Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Non-hazardous Baeyer-Villiger Oxidation of Levulinic Acid Derivatives: Sustainable Access to 3-Hydroxypropionates

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Contents

BVOx of levulinates 1–3 using organic peroxides

General Procedure

Organic peroxide was mixed with or suspended (*m*CPBA) in the solvent (0.5 mL). Peracetic acid and trifluoroperacetic acid were prepared *in situ* by addition of 50% H_2O_2 to the corresponding carboxylic acid (0.5 mL) at 0 °C and stirring for 30 min. Then the keto ester was added as solution in the same solvent (0.5 mL, final concentration 10% w/v). The resulting mixture was then stirred at the indicated temperature. Samples were taken by dilution with DCM and neutralization with aqueous sodium bicarbonate solution, and then measured via GC-MS(EI) or GC-FID.

Reagent, Solvent	**Peroxide Equivalents**	**Reaction Conditions**	**Reaction Outcome**		
			1	**2**	**3**
*m*CPBA, DCM	1.5	rt, 45 h	decomp.	decomp.	decomp.
*m*CPBA, toluene	1.5 + 5.0 (after 24 h)	rt, 48 h	n.c.	n.c.	n.c.
*t*BuOOH toluene	1.5	rt, 24 h	n.c.	n.c.	n.c.
CH_3CO_3H	2.0	rt, 48 h	n.c.	n.c.	n.c.
CF_3CO_3H	2.0	rt, 48 h	29% 79:21[a]	27% 66:34[a]	24% 80:20[a]
CF_3CO_3H	10.0	50 °C, 48 h	decomp.	decomp.	decomp.

[a] Ratio of desired products **1a–3a** to unidentified by-product; decomp.: decomposition of starting material and/or product(s), n.c.: no conversion

$CPMO_{F156L}$ crude cell extract preparation

LB_{amp} medium (10 mL) was inoculated with single colony from a LB_{amp} agar plate (<4 weeks old) and shaken at 200 rpm and 37 °C on an orbital shaker for 12–18 h in a baffled Erlenmeyer flask (50 mL). Then TB_{amp} medium (500 mL, 4 g L^{-1} D-glucose) was inoculated with 2% v/v of the overnight culture and incubated on the orbital shaker for 1.5–2.5 h at 200 rpm and 37 °C until an OD_{600} of 0.3–0.8 was reached. IPTG (0.2 mM) was added and the baffled Erlenmeyer flask (2000 mL) transferred to a 24 °C shaker and incubated at 200 rpm for 24 h. Cells were harvested by centrifugation (6 kRCF, 15 min, 4°C), resuspended in TrisHCl buffer (50 mL, 10 mM, pH 7.5) and centrifuged (6 kRCF, 15min, 4 °C). The washed cells were again resuspended in TrisHCl buffer (10 mL, 10 mM, pH 7.5). PMSF (0.2 mM, 0.2 M stock in EtOH), FAD (0.1 mM, 0.1 M stock in water) and $NADP^+$ (0.2 mM, 0.1 M stock in water) were added. Cells were lysed by sonication (50 % amplitude, 10 s pulse, 60 s pause, 6 cycles, 4–8 °C). Cell debris was then removed by centrifugation (9.5–18 kRCF depending on Falcon tube size, 60–120 min, 4 °C) and the intense yellow supernatant stored at -20 °C. Total protein concentration was determined by Bradford assay using bovine serum albumin calibration.

General procedure for analytical scale screening using whole cells

A baffled Erlenmeyer flask was charged with LB medium with appropriate antibiotics supplement (10 mL), inoculated with a bacterial single colony from an Agar plate and incubated at 37 °C in an orbital shaker o/n. The biotransformation medium, supplemented with appropriate antibiotics, was then inoculated with 2% v/v of the preculture and incubated for approx. 1–2 h under the same conditions until an optical density of 0.2–0.6 was reached. Inducing agent and β-cyclodextrin (1 equiv.) were added, the mixture was thoroughly mixed and split in 1.0 mL aliquots into 24-well plates. Substrates were added as 0.8 M solutions in 1,4-dioxane to a final concentration of 4 mM. The plates were sealed with adhesive film and incubated at the appropriate temperature in an orbital shaker for up to 24 h. Analytical samples were prepared by extraction of 0.5 mL of biotransformation culture with 1.0 mL EtOAc (supplemented with 1 mM methyl benzoate as internal standard) after centrifugal separation of the cell mass (approx. 15 kRCF, 1 min, rt).

