Supplementary Information

Protein Degradation through Covalent Inhibitor-based PROTACs

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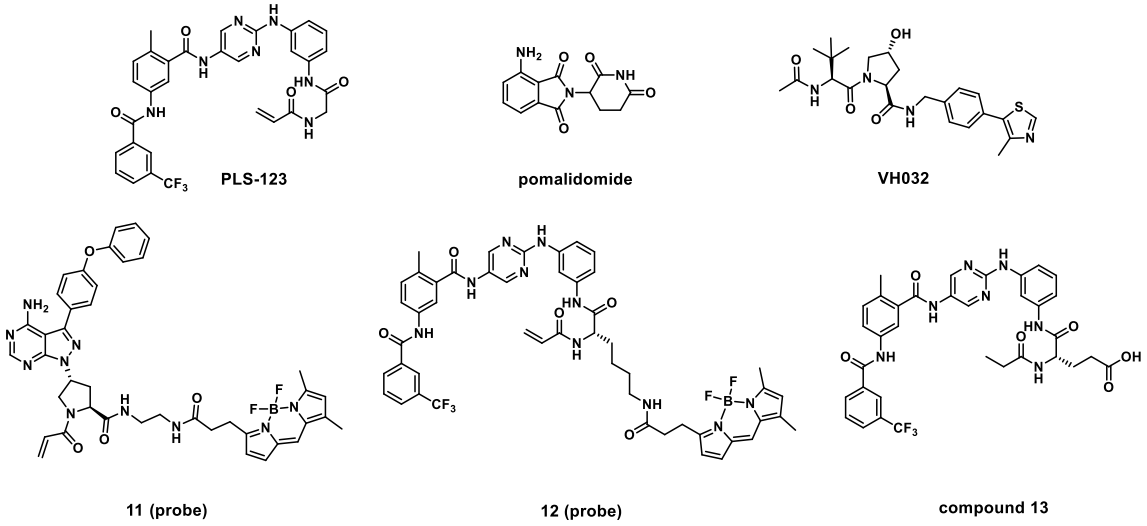


Fig. S1 Chemical structures of some compounds used in this study.

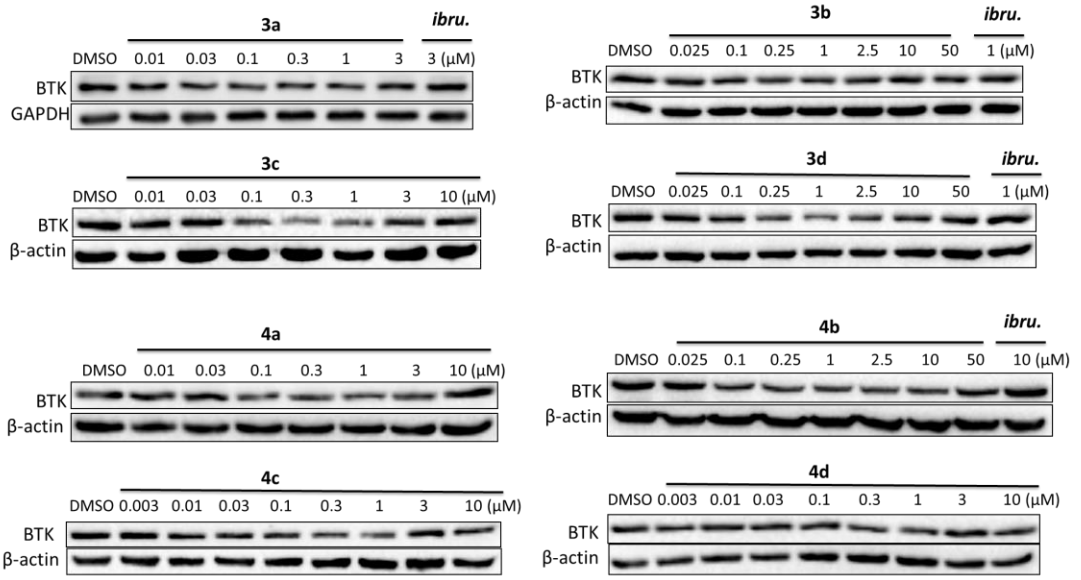


Fig. S2 The degradation of BTK by increasing concentration of PROTACs (**3**-**4**) in K562 cells for 24 h.

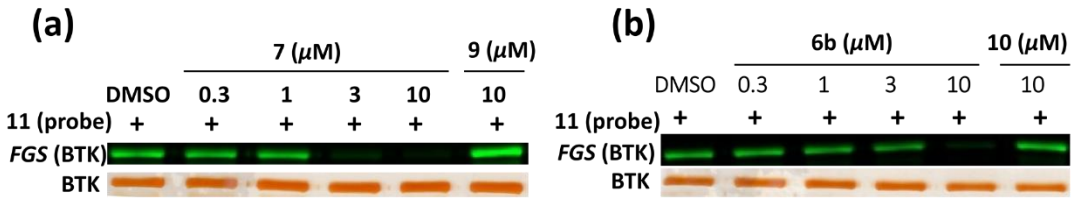


Fig. S3 Competitive assays between PROTACs and covalent fluorescent probe **11** against BTK. (a) Fluorescent gel scanning results showed that PROTAC **7** at 3 μM completely competed out the labeling of BTK by probe **11**, while **9** did not at 10 μM. (b) Fluorescent gel scanning results showed that PROTAC **6b** at 10 μM competed out the labeling of BTK by probe **11**, while **10** did not.

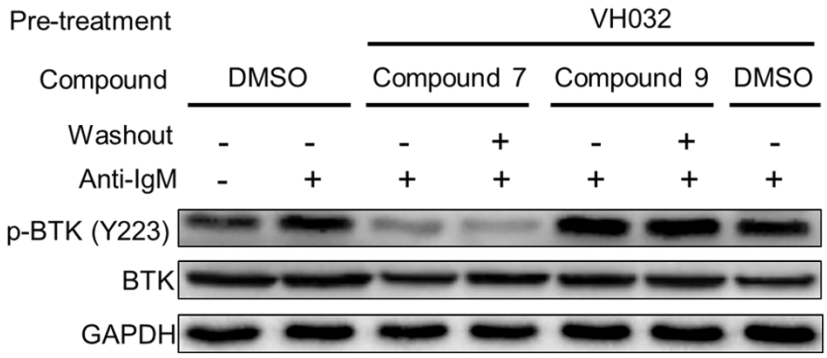


Fig. S4 Washout assay. After K562 cells were pretreated with VHL inhibitor (VH032, 30 μM) for 1.5 h, compound **7** (20 μM), compound **9** (20 μM) or DMSO was added, and the cells were incubated for another 2 h. The compounds were washed out (+) with cell incubate medium and anti-IgM was added (+) to stimulate the BCR signaling pathway for 5 min. Then the levels of p-Y223 of BTK, total BTK and GAPDH were analyzed by western blot.

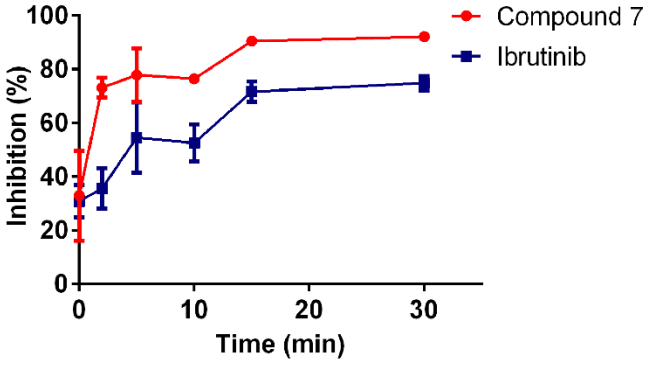


Fig. S5 Time-dependent inhibition assay. BTK kinase was preincubated with compound **7** (40 nM) or ibrutinib (1 nM) for different time (0, 2, 5, 10, 15, 30min). ATP and substrate were then added to the mixture to initiate the kinase reaction. The remaining BTK activity was detected according to the assay kit.

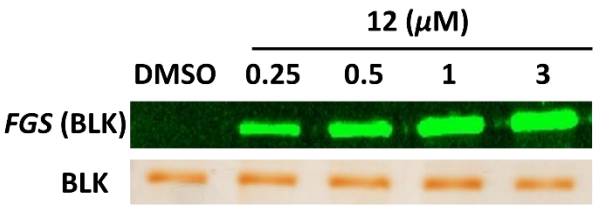


Fig. S6 BLK was labeled with various concentration of probe **12**.

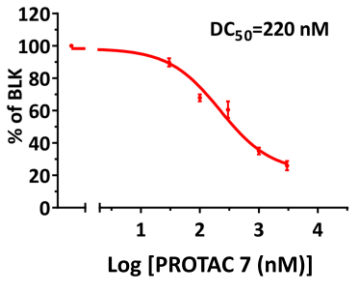


Fig.S7 BLK protein levels after cells were treated with various concentration of PROTAC **7**. Numbers were calculated by BLK/GAPDH ratio with normalization by the DMSO control as 100. The bar in graphs show the means ± standard deviation from two biological replicates.

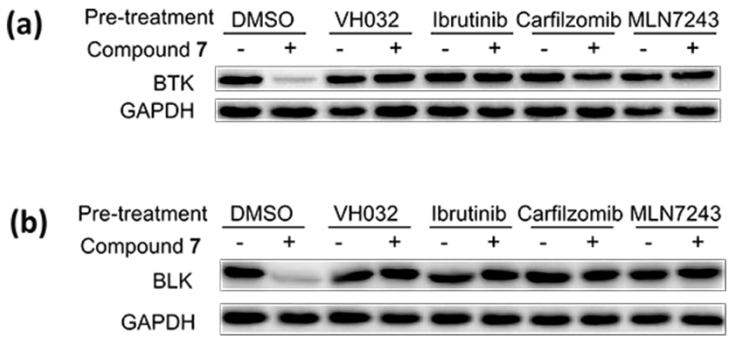


Fig.S8 The degradation of BTK and BLK induced by PROTAC **7**. (a) After K562 cells were pretreated with DMSO, VHL inhibitor (VH032, 30 μM), BTK inhibitor (ibrutinib, 10 μM), proteasome inhibitor (carfilzomib, 100 nM), or ubiquitin-like modifier activating enzyme (UAE) inhibitor (MLN7423, 200 nM) for 1.5 h, compound **7** (3 μM, +) or DMSO (-) was added and the cells were incubated for another 8 h. Then the levels of BTK protein and GAPDH were analyzed by western blot assay. (b) After Ramos cells were pretreated with DMSO, VH032 (30 μM), ibrutinib (10 μM), carfilzomib (100 nM) or (MLN7423, 200 nM) for 1.5 h, compound **7** (3 μM, +) or DMSO (-) was added and the cells were incubated for another 4 h. Then the levels of BLK protein and GAPDH were analyzed by western blot assay.

Biological Assays

Cell culture.

K562 and Ramos cells were maintained in RPMI-1640 medium (A10491, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and were cultured in an incubator at 37 °C in 5% CO2.

Western blot assay

1×10^6 cells in 1 mL culture medium were seeded in 12-well cell culture plate with different concentrations of compounds for the indicated time, followed by washing with PBS once and lysing in lysis buffer containing Beyotime P0013, 1 mM PMSF, 10 mM NaF, 100× phosphatase inhibitors and protease inhibitors. After measuring the sample protein concentrations with NanoDrop 2000 spectrophotometer, we separated the protein lysate by SDS/PAGE and transferred to PVDF membrane. Membrane was blocked in milk and incubated with primary antibodies at 4 °C overnight, followed by rabbit (Cell Signaling Technology (CST), Prod#7074S, 1:1000) secondary antibodies incubated for 1 h at room temperature. The membrane was imaged with Millpore reagent (Luminata™, Cat. No. WBLUR0500) on Chemidoc MP instrument. Analysis of band intensities was processing with Image lab software. Primary antibodies against BTK (Prod#8547S, 1:1000), BLK (Prod#3262S, 1:1000), GAPDH (Prod#2118S, 1:1000), β-actin (Prod#4970L, 1:1000) were purchased from CST.

BTK enzymatic assay.

Compounds' inhibitory potencies against BTK kinase (Carna Biosciences, 08-080) were determined with the protocols of HTRF (homogeneous time-resolved fluorescence) KinEase assays provided by Cisbio Bioassays company.

Protein labeling assay

Incubation of increasing concentrations of probe **12** with recombinant BLK (0.2 μg, life technologies, PR6635A) in PBS buffer for 2 h. The samples were separated by SDS/PAGE and the fluorescent gel was scanned by molecular imager (BIO-RAD, PharosFX™ Plus, 447BR1227). The protein content was analyzed by silver staining experiment according to the protocols of Pierce® Silver Stain for Mass Spectrometry (24600) provided by Thermo Scientific company.

Competition assay

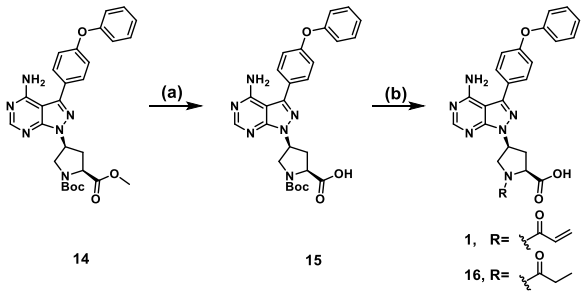
Preincubation of DMSO and various concentrations of PROTACs with BTK (0.35 μg, Carna Biosciences, 08-080) or BLK (0.2 μg, life technologies, PR6635A) in PBS buffer for the indicated time at room temperature, then probe **11** (1 μM) or probe **12** (0.5 μM) were added to the mixture for 1.5 h. The samples were analyzed and the gel was scanned as described above.

Synthetic Procedures

General Methods

Compounds **2**[1] and **24**[2] were synthesized according to references, and other starting materials and reagents were commercially available and used without further purification. DCM were distilled from calcium hydride, and other anhydrous solvents were obtained from commercial suppliers. Thin-layer chromatography (TLC) carried out on 0.25 mm Yantai silica gel plates (HSGF 254) was used to monitor reactions using UV light (254 nm). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Advance 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz), Bruker Advance 400 (^{1}H: 400 MHz; ^{13}C: 101 MHz) or Bruker Advance 500 (^{1}H: 500 MHz; ^{13}C: 126 MHz) spectrometer at ambient temperature. The chemical shift values reported in ppm were referenced to NMR solvent signals: dimethyl sulfoxide (δ_H 2.50 and δ_C 39.52), or methanol (δ_H 3.31 and δ_C 49.00), $CDCl_3$ (δ_H 7.26 and δ_C 77.16). The spectra data are reported as follows: chemical shift, signal splitting patterns (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (HZ), and number of protons. High resolution mass spectra (HRMS) were recorded on a Bruker Apex IV RTMS instrument. The purities of all final compounds were analyzed by high performance liquid chromatography (HPLC) monitored at 254 nm. The HPLC analyses were performed on an Agilent PN880975-902 ZORBAX SB-C18 4.6 × 250mm column, and the mobile phase was water and MeCN with a gradient from 20% to 95% over 15 min, flow 3 ml/min.

Scheme S1. Synthesis of compounds 1 and 16.

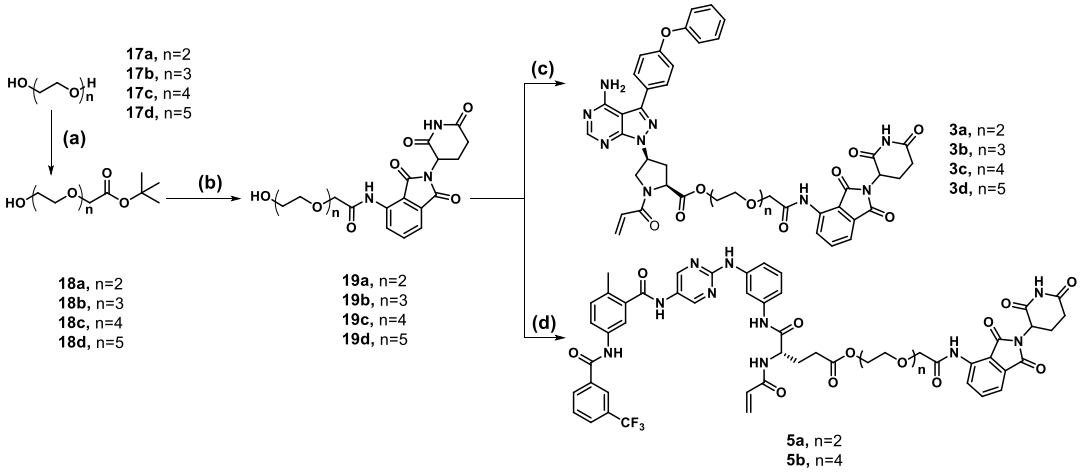


Reagents and conditions: (a) LiOH, THF/H_2O/MeOH, rt, 2h. (b) (i)TFA, DCM, rt, 2h; (ii) acryloyl chloride or propionyl chloride, Et_3N, THF, rt, 4h.

Compound **14** (1.54 g, 2.90 mmol) was dissolved in 50 ml THF: MeOH: H_2O = 3 : 1 : 1 at 0 °C. 1 N LiOH (7.05 mL, 7.05 mmol) was added slowly to the reaction mixture. After 4 hours, the mixture was concentrated and extracted with ethyl acetate and water. The aqueous layers were combined and acidified to pH = 1 with 1N HCl. The reaction mixture was extracted with ethyl acetate and water three times. The organic layers were collected and dried over anhydrous Na_2SO_4 to give the carboxylic acid intermediate **15** without further purification. Intermediate **15** was dissolved in DCM (7 mL) and TFA (7 mL) for 2 h at room temperature. The mixture solution was removed to provide yellow oil. The oil was dissolved in THF at 0 °C. TEA (1.98 ml, 14.10 mmol) and acryloyl chloride (0.51 g, 5.64 mmol) were added to the solution. The mixture was stirred for 4 hours at room temperature and based to pH > 7 with Na_2CO_3.

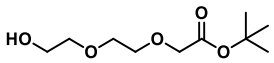
After removing THF, the residue was extracted with ethyl acetate and water. The aqueous layers were acidified to pH = 1 with 1N HCl. The reaction mixture was extracted with ethyl acetate and water three times. The organic layer was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, concentrated and purified by flash column chromatography to give 0.79 g of compound **1** as a white solid (yield 58 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 9.5 Hz, 1H), 7.51 (dd, *J* = 17.8, 8.4 Hz, 2H), 7.43 – 7.19 (m, 2H), 7.21 – 6.82 (m, 5H), 6.40 (dd, *J* = 11.8, 6.8 Hz, 2H), 5.82 – 5.57 (m, 1H), 5.56 – 5.31 (m, 1H), 4.70 (m, 1H), 4.48 – 4.08 (m, 2H), 3.28 – 2.68 (m, 2H). HRMS calculated for $C_{25}H_{23}N_6O_4$ $[M+H]^+$: 471.1775, found 471.1770. According to the procedure for the synthesis of **1**, compound **16** was synthesized as a white solid. HRMS calculated for $C_{25}H_{25}N_6O_4$ $[M+H]^+$: 473.1932, found 473.1933.

Scheme S2. **Synthesis of compounds 3 and 5**.



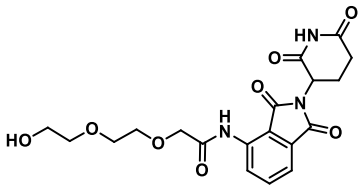
Reagents and conditions: (a) NaH, tert-Butyl 2-bromoacetate, THF. (b) (i) TFA, DCM, rt, 2h; (ii) $SOCl_2$, reflux, 2h; (iii) pomalidomide, THF, 65°C, 4h. (c) **1**, EDCI, DMAP, DCM. (d) **2**, EDCI, DMAP, DCM/DMF.

