Bio-based crotonic acid from polyhydroxybutyrate: synthesis and photocatalyzed hydroacylation

Adriano Parodi,[a] Alexandra Jorea,[b] Maurizio Fagnoni,[b] Davide Ravelli,[,b] Chiara Samorì,*[,a] Cristian Torri,[a] Paola Galletti[a]*

[a].Dipartimento di Chimica "Giacomo Ciamician", Università di Bologna, via S. Alberto 163, 48123 Ravenna (Italy)

[b] PhotoGreen Lab, Dipartimento di Chimica, Università di Pavia, viale Taramelli 12, 27100 Pavia (Italy)

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1 PHB Production and Thermal Treatments

PHB production by mixed microbial cultures. PHB-containing bacteria were produced with a 0.75 m^3 prototype consisting of a 500 L sequencing batch reactor (SBR) overflowing into a 250 L accumulation reactor (AR). The system utilized the scheme previously proposed in the literature (see Figure S1).[S1]

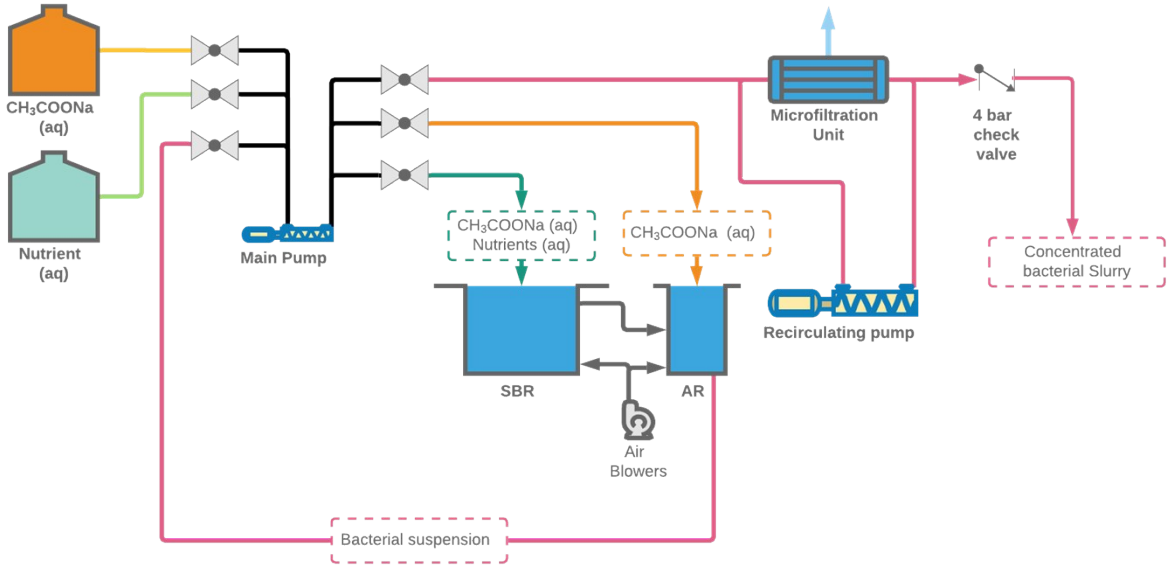


Figure S1. Scheme of the prototype used for MMC cultivation and PHB accumulation.

The system was initially inoculated with 10 g of freeze-dried mixed microbial cultures (MMC) from 5 L lab-scale SBR, acclimatized for more than 3 years, and run with variable cycles length (according to biomass growth and substrate consumption rate) for about one month; after that, cycles of 8 h (3 cycles d^{-1}) were adopted. At the beginning of each cycle, SBR was fed with 110 L of a substrate/nutrient solution (provided through an Auger pump, 50 L h^{-1}, controlled by a PLC and connected to aspiration and discharge manifolds) with the following composition: 3.9 g L^{-1} sodium acetate (2.7 g COD L^{-1}) and nutrients, consisting in 380 g L^{-1} NH_4Cl, 111 mg L^{-1} K_2HPO_4, 80 mg L^{-1} KH_2PO_4, 16 mg L^{-1} $CaCl_2 \times 2\ H_2O$, 32 mg L^{-1} $MgSO_4 \times 7\ H_2O$, 0.64 mg L^{-1} $FeCl_3 \times 6\ H_2O$, 1 mg L^{-1} Na_2ETDA, 32 µg L^{-1} $ZnSO_4$, 9.6 µg L^{-1} $MnCl_2 \times 4\ H_2O$, 96 µg L^{-1} H_3BO_3, 64 µg L^{-1} $CoCl_2 \times 6\ H_2O$, 6.4 µg L^{-1} $NiCl_2 \times 6\ H_2O$, 3.2 µg L^{-1} $CuCl_2 \times 5\ H_2O$, 9.6 µg L^{-1} $NaMoO_4 \times 2\ H_2O$. An aqueous slurry (110 L $cycle^{-1}$) with an average biomass content of 1.3±0.5 gVSS L^{-1} was overflowed from SBR to AR, in which an additional solution of the substrate (30 L of 12.7 g L^{-1} sodium acetate) was added to induce the accumulation of PHB. At the end of the cycle, the aqueous slurry, which had an

average concentration of 1.8±0.4 gVSS L^{-1}, was pressured to 4 bar into an automated tangential filtration module, composed of a ceramic filter module and an additional Auger pump (3 m^3 h^{-1}). The tangential filtration module concentrated the slurry to 50 g L^{-1}, furtherly concentrated to 200 g L^{-1} through centrifugation, and then freeze-dried.

PHB content (%) inside freeze-dried MMC cells was determined by an in-vial thermolysis procedure already described in the literature.[S2]

Thermal treatment of PHB and PHB-MMC samples.

TP1) *thermolysis-distillation*: a 250 mL single-neck round-bottom flask, equipped with a Vigreux condenser (Figure S2) was charged with PHB or PHB-MMC samples and then kept under reduced pressure (150 mbar); the flask was inserted in a heating mantle already set at 290°C and kept at this temperature for 30 min.

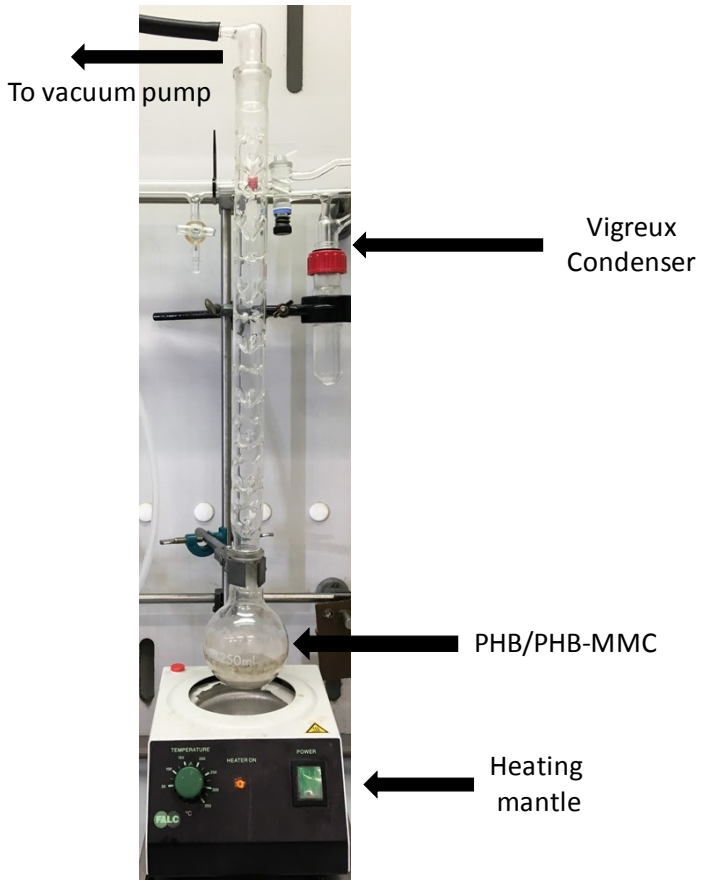


Figure S2. Apparatus adopted for processing PHB or PHB-MMC through TP1 (*thermolysis* step), TP2 (*thermolysis-thermolysis* steps) and TP3 (*thermolytic distillation* step).

During the thermolysis, reflux was observed. After 30 min, the solution was cooled down to rt and room pressure was restored, then the Vigreux column was removed and the flask equipped with a splash-guard adapter with a return hole to distill volatile products (Figure S3). Distillation was performed under reduced pressure (150 mbar) at 170°C, a condition under which further thermolysis of the volatile products could eventually occur; the distilled fraction was analyzed in terms of mass yield, as well as **CA** and by-products amount.

