Supplementary Information

First total synthesis and solution structure of a polypeptin, PE2, a cyclic lipopeptide with broad spectrum antibiotic activity.

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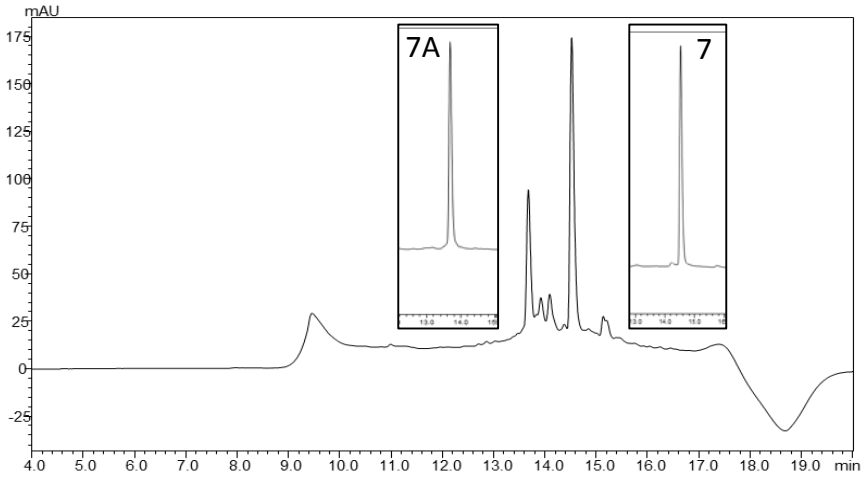
Contents

A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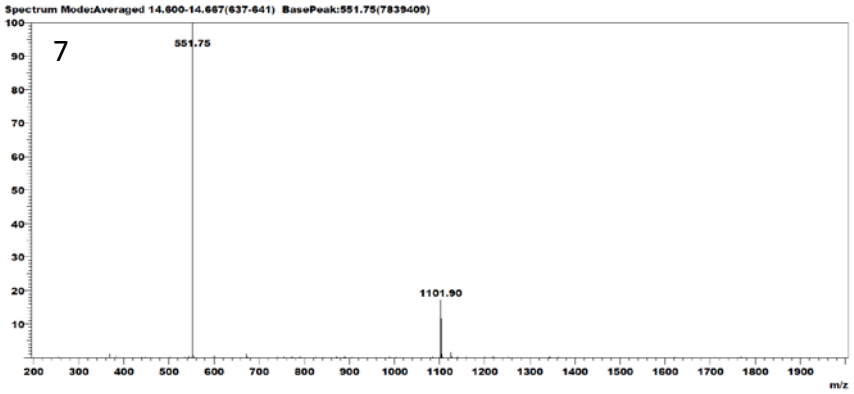
7A
7
min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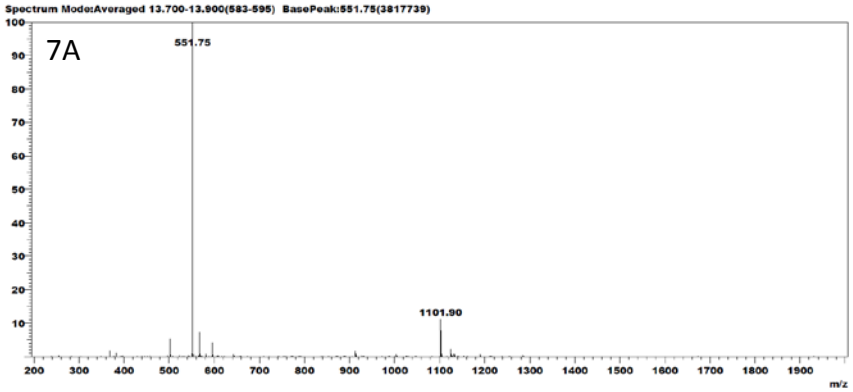
m/z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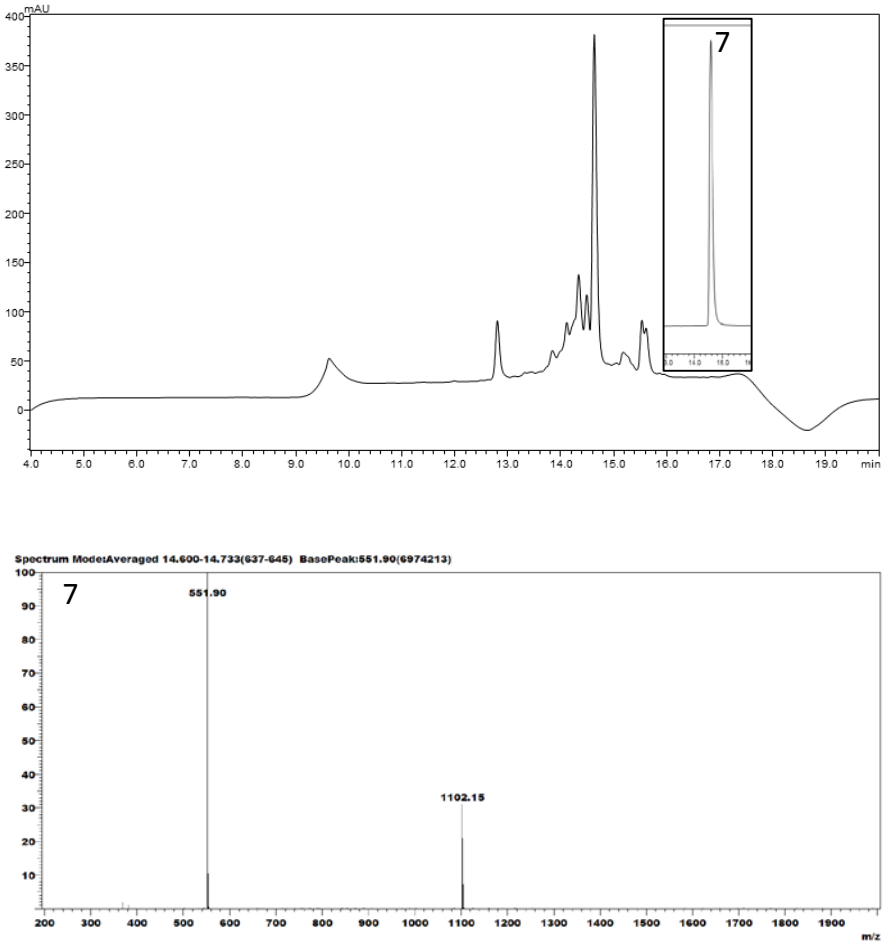


Figure S1 HPLC and ESI-MS of crude and purified (inset) peptides **7** and **7A**. (A) Synthesis from **12**. (B) Synthesis from ***S*-12.**

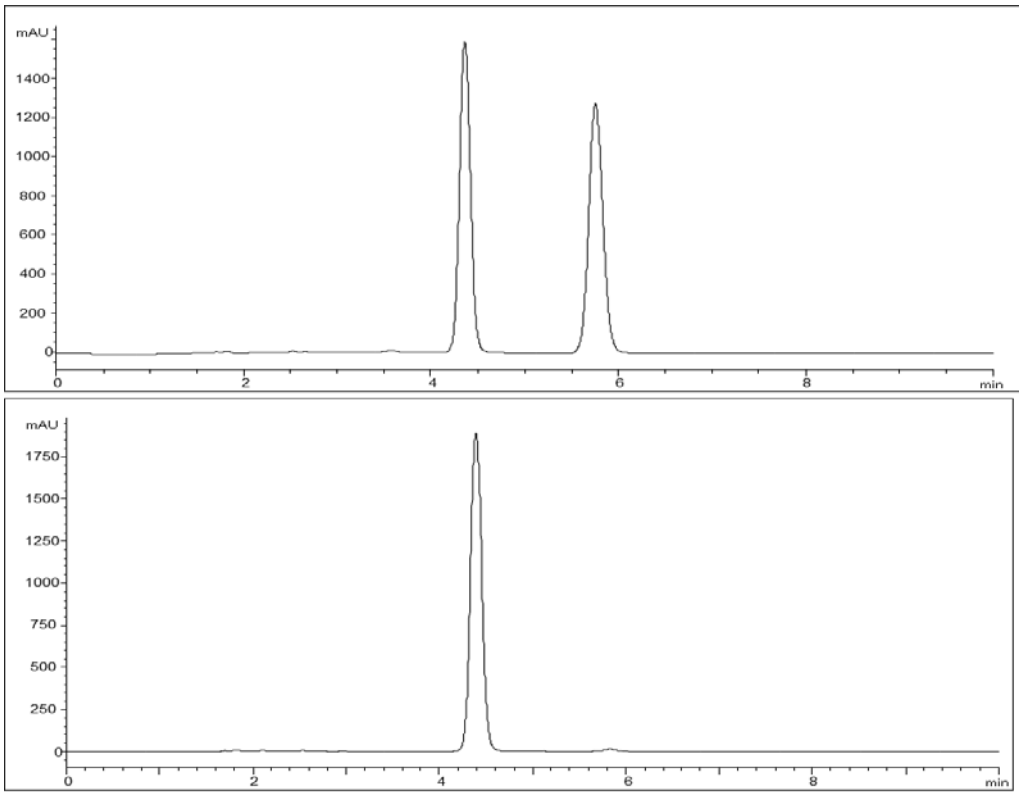


Figure S2 Chiral HPLC traces for ***RS*-15** (a) and ***S*-15** (b).

Chemical shift assignments:

Sequence specific chemical shift assignments of **7 (**FADDI-491B) and **7A** (FADDI-491A**)** peptides are provided in the supplementary information (see two excel files).

