

SUPPLEMENTARY MATERIALS

New tricholidic acid triterpenoids from the mushroom *Tricholoma ustaloides* collected in an Italian beech wood

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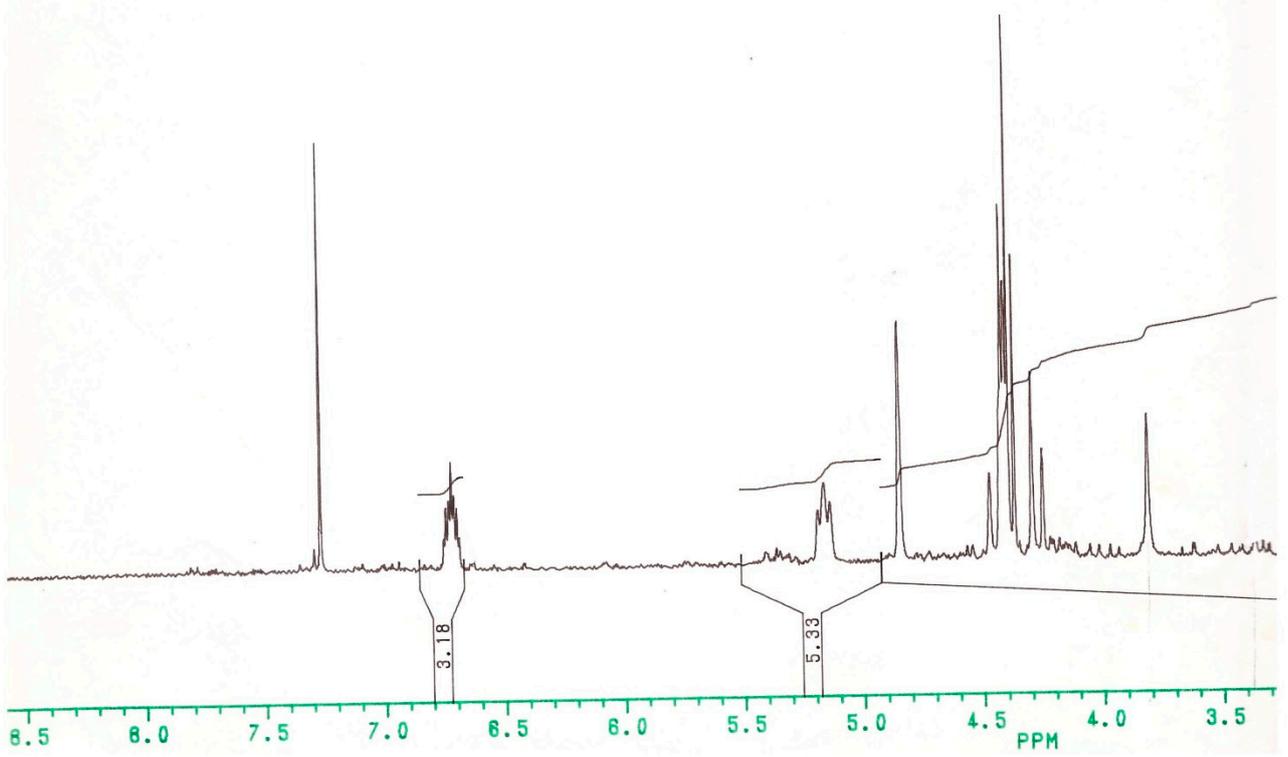
Abstract: The secondary metabolites produced by *Tricholoma ustaloides* Romagn., a mushroom species belonging to the large *Tricholoma* genus (Basidiomycota, Tricholomataceae), are unknown. Therefore, encouraged by the interesting results obtained in our previous chemical analyses of a few *Tricholoma* species collected in Italian woods, we were aimed to investigate the secondary metabolites of *Tricholoma ustaloides*. The chemical analysis involved the isolation and characterization of secondary metabolites through an extensive chromatographic study. The structures of isolated metabolites, including the absolute configuration, were established based on a detailed analysis of MS, NMR spectroscopic, optical rotation and circular dichroism data, and on comparison with those of related compounds reported in the literature. Two novel lanostane triterpenoids, named tricholidic acids B and C, together with triglycerides, a mixture of free fatty acids, five unidentified metabolites, and known rare saponaceolides F and J, tricholidic acid, and tricholomenyn C, were isolated from an EtOAc extract of fruiting bodies of *Tricholoma ustaloides*, that were collected in an Italian beech wood. This is the second example of isolation of tricholidic acid derivatives from a natural source. Saponaceolides F and J exhibited high cytotoxicity (IC₅₀ values ≤ 10 μM) against a panel of five human cancer cell lines. The toxicity against myeloid leukemia (HL-60), lung cancer (A-549), hepatocellular cancer (HepG2), renal cancer (Caki-1), and breast cancer (MCF-7) cells was higher than that shown by the very well-known cytotoxic drug cisplatin.

Keywords: *Tricholoma ustaloides*; Tricholomataceae (Basidiomycota); lanostane triterpenoids; tricholidic acid derivatives; saponaceolides; tricholomenyn; cytotoxicity.

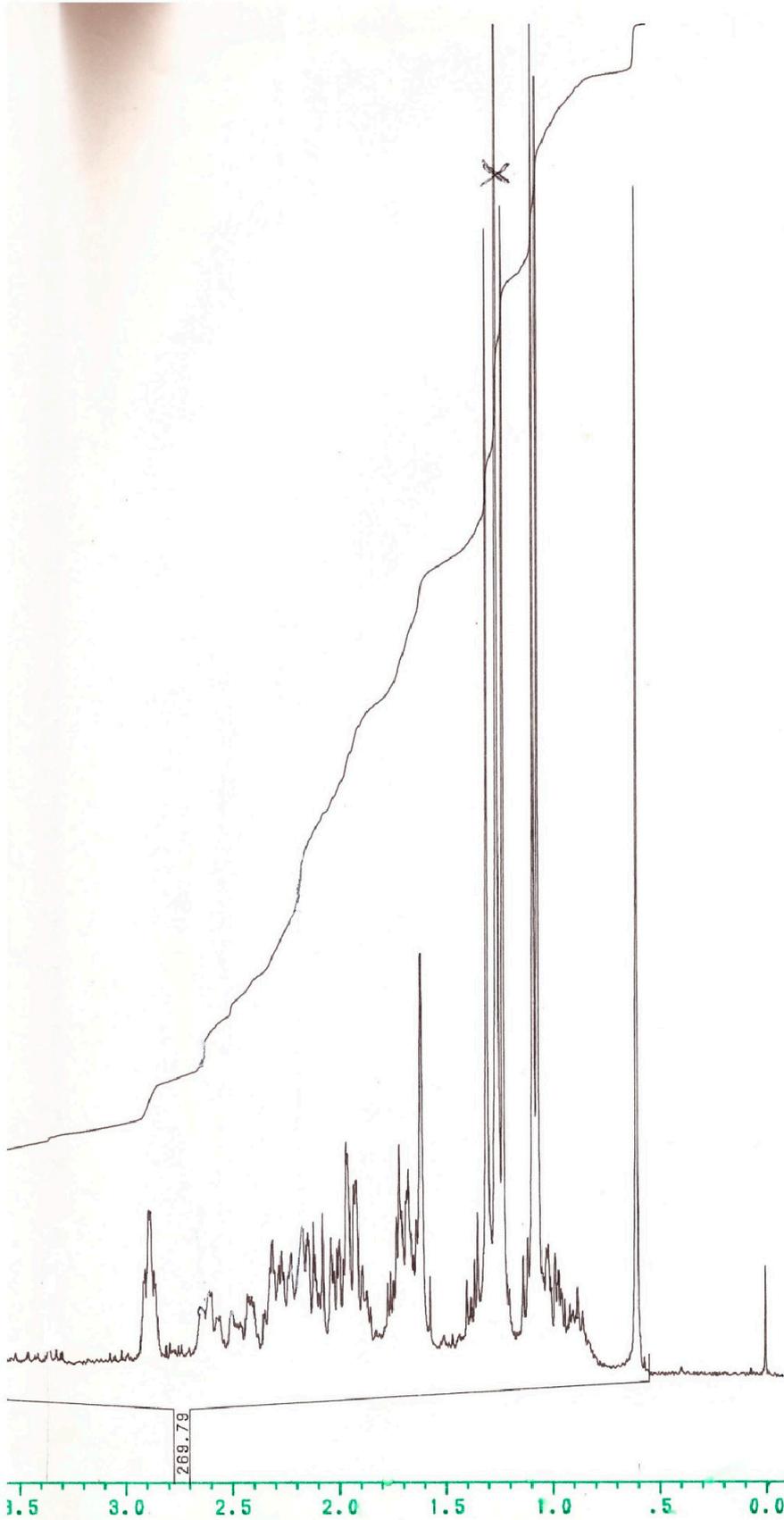
¹ H NMR spectrum of saponaceolide J (1)	S1A + S1B
¹³ C NMR spectrum of saponaceolide J (1)	S2A + S2B
¹ H NMR spectrum of saponaceolide F (2)	S3
¹³ C NMR spectrum of saponaceolide F (2)	S4
¹ H NMR spectrum of tricholidic acid (5)	S5
COSY spectrum of tricholidic acid (5)	S6
¹³ C NMR spectrum of tricholidic acid (5)	S7
DEPT (135°) of tricholidic acid (5)	S8A + S8B
¹ H NMR spectrum of tricholidic acid B (6)	S9

COSY spectrum of tricholodic acid B (6)	S10
¹ H NMR spectrum of tricholidic acid B methyl ester	S11A + S11B
¹³ C NMR spectrum of tricholidic acid B (6)	S12A + S12B
DEPT (135°) of tricholoidic acid B (6)	S13
HSQC spectrum of tricholidic acid B (6)	S14A – S14D
¹ H NMR spectrum of tricholidic acid C (7)	S15
COSY spectrum of tricholidic acid C (7)	S16A + S16B
¹³ C NMR spectrum of tricholidic acid C (7)	S17
DEPT (135°) of tricholoidic acid C (7)	S18A – S18C
HSQC spectrum of tricholidic acid C (7)	S19A – S19C

