Direct-to-Biology Accelerates PROTAC Synthesis and the Evaluation of Linker Effects on Permeability and Degradation

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Supporting Information

I. General Experimental Information

General Information. Reactions were performed in standard lab glassware or disposable borosilicate vials with stirring without exclusion of air. Direct-to-Biology experiments were performed in aluminum 96-position reactor blocks filled with 1 mL borosilicate vials containing 4.80 mm parylene coated magnetic stir bars and sealed with a film of PFA supported by a rubber mat and an aluminum backed screw-down plate cap, while stirring and heating were achieved by tumble stirring (V&P Scientific Magnetic Tumble Stirrer, 500 rpm) and heating on a temperature-controlled heating block. Solid dosing of resins was generally achieved using a 96-well plate formatted volumetric solid dispensing plate, approximate masses dosed for specific resins employed were calibrated and used without correction. Filtration was achieved using 96-well plate format PTFE filter plates (0.4 micron) and eluted under vacuum pressure. Liquid dispensing was achieved using standard single channel and multi-channel pipettes without additional calibration. Slurry transfer was achieved in parallel using a multi-channel pipette and clipped pipette tips to permit transfer of heterogenous mixtures. Purified compounds were obtained in >95% purity, as assessed by UPLC-MS and/or ^{1}H NMR, unless otherwise indicated.

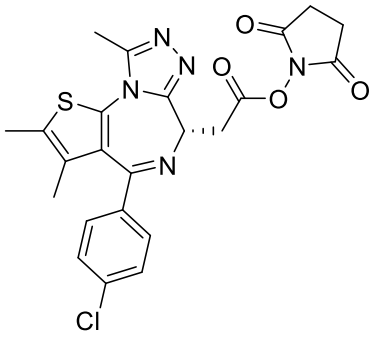
Materials. Commercially available starting materials and mono-Boc-diamine building blocks were purchased and used as received without further purification. Resin-bound scavengers MP-Trisamine and PS-Isocyanate were purchased from Biotage and used as received. DMF and DMSO were used from freshly unsealed anhydrous bottles. An authentic sample of dBet1 was purchased commercially and used as received.

Instrumentation. Analytical samples were performed on a Waters Acquity I-Class Ultra-High Pressure Liquid Chromatography-Mass Spec with a PDA detector and SQD2 mass-detection (ESI-pos/neg). Charged-Aerosol Detection was performed using a Waters Acquity I-Class UPLC-MS equipped with PDA and SQD2 detectors (ESI positive ionization mode); column: Waters CSH C18, 2.1mm x 50 mm (Waters # 186006101) with Waters ACQUITY UPLC Col. In-Line Filter Kit (Waters #205000343); Mobile Phase A: 0.1 % trifluoroacetic acid in MilliQ water, Mobile Phase B: 0.1% trifluoroacetic acid in Optima grade Acetonitrile. Corona Aerosol Detection (CAD) analysis was conducted with Thermo Corona™ Veo™ RS Charged Aerosol Detector. The flow rate was 2 mL/min using a gradient from 5-100% Acetonitrile over 2 minutes. The total run time for each injection was 2.3 minutes. Purification was performed using a Waters mass-triggered liquid chromatography using Waters XSelect CSH (C18, 5u, 19x100 mm) or Waters XBridge BEH (C18, 5u, 19x100 mm) reversed-phase columns and appropriate linear gradients of increasing concentration of acetonitrile in water, 0.1% TFA or FA. Fractions containing the desired product were combined and concentrated by centrifuge evaporation. High-throughput NMR data were collected using a Bruker AVANCE III HD solution-state NMR spectrometer equipped with a room temperature 1 mm TXI Microprobe at 400 MHz proton frequency at 298 K. The pulse sequence used was a one-dimensional (1D) proton experiment with water presaturation (zgpr). The acquisition time was 2.04 s using 32768 points for 20 ppm sweep width. The number of scans collected for each sample was determined based on its estimated weight. NMR data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad), coupling constant (Hz), and integration.

Computation. Calculated properties for molecular weight, formula, rotatable bonds were obtained from RDKit: Open Source Cheminformatics or analogous internal calculators. Calculated values for logD were obtained using BIOVIA, Dassault Systèmes, PipelinePilot, 21.2.100.53. Topological diameter was calculated using methods available in Schrödinger (Release 2020: Maestro, Schrödinger, LLC, New York, NY, 2021).

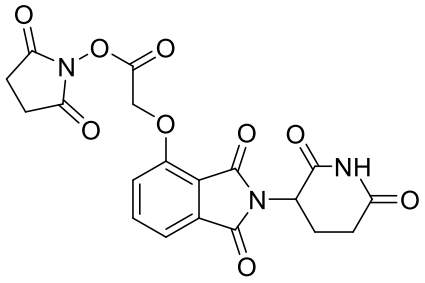
II. General Experimental Protocols

Synthesis of Starting Materials



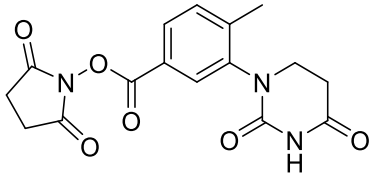
2,5-dioxopyrrolidin-1-yl-(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate (3)

A 40 mL vial was charged with (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (801 mg, 2.00 mmol, 1 equiv), *N*-hydroxysuccinimide (258 mg, 2.20 mmol, 1.1 equiv), DMAP (17 mg, 0.14 mmol, 0.07 equiv), and DCC Resin (3.60 g, [1.39 mmol/g], 5.00 mmol, 2.5 equiv), then DMF (13.3 mL) was added and the resulting slurry was agitated by stir bar until all acid starting material was observed to be dissolved and then allowed to stand at room temperature with occasional mixing so as to not degrade the resin. After 24 hours the reaction was diluted with ethyl acetate (10 mL) and the resin was removed by vacuum filtration through a pad of celite, washing with ethyl acetate (65 mL). The filtrate was further diluted in ethyl acetate (50 mL) and washed with a 1:1 brine/DI water solution (60 mL), the aqueous phase back extracted with ethyl acetate (2 x 60 mL), and the combined organic layers washed, sequentially, with DI water (4 x 60 mL), sat. $NaHCO_3$ (2 x 60 mL), and brine (60 mL) before drying over Na_2SO_4, filtering by vacuum filtration, and concentrating by rotary evaporation. The resulting solid was further dried by Genevac at high vacuum to afford the title compound (946 mg, 85%, purity 91%). 1H NMR (400 MHz, DMSO) δ 7.50 (s, 4H), 4.61 (dd, J = 7.8, 6.6 Hz, 1H), 3.79 – 3.72 (m, 2H), 2.88 (d, J = 20.8 Hz, 4H), 2.63 (s, 3H), 2.42 (s, 3H), 1.63 (s, 3H). 13C NMR (101 MHz, DMSO) δ 170.1, 166.8, 163.7, 154.0, 150.1, 136.6, 135.4, 132.5, 130.8, 130.4, 129.9, 129.4, 128.4, 53.3, 33.9, 25.5, 14.0, 12.7, 11.3. HRMS-ESI (m/z) Calcd for ($C_{23}H_{21}ClN_5O_4S$) ($[M+H]^+$): 498.0997; found: 498.1003.



2,5-dioxopyrrolidin-1-yl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (4).[1]

To a 40 mL vial add 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (300 mg, 0.90 mmol, 1 equiv), *N*-hydroxysuccinimide (116 mg, 1.76 mmol, 1.1 equiv), and DMAP (29 mg, 0.24 mmol, 0.15 equiv) and dissolve in DMF (5.0 mL) then cool by ice/water batch with stirring for at least 15 minutes. Once cooled, a slurry of EDCI (376 mg, 1.92 mmol, 1.2 equiv) in DMF (3.5 mL) was added in small portions of approx. 0.3 mL every 30 seconds until complete addition to the reaction mixture and, remaining in the ice bath, the mixture was permitted to slowly warm to room temperature overnight. The reaction was then diluted in ethyl acetate (150 mL) and washed with a 1:1 brine/DI water mixture (90 mL), the aqueous layer back extracted with ethyl acetate (2 x 60 mL), and the combined organics washed sequentially with DI water (4 x 75 mL), sat. $NaHCO_3$ (2 x 75 mL), and brine (50 mL) then dried over Na_2SO_4, before filtering by vacuum filtration, and concentrating by rotary evaporation. The resulting solid was further dried by Genevac at high vacuum to afford the title compound (283 mg, 41%). ^{1}H NMR (400 MHz, DMSO, 1H masked by DMSO) δ 11.11 (s, 1H), 7.86 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.52 (dd, *J* = 19.9, 7.9 Hz, 2H), 5.66 (s, 2H), 5.11 (dd, *J* = 12.8, 5.4 Hz, 1H), 2.83 (s, 4H), 2.70 – 2.51 (m, 2H), 2.05 (ddd, *J* = 13.6, 6.3, 3.9 Hz, 1H). LCMS-ESI (m/z) Expected ($C_{19}H_{16}N_3O_9$) ([M+H]$^+$): 430.1; observed: 429.9.

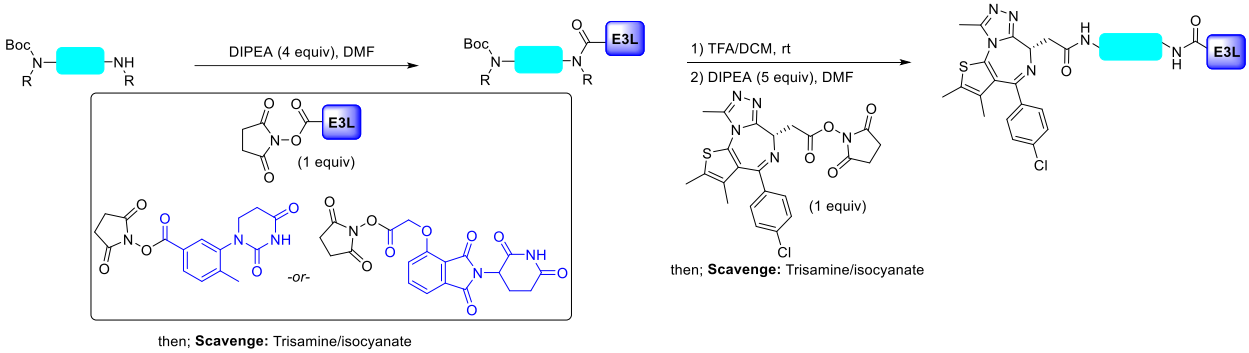


2,5-dioxopyrrolidin-1-yl 3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzoate (5).

A 40 mL vial was charged with 3-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-4-methylbenzoic acid (500 mg, 2.01 mmol, 1 equiv)[2], *N*-hydroxysuccinimide (260 mg, 2.22 mmol, 1.1 equiv), DMAP (17 mg, 0.14 mmol, 0.07 equiv), and DCC Resin (3.62 g, [1.39 mmol/g], 5.04 mmol, 2.5 equiv), then DMF (13.4 mL) was added and the resulting slurry was agitated by stir bar until all acid starting material was observed to be dissolved and then allowed to stand at room temperature with occasional mixing so as to not degrade the resin. After 24 h the reaction was filtered through a pad of celite by vacuum filtration, rinsing with copious DCM, then further diluted to a total volume of 500 mL DCM before extracting sequentially with brine (100 mL), sat. $NaHCO_3$ (2 x 100 mL), DI water (2 x 100 mL), and again with brine (50 mL) then dried over dried over Na_2SO_4, before filtering by vacuum filtration, and concentrating by rotary evaporation. The resulting solid was further dried by Genevac at high vacuum to afford the title compound (636 mg, 91%). ^{1}H NMR (400 MHz, DMSO) δ 10.45 (s, 1H), 8.04 (d, *J* = 1.9 Hz, 1H), 7.96 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 3.87 (ddd, *J* = 12.1, 9.5, 5.3 Hz, 1H), 3.58 (dt, *J* = 12.0, 5.9 Hz, 1H), 2.90 (s,

4H), 2.75 (dtd, *J* = 16.7, 11.2, 10.2, 6.0 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.7, 170.3, 161.2, 151.9, 144.7, 141.8, 131.9, 129.2, 128.9, 123.1, 44.3, 31.0, 25.5, 18.0. HRMS-ESI (m/z) Calcd for ($C_{16}H_{16}N_3O_6$) ([M+H]$^+$): 346.1034; found: 346.140.

General Procedure A: Experimental Method for Direct-to-Biology Sequence



Coupling of E3-ligase Ligand Moiety to mono-Boc Diamine Library to Afford Boc-protected E3-ligase-Ligand Linker-Amines

A 96-position aluminum reaction plate was loaded with 1 mL vials containing a stir bar and a pre-dispensed array of 91 unique *N*-Boc-diamines (6 µmol, 1.2 equiv) with 5 blank positions reserved for controls. A stock solution was generated and dosed to each vial by multi-channel pipette as E3-ligase (5 µmol, [0.1], 1 equiv) and DIPEA (3.45 µL, 20 µmol, 4 equiv) in DMF (50 µL). The plate was then sealed and either heated to 60 °C for 16 h (tDHU-NHS) or at 40 °C for 4 h with stirring before cooling to room temperature. The plate was unsealed, and using a solid-dispensing dropper plate, approx. 20 mg of MP-trisamine resin then approx. 20 mg PS-isocyanate resin were added to each vial before a multi-channel pipette was used to add an additional portion of DMF (50 µL), the plate re-sealed then stirred at room temperature for at least 5 hours. Vacuum filtration through a 0.4 micron filter plate was achieved using liquid transfer by multi-channel pipette, with pipette tips cut to prevent clogging, and rinsing with DMF (2 x 150 µL). The scavenged mixtures were concentrated by Genevac to afford the Boc-protected intermediates which were carried forward without further purification.

Boc Deprotection of Amide Intermediates

A 96-position aluminum reaction plate racked with vials containing the crude *N*-Boc-protected intermediates and a stir bar was dosed with DCM (10 µL) followed by TFA (40 µL), sealed, and

stirred at room temperature for 2 hours before unsealing and drying by Genevac* to remove volatiles. Resulting crude salts were carried forward without further manipulation.

JQ1 Moiety Coupling to E3-ligase-Ligand Linker-Amine TFA salts

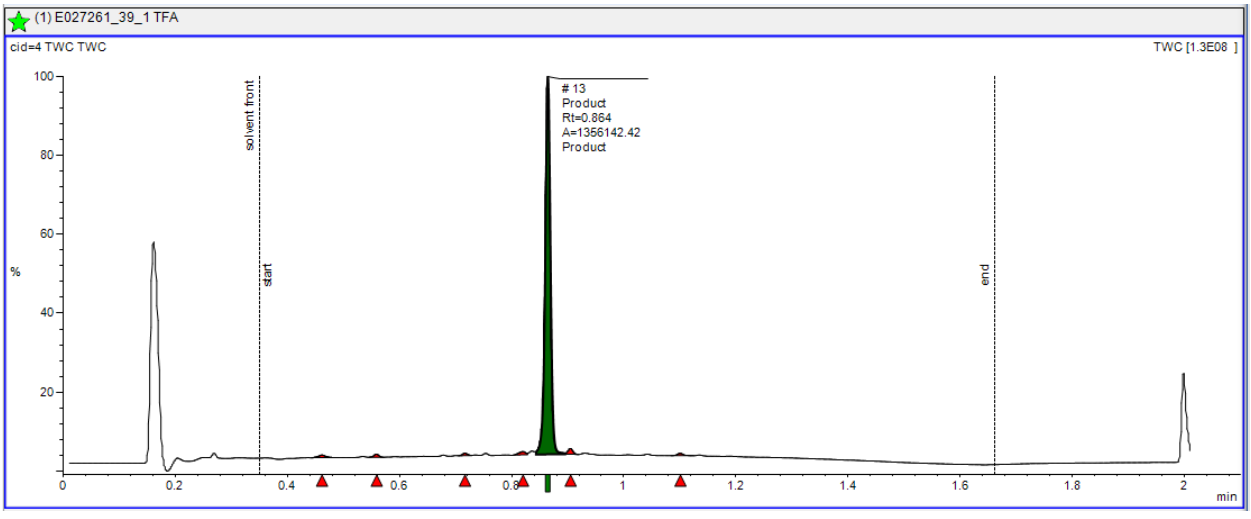
A stock solution of JQ1-NHS ester (5 µmol, 1 equiv) and DIPEA (25 µmol, 5 equiv) in DMF (50 µL) was then added to the crude TFA amine salts, the plate sealed, and the resulting mixtures placed on 40 °C heat with stirring for 16 hours before removing from heat and cooling to room temperature. The plate was unsealed, and using a solid-dispensing dropper plate, approx. 20 mg of MP-trisamine resin then approx. 20 mg PS-isocyanate resin were added to each vial before a multi-channel pipette was used to add an additional portion of DMF (50 µL), the plate re-sealed then stirred at room temperature for at least 5 hours before vacuum filtration through a 0.4 micron filter plate, rinsing with DMF (2 x 150 µL) and concentrated and dried by Genevac to afford the crude product residue. The crude material was solubilized in DMSO (250 µL) to afford at stock solution of maximally 20 mM concentration, an aliquot (5 µL) removed for quantification by CAD, and the remainder reserved for assay plating. Yield determination for each well was determined by CAD, against a calibration curve with noscapine, to determine the actual concentration of product in each well and dividing by the theoretical concentration for the reaction sequence.

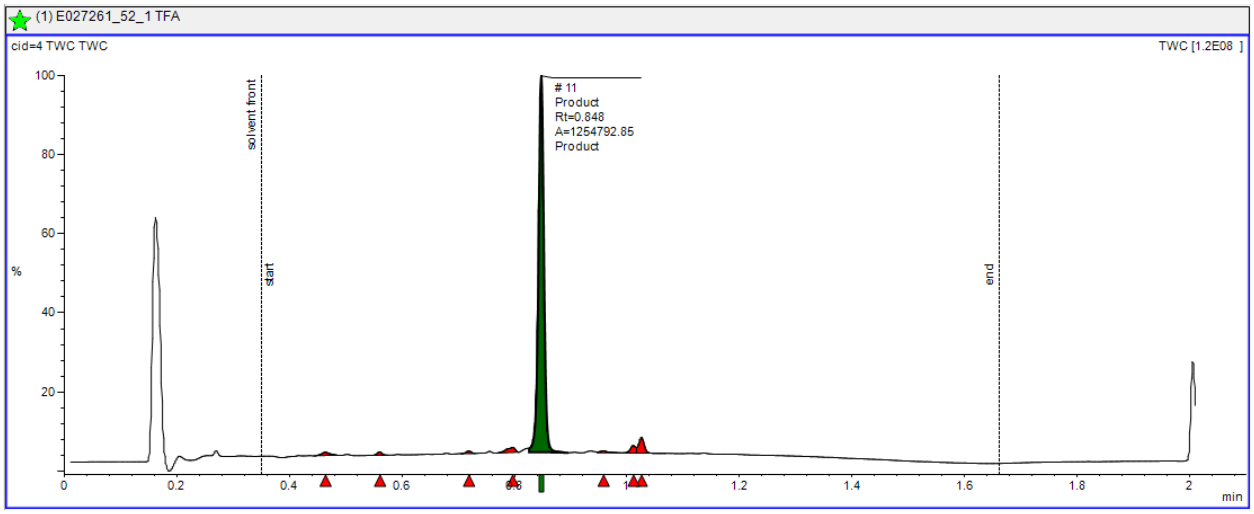
* Though the TFA/DCM mixture was typically removed within a few hours, thorough drying on high BP setting overnight was required to remove residual TFA.

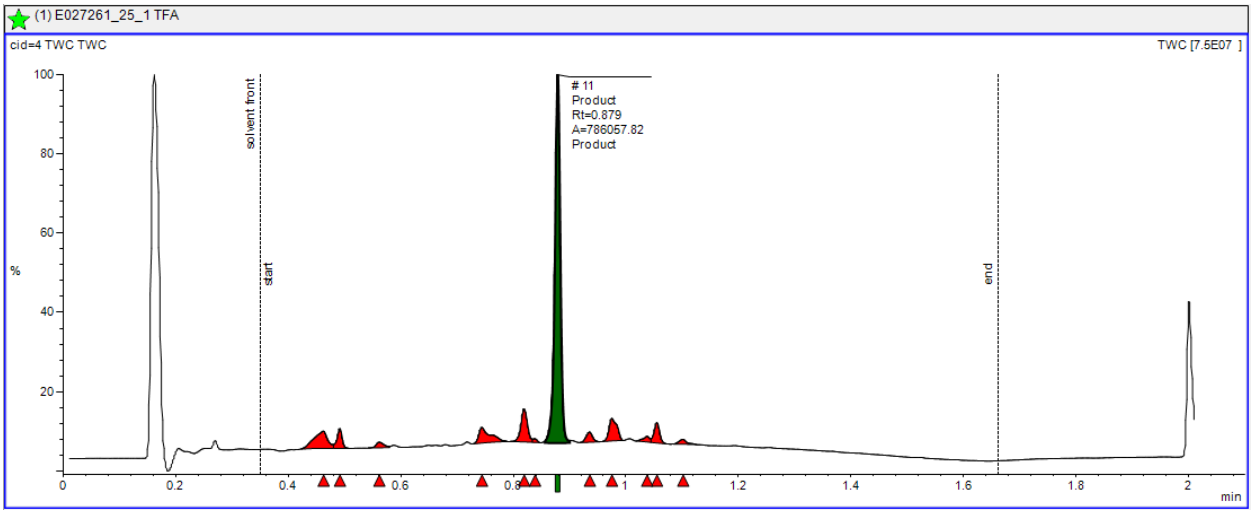
Table S1. Select Examples of Product Purity

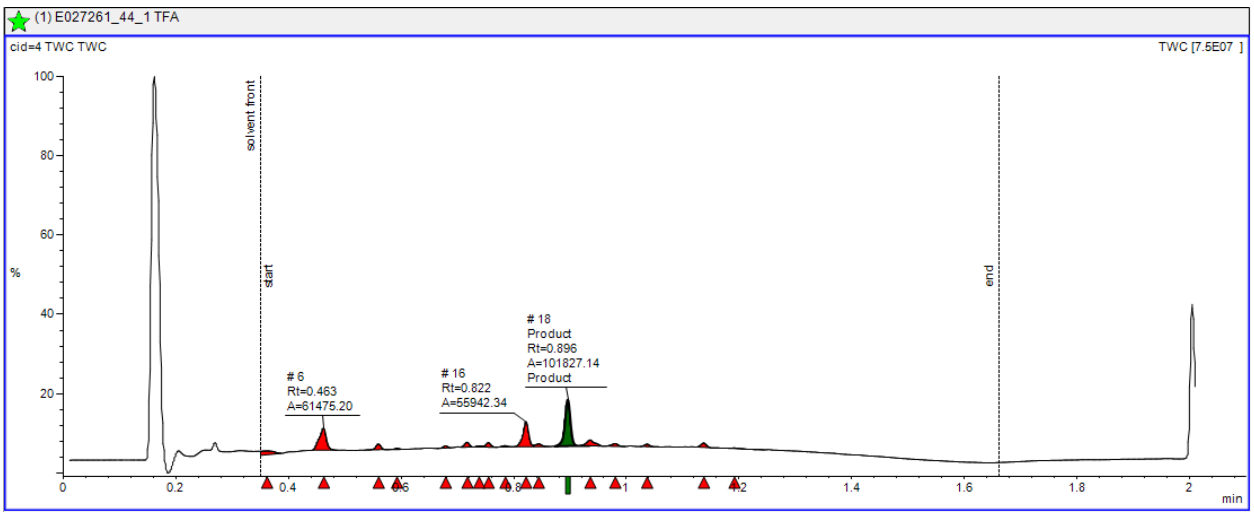
Coords.	Mass Expected	Product mg/mL	CAD Yield	Rt (min)	UV215 Area%
D03	832.2	0.622	41%	0.865	92.22
E04	822.2	0.509	34%	0.848	88.48
C01	846.2	0.314	20%	0.879	60.55
D08	824.3	0.045	3%	0.896	29.33

Figure S1. UPLC Traces of Select D2B Synthesis Samples







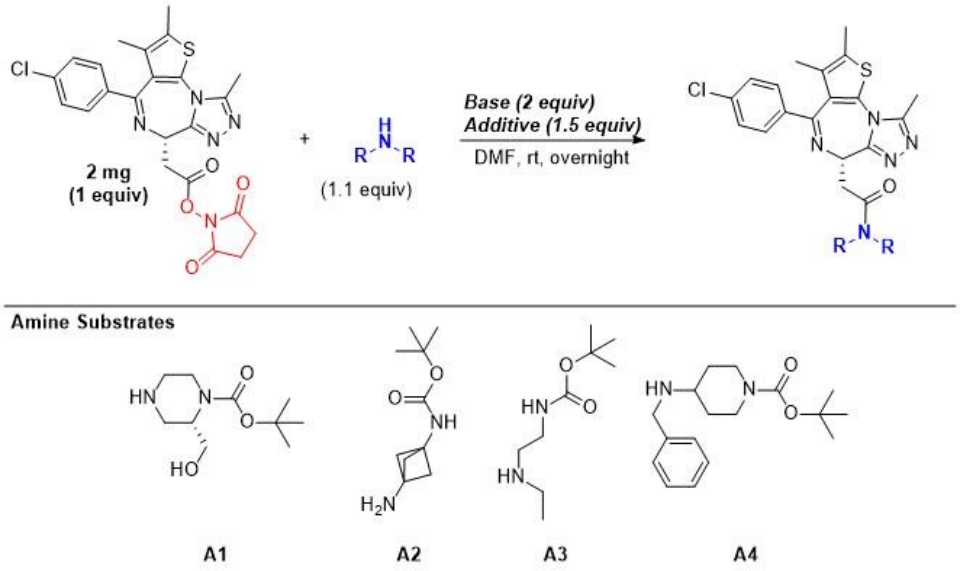


III. Chemistry Optimization Data

HTE Screening Procedure for NHS-Ester Coupling Optimization

A 24-vial aluminum reaction screening block with 1 mL vials was charged with a stock solution of the indicated amine to each well in DMF (5.5 µmol, 12.5 µL, [0.44], 1.1 equiv) followed by JQ1-NHS ester **3** in DMF (5 µmol, 12.5 µL, [0.2], 1 equiv). To the indicated wells was then added the additive as either a stock solution or slurry in DMF (12.5 µL) followed by the indicated base as either a stock solution or slurry (10 µmol, 2 equiv) and the plate was sealed and stirred at room temperature for 16 h before unsealing and quenching with a solution of Ph3N in MeCN/DMSO (250 µL, [0.002], 3:1). The crude mixtures were mixed vigorously and aliquots were removed (25 µL) for UPLC-MS analysis.

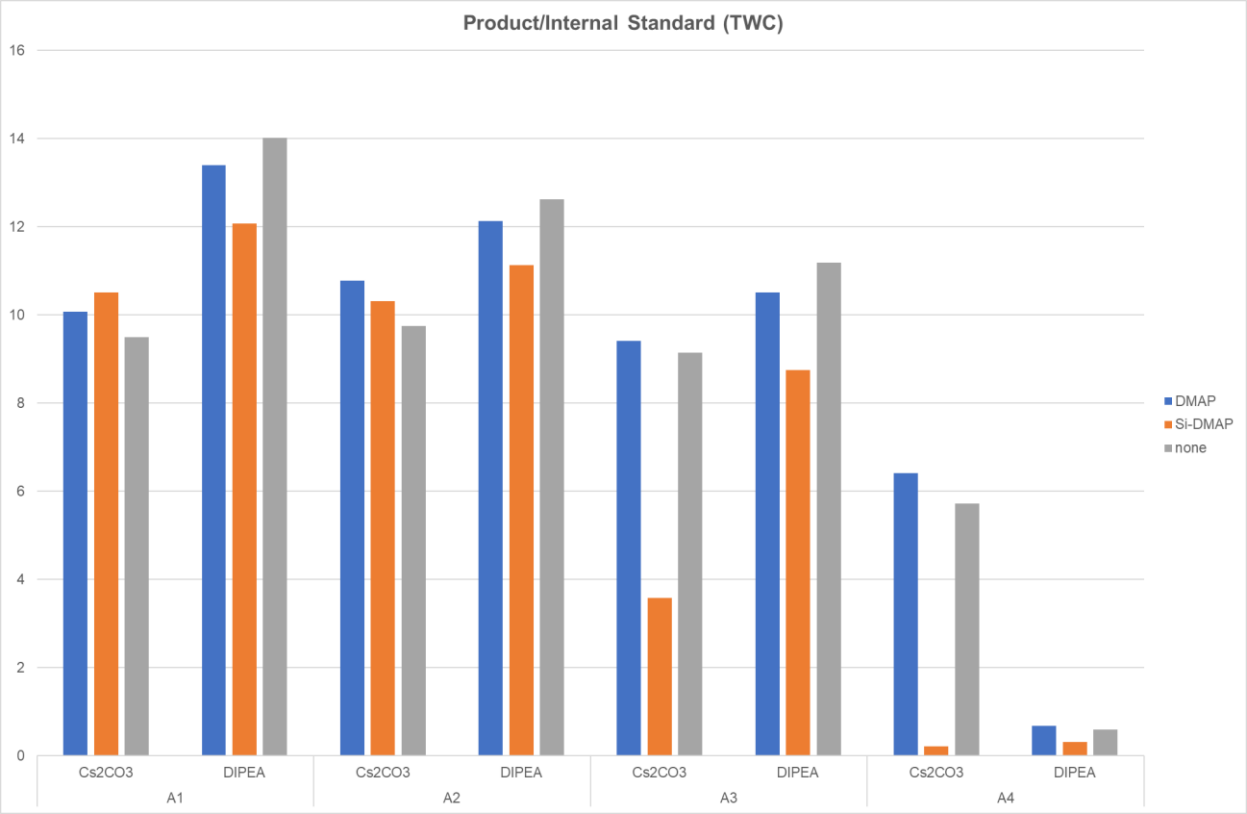
Table S2. Optimization Data for JQ1-NHS ester Coupling



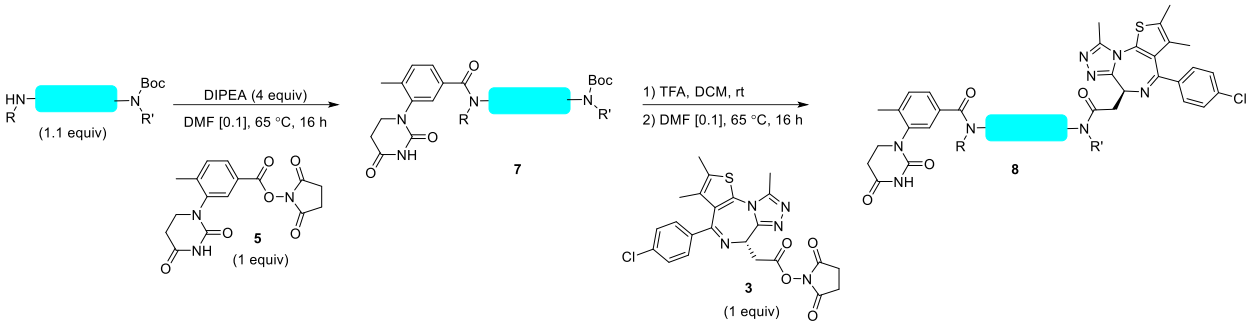
Substrate	Base	Additive	*Prod./IS*	*SM1/IS*	*JQ1-CO2H/IS*
A1	DIPEA	none	14.02	0.00	0.35
A1	DIPEA	DMAP	13.40	0.00	0.36
A1	DIPEA	Si-DMAP	12.08	0.00	0.36
A1	Cs2CO3	none	9.50	0.00	1.38
A1	Cs2CO3	DMAP	10.08	0.00	1.44
A1	Cs2CO3	Si-DMAP	10.51	0.00	0.91
A2	DIPEA	none	12.63	0.00	0.35
A2	DIPEA	DMAP	12.13	0.00	0.34
A2	DIPEA	Si-DMAP	11.13	0.00	0.29
A2	Cs2CO3	none	9.76	0.00	2.21
A2	Cs2CO3	DMAP	10.79	0.00	1.42
A2	Cs2CO3	Si-DMAP	10.32	0.00	1.02

A3	DIPEA	none	11.18	2.27	0.70
A3	DIPEA	DMAP	10.51	0.49	2.19
A3	DIPEA	Si-DMAP	8.75	0.00	0.53
A3	Cs2CO3	none	9.14	0.00	2.84
A3	Cs2CO3	DMAP	9.41	0.00	2.40
A3	Cs2CO3	Si-DMAP	3.58	0.00	2.72
A4	DIPEA	none	0.60	10.66	1.73
A4	DIPEA	DMAP	0.68	0.47	11.67
A4	DIPEA	Si-DMAP	0.31	4.17	2.13
A4	Cs2CO3	none	5.73	0.00	5.45
A4	Cs2CO3	DMAP	6.41	0.00	4.71
A4	Cs2CO3	Si-DMAP	0.21	0.00	4.47

Figure S2. Bar chart of JQ1-NHS ester coupling optimization results.



Pilot Study Procedure for Direct-to-Biology Sequence



A 24-vial aluminum reaction screening block with 1 mL vials was charged with a stock solution of the indicated amine in DMF (5.5 µmol, 28 µL, [0.2], 1.1 equiv) followed by either JQ1-NHS ester **3** in DMF (5 µmol, 25 µL, [0.2], 1 equiv), or tDHU-NHS ester **5** in DMF (5 µmol, 25 µL, [0.2], 1 equiv) then DIPEA (3.5 µL, 4 equiv) before sealing the plate, stirring at 65 °C for 16 h, and cooling to room temperature. An aliquot was removed for pre-scavenger analysis, then to the indicated wells was added MP-Trisamine resin (approx. 20 mg) and PS-isocyanate resin (approx. 20 mg) followed by additional DMF (150 µL) and the resulting slurry stirred at room temperature for 4 hours before removing the resin by vacuum filtration, washing with DMF (2 x 150 µL), removing an aliquot for analysis, transferring to clean 1 mL vials, and concentrating by Genevac to afford the crude Boc-protected amine intermediate which was carried forward without further purification.

Table S3. Results of tDHU-NHS amine coupling with and without scavenging

Amine	Product Area	tDHU-NHS Area	IS Area	P/IS	tDHU-NHS/IS
CRUDE					
1	571778	182527	601466	0.95	0.30
2	519705	53589	606438	0.86	0.09
3	855200		603450	1.42	0.00
4	383613	346398	603323	0.64	0.57
5	635491		602729	1.05	0.00
6	799539		548050	1.46	0.00
SCAVENGED					
1	734268		618621	1.19	0.00
2	505830		590482	0.86	0.00
3	912662		622034	1.47	0.00
4	386488		647899	0.60	0.00
5	709439		664493	1.07	0.00
6	888876		718373	1.24	0.00

Figure S3. Bar graph of D2B Pilot Study Product Formation

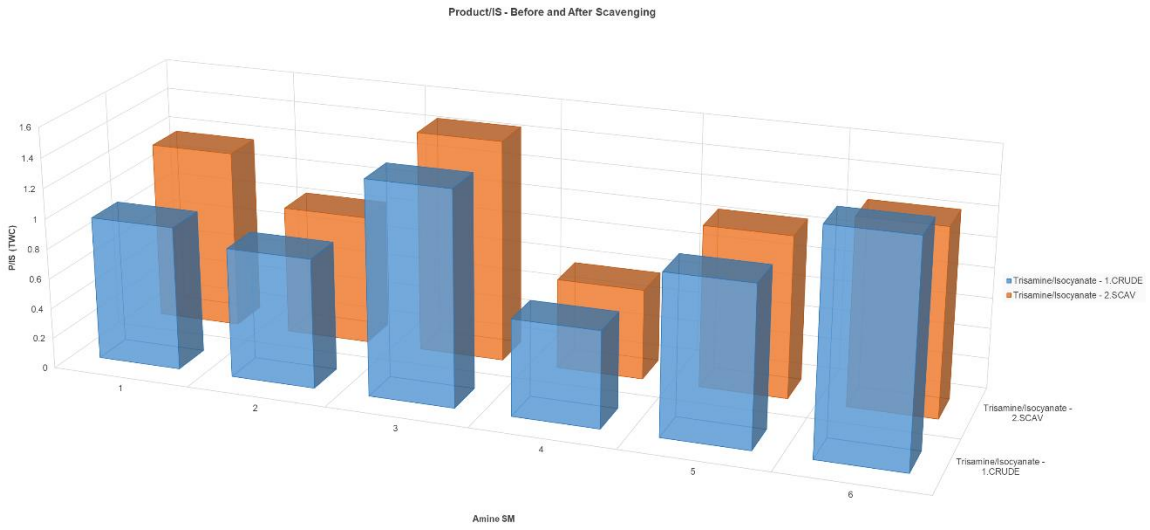
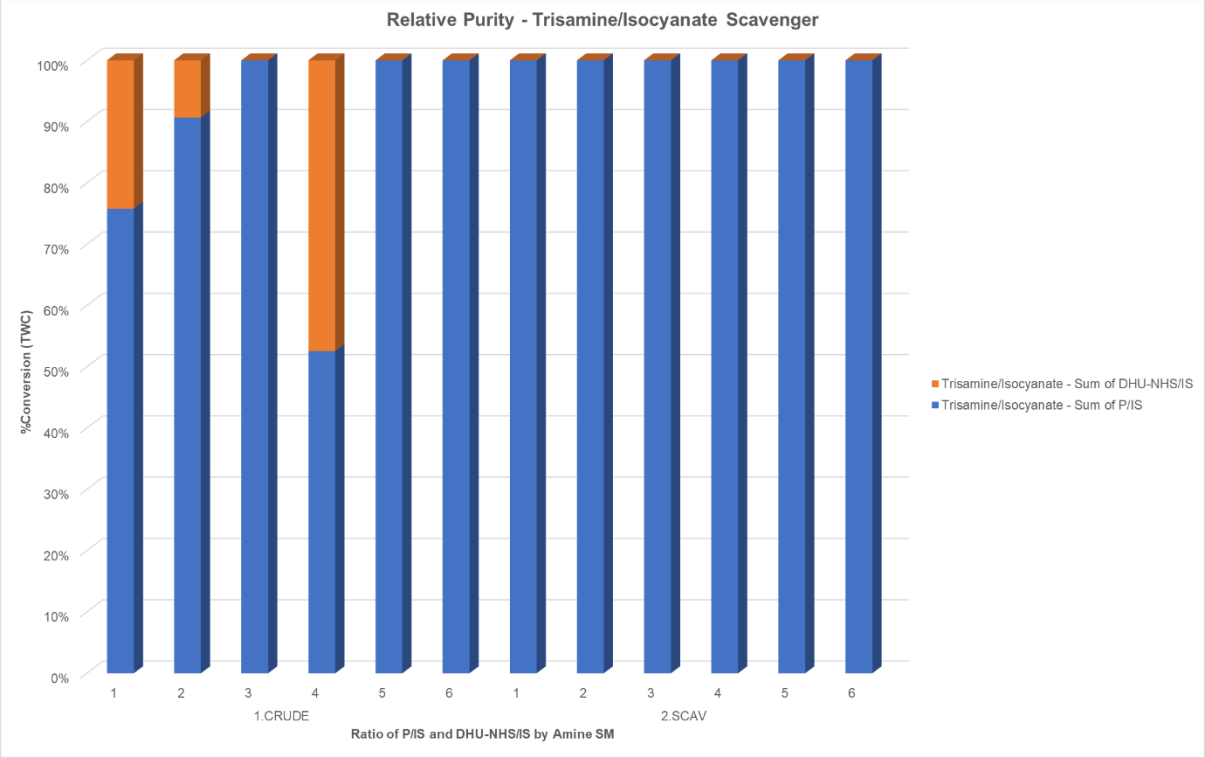


Figure S4. Stacked bar graph of relative purity after tDHU-NHS coupling step



To the vials containing the crude *N*-Boc diamines and charged with a stir bar was added DCM (15 µL) followed by TFA (35 µL) and the reactions stirred at room temperature for 4 hours before concentrating by Genevac. To the thoroughly dried crude salts was then added a stock solution of either JQ1-NHS ester **3** in DMF (5 µmol, 25 µL, [0.2], 1 equiv), or tDHU-NHS ester **5** in DMF (5 µmol, 25 µL, [0.2], 1 equiv) then DIPEA (4.3 µL, 5 equiv) before sealing the plate, stirring at 65 °C for 16 h, and cooling to room temperature. An aliquot was removed for pre-scavenger analysis, then to the indicated wells was added MP-Trisamine resin (approx. 20 mg) and PS-isocyanate resin (approx. 20 mg) followed by additional DMF (150 µL) and the resulting slurry stirred at room temperature for 4 hours before removing an aliquot for analysis.