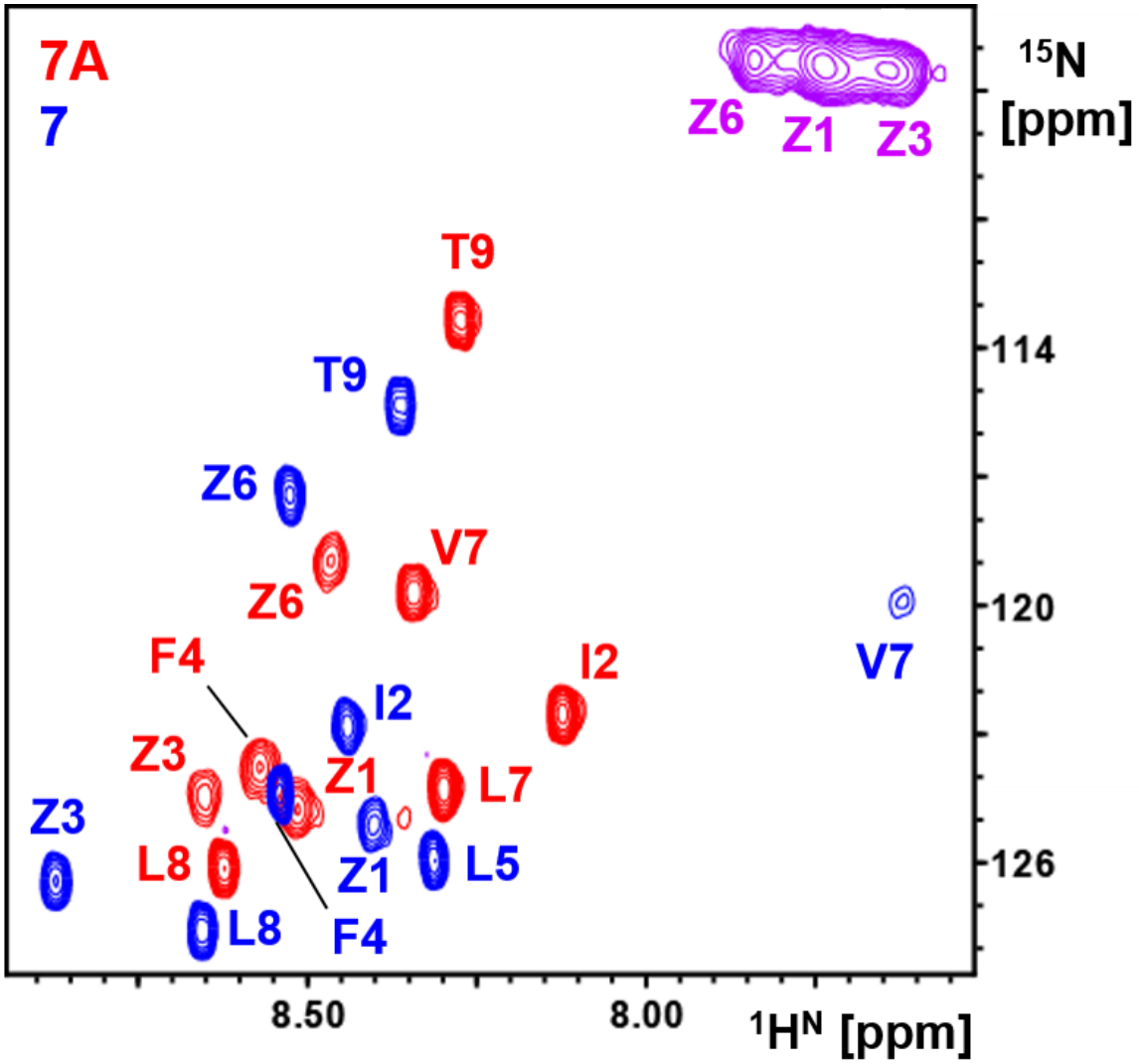


Figure S3 Overlay of 2D [^{15}N,^{1}H^{N}]-SOFAST-HMQC spectra of **7A** and **7** (3*RS*-12). Spectra were acquired from each sample with peptide concentration of ~ 1.7 mM in 7% $^{2}H_2O$ and 93% H_2O at pH 4.35. All data were collected on a 600 MHz spectrometer equipped with CryoProbe at 10 °C. The side-chain amide peaks for 7 are labelled in magenta. I, L, T, Z, V and F indicates Ile, Leu, Thr, Dab, D-Val and D-Phe residues.

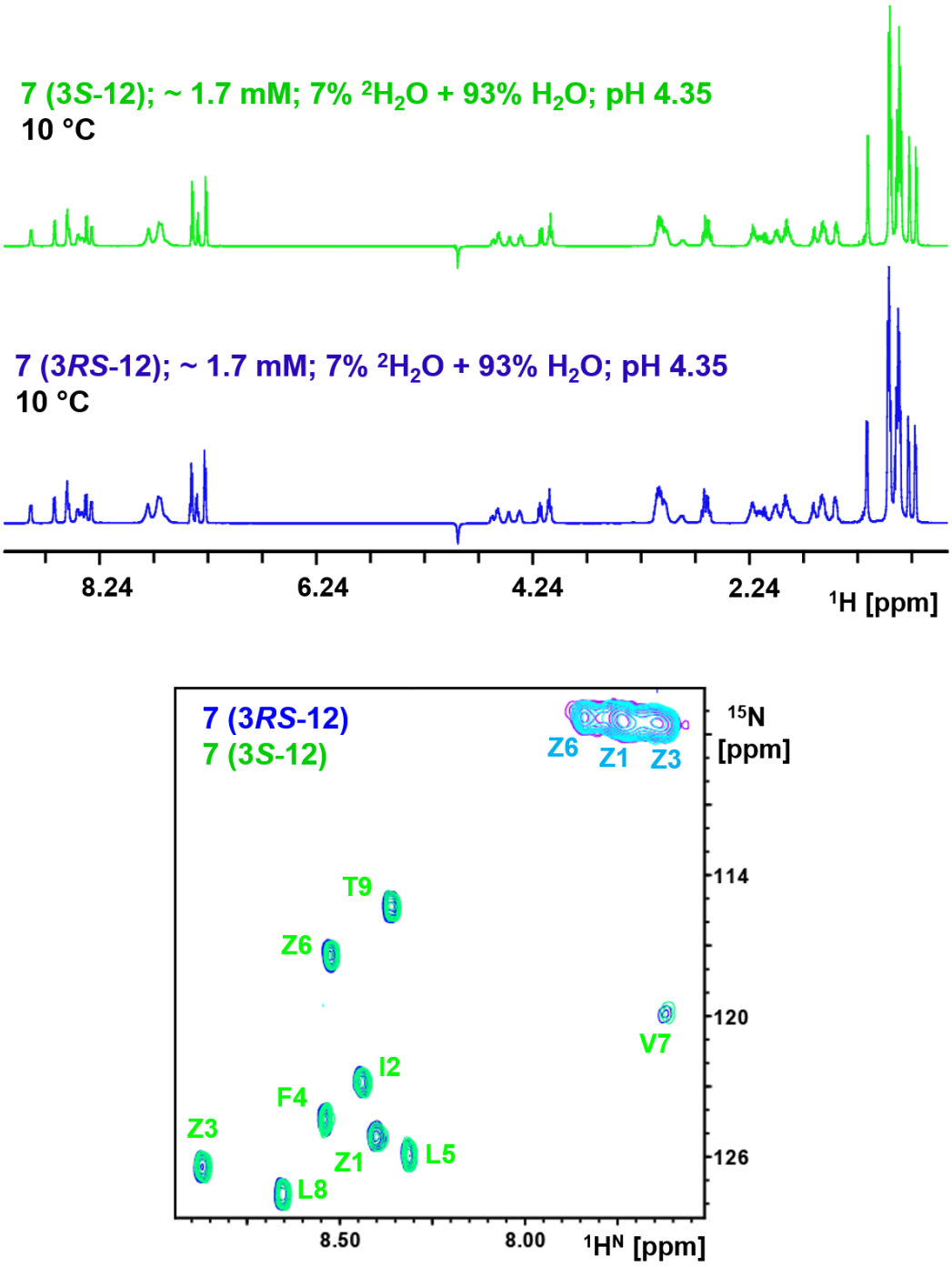


Figure S4 Overlay of 1D ^{1}H and 2D [^{15}N,$^{1}H^{N}$]-SOFAST-HMQCs spectra enabling comparison of **7** (synthesized via **12**) and **7** (synthesized via ***S*-12**). Spectra were acquired from each sample with peptide concentration of ~ 1.7 mM in 7% $^{2}H_2O$ and 93% H_2O at pH 4.35. All data were collected on a 600 MHz spectrometer equipped with CryoProbe at 10 °C. I, L, T, Z, V and F indicates Ile, Leu, Thr, Dab, D-Val and D-Phe residues.

