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Combination strategy using pure enzymes and whole cells as biocatalysts for the preparation of 2-hydroxyesters and lactones from 2-oxoglutaric acid

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Abstract—An innovative combination strategy that uses pure enzymes and whole microbial cells in the same process was used to prepare enantiomerically pure 3-carboxyalkyl-γ-butyrolactones and several alkyl esters of 2-hydroxyglutarates from 2-oxoglutaric acid. The method involves two consecutive biocatalytic steps. The first step, which converts the 2-oxoglutaric acid into the corresponding dialkyl esters, was catalyzed by a lipase. Then in the second step, by microbial reduction of the dialkyl-2-oxoglutarates, it is possible to obtain 3-carboxyalkyl-γ-butyrolactones or 2-hydroxyesters depending on the length of the chain in the alkyl moiety of the esters and on the fresh or lyophilized status of the cells.

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1. Introduction

Chiral hydroxy esters are important versatile building blocks in asymmetric synthesis. For instance, γ- and δ-hydroxy acid derivatives can be easily transformed into the corresponding lactones, which are present in a variety of natural products.[1] In addition, lactones are important building blocks for the synthesis of natural products, such as alkaloids and terpenoids[2] and continue to attract considerable attention due to their interesting pharmacological activities.[3a]

There are several examples in the literature of γ-butyrolactones, which contain a carboxylic group as a substituent of the lactonic ring. (−)-Nephrosteranic acid and (−)-roccellaric acid, known as paraconic acids, belong to this class of butyrolactones with antibacterial properties (Fig. 1).[3b]

On the other hand, some derivatives of enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic acid are natural products present in a variety of fruits and in some species of insects as components of sex-attractant pheromones.[4] For example the ethyl ester of the (*S*)-isomer of tetrahydro-5-oxo-2-furancarboxylic acid is the raw material for the synthesis of the pheromone 4-(*Z*)-6-dodecenyl-γ-butyrolactone (Fig. 2).

The synthesis of derivatives of tetrahydro-5-oxo-2-furancarboxylic acid has been reported through a series of non-‘Green Chemistry’ steps. Biocatalysis, either employing isolated enzymes or whole microbial cells, offers a greener way to many chiral building blocks.[5–7] This has been demonstrated in recent years in the production of bulk chemical such as acrylamide and in the fine chemicals and drug industry.[8]

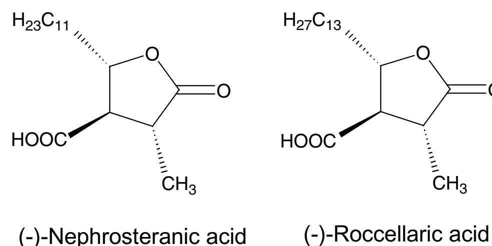

Figure 1.

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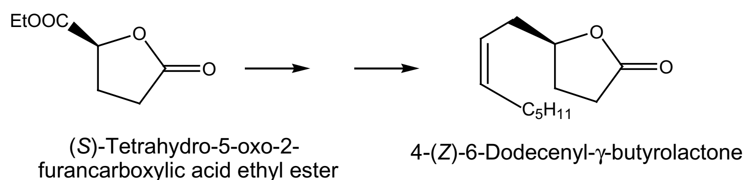


Figure 2.

There are some examples in the literature about the preparation of substituted γ-butyrolactones applying biocatalytic methods through enzyme-catalyzed lactonization of hydroxy diesters[9] or by microbial reduction of 1,4-ketoacids.[10] The synthesis of tetrahydro-5-oxo-2-furancarboxylic acid alkyl esters following a chemoenzymatic way, in which biocatalytic procedures were used in combination with reagents of the traditional synthetic approach, has also been reported.[11]

Our previous work on lipase-catalyzed esterification of carboxylic acids[12] and stereoselective reduction of β-ketoesters[13] prompted us to explore the application of the combination of both methodologies in a consecutive way, using 2-oxo-glutaric acid as the substrate. The aim of the work was to find a green alternative to traditional chemical reactions, which involve reagents and reaction conditions not friendly to the environment.