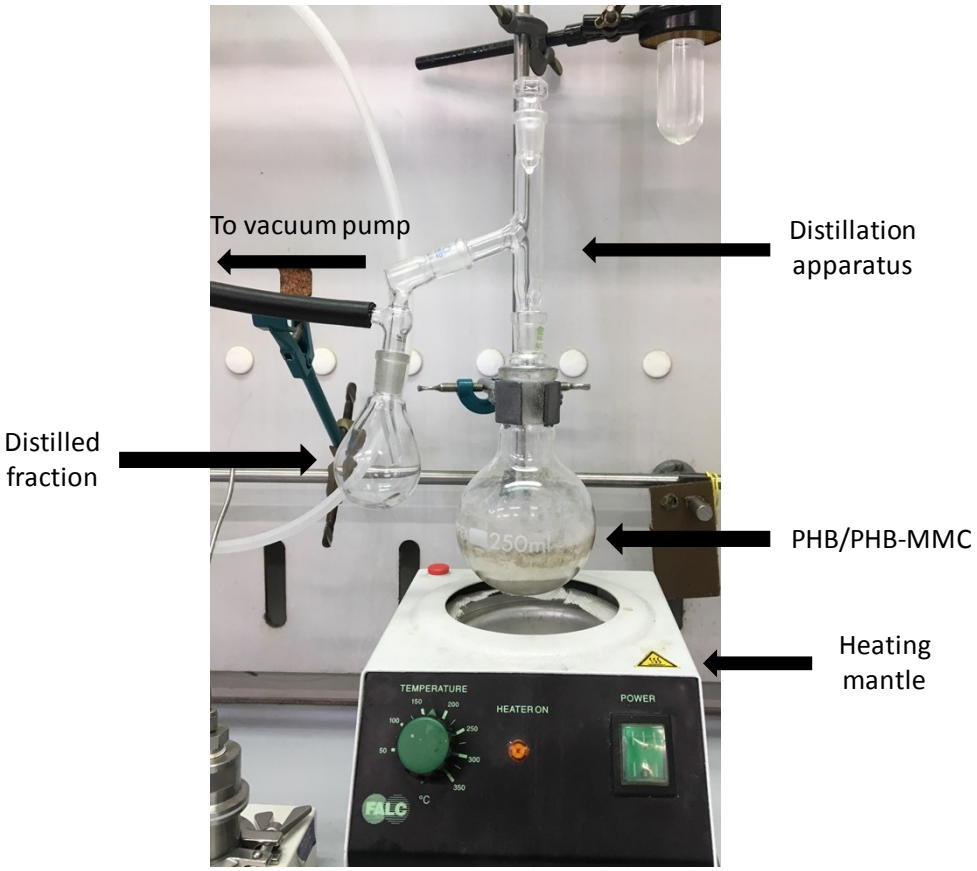


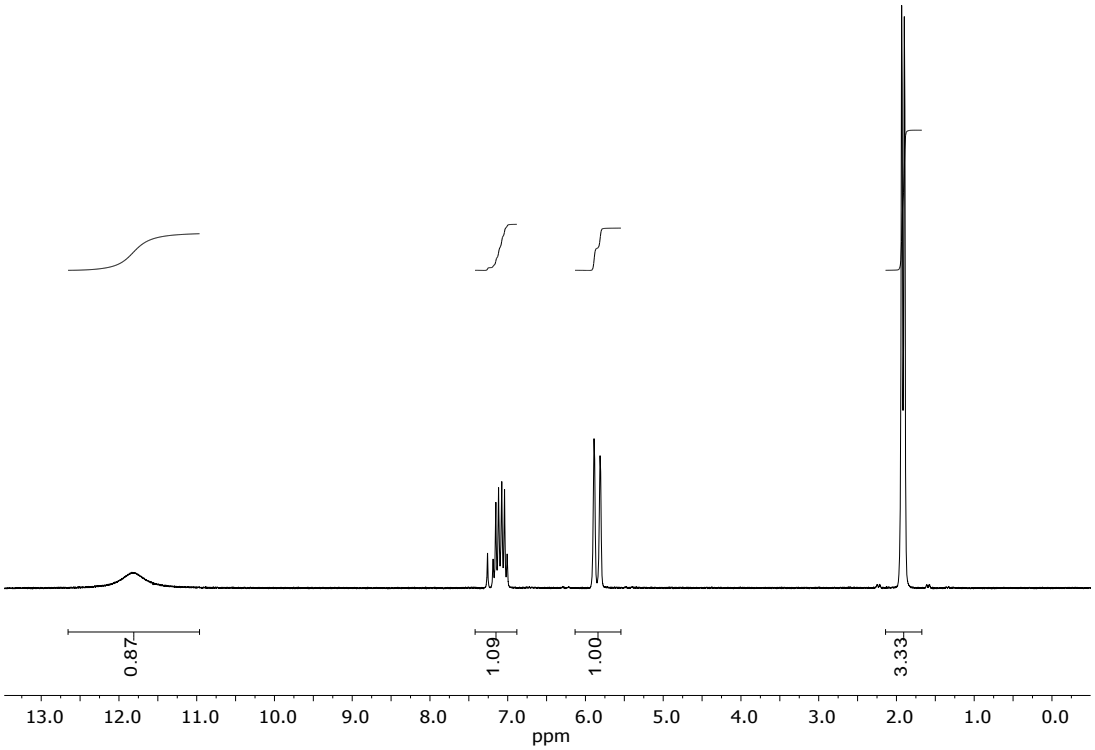
Figure S3. Apparatus adopted for distilling the volatile fraction in TP1 and TP2 (*distillation* step).

TP2) *thermolysis-thermolysis-distillation*: a 250 mL single-neck round-bottom flask equipped with a distillation apparatus (Figure S2) was charged with PHB or PHB-MMC samples and then kept under reduced pressure (150 mbar); the flask was inserted in a heating mantle already set at 240 or 290°C and kept at this temperature for 30 or 15 min. The volatile compounds generated during the thermal treatment were collected in a flask put at the end of the distillation apparatus and further treated at 290°C with a Vigreux condenser under reduced pressure (150 mbar); then, they were distilled in the same way as described in TP1 (Figure S3). The distilled fraction was analyzed in terms of mass yield, as well as **CA** and by-products amount.

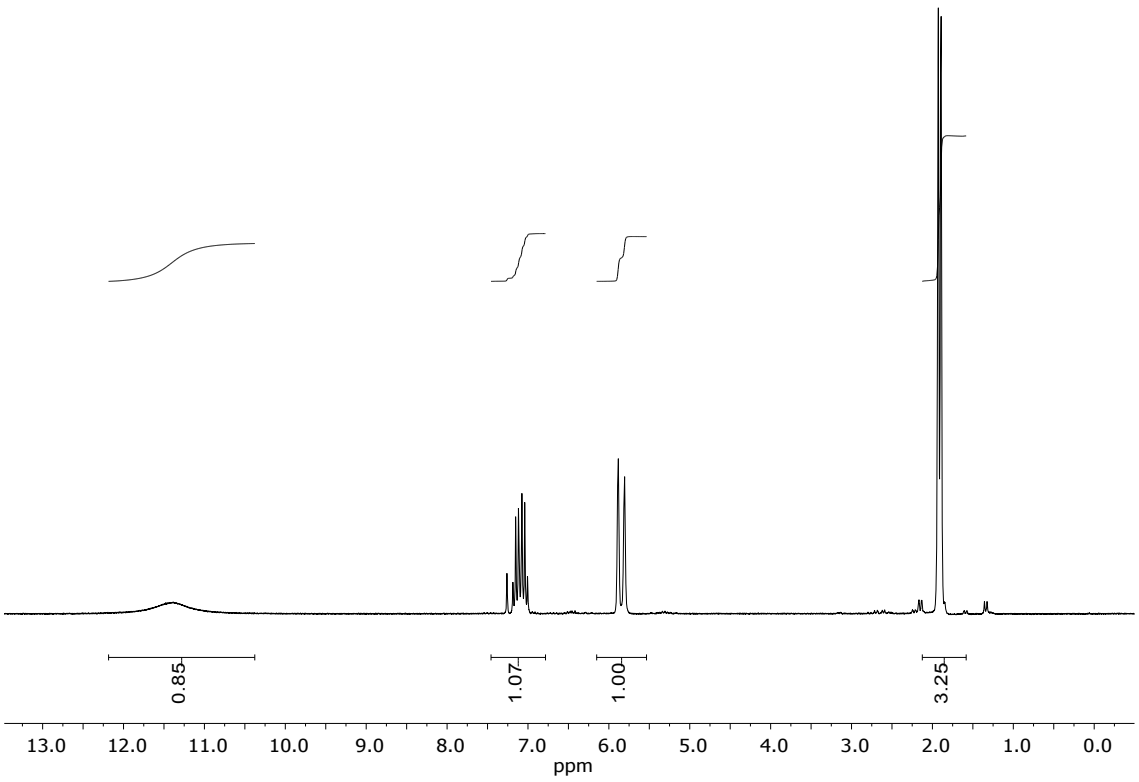
TP3) *thermolytic distillation*: a 250 mL single-neck round-bottom flask equipped with a distillation apparatus (Figure S2) was charged with PHB or PHB-MMC samples and then kept under reduced pressure (150 mbar); the flask was inserted in a heating mantle already set at 170°C and kept at this temperature until no vapors were observed (approximately 1 h). The distilled fraction collected in a flask put at the end of the apparatus was analyzed in terms of mass yield, as well as **CA** and by-products amount.

