Appendix A – Hardware and Software

Table S1: Software used in the study

S.No.	Software	Study/Method
1	SWISS-MODEL [1]	Homology Modelling
4	UCSF DOCK6 [2]	Virtual Screening
5	UCSF Chimera [3], PyMOL [4] , VMD [5],	Molecular Visualisation
6	BIOVIA Discovery Studio [6], LigPlot [7], PLIP: Protein-Ligand Interaction Profiler [8]	Receptor-ligand interactions
7	Raccoon [9]	Splitting batch file of compounds library
8	OpenBabel [10]	Molecular file format conversion
9	Avogadro [11], HyperChem [12], ArgusLab [13], ChemDraw [14]	Molecular drawing and optimization
10	GROMACS 5.1.1 [15]	MD Simulations
11	MS Office 365, MS OneNote, Joplin	Drafting research

Table 2: Hardware specifications used in the study

S. No.	Virtual Machines	Processing	RAM	Hard Disk
1	VM-1	40 CPUs	64 GB	2.2 TB
2	VM-2	20 CPUs	32 GB	1.1 TB
3	VM-3	40 CPUs	64 GB	1.1 TB
4	VM-4	20 CPUs	64 GB	1.1 TB

Appendix B – Synthesis of Fragments of the Hit Compounds

Synthesis of N-(2,4,5-trichlorophenyl)methanesulfonamide (24MSC)

2,4,5-Tricholoroaniline (2 g) was taken in a round-bottomed flask (100 mL) and dissolved into 5% Sodium Carbonate (18 mL). The pH of the reaction mixture was maintained at 8-10. The residue was dissolved in 5% Sodium Carbonate, and methane sulfonyl chloride (0.82 cm^3) was added and stirred at room temperature for 6 hours and 30 minutes. TLC (hexanes, acetate; 80:20) showed a single spot. The precipitates of products were filtered and dried.

Synthesis of N-(2-aminoethyl)-N-(2,4,5-trichlorophenyl)methanesulfonamide (M24D)

Bromoethylamine (0.18 g) was taken in a round-bottomed flask (150 mL) and dissolved into 5% DMF (15 mL). The residue was dissolved in DMF, and N-(2,4,5-trichlorophenyl)methanesulfonamide (0.4 g) was added and stirred at room temperature for 5 hours and 15 minutes. Lithium hydride (0.002 g) was also added as a catalyst. TLC (hexanes, acetate; 80:20) showed a single spot. The reaction mixture was quenched with the chilled water, the product got precipitated, filtered, and dried.

Synthesis of 1-(4-(bromomethyl)phenylsulfonyl)piperidine (BSPP)

Piperidine (0.37 cm^3) was taken in a round-bottomed flask (100 mL) and dissolved into 5% Sodium Carbonate (18 mL). The pH of the reaction mixture was maintained at 8-10. Piperidine was dissolved in 5% Sodium Carbonate, and 4-(bromoethyl)benzene-1-sulfonyl chloride (1 g) was added and stirred at room temperature for 7 hours 50 minutes. TLC (hexanes, acetate; 80:20) showed a single spot. The precipitates of products were filtered and dried.

Synthesis of N-(2,4-dichlorophenyl)methanesulfonamide (ABR1)

2,4-dichloroaniline (2 g) was taken in a round-bottomed flask (100 mL) and dissolved into 5% Sodium Carbonate (18 mL). The pH of the reaction mixture was maintained at 8-10. Next, 2,4-dichloroaniline was dissolved in 5% Sodium Carbonate methane sulfonyl chloride (1.4136 g) was added into it and stirred at room temperature for 5 hours and 30 minutes. TLC (hexanes, acetate; 80:20) showed a single spot. Finally, the precipitates of products were filtered and dried.

Synthesis of N-(2-aminoethyl)-N-(2,4-dichlorophenyl)methanesulfonamide (ABR2)

N-(2,4-dichlorophenyl)methanesulfonamide (1 g) was taken in a round-bottomed flask (150 mL) and dissolved into 5% DMF (15 mL). The residue was dissolved in DMF, and Bromoethylamine (0.51 g) was added and stirred at room temperature for 5 hours. Lithium hydride (0.002 g) was also added as a catalyst. TLC (hexanes, acetate; 80:20) showed a single spot. The reaction mixture was quenched with chilled water, and the product precipitated, filtered, and dried.

Synthesis of 4-(4-(bromomethyl)phenylsulfonyl)morpholine (BBMP)

Morpholine (0.32 cm^3) was taken in the round-bottomed flask (100 mL) and dissolved into 5% Sodium Carbonate (18 mL). The pH of the reaction mixture was maintained at 8-10. Morpholine was dissolved in 5% Sodium Carbonate, and 4-(bromomethyl) benzene-1-sulfonyl chloride (1 g) was added to it and stirred at room temperature for 6 hours and 30 minutes. TLC (hexanes, acetate; 80:20) showed a single spot. The precipitates of products were filtered and dried.

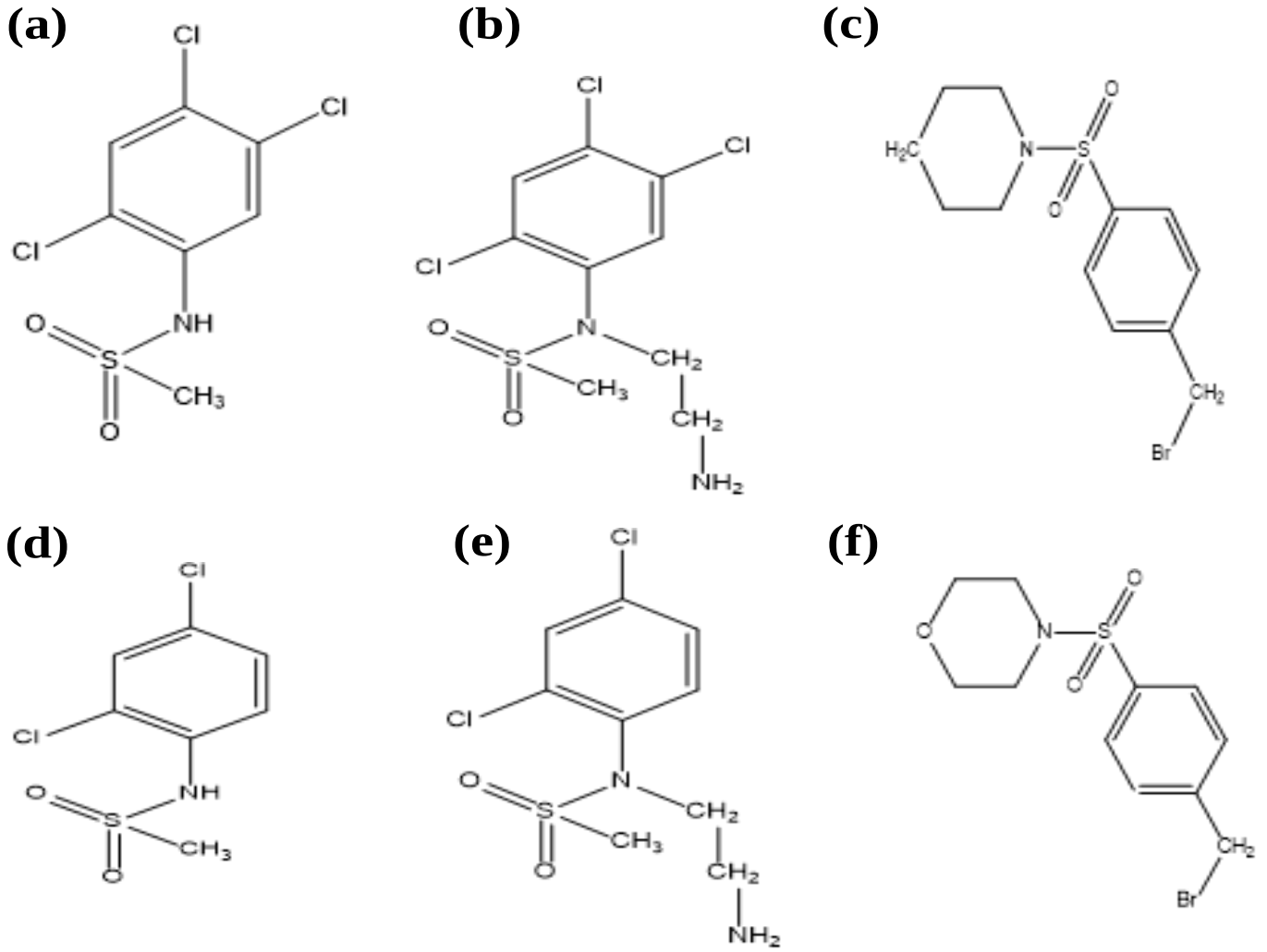


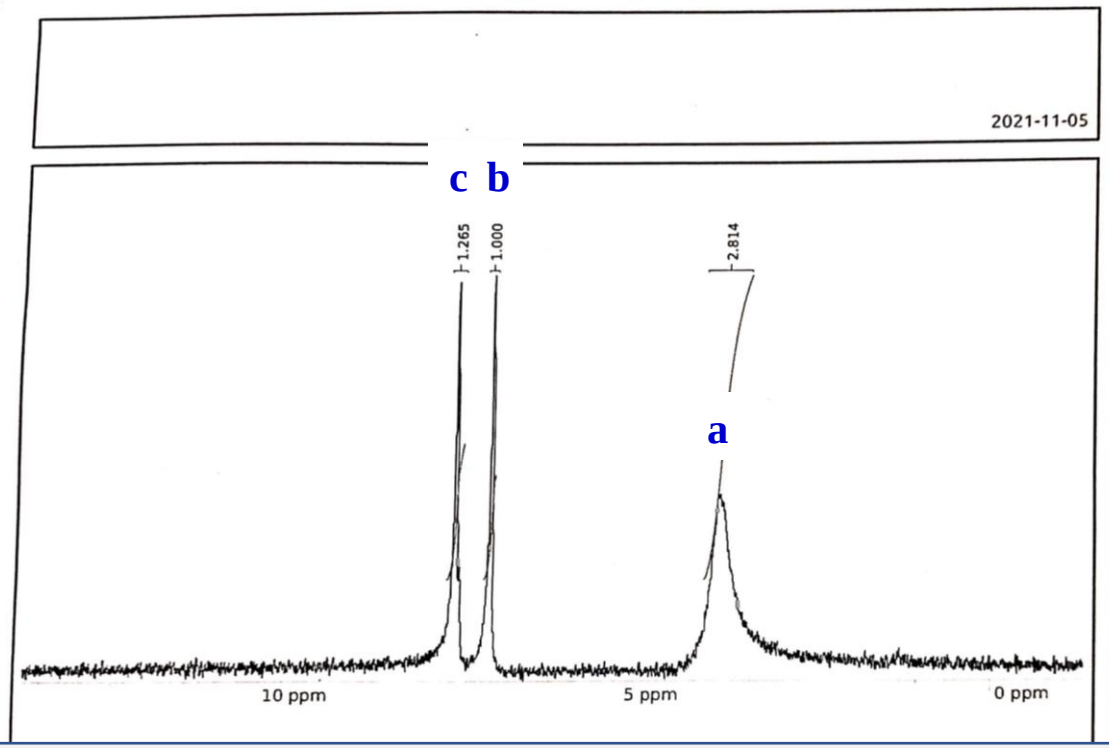
Figure 1: Structures of fragments of hit compounds TCM, TCP, DCP and DCM. The fragments are 24MSC (a), M24D (b), BSPP (c), ABR1 (d), ABR2 (e), BBMP (f)

Table 1: Physical properties of fragments of the hit compounds TCM, TCP, DCP and DCM.

