Taiwo, BJ, Popoola, TD, van Heerden, FR and Fatokun, AA

Isolation and Characterisation of Two Quercetin Glucosides with Potent Anti-Reactive Oxygen Species (ROS) Activity and an Olean-12-en Triterpene Glucoside from the Fruit of Abelmoschus esculentus (L.) Moench

Spectroscopic data

Compound 1 (Olea-12-ene 3-*O*-β-D-glucopyranoside**): ^{1}H NMR (300 MHz, MeOD):** δH: 5.31 (1H, dd, *J*=2.6, 0.2 Hz, H-12), 4.22 (1H, d, *J*= 8.2 Hz, H-1'), 3.41 (1H, d, *J*=4.1 Hz, H-3), 3.17 (1H, H-6'), 3.11 (1H, d, *J*=9.2 Hz, H-3'), 3.04 (1H, t), 2.89 (1H, t), 2.08 (1H, s, H-1), 1.86-2.02 (2H, m, H-2) 1.66 (1H, dd, *J*=1.6, 6.8 Hz, H-22), 1.60 (2H, dd, *J*=), 1.46-1.58 (2H, m, H-6) 1.23 (3H, s, H-5), 1.00 (3H, s, H-27) 0.98 (3H, s, H-29), 0.96 (3H, s, H-24), 0.90 (3H, H-25), 0.85 (3H, s,), 0.82 (3H, d, *J*=1.9 Hz, H-23), 0.81 (3H, s, H-30), 0.78 (3H, s, H-26), 0.65 (3H, s, H-28). **^{13}C NMR (75 MHz, DMSO-d6)**: δ: 140.9 (C-13), 121.7 (C-12), 101.3 (C-1'), 77.3 (C-3), 77.3 (C-2'), 77.3 (C-4'), 73.9 (C-5'), 70.6 (C-3'), 61.6 (C-6'), 55.9 (C-5), 50.1 (C-18), 49.1 (C-17), 45.6 (C-9), 42.3 (C-14), 40.2 (C-4), 39.7 (C-8), 39.4 (C-20), 39.2 (C-19), 38.8 (C-1), 36.9 (C-10), 35.9 (C-22), 31.9 (C-7), 29.7 (C-21), 29.1 (C-15), 25.9 (C-23), 25.9 (C-2), 23.1 (C-16), 23.1 (C-27), 21.1 (C-30), 19.6 (C-28), 19.6 (C-6), 19.4 (C-29), 19.1 (C-26), 12.3 (C-24), 12.1 (C-25). **TOF ES MS** *m/z* 1176.1453 $[2M]^{+}$.

Compound 2 (isoquercitrin**)**: **^{1}H NMR (300 MHz, MeOD)**: δ 7.73 (1H, d, J=2.1 Hz), 7.60 (1H, dd, J= 8.5, 2.1 Hz), 6.88 (1H, d, J=8.6 Hz), 6.41 (1H, d, J=2.1 Hz), 6.22 (1H, d, J=2.1 Hz), 5.250 (d, 1H, J = 7.6 Hz). **^{13}C NMR (75 MHz, MeOD)**: δ 179.5 (C-4), 166.0 (C-7), 163.0 (C-5), 159.0 (C-2), 158.5 (C-9), 149.9 (C-3′), 145.9 (C-4′), 135.6 (C-3), 123.2 (C-6′), 123.1 (C-1′), 117.6 (C-2′), 116.1 (C-5′), 105.7 (C-10), 104.3 (C-1″), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5″), 78.1 (C-3″), 75.7 (C-2″),71.1 (C-4″),62.6 (C-6″). **TOF ES HRMS** m/z 463.0880 [M-H]+.

Compound 3 (5,7,3′,4′-tetrahydroxy-flavonol-3-O-[*β*-D-glucopyranosyl-1→6)]-*β*-D-glucopyranoside (quercetin diglycoside**)**: **^{1}H NMR (300 MHz, MeOD)**: δH: 7.72 (1H, d, J=2.0), 7.68 (1H, dd, J=8.4, 2.0 Hz), 6.88 (1H, d, J=8.2 Hz), 6.42 (1H, d, J=1.9 Hz), 6.22 (1H, d, J=1.8 Hz), 5.25 (1H, d, J=7.3 Hz), 4.17 (1H, d, J=7.6 Hz). **TOF ES HRMS** m/z 649.1378 [M+Na]+.

Table 1: Carbon 13 NMR data for <u>Compound 1</u> (Olean-12-en-3-O-β-D-glucopyranoside) compared with literature*

SN	*β-Amyrin (125 MHz $CDCl_3$)	Compound 1 (Olean-12-en-3-O-β-D-glucopyranoside) (75 MHz-$DMSO_{d6}$)
1	38.8	38.7
2	27.4	27.8
3	79.2	76.9
4	39.0	39.0
5	55.4	55.4
6	18.6	18.9
7	32.9	31.4
8	40.2	40.1
9	47.4	48.6
10	37.2	36.8
11	23.8	22.6
12	121.9	121.2
13	145.4	140.5
14	41.9	41.8
15	26.4	25..4
16	27.1	28.7
17	32.7	31.3
18	47.8	48.6
19	47.0	45.1
20	31.3	30.7
21	37.4	36.8
22	34.9	35.5
23	15.7	11.7
24	28.3	28.7
25	15.8	11.8
26	17.0	18.6
27	26.2	25.4
28	28.6	29.3
29	33.7	35.5
30	23.9	22.6
1'		100.8
2'		76.8
3'		76.8
4'		73.5
5'		70.1
6'		61.1

* **Okoye *et al.* (2014). Pharm Biol. 52(11): 1478–1486 (Reference 21 in the manuscript):** Please note that the cited literature (Okoye et al., 2014) [21] acquired the carbon 13 data in deuterated chloroform at 125 MHz, while for compound 1 spectral data were acquired in deuterated dimethyl sulfoxide on 75 MHz spectrometer. These differences account for the occasional variations (our value vs. literature value), where such variations exist, in the chemical shift values for the carbon atoms.

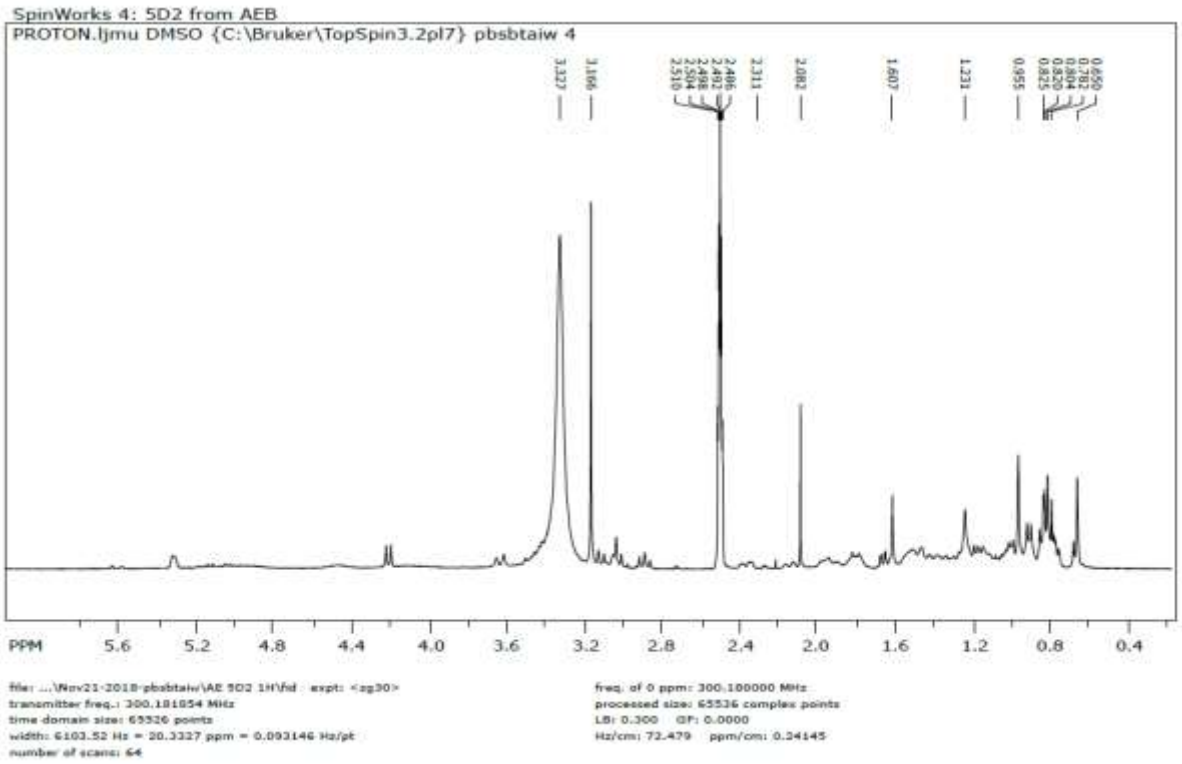
PLEASE NOTE:

Compound 1 (Olea-12-ene 3-O-β-D-glucopyranoside) is **AE 5D2**

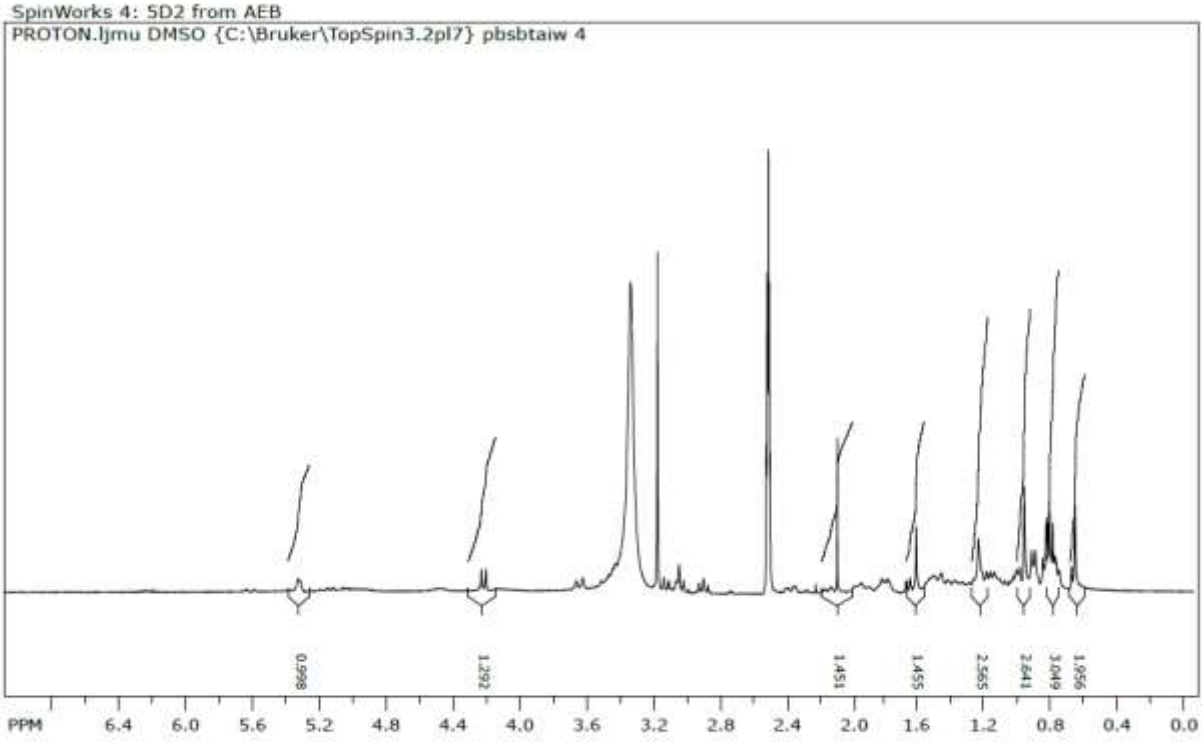
Compound 2 (isoquercitrin) is **AE 5B2**

