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Enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic esters from dialkyl 2-oxoglutarates

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Abstract

Enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic esters can be prepared either by enzymatic resolution of the racemic γ-lactones themselves or by bioreduction with baker's yeast of dialkyl 2-oxoglutarates and subsequent cyclization of the resulting dialkyl 2-hydroxyglutarates. The best results were obtained by the former route, by which the desired compounds were isolated in high enantiomeric excess. Bioreductions were less satisfactory. In fact the hydroxyester intermediates were initially formed as racemic mixtures and their final enantiomeric enrichment was reached by asymmetric destruction, occurring in the bioreaction medium, however at the same time large amounts of alkyl 4-hydroxybutanoates were formed as side products.

1. Introduction

The enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic acid **1**[1] (Scheme 1) has been widely utilized in the synthesis of γ-alkyl[1b] and γ-alkenyl-γ-lactones,[1b] which are ubiquitous natural products present in a variety of fruits,[2] milk products, fermented foods, tobacco and in some flowers as well as in some species of insects as components of sex-attractant pheromones.[1e,3] The (*S*)-**1** enantiomer has been used as a chiral derivatizing agent for alcohols[4] and also for the synthesis of (*S*)-5-hydroxymethyl γ-butyrolactone, a template for an approach to acyclic stereoselection.[5]

The literature procedure for the synthesis of lactonic acid **1** in its enantiomerically pure forms is by nitrous acid deamination of enantiomerically pure glutamic acid **2** (Scheme 1).[6] However, another synthetic strategy to the same compound might be the lactonization of 2-hydroxyglutaric acid **3**. The enantiodifferentiation could be achieved either by bioreduction of the precursor, 2-oxoglutaric acid **4**, or by kinetic resolution of chiral racemic tetrahydro-5-oxo-2-furancarboxylic esters **5**.

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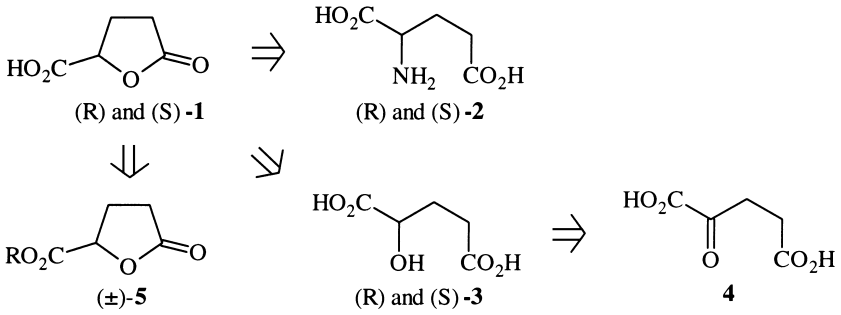
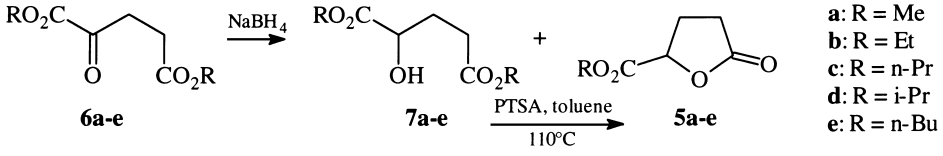


Scheme 1.

2. Results and discussion

In order to follow the enzymatic route, some racemic lactonic esters (±)-**5a**–**e**[7] (Scheme 2) were prepared by reduction with sodium borohydride of the corresponding dialkyl 2-oxoglutarates **6a**–**e**,[8] obtained from **4** by the usual procedure. The reaction furnished a 3:1 mixture of dialkyl 2-hydroxyglutarates **7a**–**e**[8] and the corresponding lactones **5a**–**e**. Lactonization was then completely achieved under acidic conditions.



Scheme 2.

2.1. Enzymatic resolutions of alkyl tetrahydro-5-oxo-2-furancarboxylic esters *5a–e*

A number of enzymes were checked, namely α-chymotrypsine (α-CT), pig liver esterase (PLE), *Candida rugosa* lipase (CRL), *Mucor miehei* lipase (MML), porcine pancreatic lipase (PPL) and horse liver acetone powder (HLAP), but only the last two systems proved able to operate an enantioselective hydrolysis of the ester groups. Under the pH conditions used (phosphate buffer, pH 7.4) the alkoxycarbonyl groups underwent chemical hydrolysis too, which however was much slower than the enzymatic one. In fact, in the absence of enzyme the pH value decreased by only 0.60 units in 15 h, whereas the enzymatic hydrolyses were over in 20 min. The reactions were stopped at about 75% conversion, which allowed the isolation of the enantiomerically pure lactonic esters **5a**–**e** in yields ranging from 10% to 24%. Attempts were also made to isolate the enantiomerically pure lactonic acid **1** at low conversion values, but they failed because the unreacted esters **5a**–**e** could never be extracted completely from the aqueous reaction solution. As a consequence, the subsequent extraction of the acidified mother liquors gave an inseparable mixture of the acid **1** and the ester **5** and therefore the enantiomeric excess of the acid could not be determined. Incidentally, this is also the reason why an evaluation of the enantiomeric ratio E[9] for the enzymes was not possible. However, enantiomerically pure lactonic acid **1** can be obtained from the corresponding methyl and ethyl esters **5a** and **5b** using bis(tributyltin) oxide (BTTO).[10]

The best results of the enzymatic resolutions are summarized in Table 1. It is interesting to note that PPL and HLAP showed opposite enantiopreferences. Furthermore, in contrast to PPL, the enantioselectivity of HLAP showed a strong dependence on the enzyme/substrate ratio, being the highest for 75 mg of enzyme per mmol of substrate. This result is indicative of the simultaneous action of several enzymes.

