# Development of Hypoxia-activated PROTAC Exerting More Potent Effect in Tumor Hypoxia than in Normoxia

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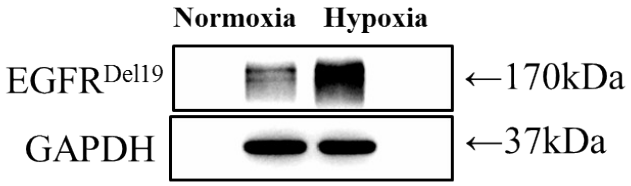



**Fig. S1** Expression of EGFR$^{Del19}$ in HCC4006 cells after 24 h incubation in normoxia and hypoxia.

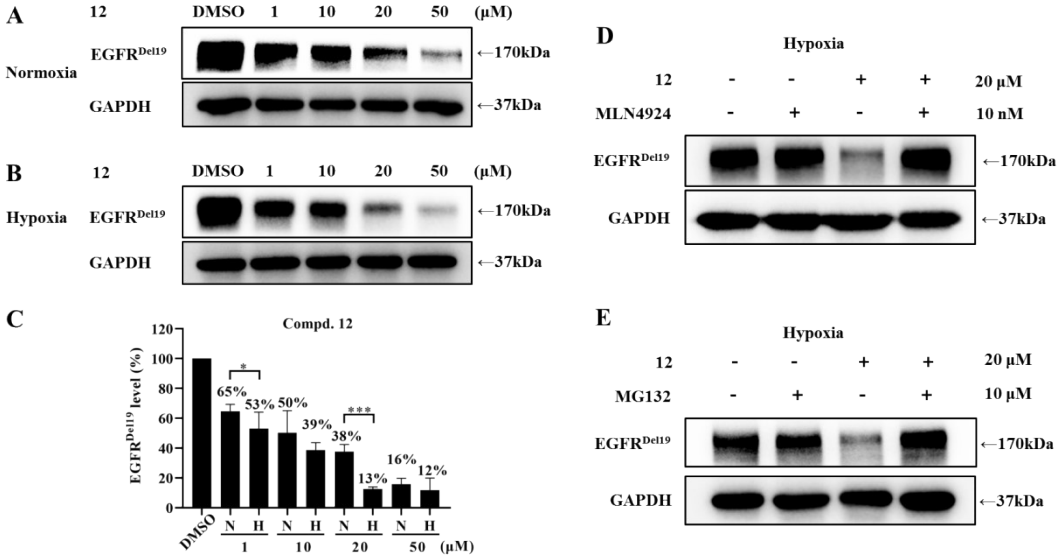



**Fig. S2** Effect of **12**, MLN4924 and MG132 on the degradation of EGFR$^{Del19}$. (A) Degradation of EGFR$^{Del19}$ by ha-PROTAC **12** in HCC4006 cells for 24 h at the four indicated concentrations in normoxia. (B) Degradation of EGFR$^{Del19}$ by ha-PROTAC **12** in HCC4006 cells for 24 h at the four indicated concentrations in hypoxia. (C) EGFR$^{Del19}$ protein levels in cells in normoxia and hypoxia. Numbers were calculated by the EGFR$^{Del19}$/GAPDH ratio with normalization by the DMSO control as 100. The bars in the graphs show the means±standard deviations from two biological replicates. *p<0.05, **p<0.01, and ***p<0.001 are from unpaired t-tests. (D) The degradation of EGFR$^{Del19}$ by MLN4924 alone, **12** alone, or MLN4924 combined with **12**. (E) The degradation of EGFR$^{Del19}$ by MG132 alone, **12** alone, or MLN4924 combined with **12**.

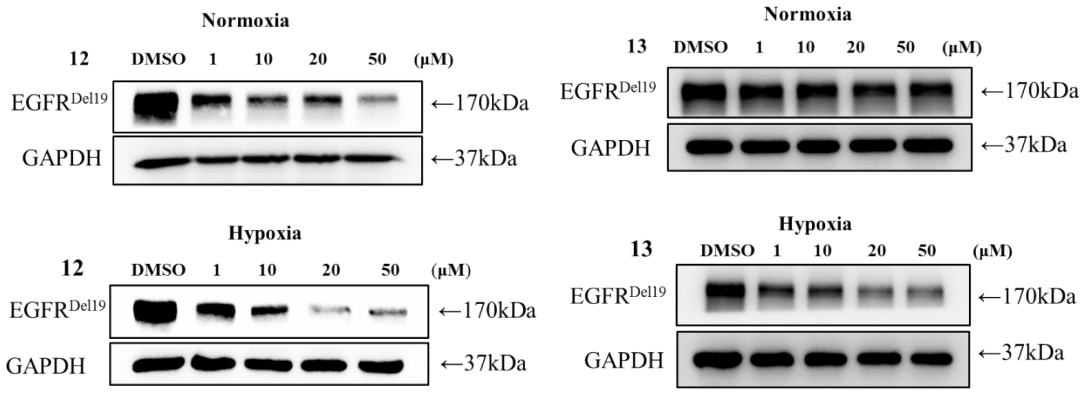



**Fig. S3** The second western blot replicate data for compounds **12** and **13**.

**In vitro enzymatic inhibitory activity assay**

In vitro $EGFR^{Del19}$ (Carna, Cat. No. 08-527) inhibitory assay was carried out by Sundia MediTech Co., Ltd in Shanghai, China, using afatinib (Selleck, Cat. No. S1011) as the reference compound ($IC_{50}$: 0.70 nM). Five compounds were screened on $EGFR^{Del19}$ kinase by mobility shift assay. The initial concentration of compounds was 75 nM, 50000 nM, or 100 nM, 3-fold dilution, 7 concentrations; or 10000 nM, 5-fold dilution, 6 concentrations. The general procedures were as the following: 1x assay buffer (modified Tris Buffer) was prepared, and candidate compounds were transferred to assay plate by Echo550 in 100% DMSO. Substrate solution was made by preparing enzyme solution in 1x assay buffer and added to assay plate, then incubated at r.t. for 10 minutes. The mixed solution of ATP and kinase substrate 22 (GL, Cat. No. 112393) was prepared and added to the plate and incubated at room temperature (r.t.) for 10 minutes. Finally, detection solution was added to stop the reaction and the conversion rate was read with Caliper EZ reader II.

**Cell lines**

Human lung adenocarcinoma cell lines HCC4006 were purchased from Cell Bank of China Science Academy (Shanghai, People's Republic of China). The cells were cultured in RPMI-1640 (Thermo Fisher Scientific) medium with heat-inactivated 10% FBS, penicillin (100 units/mL), and streptomycin (100 mg/mL) and incubated at 37°C and 5% $CO_2$.

**Western Blotting**

HCC4006 cells were seeded at $2 \times 10^6$ cells per well in a 6-well plate and treated with different compounds for 24 hours in normoxia atmosphere (20% $O_2$, 5% $CO_2$, 37 °C) or in hypoxia atmosphere (1% $O_2$, 5% $CO_2$, 37 °C). Following treatment, cells were harvested and washing twice with normal saline, the SDS lysate was mixed with a ratio of 2:100, placed at r.t. for 5 min and heated at 100 °C for

30 min, and centrifuged to obtain the whole solution, that is, the total protein of cells. The amount of protein was detected by BCA method. The protein was diluted with 5 × protein loading buffer and denatured at 100 °C for 5 min. The protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then electrophoretically transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked in 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.05% (v/v) Tween 20 containing 5% nonfat milk for 1 h at r.t.. The membrane was then probed with the primary antibody at 4 °C overnight, followed by incubation with the appropriate horseradish peroxidase (HRP) conjugated secondary antibody for 1 h at r.t.. The blots were developed by ChemiDoc XRS+ (Bio-Rad). The gray value analysis was used Image J software.

**UPLC-MS/MS detection of the HALG cleavage of ha-PROTAC 13**

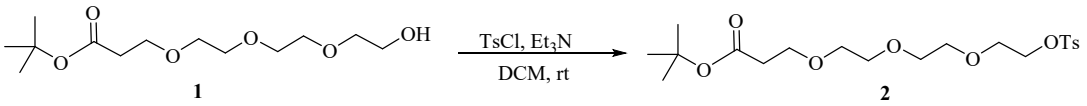
Compound **13** (1.0 μM) was dissolved in PBS (pH 7.4, Servicebio, Wuhan, China) and then incubated with NADPH (1.0 mM, Sigma-Aldrich) and nitroreductase (50 μg/mL, Sigma-Aldrich) at 37 °C. $N_2$ was used to displace $O_2$ from the reaction system to maintain hypoxia. At 0 (before enzyme addition) and 20 min, 100 μL of reaction mixture was taken out and quenched by 300 μL of methanol (HPLC grade, Fisher Scientific) and then transferred to a vial for analysis using UPLC-MS/MS.

The UPLC was performed on an ExionLCTManalytical (UPLC) system (AB Sciex, USA). Chromatographic separation was carried out on Agela Venusil MP C18 column (2.1 mm × 100 mm, 3 μm). The flow rate was 0.3 mL/min, and the mobile phase consisted of water containing 0.1% formic acid (solvent A) and methanol (solvent B) in a linear gradient. The gradient program was as follows: 0 to 1min, 95% A; 1 to 2 min, 95 to 10% A; 2 to 3 min, 10% A to 2% A; 3 to 4 min, 2 to 95% A; 4 to 5 min, 95% A. The column temperature was maintained at 40 ◦C. The temperature of the autosampler was set at 4 ◦C, and the injection volume was 5 μL.

MS/MS analysis was carried out on a Qtrap 4500 mass spectrometer (AB Sciex, USA) equipped with Turbo Ionspray interface operating in positive ESI mode. The instrument was operated with an ion spray voltage of 4.5 kV and a heater gas temperature of 500◦C. A nebulizer gas (gas 1) of 40 psi, a heater gas (gas 2) of 50 psi, a curtain gas of 20 psi, and a medium collision gas were used. Mass-dependent parameters such as the declustering potential, entrance potential, collision energy, and collision cell exit potential, were set to the optimal values obtained by automated optimization. Multiple reaction monitoring (MRM) was employed for data acquisition. Data acquisition was performed with Analyst 1.6.2 software (Applied Biosystems, USA).

**General methods/instruments**

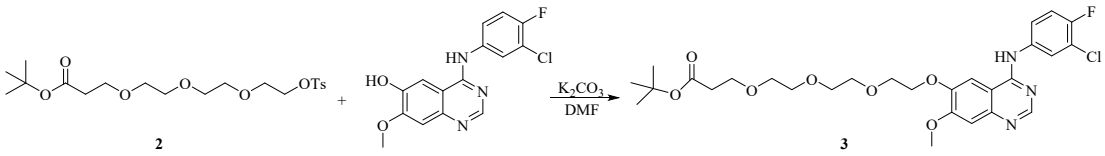
$^{1}H$ NMR were recorded on a AVII 400 MHz and $^{13}C$ NMR were recorded on a BRUKER AVII 100 MHz spectrometer with TMS as the internal standard. Proton chemical shifts are expressed in parts per million (ppm) and coupling constants in Hz. Mass spectra (ESI-MS) were performed on the AB Sciex TripleTOF 6600 mass spectrometry.




1 2

**tert-Butyl 3-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)propanoate (2)**

4-methylbenzenesulfonyl chloride (0.75g, 3.95 mmol) was added to a stirred mixture of tert-butyl 3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)propanoate (1.0g, 3.59 mmol) and triethylamine (1.45g, 14.36 mmol) in DCM (10 mL). The mixture was stirred at r.t. for about 2 h. After the reaction was completed, the reaction mixture was diluted with DCM (20 mL), washed with saturated salt water (3x20 mL), the organic solvent was dried over sodium sulfate and concentrated to obtain crude product, which was further purified by column chromatography (petroleum ether (PE): EtOAc=3:1) to get pure material as colorless oil (1.47g, 94.8% yield). $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 7.78 (d, $J$ = 8.1 Hz, 2H), 7.32 (d, $J$ = 8.0 Hz, 2H), 4.18 – 4.10 (m, 2H), 3.81 – 3.48 (m, 12H), 2.59 – 2.37 (m, 5H), 1.42 (s, 9H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 170.90, 144.80, 132.97, 129.83 (2C), 127.98 (2C), 80.52, 70.73, 70.52 (2C), 70.34, 69.25, 68.66, 66.88, 36.24, 28.08 (3C), 21.64. HRMS (ESI): m/z calcd for $(C_{20}H_{32}O_8S + NH_4)^+$: 450.2156; found: 450.2167.

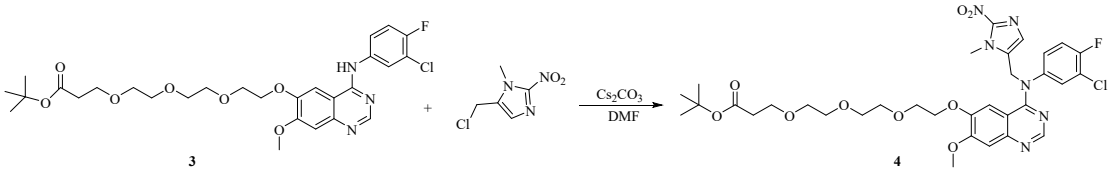



2 3

**tert-Butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (3)**

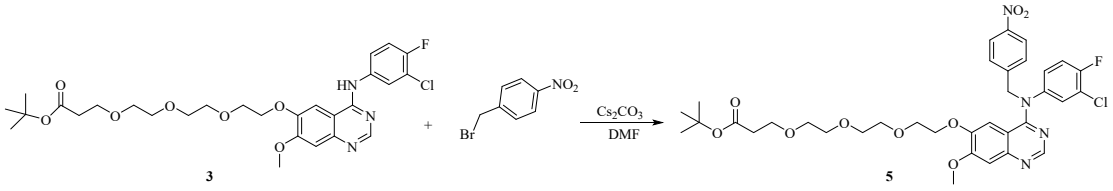
tert-Butyl 3-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)propanoate (**2**, 1.0g, 2.31 mmol) was added to a stirred mixture of 4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-ol (0.70g, 2.20 mmol) and potassium carbonate (0.18g, 1.32 mmol) in anhydrous DMF (10 mL). The reaction mixture was heated to 80 °C and stirred for about 1 h. After the reaction was completed, the reaction mixture was concentrated under vacuum and extracted with EtOAc (30 mL)/saturated salt water (6x20 mL), the organic solvent was dried over sodium sulfate and concentrated to obtain

crude product, which was further purified by column chromatography (DCM : MeOH=100 : 3) to get pure material as colorless oil (1.26g, 98.5% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.45 (s, 1H), 7.94 – 7.89 (m, 1H), 7.67 – 7.60 (m, 1H), 7.52 (s, 1H), 7.28 (s, 1H), 7.20 – 7.06 (m, 2H), 4.27 – 4.21 (m, 2H), 3.91 – 3.84 (m, 5H), 3.74 – 3.64 (m, 4H), 3.63 – 3.57 (m, 4H), 3.57 – 3.52 (m, 2H), 2.42-2.35 (m, 2H), 1.38 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.98, 156.68, 155.05, 154.57 (d, $J_{C\text{-}F}$ = 245.8 Hz), 153.49, 148.47, 147.48, 135.86 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.41, 122.09 (d, $J_{C\text{-}F}$ = 6.8 Hz), 120.69 (d, $J_{C\text{-}F}$ = 18.7 Hz), 116.36 (d, $J_{C\text{-}F}$ = 21.9 Hz), 109.14, 107.42, 103.12, 80.78, 70.57, 70.55, 70.32, 70.28, 69.74, 68.83, 66.79, 56.00, 36.08, 28.02 (3C). HRMS (ESI): m/z calcd for $(C_{28}H_{35}ClFN_3O_7 + H)^+$: 580.2220; found: 580.2221.



