Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis and Biological Evaluation of Novel Acyclic and Cyclic Glyoxamide derivatives as Bacterial Quorum Sensing and Biofilm Inhibitors

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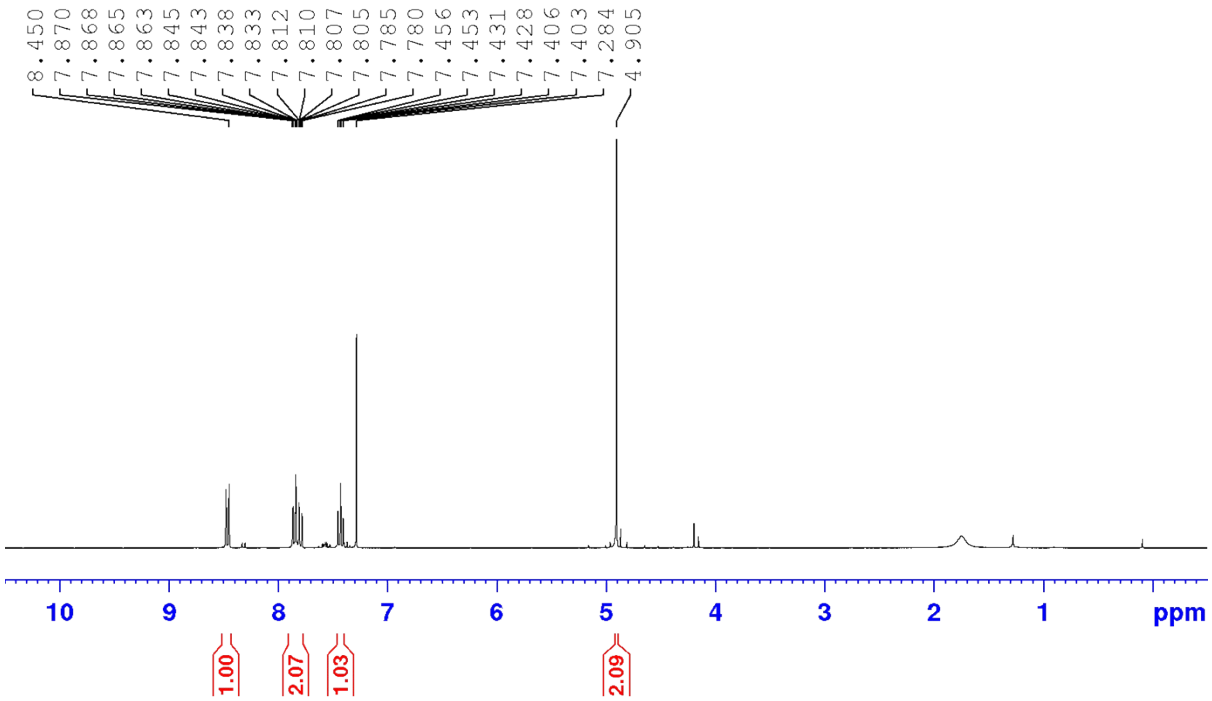

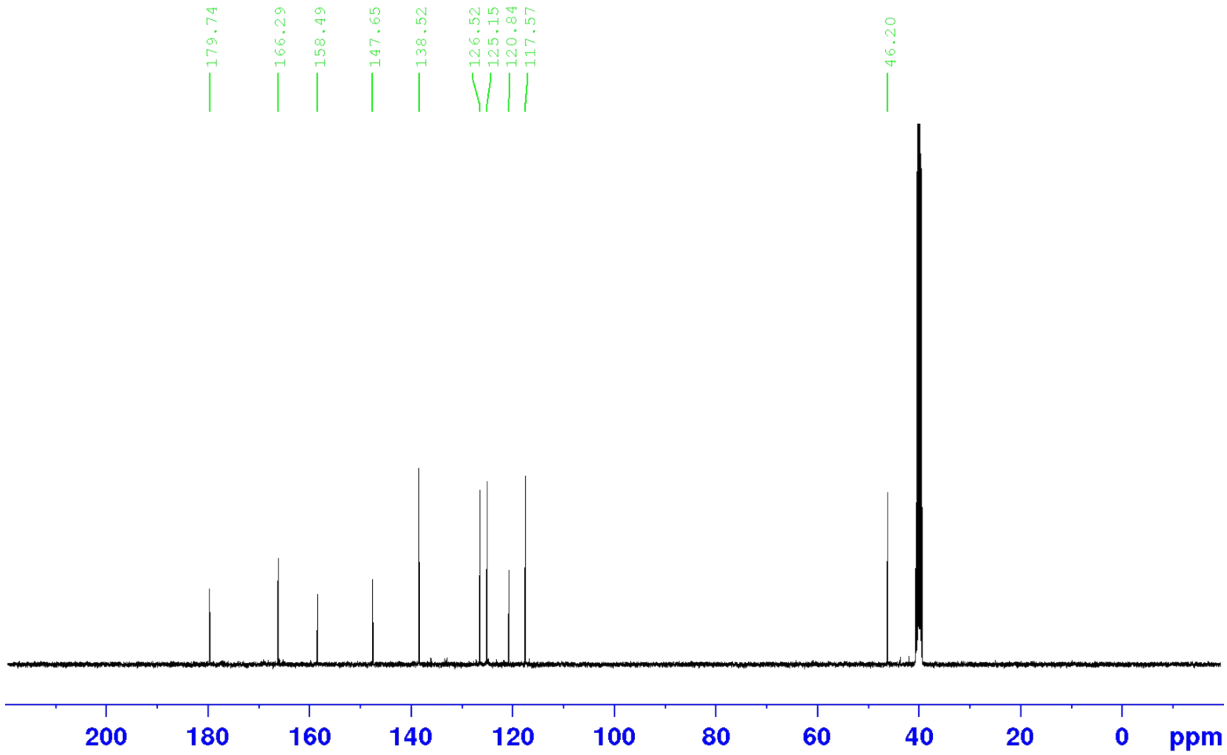

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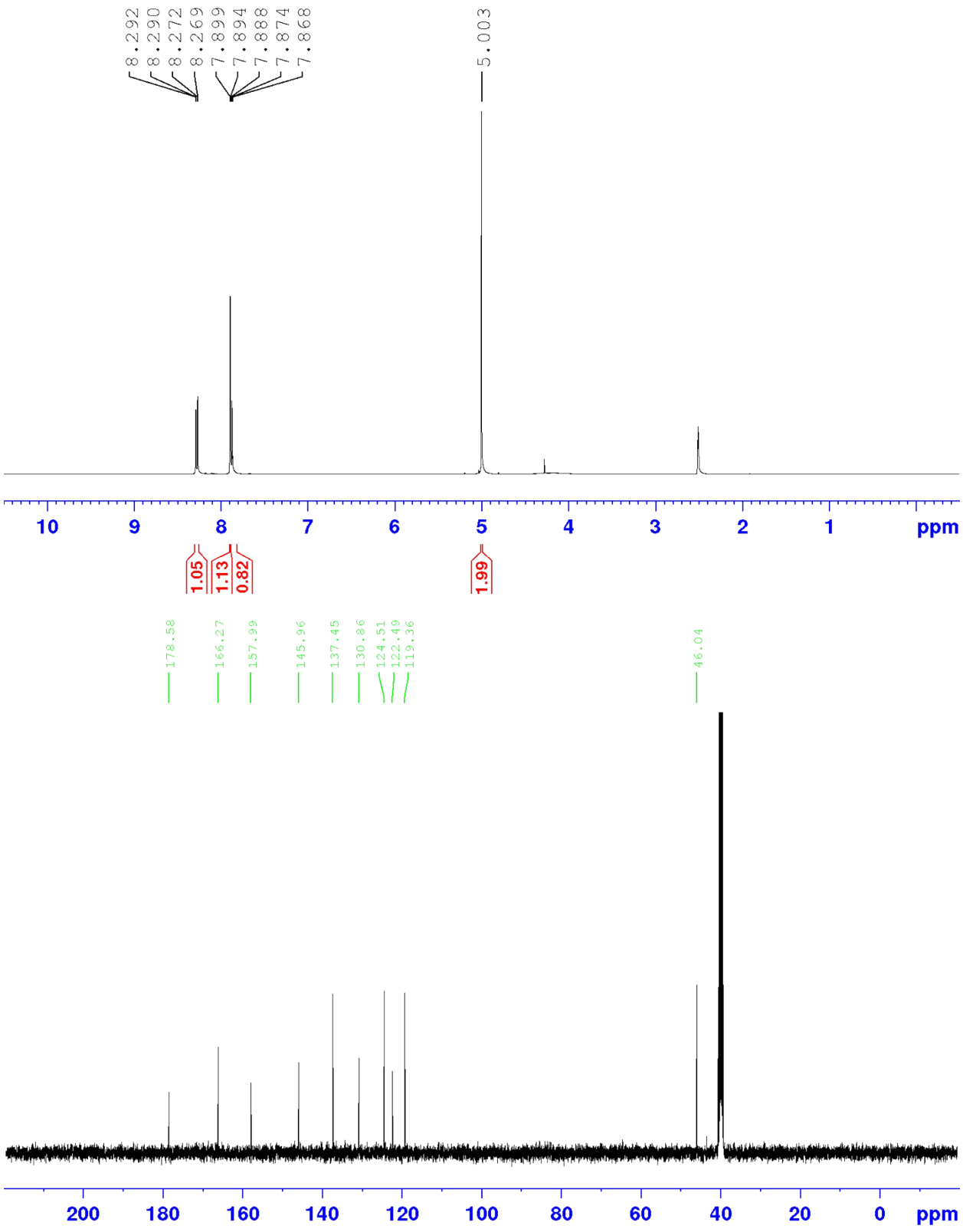

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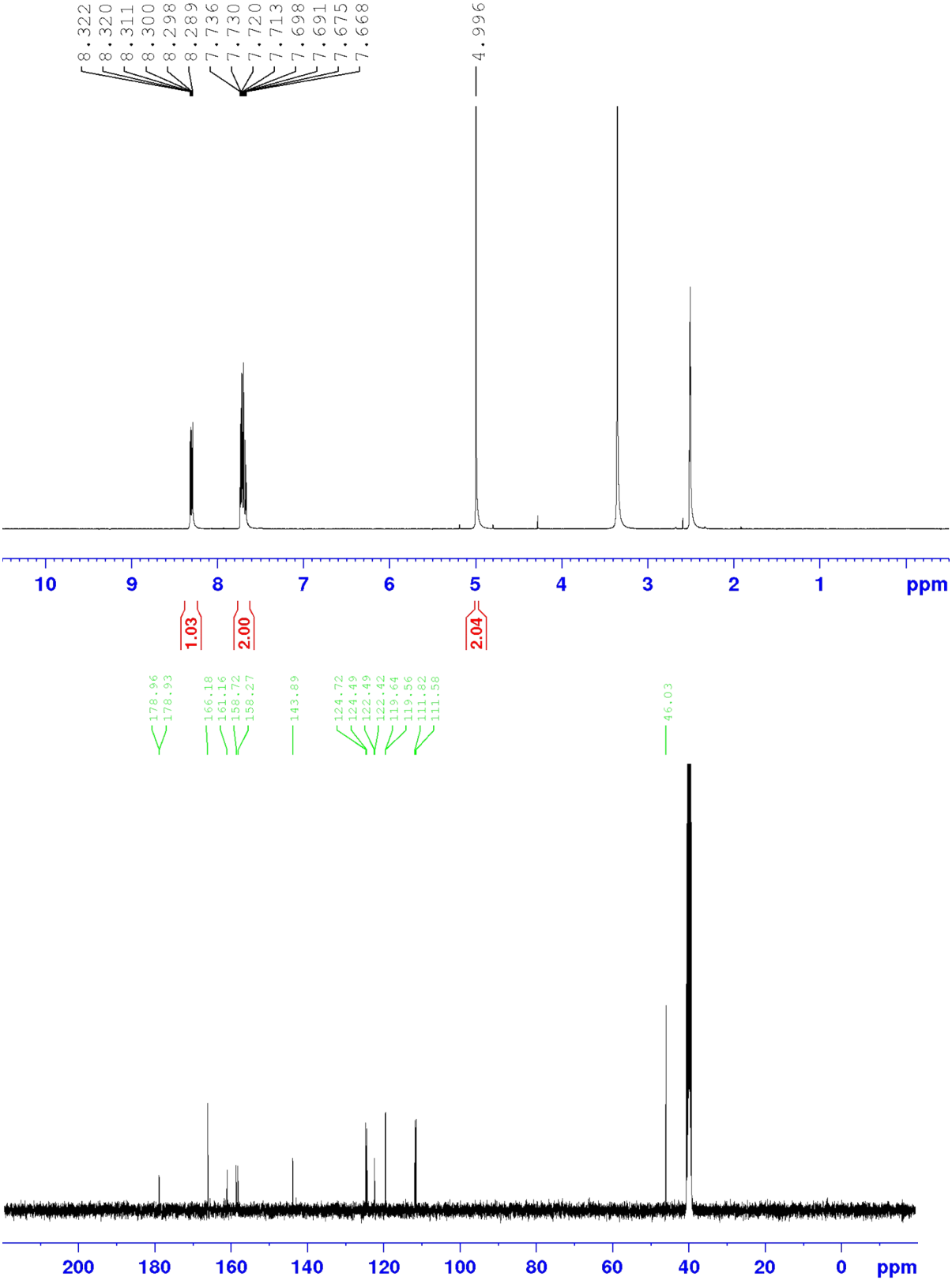

