Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Armeniaspirol analogues with more potent Gram-positive antibiotic activity show enhanced inhibition of the ATP-dependent proteases ClpXP and ClpYQ

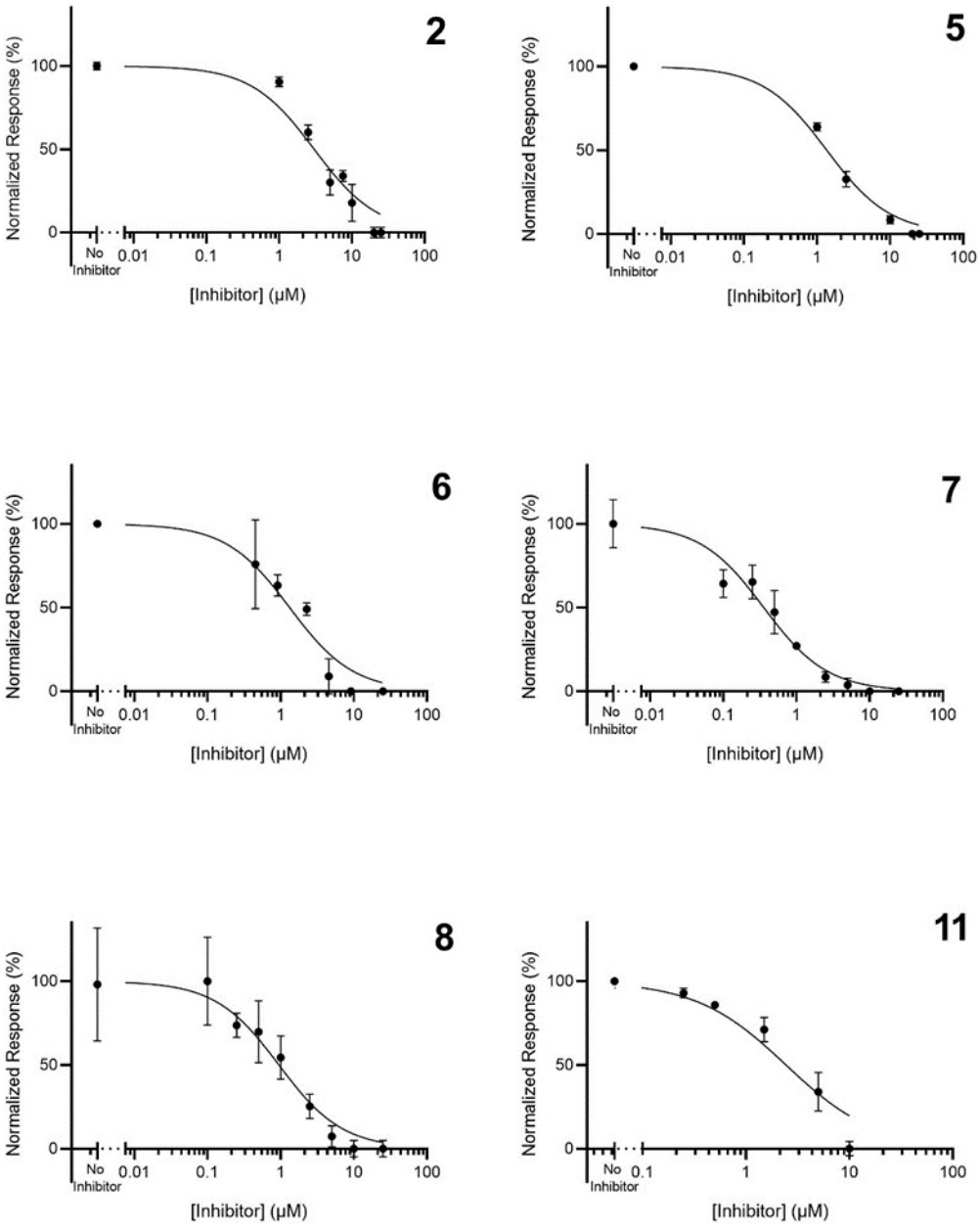
Michael G. Darnowski,[1] Taylor Lanosky,[1] Puneet Labana[1], Jordan Brazeau-Henrie,[1] Nicholas D. Calvert,[1] Mark H. Dornan,[1] Claudia Natola,[1] André R. Paquette,[1] Adam J. Shuhendler,[1] and Christopher N. Boddy[1]*

[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, ON Canada, K1N 6N5

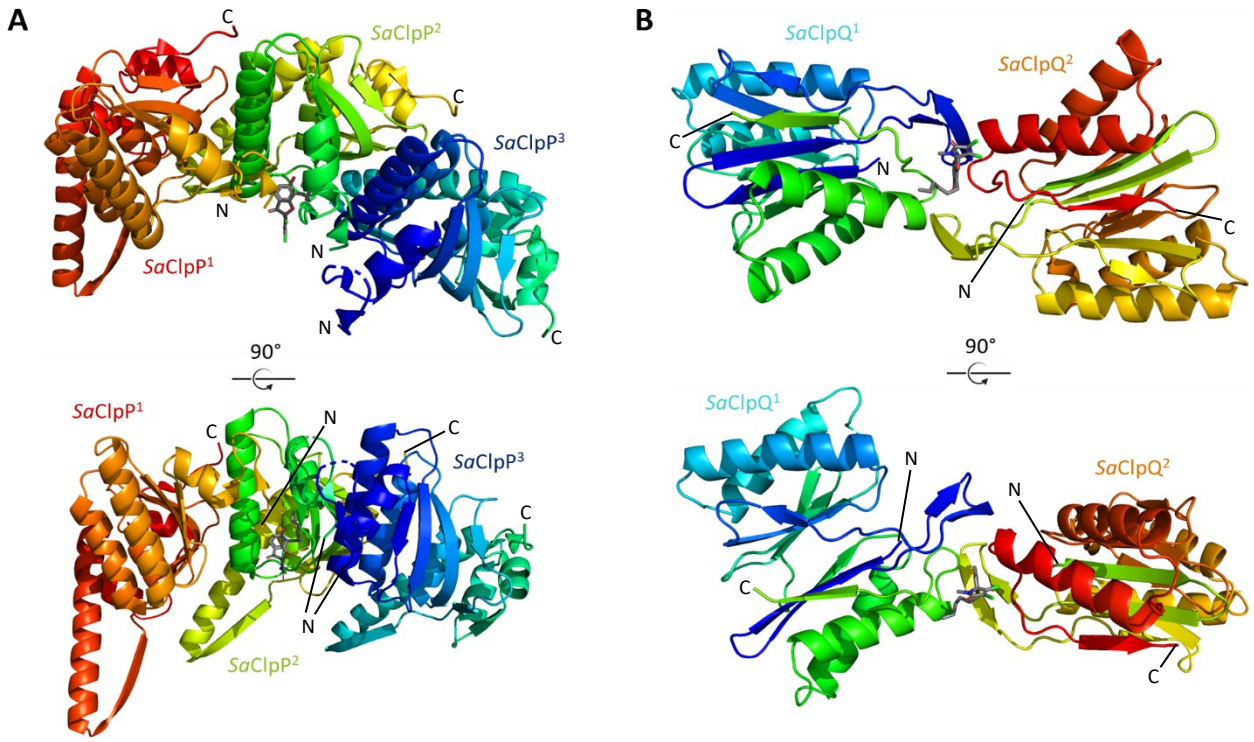
*Corresponding author: Christopher N. Boddy, Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, ON Canada, K1N 6N5, cboddy@uottawa.ca 613-762-8900

Experimental

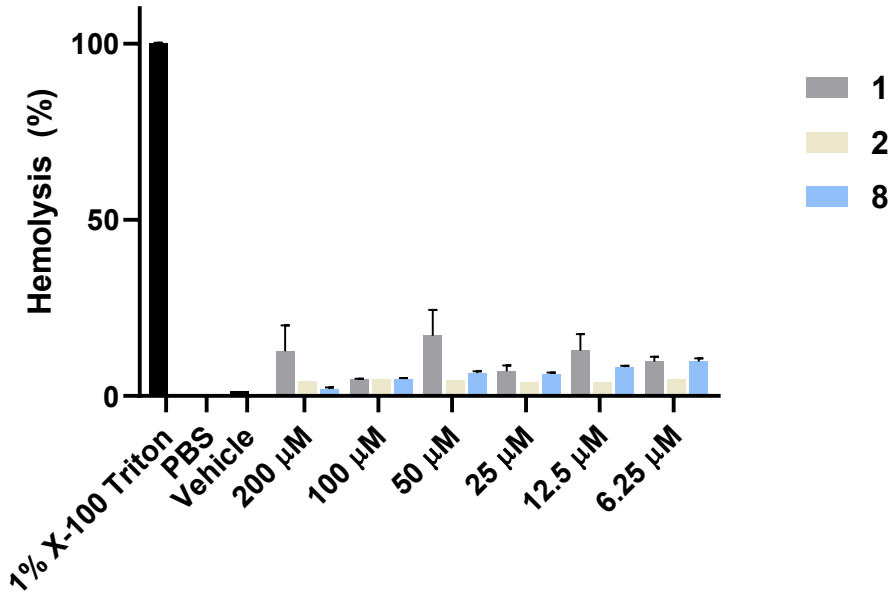
Supplemental Figures and Tables



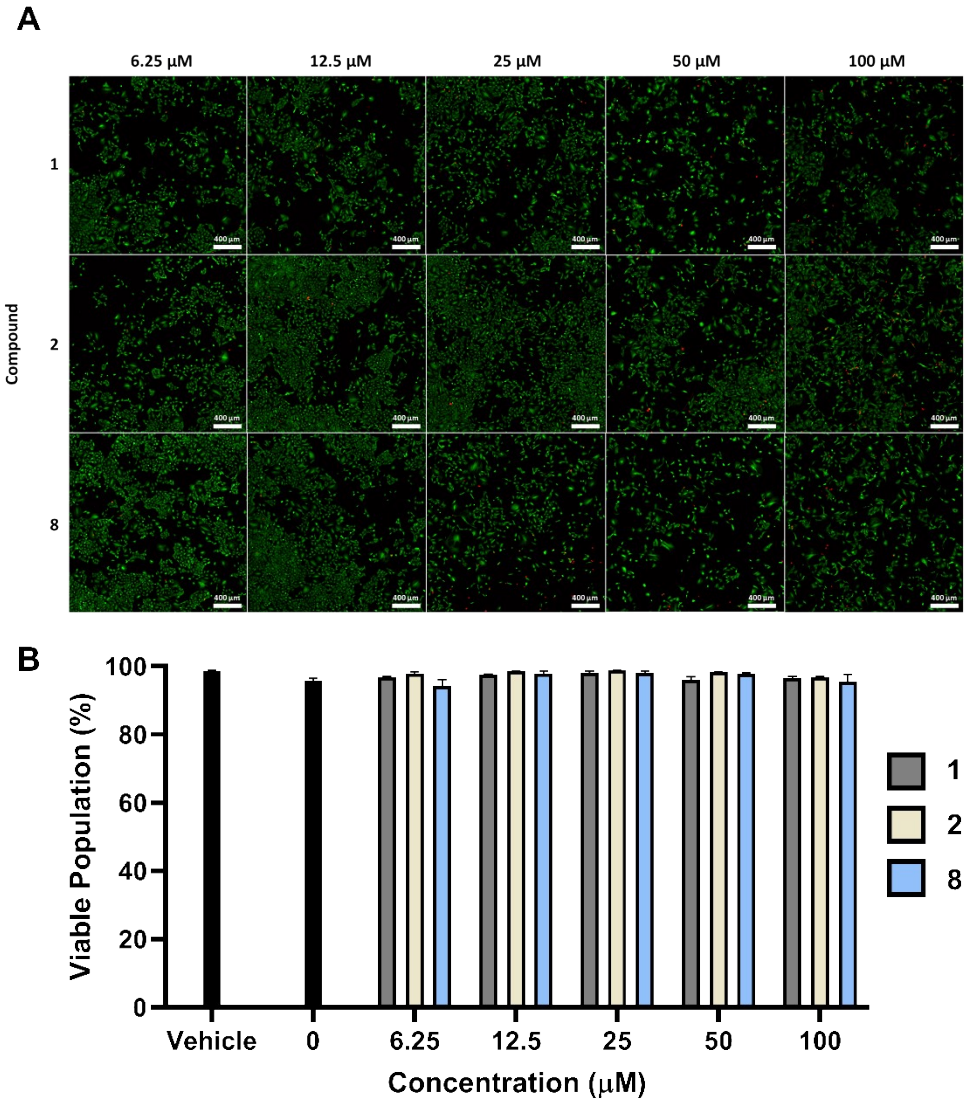
Supplemental Figure S1. ClpYQ inhibition curves showing fit to an IC_{50}



Supplemental Figure S2. Alternate armeniaspirol binding site in *S. aureus* ClpP and ClpQ. (A) Top and side views of armeniaspirol's alternate binding site on *S. aureus* ClpP (SaClpP) trimer. (B) Top and side views of armeniapsirol's alternate binding site on *S. aureus* ClpQ (*Sa*ClpQ) dimer. In both panels, each monomer is coloured differently and labelled with armeniaspirol shown as grey sticks.



Supplemental Figure S3. Hemolyses of sheep red blood cells by armeniaspirol analogs.



Supplemental Figure S4. Toxicity of armeniaspirol analogues against mammalian A549 cells. (A) Human lung epithelial carcinoma cells (A549) cultured with 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, or 0 µM of compound **1**, **2**, or **8** for 24 hrs and stained with 1 µM calcein-AM and 2 µM ethidium homodimer-1. Images were acquired on a Zeiss LSM 880 confocal microscope. (B) The viable cell population for each condition, including a 0.5 % DMSO control (vehicle), was determined by comparing the total number of singly-stained, calcein-AM-positive cell counts to the total number of cells that were singly-stained as positive for live or dead as determined by flow cytometry using a 488 nm excitation with 525/40 bandpass for calcein-AM (live cells, green) and 620/20 bandpass for ethidium homodimer-1 (dead cells, red).

Supplemental Table S1. Minimum bactericidal concentration of analogues.

	Minimum Bactericidal Concentration (μg/ml)													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Gram-positive														
S. aureus IA116-USA100	32	8	16	32	8	>32	8	8	>32	32	4	>32	>32	>32
S. aureus MN8-USA200	32	16	16	32	>32	32	16	16	>32	>32	8	>32	>32	>32
S. aureus LAC-Fitz-USA300	>32	8	32	>32	>32	16	16	8	>32	>32	2	>32	>32	>32
S. aureus MW2-USA400	>32	8	16	>32	>32	16	16	8	>32	>32	8	>32	>32	>32
E. faecalis NJ3	>32	32	>32	>32	>32	>32	32	32	>32	>32	8	>32	>32	>32
Gram-negative														
P. aeruginosa PA01	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32

Supplemental Table S2. Clp YQ kinetics characterization data

Compound	IC50 (± std dev) (μM)	R^2	K_i (μM)
7	0.3562 ± 0.04095	0.9355	0.1532
8	0.9355 ± 0.1663	0.8786	0.4067
6	1.325 ± 0.2319	0.8759	0.5700
5	1.353 ± 0.1131	0.8759	0.5821
2	3.022± 0.3659	0.9209	1.3001
11	2.435 ± 0.3659	0.9095	1.0475

Supplemental Table S3. MEC of Clp XP of analogues in urease assay

Compound	ClpXP MEC (μg/mL)
1	1
2	0.067
3	1
4	1
5	0.25
6	1
7	0.25
8	0.25
9	>1
10	>1
11	0.5
12	>1
13	>1
14	>1

Supplementary Table 4. Additional minimum inhibitory concentration of 1 and 2.

Minimum inhibitory Concentration (μg/ml)		
Bacteria	**1**	**2**
S. aureus LAC-Fitz-USA300	4	1
S. aureus LAC-Fitz-USA300 + 0.5% BSA	>32	32
S. aureus LAC-Fitz-USA300 + 0.01% Triton X-100	8	2
E. coli Δ*tolC*	8	nd
E. coli BW25113	>32	>32

ClpYQ protein purification

The ClpQ expression vector (pPL29, pET21c-based, C-terminal His tag, AmpR) was transformed into chemically competent *E. coli* BL21(DE3) for protein expression. 400 mL LB media was inoculated with 0.5% (*v/v*) of an overnight pre-culture. The culture was grown at 37 °C (200 rpm) and expression was induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside at an optical density (OD_{600}) of 0.5. The culture was grown at 37 °C (200 rpm) for 4 h. The cells were pelleted at 4,000 rpm for 20 min and re-suspended in lysis buffer (50 mM Tris, 100 mM NaCl, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, 1 mg/mL lysozyme, 10% glycerol, pH 8.0). Mechanical cell lysis was achieved by 3 cycles of 3 s sonication then 2 s incubation on ice followed by a 1 min incubation on ice. The cell debris was pelleted at 10,000 rpm for 60 min and the supernatant was incubated with 800 μL 50% Ni-NTA agarose resin (QIAGEN) for 30 min at 4 °C with gentle shaking. The lysate was loaded onto a column and the flow-through was collected. The resin was washed sequentially with 5 mL elution buffer (100 mM Tris, 300 mM NaCl, pH 8.0) containing 0 mM, 20 mM, 100 mM and 250 mM imidazole. Fractions were analyzed by SDS-PAGE (4-20% Mini-PROTEAN TGX Precast Gels; Bio-Rad). ClpQ-containing fractions were pooled and additionally purified by FPLC size exclusion using a Superdex 200 10/30 GL column (GE Healthcare). Buffer used in FPLC purification included 50 mM Tris, 250 mM NaCl, pH 8.0. ClpQ-containing fractions were concentrated using a 10 kDa Amicon Ultra-15 centrifugal filter unit (Millipore Sigma). The concentrated protein was stored at -80 °C with 30% (*w/w*) glycerol. The *B. subtilis* clpY gene was cloned into pET28B (N-terminal His tag, KanR).

ClpY was expressed and purified from the resulting plasmid (pPL31) as described above with the following modifications. The culture was grown at 16 °C overnight (200 rpm) post-induction. The column fraction containing the highest concentration of ClpY (with 100 mM imidazole) was additionally purified by FPLC size exclusion using a buffer containing 300 mM NaCl, 100 mM Tris, and 100 mM imidazole, pH 8.0. Imidzaole was added to the FPLC buffer to improve protein solubility since ClpY has the tendency to aggregate and must be treated carefully during purification. Accordingly, ClpY-containing fractions obtained from FPLC purification were pooled but not concentrated. All protein concentrations were determined by Bradford assay.

Peptide hydrolysis assays

Peptide hydrolysis was assayed using the Cbz-Gly-Gly-Leu-AMC (Millipore Sigma) substrate. 0.1 mL reaction assays were done in Nunc 96-well microplates for fluorescence-based assays (ThermoFisher Scientific). Assays were composed of purified bsClpQ and bsClpY protein, 0.1 M Tris (pH 8.0), 0.1 mM Cbz-GGL-AMC, 10 mM MgCl2, 1 mM ATP, 1 mM TCEP, and 1 mM EDTA. A continuous assay of AMC release was monitored at 37 °C using a Synergy H4 microplate reader (BioTek). Excitation and emission for Cbz-GGL-AMC were measured at 355 nm and 460 nm, respectively. Inhibition was observed with varying concentrations of analogs.

Minimum inhibitory/bactericidal concentration

Minimum inhibitory concentration (MIC) was carried out in Mueller-Hinton broth with indicated supplements (BSA, Triton X-100) in 100 μL assays in 96-well plates. Sequential concentrations of compound were pipetted into each column through two-fold serial dilutions. 5 μL of bacterial culture (OD_{600} 0.07-0.1) was inoculated into each well and incubated at 37°C for 16 h. Growth was observed by OD_{600} readings using a Synergy H4 microplate reader (BioTek). The MIC was determined by the lowest concentration of compound that prevented bacterial growth.

Minimum bactericidal concentration (MBC) was performed by streaking 100 μL of each MIC bacterial subculture on individual petri dishes of non-antibiotic containing Mueller-Hinton Agar. The MBC was determined by a colony count resulting in a 3-log reduction (99.9%) of bacterial growth. A compound is considered bactericidal if it has an MBC less than 4 × the MIC.

Staphylococcus aureus urease activity assay

Christensen urease broth (pH 6.8) was prepared as follows: 1 g/L peptone, 1 g/L glucose, 5 g/L NaCl, 1.2 g/L Na_2HPO_4, 0.8 g/L KH_2PO_4, 0.004 g/L phenol red. 5 mL of 20% (w/v) urea was then added to a fresh 95 mL of Christensen urease broth. Christensen urease broth was inoculated with 1% (v/v) of an overnight MRSA USA300 pre-culture and 200 μL was added to each well. Compounds **1**-**14** were added (1 μg/mL to 0.067 μg/mL). Cultures were grown overnight at 37°C. Cultures were spun down at 4000 rpm for 30 minutes. The supernatant was aspirated into a fresh 96 well plate and resulting colour change was visualized using a Synergy H4 microplate reader (BioTek) at 560nm.

Preparation of ClpP, ClpQ and ligand structures.

Crystal structures for ClpP and ClpQ (HlsV) from *Staphylococcus aureus* were taken from the PDB database using codes 3V5E and 6KUI respectively. Structures were prepared in the graphical user interface program AutoDock Tools (ADT). ADT removed water molecules, assigned polar hydrogens, computed Gasteiger charges and wrote prepared structures in a PDBQT file format. ChemDraw for armeniaspirol was converted to a MOL2 file format and pre-processed in Avogadro[1] by assigning polar hydrogens and performing structure minimization with default settings. Resulting pre-processed structure was opened in ADT to assign rotatable bonds and convert into a PDBQT file format[2–4].

Docking methodology.

Molecular docking was performed using the AutoDock Vina program[2,4]. Armeniaspirol was docked to both ClpP and ClpQ within grid coordinates (grid center) and grid boxes centered around the oligomers axis of symmetry using ADT. Armeniaspirol was in a flexible condition, with 6 rotatable bonds, when interacting with the proteins under rigid conditions. The grid size for ClpP was set to 70 × 70 × 58 *xyz* points with a grid spacing of 1 Å and a grid center designated at dimensions -44.762*x*, -12.455*y* and -19.341*z*. The grid size for ClpQ was set to 68 × 46 × 54 *xyz* points with a grid spacing of 1 Å and a grid center designated at dimensions 1.02*x*, 19.085*y* and 40.824*z*. Ligand-binding affinities were predicted as Gibbs free energy scores (ΔG, kcal/mol) using the AutoDock Vina scoring function. Docked ligand poses with the best score were chosen for post-docking analyses in PyMOL.

Hemolysis assay.

Compounds **1**, **2**, and **8** were diluted to 400 μM in PBS buffer from 50 mM DMSO stock. 1% Triton X-100 was used as the positive control, PBS was used as a negative control and 0.78% DMSO was used as the vehicle control. 75 μL of compound in PBS were added to 75 μL of sheep red blood cells (sRBC) in a 10% suspension in PBS buffer in a flat bottomed black 96-well plate. The plate was sealed and agitated at 37°C for 1 h. The plate was centrifuged for 10 mins at RT at 1000 x g and 35 μL of the supernatant was removed. A 10 μL aliquot from the supernatant was added to 90 μL PBS in a flat bottomed black 96-well plate and measured absorbance at 414 nm on a Synergy H1 Hybrid Multi-Mode Reader (Biotek, Winooski, VT). % hemolysis was calculated, hemolysis (%) = (abs sample – abs neg average)/(abs pos average – abs neg average) x 100%. The triplicate data was analyzed in GraphPad Prism 8.

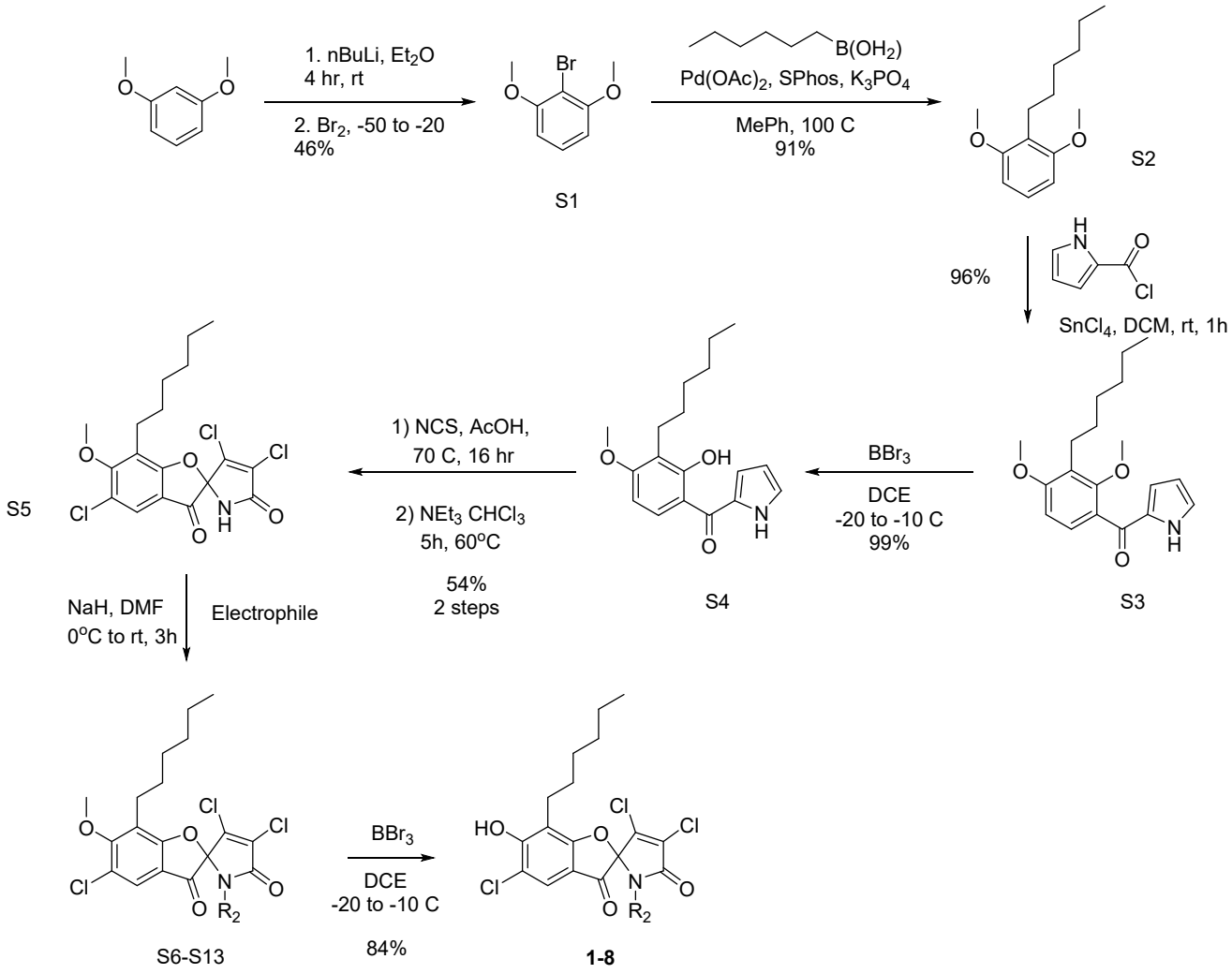
Mammalian cell culture toxicity.

Human lung epithelial carcinoma cells (A549) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin were grown and maintained under cell culture conditions (37°C, 5% CO_2, and in a humidified environment) in a 24-well plate. At confluency, fresh DMEM was added containing 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, or 0 μM of compound **1**, **2**, or **8** (from a 50 mM stock in DMSO) in triplicate wells and incubated under cell culture conditions for 24 hrs. Following incubation, fresh DMEM without phenol red containing 1 μM calcein-AM and 2 μM ethidium homodimer-1 was added and incubated under cell culture conditions for 30 min. Three-by-three tiled images were acquired on a Zeiss LSM 880 confocal microscope while maintaining cell culture conditions. Following microscopy, the media was set aside and cells were detached from the plate with trypsin-EDTA, centrifuged (5min, 1000 × g, 4°C), then resuspended in the dye-containing media. Cell populations were examined using flow cytometry using a 488 nm excitation with 525/40 bandpass for calcein-AM (live cells, green) and 620/20 bandpass for ethidium homodimer-1 (dead cells, red). The viable cell population for each condition, including a 0.5 % DMSO control (vehicle), was determined by comparing the total number of singly-stained, calcein-AM-positive cell counts to the total number of cells that were singly-stained as positive for live or dead.

Synthesis

General Synthetic Protocols

All reagents were purchased from Sigma-Aldrich at the highest available purity and used without further purification. All solvents were purchased from Fisher Scientific. All reactions were conducted using dry solvents under an argon atmosphere unless otherwise noted. NMR spectroscopy was performed with a Bruker Avance II, operating at 400 MHz for 1H spectra, and 100 MHz for 13C spectra or Bruker Avance III, with cryoprobe operating at 600 MHz for 1H spectra, and 150 MHz for 13C spectra. Preparatory TLC was performed using Merck Millipore 20x20cm silica gel 60 F254 plates. High-resolution mass spectroscopy (HRMS) was conducted on a Micromass Q-TOF I for ESI measurements and a Kratos Concept 1S High Resolution Mass Spectrometer for EI measurements (John L. Holmes Mass Spectroscopy Facility)



Scheme S1. Synthesis of hexyl R1 series of analogues

S1

1,3-dimethoxybenzene (14.40 g, 0.104 mol, 1.0 equiv.) was dissolved in Et_2O (375 mL) in a round bottom flask. n-BuLi (50 mL of a 2.5 M solution, 1.2 equiv.) was added and the solution was allowed to stir at ambient temperature for 4 hours. The reaction mixture was then cooled to – 50 °C using a dry ice/acetone bath. Bromine (18.65 g, 0.117 mol, 1.1 equiv.) was added dropwise. The solution was then heated to room temperature and allowed to react for another two hours. To the resulting mixture, 250 mL of a 10 % sodium thiosulfate solution was added and the resulting mixture was allowed to stir for 1 hour. The solution was extracted 2 × with Et_2O. The resulting organic fractions were combined and washed with brine. The organic phase was then dried with Na_2SO_4 and concentrated to yield the desired compound **S1** (9.45 g, 43.5 mmol, 42 % yield) which

was used without further purification. The NMR data were consistent with literature values.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.21 (t, *J* = 8.3 Hz, 1H), 6.56 (d, *J* = 8.4 Hz, 2H), 3.88 (s, 6H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 157.21, 128.24, 104.70, 100.97, 56.45.