Herein we report the results obtained by lipase-catalyzed esterification of 2-oxoglutaric acid with several alcohols and the following reduction of the obtained α-ketoesters by whole cells of the fungus *Mucor rouxii* as source of reductase activity (Scheme 1).

2. Results and discussion

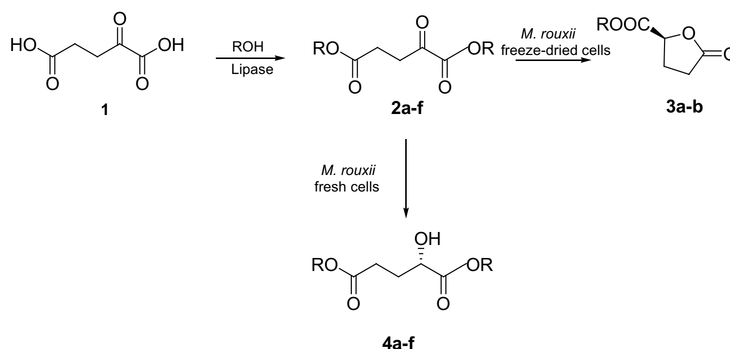
We have prepared, under mild reaction conditions, a variety of alkyl derivatives of 2-hydroxyglutaric acid in a chemo- and stereoselective manner, and in moderate to high yield. The preparation was performed in two consecutive enzymatic steps: (1) lipase-catalyzed esterification of 2-oxoglutaric acid and (2) chemo- and stereoselective microbial reduction of 2-oxoglutarate alkyl esters.

2.1. First enzymatic step: lipase-catalyzed esterification of 2-oxoglutaric acid

Lipases in nature catalyze the hydrolysis of triacylglycerides, and interestingly also catalyze related reactions such as esterification or transesterification in non-natural reaction conditions like anhydrous organic media. In previous work, we have applied this methodology in the esterification of carboxylic acids of variable chain length including fatty acids with ethanol[12,14] and some natural alcoholism such as steroids and vitamin B_6.[15,16]

Herein, compounds **2a**–**f** were obtained through esterification of the 2-oxoglutaric acid **1** catalyzed by lipases from several sources: from yeast: *Candida rugosa* lipase (CRL), *Candida antarctica* lipase (CAL); Lipozyme from the fungus *Mucor miehei* (LIP), and lipase PS (PSL) and PS-C (PSL-C) from *Pseudomonas* sp. The five commercial lipases were tested in the esterification of **1** with ethanol to obtain **2b**.The lipase from *Candida antarctica* (CAL) gave the most satisfactory results at both temperatures. The other four lipases were also active but gave a lower performance. Without enzymes, 2-oxoglutaric acid did not react at all. To optimize the reaction conditions, we performed several experiments by changing reaction parameters such as temperature (30 °C and 55 °C) and enzyme–substrate ratio (E/S). We observed that an increase in temperature did not improve the results, not even in the case of PSL and PSL-C which showed low reactivity towards the esterification reaction.

Regarding the influence of the enzyme:substrate ratio, it can be observed that an enzyme:substrate ratio of 5:1 gave the best results. Taking into consideration the above mentioned work,[12] we have chosen the following



Scheme 1. Lipase-catalyzed esterification of 2-oxoglutaric acid and subsequent *M. rouxii* reduction. **a**: R = CH_3–; **b**: R = CH_3CH_2–; **c**: R = $CH_3(CH_2)_2$–; **d**: R = $(CH_3)_2CH$–; **e**: R = $CH_3(CH_2)_3$–; **f**: R = $(CH_3)_2CHCH_2$–.

standard conditions for the biocatalytic esterification: CAL, excess of alcohol, temperature of 30 °C and an E/S ratio of 5/1.

