Supporting Information

Discovery of Dual Aβ/Tau Inhibitors and Evaluation of Their Therapeutic Effect on a *Drosophila* Model of Alzheimer's Disease

Annachiara Gandini,[a, b] Ana Elisa Gonçalves,[a, c] Silvia Strocchi,[a] Claudia Albertini,[a] Jana Janočková,[d] Anna Tramarin,[a] Daniela Grifoni,[a, e] Eleonora Poeta,[a] Ondrej Soukup,[d] Diego Muñoz-Torrero,[f] Barbara Monti,[c] Raimon Sabaté,[g] Manuela Bartolini,[a] Giuseppe Legname,[b] Maria Laura Bolognesi[a]*

(a) Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy; *Email: marialaura.bolognesi@unibo.it (b) Department of Neuroscience, Laboratory of Prion Biology, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Via Bonomea 265, I-34136 Trieste, Italy; (c) Pharmaceutical Sciences Postgraduate Program, Center of Health Sciences, Universidade do Vale do Itajaí, Rua Uruguai 458, 88302-202, Itajaí, Santa Catarina, Brazil; (d) Biomedical Research Center, University Hospital Hradec Kralove, 500 00, Hradec Kralove, Czech Republic; (e) Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio, Coppito II, 67100 L'Aquila, Italy; (f) Laboratory of Medicinal Chemistry (CSIC Associated Unit), Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona (UB), Av. Joan XXIII 27-31, E-08028 Barcelona, Spain; (g) Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Av Joan XXIII 27-31, E-08028 Barcelona, Spain.

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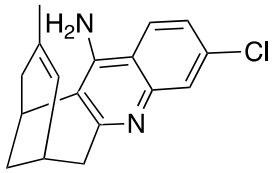
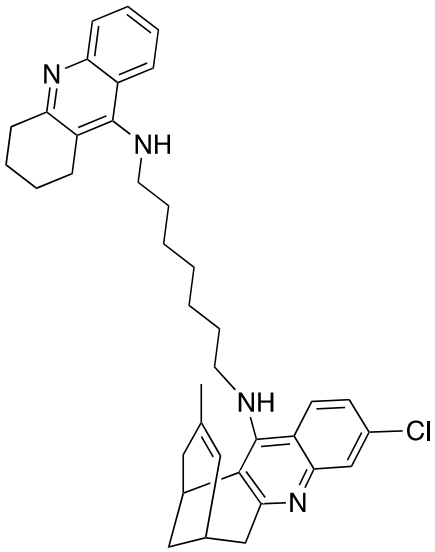
Table S1. Prediction of BBB penetration of the studied compounds expressed as Pe ± SEM (n=3)

	BBB Penetration Estimation	
Compound	**Pe ± SEM (× 10^{-6} cm s^{-1})**	**CNS (+/-)**[a]
7	11.36 ± 0.49[b]	CNS +
10	5.79 ± 1.16[b]	CNS +
16	0.55 ± 0.12	CNS -
17	0.88 ± 0.20	CNS -
22	5.30 ± 1.17	CNS +
23	18.9 ± 5.39	CNS +
Furosemide	0.19 ± 0.07	CNS -
Chlorothiazide	1.14 ± 0.53	CNS -
Cefuroxime	0.62 ± 0.16	CNS -
Donepezil	21.49 ± 2.05	CNS +
Rivastigmine	20.00 ± 2.07	CNS +
Tacrine	5.96 ± 0.59	CNS +

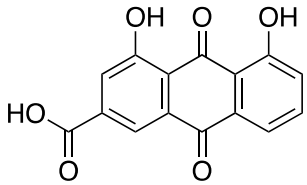
[a] CNS + (high BBB permeation predicted): *Pe* (×10^{-6} cm s^{-1}) > 4.0; CNS - (low BBB permeation predicted): *Pe* (×10^{-6} cm s^{-1}) < 2.0; CNS +/- (uncertain BBB permeation): *Pe* (×10^{-6} cm s^{-1}) from 4.0 to 2.0. [b] Study monitoring the compound stability in the PAMPA buffer solution showed a slow degradation over time (< 20% after 6h). However, the slow degradation was observed in both donor and acceptor wells, indicating that it has negligible relevance for the permeability results.



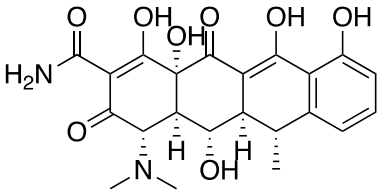
(±)-huprine Y



HUP7TH



rhein



doxycycline

Figure S1. Chemical structures of reference compounds.

Table S2. Inhibition of $Tau_{(306-336)}$ peptide self-aggregation

Compound[1]	**Inhibition of $Tau_{(306-336)}$ peptide self - aggregation (%) ± SEM** [*]
Doxycycline	61.5 ± 0.8
22	NS
23	51.8 ± 11.7

[*] The results are the mean of at least two independent measurements each performed in duplicate.
[1] Compounds were measured at 50 μM.
NS = not soluble in the assay conditions

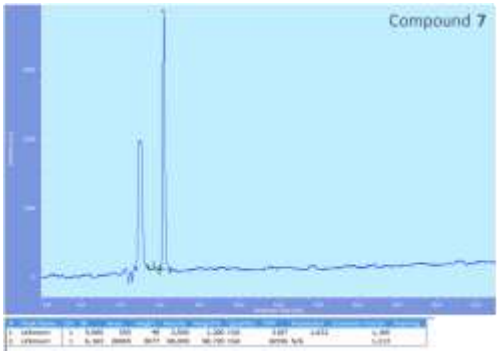
Compounds' purity.

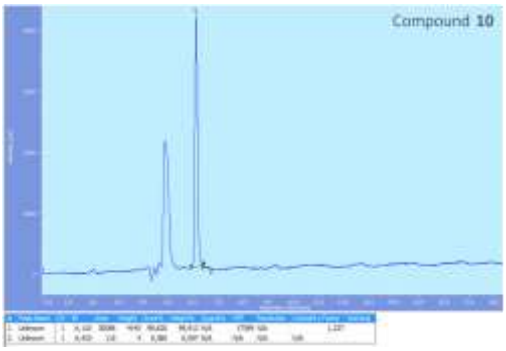
Purity of final compounds **1-24** was determined using a Waters Spherisorb® ODS2 HPLC column (5µm, 250 × 4.6 mm) and a HPLC Jasco Corporation (Tokyo, Japan) instrument, model PU-1585 UV equipped with a 20 µL loop valve. HPLC parameters were the following: water with 0.05% trifluoroacetic acid (eluent A), and acetonitrile with 0.05% trifluoroacetic acid (eluent B); detection UV-Vis Abs at 254 nm. Two different elution conditions were used. Condition 1 (compounds **1-18**): flow rate 0.6 mL/min; elution type isocratic; 75% eluent A and 25% eluent B. Condition 2 (compounds **19-24)**: flow rate 0.4 mL/min; elution type isocratic 80% eluent A and 20% eluent B. All samples were dissolved in DMSO (10 µg/mL).

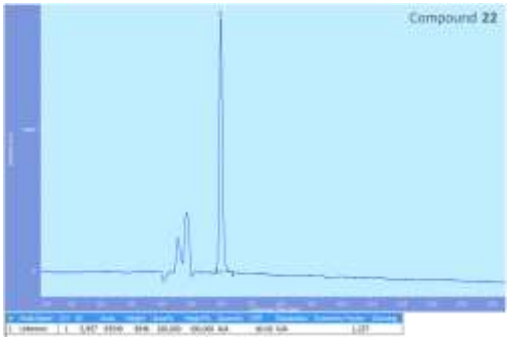
Table S3. Compounds' Purity by HPLC.

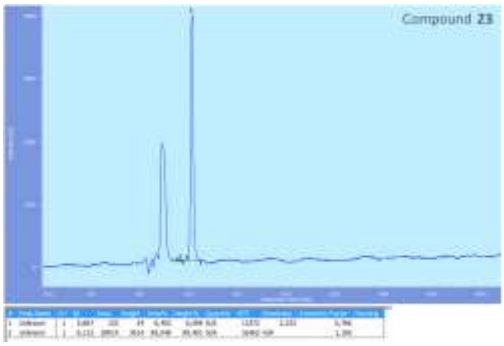
Compound	Purity (%)	Compound	Purity (%)	Compound	Purity (%)
1	98.27	**9**	97.75	**17**	99.66
2	99.53	**10**	99.62	**18**	96.32
3	96.82	**11**	98.92	**19**	96.58
4	96.41	**12**	95.88	**20**	98.14
5	97.03	**13**	100.00	**21**	98.47
6	98.36	**14**	96.90	**22**	100.00
7	98.00	**15**	96.37	**23**	99.55
8	99.74	**16**	98.98	**24**	97.84

Copies of representative chromatograms.



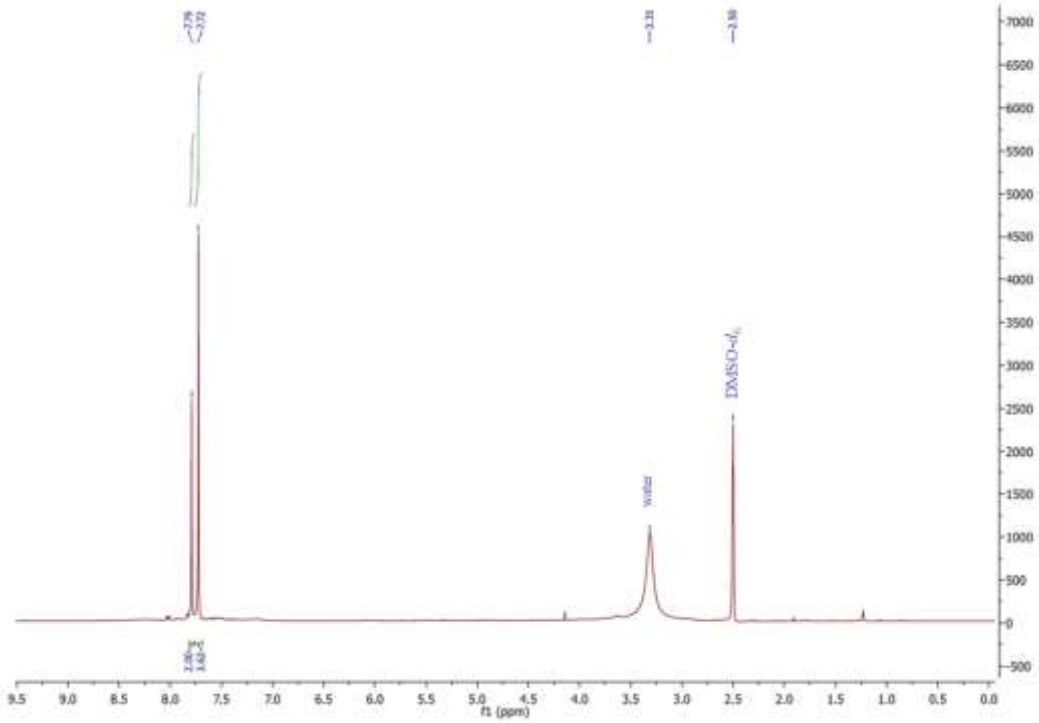






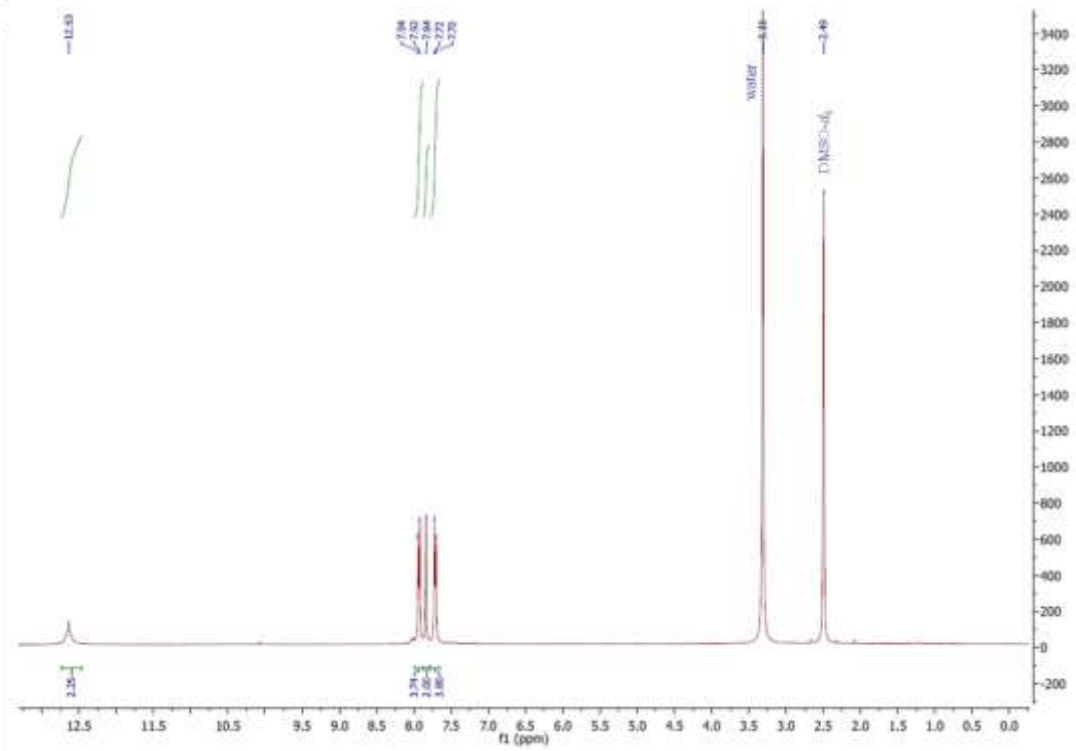
Copies of ^{1}H-NMR and ^{13}C-NMR spectra.

Compound **1**



We were unable to acquire ^{13}C-NMR, due to the low solubility of the compound.

Compound **2**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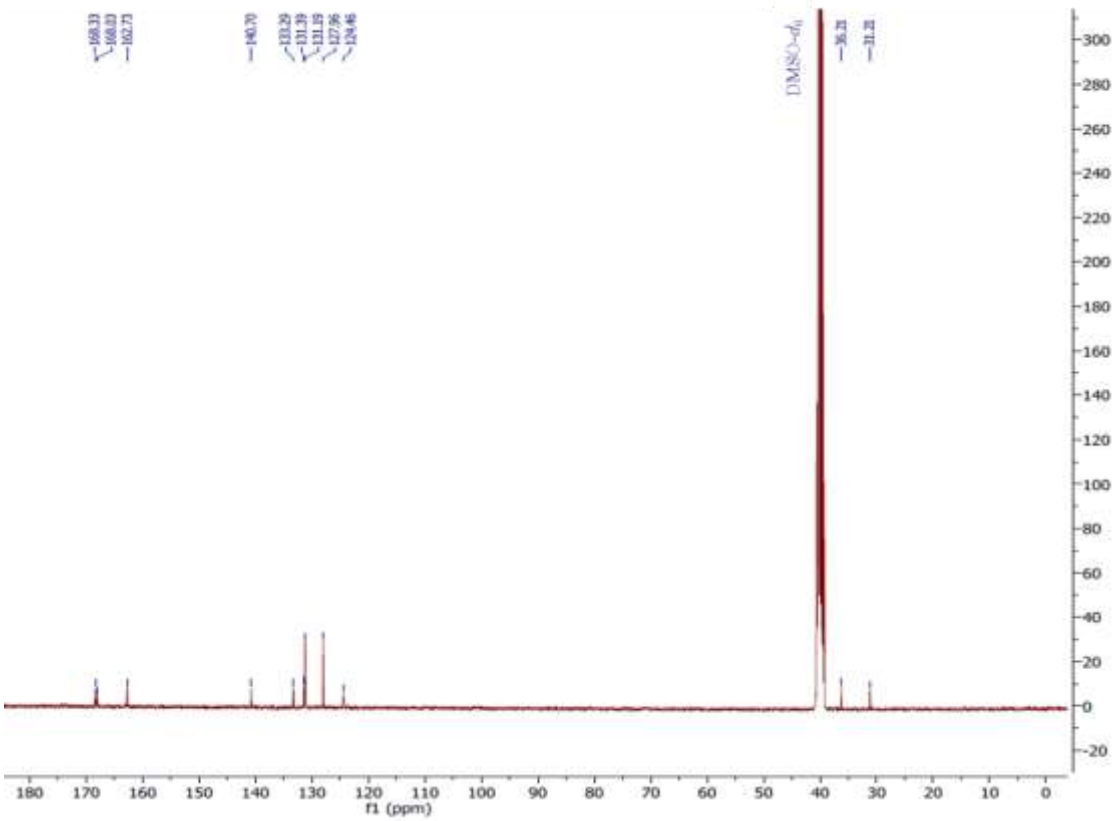
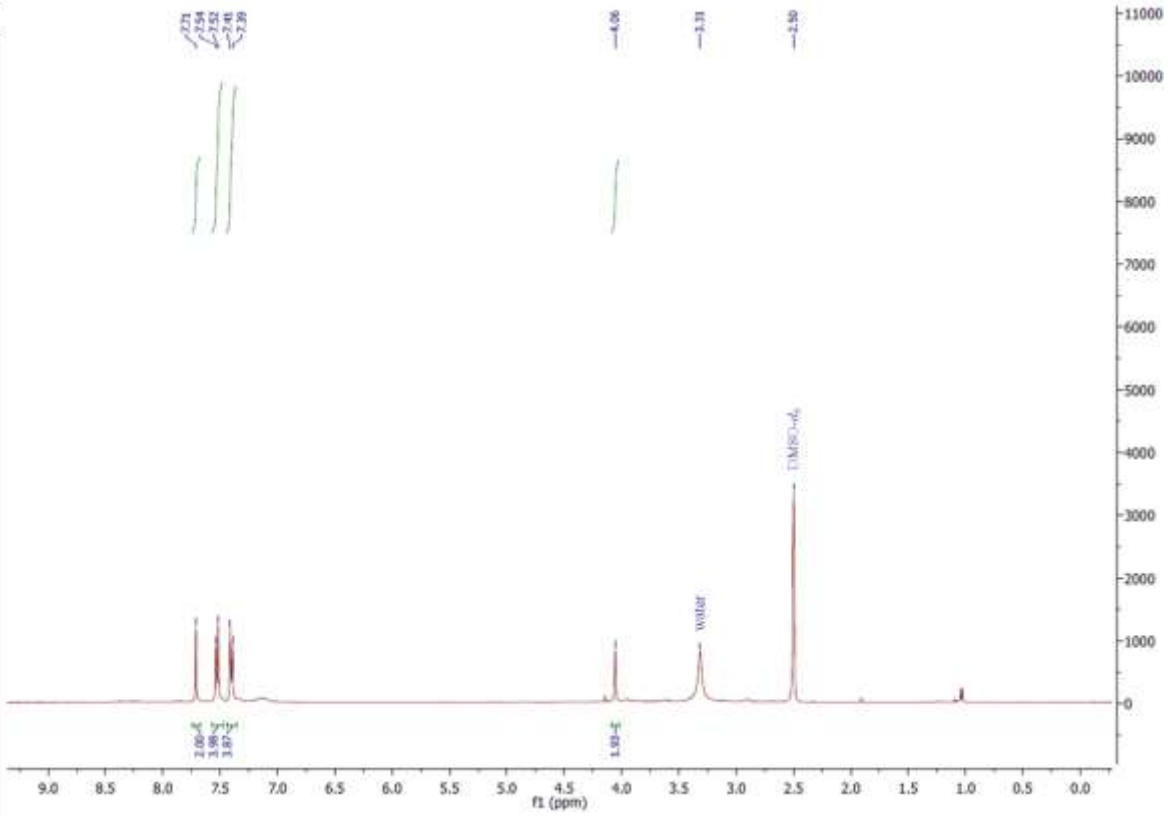
7.92
7.84
7.72
7.70
water
3.40
2.49
DMSO-d6
f1 (ppm)


