

Electronic Supporting Information

Improved synthesis of (*E*)-12-nitrooctadec-12-enoic acid, a potent PPAR γ receptor activator.

Development of a “*buffer-free*” enzymatic method for hydrolysis of methyl esters

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General

All solvents were of commercial quality and were purified by distillation over the drying agents indicated: THF (Na/benzophenone), CH_2Cl_2 , hexane, Et_3N (CaH_2), toluene (Na/K). All other reagents were used as supplied. All moisture-sensitive reactions were carried out under a positive static atmosphere of Ar in flame-dried glassware. Syringes and needles for the transfer of reagents were dried at 140 °C and allowed to cool in a desiccator over P_2O_5 before use. Routine monitoring of reactions was performed using silica gel 60 (0.25 mm), aluminum-supported TLC plates. Compounds were visualized by UV irradiation at a wavelength of 254 nm, or stained by exposure to a 0.5% soln. of vanillin in $\text{H}_2\text{SO}_4/\text{EtOH}$, followed by charring. Flash column chromatography was performed on silica gel (40–63 μm). 2-Nitrocyclododecanone was purchased from Sigma-Aldrich and used as received. CAL-B Lipase immobilized from *Candida antarctica*, beads, slightly brown, >2 units/mg IUBMB 3.1.1.3, EC number 232-619-9, batch 1241096 41708197, was purchased from Sigma-Aldrich.

^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, in the solvents indicated; chemical shifts (δ) are given in ppm relative to TMS, coupling constants (J) in Hz. The solvent signals were used as references and the chemical shifts converted to the TMS scale (CDCl_3 : δ_{C} 77.16; residual CHCl_3 in CDCl_3 : δ_{H} 7.26; CD_2Cl_2 : δ_{C} 53.8; residual ^1H : δ_{H} 5.32 ppm. The number of H-atoms attached to each C-atom (s = 0H, d = 1H, t = 2H, q = 3H) was determined by DEPT experiments. Optical rotations were recorded at 589 nm, concentration (c) in g/100 mL.

Synthesis of (*E*)-10-nitrooleic acid

Step 1: synthesis of methyl 9-hydroxy-10-nitrooctadecanoate¹

Methyl 9-oxononanoate² (300 mg, 1.611mmol) and the 1-nitro nonane (279 mg, 1.611 mmol) were stirred under Ar without solvent in a dry bottom flask. To a this mixture was added DBU (0.161 mmol) and the mixture was stirred for 24h at room temperature. The reaction was diluted with EtOAc and was quenched with HCl 1N (8 ml). The layers were separated and the aq layer was extracted with Et₂O. The organic layers reunited were dried on Na₂SO₄, filtered and concentrated under vacuum. The residue was purified on silica gel using hexane/EtOAc 9:1 as eluent (*R*_f = 0.30): methyl 9-hydroxy-10-nitrooctadecanoate, 423 mg (yield=73%).

¹H NMR (300MHz, CDCl₃): δ 0.85 (t, *J*=6.8 Hz, 3H), 1.2-1.6 (m, 25H), 1.75 (m, 1H), 2.10 (m, 1H), 2.30 (t, *J*=7.5 Hz, 2H), 3.70 (s, 3H), 3.85 (bm, 0.5) 4.05 (bm, 0.5H), 4.45 (m, 1H).

¹³C NMR (75MHz, CDCl₃): δ 174.26 (C), 92.92 (CH), 92.38 (CH), 72.30 (CH), 71.98 (CH), 51.40 (CH₃), 33.96 (CH₂), 33.45 (CH₂), 33.11 (CH₂), 31.70 (CH₂), 30.37 (CH₂), 29.13 (CH₂), 29.03 (CH₂), 28.96 (CH₂), 28.88 (CH₂), 28.04 (CH₂), 25.92 (CH₂), 25.63 (CH₂), 25.44 (CH₂), 25.10 (CH₂), 24.76 (CH₂), 22.54 (CH₂), 13.99 (CH₃).

IR (CH₂Cl₂): 3474.2, 2925.2, 2854.9, 1736.9, 1555.9, 1438.2, 1365.7, 1200.6, 1107.9, 854.1, 724.1 cm⁻¹.

HRMS-ESI, *m/z* for C₁₉H₃₇NO₅ [M+H]⁺ calcd 360.2750, found 360.2775.

Step 2: synthesis of (*E*)-methyl 10-nitrooctadec-9-enoate¹

To a magnetically-stirred solution of methyl 9-hydroxy-10-nitrooctadecanoate (75 mg, 0.209 mmol) in dry CH₂Cl₂ (0.3 ml) under Ar was added trifluoroacetic anhydride (0.219 mmol) and triethylamine (0.439 mmol) was added dropwise at 0°C. The reaction was allowed to reach room temperature and after 3-4 hours was quenched with aq NH₄Cl. The mixture was diluted with CH₂Cl₂ and the two layers were separated. The aqueous layer was extracted with CH₂Cl₂ and the organic layers reunited were washed with Brine, dried on Na₂SO₄, filtered, and concentrated under vacuum. The residue was purified on silica gel using hexane /EtOAc 95:5 as eluent (*R*_f = 0.28) . (*E*)-methyl 10-nitrooctadec-9-enoate was thus obtained in 65% yield, 47 mg.

¹H NMR (300MHz, CDCl₃): δ 0.90 (t, *J*=6.4 Hz, 3H), 1.25-1.40 (m, 16H), 1.55 (m, 4H), 1.65 (m, 2H), 2.25 (dt, *J*₁=7.7 Hz, *J*₂=7.3 Hz, 2H), 2.35 (t, *J*=7.4 Hz, 2H), 2.60 (t, *J*=7.3 Hz, 2H), 3.65 (s, 3H), 7.08 (t, *J*=7.9 Hz, 1H).

¹³C NMR (75MHz, CDCl₃): δ 174.15 (C), 151.95 (C), 136.17 (CH), 51.45 (CH₃), 33.99 (CH₂), 31.79 (CH₂), 29.22 (CH₂), 29.14 (CH₂), 28.96 (CH₂), 28.45 (CH₂), 27.94 (CH₂), 27.89 (CH₂), 26.34 (CH₂), 24.83 (CH₂), 22.61 (CH₂), 14.06 (CH₃).

IR (CH₂Cl₂) : 2928.0, 2855.7, 1740.4, 1522.8, 1458.6, 1436.9, 1336.5, 1198.0, 1171.2, 1112.9, 852.7, 724.2 cm⁻¹.

HRMS-ESI, *m/z* for C₁₉H₃₅NO₄ [M+H]⁺ calcd 342.2644, found 342.2670

Step 3: synthesis of (*E*)-10-nitrooleic acids¹

To a magnetically-stirred solution of (*E*)-methyl 10-nitrooctadec-9-enoate (17 mg, 0.050 mmol) in tert-butyl methyl ether (0.4ml) were added water (2.5 mmol, 50 equiv.) and lipasi immobilized from *Candida Antartctica* B. The solution was stirred at room temperature. The reaction was filtered and concentrated under vacuum. The residue was purified on silica gel using hexane/EtOAc 7:3 as eluent ($R_f = 0.25$) : (*E*)-10-nitrooleic acids, 16 mg (yield 98%).

¹H NMR (300 MHz, CDCl₃): δ 0.90 (t, $J=6.9$ Hz, 3H), 1.25-1.40 (m, 16H), 1.55 (m, 4H), 1.65 (m, 2H), 2.25 (dt, $J_1=7.7$ Hz, $J_2=7.4$ Hz, 2H), 2.40 (t, $J=7.4$ Hz, 2H), 2.60 (t, $J=7.3$ Hz, 2H), 7.08 (t, $J=7.9$ Hz, 1H).

¹³C NMR (75MHz, CDCl₃): δ 179.27 (C), 151.98 (C), 136.15 (CH), 33.81 (CH₂), 31.79 (CH₂), 29.21 (CH₂), 29.14 (CH₂), 28.95 (CH₂), 28.85 (CH₂), 28.44 (CH₂), 27.90 (CH₂), 26.34 (CH₂), 24.55 (CH₂), 22.61 (CH₂), 14.05 (CH₃).

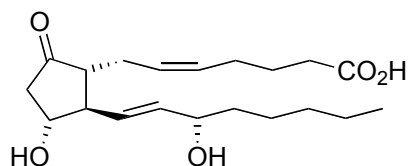
IR (CH₂Cl₂) : 2927.8, 2855.6, 1712.0, 1519.0, 1462.8, 1336.6, 1112.9, 937.5, 724.2 cm⁻¹

HRMS-ESI, m/z for C₁₈H₃₃NO₄ [M+H]⁺ calcd 328.2488, found 328.2512.

Enzymatic Hydrolysis

Following the general procedure described in the manuscript, methyl esters afforded the corresponding acids, the ^1H and ^{13}C NMR spectra which were in agreement with those reported in literature have been reported.

*(-)*Prostaglandin- E_2 (**10**)



Prostaglandin- E_2 methyl ester,⁴ 8.2 mg (22.4 μmol) afforded *(-)*-prostaglandin- E_2 (**10**), 7.5 mg, yield 95% (3% MeOH in EtOAc, $R_f = 0.2$).

^1H NMR (300MHz, CD_3CN): δ 5.59 (dd, $J_1=3.7$, $J_2=2.6$ Hz, 2H), 5.48-5.04 (m, 2H), 4.04 (q, $J = 9.0$ Hz, 2H), 2.63 (dd, $J_1=18.3$, $J_2=7.4$ Hz, 1H), 2.41 - 2.19 (m, 5H), 2.18 - 1.87 (m, 5H), 1.70 - 1.56 (m, 2H), 1.54 - 1.19 (m, 7H), 1.04 - 0.77 (m, 3H). .

