

**Scaffold Hopping Based Designing of Selective ALDH1A1 Inhibitors to Overcome  
Cyclophosphamide Resistance: Synthesis and Biological Evaluation**

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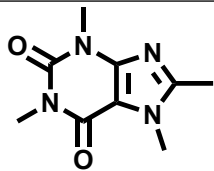
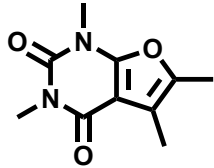
<sup>4</sup>*Department of Botany and Environmental Sciences, Guru Nanak Dev University, Amritsar,  
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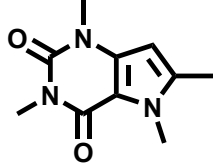
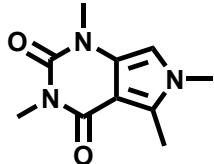
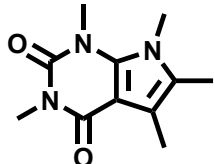
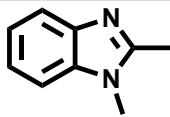
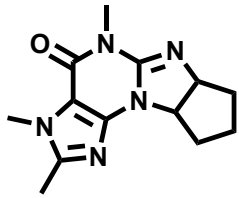
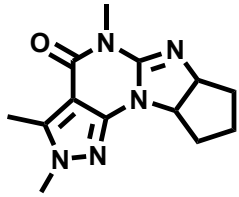
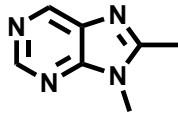
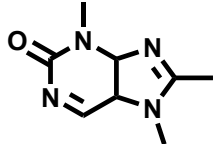
\*Corresponding authors E-mail: [mm1pup73@gmail.com](mailto:mm1pup73@gmail.com); Tel: +91-9501542696; Fax: +91  
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## Mafosfamide sensitivity assay description

Enhancement of tumor cell sensitivity to mafosfamide with ALDH1A1 inhibitors involved simultaneous treatment of cells with mafosfamide (Maf) in combination with ALDH1A1 inhibitors. To determine the approximate IC<sub>50</sub> value of mafosfamide, an initial concentration of 1000  $\mu$ M was employed for A549 and Mia PaCa-2 cells. The IC<sub>50</sub> maf in A549 and Mia PaCa-2 cells was observed as 276.09  $\mu$ M and 256.17  $\mu$ M. After optimization, A549 (5000 cells per well) and Mia PaCa-2 (5000 cells per well) cells were seeded in 96-well plates. Following a 29-hour incubation period, A549 and Mia PaCa-2 cells were treated with ALDH1A1 inhibitors (A1, A2, and A3) alone. Notably, compounds A2 and A3 exhibited no significant anticancer activity, while compound A1 demonstrated anticancer activity at approximately 370  $\mu$ M against both A549 and Mia PaCa-2 cells. In subsequent combination studies, Compound A1 was used at a concentration lower than 370  $\mu$ M as the initial stock preparation, with further dilutions made from this stock. Because at <370  $\mu$ M concentration, compound A1 doesn't have anti-cancer activity, so, that concentration will not affect the original maf anti-cancer activity when treated in the combination. In contrast, compounds A2, A3, and maf were prepared as stocks with a concentration of 1000  $\mu$ M, and subsequent dilutions were made from this initial stock concentration. Then, the MTT assay was conducted after a 19-hour incubation with the inhibitors (0.25% DMSO) and maf. The relative percentage of cell proliferation was calculated in comparison to DMSO-treated (0.25%) controls.

**Table S1:** The top ten scaffolds identified through scaffold hopping

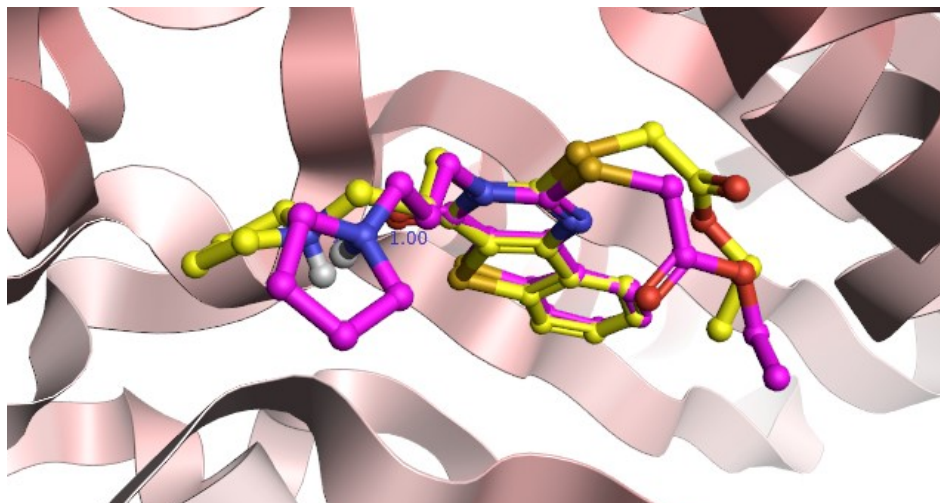
S. No.	Scaffold	Score@	Field Score <sup>s</sup>	Shape Score <sup>#</sup>
1		0.998	0.997	0.998
2		0.966	0.936	0.996

3		0.964	0.936	0.996
4		0.960	0.927	0.992
5		0.941	0.906	0.976
6		0.935	0.914	0.956
7		0.930	0.899	0.960
8		0.924	0.911	0.937
9		0.923	0.887	0.959
10		0.919	0.858	0.980

@The similarity of the resultant molecule to the reference in terms of electrostatic and steric field

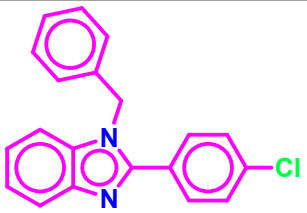
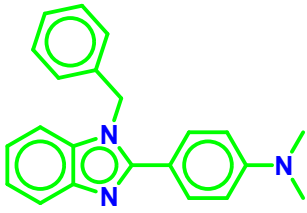
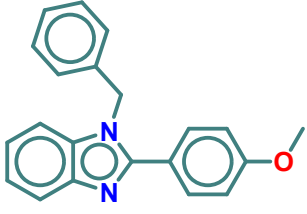
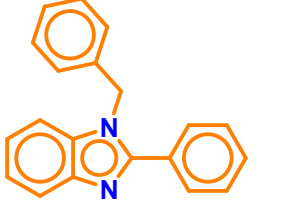
§Similarity of the results molecule to the reference in terms of Shape

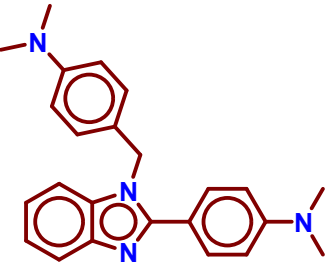
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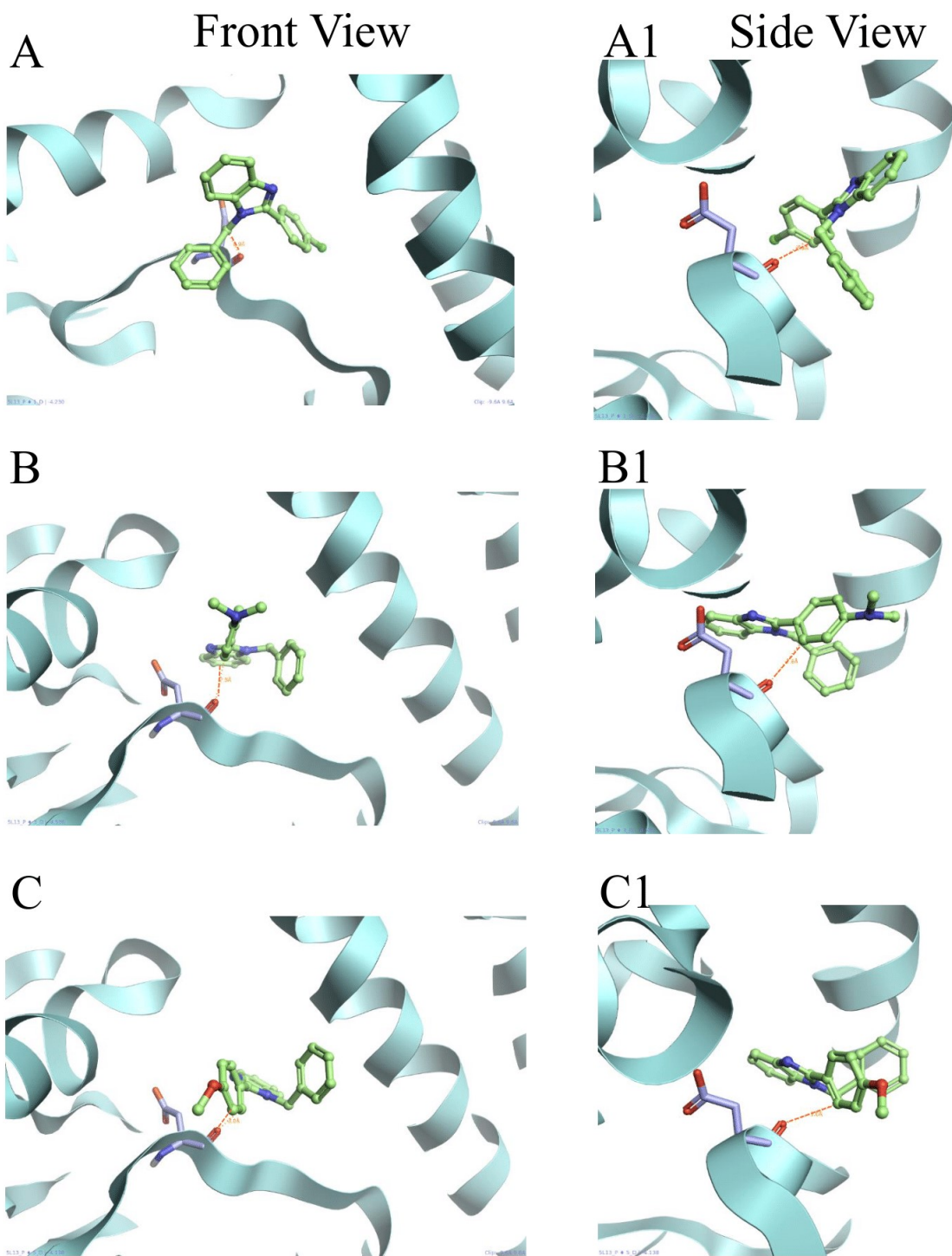