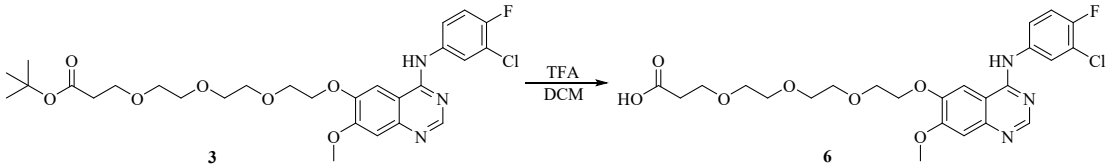

**tert-Butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)((1-methyl-2-nitro-1H-imidazol-5-yl)methyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (4)**

tert-Butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (350 mg, 0.60 mmol) and 5-(chloromethyl)-1-methyl-2-nitro-1H-imidazole (316 mg, 1.8 mmol) were added to a stirred mixture of $Cs_2CO_3$ (315 mg, 0.63 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred at r.t. for about 3 h. After the reaction was completed, the reaction mixture was added EtOAc (40 mL) and washed with saturated salt water (6x20 mL), the organic solvent was dried over sodium sulfate and concentrated to obtain crude product, which was further purified by column chromatography (DCM : EtOAc =2 : 1) to get pure material as light yellow gum (364 mg, 84.3% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.24 – 7.15 (m, 3H), 7.01 – 6.93 (m, 2H), 6.43 (s, 1H), 5.32 (s, 2H), 4.11 (s, 3H), 3.96 (s, 3H), 3.76 – 3.55 (m, 14H), 2.49 (t, $J$ = 6.5 Hz, 2H), 1.44 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.92, 159.03, 156.43 (d, $J_{C\text{-}F}$ = 251.2 Hz), 154.97, 152.47, 149.79, 147.95, 145.73, 143.19 (d, $J_{C\text{-}F}$ = 3.7 Hz), 134.27, 128.91, 128.35, 126.14 (d, $J_{C\text{-}F}$ = 7.1 Hz), 122.72 (d, $J_{C\text{-}F}$ = 19.0 Hz), 118.03 (d, $J_{C\text{-}F}$ = 22.1 Hz), 110.73, 107.63, 104.79, 80.55, 70.83, 70.56, 70.49, 70.35, 68.96, 68.00, 66.88, 56.15, 46.70, 36.23, 34.52, 28.08 (3C). HRMS (ESI): m/z calcd for $(C_{33}H_{40}ClFN_6O_9 + H)^+$: 719.2602; found: 719.2624.




**tert-Butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (5)**

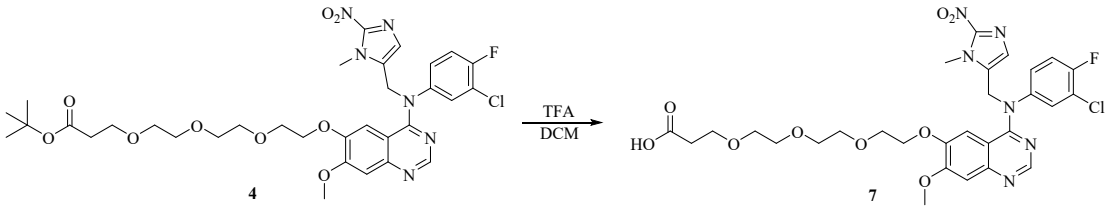
tert-Butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (**3**, 350 mg, 0.60 mmol) and 1-(bromomethyl)-4-nitrobenzene (391 mg, 1.8 mmol) were added to a stirred mixture of $Cs_2CO_3$ (315 mg, 0.63 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred at r.t. for about 3 h. After the reaction was completed, the reaction mixture was added EtOAc (40 mL) and washed with saturated salt water (6x20 mL), the organic solvent was dried over sodium sulfate and concentrated to obtain crude product, which was further purified by column chromatography (DCM : EtOAc =2 : 1) to get pure material as light yellow oil (271mg, 63.2% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.18 – 8.13 (m, 2H), 7.59 – 7.52 (m, 2H), 7.22 (s, 1H), 7.20 – 7.16 (m, 1H), 7.14 – 7.08 (m, 1H), 6.98 – 6.92 (m, 1H), 6.49 (s, 1H), 5.45 (s, 2H), 3.97 (s, 3H), 3.76 – 3.55 (m, 14H), 2.52-.48 (m, 2H), 1.44 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.90, 162.53, 159.37, 155.77 (d, $J_{C-F}$ = 250.1 Hz), 154.88, 153.00, 149.80, 147.76, 147.28, 145.42, 128.74 (2C), 127.34, 125.11 (d, $J_{C-F}$ = 7.1 Hz), 123.79 (2C), 122.33 (d, $J_{C-F}$ = 18.7 Hz), 117.70 (d, $J_{C-F}$ = 22.1 Hz), 110.84, 107.63, 105.12, 80.54, 70.84, 70.57, 70.51, 70.36, 68.96, 67.98, 66.89, 56.14 (2C), 36.49, 36.23, 28.08 (3C). HRMS (ESI): m/z calcd for $(C_{35}H_{40}ClFN_4O_9 + H)^+$: 715.2541; found: 715.2558.




**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (6)**

TFA (1 mL) was added dropwise to a stirred solution of tert-butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (**3**, 260 mg) in DCM (5 mL). The reaction mixture was stirred at r.t. for about 1 h. After the reaction was completed,

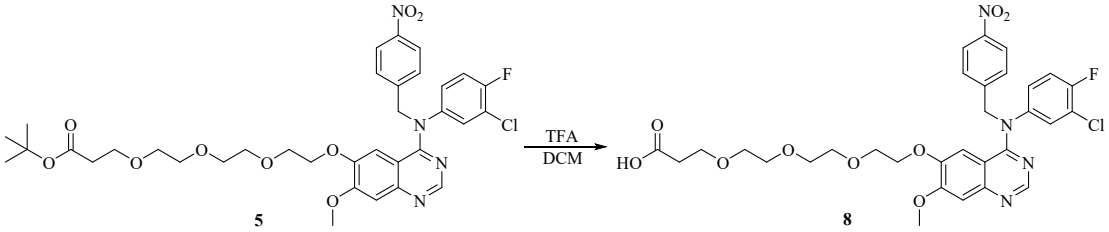
the reaction mixture was concentrated and the residue was purified by column chromatography (DCM : MeOH = 100 : 4) to get the pure material as yellow oil with 100% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.60 (s, 1H), 8.59 (s, 1H), 8.17 – 7.97 (m, 1H), 7.81 – 7.49 (m, 2H), 7.22 – 7.11 (m, 1H), 7.05 (s, 1H), 4.30 – 4.12 (m, 2H), 4.03 – 3.90 (m, 2H), 3.88 – 3.58 (m, 13H), 2.69 – 2.57 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 176.28, 157.14, 156.50, 155.80 (d, $J_{C\text{-}F}$ = 248.6 Hz), 149.65, 148.74, 135.60, 133.62 (d, $J_{C\text{-}F}$ = 3.3 Hz), 125.92, 123.26 (d, $J_{C\text{-}F}$ = 7.2 Hz), 120.72 (d, $J_{C\text{-}F}$ = 18.6 Hz), 116.33 (d, $J_{C\text{-}F}$ = 22.1 Hz), 106.75, 102.83, 100.02, 70.61, 70.50, 70.44, 70.21, 69.18, 69.03, 66.84, 56.43, 35.54. HRMS (ESI): m/z calcd for $(C_{24}H_{27}ClFN_3O_7 + H)^+$: 524.1594; found: 524.1610.




4 7

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)((1-methyl-2-nitro-1H-imidazol-5-yl)methyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (7)**

Compound **7** was prepared in the same way as compound **6**, but the start material was changed as tert-butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)((1-methyl-2-nitro-1H-imidazol-5-yl)methyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (**4**). Yellow solid, 100% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.68 (s, 1H), 7.46 (dd, $J$ = 6.1, 2.6 Hz, 1H), 7.38 (t, $J$ = 8.5 Hz, 1H), 7.22 – 7.15 (m, 1H), 7.02 (s, 1H), 6.38 (s, 1H), 5.52 (s, 2H), 4.06 (s, 3H), 4.03 (s, 3H), 3.76 – 3.55 (m, 14H), 2.53 (t, $J$ = 5.9 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 173.58, 159.76, 157.98 (d, $J_{C\text{-}F}$ = 255.2 Hz), 157.38, 149.14, 147.83, 145.93, 140.25, 139.85 (d, $J_{C\text{-}F}$ = 4.0 Hz), 131.99, 129.71, 129.26, 127.70 (d, $J_{C\text{-}F}$ = 7.3 Hz), 123.69 (d, $J_{C\text{-}F}$ = 19.1 Hz), 118.91 (d, $J_{C\text{-}F}$ = 22.1 Hz), 108.02, 105.95, 101.81, 70.81, 70.50, 70.23, 70.04, 68.98, 68.32, 66.44, 57.18, 47.65, 34.73, 34.57. HRMS (ESI): m/z calcd for $(C_{29}H_{32}ClFN_6O_9 + H)^+$: 663.1976; found: 663.1986.

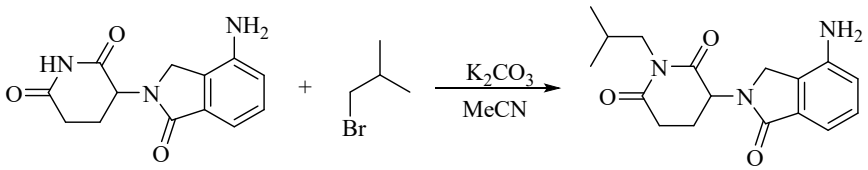



5 8

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (8)**

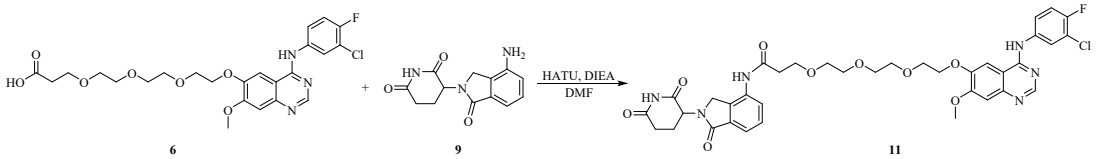
Compound **8** was prepared in the same way as compound **6**, but the start material was changed as tert-butyl 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoate (**5**). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.16 (d, *J* = 8.6 Hz, 2H), 7.68 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.43 – 7.37 (m, 1H), 7.36 – 7.28 (m, 1H), 7.16 – 7.09 (m, 1H), 6.30 (s, 2H), 5.57 (s, 2H), 4.00 (s, 3H), 3.79 – 3.48 (m, 14H), 2.59 – 2.44 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ174.00, 160.01, 157.79 (d, $J_{C-F}$ = 254.6 Hz), 157.37, 148.96, 147.81, 147.56, 142.26, 140.03 (d, $J_{C-F}$ = 4.0 Hz), 139.34, 129.59, 129.45 (2C), 127.50 (d, $J_{C-F}$ = 7.3 Hz), 124.06 (2C), 123.51 (d, $J_{C-F}$ = 19.3 Hz), 118.72 (d, $J_{C-F}$ = 22.2 Hz), 107.47, 106.00, 101.35, 70.85, 70.51, 70.26, 70.13, 68.89, 68.20, 66.47, 57.51, 57.21, 34.79. HRMS (ESI): m/z calcd for ($C_{31}H_{32}ClFN_4O_9$+ H)$^+$: 659.1915; found: 659.1928.

**3-(4-amino-1-oxoisoindolin-2-yl)-1-isobutylpiperidine-2,6-dione (10)**




**9** **10**

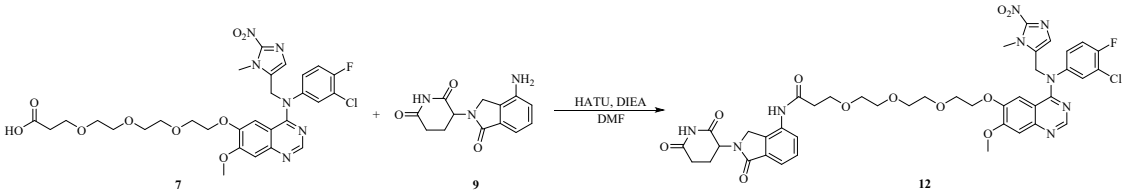
To a stirred solution of 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**9**, 1.0g, 3.86 mmol) in MeCN (50 mL) were added $K_2CO_3$ (0.53 g, 3.86 mmol) and 1-bromo-2-methylpropane (0.63 g, 4.63 mmol). Then the reaction mixture was stirred at reflux temperature for 5 h. When the reaction was no longer further continued, the reaction mixture was concentrated and purified by column chromatography (DCM: EtOAc=2:1) to obtain the target compound as a white solid (0.18g, 14.8% yield). $^1$H NMR (400 MHz, DMSO-d6) δ 7.16 (t, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 7.3 Hz, 1H), 6.77 (d, *J* = 7.9 Hz, 1H), 5.40 (s, 2H), 5.16 (dd, *J* = 13.4, 5.0 Hz, 1H), 4.19 (d, *J* = 16.8 Hz, 1H), 4.04 (d, *J* = 16.9 Hz, 1H), 3.56 – 3.40 (m, 2H), 3.10 – 2.92 (m, 1H), 2.84 – 2.69 (m, 1H), 2.27 (qd, *J* = 13.3, 4.4 Hz, 1H), 2.08 – 1.98 (m, 1H), 1.93 – 1.77 (m, 1H), 0.80 (s, 3H), 0.79 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-d6) δ 172.04, 170.95, 168.95, 143.66, 132.29, 128.89, 125.60, 116.47, 110.47, 52.24, 46.26, 45.66, 31.51, 26.71, 22.11, 20.10, 20.07. HRMS (ESI): m/z calcd for ($C_{17}H_{21}N_3O_3$+ H)$^+$: 316.1656; found: 316.1659.