Table S4. Pilot D2B Results for JQ1-NHS ester coupling step with and without Scavenging.

Amine	P/IS	JQ1-CO2H/IS	JQ1-NHS/IS
CRUDE			
1	2.70	0.00	0.66
2	2.36	0.00	0.20
3	3.14	0.00	0.23
4	1.61	0.47	1.04
5	2.93	0.29	0.36
6	2.92	0.31	0.28
SCAVENGED			
1	4.01	0.00	0.00
2	2.34	0.00	0.00
3	3.40	0.00	0.00
4	1.69	0.39	0.00
5	2.97	0.25	0.00
6	4.59	0.43	0.00

Figure S5. Bar graph of product vs. internal standard before and after scavenging

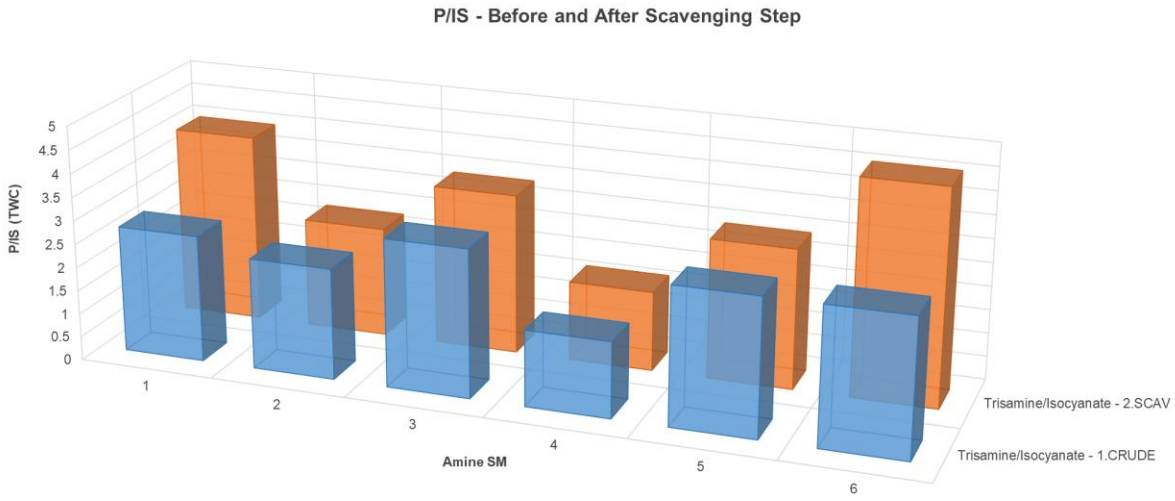
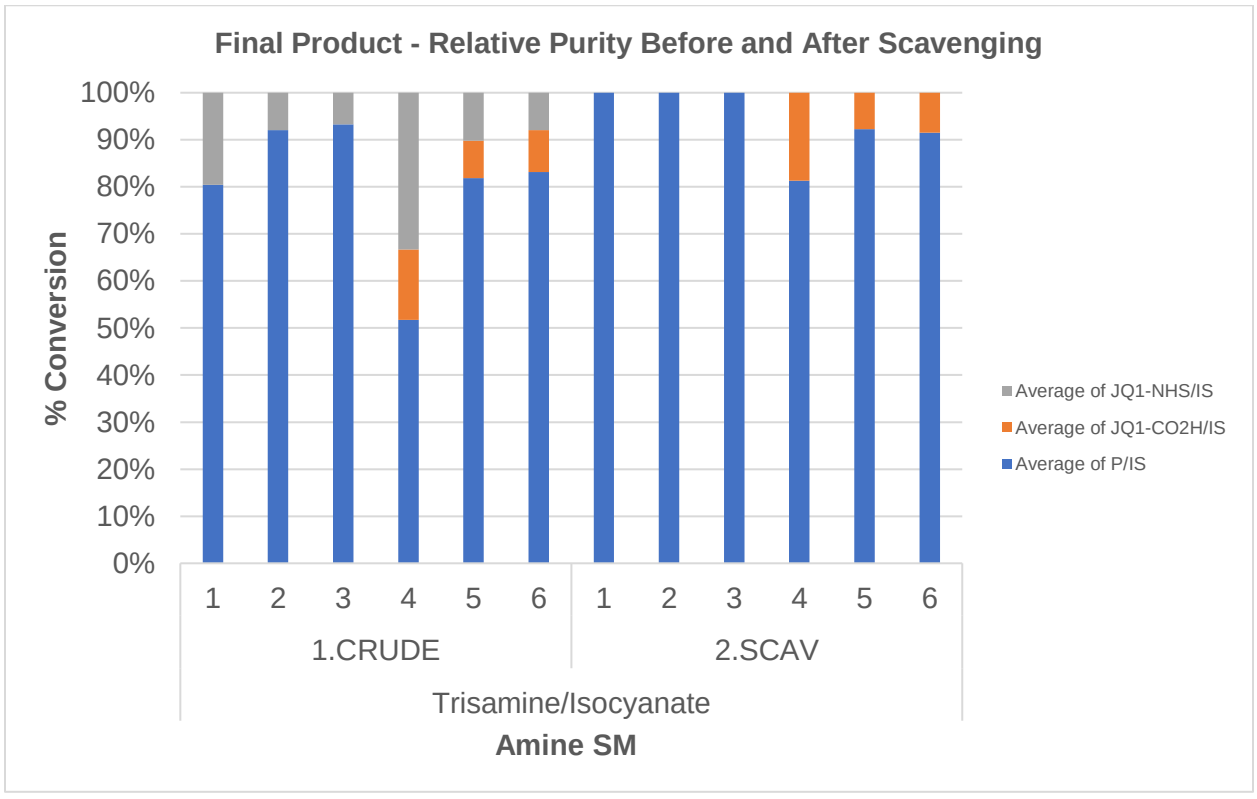
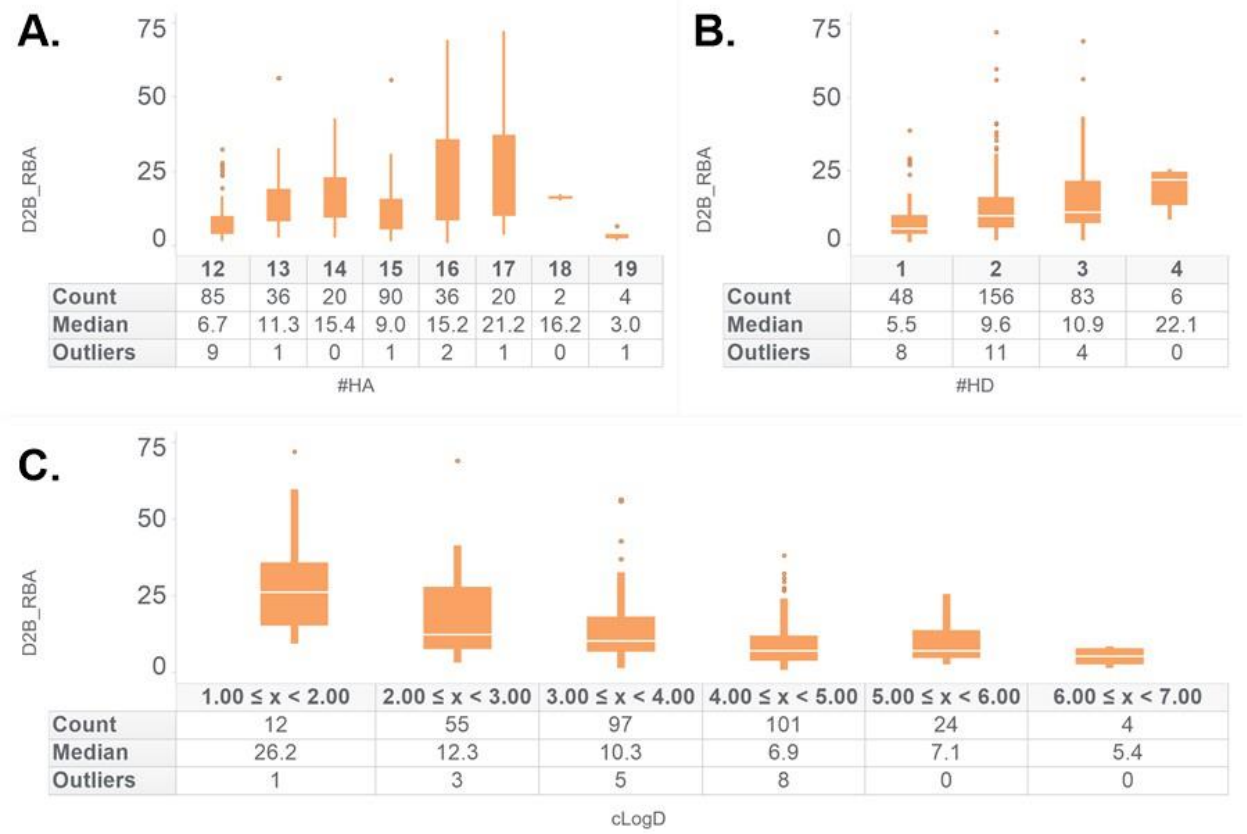


Figure S6. Stacked bar-graph showing relative purity of product before and after scavenging.



IV. Property Trends Observed from D2B Library Data

Figure S7. Box plots representing RBA versus H-Bond acceptor count, H-Bond donor count, and clogD.



#HA	12	13	14	15	16	17	18	19
Count	85	36	20	90	36	20	2	4
Median	6.7	11.3	15.4	9.0	15.2	21.2	16.2	3.0
Outliers	9	1	0	1	2	1	0	1

#HD	1	2	3	4
Count	48	156	83	6
Median	5.5	9.6	10.9	22.1
Outliers	8	11	4	0

cLogD	1.00 ≤ x < 2.00	2.00 ≤ x < 3.00	3.00 ≤ x < 4.00	4.00 ≤ x < 5.00	5.00 ≤ x < 6.00	6.00 ≤ x < 7.00
Count	12	55	97	101	24	4
Median	26.2	12.3	10.3	6.9	7.1	5.4
Outliers	1	3	5	8	0	0

V. Product Synthesis and Characterization

General Procedure B: Library Synthesis of Degraders using NHS-Esters

*Synthesis of Stock Solution of JQ1-NHS (**3**)*

To a 20 mL vial add (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (500 mg, 1.25 mmol, 1 equiv), *N*-hydroxysuccinimide (161 mg, 1.37 mmol, 1.1 equiv), DMAP (11 mg, 0.09 mmol, 0.07 equiv), and PS-DCC resin (1.49 g, 1.67 mmol/g, 2.49 mmol, 2 equiv) followed by DMF (8.3 mL) and the resulting slurry was stirred until all solid acid was dissolved then allowed to stand at room temperature overnight after which time the resin was removed by vacuum filtration, rinsing with minimal DCM. Volatiles were removed by rotary evaporation to afford the crude product as a solution in DMF which was immediately carried forward without further purification as a stock solution in DMF ([0.21]) with presumed quantitative yield and volume of remaining DMF.

*Synthesis of Stock Solution of O-Pom-NHS (**4**)*

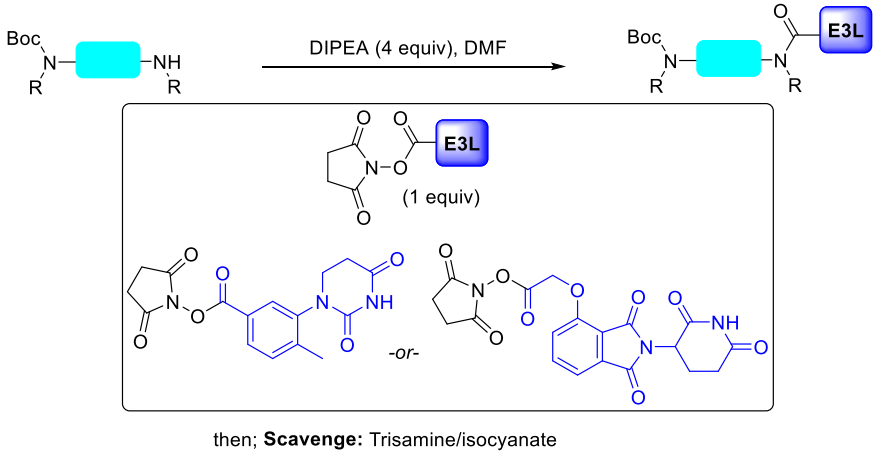
To a 40 mL vial add 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (731 mg, 2.20 mmol, 1 equiv), *N*-hydroxysuccinimide (284 mg, 2.42 mmol, 1.1 equiv), DMAP (40 mg, 0.33 mmol, 0.15 equiv), and PS-DCC resin (2.65 g, 2.61 mmol/g, 6.92 mmol, 3.14 equiv) followed by DMF (14.7 mL) and the resulting slurry was stirred until all solid acid was dissolved then allowed to stand at room temperature overnight after which time the resin was removed by vacuum filtration to afford the crude product as a solution in DMF. The resulting product solution was immediately carried forward without further purification as a stock solution in DMF ([0.15]) with presumed quantitative yield.

*Synthesis of Stock Solution of tDHU-NHS (**5**)*

To a 20 mL vial add 3-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-4-methylbenzoic acid (350 mg, 1.41 mmol, 1 equiv), *N*-hydroxysuccinimide (182 mg, 1.55 mmol, 1.1 equiv), DMAP (12 mg, 0.1 mmol, 0.07 equiv), and PS-DCC resin (2.53 g, 1.67 mmol/g, 4.23 mmol, 3 equiv) followed by DMF (9.4 mL) and the resulting slurry was stirred until all solid acid was dissolved then allowed to stand at room temperature overnight after which time the resin was removed by vacuum filtration, rinsing with minimal DCM. The DCM was distilled off by rotary evaporation at moderate vacuum to afford the crude product as a solution in DMF which was immediately carried forward without further purification as a stock solution in DMF ([0.15]) with presumed quantitative yield.

Synthesis of Degraders

Step 1: E3-ligase ligand coupling to afford Boc-protected intermediates S



then; **Scavenge:** Trisamine/isocyanate

To a 24-vial reactor block containing 1 dram vials with pre-dispensed mono-Boc-diamines (80 µmol, 1.07 equiv) was added a stock solution of either O-Pom-NHS **4** or tDHU-NHS **5** (0.52 mL, [0.15], 75 µmol, 1 equiv) followed by DIPEA (52 µL, 0.30 mmol, 4 equiv) before the vials were sealed, placed on either 40 ℃ (O-Pom-NHS) or 65 ℃ (tDHU-NHS) heat and stirred vigorously for 24 hours before being cooled to room temperature.

Workup: OPom-NHS

The reaction vials were unsealed and to each of the crude mixtures was added MP-trisamine resin (0.1 g) and an additional aliquot of DMF (0.4 mL) before the resulting slurries were stirred, gently, for 4 hours at room temperature. The contents of each vial were then transferred to a vacuum filtration plate, rinsing each source vial with DMF (0.5 mL), filtered, and the resulting filtrate transferred to fresh 1-dram vials before concentrating to dryness by centrifuge evaporation overnight to afford the crude amide intermediate.

Workup: tDHU-NHS

The reaction vials were unsealed and to each of the crude mixtures was added MP-isocyanate resin (0.1 g) and PS-tosyl-hydrazine resin (0.1 g) followed by an additional aliquot of DMF (0.4 mL) before the resulting slurries were stirred, gently, overnight at room temperature. The contents of each vial were then transferred to a vacuum filtration plate, rinsing each source vial with DMF (0.5 mL), filtered, and the resulting filtrate transferred to fresh 1-dram vials before concentrating to dryness by centrifuge evaporation overnight to afford the crude amide intermediate.

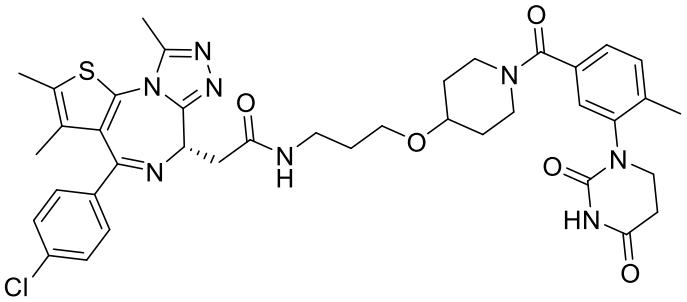
Step 2: Boc-deprotection

To a reactor block containing 1 dram vials with the crude Boc-amines, to each vial was added DCM (0.1 mL) followed by TFA (0.3 mL), the vials sealed, and the resulting mixtures stirred at room temperature for either 3 hours (O-Pom substrates) or overnight (DHU substrates) before unsealing and concentrating to dryness by centrifuge evaporation.

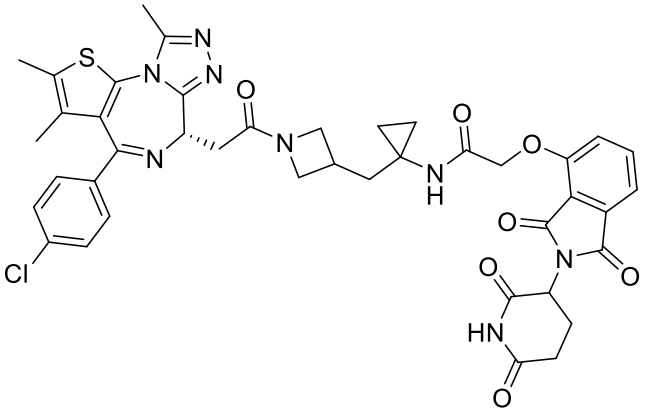
Step 3: JQ1-NHS ester coupling

To a reactor block containing 1 dram vials with crude TFA amine salts (75 µmol, 1.07 equiv) was added a crude stock solution of JQ1-NHS ester **3** ([0.21], 0.34 mL, 70 µmol, 1 equiv) followed by DIPEA (60 µL, 0.35 mmol, 5 equiv) and stirred at (for O-Pom substrates) 40 ℃ for 16 h or (for tDHU substrates) room temperature for 72 h. The crude mixtures were treated with (O-Pom substrates) MP-trisamine (0.1 g) or (for tDHU substrates) Si-carbonate (0.1 g) and stirred gently for 5 h before transferring the contents of each vial to a vacuum filtration plate, rinsing with DMF (0.5 mL), and filtering to afford the crude reaction mixtures in DMF which were directly injected for purification using Mass-Directed Liquid Chromatography to afford purified products.

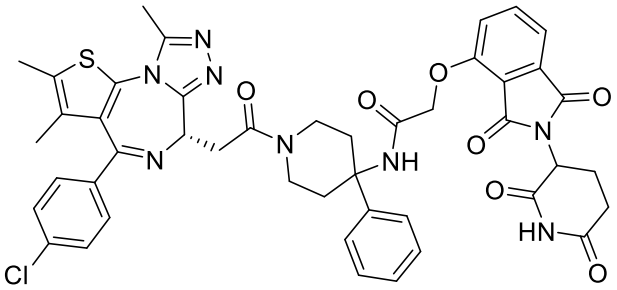
Product Characterization of Select Purified Validation Compounds



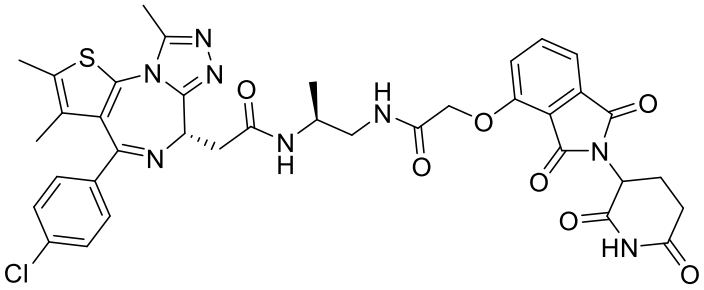
(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-(3-((1-(3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzoyl)piperidin-4-yl)oxy)propyl)acetamide (9). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (37.5 mg, 62% yield). 1H NMR (400 MHz, MeOD) δ 7.52 – 7.38 (m, 5H), 7.37 – 7.27 (m, 2H), 4.70 (dd, J = 8.5, 5.6 Hz, 1H), 3.88 (dd, J = 33.9, 15.8 Hz, 3H), 3.74 – 3.34 (m, 10H), 2.97 – 2.67 (m, 4H), 2.63 – 2.17 (m, 8H), 2.11 – 1.44 (m, 8H); HRMS-ESI (m/z) Calcd for ($C_{39}H_{44}ClN_8O_5S$) ([M+H]$^+$): 771.2838; found: 771.2843.



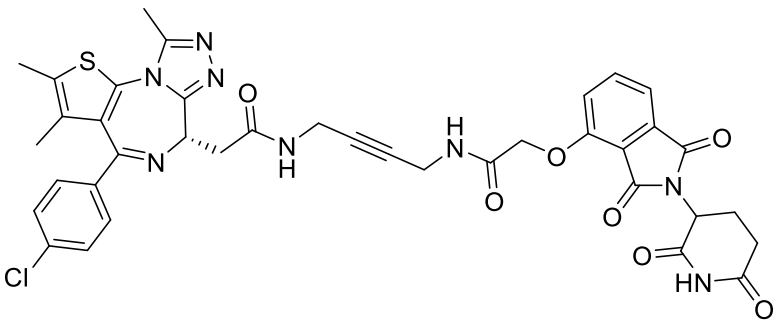
***N*-(1-((1-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)azetidin-3-yl)methyl)cyclopropyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (10).** Purified standard synthesized using General Procedure B with OPom-NHS to give title compound (22.6 mg, 37% yield). ^{1}H NMR (400 MHz, DMSO, glutarimide N-H silent) as a mixture of diastereomers δ 11.13 (s, 1H), 8.25 (d, *J* = 7.5 Hz, 1H), 7.88 – 7.78 (m, 1H), 7.61 – 7.28 (m, 6H), 5.26 – 5.05 (m, 1H), 4.74 (s, 2H), 4.58 – 4.33 (m, 2H), 4.25 – 3.87 (m, 2H), 3.28 – 3.04 (m, 2H), 3.00 – 2.71 (m, 2H), 2.61 (d, *J* = 2.3 Hz, 4H), 2.42 (s, 3H), 2.11 – 1.79 (m, 3H), 1.63 (d, *J* = 2.6 Hz, 3H), 0.66 (d, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO) as a mixture of diastereomers δ 172.8, 169.9, 169.6, 167.5, 167.5, 166.8, 165.6, 165.5, 163.3, 163.2, 155.2, 155.0, 150.1, 137.0, 136.7, 135.3, 133.0, 132.2, 130.9, 130.2, 129.9, 129.6, 129.6, 128.5, 120.5, 116.8, 116.8, 116.0, 67.6, 55.3, 53.8, 53.7, 53.0, 48.8, 33.1, 31.2, 31.1, 30.9, 26.6, 22.0, 14.1, 12.7, 12.6, 11.3. HRMS-ESI (m/z) Calcd for ($C_{41}H_{40}ClN_8O_7S$) ([M+H]$^+$): 823.2429; found: 823.2425.



***N*-(1-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-*6H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)-4-phenylpiperidin-4-yl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (11).** Purified standard synthesized using General Procedure B with OPom-NHS to give title compound (3.0 mg, 4% yield). 1H NMR (401 MHz, DMSO, 6H not observed due to suppression of water signal) δ 11.05 (dd, J = 12.4, 6.2 Hz, 1H), 7.96 (s, 1H), 7.77 – 7.63 (m, 1H), 7.50 – 6.91 (m, 10H), 5.01 (s, 1H), 4.76 (d, J = 21.0 Hz, 1H), 4.68 – 4.50 (m, 3H), 4.32 – 3.96 (m, 1H), 3.53 (t, J = 66.4 Hz, 3H), 2.91 – 2.65 (m, 1H), 2.65 – 2.53 (m, 3H), 2.38 (d, J = 4.3 Hz, 3H), 1.98 (s, 1H), 1.58 (q, J = 5.5, 5.0 Hz, 3H).. HRMS-ESI (m/z) Calcd for ($C_{45}H_{42}ClN_8O_7S$) ([M+H]$^+$): 873.2580; found: 873.2586.

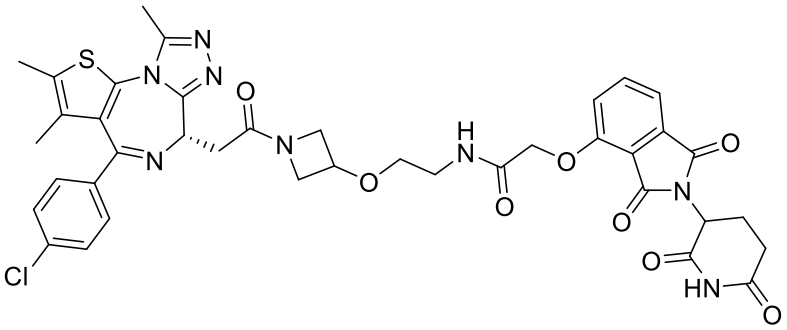


2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-((2*S*)-1-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)propan-2-yl)acetamide (S1). Purified standard synthesized using General Procedure B with OPom-NHS to give title compound (6.1 mg, 10% yield). ^{1}H NMR (401 MHz, DMSO, 2H masked by DMSO peak) as a mixture of diastereomers δ 11.07 (s, 1H), 8.73 (s, 1H), 7.79 (q, J = 8.1, 6.7 Hz, 1H), 7.60 – 7.11 (m, 7H), 5.03 (q, J = 5.4 Hz, 1H), 4.73 (d, J = 4.3 Hz, 2H), 4.51 (s, 1H), 3.50 (d, J = 35.6 Hz, 3H), 3.33 (s, 1H), 2.86 (d, J = 57.0 Hz, 2H), 2.55 (t, J = 3.0 Hz, 3H), 2.34 (d, J = 4.3 Hz, 3H), 2.12 (d, J = 51.8 Hz, 2H), 1.80 – 1.31 (m, 5H). HRMS-ESI (m/z) Calcd for ($C_{37}H_{36}ClN_8O_7S$) ([M+H]$^+$): 771.2111; found: 771.2123.

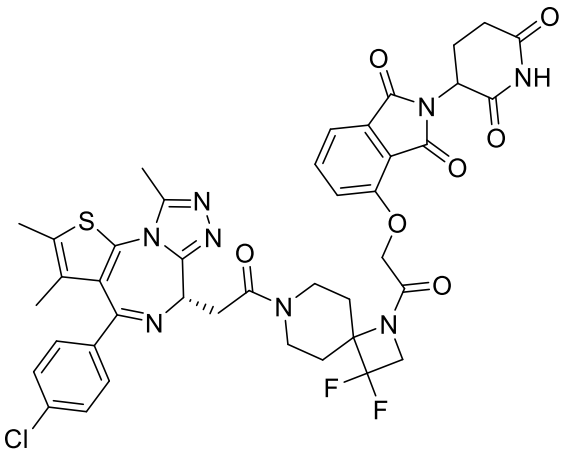


2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)but-2-yn-

1-yl)acetamide (S2). Purified standard synthesized using General Procedure B with OPom-NHS to give title compound (2 mg, 3% yield). 1H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 8.68 (t, J = 5.5 Hz, 1H), 8.46 (t, J = 5.6 Hz, 1H), 7.81 (dd, J = 8.5, 7.3 Hz, 1H), 7.53 – 7.46 (m, 3H), 7.41 (dt, J = 13.6, 8.0 Hz, 3H), 5.12 (dd, J = 12.9, 5.4 Hz, 1H), 4.84 (s, 2H), 4.51 (dd, J = 8.0, 6.2 Hz, 1H), 4.03 – 3.92 (m, 3H), 3.33 – 3.13 (m, 2H), 2.96 – 2.82 (m, 1H), 2.59 (s, 4H), 2.41 (s, 3H), 2.07 – 1.98 (m, 1H), 1.61 (s, 3H). 13C NMR (100 MHz, DMSO) δ 172.8, 169.9, 169.3, 166.8, 166.8, 165.4, 163.2, 155.1, 155.0, 150.0, 136.9, 136.7, 135.3, 133.1, 132.2, 130.9, 130.2, 129.9, 129.6, 128.5, 120.4, 116.8, 116.1, 79.3, 78.5, 67.4, 53.7, 48.8, 37.3, 31.0, 28.0, 28.0, 22.0, 14.1, 12.7, 11.3. HRMS-ESI (m/z) Calcd for ($C_{38}H_{34}ClN_8O_7S$) ([M+H]$^+$): 781.1960; found: 781.1958.

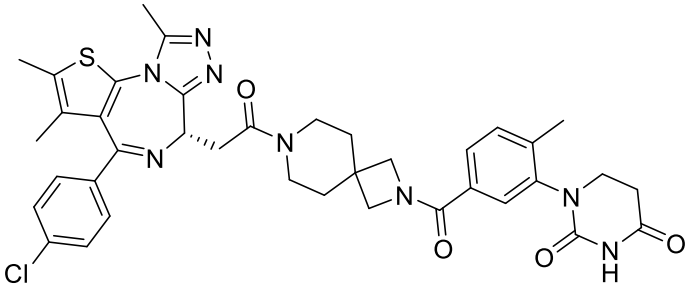


***N*-(2-((1-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)azetidin-3-yl)oxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (S3).** Purified standard synthesized using General Procedure B with OPom-NHS to give title compound (6 mg, 10% yield). 1H NMR (401 MHz, DMSO) as a mixture of diastereomers δ 11.10 (s, 1H), 7.76 (dt, J = 11.0, 8.0 Hz, 1H), 7.52 – 7.22 (m, 8H), 5.14 (d, J = 8.7 Hz, 2H), 5.05 (dd, J = 12.8, 5.4 Hz, 1H), 4.53 (t, J = 6.8 Hz, 1H), 3.87 – 3.80 (m, 1H), 3.77 – 3.34 (m, 2H), 3.01 (s, 3H), 2.57 (s, 4H), 2.37 (s, 3H), 2.15 – 1.93 (m, 1H), 1.59 (s, 3H). HRMS-ESI (m/z) Calcd for ($C_{39}H_{38}ClN_8O_8S$) ([M+H]$^+$): 813.2222; found: 813.2232.

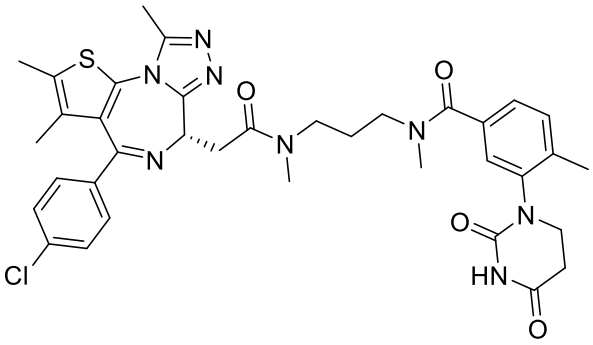


4-(2-(7-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)-3,3-difluoro-1,7-diazaspiro[3.5]nonan-1-yl)-2-oxoethoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (S4). Purified standard synthesized using General Procedure B with OPom-NHS to give title compound (16 mg, 27% yield, 90% purity). 1H NMR (400 MHz, MeOD, 6H masked by DMSO and water peaks) as a mixture of diastereomers δ 7.74 (s, 1H), 6.97 (dd, J = 8.5, 7.3 Hz, 1H), 6.76 – 6.51 (m, 6H), 4.32 (dd, J = 12.6, 5.4 Hz, 1H),

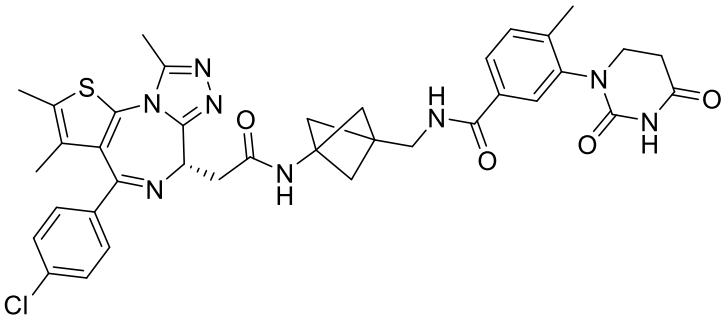
4.14 – 4.07 (m, 2H), 3.89 (td, J = 5.9, 2.6 Hz, 1H), 3.79 (d, J = 14.9 Hz, 1H), 3.57 (t, J = 13.4 Hz, 1H), 2.99 – 2.85 (m, 1H), 2.78 – 2.53 (m, 2H), 2.23 – 1.76 (m, 6H), 1.71 – 1.59 (m, 3H), 1.58 – 1.24 (m, 2H), 0.91 – 0.87 (m, 3H); HRMS-ESI (m/z) Calcd for ($C_{41}H_{38}ClF_2N_8O_7S$) ([M+H]$^+$): 859.2241; found: 859.2237.



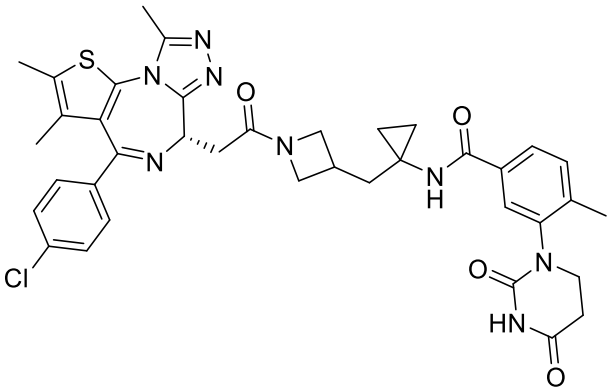
(*S*)-1-(5-(7-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)-2,7-diazaspiro[3.5]nonane-2-carbonyl)-2-methylphenyl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (S5). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (42 mg, 81% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.63 – 7.57 (m, 2H), 7.51 – 7.39 (m, 6H), 4.75 (t, *J* = 6.9 Hz, 1H), 4.21 (d, *J* = 4.3 Hz, 2H), 4.01 – 3.84 (m, 3H), 3.82 – 3.48 (m, 6H), 2.96 – 2.78 (m, 2H), 2.77 – 2.72 (m, 3H), 2.46 (s, 3H), 2.33 (s, 3H), 2.06 – 1.92 (m, 2H), 1.83 (t, *J* = 5.8 Hz, 2H), 1.71 (s, 3H). HRMS-ESI (m/z) Calcd for ($C_{38}H_{40}ClN_8O_4S$) ([M+H]$^+$): 739.2576; found: 739.2584.



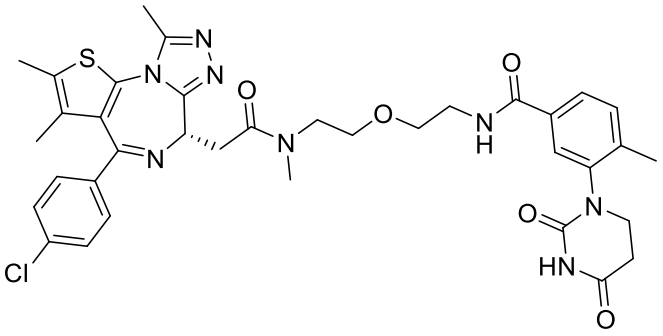
(*S*)-*N*-(3-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-methylacetamido)propyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-*N*,4-dimethylbenzamide (S6). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (37 mg, 73% yield). ^{1}H NMR (400 MHz, MeOD) δ 10.36 (s, 1H), 7.63 – 7.08 (m, 7H), 4.54 (d, *J* = 40.7 Hz, 2H), 3.79 (s, 1H), 3.45 (d, *J* = 39.0 Hz, 6H), 3.20 (s, 1H), 2.96 (d, *J* = 26.6 Hz, 5H), 2.61 (s, 4H), 2.43 (s, 4H), 2.20 (s, 4H), 2.01 (d, *J* = 12.1 Hz, 1H), 1.86 – 1.56 (m, 4H). HRMS-ESI (m/z) Calcd for ($C_{36}H_{40}ClN_8O_4S$) ([M+H]$^+$): 715.2582; found: 715.2581.



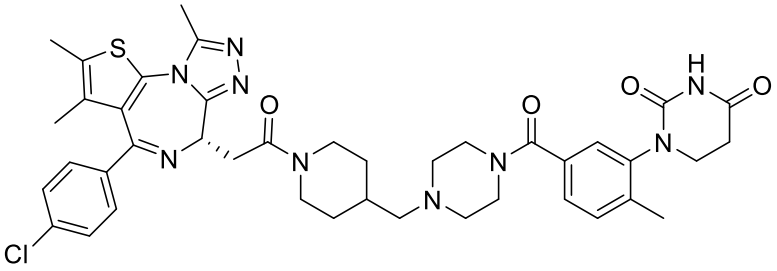
(*S*)-*N*-((3-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)bicyclo[1.1.1]pentan-1-yl)methyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S7). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (33 mg, 66% yield) as a TFA salt. ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s, 1H), 8.67 (s, 1H), 8.46 (t, *J* = 6.0 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.56 – 7.30 (m, 5H), 4.47 (t, *J* = 7.0 Hz, 1H), 3.82 (ddd, *J* = 14.6, 9.3, 5.2 Hz, 1H), 3.55 (dt, *J* = 12.2, 6.0 Hz, 1H), 3.43 (q, *J* = 8.3, 7.0 Hz, 2H), 3.25 – 3.11 (m, 2H), 2.86 – 2.65 (m, 2H), 2.60 (s, 3H), 2.41 (s, 3H), 2.23 (s, 3H), 1.90 (s, 6H), 1.62 (s, 3H). 13C NMR (101 MHz, DMSO) δ 170.7, 169.8, 165.3, 163.1, 158.4, 158.0, 155.0, 151.8, 149.9, 140.8, 138.9, 136.7, 135.3, 133.4, 132.2, 130.8, 130.4, 130.2, 129.9, 129.6, 128.5, 126.2, 126.1, 116.9, 114.0, 53.6, 52.1, 45.5, 44.5, 37.5, 36.7, 31.1, 17.5, 14.1, 12.7, 11.3. HRMS-ESI (m/z) Calcd for ($C_{37}H_{38}ClN_8O_4S$) ([M+H]$^+$): 725.2425; found: 725.2421.