S. No.	Properties of Compounds	24MSC	M24D	BSPP	ABR1	ABR2	BBMP
1	**Physical appearance**	Solid	Solid	Solid	Solid	Solid	Solid
2	**Colour**	Beige	Vivid white	Cream	White	White	Pure white
3	**Chemical formula**	$C_7H_6Cl_3NO_2S$	$C_9H_{11}Cl_3N_2O_2S$	$C_{12}H_{16}BrNO_2S$	$C_7H_7Cl_2NO_2S$	$C_9H_{12}Cl_2N_2O_2S_2$	$C_{11}H_{14}BrNO_3S$
4	**Molecular weight**	274.55 g/mol	317.62 g/mol	320.20 g/mol	240.11.20 g/mol	283.17 g/mol	318.23 g/mol
5	**Solubility**	Chloroform DMSO	Chloroform DMSO	Chloroform DMSO	Chloroform DMSO	Chloroform DMSO	Chloroform DMSO
6	**Melting Point**	100 - 103 ℃	94 - 96 ℃	100 - 102 ℃	125 - 128 ℃	128 -130 ℃	150 -153 ℃

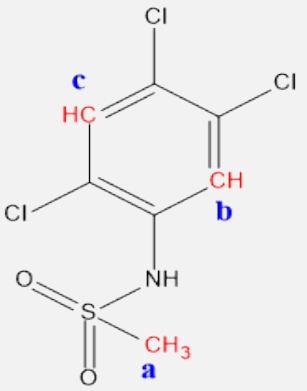
Appendix C – NMR spectra of fragments of the hit compounds

NMR Spectra of the fragment N-(2,4,5-trichlorophenyl)methanesulfonamide (24MSC)



Acquisition and Processing Parameters

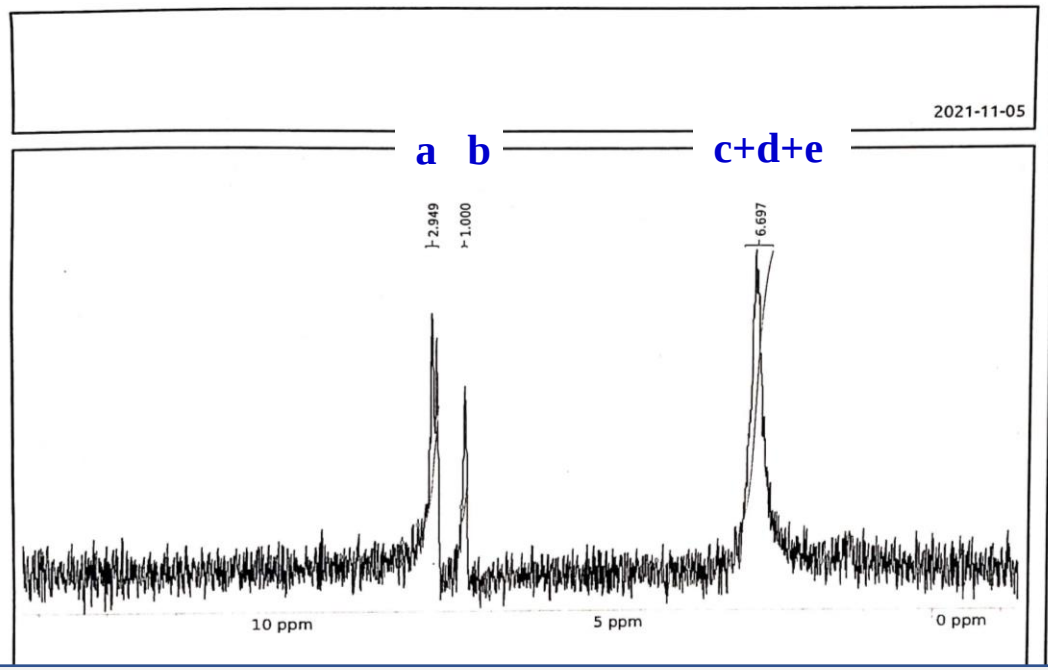
Filename:
rashid 24msc
Acquisition Time:
2021/08/24 15:10:36+0500
Nucleus: ^1H
Solvent: Chloroform-d
TX Frequency: 59.57 MHz
Scans: 100
Time Per Scan: 4.4 sec
Spectral Width: 14 ppm
Dwell Time: 0.34 uS
Digital Resolution: 0.05 Hz
NPoints (Complex): 2048
Zero Filling: 7
Apodization: 0.05
Receiver Gain: 12.8807487488
Pulse Width: 14.1 us
Peaks:
1: 218 Hz (3.67 ppm)
2: 230 Hz (3.86 ppm)
3: 406 Hz (6.83 ppm)
4: 431 Hz (7.25 ppm)
5: 435 Hz (7.31 ppm)



N-(2,4,5-trichlorophenyl)methanesulfonamide

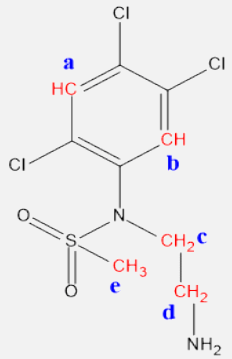
Labeled H-atom	No. of hydrogen atom	Chemical shift value(ppm)	Multiplicity
a	3	3.5	s
b	1	7.3	d
c	1	7.6	d

NMR Spectra of the fragment N-(2-aminoethyl)-N-(2,4,5-trichlorophenyl)methanesulfonamide (M24D)



Acquisition and Processing Parameters

Filename:
rashid m24d
Acquisition Time:
2021/08/24 15:21:15+0500
Nucleus: ^1H
Solvent: Chloroform-d
TX Frequency: 59.57 MHz
Scans: 100
Time Per Scan: 4.4 sec
Spectral Width: 14 ppm
Dwell Time: 0.34 uS
Digital Resolution: 0.05 Hz
NPoints (Complex): 2048
Zero Filling: 7
Apodization: 0.05
Receiver Gain: 12.8807487488
Pulse Width: 14.1 us
Peaks:
1: 135 Hz (2.27 ppm)
2: 141 Hz (2.37 ppm)
3: 154 Hz (2.59 ppm)
4: 407 Hz (6.84 ppm)
5: 431 Hz (7.25 ppm)
6: 435 Hz (7.32 ppm)

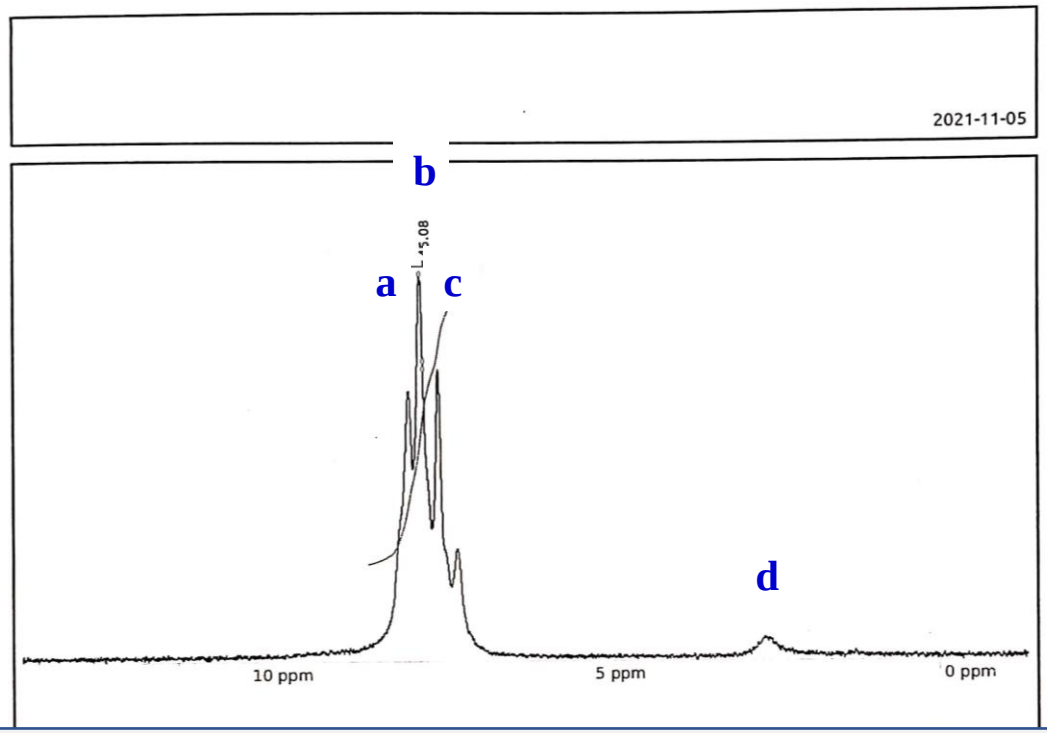


N-(2-aminoethyl)-*N*-(2,4,5-trichlorophenyl)methanesulfonamide

Labeled H-atom	No. of hydrogen atom	Chemical shift value(ppm)	Multiplicity
a	1	7.30	d
b	1	6.83	d
c	2	2.8-3.9	t
d	2	2.8-3.9	t
e	3	2.8-3.9	s

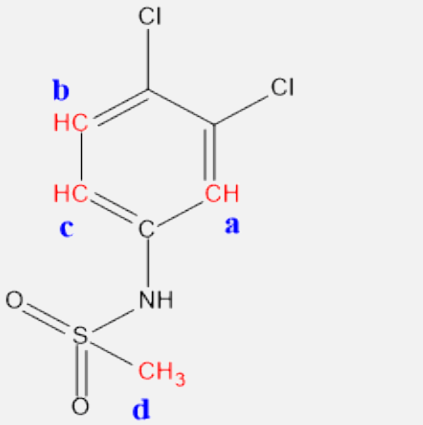
The peak at 7.24ppm is of chloroform

NMR spectra of the fragment N-(2,4-dichlorophenyl)methanesulfonamide (ABR1)



Acquisition and Processing Parameters

Filename:
rashid hussain abr1
Acquisition Time:
2021/09/16 10:25:20+0500
Nucleus: ^1H
Solvent: Chloroform-d
TX Frequency: 59.57 MHz
Scans: 100
Time Per Scan: 4.4 sec
Spectral Width: 14 ppm
Dwell Time: 0.34 uS
Digital Resolution: 0.05 Hz
NPoints (Complex): 2048
Zero Filling: 7
Apodization: 0.05
Receiver Gain: 12.8807487488
Pulse Width: 14.1 us
Peaks:
1: 148 Hz (2.50 ppm)
2: 413 Hz (6.95 ppm)
3: 431 Hz (7.24 ppm)
4: 447 Hz (7.51 ppm)
5: 457 Hz (7.68 ppm)
6: 488 Hz (8.20 ppm)
7: 495 Hz (8.32 ppm)