Compound 3 (5,7,3′,4′-tetrahydroxy-flavonol-3-O-[β-D-glucopyranosyl-1→6)]-β-D-glucopyranoside) is **AEBiii**

NMR for Compound 1



SpinWorks 4: 5D2 from AEB



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number of scans: 64

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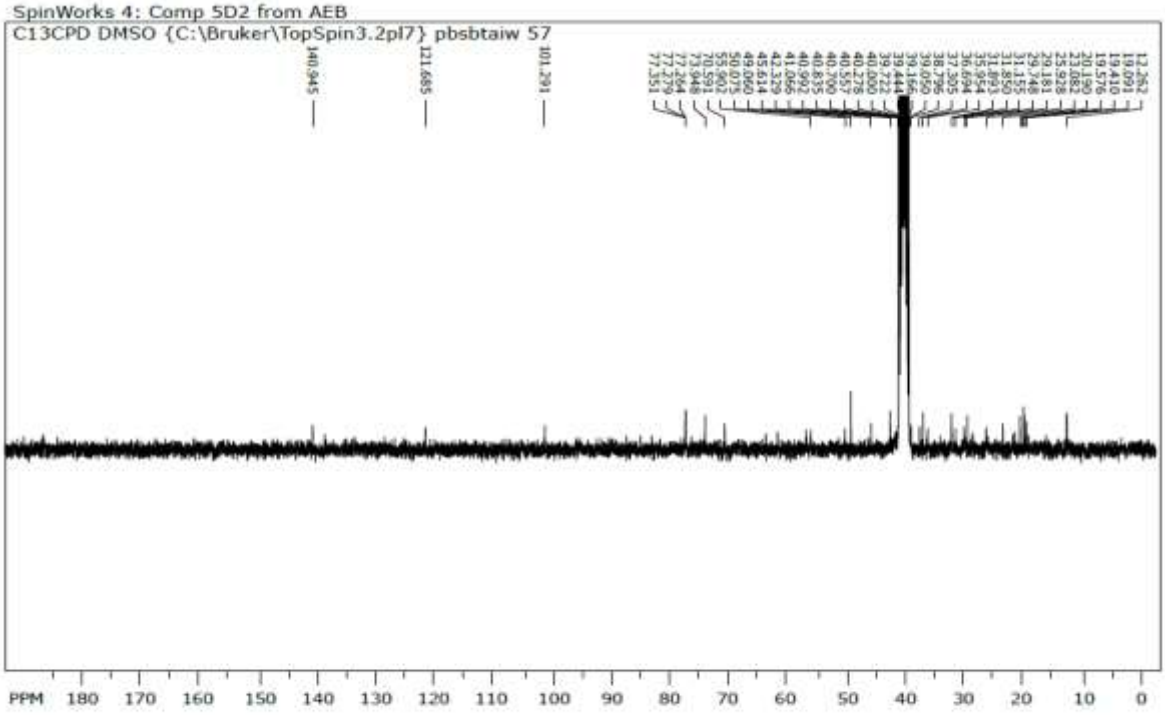
Hz/cm: 86.103 ppm/cm: 0.28684

SpinWorks 4: Comp 5D2 from AEB



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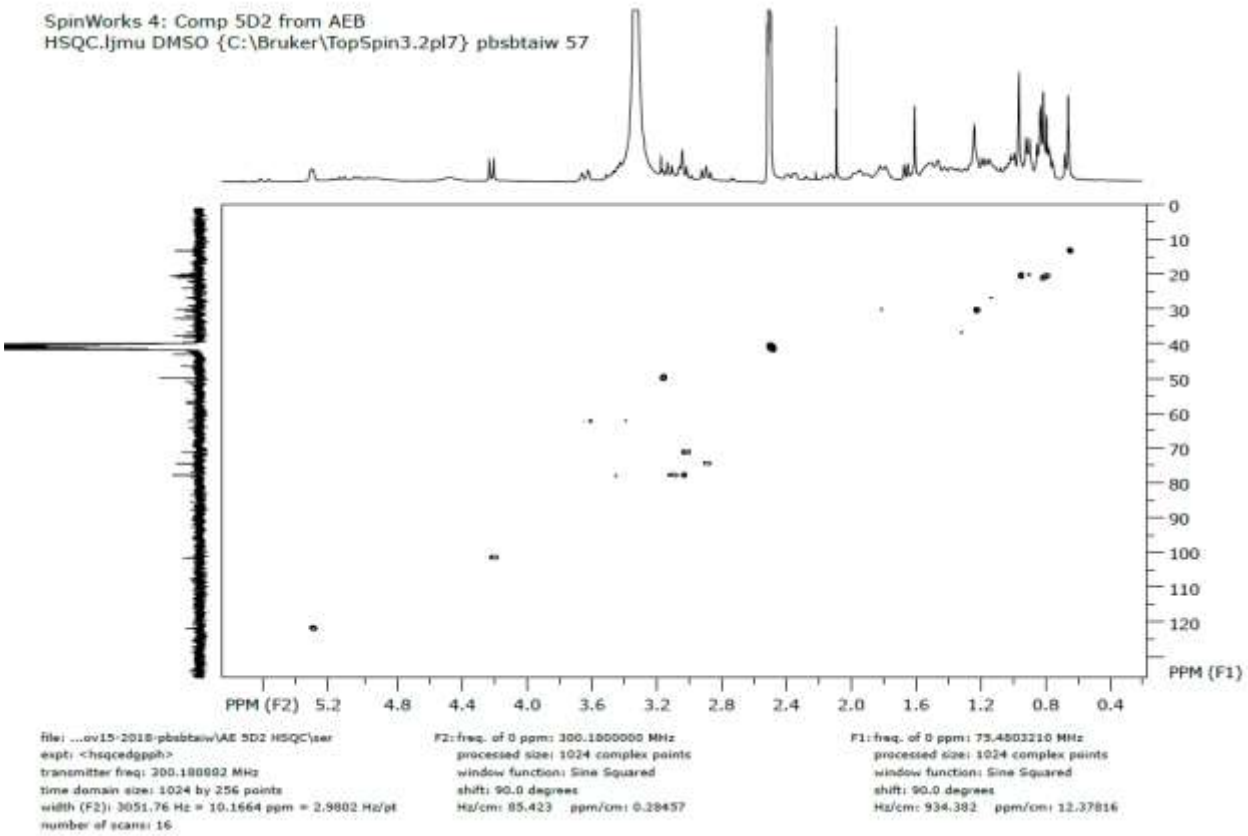
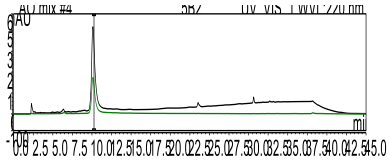
time domain size: 65532 points
width: 24414.06 Hz = 323.4170 ppm = 0.372552 Hz/pt
number of scans: 15400

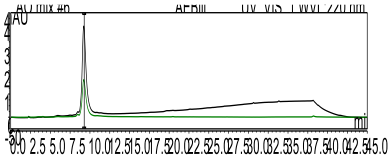
freq. of 0 ppm: 75.480321 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 618.026 ppm/cm: 8.18709



