A study on the enzyme catalysed enantioselective hydrolysis of methyl 2-methyl-4-oxopentanoate, a precursor of chiral γ-butyrolactones

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Supporting Information

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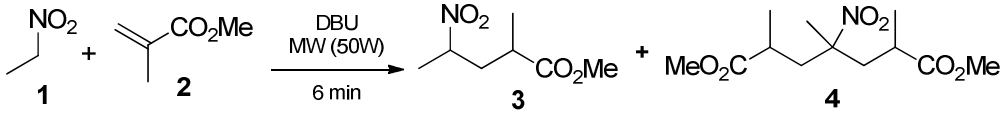
S1. ***Synthesis of diastereomeric methyl 2-methyl-4-nitropentanoate (3)***

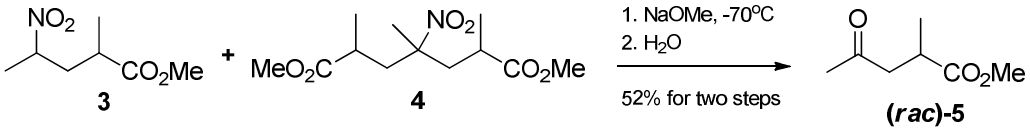


1 2 3 4

Compound (**3**) was synthesized according to a previous reported procedure with some modification as follows (Escalante and Díaz-Coutiño 2009). In a 100 mL, one neck round-bottomed flask equipped with magnetic stirring, a mixture of nitroethane (**1**) (50 mmol, 3.76 g, 3.56 mL), methyl methacrylate (**2**) (45 mmol, 4.50 g, 4.81 mL) and DBU (1.0 mmol, 0.142 g, 0.150 mL) was added. Then, a 15 cm vigreaux column was connected to the flask, and the mixture was submitted to microwave irradiation (50W, maximum temperature of 75°C) under stirring for 6 minutes. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure leading to a yellow oil. The crude product was filtered in a short pad of silica-gel and washed with ethyl acetate (50%) to remove DBU. The solvent was removed under reduced pressure to afford a diastereomeric mixture of methyl 2-methyl-4-nitropentanoate (85.2%) (**3**) and dimethyl 2,4,6-trimethyl-4-nitroheptanedioate (14.8%) (**4**) as a pale yellow oil.

Compound (**3**): MS (70 eV, EI): m/z (%) 144 (16), 129 (25), 73 (51), 69 (93), 59 (94), 55 (35), 43 (74), 41(100), CG t_R = 7.25 min and 7.92 min (diastereoisomers), CP-Sil 5 CB capillary column (30 m×0.25 mm×0.25 µm), temperature gradient: 50 °C (5 min., iso), 10 °C/min to 250 °C. Compound (**4**): MS (70 eV, EI): m/z (%) 244 (0.4), 229 (2), 216 (0.1), 169 (52), 137 (54), 109 (100), 83 (29), 67 (32), 55 (46), 43 (42), 41 (69), CG t_R = 14.39 min, CP-Sil 5 CB capillary column (30 m×0.25 mm×0.25 µm), temperature gradient: 50 °C (5 min iso), 10 °C/min to 250 °C.

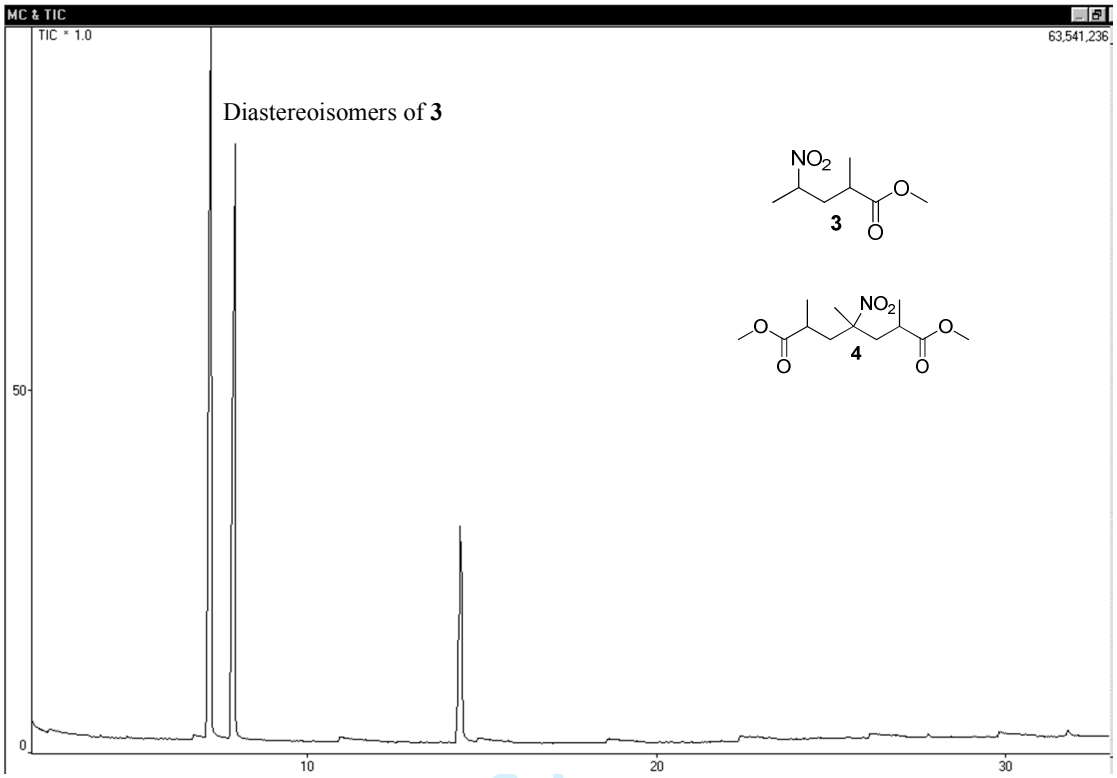
S2. ***Synthesis of racemic methyl 2-methyl-4-oxopentanoate (5)***