2 ^{1}H NMR Analysis of Samples CA_C, CA_{PHB}, CA_{60} and CA_{30}

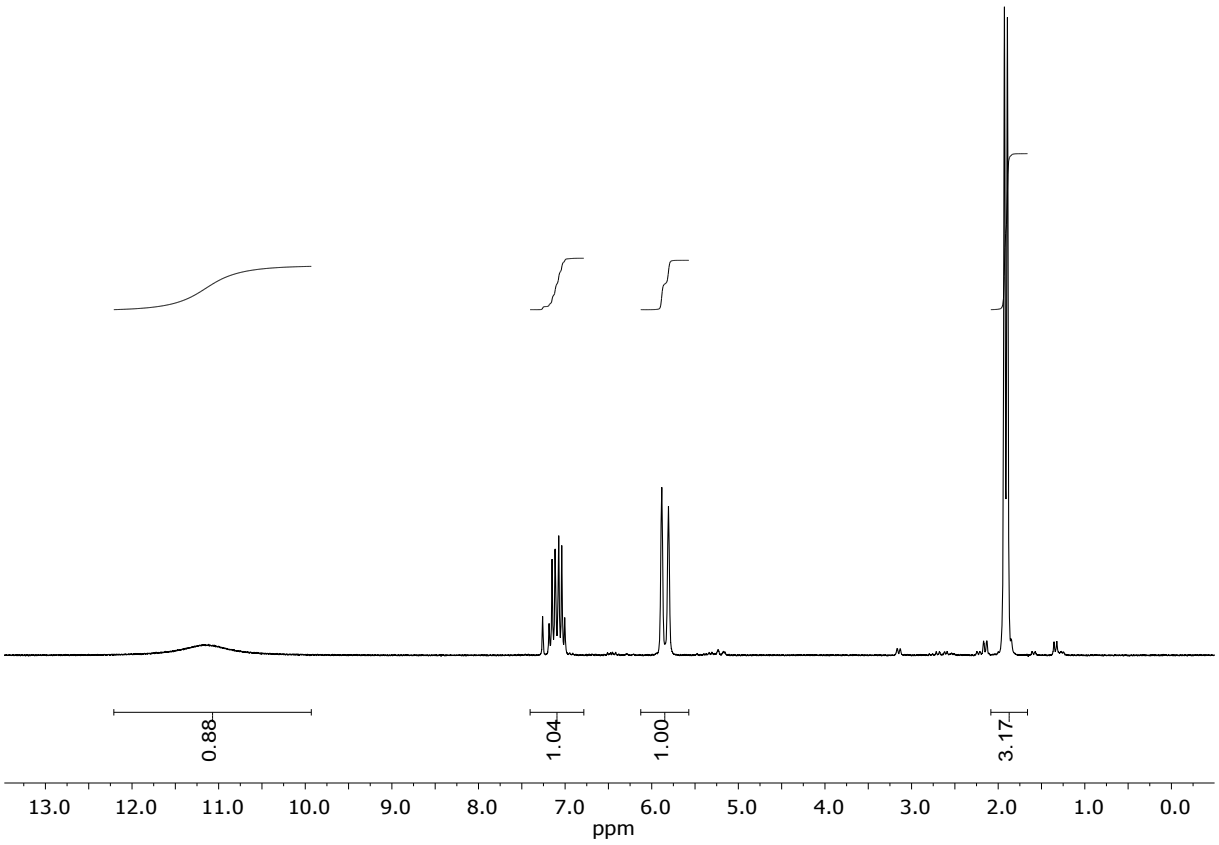
CA_C



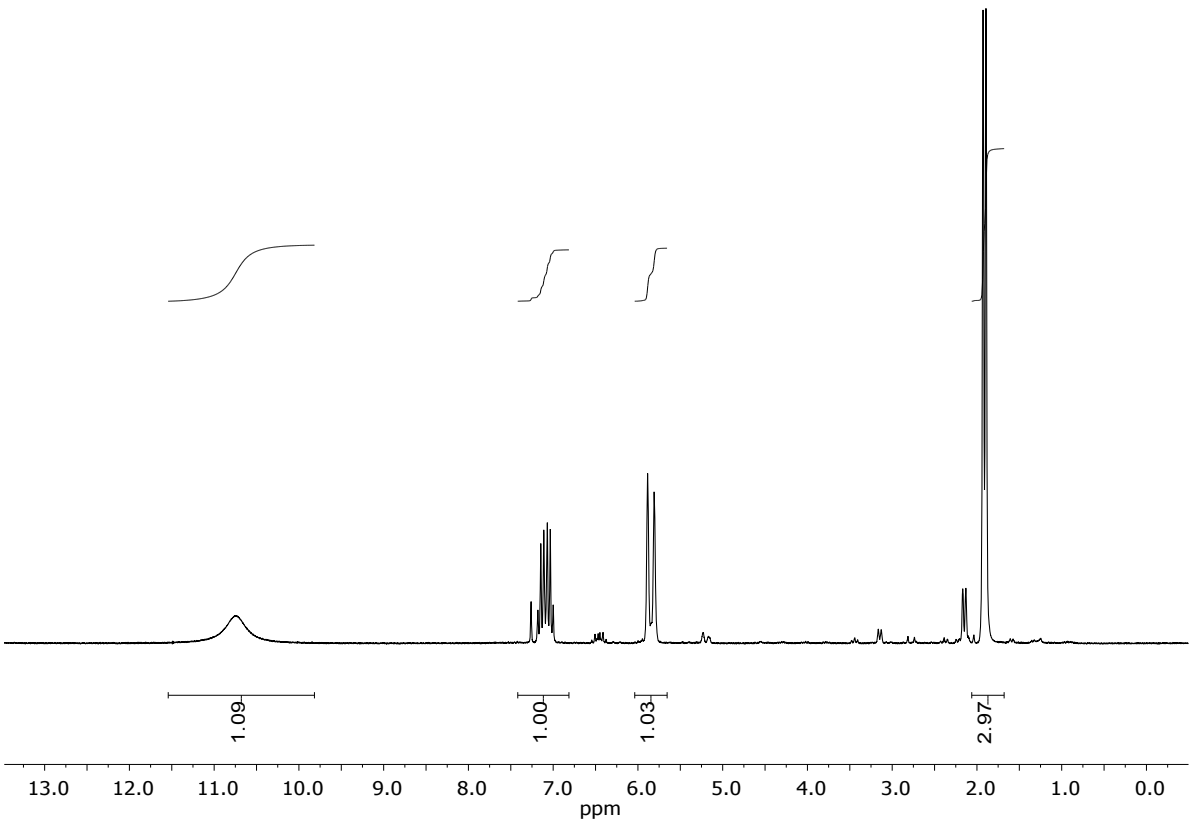
CA_{PHB}



CA_{60}


0.88
1.04
1.00
3.17
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm

CA_{30}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1.00
1.03
2.97
13.0
12.0
11.0
10.0
9.0
8.0
7.0
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5.0
4.0
3.0
2.0
1.0
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ppm

3 UV–Vis Spectra

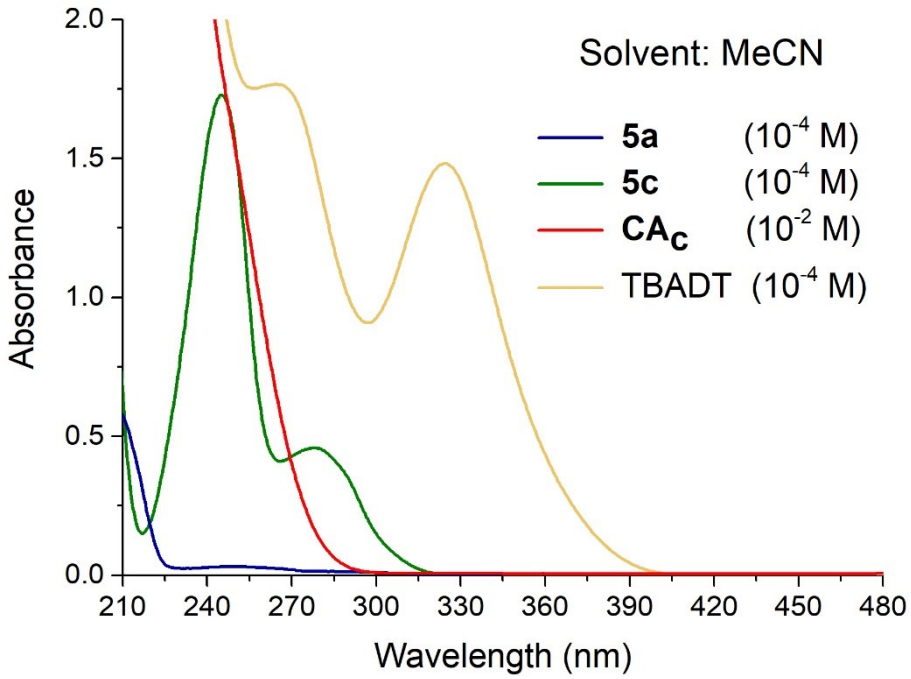


Figure S4. UV-Vis spectra of reaction components under diluted conditions.

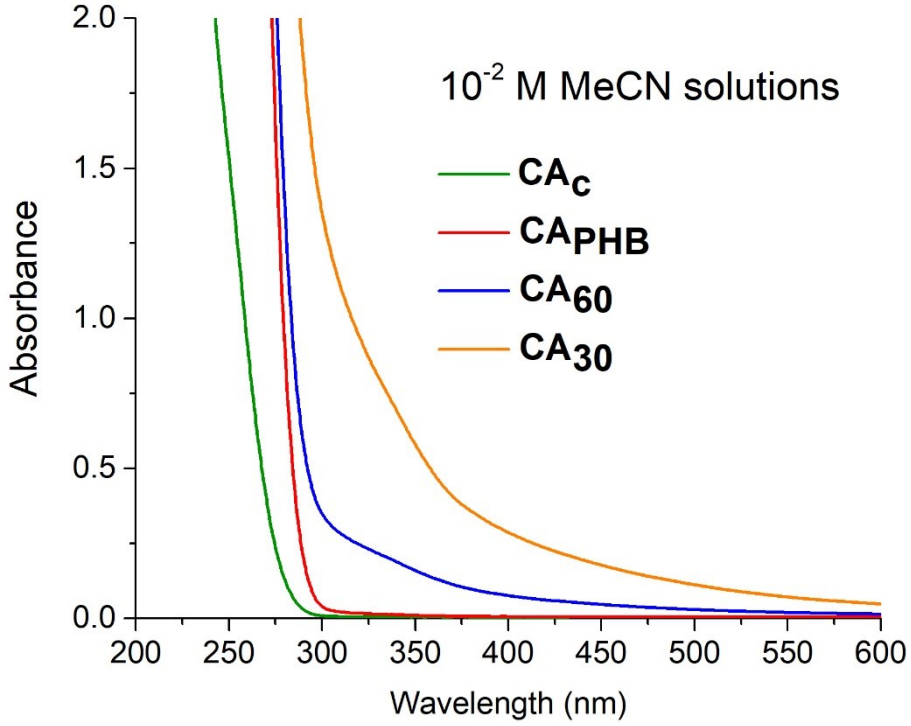


Figure S5. UV-Vis spectra of the different samples of **CA** used in this work (10^{-2} M solution in MeCN).