**Figure S1:** Comparison between ALDH1A1 cocrystal ligand (pink) and its redocked ligand pose (yellow)

**Table S2:** Molecular docking scores of top five molecules identified towards ALDH2 and ALDH3A1

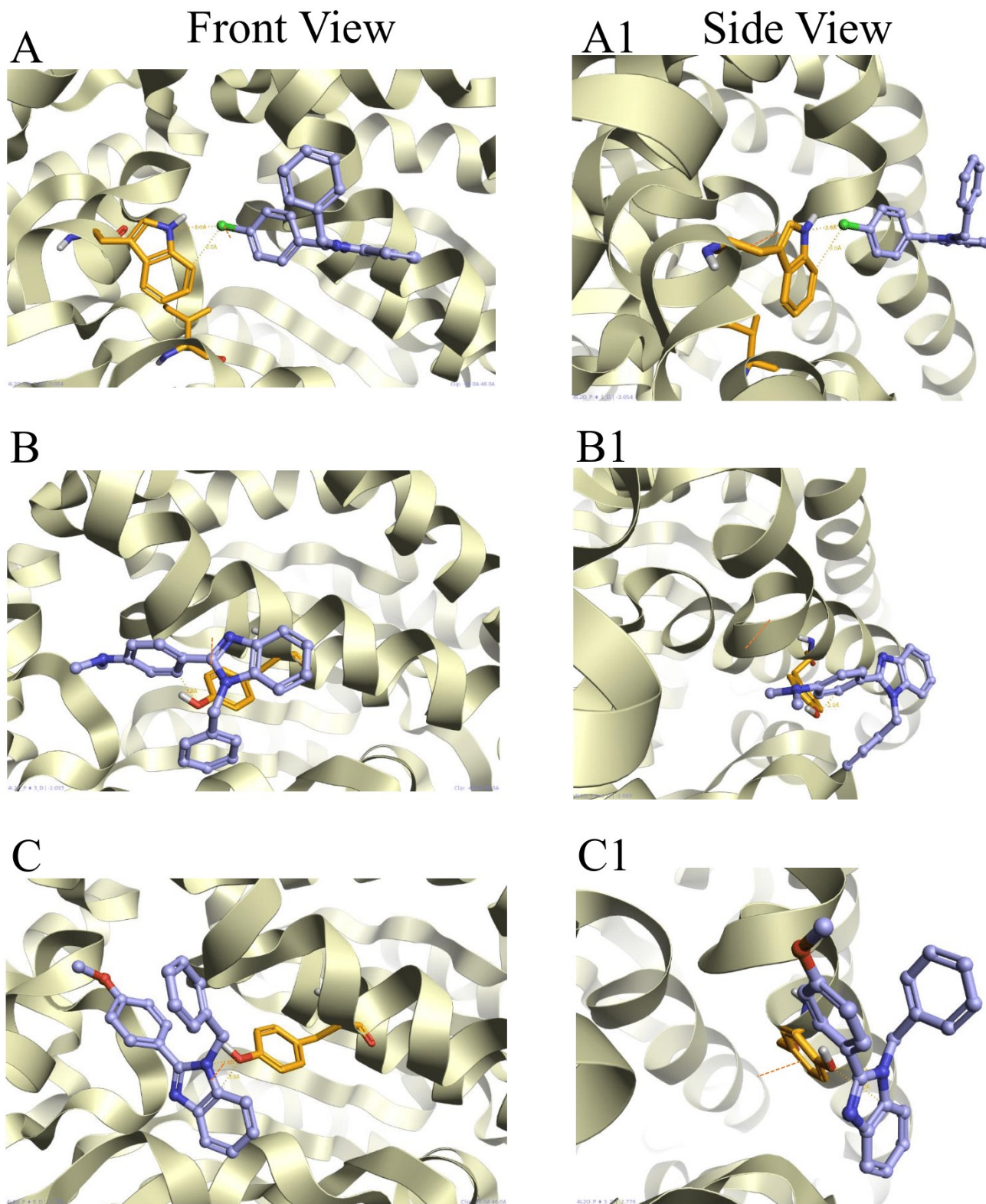
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		LF rank score <sup>@</sup>	LD dG score <sup>#</sup>	LF VS score <sup>s</sup>	LF rank score <sup>@</sup>	LD dG score <sup>#</sup>	LF VS score <sup>s</sup>
		kcal/mol			Kcal/mol		
A1		-1.25	-2.65	2.65	2.65	1.95	2.65
A2		-0.95	2.25	-1.42	-0.56	1.58	0.87
A3		-1.36	-2.95	-2.05	1.65	-2.85	0.54
A4		-3.01	-3.38	-2.86	2.15	2.65	3.14

A5		-1.51	-1.87	-1.65	3.56	-2.65	-3.58
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**Figure S2:** The binding poses of the top three compounds, **A** and **A1**) compound A1, **B** and **B1**) compound A2, **C** and **C1**) compound A3 in the active site of ALDH2



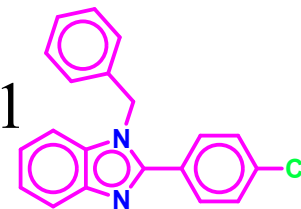


**Figure S3:** The binding poses of the top three compounds, **A** and **A1**) compound A1, **B** and **B1**) compound A2, **C** and **C1**) compound A3 in the active site of ALDH3A1.



