Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Traceless Staudinger Ligation Enabled Parallel Synthesis of Proteolysis Targeting Chimera Linker Variants

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A. Supporting Figures.

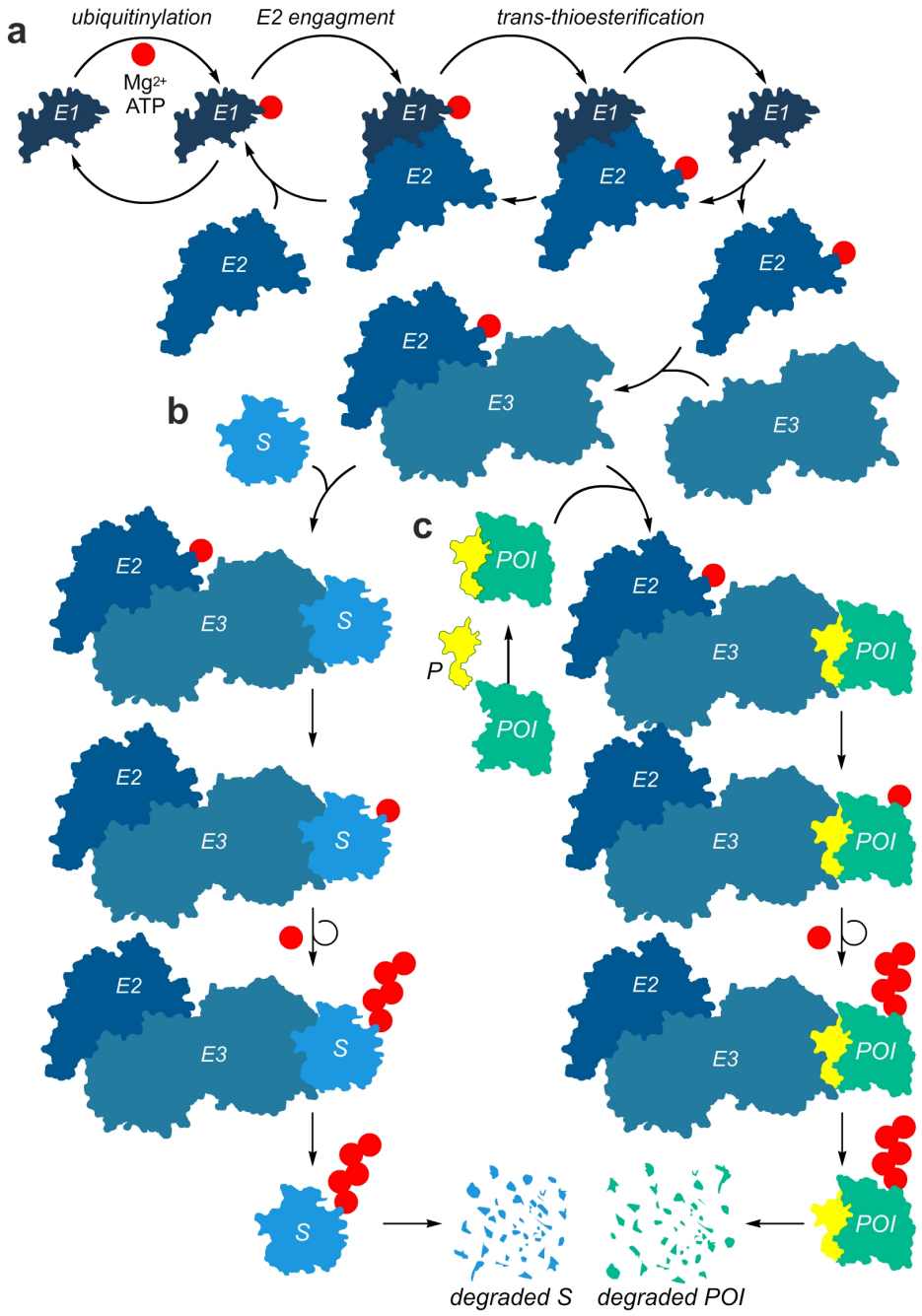


Fig. S1. Ubiquitin conjugation and associated-protein degradation. **a)** Schematic representation of the activation of an E3 (blue) ligase by ubiquitinylation of E1 (dark blue) followed by E2 engagement and trans-thioesterification. **b)** Schematic representation of how a normal E3 ligase targets its substrate (S, light blue) for proteolysis. **c)** Complementary process where a heterobifunctional molecule or PROTAC (p, yellow) is used to target the degradation of a protein of interest (POI, green). In this process, the PROTAC contains motifs that bind to both POI and E3 ligase.

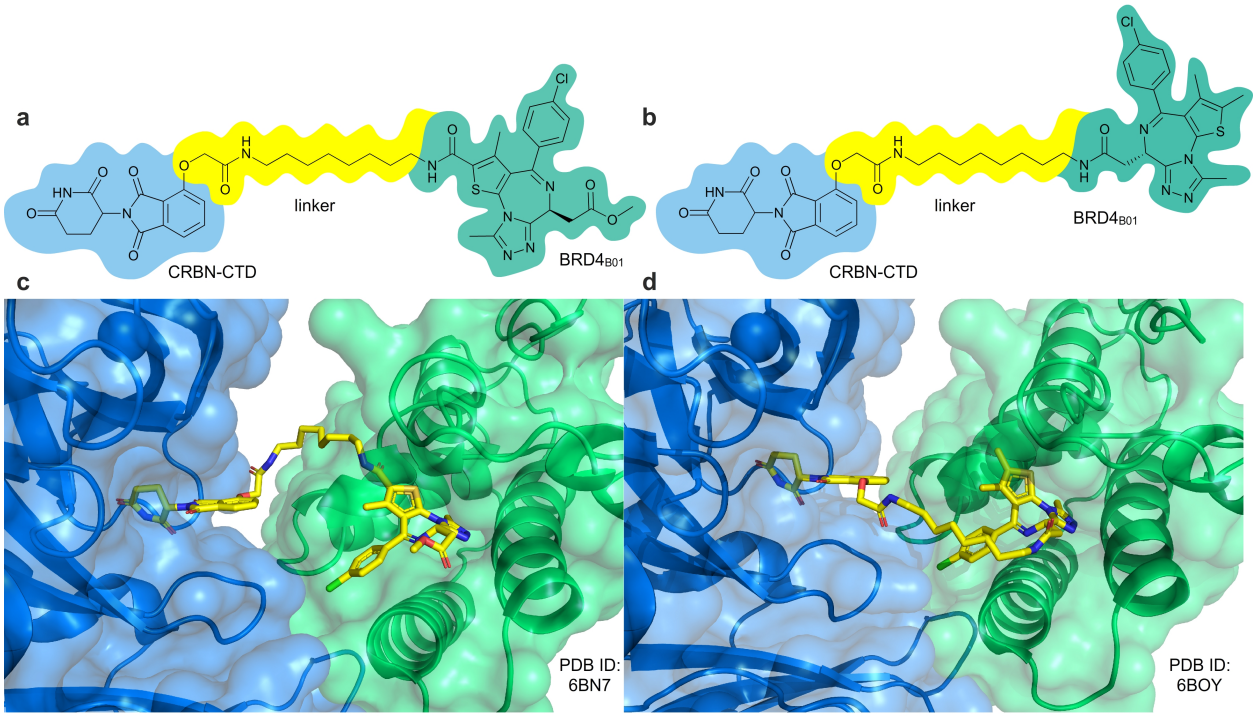
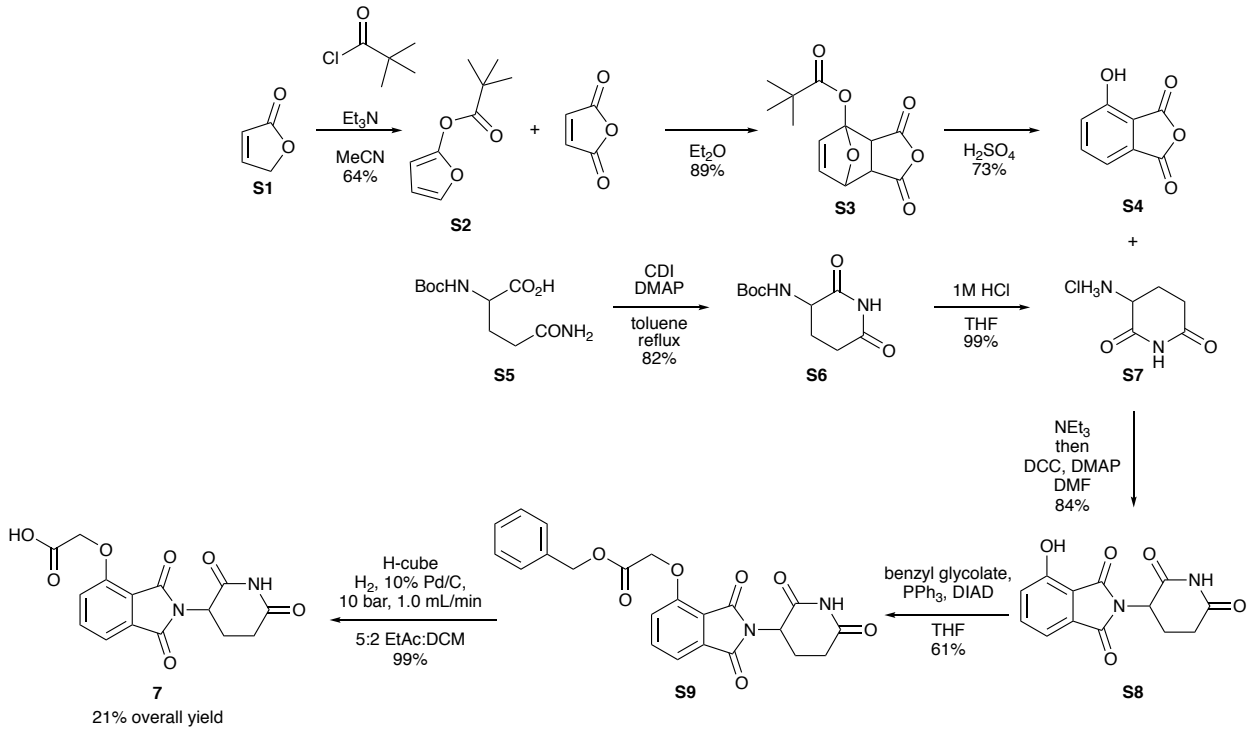


Fig. S2. Structural-basis for PROTAC diversification. **a)** Structure of the dBET23 ligand. **b)** Structure of the dBET6 ligand. **c)** X-ray crystal structure (PDB = 6BN7)[1] depicting the binding of the dBE23 ligand (yellow) between the E3 ligase CRBN-CTD (blue) and protein of interest, $BRD4^{B01}$. **d)** X-ray crystal structure (PDB = 6BOY)[1] depicting the binding of the dBE6 ligand (yellow) between the E3 ligase CRBN-CTD (blue) and protein of interest, $BRD4^{B01}$.

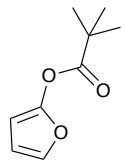
B. General Materials and Methods.

Chemical reagents were purchased from Acros, Fluka, Sigma-Aldrich, or TCI and any further purifications will be denoted in the following section. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories. All reactions were conducted with rigorously dried anhydrous solvents that were obtained by passing through a solvent column composed of activated A1 alumina and dispensed under an atmosphere of argon. An exception was N,N-dimethylformamide (DMF), which was purchased anhydrous (EMD Millipore) and used without further purification via provided septa. Acetonitrile and amines were dried via storage over molecular sieves and used as provided. DABCO was purified fresh before use through sublimation under reduced pressure at 45 °C. All reactions were performed under a positive pressure of argon via balloons unless otherwise noted and glassware was oven-dried and sealed with septa. Stirring was accomplished using Teflon coated stir-bars using an IKA RCT-basic mechanical stirrer. Solutions were heated using silicon oil baths. Analytical Thin Layer Chromatography (TLC) was performed on Silica Gel 60 F254 precoated glass plates (EMD Millipore). Preparative TLC (pTLC) was conducted on Silica Gel 60 F254 plates (EMD Millipore) that were pre-run with chloroform in order to minimize binder grease. Visualization was achieved with UV light (254 nm, 365 nm) and/or $KMnO_4$. Flash column chromatography was carried out with Geduran Silica Gel 60 (230-400 mesh) from Fischer Scientific. ^{1}H NMR spectra were recorded on Varian Mercury Plus 400 or Varian VX500 spectrometers. ^{13}C NMR spectra were recorded at 125 MHz on a Varian VX500 spectrometer equipped with an Xsens Cold probe or at 100 MHz on a Varian Mercury 400 Plus spectrometers. Chemical shifts for ^{1}H NMR and ^{13}C NMR were referenced to the reported values of Gottlieb[2] using the signal from the residual solvent ^{1}H or ^{13}C signals from the deuterated solvent. Chemical shift δ values for ^{1}H and ^{13}C spectra are reported in parts per million (ppm) relative to these referenced values. Multiplicities are abbreviated as; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad signal. All ^{13}C spectra were recorded with complete proton decoupling. FID files were processed using MestreNova 14.0.1 (MestreLab Research). LC-MS was performed on a Waters SQ detector (quadrupole) in either positive or negative electrospray ionization (ESI) modes with a serial Waters Acquity TUV detector, Waters Acquity column manager and Waters Acquity binary solvent manager. Spectral data and procedures are provided for all compounds and spectral data for key compounds are explicitly provided. Compounds not cited in the paper from here on will be numbered starting with **S1**.

C. Synthesis of thalidomide acid 7. Samples of acid **7** were synthesized from furan-2(5H)-one (**S1**) and Boc-glutamine (**S5**) in 8 steps as shown in Fig. S1.



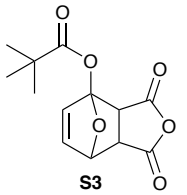
Scheme S1. Synthetic route used to synthesize thalidomide acid **7**, adapted from the conditions outlined by Lohbeck and Millller.[3]



S2

Furan-2-yl pivalate (S2). Furan-2(5*H*)-one (**S1**) (1.7 mL, 23.8 mmol) and pivaloyl chloride (3.5 mL, 28.6 mmol) were dissolved in dry CH_3CN (10 mL) under an Ar atmosphere. Et_3N (4.0 mL, 28.6 mmol) dissolved in dry CH_3CN (5 mL) and added drop wise to the reaction over the course of 5 min *via* a pressure equalizing addition funnel. The reaction was stirred 19 h resulting a brown, opaque solution. The precipitate was filtered off and washed with Et_2O. The organic phase was then washed with 10% w/v Na_2CO_3, dried over Na_2SO_4 and concentrated via nitrogen flow. CAUTION: product is volatile. Crude material was then purified via flash column chromatography (1:20 EtOAc/hexanes), yielding 2.6 g of **S2** (64%), a clear, colorless to pale yellow oil.

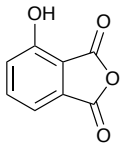
Furan **S2**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (m, 1H), 6.36 (m, 1H), 5.86 (m, 1H), 1.34 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 151.6, 135.4, 111.3, 92.3, 39.3, 27.0. Spectral signals matched those by Lohbeck and Miller.[3]



S3

1,3-Dioxo-3,3a,7,7a-tetrahydro-4,7-epoxyisobenzofuran-4(1*H*)-yl pivalate (S3). Furan **S2** (2.58 g, 15.3 mmol) was dissolved in Et_2O (10 mL) under an atmosphere of Ar. Maleic anhydride (1.65 g, 16.9 mmol) was added and the reaction was allowed to stir for 72 h. Yellow solid **S3** (3.63 g, 89%) was collected *via* vacuum filtration, washed with cold Et_2O.

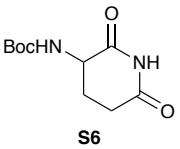
Cycloadduct **S3**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.72 (m, 2H), 5.34 (m, 1H), 3.79 (m, 1H), 3.44 (m, 1H), 1.32 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 169.4, 166.0, 138.1, 137.2, 111.7, 77.3, 52.7, 49.0, 39.2, 27.0. Spectral signals matched those by Lohbeck and Miller.[3]



S4

4-Hydroxyisobenzofuran-1,3-dione (S4). Concentrated H_2SO_4 (3 mL) was cooled to -30 °C. Cycloadduct **S3** (1.12 g, 4.20 mmol) was slowly added and stirred for 5 min. The reaction mixture was then poured over crushed ice (3.3 g) and the resulting brown precipitate **S4** (500 mg, 73%) was collected *via* vacuum filtration and dried overnight under vacuum.

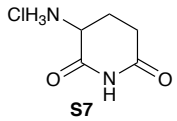
Anhydride **S4**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 7.78 (dd, J = 8.4, 7.3 Hz, 1H), 7.46 (d, J = 7.3 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.5, 160.9, 157.1, 138.2, 132.8, 124.4, 116.1, 114.5. HRMS (+ESI) calc. for $[C_8H_5O_4]^+$ 165.0182 ($[M+H]^+$), found *m/z* 165.0179. Spectral signals matched those by Lohbeck and Miller.[3]



S6

***tert*-Butyl (2,6-dioxopiperidin-3-yl)carbamate (S6). Boc-**glutamine **S5** (2.50 g, 10.15 mmol) was dissolved in THF (12 mL). *N*,*N*-Carbonyldiimidazole (1.86 g, 11.2 mmol) and DMAP (124.0 mg, 1.02 mmol) were added. The solution was brought to reflux for 22 h. The reaction was then cooled to rt and further cooled in an ice bath. The precipitate was filtered off, washed with cold THF yielding 1.89 g of **S6** (82%), a colorless solid.

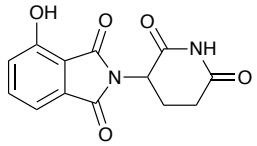
Carbamate **S6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 7.13 (d, J = 8.6 Hz, 1H), 4.20 (m, 1H), 2.71 (m, 1H), 2.46 (m, 1H), 1.90 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 172.1, 155.4, 78.2, 50.4, 31.0, 28.0, 23.8. HRMS (+ESI) calc. for $[C_{10}H_{16}N_2O_4Na]^+$ 251.1002 ($[M+Na]^+$), found *m/z* 251.1005. Spectral signals matched those by Lohbeck and Miller.[3]



S7

3-Aminopiperidine-2,6-dione hydrochloride (S7). HCl (12 M, 35 mL) was added slowly to MeOH (120 mL) with stirring. *tert*-Butyl (2,6-dioxopiperidin-3-yl)carbamate (**S6**) (1.06 g, 4.66 mmol) was added and the reaction was stirred for 2 h. The solvent was then removed by rotary evaporation yielding 770 mg of **S7** (99%), as a white powder.

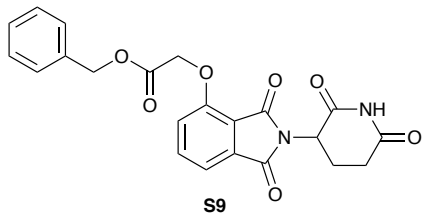
Glutarimide **S7**. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 8.67 (s, 3H), 4.20 (dd, J = 8.2, 4.5 Hz, 1H), 2.65 (m, 2H), 2.22 (m, 1H), 2.00 (m, 1H). Spectral signals matched those by Lohbeck and Miller.[3]



S8

2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (S8). Anhydride **S4** (1.04 g, 6.35 mmol) and glutarimide **S7** (0.86 g, 5.20 mmol) and Et_3N (0.76 mL, 5.46 mmol) were dissolved in dry THF (25 mL). The mixture was stirred for 15 min and then brought to reflux for 5 h. After cooling, *N*,*N*'-dicyclohexylcarbodiimide (1.28 g, 5.85 mmol) and DMAP (64 mg, 0.52 mmol) were added. The reaction mixture was then refluxed overnight. The heat was removed and the reaction was cooled in an ice bath. The precipitate was filtered off via vacuum filtration and washed with THF. The filtrate was concentrate on a rotary evaporator. The crude product was then purified via trituration (CH_2Cl_2/MeOH) yielding 1.19 g of hydroxy-thalidomide **S8** (84%), as a yellow solid. Spectral signals matched those by Lohbeck and Miller.[3]

Hydroxy-thalidomide **S8**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.24 (br s, 1H), 11.12 (s, 1H), 7.64 (dd, J = 8.3, 7.2 Hz, 1H), 7.31 (d, J = 7.2 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 5.07 (dd, J = 12.9, 5.4 Hz, 1H), 2.88 (m, 1H), 2.55 (m, 2H), 2.04-1.97 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.9, 170.2, 167.2, 166.0, 156.0, 136.3, 133.2, 124.0, 114.2, 113.8, 48.6, 31.0, 22.1.

