Supplementary Information for

Implementation of permeation rules leads to a FabI inhibitor with activity against Gram-negative pathogens

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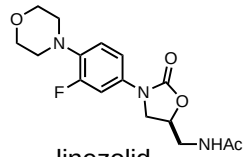
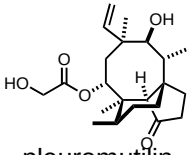
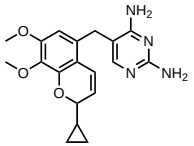
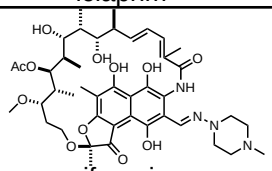
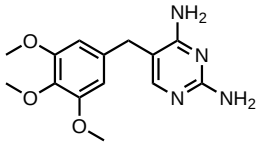
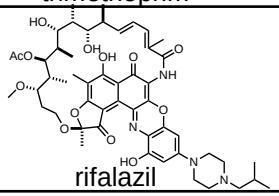
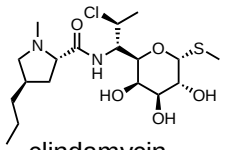
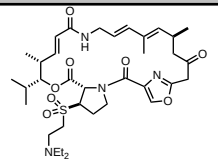
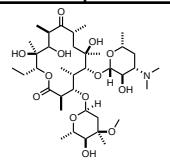
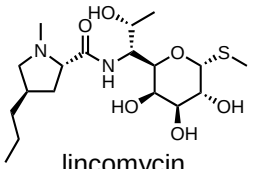
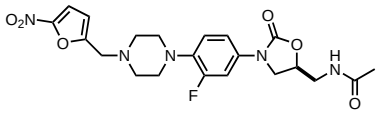
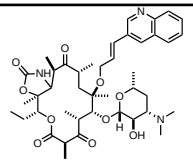
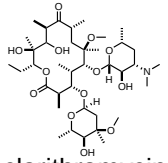
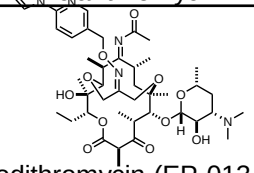
Table of Contents

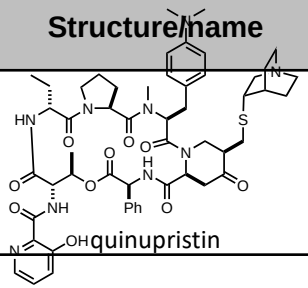
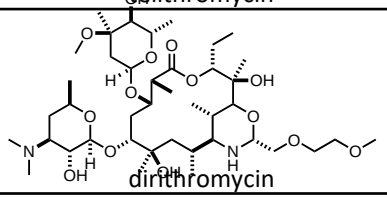
Supplementary Table 1. Structures and physicochemical properties of candidates for conversion. SMILES strings were generated using Open Babel with canonicalized atom order. If known, the target of each antibiotic was included. If available, PDB accession codes are included for co-crystal structures of antibiotic bound to protein target. * indicates PDB accession for close analogue.

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 linezolid	Approved	4	0.058	Yes	50S ribosome	PDB: 3CPW, 3DLL, 4WFA
 pleuromutilin	Approved	4	0.370	Yes	50S ribosome	PDB: 1XBP, 2OGM, 2OGN, 2OGO
 iclaprim	Approved	5	0.166	Yes	DHFR	PDB: 3FRA, 3FRF
 rifampin	Approved	5	0.367	Yes	RNA polymerase	PDB: 6CCV, 5UAC, 4KMU
 trimethoprim	Approved	5	0.154	Yes	DHFR	PDB: 2W9H, 3FRE
 rifalazil	Phase 3, Terminated	6	0.337	Yes	RNA polymerase	none
 clindamycin	Approved	7	0.157	Yes	50S ribosome	PDB: 1JZX, 4V7V

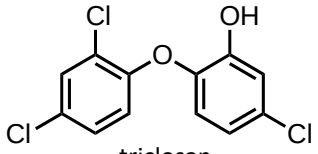
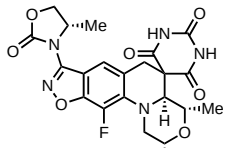
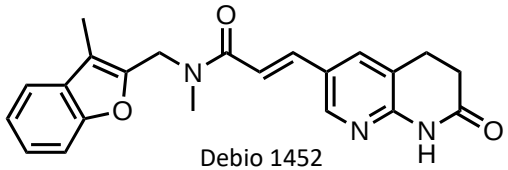
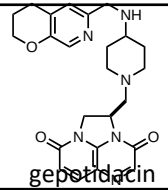
*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 dalfopristin	Approved	7	0.384	Yes	50S ribosome	PDB: 4U24, 4U26
 erythromycin	Approved	7	0.283	Yes	50S ribosome	PDB: 4V7U
 lincomycin	Approved	7	0.213	Yes	50S ribosome	PDB: 5HKV
 ranbezolid	Phase 1	7	0.115	Yes	50S ribosome	none
 cethromycin	Approved	8	0.263	Yes	50S ribosome	PDB: 1NWX
 clarithromycin	Approved	8	0.200	Yes	50S ribosome	PDB: 1J5A
 Modithromycin (EP-013420)	Phase 1	8	0.204			none

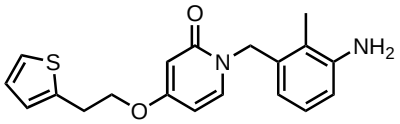
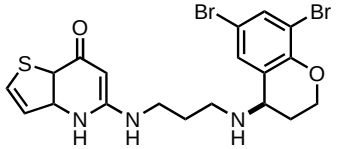
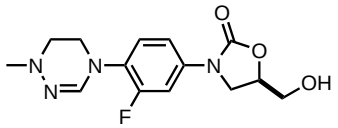
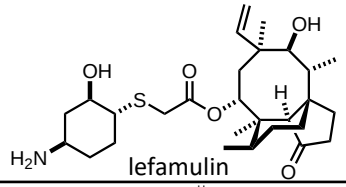
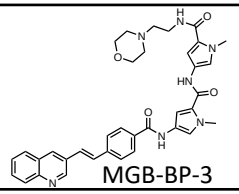
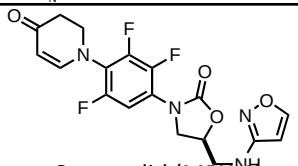
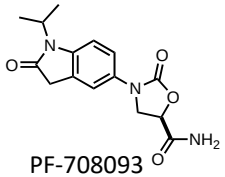
*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 quinupristin	Approved	10	0.364		50S ribosome	PDB: 1SM1, 1YJW, 4U1U, 4U26
Structure not shown telithromycin	Approved	11	0.335			PDB: 4V7S, 4WF9
 dirithromycin	Approved	12	0.290			none
Structure not shown roxithromycin	Approved	13	0.280	Yes	50S ribosome	PDB: 1JZZ
Structure not shown vancomycin	Approved	13	0.281	Yes	D-ala-D-ala	PDB: 1FVM, 3RUN
Structure not shown oritavancin	Approved	19	0.299	Yes	D-ala-D-ala	none
Structure not shown teicoplanin	Approved	19	0.287		D-ala-D-ala	none

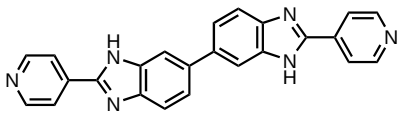
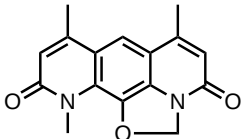
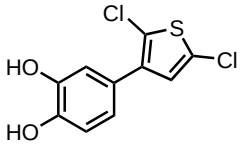
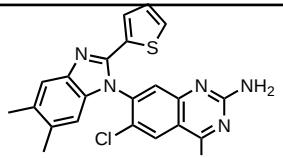
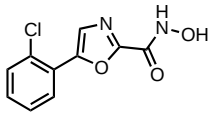
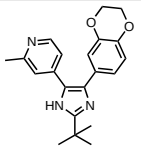
*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
dalbavancin	Approved	22	0.331	Yes	D-ala-D-ala	PDB: 3RUL
telavancin	Approved	30	0.298	Yes	D-ala-D-ala	none
daptomycin	Approved	35	0.380	Limited	Cell membrane	none
 triclosan	Approved	2	0.069	Yes	FabI	PDB: 1QSG, 4ALI
 zoliflodacin	Phase 3	1	0.087	Yes	DNA gyrase	PDB: 5CDM*
 Debio 1452	Phase 2	4	0.093	Yes	FabI	PDB: 4JQC, 4FS3
 gepotidacin	Phase 2	5	0.115	Yes	DNA gyrase	PDB: 5IWI,* 2XCS*

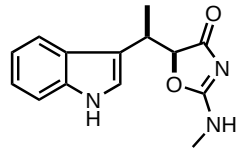
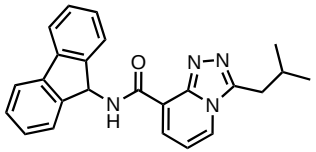
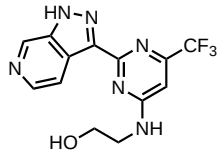
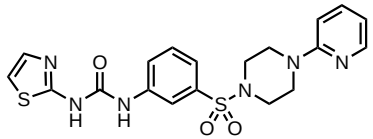
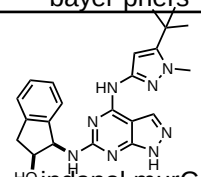
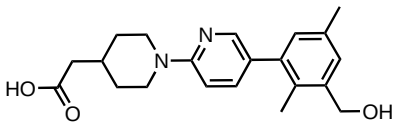
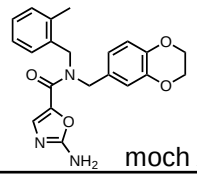
*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 CG400549	Phase 2	6	0.110	Yes	FabI	PDB: 4CV1, 4CV2
 CRS3123 (REP3123)	Phase 1	6	0.307	Yes	MetRS	PDB: 4ZT6,* 4ZT7*
 Delpazolid (LCB01-0371)	Phase 1	3	0.046	Yes	50S ribosome	none
 lefamulin	Phase 3	6	0.266	Yes	50S ribosome	PDB: 5HL7
 MGB-BP-3	Phase 1	10	0.145	Limited	DNA minor groove	none
 Contezolid (MRX-1)	Phase 2	5	0.059	Yes	50S ribosome	none
 PF-708093	Phase 1	3	0.069	Yes	50S ribosome	none

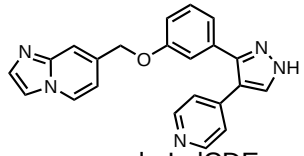
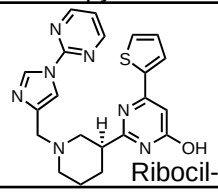
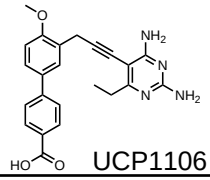
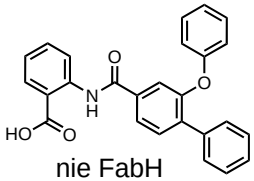
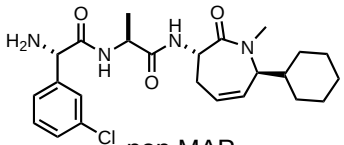
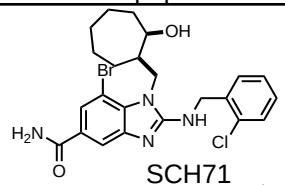
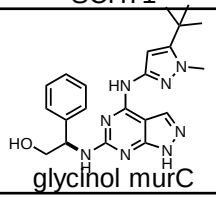
*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
Not shown ramoplanin	Phase 2	35	0.546	No	Lipid II	CSD: 729786
 Ridinilazole (SMT19969)	Phase 2	3	0.020	No	No	none
 DNM	Preclinical	0	0.024	Yes	Yes DNA gyrase	none
 cat MAP	Preclinical	1	0.057	Yes	Methionine aminopeptidase	PDB: 3D27*
 diaminoquinazoline antifolate	Preclinical	2	0.118	Yes	Yes DHFR	PDB: 4LAE
 HA7	Preclinical	2	0.021	Yes	Methionine aminopeptidase	PDB: 4A6W
 imidazole LolCDE	Preclinical	3	0.088	Limited	Yes LolCDE	none

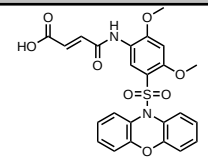
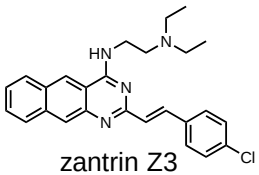
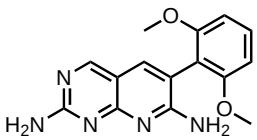
*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 indolmycin	Preclinical	3	0.188	Yes	TrpRS	PDB: 5DK4
 MRL-436	Preclinical	4	0.171	No	RNAP	none
 6-azaindazole	Preclinical	5	0.042	Limited	DNA ligase	PDB: 4CC6*
 bayer phers	Preclinical	5	0.136	Yes	PheRS	PDB: 4P73
 indanol murC	Preclinical	5	0.100	Yes	MurC	none
 mckinney fabH	Preclinical	5	0.057	Yes	FabH	PDB: 5BQS*
 moch AccC	Preclinical	5	0.180	Limited	AccC	PDB: 2W6N*

*structure of analog

Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 pyrazole LolCDE	Preclinical	5	0.155	Limited	LolCDE	No
 Ribocil-C	Preclinical	5	0.193	Limited	*ribA* riboswithc	PDB: 5KX9
 UCP1106	Preclinical	6	0.117	Yes	DHFR	PDB: 5IST
 nie FabH	Preclinical	6	0.060	Yes	FabH	PDB: 3IL6*
 pep MAP	Preclinical	6	0.174	Yes	Methionine aminopeptidase	PDB: 4Z7M*
 SCH71	Preclinical	6	0.180	Yes	AccC	PDB: 3JZ1
 glycinol murC	Preclinical	7	0.095	Yes	MurC	none

*structure of analog

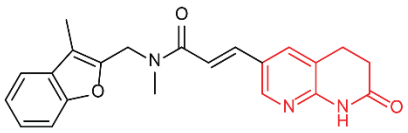
Structure/name	Phase	# RB	Glob	SAR	Validated Target	X-ray structure
 sulfonamide GlmU	Preclinical	7	0.195	Yes	GlmU	PDB: 4AC3
 zantrin Z3	Preclinical	8	0.088	Yes	No	none
 Pfizer AccC	Preclinical	4	0.143	Limited	Resistance	PDB: 2J9G

*structure of analog

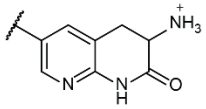
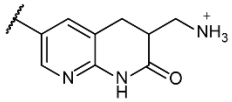
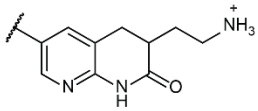
Supplementary Table 2. Physiochemical properties of Debio-1452 and amine analogues. eNTRy rule guidelines (RBs, Globularity, Functional Group) were calculated using eNTRyway. For calculation of vsurf_A, chemical structures were created and managed using Canvas (Version 2.6, Schrödinger, LLC). Initial structure preparation and 3D minimization was performed with LigPrep (Version 3.6, Schrödinger, LLC) using OPLS_2005 force fields. Tautomeric and protonation states were determined using Epik (Version 3.4, Schrödinger, LLC) at pH 7.4 (*J. Comput. Aided Mol. Des.* **24**, 591-604 (2010); *J. Comput. Aided Mol. Des.* **21**, 681-691 (2007)). Generation of ensembles of conformations was performed using Conformational Search in MOE 2015.10 (*J. Chem. Inf. Model* **50**, 792-800 (2010)) using the LowModeMD method with default settings. The vsurf_A value obtained for individual stereoisomers was then averaged.

Compound	Number of RBs	Globularity	Functional Group	Vsurf_A
Debio-1452	4	0.093	No Amine	6.44
Debio-1452-NH3	4	0.061	Primary Amine	6.73
Compound 2	5	0.059	Primary Amine	6.84
Compound 3	6	0.082	Primary Amine	6.43

Supplementary Table 3. Results from molecular docking of Debio-1452 derivatives into FabI. *S. aureus* FabI (PDB: 4FS3) was prepared as a receptor using Schrodinger Protein Prep Wizard. Ligands were prepared using LigPrep and docked using Glide XP (rigid receptor, flexible ligand). Amine-containing derivatives were docked as protonated forms. Top ranked poses were refined using MM-GBSA with Prime (VSGB solvation model, OPLS3e force field, flexibility allowed 5 Å around ligands with hierarchical sampling).



n.c. = no change

Name	R	Docking Score (kcal/mol)		MM-GBSA $\Delta\Delta G_{bind}$ (kcal/mol)	
		E.coli	*S. aureus*	*E.coli*	*S. aureus*
Debio-1452	n.c.	n.a.	n.a.	0	0
Debio-1452-NH3		-15.25	-15.18	-4.29	-5.76
2		-15.19	-14.84	-3.11	-1.50
3		-14.59	-14.33	-7.57	-4.62

Supplementary Table 4. Antimicrobial susceptibility of clinical isolates to Debio-1452 and derivatives. The aqueous solubility limit of Debio-1452 prevents determining actual MIC values that are above 32 µg/mL. Compounds were evaluated against a panel of Gram-positive and Gram-negative organisms. MIC values were determined using the micro-dilution broth method as outlined by the Clinical and Laboratory Standards Institute (CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018) and are listed in µg/mL. All experiments were performed in biological triplicate.

Bacterial Strain	**MIC (µg/mL)**				
	Debio-1452	**Debio-1452-NH3**	**2**	**3**	**8**
WT Gram-positive					
S. aureus ATCC 29213	0.008-0.016	0.03	0.03	0.125	0.016
S. aureus ATCC 29213 (+50% Human serum)	0.125	0.062			
Gram-negative permeability mutant					
E. coli Δ*tolC* JW5503	0.031	0.062	0.125	0.062	0.125
E. coli Δ*rfaC* JW3596	0.5	0.25	0.5	0.5	0.25
WT Gram-negative					
E. coli MG1655	>32	4	8	8	>32
E. coli MG1655 (+ 4% Human serum albumin)	>32	16			
E. coli BAA-2340	>32	4			>32
E. coli BAA-2469	4	2	2		
E. coli BAA-2471	8	4	4		
E. coli F20987	>32	4			
E. coli M66623	>32	8			
E. coli AR-0048	>32	32			>32
E. coli AR-0058	32	8			>32
E. coli AR-0085	>32	16			>32
E. coli AR-0114	>32	8			>32
E. coli AR-0137	>32	8			>32
E. coli AR-0151	>32	8			>32
E. coli AR-0162	>32	8			>32
E. coli AR-0346	>32	8	4		
E. coli AR-0349	4	2	2		
E. coli AR-0493	>32	4	4		>32
E. coli AR-0495	8	4	2		
E. coli AR-0541	>32	8			>32

E. coli AR-0543	>32	8		>32
E. coli AR-0559	32	8		>32
E. cloacae ATCC 29893	>32	8		>32
E. cloacae BAA-2341	>32	8	16	
E. cloacae BAA-2468	>32	8	8	
E. cloacae S28901.1	>32	16	8	
K. pneumoniae AR-0034	16	8		>32
K. pneumoniae AR-0066	>32	32		>32
K. pneumoniae AR-0098	>32	32		>32
K. pneumoniae AR-0113	>32	32		>32
K. pneumoniae AR-0139	>32	16		>32
K. pneumoniae AR-0141	>32	8		>32
K. pneumoniae AR-0347	>32	16	8	
K. pneumoniae AR-0542	>32	16		>32
K. pneumoniae AR-0548	>32	32		>32
K. pneumoniae AR-0555	>32	32		>32
K. pneumoniae AR-0560	>32	32		>32
K. pneumoniae BAA-1705	>32	8	16	
K. pneumoniae BAA-2342	>32	16	16	
K. pneumoniae BAA-2470	8	4	4	
K. pneumoniae BAA-2472	>32	16	16	
K. pneumoniae BAA-2473	>32	16	16	
K. pneumoniae M14723	>32	16	16	
K. pneumoniae M67198	>32	32	32	
K. pneumoniae M67297	>32	32	32	
K. pneumoniae S20595	>32	16	16	
K. pneumoniae S47889	>32	8	8	>32
A. baumannii AR-0033	>32	32		>32
A. baumannii AR-0078	>32	16		>32
A. baumannii AR-0083	>32	16		>32
A. baumannii AR-0273	>32	32		>32
A. baumannii AR-0278	>32	32		>32
A. baumannii AR-0288	>32	32		>32
A. baumannii AR-0299	>32	32		>32
A. baumannii AR-0311	8	8		>32
A. baumannii AR-0312	>32	16		>32

A. baumannii AR-0313	>32	32			>32
A. baumannii W41979	>32	4	16		>32
A. baumannii F19521	>32	4	16		
A. baumannii KB304	>32	64	64		
A. baumannii KB343	>32	64	>64		
A. baumannii KB357	>32	64	>64		
A. baumannii M13100	>32	16	16		
A. baumannii WO22	>32	64	>64		
P. aeruginosa PA01	>32	>64			>32
Mammalian					
H. sapiens IMR-90	16%	30%	48%	12%	
(% inhibition at 30 μM)					
H. sapiens IMR-90 IC_{50}		52.2 ± 3.6			

Supplementary Table 5. Antimicrobial susceptibility of triclosan to Debio-1452-NH3-resistant colonies. MIC values were determined using the micro-dilution broth method as outlined by the Clinical and Laboratory Standards Institute (http://clsi.org/) (CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018) and are listed in μg/mL. All experiments were performed in biological triplicate.

Bacterial Strain	**MIC (μg/mL)**	
	Triclosan	**Debio-1452-NH3**
WT Gram-negative		
E. coli MG1655	0.5	4
Debio-1452-NH3 Resistant Strains		
E. coli MG1655 A116V colony 1	0.5	64
E. coli MG1655 A116V colony 5	0.5	64
E. coli MG1655 G148S colony 1	2	>64
E. coli MG1655 G148S colony 2	2	>64

Supplementary Table 6. Compound test set for calculation of eNTRyway accuracy. The confusion matrix to calculate eNTRyway accuracy was generated by using eNTRyway to reanalyze the diverse compound collection published in reference 14 (Tables S2-S4 of reference 14; 188 compounds). The predicted results using eNTRyway for this compound test set was compared to experimental results published in reference 14 (Tables S2-S4 of reference 14; 188 compounds) for whole-cell accumulation in *E. coli*.

Materials and Methods for Chemical Synthesis:

All reactions were performed under inert atmosphere using nitrogen gas unless otherwise specified. Chemical reagents were purchased from commercial sources and used without further purification. Debio-1452 used for *in vitro* and cell-based studies was purchased from MedChemExpress. Anhydrous solvents were either purchased from commercial suppliers or dried after being passed through columns packed with activated alumina under positive pressure of nitrogen using a PureSolv MD-5 (Inert, previously Innovative Technology Inc.) solvent purification system. Final compounds were dried in an Abderhalden drying pistol to remove any residual solvents. ^{1}H NMR, ^{13}C NMR, and 2D NMR experiments for prepared intermediates and products were recorded on a Varian Unity Inova 600 MHz NMR system equipped with an autoX broadband probe and/or a Bruker Avance III HD 500 MHz NMR system equipped with a CryoProbe. Spectra were obtained in the following solvents (reference peaks also included for ^{1}H and ^{13}C NMRs: Deuterated Chloroform-*d* (^{1}H NMR 7.26 ppm; ^{13}C NMR 77.16 ppm), DMSO-d_6 (^{1}H NMR 2.50 ppm; ^{13}C NMR 39.52 ppm) [1]. All the chemical shifts are expressed in ppm (δ), coupling constants (*J*, Hz) and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). High resolution mass spectra (HRMS) were obtained in the School of Chemical Sciences Mass Spectrometry Laboratory on a Waters Q-TOF Ultima quadrupole time of flight spectrometer using electrospray ionization ESI. Purity of the final compounds were purified to ≥ 95% as assessed by an Agilent Technologies 1290 Infinity II UHPLC equipped with a Phenomenex Kinetex column (2.1 mm ID x 50 mm, 1.7 µm particle size, 100 Å pore size).

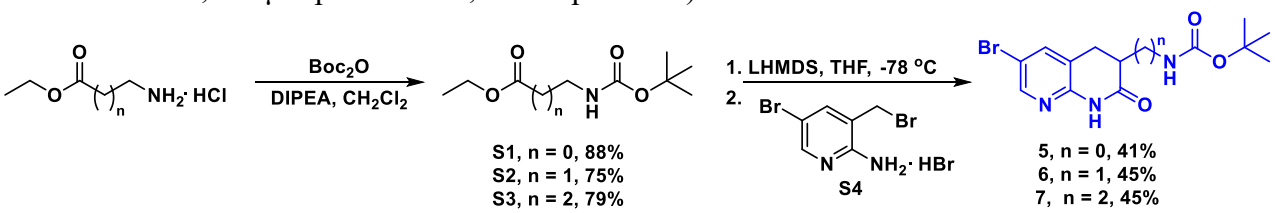
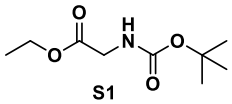


Figure S1. Synthesis of Naphthyridinone Precursors and Debio-1452 Amine Containing Analogues
Boc_2O, di-*tert*-butyl dicarbonate; DIPEA, *N*,*N*-diisopropylethylamine ; LHMDS, lithium bis(trimethylsilyl)amide; THF, tetrahydrofuran

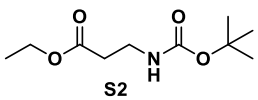

S1

ethyl (tert-butoxycarbonyl)glycinate (**S1**)- *N*,*N*-diisopropylethylamine (2.2 eq, 44 mmol) was added dropwise to a solution of glycine ethyl ester hydrochloride (1 eq, 20 mmol) in CH_2Cl_2 (80 mL) at 0 °C followed by the dropwise addition of di-*tert*-butyl dicarbonate (1.1 eq, 22 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. The reaction was quenched with saturated aqueous ammonium chloride and extracted with dichloromethane. The combined organic extracts were washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated under reduced pressure. Purification by flash purification column chromatography (10:40:50, EtOAc:CH_2Cl_2:Hexanes) yielded ethyl (*tert*-butoxycarbonyl)glycinate (**S1,** 3.58 g, 17.6 mmol, 88%) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 5.00 (s, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.90 (d, *J* = 5.6 Hz, 2H), 1.45 (s, 9H), 1.28 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*): 170.49, 155.83, 80.11, 61.48, 42.62, 28.47, 14.31.

Experimental information for the above compound has been previously reported [2].



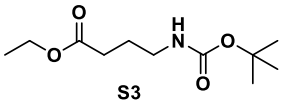
S2

ethyl 3-((tert-butoxycarbonyl)amino)propanoate (**S2**)- *N,N*-diisopropylethylamine (2.2 eq, 44 mmol) was added dropwise to a solution of β-alanine ethyl ester hydrochloride (1 eq, 20 mmol) in CH_2Cl_2 (80 mL) at 0 ºC followed by the dropwise addition of di-*tert*-butyl dicarbonate (1.1 eq, 22 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. The reaction was quenched with saturated aqueous ammonium chloride and extracted with dichloromethane. The combined organic extracts were washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated under reduced pressure. Purification by flash purification column chromatography (10:40:50, EtOAc:CH_2Cl_2:Hexanes) yielded ethyl 3-((*tert*-butoxycarbonyl)amino)propanoate (**S2,** 3.25 g, 15.0 mmol, 75%) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 5.01 (s, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 3.48 – 3.27 (m, 2H), 2.51 (t, *J* = 6.1 Hz, 2H), 1.43 (s, 9H), 1.26 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 172.64, 155.92, 79.48, 60.78, 36.26, 34.81, 28.54, 14.35.

Experimental information for the above compound has been previously reported [3].



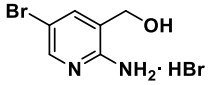
S3

ethyl 4-((tert-butoxycarbonyl)amino)butanoate (**S3**)- *N,N*-diisopropylethylamine (2.2 eq, 44 mmol) was added dropwise to a solution of ethyl 4-aminobutyrate hydrochloride (1 eq, 20 mmol) in CH_2Cl_2 (80 mL) at 0 ºC followed by the dropwise addition of di-*tert*-butyl dicarbonate (1.1 eq, 22 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. The reaction was quenched with saturated aqueous ammonium chloride and extracted with dichloromethane. The combined organic extracts were washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated under reduced pressure. Purification by flash purification column chromatography (10:40:50, EtOAc:CH_2Cl_2:Hexanes) yielded ethyl 4-((*tert*-butoxycarbonyl)amino)butanoate (**S3,** 3.66 g, 15.8 mmol, 79%) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 4.62 (s, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.25 – 3.06 (m, 2H), 2.34 (t, *J* = 7.3 Hz, 2H), 1.81 (p, *J* = 7.2 Hz, 2H), 1.43 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 173.42, 156.06, 79.34, 60.59, 40.11, 31.77, 28.55, 25.45, 14.37.

Experimental information for the above compound has been previously reported [4].



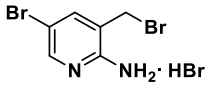
S4 Precursor

(2-amino-5-bromopyridin-3-yl)methanol hydrobromide (**S4 Precursor**)- Bromine (1.01 eq, 39.02 mmol) was added dropwise to a solution of 2-amino-3-(hydroxymethyl)pyridine (1 eq, 38.6 mmol) in glacial acetic acid (60 mL) cooled in an ice bath. After the addition of bromine was complete, the reaction mixture was returned to room temperature. After stirring overnight, the reaction mixture was filtered and washed several times with ether to yield (2-amino-5-bromopyridin-3-yl)methanol hydrobromide (**S4 Precursor**, 10.01 g, 35.5 mmol, 92% yield) as a yellow solid. (HBr Salt)

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.17 (d, *J* = 2.3 Hz, 1H), 7.97 – 7.93 (m, 1H), 4.41 (s, 2H).

^{13}C NMR (126 MHz, DMSO-d_6): δ 151.39, 141.11, 135.60, 127.72, 104.27, 57.98.

