Supporting Information

Synthesis of Haptens and Development of an Immunoassay for the Olive Fruit Fly Pheromone.

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Contents

2-Chloromethyltetrahydropyran (5): To a mixture of tetrahydropyran-2-methanol **4** (26.25g, 0.226 mol) and freshly distilled pyridine (22 mL, 0.271 mol), was added freshly distilled thionyl chloride (11 mL, 0.271 mol) at a rate to maintain a reaction temperature of 40-45 ºC. When the addition was complete, the mixture was heated for 8 h at 45 ºC. The resulting heavy brown oil was extracted with diethylether (6 x 50 mL). The combined ether solutions were washed with water (4 x 30 mL), saturated aqueous sodium bicarbonate (3 x 30 mL), and brine (3 x 30 mL). The ether phase was dried over anhydrous sodium sulfate, and the solvent was removed at reduced pressure. Distillation of the residue gave the chloride **5** as a colorless liquid (17.85g, 68%); bp 60-64 ºC (7 mmHg) [lit.[8] 55.0-55.5 ºC (6 mmHg)]; IR (cm^{-1}) 2944, 2853, 1656, 1097, 1084, 1046; ^{1}H NMR δ 1.25-1.90 (m, 2H), 3.38-3.52 (m, 4H), 3.98-4.06 (m, 2H).

2-Methylenetetrahydropyran (6): A mixture of 2-chloromethyltetrahydropyran **5** (17.85 g, 0.132 mol) and powdered potassium hydroxide (15.37 g, 0.274 mol) was heated under reflux for 3.5 h with vigorous stirring. The crude product was then distilled through a base washed (KOH) oven dried Vigreux column and condenser, into a base washed receiver flask, cooled to 0ºC, containing potassium hydroxide pellets (3.0 g). (Note: the temperature of the oil bath was held at 190 ºC, while the distillation head temperature should not exceed 110 ºC). Distillate at 82-108 ºC was collected as colorless oil (16.60 g, 93%); IR(cm^{-1}) 3114, 1655; ^{1}H NMR δ 1.68-1.76 (4H, m), 2.24 (2H, m), 3.86-3.91 (2H, m), 4.04 (1H, s), 4.31 (1H, s); ^{13}C NMR δ 23.00, 25.02, 29.22, 69.52, 77.20, 90.94.

Methyl 5-hydroxypentanoate (8): Concentrated sulfuric acid (0.7 mL) was added to a magnetically stirred solution of δ-valerolactone (40.26 g, 0.402 mol) in methanol (54 mL) and the mixture was boiled under reflux for 10 h. The mixture was then cooled in an ice/salt bath and sodium hydrogen carbonate (0.3 g) was added. The mixture stirred for 10 min, the excess solid removed by filtration, and the solvent removed under reduced pressure while the temperature did not exceed 25 ºC. Distillation of the residue gave product **8** as a colorless liquid (24.6 g, 62%). bp 82 ºC /1.5 mmHg [lit.[9] 67-71 ºC/0.2 mmHg]; IR (cm^{-1}) 3639, 3461, 1739.

Methyl 5-oxopentanoate (9): Pyridinium chlorochromate (60.56 g, 0.281 mol) in dry dichlomethane (110 mL) was stirred mechanically at room temperature and a solution of methyl 5-hydroxypentanoate **8** (24.96 g, 0.189 mol) in dichloromethane (110 mL) was added in one portion. The mixture was stirred for 2 h at room temperature and then dry diethyl ether (150 mL) was added. The solution was decanted off and the residue washed with diethyl ether (3 x 50 mL). The combined organic phase was then filtered through Florisil and the solvent removed under reduced pressure. Pure product **9** is collected (20.22 g, 81%); IR (cm^{-1}) 2822, 2719, 1730.

Methyl 2-Methylene-5-oxopentanoate (10): A mixture of methyl 5-oxopentanoate **9** (20.22 g, 0.155 mol), dimethylammonium chloride (18.92 g, 0.232 mol) and aqueous 37% formaldehyde (14 mL, 0.170 mol) was stirred at 80 ºC for 3 h. The organic products were extracted by diethyl ether and washed with water. The crude product is dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography (petroleum ether : ether 4 : 1) to give compound **10** (9.1 g, 45%); IR (cm^{-1}) 2999, 2819, 2703, 1744, 1695, 1654; ^{1}H NMR δ 2.30-2.59 (4H, m), 3.62 (3H, s), 6.01 (1H, s), 6.27 (1H, s), 9.49 (1H, s); ^{13}C NMR δ 23.37, 31.89, 51.48, 134.69, 148.25, 172.83, 193.98.

5-Bromopentanol (14): To a mixture of 1,5-pentanediol **13** (18.44 g, 0.177 mol) in toluene (55 mL), were added concentrated HBr (30.5 ml of a 47% aqueous solution, 0.177 mol) and concentrated H_2SO_4 (1 mL). The heterogeneous mixture was stirred and heated under reflux for 4h. The reaction mixture is allowed to cool to room temperature and the phases were separated. The organic phase was washed successively by water, $NaHCO_3$ (5%) and water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The crude product (12.32 g) was collected as yellow oil. Column chromatography on silica gel 60 of the crude product (petroleum ether 40-60 °C : ether 4 : 1) provided pure compound **14** (7.56 g, 41%); IR (film, cm^{-1}) 3345, 642; ^{1}H NMR δ 1.35-1.60 (4H, m), 1.75-1.89 (2H, m), 3.06 (1H, s), 3.36 (2H, t, J= 10.0Hz), 3.57 (2H, t, J= 8.0Hz).

5-Cyanopentanol (15): In a three-necked flask were added, H_2O (25 mL), NaOH (0.12 g, 0.003 mol), KCN (4.40 g, 0.068 mol), $Bu_4N(HSO_4)$ (0.44 g, 0.0013 mol) and 5-bromo-pentanol **14** (7.56 g, 0.045 mol). The mixture was heated at 60 °C for 4,5 h. CH_2Cl_2 (100 mL) was added to the reaction mixture, the organic phase was washed by H_2O (50 ml) and brine (50 ml), dried over anhydrous Na_2SO_4 and the solvent was concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel 60 (petroleum ether : ether 1 : 1) to give pure product **15** (7.33 g, 97%); IR (film, cm^{-1}) 3389, 2248; ^1H NMR δ 1,36-1,77 (6H, m), 2,38 (2H, t, J= 10.7Hz), 2.68 (1H. s), 3.62 (2H, t, J= 8.6Hz).

5-Cyano-pentanal (16): Pyridinium chlorochromate (21.01 g, 0.0975 mol) in dry CH_2Cl_2 (40 mL) was stirred mechanically and a solution of **15** (7.33 g, 0.065 mol) in CH_2Cl_2 (40 mL) was added in one portion. The mixture was stirred for 2,5 h at room temperature and then dry diethyl ether (80 mL) was added. The solution is decanted and the residue washed with diethyl ether (3 x 50 mL). The combined organic phase was filtered through Florisil and the solvent removed under reduced pressure to give pure compound **16** (5.35 g, 73%); IR (film, cm^{-1}) 2732, 2247, 724; ^1H NMR δ 1.60 (4H, m), 2.34 (2H, t, J= 10.0Hz), 2.50 (2H, t, J= 8.0Hz), 9.73 (1H, s).