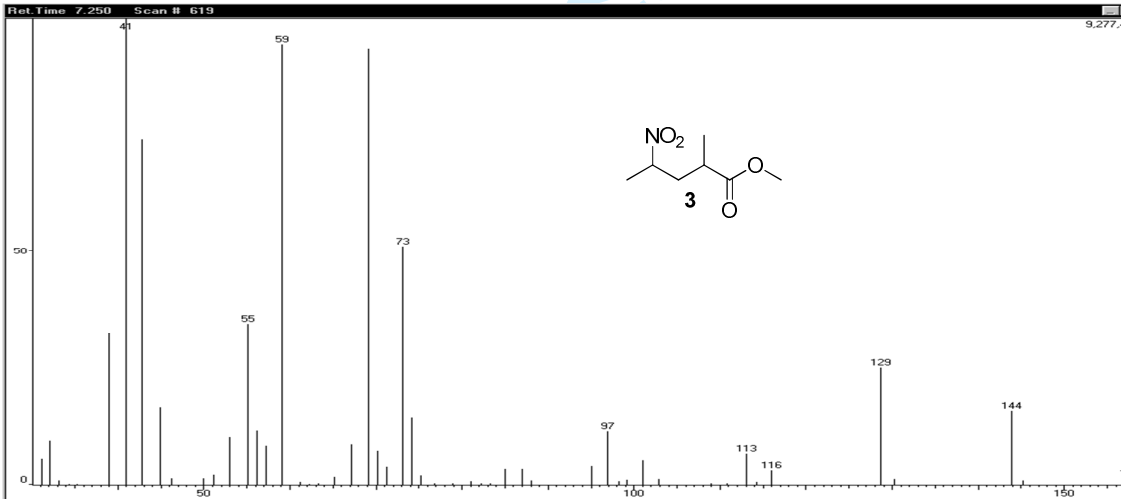
Preparation of compound (**5**) was made using the same methodology described previously with minor modifications (Ballini 1993). In a two-necked round bottomed flask, a methanolic sodium methoxide solution was prepared by addition of metallic sodium (47.21 mmol, 1.70 g) to an ice cooled dry methanol (110 mL) under N_2 atmosphere. After consumption of sodium, the crude mixture of nitro compounds **3** and **4** (7.39 g) was added under stirring, and the mixture was allowed to react for 15 minutes at room temperature. Next, this solution was transferred dropwise under N_2 atmosphere to a solution of H_2SO_4 (421.10 mmol, 22.5 mL) in methanol (110 mL) at 0°C for 30 minutes. This was then stirred for more than 10 minutes to yield a colorless solution. The work-up was done by addition of 70 mL of water, and the colorless solution became light blue. Methanol was removed under reduced pressure, and the aqueous phase was washed with CH_2Cl_2 (3x40 mL). The organic phases were combined and washed with 10% aqueous NaOH (40 mL), brine (40 mL) and dried with $MgSO_4$. The mixture was filtered and concentrated under reduced pressure to afford racemic methyl 2-methyl-4-oxopentanoate (**5**) as a pale yellow oil. Purification was done by Kugelrohr distillation at 160°C using a pressure of 12 mbar affording a colorless oil (3.42 g, 52% yield after two steps).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H), 2.98-2.88 (m, 2H), 2.54-2.44 (m, 1H), 2.16 (s, 3H), 1.18 (d, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.6 (C=O), 176.3(C=O), 51.9 (CH_3), 46.7 (CH_2), 34.7 (CH), 30.1 (CH_3), 17.1 (CH_3); IR (neat) 1730, 1713, 1157 cm^{-1}; MS (70 eV, EI): m/z (%) 144 (0.8), 129 (4), 113 (11), 112 (15), 87 (23), 69 (5), 59 (33), 43 (100), 39 (11), *Rf* 0.48 (hexanes/EtOAc, 1:1), CG t_R = 4.90 min, CP-Sil 5 CB capillary column (30 m×0.25 mm×0.25 μm), temperature gradient: 50 °C (5 min iso), 10 °C/min to 250 °C.