CDCL3

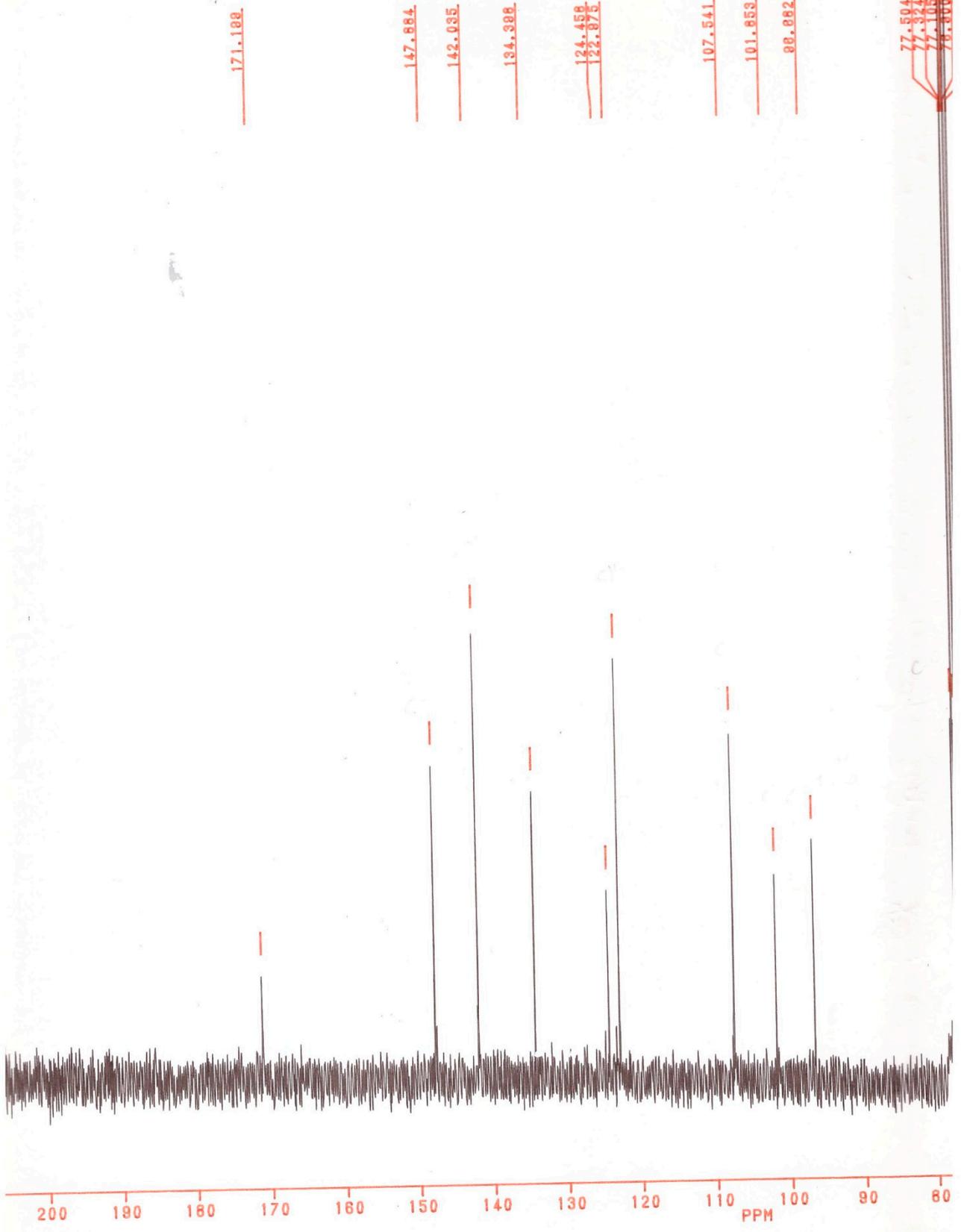


S1A

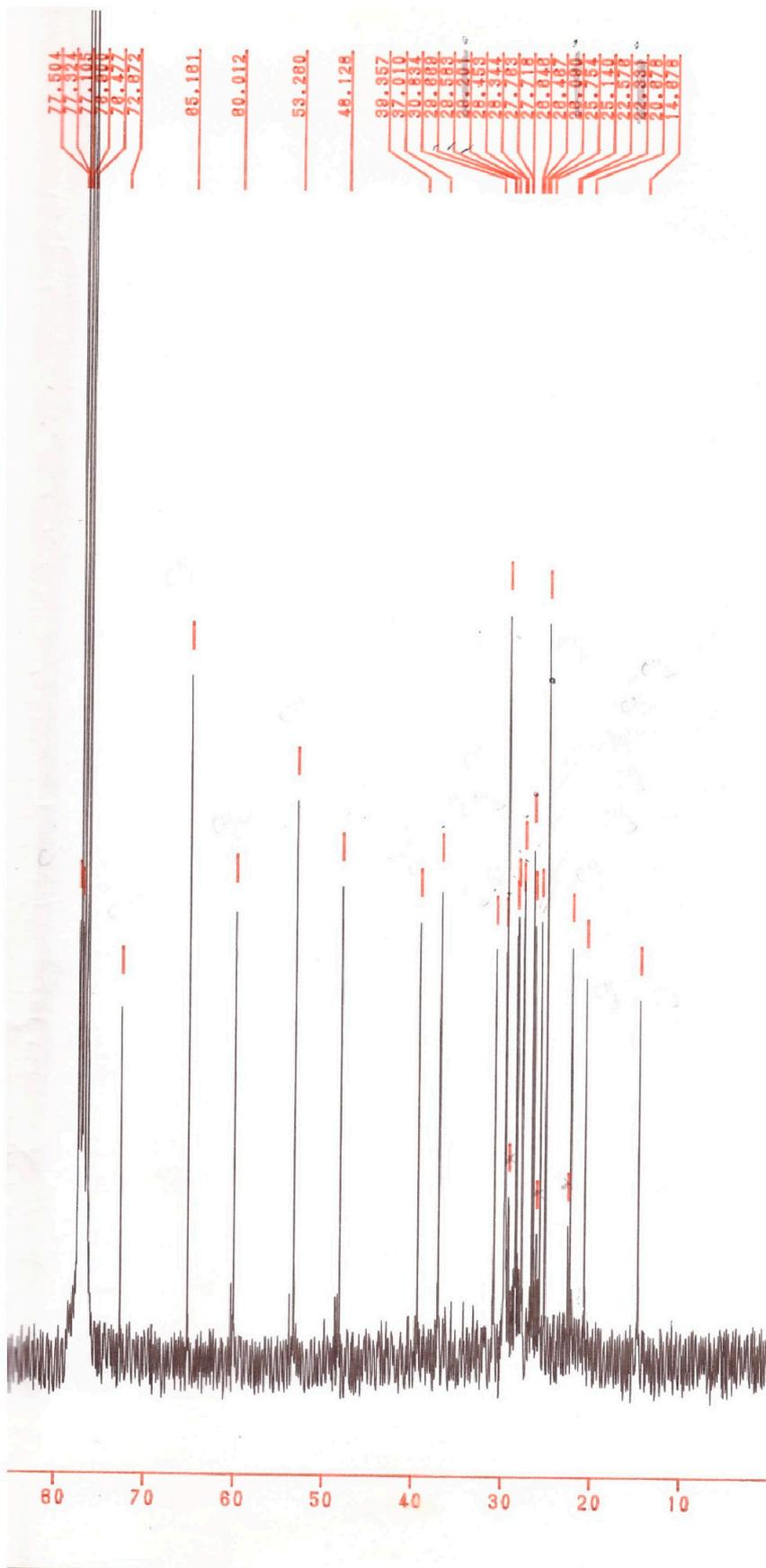


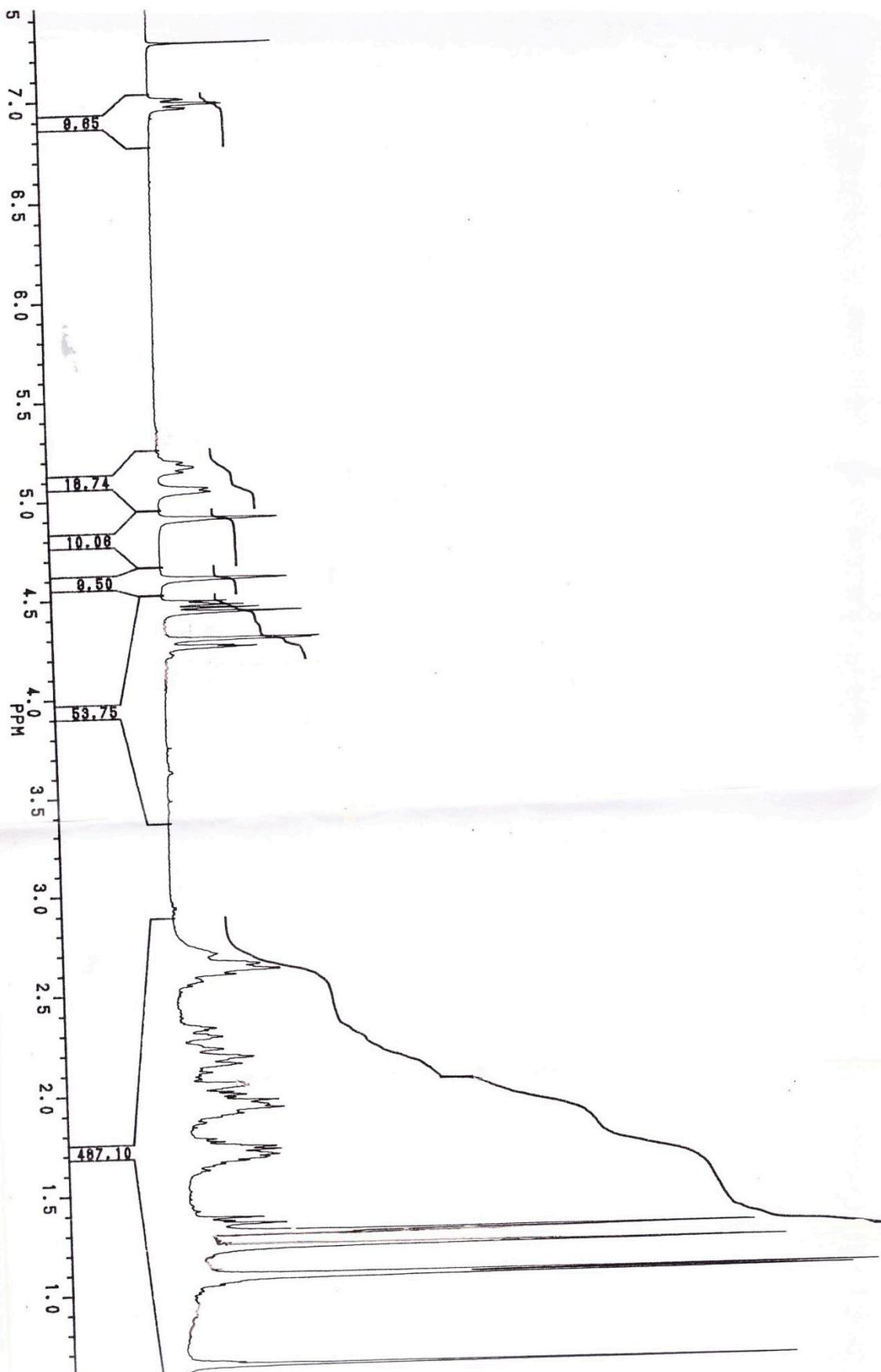
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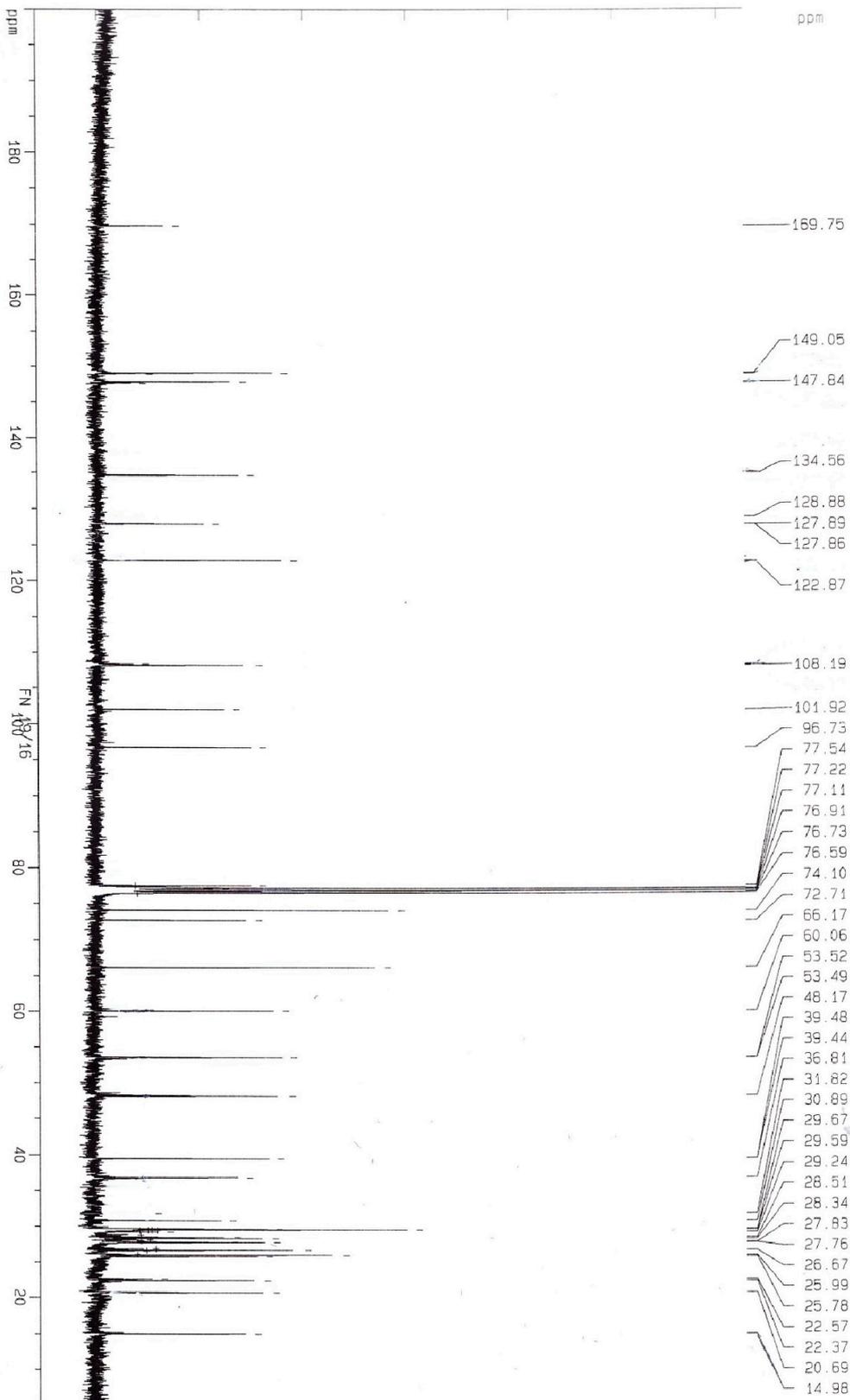
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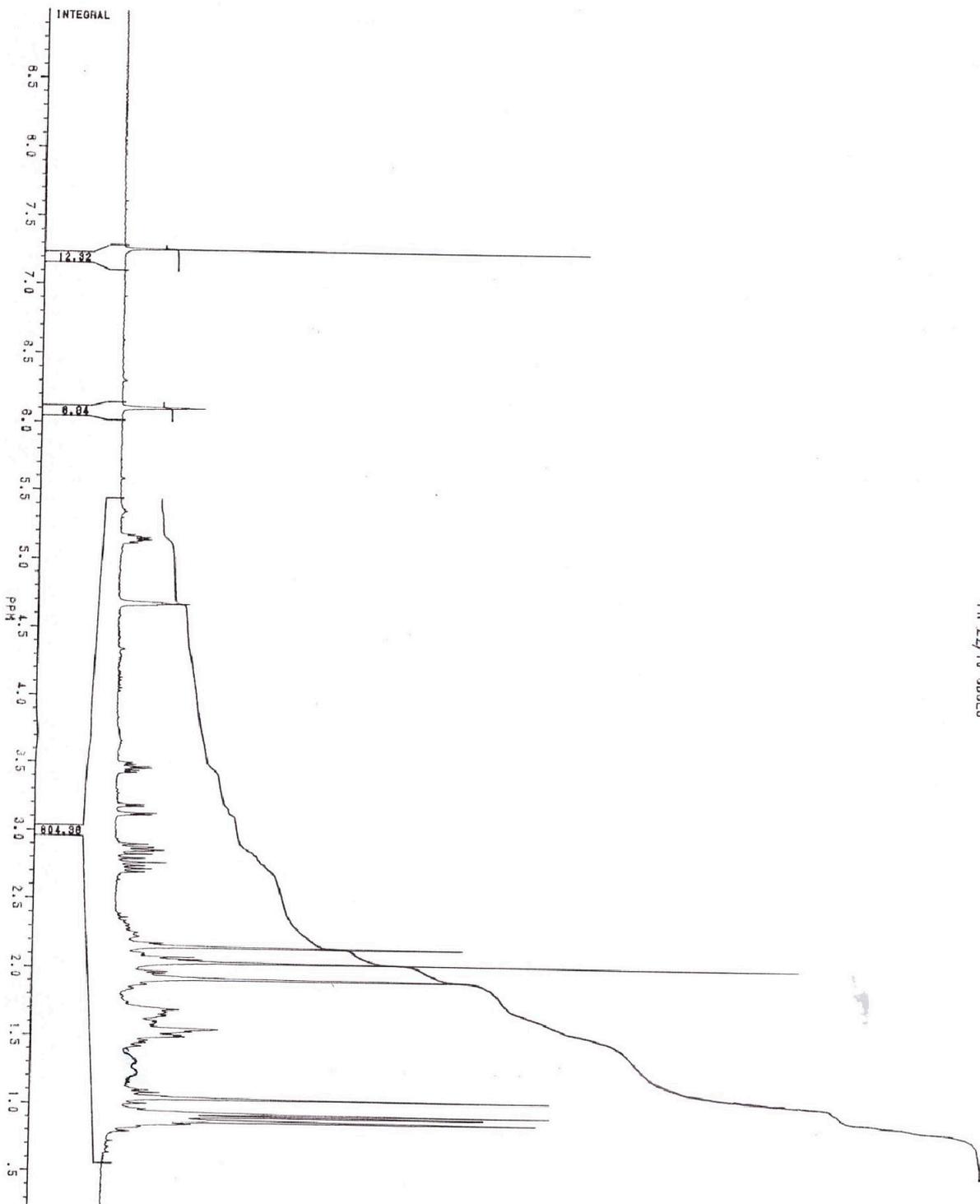
S2A