Table 1
Kinetic resolutions of **5a–e**[a]

PPL			HLAP		
Product	e.e. (%)	yield (%)[b]	Product	e.e. (%)	yield (%)[b]
(S)-(+)-**5a**	95[c]	15	(R)-(–)-**5a**	73[c]	10
(S)-(+)-**5b**	98[c]	18	(R)-(–)-**5b**	87[c]	18
(S)-(+)-**5c**	97[d]	22	(R)-(–)-**5c**	96[d]	17
(S)-(+)-**5d**	95[e]	24	(R)-(–)-**5d**	96[e]	10
(S)-(+)-**5e**	94[c]	22	(R)-(–)-**5e**	53[c]	23

[a]Reaction conditions: mg of enzyme/mmole of substrate: PPL, 150; HLAP, 75; phosphate buffer, pH = 7.4 (10 ml); 25 °C; [b]after chromatographic purification; [c]Determined by HRGC (γ-CDX, 150°C); [d]Determined by HRGC (γ-CDX, 10 min at 100°C, 3°C/min up to 150°C); [e]Determined by HRGC (β-CDX, 20 min at 80°C, 3°C/min up to 150°C).

An attempt was also made to perform an asymmetric lactonization of racemic dialkyl 2-hydroxydiesters **7a–e**, under the conditions used by Gutman and co-workers[11] for the enantioselective lactonization of racemic γ- and δ-hydroxyesters, however, this was unsuccessful.

The absolute configurations of the lactones **5b–e** were assigned by comparison of their CD spectra with that of **5a**, obtained by esterification with diazomethane of commercially available (*S*)-(+)-**1**. All (*S*)-(+)-lactonic esters **5a–e** showed a positive Cotton effect as did the acid (*S*)-(+)-**1** (Fig. 1).

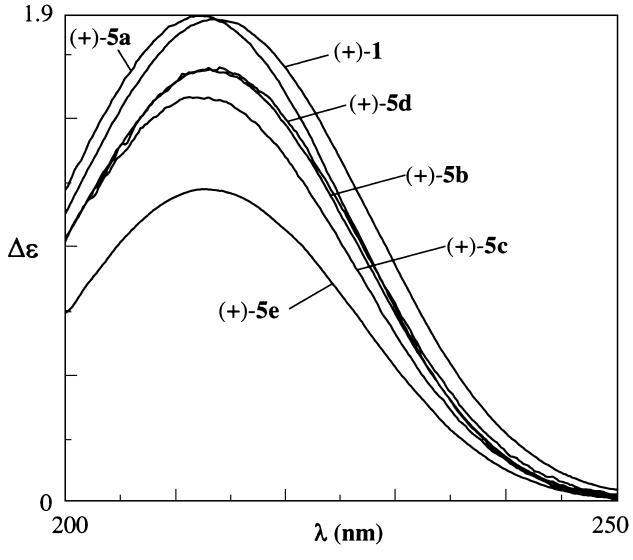


Figure 1. CD spectra of lactones (+)-**1** and (+)-**5a–e**

2.2. Bioreductions with baker's yeast of dialkyl 2-oxoglutarates **6a–e**

Dialkyl 2-oxoglutarates **6a–e** were reduced with baker's yeast with the aim of obtaining the corresponding enantiomerically pure dialkyl 2-hydroxyglutarates **7a–e** whose lactonization would have given the corresponding enantiomerically enriched lactonic esters **5a–e** (Table 2).

Bioreductions were performed using raw and dry baker's yeast. The isolation from cells of baker's yeast of several enzymes responsible for reduction of ketoesters to the corresponding hydroxyesters has recently been reported.[12] Some of the yeast ketoester reductases (YKERs) were specific for the reduction of α-ketoesters,[12,13] others were specific for β-ketoesters[12,14] and one of them called YKER-III, which depends on NADH, catalyzed ethanol–acetaldehyde oxidation–reduction process.[12] Among them, YKER-I is of great importance for the enantioselective synthesis of alcohols, because of its availability as the pure enzyme and excellent stereoselectivity.[15] It is therefore evident that, owing to the complexity of this multienzymatic system, the results may be strongly influenced by both physical and chemical parameters, such as thermal pre-treatment of the yeast,[16] concentration of the substrate,[17] pH,[18] presence of organic solvent,[19] nutrients,[20] or inhibitors.[20a,21] Moreover, it has been found that when some α-ketoesters[19d] were reduced by baker's yeast in water, side-products were formed whose nature of substituted primary alcohols had been proposed by Neuberg[22] and recently demonstrated by Nakamura and co-workers[21a] for the biotransformation of several alkyl 2-oxo-4-phenylbutanoates. Also in the present case, bioreductions carried out on 2-oxoglutarates **6a–e** were complicated by the formation of large amounts of the corresponding alkyl 4-hydroxybutanoates **8a–e**.