**6** **9** **11**

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (11)**

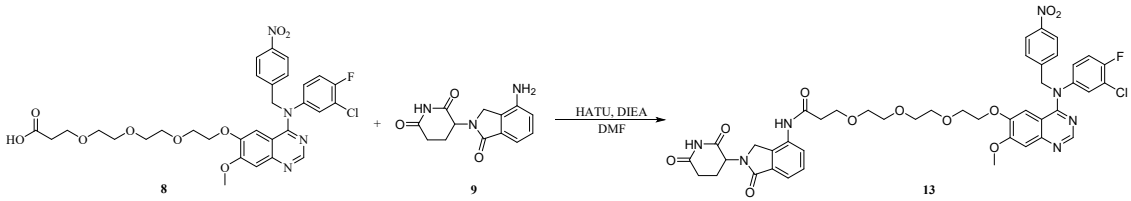
2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 171 mg, 0.45 mmol) and N,N-diisopropylethylamine (DIEA, 116 mg, 0.90 mmol) were added to a stirred solution of 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (**6**, 157 mg, 0.30 mmol) and 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**9**, 82 mg, 0.32 mmol) in anhydrous DMF (3 mL). The reaction mixture was stirred at r.t. for about 5 h. After the reaction was completed, the reaction mixture was added EtOAc (20 mL) and washed with saturated salt water (6x10 mL), the organic solvent was dried over sodium sulfate and concentrated, the obtained crude product was further purified by column chromatography (DCM : MeOH =100 : 3) to get pure **11** as white solid (99mg, 43.1% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 10.26 (brs, 1H), 9.03 (s, 1H), 8.92 (s, 1H), 8.54 (s, 1H), 8.04 – 7.96 (m, 1H), 7.86 (d, $J$ = 7.9 Hz, 1H), 7.75 – 7.66 (m, 1H), 7.59 (d, $J$ = 7.5 Hz, 1H), 7.38 (t, $J$ = 7.8 Hz, 1H), 7.33 (s, 1H), 7.17 (s, 1H), 7.09 (t, $J$ = 8.8 Hz, 1H), 4.94 (dd, $J$ = 13.1, 5.0 Hz, 1H), 4.45 (dd, $J$ = 34.2, 16.7 Hz, 2H), 3.93 – 3.75 (m, 7H), 3.73 – 3.32 (m, 10H), 2.73 – 2.47 (m, 4H), 2.34 – 2.01 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 171.88, 170.64, 170.13, 169.51, 156.47, 154.69, 154.20 (d, $J_{C\text{-}F}$ = 245.2 Hz), 153.17, 148.17, 147.06, 136.22 (d, $J_{C\text{-}F}$ = 3.0 Hz), 134.13, 133.10, 132.31, 129.24, 126.39, 123.76, 121.65 (d, $J_{C\text{-}F}$ = 6.5 Hz), 120.43 (d, $J_{C\text{-}F}$ = 18.3 Hz), 120.42, 116.30 (d, $J_{C\text{-}F}$ = 21.9 Hz), 109.07, 107.22, 102.33, 70.29, 70.10, 69.69, 69.65, 69.32, 67.99, 66.52, 56.10, 52.53, 46.82, 37.05, 31.48, 23.06. HRMS (ESI): m/z calcd for $(C_{37}H_{38}ClFN_6O_9 + H)^+$: 765.2446; found: 765.2463.




7 9 12

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)((1-methyl-2-nitro-1H-imidazol-5-yl)methyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (12)**

Compound **12** was prepared in the same way as compound **11**, but the start material **6** was changed as 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)((1-methyl-2-nitro-1H-imidazol-5-yl)methyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (**7**). Yellow solid, 26.3% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.77 (s, 1H), 8.75 (s, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 7.4 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.24 (s, 1H), 7.21 – 7.09 (m, 2H), 7.01 – 6.89 (m, 2H), 6.34 (s, 1H), 5.31 (s, 2H), 5.15 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.40 (s, 2H), 4.09 (s, 3H), 3.92 (s, 3H), 3.82 (t, *J* = 5.5 Hz, 2H), 3.73 – 3.49 (m, 12H), 2.92 – 2.08 (m, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 171.45, 170.16, 169.79, 169.02, 159.07, 156.38 (d, $J_{C-F}$ = 251.3 Hz), 154.79, 152.59, 149.75, 147.66, 145.76, 143.21, 134.36, 134.07, 133.04, 132.55, 128.90 (d, $J_{C-F}$ = 6.0 Hz), 128.37, 126.28 (d, $J_{C-F}$ = 7.0 Hz), 126.02, 122.56 (d, $J_{C-F}$ = 18.9 Hz), 120.67, 117.99 (d, $J_{C-F}$ = 22.2 Hz), 115.11, 110.72, 107.69, 104.86, 70.58, 70.32, 70.14, 70.09, 68.86, 67.80, 66.79, 56.17, 51.89, 46.64, 46.52, 37.27, 34.54, 31.53, 23.33. HRMS (ESI): m/z calcd for $(C_{42}H_{43}ClFN_9O_{11} + H)^+$: 904.2827; found: 904.2831.

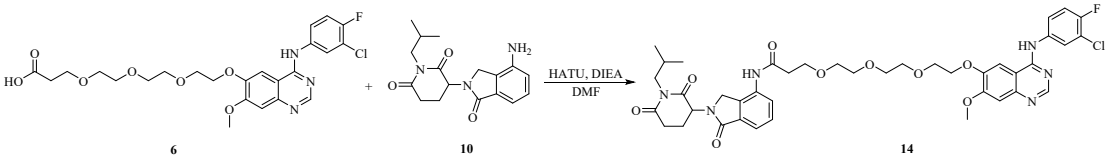



8 9 13

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (13)**

Compound **13** was prepared in the same way as compound **11**, but the start material **6** was changed as 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (**8**). Brown solid, 26.7% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.76 (s, 1H), 8.70 (s, 1H), 8.16 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.67 (d, *J* = 7.5 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.26 (s, 1H), 7.20 (dd, *J* = 6.4, 2.7 Hz, 1H), 7.10 (t, *J* = 8.6 Hz, 1H), 7.00 – 6.93 (m, 1H), 6.41 (s, 1H), 5.47 (s, 2H), 5.18 (dd, *J* = 13.2, 5.2 Hz, 1H), 4.42 (s, 2H), 3.94 (s, 3H), 3.84 (t, *J* = 5.5 Hz, 2H), 3.77 – 3.47 (m, 12H), 2.91 – 2.13 (m, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 171.33, 170.14, 169.68, 169.03, 159.38, 155.75 (d, $J_{C-F}$ = 249.8 Hz), 154.68, 153.09, 149.74, 147.45, 147.28, 145.36, 143.67 (d, $J_{C-F}$ = 3.6 Hz), 134.02, 133.00, 132.57, 128.98, 128.82 (2C), 127.46, 125.97, 125.32 (d, $J_{C-F}$ = 6.9 Hz), 123.79 (2C), 122.23 (d, $J_{C-F}$ = 18.7 Hz), 120.77, 117.68 (d, $J_{C-F}$ = 22.1 Hz), 110.74, 107.70, 105.13, 70.62, 70.31, 70.16, 70.11, 68.88,

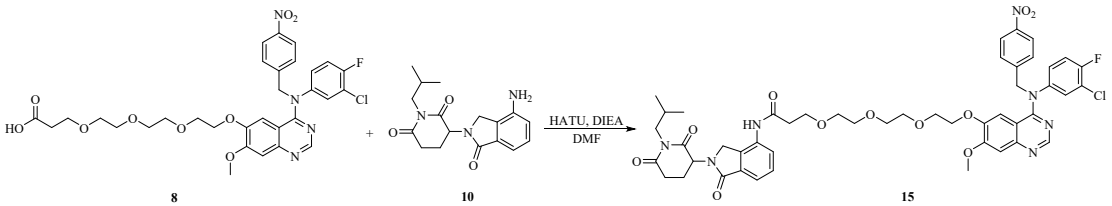
67.78, 66.98, 56.14, 53.46, 51.87, 46.48, 37.30, 31.54, 23.36. HRMS (ESI): m/z calcd for $(C_{44}H_{43}ClFN_7O_{11}+H)^+$: 900.2766; found: 900.2790.




6 10 14

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)-N-(2-(1-isobutyl-2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (14)**

Compound **14** was prepared in the same way as compound **11**, but the start material **9** was changed as 3-(4-amino-1-oxoisoindolin-2-yl)-1-isobutylpiperidine-2,6-dione (**10**). White solid, 35.9% yield. $^1$H NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 9.51 (s, 1H), 8.51 (s, 1H), 8.12 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.90 – 7.70 (m, 3H), 7.57 – 7.36 (m, 3H), 7.21 (s, 1H), 5.23 (dd, *J* = 13.4, 5.0 Hz, 1H), 4.49 – 4.16 (m, 4H), 3.94 (s, 3H), 3.89 – 3.78 (m, 2H), 3.71 (t, *J* = 6.2 Hz, 2H), 3.65 – 3.41 (m, 10H), 3.14 – 2.96 (m, 1H), 2.86 – 2.70 (m, 1H), 2.61 (t, *J* = 6.2 Hz, 2H), 2.33 (qd, *J* = 13.2, 4.3 Hz, 1H), 2.14 – 2.02 (m, 1H), 1.94 – 1.78 (m, 1H), 0.85 – 0.75 (m, 6H). $^{13}$C NMR (100 MHz, DMSO) δ 171.93, 170.75, 169.38, 167.84, 156.01, 154.42, 153.13 (d, $J_{C-F}$ = 242.7 Hz), 152.70, 148.10, 147.04, 136.81, 133.66, 133.59, 132.69, 128.66, 125.20, 123.37, 122.19 (d, $J_{C-F}$ = 6.9 Hz), 119.09, 118.79 (d, $J_{C-F}$ = 18.4 Hz), 116.53 (d, $J_{C-F}$ = 21.7 Hz), 108.72, 107.37, 102.56, 70.00, 69.82, 69.73, 69.66, 68.64, 68.27, 66.59, 55.84, 52.25, 46.56, 46.30, 36.60, 31.47, 26.69, 21.98, 20.07, 20.04. HRMS (ESI): m/z calcd for $(C_{41}H_{46}ClFN_6O_9+H)^+$: 822.3079; found: 821.3051.

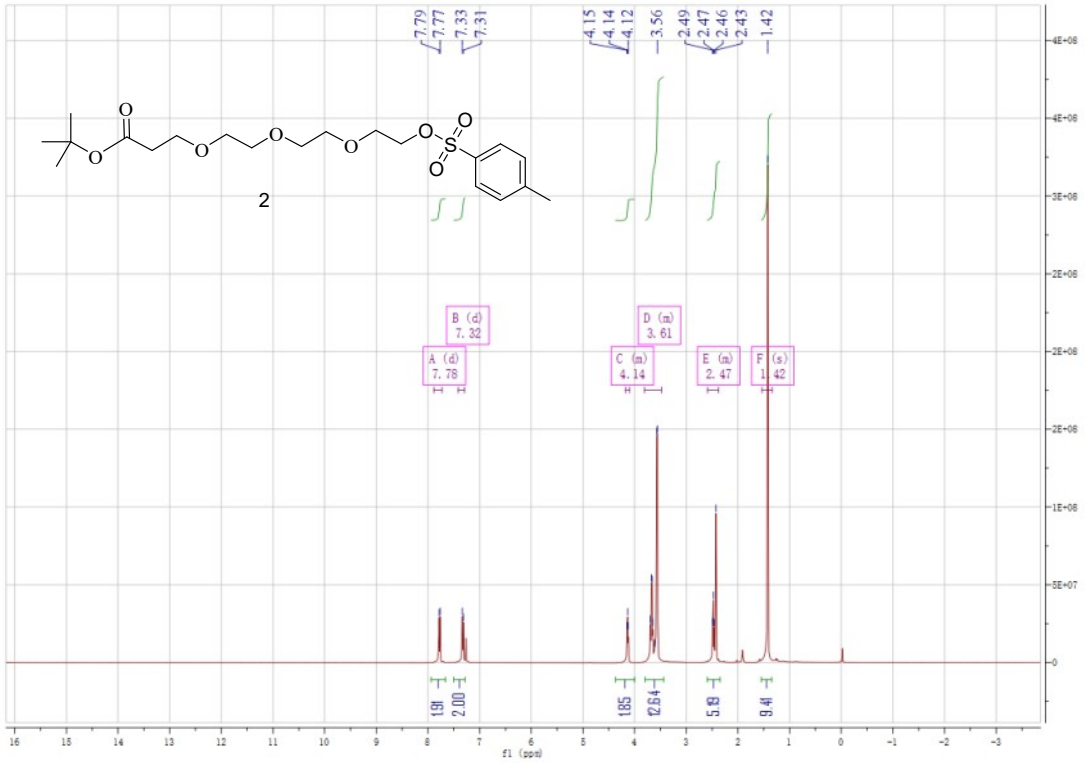


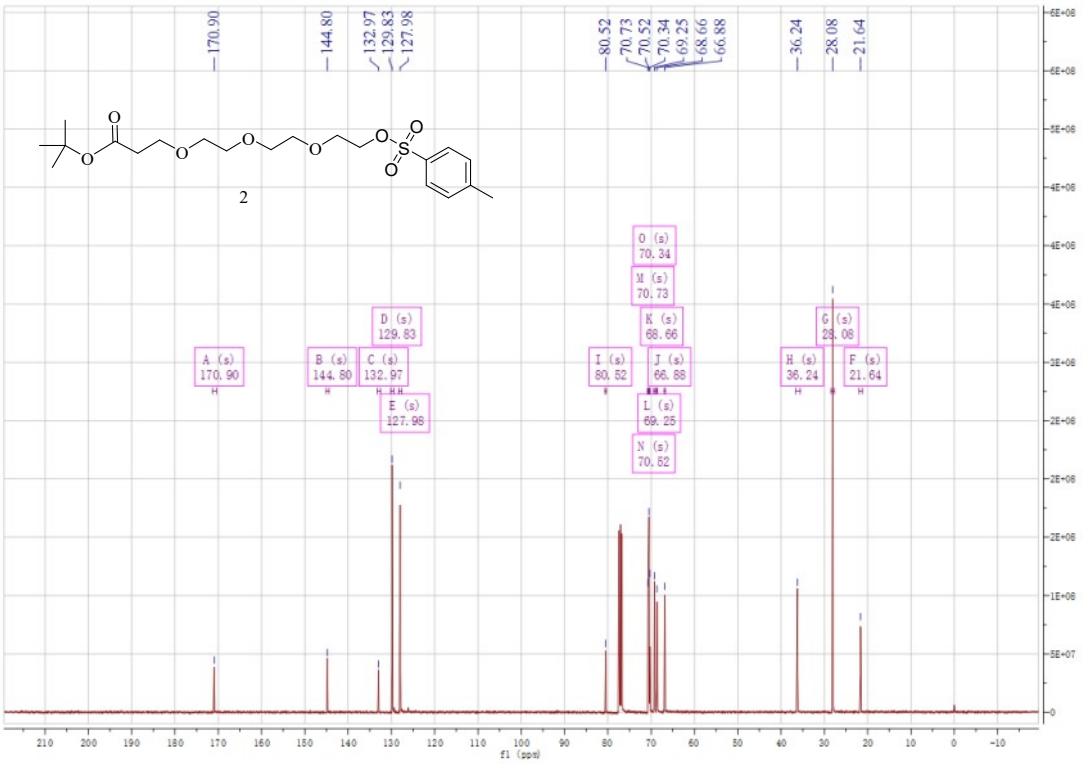

8 10 15

**3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)-N-(2-(1-isobutyl-2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (15)**