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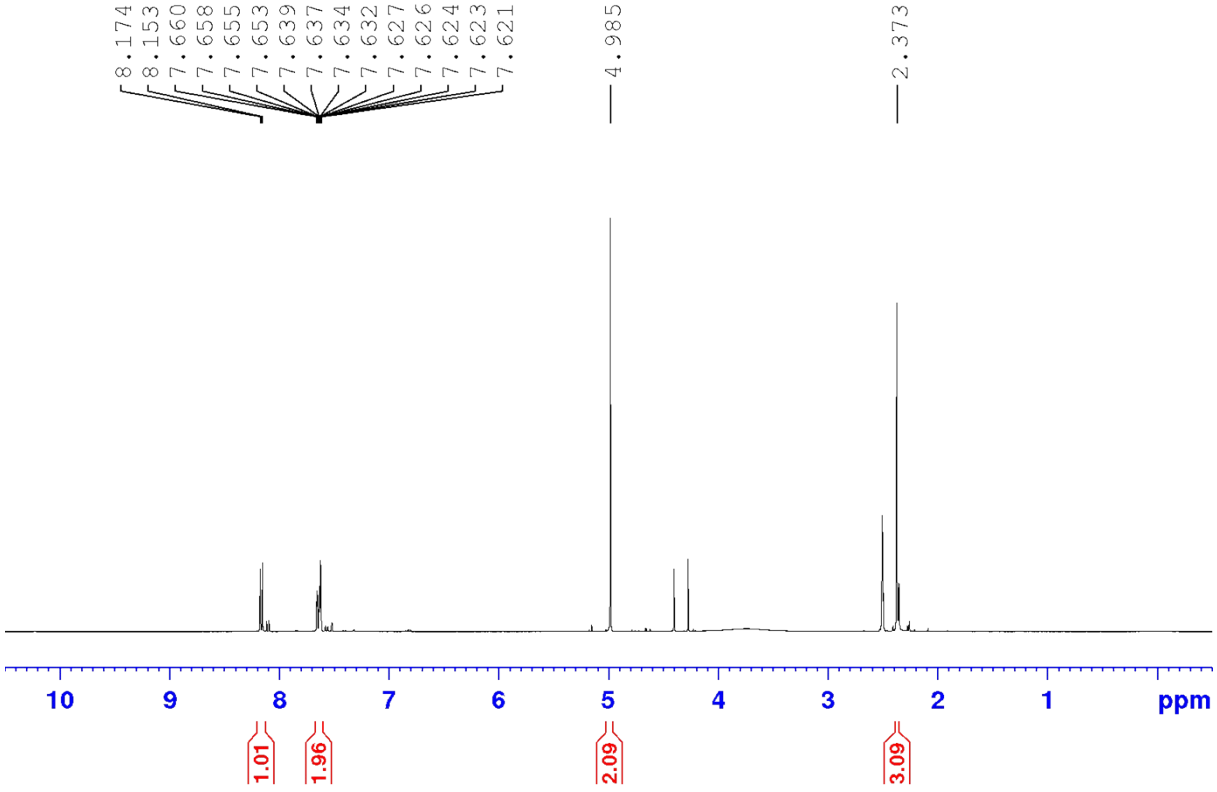

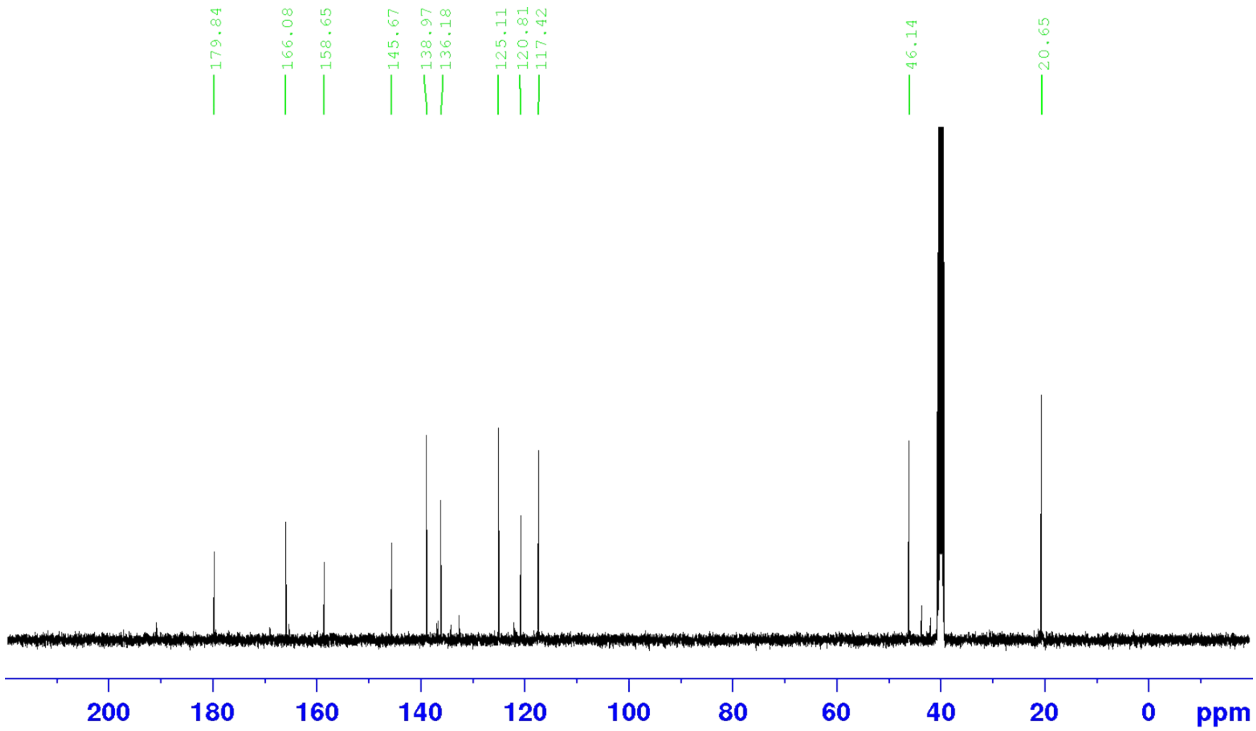

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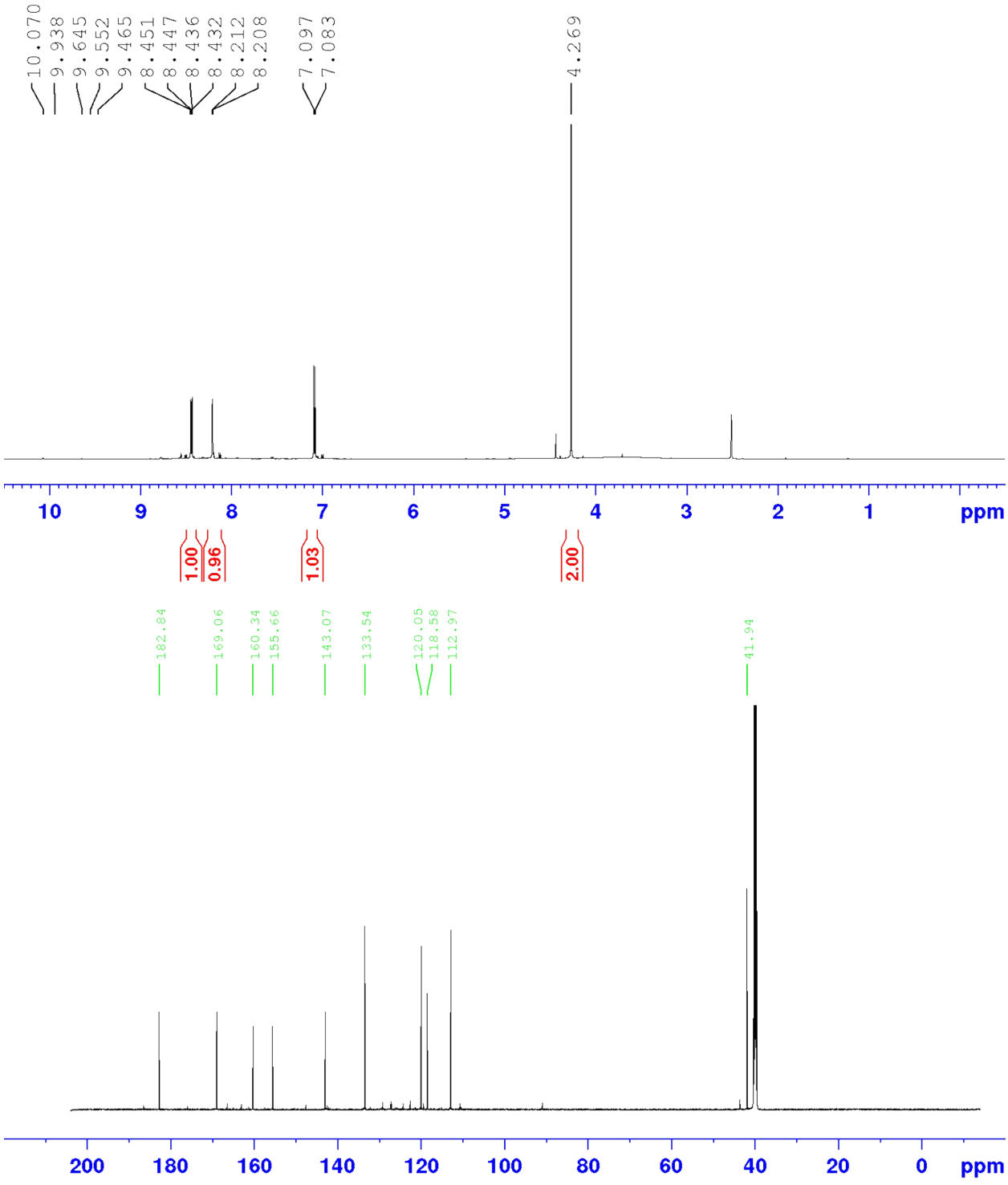

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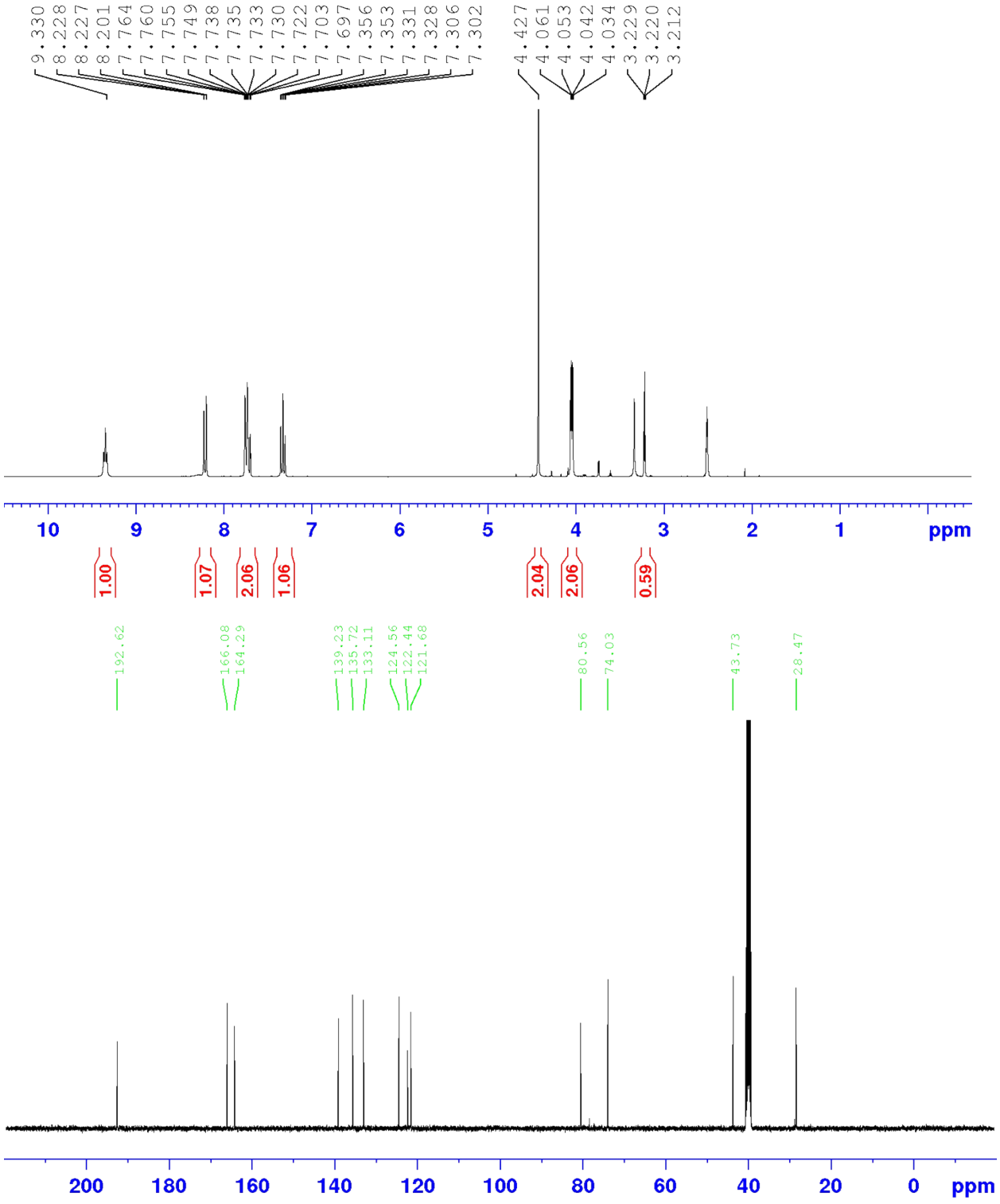