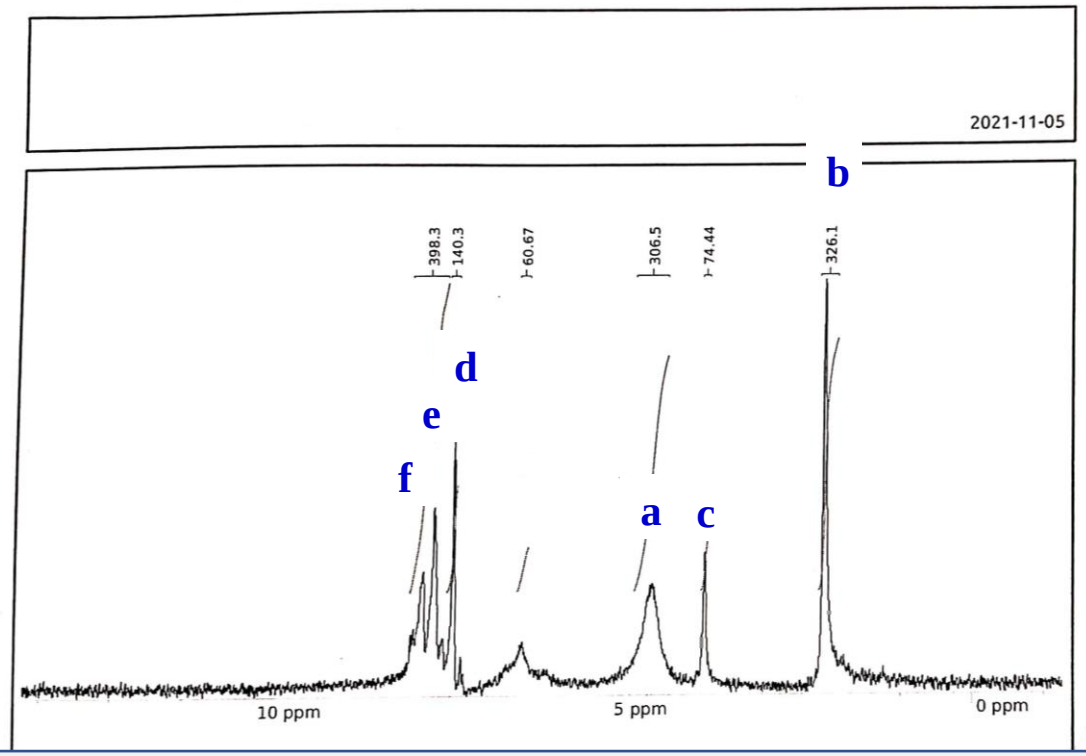


N-(3,4-dichlorophenyl)methanesulfonamide

Labeled H-atom	No. of hydrogen atom	Chemical shift value(ppm)	Multiplicity
a	1	7.68	dd
b	1	7.51	dd
c	1	6.95	dd
d	3	3.15	dd

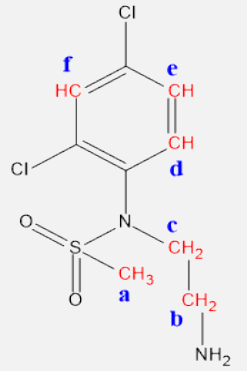
The peak at 7.24 is of chloroform

NMR spectra of the fragment N-(2-aminoethyl)-N-(2,4-dichlorophenyl)methanesulfonamide (ABR2)



Acquisition and Processing Parameters

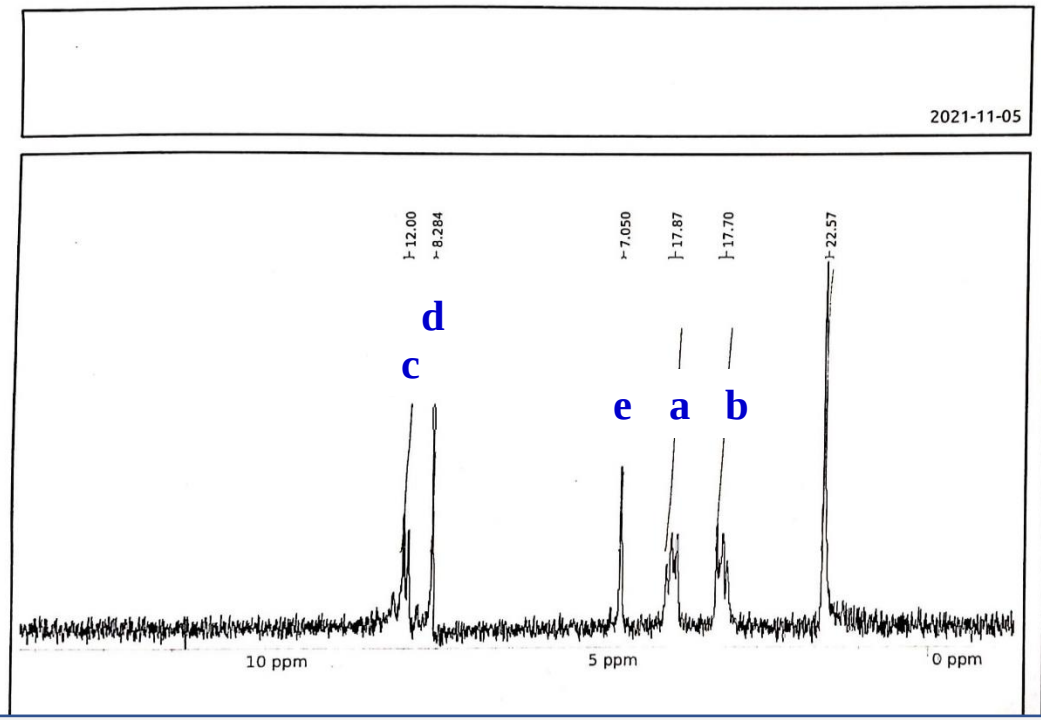
Filename:
rashid hussain abr2
Acquisition Time:
2021/09/16 14:38:55+0500
Nucleus: ^1H
Solvent: Chloroform-d
TX Frequency: 59.57 MHz
Scans: 100
Time Per Scan: 4.4 sec
Spectral Width: 14 ppm
Dwell Time: 0.34 uS
Digital Resolution: 0.05 Hz
NPoints (Complex): 2048
Zero Filling: 7
Apodization: 0.05
Receiver Gain: 12.880748748
Pulse Width: 14.1 us
Peaks:
1: 106 Hz (1.79 ppm)
2: 122 Hz (2.05 ppm)
3: 220 Hz (3.71 ppm)
4: 264 Hz (4.44 ppm)
5: 372 Hz (6.25 ppm)
6: 424 Hz (7.13 ppm)
7: 431 Hz (7.24 ppm)
8: 440 Hz (7.39 ppm)
9: 448 Hz (7.53 ppm)
10: 456 Hz (7.66 ppm)
11: 464 Hz (7.79 ppm)
12: 466 Hz (7.83 ppm)



N-(2-aminoethyl)-*N*-(2,4-dichlorophenyl)methanesulfonamide

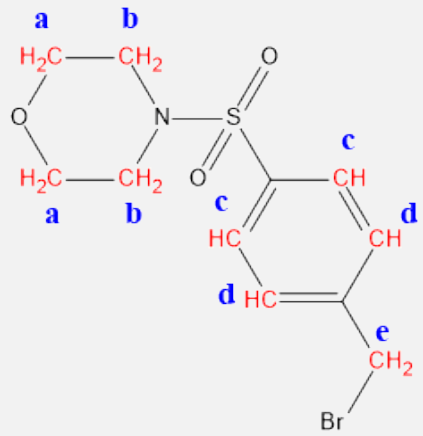
Labeled H-atom	No. of hydrogen atom	Chemical shift value(ppm)	Multiplicity
a	3	4.44	s
b	2	2.05	t
c	2	3.71	t
d	1	7.66	dd
e	1	7.79	dd
f	1	7.83	dd

NMR spectra of the fragment 4-(4-(bromomethyl)phenylsulfonyl)morpholine (BBMP)



Acquisition and Processing Parameters

Filename:
rashid bbmp
Acquisition Time:
2021/08/26 11:13:29+0500
Nucleus: ^1H
Solvent: Chloroform-d
TX Frequency: 59.57 MHz
Scans: 100
Time Per Scan: 4.4 sec
Spectral Width: 14 ppm
Dwell Time: 0.34 uS
Digital Resolution: 0.05 Hz
NPoints (Complex): 2048
Zero Filling: 7
Apodization: 0.05
Receiver Gain: 12.8807487488
Pulse Width: 14.1 us
Peaks:
1: 90 Hz (1.52 ppm)
2: 173 Hz (2.92 ppm)
3: 177 Hz (2.98 ppm)
4: 183 Hz (3.07 ppm)
5: 217 Hz (3.65 ppm)
6: 222 Hz (3.73 ppm)
7: 226 Hz (3.81 ppm)
8: 267 Hz (4.49 ppm)
9: 431 Hz (7.24 ppm)
10: 452 Hz (7.60 ppm)
11: 456 Hz (7.67 ppm)
12: 465 Hz (7.81 ppm)

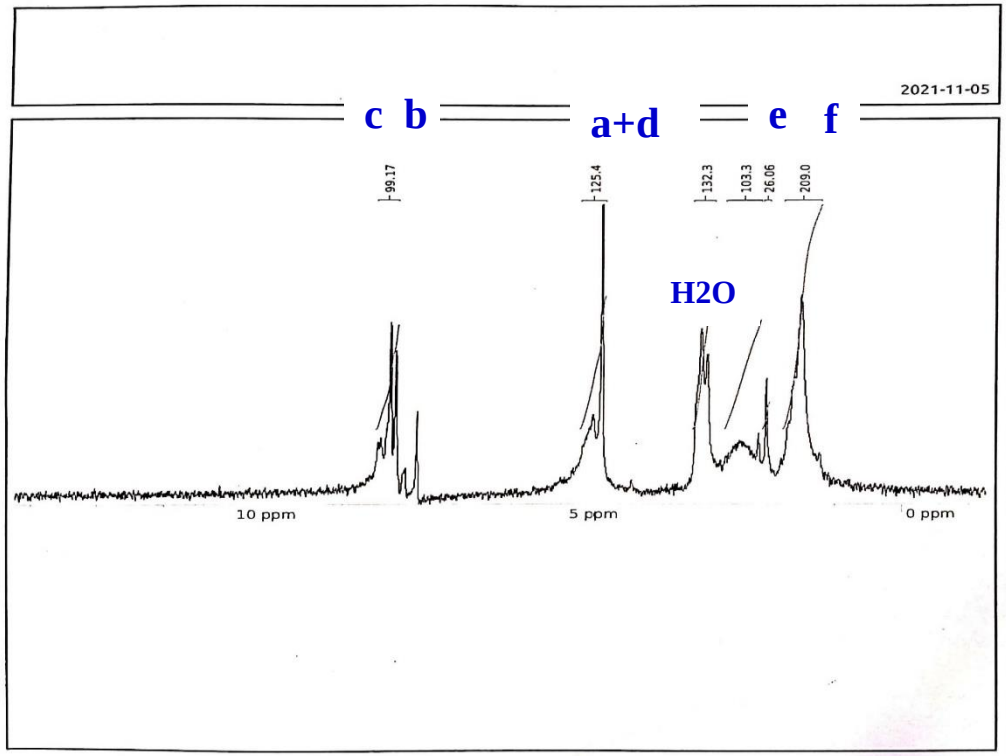


4-(4-(bromomethyl)phenylsulfonyl)morpholine

Labeled H-atom	No. of hydrogen atom	Chemical shift value(ppm)	Multiplicity
a	4	3.85	m
b	4	3.60	m
c	2	7.55	m
d	2	7.35	m
e	2	4.51	m

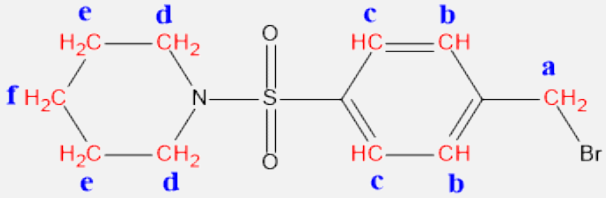
The peak is at 7.34 chloroform

NMR spectra of fragment 1-(4-(bromomethyl)phenylsulfonyl)piperidine (BSPP)