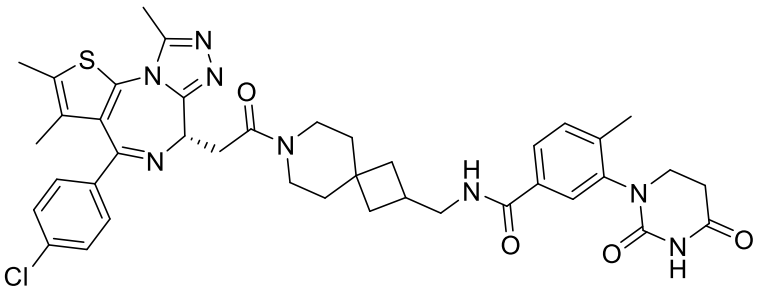
(*S*)-*N*-(1-((1-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)azetidin-3-yl)methyl)cyclopropyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S8). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (39 mg, 69% yield, 90% purity). ^{1}H NMR (400 MHz, MeOD, N-H protons silent, 2H masked by MeOD signal) δ 7.83 – 7.61 (m, 2H), 7.57 – 7.34 (m, 5H), 4.69 – 4.50 (m, 2H), 4.34 – 4.07 (m, 2H), 3.93 – 3.56 (m, 2H), 3.35 (s, 0H), 3.30 – 3.10 (m, 1H), 2.99 (t, *J* = 7.0 Hz, 1H), 2.90 – 2.67 (m, 5H), 2.45 (s, 3H), 2.35 – 2.25 (m, 3H), 2.06 (dd, *J* = 11.8, 7.4 Hz, 2H), 1.69 (d, *J* = 2.1 Hz, 3H), 1.13 – 0.66 (m, 4H); HRMS-ESI (m/z) Calcd for ($C_{38}H_{40}ClN_8O_4S$) ([M+H]$^+$): 739.2576; found: 739.2578.



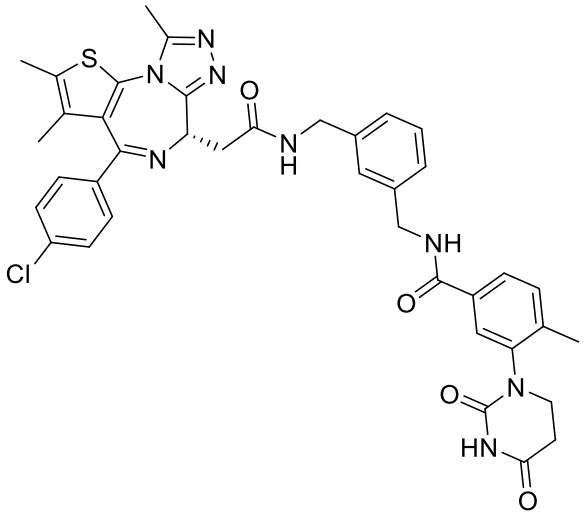
(*S*)-*N*-(2-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-methylacetamido)ethoxy)ethyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S9). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (19 mg, 38% yield. ^{1}H NMR (400 MHz, DMSO, 2H masked by NMR solvent) as a mixture of diastereomers δ 10.41 – 10.31 (m, 1H), 8.59 – 8.39 (m, 1H), 7.77 – 7.61 (m, 2H), 7.52 – 7.39 (m, 4H), 7.30 (ddd, *J* = 30.4, 8.1, 3.2 Hz, 1H), 4.56 (dt, *J* = 12.8, 6.3 Hz, 1H), 3.84 – 3.36 (m, 11H), 3.17 (s, 1H), 2.94 – 2.54 (m, 6H), 2.44 – 2.39 (m, 3H), 2.28 – 2.09 (m, 3H), 1.69 – 1.60 (m, 3H). HRMS-ESI (m/z) Calcd for ($C_{36}H_{40}ClN_8O_5S$) ([M+H]$^+$): 731.2525; found: 731.2528.



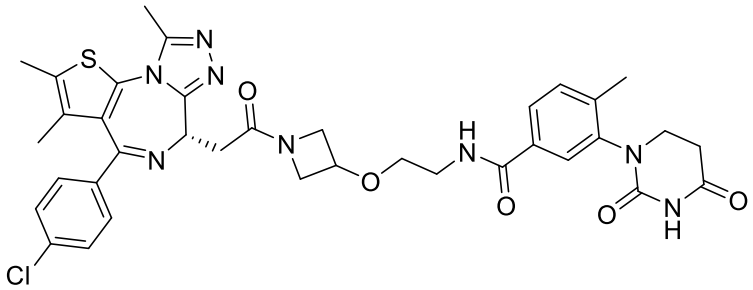
(*S*)-1-(5-(4-((1-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)piperidin-4-yl)methyl)piperazine-1-carbonyl)-2-methylphenyl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (S10). Purified standard synthesized using HATU (1.05 equiv) and DIPEA (5 equiv) from JQ1-CO_2H (60 umol, 1 equiv) to give title compound (8.5 mg, 17% yield). ^{1}H NMR (400 MHz, MeOD, N-H proton is silent, 9H missing due to broadening) δ 7.59 – 7.33 (m, 7H), 4.71 (t, J = 6.9 Hz, 1H), 4.60 (d, J = 13.3 Hz, 1H), 4.31 (t, J = 16.2 Hz, 1H), 3.87 (ddd, J = 12.4, 9.6, 5.4 Hz, 1H), 3.76 – 3.44 (m, 3H), 3.18 (d, J = 6.9 Hz, 2H), 2.95 – 2.66 (m, 6H), 2.46 (d, J = 0.8 Hz, 3H), 2.35 (s, 3H), 2.26 (dd, J = 16.6, 9.3 Hz, 1H), 2.04 – 1.82 (m, 2H), 1.70 (d, J = 0.9 Hz, 3H), 1.64 – 1.36 (m, 1H), 1.36 – 1.16 (m, 1H).; HRMS-ESI (m/z) Calcd for ($C_{41}H_{46}ClN_9O_4S$) ([M+H]$^+$): 796.3155; found: 796.3154.



(*S*)-*N*-((7-(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)acetyl)-7-azaspiro[3.5]nonan-2-yl)methyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S11). Purified standard synthesized using HATU (1.05 equiv) and DIPEA (5 equiv) from JQ1-CO_2H (60 umol, 1 equiv) to give the title compound (25.1 mg, 52%). ^{1}H NMR (400 MHz, MeOD, N-H protons silent) δ 7.82 – 7.65 (m, 2H), 7.59 – 7.30 (m, 5H), 4.75 (dd, *J* = 7.4, 6.3 Hz, 1H), 3.89 (ddd, *J* = 12.6, 9.4, 5.6 Hz, 1H), 3.76 – 3.48 (m, 7H), 3.44 (d, *J* = 7.2 Hz, 2H), 2.96 – 2.76 (m, 2H), 2.74 (s, 3H), 2.64 (p, *J* = 7.8 Hz, 1H), 2.46 (d, *J* = 0.9 Hz, 3H), 2.33 (s, 3H), 2.10 – 1.99 (m, 2H), 1.83 – 1.76 (m, 1H), 1.73 – 1.60 (m, 7H), 1.56 (t, *J* = 5.8 Hz, 1H); HRMS-ESI (m/z) Calcd for ($C_{40}H_{44}ClN_8O_4S$) ([M+H]$^+$): 767.2889; found: 767.2891.

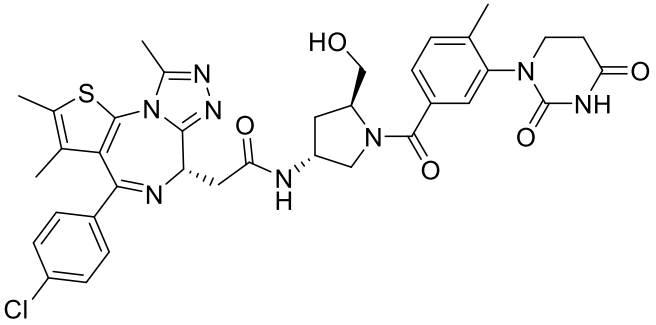


(*S*)-*N*-(3-((2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)methyl)benzyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S12). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (29 mg, 55% yield). ^{1}H NMR (400 MHz, MeOD, N-H protons silent, 1H masked by residual NMR solvent peak) δ 7.80 – 7.70 (m, 2H), 7.37 (d, *J* = 7.0 Hz, 6H), 7.34 – 7.22 (m, 3H), 4.71 – 4.31 (m, 5H), 3.84 (ddt, *J* = 13.8, 9.6, 4.9 Hz, 1H), 3.64 (dtd, *J* = 12.4, 6.1, 2.8 Hz, 1H), 3.46 (dd, *J* = 14.9, 8.8 Hz, 1H), 3.36 (d, *J* = 5.7 Hz, 1H), 2.91 – 2.64 (m, 5H), 2.44 (s, 3H), 2.30 (s, 3H), 1.68 (s, 3H); HRMS-ESI (m/z) Calcd for ($C_{39}H_{38}ClN_8O_4S$) ([M+H]$^+$): 749.2425; found: 749.2428.

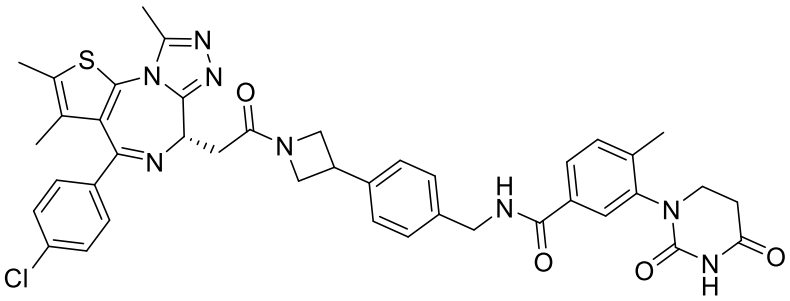


(*S*)-*N*-(2-((1-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)azetidin-3-yl)oxy)ethyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S13). Purified standard synthesized using General Procedure B with

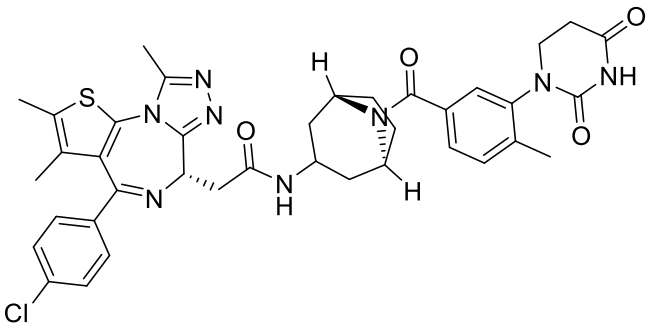
tDHU-NHS to give title compound (38 mg, 75% yield). ^{1}H NMR (400 MHz, MeOD, N-H protons are silent) δ 7.75 (d, *J* = 6.4 Hz, 2H), 7.60 – 7.31 (m, 5H), 4.72 – 4.56 (m, 2H), 4.48 (dq, *J* = 6.8, 3.4 Hz, 1H), 4.37 – 4.16 (m, 2H), 3.89 (dddd, *J* = 16.7, 12.7, 9.3, 3.6 Hz, 2H), 3.64 (ddd, *J* = 16.3, 7.2, 4.8 Hz, 4H), 3.46 – 3.33 (m, 2H), 3.29 – 3.20 (m, 1H), 2.96 – 2.68 (m, 5H), 2.46 (s, 3H), 2.31 (d, *J* = 1.9 Hz, 3H), 1.69 (s, 3H); HRMS-ESI (m/z) Calcd for ($C_{36}H_{38}ClN_8O_5S$) ([M+H]$^+$): 729.2369; found: 729.2375.



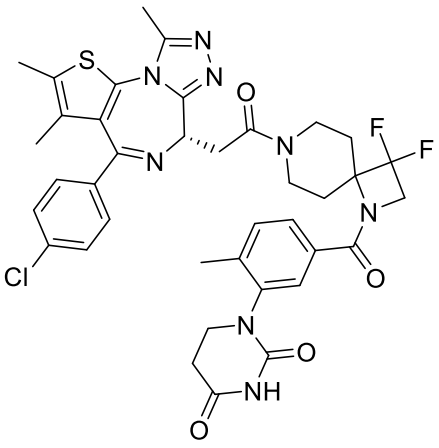
2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-((3*R*,5*S*)-1-(3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzoyl)-5-(hydroxymethyl)pyrrolidin-3-yl)acetamide (S14). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (36 mg, 58% yield). ^{1}H NMR (400 MHz, MeOD, N-H and O-H protons silent, 1H masked by residual NMR solvent signal) δ 7.59 – 7.25 (m, 7H), 4.70 – 4.31 (m, 4H), 4.06 – 3.54 (m, 3H), 3.49 (t, J = 10.2 Hz, 1H), 2.97 – 2.61 (m, 5H), 2.44 (s, 4H), 2.32 (s, 5H), 2.15 (d, J = 10.1 Hz, 1H), 1.67 (t, J = 8.2 Hz, 4H); HRMS-ESI (m/z) Calcd for ($C_{36}H_{38}ClN_8O_5S$) ([M+H]$^+$): 729.2374; found: 729.2370.



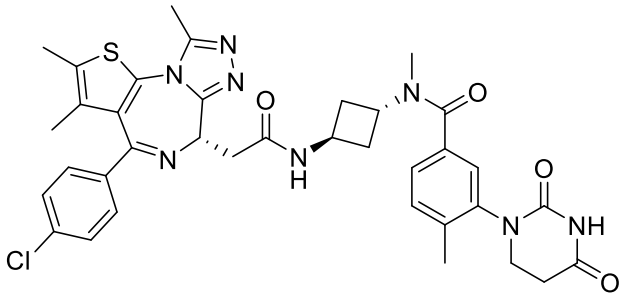
(*S*)-*N*-(4-(1-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)azetidin-3-yl)benzyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S15). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (36 mg, 66% yield). ^{1}H NMR (400 MHz, MeOD, N-H protons silent, 1H masked by residual NMR solvent signal) δ 7.78 (dd, *J* = 6.8, 1.8 Hz, 2H), 7.65 – 7.29 (m, 9H), 4.78 – 4.64 (m, 1H), 4.64 – 4.39 (m, 4H), 4.22 – 3.81 (m, 3H), 3.67 (ddd, *J* = 13.0, 6.6, 5.2 Hz, 1H), 3.56 – 3.36 (m, 2H), 3.28 – 3.20 (m, 0H), 2.95 – 2.70 (m, 5H), 2.46 (s, 3H), 2.33 (s, 3H), 1.70 (d, *J* = 5.9 Hz, 3H); HRMS-ESI (m/z) Calcd for ($C_{41}H_{40}ClN_8O_4S$) ([M+H]$^+$): 775.2576; found: 775.2583.



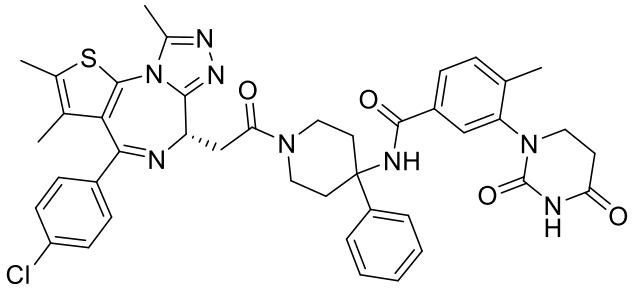
2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-*N*-((1*S*,5*S*)-8-(3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzoyl)-8-azabicyclo[3.2.1]octan-3-yl)acetamide (S16). ^{1}H NMR (400 MHz, MeOD) *N*-H protons not observed, 2H missing δ 7.46 (d, *J* = 6.9 Hz, 7H), 4.79 (d, *J* = 13.8 Hz, 1H), 4.68 (dd, *J* = 8.9, 5.4 Hz, 1H), 4.44 (tt, *J* = 11.6, 5.8 Hz, 1H), 4.25 (s, 1H), 3.90 (s, 1H), 3.71 (s, 1H), 3.41 (dd, *J* = 15.0, 9.0 Hz, 1H), 3.31 – 3.24 (m, 1H), 3.04 – 2.68 (m, 5H), 2.48 (s, 3H), 2.36 (s, 3H), 2.27 – 1.80 (m, 6H), 1.73 (s, 3H). HRMS-ESI (m/z) Calcd for ($C_{38}H_{40}ClN_8O_4S$) ([M+H]$^+$): 739.2582; found: 739.2577.



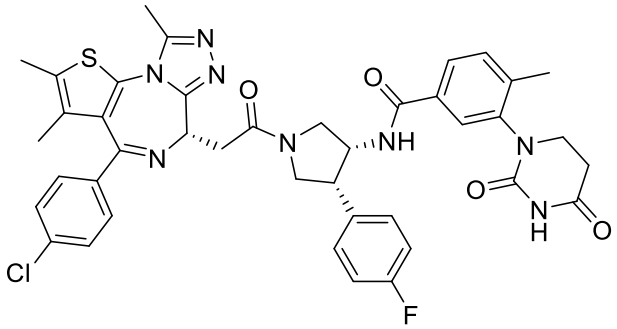
(*S*)-1-(5-(7-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetyl)-3,3-difluoro-1,7-diazaspiro[3.5]nonane-1-carbonyl)-2-methylphenyl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (S17). Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (14 mg, 26% yield). ^{1}H NMR (400 MHz, DMSO) δ 10.40 (s, 1H), 7.80 – 7.17 (m, 7H), 4.95 – 4.57 (m, 1H), 4.55 – 4.28 (m, 1H), 3.82 (d, *J* = 12.2 Hz, 1H), 3.58 (ddd, *J* = 22.8, 14.8, 6.8 Hz, 3H), 3.23 (q, *J* = 12.6 Hz, 1H), 2.74 (s, 2H), 2.61 (s, 3H), 2.47 – 2.06 (m, 8H), 1.65 (s, 3H). HRMS-ESI (m/z) Calcd for ($C_{38}H_{38}ClF_2N_8O_4S$) ([M+H]$^+$): 775.2393; found: 775.2390.



***N*-((1*S*,3r)-3-(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)acetamido)cyclobutyl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-*N*,4-dimethylbenzamide (S18).** Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (12 mg, 23% yield). ^{1}H NMR (400 MHz, MeOD, *N*-H signals not observed, 2H missing due to broadening) δ 7.38 (d, *J* = 28.6 Hz, 7H), 4.60 (dd, *J* = 13.6, 6.5 Hz, 1H), 4.25 (s, 1H), 3.87 (td, *J* = 12.1, 9.5, 5.6 Hz, 1H), 3.68 (dq, *J* = 12.0, 5.9 Hz, 1H), 3.43 (d, *J* = 42.1 Hz, 2H), 3.10 (s, 3H), 2.99 – 2.62 (m, 7H), 2.44 (s, 3H), 2.34 (s, 3H), 1.69 (s, 3H); HRMS-ESI (m/z) Calcd for ($C_{36}H_{38}ClN_8O_4S$) ($[M+H]^+$): 713.2425; found: 713.2424.



(*S*)-*N*-(1-(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)acetyl)-4-phenylpiperidin-4-yl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S19). Purified standard synthesized using HATU (1.05 equiv) and DIPEA (5 equiv) from JQ1-CO2H (32 umol, 1 equiv) to give the title compound (18.7 mg, 74%). ^{1}H NMR (400 MHz, MeOD) δ 7.93 – 7.64 (m, 2H), 7.64 – 7.30 (m, 10H), 7.24 (q, *J* = 7.6 Hz, 1H), 4.72 (dd, *J* = 7.8, 6.0 Hz, 1H), 4.49 (d, *J* = 13.8 Hz, 1H), 4.23 (d, *J* = 14.2 Hz, 1H), 3.89 (d, *J* = 11.7 Hz, 1H), 3.81 – 3.48 (m, 4H), 3.19 (q, *J* = 15.7, 14.4 Hz, 1H), 3.08 – 2.61 (m, 7H), 2.45 (s, 3H), 2.35 (d, *J* = 3.4 Hz, 4H), 2.03 (s, 1H), 1.70 (s, 3H); HRMS-ESI (m/z) Calcd for ($C_{42}H_{42}ClN_8O_4S$) ($[M+H]^+$): 789.2738; found: 789.2733.



***N*-((3*R*,4*R*)-1-(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)acetyl)-4-(4-fluorophenyl)pyrrolidin-3-yl)-3-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-4-methylbenzamide (S20).** Purified standard synthesized using General Procedure B with tDHU-NHS to give title compound (36 mg, 58% yield, 90% purity). ^{1}H NMR (400 MHz, DMSO, NH silent, 1H masked by DMSO) δ 10.83 (s, 1H), 7.83 – 7.64 (m, 2H), 7.59 – 7.14 (m, 6H), 7.10 – 7.00 (m, 2H), 5.06 (dt, J = 11.7, 5.7 Hz, 1H), 4.81 (d, J = 28.4 Hz, 1H), 4.65 (td, J = 5.3, 2.2 Hz, 3H), 4.42 – 4.03 (m, 1H), 3.95 – 3.31 (m, 5H), 2.92 – 2.75 (m, 1H), 2.70 – 2.55 (m, 4H), 2.52 (d, J = 1.9 Hz, 2H), 2.43 (s, 3H), 2.15 – 1.95 (m, 1H), 1.66 (d, J = 4.9 Hz, 3H); HRMS-ESI (m/z) Calcd for ($C_{41}H_{39}ClFN_8O_4S$) ([M+H]$^+$): 793.2482; found: 793.2491.

VI. Assay Experimental Procedures

BRD4-HiBiT and CellTiterGlo assays:

The HiBiT and CellTiterGlo (CTG) assays were run in parallel using the same cell line, HEK293 HiBiT-BRD4 knock in, which was purchased from Promega (CS3023269). Frozen cells were thawed in a 37℃ water bath (cryopreservation media: Gibco 12648-010). The cells were added to 10 mL growth media, DMEM (Gibco 11995-065) with 10% FBS (Gibco 16000-044), then spun down @ 1000 rpm for 4 min. The media was aspirated, and cells were re-suspended in assay media (99% DMEM 1% FBS, Gibco 10566-016, Gibco 16000-044) to a final concentration of 5x10e5 cells/mL. Using a multidrop combi, 20 uL of the cell suspension was dispensed into each well of columns 1-23, and 20 uL of assay media was dispensed to each well of column 24 of a 384 well assay plate (Corning 3570) (Two assay plates per compound source plate, one for the HiBiT assay, the other for the CTG assay). The assay plates were then centrifuged at 1000 rpm for 1 min and left in an incubator for 2 h (37C 5% CO2).

D2B compounds were dissolved in DMSO to a theoretical concentration of 20 mM (assuming reactions were 100% productive). 11pt, 3-fold titrations were made from each compound (20 mM theoretical starting concentration). 50 nL of compound titrations or DMSO were dosed into assay plates using an acoustic liquid handler (Echo 655). After dosing, the plates were centrifuged at 1000rpm for 1 min and returned to the incubator for 24 h.

Detecting assay plates: 30 min prior to adding detection reagents, the assay plates were removed from the incubator and allowed to equilibrate to room temperature.

HiBiT-BRD4 detection: HiBiT detection reagents were thawed and allowed to come to room temperature (Promega N3050). LgBiT protein was diluted 1:100, and Nano-Glo substrate was diluted 1:50 in lytic buffer (~9mL per plate). Using a multidrop combi, 20uL of HiBiT detection reagent were added to each well of the 384 well plate. The plate(s) were then incubated in the dark for 30 min before reading.

CTG detection: CTG reagent was thawed and allowed to come to room temperature (Promega G7571). Using a multidrop combi, 20 uL of CTG reagent were added to each well of the 384 well plate. The plate(s) were then incubated in the dark for 30 min before reading.

Detection: Luminescence was read on a PHERAstar FSX using the LUM plus setting with a 0.2 second measurement interval.

CRBN-tracer NanoBRET assay:

Generation of assay ready cells:

The CRBN-tracer nanoBRET assay was run using frozen assay ready transiently transfected cells. To generate assay ready cells, HEK293 cells were grown in T225 flasks with growth media (DMEM (Gibco 10566-016) with 10% FBS (Gibco 16000-044)). To harvest cells, the media was aspirated, then each flask was rinsed with 10mL PBS (Corning, 21-040-CV). 4 mL of warm 0.05% trypsin (Corning 25-051-CI) was added to each plate and incubated at 37C for 3 minutes. 10ml of growth media was added to each flask, and cells were dispersed. The cells were transferred to centrifuge tubes and spun down at 1000rpm for 5 min. The media was aspirated, and the cells were resuspended in growth media to a concentration of 400,000 cells/mL. A 20x transfection mix of Lipid:DNA complexes using plasmids encoding NanoLuc-CRBN and DDB1 (Promega, CRBN-31K, DDB1-5K) was generated in optimem (Gibco 31985-070). For each mL of optimem, 18 ug of DDB1 plasmid and 2 ug of NanoLuc-CRBN was added. Then 60 uL of FuGENE was added, and the tube was gently mixed by inversion 5-10 times. This mixture was incubated at room temperature for 15 min before adding to cells (20x). Cells were grown in T225 flasks overnight and harvested and frozen the next morning. Cells were detached following the same procedure described above. After spinning down and removing the supernatant, the cells were re-suspended in cryopreservation media (Gibco 12648-010) and frozen in a corning coolcell FTS30 chiller.

Running the CRBN-tracer nanoBRET assay:

This assay is run in live and permeabilized modes in parallel. For each compound source plate two assay plates are generated, on for the live mode and one for the permeabilized mode.

Dosing assay plates: 384 well assay plates (corning 3574) were dosed with CRBN-tracer (Promega CS:1810C141) using an Echo655 (25 nL for live plates 7.5 nL for permeabilized plates). Then 50 nL of 11pt, 3-fold titrations of D2B compounds were dosed to each well (20 mM theoretical starting source concentration, 50 uM starting assay concentration).

Preparing cells: Frozen transfected cells were thawed in a 37℃ water bath (cryopreservation media: Gibco 12648-010). The cells were added to 10 mL growth media, DMEM (Gibco 11995-065) with 10% FBS (Gibco 16000-044), then spun down @ 1000 rpm for 4 min. The media was aspirated, and cells were re-suspended in warm optimem without phenol red (Gibco 11058-021) to 500,000 cells/mL.

Adding cells to assay plates: Using a multidrop combi 20 uL of cells were added to the live cell plate(s), and 18 uL of cells were added to the permeabilized plate(s). The plates were spun down at 1000 rpm for 1 min. The live cell plates were incubated at 37℃ for 2 hr. 2 uL of 0.5 mg/mL digitonin (MP Biomedicals 0215948082) in optimem (Gibco 11058-021) was added to each well of the permeabilized plate (50 ug/mL digitonin was dissolved in DMSO, this stock was diluted in optimem to generate a 0.5mg/mL stock). The permeabilized centrifuged at 1000rpm for 1 min and incubated at room temp for 30 min.

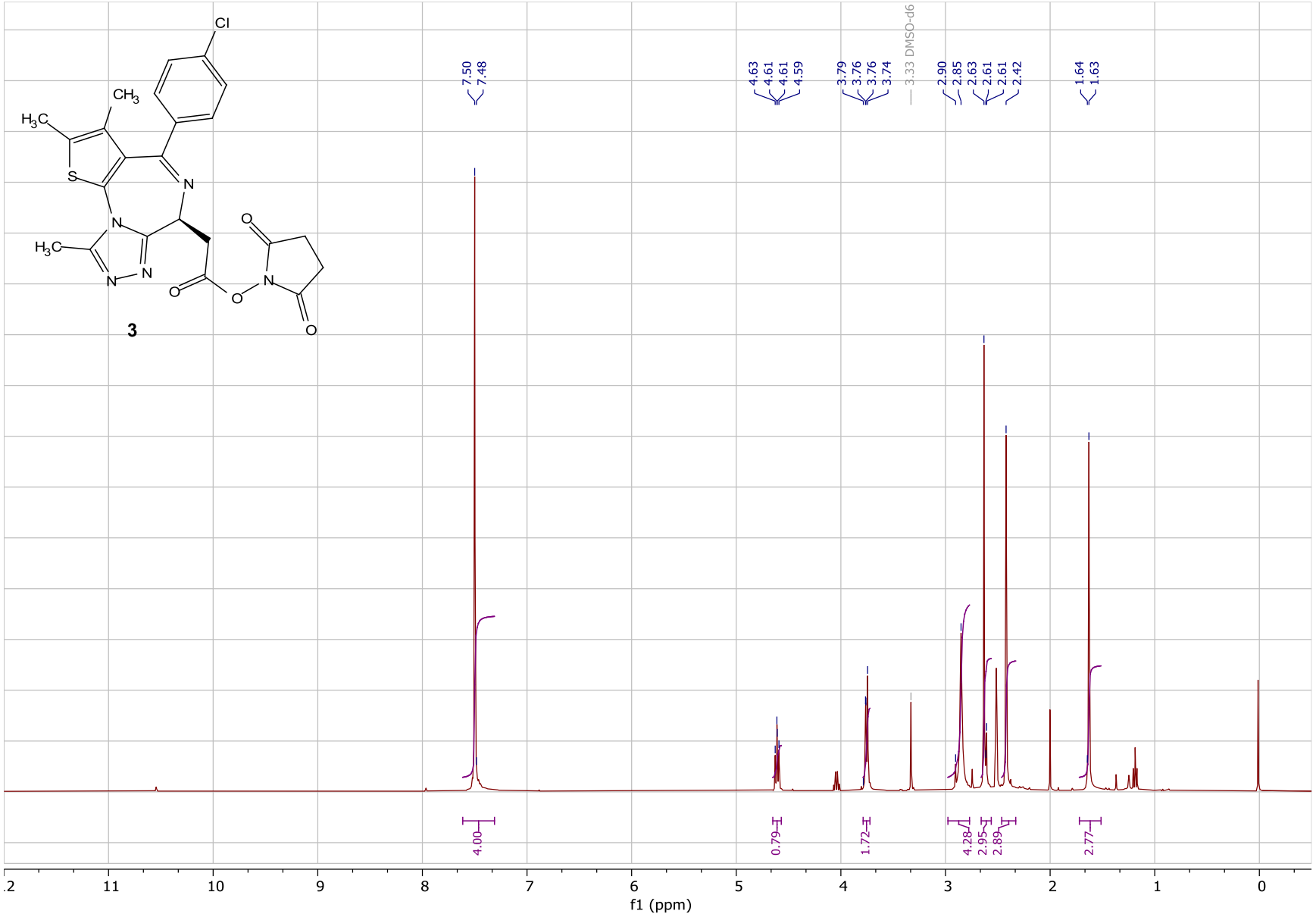
Detection: after their incubation periods, 10 uL of 3X nanoBRET detection reagent was added to each well (Promega N2161). For the permeabilized plate nanoBRET Glo substrate was diluted 1:166 in optimem. For the live cell plate, nanoBRET Glo substrate was diluted 1:166 and

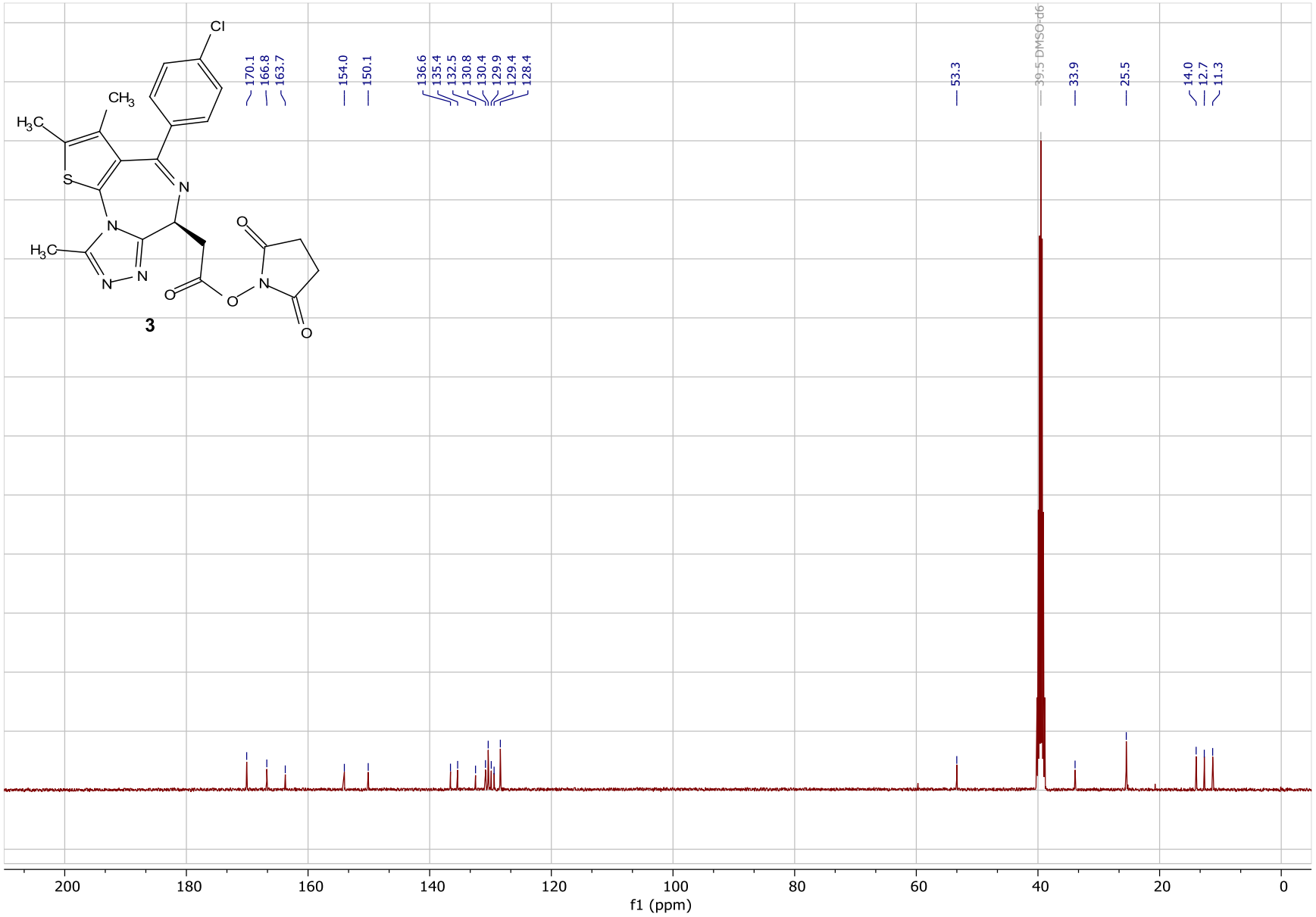
extracellular inhibitor was diluted 1:500 in optimem. The plates were centrifuged at 1000 rpm for 1 min then detected using a PHERAstar FSX using LUM 2007H1 module (610-LP, 450-480nM).