Compound **15** was prepared in the same way as compound **14**, but the start material **6** was changed as 3-(2-(2-(2-((4-((3-chloro-4-fluorophenyl)(4-nitrobenzyl)amino)-7-methoxyquinazolin-6-yl)oxy)ethoxy)ethoxy)ethoxy)propanoic acid (**8**). White solid, 73.9% yield. $^1$H NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 8.66 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 7.3 Hz, 1H), 7.75 – 7.56 (m,

3H), 7.55 – 7.43 (m, 2H), 7.39 (t, $J$ = 8.9 Hz, 1H), 7.28 – 7.14 (m, 2H), 6.45 (s, 1H), 5.52 (s, 2H), 5.24 (dd, $J$ = 13.2, 4.0 Hz, 1H), 4.35 (dd, $J$ = 44.7, 17.5 Hz, 2H), 3.91 (s, 3H), 3.71 (t, $J$ = 6.0 Hz, 2H), 3.65 – 3.56 (m, 2H), 3.56 – 3.41 (m, 12H), 3.15 – 2.95 (m, 1H), 2.86 – 2.70 (m, 1H), 2.66 – 2.55 (m, 2H), 2.34 (qd, $J$ = 13.0, 3.7 Hz, 1H), 2.16 – 1.99 (m, 1H), 1.97 – 1.78 (m, 1H), 0.91 – 0.67 (m, 6H). $^{13}$C NMR (100 MHz, DMSO) δ = 172.40, 171.21, 169.83, 168.30, 159.69, 155.22 (d, $J_{C\text{-}F}$=246.5 Hz), 154.68, 153.10, 149.64, 147.24, 147.02, 146.56, 143.74 (d, $J_{C\text{-}F}$=3.3 Hz), 134.13, 134.05, 133.15, 129.37 (2C), 129.10, 127.85, 126.62 (d, $J_{C\text{-}F}$=7.1 Hz), 125.65, 123.87 (2C), 120.92 (d, $J_{C\text{-}F}$=18.3 Hz), 119.54, 118.19 (d, $J_{C\text{-}F}$=22.1 Hz), 110.56, 107.88, 105.56, 70.35, 70.18, 70.16, 70.10, 68.60, 67.72, 67.06, 56.36, 55.58, 52.71, 47.02, 46.76, 37.08, 31.94, 27.16, 22.45, 20.52, 20.50. HRMS (ESI): m/z calcd for $(C_{48}H_{51}ClFN_7O_{11}+H)^+$: 956.3392; found: 956.3395.

