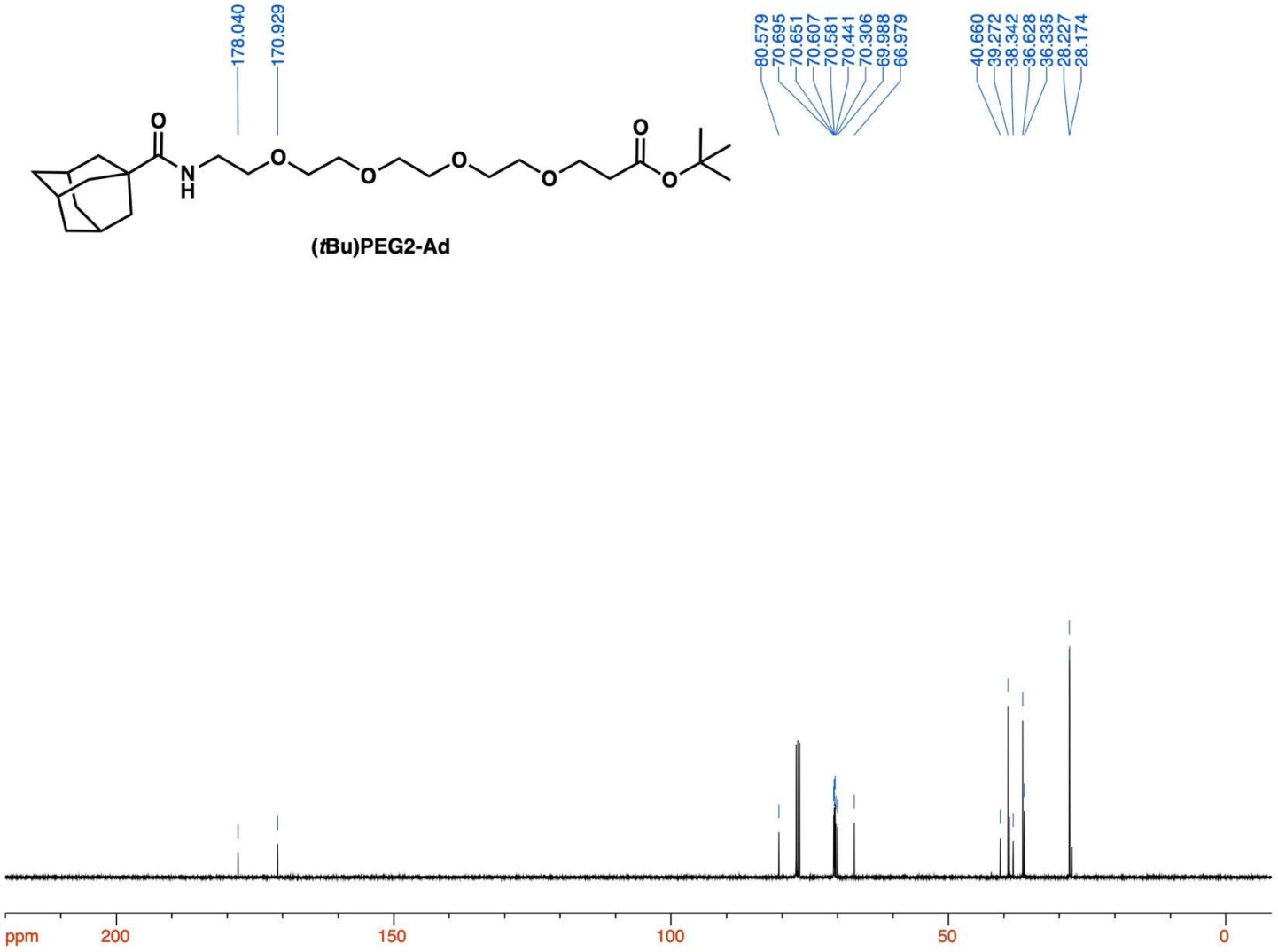


Figure S15. ^{13}C-NMR spectrum of (*t*Bu)PEG2-Ad (101 MHz; $CDCl_3$).