Table 2 lists the results of the bioreductions of dialkyl 2-oxoglutarates **6a–e** with raw baker's yeast in water. The reactions were run for two preincubation times of baker's yeast and monitored at regular intervals. The ratios between 2-hydroxydiesters **7a–e** and their corresponding 4-hydroxybutanoates **8a–e** were reported as a function of time at each conversion value, in order to stress the fact that it is just the destruction of **7a–e**, when asymmetric, that is responsible for the final enantiomeric excess of the remaining 2-hydroxydiesters and their yields. In the same columns the enantiomeric excesses of **7a–e** are also given, together with their absolute configurations, determined as indicated in the legend to Table 2.

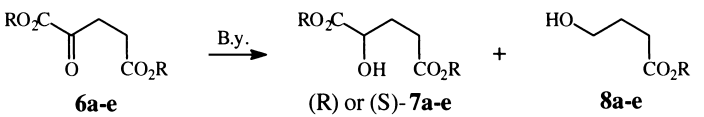
First of all it is evident that the preincubation time greatly affects both the enantiomeric excess of **7** and the amount of the side-product **8**. Bioreduction without preliminary preincubation was checked only for dimethyl 2-oxoglutarate **6a**. In that case the e.e. of **7a** was very low and its destruction to **8a** very rapid and poorly asymmetric. On the contrary, the thermal treatment of the yeast for 1 h was very efficient in deactivating the (*R*)-selective enzymes, and after 5 h the e.e. of the resulting 2-hydroxydiester (*S*)-(−)-**7a** was 96% (30% relative yield).

In the bioreduction of diethyl 2-oxoglutarate **6b** the best result was obtained by preincubating the yeast for 30 min. Bioreduction was over in 1 h but with complete lack of enantioselectivity. However in 24 h the asymmetric degradation of **7b** left (*S*)-(−)-**7b** with 99% e.e. (24% relative yield). When preincubation was prolonged for 1 h, (*R*)-(+)-**7b** (25% e.e.) was obtained directly and not as a result of an asymmetric destruction.

A completely parallel behaviour was observed for di-*i*-propyl 2-oxoglutarate **6d**. Using baker's yeast preincubated for 30 min, **7d**, initially formed as a racemic compound, was asymmetrically destroyed and after 24 h, (*S*)-(−)-**7d** was obtained with 96% e.e.

Bioreductions of di-*n*-propyl and di-*n*-butyl 2-oxodiesters **6c** and **6e**, respectively, were not enantioselective and the asymmetric destructions of the corresponding 2-hydroxydiesters **7c** and **7e** were too slow to lead to a high enantiodifferentiation within 24 h. It is interesting that when baker's yeast was preincubated for 30 min, **7c** was initially formed as the (*R*)-enantiomer (12% e.e.) while after 24 h it was recovered as the (*S*)-enantiomer (36% e.e). The same occurred for the reduction of **6e** when preincubation

Table 2. Reduction of the ketodiesters **6a–e** with raw baker's yeast in water[a]



6a-e (R) or (S)-**7a-e** **8a-e**

	6a (R = Me)						**6b** (R = Et)				**6c** (R = *n*-Pr)				**6d** (R = *i*-Pr)				**6e** (R = *n*-Bu)			
	Preincubation time (50°C)						Preincubation time (50°C)				Preincubation time (50°C)				Preincubation time (50°C)				Preincubation time (50°C)			
	0 h		0.5 h		1 h		0.5 h		1 h		0.5 h		1 h		0.5 h		1 h		0.5 h		1 h	
React. time (h)	Conv. %	**7a/8a** (% ee,[b] conf.)	Conv. %	**7a/8a** (% ee, conf.)	Conv. %	**7a/8a** (% ee, conf.)	Conv. %	**7b/8b** (% ee,[c] conf.)	Conv. %	**7b/8b** (% ee, conf.)	Conv. %	**7c/8c** (% ee,[d] conf.)	Conv. %	**7c/8c** (% ee, conf.)	Conv. %	**7d/8d** (% ee,[e] conf.)	Conv. %	**7d/8d** (% ee, conf.)	Conv. %	**7e/8e** (% ee,[f] conf.)	Conv. %	**7e/8e** (% ee, conf.)
1	100	50/50 (13, S)	100	67/33 (10, S)	87	31/69 (93, S)	100	74/26 (0)	37	65/35 (67, S)	90	80/20 (12, R)	45	73/27 (12, R)	100	82/18 (0)	32	84/16 (25, R)	65	46/54 (0)	10	3/97 (0)
2	100	39/61 (17, S)	100	63/37 (13, S)	100	33/67 (91, S)	100	69/31 (17, S)	71	62/38 (66, S)	94	78/22 (12, R)	88	68/32 (7, R)	100	68/32 (63, S)	63	84/16 (26, R)	59	64/36 (38, R)	58	26/74 (42, R)
5	100	31/69 (38, S)	100	54/46 (30, S)	100	30/70 (96, S)	100	57/43 (60, S)	98	58/42 (61, S)	100	70/30 (6, S)	95	69/31 (7, R)	100	49/51 (98, S)	94	82/18 (28, R)	79	66/34 (41, R)	73	30/70 (33, R)
24	100	4/96 (S)	100	21/79 (35, S)	100	14/86 (90, S)	100	24/76 (99, S)	100	50/50 (58, S)	100	50/50 (36, S)	100	67/33 (11, R)	100	13/87 (96, S)	100	79/21 (28, R)	94	50/50 (9, R)	93	35/65 (11, S)