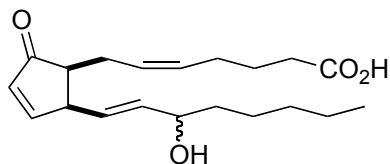
^{13}C NMR (75MHz, CD_3CN): δ 215.7 (C), 175.0 (C), 137.5 (CH), 131.4 (CH), 131.0 (CH), 128.0 (CH), 72.9 (CH), 72.6 (CH), 55.0 (CH), 53.9 (CH), 47.3 (CH_2), 38.3 (CH_2), 33.6 (CH_2), 32.5 (CH_2), 27.2 (CH_2), 26.0 (CH_2), 25.6 (CH_2), 25.5 (CH_2), 23.4 (CH_2), 14.3 (CH_3).

IR (CH_2Cl_2) : 3379, 2931, 2856, 1732, 1709, 1398, 1204, 1158, 1120, 1084, 1033, 973 cm^{-1}

HRMS-ESI, m/z for $\text{C}_{20}\text{H}_{32}\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 353.2328, found 353.2353.

$[\alpha]_{\text{D}}^{20} = -71.4$ (c 0.7 EtOH).

rac-Isoprostane-A₂ (**11**)



Isoprostane-A₂ methyl ester

To a stirred solution of rac-isoprostane A₂ 35 mg (0.105 mmol) in ether (5 ml) cooled at -20 °C, was added a saturated ethereal solution of CH₂N₂ until persistent light yellow colour. Evaporation in vacuo at rt followed by silica gel purification (5g, hexane:EtOAc 7:3, *R_f* = 0.27) afforded 35 mg of the corresponding methyl ester in 97% yield.

¹H NMR (300MHz, CD₂Cl₂): δ 0.9 (t, *J* = 6.62 Hz, 3H); 1.3 (bs, 6H); 1.48-1.62 (m, 2H); 1.70 (m, 3H); 2.1 (q, *J* = 7.4 Hz, 3H); 2.25-2.3 (t, *J* = 7.44 Hz, 2H); 2.50 (m, 2H); 3.65 (s, 3H); 3.72 (m, 1H); 4.14 (q, *J* = 6.48 Hz, 1H); 5.3-5.7 (m, 4H); 6.21-6.27 (dd, *J*₁ = 1.83 Hz, *J*₂ = 5.75 Hz, 1H); 7.50-7.56 (dd, *J*₁ = 2.84 Hz, *J*₂ = 5.69 Hz, 1H).

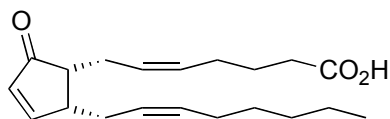
¹³C NMR (75MHz, CD₂Cl₂): δ 210.71 (C), 174.74 (C), 165.65 (CH), 138.15 (CH), 133.45 (CH), 130.54 (CH), 129.21 (CH), 128.51 (CH), 73.11 (CH), 52.11 (CH₃), 50.44 (CH), 48.09 (CH), 38.07 (CH₂), 34.10 (CH₂), 32.51 (CH₂), 27.53 (CH₂), 25.88 (CH₂), 25.39 (CH₂), 23.38 (CH₂), 14.56 (CH₃).

IR (CH₂Cl₂) : 3445, 2929, 1738, 1713, 1699, 1694, 1559, 1456 cm⁻¹

HRMS-ESI, *m/z* for C₂₁H₃₂O₄ [M+H]⁺ calcd 349.2379 found 349.2403.

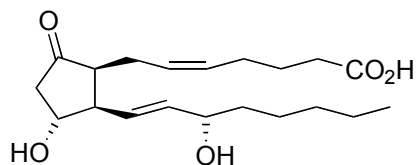
Hydrolysis: 30.8 mg (88.4 μmol) of methyl ester afforded isoprostane-A₂ (**11**), 27.2 mg, yield 92% (eluent: hexane/EtOAc 1:1, *R_f* = 0.27). The NMR data of compound **11** were in perfect agreement with those reported in the literature³

(-)-Preclavulone-A (**12**)



Preclavulone-A methyl ester,⁵ 10.1 mg (30.3 μ mol), afforded preclavulone-A (**12**), 9 mg, yield 93% (eluent: hexane/EtOAc 4:1, R_f = 0.23). The NMR data of compound **12** were in perfect agreement with those reported in the literature.⁵

(+)-Isoprostane-E_{2t} (**13**)



Isoprostane-E_{2t} methyl ester,⁶ 5.6 mg (15.3 μ mol), afforded isoprostane-E_{2t} (**13**), 4.9 mg, yield 92% (3% MeOH in EtOAc, R_f = 0.2).

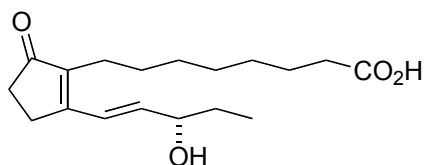
¹H NMR (300MHz, CD₃OD): δ 5.7-5.6 (dd, J_1 =15.3 Hz, J_2 =6.6 Hz, 1H), 5.5-5.3 (m, 2H), 5.3 (dd, J_1 =15.3, J_2 =9.9 Hz, 1H), 4.3-4.2 (m, 1H), 4.0 (m, 1H), 3.0 (m, 1H), 2.7-2.6 (m, 1H), 2.6-2.5 (dd, J_1 =19.2, J_2 =5.7 Hz, 1H), 2.5-2.3 (m, 1H), 2.3-2.2 (t, J =7.5Hz, 2H), 2.3-2.2 (m, 1H), 2.1-1.9 (m, 3H), 1.7-1.6 (quint., J =7.5Hz, 2H), 1.6-1.2 (m, 8H), 0.9 (t, J =6.6Hz, 3H).

¹³C NMR (75MHz, CD₃OD): δ 219.70 (C), 177.49 (C), 138.66 (CH), 131.12 (CH), 129.11 (CH), 128.23 (CH), 73.32 (CH), 72.85 (CH), 52.45 (CH), 51.74 (CH), 45.49 (CH₂), 38.40 (CH₂), 34.34 (CH₂), 32.91 (CH₂), 27.76 (CH₂), 26.17 (CH₂), 25.93 (CH₂), 24.22 (CH₂), 23.64 (CH₂), 14.29 (CH₃).

$[\alpha]_D^{25} = +87$ (c 0.057, MeOH).

HRMS-ESI, m/z for $C_{20}H_{32}O_5$ $[M+H]^+$ calcd 353.2328, found 353.2349.

Phytoprostane- B_1 Type I (**14**)



Phytoprostane- B_1 Type I methyl ester,⁷ 12.1 mg (37.5 μ mol), afforded phytoprostane- B_1 Type I (**14**), 10.4 mg, yield 90% (eluent: hexane/EtOAc 1:1, R_f = 0.25).

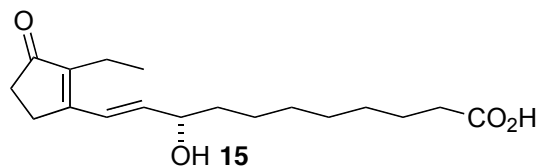
1H NMR (300MHz, $CDCl_3$): δ 1.00 (t, $J=7.4$ Hz, 3H), 1.25-1.46 (m, 8H), 1.57-1.73 (m, 4H), 2.25-2.47 (m, 6H), 2.66 (bt, 2H), 4.28-4.35 (q, $J=5.8$ Hz, 1H), 6.26 (dd, $J_1=5.8$ Hz, $J_2=15.8$ Hz, 1H), 6.82 (d, $J=15.8$ Hz, 1H).

^{13}C NMR (75MHz, $CDCl_3$): δ 209.77 (C), 178.12 (C), 163.21 (C), 141.45 (C), 139.64 (CH), 124.11 (CH), 73.64 (CH), 33.80 (CH_2), 30.06 (CH_2), 28.96 (CH_2), 28.80 (CH_2), 28.53 (CH_2), 28.50 (CH_2), 28.39 (CH_2), 25.61 (CH_2), 24.52 (CH_2), 22.77 (CH_2), 9.63 (CH_3).

IR (CH_2Cl_2): 3400, 1695, 1645, 1600, 970 cm^{-1} .

HRMS-ESI, m/z for $C_{18}H_{28}O_4$ $[M+H]^+$ calcd 309.2066, found 309.2093.

(+)-Phytoprostane-B₁ Type II (**15**)



Phytoprostane-B₁ Type II methyl ester,⁷ 10.3 mg (32 μ mol), afforded phytoprostane-B₁ Type II (**15**), 8.6 mg, yield 87% (eluent: hexane/EtOAc 1:1, R_f = 0.25).

¹H NMR (300MHz, CDCl₃): δ 1.01 (t, J =7.6 Hz, 3H), 1.28-1.55 (m, 9H), 1.60-1.74 (m, 4H), 2.27-2.40 (m, 4H), 2.42-2.50 (m, 2H), 2.65-2.71 (m, 2H), 4.32 (q, J =6.1 Hz, 1H), 6.26 (dd, J_1 =5.98 Hz, J_2 =15.8 Hz, 1H), 6.80 (d, J =15.7 Hz, 1H).

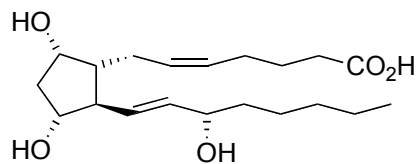
¹³C NMR (75MHz, CDCl₃): δ 209.41 (C), 178.36 (C), 162.49 (C), 142.61 (C), 139.80 (CH), 123.62 (CH), 72.20 (CH), 36.97 (CH₂), 33.64 (CH₂), 33.58 (CH₂), 29.01 (CH₂), 28.86 (CH₂), 28.68 (CH₂), 25.39 (CH₂), 25.02 (CH₂), 24.39 (CH₂), 16.10 (CH₂), 13.29 (CH₃).

