# Non-linear Tendency Between Acyl Chain Length and Selectivity in Enzymatic Deacylation of Carboxylic Esters in Batch and Continuous-Flow

Tendência Não-linear entre Tamanho de Cadeia e Seletividade na Deacilação Enzimática de Ésteres Carboxílicos em Batelada e Fluxo Contínuo

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## Supplementary Material

### General Information

$^{1}$H NMR spectra were recorded on a Bruker DPX 200 MHz spectrometer. $^{13}$C NMR spectra were recorded on the same instrument at 50 MHz. The chemical shifts of $^{1}$H and $^{13}$C NMR were quoted relative to internal tetramethylsilane ($\delta_{TMS}$ = 0.00). $^{1}$H NMR data are reported as follows: chemical shift in ppm (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz) and relative intensity (integral). $^{13}$C NMR data are reported as chemical shift in ppm (δ).

Infrared spectra were recorded from KBr discs on a Bomem MB100 spectrometer coupled with FTIR. Maximum absorption ($\nu_{max}$) is reported in wavenumber ($cm^{-1}$).

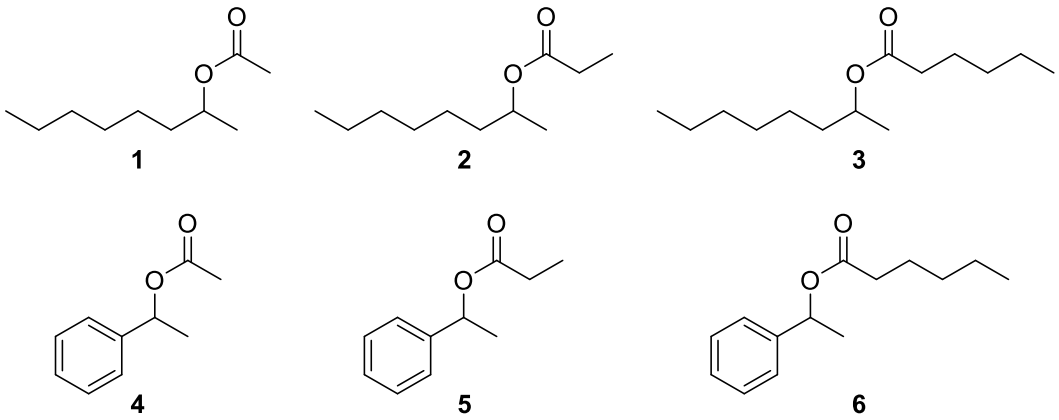
Chiral GC analyses were performed on a Shimadzu model GC-17A chromatograph (FID) equipped with Beta Dex 325 column (30 m × 0.25 mm diameter, 0.25 µm film thickness). The split ratio was 1:50 and $N_2$ was used as carrier gas. The injector and detector temperatures were set at 220°C. 2-octanol and 1-phenyl ethanol were derivatized to their corresponding propionates or acetates before analysis.

*Temperature programming for compound **2**:* 40 °C for 1 min, increasing in 2 °C $min^{-1}$ up to 120 °C, kept at 120 °C for 3 min. Retention times (min): (*S*)-**1**: 33.7, (*R*)-**1**: 35.2, (*S*)-**2**: 39.6, (*R*)-**2**: 40.1.

*Temperature programming for compound 3:* 60 °C for 1 min, increasing in 1 °C $min^{-1}$ up to 200 °C. Retention times (min): (*S*)-**1**: 34.4, (*R*)-**1**: 37.1, (*S*)-**2**: 79.6, (*R*)-**2**: 79.9.

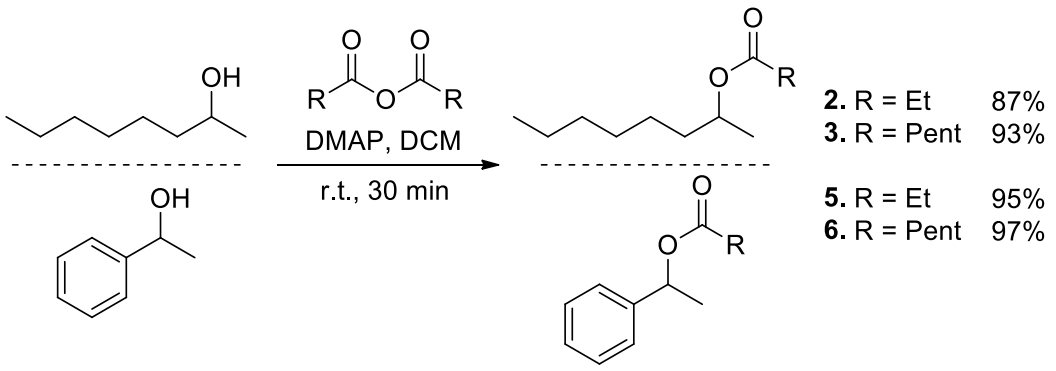
*Temperature programming for compound **5**:* 80 °C for 1 min, increasing in 0.5 °C $min^{-1}$ up to 200 °C. Retention times (min): (*S*)-**4**: 38.4, (*R*)-**4**: 40.7, (*S*)-**5**: 53.6, (*R*)-**5**: 54.1.

*Temperature programming for compound **6**:* 110 °C, increasing in 1 °C $min^{-1}$ up to 120 °C, kept at 120 °C for 5 min, increasing in 10 °C $min^{-1}$ up to 210 °C, kept at 210 °C for 20 min. Retention times (min): (*S*)-**4**: 13.8, (*R*)-**4**: 14.3, (*RS*)-**6**: 22.4.




**Experimental Procedures**

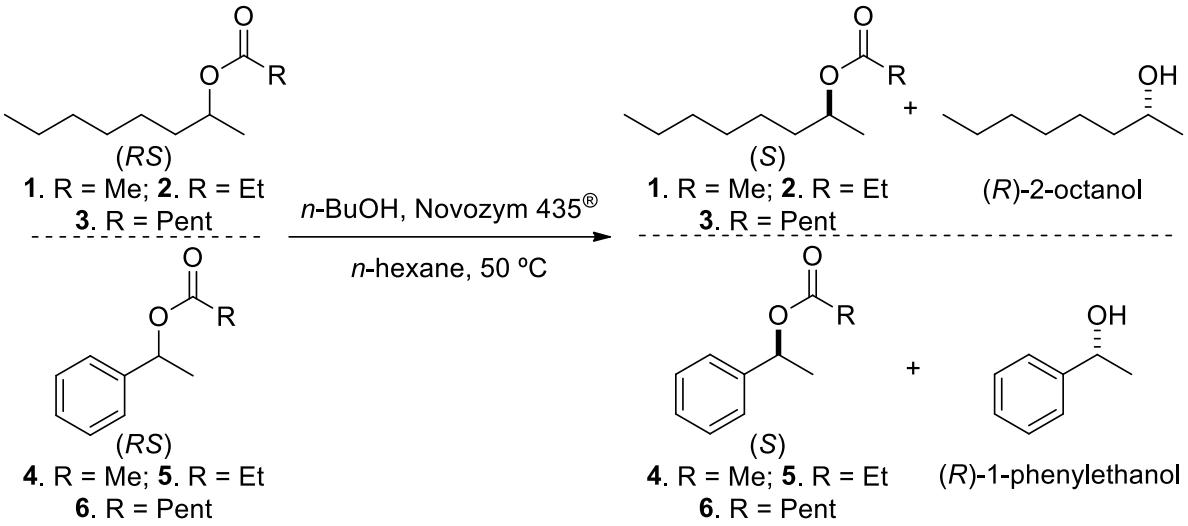
**Syntheses of Racemic Esters 2, 3, 5 and 6**




To a solution of corresponding alcohol (2-octanol, 3 mmol, 390 mg; 1-phenylethanol, 3 mmol, 366 mg) in dichloromethane (5 mL), propionic anhydride (4 mmol, 0.51 mL) or hexanoic anhydride (4 mmol, 0.92 mL) and DMAP (1 crystal) were added. The reaction mixture was kept under magnetic stirring for 30 min at room temperature, then neutralized with aqueous $NaHCO_3$ (3 x 5

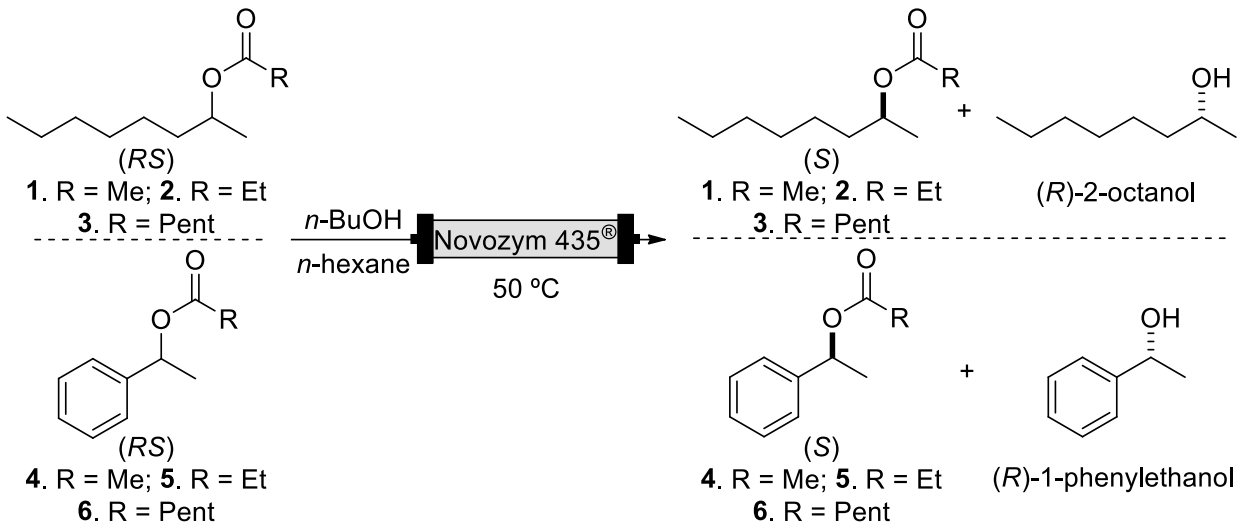
mL). The organic phase was dried over anhydrous $MgSO_4$, filtered through silica and the solvent was evaporated under reduced pressure.

**Enzymatic Kinetic Resolution in Batch Mode**



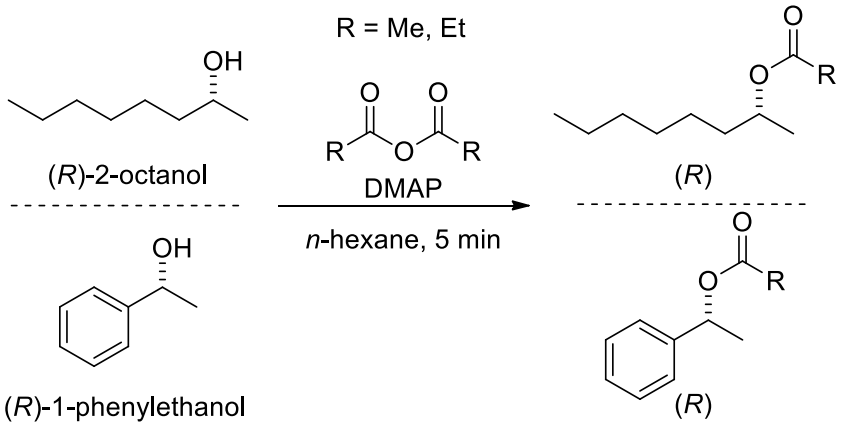

In a sealed vial (4 mL), esters **1–6** (0.1 mmol) were dissolved in *n*-hexane (2 mL). *n*-butanol (37 µL, 0.4 mmol) and the supported enzyme CAL-B (Novozym 435®; 20 mg) were then added to this solution. The reaction mixture was stirred at constant temperature (50 °C), and aliquots of 200 µL were periodically taken, diluted in 300 µL of *n*-hexane, derivatized and analyzed by chiral GC.

**Enzymatic Kinetic Resolution in Continuous-Flow Mode**




Esters **1–6** (0.5 mmol) and *n*-butanol (0.18 mL, 2 mmol) were dissolved in *n*-hexane (5 mL) and the solution was eluted through a packed-bed column with the biocatalyst (200 mg) with a flow rate of 0.1 mL $min^{-1}$ for two cycles at 50 °C. Aliquots from each cycle were collected, derivatized and analyzed by chiral GC. The internal volume of the reactor was equal to 0.43 mL.

Derivatization to Acetate or Propionate




Acetic anhydride or propionic anhydride (5 µL) and DMAP (1 crystal) were added directly to the reaction aliquot and it was maintained under magnetic stirring for 5 min. The aliquot was neutralized with aqueous $NaHCO_3$ and the organic layer was dried over anhydrous $MgSO_4$ before analysis.