Chromatogram for Compound 2

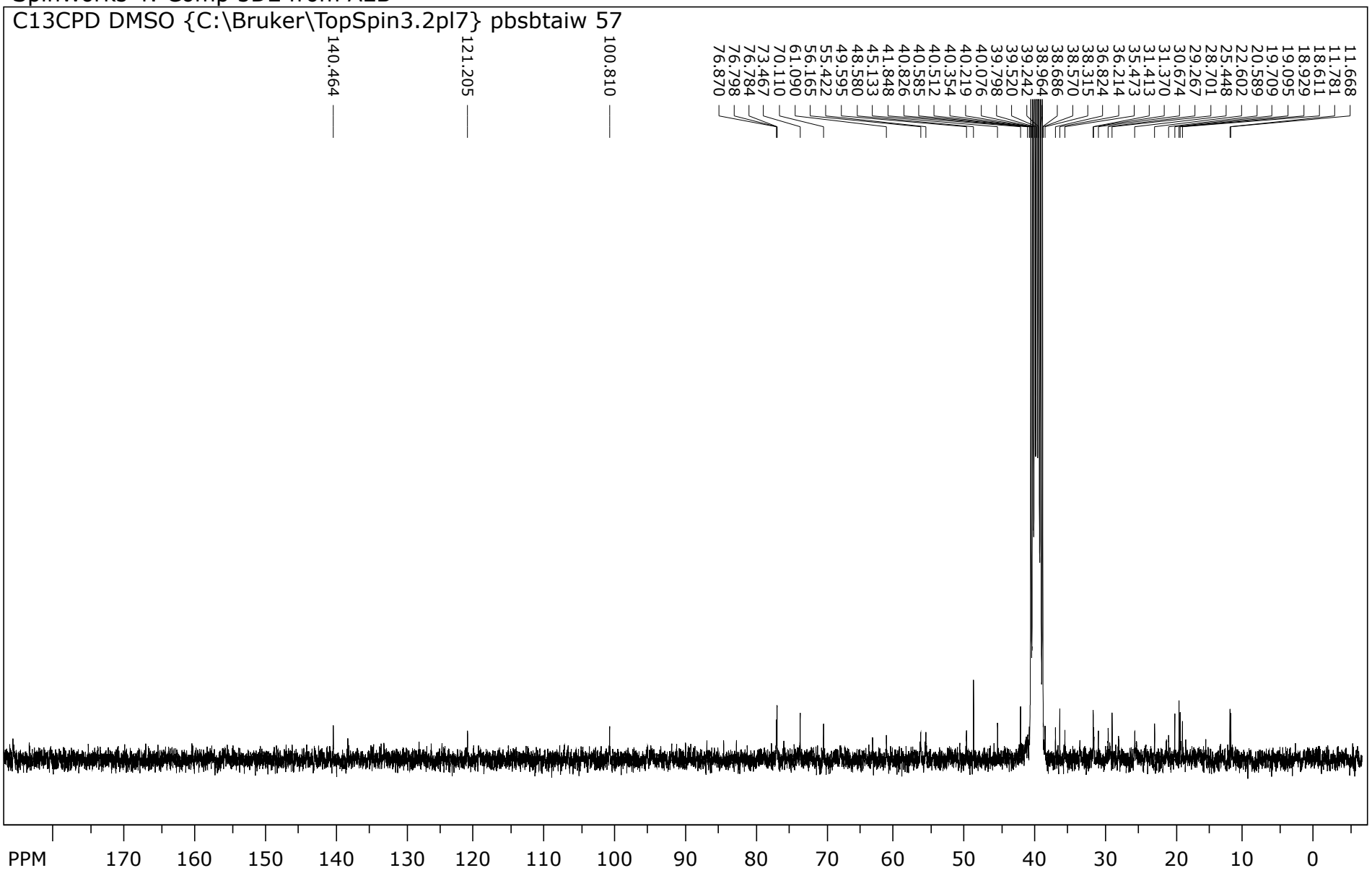


Chromatogram for Compound 3



SpinWorks 4: Comp 5D2 from AEB

C13CPD DMSO {C:\Bruker\TopSpin3.2pl7} pbsbtaiw 57



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number of scans: 15400

freq. of 0 ppm: 75.480356 MHz
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LB: 1.000 GF: 0.0000
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SpinWorks 4: 5D2 from AEB
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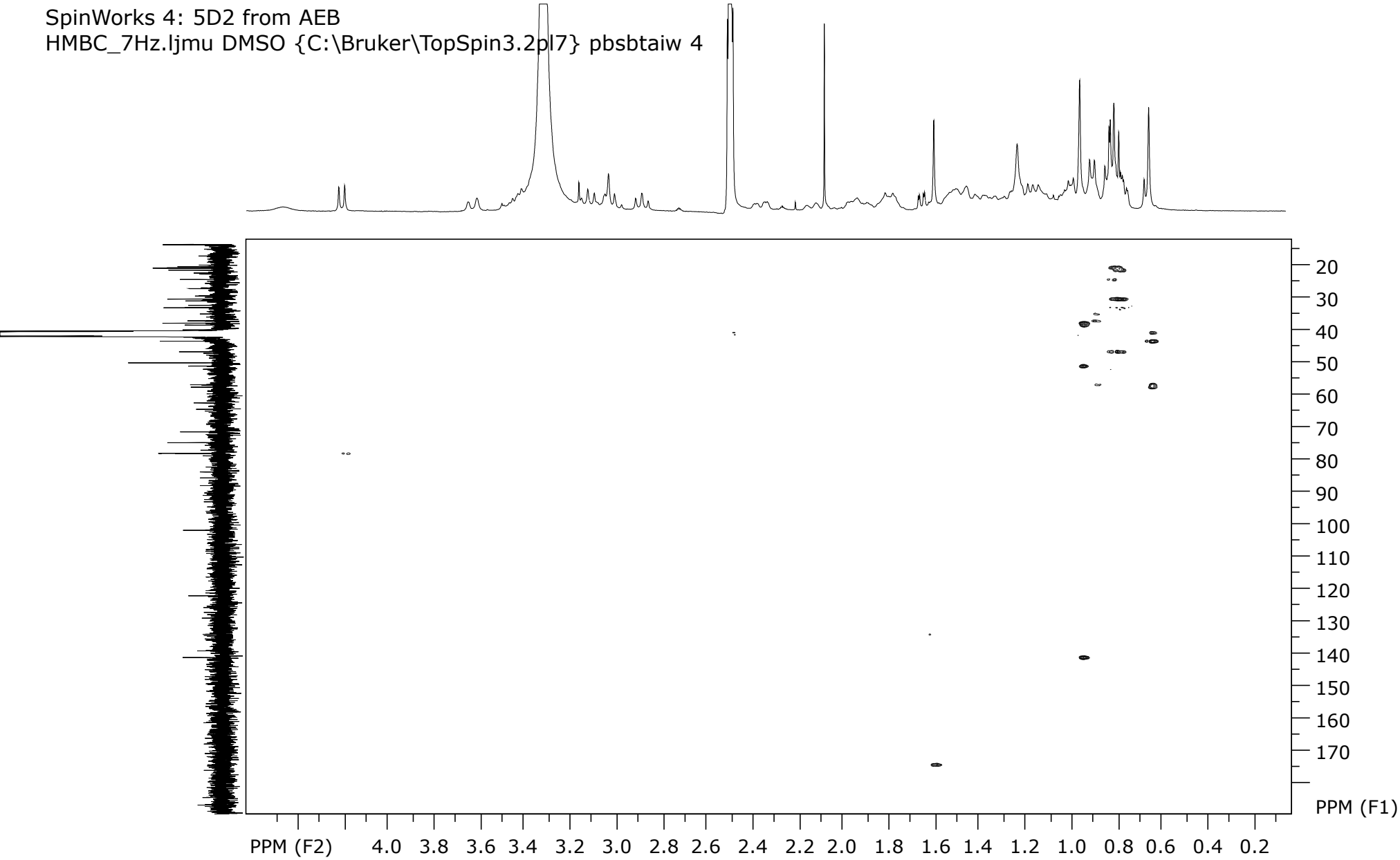

file: ...ov21-2018-pbsbtaiw\AE 5D2 HMBC\ser
expt: <hmbcgplpndqf>
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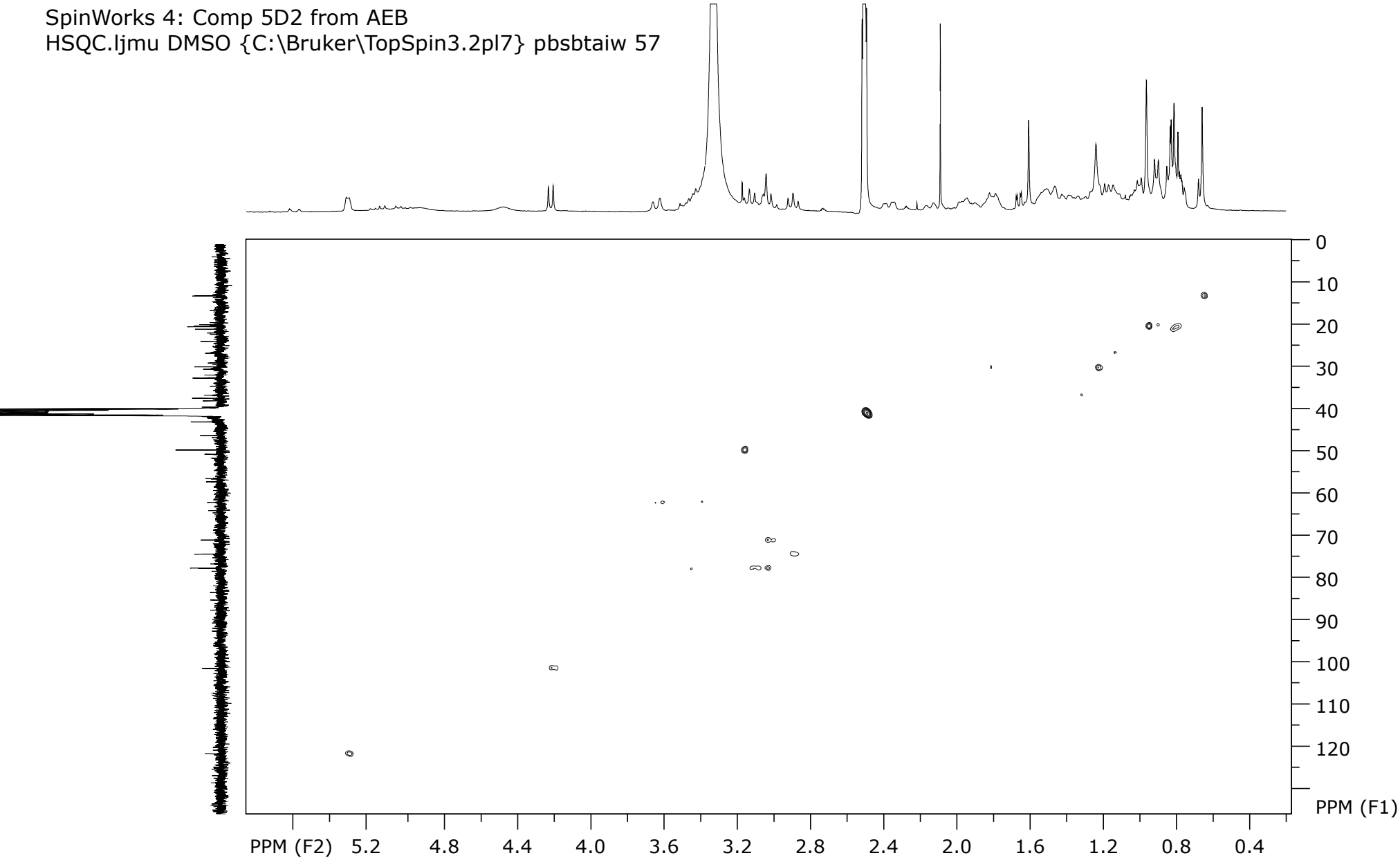
time domain size: 2048 by 512 points
width (F2): 3051.76 Hz = 10.1664 ppm = 1.4901 Hz/pt
number of scans: 32

F2: freq. of 0 ppm: 300.1800000 MHz
processed size: 2048 complex points
window function: Sine
shift: 0.0 degrees
Hz/cm: 68.958 ppm/cm: 0.22972

F1: freq. of 0 ppm: 75.4803210 MHz
processed size: 1024 complex points
window function: Sine
shift: 0.0 degrees
Hz/cm: 1218.528 ppm/cm: 16.14195

SpinWorks 4: Comp 5D2 from AEB
HSQC.ljmu DMSO {C:\Bruker\TopSpin3.2pl7} pbsbtaiw 57



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number of scans: 16

F2: freq. of 0 ppm: 300.1800000 MHz
processed size: 1024 complex points
window function: Sine Squared
shift: 90.0 degrees
Hz/cm: 85.423 ppm/cm: 0.28457