2-Methylene-5-cyano-pentanal (17). A mixture of 5-cyanopentanal **16** (5.35 g, 0.048 mol), dimethyl-ammonium chloride (5.87 g, 0.072 mol) and aqueous 37% formaldehyde (4mL, 0.0528 mol) was stirred at 80 ºC for 3 h. The reaction mixture was extracted by diethylether and the organic phase was washed by water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was purified by column chromatography (petroleum ether : ether 1 : 1) to give a rather pure compound **17,** which was further purified by flash chromatography (dichromethane). Pure product **17** was collected (2.62 g, 49%); IR (cm^{-1}) 2703, 2251, 1695, 1631; ^1H NMR δ 1.77-1.87 (2H, m), 2.32-2.43 (4H, m), 6.09 (1H, s), 6.33 (1H, s), 9.53 (1H, s).

Table. Features of the ELISA displacement curve.

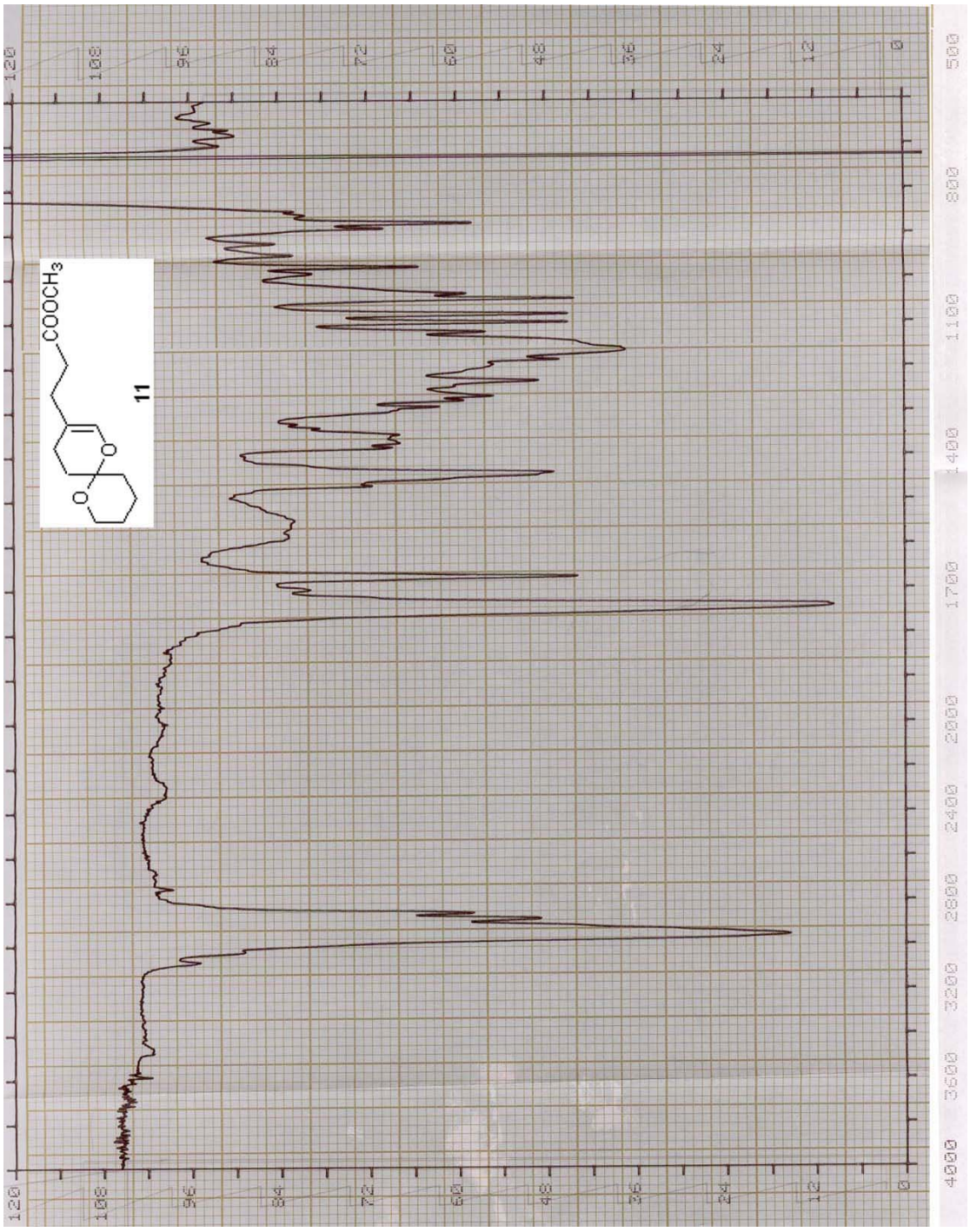
A[a] st0 (Aref)	1.015 ± 0.059[b] (CV:5.8%)
Ast1 (Amin)	0.213 ± 0.024 (CV:11.5%)
Ast2	0.279 ± 0.027 (CV:9.8%)
Ast3	0.392 ± 0.045 (CV:11.45%)
Ast4	0.553 ± 0.055 (CV:10.0 %)
Ast5	0.713 ± 0.059 (CV:8.3%)
Ast6	0.846 ± 0.047 (CV: 5.5%)
Ast7	0.919 ± 0.036 (CV:3.9%)
Ast8 (Amax)	0.956 ± 0.025 (CV:2.6%)
Ablank	0.097 ± 0.005 (CV:5.4%)
IC_{50}[c] (μg/ml)	1.237 ± 0.139 (CV:11.3%)
working range	80ng/ml - 10μg/ml
r^2	0.9927 ± 0.003 (CV:0.3%)
slope	-0.4625 ± 0.030 (CV:6.6%)

([a])Optical absorption measured at 405 nm for each standard pheromone solution (st0=no pheromone, st1=10 μg/ml, st2=5 μg/ml, st3=2.5 μg/ml, st4=1.25 μg/ml, st5=625 ng/ml, st6=312.5 ng/ml, st7=156 ng/ml, st8=80 ng/ml)

([b])Mean value ± SD

([c])IC_{50} concentration of free pheromone which causes 50% displacement of the antibody binding to the ELISA microwells.