Experimental information for the above compound has been previously reported [5].



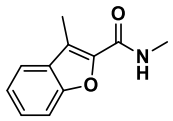
S4

5-bromo-3-(bromomethyl)pyridin-2-amine hydrobromide (**S4**)-A suspension of (2-amino-5-bromopyridin-3-yl)methanol hydrobromide (1 eq, 35.47 mmol) in 48% hydrobromic acid (70 mL) was refluxed for 10 h. After 10 h, the reaction mixture was allowed to slowly cool to room with stirring, filtered, and rinsed with ethyl acetate. The solid was triturated with ethyl acetate to yield 5-bromo-3-(bromomethyl)pyridin-2-amine hydrobromide (**S4**, 10.226 g, 29.7 mmol, 84%) as a light beige solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.18 (d, *J* = 2.4 Hz, 1H), 8.15 (d, *J* = 2.4 Hz, 1H), 4.72 (s, 2H).

^{13}C NMR (126 MHz, DMSO): δ 153.04, 144.29, 141.01, 121.66, 104.11, 29.13.

Experimental information for the above compound has been previously reported [5].



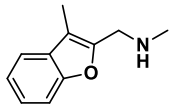
4 Precursor a

N,3-dimethylbenzofuran-2-carboxamide (**4 Precursor a**)-To a solution of 3-methylbenzo[b]furan-2-carboxylic acid (1 eq, 52 mmol), methylamine hydrochloride (1.1 eq, 57.52 mmol), *N*,*N*-diisopropylethylamine (2.2 eq, 114.4 mmol), and HOBt (1.1 eq, 57.52 mmol) in DMF (150 mL) was added EDC (1.1 eq, 57.52 mmol). The reaction mixture was heated to 70 ºC overnight. The solvent was reduced to a few mL. The crude reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate. The organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash column chromatography (**4 Precursor a**, 20:50:30, EtOAc:CH_2Cl_2:hexanes) yielded *N*,3-dimethylbenzofuran-2-carboxamide (9.24 g, 48.9 mmol,94%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*): δ7.61 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.29 (ddd, *J* = 8.0, 6.4, 1.7 Hz, 1H), 6.64 (s, 1H), 3.03 (d, *J* = 5.0 Hz, 3H), 2.63 (s, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 161.10, 153.35, 142.96, 129.95, 127.04, 123.19, 122.19, 121.07, 111.55, 25.86, 9.00.

Experimental information for the above compound has been previously reported [6].



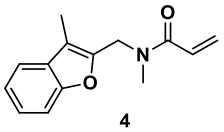
4 Precursor b

N-methyl-1-(3-methylbenzofuran-2-yl)methanamine (**4 Precursor b**)-Lithium aluminum hydride (3 eq, 47.6 mmol) was added portionwise to a solution of *N*,3-dimethylbenzofuran-2-carboxamide (1 eq, 15.86 mmol) in THF (75 mL) at room temperature. The reaction mixture was refluxed for 11 h. After reaction completion, the reaction mixture was cooled to 0 ^{o}C and slowly quenched by the sequential dropwise addition of 2 mL water, 2 mL 15% sodium hydroxide, 6 mL water at 15-30 min intervals. The mixture was filtered through a pad of celite rinsed several times with ethyl acetate. Purification by flash column chromatography (5:95, MeOH:CH_2Cl_2) yielded *N*-methyl-1-(3-methylbenzofuran-2-yl)methanamine (**4 Precursor b**, 2.513 g, 14.3 mmol, 91%).

^{1}H NMR (500 MHz, Chloroform-*d*): δ 7.49 – 7.44 (m, 1H), 7.43 – 7.37 (m, 1H), 7.27 – 7.23 (m, 1H), 7.22 (td, *J* = 7.3, 1.3 Hz, 1H), 3.87 (s, 2H), 2.45 (s, 3H), 2.23 (s, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 154.25, 151.34, 130.08, 124.01, 122.26, 119.28, 112.33, 111.04, 46.23, 35.80, 8.07.

Experimental information for the above compound has been previously reported [6].



4

N-methyl-N-((3-methylbenzofuran-2-yl)methyl)acrylamide (**4**)- *N*,*N*-diisopropylethylamine (1.5 eq, 15.4 mmol) was added dropwise to a solution of *N*-methyl-1-(3-methylbenzofuran-2-yl)methanamine (1 eq, 10.3 mmol) in CH_2Cl_2 (75 mL) at room temperature. After 10 min, acryloyl chloride (2 eq, 20.6 mmol) was added dropwise and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure and purification by flash column chromatography (1:99 to 3:97, MeOH:CH_2Cl_2) yielded *N*-methyl-*N*-((3-methylbenzofuran-2-yl)methyl)acrylamide (**4**, 1.861 g, 8.12 mmol, 79%) as a colorless oil.

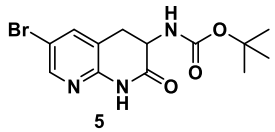
Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 35:65 and is reflected in the reported integral values.

^{1}H NMR (500 MHz, Chloroform-*d*): 7.51 – 7.45 (m, 1H), 7.43 – 7.36 (m, 1H), 7.32 – 7.18 (m, 2H), 6.85 (dd, *J* = 16.8, 10.6 Hz, 0.35H), 6.59 (dd, *J* = 16.7, 10.4 Hz, 0.65H), 6.42 – 6.33 (m, 1H), 5.80 – 5.67 (m, 1H), 4.77 (s, 1.3H), 4.62 (s, 0.7H), 3.13 (s, 1.95H), 3.02 (s, 1.05H), 2.29 (s, 1.95H), 2.25 (s, 1.05H).

Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature which results in doubling of signals for most ^{13}C nuclei.

^{13}C NMR (126 MHz, Chloroform-*d*): 167.07, 166.29, 154.26, 154.23, 148.93, 147.52, 129.84, 129.48, 128.48, 128.22, 128.04, 127.54, 124.75, 124.30, 122.59, 122.37, 119.49, 113.74, 113.36, 111.20, 111.07, 45.21, 42.26, 35.36, 33.64, 7.95.

Experimental information for the above compound has been previously reported [7].



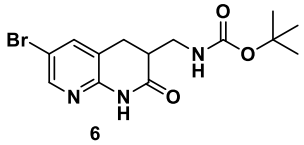
5

tert-butyl (6-bromo-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)carbamate (**5**). To a solution of LHMDS (1 M in THF, 4 eq, 33.4 mmol) cooled to -78 ^{o}C was added a solution of ethyl (*tert*-butoxycarbonyl)glycinate (**S1**, 2 eq, 16.72 mmol) in THF (34 mL) dropwise. The reaction mixture was stirred for 1 h followed by the portionwise addition (3 portions at 15 min intervals) of 5-bromo-3-(bromomethyl)pyridin-2-amine hydrobromide (**S4**, 1 eq, 8.36 mmol) via a solid addition tube kept under N_2. The reaction mixture was kept at -78 ^{o}C for several hours and allowed to warm to -40 ^{o}C overnight. The reaction mixture was quenched with 0.5M HCl (aq) and extracted with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (01:99 to 10:90, THF:CH_2Cl_2) followed by trituration with ether/n-pentane yielded *tert*-butyl (6-bromo-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)carbamate (**5**, 1.48 g, 3.46 mmol, 41%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 9.68 (s, 1H), 8.32 (s, 1H), 7.65 (s, 1H), 5.63 (s, 1H), 4.42 – 4.30 (m, 1H), 3.52 (dd, *J* = 16.4, 6.4 Hz, 1H), 2.83 (t, *J* = 14.9 Hz, 1H), 1.48 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 169.16, 155.72, 148.68, 147.95, 139.50, 119.83, 114.37, 80.54, 49.85, 31.17, 28.48.

HRMS (ESI): m/z calc for $C_{13}H_{16}BrN_3O_3$ $[M+H]^+$: 342.0448, found: 342.0451.



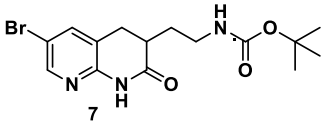
6

tert-butyl ((6-bromo-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)methyl)carbamate (**6**). To a solution of LHMDS (1 M in THF, 4 eq, 36 mmol) cooled to -78 ^{o}C was added a solution ethyl 3-((*tert*-butoxycarbonyl)amino)propanoate (**S2**, 2 eq, 18 mmol) in THF (36 mL) dropwise. The reaction mixture was stirred for 1.5 h followed by the portionwise addition (3 portions at 15 min intervals) of 5-bromo-3-(bromomethyl)pyridin-2-amine hydrobromide (**S4**, 1 eq, 9 mmol) via a solid addition tube kept under N_2. The reaction mixture was kept at -78 ^{o}C for several hours and allowed to warm to -40 ^{o}C overnight. The reaction mixture was quenched with 0.5M HCl (aq) and extracted with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (01:99 to 10:90, THF:CH_2Cl_2) followed by trituration with ether/n-pentane yielded *tert*-butyl ((6-bromo-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)methyl)carbamate (**6**, 1.431 g, 4.02 mmol, 45%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 9.38 (s, 1H), 8.28 (s, 1H), 7.63 (s, 1H), 5.31 (d, *J* = 6.9 Hz, 1H), 3.71 – 3.55 (m, 1H), 3.48 (dt, *J* = 13.7, 6.3 Hz, 1H), 2.95 (dd, *J* = 16.1, 6.9 Hz, 1H), 2.89 (t, *J* = 14.5 Hz, 1H), 2.74 (ddt, *J* = 13.0, 6.7, 3.4 Hz, 1H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 172.23, 156.45, 149.34, 147.35, 138.99, 120.65, 113.89, 79.69, 40.77, 40.01, 28.53, 27.84.

HRMS (ESI): m/z calc for $C_{14}H_{18}BrN_3O_3$ $[M+H]^+$: 356.0604, found: 356.0609.



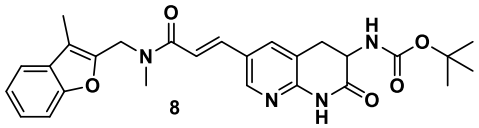
7

tert-butyl (2-(6-bromo-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)ethyl)carbamate (**7**). To a solution of LHMDS (1 M in THF, 4 eq, 18 mmol) cooled to -78 °C was added a solution ethyl 4-((*tert*-butoxycarbonyl)amino)butanoate (**S3**, 2 eq, 9 mmol) in THF (18 mL) dropwise. The reaction mixture was stirred for 1.5 h followed by the portionwise addition (2 portions at 15 min intervals) of 5-bromo-3-(bromomethyl)pyridin-2-amine hydrobromide (**S4**, 1 eq, 4.5 mmol) via a solid addition tube kept under N_2. The reaction mixture was kept at -78 °C for several hours and allowed to warm to -40 °C overnight. The reaction mixture was quenched with 0.5M HCl (aq) and extracted with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (01:99 to 10:90, THF:CH_2Cl_2) followed by trituration with ether/n-pentane yielded *tert*-butyl (2-(6-bromo-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)ethyl)carbamate (**7**, 0.740 g, 2.01 mmol, 45%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.73 (s, 1H), 8.25 (d, *J* = 2.2 Hz, 1H), 7.62 (d, *J* = 2.1 Hz, 1H), 4.81 (s, 1H), 3.44 – 3.29 (m, 1H), 3.28 – 3.17 (m, 1H), 3.08 (dd, *J* = 16.0, 6.1 Hz, 1H), 2.78 (dd, *J* = 16.0, 9.8 Hz, 1H), 2.65 (dq, *J* = 9.8, 6.6 Hz, 1H), 2.00 (dq, *J* = 13.8, 6.9 Hz, 1H), 1.82 – 1.67 (m, 1H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*): δ 172.83, 156.19, 149.31, 147.47, 138.98, 120.11, 113.80, 79.53, 38.23, 37.37, 30.33, 29.67, 28.55.

HRMS (ESI): m/z calc for $C_{15}H_{20}BrN_3O_3$ $[M+H]^+$: 370.0761, found: 370.0766.



8

tert-butyl(E)-(6-(3-(methyl((3-methylbenzofuran-2-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)carbamate (**8**). Anhydrous DMA (10 mL, sparged with N_2 before using) was added to a flask containing **4** (1.5 eq, 1.875 mmol), **5** (1 eq, 1.25 mmol), palladium(II) acetate (0.2 eq, 0.25 mmol), and tricyclohexylphosphine tetrafluoroborate (0.4 eq, 0.5 mmol) followed by the addition of *N*,*N*-diisopropylethylamine (2 eq, 2.5 mmol, distilled and sparged with N_2 before using). The reaction mixture was heated to 90-100 °C for 24 h. After reaction completion, the reaction mixture was diluted with ethyl acetate and filtered through a pad of celite and the filtrate was washed with saturated sodium bicarbonate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (10:90 to 20:00, THF:CH_2Cl_2) followed by trituration with ether/n-pentane

yielded *tert*-butyl (*E*)-(6-(3-(methyl((3-methylbenzofuran-2-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)carbamate (**8**, 0.374 g, 0.762 mmol, 61%) as a white solid.

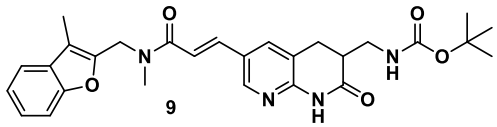
Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 10.82 (s, 1H), 8.48 – 8.33 (m, 1H), 8.17 – 8.02 (m, 1H), 7.60 – 7.54 (m, 1H), 7.54 – 7.44 (m, 2.4H), 7.30 – 7.22 (m, 2H), 7.20 (d, *J* = 15.4 Hz, 0.6H), 7.12 – 6.97 (m, 1H), 4.98 (s, 0.8H), 4.79 (s, 1.2H), 4.40 – 4.17 (m, 1H), 3.18 (s, 1.8H), 3.08 – 2.91 (m, 3.2H), 2.26 (s, 3H), 1.41 (s, 9H).

^{1}H NMR (600 MHz, DMSO-d_6, 115 °C): δ 10.33 (s, 1H), 8.36 (d, *J* = 2.1 Hz, 1H), 7.97 (s, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 15.4 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.22 – 7.16 (m, 1H), 6.57 (d, *J* = 7.8 Hz, 1H), 4.85 (s, 2H), 4.26 (dt, *J* = 13.9, 7.2 Hz, 1H), 3.16 – 3.06 (m, 4H), 3.05 – 2.98 (m, 1H), 2.27 (s, 3H), 1.44 (s, 9H).

^{13}C NMR (151 MHz, DMSO-d_6, 115 °C): δ 168.63, 165.20, 154.67, 153.13, 150.96, 148.64, 146.37, 137.28, 133.72, 128.96, 125.41, 123.61, 121.76, 118.74, 117.92, 117.39, 112.12, 110.10, 77.93, 49.08, 42.28 (brs, see HSQC) 33.72 (brs), 29.87, 27.66, 6.61.

HRMS (ESI): m/z calc for $C_{27}H_{30}N_4O_5$ $[M+H]^+$:491.2289, found: 491.2302.



9

tert-butyl(E)-((6-(3-(methyl((3-methylbenzofuran-2-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)methyl)carbamate (**9**). Anhydrous DMA (32 mL, sparged with N_2 before using) was added to a flask containing **4** (1.5 eq, 6 mmol), **6** (1 eq, 4 mmol), palladium(II) acetate (0.2 eq, 0.8 mmol), and tricyclohexylphosphine tetrafluoroborate (0.4 eq, 1.6 mmol) followed by the addition of *N*,*N*-diisopropylethylamine (2 eq, 8 mmol, distilled and sparged with N_2 before using). The reaction mixture was heated to 90-100 °C for 24 h. After reaction completion, the reaction mixture was diluted with ethyl acetate and filtered through a pad of celite and the filtrate was washed with saturated sodium bicarbonate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (10:90 to 20:00, THF:CH_2Cl_2) followed by trituration with ether/n-pentane yielded *tert*-butyl (*E*)-((6-(3-(methyl((3-methylbenzofuran-2-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)methyl)carbamate (**9**, 1.285 g, 2.55 mmol, 64%) as a white solid.

Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

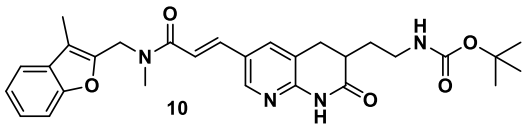
^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 10.72 (s, 1H), 8.47 – 8.32 (m, 1H), 8.15 – 8.02 (m, 1H), 7.59 – 7.54 (m, 1H), 7.54 – 7.43 (m, 2.4H), 7.31 – 7.26 (m, 1H), 7.26 – 7.17 (m, 1.6H), 6.91 – 6.81 (m, 1H), 4.99 (s, 0.8H), 4.79 (s, 1.2H), 3.51 – 3.36 (m, 1H), 3.18 (s, 1.8H), 3.12 – 2.96 (m, 2H), 2.93 (s, 1.2H), 2.81 – 2.70 (m, 1H), 2.69 – 2.59 (m, 1H), 2.26 (s, 3H), 1.45 – 1.28 (m, 9H).

^{1}H NMR (600 MHz, DMSO-d_6, 120 °C): δ 10.12 (s, 1H), 8.34 (s, 1H), 7.93 (s, 1H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 15.5 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.28 (td, *J* = 8.1, 7.6, 1.5 Hz, 1H), 7.24 (td, *J* = 7.4, 1.1 Hz,

1H), 7.19 (d, *J* = 15.4 Hz, 1H), 6.27 (s, 1H), 4.85 (s, 2H), 3.43 (dt, *J* = 13.6, 5.5 Hz, 1H), 3.18 – 3.08 (m, 4H), 3.04 (dd, *J* = 15.9, 6.1 Hz, 1H), 2.79 (dd, *J* = 15.8, 10.4 Hz, 1H), 2.75 – 2.68 (m, 1H), 2.27 (s, 3H), 1.40 (s, 9H).

^{13}C NMR (151 MHz, DMSO-d_6, 120 °C): δ 170.73, 165.23, 155.00, 153.12, 151.17, 148.62, 146.07, 137.33, 133.52, 128.94, 125.39, 123.55, 121.71, 118.68, 117.74, 117.70, 112.05, 110.04, 77.38, 43.32 (brs, see HSQC), 39.58, 39.29 (solvent overlap, see HSQC), 33.69 (brs), 27.66, 26.66, 6.54.

HRMS (ESI): m/z calc for $C_{28}H_{32}N_4O_5$ $[M+H]^+$: 505.2445, found: 505.2443.



10

tert-butyl (E)-(2-(6-(3-(methyl((3-methylbenzofuran-2-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)ethyl)carbamate (**10**). Anhydrous DMA (10 mL, sparged with N_2 before using) was added to a flask containing **4** (1.5 eq, 1.875 mmol), **7** (1 eq, 1.25 mmol), palladium(II) acetate (0.2 eq, 0.25 mmol), and tricyclohexylphosphine tetrafluoroborate (0.4 eq, 0.5 mmol) followed by the addition of *N*,*N*-diisopropylethylamine (2 eq, 2.5 mmol, distilled and sparged with N_2 before using). The reaction mixture was heated to 90-100 °C for 24 h. After reaction completion, the reaction mixture was diluted with ethyl acetate and filtered through a pad of celite and the filtrate was washed with saturated sodium bicarbonate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (10:90 to 20:80, THF:CH_2Cl_2) followed by trituration with ether/n-pentane yielded *tert*-butyl (*E*)-(2-(6-(3-(methyl((3-methylbenzofuran-2-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl)ethyl)carbamate (**10**, 0.364 g, 0.702 mmol, 56%) as a white solid.

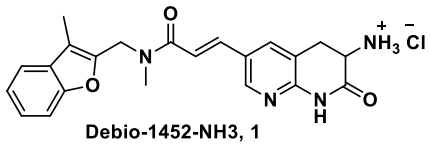
Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 10.70 (s, 1H), 8.52 – 8.29 (m, 1H), 8.21 – 8.01 (m, 1H), 7.60 – 7.55 (m, 1H), 7.54 – 7.43 (m, 2.4H), 7.33 – 7.26 (m, 1H), 7.26 – 7.22 (m, 1H), 7.21 (d, *J* = 16.4 Hz, 0.6H), 6.87 (t, *J* = 5.8 Hz, 1H), 5.00 (s, 0.8H), 4.79 (s, 1.2H), 3.18 (s, 1.8H), 3.12 – 2.95 (m, 4H), 2.92 (s, 1.2H), 2.78 – 2.67 (m, 1H), 2.33 – 2.22 (m, 3H), 1.94 – 1.84 (m, 1H), 1.47 – 1.39 (m, 1H), 1.38 – 1.28 (m, 9H).

^{1}H NMR (600 MHz, DMSO-d_6, 120 °C): δ 10.05 (s, 1H), 8.34 (s, 1H), 7.93 (s, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 15.5 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.32 – 7.26 (m, 1H), 7.24 (t, *J* = 7.3 Hz, 1H), 7.19 (d, *J* = 15.4 Hz, 1H), 6.29 (s, 1H), 4.85 (s, 2H), 3.14 – 3.08 (m, 2H), 3.05 (dd, *J* = 15.9, 6.1 Hz, 1H), 2.88 (s, 3H), 2.75 (dd, *J* = 15.9, 10.1 Hz, 1H), 2.63 – 2.53 (m, 1H), 2.27 (s, 3H), 1.94 (dq, *J* = 13.6, 7.0 Hz, 1H), 1.51 (dq, *J* = 13.9, 6.9 Hz, 1H), 1.39 (s, 9H).

^{13}C NMR (151 MHz, DMSO-d_6, 120 °C):): δ 171.96, 165.22, 154.95, 153.11, 151.32, 148.62, 146.10, 137.34, 133.32, 128.94, 125.28, 123.55, 121.71, 118.68, 117.92, 117.68, 112.05, 110.04, 77.01, 42.26, (brs, see HSQC) 37.59, 36.76, 33.66 (brs), 29.37, 28.53, 27.68, 6.54.

HRMS (ESI): m/z calc for $C_{29}H_{34}N_4O_5$ $[M+H]^+$: 519.2602, found: 519.2616.



Debio-1452-NH3, 1

(E)-3-(6-amino-7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)-N-methyl-N-((3-methylbenzofuran-2-yl)methyl)acrylamide hydrochloride (**Debio-1452-NH3**, **1**). Anhydrous 4M HCl in dioxane (1 mL) was added dropwise to a solution of **8** (1 eq, 0.652 mmol) in dioxane (3 mL). The reaction mixture was stirred at room temperature. After 4 h, the reaction mixture was concentrated from CH_2Cl_2 several times followed by trituration with ether/n-pentane to afford (*E*)-3-(6-amino-7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)-*N*-methyl-*N*-((3-methylbenzofuran-2-yl)methyl)acrylamide hydrochloride (**Debio-1452-NH3**, **1**, 244 mg, 0.571 mmol, 88%) as a white solid.

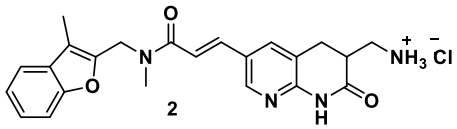
Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

^{1}H NMR (600 MHz, DMSO-d_6, 25 ^{o}C): δ 11.33 (s, 1H), 8.79 – 8.66 (m, 3H), 8.55 – 8.43 (m, 1H), 8.31 – 8.20 (m, 1H), 7.62 – 7.55 (m, 1H), 7.55 – 7.45 (m, 2.4H), 7.33 – 7.26 (m, 1.6H), 7.26 – 7.21 (m, 1H), 5.01 (s, 0.8H), 4.79 (s, 1.2H), 4.44 – 4.28 (m, 1H), 3.35 – 3.24 (m, 1H), 3.24 – 3.06 (m, 2.8H), 2.92 (s, 1.2H), 2.26 (s, 3H).

^{1}H NMR (600 MHz, DMSO-d_6, 120 ^{o}C): δ 10.90 (s, 1H), 8.63 (s, 3H), 8.44 (s, 1H), 8.08 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 15.4 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.36 – 7.18 (m, 3H), 4.86 (s, 2H), 4.28 (dd, *J* = 14.1, 6.9 Hz, 1H), 3.38 (dd, *J* = 15.6, 6.7 Hz, 1H), 3.21 (t, *J* = 14.7 Hz, 1H), 3.11 (s, 3H), 2.27 (d, *J* = 2.3 Hz, 3H).

^{13}C NMR (151 MHz, DMSO, 120 ^{o}C): δ 166.20, 165.14, 153.13, 150.23, 148.61, 146.69, 136.90, 134.20, 128.95, 126.14, 123.61, 121.76, 118.74, 118.63, 115.77, 112.13, 110.07, 47.37, 42.37 (brs, see HSQC), 33.54 (brs, see HSQC), 27.44, 6.59.

HRMS (ESI): m/z calc for $C_{22}H_{22}N_4O_3$ $[M+H]^+$ (**Note:** hydrochloride salt not observed): 391.1765, found: 391.1773.



2

(E)-3-(6-(aminomethyl)-7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)-N-methyl-N-((3-methylbenzofuran-2-yl)methyl)acrylamide hydrochloride (**2**) -Anhydrous 4M HCl in dioxane (1 mL) was added dropwise to a solution of **9** (1 eq, 0.6 mmol) in dioxane (3 mL). The reaction mixture was stirred at room temperature. After 4 h, the reaction mixture was concentrated from CH_2Cl_2 several times followed by trituration with ether/n-pentane to afford (*E*)-3-(6-(aminomethyl)-7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)-*N*-methyl-*N*-((3-methylbenzofuran-2-yl)methyl)acrylamide hydrochloride (**2**, 256 mg, 0.581 mmol, 97%) as a white solid.

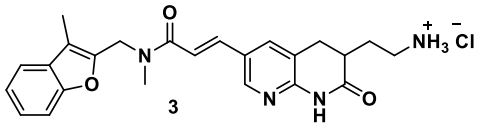
Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 11.03 (s, 1H), 8.49 – 8.40 (m, 1H), 8.21 – 8.04 (m, 4H), 7.59 – 7.55 (m, 1H), 7.55 – 7.46 (m, 2.4H), 7.31 – 7.21 (m, 2.6H), 5.01 (s, 0.8H), 4.79 (s, 1.2H), 3.29 – 3.21 (m, 1H), 3.19 (s, 1.8H), 3.08 – 2.97 (m, 3H), 2.95 – 2.85 (m, 2.2H), 2.32 – 2.19 (m, 3H).

^{1}H NMR (600 MHz, DMSO-d_6, 120 °C): δ 10.49 (s, 1H), 8.40 (d, *J* = 2.2 Hz, 1H), 8.15 (s, 3H), 7.96 (s, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 15.5 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.28 (td, *J* = 8.1, 7.7, 1.5 Hz, 1H), 7.26 – 7.12 (m, 2H), 4.85 (s, 2H), 3.31 – 3.27 (m, 1H), 3.14 – 3.02 (m, 6H), 2.96 – 2.90 (m, 1H), 2.27 (s, 3H).

^{13}C NMR (151 MHz, DMSO-d_6, 120 °C): δ 170.24, 165.20, 153.12, 150.80, 148.63, 146.25, 137.15, 133.57, 128.94, 125.74, 123.59, 121.74, 118.72, 118.20, 117.47, 112.10, 110.06, 42.40 (brs, see HSQC), 38.34, 36.80, 33.73 (brs), 26.66, 6.58.

HRMS (ESI): m/z calc for $C_{23}H_{24}N_4O_3$ $[M+H]^+$ (**Note:** hydrochloride salt not observed): 405.1921, found: 405.1927



3

(E)-3-(6-(aminoethyl)-7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)-N-methyl-N-((3-methylbenzofuran-2-yl)methyl)acrylamide hydrochloride (**3**). Anhydrous 4M HCl in dioxane (1 mL) was added dropwise to a solution of **10** (1 eq, 0.6 mmol) in dioxane (3 mL). The reaction mixture was stirred at room temperature. After 4 h, the reaction mixture was concentrated from CH_2Cl_2 several times followed by trituration with ether/pentane to afford (*E*)-3-(6-(aminoethyl)-7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)-*N*-methyl-*N*-((3-methylbenzofuran-2-yl)methyl)acrylamide hydrochloride (**3**, 244 mg, 0.536 mmol, 89%) as a white solid.

Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 10.80 (s, 1H), 8.47 – 8.37 (m, 1H), 8.17 – 8.09 (m, 1H), 8.06 – 7.95 (m, 3H), 7.60 – 7.54 (m, 1H), 7.54 – 7.43 (m, 2.4H), 7.30 – 7.26 (m, 1H), 7.26 – 7.17 (m, 1.6H), 4.99 (s, 0.8H), 4.79 (s, 1.2H), 3.18 (s, 1.8H), 3.03 – 2.97 (m, 1H), 2.96 – 2.89 (m, 3.2H), 2.80 – 2.67 (m, 2H), 2.30 – 2.22 (m, 3H), 2.10 – 2.00 (m, 1H), 1.71 – 1.63 (m, 1H).

^{1}H NMR (600 MHz, DMSO-d_6, 115 °C): δ 10.27 (s, 1H), 8.38 (s, 1H), 7.96 (s, 1H), 7.83 (s, 3H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 15.4 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 1H), 7.24 (t, *J* = 7.4 Hz, 1H), 7.20 (d, *J* = 15.4 Hz, 1H), 4.85 (s, 2H), 3.11 (s, 3H), 3.05 (dd, *J* = 15.4, 5.7 Hz, 1H), 3.03 – 2.89 (m, 2H), 2.79 (dd, *J* = 15.4, 11.3 Hz, 1H), 2.76-2.69 (m, 1H), 2.27 (s, 3H), 2.10 (dq, *J* = 14.4, 7.4 Hz, 1H), 1.77 (dq, *J* = 13.8, 7.0 Hz, 1H).

^{13}C NMR (151 MHz, DMSO-d_6, 115 °C): δ 171.63, 165.22, 153.13, 151.19, 148.64, 146.21, 137.35, 133.50, 128.96, 125.46, 123.63, 121.78, 118.76, 117.92, 117.85, 112.13, 110.09, 42.25 (brs, see HSQC), 36.76, 36.57, 33.71 (brs), 28.73, 27.07, 6.62.

