Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Solid-phase Synthesis for Thalidomide-based Proteolysis-Targeting Chimeras (PROTAC)

Soňa Krajčovičová,[a] Radek Jorda,[b] Denisa Hendrychová,[b] Vladimír Kryštof,*[b] and Miroslav Soural*[c]

[a] *Department of Organic Chemistry, Faculty of Science, Palacký University, Tr. 17. listopadu 12, 771 46 Olomouc, Czech Republic*

[b] *Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University and Institute of Experimental Botany AS CR, Šlechtitelů 27, 78371 Olomouc, Czech Republic*

[c] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 5, 779 00, Olomouc, Czech Republic*

**Corresponding author*

E-mail: vladimir.krystof@upol.cz, miroslav.soural@upol.cz

Table of Contents

1. General information S-2
2. Biological assay S-3
3. Experimental procedures S-3
4. Analytical data of final compounds S-6
5. Spectral data of final compounds S-10

1. General information

All reagents were of reagent grade and used without further purification. Solvents and chemicals were purchased from Sigma-Aldrich (Milwaukee, US, www.sigmaaldrich.com), Acros Organics (Geel, Belgium, www.acros.cz) or Fluorochem (Derbyshire, UK, www.fluorochem.co.uk). CDK inhibitors were purchased from Arctom Chemicals (Cambridge, US, www.arctomchem.com). Dry solvents were dried over 4Å molecular sieves or stored as received from commercial suppliers. Aminomethyl resin (100-200 mesh, 1% DVB, 0.98 mmol/g) was obtained from AAPPTec (Louisville, US, www.aapptec.com).

The LC/MS analyses were carried out on UHPLC-MS system consisting of UHPLC chromatograph Acquity with photodiode array detector and single quadrupole mass spectrometer (Waters), using X-Select C18 column at 30 °C and flow rate of 600 µL/min. Mobile phase was (A) 0.01 M ammonium acetate in H_2O, and (B) CH_3CN, linearly programmed from 10% A to 80% B over 2.5 min, kept for 1.5 min. The column was re-equilibrated with 10% of solution B for 1 min. The ESI source operated at discharge current of 5 µA, vaporizer temperature of 350 °C and capillary temperature of 200 °C.

Purification was carried out on C18 reverse phase column (YMC Pack ODS-A, 20× 100 mm, 5 µm particles), gradient was formed from 10 mM aqueous ammonium acetate and CH_3CN, flow rate 15 mL/min. For lyophilization of residual solvents at -110 °C the ScanVac Coolsafe 110-4 was used.

NMR spectra were recorded on JEOL ECX500 spectrometer at magnetic field strengths of 11.75 T (with operating frequencies 500.16 MHz for ^{1}H and 125.77 MHz for ^{13}C). Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*) are reported in Hertz (Hz). The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the residual signals of CD_3CN [1.94 (^{1}H) and 118.26 (^{13}C)] or $CDCl_3$ [7.26 (^{1}H) and 77.16 (^{13}C)]. The residual signal of DMSO (from biological tests) exhibited signal at 2.61 ppm (^{1}H) and 41.1 ppm (^{13}C). Abbreviations in NMR spectra: app d – apparent doublet, app s – apparent singlet, app t – apparent triplet, br s – broad singlet, d – doublet, dd – doublet of doublets, m – multiplet, s – singlet, sp – septet, t – triplet.

HRMS analysis was performed using LC-MS an Orbitrap Elite high-resolution mass spectrometer (Dionex Ultimate 3000, Thermo Exactive plus, MA, US) operating at positive full scan mode (120 000 FWMH) in the range of 100-1000 m/z. The settings for electrospray ionization were as follows: oven temperature of 150 °C and source voltage of 3.6 kV. The acquired data were internally calibrated with phthalate as a contaminant in CH_3OH (m/z 297.15909). Samples were diluted to a final concentration of 0.1 mg/mL in H_2O and CH_3OH (50:50, v/v). Before HPLC separation (column Phenomenex Gemini, 50 × 2.00 mm, 3 µm particles, C18), the samples were injected by direct infusion into the mass spectrometer using autosampler. Mobile phase was isocratic CH_3CN/IPA/0.01 M ammonium acetate (40:5:55) and flow 0.3 mL/min.

Reactions were carried out in plastic reaction vessels (syringes, each equipped with a porous disk) using a manually operated synthesizer (Torviq, Niles, US, www.torviq.com) or in dried glassware, unless stated otherwise. The volume of wash solvent was 10 mL per 1 g of resin. For washing, resin slurry was shaken with the fresh solvent for at least 1 min before changing the solvent. Resin-bound intermediates were dried under a stream of nitrogen for prolonged storage and/or quantitative analysis. For the LC/MS analysis a sample of resin (~5 mg) was treated with CH_2Cl_2/TFA (1:1, 1 mL, v/v), the cleavage cocktail was evaporated under a stream of nitrogen, and cleaved compounds extracted into CH_3CN (1 mL).

Yields of final compounds were calculated from loading of compound **6** (0.05 – 0.2 mmol/g) according to published procedure.[1]

2. Biological assay

- **Cell line**

Ramos cell line was obtained from DSMZ and was cultivated according to the provider's instructions. The cells were grown in Iscove's Modified Dulbecco's medium supplemented with 10% fetal bovine serum, 4 mM glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin and were cultivated in a humidified CO_2 incubator at 37 °C. BTK activation in cells was induced by treatment with goat $F(ab')_2$ Anti Human IgM purchased from Southern Biotech. Ibrutinib was a generous gift from dr. P. Klener (Charles University, Prague, Czech Republic).

- **Immunoblotting**

Cell lysates were prepared, and then proteins were separated on SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. After blocking, overnight incubation with specific primary antibodies, and 1 h incubation with peroxidase-conjugated secondary antibodies, peroxidase activity was visualised with Super- Signal West Pico reagents (Thermo Scientific) using a CCD camera LAS-4000 (Fujifilm). Antibodies specific for anti-BTK (clone C82B8), anti-phospho-BTK (Y323), anti-SYK (clone D3Z1E) and anti-Src (clone 36D10) were purchased from Cell Signaling, α-tubulin from Merck and anti-PCNA (clone PC-10) was generously gifted by Dr. B. Vojtěšek (Masaryk Memorial Cancer Institute, Brno, Czech Republic).

- **Kinase reaction**

The BTK (ProQinase, GST-tagged) kinase reactions were assayed with peptide substrate poly(Glu,Tyr) (4:1, 1 mg/mL, Merck) in the presence of 15 µM ATP, 0.05 µCi [γ-^{33}P]ATP, and the test compound in a final volume of 10 µL, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 2.5 µg/50 µL $PEG_{20.000}$). The reactions were stopped by adding 5 µL of 3% aq. H_3PO_4. Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3× with 0.5% aq. H_3PO_4, and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer. The concentration of the test compounds required to reduce the kinase's activity by 50% was determined from dose−response curves and reported as the IC_{50} value.

3. Experimental procedures

- **Preparation of BAL resin**

Aminomethyl polystyrene resin (1 g, loading 0.98 mmol/g) was swollen in CH_2Cl_2 (10 mL) for 30 min, washed with DMF (3x 10 mL), neutralized in DMF/piperidine (5:1, 10 mL) for additional 30 min and then again washed with DMF (5x 10 mL). Backbone amide linker (700 mg, 2.94 mmol) and HOBt (450 mg, 2.94 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 10 mL, v/v) and DIC (460 µL, 2.94 mmol) was added. The resulting solution was added to polypropylene fritted syringe with aminomethyl resin. The reaction slurry was shaken at ambient temperature overnight, followed by wash with DMF (3x 10 mL) and CH_2Cl_2 (3x 10 mL). Bromphenol blue test confirmed quantitative acylation of amino groups.

- **Procedure for reductive amination**

BAL resin **1** (1 g, loading 0.98 mmol/g) was swollen in CH_2Cl_2 (10 mL) for 30 min, then washed with dry THF (3x 10 mL) and dry DMF (3x 10 mL). The solution of 2-(2-aminoethoxy)ethanol (490 µL, 4.9 mmol) in DMF/AcOH (10:1, 10 mL, v/v) was added to polypropylene fritted syringe with BAL resin and it was shaken overnight at ambient temperature. Then, $NaBH(OAc)_3$ (210 mg, 2.94 mmol) in DMF/AcOH (20:1, 5 mL, v/v) was added portionwise to the reaction mixture during period of 4 h, followed by washing with DMF (5x 10 mL) and CH_2Cl_2 (3x 10 mL) and neutralization with DMF/TEA (10:1, 10 mL, v/v) for additional 30 min to obtain resin **2**.

- **Procedure for protection with Fmoc**

Resin **2** (1 g) was swollen in CH_2Cl_2 (10 mL) for 30 min and then washed with CH_2Cl_2 (3x 15 mL). Fmoc-OSu (2 g, 6 mmol) was dissolved in CH_2Cl_2 (10 mL) and added to polypropylene fritted syringe with the resin. The reaction slurry was shaken at ambient temperature overnight, followed by washing with CH_2Cl_2 (5x 10 mL). Subsequent cleavage from the resin and UHPLC-MS analysis confirmed the presence of desired product **3** (crude purity: 99%). Loading was determined according to published procedure[1] (0.40 – 0.50 mmol/g). MS (ESI): *m/z* $[M+H]^+$ = 328.

- **Procedure for Mitsunobu reaction**

Resin **3** (500 mg, 0.40 mmol/g) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (3x 5 mL) and dry THF (3x 5 mL). Solution of PPh_3 (393 mg, 1.5 mmol), 3-hydroxyphthalic anhydride (246 mg, 1.5 mmol) in dry THF (4 mL) were added to the resin and the polypropylene fritted syringe was connected with second syringe, which included solution of DIAD (295 µL, 1.5 mmol) in dry THF (1 mL), through joint. The syringes were put into freezer (-20 °C) for 30 min. Then solution of DIAD in THF was slowly added to the polypropylene fritted syringe with resin. The reaction mixture was shaken at ambient temperature for 16 h, followed by wash with THF (3x 5 mL) and CH_2Cl_2 (5x 5 mL). Subsequent cleavage from the resin and UHPLC-MS analysis confirmed the presence of desired product **4** (crude purity: 80 – 85%). Loading was determined according to published procedure[1] (0.20 – 0.30 mmol/g). MS (ESI): *m/z* $[M+H]^+$ = 474.

- **Procedure leading to the thalidomide ring formation**

To a sealed vial with resin **4** (250 mg, 0.21 mmol/g) was added dry toluene (2.5 mL), 3-aminopiperidine-2,6-dione hydrochloride (10 mg, 0.06 mmol) and TEA (9 µL, 0.06 mmol). Reaction mixture was stirred at reflux for 16 h, followed by wash with DMF (3x 3 mL) and CH_2Cl_2 (5x 3 mL) at ambient temperature. Subsequent cleavage from the resin and UHPLC-MS analysis showed sometimes the ratio of desired product **5** and starting material **4** as 2:1 (according to UV traces at 300 nm). In such case, the resin (250 mg) was again added to a sealed vial and additional dry toluene (2.5 mL), 3-aminopiperidine-2,6-dione hydrochloride (5 mg, 0.03 mmol) and TEA (5 µL, 0.03 mmol) were added. The reaction mixture was stirred at reflux for another 6 h. Subsequent wash with DMF (3x 3 mL), CH_2Cl_2 (5x 3 mL) at ambient temperature, cleavage from the resin and UHPLC-MS analysis confirmed the full conversion to desired product **5** (crude purity: 85 – 90%). Loading was determined according to published procedure[1] (0.05 – 0.25 mmol/g). MS (ESI): *m/z* $[M+H]^+$ = 585.

- **Procedure for deprotection of Fmoc**

Resin (250 mg) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with DMF (3x 3 mL). The freshly prepared solution of CH_2Cl_2/DBU (1:1, 3 mL, v/v) was added to polypropylene fritted syringe with the resin. The reaction slurry was shaken at ambient temperature for 15 min, followed by wash with CH_2Cl_2 (3x 3 mL), THF (3x 3 mL) and CH_2Cl_2 (3x 3 mL). Resin was used in the next step without further analysis.

- **Procedure for acylation with FAEEAA**

Fmoc deprotected resins **5** (250 mg, 0.10 mmol/g) or **6** (250 mg, 0.15 mmol/g) were swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with DMF (3x 3 mL) and CH_2Cl_2 (3x 3 mL), respectively. [2-[2-(Fmoc-amino)ethoxy]ethoxy]acetic acid (334 mg, 0.9 mmol) and HOBt (137 mg, 0.9 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 3 mL, v/v) and DIC (140 µL, 0.9 mmol) was added. The resulting solution was added to polypropylene fritted syringes with the resins. The reaction slurry was shaken at ambient temperature for 16 h, followed by washing with DMF (10x 3 mL) and CH_2Cl_2 (10x 3 mL). Subsequent cleavage from the resin and UHPLC-MS analysis confirmed the presence of desired products (crude purities: 90 – 95%). Loading after this step was determined according to published procedure[1] (0.05 – 0.20 mmol/g). MS (ESI): *m/z* $[M+H]^+$ = 730 (**6**), 875.