The data presented correspond to the average of seven displacement curves run on seven different weeks. Each curve was constructed using three well replicates.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1.5
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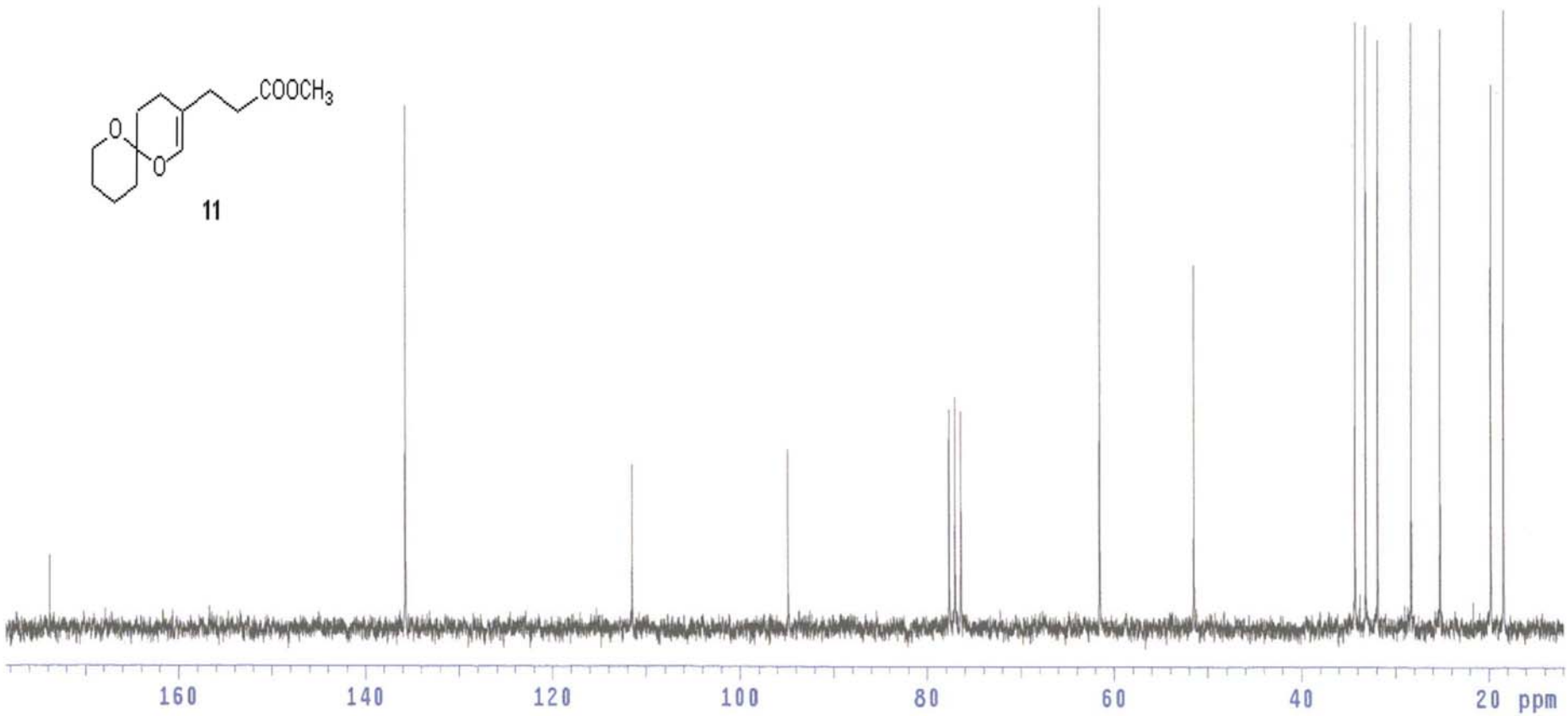
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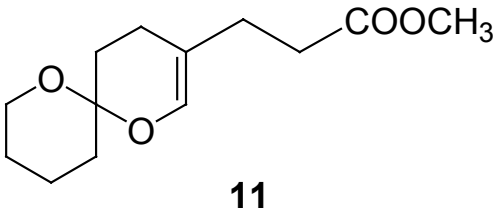
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O