S2

In a round-bottom flask, **S1** (5.0 g, 23.0 mmol, 1.0 equiv.) was dissolved in toluene (230 mL). To the resulting solution, n-hexylboronic acid (5.98 g, 46 mmol, 2.0 equiv.), $K_3PO_4 \cdot H_2O$ (10.6 g, 46 mmol, 2.0 equiv.), $Pd(OAc)_2$ (516 mg, 2.3 mmol, 0.1 equiv.), and SPhos (1.89 g, 4.6 mmol, 0.2 equiv.) were added at ambient temperature. The mixture was stirred at 100 °C for 15 hours. After cooling, the reaction mixture was quenched with NH_4Cl (aq) and extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4 and concentrated. The desired compound S2(4.65 g, 20.9 mmol, 91 % yield) was purified from the crude mixture by silica column chromatography (100 % hexanes). The NMR data were consistent with literature values.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.08 (t, *J* = 8.3 Hz, 1H), 6.51 (d, *J* = 8.3 Hz, 2H), 3.78 (s, 6H), 2.73 – 2.50 (m, 2H), 1.43 (q, *J* = 7.2 Hz, 2H), 1.37 – 1.22 (m, 6H), 0.89 – 0.81 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 158.30, 126.33, 119.75, 103.71, 55.70, 31.81, 29.53, 29.25, 22.91, 22.71, 14.17.

S3

Pyrrole 2-carboxylic acid (0.721g, 6.49 mmol, 2 eq) was dissolved in 10 ml of DCM. oxalyl chloride (9.7 mL, 19.48 mmol, 3 equiv) was added dropwise, 1 drop of DMF was added. The following mixture was stirred for 1 hour at room temperature. The solvent was removed efn vacuo. The intermediate acid chloride was resuspended in DCM In a round-bottom flask, **S2** (0.5 g, 3.246 mmol, 1.0 equiv.) was added and cooled to 0 °C with an ice bath. added, followed by $SnCl_4$ (16.2

mL of a 1.0 M solution, 5 equiv.). The mixture was stirred for 1 hour at 0 °C, then warmed to ambient temperature. The reaction mixture was quenched with a saturated $NaHCO_3$(aq) and extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4 and concentrated. The desired compound (0.640 g, 2.519 mmol, 78 % yield) was purified from the crude mixture by silica column chromatography (30 % EtOAc in Hexanes). The NMR data were consistent with literature values

^{1}H NMR (400 MHz, Acetone-d_6) δ 7.30 (d, J = 8.5 Hz, 1H), 7.19 (dq, J = 2.9, 1.5 Hz, 1H), 6.80 (d, J = 8.6 Hz, 1H), 6.59 (ddd, J = 3.8, 2.2, 1.4 Hz, 1H), 6.23 (dt, J = 3.8, 2.3 Hz, 1H), 3.89 (s, 3H), 3.67 (s, 3H), 2.70 – 2.57 (m, 2H), 1.52 (q, J = 7.7 Hz, 2H), 1.42 – 1.24 (m, 6H), 0.93 – 0.82 (m, 3H).

^{13}C NMR (100 MHz, Acetone-d_6) δ 183.59, 160.13, 157.54, 132.64, 128.20, 125.82, 125.16, 124.37, 118.53, 109.94, 105.12, 61.89, 55.29, 31.56, 29.43, 23.38, 22.43, 13.49.

S4

In a round-bottom flask, **S2** (0.830 g, 2.63 mmol, 1.0 equiv.) was dissolved in 1,2-dichloroethane (3 mL). The solution was cooled to -20 °C using a dry ice/acetone bath. BBr3 (3.13 mL of a 1 M solution, 1.2 equiv.) was added dropwise, and the reaction mixture was stirred at -20 to – 10 °C for 2 hours. Subsequently, Et3N / water was added, and the solution was extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na2SO4, and concentrated. The desired compound (0.788g, 2.61 mmol, 99 % yield) was used without purification. The NMR data were consistent with literature values

^{1}H NMR (400 MHz, Acetone-d_6) δ 12.76 (s, 1H), 8.05 (dd, J = 8.9, 0.9 Hz, 1H), 7.27 (ddd, J = 3.0, 2.5, 1.4 Hz, 1H), 7.05 (dtd, J = 4.2, 2.8, 1.3 Hz, 1H), 6.67 (d, J = 9.0 Hz, 1H), 6.36 (ddd, J = 3.9, 2.9, 2.0 Hz, 1H), 3.93 (s, 3H), 2.73 – 2.58 (m, 2H), 1.59 – 1.42 (m, 2H), 1.40 – 1.26 (m, 6H), 0.91 – 0.82 (m, 3H).

^{13}C NMR (100 MHz, Acetone-d_6) δ 186.18, 162.83, 161.95, 130.68, 129.95, 125.49, 118.54, 117.86, 113.36, 110.70, 102.20, 55.34, 31.64, 29.28, 28.60, 22.45, 22.23, 13.50.

S6- S13 we prepared following the same procedure

Separately in a round-bottom flasks, **S4** (1.0 equiv.) were dissolved in acetic acid (0.2M). N-chlorosuccinimide (2.0 equiv.) was added and the mixture was stirred at ambient temperature for 2 hours. Following this, N-chlorosuccinimide (4.0 equiv.) was added and the resulting mixture was heated to 70 °C for 16 hours. The reaction mixture was then quenched with a 10 % K_2CO_3(aq) and extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4 and concentrated. The resulting oil was dissolved in $CHCl_3$ (0.2M) and Et_3N (3 equiv.) was added. The mixture was heated at 60 °C for 5 hours. The solution was cooled to ambient temperature, concentrated, and the spiro- intermediates were isolated from the crude mixture by silica column chromatography (10 % EtOAc in hexanes). The spiro- intermediate (1.0 equiv.) was dissolved in DMF (0.2M). The solution was cooled to 0 °C using an ice bath and NaH (1.5 equiv.) was added and allowed to stir for 15 min. Subsequently, the desired electrophile (1.3 equiv.) was

added dropwise and stirring was continued at 0 °C to ambient temperature for 5 hours. The mixture was quenched with NH_4Cl(aq), extracted 3 × with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated. The desired compounds **S6- S13** (41-54%) were purified from the crude mixture by silica column chromatography (25 % EtOAc in hexanes)

S6

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 4.00 (s, 3H), 2.79 (s, 3H), 2.76 – 2.70 (m, 2H), 1.64 – 1.55 (m, 3H), 1.41 – 1.24 (m, 6H), 0.88 (t, J = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 189.88, 170.33, 163.79, 163.16, 138.24, 129.38, 124.84, 124.33, 123.73, 115.65, 97.05, 61.92, 31.73, 29.46, 29.38, 25.96, 24.00, 22.71, 14.19.

HRMS (ESI): Exact mass calculated for $C_{19}H_{20}Cl_3NNaO_4$ [M + Na] + : 454.0350. Found: 454.0356

S7

1H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 4.00 (s, 3H), 3.42 (dt, J = 14.5, 7.3 Hz, 1H), 3.00 (dt, J = 14.5, 7.3 Hz, 1H), 2.78 – 2.64 (m, 2H), 1.65 – 1.53 (m, 2H), 1.48 – 1.12 (m, 14H), 0.88 (t, J = 6.9 Hz, 3H), 0.83 (t, J = 6.8 Hz, 3H).

13C NMR (100 MHz, $CDCl_3$) δ 190.25, 170.00, 163.71, 163.46, 138.25, 129.29, 124.75, 124.33, 123.72, 115.84, 97.38, 61.93, 41.67, 31.74, 31.36, 29.54, 29.47, 28.76, 26.47, 24.00, 22.71, 22.56, 14.20, 14.10.

HRMS (ESI): Exact mass calculated for $C_{24}H_{30}Cl_3NNaO_4$ [M + Na] + : 524.1133. Found: 524.1134

S8

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.62 (s, 1H), 3.98 (s, 3H), 3.40 (dt, *J* = 14.7, 7.5 Hz, 1H), 2.98 (dt, *J* = 14.6, 7.5 Hz, 1H), 2.77 – 2.61 (m, 2H), 1.64 – 1.54 (m, 2H), 1.45 – 1.13 (m, 26H), 0.87 (dt, *J* = 5.9, 3.8 Hz, 6H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 190.11, 169.85, 163.55, 163.32, 138.10, 129.14, 124.60, 124.18, 123.58, 115.69, 97.23, 61.79, 41.54, 31.92, 31.61, 31.60, 29.64, 29.63, 29.57, 29.44, 29.41, 29.35, 29.08, 28.66, 26.67, 23.87, 22.70, 22.59, 14.13, 14.08.

HRMS (ESI): Exact mass calculated for $C_{30}H_{42}Cl_3NNaO_4$ [M + Na] + : 608.2077. Found: 608.2056

S9

^{1}H NMR (400 MHz, Acetone-d_6) δ 7.72 (s, 1H), 5.70 (ddt, *J* = 16.7, 10.1, 6.3 Hz, 1H), 5.04 – 4.91 (m, 2H), 4.07 (ddt, *J* = 15.9, 6.3, 1.5 Hz, 1H), 4.02 (s, 3H), 3.81 (ddt, *J* = 15.9, 6.4, 1.4 Hz, 1H), 2.80 – 2.74 (m, 2H), 1.72 – 1.59 (m, 2H), 1.44 – 1.27 (m, 6H), 0.87 (td, *J* = 5.8, 4.7, 2.8 Hz, 3H).

^{13}C NMR (100 MHz, Acetone-d_6) δ 189.69, 169.85, 163.50, 162.05, 138.79, 132.15, 128.57, 124.33, 124.17, 123.24, 118.31, 115.95, 96.66, 61.53, 43.32, 31.44, 29.05, 29.00, 23.48, 22.33, 13.43.

HRMS (ESI): Exact mass calculated for $C_{21}H_{22}Cl_3NNaO_4$ [M + Na] +: 480.0512. Found: 480.0502

S10

^{1}H NMR (400 MHz, Acetone-d_6) δ 7.61 (s, 1H), 7.22 – 7.17 (m, 3H), 7.11 – 7.07 (m, 2H), 4.80 – 4.72 (d, J = 15.5 Hz, 1H), 4.23 (d, J = 15.5 Hz, 1H), 3.98 (s, 3H), 2.59 (ddd, J = 13.3, 9.1, 6.1 Hz, 1H), 2.40 (ddd, J = 13.3, 9.2, 6.3 Hz, 1H), 1.49 – 1.38 (m, 2H), 1.27 (d, J = 8.2 Hz, 6H), 0.88 – 0.79 (m, 3H).

^{13}C NMR (100 MHz, Acetone-d_6) δ 189.40, 169.73, 163.35, 162.53, 138.86, 135.30, 128.65, 128.49, 128.25, 127.86, 124.23, 124.14, 123.12, 115.70, 96.83, 61.43, 44.58, 31.33, 29.40, 29.01, 23.32, 22.33, 13.44.

HRMS (ESI): Exact mass calculated for $C_{25}H_{24}Cl_3NNaO_4$ [M + Na] +: 530.0669 Found: 530.0685

S11

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.72 (s, 1H), 7.66 – 7.63 (m, 2H), 7.46 (ddt, J = 8.1, 1.5, 0.8 Hz, 2H), 5.00 – 4.93 (m, 1H), 4.39 (d, J = 16.0 Hz, 1H), 4.04 (s, 3H), 2.64 (ddd, J = 13.3, 9.5, 6.0 Hz, 1H), 2.42 (ddd, J = 13.3, 9.6, 6.2 Hz, 1H), 1.38 – 1.25 (m, 8H), 0.95 – 0.89 (m, 3H).

^{13}C NMR (150 MHz, Acetone-d_6) δ 189.37, 169.78, 163.55, 162.61, 140.37, 138.98, 128.96, 128.65, 125.21, 125.19, 125.16, 124.44, 124.12, 123.35, 115.58, 96.90, 61.50, 43.98, 31.28, 23.24, 22.35, 13.42.

HRMS (ESI): Exact mass calculated for $C_{26}H_{23}Cl_3F_3NNaO_4$ [M + Na] +: 598.0542 Found: 598.0552

S12

^{1}H NMR (600 MHz, Chloroform-d) δ 7.57 (s, 1H), 7.18 – 7.08 (m, 3H), 7.01 – 6.96 (m, 2H), 3.94 (s, 3H), 3.54 (ddd, J = 14.5, 10.1, 6.2 Hz, 1H), 3.18 (ddd, J = 14.5, 10.1, 5.9 Hz, 1H), 2.82 – 2.69 (m, 2H), 2.66 (dt, J = 13.2, 7.4 Hz, 1H), 2.58 (dt, J = 13.2, 7.5 Hz, 1H), 1.53 – 1.48 (m, 3H), 1.32 – 1.29 (m, 2H), 1.23 – 1.18 (m, 6H), 0.79 (td, J = 5.8, 4.8, 2.4 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 189.93, 169.85, 163.65, 163.22, 138.31, 137.69, 129.12, 128.56, 128.55, 126.71, 124.72, 124.22, 123.63, 115.64, 97.17, 61.82, 42.89, 34.81, 31.57, 29.41, 29.31, 23.87, 22.56, 14.06.

HRMS (ESI): Exact mass calculated for $C_{26}H_{26}Cl_3NNaO_4$ [M + Na] +: 544.0825 Found: 544.0830

S13

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.45 (s, 1H), 6.93 – 6.90 (m, 2H), 6.90 – 6.86 (m, 2H), 4.65 (d, *J* = 15.1 Hz, 1H), 4.19 (d, *J* = 15.1 Hz, 1H), 3.95 (s, 3H), 2.53 (ddd, *J* = 13.2, 9.6, 5.8 Hz, 1H), 2.35 (ddd, *J* = 13.2, 9.6, 6.1 Hz, 1H), 2.25 (s, 3H), 1.45 – 1.36 (m, 2H), 1.34 – 1.24 (m, 8H), 0.88 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 189.67, 169.55, 163.35, 163.13, 138.81, 137.84, 131.25, 129.03, 128.88, 124.23, 124.06, 123.11, 115.75, 96.80, 61.71, 44.93, 31.55, 29.32, 29.12, 23.58, 22.61, 21.10, 14.10.

HRMS (ESI): Exact mass calculated for $C_{26}H_{26}Cl_3NNaO_4$ [M + Na] +: 544.0825 Found: 544.0830

1-8 we prepared following the same procedure

In separate round-bottom flasks, **1-8** (1.0 equiv.) were dissolved in 1,2- dichloroethane (0.4M). The mixture was cooled to 0 °C using an ice bath and BBr_3 (3.0 equiv.) was added dropwise. The reaction was allowed to proceed for 4 hours from 0 °C to ambient temperature. Et_3N / water was added, and the solution was extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4, and concentrated. The desired compounds **1-8** (63-84%) were purified from the crude mixture using Preparative TLC (20 to 30 % EtOAc in hexanes)

1

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 6.62 (s, 1H), 2.79 (s, 3H), 2.76 (t, J = 7.6 Hz, 2H), 1.66 – 1.56 (m, 2H), 1.39 – 1.24 (m, 6H), 0.87 (t, J = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 188.93, 170.34, 163.21, 158.77, 138.43, 129.27, 122.56, 117.67, 115.79, 112.66, 97.16, 31.77, 29.22, 28.47, 25.91, 23.37, 22.74, 14.21.

HRMS (ESI): Exact mass calculated for C18H17Cl3NO4 [M - H] - : 416.0229. Found: 416.0223

2

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 6.51 (s, 1H), 3.46 – 3.36 (m, 1H), 3.04 – 2.91 (m, 1H), 2.80 – 2.63 (m, 2H), 1.65 – 1.11 (m, 16H), 0.86 (t, J = 7.1 Hz, 3H), 0.81 (t, J = 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 189.33, 170.01, 163.50, 158.65, 138.42, 129.20, 122.54, 117.57, 115.82, 112.88, 97.50, 41.65, 31.79, 31.39, 29.31, 28.76, 28.56, 26.50, 23.38, 22.74, 22.58, 14.22, 14.10.

HRMS (ESI): Exact mass calculated for C23H27Cl3NO4 [M - H]- : 486.1001. Found: 486.1006

3

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.42 (s, 1H), 3.58 (dt, *J* = 14.2, 7.5 Hz, 1H), 3.06 (ddd, *J* = 14.1, 7.6, 6.3 Hz, 3H), 2.68 – 2.61 (m, 2H), 1.62 (m, 30H), 1.02 – 0.96 (m, 6H).

^{13}C NMR (150 MHz, Acetone-d_6) δ 180.35, 174.82, 169.38, 162.81, 141.26, 126.57, 125.15, 121.07, 112.12, 100.69, 97.74, 40.79, 31.99, 31.79, 29.52, 29.52, 29.47, 29.38, 29.08, 26.69, 23.18, 22.61, 22.48, 13.63, 13.52.

HRMS (+EI): Exact mass calculated for $C_{29}H_{40}Cl_3NO_4$: 571.2023 Found: 571.2015

4

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.27 (s, 1H), 5.73 (ddt, *J* = 17.1, 10.2, 6.0 Hz, 1H), 5.06 (dq, *J* = 17.1, 1.6 Hz, 1H), 4.96 (dq, *J* = 10.2, 1.4 Hz, 1H), 4.04 (ddt, *J* = 16.0, 5.9, 1.5 Hz, 1H), 3.51 (dd, *J* = 10.9, 5.8 Hz, 1H), 2.52 – 2.47 (m, 2H), 1.51 – 1.47 (m, 2H), 1.29 (q, *J* = 9.0, 8.0 Hz, 8H), 0.86 (td, *J* = 7.1, 2.7 Hz, 3H).

^{13}C NMR (150 MHz, Acetone d_6) δ 179.76, 174.75, 169.29, 162.42, 141.74, 132.94, 126.43, 125.25, 121.00, 116.72, 111.97, 100.57, 97.49, 43.01, 31.95, 29.50, 28.77, 23.11, 22.56, 13.60.

HRMS (+EI): Exact mass calculated for $C_{20}H_{20}Cl_3NO_4$: 443.0458 Found: 443.0460

5

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.41 (s, 1H), 7.13 – 7.06 (m, 3H), 6.97 – 6.94 (m, 2H), 4.63 (d, *J* = 15.3 Hz, 1H), 4.12 (d, *J* = 15.3 Hz, 1H), 2.48 (ddd, *J* = 13.3, 9.1, 5.9 Hz, 1H), 2.31 (ddd, *J* = 13.2, 9.0, 6.5 Hz, 1H), 1.41 – 1.25 (m, 3H), 1.22 – 1.18 (m, 7H), 0.80 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 188.67, 169.56, 163.35, 158.19, 138.96, 134.79, 128.95, 128.57, 128.28, 127.98, 122.00, 117.17, 115.58, 112.76, 107.21, 97.00, 44.98, 31.58, 29.13, 28.17, 23.03, 22.61, 14.10.

HRMS (+EI): Exact mass calculated for $C_{24}H_{22}Cl_3NO_4$: 493.0614 Found: 493.0619

6

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.72 – 7.66 (m, 2H), 7.57 – 7.45 (m, 2H), 7.41 – 7.40 (m, 1H), 4.90 (ddd, *J* = 16.0, 7.9, 2.1 Hz, 1H), 4.27 – 4.19 (m, 1H), 2.55 – 2.48 (m, 1H), 2.34 (dddd, *J* = 12.6, 9.0, 6.5, 4.0 Hz, 1H), 1.54 – 1.42 (m, 2H), 1.38 – 1.26 (m, 8H), 0.97 – 0.94 (m, 3H).

^{13}C NMR (150 MHz, Acetone) δ 179.89, 174.72, 169.29, 162.93, 141.85, 128.45, 128.37, 128.10, 126.56, 125.19, 125.05, 125.02, 125.00, 124.14, 121.10, 112.31, 100.87, 97.71, 43.84, 31.80, 29.50, 29.14, 22.97, 22.59, 13.54.

HRMS (+EI): Exact mass calculated for $C_{25}H_{21}Cl_3F_3NO_4$: 561.0488 Found: 561.0479

7

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.33 (s, 1H), 7.16 – 7.12 (m, 2H), 7.10 – 7.06 (m, 2H), 4.69 (d, J = 15.5 Hz, 1H), 4.01 (d, J = 15.6 Hz, 1H), 2.47 (ddd, J = 12.8, 9.4, 5.0 Hz, 1H), 2.38 – 2.33 (m, 1H), 1.50 – 1.41 (m, 2H), 1.32 – 1.25 (m, 7H), 0.90 (t, J = 7.0 Hz, 4H).

^{13}C NMR (150 MHz, Acetone) δ 179.91, 174.81, 169.32, 162.77, 141.61, 136.55, 134.05, 128.69, 128.00, 126.54, 125.18, 121.00, 112.20, 100.72, 97.83, 44.20, 31.91, 29.51, 29.12, 23.10, 22.61, 20.27, 13.62.

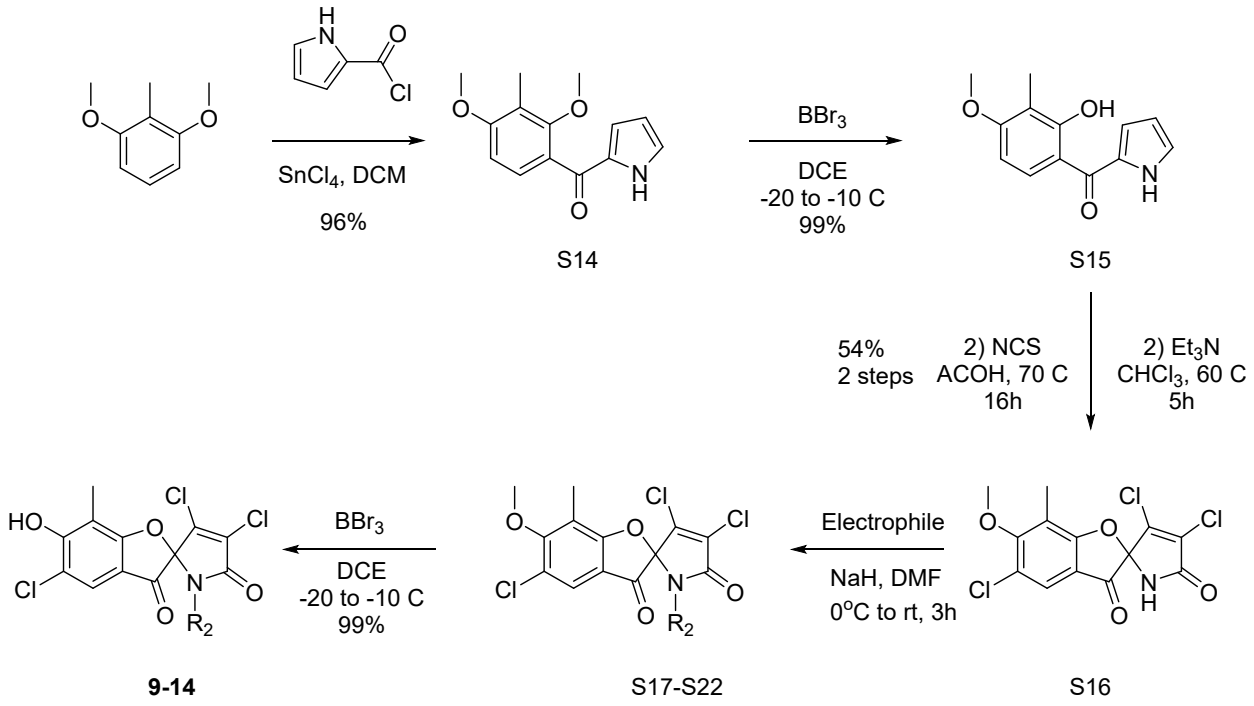
HRMS (+EI): Exact mass calculated for $C_{25}H_{24}Cl_3NO_4$: 507.0771 Found: 507.0768

8

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.37 (s, 1H), 7.26 – 7.21 (m, 2H), 7.18 – 7.11 (m, 3H), 3.69 – 3.61 (m, 1H), 3.18 (ddd, J = 14.2, 9.5, 5.6 Hz, 1H), 2.86 – 2.76 (m, 3H), 2.56 (qdd, J = 12.7, 8.5, 6.5 Hz, 2H), 1.59 – 1.47 (m, 2H), 1.31 (ddd, J = 6.5, 3.5, 1.5 Hz, 2H), 1.24 – 1.19 (m, 4H), 0.82 – 0.79 (m, 3H).

^{13}C NMR (150 MHz, Acetone) δ 180.64, 174.89, 169.52, 162.60, 141.30, 138.70, 128.61, 128.39, 126.67, 126.32, 125.20, 121.20, 112.37, 100.06, 97.70, 42.21, 34.65, 31.92, 29.38, 28.83, 23.16, 22.54, 13.58.

HRMS (+EI): Exact mass calculated for $C_{25}H_{24}Cl_3NO_4$: 507.0771 Found: 507.0777



Scheme S2. Synthesis of r1 methyl series of analogues

S14

Pyrrole 2-carboxylic acid (0.721g, 6.49 mmol, 2 eq) was dissolved in 10 ml of DCM. oxalyl chloride (9.7 mL, 19.48 mmol, 3 equiv) was added dropwise, 1 drop of DMF was added. The following mixture was stirred for 1 hour at room temperature. The solvent was removed en vacuo. The intermediate acid chloride was resuspended in DCM In a round-bottom flask, 2,6 dimethoxy toluene (0.5 g, 3.246 mmol, 1.0 equiv.) was added and cooled to 0 °C with an ice bath. added, followed by $SnCl_4$ (16.2 mL of a 1.0 M solution, 5 equiv.). The mixture was stirred for 1 hour at 0 °C, then warmed to ambient temperature. The reaction mixture was quenched with a saturated $NaHCO_3$(aq) and extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4 and concentrated. The desired compound (0.640 g, 2.519 mmol, 78 % yield) was purified from the crude mixture by silica column chromatography (30 % EtOAc in Hexanes.

^{1}H NMR (400 MHz, Acetone-d_6) δ 7.30 (dq, J = 8.5, 0.6 Hz, 1H), 7.20 (dq, J = 2.9, 1.5 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 6.57 (ddd, J = 3.7, 2.2, 1.4 Hz, 1H), 6.23 (dt, J = 3.8, 2.3 Hz, 1H), 3.90 (s, 3H), 3.69 (s, 3H), 2.13 (d, J = 0.5 Hz, 3H).

^{13}C NMR (100 MHz, Acetone) δ 183.47, 160.22, 157.53, 132.70, 127.67, 126.21, 125.16, 119.56, 118.55, 109.91, 104.90, 61.41, 55.29, 8.14.

HRMS (+EI) : calcd for: $C_{14}H_{15}NO_3$ Exact Mass: 245.1052. Found: 245.1043

S15

In a round-bottom flask, **S14** (0.641 g, 2.61 mmol, 1.0 equiv.) was dissolved in 1,2-dichloroethane (3 mL). The solution was cooled to -20 °C using a dry ice/acetone bath. BBr_3 (3.13 mL of a 1 M solution, 1.2 equiv.) was added dropwise and the reaction mixture was stirred at -20 to – 10 °C for 2 hours. Subsequently, Et_3N / water was added and the solution was extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4, and concentrated. The desired compound (0.600g, 2.586 mmol, 99 % yield) was used without purification.

^{1}H NMR (400 MHz, Acetone-d_6) δ 8.05 (dd, J = 9.0, 0.7 Hz, 1H), 7.27 (ddd, J = 3.0, 2.5, 1.3 Hz, 1H), 7.05 (ddd, J = 3.9, 2.5, 1.3 Hz, 1H), 6.67 (d, J = 9.0 Hz, 1H), 6.35 (dt, J = 3.8, 2.4 Hz, 1H), 3.93 (s, 3H), 2.06 (d, J = 0.5 Hz, 3H).

^{13}C NMR (100 MHz, Acetone) δ 186.09, 162.86, 161.63, 130.47, 129.99, 125.48, 118.52, 113.27, 112.69, 110.67, 100.98, 55.35, 7.00.

HRMS (+EI) : calcd for: $C_{13}H_{13}NO_3$ Exact Mass: 231.0895 Found: 231.0880

S17- S22 we prepared following the same procedure

Separately in a round-bottom flasks, **S15** (1.0 equiv.) were dissolved in acetic acid (0.2M). N-chlorosuccinimide (2.0 equiv.) was added and the mixture was stirred at ambient temperature for 2 hours. Following this, N-chlorosuccinimide (4.0 equiv.) was added and the resulting mixture was heated to 70 °C for 16 hours. The reaction mixture was then quenched with a 10 % K_2CO_3(aq) and extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4 and concentrated. The resulting oil was dissolved in $CHCl_3$ (0.2M) and Et_3N (3 equiv.) was added. The mixture was heated at 60 °C for 5 hours. The solution was cooled to ambient temperature, concentrated, and the spiro- intermediates were isolated from the crude mixture by silica column chromatography (10 % EtOAc in hexanes). The spiro- intermediate (1.0 equiv.) was dissolved in DMF (0.2M). The solution was cooled to 0 °C using an ice bath and NaH (1.5 equiv.) was added and allowed to stir for 15 min. Subsequently, the desired electrophile (1.3 equiv.) was added dropwise and stirring was continued at 0 °C to ambient temperature for 5 hours. The mixture was quenched with NH_4Cl(aq), extracted 3 × with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated. The desired compounds **S17- S22** (44-54% over 2 steps) were purified from the crude mixture by silica column chromatography (25 % EtOAc in hexanes)

S17

^{1}H NMR (600 MHz, Chloroform-d) δ 7.64 (s, 1H), 3.98 (s, 3H), 2.80 (s, 3H), 2.31 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 189.71, 170.16, 163.75, 163.03, 138.08, 129.25, 124.65, 123.36, 119.07, 115.43, 97.00, 61.08, 25.88, 8.75.

HRMS (ESI): Exact mass calculated for $C_{14}H_{10}Cl_3NNaO_4$ [M + Na] +: 383.9573. Found: 383.9566

S18

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.64 (d, *J* = 0.7 Hz, 1H), 3.99 (s, 3H), 3.42 (ddd, *J* = 14.6, 8.3, 6.5 Hz, 1H), 3.00 (ddd, *J* = 14.5, 8.2, 6.3 Hz, 1H), 2.29 (d, *J* = 0.7 Hz, 3H), 1.50 – 1.35 (m, 3H), 1.25 – 1.12 (m, 7H), 0.83 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 190.24, 170.02, 163.82, 163.43, 138.17, 129.35, 124.72, 123.51, 119.16, 115.74, 97.41, 61.23, 41.67, 31.37, 28.68, 26.41, 22.55, 14.11, 8.82.

HRMS (ESI): Exact mass calculated for $C_{19}H_{20}Cl_3NO_4$ [M + Na]+: 454.0386 Found: 454.0347

S19

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.64 (d, *J* = 0.8 Hz, 1H), 3.98 (s, 3H), 3.42 (ddd, *J* = 14.7, 8.4, 6.4 Hz, 1H), 3.00 (ddd, *J* = 14.6, 8.3, 6.3 Hz, 1H), 2.29 (d, *J* = 0.6 Hz, 3H), 1.48 – 1.36 (m, 3H), 1.30 – 1.16 (m, 20H), 0.89 – 0.86 (m, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 190.09, 169.86, 163.66, 163.27, 138.01, 129.18, 124.57, 123.36, 119.01, 115.57, 97.24, 61.09, 41.52, 31.91, 29.62, 29.55, 29.40, 29.35, 29.34, 29.06, 28.57, 26.59, 22.69, 14.13, 8.69.