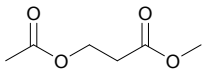
General procedure for analytical scale screening using crude cell extract

CCE of CPMOB1-A3 was prepared as described above. Reactions were performed in 96-well plates (200 µL working volume) or 24-well plates (0.5–1.0 mL working volume). $CPMO_{F156L}$ CCE (solution in 10 mM TrisHCl pH 7.5), $NADP^+$ (0.2 mM, 0.1 M solution in water), glucose-6-phsosphate (0.5 equiv., 0.5 M solution in water) and glucose-6-phosphate dehydrogenase (5 U mL^{-1}, 0.26 µL U^{-1} ammonium sulfate suspension) were added to aqueous TrisHCl buffer (50 mM, pH 7.1). Ketone **2** was either added neat or as solution in MeOH (2% v/v MeOH final concentration in the biotransformation; substrate stock solutions were prepared accordingly) to the reaction wells using a piston pipette (calibrated for neat additions). Multi-well plates were agitated on a benchtop orbital shaker (250–350 rpm) at rt. Samples were taken by extraction of an aliquot of reaction mixture with 1–5x volume equivalents of EtOAc (supplemented with 1.0 mM methyl benzoate as IS). The extracts were centrifuged and analyzed by GC-FID.

General procedure for shakeflask biotransformations

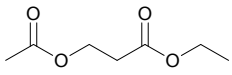
LB growth medium was inoculated, induced, and charged with additives as described above. The substrate was then added directly to the shakeflask as approx. 10% (w/v) solution in dioxane. Incubation at 24 °C was carried out until full conversion was determined via GC control. The aqueous solution was then centrifuged (17 kRCF, 15 min 4 °C) and the supernatant was extracted with Et_2O. The pooled organic layers were washed with brine, dried and concentrated. The crude compounds were purified by chromatography on silica using LP/Et_2O mixtures.

Methyl 3-acetoxypropionate 1a



According to the general procedure, keto ester **1** (50 mg) was oxidized using $CPMO_{Coma}$ (2 mM substrate concentration, 25 h reaction time). Compound **1a**[1] was obtained in 80% yield (45 mg) as colorless liquid after column chromatography (LP/Et_2O 3:1). ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.02 (s, 3H), 2.63 (t, 2H, *J* = 6.4 Hz), 3.69 (s, 3H), 4.31 (t, 2H, *J* = 6.4 Hz) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 20.9 (q), 33.7 (t), 52.0 (q), 59.9 (t), 170.9 (s), 171.2 (s) ppm.

Gram scale Baeyer-Villiger oxidation of ethyl levulinate 2 to ethyl 3-acetoxypropionate 2a



In a New Brunswick benchtop fermentor LB_{amp} medium (1.8 L) was inoculated from an overnight culture of $CPMO_{F156L}$ (36 ml, 2% v/v) at 37 °C. The solution was stirred (250 rpm) and sparged wit air (1 vvm) at 37 °C and pH 7.0 for 2 h. Temperature was then set to 24 °C and BVMO overexpression was induced by addition of IPTG (429 µL, 839 mM stock in water, 0.2 mM final concentration). LevOEt **2** (2.60 g, 18 mmol, 10 mM) was added neat along with β-cyclodextrin (2.04 g, 1.8 mmol, 0.1 equiv.). The culture was agitated under the same conditions for 20 h. Cell mass was separated by centrifugation (17 kRCF, 15 min, 4 °C) and the supernatant extracted with DCM (6 x 250 mL). The pooled organic layers were dried and concentrated under reduced pressure, yielding **2a**[2] as yellow liquid with minor impurities (1.9 g, 66%, purity >90% via GC-FID). ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.16 (t, 3H, *J* = 7.1 Hz), 1.94 (s, 3H), 2.53 (t, 2H, *J* = 6.4 Hz), 4.06 (q, 2H, *J* = 7.1 Hz), 4.23 (t, 2H, *J* = 6.3 Hz) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 14.0 (q), 20.7 (q), 33.7 (t), 59.7 (t), 60.6 (t), 170.5 (s), 170.6 (s) ppm.