IR (CH₂Cl₂): 3400, 1691, 1644, 1601, 969 cm⁻¹.

HRMS-ESI, m/z for C₁₈H₂₈O₄ [M+H]⁺ calcd 309.2066, found 309.2092.

$[\alpha]_D^{25}$ = +16.7 (c 0.054, EtOAc).

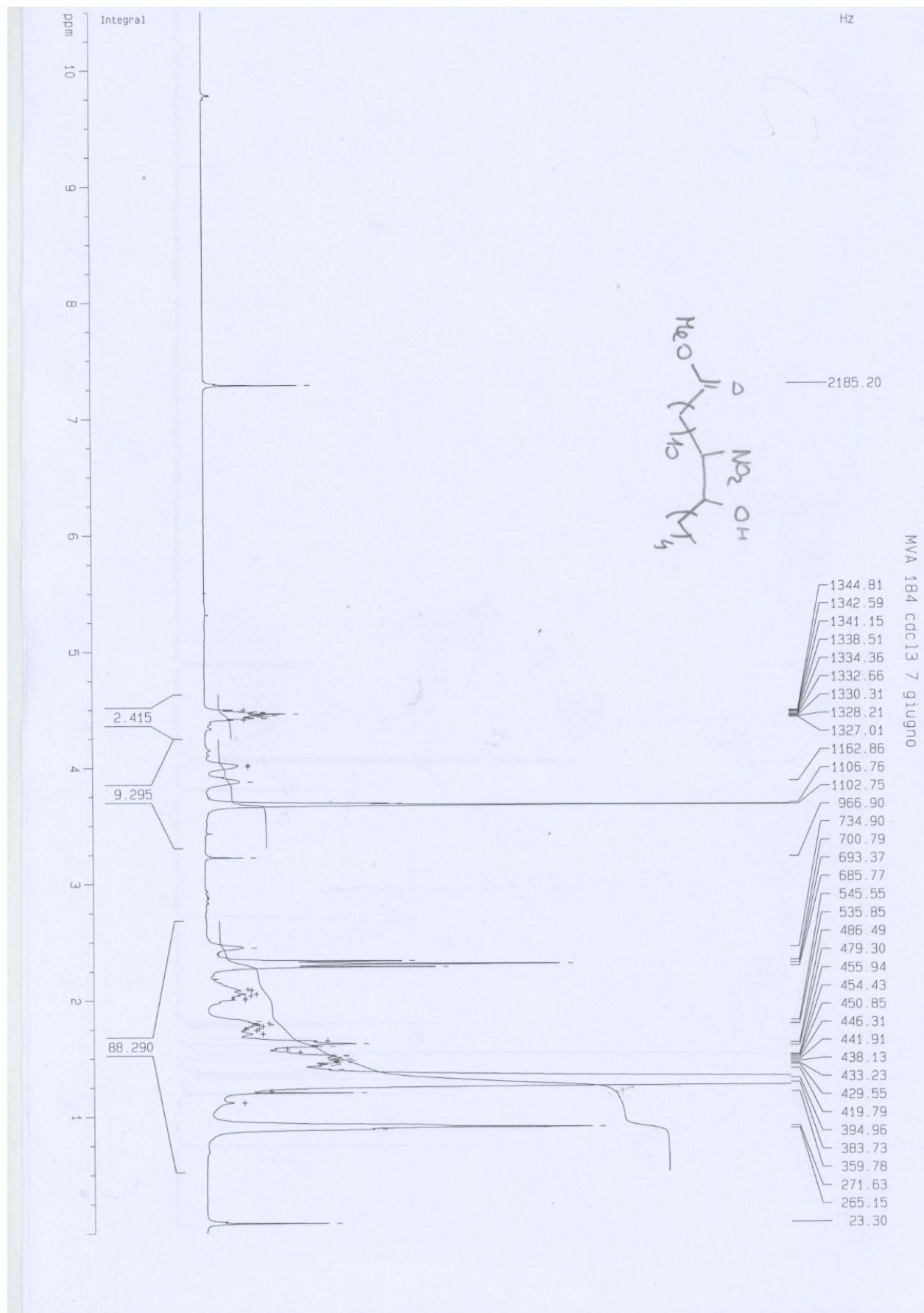
(+)-Prostaglandin- $F_{2\alpha}$ (**16**)



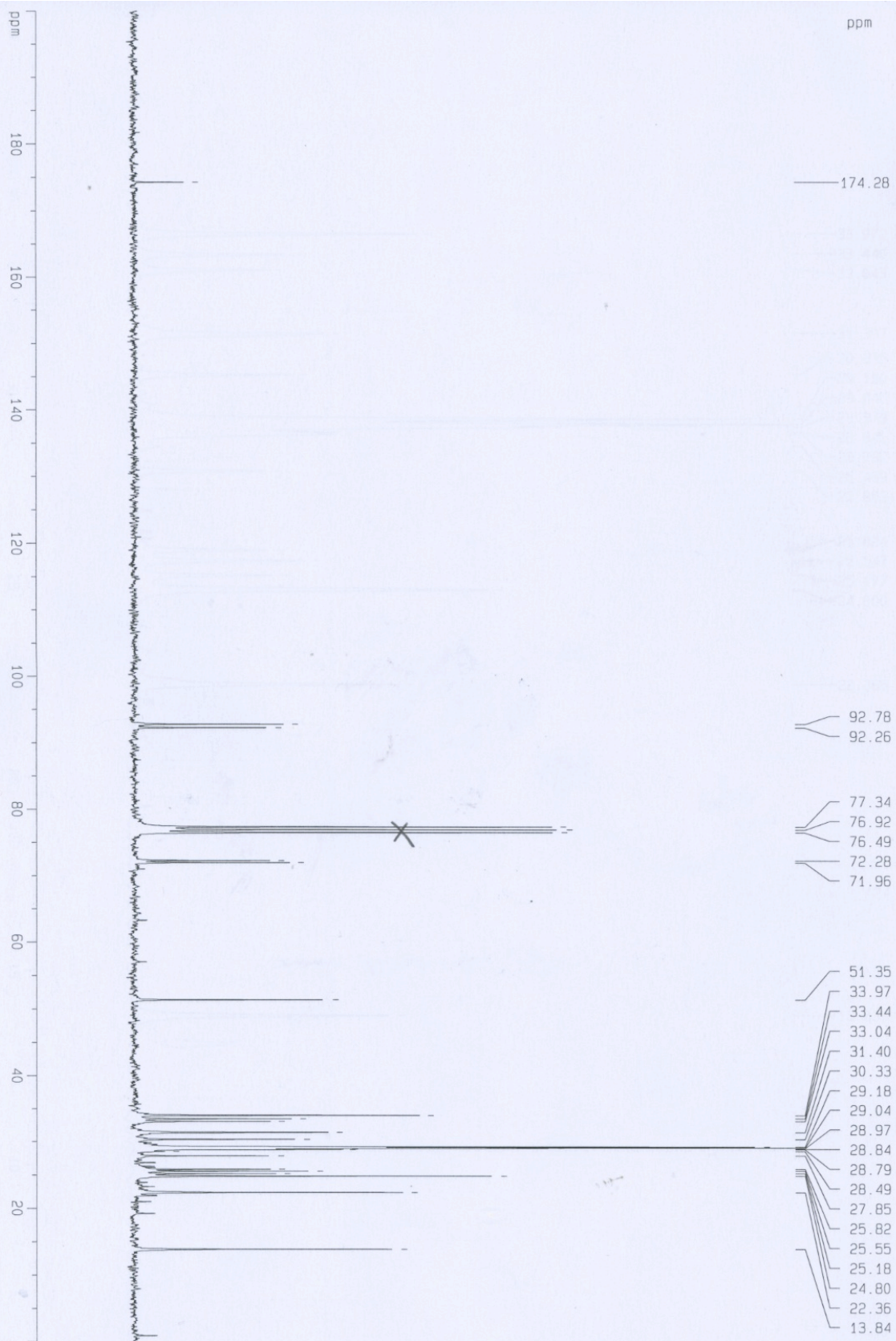
Prostaglandin- $F_{2\alpha}$ methyl ester,⁸ 2.5 mg (6.8 μ mol), afforded prostaglandin- $F_{2\alpha}$ (**16**), 2.2 mg, yield 90% (3% MeOH in EtOAc, R_f = 0.2). The NMR data of compound **16** were in perfect agreement with those reported in the literature.⁹