HRMS (ESI): m/z calc for $C_{24}H_{26}N_4O_3$ $[M+H]^+$ (**Note:** hydrochloride salt not observed): 419.2078, found: 419.2071.

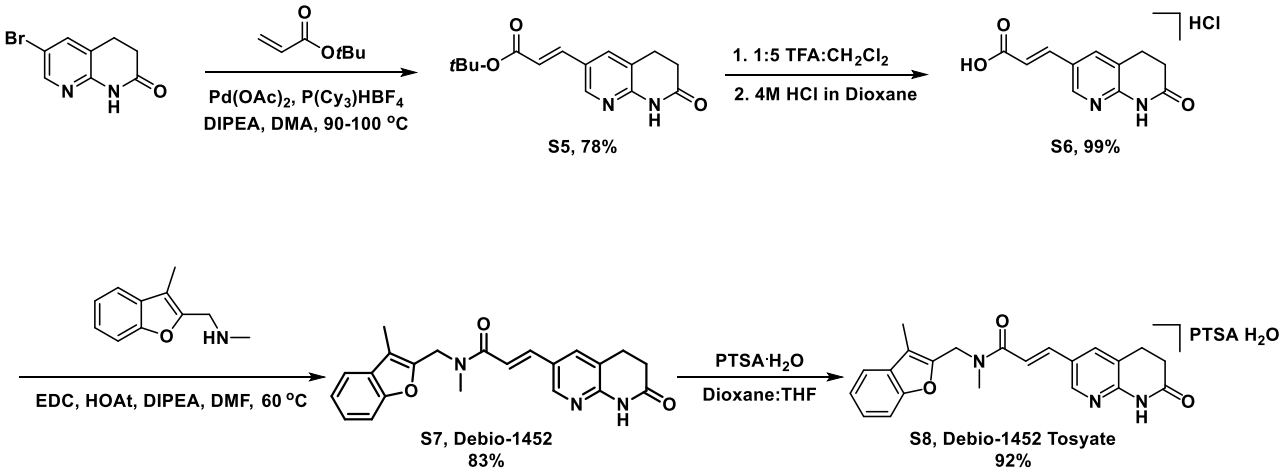
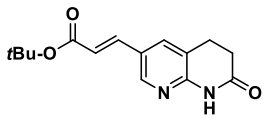


Figure S2. Synthesis of Debio-1452 Tosylate
Cy, cyclohexyl; DIPEA, *N*,*N*-diisopropylethylamine ; TFA, trifluoroacetic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOAt, 1-hydroxy-7-azabenzotriazole; DMF, *N*,*N*-dimethylformamide; PTSA, *p*-toluene sulfonic acid; THF, tetrahydrofuran



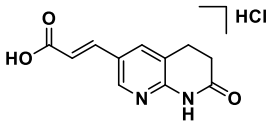
S5

tert-butyl (E)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylate (**S5**). Anhydrous DMA (32 mL, sparged with N_2 before using) was added to a flask containing 6-bromo-3,4-dihydro-1,8-naphthyridin-2(*1H*)-one (1 eq, 10 mmol), palladium(II) acetate (0.05 eq, 0.5 mmol), and tricyclohexylphosphine tetrafluoroborate (0.1 eq, 1.0 mmol) followed by the addition of *tert*-butyl acrylate (1.5 eq, 15 mmol, sparged with N_2 before using), *N*,*N*-diisopropylethylamine (2 eq, 20 mmol, distilled and sparged with N_2 before using). The reaction mixture was heated to 90-100 ^oC for 24 h. After reaction completion, the reaction mixture was diluted with ethyl acetate and filtered through a pad of celite and the filtrate was washed with saturated sodium bicarbonate. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash purification column chromatography (10:90 to 30:70, EtOAc:CH_2Cl_2) followed by trituration with ether/n-pentane yielded *tert*-butyl (*E*)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylate (**S5**, 2.151 g, 7.85 mmol, 78%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.94 (s, 1H), 8.32 (d, *J* = 2.1 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 7.51 (d, *J* = 16.0 Hz, 1H), 6.33 (d, *J* = 16.0 Hz, 1H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.71 (dd, *J* = 8.4, 6.8 Hz, 2H), 1.53 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*): 170.97, 165.97, 151.95, 147.37, 139.43, 134.05, 126.16, 120.57, 118.84, 80.99, 77.36, 30.40, 28.34, 24.22.

Experimental information for the above compound has been previously reported [8].



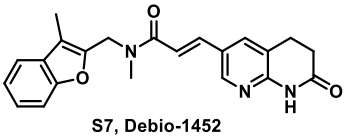
S6

(E)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylic acid hydrochloride (**S6**). *E*)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylate (**S5**) was dissolved trifluoroacetic acid:CH_2Cl_2 (8mL:40mL) and stirred at room temperature. After 2h, the reaction mixture was concentrated several times from CH_2Cl_2. The crude material was suspended in 4 M HCl in dioxane (20 mL), stirred for 30 min, filtered, and rinsed with ether to afford (*E*)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylic acid hydrochloride (**S6**, 7.67 mmol, 99%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.68 (s, 1H), 8.35 (d, *J* = 2.2 Hz, 1H), 8.02 (d, *J* = 2.1 Hz, 1H), 7.54 (d, *J* = 16.0 Hz, 1H), 6.51 (d, *J* = 16.0 Hz, 1H), 2.91 (t, *J* = 7.6 Hz, 2H), 2.53 (dd, *J* = 8.5, 6.8 Hz, 2H).

^{13}C NMR (126 MHz, DMSO): δ 171.01, 167.47, 152.77, 147.33, 140.62, 133.78, 124.72, 119.20, 118.34, 29.97, 23.27.

Experimental information for the above compound has been previously reported [8].



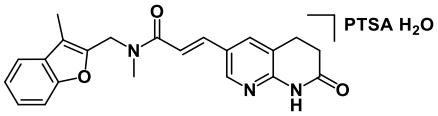
S7, Debio-1452

(E)-N-methyl-N-((3-methylbenzofuran-2-yl)methyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide (**S7, Debio-1452**)-To a solution of *N*-methyl-1-(3-methylbenzofuran-2-yl)methanamine (1.1 eq, 6.93 mmol), (*E*)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylic acid hydrochloride (1 eq, 6.3 mmol), 1-hydroxy-7-azabenzotriazole (1.1 eq, 6.93 mmol), in *N*,*N*-dimethylformamide (32 mL) was added *N*,*N*-diisopropylethylamine (2.2 eq, 13.86 mmol) followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.1 eq, 6.93 mmol). The reaction mixture was heated to 60 °C for 6 h. The crude reaction mixture was diluted with water, filtered, rinsed with water, rinsed with ether, and dried to afford (*E*)-*N*-methyl-*N*-((3-methylbenzofuran-2-yl)methyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide (**S7, Debio-1452,** 1.970 g, 5.25 mmol, 83%) as a light beige solid.

Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.65 (s, 1H), 8.41 – 8.34 (m, 1H), 8.18 – 8.00 (m, 1H), 7.59 – 7.54 (m, 1H), 7.54 – 7.45 (m, 2.4H), 7.31 – 7.26 (m, 1H), 7.26 – 7.22 (m, 1H), 7.22 – 7.16 (m, 0.6H), 4.99 (s, 0.8H), 4.79 (s, 1.2H), 3.18 (s, 1.8H), 2.92 (s, 1.2H), 2.92 – 2.87 (m, 2H), 2.57 – 2.51 (m, 2H), 2.26 (s, 3H).

Experimental information for the above compound has been previously reported [6].



S8, Debio-1452 Tosyate

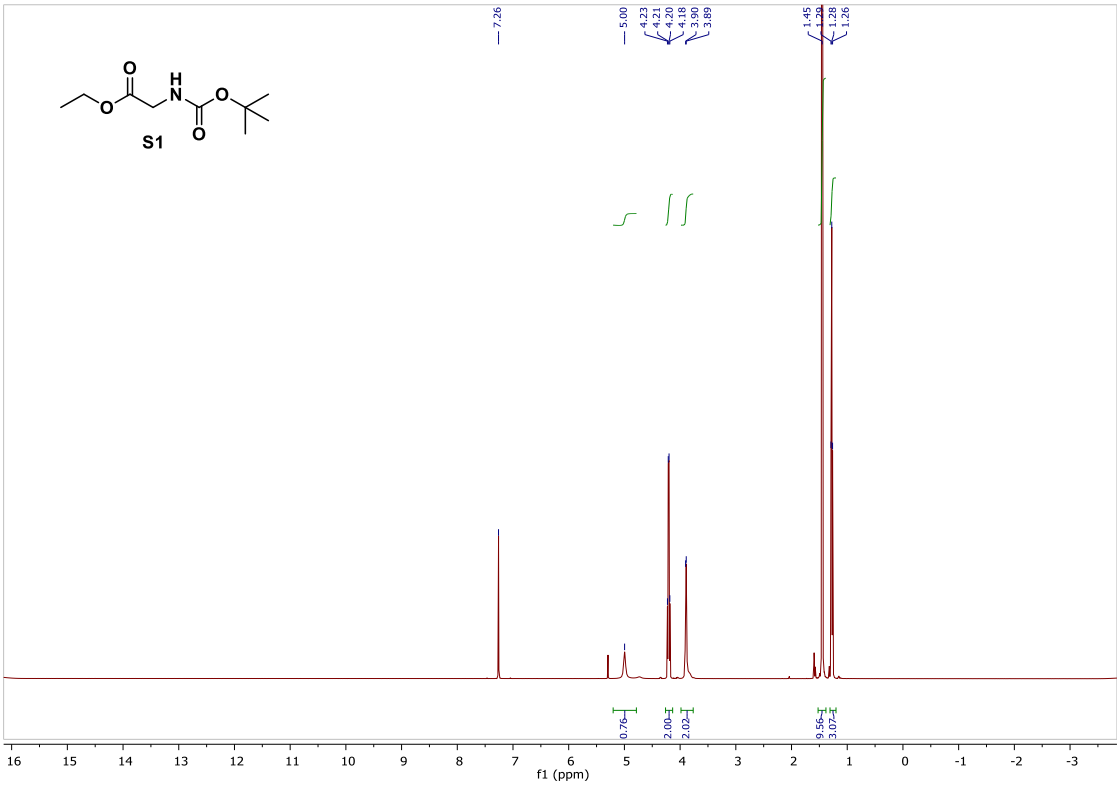
(E)-N-methyl-N-((3-methylbenzofuran-2-yl)methyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide p-toluenesulfonic acid monohydride (**S8, Debio-1452 Tosylate**)- **S7, Debio-1452** (1 eq, 1.5 mmol) was suspended in THF (120 mL) and heated to reflux. After 30 min, *p*-toluene sulfonic acid monohydrate (1.05 eq, 1.58 mmol) in dioxane (12 mL) was added to the reaction mixture and stirred for 1h. The reaction mixture was allowed to cool to room temperature and diluted with a mixture of 1:1 ether:*n*-pentane (80 mL), filtered, rinsed with 1:1 ether:*n*-pentane, and dried to afford (*E*)-*N*-methyl-*N*-((3-methylbenzofuran-2-yl)methyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide *p*-toluenesulfonic acid (**S8, Debio-1452 Tosylate,** 0.756 g, 1.38 mmol, 92%) as a white solid. The product was further processed for *in vivo* efficacy studies to improve solubility. For these studies, **Debio-1452 Tosylate** was ground in a mortar and pestle and then sieved through a 75 µM mesh.

Note: Tabulated NMR data for acrylamide derivatives consist of two rotamers that exist at room temperature in a ratio of 40:60 and is reflected in the reported integral values.

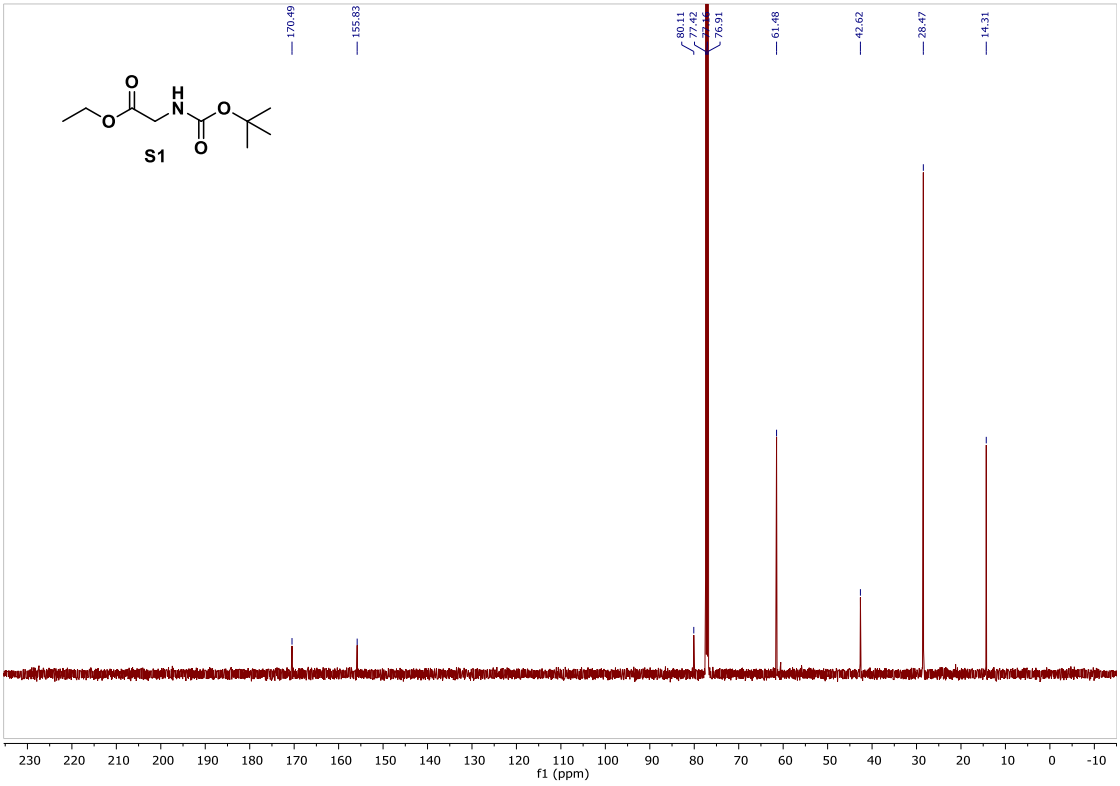
^{1}H NMR (500 MHz, DMSO-d_6): δ 10.69 (s, 1H), 9.49 (brs, 1H), 8.42 – 8.32 (m, 1H), 8.17 – 8.05 (m, 1H), 7.59 – 7.54 (m, 1H), 7.54 – 7.43 (m, 4.4H), 7.31 – 7.22 (m, 2H), 7.21 (d, J = 12.6 Hz, 0.6H), 7.15 – 7.09 (m, 2H), 4.99 (s, 0.8H), 4.79 (s, 1.2H), 3.18 (s, 1.8H), 2.96 – 2.89 (m, 3.2H), 2.57 – 2.51 (m, 2H), 2.29 (s, 3H), 2.26 (s, 3H).

Experimental information for the above compound has been previously reported [6].

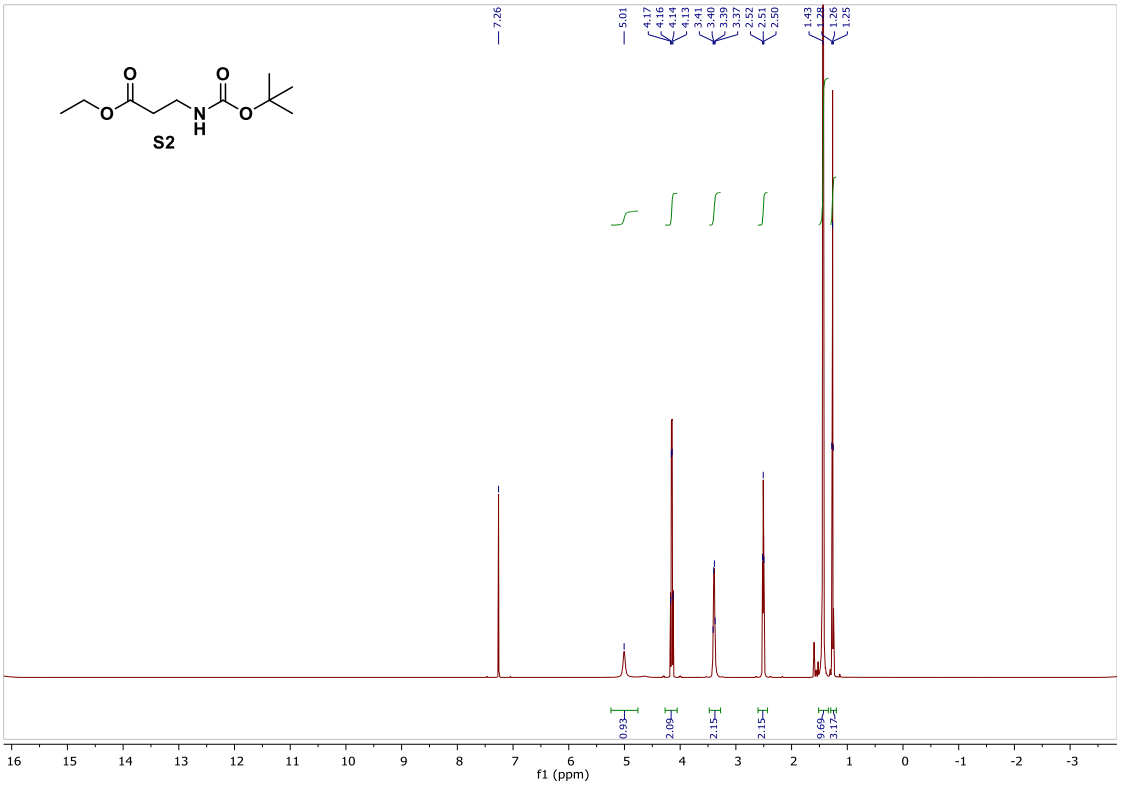
^{1}H NMR (500 MHz, chloroform-*d*):



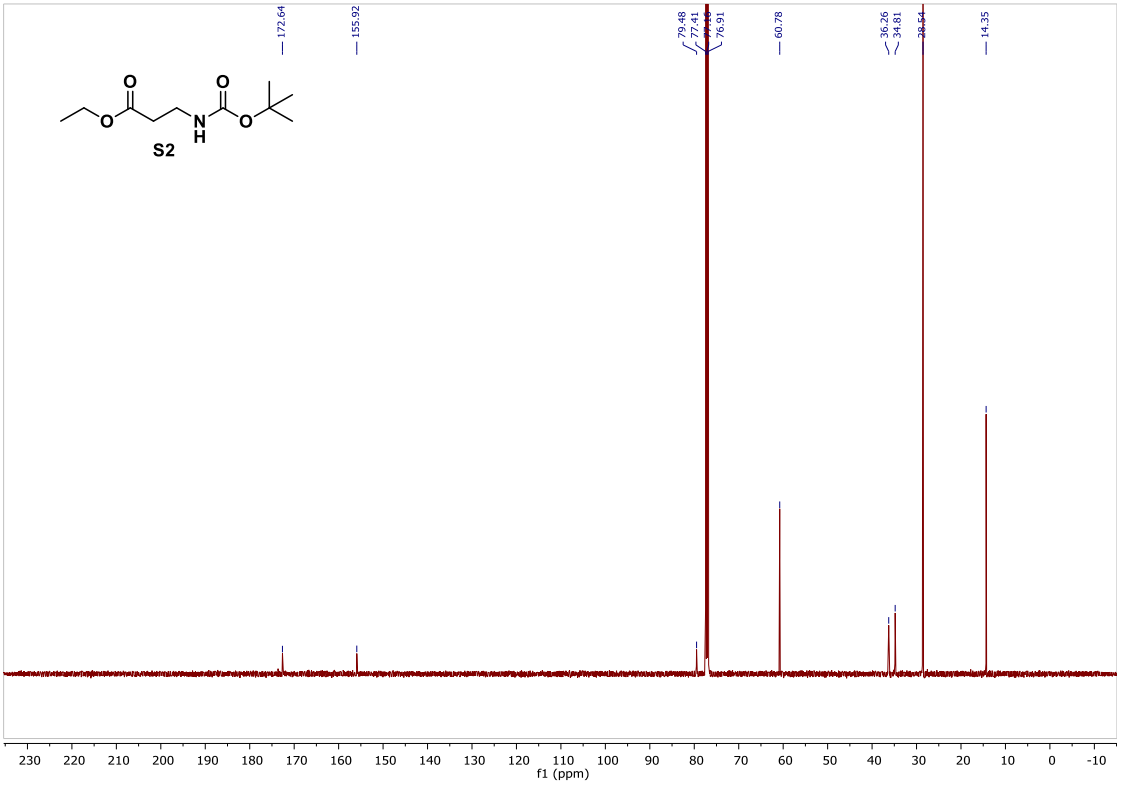
^{13}C NMR (126 MHz, chloroform-*d*):



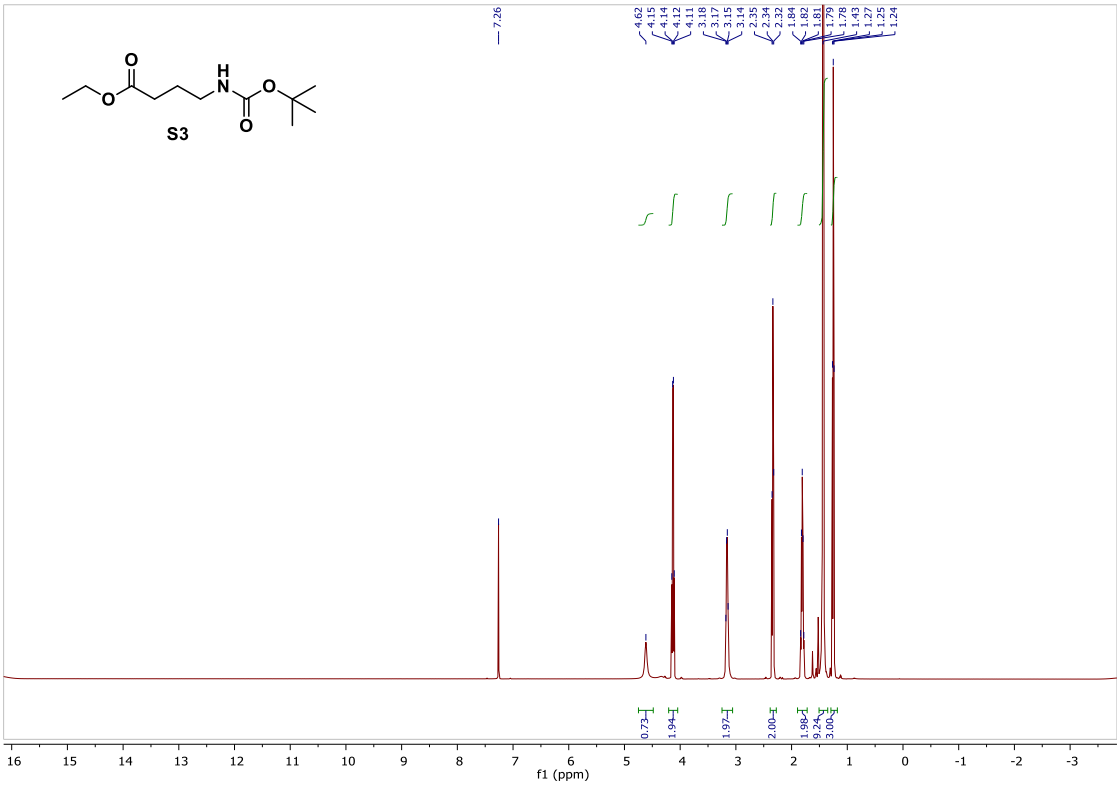
^{1}H NMR (500 MHz, chloroform-*d*):



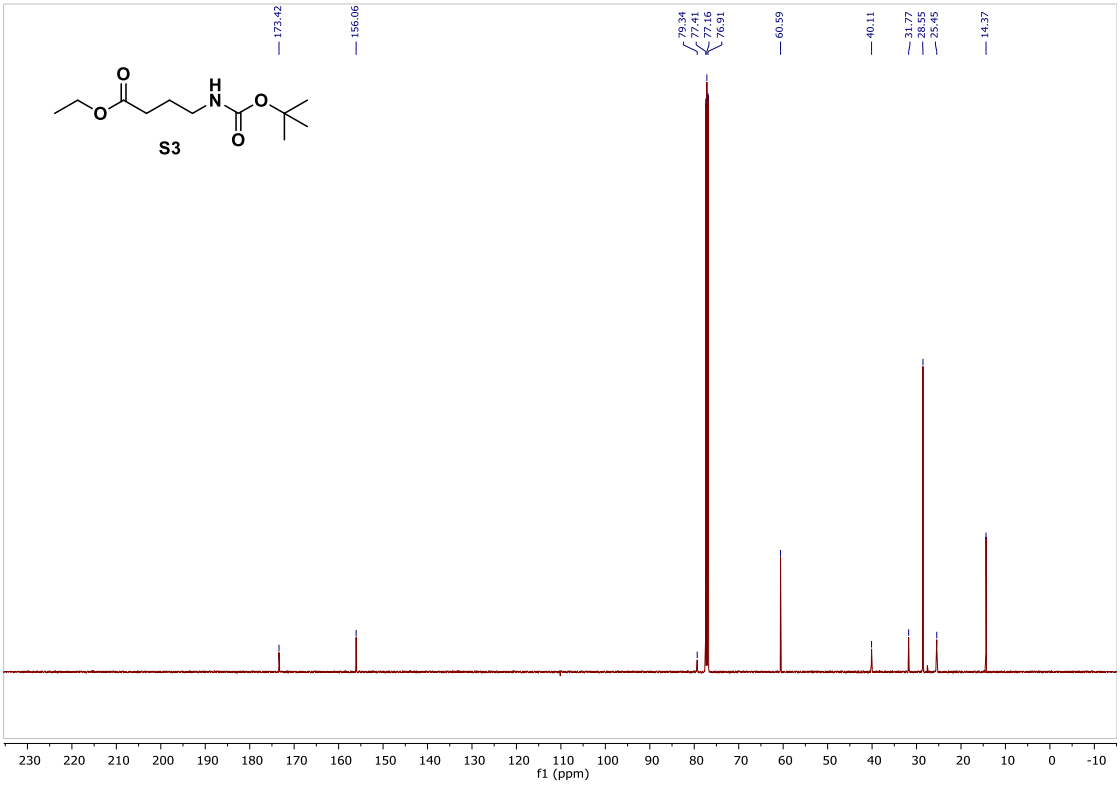
^{13}C NMR (126 MHz, chloroform-*d*):



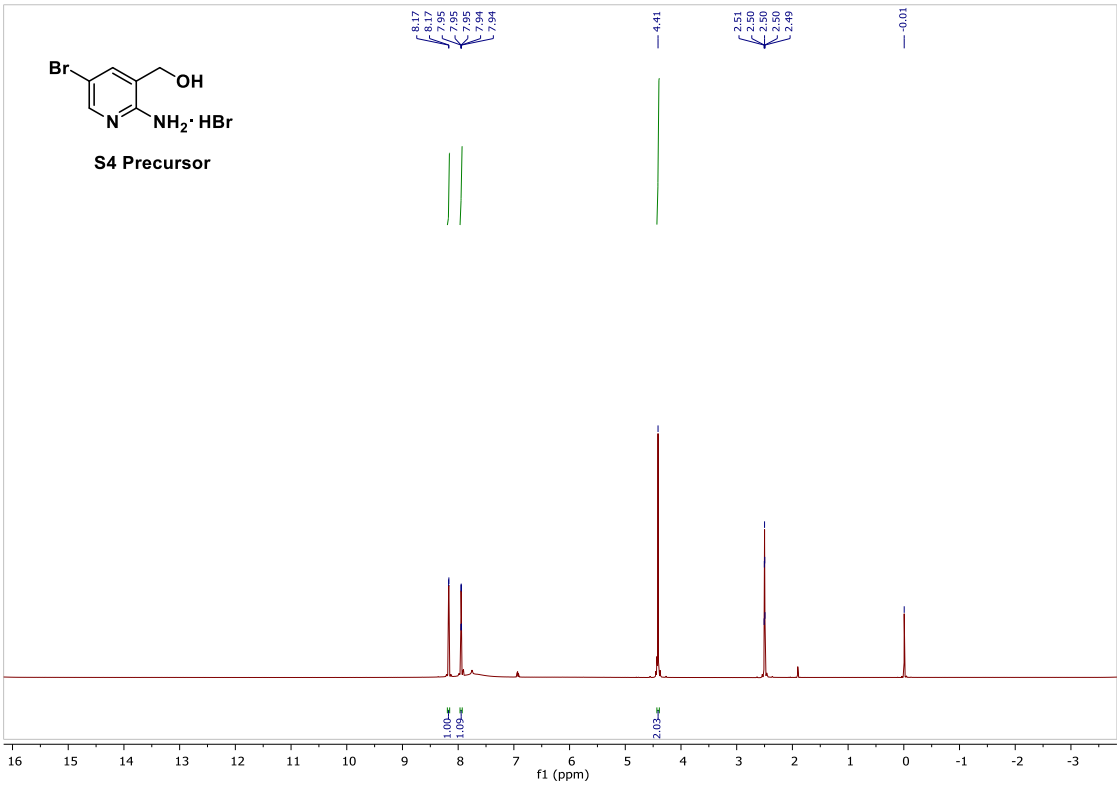
^{1}H NMR (500 MHz, chloroform-*d*):