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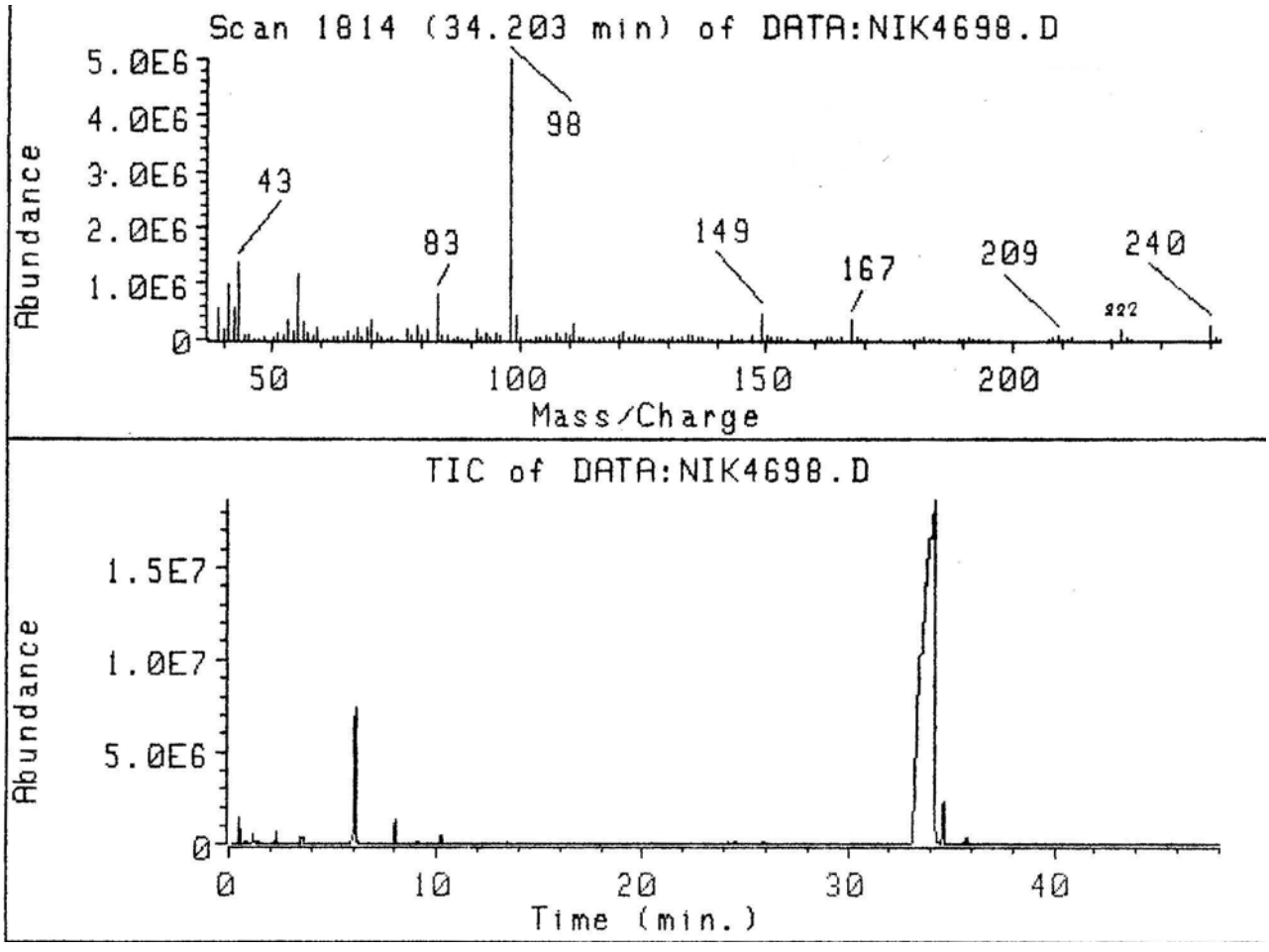
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98
149
167
209
222
240
50
100
150
200
Mass/Charge
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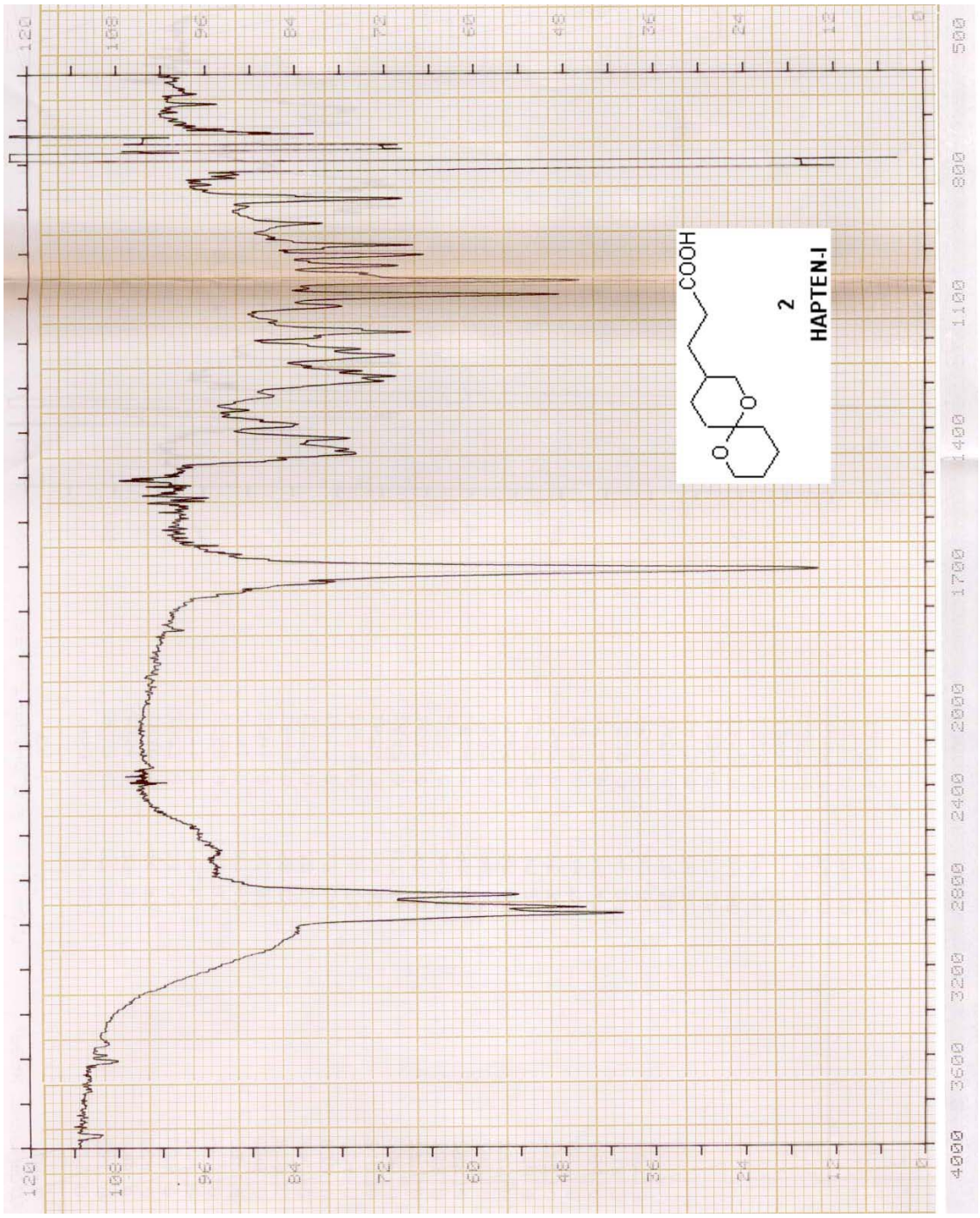
0
10
20
30
40
Time (min.)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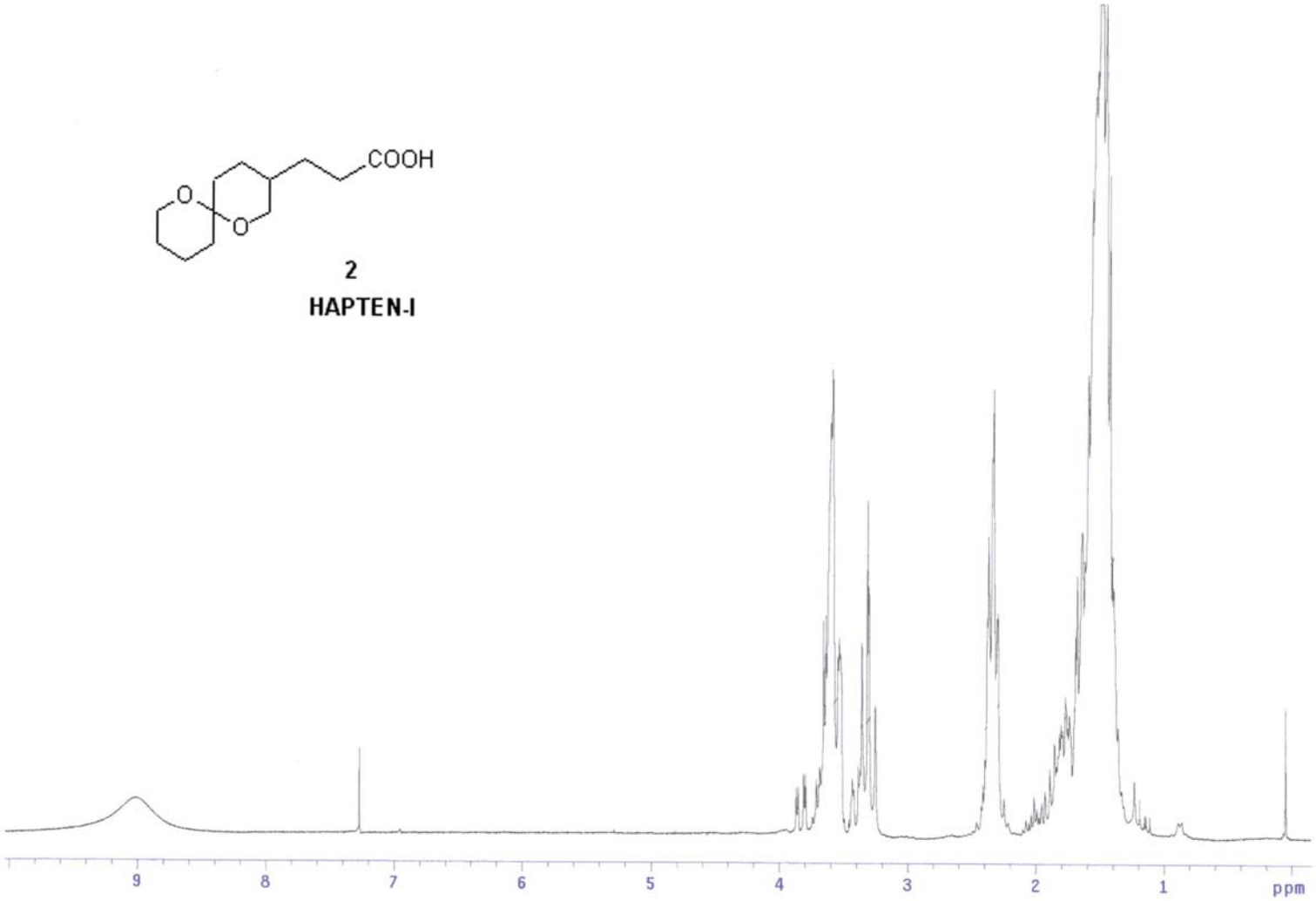
2
HAPTEN-I