***tert*-butyl 2-(2-(2-hydroxyethoxy)ethoxy)acetate (18a)**



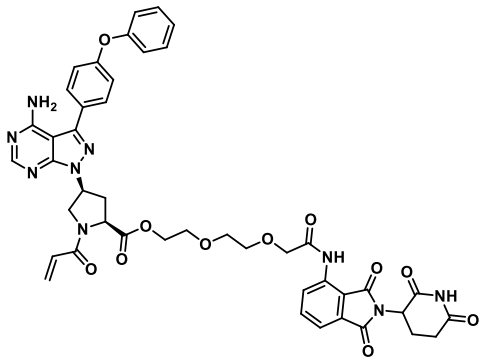
A solution of compound **17a** (1 g, 9.43 mmol) in THF was cooled to 0 °C in ice water bath. Then NaHMDS (1 M in THF, 2.35 mL, 2.35 mmol) was added into the solution. The mixture was stirred for 1h, then tert-butyl 2-bromoacetate (0.32 mL, 1.97 mmol) was added, followed by the ice water bath was removed and the reaction mixture was stirred for another 1h. After the reaction was quenched with water, the mixture was extracted with ethyl acetate. The combined extract was washed with brine and dried over Na_2SO_4 and the solvent was removed under reduced pressure. After concentration, the crude residue was purified by column chromatography to provide 0.13 g (30%) of **18a** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.00 (s, 2H), 3.75 – 3.70 (m, 2H), 3.70 – 3.66 (m, 4H), 3.60 (dd, *J* = 5.7, 3.4 Hz, 2H), 2.67 (s, 1H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.61, 81.75, 72.64, 70.84, 70.32, 68.98, 61.69, 28.10. HRMS calculated for $C_{10}H_{20}O_5Na$ $[M+Na]^+$: 243.1203, found 243.1200.

***N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-2-(2-(2-hydroxyethoxy)ethoxy)acetamide (19a)**



18a (470 mg, 2.14 mmol) was dissolved in DCM (2 mL) and TFA (2 mL) and the reaction mixture was stirred at room temperature for 2 h. The mixture solution was concentrated by rotary evaporator to provide yellow oil. The yellow oil was dissolved in $SOCl_2$ (2 mL), and the reaction mixture was heated to reflux for 2 h. After cooling down, the solvent was removed by rotary evaporator. The residue was dissolved in dry THF (5 mL) and pomalidomide (419 mg, 1.53 mmol) was added to the solution. The reaction mixture was heated to 65 °C for 4 h, then the reaction was quenched with water. The mixture solution was extracted with EtOAc. The combined organic phase was washed with brine and dried over Na_2SO_4 and the solvent was removed by rotary evaporator. After concentration, the crude residue was purified by column chromatography with DCM/MeOH to provide 0.38 g (59%) of **19a** as a light green solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.54 (s, 1H), 8.86 (d, *J* = 8.4 Hz, 1H), 8.37 (s, 1H), 7.79 – 7.68 (m, 1H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.26 (s, 1H), 4.98 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.24 – 4.13 (m, 2H), 3.80 (dq, *J* = 8.2, 3.8 Hz, 4H), 3.75 – 3.70 (m, 2H), 3.65 – 3.60 (m, 2H), 2.96 – 2.81 (m, 2H), 2.81 – 2.70 (m, 2H), 2.15 (dt, *J* = 7.3, 3.4 Hz, 1H).^{13}C NMR (126 MHz, $CDCl_3$) δ 170.88, 169.22, 168.94, 167.93, 166.72, 136.86, 136.44, 131.34, 125.30, 118.88, 116.18, 72.86, 71.48, 71.01, 70.29, 61.68, 49.37, 31.37, 22.70. HRMS calculated for $C_{19}H_{22}N_3O_8$ $[M+H]^+$: 420.1401, found 420.1369.

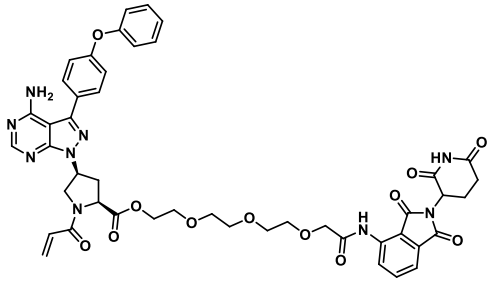
2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethyl (2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pyrrolidine-2-carboxylate (3a)



In a round-bottom flask, **19a** (85 mg, 0.20 mmol), **1** (113mg, 0.24 mmol), EDCI (119 mg, 0.62 mmol), and catalytic amount of DMAP (10mg) were added to DCM (5 mL). The mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure and the crude was purified by preparative TLC and HPLC to provide 88 mg (51%) of title compound as a white solid. ^{1}H NMR (500 MHz, DMSO) δ 11.12 (s, 1H), 10.27 (d, *J* = 14.0 Hz, 1H), 8.64 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 4.6 Hz,

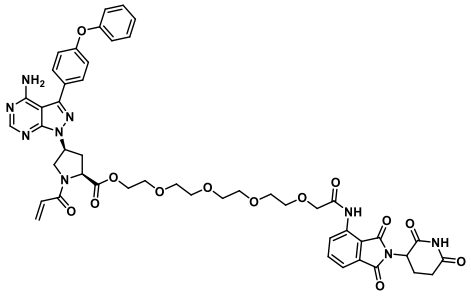
1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.68 – 7.50 (m, 3H), 7.40 (t, *J* = 7.8 Hz, 2H), 7.20 – 6.97 (m, 5H), 6.51 (m, 1H), 6.15 (dd, *J* = 9.0, 7.5 Hz, 1H), 5.66 (dd, *J* = 36.3, 11.1 Hz, 1H), 5.50 (dd, *J* = 36.2, 28.1 Hz, 1H), 5.16 – 4.98 (m, 1H), 4.64 – 4.52 (m, 0.5H), 4.36 – 4.22 (m, 0.5H), 4.23 – 4.06 (m, 3H), 3.97 (dd, *J* = 20.6, 11.6 Hz, 1H), 3.84 – 3.48 (m, 5H), 2.98 – 2.68 (m, 3H), 2.58 (t, *J* = 13.4 Hz, 2H), 2.09 – 1.98 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 173.20, 171.49, 170.21, 169.74, 168.71, 167.16, 164.86, 164.12, 158.36, 157.76, 157.65, 156.79, 156.74, 155.97, 154.90, 144.38, 136.99, 136.95, 136.41, 131.75, 130.61, 130.58, 129.26, 128.66, 128.07, 124.81, 124.30, 124.23, 119.47, 119.45, 119.35, 118.78, 116.49, 98.12, 71.13, 70.62, 70.24, 70.12, 68.76, 68.55, 64.67, 64.40, 58.20, 57.95, 54.36, 53.31, 50.64, 49.46, 35.41, 33.30, 31.41, 22.43. Purity > 99%. HRMS: m/z calculated for $C_{44}H_{42}N_9O_{11}$ $[M+H]^+$ =872.2998, found $[M+H]^+$ =872.2997.

2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethyl (2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pyrrolidine-2-carboxylate (3b)



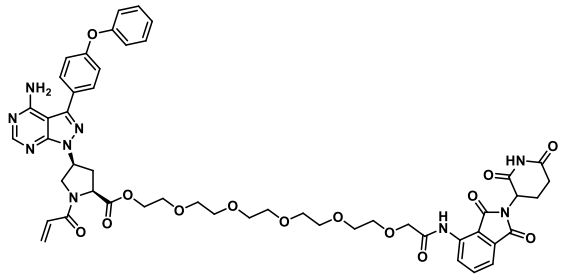
According to the procedure for the synthesis of **3a**, compound **3b** was synthesized (38% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.46 (d, *J* = 5.2 Hz, 1H), 8.83 (d, *J* = 8.1 Hz, 1H), 8.39 (d, *J* = 2.0 Hz, 1H), 7.77 – 7.66 (m, 1H), 7.66 – 7.51 (m, 3H), 7.38 (dd, *J* = 8.3, 7.6 Hz, 2H), 7.22 – 6.99 (m, 5H), 6.52 – 6.26 (m, 2H), 5.94 (s, 2H), 5.79 – 5.64 (m, 1H), 5.49 (dd, *J* = 14.4, 7.1 Hz, 1H), 5.06 – 4.88 (m, 1H), 4.82 – 4.63 (m, 1H), 4.44 –4.03 (m, 6H), 3.83 – 3.35 (m, 10H), 3.03 – 2.64 (m, 5H), 2.22 – 2.06 (m, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 172.09, 172.01, 170.77, 170.76, 170.74, 169.39, 169.34, 169.02, 168.68, 168.47, 166.85, 164.79, 164.73, 158.75, 158.73, 157.92, 156.21, 155.78, 155.74, 154.75, 154.72, 144.38, 136.73, 136.70, 136.32, 136.26, 131.40, 130.00, 129.23, 127.71, 127.67, 127.20, 125.22, 124.15, 119.63, 119.61, 119.06, 118.77, 116.18, 98.83, 71.63, 71.59, 70.92, 70.82, 70.74, 70.60, 70.55, 70.48, 70.42, 68.91, 68.85, 64.33, 57.69, 54.96, 50.37, 49.26, 49.23, 33.34, 33.30, 31.46, 31.42, 22.75. Purity: 95%. HRMS: m/z calculated for $C_{46}H_{46}N_9O_{12}$ $[M+H]^+$ = 916.3260, found $[M+H]^+$ =916.3209.

14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-14-oxo-3,6,9,12-tetraoxatetradecyl (2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pyrrolidine-2-carboxylate (3c)



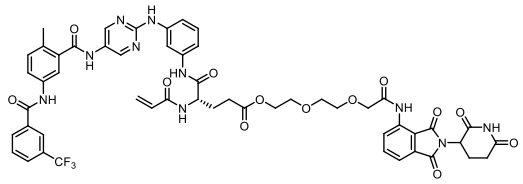
According to the procedure for the synthesis of **3a**, compound **3c** was synthesized (49% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.01 (s, 1H), 10.47 (s, 1H), 8.82 (d, *J* = 8.4 Hz, 1H), 8.39 (s, 1H), 7.79 – 7.47 (m, 4H), 7.37 (t, *J* = 7.9 Hz, 2H), 7.19 – 6.96 (m, 5H), 6.57 – 6.27 (m, 2H), 6.10 (s, 2H), 5.71 (dd, *J* = 8.8, 3.4 Hz, 1H), 5.49 (td, *J* = 8.1, 3.3 Hz, 1H), 4.98 (dd, *J* = 11.8, 5.0 Hz, 1H), 4.81 – 4.62 (m, 1H), 4.48 – 3.92 (m, 6H), 3.76 (s, 4H), 3.67 – 3.42 (m, 10H), 3.13 – 2.64 (m, 5H), 2.14 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.29, 172.20, 170.81, 169.38, 169.12, 168.98, 168.48, 166.86, 165.31, 164.69, 164.66, 158.69, 158.56, 157.96, 156.20, 155.74, 155.54, 154.74, 154.40, 144.44, 144.02, 136.70, 136.24, 131.39, 130.00, 129.95, 129.18, 128.46, 127.85, 127.73, 127.69, 127.45, 127.29, 125.16, 124.13, 119.59, 119.04, 118.96, 118.77, 116.16, 98.74, 71.58, 71.55, 70.88, 70.63, 70.55, 70.49, 70.40, 70.26, 68.92, 68.60, 64.68, 64.35, 58.45, 57.73, 54.70, 53.19, 50.44, 49.26, 35.80, 33.28, 31.46, 29.69, 22.76. Purity: 94%. HRMS: m/z calculated for $C_{48}H_{50}N_9O_{13}$ $[M+H]^+$ = 960.3523, found $[M+H]^+$ =960.3481.

17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-17-oxo-3,6,9,12,15-pentaoxaheptadecyl (2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pyrrolidine-2-carboxylate (3d)



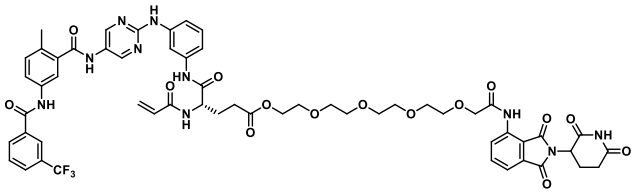
According to the procedure for the synthesis of **3a**, compound **3d** was synthesized (37% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.88 (s, 1H), 10.48 (s, 1H), 8.83 (d, *J* = 8.5 Hz, 1H), 8.41 (d, *J* = 4.7 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.64 – 7.52 (m, 3H), 7.37 (dd, *J* = 11.4, 4.4 Hz, 2H), 7.22 – 7.00 (m, 5H), 6.59 – 6.28 (m, 2H), 6.00 (s, 1H), 5.71 (m, 1H), 5.58 – 5.43 (m, 1H), 5.05 – 4.90 (m, 1H), 4.74 (m, 1H), 4.48 – 4.01 (m, 6H), 3.79 (m, 4H), 3.57 (m, 14H), 3.05 – 2.69 (m, 5H), 2.20 – 2.08 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.04, 171.82, 170.84, 169.38, 169.34, 169.06, 168.73, 168.51, 166.85, 165.35, 164.74, 158.77, 157.96, 156.29, 155.82, 154.86, 144.51, 136.79, 136.25, 131.46, 130.09, 130.01, 129.06, 127.82, 127.36, 125.24, 124.15, 119.62, 119.08, 119.00, 118.78, 116.24, 98.78, 71.65, 70.94, 70.69, 70.52, 70.48, 70.44, 70.40, 68.94, 68.65, 64.73, 64.38, 58.51, 57.79, 54.68, 54.57, 50.47, 49.34, 35.82, 33.44, 33.38, 31.50, 29.70, 22.80. Purity: 97%. HRMS: m/z calculated for $C_{50}H_{54}N_9O_{14}$ $[M+H]^+$ =1004.3785, found $[M+H]^+$ =1004.3737.

2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethyl (4*S*)-4-acrylamido-5-((3-((5-(2-methyl-5-(3-(trifluoromethyl)benzamido)benzamido)pyrimidin-2-yl)amino)phenyl)amino)-5-oxopentanoate (5a)



According to the procedure for the synthesis of **3a**, compound **2** instead of **1** was added into the solvent of DCM:DMF = 5:1, compound **5a** was synthesized (21% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 11.15 (s, 1H), 10.61 (s, 1H), 10.44 (s, 1H), 10.33 (s, 1H), 10.12 (s, 1H), 9.65 (s, 1H), 8.81 (s, 2H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.45 (d, *J* = 7.3 Hz, 1H), 8.37 – 8.18 (m, 2H), 8.12 – 7.72 (m, 6H), 7.61 (d, *J* = 7.3 Hz, 1H), 7.44 – 7.24 (m, 3H), 7.17 (t, *J* = 8.1 Hz, 1H), 6.37 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.11 (d, *J* = 17.0 Hz, 1H), 5.72 – 5.51 (m, 1H), 5.14 (dd, *J* = 12.9, 5.3 Hz, 1H), 4.55 (d, *J* = 6.2 Hz, 1H), 4.18 (s, 2H), 4.11 (s, 2H), 3.68 (m, 6H), 3.03 – 2.77 (m, 1H), 2.69 – 2.52 (m, 2H), 2.36 (m, 5H), 2.14 – 1.82 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.17, 172.61, 170.33, 170.18, 169.81, 168.72, 168.08, 167.16, 165.17, 164.48, 157.05, 150.34, 141.44, 139.44, 137.05, 137.01, 136.96, 136.47, 136.01, 132.32, 131.87, 131.80, 131.46, 131.40, 130.30, 129.88, 129.62, 129.06, 128.72, 126.87, 126.21, 125.55, 124.93, 124.66, 123.39, 122.35, 119.83, 118.79, 116.58, 114.56, 113.14, 110.14, 71.20, 70.72, 70.15, 68.82, 63.80, 56.53, 53.22, 49.51, 31.43, 30.67, 27.95, 22.45, 19.29, 19.01. Purity > 99%. HRMS: m/z calculated for $C_{53}H_{50}F_3N_{10}O_{13}$ $[M+H]^+$ =1091.3505, found $[M+H]^+$ =1091.3448.

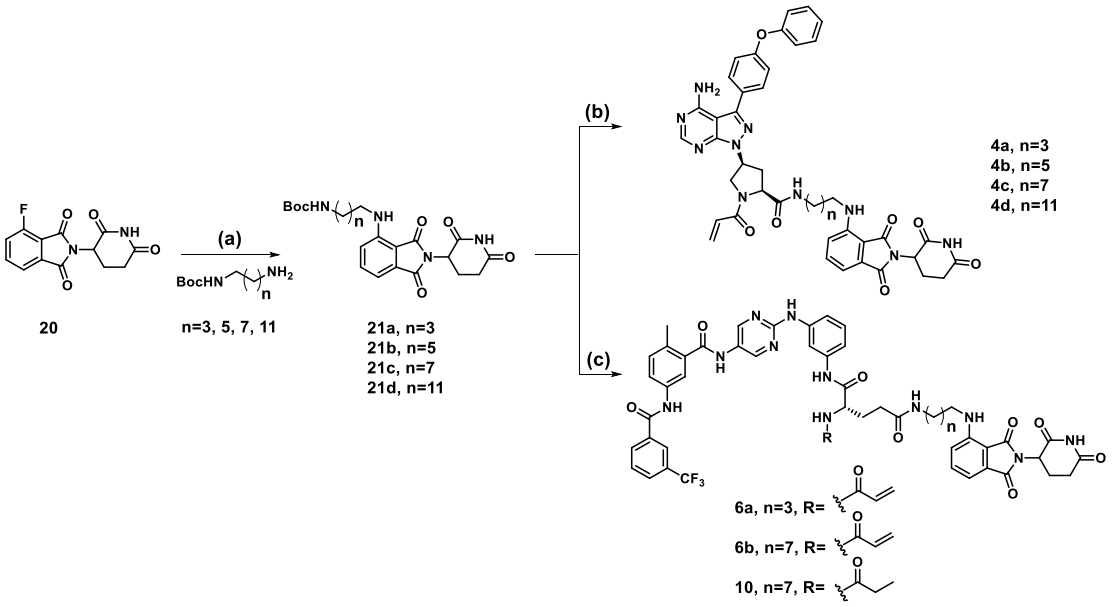
14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-14-oxo-3,6,9,12-tetraoxatetradecyl (4*S*)-4-acrylamido-5-((3-((5-(2-methyl-5-(3-(trifluoromethyl)benzamido)benzamido)pyrimidin-2-yl)amino)phenyl)amino)-5-oxopentanoate (5b)



According to the procedure for the synthesis of **5a**, compound **5b** was synthesized as a white solid (27% yield). ^{1}H NMR (300 MHz, DMSO) δ 11.15 (s, 1H), 10.61 (d, *J* = 5.3 Hz, 1H), 10.44 (s, 1H), 10.33 (s, 1H), 10.12 (s, 1H), 9.64 (s, 1H), 8.80 (s, 2H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.45 (d, *J* = 7.1 Hz, 1H), 8.29 (d, *J* = 13.5 Hz, 2H), 7.88 (m, 6H), 7.60 (d, *J* = 7.3 Hz, 1H), 7.45 – 7.24 (m, 3H), 7.17 (t, *J* = 8.0 Hz, 1H), 6.37 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.11 (d, *J* = 17.0 Hz, 1H), 5.69 – 5.52 (m, 1H), 5.14 (dd, *J* = 12.8, 5.1 Hz, 1H), 4.55 (d, *J* = 7.0 Hz, 1H), 4.17 (s, 2H), 4.14 – 4.00 (m, 2H), 3.81 – 3.44 (m, 14H), 2.99 – 2.77 (m, 1H), 2.56 (m, 2H), 2.37 (s, 5H), 2.00 (m, 3H). ^{13}C NMR (75 MHz, DMSO) δ 173.22, 172.56, 170.34, 170.21, 169.85, 168.68, 168.03, 167.15, 165.09, 164.44, 156.97, 150.25, 141.42, 139.44, 136.97, 136.42, 135.93, 132.34, 131.81, 131.76, 131.46, 131.36, 130.29, 129.89, 129.46, 129.07, 128.77, 126.85, 126.25,

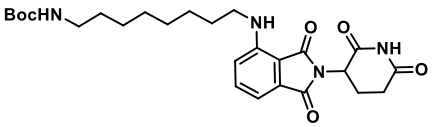
124.86, 124.69, 122.63, 122.29, 119.77, 118.78, 116.51, 114.44, 113.00, 109.94, 71.23, 70.66, 70.29, 70.19, 70.13, 68.65, 63.77, 53.17, 49.43, 31.39, 30.67, 27.97, 22.41, 19.30. Purity: 94%. HRMS: m/z calculated for $C_{57}H_{58}F_3N_{10}O_{15}$ $[M+H]^+$ =1179.4030, found $[M+H]^+$ =1179.3981.