[a]Conditions used: substrate, 0.5 mmol; b.y., 5 g in water (10 ml), 25 °C; [b]Determined by HRGC (β-CDX, 15 min at 110°C, 3°C/min up to 150°C); [c]Monitored by HRGC on the TFA derivative of **7b** (γ-CDX, 100°C); [d] Determined on the lactone **5c** by HRGC (γ-CDX, 10 min at 100°C, 3°C/min up to 150°C); [e] Determined by HRGC (β-CDX, 15 min at 120°C, 3°C/min up to 150°C); [f] determined on the lactone **5e** by HRGC (γ-CDX, 150°C).

was prolonged for 1 h: asymmetric degradation of **6e**, initially in (*R*)-configuration (42% e.e.), led, after 24 h, to the (*S*)-enantiomer, albeit with very low enantiomeric excess (11%).

As is evident from the above results, a period of preincubation is necessary for the deactivation of some dehydrogenases, as already emphasised by Nakamura.[16] While preincubation of baker's yeast for 30 min allowed the asymmetric degradation of the 2-hydroxydiesters **7** to the corresponding 4-hydroxyesters **8** to occur, preincubation for 1 h prevented it. In fact the ratio of **7** to **8** remained practically unchanged within 24 h in all cases with the exception of **7e**.

The use of inhibitors such as allyl bromide[21b] and methyl vinyl ketone,[23] had no effect on the enantioselectivity of the reaction, whereas it greatly enhanced the formation of 4-hydroxyesters **8a–e**. It is likely that they act as inhibitors of both (*R*)- and (*S*)-selective enzymes, whereas they had no effect on the activity of the enzyme system responsible for the formation of the side-products **8a–e**.

The use of an apolar organic solvent is usually of great advantage in bioreductions with baker's yeast.[19,21a] Reagents and products, in fact, can be fairly soluble in water and since bioreduction occurs at the interphase between water, where baker's yeast is present, and the organic solvent, undesired side-reactions are minimized. Among the organic solvents benzene was used and since the water content is critical, a buffer solution was added (pH 5, 0.6 ml of water/g of dry baker's yeast). The results are summarized in Table 3.

Table 3
Reduction of the ketodiesters **6a–e** with dry baker's yeast in benzene

	6a (R = Me)				**6b** (R = Et)		**6c** (R = *n*-Pr)	
	No preincub.		Preincub. 50°C, 1 h		No preincub.		No preincub.	
React. time (h)	Conv. %	**7a/8a** (% ee,[a] conf.)	Conv. %	**7a/8a** (% ee,[a] conf.)	Conv. %	**7b/8b** (% ee,[b] conf.)	Conv. %	**7c/8c** (% ee,[c] conf.)
0.5	12	8/92 (0)	7	71/29[d]	6	0/100	2	100/0[d]
1	33	27/73 (29, R)	12	75/25 (35, S)	14	14/86[d]	7	100/0[d]
2	71	53/47 (28, R)	22	73/27 (41, S)	31	52/48 (56, R)	18	89/11 (72, R)
5	100	61/39 (29, R)	45	71/29 (38, S)	70	71/29 (50, R)	44	91/9 (72, R)
24	100	51/49 (13, R)	93	69/31 (39, S)	100	71/29 (40, R)	73	90/10 (75, R)

[a]Determined by HRGC (β-CDX, 15 min at 110°C, 3°C/min up to 150°C); [b]Monitored by HRGC on the TFA derivative of **7b** (γ-CDX, 100°C); [c]Determined on the lactone **5c** by HRGC (γ-CDX, 10 min at 100°C, 3°C/min up to 150°C); [d]The e.e. was not determined because conversion was too low.