2

HAPTEN-I



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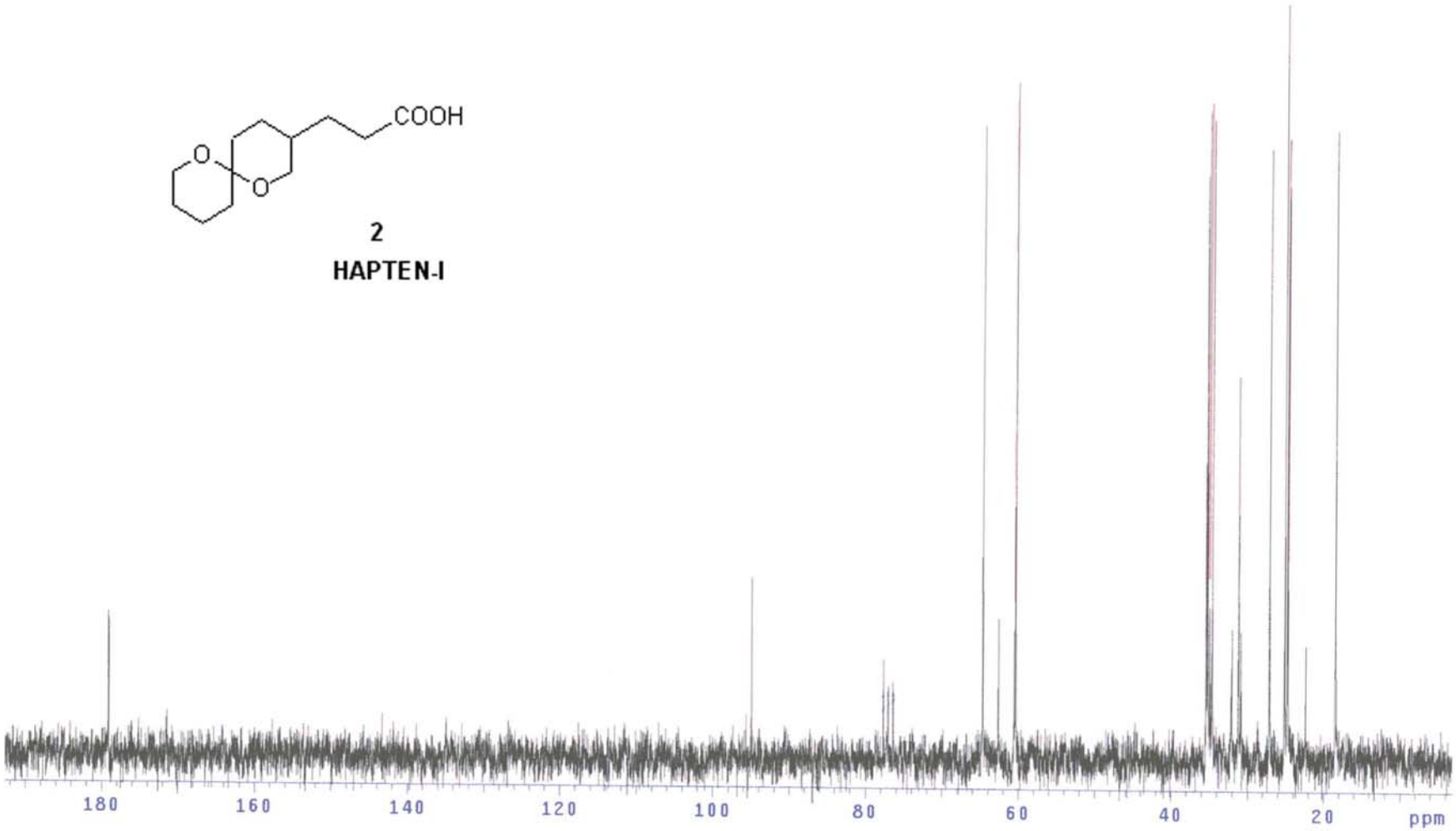