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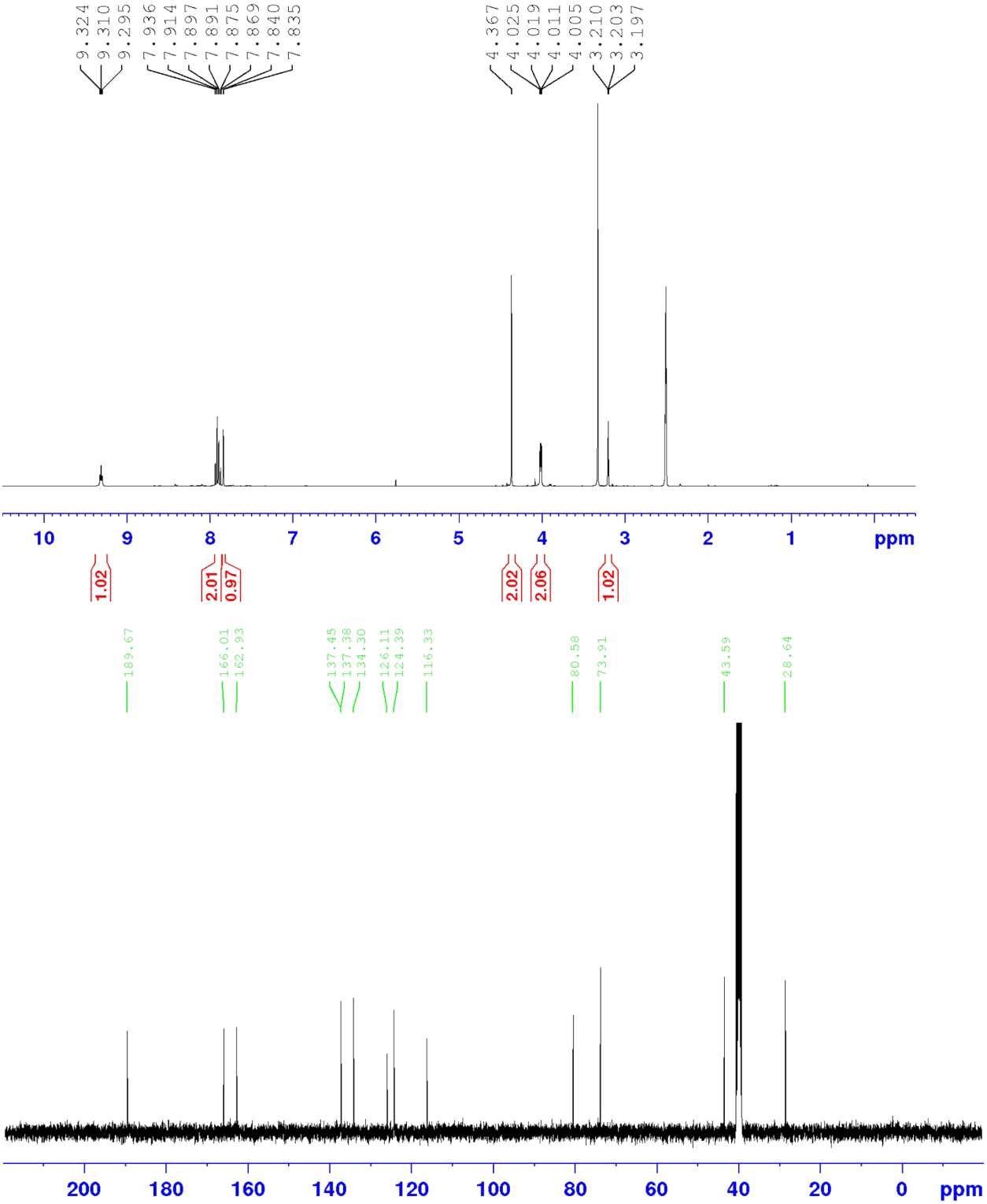

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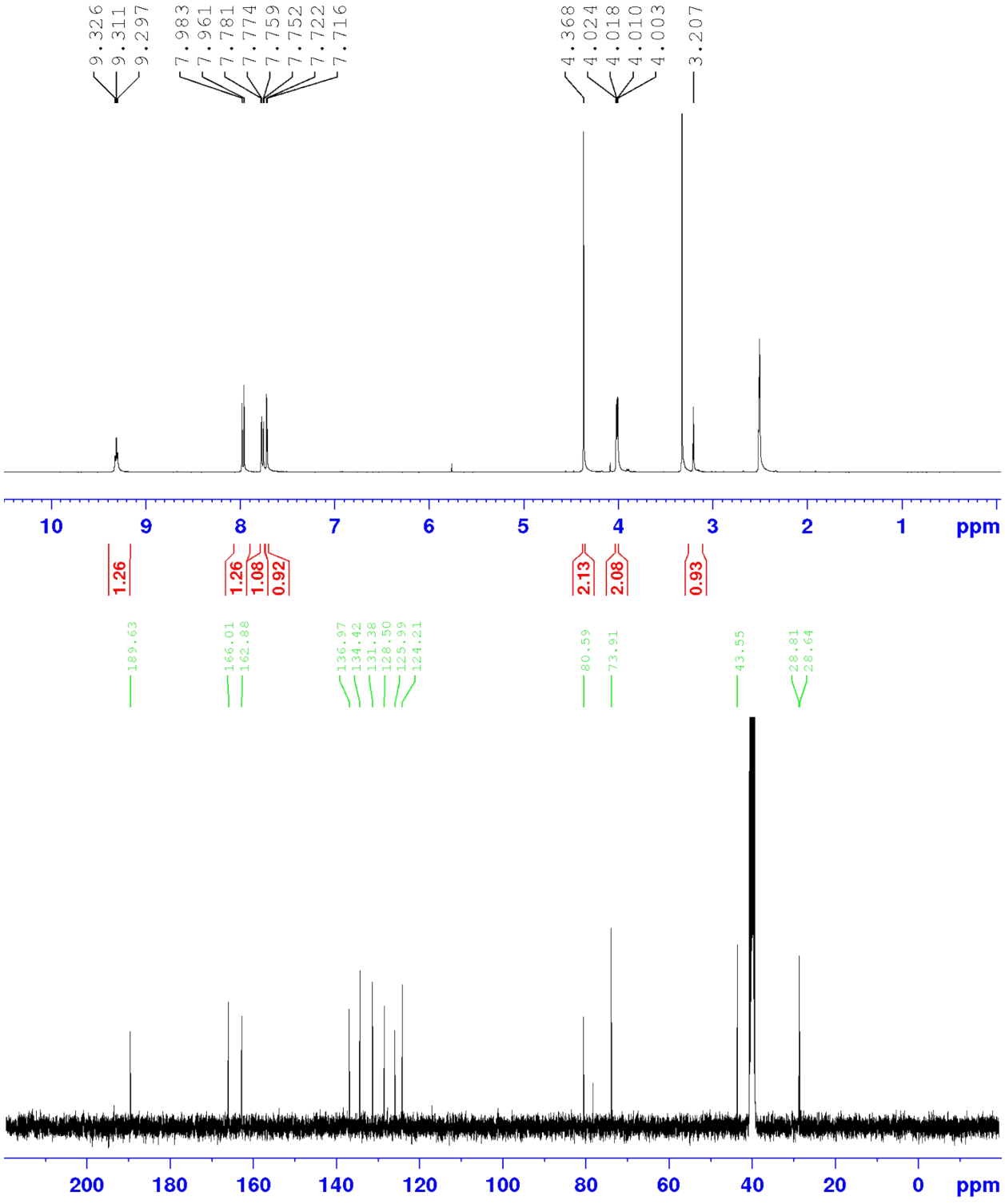

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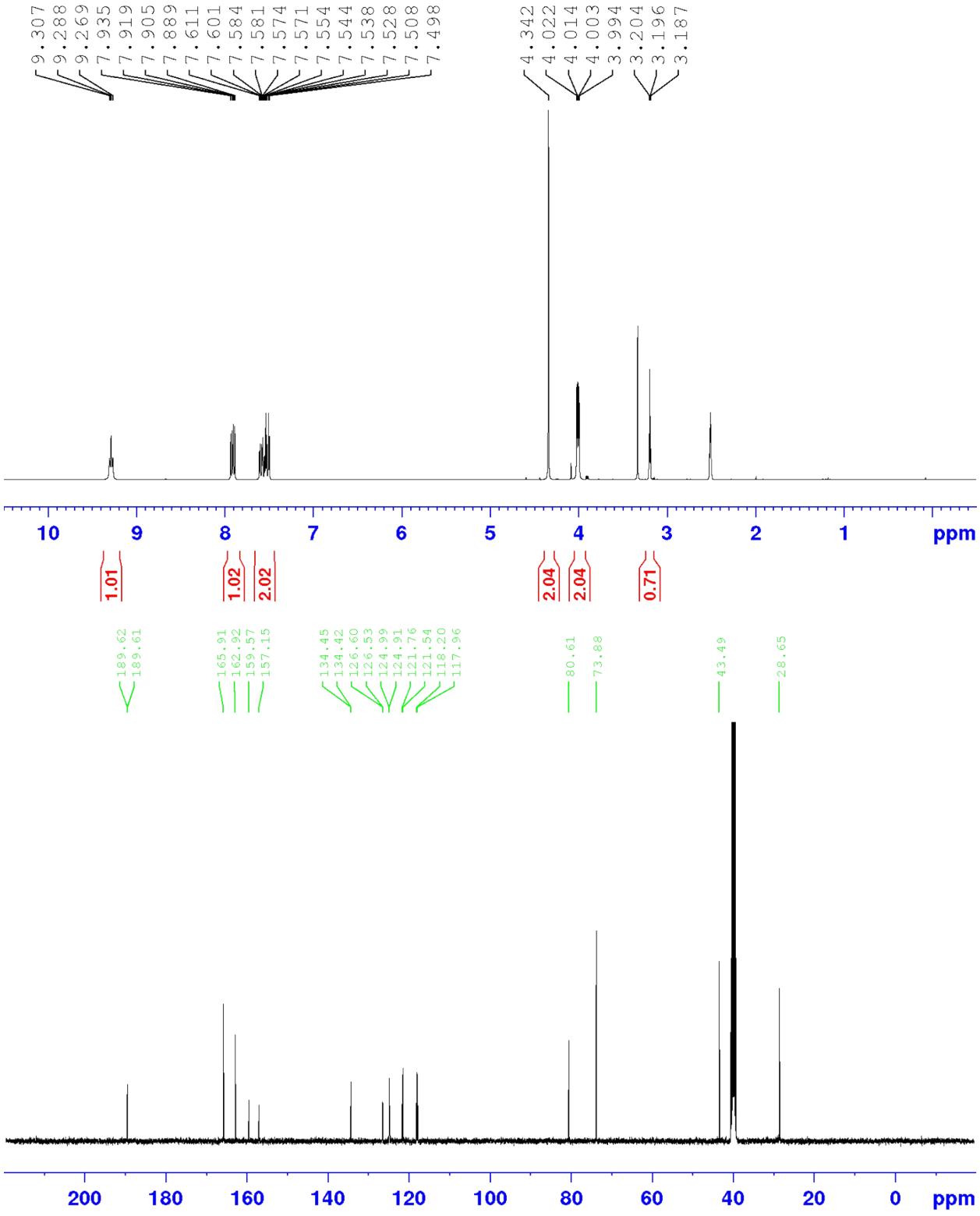