Table S5. Performance statistics of assays evaluated in D2B workflow

	Average RZ'	Average S/B	min RZ'	min S/B	Ctrl cmpd. IC50 (StDev)
HiBiT-BRD4 (ZXH-3-26)[3]	0.89	538.77	0.84	291.9	4.1 nM (0.18nM)
CellTiterGlo	0.92	344.52	0.88	146.9	
CRBN nanoBRET - Live (CC885)[4]	0.78	4.65	0.66	4.36	10 nM (1.2 nM)
CRBN nanoBRET - Perm (CC885)[4]	0.71	3.46	0.63	3.11	18 nM (2.9 nM)

VII. NMR Spectra

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H3C
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O
O
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O
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154.0
150.1
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135.4
132.5
130.8
130.4
129.9
129.4
128.4
53.3
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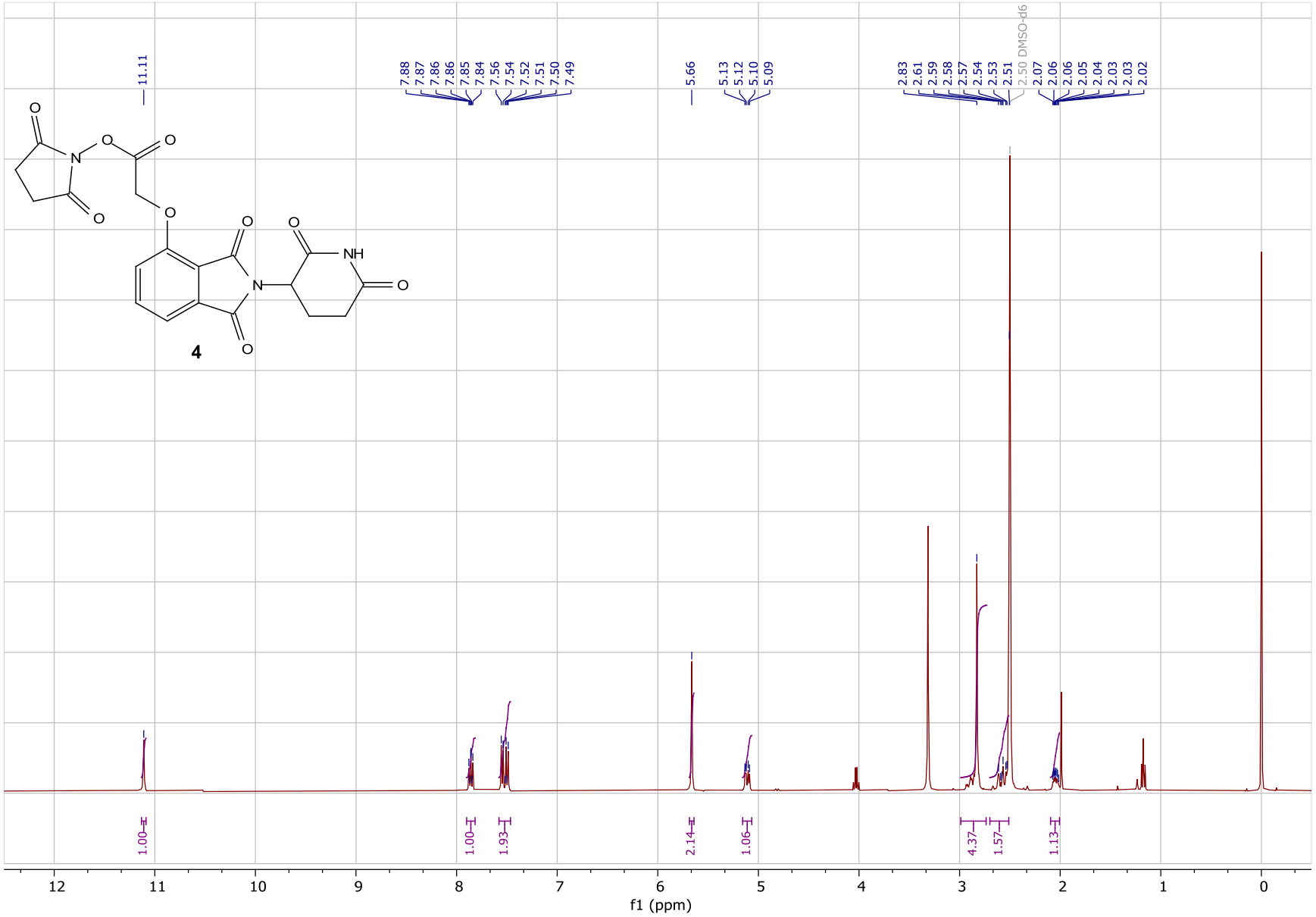
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2.51
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2.06
2.06
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2.03
2.02
4
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1.06
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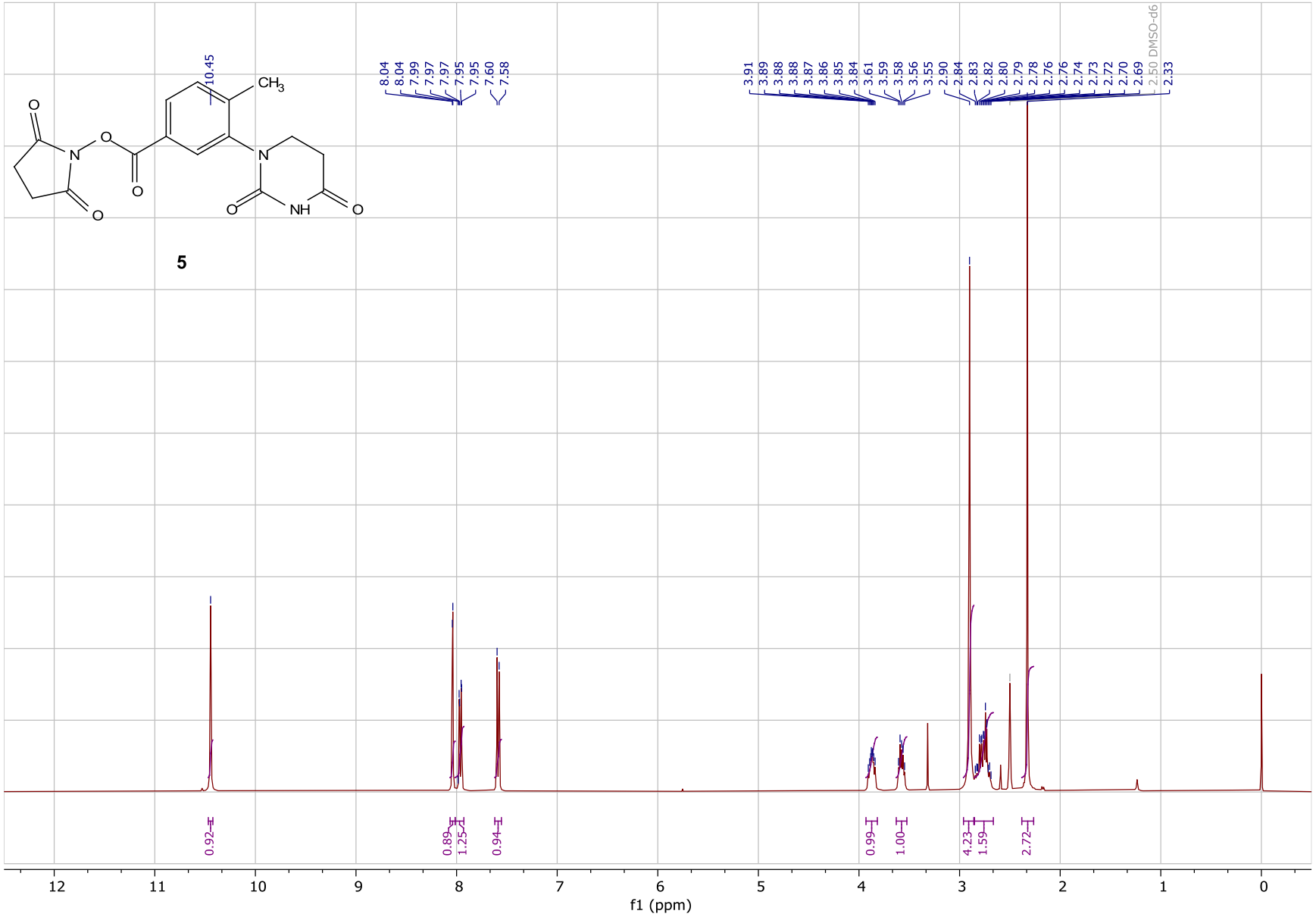
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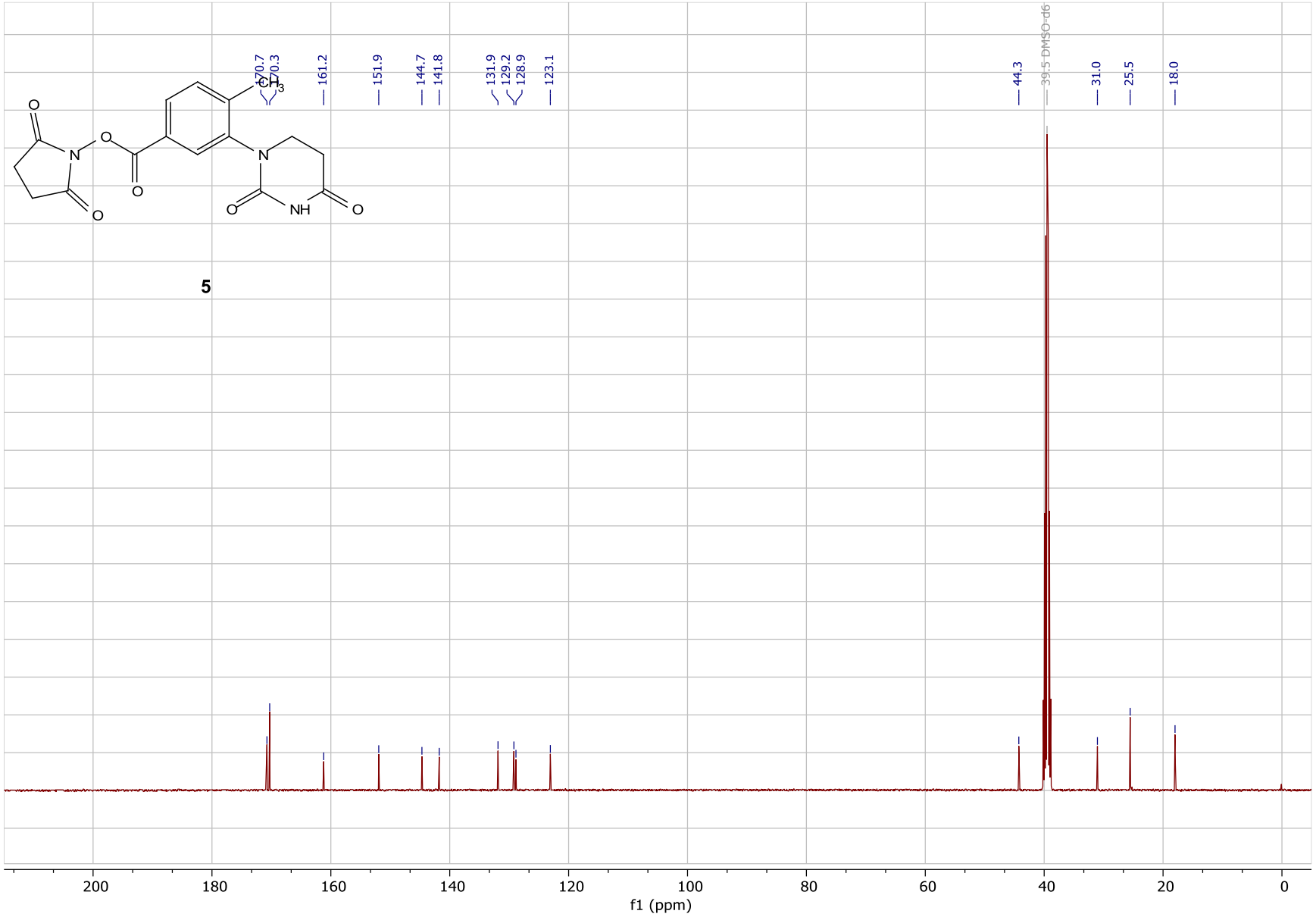
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18.0
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O
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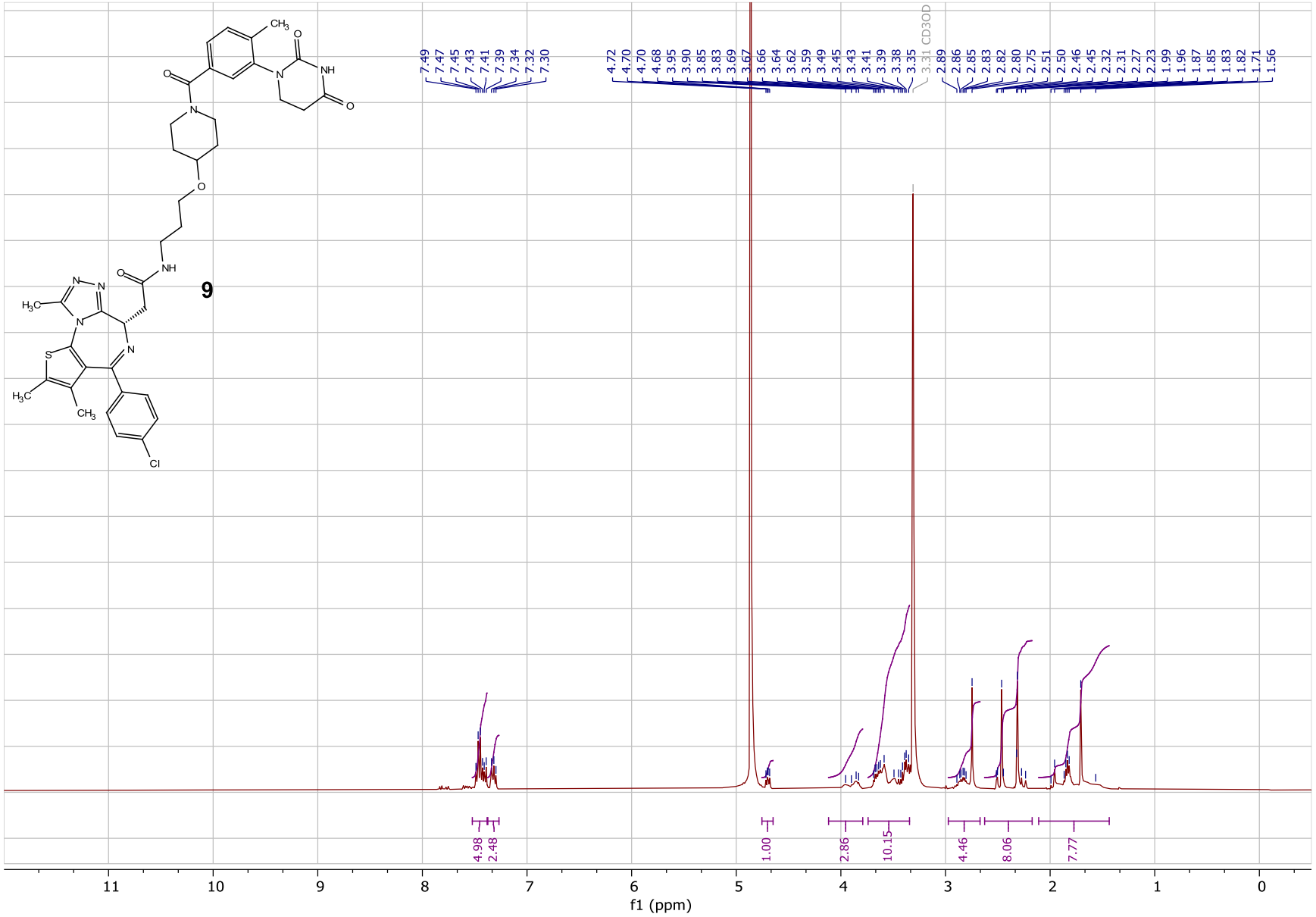
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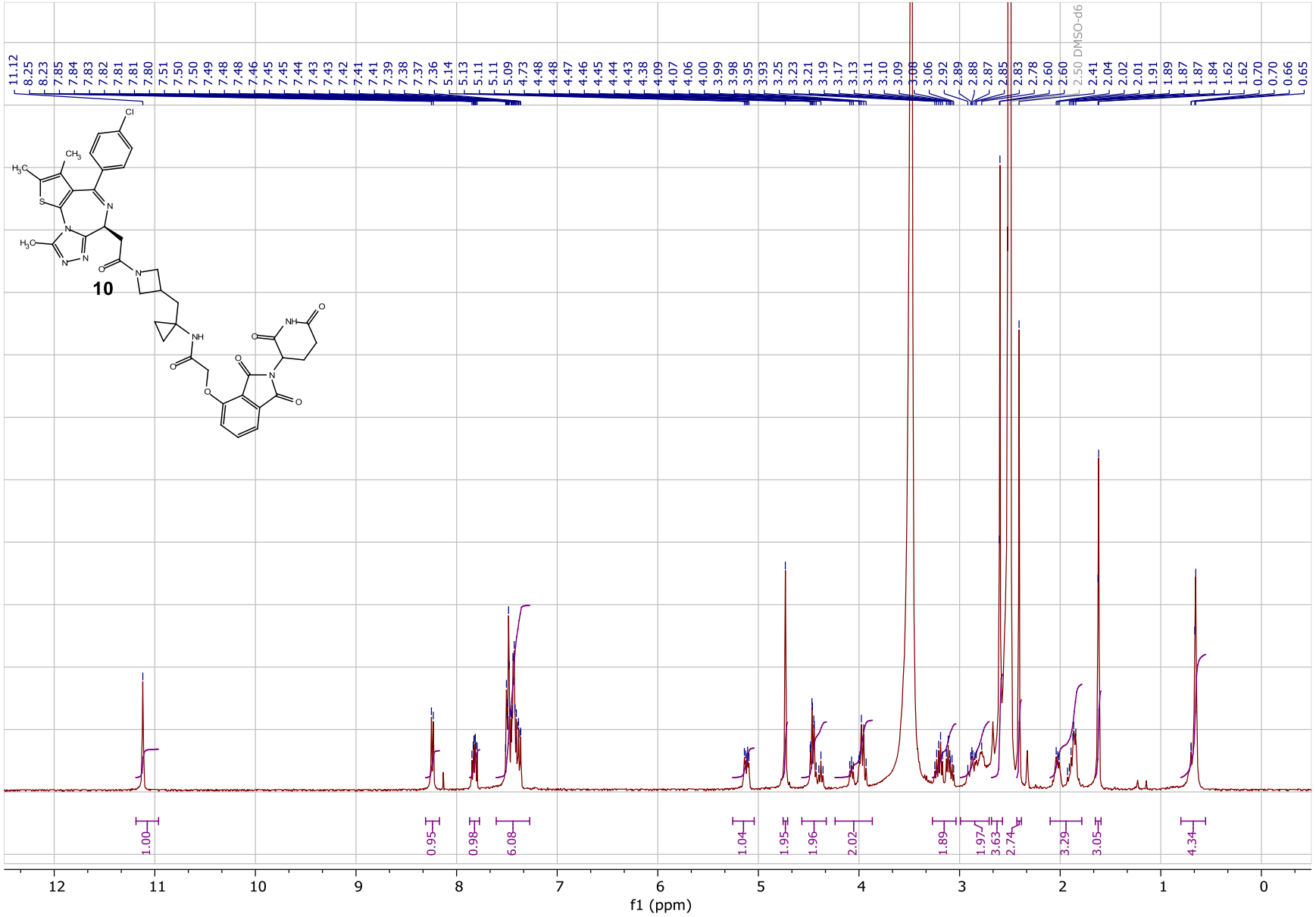
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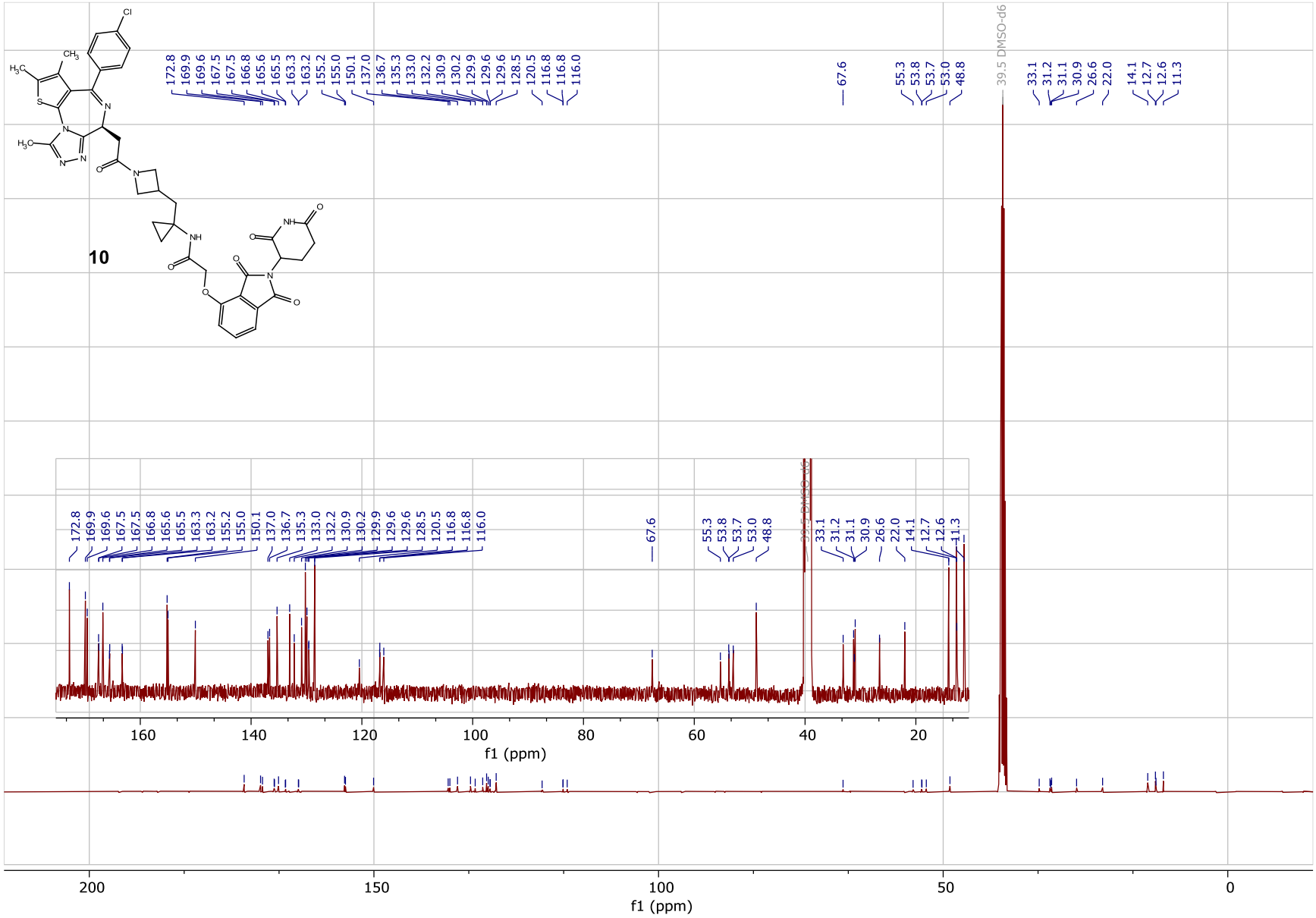
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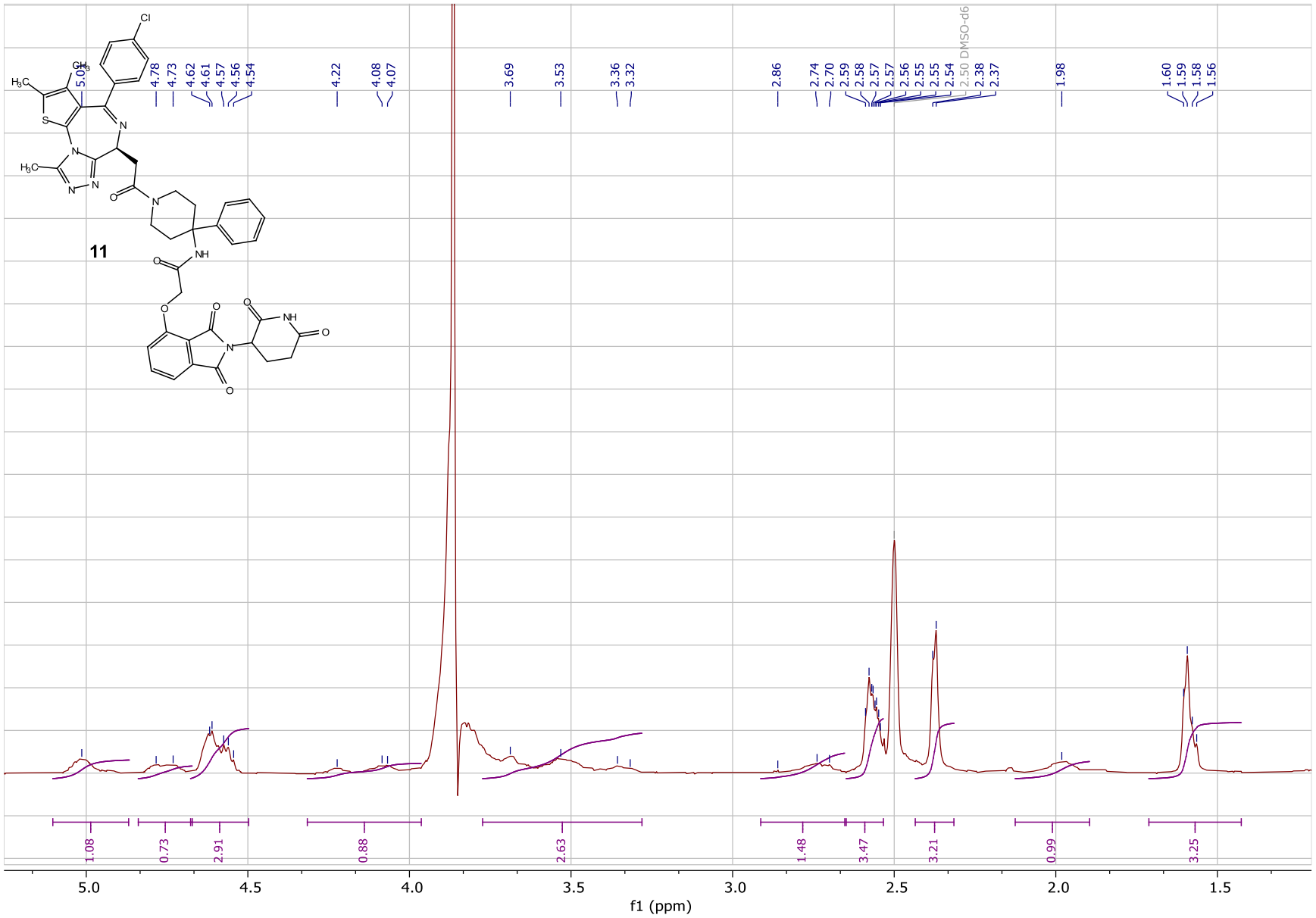
f1 (ppm)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2.58
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2.56
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2.54
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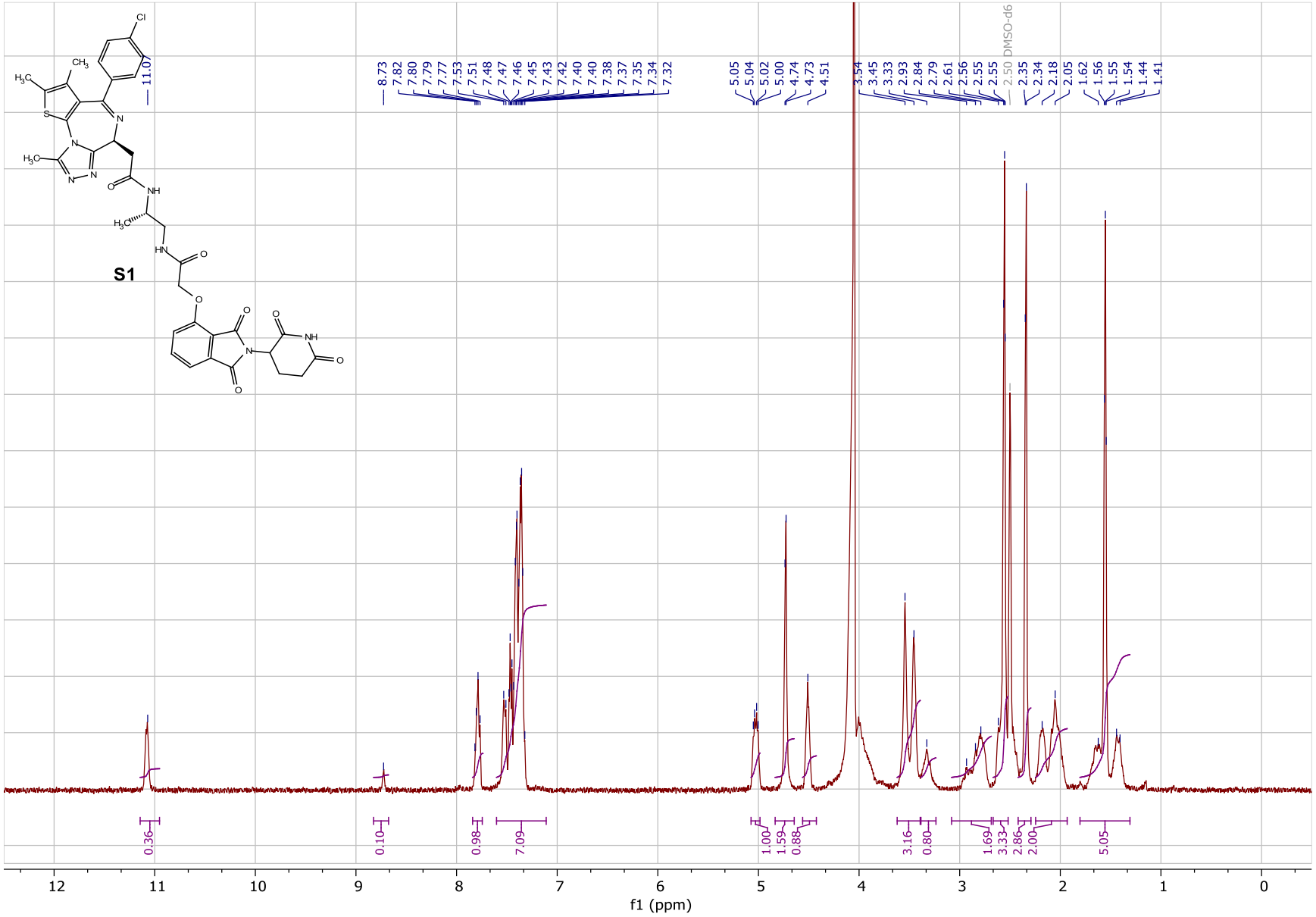
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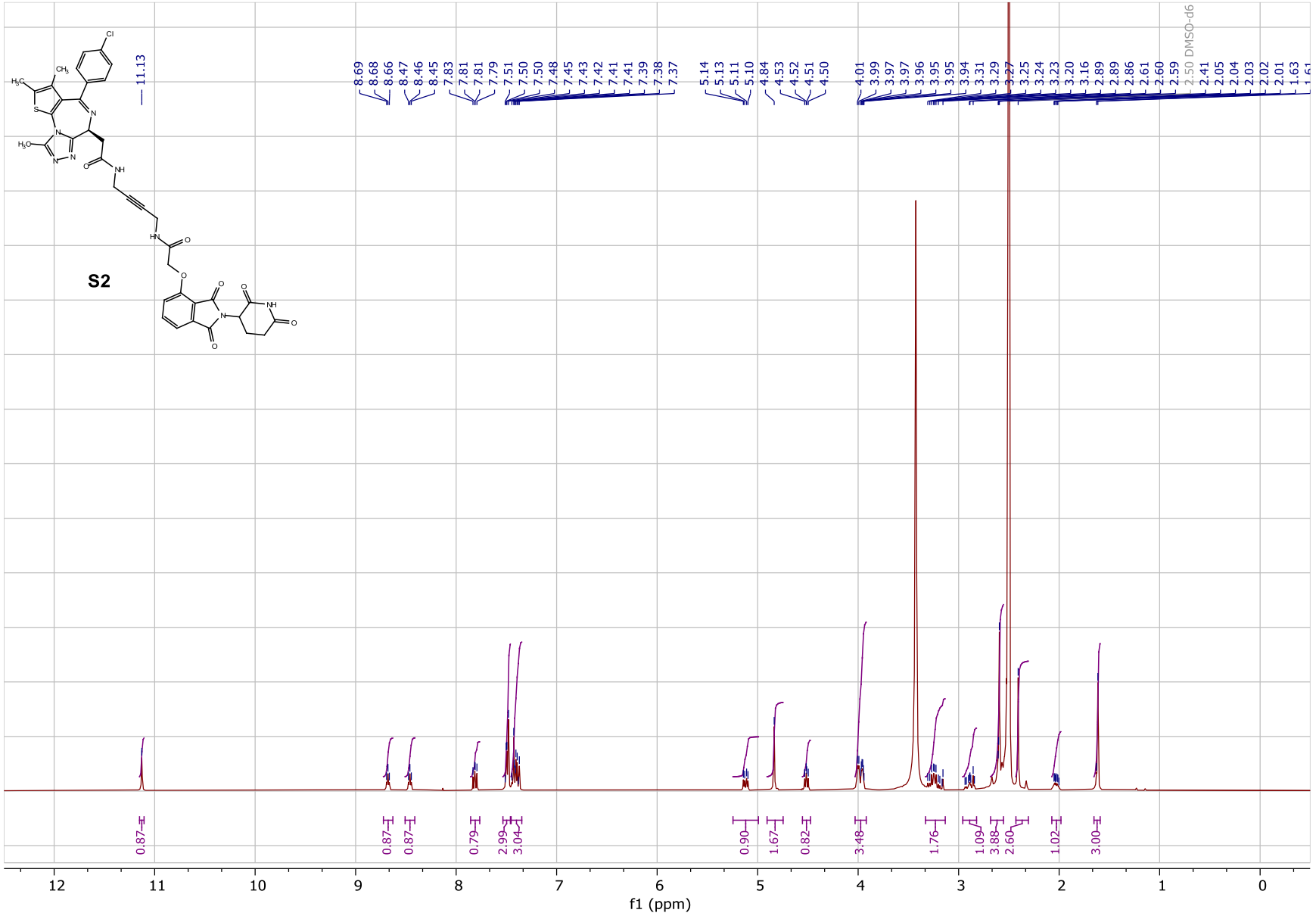
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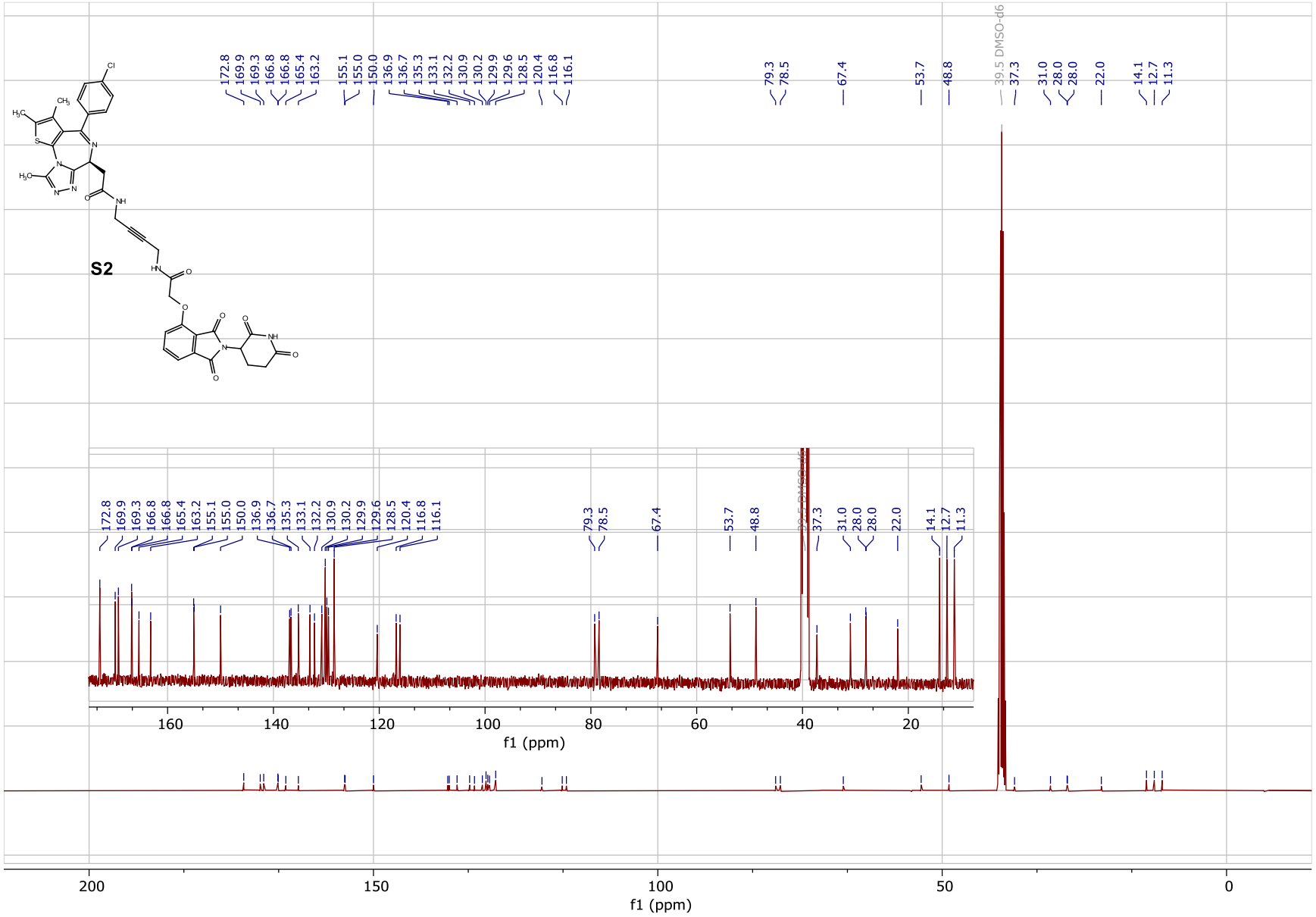
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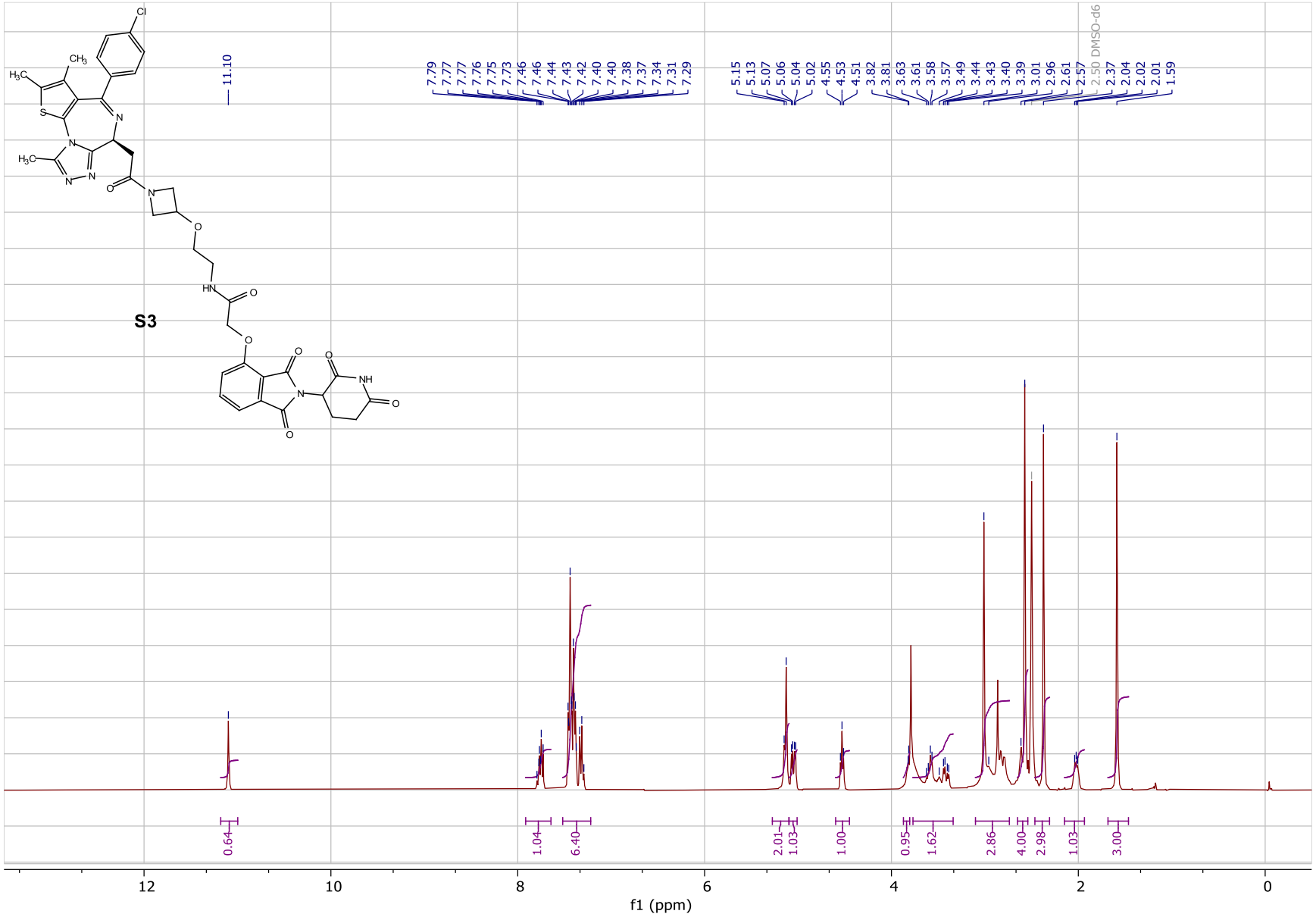
f1 (ppm)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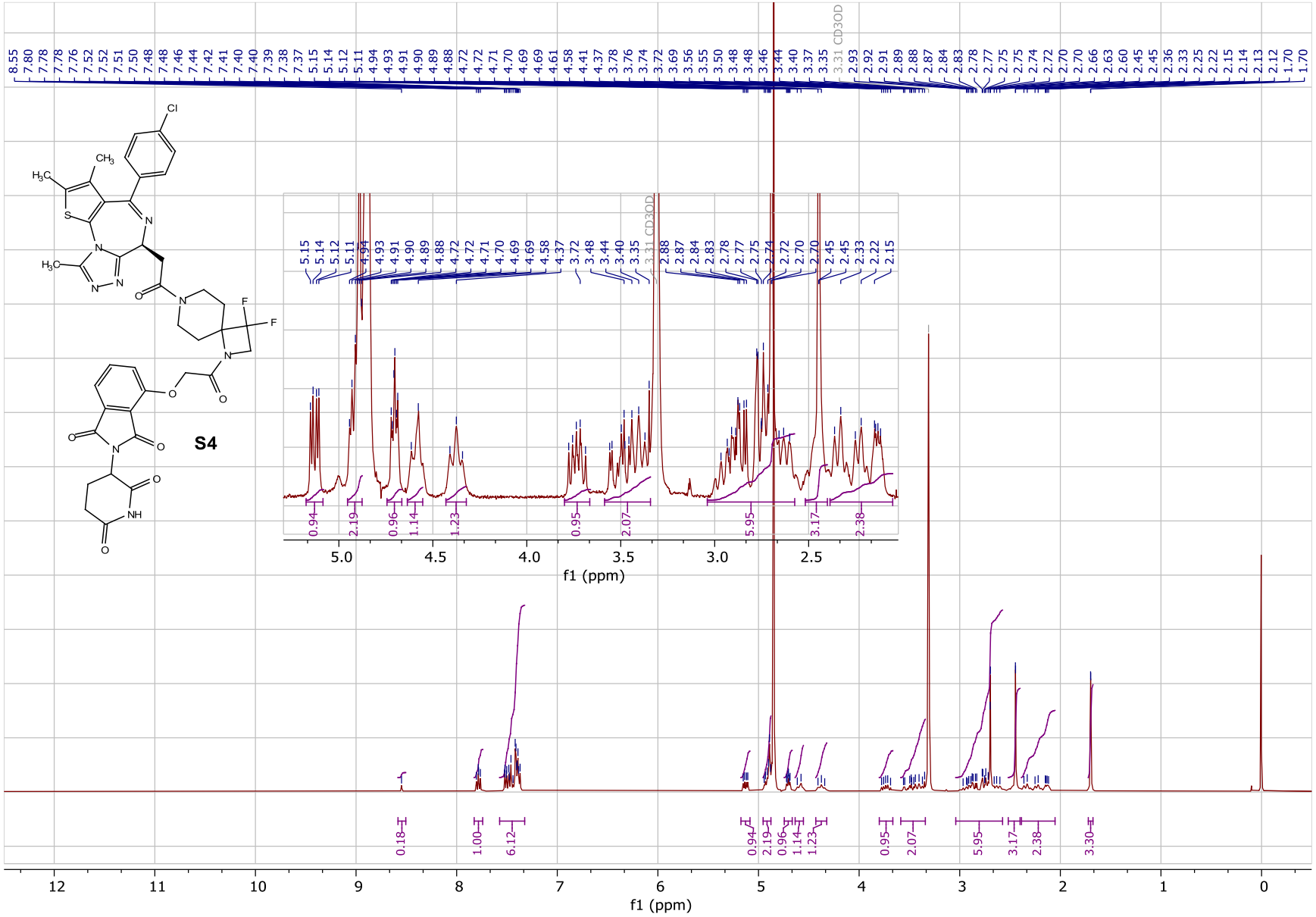
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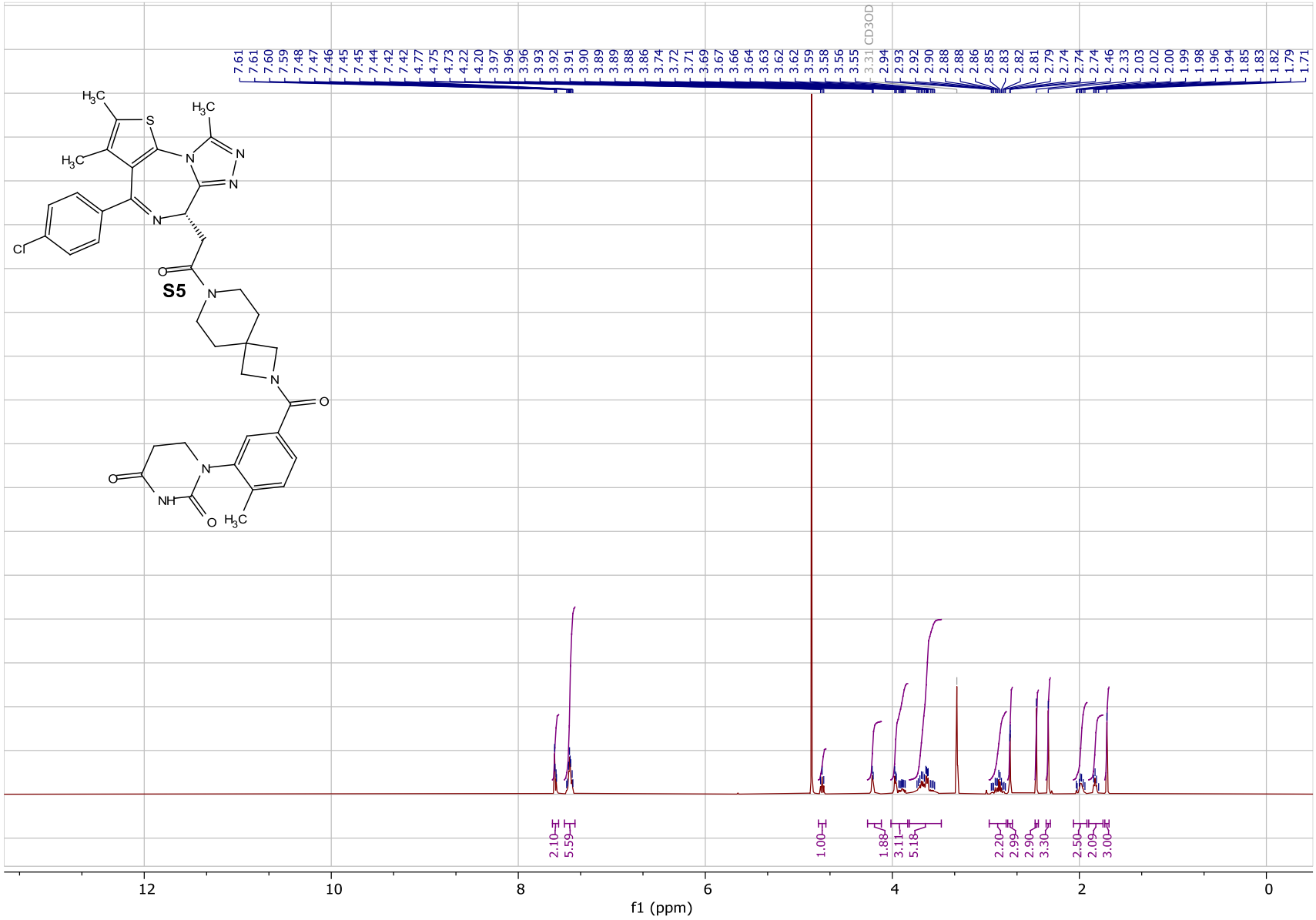
f1 (ppm)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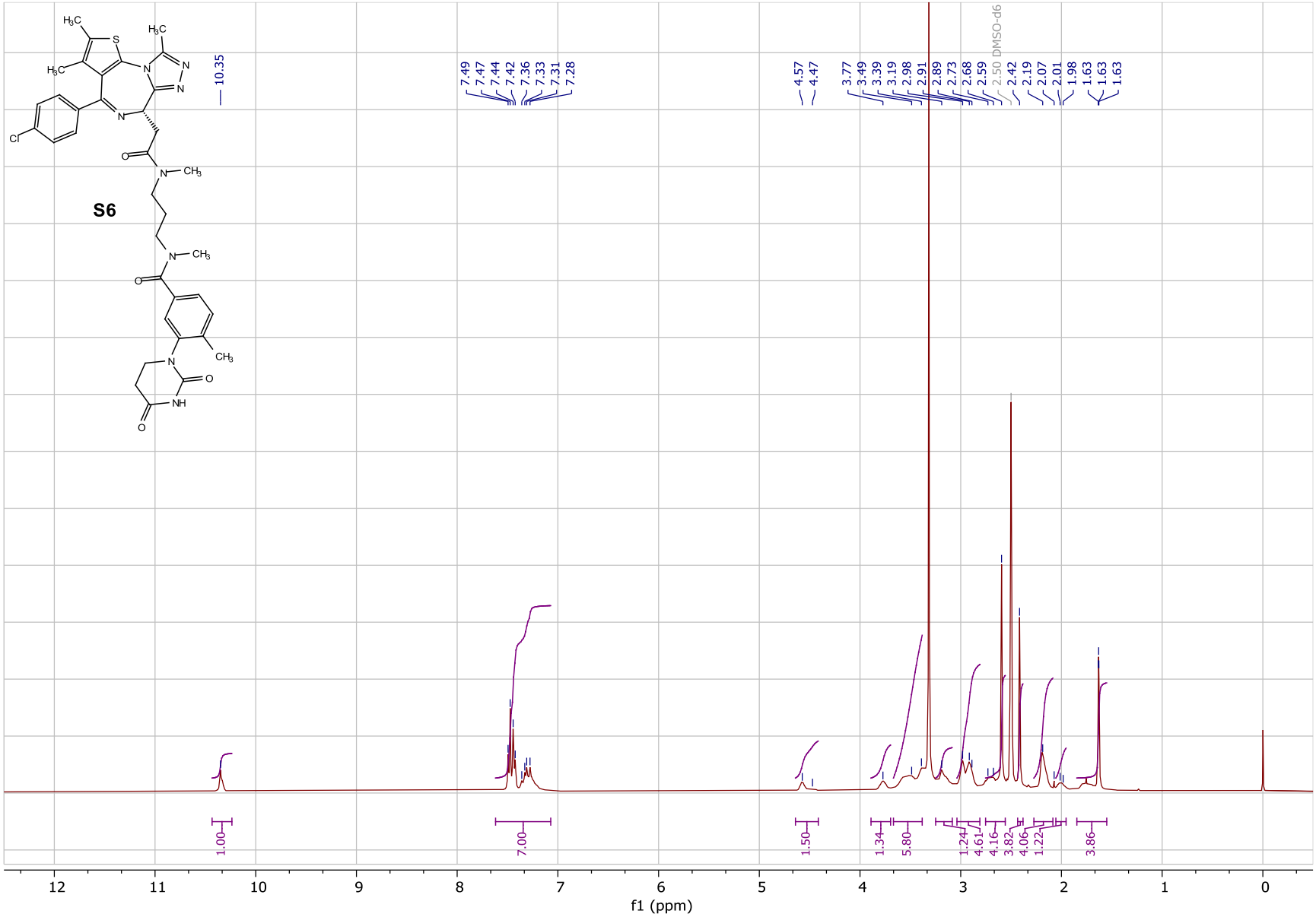
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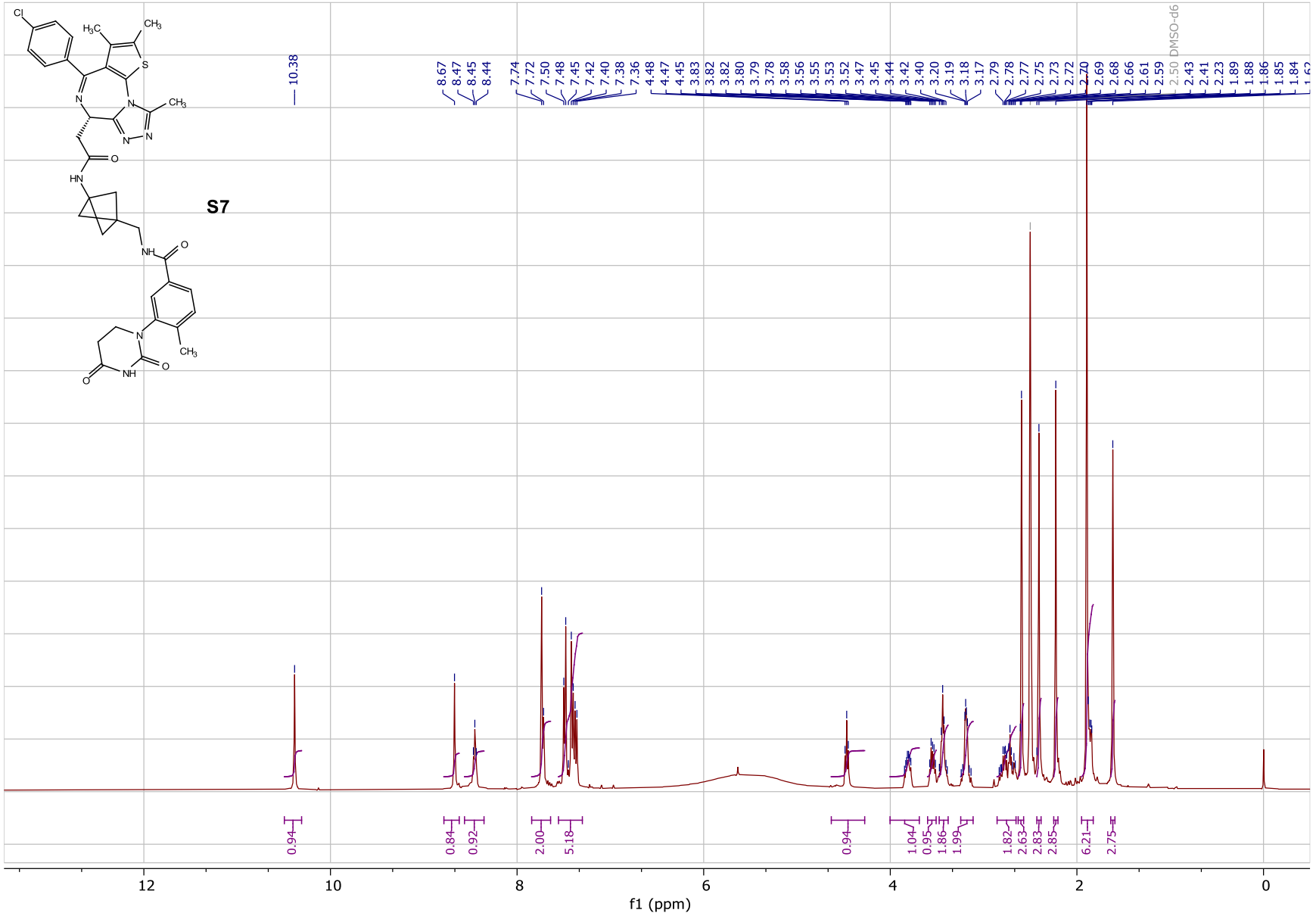
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2.72
2.70
2.69
2.68
2.66
2.61
2.59
2.50 DMSO-d6
2.43
2.41
2.23
1.89
1.88
1.86
1.85
1.84
0.94
0.84
0.92
2.00
5.18
0.94
1.04
0.95
1.86
1.99
1.82
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2.83
2.85
6.21
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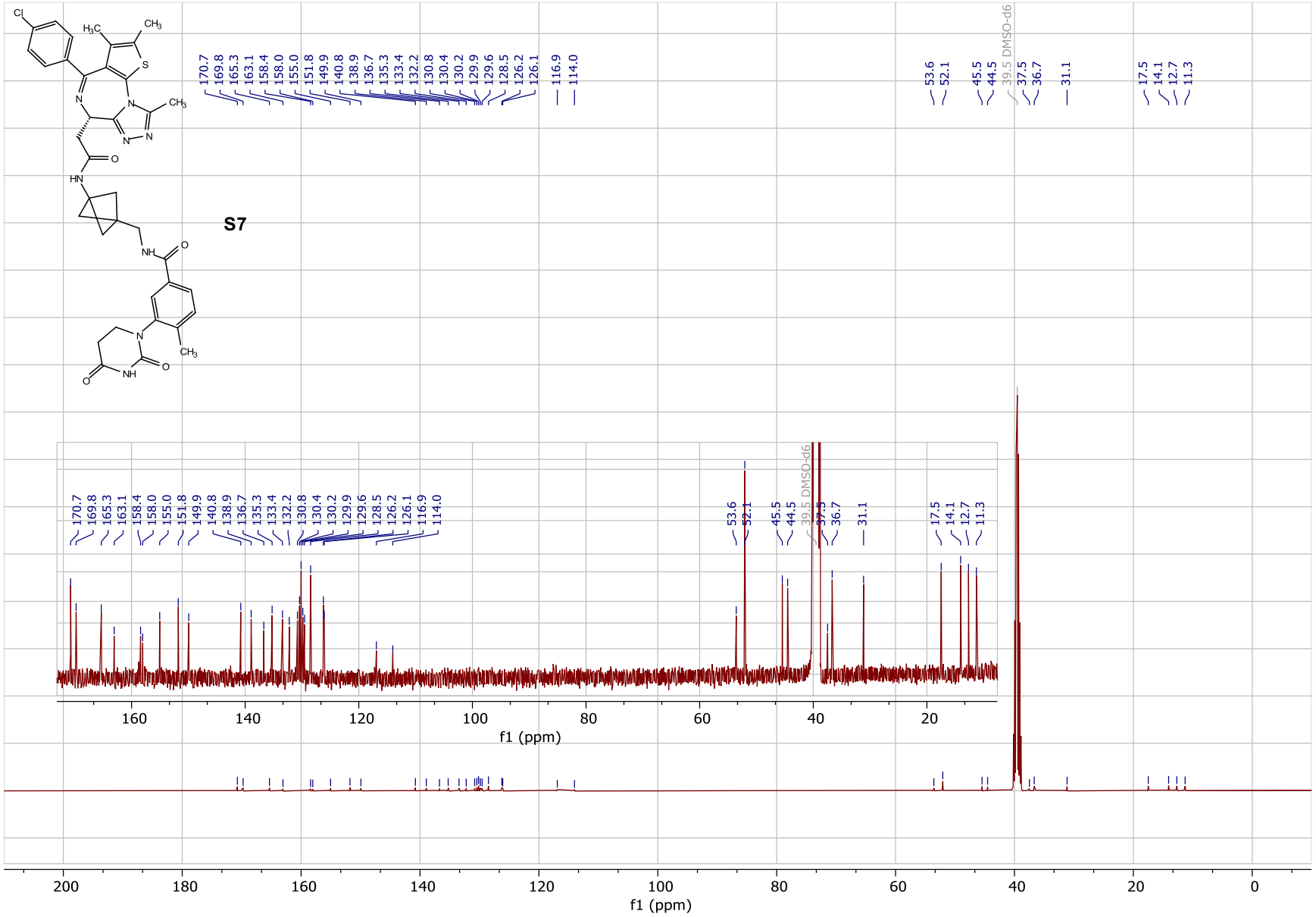
12
10
8
6
4
2
0
f1 (ppm)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135.3
133.4
132.2
130.8
130.4
130.2
129.9
129.6
128.5
126.2
126.1
116.9
114.0
53.6
52.1
45.5
44.5
39.5 DMSO-d6
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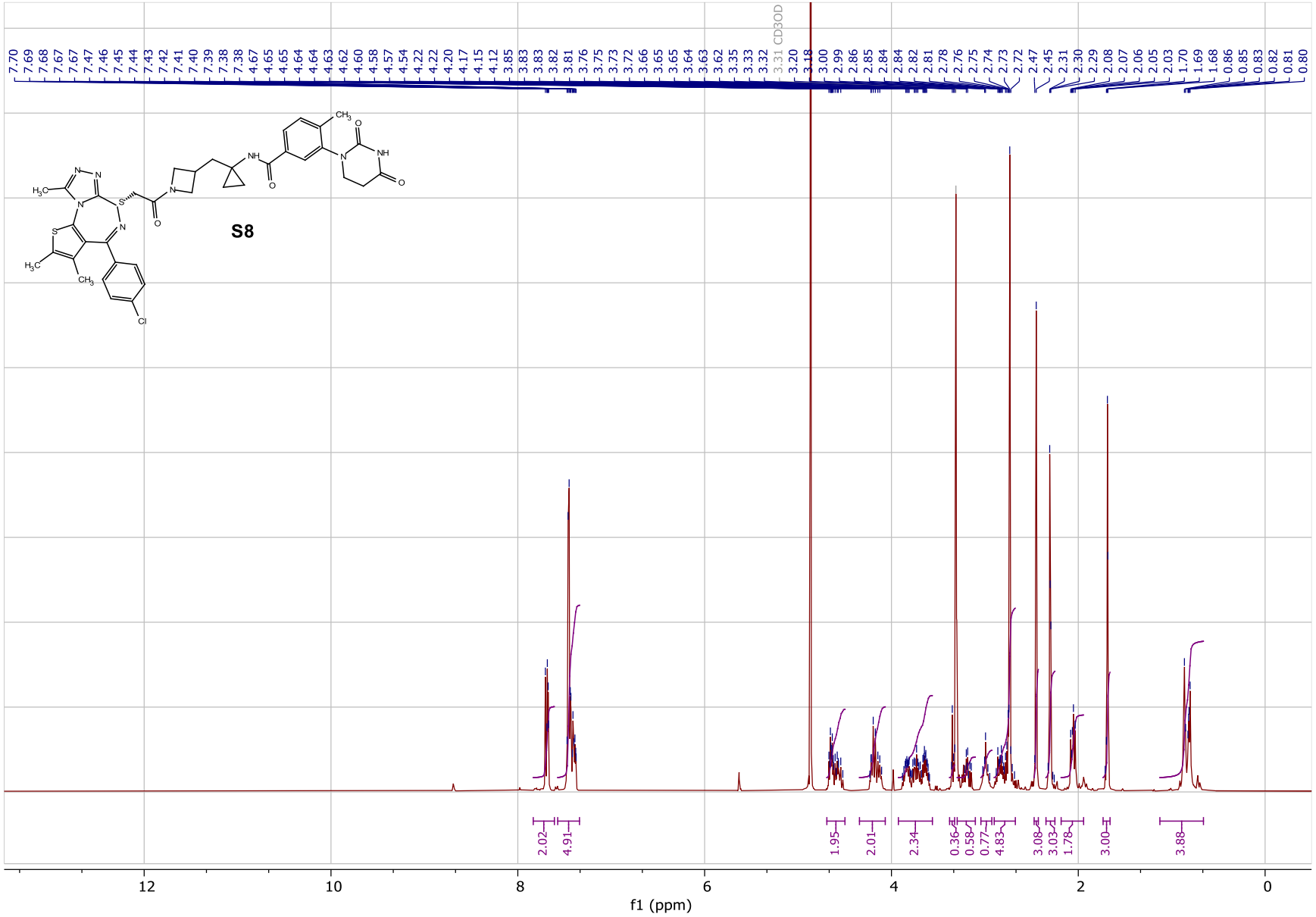
12.7
11.3
f1 (ppm)