## Chromatograms

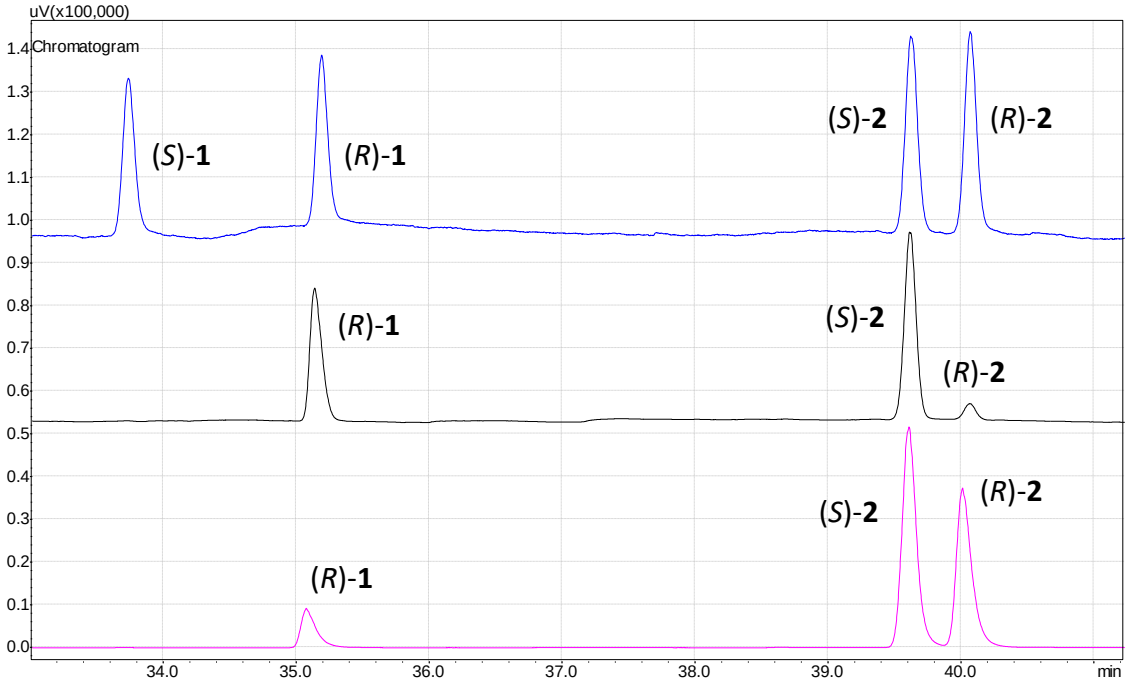



**Figure S1.** Chromatograms of racemic standards (blue), batch EKR aliquot (black) and continuous-flow EKR aliquot (pink) of compound **2**

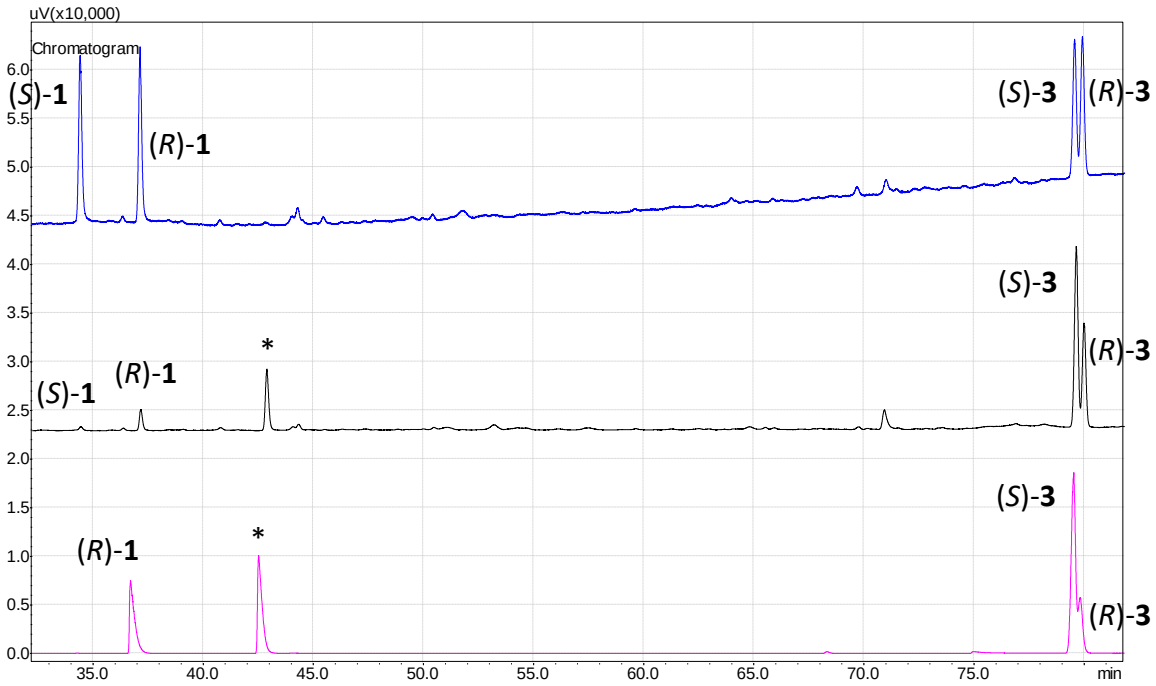



**Figure S2.** Chromatograms of racemic standards (blue), batch EKR aliquot (black) and continuous-flow EKR aliquot (pink) of compound **3**. * acetic acid from derivatization

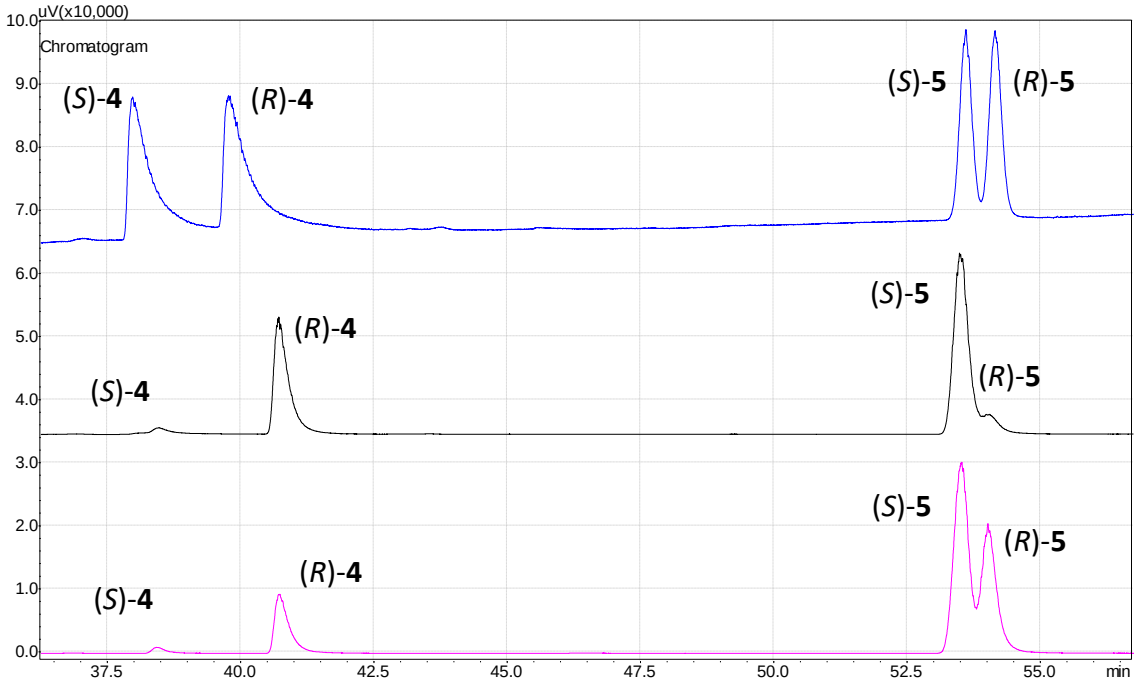



**Figure S3.** Chromatograms of racemic standards (blue), batch EKR aliquot (black) and continuous-flow EKR aliquot (pink) of compound **5**

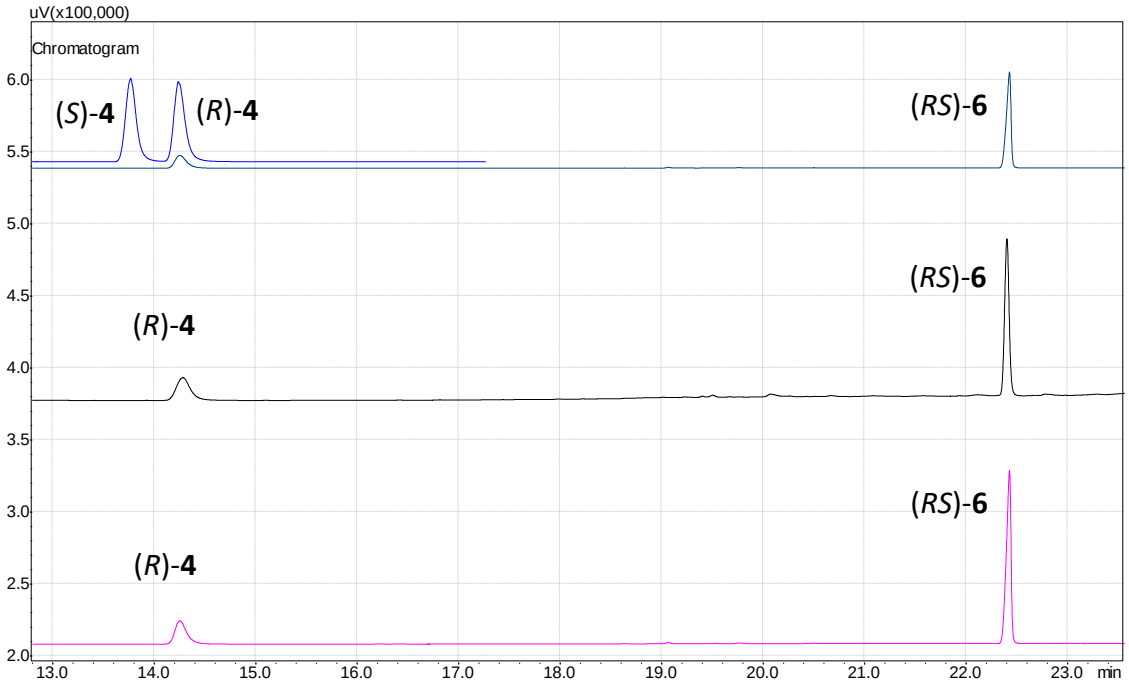
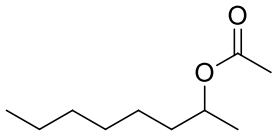



**Figure S4.** Chromatograms of racemic standards (blue), batch EKR aliquot (black) and continuous-flow EKR aliquot (pink) of compound **6**



$C_{10}H_{20}O_2$
M.M: 172.26 g $mol^{-1}$

2-Octyl acetate (**1**)

GC-MS (70 eV), *m/z* (relative intensity) 129 (1%), 112 (11%), 102 (5%), 97 (7%), 87 (54%), 83 (17%), 70 (24%), 58 (10%), 55 (17%), 43 (100%).

$^1$H NMR (200 MHz, $CDCl_3$, TMS), $\delta$ 0.88 (t, $J$ = 6.3 Hz, 3H), 1.20 (d, $J$ = 6.3 Hz, 3H), 1.28-1.57 (m, 10H), 2.02 (s, 3H), 4.81-4.97 (m, 1H).

$^{13}$C NMR (50 MHz - $CDCl_3$), $\delta$ 14.0, 20.0, 21.3, 22.5, 25.3, 29.1, 31.7, 35.9, 71.0, 170.7.

IR (KBr) $\nu$/$cm^{-1}$ 2929, 2863, 1738, 1467, 1371, 1238.