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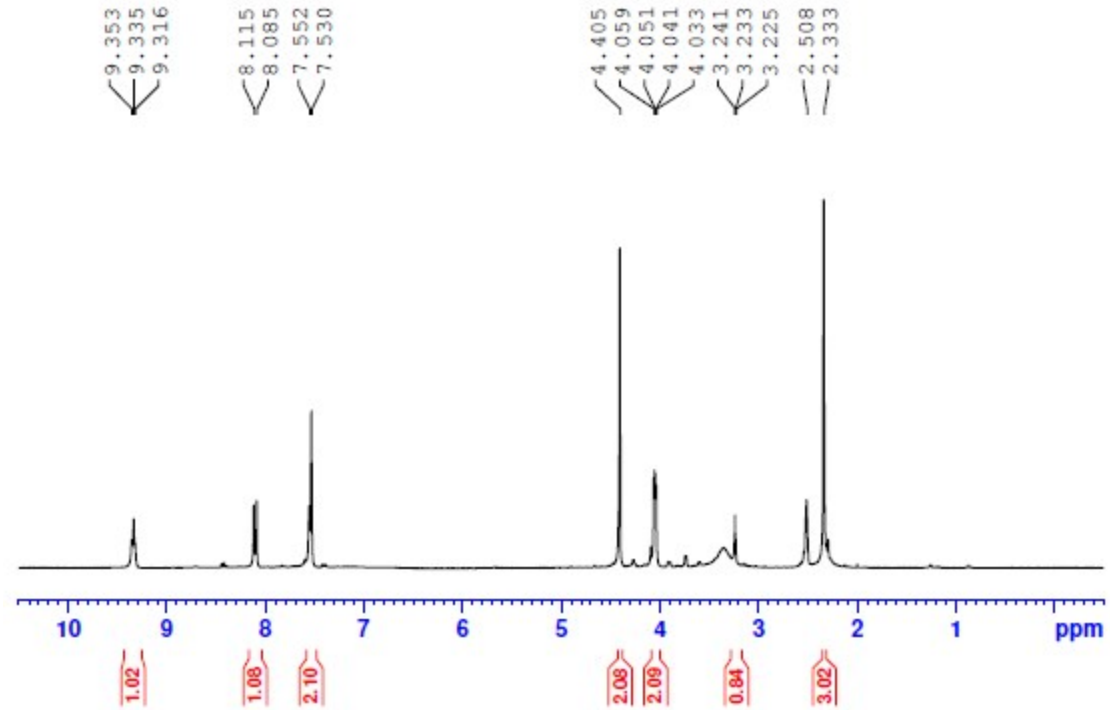

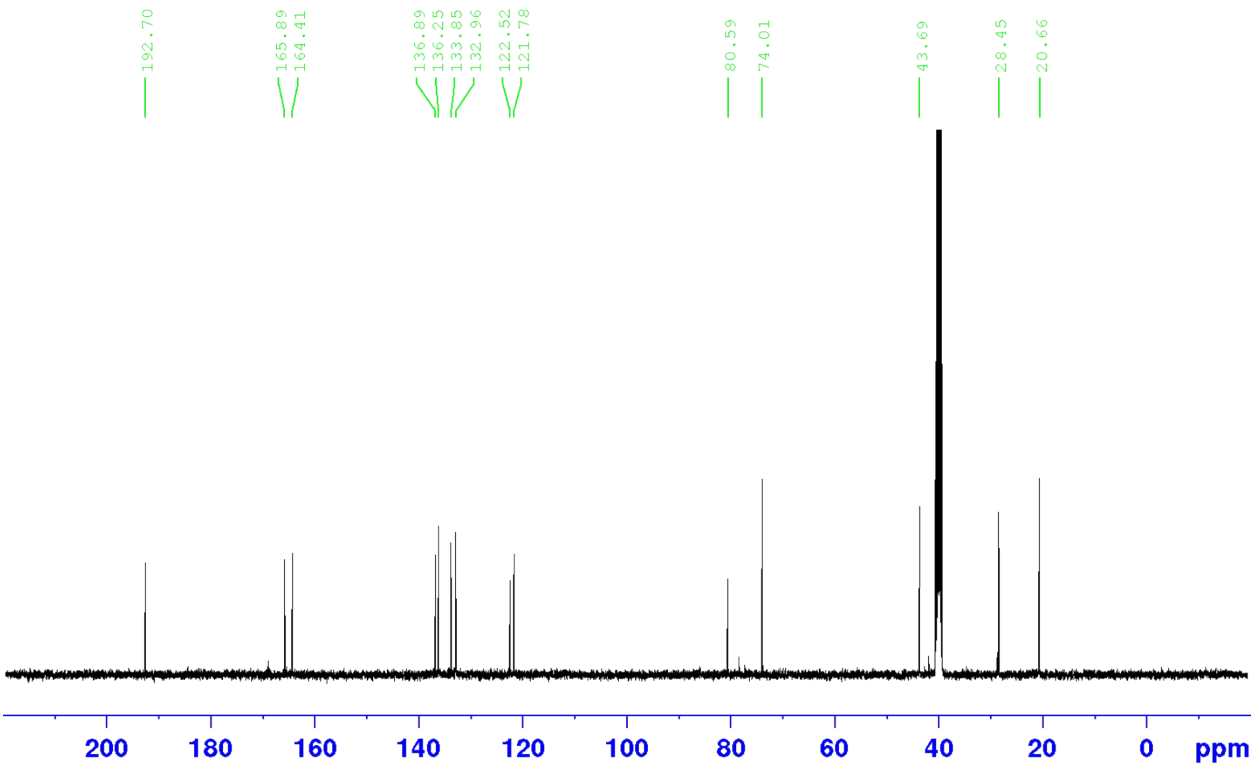

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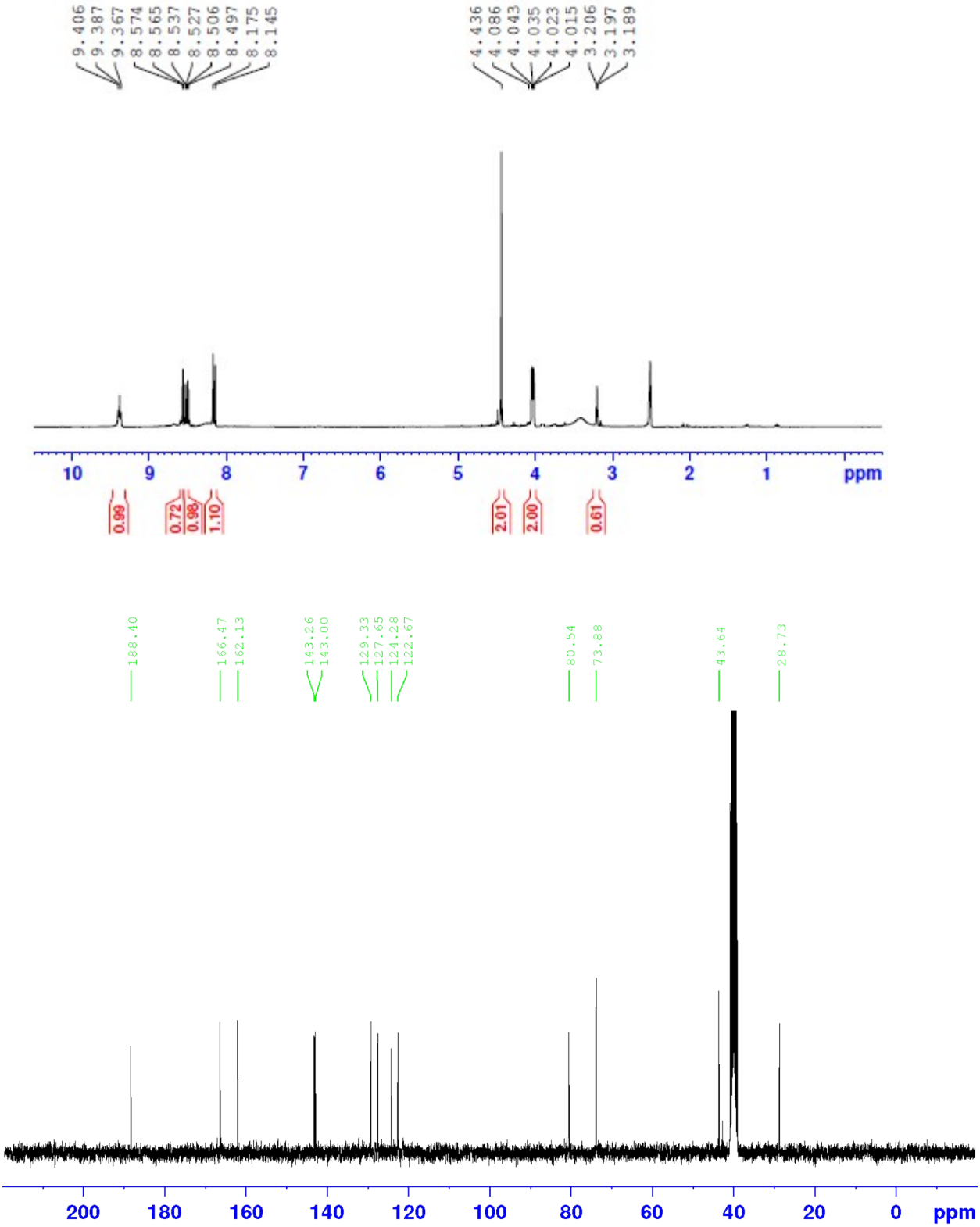

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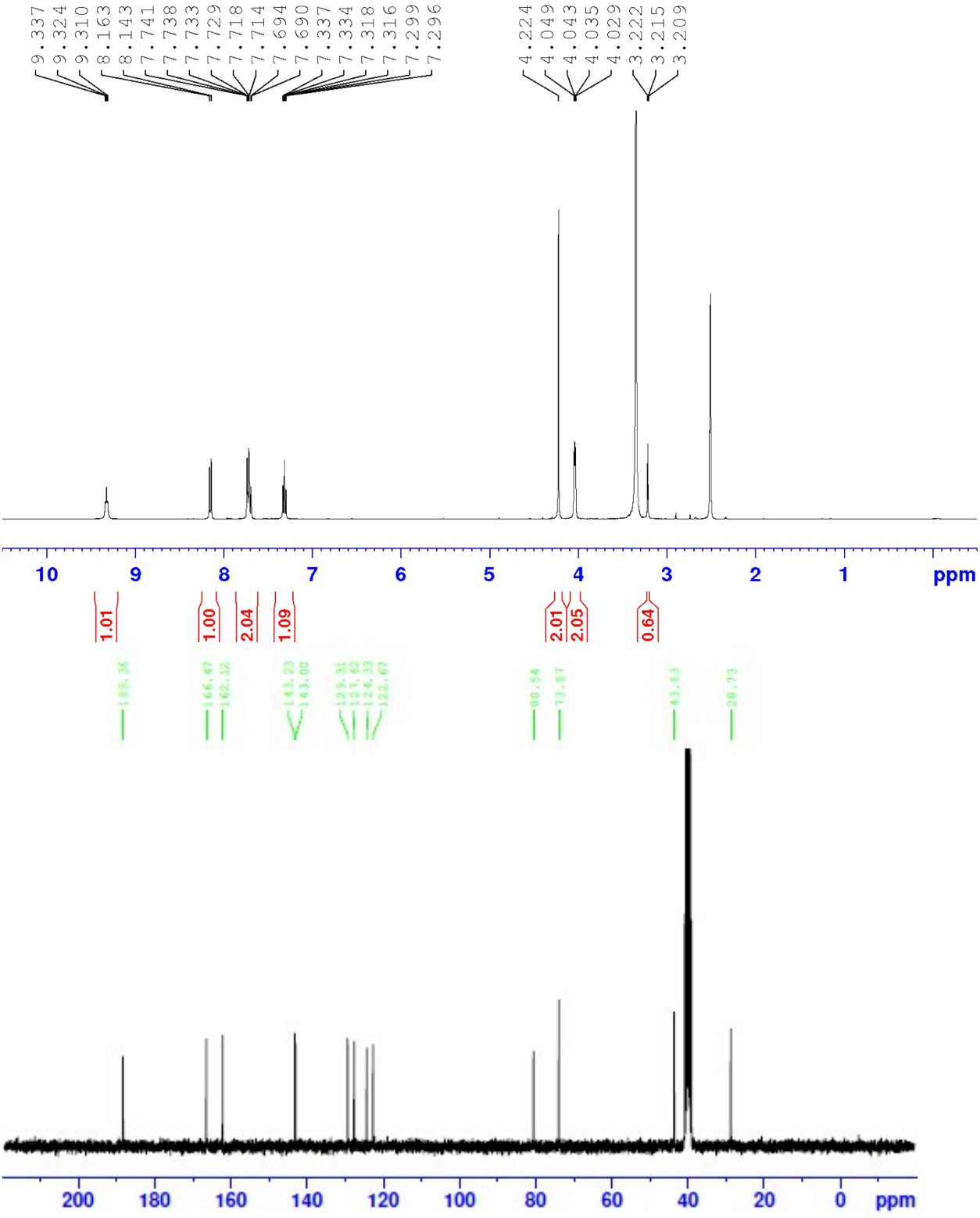