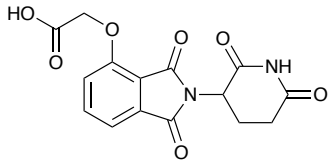


S9

Benzyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (S9). 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (**S8**) (49.4 mg, 0.18 mmol) and PPh_3 (72.9 mg, 0.28 mmol) were dissolved in THF (1 mL) under argon with 4Å molecular sieves. Benzyl glycolate (30.0 µL, 0.20 mmol) was added and the mixture was sonicated for 10 min. DIAD (40 µL, 0.20 mmol) was added drop wise to the solution and sonication continued for an additional 30 min. The reaction was then moved to a stir plate and allowed to stir for an additional 20 h. The sieves were filtered off and washed with EtOAc (1 mL) and the reaction mixture was partitioned between EtOAc (30 mL) and water (20 mL). Aqueous layer was extracted with EtOAc (30 mL) and the combined organic phases were dried over Na_2SO_4 and concentrated on

a rotary evaporator. The crude material was further purified *via* flash column chromatography (65% EtOAc/hexanes) to yield 46.7 mg of benzyl-thalidomide **S9** (61%), as a white foam.

Benzyl-thalidomide **S9.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.61 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.50 (d, *J* = 7.3 Hz, 1H), 7.34 (m, 5H), 7.07 (d, *J* = 8.4 Hz, 1H), 4.89 (m, 3H), 2.80 (m, 3H), 2.11 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 168.1, 167.9, 166.9, 165.5, 155.4, 136.5, 135.0, 134.0, 128.8, 128.8, 128.7, 126.9, 120.1, 117.8, 117.3, 67.5, 66.4, 49.3, 31.5, 22.7; HRMS (+ESI) calc. for $[C_{22}H_{18}N_2O_7NH_4]^+$ 440.1452 ($[M+NH_4]^+$), found *m/z* 440.1452, calc. for $[C_{22}H_{18}N_2O_7Na]^+$ 445.1006 ($[M+Na]^+$), found *m/z* 445.1005. Spectral signals matched those by Lohbeck and Miller.[3]

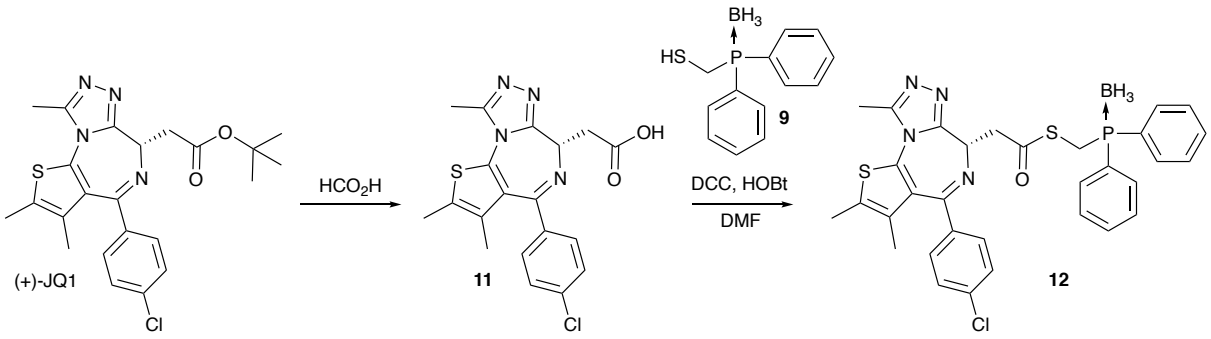


7

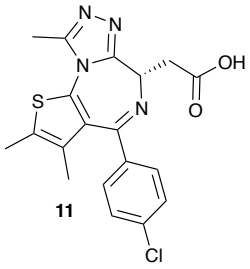
2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (7). Benzyl-thalidomide **S9** (258.8 mg, 0.6 mmol) was dissolved in a 5:3 mixture of EtOAc:CH_2Cl_2 (150 mL) and eluted through an H-cube flow hydrogenolysis apparatus (10% Pd/C, 10 bar, 1.0 mL/min). Receiver flask was concentrated on a rotary evaporator yielding 208.6 mg of acid **7** (99%), as a white powder.

Acid **7**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.23 (br s, 1H), 11.13 (s, 1H), 7.79 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 5.11 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.99 (s, 2H), 2.93-2.85 (m, 1H), 2.63-2.47 (m, 2H), 2.06-2.01 (m, 1H). HRMS (-ESI) calc. for $[C_{15}H_{11}N_2O_7]^-$ 331.0569 (M^-), found *m/z* 331.0569. Spectral signals matched those by Lohbeck and Miller.[3]

D. Synthesis of (+)-JQ1 thioester 12. A two-step procedure was used to prepare thioester **12** from (+)-JQ1 as shown in Supplementary Scheme S2.



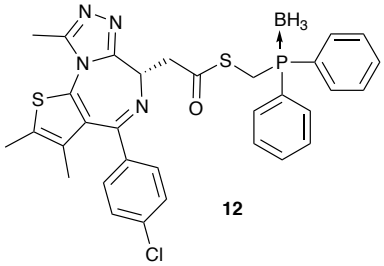
Scheme S2. Synthetic route to (+)-JQ1 thioester **12.** Synthetic route of **11** adapted from Winter *et. al*.[4]



(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetic acid (11).

(+)-JQ1 (24.3 mg, 0.053 mmol) was stirred in anhydrous HCO_2H (4 mL) under an Ar atmosphere for 48 h. Solvent was removed by rotary evaporation yielding 22.8 mg of acid **11** (99%) and was used without further purification.

(+)-JQ1 acid **11**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.61 (t, *J* = 8 Hz, 1H), 3.66 (m, 2H), 2.70 (s, 3H), 2.41 (s, 3H), 1.69 (s, 3H); LRMS (-ESI) calc. for $[C_{19}H_{16}ClN_4O_2S]^-$ 399.07 ($[M-H]^-$), found *m/z* 398.95. Spectral signals matched those by Winter *et. al.*[4]

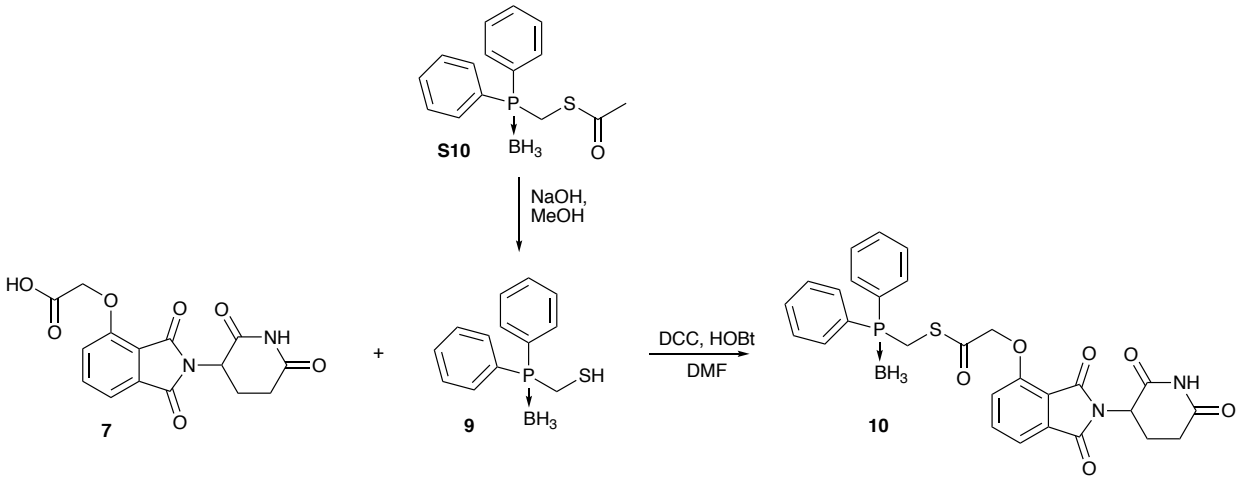


***S*-((diphenylphosphaneyl)methyl) (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)ethanethioate borane complex (12).** Acid **11** (10.0 mg, 0.025 mmol) and HOBt (3.7 mg, 0.027 mmol) were dissolved in DMF (0.3 mL) under an Ar atmosphere. DCC (5.7 mg, 0.027 mmol) was added. After stirring at rt overnight, thiol **9** (6.8 mg, 0.027 mmol) was added as a solution in CH_2Cl_2 (0.2 mL). After stirring overnight, the solvent

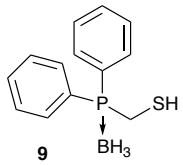
was removed *via* vacuum and the crude material was purified on a microcolumn containing 1 g of silica gel. Column was eluted first with Et_2O (10 mL) and then EtOAc (10 mL). Rotary evaporation of the EtOAc fraction returned at 5.0 mg of **12** (32%). The reaction product could be used without silica gel purification.

Thioester **12**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (m, 4H), 7.46 (m, 6H), 7.36 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 2H), 4.57 (dd, *J* = 9.0, 4.5 Hz, 1H), 3.88 (dd, *J* = 16.1, 9.1 Hz, 1H), 3.80 (m, 2H), 3.68 (dd, *J* = 16.1, 4.5 Hz, 1H), 2.66 (s, 3H), 2.42 (s, 3H), 1.69 (s, 3H), 0.92-0.80 (BH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.4, 165.0, 154.5, 150.1, 137.0, 136.5, 132.6 (d, *J* = 9.5 Hz), 132.6 (d, *J* = 9.5 Hz), 132.3, 132.0 (d, *J* = 2.5 Hz), 131.9 (d, *J* = 2.5 Hz), 131.3 (d, *J* = 9.7 Hz), 131.1 (d, *J* = 10.4 Hz), 130.4, 130.1, 129.5 (d, *J* = 10.5 Hz), 129.3 (d, *J* = 10.2 Hz), 129.1 (d, *J* = 10.0 Hz), 129.0 (d, *J* = 10.0 Hz), 128.8, 127.9 (d, *J* = 55.1 Hz), 127.6 (d, *J* = 55.3 Hz), 124.0, 118.7, 111.2, 53.8, 45.8, 23.7 (d, *J* = 34.5 Hz), 14.6, 13.29, 12.0; LC-MS (+ESI) calc. for $[C_{32}H_{32}BClN_4OPS_2]^+$ 629.15 ($[M+H]^+$), found *m/z* 629.26.

E. Synthesis of thalidomide thioester 10. A two-step procedure was used to prepare thioester **10** from acid **7** and thioester **S10** as shown in Supplementary Scheme S3.

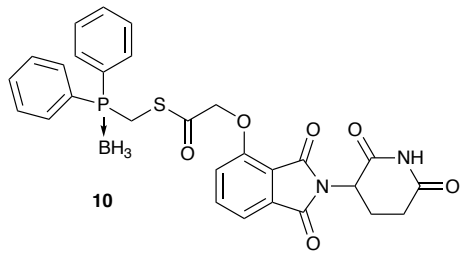


Scheme S3. Synthesis of thalidomide thioester **10** adapted from Mühlberg *et. al*.[5]



Diphenylphosphaneylmethanethiol borane complex (9). Thioester **S10** (91.8 mg, 0.319 mmol) was dissolved in MeOH (2 mL). NaOH (25.1 mg, 0.637 mmol) was added as a solid. After stirring for 3 h at rt, the solvent was removed *via* rotary evaporation. The crude residue partitioned between CH_2Cl_2 (30 mL) and 0.5 M HCl (30 mL). Organic phase was washed with 0.5 M HCl (30 mL), brine (30 mL) and then dried over Na_2SO_4 and concentrated *via* rotary evaporation affording 84.2 mg of **9** (99%), which was used without further purification.

Thiol **9**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (m, 10H), 3.19 (dd, *J* = 8.2, 6.1 Hz, 2H), 1.89 (td, *J* = 8.2, 8.1, 6.7 Hz, 1H), 1.40-0.72 (br m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 132.6 (d, *J* = 9.0 Hz), 131.8 (d, *J* = 2.4 Hz), 129.1 (d, *J* = 10.0 Hz), 127.8 (d, *J* = 55.2 Hz), 19.8 (d, *J* = 32.6 Hz).

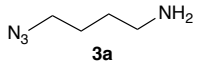


***S*-((diphenylphosphaneyl)methyl) 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethanethioate borane complex (10).** Acid **7** (31.5 mg, 0.095 mmol) and HOBt (14.5 mg, 0.100 mmol) were dissolved in DMF (0.4 mL) under an Ar atmosphere. DCC (24.8 mg, 0.120 mmol) was added. After stirring at rt for 30 min, thiol **9** (21.2 mg, 0.086 mmol) was added as a solution in DMF (0.35 mL). The resulting solution was stirred at rt for 18 h. The solution was filtered to remove the precipitate and filtrate concentrated on a rotary evaporator. Note: do

not attempt aqueous workup or thioester will hydrolyze. The crude product was then purified by flash column chromatography 7:3 EtOAc/hexanes to yielding 35.3 mg of **10** (73%), as a white wax.

Thioester **10**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.69 (m, 4H), 7.63 (m, 1H), 7.53 (m, 3H), 7.45 (dt, *J* = 7.6, 2.3 Hz, 4H), 6.98 (d, *J* = 8.4 Hz, 1H), 4.97 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.87 (s, 2H), 3.77 (d, *J* = 6.9 Hz, 2H), 2.90 (m, 1H), 2.79 (m, 2H), 2.16 (m, 1H), 1.93-1.17 (BH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.6, 171.0, 168.0, 166.7, 165.3, 154.8, 136.7, 134.0, 132.6 (d, *J* = 9.5 Hz), 132.0 (d, *J* = 2.5 Hz), 129.1 (d, *J* = 10.2 Hz), 127.4 (d, *J* = 55.4 Hz), 120.5, 118.1, 117.9, 73.6, 49.4, 31.5, 22.8 (d, *J* = 32.4 Hz), 22.7; LRMS **(**+ESI) calc. for $[C_{28}H_{24}N_2O_6PS]^+$ 547.11 ($[M\text{-}BH_3+H]^+$), found *m/z* 547.18, $[C_{28}H_{23}N_2NaO_6PS]^+$ 569.09 ($[M\text{-}BH_3+Na]^+$), found *m/z* 569.09, $[C_{28}H_{26}BN_2NaO_6PS]^+$ 583.12 ($[M+Na]^+$), found *m/z* 583.07.

F. Synthesis of thalidomide azides 8a and 8c. The following methods were used to prepare thalidomide azides **8a** and **8c**.

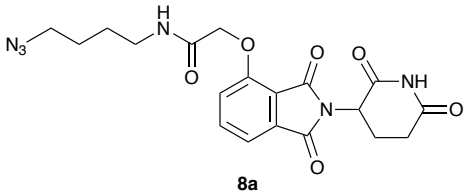


3a

4-azidobutan-1-amine (3a).

Azidoamine **3a** was synthesized using previously published methods and spectral signals matched those provided.[7]

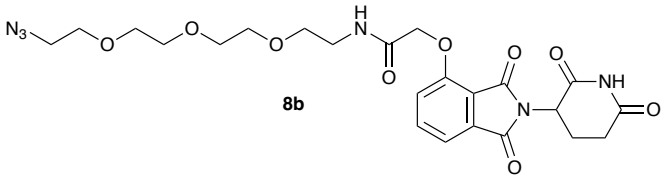
Amine **3a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 3.28 (t, *J* = 6.7 Hz, 2H), 2.73 (t, *J* = 6.9 Hz, 2H), 2.67 (br s, 2H), 1.58 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.4, 41.4, 30.2, 26.4; HRMS (+ESI) calc. for $[C_4H_{11}N_4]^+$ 115.0978 ($[M+H]^+$), found *m/z* 115.0976.



8a

***N*-(4-azidobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (8a).** Acid **7** (8.2 mg, 0.025 mmol), azidoamine **3a** (3.4 mg, 0.030 mmol) and Et*i*Pr$_2$N (10 µL, 0.062 mmol) were dissolved in DMF (0.2 mL) under an Ar atmosphere. HATU (12.2 mg, 0.045 mmol) was added and the reaction stirred for 24 h. Reaction was diluted with EtOAc (10 mL). The organic phase was then washed with sat. NH_4Cl (10 mL), 10% w/v Na_2CO_3 (10 mL) and brine (10 mL). The organic layer was then dried over Na_2SO_4 and concentrated on a rotary evaporator to yield 5.4 mg of azide **8a** (50%).

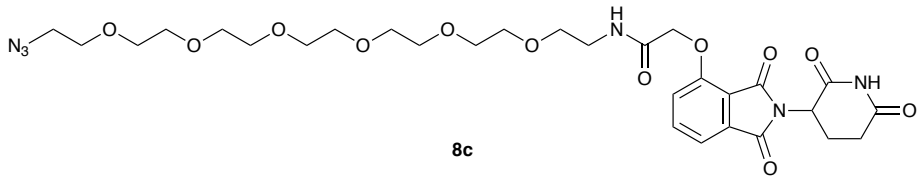
Azide **8a**. ^{1}H NMR (500MHz, $CDCl_3$) δ 8.21 (br s, 1H), 7.75 (m, 1H), 7.55 (m, 2H), 7.20 (d, *J* = 8.3 Hz, 1H), 4.98 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.65 (s, 2H), 3.42 (q, *J* = 6.2 Hz, 2H), 3.32 (t, *J* = 6.2 Hz, 2H), 2.80 (m, 3H), 2.18 (m, 1H), 1.70 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 168.1, 167.0, 166.7, 166.3, 154.6, 137.3, 133.6, 119.8, 118.3, 117.7, 68.3, 51.2, 49.4, 38.8, 31.5, 26.7, 26.4, 22.7; LC-MS (+ESI) calc. for $[C_{19}H_{21}N_6O_6]^+$ 429.15 ($[M+H]^+$), found *m/z* 429.19. Spectral signals matched those previously reported.[6]



8b

N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (8b). Acid **7** (17.3 mg, 0.052 mmol), azidoamine **3b** (14.8 mg, 0.068 mmol) and Et*i*Pr$_2$N (23 µL, 0.130 mmol) were dissolved in DMF (0.2 mL) under an Ar atmosphere. HATU (25.7 mg, 0.068 mmol) was added and the reaction stirred for 24 h. Reaction was diluted with EtOAc (10 mL). The organic phase was then washed with sat. NH_4Cl (10 mL), 10% w/v Na_2CO_3 (10 mL) and brine (10 mL). The organic layer was then dried over Na_2SO_4 and concentrated on a rotary evaporator to yield 13.1 mg of azide **8b** (47%).

Azide **8b**. ^{1}H NMR (500MHz, $CDCl_3$) δ 8.70 (br s, 1H), 7.74 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.63 (m 1H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 4.95 (dd, *J* = 12.2, 5.5 Hz, 1H), 4.65 (s, 2H), 3.63 (m, 14H) 3.40 (m, 2H), 2.89 (t, *J* = 6.2 Hz, 1H), 2.78 (m, 2H), 2.15 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 168.2, 167.0, 166.8, 165.9, 154.5, 137.1, 133.7, 119.4, 118.1, 117.5, 72.6, 70.9, 70.6, 70.5, 70.3, 70.0, 69.5, 68.0, 61.9, 50.7, 49.3, 39.2, 31.5, 22.8; HRMS (+ESI) calc. for $[C_{23}H_{29}N_6O_9]^+$ 533.1991 ($[M+H]^+$), found *m/z* 533.1994.