F1: freq. of 0 ppm: 75.4803210 MHz
processed size: 1024 complex points
window function: Sine Squared
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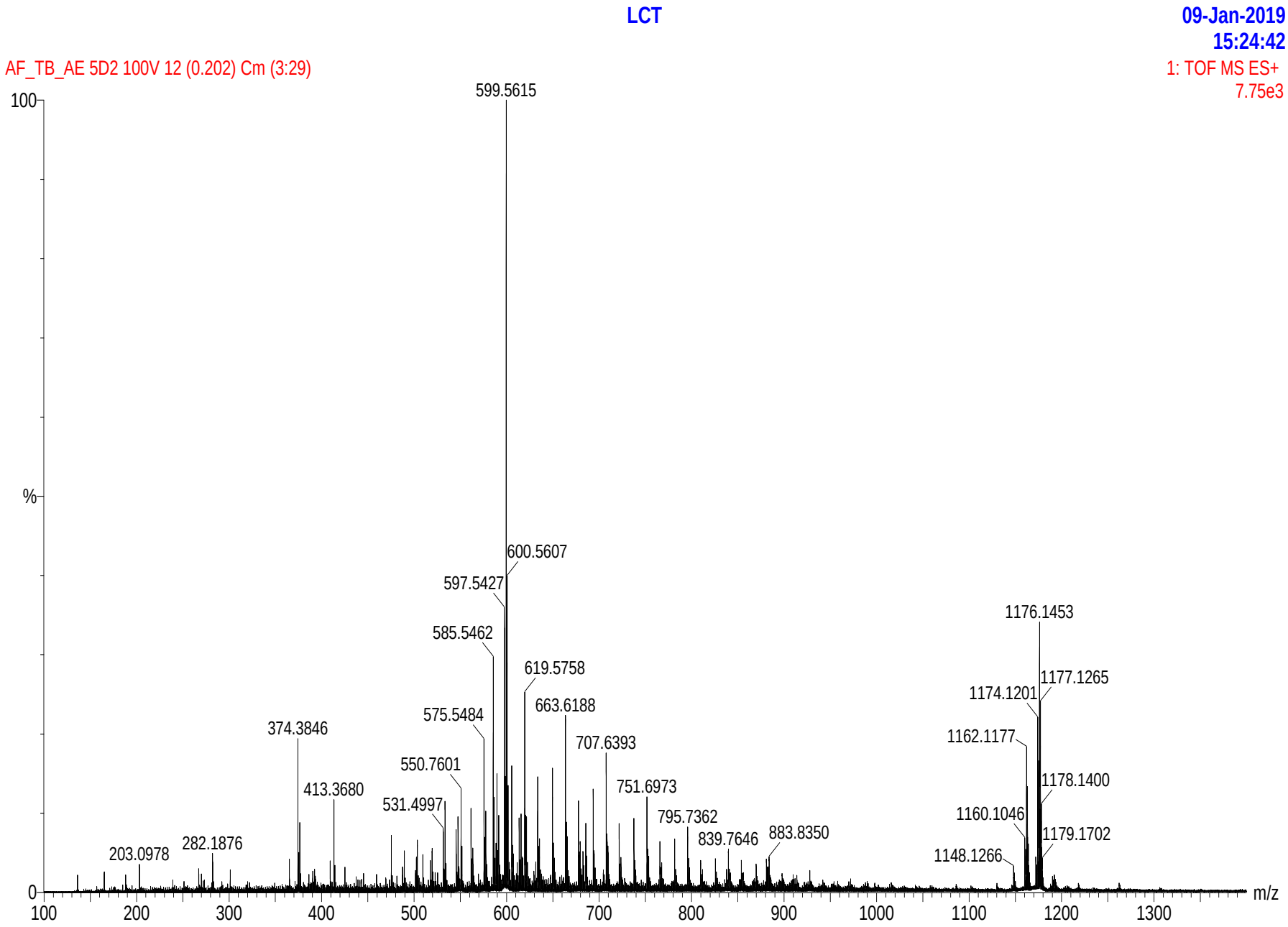
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09-Jan-2019
15:24:42
AF_TB_AE 5D2 100V 12 (0.202) Cm (3:29)
1: TOF MS ES+
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100
%
0
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600.5607
597.5427
585.5462
619.5758
663.6188
575.5484
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707.6393
550.7601
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1176.1453
1177.1265
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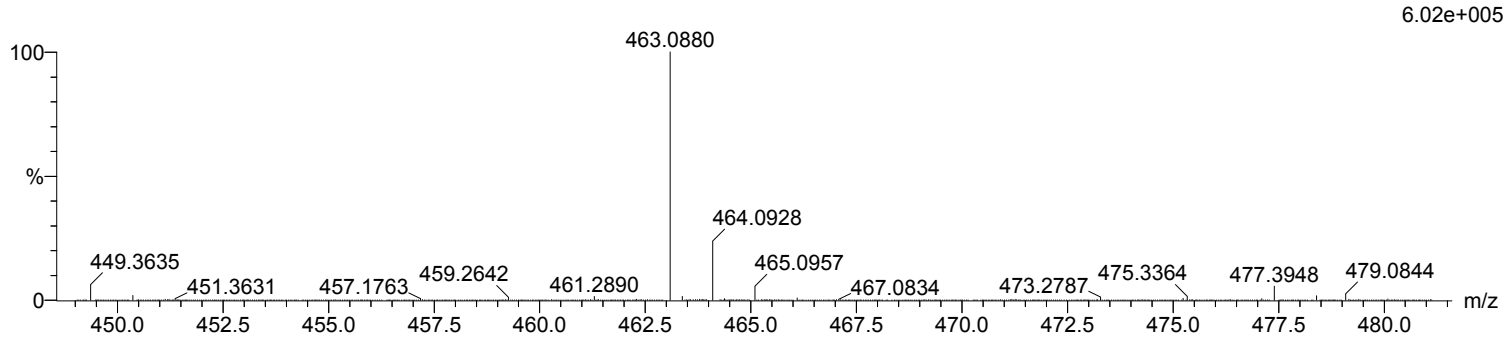
1162.1177
1178.1400
1160.1046
1179.1702
1148.1266
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
m/z

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1860 formula(e) evaluated with 11 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-50 O: 0-50

AEB 2a 18 (0.574) Cm (1:61)
TOF MS ES-



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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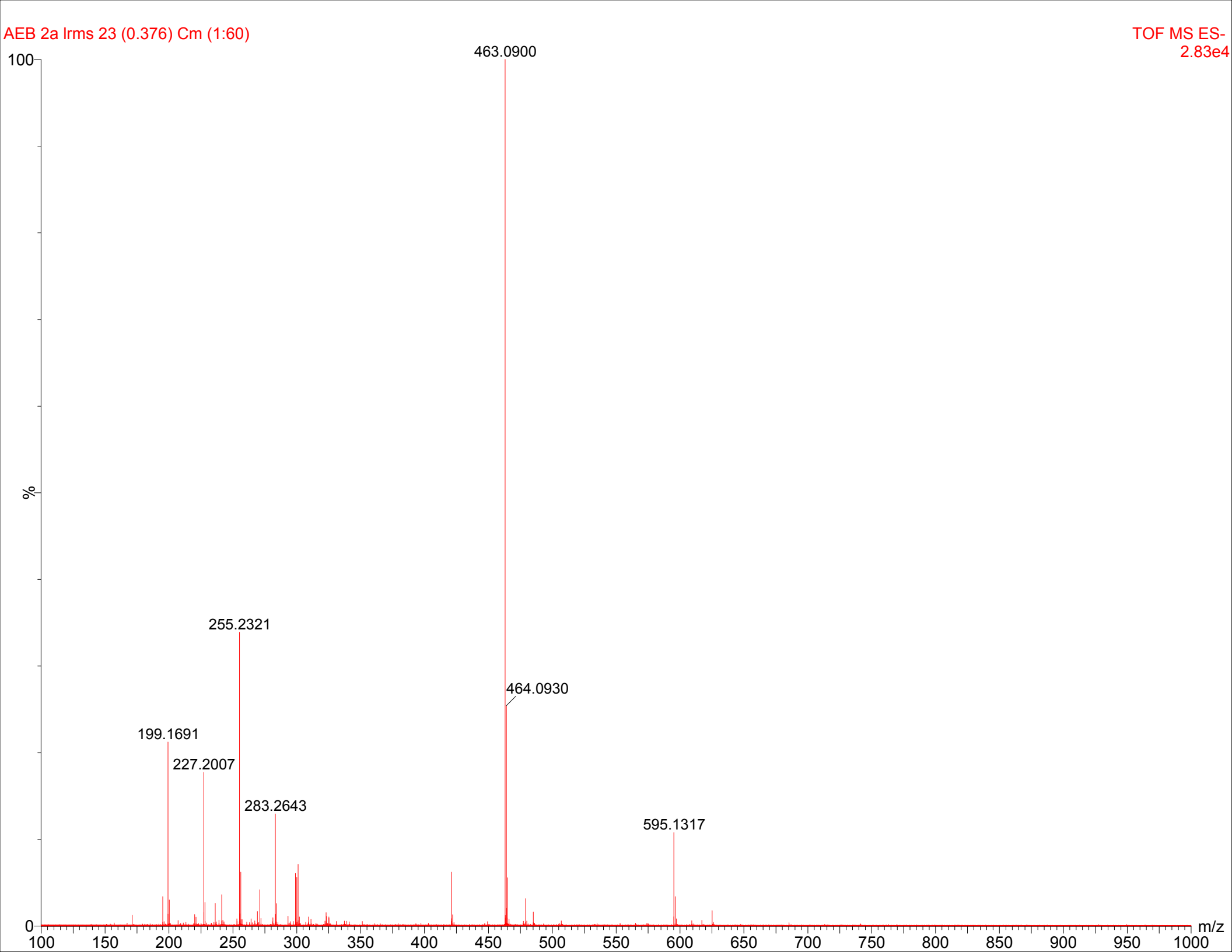
	463.0882	-0.2	-0.4	5.5	572.7	14.0	C6 H15 N12 O13
	463.0877	0.3	0.6	12.5	558.7	0.0	C21 H19 O12
	463.0876	0.4	0.9	23.5	568.0	9.2	C19 H7 N14 O2
	463.0871	0.9	1.9	30.5	569.4	10.6	C34 H11 N2 O
	463.0890	-1.0	-2.2	17.5	564.0	5.2	C22 H15 N4 O8
	463.0868	1.2	2.6	0.5	573.3	14.6	C5 H19 N8 O17
	463.0868	1.2	2.6	11.5	574.9	16.1	C3 H7 N22 O7
	463.0895	-1.5	-3.2	-0.5	570.9	12.2	C9 H23 N2 O19
	463.0895	-1.5	-3.2	10.5	572.3	13.6	C7 H11 N16 O9
	463.0863	1.7	3.7	18.5	566.6	7.9	C18 H11 N10 O6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TOF MS ES-
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464.0930
199.1691
227.2007
283.2643
595.1317
m/z
100
150
200
250
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350
400
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550
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900
950
1000