Scheme S3. **Synthesis of compounds 4, 6 and 10**.



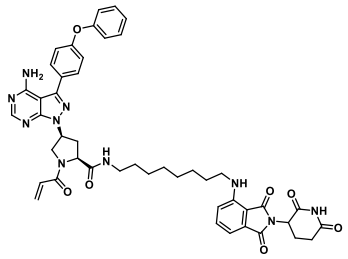
Reagents and conditions: (a) TEA, DMF, 90°C, 12h. (b) (i) TFA, DCM, rt, 2h; (ii) **1**, EDCI, HOBT, TEA, DCM. (c) (i) TFA, DCM, rt, 2h; (ii) **2 or 13**, HATU, DIEA, DMF.

***tert*-butyl (8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)carbamate (21c)**



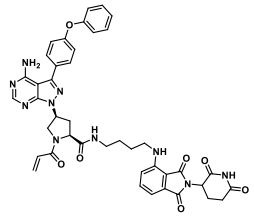
To a solution of **20** (200 mg, 0.72 mmol) and TEA (0.25 mL, 1.75 mmol) in DMF (5 mL), tert-butyl (8-aminooctyl)carbamate (223 mg, 0.91mmol) was added. The resulting solution was stirred at 90 °C for 12 h. After the reaction cooled to room temperature, the mixture solution was poured into water and extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4 and the solvent was removed by rotary evaporator. After concentration, the residue was purified by column chromatography to provide 64 mg (18%) of **21** as a green solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.48 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.08 (d, *J* = 7.0 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.23 (t, *J* = 5.3 Hz, 1H), 4.91 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.53 (s, 1H), 3.25 (dd, *J* = 12.8, 6.8 Hz, 2H), 3.10 (d, *J* = 6.6 Hz, 2H), 2.93 – 2.66 (m, 3H), 2.20 – 2.03 (m, 1H), 1.65 (m, 3H), 1.44 (s, 9H), 1.30 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.88, 169.54, 168.30, 167.61, 155.96, 147.11, 136.08, 132.61, 116.62, 111.37, 110.04, 48.96, 42.67, 31.43, 30.06, 29.16, 29.12, 28.45, 26.82, 26.67, 22.87. HRMS calculated for $C_{26}H_{36}N_4O_6Na$ $[M+Na]^+$: 523.2527, found 523.2527.

(2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-*N*-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)pyrrolidine-2-carboxamide (4c)



In a round-bottom flask, the compound **21c** (64 mg, 0.128 mmol) was dissolved in DCM (3 mL) and TFA (3 mL) and the reaction mixture was stirred at room temperature for 2 h. The mixture solution was concentrated by rotary evaporator. The solution of the residue and TEA (54 uL, 0.384 mmol) in DCM (1 mL) was added to the solution of compound **1** (73 mg, 0.154 mmol), EDCI (74 mg, 0.384 mmol), HOBT (22.5 mg, 0.166 mmol) in DCM (2 mL) at 0 °C in ice water bath. After removing the ice water, the reaction mixture was stirred at rt for 2 h. The resulting mixture was poured into water extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4 and the solvent was removed by rotary evaporator. After concentration, the crude was purified by HPLC to provide 23.2 mg (21%) of title compound as a green solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (d, *J* = 2.4 Hz, 1H), 7.61 (t, *J* = 10.2 Hz, 2H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 2H), 7.21 – 7.02 (m, 6H), 6.89 (dt, *J* = 30.4, 14.1 Hz, 2H), 6.57 – 6.29 (m, 2H), 6.23 (d, *J* = 4.6 Hz, 1H), 5.74 (d, *J* = 15.4 Hz, 1H), 5.45 (dd, *J* = 14.3, 7.3 Hz, 1H), 4.91 (dd, *J* = 11.5, 5.3 Hz, 1H), 4.73 (m, 0.5H), 4.61 – 4.51 (m, 0.5H), 4.43 – 4.26 (m, 1H), 4.26 – 4.07 (m, 1H), 3.28 – 2.98 (m, 5H), 2.91 – 2.59 (m, 4H), 2.12 (dd, *J* = 7.7, 5.3 Hz, 1H), 1.67 – 1.56 (m, 2H), 1.41 – 1.04 (m, 10H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.50, 169.91, 169.58, 168.88, 167.68, 166.12, 158.65, 157.91, 156.39, 155.88, 147.10, 144.13, 136.07, 132.59, 129.99, 129.23, 128.17, 127.63, 124.08, 119.56, 119.06, 116.69, 111.35, 110.02, 98.97, 77.27, 77.02, 76.76, 59.30, 50.91, 48.97, 42.58, 39.68, 32.79, 31.48, 29.27, 28.97, 26.69, 26.62, 22.88. Purity > 99%. HRMS: m/z calculated for $C_{46}H_{49}N_{10}O_7$ $[M+H]^+$ =853.3780, found $[M+H]^+$ =853.3735.

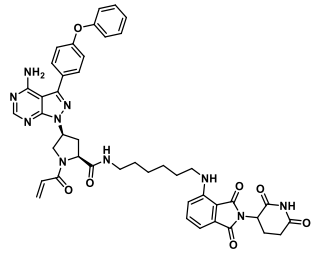
(2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-*N*-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)pyrrolidine-2-carboxamide (4a)



According to the procedure for the synthesis of **4c**, compound **4a** was synthesized (61% yield) from **21a** as a green solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.66 (s, 1H), 8.36 (s, 1H), 7.63 – 7.48 (m, 2H), 7.43 – 7.29 (m, 3H), 7.22 – 6.91 (m, 6H), 6.74 (m, 1H), 6.52 – 6.27 (m, 2H), 6.23 – 6.04 (m, 1H), 5.71 (m, 1H), 5.58 – 5.35 (m, 1H), 5.00 – 4.85 (m, 1H), 4.64 (m, 1H), 4.42 – 4.13 (m, 2H), 3.12 (m, 5H), 2.75 (m, 4H), 2.06 (m, 1H), 1.60 – 1.22 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.25, 170.43, 169.62, 169.52, 167.71, 166.05, 158.64, 158.00, 156.30, 155.78, 154.71, 146.87, 144.31, 136.09, 132.46, 130.00, 129.96, 129.40,

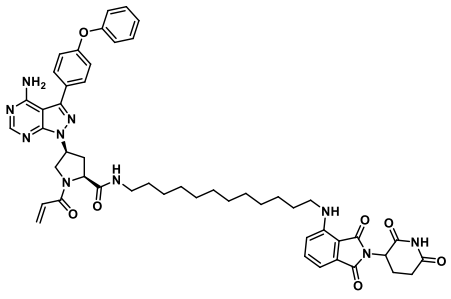
128.08, 127.50, 124.10, 119.55, 119.07, 116.72, 111.38, 109.91, 98.73, 61.02, 59.45, 54.74, 51.02, 48.94, 42.23, 39.08, 33.03, 31.53, 29.69, 26.79, 26.48, 22.82. Purity: 90%. HRMS: m/z calculated for $C_{42}H_{41}N_{10}O_7$ $[M+H]^+$ =797.3154, found $[M+H]^+$ =797.3165.

(2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-*N*-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)pyrrolidine-2-carboxamide (4b)



According to the procedure for the synthesis of **4c**, compound **4b** was synthesized (43% yield) from **21b** as a green solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.46 (m, 1H), 8.37 (s, 1H), 7.59 (m, 2H), 7.40 (m, 3H), 7.22 – 6.89 (m, 7H), 6.82 (m, 1H), 6.57 – 6.27 (m, 2H), 6.20 (m, 1H), 5.81 – 5.67 (m, 1H), 5.56 – 5.36 (m, 1H), 4.91 (dd, *J* = 9.9, 5.0 Hz, 1H), 4.78 – 4.52 (m, 1H), 4.30 (m, 2H), 3.32 – 2.91 (m, 5H), 2.72 (m, 4H), 2.14 – 2.05 (m, 1H), 1.56 (m, 2H), 1.43 – 1.13 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.22, 172.17, 171.47, 170.11, 169.56, 169.45, 167.73, 166.03, 158.69, 158.57, 157.96, 156.27, 155.72, 154.65, 146.96, 144.26, 136.09, 132.47, 129.99, 129.43, 128.06, 127.47, 127.24, 124.18, 124.07, 119.51, 119.05, 116.70, 111.34, 109.85, 98.75, 60.83, 59.31, 54.78, 54.67, 53.86, 50.93, 48.89, 45.84, 42.47, 39.47, 37.37, 32.89, 31.51, 29.17, 28.94, 26.48, 26.43, 22.83. Purity > 99%. HRMS: m/z calculated for $C_{44}H_{45}N_{10}O_7$ $[M+H]^+$ =825.3467, found $[M+H]^+$ =825.3419.

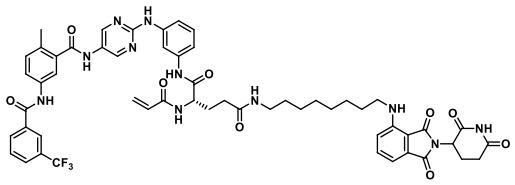
(2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-*N*-(12-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)dodecyl)pyrrolidine-2-carboxamide (4d)



According to the procedure for the synthesis of **4c**, compound **4d** was synthesized (77% yield) from **21d** as a green solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.48 (s, 1H), 8.41 (d, *J* = 1.3 Hz, 1H), 7.61 (t, *J* = 8.1 Hz, 2H), 7.54 – 7.43 (m, 1H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.23 – 7.02 (m, 6H), 6.91 (m, 2H), 6.56 – 6.28 (m, 2H), 6.23 (t, *J* = 5.5 Hz, 1H), 5.76 (dd, *J* = 12.4, 5.6 Hz, 1H), 5.47 (dd, *J* = 14.9, 7.4 Hz, 1H), 4.92 (dd, *J* = 11.9, 5.2 Hz, 1H), 4.74 (m, 0.5H), 4.59 (m, 0.5H), 4.52 – 4.15 (m, 2H), 3.33 – 2.96 (m, 5H), 2.91 – 2.61 (m, 4H), 2.16 – 2.06 (m, 1H), 1.61 (m, 1H), 1.40 – 0.84 (m, 19H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.15, 171.31, 169.92, 169.56, 169.44, 167.73, 166.07, 158.79, 158.57, 158.00, 156.29, 156.11,

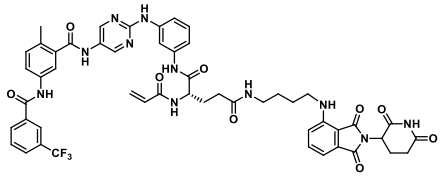
155.97, 155.74, 154.67, 154.46, 147.02, 144.19, 136.08, 132.49, 129.98, 129.35, 128.09, 127.52, 127.29, 124.21, 124.06, 119.62, 119.54, 119.02, 116.65, 111.29, 109.80, 98.77, 60.84, 59.27, 54.71, 53.63, 50.90, 48.88, 42.65, 39.73, 39.51, 32.76, 31.53, 29.41, 29.32, 29.25, 29.19, 26.89, 26.84, 22.85, 22.63. Purity: 97%. HRMS: m/z calculated for $C_{50}H_{57}N_{10}O_7$ $[M+H]^+$ =909.4406, found $[M+H]^+$ =909.4409.

(2*S*)-2-acrylamido-*N*^{5}-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-*N*^{1}-(3-((5-(2-methyl-5-(3-(trifluoromethyl)benzamido)benzamido)pyrimidin-2-yl)amino)phenyl)pentanediamide (6b)



According to the procedure for the synthesis of **4c**, compound **2 (**instead of **1**) was added into the solution of HATU in DMF, compound **6b** was synthesized (26% yield) as a green solid. ^{1}H NMR (300 MHz, DMSO) δ 11.09 (s, 1H), 10.58 (s, 1H), 10.43 (s, 1H), 10.10 (s, 1H), 9.65 (s, 1H), 8.81 (s, 2H), 8.42 (d, *J* = 7.7 Hz, 1H), 8.34 – 8.22 (m, 2H), 8.06 (s, 1H), 7.99 – 7.90 (m, 2H), 7.88 – 7.73 (m, 3H), 7.61 – 7.48 (m, 1H), 7.33 (dd, *J* = 17.4, 9.0 Hz, 3H), 7.17 (t, *J* = 8.1 Hz, 1H), 7.02 (dd, *J* = 17.3, 7.8 Hz, 2H), 6.49 (t, *J* = 5.7 Hz, 1H), 6.37 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.18 – 6.03 (m, 1H), 5.61 (dd, *J* = 10.2, 2.0 Hz, 1H), 5.03 (dd, *J* = 12.8, 5.3 Hz, 1H), 4.52 (d, *J* = 6.1 Hz, 1H), 3.25 (d, *J* = 6.4 Hz, 2H), 2.99 (d, *J* = 5.9 Hz, 2H), 2.93 – 2.74 (m, 1H), 2.56 (m, 2H), 2.38 (s, 3H), 2.23 – 2.09 (m, 2H), 2.06 – 1.77 (m, 3H), 1.52 (m, 2H), 1.29 (m, 10H). ^{13}C NMR (75 MHz, DMSO) δ 173.29, 171.47, 170.63, 170.58, 169.42, 168.02, 167.77, 164.99, 164.43, 156.99, 150.25, 146.88, 141.42, 139.54, 137.00, 136.73, 135.94, 132.64, 132.33, 131.95, 131.47, 131.36, 130.30, 129.90, 129.47, 129.05, 128.77, 126.84, 126.25, 126.09, 124.67, 124.62, 122.64, 122.26, 119.74, 117.62, 114.39, 112.99, 110.83, 109.92, 109.45, 53.64, 49.00, 42.28, 32.31, 31.44, 29.51, 29.18, 29.13, 28.83, 26.84, 26.75, 22.62, 19.29. Purity > 99%. HRMS: m/z calculated for $C_{55}H_{57}F_3N_{11}O_9$ $[M+H]^+$ =1072.4287, found $[M+H]^+$ =1072.4302.

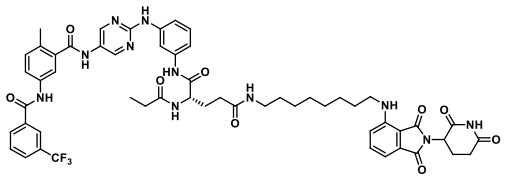
(2*S*)-2-acrylamido-*N*^{5}-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)-*N*^{1}-(3-((5-(2-methyl-5-(3-(trifluoromethyl)benzamido)benzamido)pyrimidin-2-yl)amino)phenyl)pentanediamide (6a)



According to the procedure for the synthesis of **6b**, compound **6a** was synthesized (22% yield) from **21a** as a green solid. ^{1}H NMR (400 MHz, DMSO) δ 11.07 (s, 1H), 10.56 (s, 1H), 10.41 (s, 1H), 10.09 (s, 1H), 9.64 (s, 1H), 8.80 (s, 2H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.35 – 8.21 (m, 2H), 8.04 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 2.1 Hz, 1H), 7.90 – 7.75 (m, 3H), 7.54 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.42 – 7.24

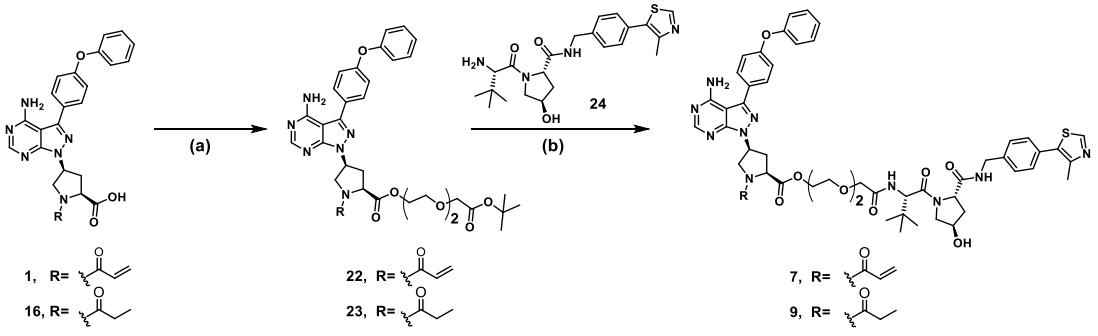
(m, 3H), 7.17 (t, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 1H), 6.98 (d, *J* = 7.0 Hz, 1H), 6.52 (t, *J* = 5.8 Hz, 1H), 6.37 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.10 (dd, *J* = 17.1, 2.0 Hz, 1H), 5.60 (dd, *J* = 10.2, 2.0 Hz, 1H), 5.02 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.51 (dd, *J* = 13.8, 7.9 Hz, 1H), 3.30 – 3.22 (m, 2H), 3.05 (d, *J* = 6.0 Hz, 2H), 2.86 (ddd, *J* = 17.3, 14.1, 5.4 Hz, 1H), 2.56 (d, *J* = 17.3 Hz, 2H), 2.37 (s, 3H), 2.23 – 2.05 (m, 2H), 2.05 – 1.91 (m, 2H), 1.90 – 1.77 (m, 1H), 1.52 (dd, *J* = 14.3, 7.2 Hz, 2H), 1.44 (dd, *J* = 14.2, 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 173.25, 171.53, 170.60, 170.55, 169.37, 167.99, 167.74, 164.96, 164.40, 156.98, 150.24, 146.84, 141.40, 139.52, 137.00, 136.70, 135.94, 132.65, 132.31, 131.95, 131.45, 131.33, 130.28, 129.83, 129.51, 129.03, 128.75, 126.82, 126.05, 125.78, 124.64, 124.60, 123.07, 122.23, 119.72, 117.65, 114.38, 112.97, 110.82, 109.92, 109.46, 53.60, 48.98, 41.97, 38.58, 32.30, 31.42, 28.82, 26.93, 26.62, 22.60, 19.27. Purity: 91%. HRMS: m/z calculated for $C_{51}H_{49}F_3N_{11}O_9$ $[M+H]^+$ =1016.3661, found $[M+H]^+$ =1016.3684.