8c

***N*-(20-azido-3,6,9,12,15,18-hexaoxaicosyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (8c).** A solution of **3c** (8.8 mg, 0.025 mmol) in DMF (0.2 mL) was added to thioester **10** (7.0 mg, 0.012 mmol) under an Ar atmosphere. After stirring for 24 h at rt, the reaction was diluted with EtOAc (10 mL). The organic phase was washed with sat. NH_4Cl (10 mL), water (10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated on a rotary evaporator. Crude material was purified on silica microcolumn containing 1 g of silica gel (EtOAc to 1:4 MeOH/EtOAc) to yield 6.3 mg of azide **8c** (73%).

Azide **8c**. ^{1}H NMR (500MHz, $CDCl_3$) δ 9.02 (br s, 1H), 7.74 (dd, *J* = 8.4, 7.4 Hz), 7.68 (br t, *J* = 5.3 Hz, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 4.95 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.65 (s, 2H), 3.64 (m, 26H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.88 (m, 1H), 2.78 (m, 2H), 2.15 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 168.38, 166.9, 166.9, 165.9, 154.5, 137.1, 133.8, 119.3, 118.1, 117.4, 70.8, 70.7, 70.7, 70.6, 70.6, 70.5, 70.4, 70.4, 70.1, 69.6, 67.9, 50.8, 49.4, 39.2, 31.6, 22.8; HRMS (+ESI) calc. for $[C_{29}H_{41}N_6O_{12}]^+$ 665.2777 ($[M+H]^+$), found *m/z* 665.2782.

G. Procedures for the one-pot synthetic approach. The following section provides the general synthetic procedure of the one-pot reaction. For all reactions, the solvents were purged with Ar, all reagents evaporated from benzene to minimize water content and all solutions were made under an atmosphere of Ar in order to minimize introduction of oxygen to one-pot reaction. Procedures are given using the compound numbering in Scheme 1. Anhydrous DMF was used for all reactions.

Preliminary Method. Three methods were examined the first or preliminary method used CDI to engage the coupling of **4** to **3a**-**3b** (Scheme 1). It begins by preparation of stock solutions and then uses these solutions to conduct reactions in parallel using 20 mL vials fitted with rubber septa. It is demonstrated for the preparation of **1a**-**1c**.

Ligand A and CDI (4, Scheme 1): 55 mM **7** with 85 mM CDI in DMF

Ligand B (6, Scheme 1): 43 mM **6** in DMF

Linkers and DMAP (3a-3c, Scheme 1): 100 mM **3a** with 10 mM DMAP in DMF, 100 mM **3b** with 10 mM DMAP in DMF or 100 mM **3c** with 10 mM DMAP in DMF

DABCO: 460 mM DABCO in DMF

Protocol: Stock solutions were made 24 h within use and stored in rubber septa capped vials. Ligand A **4** and CDI (0.2 mL, 1.1 and 1.5 eq, respectively) added to a reaction vial and stirred at rt for 1 h. Linkers **3** and DMAP (0.1 mL, 1.0 and 0.1 eq, respectively) were added via syringe and the resulting solution was stirred for 3 h. Ligand B **6** (0.3 mL, 1.5 eq) was added *via* syringe, followed by addition of DABCO (0.1 mL, 4.5 eq). The reaction was then heated to 40 °C and stirred for 18 h. After cooling, the reaction was diluted with EtOAc (30 mL). The organic phase was then washed with sat. NH_4Cl (20 mL), 10% w/v Na_2CO_3(20 mL) and brine (20 mL). The organic layer was then dried over Na_2SO_4 and concentrated on a rotary evaporator. Products were then purified via preparative TLC (chamber contained EtOAc), Note: TLC plates had to be loaded as a concentrated solution in DMF due to solubility issues. The plates were then dried under N_2 flow prior to developing them.

Method A. The second or method A used HATU to engage the coupling of **4** to **3b** (Scheme 1). It begins by preparation of stock solutions and then uses these solutions to conduct reactions in parallel using 20 mL vials capped with rubber septa. It is demonstrated for the preparation of **2b**.
Stock solutions (applied to prepare 2b):

Linker: 110 mM **3b** in DMF

Ligand B (6, Scheme 1): 73 mM **12** in DMF

DABCO: 900 mM DABCO in DMF

Protocol: Stock solutions were made 24 h within use and stored in rubber septa sealed vials. Ligand A **4** (4.8 mg, 0.014 mmol, 1.3 eq) and HATU (5.5 mg, 0.014 mmol, 1.3 eq) were dissolved in linker **3** stock solution (0.1 mL 1.0 eq) under an Ar atmosphere. DABCO (0.05 mL, 4 eq) was added via syringe and the reaction mixture was stirred at rt for 1.5 h. Ligand B **6** (0.15 mL, 1.0 eq) was added *via* syringe. The reaction was then heated to 40 °C and stirred for 18 h. After cooling, the reaction was diluted with EtOAc (30 mL). The organic phase was then washed with sat. NH_4Cl (20 mL), 10% w/v Na_2CO_3 (20 mL) and brine (20 mL). The organic layer was then dried over Na_2SO_4 and concentrated on a rotary evaporator. The product was then purified via flash column chromatography (EtOAc to 1:4 MeOH/EtOAc).

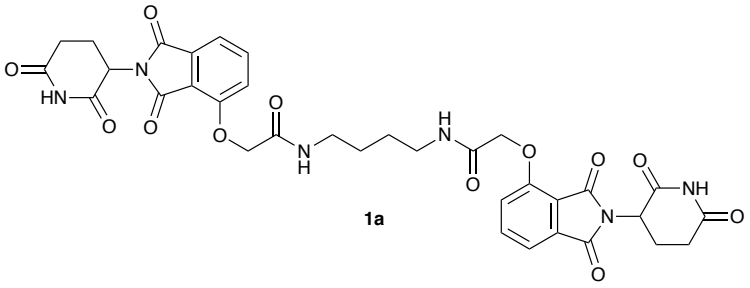
Method B (used for compounds 2a and 2c). The third or method B uses purified ligand A azide **5** to engage the coupling of **6** (Scheme 1). It begins by preparation of stock solutions and then uses these solutions to conduct reactions in parallel 20 mL vials capped with rubber septa. It is demonstrated for the preparation of compounds **2a**-**2c**.

Ligand A (5, Scheme 1): 87 mM **8a** in DMF or 87 mM **8c** in DMF

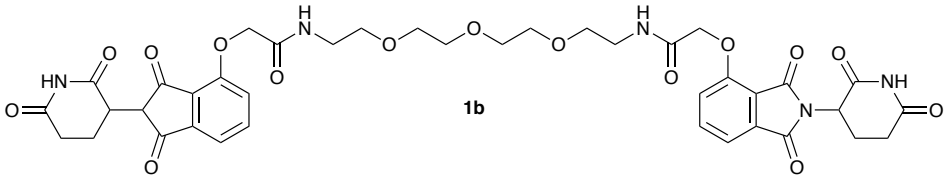
Ligand B (6, Scheme 1): 87 mM **12** in DMF

DABCO: 760 mM DABCO in DMF

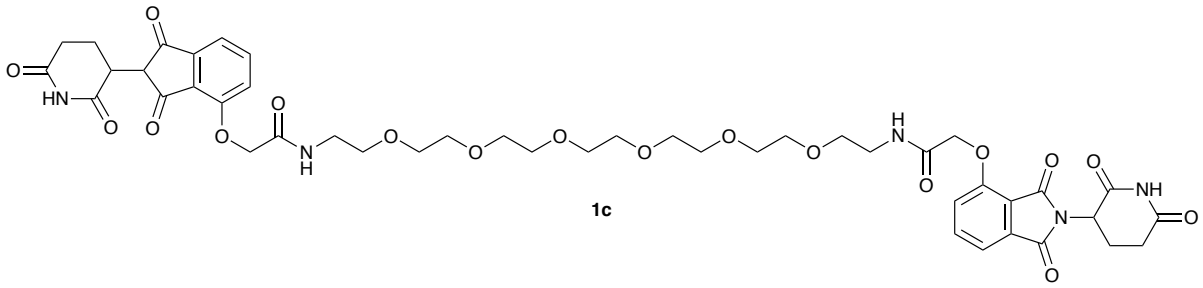
Ligand A **5** (0.15 mL, 1.0 eq) and Ligand B **6** (0.15 mL, 1.0 eq) were added to a vial under an Ar atmosphere. DABCO (0.05 mL, 3 eq) was added *via* syringe. The reaction was then heated to 40 °C and stirred for 18 h. After cooling, the reaction was diluted with EtOAc (30 mL). The organic phase was then washed with sat. NH_4Cl (20 mL), 10% w/v Na_2CO_3 (20 mL) and brine (20 mL). The organic layer was then dried over Na_2SO_4 and concentrated on a rotary evaporator. The product was then purified via flash column chromatography (EtOAc to 1:4 MeOH/EtOAc).



***N*,*N*'-(butane-1,4-diyl)bis(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide) (1a).** Preliminary study was a LCMS based study, and there is no isolated yield to report due to the product being inseparable from the reaction mixture on silica (see Fig. S3). Compound synthesized using general one-pot preliminary method A. HRMS (+ESI) calc. for $[C_{34}H_{33}N_6O_{12}]^+$ 717.2151 ($[M+H]^+$), found *m/z* 717.2157.

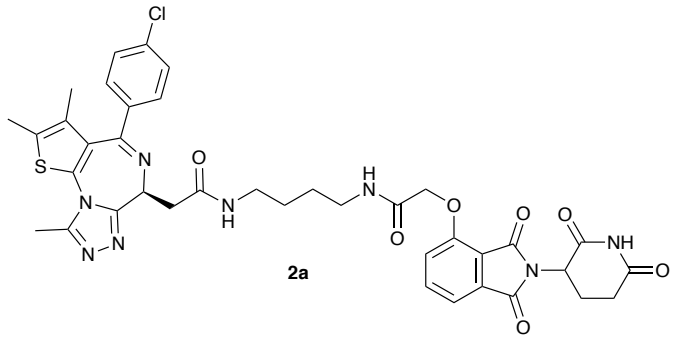


***N*,*N*'-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide) (2b).** Preliminary study was a LCMS based study, and there is no isolated yield to report due to the product being inseparable from the reaction mixture on silica (see Fig. S4). Compound synthesized using general one-pot preliminary method A. HRMS (+ESI) calc. for $[C_{38}H_{40}N_6O_{15}Na]^+$ 843.2444 ($[M+Na]^+$), found *m/z* 843.2434.



1c

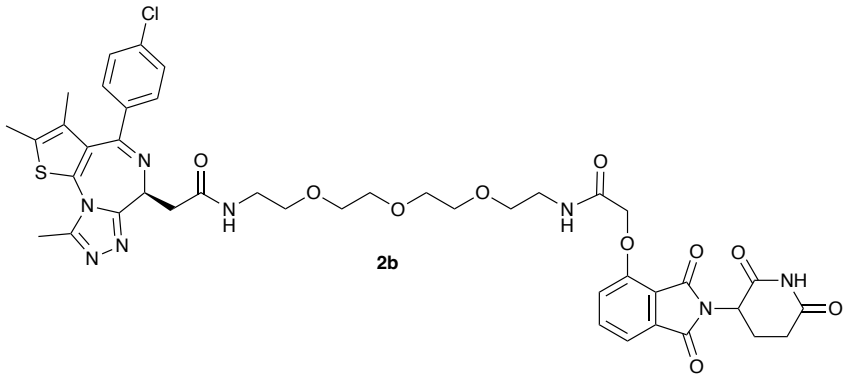
***N*,*N*'-(3,6,9,12,15,18-hexaoxaicosane-1,20-diyl)bis(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide) (1c).** Compound synthesized using general one-pot preliminary method A. Yield: 1.1 mg (10%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (s, 1H), 7.74 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.66 (t, *J* = 5.4 Hz, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 4.96 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.65 (s, 2H), 3.60 (m, 12H), 3.38 (m, 2H), 2.81 (m, 3H), 2.50 (m, 2H), 2.14 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 168.3, 166.9, 166.8, 165.9, 154.5, 137.1, 133.8, 119.8, 118.1, 117.4, 71.1, 70.8, 70.8, 70.8, 70.7, 70.6, 70.6, 70.5, 70.5, 70.4, 70.1, 69.6, 67.9, 50.8, 49.9, 39.2, 32.1, 22.9; HRMS (+ESI) calc. for $[C_{44}H_{52}N_6O_{18}NH_4]^+$ 970.3676 ($[M+NH_4]^+$), found *m/z* 970.3647, calc. for $[C_{44}H_{52}N_6O_{18}Na]^+$ 975.3236 ($[M+Na]^+$), found *m/z* 975.3230.



2a

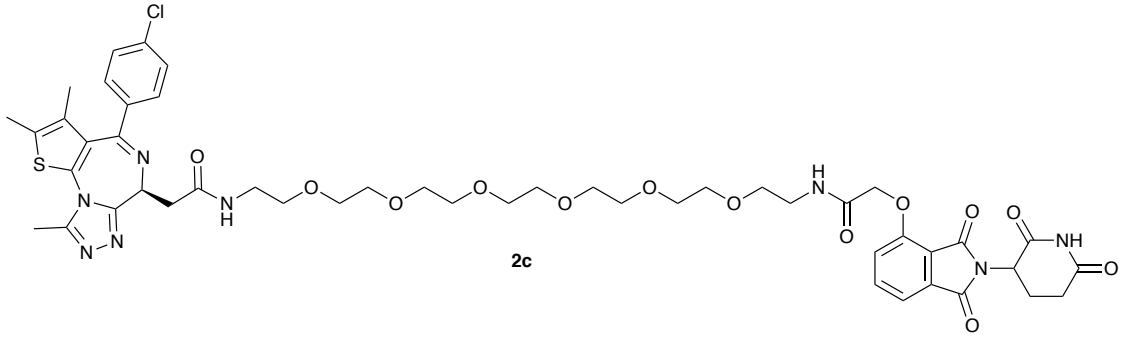
2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)acetamide (2a).

Compound synthesized using general one-pot method B. Yield: 5.4 mg (54%). Spectral signals matched those previously reported.[4] ^{1}H NMR (500MHz, CD_3OD) δ 7.81 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.49 (m, 5H), 5.10 (m, 1H), 4.77 (s, 2H), 4.62 (dd, *J* = 8.9, 5.3 Hz, 1H), 3.41 (m, 3H), 3.27 (m, 2H), 2.73 (m, 7H), 2.44 (s, 3H), 2.09 (m, 1H), 1.61 (m, 7H). ^{13}C NMR (125 MHz, D_3COD) δ 174.5, 172.7, 171.4, 170.0, 168.4, 167.8, 166.3, 157.0, 156.3, 152.2, 138.3, 138.1, 138.0, 135.0, 133.6, 133.2, 132.0, 132.0, 131.4, 130.0, 129.4, 121.9, 119.4, 118.0, 69.5, 55.2, 50.5, 40.1, 39.8, 38.8, 32.1, 27.8, 27.6, 23.6, 14.5, 12.9, 11.5; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.14 (br s, 1H), 8.23 (t, *J* = 5.8 Hz, 1H), 8.01 (t, *J* = 5.8 Hz, 1H), 7.81 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.48 (m, 3H), 7.39 (m, 3H), 5.12 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.78 (s, 2H), 4.49 (dd, *J* = 8.1, 6.1 Hz, 1H), 4.40 (d, *J* = 9.3 Hz, NH), 3.15 (m, 6H), 2.88 (m, 1H), 2.53 (m, 5H), 2.40 (m, 3H), 2.03 (m, 1H), 1.61 (s, 3H), 1.56 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.9, 169.4, 168.1, 167.0, 166.8, 166.8, 165.6, 163.1, 155.2, 155.1, 149.9, 137.0, 136.8, 135.3, 133.1, 132.3, 130.8, 130.2, 129.9, 129.6, 129.1, 128.5, 120.4, 116.8, 116.1, 66.9, 67.6, 53.9, 48.8, 44.1, 31.3, 26.6, 26.6, 22.2, 14.1, 12.8, 11.4; HRMS (+ESI) calc. for $[C_{38}H_{38}ClN_8O_7S]^+$ 785.2267 ($[M+H]^+$), found *m/z* 785.2264; LC-MS (+ESI) calc. for $[C_{38}H_{38}ClN_8O_7S]^+$ 785.23 ($[M+H]^+$), found *m/z* 785.37.



2b

2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)acetamide (2b). Yield: 4.8 mg (48%). Note: The reported yield is not fully purified, but represents the mass of the material shown in Fig. S10. For further characterization, this compound was synthesized using general one-pot method B. Yield: 4.2 mg (39%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.14 (s, 1H), 8.31 (t, *J* = 5.7 Hz, 1H), 8.07 (t, *J* = 5.5 Hz, 1H), 7.80 (m, 1H), 7.48 (m, 3H), 7.41 (m, 3H), 5.11 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.79 (s, 2H), 4.49 (dd, *J* = 8.2, 6.2 Hz, 1H), 3.50 (m, 10H), 3.26 (m, 6H), 2.89 (m, 2H), 2.81 (dd, *J* = 9.3, 5.8 Hz, 1H), 2.72 (dd, *J* = 9.1, 6.0 Hz, 1H), 2.54 (m, 2H), 2.40 (m, 3H), 2.03 (m, 1H), 1.61 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.8, 170.0, 169.7, 167.0, 166.8, 165.5, 163.1, 155.1, 155.0, 149.9, 137.0, 136.8, 135.3, 133.1, 132.3, 130.7, 130.2, 129.9, 129.6, 128.5, 120.3, 116.7, 116.1, 72.4, 69.7, 69.2, 68.9, 67.5, 60.2, 53.9, 51.2, 50.7, 48.8, 45.5, 38.6, 38.4, 37.5, 31.0, 22.0, 14.1, 12.7, 11.4; HRMS (+ESI) calc. for $[C_{42}H_{46}ClN_8O_{10}S]^+$ 889.2741 ($[M+H]^+$), found *m/z* 889.2730, calc. for $[C_{42}H_{45}ClN_8O_{10}SNa]^+$ 911.2560 ($[M+Na]^+$), found *m/z* 911.2558; LC-MS (+ESI) calc. for $[C_{42}H_{46}ClN_8O_{10}S]^+$ 889.27 ($[M+H]^+$), found *m/z* 889.32.



2c

2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-6,9,12,15,18,21-hexaoxa-3-azatricosan-23-yl)acetamide (2c). Compound synthesized using general one-pot method B. Yield: 9.1 mg (85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.14 (br s, 1H), 8.31 (t, *J* = 5.7 Hz, 1H), 8.03 (t, *J* = 5.7 Hz, 1H), 7.80 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.49 (m, 2H), 7.39 (m, 2H) 6.93 (m, 1H), 5.11 (dd, *J* = 12.9, 5.5 Hz, 1H), 5.02 (s, 1H), 4.79 (s, 1H), 4.49 (dd, *J* = 8.2, 6.0 Hz, 1H), 4.40 (m, 1H), 3.48 (m, 18H), 3.29 (m, 10H), 2.90 (m, 2H), 2.54 (m, 3H), 2.38 (m, 3H), 2.03 (m, 2H), 1.77 (dt, *J* = 14.3, 6.8 Hz, 1H), 1.59 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.7, 173.2, 171.5, 167.7, 166.9, 166.2, 164.0, 155.7, 155.4, 150.8, 137.7, 137.2, 136.0, 133.5, 132.6, 131.7, 130.8, 130.5, 130.3, 129.4, 120.9, 117.2, 117.0, 77.4, 72.7, 70.3, 70.3, 70.1, 69.6, 69.2, 68.1, 63.3, 60.6, 55.9, 54.3, 49.4, 44.8, 36.1, 34.2, 33.9, 31.4, 22.7, 13.2, 11.8, 9.4; HRMS (+ESI) calc. for $[C_{48}H_{58}ClN_8O_{13}S]^+$ 1021.3527 ($[M+H]^+$), found *m/z* 1021.3530; LC-MS (+ESI) calc. for $[C_{48}H_{58}ClN_8O_{13}S]^+$ 1021.35 ($[M+H]^+$), found *m/z* 1021.42.