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28.08
21.64
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O
O
O
O
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O
O
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C (s) 132.97
D (s) 129.83
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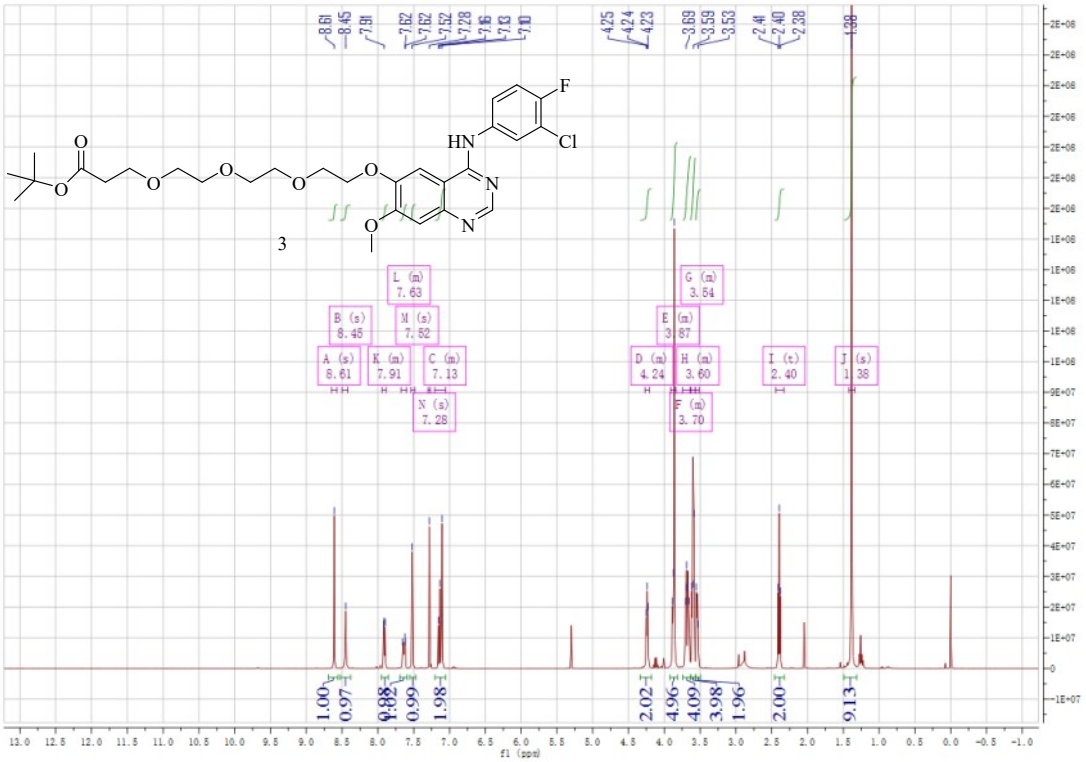
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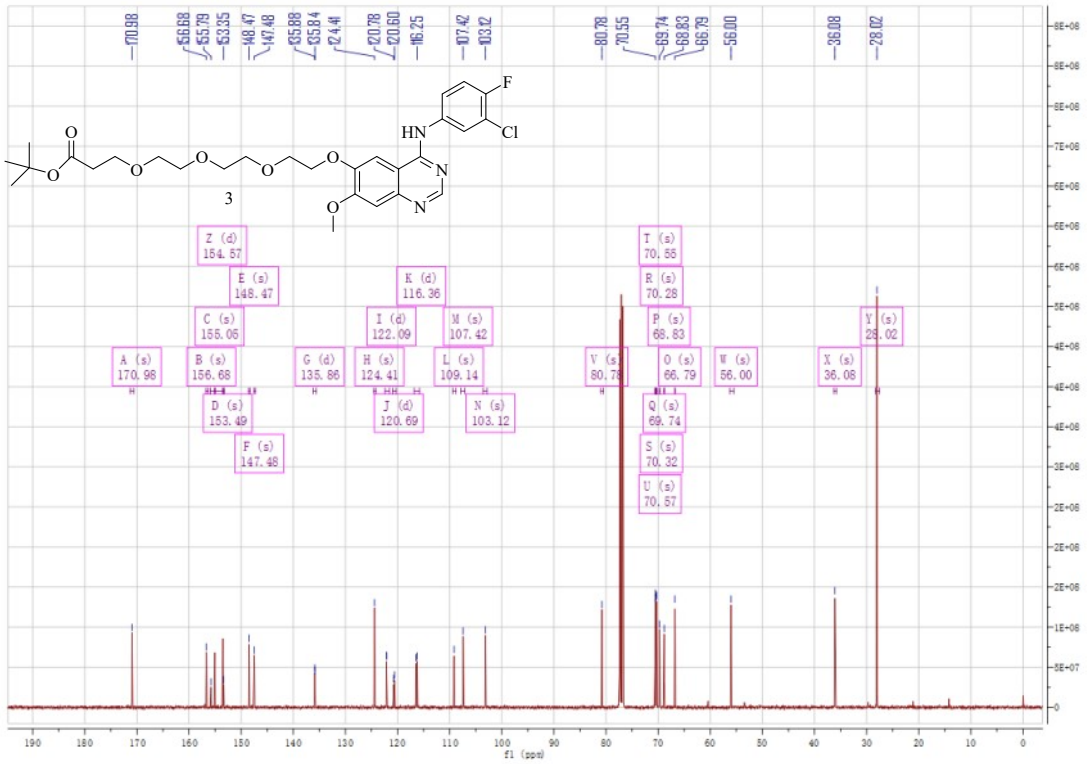
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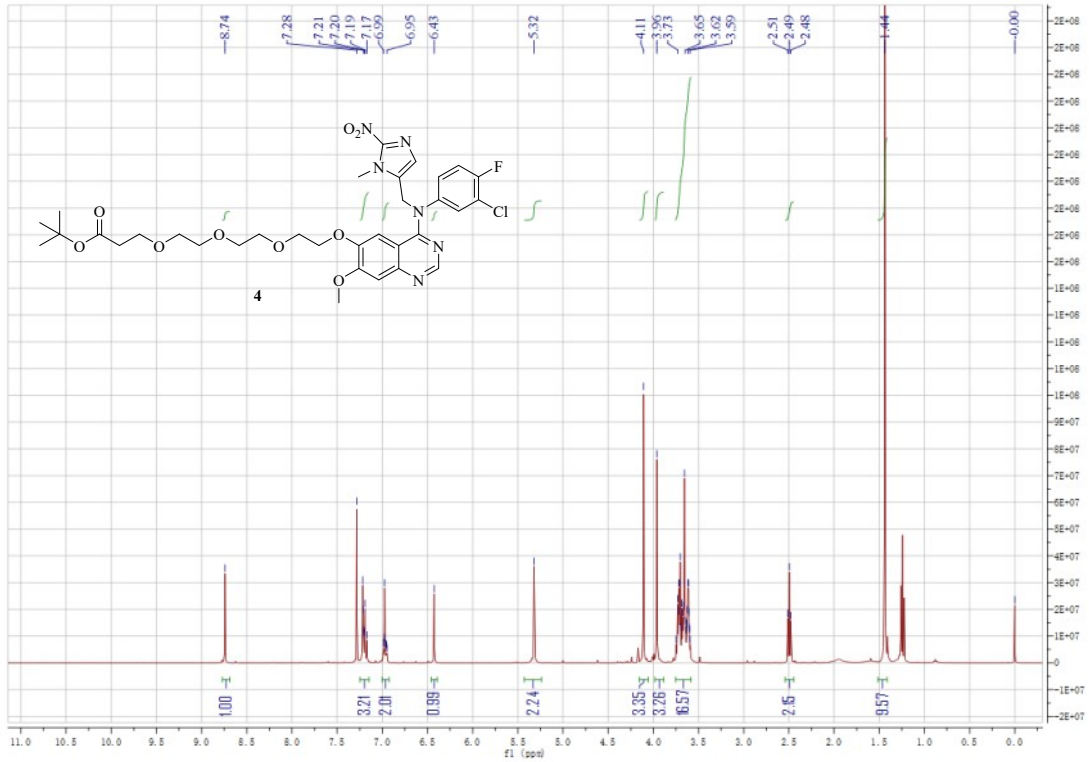
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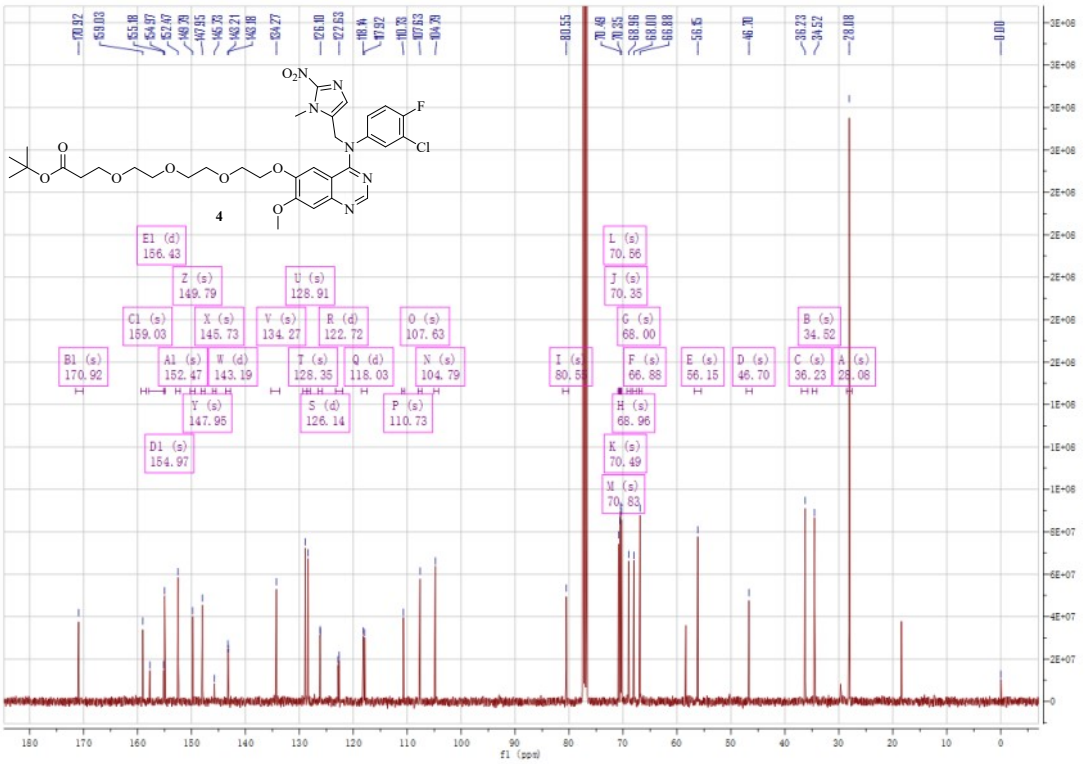
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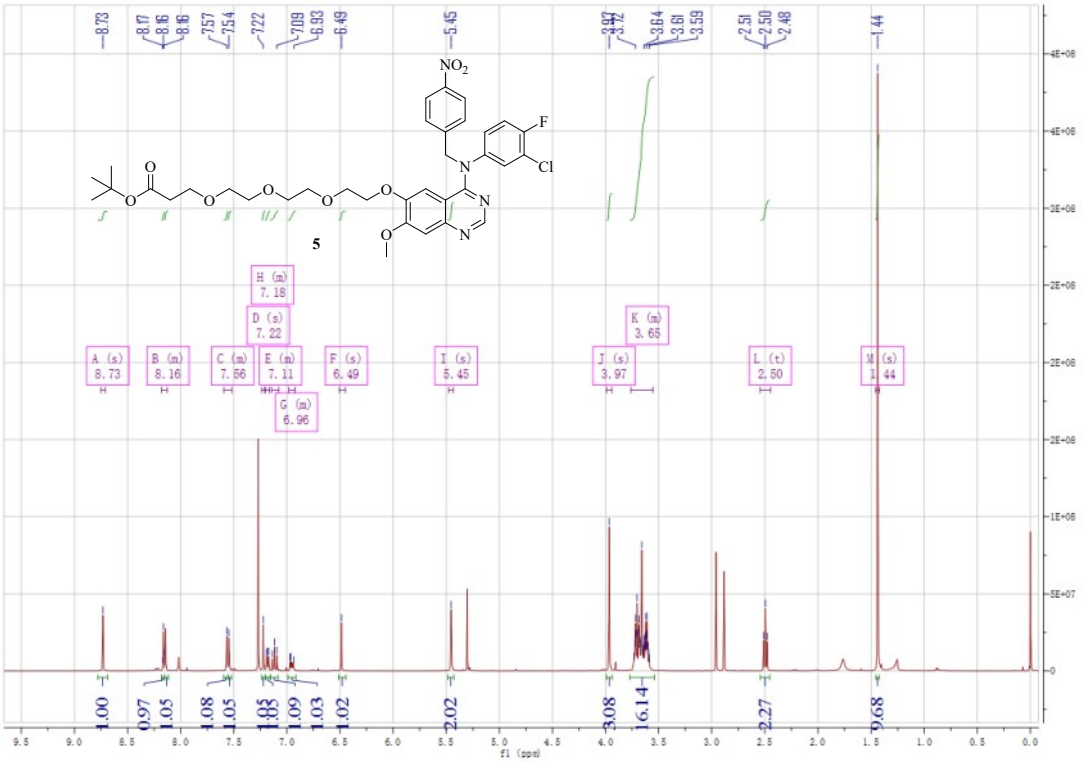
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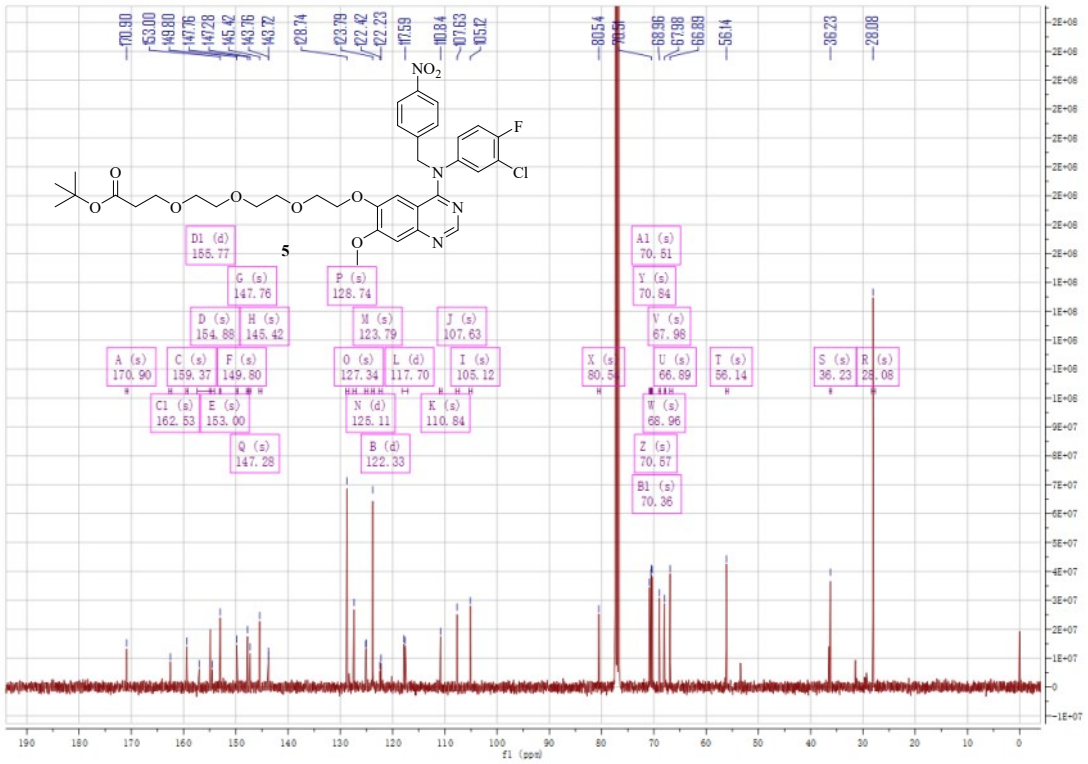
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N
O
N
O
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P (s) 128.74
M (s) 123.79
O (s) 127.34
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N (d) 125.11
B (d) 122.33
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X (s) 80.54
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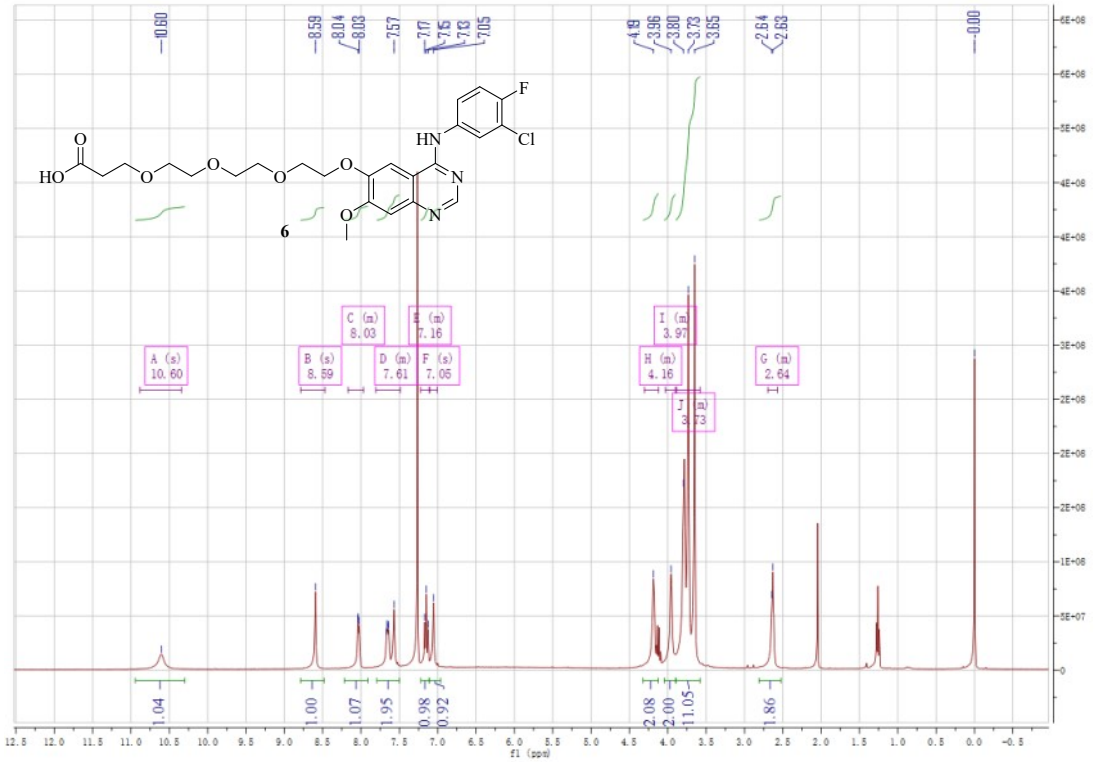
f1 (ppm)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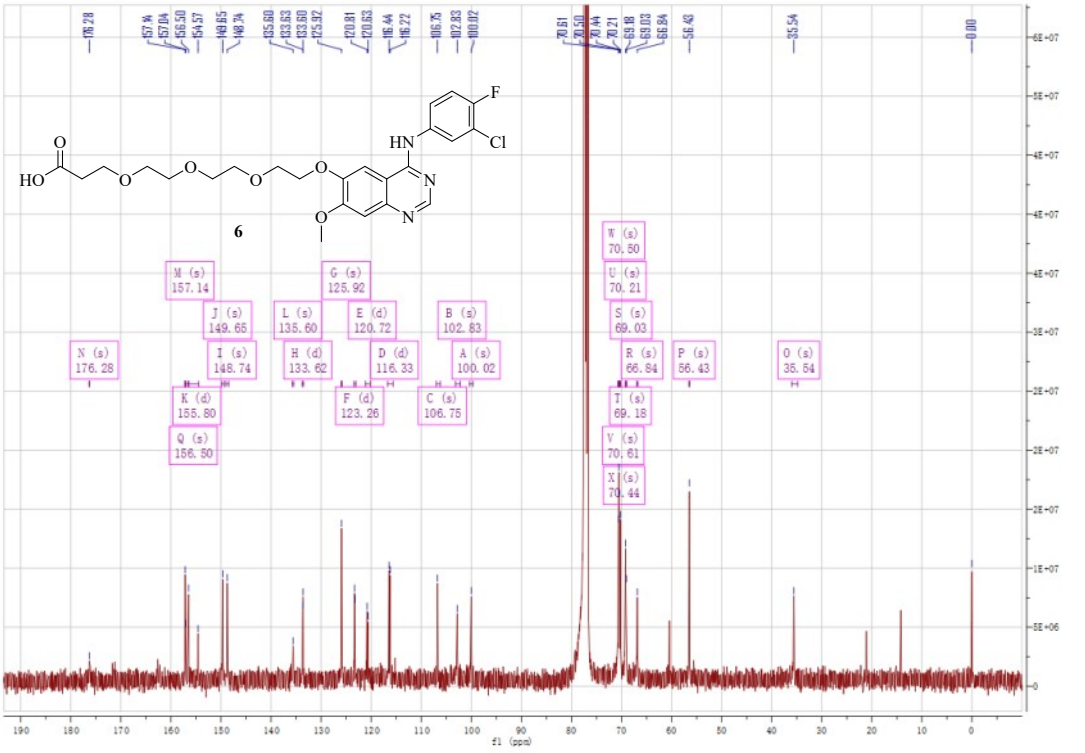
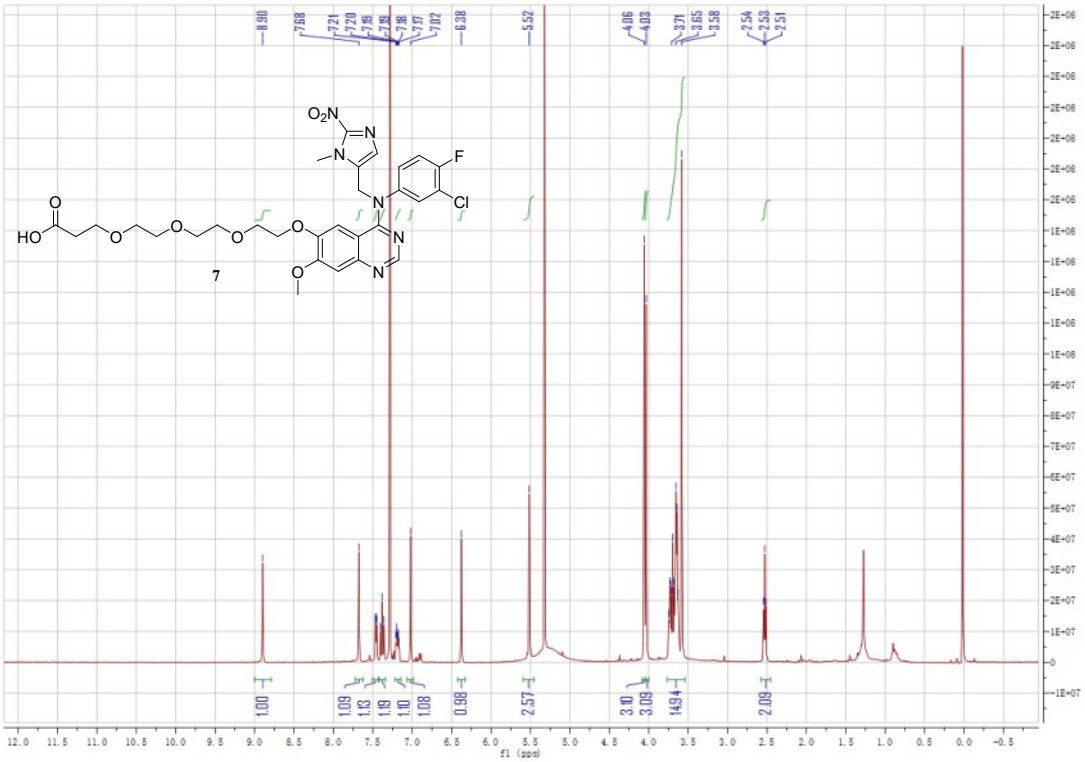
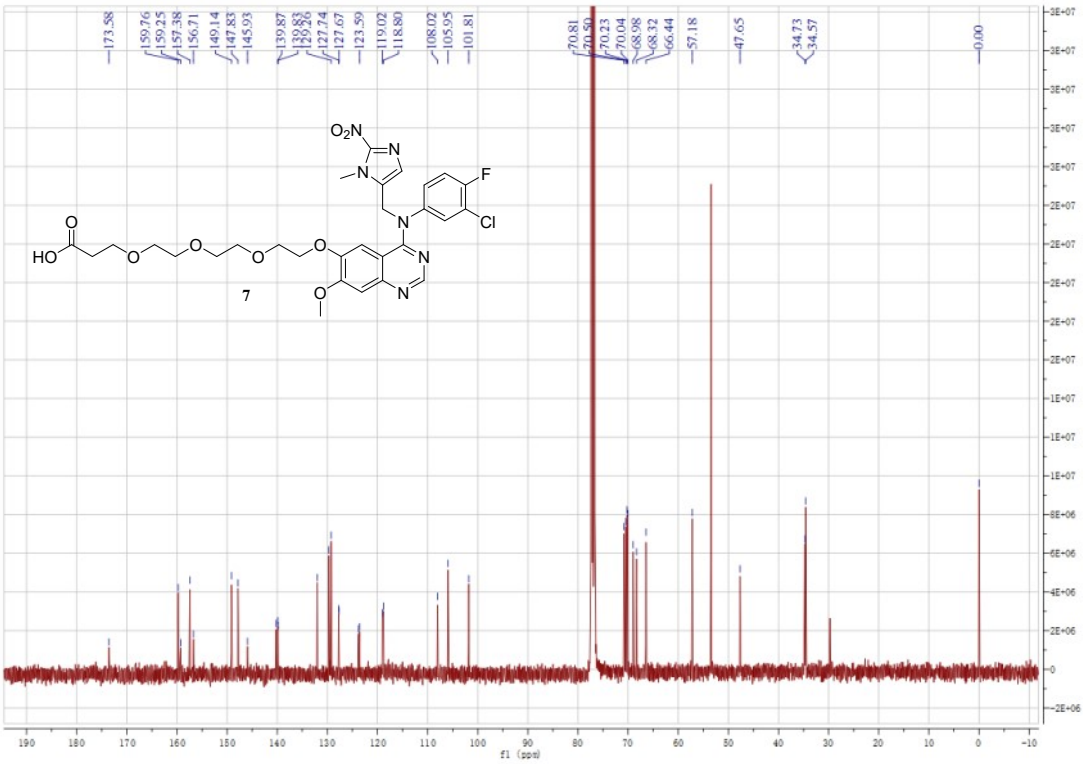
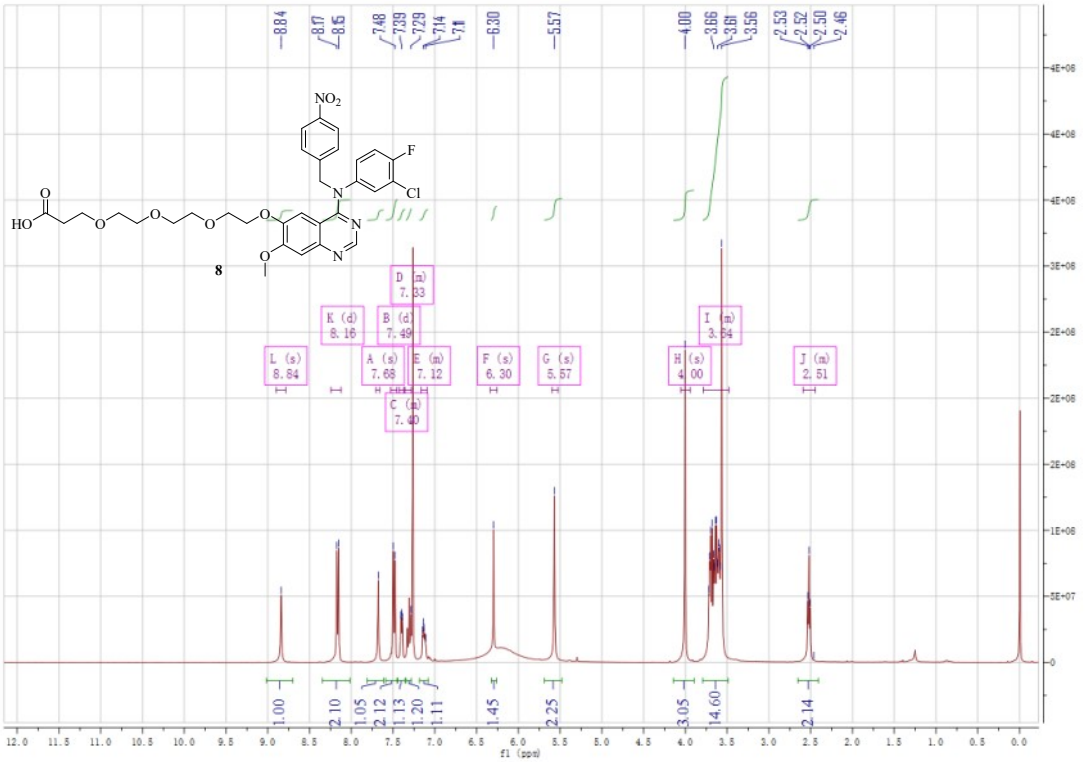
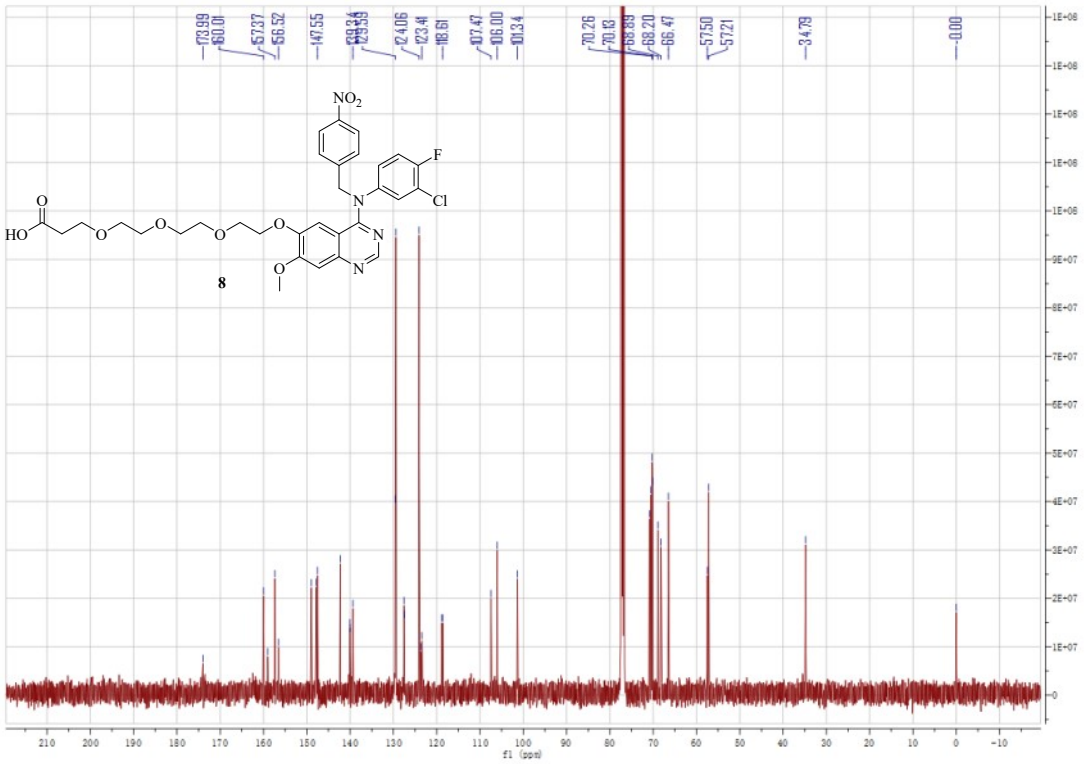
F (s) 7.05
H (m) 4.16
I (m) 3.97
J (m) 3.73
G (m) 2.64
1.04
1.00
1.07
1.95
0.98
0.92
2.08
2.00
11.05
1.86
f1 (ppm)