Acquisition and Processing Parameters

Filename:
rashid bspp
Acquisition Time:
2021/08/26 12:29:50+0500
Nucleus: ^1H
Solvent: Chloroform-d
TX Frequency: 59.57 MHz
Scans: 100
Time Per Scan: 4.4 sec
Spectral Width: 14 ppm
Dwell Time: 0.34 uS
Digital Resolution: 0.05 Hz
NPoints (Complex): 2048
Zero Filling: 7
Apodization: 0.05
Receiver Gain: 12.8807487488
Pulse Width: 14.1 us
Peaks:
1: 72 Hz (1.23 ppm)
2: 89 Hz (1.49 ppm)
3: 106 Hz (1.79 ppm)
4: 120 Hz (2.02 ppm)
5: 126 Hz (2.13 ppm)
6: 140 Hz (2.36 ppm)
7: 144 Hz (2.42 ppm)
8: 153 Hz (2.58 ppm)
9: 155 Hz (2.61 ppm)
10: 160 Hz (2.70 ppm)
11: 171 Hz (2.89 ppm)
12: 177 Hz (2.98 ppm)
13: 266 Hz (4.47 ppm)
14: 274 Hz (4.60 ppm)
15: 431 Hz (7.24 ppm)
16: 450 Hz (7.56 ppm)
17: 455 Hz (7.65 ppm)
18: 463 Hz (7.79 ppm)
19: 466 Hz (7.84 ppm)



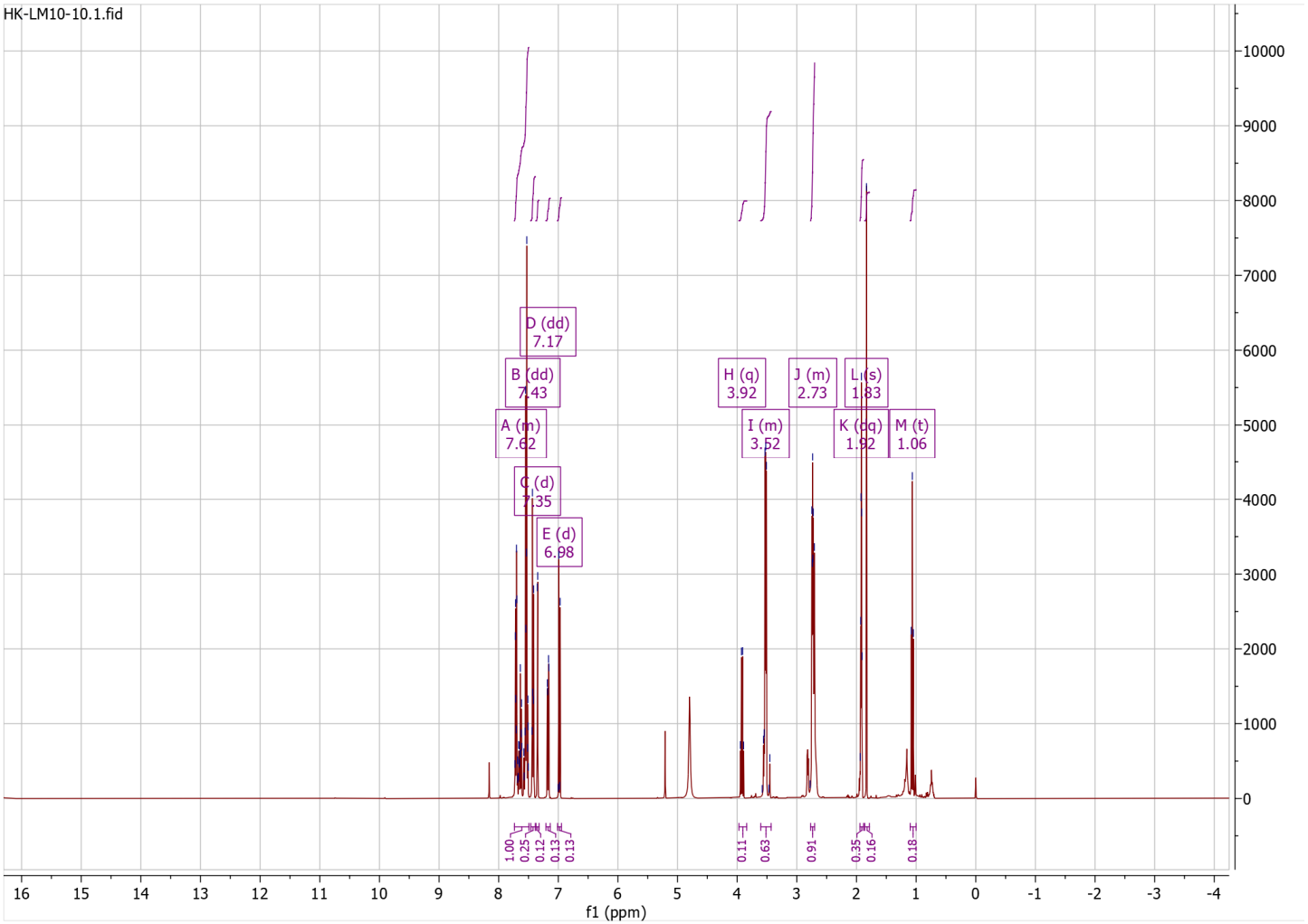
1-(4-(bromomethyl)phenylsulfonyl)piperidine

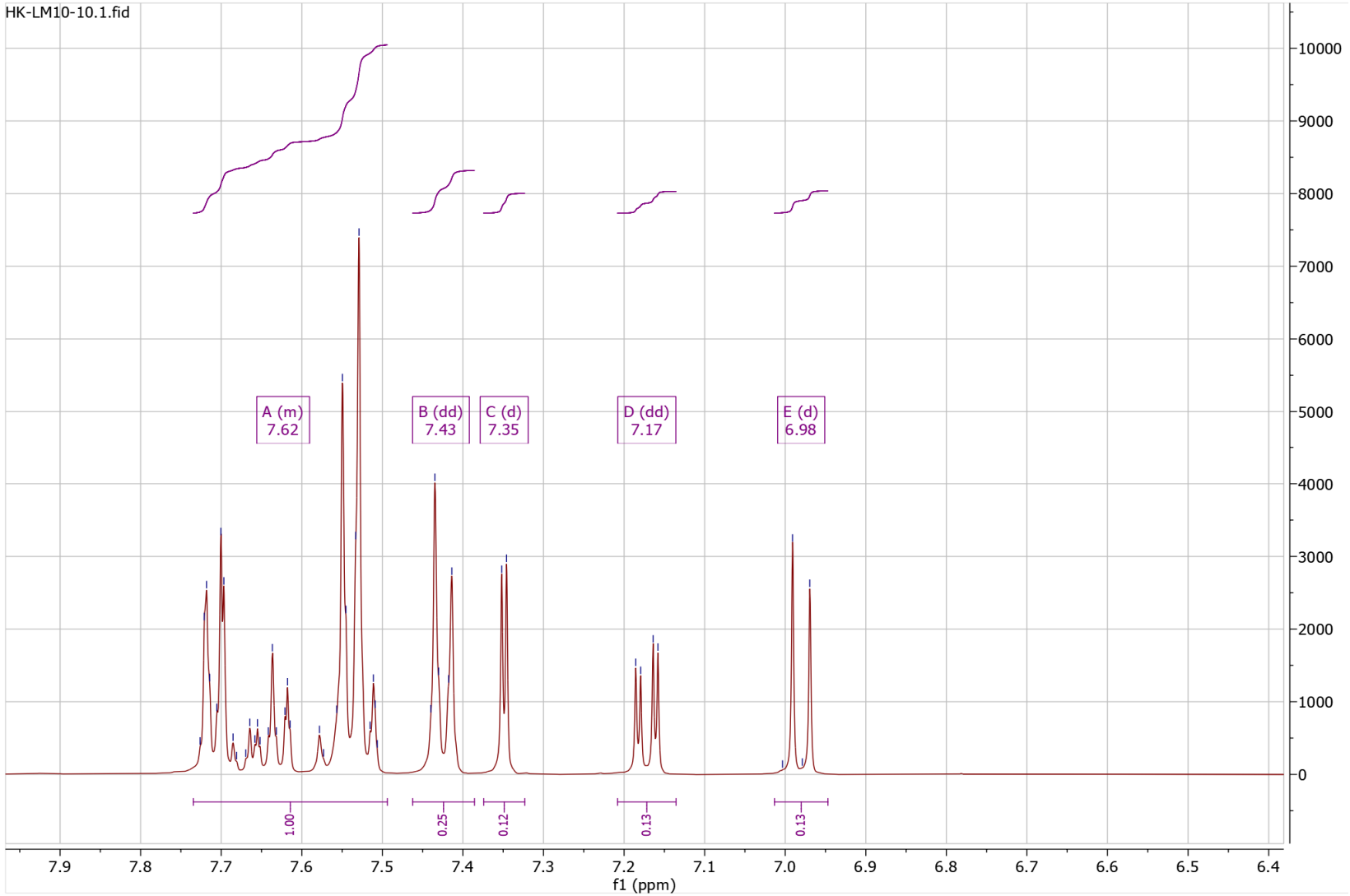
Labeled H-atom	No. of hydrogen atom	Chemical shift value(ppm)	Multiplicity
a + d	6	4.47 – 4.60	m
b	2	7.41	m
c	2	7.56	m
e	4	2.02	m
f	2	1.49	m

