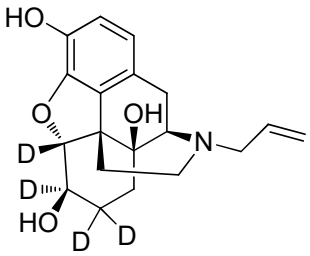
Certificate of Analysis

Issued: January 20th, 2022
Re-Test: January 20th, 2025

Compound Name	β-Naloxol labeled d_4
Potency	920 µg of β-Naloxol labeled d_4 per mg
Physical Description	Off-white solid
Chemtos Lot Number	C5-168-093
Certificate Number	C5-168-093
Chemical Structure	
Empirical Formula	$C_{19}H_{19}D_4NO_4$
Molecular Weight	333.41
Exact Mass	333.19
Mass Spectrometry	Electrospray MS(ES+): m/z 334.2 $(M+H)^+$ Data consistent to that of the title compound
Isotopic Purity	The following table gives the normalized isotopic mass distribution, including the naturally occurring higher isotopic masses.

Abundance	m/z		Abundance	m/z
Undetected	330.2 (Unlabeled)		31.9	335.0 (+5)
Undetected	331.2 (+1)		3.5	336.2 (+6)
1.7	332.2 (+2)		0.5	336.8
24.4	333.2 (+3)		0.5	337.2 (+7)
100.0	**334.2 (+4)**			

HPLC Purity
The product was examined by analytical HPLC using a diode array detector. Column: Chromolith Performance C_{18} 4.6 mm X 100 mm; Flow Rate: 1 ml/min; Solvents: Water (0.1% TFA) and acetonitrile; Gradient: 5% to 100% acetonitrile over 20 min, return to 95% water over 10 minutes. The chromatogram used for purity and homogeneity assessment was the summed absorbance between 210 nm and 230 nm. Purity was determined as the area percent of the major peak after integration of any impurities judged to be authentic by the analyst. Using this method the purity was determined at **99.6%**.

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Quantitative ^{1}H NMR

Proton Nuclear magnetic resonance spectra were acquired in DMSO-d_6 at 500 MHz. An internal reference standard 2,3,5,6-Tetrachloronitrobenzene was utilized to obtain a quantitative NMR potency value. A weighed quantity of β-Naloxol labeled d_4 and 2,3,5,6-Tetrachloronitrobenzene were dissolved in DMSO-d_6 and the proton spectrum was obtained. The integration value of single proton resonance of 2,3,5,6-Tetrachloronitrobenzene at ^{1}H δ 8.5 ppm was compared to the integration value of β-Naloxol labeled d_4 proton resonances from ^{1}H δ 6.7 to ^{1}H δ 6.4 ppm. The peaks were normalized based on calculated moles and predetermined purity value for 2,3,5,6-Tetrachloronitrobenzene. NMR analysis was performed twice and potency was determined as the summed average of the two measurements.

Method	Experiment No	Potency (%)	Average potency (%)
Quantitative ^{1}H NMR	C5-168-093-1	91.99	92.0
	C5-168-093-2	92.01	

Using this method, the potency was determined at **92.0%**. The NMR data appears to be consistent with the molecular structure expected for this compound.

Storage Conditions

- Samples should be stored in an air tight vial.
- Samples should be stored at ≤ 0˚ Celsius and minimize contact with moisture.

Caution

This information is provided as an indication of the quality of the underlying material when examined by a specific technique. The reported values are subject to normal experimental error and should be treated as estimates. The absence of undetected impurities cannot be guaranteed by this or any other general approach and this certificate does not certify the absence of such substances in the sample.

Intended Use

This analysis report does not qualify compound use in humans. It may be pharmaceutically unrefined, may contain uncharacterized toxic impurities, and has not been adequately analyzed to assure safe use in humans. Responsibility for proper use of this compound and compliance with all federal laws rests solely with the user.

GLP Compliance

These analyses were performed and reviewed in compliance with the applicable requirements of 21 CFR 58 pertaining to an analytical chemistry laboratory supporting a study under Good Laboratory Practices. Raw data has been archived at Chemtos' Texas facility.

Preparer	Amrutha. Digitally signed by Amrutha Mekala Date: 2022.01.20 16:21:23 -06'00'	
Analytical Review and Approval	Tesfay Tesfatsion 2022.01.20 16:21:55 -06'00'	
QA Review	Digitally signed by Khalid Thakur Date: 2022.01.20 16:34:49 -06'00'	

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Quantitative NMR	Weighed amount (mg)	Ref Std CoA Potency	Dry weight (mg)	MW	mmoles	Normalized mmoles		integration value	Protons	Integration/ proton	Normalized
2,3,5,6-Tetrachloronitrobenzene (98.3%)	1.10	98.3%	1.0775	260.89	0.0041	1		100.00	1	100.00	1
β-Naloxol labeled d4(as free base)	1.01			333.41	0.0030	0.736		135.39	2	67.70	0.6770
C5-168-093											
Calculated potency	91.99%										

	Empty vial (mg)	**Vial+sample(mg)**	**Sample+ref std (mg)**	**Sample (mg)**	**Ref Std (mg)**
	2730.12	2731.14	2732.24		
	2730.12	2731.14	2732.22		
	2730.12	2731.12	2732.23		
Average	2730.12	2731.13	2732.23	1.01	1.10

Quantitative NMR	Weighed amount (mg)	Ref Std CoA Potency	Dry weight (mg)	MW	mmoles	Normalized mmoles		integration value	Protons	Integration/ proton	Normalized
2,3,5,6-Tetrachloronitrobenzene (98.3%)	1.30	98.3%	1.2772	260.89	0.0049	1		100.00	1	100.00	1
β-Naloxol labeled d4(as free base)	0.97			333.41	0.0029	0.594		109.36	2	54.68	0.5468
C5-168-093											
Calculated potency	92.01%										

	Empty vial (mg)	**Vial+sample(mg)**	**Sample+ref std (mg)**	**Sample (mg)**	**Ref Std (mg)**
	2779.34	2780.31	2781.61		
	2779.35	2780.32	2781.61		
	2779.35	2780.32	2781.63		
Average	2779.35	2780.32	2781.62	0.97	1.30