HRMS (ESI): Exact mass calculated for $C_{25}H_{32}Cl_3NO_4$ [M + Na]+: 538.1295 Found: 538.1308

S20

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.60 (t, *J* = 0.7 Hz, 1H), 5.67 (ddt, *J* = 16.7, 10.1, 6.5 Hz, 1H), 4.97 – 4.84 (m, 2H), 4.04 (ddt, *J* = 15.7, 6.4, 1.4 Hz, 1H), 3.97 (s, 4H), 3.77 (ddt, *J* = 15.8, 6.6, 1.4 Hz, 1H), 2.27 (d, *J* = 0.7 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 189.95, 169.72, 163.53, 162.87, 138.60, 131.76, 129.08, 124.47, 123.12, 118.97, 118.96, 115.83, 96.74, 61.11, 43.70, 8.65.

HRMS (ESI): Exact mass calculated for $C_{16}H_{12}Cl_3NNaO_4$ [M + Na]+: 409.9730 Found: 409.9709

S21

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.50 (q, *J* = 0.6 Hz, 1H), 7.17 (d, *J* = 7.3 Hz, 1H), 7.15 – 7.10 (m, 2H), 7.02 – 6.97 (m, 2H), 4.86 (d, *J* = 15.1 Hz, 1H), 4.04 (d, *J* = 15.2 Hz, 1H), 3.91 (s, 3H), 1.93 (d, *J* = 0.6 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 189.71, 169.68, 163.30, 163.28, 138.69, 134.68, 129.10, 128.73, 128.20, 128.01, 124.31, 122.86, 119.04, 115.54, 96.91, 60.95, 45.10, 8.34.

HRMS (ESI): Exact mass calculated for $C_{20}H_{14}Cl_3NNaO_4$ [M + Na]+: 459.9886 Found: 459.9895

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.54 (q, *J* = 0.6 Hz, 1H), 7.44 – 7.40 (m, 2H), 7.18 – 7.14 (m, 2H), 5.00 – 4.91 (m, 1H), 4.03 (d, *J* = 15.5 Hz, 1H), 3.91 (s, 3H), 1.91 (d, *J* = 0.6 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 189.46, 169.56, 163.68, 163.34, 138.96, 138.93, 129.02, 128.89, 125.20, 125.17, 125.15, 125.12, 124.63, 123.03, 118.96, 115.28, 96.90, 61.01, 44.53, 8.19.

HRMS (ESI): Exact mass calculated for $C_{21}H_{13}Cl_3F_3NNaO_4$ [M + Na]+: 527.9760 Found: 527.9769

9-14 we prepared following the same procedure

In separate round-bottom flasks, **9-14** (1.0 equiv.) wer dissolved in 1,2- dichloroethane (0.4M). The mixture was cooled to 0 °C using an ice bath and BBr_3 (3.0 equiv.) was added dropwise. The reaction was allowed to proceed for 4 hours from 0 °C to ambient temperature. Et_3N / water was added, and the solution was extracted 3 × with EtOAc. The organic fractions were combined, washed with brine, dried over Na_2SO_4, and concentrated. The desired compounds **9-14** (88-99%) were purified from the crude mixture using Preparative TLC (20 to 40 % EtOAc in hexanes)

9

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.32 (d, *J* = 0.4 Hz, 1H), 2.71 (s, 3H), 1.94 (d, *J* = 0.3 Hz, 3H).

^{13}C NMR (150 MHz, Acetone) δ 180.35, 175.27, 169.69, 162.51, 141.05, 126.79, 124.62, 121.04, 106.96, 100.86, 97.32, 24.83, 7.77.

HRMS (+EI): Exact mass calculated for $C_{13}H_8Cl_3NO_4$: 346.9519 Found: 346.9522

10

^{1}H NMR (600 MHz, Methanol-d_4) δ 7.43 (s, 1H), 3.45 (ddd, *J* = 14.6, 8.0, 6.9 Hz, 1H), 2.98 (ddd, *J* = 14.2, 7.9, 6.1 Hz, 1H), 2.01 (s, 3H), 1.53 – 1.41 (m, 2H), 1.27 – 1.21 (m, 5H), 0.85 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (150 MHz, MeOD) δ 183.53, 176.13, 170.25, 163.74, 140.58, 127.13, 125.17, 121.35, 108.36, 103.17, 97.82, 40.73, 30.98, 28.04, 26.00, 22.08, 12.96, 7.12.

HRMS (+EI): Exact mass calculated for $C_{18}H_{18}Cl_3NO_4$: 417.0301 Found: 417.0310

11

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 6.51 (s, 1H), 3.46 – 3.36 (m, 1H), 3.04 – 2.91 (m, 1H), 2.80 – 2.63 (m, 2H), 1.65 – 1.11 (m, 16H), 0.86 (t, J = 7.1 Hz, 3H), 0.81 (t, J = 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 189.33, 170.01, 163.50, 158.65, 138.42, 129.20, 122.54, 117.57, 115.82, 112.88, 97.50, 41.65, 31.79, 31.39, 29.31, 28.76, 28.56, 26.50, 23.38, 22.74, 22.58, 14.22, 14.10.

HRMS (+EI): Exact mass calculated for $C_{24}H_{30}Cl_3NO_4$: 501.1240 Found: 501.1233

12

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.30 (s, 1H), 5.71 (dddd, *J* = 17.1, 10.2, 6.3, 5.8 Hz, 1H), 5.03 (dq, *J* = 17.1, 1.6 Hz, 1H), 4.95 (dq, *J* = 10.2, 1.4 Hz, 1H), 4.04 (ddt, *J* = 16.0, 5.9, 1.5 Hz, 1H), 3.60 – 3.58 (m, 1H), 3.57 – 3.55 (m, 1H), 1.91 (s, 3H).

^{13}C NMR (150 MHz, Acetone-d_6) δ 181.25, 176.03, 170.26, 163.20, 142.30, 133.71, 127.50, 125.45, 121.81, 117.55, 107.84, 100.90, 98.30, 43.82, 8.53.

HRMS (+EI): Exact mass calculated for $C_{15}H_{10}Cl_3NO_4$: 372.9675 Found: 372.9675

13

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.30 (s, 1H), 7.21 – 7.16 (m, 5H), 4.74 (d, *J* = 15.5 Hz, 1H), 3.94 (d, *J* = 15.5 Hz, 1H), 1.72 (s, 3H).

^{13}C NMR (150 MHz, Acetone-d_6) δ 180.15, 175.14, 169.30, 162.70, 141.43, 137.04, 128.13, 128.03, 127.14, 126.68, 124.67, 120.94, 107.04, 100.95, 97.79, 44.38, 7.57.

HRMS (+EI): Exact mass calculated for $C_{19}H_{12}C_{l3}NO_4$: 422.9832 Found: 422.9840

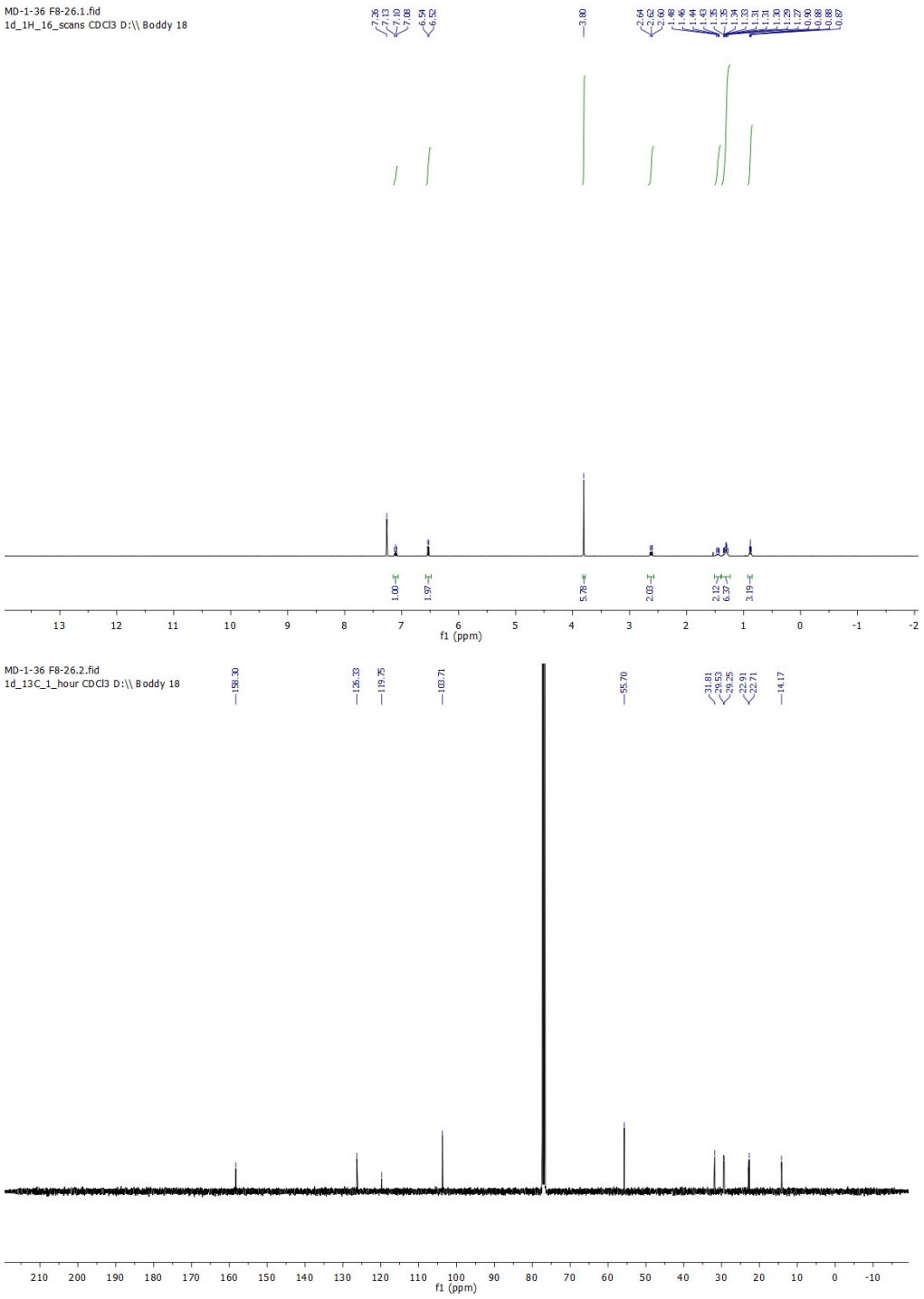
14

^{1}H NMR (600 MHz, Acetone-d_6) δ 7.56 (dd, *J* = 19.3, 8.2 Hz, 2H), 7.39 (dd, *J* = 22.2, 8.1 Hz, 2H), 7.29 (s, 1H), 4.87 – 4.80 (m, 1H), 4.07 (d, *J* = 15.9 Hz, 1H), 1.68 (s, 3H).

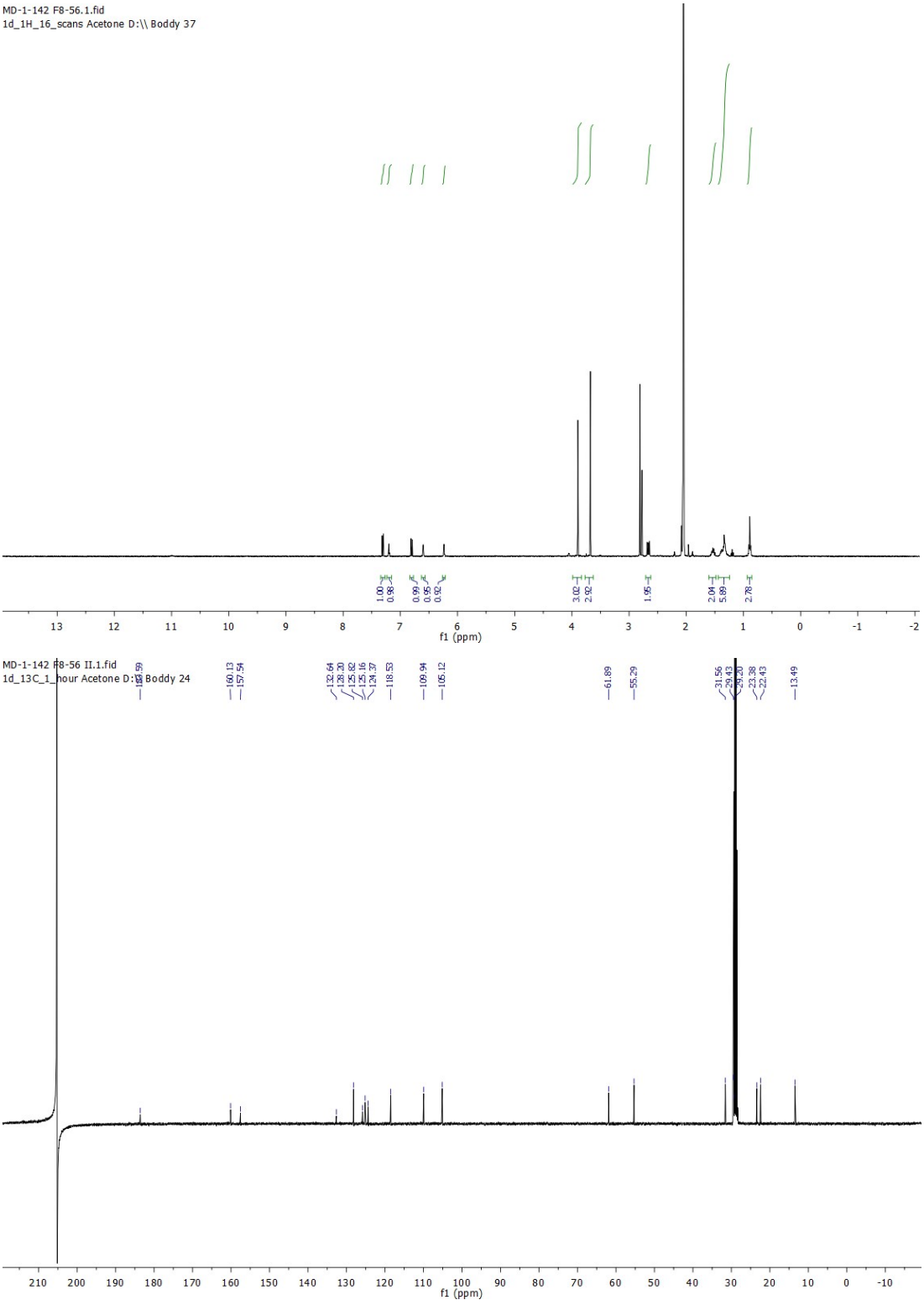
^{13}C NMR (150 MHz, Acetone-d_6) δ 179.74, 175.16, 169.11, 162.80, 141.72, 128.67, 128.55, 126.61, 124.97, 124.94, 124.92, 124.89, 124.80, 120.99, 106.95, 100.76, 97.69, 43.76, 25.31, 7.45.

HRMS (+EI): Exact mass calculated for $C_{20}H_{11}Cl_3F_3NO_4$: 490.9706 Found 490.9701

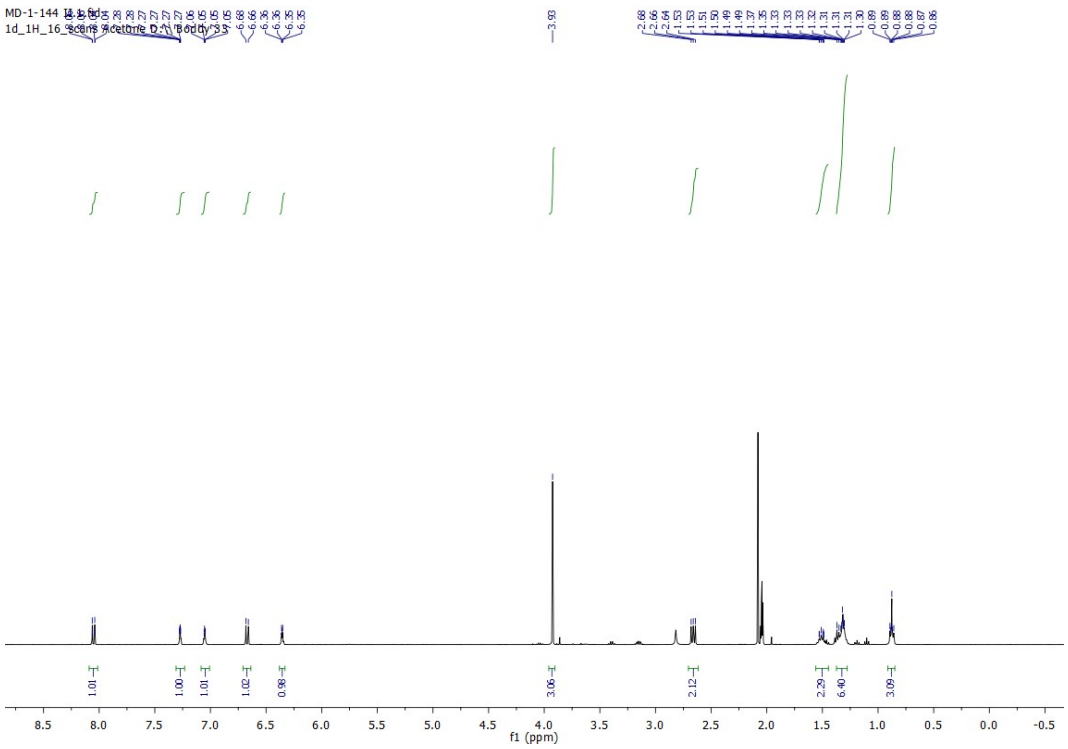
NMR Spectra

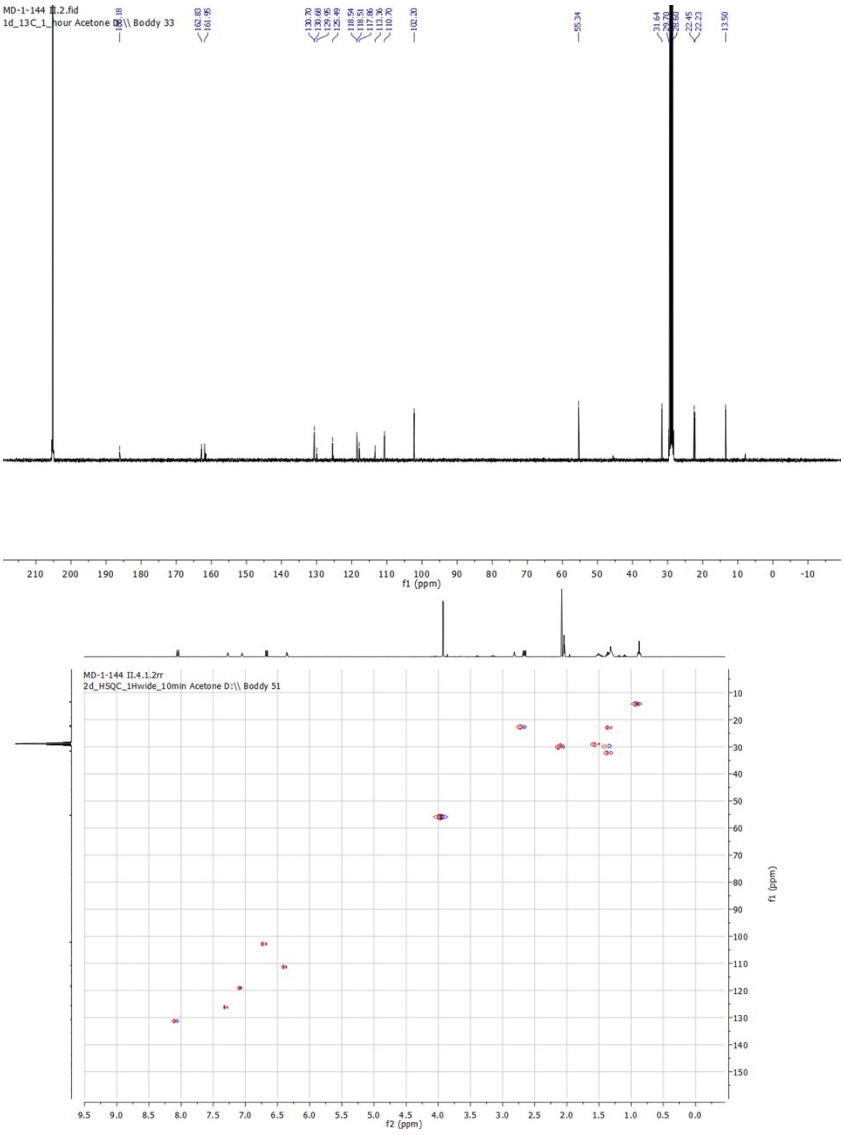


^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) of S2

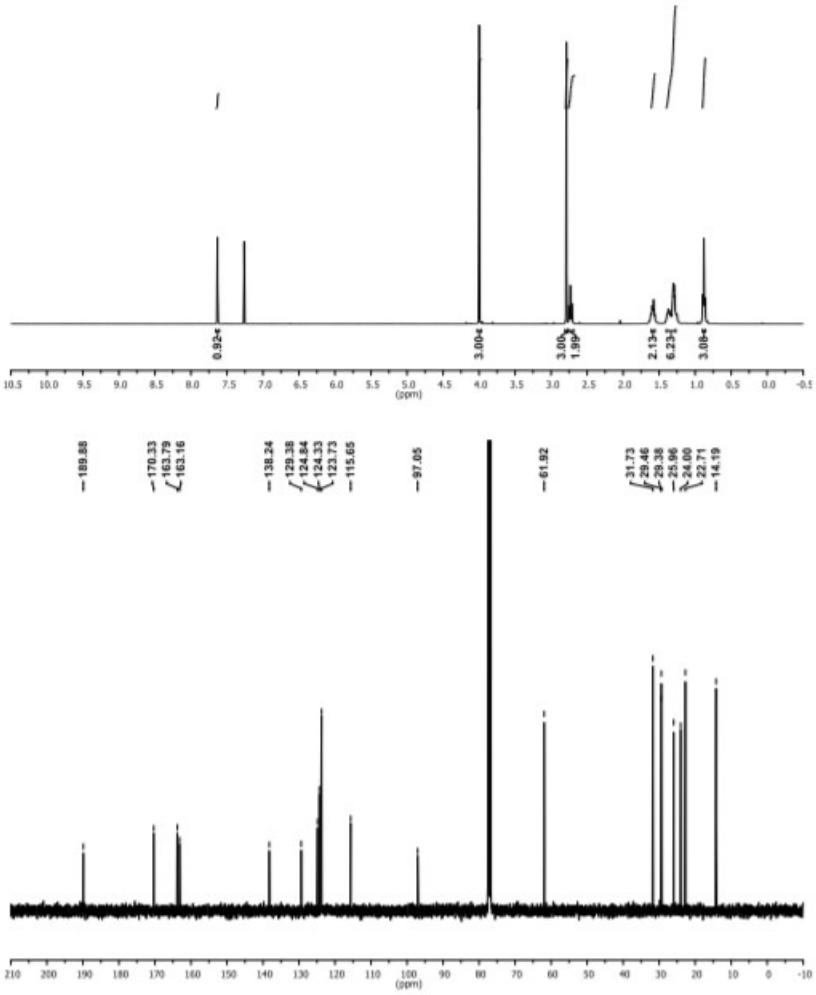


^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) of S3

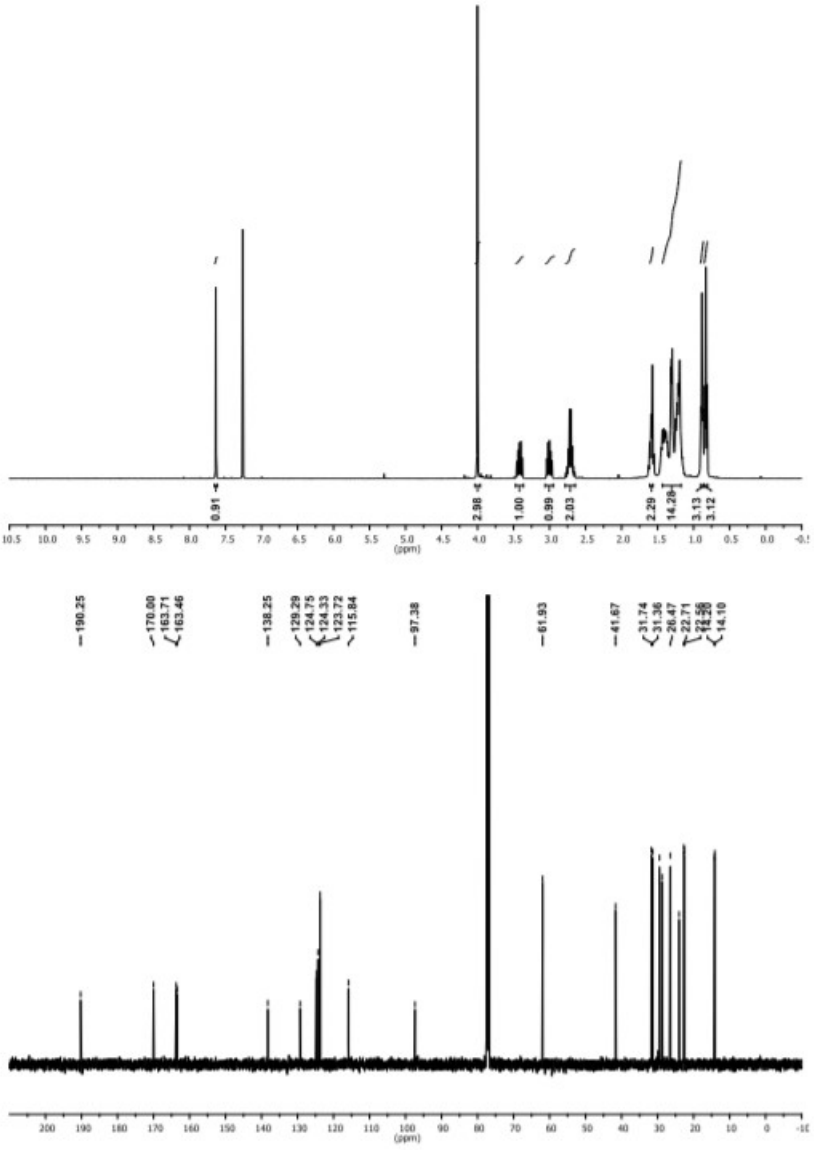

MD-1-144
1d_1H_16_acetone D:\Boddy 33
7.27
7.05
6.68
6.66
6.36
6.35
3.93
2.68
2.66
2.64
1.53
1.51
1.50
1.49
1.37
1.35
1.33
1.32
1.31
1.30
0.89
0.88
0.87
0.86
1.01
1.00
1.01
1.02
0.98
3.06
2.12
2.29
6.40
3.09
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



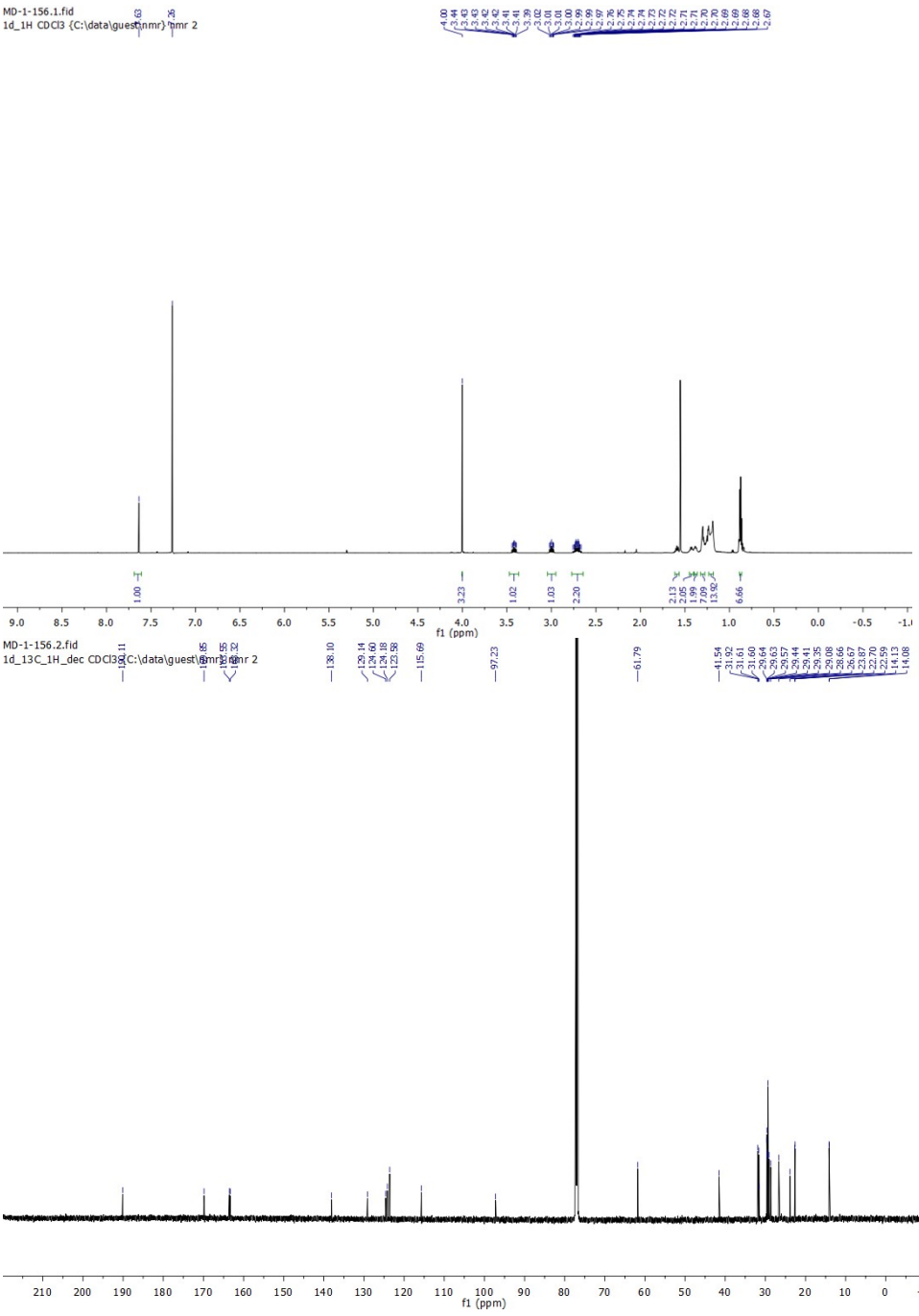
^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) and HSQC of S4

