

Supporting Information

Regiospecific Synthesis of Ring A Fused Withaferin A Isoxazoline Analogues: Induction of Premature Senescence by W-2b in Proliferating Cancer Cells

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³Cancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Jammu-180001, India.

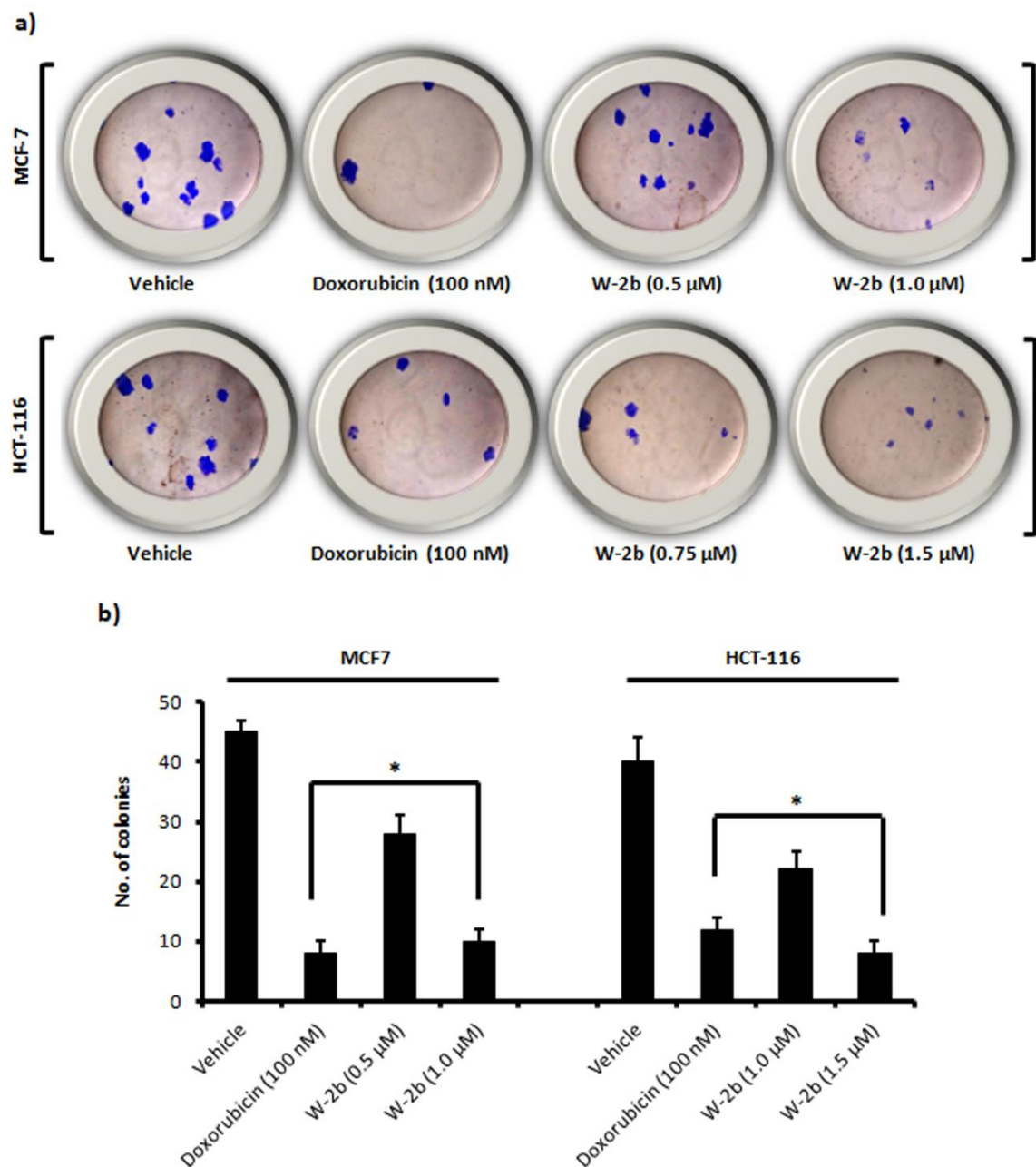
⁴Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Srinagar-190005, India.

⁵Natural Product Chemistry-Plants, CSIR-Indian Institute of Integrative Medicine, Jammu-180001, India.

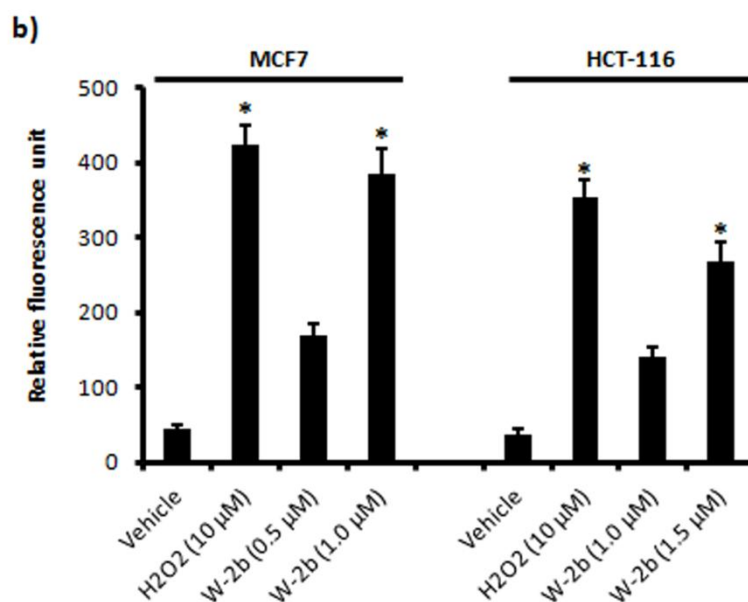
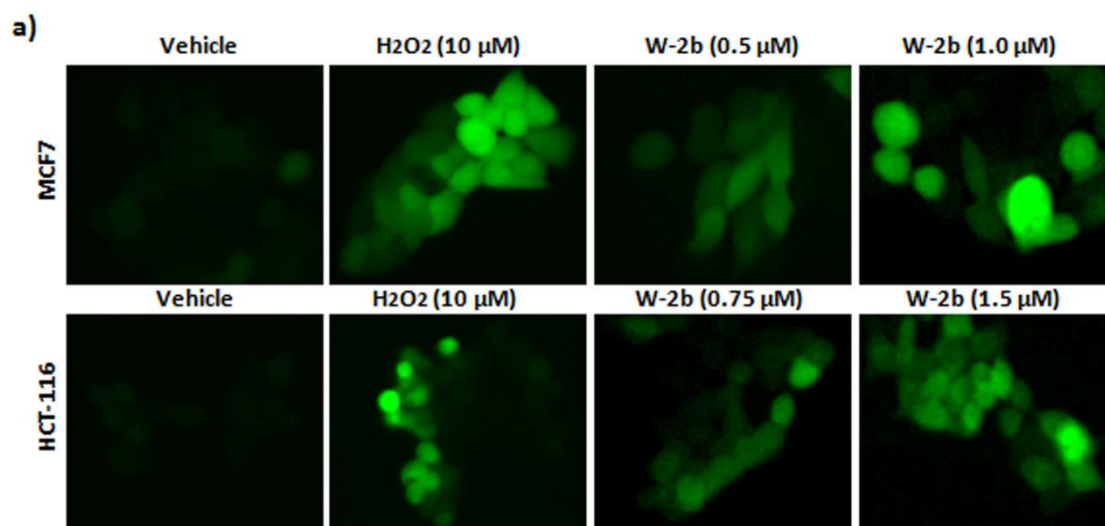
Table of Contents:

Sl. No.	Contents	Page No.
1	Supporting figures	S2-S3
2	HPLC of W-2b	S4
3	HRMS OF W-2b	S5
4	Copies of ¹ H NMR, ¹³ C NMR	S6-S43
5	2D NMR	S44-S51

1. Supplementary figures



Supplementary Figure S1. W-2b inhibits cell proliferation. (a) Effect of vehicle, doxorubicin and increasing concentrations of **W-2b** on colony formation ability of MCF7 and HCT-116 cells. Original magnification 20x. (b) Bar graph showing the quantification of number of colonies in vehicle and each treatment conditions. Error bars: mean \pm s.d., * $P < 0.05$.



Supplementary Figure S2. Induction of ROS generation by **W-2b**. (a) Effects of vehicle, H₂O₂ (10 μM) and increasing concentrations of **W-2b** on ROS generation in MCF7 and HCT-116 cells. Original magnification 20x. (b) Bar graph showing the quantification of ROS generation in terms of relative fluorescence units (RFU) in each case. Error bars: mean ± s.d. of three independent experiments. **P* < 0.05.

HPLC of W-2b

NPC DIVISION IIIM JAMMU

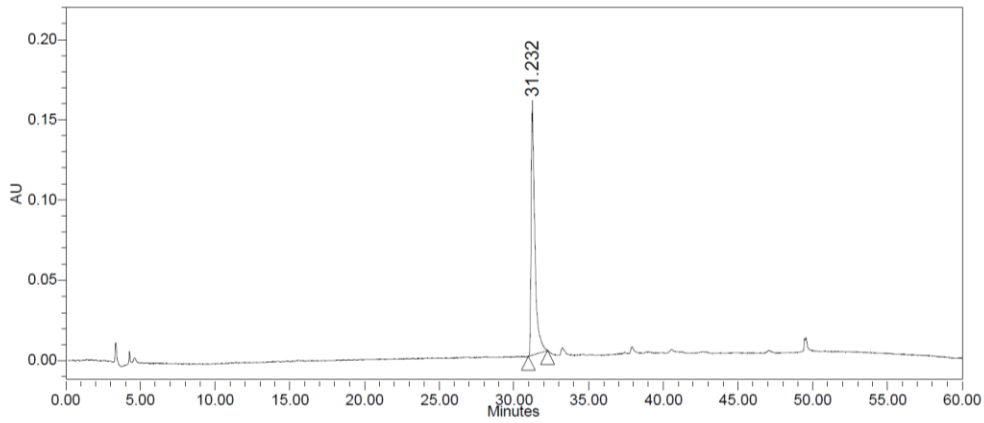
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Project Name: NP

SAMPLE INFORMATION

Sample Name:	W-2a	Acquired By:	System
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Vial:	5	Acq. Method Set:	Withania
Injection #:	1	Date Processed:	5/15/2015 4:07:11 PM
Injection Volume:	5.00 ul	Processing Method:	Withaferin A
Run Time:	60.0 Minutes	Channel Name:	W2996 237.0nm-1.2
Sample Set Name:	Withania	Proc. Chnl. Descr.:	W2996 PDA 237.0 nm at 1.2
column_name	RP-18,5um	Flow rate:	0.70 ml/min
		Sample conc:	1 mg/ml
		Mobile phase:	ACN:Water(gradient)

Auto-Scaled Chromatogram



	RT	Area ($\mu\text{V}\cdot\text{sec}$)	% Area	Height (μV)
1	31.232	2717828	100.00	152379

HRMS of W-2b

Qualitative Compound Report

Data File W-2b **Sample Name** W-2b
Sample Type Sample **Position** Vial 7
Instrument Name Instrument 1 **User Name**
Acq Method vishal_12-01-13.m **Acquired Time** 17-06-2015 PM 12:52:38
IRM Calibration Status Success **DA Method** daily_report.m
Comment

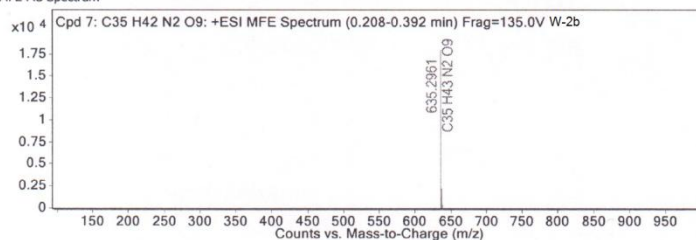
Sample Group Info.
Acquisition SW 6200 series TOF/6500 series
Version Q-TOF B.05.01 (B5125)

Compound Table

Compound Label	RT	Mass	Formula	MFG Formula	MFG Diff (ppm)	DB Formula
Cpd 7: C35 H42 N2 O9	0.265	634.2888	C35 H42 N2 O9	C35 H42 N2 O9	0.36	C35 H42 N2 O9

Compound Label	m/z	RT	Algorithm	Mass
Cpd 7: C35 H42 N2 O9	635.2961	0.265	Find by Molecular Feature	634.2888

MFE MS Spectrum



MS Spectrum Peak List

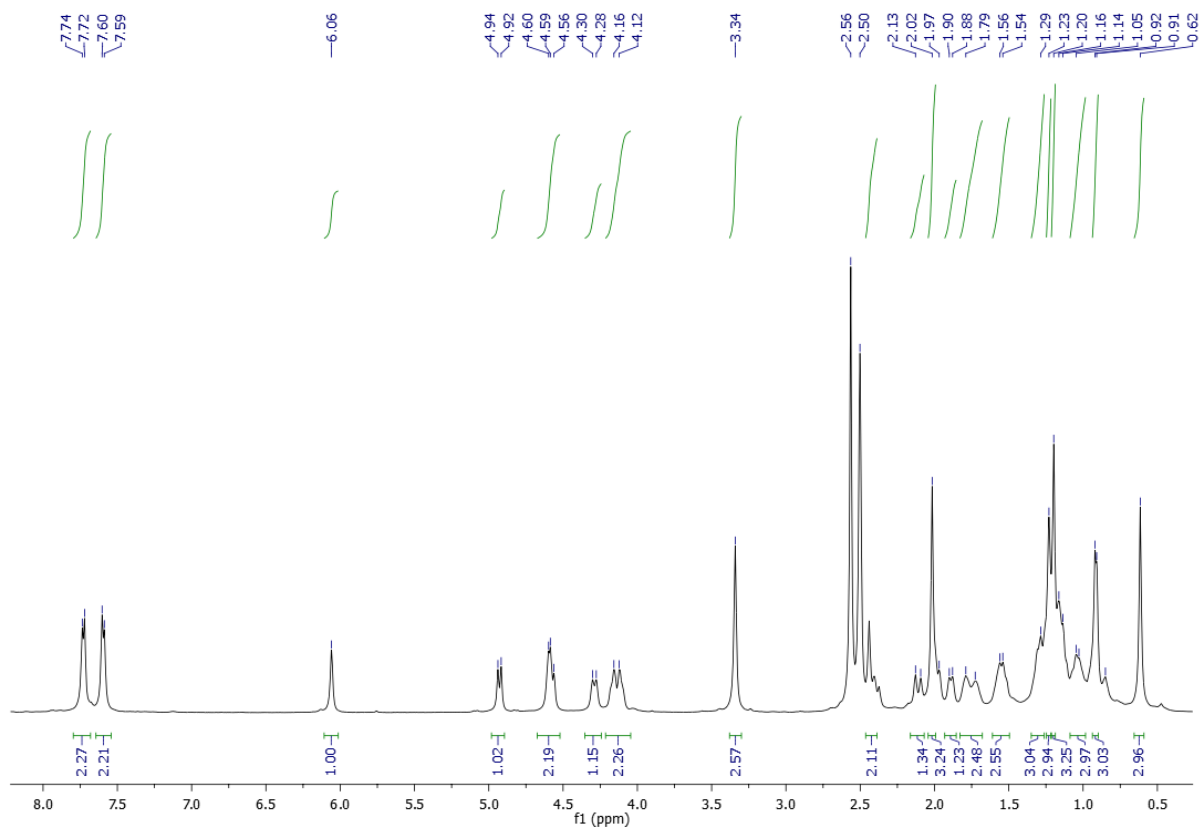
m/z	z	Abund	Formula	Ion
635.2961	1	17890.38	C35 H43 N2 O9	(M+H)+
636.2992	1	6736.49	C35 H43 N2 O9	(M+H)+
637.3036	1	2241.15	C35 H43 N2 O9	(M+H)+
638.3013	1	443.86	C35 H43 N2 O9	(M+H)+

Predicted Isotope Match Table

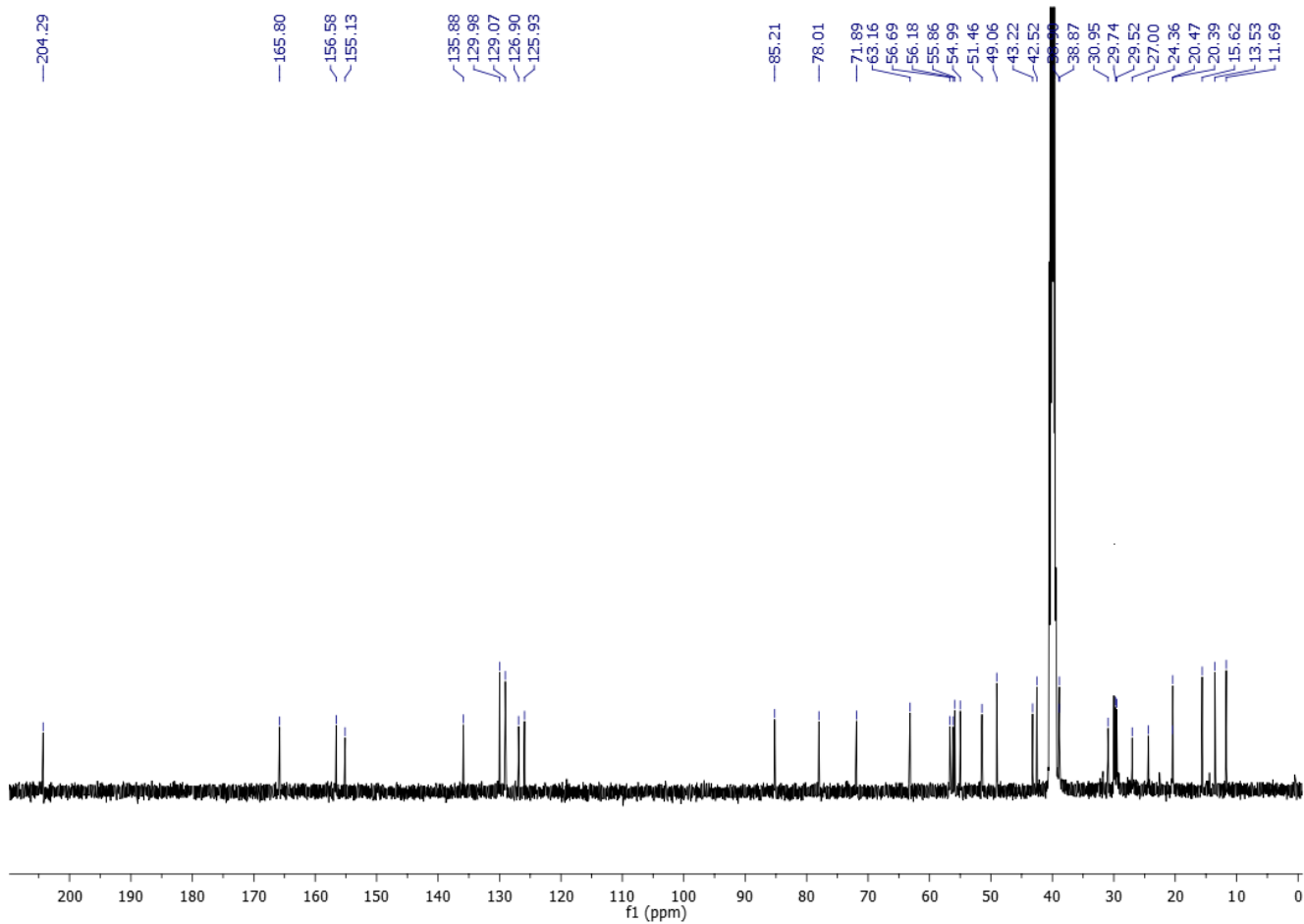
Isotope	m/z	Calc m/z	Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund Sum %
1	635.2961	635.2963	0.38	100	100	65.5	66.44
2	636.2992	636.2996	0.66	37.65	39.42	24.67	26.19
3	637.3036	637.3024	-1.82	12.53	9.41	8.21	6.26
4	638.3013	638.3051	6.06	2.48	1.67	1.63	1.11