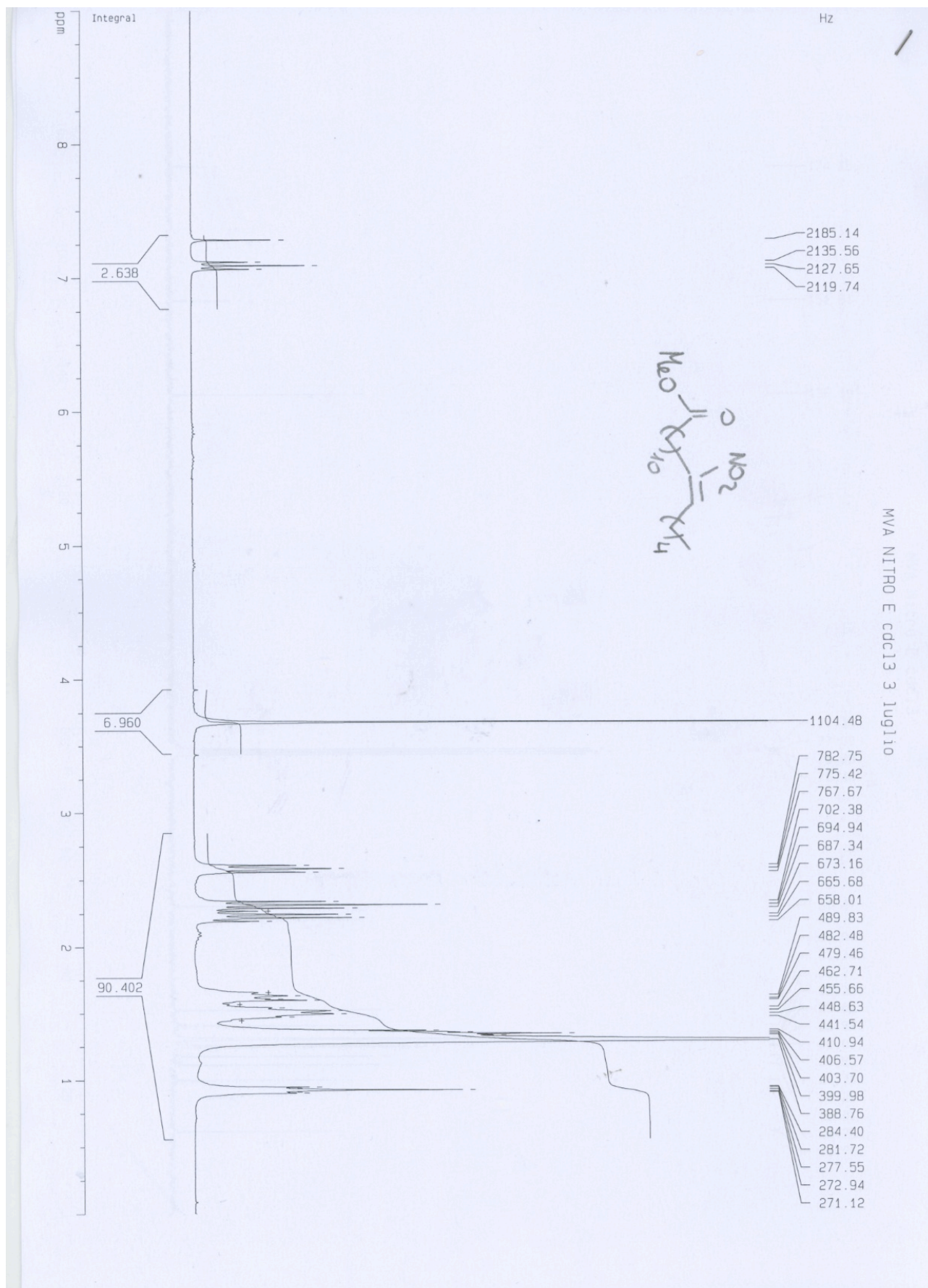
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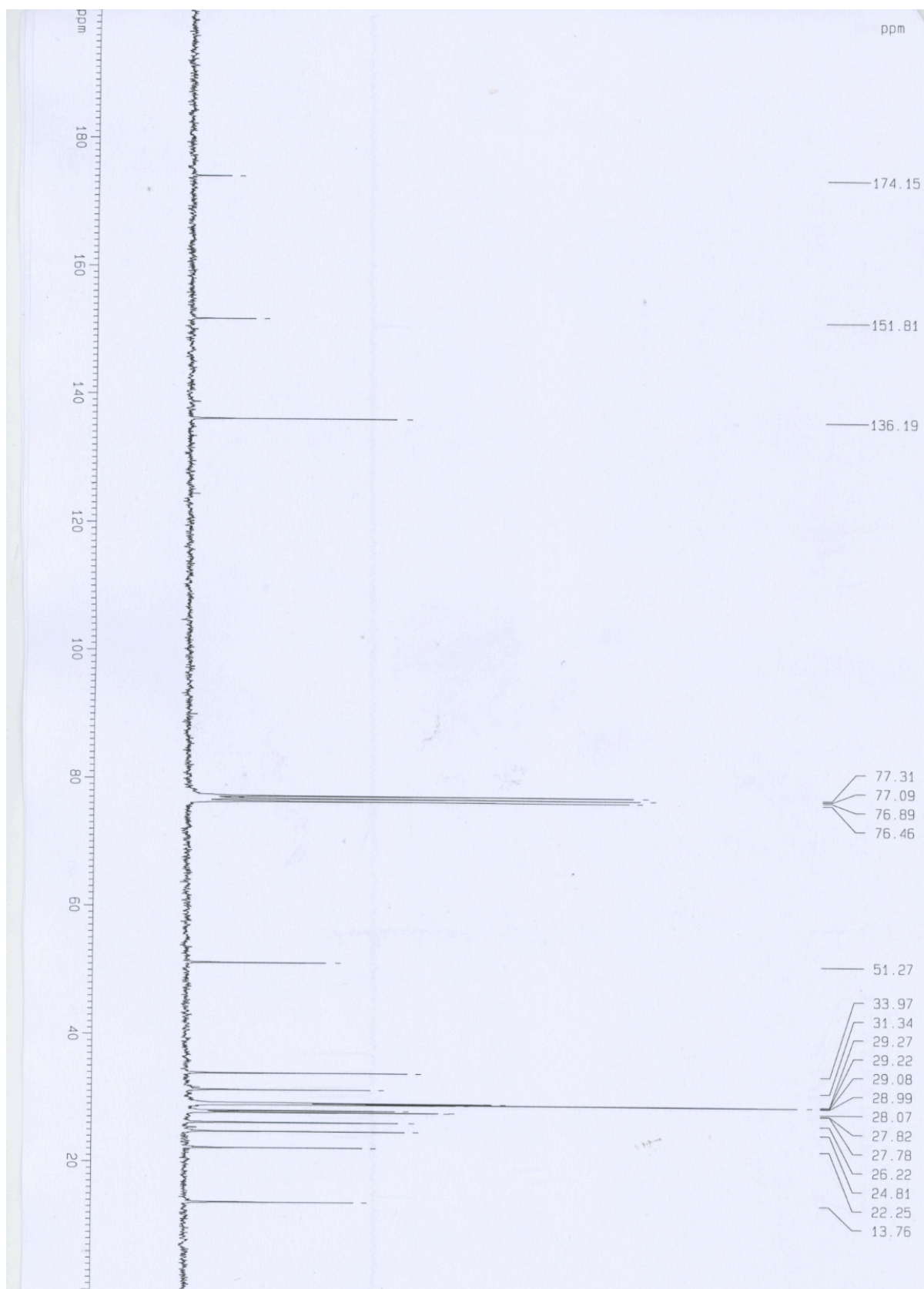