H. Additional references

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S5 M. Mühlberg, D. M. M. Jaradat, R. Kleineweischede, I. Papp, D. Dechtrirat, S. Muth, M. Broncel and C. P. R. Hackenberger, *Bioorganic & Medicinal Chemistry*, 2010, **18**, 3679-3686.

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S7 Y. Zhang, D. Duan, Y. Zhong, X. Guo, J. Guo, J. Guo, Z. Gao and B. Yu, *Org. Lett.*, 2019, **21**, 4960-4965.

Representative LC/MS data. Copies of LC-MS in Fig. S3-S12 has been provided for reactions and purified compounds. LC-MS runs were conducted using the following solvent: A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile. Total run time was 8 min. Runs were conducted using an Acquity UPLC Beh C18 column (130Å, 1.7 µm, 2.1 mm × 50 mm) with a flow rate of 0.6 mL/min. Samples were dissolved in acetonitrile.

LC-MS Methods

Method A

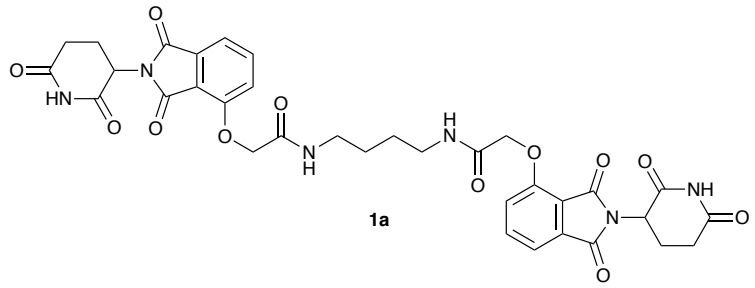
Equilibration conditions: 95%A:5%B

time (min)	%A	%B
Initial	95	5
0.50	95	5
4.00	15	85
6.00	5	95
8.00	95	5

Method B

Equilibration conditions: 95%A:5%B

time (min)	%A	%B
Initial	95	5
0.50	95	5
8.00	5	95



1a

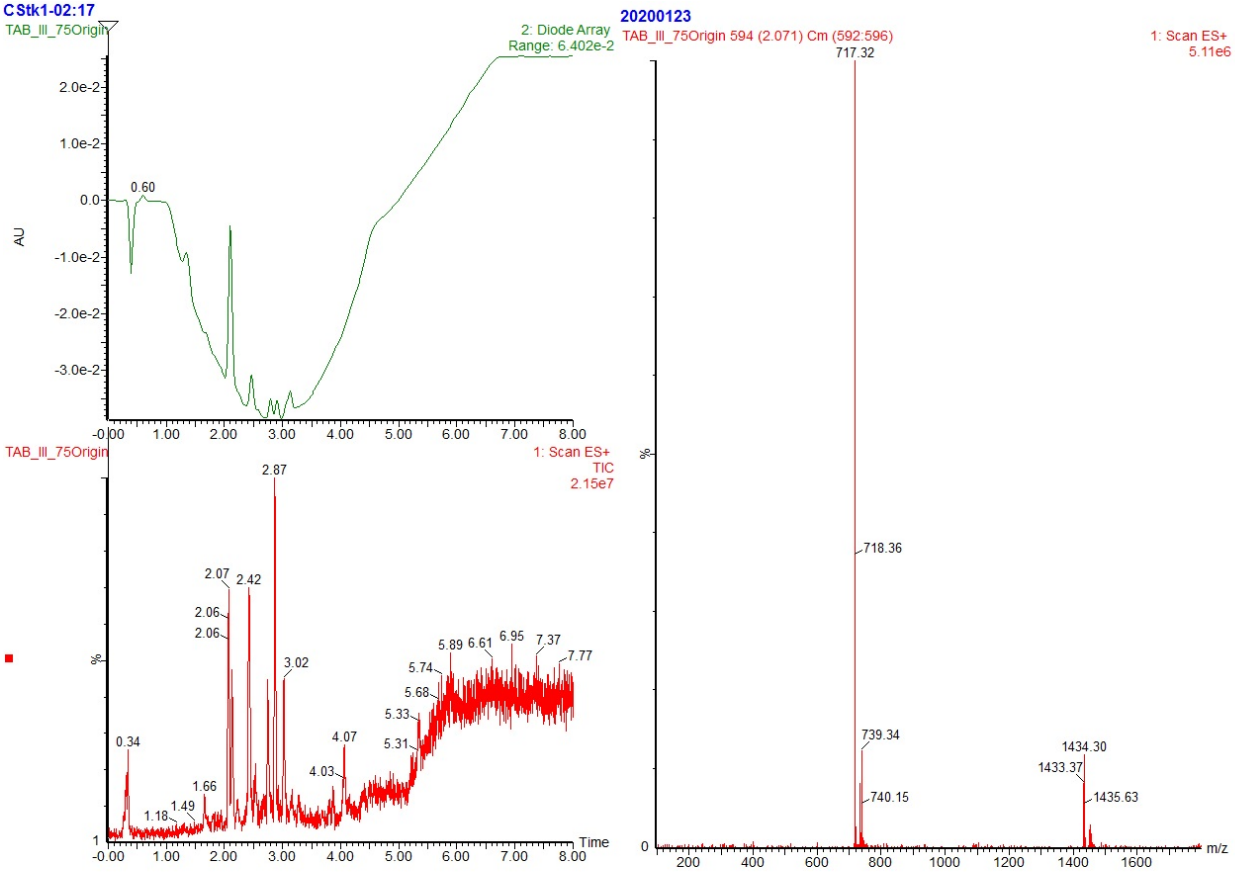


Fig. S3. LC-MS trace of **1a** using LC-MS method A. UV-vis detection was at λ = 254 nm. Peak at 2.07 min corresponds to the product. (Note: Peak at 2.15 min corresponds to inseparable intermediate **8a**.)

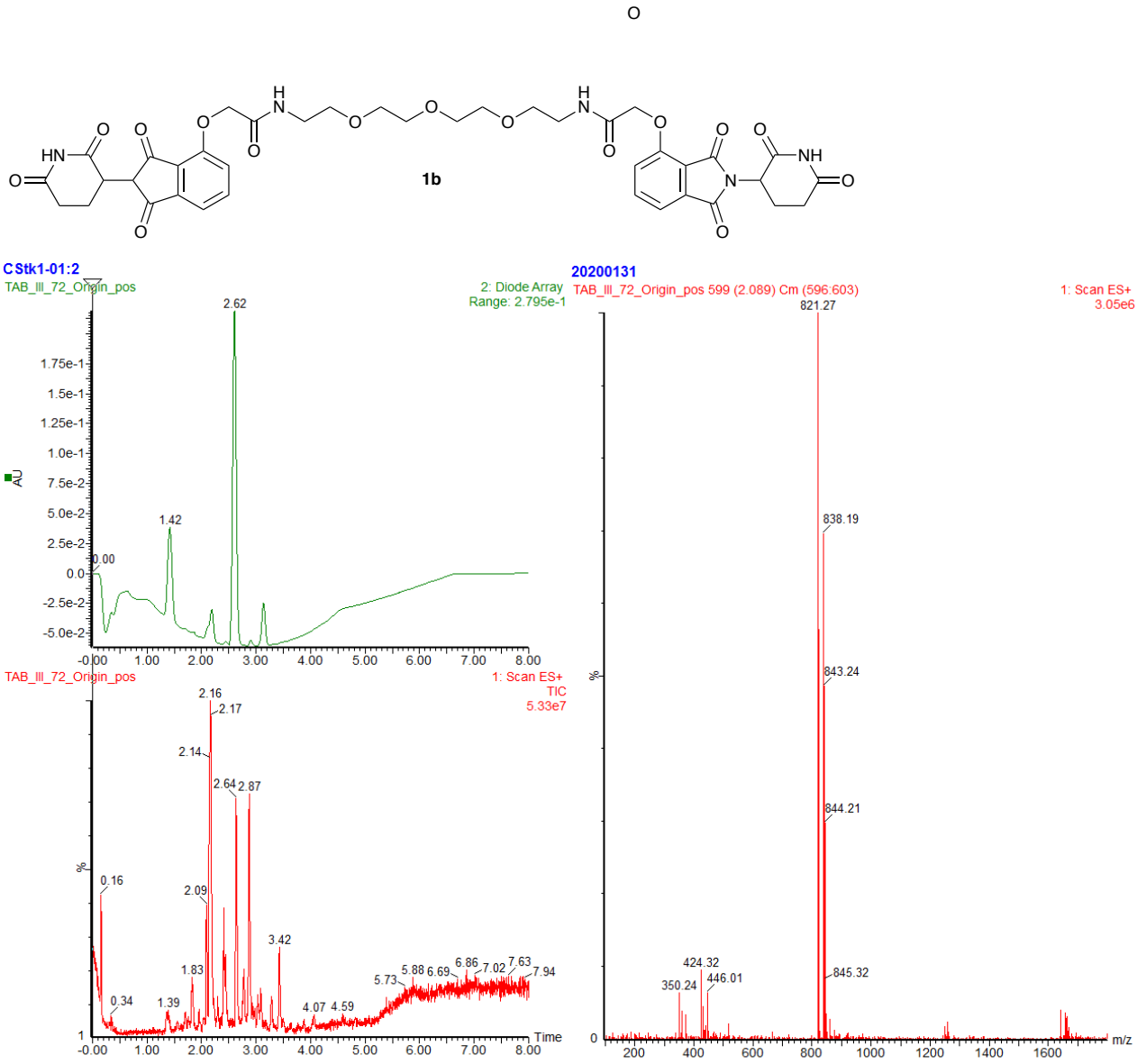
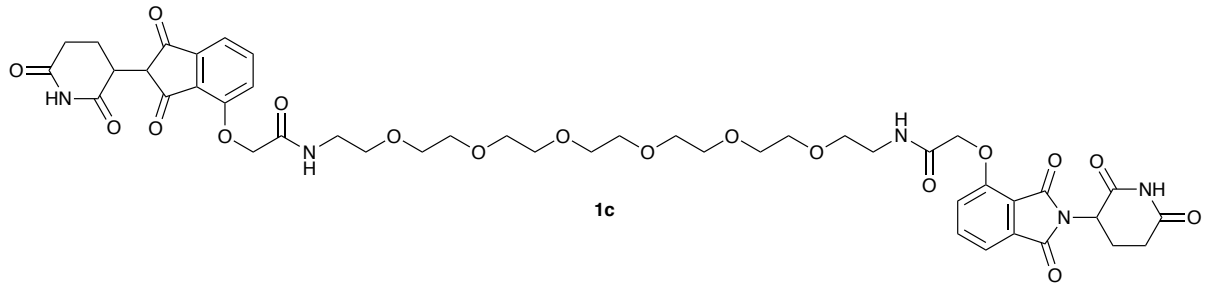


Fig. S4. LC-MS trace of **1b** using LC-MS method A. UV-vis detection was at λ = 254 nm. Peak at 2.09 min corresponds to the product. (Note: Peak at 2.16 min corresponds to inseparable intermediate **8b**.)



1c

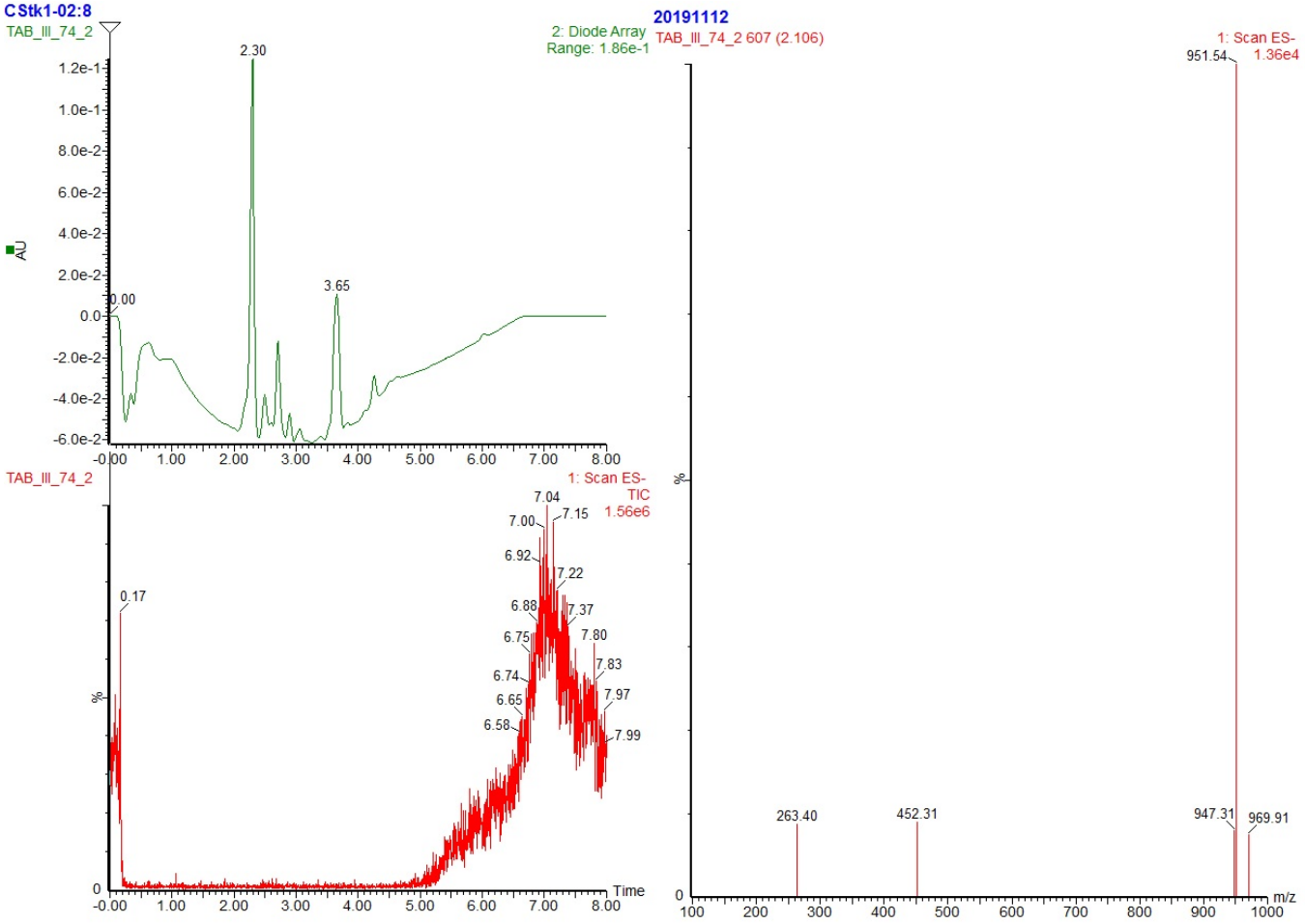


Fig. S5. LC-MS trace of **1c** using LC-MS method A. UV-vis detection was at λ = 254 nm. Peak at 2.11 min (front shoulder of large UV signal at 2.30 min) corresponds to the product. The large peak at 2.30 min corresponds to accumulation of intermediate **8c**. Note: compound **1c** was difficult to detect on ES+ detection mode so ES- was used instead.

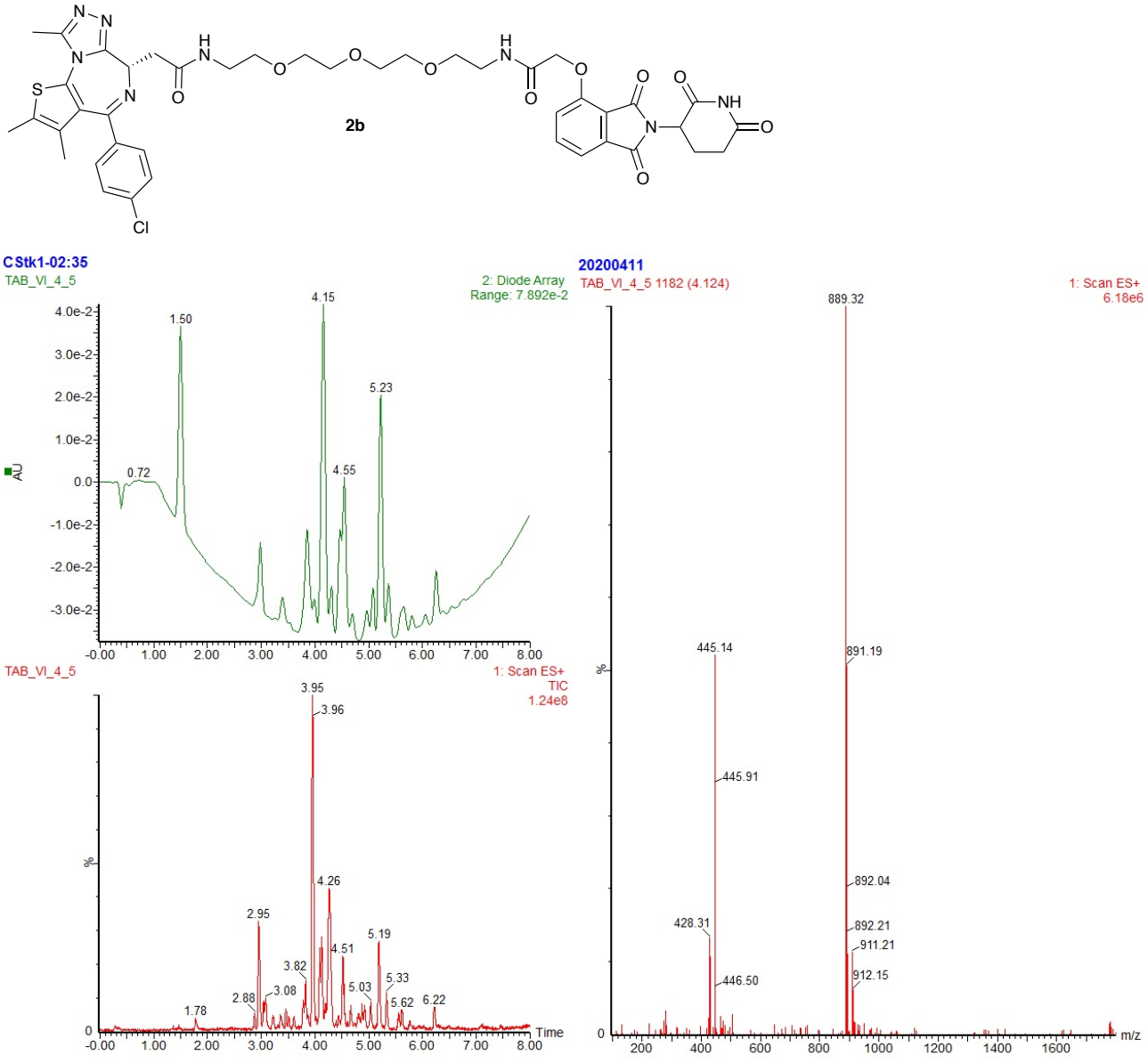
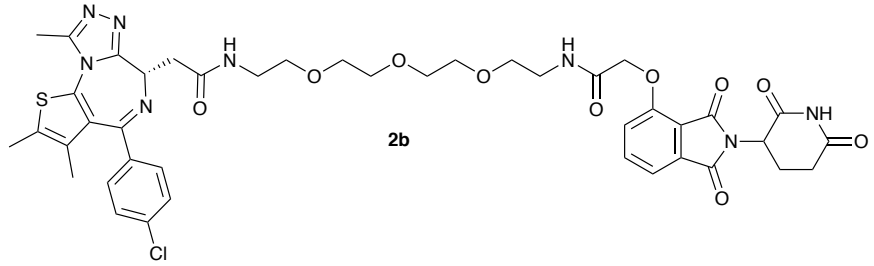


Fig. S6. LC-MS trace of **2b** using LC-MS method B. UV-vis detection was at λ = 254 nm. Peak at 4.12 min corresponds to the product.