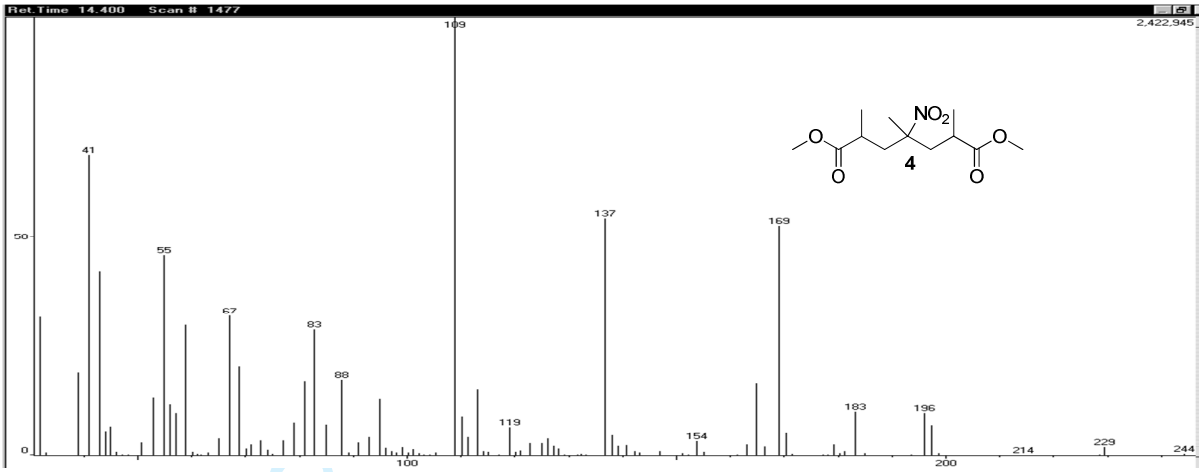
S3. Gas chromatogram of diastereoisomers of nitroester **3** and **4**.



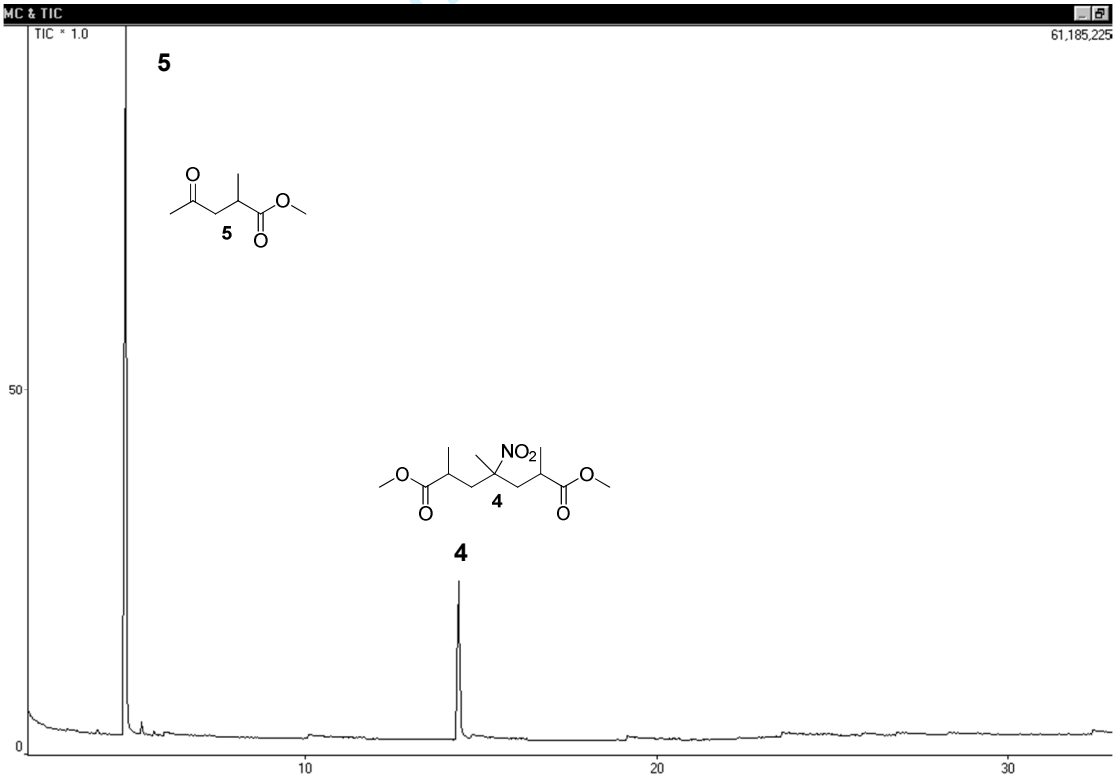
S4. Mass spectrum of nitroester **3**.



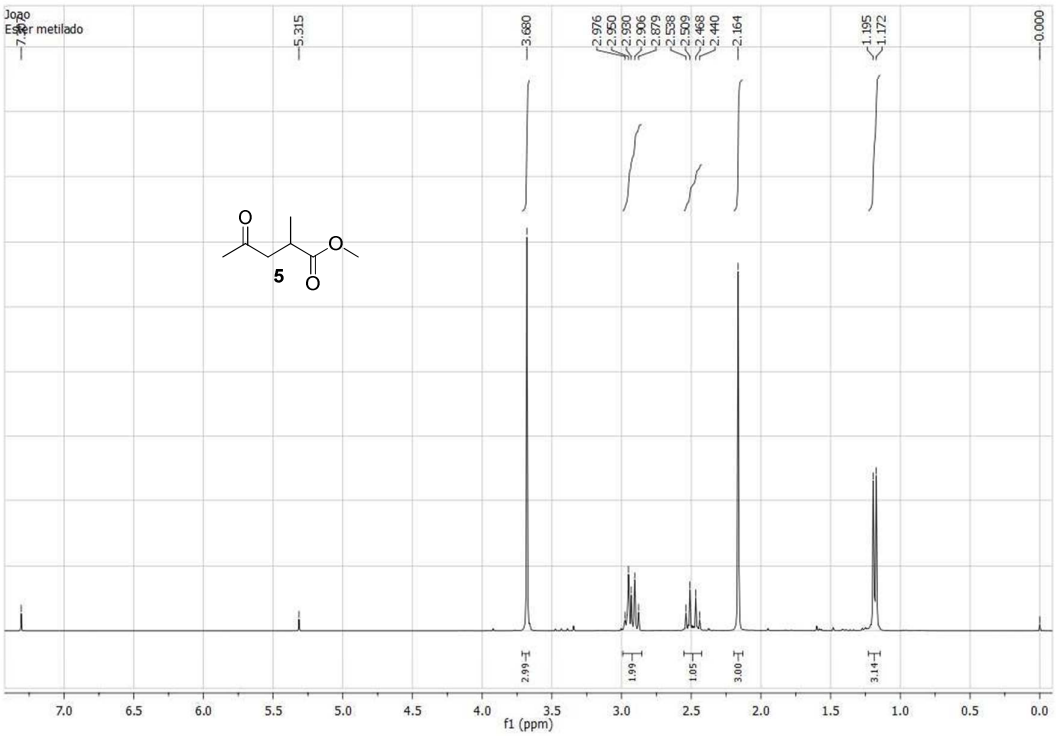
S5. Mass spectrum of nitroester **4**.