- **Procedure for acylation with iodoacetic acid**

Fmoc deprotected resin (250 mg, 0.1 – 0.2 mmol/g) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (3x 3 mL). Iodoacetic acid (121 mg, 0.75 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and DIC (58 µL, 0.37 mmol) was added. The reaction mixture was stirred for 15 min at ambient temperature with precipitation of diisopropylurea. The resulting solution was added to polypropylene fritted syringe with resin. The reaction mixture was shaken at ambient temperature for 90 min, followed by wash with DMF (3x 3 mL) and CH_2Cl_2 (3x 3 mL). Resins were used directly in the next step without further quantification. MS (ESI): *m/z* $[M+H]^+$ = 530 (**9**), 675 (**7**), 820 (**11**).

- **Procedure for acylation with CDK inhibitors**

Resins **7, 9** or **11** (200 mg, respectively) were swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (3x 3 mL). To each solution of CDK inhibitors (0.06 M) in DMSO (3 mL) was added DIPEA (313 µL, 0.6 M) and the resulting mixture was added to polypropylene fritted syringes with starting materials. The reaction slurries were shaken at ambient temperature for 16 h, followed by wash with CH_2Cl_2 (10x 3 mL), DMF (5x 3 mL) and THF (5x 3 mL).

- **General procedure for cleavage from the resin**

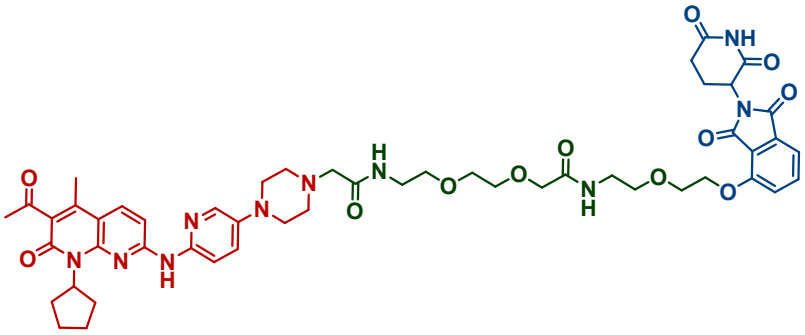
Cleavage of the intermediates **3 – 8a-e** and **12** in analytical scale (~5 mg) prior to analysis was carried out in CH_2Cl_2/TFA (1:1, 1 mL, v/v) for 30 min according to the General Information. Cleavage of intermediate **10** in analytical scale prior to analysis was carried out in neat TFA (2 mL) for 1 h.
Cleavage of the final compounds **8a-e** and **12** in preparative scale (200 mg): The corresponding resin was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (5x 3 mL). Solution of CH_2Cl_2/TFA (1:1, 6 mL, v/v) was added to each polypropylene fritted syringe with resin. The reaction slurry was shaken at ambient temperature for 2 h and then washed with CH_2Cl_2/TFA (1:1, 3x 3 mL, v/v) and CH_2Cl_2 (3x 3 mL). The cleavage cocktail with combined washes was evaporated under a stream of nitrogen,

the crude products were dissolved in CH_3CN (3 mL) and purified by RP-HPLC to afford final compounds **8a-e** and **12.**

Cleavage of the final compound **10** in preparative scale (200 mg): The corresponding resin was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (5x 3 mL). Neat TFA (5 mL) was added to vial with resin and the reaction slurry was stirring at reflux for 2 h. Then was the reaction mixture cooled down to ambient temperature and the crude product was dissolved in CH_3CN (3 mL) and purified by RP-HPLC to afford final compound **10.**

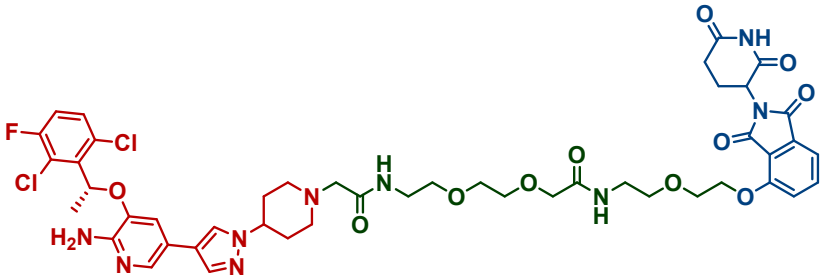
4. Analytical data of final compounds

PROTAC conjugate 8a



Yellow amorphous solid (10.0 mg, 50% yield). ^{1}H NMR (500 MHz, CD_3CN): δ 9.50 (br s, 1H), 8.85 (s, 1H), 8.68 (s, 1H), 8.06 (d, *J* = 9.1 Hz, 1H), 8.02 (d, *J* = 3.1 Hz, 1H), 7.71 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.42 – 7.38 (m, 3H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.07 (s, 1H), 5.88 (m, 1H), 4.96 (dd, *J* = 12.6, 5.4 Hz, 1H), 4.31 – 4.27 (m, 2H), 3.86 (s, 2H), 3.84 – 3.81 (m, 2H), 3.61 (t, *J* = 5.6 Hz, 2H), 3.58 – 3.55 (m, 2H), 3.53 – 3.51 (m, 2H), 3.49 (t, *J* = 5.5 Hz, 2H), 3.39 – 3.34 (m, 4H), 3.17 (dd, *J* = 6.1, 4.0 Hz, 4H), 2.98 (s, 2H), 2.82 – 2.65 (m, 4H), 2.64 – 2.62 (m, 4H), 2.43 (s, 3H), 2.31 (s, 3H), 2.16 – 2.15 (m, 9H, *overlap with solvent*) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ 173.3, 170.6, 170.6, 170.5, 168.1, 166.7, 162.3, 159.3, 158.7, 157.2, 156.4, 145.7, 144.7, 142.7, 137.7, 136.7, 134.8, 131.5, 126.6, 120.6, 117.9, 116.4, 115.0, 108.5, 71.7, 71.1, 70.6, 70.4, 70.1, 69.6, 62.2, 54.8, 53.9, 50.1, 49.9, 39.2, 39.2, 32.0, 31.6, 28.7, 26.4, 23.2, 14.2 ppm. HRMS (ESI): *m/z* calcd for $C_{50}H_{61}N_{10}O_{12}$ $[M+H]^+$ = 993.4465, found $[M+H]^+$ = 993.4410.

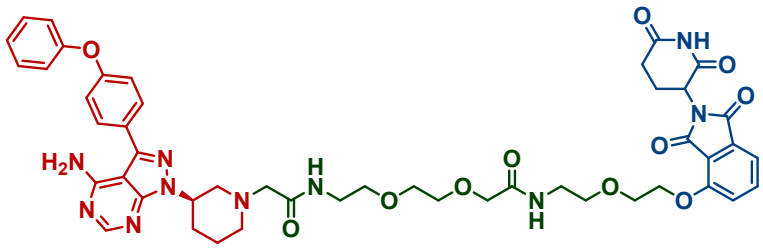
PROTAC conjugate 8b



White amorphous solid (17.6 mg, 85% yield). ^{1}H NMR (500 MHz, CD_3CN): δ 9.76 (br s, 1H), 7.73 (d, *J* = 1.9 Hz, 1H), 7.71 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.67 (s, 1H), 7.52 – 7.50 (m, 1H), 7.45 – 7.38 (m, 3H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.17 (t, *J* = 8.7 Hz, 1H), 7.14 – 7.09 (m, 1H), 6.91 (d, *J* = 2.0 Hz, 1H), 6.13 (q, *J* = 6.7 Hz, 1H), 5.21 (br s, 2H), 4.97 (dd, *J* = 12.2, 5.4 Hz, 1H), 4.29 – 4.26 (m, 2H), 4.12 – 4.05 (m, 1H), 3.86 (s, 2H),

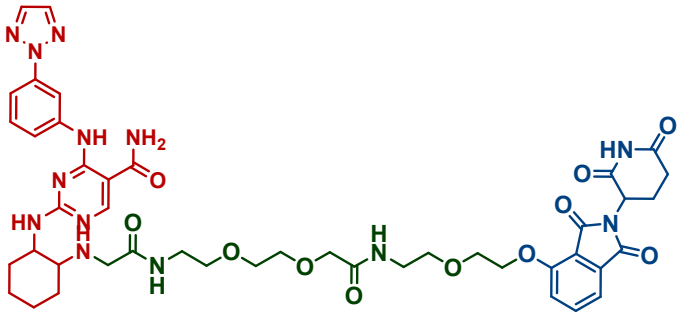
3.83 – 3.80 (m, 2H), 3.61 (t, *J* = 5.5 Hz, 2H), 3.57 – 3.54 (m, 2H), 3.52 (d, *J* = 3.2 Hz, 1H), 3.50 – 3.45 (m, 4H), 3.36 (dq, *J* = 17.1, 5.7 Hz, 4H), 2.94 (s, 2H), 2.91 – 2.88 (m, 2H), 2.81 – 2.65 (m, 4H), 2.31 – 2.24 (m, 3H), 2.12 – 2.05 (m, 2H), 2.04 – 1.99 (m, 4H), 1.96 (d, *J* = 1.2 Hz, 2H), 1.82 (d, *J* = 6.7 Hz, 3H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ 173.5, 173.4, 172.9, 171.0, 170.6, 170.5, 166.4 (d, $^{1}J_{C\text{-}F}$ = 174.5 Hz), 159.5, 157.5, 157.2, 150.4, 140.4, 138.0, 137.7, 136.0, 134.7, 131.3 (d, $^{3}J_{C\text{-}F}$ = 5.4 Hz), 130.0 (d, $^{3}J_{C\text{-}F}$ = 3.4 Hz), 124.3, 122.6 (d, $^{2}J_{C\text{-}F}$ = 19.3 Hz), 120.6, 120.4, 119.6, 117.8 (d, $^{2}J_{C\text{-}F}$ = 23.8 Hz), 116.4, 116.1, 73.5, 71.7, 71.1, 70.7, 70.6, 70.4, 70.1, 69.7, 62.1, 59.5, 53.5, 50.1, 39.2, 39.1, 33.2, 32.1, 23.1, 20.7, 19.1 ppm. HRMS (ESI): *m/z* calcd for $C_{46}H_{51}Cl_2FN_9O_{11}$ $[M\text{-}H]^-$ = 994.3075, found $[M\text{-}H]^-$ = 994.3064.

PROTAC conjugate 8c



White amorphous solid (24 mg, 83% yield). ^{1}H NMR (500 MHz, CD_3CN): δ 9.44 (br s, 1H), 8.28 (s, 1H), 7.72 – 7.63 (m, 4H), 7.43 – 7.40 (m, 2H), 7.39 (d, *J* = 7.3 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.21 – 7.17 (m, 2H), 7.16 – 7.13 (m, 2H), 7.12 – 7.09 (m, 3H), 5.12 (br s, 1H), 4.95 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.30 – 4.26 (m, 2H), 3.84 (s, 2H), 3.83 – 3.80 (m, 2H), 3.60 (t, *J* = 5.5 Hz, 2H), 3.53 – 3.47 (m, 5H), 3.44 (t, *J* = 5.4 Hz, 2H), 3.38 - 3.35 (m, 3H), 3.34 – 3.30 (m, 2H), 3.07 - 3.05 (m, 2H), 2.80 – 2.60 (m, 6H), 2.12 – 2.03 (m, 3H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ 173.1, 170.8, 170.5, 168.0, 166.7, 162.1, 161.8, 159.2, 158.0, 157.5, 157.2, 154.2, 153.9, 145.7, 137.7, 134.7, 131.1, 131.0, 128.2, 124.9, 120.6, 120.2, 117.8, 116.4, 98.7, 71.5, 71.1, 70.7, 70.5, 70.1, 70.0, 69.6, 60.6, 57.1, 54.1, 53.5, 50.0, 39.6, 39.2, 31.9, 29.2, 23.1 ppm. HRMS (ESI): *m/z* calcd for $C_{47}H_{51}N_{10}O_{11}$ $[M\text{-}H]^-$ = 931.3744, found $[M\text{-}H]^-$ = 931.3738.