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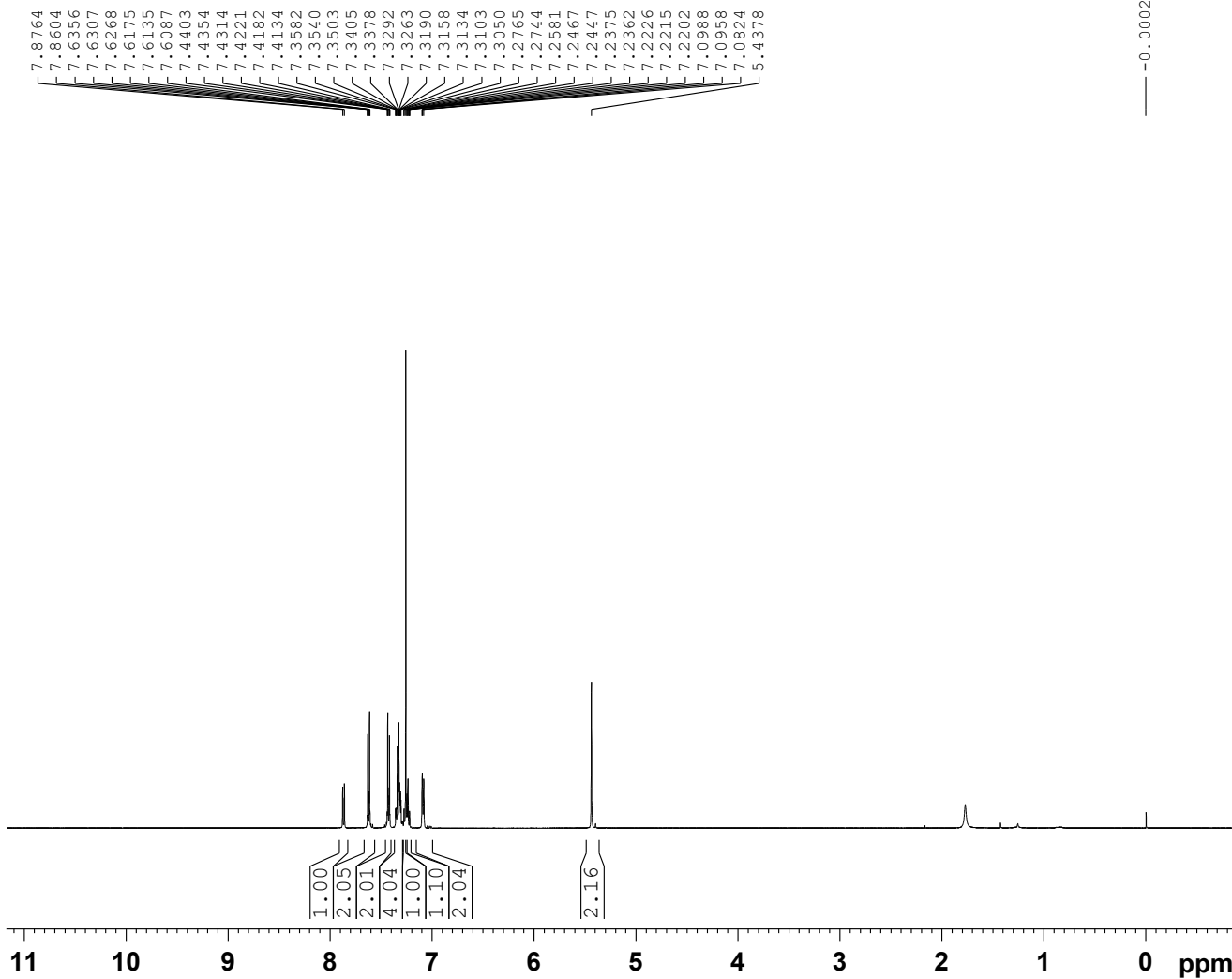
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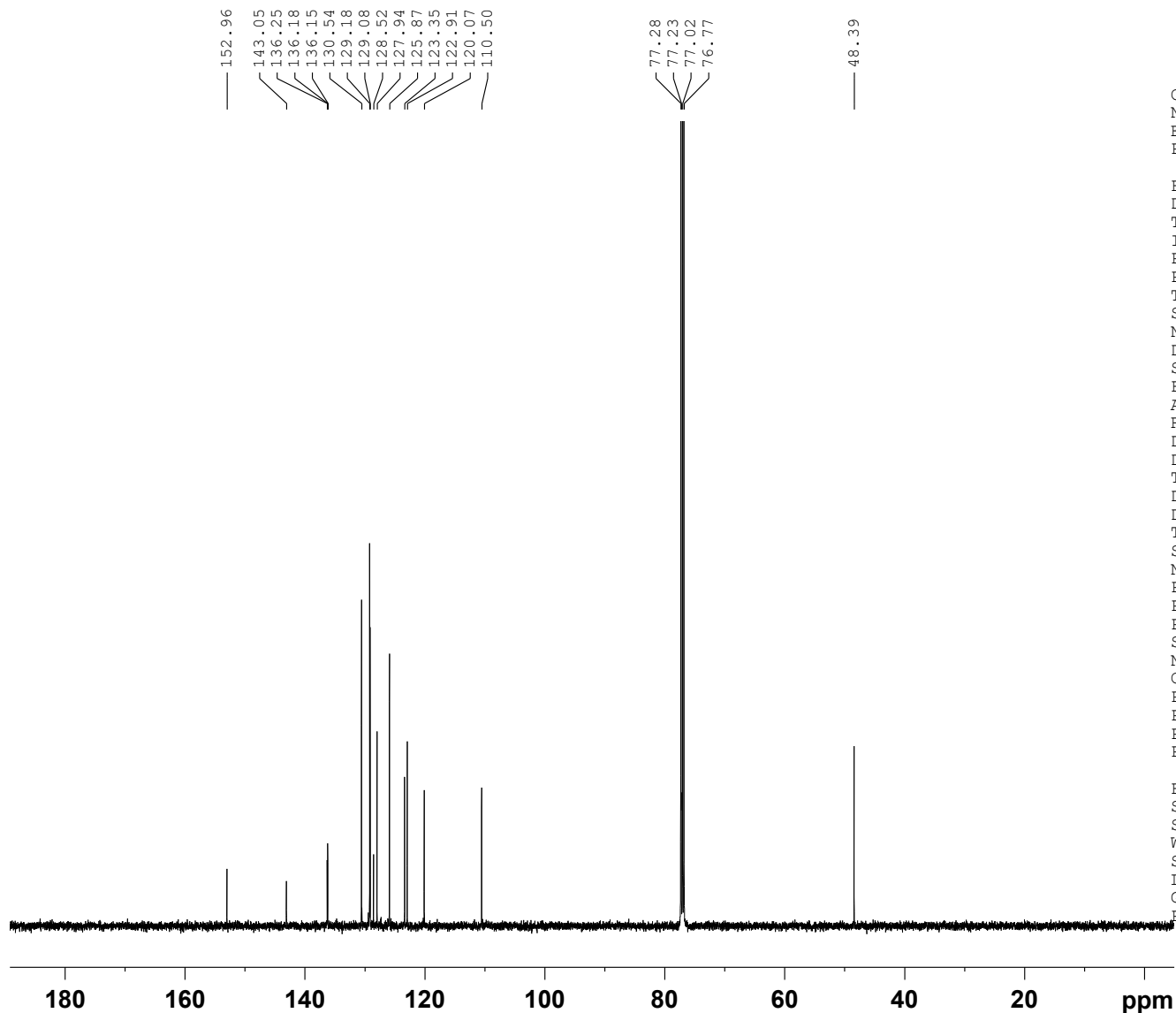
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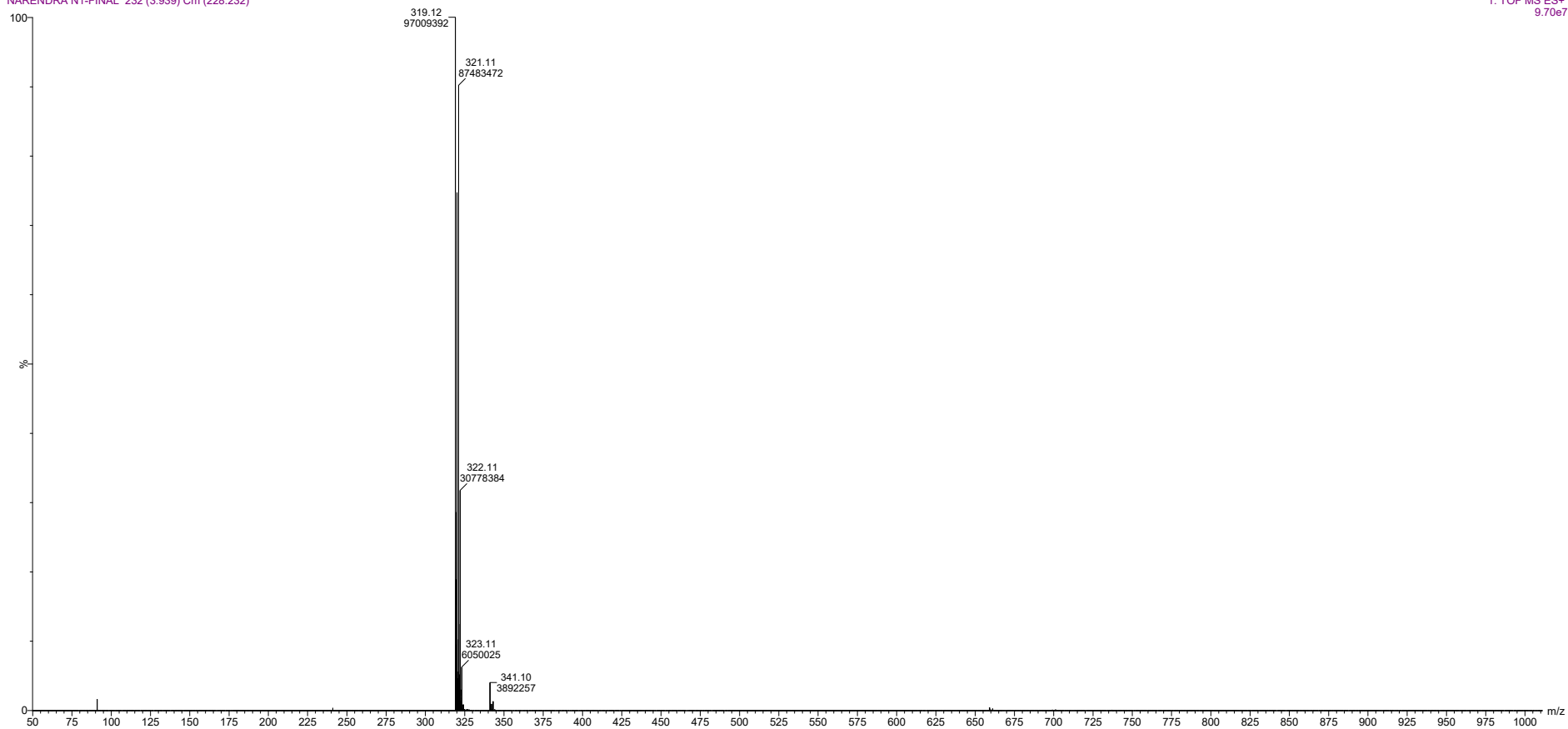
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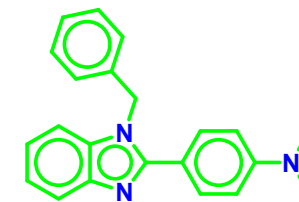