--- End Of Report ---

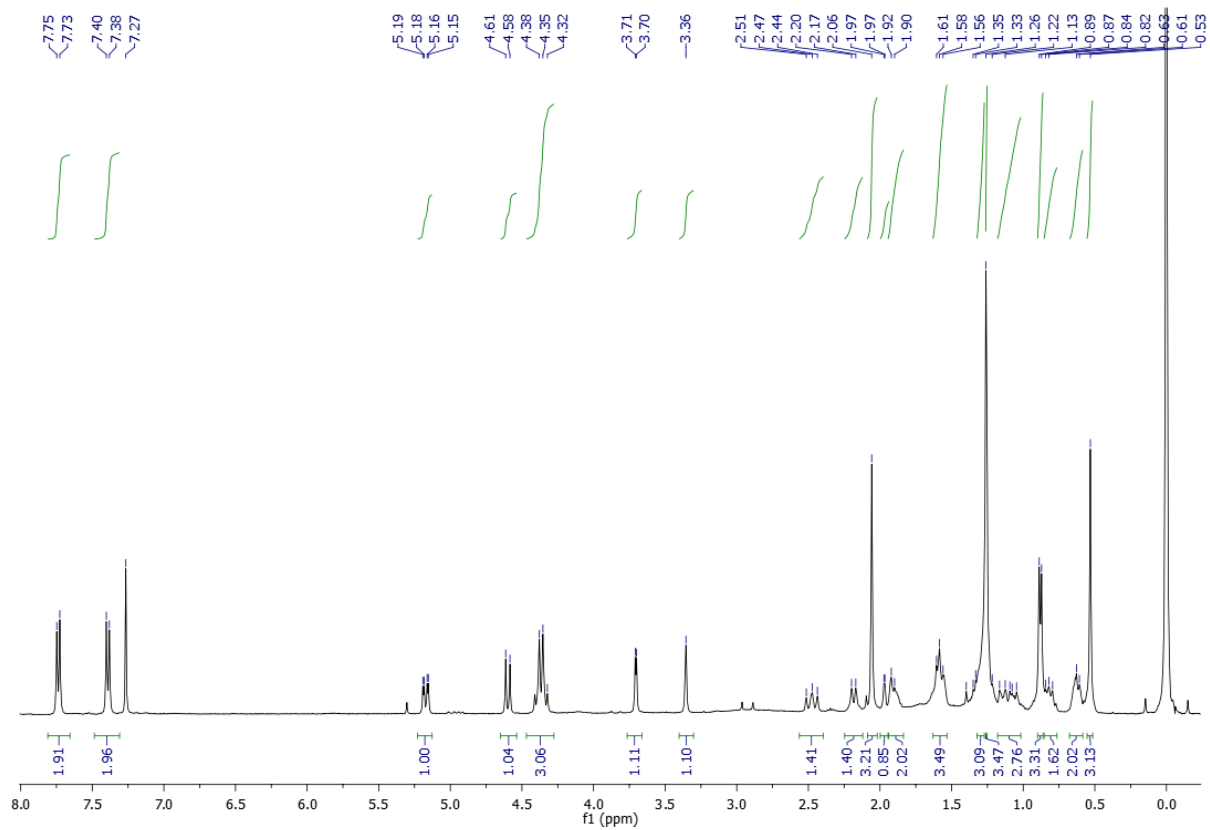
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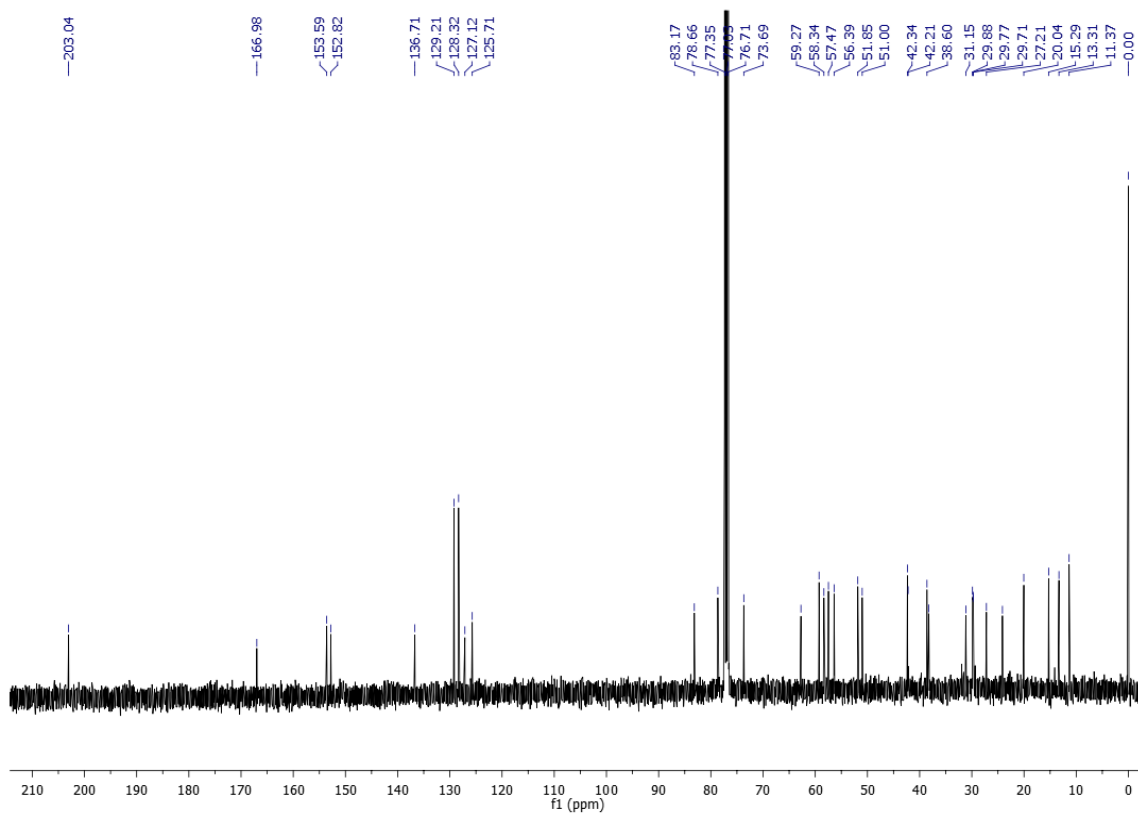
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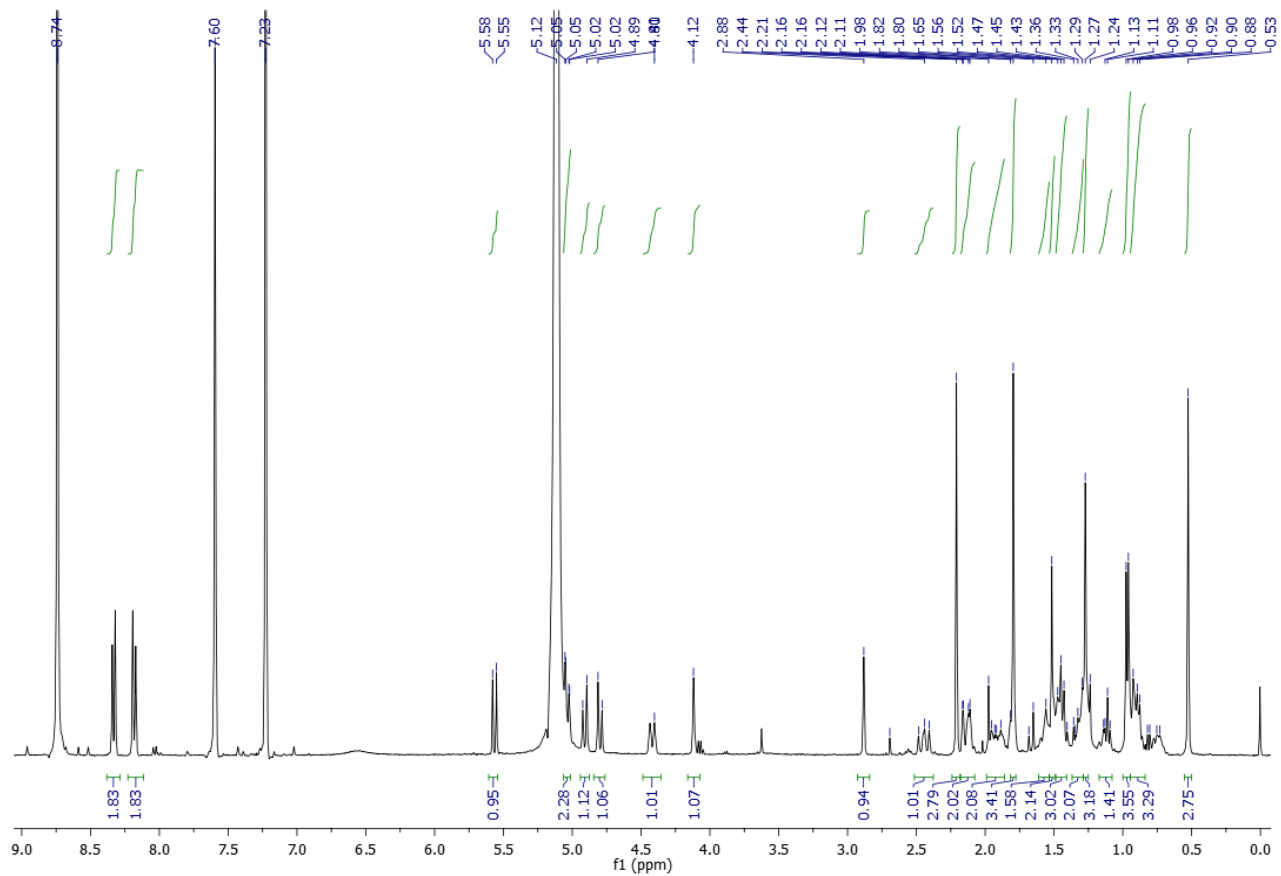
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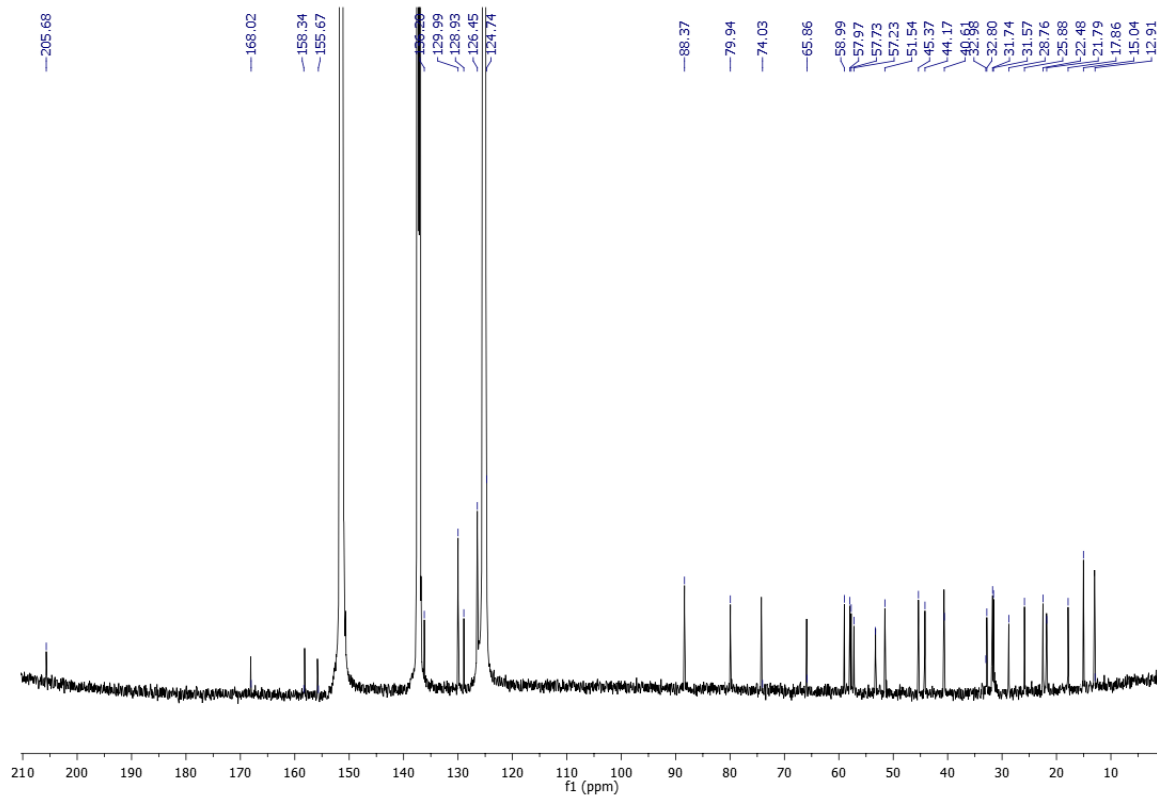
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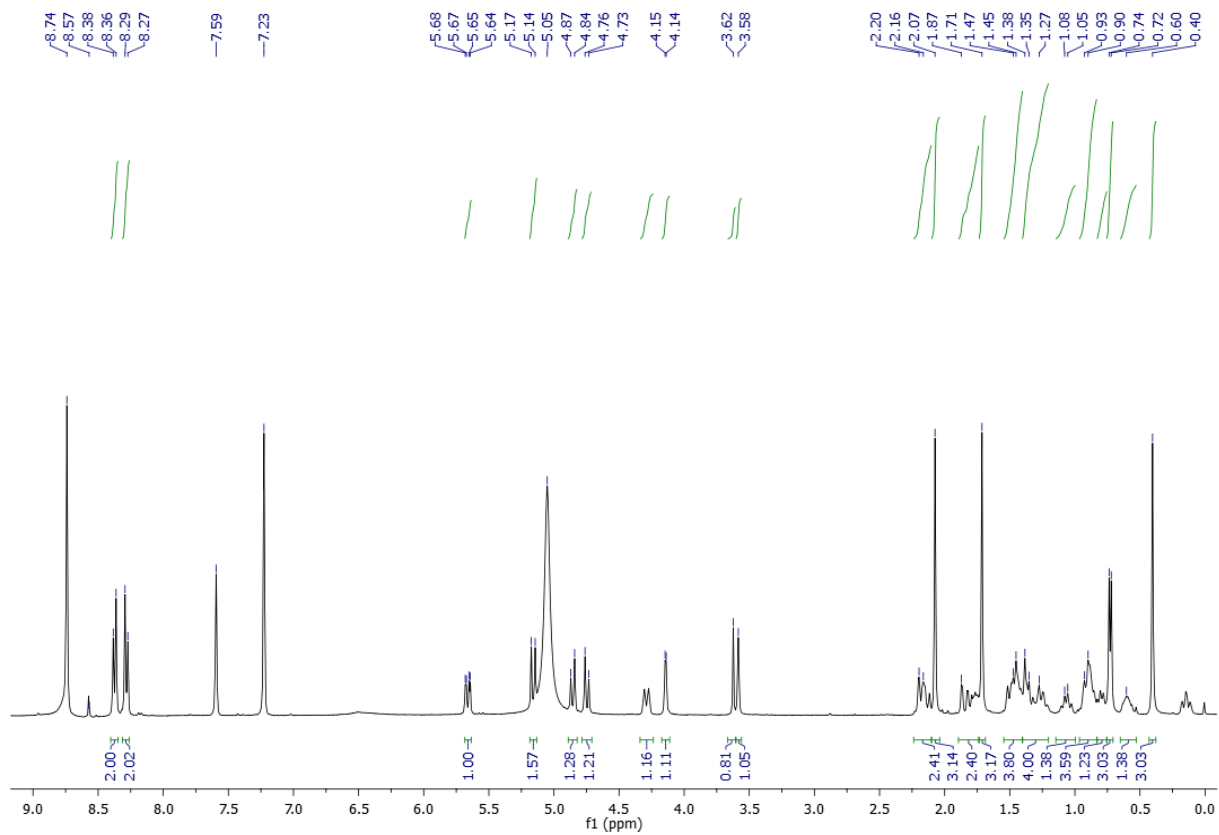
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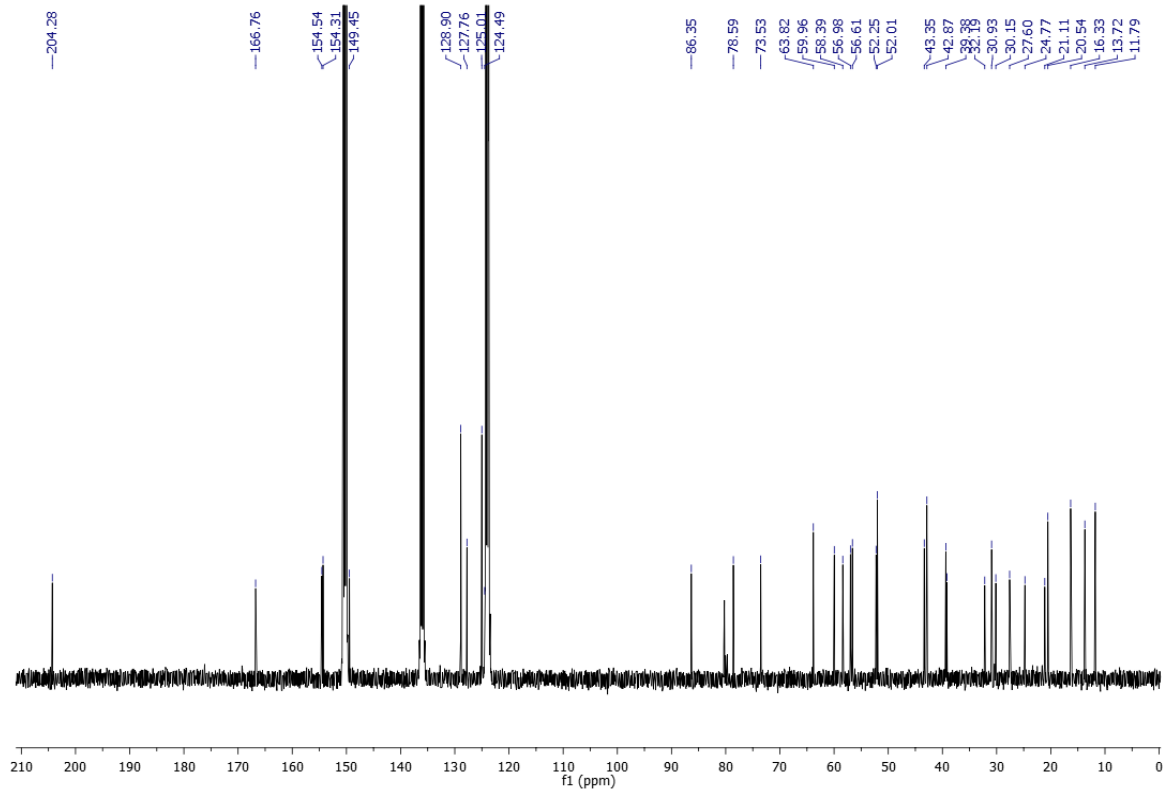
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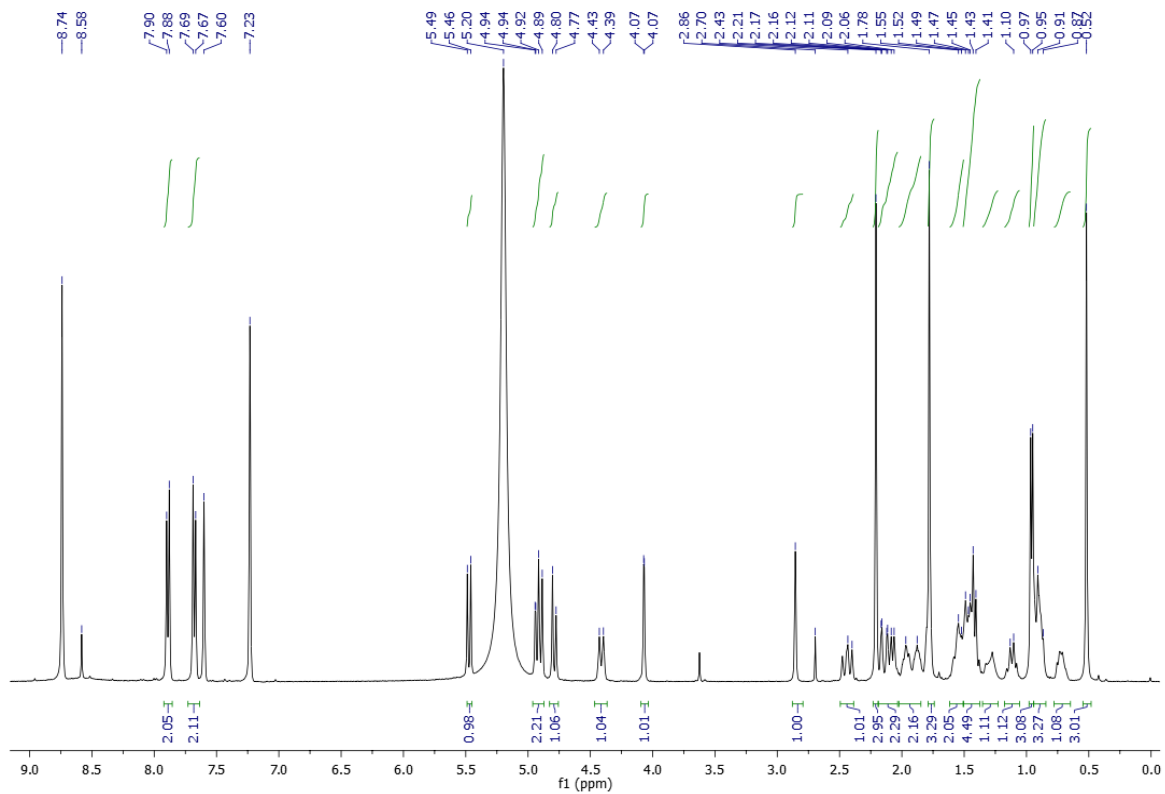