SpinWorks 4: AE 5B2

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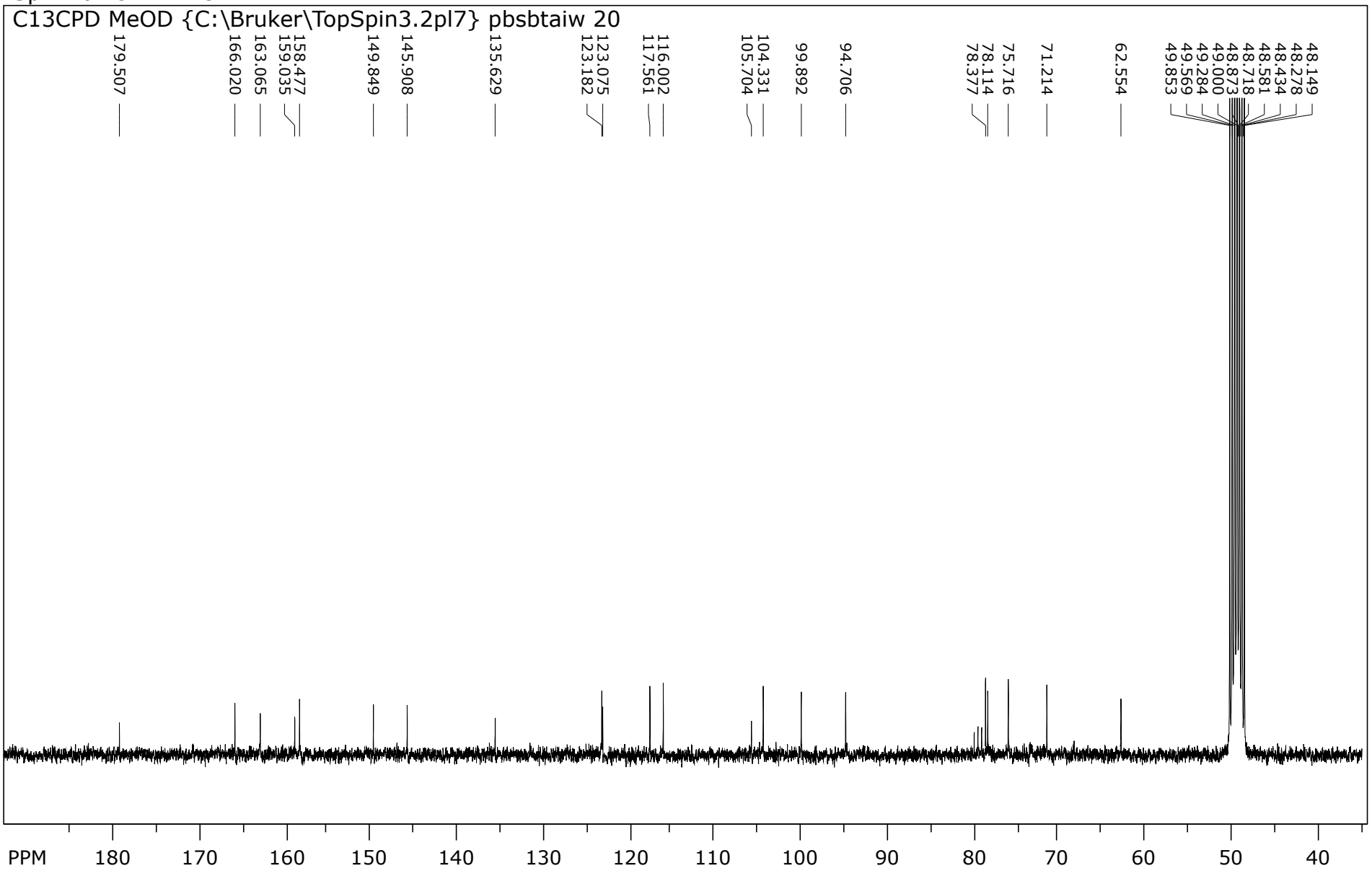

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number of scans: 9216

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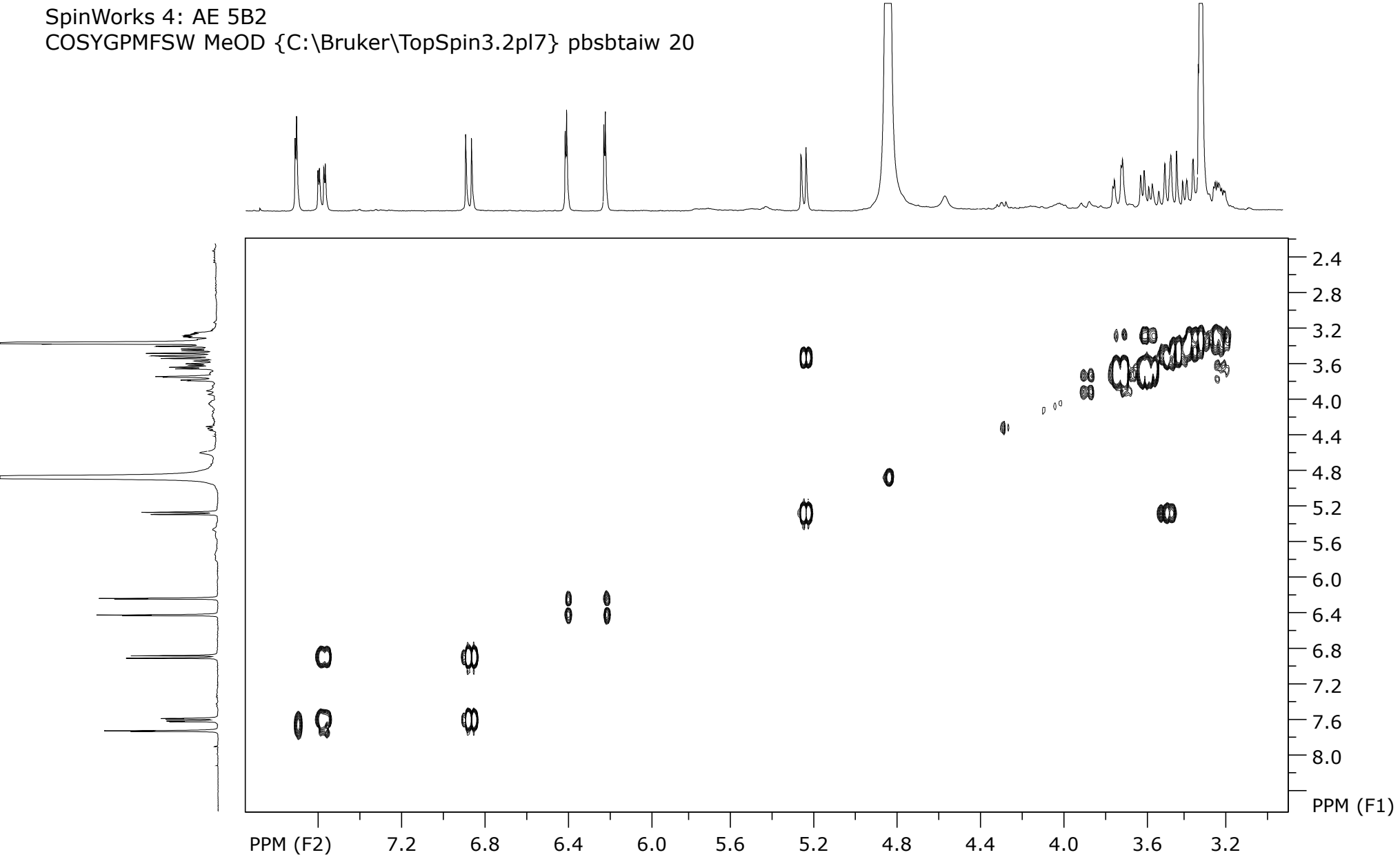
SpinWorks 4: AE 5B2
COSYGPMFSW MeOD {C:\Bruker\TopSpin3.2pl7} pbsbtaiw 20



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time domain size: 2048 by 128 points
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number of scans: 24

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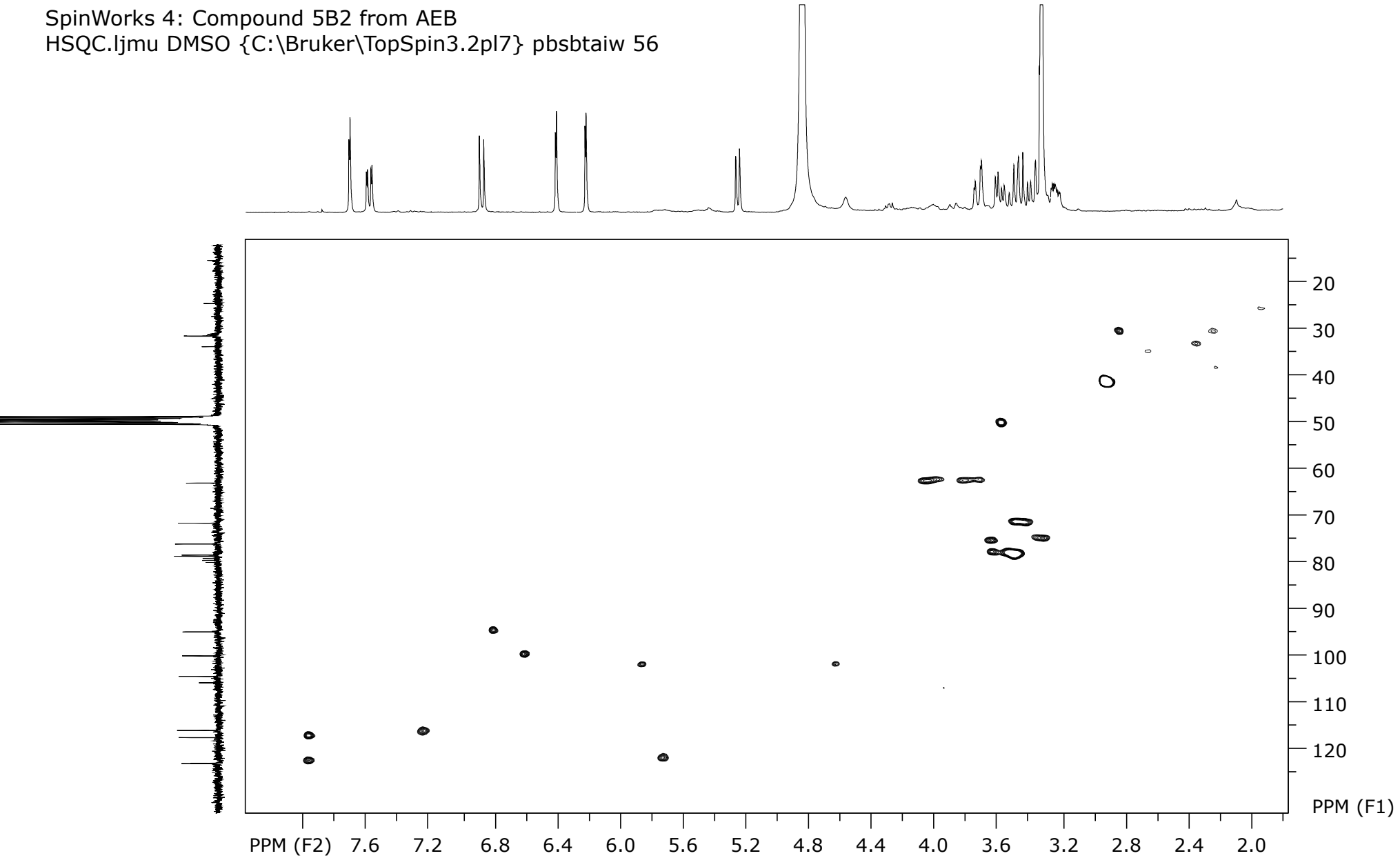
processed size: 1024 complex points
window function: Sine
shift: 0.0 degrees
Hz/cm: 75.805 ppm/cm: 0.25253