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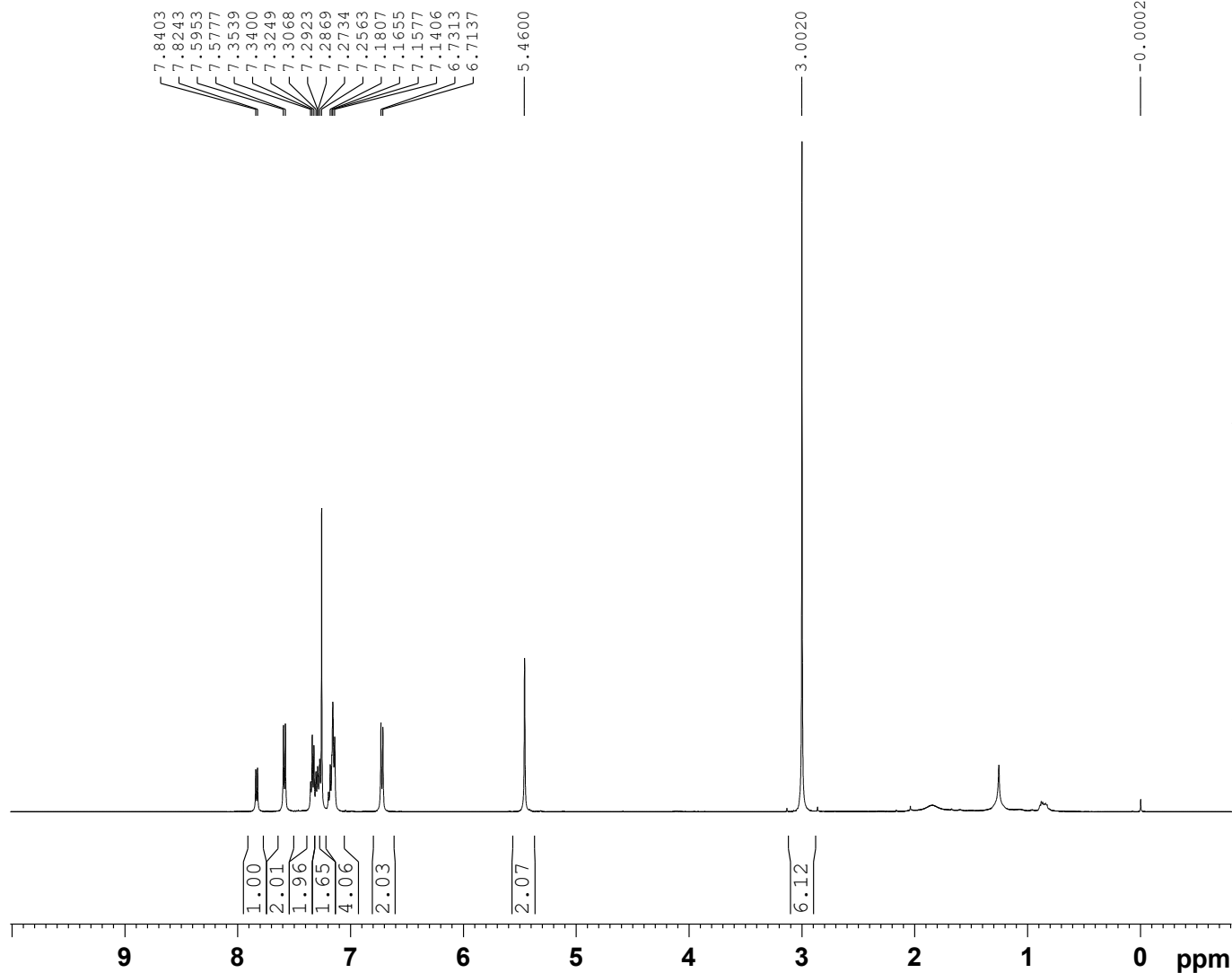