168.33
168.03
162.73
140.70
132.29
131.39
131.35
127.96
124.46
DMSO-d6
36.21
31.21
f1 (ppm)

Compound **3**


7.71
7.54
7.52
7.41
7.39
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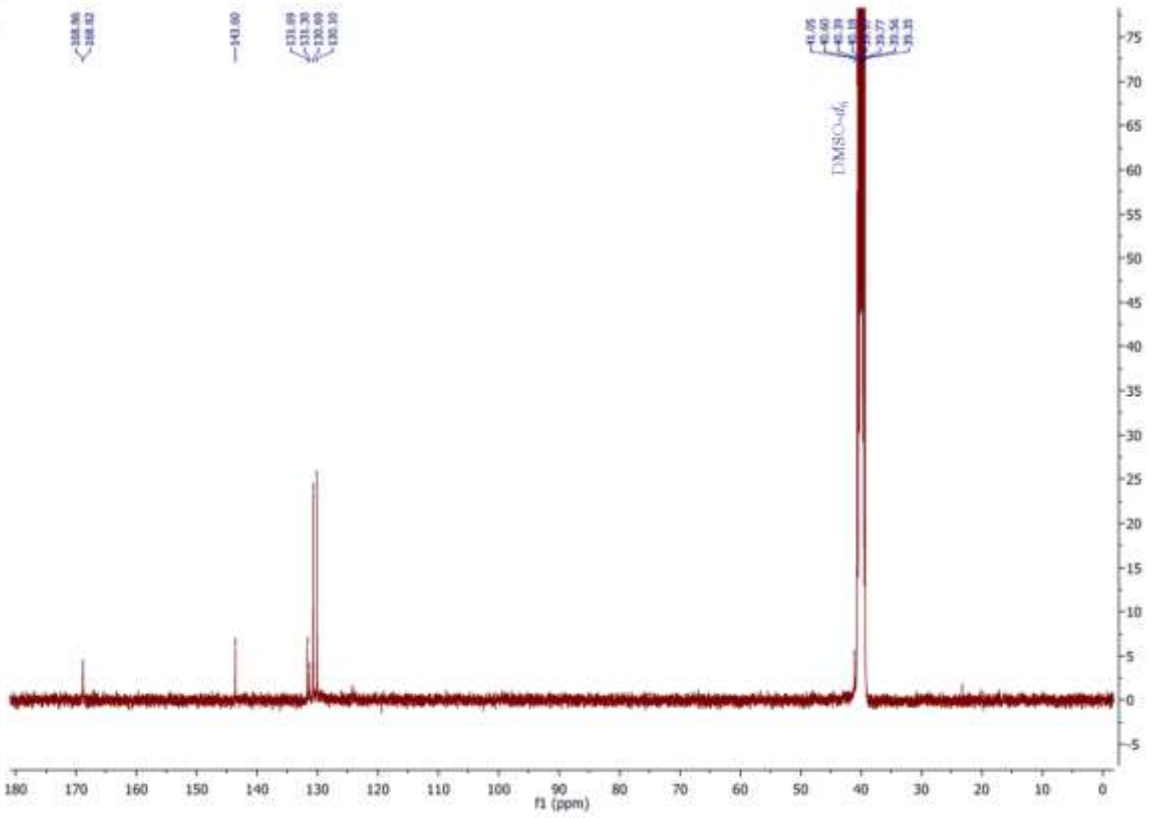
3.33
2.50
DMSO-d_6
water
2.00
3.98
3.87
1.93
f1 (ppm)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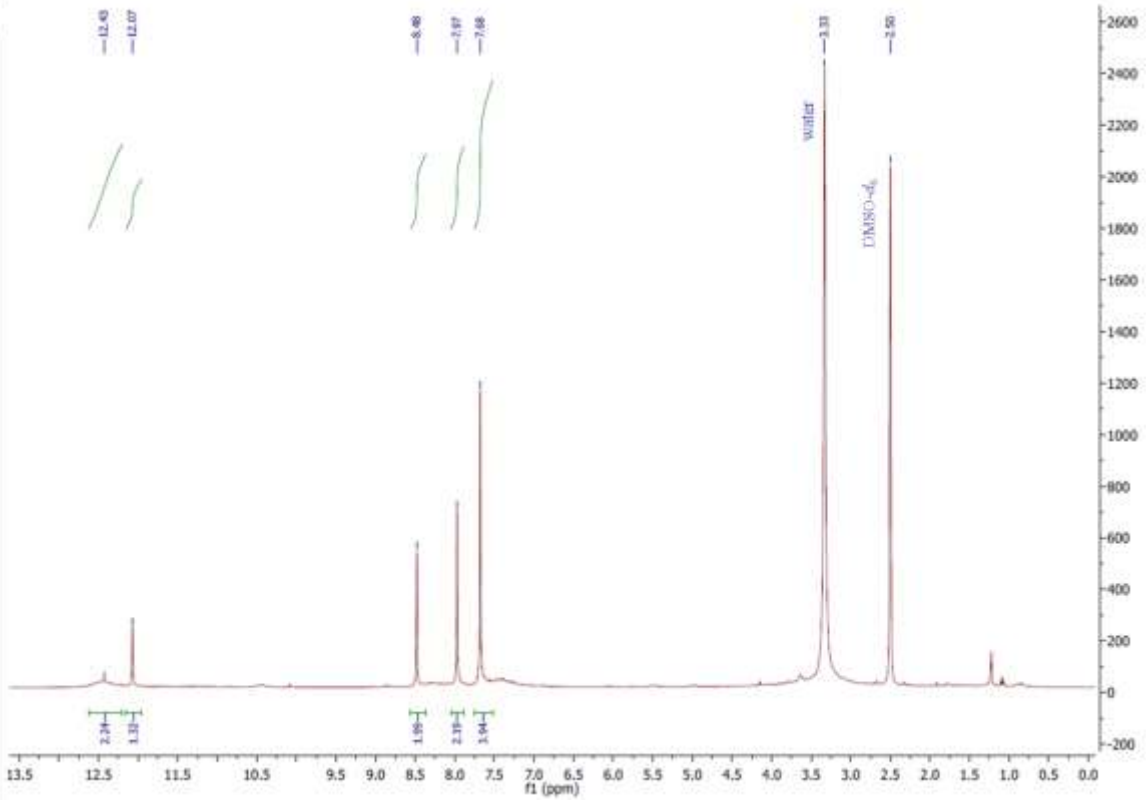
168.92
143.60
131.69
131.30
130.69
130.10
41.05
40.60
40.39
40.18
39.77
39.56
39.35
DMSO-d_6
f1 (ppm)

Compound **4**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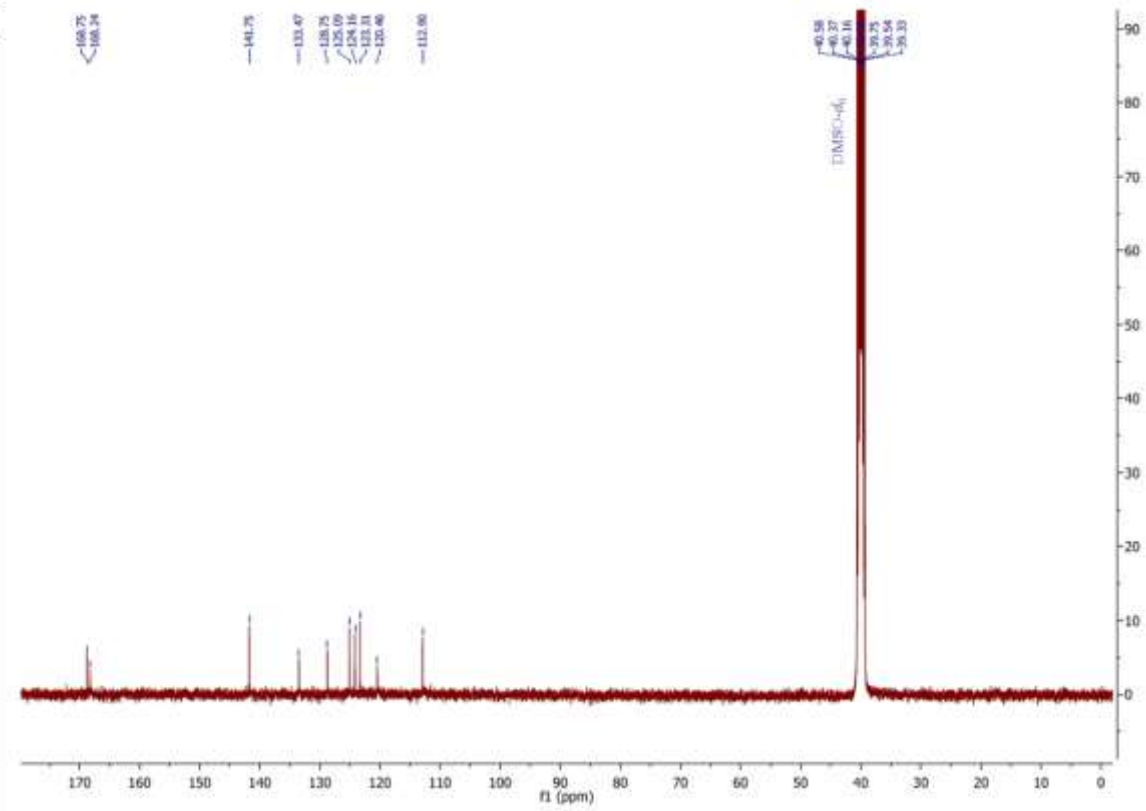
12.07
8.48
7.97
7.68
3.33
2.50
water
DMSO-d6
2.24
1.33
1.96
2.19
3.94
f1 (ppm)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168.24
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128.75
125.09
124.16
123.31
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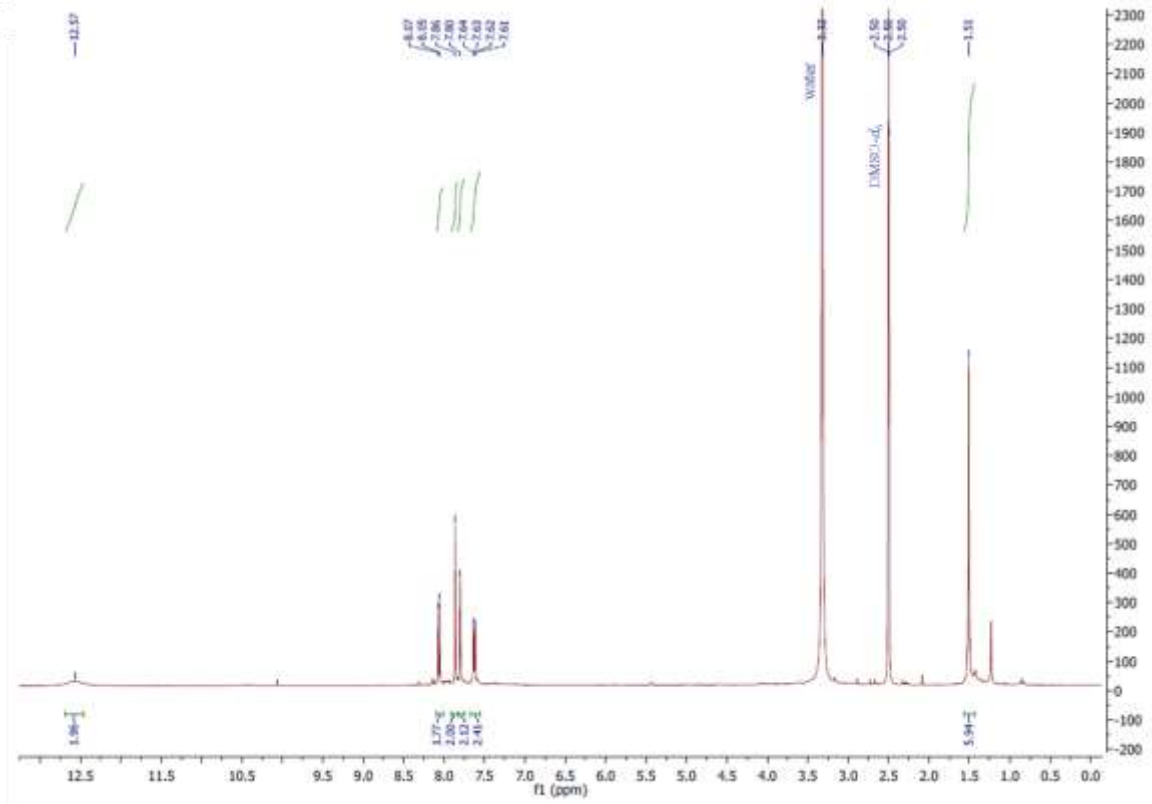
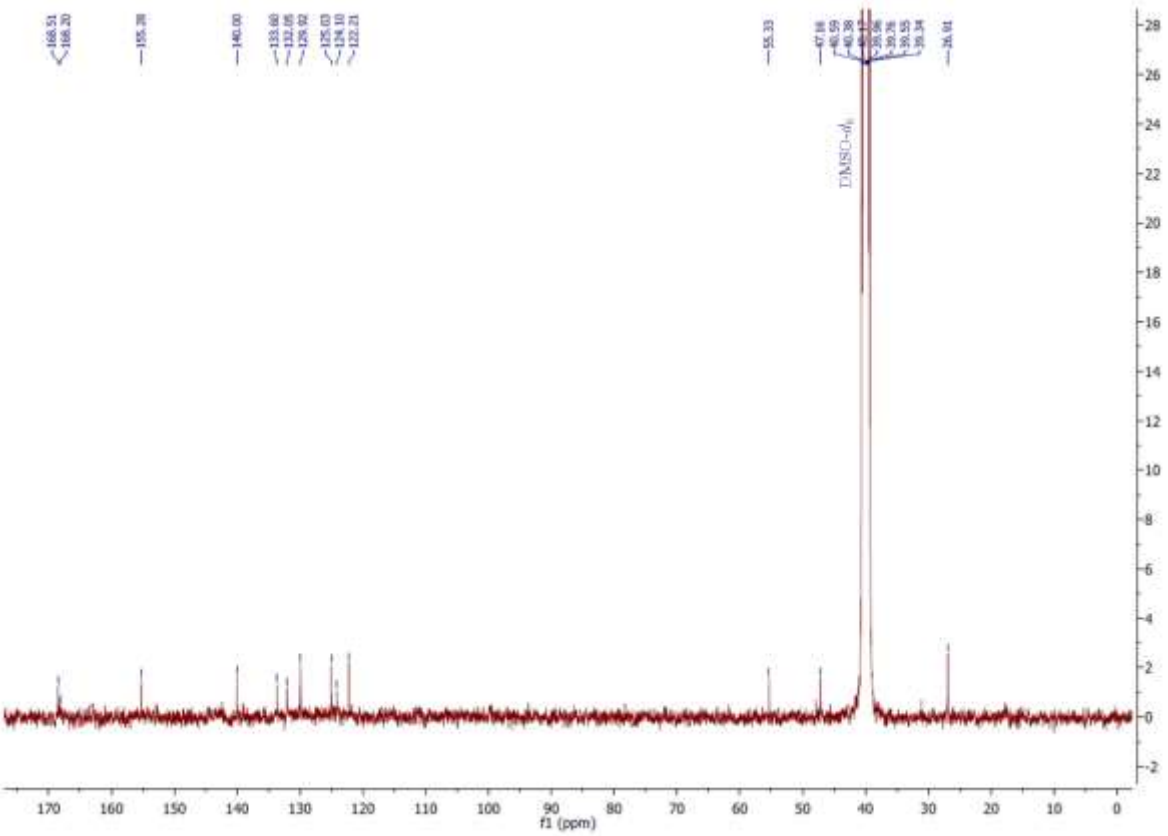
40.16
39.75
39.54
39.33
DMSO-d6
f1 (ppm)