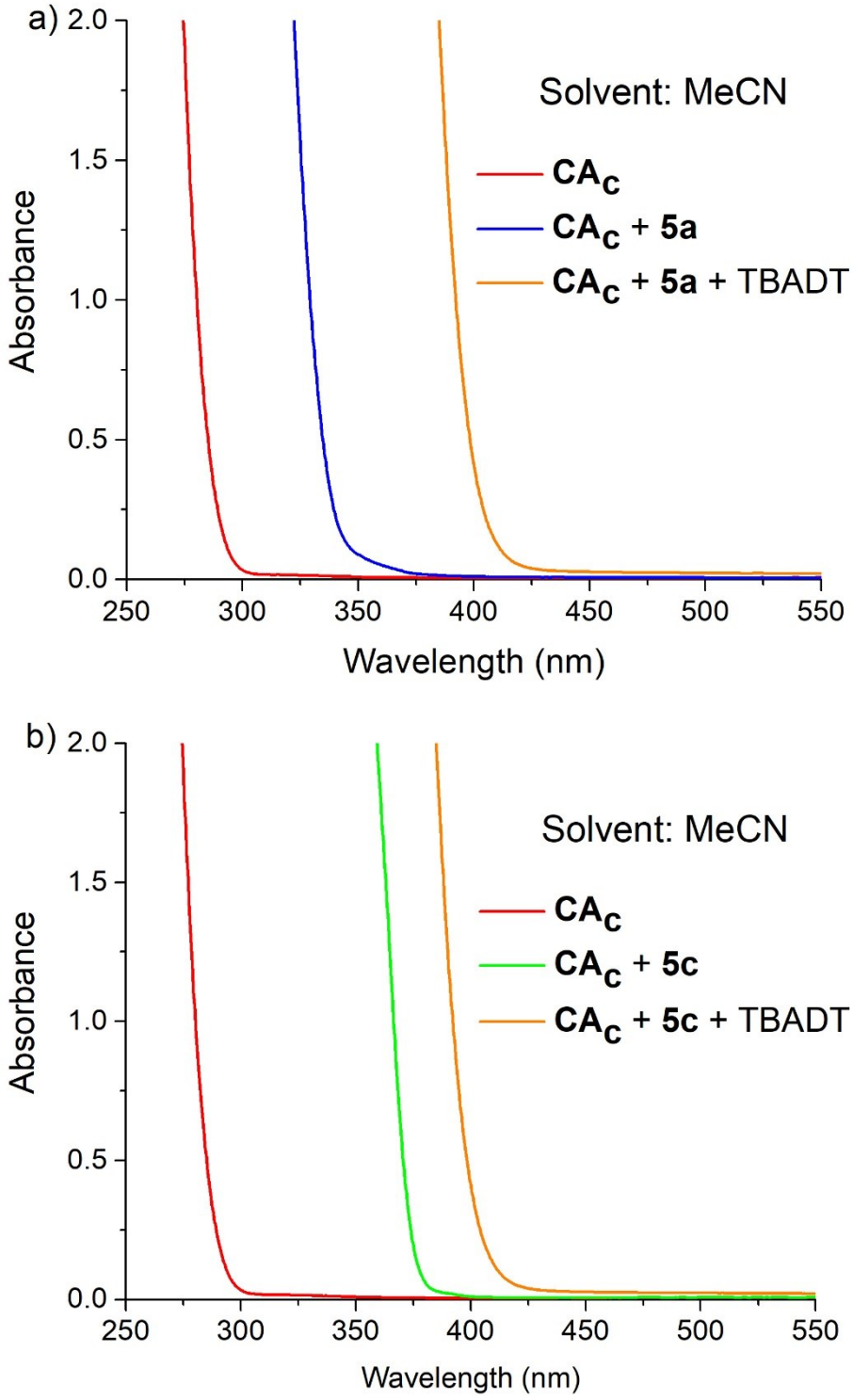
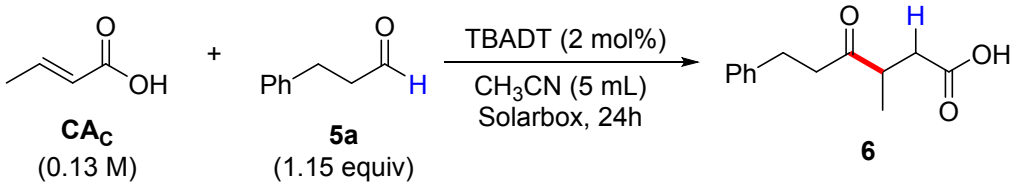


Figure S6. UV-Vis spectra of the different components of the reaction mixtures adopted in the preparation of: **6** (a) and **8** (b).

4 Control Experiments

Table S1. Control experiments for the photocatalytic hydroacylation of **CA$_C$** with aldehyde **5a**.



CA$_C$ (0.13 M) **5a** (1.15 equiv) **6**

Variation from optimized conditions	Product [a]
Reaction in the dark (hν omitted)	**6**, n.d.
TBADT omitted	**6**, n.d.
Additive: TEMPO (1.15 equiv.)	**6**, 36%

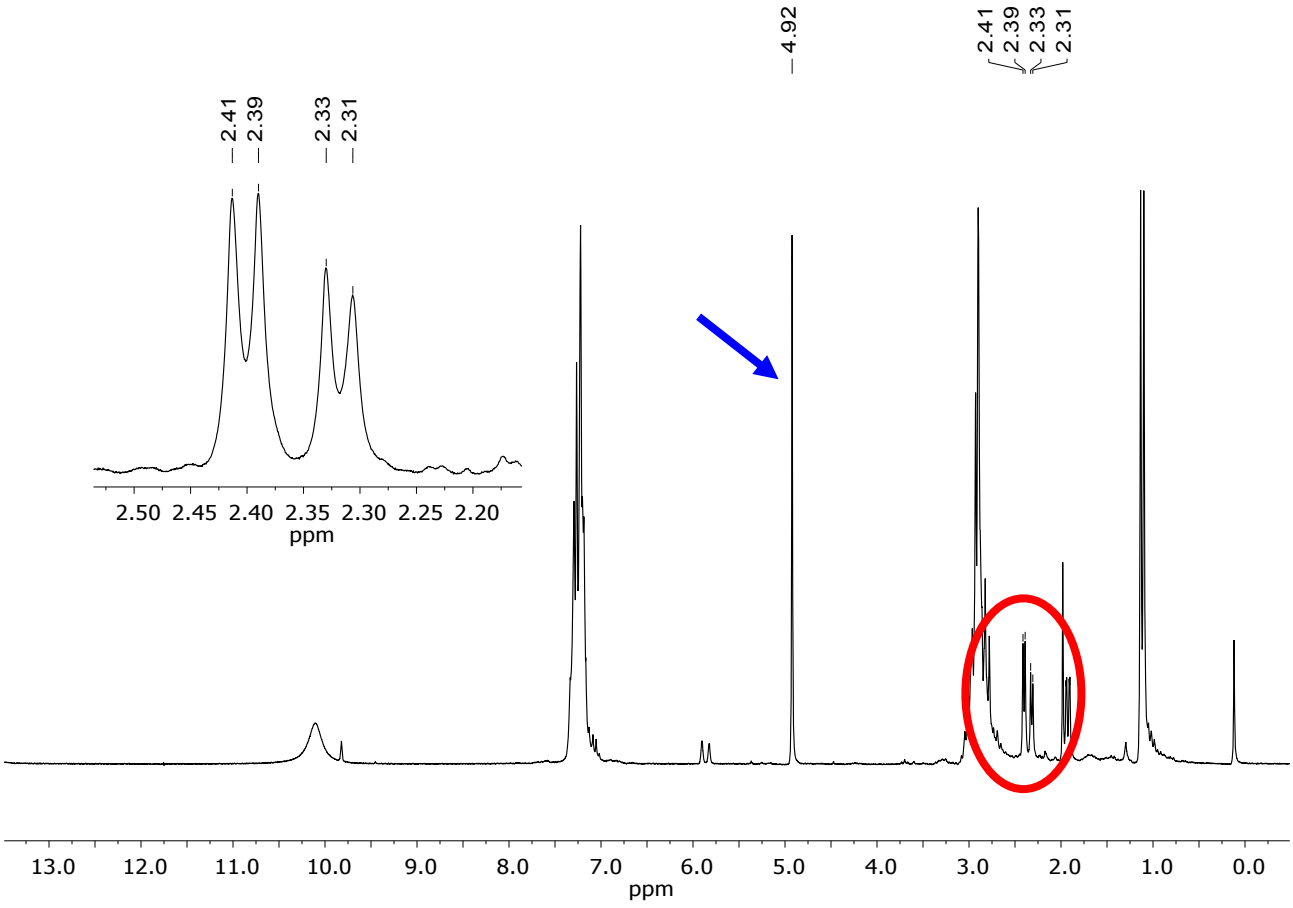
[a] NMR yield.

5 Yield Determination via NMR Spectra Analysis

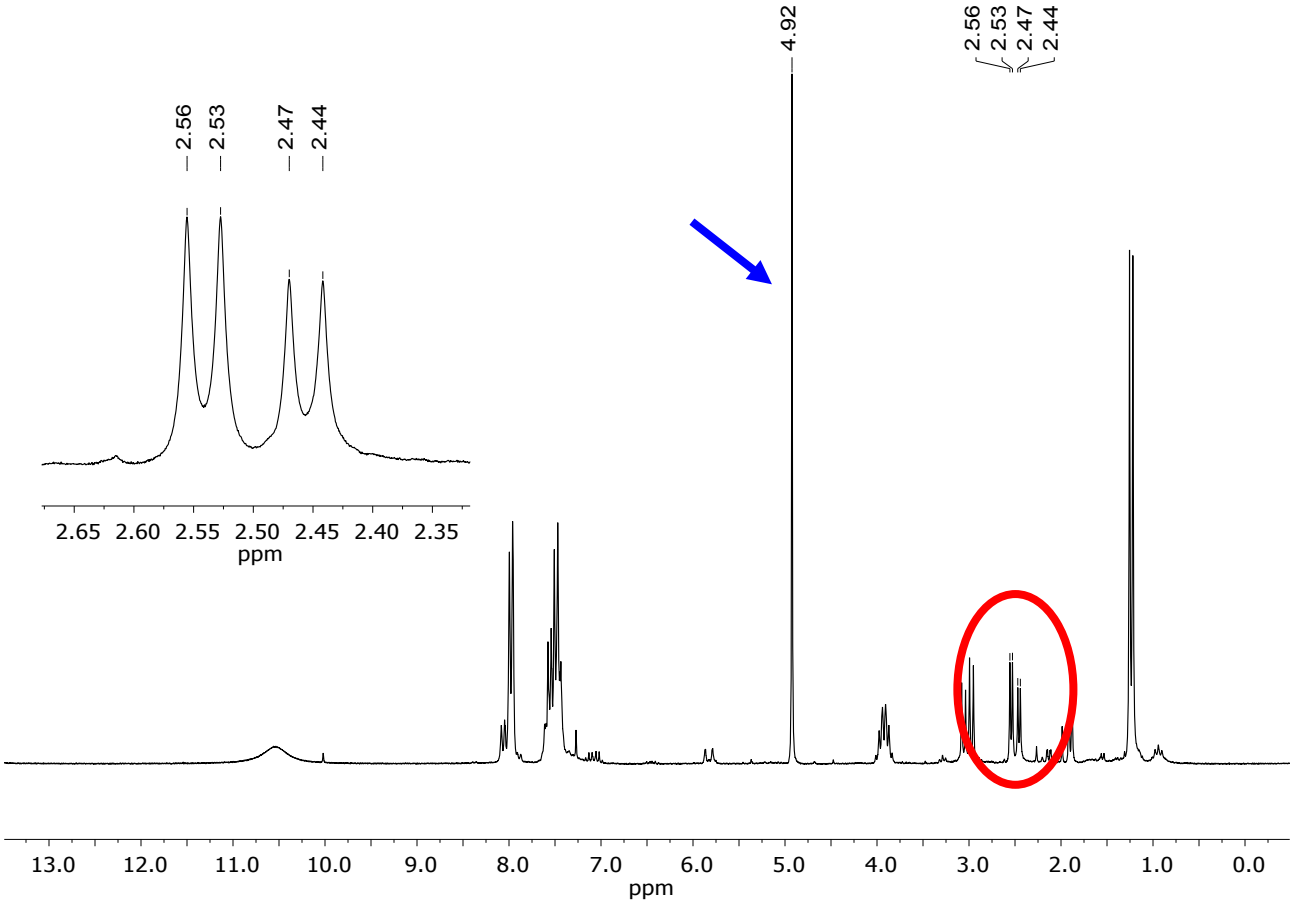
The comparison among the reactivity profiles offered by the different samples of **CA** has been performed via ^{1}H NMR (200 MHz) by adding dibromomethane (CH_2Br_2) as an external standard. A couple of representative NMR charts are reported below. Specifically, the integrals of the following signals have been compared:

- Dibromomethane singlet at *ca.* 4.90 ppm, 2H; (see blue arrow)
- Adduct double doublet at 2.36 ppm for **6** or 2.50 for **8**, 1H (see insets and red circle)

NMR spectrum of the raw mixture obtained in the reaction between CA_C and 5a to give 6



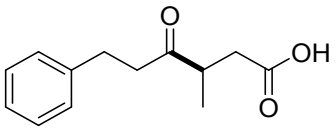
NMR spectrum of the raw mixture obtained in the reaction between CA_C and 5c to give 8



6 Preparation and Characterization of Products 6-12

General procedure for CA acylation. A degassed solution (by argon bubbling for 10 minutes) of **CA** (0.65 mmol, 0.13 M, 56 mg), the chosen aldehyde **5a-g** (0.75 mmol, 0.15M) and TBADT (2 mol%, 43 mg) in 5 mL of acetonitrile, was exposed for 24 h to simulated sunlight in a closed pyrex vessel, using a Solarbox (Solarbox 1500e; CO.FO.ME.GRA., Italy, equipped with a 1.5 kW Xenon lamp; light intensity: 500 W m^{-2}; the emission spectrum of the employed lamp is available online at: https://cofomegra.it/). The progress of the reaction was monitored by GC-FID and, upon completion, the crude mixture was poured into a round-bottom flask and the solvent removed via rotary evaporation. Then, the reaction product was isolated by column chromatography using SiO_2 as the stationary phase and mixtures of cyclohexane/ethyl acetate as eluants. Column chromatography was performed on an Isolera Spektra One (Biotage, Sweden), using Sepachrom Purezza Daily - Open Load cartridges (Sepachrom Srl, Italy).

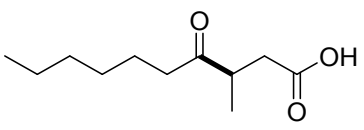
3-Methyl-4-oxo-6-phenylhexanoic acid (6)



Prepared from $\mathbf{CA_C}$ (0.65 mmol, 56 mg) and hydrocinnamaldehyde (**5a**, 0.75 mmol, 1.15 equiv, 99 μL, ρ = 1.019 g mL^{-1}) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 5:5) to afford **6** (102 mg, 71% yield) as a yellowish solid (mp: 52-54 °C). The reaction was repeated on a 3.25 mmol scale (25 mL solution) by using $\mathbf{CA_C}$ and $\mathbf{CA_{60}}$ (280 mg). In such case, product **6** was isolated in 88% (630 mg) and 85% (612 mg) yield, respectively, from $\mathbf{CA_C}$ and $\mathbf{CA_{60}}$. Spectroscopic data of **6** (^{1}H NMR) are in accordance with the literature.[S3]

^{1}H NMR (300 MHz, $CDCl_3$):[S3] δ 11.20 (bs, 1H), 7.31–7.13 (m, 5H), 3.03–2.74 (m, 6H), 2.35 (dd, *J* = 17, 5 Hz, 1H), 1.11 (d, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.6, 177.9, 141.0, 128.4, 128.2, 126.0, 42.6, 41.8, 36.4, 29.5, 16.4. IR (ν/cm^{-1}): 3167, 1709. Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.8; H, 7.3.

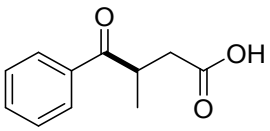
3-Methyl-4-oxodecanoic acid (7)[S4]



Prepared from $\mathbf{CA_C}$ (0.65 mmol, 56 mg) and heptaldehyde (**5b**, 0.75 mmol, 1.15 equiv, 105 μL, ρ = 0.817 g mL^{-1}) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 7:3) to afford **7** (76 mg, 58% yield) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (bs, 1H), 2.96 (ddd, *J* = 14, 10, 6 Hz, 1H), 2.81 (dd, *J* = 17, 9 Hz, 1H), 2.57–2.44 (m, 2H), 2.33 (dd, *J* = 17, 5 Hz, 1H), 1.57 (t, *J* = 7 Hz, 2H), 1.36-1.21 (m, 6H), 1.14 (d, *J* = 7 Hz, 3H), 0.86 (t, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.8, 177.7, 41.7, 40.9, 36.4, 31.5, 28.8, 23.4, 22.4, 16.6, 13.9. IR (ν/cm^{-1}): 2931, 1701. Anal. Calcd. for $C_{11}H_{20}O_3$: C, 65.97; H, 10.07. Found: C, 65.9; H, 10.1.

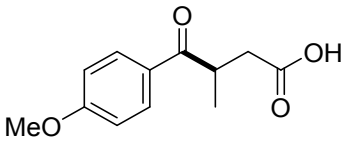
3-Methyl-4-oxo-4-phenylbutanoic acid (8)



Prepared from **CA$_C$** (0.65 mmol, 56 mg) and benzaldehyde (**5c**, 0.75 mmol, 1.15 equiv, 76 μL, ρ = 1.044 g mL^{-1}) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 9:1) to afford **8** (87 mg, 70% yield) as a syrup (mp: Lit. 56-57 °C).[S5] Spectroscopic data of **8** (^{1}H NMR) are in accordance with the literature.[S5]

^{1}H NMR (300 MHz, $CDCl_3$):[S5] δ 10.01 (bs, 1H), 8.00-7.90 (m, 2H), 7.60-7.52 (m, 1H), 7.50-7.41 (m, 2H), 3.99-3.80 (m, 1H), 2.99 (dd, *J* = 17, 8 Hz, 1H), 2.48 (dd, *J* = 17, 6 Hz, 1H), 1.22 (d, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 202.2, 178.1, 135.3, 132.9, 128.4, 128.2, 36.8, 175. IR (ν/cm^{-1}): 2976, 1713, 1240. Anal. Calcd. for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.8; H, 6.2.

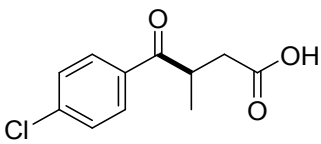
4-(4-Methoxyphenyl)-3-methyl-4-oxobutanoic acid (9)



Prepared from **CA$_C$** (0.65 mmol, 56 mg) and *p*-anisaldehyde (**5d**, 0.75 mmol, 1.15 equiv, 91 μL, ρ = 1.119 g mL^{-1}) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 7:3) to afford **9** (95 mg, 66% yield) as a syrup (mp: Lit. 65-67 °C).[S5] Spectroscopic data of **9** (^{1}H NMR) are in accordance with the literature.[S5]

^{1}H NMR (300 MHz, $CDCl_3$):[S5] δ 8.25 (bs, 1H), 7.96 (d, *J* = 9 Hz, 2H), 6.94 (d, *J* = 9 Hz, 2H), 3.92-3.78 (m, 4H), 2.97 (dd, *J* = 17, 8 Hz, 1H), 2.47 (dd, *J* = 17, 6 Hz, 1H), 1.23 (d, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.8, 177.5, 163.3, 130.5, 128.2, 113.6, 55.2, 36.9, 36.4, 17.8. IR (ν/cm^{-1}): 2973, 1709, 1673, 1246. Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.9; H, 6.4.

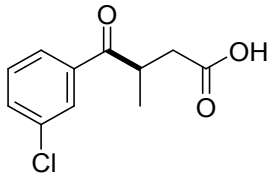
4-(4-Chlorophenyl)-3-methyl-4-oxobutanoic acid (10)



Prepared from **CA$_C$** (0.65 mmol, 56 mg) and *p*-chlorobenzaldehyde (**5e**, 0.75 mmol, 1.15 equiv, 105 mg) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 7:3) to afford **10** (85 mg, 58% yield) as a yellowish solid (mp: 81-82 °C; Lit. 80-81 °C).[S6] Spectroscopic data of **10** (^{1}H NMR) are in accordance with the literature.[S6]

^{1}H NMR (300 MHz, $CDCl_3$):[S6] δ 9.72 (bs, 1H), 7.85 (d, *J* = 9 Hz, 2H), 7.43 (d, *J* = 9 Hz, 2H), 3.91-3.75 (m, 1H), 2.99 (dd, *J* = 17, 9 Hz, 1H), 2.48 (dd, *J* = 17, 5 Hz, 1H), 1.21 (d, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.9, 178.8, 140.2, 134.5, 130.4, 129.6, 37.6, 37.6, 18.4. IR (ν/cm^{-1}): 3066, 2973, 1713, 1696, 1590, 839. Anal. Calcd. for $C_{11}H_{11}ClO_3$: C, 58.29; H, 4.89. Found: C, 58.3; H, 4.9.