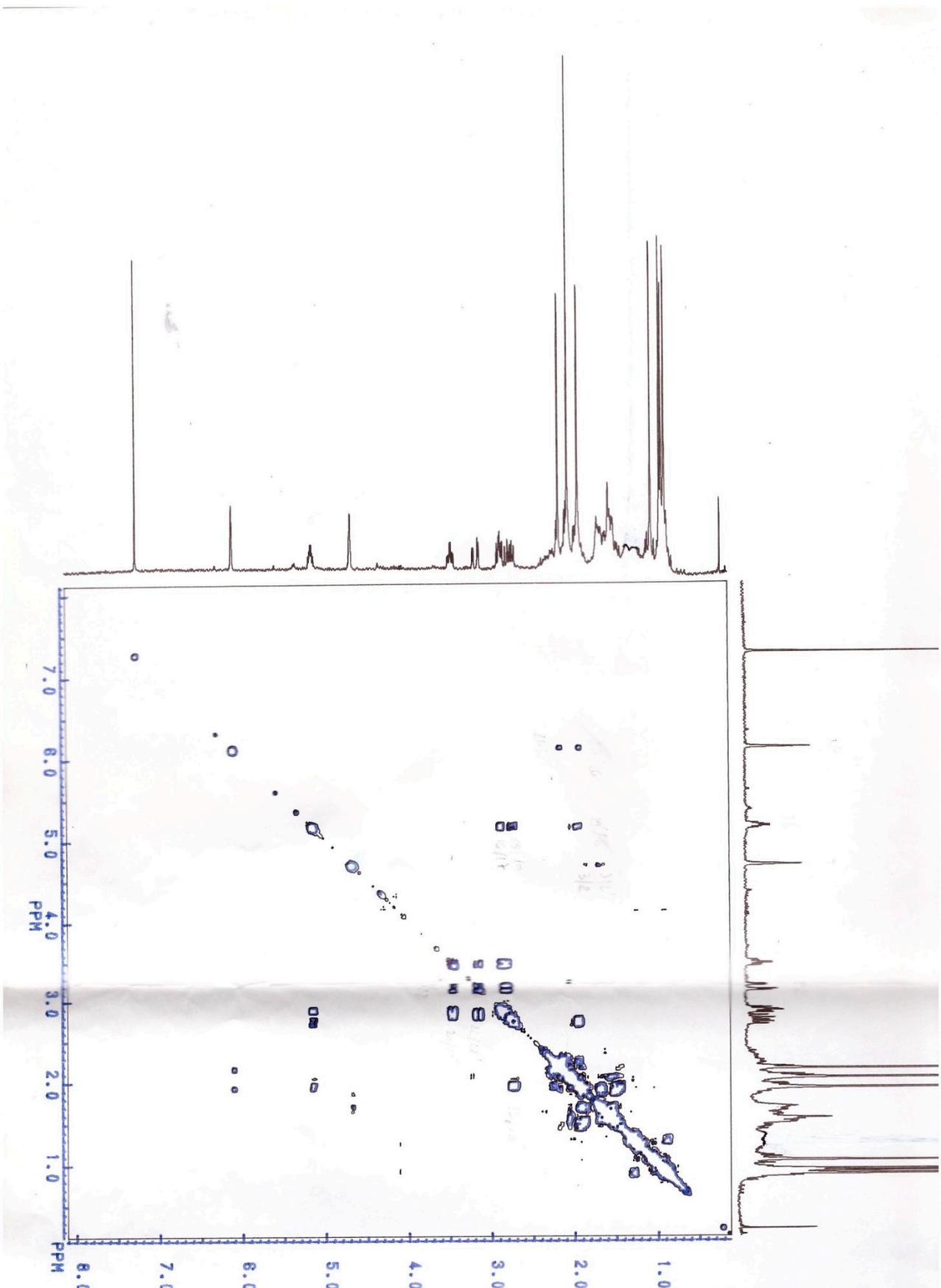


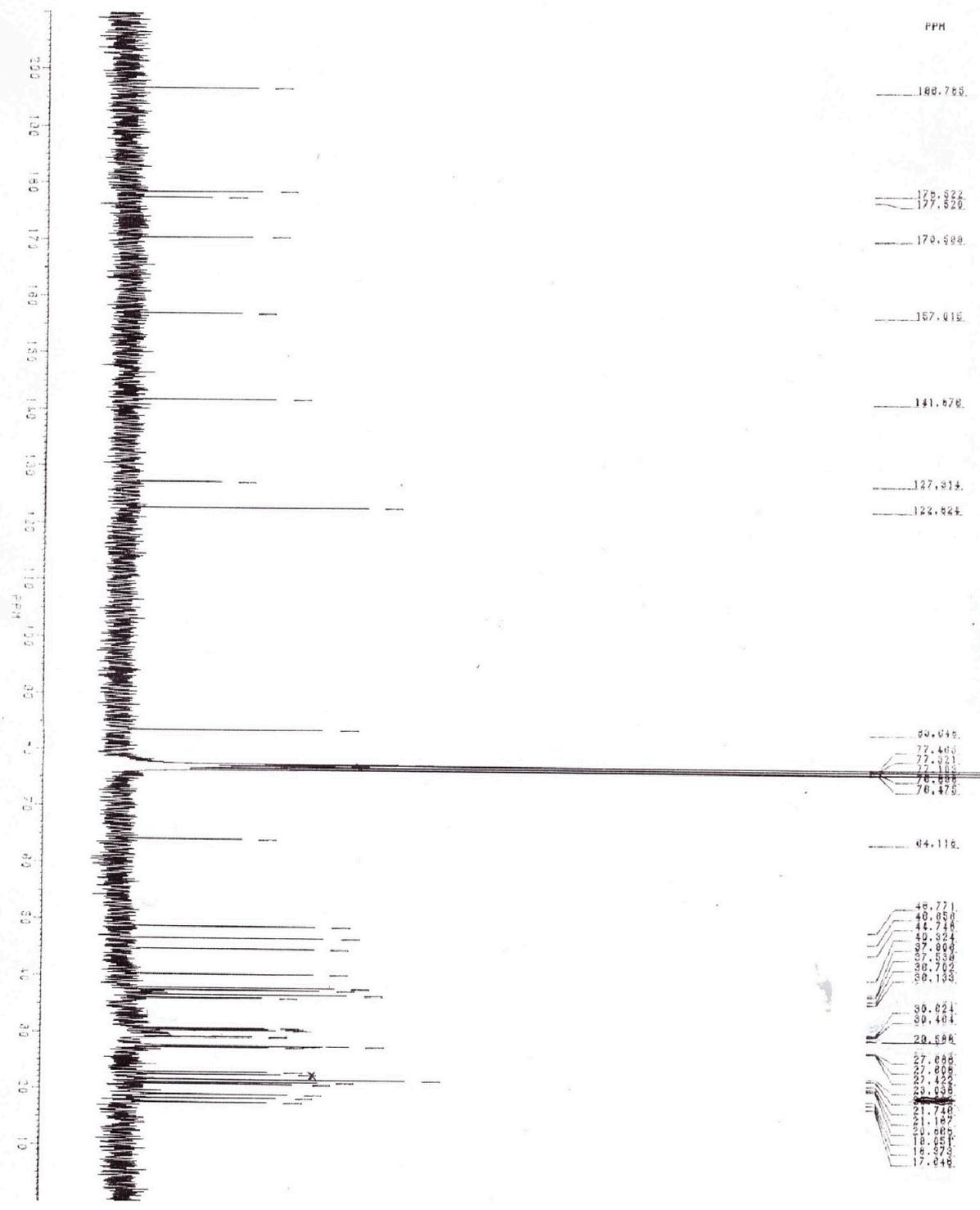


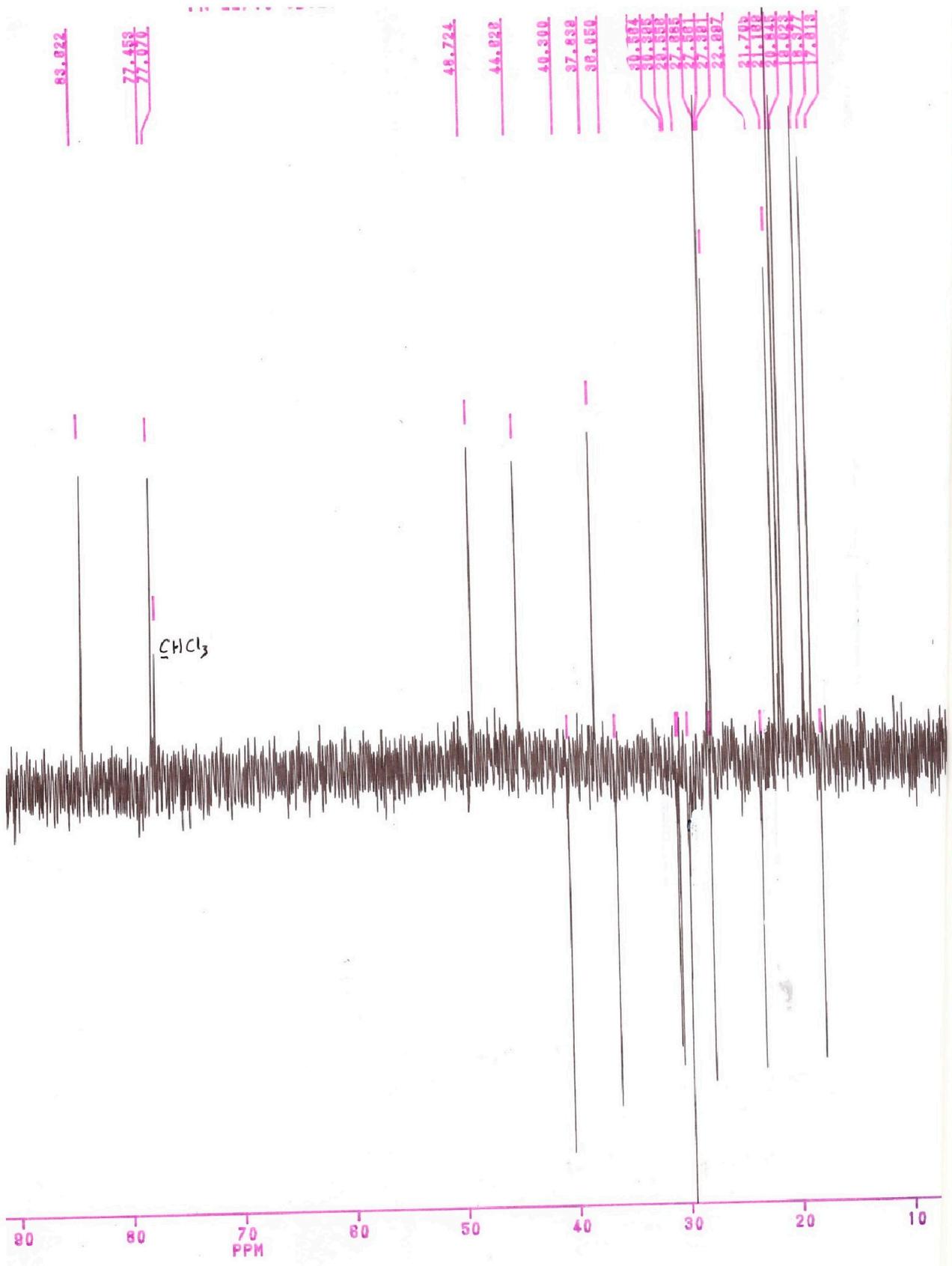
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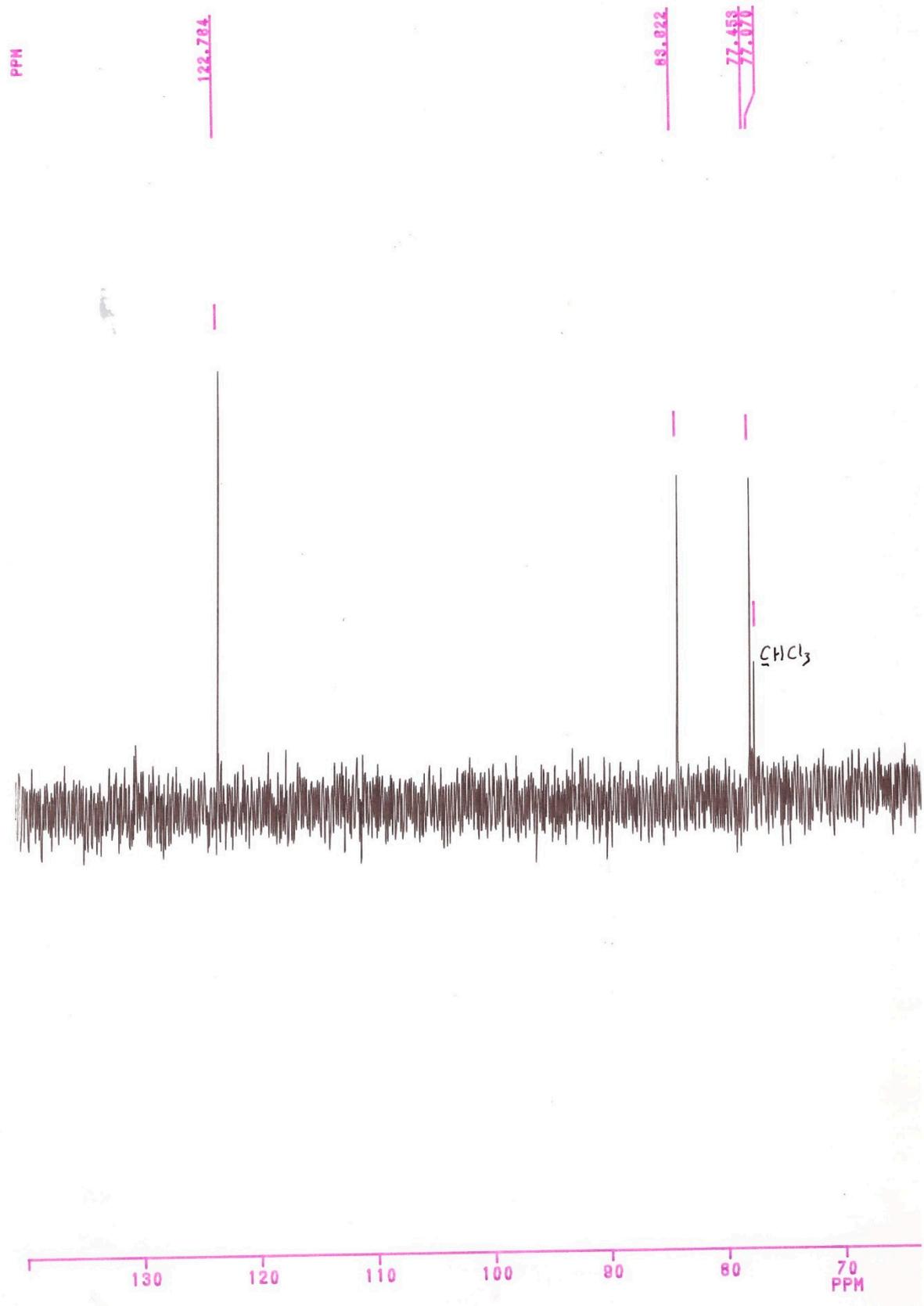
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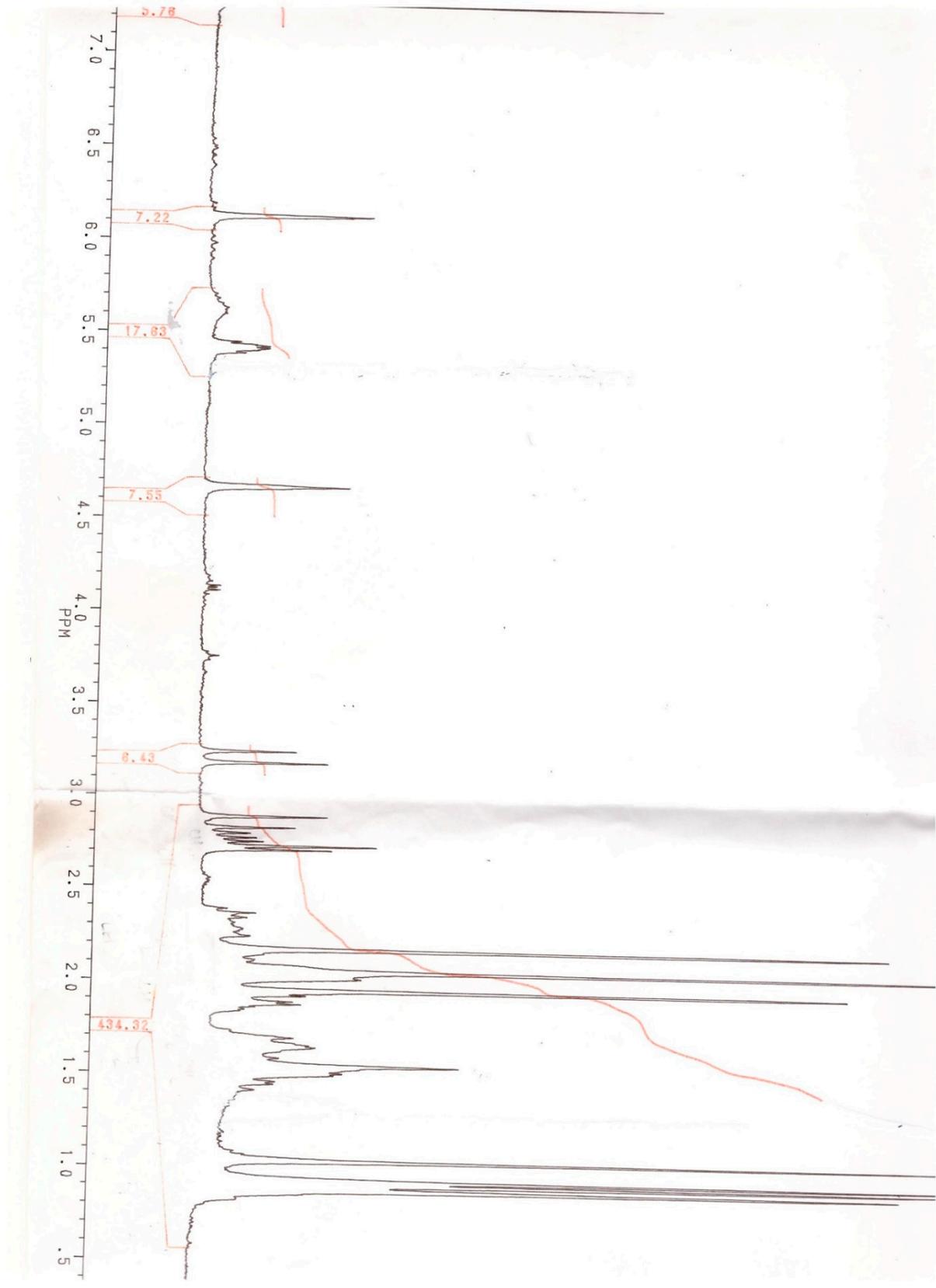