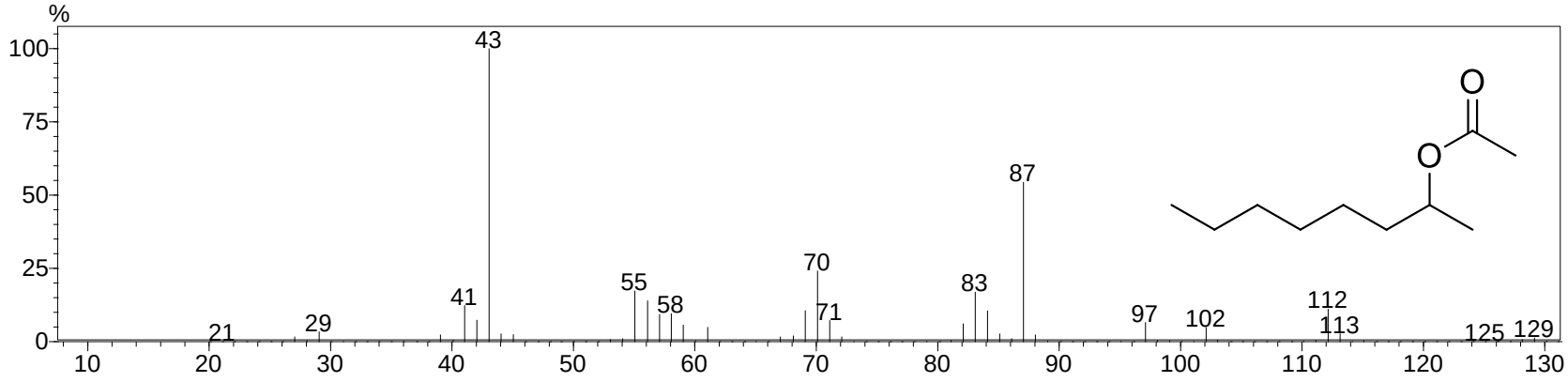



**Figure S5.** Mass spectrum of compound **1**

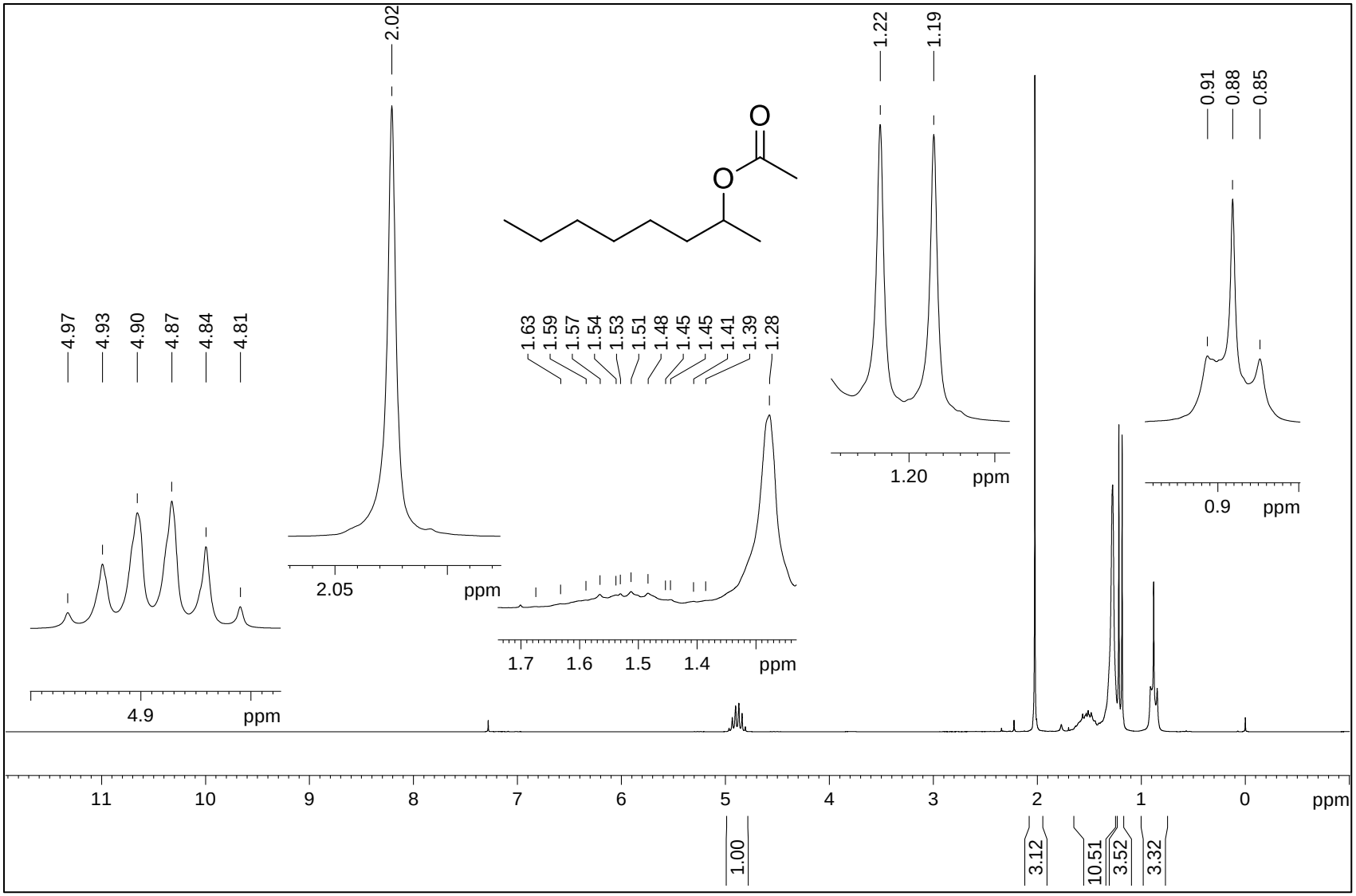



**Figure S6.** $^{1}H$ NMR (200 MHz, $CDCl_3$, TMS) spectrum of compound **1**

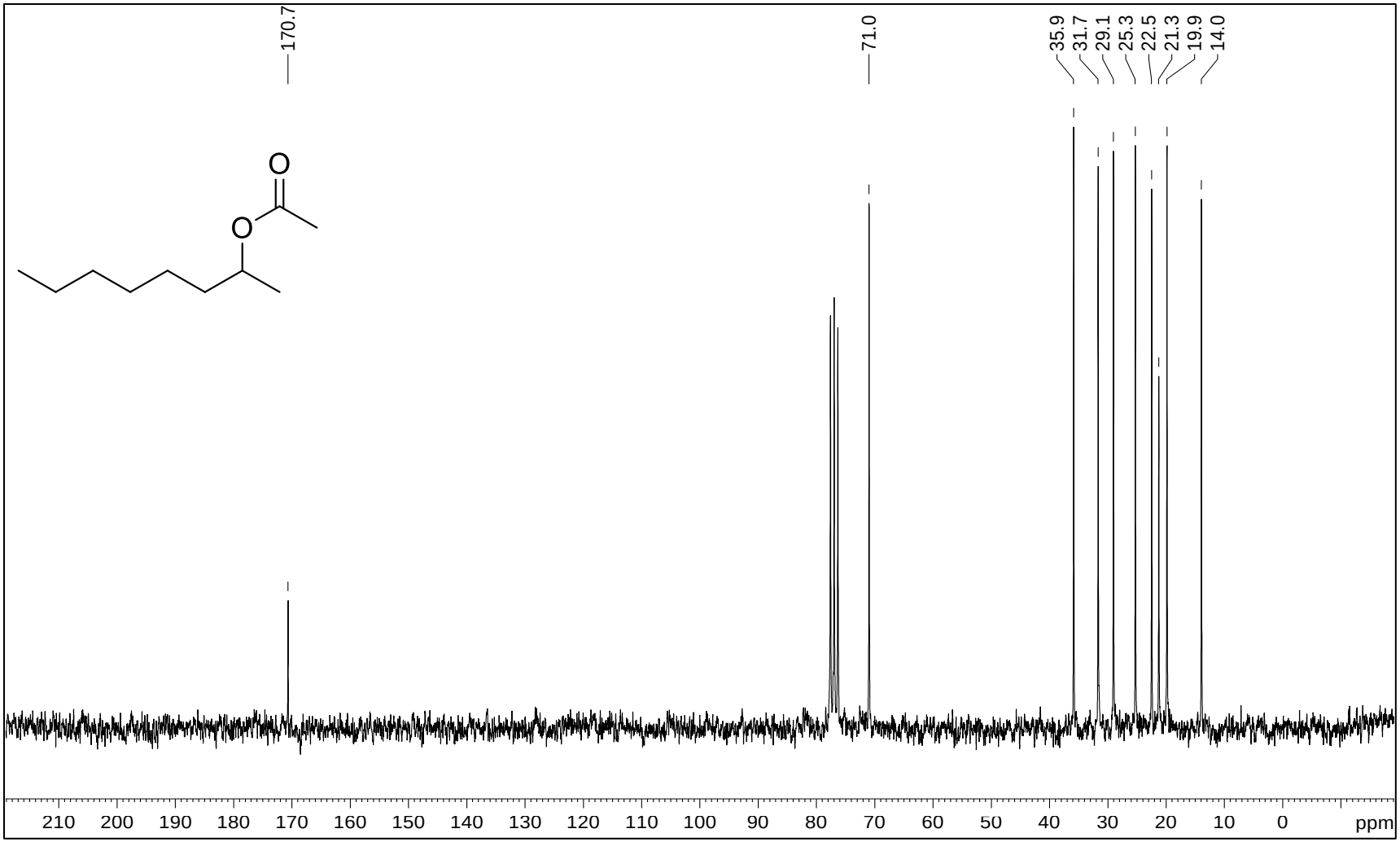



**Figure S7.** $^{13}C$ NMR (50 MHz, $CDCl_3$) spectrum of compound **1**

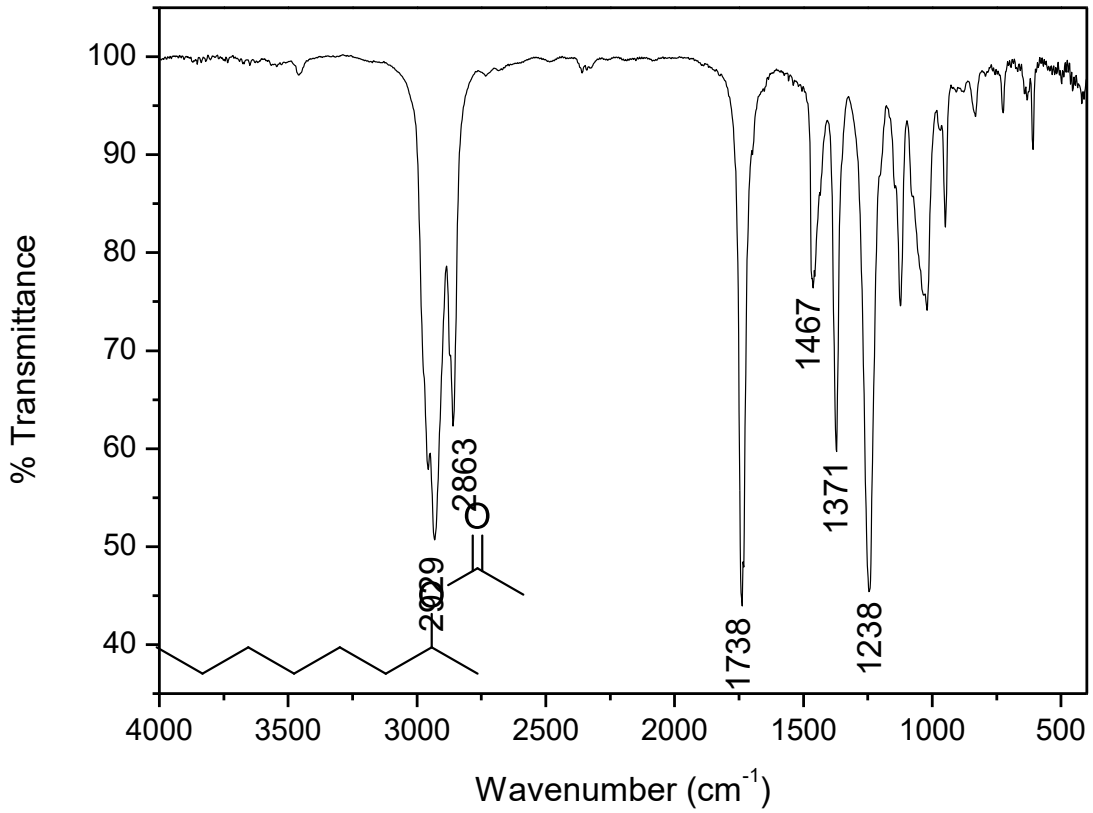
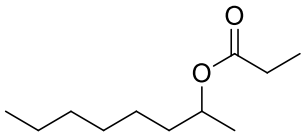



**Figure S8.** IR spectrum of compound **1**




$C_{11}H_{22}O_2$
186.29

2-Octyl propionate (**2**)

Yield: 87%. Colourless liquid.

GC-MS (70 eV), *m/z* (relative intensity) 129 (1%), 116 (1%), 112 (10%), 101 (27%), 97 (3%), 83 (12%), 75 (8%), 70 (18%), 57 (100%), 43 (11%), 29 (9%).

$^{1}$H NMR (200 MHz, $CDCl_3$, TMS), $\delta$ 0.89 (t, $J$ = 6.3 Hz, 3H); 1.14 (t, $J$ = 7.5 Hz, 3H); 1.21 (d, $J$ = 6.2 Hz, 3H); 1.24–1.70 (m, 10H); 2.30 (q, $J$ = 7.5 Hz, 2H); 4.91 (sext, $J$ = 6.2 Hz, 1H).

$^{13}$C NMR (50 MHz, $CDCl_3$), $\delta$ 9.1; 13.9; 19.9; 22.5; 25.3; 27.9; 29.0; 31.7; 35.9; 70.7; 174.1.

IR (KBr) $\nu$/cm$^{-1}$ 2936, 2856, 1731, 1459, 1187.