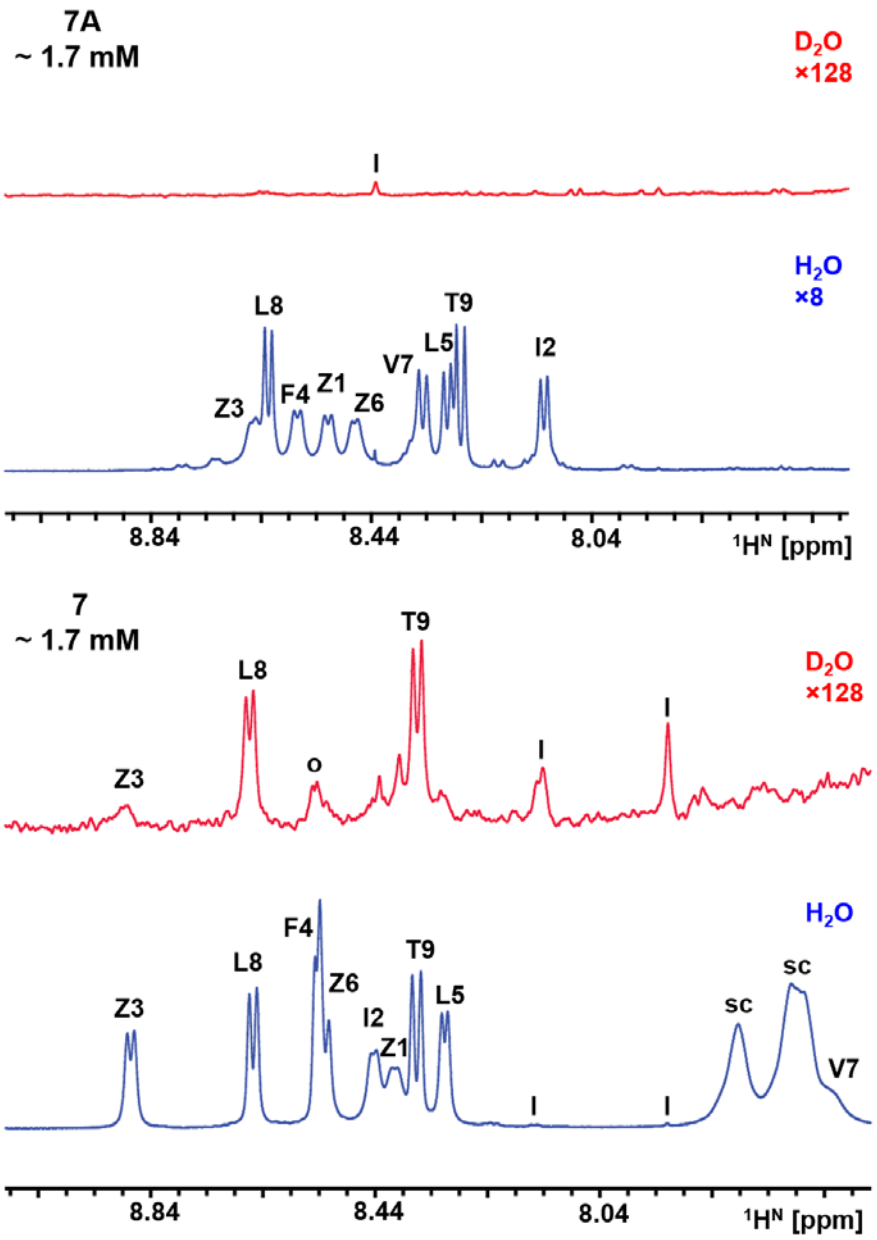
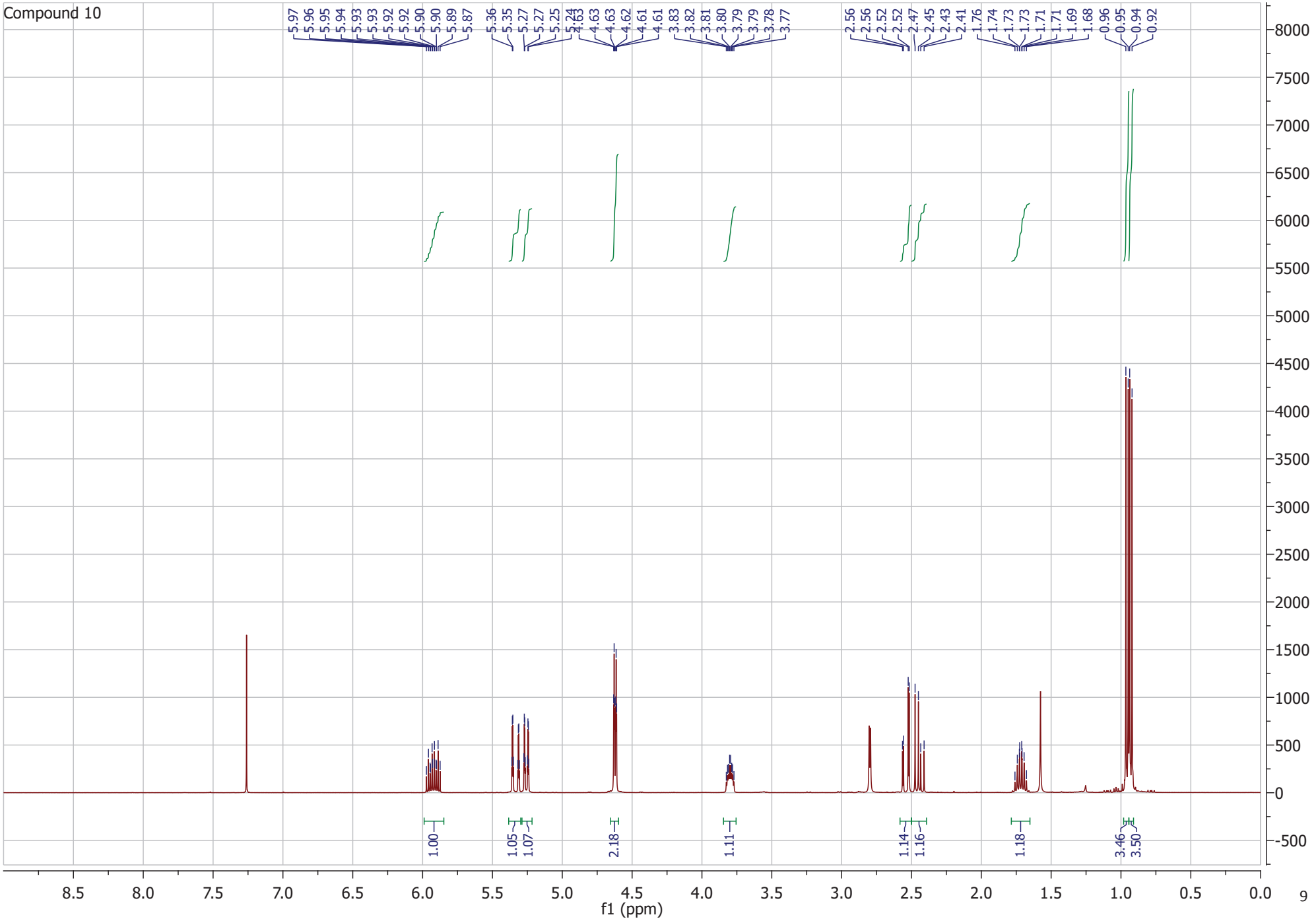


Figure S5 1D ^{1}H-NMR spectra of **7** and **7A** in water and D_2O. Only amide region is shown for clarity. Spectra were acquired from each sample with peptide concentration of ~ 1.7 mM. The dead time between the addition of D_2O to the lyophilized peptides and the beginning of data acquisition was ~ 8 minutes. The data acquisition time of each spectrum took ~ 7 minutes. All data were collected on a 600 MHz spectrometer equipped with CryoProbe at 10 °C. I, O and sc indicate unassigned peaks from sample impurities, overlapped peaks and side-chain amide peaks, respectively. I, L, T, Z, V and F indicates Ile, Leu, Thr, Dab, D-Val and D-Phe residues.