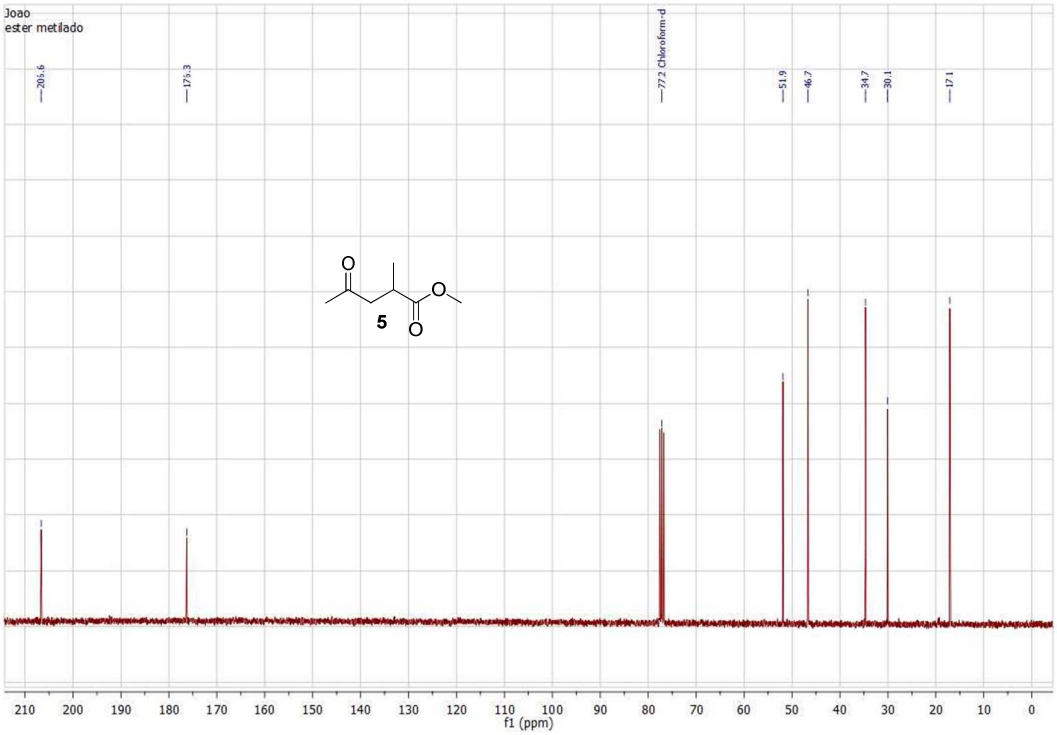
S6. Gas chromatogram of keto-ester **5**.



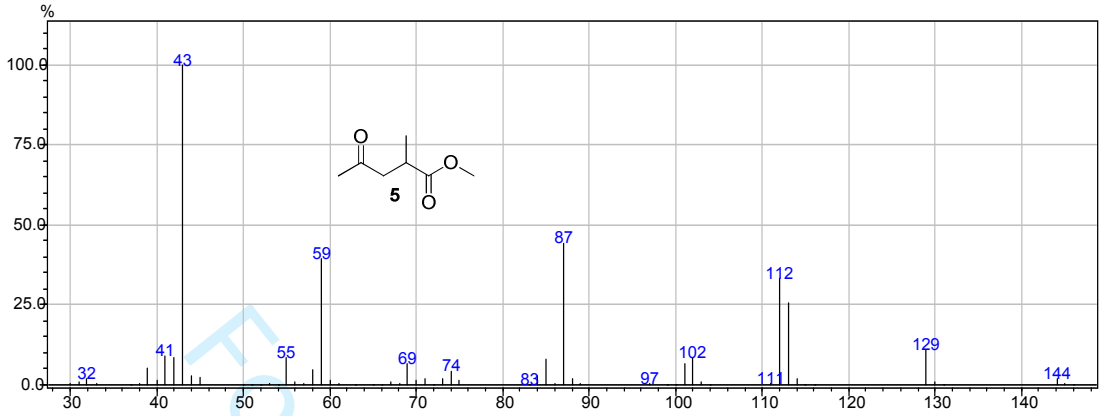
S7. ^{1}H NMR spectrum of keto-ester **5**.