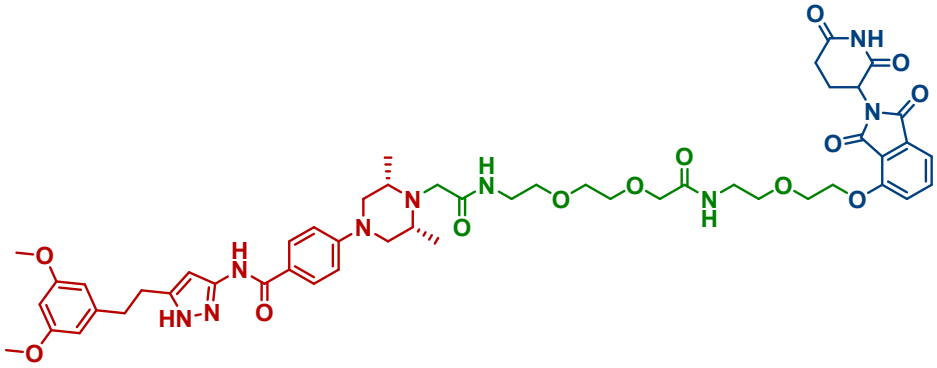
PROTAC conjugate 8d



White amorphous solid (9.0 mg, 49% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.99 – 8.59 (m, 2H), 7.88 – 7.74 (m, 3H), 7.71 – 7.65 (m, 1H), 7.50 – 7.38 (m, 3H), 7.34 – 7.29 (m, 1H), 7.23 – 7.20 (m, 1H), 5.02 – 4.95 (m, 1H), 4.34 – 4.26 (s, 2H), 4.02 – 3.89 (m, 4H), 3.80 – 3.64 (m, 5H), 3.58 – 3.33 (m, 11H), 2.88 – 2.78 (m, 3H), 2.16 – 2.10 (m, 1H), 1.94 – 1.88 (m, 2H), 1.73 – 1.58 (m, 4H), 1.49 – 1.31 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9, 170.7, 167.2, 166.0, 164.7, 156.3, 152.5, 151.4, 147.3, 143.7, 140.7, 135.8, 135.7, 133.9, 131.6, 129.5, 127.3, 125.7, 119.2, 117.3, 108.1, 81.6, 71.1, 70.7, 70.6, 70.2,

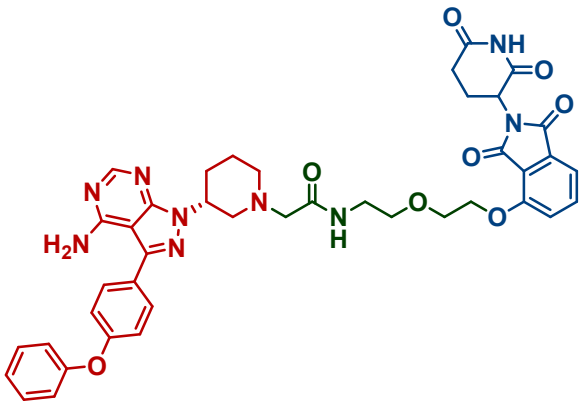
69.6, 69.0, 68.9, 63.2, 56.5, 53.7, 52.8, 51.4, 48.8, 39.0, 38.9, 36.9, 31.6, 29.8, 26.1, 23.4, 20.7 ppm. HRMS (ESI): *m/z* calcd for $C_{44}H_{52}N_{13}O_{11}$ $[M-H]^-$ = 938.3915, found $[M-H]^-$ = 938.3905.

PROTAC conjugate 8e



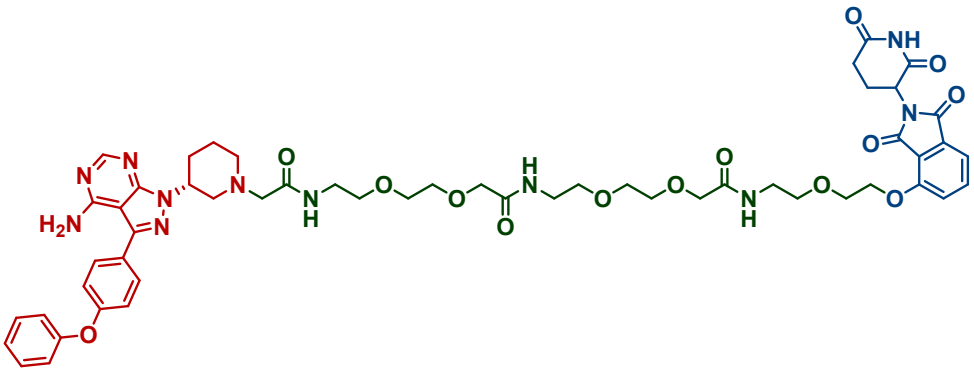
White amorphous solid (9.6 mg, 24% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 – 7.74 (m, 2H), 7.74 – 7.62 (m, 2H), 7.44 – 7.42 (m, 1H), 7.32 – 7.29 (m, 1H), 7.23 – 7.21 (m, 2H), 6.91 – 6.85 (m, 2H), 6.45 – 6.38 (m, 2H), 6.34 – 6.30 (m, 1H), 4.98 – 4.93 (m, 1H), 4.33 – 4.28 (m, 4H), 4.02 – 3.97 (m, 4H), 3.92 – 3.86 (m, 5H), 3.79 – 3.76 (m, 8H), 3.73 – 3.69 (m, 8H), 3.61 – 3.57 (m, 9H), 3.51 – 3.45 (m, 6H), 3.02 – 2.99 (m, 2H), 2.84 – 2.75 (m, 2H), 2.12 – 2.10 (m, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.0, 169.4, 167.0, 165.9, 164.7, 164.4, 162.9, 161.0, 156.4, 145.4, 143.2, 136.7, 133.9, 132.7, 129.3, 119.7, 119.5, 117.4, 116.4, 114.5, 106.6, 106.5, 98.5, 98.4, 70.9, 70.7, 70.6, 70.3, 70.0, 69.4, 69.2, 69.1, 69.0, 55.4, 49.3, 38.8, 38.7, 35.5, 31.5, 29.8, 28.06, 22.9, 22.8 ppm. HRMS (ESI): *m/z* calcd for $C_{51}H_{64}N_9O_{13}$ $[M+H]^+$ = 1010.4618, found $[M+H]^+$ = 1010.4622.

PROTAC conjugate 10



White amorphous solid (6.0 mg, 76% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 18.1 Hz, 1H), 7.67 – 7.63 (m, 1H), 7.63 – 7.59 (m, 2H), 7.47 – 7.43 (m, 1H), 7.41 – 7.36 (m, 2H), 7.24 – 7.20 (m, 1H), 7.19 – 7.16 (m, 1H), 7.15 – 7.12 (m, 2H), 7.10 – 7.06 (m, 2H), 5.04 – 5.00 (m, 1H), 4.34 – 4.26 (m, 2H), 3.91 – 3.86 (m, 2H), 3.71 – 3.64 (m, 2H), 3.50 – 3.44 (m, 2H), 3.32 – 3.23 (m, 2H), 3.20 – 3.05 (m, 2H), 2.90 – 2.78 (m, 4H), 2.17 – 2.09 (m, 3H), 1.95 – 1.87 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.9, 171.6, 168.8, 167.1, 167.1, 158.9, 156.4, 153.7, 153.6, 136.6, 133.9, 133.9, 130.1, 130.1, 130.0, 127.3, 124.2, 124.2, 117.5, 117.5, 116.4, 98.3, 70.2, 69.9, 69.2, 69.2, 69.2, 68.9, 68.9, 56.9, 53.2, 49.49, 49.4, 39.1, 39.0, 22.8, 22.7 ppm. HRMS (ESI): *m/z* calcd for $C_{41}H_{40}N_9O_8$ $[M-H]^-$ = 786.3005, found $[M-H]^-$ = 786.2999.

PROTAC conjugate 12



White amorphous solid (22.5 mg, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 0.8 Hz, 1H), 7.66 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.63 – 7.59 (m, 2H), 7.45 (dt, *J* = 7.3, 0.6 Hz, 1H), 7.39 – 7.35 (m, 2H), 7.24 (dd, *J* = 8.6, 0.7 Hz, 1H), 7.20 – 7.14 (m, 3H), 7.13 – 7.10 (m, 2H), 7.08 – 7.04 (m, 2H), 5.02 – 4.98 (m, 1H), 4.97 – 4.92 (m, 1H), 4.32 – 4.30 (m, 2H), 3.97 – 3.92 (m, 4H), 3.90 – 3.86 (m, 2H), 3.70 – 3.68 (m, 2H), 3.66 – 3.56 (m, 7H), 3.55 – 3.46 (m, 9H), 3.46 – 3.41 (m, 2H), 3.40 – 3.34 (m, 1H), 3.11 (br s, 2H), 2.91 – 2.69 (m, 5H), 2.16 – 2.05 (m, 3H), 1.93 – 1.90 (m, 1H), 1.86 – 1.79 (m, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.7, 172.6, 170.1, 169.1, 169.1, 167.1, 165.8, 158.6, 157.9, 156.4, 155.1, 154.0, 144.2, 136.6, 133.9, 130.0, 127.7, 124.1, 119.6, 119.5, 119.2, 117.4, 116.3, 98.3, 70.9, 70.9, 70.6, 70.3, 70.1, 70.0, 69.9, 69.8, 69.3, 69.1, 61.5, 57.9, 53.6, 53.4, 53.4, 49.3, 38.7, 38.7, 38.6, 31.6, 29.2, 24.5, 22.7 ppm. HRMS (ESI): *m/z* calcd for $C_{53}H_{64}N_{11}O_{14}$ $[M+H]^+$ = 1078.4629, found $[M+H]^+$ = 1078.4622.

References:

1 P. Králová, V. Fülöpová, M. Maloň, T. Volná, I. Popa and M. Soural, *ACS Comb. Sci.*, 2017, **19**, 173–180.

5. Spectral data of final compounds

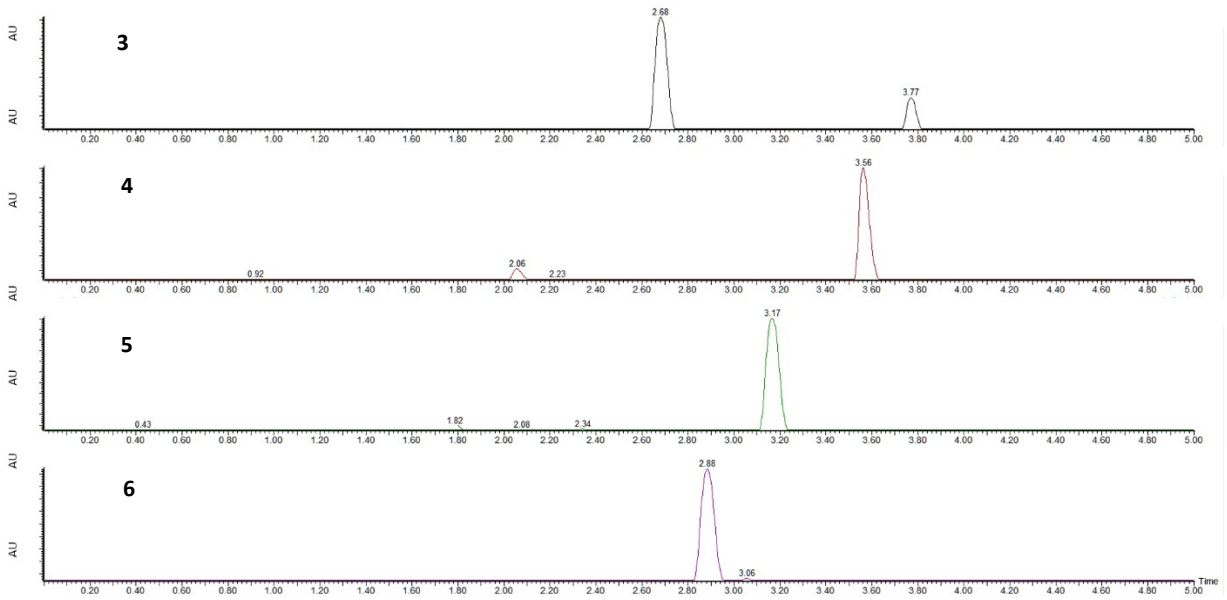


Figure 1. Crude purities of the intermediates.