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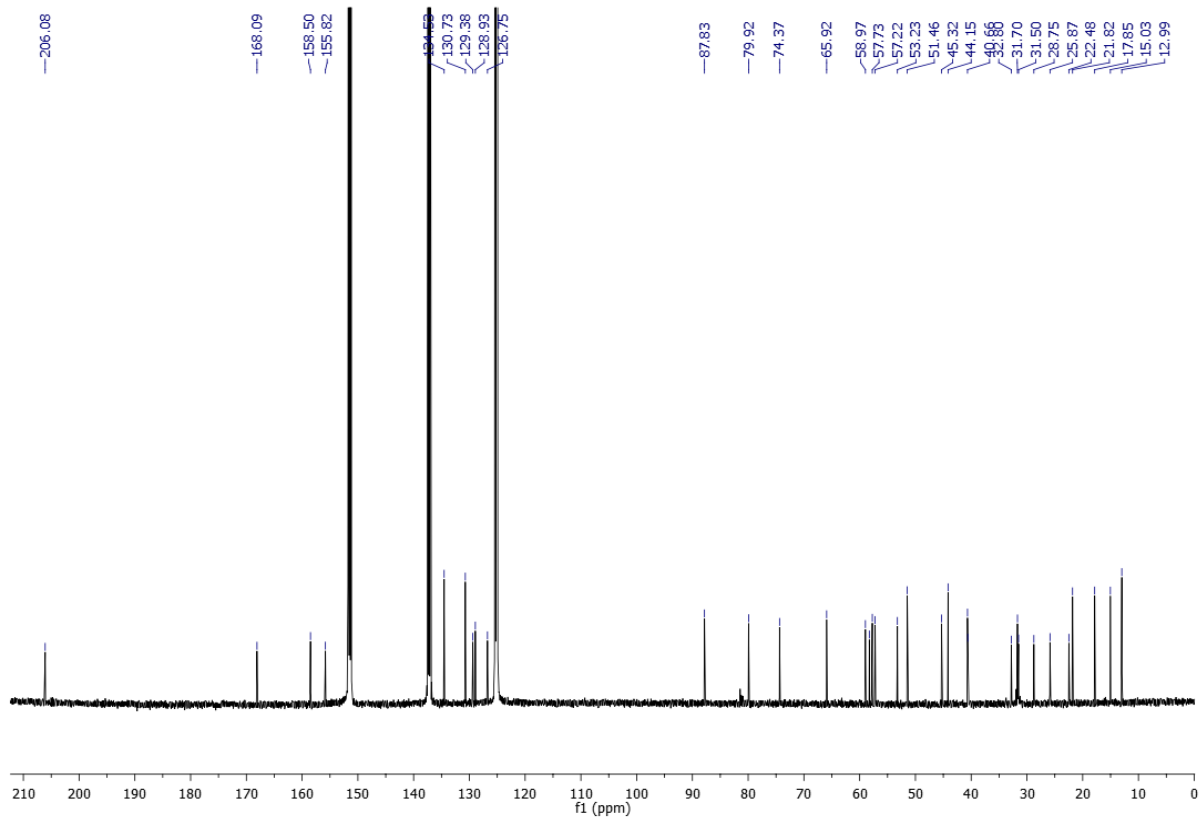
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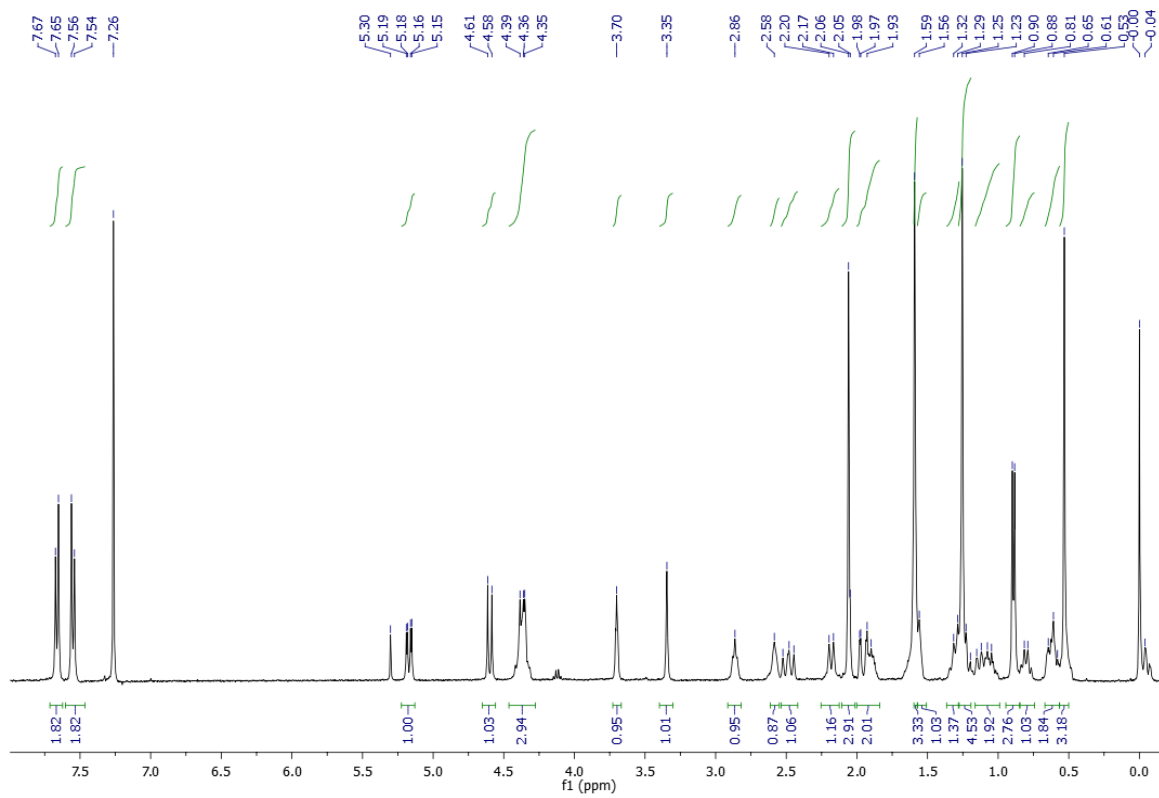
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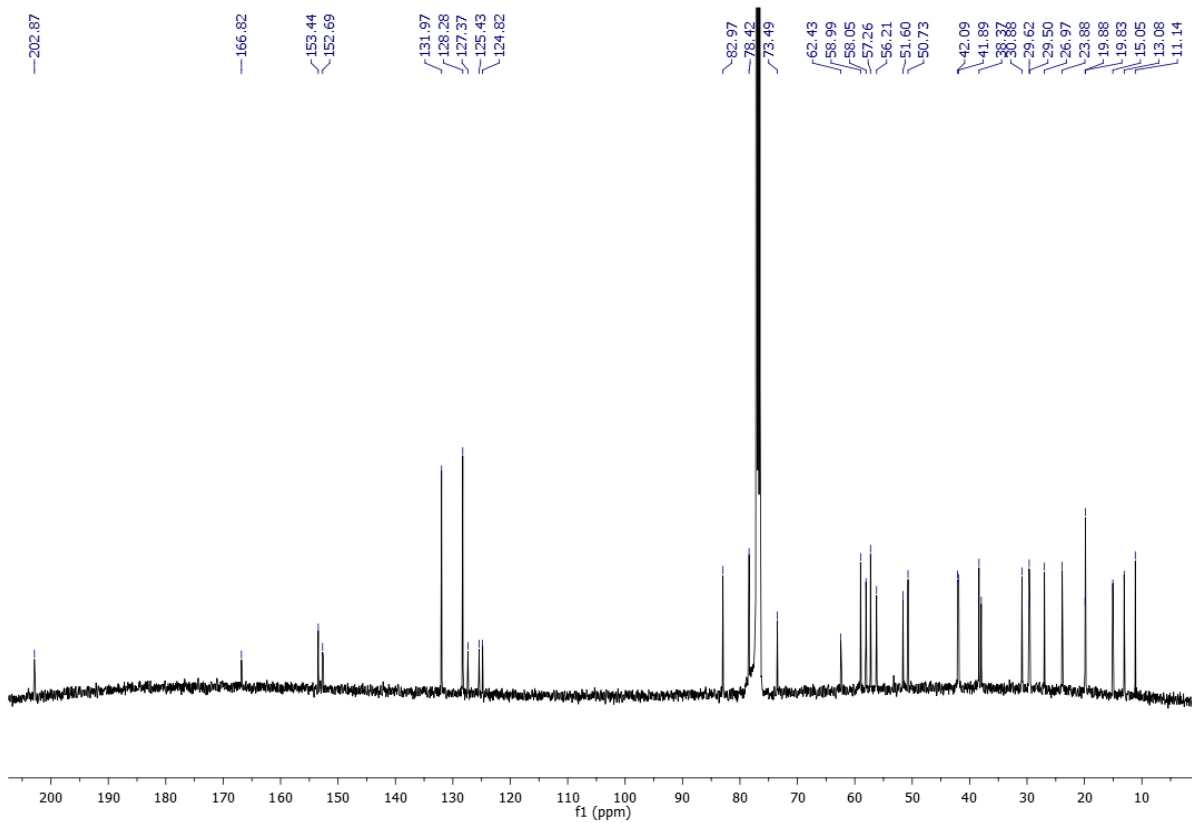
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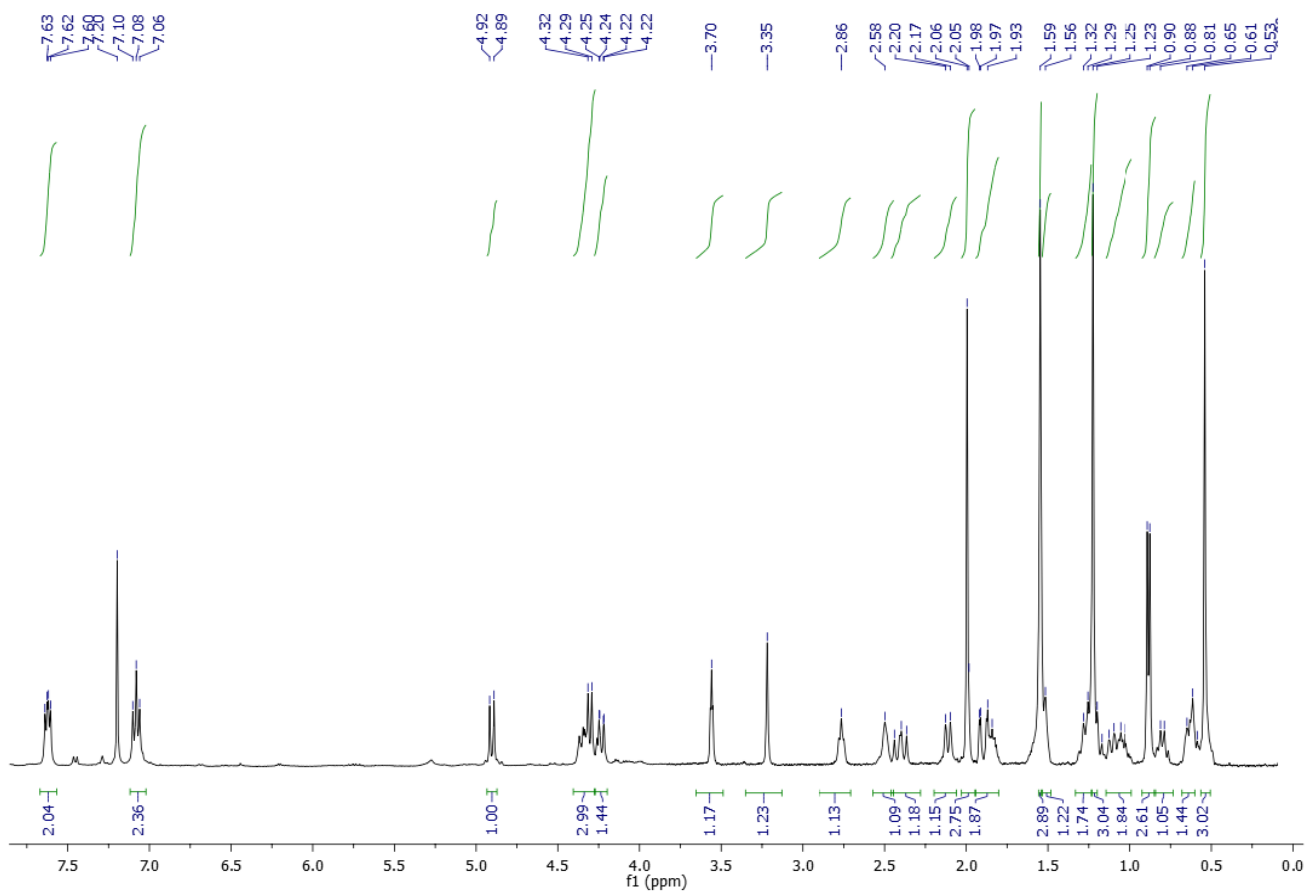
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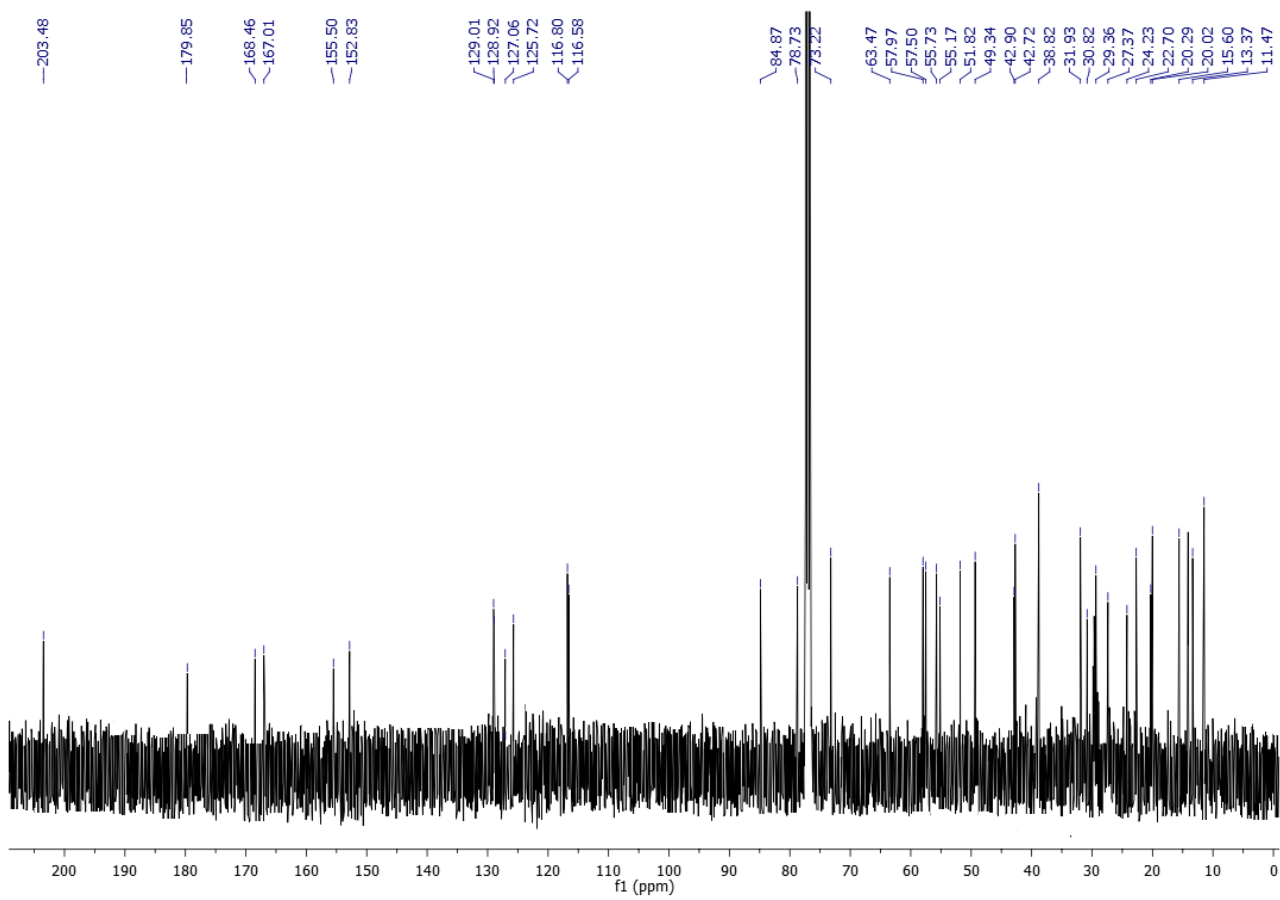
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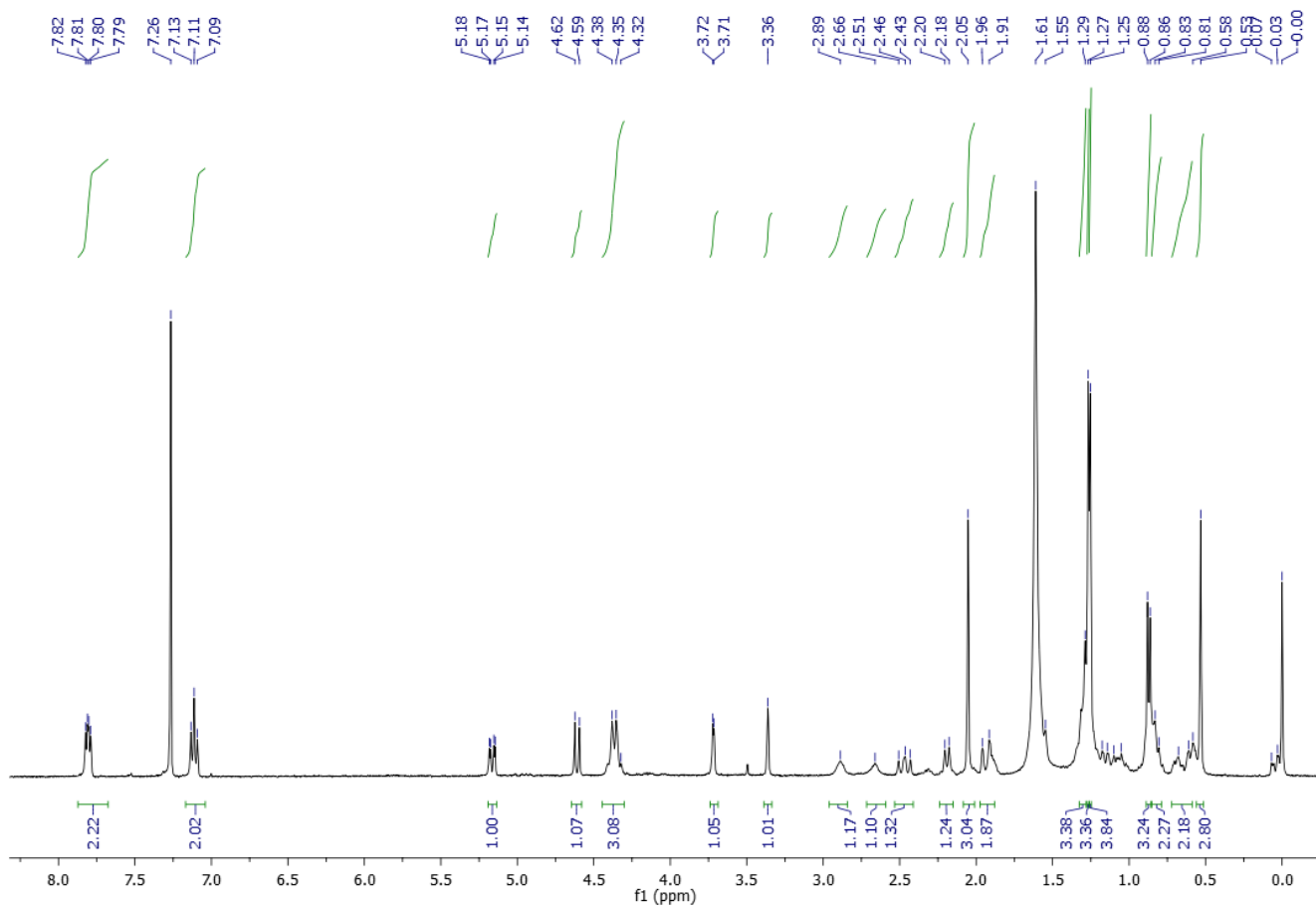