Visualization of $CPMO_{F156L}$ in comparison with wild-type $CPMO_{Coma}$

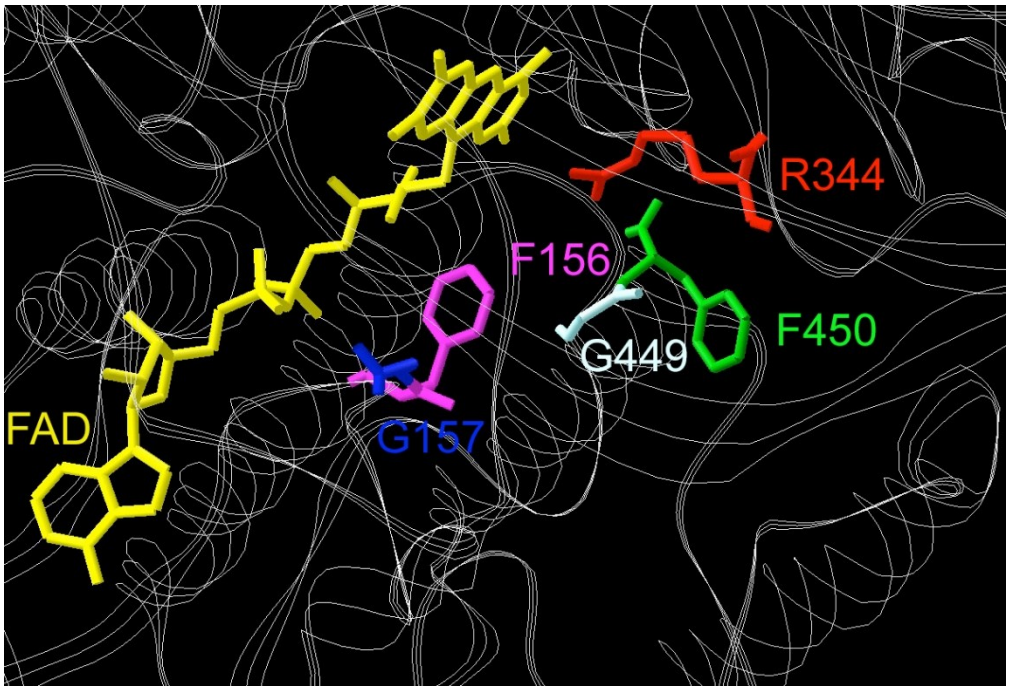


Figure S 1 Wild-type $CPMO_{Coma}$

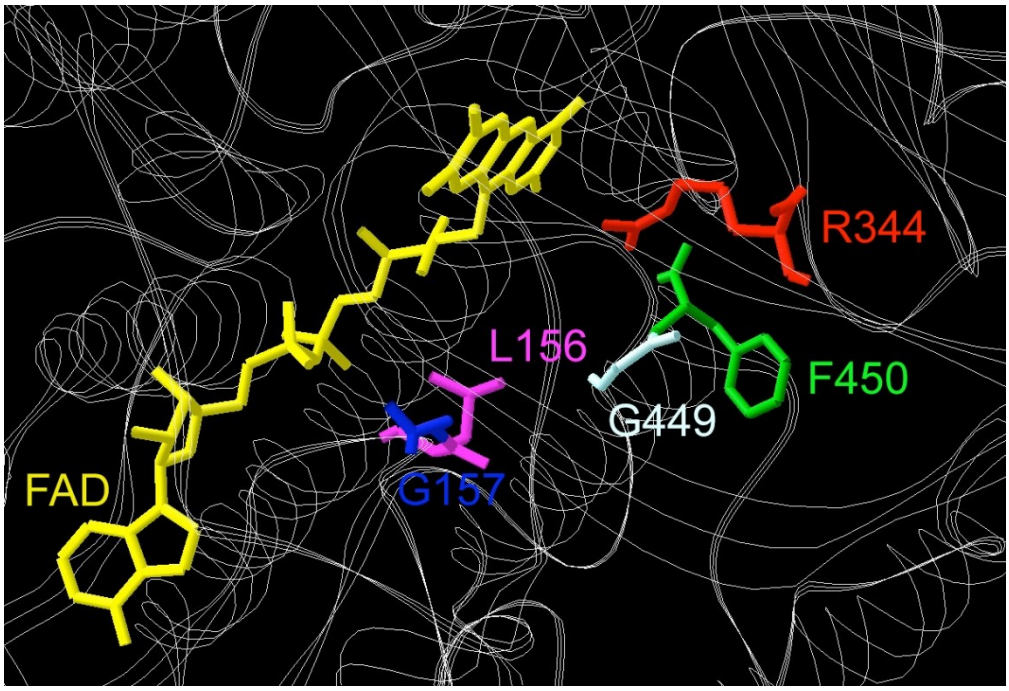


Figure S 2 CPMO F156L variant

Results from analytical scale screening of CPMO variants