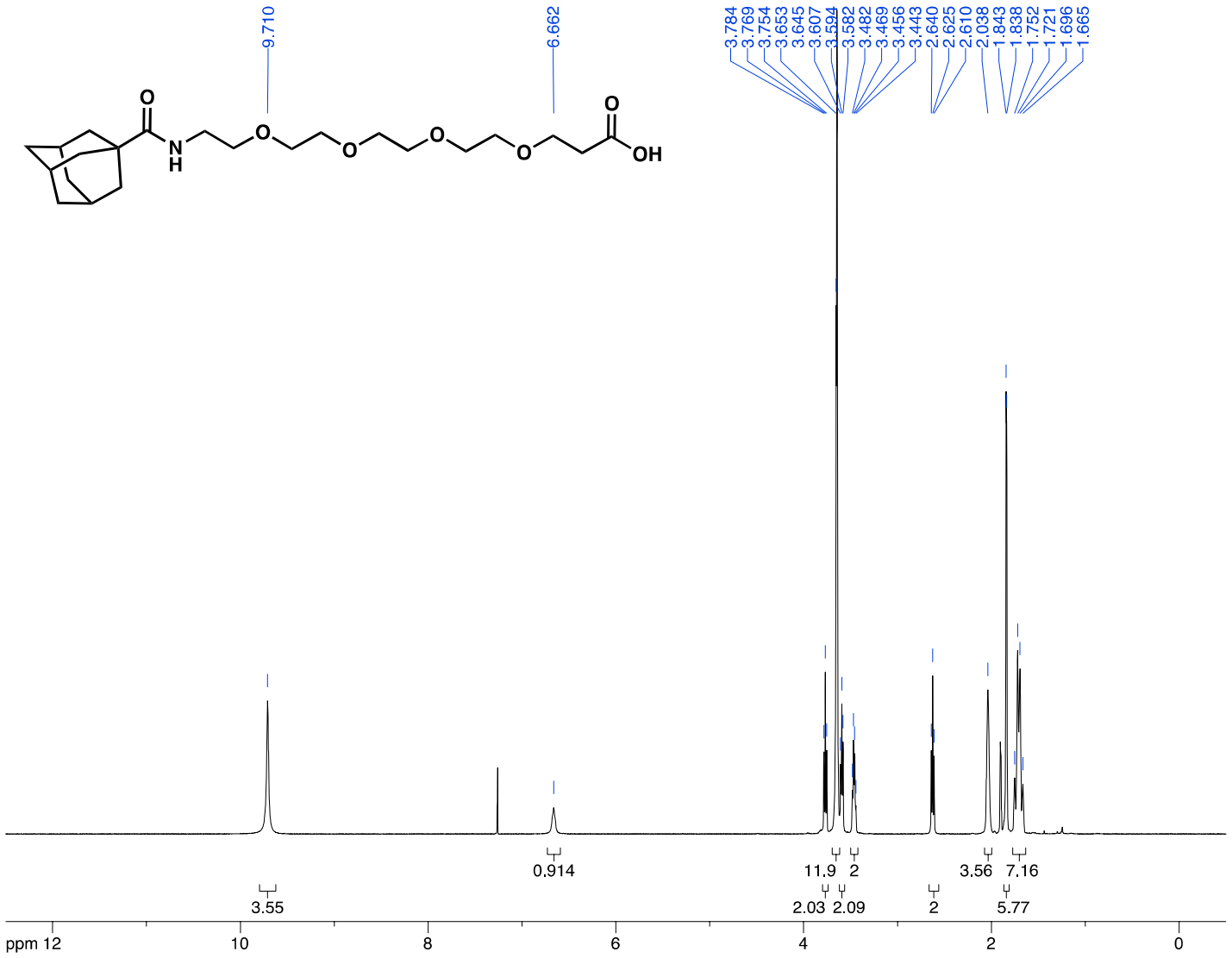


Figure S15. ^{1}H-NMR spectrum of (acid)PEG2-Ad (400 MHz; $CDCl_3$).

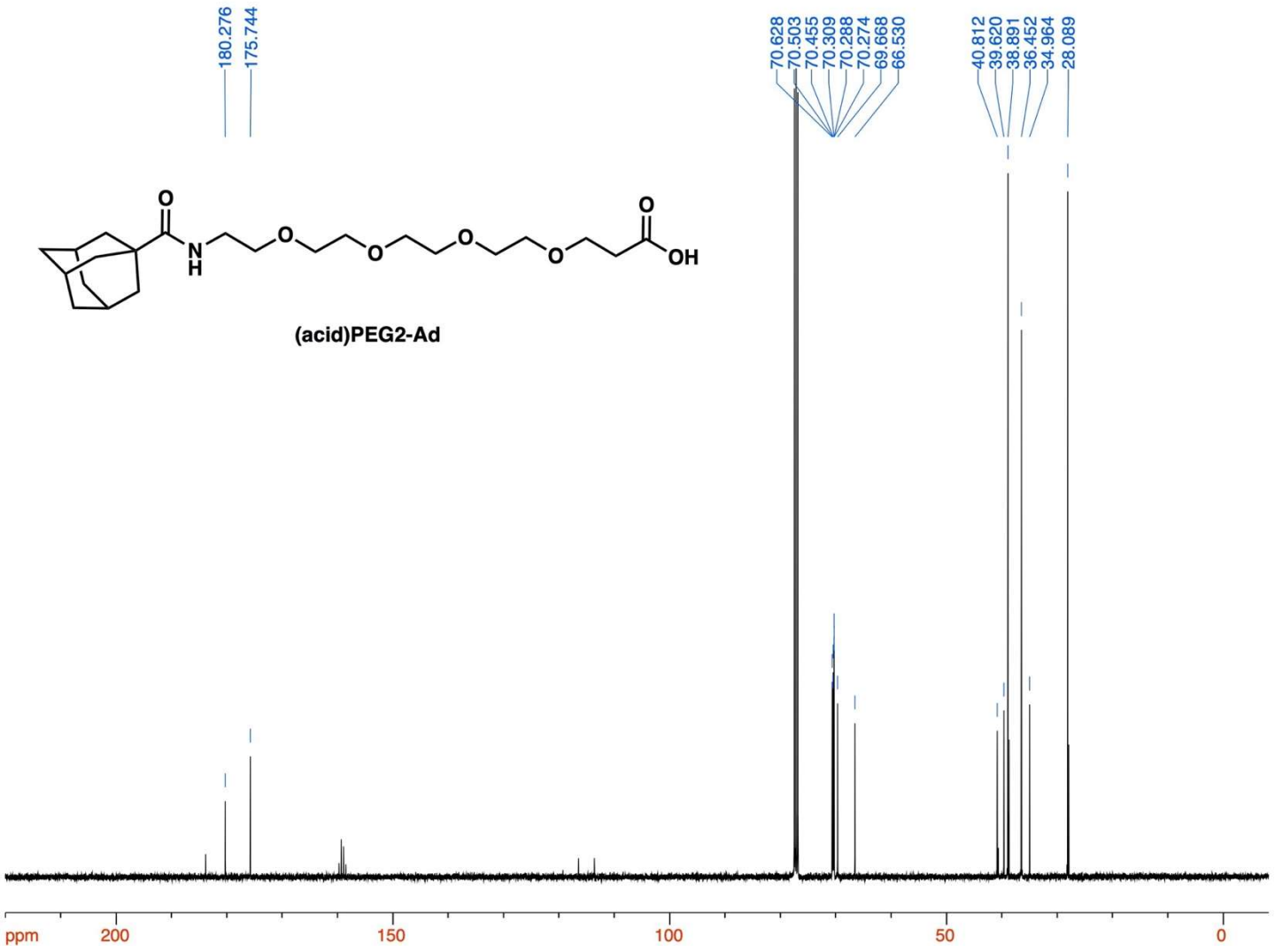


Figure S17. ^{13}C-NMR spectrum of (acid)PEG2-Ad (101 MHz; $CDCl_3$).