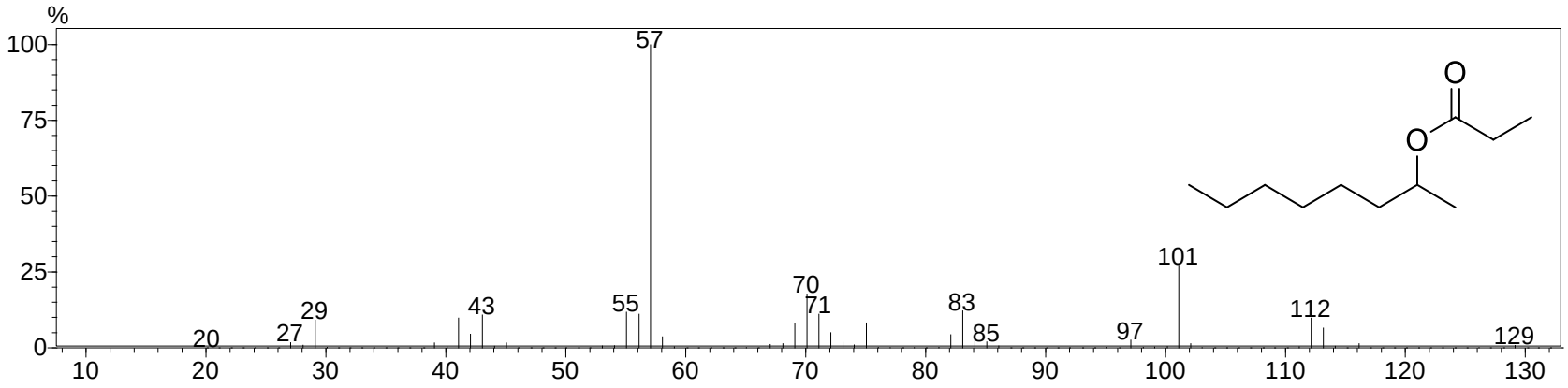



**Figure S9.** Mass spectrum of compound **2**

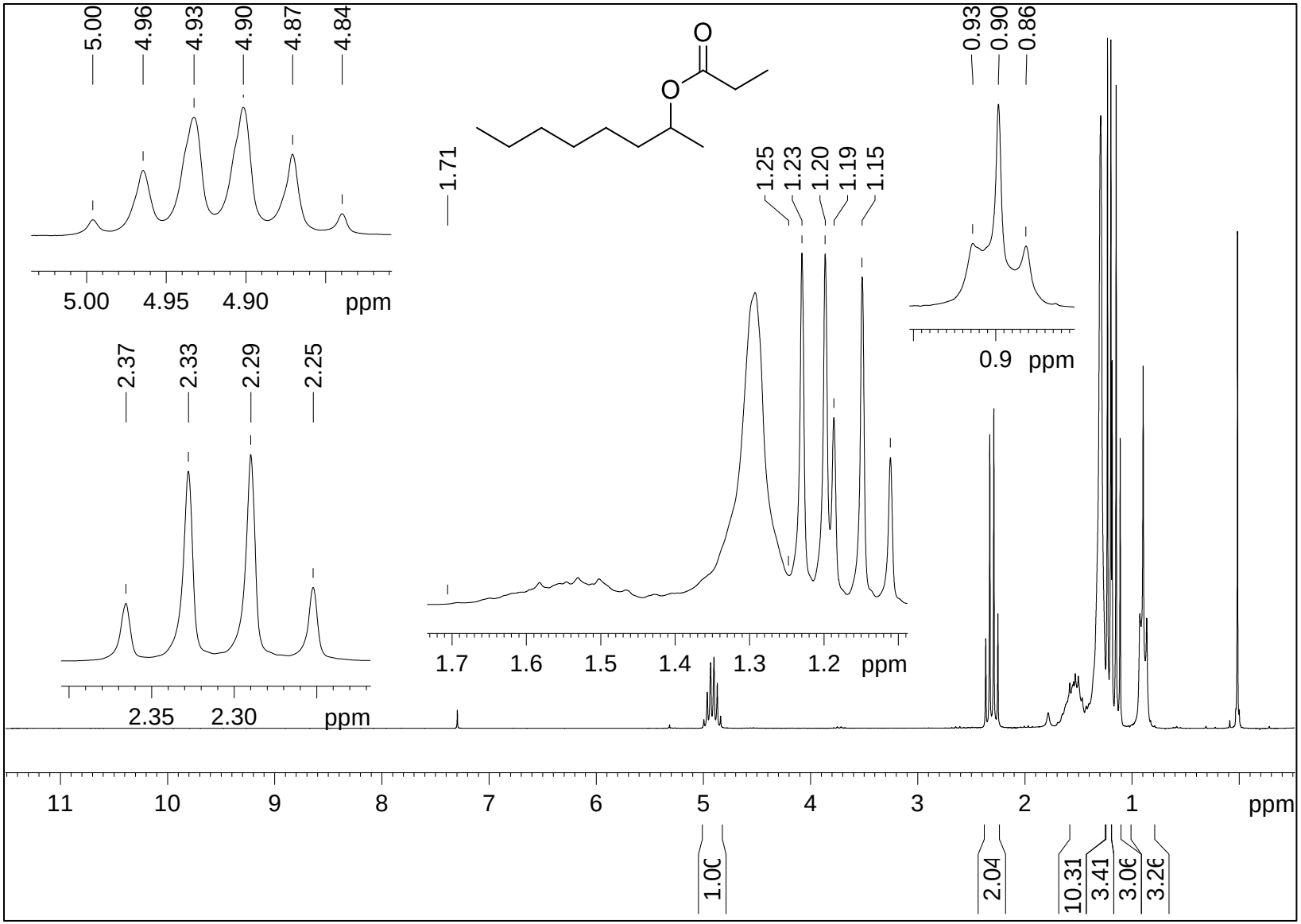



**Figure S10.** $^{1}H$ NMR spectrum (200 MHz, $CDCl_3$, TMS) of compound **2**

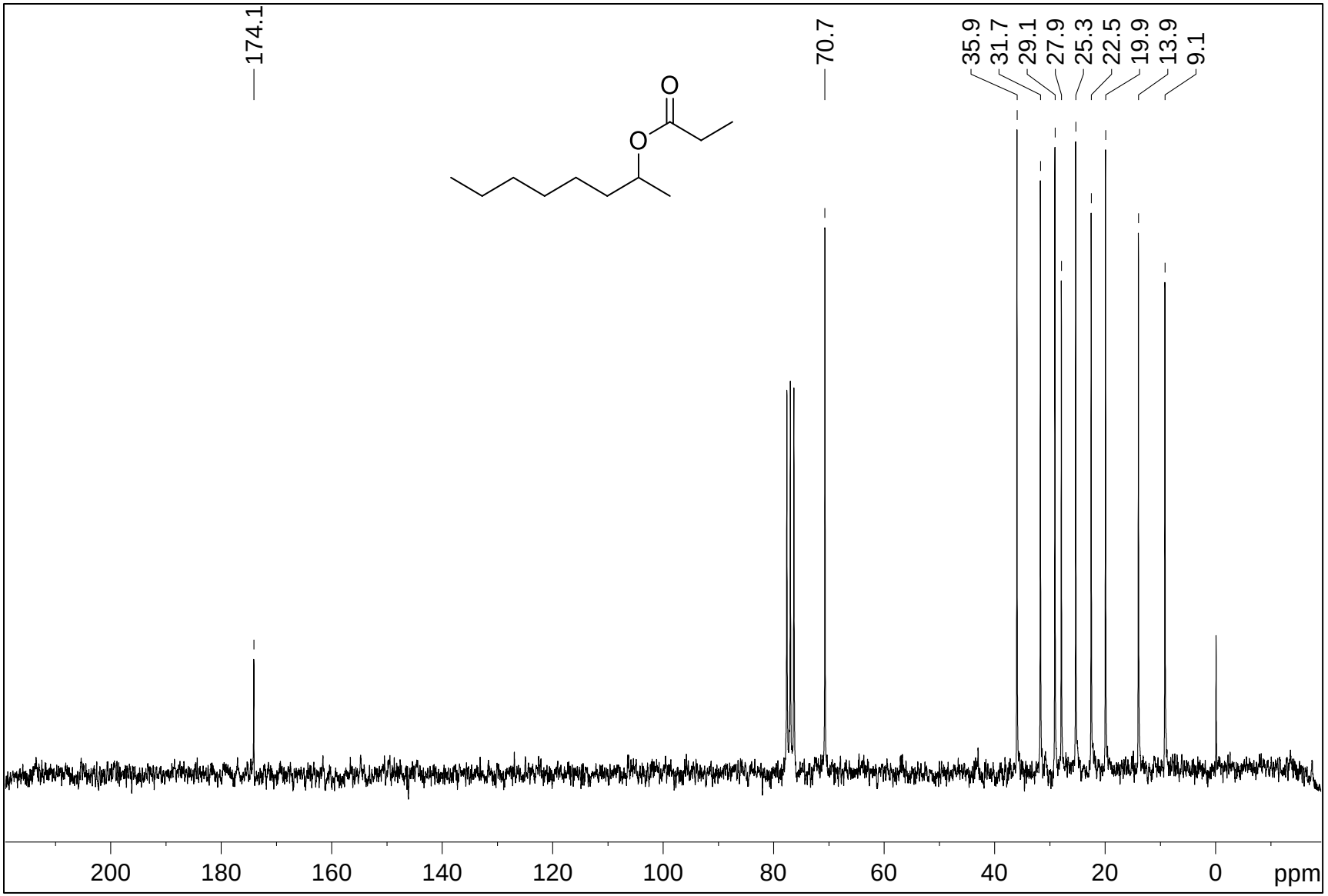



**Figure S11.** $^{13}C$ NMR spectrum (50 MHz, $CDCl_3$) of compound **2**

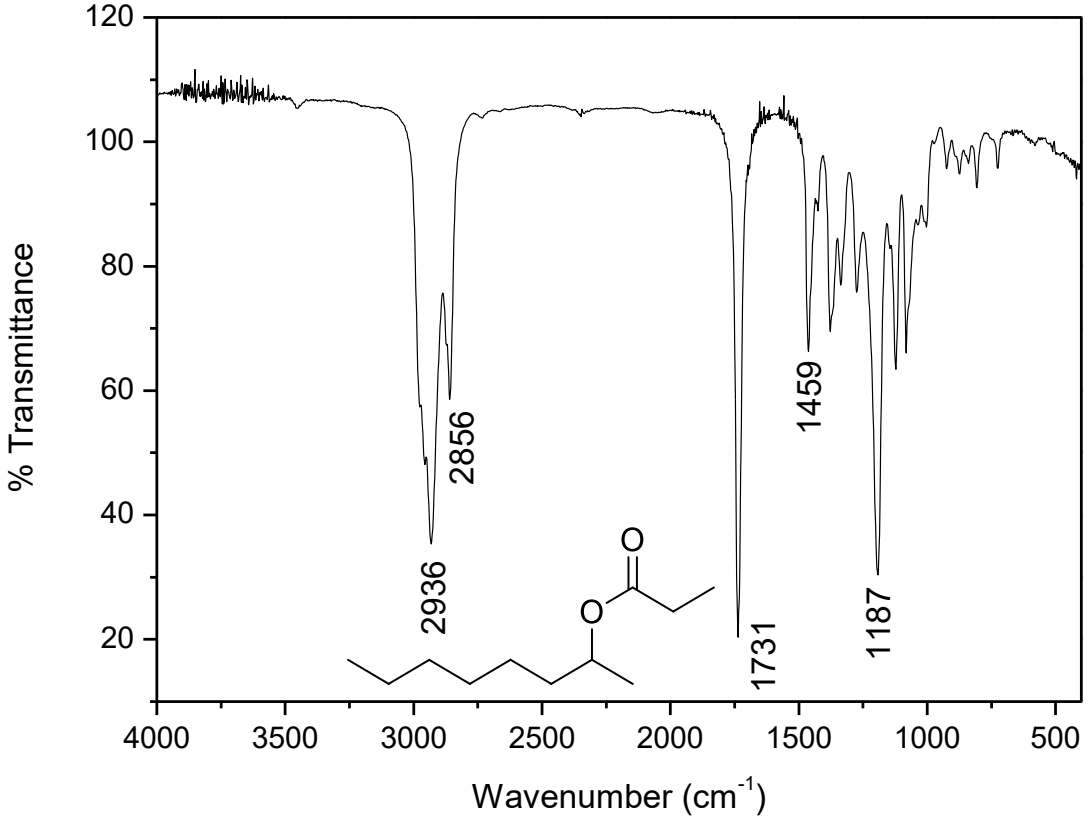
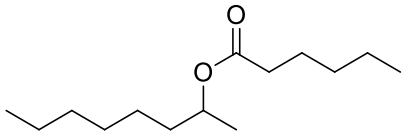



**Figure S12.** IR spectrum of compound **2**




$C_{14}H_{28}O_2$
228.37

2-Octyl hexanoate (**3**)

Yield: 93%. Colourless liquid.

GC-MS (70 eV), *m/z* (relative intensity) 143 (8%), 129 (2%), 117 (19%), 112 (16%), 99 (100%), 83 (17%), 71 (37%), 60 (9%), 57 (17%), 43 (27%), 29 (3%).

$^1$H NMR (200 MHz, $CDCl_3$, TMS), $\delta$ 0.85-0.93 (m, 6H); 1.20 (d, $J$ = 6.2 Hz, 3H); 1.28-1.67 (m, 15H); 2.23-2.31 (m, 3H); 4.90 (sext, $J$ = 6.2 Hz, 1H).

$^{13}$C NMR (50 MHz, $CDCl_3$), $\delta$ 13.8; 13.9; 19.9; 22.2; 22.5; 24.7; 25.3; 29.0; 31.2; 31.7; 34.6; 35.9; 70.7; 173.6.

IR (KBr) $\nu$/cm$^{-1}$ 2957, 2930, 2858, 1734, 1464, 1377, 1178.