Data File Z:\1102\1103200407.D
Sample Name: C5-168-093 b

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Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 1/19/2022 4:02:39 PM
                                                 Inj Volume : 5.0 µl
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Last changed    : 1/19/2022 4:01:39 PM by ABM
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\DEF_LC.M
Last changed    : 1/20/2022 10:49:27 AM
                  (modified after loading)
Additional Info : Peak(s) manually integrated
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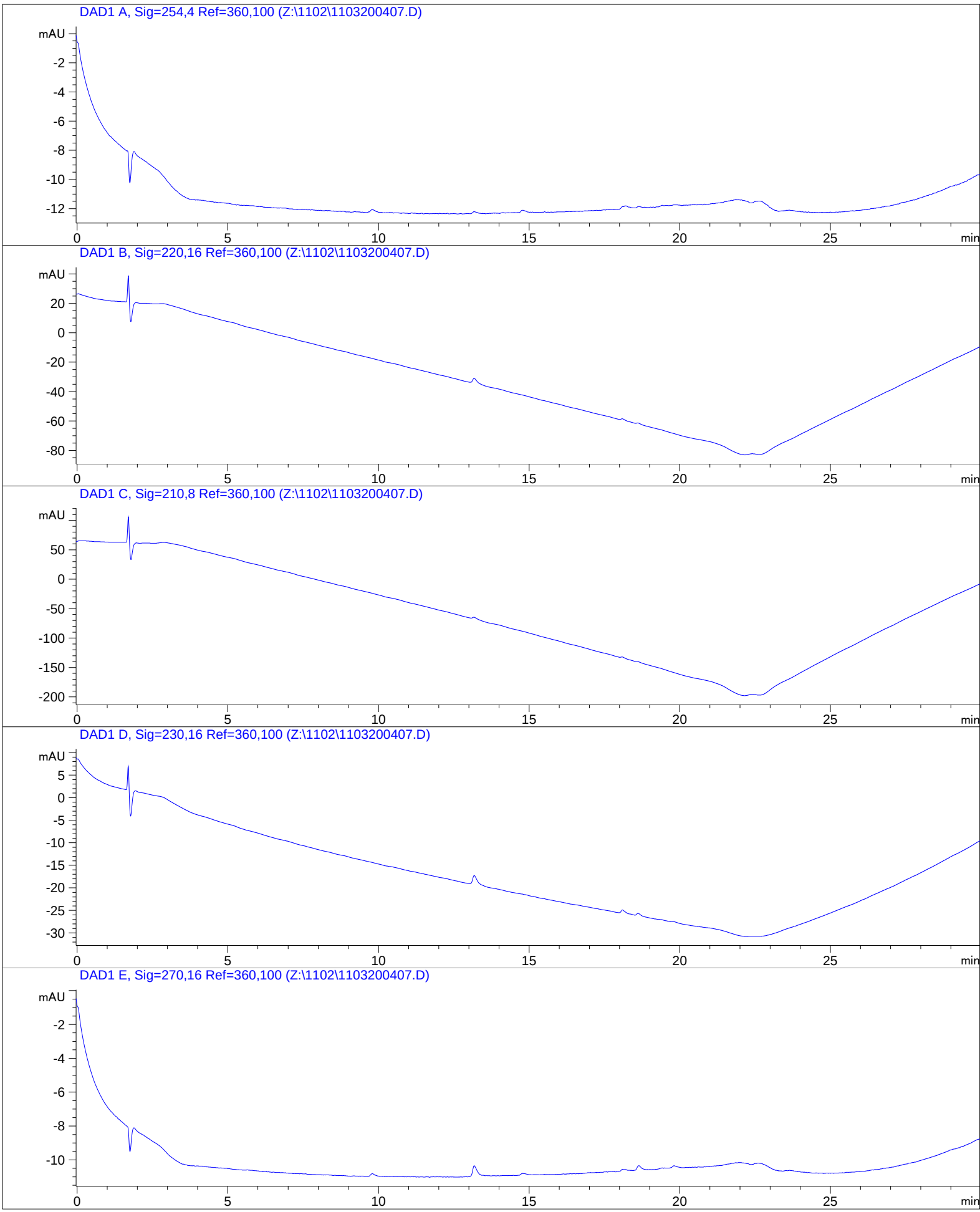
Data File Z:\1102\1103200407.D
Sample Name: C5-168-093 b



Data File Z:\1102\1103200407.D
Sample Name: C5-168-093 b

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Area Percent Report

==========

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

==========

*** End of Report ***

Data File Z:\1102\1103200408.D
Sample Name: C5-168-093

```
   ==========================================================================
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   Acq. Instrument : Instrument 1                    Location : Vial 1
   Injection Date  : 1/19/2022 4:38:44 PM
                                                  Inj Volume : 5.0 µl
   Acq. Method     : C:\CHEM32\1\METHODS\CHEMTOS_LCMS.M
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                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\DEF_LC.M
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                     (modified after loading)
   Additional Info : Peak(s) manually integrated
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Data File Z:\1102\1103200408.D
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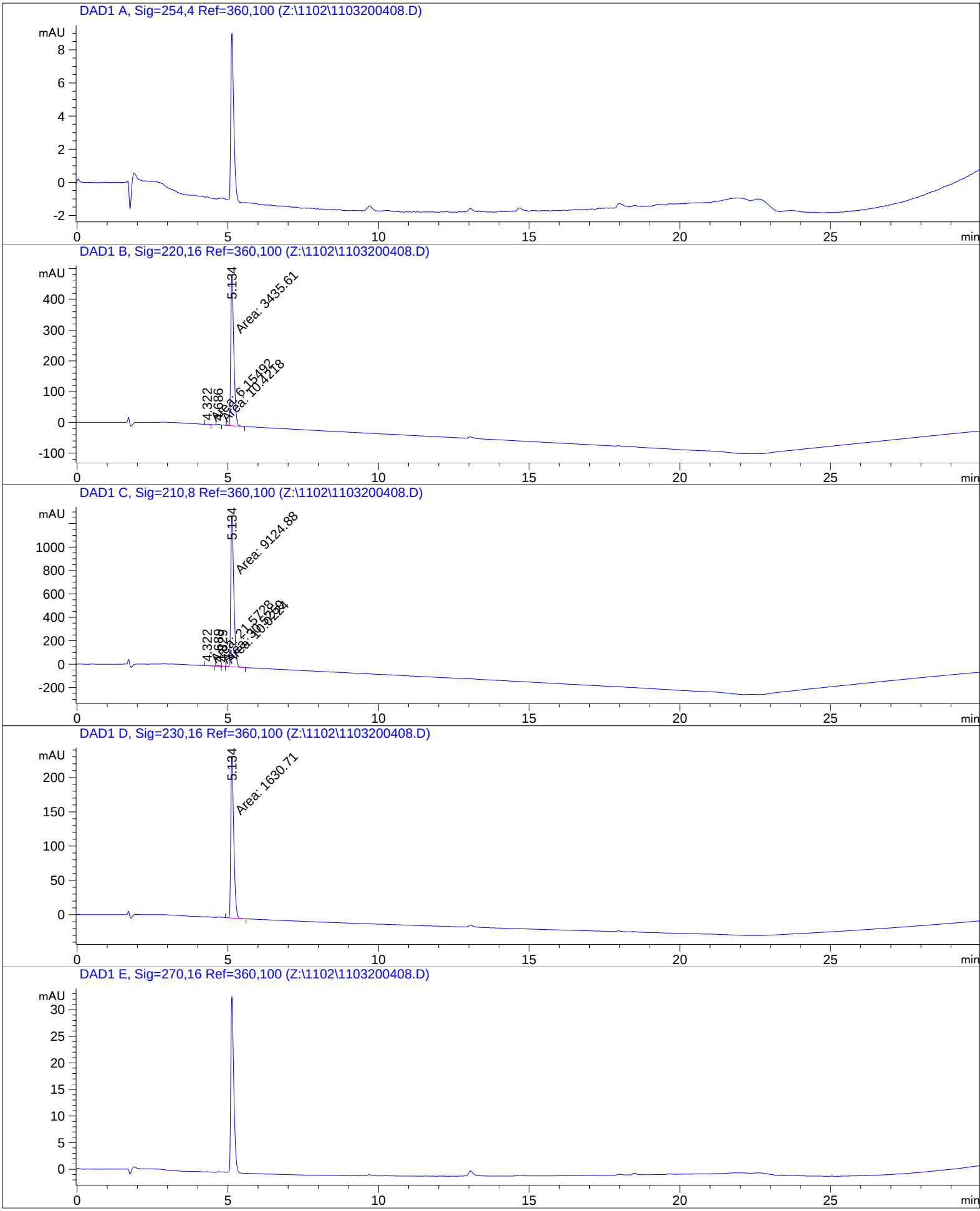

Data File Z:\1102\1103200408.D
Sample Name: C5-168-093

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Signal 2: DAD1 B, Sig=220,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.322	MM	0.0985	6.15492	1.04128	0.1783
2	4.686	MM	0.0831	10.42175	2.08969	0.3019
3	5.134	MM	0.1168	3435.61353	490.43970	99.5198
Totals :				3452.19020	493.57066	

Signal 3: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.322	MM	0.1411	21.57277	2.54754	0.2348
2	4.689	MF	0.0917	30.52593	5.54529	0.3323
3	4.829	FM	0.0718	10.02243	2.32658	0.1091
4	5.134	MM	0.1178	9124.87500	1291.17957	99.3238
Totals :				9186.99614	1301.59897	

Signal 4: DAD1 D, Sig=230,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.134	MM	0.1154	1630.71082	235.43799	100.0000
Totals :				1630.71082	235.43799	

Signal 5: DAD1 E, Sig=270,16 Ref=360,100

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*** End of Report ***

Data File Z:\1102\1103200411.D
Sample Name: C5-168-093