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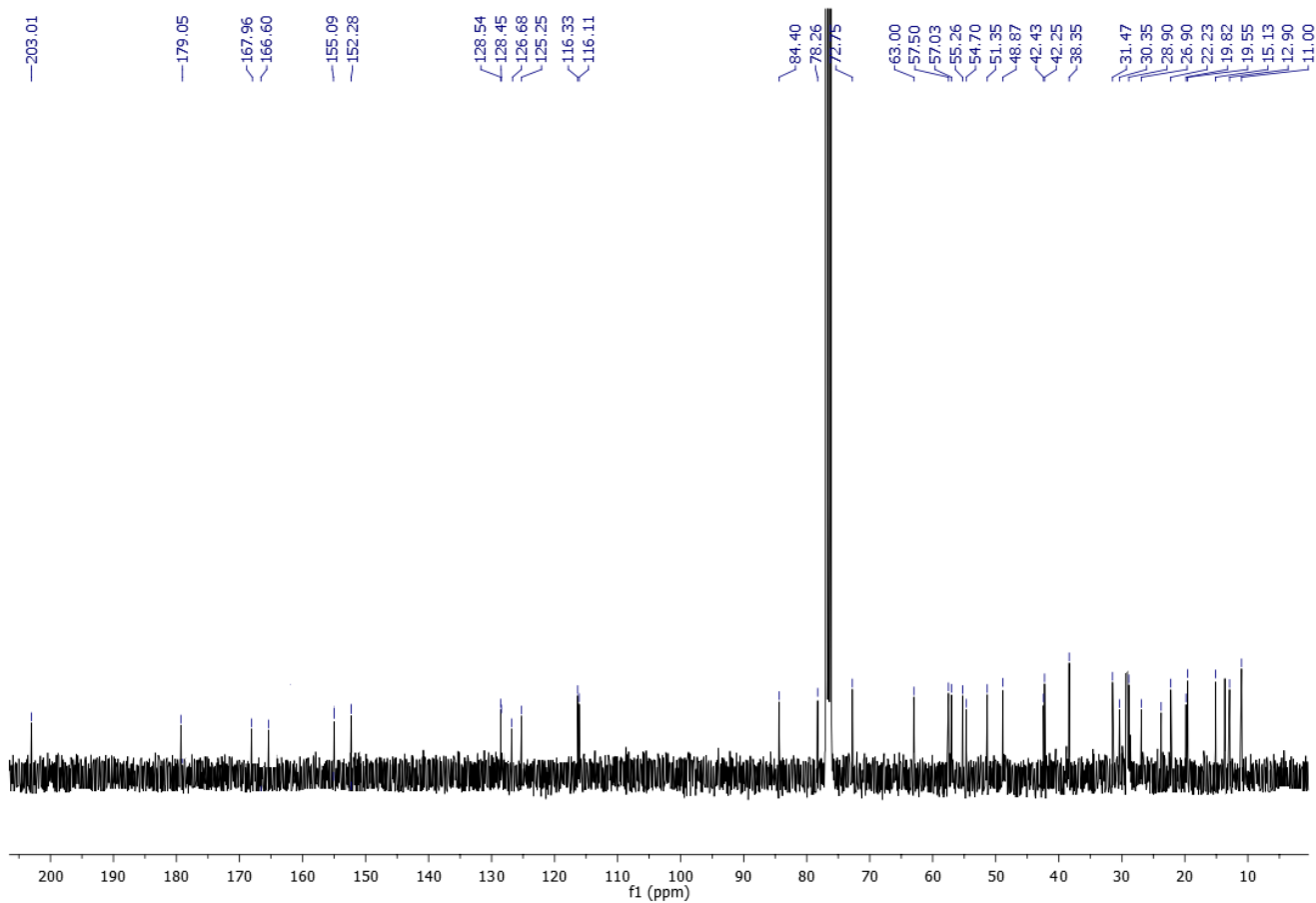
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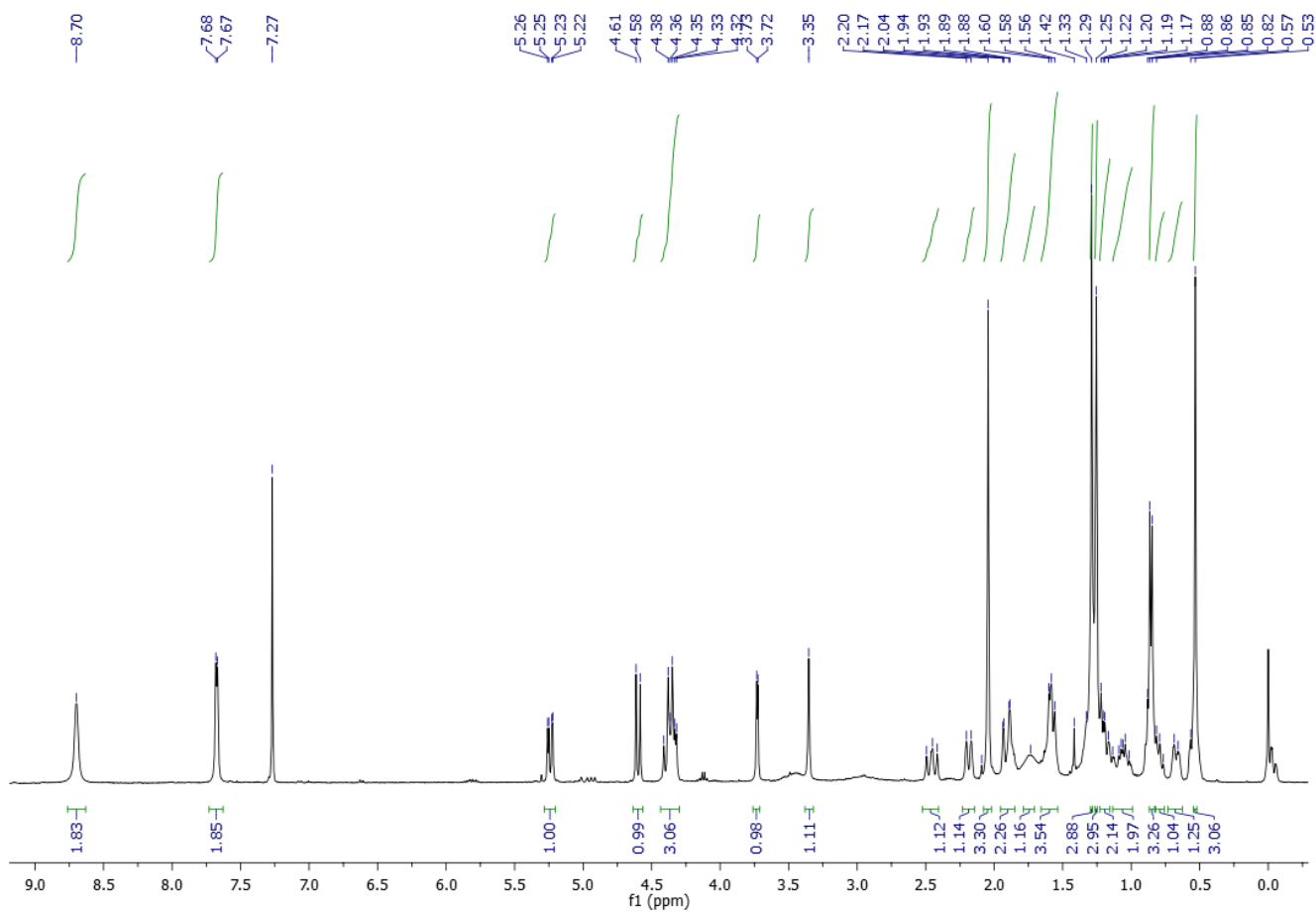
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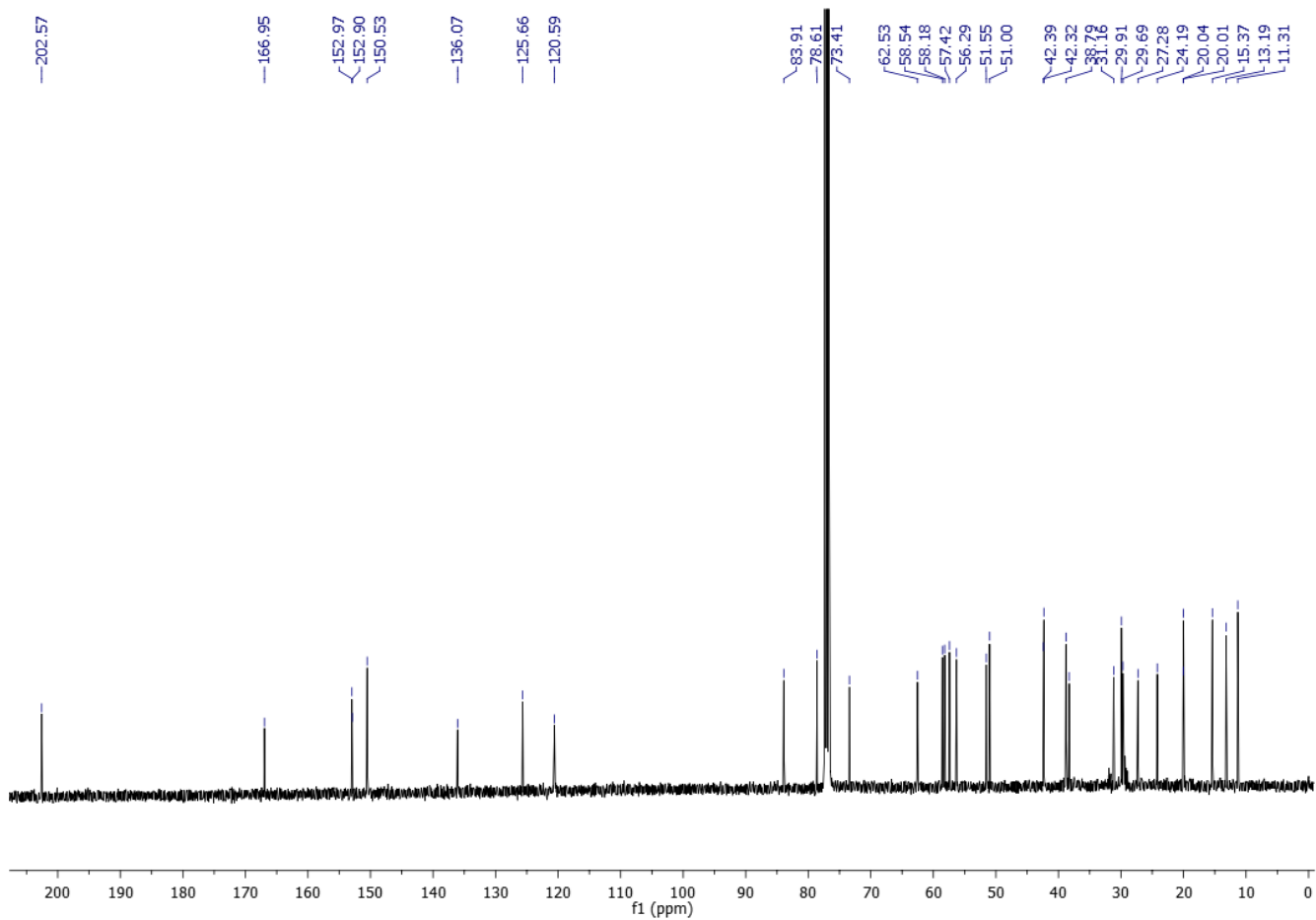
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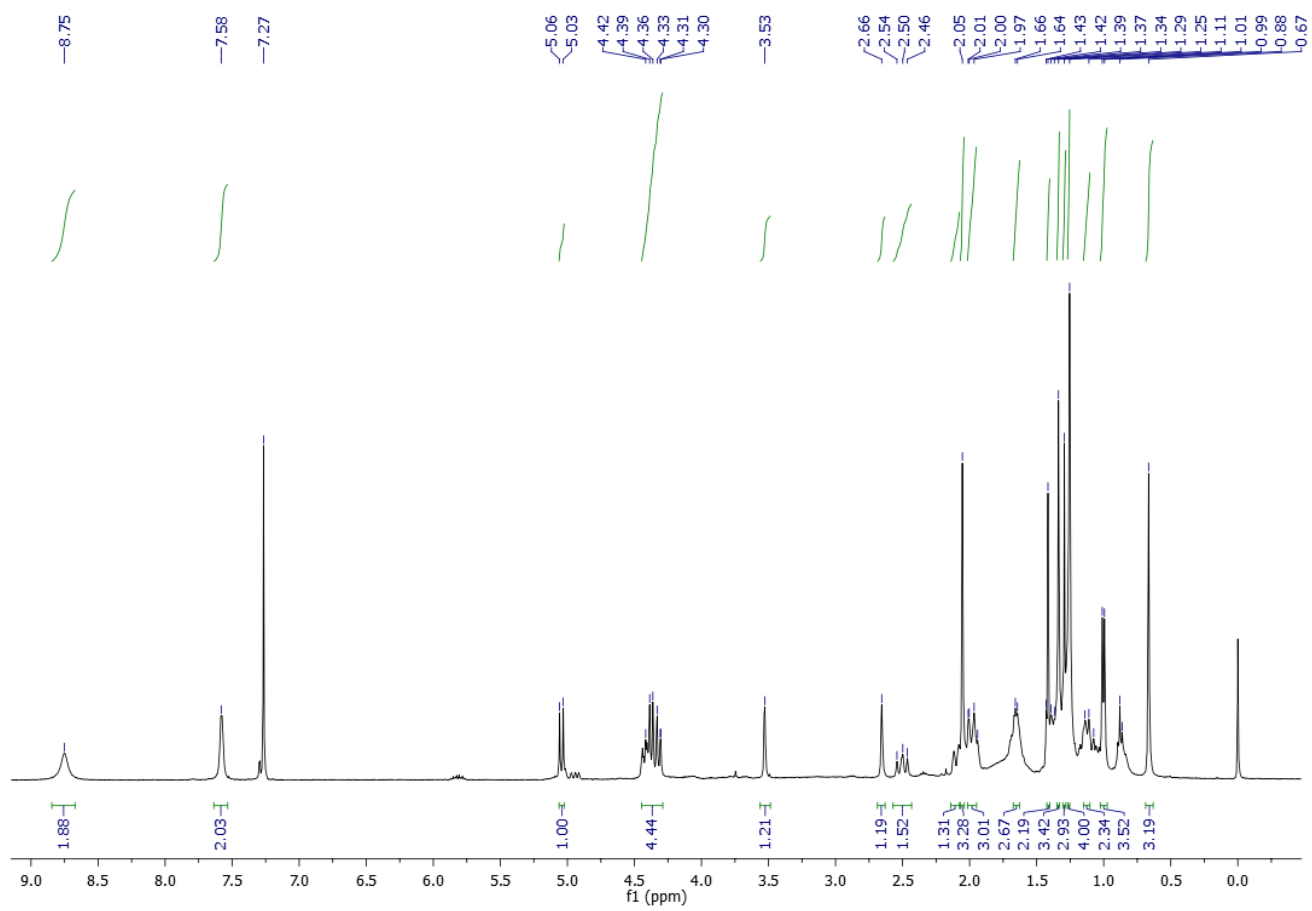
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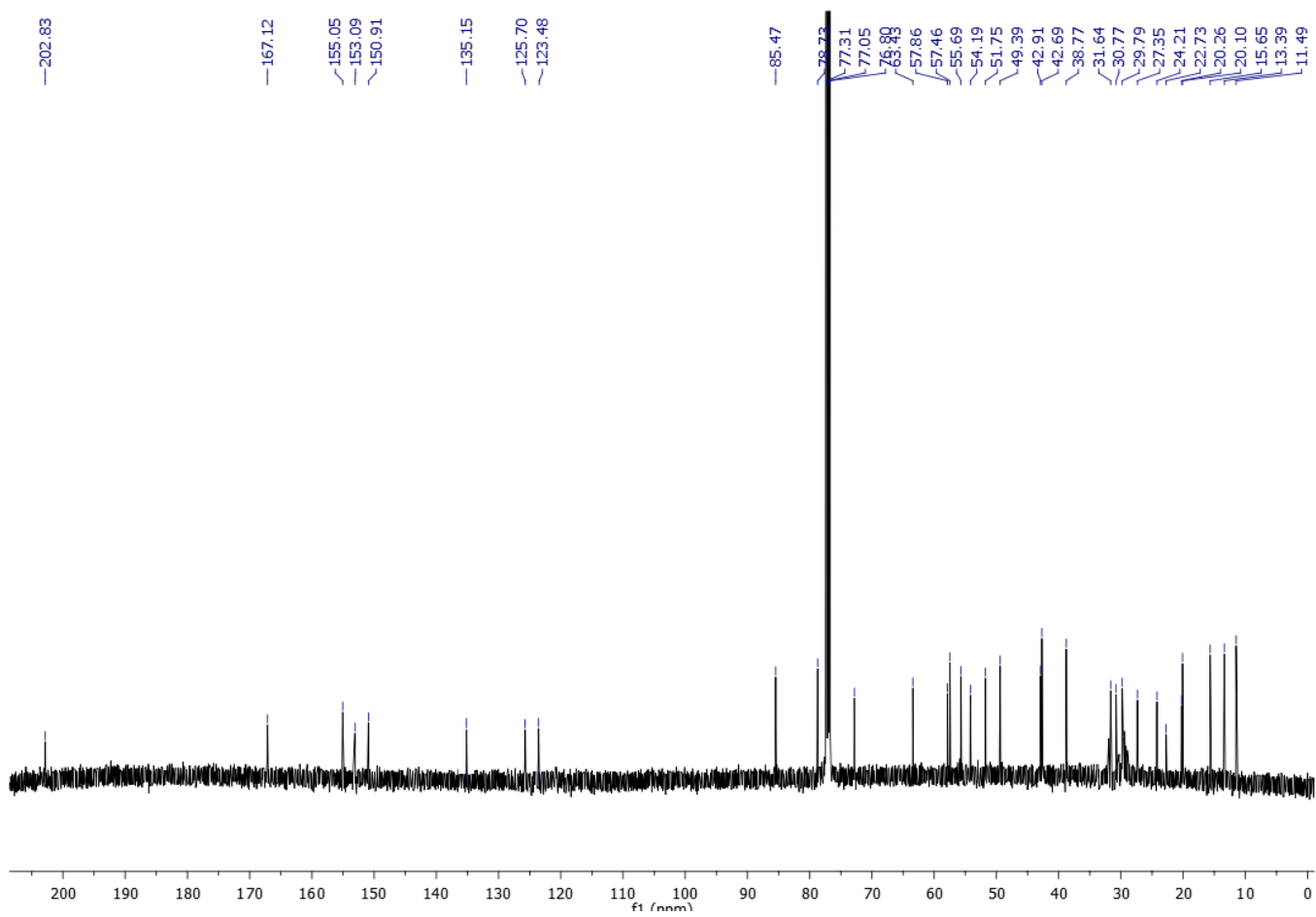
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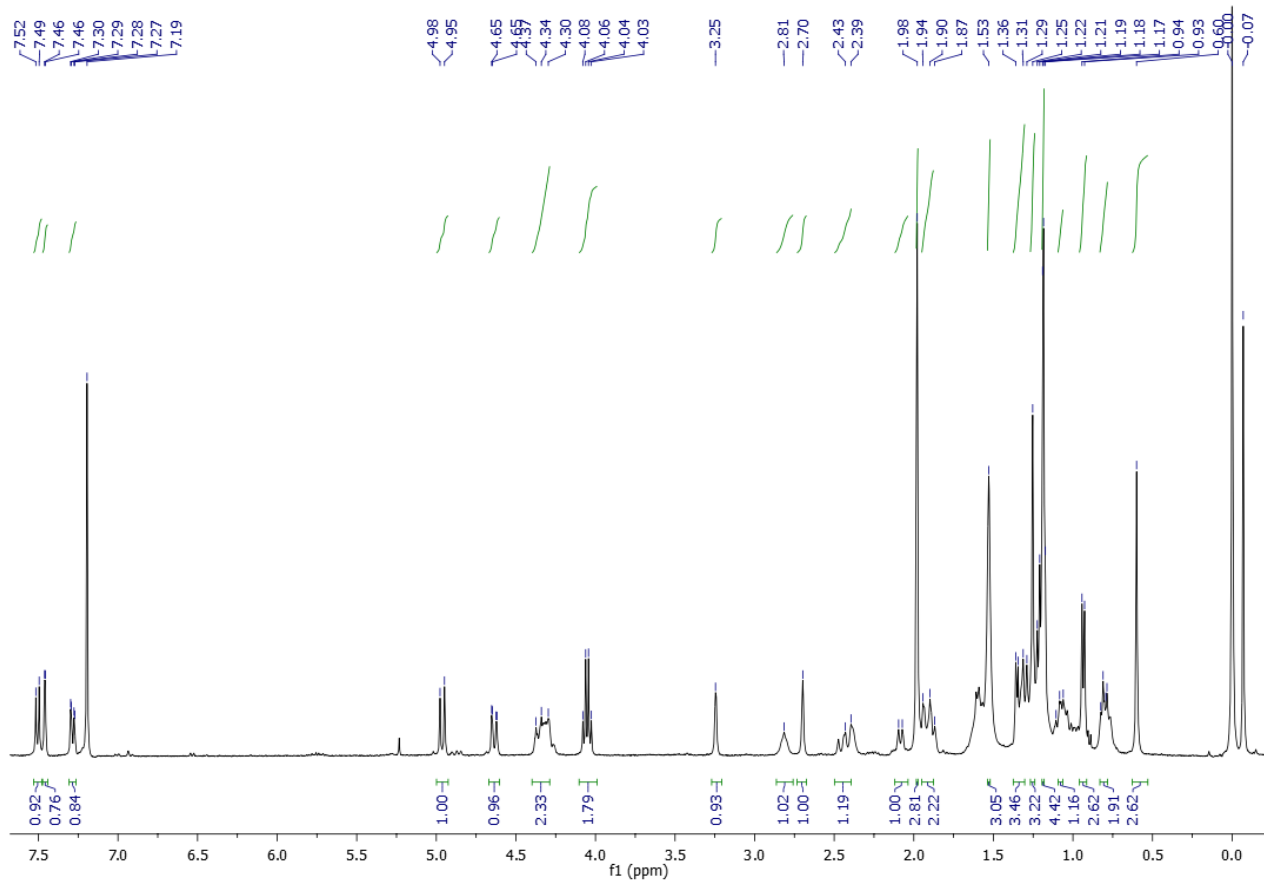
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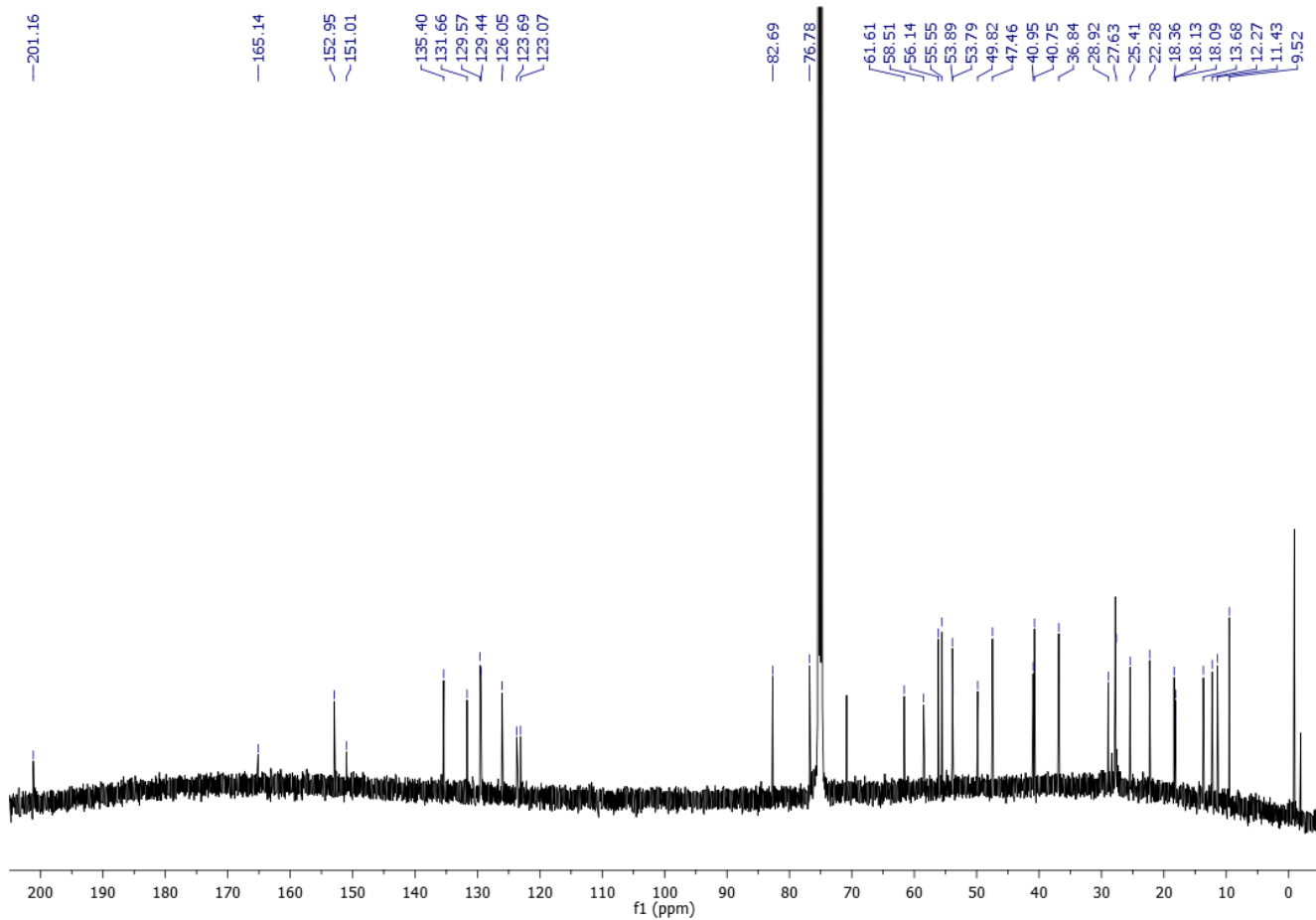