(2*S*)-*N*^{5}-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-*N*^{1}-(3-((5-(2-methyl-5-(3-(trifluoromethyl)benzamido)benzamido)pyrimidin-2-yl)amino)phenyl)-2-propionamidopentanediamide (10)



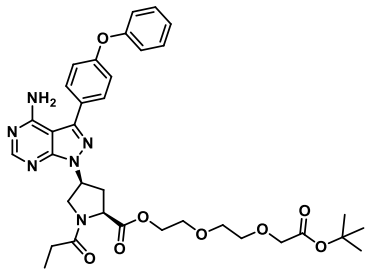
According to the procedure for the synthesis of **6b** with **13** instead of **2**, compound **10** was synthesized (46% yield) as a green solid. ^{1}H NMR (300 MHz, DMSO) δ 11.10 (s, 1H), 10.66 (d, *J* = 6.0 Hz, 1H), 10.46 (s, 1H), 10.03 (s, 1H), 9.64 (s, 1H), 8.82 (s, 2H), 8.31 (d, 2H), 8.08 (d, *J* = 13.4 Hz, 2H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.90 – 7.73 (m, 3H), 7.59 – 7.50 (m, 1H), 7.33 (dd, *J* = 18.0, 9.4 Hz, 3H), 7.17 (t, *J* = 8.1 Hz, 1H), 7.03 (dd, *J* = 18.8, 7.8 Hz, 2H), 6.50 (t, *J* = 5.7 Hz, 1H), 5.04 (dd, *J* = 12.8, 5.3 Hz, 1H), 4.40 (d, *J* = 6.1 Hz, 1H), 3.26 (m, 2H), 3.00 (m, 2H), 2.89 – 2.77 (m, 1H), 2.57 (m, 2H), 2.38 (s, 3H), 2.16 (dd, *J* = 15.0, 7.5 Hz, 4H), 2.07 – 1.72 (m, 3H), 1.53 (d, *J* = 6.2 Hz, 2H), 1.30 (dd, *J* = 24.9, 8.5 Hz, 10H), 0.99 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 173.55, 173.30, 171.63, 170.96, 170.58, 169.41, 168.04, 167.78, 164.44, 156.99, 150.27, 146.88, 141.38, 139.59, 137.01, 136.96, 136.75, 135.92, 132.63, 132.36, 131.44, 131.34, 130.28, 129.88, 129.45, 129.01, 128.77, 126.84, 126.25, 124.71, 122.64, 122.30, 119.79, 117.64, 114.35, 113.01, 110.83, 109.97, 109.43, 70.24, 53.58, 48.99, 42.28, 32.39, 31.44, 29.52, 29.18, 28.68, 26.84, 26.75, 22.61, 19.31, 10.31. Purity > 99%. HRMS: m/z calculated for $C_{55}H_{59}F_3N_{11}O_9$ $[M+H]^+$ =1074.4444 found $[M+H]^+$ =1074.4478.

Scheme S4. **Synthesis of compounds 7 and 9**.



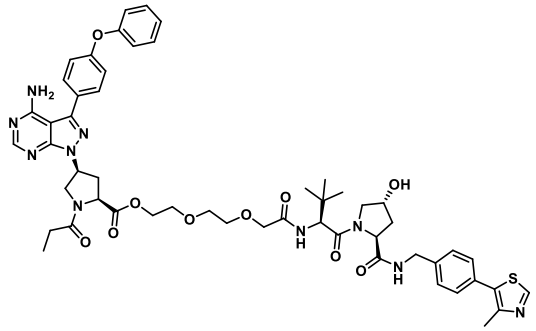
Reagents and conditions: (**a**) **18a**, EDCI, DMAP, DCM, overnight, rt. (**b**) (i) TFA, DCM, rt, 2h; (ii) **24**, HATU, DIEA, DMF, 2h, rt.

2-(2-(2-(*tert*-butoxy)-2-oxoethoxy)ethoxy)ethyl (2*S*,4*S*)-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-1-propionylpyrrolidine-2-carboxylate (23)



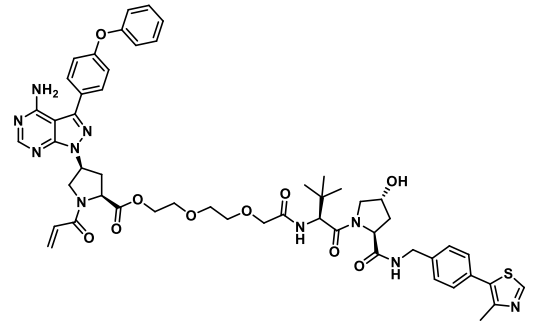
In a round-bottom flask, **16** (200mg, 0.42mmol), **18a** (140mg, 0.64mmol), EDCI (244 mg, 1.27 mmol), and catalytic amount of DMAP (28mg) were added to DCM (5 mL). The mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure and the crude was purified by preparative TLC to provide 51 mg (18%) of title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (d, *J* = 7.6 Hz, 1H), 7.66 – 7.54 (m, 2H), 7.40 – 7.30 (m, 2H), 7.18 – 6.98 (m, 5H), 5.82 (d, *J* = 3.1 Hz, 1H), 5.52 – 5.40 (m, 1H), 4.62 (t, *J* = 8.4 Hz, 1H), 4.42 – 4.20 (m, 2H), 4.08 (dd, *J* = 8.2, 2.5 Hz, 2H), 3.93 (d, *J* = 12.5 Hz, 2H), 3.71 – 3.51 (m, 6H), 3.02 – 2.74 (m, 2H), 2.37 – 2.22 (m, 2H), 1.41 (d, *J* = 2.2 Hz, 9H), 1.11 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.64, 171.25, 169.66, 158.77, 157.94, 156.27, 155.93, 154.88, 144.44, 130.03, 130.00, 127.43, 124.18, 119.65, 119.06, 118.95, 98.83, 81.58, 70.68, 70.57, 70.51, 69.05, 64.60, 64.31, 58.53, 57.54, 54.40, 52.93, 50.50, 35.80, 33.33, 29.72, 28.13, 27.75, 26.93, 9.07, 8.72. MS: m/z calculated for $C_{35}H_{43}N_6O_8$ $[M+H]^+$ =675, found $[M+H]^+$ =675.

2-(2-(2-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)ethyl (2*S*,4*S*)-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-1-propionylpyrrolidine-2-carboxylate (9)



In a round-bottom flask, the compound **23** (51 mg, 0.076 mmol) was dissolved in DCM (1 mL) and TFA (1 mL) and the reaction mixture was stirred at room temperature for 2 h. The mixture solution was concentrated by rotary evaporator. The solution of the residue and DIEA (41 uL, 0.25 mmol) in DMF (1 mL) was added to the solution of **24** (42 mg, 0.098 mmol), HATU (44 mg, 0.114 mmol) in DMF (2 mL) at 0 °C in ice water bath. After removing the ice water, the reaction mixture was stirred at rt for 2 h. The resulting mixture was poured into water and extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4 and the solvent was removed by rotary evaporator. After concentration, the crude was purified by preparative HPLC to provide 22 mg (28%) of title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.31 (s, 1H), 7.59 (t, *J* = 8.0 Hz, 3H), 7.43 – 7.26 (m, 7H), 7.18 – 6.99 (m, 5H), 5.67 (s, 2H), 5.53 – 5.39 (m, 1H), 4.69 – 4.57 (m, 2H), 4.57 – 4.46 (m, 3H), 4.40 – 4.26 (m, 2H), 4.23 – 4.12 (m, 1H), 4.11 – 3.94 (m, 4H), 3.89 (d, *J* = 15.4 Hz, 1H), 3.71 – 3.44 (m, 8H), 2.87 – 2.72 (m, 2H), 2.47 (s, 3H), 2.40 – 2.33 (m, 1H), 2.29 (q, *J* = 7.4 Hz, 2H), 2.16 (m, 1H), 1.08 (t, *J* = 7.4 Hz, 3H), 0.93 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.97, 171.50, 171.34, 171.12, 171.02, 170.14, 158.82, 157.89, 156.23, 155.92, 154.75, 150.28, 148.46, 144.45, 138.46, 131.71, 130.80, 130.05, 129.98, 129.42, 128.10, 127.34, 124.22, 119.65, 119.06, 118.99, 98.88, 71.07, 70.62, 70.52, 70.23, 68.92, 64.32, 58.86, 57.59, 57.02, 54.43, 50.57, 43.16, 36.49, 35.69, 33.37, 27.77, 26.46, 16.08, 9.07, 8.73. Purity: 99%. HRMS: m/z calculated for $C_{53}H_{63}N_{10}O_{10}S$ $[M+H]^+$ =1031.4444, found $[M+H]^+$ =1031.4450.

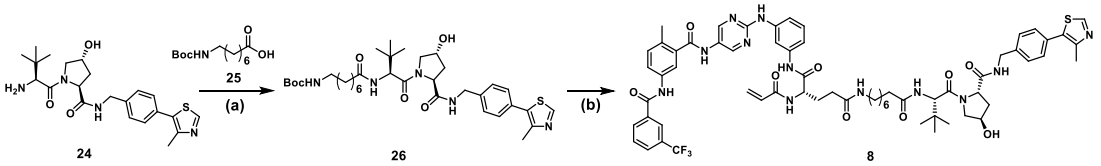
2-(2-(2-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)ethyl (2*S*,4*S*)-1-acryloyl-4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pyrrolidine-2-carboxylate (7)



According to the procedure for the synthesis of **9**, using compound **1**, compound **7** was synthesized (35% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.34 (s, 1H), 7.71 – 7.57 (m, 3H), 7.48 – 7.31 (m, 7H), 7.22 – 6.99 (m, 5H), 6.38 (qd, *J* = 16.8, 6.0 Hz, 2H), 5.72 (dd, *J* = 9.9, 2.1 Hz, 1H), 5.64 (s, 2H), 5.58 – 5.44 (m, 1H), 4.77 (t, *J* = 8.5 Hz, 1H), 4.63 (t, *J* = 8.2 Hz, 1H), 4.60 – 4.49 (m, 3H), 4.41 – 4.32 (m, 2H), 4.20 (m, 4H), 4.03 (dd, *J* = 13.4, 8.0 Hz, 2H), 3.92 (d, *J* = 15.5 Hz, 1H), 3.73 – 3.51

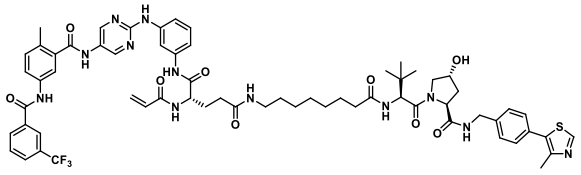
(m, 8H), 2.91 – 2.79 (m, 2H), 2.50 (s, 3H), 2.38 (dd, *J* = 8.7, 4.5 Hz, 1H), 2.13 (dd, *J* = 13.4, 7.6 Hz, 1H), 0.97 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.31, 171.15, 171.03, 170.13, 164.92, 158.83, 157.89, 156.24, 156.02, 154.83, 150.21, 148.47, 144.47, 138.46, 131.70, 130.81, 130.03, 129.97, 129.42, 129.28, 128.09, 127.70, 127.35, 124.21, 119.64, 119.05, 98.91, 71.08, 70.62, 70.51, 70.26, 68.91, 64.46, 58.83, 57.83, 57.03, 54.43, 50.66, 43.17, 36.43, 35.64, 33.25, 26.46, 16.06. Purity > 99%. HRMS: m/z calculated for $C_{53}H_{61}N_{10}O_{10}S$ $[M+H]^+$ =1029.4287, found $[M+H]^+$ =1029.4335.

Scheme S5. **Synthesis of compound 8**.



Reagents and conditions: (**a**) **25**, EDCI, HOBT, TEA, DCM, 2h, rt. (**b**) (i) TFA, DCM, rt, 2h; (ii) **2**, HATU, DIEA, DMF, 2h, rt.

(*S*)-2-acrylamido-*N*^{5}-(8-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-8-oxooctyl)-*N*^{1}-(3-((5-(2-methyl-5-(3-(trifluoromethyl)benzamido)benzamido)pyrimidin-2-yl)amino)phenyl)pentanediamide (8)

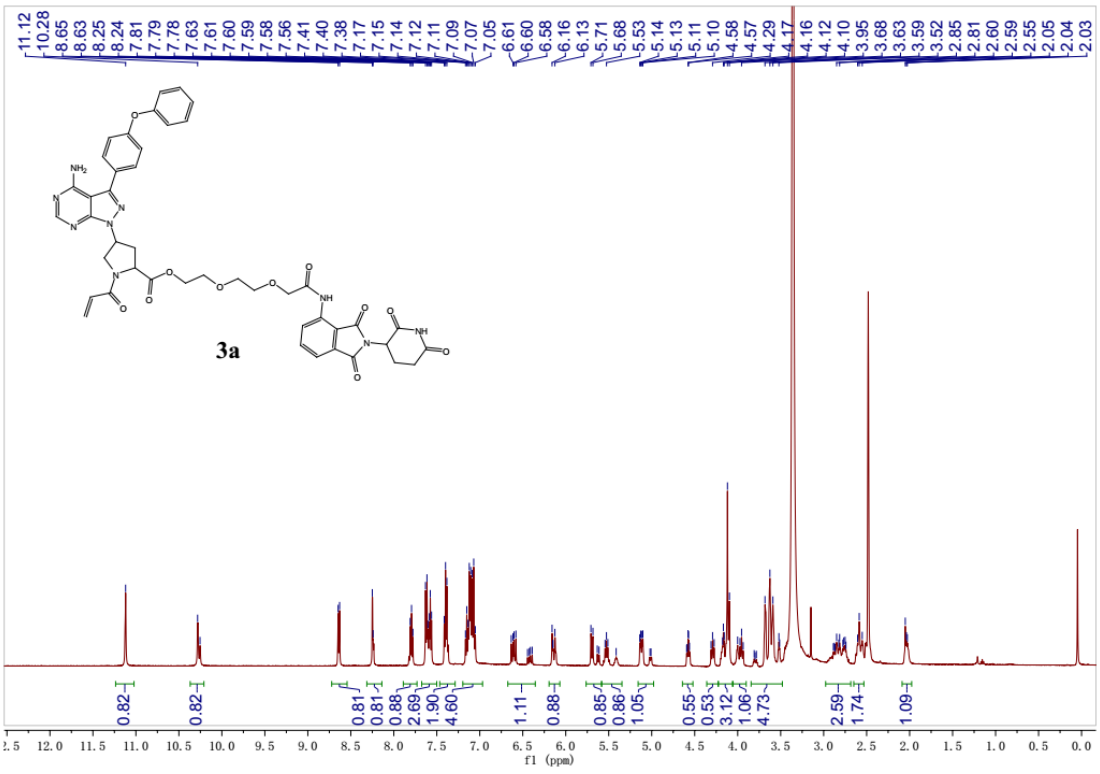


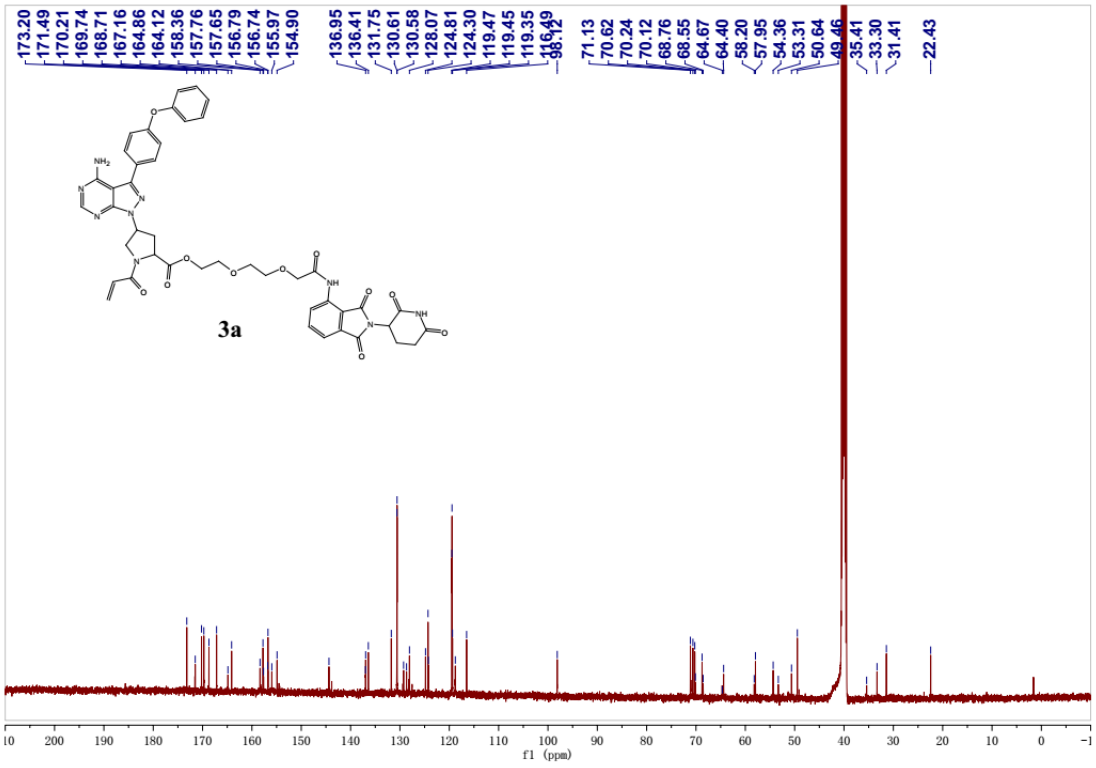
Compound **24** (65 mg, 0.15 mmol) and TEA (106 uL, 0.76 mmol) in DCM (1 mL) was added to the solution of compound **25** (46 mg, 0.18 mmol), EDCI (87 mg, 0.453 mmol), HOBT (31 mg, 0.23 mmol) in DCM (2 mL) at 0 °C in ice water bath. After removing the ice water, the reaction mixture was stirred at rt for 2 h. The resulting mixture was poured into water extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4 and the solvent was removed by rotary evaporator. After concentration, the crude was purified by preparative TLC to provide 78 mg (77%) of title compound as a white solid. MS calculated for $C_{35}H_{54}N_5O_6S$ $[M+H]^+$ =672, found $[M+H]^+$ =672.

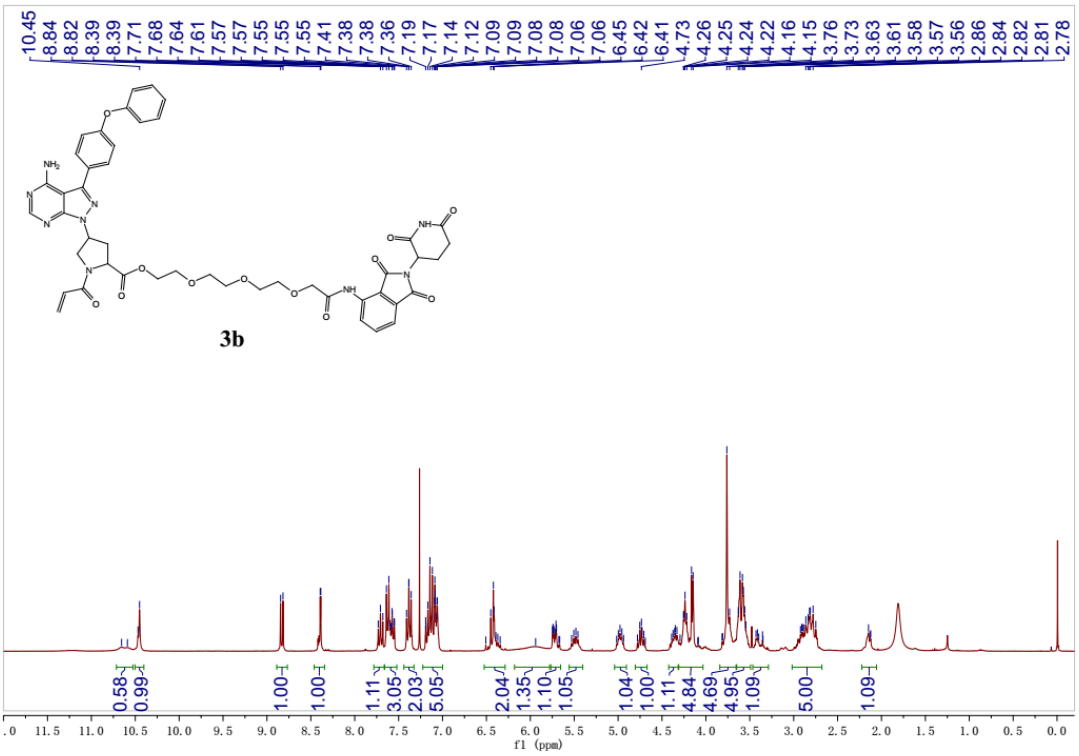
In a round-bottom flask, the compound **26** (39 mg, 0.058 mmol) was dissolved in DCM (1 mL) and TFA (1 mL) and the reaction mixture was stirred at room temperature for 2 h. The mixture solution was concentrated by rotary evaporator. The solution of the residue and DIEA (52 uL, 0.31 mmol) in DMF (1 mL) was added to the solution of **2** (48 mg, 0.069 mmol), HATU (33 mg, 0.087 mmol) in DMF (1 mL) at 0 °C in ice water bath. After removing the ice water, the reaction mixture was stirred at rt for 2 h. The resulting mixture was poured into water extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4 and the solvent was removed by rotary evaporator. After concentration, the crude

was purified by preparative HPLC to provide 20.4 mg (28%) of the title compound as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 8.84 (s, 1H), 8.76 (s, 2H), 8.31 – 8.13 (m, 2H), 8.06 (t, *J* = 1.8 Hz, 1H), 7.91 (dd, *J* = 18.7, 4.4 Hz, 4H), 7.78 – 7.62 (m, 2H), 7.47 – 7.13 (m, 9H), 6.30 (qd, *J* = 17.1, 6.0 Hz, 1H), 5.68 (dd, *J* = 9.8, 2.1 Hz, 1H), 4.69 – 4.54 (m, 3H), 4.52 – 4.43 (m, 2H), 4.35 (t, *J* = 9.5 Hz, 1H), 3.90 (d, *J* = 11.1 Hz, 1H), 3.78 (dd, *J* = 10.9, 3.7 Hz, 1H), 3.19 – 3.02 (m, 2H), 2.45 (s, 3H), 2.43 (s, 3H), 2.38 – 1.92 (m, 9H), 1.56 (s, 2H), 1.44 (s, 2H), 1.27 (s,7H), 1.01 (s, 9H). ^{13}C NMR (75 MHz, MeOD) δ 174.64, 173.20, 173.07, 171.02, 170.50, 169.31, 166.60, 165.64, 157.08, 151.42, 150.47, 147.57, 140.74, 138.76, 138.44, 136.15, 136.11, 135.64, 132.19, 132.01, 131.11, 130.98, 130.82, 130.39, 130.28, 130.07, 129.31, 128.92, 128.56, 128.07, 127.56, 126.07, 125.94, 125.76, 124.21, 124.15, 122.65, 122.16, 119.69, 114.89, 113.63, 110.63, 69.68, 59.50, 57.58, 56.67, 53.67, 42.33, 39.07, 37.55, 35.16, 31.77, 28.79, 28.64, 28.54, 28.01, 26.35, 25.67, 25.43, 17.95, 14.45. Purity: 97%. HRMS: m/z calculated for $C_{64}H_{74}F_3N_{12}O_9S$ $[M+H]^+$ =1243.5375, found $[M+H]^+$ =1243.5408.