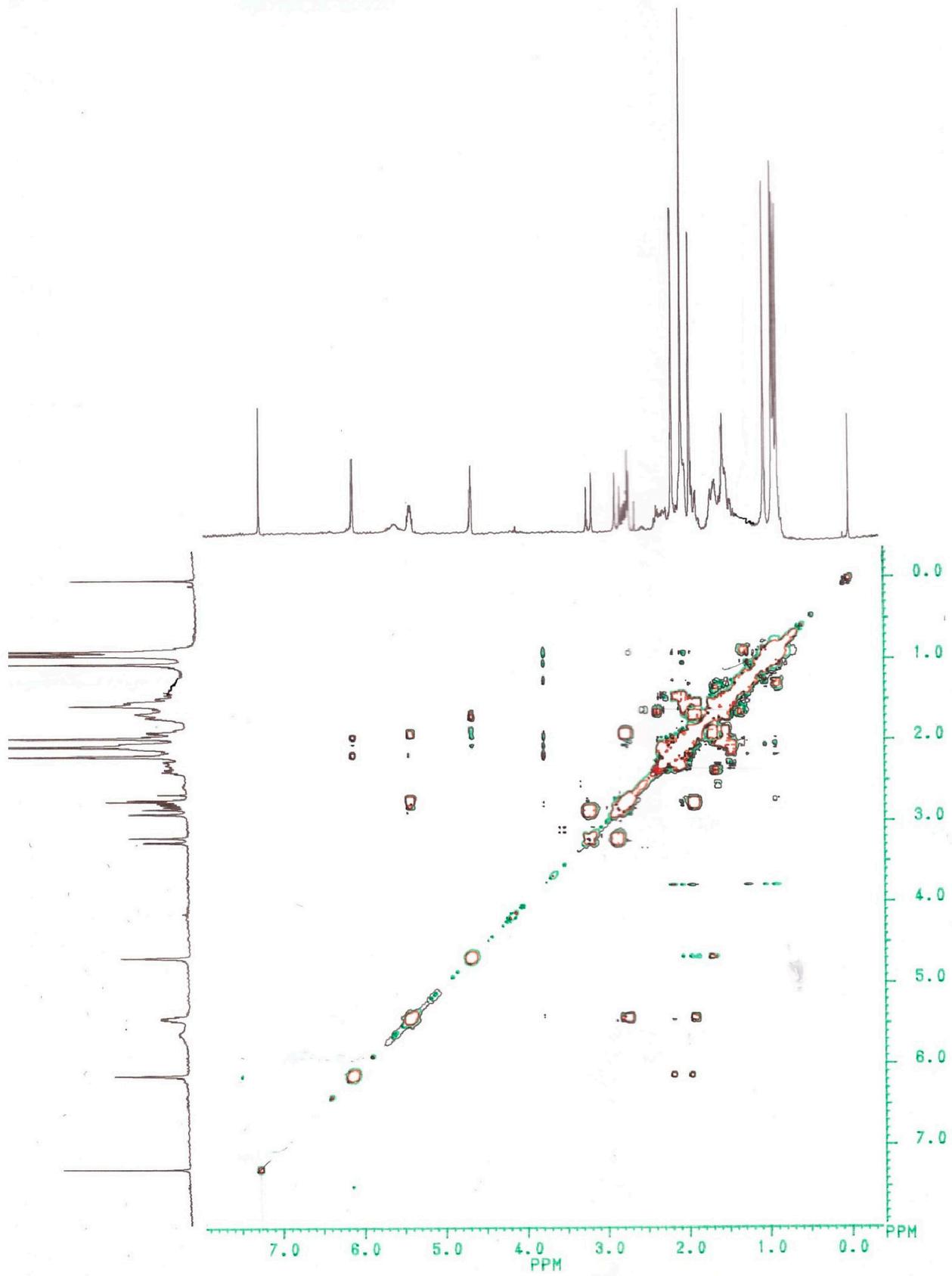


S8A

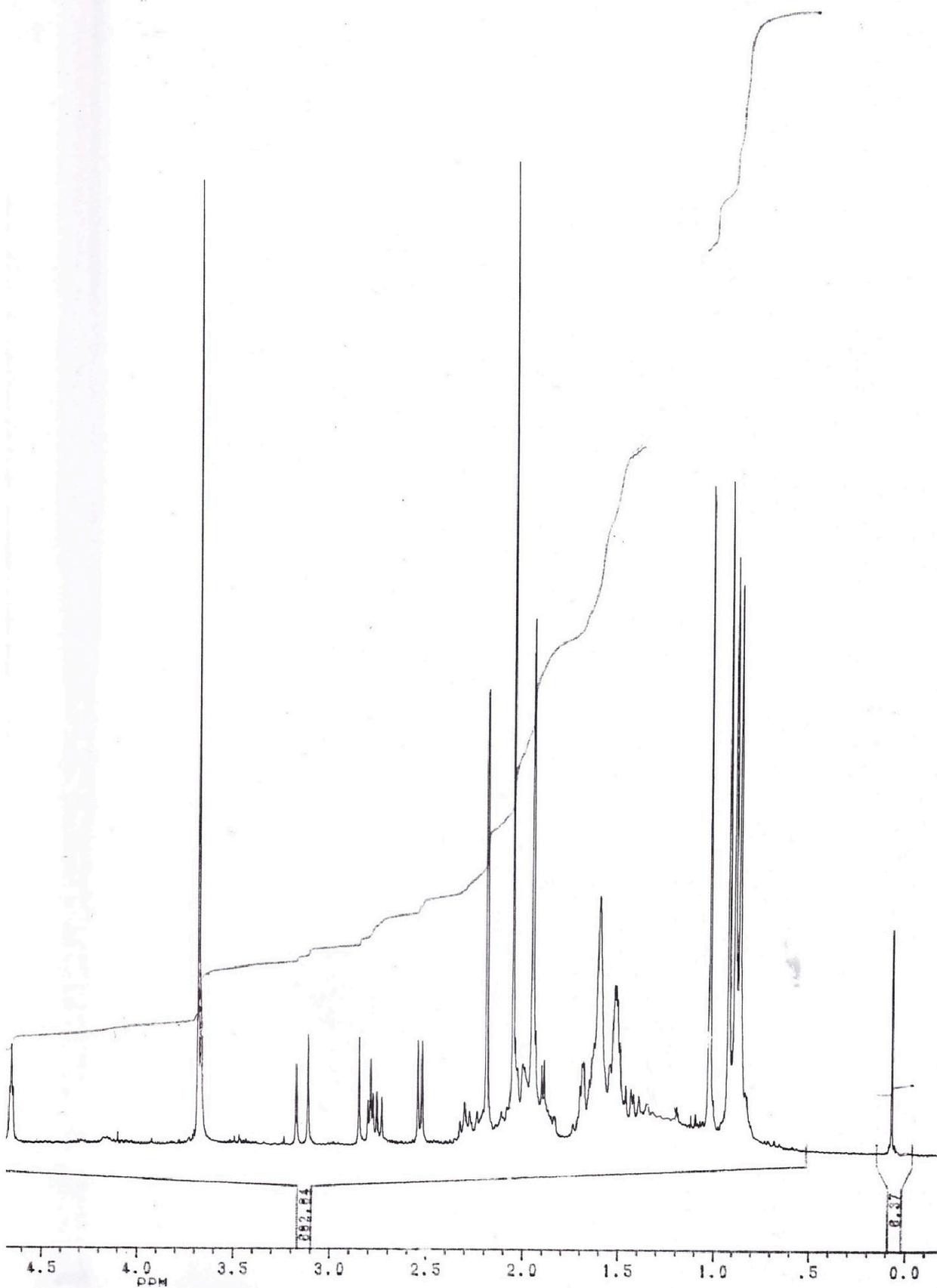


S8B

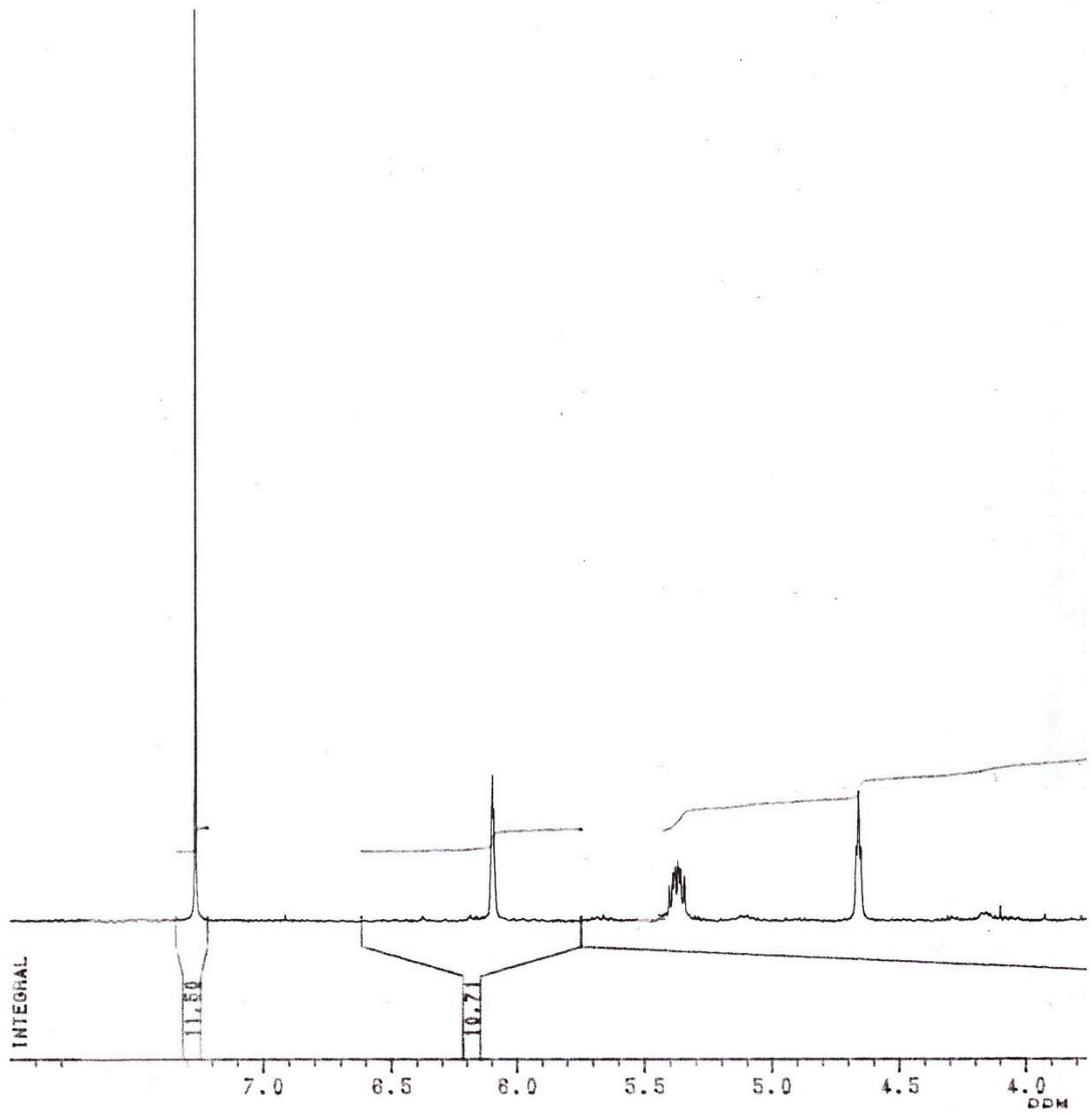




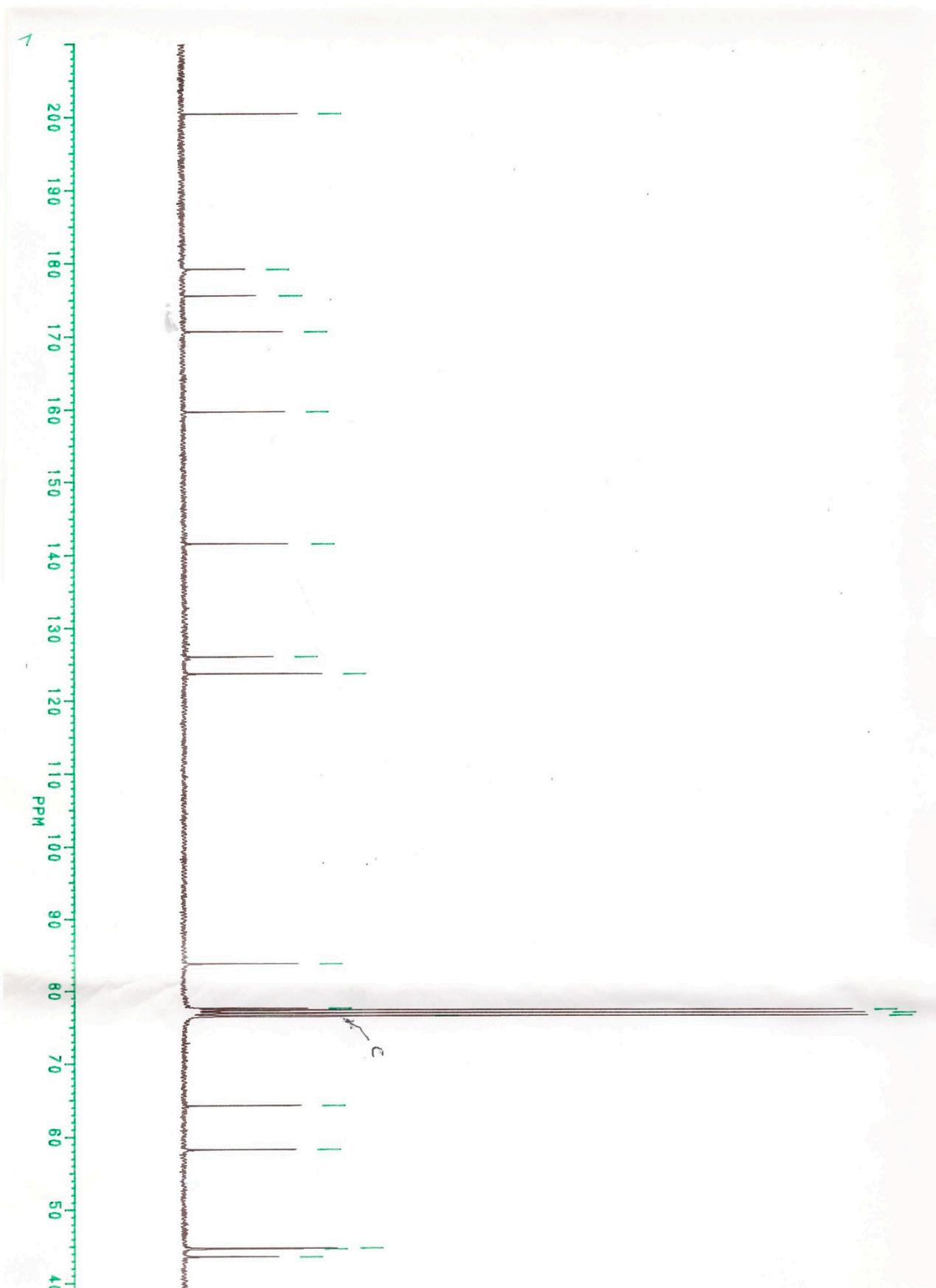
S10



S11A

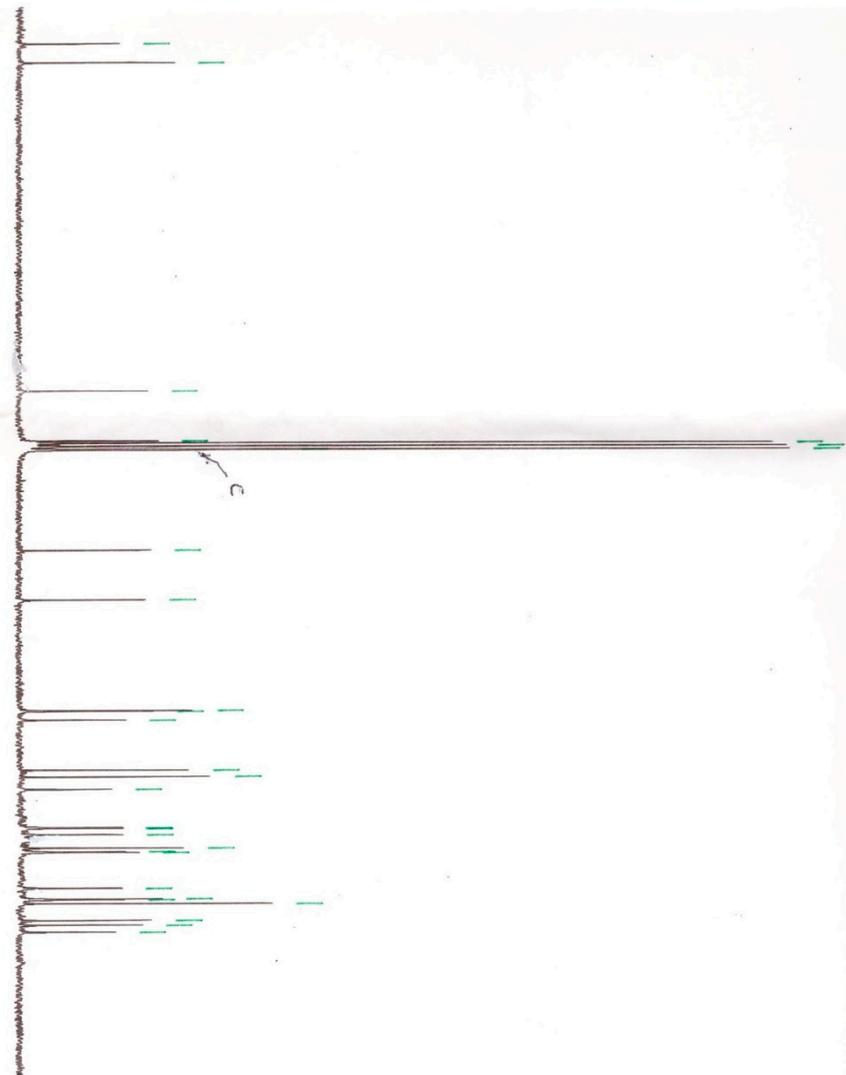