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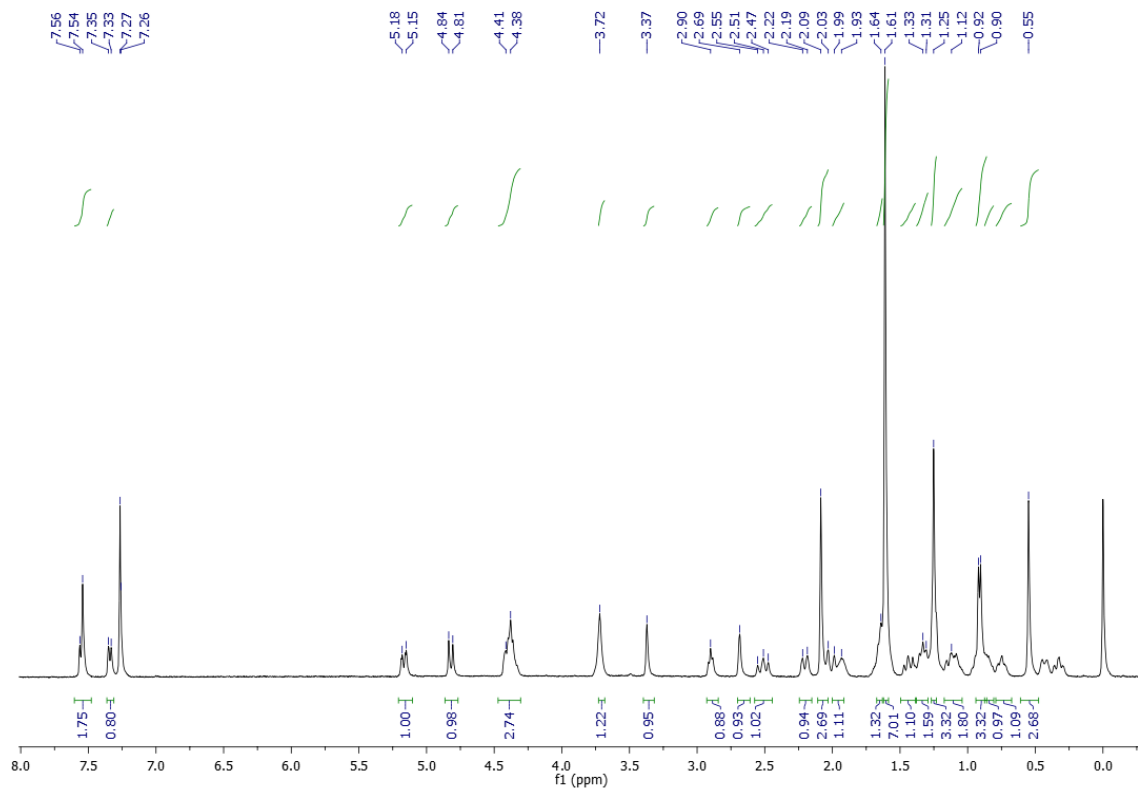
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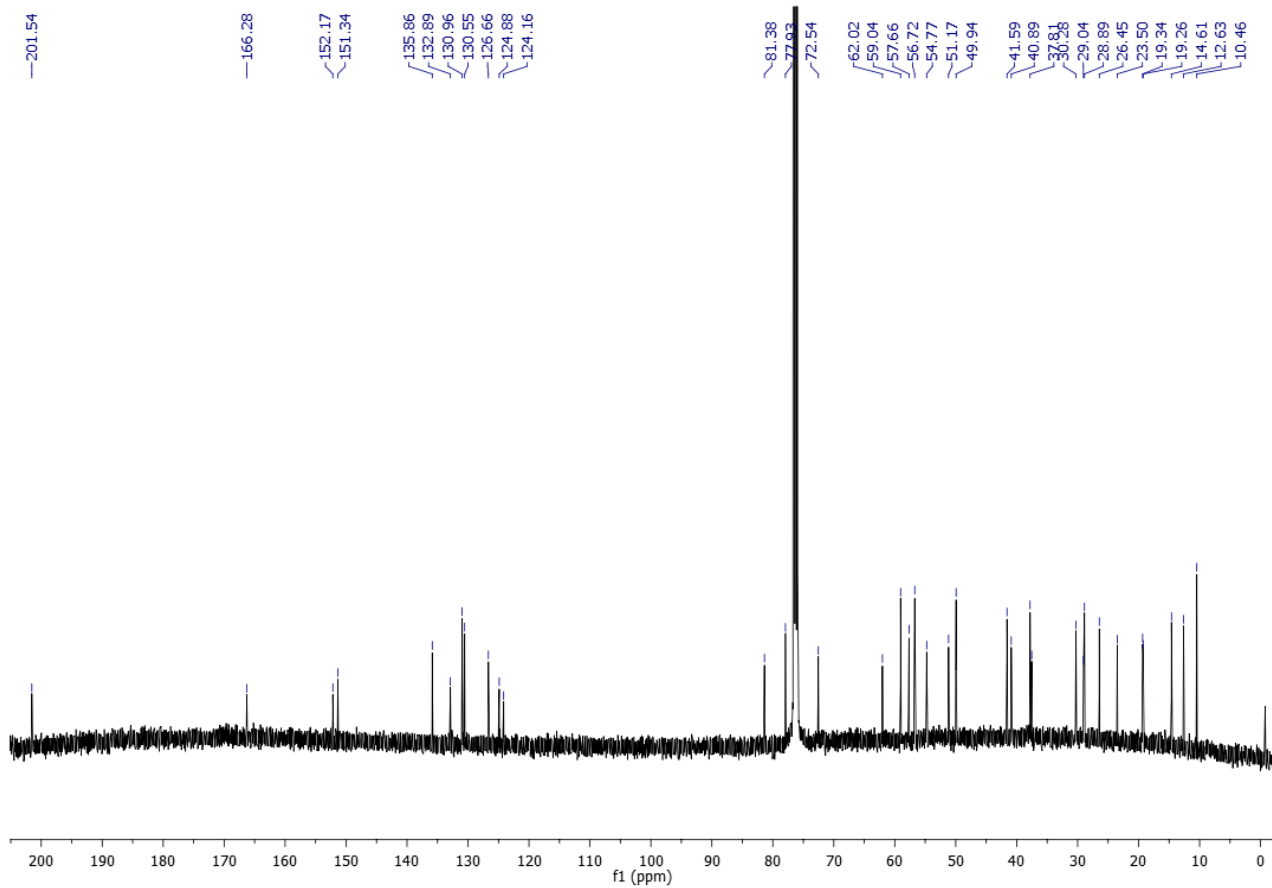
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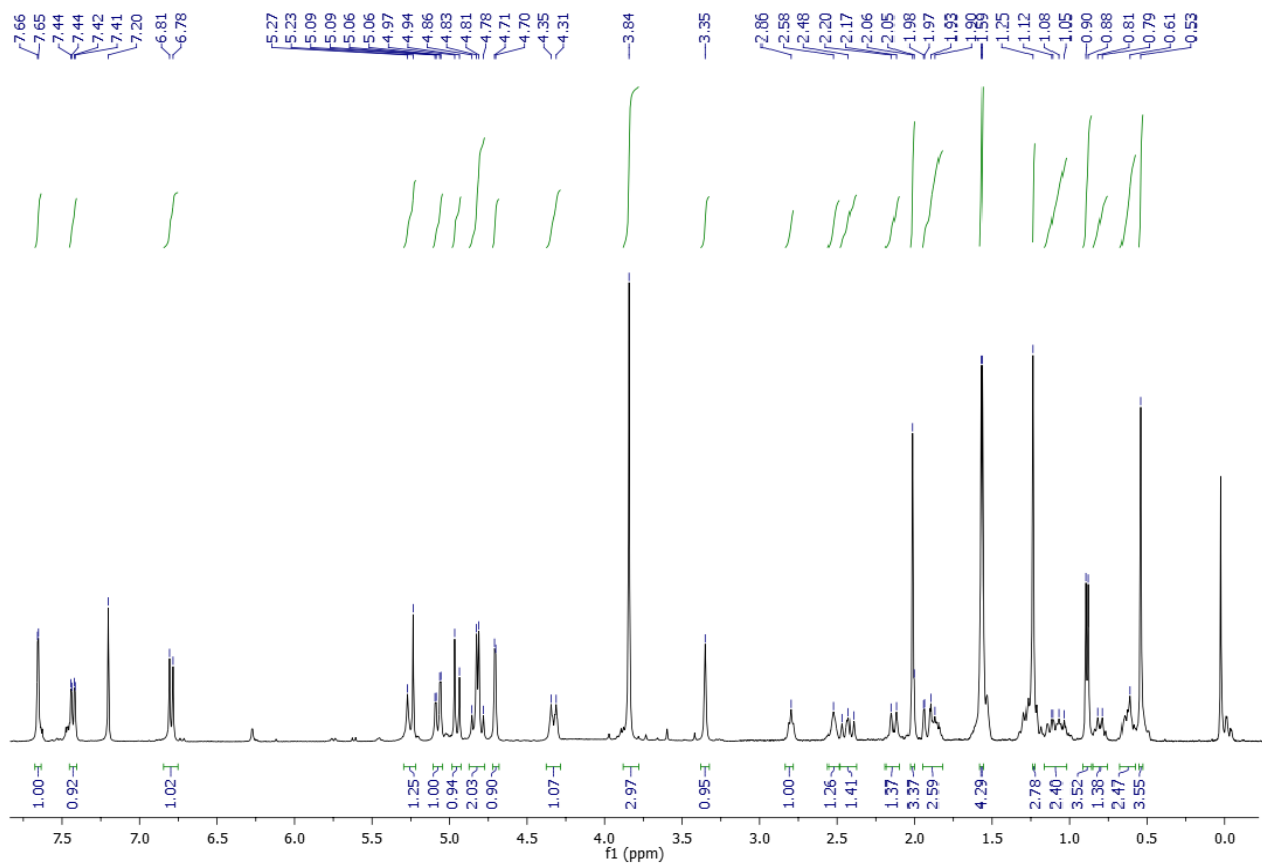
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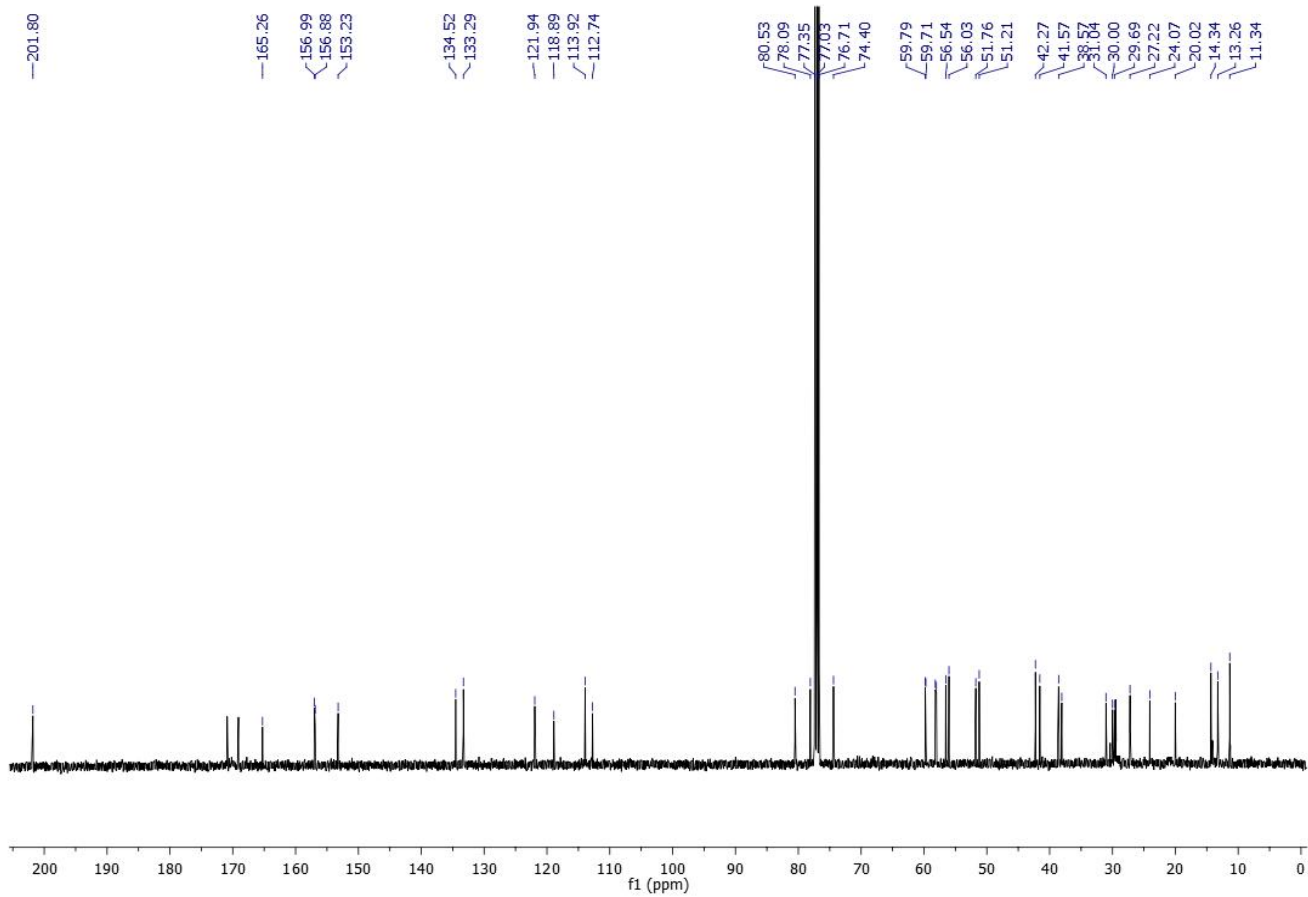
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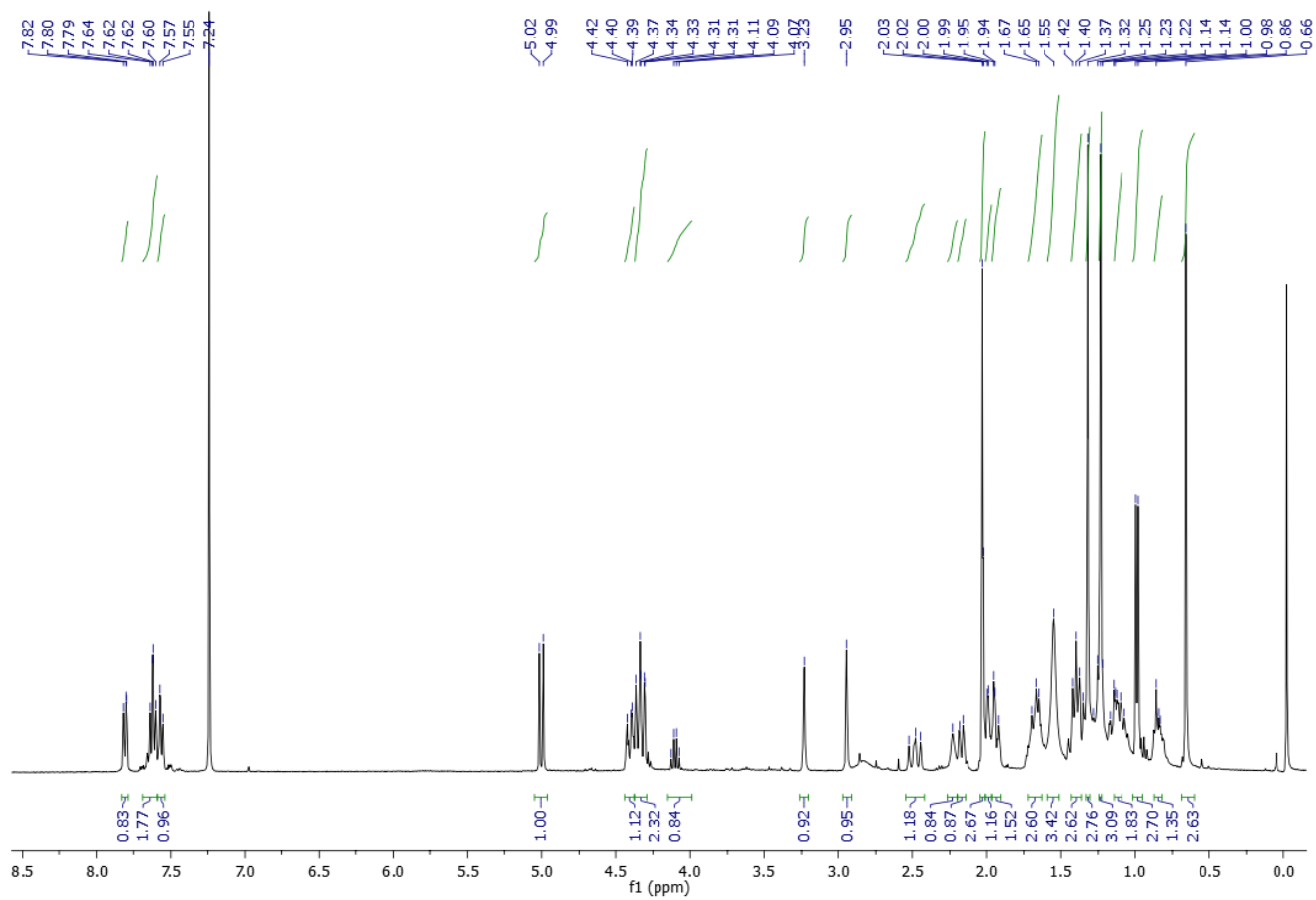
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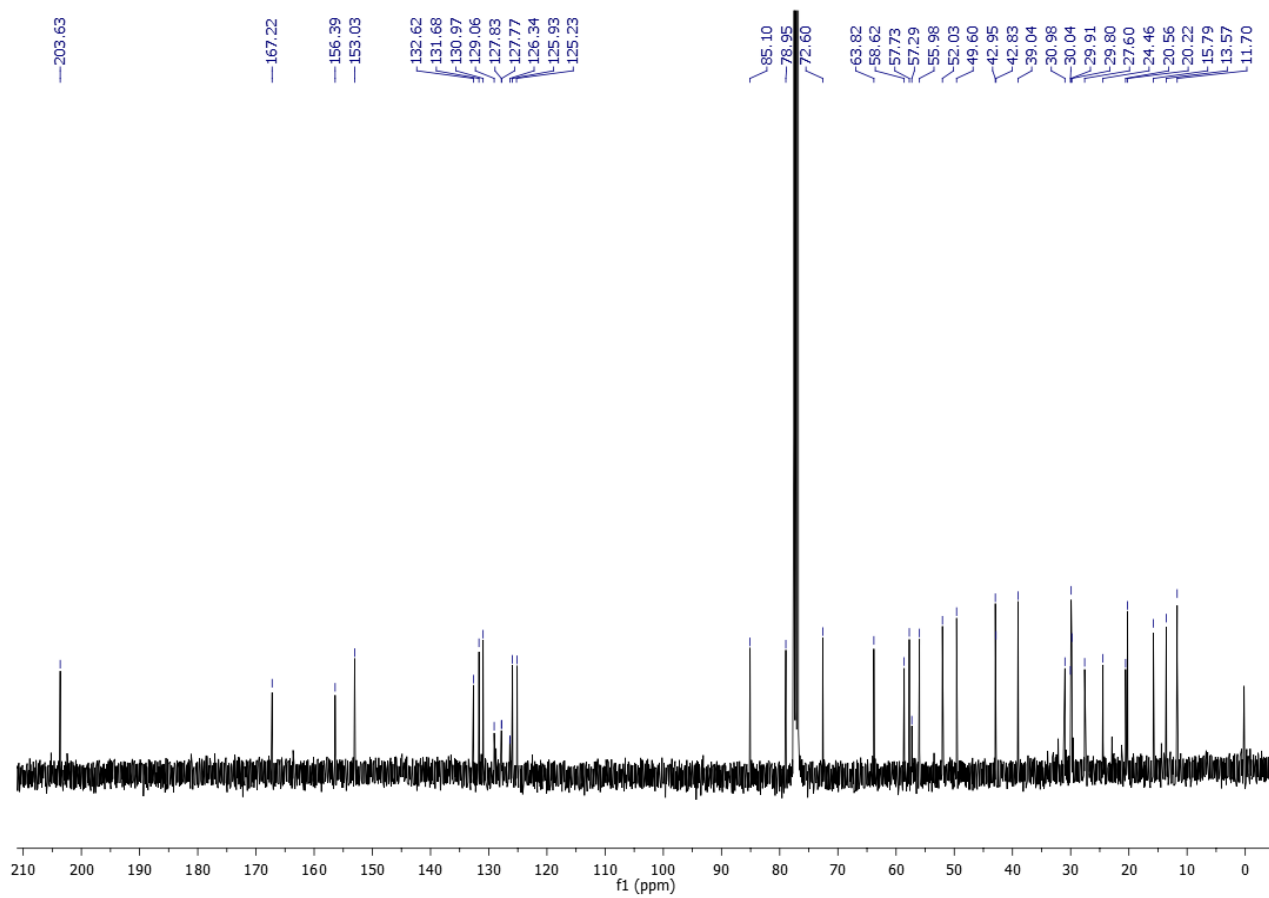
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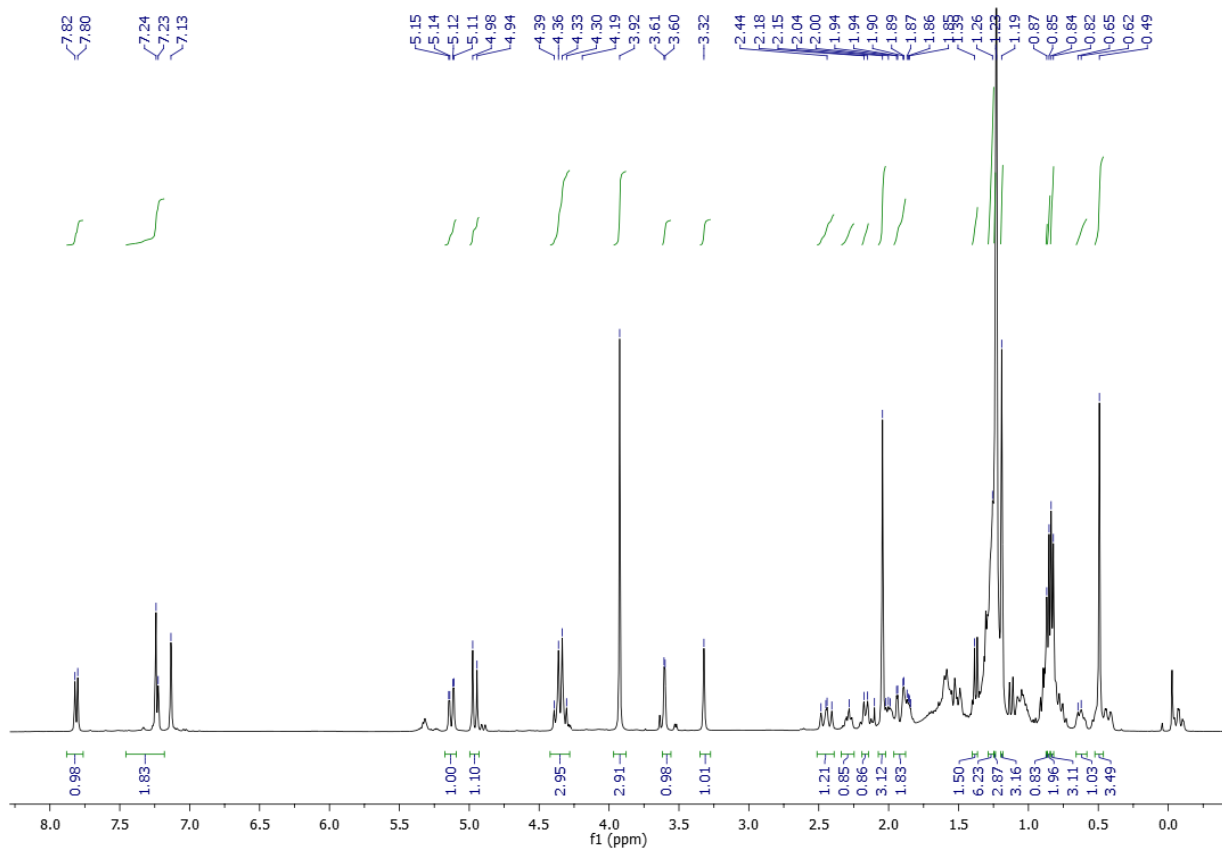