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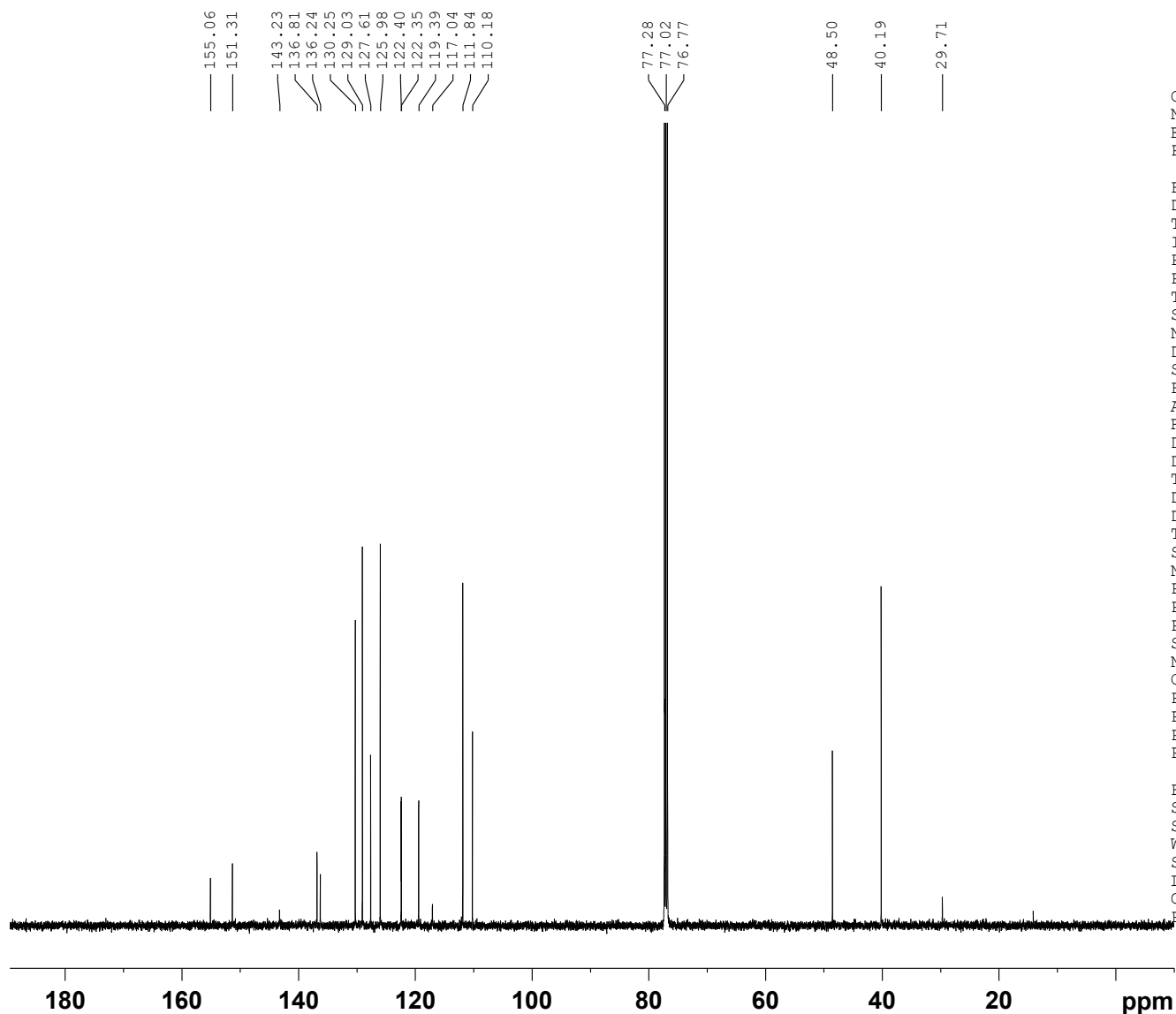
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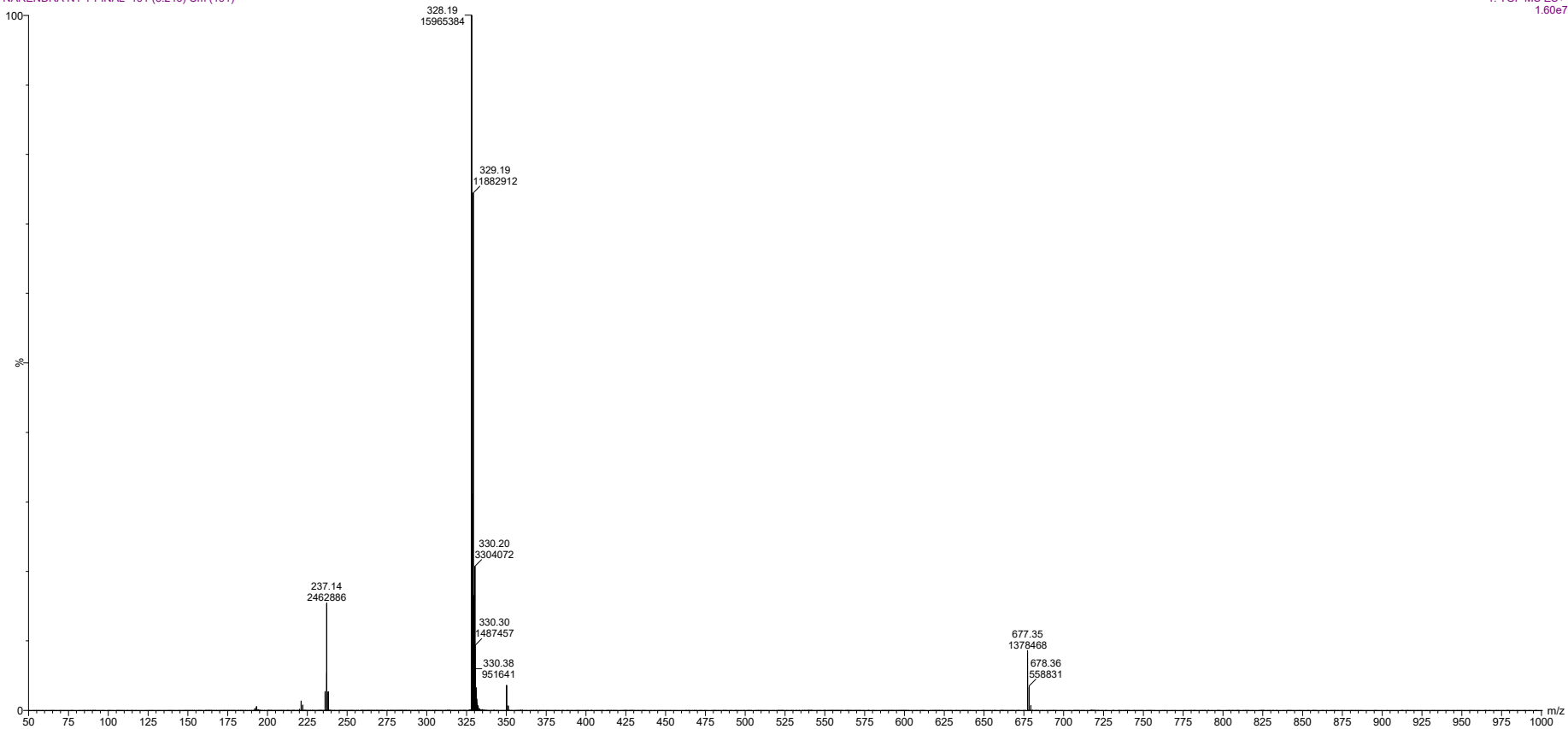
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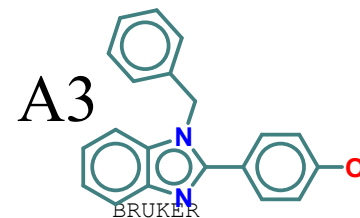
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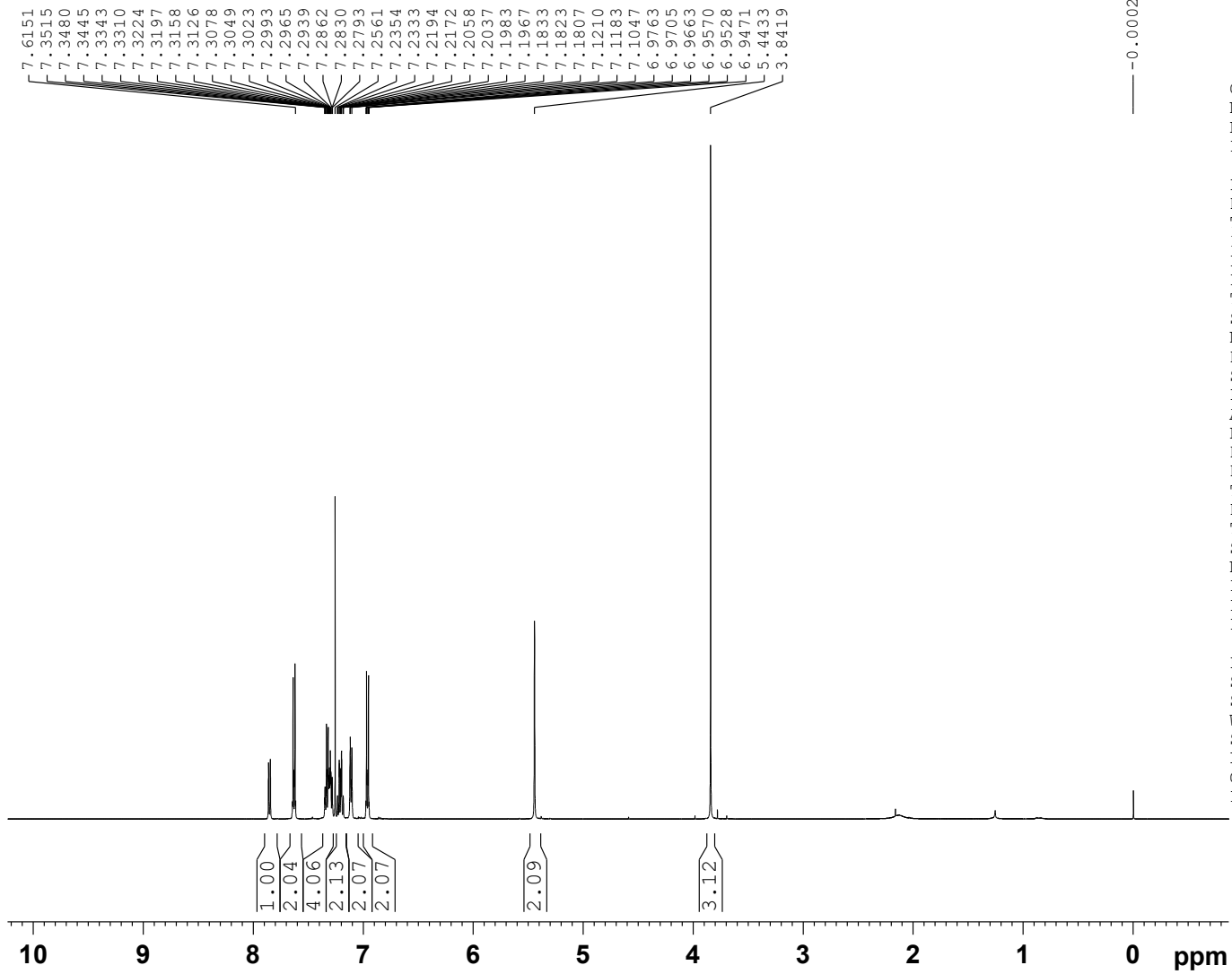