```
   ==========================================================================
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   Acq. Instrument : Instrument 1                    Location : Vial 1
   Injection Date  : 1/20/2022 12:00:55 PM                Inj :   1
                                                  Inj Volume : 5.0 µl
   Acq. Method     : C:\CHEM32\1\METHODS\CHEMTOS_LCMS.M
   Last changed    : 1/20/2022 11:59:56 AM by ABM
                     (modified after loading)
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   Last changed    : 1/20/2022 12:22:21 PM
                     (modified after loading)
   Additional Info : Peak(s) manually integrated
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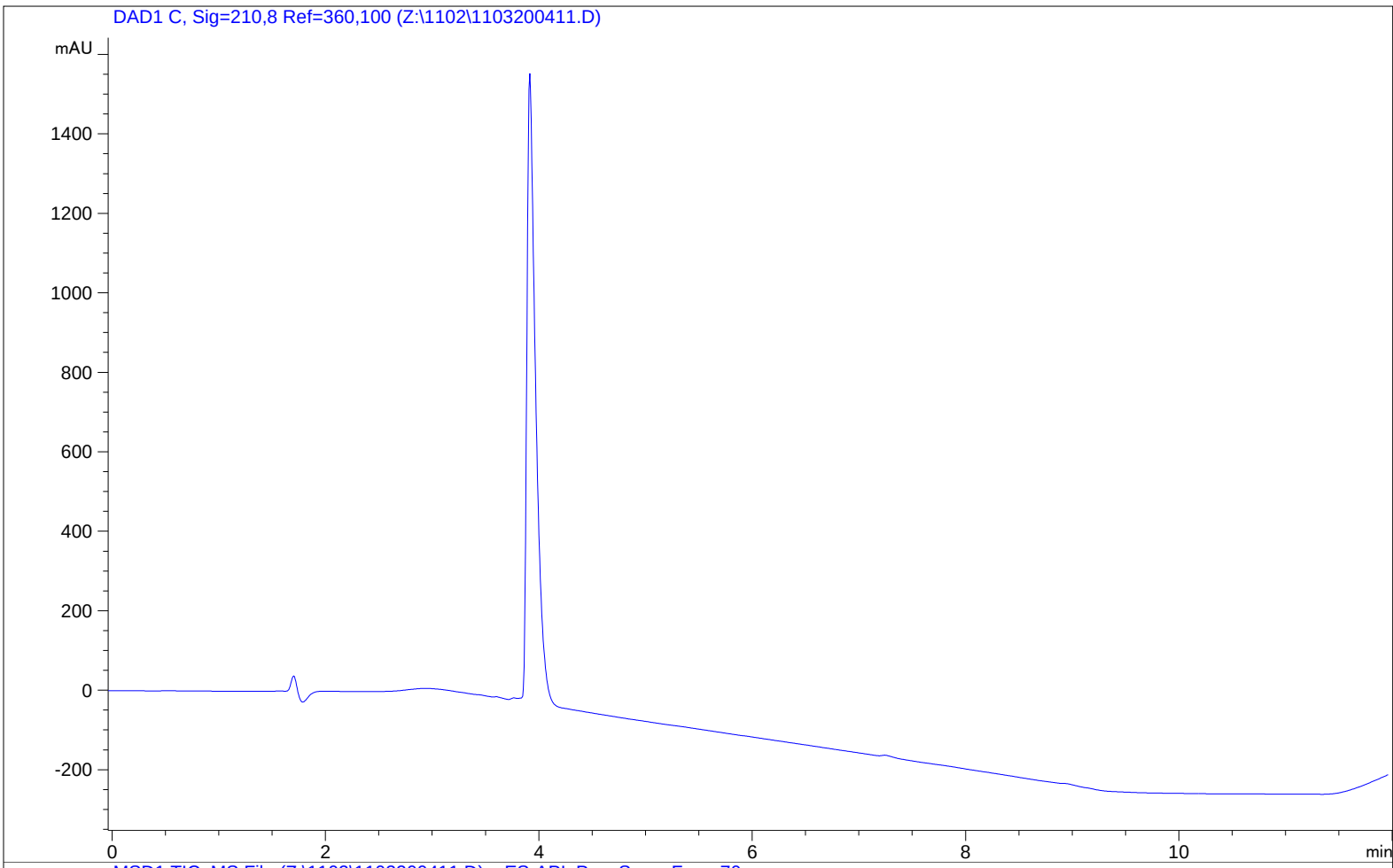
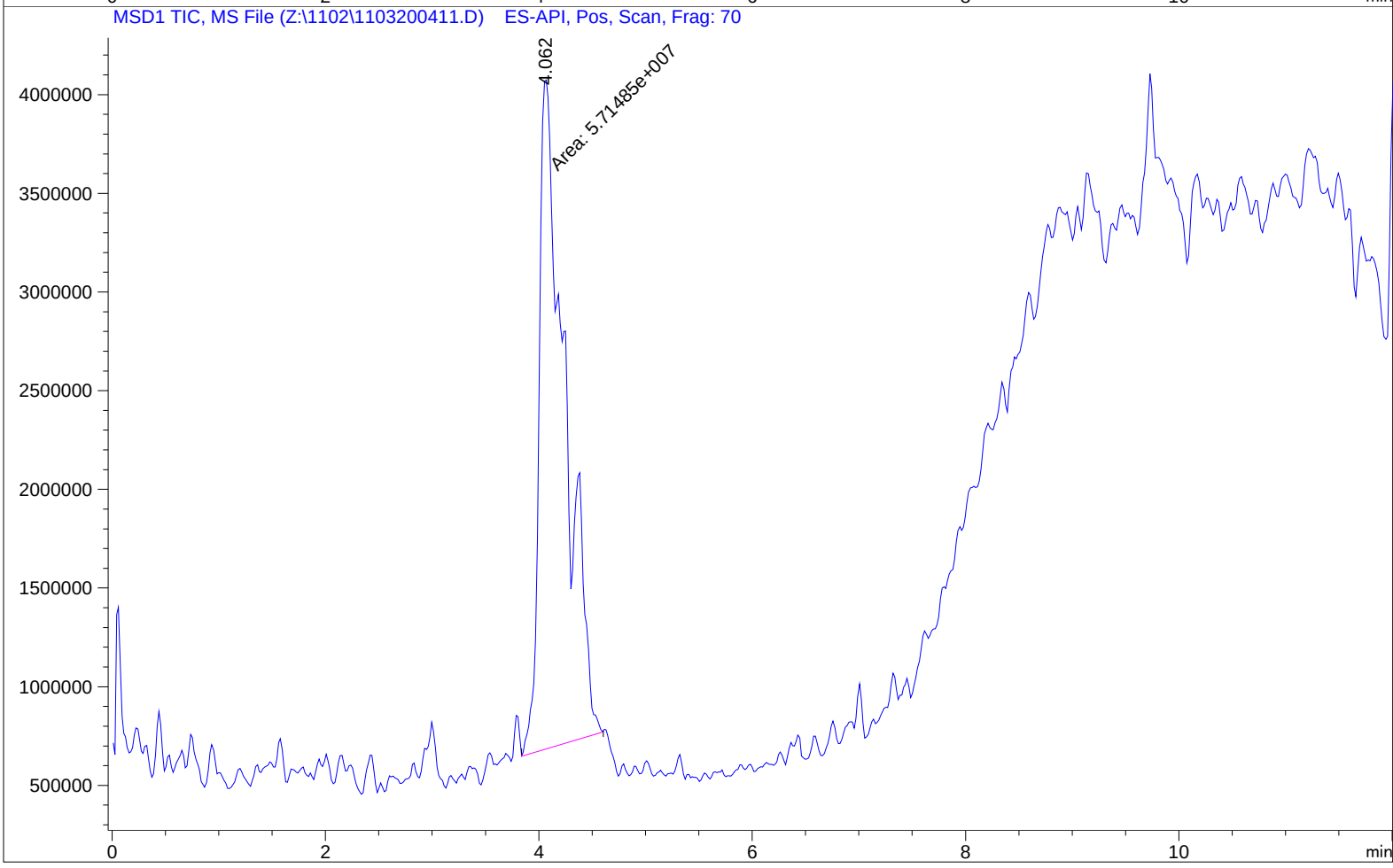
Data File Z:\1102\1103200411.D
Sample Name: C5-168-093