Compound **5**





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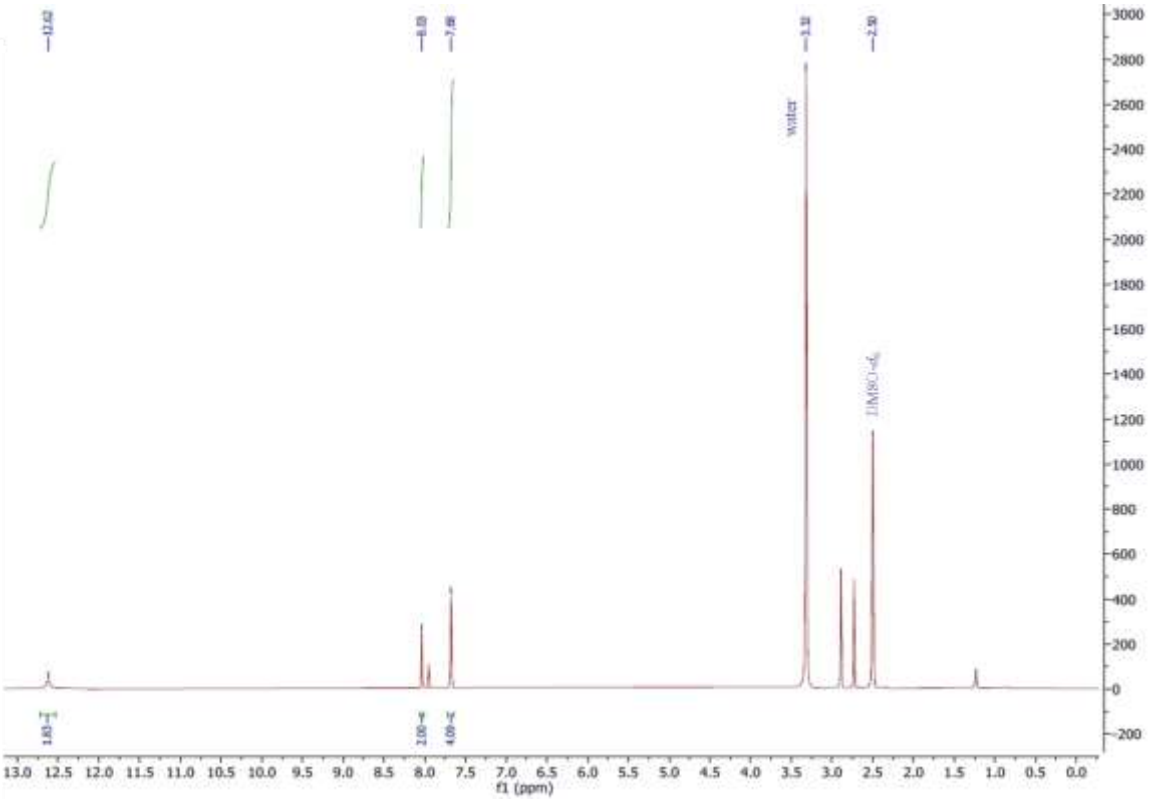
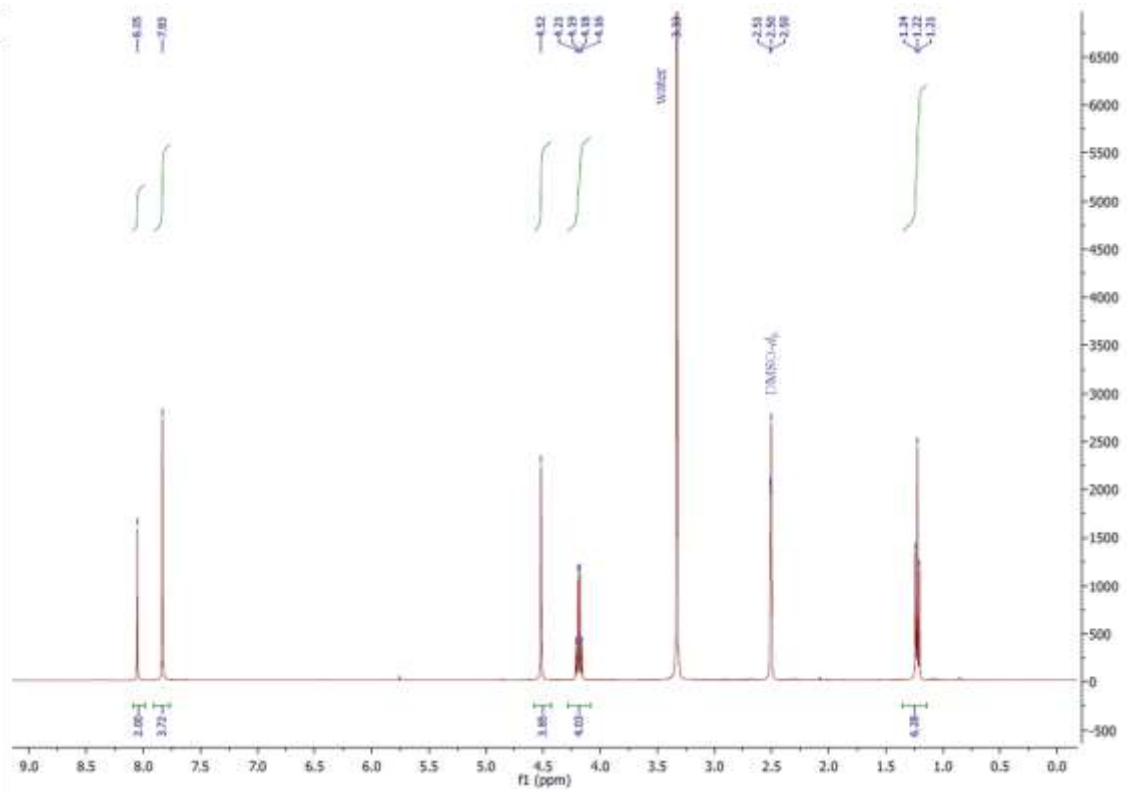
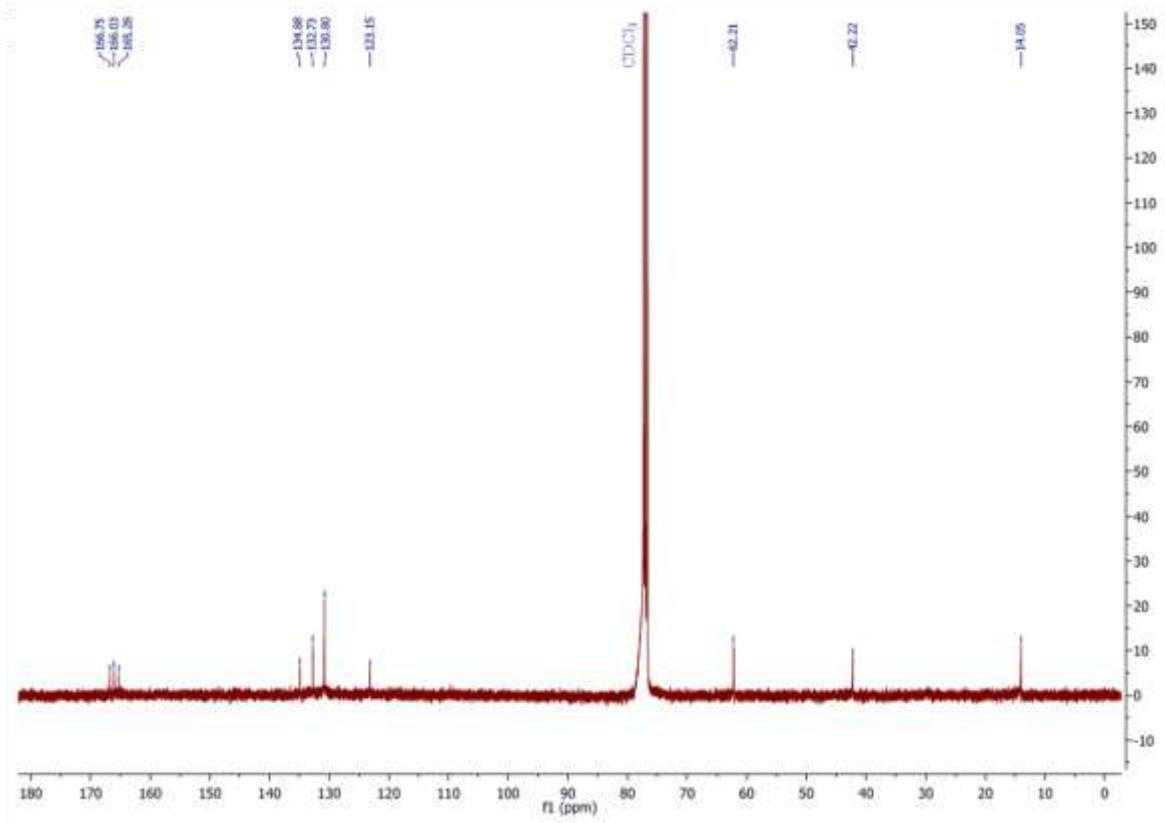


We were unable to acquire ^{13}C-NMR, due to the low solubility of the compound.

Compound **7**





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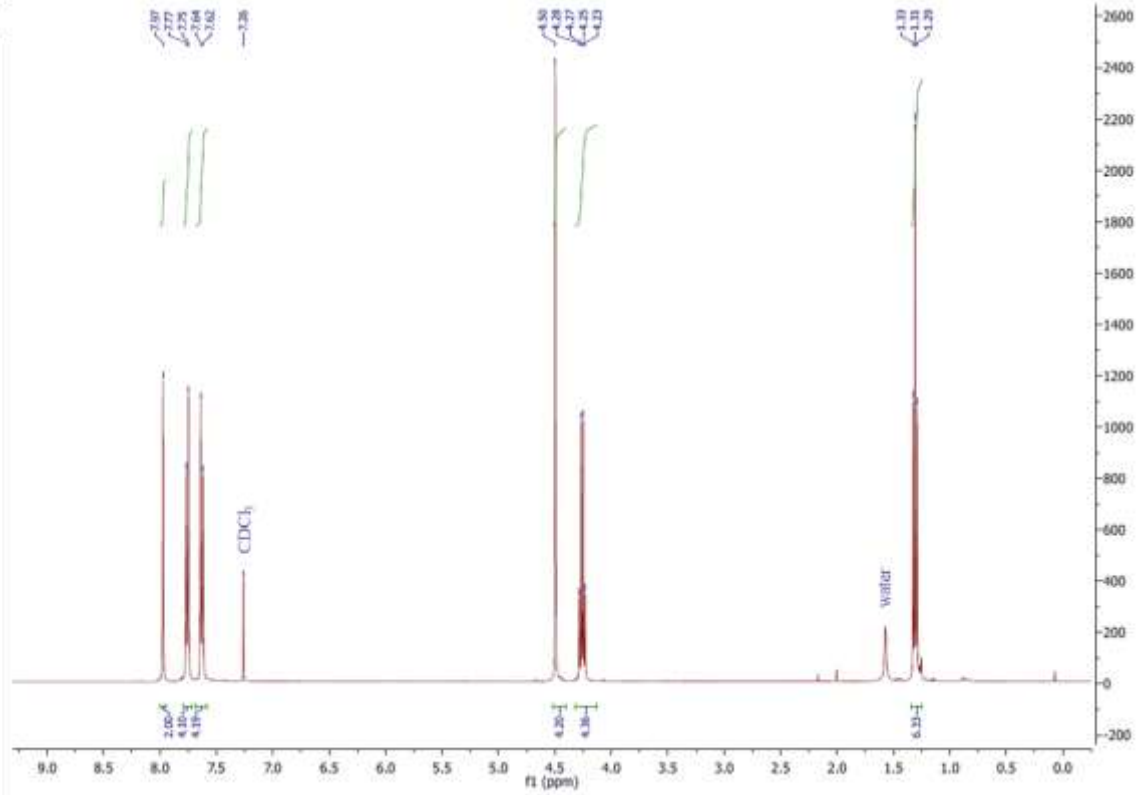

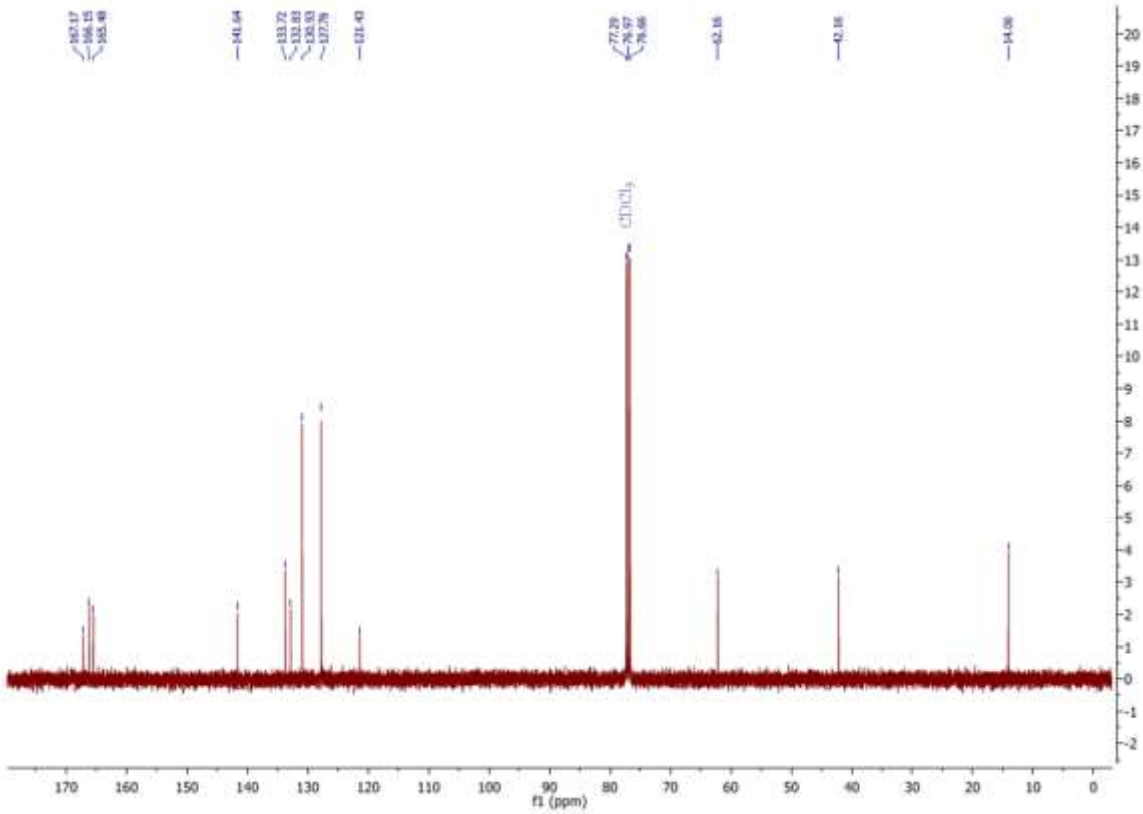

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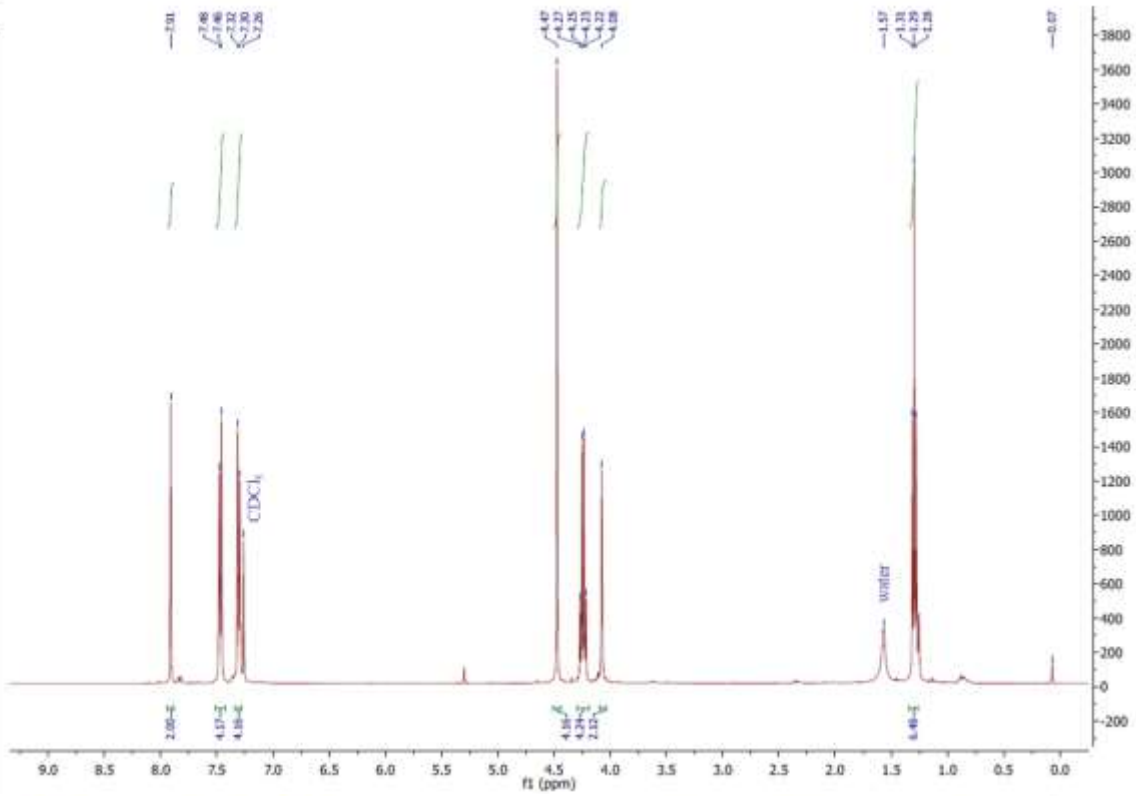