^{1}H and ^{13}C NMR Spectra of final compounds



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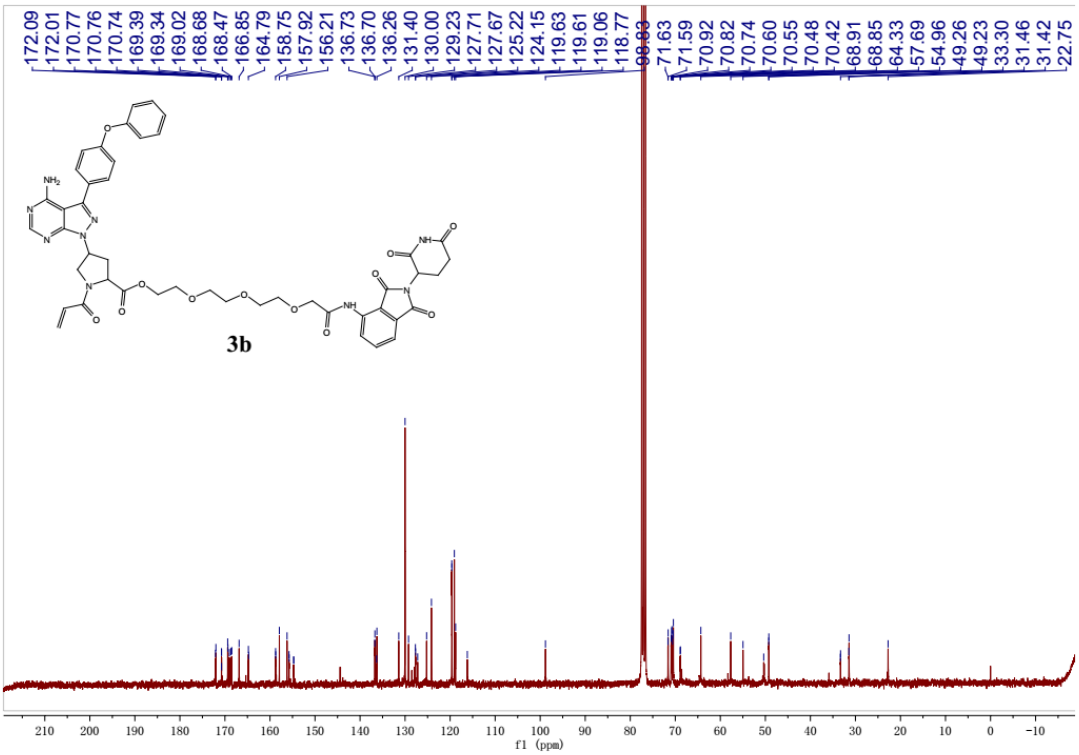
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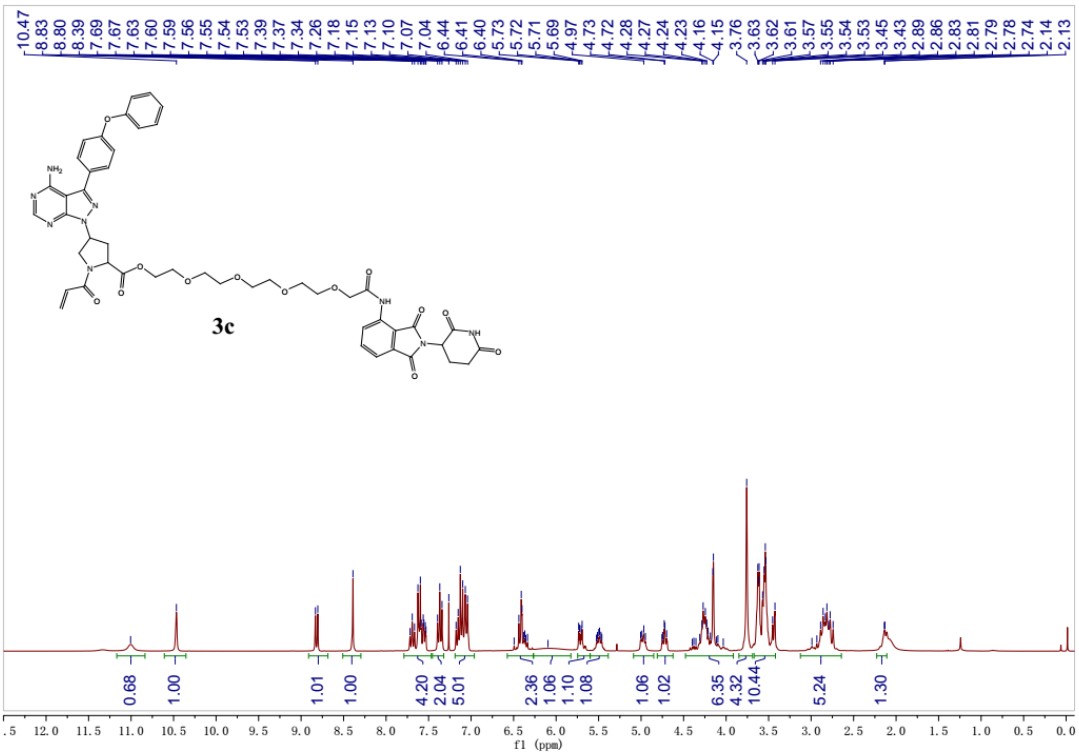
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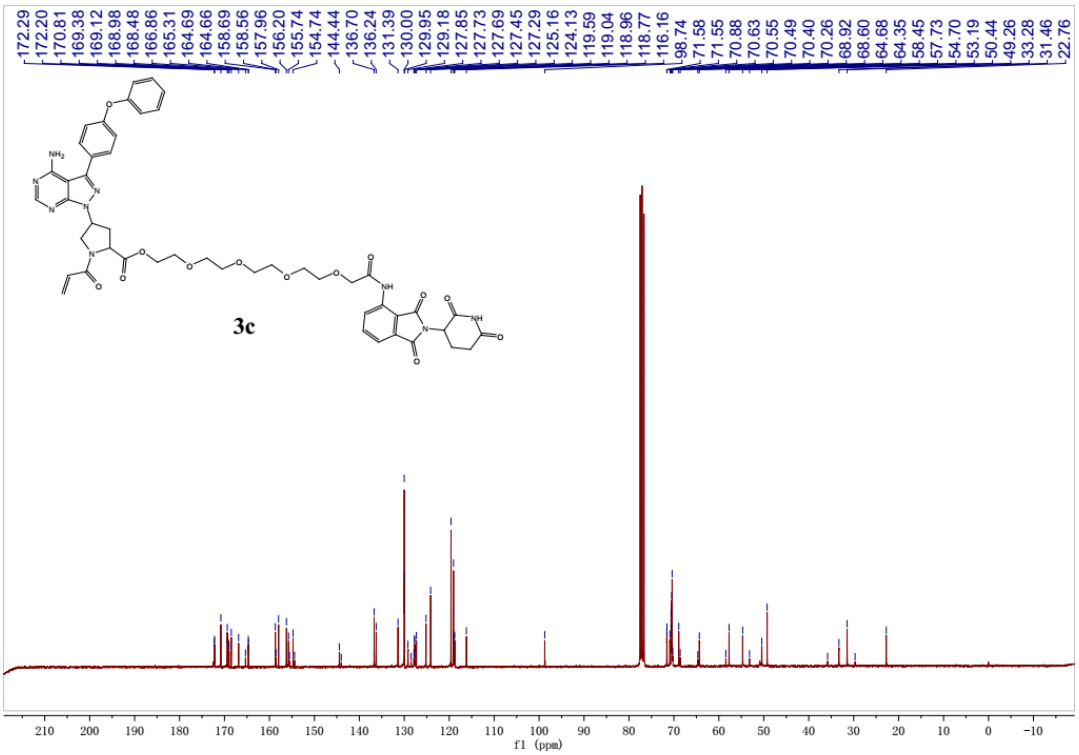
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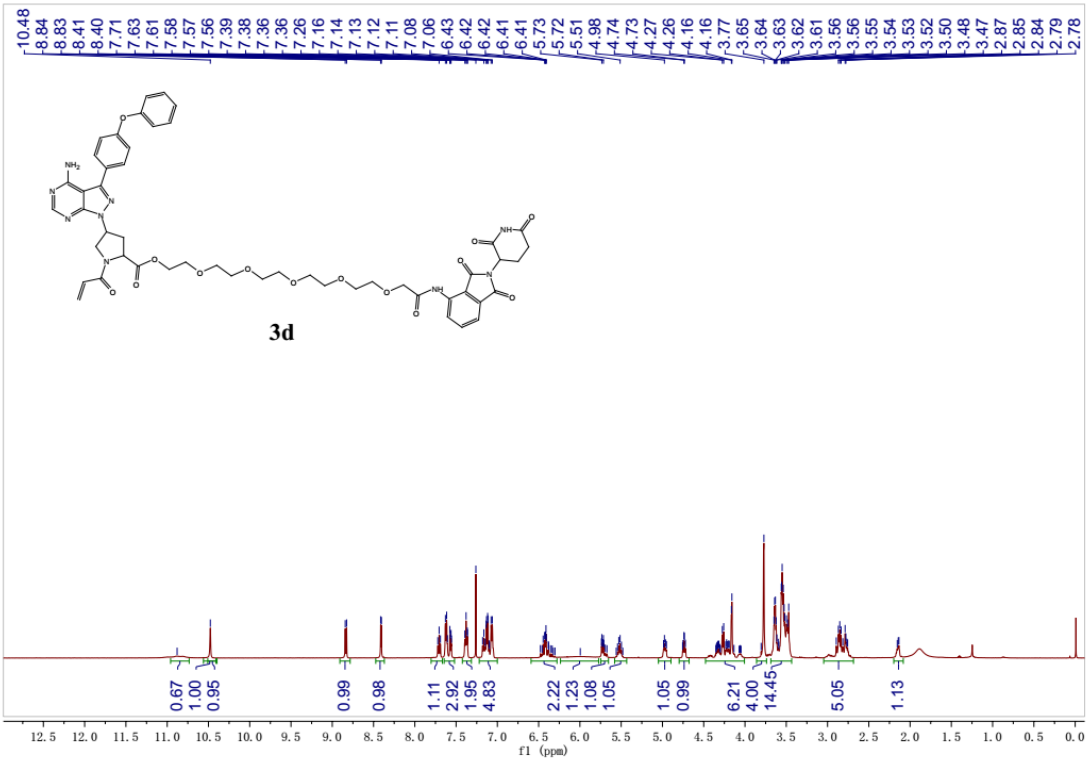
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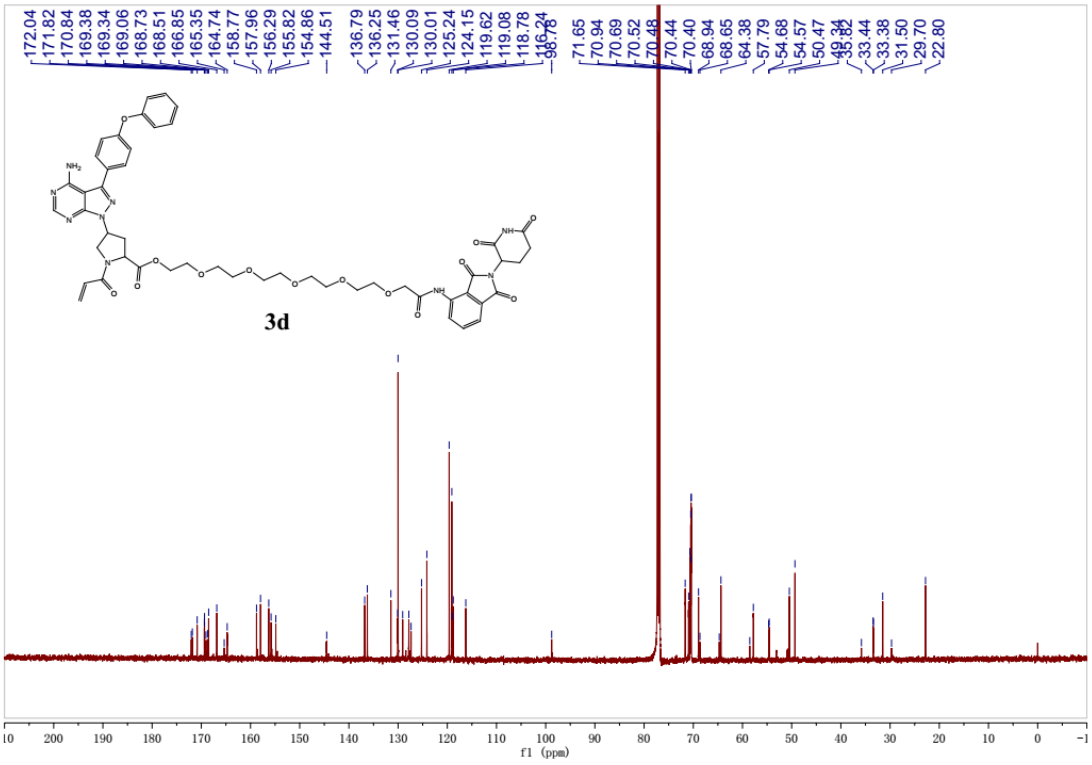
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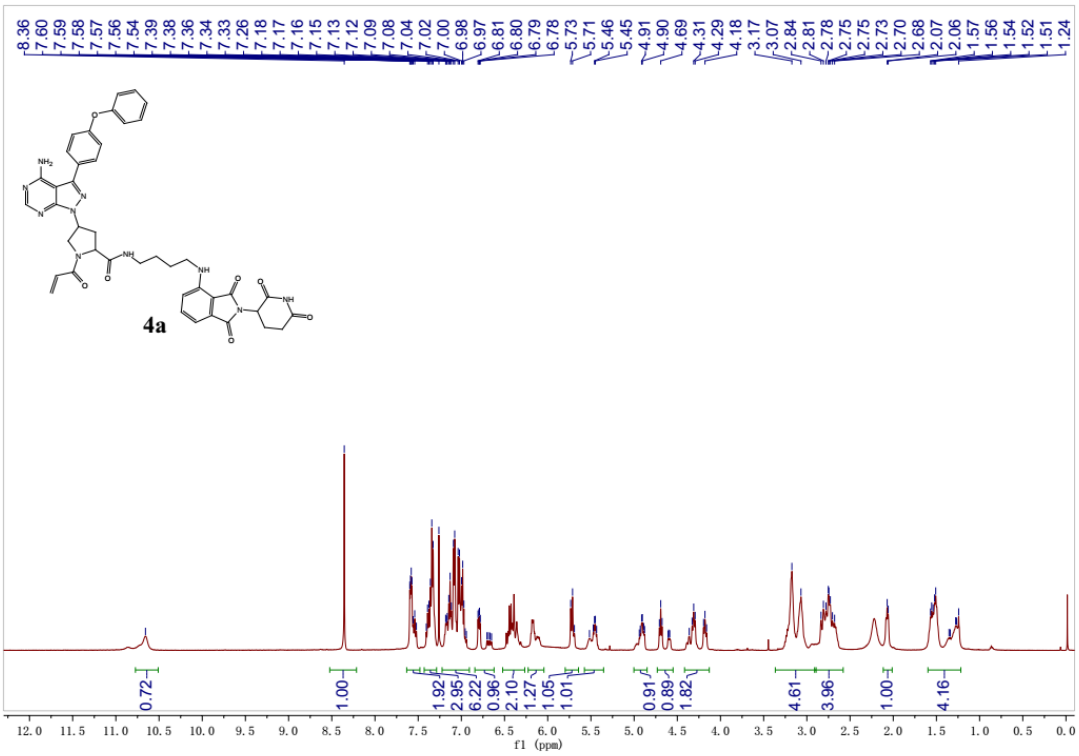
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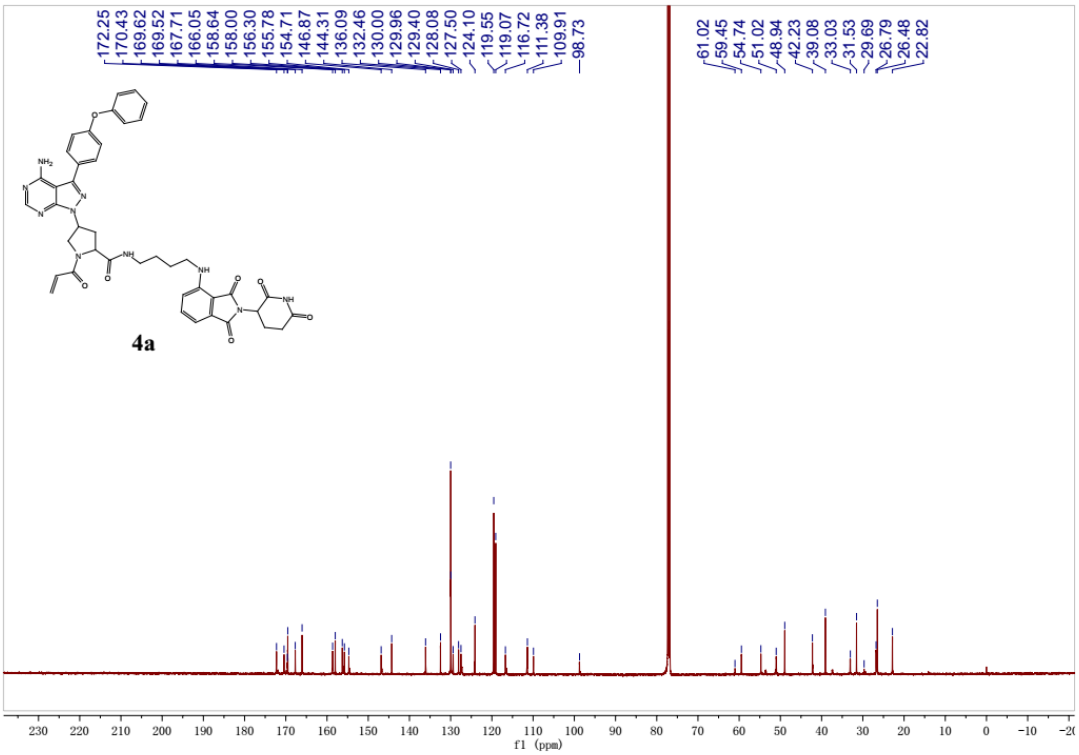
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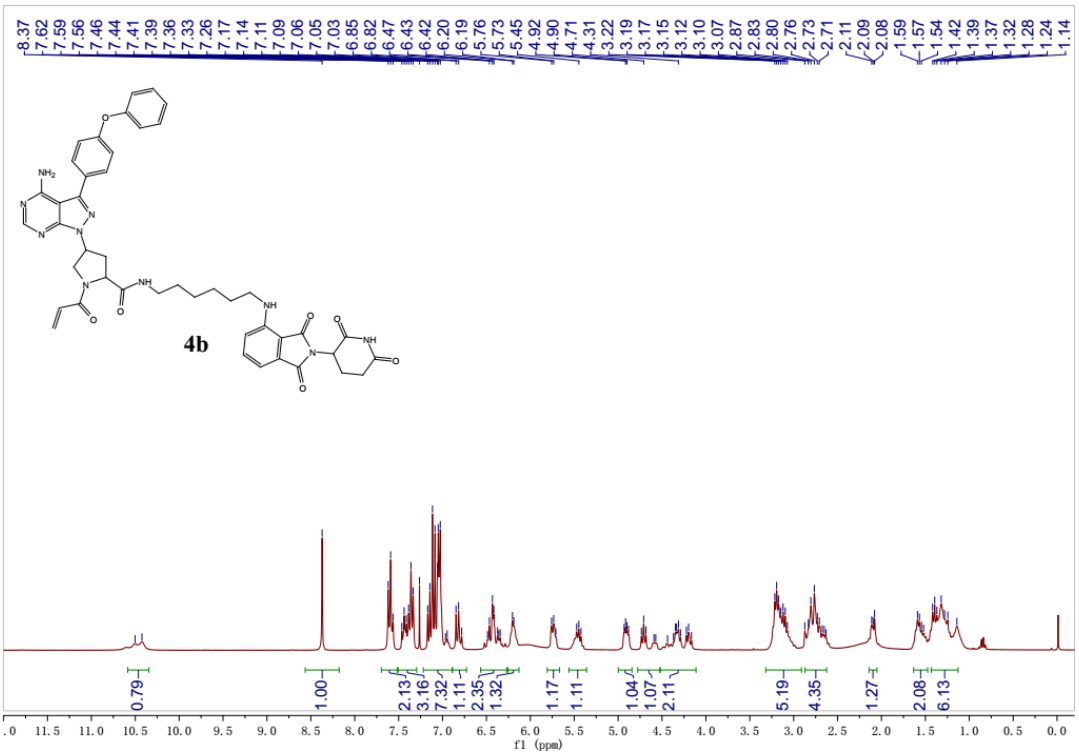
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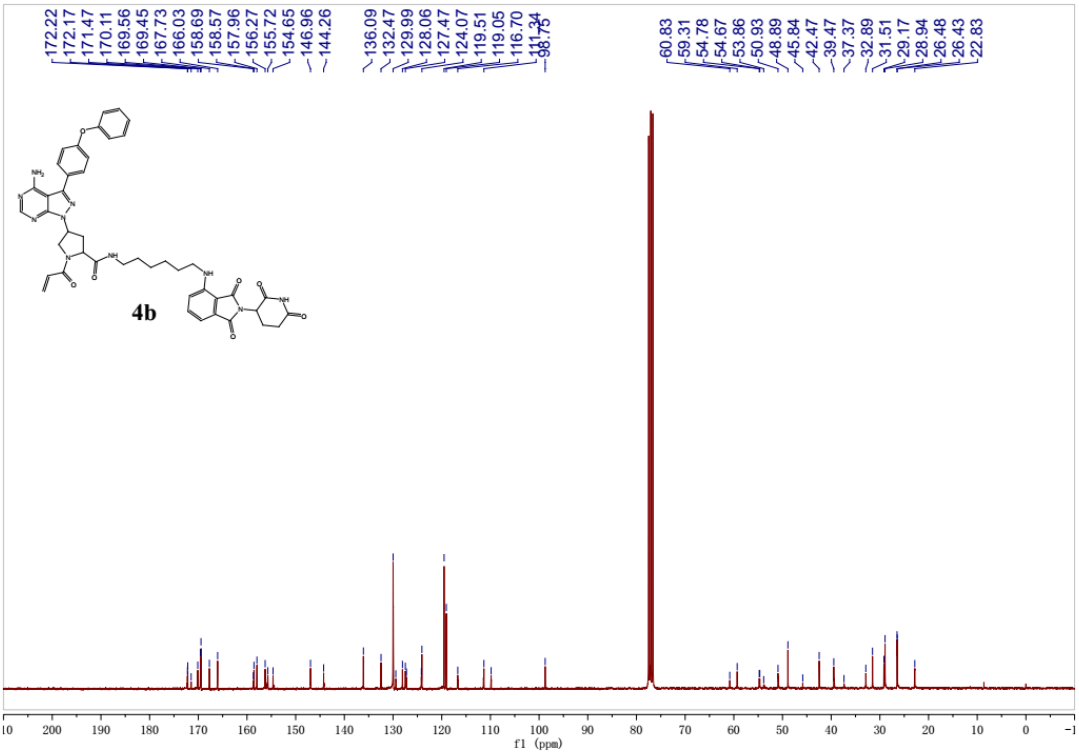
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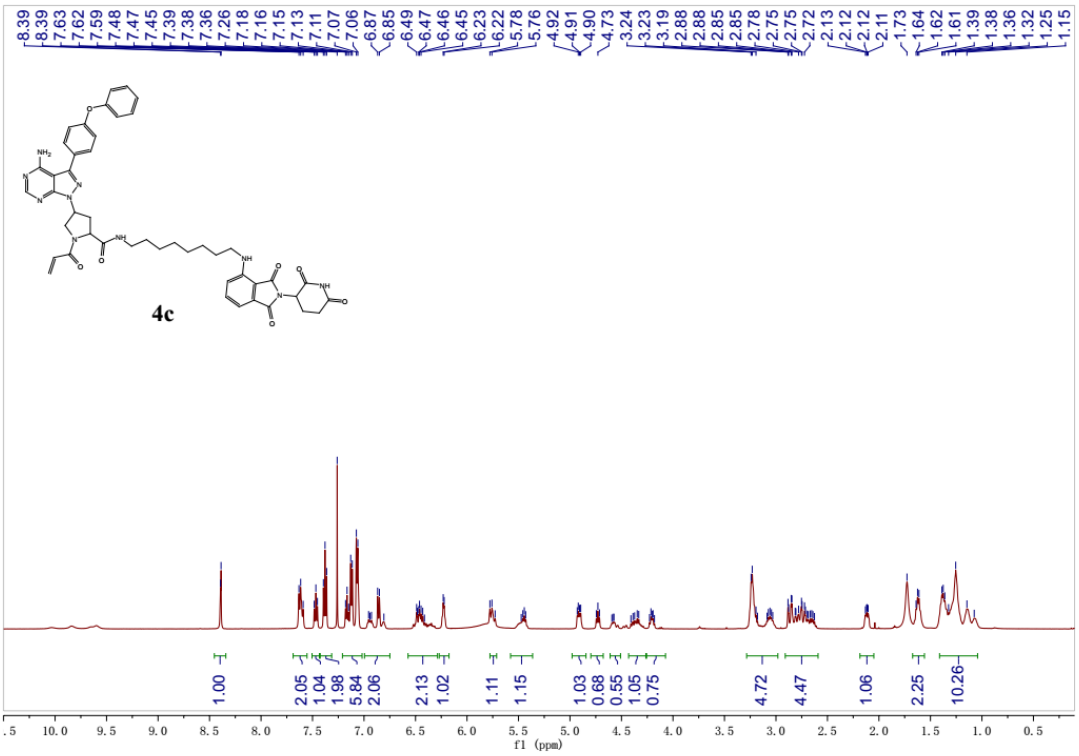
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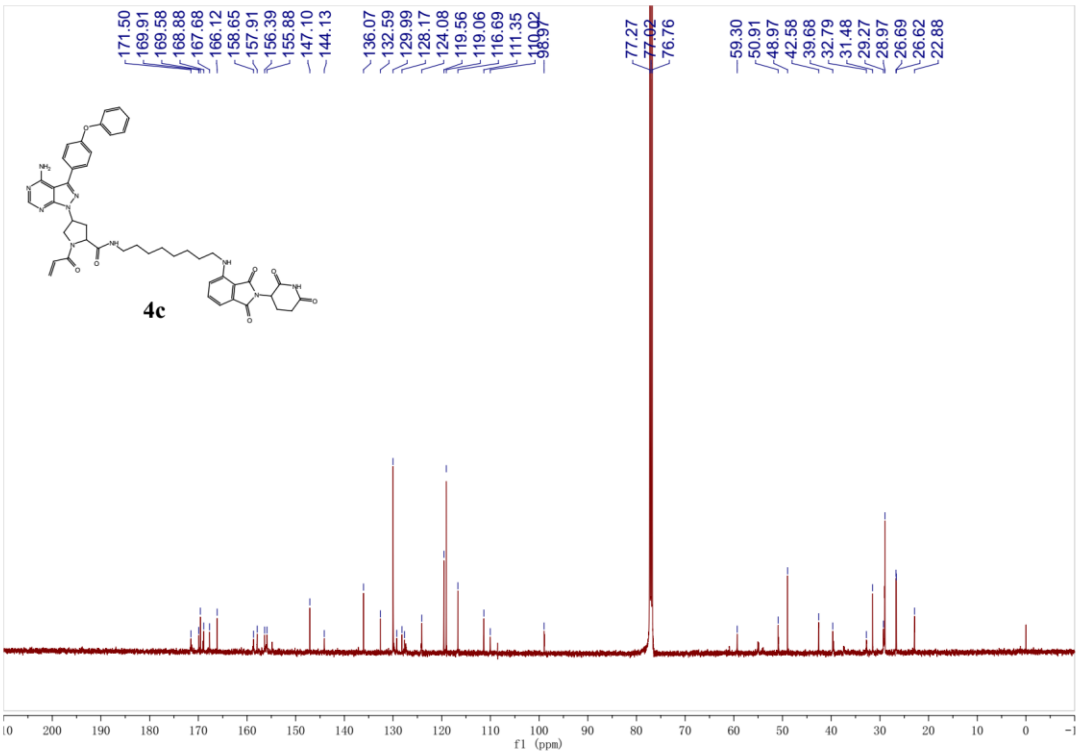
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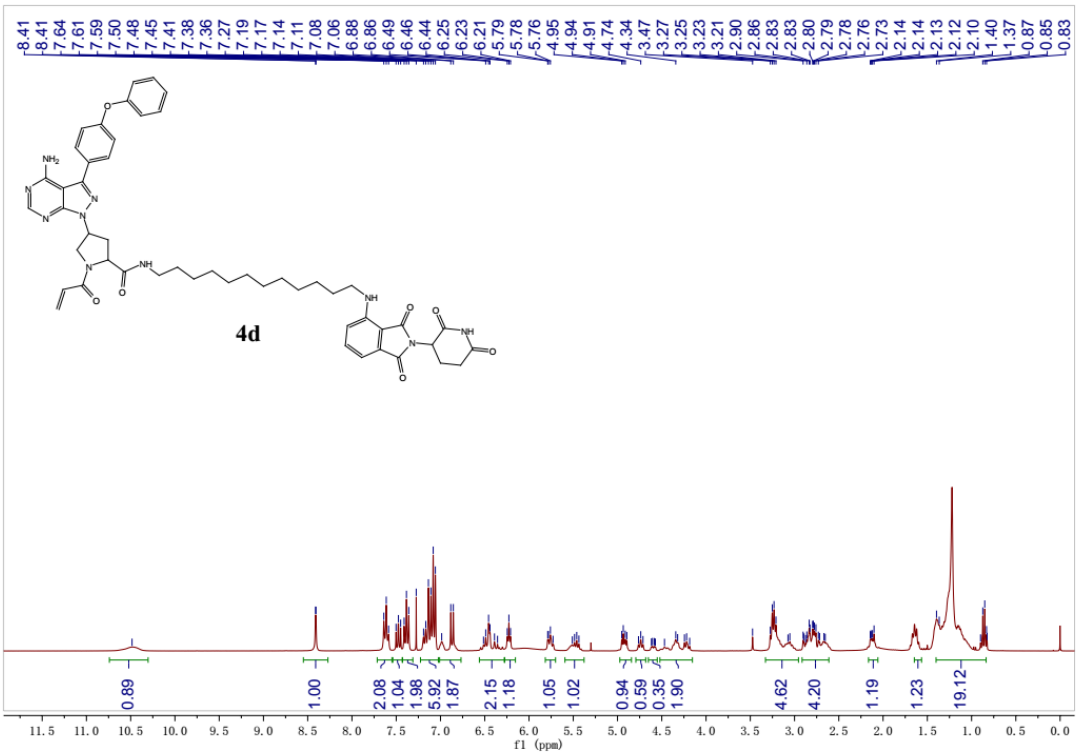
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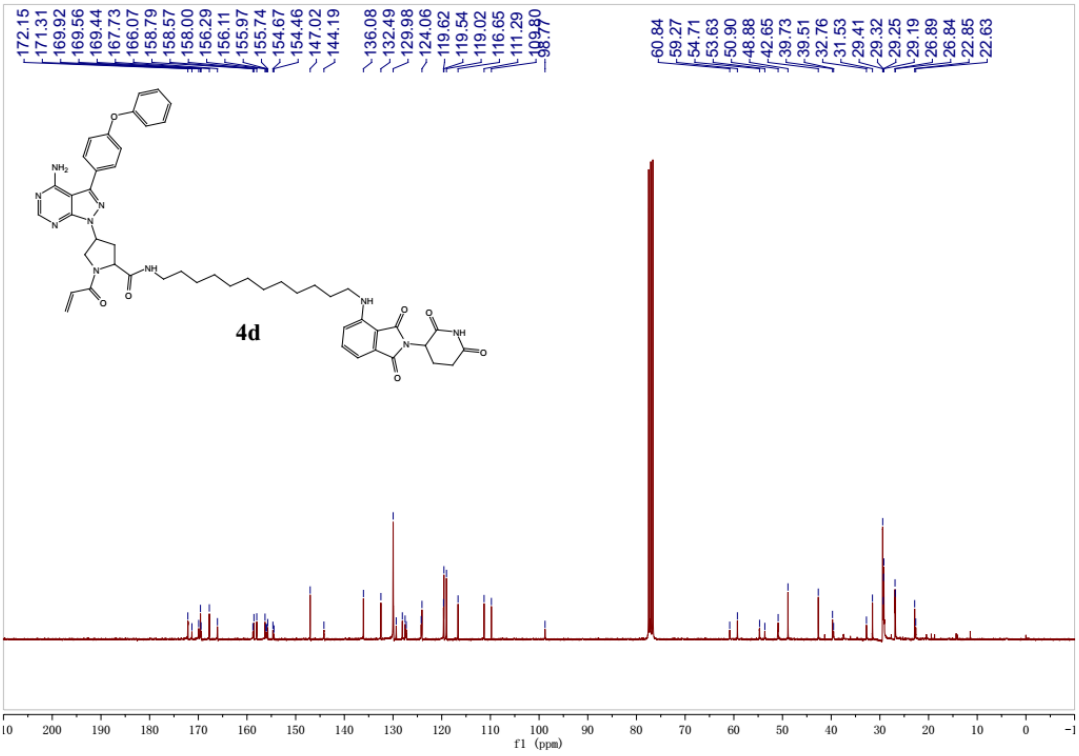
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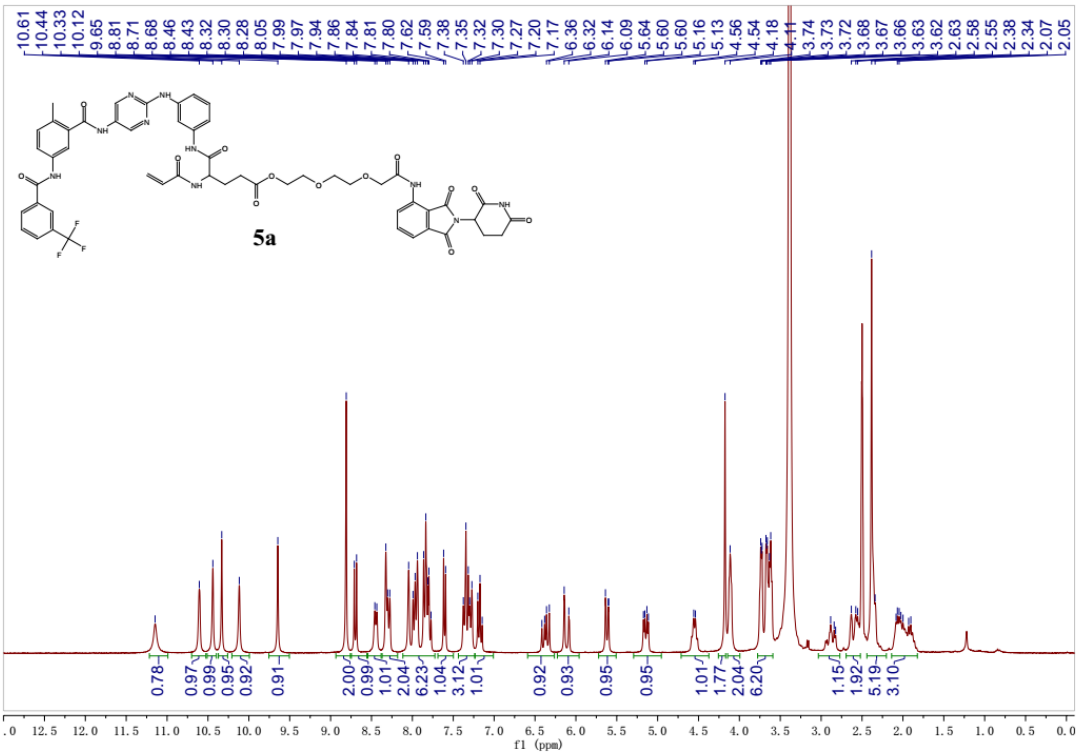
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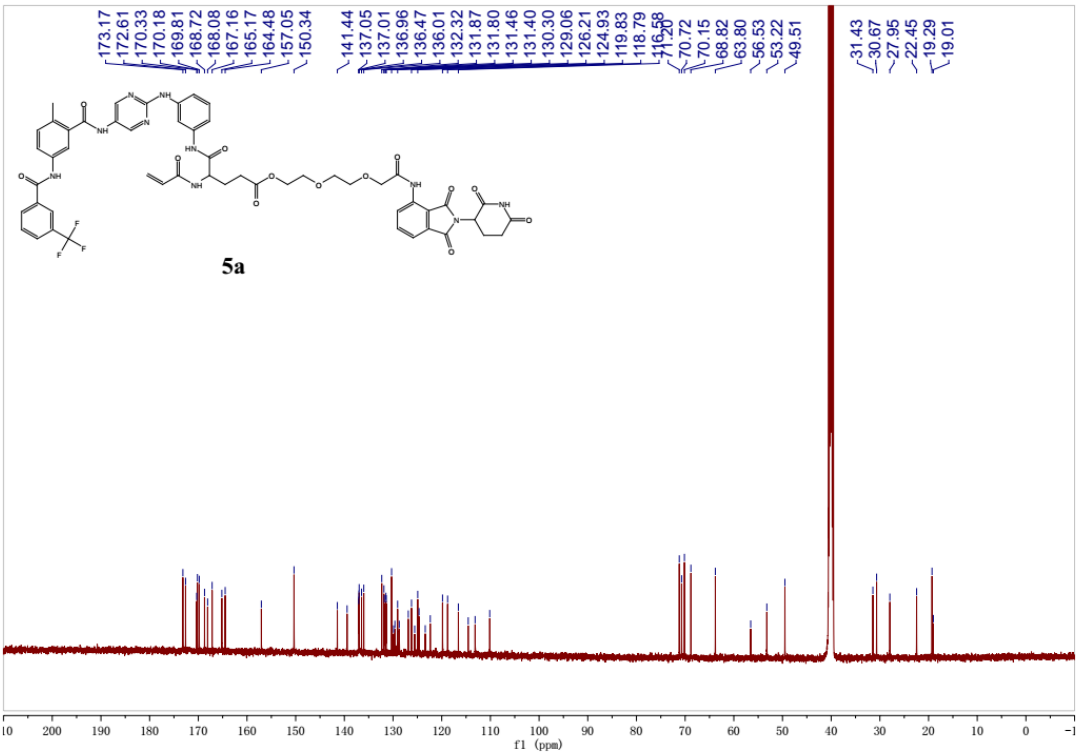