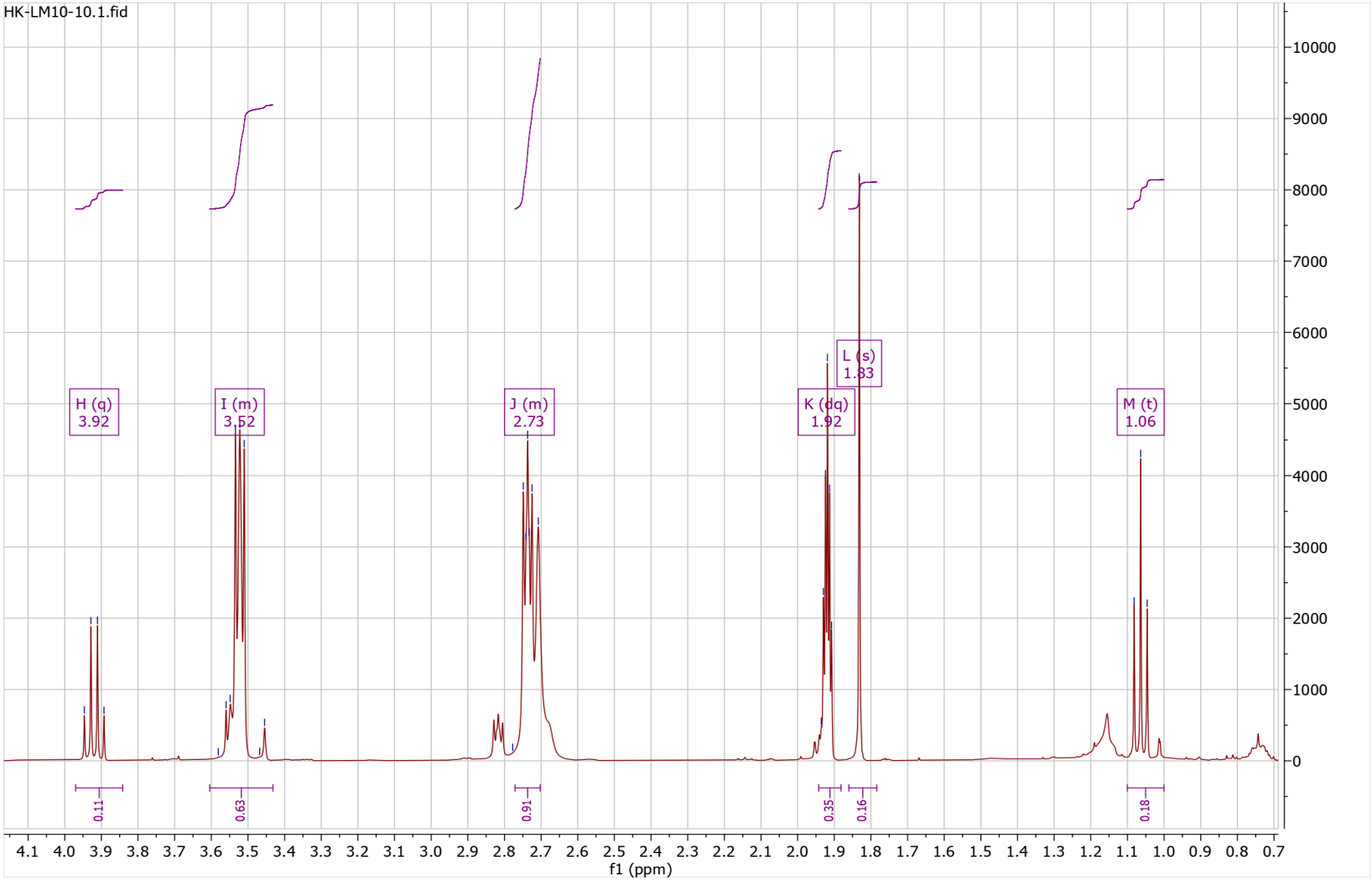
Chloroform peak is at 7.24 ppm

Appendix D – NMR spectra of the hit compounds

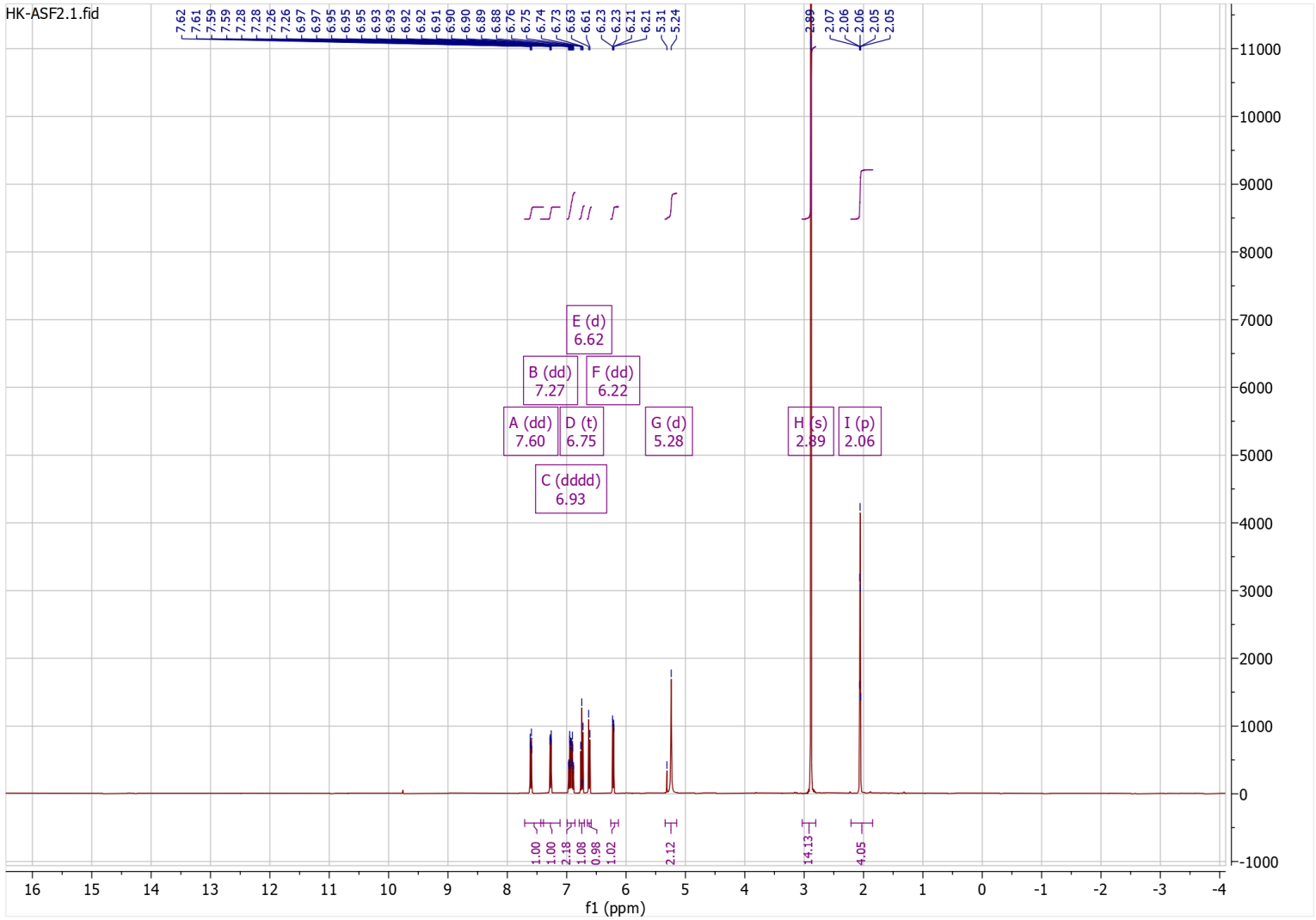
^{1}H NMR spectrum of DCM

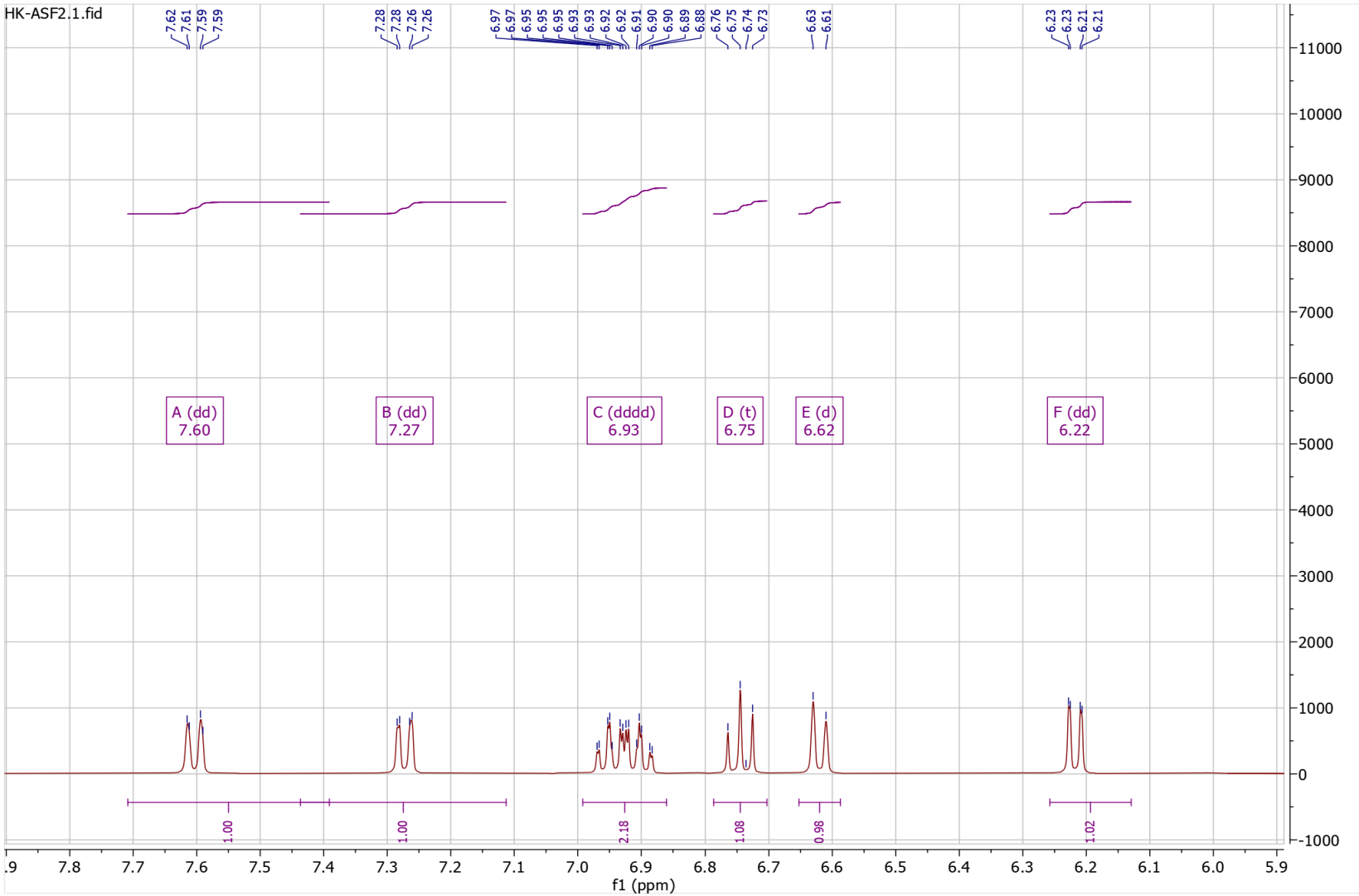


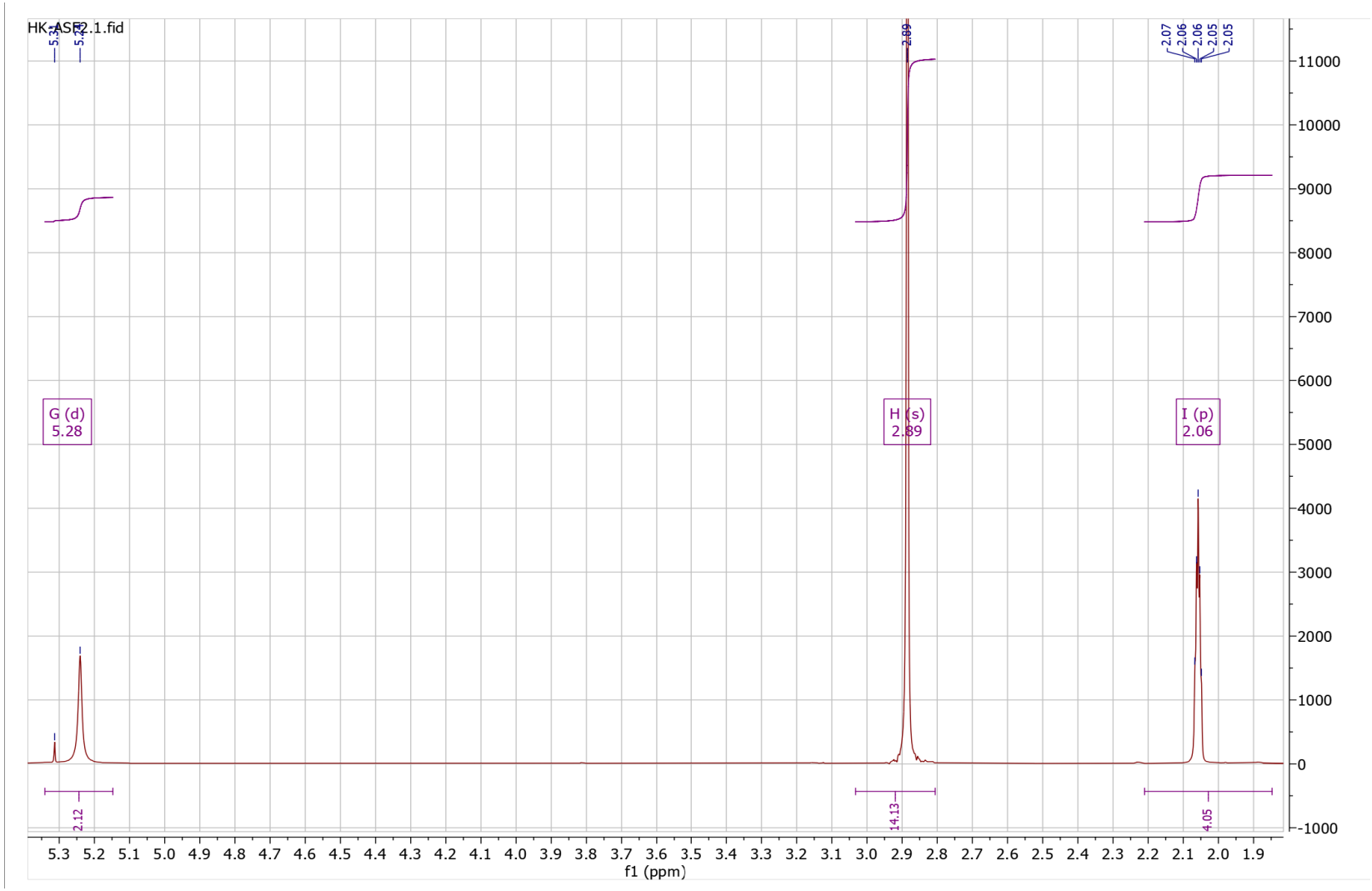

HK-LM10-10.1.fid
A (m)
7.62
B (dd)
7.43
C (d)
7.35
D (dd)
7.17
E (d)
6.98
1.00
0.25
0.12
0.13
0.13
f1 (ppm)