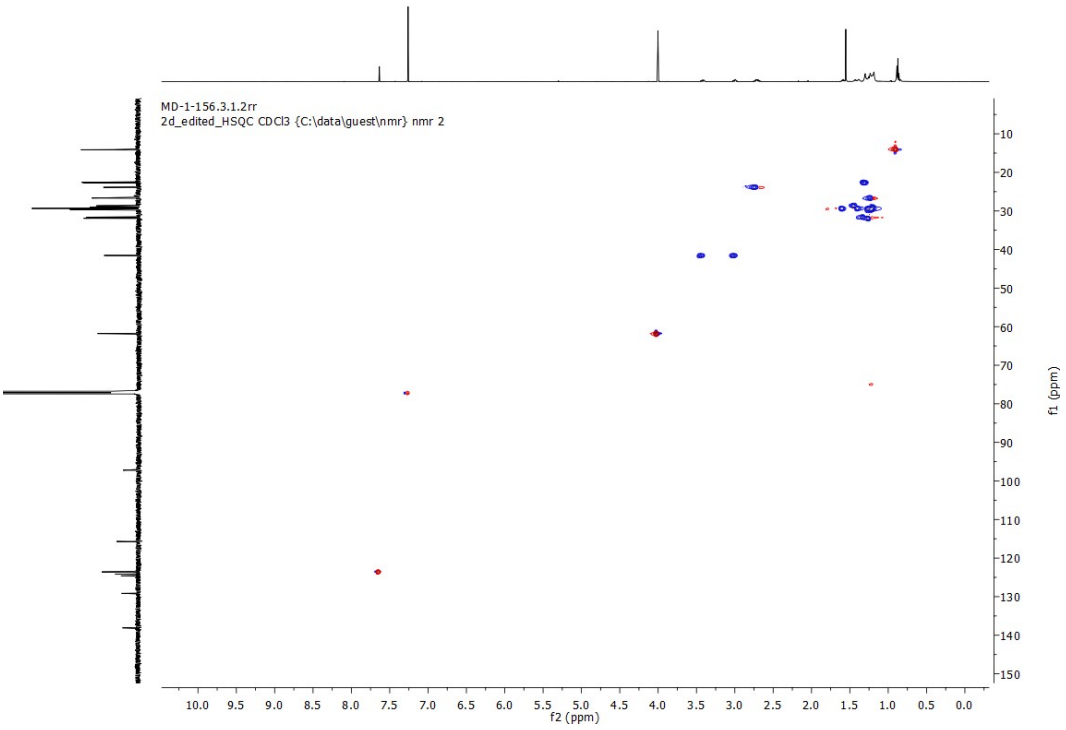


^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) of S6

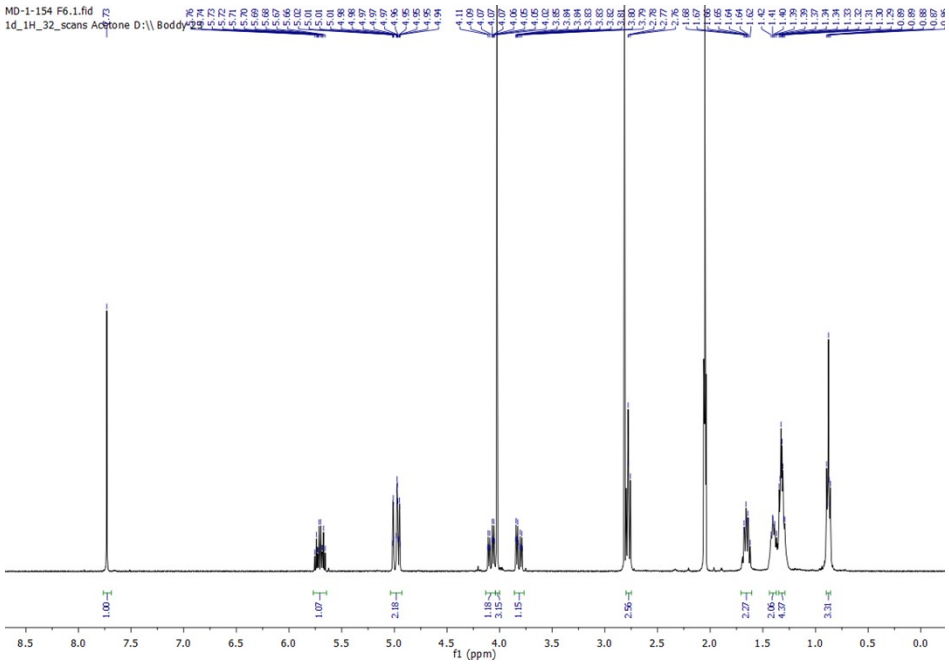


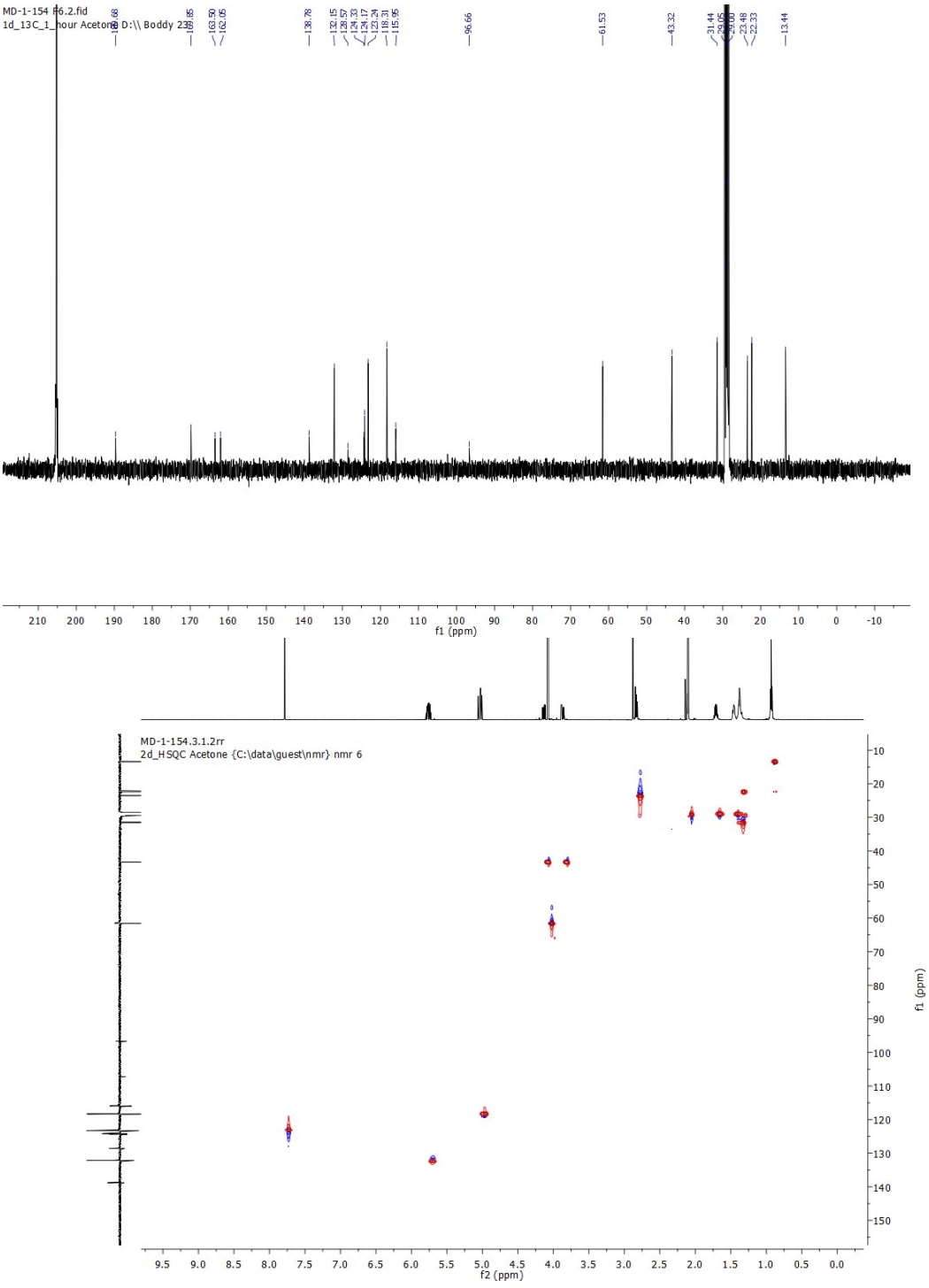
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) of S7


MD-1-156.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 2
MD-1-156.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\6nmr} nmr 2
f1 (ppm)
f1 (ppm)

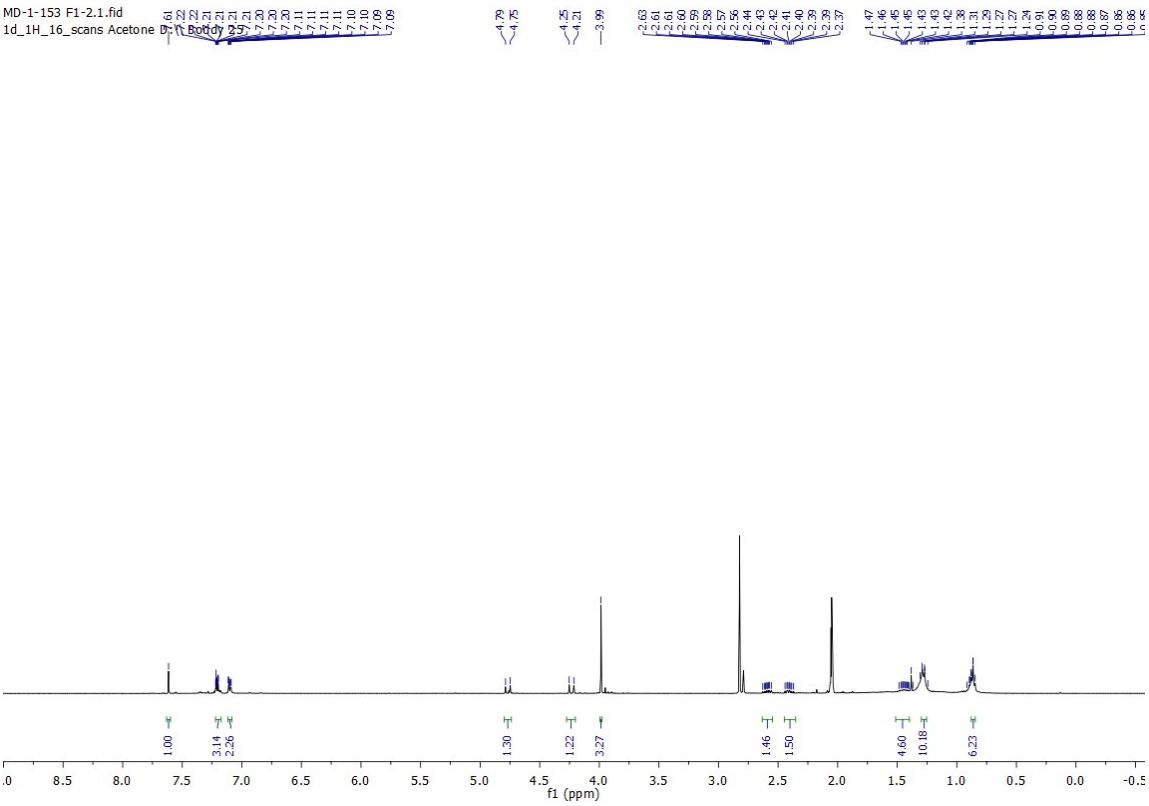


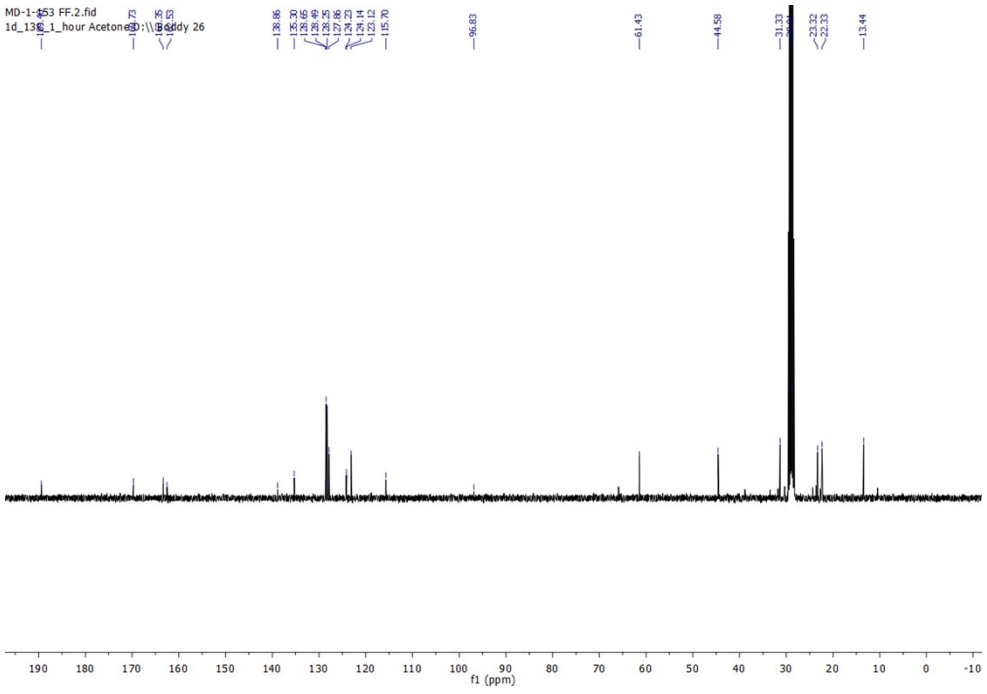
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of S8


MD-1-154 F6.1.fid
1d_1H_32_scans Acetone D:\\ Boddy
1.00
1.07
2.18
1.18
3.15
1.15
2.56
2.27
2.06
4.37
3.31
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

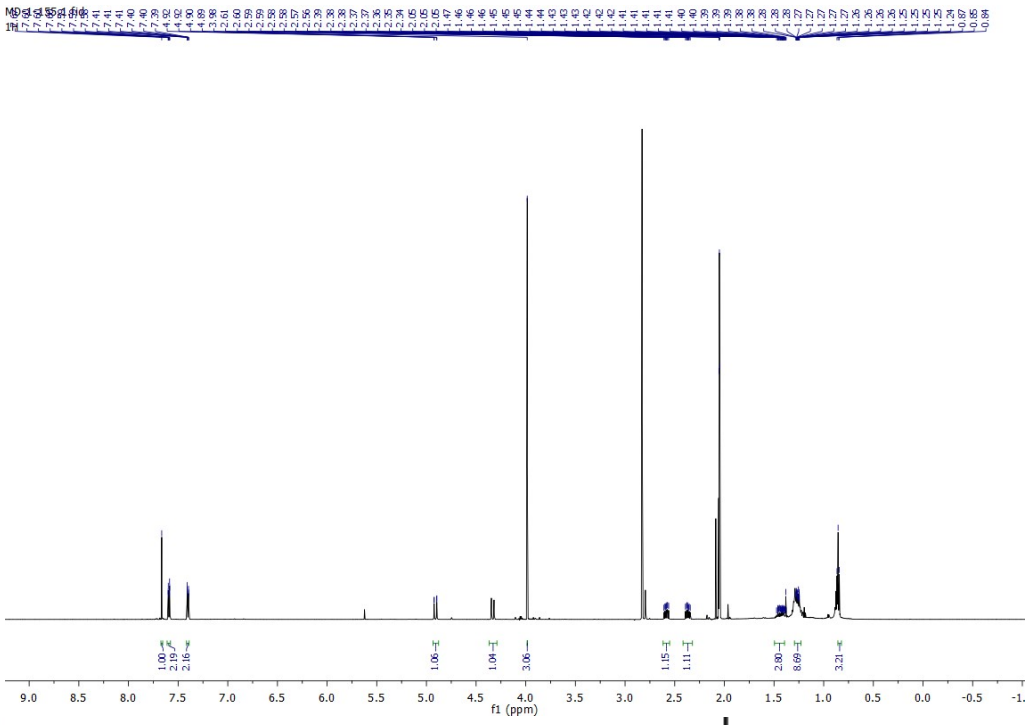


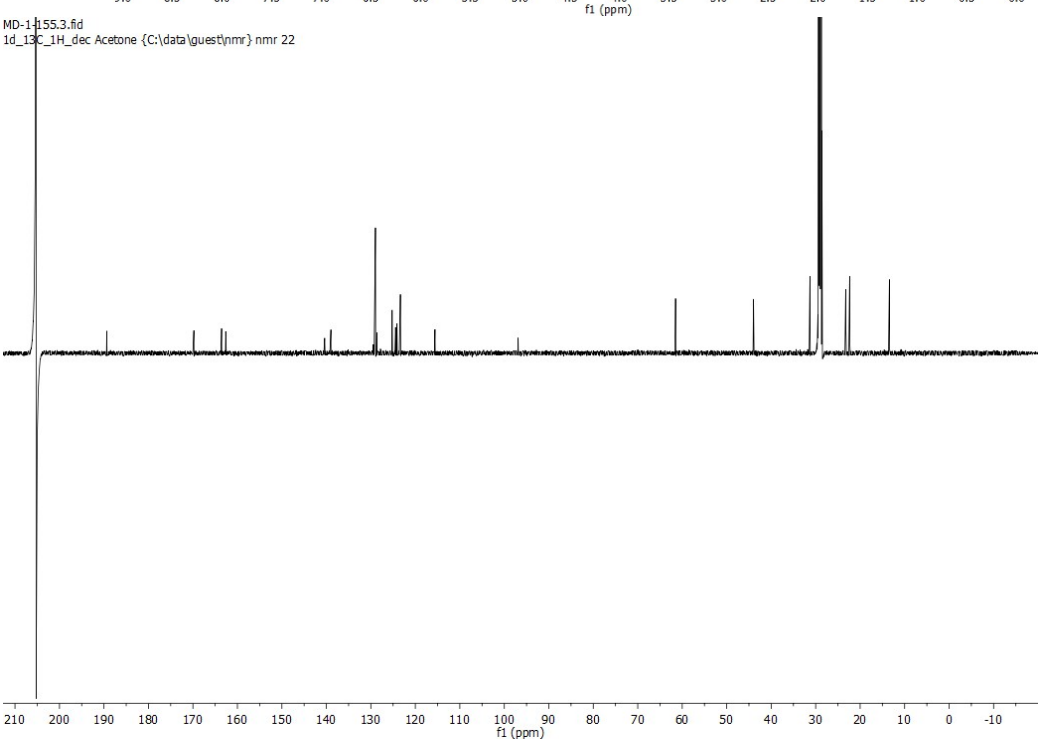
^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) and HSQC of S9

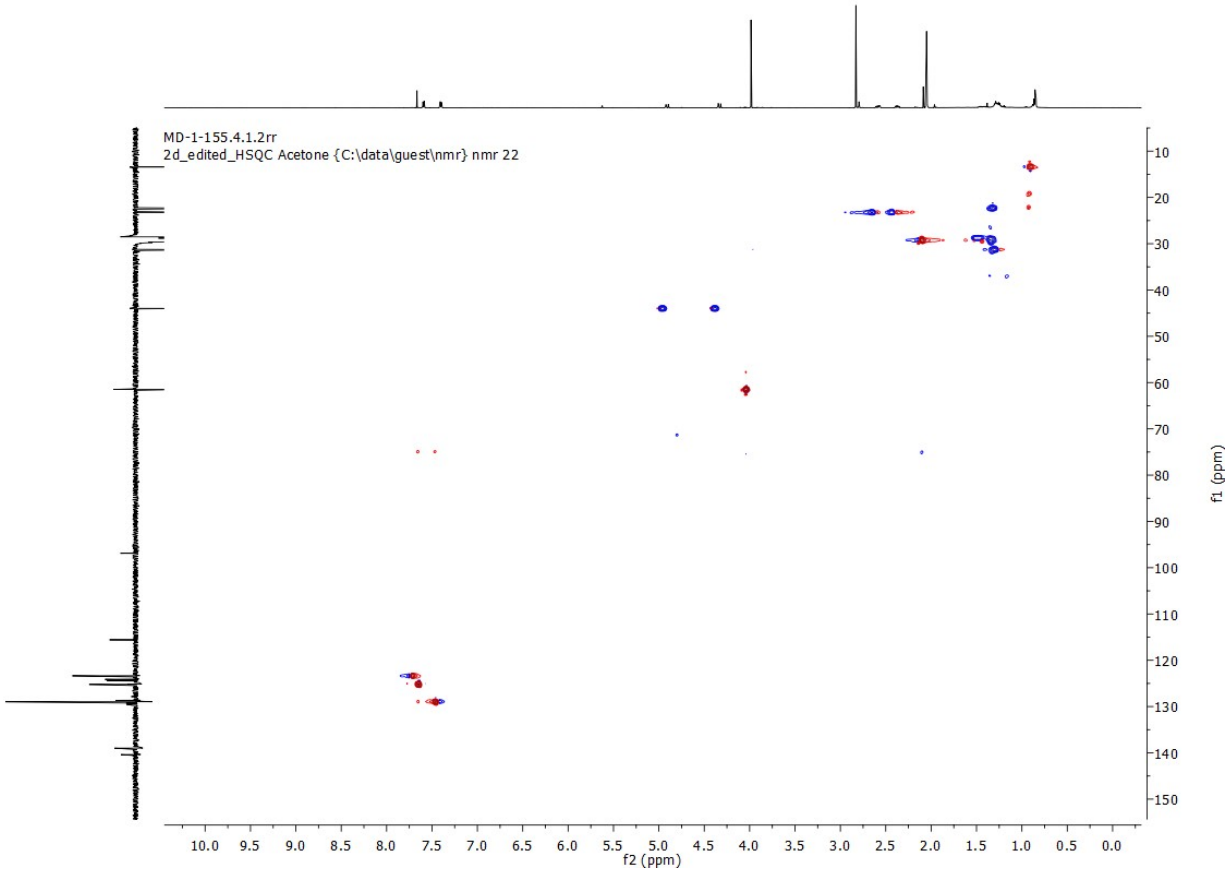




^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) of S10

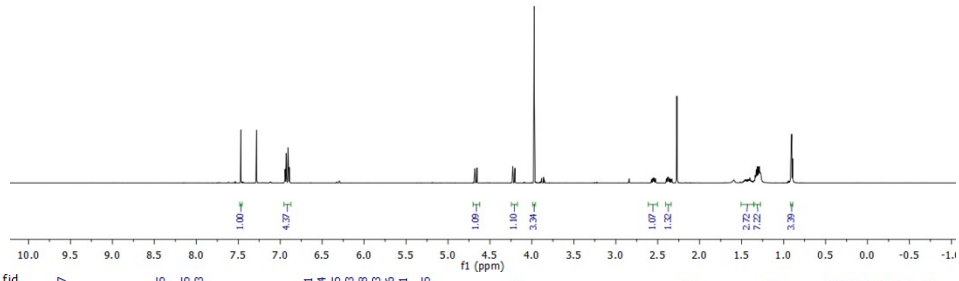

1.00
2.19
2.16
1.06
1.04
3.06
1.15
1.11
2.80
8.69
3.21
f1 (ppm)


MD-1-155.3.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 22
f1 (ppm)

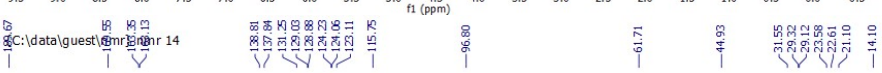


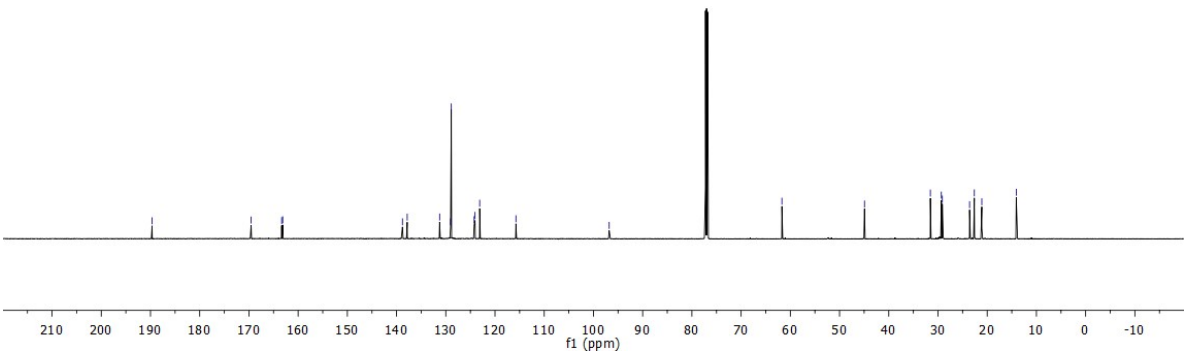
^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) and HSQC of S11

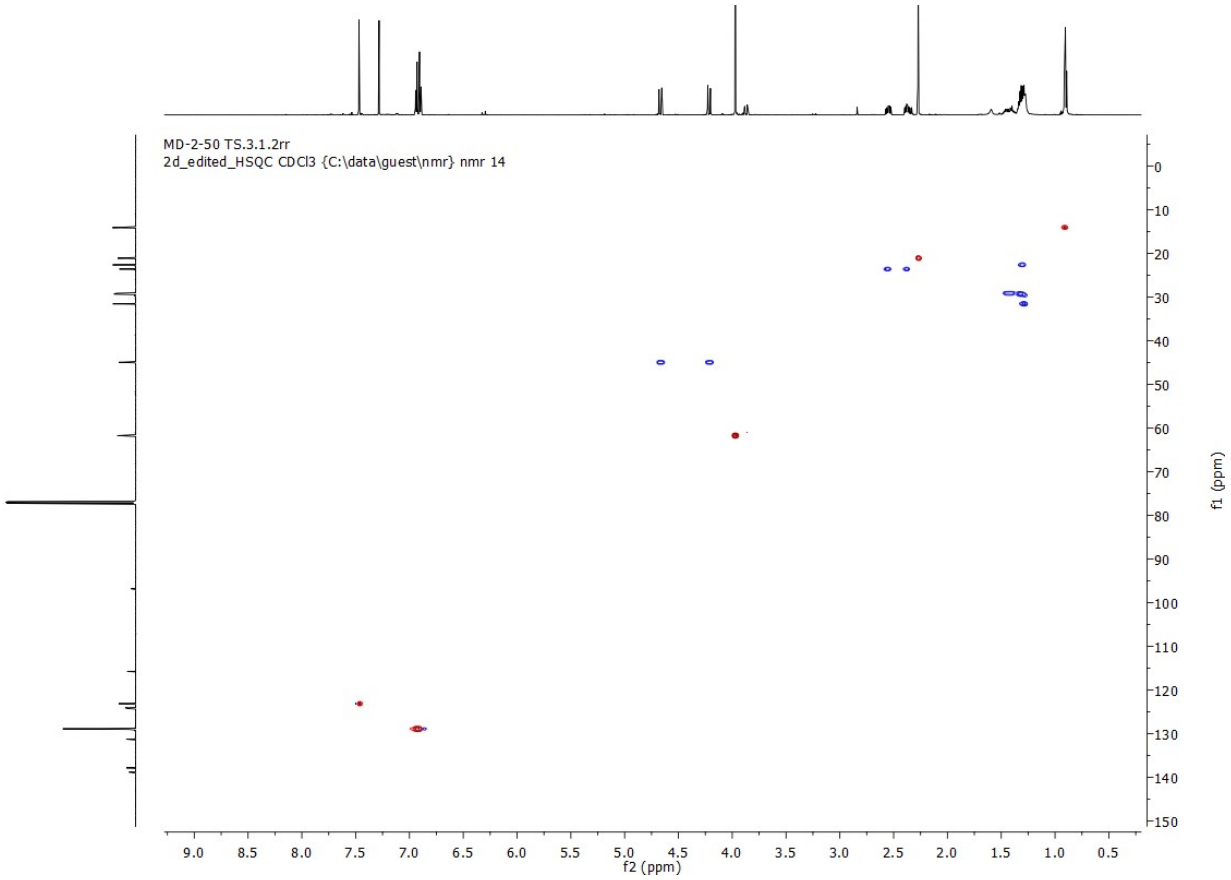
MD-2-50 TS.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 14



MD-2-50 TS.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} nmr 14

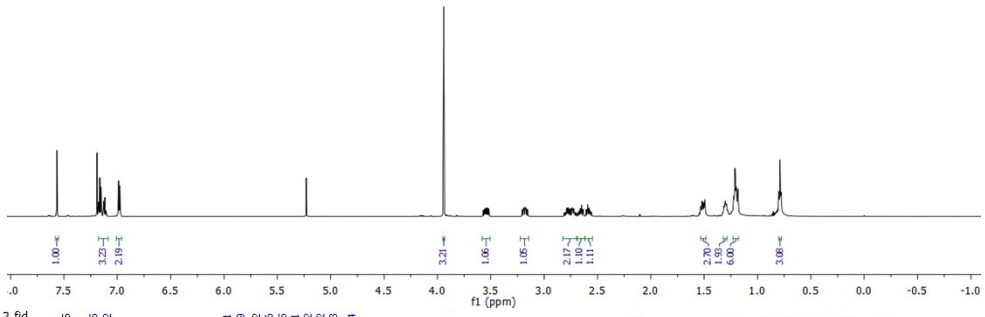




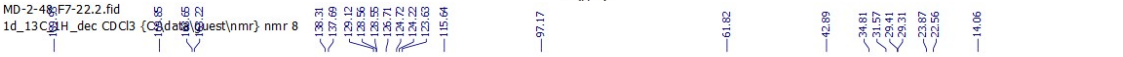


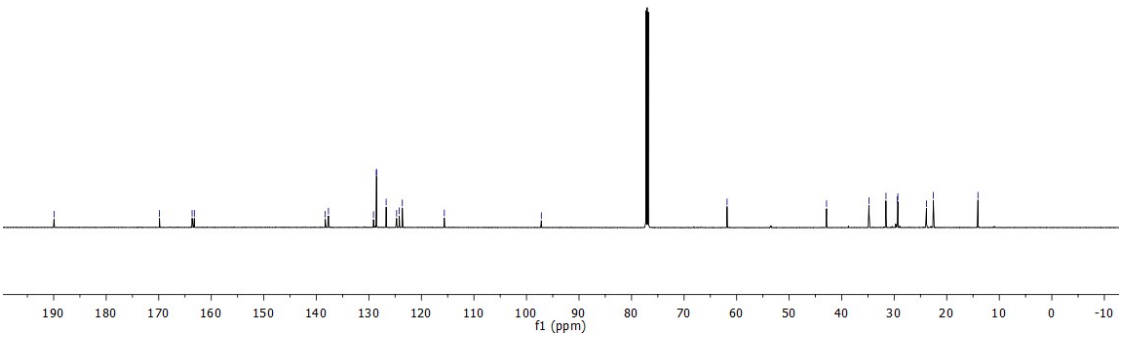
^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) and HSQC of S12