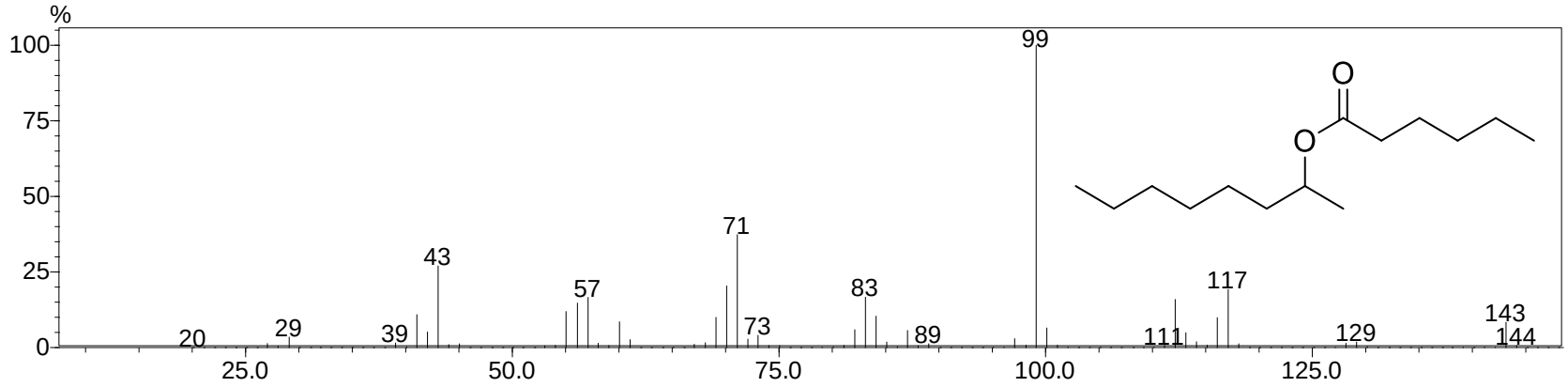



**Figure S13.** Mass spectrum of compound **3**

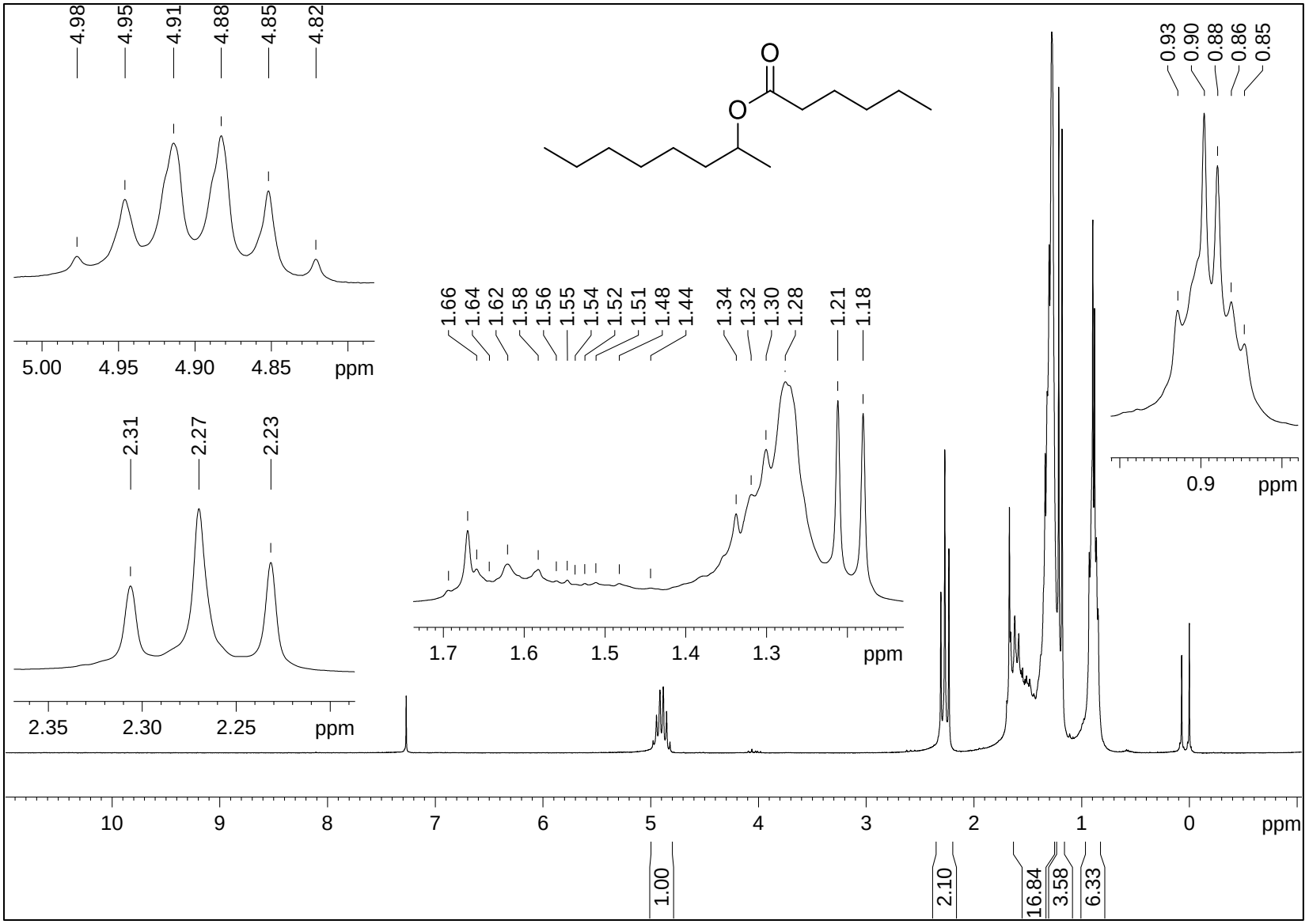



**Figure S14.** $^{1}H$ NMR spectrum (200 MHz, $CDCl_3$, TMS) of compound **3**

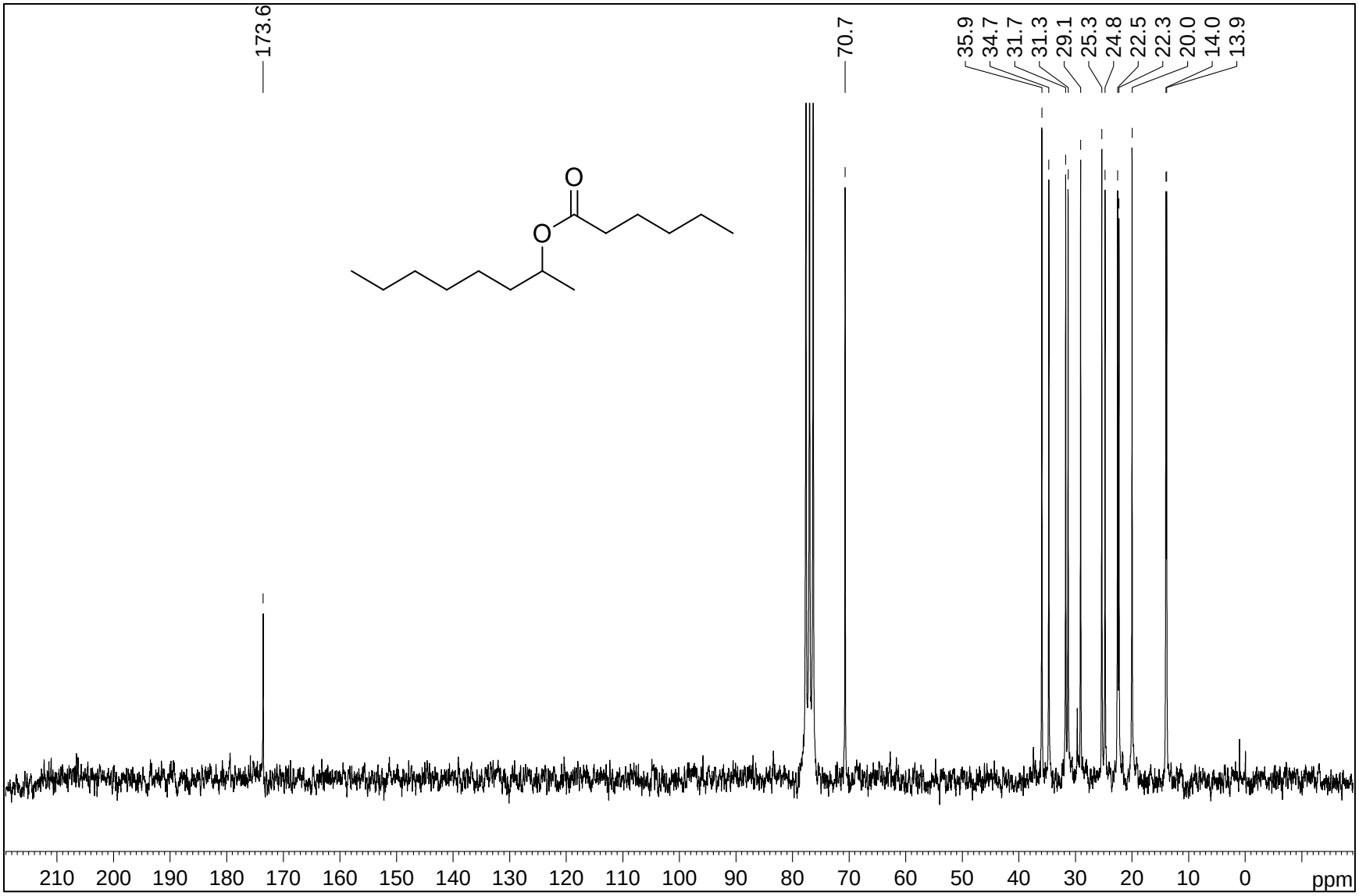



**Figure S15.** $^{13}C$ NMR spectrum (50 MHz, $CDCl_3$) of compound **3**

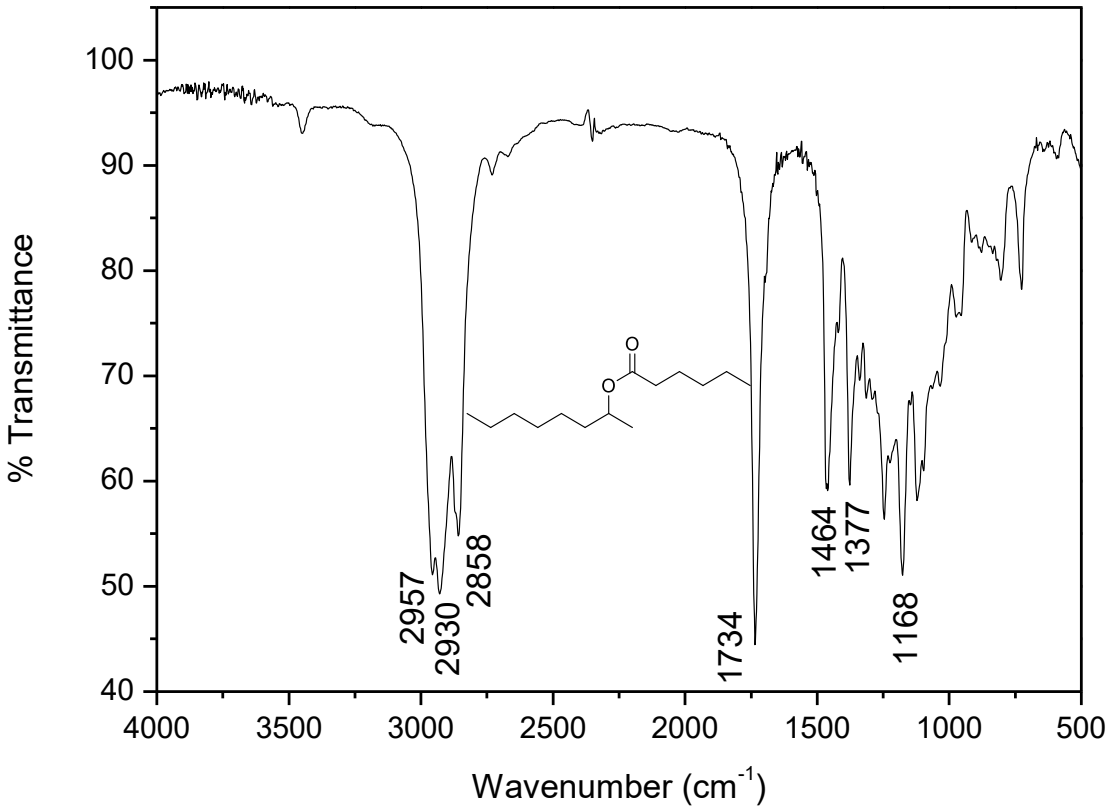
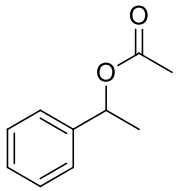



**Figure S16.** IR spectrum of compound **3**



$C_{10}H_{12}O_2$
M.M. 164.08 g $mol^{-1}$

1-Phenylethyl acetate (**4**). Colourless liquid.

GC-MS (70 eV), *m/z* (relative intensity) 164 ($M^{\bullet+}$, 23%), 122 (100%), 105 (67%), 104 (89%), 107 (36%), 51 (15%), 43 (55%).

$^1$H NMR (200 MHz, $CDCl_3$, TMS), $\delta$ 1.53 (d, $J$ = 6.6 Hz, 3H), 2.06 (s, 3H), 5.88 (q, $J$ = 6.6 Hz, 1H), 7.24-7.31 (m, 5H).

$^{13}$C NMR (50 MHz - $CDCl_3$), $\delta$ 21.3, 22.2, 72.3, 126.1, 127.8, 128.5, 141.7, 170.3.

IR (KBr) $\nu$/$cm^{-1}$ 3066, 3035, 2983, 2934, 1743, 1374, 1232, 1069, 760, 696.