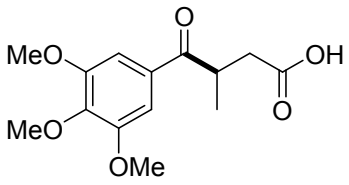
4-(3-Chlorophenyl)-3-methyl-4-oxobutanoic acid (11)



Prepared from **CA$_C$** (0.65 mmol, 56 mg) and *m*-chlorobenzaldehyde (**5f**, 0.75 mmol, 1.15 equiv, 85 μL, ρ = 1.241 g mL^{-1}) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 7:3) to afford **11** (131 mg, 89% yield) as a yellowish oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.54 (bs, 1H), 7.94 (s, 1H), 7.85 (d, *J* = 8 Hz, 1H), 7.67-7.53 (m, 1H), 7.45 (t, *J* = 8 Hz, 1H), 3.93-3.78 (m, 1H), 3.04 (dd, *J* = 17, 9 Hz, 1H), 2.53 (dd, *J* = 17, 5 Hz, 1H), 1.26 (d, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.2, 177.9, 137.2, 135.0, 133.0, 129.9, 128.4, 126.4, 37.1, 36.8, 17.6. IR (ν/cm^{-1}): 3071, 2977, 1712, 1674, 977. Anal. Calcd. for $C_{11}H_{11}ClO_3$: C, 58.29; H, 4.89. Found: C, 58.2; H, 5.0.

3-Methyl-4-oxo-4-(3,4,5-trimethoxyphenyl)butanoic acid (12)



Prepared from **CA$_C$** (0.65 mmol, 56 mg) and 3,4,5-trimethoxybenzaldehyde (**5g**, 0.75 mmol, 1.15 equiv, 147 mg) according to the general procedure. The crude mixture was purified through column chromatography (SiO_2; cyclohexane/ethyl acetate 1:1) to afford 9 (127 mg, 69% yield) as a syrup.

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.01 (bs, 1H), 7.26 (s, 2H), 3.94 (s, 9H), 3.89-3.80 (m, 1H), 3.01 (dd, *J* = 17, 8 Hz, 1H), 2.51 (dd, *J* = 17, 5 Hz, 1H), 1.27 (d, *J* = 7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.0, 177.7, 152.9, 142.5, 130.5, 105.7, 60.6, 56.0, 36.9, 36.6, 17.8. IR (ν/cm^{-1}): 2966, 1709, 1679. Anal. Calcd. for $C_{14}H_{18}O_6$: C, 59.57; H, 6.43. Found: C, 59.6; H, 6.4.

7 References

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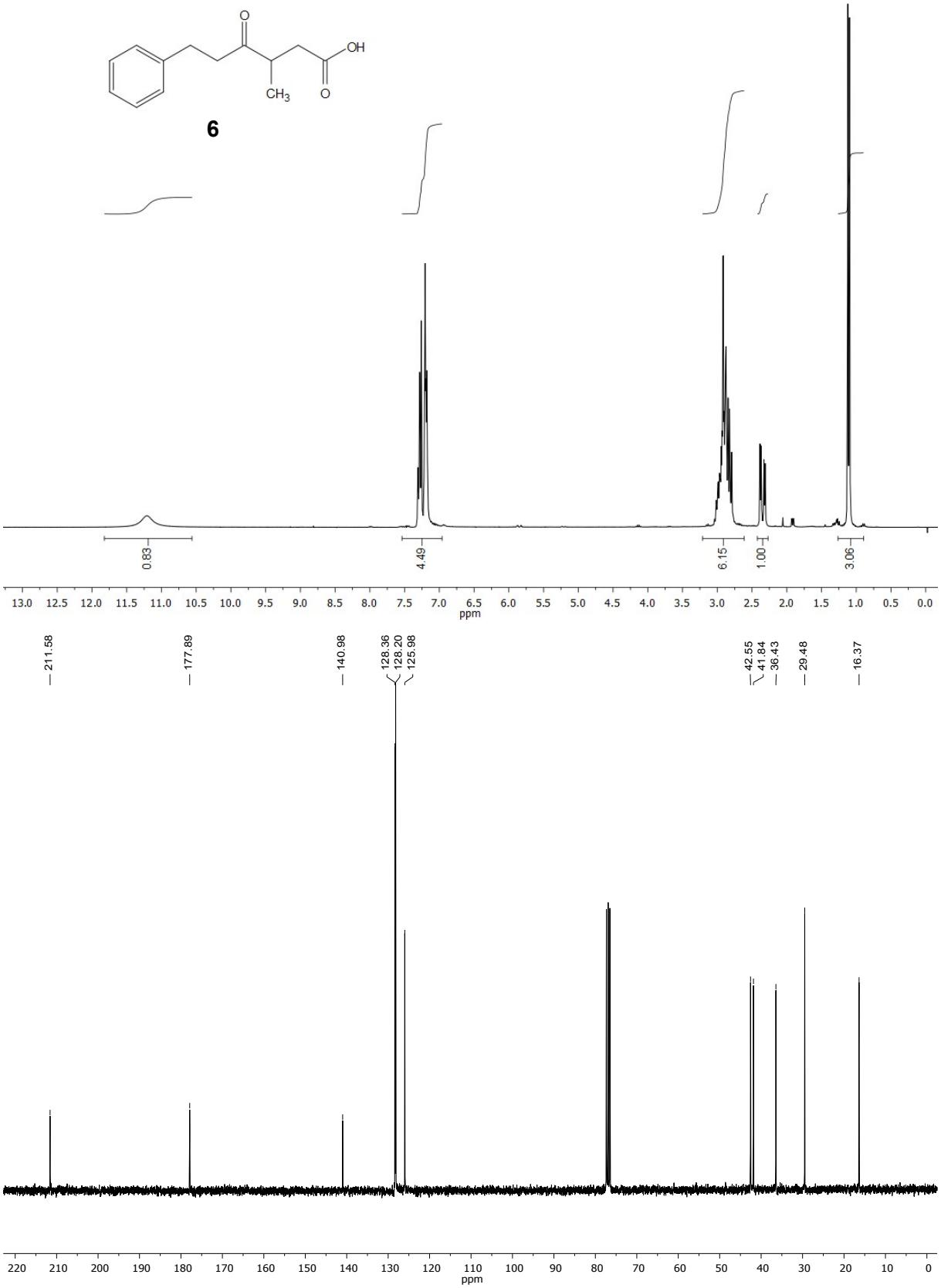
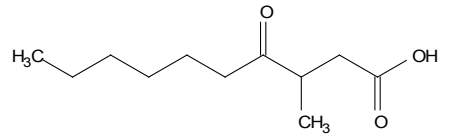
S4 H. Schick, F. Theil, H. Jablokoff and S. Schwarz, *Z. Chem.*, 2010, **21**, 68–69.

S5 R. Frenette, M. Kakushima, R. Zamboni, R. N. Young and T. R. Verhoeven, *J. Org. Chem.*, 1987, **52**, 304–307.

S6 R. Jonas, M. Klockow, I. Lues, H. Prücher, H. Schliep and H. Wurziger, *Eur. J. Med. Chem.*, 1993, **28**, 129–140.

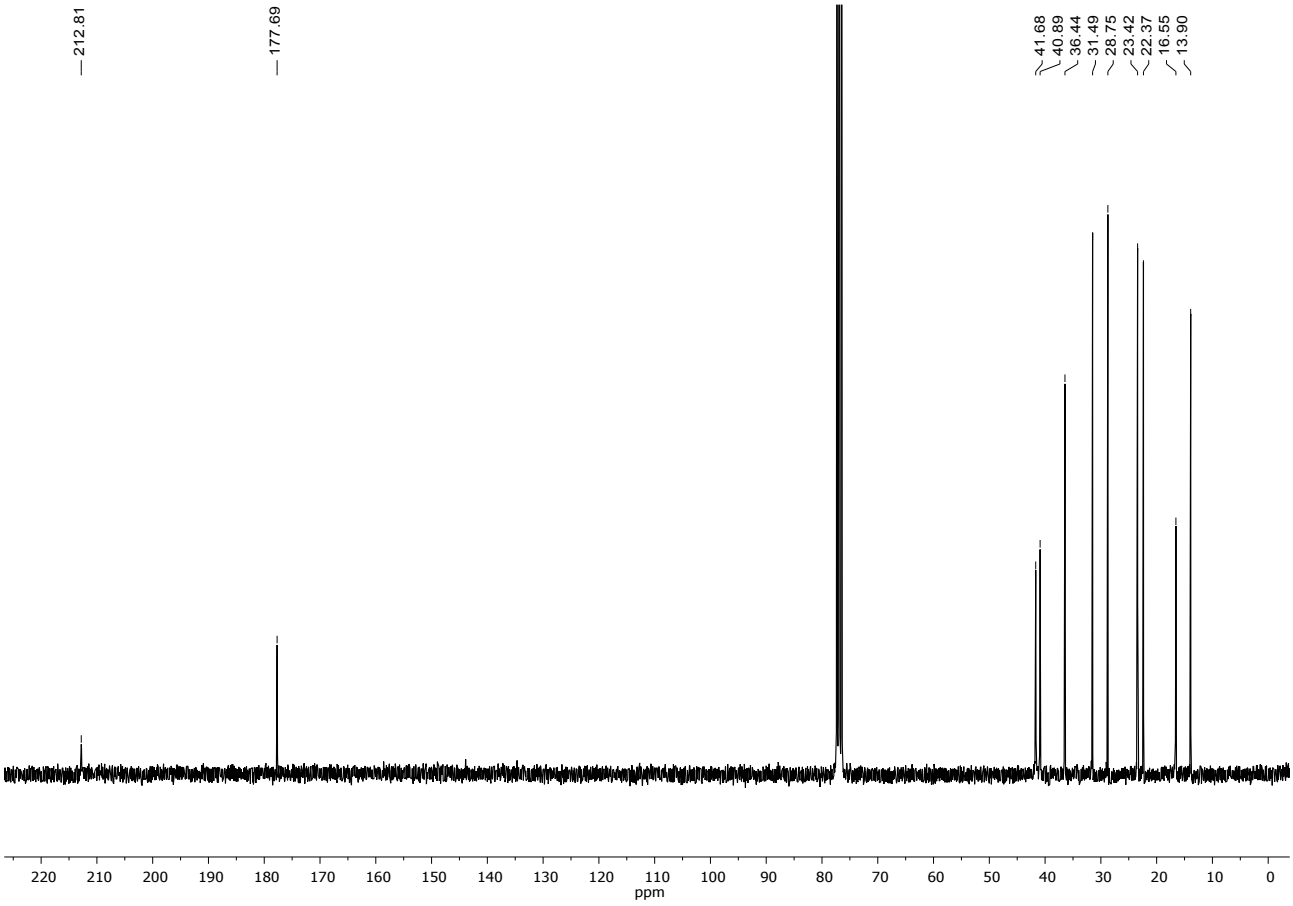
8 Copy of ^{1}H-NMR and ^{13}C-NMR Spectra of Compounds 6-12