Table S1 MIC values for 7A and polymyxin B

Bacterial species	Strain	7A MIC (μg/mL)	Polymyxin B MIC (μg/mL)
Gram Negative *P. aeruginosa*	Pa ATCC 27853	>32	0.5
	FADDI-PA021	>32	0.5
	FADDI-PA025	>32	1
	FADDI-PA070	>32	>32
	FADDI-PA060	>32	4
	FADDI-PA090	>32	2
Gram Negative *A. baumannii*	Ab ATCC 19606	>32	0.5
	FADDI-AB34	>32	0.5
	Ab ATCC 17978	>32	1
	FADDI-AB065	>32	>32
	FADDI-AB156	>32	8
	FADDI-AB167	>32	8
Gram Negative *K. pneumonia*	Kp ATCC 13883	>32	1
	FADDI-KP032	16	0.5
	FADDI-KP027	>32	>32
	FADDI-KP003	>32	>32
	FADDI-KP012	>32	>32
Gram Negative *E. cloacae*	FADDI-EC006	32	0.5
	FADDI-EC001	32	0.25
	FADDI-EC003	32	0.25
Gram Positive	VRE[a] ATCC 700221	>32	>32
	MRSA ATCC 43300	>32	>32
	VISA ATCC 700698	>32	>32
	VRSA ATCC 700699	>32	>32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5.92
5.92
5.90
5.90
5.89
5.87
5.36
5.35
5.27
5.27
5.25
5.24
4.63
4.63
4.62
4.61
4.61
3.83
3.82
3.81
3.80
3.79
3.79
3.78
3.77
2.56
2.56
2.52
2.52
2.47
2.45
2.43
2.41
1.76
1.74
1.73
1.73
1.71
1.71
1.69
1.68
0.96
0.95
0.94
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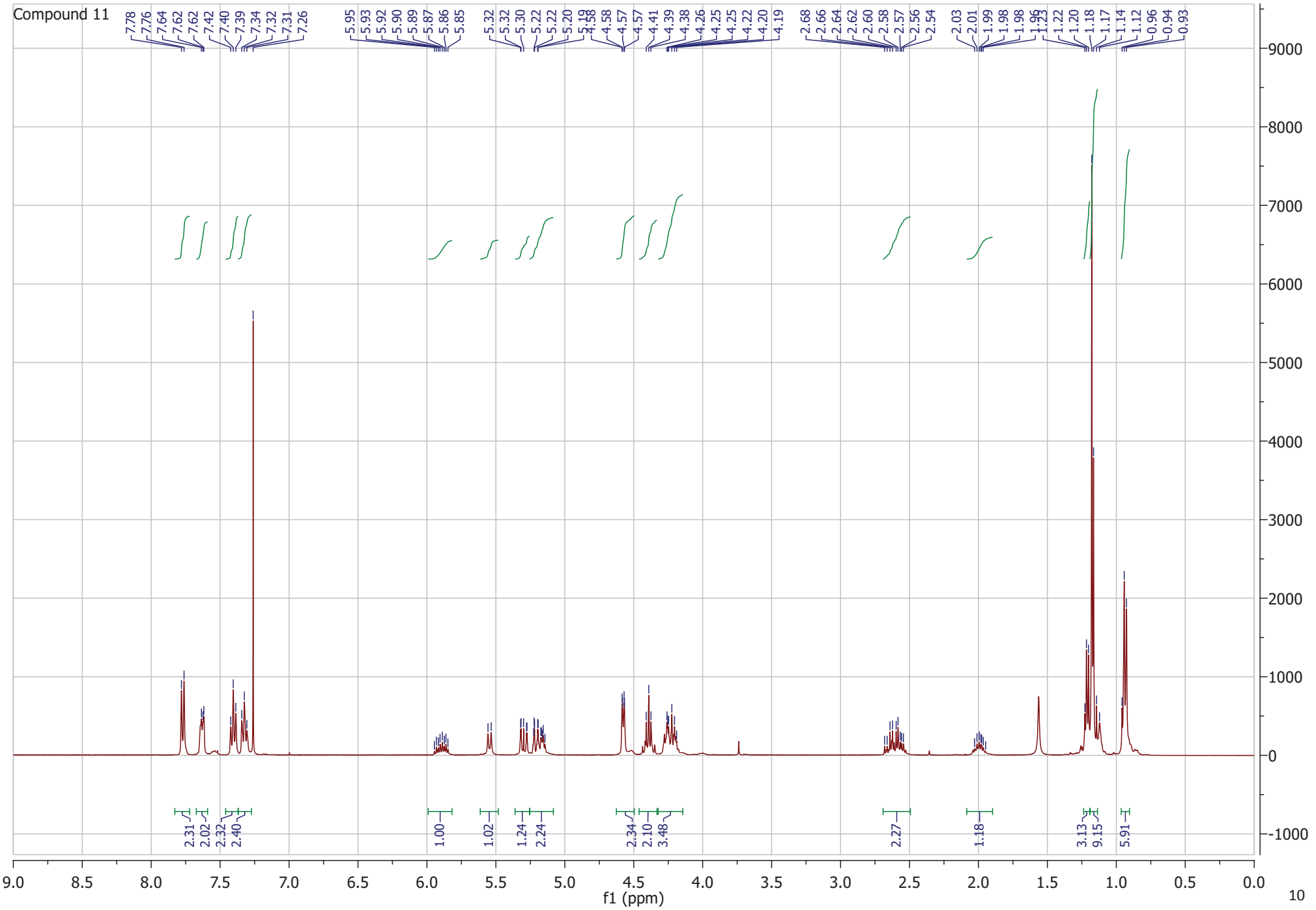
f1 (ppm)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5.95 5.93 5.92 5.90 5.89 5.87 5.86 5.85
5.32 5.32 5.30 5.22 5.22 5.20 5.19 4.58 4.58 4.57 4.57
4.41 4.39 4.38 4.26 4.25 4.25 4.22 4.20 4.19
2.68 2.66 2.64 2.62 2.60 2.58 2.57 2.56 2.54
2.03 2.01 1.99 1.98 1.98 1.96 1.22 1.20 1.18 1.17 1.14 1.12 0.96 0.94 0.93
2.31 2.02 2.32 2.40 1.00 1.02 1.24 2.24 2.34 2.10 3.48 2.27 1.18 3.13 9.15 5.91
f1 (ppm)
9000 8000 7000 6000 5000 4000 3000 2000 1000 0 -1000
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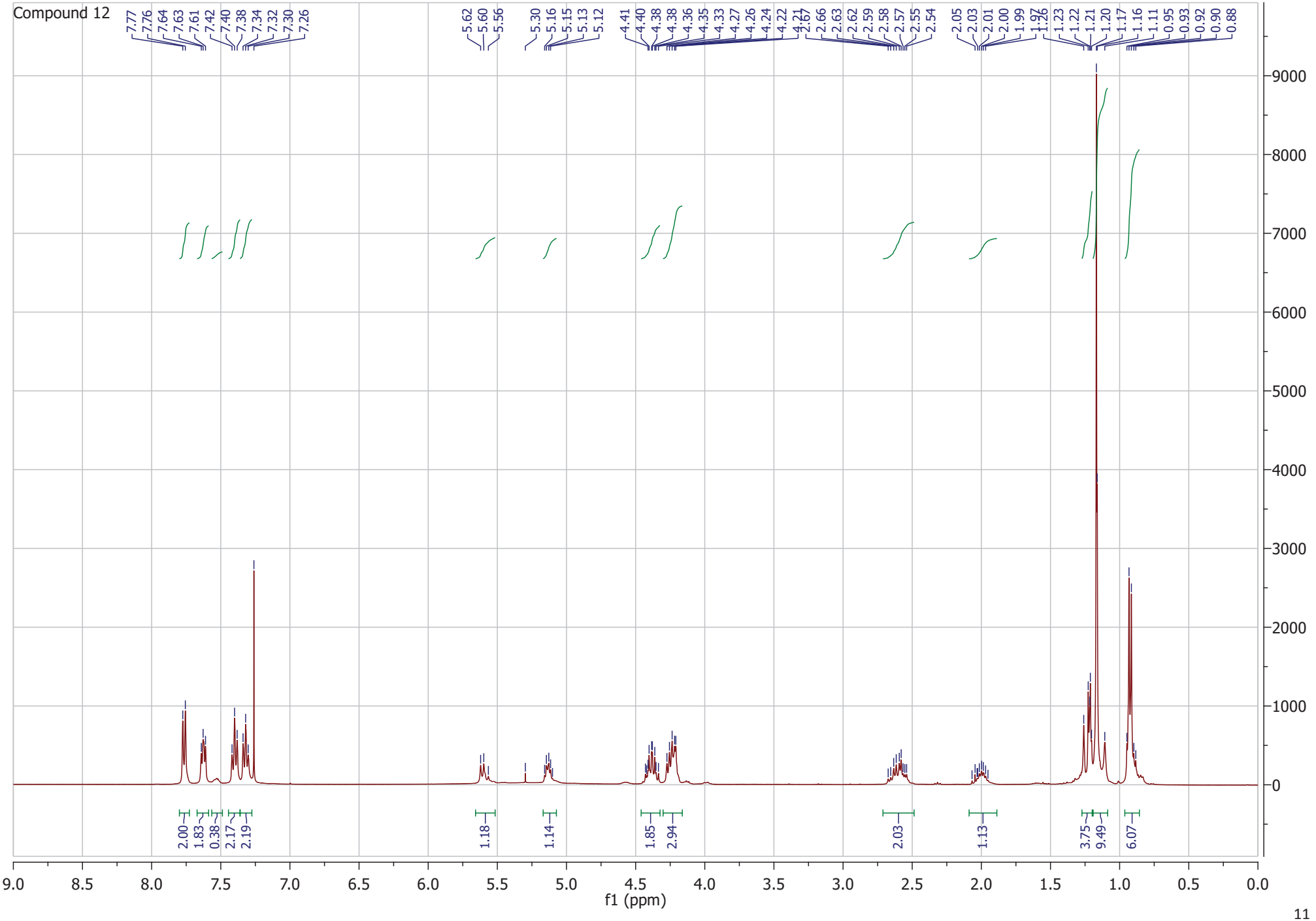