The treatment of 2-oxoglutaric acid with several primary alcohols in the presence of CAL afforded the corresponding 2-oxoglutarate dialkyl esters in almost quantitative yields (94–100%).

The preparation of compounds **2a–e** has been previously reported according to the known chemical procedures by refluxing the acid with the corresponding alcohol in toluene with p-toluenesulfonic acid as a catalyst.[11] Due to α-ketoacids, such as 2-oxoglutaric acid, undergoing decarboxylation fairly readily, yields were not very high. The enzymatic approach we have applied for the first time on this substrate gave the products in almost quantitative yield. The procedure is simpler, can be carried out at room temperature and only uses alcohol as a solvent and esterifying agent.

2.2. Second enzymatic step: chemo- and stereoselective microbial reduction of 2-oxoglutarate dialkyl esters

In this step we studied the enzymatic reduction of the dialkyl 2-oxoglutarates. Considering the good performance shown by the fungus *M. rouxii* in the reduction of β-ketoesters,[13] we decided to use it as a microbial source. *M. rouxii* is a dimorphic fungus that can grow as a cenocytic mycelium under aerobic conditions or as yeast cells under anaerobiosis and high glucose levels in the culture medium. Herein we used fresh and freeze-dried cells from *M. rouxii* cultures, derived from anaerobic (yeast) growth conditions, as a source of reductase activity. The results using fresh cultures of the yeast cells of the fungus are shown in Tables 1 and 2.

Table 1. *M. rouxii* yeast-like cells-reduction of diethyl 2-oxoglutarate **2b**,[a] solvent effect

Solvent	Biomass/substrate (g/mmol)	Time (h)	Conversion (%)	Ee (%)
Water	8	24	100	48 (*S*)
Hexane	17	24	100	98 (*S*)
Hexane–water	17	24	100	79 (*S*)
Toluene	17	24	63	75 (*S*)
Toluene–water	17	24	76	63 (*S*)
Dioxane	17	36	0	—
Dioxane–water	17	36	0	—
Ethyl acetate	17	36	0.3	—
Ethyl acetate-water	17	36	0.9	—

[a]Reaction conditions are described in the Experimental. The biomass was obtained from 18–20 h anaerobic cultures. Solvent: hexane.

It was observed that the carbonyl group of the ketoesters was chemoselectively reduced to give the corresponding α-hydroxyesters **4a–f**, while the ester carbonyl groups remained unchanged.

Yeast cells were active both in organic media and in biphasic systems, as can be observed in Table 1. In every case, only the α-hydroxyester was obtained. No decomposition product was detected. The best results were obtained in low polar organic solvents such as pure hexane, with high enantioselectivity of the (*S*)-enantiomer. In the presence of more polar solvents, such as dioxane or ethyl acetate, only the starting material was recovered. We observed that conversion decreased by increasing the polarity of the solvent. The use of water-organic solvent media was only effective in mixtures of hexane–water and toluene–water, but although the degree of conversion obtained was similar or even better to that of the organic solvent alone, the stereoselectivity decreased (Table 1). The (*S*)-α-hydroxyester was the isolated enantiomer in all cases.

Table 2. *M. rouxii* yeast-like cells catalyzed reduction of alkyl 2-oxoglutarates

Substrate	Product	Time (h)	Conversion (%)	Ee (%)
2a	**4a**	24	100	95 (*S*)
2b	**4b**	24	100	98 (*S*)
2c	**4c**	24	100	94 (*S*)
2d	**4d**	24	98	99 (*S*)
2e	**4e**	40	40	93 (*S*)
2f	**4f**	40	26	95 (*S*)
2g[a]	**4g**	24	96	91 (*S*)

Reaction conditions are described in the Experimental. The biomass was obtained from 18–20 h anaerobic cultures. Solvent: hexane.
[a] **2g**: Ethyl 2-oxo-4-phenylbutanoate; **4g**: Ethyl-2-hydroxy-4-phenylbutanoate.