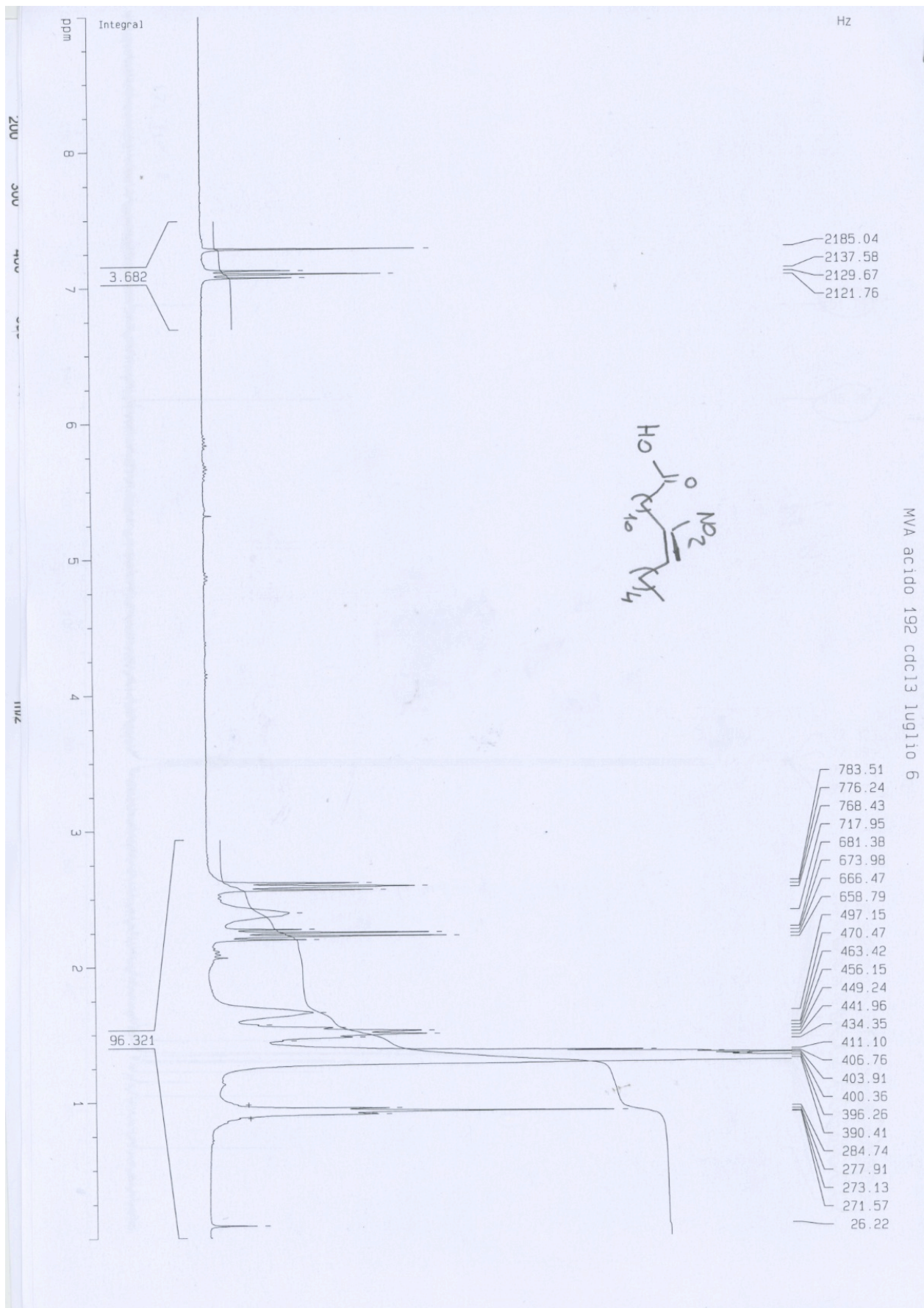
MVA 184 Cdc13



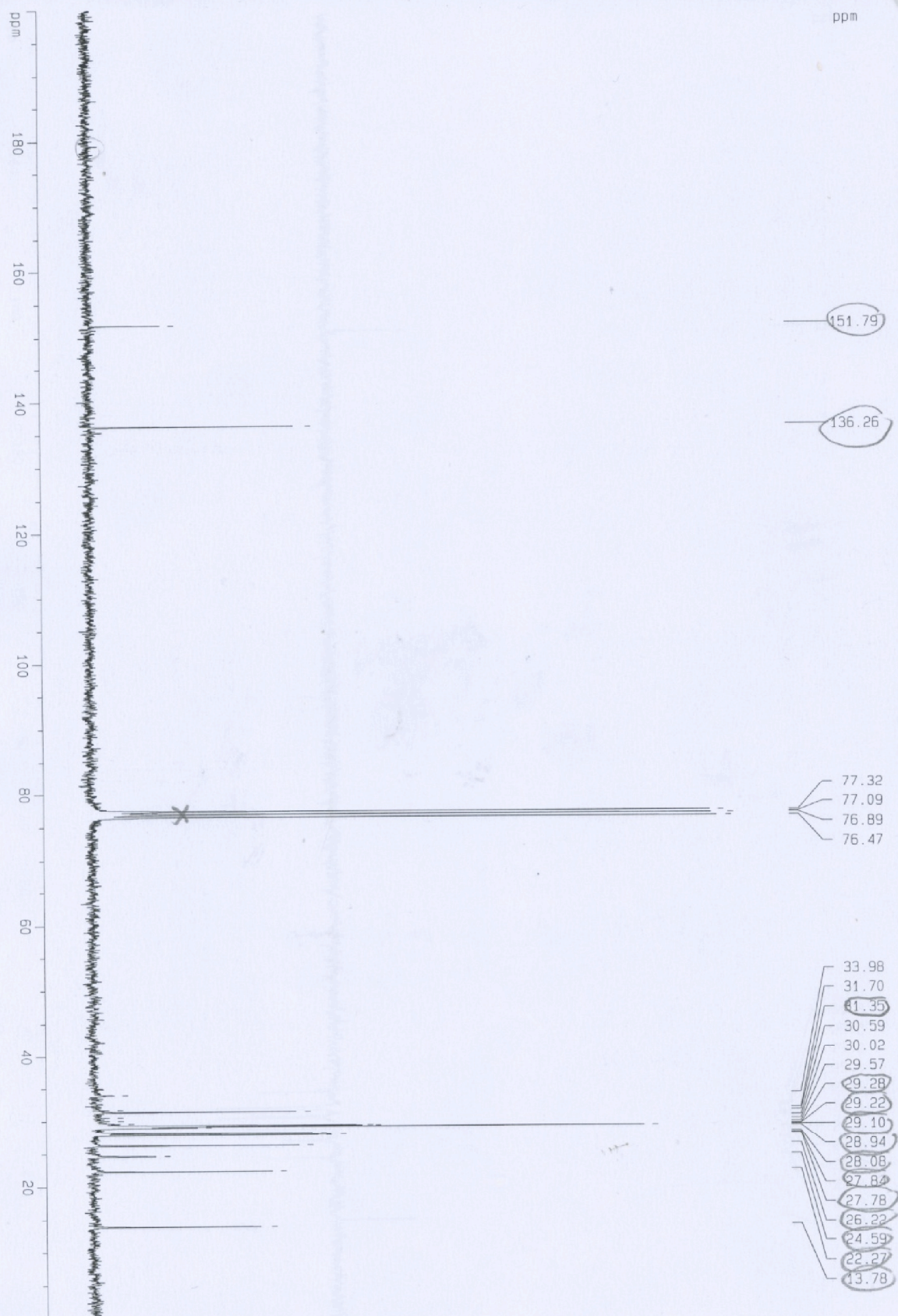


MVA Nitro E cdCl3

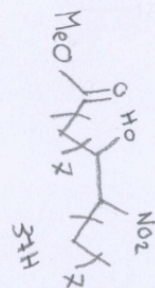




MVA Acido 192 cdcl3