S8
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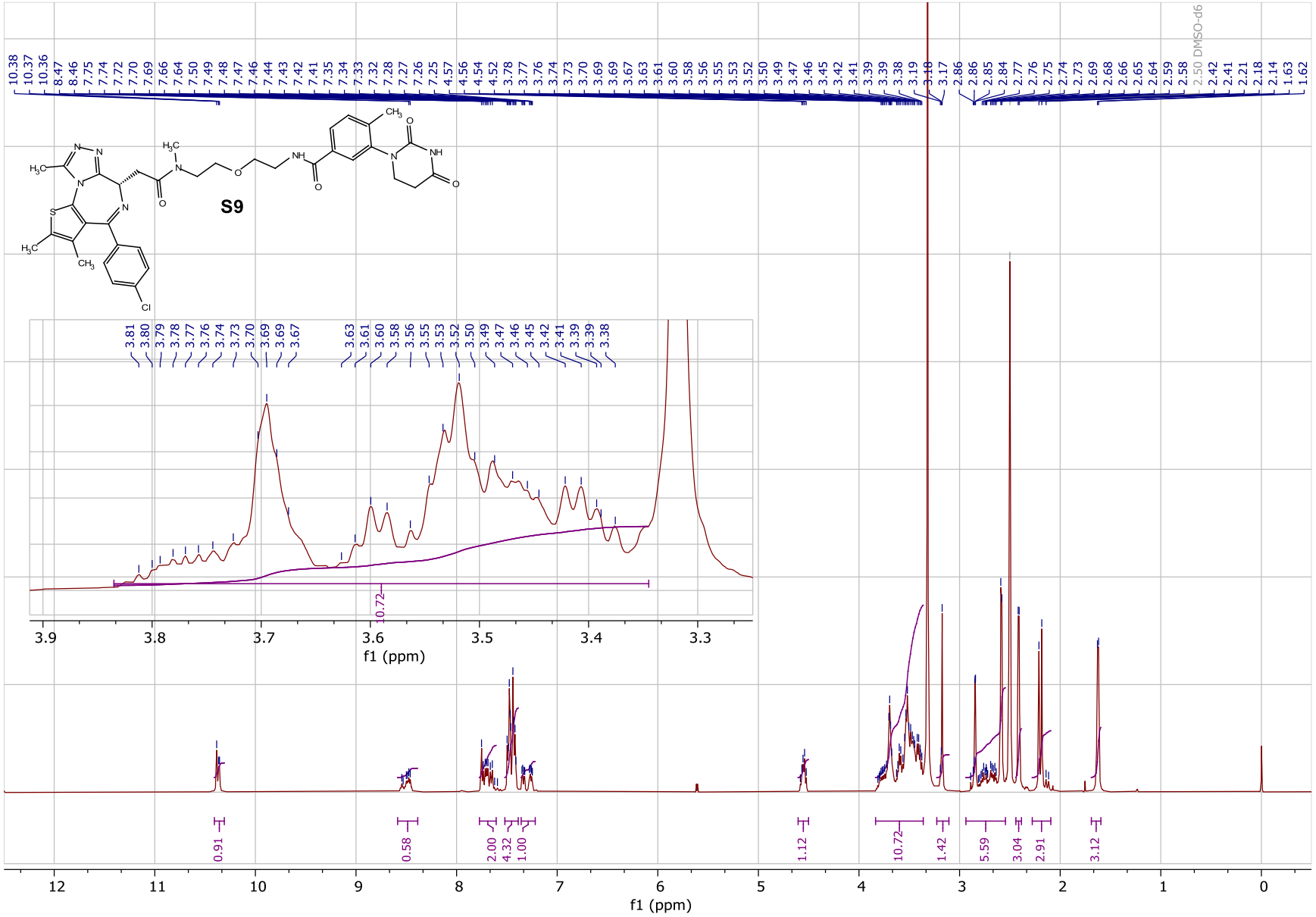
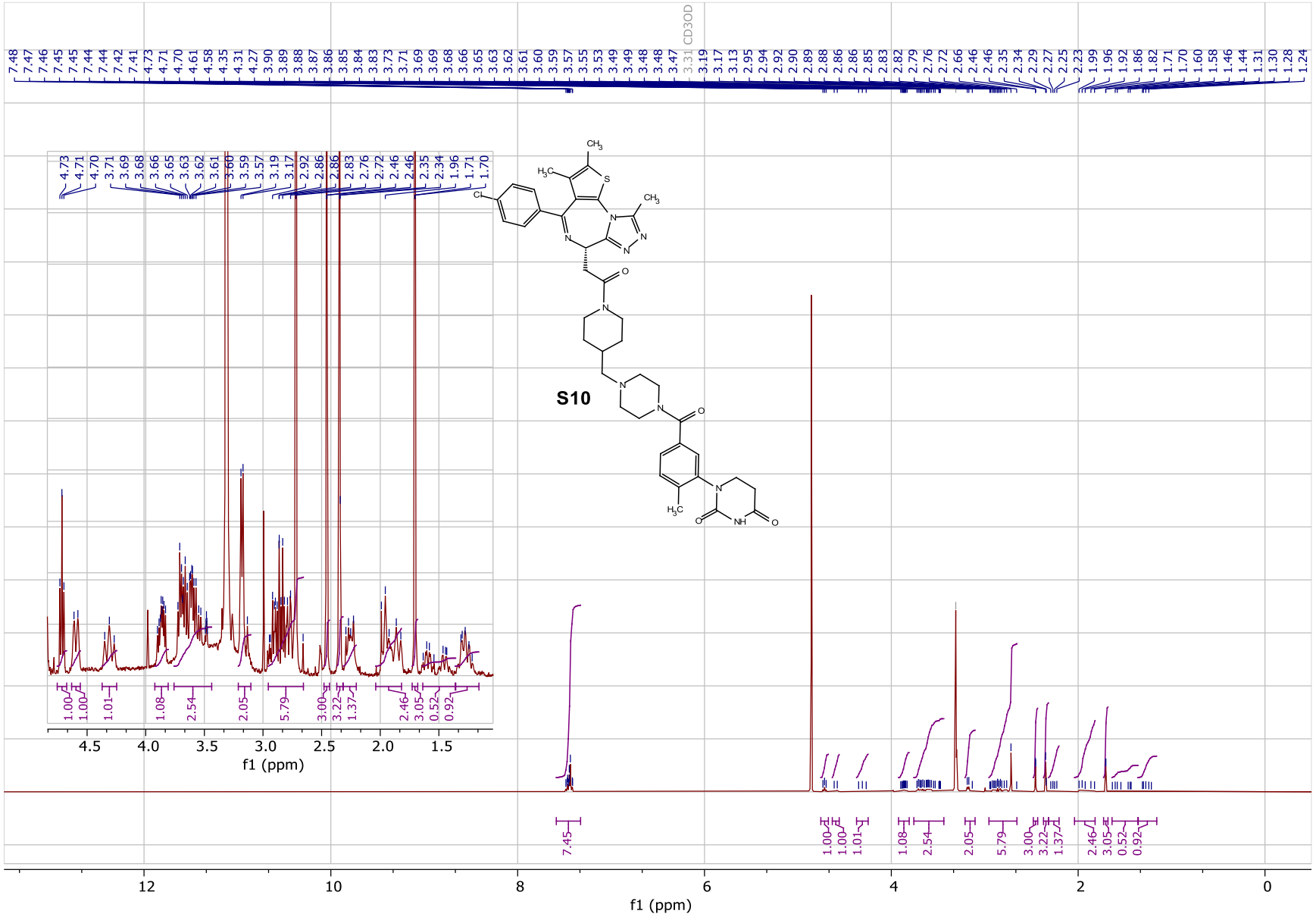
2.02 4.91 1.95 2.01 2.34 0.36 0.58 0.77 4.83 3.08 3.03 1.78 3.00 3.88
12 10 8 6 4 2 0
f1 (ppm)


S9
f1 (ppm)
f1 (ppm)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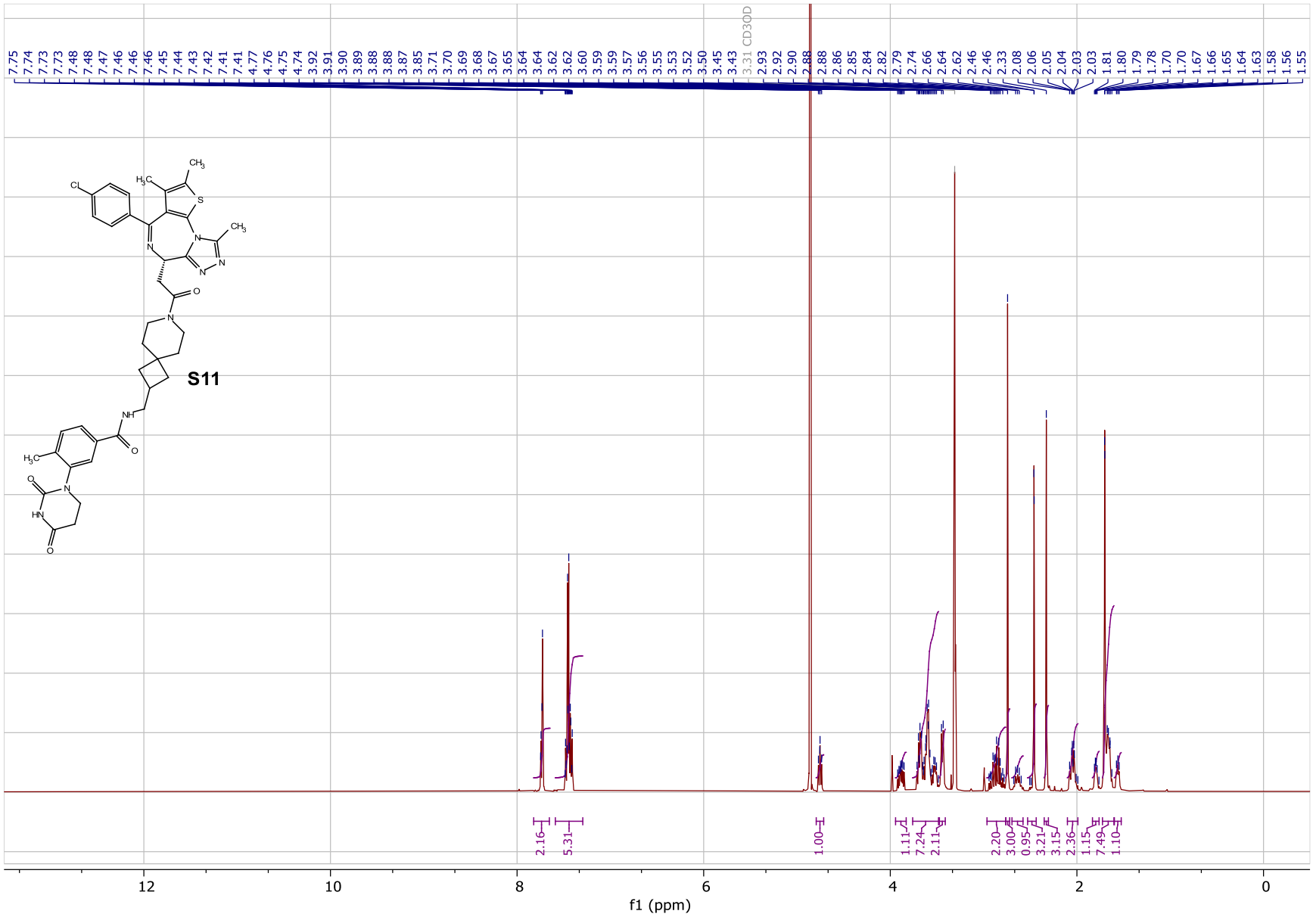
f1 (ppm)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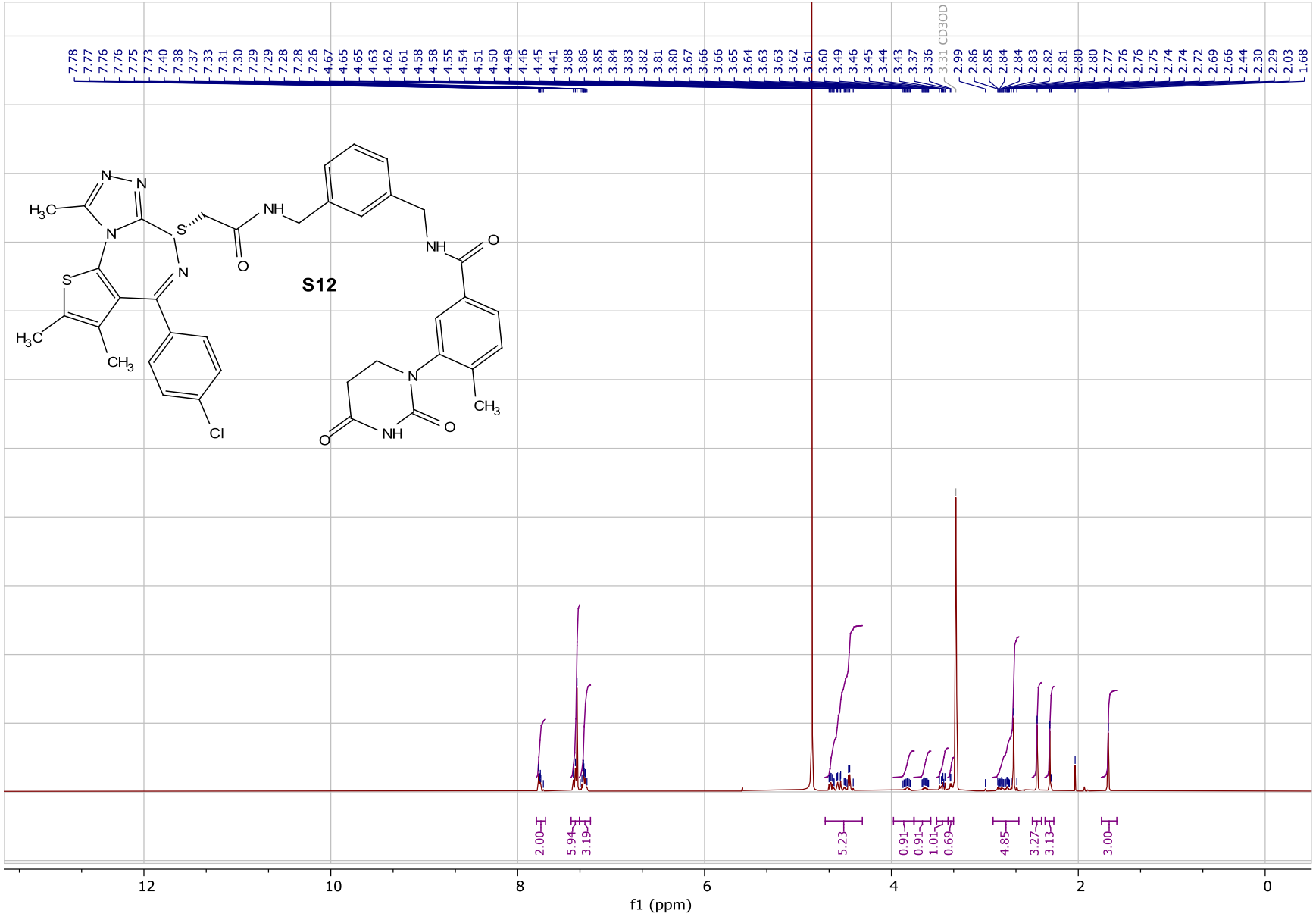
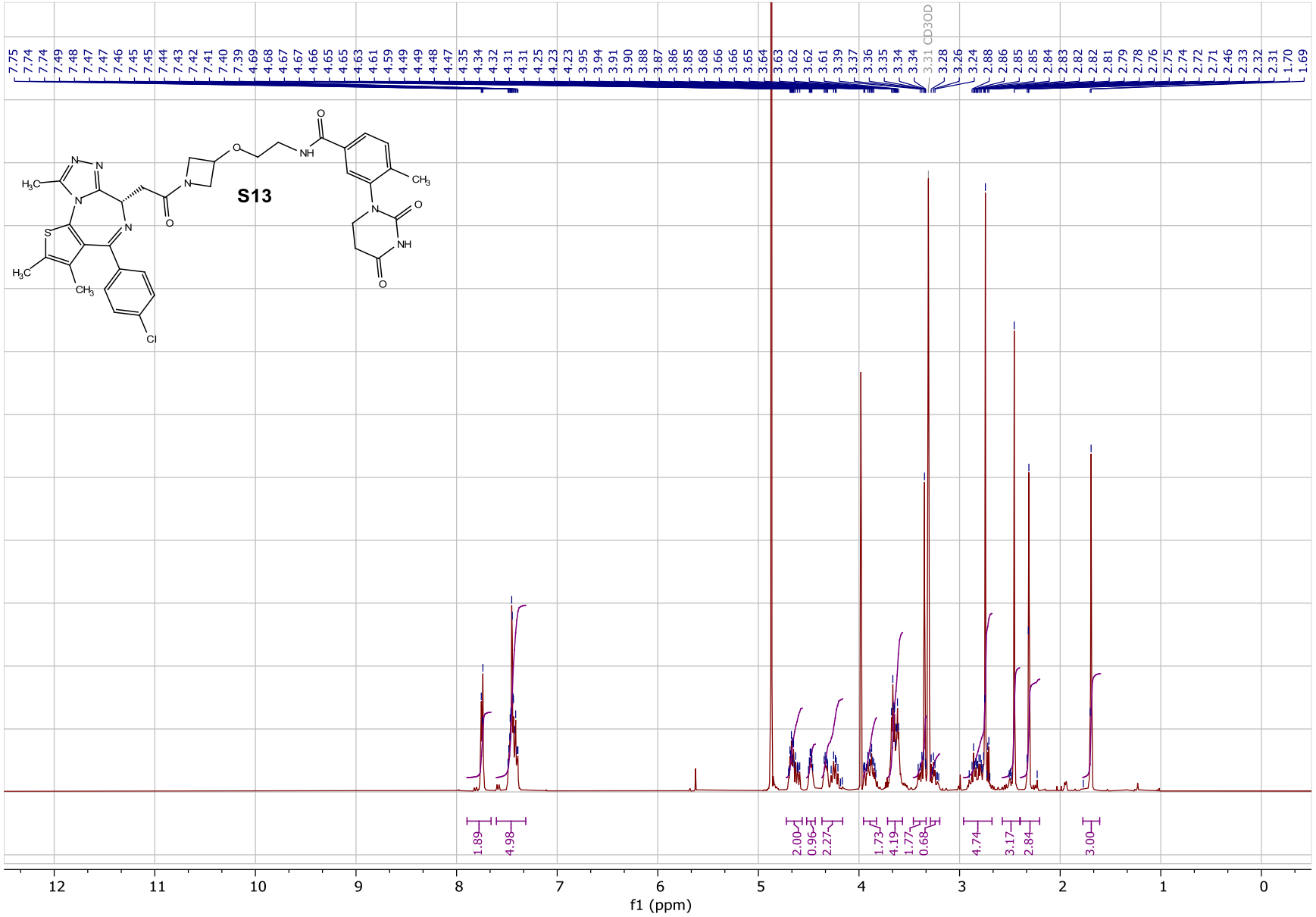
f1 (ppm)