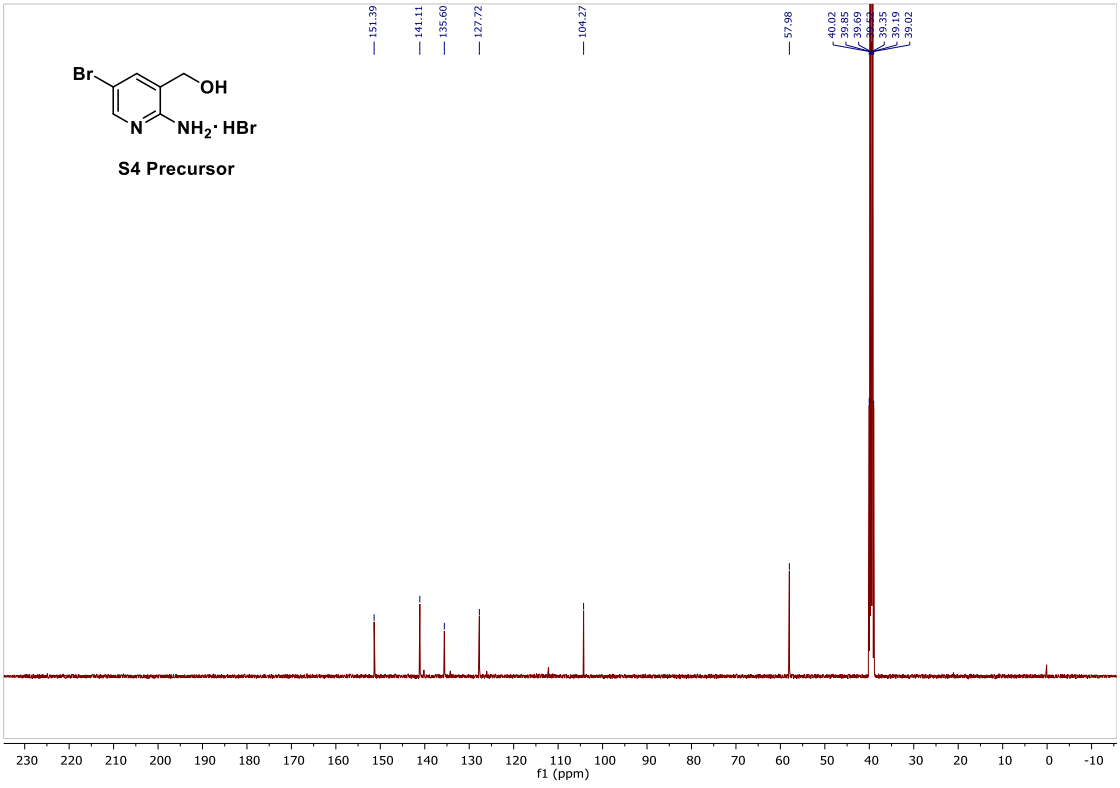
^{13}C NMR (126 MHz, chloroform-*d*):



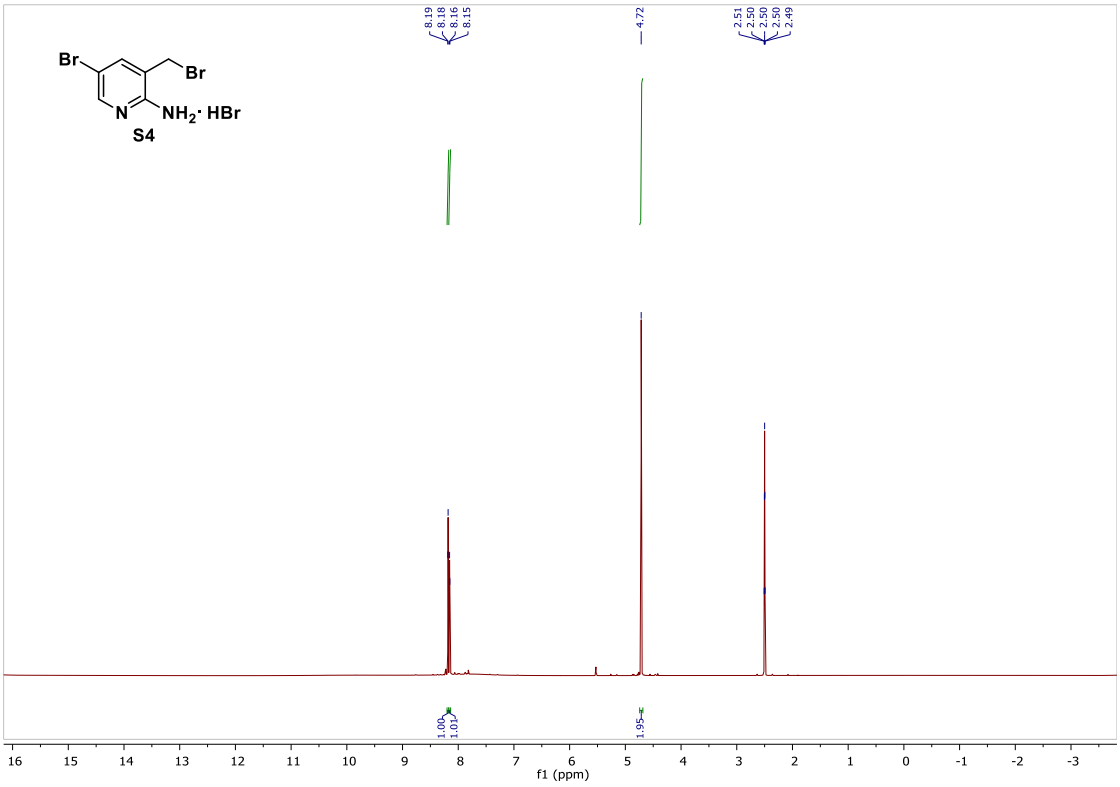
^{1}H NMR (500 MHz, DMSO-d_6):



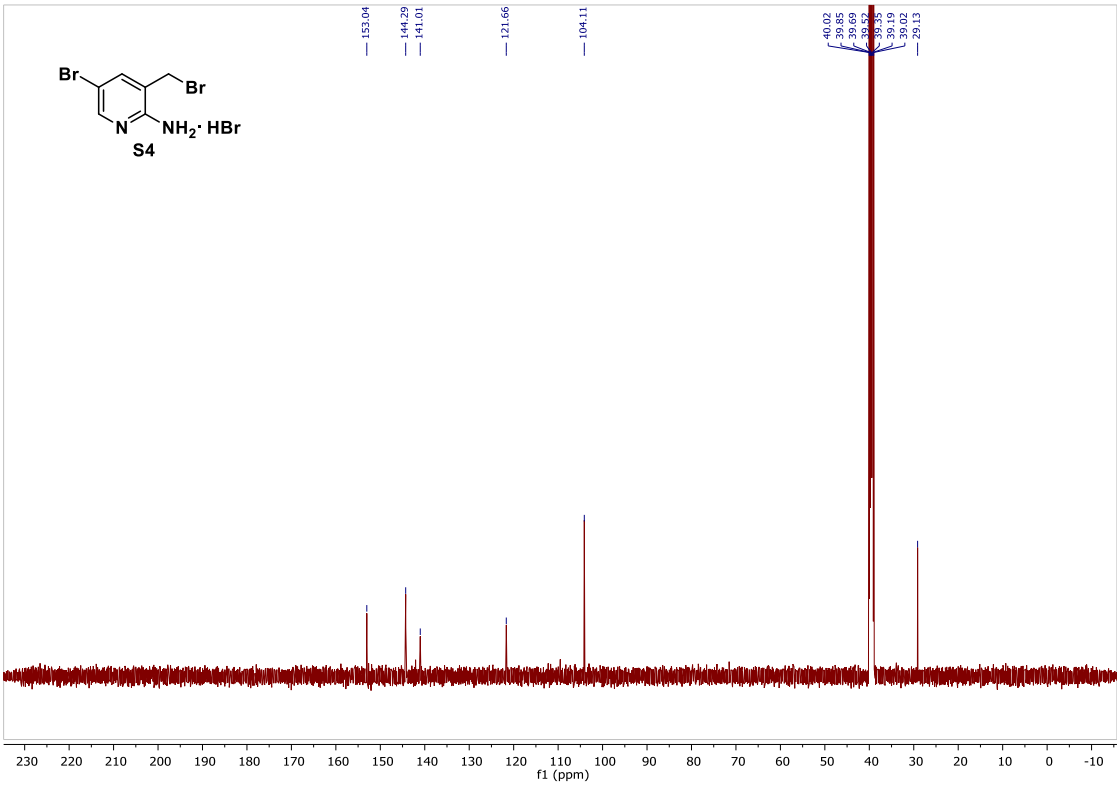
^{13}C NMR (126 MHz, DMSO-d_6):



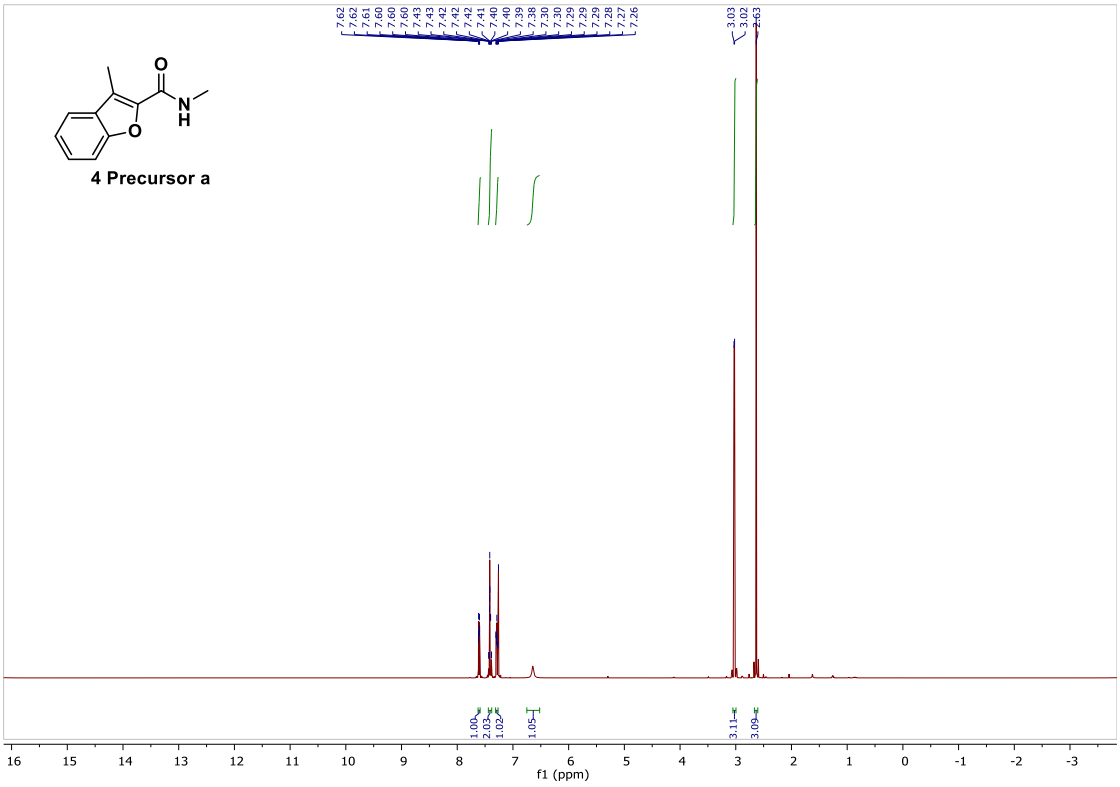
^{1}H NMR (500 MHz, DMSO-d_6):



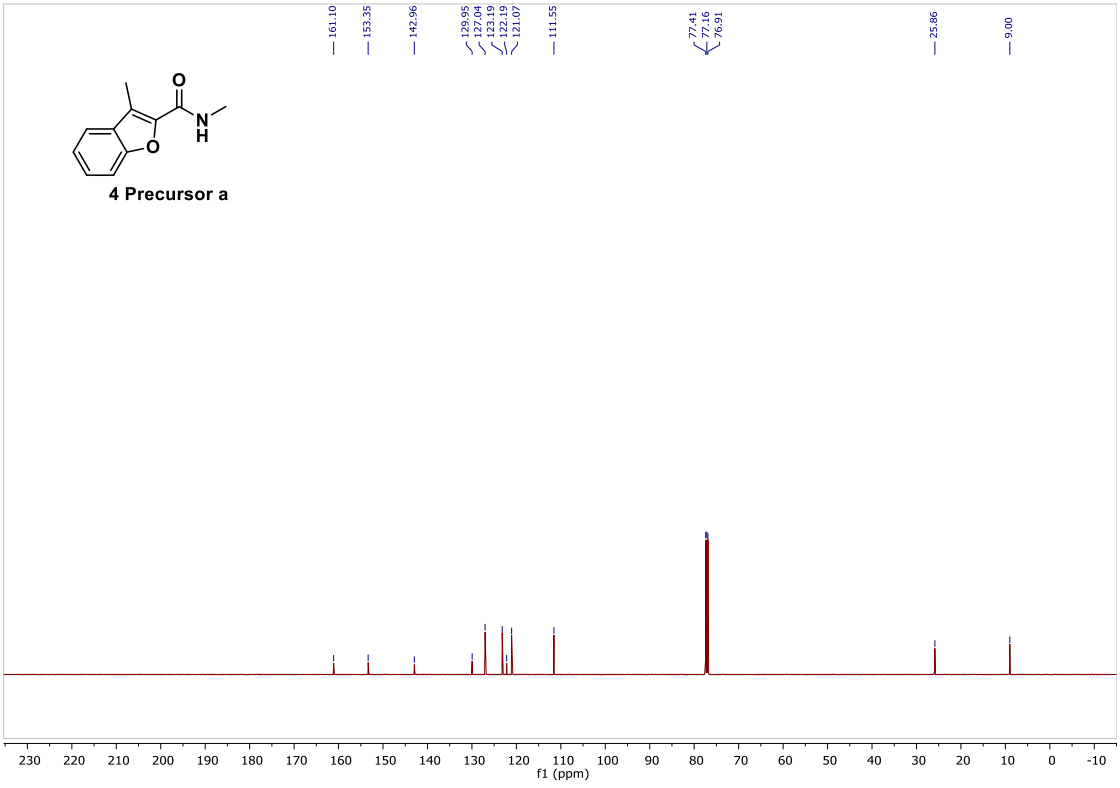
^{13}C NMR (126 MHz, DMSO-d_6)



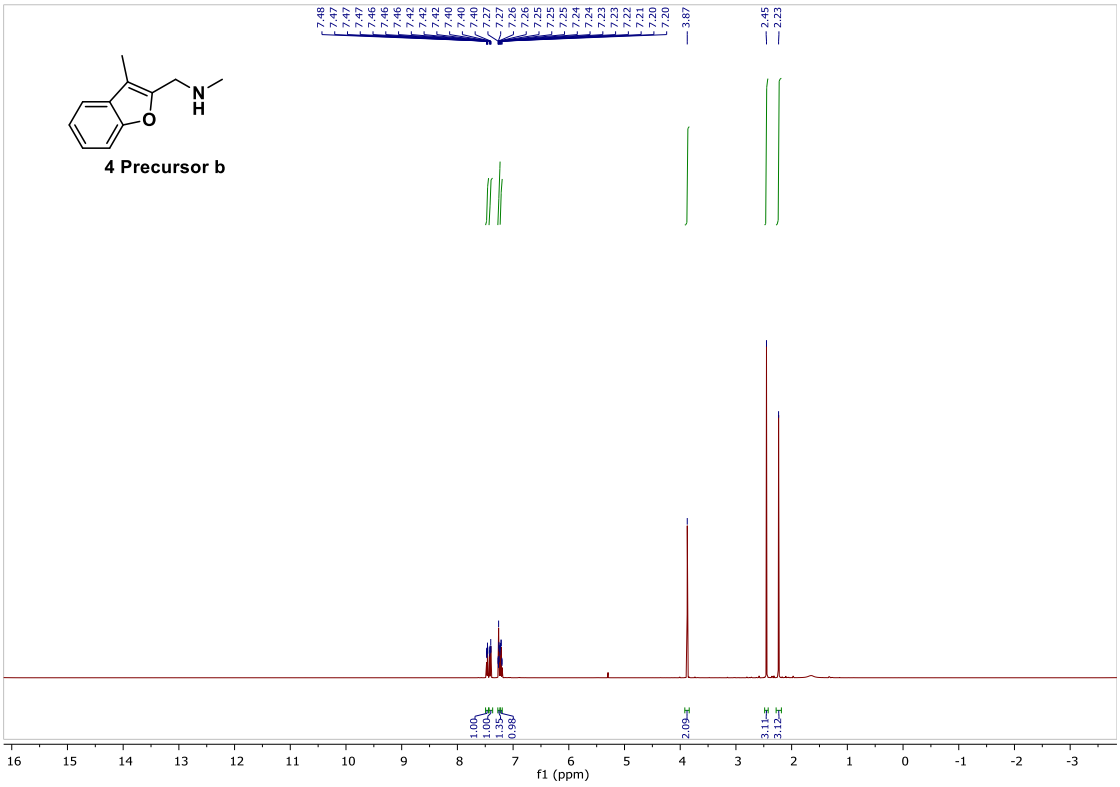
^{1}H NMR (500 MHz, chloroform-*d*):



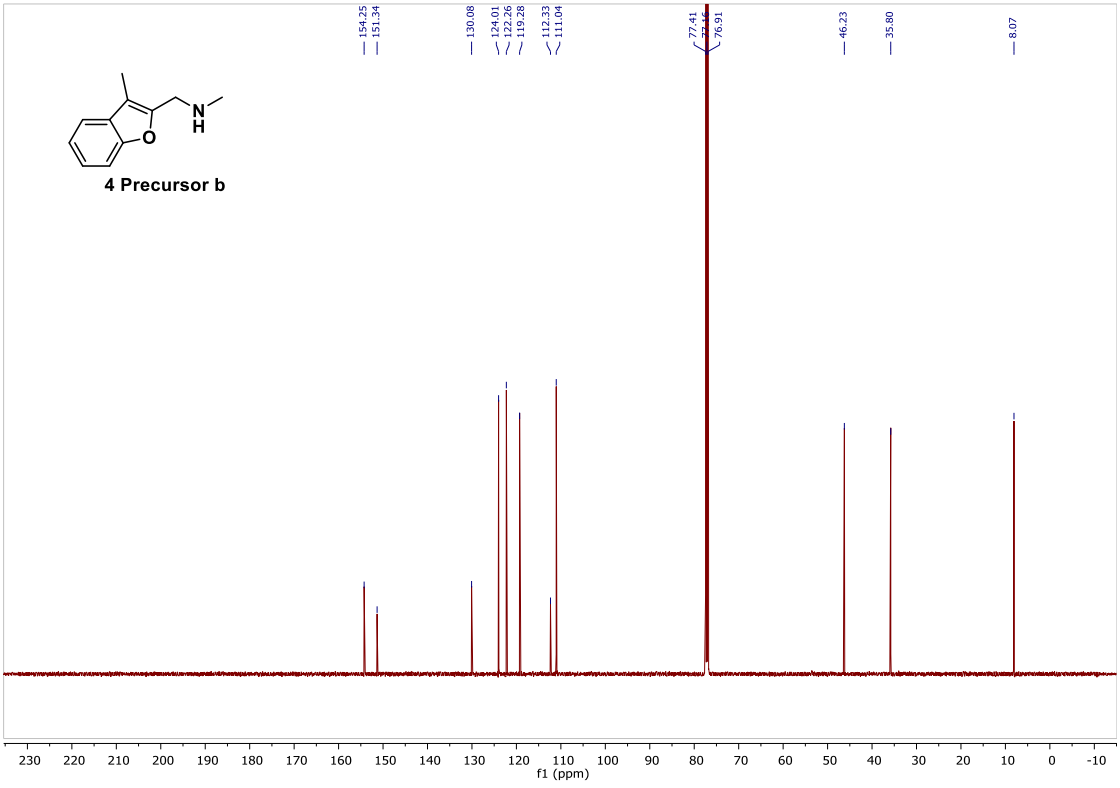
^{13}C NMR (126 MHz, chloroform-*d*):



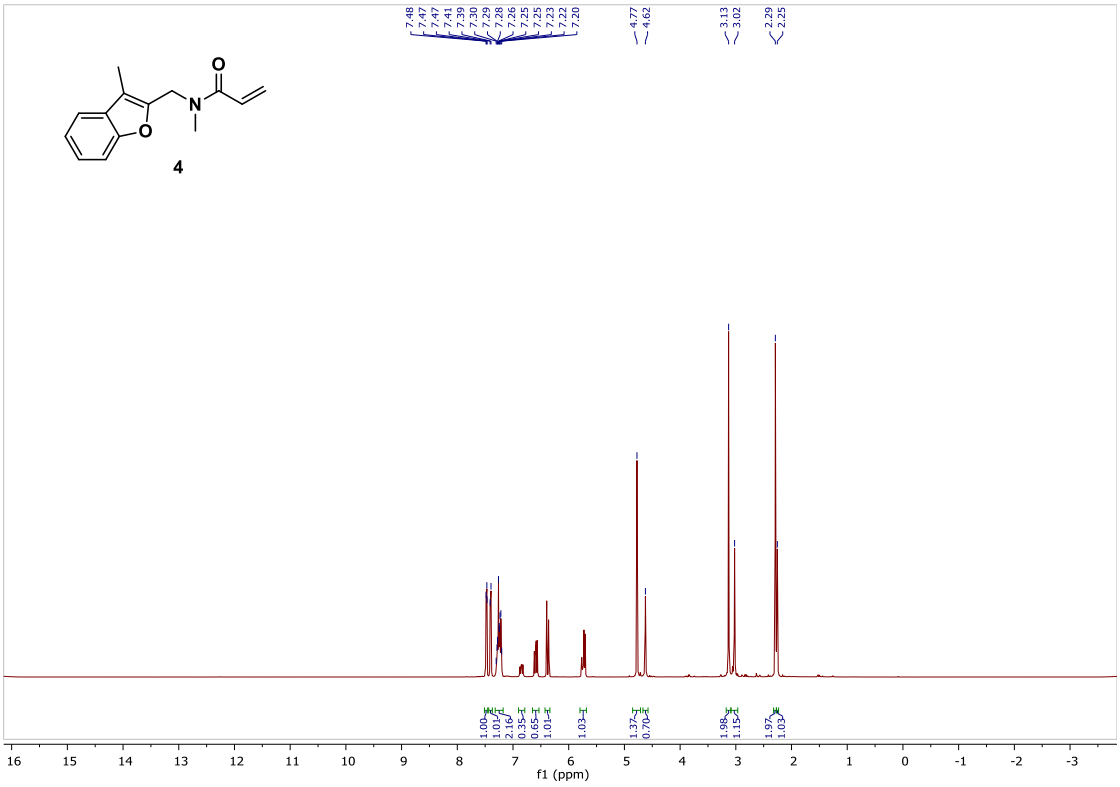
^{1}H NMR (500 MHz, chloroform-*d*):



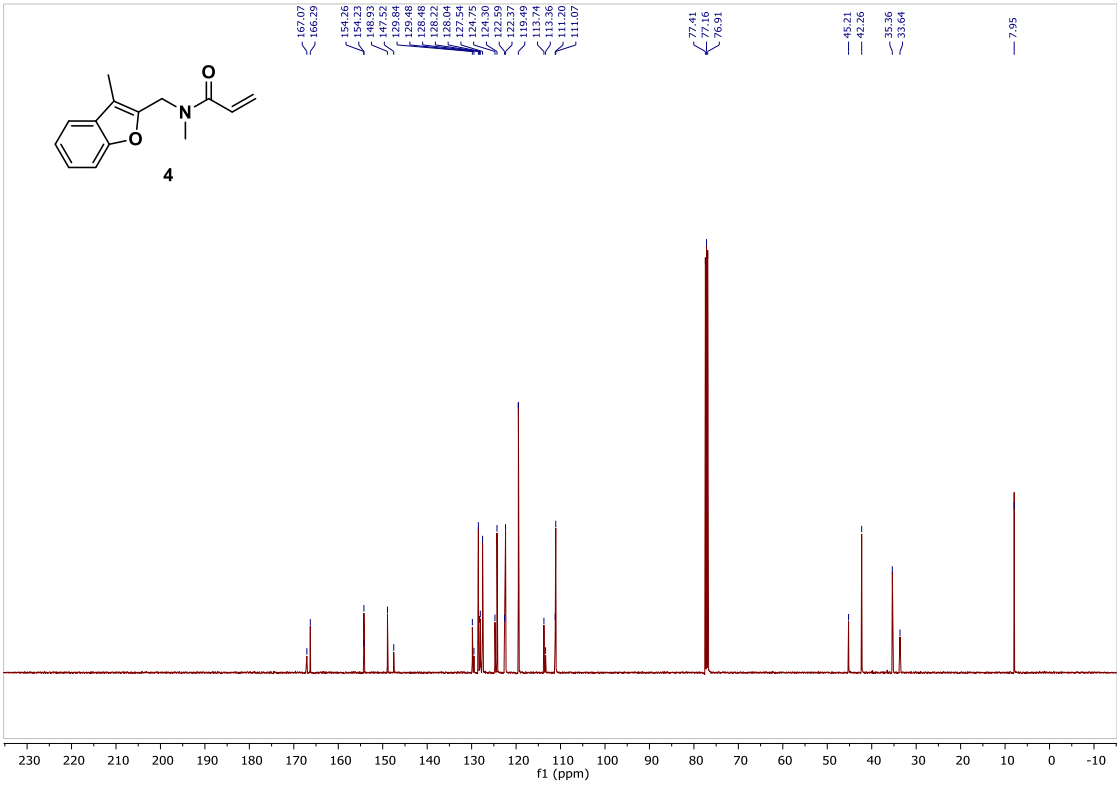
^{13}C NMR (126 MHz, chloroform-*d*):



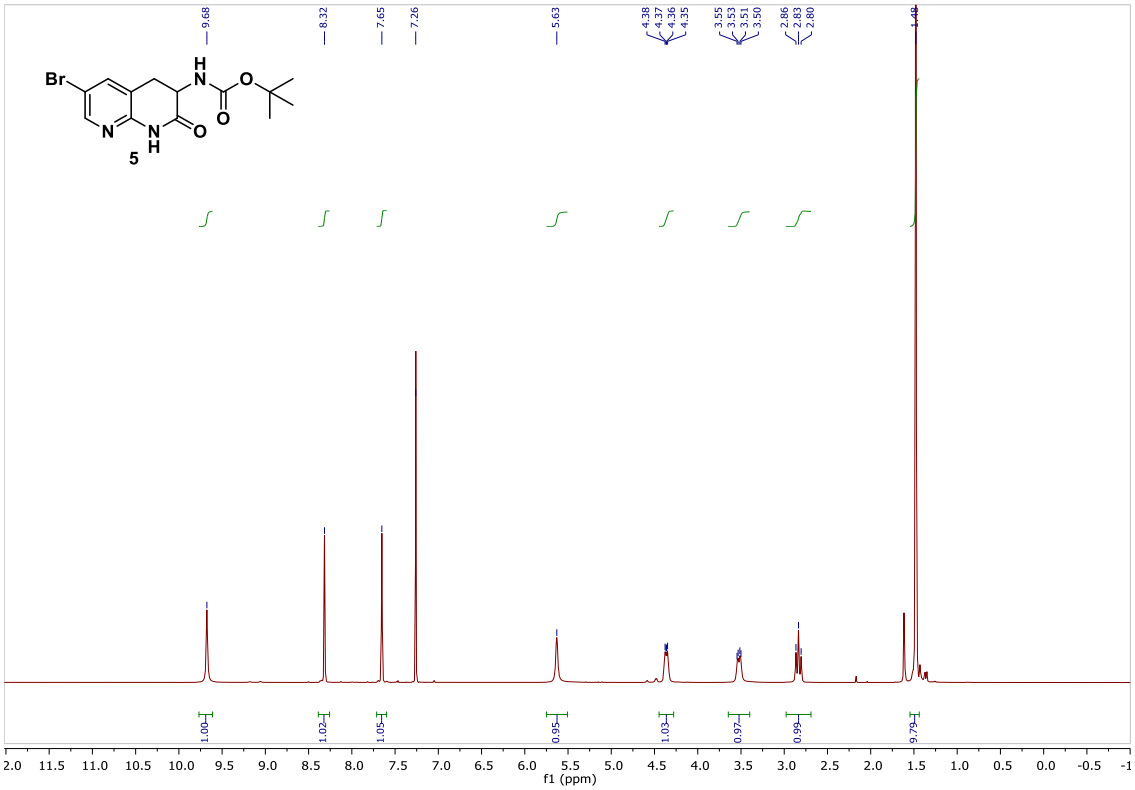
^{1}H NMR (500 MHz, chloroform-*d*):



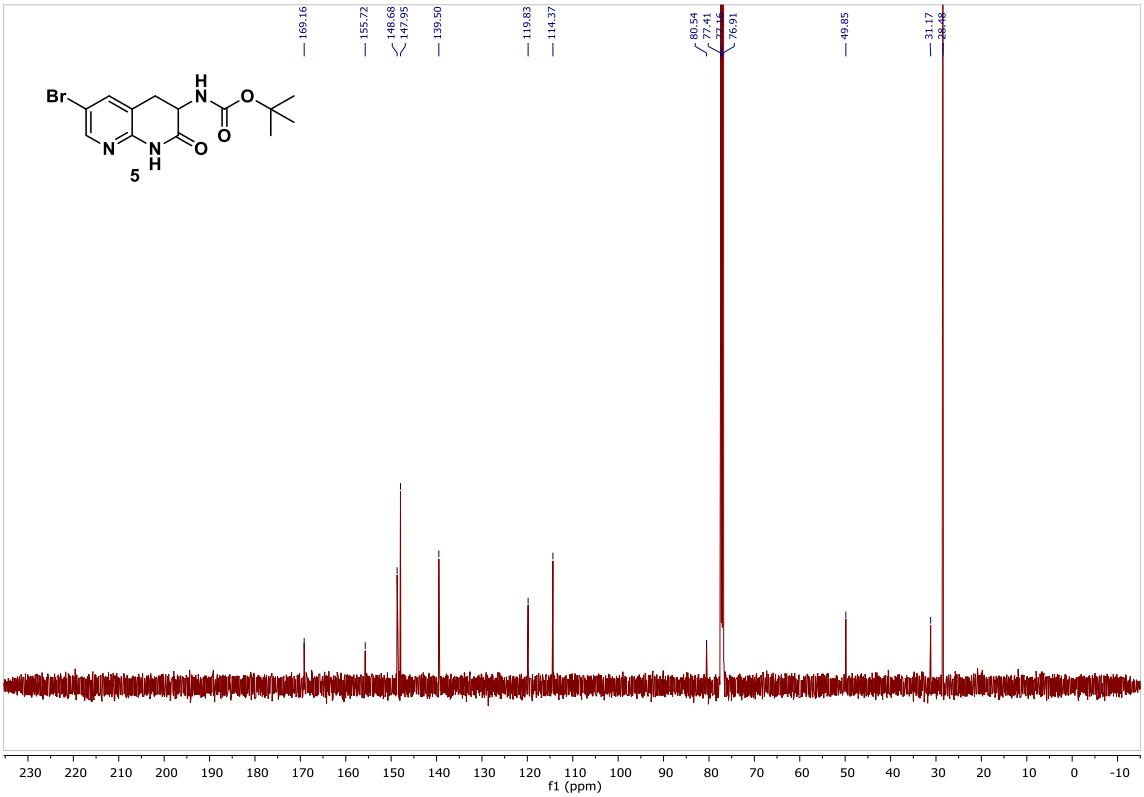
^{13}C NMR (126 MHz, chloroform-*d*):