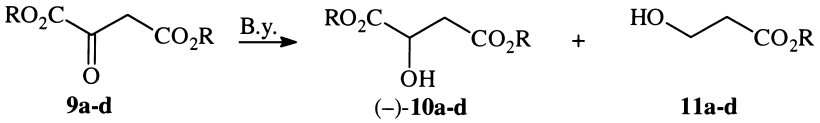
As already found for the bioreductions of α-ketoesters in benzene,[19d] in all our cases but one the absolute configuration of the carbinol carbon atom was (*R*). The exception was the product **7a** obtained from **6a** using baker's yeast preincubated at 50°C for 1 h (Table 3). The enantiopreference for the (*R*)-configuration was attributed to an accelerated activity of the (*R*)-selective enzymes with respect to the

(*S*)-selective enzymes, caused by the extremely low concentration of the substrate in water.[19c] This observation is confirmed by the lack of reduction observed for **6d** and **6e** under the same conditions.

A final experiment was carried out on the 2-hydroxydiesters **7a**–**e**. When kept under the same conditions as those used for bioreductions of dialkyl 2-oxoglutarates (5 g of baker's yeast:10 ml H_2O, preincubated at 50°C for 0.5 h), the asymmetric destruction was significant only for **7b** and **7d**. The respective (*S*)-(−)-enantiomer was obtained in 92% e.e. after 24 h in the former case and in 53% e.e. after 48 h in the latter case.

2.3. *Bioreductions of dialkyl 2-oxosuccinates **9a**–**d***

Ethyl 2-hydroxypropionate, ethyl 2-hydroxybutyrate, and diethyl 2-hydroxyhexandioate had already been found to undergo an enantioselective decomposition in the reaction medium with consumption of the (*R*)-enantiomer.[12,24] In order to investigate more thoroughly the formation of the side-products **8a**–**e** from the corresponding dialkyl 2-hydroxyglutarates **7a**–**e** in the bioreduction medium, we examined the reduction of dialkyl 2-oxosuccinates **9a**–**d**[25,26] under the same conditions (Scheme 3).



9a-d (−)-**10a-d** **11a-d**

Scheme 3.

Baker's yeast reduction of diethyl 2-oxosuccinate **9b** had already been investigated by Santaniello and co-workers:[26] the sodium enolate of the diester **9b** was reduced by baker's yeast in water, with sucrose added, affording, after 24 h, the corresponding alcohol (*S*)-(−)-**10b** with 98% e.e. However, when the reaction was repeated and monitored every hour by HRGC, we observed that bioreduction was over after 1 h and that the enantiomeric excess of the resulting alcohol (*S*)-(−)-**10b** was 56%. On standing in the reaction medium for 24 h, its enantiomeric excess increased to 98%, while the decomposition product **11b**, identified in the crude reaction mixture, was present in 15% relative yield. Evidently an asymmetric destruction of **10b** took place. Actually, when (±)-**10b** was kept under the same conditions for 24 h, (*S*)-(−)-**10b** was obtained with 98% e.e. However at the same time the amount of decomposition product **11b** also increased, being, after 48 h, the only product.

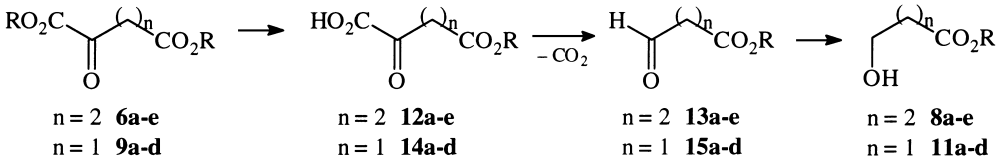
The same experiment was repeated under the conditions used for bioreductions of the dialkyl 2-oxoglutarates **6a**–**e**, that is preincubating baker's yeast at 50°C for 30 min and with no nutrients added. Under these conditions, reduction of **9b** was over in 3 h and the corresponding alcohol (*S*)-(−)-**10b** had 36% e.e. which increased to 95% when the product was kept in the reaction medium for 72 h. The same result was obtained using dry yeast preincubated at 50°C for 30 min (1 g/mmol of substrate). After 16 h the reduction was complete and the resulting alcohol (*S*)-(−)-**10b** had 64% e.e., which increased to 90% after six days.

The same behaviour was observed for the di-*i*-propyl derivative **9d**, whose bioreduction was over in 3 h leading to the corresponding alcohol (*S*)-(−)-**10d** with 28% e.e. However after 48 h its e.e. was 76%, as a result of the asymmetric destruction of **10d**. In fact when racemic alcohol **10d** was kept under the same conditions, the corresponding pure (*S*)-(−)-enantiomer (99% e.e.) was obtained after 48 h.

Dimethyl and di-*n*-propyl 2-oxosuccinates **9a** and **9c** showed a different behaviour. In fact they underwent a rapid reduction with raw baker's yeast, preincubated at 50°C for 30 min, to furnish the corresponding alcohols (*S*)-(−)-**10a** and (*S*)-(−)-**10c**, having 81% and 94% e.e., respectively. Their enantiomeric excesses did not vary with time. However destruction of **10a** and **10c** occurred, although

not enantioselectively. In fact when (±)-**10a** and (±)-**10c** were kept under the same reaction conditions, no enantiopreference in the destruction was observed and **11a** and **11c** were eventually the only products.