S11B

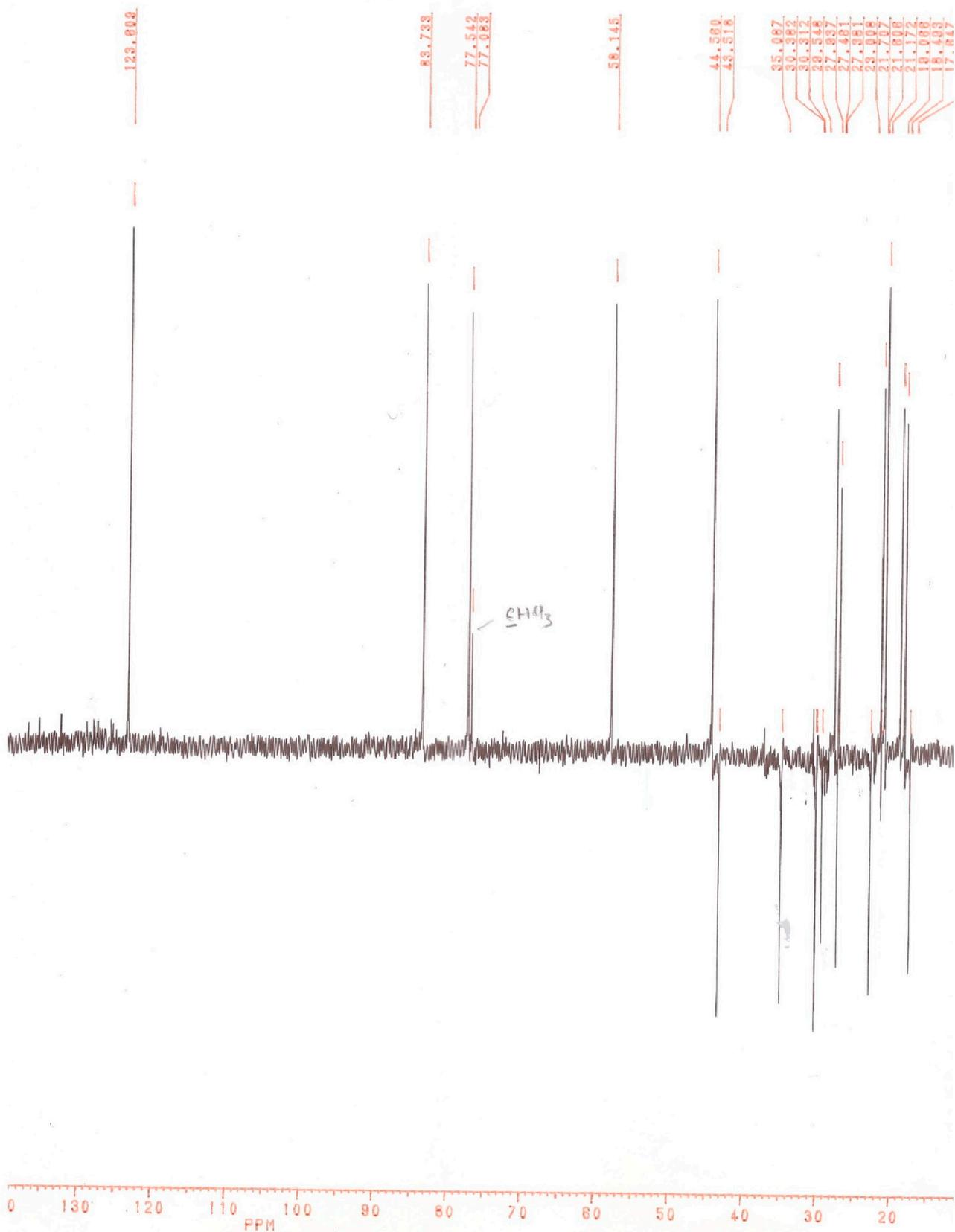


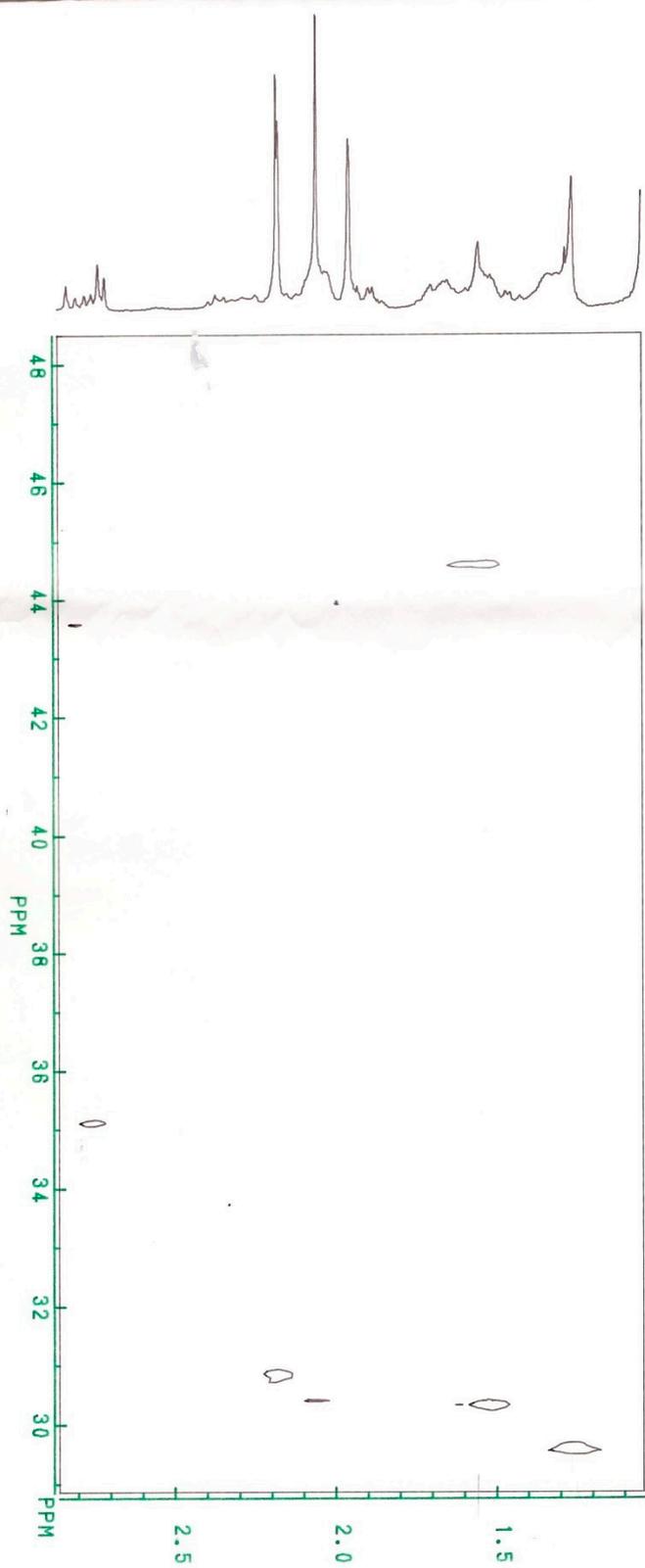
S12A

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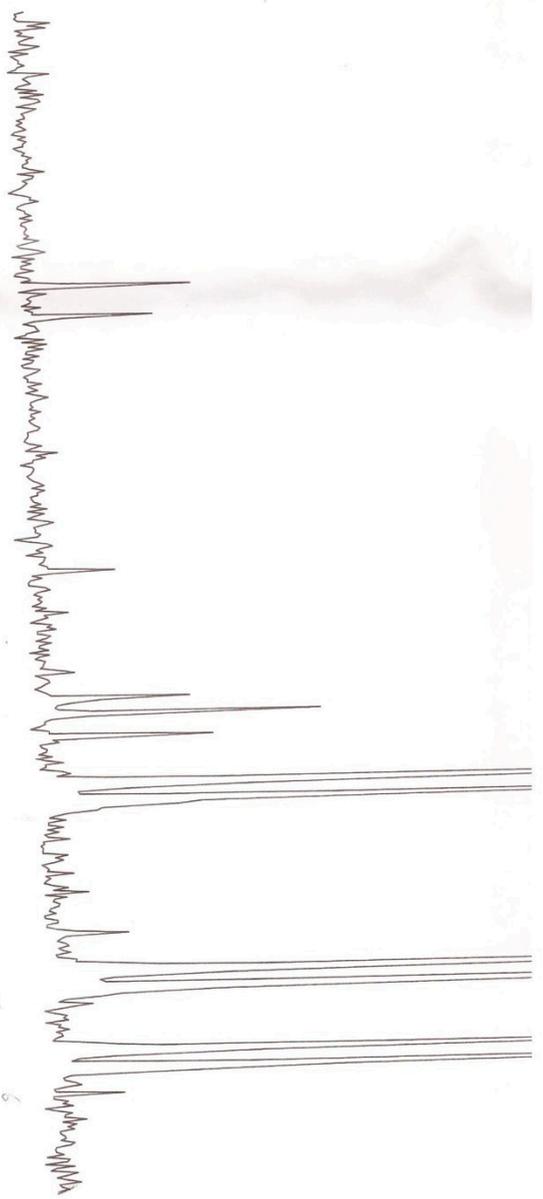
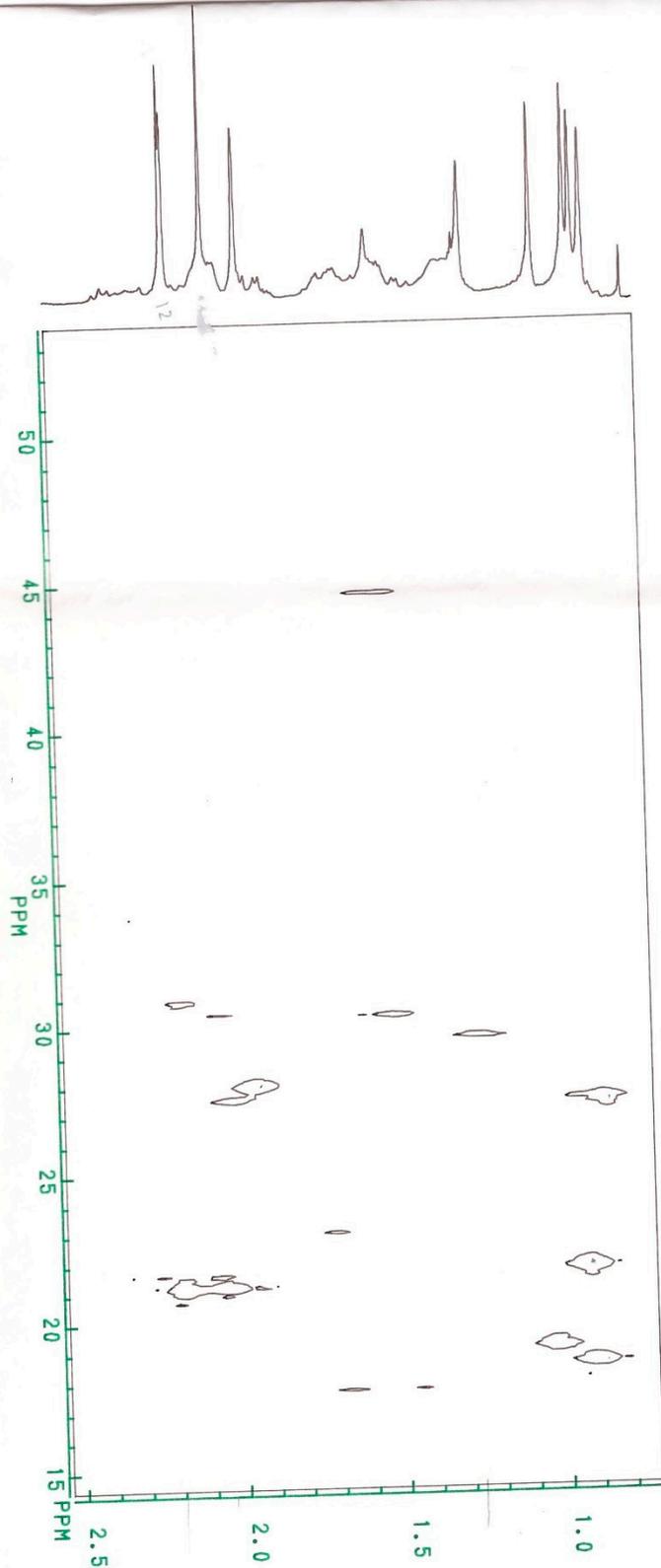