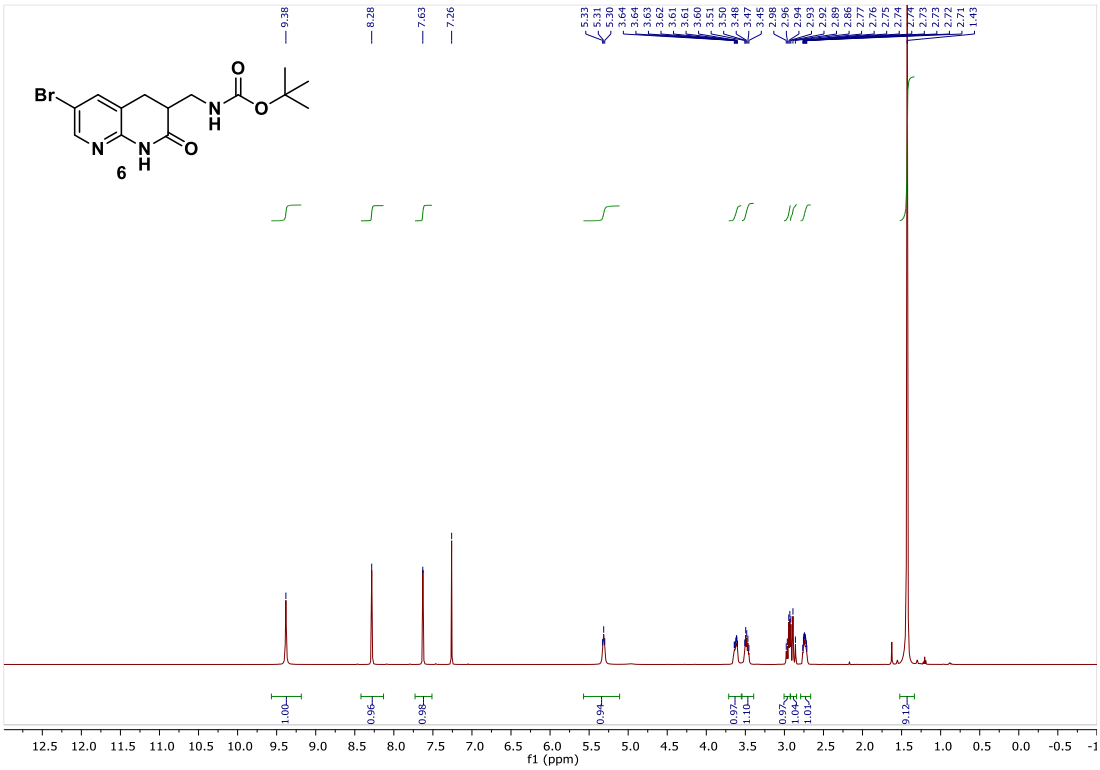
^{1}H NMR (500 MHz, chloroform-*d*):



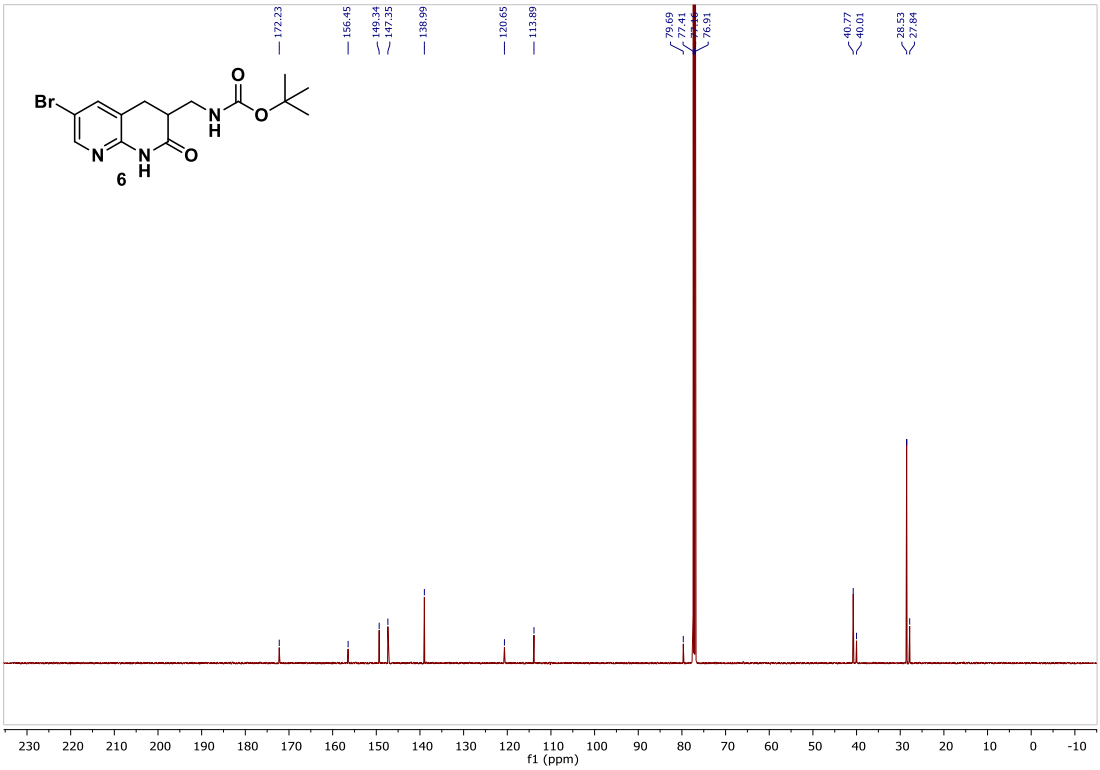
^{13}C NMR (126 MHz, chloroform-*d*):



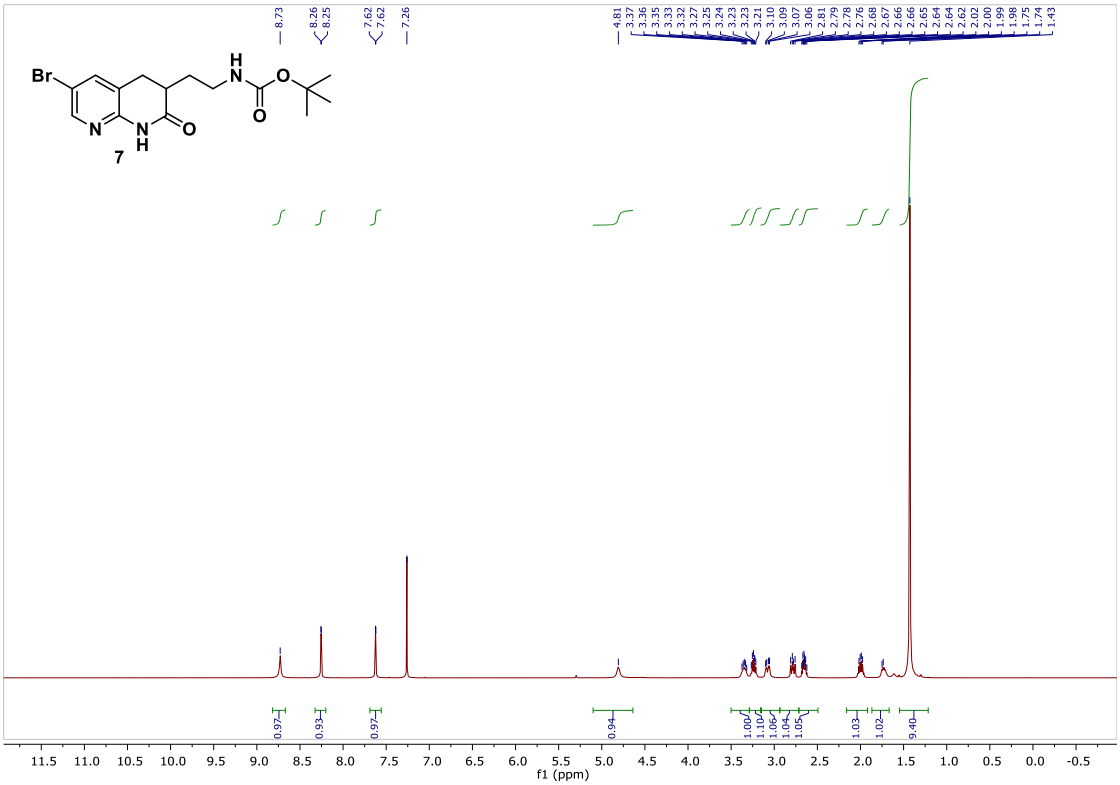
^{1}H NMR (500 MHz, chloroform-*d*):



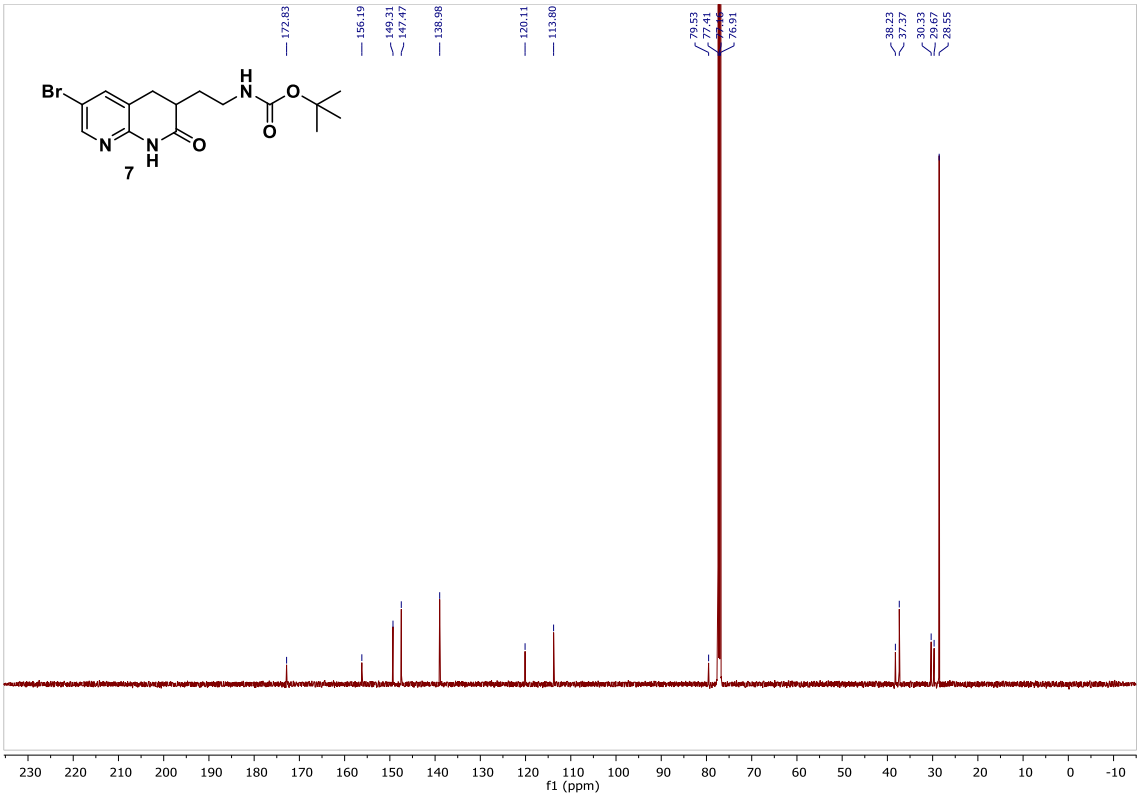
^{13}C NMR (126 MHz, chloroform-*d*):



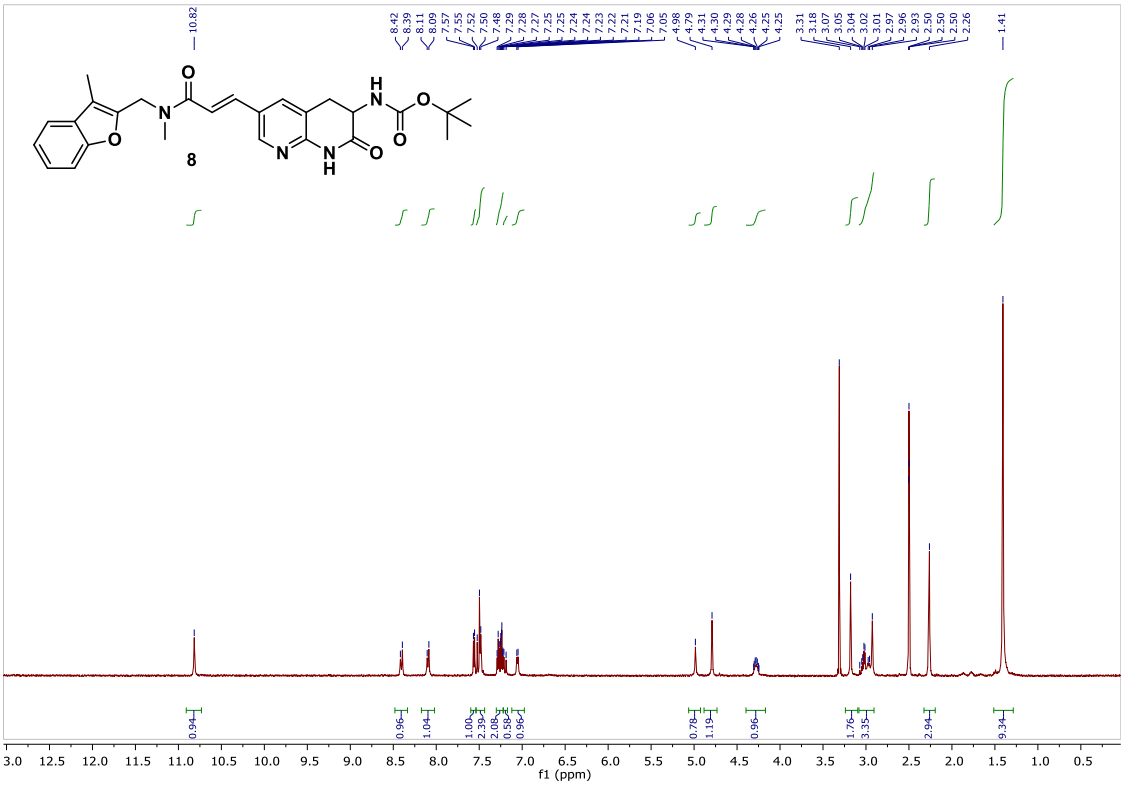
^{1}H NMR (500 MHz, chloroform-*d*):



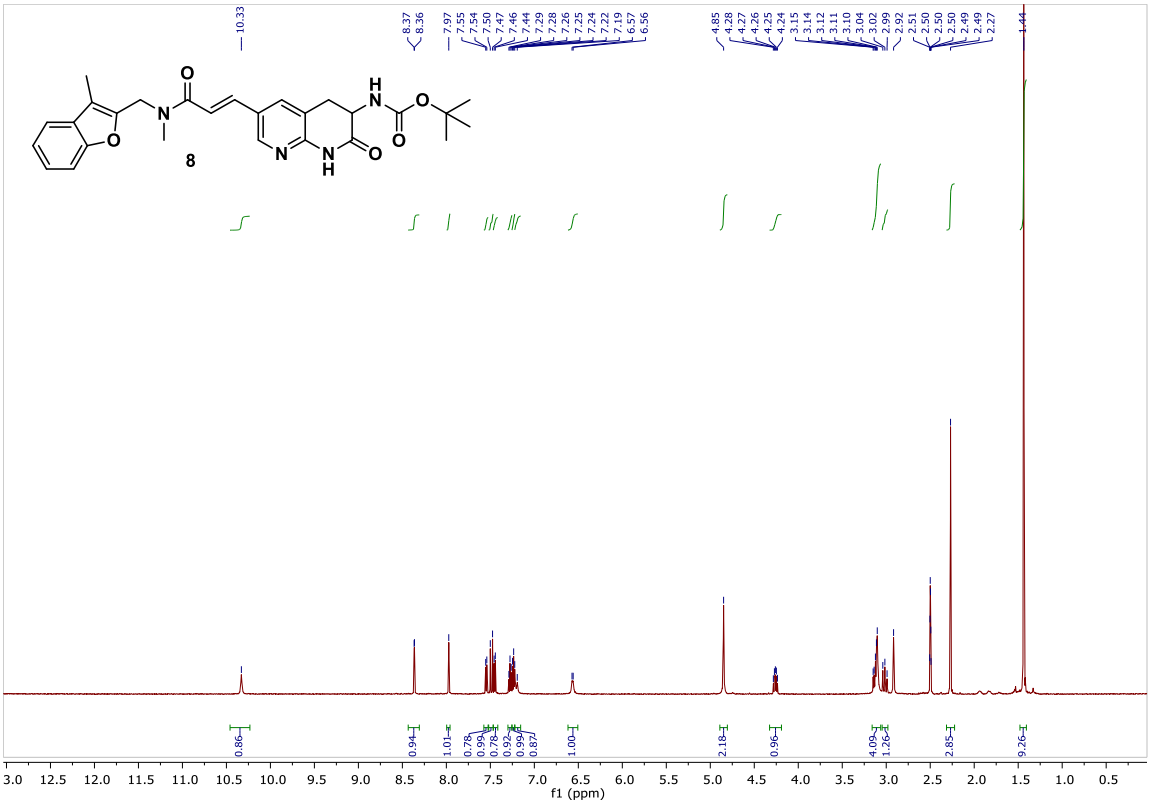
^{13}C NMR (126 MHz, chloroform-*d*):



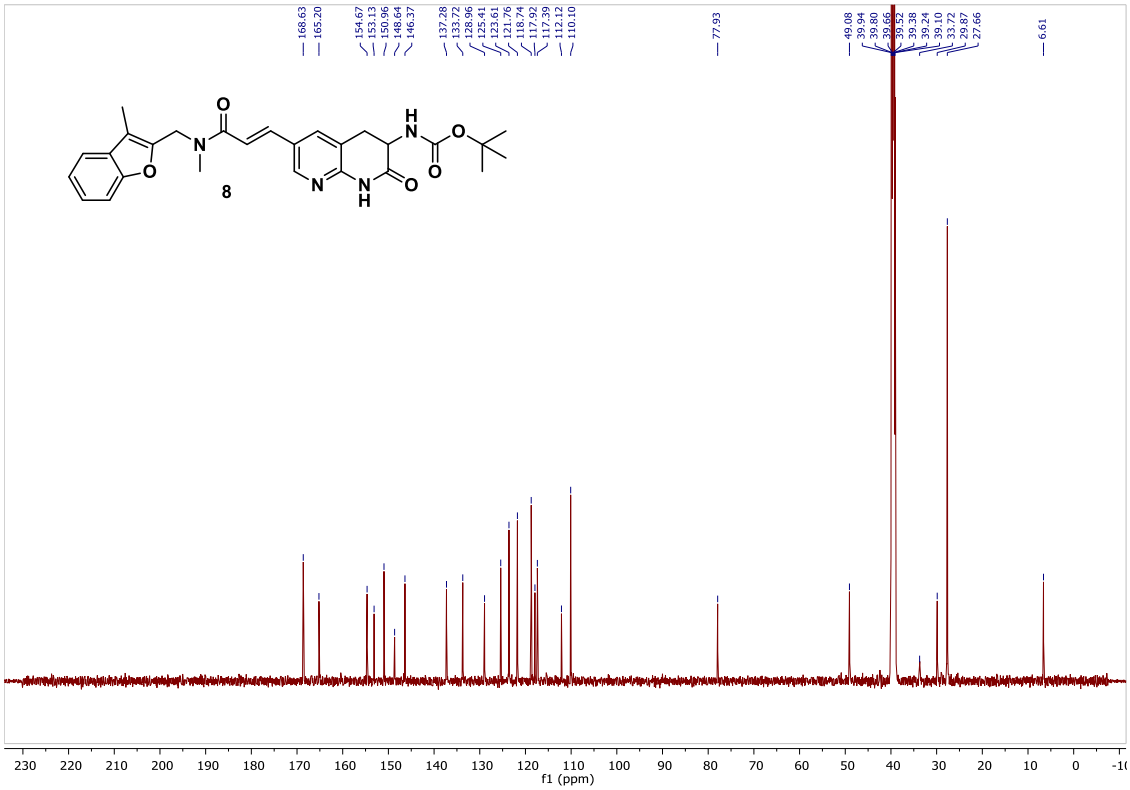
^{1}H NMR (600 MHz, DMSO-d_6, 25 ^{o}C):



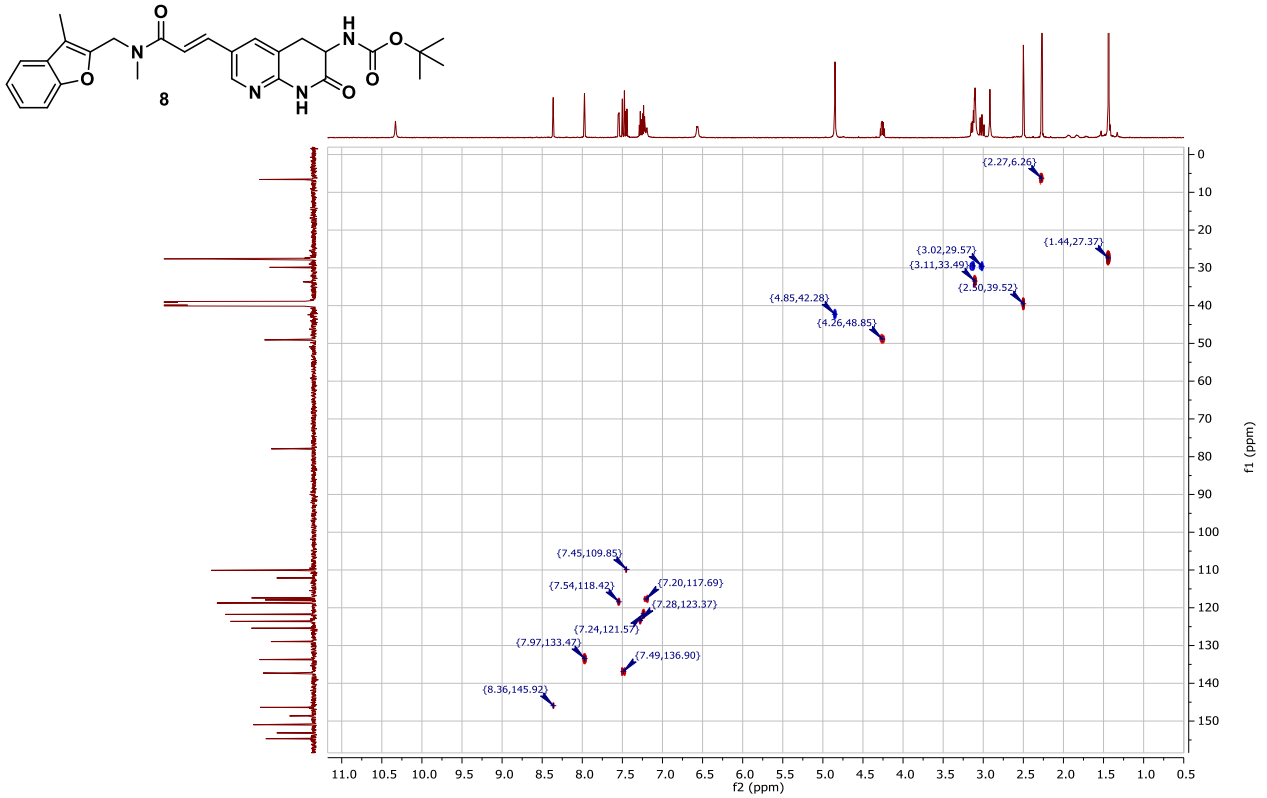
^{1}H NMR (600 MHz, DMSO-d_6, 115 ^{o}C):



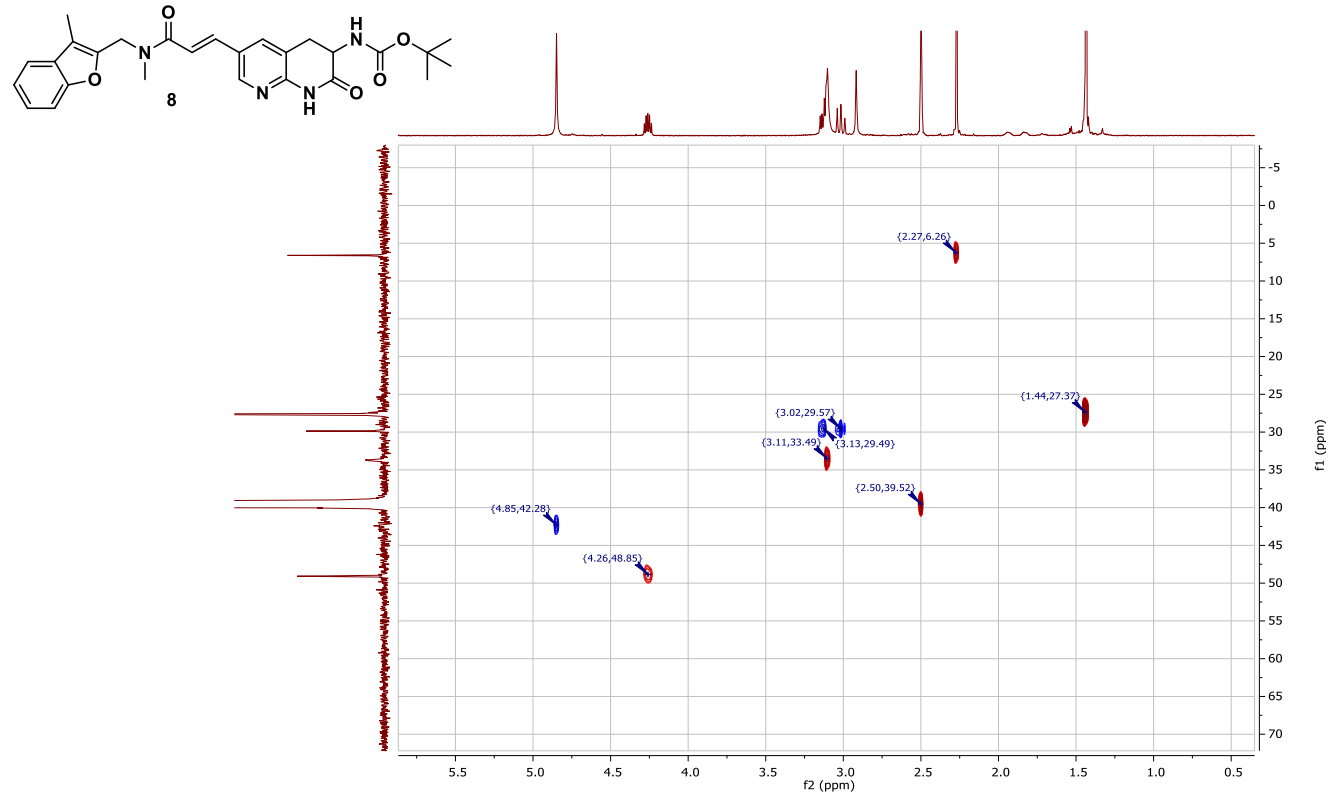
^{13}C NMR (151 MHz, DMSO-d_6, 115 ^{o}C):



gHSQC (DMSO-d_6, 115 °C):



gHSQC (DMSO-d_6, 115 °C):