Under our experimental conditions *M. rouxii* reductases showed a better stereoselectivity than the reported on reductions using baker's yeast.[17] This result can be explained by considering not only the nature of *M. rouxii* yeast cells but also the influence of the organic medium. It has been reported that side products were formed when some α-ketoesters were reduced by baker's yeast in water.[18] Nakamura et al. have demonstrated that these side products are primary alcohols such as 4-hydroxybutanoates, in the biotransformation of several alkyl 2-oxo-4-phenylbutanoates.[19] To compare this result, we tested the reduction of ethyl 2-oxo-4-phenylbutanoate **2g** with yeast cells of *M. rouxii* in hexane and obtained the corresponding (*S*)-α-hydroxyester **4g** as the only product with 96% conversion and 91% ee.

Considering that hexane was the best solvent in the case of substrate **2b**, we studied the course of its conversion and enantiomeric excess by *M. rouxii* yeast cells under these conditions. An enantiomeric excess of around 90–98% was obtained throughout, independently of the degree of conversion. Table 2 shows the results of yeast-catalyzed reduction of the rest of the substrates **2a–f**, using a suspension of cells in hexane.

The data show that the alkoxy chain in the ketoglutarate esters affected the degree of conversion of substrates but not the enantiomeric purity of the product. The cellular reductase activity was sensitive to the presence of a bulky group as substituent in R such as butyl **2e** and isobutyl **2f** with only 40% and 26% of conversion being observed in these cases. Di-isobutyl-2-hydroxyglutarate **4f** had not been previously reported in literature. This novel compound was completely identified

by spectroscopic methods: FTIR, ^{1}H and ^{13}C NMR and HR-MS.

The absolute configuration was determined by converting **4f** to 2-hydroxyglutaric acid[20] through a CAL-catalyzed hydrolysis. To be sure that the enzymatic hydrolysis of the ester did not affect the absolute configuration at carbon two, the method was previously performed using di-isopropyl-(2*S*)-hydroxyglutarate **4d** of known configuration: $[\alpha]_D^{25} = -1.2$.[11] The CAL catalyzed hydrolysis of **4d** produced (2*S*)-hydroxyglutaric acid; $[\alpha]_D^{25} = -2.0$. Treating **4f**, $[\alpha]_D^{25} = -5.2$, in the same conditions gave again (2*S*)-hydroxyglutaric acid, $[\alpha]_D^{25} = -1.85$. It can be concluded that the absolute configuration of C-2 in **4f** is (*S*).

Next we studied the behavior of the microbial system with freeze-dried yeast cells. The cells were rehydrated with water before the addition of hexane as solvent. Table 3 shows the results.

Table 3. *M. rouxii* freeze-dried yeast-like cells catalyzed reduction of alkyl 2-oxoglutarates

Substrate	Product	Time (h)	Conversion (%)	Ee (%)
2a	**3a**	24	88	97 (*S*)
2b	**3b**	24	100	98 (*S*)
2c	**4c**	24	100	97 (*S*)
2d	**4d**	24	100	98 (*S*)
2e	**4e**	40	35	94 (*S*)
2f	**4f**	40	14	92 (*S*)

Reaction conditions are described in the Experimental. Biomass/substrate: 20 g/mmol. The dehydrated biomass was obtained from 18–20 h anaerobic cultures and added to the reaction system according to the fresh:dehydrated ratio as explained in Experimental.

Depending on the size of the alkyl chain in the 2-oxoglutarate alkyl ester, we have obtained different products. With the largest groups of the three carbons (*n*-propyl **2c** and isopropyl **2d**) and four carbons (*n*-butyl **2e** and isobutyl **2f**) the freeze-dried yeast cells produced the corresponding 2-hydroxyesters **4c** to **4f**. With these substrates, freezed-dried cells and fresh cultures work in the same way. However in the case of small alkyl groups in alkyl 2-oxoglutarates, such as methyl **2a** and ethyl **2b**, we obtained the corresponding γ-butyrolactones **3a** and **3b**. Although the reactivity was better, as in the case of the ethyl derivative **3b**, we found that the steric course of the reaction was the same as in the reduction with fresh cells, giving the (*S*)-stereoisomers for **3a** and **3b**. Both γ-butyrolactones were obtained in one step by treating the methyl and ethyl 2-oxoglutarate with the freeze-dried yeast cells from *M. rouxii*.