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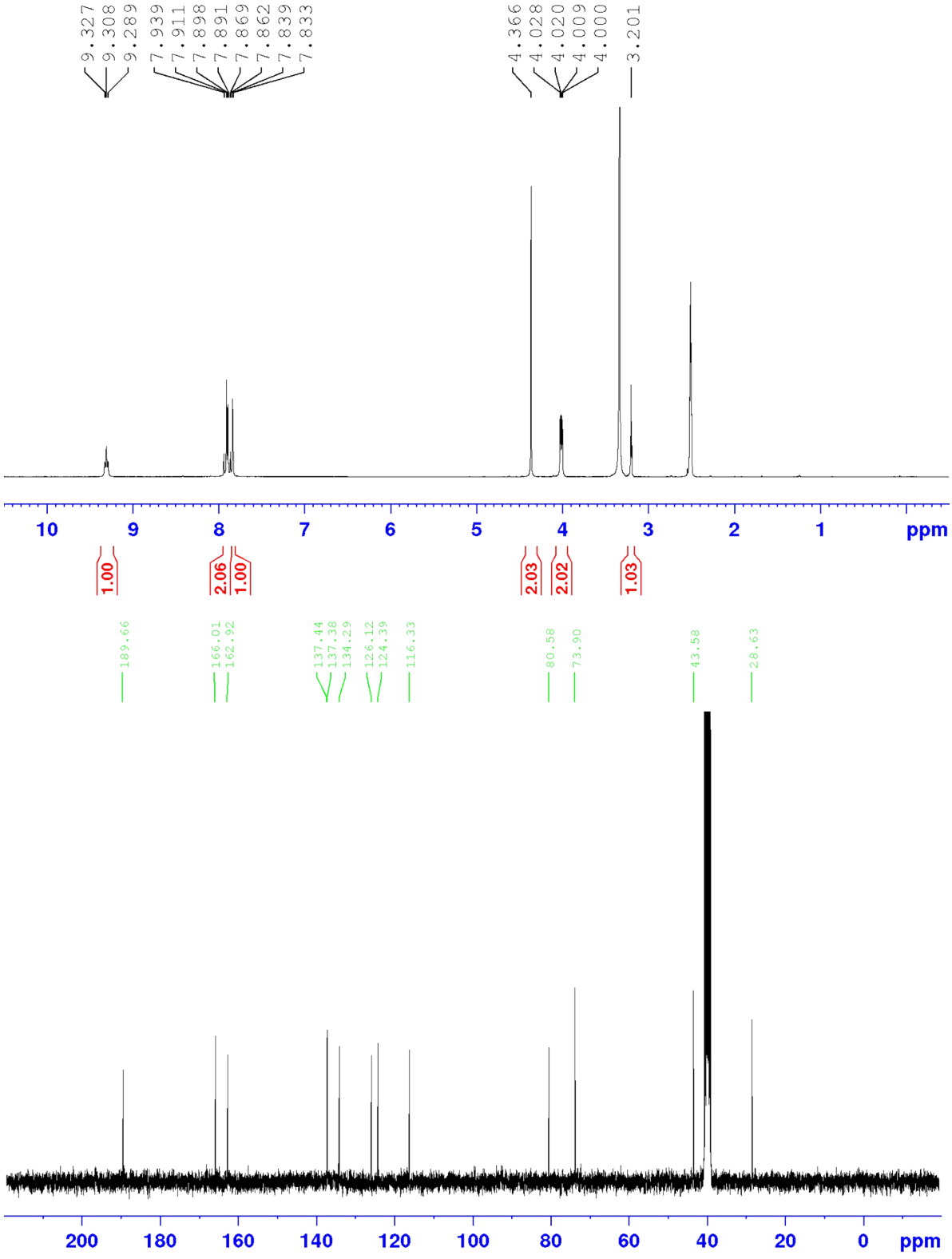

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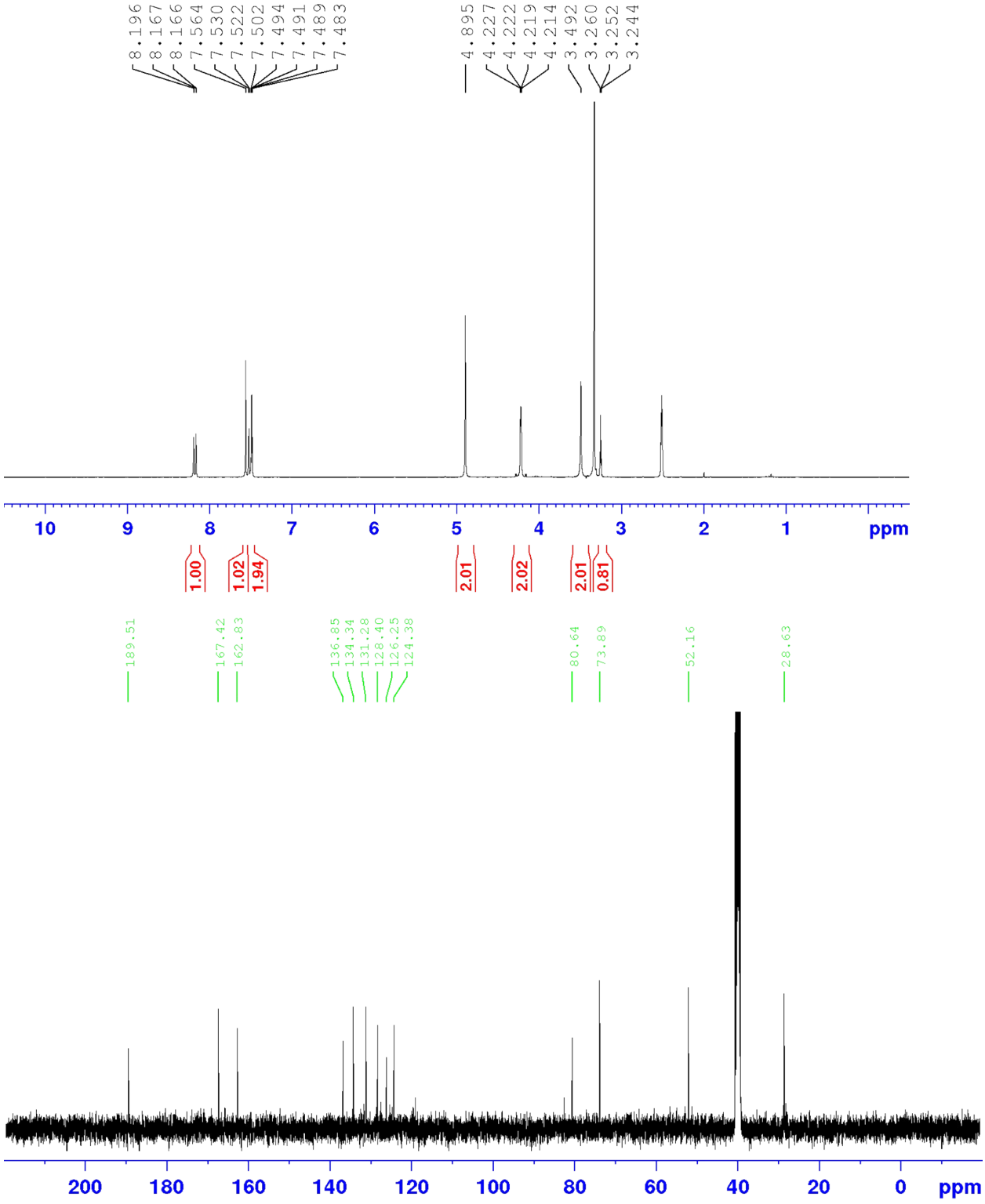

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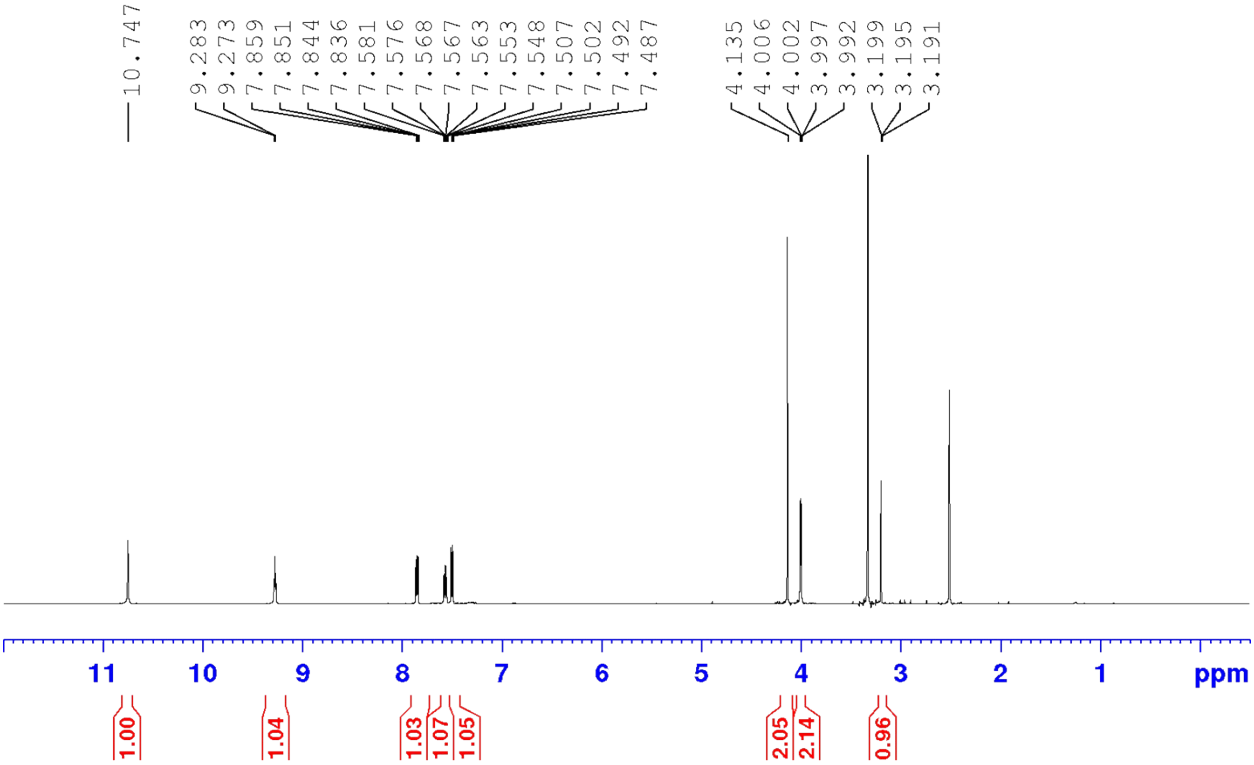