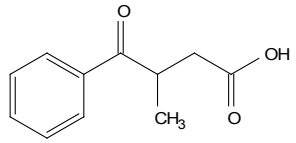



O
H3C
OH
CH3
O

7

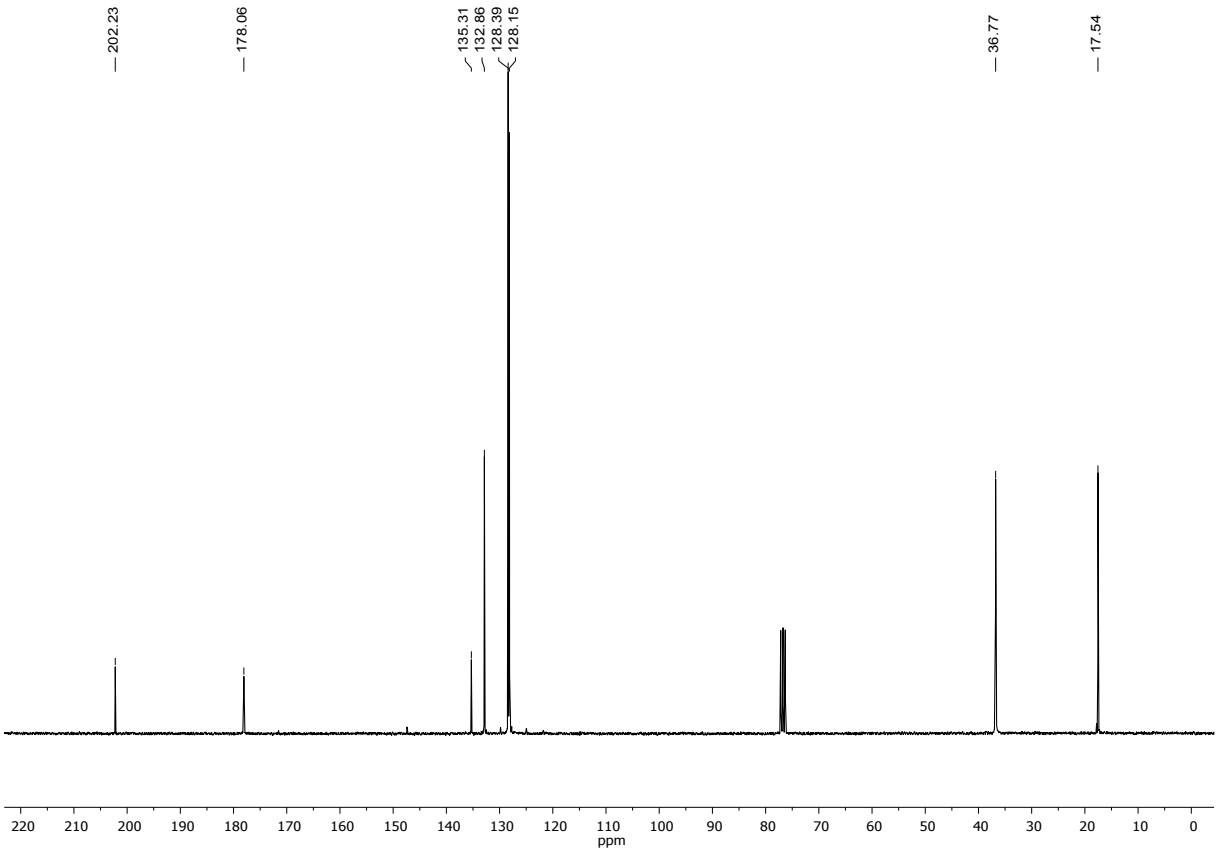
0.90
1.00
1.02
2.08
1.08
2.12
6.28
3.12
3.06
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm

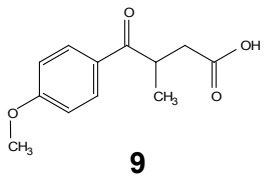

212.81
177.69
41.68
40.89
36.44
31.49
28.75
23.42
22.37
16.55
13.90
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


O
OH
CH3
O

8

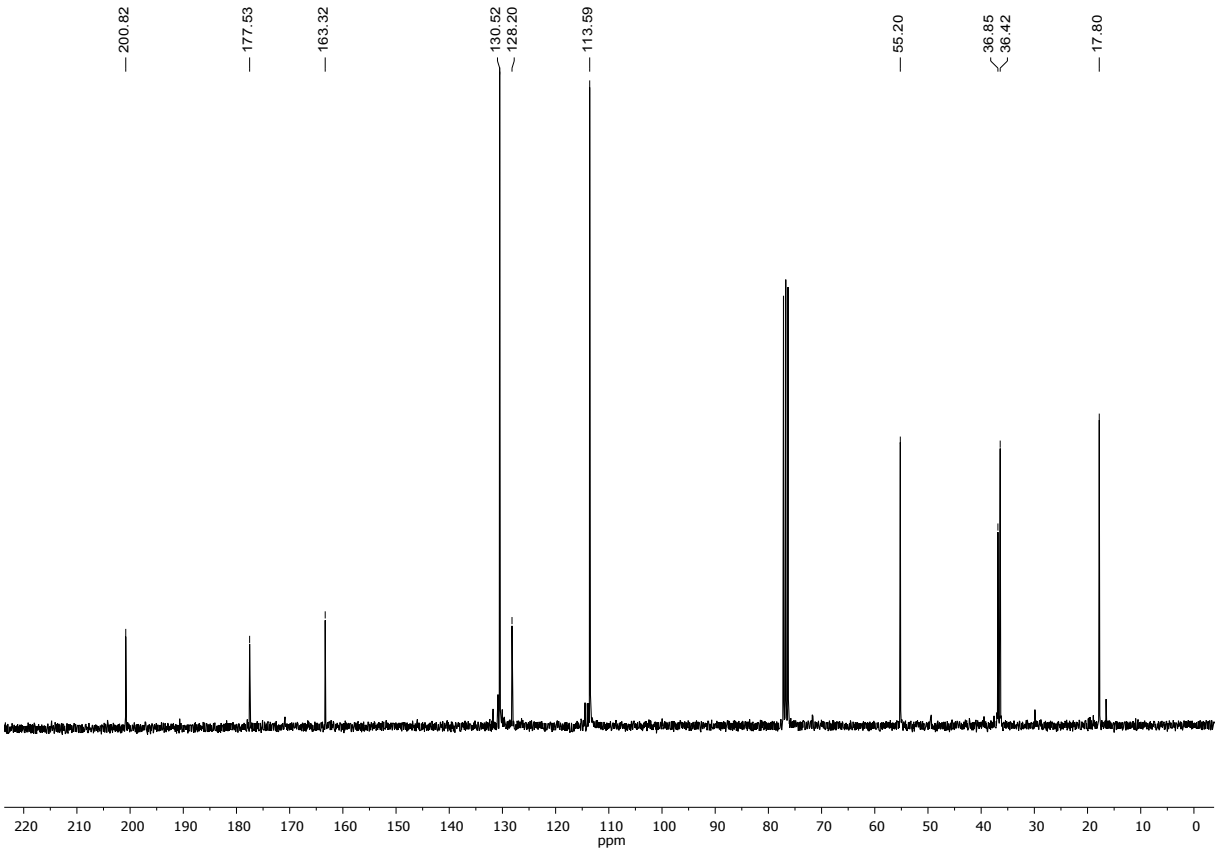
1.09
2.04
1.00
2.01
1.04
1.05
1.06
3.28
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm

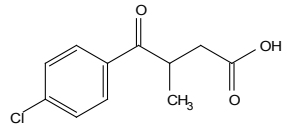

202.23
178.06
135.31
132.86
128.39
128.15
36.77
17.54
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