S12B

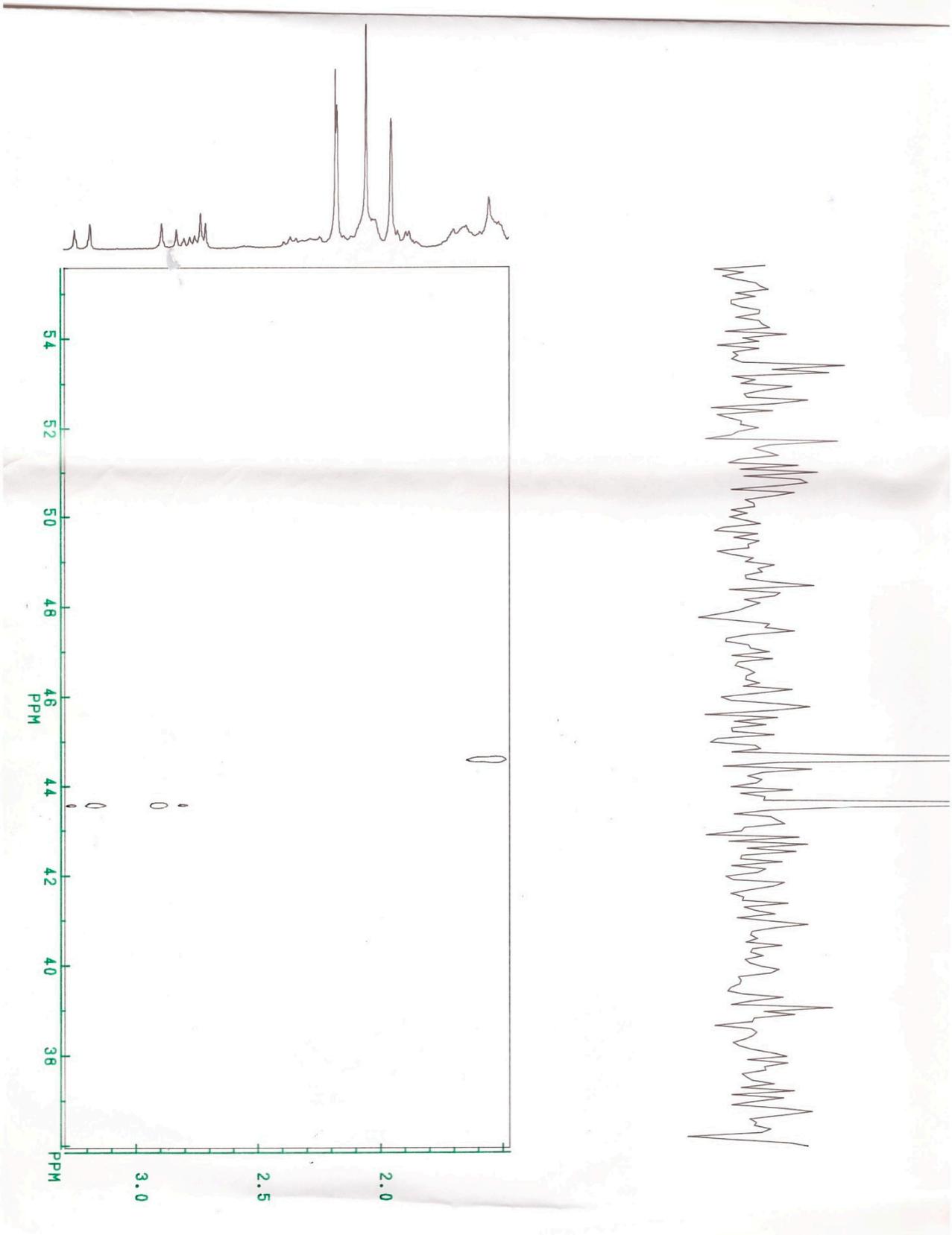




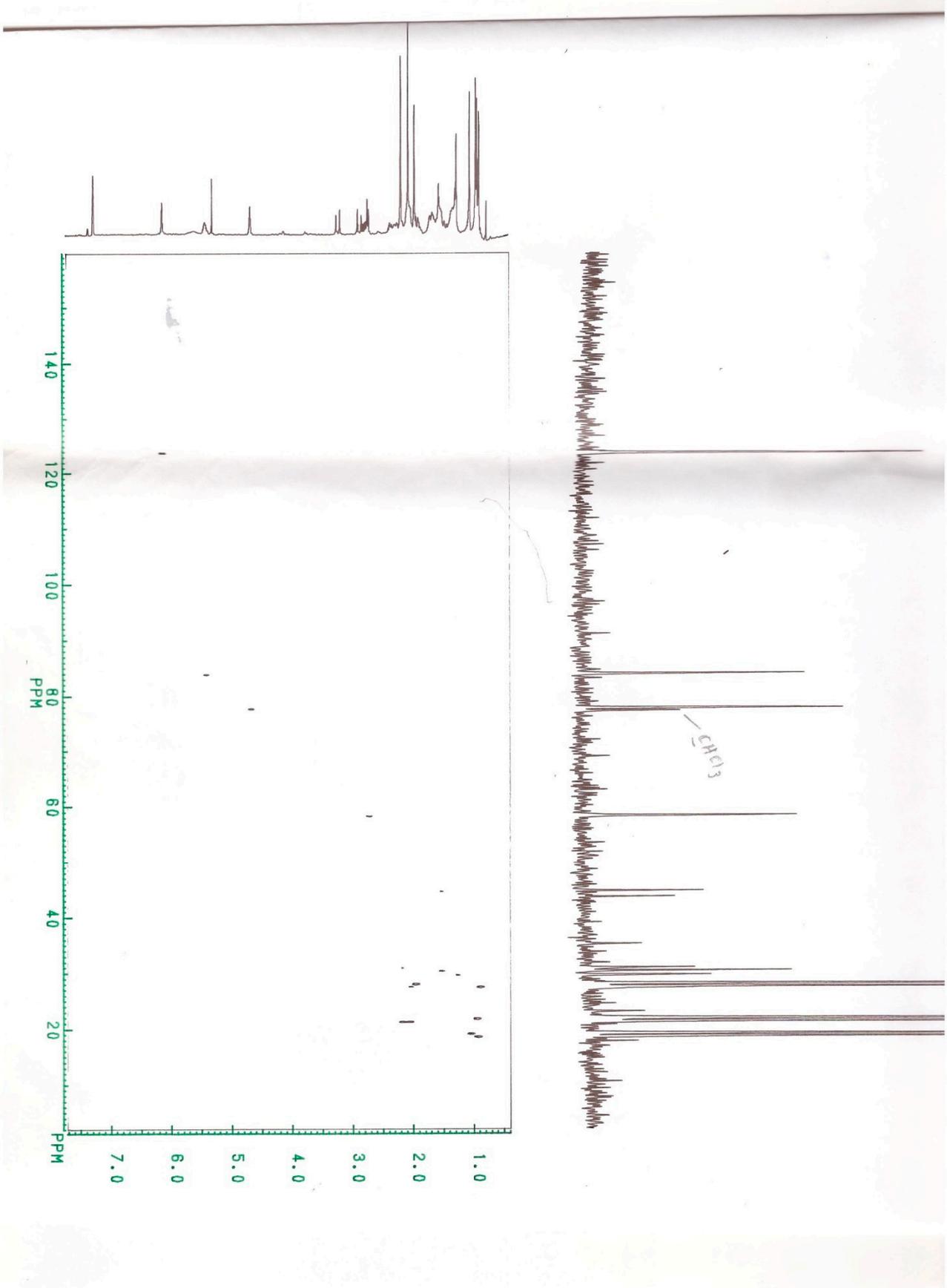
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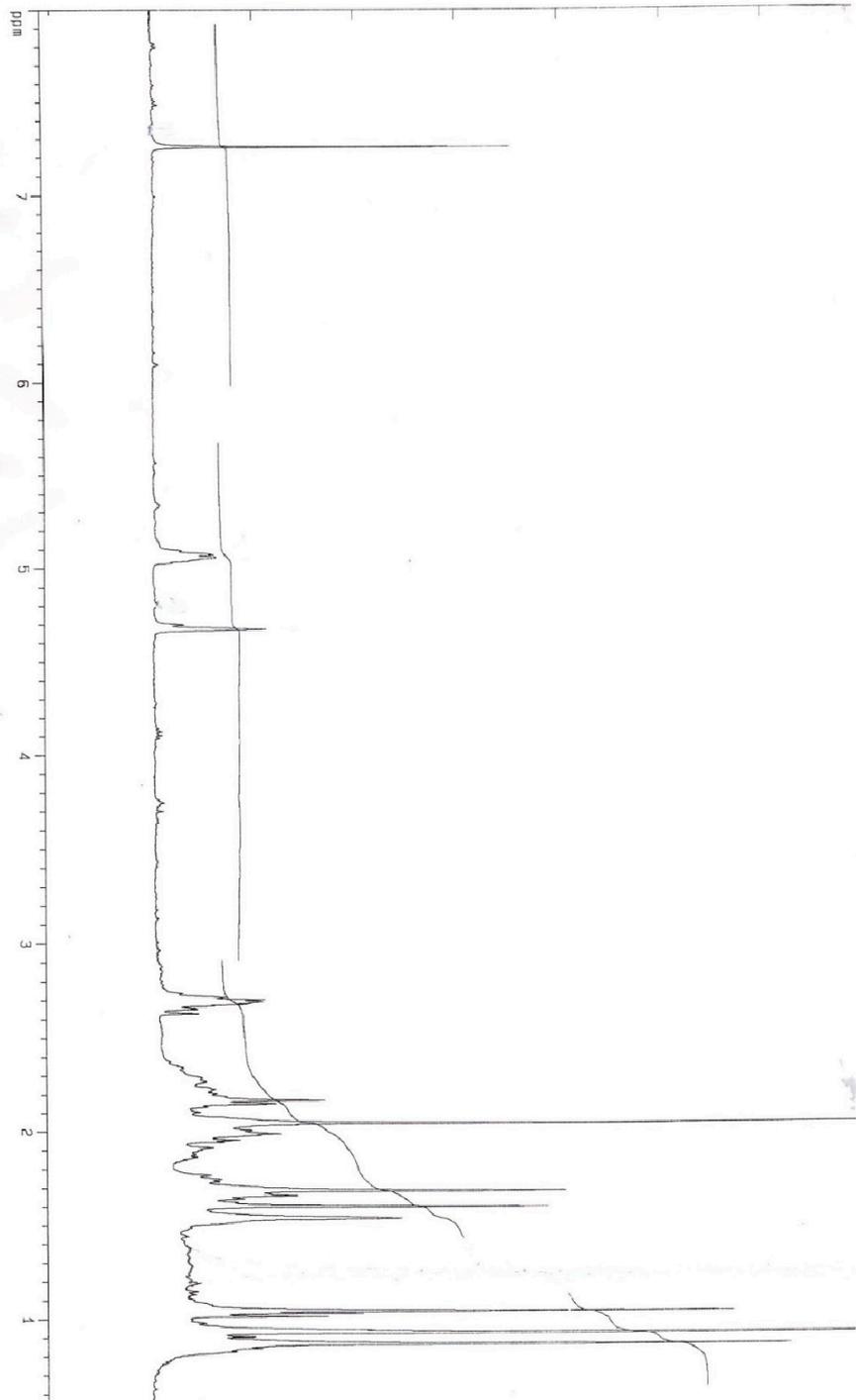
S14B