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7.61
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7.40
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7.34
7.32
7.30
7.26
5.62
5.60
5.56
5.30
5.16
5.15
5.13
5.12
4.41
4.40
4.38
4.38
4.36
4.35
4.33
4.27
4.26
4.24
4.22
4.21
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1.97
1.26
1.23
1.22
1.21
1.20
1.17
1.16
1.11
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0.92
0.90
0.88
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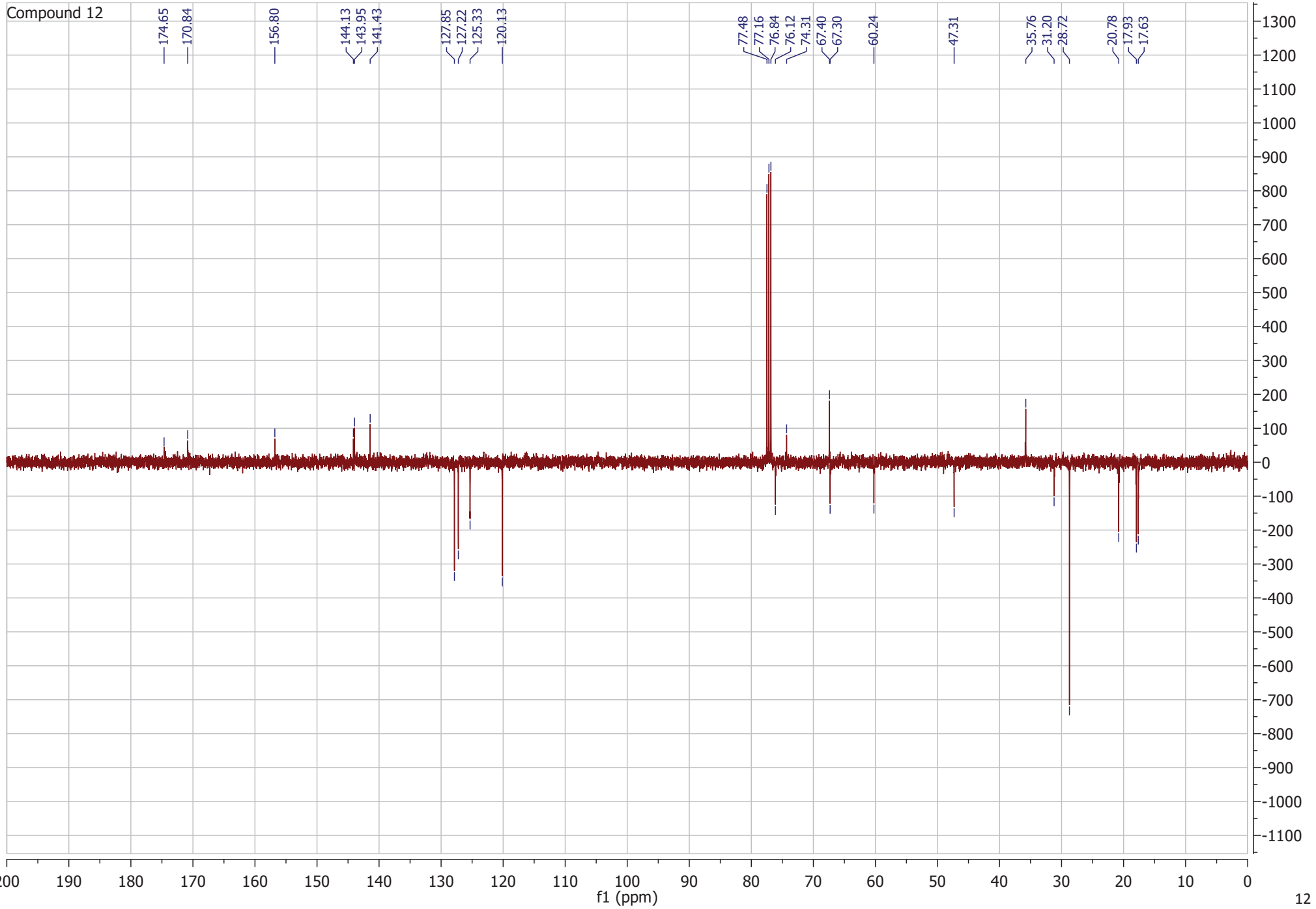
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76.84
76.12
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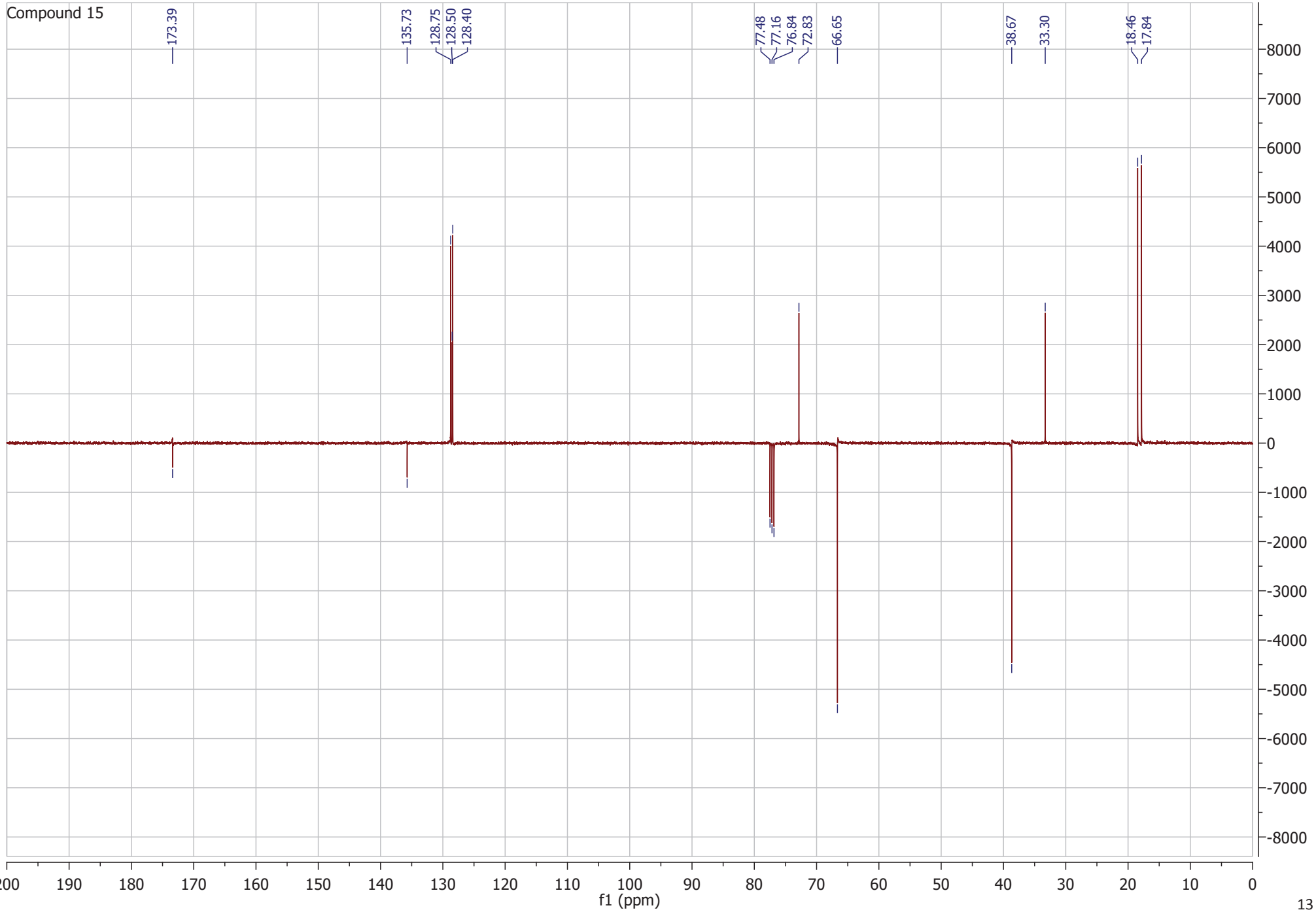
f1 (ppm)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33.30
18.46
17.84
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6000
5000
4000
3000
2000
1000
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-4000
-5000
-6000
-7000
-8000
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180
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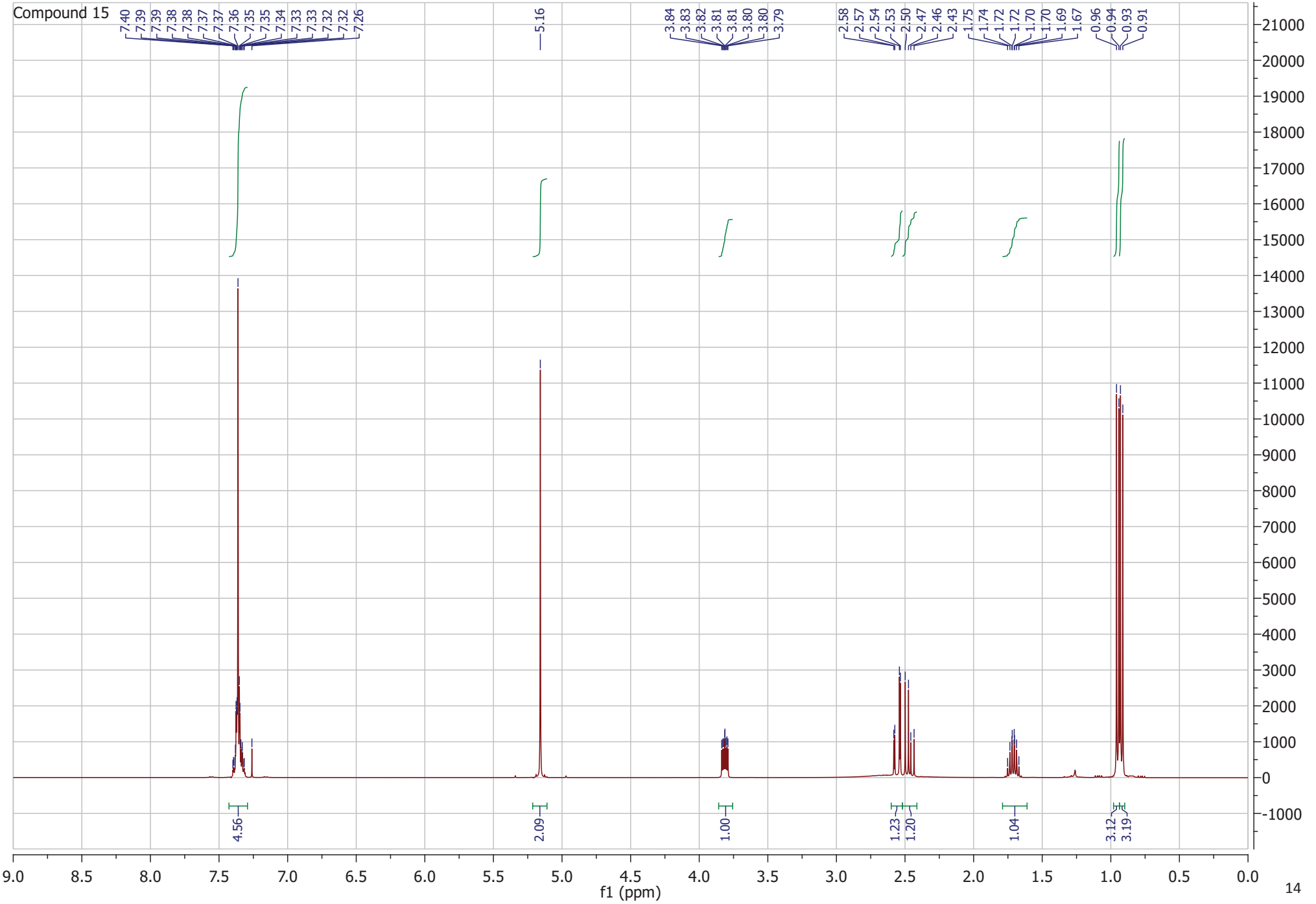
0
f1 (ppm)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7.33
7.32
7.32
7.26
5.16
3.84
3.83
3.82
3.81
3.81
3.80
3.80
3.79
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2.57
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2.43
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1.72
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1.70
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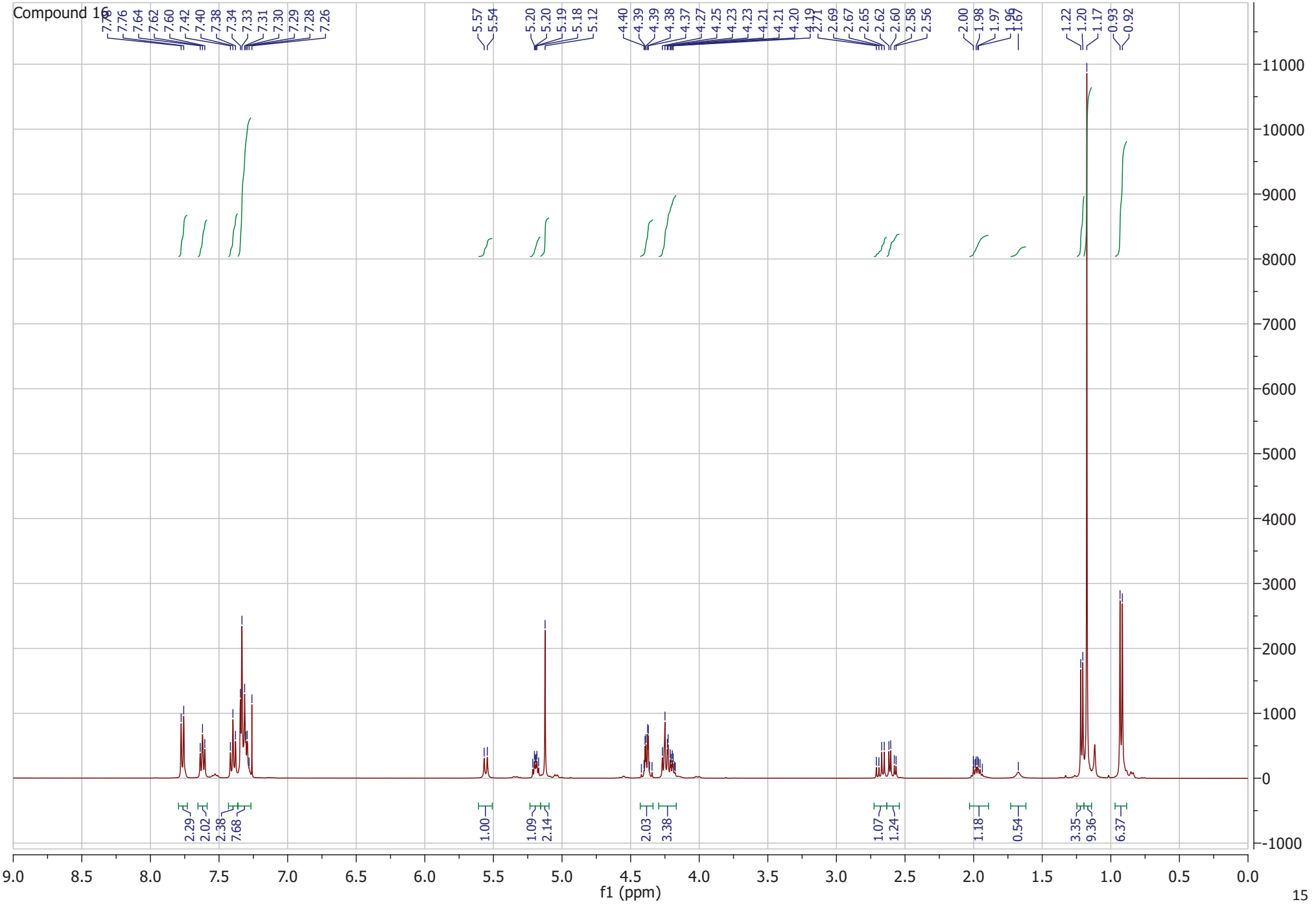
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7.29
7.28
7.26