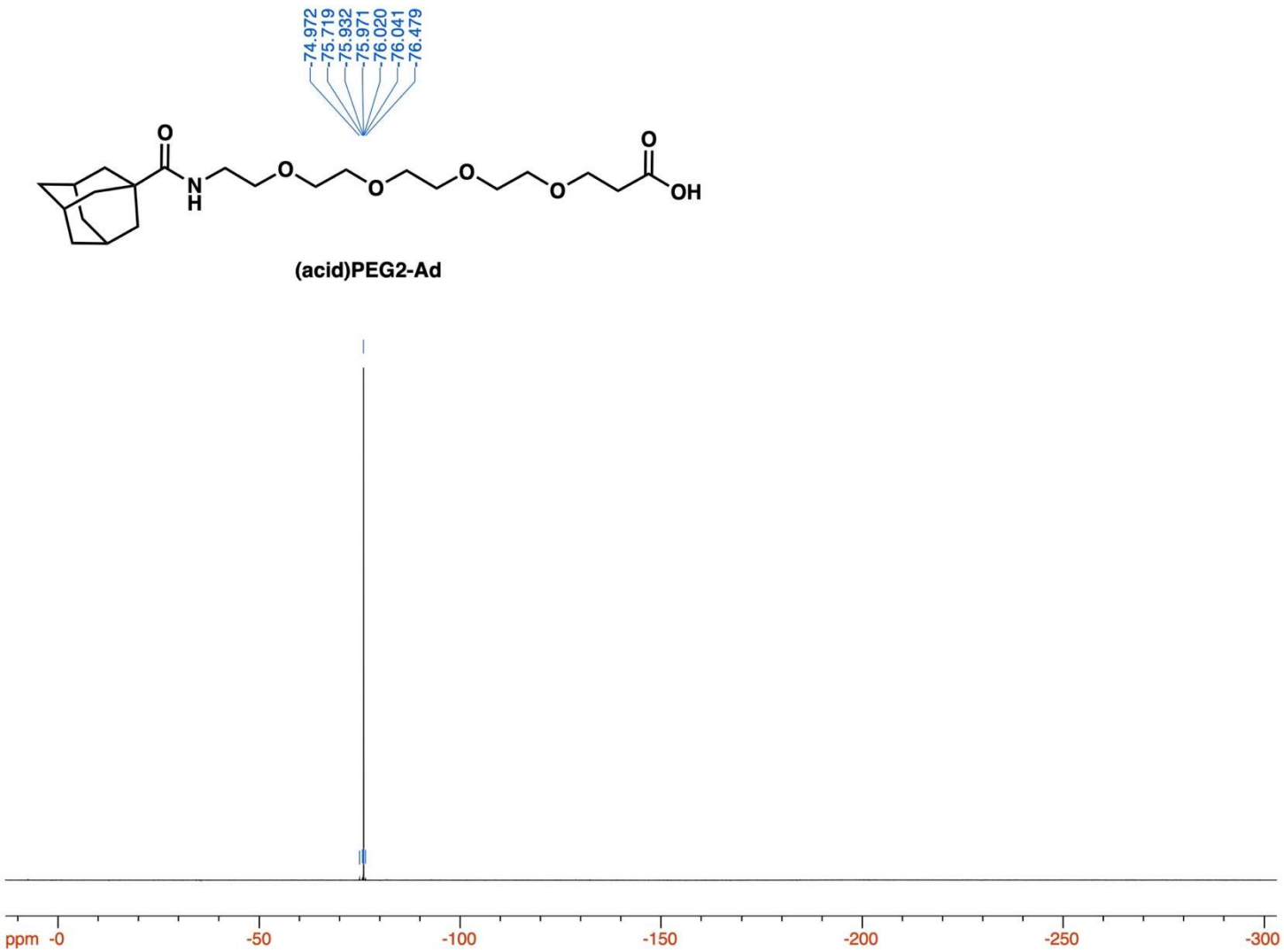


Figure S18. ^{19}F-NMR spectrum of (acid)PEG2-Ad (376 MHz; $CDCl_3$).

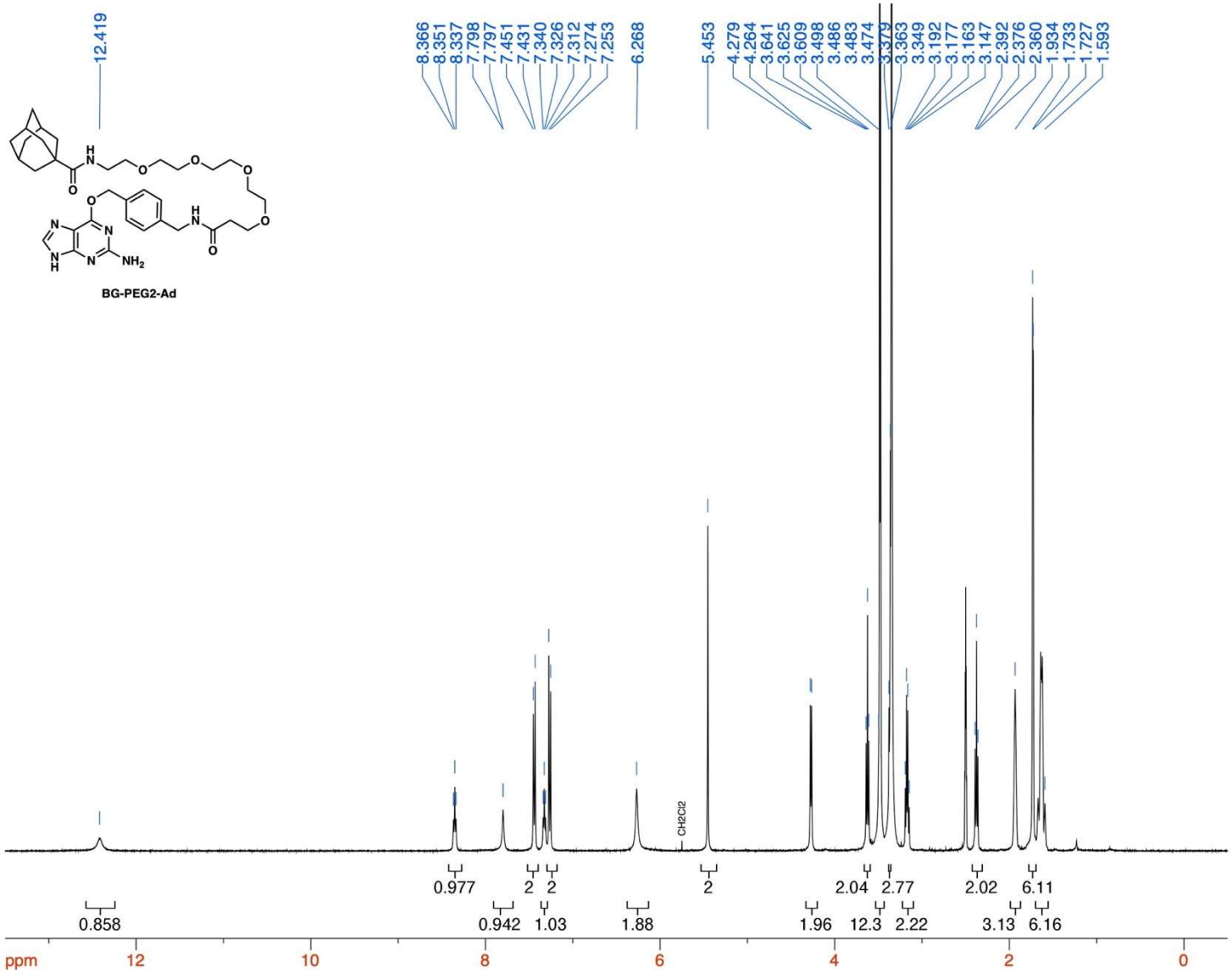


Figure S19. ^{1}H-NMR spectrum of BG-PEG2-Ad (400 MHz; DMSO-d_6).