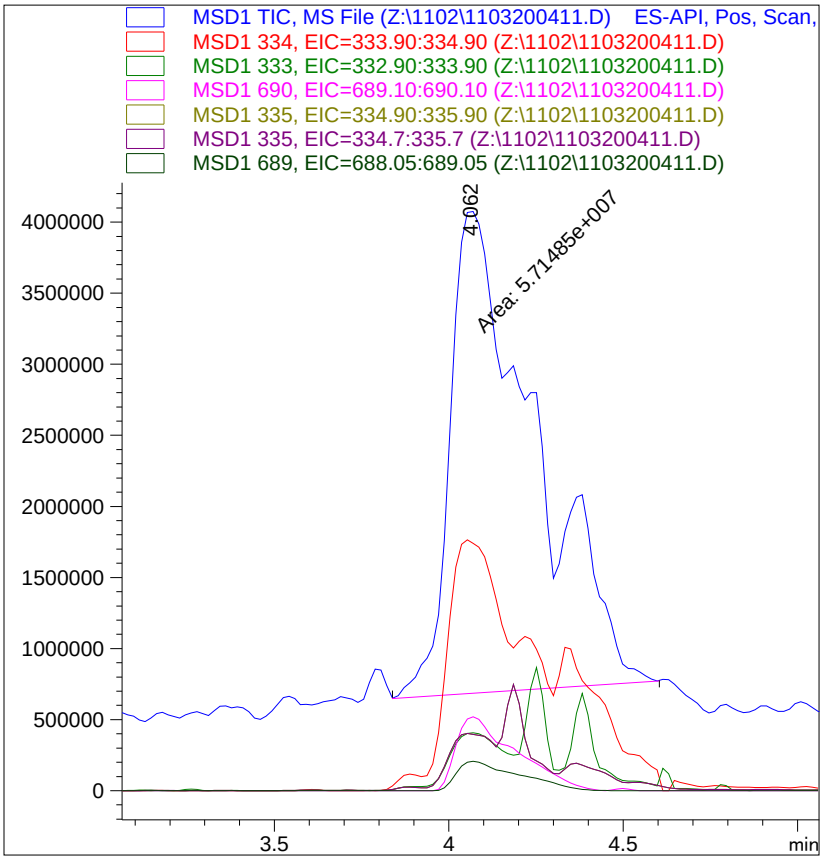


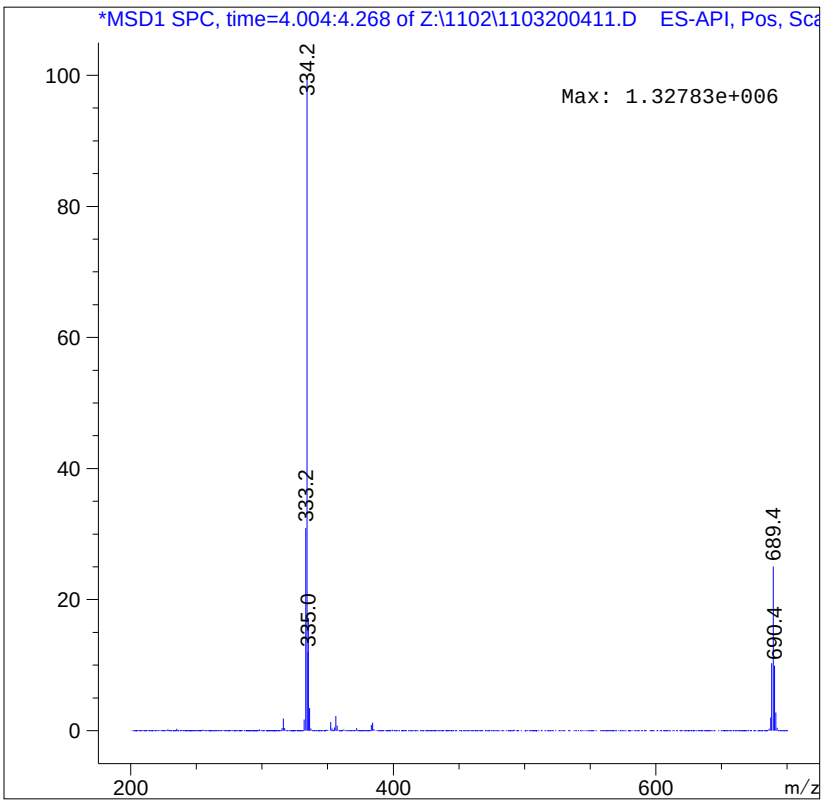
Data File Z:\1102\1103200411.D
Sample Name: C5-168-093

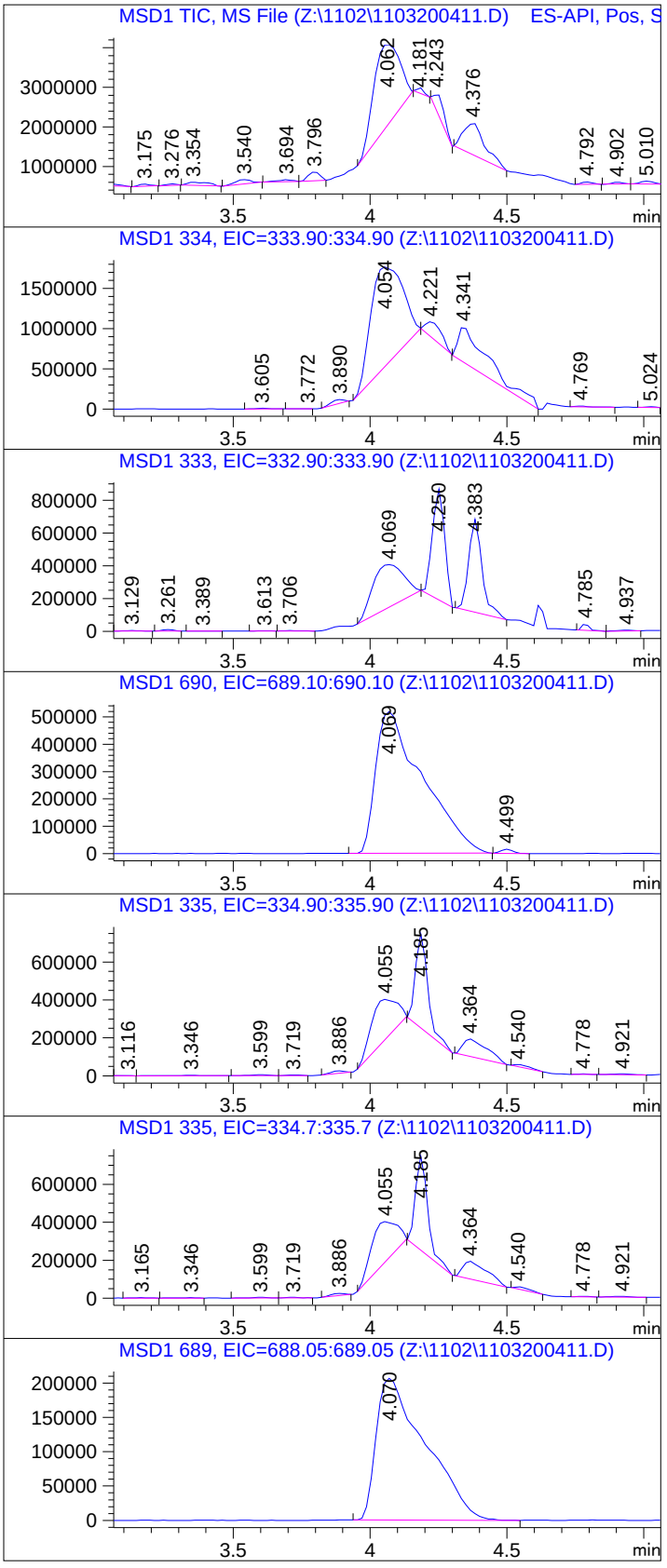
Spectra averaged over upper half of peaks.
Number of ions per peak: 6
Display Time Range(+- mins): 1.0