HK-LM10-10.1.fid
H (q)
3.92
I (m)
3.52
J (m)
2.73
K (dq)
1.92
L (s)
1.83
M (t)
1.06
0.11
0.63
0.91
0.35
0.16
0.18
f1 (ppm)

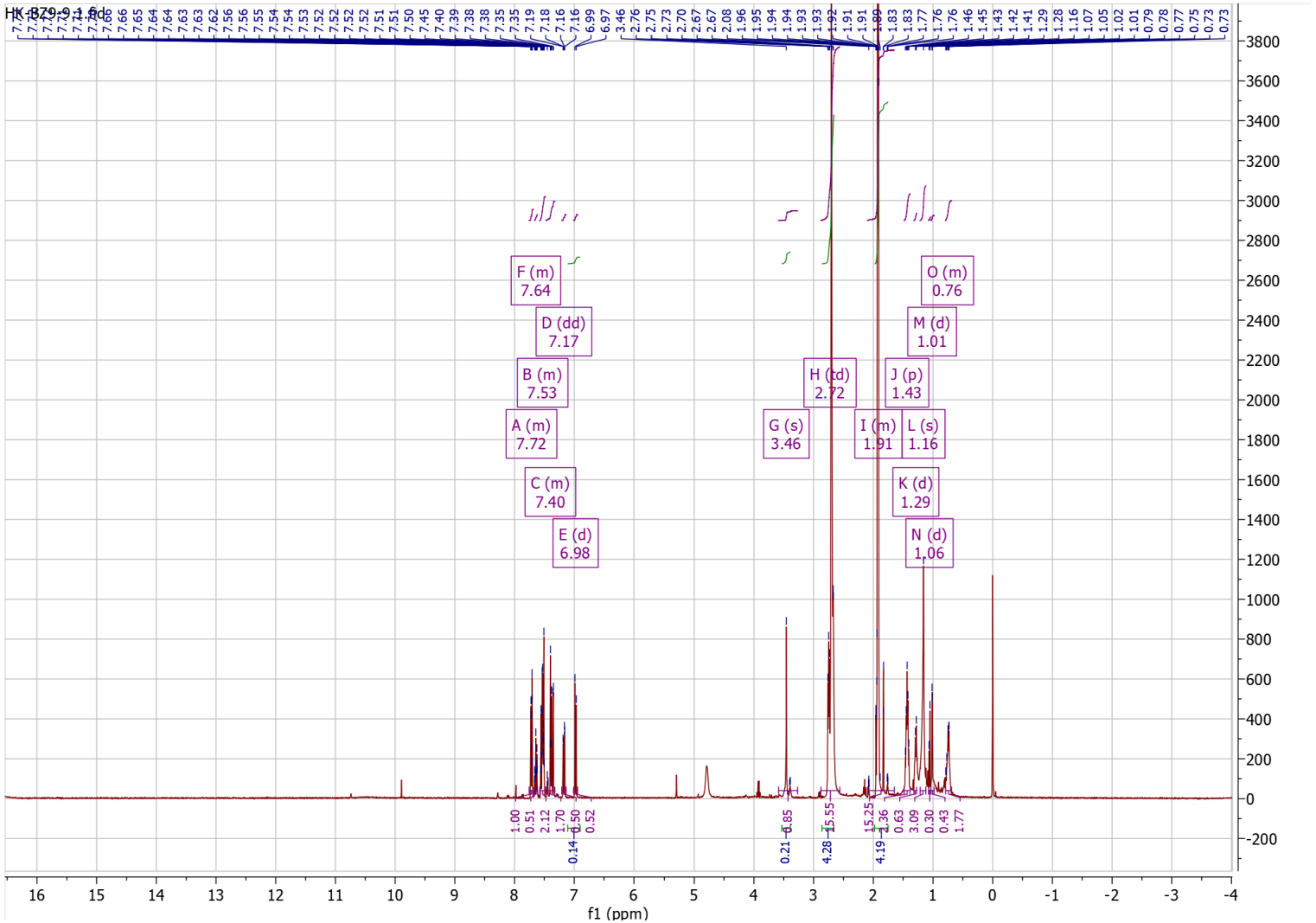
^{1}H NMR spectrum of TCM

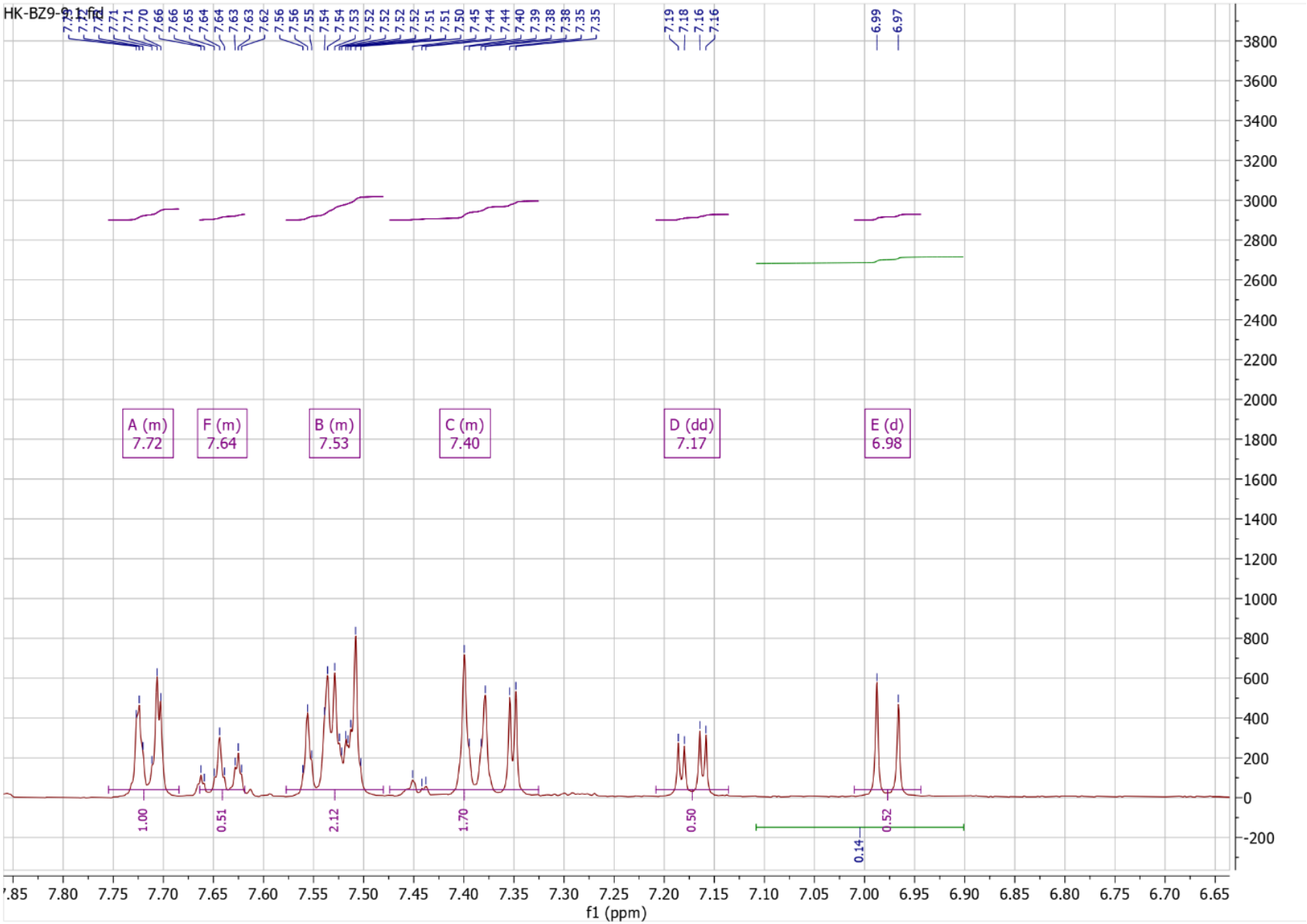



HK-ASF2.1.fid
7.62
7.61
7.59
7.59
7.28
7.28
7.26
7.26
6.97
6.97
6.95
6.95
6.95
6.93
6.92
6.92
6.91
6.90
6.90
6.89
6.88
6.76
6.75
6.74
6.73
6.63
6.61
6.23
6.23
6.21
6.21
A (dd)
7.60
B (dd)
7.27
C (dddd)
6.93
D (t)
6.75
E (d)
6.62
F (dd)
6.22
1.00
1.00
2.18
1.08
0.98
1.02
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
f1 (ppm)