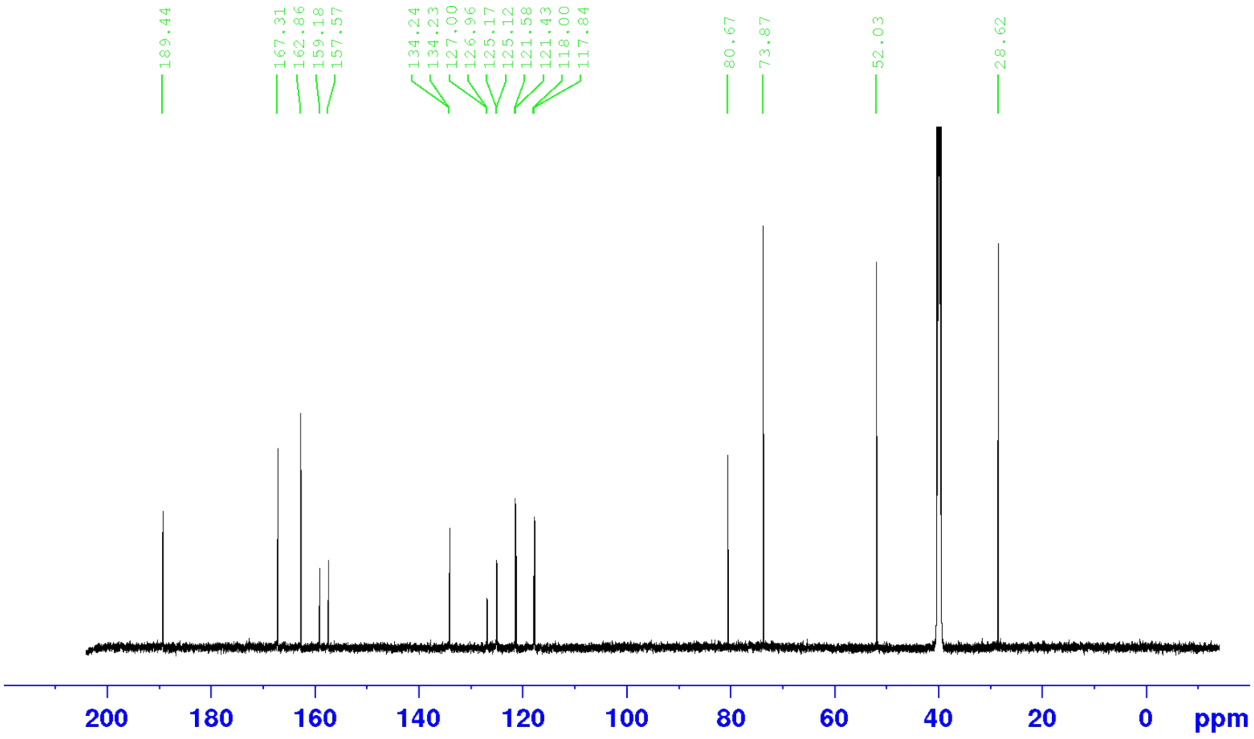

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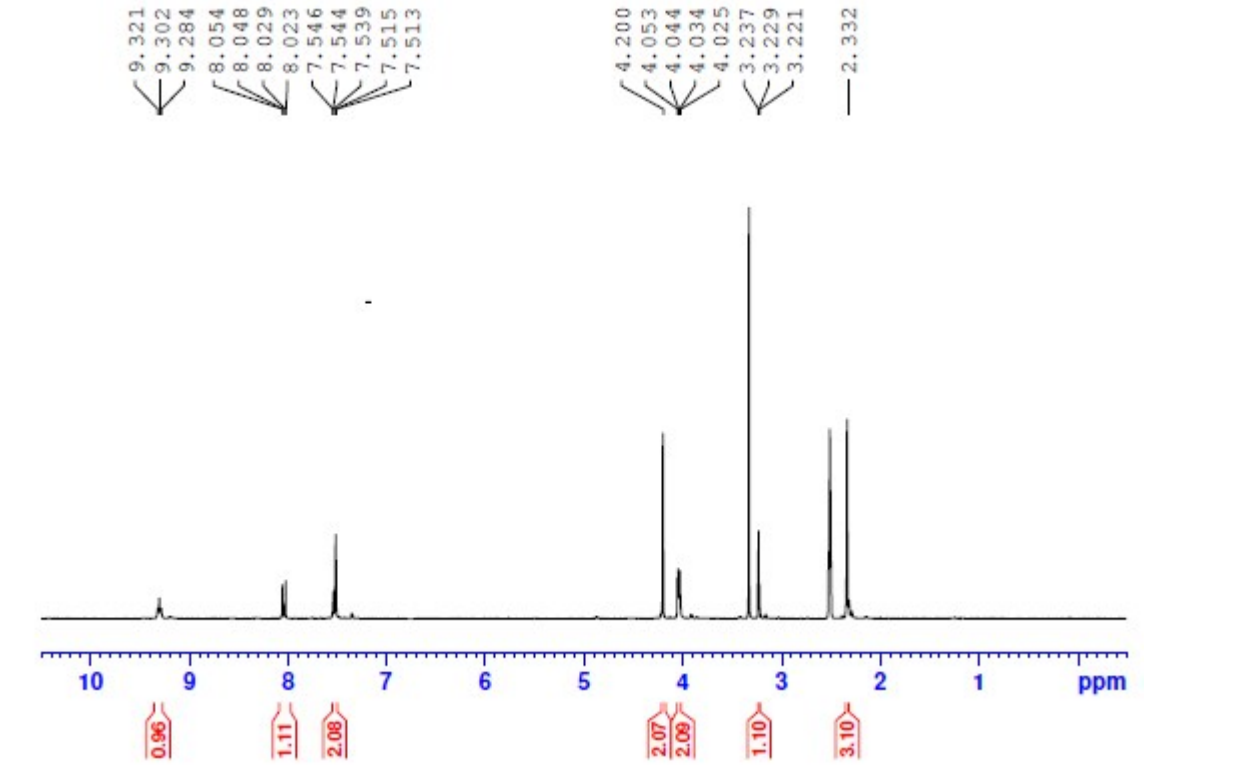

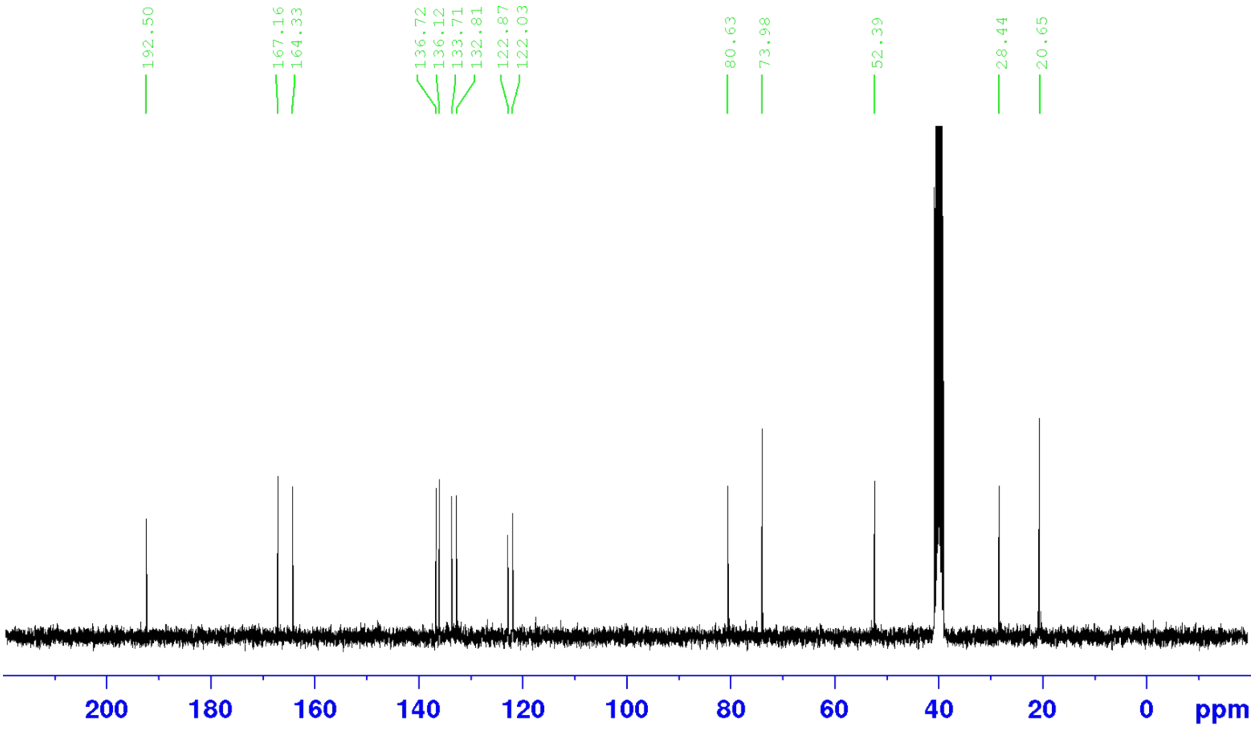

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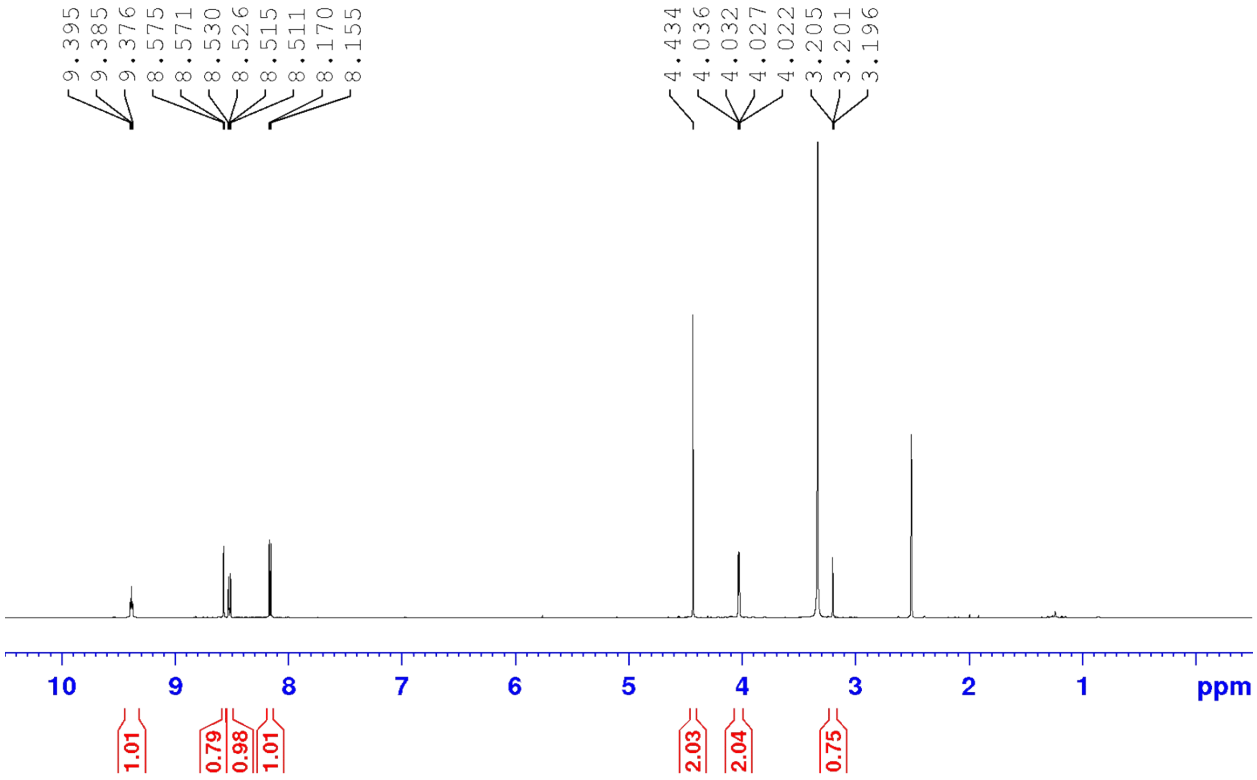
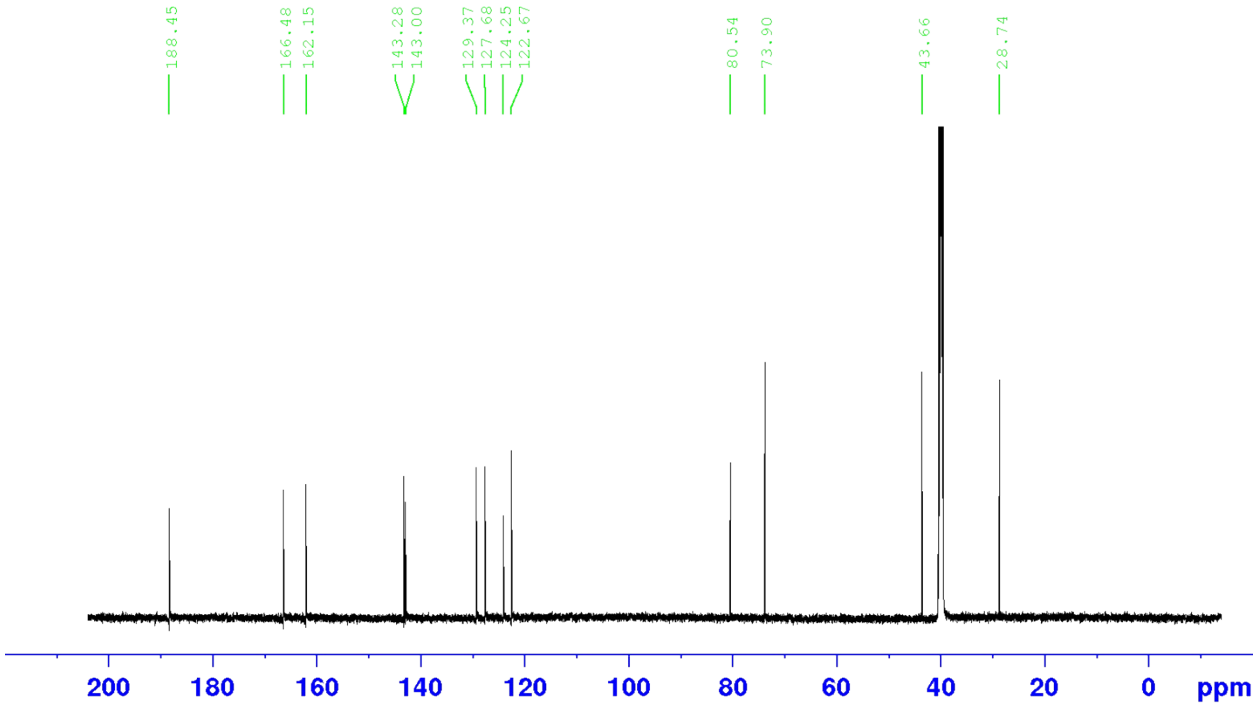
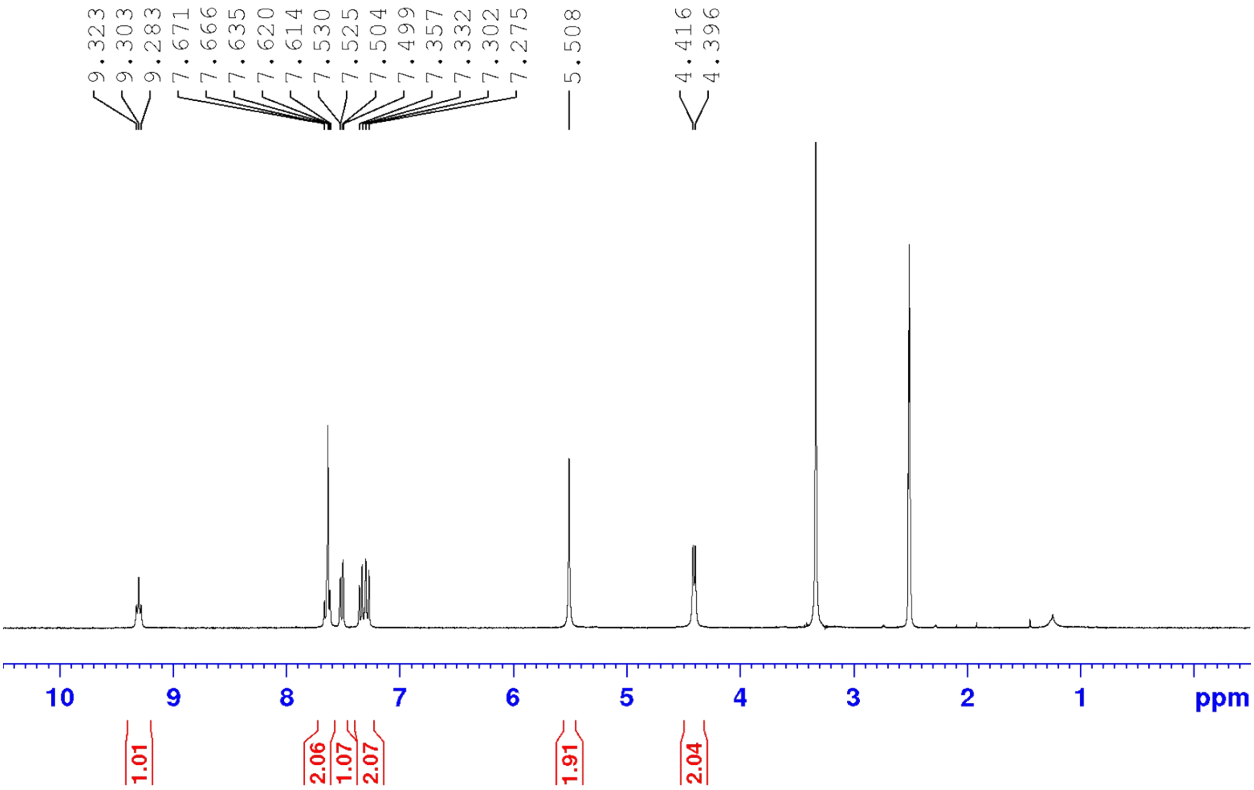
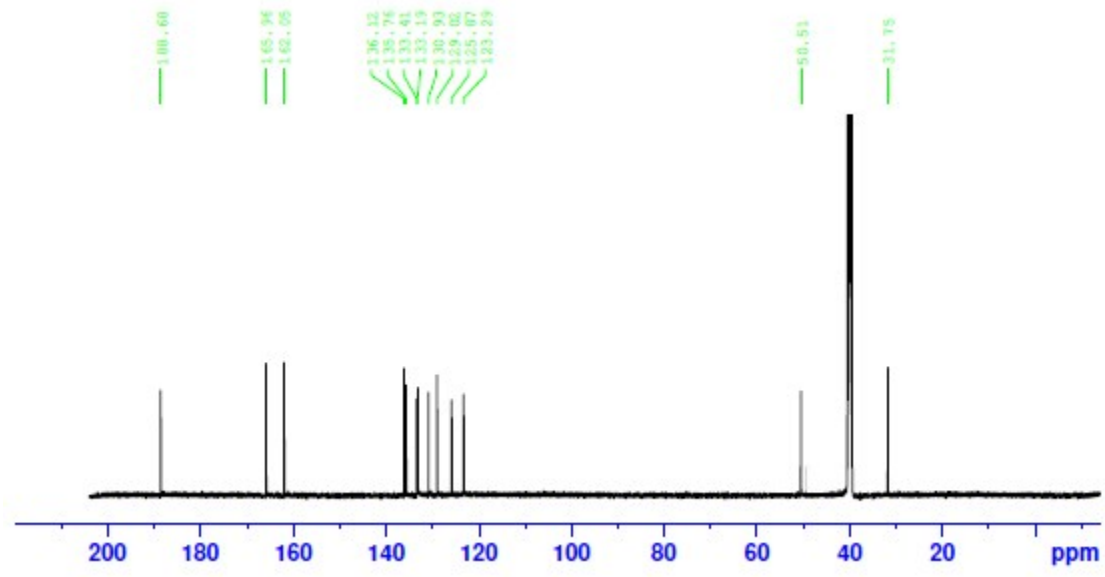