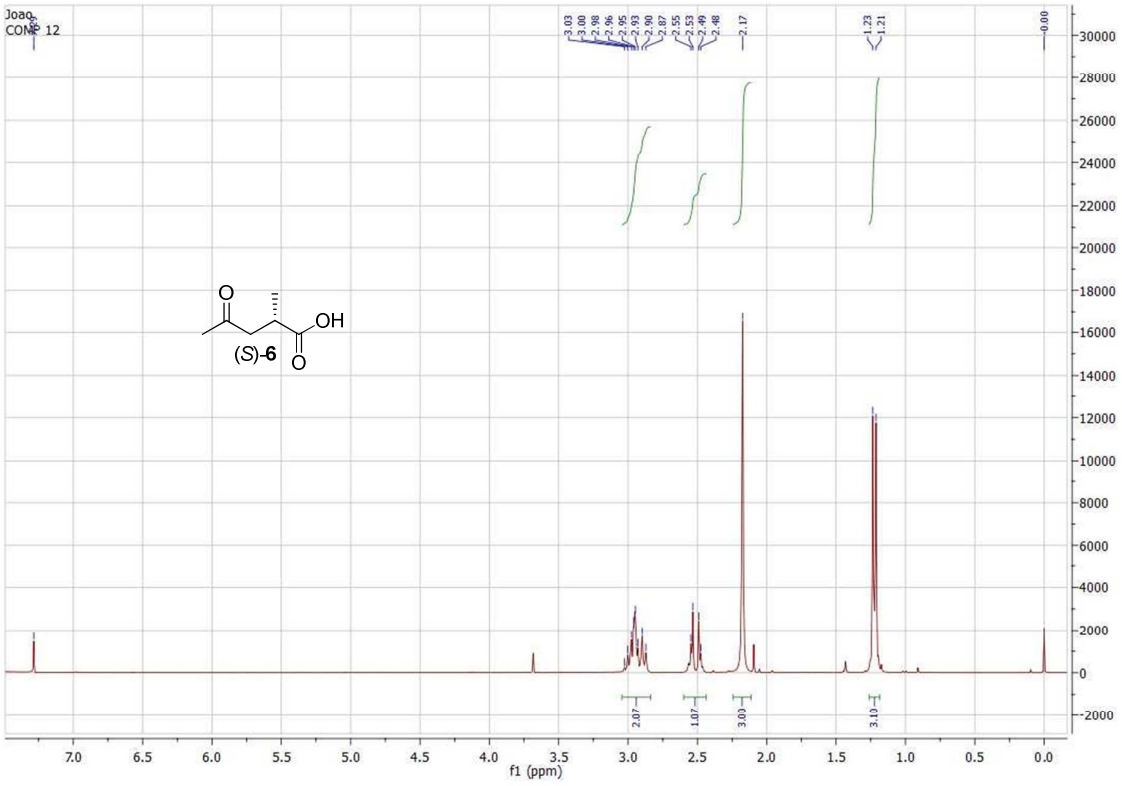
S8. ^{13}C NMR spectrum of keto-ester **5**.



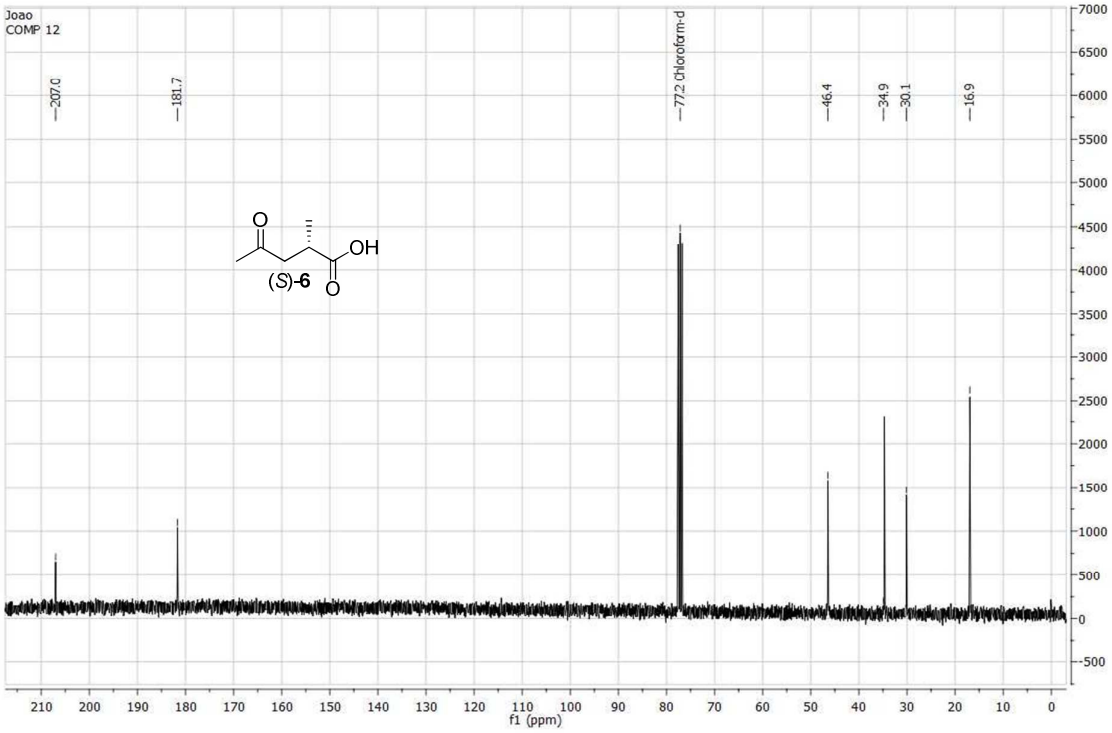
S9. Mass spectrum of keto-ester **5**.