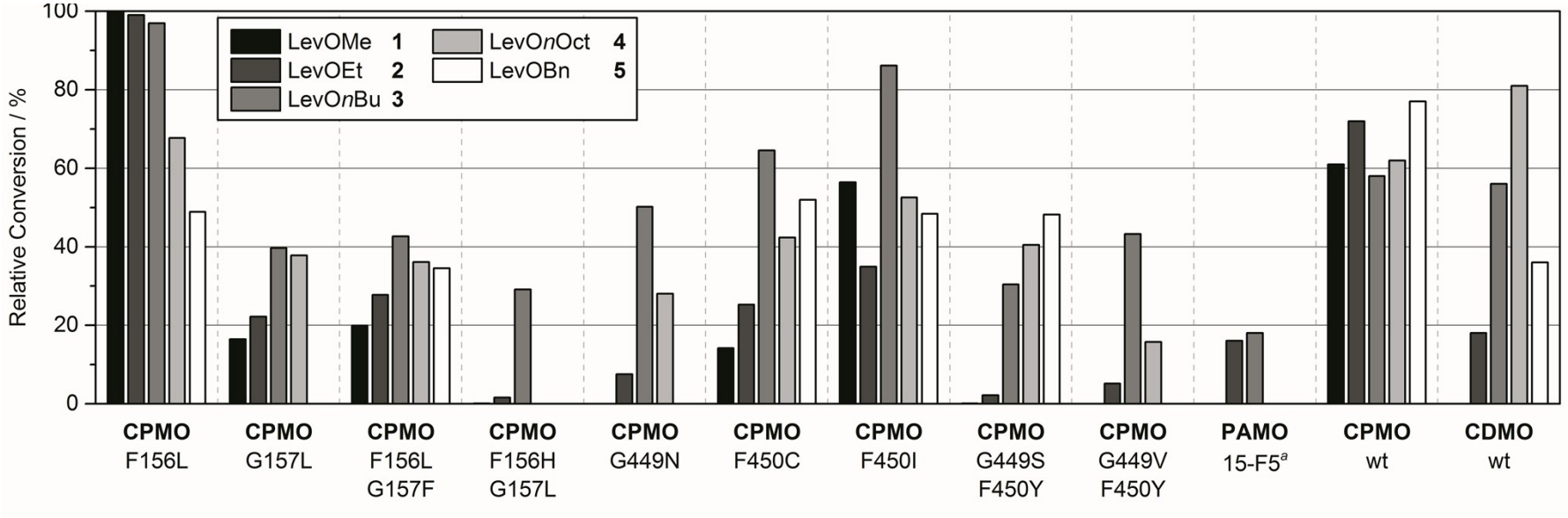
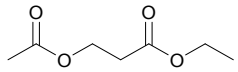
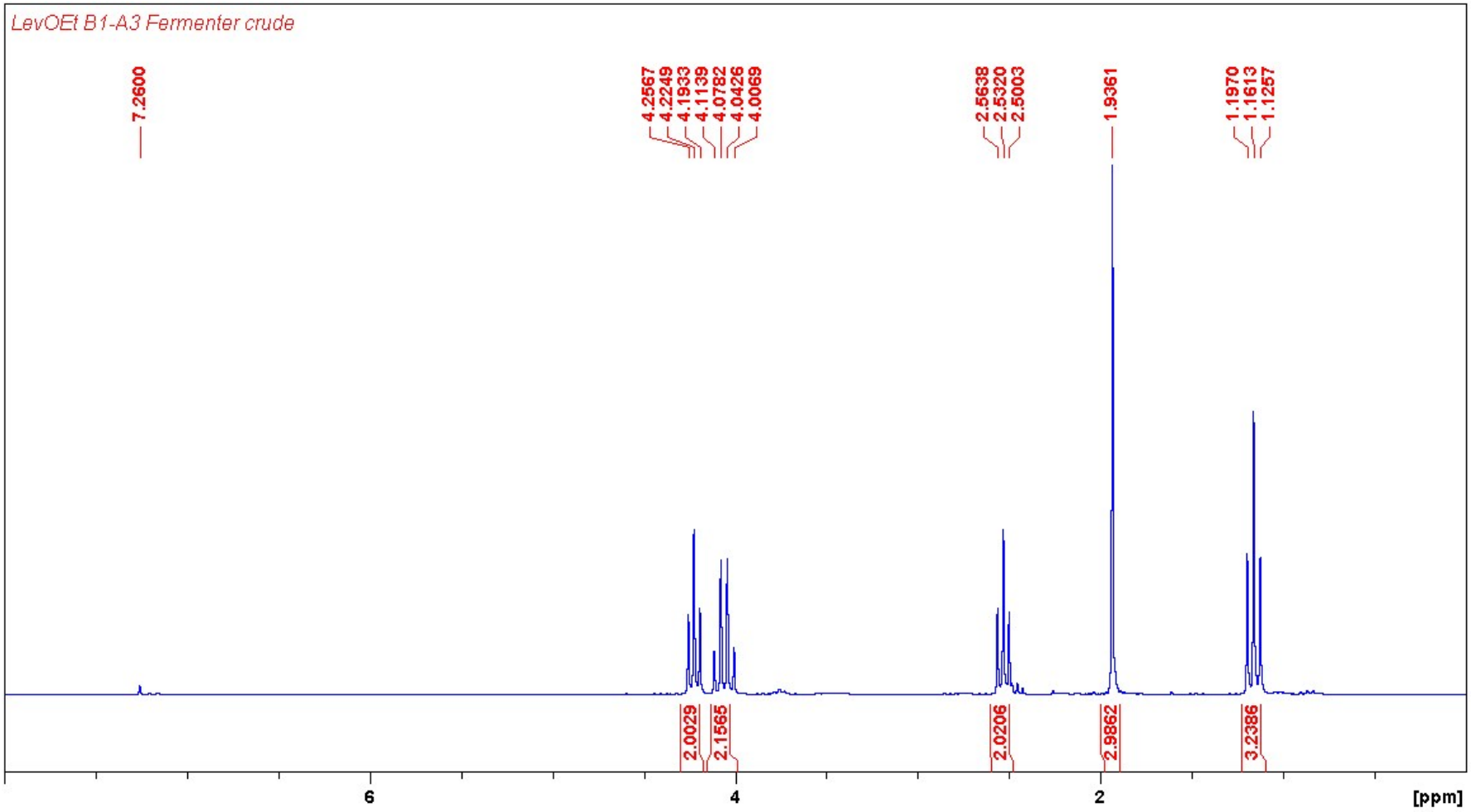


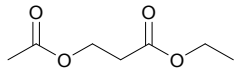
Figure S 3 Analytical scale screening of CAST variants of $CPMO_{Coma}$ using levulinates **1–5**; relative conversion as determined by GC-FID; reaction conditions: BVMO in recombinant E. coli, air, LB medium, 8 mM substrate, 0.5% v/v 1,4-dioxane, 1 mL reaction volume, 24 °C, 24 h;
[a] $CPMO_{Coma}$-templated variant of $PAMO_{Thermo}$, tested at 4 mM substrate concentration (amino acid exchanges: P253F, G254A, R258M, L443F).