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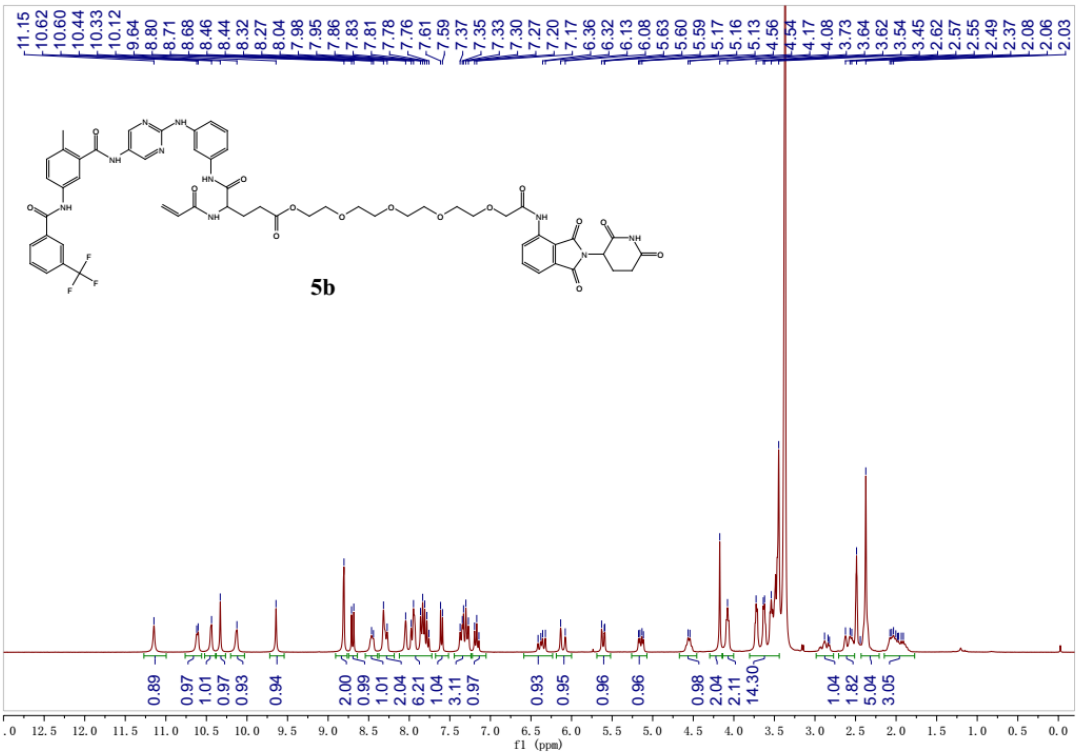
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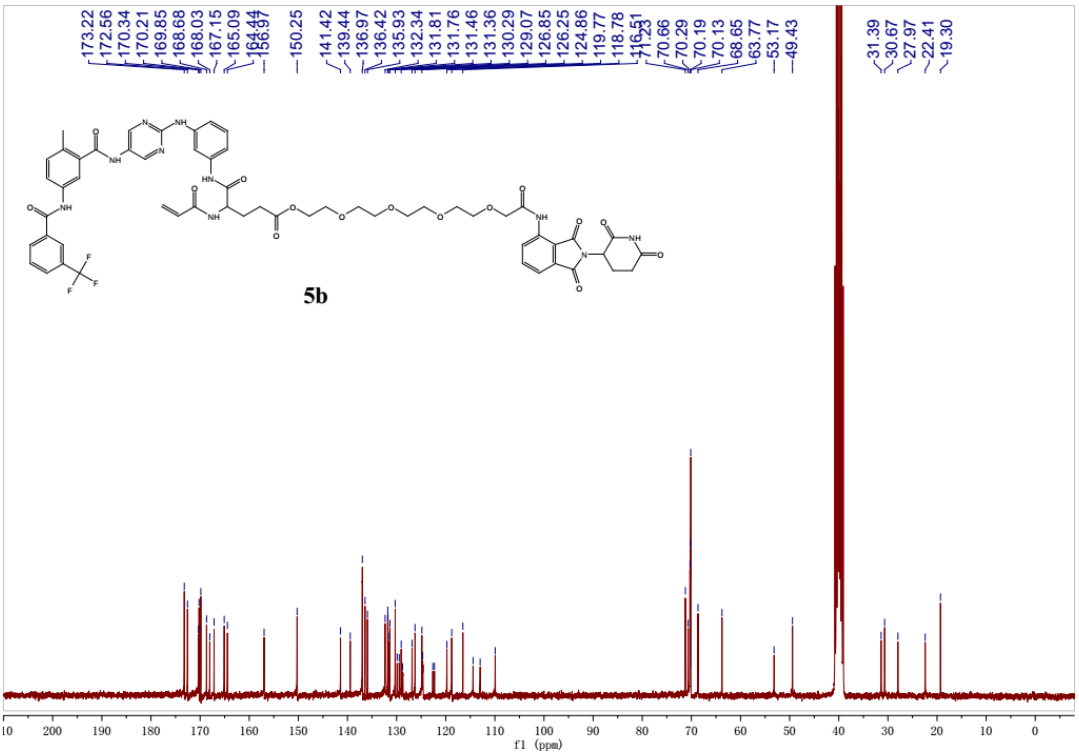
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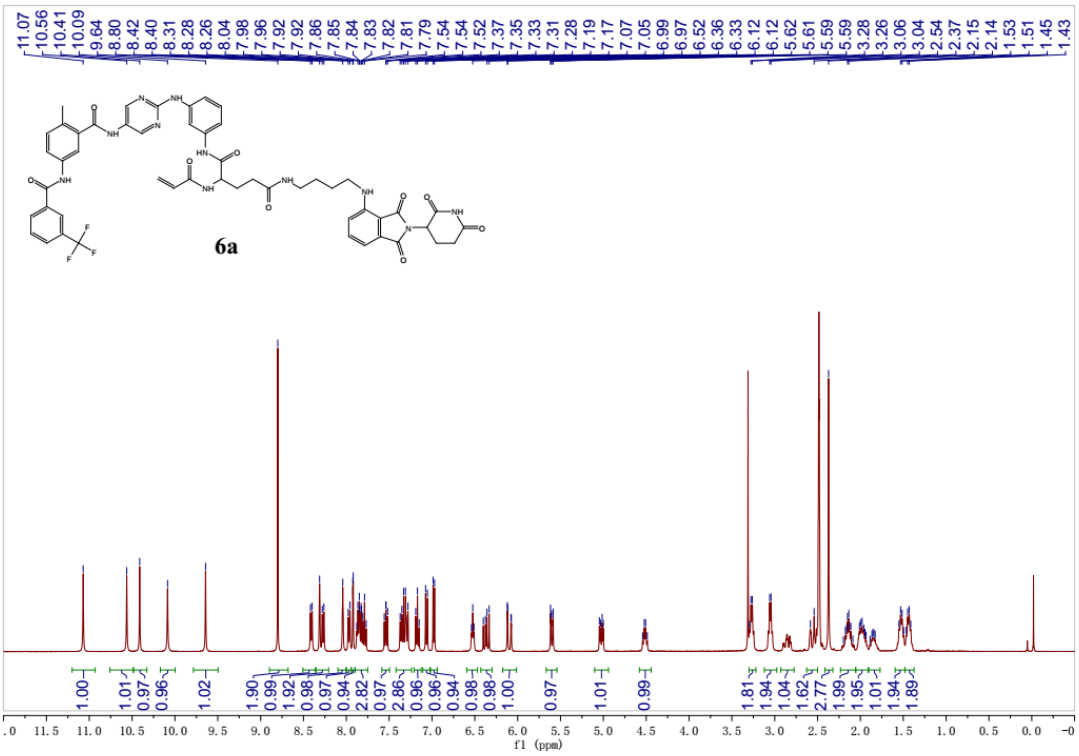
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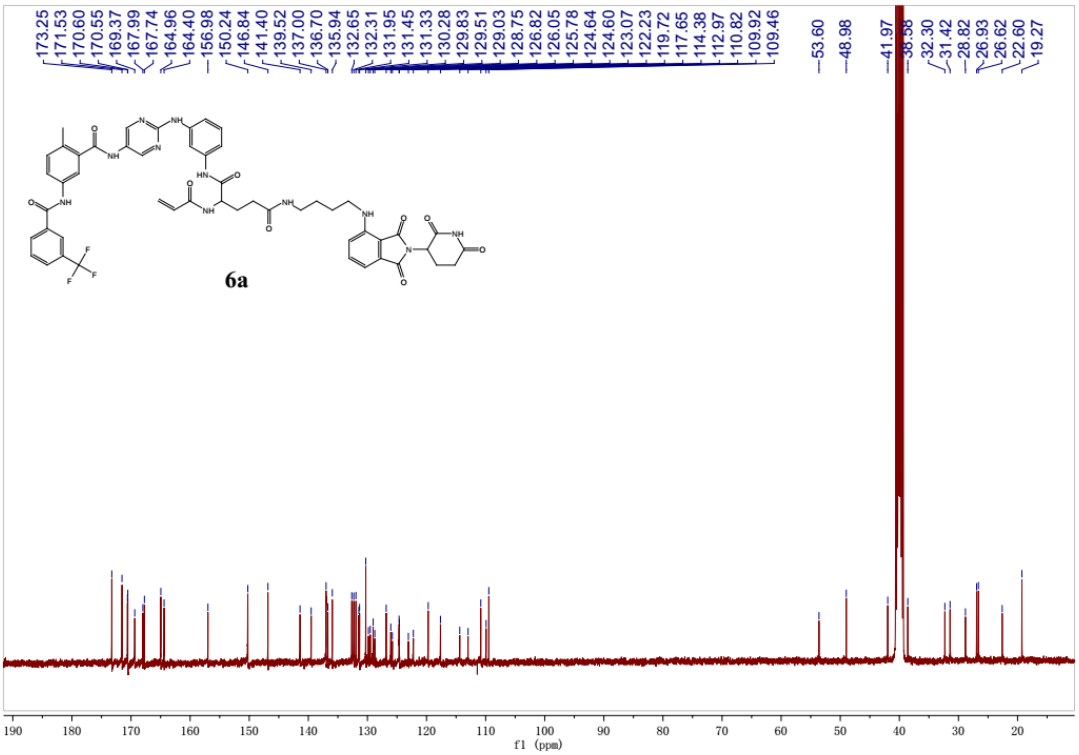
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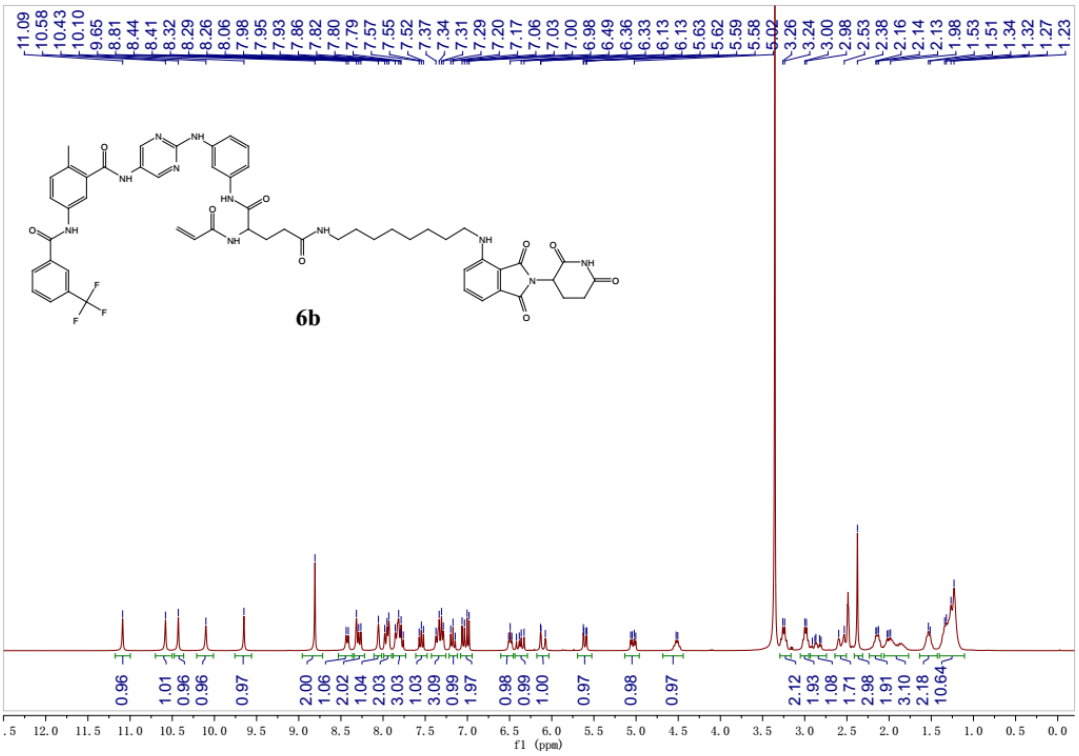
f1 (ppm)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7.31
7.29
7.20
7.17
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7.03
7.00
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6.13
6.13
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5.62
5.59
5.58
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2.53
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2.13
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1.32
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1.23
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0.96
0.96
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1.06
2.02
1.04
2.03
3.03
1.03
3.09
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0.98
0.99
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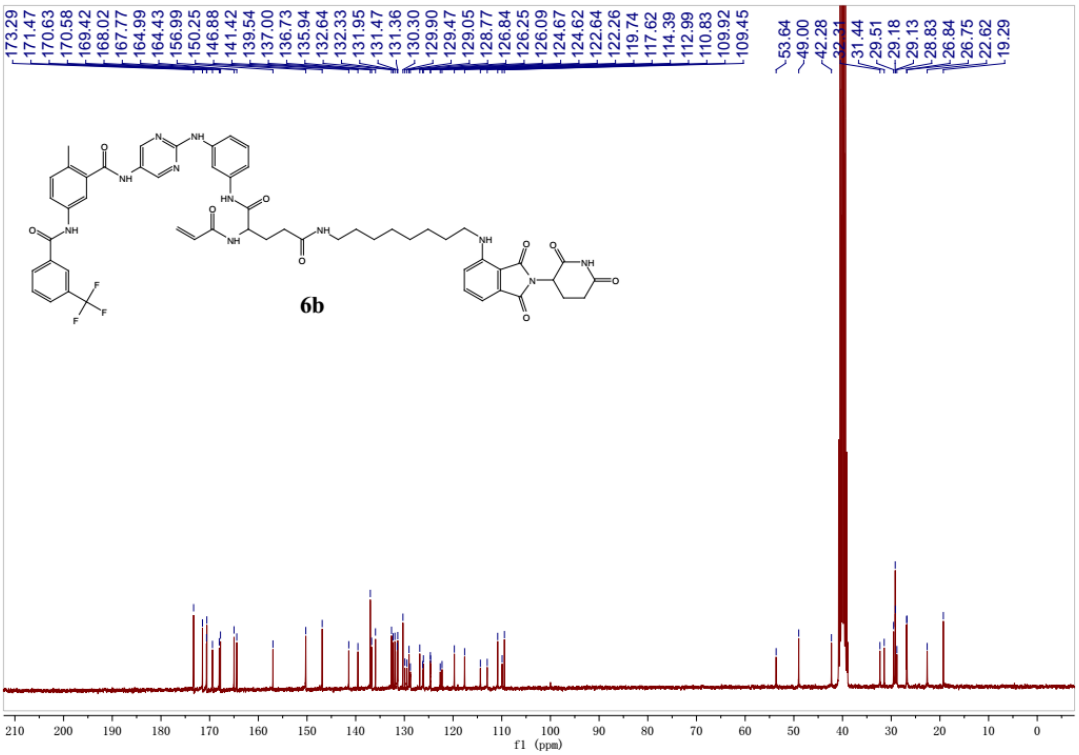
f1 (ppm)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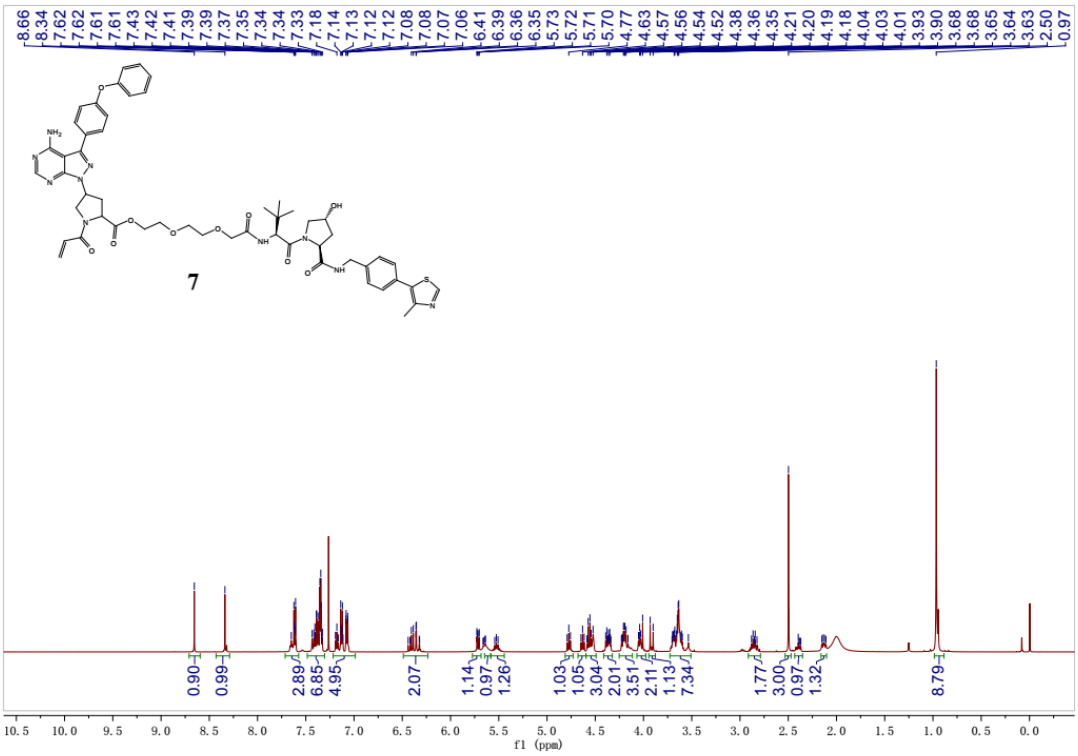
6b
f1 (ppm)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7.34
7.33
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7.14