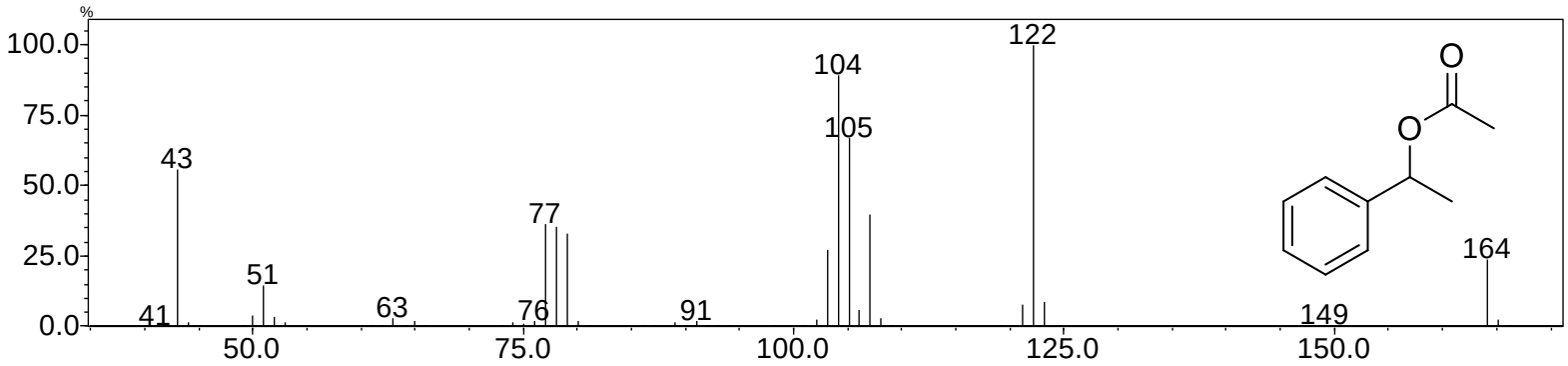



**Figure S17.** Mass spectrum of compound **4**

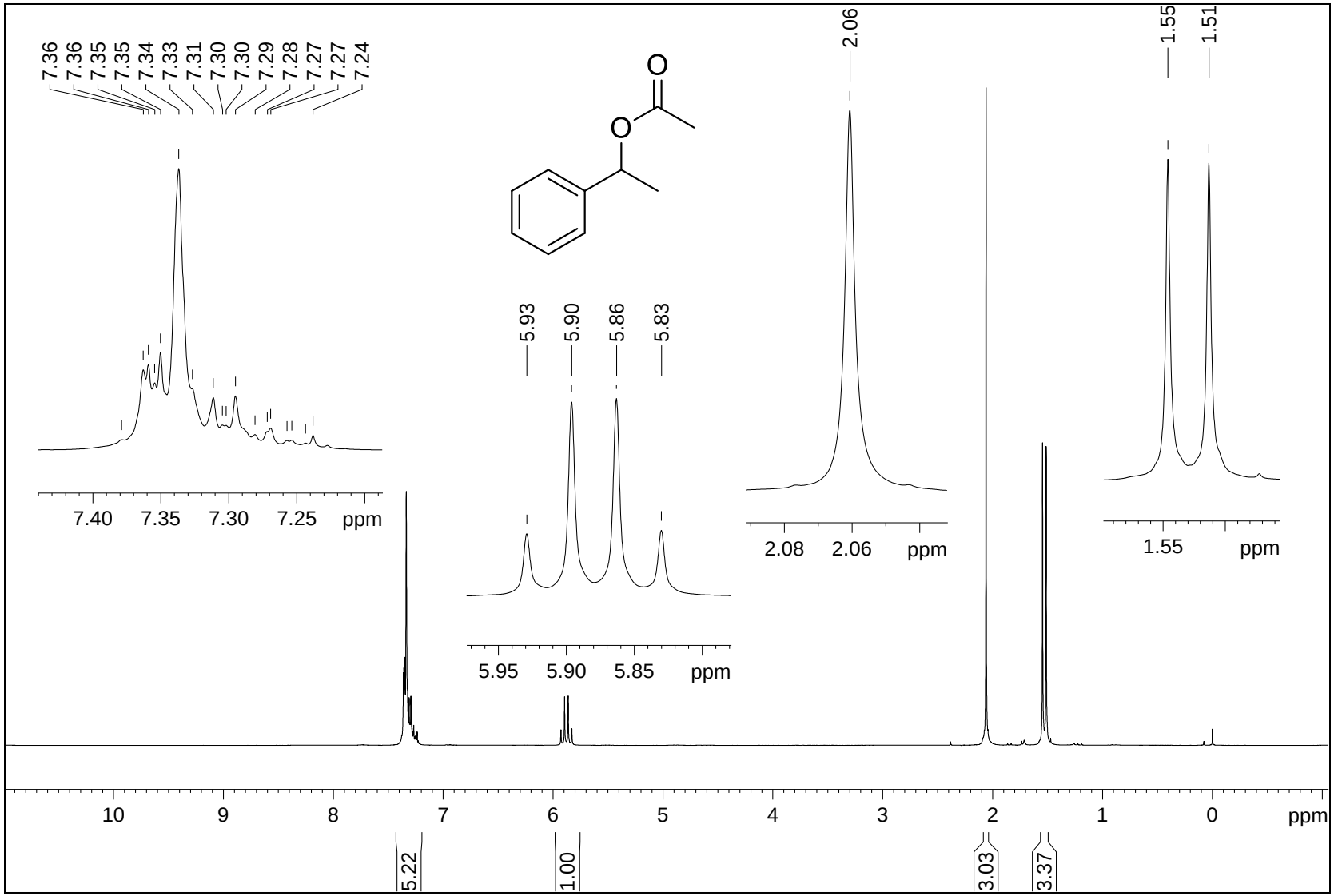



**Figure S18.** $^{1}H$ NMR (200 MHz, $CDCl_3$, TMS) spectrum of compound **4**

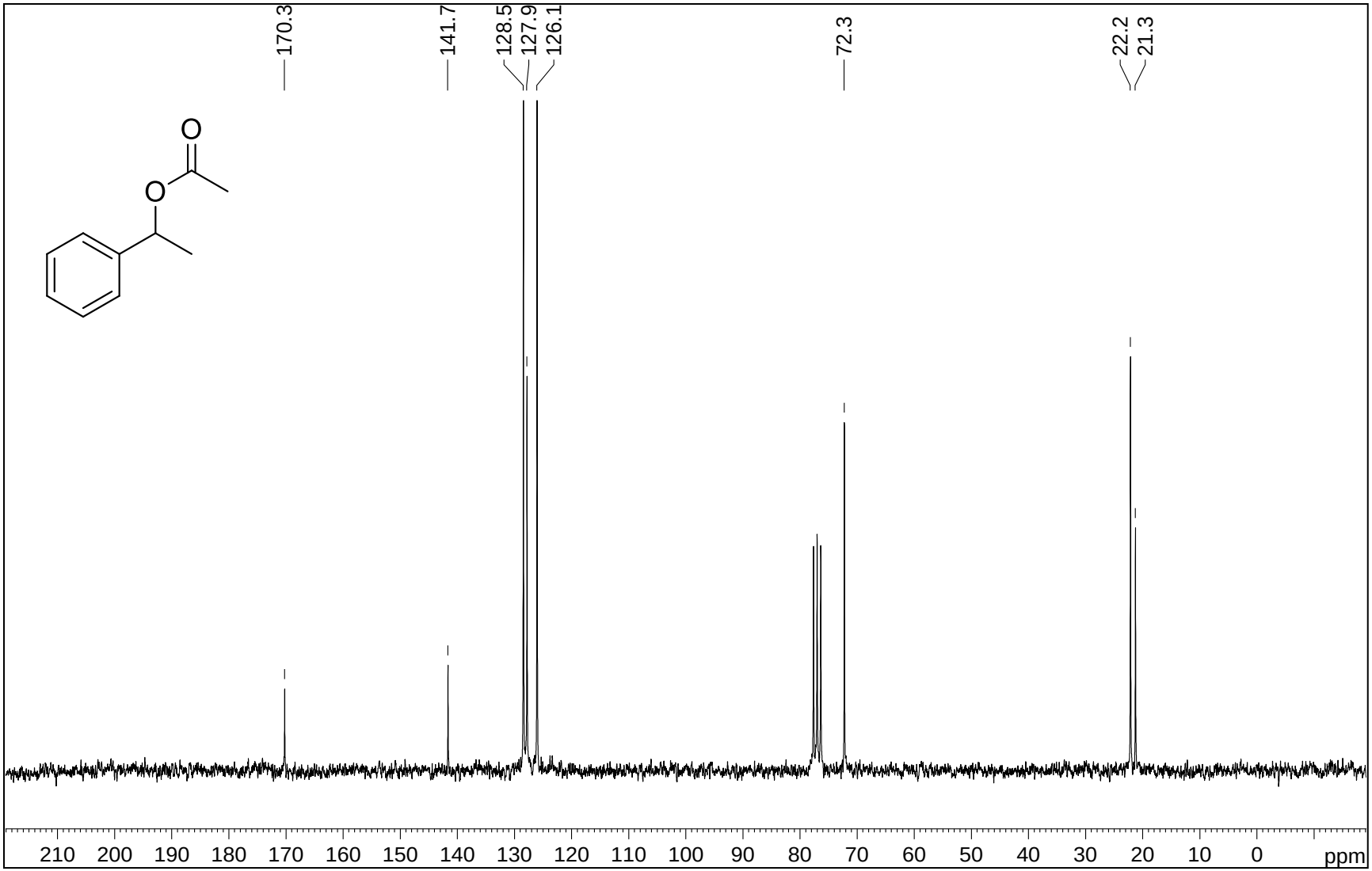



**Figure S19.** $^{13}C$ NMR (50 MHz, $CDCl_3$) spectrum of compound **4**

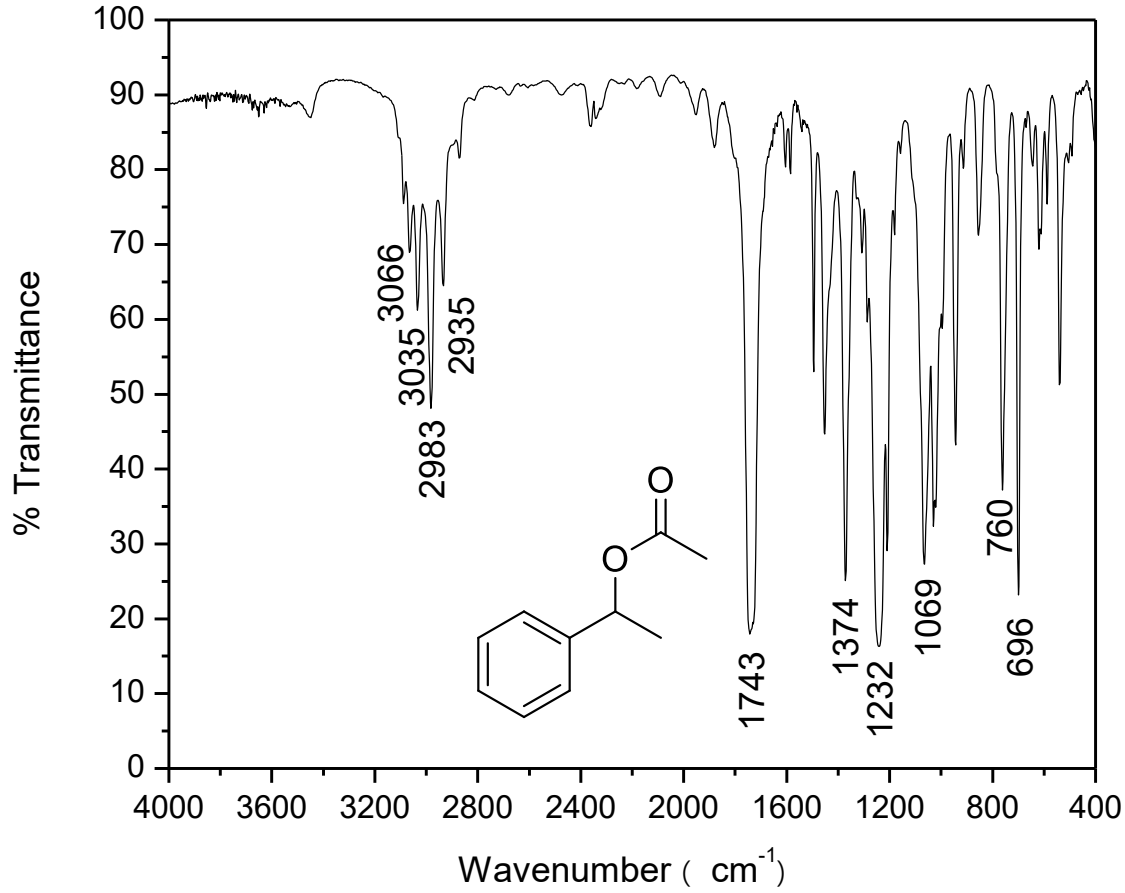
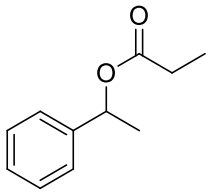



**Figure S20.** IR spectrum of compound **4**



$C_{11}H_{14}O_2$
178.23

1-Phenyl ethyl propionate (**5**)

Yield: 95%. Colourless liquid.

GC-MS (70 eV), *m/z* (relative intensity) 178 ($M^{\bullet+}$, 26%), 122 (100%), 105 (97%), 91 (2%), 77 (30%), 63 (2%), 57 (53%), 51 (10%), 43 (7%).

$^1$H NMR (200 MHz, $CDCl_3$, TMS), $\delta$ 1.14 (t, $J$ = 7.6 Hz, 3H); 1.53 (d, $J$ = 6.6 Hz, 3H); 2.30-2.41 (m, 2H); 5.89 (q, $J$ = 6.6 Hz, 1H); 7.26- 7.38 (m, 5H).

$^{13}$C NMR (50 MHz, $CDCl_3$), $\delta$ 9.1; 22.3; 27.9; 72.1; 126.0; 127.8; 128.5; 141.9; 173.7.

IR (KBr) $\nu$/cm$^{-1}$ 3064, 3034, 2983, 2935, 1740, 1452, 1369, 1190, 1064, 759, 699.