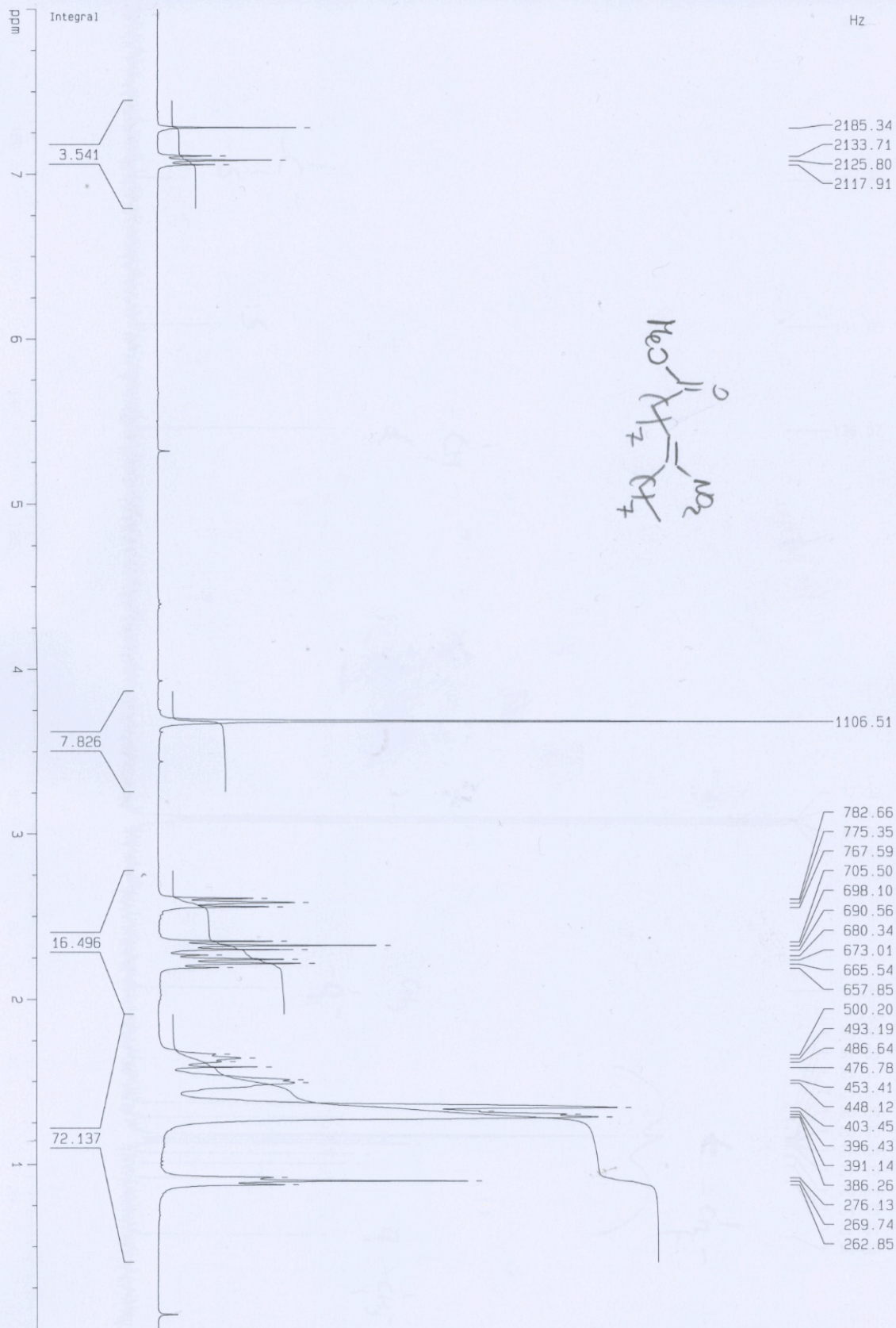


MVA - Nitro OH CC13

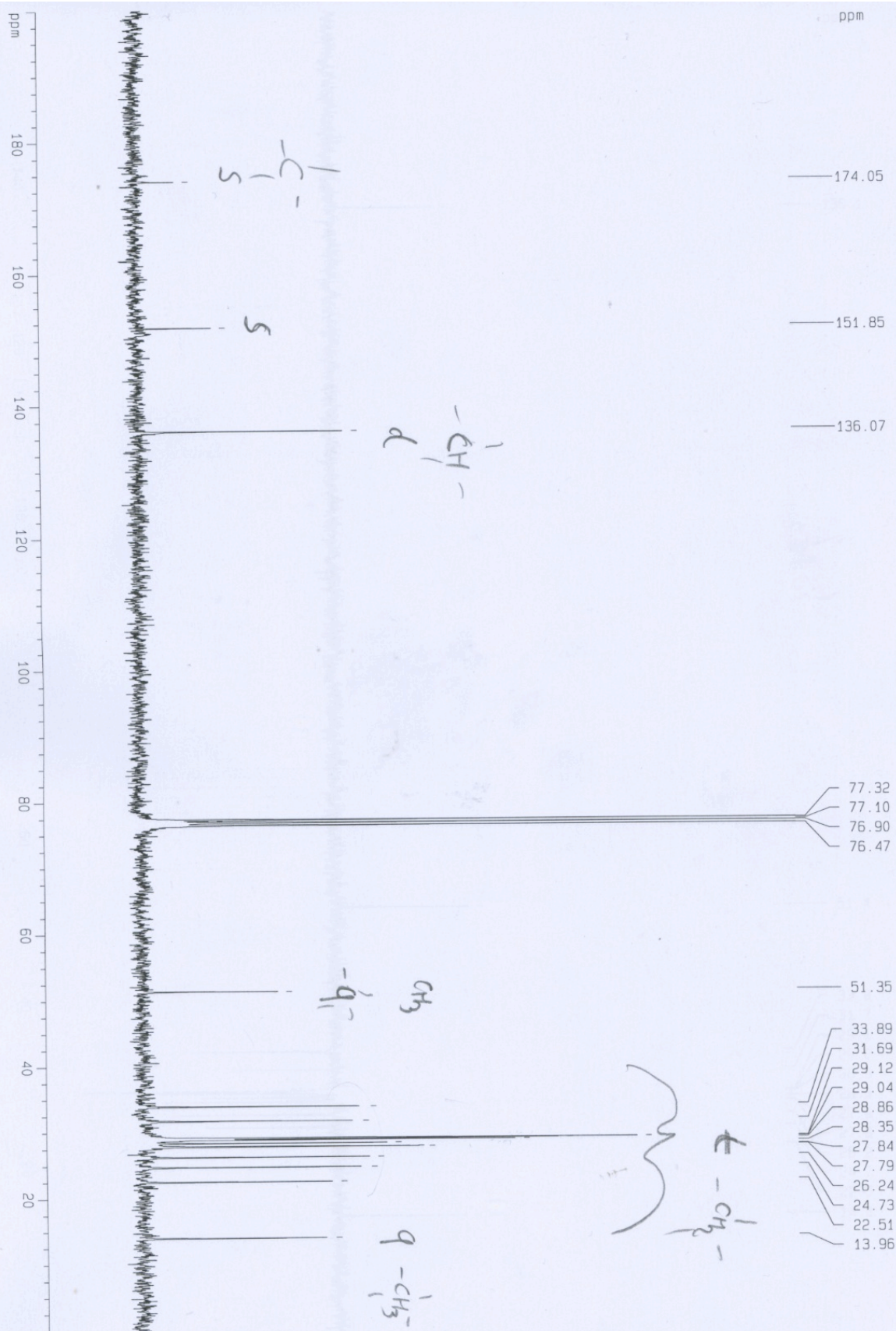


MVA Nitro-OH cdc13

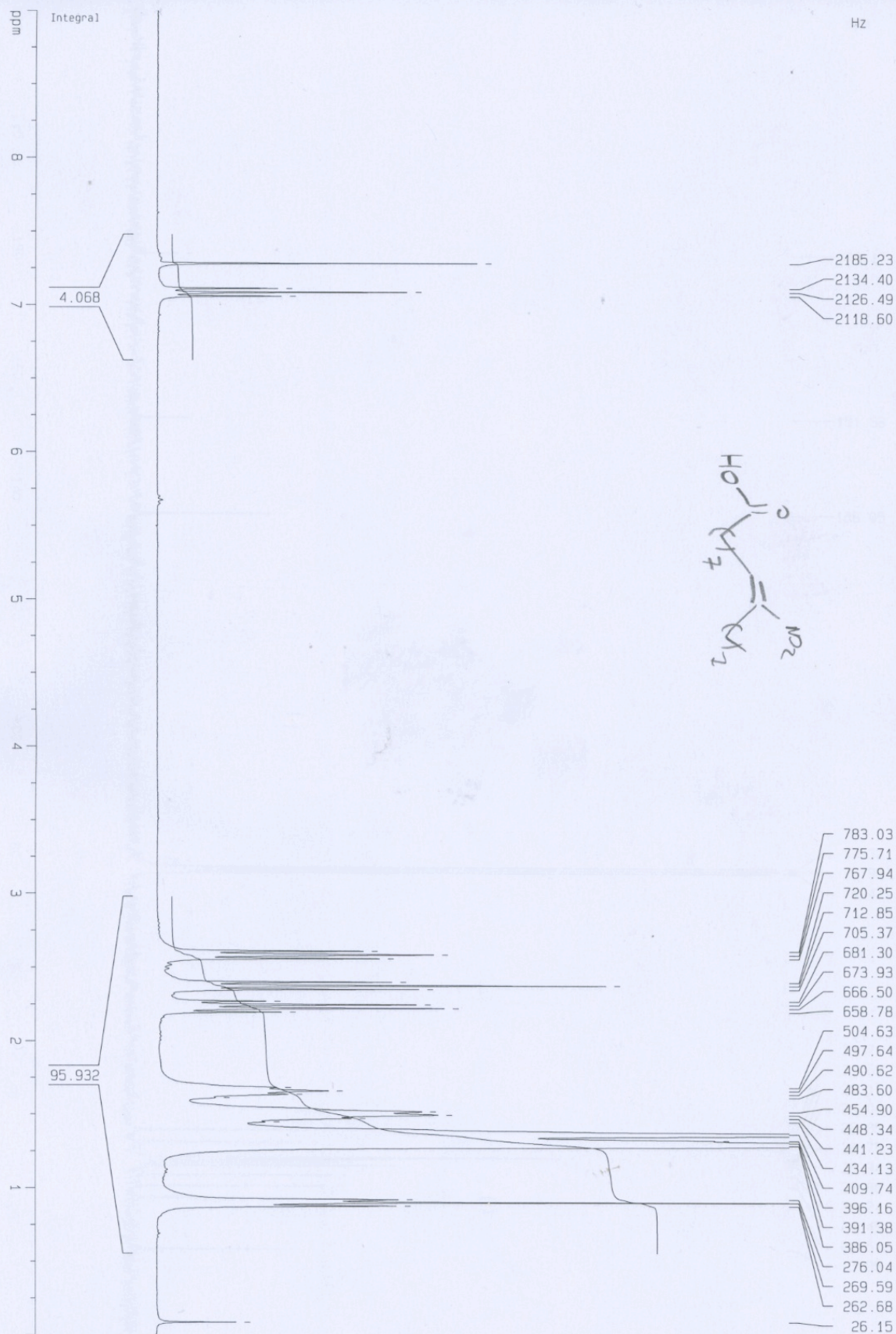
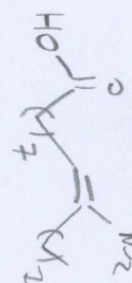