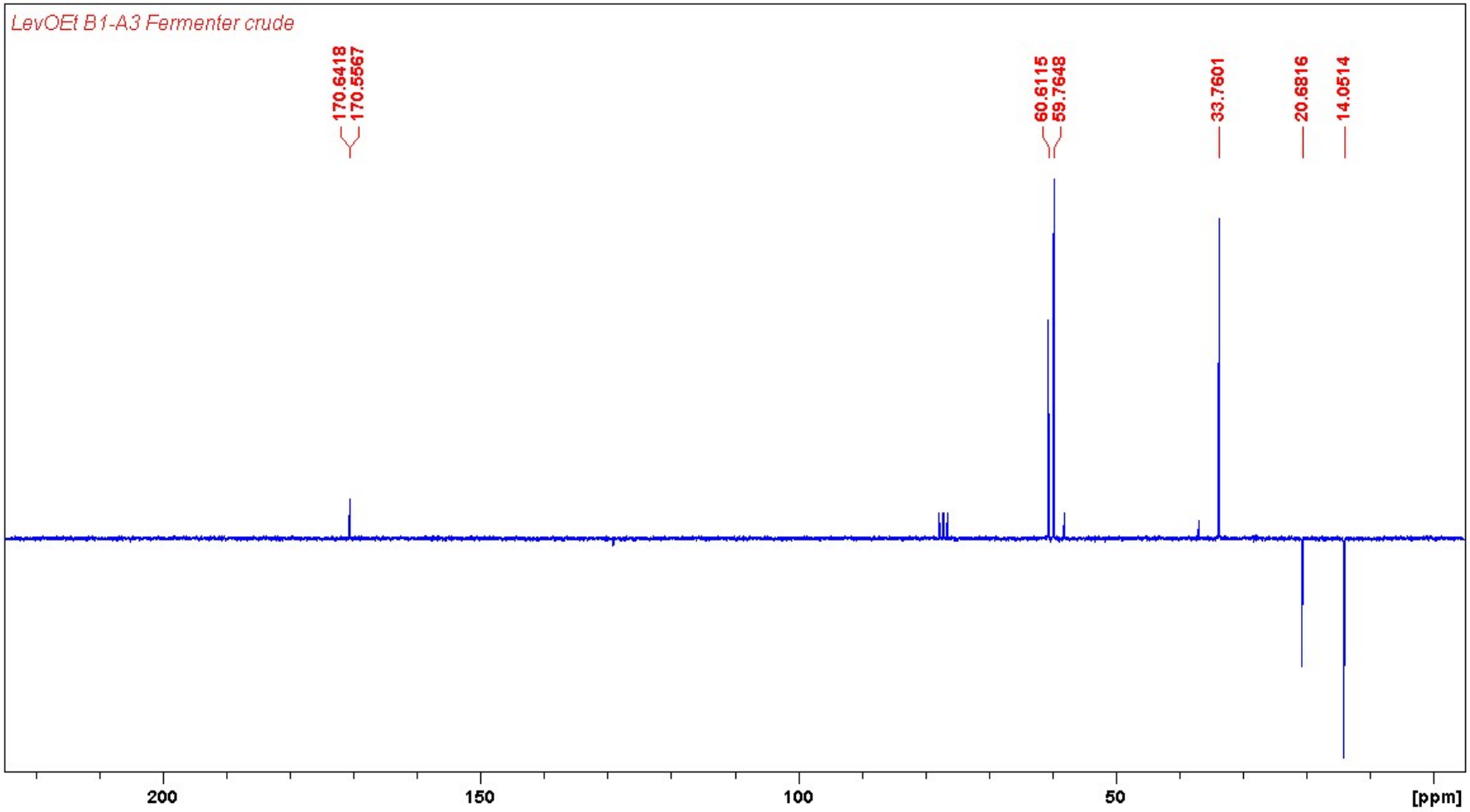
^{1}H-NMR of ethyl 3-acetoxypropionate 2a after extractive workup





^{13}C-NMR-APT of ethyl 3-acetoxypropionate 2a after extractive workup





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2. Fein and Fischer, *J. Org. Chem.*, 1948, **13**, 750,751; Gresham, Jansen and Shaver, *J. Am. Chem. Soc.*, 1948, **70**, 1002; I. I. Lapkin and Z. D. Belykh, *Journal of Organic Chemistry USSR (English Translation)*, 1968, **4**, 1120 - 1121.