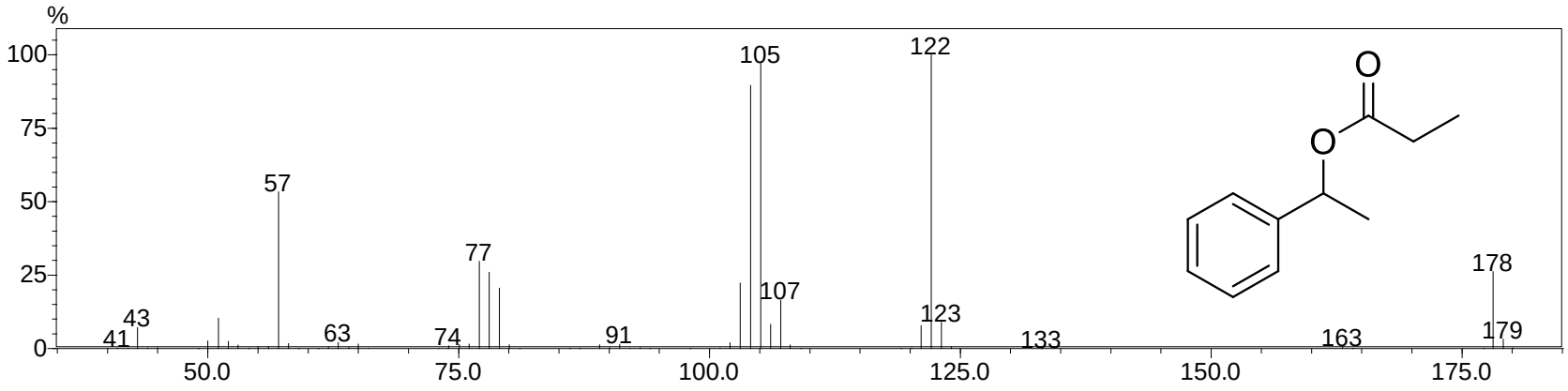



**Figure S21.** Mass spectrum of compound **5**

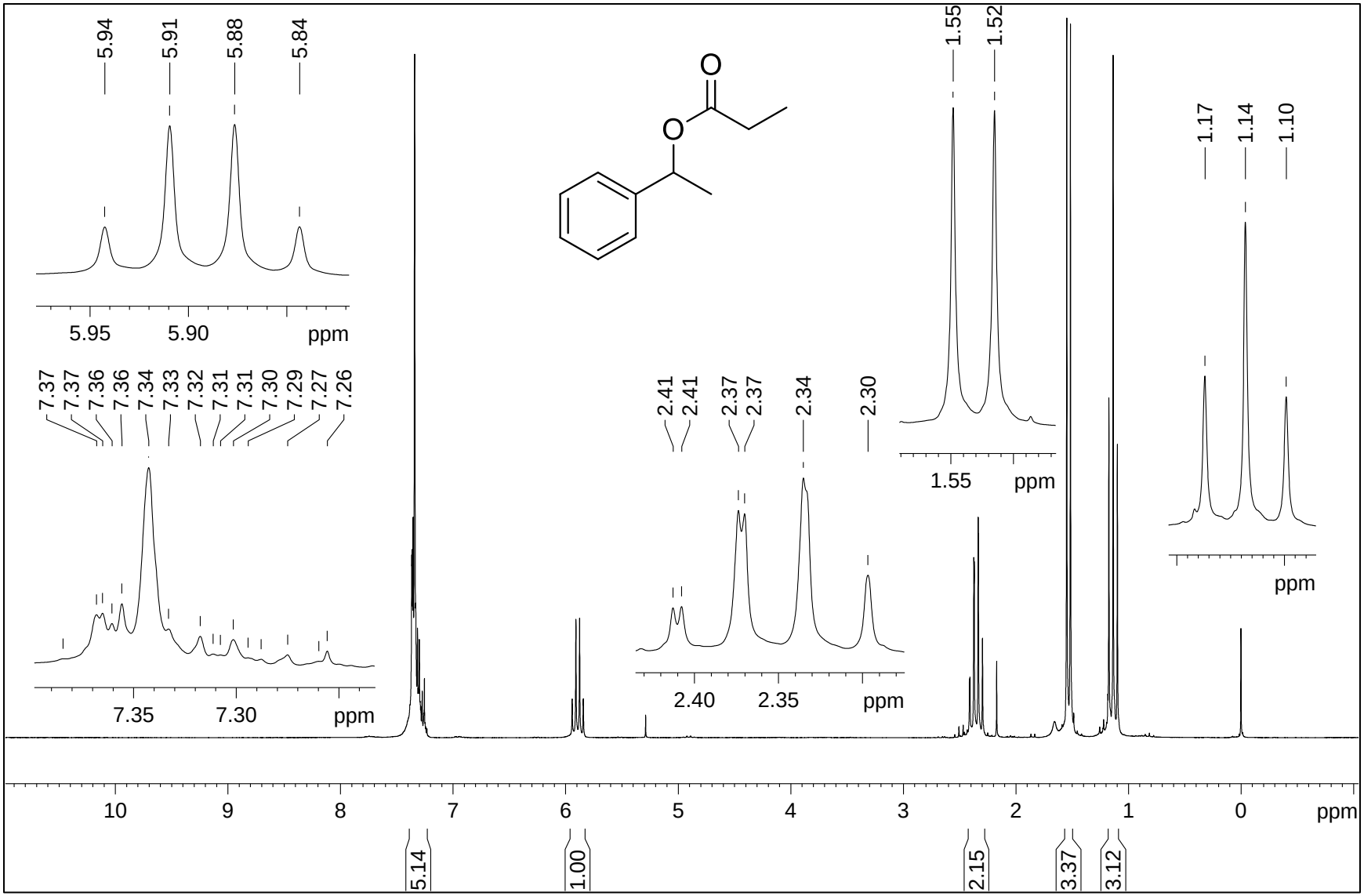



**Figure S22.** $^{1}H$ NMR spectrum (200 MHz, $CDCl_3$, TMS) of compound **5**

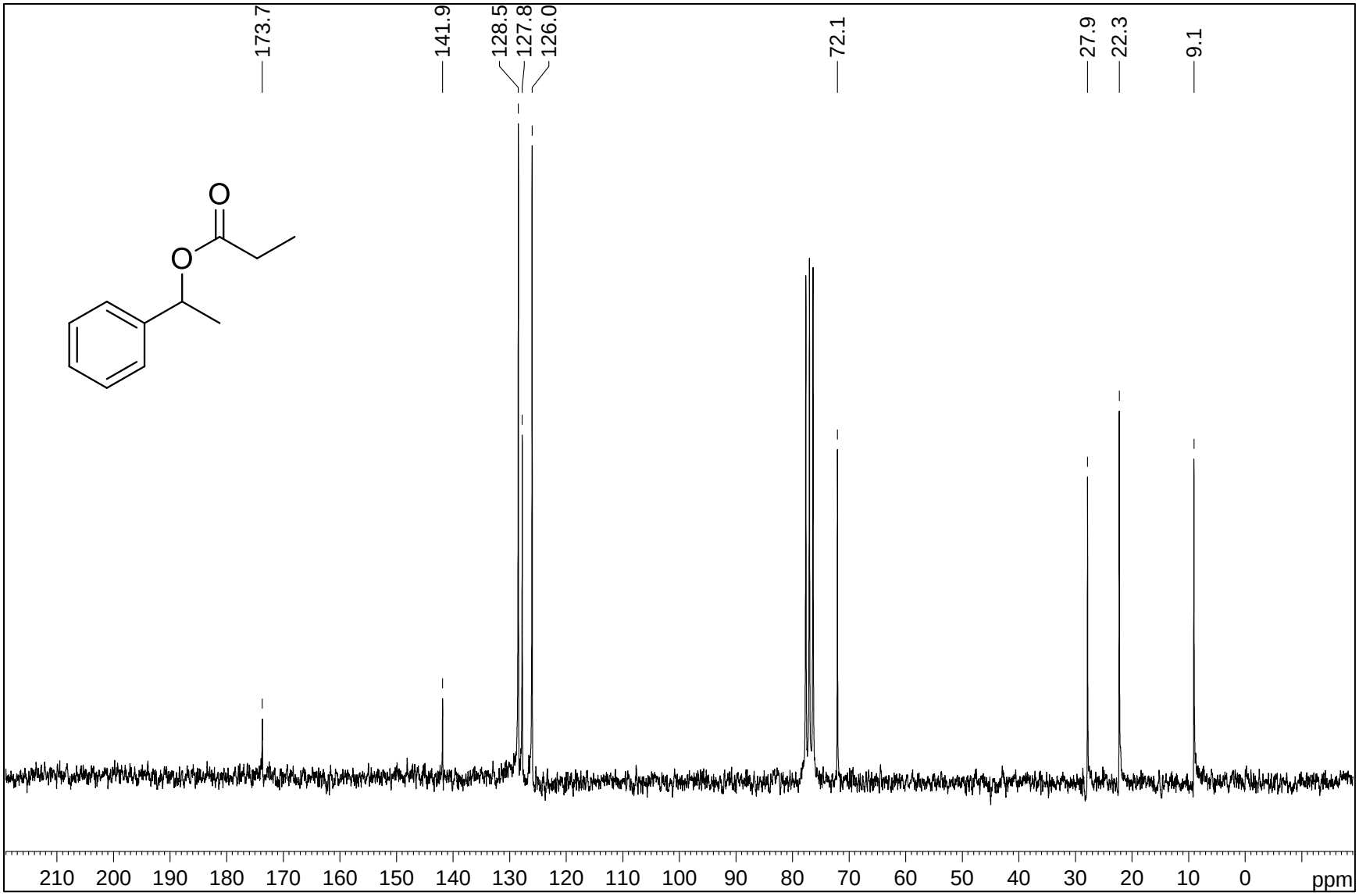



**Figure S23.** $^{13}C$ NMR spectrum (50 MHz, $CDCl_3$) of compound **5**

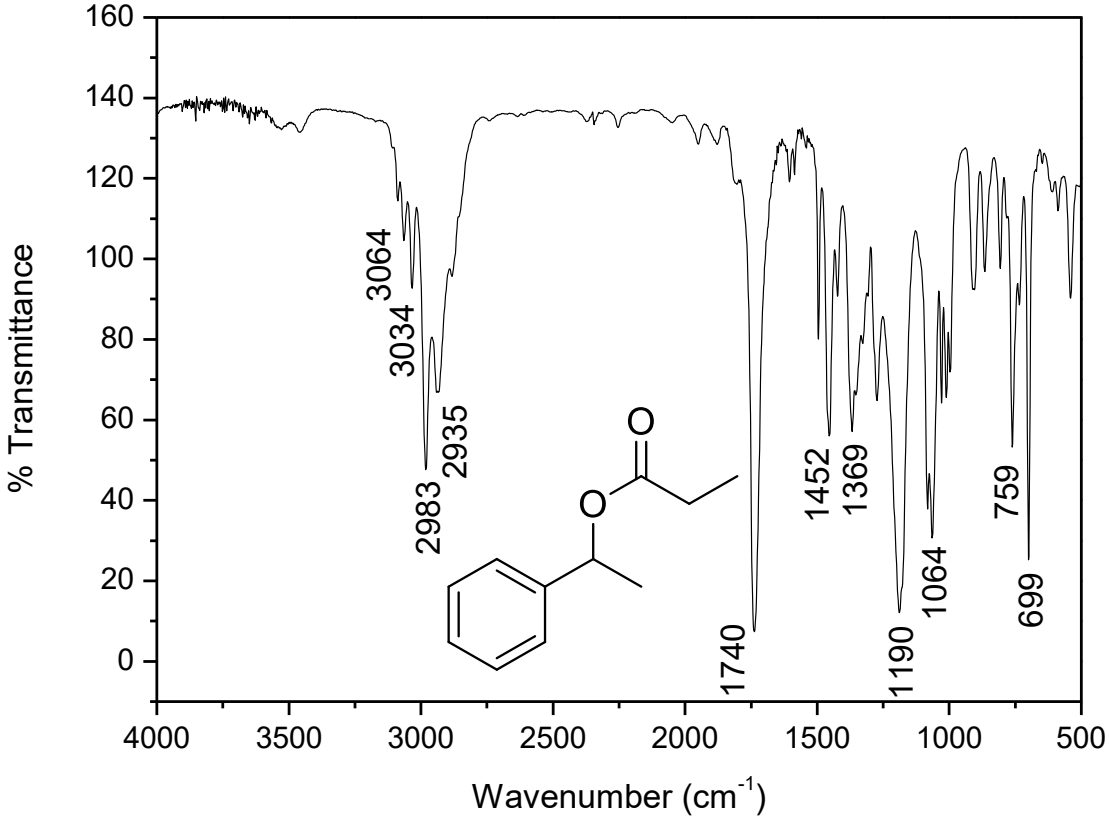
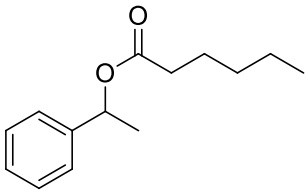



**Figure S24.** IR spectrum of compound **5**



$C_{14}H_{20}O_2$
220.31

1-Phenyl ethyl hexanoate (**6**)

Yield: 97%. Colourless liquid.

GC-MS (70 eV), *m/z* (relative intensity) 220 ($M^{\bullet+}$, 5%), 122 (91%), 115 (7%), 105 (100%), 99 (16%), 91 (2%), 77 (19%), 71 (14%), 55 (4%), 51 (5%), 43 (22%).