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5.54
5.20
5.20
5.19
5.18
5.12
4.40
4.39
4.39
4.38
4.37
4.27
4.25
4.23
4.23
4.21
4.21
4.20
4.19
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2.69
2.67
2.65
2.62
2.60
2.58
2.56
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1.96
1.67
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0.92
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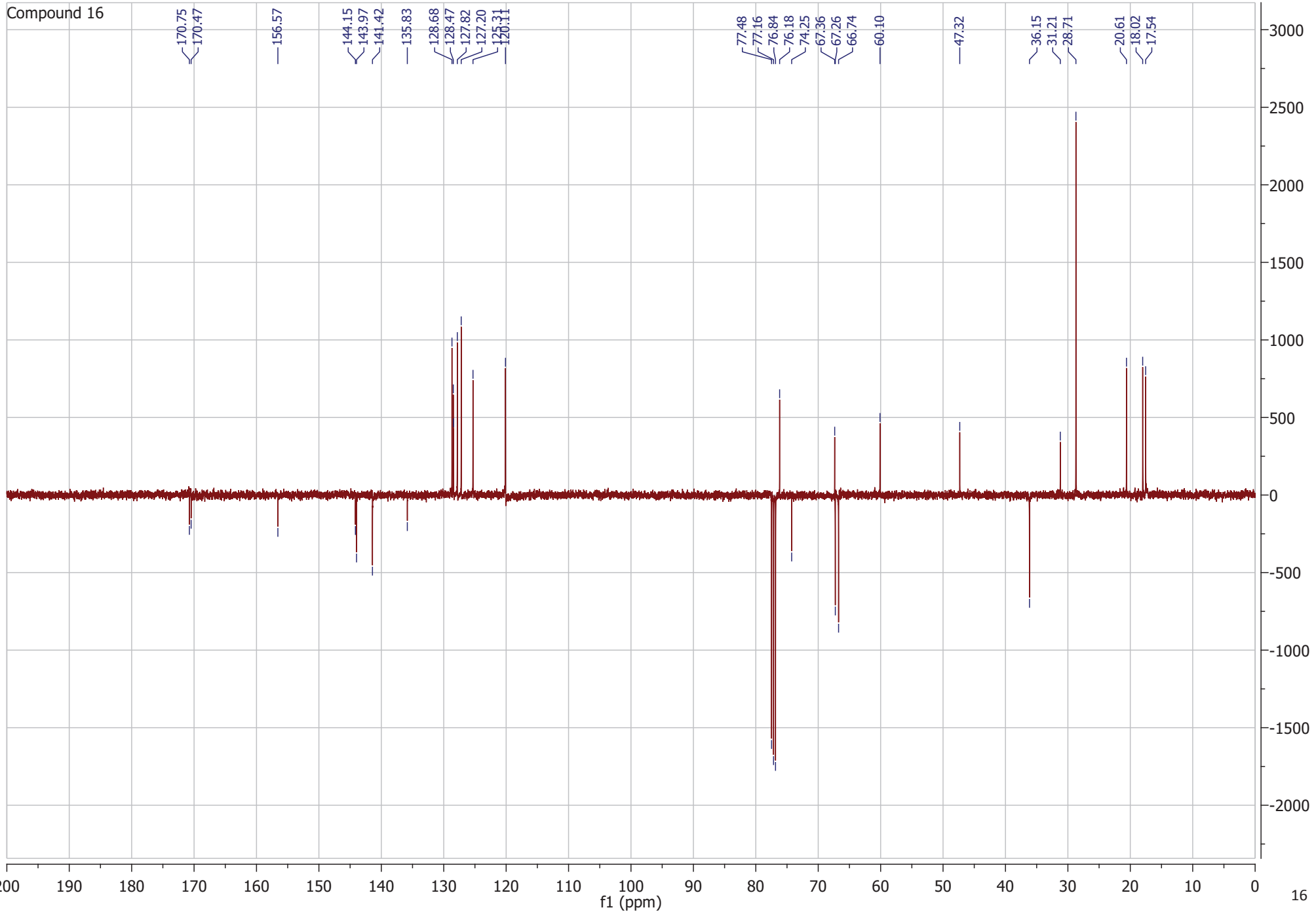
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76.84
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67.26
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36.15
31.21
28.71
20.61
18.02
17.54
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2000
1500
1000
500
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-1000
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180
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f1 (ppm)
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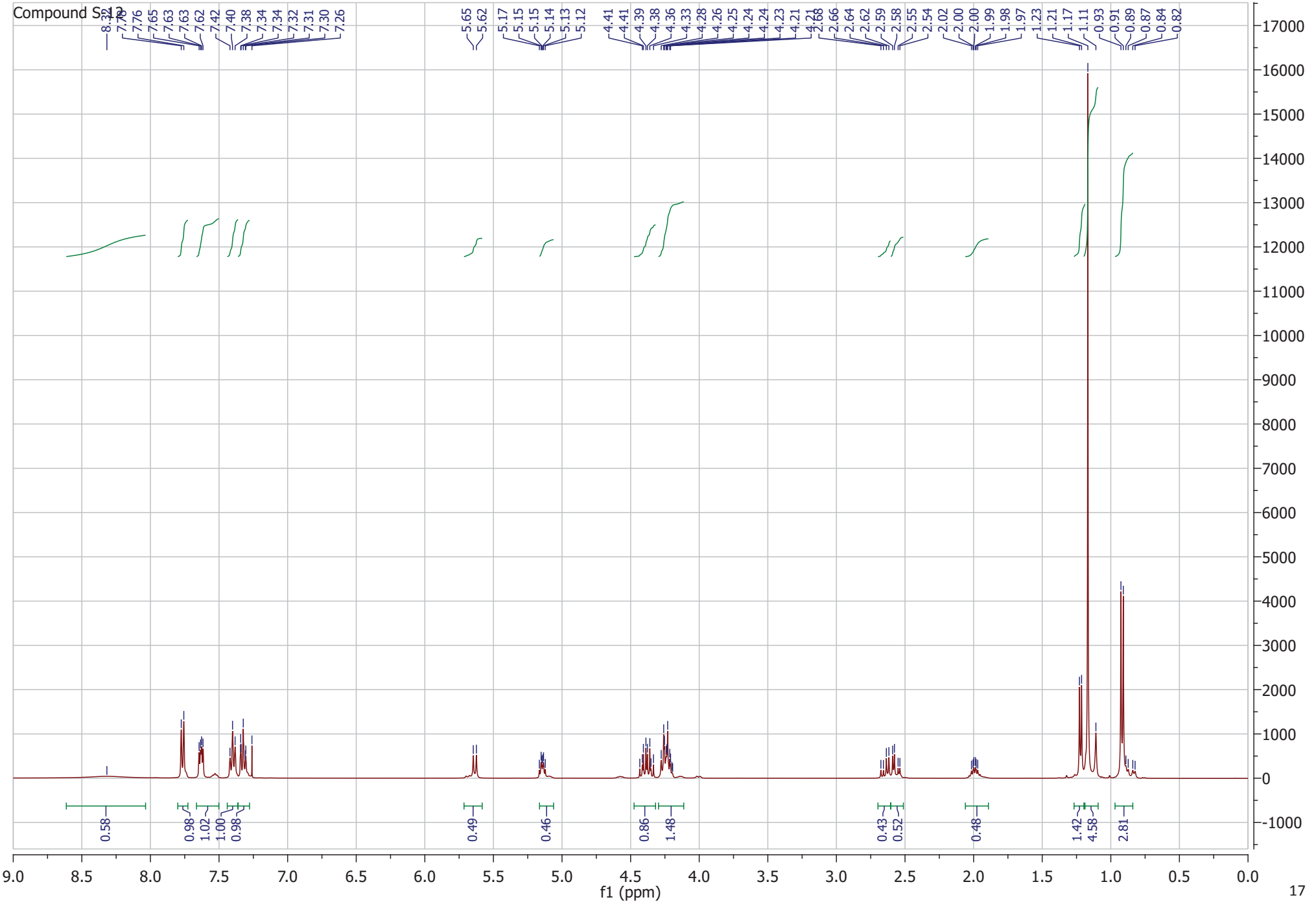
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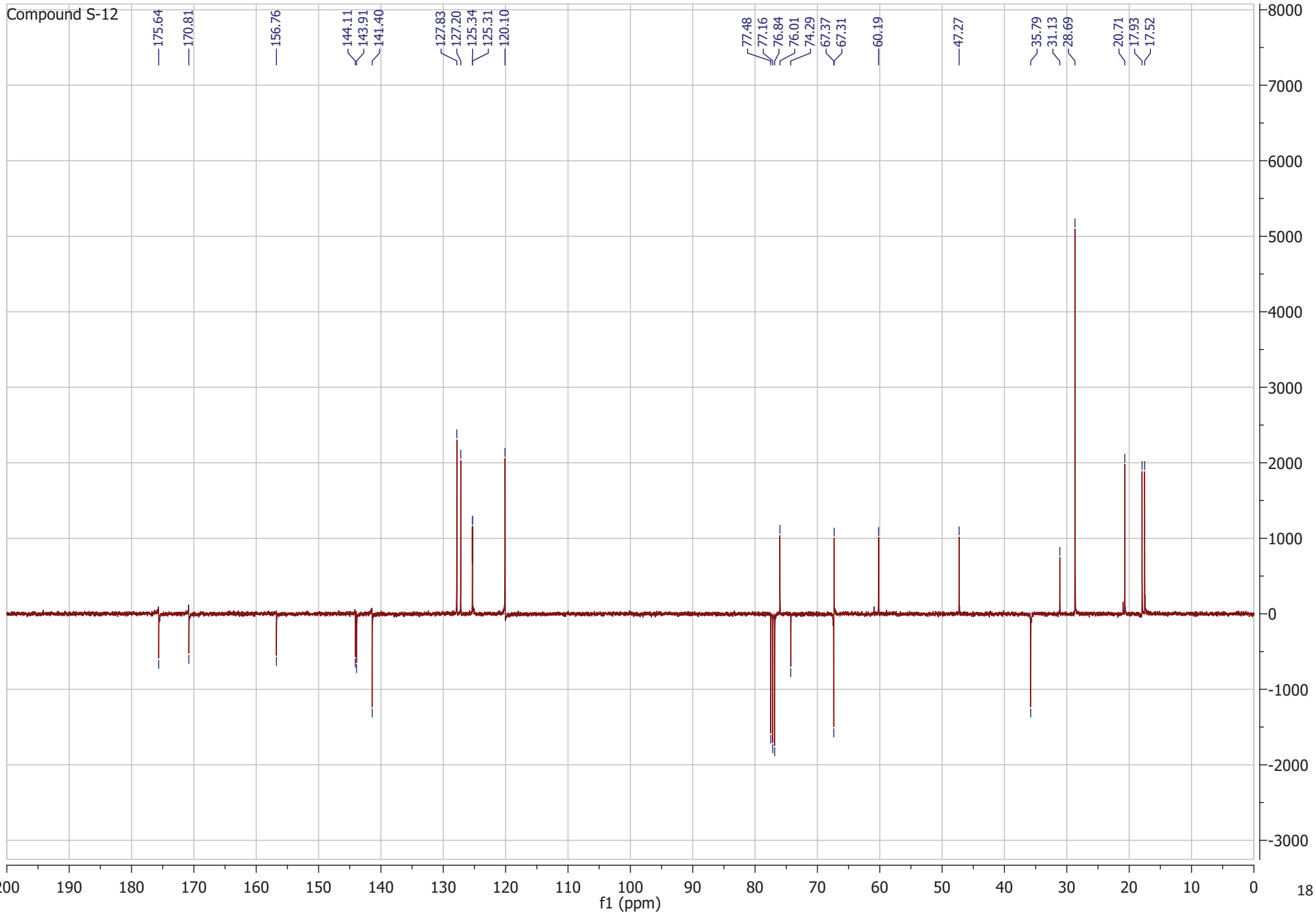
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77.16
76.84
76.01
74.29
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67.31
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47.27
35.79
31.13
28.69
20.71
17.93
17.52
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5000
4000
3000
2000
1000
0
-1000
-2000
-3000
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190
180
170
160
150
140
130
120
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90
80
70
60
50
40
30
20
10
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f1 (ppm)