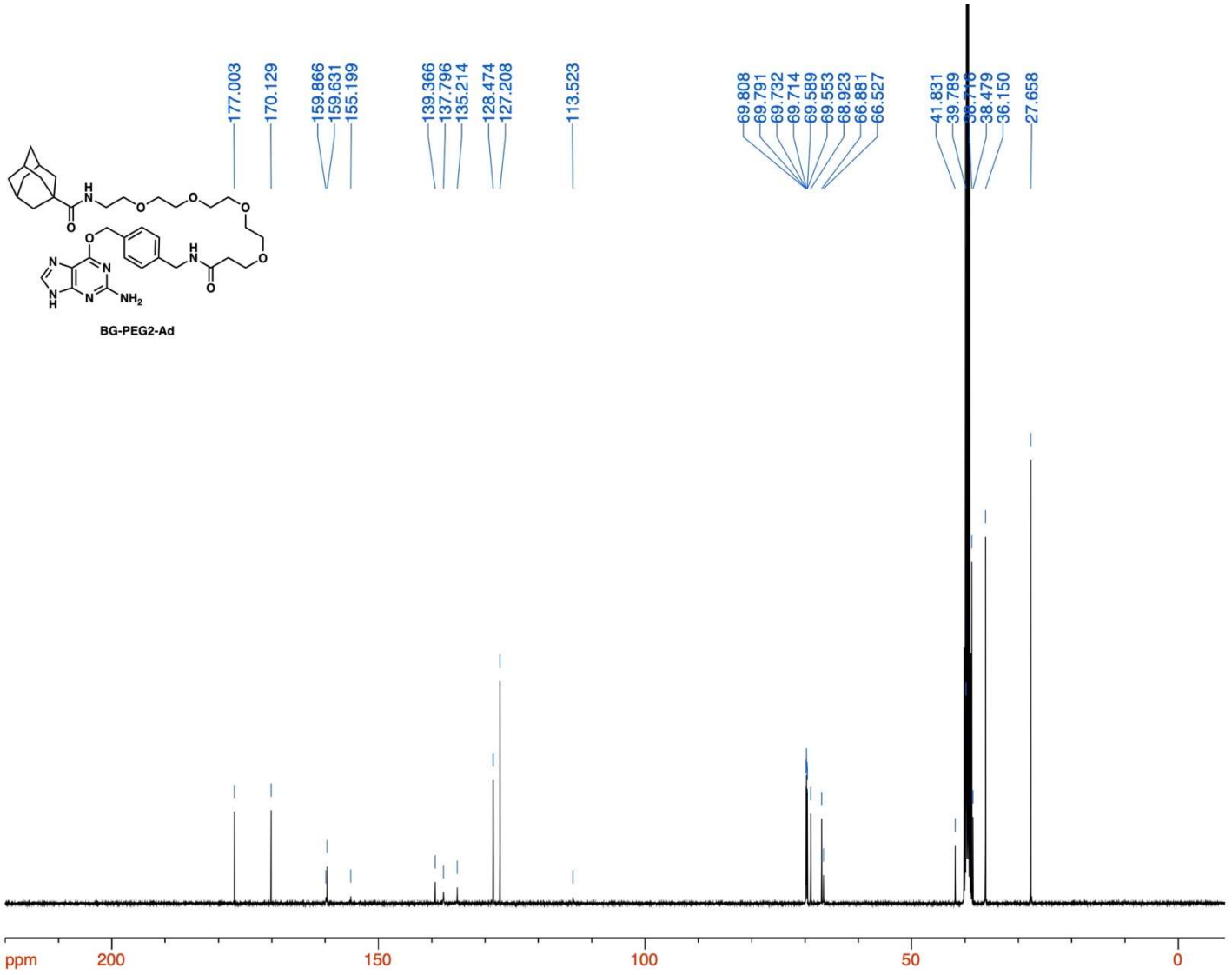


Figure S20. ^{13}C-NMR spectrum of BG-PEG2-Ad (101 MHz; DMSO-d_6).