$^1$H NMR (200 MHz, $CDCl_3$, TMS), $\delta$ 0.84-0.91 (m, 3H); 1.24-1.32 (m, 4H); 1.53 (d, $J$ = 6.6 Hz, 3H); 1.59-1.70 (m, 2H); 2.28-2.36 (m, 2H); 5.90 (q, $J$ = 6.6 Hz, 1H); 7.26-7.36 (m, 5H).

$^{13}$C NMR (50 MHz, $CDCl_3$), $\delta$ 13.9; 22.2; 24.6; 31.2; 33.9; 34.6; 72.0; 126.0; 127.8; 128.4; 141.9; 173.1.

IR (KBr) $\nu$/cm$^{-1}$ 3088, 3065, 3034, 2957, 2932, 2864, 1735, 1453, 1372, 1171, 1057, 760, 698.

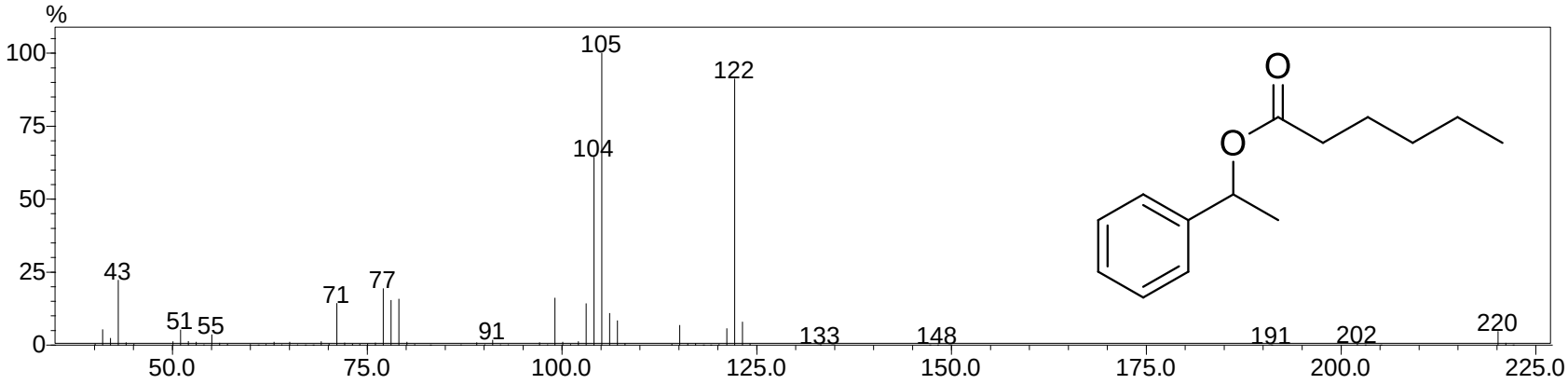



**Figure S25.** Mass spectrum of compound **6**

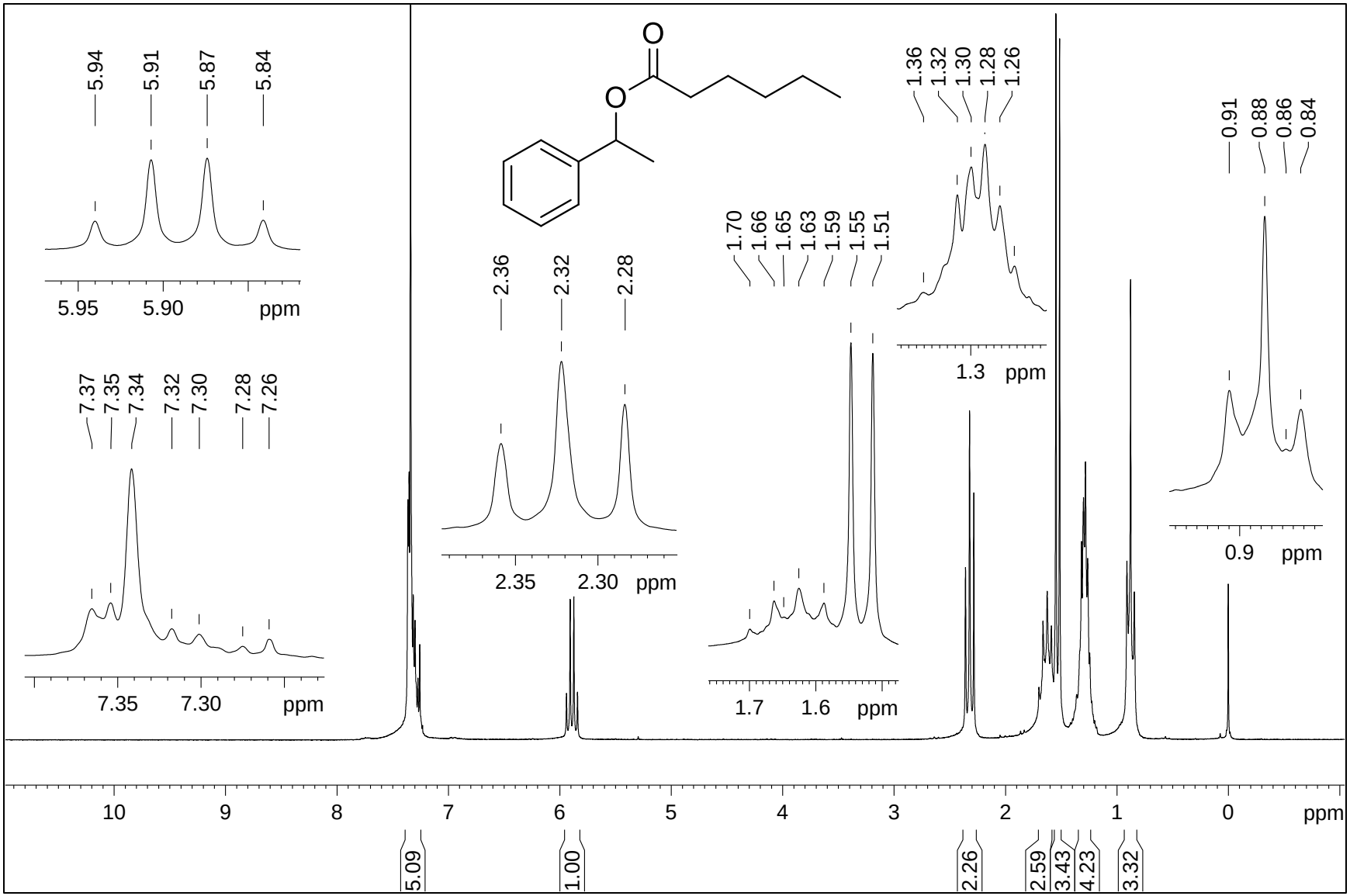



**Figure S26.** $^{1}H$ NMR spectrum (200 MHz, $CDCl_3$, TMS) of compound **6**

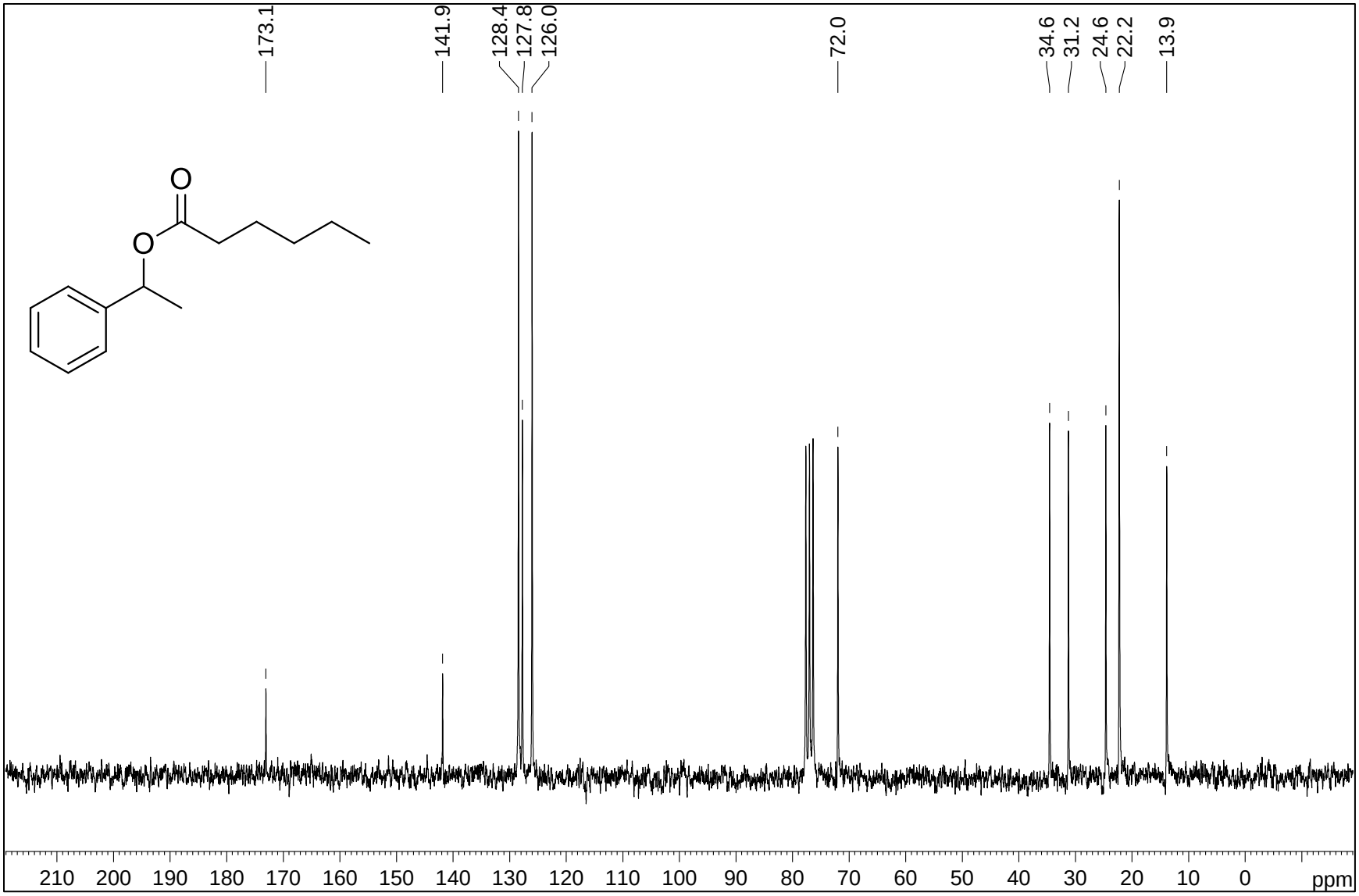



**Figure S27.** $^{13}C$ NMR spectrum (50 MHz, $CDCl_3$) of compound **6**

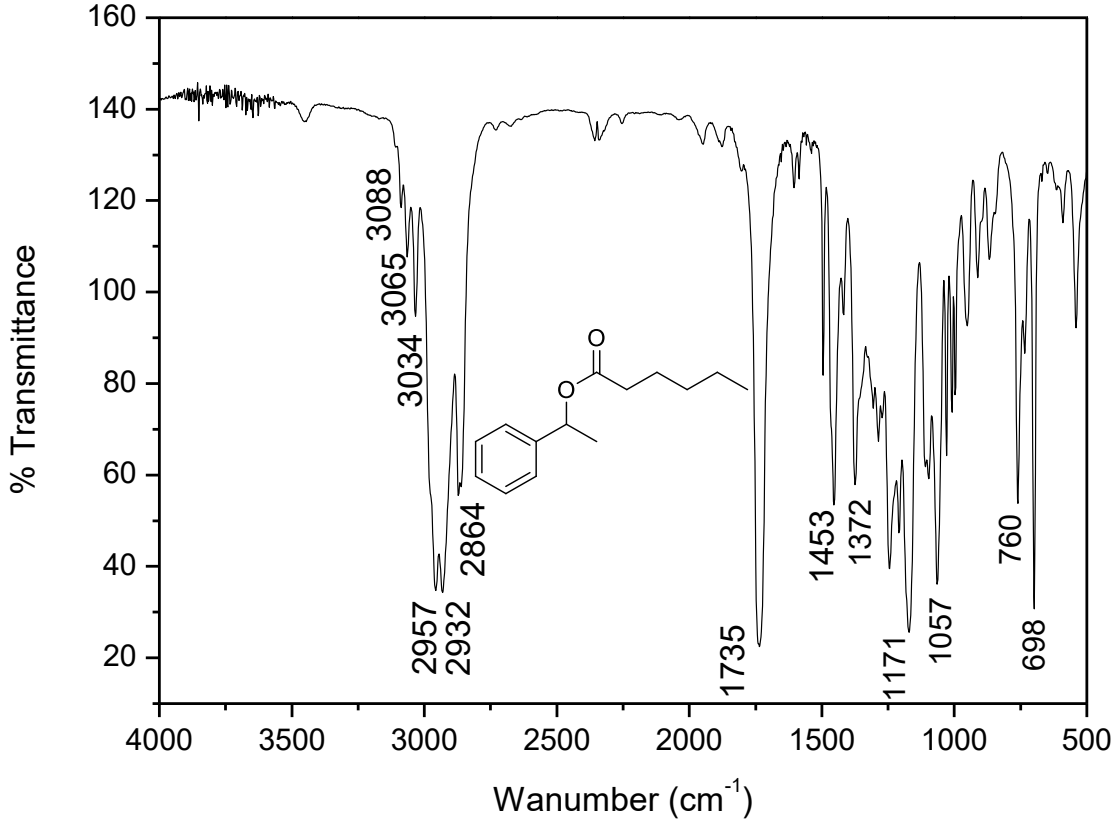



**Figure S28.** IR spectrum of compound **6**