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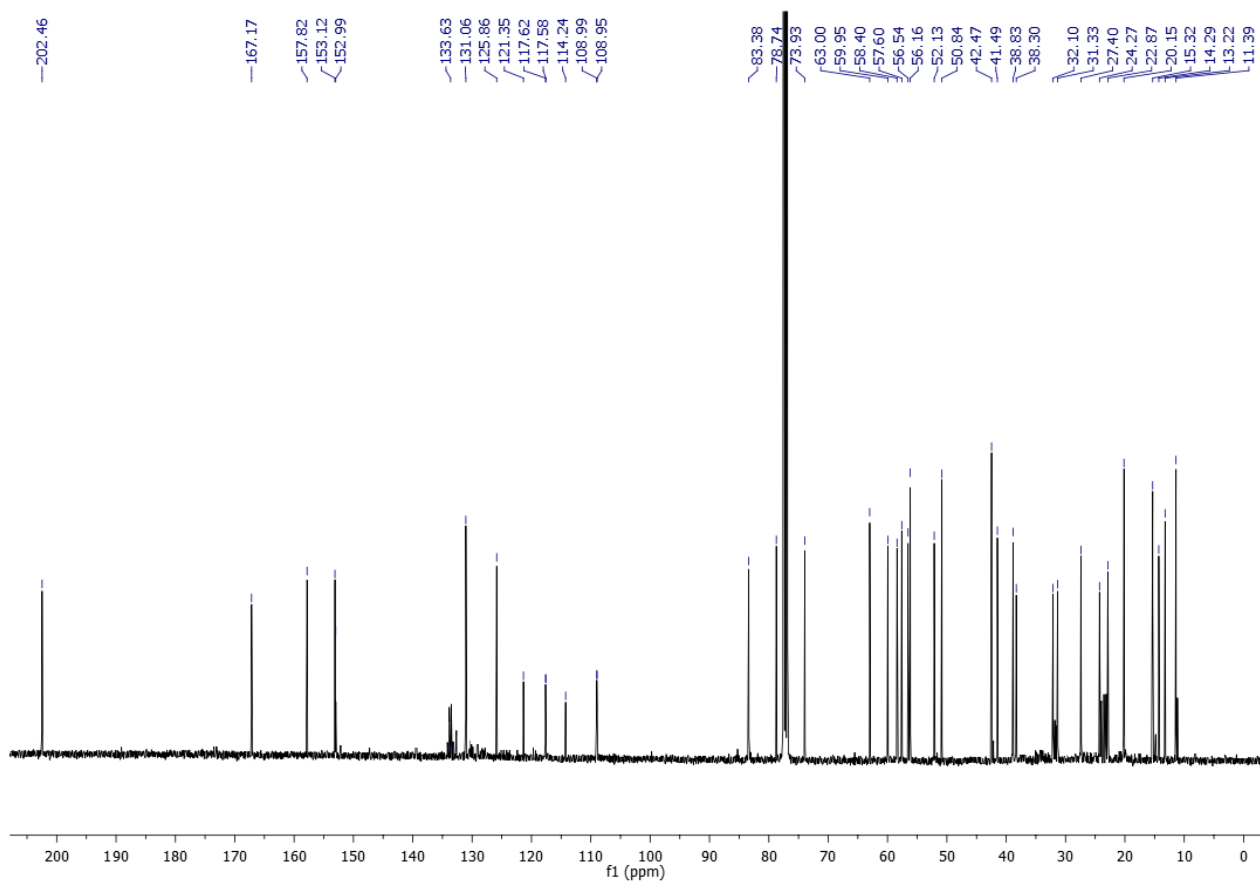
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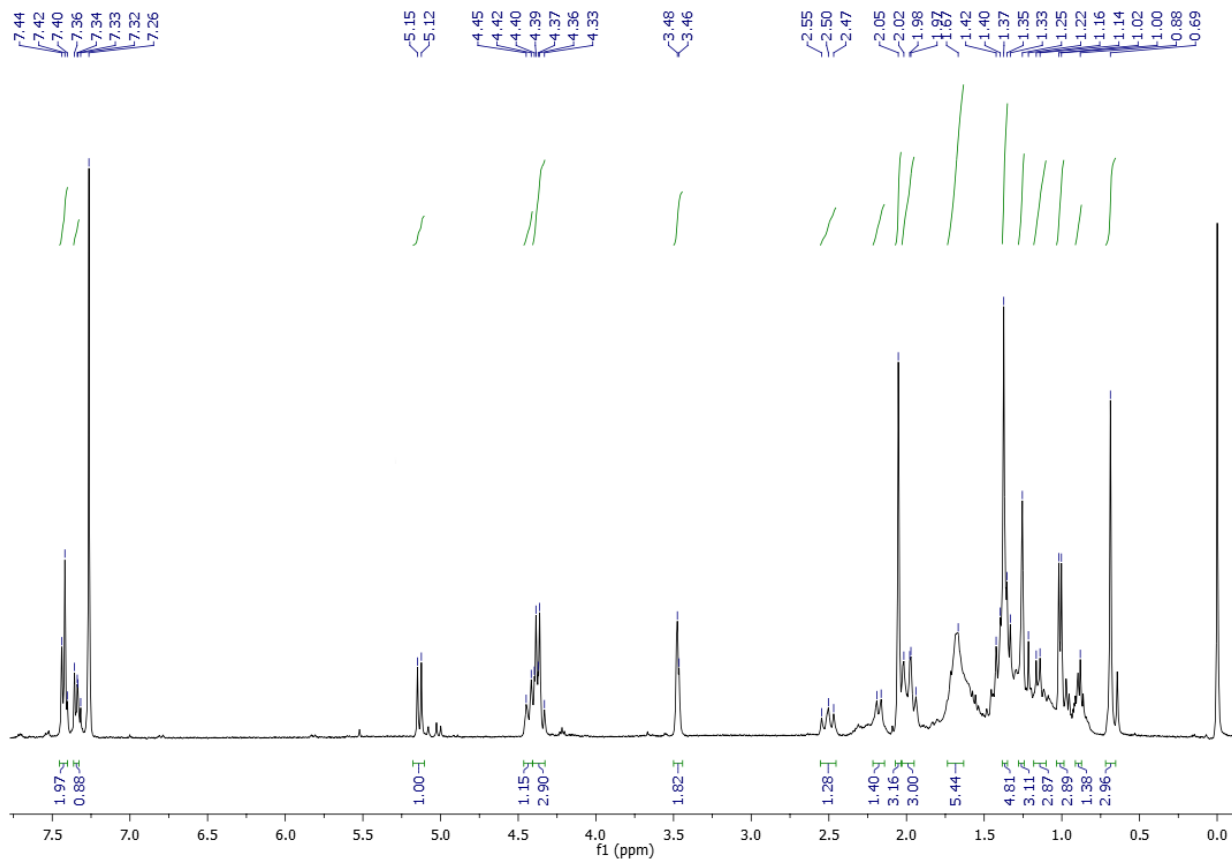
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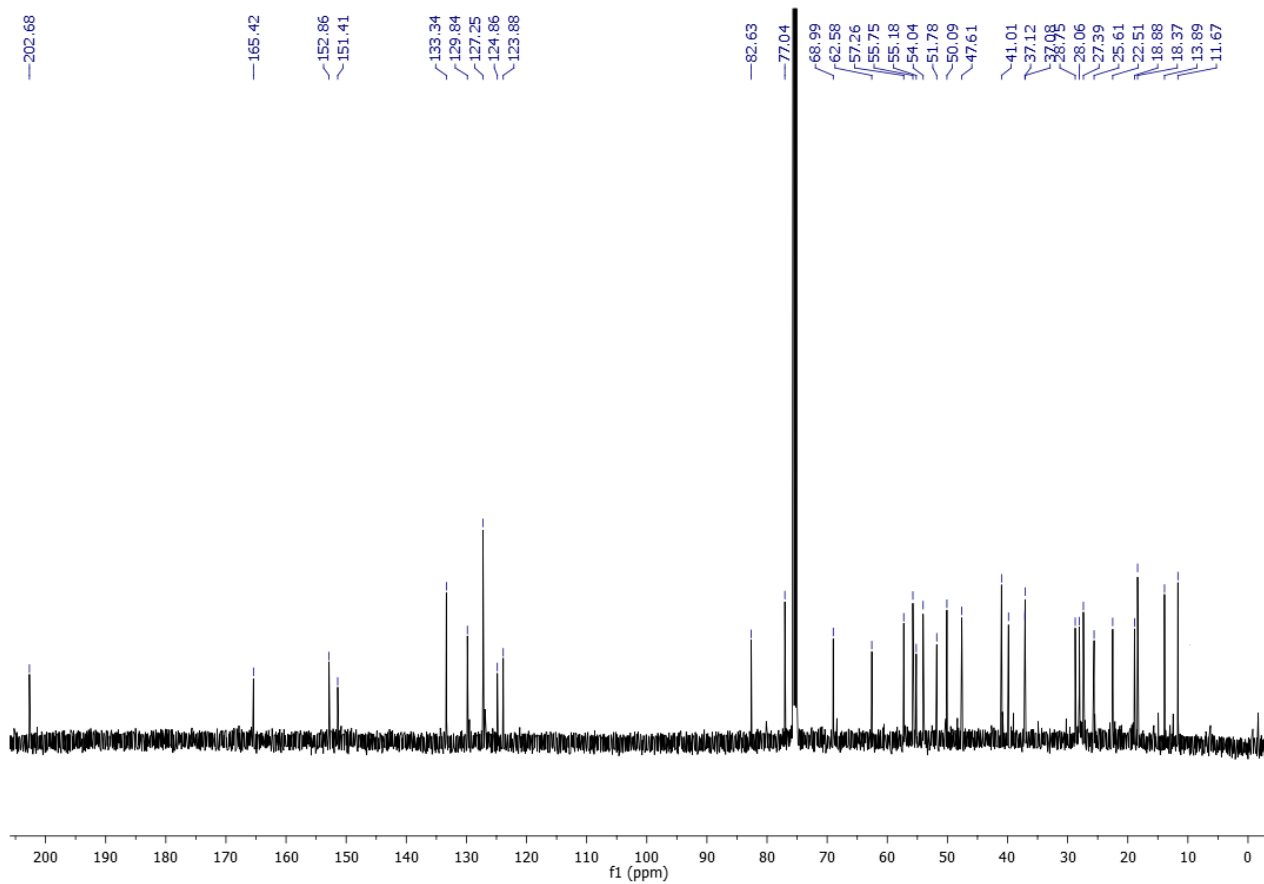
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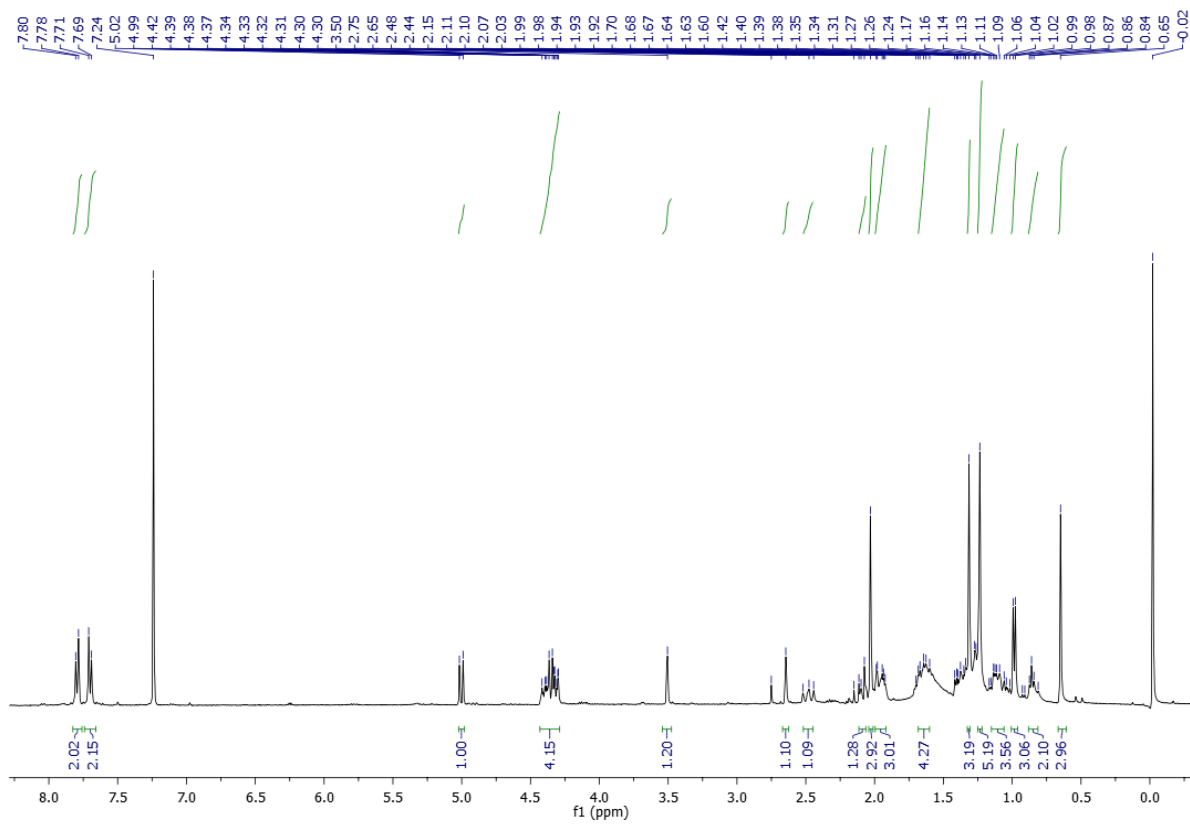
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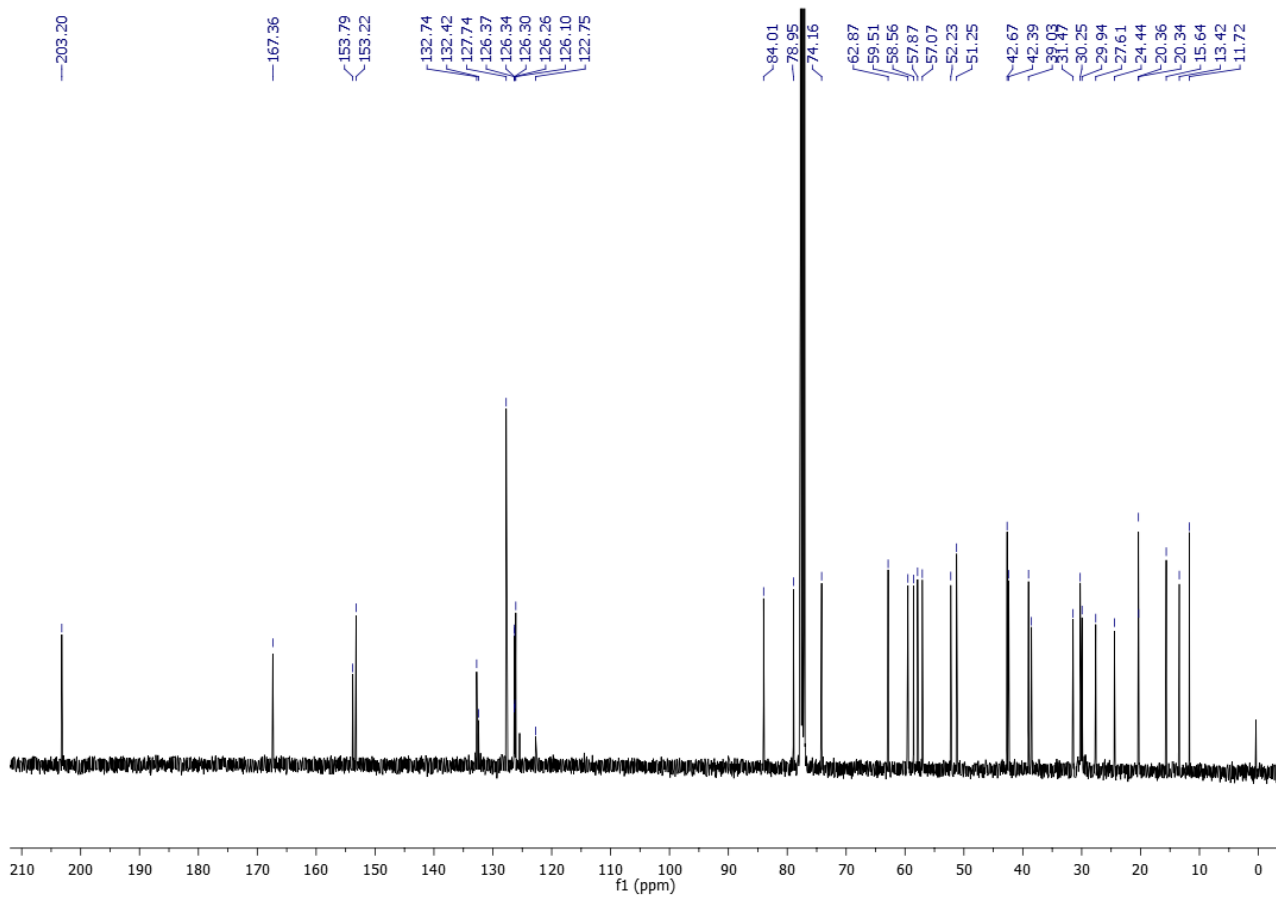
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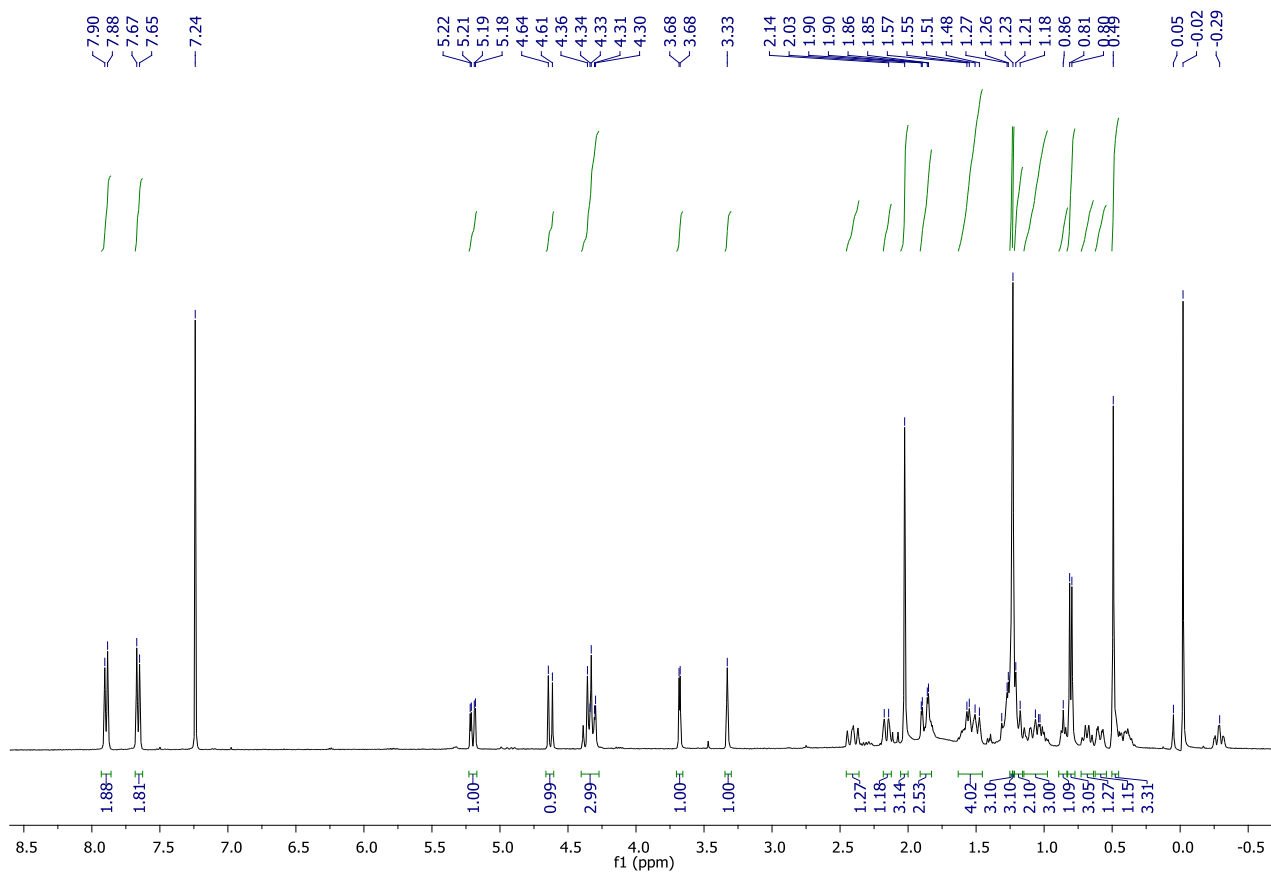
¹H NMR of W-11a