S12
f1 (ppm)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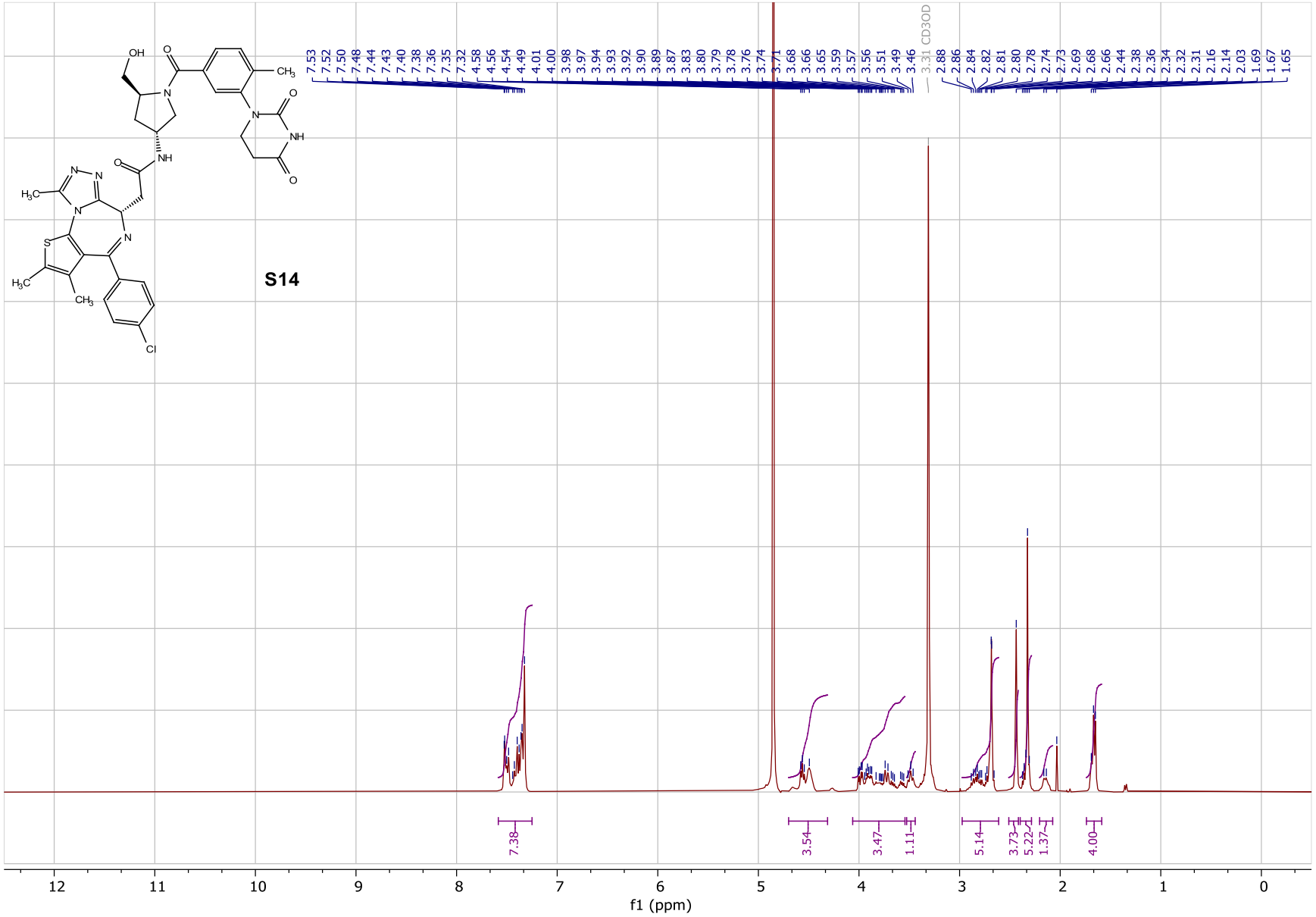
1.89 4.98 2.00 0.96 2.27 1.73 4.19 1.77 0.68 4.74 3.17 2.84 3.00
12 11 10 9 8 7 6 5 4 3 2 1 0
f1 (ppm)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7.32
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4.54
4.49
4.01
4.00
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3.97
3.94
3.93
3.92
3.90
3.89
3.87
3.83
3.80
3.79
3.78
3.76
3.74
3.71
3.68
3.66
3.65
3.59
3.57
3.56
3.51
3.49
3.46
3.31 CD3OD
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2.86
2.84
2.82
2.81
2.80
2.78
2.74
2.73
2.69
2.68
2.66
2.44
2.38
2.36
2.34
2.32
2.31
2.16
2.14
2.03
1.69
1.67
1.65
7.38
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3.47
1.11
5.14
3.73
5.22
1.37
4.00
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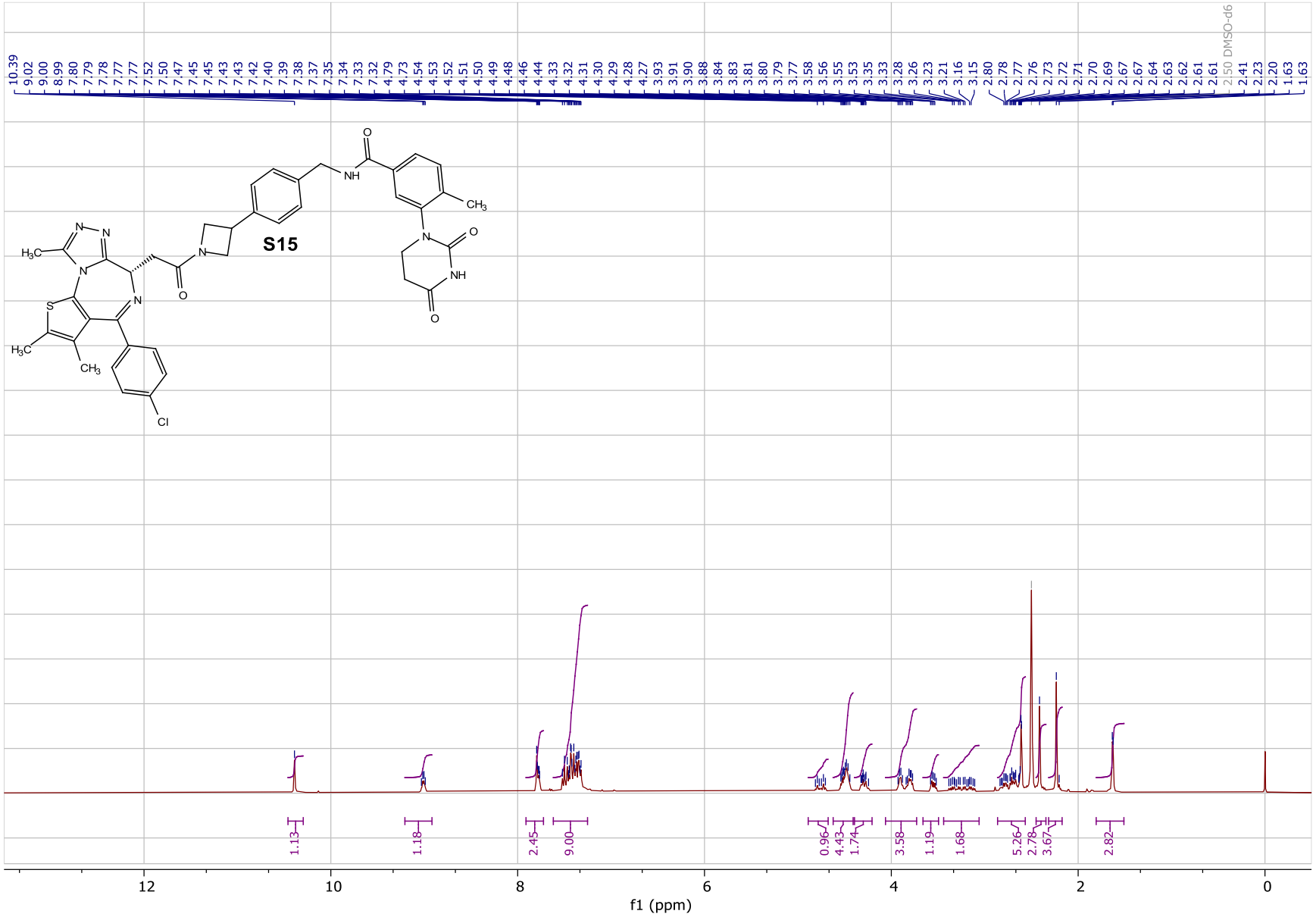
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)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7.77
7.52
7.50
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3.91
3.90
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3.84
3.83
3.81
3.80
3.79
3.77
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3.56
3.55
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3.35
3.33
3.28
3.26
3.23
3.21
3.16
3.15
2.80
2.78
2.77
2.76
2.73
2.72
2.71
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2.69
2.67
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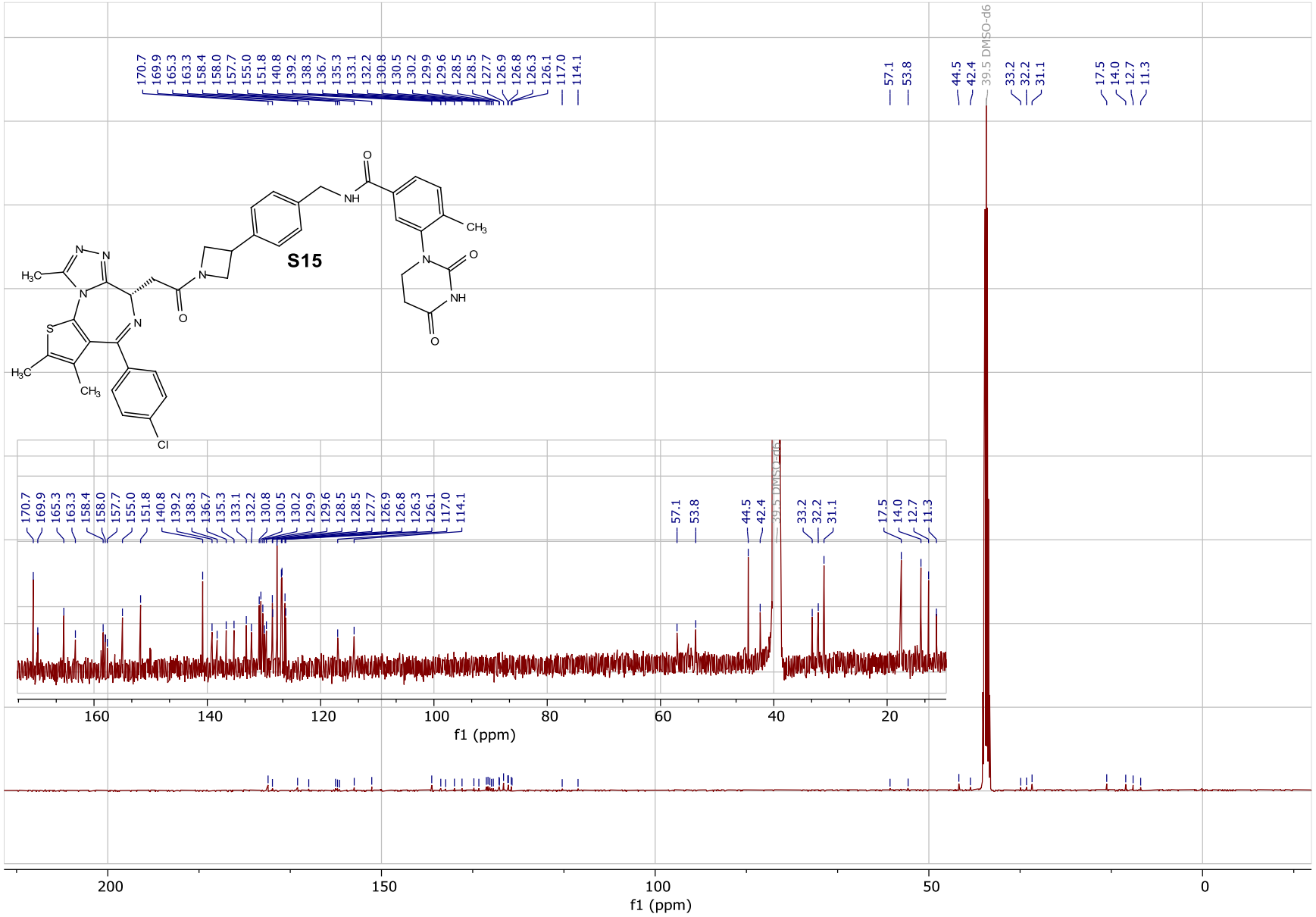
2.61
2.61
2.50 DMSO-d6
2.41
2.23
2.20
1.63
1.63
1.13
1.18
2.45
9.00
0.96
4.43
1.74
3.58
1.19
1.68
5.26
2.78
3.67
2.82
12
10
8
6
4
2
0
f1 (ppm)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158.0
157.7
155.0
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138.3
136.7
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133.1
132.2
130.8
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129.6
128.5
128.5
127.7
126.9
126.8
126.3
126.1
117.0
114.1
57.1
53.8
44.5
42.4
39.5 DMSO-d6
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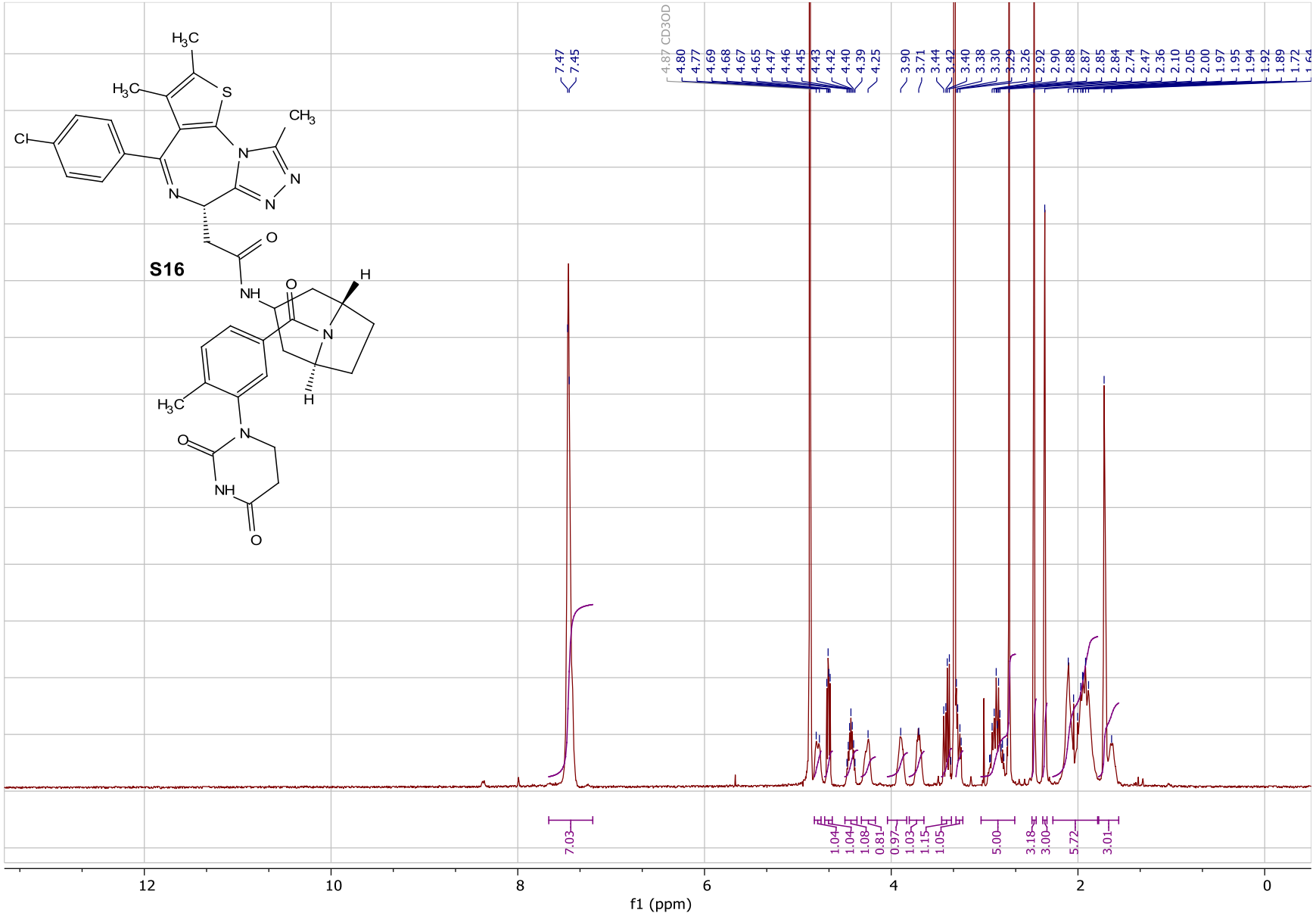
32.2
31.1
17.5
14.0
12.7
11.3
S15
f1 (ppm)
f1 (ppm)


S16
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4.43
4.42
4.40
4.39
4.25
3.90
3.71
3.44
3.42
3.40
3.38
3.30
3.29
3.26
2.92
2.90
2.88
2.87
2.85
2.84
2.74
2.47
2.36
2.10
2.05
2.00
1.97
1.95
1.94
1.92
1.89
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1.64
7.03
1.04
1.04
1.08
0.81
0.97
1.03
1.15
1.05
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3.18
3.00
5.72
3.01
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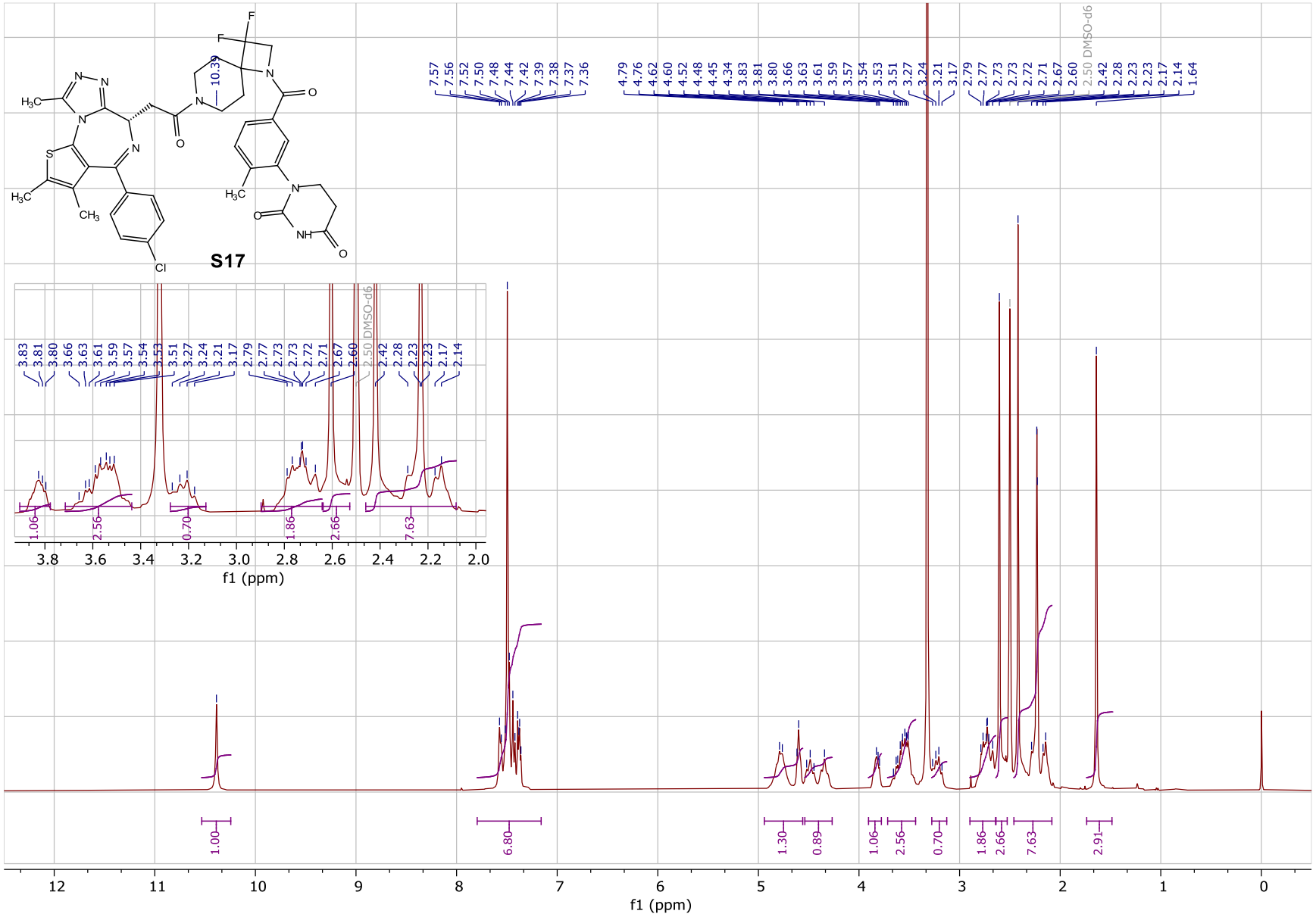
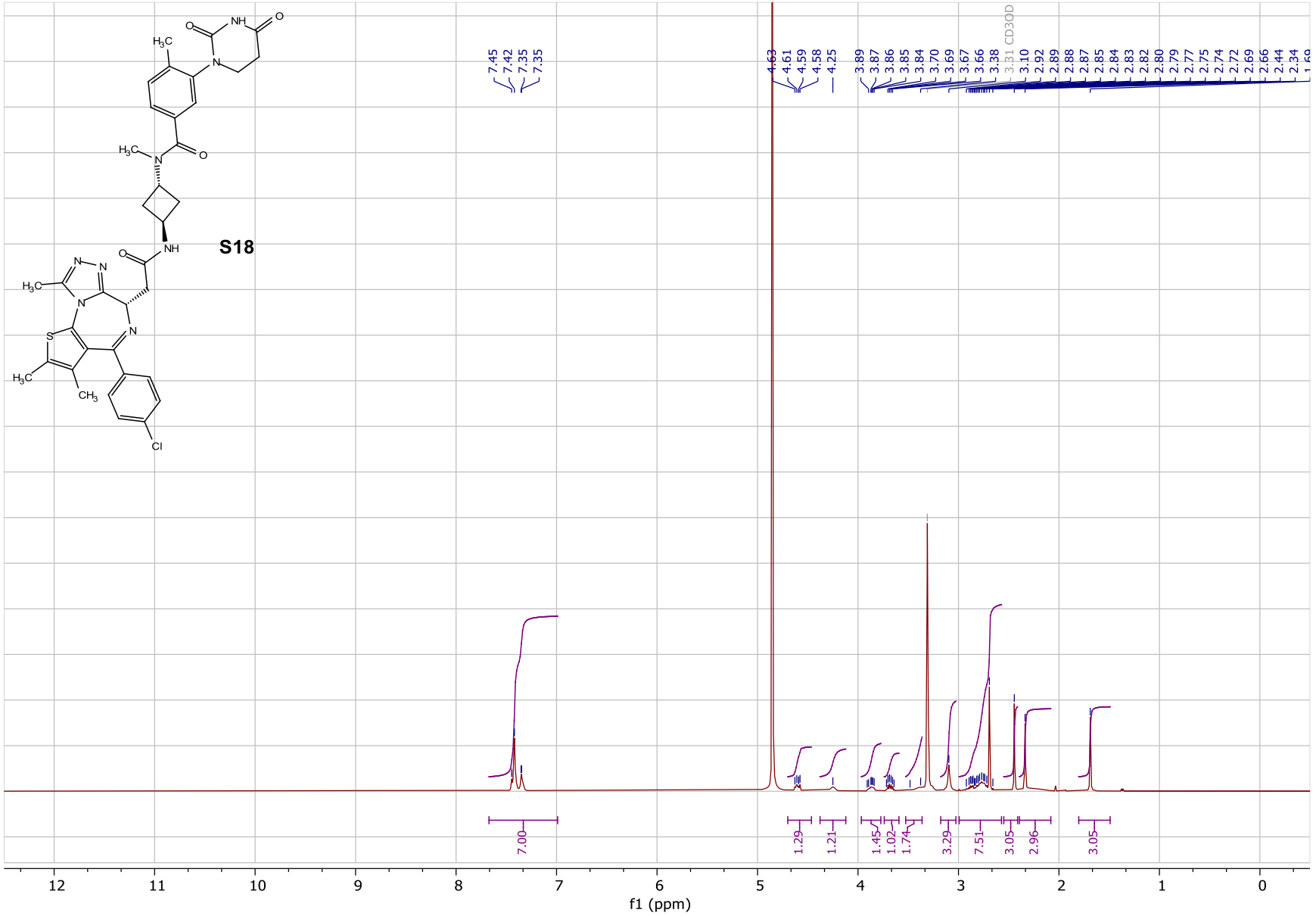
10
8
6
4
2
0
f1 (ppm)


S17
f1 (ppm)
DMSO-d6


S18
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7.42
7.35
7.35
4.63
4.61
4.59
4.58
4.25
3.89
3.87
3.86
3.85
3.84
3.70
3.69
3.67
3.66
3.38
3.31 CD3OD
3.10
2.92
2.89
2.88
2.87
2.85
2.84
2.83
2.82
2.80
2.79
2.77
2.75
2.74
2.72
2.69
2.66
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1.21
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1.02
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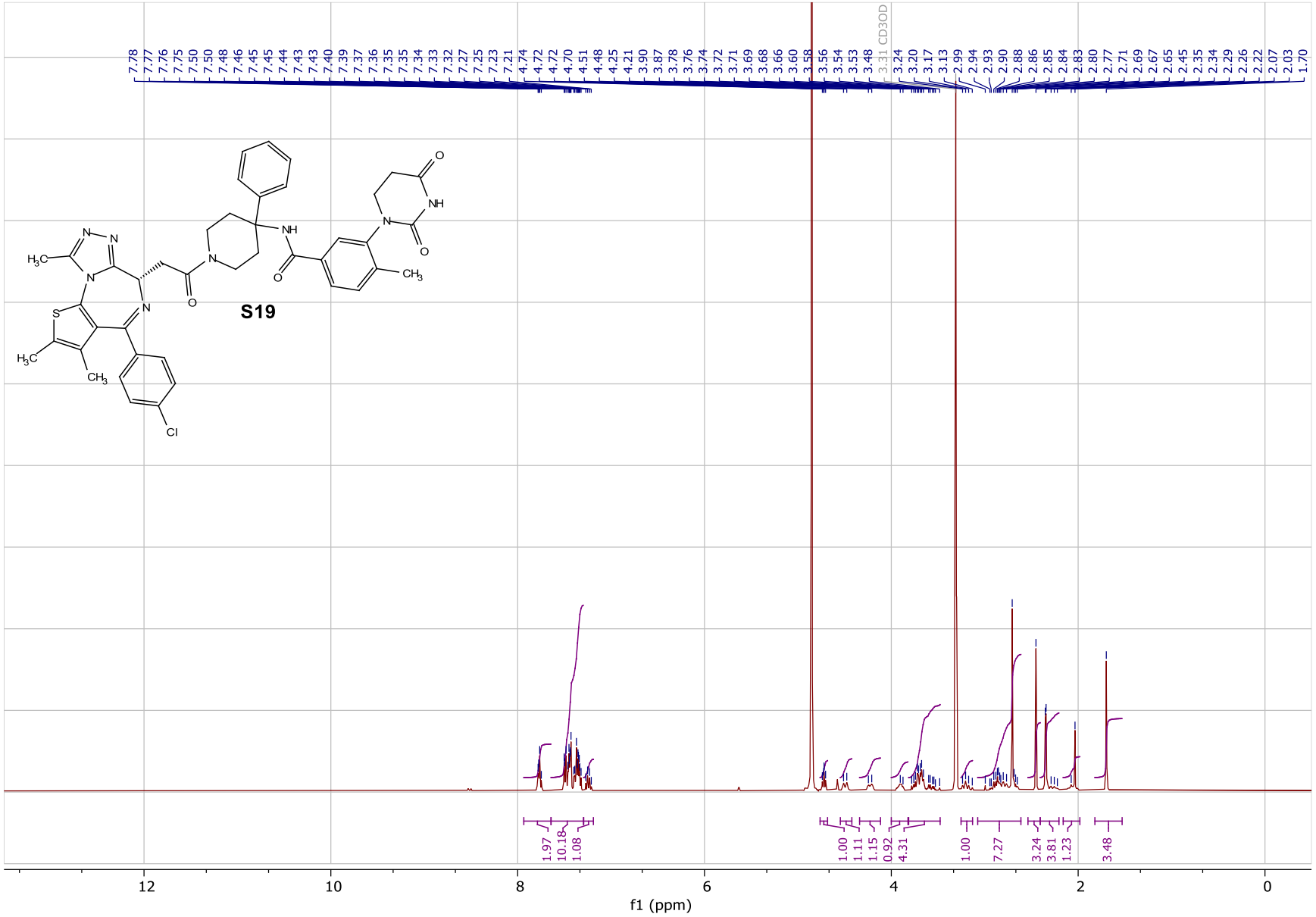
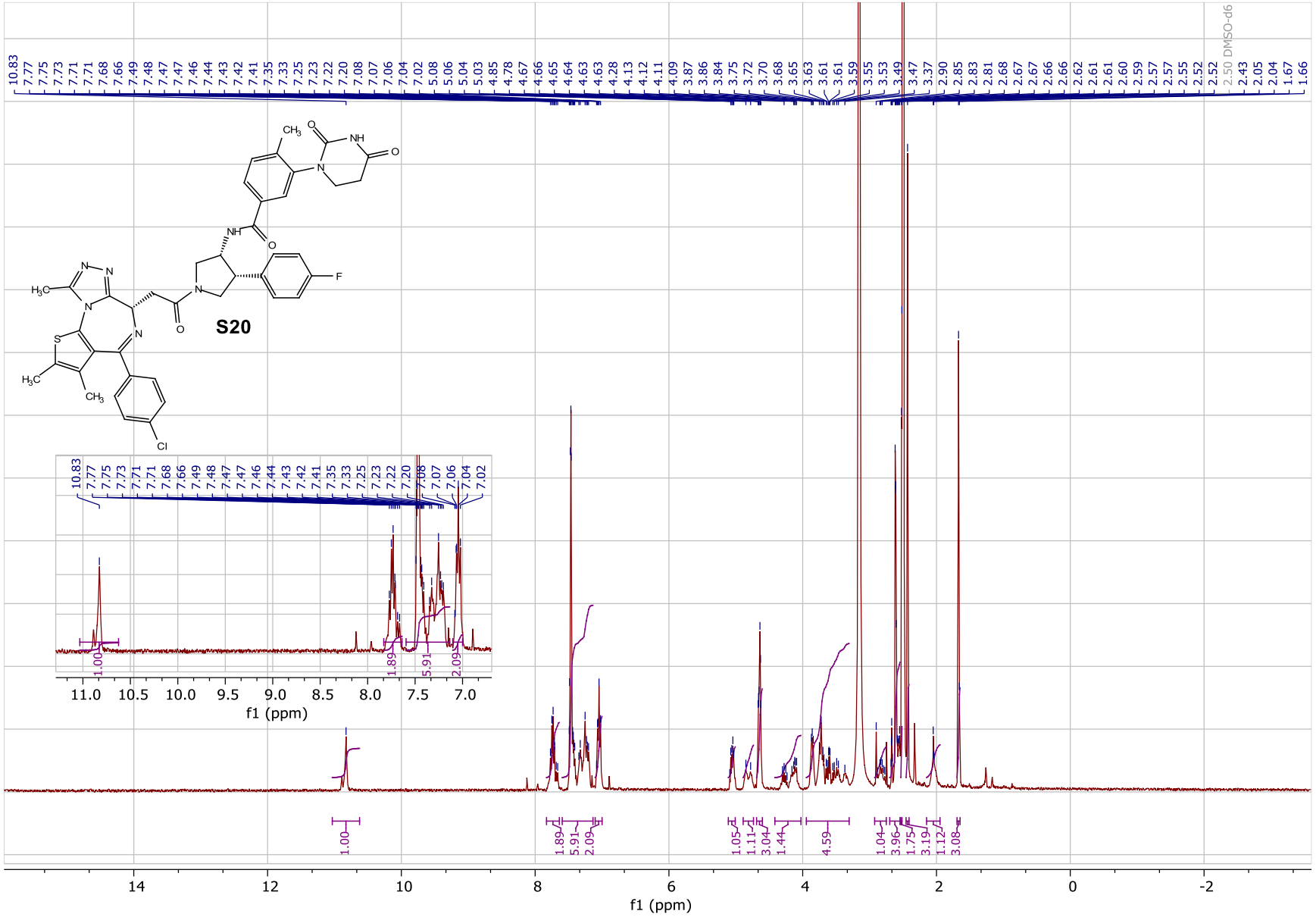
3.29
7.51
3.05
2.96
3.05
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


S19
f1 (ppm)


S20
f1 (ppm)
14
12
10
8
6
4
2
0
-2
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0

VIII. References

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3. Nowak, R. P.; DeAngelo, S. L.; Buckley, D.; He, Z.; Donovan, K. A.; An, J.; Safaee, N.; Jedrychowski, M. P.; Ponthier, C. M.; Ishoey, M.; Zhang, T.; Mancias, J. D.; Gray, N. S.; Bradner, J. E.; Fischer, E. S. Plasticity in binding confers selectivity in ligand-induced protein degradation. **2018,** *14*, 706-714.
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IX. D2B Synthesis Data and Linker Tables

CAD Quantification Tables

Table S6. O-Pom Plate 1 CAD Results

Location	Mass Expected	Product Conc (mg/mL)	CAD Yield	Rt (min)	Purity %
A01	930.3	0.000	0		
A02	810.2	0.000	0		
A03	905.3	0.082	18	0.840	85
A04	902.3	0.061	14	0.814	72
A05	893.3	0.097	22	0.819	84
A06	888.3	0.039	9	0.796	49
A07	888.3	0.055	13	0.788	61
A08	887.3	0.000	0		
A09	879.3	0.103	24	0.828	89
A10	878.3	0.088	21	0.963	57
A11	877.2	0.042	10	0.871	49
A12	876.2	0.136	32	0.947	92
B01	872.3	0.045	11	0.970	28
B02	812.2	0.087	22	0.843	86
B03	858.2	0.071	17	0.890	47
B04	858.2	0.000	0		
B05	858.2	0.000	0		
B06	858.2	0.143	34	0.915	95
B07	854.3	0.141	34	0.847	83
B08	852.2	0.000	0	0.835	12
B09	852.2	0.113	27	0.849	84
B10	851.2	0.000	0	0.830	18
B11	848.2	0.000	0		
B12	848.2	0.000	0		
C01	846.2	0.098	24	0.877	79
C02	846.2	0.072	17	0.830	82
C03	846.2	0.000	0		
C04	845.2	0.142	35	0.835	91
C05	840.2	0.084	20	0.816	59
C06	839.3	0.138	34	0.775	92
C07	837.2	0.046	11	0.785	51
C08	836.3	0.191	47	0.911	54
C09	836.3	0.109	27	0.892	11
C10	836.3	0.182	45	0.855	85
C11	832.2	0.091	22	0.886	78
C12	832.2	0.071	18	0.945	53
D01	810.2	0.101	26	0.836	69
D02	832.2	0.061	15	0.917	70
D03	832.2	0.148	36	0.865	94
D04	832.2	0.119	29	0.905	89

D05	826.2	0.147	36	0.795	5
D06	864.3	0.072	17	0.912	53
D07	840.3	0.067	16	0.922	70
D08	824.3	0.033	8	0.895	37
D09	824.2	0.064	16	0.824	46
D10	824.2	0.000	0		
D11	822.2	0.147	37	0.861	91
D12	822.2	0.142	35	0.858	89
E01	822.2	0.000	0		
E02	822.2	0.028	7	0.875	46
E03	822.2	0.000	0	0.876	40
E04	822.2	0.117	29	0.845	88
E05	814.2	0.111	28	0.827	86
E06	812.2	0.130	33	0.835	81
E07	812.2	0.076	19	0.812	53
E08	812.2	0.114	29	0.784	78
E09	811.2	0.074	19	0.794	82
E10	810.2	0.155	39	0.878	97
E11	810.2	0.095	24	0.859	84
E12	810.2	0.063	16	0.887	4
F01	810.2	0.102	26	0.861	76
F02	810.2	0.056	14	0.888	66
F03	810.2	0.097	25	0.855	76
F04	808.2	0.207	53	0.855	28
F05	808.2	0.192	49	0.865	94
F06	808.2	0.118	30	0.878	77
F07	808.2	0.104	26	0.861	76
F08	800.2	0.141	36	0.851	33
F09	798.2	0.137	35	0.849	87
F10	798.2	0.101	26	0.817	83
F11	798.2	0.074	19	0.878	65
F12	798.2	0.071	18	0.861	47
G01	796.2	0.000	0	0.865	28
G02	796.2	0.107	28	0.873	86
G03	796.2	0.038	10	0.861	59
G04	796.2	0.053	14	0.821	65
G05	796.2	0.129	33	0.835	87
G06	796.2	0.144	37	0.834	95
G07	794.2	0.115	30	0.836	87
G08	784.2	0.147	38	0.818	89
G09	782.2	0.070	18	0.843	48
G10	780.2	0.060	16	0.841	59
G11	770.2	0.063	17	0.839	63
G12	850.3	0.000	0	0.910	8
H01	865.3	0.134	32	0.770	87
H02	879.3	0.036	8	0.771	31
H03	959.3	0.082	17	0.905	37
H04	919.3	0.044	10	0.871	39
H05	784.2	0.000	0		

H06	800.2	0.000	0	0.816	11
H07	844.2	0.096	23	0.819	66
H08	497.1	0.125	52	0.935	76
H09	429.1	0.000	0		
H10	400.1	0.125	64	0.820	95
H11	332.1	0.086	53	0.462	77
H12	115.0	0.000	0	1.035	5

Table S7. O-Pom Plate 2 CAD Results

Location	Mass Expected	Product Conc (mg/mL)	CAD Yield (%)	Rt (min)	Purity %
A01	930.3	0.266	16	0.815	61
A02	810.2	0.000	0		
A03	905.3	0.340	21	0.840	76
A04	902.3	0.177	11	0.814	71
A05	893.3	0.375	23	0.820	84
A06	888.3	0.255	16	0.795	73
A07	888.3	0.489	30	0.787	74
A08	887.3	0.494	31	0.805	75
A09	879.3	0.253	16	0.825	74
A10	878.3	0.271	17	0.964	37
A11	877.2	0.162	10	0.873	47
A12	876.2	0.000	0		
B01	872.3	0.093	6	0.970	20
B02	812.2	0.279	19	0.844	81
B03	858.2	0.299	19	0.890	59
B04	858.2	0.000	0	0.981	12
B05	858.2	0.000	0		
B06	858.2	0.389	25	0.915	72
B07	854.3	0.620	40	0.849	78
B08	852.2	0.040	3		
B09	852.2	0.267	17	0.849	68
B10	851.2	0.091	6	0.831	18
B11	848.2	0.145	9	0.804	49
B12	848.2	0.000	0		
C01	846.2	0.314	20	0.879	61
C02	846.2	0.212	14	0.830	44
C03	846.2	0.598	39	0.834	82
C04	845.2	0.507	33	0.835	90
C05	840.2	0.162	11	0.817	31
C06	839.3	0.699	46	0.775	92
C07	837.2	0.221	15	0.785	51
C08	836.3	0.000	0	0.889	26
C09	836.3	0.224	15	0.933	61
C10	836.3	0.180	12	0.856	52
C11	832.2	0.428	28	0.887	81
C12	832.2	0.000	0		