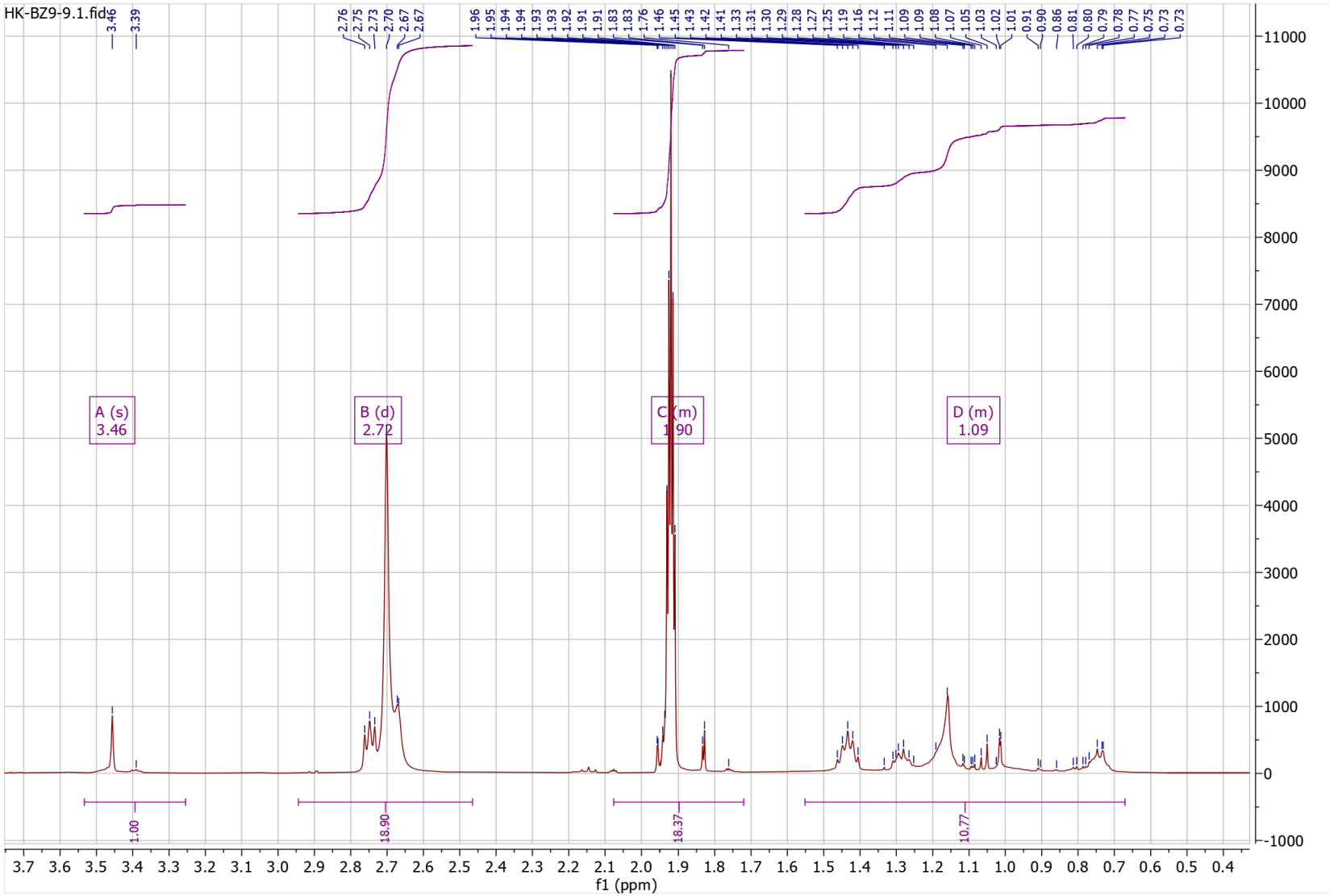

HK-ASP2.1.fid
5.28
5.27
2.89
2.07
2.06
2.06
2.05
2.05
G (d)
5.28
H (s)
2.89
I (p)
2.06
2.12
14.13
4.05
f1 (ppm)