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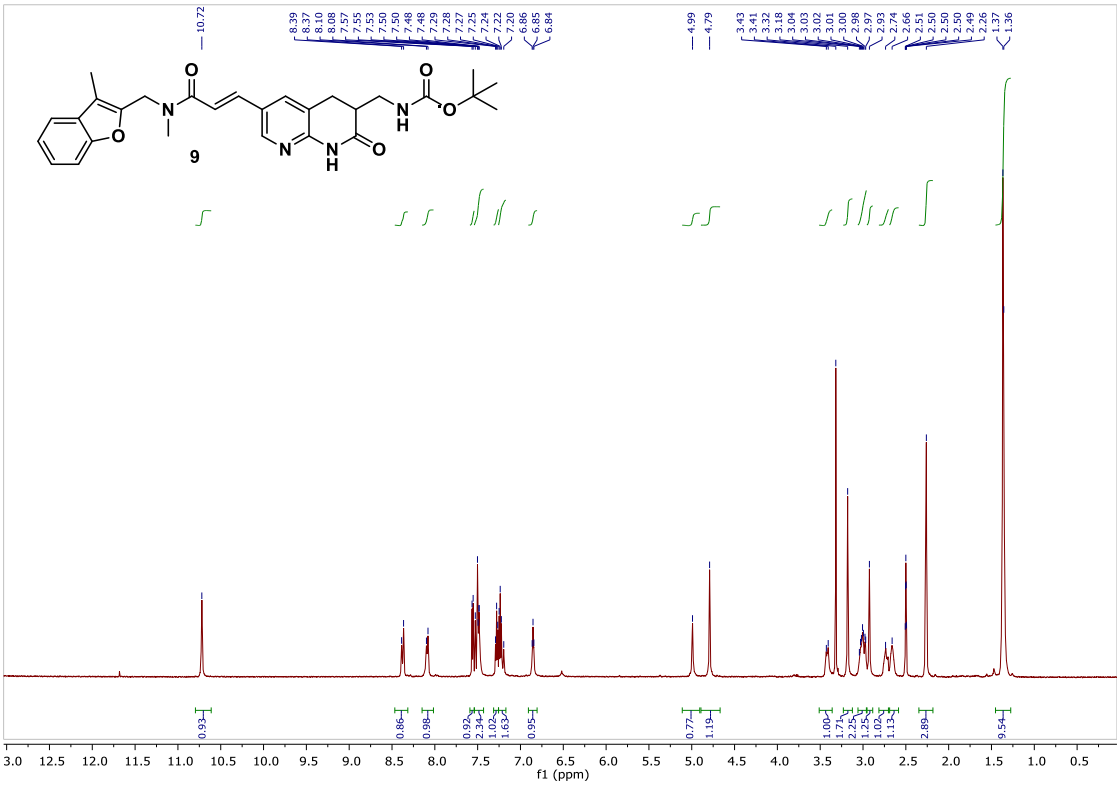
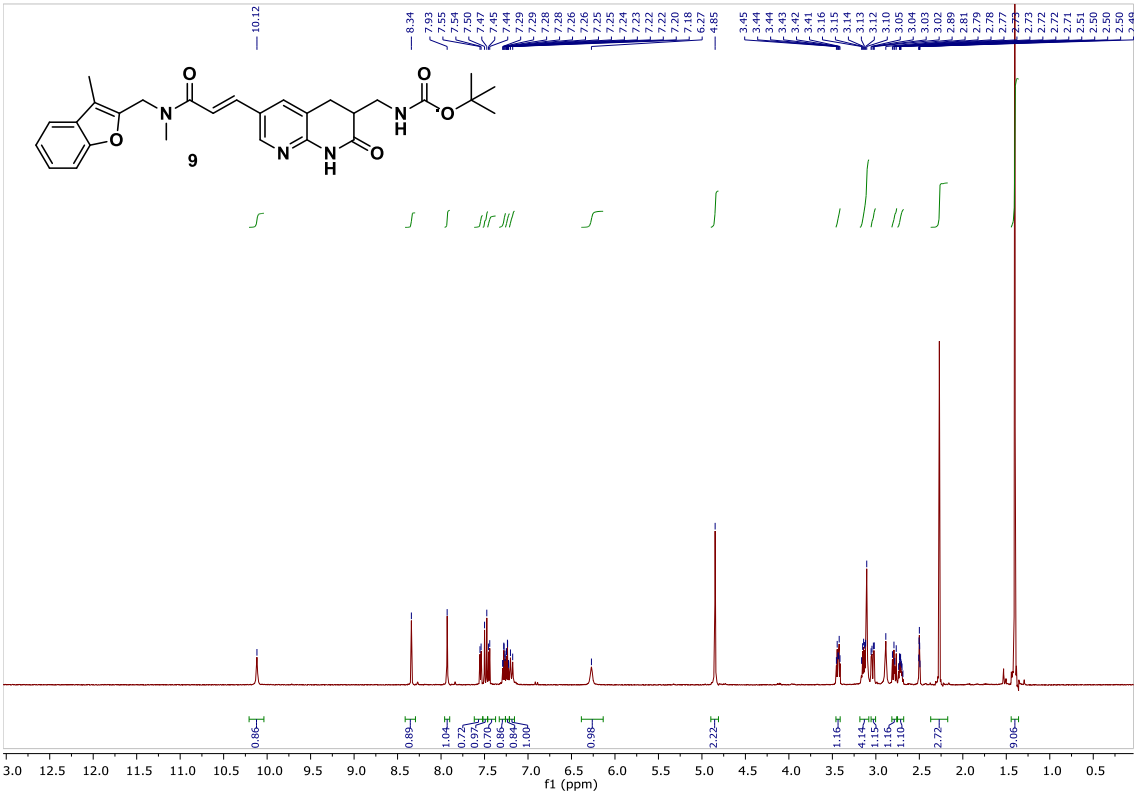
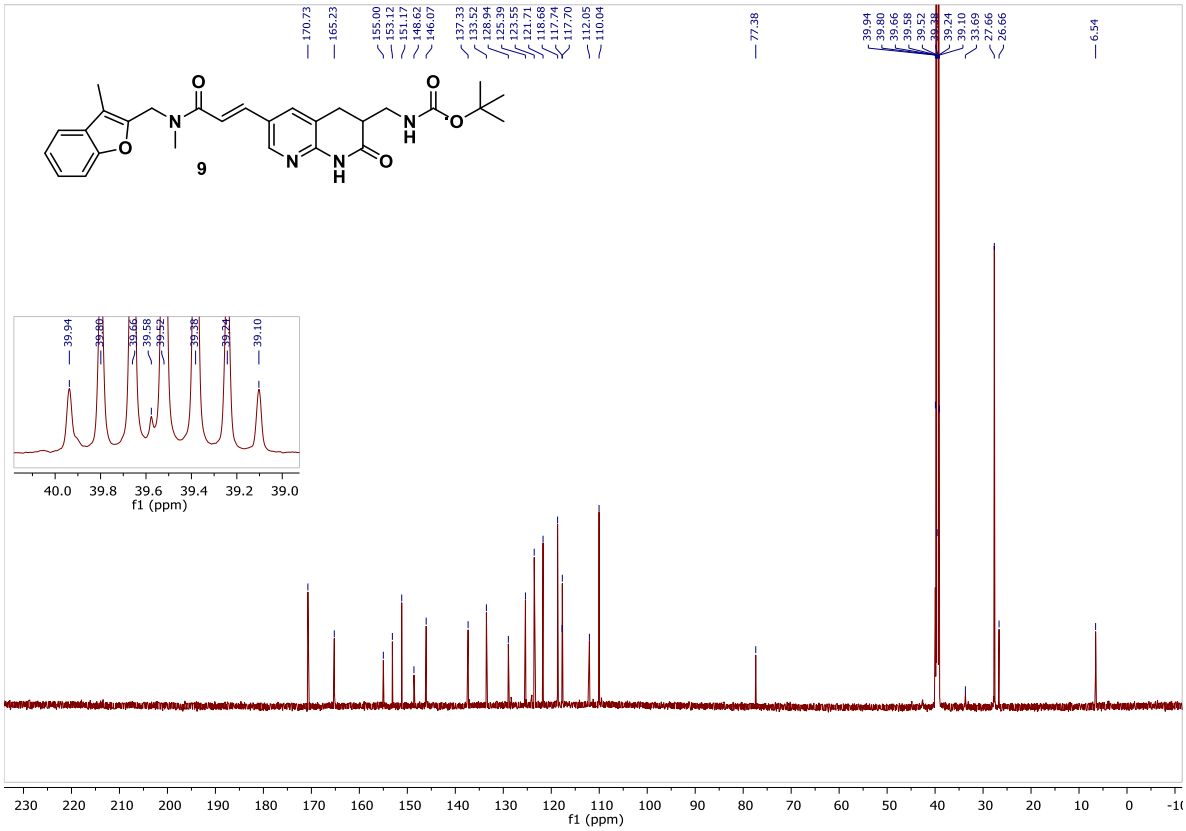


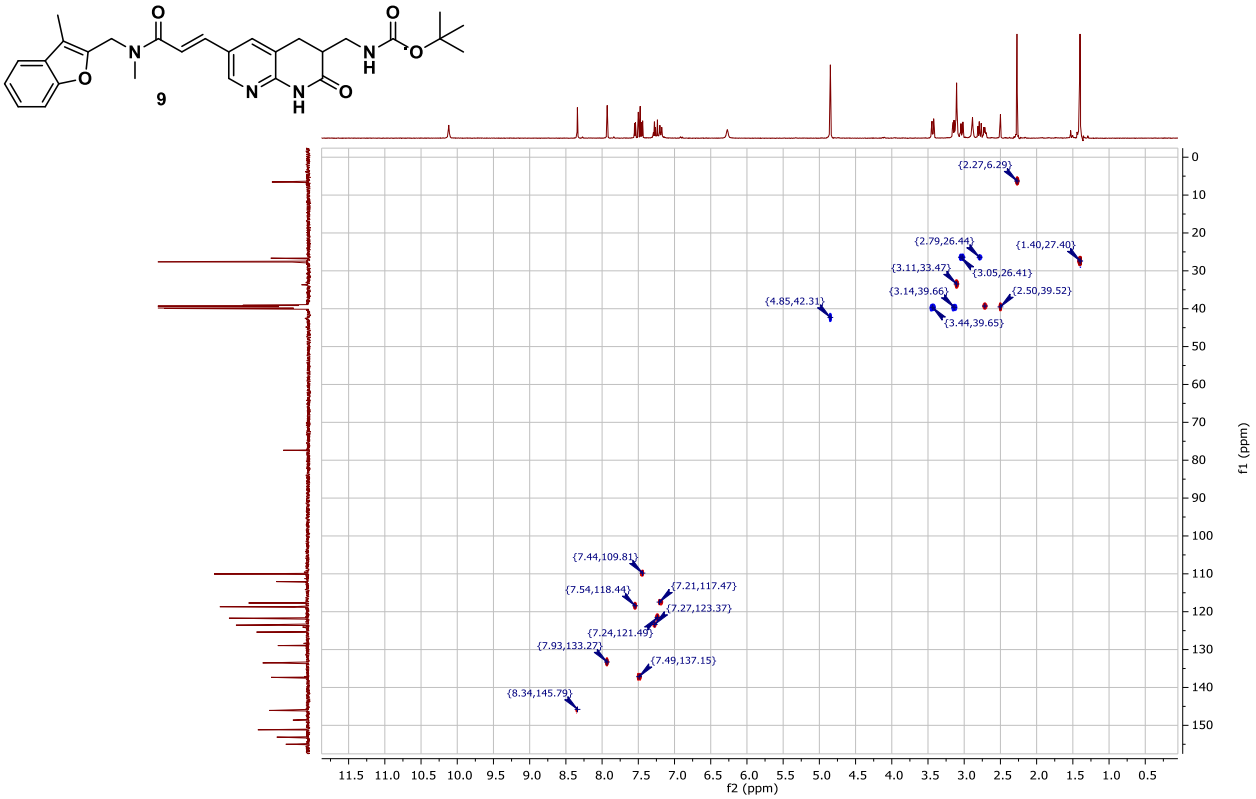
^{1}H NMR (600 MHz, DMSO-d_6, 120 ^{o}C):



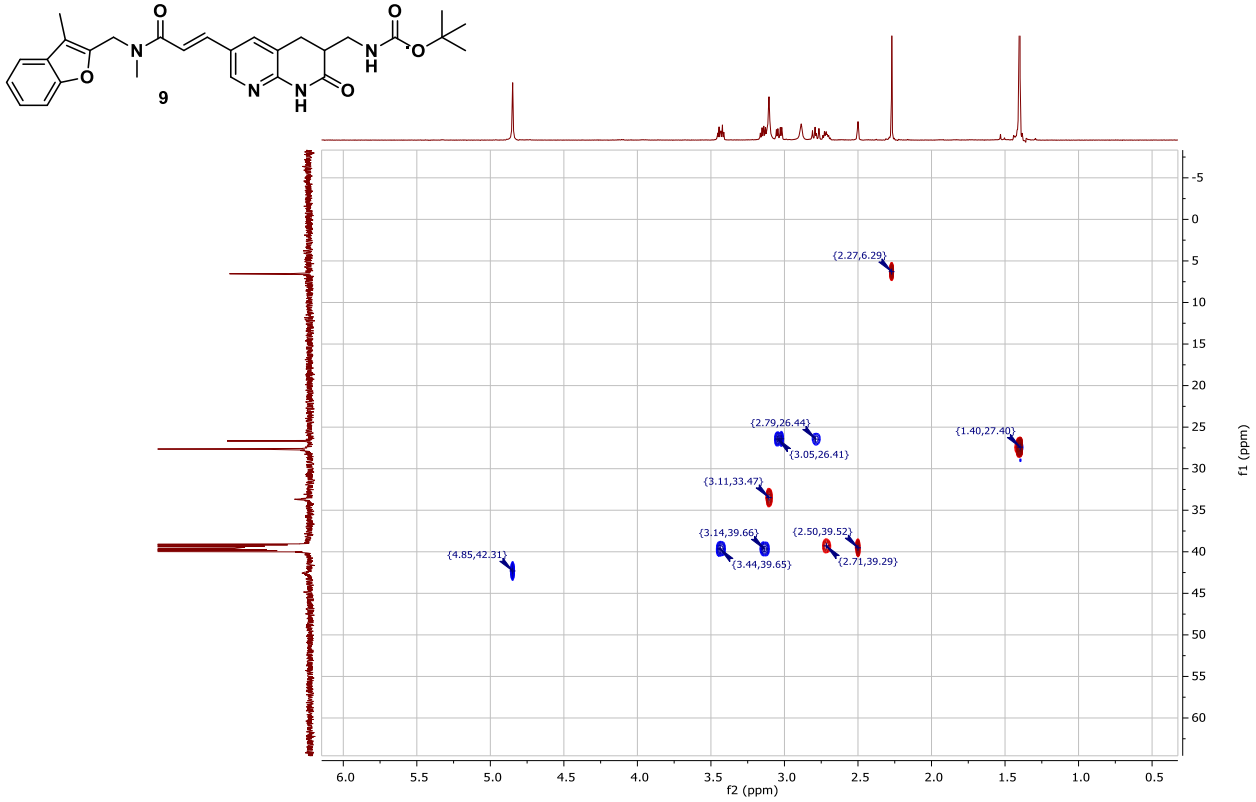
^{13}C NMR (151 MHz, DMSO-d_6, 120 ^{o}C):



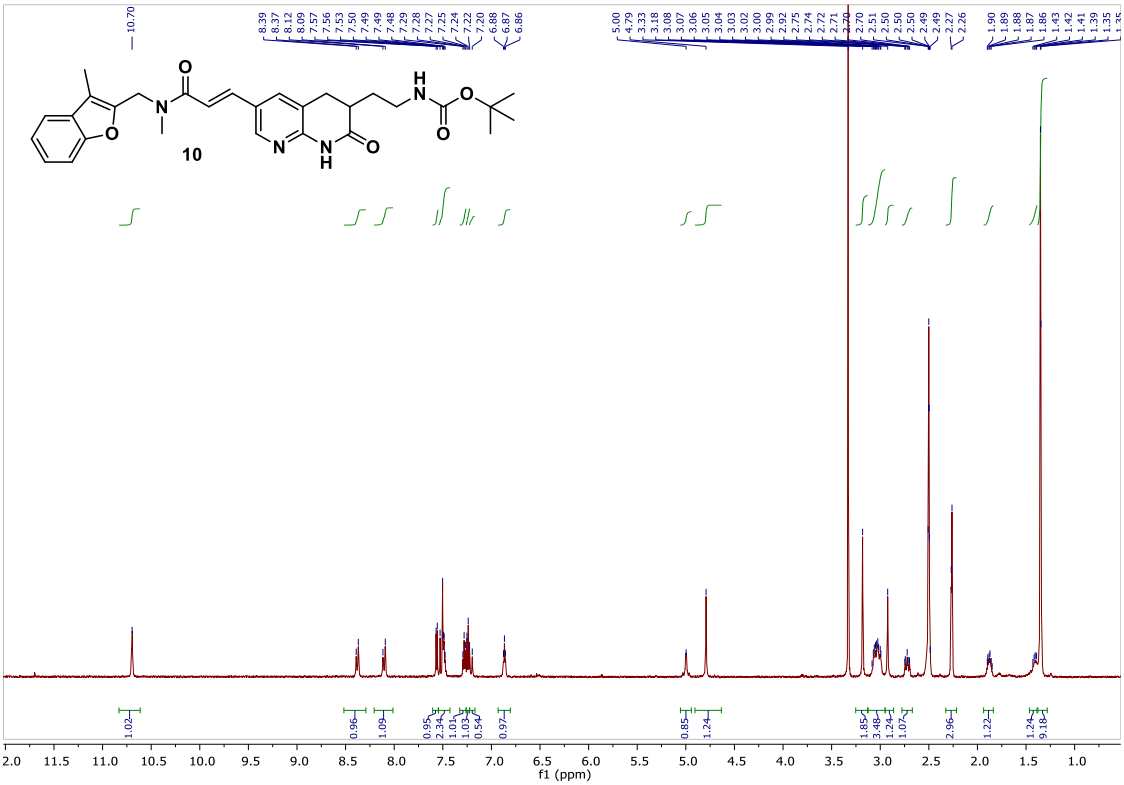
gHSQC (DMSO-d_6, 115 °C):



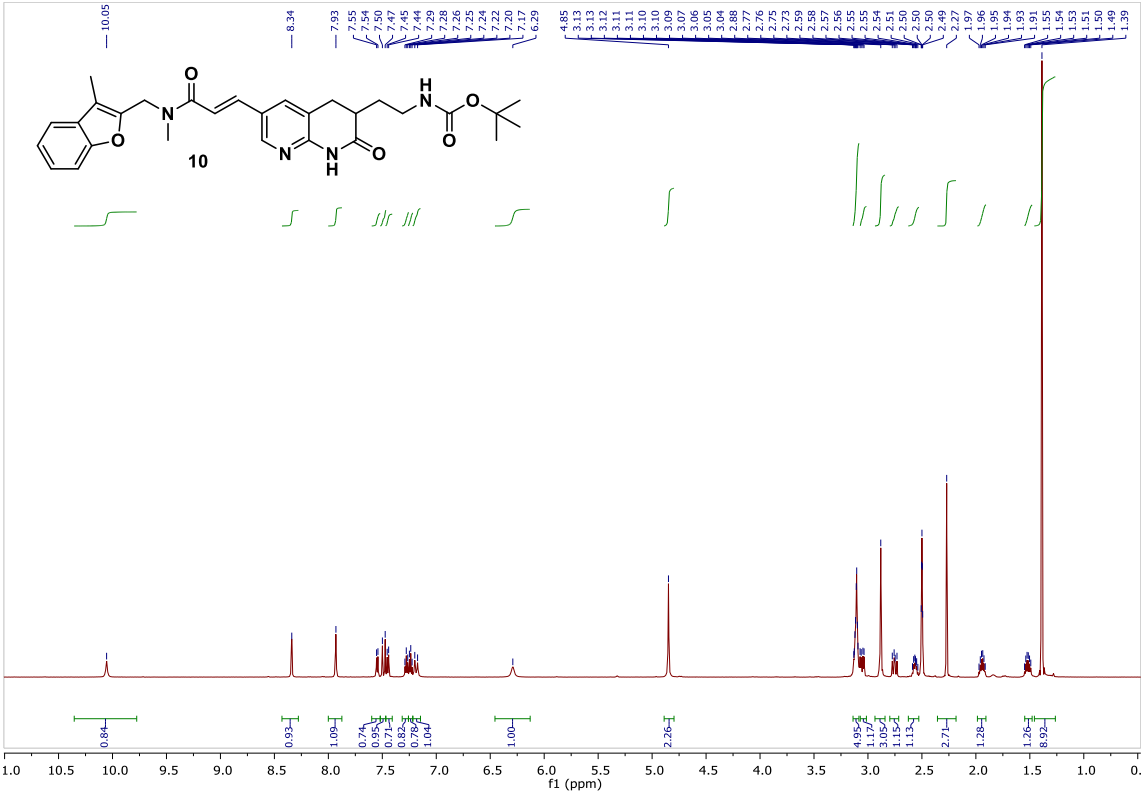
gHSQC (DMSO-d_6, 115 °C):



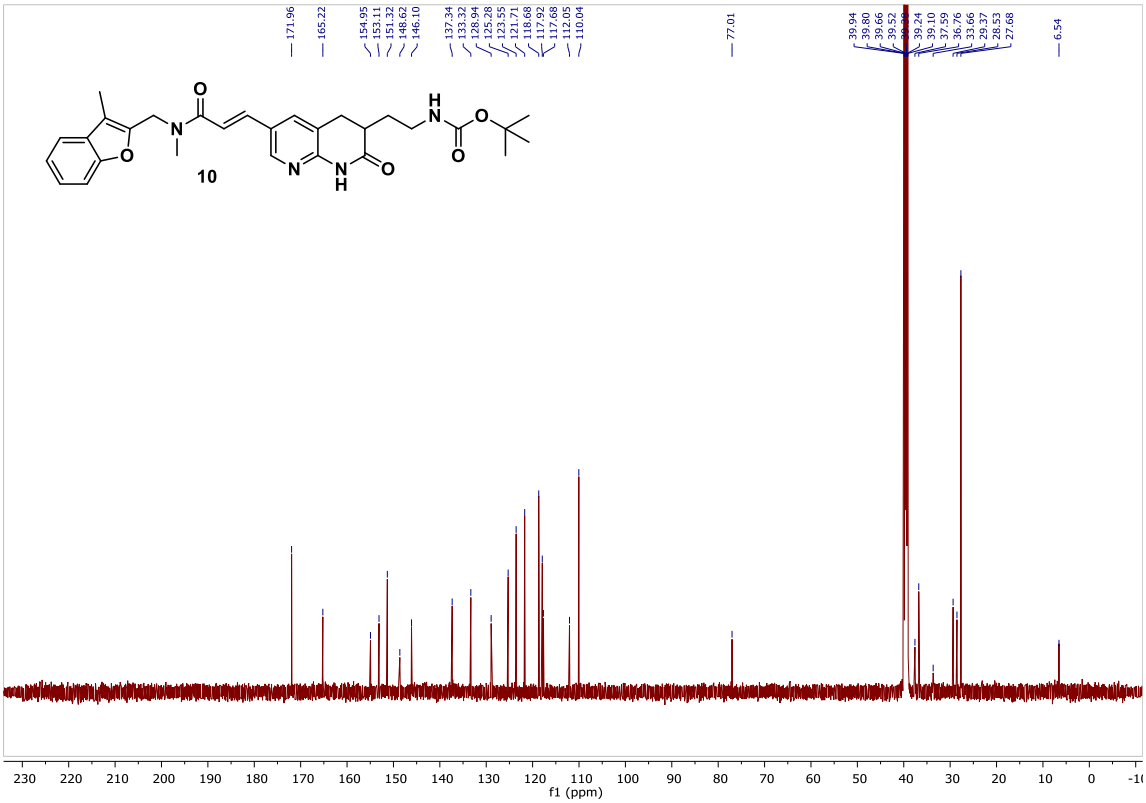
^{1}H NMR (600 MHz, DMSO-d_6, 25 ^{o}C):



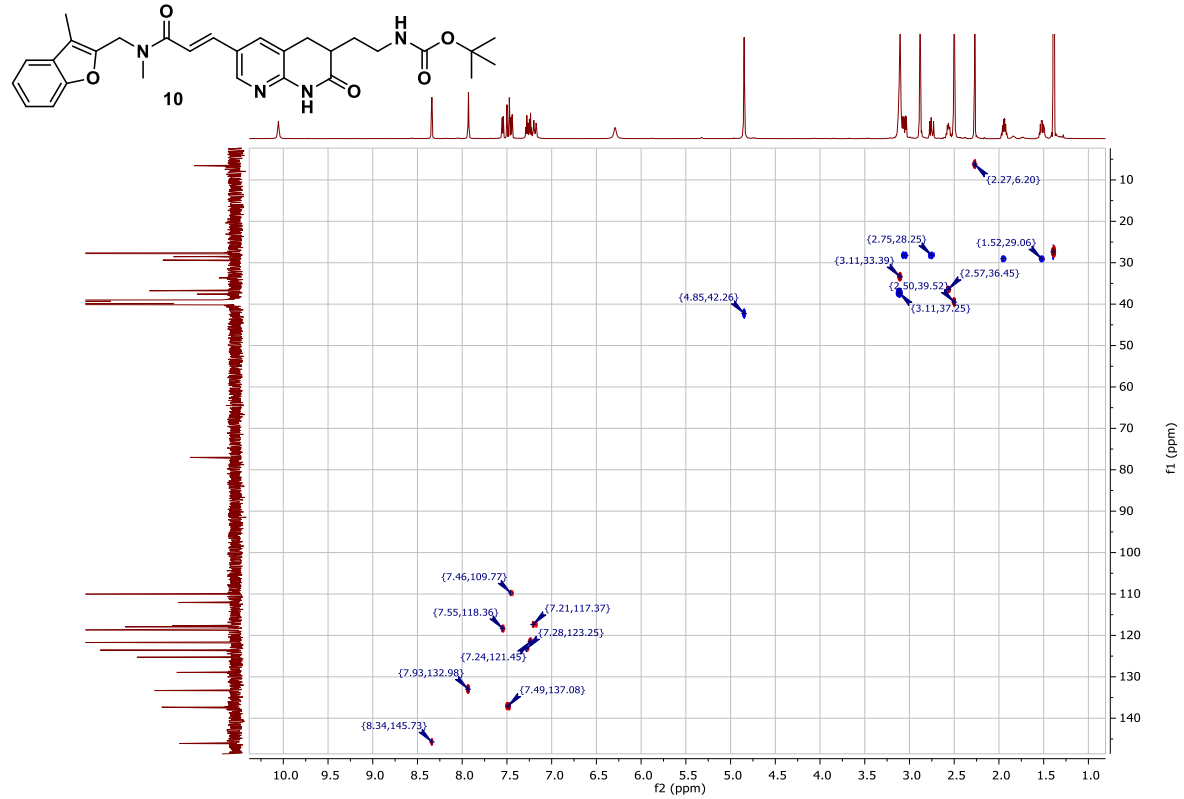
^{1}H NMR (600 MHz, DMSO-d_6, 120 ^{o}C):



^{13}C NMR (151 MHz, DMSO-d_6, 120 ^{o}C):



gHSQC (DMSO-*d*$_6$, 115^{o}C):