7.13
7.12
7.12
7.08
7.08
7.07
7.06
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6.39
6.36
6.35
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5.72
5.71
5.70
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4.63
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4.56
4.54
4.52
4.38
4.36
4.35
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4.20
4.19
4.18
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4.03
4.01
3.93
3.90
3.68
3.68
3.65
3.64
3.63
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0.99
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6.85
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1.14
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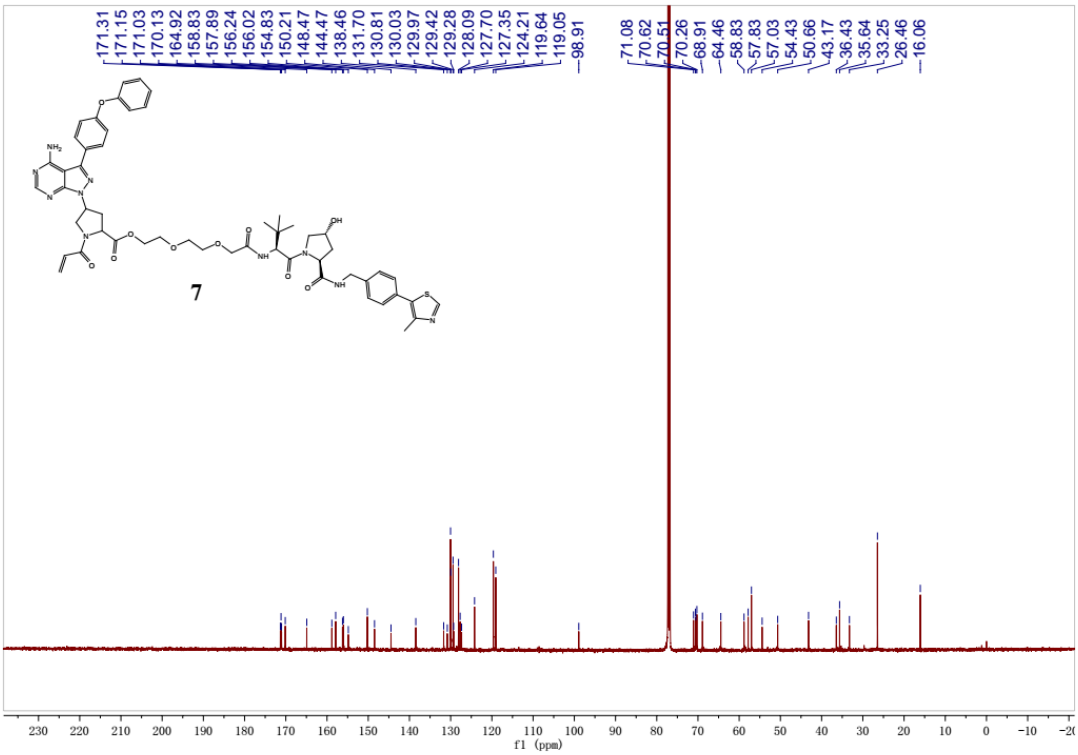
f1 (ppm)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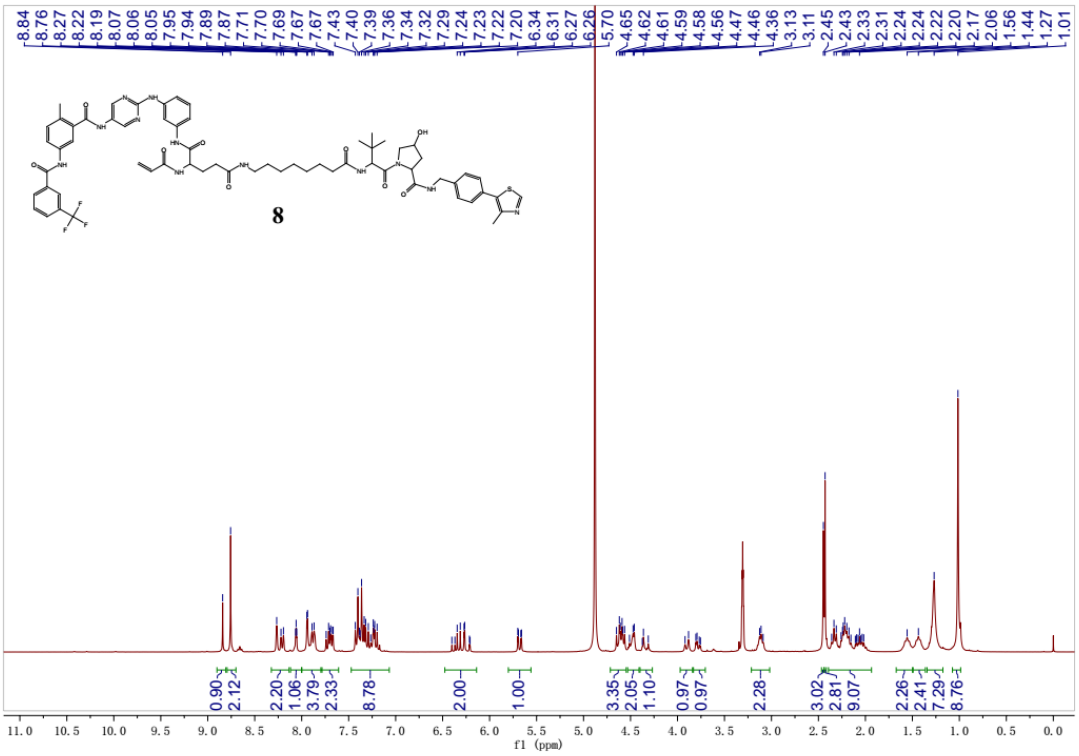
f1 (ppm)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7.67
7.67
7.43
7.40
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7.36
7.34
7.32
7.29
7.24
7.23
7.22
7.20
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6.26
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4.61
4.59
4.58
4.56
4.47
4.46
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2.31
2.24
2.24
2.22
2.20
2.17
2.06
1.56
1.44
1.27
1.01
8
0.90
2.12
2.20
1.06
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2.33
8.78
2.00
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2.05
1.10
0.97
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2.28