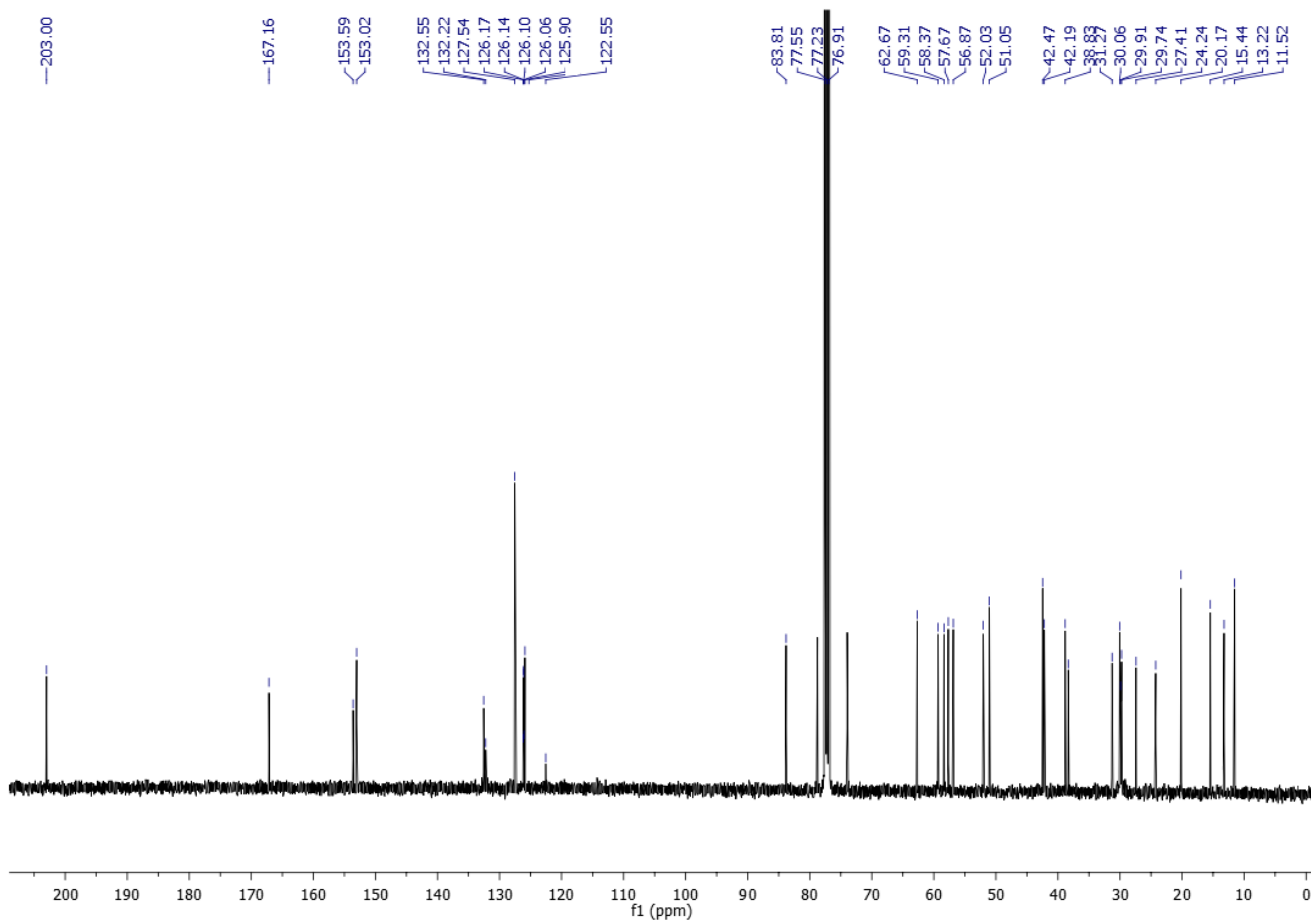
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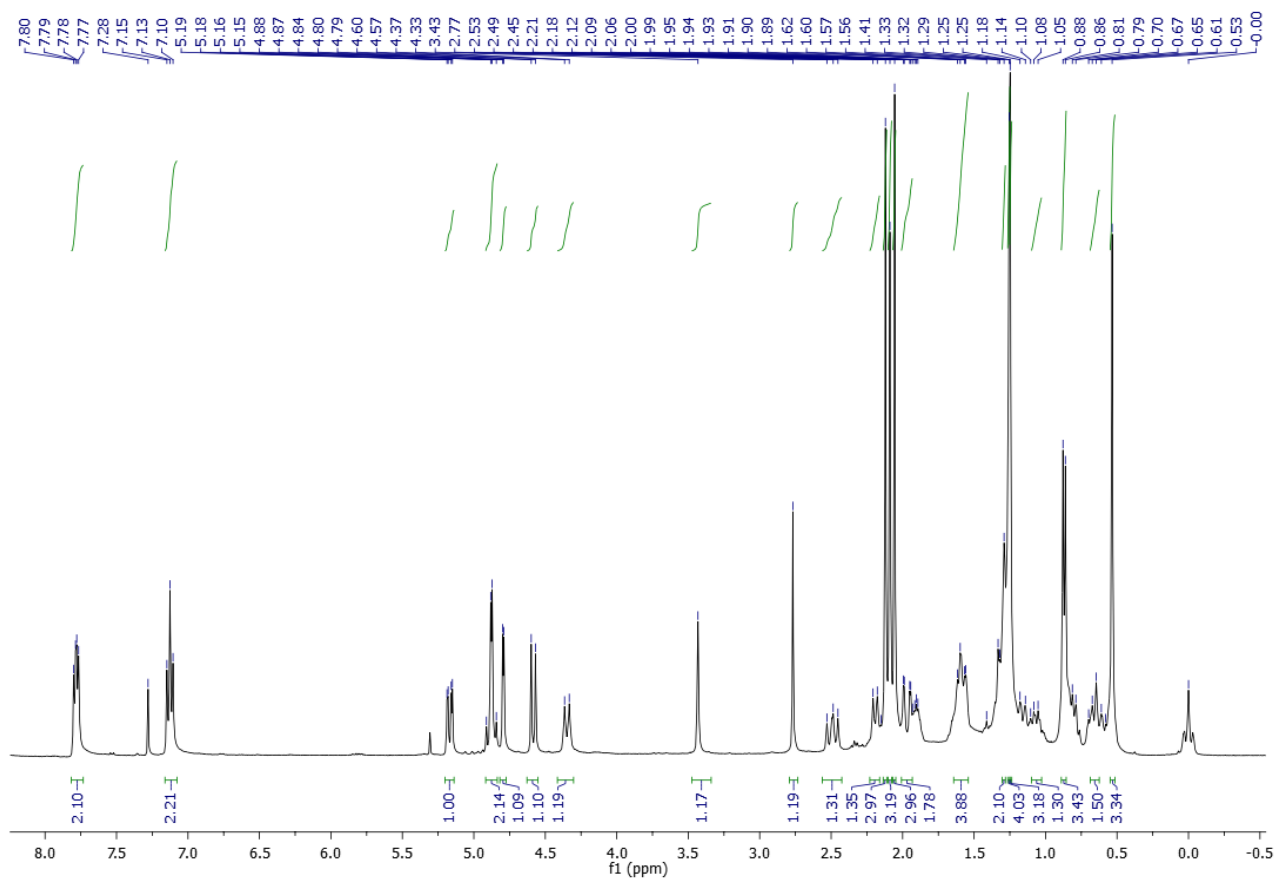
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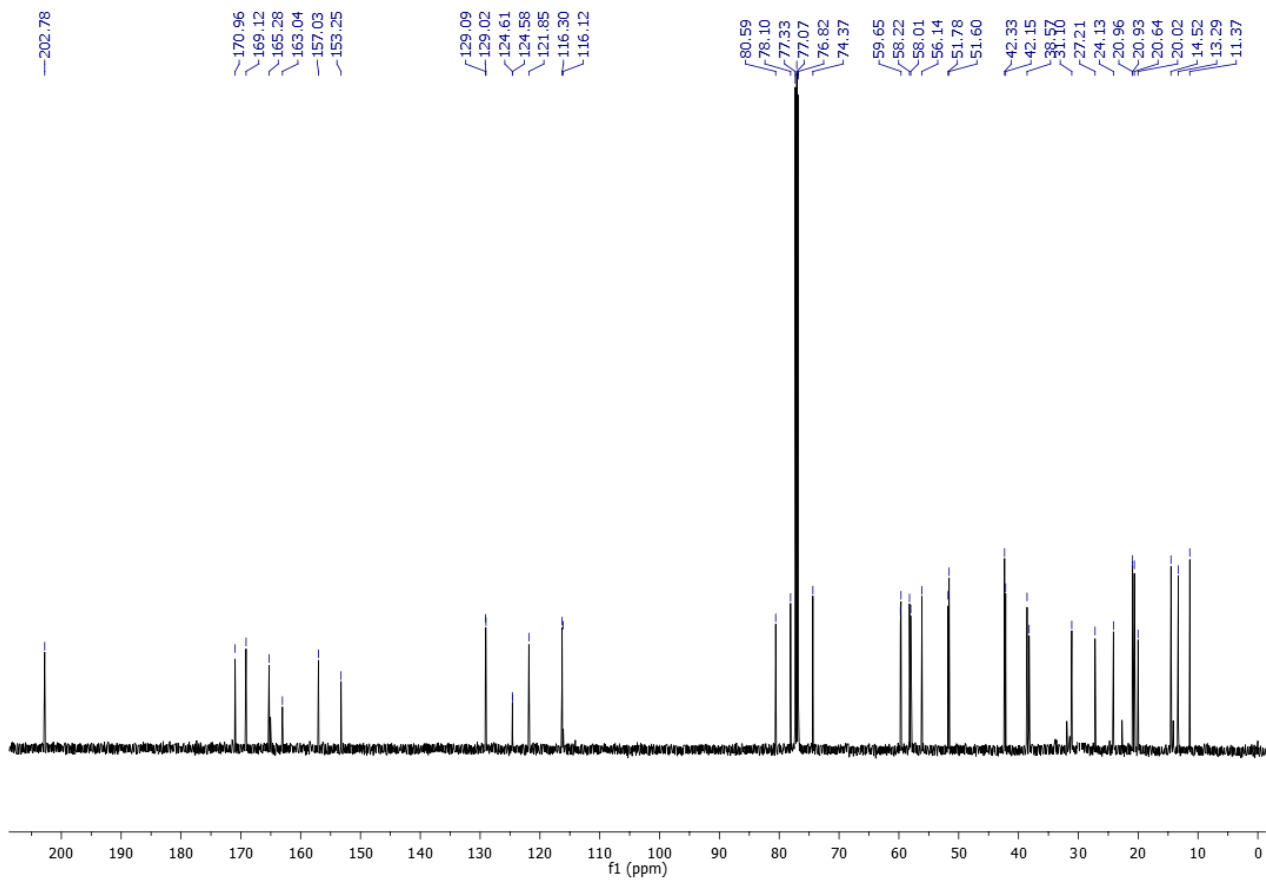
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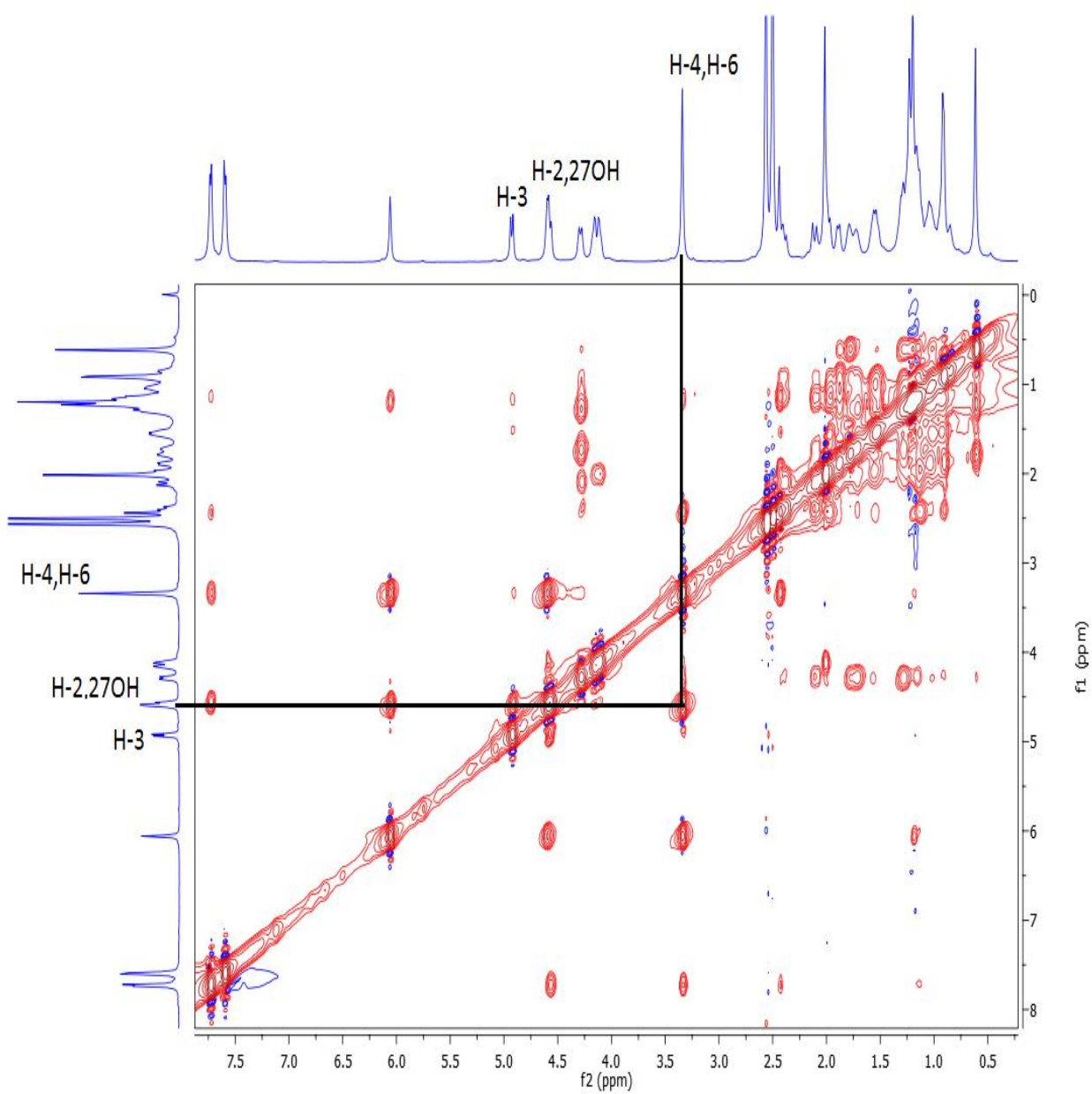
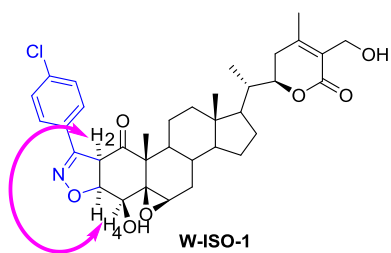
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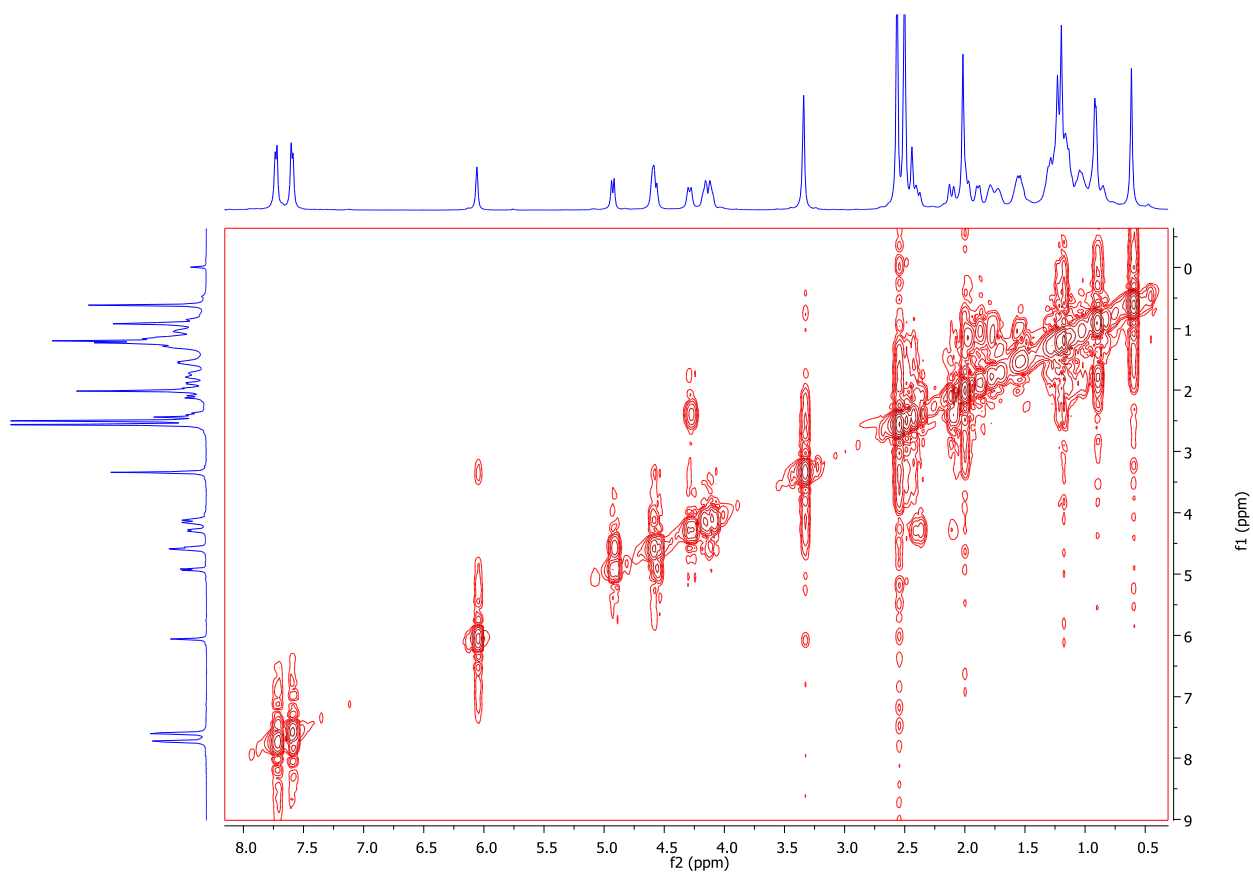