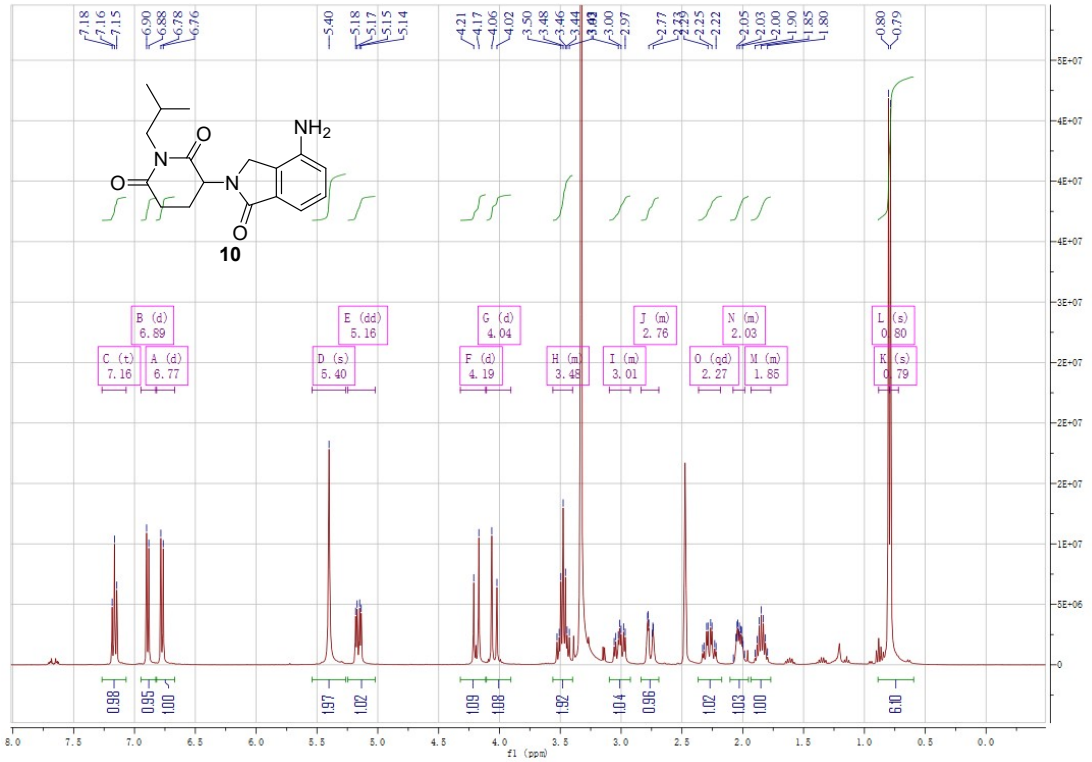

6
N (s) 176.28
M (s) 157.14
J (s) 149.65
I (s) 148.74
K (d) 155.80
Q (s) 156.50
L (s) 135.60
H (d) 133.62
G (s) 125.92
E (d) 120.72
D (d) 116.33
F (d) 123.26
B (s) 102.83
A (s) 100.02
C (s) 106.75
W (s) 70.50
U (s) 70.21
S (s) 69.03
R (s) 66.84
T (s) 69.18
V (s) 70.61
X (s) 70.44
P (s) 56.43
O (s) 35.54
f1 (ppm)

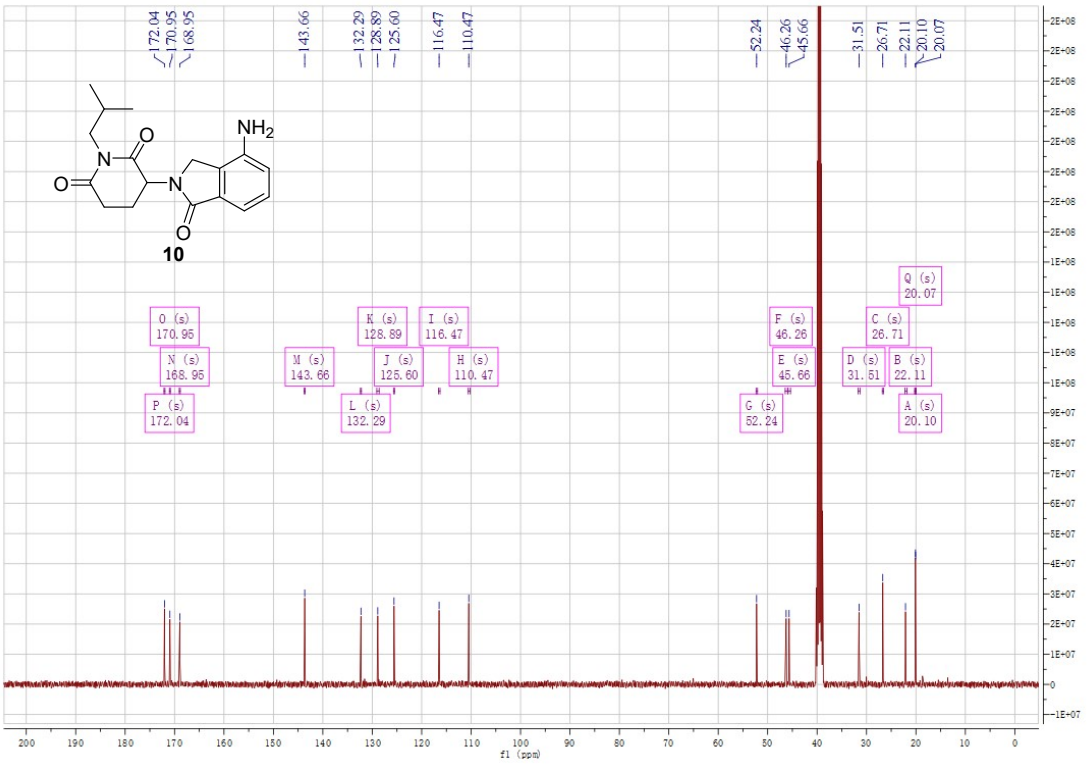

7
8.90
7.88
7.72
7.71
7.20
7.19
7.18
7.17
7.02
6.38
5.52
4.06
4.03
3.71
3.65
3.58
2.54
2.53
2.51
1.00
1.09
1.63
1.19
1.10
1.08
0.98
2.57
3.10
3.09
14.94
2.09
f1 (ppm)

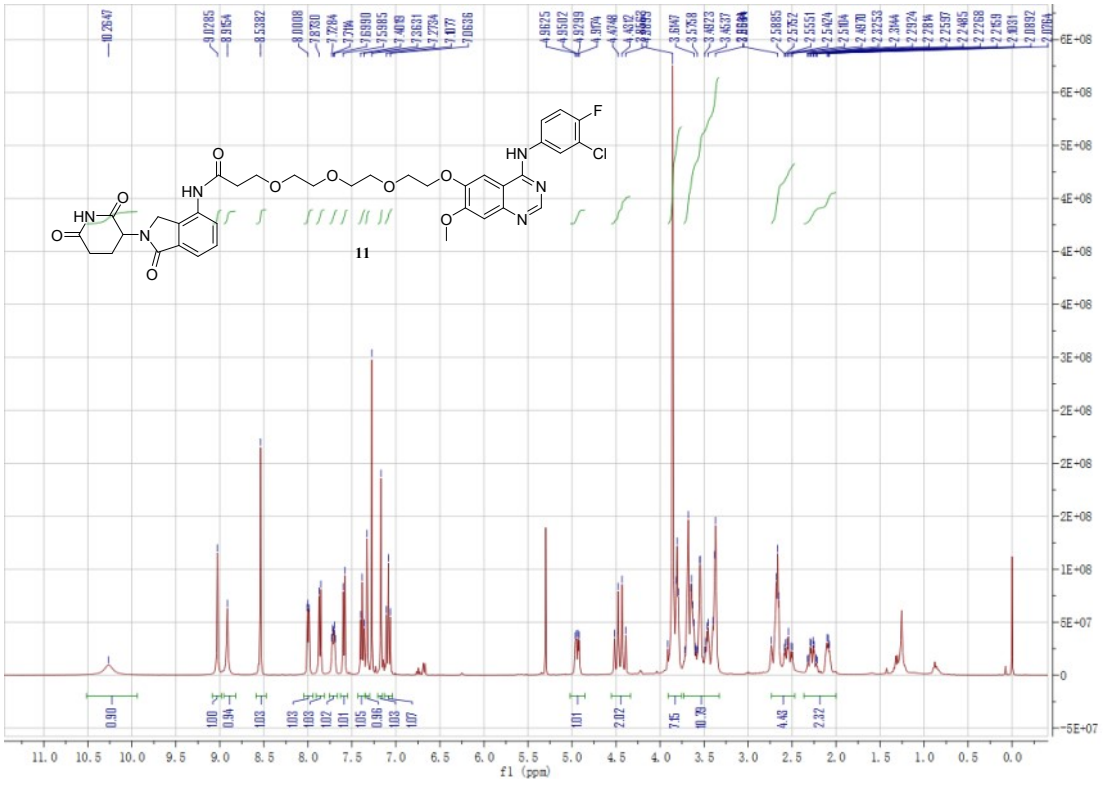

O2N
N
N
F
Cl
N
HO
O
O
O
O
O
O
N
N
O
7
f1 (ppm)