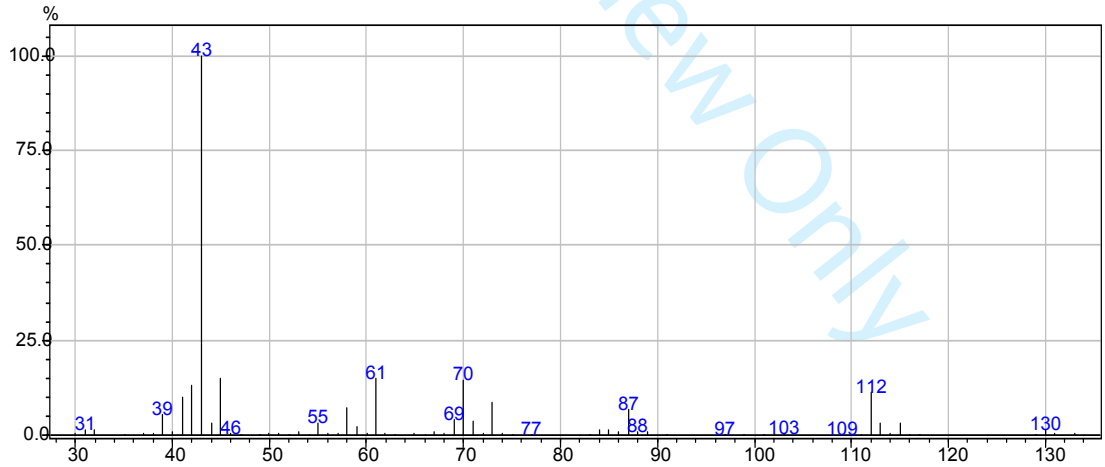
S10. ^{1}H NMR spectrum of keto acid (*S*)-**6**.



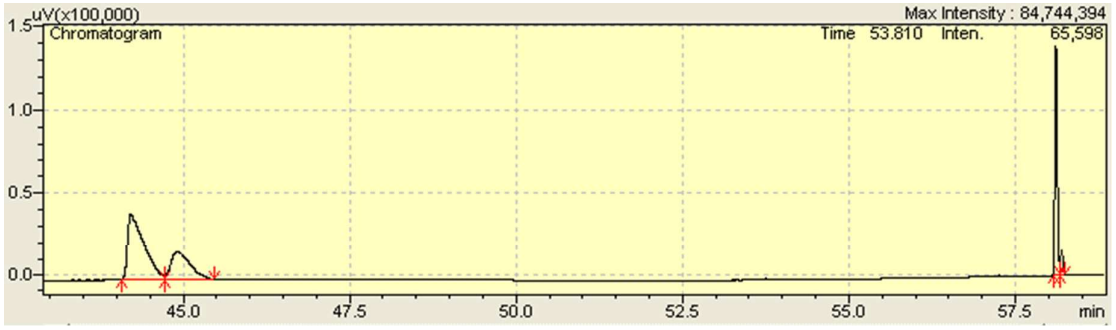
S11. ^{13}C NMR spectrum of keto acid (*S*)-**6**.



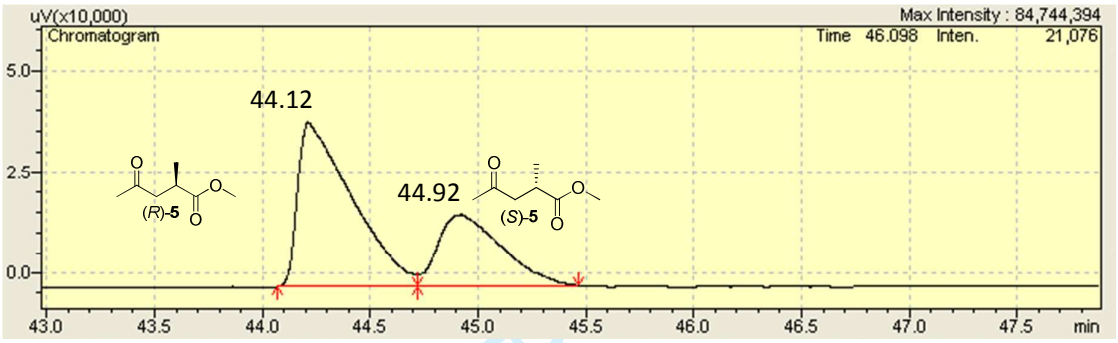
S12. Mass spectrum of keto acid (*S*)-**6.**



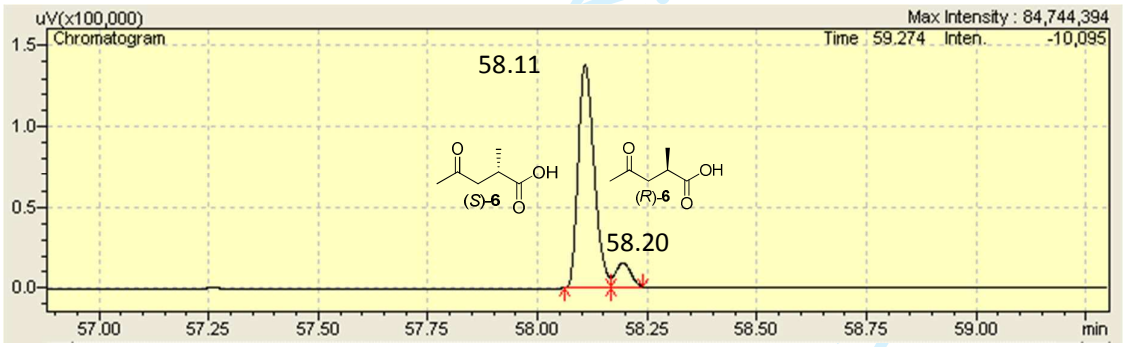
S13. Gas chromatogram of keto-ester **5** and keto-acid **6** after 2 hours of reaction with PPL.