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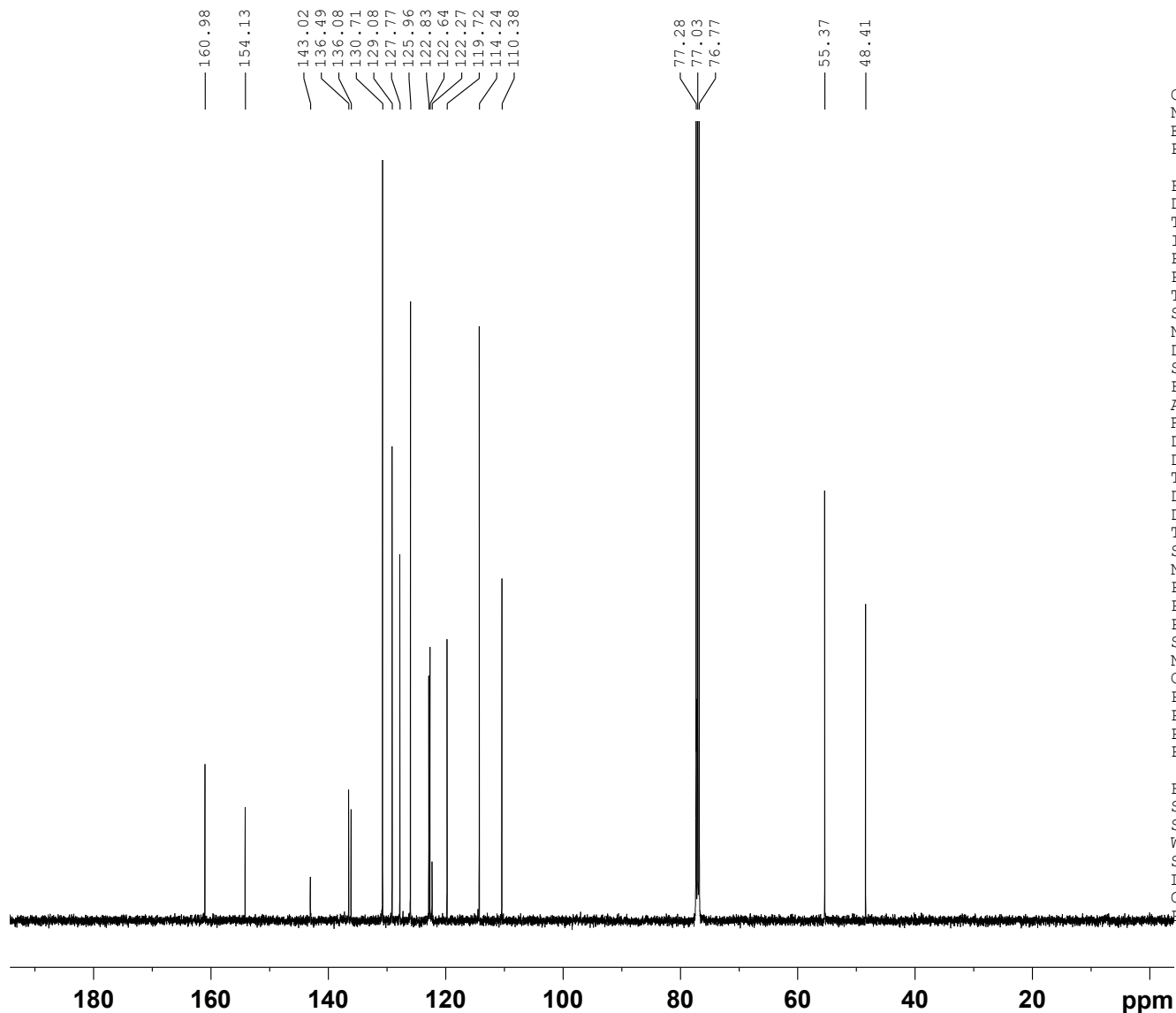
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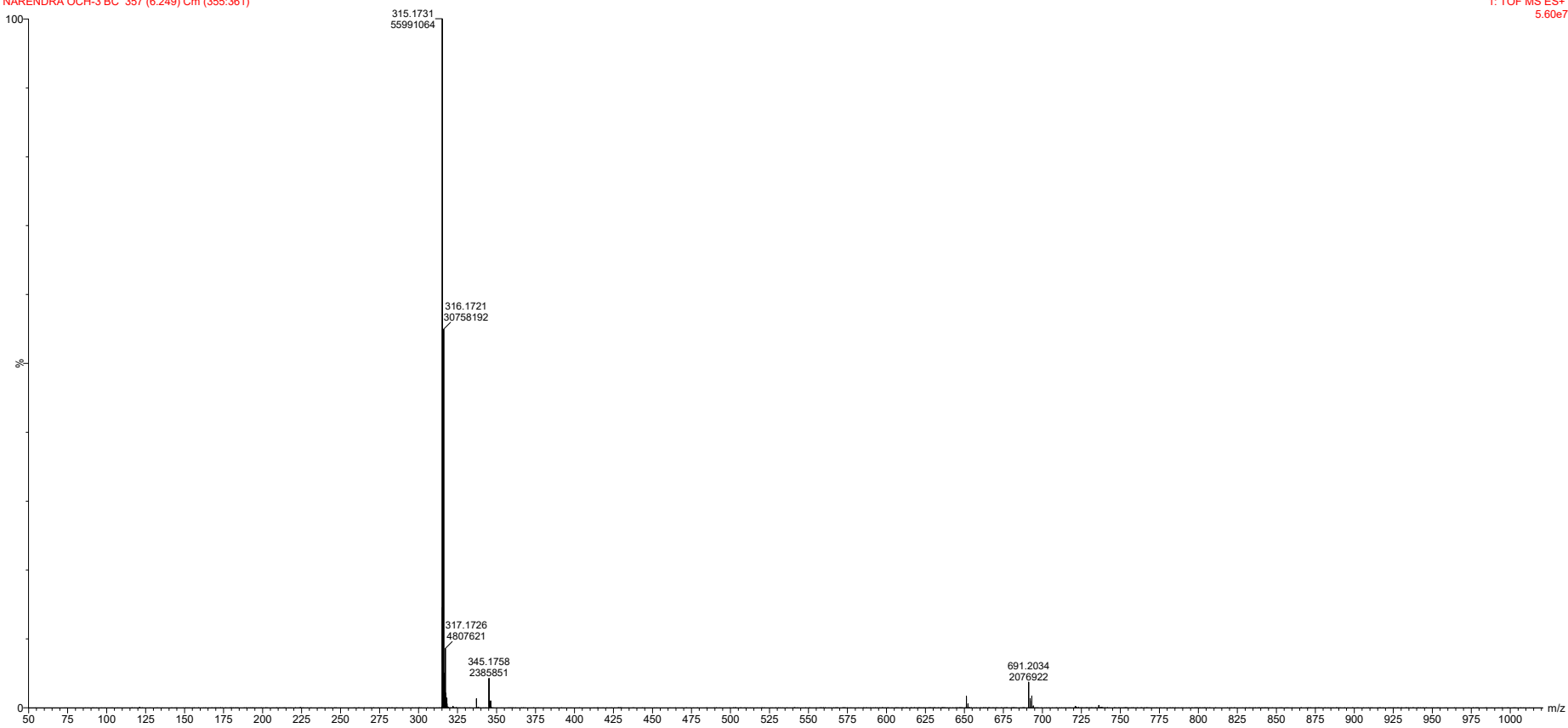