3.02
2.81
9.07
2.26
2.41
7.29
8.76
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
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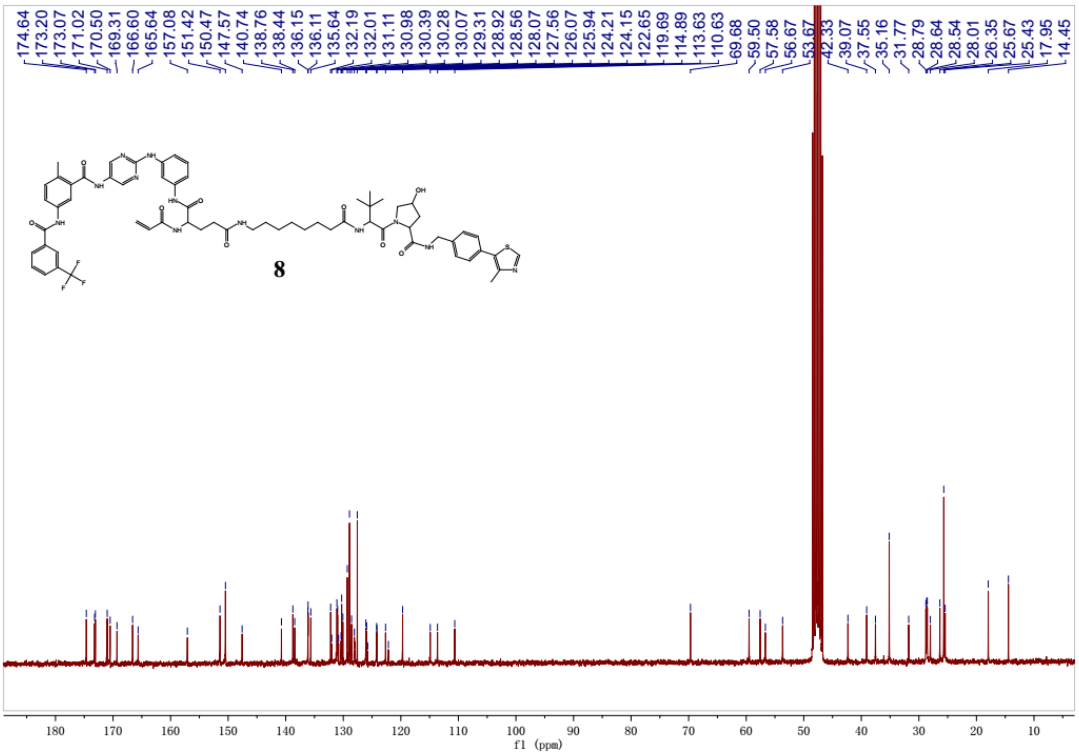
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8
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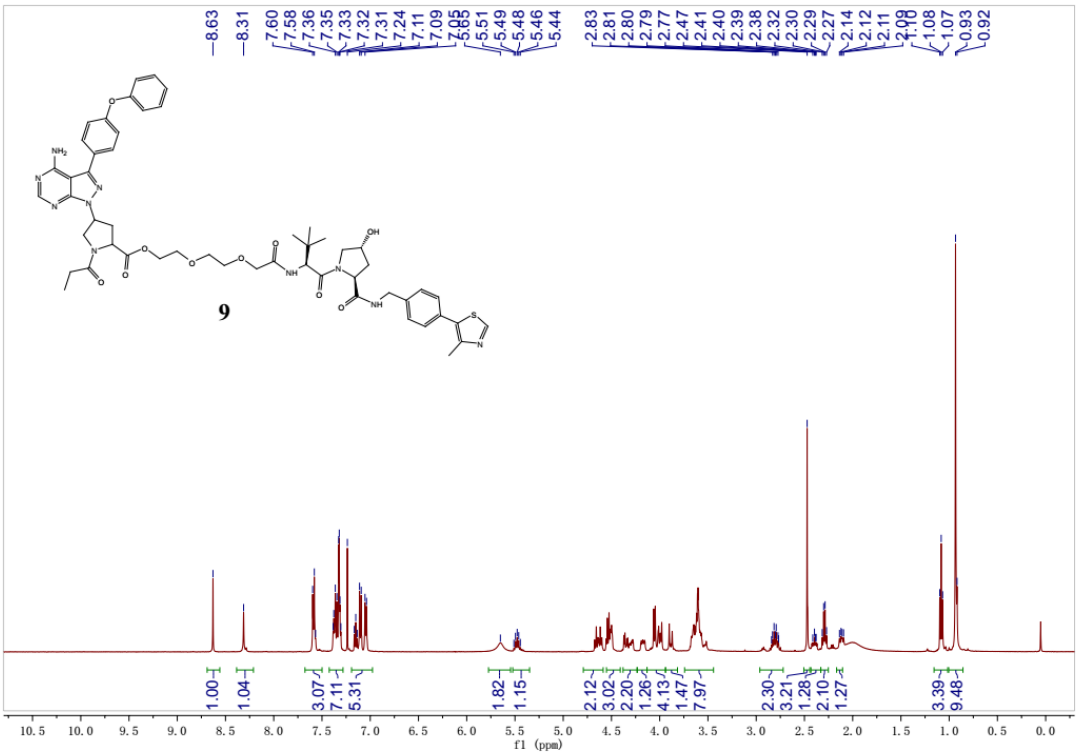
f1 (ppm)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5.51
5.49
5.48
5.46
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2.39
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2.27
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2.12
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2.08
1.10
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9
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1.15
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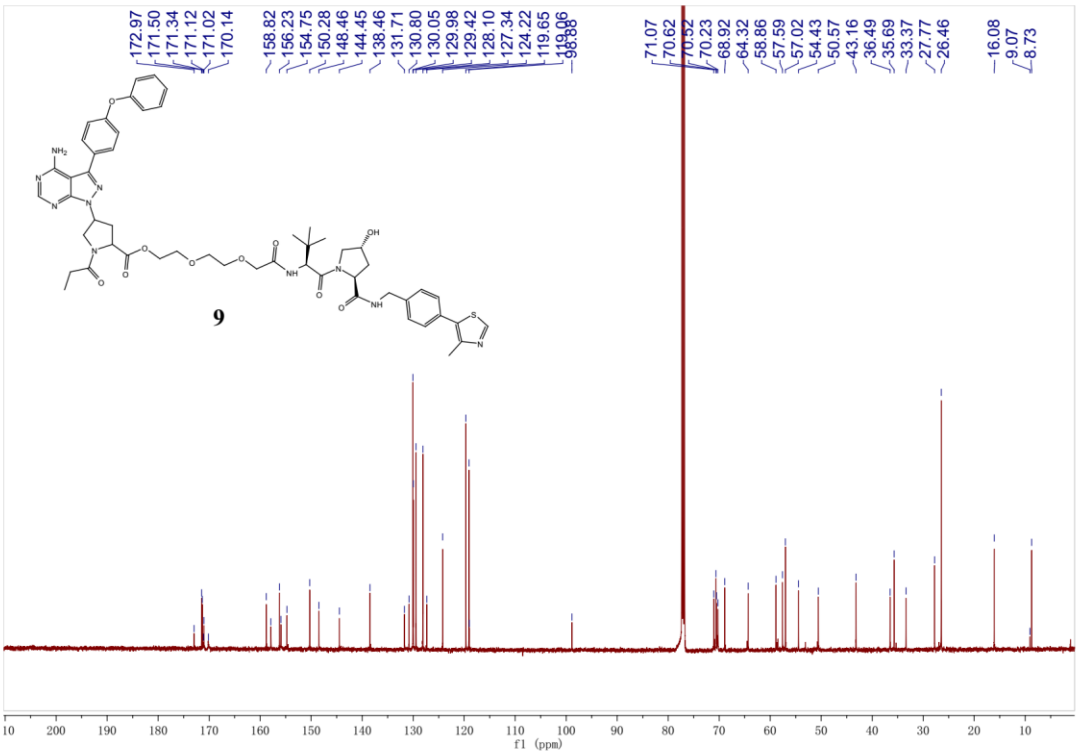
f1 (ppm)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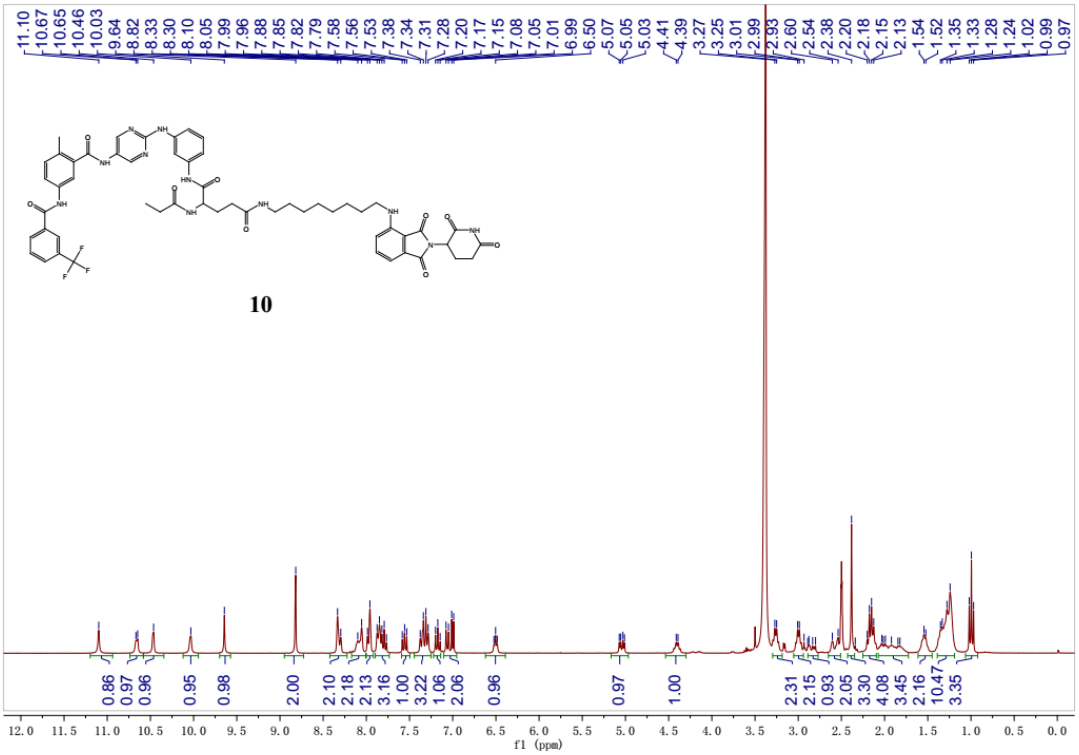
f1 (ppm)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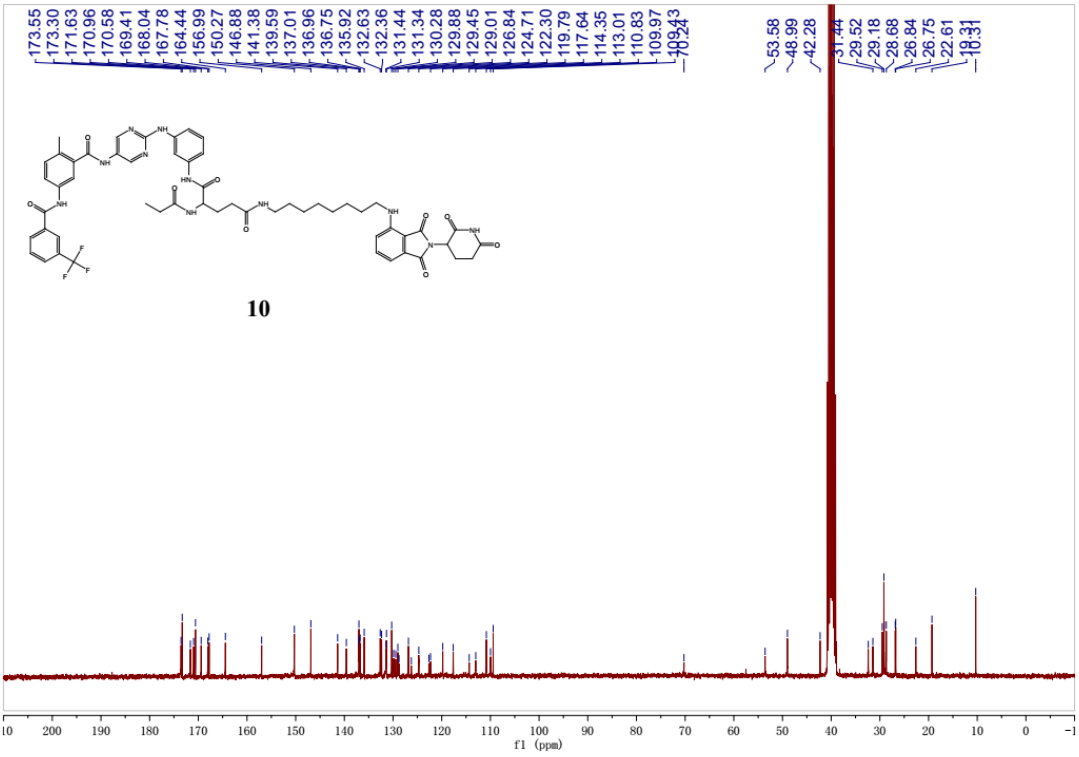
f1 (ppm)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10.11
10
f1 (ppm)

Reference:

1. X. Li, Y. Zuo, G. Tang, Y. Wang, Y. Zhou, X. Wang, T. Guo, M. Xia, N. Ding and Z. Pan, *Journal of medicinal chemistry*, 2014, **57**, 5112-5128.
2. C. Galdeano, M. S. Gadd, P. Soares, S. Scaffidi, I. Van Molle, I. Birced, S. Hewitt, D. M. Dias and A. Ciulli, *Journal of medicinal chemistry*, 2014, **57**, 8657-8663.