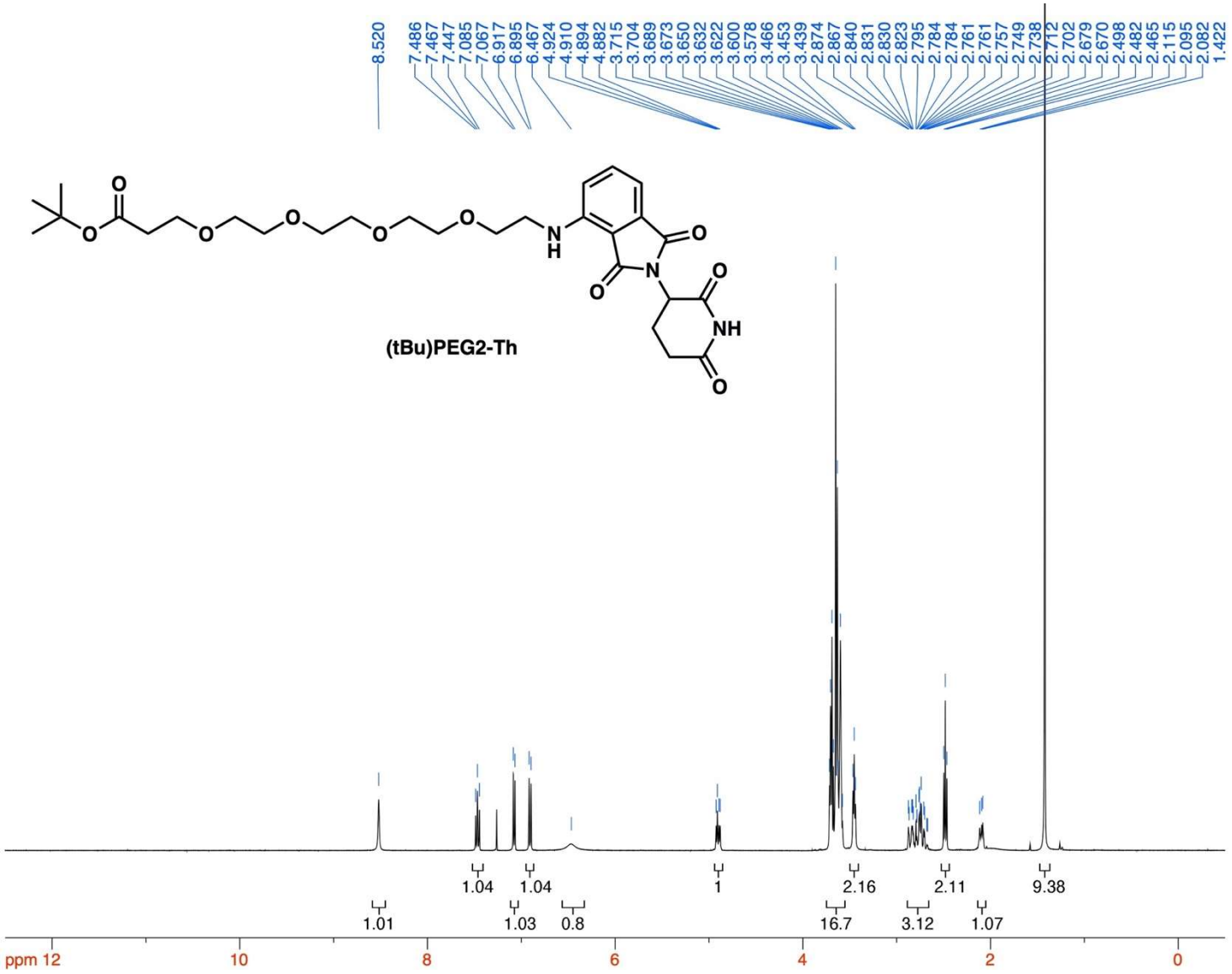


Figure S21. ^{1}H-NMR spectrum of (*t*Bu)PEG2-Th (400 MHz; $CDCl_3$).

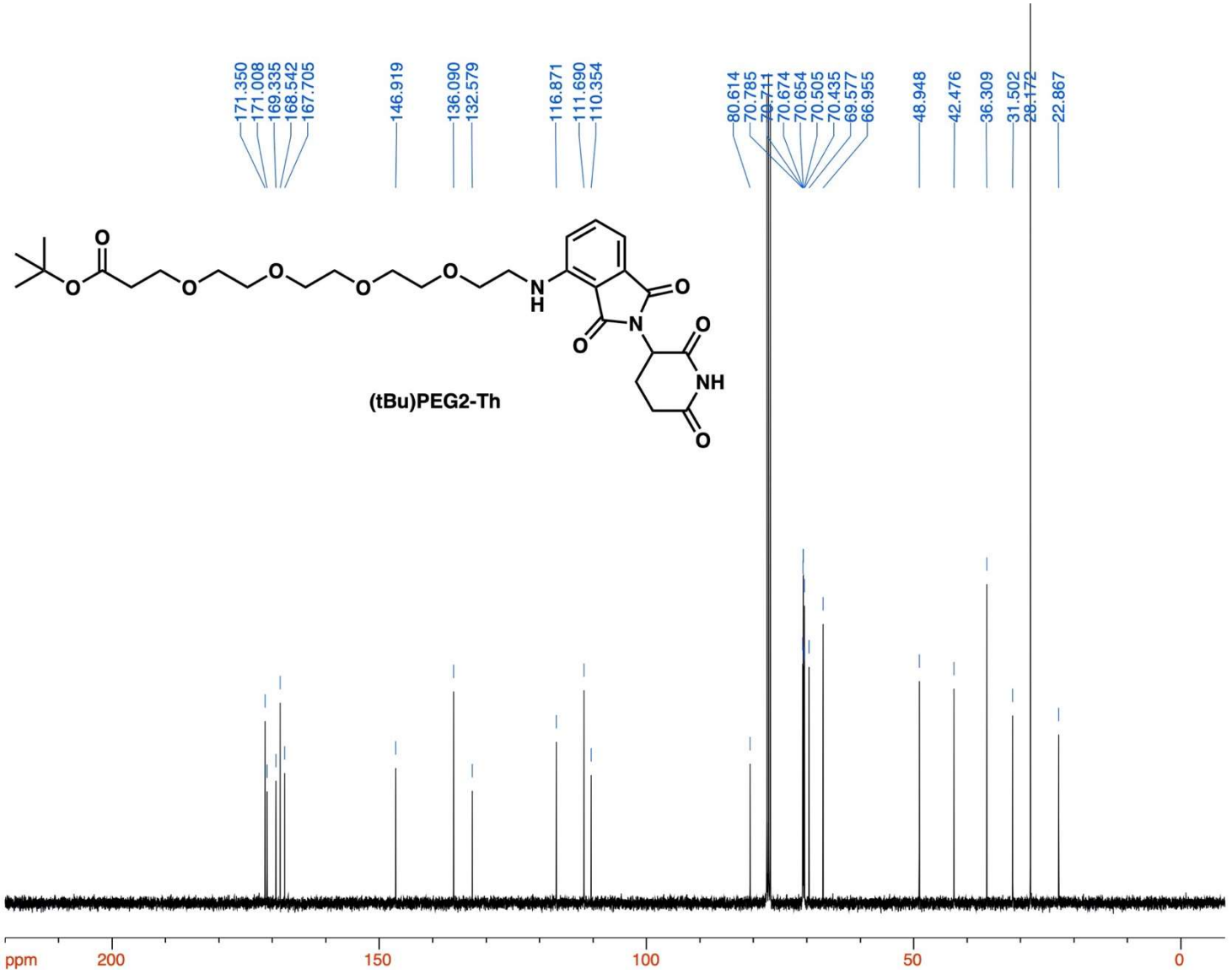


Figure S22. ^{13}C-NMR spectrum of (*t*Bu)PEG2-Th (101 MHz; $CDCl_3$).