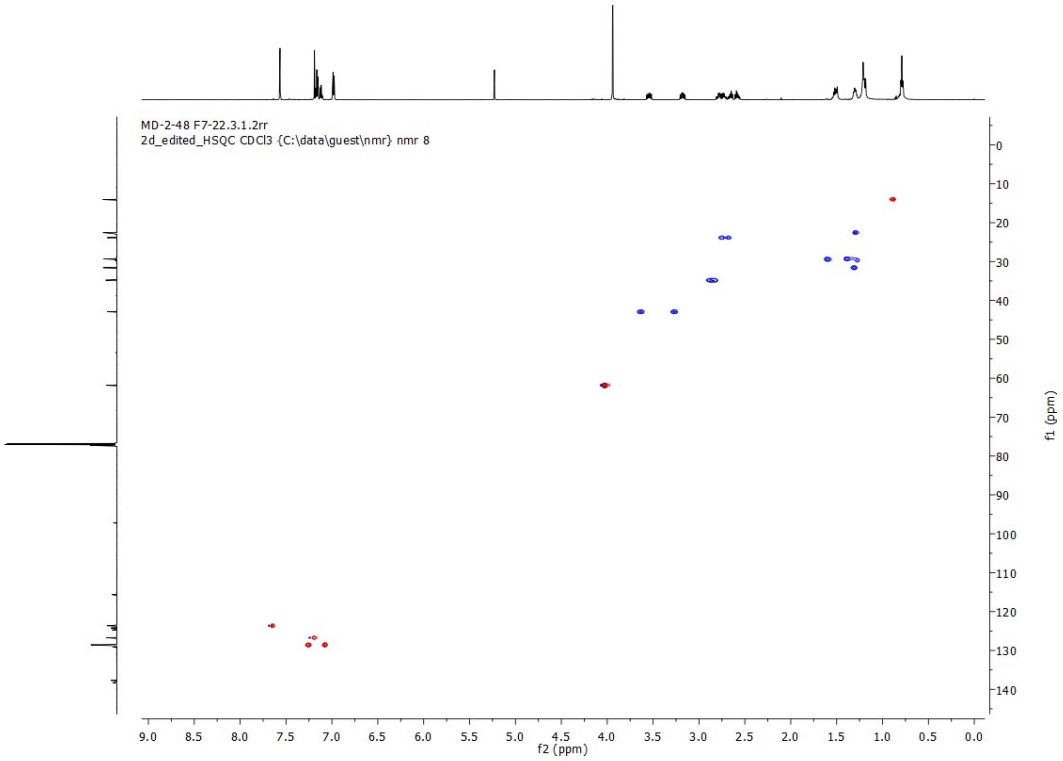
MD-2-48 F7-22.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 8



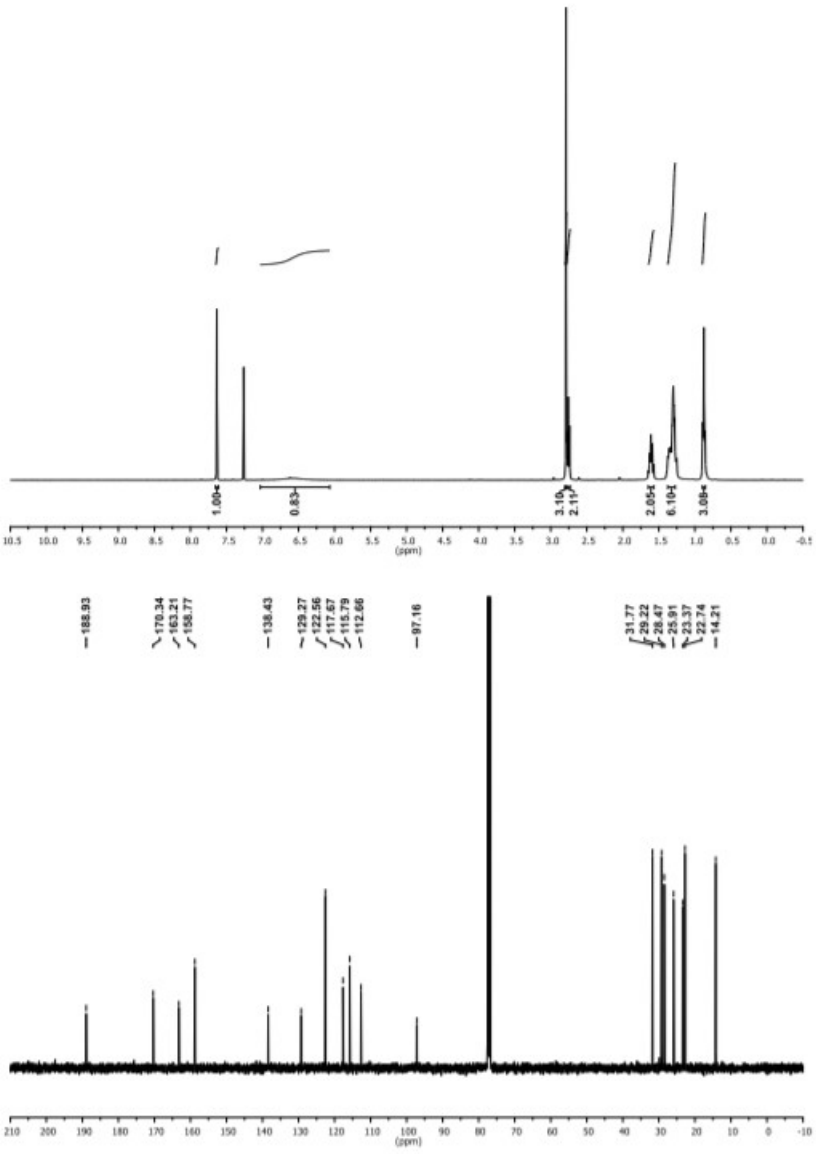
MD-2-48 F7-22.2.fid
1d_13C{1H_dec CDCl3 {C:\data\guest\nmr} nmr 8



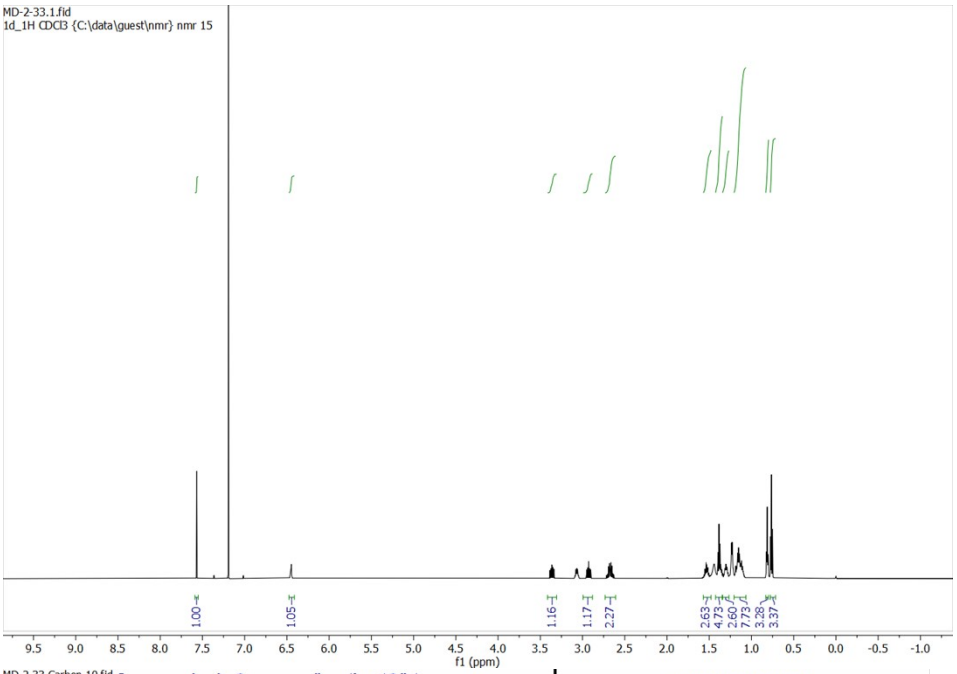


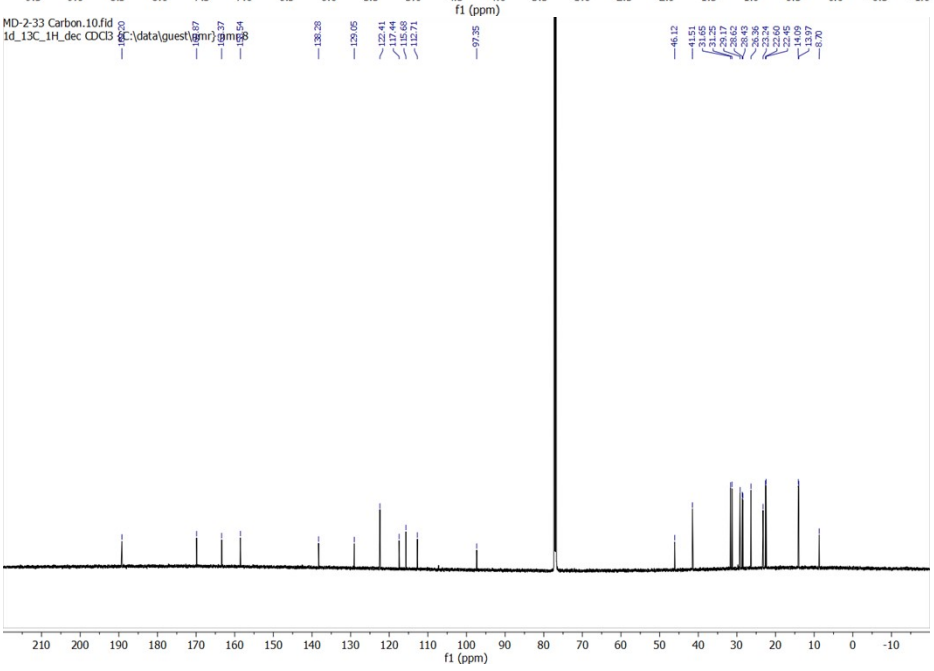


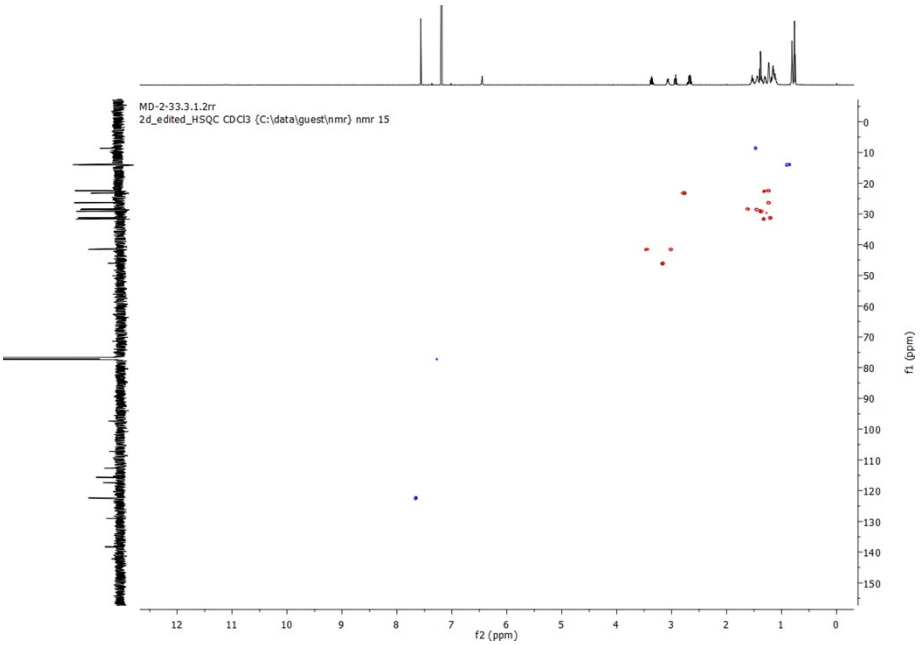
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of S13



^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) of **1**

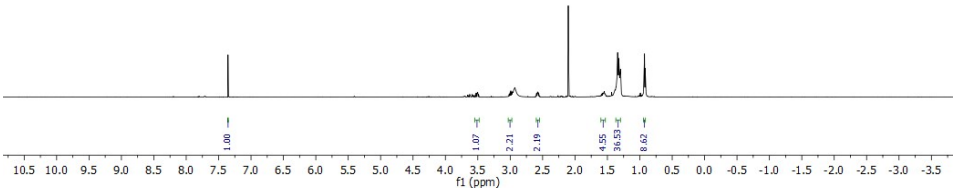

MD-2-33.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 15
1.00
1.05
1.16
1.17
2.27
2.63
4.73
2.60
7.73
3.28
3.37
f1 (ppm)

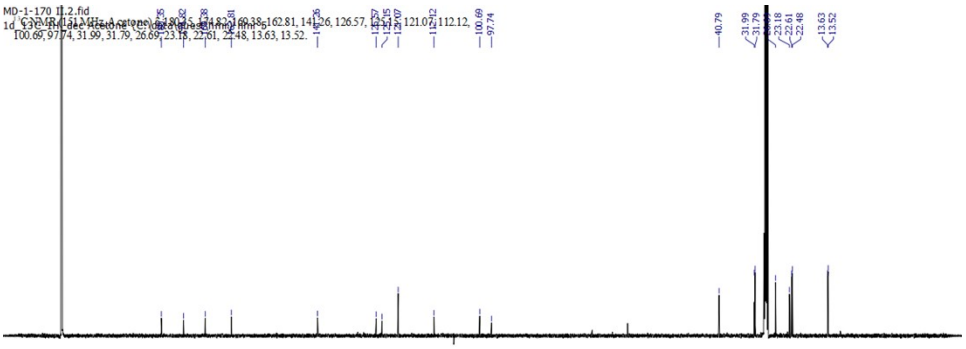

MD-2-33 Carbon.10.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} mmp8
138.28
129.05
122.41
117.44
115.68
112.71
97.35
46.12
41.51
31.65
31.25
29.17
28.92
28.43
26.36
23.24
22.60
22.46
14.09
13.97
8.70
f1 (ppm)

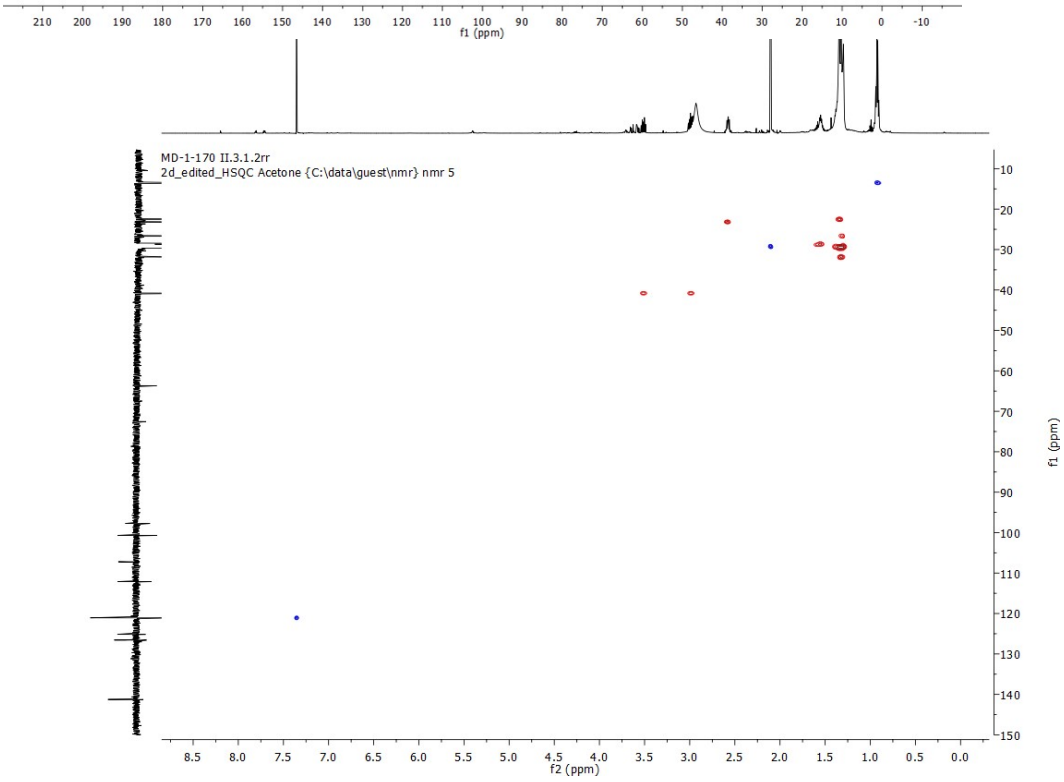


^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of **2**

MD-1-170 II.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 5

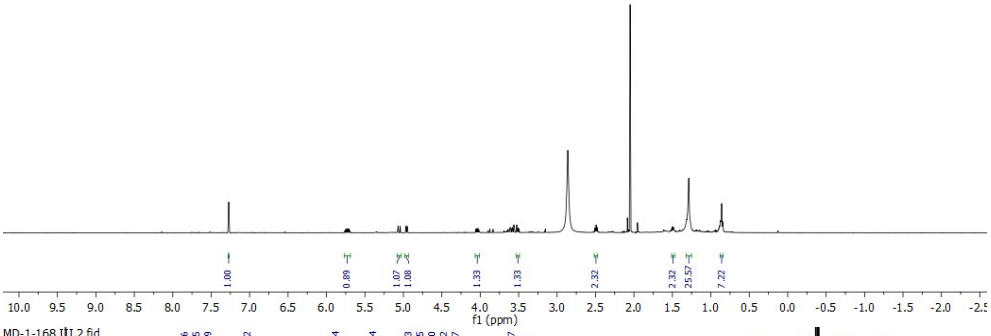




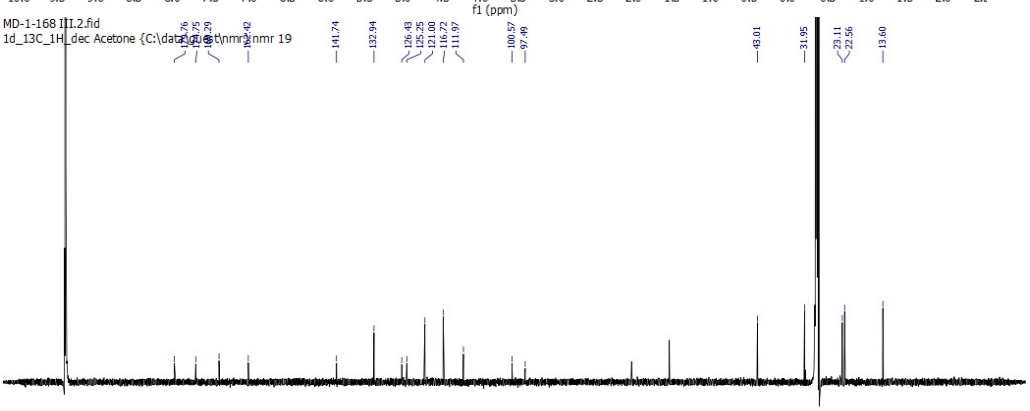


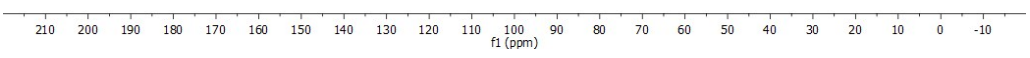
^{1}H NMR (600 MHz, Acetone-D_6) and ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **3**

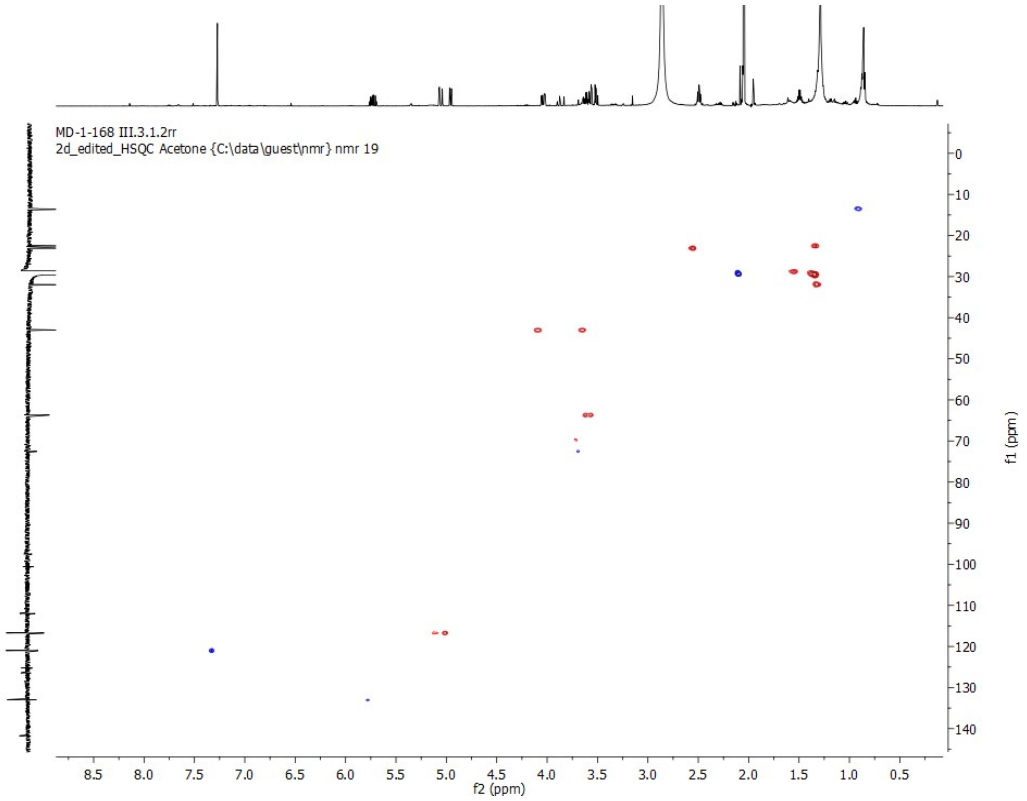
MD-1-168 III.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 19



MD-1-168 III.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 19

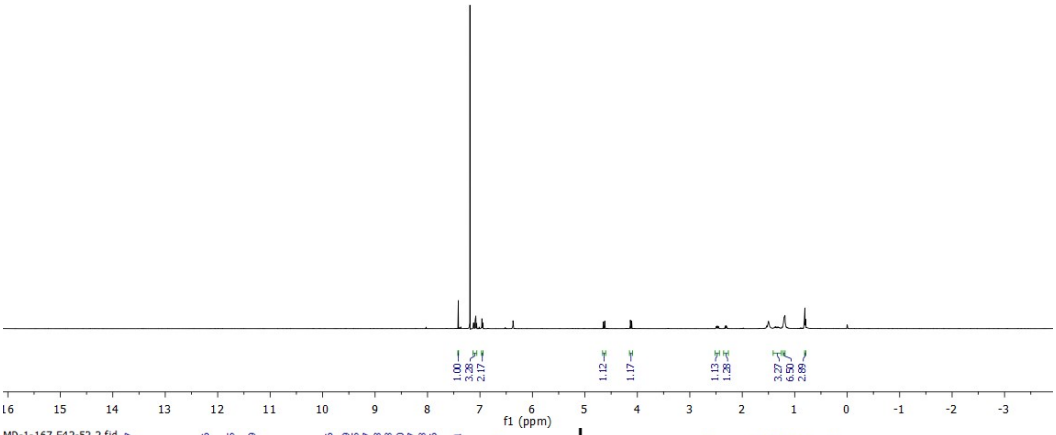




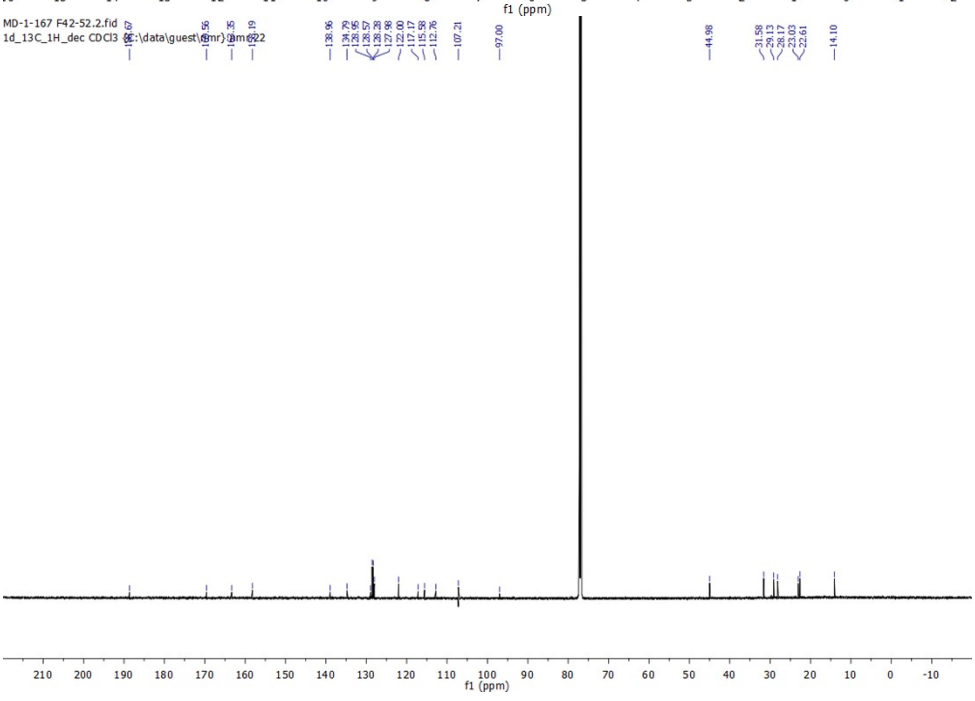


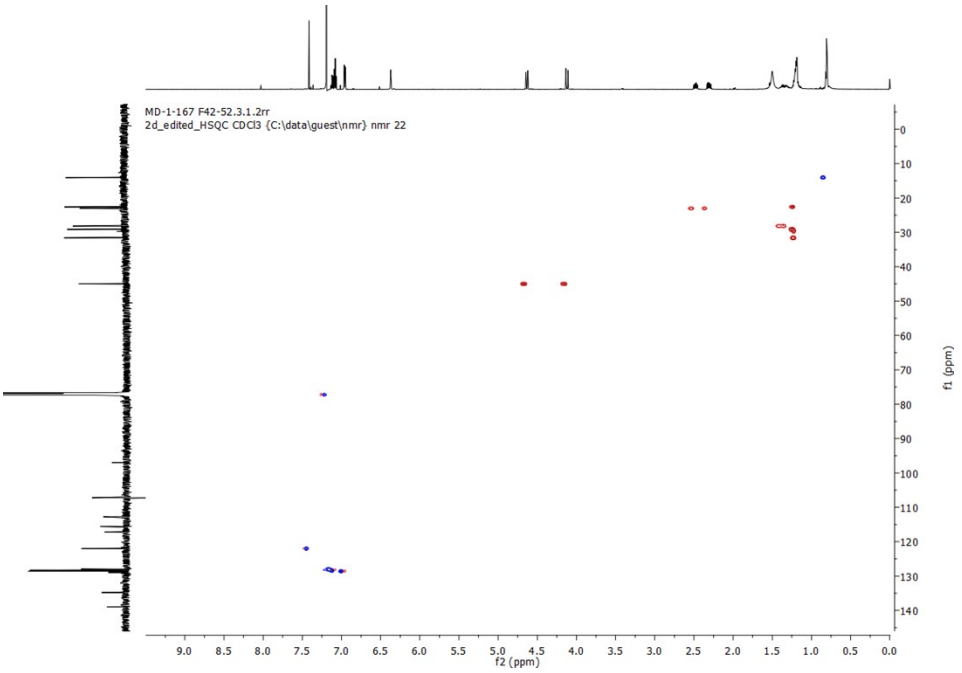
^{1}H NMR (600 MHz, Acetone-D_6) and ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **4**

MD-1-167 F42-52.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 22



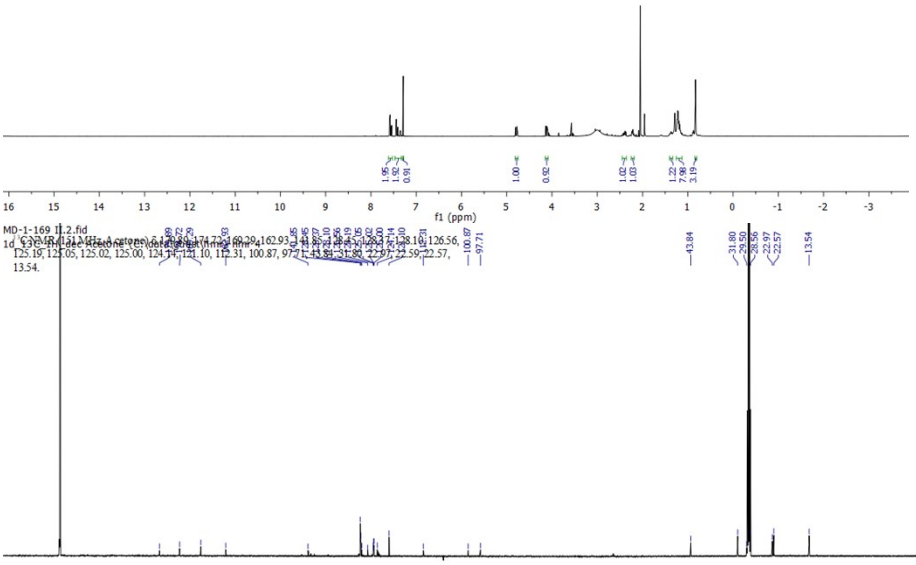
MD-1-167 F42-52.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} nmr 22



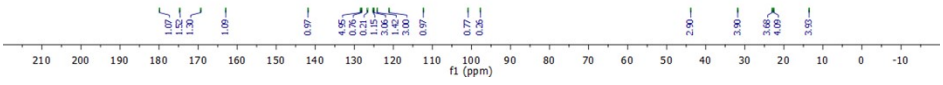


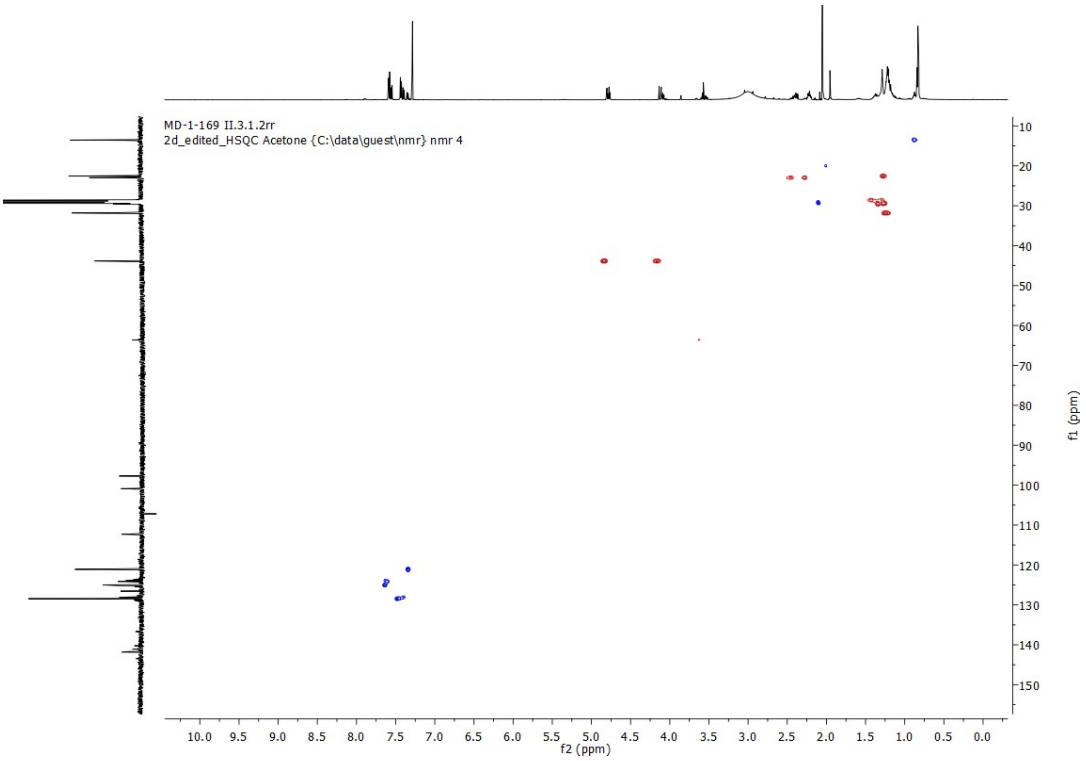
^{1}H NMR (600 MHz, Acetone-D_6) and ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **5**