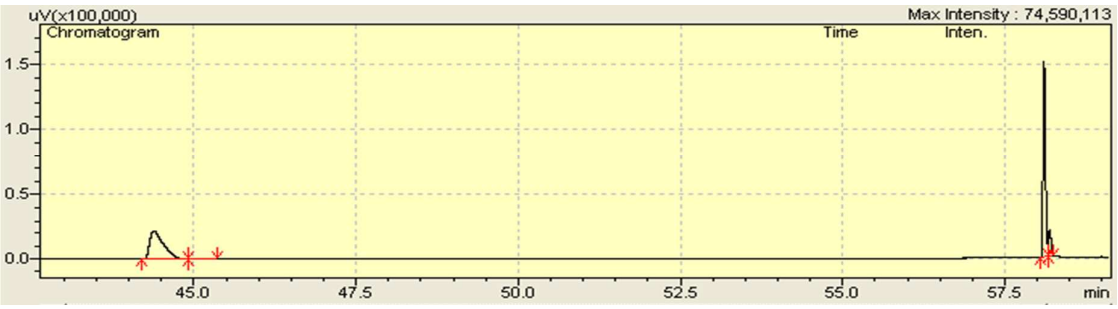
S14. Expansion of gas chromatogram at region of keto-ester **5** after 2 hours of reaction with PPL.



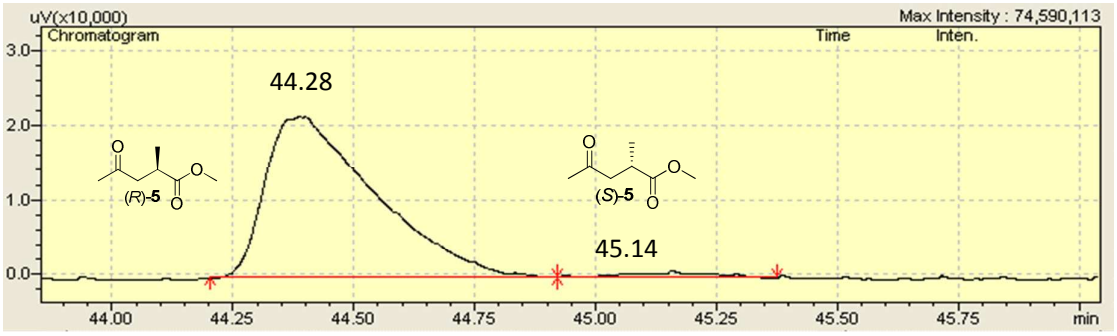
S15. Expansion of gas chromatogram at region of keto-acid **6** after 2 hours of reaction with PPL.



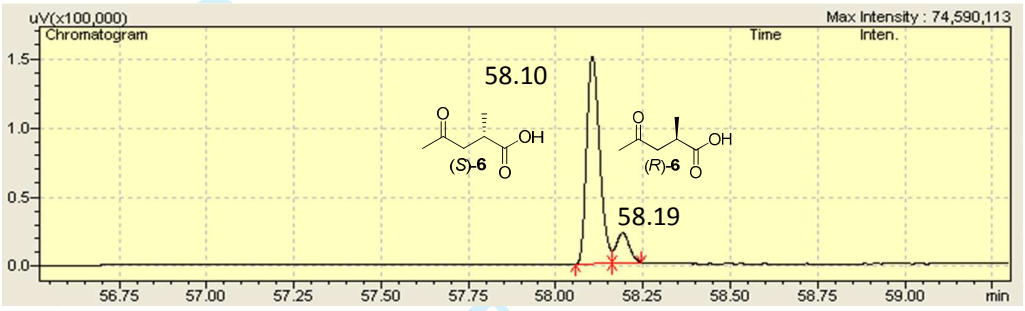
S16. Gas chromatogram of keto-ester **5** and keto-acid **6** after 2 hours of reaction with PPL.



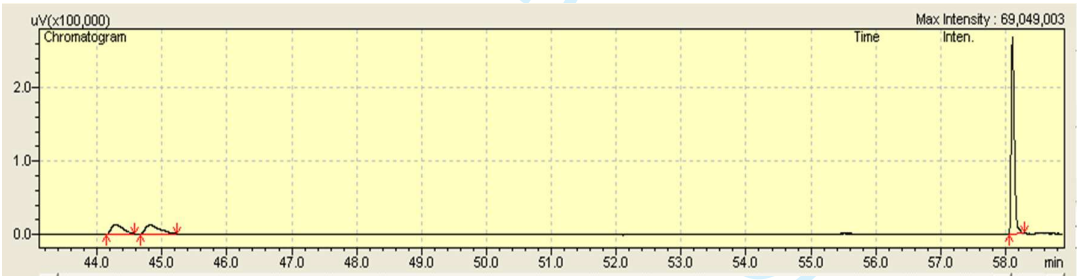
S17. Expansion of gas chromatogram at region of keto-ester **5** after 2 hours of reaction with PPL.