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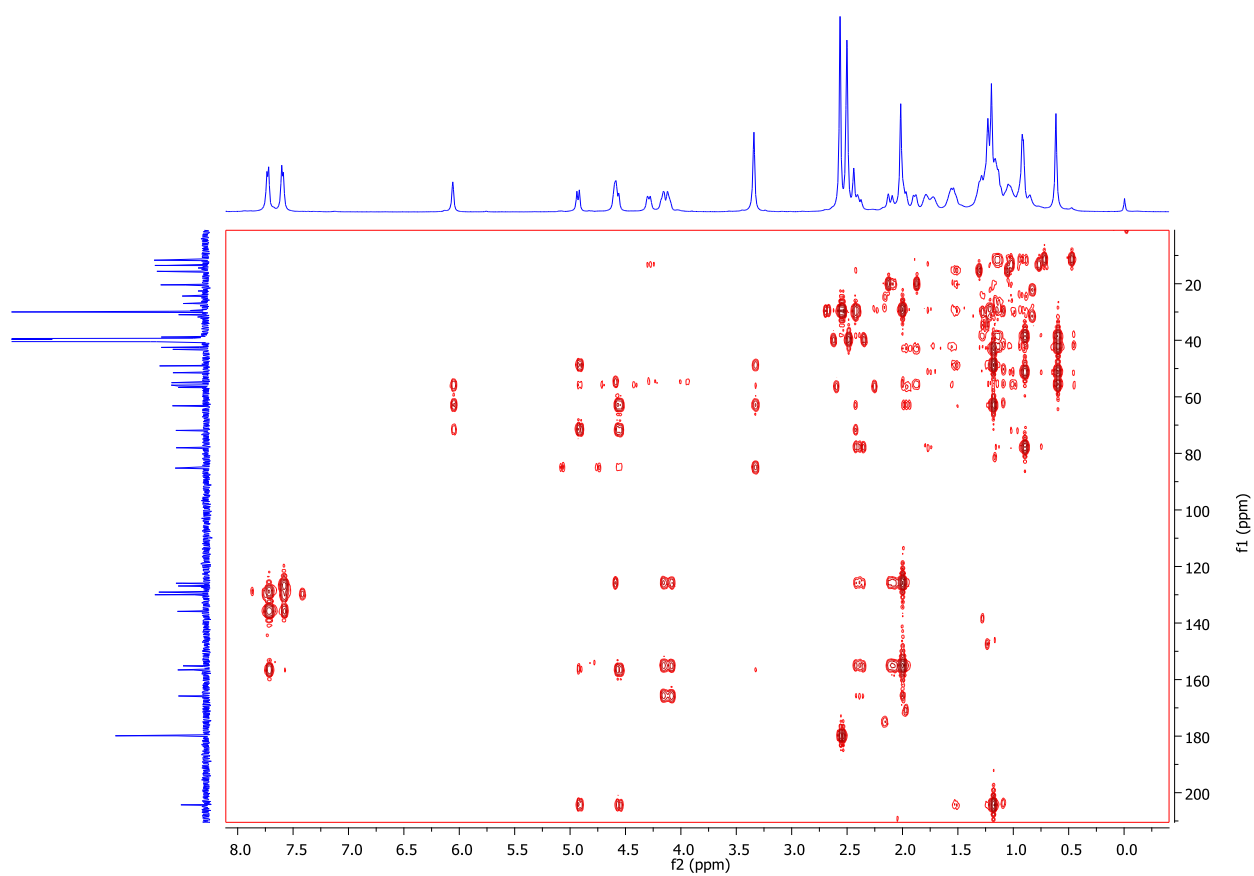
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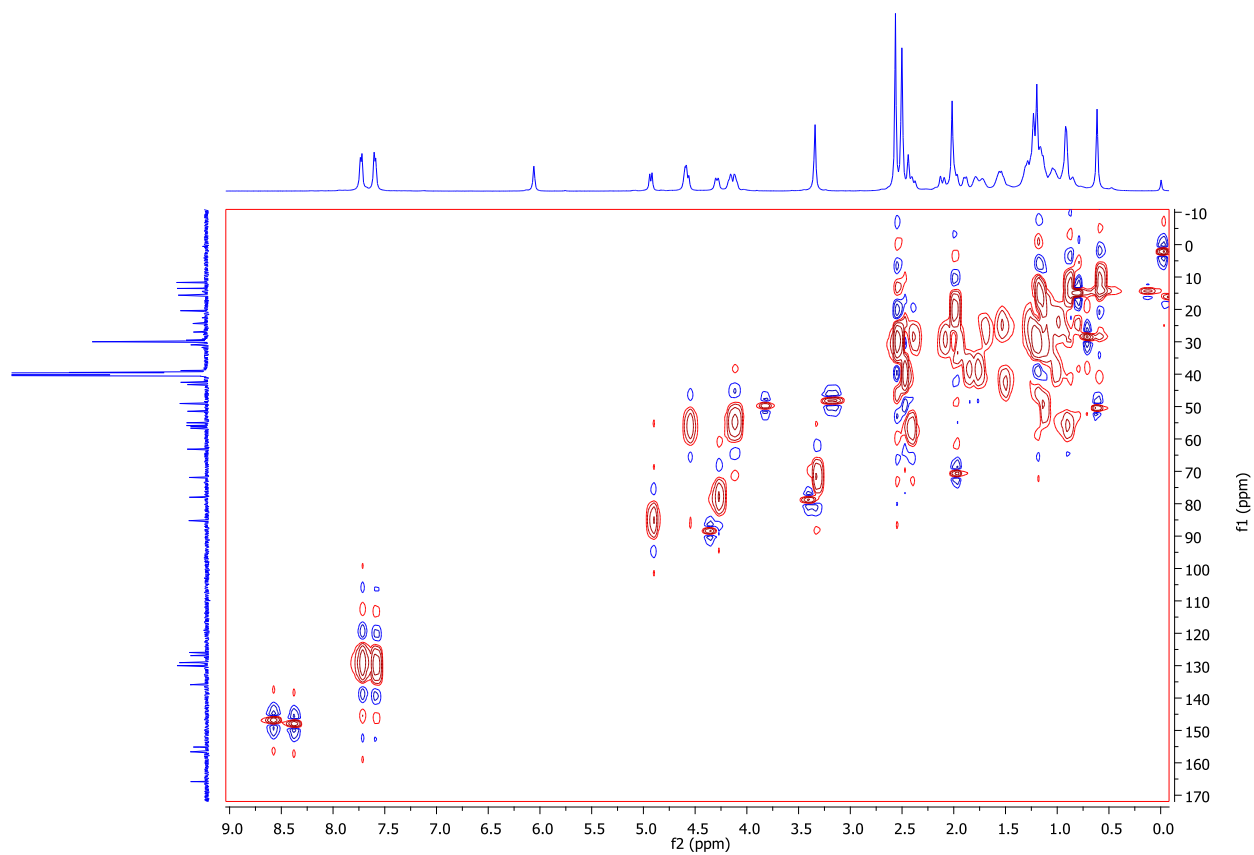
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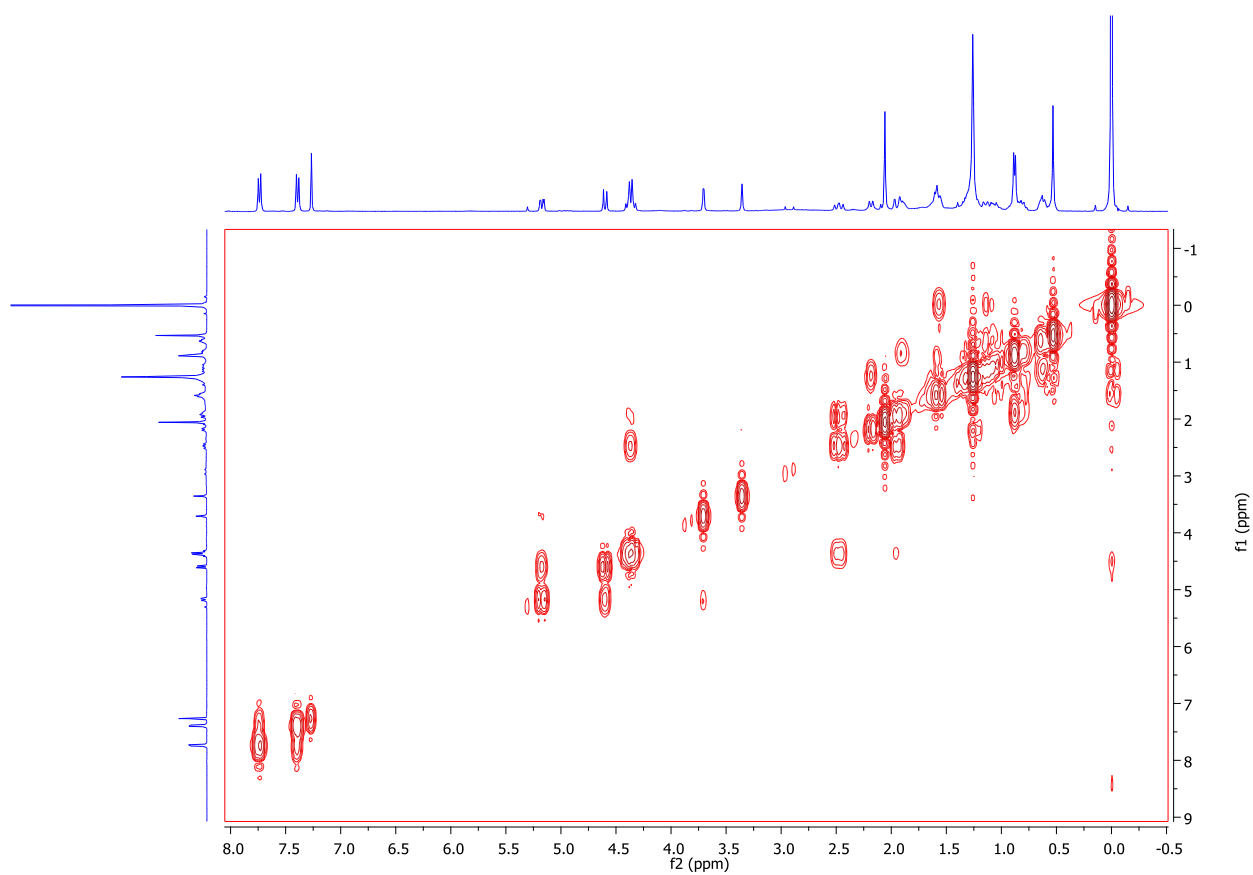
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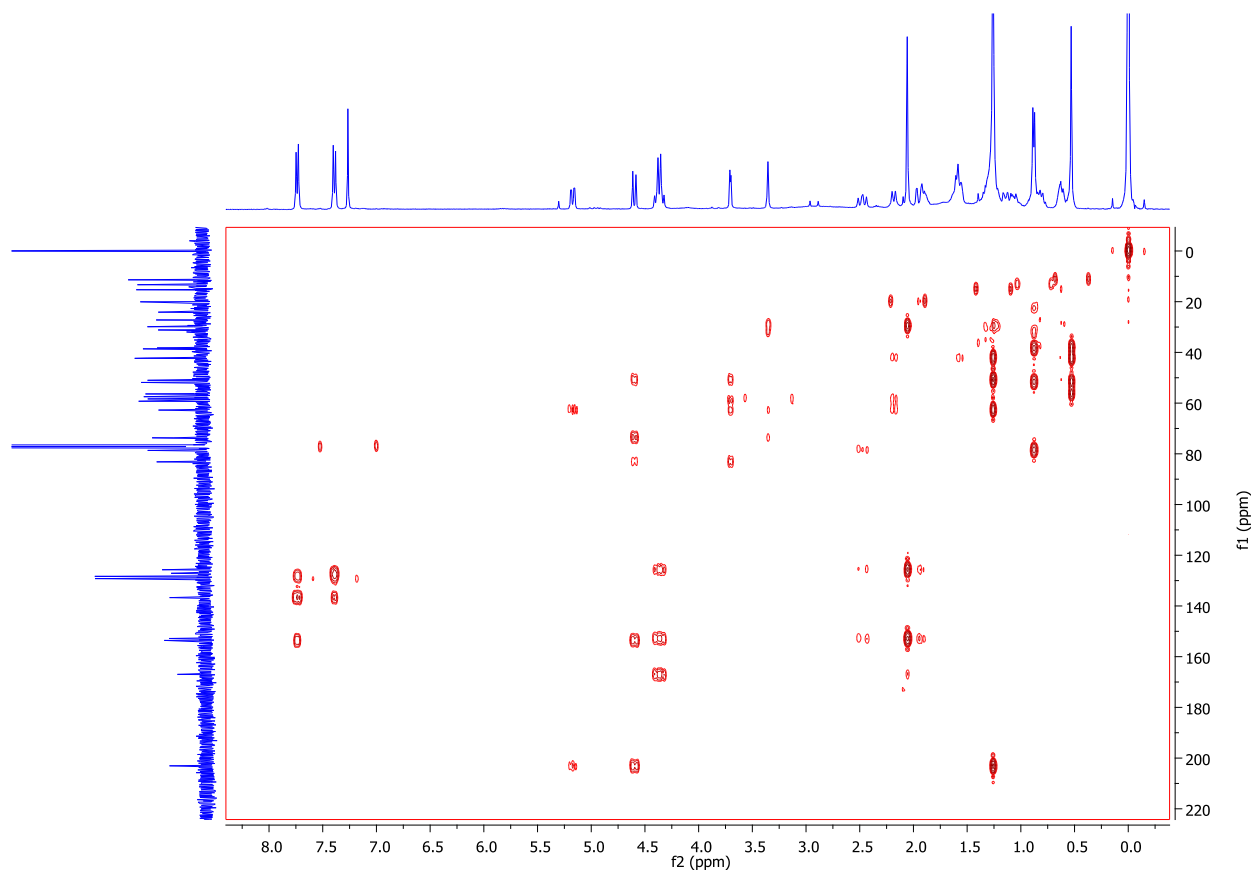
HSQC of W-1a



COSY of W-1b



HSQC of W-1b



HMBC of W-1b