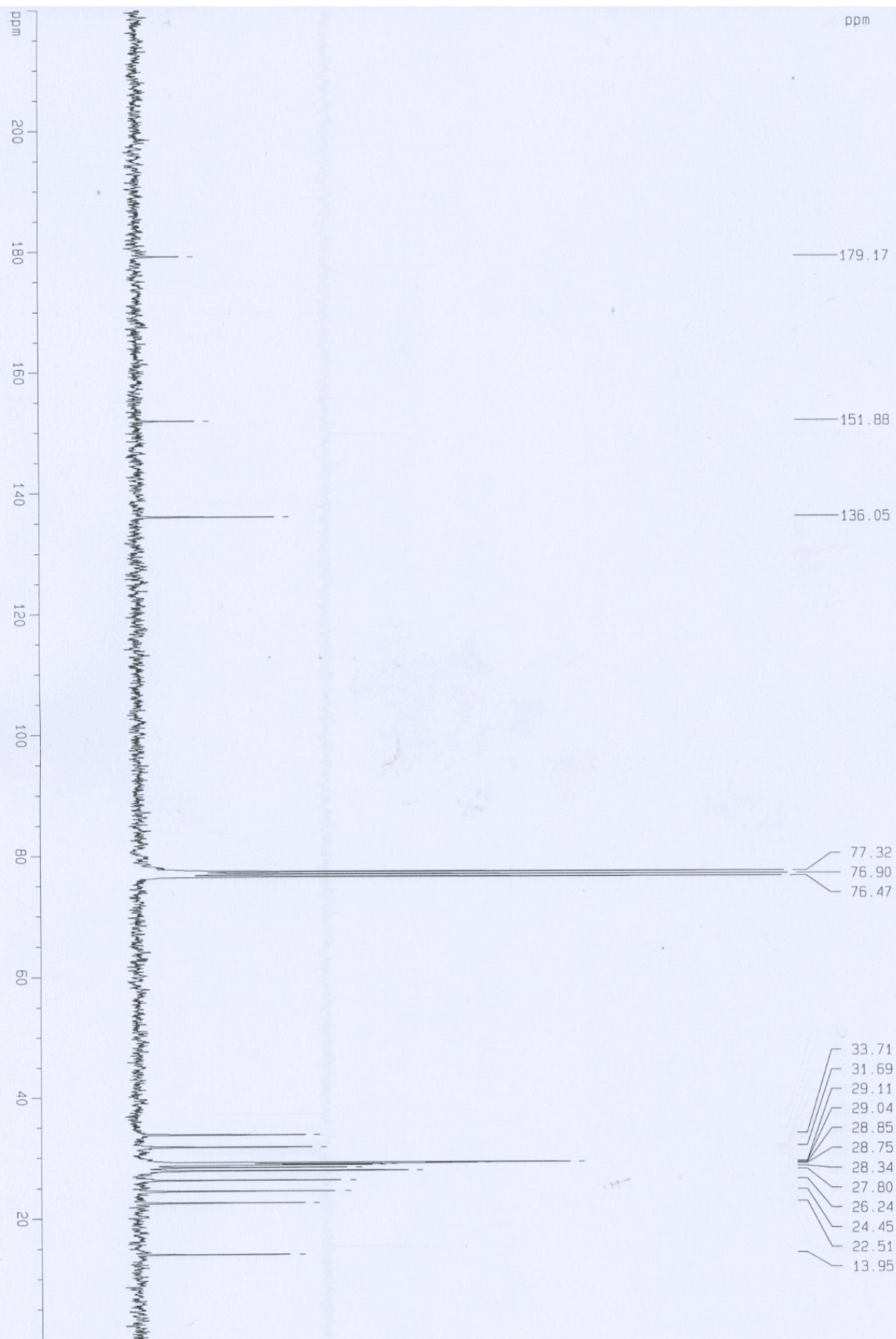
MVA 172" CDCl₃

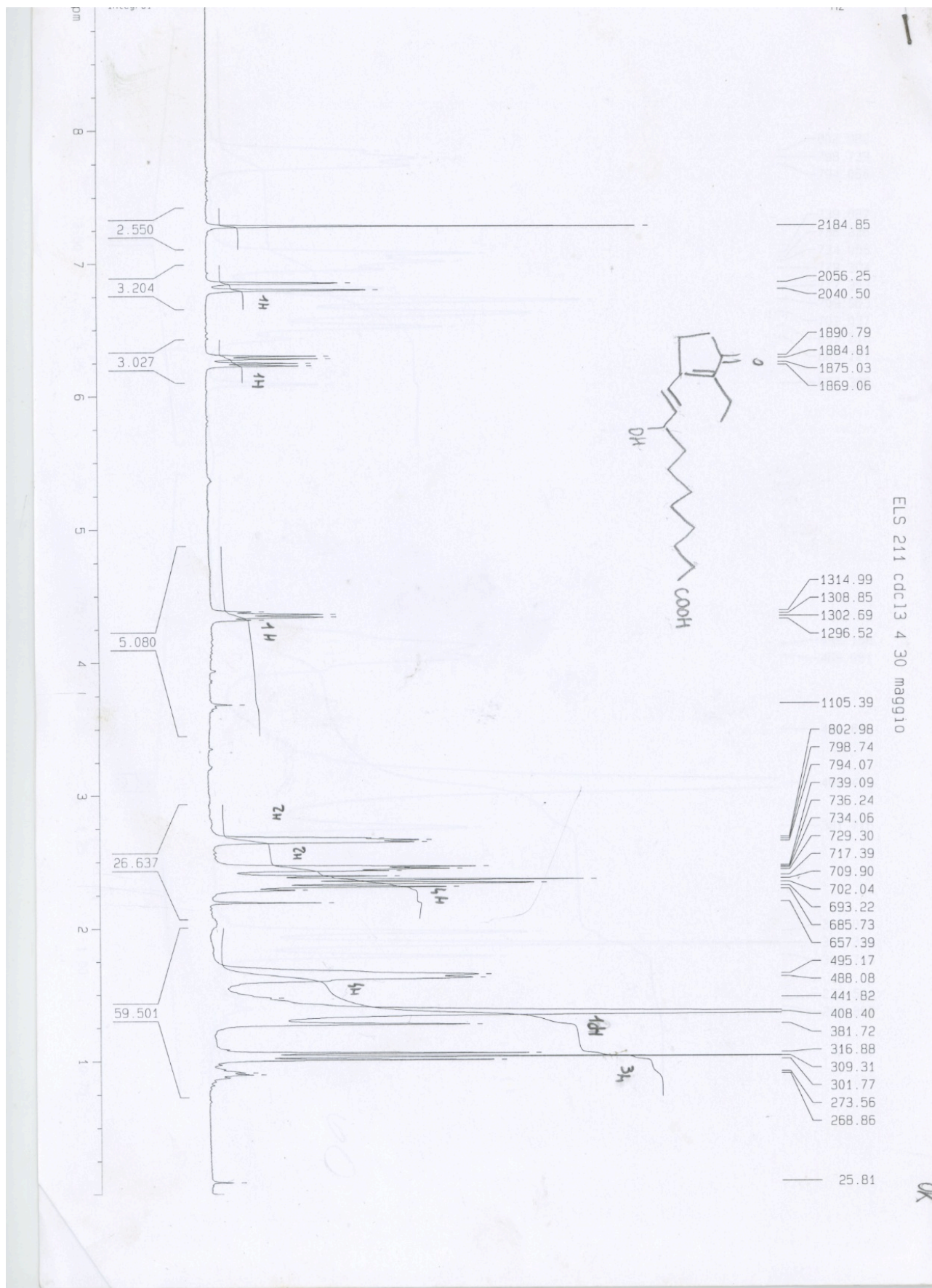


MVA 175" giugno 3 cdcl3

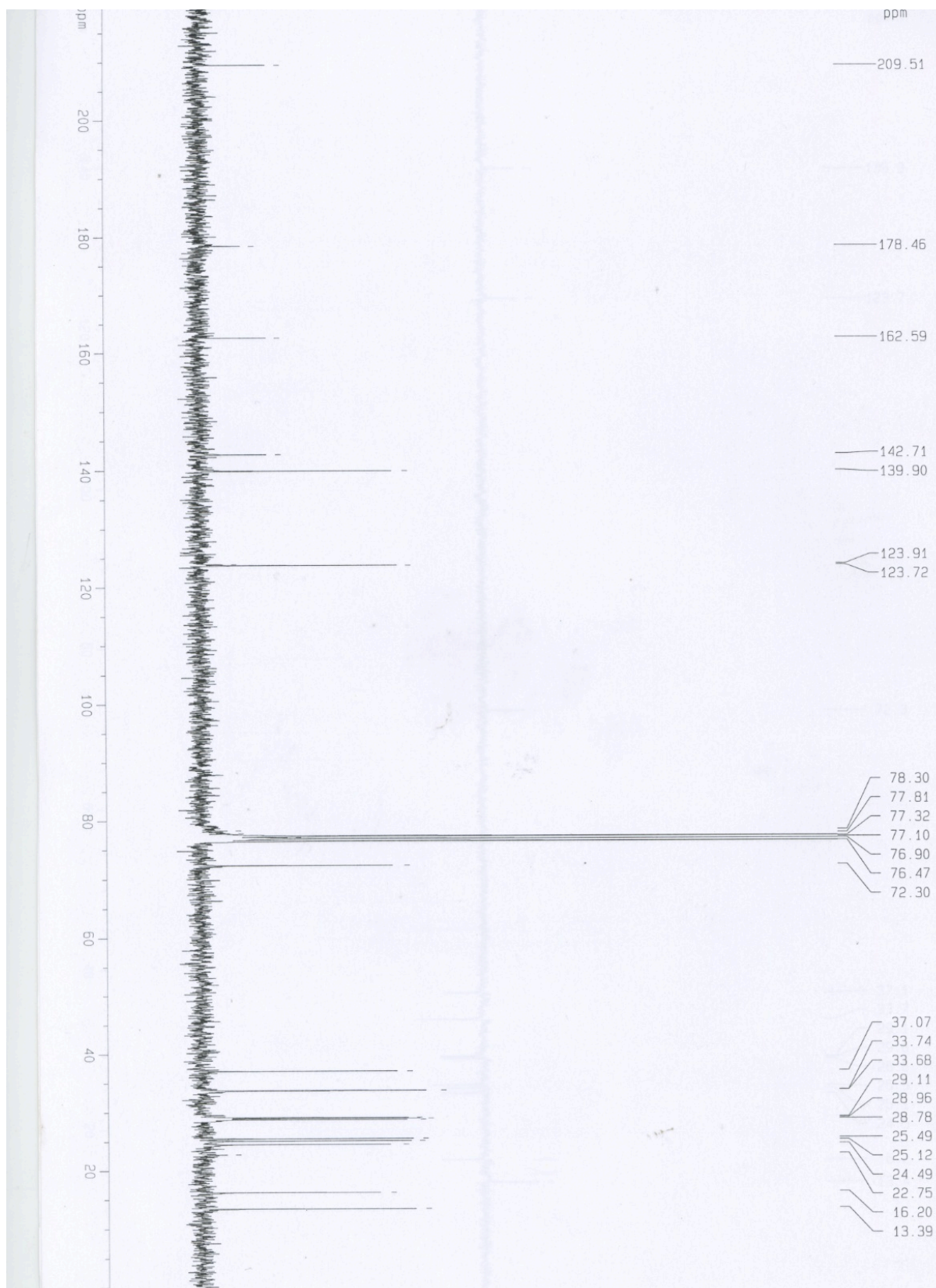


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