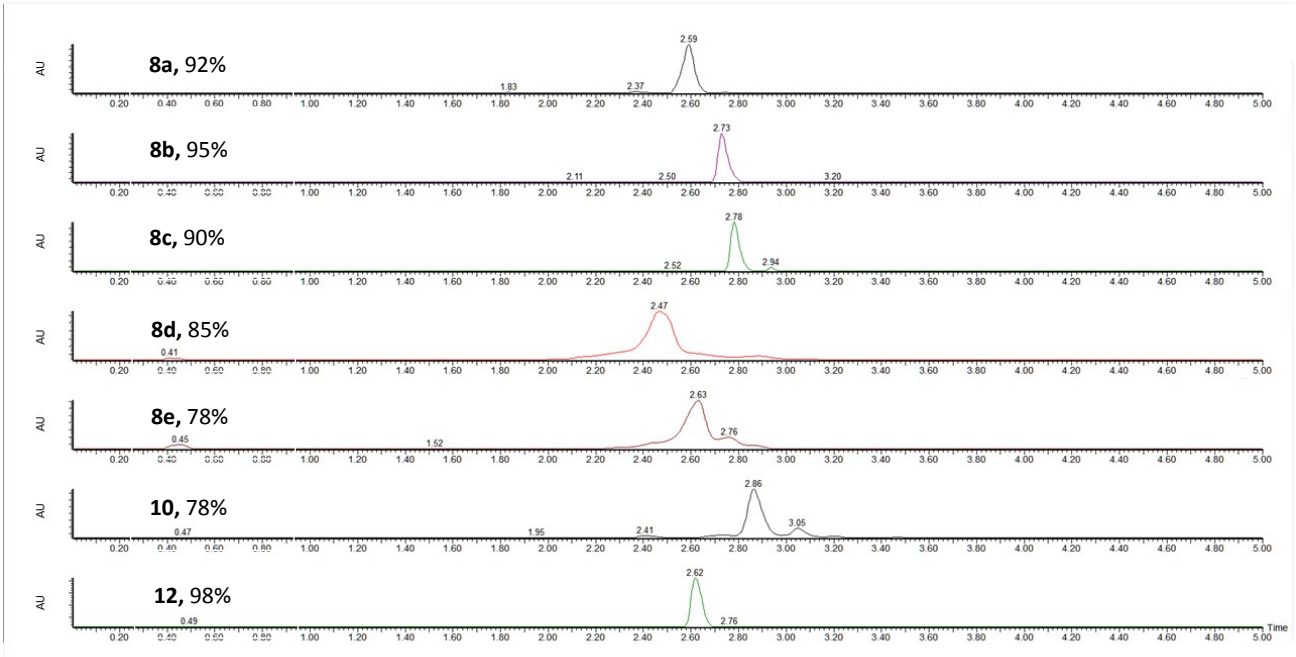
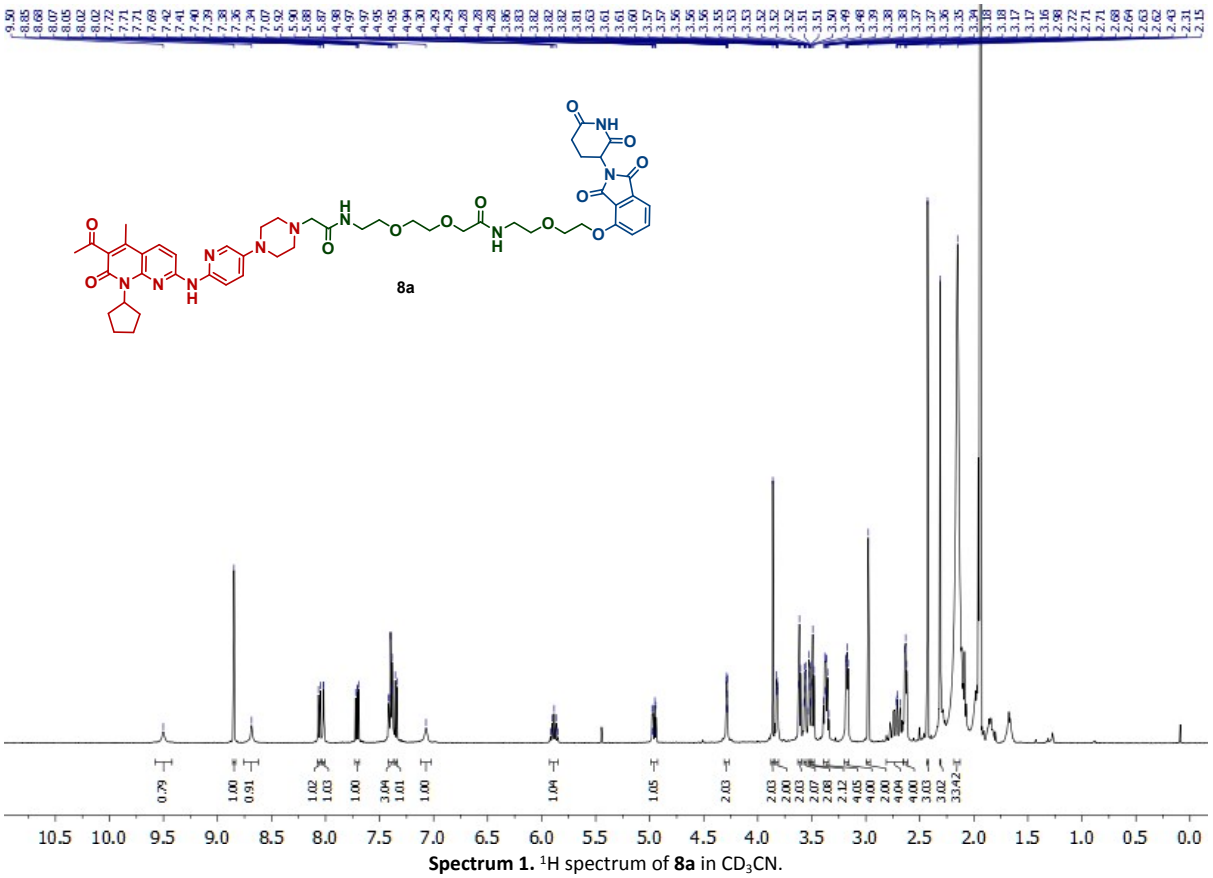
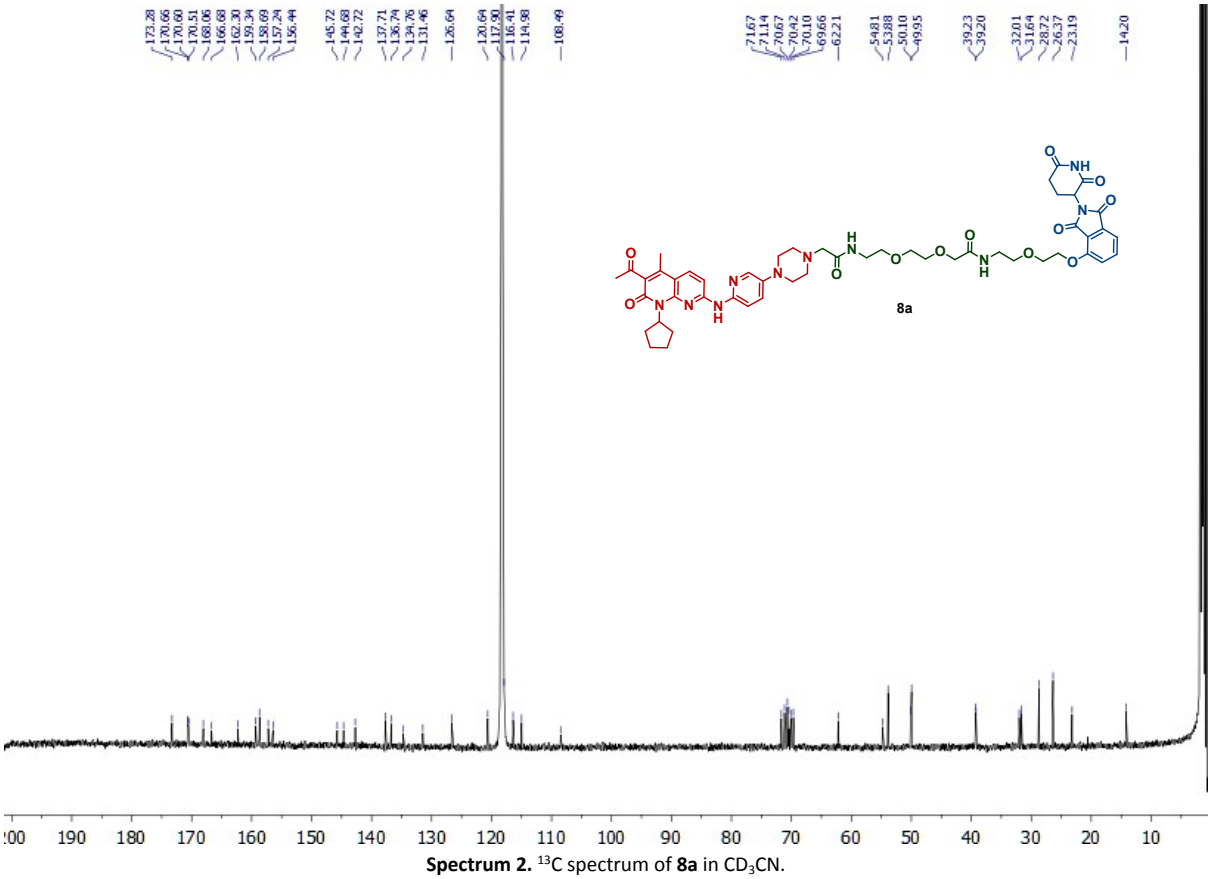


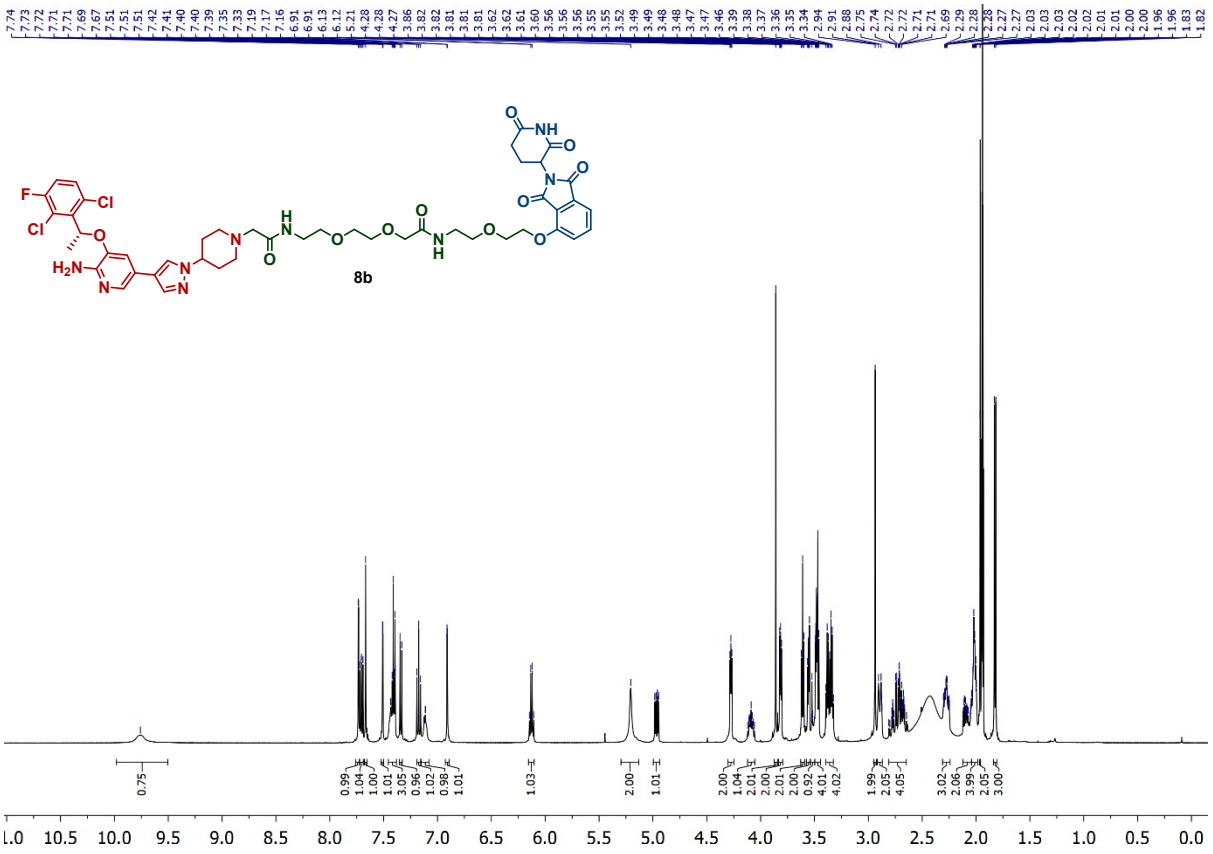
Figure 2. Crude purities of the final compounds after cleavage from the resin.



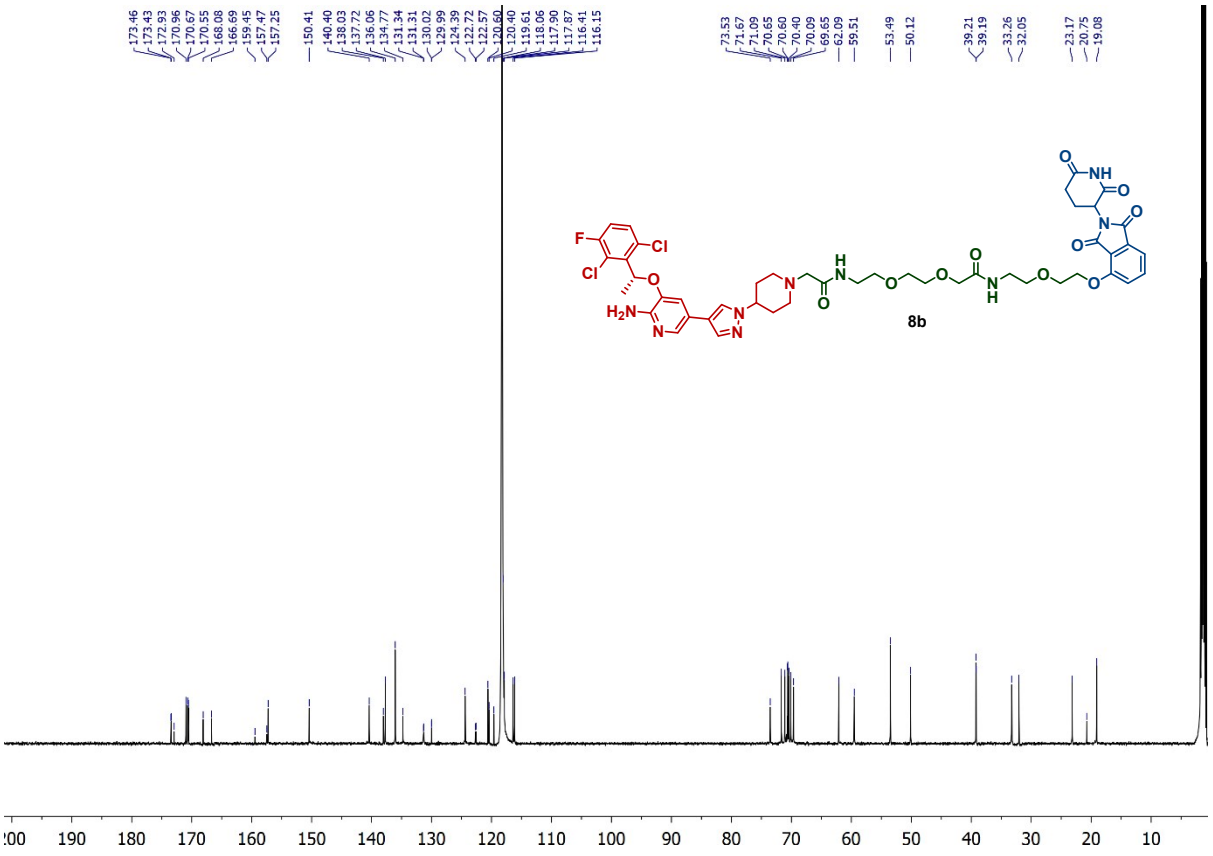
Spectrum 1. ^{1}H spectrum of **8a** in CD_3CN.