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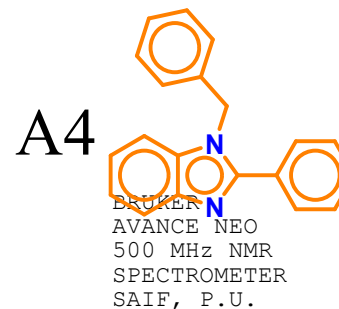
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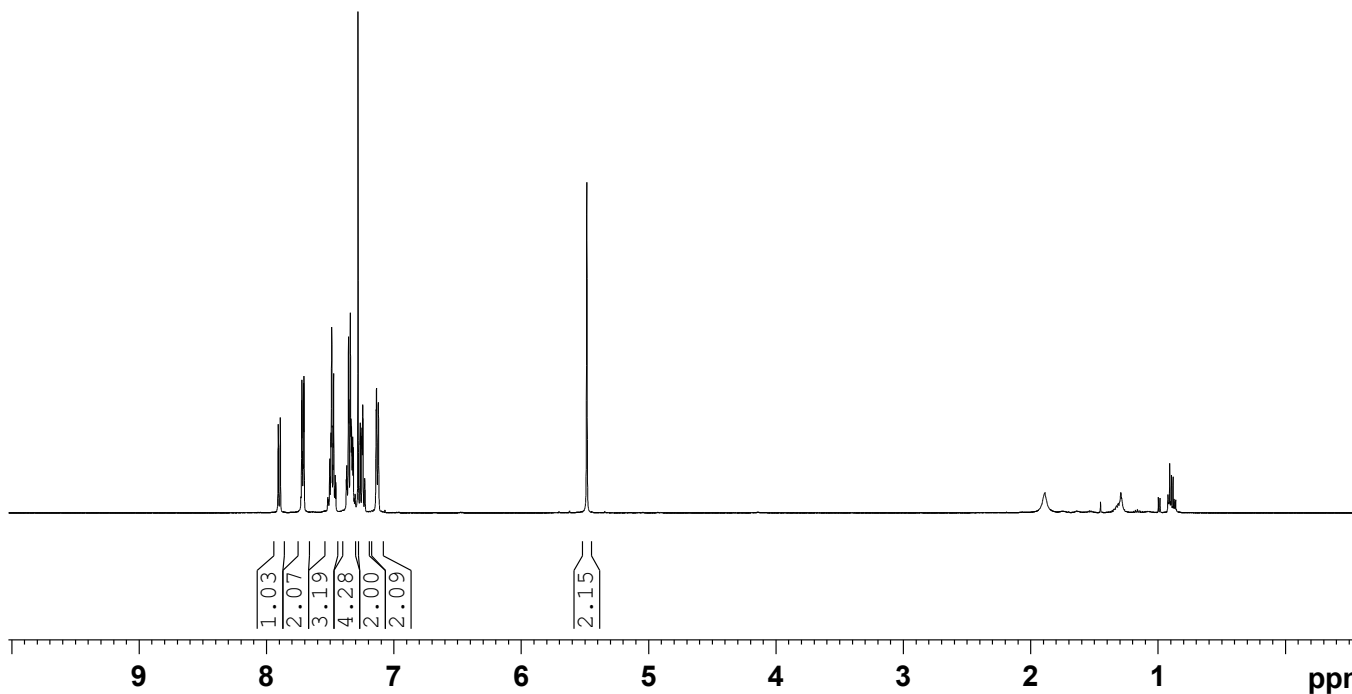
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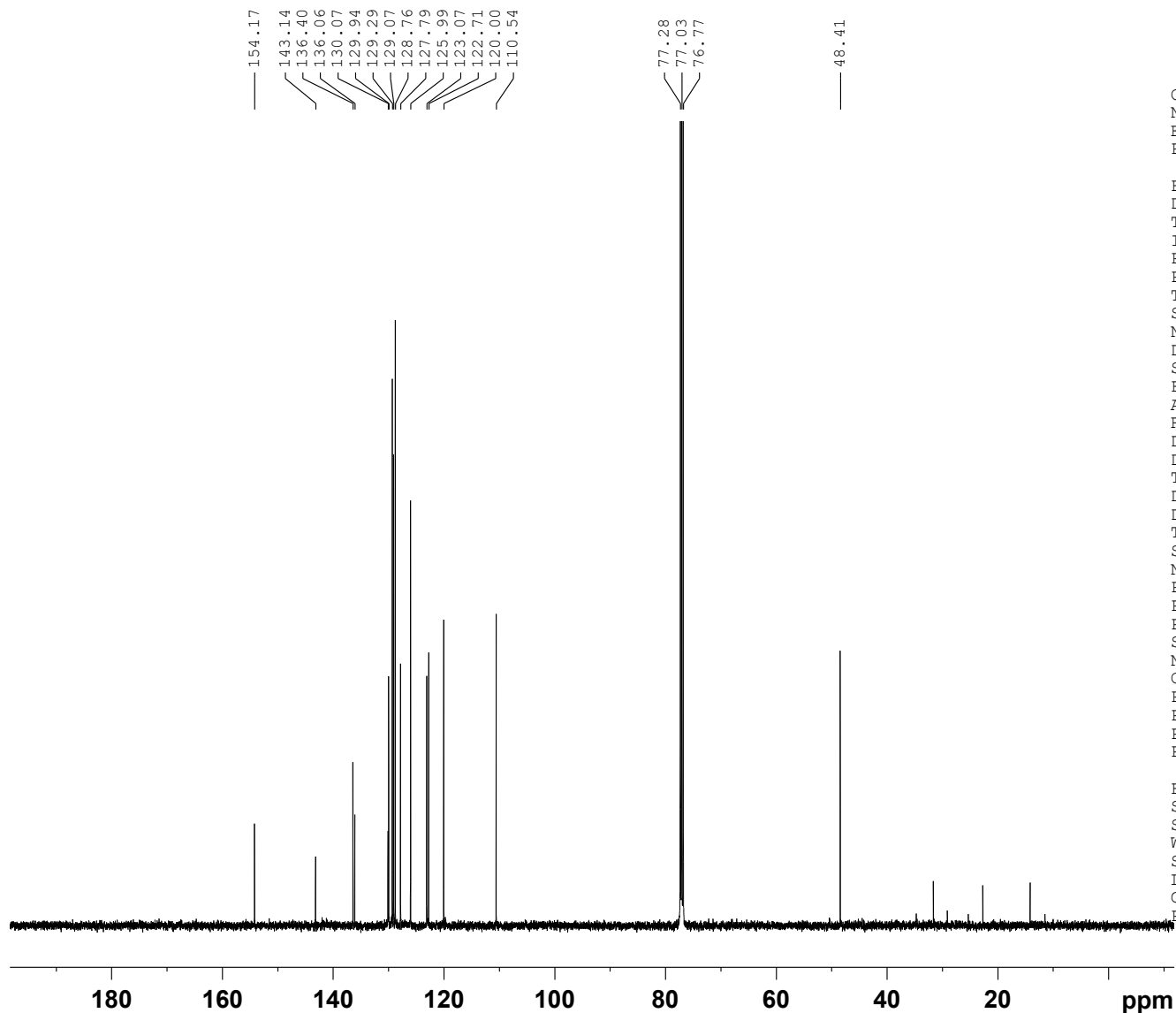
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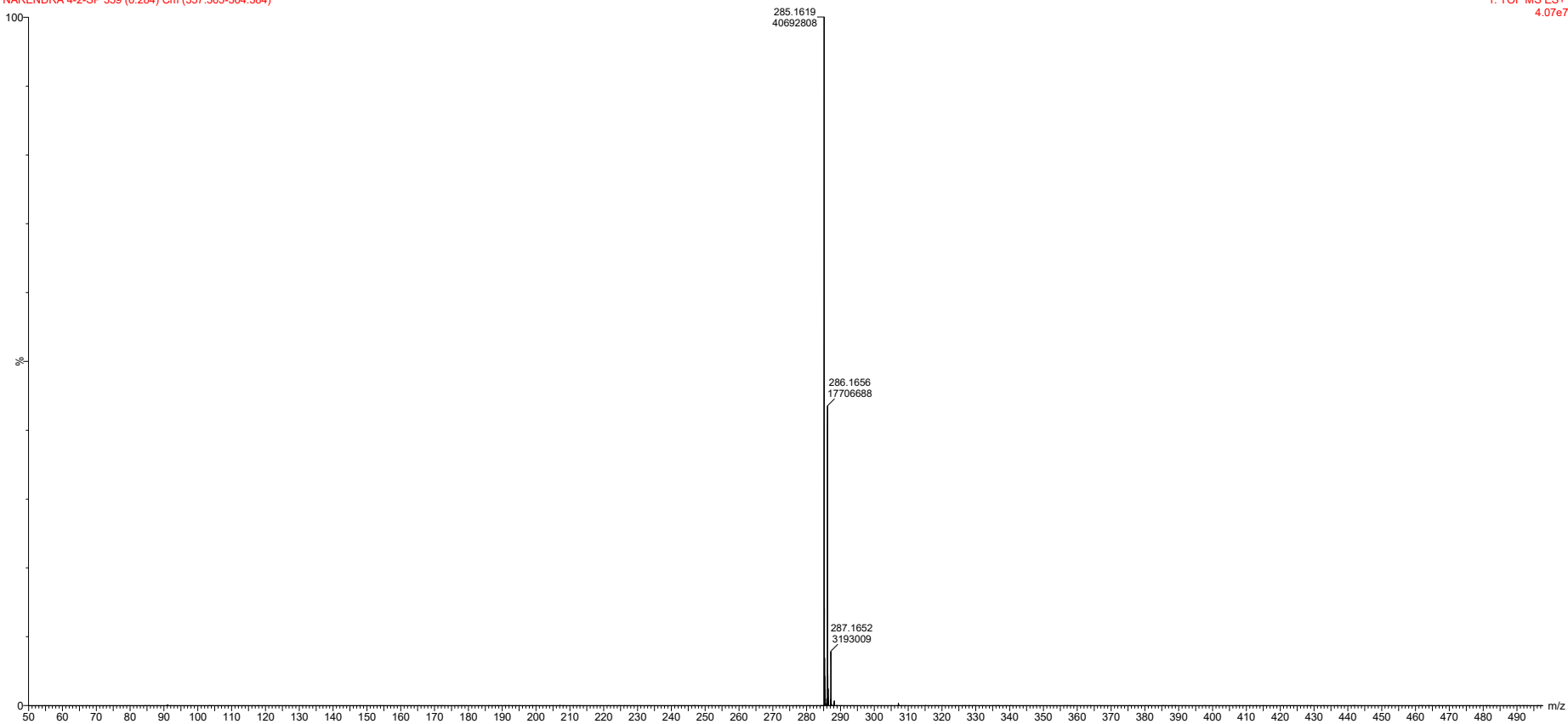


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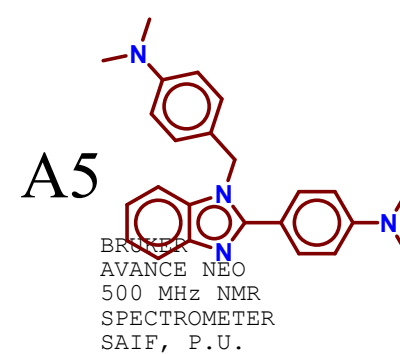
SYNAPT-XS#DBA064

05-Aug-2021  
11:26:45  
1: TOF MS ES+  
4.07e7

NARENDRA 4-2-SP 359 (6.284) Cm (357:363-364:384)