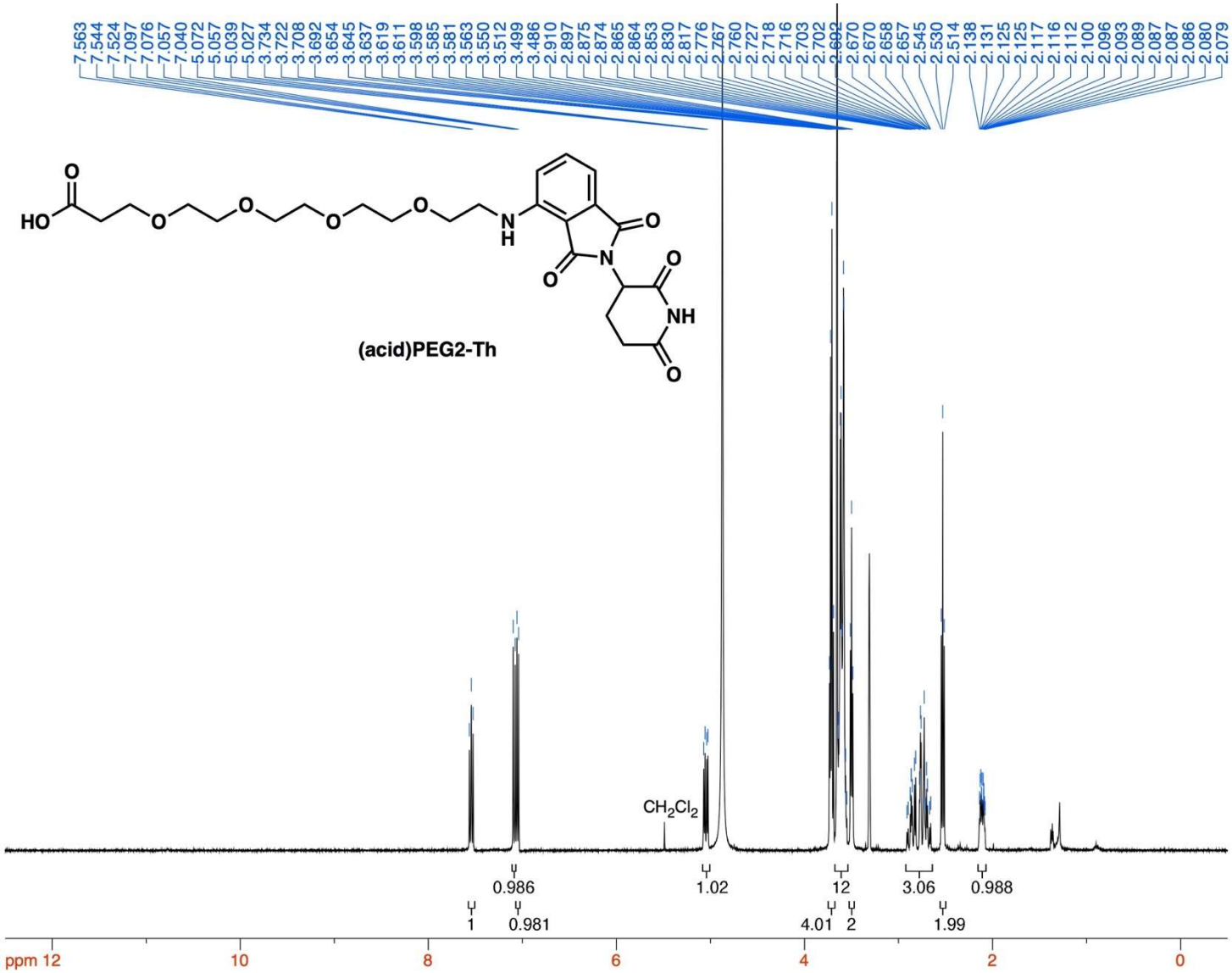


Figure S23. ^{1}H-NMR spectrum of (acid)PEG2-Th (400 MHz; CD_3OD).

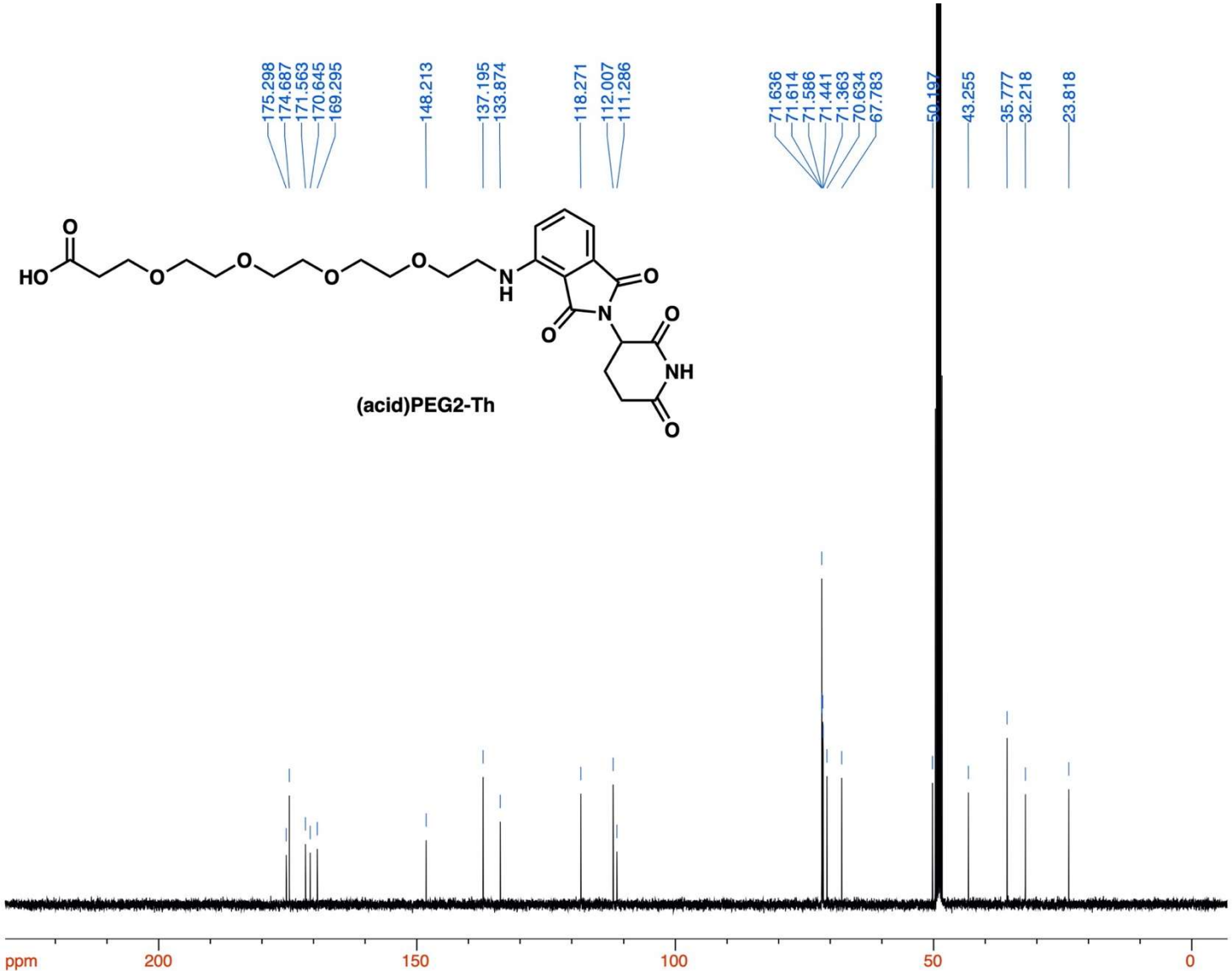


Figure S24. ^{13}C-NMR spectrum of (acid)PEG2-Th (101 MHz; CD_3OD).