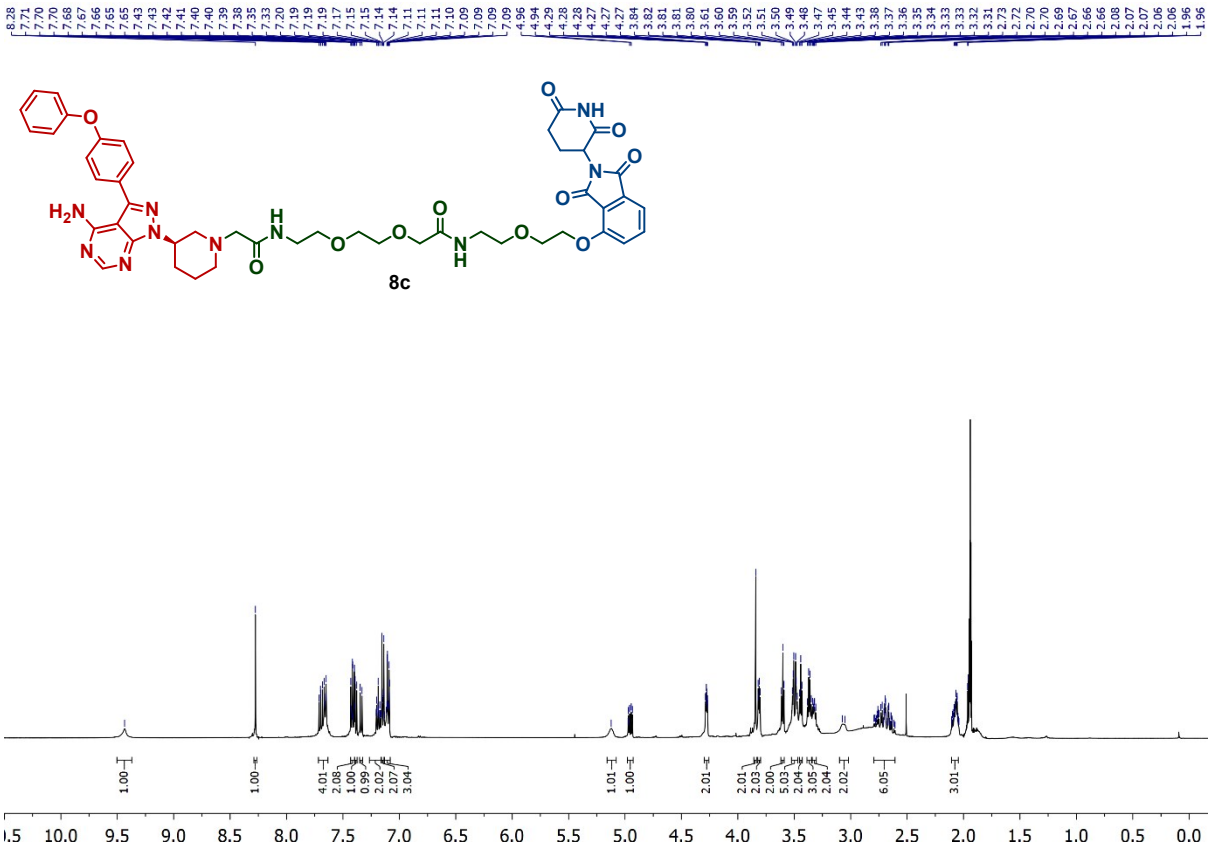
Spectrum 2. ^{13}C spectrum of **8a** in CD_3CN.



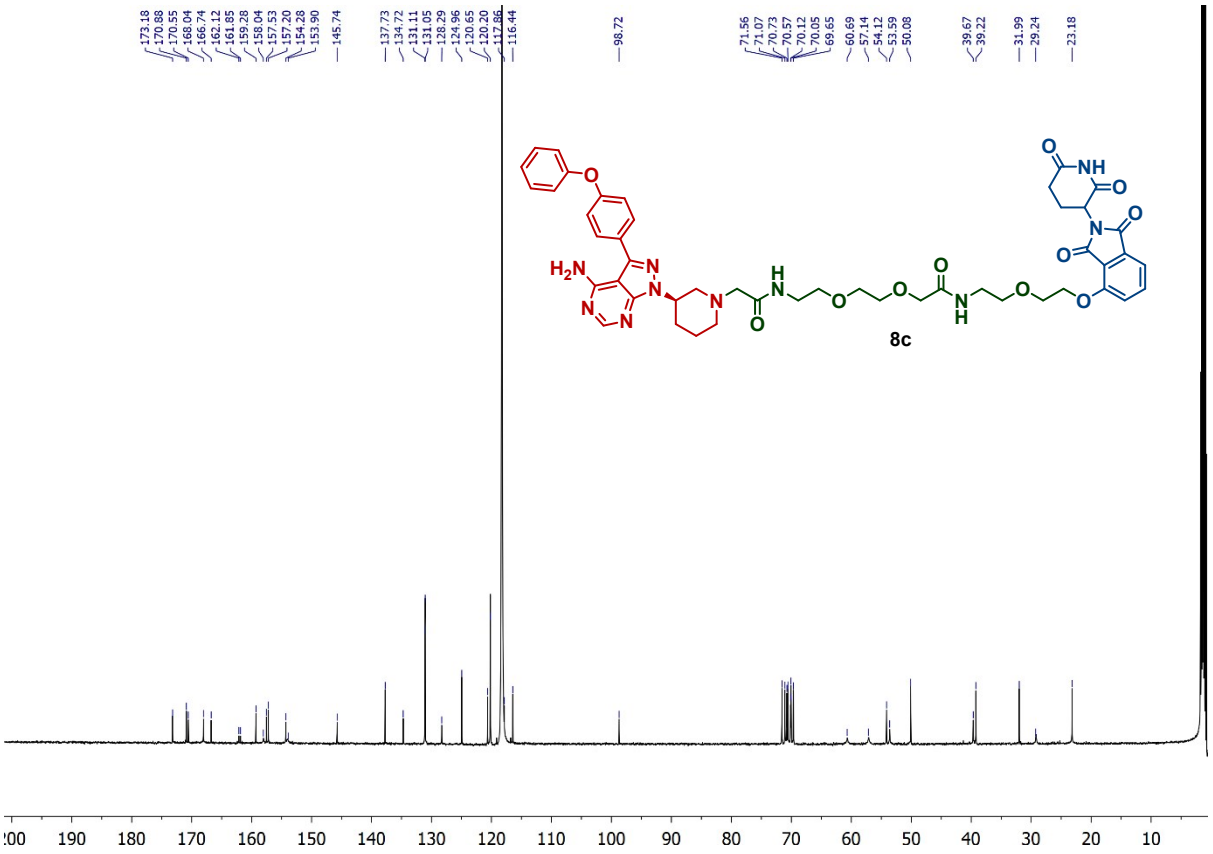
Spectrum 3. ^{1}H spectrum of **8b** in CD_3CN.



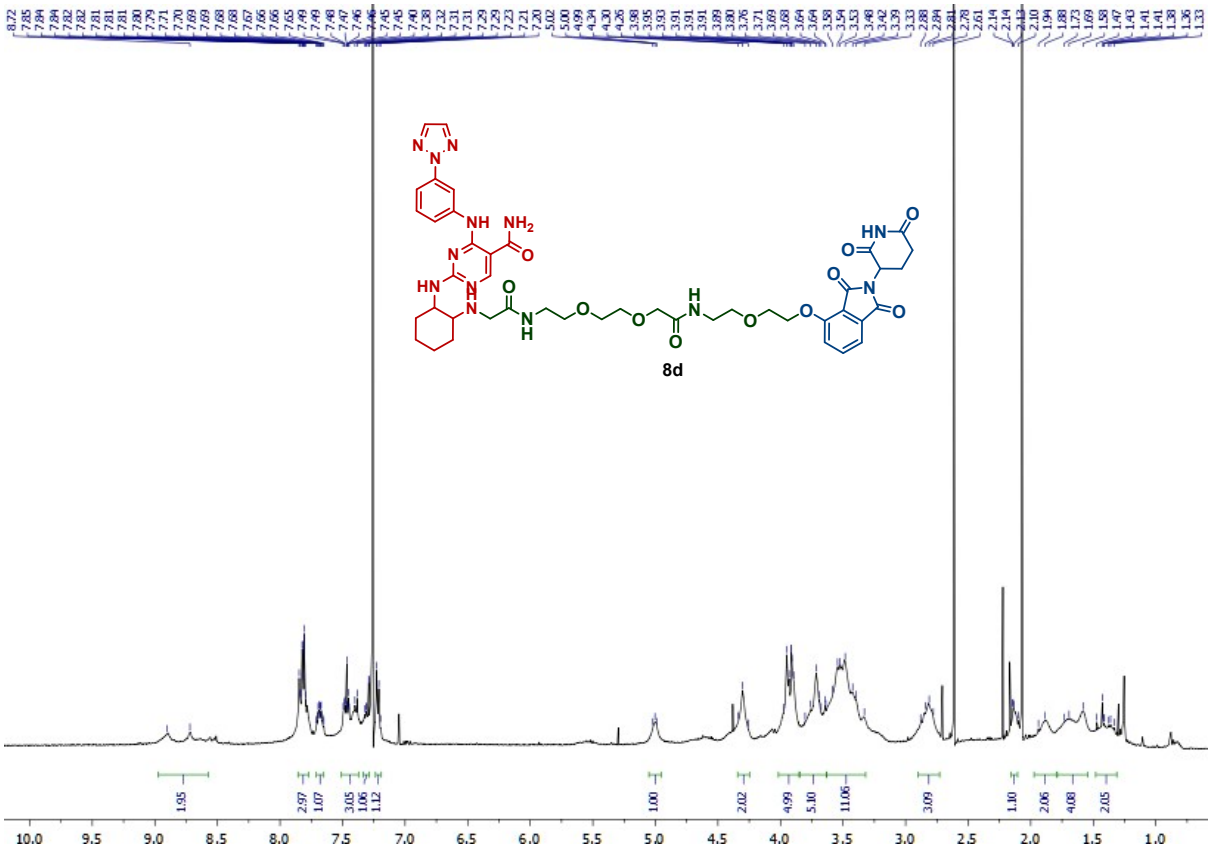
Spectrum 4. ^{13}C spectrum of **8b** in CD_3CN.



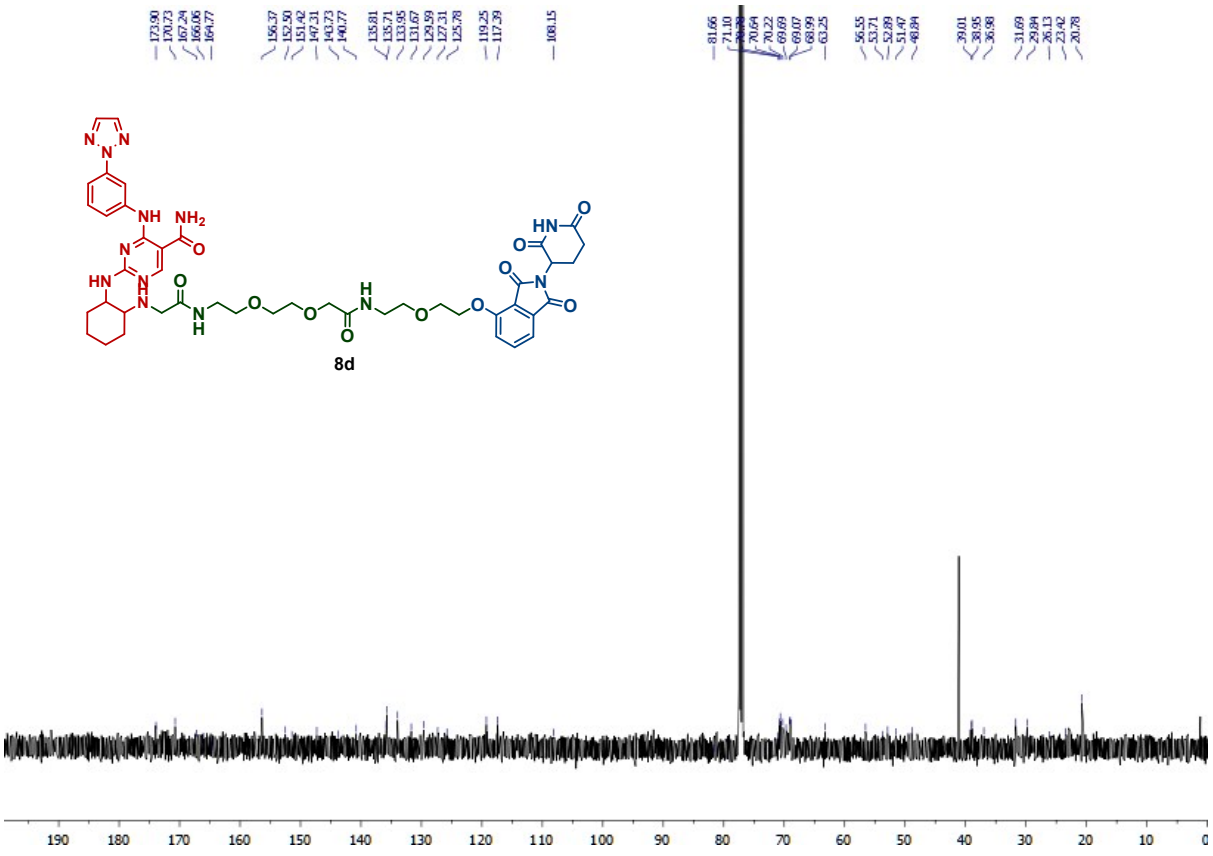
Spectrum 5. ^{1}H spectrum of **8c** in CD_3CN.