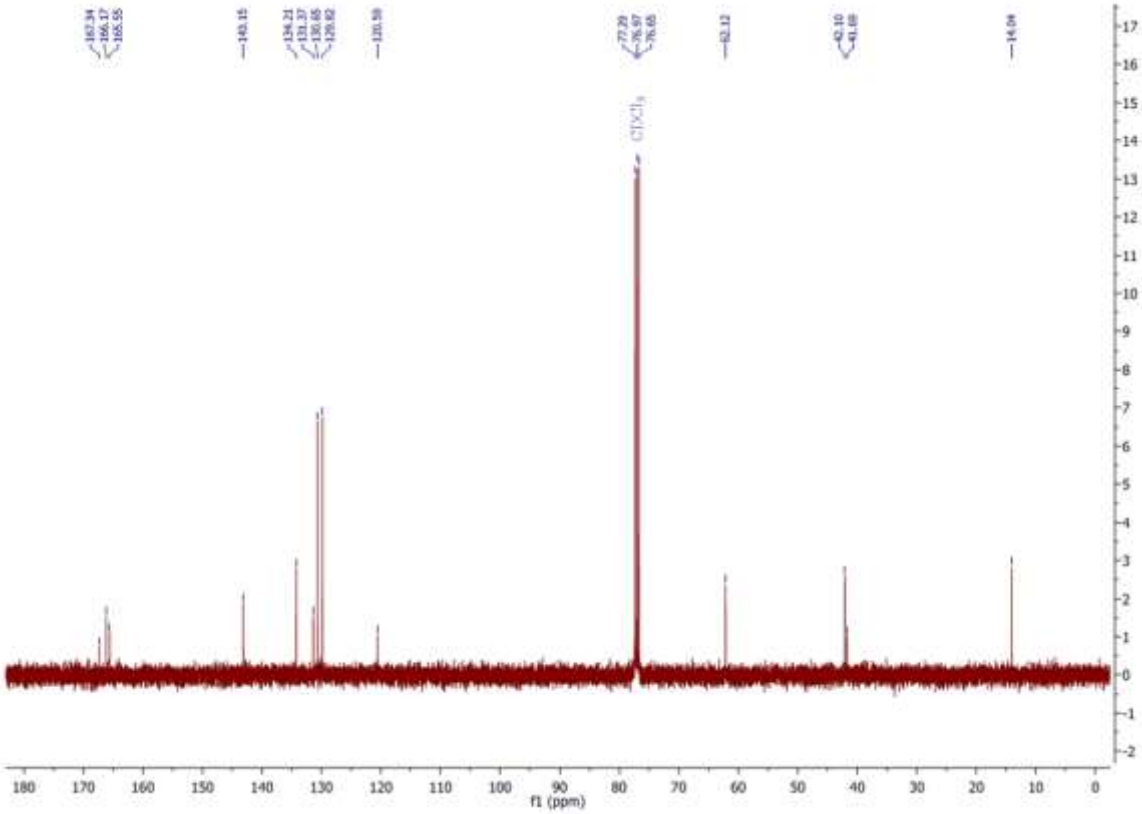

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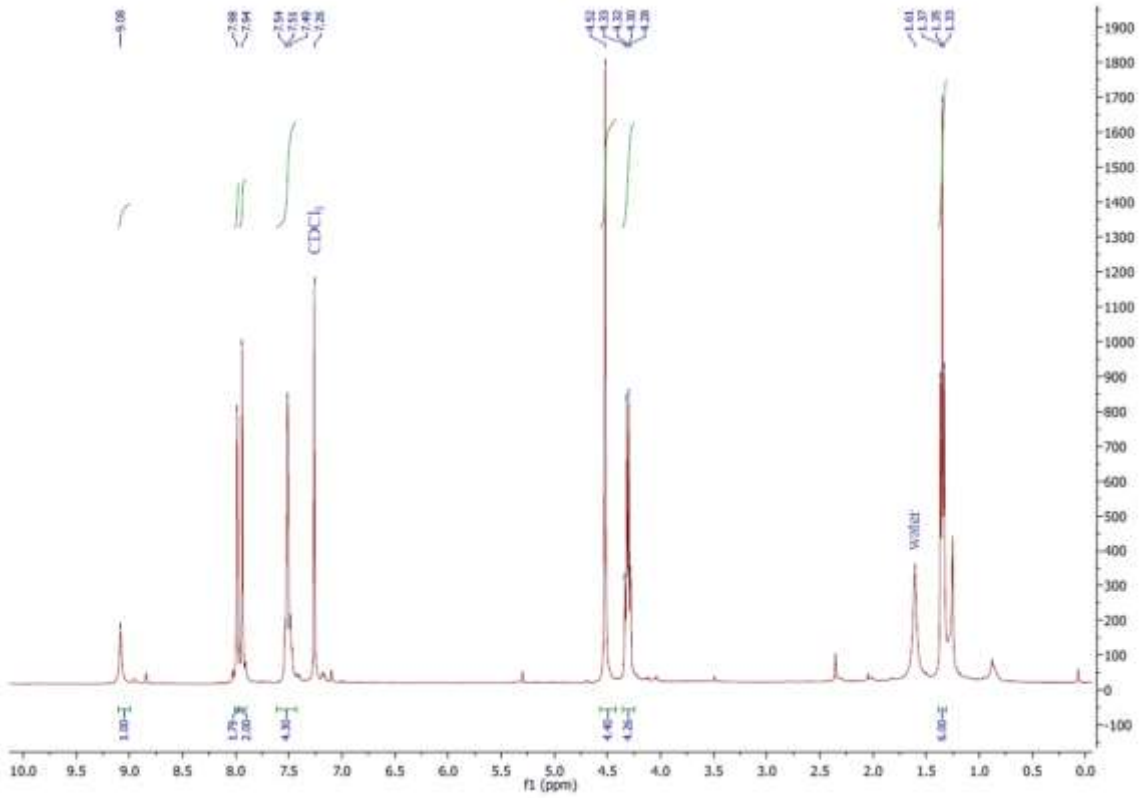

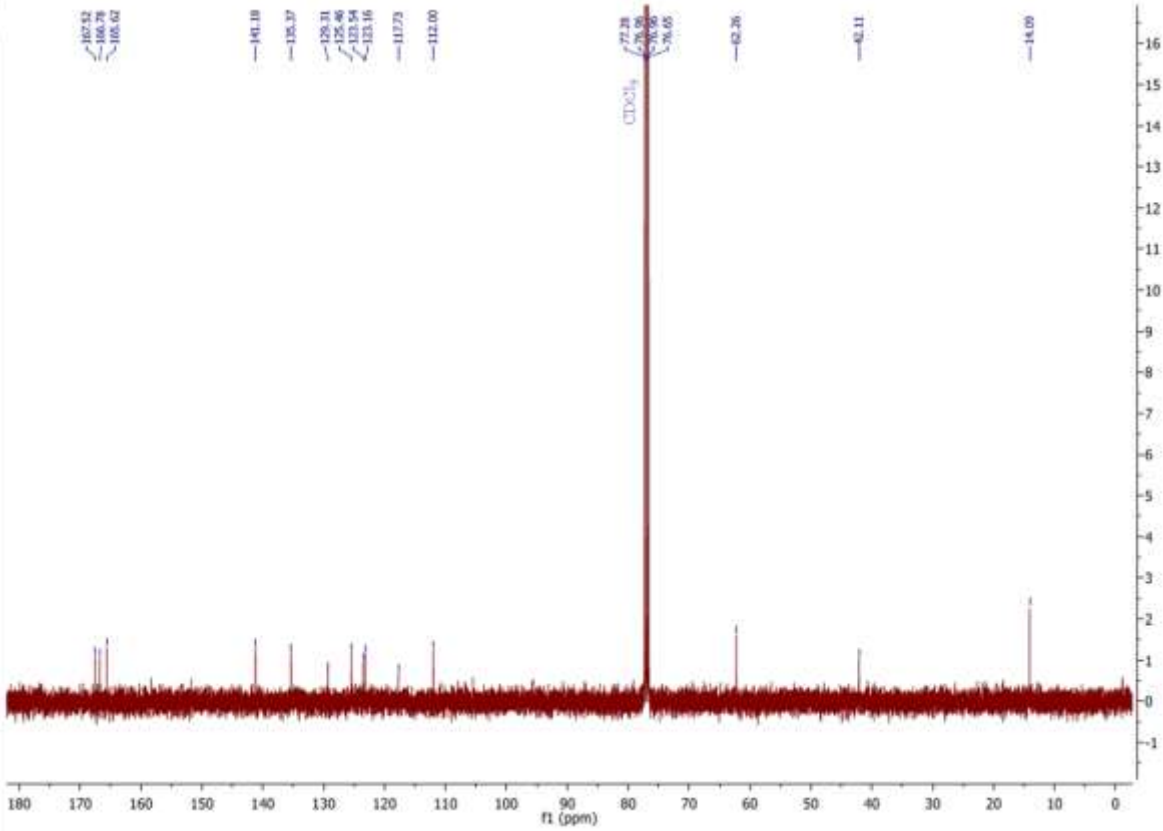

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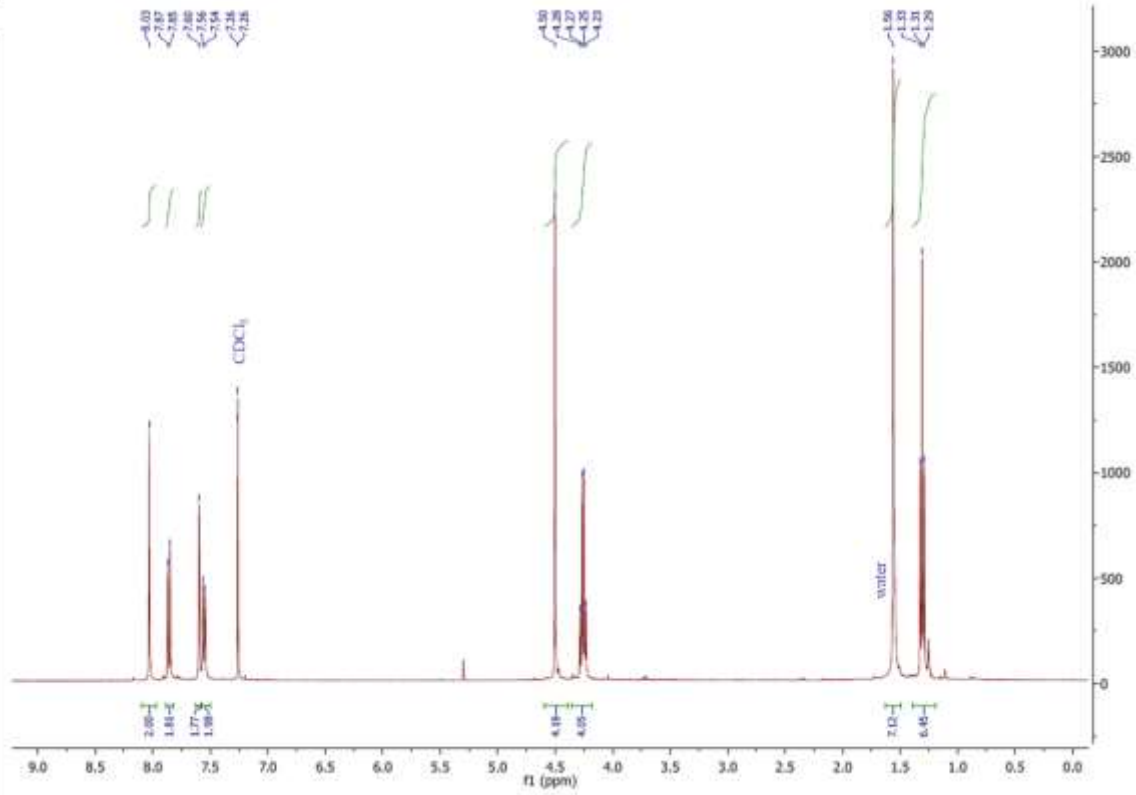