Qualitative Compound Report

Data File	SJM-402-50.d	**Sample Name**	SJM-402-50
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Acq Method	Monash_Direct.m	**Acquired Time**	23-Feb-15 11:24:29 AM
IRM Calibration Status	Success	**DA Method**	Monash_Accuracy.m
Comment			

Sample Group		**Info.**	
Formula	C29H37NO7	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
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Compound Label	*m/z*	RT	Algorithm	Mass
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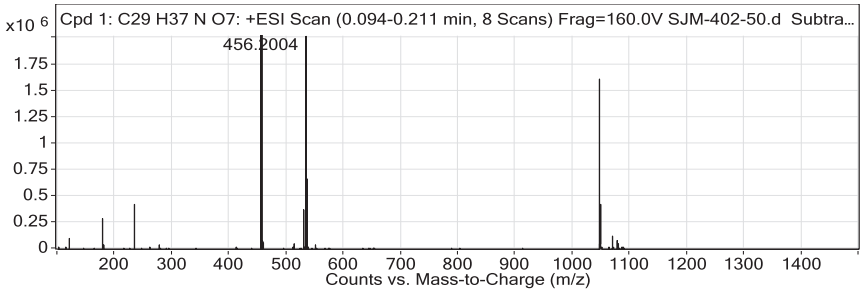
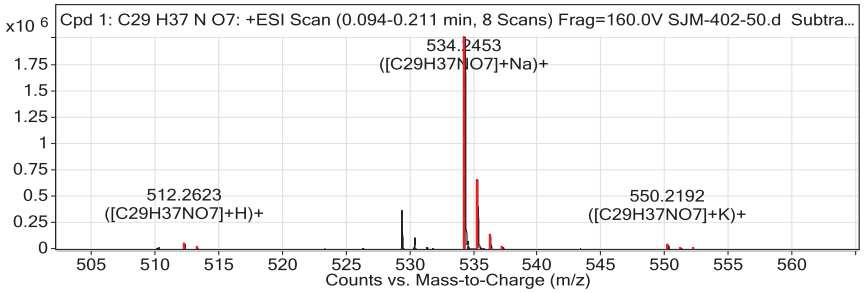
MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
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534.2453	534.2462	1.73	1	2025250.44	C29H37NO7	(M+Na)+
550.2192	550.2202	1.81	1	45588.23	C29H37NO7	(M+K)+