MD-1-169 II.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 4



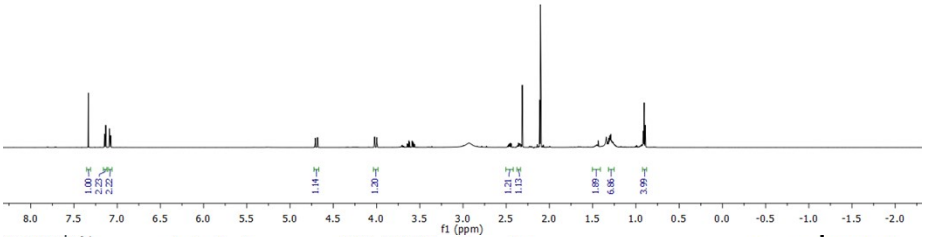
MD-1-169 II.2.fid
1d_13C-RAPID 51 MHz 4 acetone {C:\data\guest\nmr} nmr 4



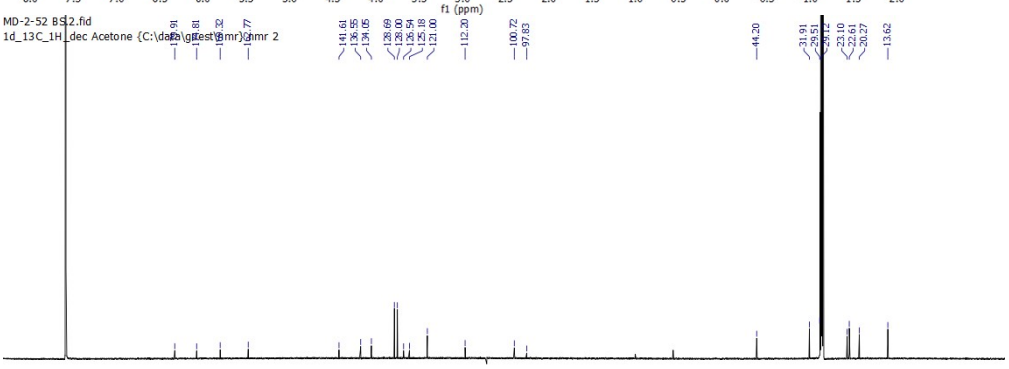


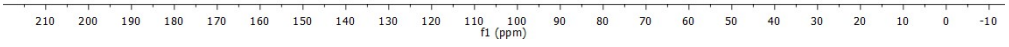
^{1}H NMR (600 MHz, Acetone-D_6) and ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **6**

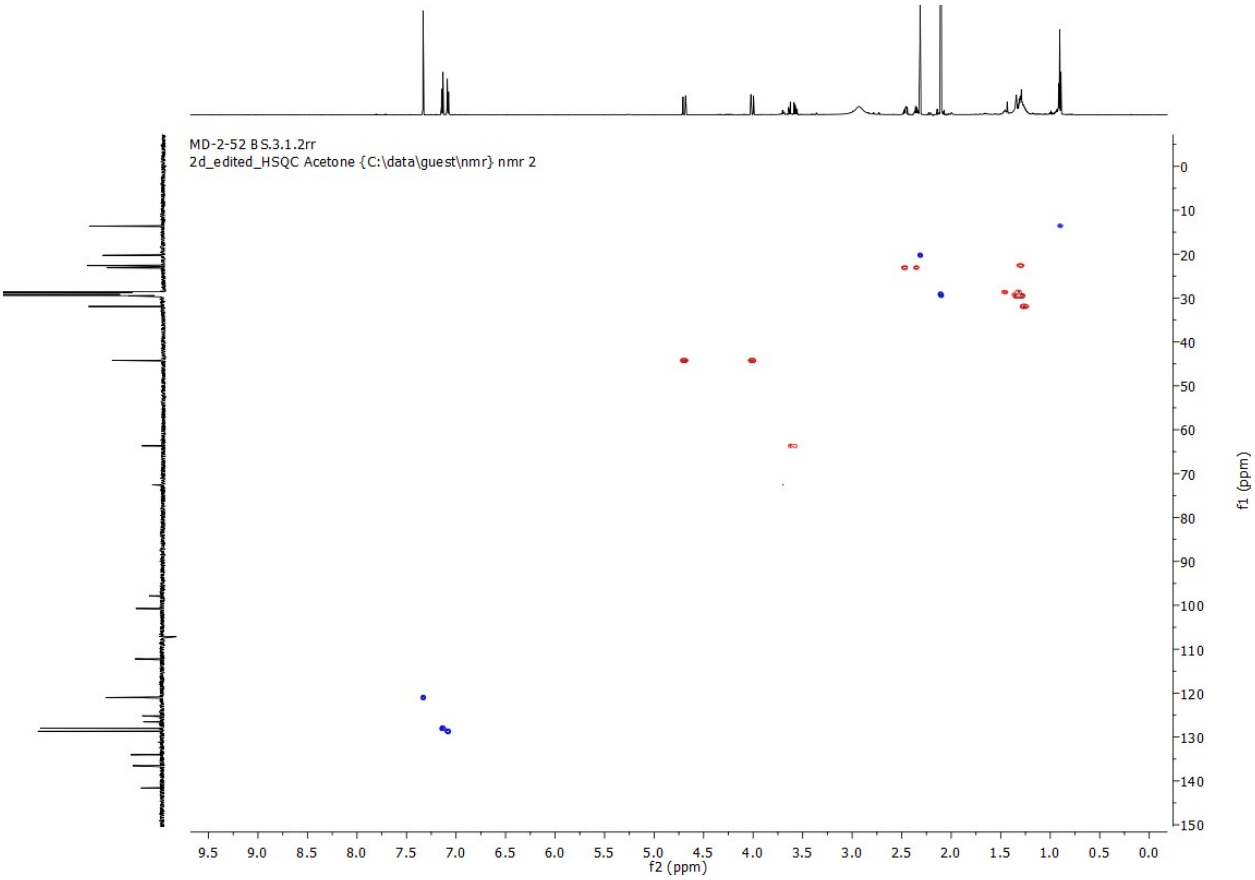
MD-2-52 BS.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 2



MD-2-52 BS.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 2

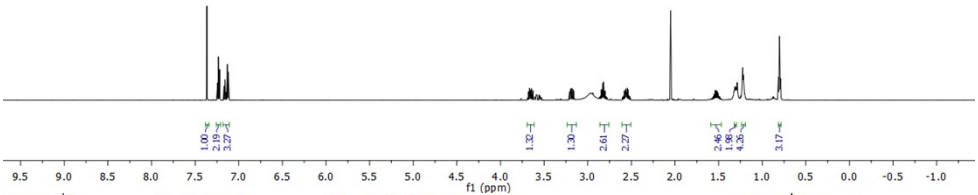




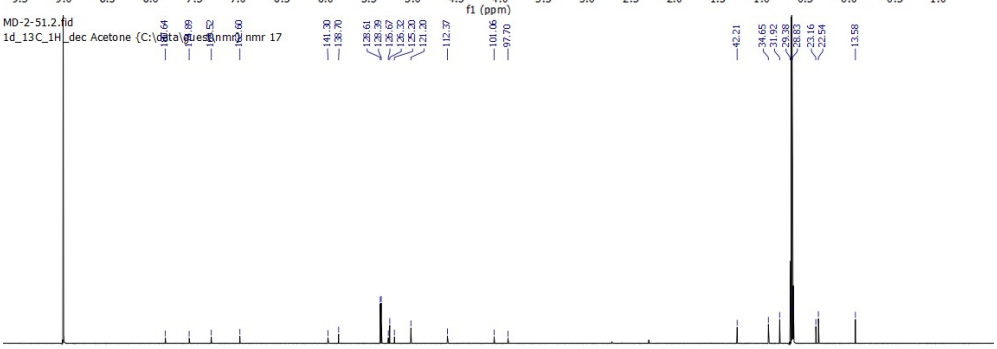


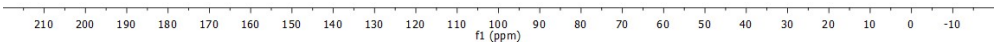
^{1}H NMR (600 MHz, Acetone-D_6) and ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **7**

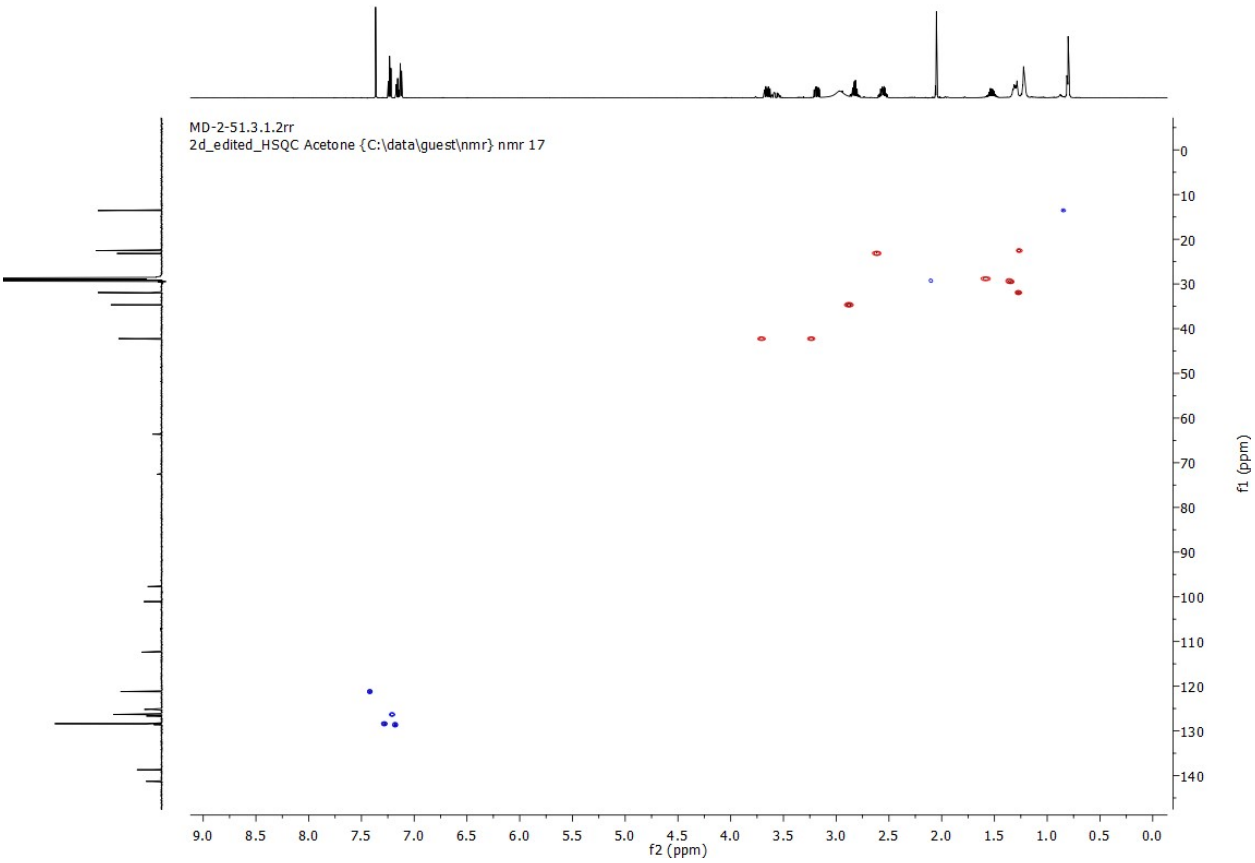
MD-2-51.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 17



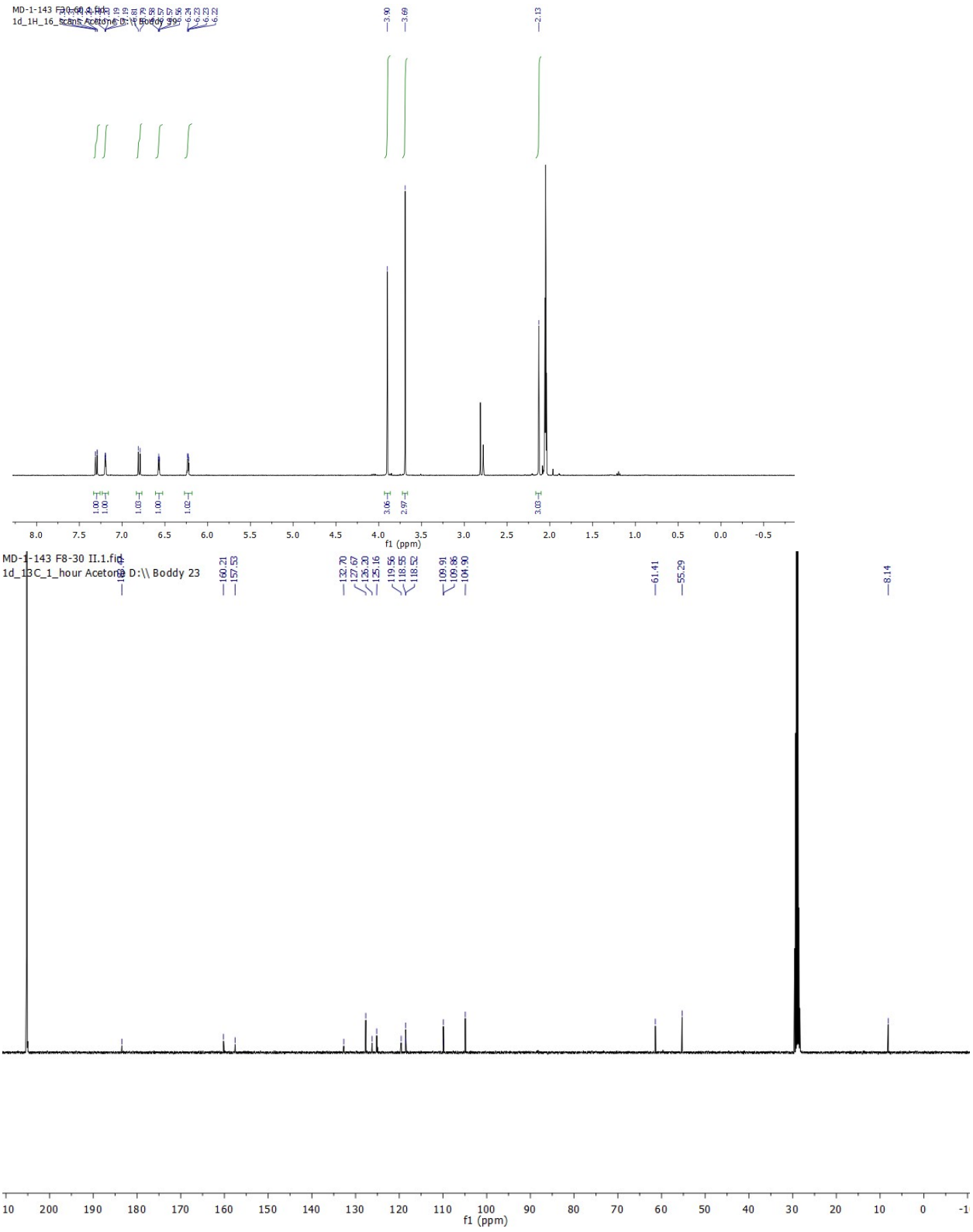
MD-2-51.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 17



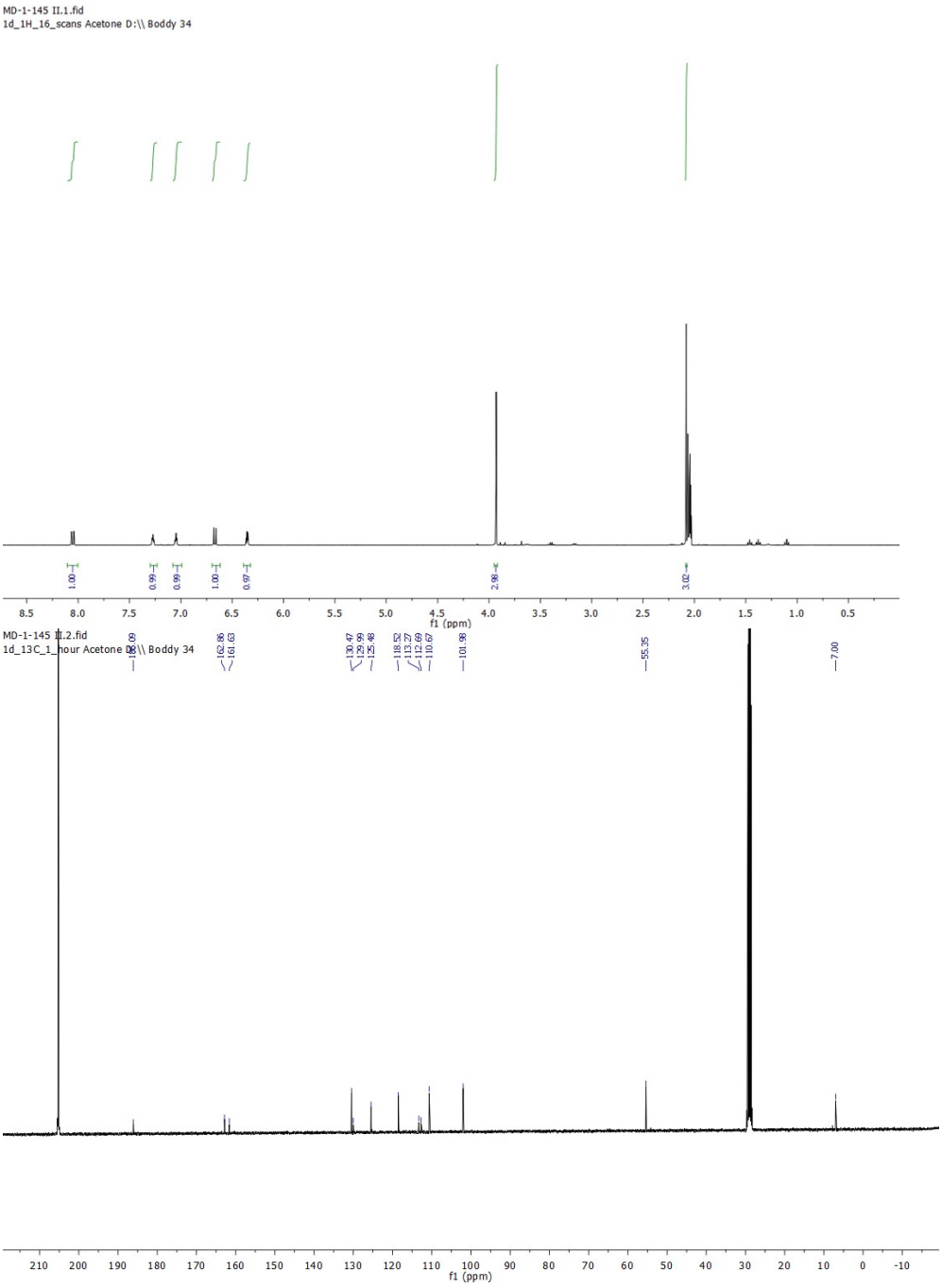




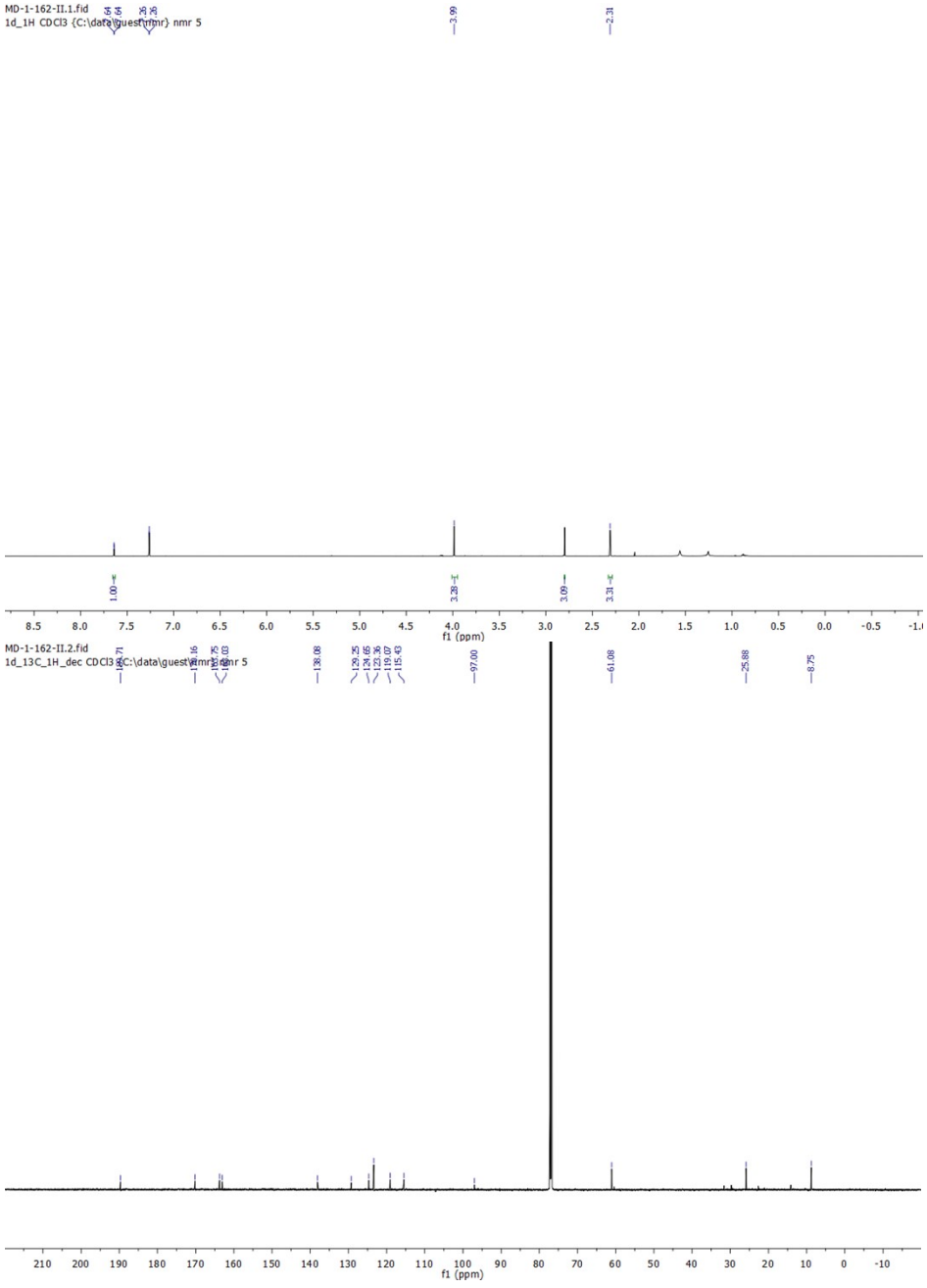
^{1}H NMR (600 MHz, Acetone-D_6) and ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **8**

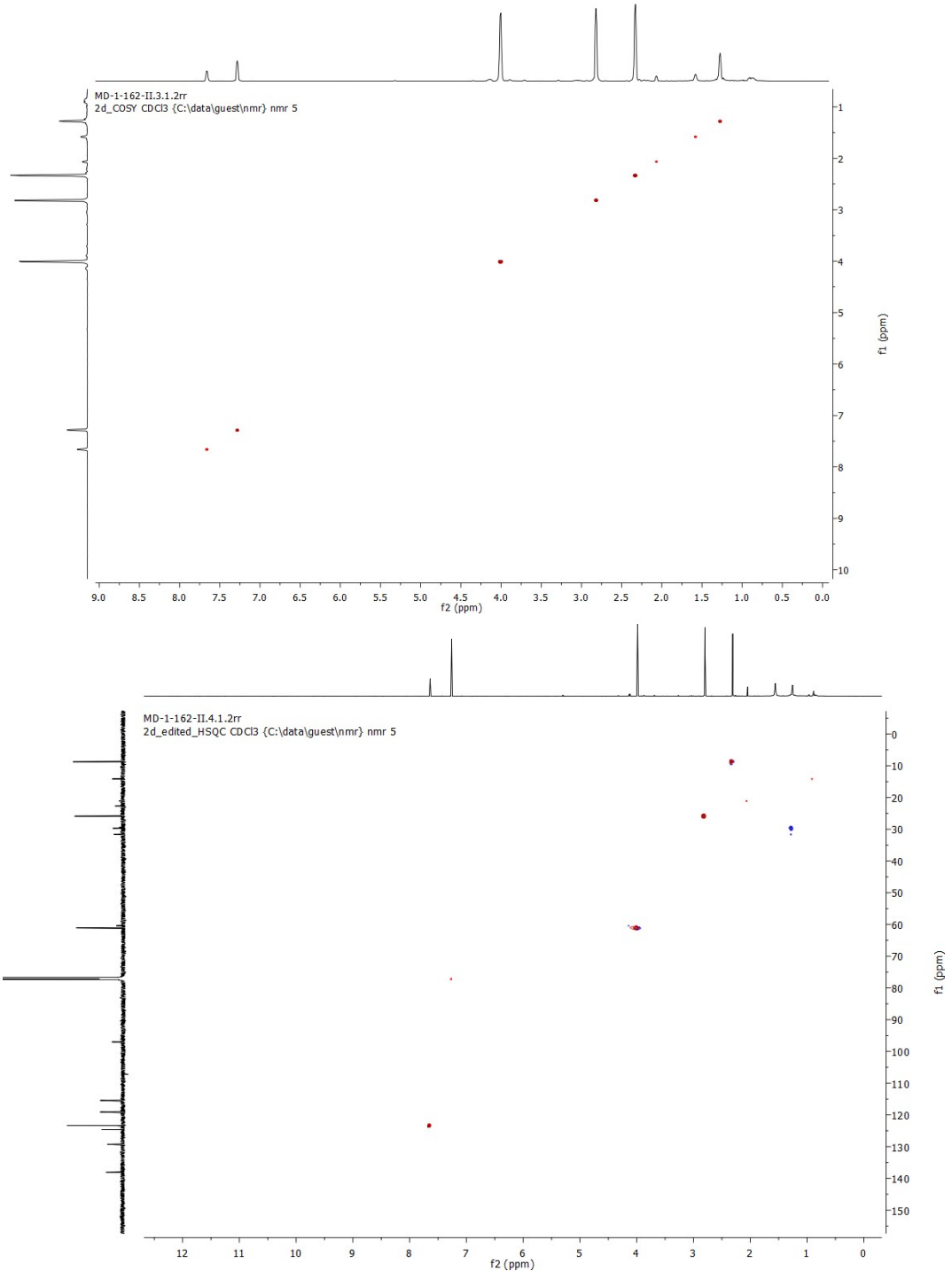


^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) of S14



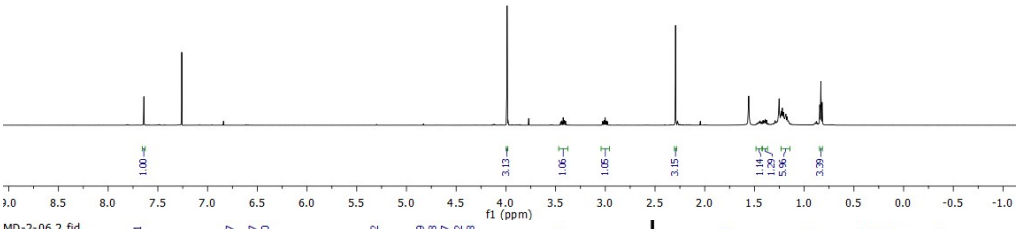
^{1}H NMR (400 MHz, Acetone-D_6) and ^{13}C NMR (100 MHz, Acetone-D_6) of S15


MD-1-162-II.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 5
7.64
7.64
7.26
7.26
3.99
2.31
1.00
3.28
3.09
3.31
f1 (ppm)
MD-1-162-II.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} nmr 5
167.71
153.16
138.08
129.25
124.85
121.35
119.07
115.43
97.00
61.08
25.88
8.75
f1 (ppm)