Data File Z:\1102\1103200411.D
Sample Name: C5-168-093

DataPath : Z:\1102\1103200411.D
DateTime: 20 Jan 22 12:01 pm -0600
Operator : ABM Vial : 1
Sample : C5-168-093
Retention : 4.062 minutes

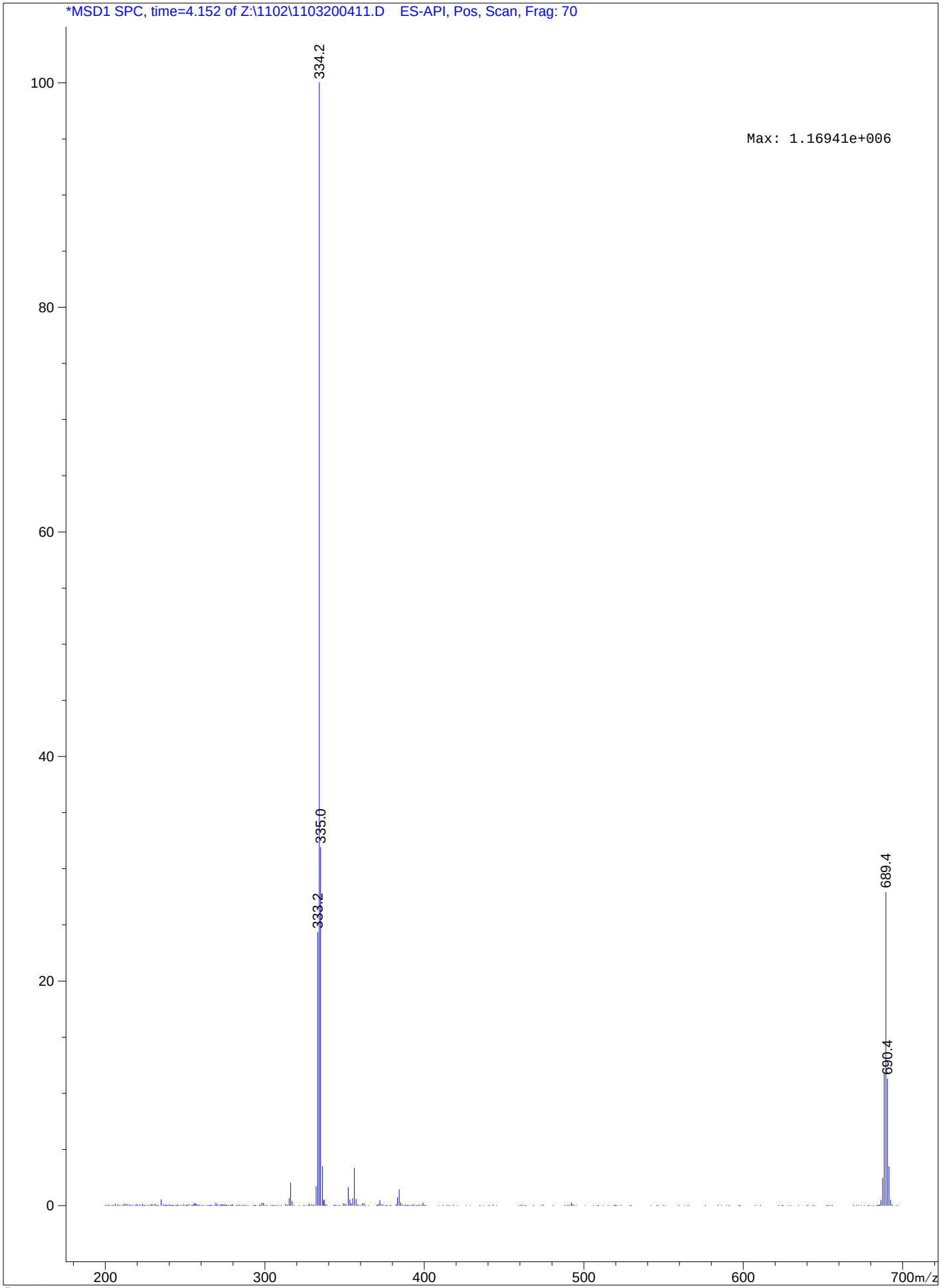






*** End of Report ***

MS Spectrum

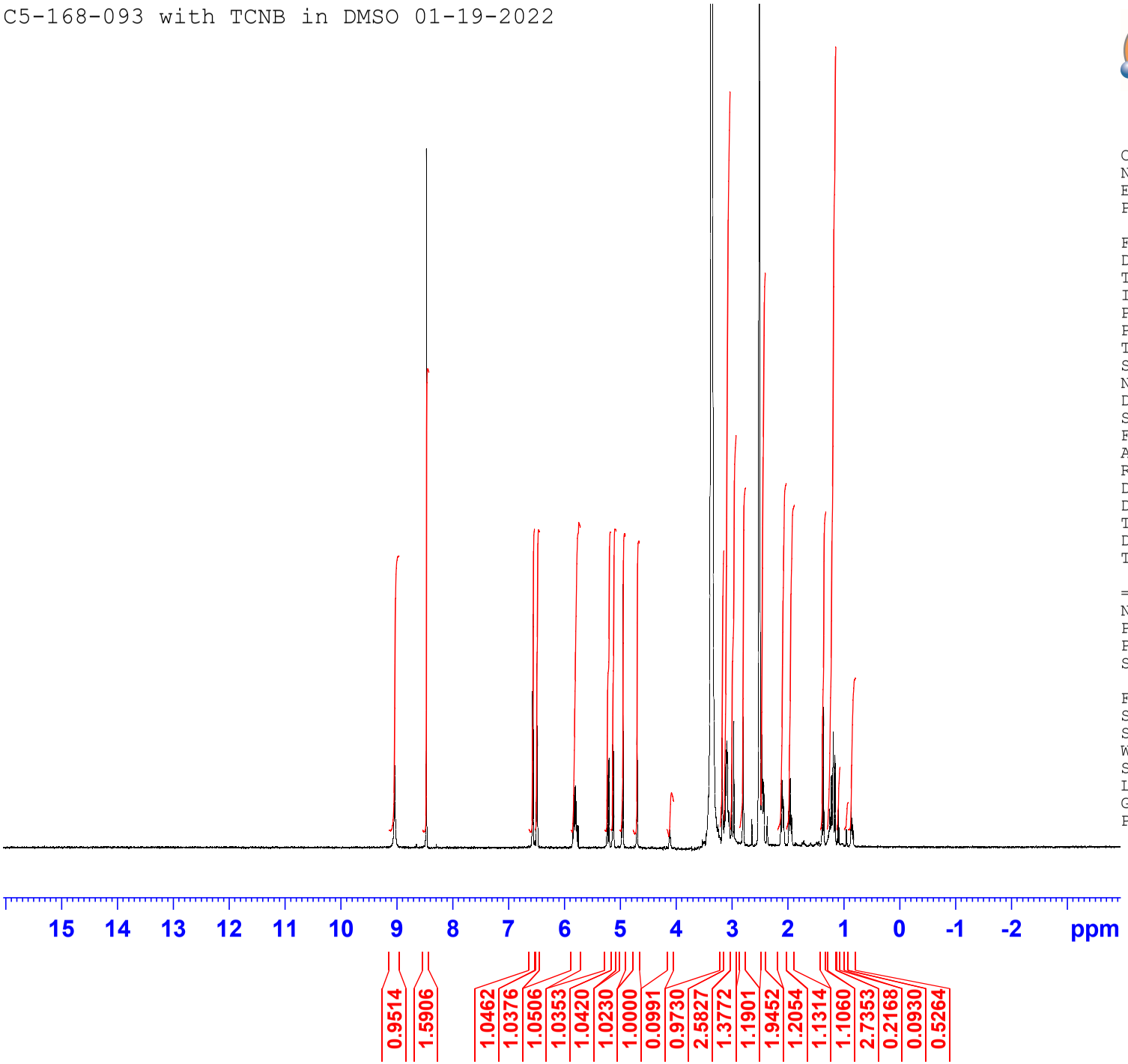

*MSD1 SPC, time=4.152 of Z:\1102\1103200411.D ES-API, Pos, Scan, Frag: 70
Max: 1.16941e+006
334.2
335.0
333.2
689.4
690.4
100
80
60
40
20
0
200
300
400
500
600
700m/z