1HNMR and 13CNMR of 14,15,29,30-Tetrahydro-5H,20H-dibenzo[h,s]bis([1,2,3]triazolo)[5,1-c:5',1'-n][1,4,7,12,15,18]hexaazacyclodocosine-6,12,13,21,27,28(7H,22H)-hexaone (30)





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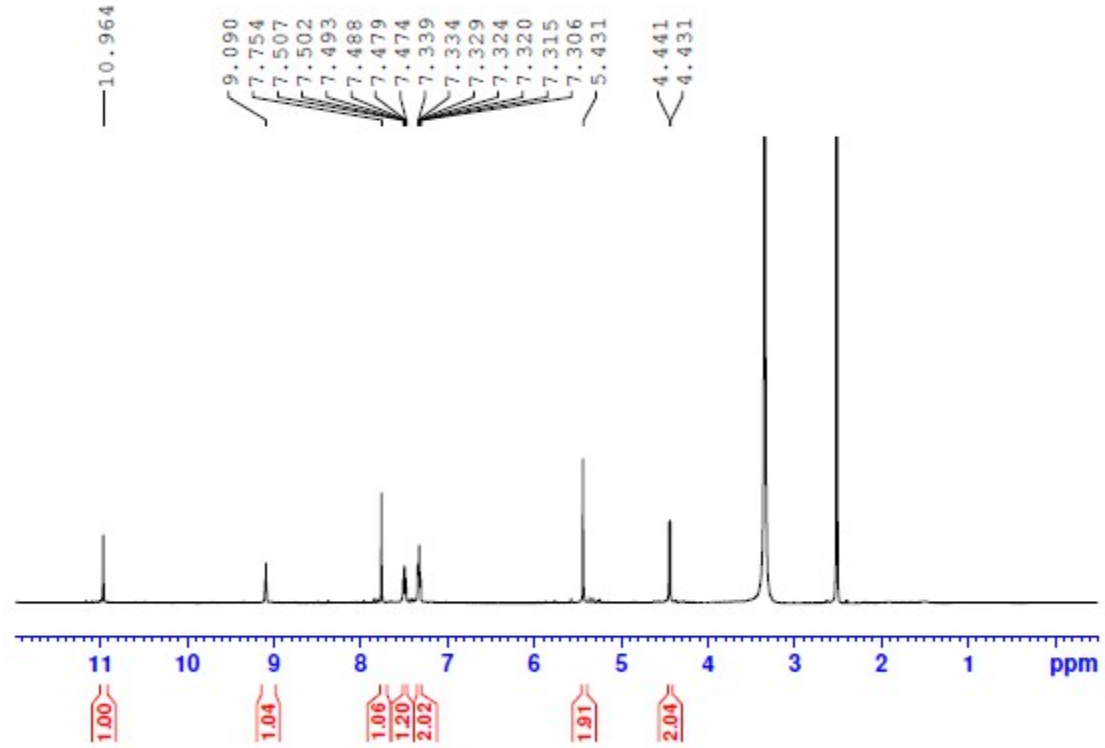

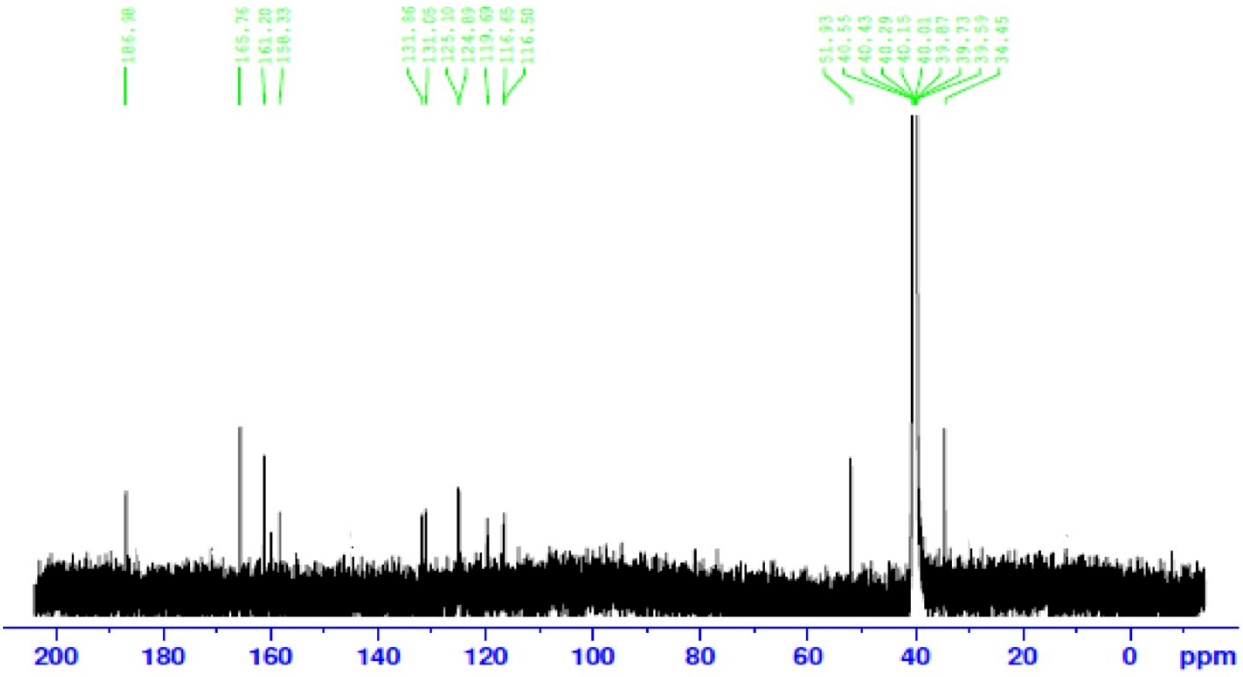

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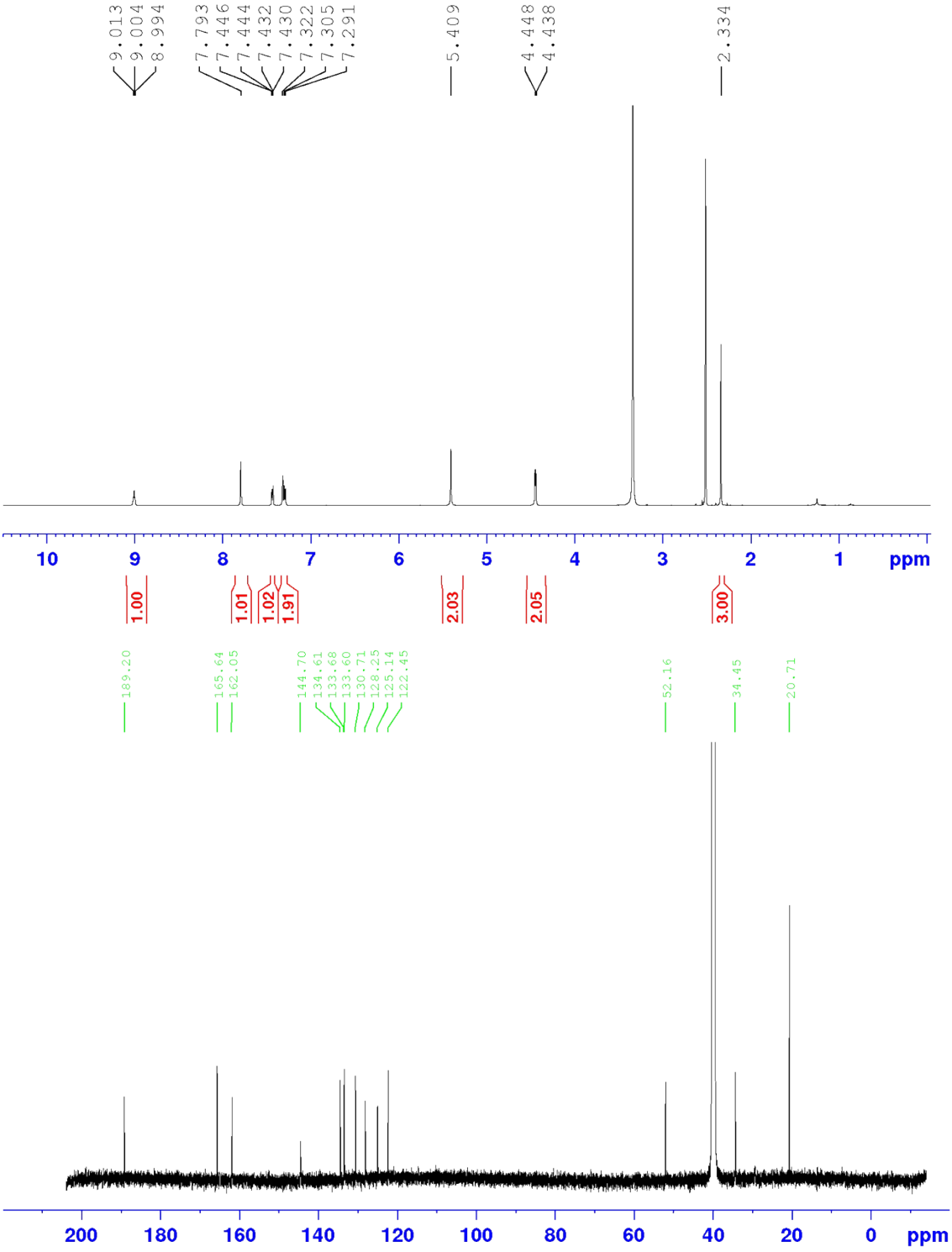

Optical Density (OD) Measurements

Table 3: Growth inhibition by the synthesized compounds against the PAMH602 and *E. coli* MT102 strains at three different concentrations.