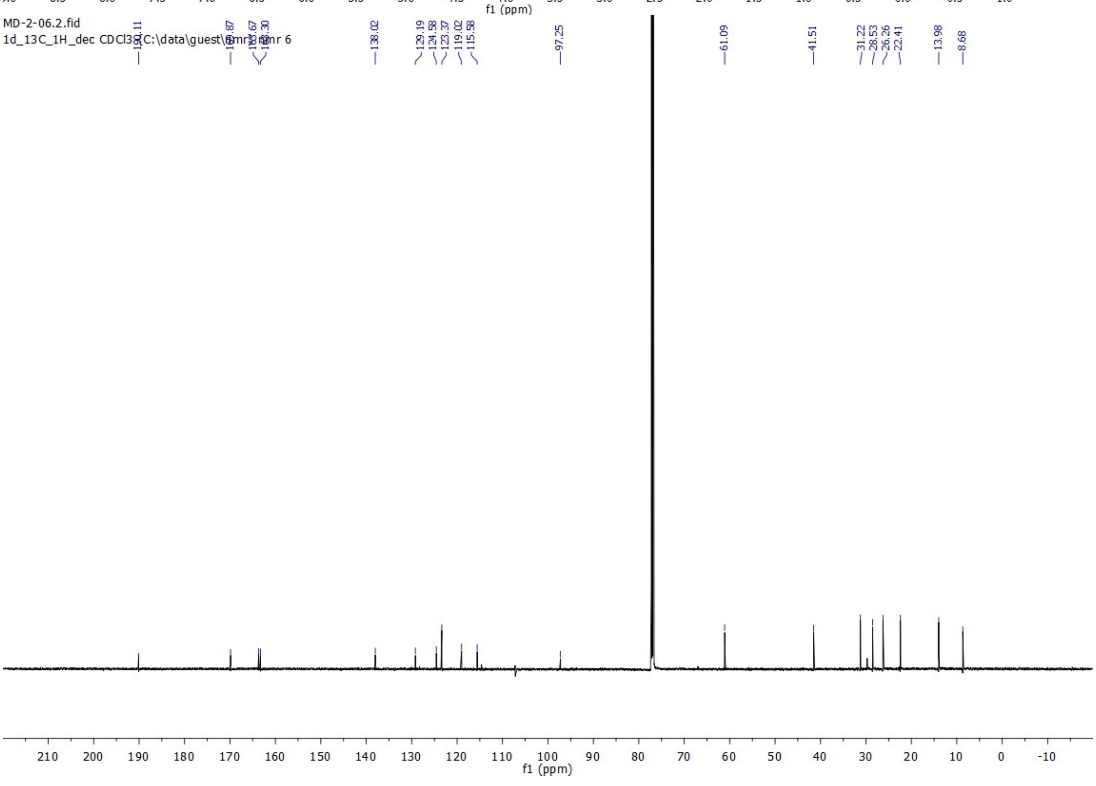


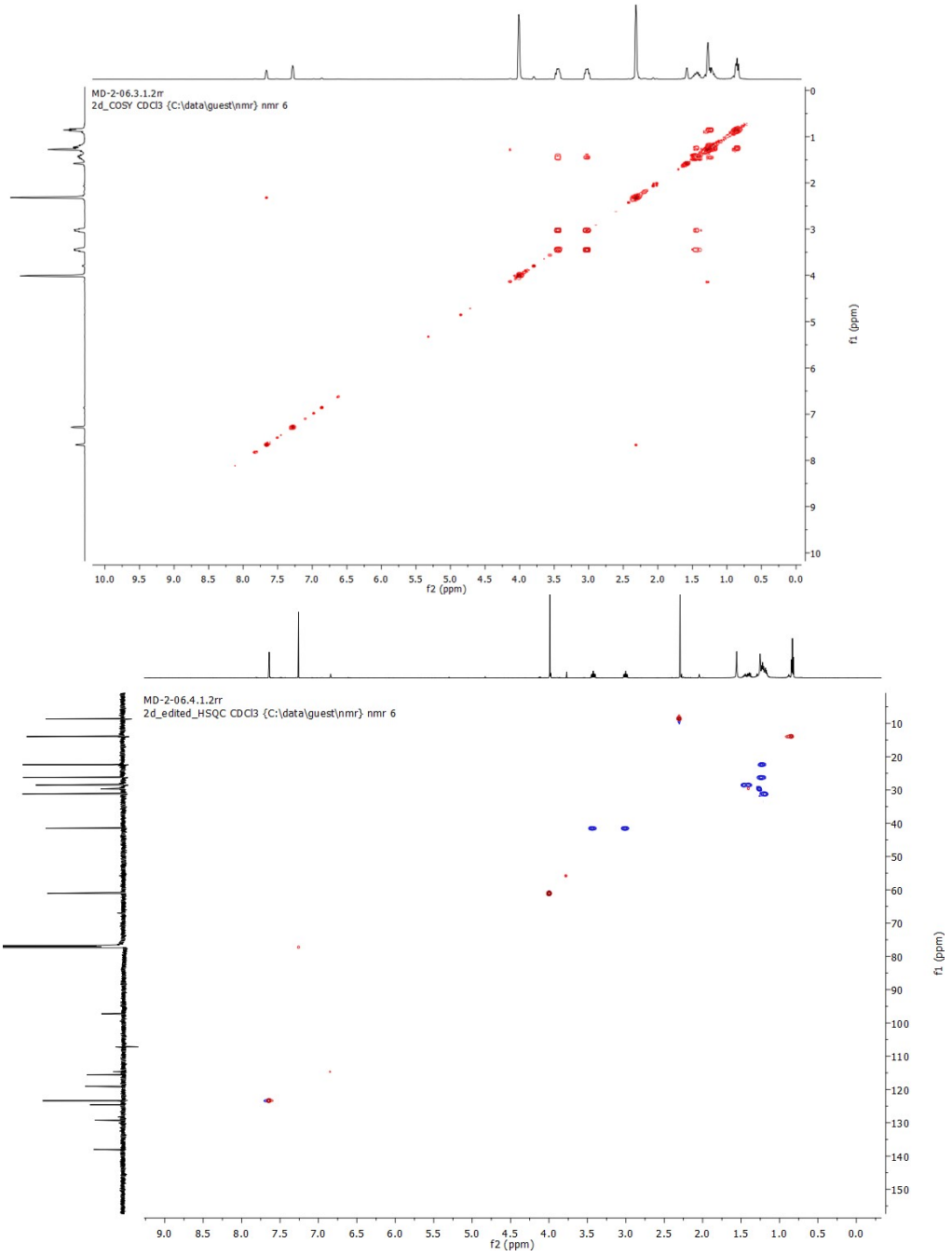
^{1}H NMR (400 MHz, $CDCl_3$) , ^{13}C NMR (100 MHz, $CDCl_3$) COSY and HSQC of S17

MD-2-06.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 6



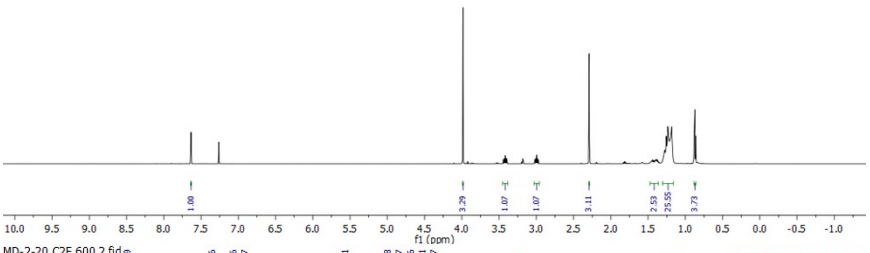
MD-2-06.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} nmr 6



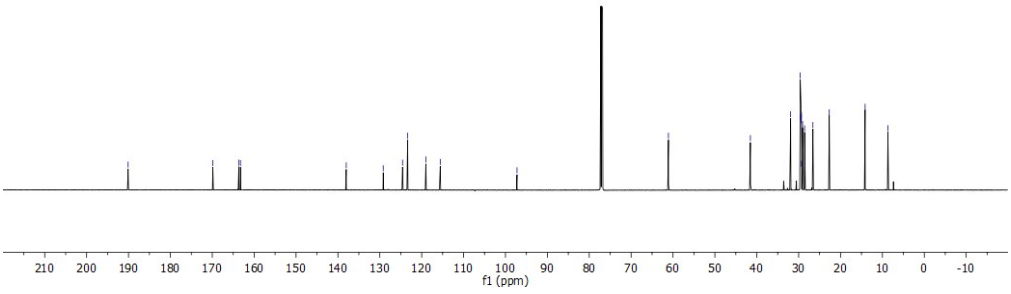


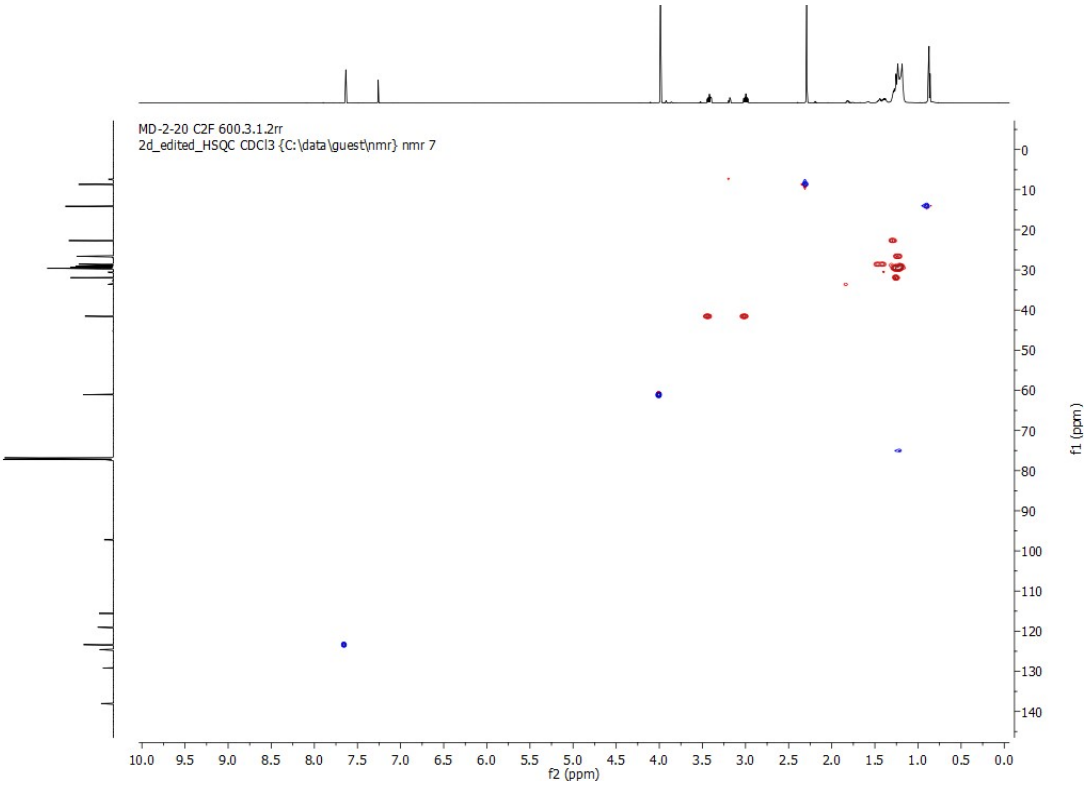
^{1}H NMR (400 MHz, $CDCl_3$) , ^{13}C NMR (100 MHz, $CDCl_3$) COSY and HSQC of S18

MD-2-20 C2F 600.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 7



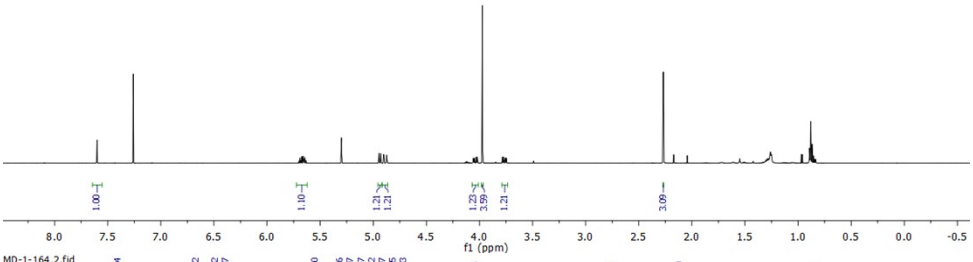
MD-2-20 C2F 600.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} nmr 7



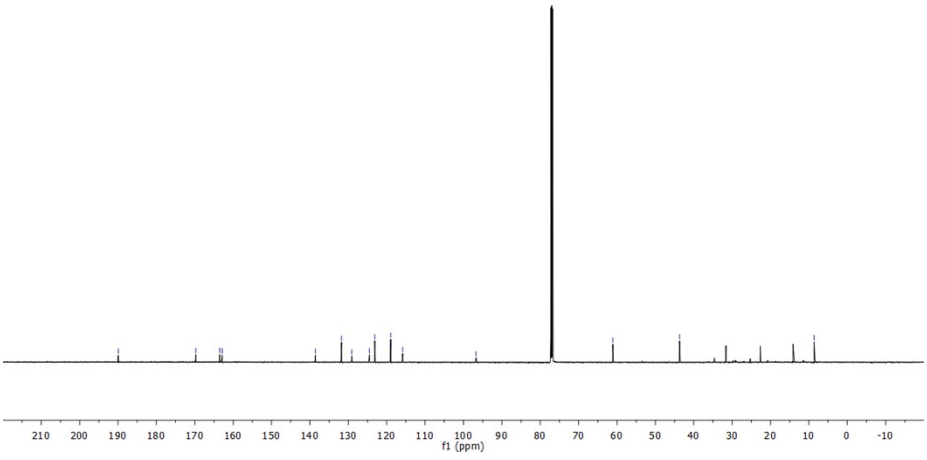


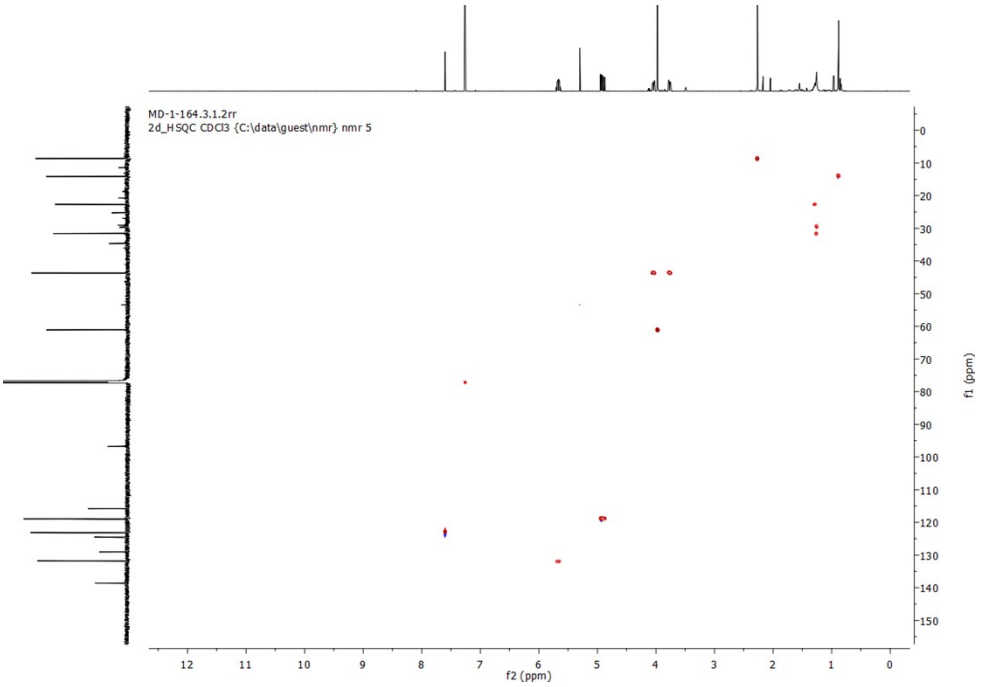
^{1}H NMR (400 MHz, $CDCl_3$) , ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of S19

MD-1-164.1.fid
1d_1H CDCl3 {C:\data\guest\nmr} nmr 5

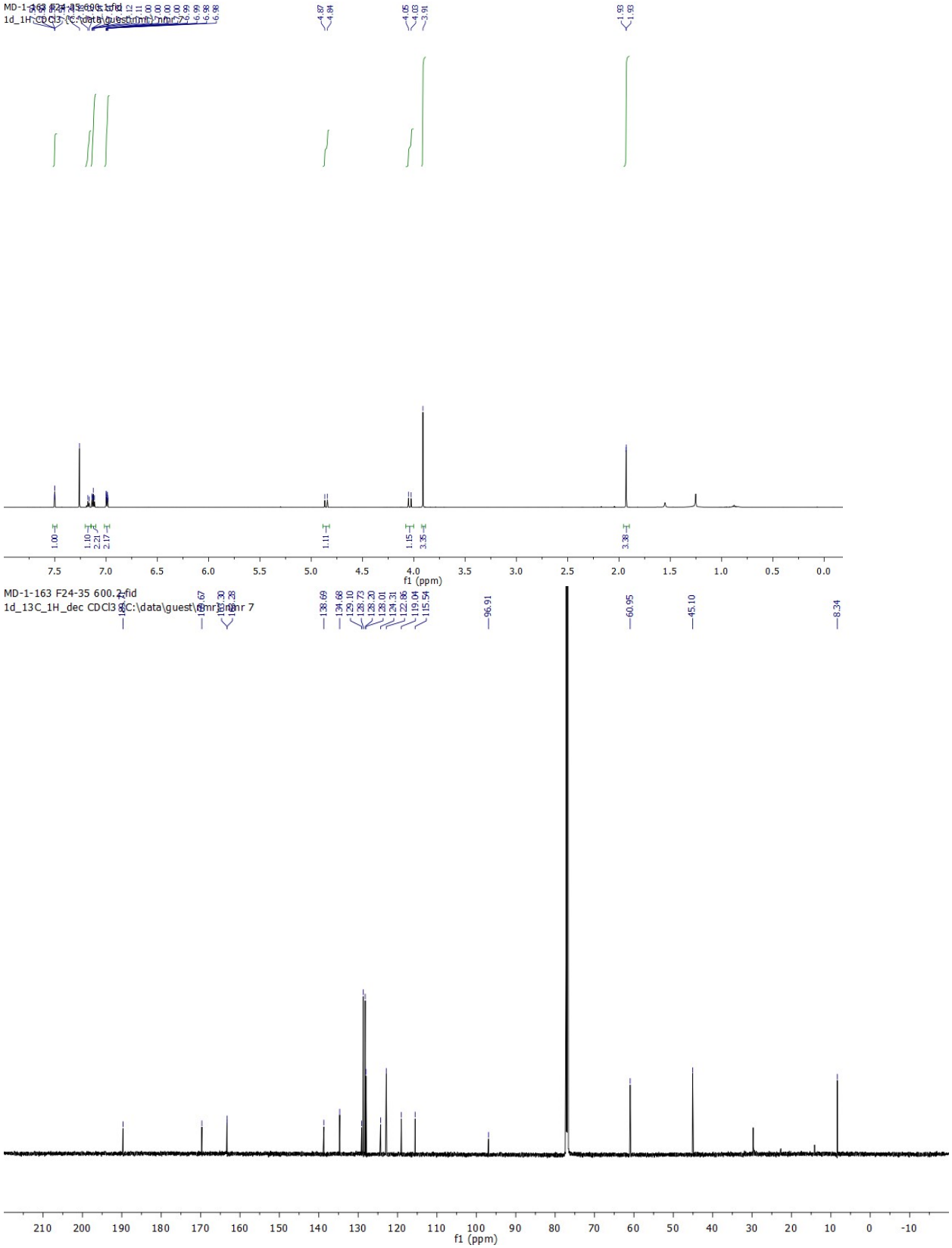


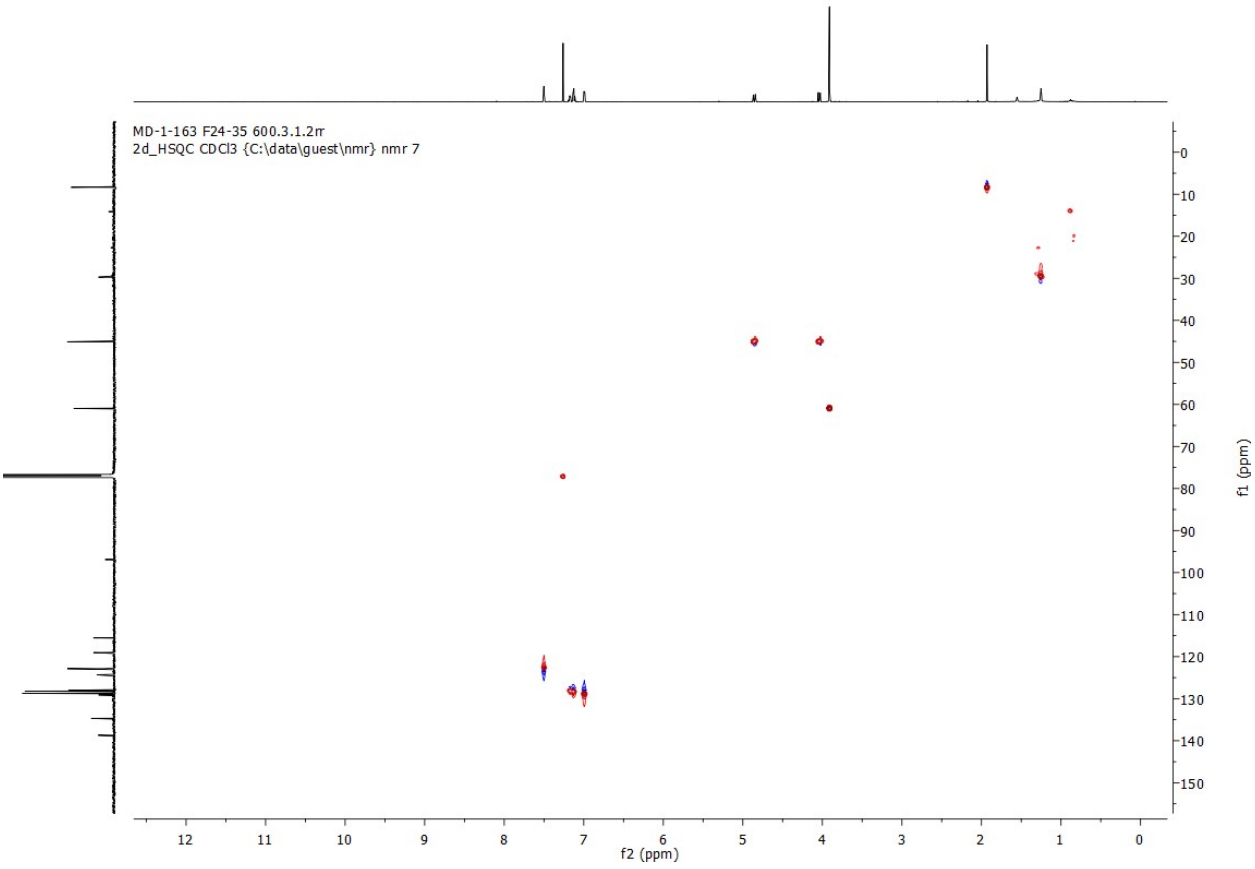
MD-1-164.2.fid
1d_13C_1H_dec CDCl3 {C:\data\guest\nmr} nmr 5



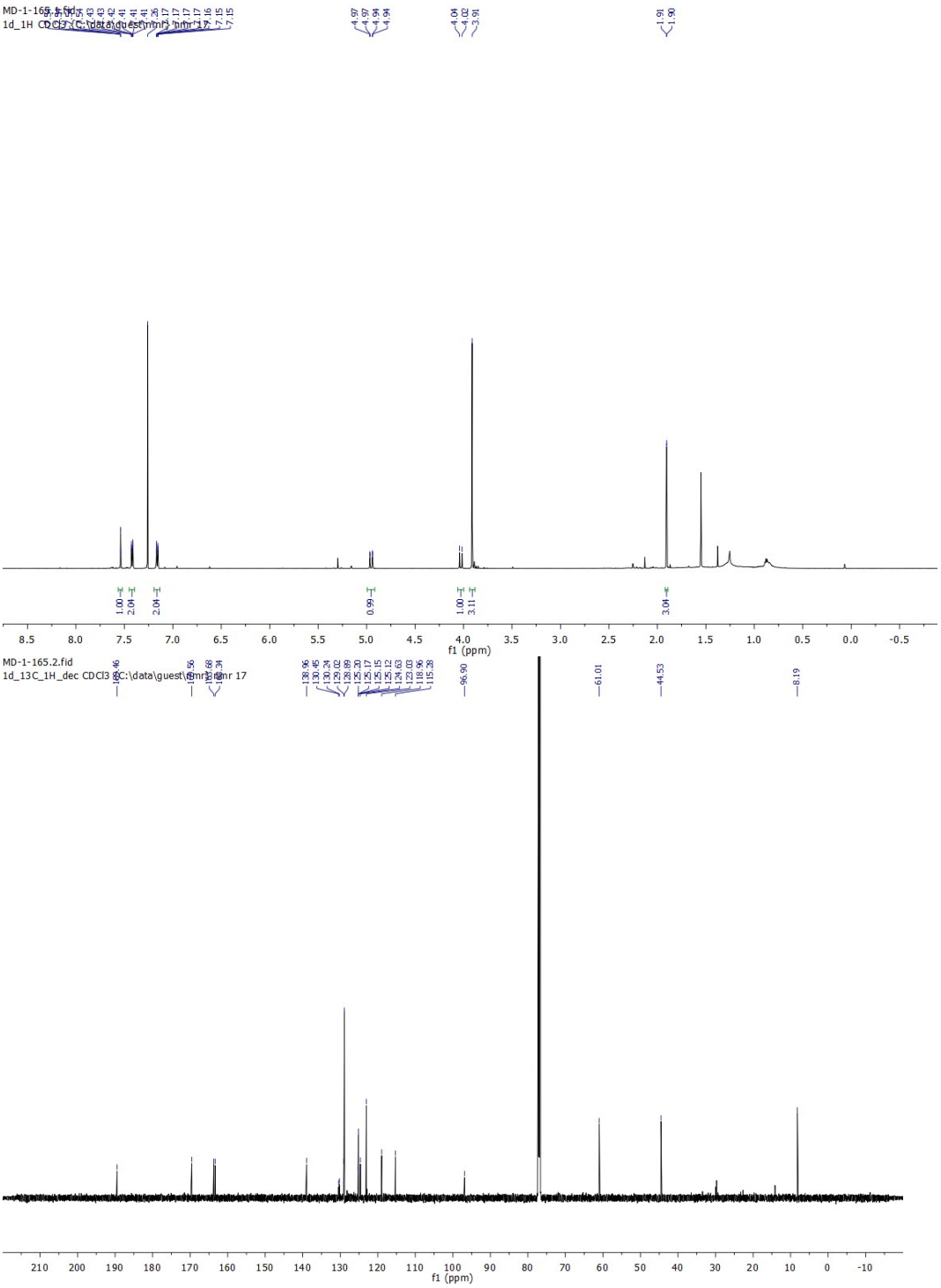


^{1}H NMR (400 MHz, $CDCl_3$) , ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of S20


1d_1H_CDCl3_C:\data\guest\nmr\nmr 7
4.87
4.84
4.05
4.03
3.91
1.93
1.93
1.00
1.10
2.21
2.17
1.11
1.15
3.35
3.38
f1 (ppm)
MD-1-163 F24-35 600.2.fid
1d_13C_1H_dec CDCl3 C:\data\guest\nmr\nmr 7
138.69
134.68
129.10
128.73
128.20
128.01
124.31
122.86
119.04
115.54
96.91
60.95
45.10
8.34
f1 (ppm)

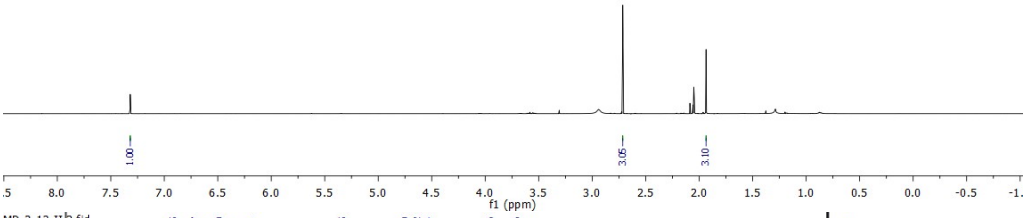


^{1}H NMR (400 MHz, $CDCl_3$) , ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of S21

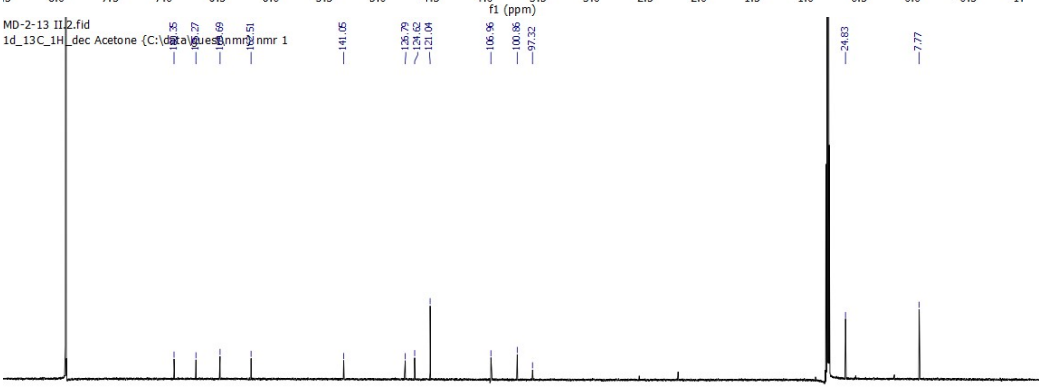


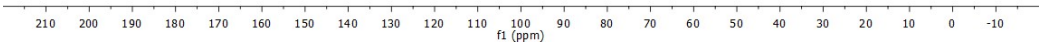
^{1}H NMR (400 MHz, $CDCl_3$) , ^{13}C NMR (100 MHz, $CDCl_3$) and HSQC of S22

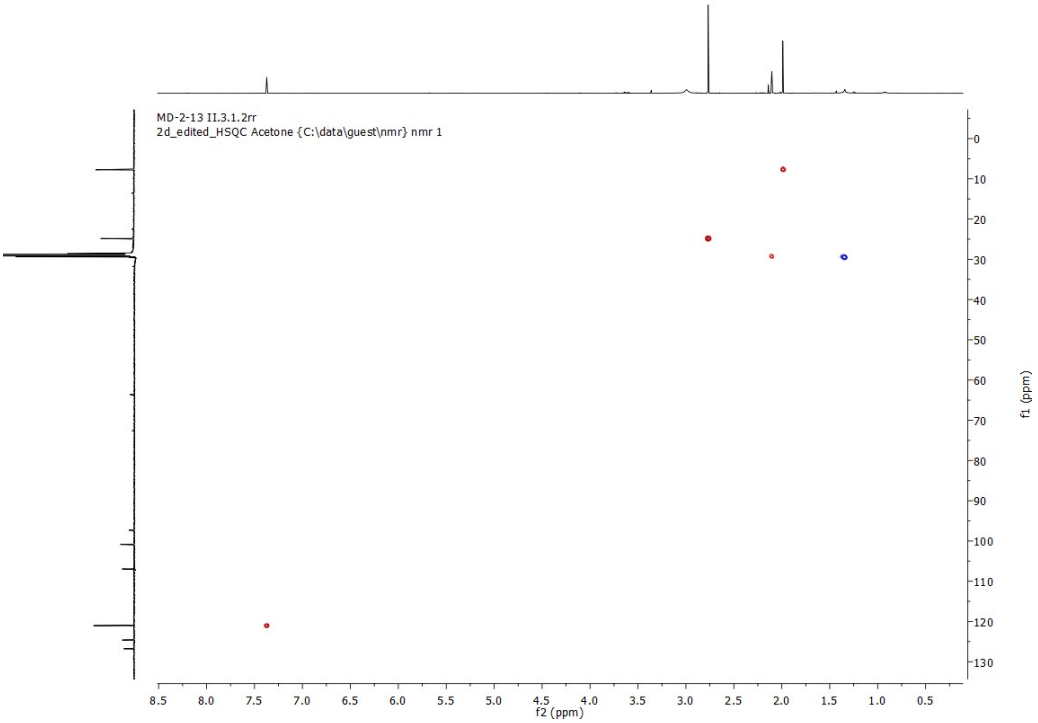
MD-2-13 II.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 1