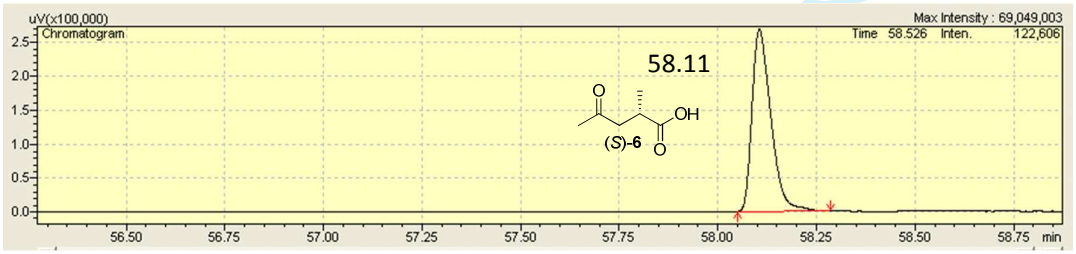
S18. Expansion of gas chromatogram at region of keto-acid **6** after 2 hours of reaction with PPL.



S19. Gas chromatogram of keto-ester **5** and keto-acid **6** after 6 hours of reaction with PPL after recycle.



S20. Expansion of gas chromatogram region of keto-acid **6** after 6 hours of reaction with PPL after recycle.



S21. Screening of enzymes to enzymatic hydrolysis of (*rac*)-**5**.

Entry	Enzyme	ee_e(%)	ee_a(%)	Conv. (%)	E	Selectivity
1	CAL-B	1.2	28.2	2.2	1.8	(*R*)-**6**
2	*Pseudomonacepacia*lipase	4.4	2.3	5.4	1.1	(*S*)-**6**
3	*Porcine pancreas* lipase	9.0	59.5	10.0	4.3	(*S*)-**6**
4	Amano PS	0.7	1.3	1.7	1.0	(*S*)-**6**
5	*Candida rugosa* lipase	1.5	9.4	2.5	1.2	(*S*)-**6**
6	*Rhizopus niveus* lipase	1.5	8.0	2.5	1.2	(*S*)-**6**
7	Esterase from *Horse* liver	45.2	30.9	46.2	2.8	(*S*)-**6**
8	Esterase from *Candida lipolytica*	5.0	44.2	6.0	2.7	(*S*)-**6**
9	Esterase from *Rhyzopus oryzae*	0.2	3.7	1.2	1.1	(*S*)-**6**
10	Esterase from *Mucor miehei*	2.9	22.5	3.9	1.6	(*S*)-**6**
11	Esterase from *Sacharomyces cerevisiae*	0.2	2.7	1.1	1.1	(*S*)-**6**

[b]Conditions: 0.1 mol L^{-1} phosphate buffer (5.0 mL / pH 7.2), temperature = 25 ºC, substrate= 25.0 mg, enzyme= 12.5 mg, reaction time= 2 hours. ee_a = enantiomeric excess of carboxylic acid. ee_e = enantiomeric excess of keto ester. E = enantiomeric ratio

S22. Effect of variation of value of pH in the reaction media[d]

pH	2 hours				4 hours			
	ee_e (%)	ee_a (%)	Conv. (%)	E	ee_e (%)	ee_a (%)	Conv. (%)	E
6.2	3.8	44.8	4.8	2.7	7.2	54.1	8.2	3.6
7.2	9.0	59.5	10.0	4.3	13.8	62.3	14.8	4.9
8.0	8.1	56.0	9.1	3.8	13.2	60.4	14.2	4.6

[d]Conditions: Phosphate buffer 0.1 M (5.0 mL, temperature=25ºC, substrate= 50mg, enzyme= 25 mg.
ee_a = enantiomeric excess of carboxylic acid.
ee_e = enantiomeric excess of keto ester.
E = enantiomeric ratio.

S23. Effect of addition of co-solvents (10%) (V/V) in the reaction media[c]

Co-solvent	2 hours				4 hours			
	ee_e (%)	ee_a (%)	Conv. (%)	E	ee_e (%)	ee_a (%)	Conv. (%)	E
THF	0.5	4.7	1.6	1.1	2.0	12.1	3.0	1.3
MeOH	1.5	31.4	2.5	1.9	3.9	42.2	4.9	2.6
Acetonitrile	0.4	6.4	1.4	1.1	1.7	17.0	2.7	1.4
AcOEt	1.2	11.2	2.2	1.3	3.0	16.8	4.0	1.4
Acetone	0.8	23.5	1.7	1.6	*	*	*	*
Diethyl ether	3.3	39.4	4.3	2.4	*	*	*	*

[c]Conditions: Phosphate buffer 0.1 M (4.5 mL / pH=7.2), co-solvent= 0.5 mL, temperature=25ºC, substrate= 50mg, enzyme= 25 mg.
ee_a = enantiomeric excess of carboxylic acid.
ee_e = enantiomeric excess of keto ester.
E = enantiomeric ratio.
* Data not available.

REFERENCES

Ballini R. 1993. 5-nitro-1-pentene as a precursor for the synthesis of allylrethrone. Synthesis. 7: 687-688.

Escalante J, Díaz-Coutiño FD.2009. Synthesis of γ-Nitro Aliphatic Methyl Esters Via Michael Additions Promoted by Microwave Irradiation. Molecules. 14:1595-1604.