D01	810.2	0.444	30	0.837	81
D02	832.2	0.099	7	0.917	51
D03	832.2	0.622	41	0.865	92
D04	832.2	0.589	39	0.907	90
D05	826.2	0.682	45	0.824	79
D06	864.3	0.356	23	0.911	64
D07	840.3	0.425	28	0.923	83
D08	824.3	0.045	3	0.896	29
D09	824.2	0.181	12	0.824	37
D10	824.2	0.000	0	0.840	15
D11	822.2	0.683	46	0.862	90
D12	822.2	0.000	0		
E01	822.2	0.000	0		
E02	822.2	0.080	5	0.876	48
E03	822.2	0.570	38	0.864	38
E04	822.2	0.509	34	0.848	88
E05	814.2	0.636	43	0.829	87
E06	812.2	0.274	19	0.835	48
E07	812.2	0.516	35	0.813	78
E08	812.2	0.516	35	0.785	78
E09	811.2	0.385	26	0.795	79
E10	810.2	0.260	18	0.879	66
E11	810.2	0.440	30	0.860	76
E12	810.2	0.000	0		
F01	810.2	0.198	13	0.863	38
F02	810.2	0.201	14	0.890	75
F03	810.2	0.487	33	0.855	78
F04	808.2	0.517	35	0.855	25
F05	808.2	0.000	0		
F06	808.2	0.108	7	0.879	33
F07	808.2	0.535	36	0.863	77
F08	800.2	0.259	18	0.852	26
F09	798.2	0.659	45	0.849	90
F10	798.2	0.286	20	0.818	78
F11	798.2	0.241	17	0.879	58
F12	798.2	0.000	0		
G01	796.2	0.125	9	0.867	57
G02	796.2	0.392	27	0.872	74
G03	796.2	0.203	14	0.861	69
G04	796.2	0.596	41	0.822	84
G05	796.2	0.563	39	0.835	86
G06	796.2	0.612	42	0.835	90
G07	794.2	0.654	45	0.837	89
G08	784.2	0.467	33	0.818	69
G09	782.2	0.364	26	0.844	68
G10	780.2	0.278	20	0.843	64
G11	770.2	0.219	16	0.840	49
G12	850.3	0.000	0		
H01	865.3	0.642	41	0.770	77

H02	879.3	0.220	14	0.771	51
H03	959.3	0.218	13	0.902	73
H04	919.3	0.185	11	0.870	55
H05	784.2	0.346	24	0.829	63
H06	800.2	0.094	6	0.815	23
H07	844.2	0.449	29	0.819	74
H08	497.1	0.345	38	0.935	61
H09	429.1	0.000	0		
H10	400.1	0.000	0		
H11	332.1	0.302	50	0.464	95

Table S8. tDHU Plate 1 CAD Results

Location	Mass Expected	Product Conc (mg/mL)	CAD Yield	Rt (min)	Purity %
A01	846.3	0.103	24	1.234	79
A02	726.3	0.000	0		
A03	821.3	0.209	51	1.244	91
A04	775.2	0.126	32	1.149	86
A05	809.3	0.159	39	1.228	77
A06	804.3	0.000	0		
A07	804.3	0.135	34	1.159	87
A08	803.3	0.000	0		
A09	795.3	0.142	36	1.243	84
A10	794.3	0.134	34	1.583	78
A11	793.3	0.110	28	1.345	64
A12	792.2	0.000	0	1.534	86
B01	788.3	0.147	37	1.560	80
B02	728.2	0.177	49	1.255	92
B03	774.3	0.201	52	1.403	93
B04	774.3	0.092	24	1.598	59
B05	774.3	0.000	0		
B06	774.2	0.096	25	1.486	78
B07	770.3	0.164	43	1.287	81
B08	768.3	0.000	0		
B09	768.3	0.184	48	1.327	86
B10	767.3	0.086	23	1.249	56
B11	764.2	0.105	27	1.177	86
B12	764.2	0.104	27	1.237	61
C01	762.3	0.086	23	1.375	49
C02	762.2	0.057	15	1.180	41
C03	762.2	0.054	14	1.250	40
C04	761.2	0.129	34	1.215	78
C05	756.3	0.113	30	1.200	71
C06	755.3	0.103	27	1.135	77
C07	753.2	0.000	0		
C08	752.3	0.084	22	1.337	30

C09	752.3	0.126	34	1.514	70
C10	752.3	0.137	37	1.323	77
C11	748.2	0.123	33	1.386	73
C12	748.2	0.057	15	1.539	58
D01	726.3	0.166	46	1.244	85
D02	748.2	0.129	34	1.474	75
D03	748.2	0.000	0		
D04	748.2	0.000	0		
D05	742.2	0.091	24	1.201	43
D06	780.3	0.077	20	1.446	48
D07	756.3	0.156	41	1.496	89
D08	740.3	0.000	0		
D09	740.2	0.038	10	1.111	37
D10	740.2	0.000	0		
D11	738.3	0.073	20	1.304	67
D12	738.3	0.135	37	1.312	84
E01	738.3	0.113	31	1.366	70
E02	738.3	0.135	37	1.339	80
E03	738.3	0.102	28	1.380	68
E04	738.3	0.137	37	1.301	75
E05	730.2	0.148	40	1.243	84
E06	728.2	0.151	42	1.223	89
E07	728.2	0.090	25	1.185	67
E08	728.2	0.091	25	1.151	70
E09	727.2	0.000	0		
E10	726.3	0.201	55	1.400	95
E11	726.3	0.098	27	1.296	72
E12	726.3	0.000	0		
F01	726.3	0.051	14	1.305	28
F02	726.3	0.114	31	1.379	68
F03	726.3	0.138	38	1.305	82
F04	724.2	0.000	0		
F05	724.2	0.000	0		
F06	724.2	0.075	21	1.346	41
F07	724.2	0.167	46	1.288	90
F08	716.2	0.113	32	1.210	66
F09	714.3	0.188	53	1.268	91
F10	714.3	0.129	36	1.259	75
F11	714.3	0.000	0		
F12	714.3	0.000	0	1.360	20
G01	712.2	0.044	12	1.300	45
G02	712.2	0.114	32	1.386	77
G03	712.2	0.000	0		
G04	712.2	0.162	46	1.215	85
G05	712.2	0.135	38	1.246	83
G06	712.2	0.143	40	1.269	78
G07	710.2	0.144	41	1.246	86
G08	700.2	0.059	17	1.208	60
G09	698.2	0.160	46	1.277	83

G10	740.3	0.201	54	1.303	93
G11	686.2	0.130	38	1.292	79
G12	766.3	0.174	45	1.431	88
H01	781.3	0.132	34	1.103	81
H02	795.3	0.109	27	1.120	74
H03	875.3	0.119	27	1.443	79
H04	835.3	0.092	22	1.340	56
H05	700.2	0.129	37	1.213	71
H06	716.2	0.167	47	1.200	91
H07	760.3	0.190	50	1.219	91
H08	497.1	0.169	68	1.517	88
H09	345.1	0.080	46	0.591	87
H10	400.1	0.000	0		
H11	248.1	0.000	0	0.465	53
H12	115.0	0.000	0		

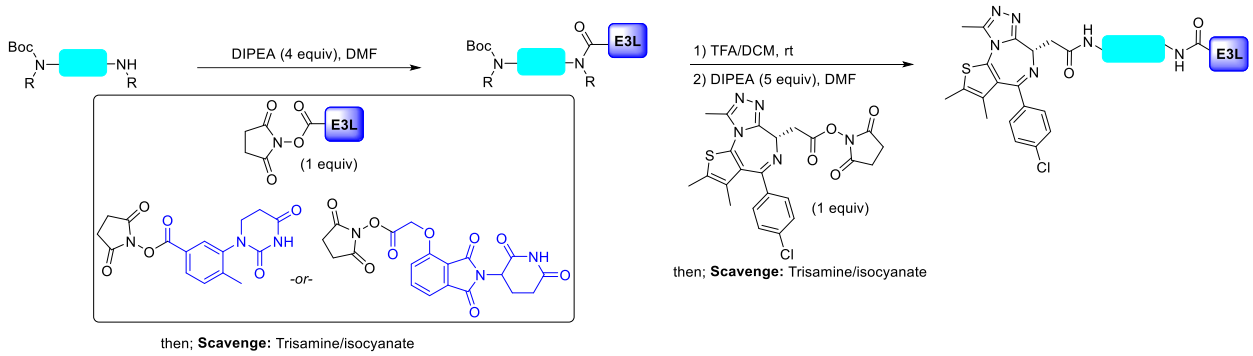
Table S9. tDHU Plate 2 CAD Results

Location	Mass Expected	Product Conc (mg/mL)	CAD Yield	Rt (min)	Purity %
A01	846.3	0.122	29	1.230	84
A02	726.3	0.000	0		
A03	821.3	0.183	44	1.243	91
A04	775.2	0.084	22	1.148	63
A05	809.3	0.146	36	1.225	77
A06	804.3	0.129	32	1.162	86
A07	804.3	0.128	32	1.156	76
A08	803.3	0.000	0		
A09	795.3	0.140	35	1.241	86
A10	794.3	0.130	33	1.580	73
A11	793.3	0.153	39	1.342	81
A12	792.2	0.219	55	1.532	92
B01	788.3	0.147	37	1.560	86
B02	728.2	0.161	44	1.254	88
B03	774.3	0.179	46	1.400	90
B04	774.3	0.000	0		
B05	774.3	0.000	0		
B06	774.2	0.084	22	1.483	71
B07	770.3	0.155	40	1.286	75
B08	768.3	0.000	0	1.113	12
B09	768.3	0.114	30	1.327	81
B10	767.3	0.122	32	1.250	76
B11	764.2	0.094	25	1.176	82
B12	764.2	0.097	25	1.236	75
C01	762.3	0.137	36	1.375	77
C02	762.2	0.048	13	1.180	42
C03	762.2	0.080	21	1.249	49
C04	761.2	0.100	26	1.215	70
C05	756.3	0.127	34	1.200	62

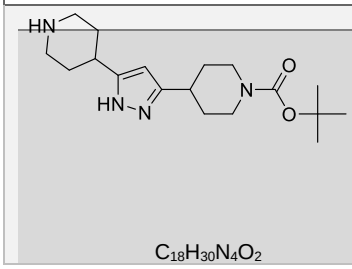
C06	755.3	0.114	30	1.134	77
C07	753.2	0.000	0		
C08	752.3	0.000	0		
C09	752.3	0.069	18	1.515	61
C10	752.3	0.165	44	1.243	5
C11	748.2	0.122	32	1.386	77
C12	748.2	0.121	32	1.539	76
D01	726.3	0.154	42	1.244	85
D02	748.2	0.074	20	1.474	67
D03	748.2	0.000	0	1.343	14
D04	748.2	0.000	0		
D05	742.2	0.124	33	1.202	55
D06	780.3	0.095	24	1.448	51
D07	756.3	0.144	38	1.495	87
D08	740.3	0.000	0	1.392	11
D09	740.2	0.093	25	1.110	68
D10	740.2	0.000	0		
D11	738.3	0.000	0		
D12	738.3	0.152	41	1.312	82
E01	738.3	0.071	19	1.366	58
E02	738.3	0.160	43	1.339	79
E03	738.3	0.102	28	1.380	68
E04	738.3	0.091	25	1.301	63
E05	730.2	0.119	33	1.242	78
E06	728.2	0.171	47	1.224	90
E07	728.2	0.117	32	1.184	75
E08	728.2	0.071	20	1.150	68
E09	727.2	0.000	0	1.134	24
E10	726.3	0.152	42	1.400	85
E11	726.3	0.091	25	1.295	75
E12	726.3	0.069	19	1.525	60
F01	726.3	0.000	0		
F02	726.3	0.129	36	1.378	77
F03	726.3	0.101	28	1.305	76
F04	724.2	0.108	30	1.242	36
F05	724.2	0.000	0		
F06	724.2	0.078	22	1.347	41
F07	724.2	0.136	37	1.289	81
F08	716.2	0.109	30	1.209	61
F09	714.3	0.139	39	1.267	92
F10	714.3	0.124	35	1.258	74
F11	714.3	0.000	0		
F12	714.3	0.000	0		
G01	712.2	0.099	28	1.300	79
G02	712.2	0.083	23	1.386	66
G03	712.2	0.155	44	1.370	60
G04	712.2	0.126	35	1.216	75
G05	712.2	0.098	28	1.246	78
G06	712.2	0.155	44	1.270	85

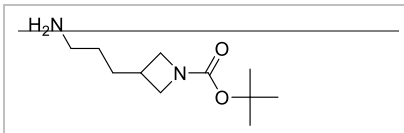
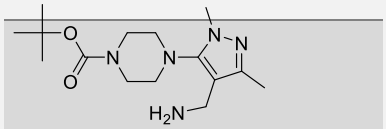
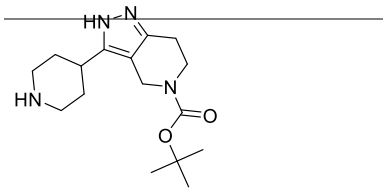
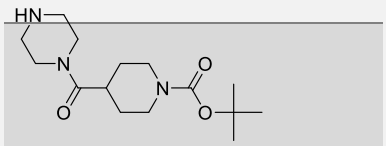
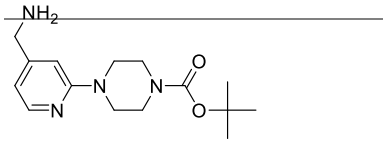
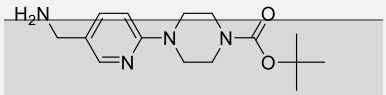
G07	710.2	0.158	44	1.246	87
G08	700.2	0.138	39	1.208	80
G09	698.2	0.146	42	1.276	86
G10	740.3	0.197	53	1.302	92
G11	686.2	0.112	33	1.292	72
G12	766.3	0.171	45	1.431	89
H01	781.3	0.194	50	1.102	80
H02	795.3	0.150	38	1.120	80
H03	875.3	0.162	37	1.442	82
H04	835.3	0.074	18	1.340	58
H05	700.2	0.124	35	1.213	85
H06	716.2	0.141	40	1.200	88
H07	760.3	0.185	49	1.219	88
H08	497.1	0.170	68	1.517	88
H09	345.1	0.076	44	0.593	87
H10	400.1	0.150	75	1.250	94
H11	248.1	0.085	69	0.364	2
H12	115.0	0.000	0		

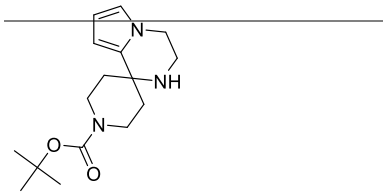
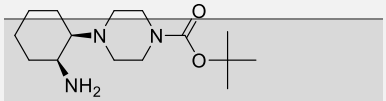
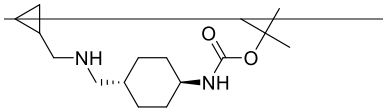
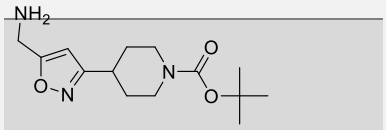
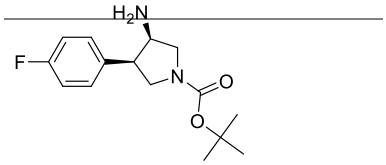
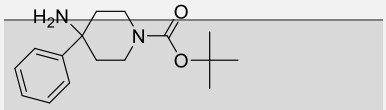
Table S10. Results of D2B Synthesis by N-Boc-diamine linker

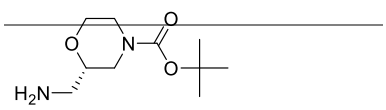
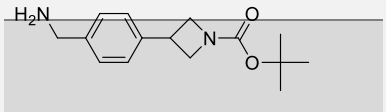
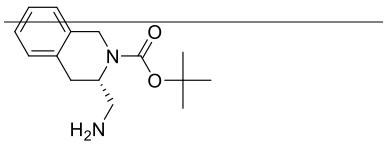
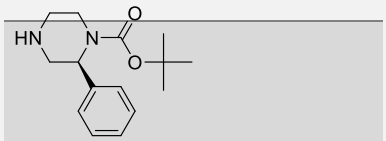
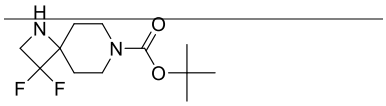
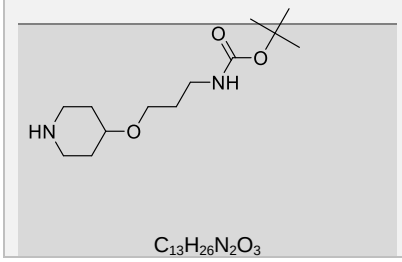


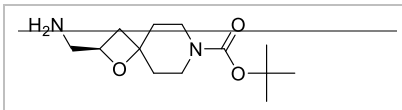
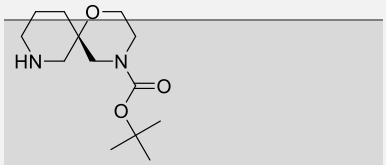
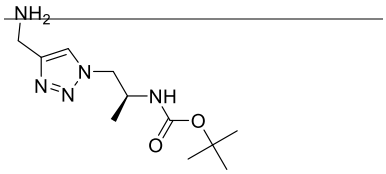
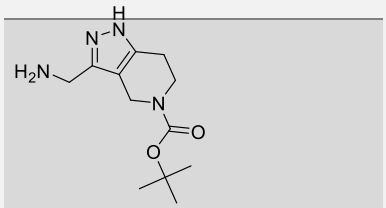
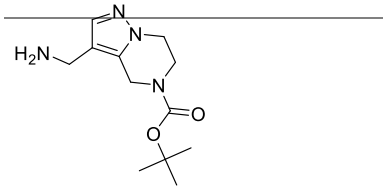
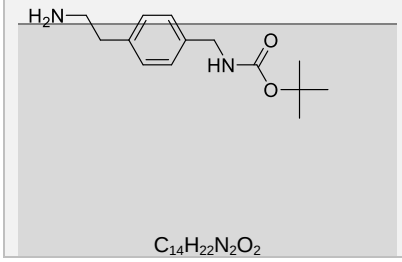
Yields as determined by CAD. Purity as determined by UV-Vis (TWC). Yields and Purity reported as decimal values.

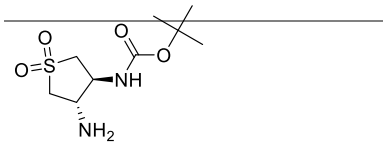
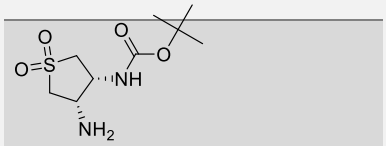
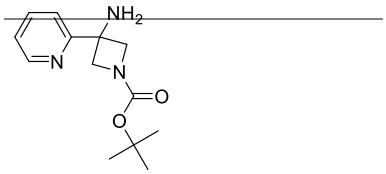
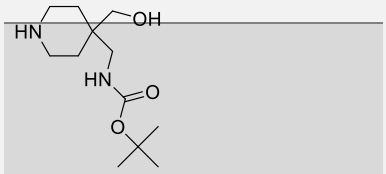
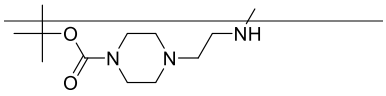
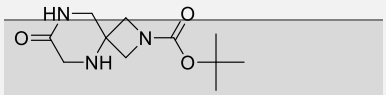
Structure	Plate ID	well location	CAS	MFCD	(Run 1) Yield	(Run 1) Purity	(Run 2) Yield	(Run 2) Purity
 $C_{18}H_{30}N_4O_2$	O-Pom	A01		MFCD29054895	0.00	0.00	0.16	0.61

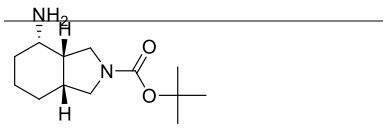
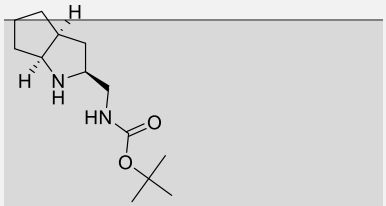
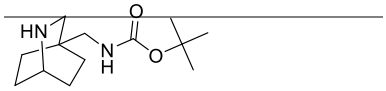
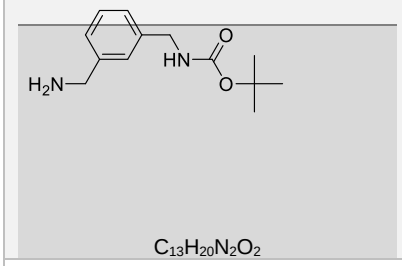
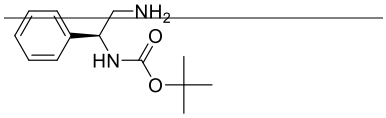
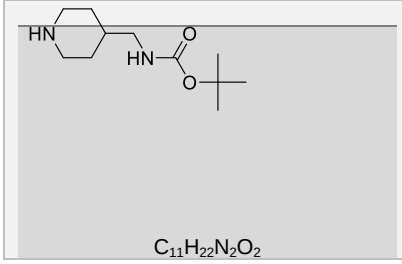
 $C_{11}H_{22}N_2O_2$	O-Pom	A02	1205750-48-6	MFCD14582364	0.00	0.00	0.00	0.00
 $C_{15}H_{27}N_5O_2$	O-Pom	A03			0.18	0.85	0.21	0.76
 $C_{16}H_{26}N_4O_2$	O-Pom	A04	2138071-17-5		0.14	0.72	0.11	0.71
 $C_{15}H_{27}N_3O_3$	O-Pom	A05	887587-18-0	MFCD07367784	0.22	0.84	0.23	0.84
 $C_{15}H_{24}N_4O_2$	O-Pom	A06	910036-87-2	MFCD09787492	0.09	0.49	0.16	0.73
 $C_{15}H_{24}N_4O_2$	O-Pom	A07	874880-91-8	MFCD08059435	0.13	0.61	0.3	0.74

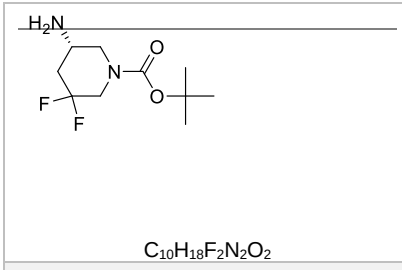
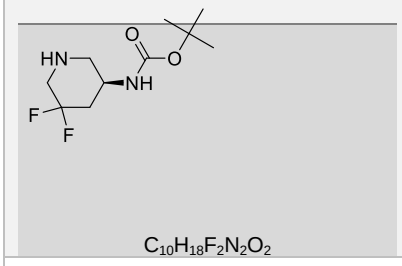
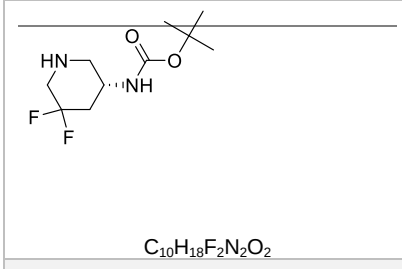
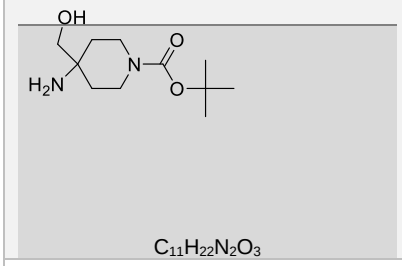
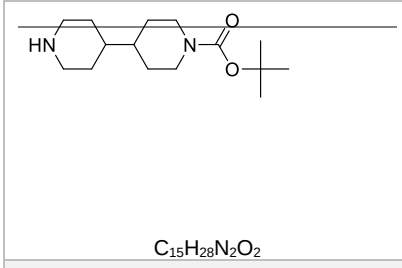
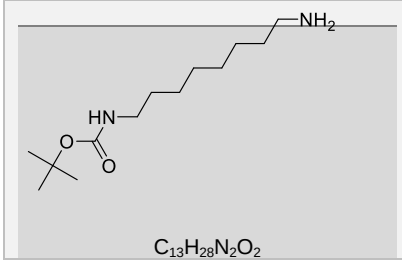
 $C_{16}H_{25}N_3O_2$	O-Pom	A08	1392466-65-7	MFCD28714538	0.00	0.00	0.31	0.75
 $C_{15}H_{29}N_3O_2$	O-Pom	A09			0.24	0.89	0.16	0.74
 $C_{16}H_{30}N_2O_2$	O-Pom	A10			0.21	0.57	0.17	0.37
 $C_{14}H_{23}N_3O_3$	O-Pom	A11	2260935-83-7		0.1	0.49	0.1	0.47
 $C_{15}H_{21}FN_2O_2$	O-Pom	A12	1218764-14-7	MFCD28502837	0.32	0.92	0.37	0.94
 $C_{16}H_{24}N_2O_2$	O-Pom	B01	1211581-86-0	MFCD22681611	0.11	0.28	0.06	0.2

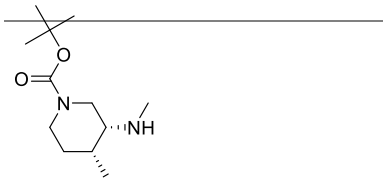
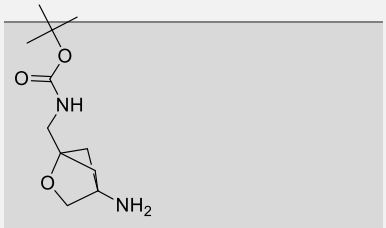
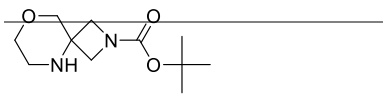
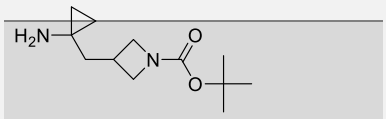
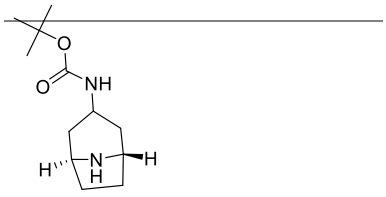
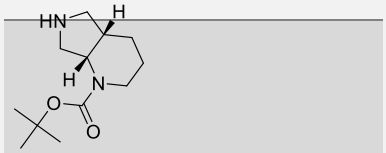
 $C_{10}H_{20}N_2O_3$	O-Pom	B02	879403-42-6	MFCD11707017	0.22	0.86	0.19	0.81
 $C_{15}H_{22}N_2O_2$	O-Pom	B03			0.17	0.47	0.19	0.59
 $C_{15}H_{22}N_2O_2$	O-Pom	B04	150417-17-7	MFCD11506157	0.00	0.00	0.00	0.12
 $C_{15}H_{22}N_2O_2$	O-Pom	B05	1240583-48-5	MFCD08685943	0.00	0.00	0.00	0.00
 $C_{12}H_{20}F_2N_2O_2$	O-Pom	B06	1935075-16-3	MFCD29042916	0.34	0.95	0.25	0.72
 $C_{13}H_{26}N_2O_3$	O-Pom	B07	1376134-81-4	MFCD22056502	0.34	0.83	0.4	0.78

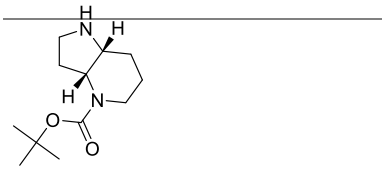
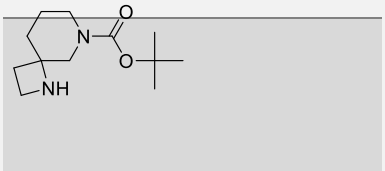
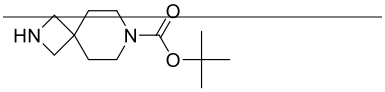
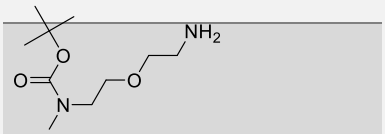
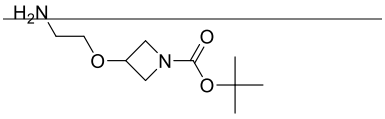
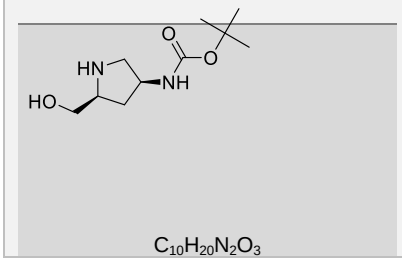
 $C_{13}H_{24}N_2O_3$	O-Pom	B08	1403766-68-6	MFCD22628730	0.00	0.12	0.03	0.00
 $C_{13}H_{24}N_2O_3$	O-Pom	B09			0.27	0.84	0.17	0.67
 $C_{11}H_{21}N_5O_2$	O-Pom	B10			0.00	0.18	0.06	0.18
 $C_{12}H_{20}N_4O_2$	O-Pom	B11	1251000-58-4	MFCD17017184	0.07	0.56	0.09	0.49
 $C_{12}H_{20}N_4O_2$	O-Pom	B12	1391733-77-9	MFCD23378346	0.00	0.00	0.00	0.00
 $C_{14}H_{22}N_2O_2$	O-Pom	C01	187283-19-8	MFCD06804563	0.24	0.79	0.2	0.61

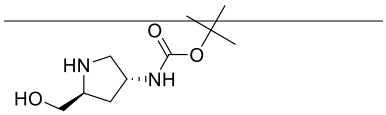
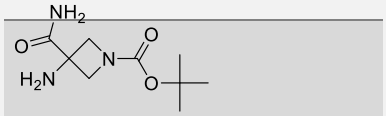
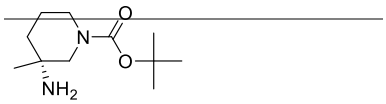
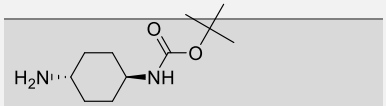
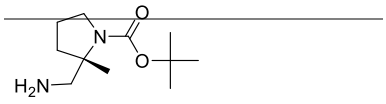
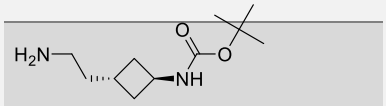
 $C_9H_{18}N_2O_4S$	O-Pom	C02		MFCD30343000	0.17	0.82	0.14	0.44
 $C_9H_{18}N_2O_4S$	O-Pom	C03		MFCD30492185	0.13	0.32	0.39	0.82
 $C_{13}H_{19}N_3O_2$	O-Pom	C04	2167677-73-6		0.35	0.91	0.33	0.9
 $C_{12}H_{24}N_2O_3$	O-Pom	C05	493026-47-4	MFCD22397604	0.2	0.59	0.11	0.31
 $C_{12}H_{25}N_3O_2$	O-Pom	C06	539822-98-5	MFCD09758985	0.34	0.92	0.46	0.92
 $C_{11}H_{19}N_3O_3$	O-Pom	C07	1839060-95-5	MFCD29043065	0.11	0.51	0.15	0.51

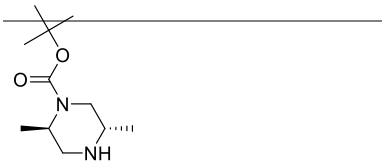
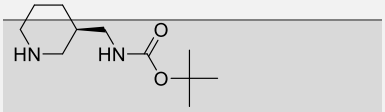
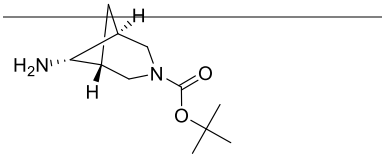
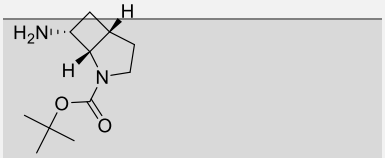
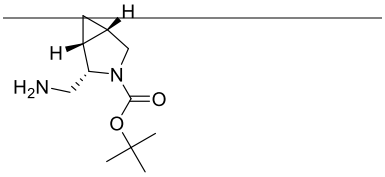
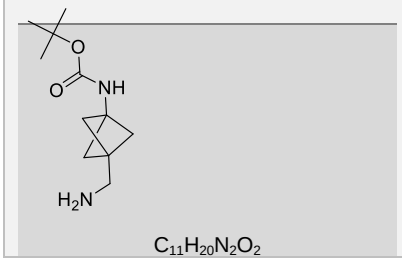
 $C_{13}H_{24}N_2O_2$	O-Pom	C08	1251001-17-8	MFCD09753917	0.47	0.54	0.53	0.92
 $C_{13}H_{24}N_2O_2$	O-Pom	C09			0.27	0.82	0.15	0.7
 $C_{13}H_{24}N_2O_2$	O-Pom	C10	2361822-74-2		0.45	0.85	0.12	0.52
 $C_{13}H_{20}N_2O_2$	O-Pom	C11	108467-99-8	MFCD01317800	0.22	0.78	0.28	0.81
 $C_{13}H_{20}N_2O_2$	O-Pom	C12	137102-30-8	MFCD09885895	0.18	0.53	0.3	0.81
 $C_{11}H_{22}N_2O_2$	O-Pom	D01	135632-53-0		0.26	0.69	0.3	0.81

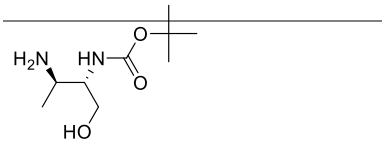
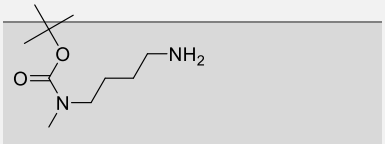
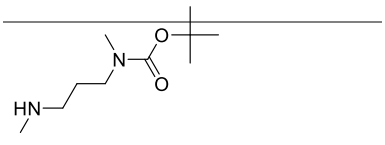
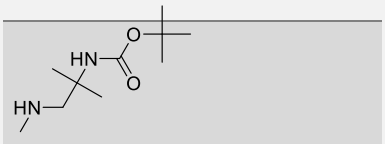
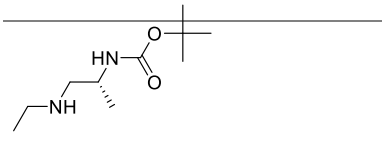
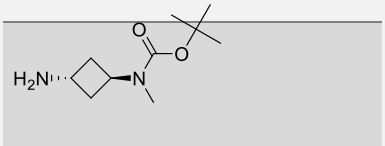
 $C_{10}H_{18}F_2N_2O_2$	O-Pom	D02	2055848-76-3	MFCD30531337	0.15	0.7	0.07	0.51
 $C_{10}H_{18}F_2N_2O_2$	O-Pom	D03	1932109-72-2	MFCD30097677	0.36	0.94	0.41	0.92
 $C_{10}H_{18}F_2N_2O_2$	O-Pom	D04	2089320-98-7		0.29	0.89	0.39	0.9
 $C_{11}H_{22}N_2O_3$	O-Pom	D05	203186-96-3	MFCD11845683	0.36	0.81	0.45	0.79
 $C_{15}H_{28}N_2O_2$	O-Pom	D06	171049-35-7	MFCD02179169	0.17	0.53	0.23	0.63
 $C_{13}H_{28}N_2O_2$	O-Pom	D07	88829-82-7	MFCD02094499	0.16	0.7	0.28	0.83

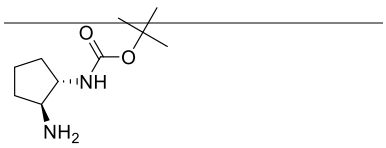
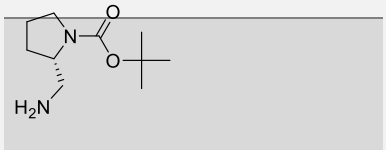
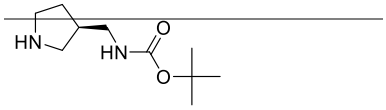
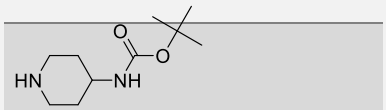
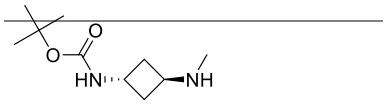
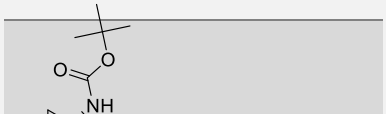
 $C_{12}H_{24}N_2O_2$	O-Pom	D08	1312762-44-9	MFCD26954785	0.08	0.37	0.03	0.29
 $C_{11}H_{20}N_2O_3$	O-Pom	D09	2241138-06-5		0.16	0.46	0.12	0.37
 $C_{11}H_{20}N_2O_3$	O-Pom	D10	1251002-01-3	MFCD14581204	0.06	0.14	0.00	0.15
 $C_{12}H_{22}N_2O_2$	O-Pom	D11	1784225-34-8		0.37	0.91	0.46	0.9
 $C_{12}H_{22}N_2O_2$	O-Pom	D12			0.35	0.89	0.00	0.00
 $C_{12}H_{22}N_2O_2$	O-Pom	E01	159991-07-8	MFCD11973767	0.00	0.00	0.00	0.00

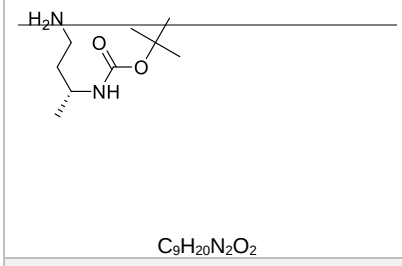
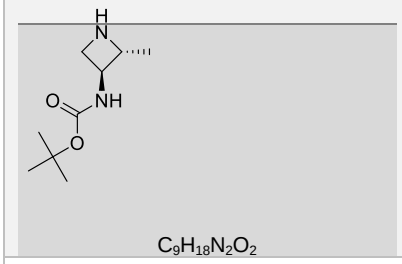
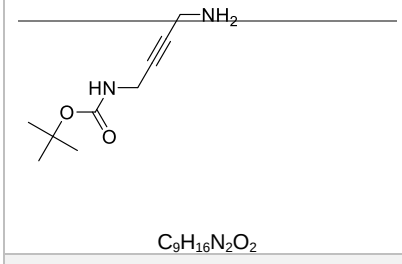
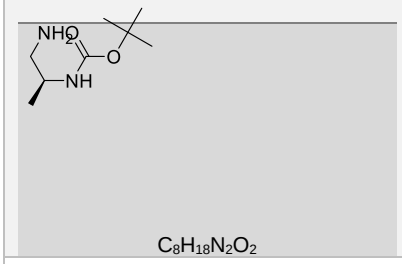
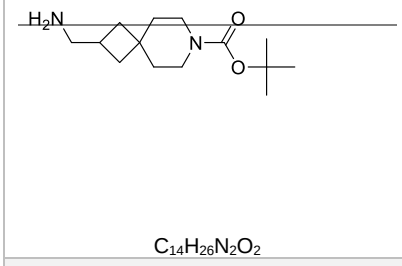
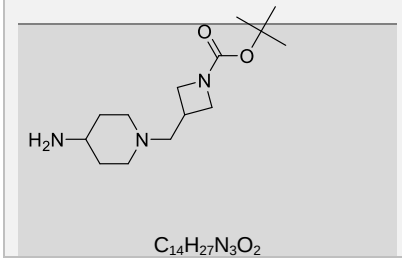
 $C_{12}H_{22}N_2O_2$	O-Pom	E02	1277168-52-1	MFCD22394610	0.07	0.46	0.05	0.48
 $C_{12}H_{22}N_2O_2$	O-Pom	E03	1251002-00-2	MFCD14581197	0.16	0.7	0.38	0.38
 $C_{12}H_{22}N_2O_2$	O-Pom	E04	896464-16-7	MFCD09839640	0.29	0.88	0.34	0.88
 $C_{10}H_{22}N_2O_3$	O-Pom	E05	436857-02-2	MFCD24465872	0.28	0.86	0.43	0.87
 $C_{10}H_{20}N_2O_3$	O-Pom	E06	898271-41-5	MFCD24637702	0.33	0.81	0.19	0.48
 $C_{10}H_{20}N_2O_3$	O-Pom	E07	663948-85-4	MFCD08704519	0.19	0.53	0.35	0.78