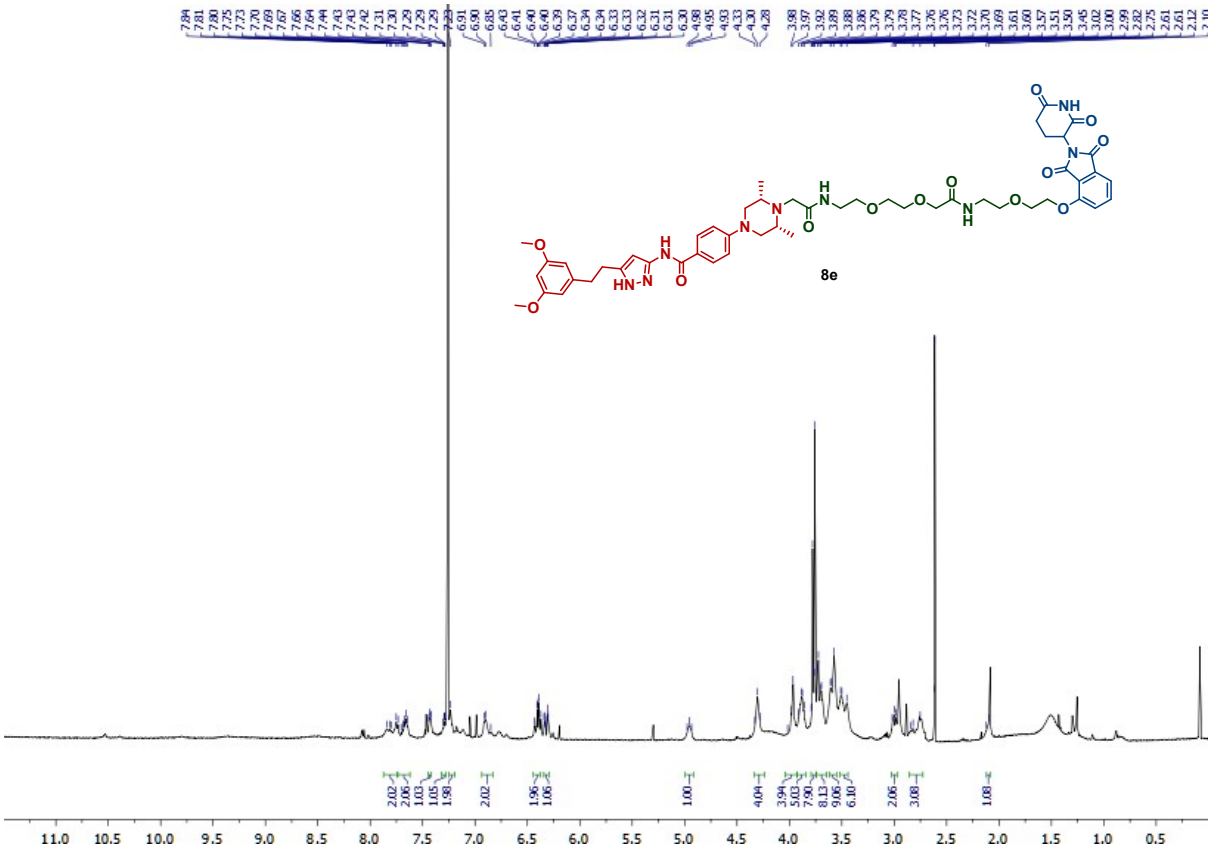
Spectrum 6. ^{13}C spectrum of **8c** in CD_3CN.



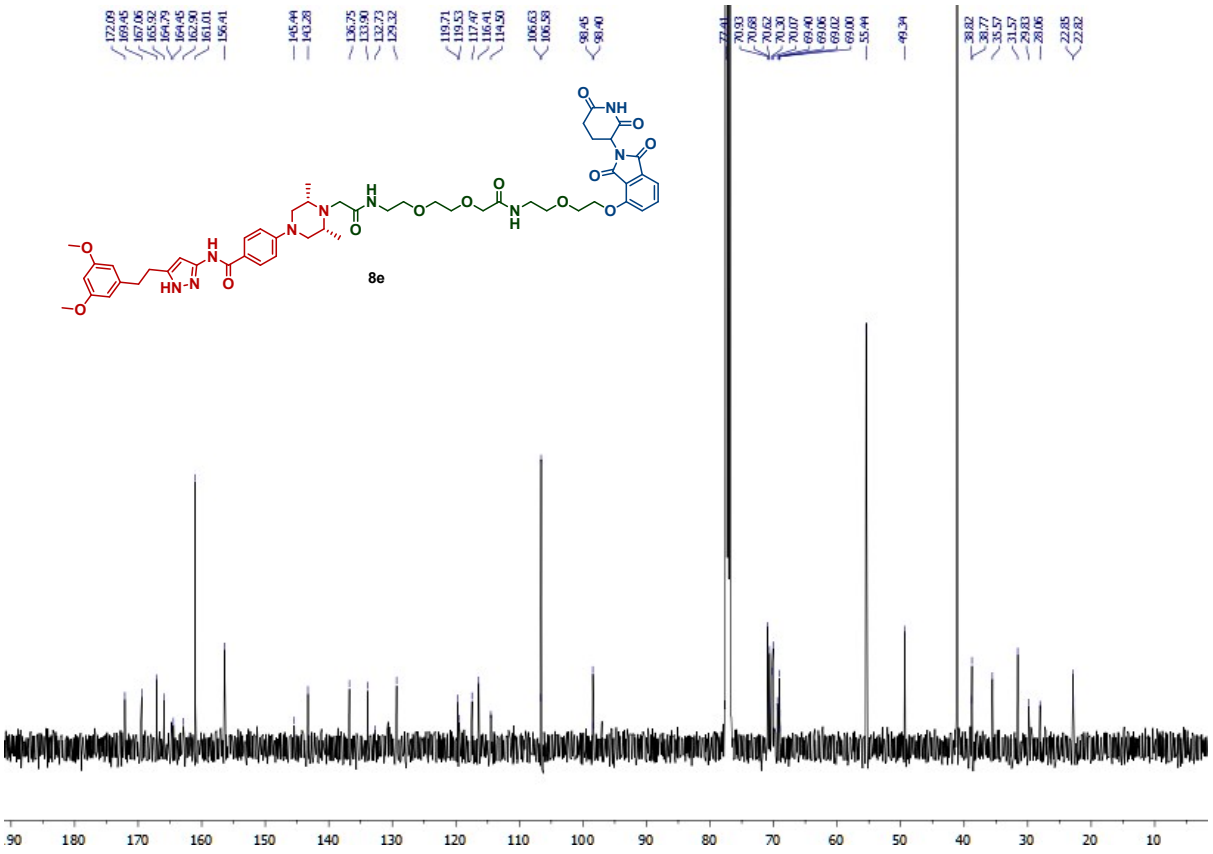
Spectrum 7. ^{1}H spectrum of **8d** in $CDCl_3$.



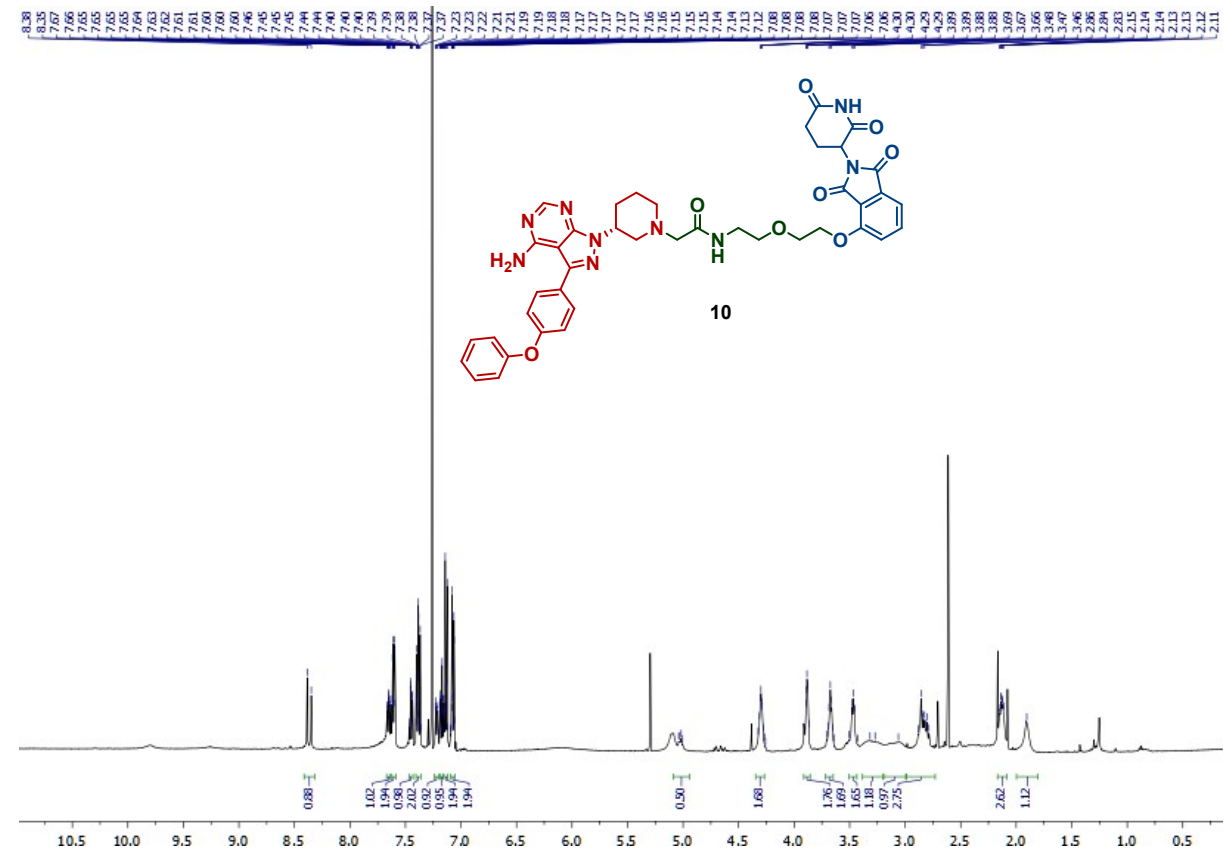
Spectrum 8. ^{13}C spectrum of **8d** in $CDCl_3$.