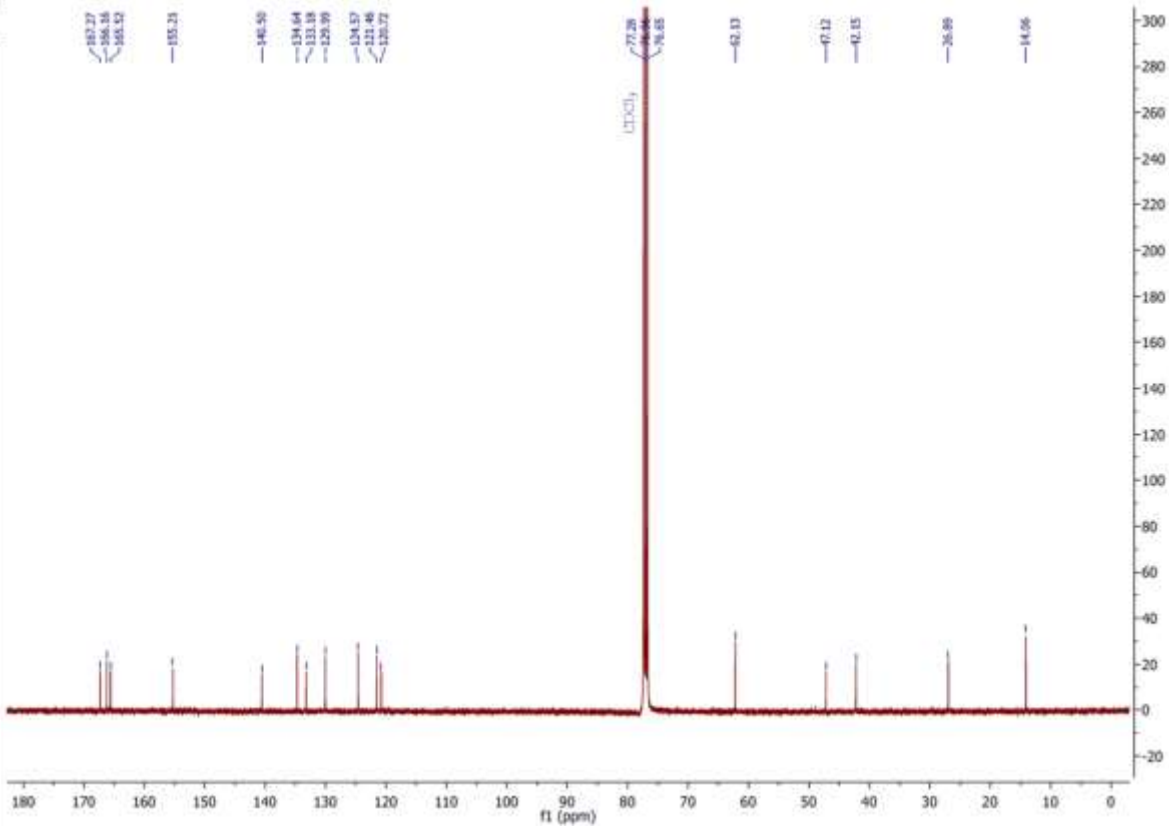

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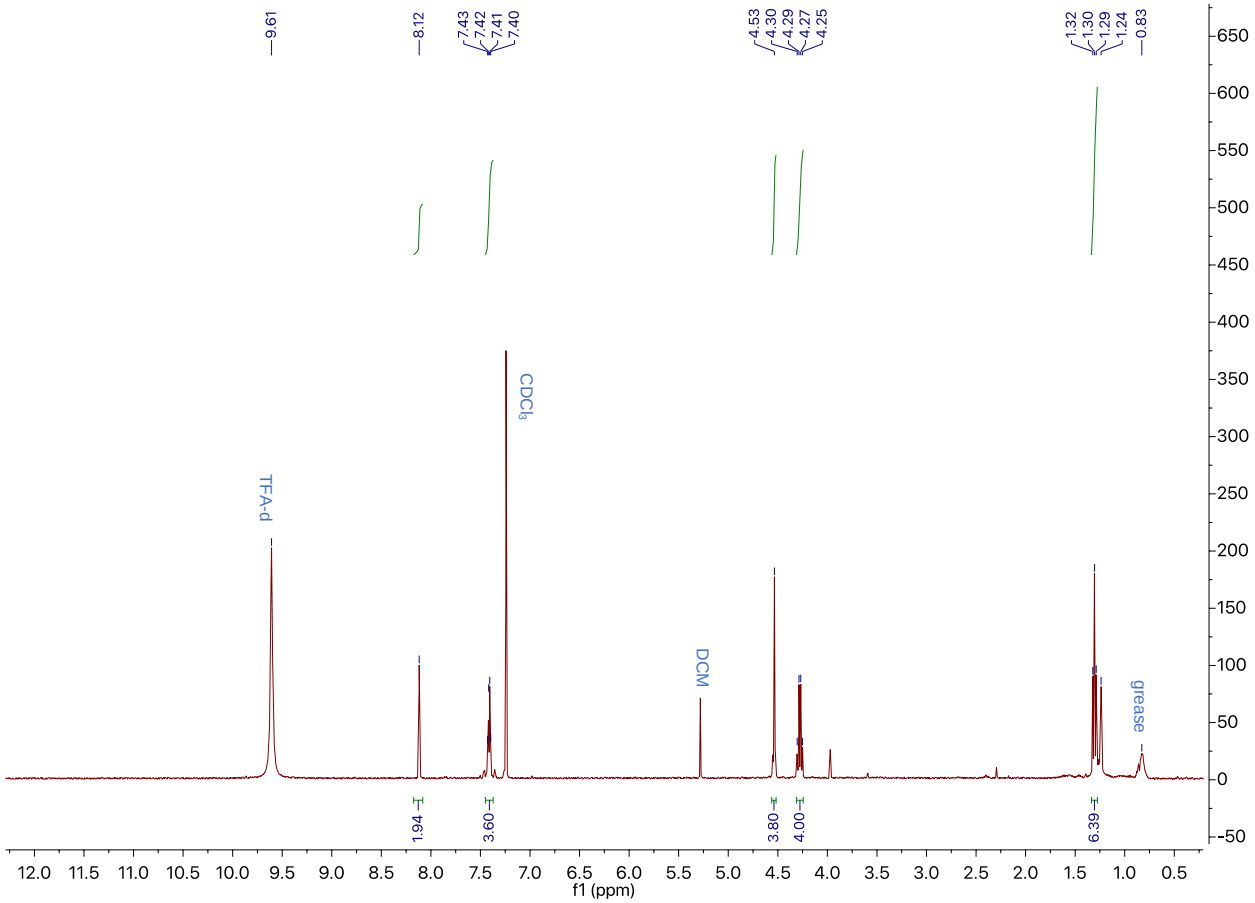
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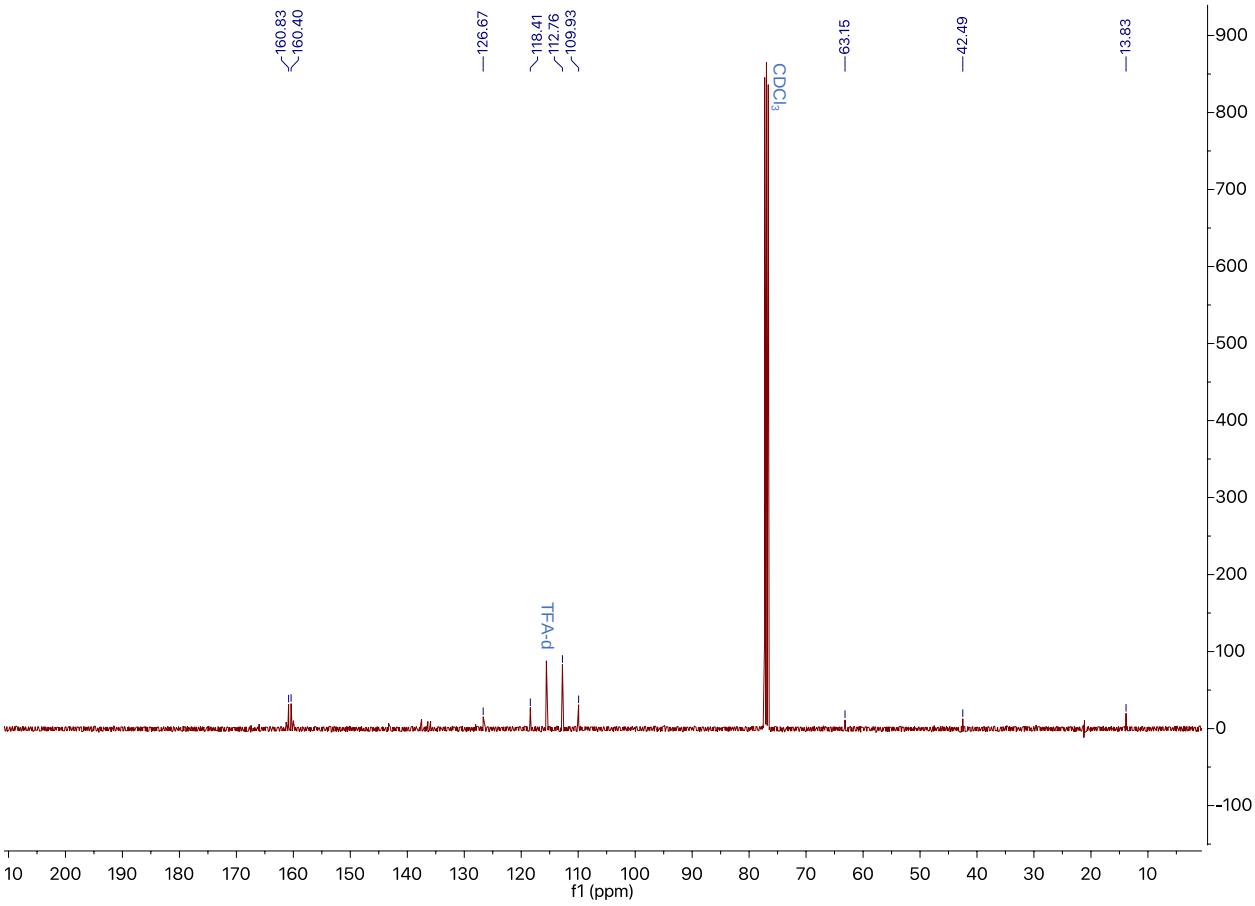
7.40
4.53
4.30
4.29
4.27
4.25
1.32
1.30
1.29
1.24
0.83
TFA-d
CDCl3
DCM
grease
1.94
3.60
3.80
4.00
6.39
f1 (ppm)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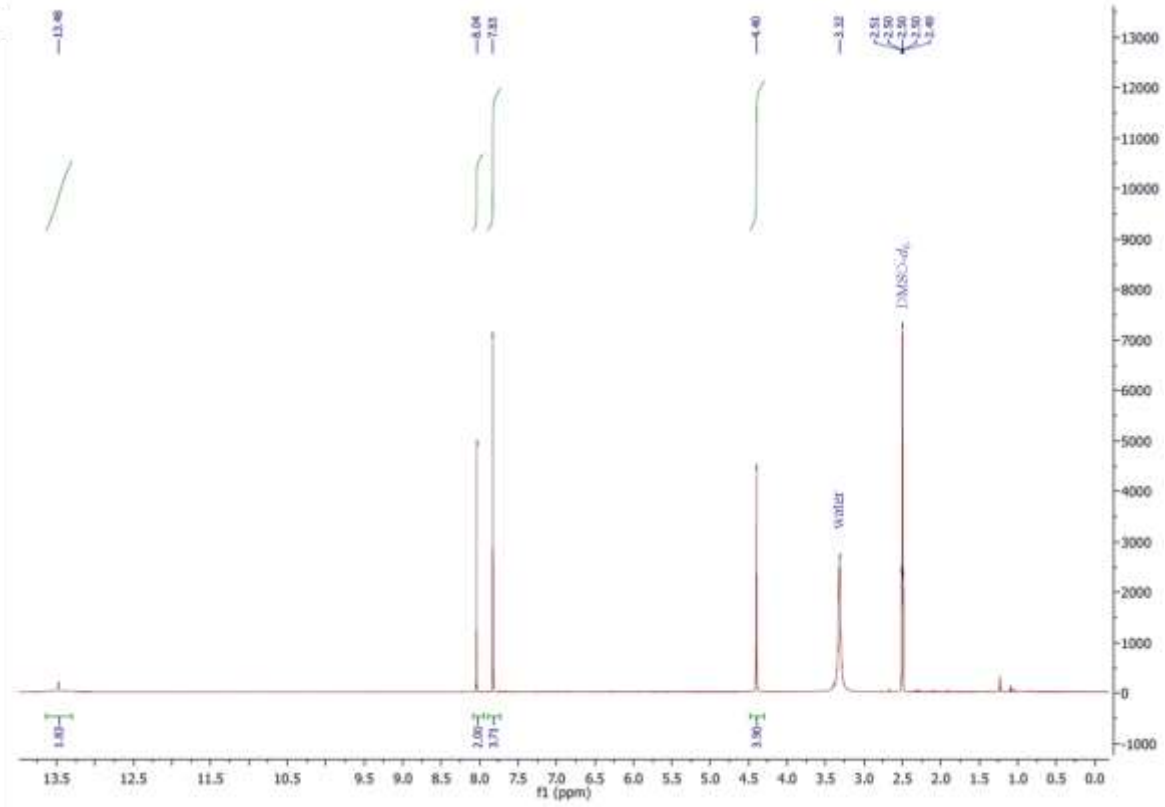
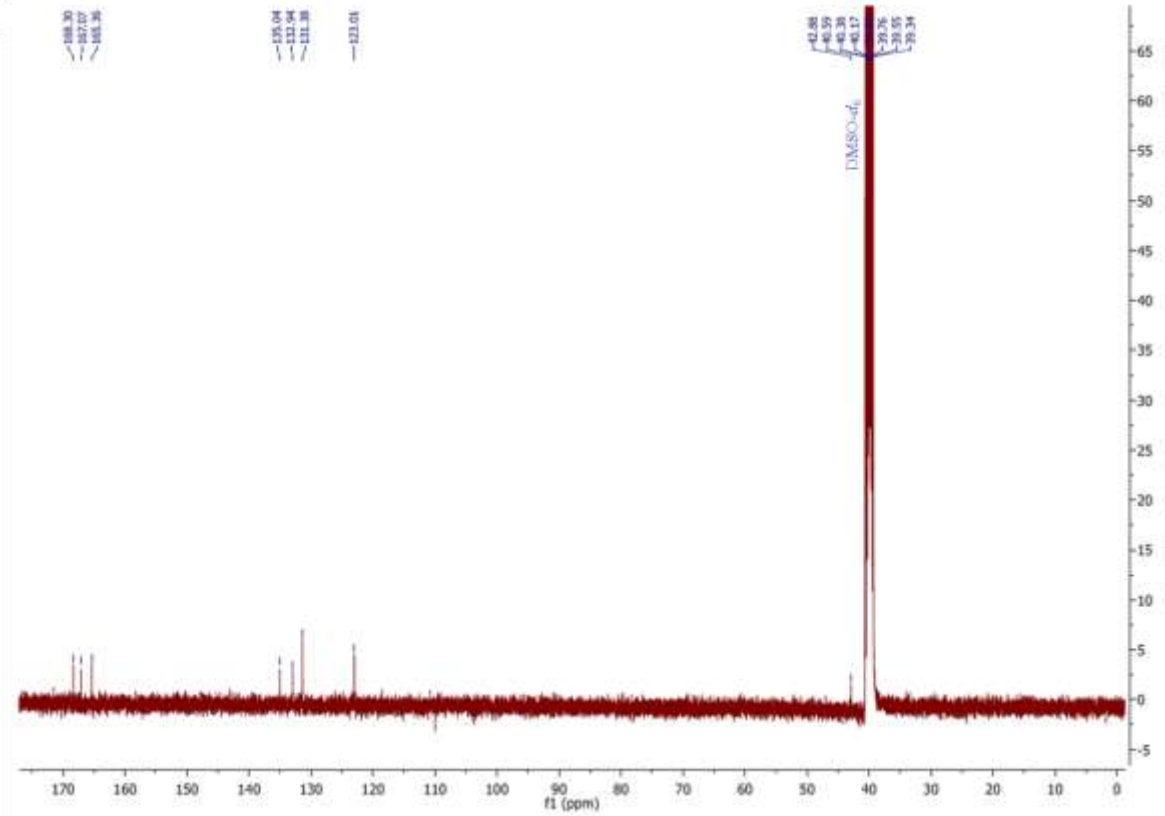
CDCl3
TFA-d
f1 (ppm)