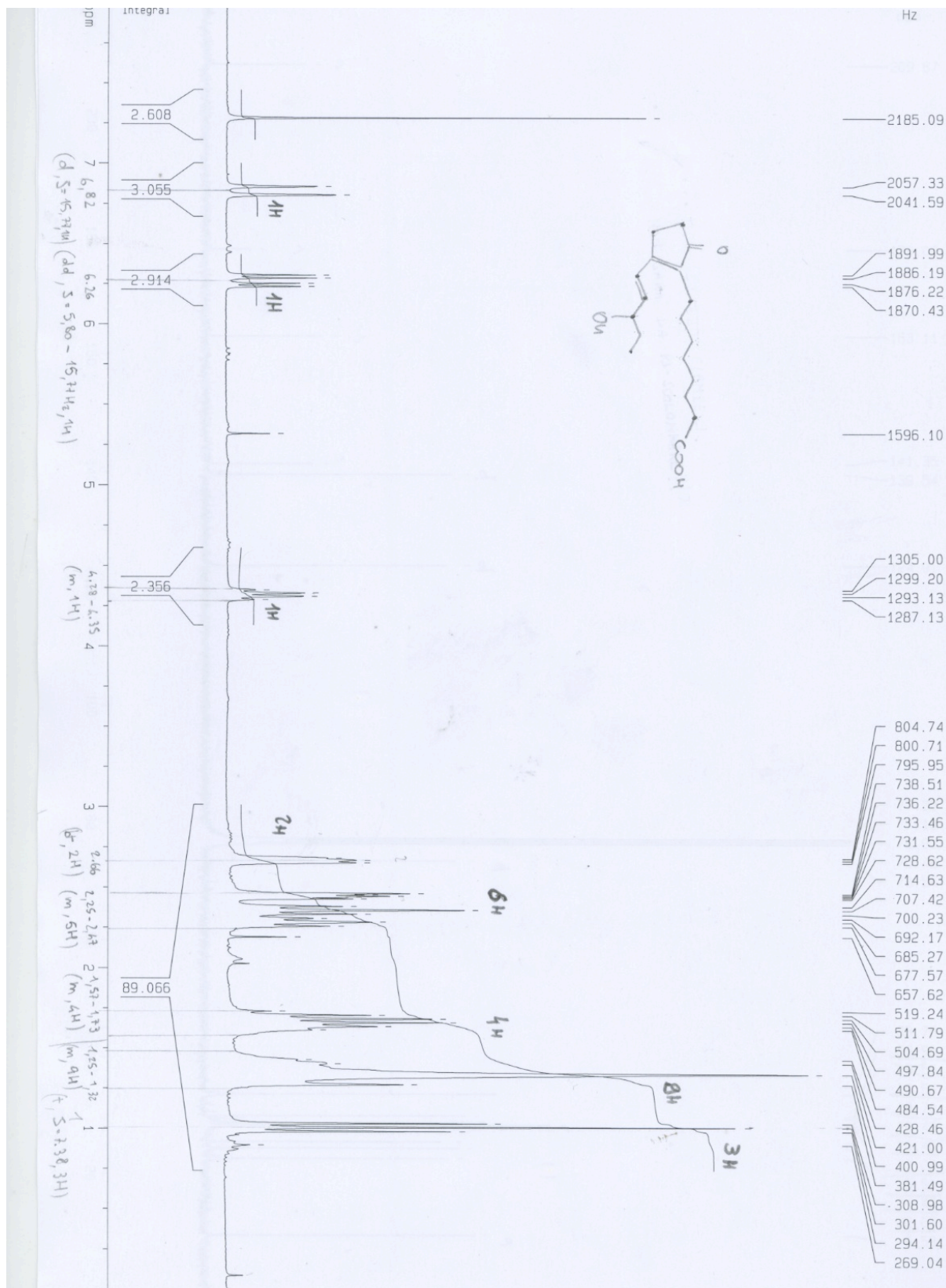




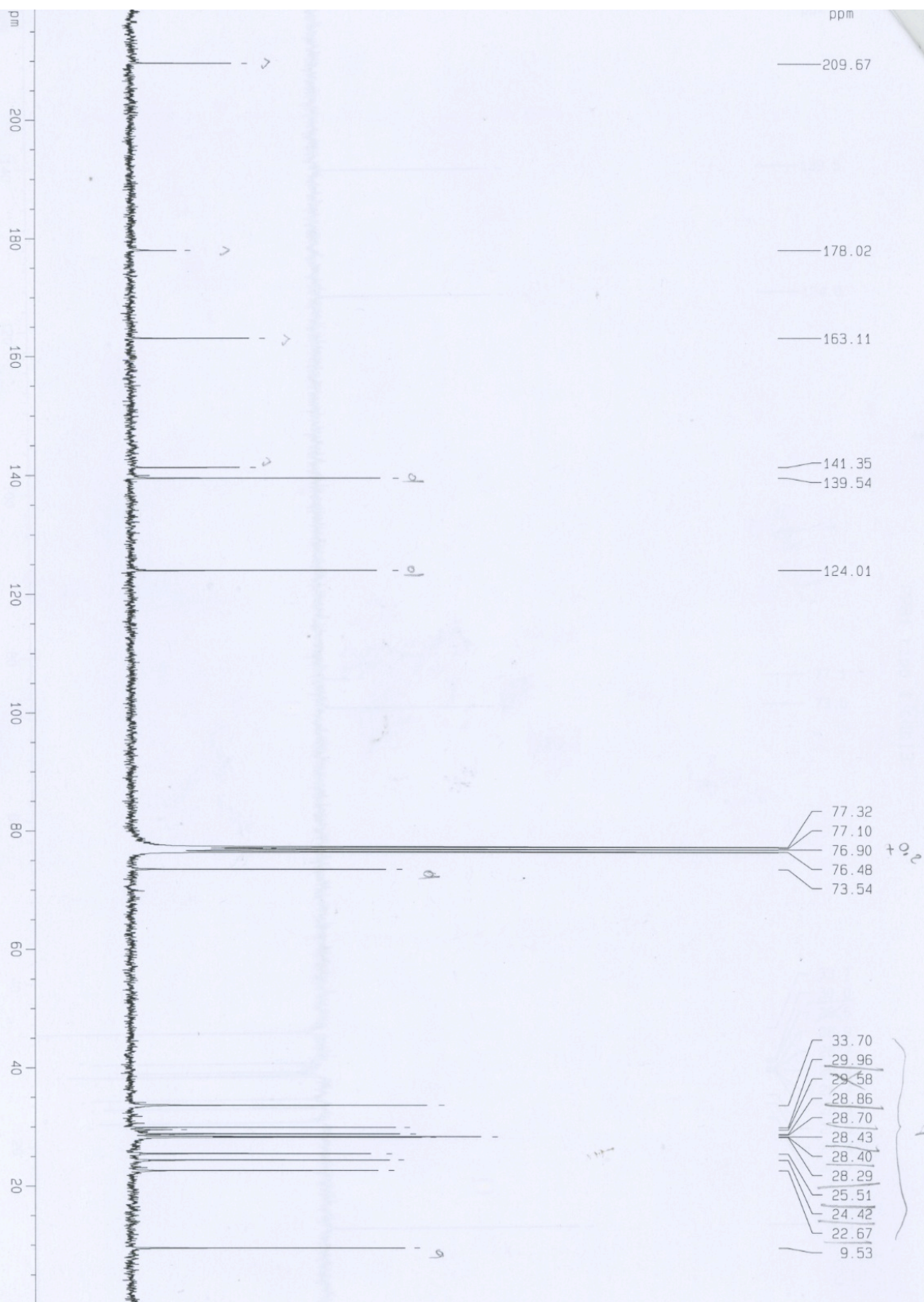
ELS 214 cdc13

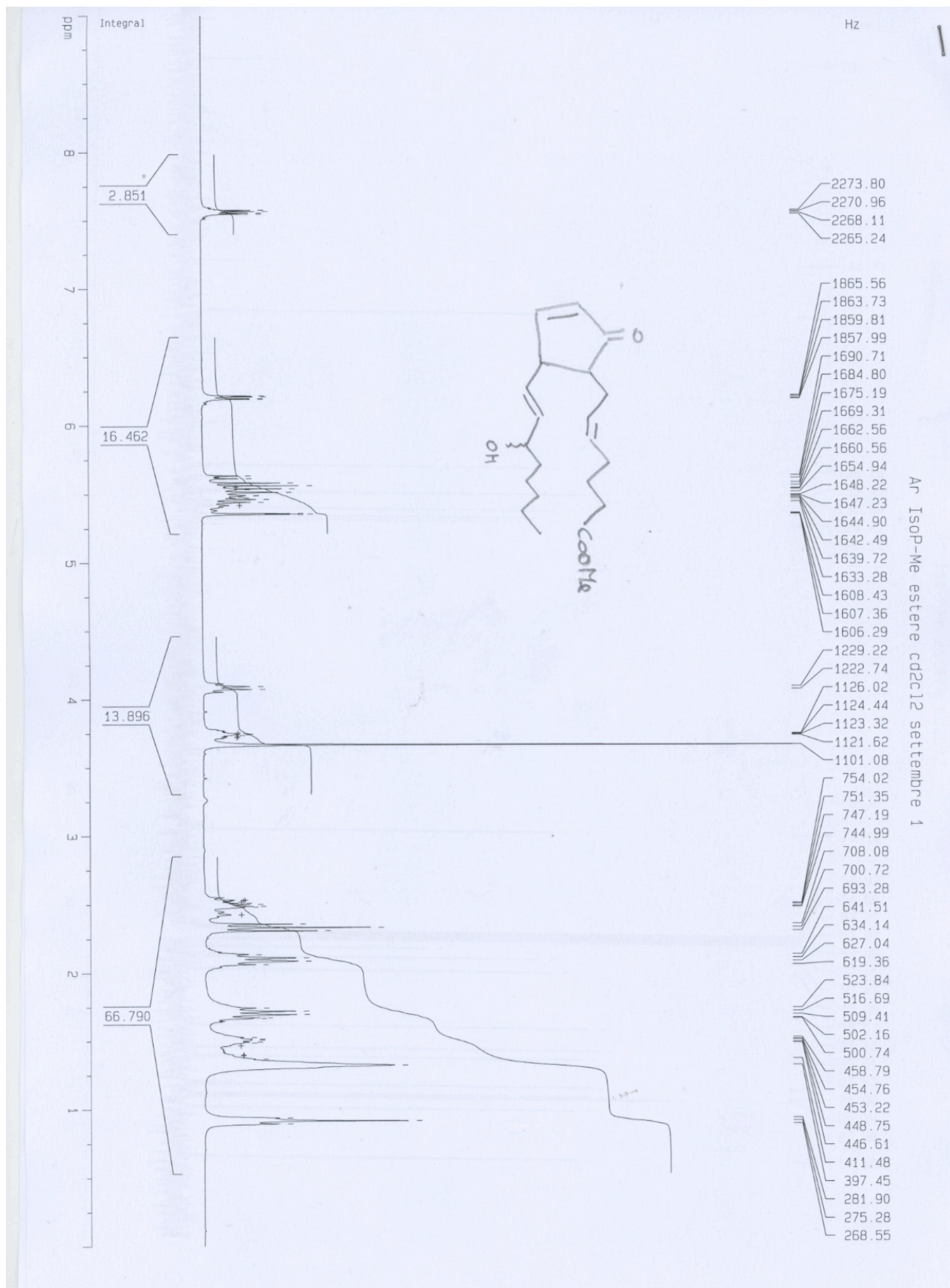


PPB4 tipo 1 cdc13 6 1 settembre



PPB1 tipo 1 cdc13





Ac-Isop-Me estere cd2c12