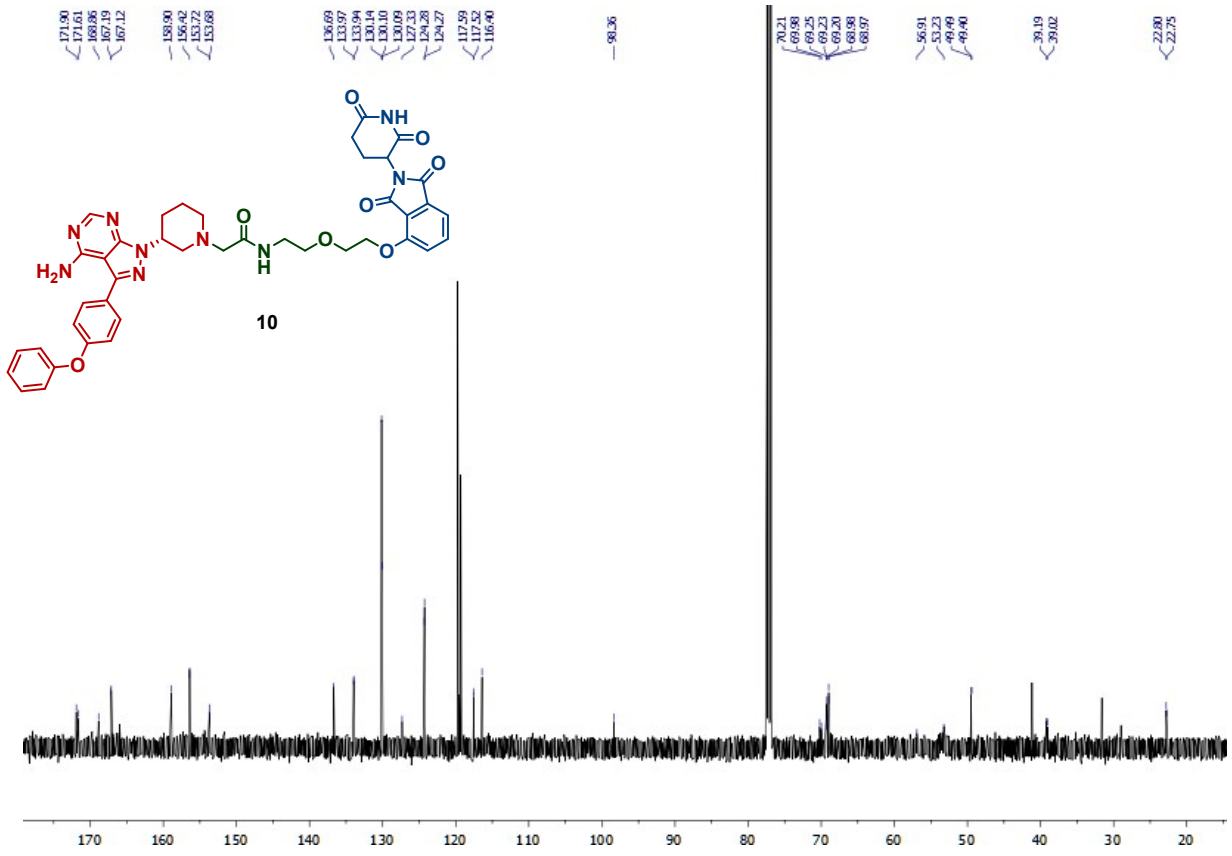
Spectrum 9. ^{1}H spectrum of **8e** in $CDCl_3$.



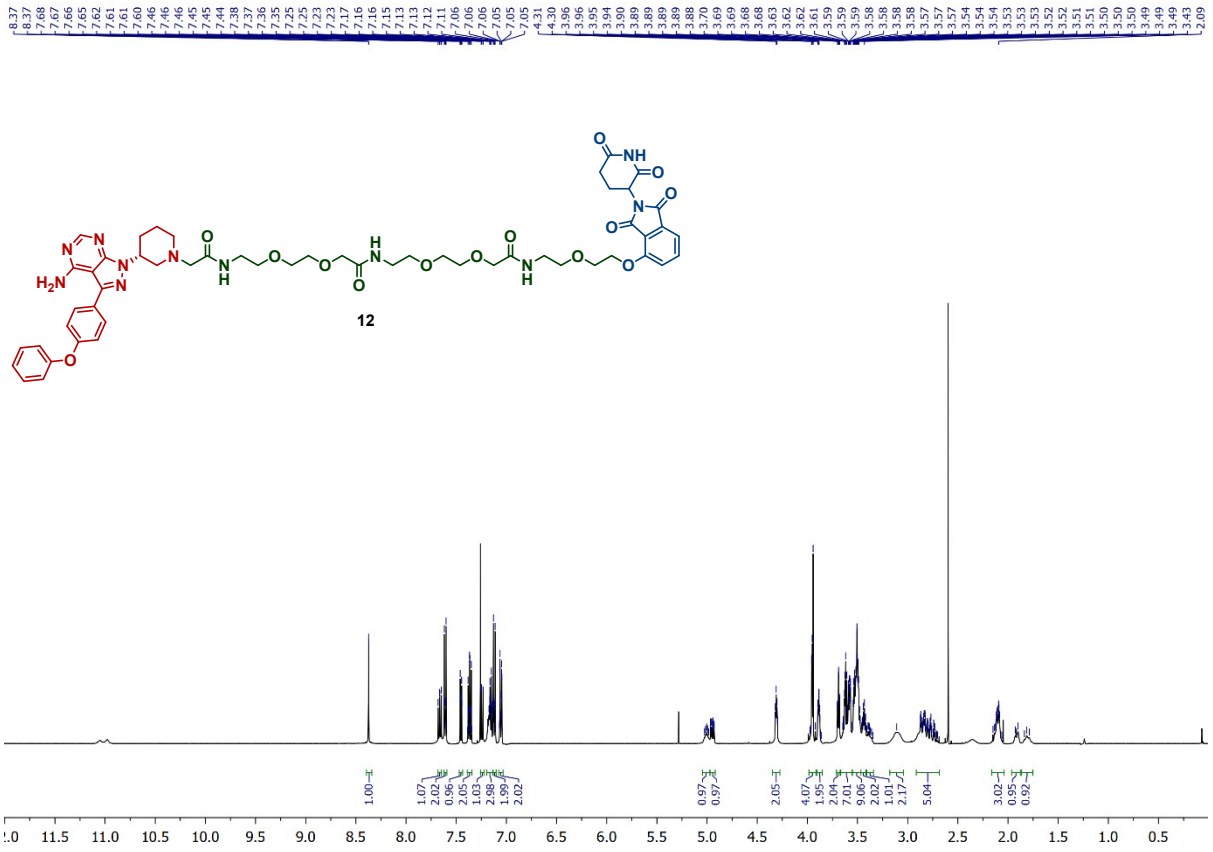
Spectrum 10. ^{13}C spectrum of **8e** in $CDCl_3$.



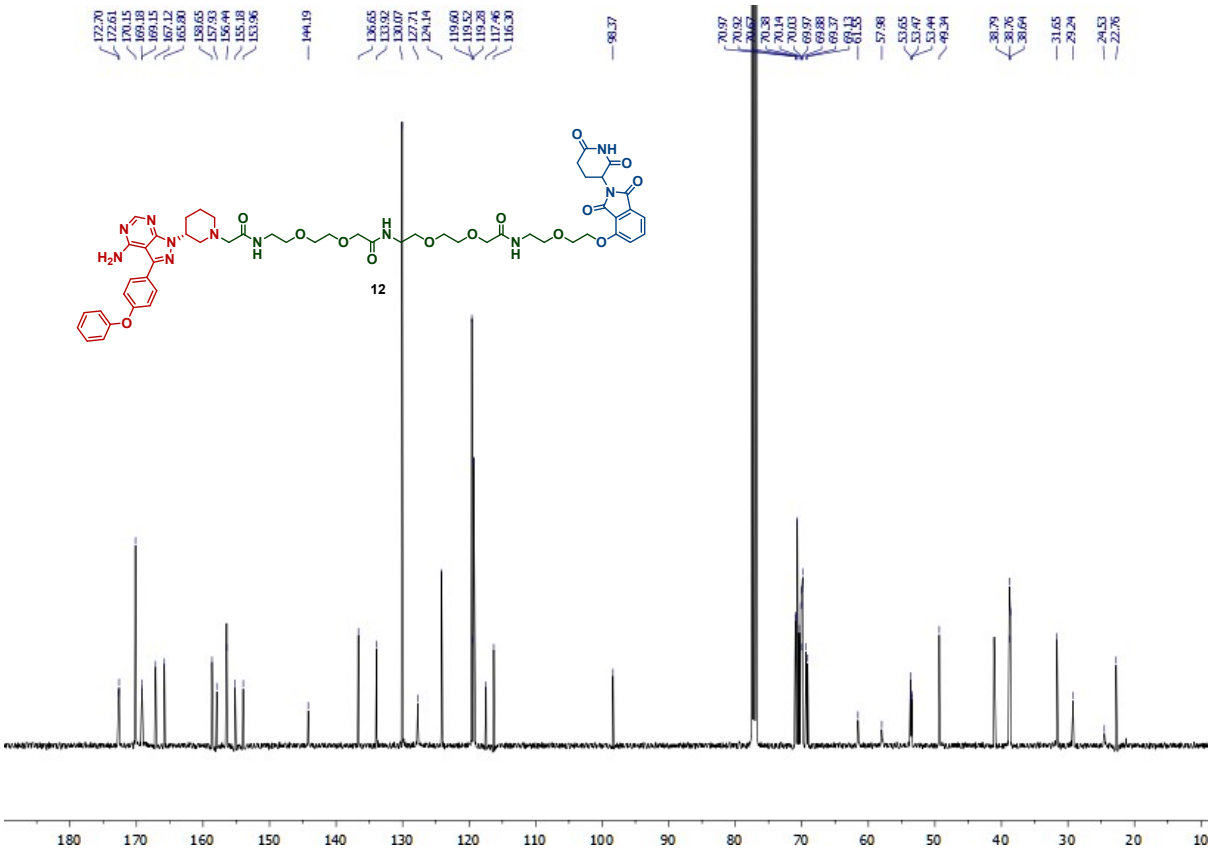
Spectrum 11. ^{1}H spectrum of **10** in $CDCl_3$.



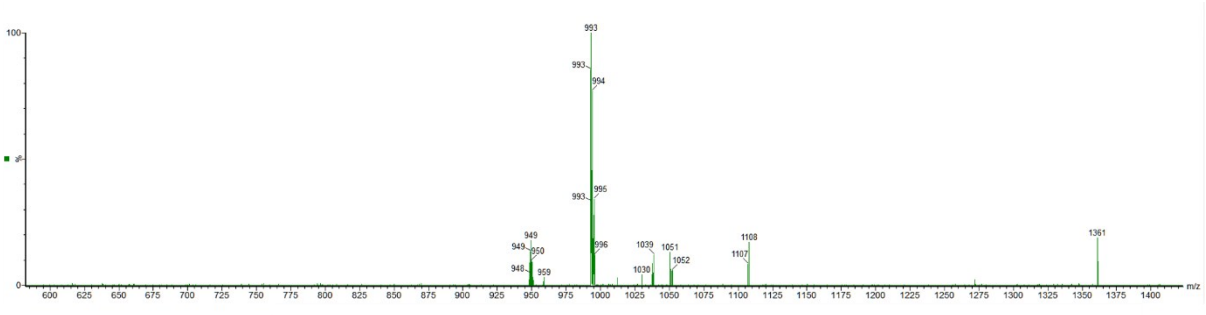
Spectrum 12. ^{13}C spectrum of **10** in $CDCl_3$.



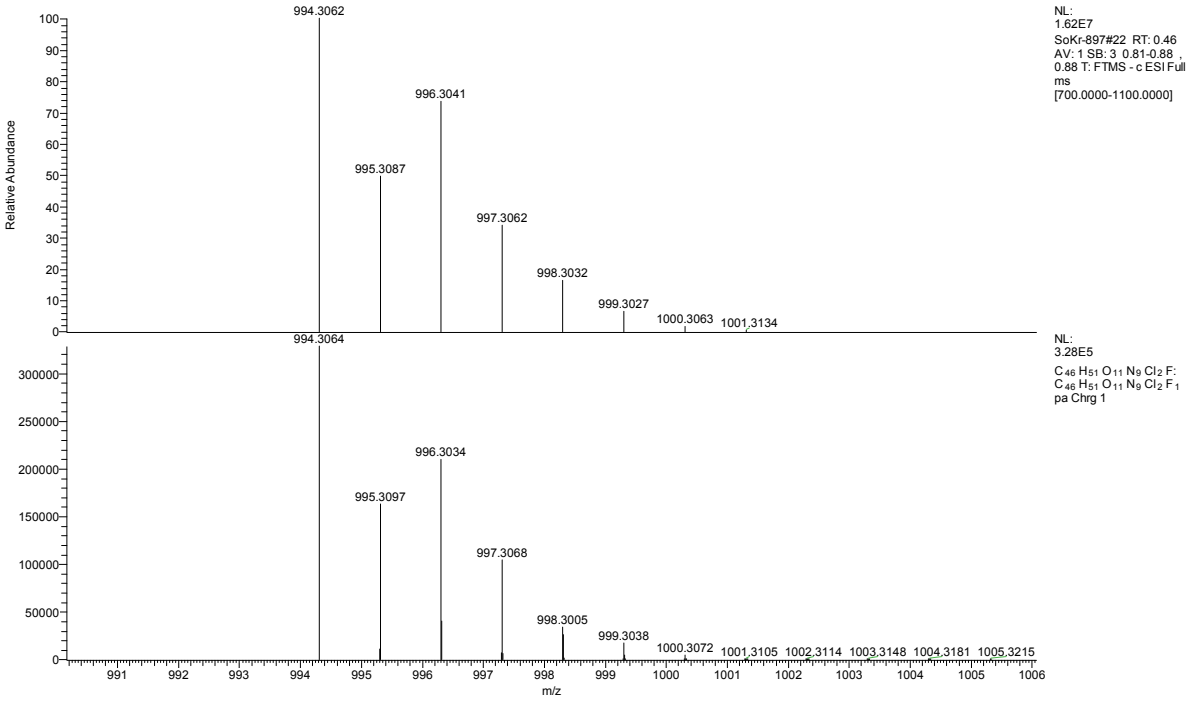
Spectrum 13. ^{1}H spectrum of **12** in $CDCl_3$.