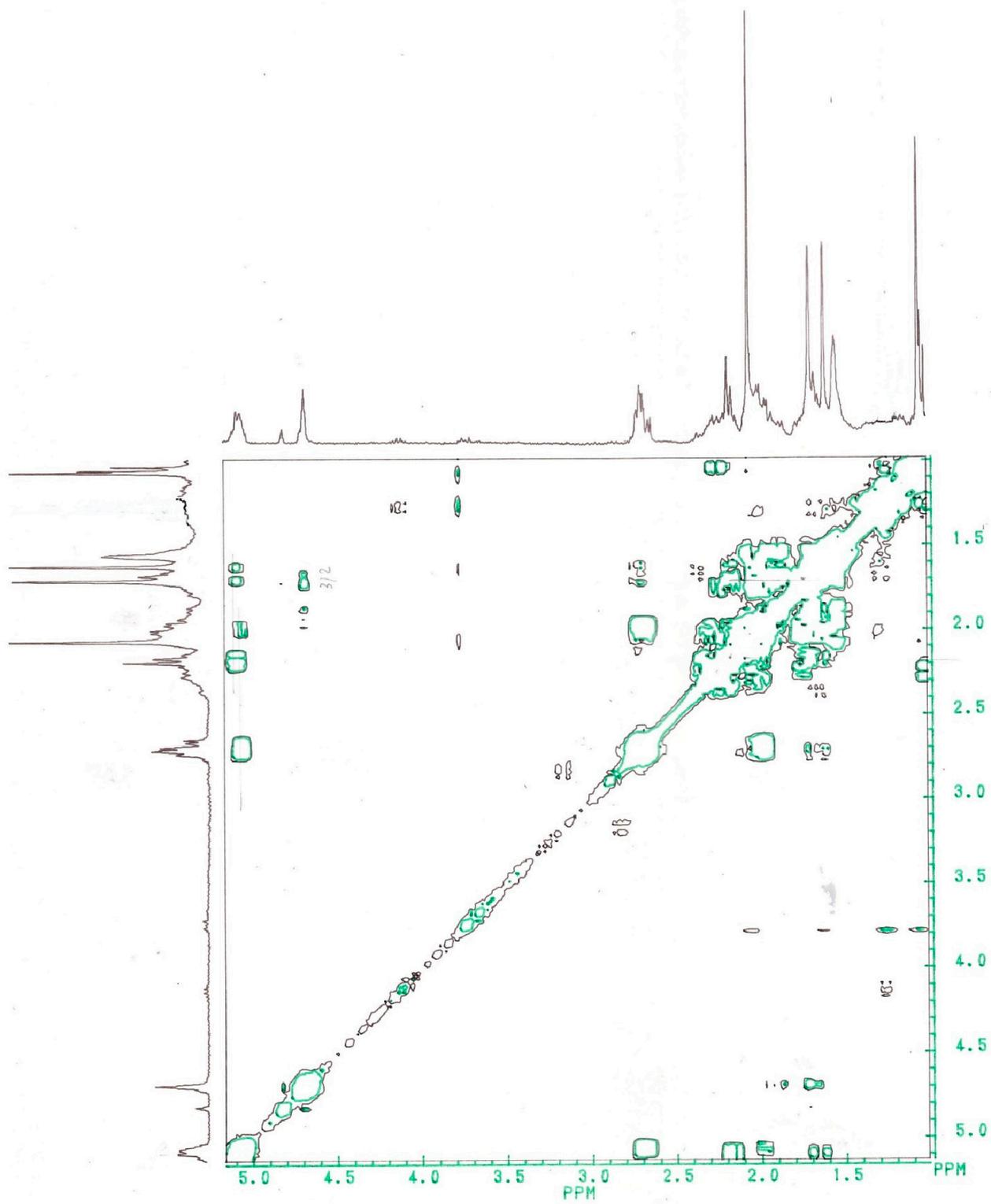
S14C



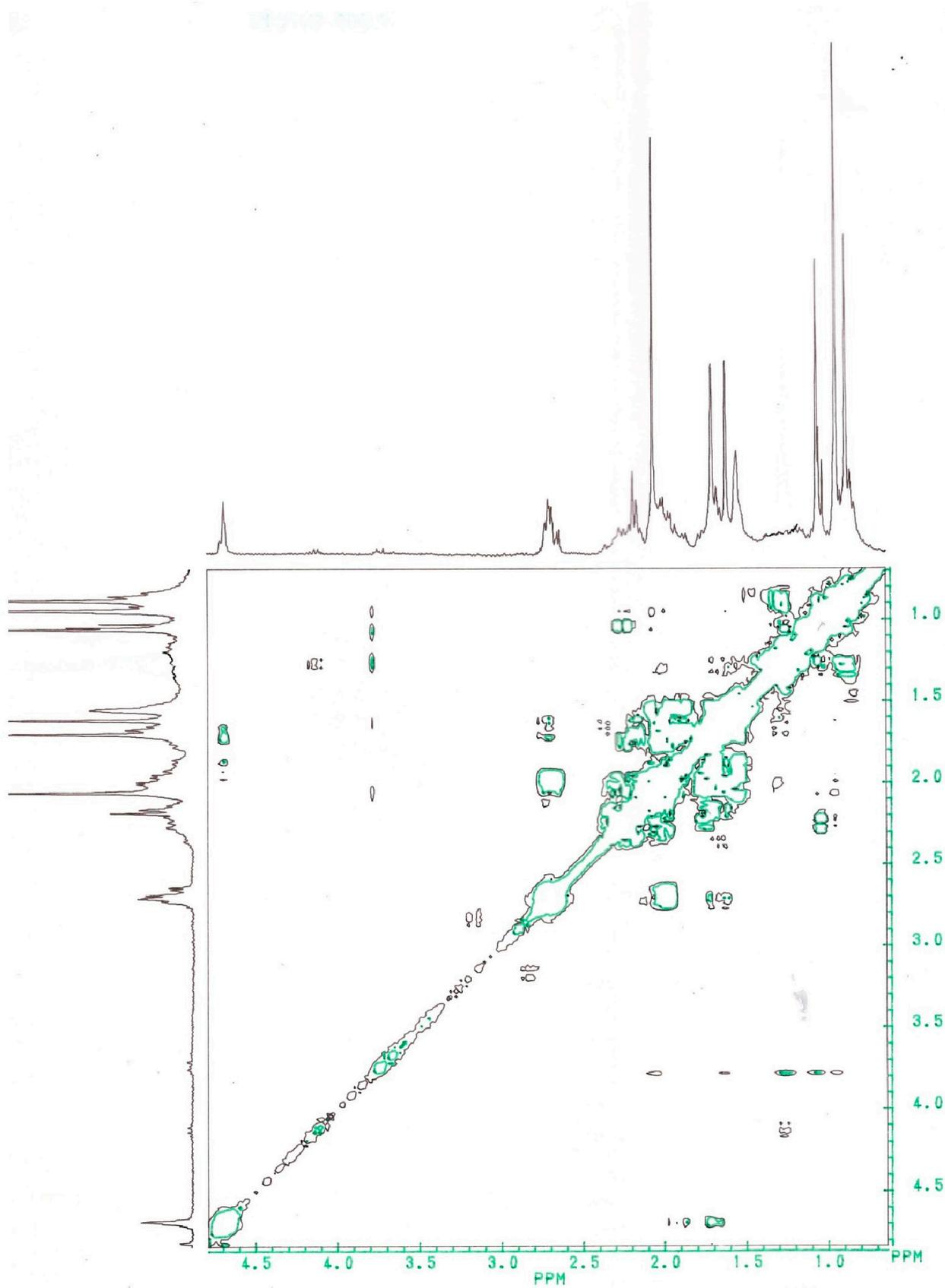
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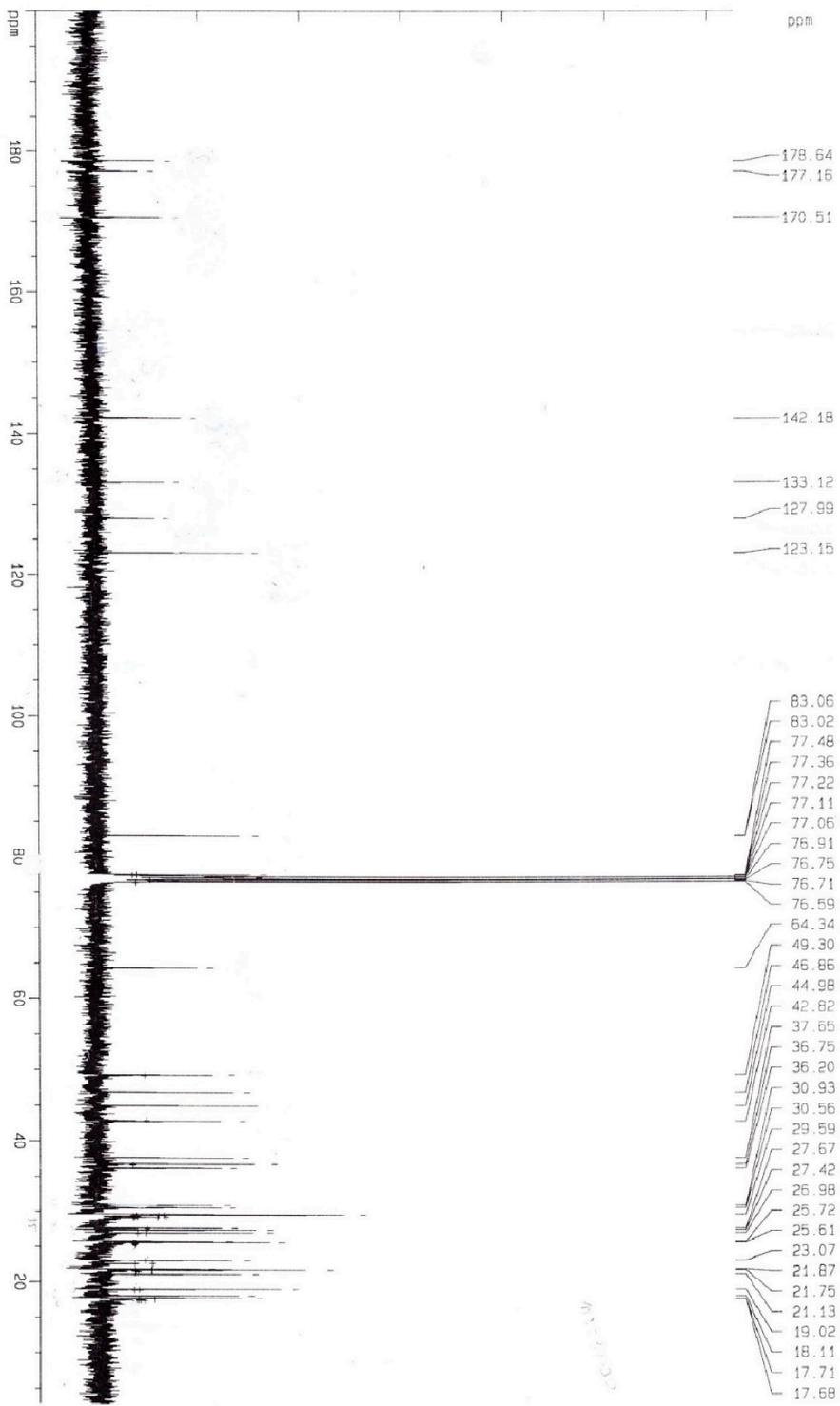
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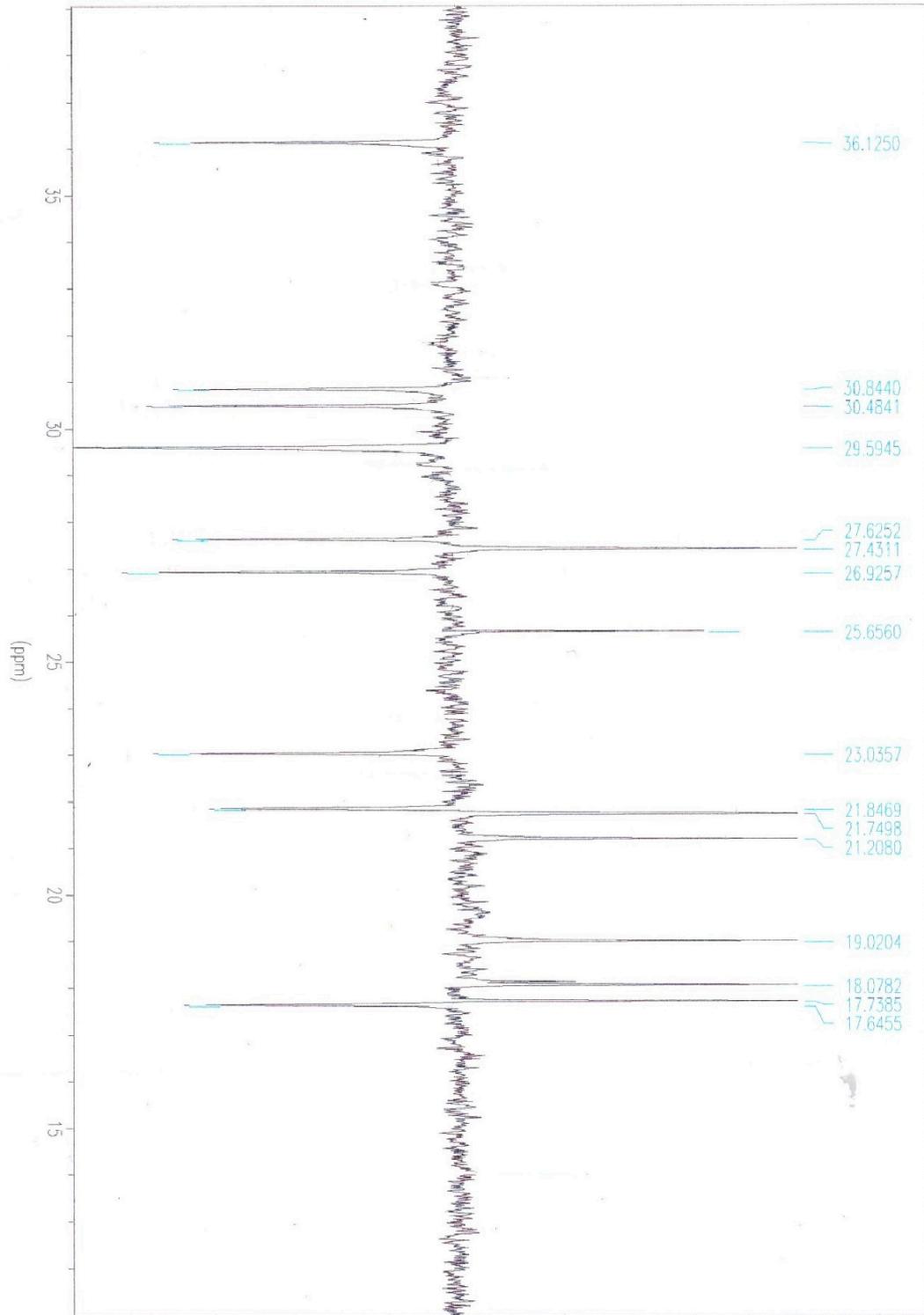


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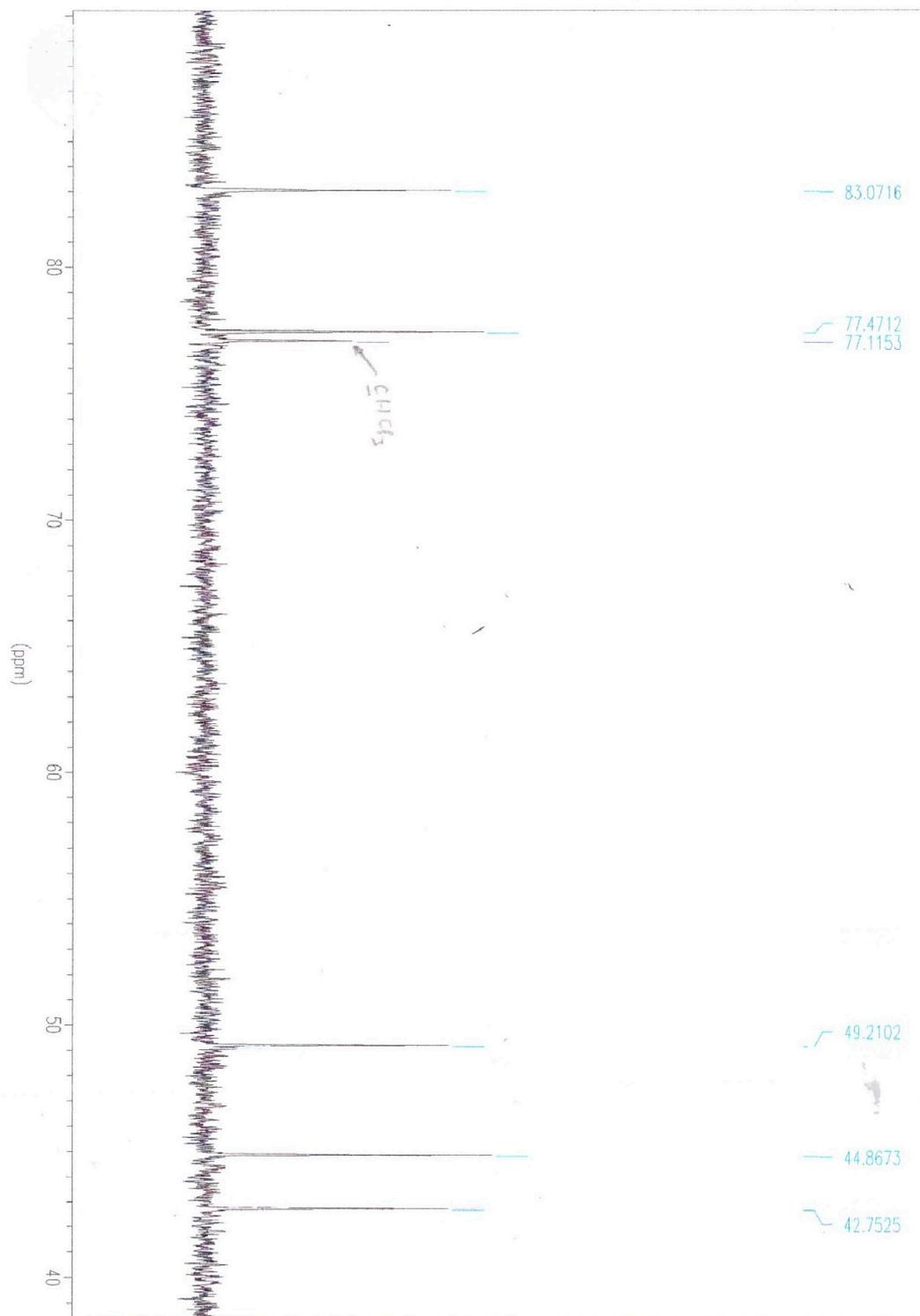