Compound **13**





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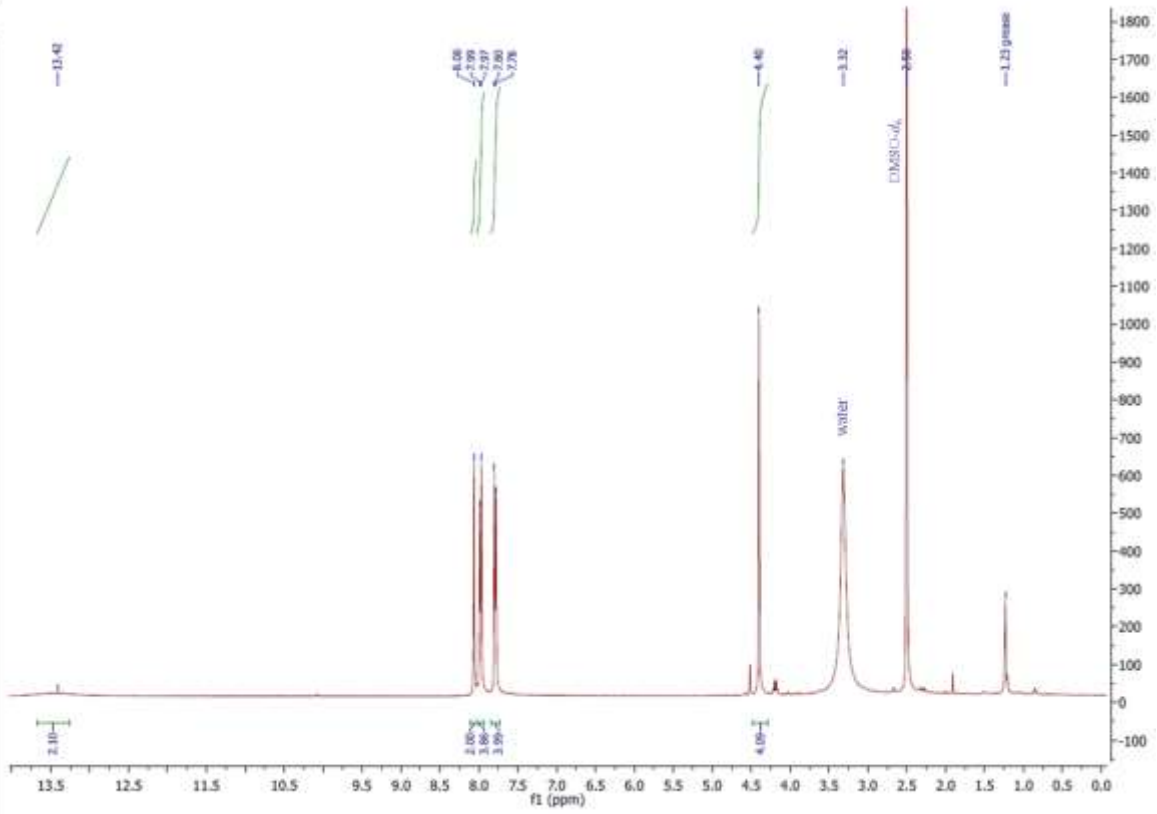
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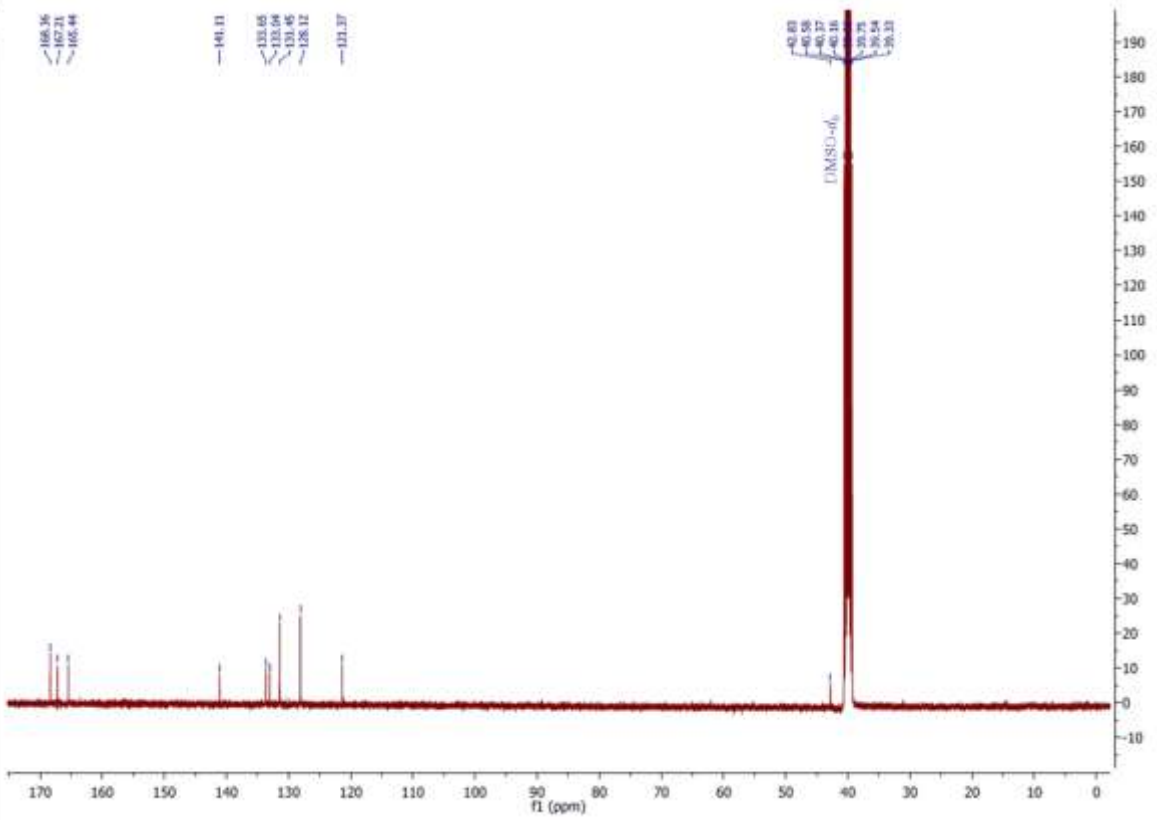
7.99
7.97
7.80
7.78
4.48
3.32
2.50
1.23 grease
DMSO-d_6
water
2.10
2.00
3.88
3.99
4.09
f1 (ppm)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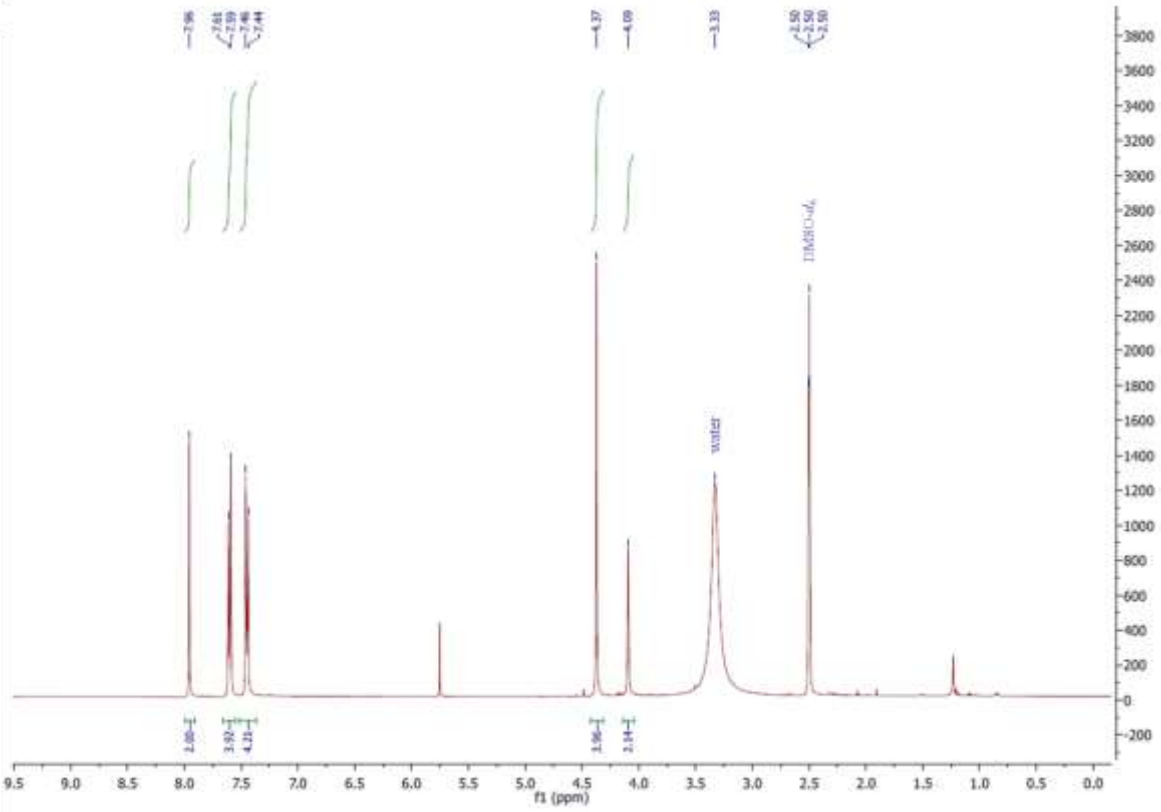
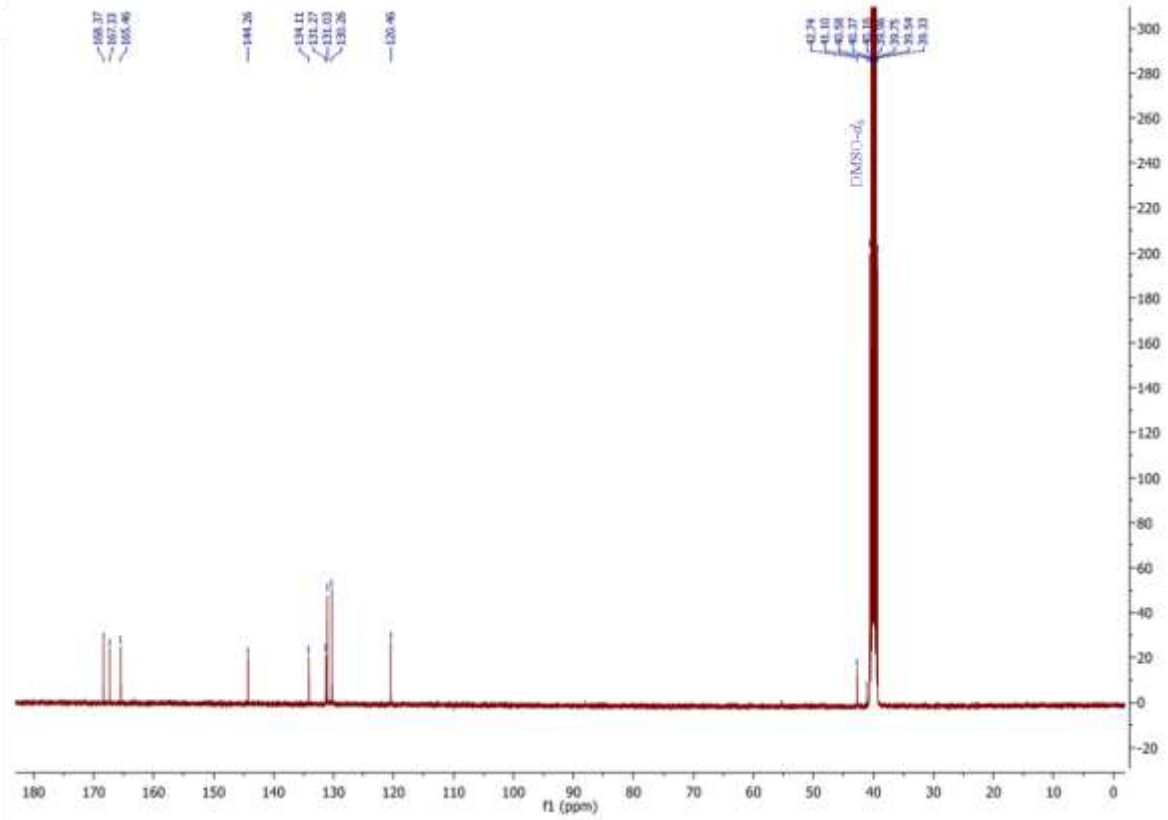
133.65
133.04
131.45
128.12
121.37
42.83
40.58
40.37
40.16
39.75
39.54
39.33
DMSO-d_6
f1 (ppm)

Compound **15**





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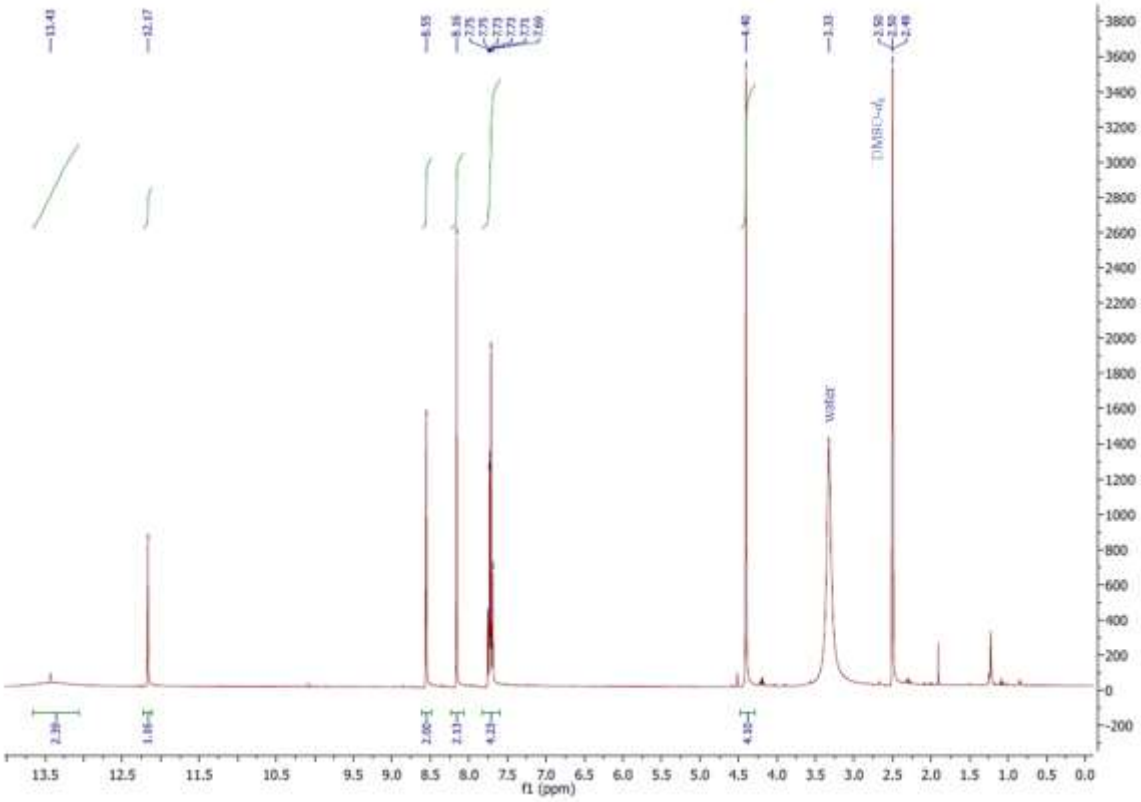

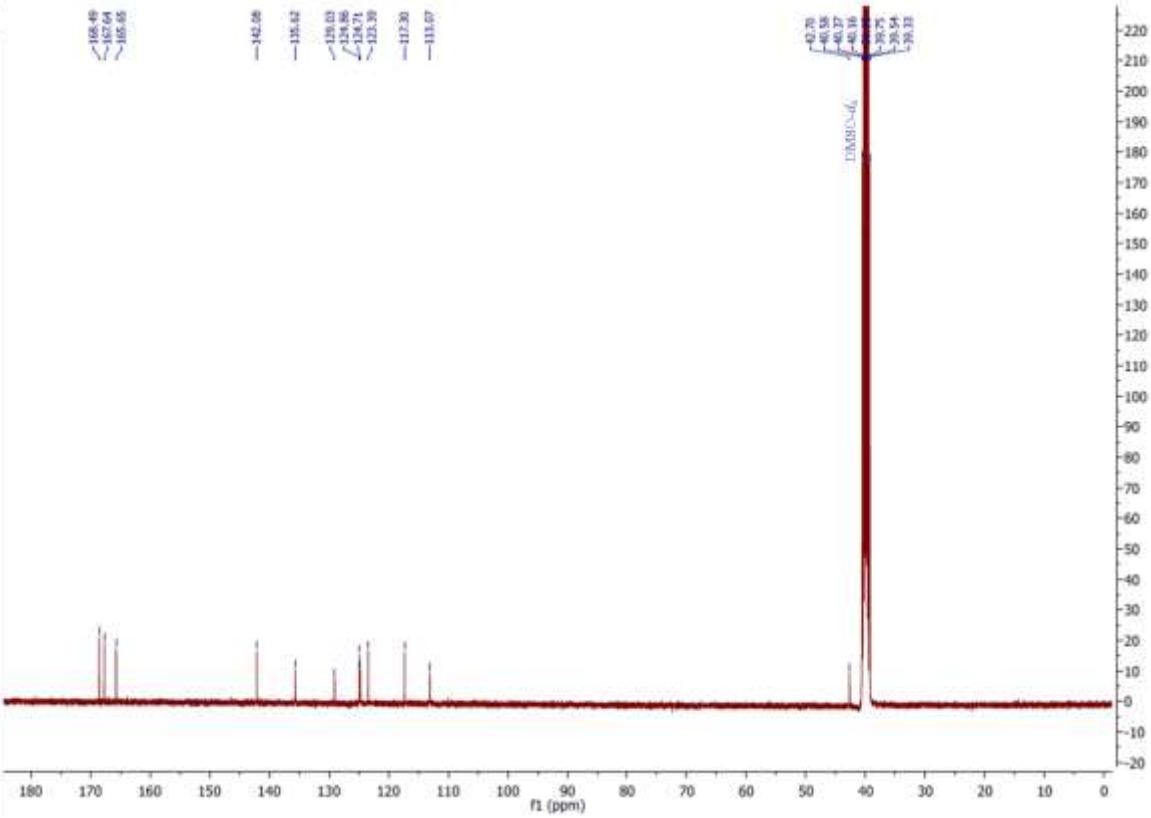

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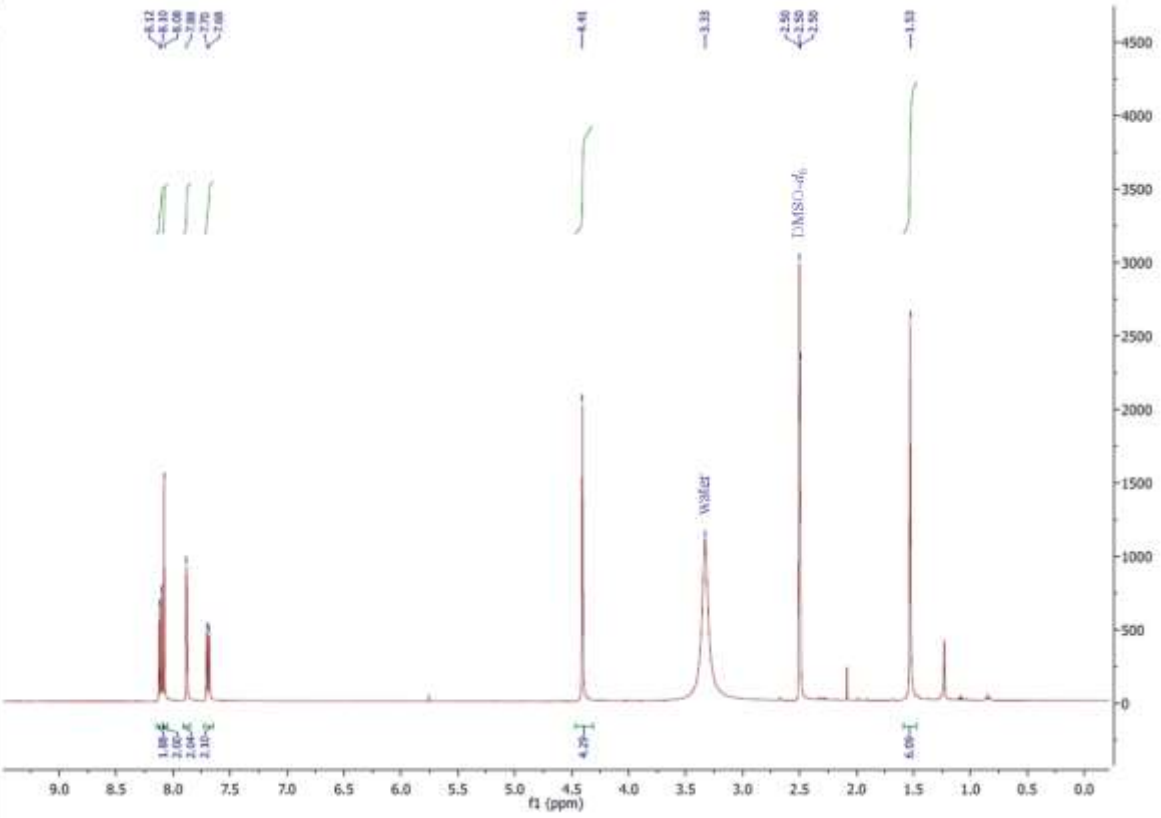