--- End Of Report ---

Qualitative Compound Report

HR-MS Compound 3S-12

Data File	SJM-402-82.d	**Sample Name**	SJM-402-82
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IRM Calibration Status	Success	**DA Method**	Monash_Accuracy.m
Comment			
Sample Group		**Info.**	
Formula	C29H37NO7	**Stream Name**	LC 1
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.06.01 (B6172 SP1)		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
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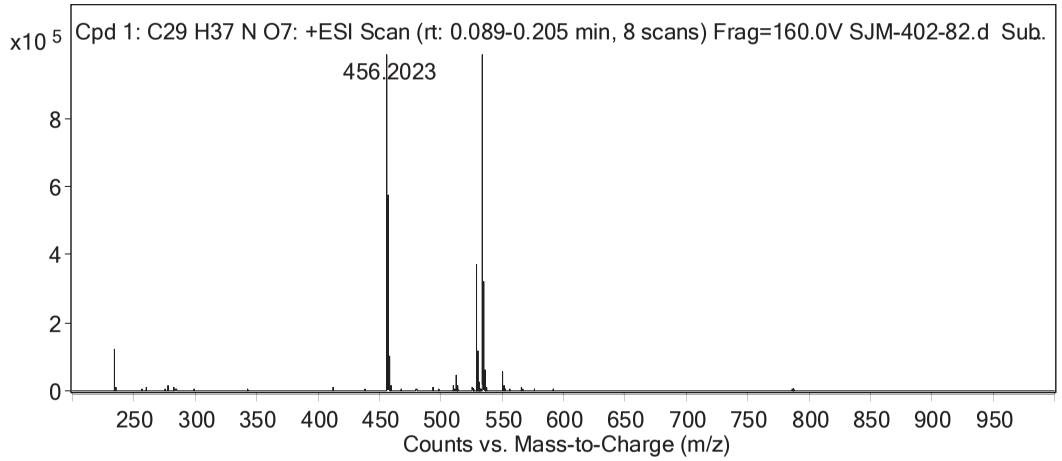
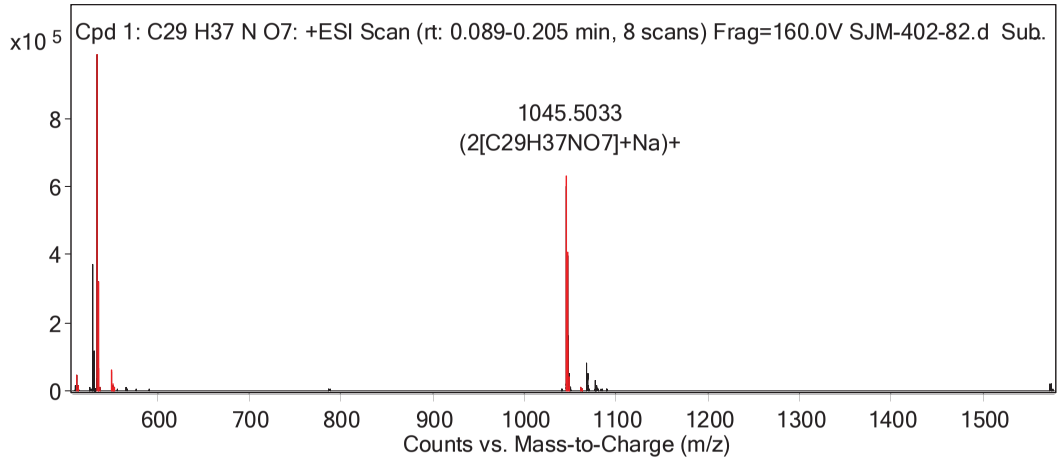
MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

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550.2196	550.2202	1.03	1	58800.23	C29H37NO7	(M+K)+
1045.5033	1045.5032	-0.07	1	603627.94	C29H37NO7	(2M+Na)+

--- End Of Report ---

Qualitative Compound Report

HR-MS Compound 16

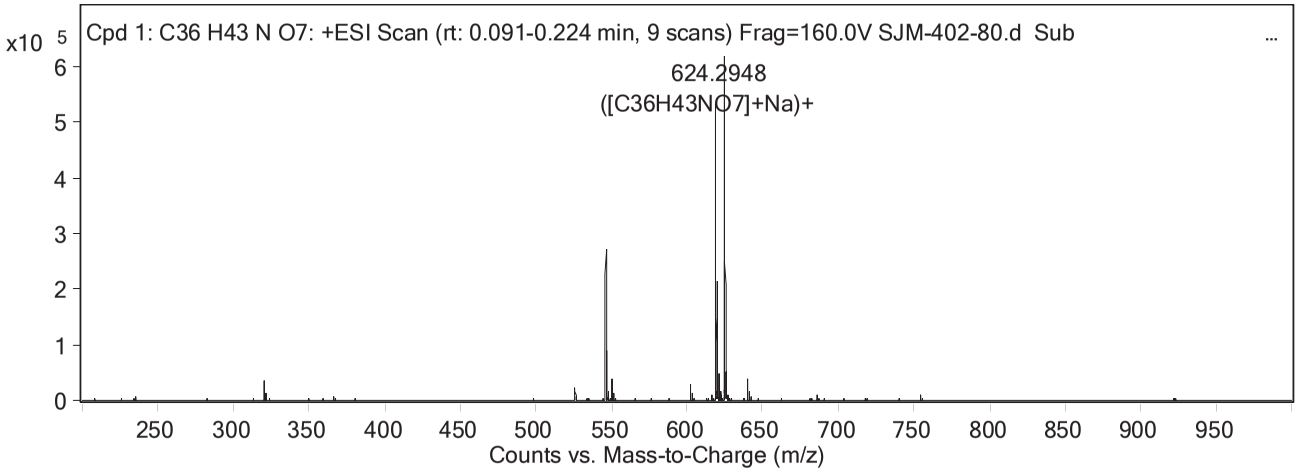
Data File	SJM-402-80.d	**Sample Name**	SJM-402-80
Sample Type	Sample	**Position**	P2-B4
Instrument Name	Instrument 1	**User Name**	Dr Jason Dang
Acq Method	Monash_Direct.m	**Acquired Time**	29-Nov-16 11:37:42 PM
IRM Calibration Status	Success	**DA Method**	Monash_Accuracy.m
Comment			
Sample Group		**Info.**	
Formula	C36H43NO7	**Stream Name**	LC 1
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.06.01 (B6172 SP1)		

Compound Table

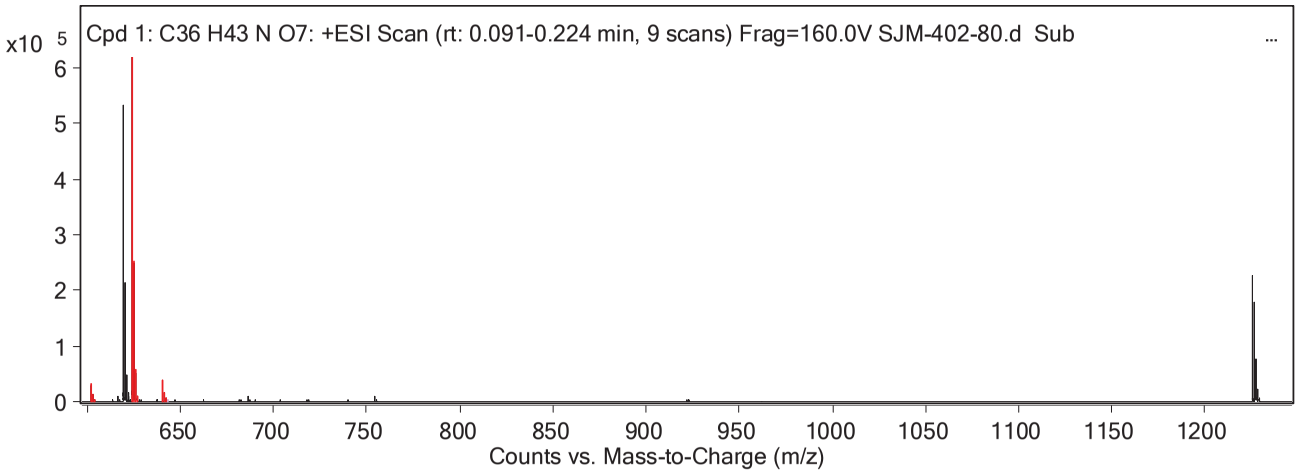
Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C36 H43 N O7	0.124	601.3052	30568	C36 H43 N O7	601.304	2.12

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C36 H43 N O7	602.3113	0.124	Find By Formula	601.3052

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	Calc *m/z*	Diff(ppm)	z	Abund	Formula	Ion
624.2948	624.2932	-2.65	1	632140.37	C36H43NO7	(M+Na)+
640.2675	640.2671	-0.59	1	37994.11	C36H43NO7	(M+K)+
1225.5987	1225.5971	-1.25	1	228312.75	C36H43NO7	(2M+Na)+

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