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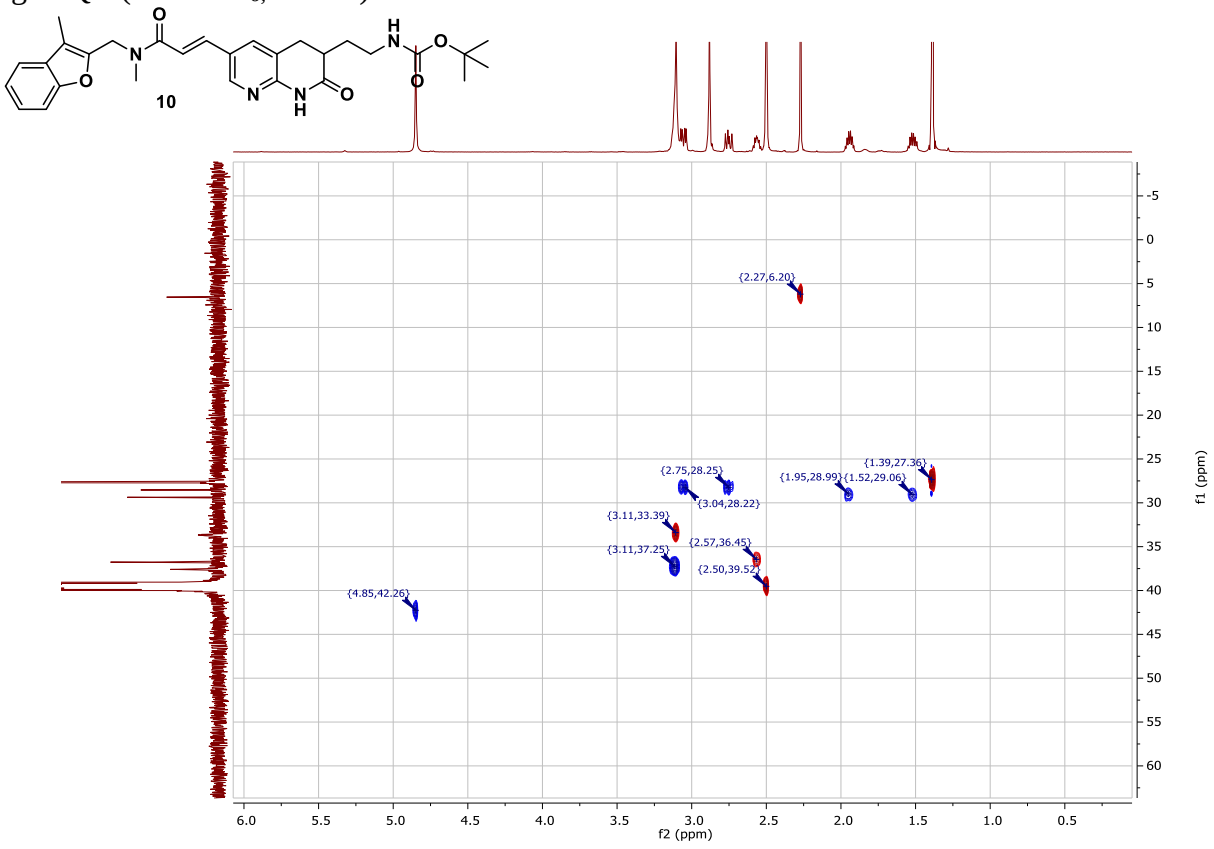
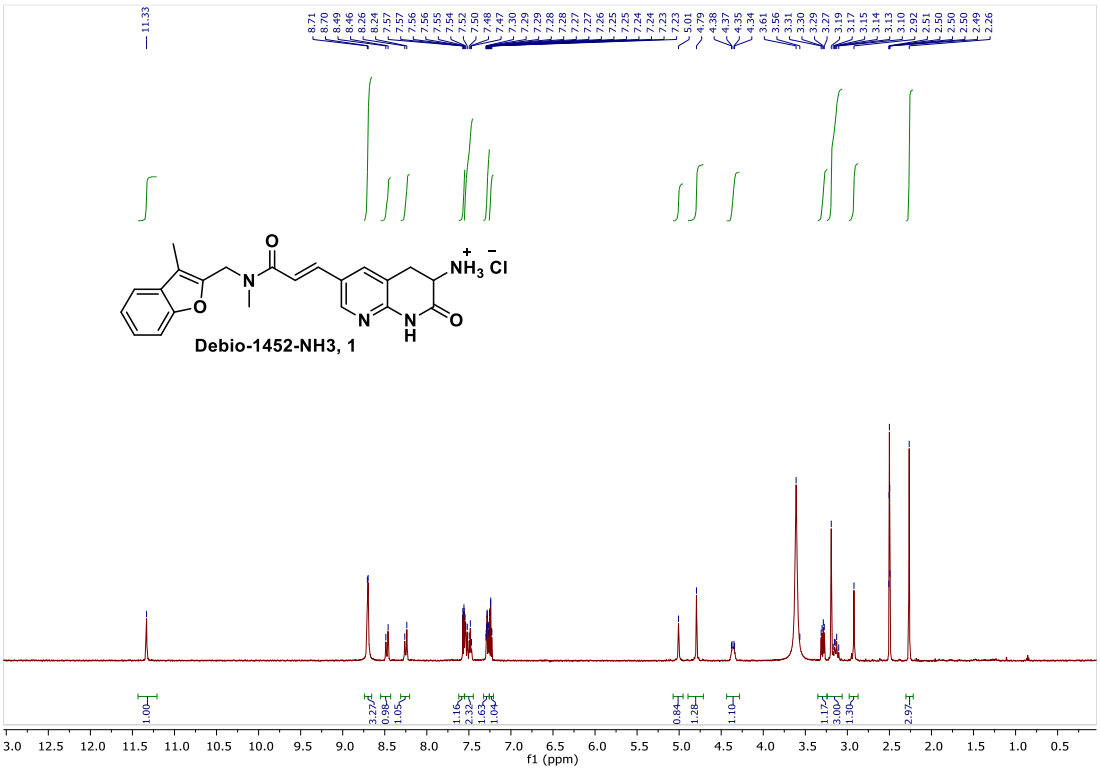
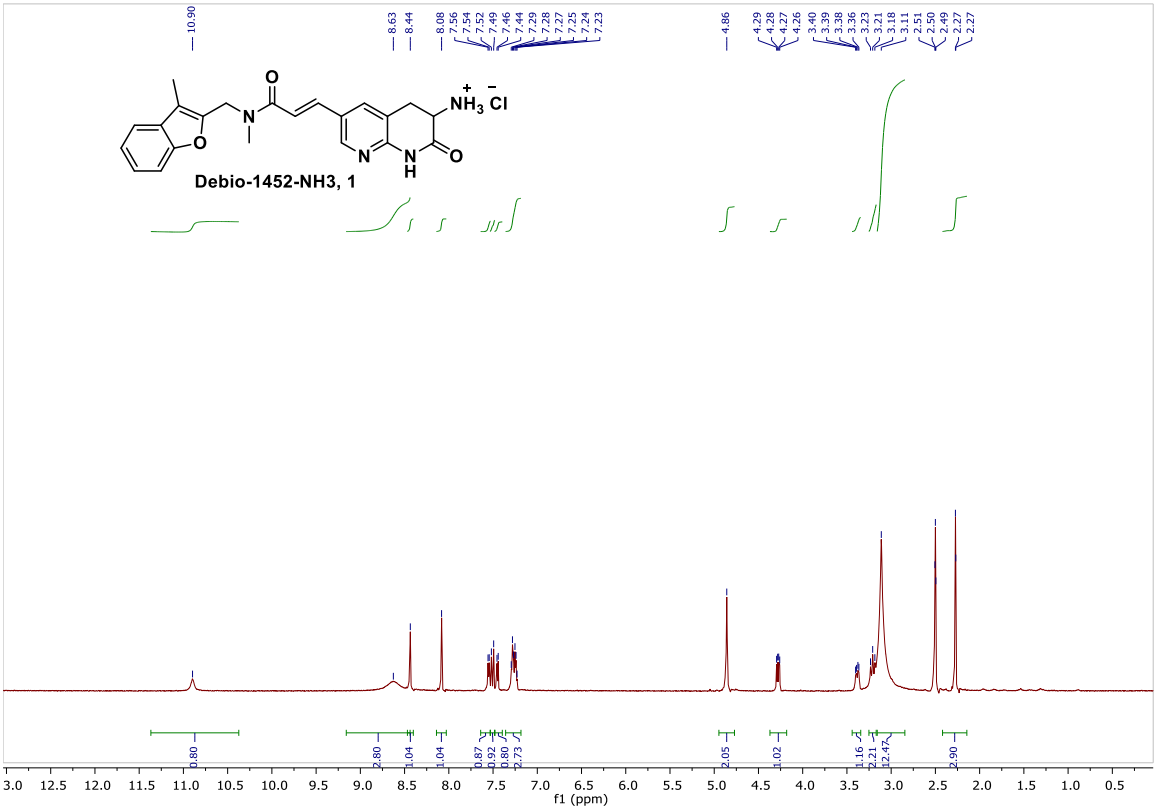


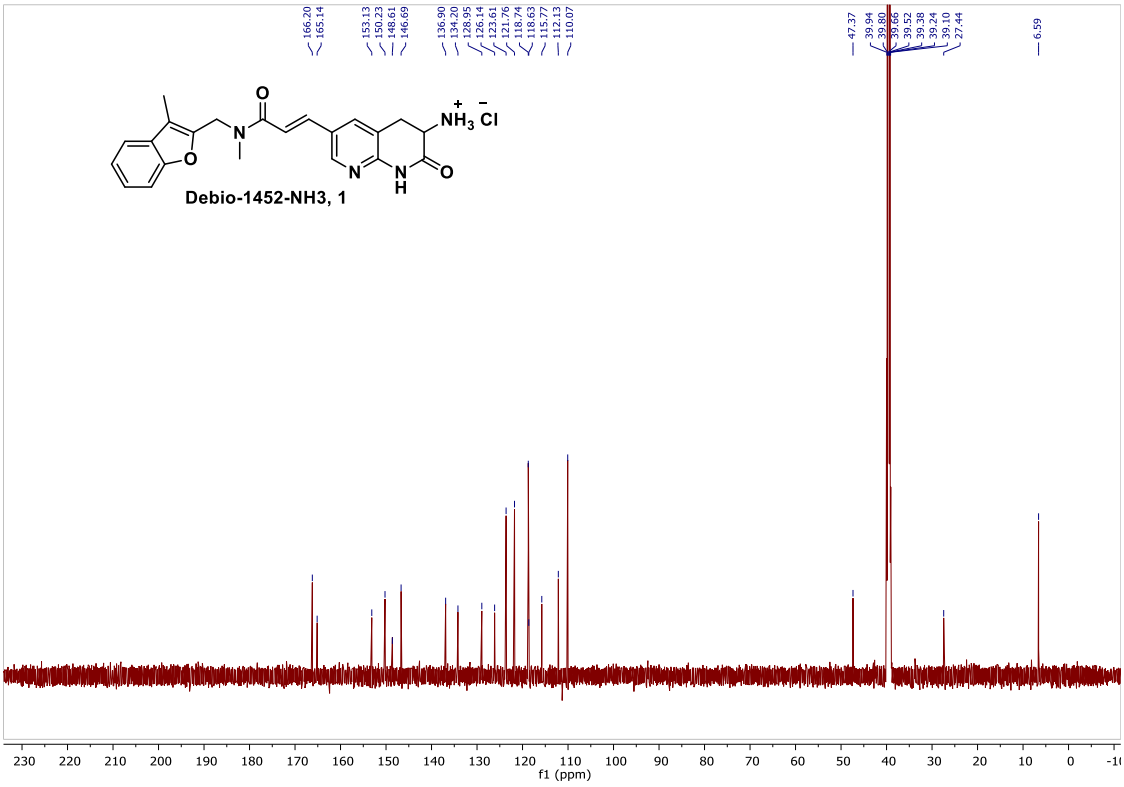
^{1}H NMR (600 MHz, DMSO-d_6, 25 ^{o}C):



^{1}H NMR (600 MHz, DMSO-d_6, 120 ^{o}C):



^{13}C NMR (151 MHz, DMSO-d_6, 120 ^{o}C):



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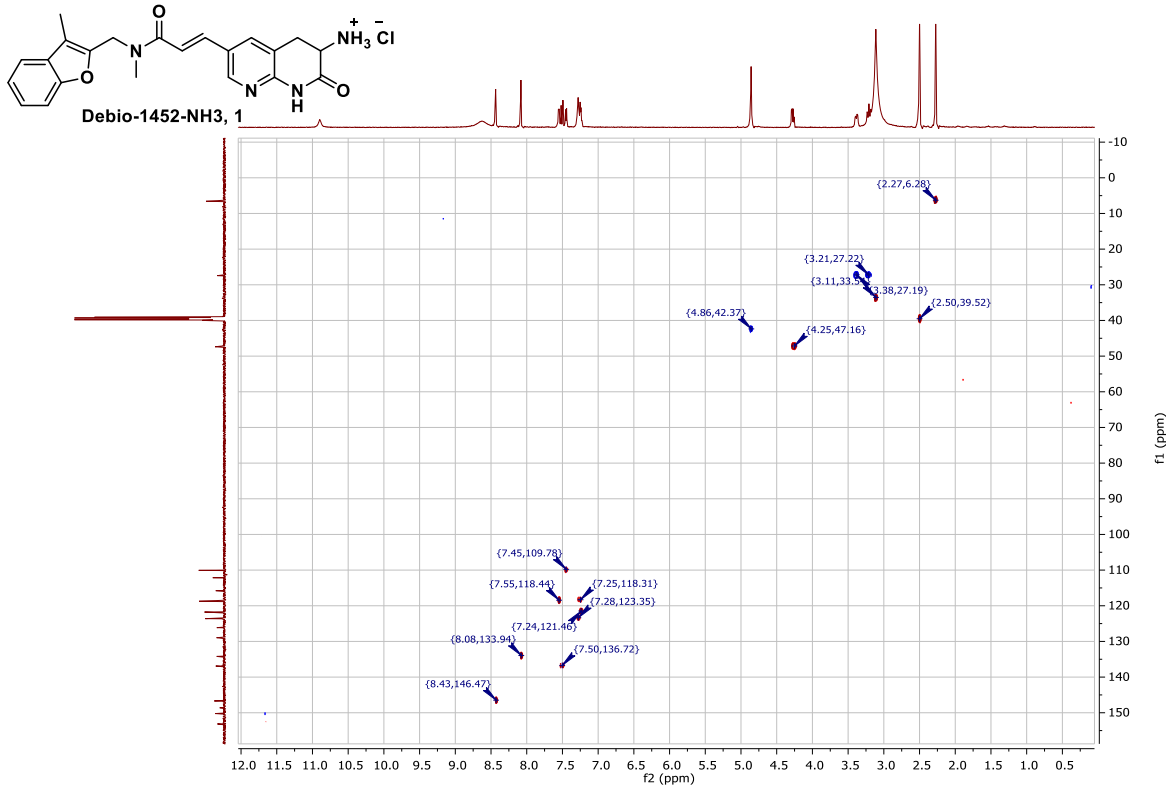

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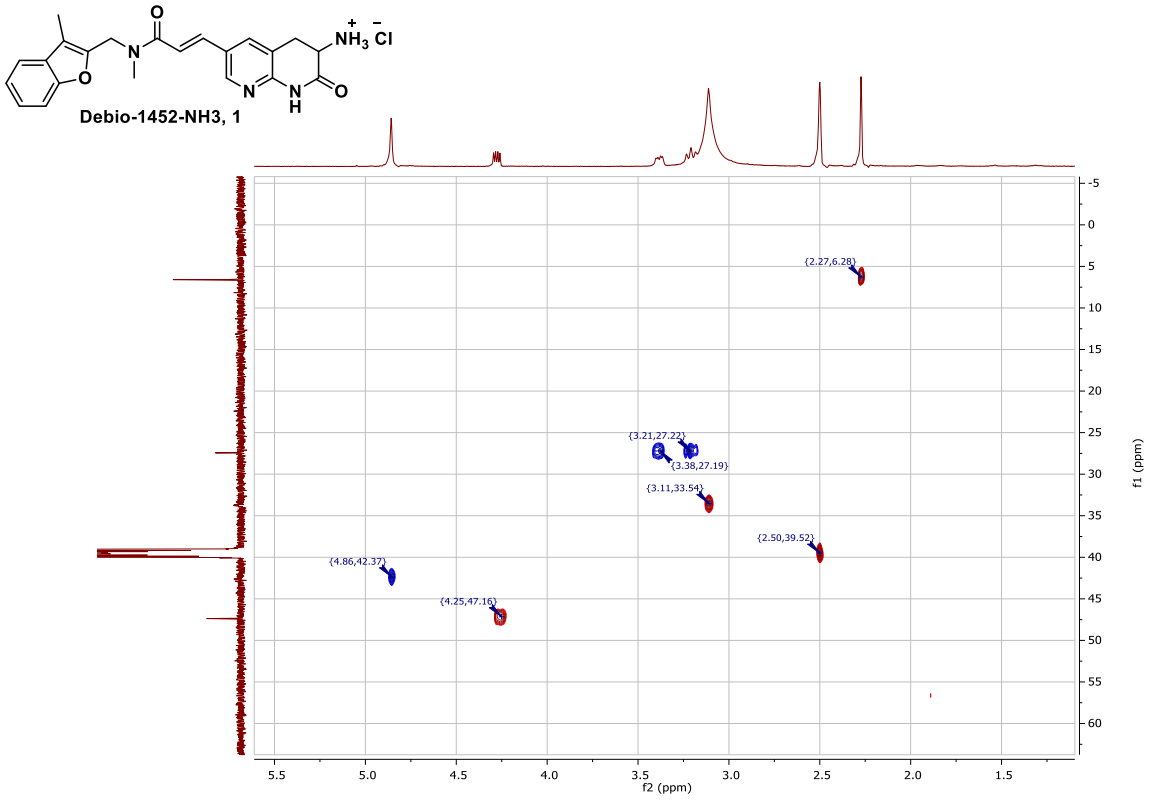
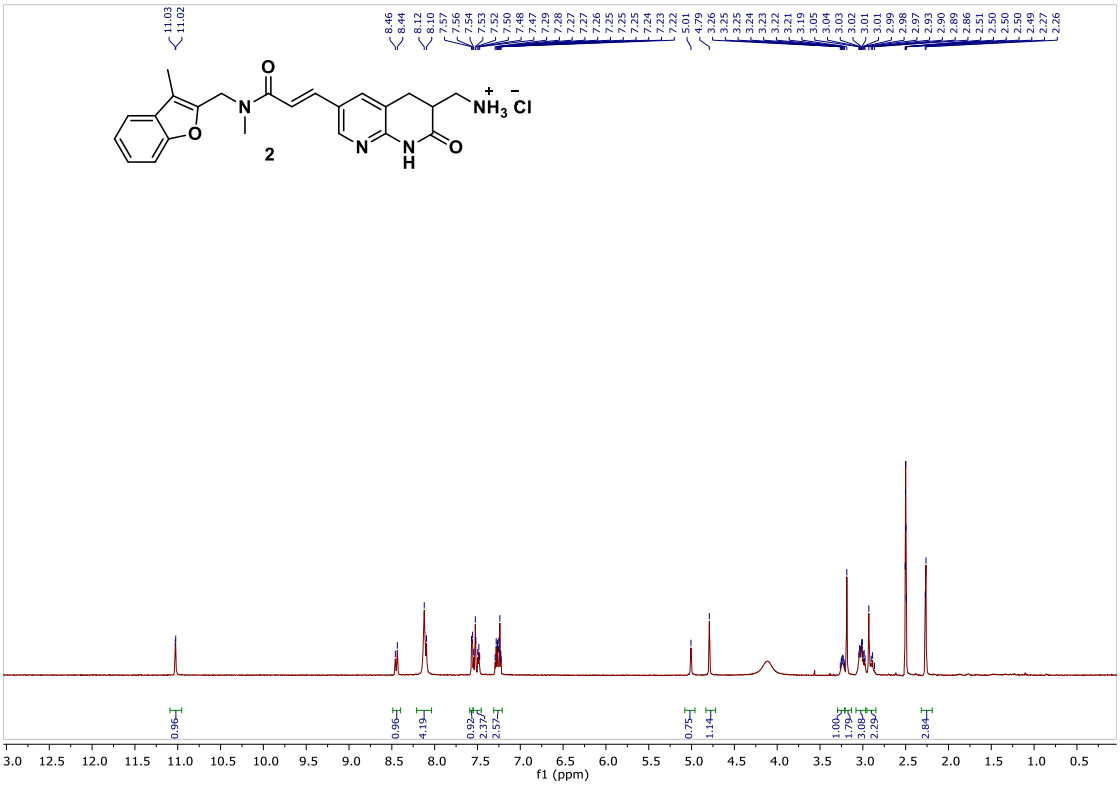


^{1}H NMR (600 MHz, DMSO-d_6, 25 ^{o}C):



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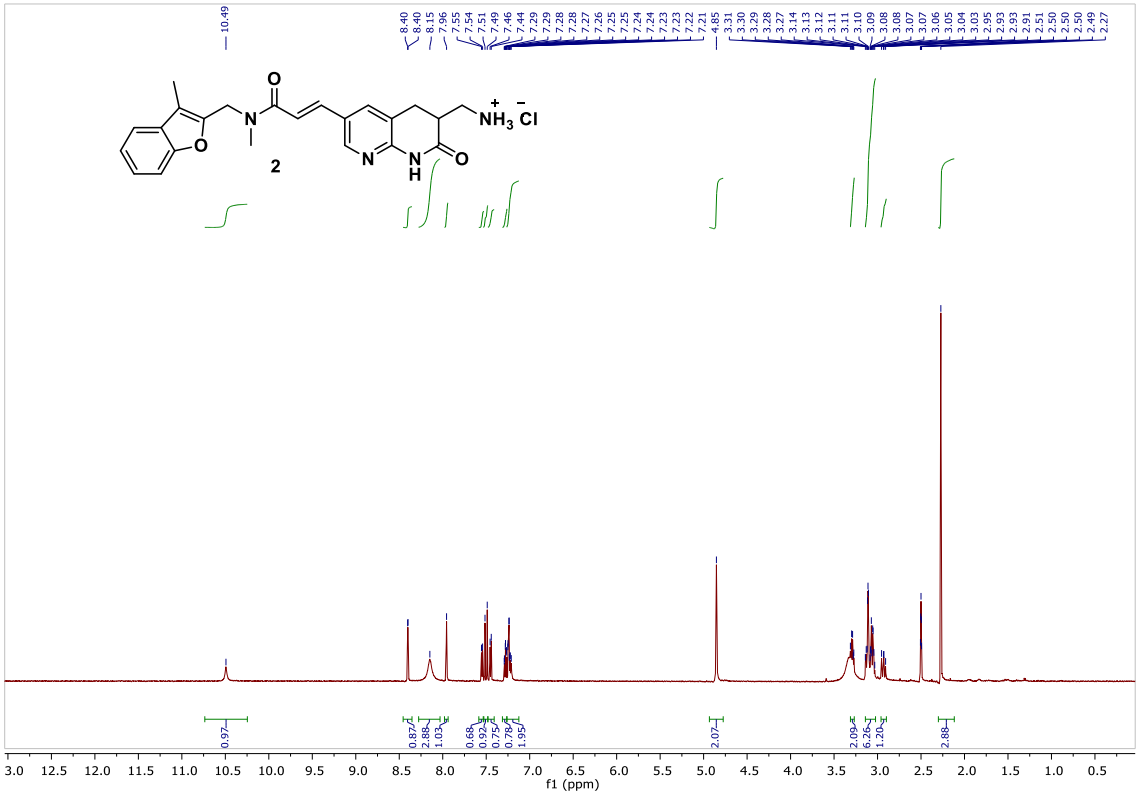

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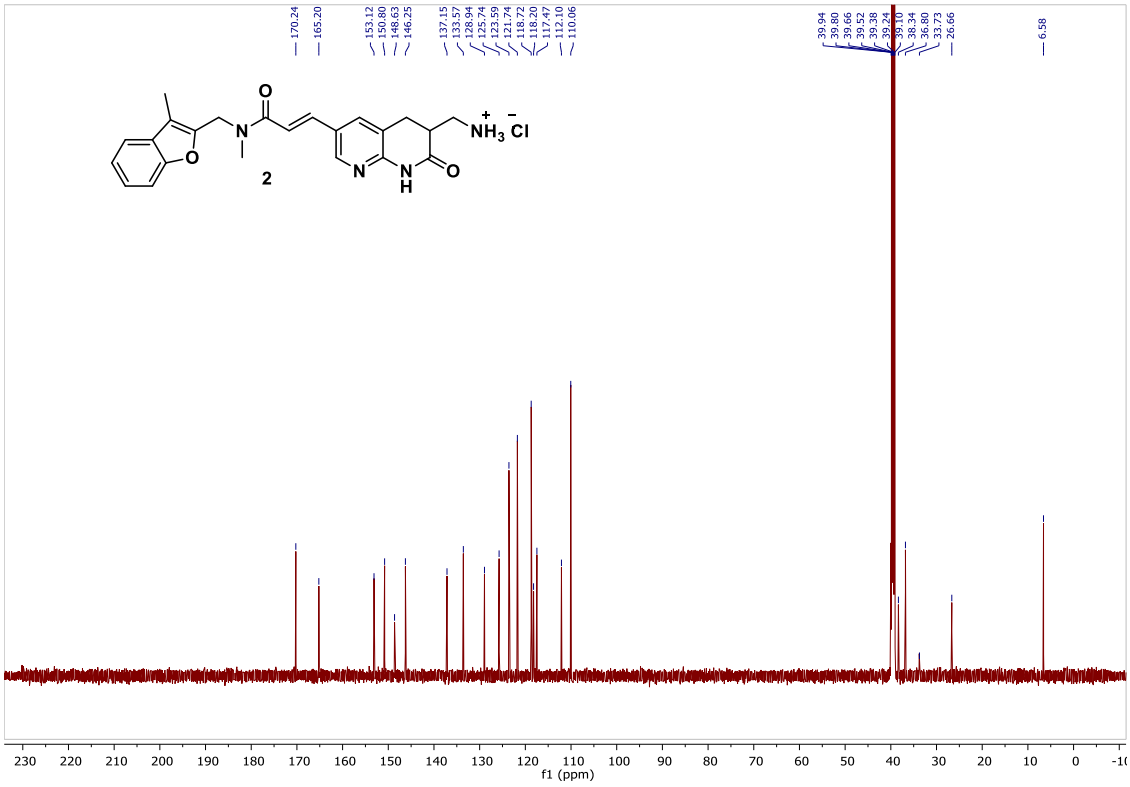

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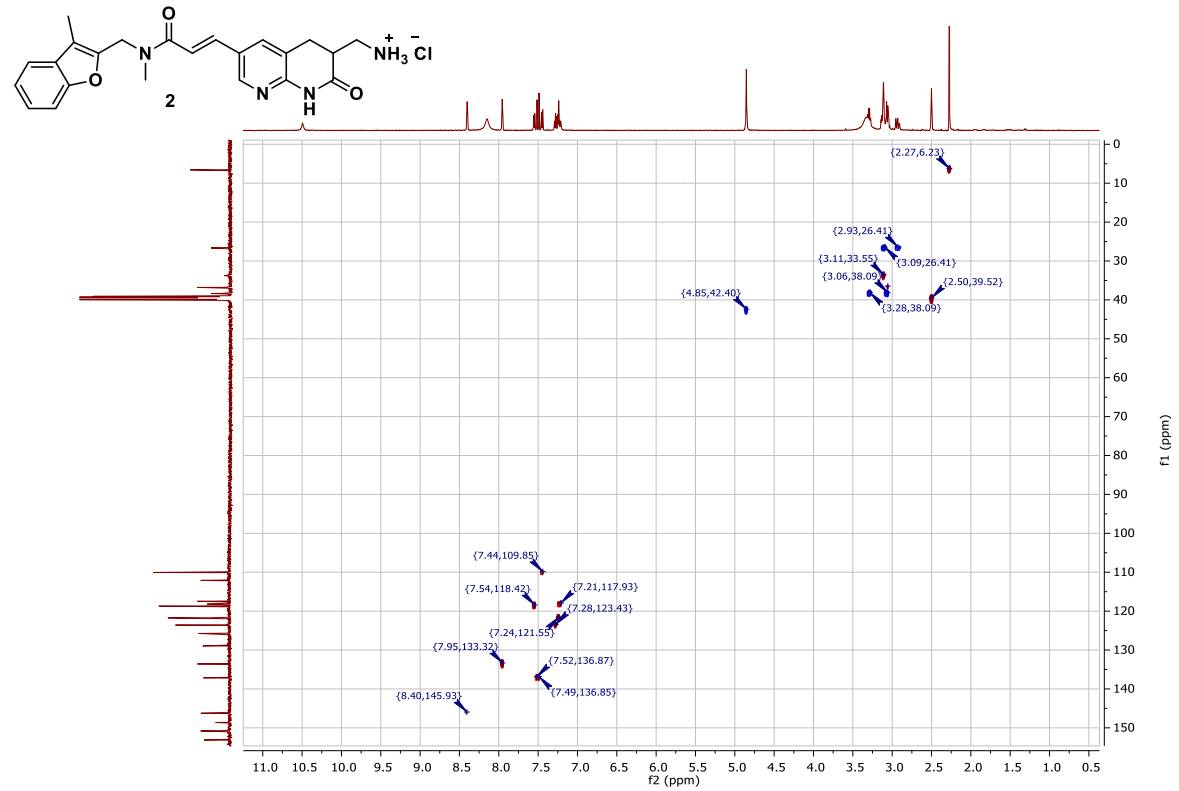

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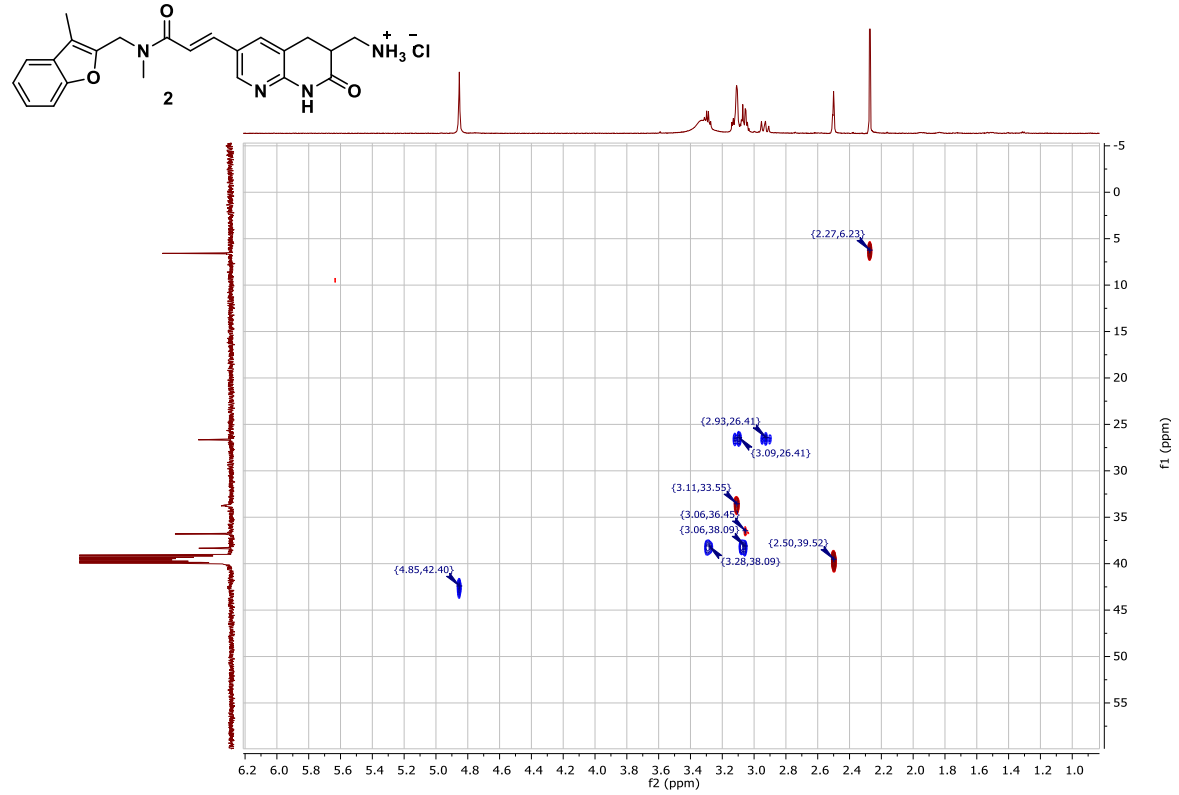

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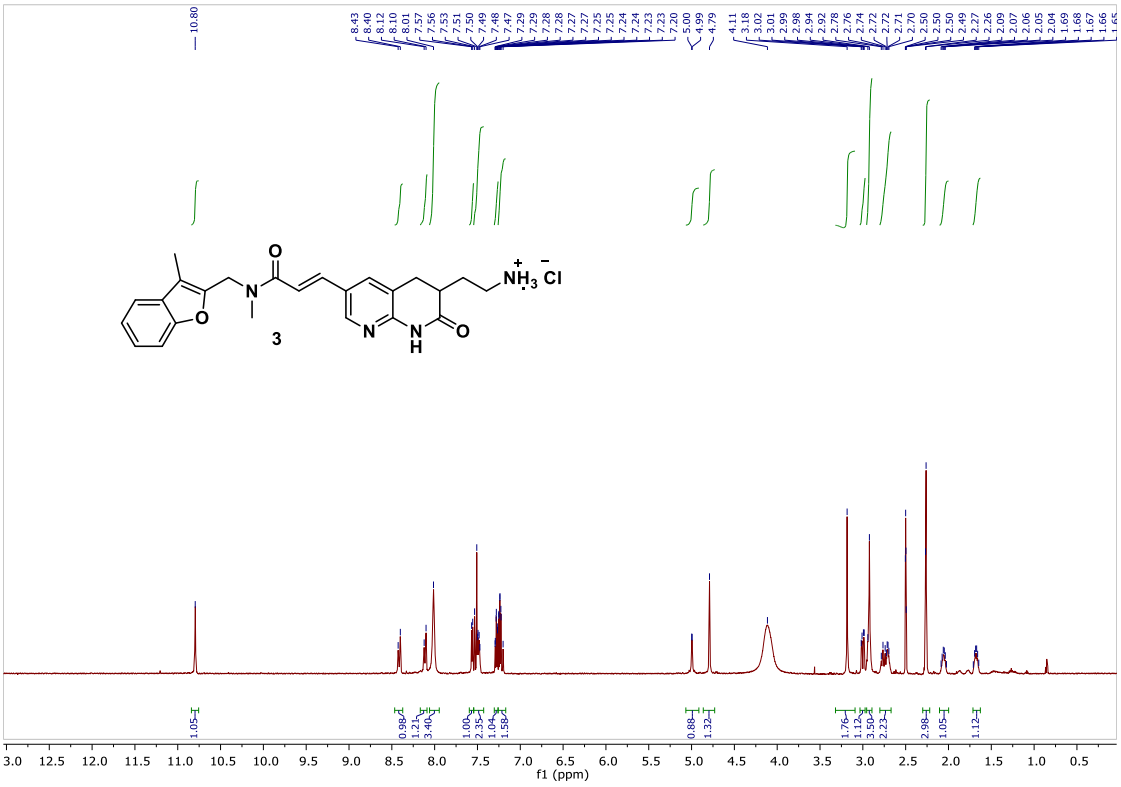