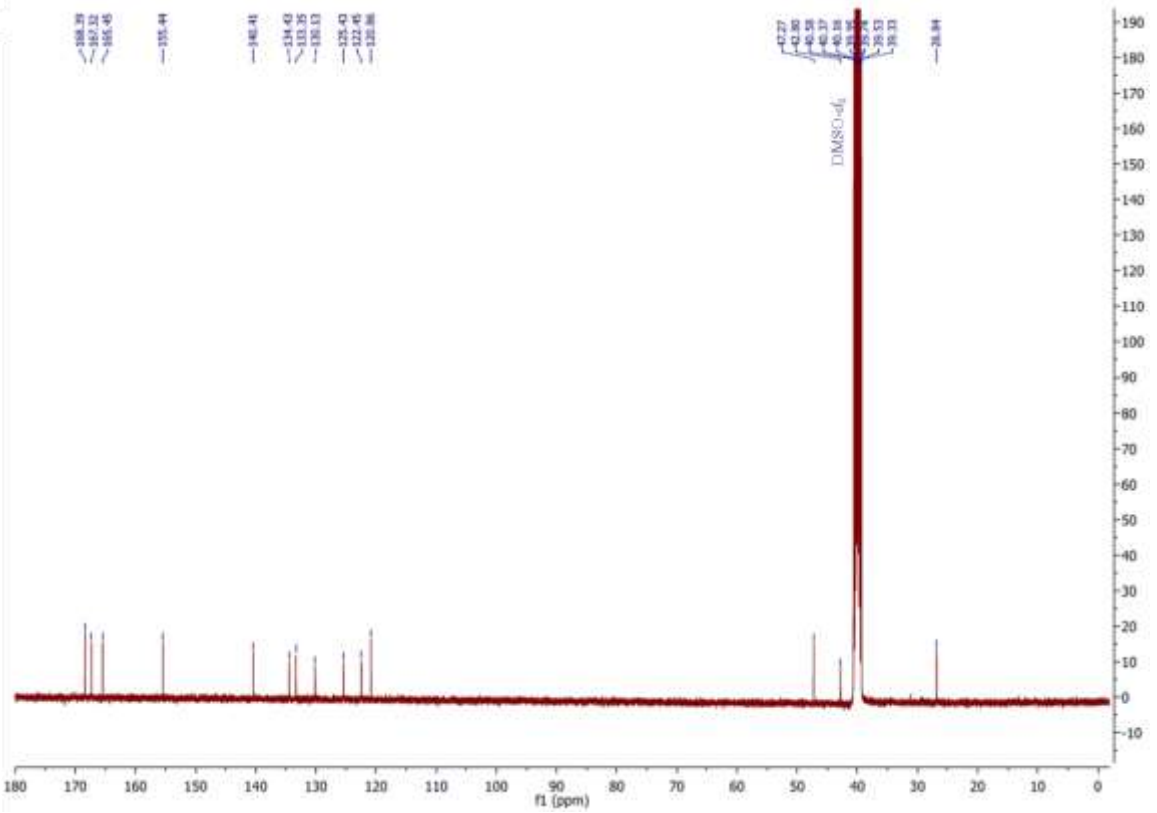

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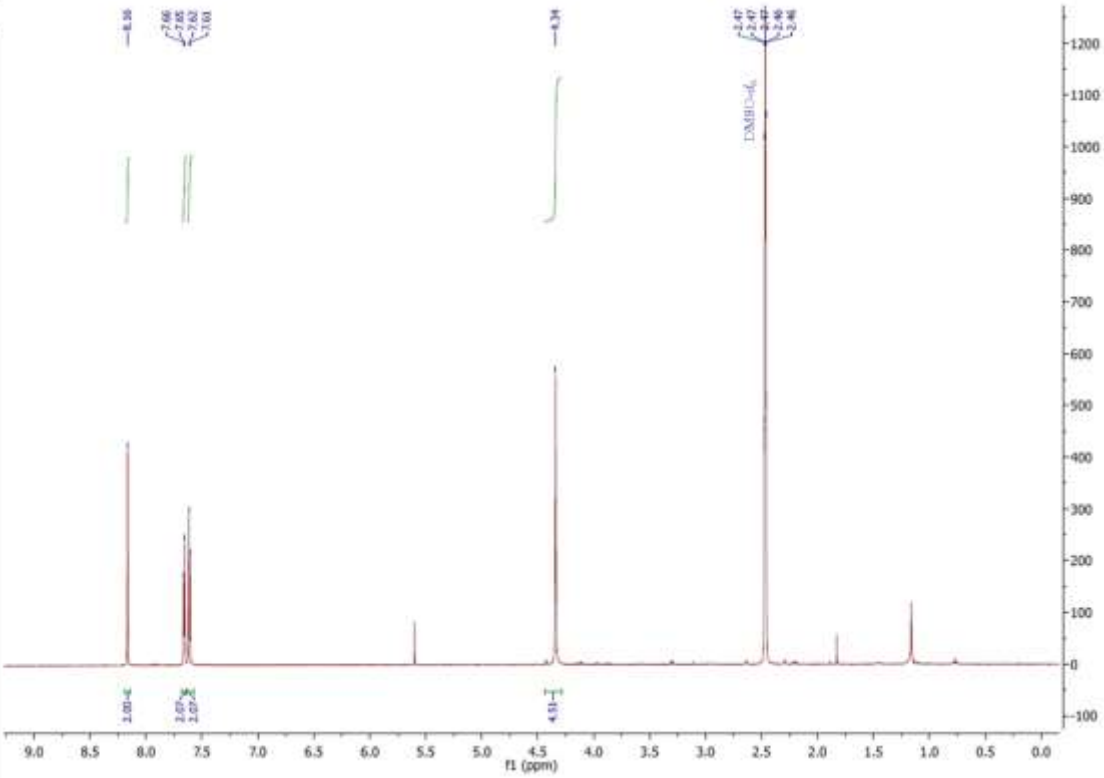
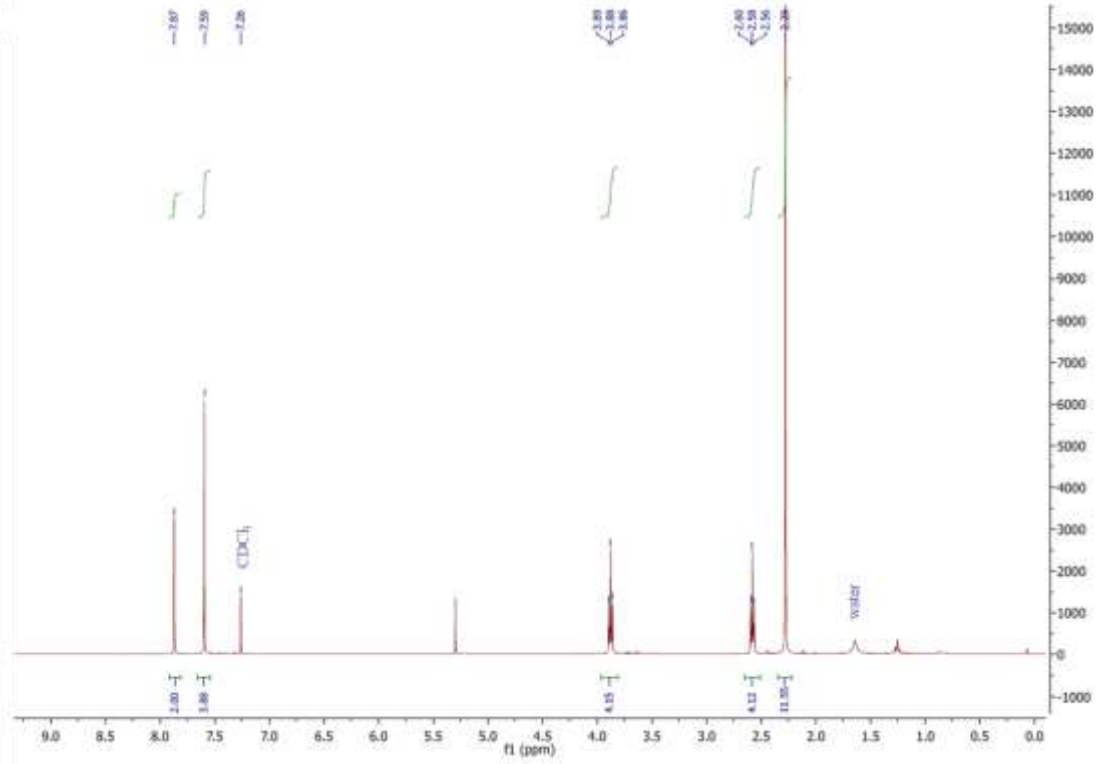
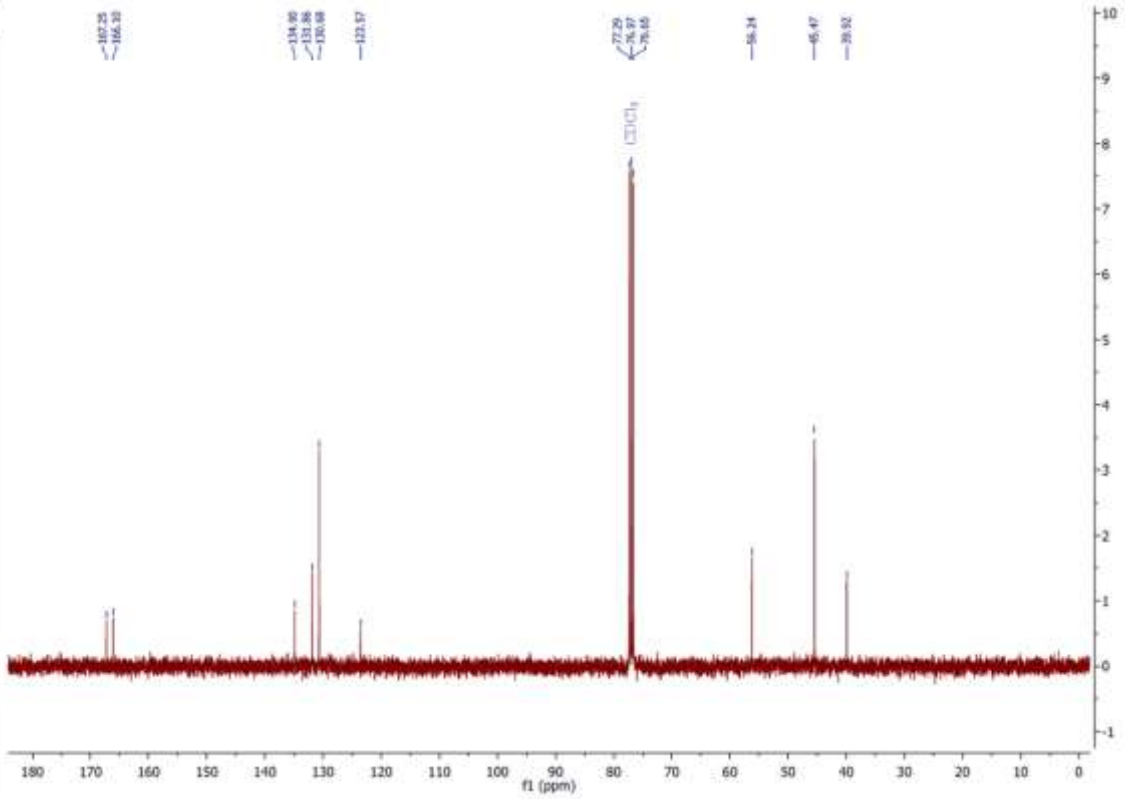


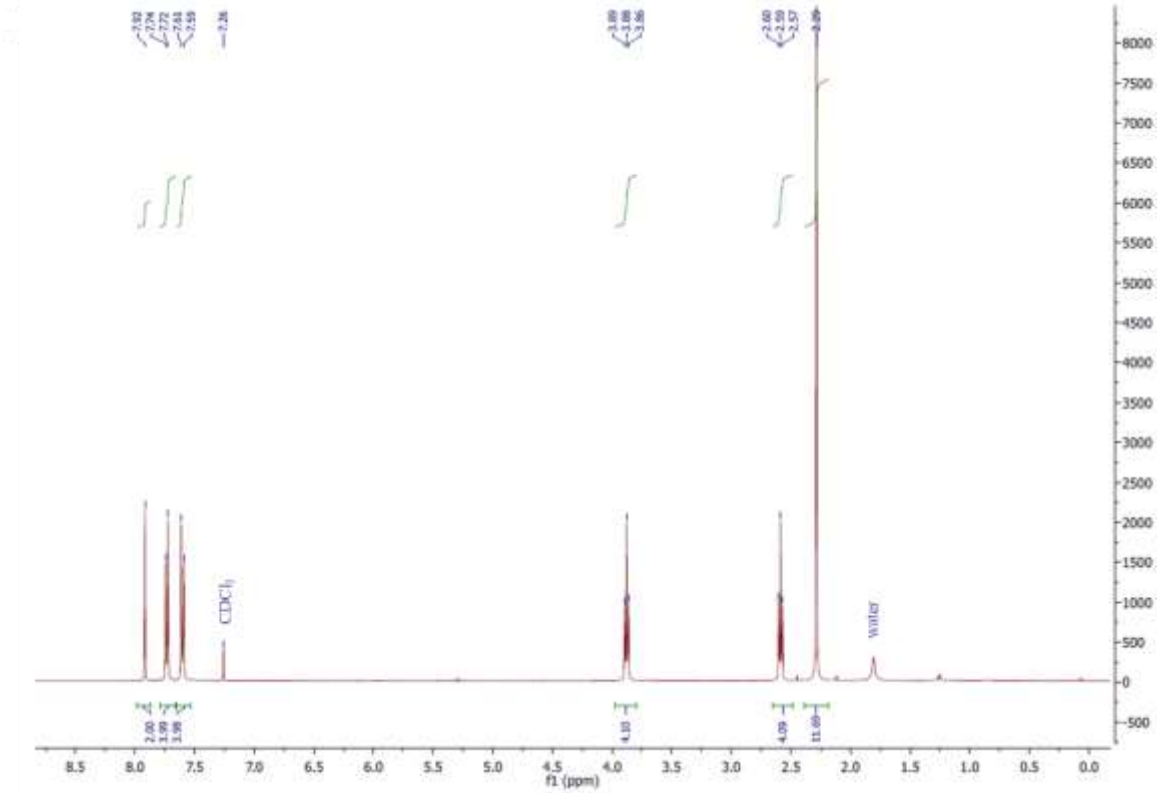
We were unable to acquire ^{13}C-NMR, due to the low solubility of the compound.