		P. aeruginosa MH602			*E. coli* MT102		
		Concentrations (μM)			Concentrations (μM)		
	Compound	250	125	62.5	250	125	62.5
***N*-chloro-acetylisatins**	**12**	10.46±1.69	2.01±1.74	0.44±0.76	0.35±0.49	0.00	0.00
	13	7.91±0.75	1.02±3.34	0.00	15.30±9.02	0.00	0.00
	14	2.75±2.13	0.00	0.42±0.72	24.34±2.55	2.12±2.99	5.50±4.86
	15	4.56±6.41	1.97±3.41	2.19±3.80	0.00	0.00	0.00
	16	1.63±1.55	0.00	0.00	31.14±2.39	12.38±0.75	6.83±5.96
	17	1.53±1.85	0.00	0.00	25.50±4.19	0.00	0.00
	18	6.24±0.20	0.00	0.00	25.08±4.24	4.55±2.26	0.00
Alkyno-phenylglyoxamides	**19**	5.26±1.80	4.31±4.39	2.17±3.77	0.00	0.00	0.00
	20	7.59±0.44	1.77±7.16	0.00	0.00	0.00	0.00
	21	9.09±0.85	0.00	5.50±6.69	8.13±1.87	5.54±1.33	6.89±11.93
	22	3.98±0.38	0.00	0.00	16.55±8.81	17.71±2.75	13.33±6.45
	23	1.05±1.48	0.00	0.76±1.32	0.00	0.00	0.00
	24	4.74±4.45	0.00	0.00	6.54±4.85	4.40±3.70	5.59±8.15
Azido-alkyno-phenylglyoxamides	**25**	4.40±6.22	2.87±4.97	4.31±4.29	0.00	0.00	0.00
	26	8.31±2.81	2.09±3.62	4.16±6.27	10.49±9.84	15.37±1.70	0.93±0.81
	27	4.60±6.50	0.00	3.40±4.48	17.65±3.08	3.07±3.42	0.00
	28	5.40±3.74	0.00	0.00	4.80±19.7	1.49±7.08	2.18±9.12
	29	7.31±2.77	0.35±7.82	0.00	15.94±3.08	6.01±4.61	3.22±12.41
Cyclic-phenyl glyoxamides	**30**	17.04±6.01	0.00	0.00	9.62±6.54	0.00	0.00
	31	4.24±2.65	3.71±6.29	2.38±2.21	0.00	0.00	0.00
	32	5.46±7.72	0.37±9.53	0.55±0.78	0.00	0.00	0.00
	Fu-30	88.11±	79.34±	1.79±12.5	98.8±11.7	99.7±0.3	75.6±0.5

Growth inhibition ± standard deviation of mean from at least two independent experiments. Compounds tested thrice in triplicate. 0 = No growth inhibition.

The crystal data, data collection and refinements

Table 4. The data collection and refinements.

Crystal data		
	Compound **30** complex with DMSO	Compound **30** complex with H_2O
Chemical formula	$C_2H_6OS{\cdot}0.25(C_{26}H_{22}N_{10}O_6)$	$C_{26}H_{22}N_{10}O_6{\cdot}2(H_2O)$
M_r	220.76	606.57
Crystal system, space group	Triclinic, $P\bar{1}$	Triclinic, $P\bar{1}$
Temperature (K)	154	158
a, *b*, *c* (Å)	9.7434 (5), 9.8225 (5), 12.0241 (6)	7.428 (4), 10.364 (7), 10.401 (6)
□, □, □ (°)	92.357 (2), 104.697 (3), 106.384 (2)	60.81 (2), 86.52 (3), 88.14 (3)
V (Å^3)	1060.01 (9)	697.8 (7)
Z	4	1
Radiation type	Mo *K*□	Mo *K*□
□ (mm^{-1})	0.29	0.11
Crystal size (mm)	0.10 × 0.06 × 0.06	0.11 × 0.09 × 0.05
Data collection		
Diffractometer	Bruker *APEX*-II CCD	Bruker *APEX*-II CCD
Absorption correction	Multi-scan *SADABS2014*/5 (Bruker,2014/5) was used for absorption correction. wR2(int) was 0.1351 before and 0.0522 after correction. The Ratio of minimum to maximum transmission is 0.9050. The □/2 correction factor is 0.00150.	Multi-scan *SADABS2014*/5 (Bruker,2014/5) was used for absorption correction. wR2(int) was 0.1810 before and 0.0610 after correction. The Ratio of minimum to maximum transmission is 0.7174. The □/2 correction factor is 0.00150.
T_{min}, T_{max}	0.675, 0.746	0.535, 0.746
No. of measured, independent and observed [$I > 2\square(I)$] reflections	14261, 3686, 2869	6795, 2401, 1069
R_{int}	0.043	0.103
(sin □/□)$_{max}$ (Å^{-1})	0.595	0.595
Refinement		
$R[F^2 > 2\square(F^2)]$, $wR(F^2)$, *S*	0.039, 0.103, 1.04	0.066, 0.167, 0.92
No. of reflections	3686	2401
No. of parameters	268	207
H-atom treatment	H-atom parameters constrained	H atoms treated by a mixture of independent and constrained refinement
□□$_{max}$, □□$_{min}$ (e Å^{-3})	0.23, -0.35	0.30, -0.30

Biofilm inhibition activity in *P. aeruginosa*

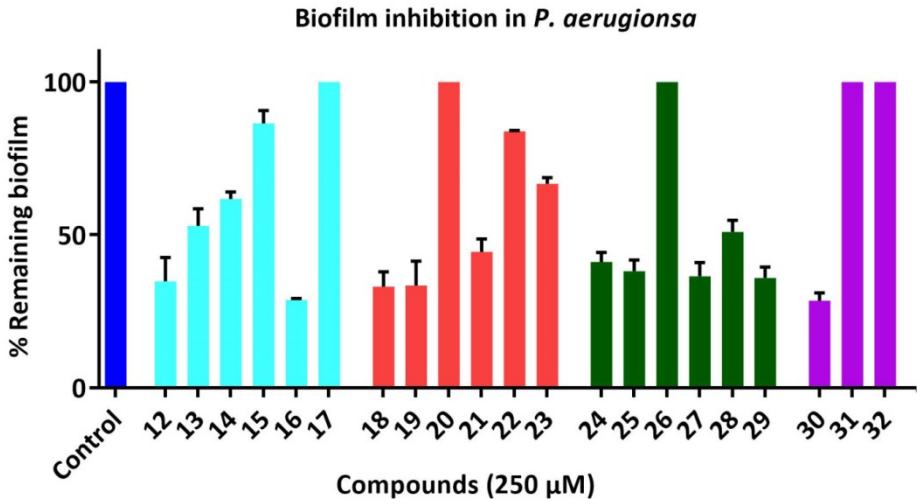

Fig. 5 Inhibition of biofilm formation in *P. aeruginosa* after 24 h treatment with 250 µM of glyoxamide compounds. The control represents the biofilms formation without any compounds. Error bars indicate the standard error of the mean (SEM) of three independent experiments.

Biofilm inhibition activity in *E. coli*

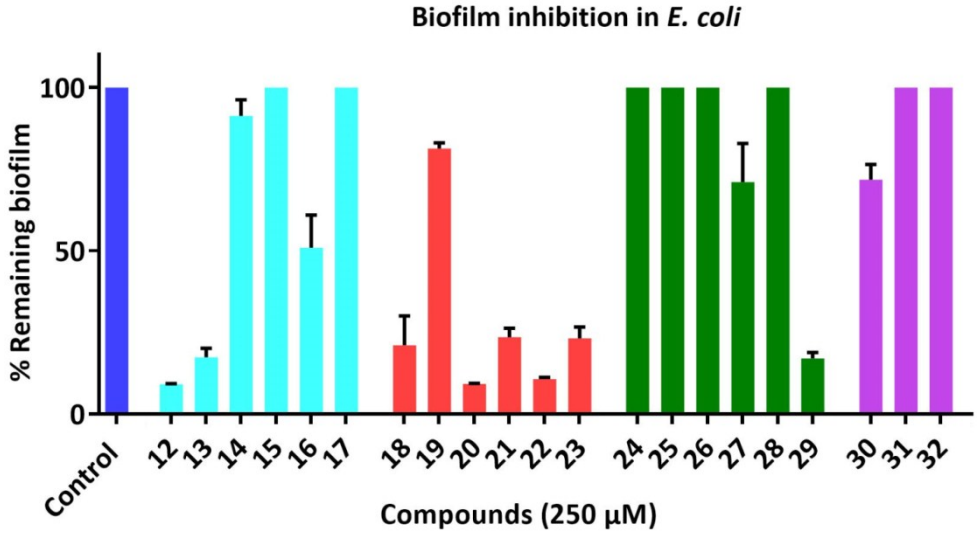

Fig. 6 Inhibition of biofilm formation in *E. coli* after 24 h treatment with 250 µM of glyoxamide compounds. The control represents the biofilms formation without any compounds. Error bars indicate the standard error of the mean (SEM) of three independent experiments.

Toxicity against human MRC-5 lung fibroblast cells

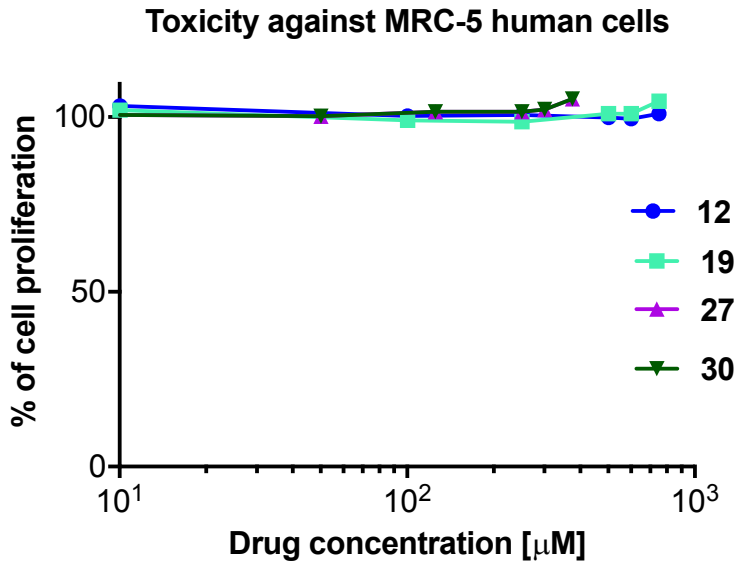

Fig. 7 *In vitro* anti-proliferative properties of compounds **12**, **19**, **27**, and **30** against MRC-5 normal human lung fibroblasts after 72 h incubation, relative to a DMSO control. The points represent the mean of at least three individual experiments ± standard error of the mean (SEM).

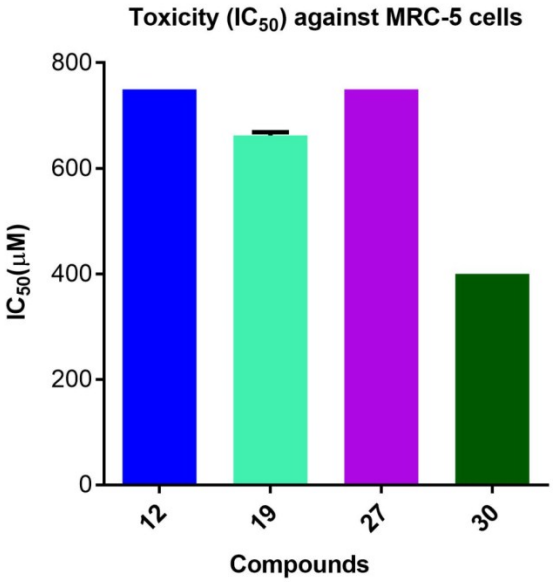

Fig. 8 The bar graph represents the IC_{50} of some of the active compounds **12**, **19**, **27**, and **30** against MRC-5 normal human lung fibroblast cells. The concentration of compounds was tested between 100-750 μM. Error bars represent the mean of minimum three independent experiments ± Standard error of the mean (SEM).