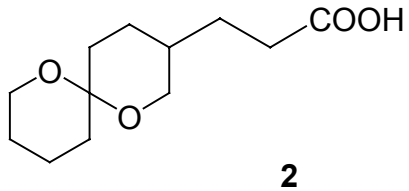
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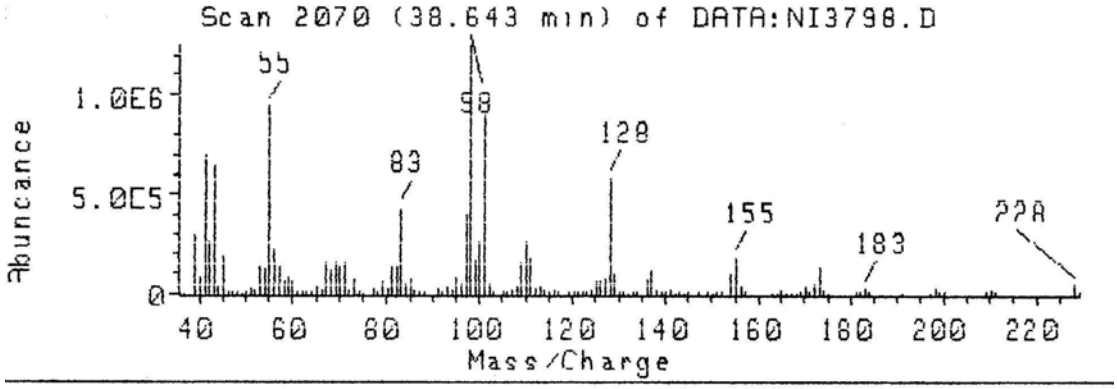
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160
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200
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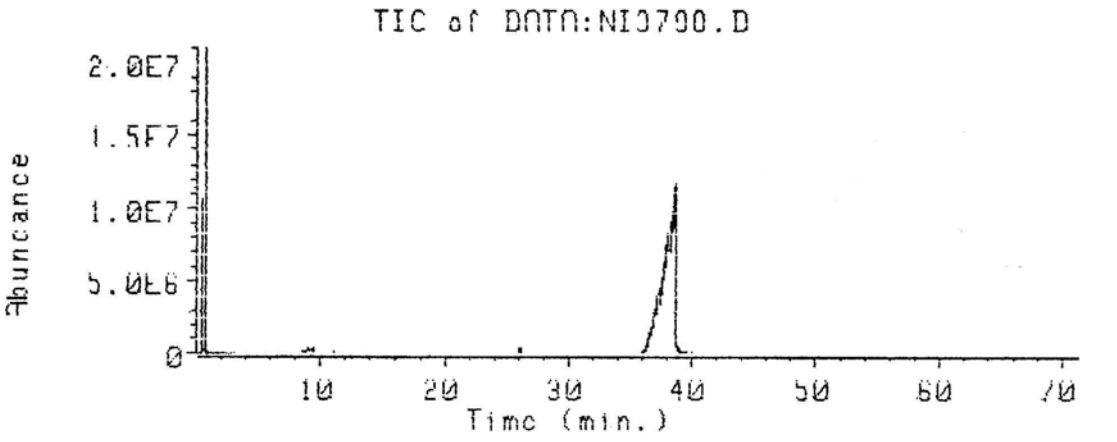