MSD1 SPC, scan=252, time=4.152 of 1103200411.D

m/z	Abundance	m/z	Abundance	m/z	Abundance	m/z	Abundance
200.10	0.1	254.90	0.1	313.00	0.2	385.20	0.3
201.10	0.0	255.80	0.2	314.00	0.1	386.30	0.1
202.00	0.1	256.40	0.2	315.30	0.7	387.20	0.0
202.90	0.0	257.00	0.1	316.20	2.0	388.10	0.1
204.20	0.0	257.90	0.1	317.10	0.4	389.00	0.0
205.10	0.1	258.70	0.0	318.30	0.0	389.50	0.0
206.30	0.2	259.30	0.0	321.80	0.0	390.00	0.0
207.30	0.0	260.20	0.0	324.20	0.0	390.40	0.0
208.00	0.1	261.20	0.0	325.10	0.0	391.30	0.0
208.90	0.0	262.30	0.0	327.10	0.0	392.30	0.0
210.10	0.0	264.20	0.0	327.80	0.1	393.10	0.1
211.20	0.1	265.00	0.0	328.20	0.0	394.10	0.0
212.20	0.2	265.40	0.0	329.40	0.1	395.00	0.0
213.00	0.1	266.20	0.1	330.40	0.1	396.10	0.0
213.90	0.1	266.90	0.0	330.80	0.1	397.10	0.1
215.30	0.1	268.20	0.1	332.20	1.7	398.30	0.1
216.00	0.0	269.20	0.2	333.20	24.4	399.20	0.3
217.20	0.1	270.00	0.1	334.20	100.0	400.00	0.1
218.40	0.0	271.10	0.1	335.00	31.9	400.40	0.0
219.30	0.1	272.20	0.1	336.20	3.5	401.60	0.0
220.10	0.1	272.90	0.1	336.80	0.5	408.90	0.0
221.30	0.1	273.40	0.1	337.20	0.5	411.70	0.1
221.80	0.0	273.80	0.1	338.20	0.1	414.20	0.0
223.20	0.2	274.40	0.1	338.90	0.0	415.20	0.0
223.90	0.0	275.10	0.0	343.40	0.0	416.10	0.0
224.30	0.0	275.80	0.0	343.80	0.1	418.30	0.1
225.00	0.0	276.20	0.1	344.20	0.0	420.50	0.0
226.20	0.0	277.30	0.0	345.00	0.0	426.30	0.0
227.10	0.1	278.20	0.0	346.30	0.0	428.90	0.0
228.30	0.1	279.30	0.1	347.10	0.0	434.60	0.0
229.10	0.1	279.90	0.1	349.20	0.2	437.20	0.0
230.10	0.1	282.10	0.0	350.20	0.2	440.20	0.0
231.10	0.1	282.60	0.0	351.10	0.1	441.00	0.0
232.00	0.0	283.30	0.0	352.30	1.6	443.20	0.1
232.90	0.0	284.20	0.1	353.30	0.5	445.10	0.0
233.30	0.0	285.40	0.0	354.10	0.2	459.20	0.0
235.00	0.5	286.20	0.0	355.10	0.6	460.20	0.0
236.30	0.1	287.10	0.1	356.20	3.4	461.10	0.1
237.30	0.0	287.50	0.0	357.20	0.6	462.10	0.0
237.80	0.0	288.40	0.0	358.20	0.1	463.40	0.0
238.20	0.1	289.40	0.0	359.00	0.0	468.20	0.0
239.10	0.1	292.90	0.0	359.90	0.0	473.30	0.0
240.10	0.1	293.50	0.1	361.10	0.2	474.40	0.1
240.70	0.0	294.10	0.0	362.20	0.2	480.70	0.0
241.40	0.0	294.50	0.0	363.10	0.1	488.20	0.0
242.00	0.0	296.30	0.0	365.20	0.0	490.00	0.0
242.40	0.0	297.00	0.1	370.10	0.0	490.90	0.1
242.90	0.0	298.10	0.2	370.50	0.1	492.20	0.3
243.90	0.0	299.10	0.2	371.20	0.2	493.00	0.1
244.70	0.0	300.00	0.0	372.20	0.5	494.20	0.0
245.30	0.1	301.30	0.1	373.00	0.1	495.10	0.0
246.20	0.0	303.60	0.0	374.10	0.1	500.90	0.0
247.20	0.0	304.40	0.0	375.30	0.0	506.00	0.0
247.90	0.1	305.00	0.0	375.90	0.0	508.20	0.0
249.20	0.1	305.40	0.0	376.90	0.0	509.00	0.0
250.10	0.0	306.30	0.1	378.40	0.0	512.30	0.0
251.10	0.1	307.10	0.0	379.10	0.0	515.30	0.0
251.60	0.0	308.00	0.0	382.30	0.1	518.70	0.0
252.50	0.1	308.90	0.0	383.20	0.7	519.20	0.0
254.10	0.0	310.30	0.1	384.20	1.4	519.80	0.0

m/z	Abundance	m/z	Abundance
520.30	0.0	692.50	0.5
521.10	0.0	693.40	0.1
523.40	0.1	696.10	0.0
529.00	0.0	697.40	0.0
529.70	0.0		
542.20	0.0		
545.60	0.0		
547.00	0.0		
550.20	0.0		
551.30	0.0		
559.80	0.1		
563.00	0.0		
565.20	0.0		
565.90	0.0		
576.10	0.0		
584.10	0.1		
586.30	0.0		
589.50	0.0		
590.90	0.0		
597.10	0.0		
597.50	0.0		
598.30	0.0		
607.10	0.0		
608.40	0.0		
610.60	0.0		
622.20	0.1		
624.30	0.0		
624.70	0.0		
627.90	0.0		
629.10	0.0		
630.20	0.0		
634.70	0.0		
639.60	0.0		
640.50	0.0		
643.30	0.0		
644.30	0.0		
652.30	0.0		
653.40	0.0		
654.20	0.0		
655.60	0.0		
669.30	0.0		
671.00	0.0		
672.40	0.0		
674.30	0.0		
675.90	0.0		
678.10	0.0		
678.70	0.0		
680.00	0.0		
681.40	0.0		
683.50	0.0		
684.10	0.0		
684.60	0.0		
685.10	0.1		
685.50	0.1		
686.40	0.5		
687.40	2.5		
688.40	11.8		
689.40	27.9		
690.40	11.3		
691.40	3.5		

C5-168-093 with TCNB in DMSO 01-19-2022



chemtos

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Current Data Parameters
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EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20220119
Time              14.02
INSTRUM          500DRX
PROBHD   5 mm PABBO BB/
PULPROG            zg60
TD                65536
SOLVENT            DMSO
NS                   32
DS                    0
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 80.6
DW               48.400 usec
DE                6.00 usec
TE                300.2 K
D1           60.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                10.00 usec
PL1                0.50 dB
SFO1        500.0030877 MHz