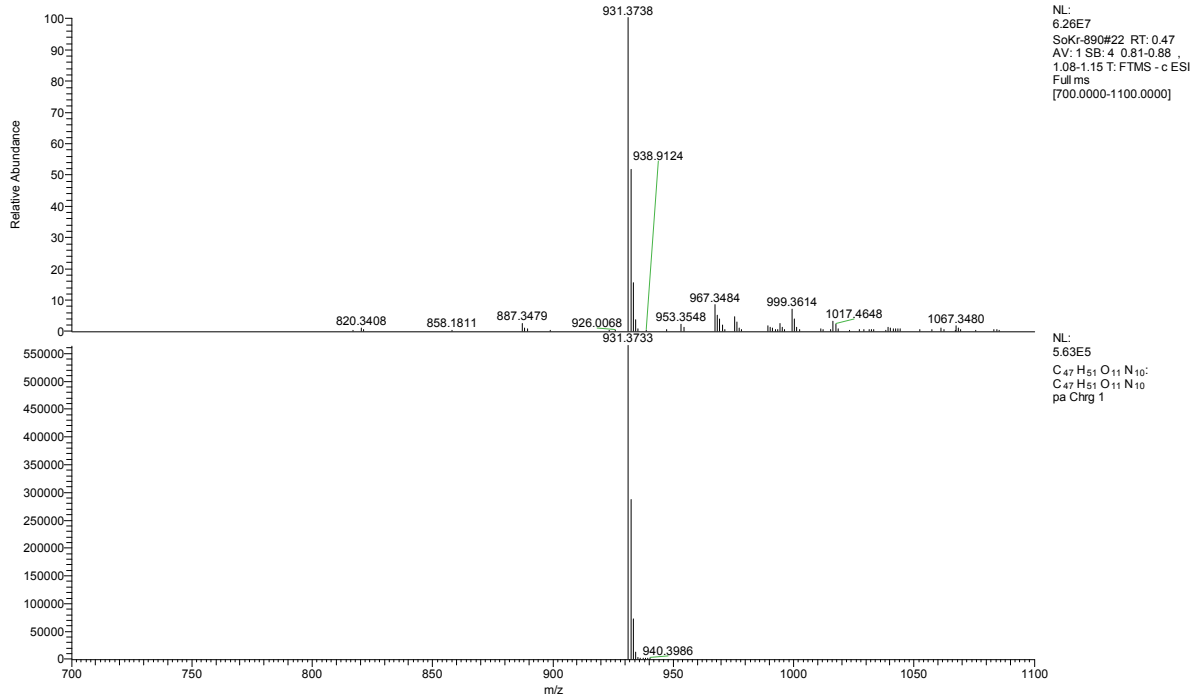
Spectrum 14. ^{13}C spectrum of **12** in $CDCl_3$.



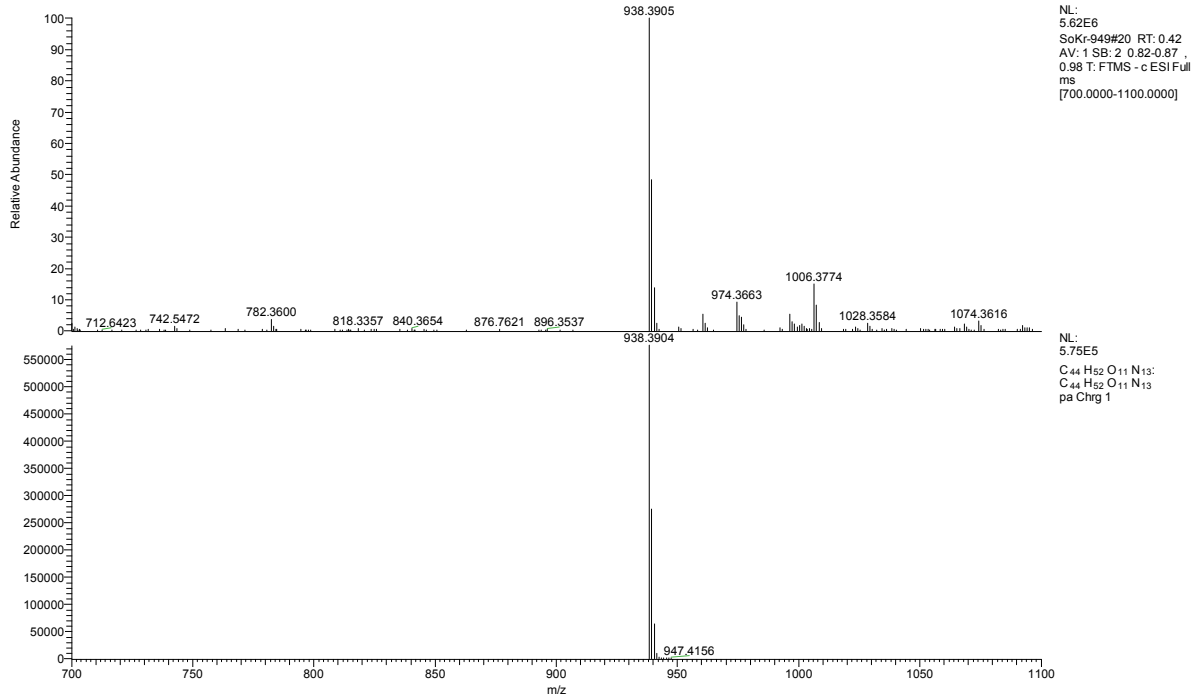
Spectrum 15. MS spectrum of **8a**.



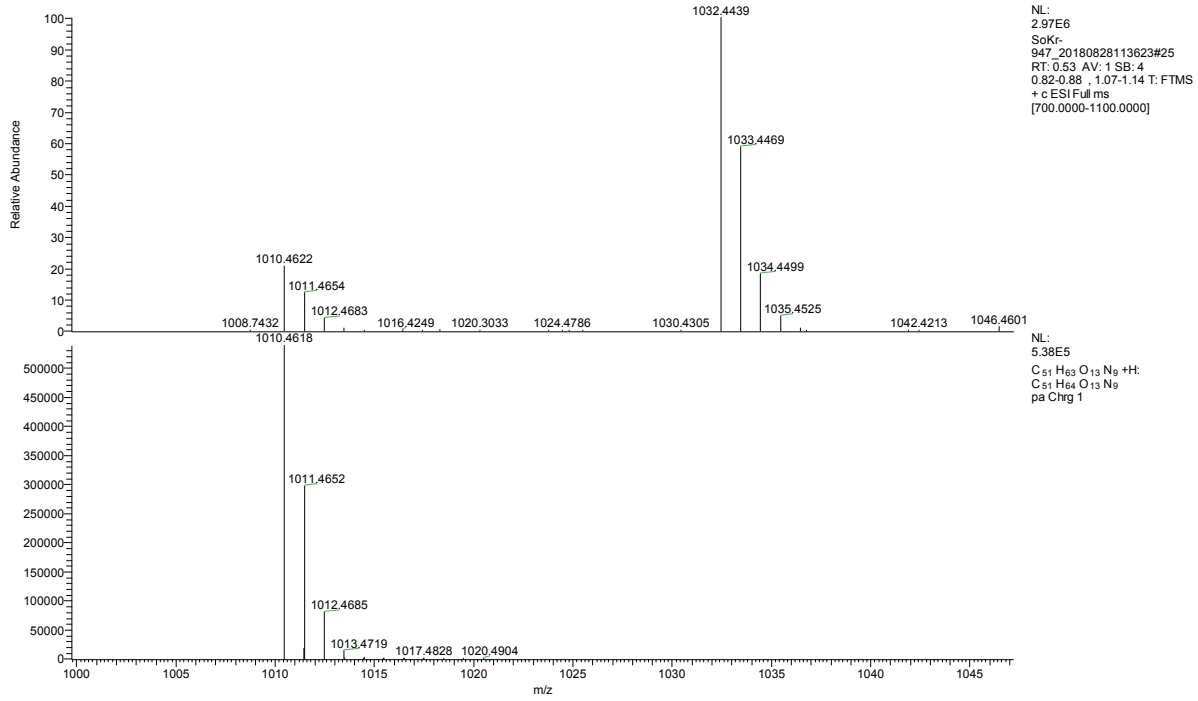
Spectrum 16. HRMS spectrum of **8b**.



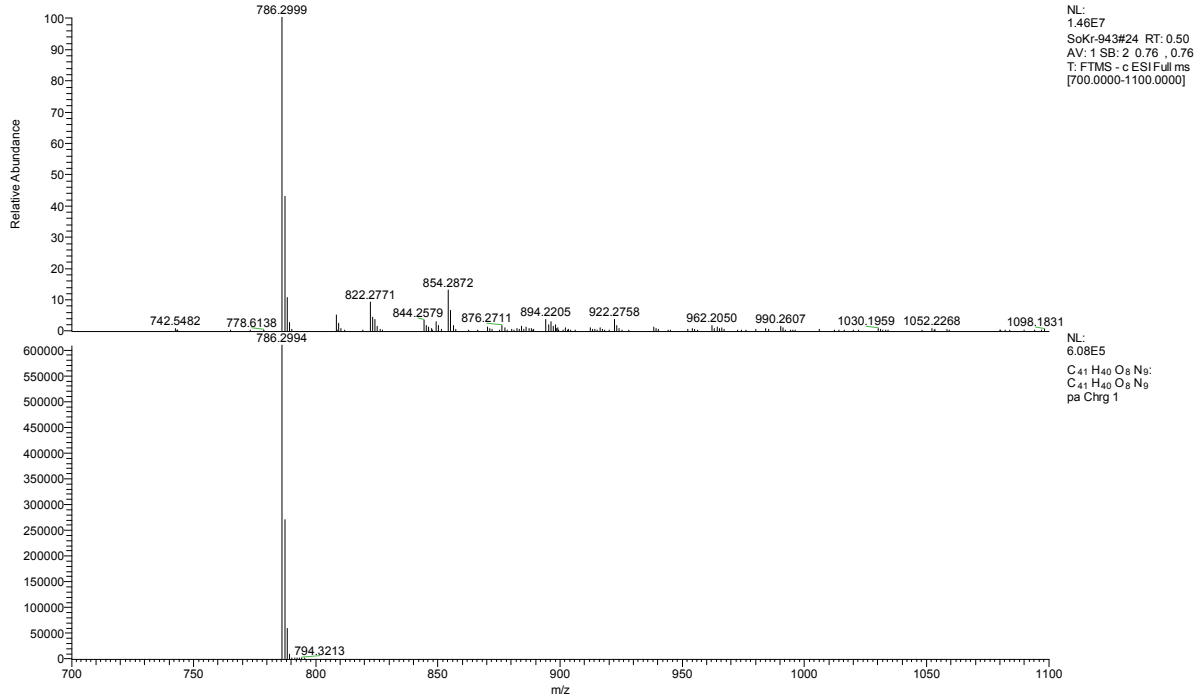
Spectrum 17. HRMS spectrum of **8c**.



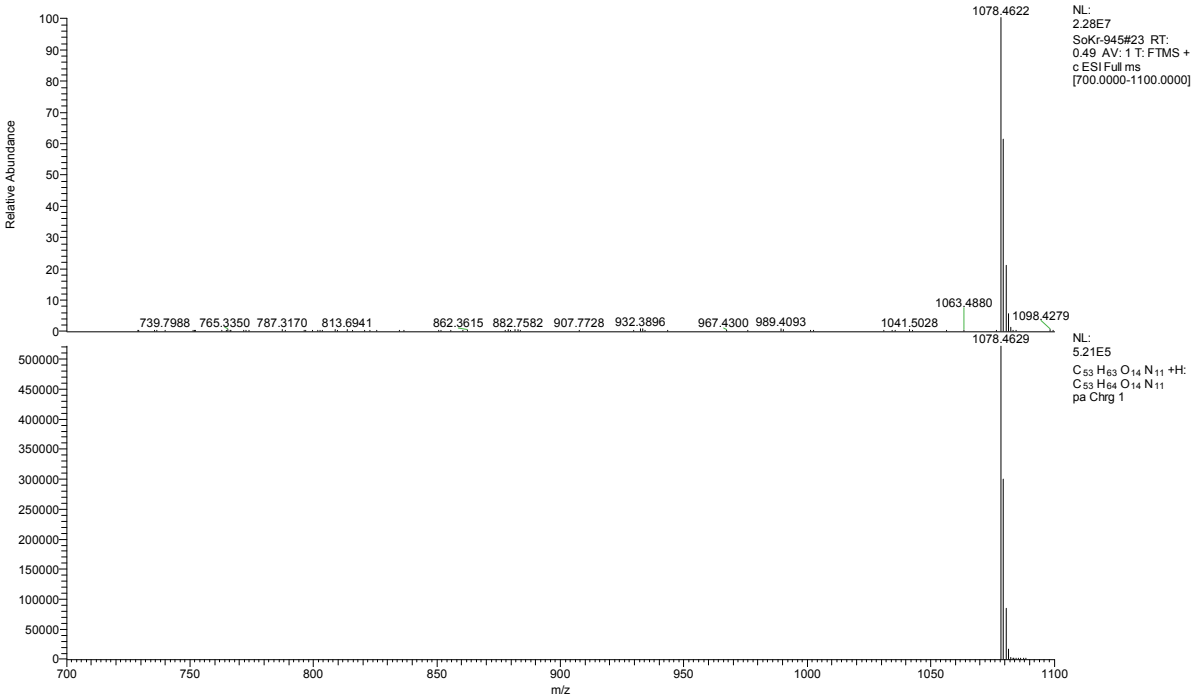
Spectrum 18. HRMS spectrum of **8d**.



Spectrum 19. HRMS spectrum of **8e**.



Spectrum 20. HRMS spectrum of **10**.



Spectrum 21. HRMS spectrum of **12**.