3. Conclusions

A new efficient enzymatic pathway has been developed for the synthesis of enantiopure (*S*)-3-carboxymethyl and (*S*)-3-carboxyethyl-γ-butyrolactones and several enantiopure dialkyl (*S*)-2-hydroxyglutarates from 2-oxoglutaric acid. We have described for the first time a fully enzymatic strategy from the 2-oxocarboxylic acid to the reduction products, involving the use of a lipase-catalyzed esterification of 2-oxoglutaric acid and the following reduction of the esters with yeast cells of the fungus *M. rouxii*. Fresh cultures of yeast cells produced (*S*)-2-hydroxyesters and freeze-dried cells produced (*S*)-3-carboxymethyl and (*S*)-3-carboxyethyl-γ-butyrolactones or (*S*)-2-hydroxyesters depending on the length of the alkyl chain in the ester. This synthetic way constitutes a green alternative to traditional chemical reactions.

4. Experimental

4.1. General

All solvents and reagents were reagent grade and used without purification. Lipase from *C. rugosa* CRL (905 units/mg solid), were purchased from Sigma Chemical Co.; *Candida antarctica* lipase B: Novozym 435 CAL (7400 PLU/g) and Lipozyme RM 1M (7800 U/g) were generous gifts of Novozymes Latinoamerica Ltda and Novozymes A/S; *Pseudomonas* lipase: Lipase PS-C Amano II PSL-C(804 U/g) and Lipase PS Amano PSL (33,200 U/g) were purchased to Amano Pharmaceutical Co. All enzymes were used 'straight from the bottle'.

Enzymatic reactions were carried out on an Innova 4000 digital incubator shaker, New Brunswick Scientific Co. at 30 °C and 200 rpm. IR spectra were measured on a Nicolet-Magna-550-FT/IR spectrophotometer; in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 MHz using a Bruker AC-200 spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) as the internal standard, using $CDCl_3$ and D_2O as solvents. EI-MS were obtained at 70 eV using a TRIO-2 VG Masslab and Shimadzu QP-5000 mass spectrometers, in *m/z* (%). High resolution mass spectra were recorded on a ZAB BEqQ instrument. Optical purity of products was determined by the specific rotation using H_2O or CH_3OH as solvents with a Perkin Elmer 343 polarimeter. GLC analysis were obtained on a Finnigan Focus GC, Thermo Electron Co. instrument, the capillary column being Carbowax 20H-022, 30 m × 0.2 mm, film thickness 0.2 μm, (1 min at 80 °C, 2 °C/min, 200 °C). For enantiomeric excess determination HP Chiraldex G-TA capillary column, 40 m × 0.32 mm, (15 min at 80 °C, 2 °C/min, 100 °C) was used and compared with the corresponding racemic products. The absolute configuration of products **3a** and **b** and **4a–e** was determined by the comparison with the sign of the specific rotation reported in the literature (circular dichroism).[11] To determine its absolute configuration, di-*i*-butyl-2-hydroxylglutarate **4f**, was enzymatically hydrolyzed to 2-hydroxyglutaric acid and the specific rotation of the product compared with literature.