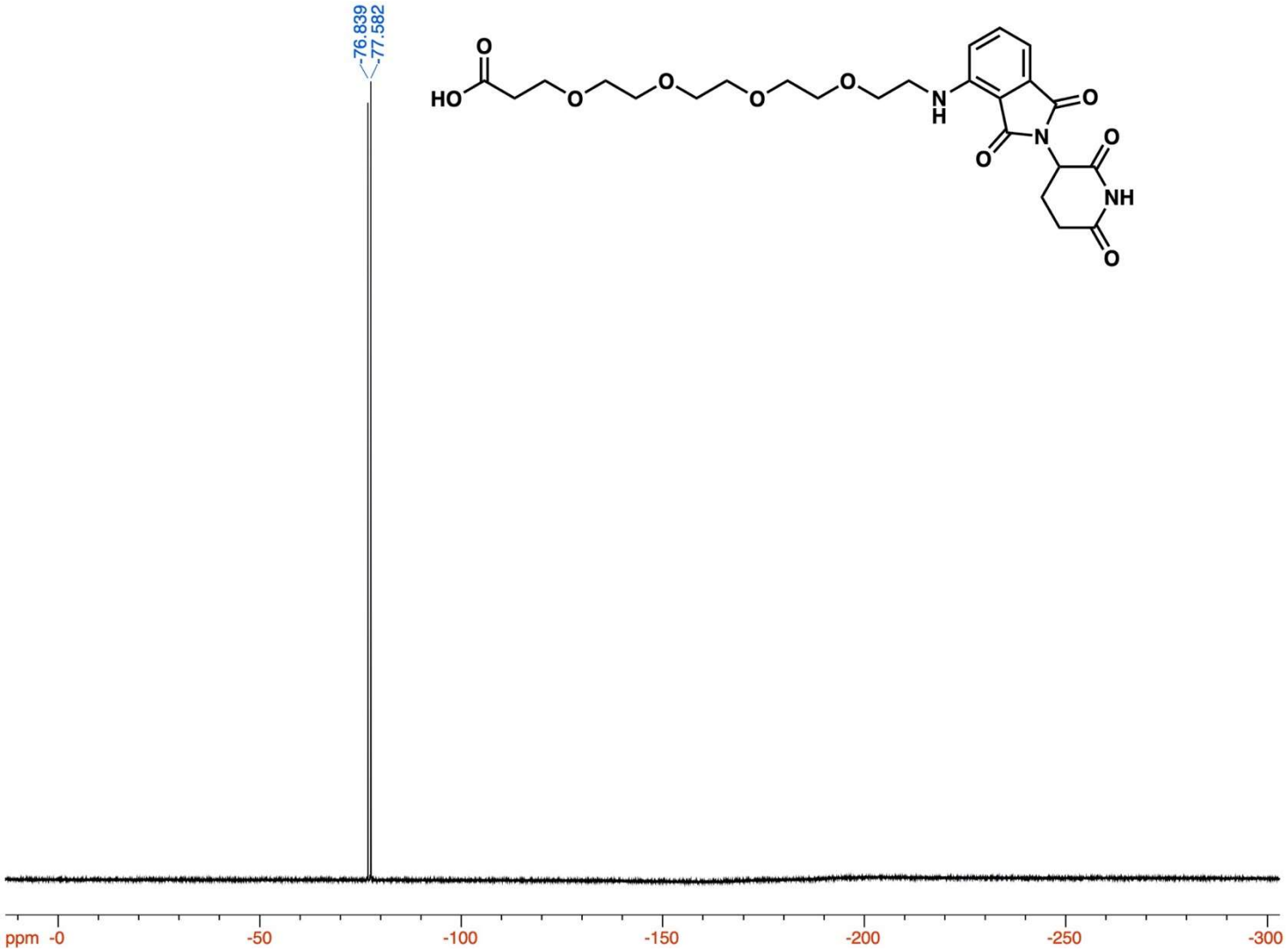


Figure S25. ^{19}F-NMR spectrum of (acid)PEG2-Th (376 MHz; CD_3OD).