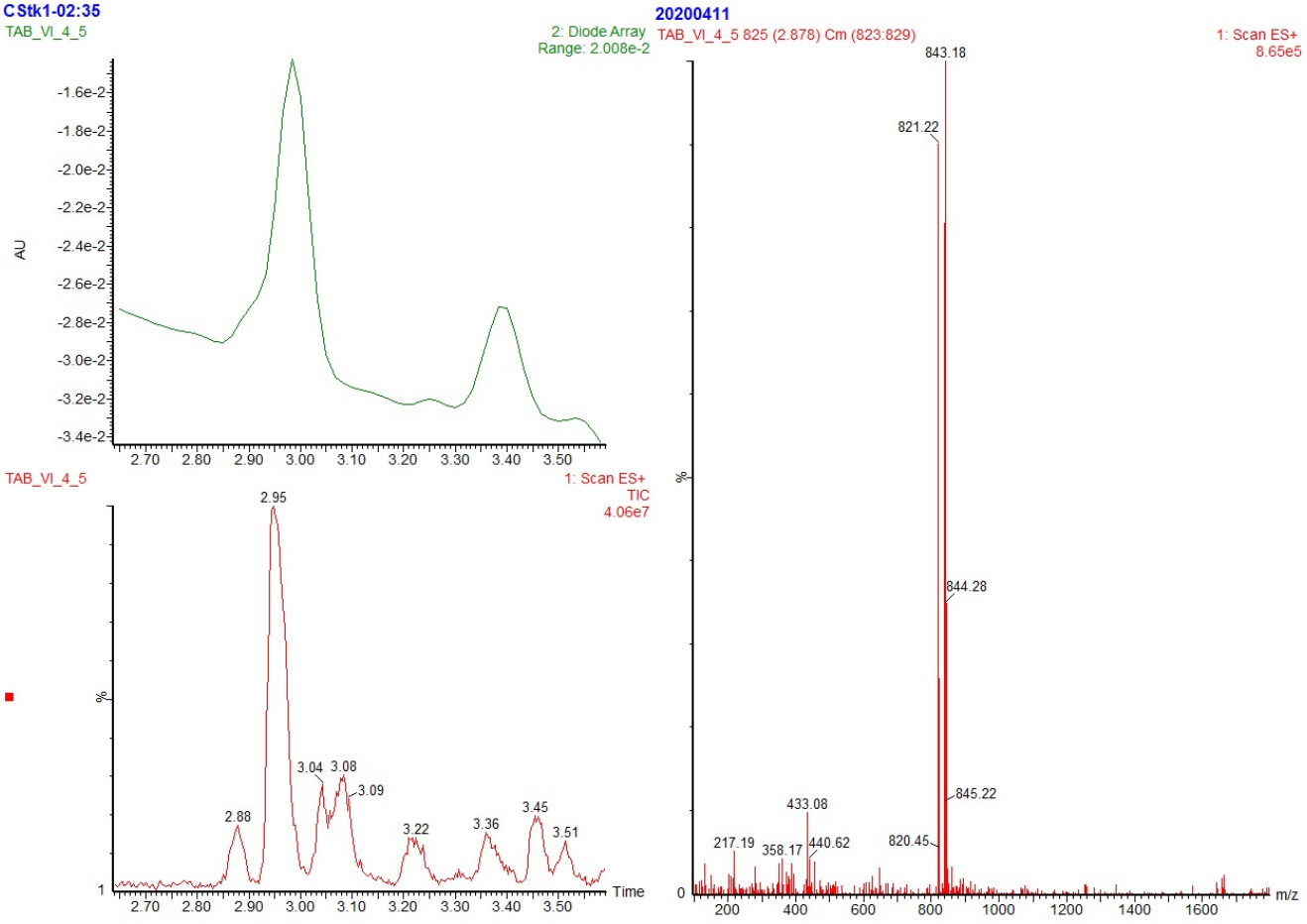


Fig. S7. Impurity profile examined via LC-MS during one-pot synthesis of **2b** using LC-MS method B (zoomed in on trace Fig. S6) . UV-vis detection was at λ = 254 nm. Peak at 2.88 min corresponds to the aberrant homo-coupled product **1b**. Note: this type of impurity is a liability to biological evaluation, however with our LC method the difference in retention time was 1 min, so it is likely this impurity could be removed via prep-HPLC techniques.

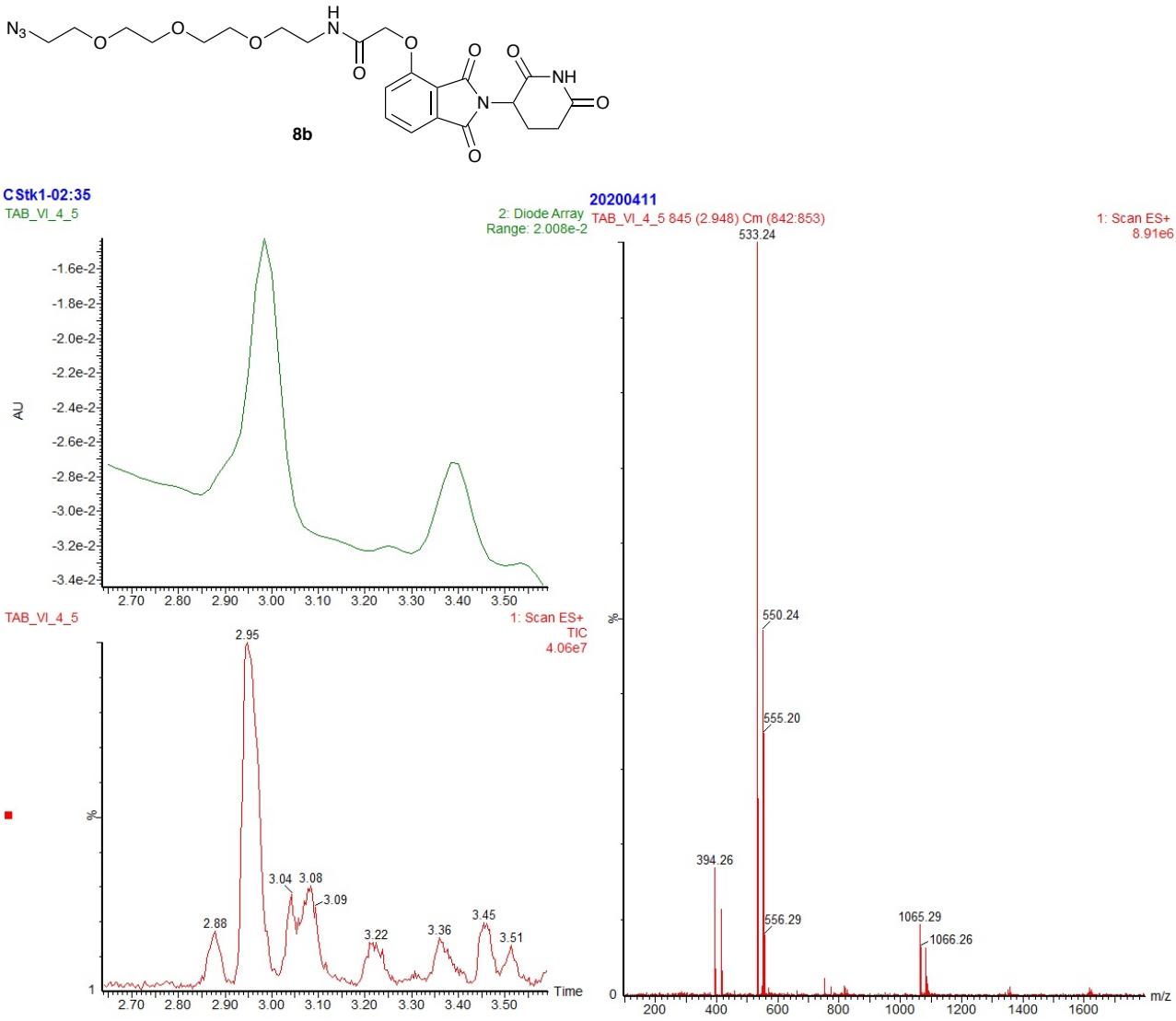


Fig. S8. Impurity profile examined via LC-MS during one-pot synthesis of **2b** using LC-MS method B (zoomed in on trace Fig. S6) . UV-vis detection was at λ = 254 nm. Peak at 2.95 min corresponds to intermediate **8b**. Note: this type of impurity is a liability to biological evaluation, however with our LC method the difference in retention time was 1 min, so it is likely this impurity could be removed via prep-HPLC techniques.

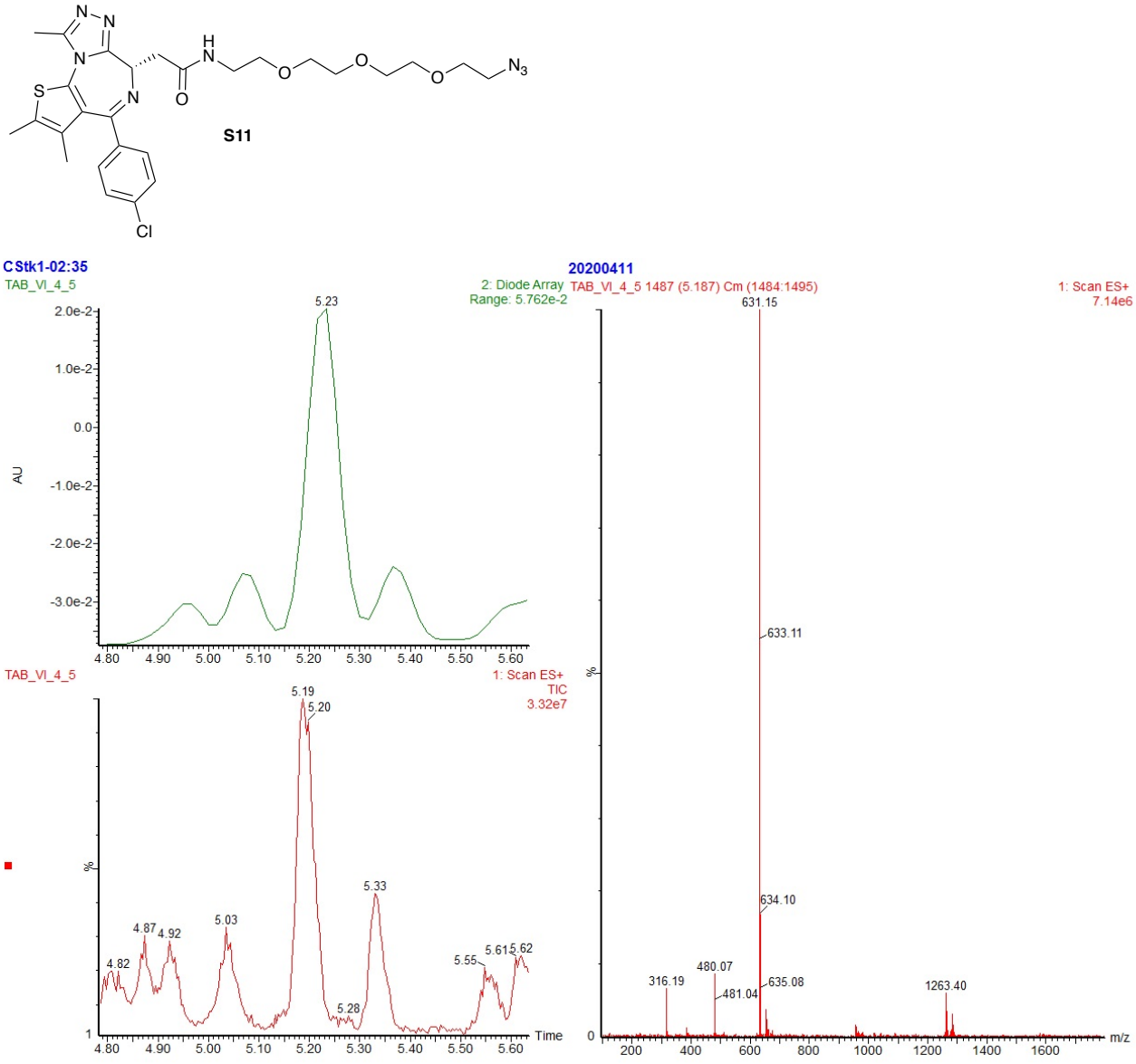


Fig. S9. Impurity profile examined via LC-MS during one-pot synthesis of **2b** using LC-MS method B (zoomed in on trace Fig. S6) . UV-vis detection was at λ = 254 nm. Peak at 5.19 min corresponds to aberrant formation of **S11.** Note: this type of impurity is a liability to biological evaluation, however with our LC method the difference in retention time was 1 min, so it is likely this impurity could be removed via prep-HPLC techniques.

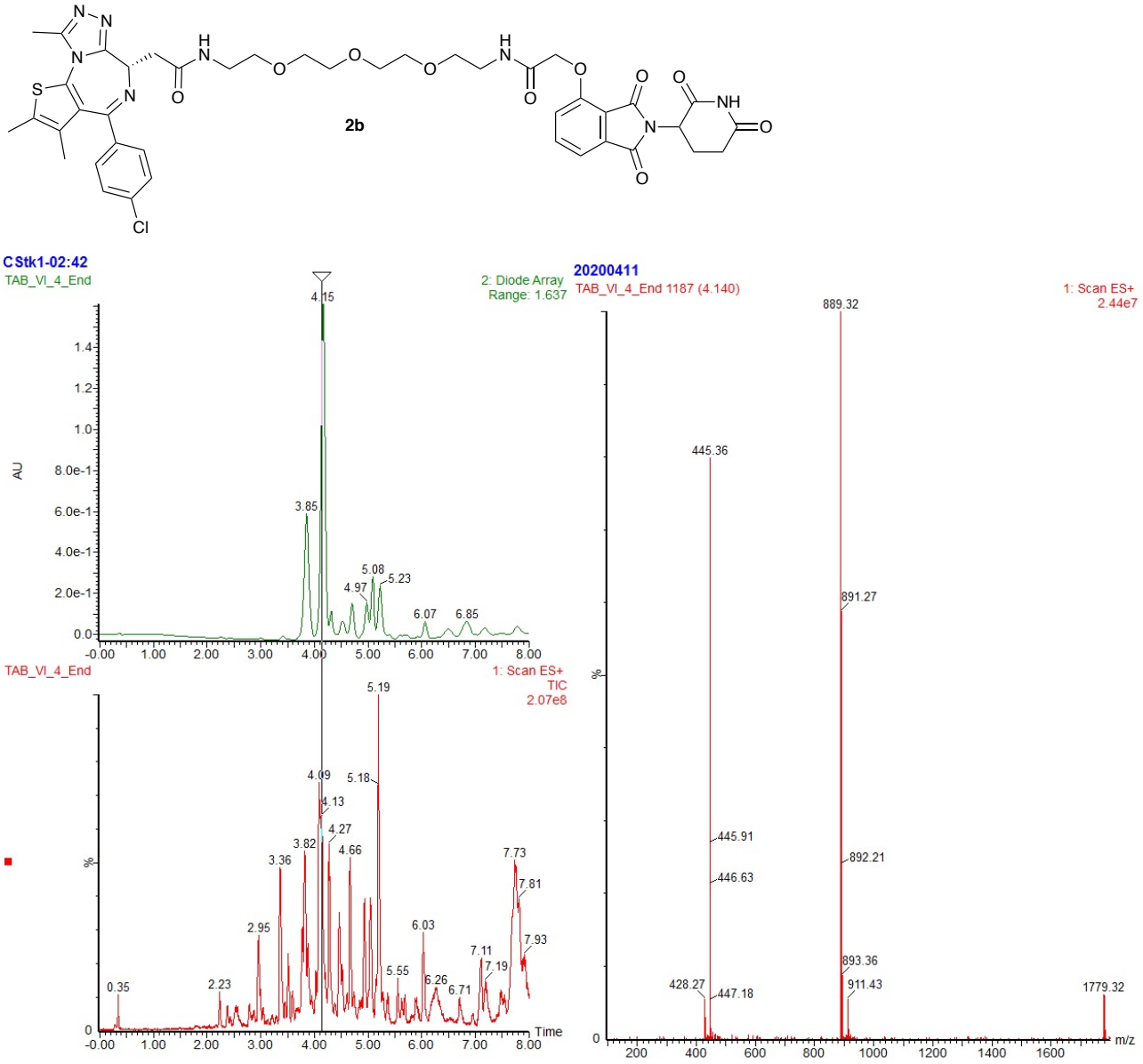


Fig. S10. Impurity profile examined via LC-MS during one-pot synthesis of **2b** using LC-MS method B, after flash column chromatography (EtOAc to 1:9 MeOH/EtOAc). UV-vis detection was at λ = 254 nm. Note: Impurities **1b** and **8b** (described in Figs. S7-S8), were able to be largely reduced/removed, however impurity **S11** (Fig. S9) appears to be present.

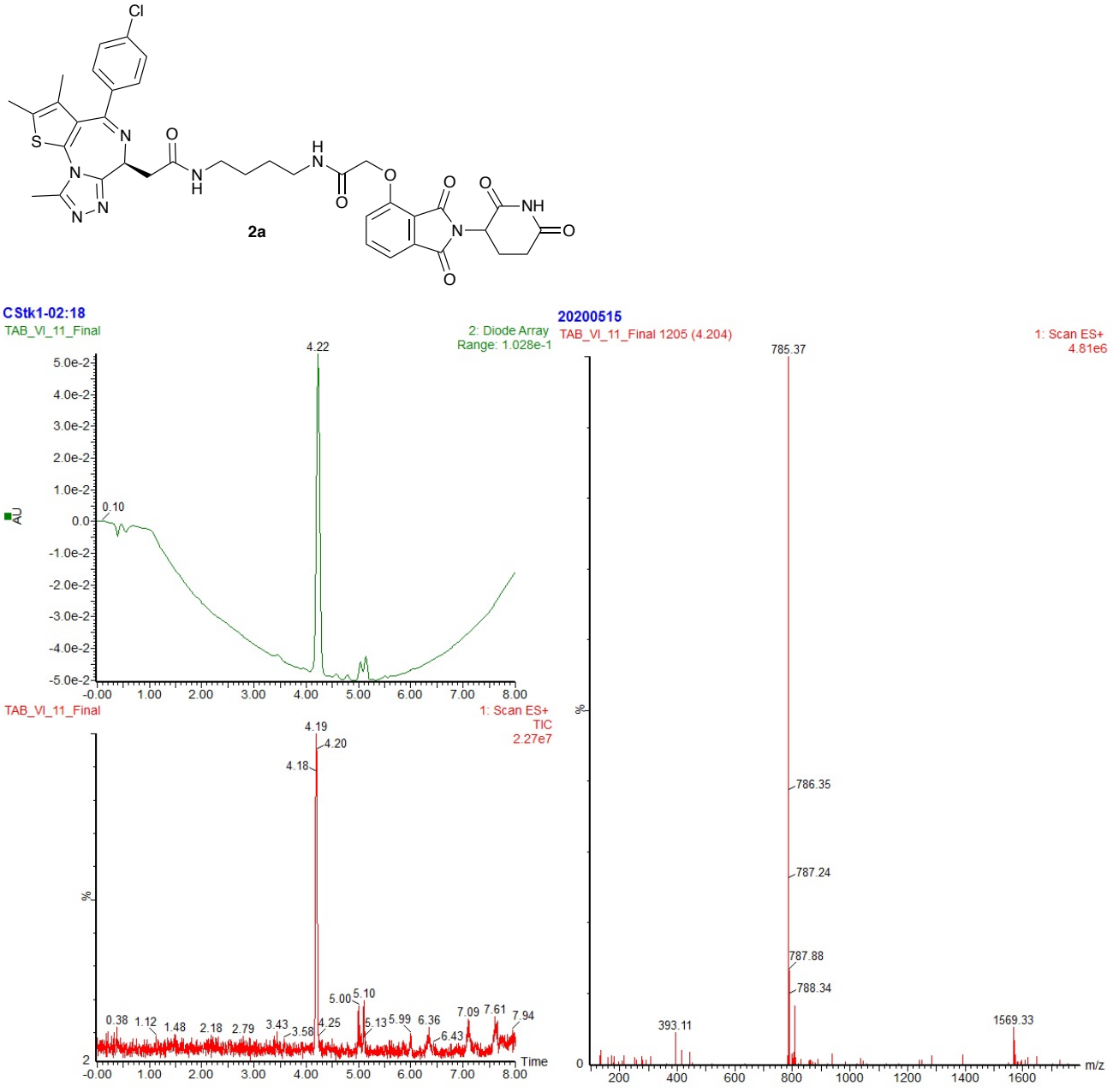


Fig. S11. LC-MS trace of **2a** using LC-MS method B. UV-vis detection was at λ = 254 nm. Peak at 4.20 min corresponds to the product. Note: This sample was purified via flash column chromatography (EtOAc to 1:9 MeOH/EtOAc) and does not contain the analogous competitive binders/degraders observed when using one-pot synthetic method B (See Figs. S7-S9).