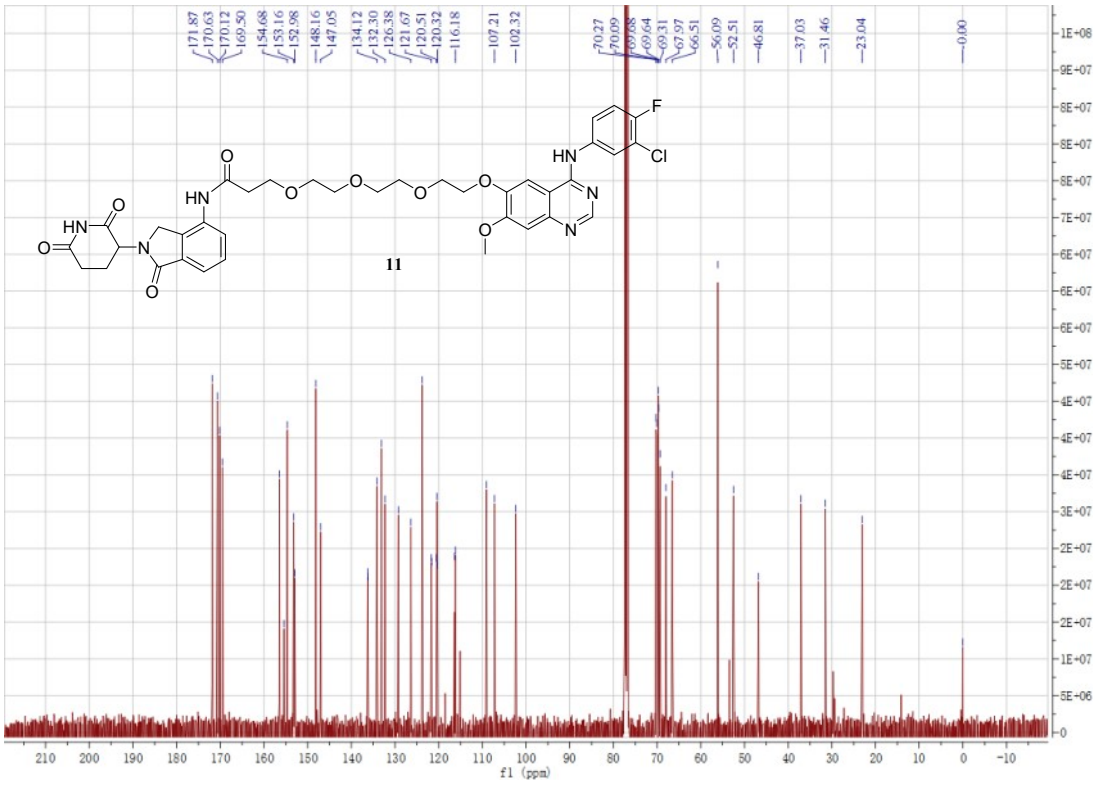

NO2
F
Cl
N
HO
O
O
O
O
O
N
N
O
8
f1 (ppm)

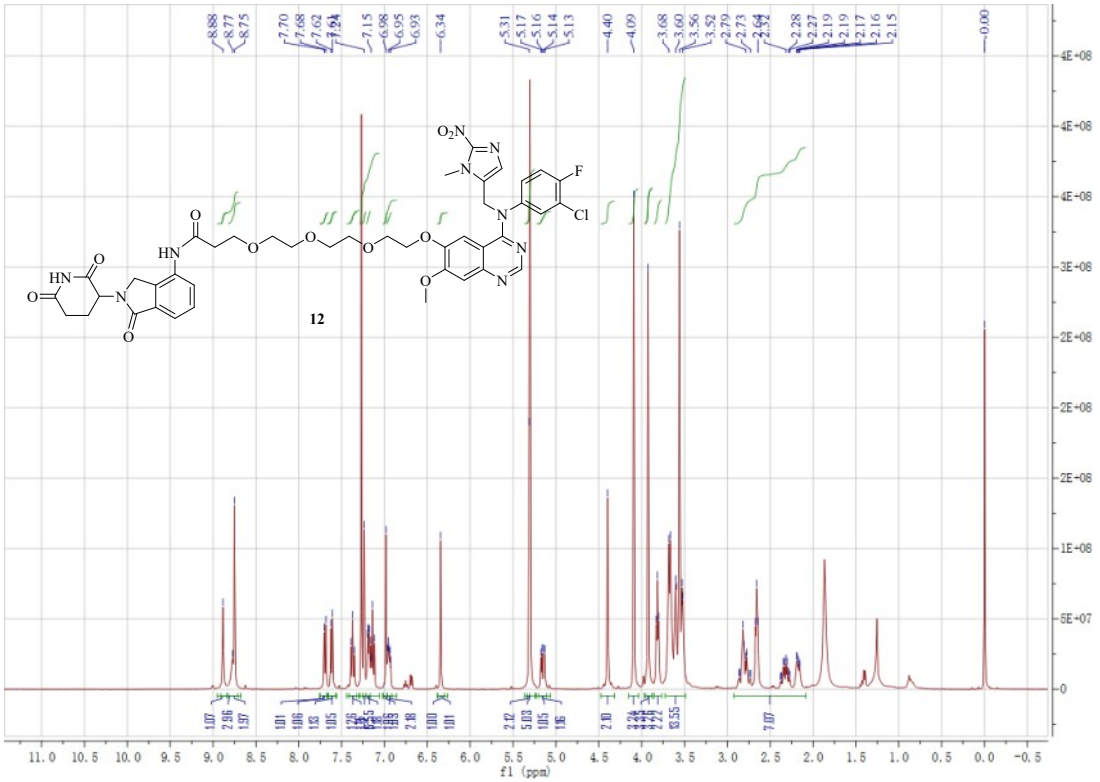

NO2
F
Cl
N
HO
O
O
O
O
O
O
N
N
O
8
f1 (ppm)

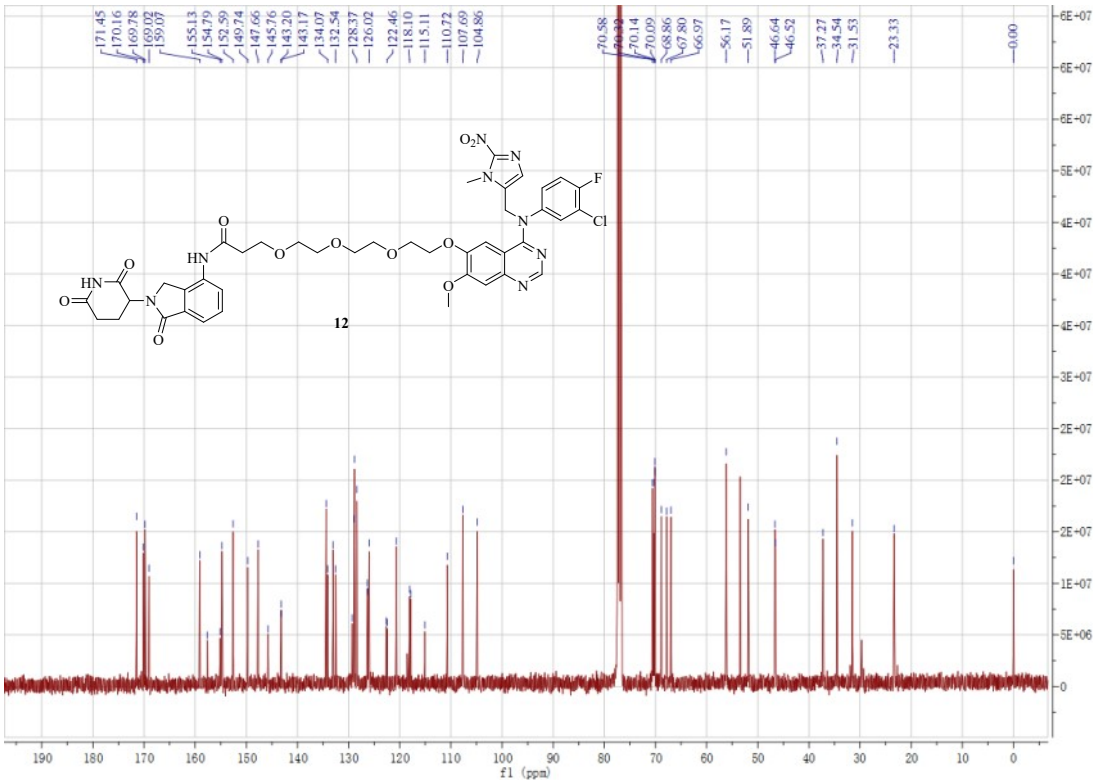

NH2
N
O
O
N
O
10
B (d) 6.89
C (t) 7.16
A (d) 6.77
E (dd) 5.16
D (s) 5.40
G (d) 4.04
F (d) 4.19
H (m) 3.48
I (m) 3.01
J (m) 2.76
O (qd) 2.27
M (m) 1.85
N (m) 2.03
L (s) 0.80
K (s) 0.79
f1 (ppm)


172.04
170.95
168.95
143.66
132.29
128.89
125.60
116.47
110.47
52.24
46.26
45.66
31.51
26.71
22.11
20.10
20.07
NH2
10
O (s) 170.95
N (s) 168.95
P (s) 172.04
M (s) 143.66
K (s) 128.89
J (s) 125.60
L (s) 132.29
I (s) 116.47
H (s) 110.47
G (s) 52.24
F (s) 46.26
E (s) 45.66
D (s) 31.51
C (s) 26.71
B (s) 22.11
A (s) 20.10
Q (s) 20.07
f1 (ppm)


F
Cl
HN
HN
HN
O
N
11
f1 (ppm)


171.87
170.63
170.12
169.50
154.68
153.16
152.98
148.16
147.05
134.12
132.30
126.38
121.67
120.51
120.32
116.18
107.21
102.32
70.27
70.09
69.68
69.64
69.31
67.97
66.51
56.09
52.51
46.81
37.03
31.46
23.04
0.00
11
f1 (ppm)


8.88
8.77
8.75
7.70
7.68
7.62
7.15
6.98
6.95
6.93
6.34
5.31
5.17
5.16
5.14
5.13
4.40
4.09
3.68
3.60
3.56
3.52
2.79
2.73
2.64
2.28
2.27
2.19
2.19
2.17
2.16
2.15
0.00
12
f1 (ppm)