O
OH
CH3
O
O
CH3

9

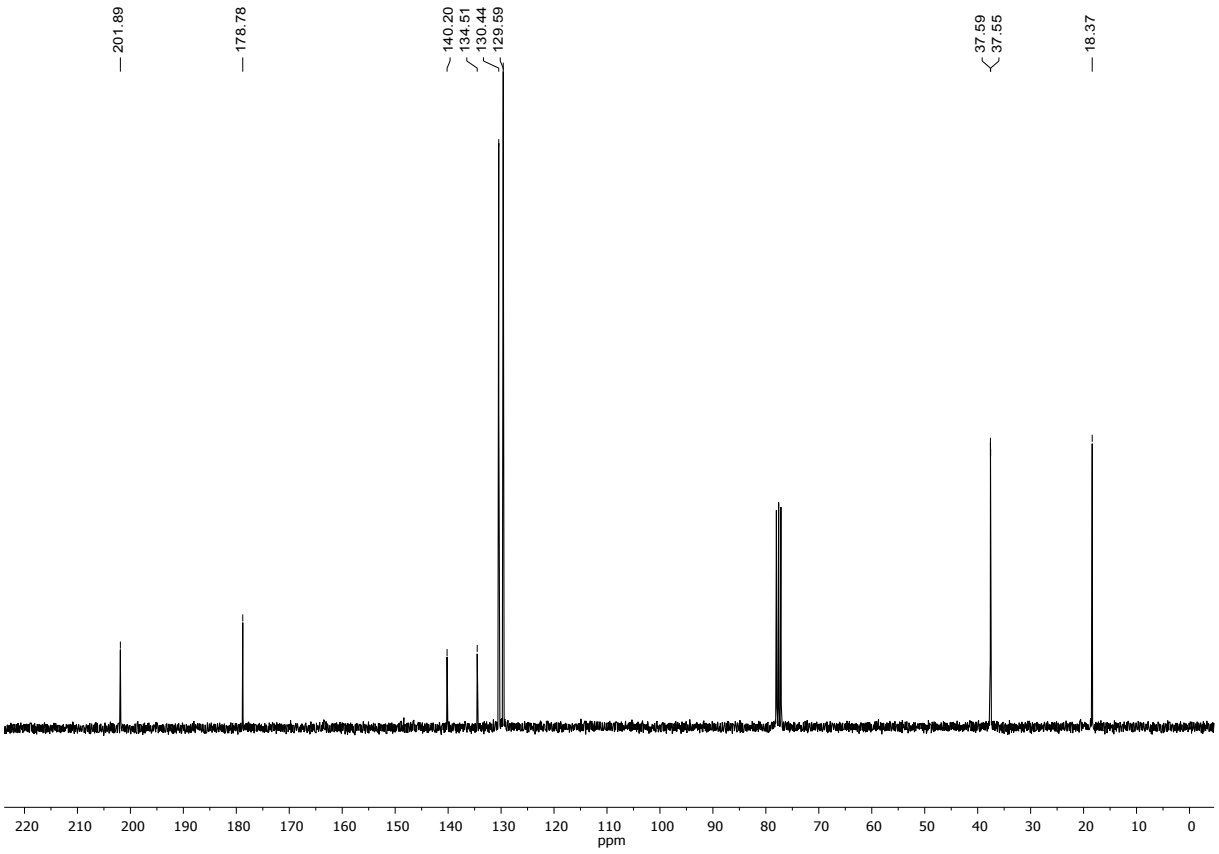
6.30
4.46
1.00
1.16
3.13
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm

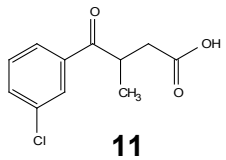

200.82
177.53
163.32
130.52
128.20
113.59
55.20
36.85
36.42
17.80
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


O
OH
CH3
O
Cl

10

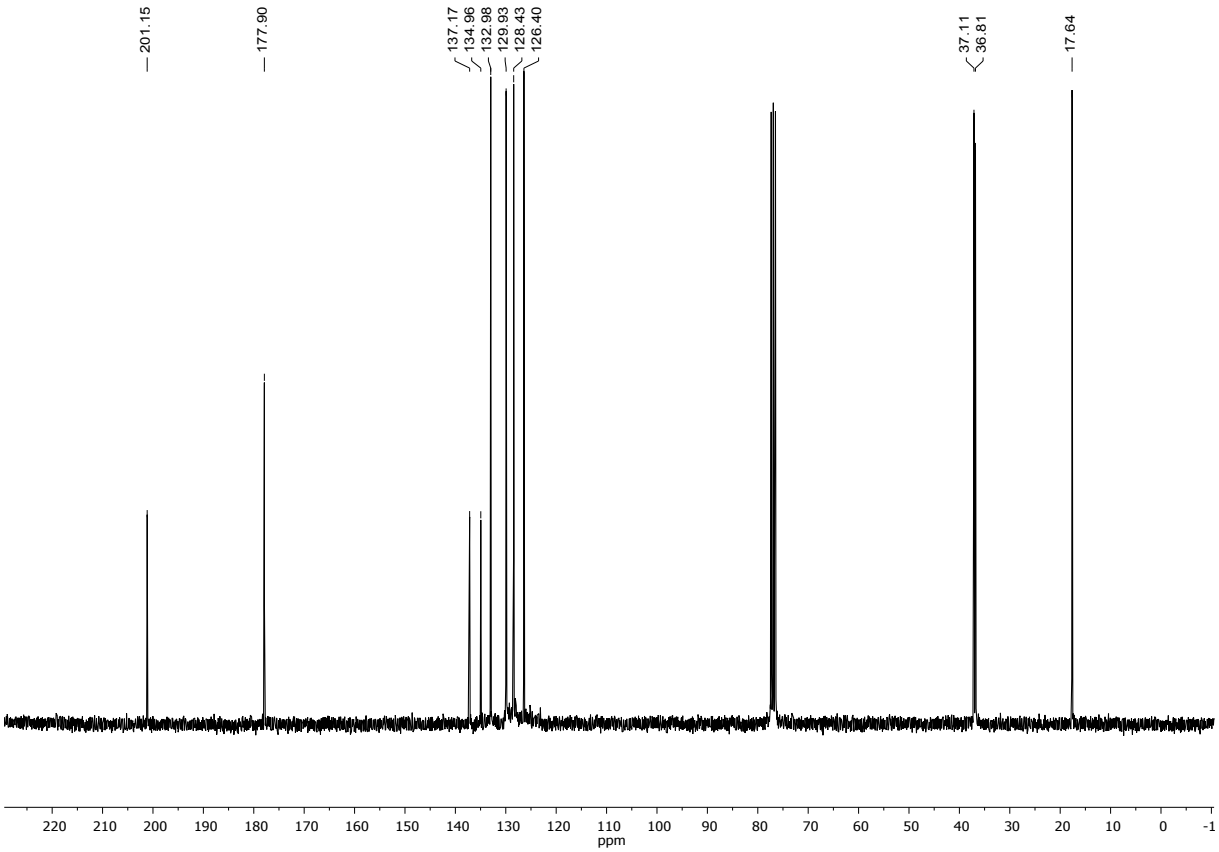
1.02
2.03
1.92
1.01
1.02
1.03
3.04
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm

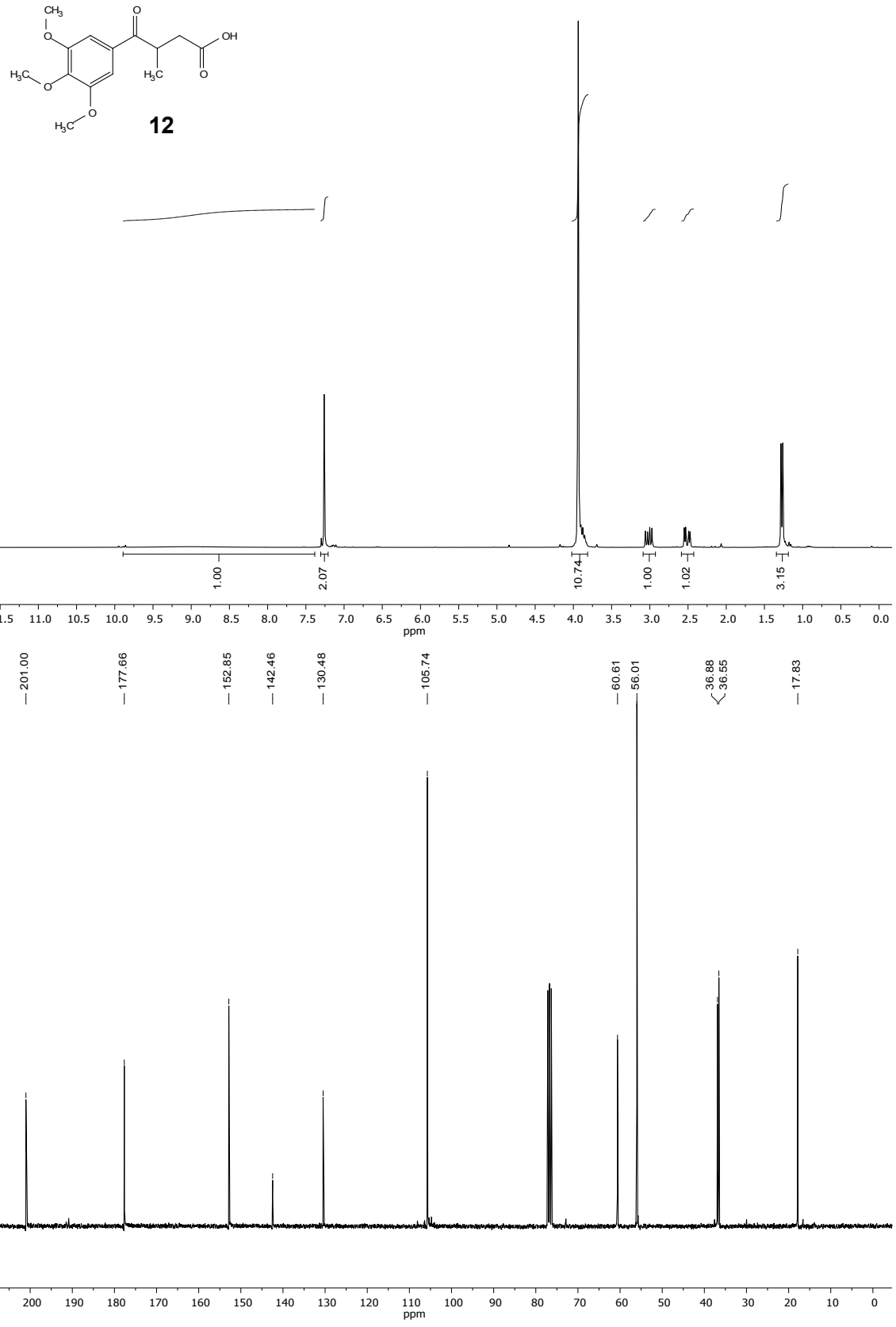

201.89
178.78
140.20
134.51
130.44
129.59
37.59
37.55
18.37
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


O
OH
CH3
O
Cl

11

1.03
1.04
1.02
1.03
1.19
1.00
1.00
1.03
3.12
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm


201.15
177.90
137.17
134.96
132.98
129.93
128.43
126.40
37.11
36.81
17.64
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
ppm


12
1.00
2.07
10.74
1.00
1.02
3.15
ppm
201.00
177.66
152.85
142.46
130.48
105.74
60.61
56.01
36.88
36.55
17.83
ppm