F1: freq. of 0 ppm: 300.1800000 MHz
processed size: 1024 complex points
window function: Sine
shift: 0.0 degrees
Hz/cm: 176.091 ppm/cm: 0.58661

SpinWorks 4: Compound 5B2 from AEB
HSQC.ljmu DMSO {C:\Bruker\TopSpin3.2pl7} pbsbtaiw 56



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number of scans: 32

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processed size: 1024 complex points
window function: Sine Squared
shift: 90.0 degrees
Hz/cm: 99.149 ppm/cm: 0.33029

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processed size: 1024 complex points
window function: Sine Squared
shift: 90.0 degrees
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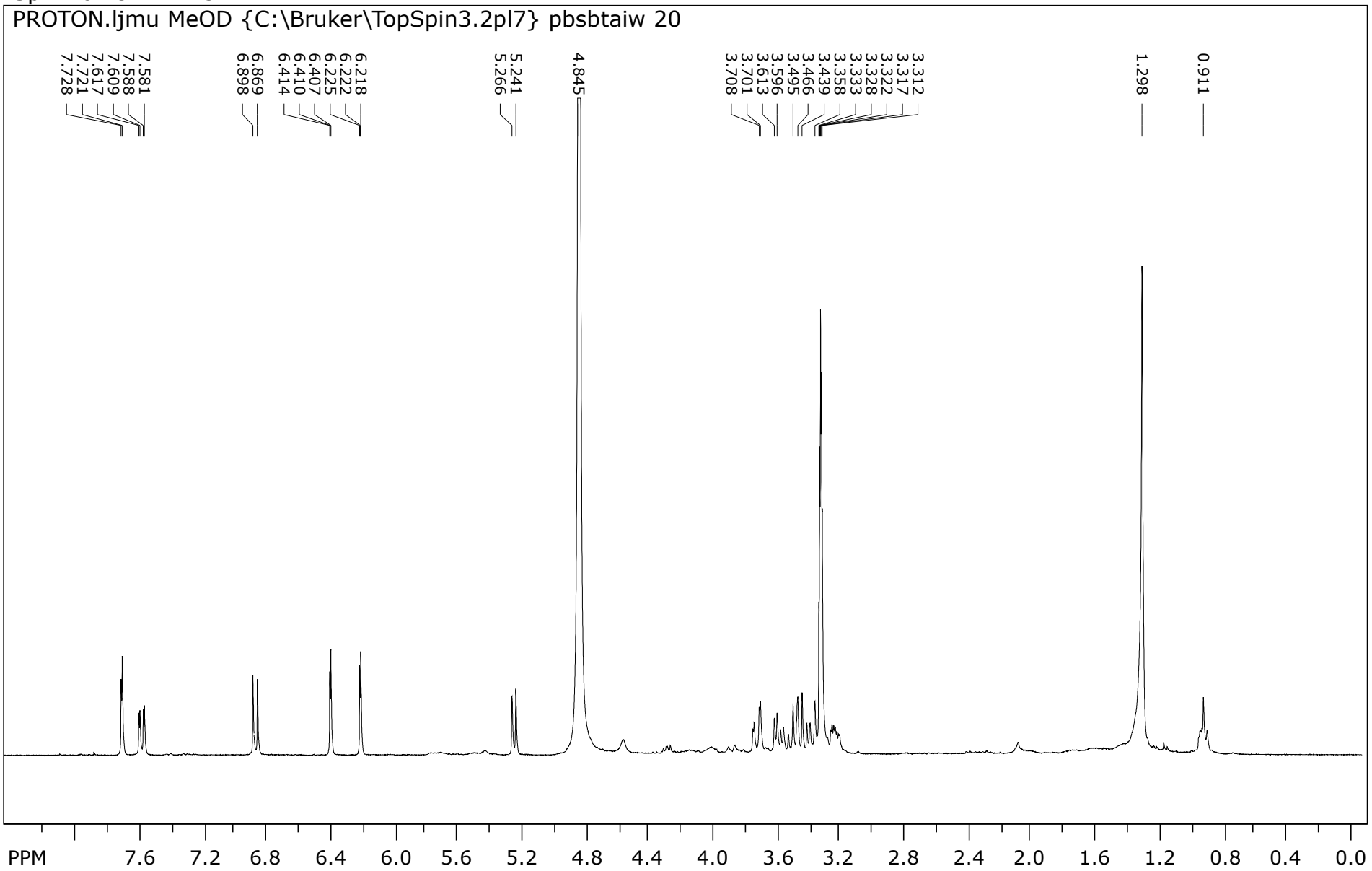
SpinWorks 4: AE 5B2

PROTON.ljmu MeOD {C:\Bruker\TopSpin3.2pl7} pbsbtaiw 20



file: ...\Dec20-2018-pbsbtaiw\AE 5B2 1H\fid expt: <zg30>
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number of scans: 128

freq. of 0 ppm: 300.180000 MHz
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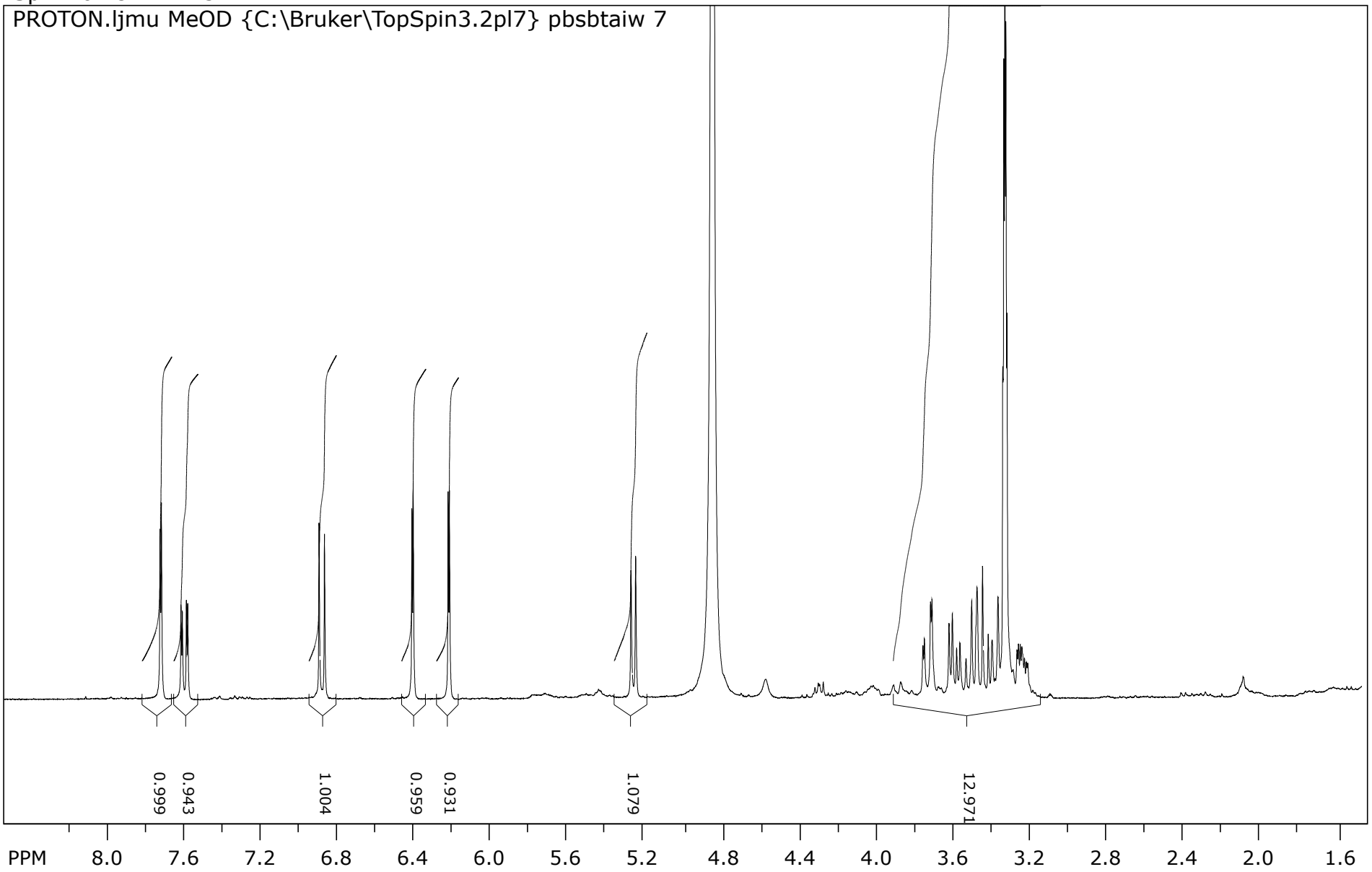
SpinWorks 4: AE 5B2



file: ...\Dec06-2018-pbsbtaiw\AE 5B2 1H\fid expt: <zg30>
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width: 6103.52 Hz = 20.3327 ppm = 0.093146 Hz/pt
number of scans: 128

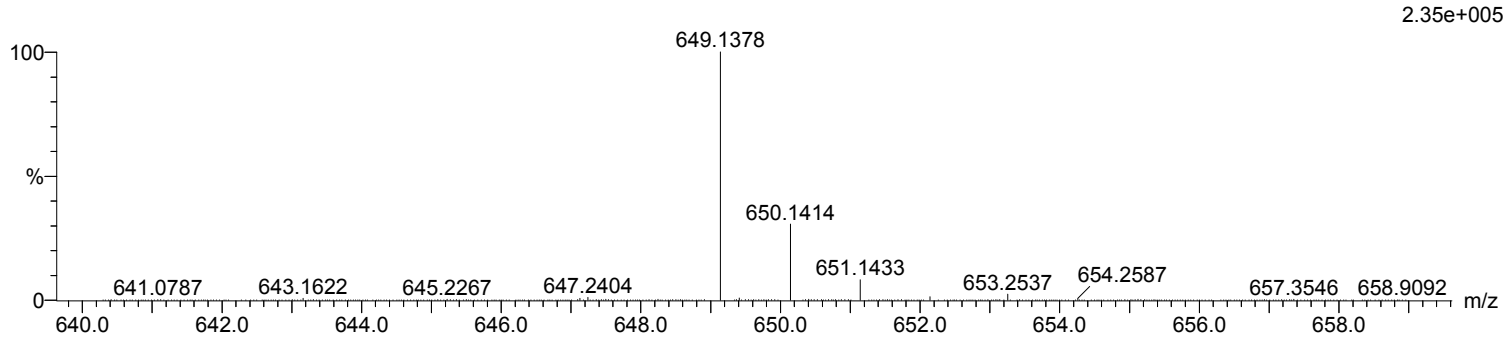
freq. of 0 ppm: 300.180000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 85.038 ppm/cm: 0.28329

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
4038 formula(e) evaluated with 23 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-50 O: 0-50 Na: 1-1