171.45
170.16
169.78
169.02
159.07
155.13
154.79
152.59
149.74
147.66
145.76
143.20
143.17
134.07
132.54
128.37
126.02
122.46
118.10
115.11
110.72
107.69
104.86
70.58
70.14
70.09
68.86
67.80
66.97
56.17
51.89
46.64
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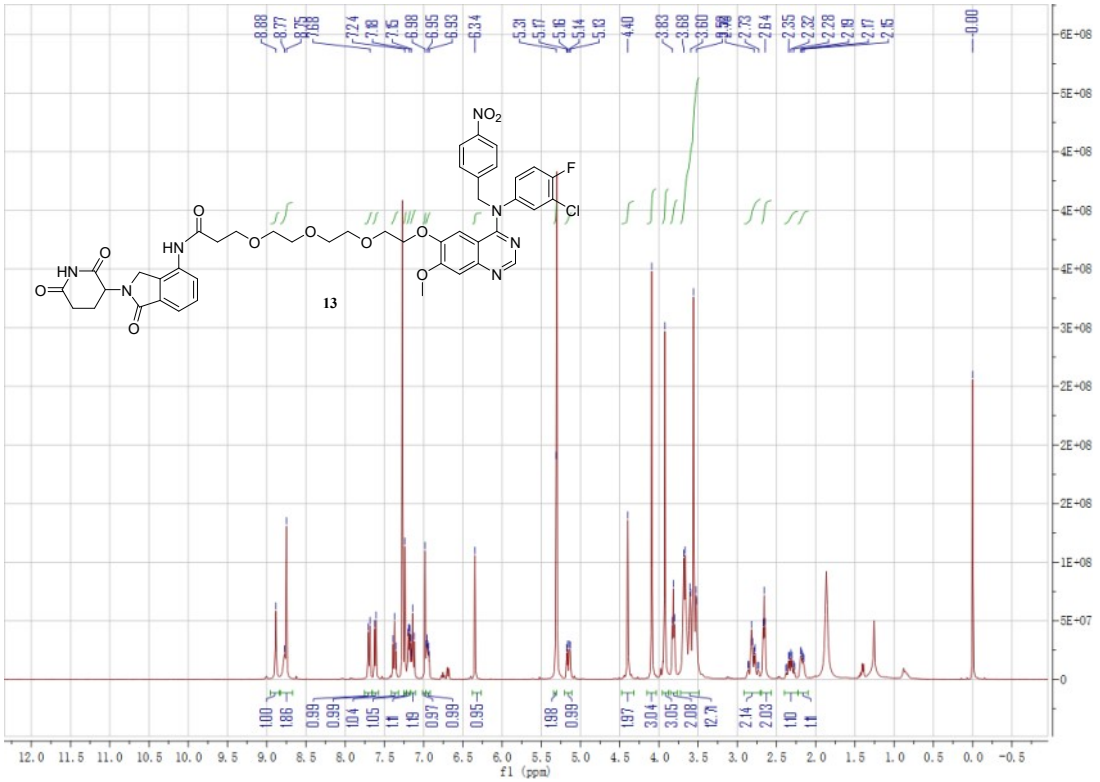
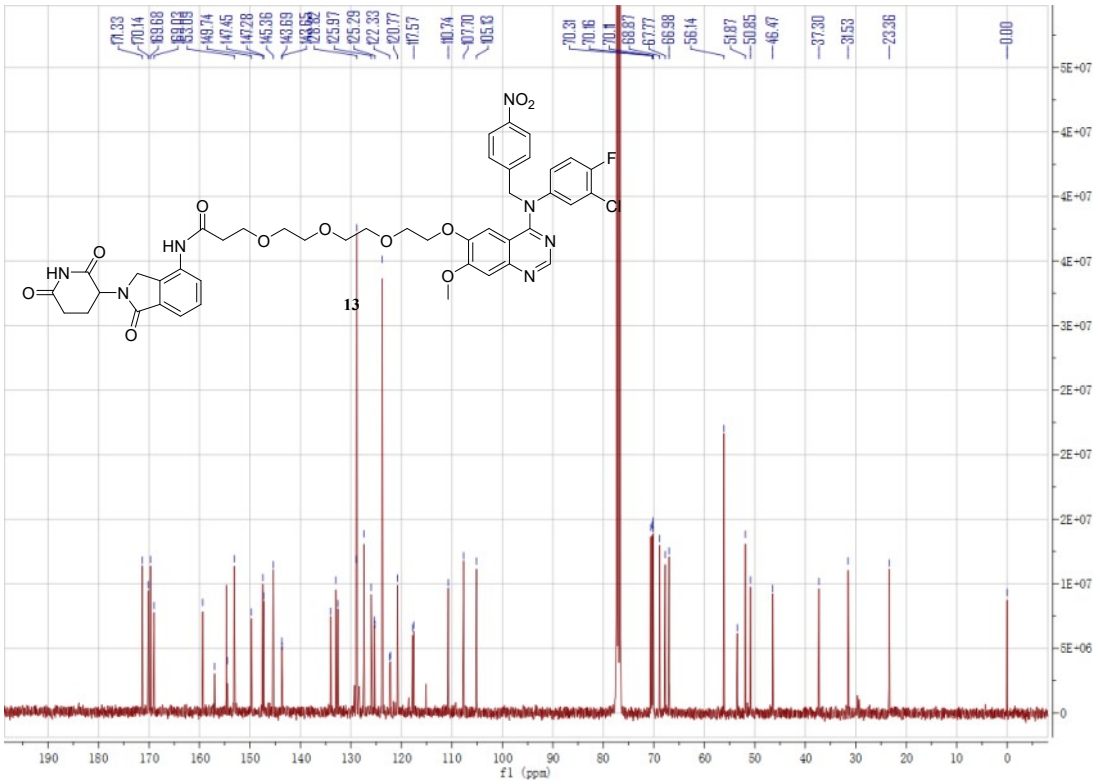
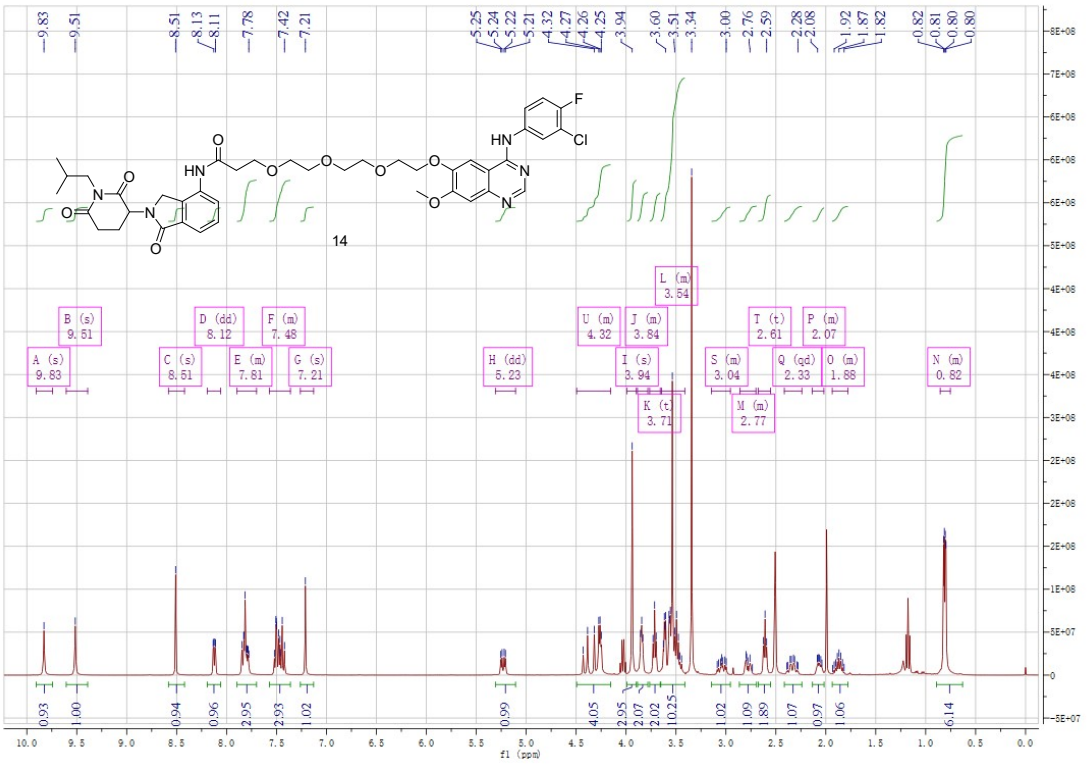
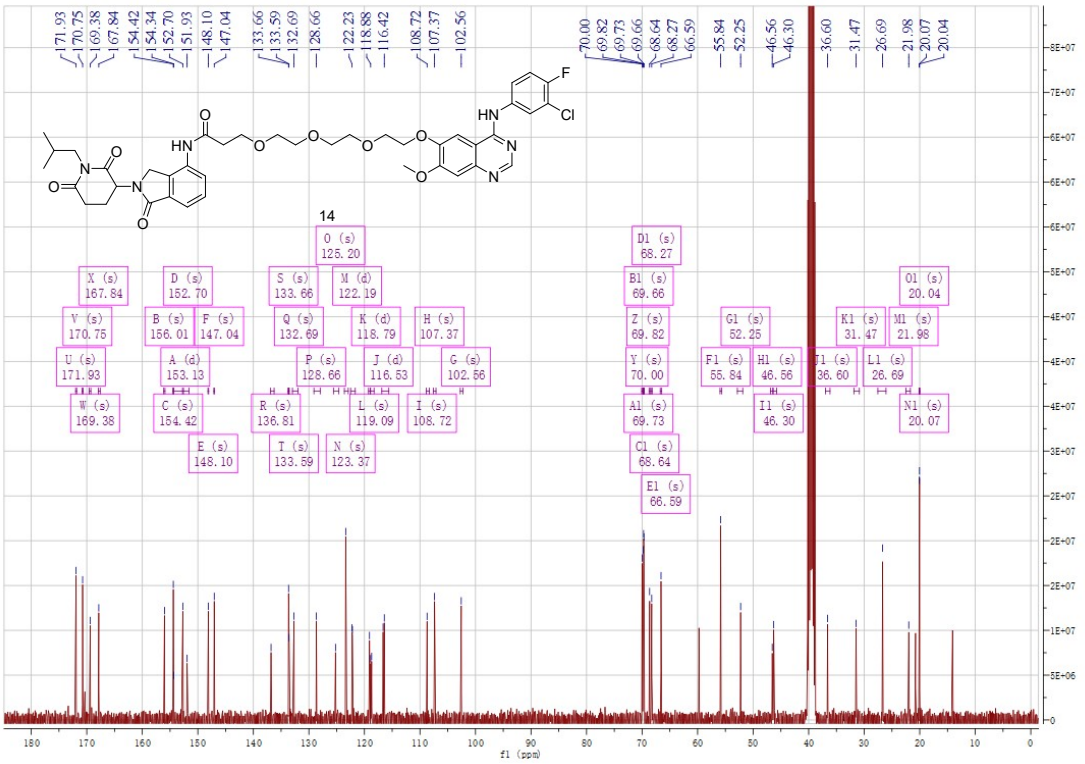
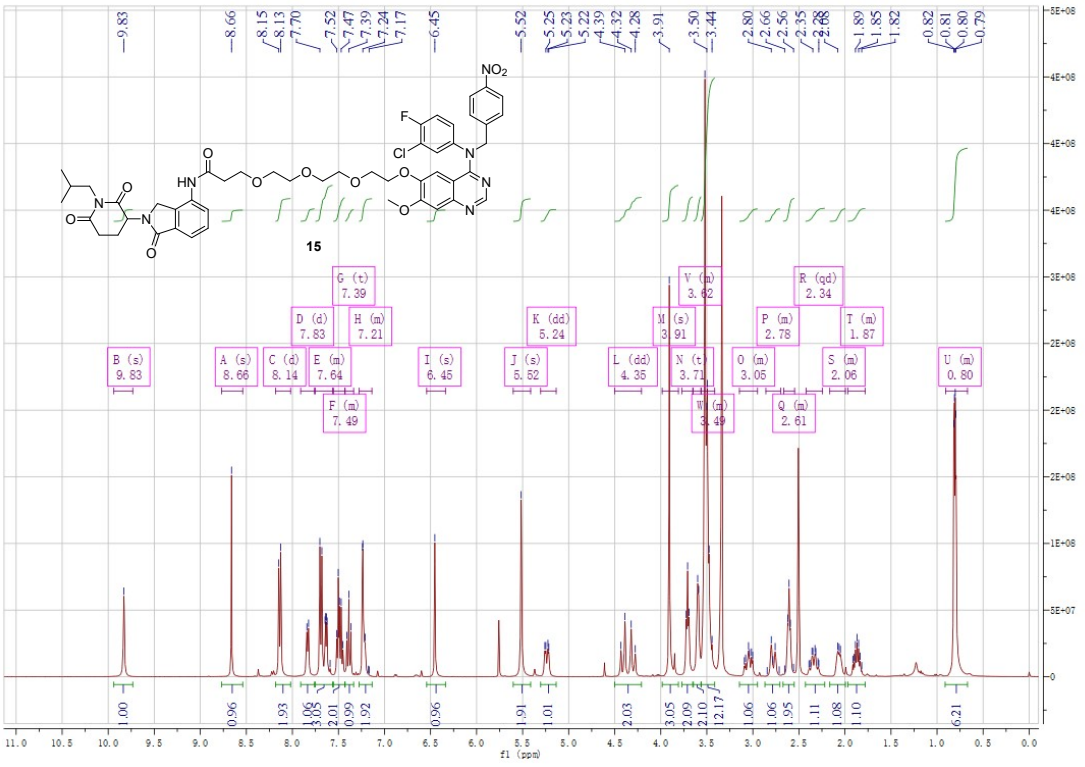
37.27
34.54
31.53
23.33
0.00
12
f1 (ppm)

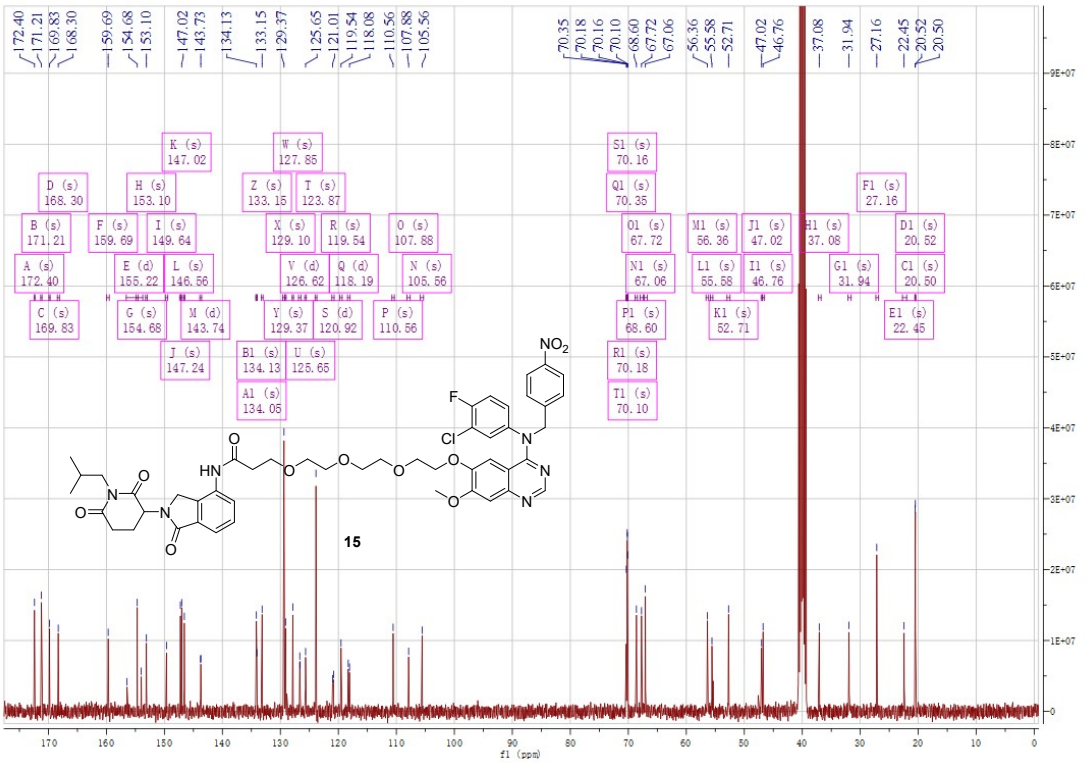

8.88
8.77
8.75
7.68
7.24
7.18
7.15
6.98
6.95
6.93
6.34
5.31
5.17
5.16
5.14
5.13
4.40
3.83
3.68
3.60
2.73
2.64
2.35
2.32
2.28
2.19
2.17
2.15
0.00
13
f1 (ppm)


NO2
F
Cl
N
HN
O
N
13
f1 (ppm)


F
Cl
HN
N
O
14
A (s) 9.83
B (s) 9.51
C (s) 8.51
D (dd) 8.12
E (m) 7.81
F (m) 7.48
G (s) 7.21
H (dd) 5.23
I (s) 3.94
J (m) 3.84
K (t) 3.71
L (m) 3.54
M (m) 2.77
N (m) 0.82
O (m) 1.88
P (m) 2.07
Q (qd) 2.33
S (m) 3.04
T (t) 2.61
U (m) 4.32
f1 (ppm)


14
f1 (ppm)


15
f1 (ppm)


172.40
171.21
169.83
168.30
159.69
154.68
153.10
147.02
143.73
134.13
133.15
129.37
125.65
121.01
119.54
118.08
110.56
107.88
105.56
70.35
70.18
70.16
70.10
68.60
67.72
67.06
56.36
55.58
52.71
47.02
46.76
37.08
31.94
27.16
22.45
20.52
20.50
K (s) 147.02
W (s) 127.85
S1 (s) 70.16
D (s) 168.30
H (s) 153.10
Z (s) 133.15
T (s) 123.87
Q1 (s) 70.35
F1 (s) 27.16
B (s) 171.21
F (s) 159.69
I (s) 149.64
X (s) 129.10
R (s) 119.54
O (s) 107.88
O1 (s) 67.72
M1 (s) 56.36
J1 (s) 47.02
H1 (s) 37.08
D1 (s) 20.52
A (s) 172.40
E (d) 155.22
L (s) 146.56
V (d) 126.62
Q (d) 118.19
N (s) 105.56
N1 (s) 67.06
L1 (s) 55.58
I1 (s) 46.76
G1 (s) 31.94
C1 (s) 20.50
C (s) 169.83
G (s) 154.68
M (d) 143.74
Y (s) 129.37
S (d) 120.92
P (s) 110.56
P1 (s) 68.60
K1 (s) 52.71
E1 (s) 22.45
J (s) 147.24
B1 (s) 134.13
U (s) 125.65
R1 (s) 70.18
A1 (s) 134.05
T1 (s) 70.10
15
f1 (ppm)