MD-2-13 II.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 1

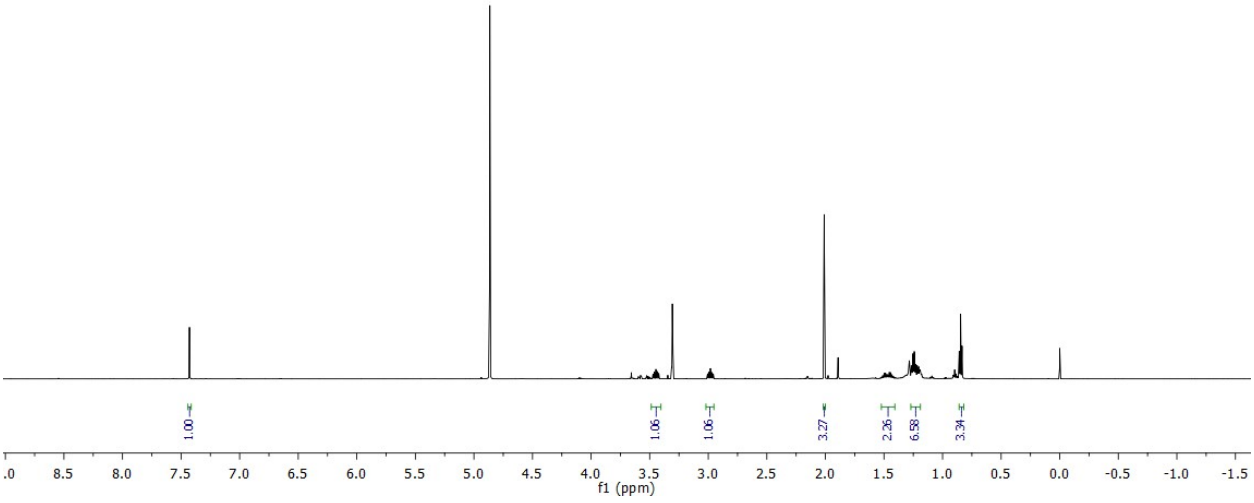


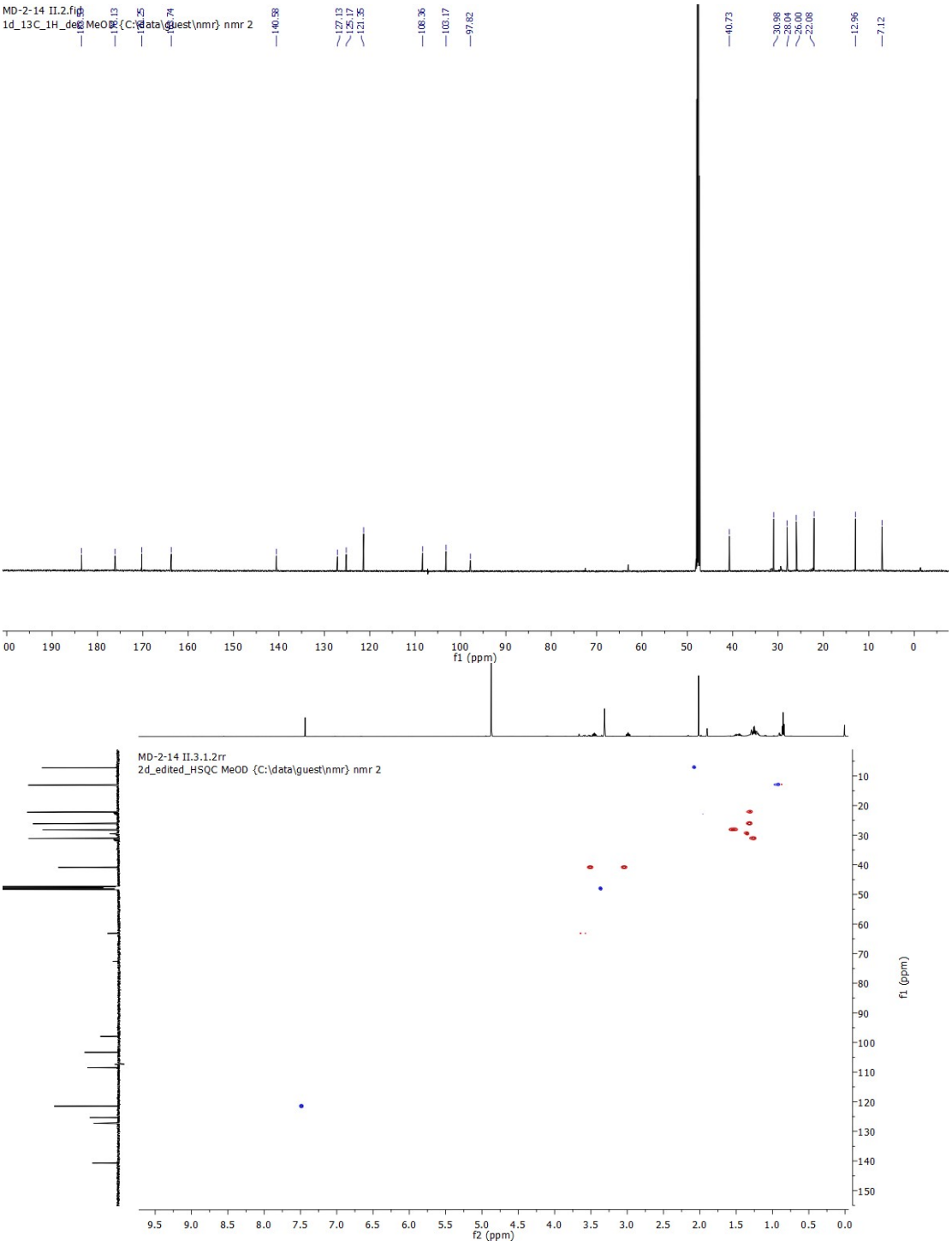




^{1}H NMR (600 MHz, Acetone-D_6) , ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **9**

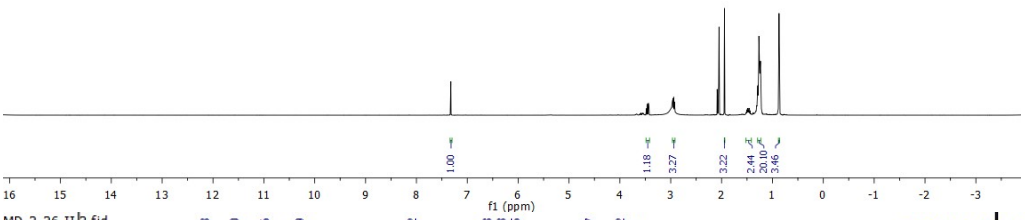
MD-2-14 II.1.fid
1d_1H MeOD {C:\data\guest\nmr} nmr 2



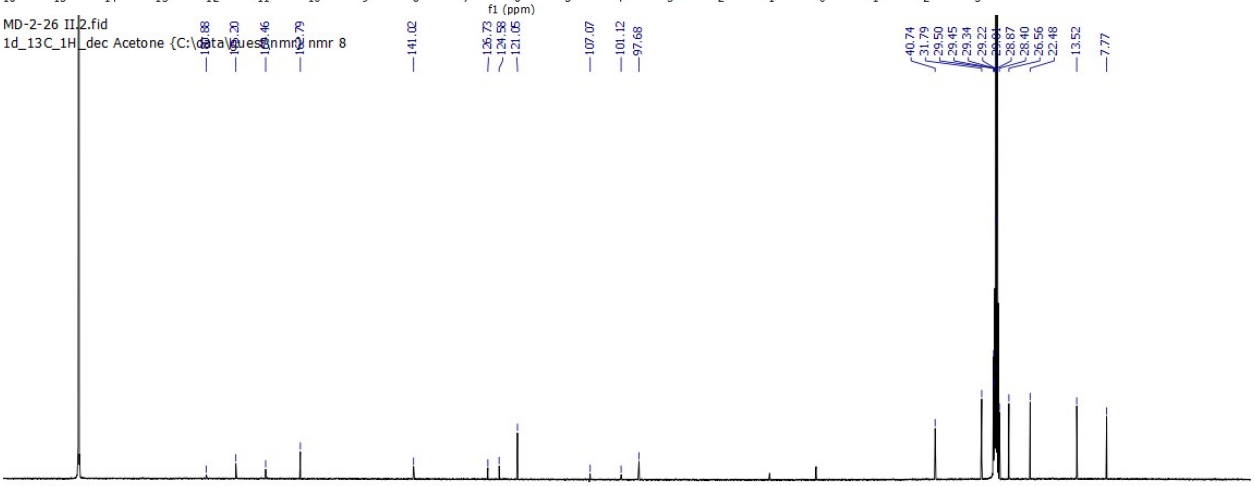


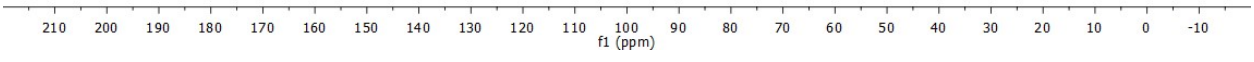
^{1}H NMR (600 MHz, MeOD-d4) , ^{13}C NMR (150 MHz, MeOD – d4) and HSQC of **10**

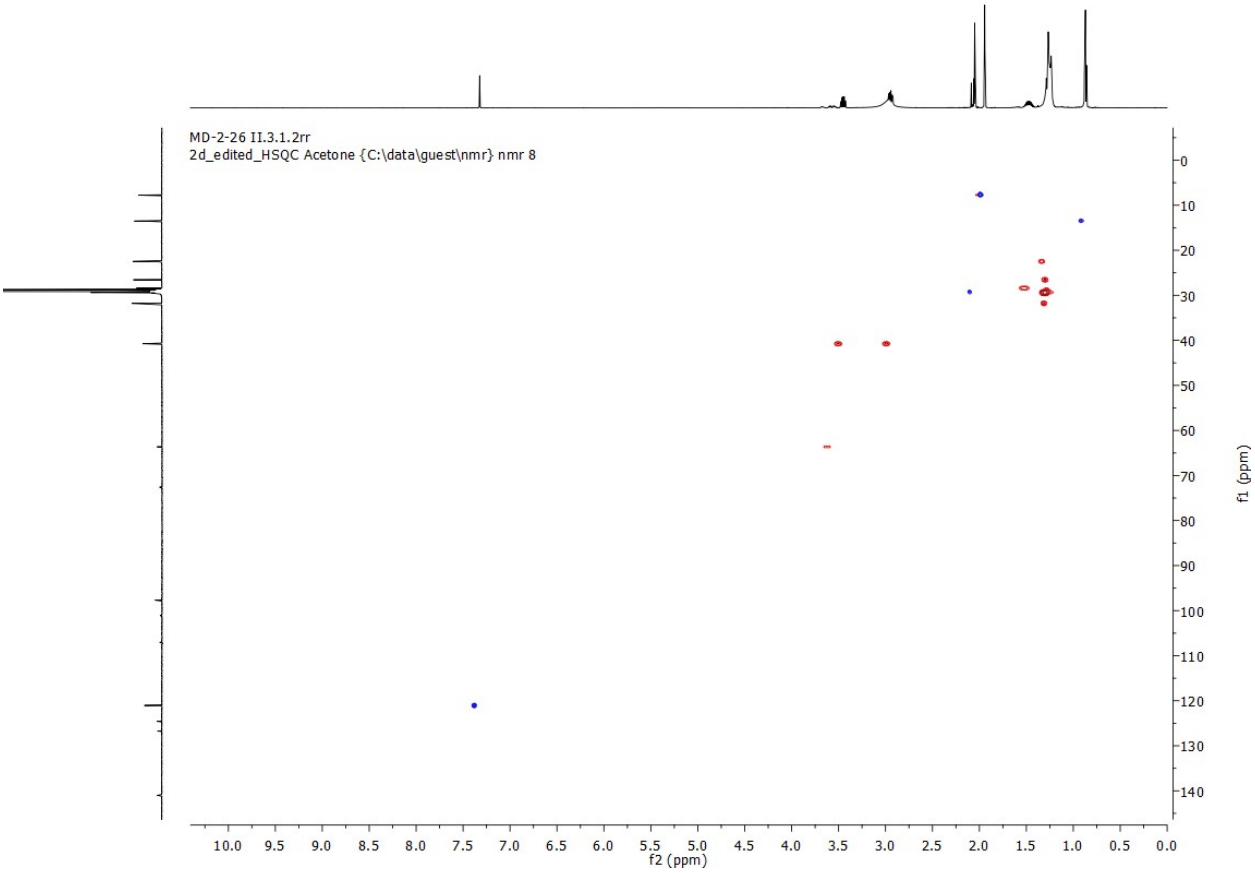
MD-2-26 II.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 8



MD-2-26 II.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 8

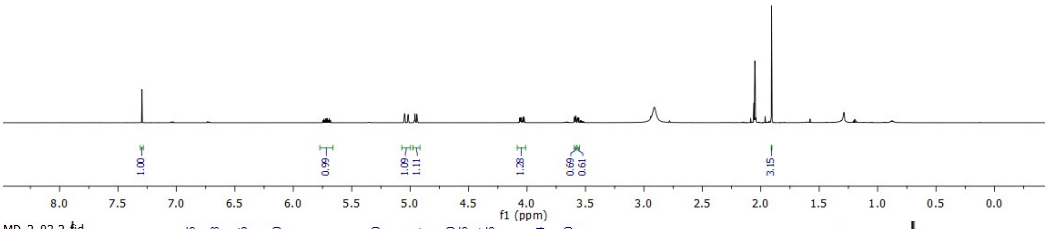




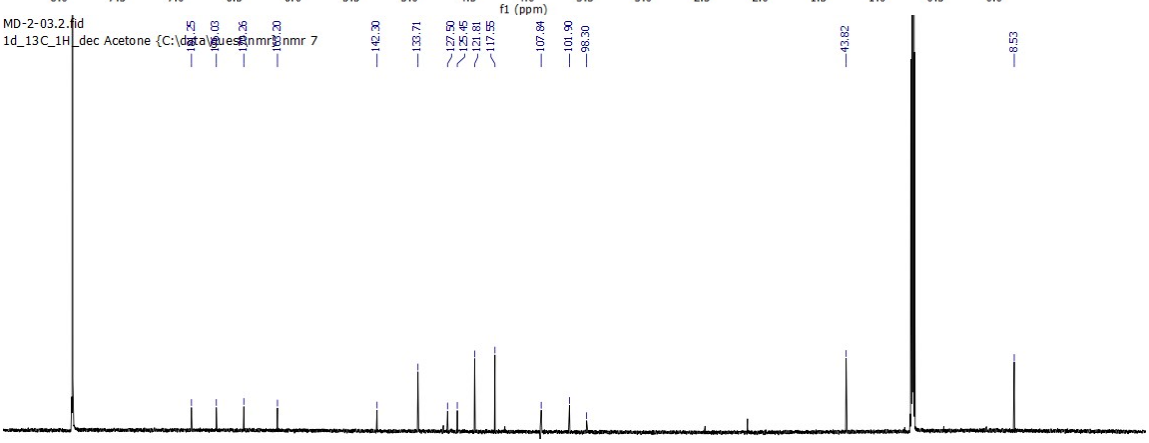


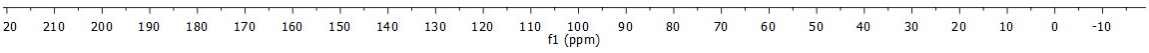
^{1}H NMR (600 MHz, Acetone-D_6) , ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **11**

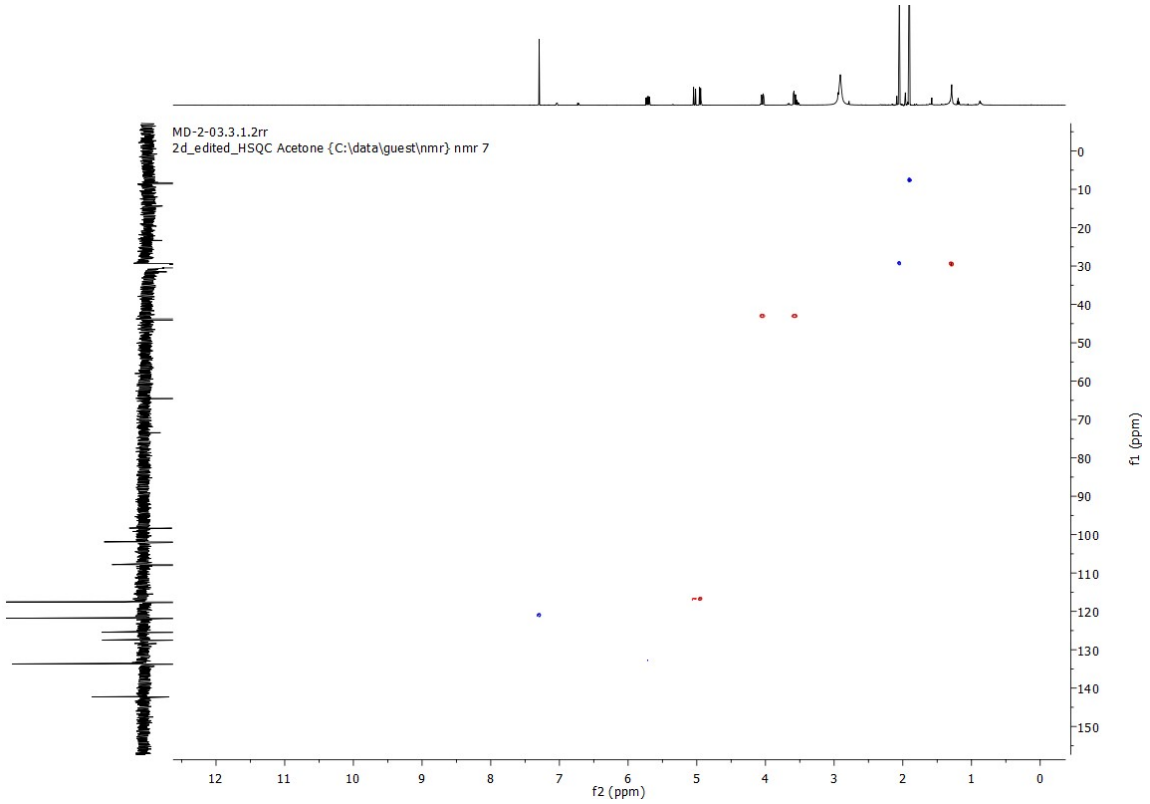
MD-2-03.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 7



MD-2-03.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 7

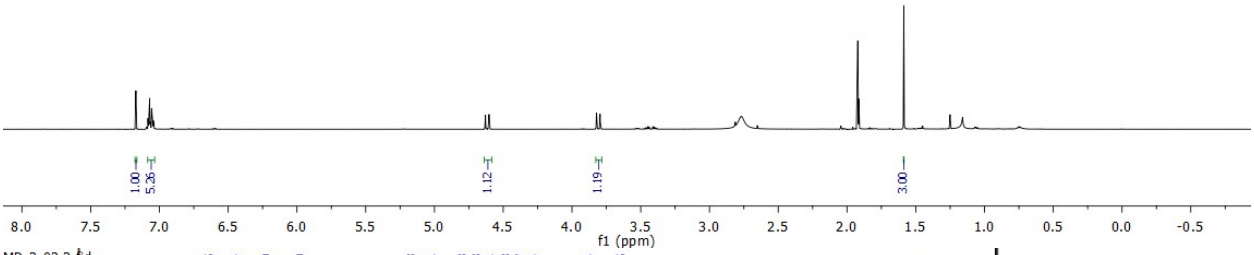




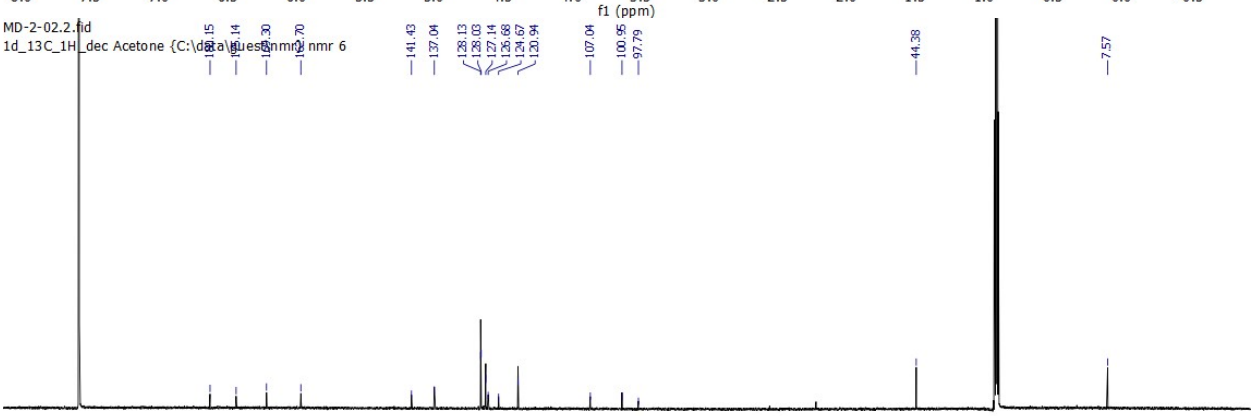


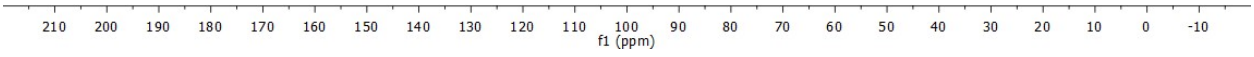
^{1}H NMR (600 MHz, Acetone-D_6) , ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **12**

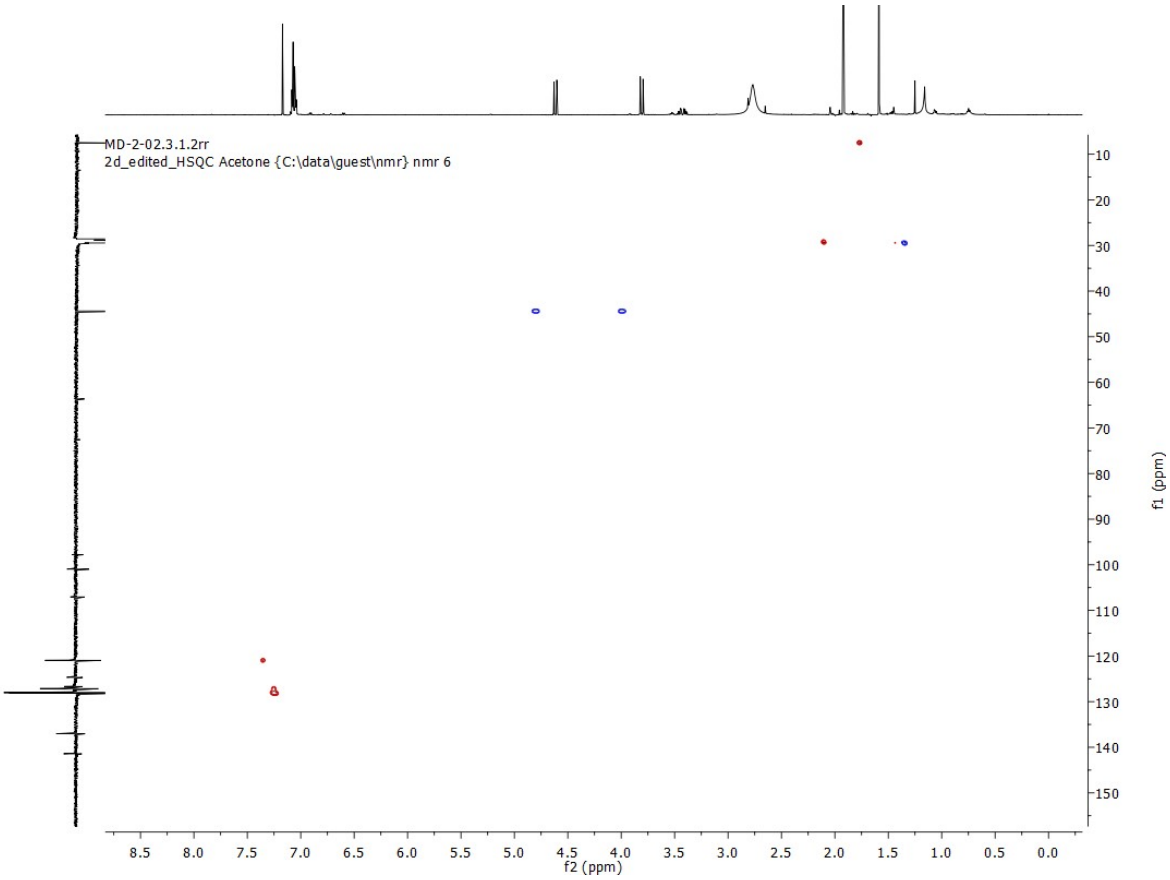
MD-2-02.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 6



MD-2-02.2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 6

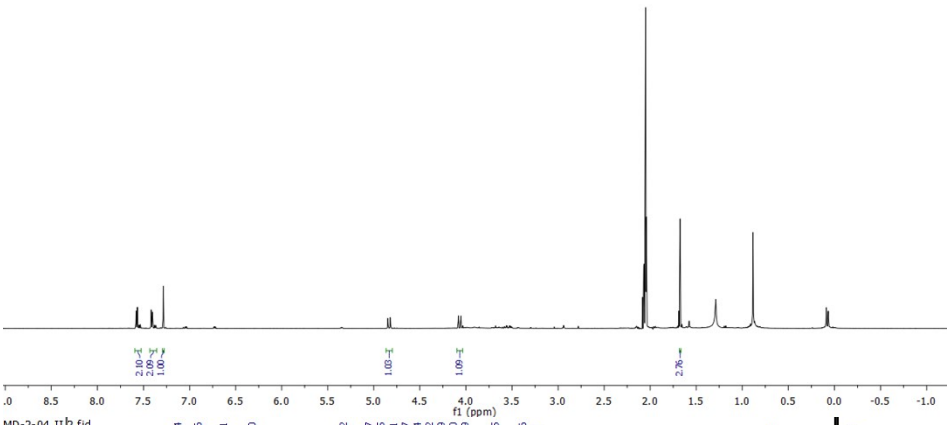




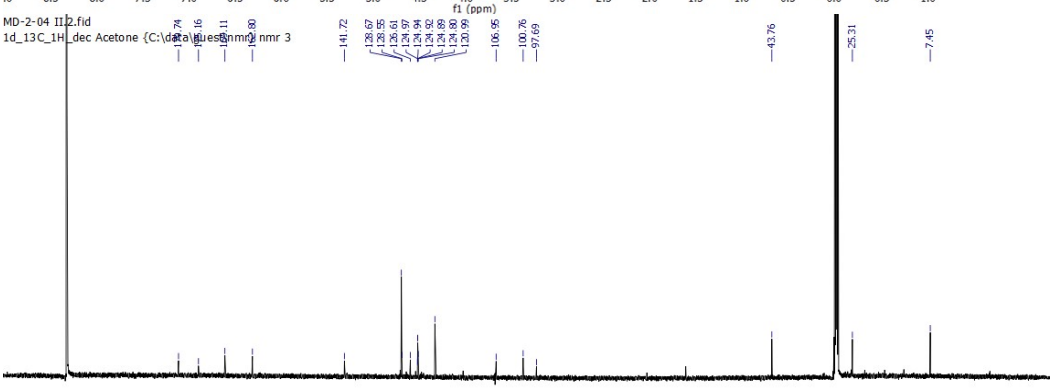


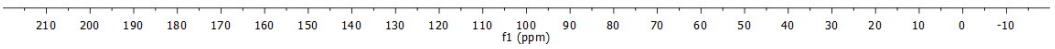
^{1}H NMR (600 MHz, Acetone-D_6) , ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **13**

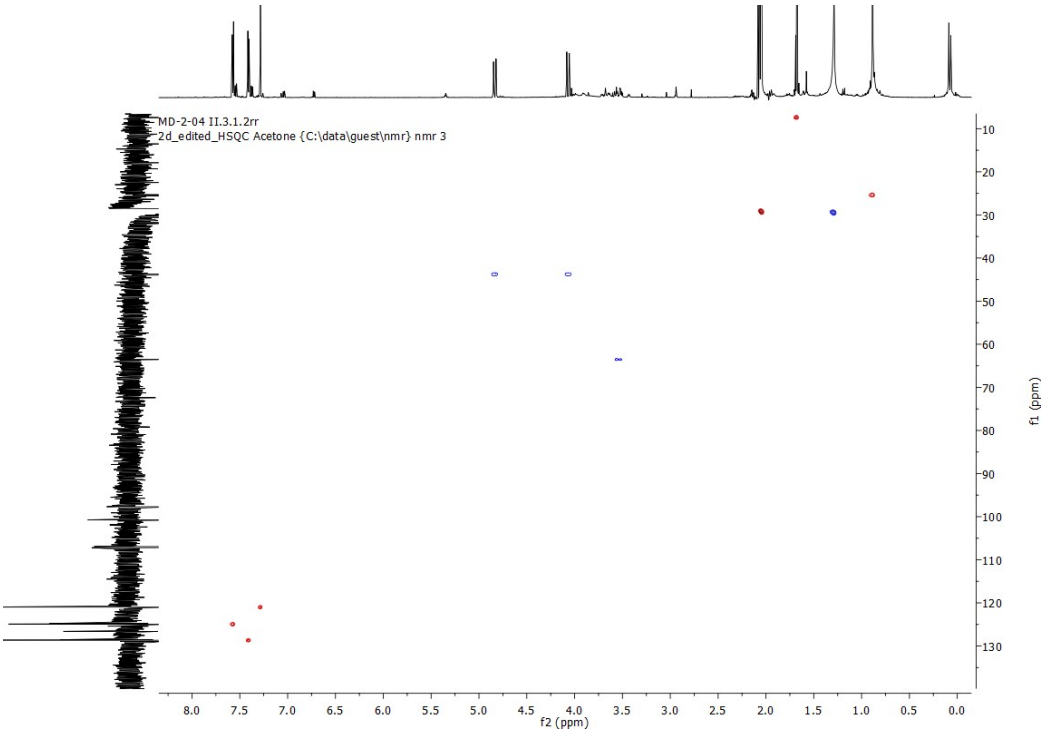
MD-2-04 II.1.fid
1d_1H Acetone {C:\data\guest\nmr} nmr 3



MD-2-04 II2.fid
1d_13C_1H_dec Acetone {C:\data\guest\nmr} nmr 3







^{1}H NMR (600 MHz, Acetone-D_6) , ^{13}C NMR (150 MHz, Acetone-D_6) and HSQC of **14**

REFERENCES

(1) Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform. *J. Cheminform.* **2012**, *4* (17), 1–17.

(2) Trott, O.; Olson, A. J. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J. Comput. Chem.* **2009**, *31* (2), 455–461. https://doi.org/10.1002/jcc.

(3) Garrett, M. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009**, *30* (16), 2785–2791. https://doi.org/10.1002/jcc.

(4) Jaghoori, M. M.; Bleijlevens, B.; Olabarriaga, S. D. 1001 Ways to Run AutoDock Vina for Virtual Screening. *J. Comput. Aided. Mol. Des.* **2016**, *30* (3), 237–249. https://doi.org/10.1007/s10822-016-9900-9.