F2 - Processing parameters
SI                 65536
SF          500.0000000 MHz
WDW                   EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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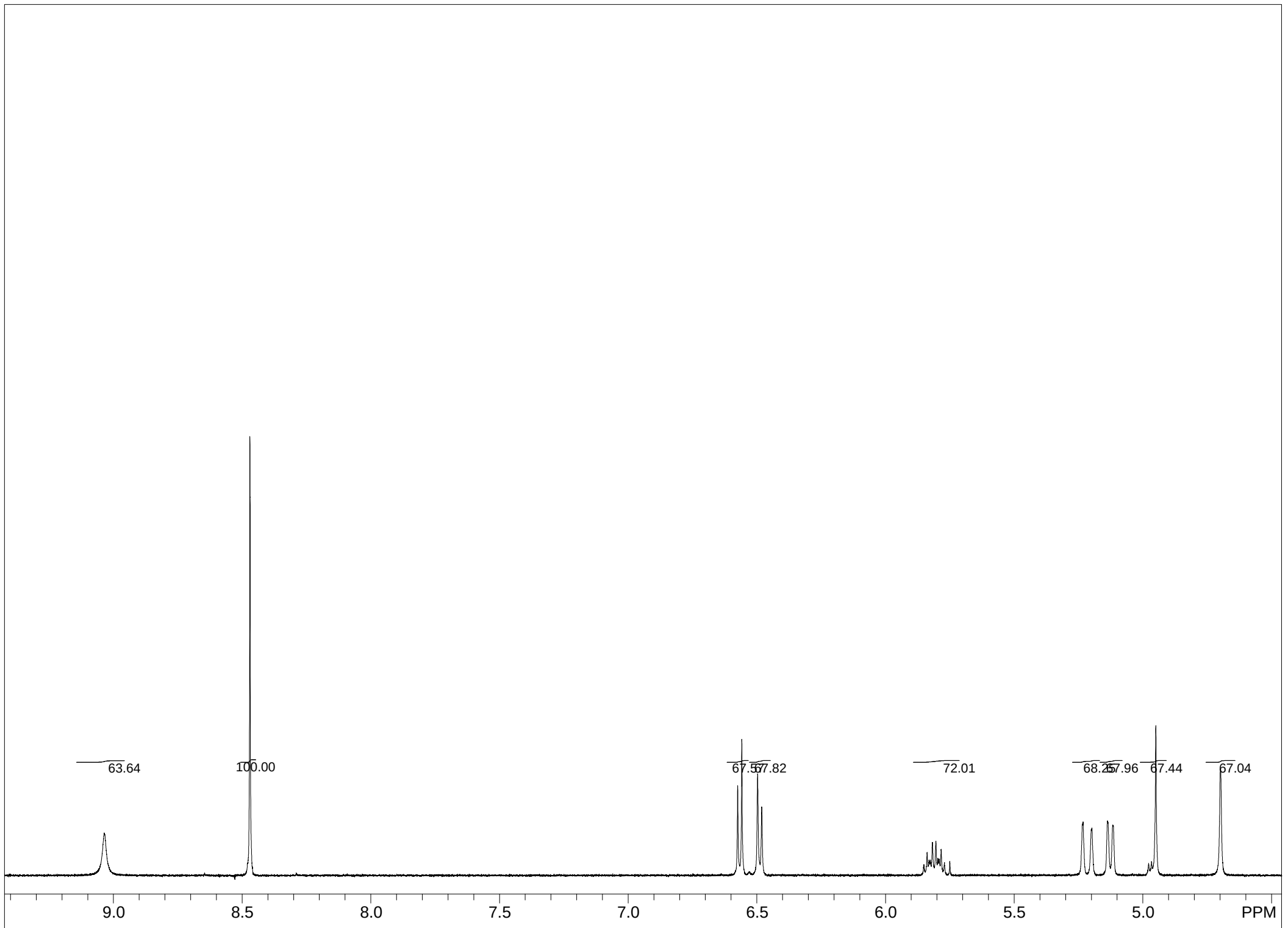

500DRX, DMSO,

USER: root -- DATE: Wed Jan 19 20:02:55 2022

F1: 500.003	F2: 1.000	SW1: 10331		OF1: 3087.8		PTS1d: 131072	
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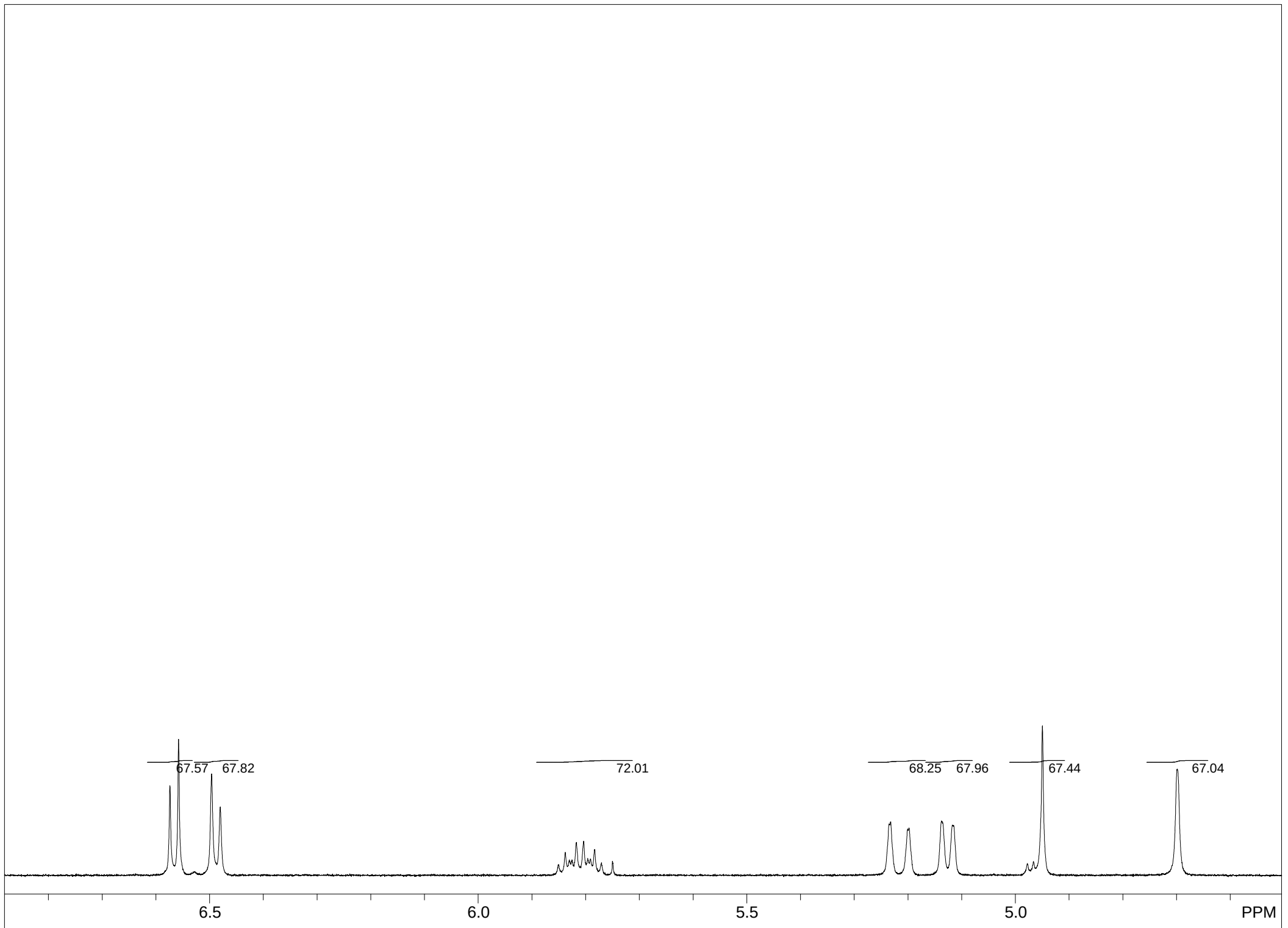
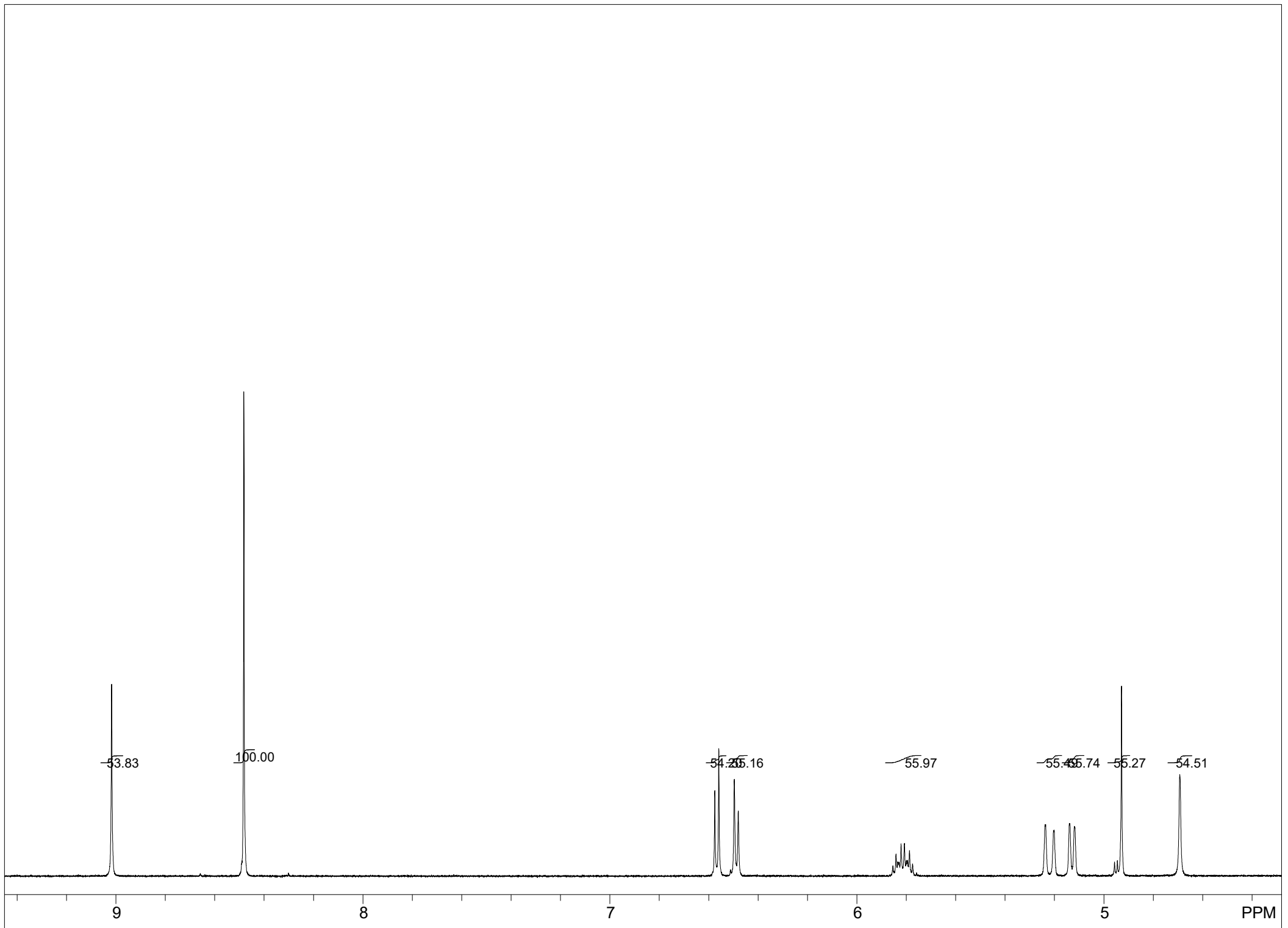