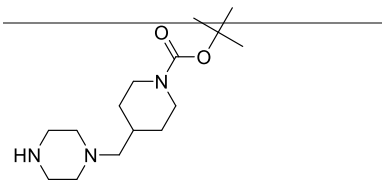
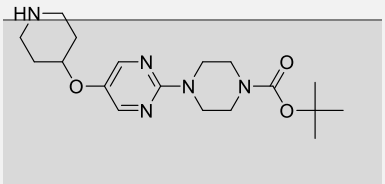
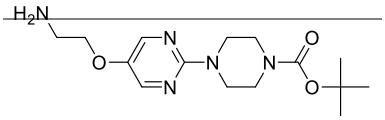
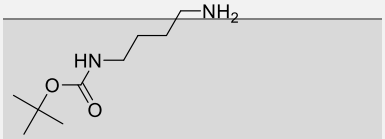
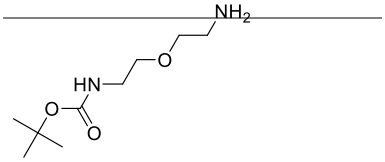
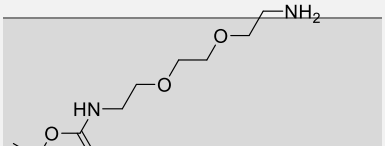
 $C_{10}H_{20}N_2O_3$	O-Pom	E08	1217975-63-7	MFCD08704528	0.29	0.78	0.35	0.78
 $C_9H_{17}N_3O_3$	O-Pom	E09	1254120-14-3	MFCD21842621	0.19	0.82	0.26	0.79
 $C_{11}H_{22}N_2O_2$	O-Pom	E10			0.39	0.97	0.18	0.66
 $C_{11}H_{22}N_2O_2$	O-Pom	E11	177906-48-8	MFCD03001719	0.24	0.84	0.3	0.76
 $C_{11}H_{22}N_2O_2$	O-Pom	E12	1408057-41-9	MFCD23106103	0.16	0.78	0.22	0.76
 $C_{11}H_{22}N_2O_2$	O-Pom	F01			0.26	0.76	0.13	0.38

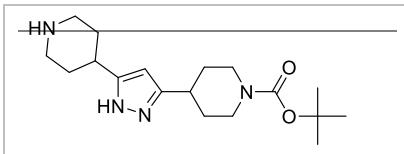
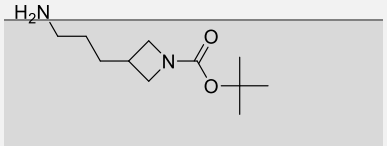
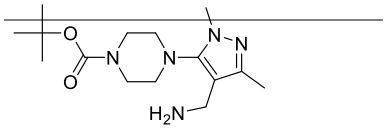
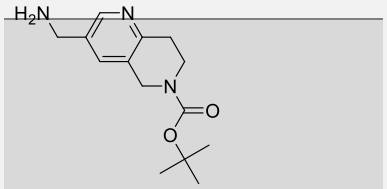
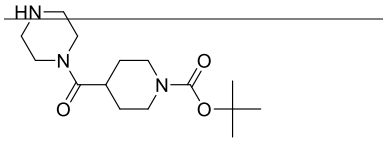
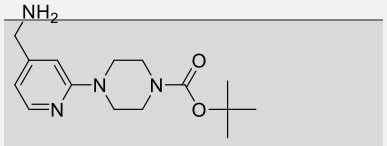
 $C_{11}H_{22}N_2O_2$	O-Pom	F02	309915-46-6	MFCD06797728	0.14	0.66	0.14	0.75
 $C_{11}H_{22}N_2O_2$	O-Pom	F03	879275-33-9	MFCD03093384	0.25	0.76	0.33	0.78
 $C_{11}H_{20}N_2O_2$	O-Pom	F04	1250997-08-0	MFCD20231336	0.53	0.96	0.35	0.25
 $C_{11}H_{20}N_2O_2$	O-Pom	F05			0.49	0.94	0.00	0.00
 $C_{11}H_{20}N_2O_2$	O-Pom	F06			0.3	0.77	0.07	0.33
 $C_{11}H_{20}N_2O_2$	O-Pom	F07	1638765-05-5	MFCD27987305	0.26	0.76	0.36	0.77

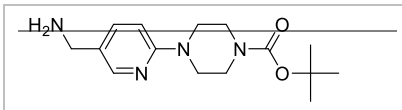
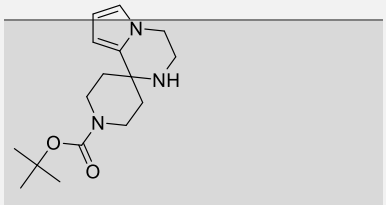
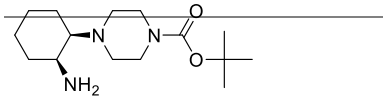
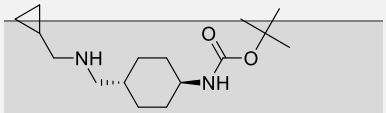
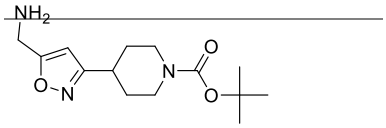
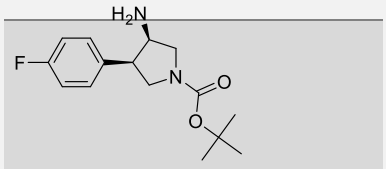
 $C_9H_{20}N_2O_3$	O-Pom	F08			0.36	0.69	0.13	0.55
 $C_{10}H_{22}N_2O_2$	O-Pom	F09	144222-23-1	MFCD03001681	0.35	0.87	0.45	0.9
 $C_{10}H_{22}N_2O_2$	O-Pom	F10	123183-72-2	MFCD14155832	0.26	0.83	0.2	0.78
 $C_{10}H_{22}N_2O_2$	O-Pom	F11	134597-95-8	MFCD14529626	0.19	0.65	0.17	0.58
 $C_{10}H_{22}N_2O_2$	O-Pom	F12	869901-70-2	MFCD18909493	0.18	0.47	0.31	0.59
 $C_{10}H_{20}N_2O_2$	O-Pom	G01	1392803-14-3	MFCD23106178	0.00	0.28	0.09	0.57

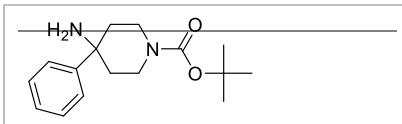
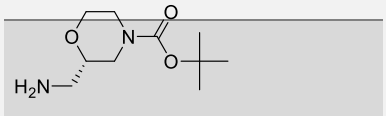
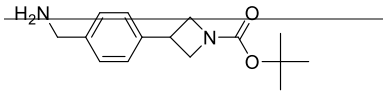
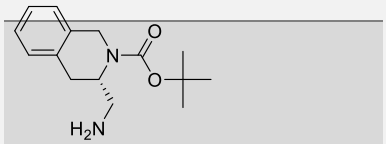
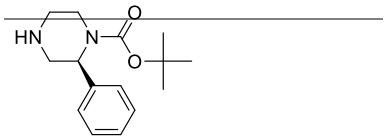
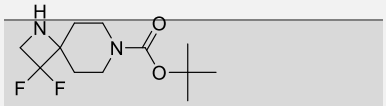
 $C_{10}H_{20}N_2O_2$	O-Pom	G02	586961-34-4	MFCD11040590	0.28	0.86	0.27	0.74
 $C_{10}H_{20}N_2O_2$	O-Pom	G03	119020-01-8	MFCD03419257	0.1	0.59	0.14	0.69
 $C_{10}H_{20}N_2O_2$	O-Pom	G04	173340-25-5	MFCD06796615	0.14	0.65	0.41	0.84
 $C_{10}H_{20}N_2O_2$	O-Pom	G05	73874-95-0	MFCD00798171	0.33	0.87	0.39	0.86
 $C_{10}H_{20}N_2O_2$	O-Pom	G06			0.37	0.95	0.42	0.9
 $C_{10}H_{18}N_2O_2$	O-Pom	G07	181941-45-7	MFCD18431936	0.3	0.87	0.45	0.89

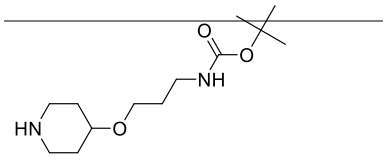
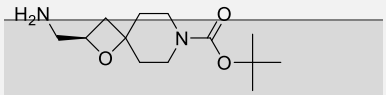
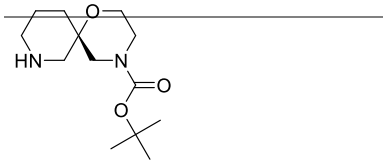
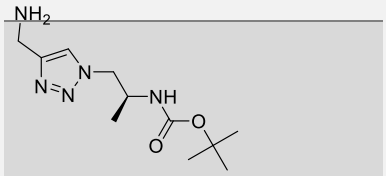
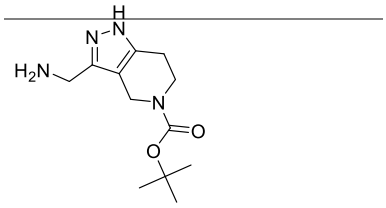
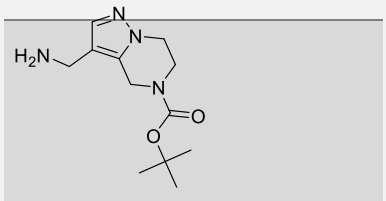
 $C_9H_{20}N_2O_2$	O-Pom	G08	170367-69-8	MFCD06804538	0.38	0.89	0.33	0.69
 $C_9H_{18}N_2O_2$	O-Pom	G09			0.18	0.48	0.26	0.68
 $C_9H_{16}N_2O_2$	O-Pom	G10		MFCD20694702	0.16	0.59	0.2	0.64
 $C_8H_{18}N_2O_2$	O-Pom	G11	146552-71-8	MFCD08726031	0.17	0.63	0.16	0.49
 $C_{14}H_{26}N_2O_2$	O-Pom	G12	1160247-15-3		0.00	0.00	0.00	0.00
 $C_{14}H_{27}N_3O_2$	O-Pom	H01			0.32	0.87	0.41	0.77

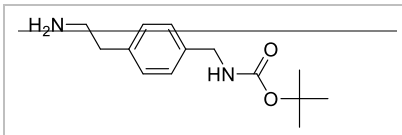
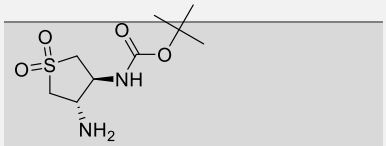
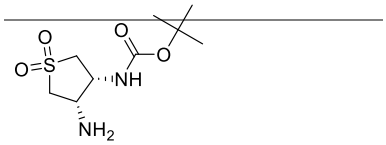
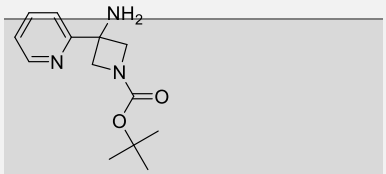
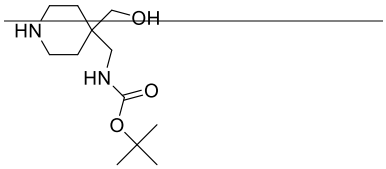
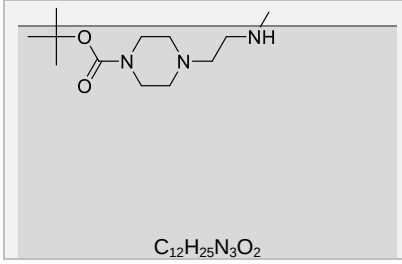
 $C_{15}H_{29}N_3O_2$	O-Pom	H02	381722-48-1		0.08	0.31	0.14	0.51
 $C_{18}H_{29}N_5O_3$	O-Pom	H03			0.17	0.37	0.13	0.73
 $C_{15}H_{25}N_5O_3$	O-Pom	H04			0.1	0.39	0.11	0.55
 $C_9H_{20}N_2O_2$	O-Pom	H05	68076-36-8	MFCD00210019	0.00	0.00	0.24	0.63
 $C_9H_{20}N_2O_3$	O-Pom	H06	127828-22-2	MFCD12031501	0.00	0.11	0.06	0.23
 $C_{11}H_{24}N_2O_4$	O-Pom	H07	153086-78-3	MFCD03788155	0.23	0.66	0.29	0.74

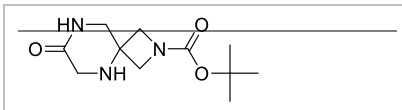
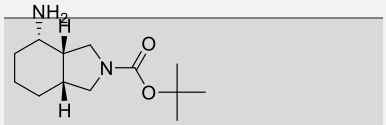
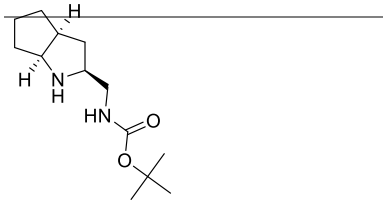
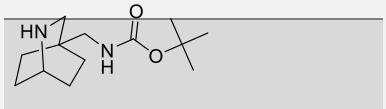
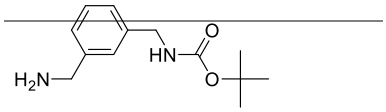
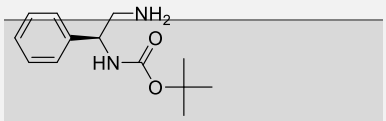
 $C_{18}H_{30}N_4O_2$	tDHU	A01		MFCD29054895	0.24	0.79	0.29	0.84
 $C_{11}H_{22}N_2O_2$	tDHU	A02	1205750-48-6	MFCD14582364	0.00	0.00	0.00	0.00
 $C_{15}H_{27}N_5O_2$	tDHU	A03			0.51	0.91	0.45	0.92
 $C_{14}H_{21}N_3O_2$	tDHU	A04			0.33	0.86	0.22	0.63
 $C_{15}H_{27}N_3O_3$	tDHU	A05	887587-18-0	MFCD07367784	0.39	0.77	0.36	0.78
 $C_{15}H_{24}N_4O_2$	tDHU	A06	910036-87-2	MFCD09787492	0.27	0.79	0.32	0.85

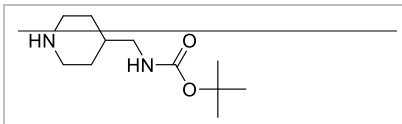
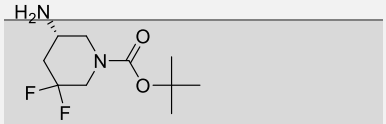
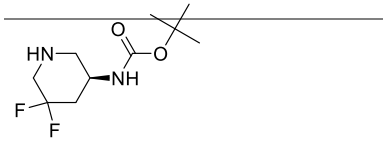
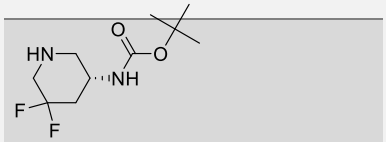
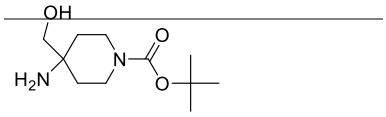
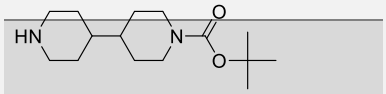
 $C_{15}H_{24}N_4O_2$	tDHU	A07	874880-91-8	MFCD08059435	0.34	0.87	0.32	0.78
 $C_{16}H_{25}N_3O_2$	tDHU	A08	1392466-65-7	MFCD28714538	0.00	0.00	0.00	0.00
 $C_{15}H_{29}N_3O_2$	tDHU	A09			0.36	0.84	0.35	0.86
 $C_{16}H_{30}N_2O_2$	tDHU	A10			0.34	0.78	0.33	0.81
 $C_{14}H_{23}N_3O_3$	tDHU	A11	2260935-83-7		0.28	0.64	0.39	0.82
 $C_{15}H_{21}FN_2O_2$	tDHU	A12	1218764-14-7	MFCD28502837	0.00	0.86	0.56	0.91

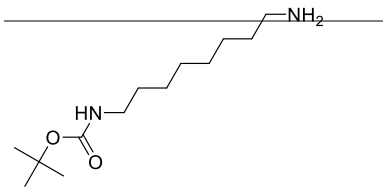
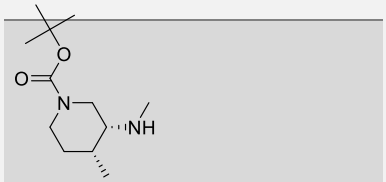
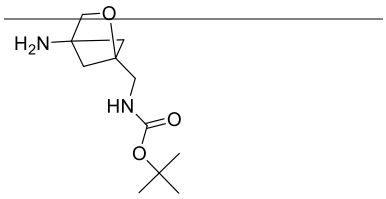
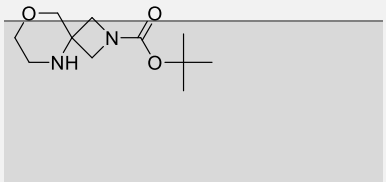
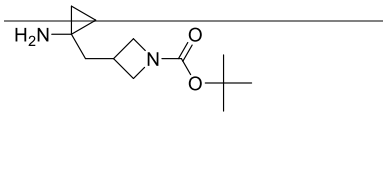
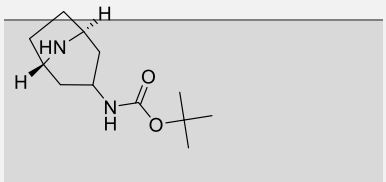
 $C_{16}H_{24}N_2O_2$	tDHU	B01	1211581-86-0	MFCD22681611	0.37	0.8	0.37	0.86
 $C_{10}H_{20}N_2O_3$	tDHU	B02	879403-42-6	MFCD11707017	0.49	0.92	0.44	0.9
 $C_{15}H_{22}N_2O_2$	tDHU	B03			0.52	0.93	0.46	0.9
 $C_{15}H_{22}N_2O_2$	tDHU	B04	150417-17-7	MFCD11506157	0.24	0.59	0.00	0.23
 $C_{15}H_{22}N_2O_2$	tDHU	B05	1240583-48-5	MFCD08685943	0.00	0.00	0.00	0.00
 $C_{12}H_{20}F_2N_2O_2$	tDHU	B06	1935075-16-3	MFCD29042916	0.25	0.78	0.22	0.71

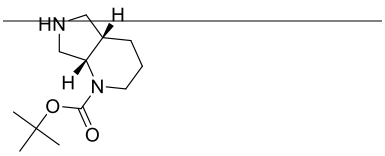
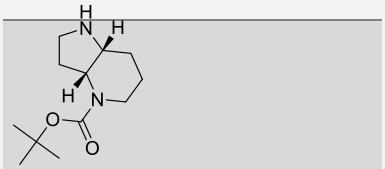
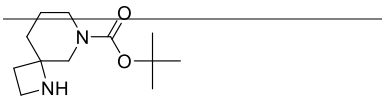
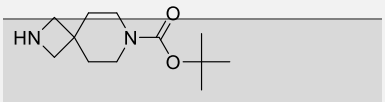
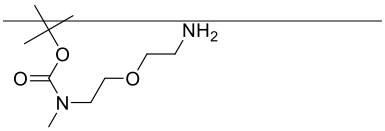
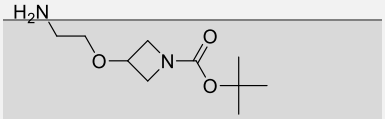
 $C_{13}H_{26}N_2O_3$	tDHU	B07	1376134-81-4	MFCD22056502	0.43	0.81	0.4	0.77
 $C_{13}H_{24}N_2O_3$	tDHU	B08	1403766-68-6	MFCD22628730	0.00	0.00	0.00	0.00
 $C_{13}H_{24}N_2O_3$	tDHU	B09			0.48	0.86	0.3	0.82
 $C_{11}H_{21}N_5O_2$	tDHU	B10			0.23	0.56	0.32	0.77
 $C_{12}H_{20}N_4O_2$	tDHU	B11	1251000-58-4	MFCD17017184	0.28	0.86	0.25	0.81
 $C_{12}H_{20}N_4O_2$	tDHU	B12	1391733-77-9	MFCD23378346	0.27	0.61	0.25	0.75

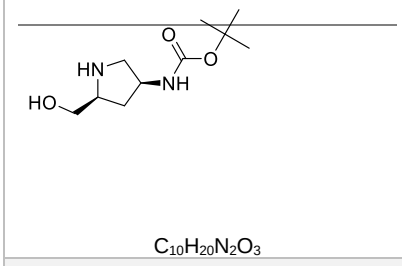
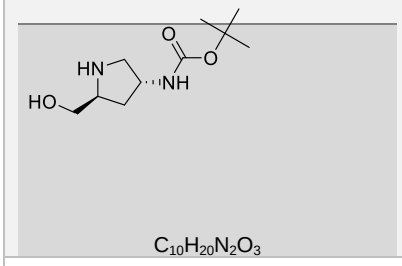
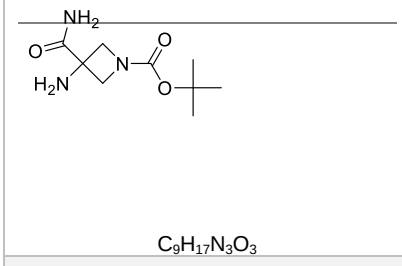
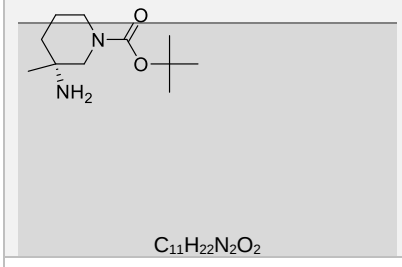
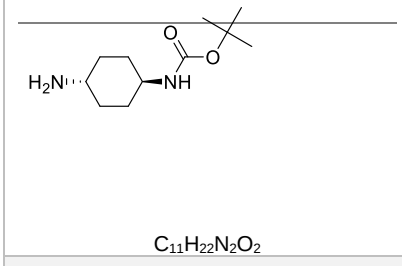
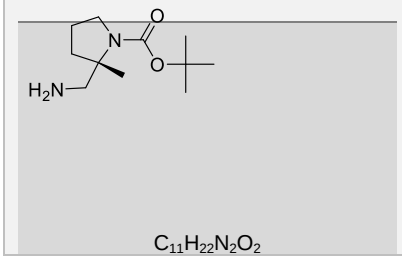
 $C_{14}H_{22}N_2O_2$	tDHU	C01	187283-19-8	MFCD06804563	0.23	0.49	0.36	0.78
 $C_9H_{18}N_2O_4S$	tDHU	C02		MFCD30343000	0.17	0.77	0.12	0.71
 $C_9H_{18}N_2O_4S$	tDHU	C03		MFCD30492185	0.14	0.67	0.21	0.86
 $C_{13}H_{19}N_3O_2$	tDHU	C04	2167677-73-6		0.34	0.78	0.26	0.68
 $C_{12}H_{24}N_2O_3$	tDHU	C05	493026-47-4	MFCD22397604	0.3	0.71	0.34	0.61
 $C_{12}H_{25}N_3O_2$	tDHU	C06	539822-98-5	MFCD09758985	0.27	0.77	0.3	0.77

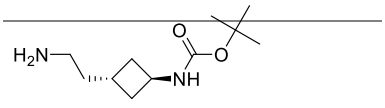
 $C_{11}H_{19}N_3O_3$	tDHU	C07	1839060-95-5	MFCD29043065	0.00	0.00	0.00	0.00
 $C_{13}H_{24}N_2O_2$	tDHU	C08	1251001-17-8	MFCD09753917	0.22	0.76	0.27	0.88
 $C_{13}H_{24}N_2O_2$	tDHU	C09			0.34	0.7	0.18	0.61
 $C_{13}H_{24}N_2O_2$	tDHU	C10	2361822-74-2		0.37	0.77	0.44	0.82
 $C_{13}H_{20}N_2O_2$	tDHU	C11	108467-99-8	MFCD01317800	0.33	0.73	0.33	0.75
 $C_{13}H_{20}N_2O_2$	tDHU	C12	137102-30-8	MFCD09885895	0.15	0.58	0.32	0.76

 $C_{11}H_{22}N_2O_2$	tDHU	D01	135632-53-0	MFCD01631214	0.46	0.85	0.42	0.85
 $C_{10}H_{18}F_2N_2O_2$	tDHU	D02	2055848-76-3	MFCD30531337	0.35	0.75	0.2	0.69
 $C_{10}H_{18}F_2N_2O_2$	tDHU	D03	1932109-72-2	MFCD30097677	0.00	0.00	0.05	0.14
 $C_{10}H_{18}F_2N_2O_2$	tDHU	D04	2089320-98-7		0.00	0.00	0.00	0.00
 $C_{11}H_{22}N_2O_3$	tDHU	D05	203186-96-3	MFCD11845683	0.24	0.43	0.33	0.56
 $C_{15}H_{28}N_2O_2$	tDHU	D06	171049-35-7	MFCD02179169	0.2	0.48	0.24	0.51

 $C_{13}H_{28}N_2O_2$	tDHU	D07	88829-82-7	MFCD02094499	0.41	0.89	0.38	0.86
 $C_{12}H_{24}N_2O_2$	tDHU	D08	1312762-44-9	MFCD26954785	0.00	0.00	0.00	0.00
 $C_{11}H_{20}N_2O_3$	tDHU	D09	2241138-06-5		0.1	0.37	0.26	0.68
 $C_{11}H_{20}N_2O_3$	tDHU	D10	1251002-01-3	MFCD14581204	0.00	0.00	0.00	0.00
 $C_{12}H_{22}N_2O_2$	tDHU	D11	1784225-34-8		0.2	0.67	0.06	0.19
 $C_{12}H_{22}N_2O_2$	tDHU	D12			0.37	0.84	0.41	0.83

 $C_{12}H_{22}N_2O_2$	tDHU	E01	159991-07-8	MFCD11973767	0.31	0.7	0.19	0.56
 $C_{12}H_{22}N_2O_2$	tDHU	E02	1277168-52-1	MFCD22394610	0.37	0.8	0.44	0.77
 $C_{12}H_{22}N_2O_2$	tDHU	E03	1251002-00-2	MFCD14581197	0.28	0.68	0.28	0.68
 $C_{12}H_{22}N_2O_2$	tDHU	E04	896464-16-7	MFCD09839640	0.37	0.75	0.25	0.63
 $C_{10}H_{22}N_2O_3$	tDHU	E05	436857-02-2	MFCD24465872	0.41	0.84	0.33	0.79
 $C_{10}H_{20}N_2O_3$	tDHU	E06	898271-41-5	MFCD24637702	0.42	0.89	0.47	0.91

 $C_{10}H_{20}N_2O_3$	tDHU	E07	663948-85-4	MFCD08704519	0.25	0.67	0.32	0.74
 $C_{10}H_{20}N_2O_3$	tDHU	E08	1217975-63-7	MFCD08704528	0.25	0.7	0.19	0.67
 $C_9H_{17}N_3O_3$	tDHU	E09	1254120-14-3	MFCD21842621	0.08	0.36	0.03	0.24
 $C_{11}H_{22}N_2O_2$	tDHU	E10			0.56	0.95	0.42	0.86
 $C_{11}H_{22}N_2O_2$	tDHU	E11	177906-48-8	MFCD03001719	0.27	0.72	0.25	0.75
 $C_{11}H_{22}N_2O_2$	tDHU	E12	1408057-41-9	MFCD23106103	0.00	0.00	0.19	0.61

 $C_{11}H_{22}N_2O_2$	tDHU	F01			0.14	0.28	0.00	0.00
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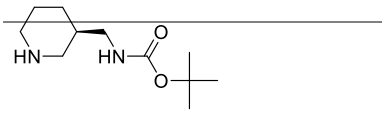
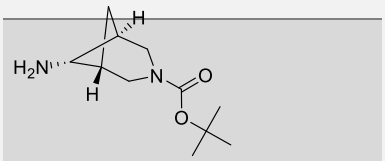
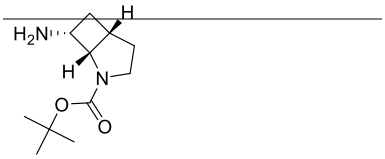
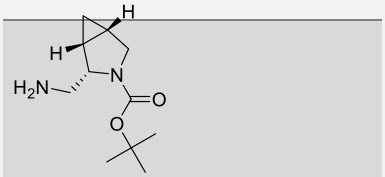
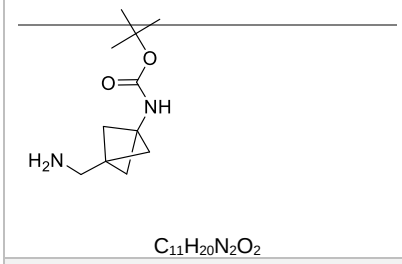
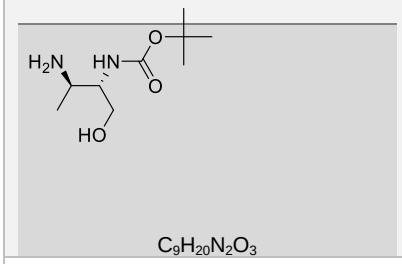
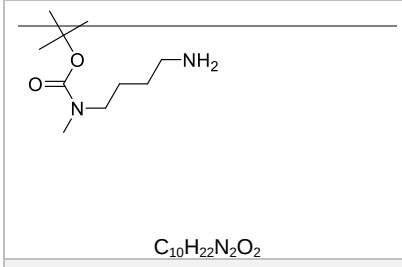
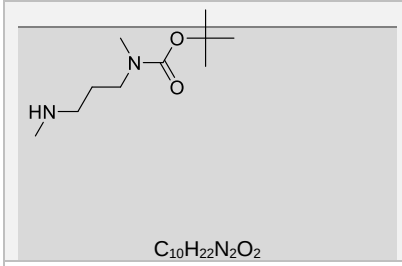
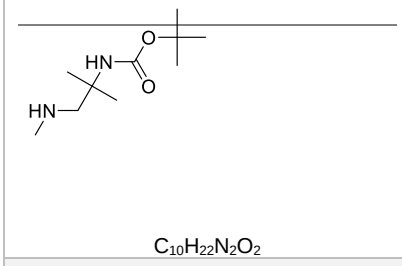
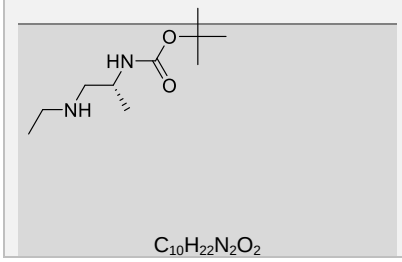
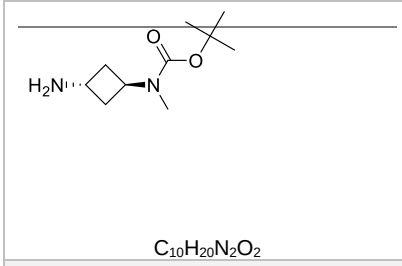
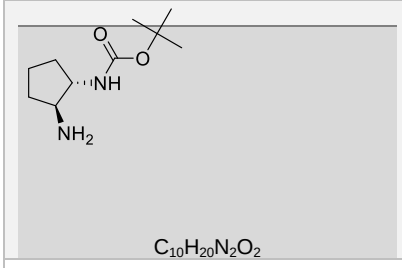
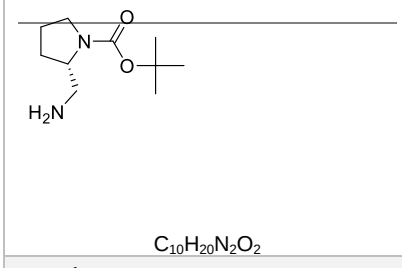
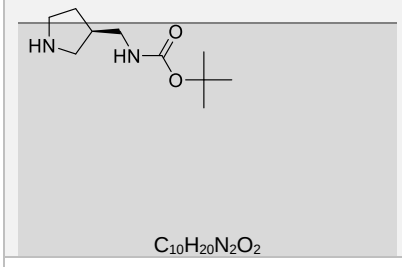
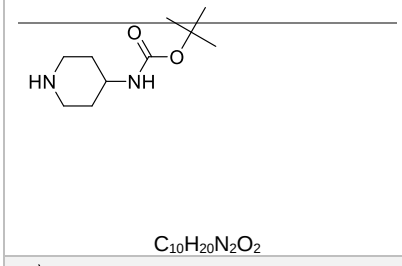
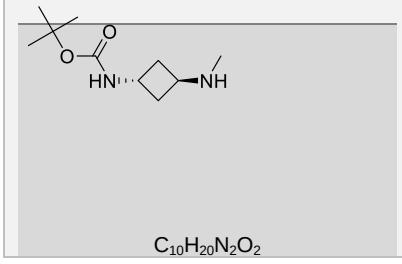
 $C_{11}H_{22}N_2O_2$	tDHU	F03	879275-33-9	MFCD03093384	0.38	0.82	0.28	0.73
 $C_{11}H_{20}N_2O_2$	tDHU	F04	1250997-08-0	MFCD20231336	0.2	0.76	0.3	0.85
 $C_{11}H_{20}N_2O_2$	tDHU	F05			0.81	0.89	0.00	0.89
 $C_{11}H_{20}N_2O_2$	tDHU	F06			0.21	0.85	0.22	0.88

 $C_{11}H_{20}N_2O_2$	tDHU	F07	1638765-05-5	MFCD27987305	0.46	0.9	0.38	0.82
 $C_9H_{20}N_2O_3$	tDHU	F08			0.32	0.66	0.31	0.61
 $C_{10}H_{22}N_2O_2$	tDHU	F09	144222-23-1	MFCD03001681	0.53	0.91	0.39	0.91
 $C_{10}H_{22}N_2O_2$	tDHU	F10	123183-72-2	MFCD14155832	0.36	0.75	0.35	0.74
 $C_{10}H_{22}N_2O_2$	tDHU	F11	134597-95-8	MFCD14529626	0.04	0.18	0.00	0.00
 $C_{10}H_{22}N_2O_2$	tDHU	F12	869901-70-2	MFCD18909493	0.32	0.69	0.32	0.39

 $C_{10}H_{20}N_2O_2$	tDHU	G01	1392803-14-3	MFCD23106178	0.12	0.45	0.28	0.75
 $C_{10}H_{20}N_2O_2$	tDHU	G02	586961-34-4	MFCD11040590	0.32	0.77	0.23	0.65
 $C_{10}H_{20}N_2O_2$	tDHU	G03	119020-01-8	MFCD03419257	0.25	0.48	0.44	0.59
 $C_{10}H_{20}N_2O_2$	tDHU	G04	173340-25-5	MFCD06796615	0.46	0.85	0.36	0.75
 $C_{10}H_{20}N_2O_2$	tDHU	G05	73874-95-0	MFCD00798171	0.38	0.83	0.28	0.78
 $C_{10}H_{20}N_2O_2$	tDHU	G06			0.4	0.78	0.44	0.84

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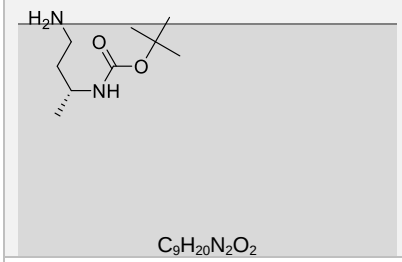
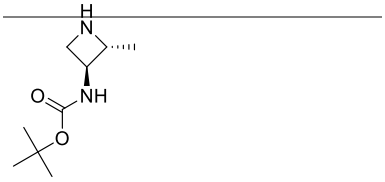
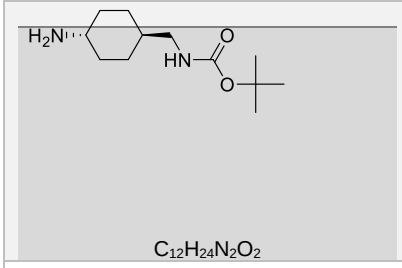
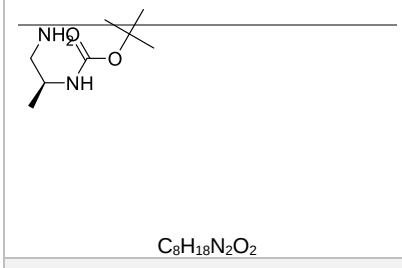
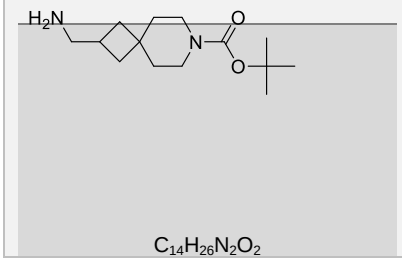
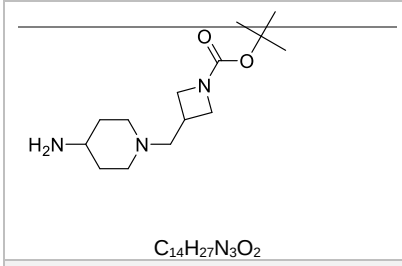
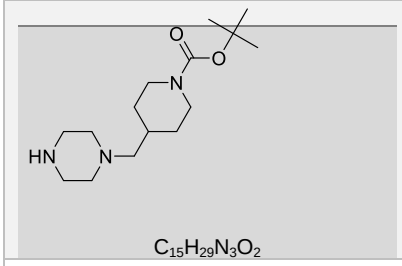
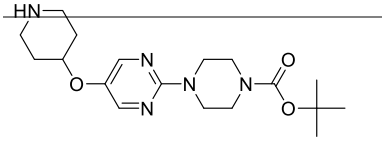
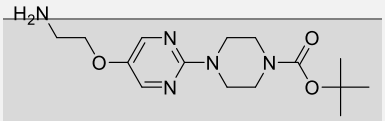
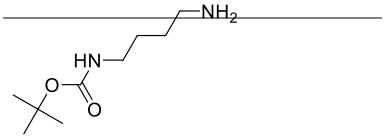
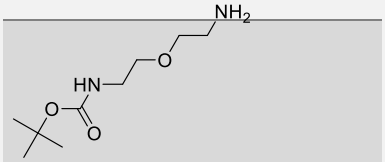
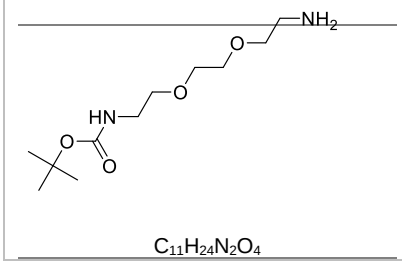
 $C_9H_{20}N_2O_2$	tDHU	G08	170367-69-8	MFCD06804538	0.17	0.6	0.4	0.82
 $C_9H_{18}N_2O_2$	tDHU	G09			0.46	0.83	0.42	0.85
 $C_{12}H_{24}N_2O_2$	tDHU	G10	192323-07-2	MFCD06657670	0.54	0.93	0.53	0.92
 $C_8H_{18}N_2O_2$	tDHU	G11	146552-71-8	MFCD08726031	0.38	0.79	0.33	0.73
 $C_{14}H_{26}N_2O_2$	tDHU	G12	1160247-15-3	MFCD12198554	0.45	0.88	0.45	0.92

 $C_{14}H_{27}N_3O_2$	tDHU	H01			0.34	0.81	0.5	0.8
 $C_{15}H_{29}N_3O_2$	tDHU	H02	381722-48-1		0.28	0.74	0.38	0.79
 $C_{18}H_{29}N_5O_3$	tDHU	H03			0.27	0.79	0.37	0.83
 $C_{15}H_{25}N_5O_3$	tDHU	H04			0.22	0.56	0.18	0.58
 $C_9H_{20}N_2O_2$	tDHU	H05	68076-36-8	MFCD00210019	0.37	0.71	0.35	0.85
 $C_9H_{20}N_2O_3$	tDHU	H06	127828-22-2	MFCD12031501	0.47	0.91	0.4	0.87

 $C_{11}H_{24}N_2O_4$	tDHU	H07	153086-78-3	MFCD03788155	0.5	0.91	0.49	0.89