Mass/Charge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50
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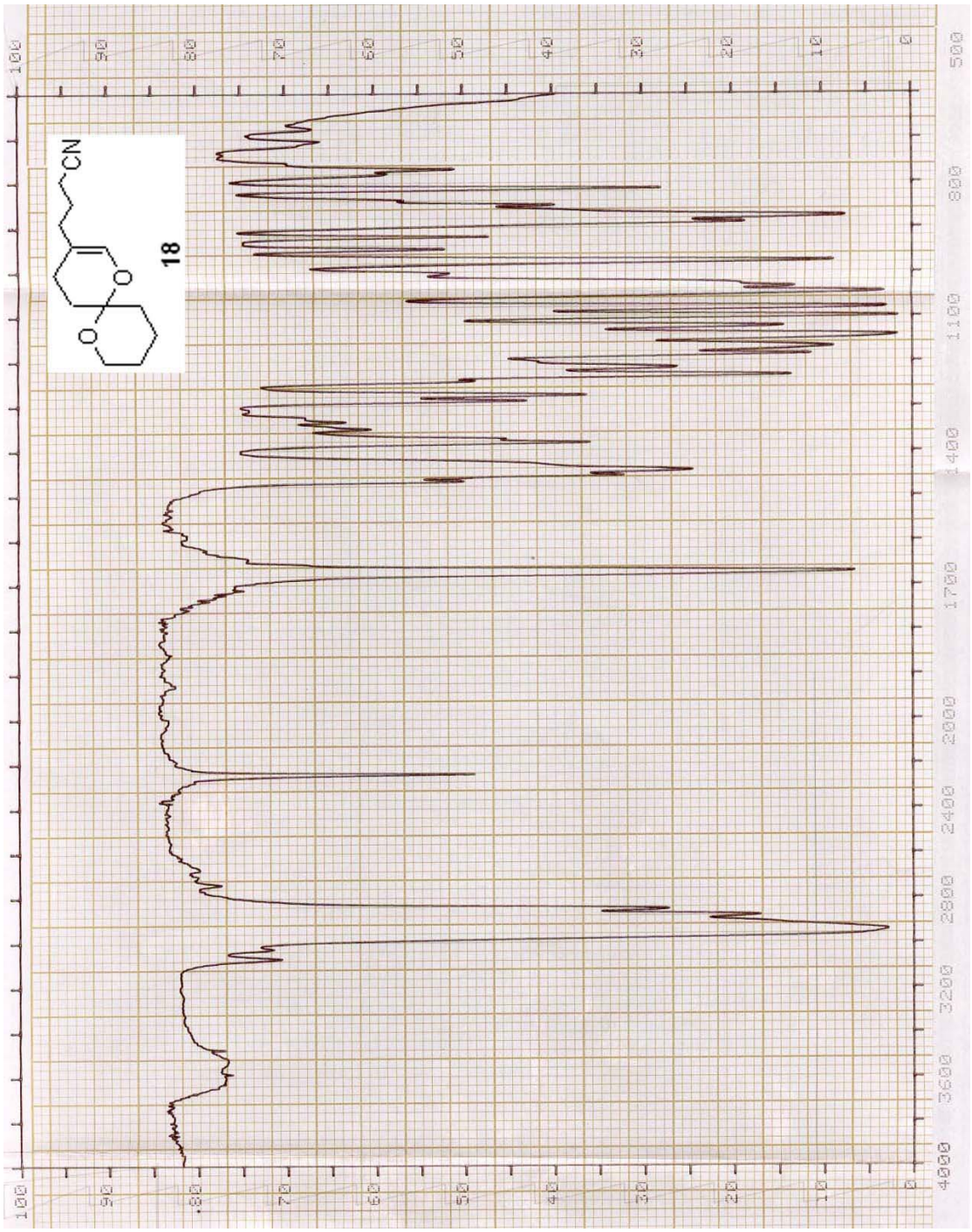
70
Time (min.)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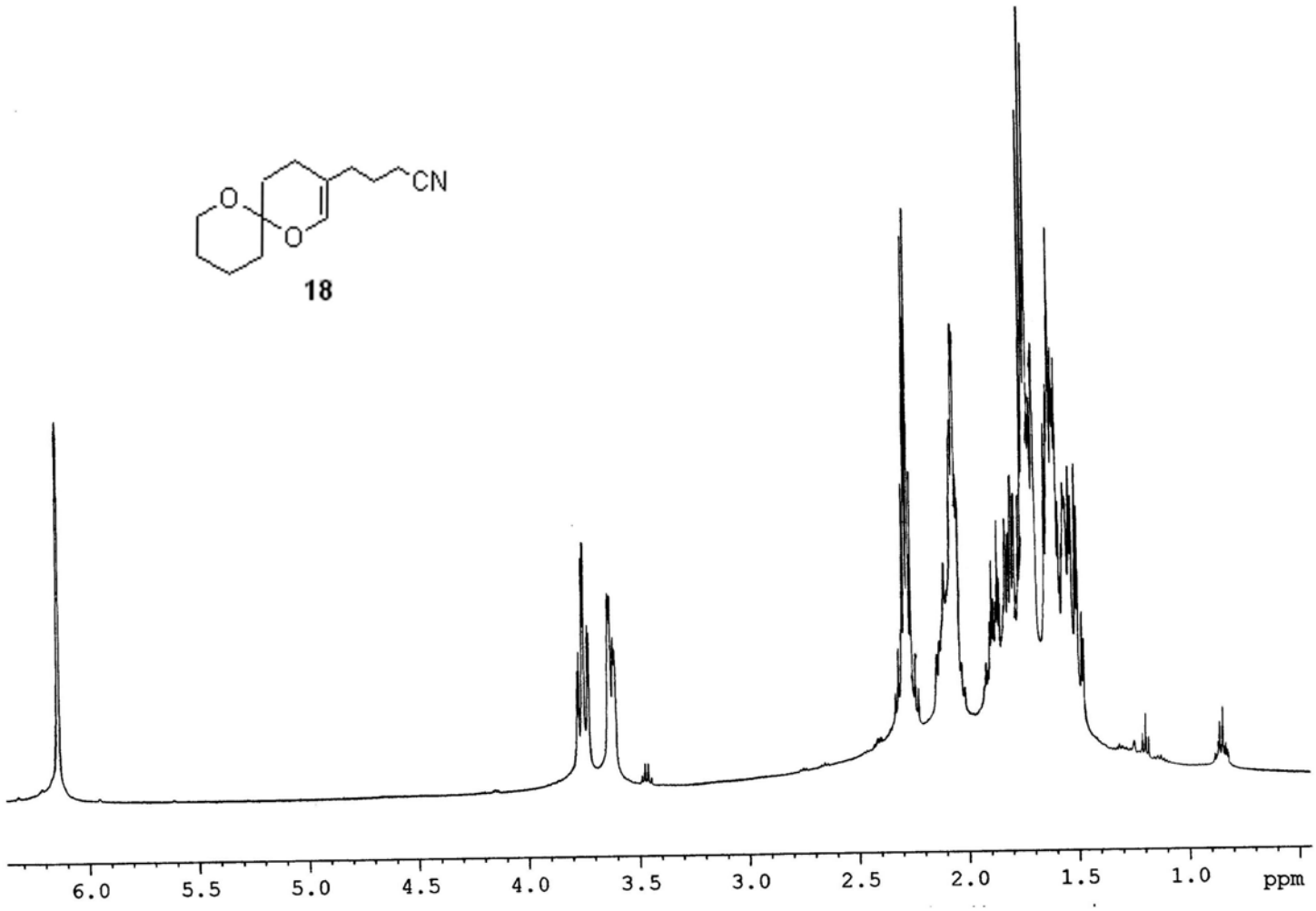