4.2. Lipase-catalyzed preparation of 2-oxoglutarate dialkyl esters

4.2.1. General procedure for lipase-catalyzed esterification of 2-oxoglutaric acid 2a–f. Lipase Novozym 435 (500 mg) was added to a solution of 2-oxoglutaric acid (3 mmol) in the corresponding alcohol (10 ml). The sus-

pension was shaken (200 rpm) at 30 °C and the progress of the reaction monitored by GLC. When the acid was converted into the alkyl ester, the enzyme was filtered off and the solvent evaporated. Products **2a–e** were identified by GC–MS and by ^{1}H and ^{13}C NMR spectroscopy. Tests with other lipases were performed under the same experimental conditions. IR, ^{1}H NMR, ^{13}C NMR and MS data of compounds **2a–e** were in accordance with those reported in the literature.[11,21,22] Yields of compounds **2a–e** are reported in Table 2.

4.2.2. Di-*i*-butyl-2-oxoglutarate 2f. 100% Yield. IR (film) cm^{-1}: 1780 (C=O), COO–(1) and (5): 1730. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.93 (d, 6H, (CH_3)$_2$-$CHCH_2OOCCH_2$–)), 0.98 (d, 6H, (CH_3)$_2CHCH_2OO$-COC–)), 1.95 (m, 1H, $(CH_3)_2CHCH_2OOCCH_2$–)), 2.05 (m, 1H, $(CH_3)_2CHCH_2OOCOC$–)), 2.70 (t, 2H, $(CH_3)_2$-$CHCH_2OOCCH_2$–)), 3.18 (t, 2H, $(CH_3)_2CHCH_2OO$-$COCCH_2$–)), 3.89 (d, 2H, $(CH_3)_2CHCH_2OOCCH_2$–)), 4.12 (d, 2H, $(CH_3)_2CHCH_2OOCOC$–)). ^{13}C NMR ($CDCl_3$): 18.96 (C3′-C4′), 27.61 (C-3), 34.27 (C-4), 70.85 (C-2′), 72.43 (C-1′), 160.56 (C-1), 161.95 (C-5), 192.54 (C-2). EI-MS (*m*/*z*,%): 258 (1), 183 (13), 101 (11), 57 (100), 41 (56). HR-MS: 258.3165 ($C_{13}H_{23}O_5^+$; calc. 258.3172).

4.3. Di-alkyl-2-oxoglutarate microbial reduction

Fresh cultures and freeze-dried yeast cells of *M. rouxii* were grown as described previously.[13] Biomass (2 g) obtained from cultures was incubated with 5 ml of organic solvents such as ethyl acetate, toluene, hexane, etc, alone or in biphasic systems mixed with 2 ml sterile water, in 25 ml sterile Erlenmeyer flasks stoppered and sealed. Water incubations were performed in 5 ml sterile water alone. The substrates were added to these systems (0.1–0.25 mmol for standard assays) and incubated at 28 °C in a rotatory shaker at 200 rpm. for different times. The reactions were stopped by centrifugation at $10{,}000 \times g$; the supernatants were removed and, when applied, water phases were extracted with ethyl acetate. The products were purified by flash chromatography (eluant: hexane:ethyl acetate, 95:5 to 75:25). All experiments were performed in duplicate.

4.4. Reduction products

IR, ^{1}H NMR, ^{13}C NMR and MS data of compounds **4a–e** and **3a** and **3b** are in accordance with those reported in literature.[11,23,24] Conversion of compounds **4a–e** obtained by *M. rouxii* yeast-like cells reduction are reported in Table 2 and of **3a** and **3b** and **4c–f** obtained by *M. rouxii* freeze-dried yeast-like cells reduction in Table 3.

4.4.1. *M. rouxii* yeast cells reduction

4.4.1.1. (*S*)-Di-methyl-2-hydroxyglutarate 4a. 100% Conversion; (*S*): 95% ee; t_R = 8.9 min (t_R (*R*) = 8.5 min; t_R (*S*) = 8.9 min). $[\alpha]_D^{25} = -5.2$ (c = 0.38, MeOH) {lit.[11] $[\alpha]_D^{25} = -4.5$ (c = 0.22, MeOH)}.