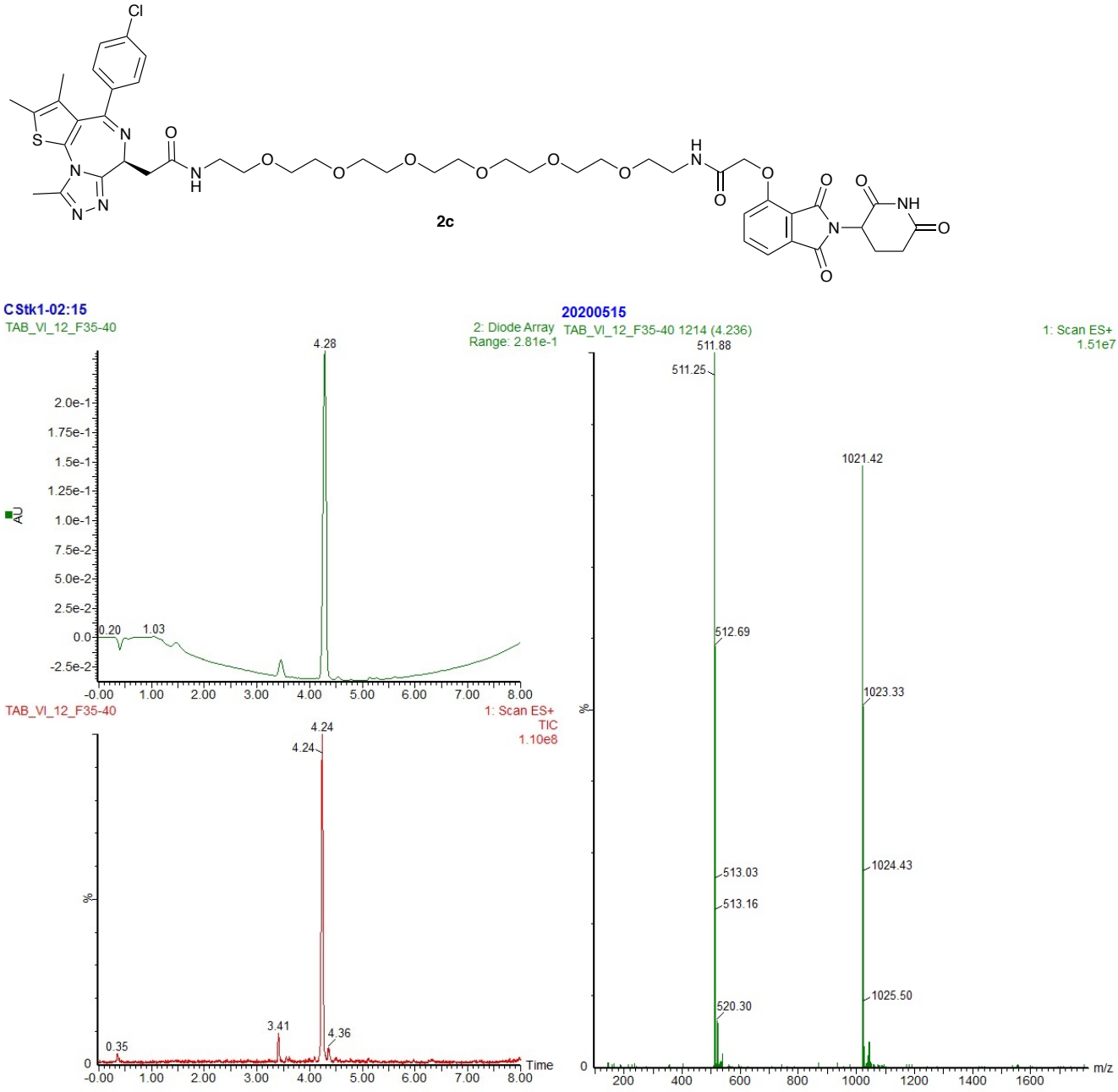
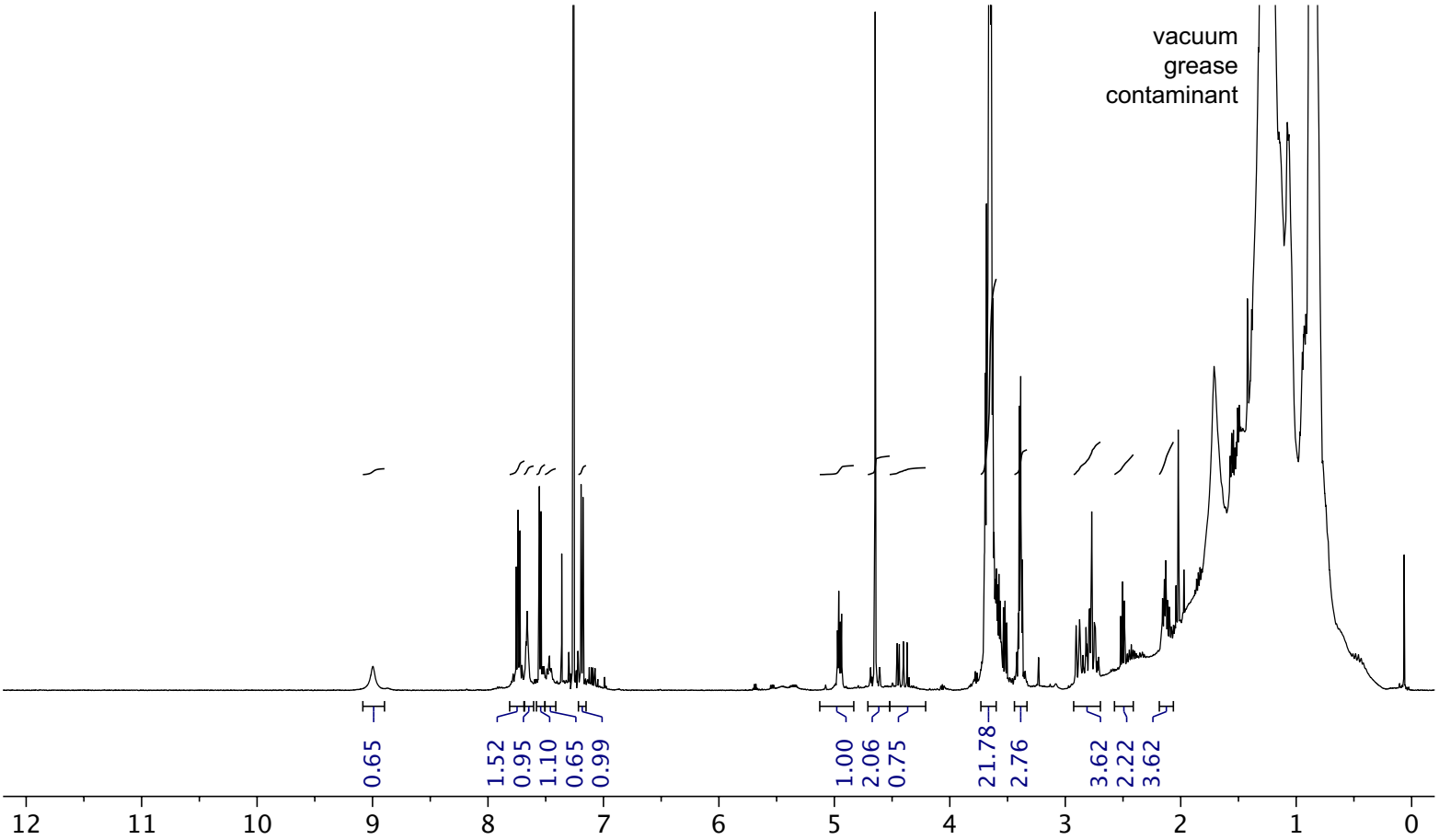
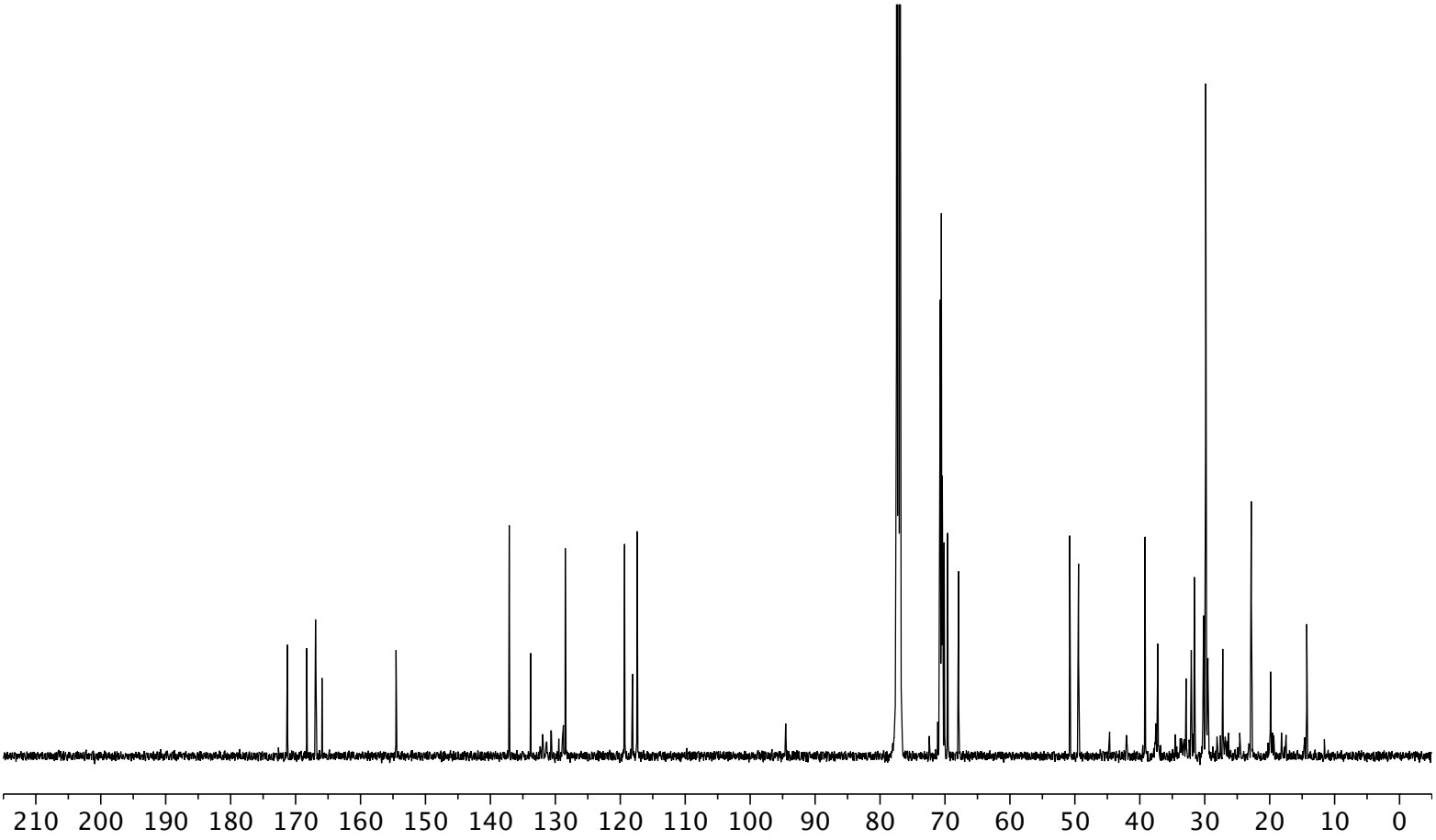


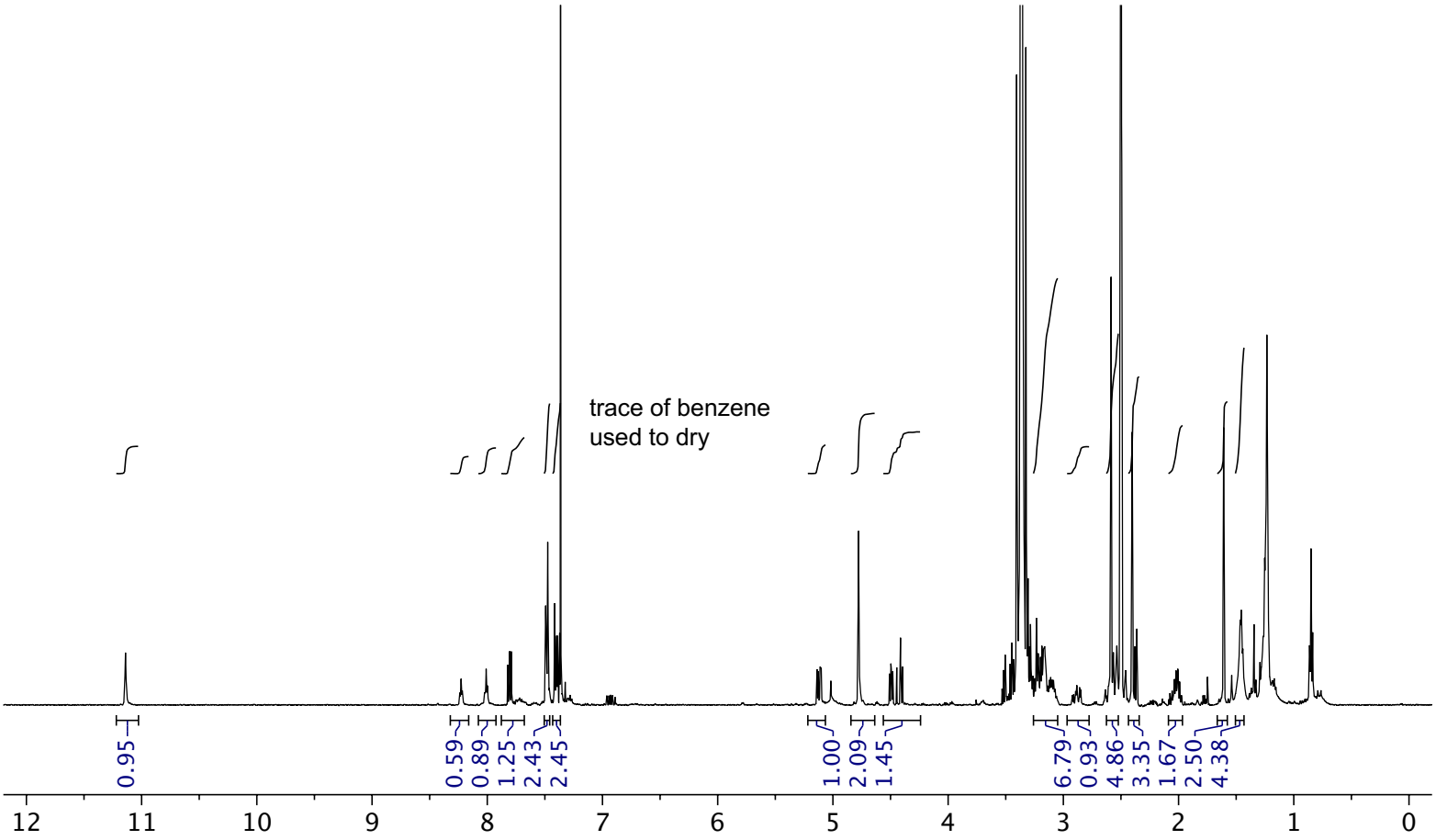
Fig. S12. LC-MS trace of **2c** using LC-MS method B. UV-vis detection was at λ = 254 nm. Peak at 4.24 min corresponds to the product. Note: This sample was purified *via* flash column chromatography (EtOAc to 1:9 MeOH/EtOAc) and does not contain the analogous competitive binders/degraders observed when using one-pot synthetic method B (see Figs. S7-S9).