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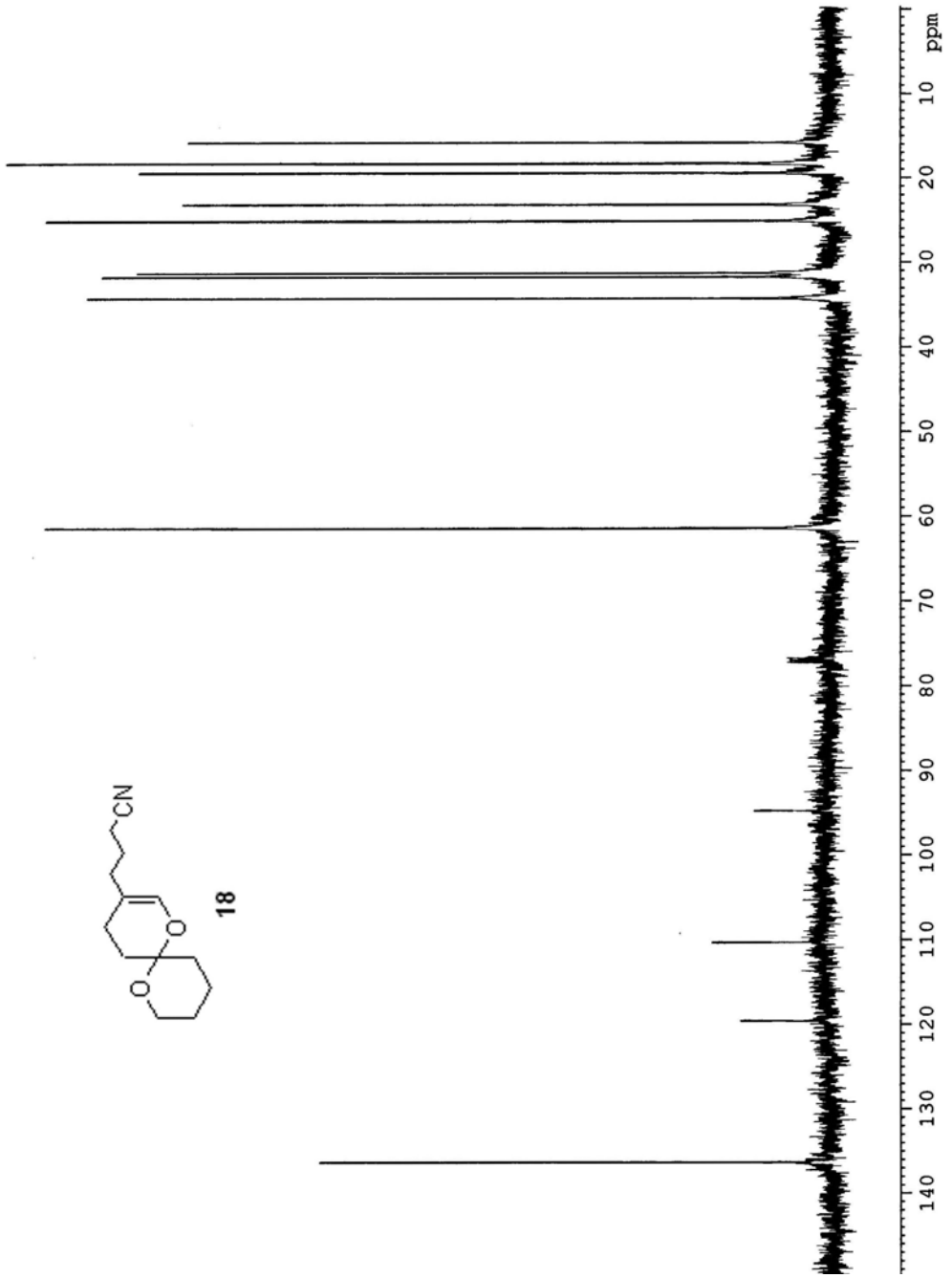
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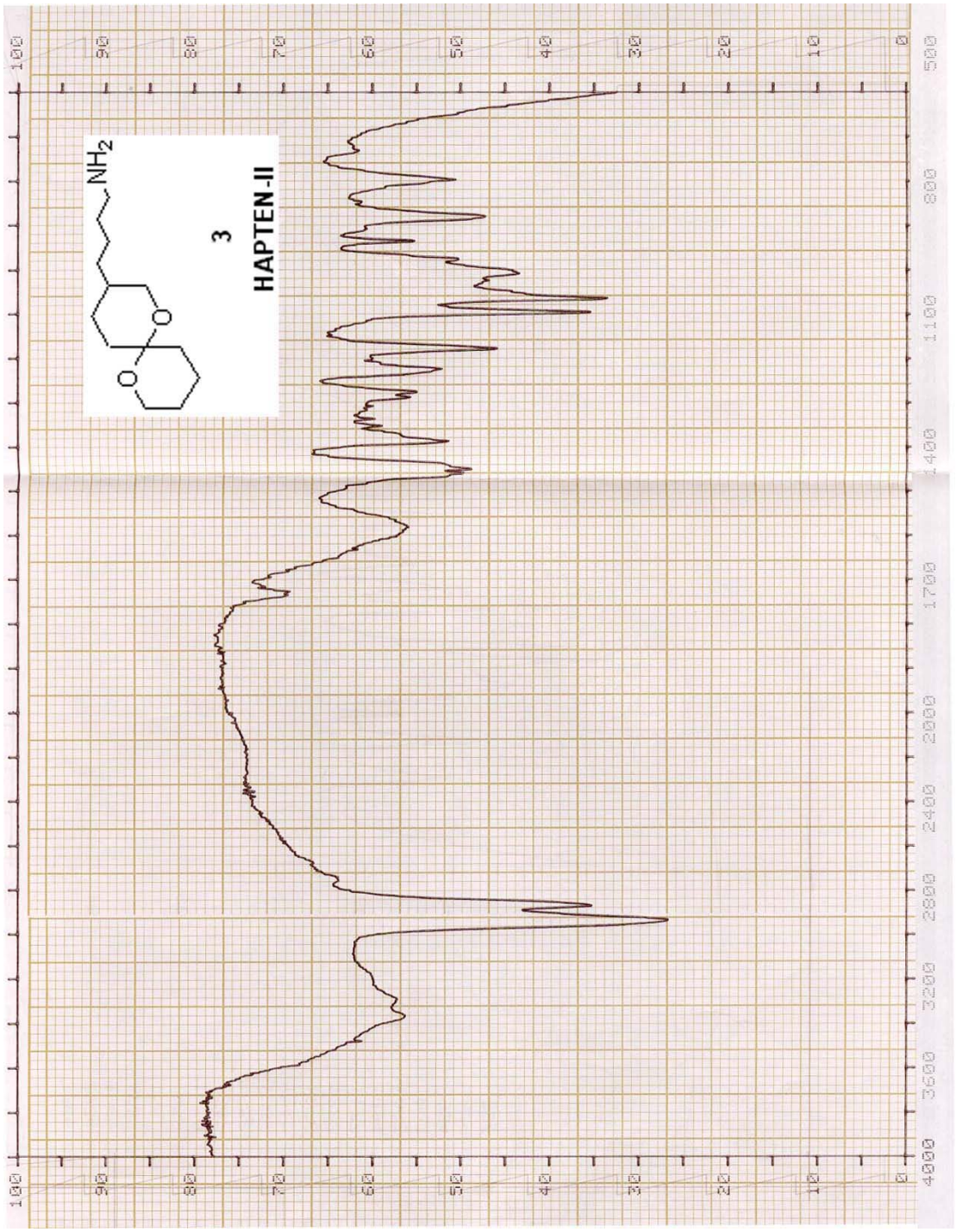
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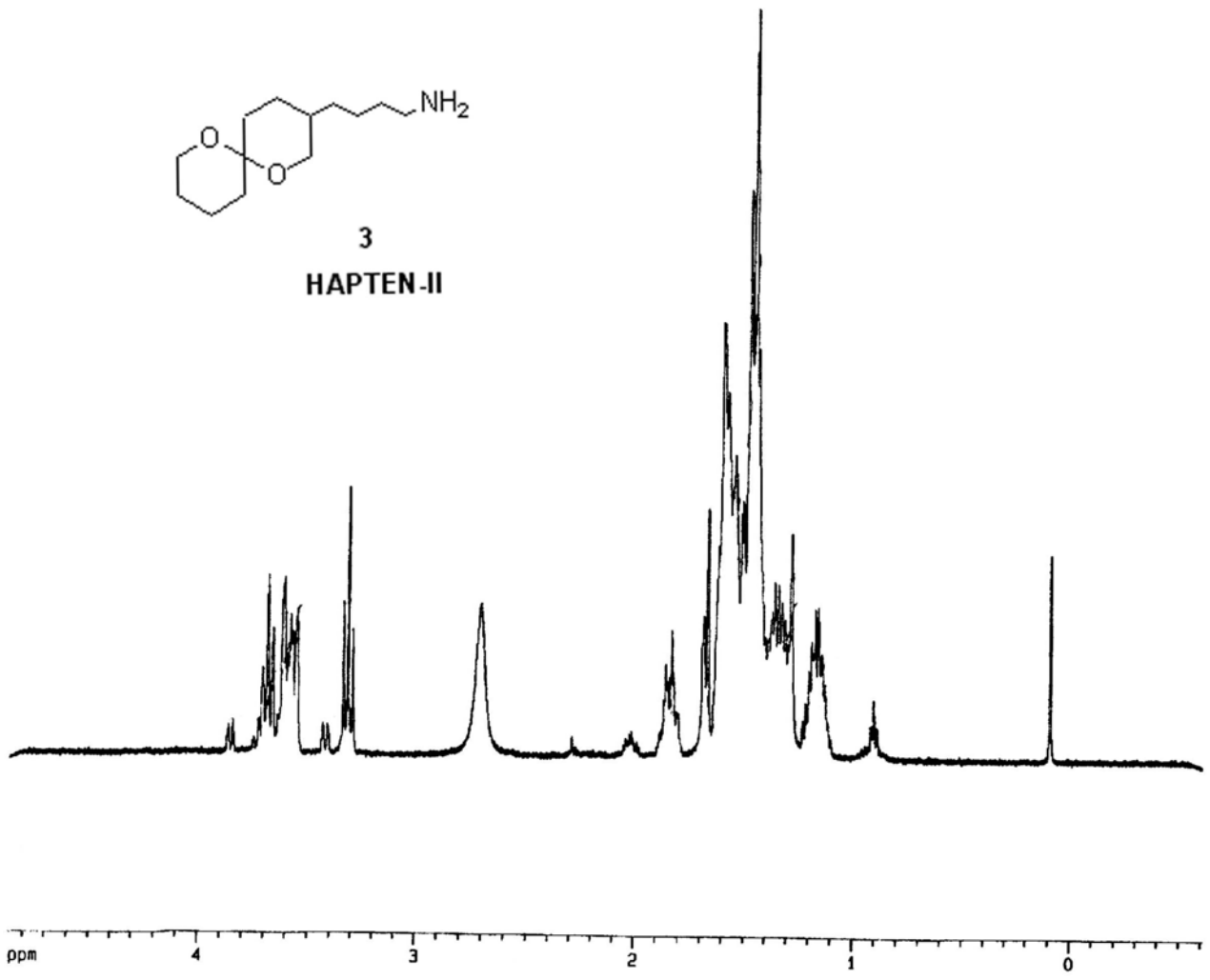

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