The mechanism of formation of the alkyl 4-hydroxybutanoates **8a–e** and of the alkyl 3-hydroxypropanoates **11a–d**, enzymatically mediated, might proceed from the parent 2-oxodiesters **6a–e** and **9a–d**, respectively, through a regioselective hydrolysis of the alkoxycarbonyl group leading to the hemiesters **12a–e** and **14a–d**, followed by decarboxylation to the corresponding aldehydes **13a–e** and **15a–d**. Reduction of the formyl group would eventually furnish **8a–e** and **11a–d** (Scheme 4).[21a]



Scheme 4.

Therefore, biotransformations of the 2-hydroxydiesters **7a–e** and **10a–d** must imply their reoxidation to the corresponding 2-oxodiesters **6a–e** and **9a–d**. However this oxidation step was not always enantioselective. In some cases the destruction observed was asymmetric, leaving the remaining 2-hydroxydiesters with high enantiomeric excess, and in some others it was symmetric, leading to the simple consumption of the substrates themselves.

3. Conclusions

Production of enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic esters is better achieved by kinetic resolution of the chiral racemic lactonic esters than by lactonization of chiral dialkyl 2-hydroxydiesters formed by bioreduction with baker's yeast of the corresponding dialkyl 2-oxoglutarates. However, both procedures allowed the isolation of both enantiomers of the γ-alkoxycarbonyl-γ-lactones. In fact the (*S*)-enantiomers with high enantiomeric excess (>94%) were obtained by kinetic resolution with PPL, or by cyclization of the products of bioreduction with baker's yeast of the corresponding dialkyl 2-oxoglutarates. In this latter case the enantiomeric excess ranged from 35% to 99%. Conversely, enantiomerically pure γ-alkoxycarbonyl-γ-lactones with the (*R*) configuration could be obtained by enzymatic hydrolysis with HLAP (53–96% e.e.) or, with poor enantiomeric excess, carrying out the bioreduction of the 2-oxodiesters in benzene and subsequent acidic cyclization. Furthermore, bioreductions of diethyl and di-isopropyl 2-oxoglutarates could be a preparative route to the corresponding enantiomerically pure 2-hydroxydiesters, which are not easy to prepare by other procedures.[8] Finally, as to the influence of the alkyl residues on bioreductions and biohydrolyses, the ethyl group seems to adapt better than the other residues to the active sites of both baker's yeast dehydrogenases and the more efficient hydrolases.

4. Experimental

4.1. General

Melting points were determined with a Büchi apparatus and are uncorrected. IR spectra were recorded in $CHCl_3$, unless otherwise stated, on a JASCO FT-IR-200 spectrometer. ^1H NMR spectra were run on a Jeol EX-400 (400.0 MHz) spectrometer using deuterochloroform as solvent and tetramethylsilane as internal standard; J values are given in hertz. ^{13}C NMR spectra were recorded on a Jeol EX-400 (100.4

MHz) instrument. Optical rotations were determined on a Perkin–Elmer Model 241 polarimeter. CD spectra were obtained on a Jasco J-700A spectropolarimeter in methanol; GLC analyses were obtained on a Carlo Erba GC 8000 instrument, the capillary column being EC-WAX, 30 m×0.32 mm (carrier gas He, 40 kPa, split 1:50, 10 min at 100°C, 3°C/min, 200°C), Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin 40 m×0.25 mm (carrier gas He, 180 kPa, split 1:100) or DMePe β-cyclodextrin 25 m×0.26 mm (carrier gas He, 110 kPa, split 1:50); mass spectra were run by the electron impact mode (20 eV and 70 eV) on a VG 7070 spectrometer. TLC was performed on Whatman K6F silica gel plates (eluant: light petroleum:ethyl acetate, 7:3). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with b.p. 40–70°C and ether to diethyl ether.

4.2. Synthesis of substrates

4.2.1. General procedure for the esterification of 2-oxoglutaric acid **4**, oxalacetic and malic acid

The acid (34 mmol) and the corresponding alcohol (120 mmol) were refluxed in toluene with *p*-toluenesulfonic acid as a catalyst in a Dean–Stark apparatus. After the usual workup the ester was isolated.

4.2.2. Dimethyl 2-oxo-pentandioate **6a**[27]

65% Yield; IR (film) ν, cm^{-1}: 1800 (sh), 1735 (CO, COO); ^1H NMR, δ, ppm: 3.89 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.17 (t, 2H, CH_2CO), 2.69 (t, 2H, CH_2CO); ^{13}C NMR, δ, ppm: 192.1 (s), 172.4 (s), 160.7 (s), 52.9 (q), 51.8 (q), 34.0 (t), 27.2 (t); MS (20 eV): 174 (1, $[M^{+\cdot}]$), 116 (10), 115 (100, $[M-COOCH_3]^+$), 101 (35), 87 (36), 59 (37, $[COOCH_3]^+$), 55 (77).