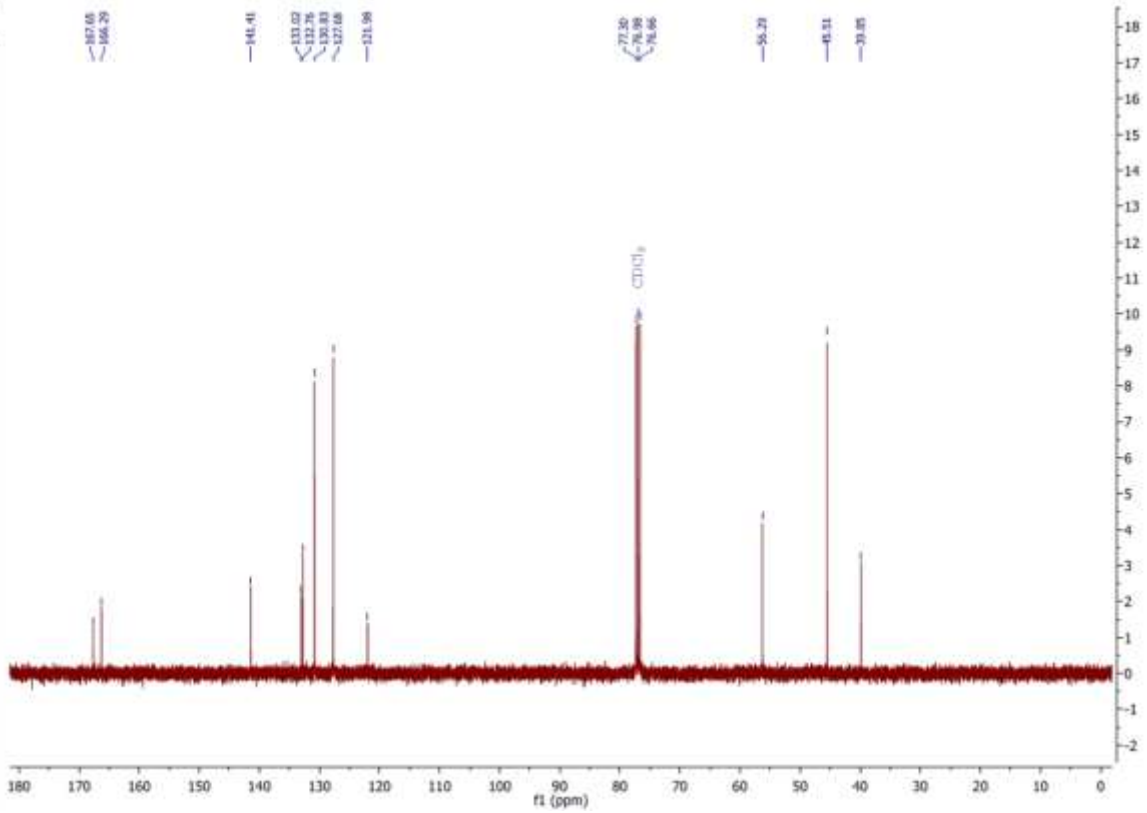
Compound **19**



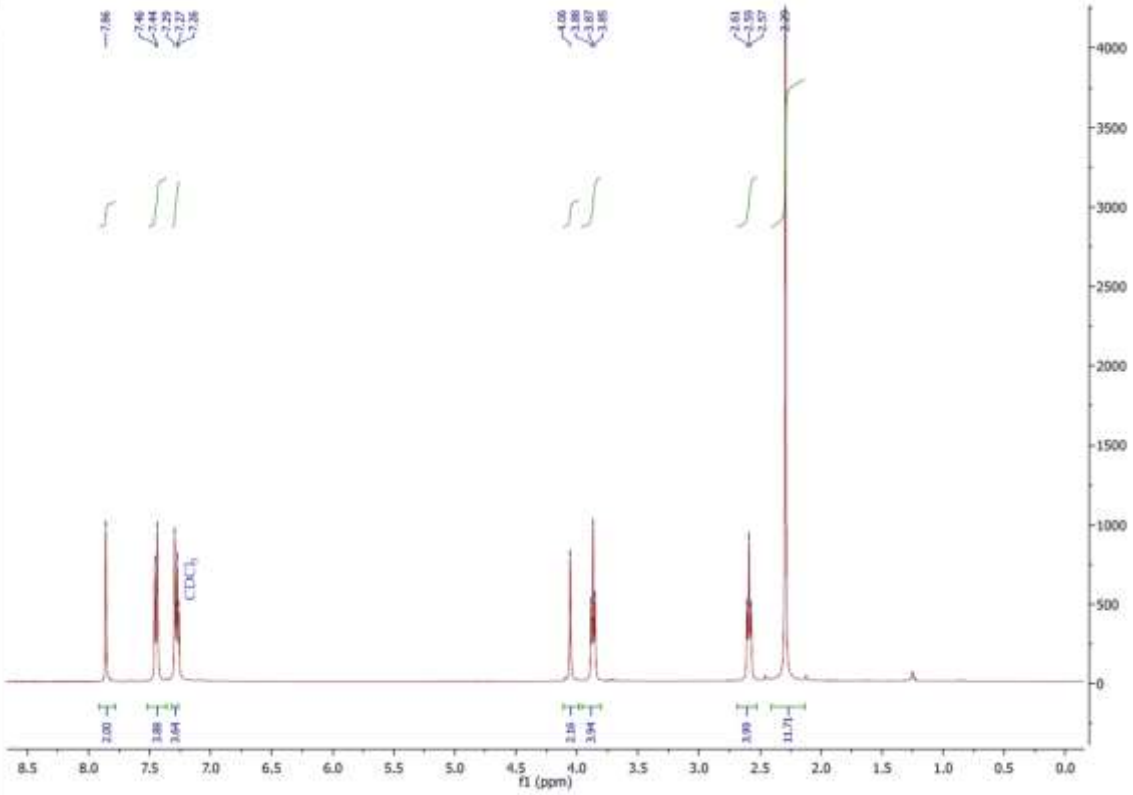


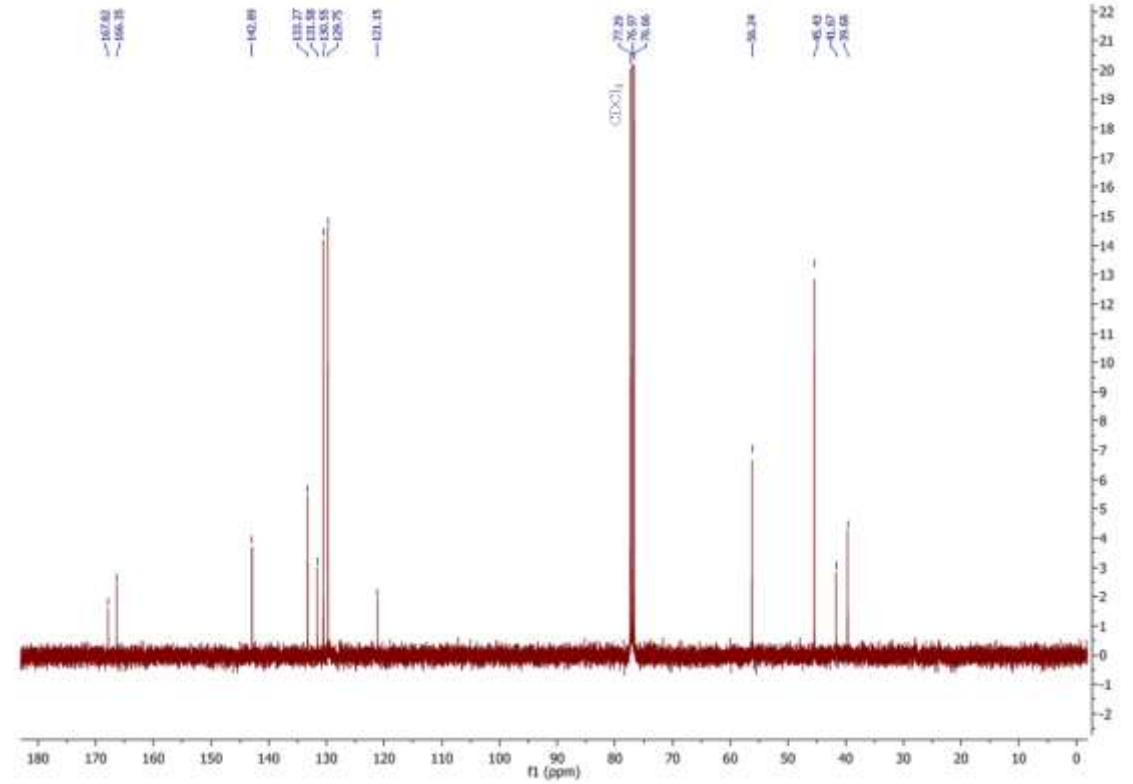
Compound **20**



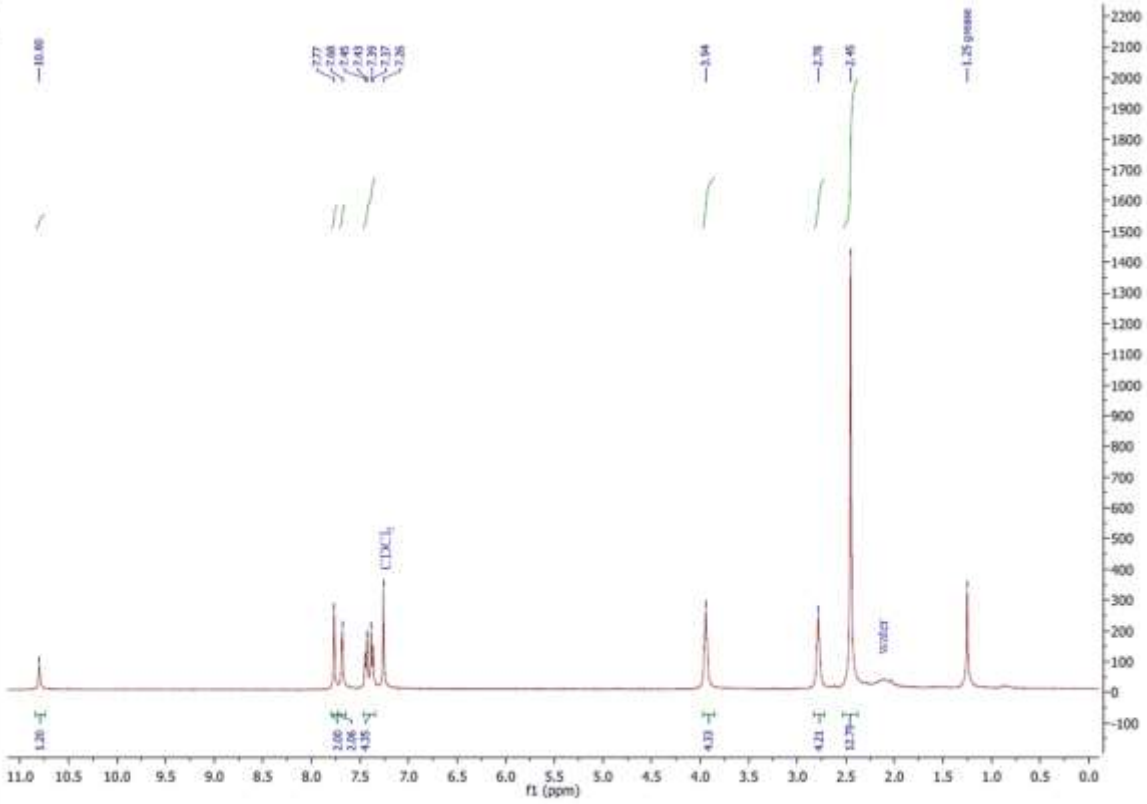


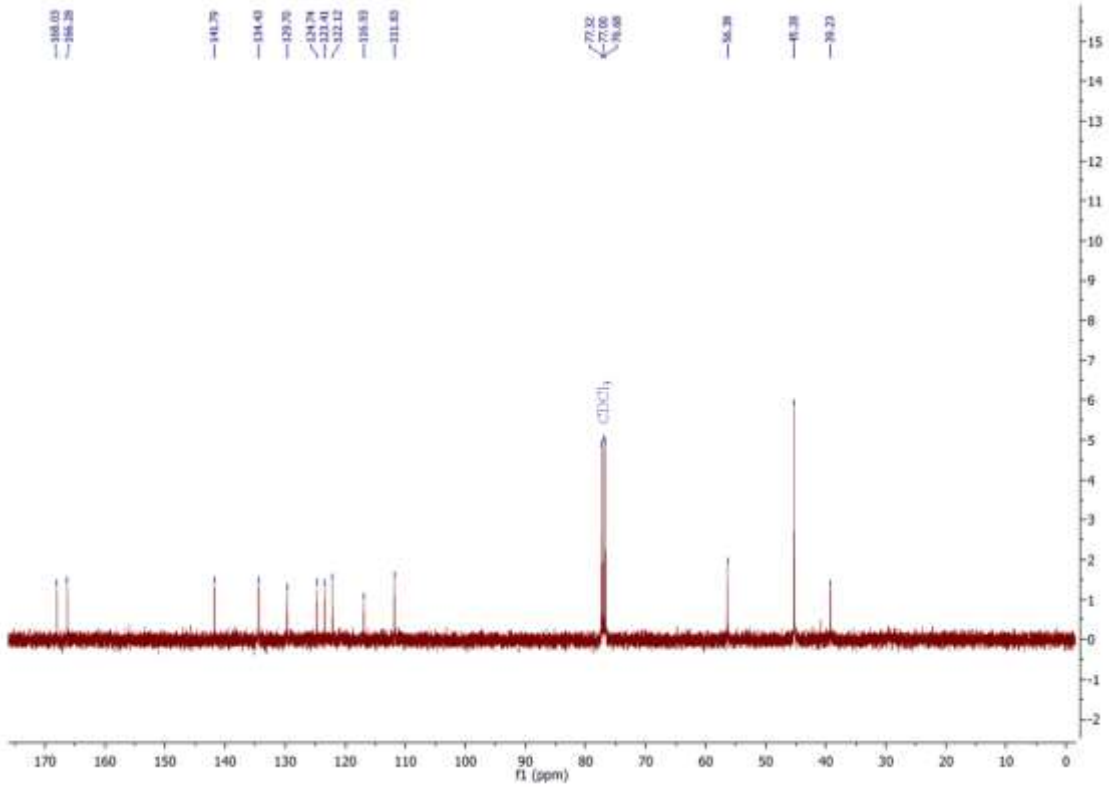
Compound **21**



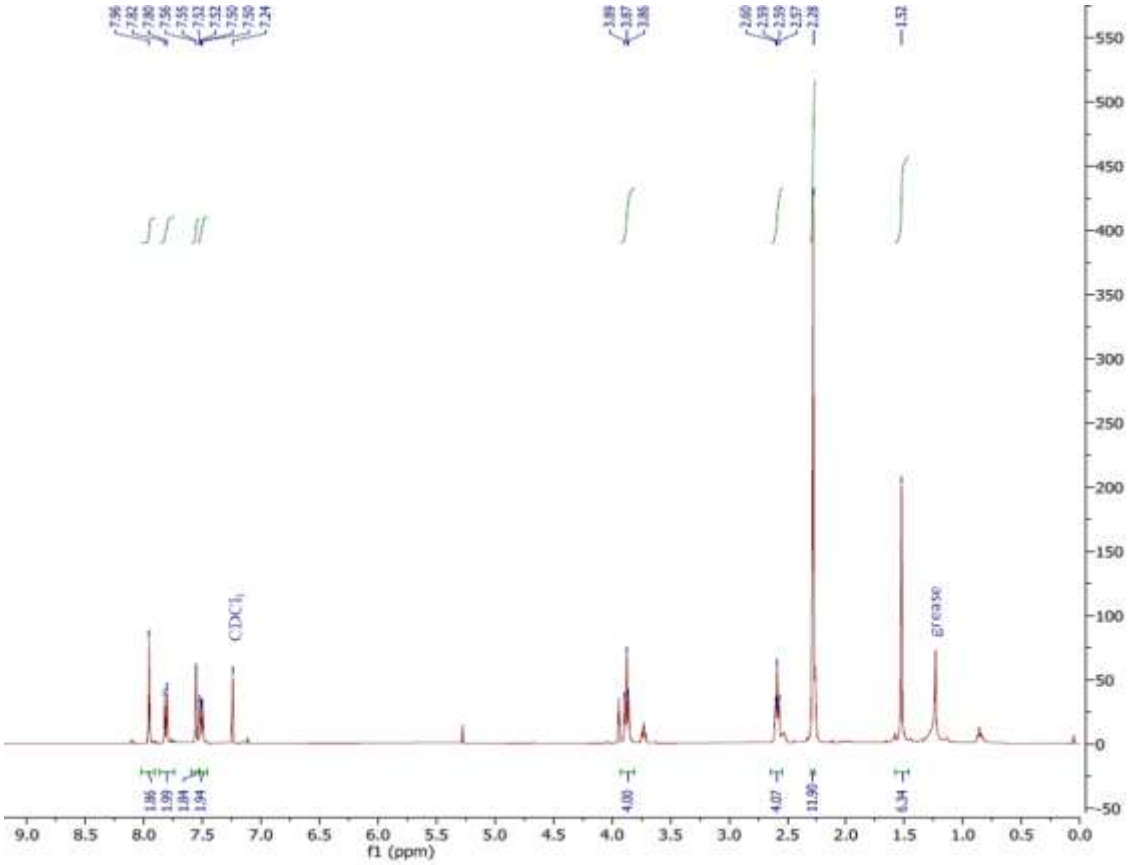


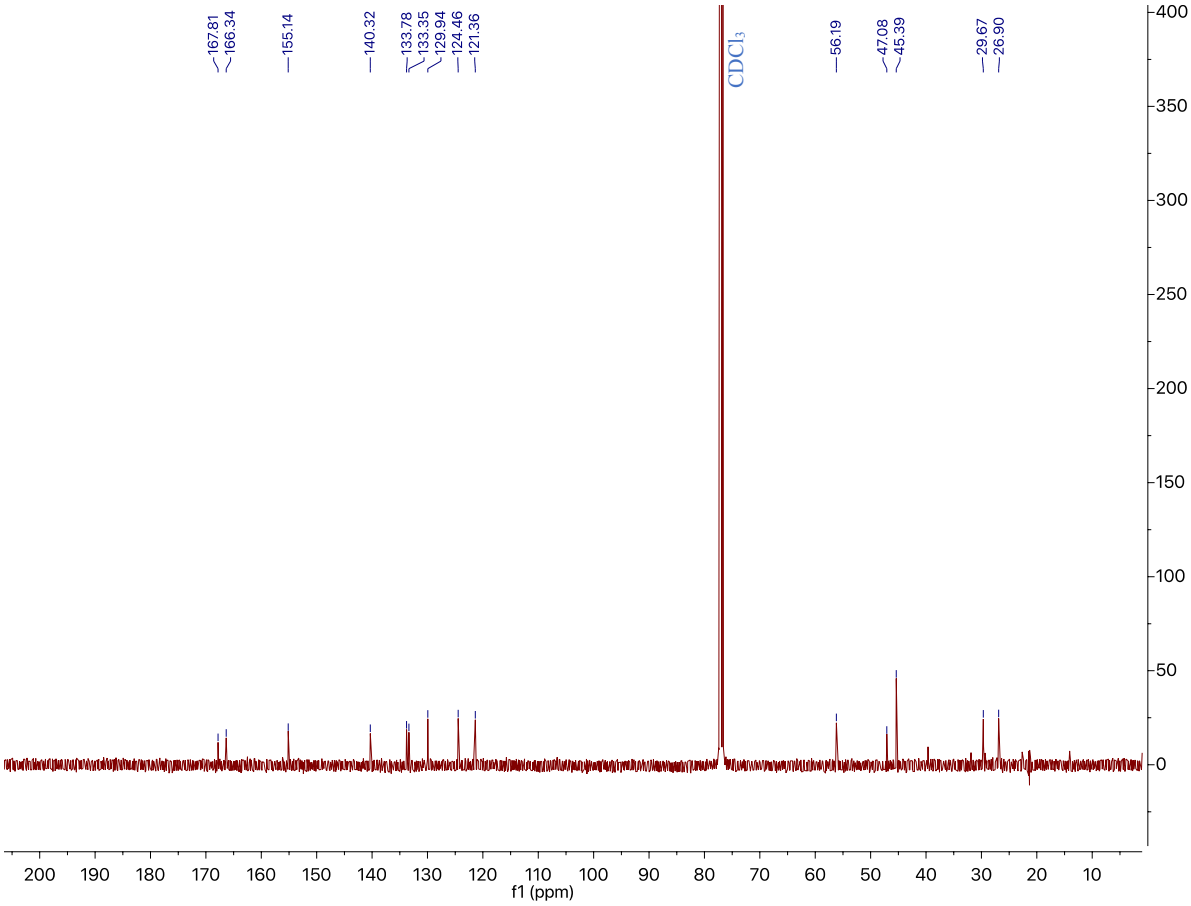
Compound **22**





Compound **23**


CDCl3
grease
f1 (ppm)


167.81
166.34
155.14
140.32
133.78
133.35
129.94
124.46
121.36
CDCl3
56.19
47.08
45.39
29.67
26.90
f1 (ppm)

Compound **24**