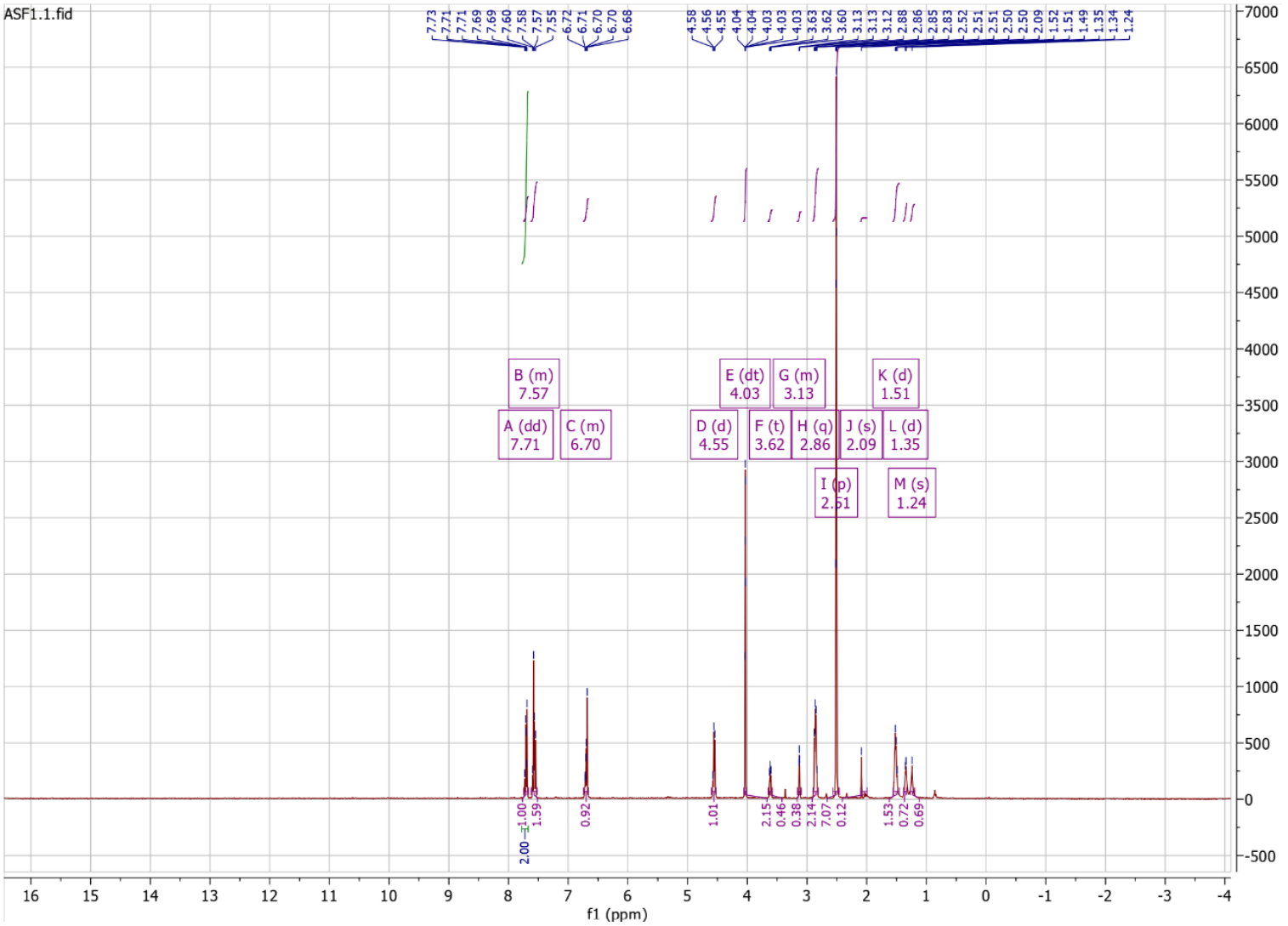
^{1}H NMR spectrum of DCP

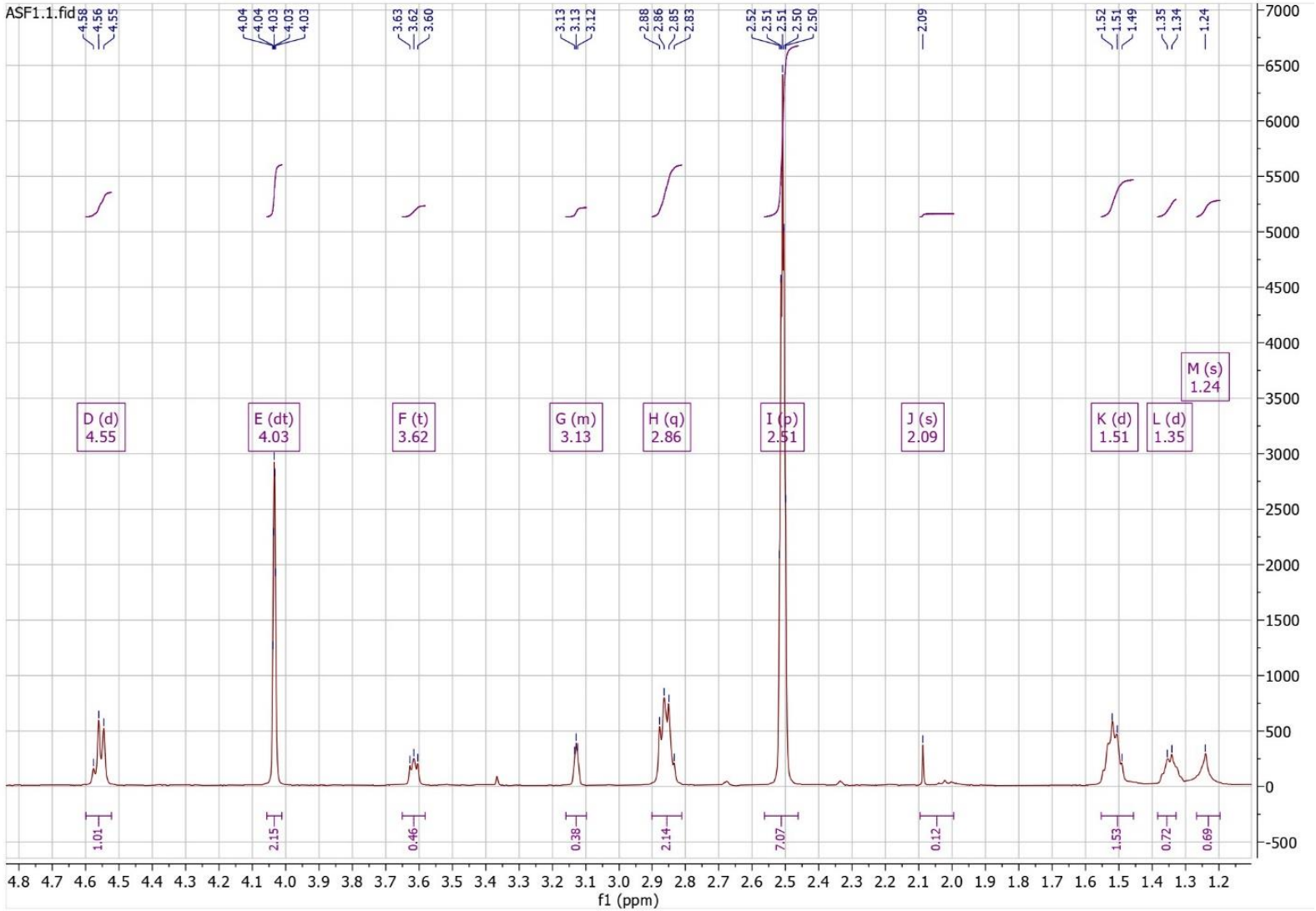


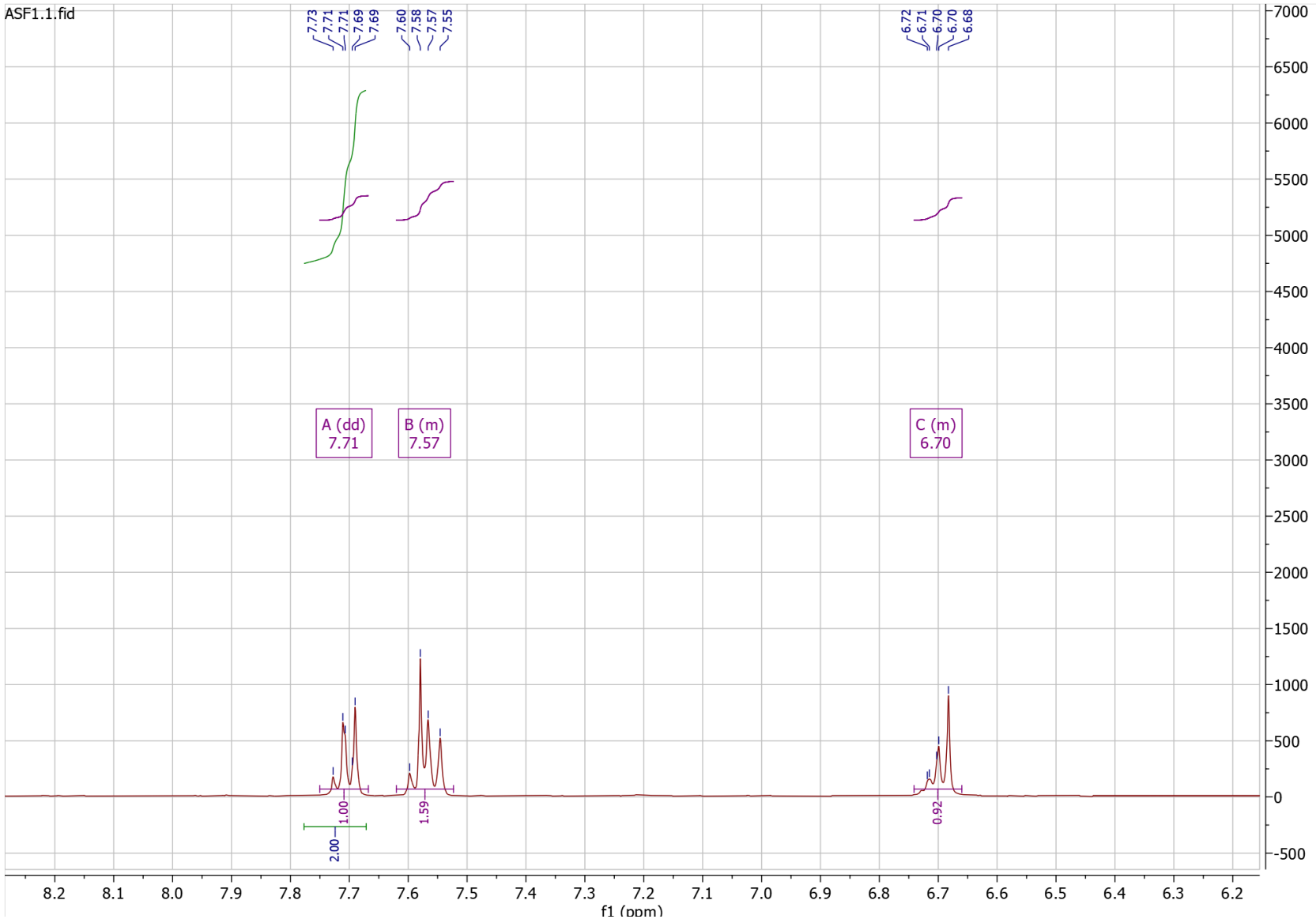

HK-BZ9-91.fid
A (m)
7.72
F (m)
7.64
B (m)
7.53
C (m)
7.40
D (dd)
7.17
E (d)
6.98
1.00
0.51
2.12
1.70
0.50
0.14
0.52
f1 (ppm)

^{1}H NMR spectrum of TCP




ASF1.1.fid
B (m) 7.57
A (dd) 7.71
C (m) 6.70
E (dt) 4.03
G (m) 3.13
K (d) 1.51
D (d) 4.55
F (t) 3.62
H (q) 2.86
J (s) 2.09
L (d) 1.35
I (p) 2.51
M (s) 1.24
f1 (ppm)


ASF1.1.fid
D (d) 4.55
E (dt) 4.03
F (t) 3.62
G (m) 3.13
H (q) 2.86
I (p) 2.51
J (s) 2.09
K (d) 1.51
L (d) 1.35
M (s) 1.24
f1 (ppm)


ASF1.1.fid
7.73
7.71
7.71
7.69
7.69
7.60
7.58
7.57
7.55
6.72
6.71
6.70
6.70
6.68
A (dd)
7.71
B (m)
7.57
C (m)
6.70
1.00
1.59
0.92
2.00
f1 (ppm)

Appendix E – pH Scouting

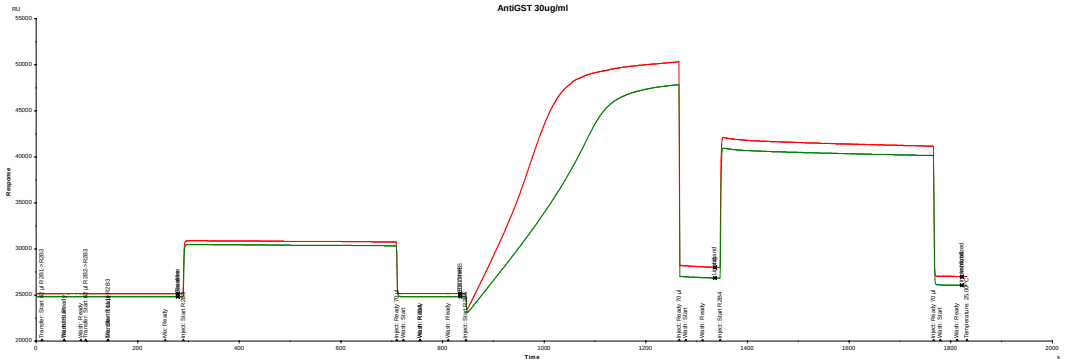


Figure 1: pH Scouting graph

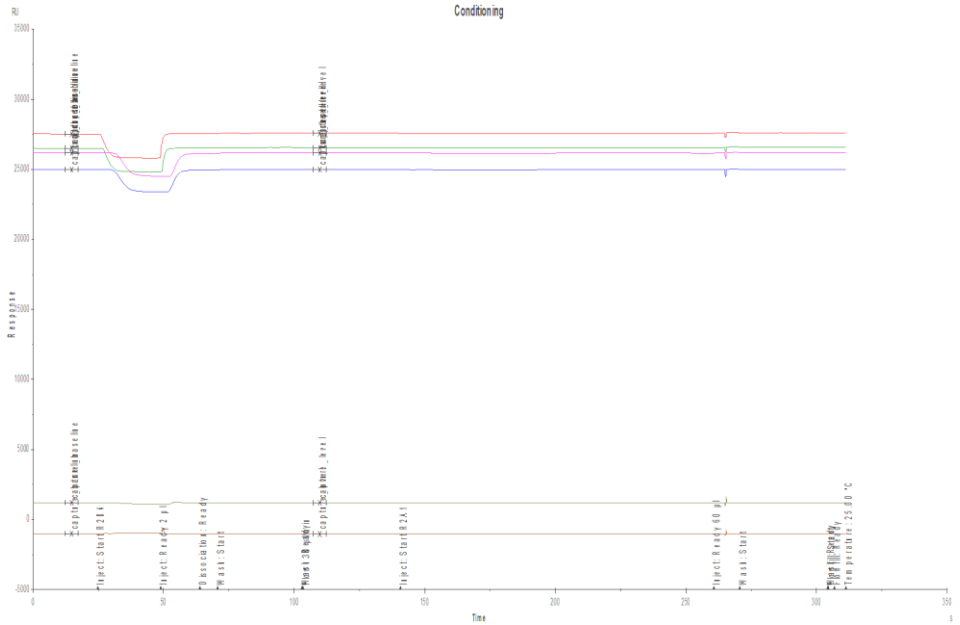


Figure 2: Graph showing Conditioning

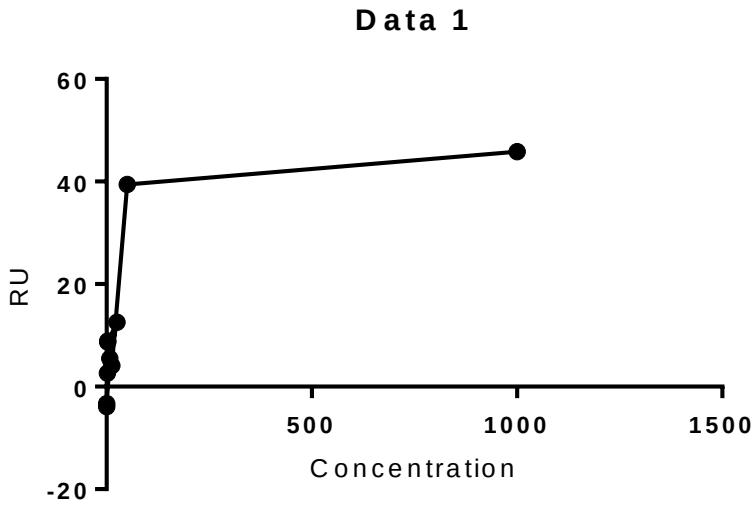


Figure 2: Data Concentration

Appendix F – SPR wizard parameters for single cell kinetics

<HtmlPreview>General settings

Temperature after run used	No
Sample compartment temperature	25°C
Sample compartment temperature varies	No
Data collection rate	10Hz
Concentration unit	nM
A	[No buffer name specified]
B	[No buffer name specified]
C	[No buffer name specified]
D	[No buffer name specified]
Detection	Multi
Flow path	2-1,4-3

Cycle Types

GST kinetics

GST conditioning

Commands in cycle type GST kinetics

Capture 1

Capture solution	GST
Contact time (s)	180
Flow rate (µl/min)	5
Flow path	1

Capture 2

Capture solution	GST-CDK2
Contact time (s)	180

Flow rate (µl/min)	5
Flow path	2
Stabilization period (s)	180

Capture 3

Capture solution	GST
Contact time (s)	60
Flow rate (µl/min)	10
Flow path	3

Capture 4

Capture solution	GST-hcv
Contact time (s)	60
Flow rate (µl/min)	10
Flow path	4
Stabilization period (s)	180

Sample 1

Type	Single Cycle Kinetics
Sample solution	Is Variable
Contact time (s)	120
Dissociation time (s)	600
Flow rate (µl/min)	30
Flow path	1,2,3,4
Extra wash solution	50% DMSO
MW	Is variable
Conc (1)	Is variable
Conc (2)	Is variable
Conc (3)	Is variable
Conc (4)	Is variable
Conc (5)	Is variable

Regeneration 1

Regeneration solution		Reg solution
Contact time (s)		120
Flow rate (µl/min)		30
Flow path	1,2,3,4	
High viscosity	No	

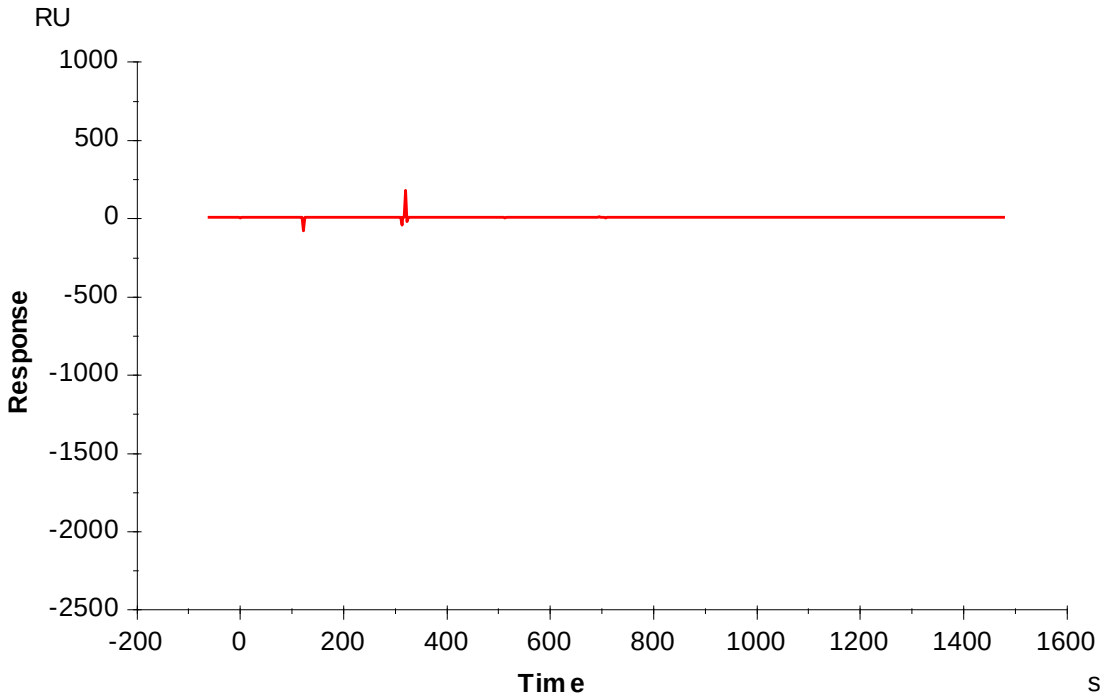


Figure 3: Response versus time graph

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