AEB 3biii 40 (1.317) Cm (1:61)
TOF MS ES+



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Maximum:		5.0	5.0	100.0			
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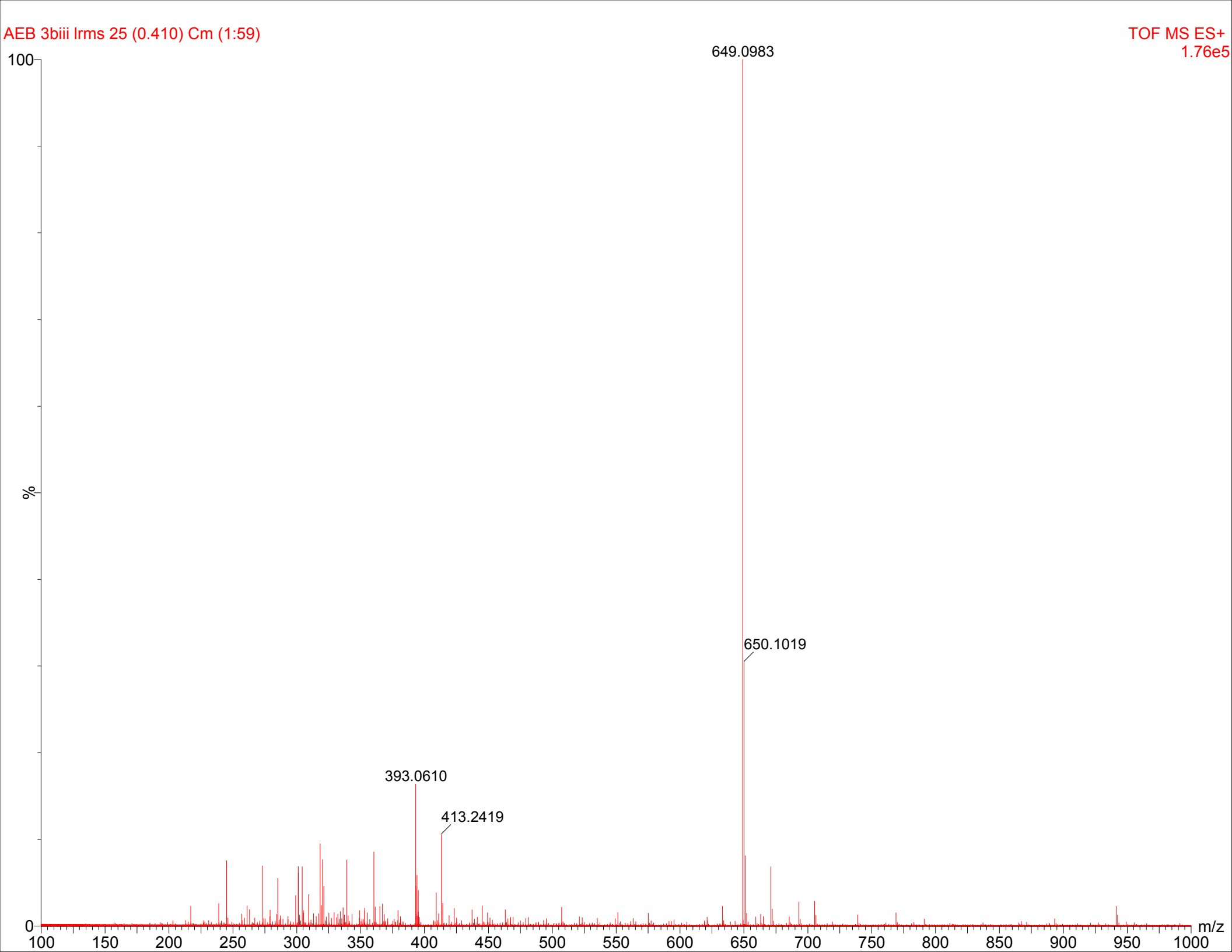
	649.1381	-0.3	-0.5	12.5	462.3	0.0	C27 H30 O17 Na
	649.1381	-0.3	-0.5	23.5	471.1	8.8	C25 H18 N14 O7 Na
	649.1372	0.6	0.9	22.5	482.5	20.2	C7 H6 N36 O2 Na
	649.1372	0.6	0.9	11.5	479.9	17.7	C9 H18 N22 O12 Na
	649.1372	0.6	0.9	0.5	477.3	15.1	C11 H30 N8 O22 Na
	649.1386	-0.8	-1.2	5.5	476.8	14.5	C12 H26 N12 O18 Na
	649.1386	-0.8	-1.2	16.5	479.6	17.3	C10 H14 N26 O8 Na
	649.1367	1.1	1.7	18.5	469.9	7.6	C24 H22 N10 O11 Na
	649.1367	1.1	1.7	29.5	475.6	13.3	C22 H10 N24 O Na
	649.1389	-1.1	-1.7	35.5	476.5	14.3	C41 H18 N6 O2 Na
	649.1394	-1.6	-2.5	17.5	469.7	7.4	C28 H26 N4 O13 Na
	649.1362	1.6	2.5	36.5	476.6	14.4	C37 H14 N12 Na
	649.1394	-1.6	-2.5	28.5	473.3	11.0	C26 H14 N18 O3 Na
	649.1359	1.9	2.9	6.5	480.7	18.4	C8 H22 N18 O16 Na
	649.1359	1.9	2.9	17.5	482.5	20.2	C6 H10 N32 O6 Na
	649.1399	-2.1	-3.2	21.5	479.7	17.4	C11 H10 N30 O4 Na
	649.1399	-2.1	-3.2	10.5	477.0	14.7	C13 H22 N16 O14 Na
	649.1399	-2.1	-3.2	-0.5	476.0	13.7	C15 H34 N2 O24 Na
	649.1354	2.4	3.7	24.5	475.3	13.1	C21 H14 N20 O5 Na

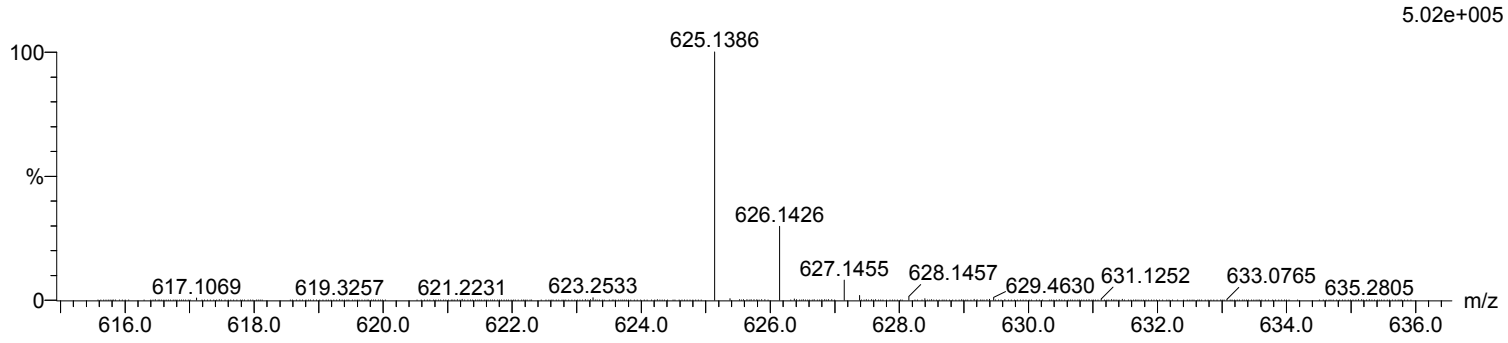

AEB 3biii lrms 25 (0.410) Cm (1:59)
TOF MS ES+
1.76e5
100
%
0
649.0983
650.1019
393.0610
413.2419
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000

Single Mass Analysis

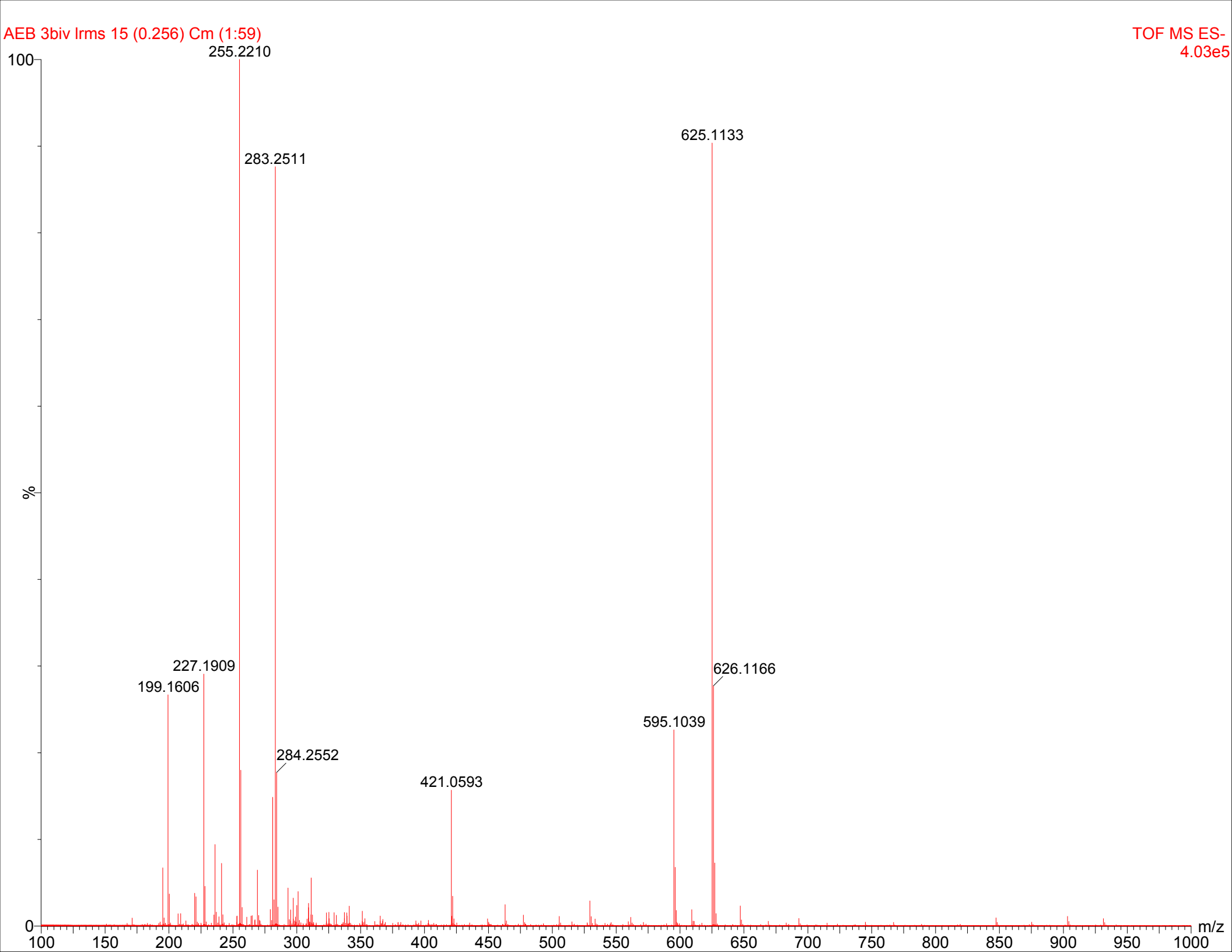
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
4157 formula(e) evaluated with 25 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-50 O: 0-50