4.2.3. Diethyl 2-oxo-pentandioate **6b**[28,29]

77% Yield; IR (film) ν, cm^{-1}: 1800 (sh), 1735 (CO, COO); ^1H NMR and ^{13}C NMR spectra were in accordance with literature;[29] MS (20 eV): 202 (2, $[M^{+\cdot}]$), 157 (18, $[M-OCH_2CH_3]^+$), 130 (17), 129 (100, $[M-COOCH_2CH_3]^+$), 102 (25), 101 (90), 73 (44, $[COOCH_2CH_3]^+$), 55 (39), 29 (34).

4.2.4. *Di*-n-*propyl 2-oxo-pentandioate* **6c**

90% Yield; IR (film) ν, cm^{-1}: 1800 (sh), 1735 (CO, COO); ^1H NMR, δ, ppm: 4.16 (t, 2H, OCH_2), 3.97 (t, 2H, OCH_2), 3.08 (t, 2H, CH_2CO), 2.60 (t, 2H, CH_2CO), 1.69, 1.57 (2m, 4H, $2CH_2CH_3$), 0.91, 0.86 (2t, 6H, $2CH_3$); ^{13}C NMR, δ, ppm: 192.6 (s, CO), 172.0, 160.6 (2s, COO), 67.9 (t), 66.4 (t), 34.2 (t), 27.6 (t), 21.8 (t), 21.7 (t), 10.2 (q), 10.1 (q); MS (70 eV): 157 (7), 101 (100), 57 (29), 41 (56).

4.2.5. *Di*-i-*propyl 2-oxo-pentandioate* **6d**

88% Yield; IR (film) ν, cm^{-1}: 1800 (sh), 1735 (CO, COO); ^1H NMR, δ, ppm: 5.06 (m, 1H, OCH), 4.91 (m, 1H, OCH), 3.05 (t, 2H, CH_2CO), 2.54 (t, 2H, CH_2CO), 1.26, 1.14 (2d, 12H, $4CH_3$); ^{13}C NMR, δ, ppm: 193.0 (s, CO), 171.5, 160.1 (2s, COO), 70.7 (d), 68.3 (d), 34.1 (t), 27.9 (t), 21.7 (q), 21.5 (q); MS (20 eV): 170 (4), 142 (13), 129 (10), 101 (100), 74 (10), 43 (32).

4.2.6. *Di*-n-*butyl 2-oxo-pentandioate* **6e**

90% Yield; IR (film) ν, cm^{-1}: 1800 (sh), 1735 (CO, COO); ^1H NMR, δ, ppm: 4.27 (t, 2H, OCH_2), 4.08 (t, 2H, OCH_2), 3.15 (t, 2H, CH_2-CO), 2.67 (t, 2H, CH_2-CO), 1.74–1.35 (m, 8H, CH_2), 0.94 (q, 3H, CH_3), 0.93 (q, 3H, CH_3); ^{13}C NMR, δ, ppm: 192.5 (s), 171.9 (s), 160.5 (s), 66.2 (t), 64.7 (t), 34.1 (t),

30.4 (t), 30.2 (t), 27.5 (t), 18.97 (t), 18.91 (t), 13.59 (q), 13.52 (q); MS (20 eV): 258 (3, $[M^{+\cdot}]$), 185 (19, $[M-OBu]^+$), 157 (100, $[M-COOBu]^+$), 129 (32), 102 (67), 101 (90), 73 (31), 56 (95), 55 (36), 43 (53).

4.2.7. Dimethyl 2-oxo-butandioate ***9a***

All spectral data were in accordance with the literature.[25]

4.2.8. Diethyl 2-oxo-butandioate ***9b***[25,26]

The title compound was purchased from Aldrich.

*4.2.9. Di-*n*-propyl 2-oxo-butandioate* ***9c***

Oil; IR (film) ν, cm^{-1}: 1745, 1730 (CO, COO); ^1H NMR, δ, ppm: 11.7 (bs, 0.8H, OH), 5.97 (s, O.8H, CH), 4.19 (t, 2H, J 6.8, OCH_2), 4.13 (t, 1.6H, J 6.8, OCH_2), 4.07 (t, 0.4H, J 6.8, OCH_2), 3.78 (s, 0.4H, $COCH_2$), 1.68 (m, 4H, CH_2CH_3), 0.94 (m, 6H, CH_2CH_3); ^{13}C NMR, δ, ppm: 171.9 (s), 161.8 (s), 159.3 (s), 96.8 (d), 67.8 (t), 66.8 (t), 45.2 (t), 21.7 (t), 10.2 (q).

*4.2.10. Di-*i*-propyl 2-oxo-butandioate* ***9d***[30]

Oil; ^1H NMR, δ, ppm: 11.7 (bs, 0.7H, OH), 5.97 (s, 0.7H, =CH), 5.16 (m, 2H, $CH(CH_3)_2$), 3.77 (s, 0.6H, $COCH_2$), 1.33 (d, 6H, J 6.1, $CH(CH_3)_2$), 1.30 (d, 6H, J 6.1, $CH(CH_3)_2$); ^{13}C NMR, δ, ppm: 122.2 (s), 160.4 (s), 159.4 (s), 96.9 (d), 70.0 (d), 68.8 (d), 45.4 (t), 21.5 (q), 21.4 (q); MS (70 eV): 157 ($[M-O^iPr]^+$, 3), 129 ($[M-COO^iPr]^+$, 24), 115 (12), 87 (100), 69 (46).