500DRX, DMSO,

USER: root -- DATE: Wed Jan 19 20:02:55 2022

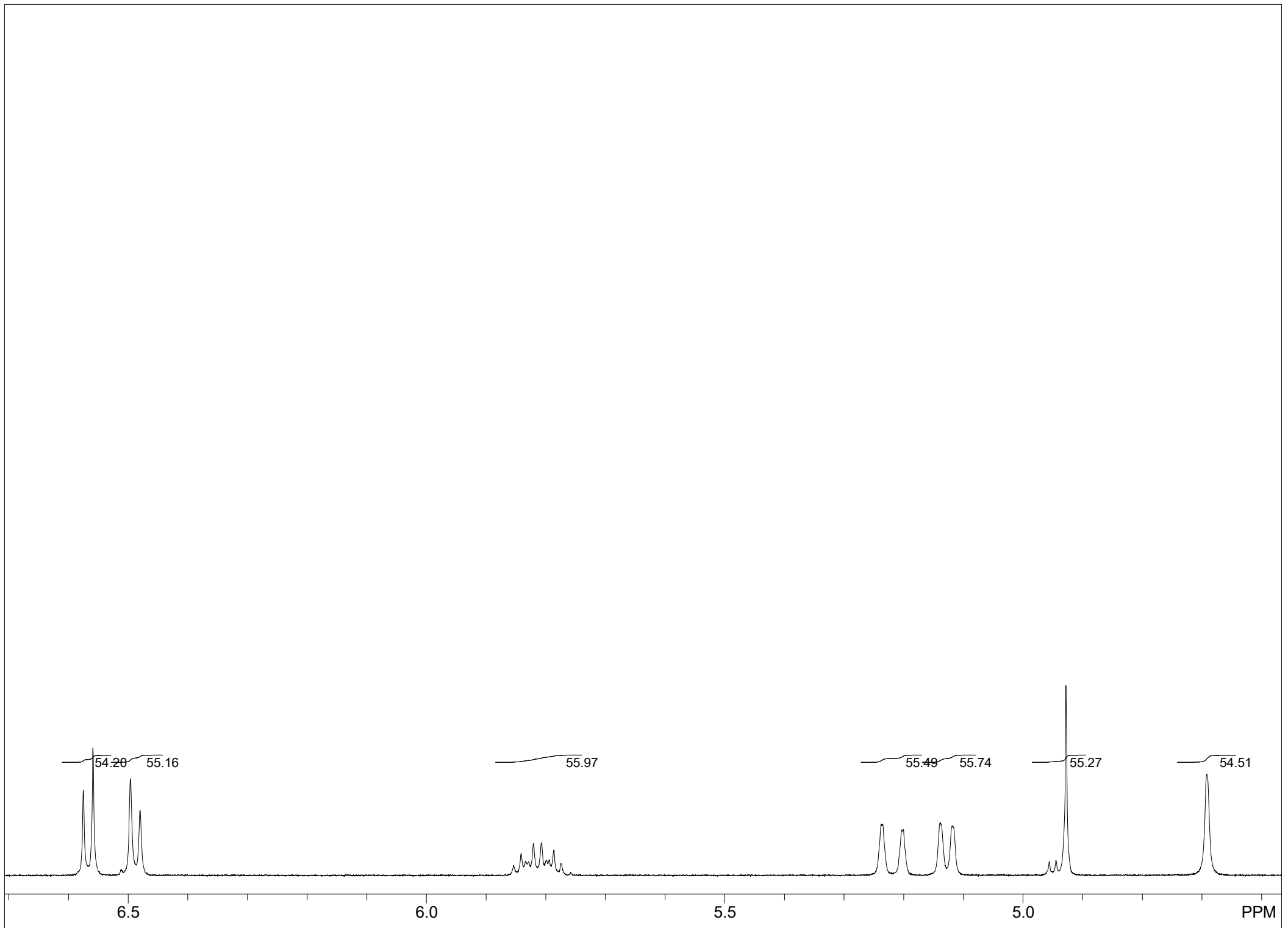
F1: 500.003	F2: 1.000	SW1: 10331		OF1: 3087.8		PTS1d: 131072	
EX: zg60		PW: 10.0 usec	PD: 0.0 sec	NA: 32	LB: 0.0	WinNuts - $C5-168-093.ft1	



500DRX, DMSO,

USER: root -- DATE: Wed Jan 19 22:43:44 2022

F1: 500.003	F2: 1.000	SW1: 10331		OF1: 3087.8		PTS1d: 65536	
EX: zg60		PW: 10.0 usec	PD: 60.0 sec	NA: 32	LB: 0.0		WinNuts - $C5-168-093



500DRX, DMSO,

USER: root -- DATE: Wed Jan 19 22:43:44 2022

F1: 500.003	F2: 1.000	SW1: 10331		OF1: 3087.8		PTS1d: 65536	
EX: zg60		PW: 10.0 usec	PD: 60.0 sec	NA: 32	LB: 0.0		WinNuts - $C5-168-093