4.4.1.2. (*S*)-Di-ethyl-2-hydroxyglutarate 4b. 100% Conversion; (*S*): 98% ee; t_R = 9.6 min (t_R (*R*) = 9.3 min; t_R (*S*) = 9.6 min). $[\alpha]_D^{25} = -4.1$ (c = 0.45, MeOH) {lit.[11] $[\alpha]_D^{25} = -4.9$ (c = 0.47, EtOH)}.

4.4.1.3. (*S*)-Di-*n*-propyl-2-hydroxyglutarate 4c. 100% Conversion; (*S*): 94% ee; t_R = 13.8 min (t_R (*R*) = 13.3 min; t_R (*S*) = 13.7 min). $[\alpha]_D^{25} = -6.9$ (c = 0.24, MeOH) {lit.[11] $[\alpha]_D^{25} = -5.5$ (c = 0.11, MeOH)}.

4.4.1.4. (*S*)-Di-*i*-propyl-2-hydroxyglutarate 4d. 98% Conversion; (*S*): 99% ee; t_R = 11,9 min (t_R (*R*) = 11.3 min; t_R (*S*) = 11.9 min). $[\alpha]_D^{25} = -3.5$ (c = 0.88, MeOH) {lit.[11] $[\alpha]_D^{25} = -1.1$ (c = 1.4, MeOH)}.

4.4.1.5. (*S*)-Di-*n*-butyl-2-hydroxyglutarate 4e. 40% Conversion; (*S*): 93% ee; t_R = 15.2 min (t_R (*R*) = 14.7 min; t_R (*S*) = 15.1 min). $[\alpha]_D^{25} = -4.6$ (c = 0.76, MeOH) {lit.[11] (*R*) $[\alpha]_D^{25} = +2.2$ (c = 0.76, MeOH)}.

4.4.1.6. Di-*i*-butyl-2-hydroxyglutarate 4f. 26% Conversion.; (*S*): 95% ee; t_R = 14.7 min; t_R (*R*) = 14.3 min; t_R (*S*) = 14.7 min; IR (film) cm^{-1}: 3455 (OH), 1735 (COO); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.92 (d, 6H, (CH_3)$_2CHCH_2OOCCH_2$–)), 0.94 (dd, 6H, (CH_3)$_2CHCH_2OOC(OH)HC$–)), 1.95 (m, 1H, $(CH_3)_2$-$CHCH_2OOCCH_2$–)), 1.81–1.99 (m, 1H, –CH_2CH_2CH-$OHCOO$–)), 2.05 (m, 1H, $(CH_3)_2CHCH_2OOCOC$)), 2.38–2.53 (dd, 2H, –$CH_2CH_2CHOHCOO$–)), 3.87 (d, 2H, $(CH_3)_2CHCH_2OOCCH_2$–)), 3.97 (dd, 2H, ((CH_3)$_2$-$CHCH_2OOC(OH)HC$–)), 4.23 (m, 1H(CH_3)$_2CH$-$CH_2OOC(OH)HC$–)). ^{13}C NMR ($CDCl_3$): 18.96 (–COOCH(CH_3)$_2$), 18.91 (–CHOHCOOCH(CH_3)$_2$), 27.7 (–COOCHCH_2(CH_3)$_2$), 29.81 (–CH_2CHOHCOO–), 29.55 (–OCO$\underline{C}H_2CH_2$CHOHCOO–), 69.55 (–CH_2CO-OCHCH_2(CH_3)$_2$), 70.56 (–CHOHCOOCHCH_2(CH_3)$_2$), 71.89 (–CHOH–), 173.18 (–CHOHCO–), 174.79 (–CH_2CO–). EI-MS (*m*/*z*, %): 259 (1), 245 (4), 187 (41), 103 (59), 59 (100). HR-MS: 260.3328 ($C_{13}H_{24}O_5^+$; calcd 260.3332) 91% ee; t_R = 14.5 min; $[\alpha]_D^{25} = -5.2$ (c = 0.68, MeOH).