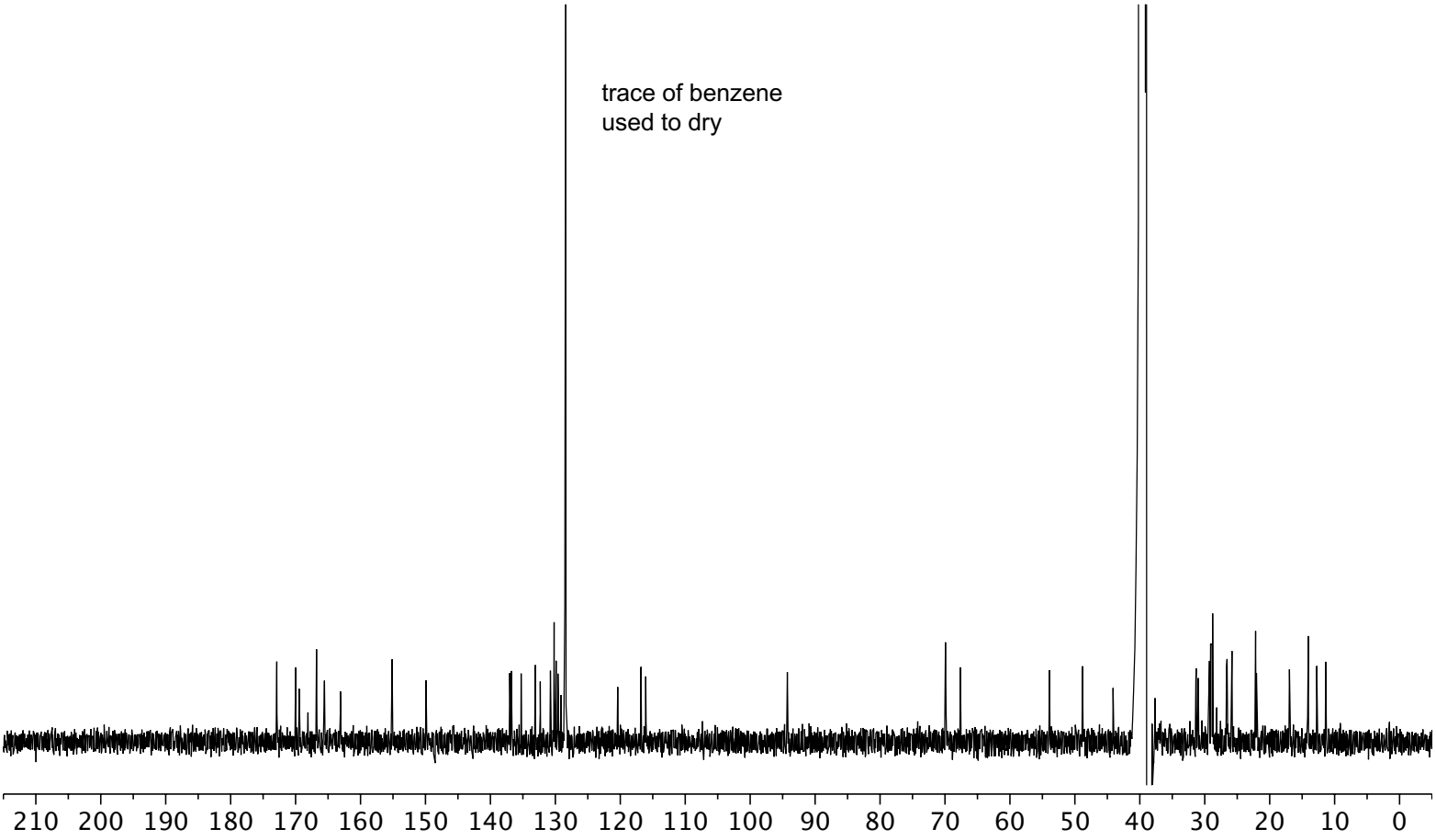
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **1c** in $CDCl_3$



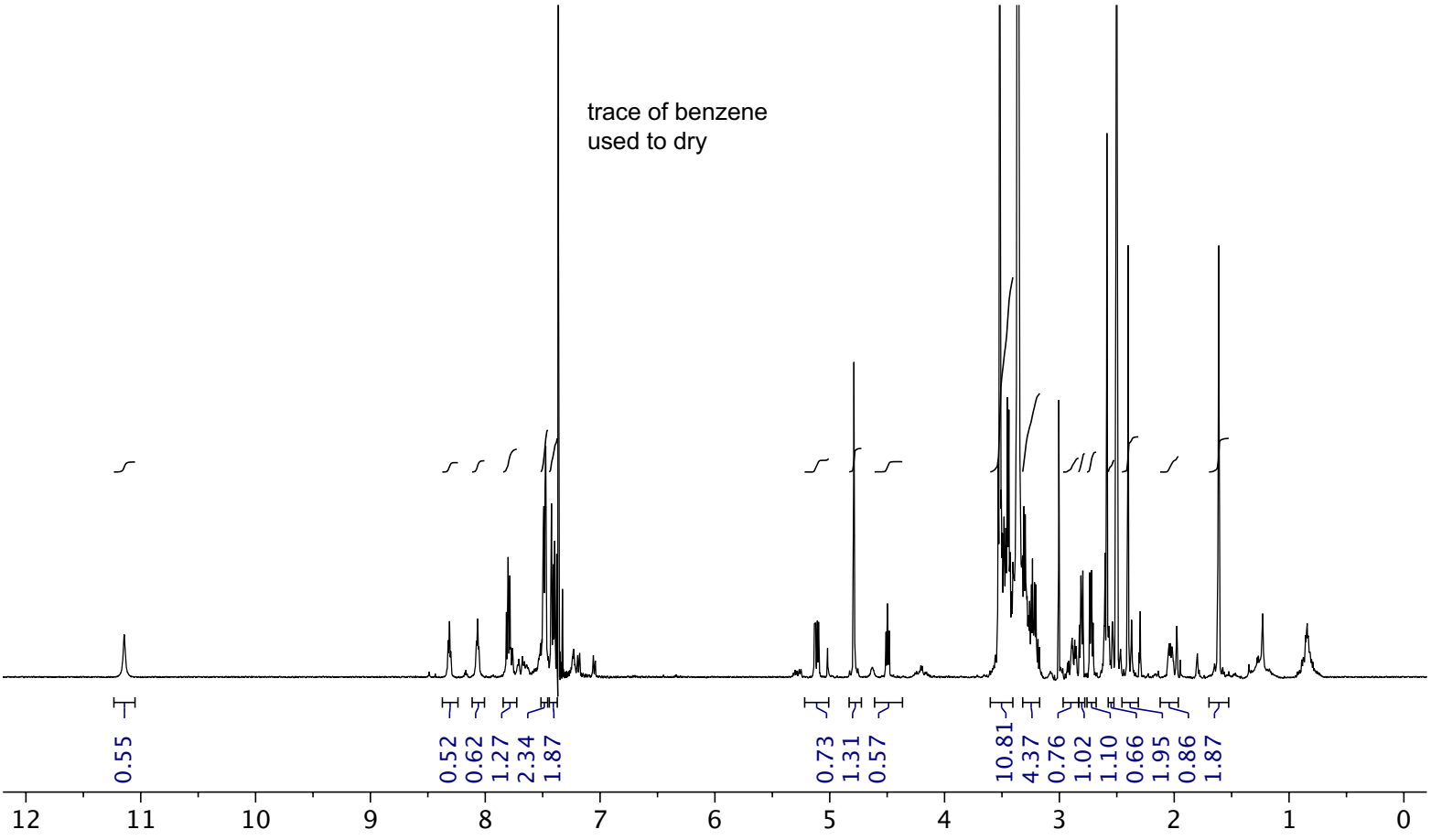


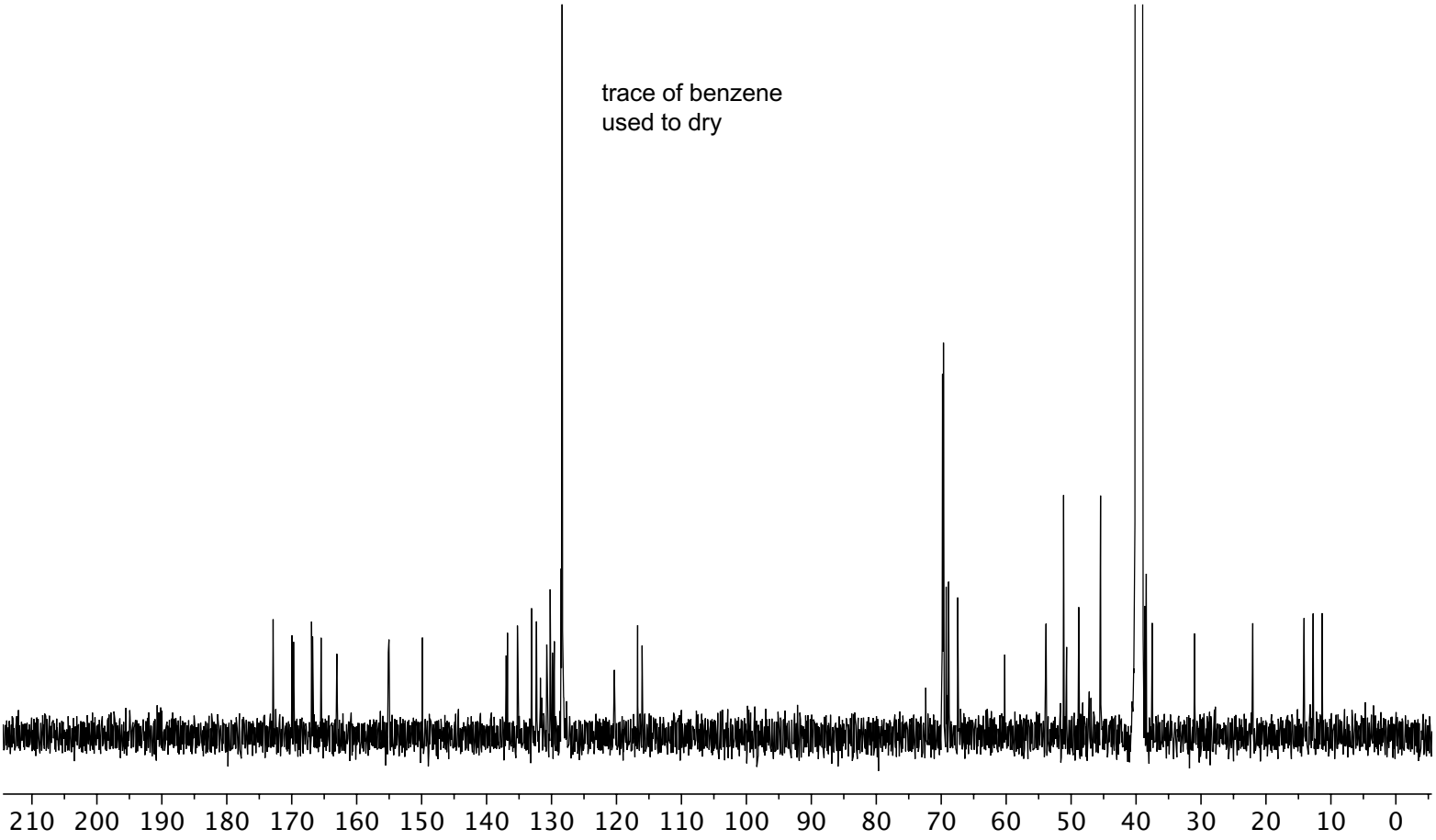
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **2a** in DMSO-d_6



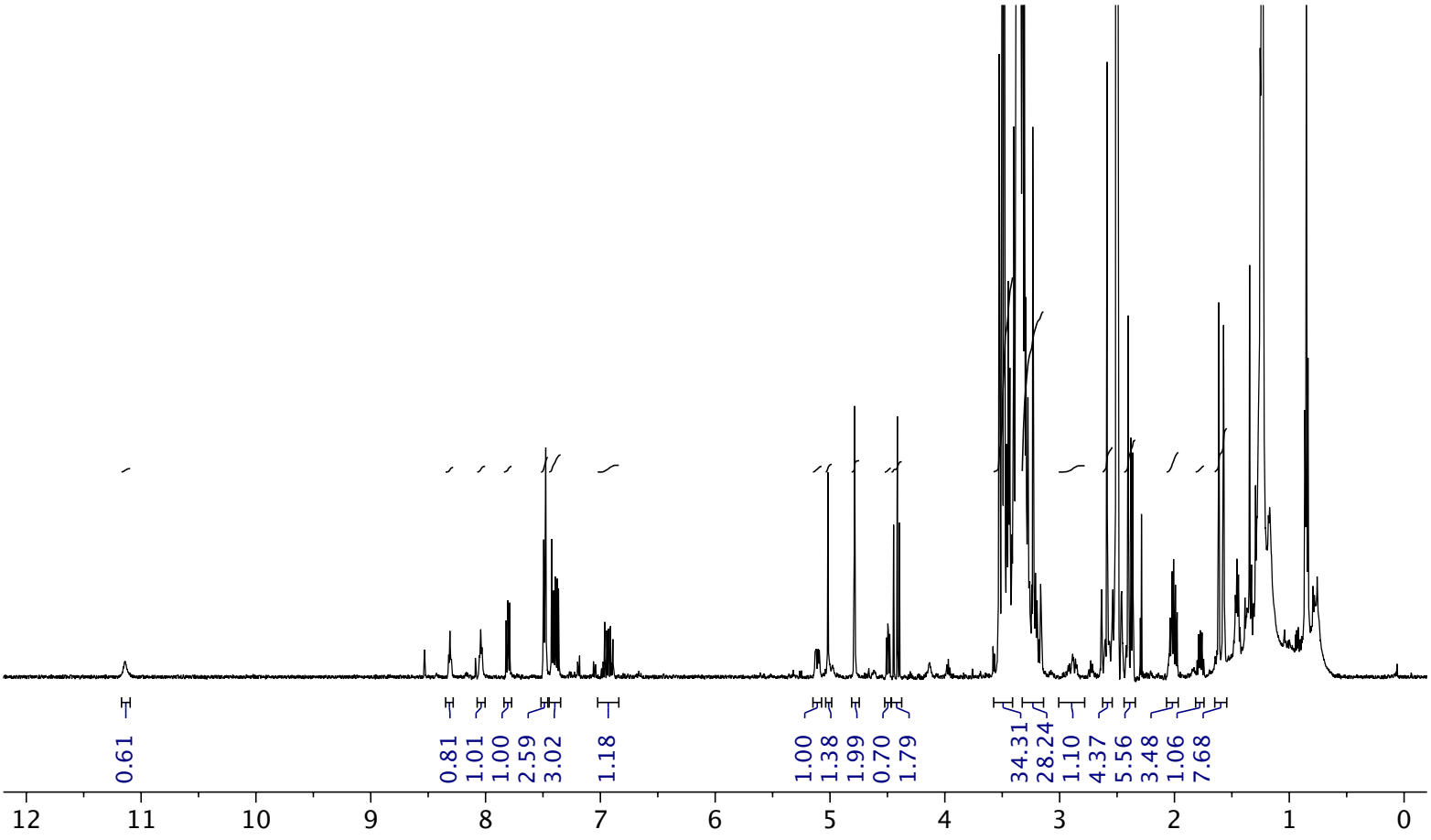


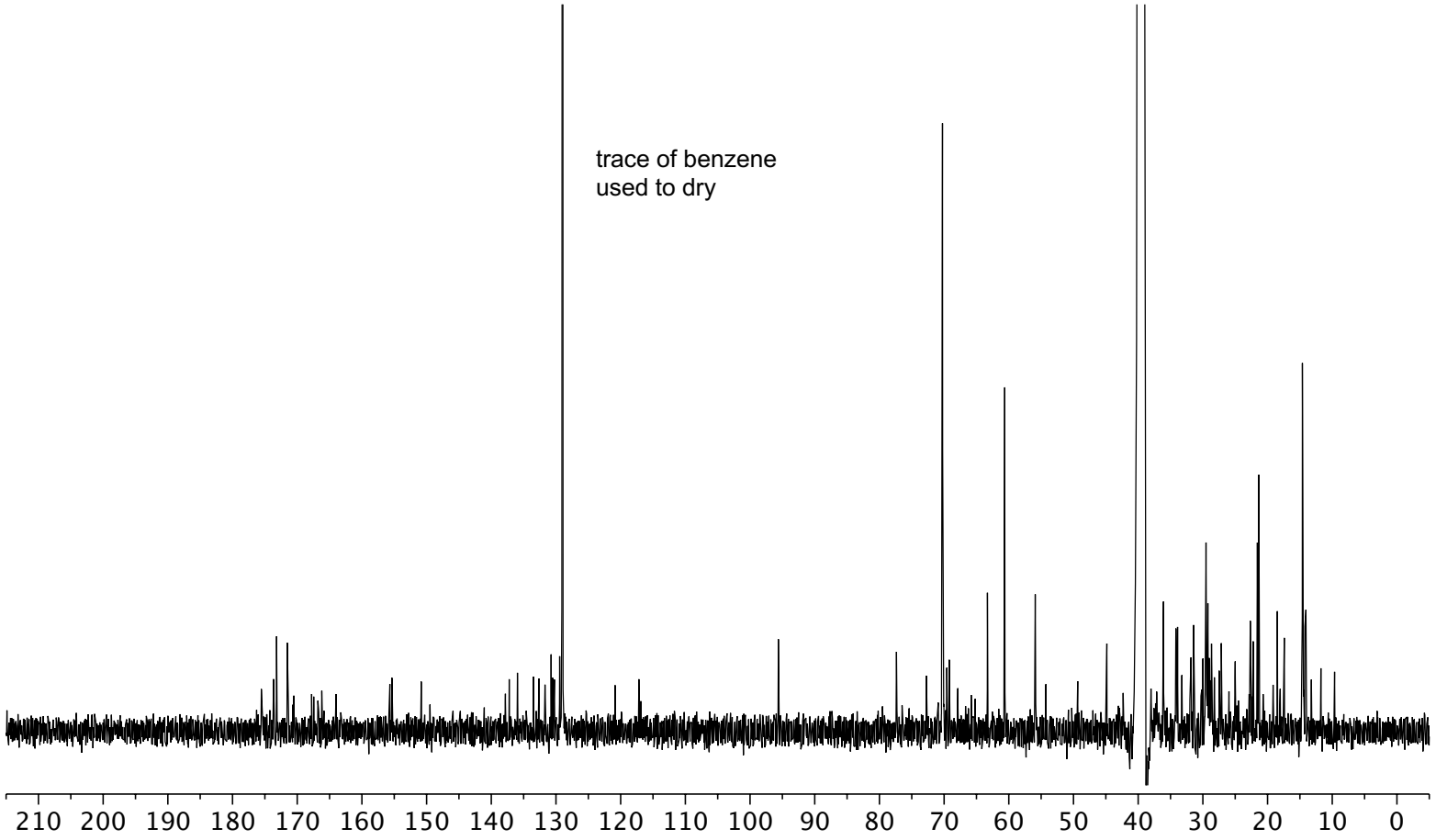
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **2b** in DMSO-d_6