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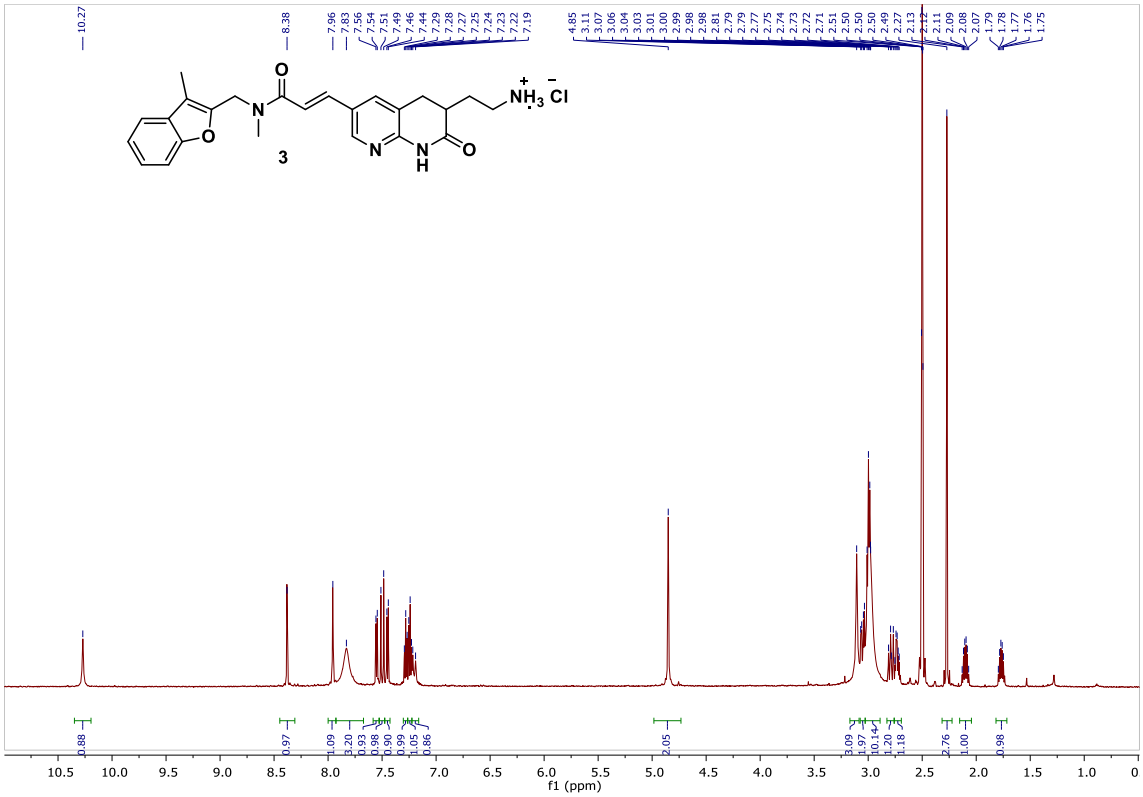

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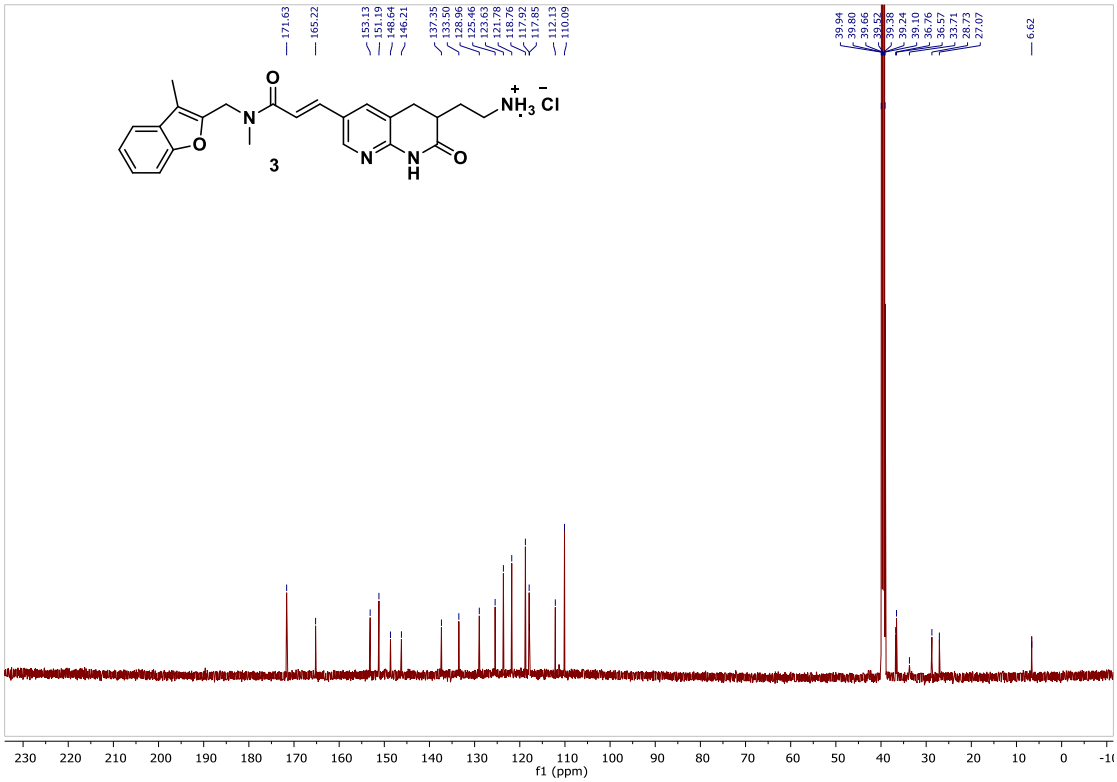

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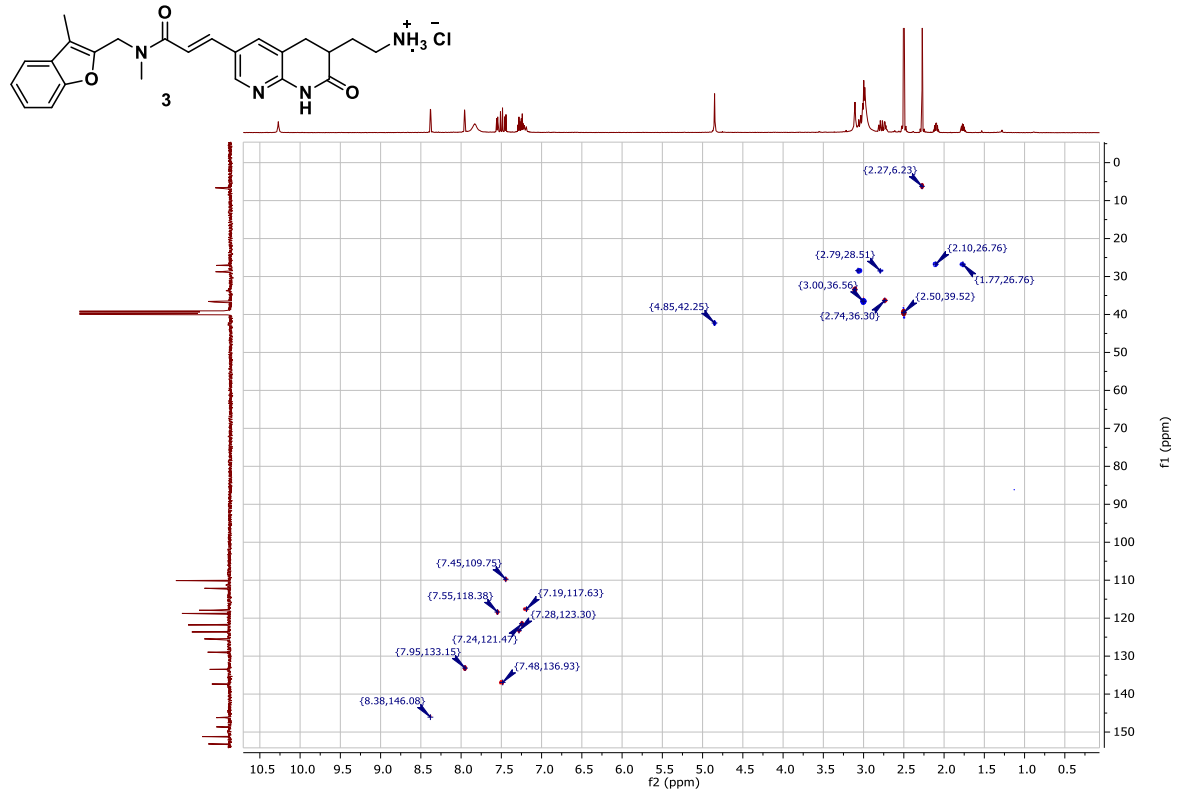

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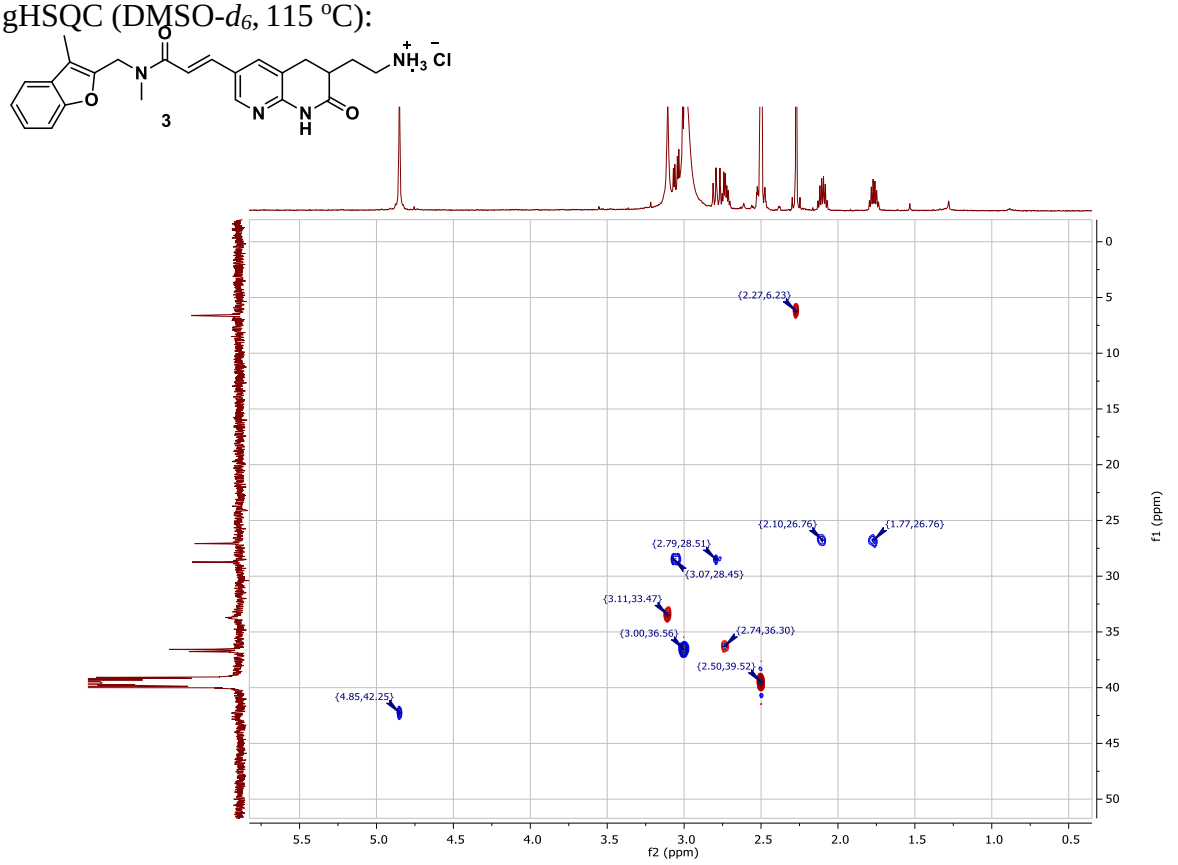

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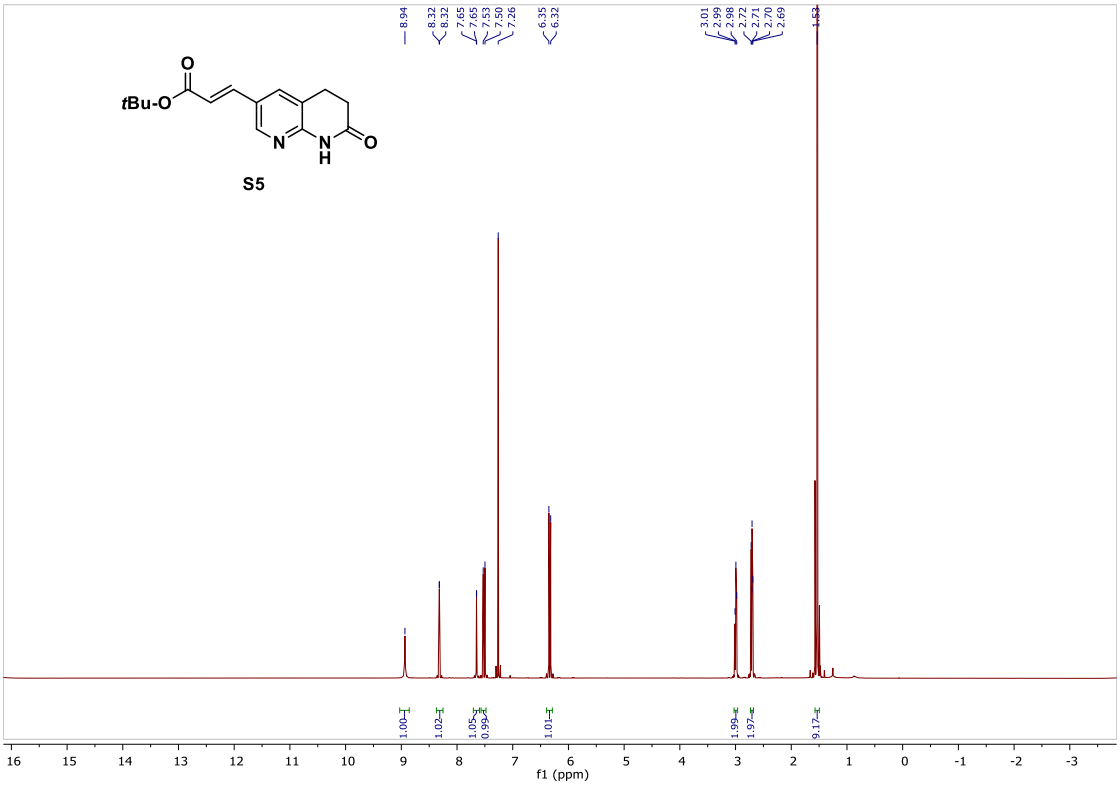
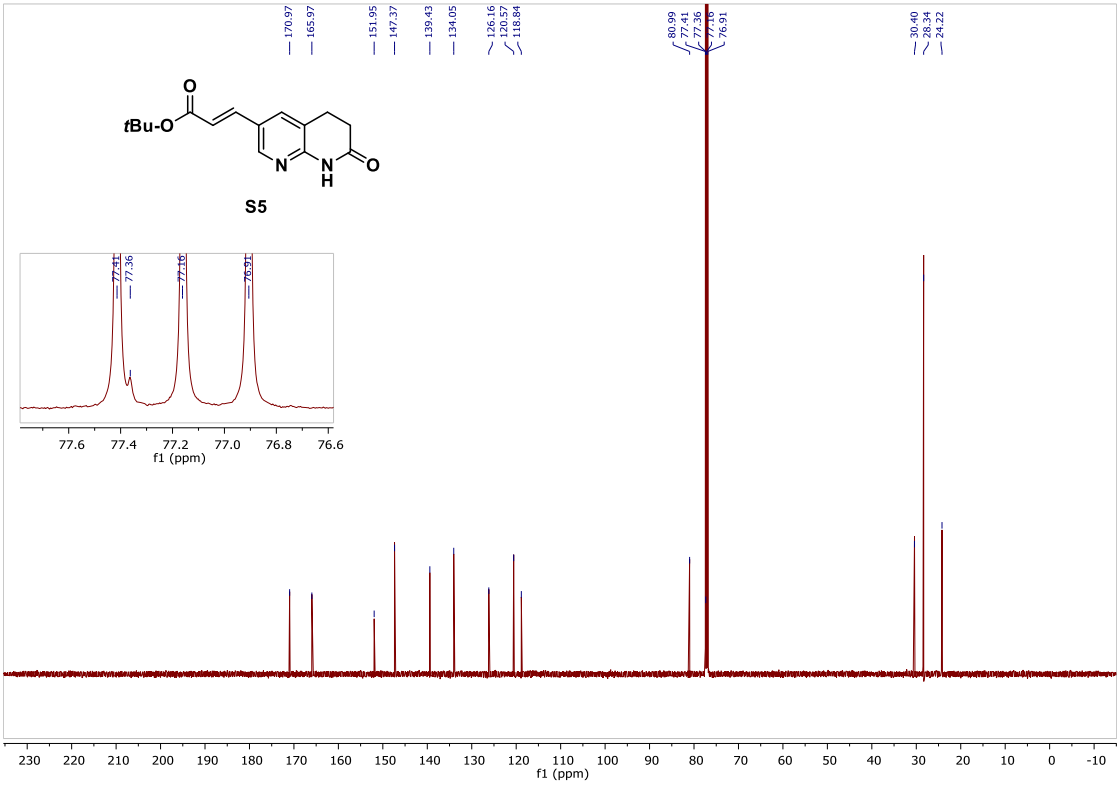


^{13}C NMR (126 MHz, chloroform-*d*):



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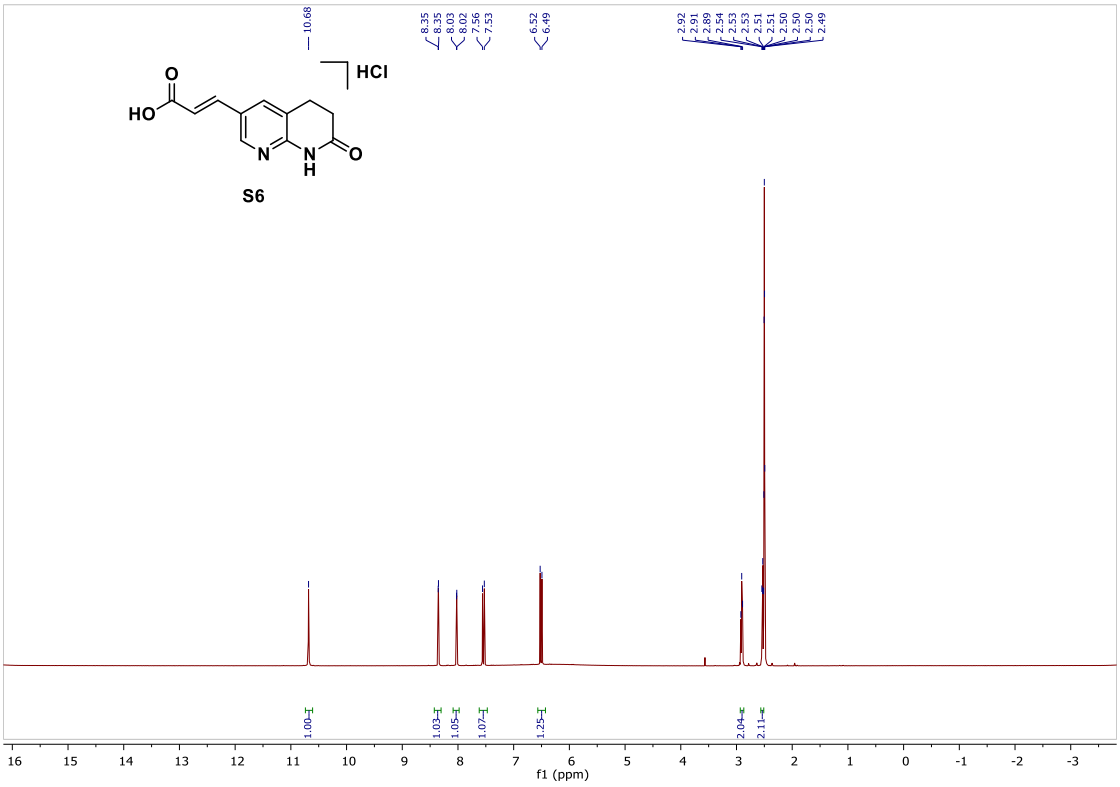

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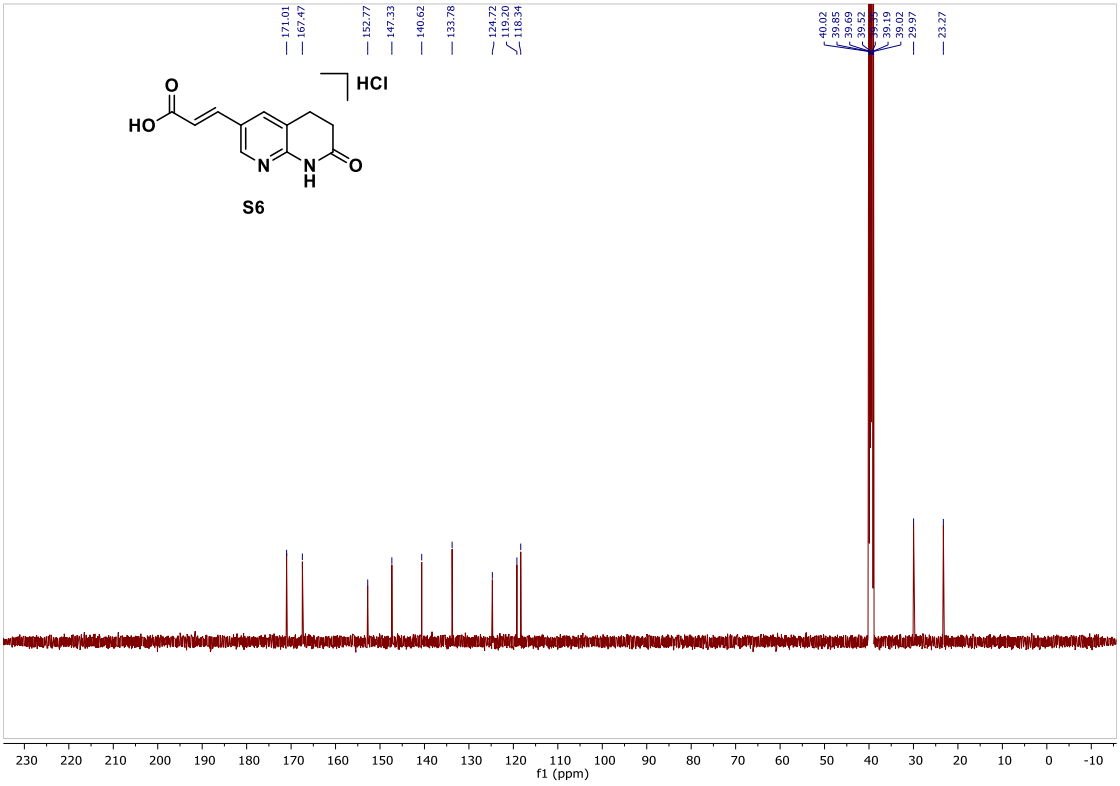

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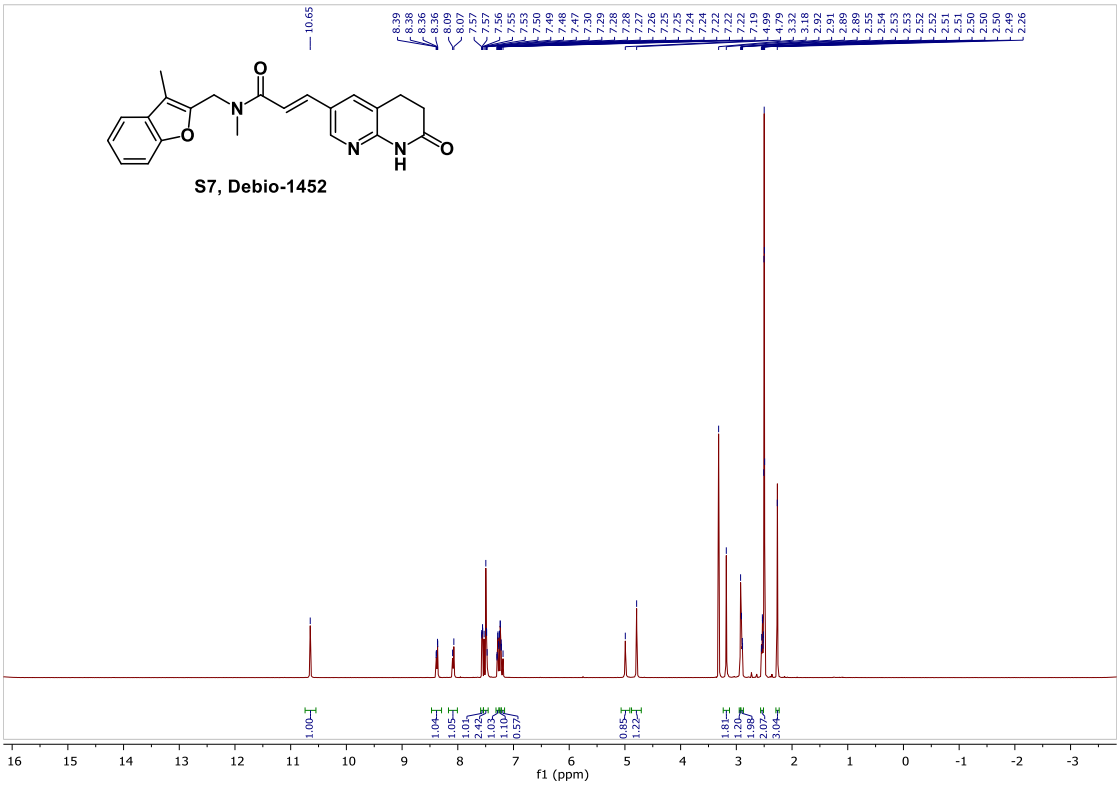

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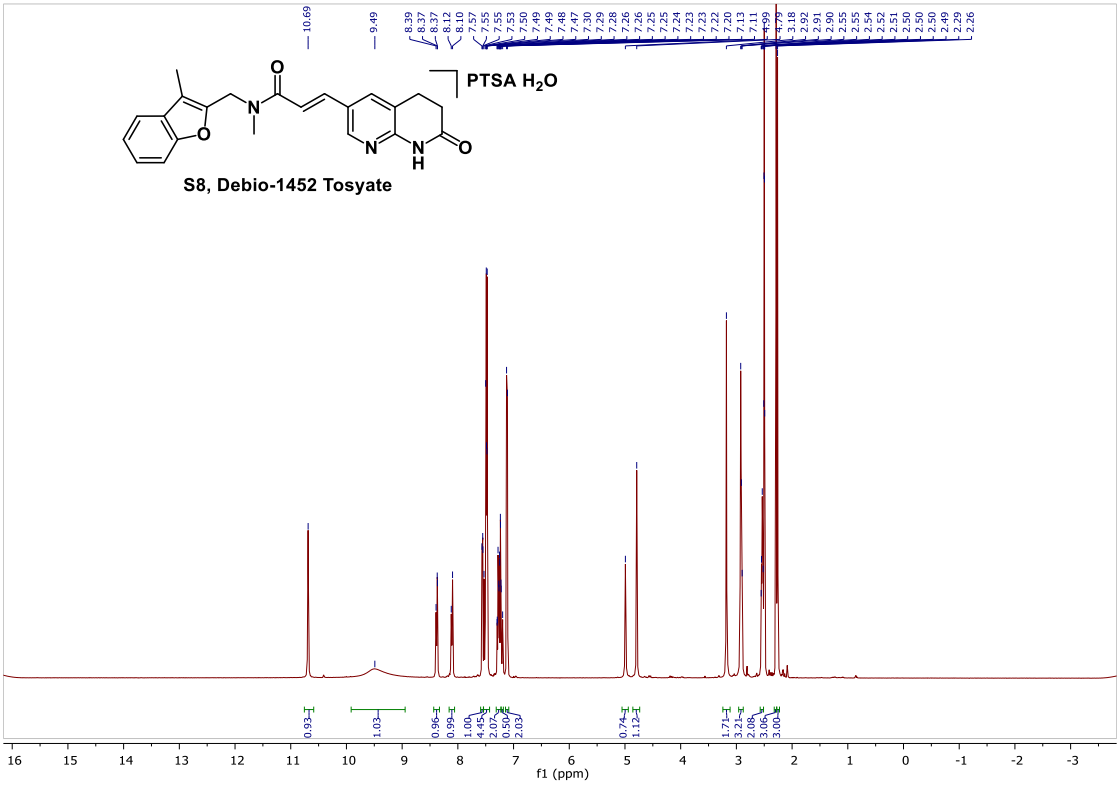

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