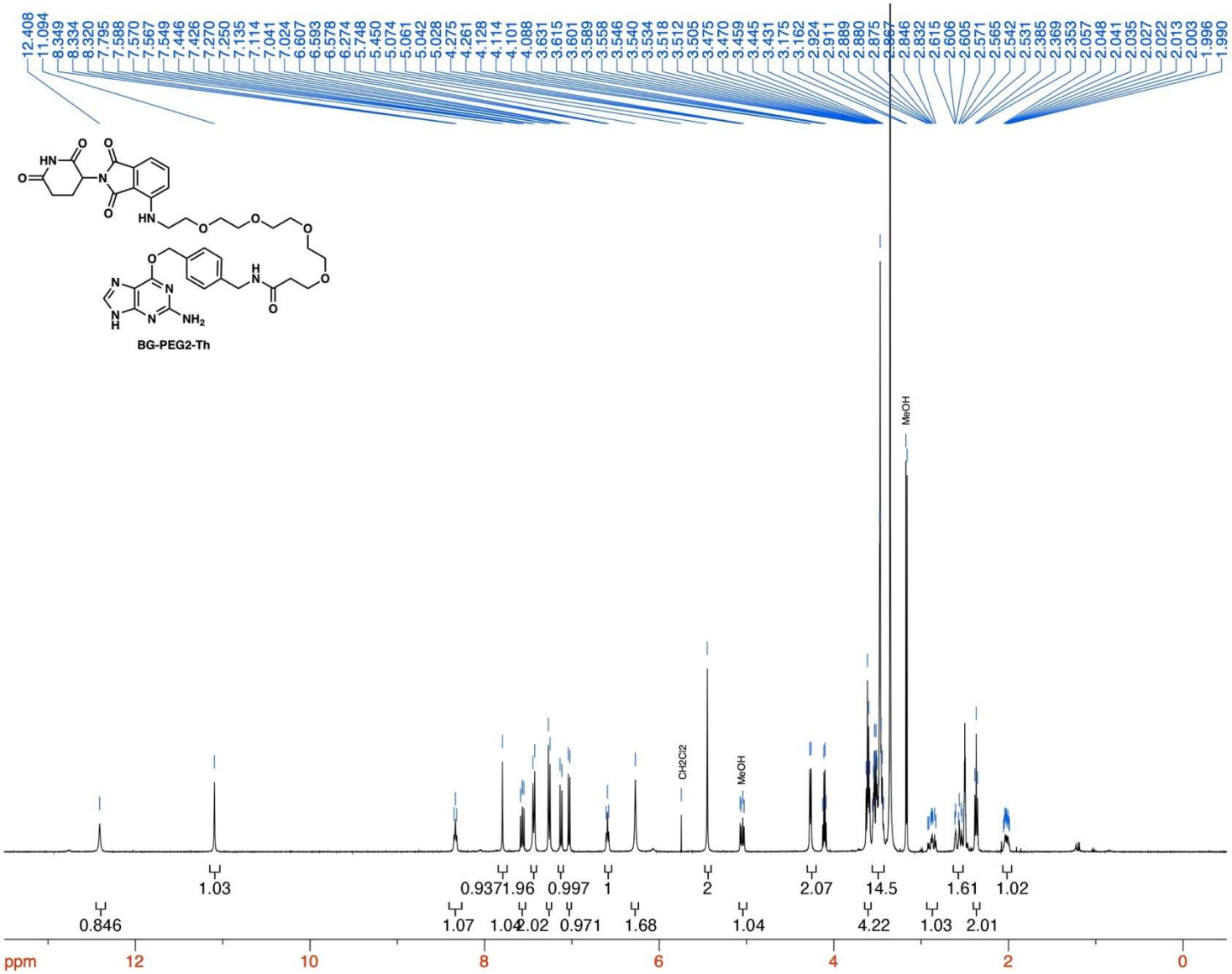


Figure S26. ^{1}H-NMR spectrum of BG-PEG2-Th (400 MHz; DMSO-d_6).

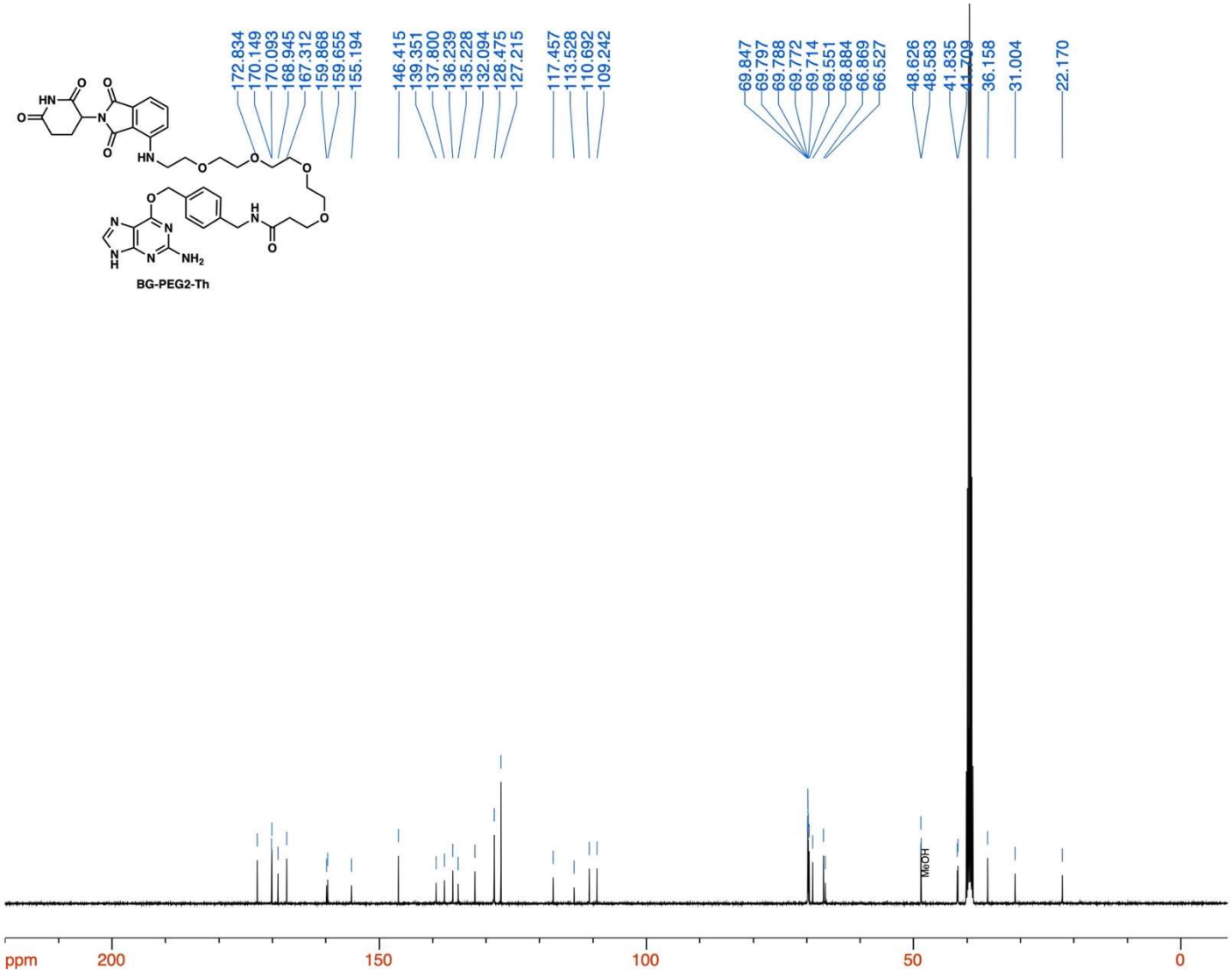


Figure S27. ^{13}C-NMR spectrum of BG-PEG2-Th (101 MHz; DMSO-d_6).

References

[1] Casini, A. *et al.* R2oDNA designer: Computational design of biologically neutral synthetic DNA sequences. *ACS Synth. Biol.* **3**, 525–528 (2014)

[2] Gibson DG. Enzymatic assembly of overlapping DNA fragments. Methods Enzymol. 2011;498:349-61. doi: 10.1016/B978-0-12-385120-8.00015-2. PMID: 21601685; PMCID: PMC7149801.

[3] Suzuki, K., Tsunekawa, Y., Hernandez-Benitez, R. *et al. In vivo* genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. *Nature* **540,** 144–149 (2016). https://doi.org/10.1038/nature20565