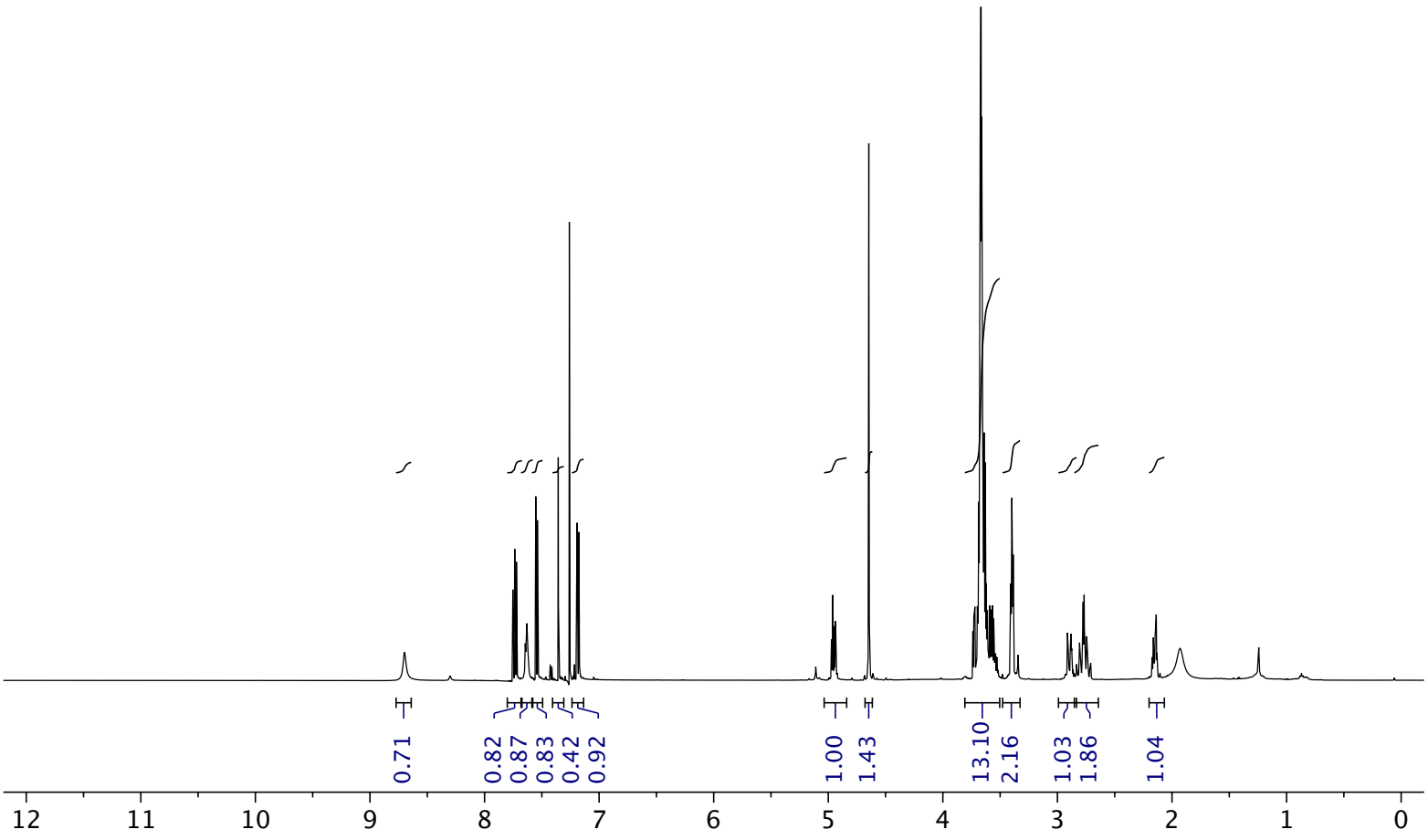


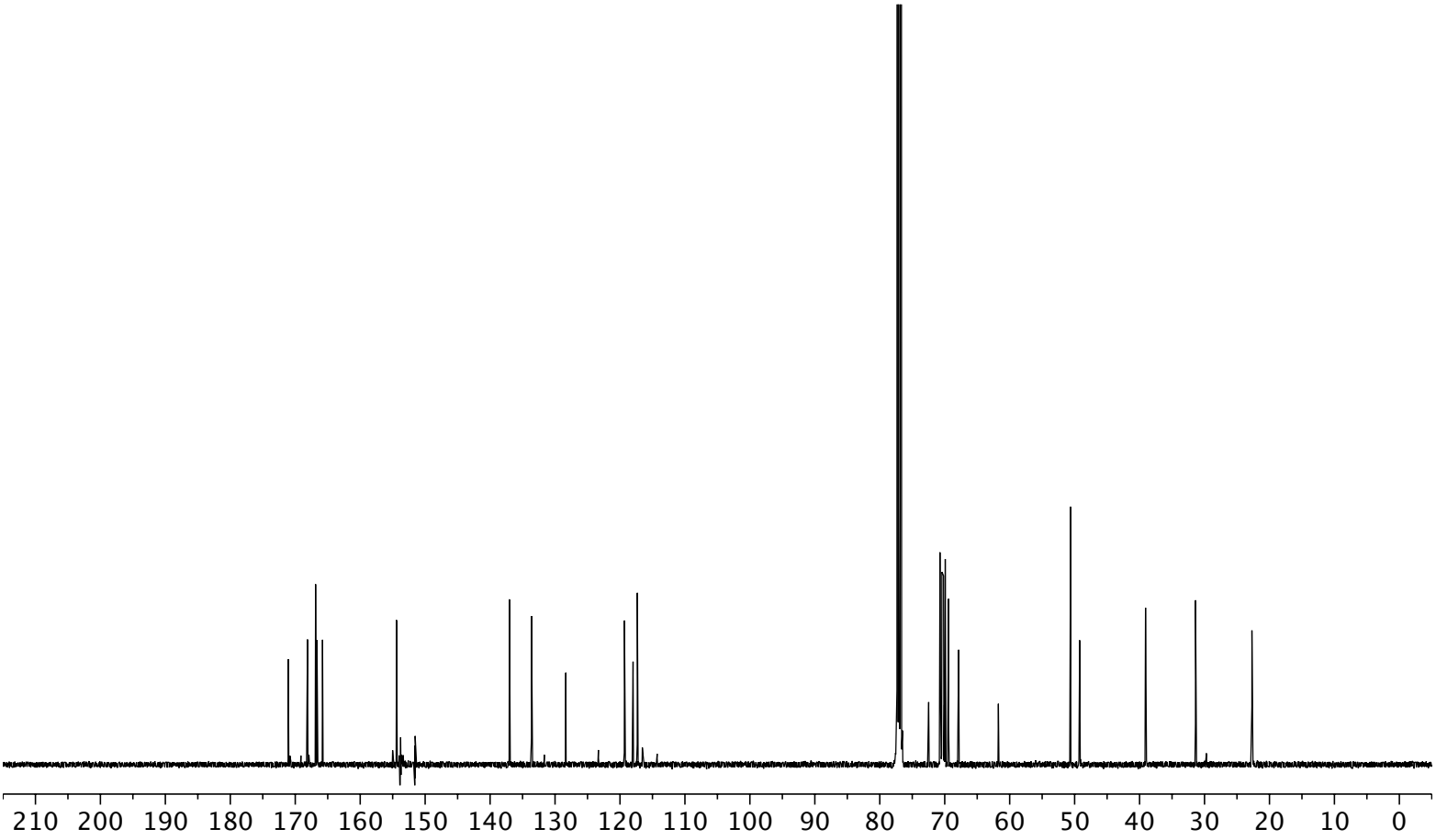
^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **2c** in DMSO-d_6





^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **8b** in $CDCl_3$





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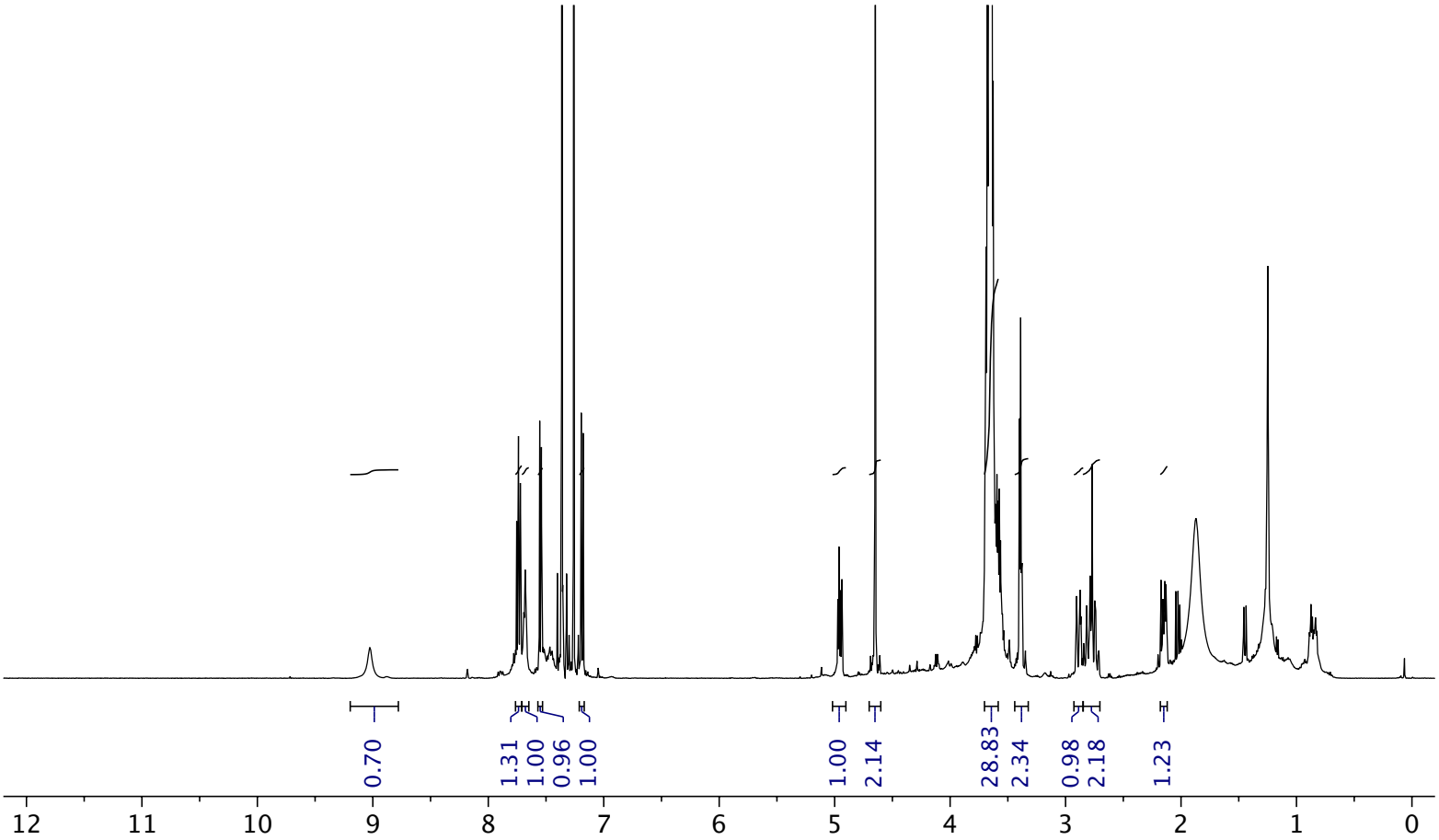
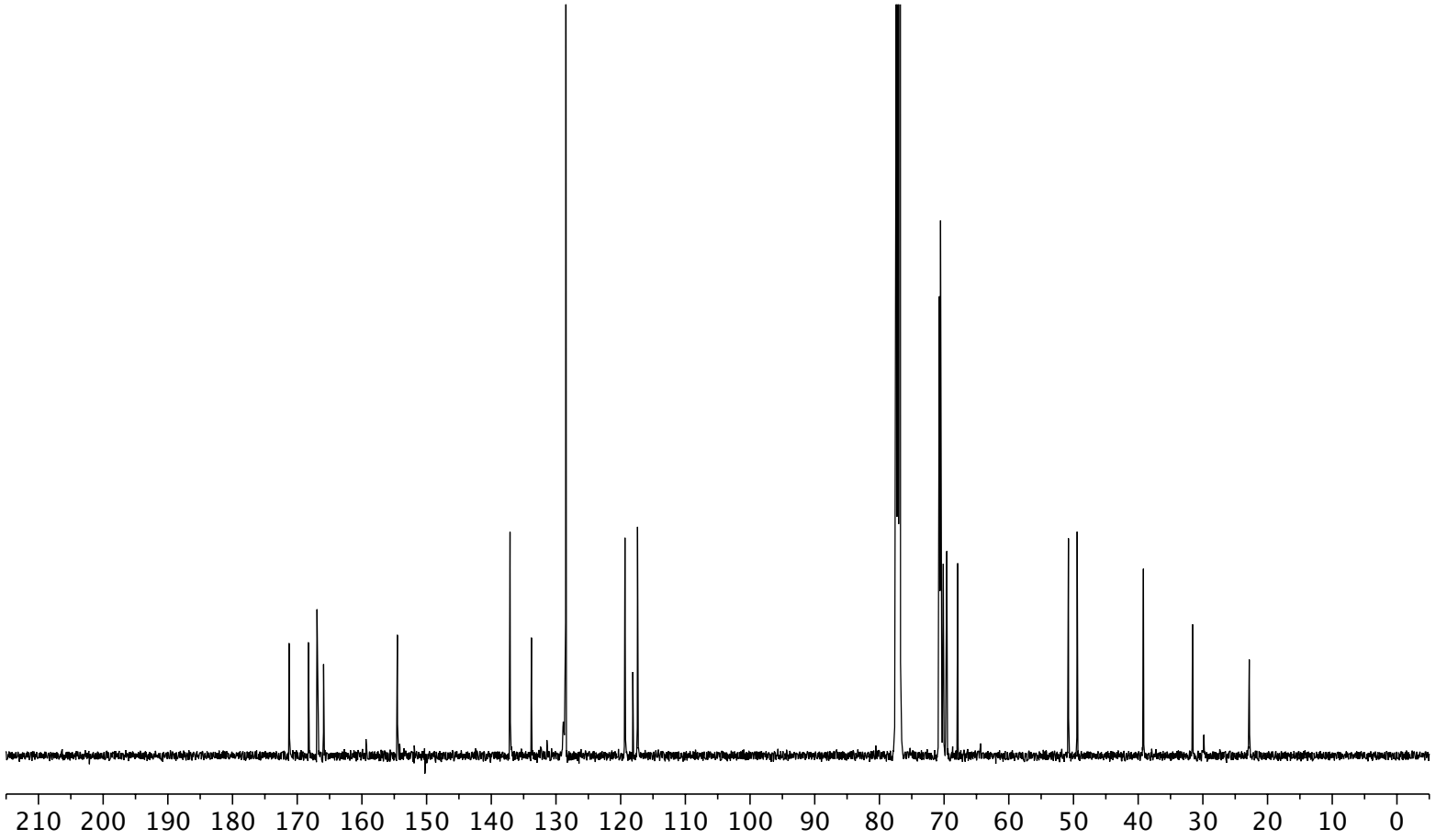
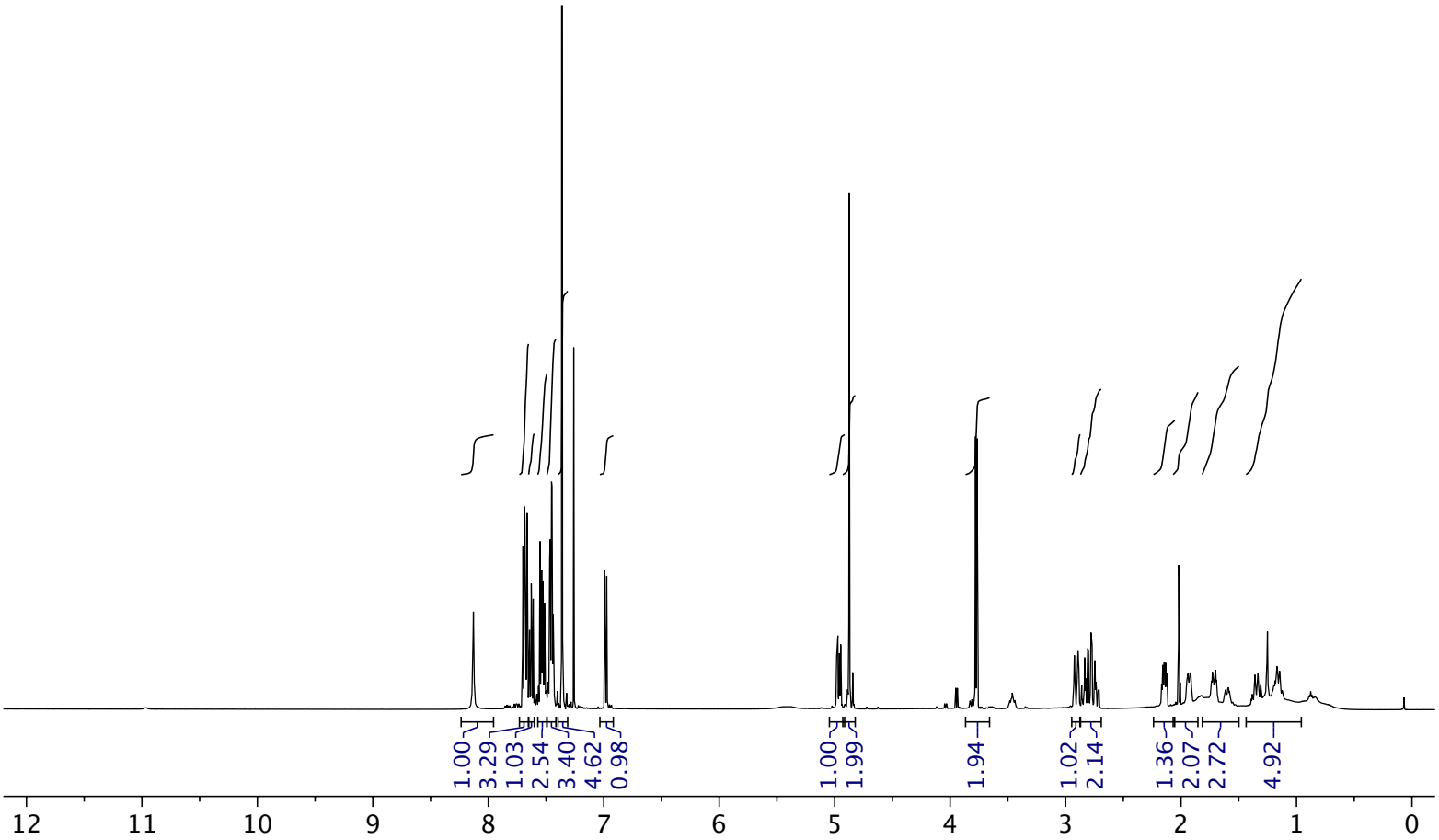
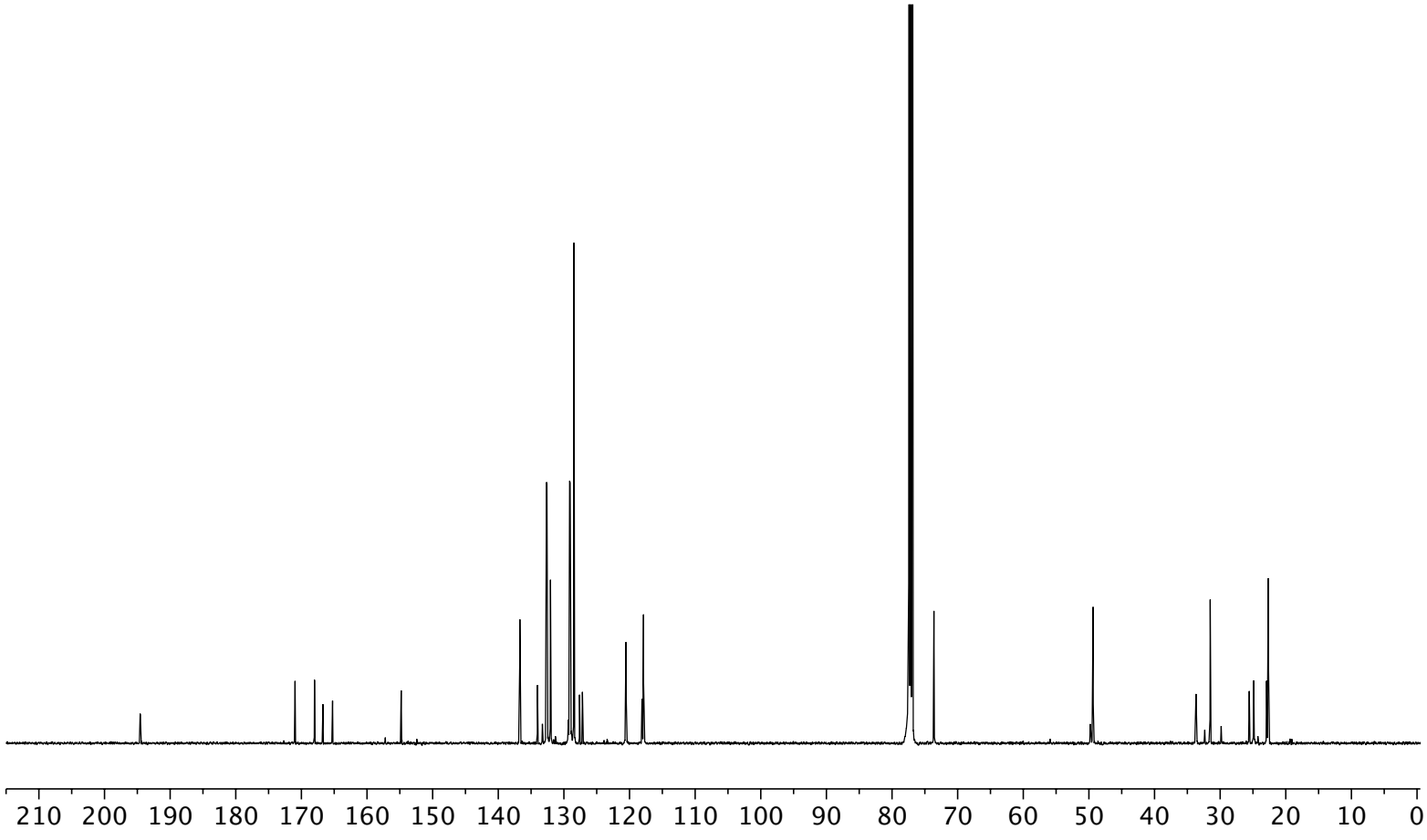




^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **10** in $CDCl_3$





^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **12** in $CDCl_3$