AEB 3biv 22 (0.708) Cm (1:61)
TOF MS ES-

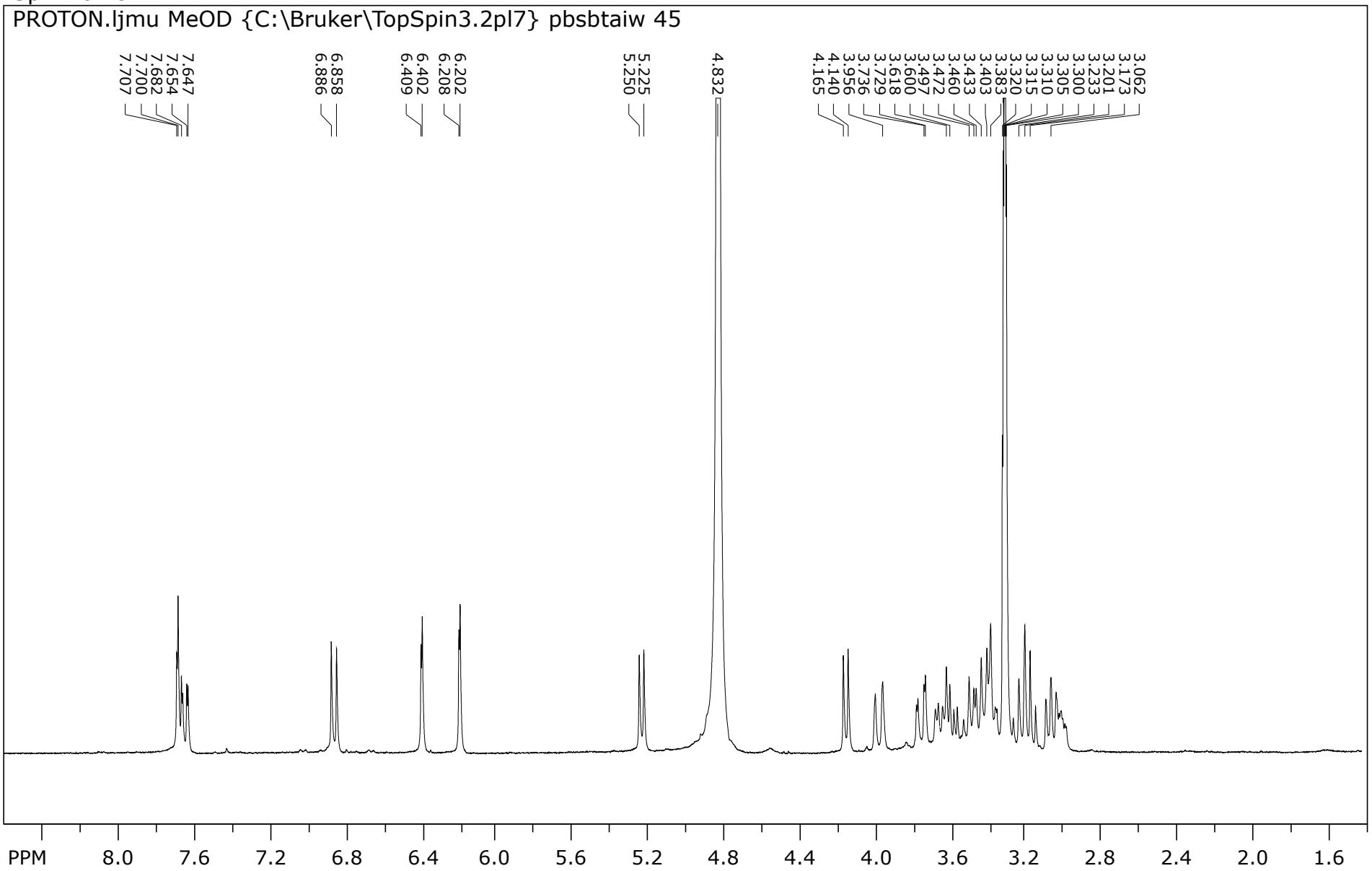


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
625.1386	625.1386	0.0	0.0	37.5	477.4	9.7	C37 H13 N12
	625.1383	0.3	0.5	18.5	483.5	15.7	C6 H9 N32 O6
	625.1383	0.3	0.5	7.5	481.6	13.8	C8 H21 N18 O16
	625.1391	-0.5	-0.8	19.5	471.9	4.1	C24 H21 N10 O11
	625.1391	-0.5	-0.8	30.5	477.5	9.7	C22 H9 N24 O
	625.1378	0.8	1.3	25.5	476.6	8.8	C21 H13 N20 O5
	625.1378	0.8	1.3	14.5	471.2	3.5	C23 H25 N6 O15
	625.1396	-1.0	-1.6	1.5	479.4	11.7	C11 H29 N8 O22
	625.1396	-1.0	-1.6	23.5	483.7	15.9	C7 H5 N36 O2
	625.1396	-1.0	-1.6	12.5	481.2	13.5	C9 H17 N22 O12
	625.1373	1.3	2.1	32.5	477.5	9.7	C36 H17 N8 O4
	625.1400	-1.4	-2.2	31.5	476.3	8.5	C40 H21 N2 O6
	625.1370	1.6	2.6	2.5	482.2	14.4	C7 H25 N14 O20
	625.1369	1.7	2.7	24.5	486.2	18.4	C3 H N42
	625.1369	1.7	2.7	13.5	483.6	15.9	C5 H13 N28 O10
	625.1405	-1.9	-3.0	24.5	474.3	6.5	C25 H17 N14 O7
	625.1405	-1.9	-3.0	13.5	467.8	0.1	C27 H29 O17
	625.1365	2.1	3.4	9.5	473.9	6.2	C22 H29 N2 O19
	625.1364	2.2	3.5	20.5	476.4	8.6	C20 H17 N16 O9
	625.1410	-2.4	-3.8	17.5	481.2	13.4	C10 H13 N26 O8


AEB 3biv Irms 15 (0.256) Cm (1:59)
TOF MS ES-
4.03e5
100
%
0
255.2210
283.2511
625.1133
227.1909
199.1606
626.1166
595.1039
284.2552
421.0593
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000

SpinWorks 4: AE Biii

PROTON.ljmu MeOD {C:\Bruker\TopSpin3.2pl7} pbsbtaiw 45



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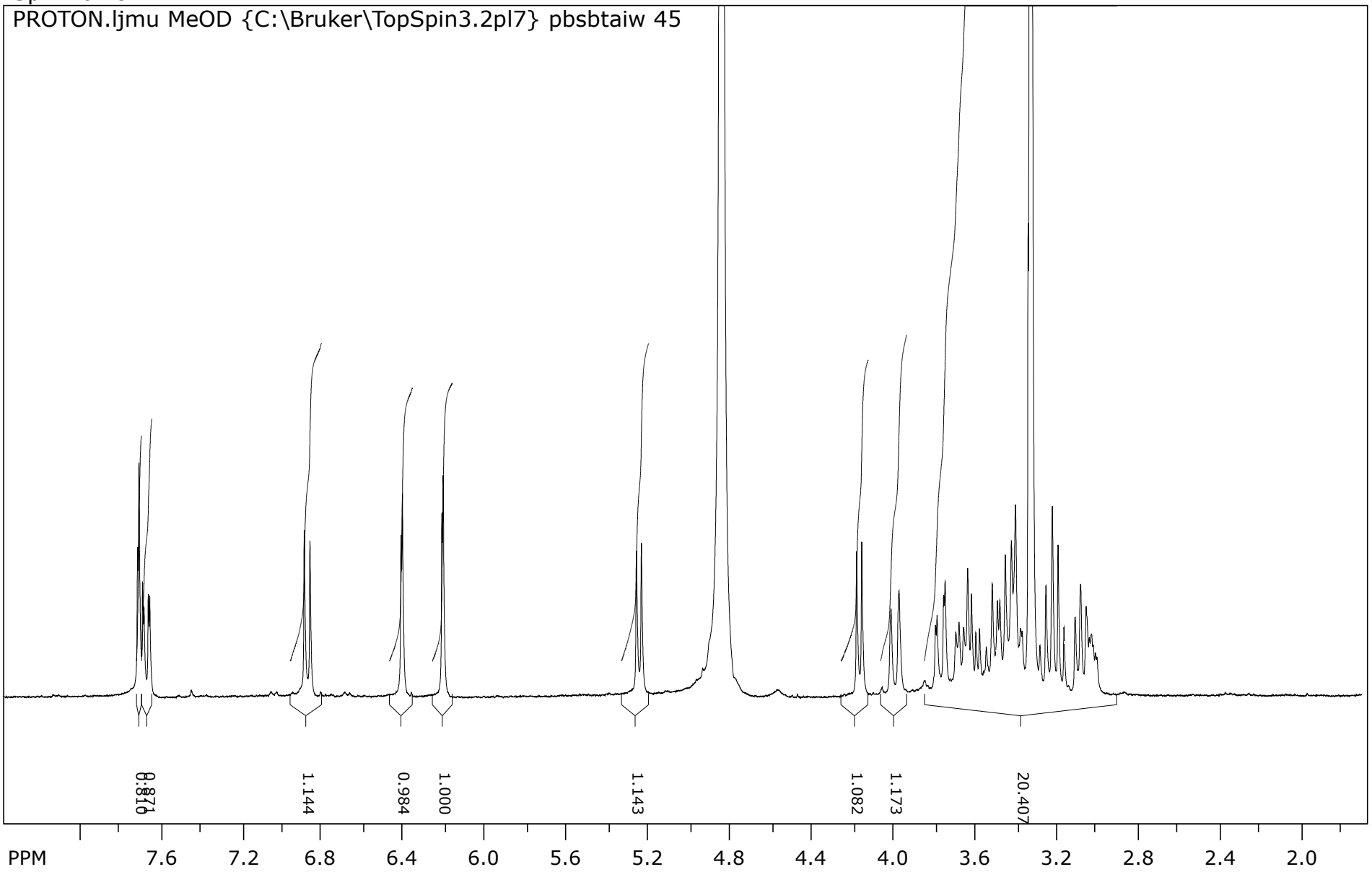
time domain size: 65526 points
width: 6103.52 Hz = 20.3327 ppm = 0.093146 Hz/pt
number of scans: 128

freq. of 0 ppm: 300.180004 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 86.648 ppm/cm: 0.28865

SpinWorks 4: AE Biii



file: ...bsbtaiw\Jan17-2019-pbsbtaiw\20\fid expt: <zg30>
transmitter freq.: 300.181854 MHz
time domain size: 65526 points
width: 6103.52 Hz = 20.3327 ppm = 0.093146 Hz/pt
number of scans: 128

freq. of 0 ppm: 300.180000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 80.335 ppm/cm: 0.26762