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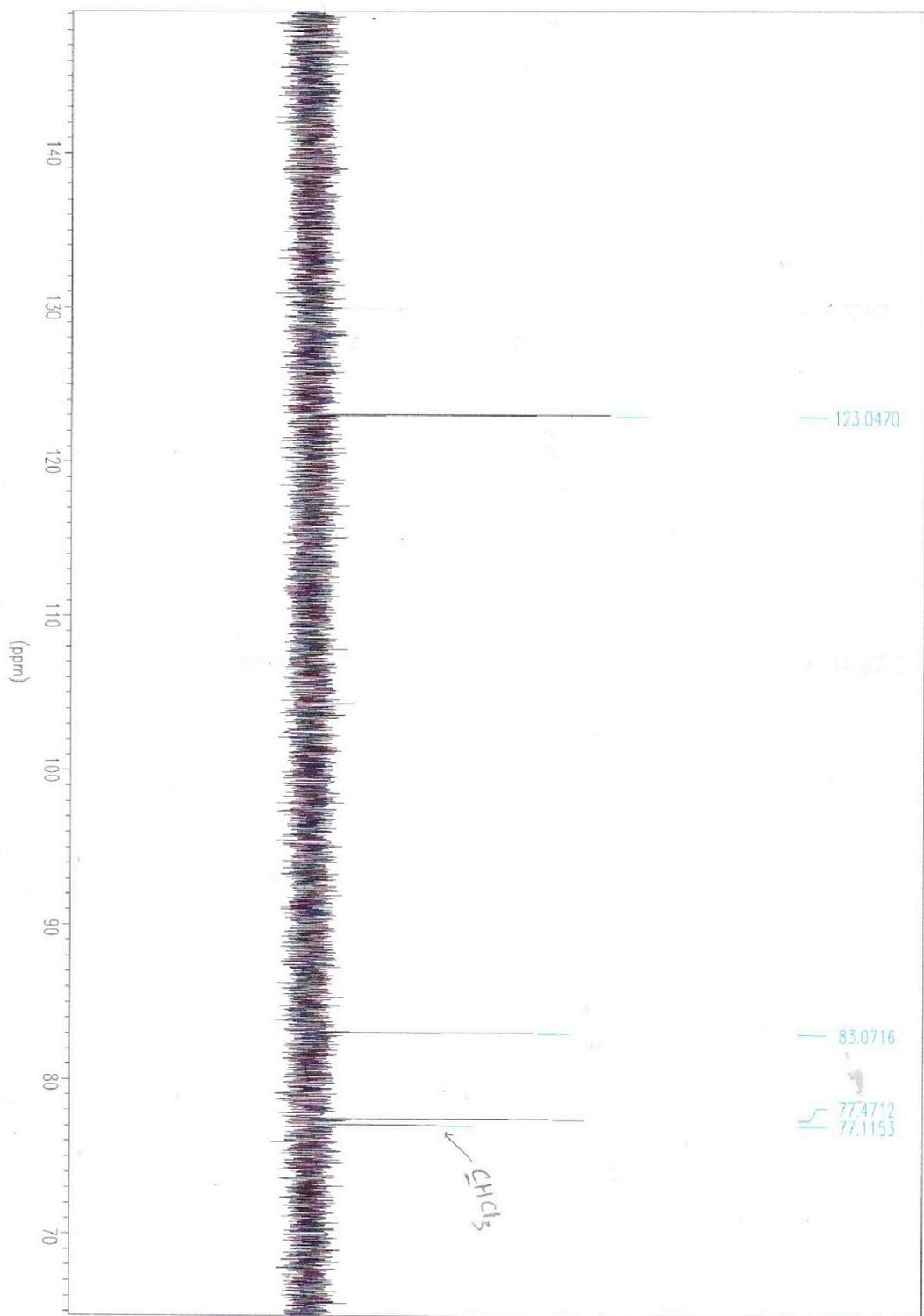




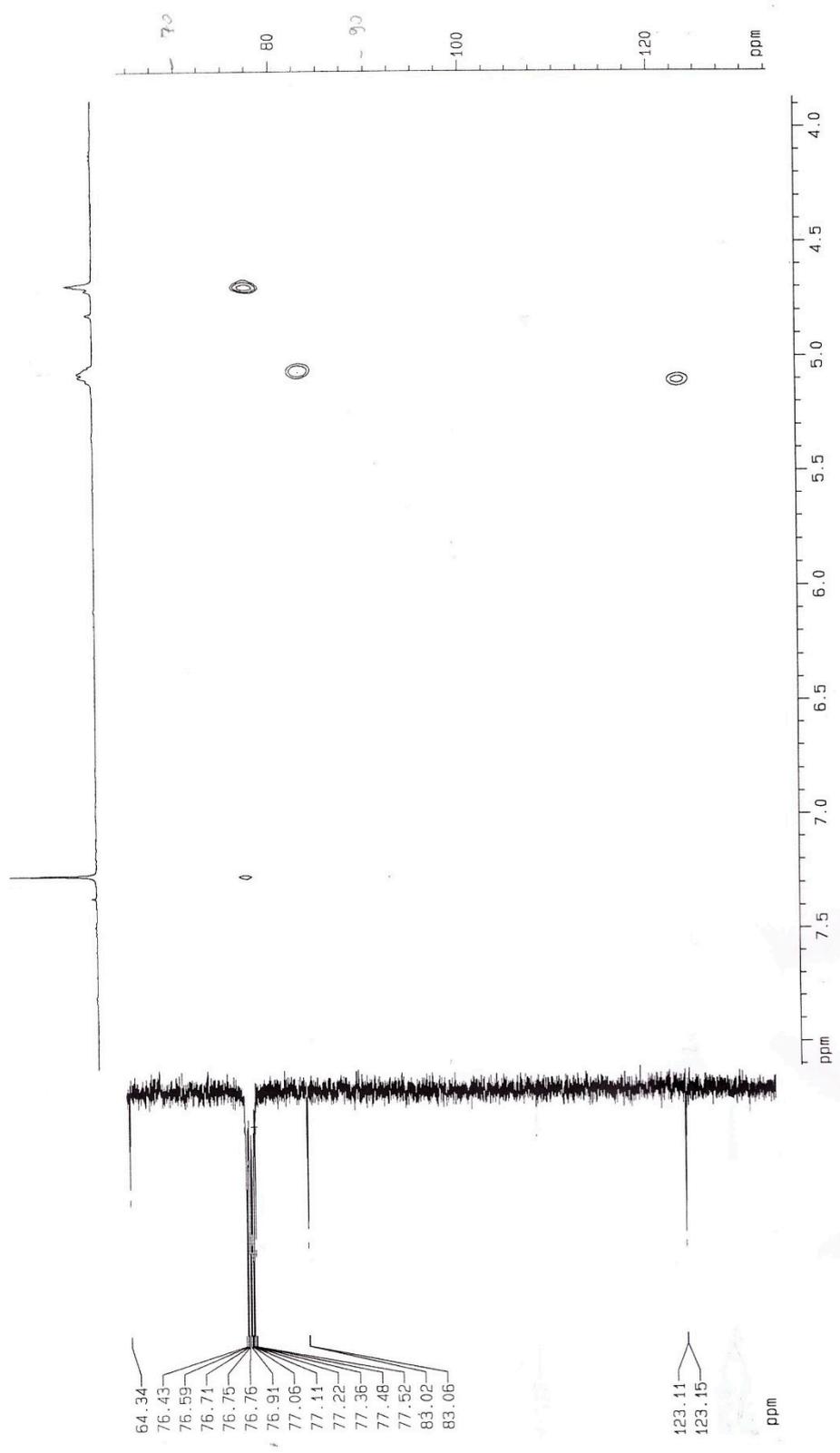
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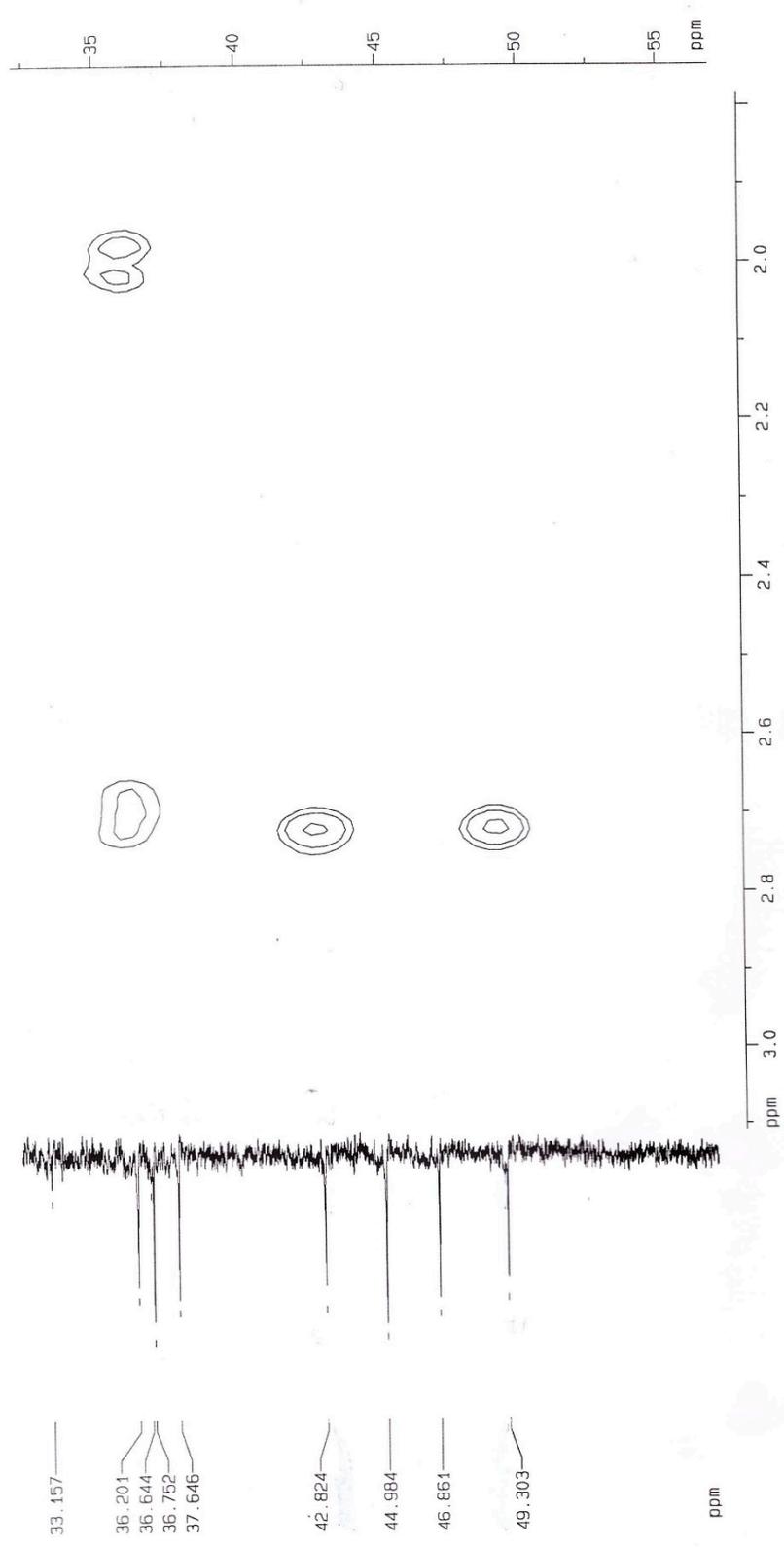
S18B



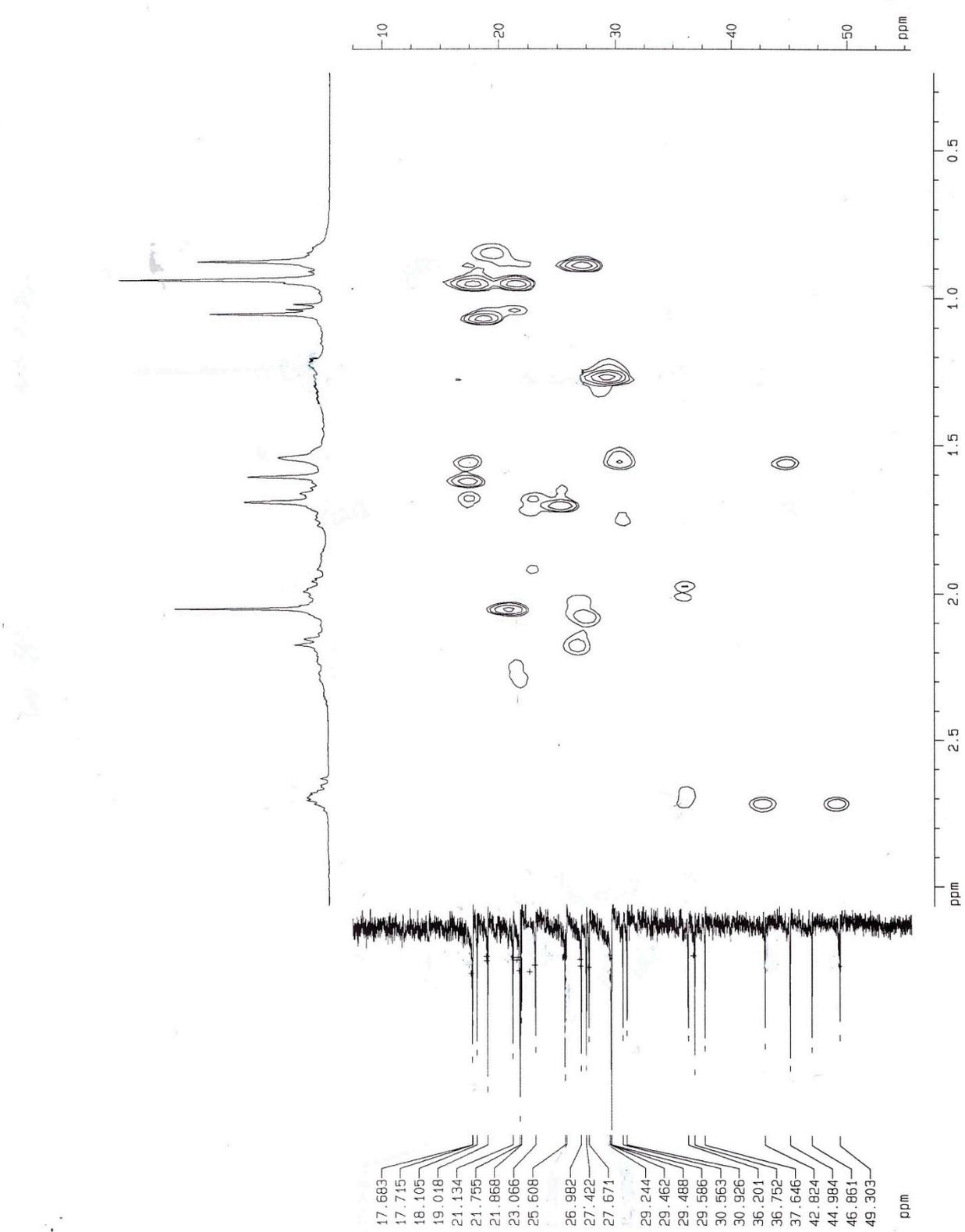
S18C



S19A



S19B



S19C