4.4.1.7. Reduction of ethyl 2-oxo-4-phenylbutanoate 2g. 96% Yield; IR (film) cm^{-1}: 3400 (OH), 1724 (COO); ^{1}H NMR ($CDCl_3$) δ (ppm) = 7.35–7.10 (m, 5H, aromatic H), 4.20 (q, 2H, –COOCH_2CH_3), 4.17 (m, 1H, –CHOH–), 2.93 (br s, 1H, OH), 2.77 (t, 2H, PhCH_2CH_2CHOHCOO–), 2.17–1.89 (m, 2H, PhCH_2CH_2CHOHCOO–), 1.28 (t, 3H, COOCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm) = 175.79 (CO), 142.29, 128.74, 128.01, 125.47 (aromatic C), 69.47 (–CHOH–), 60.91 (–COOCH_2CH_3), 34.78 (–CH_2CH_2CHOHCOO–), 32.56 (–CH_2CH_2CHOHCOO–), 14.01 (–COOCH_2CH_3). EI-MS (*m*/*z*, %): 208 (4), 190 (6), 163 (49), 145 (69), 91 (100). $[\alpha]_D^{20} = +19.4$ (c = 1.1, $CHCl_3$) {lit.[25] (*R*): $[\alpha]_D^{24} = -21.6$ (c = 1.1, $CHCl_3$)}. Ee = 91%, t_R = 15.9 min.

4.4.2. *M. rouxii* freeze-dried yeast cells reduction. Reduction reactions were performed under the conditions previously described with fresh cells. Freeze-dried biomass (0.5 g) were resuspended in 1 ml sterile water, previous to the addition of the organic solvents.

4.4.2.1. (2*S*)-Tetrahydro-5-oxo-2-furancarboxylic acid methyl ester 3a. The crude reaction product (88% conversion) was purified on silica gel (eluant: hexane:ethyl acetate, gradient from 99:1 to 70:30). mp 57–59 °C [lit.[11] mp 61 °C, lit.[24] mp 58–60 °C]. IR, ^{1}H NMR, ^{13}C NMR and MS were identical with those reported in literature.[11,25] $[\alpha]_D^{20} = +14.9$ ($c = 1.08$, MeOH); {lit.[11] $[\alpha]_D^{25} = +15.8$ ($c = 0.65$, MeOH)}.

4.4.2.2. (2*S*)-Tetrahydro-5-oxo-2-furancarboxylic acid ethyl ester 3b. The crude reaction product (100% conversion) was purified on silica gel (eluant: hexane:ethyl acetate, gradient from 99:1 to 75:25). Oil. IR, ^{1}H NMR, ^{13}C NMR and MS were identical with those reported in literature.[11,24] $[\alpha]_D^{20} = +14.6$ ($c = 0.76$ EtOH); [lit.[11] $[\alpha]_D^{25} = +13.3$ ($c = 0.56$, EtOH), lit.[22] $[\alpha]_D^{25} = +15.1$ ($c = 0.60$, EtOH)].

4.5. Enzymatic hydrolysis of 4e and 4f

To a solution of **4e** or **4f** (1 mmol) in buffer phosphate pH = 7 (16 ml), 300 mg of CAL were added. The progress of reaction was monitored by GC. When the ester was converted into the 2-hydroxyglutaric acid, the solution was acidified to pH 1 with 2 N HCl and extracted four times with diethyl ether. The organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a solid residue, which was purified by silica gel column chromatography to obtain 2-hydroxyglutaric acid. Specific rotation of the product was determined and the value corresponds to (2*S*)-hydroxyglutaric acid: 2-Hydroxyglutaric from **4e**: $[\alpha]_D^{25} = -2.0$ ($c = 1.0$, water) 2-hydroxyglutaric from **4f** $[\alpha]_D^{25} = -1.85$ ($c = 0.90$, water) {lit.[26] $[\alpha]_D^{25} = -1.1$ ($c = 1.4$, MeOH)}.

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