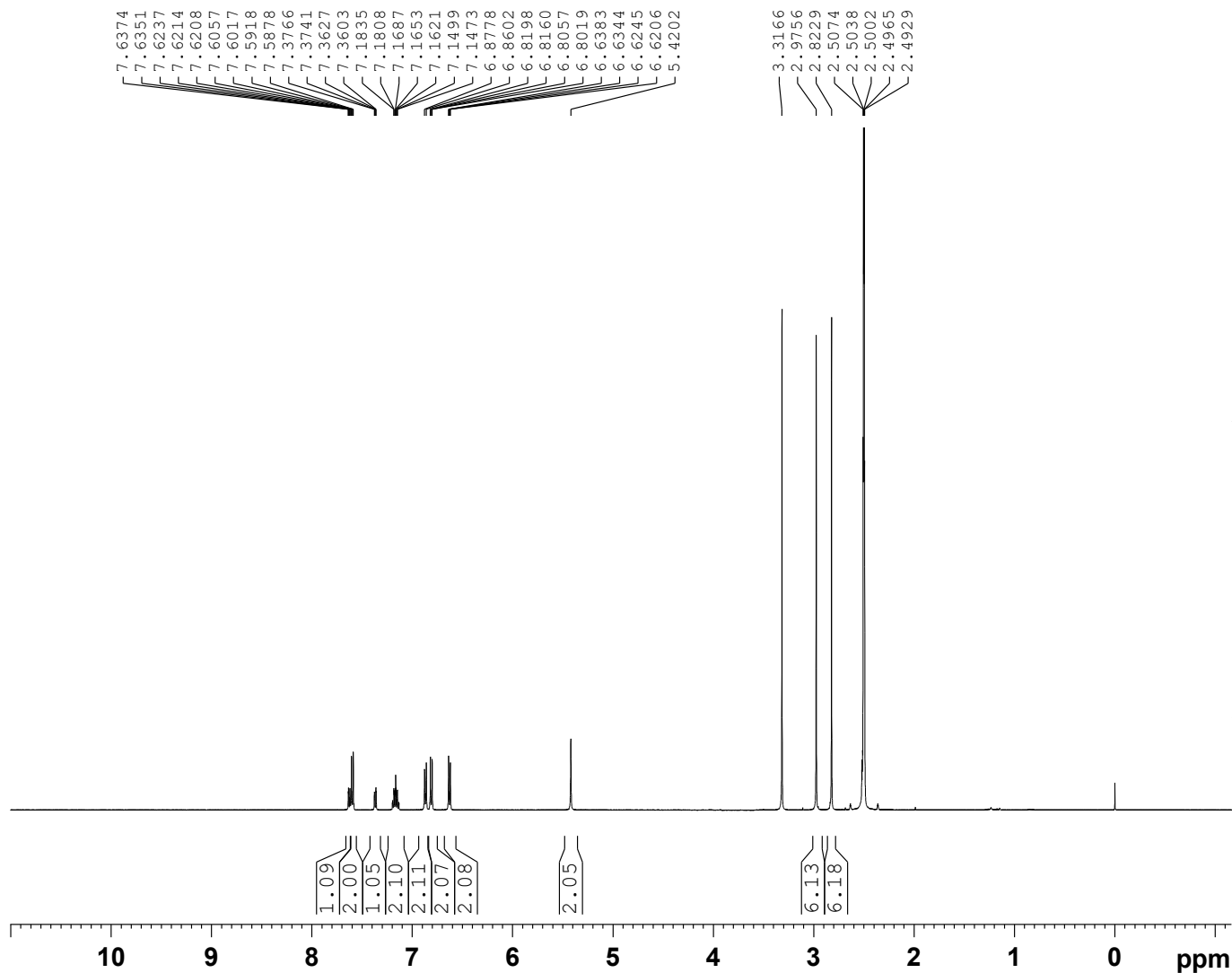
1G  
1H\_8scan DMSO {D:\Spectra} nmr 17



Current Data Parameters  
NAME Mar14-2022  
EXPNO 170  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20220314  
Time\_ 12.11 h  
INSTRUM Avance Neo 500  
PROBHD Z119470\_0333 (   
PULPROG zg30  
TD 65536  
SOLVENT DMSO  
NS 16  
DS 0  
SWH 14705.883 Hz  
FIDRES 0.448788 Hz  
AQ 2.2282240 sec  
RG 95.7854  
DW 34.000 usec  
DE 6.79 usec  
TE 300.2 K  
D1 1.00000000 sec  
TD0 1  
SFO1 500.1730885 MHz  
NUC1 1H  
P0 3.33 usec  
P1 10.00 usec  
PLW1 20.93000031 W

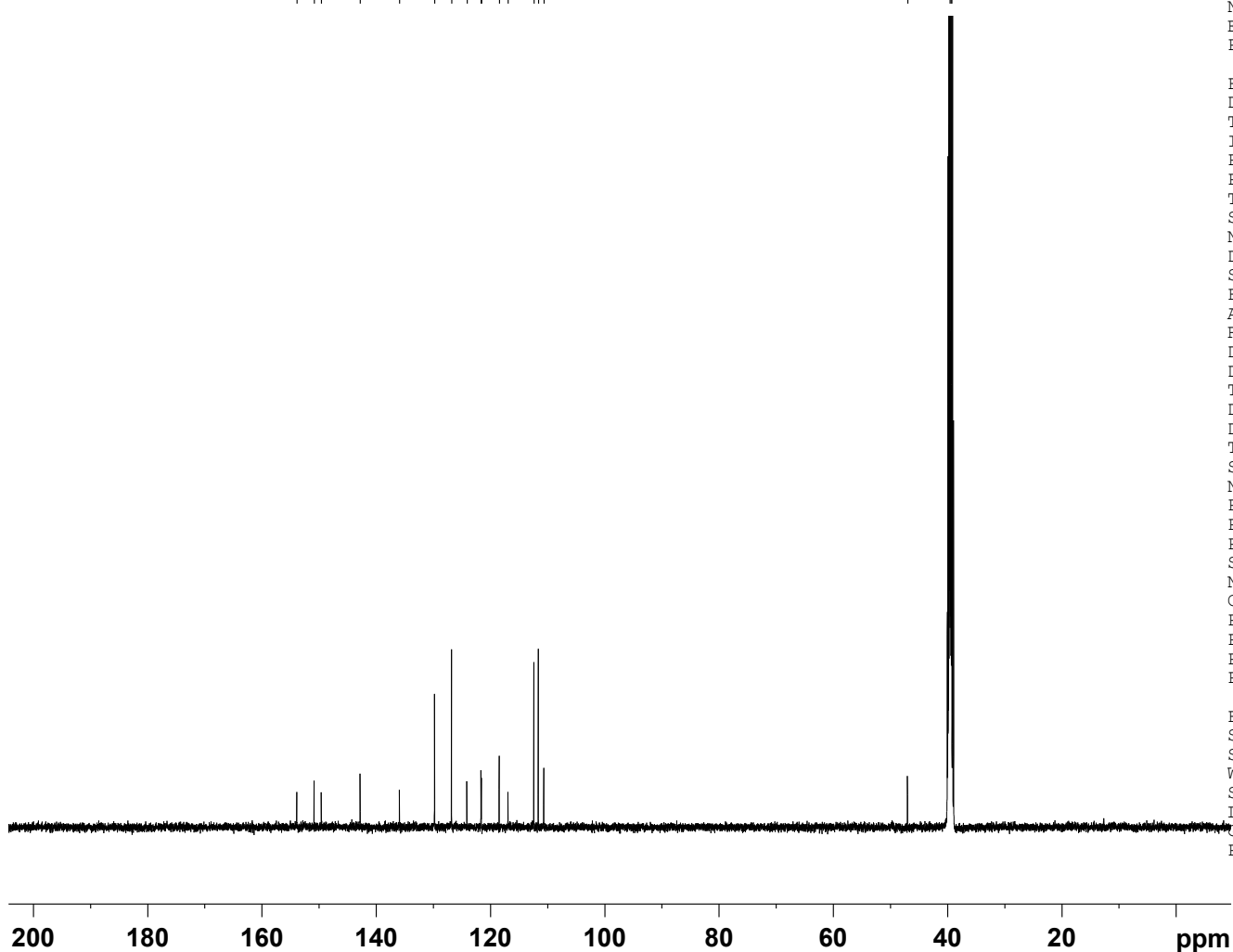
F2 - Processing parameters  
SI 65536  
SF 500.1700039 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



1G  
C13CPD DMSO {D:\Spectra} nmr 17

153.86  
150.83  
149.58  
142.78  
135.91  
129.75  
126.78  
124.10  
121.64  
121.57  
118.44  
116.91  
112.38  
111.60  
110.64

46.99  
39.58  
39.42  
39.25



BRUKER  
AVANCE NEO  
500 MHz NMR SPECTROMETER  
SAIF, PANJAB UNIVERSITY,  
CHANDIGARH

Current Data Parameters  
NAME Mar14-2022  
EXPNO 171  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20220314  
Time\_ 23.04 h  
INSTRUM Avance Neo 500  
PROBHD Z119470\_0333 (  
PULPROG zgpg30  
TD 65536  
SOLVENT DMSO  
NS 1024  
DS 4  
SWH 37037.035 Hz  
FIDRES 1.130281 Hz  
AQ 0.8847360 sec  
RG 101  
DW 13.500 usec  
DE 6.50 usec  
TE 300.2 K  
D1 2.0000000 sec  
D11 0.0300000 sec  
TD0 1  
SFO1 125.7804233 MHz  
NUC1 13C  
P0 3.33 usec  
P1 10.00 usec  
PLW1 83.14099884 W  
SFO2 500.1720007 MHz  
NUC2 1H  
CPDPRG[2] waltz65  
PCPD2 80.00 usec  
PLW2 20.93000031 W  
PLW12 0.32703000 W  
PLW13 0.16449000 W

F2 - Processing parameters  
SI 32768  
SF 125.7679214 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



NARENDRA IG 6 (0.138) Cm (5.6)