4.3. General procedure for the synthesis of lactones ***5a–e***

2-Hydroxydiesters (±)-**7a–e** were refluxed in toluene in the presence of *p*-toluenesulfonic acid as a catalyst for a time varying between 5 h and 24 h. The products (±)-**5a–e** were then purified on flash chromatography.

4.4. General procedure for enzymatic hydrolysis of lactones ***5a–e***

The lactonic ester (3 mmol) in phosphate buffer (pH 7.4, 6 ml) was reacted with the enzyme (450 mg) under stirring. The pH value was continuously adjusted with 1N NaOH. After about 75% conversion, the aqueous phase was extracted four times with diethyl ether to extract the unreacted lactonic ester. The remaining mother liquors were acidified to pH 1 with 2N HCl and extracted four times with diethyl ether which was dried on Na_2SO_4. Elimination of the solvent left an oil which was a mixture of the lactonic ester **5a–e** and the acid **1**.

4.4.1. (±)-Tetrahydro-5-oxo-2-furancarboxylic acid methyl ester ***5a***

The yellowish oily crude reaction mixture was set aside at 4°C for 6 h. The product **5a** was isolated as a yellow solid (55% yield) and it was crystallized from ethyl acetate and light petroleum: m.p. 43°C; IR (film) ν, cm^{-1}: 1780 (COO), 1746 ($COOCH_3$); ^1H NMR, δ, ppm: 4.95 (m, 1H, CHO), 3.80 (s, 3H, CH_3O), 2.63–2.30 (m, 4H, $2CH_2$); ^{13}C NMR, δ, ppm: 175.9 (s), 170.1 (s), 75.5 (d), 52.5 (q), 26.5 (t), 25.5 (t); MS (70 eV): 144 (8, $[M^{+\cdot}]$), 85 (100, $[M-COOCH_3]^+$), 59 (17, $[COOCH_3]^+$), 57 (42), 29 (91).

(*S*)-(+)-**5a**[31] was obtained in 15% yield using PPL as the hydrolytic enzyme within 15–20 min: 95% e.e. (HRGC, γ-CDX, 150°C); m.p. 61°C [lit.[31a] m.p. 58–60°C, lit.[1a] m.p. 42–44°C]; $[\alpha]_D^{25}$=+15.8 (c=0.65, MeOH); [lit.[31a] $[\alpha]_D^{25}$=+3.1 (c=4.5, H_2O), lit.[1a] $[\alpha]_D^{20}$=+6.8 (c=3.1, MeOH), lit.[31b] $[\alpha]_D^{24}$=+15.88 (c=6.4, MeOH), $[\alpha]_D^{12}$=+14.6 (c=1.7, MeOH)]; $\Delta\varepsilon_{212}$=+1.9.

(*R*)-(−)-**5a** was obtained in 10% yield using HLAP as the hydrolytic enzyme within 15–20 min: 73% e.e. (HRGC, γ-CDX, 150°C); m.p. 55°C $[\alpha]_D^{25}$=−12.0 (c=0.75, MeOH); [lit.[31b] $[\alpha]_D^{24}$=−14.89 (c=6.8, MeOH)]; $\Delta\varepsilon_{211}$=−1.2.

4.4.2. (±)-Tetrahydro-5-oxo-2-furancarboxylic acid ethyl ester ***5b***[32]

The crude reaction product was purified on silica gel (eluant: light petroleum:ethyl acetate, gradient from 95:5 to 80:20). 85% Yield, oil, IR, ^{1}H NMR and MS data were identical with those reported in the literature.[1d] ^{13}C NMR, δ, ppm: 176.0 (s), 169.7 (s), 75.6 (d), 61.8 (t), 26.5 (t), 25.6 (t), 13.9 (q).

(*S*)-(+)-**5b** was obtained in 18% yield using PPL as the hydrolytic enzyme within 15–20 min: 98% e.e. (HRGC, γ-CDX, 150°C); $[\alpha]_D^{25}$=+11.7 (c=1.17, MeOH), $[\alpha]_D^{25}$=+13.3 (c=0.56, EtOH) [lit.[1d] $[\alpha]_D^{25}$=+15.1 (c=0.6, EtOH)]; lit.[1c] $[\alpha]_D^{32}$=+11.5 (c=2.93, EtOH), lit.[31b] $[\alpha]_D^{24}$=+11.3 (c=10, EtOH)]; $\Delta\varepsilon_{213}$=+1.7.

(*R*)-(-)-**5b** was obtained in 18% yield using HLAP as the hydrolytic enzyme: 87% e.e. (HRGC, γ-CDX, 150°C); $[\alpha]_D^{25}$=−12.0 (c=0.46, EtOH) [lit.[1d] $[\alpha]_D^{25}$=−14.7 (c=0.4, EtOH), lit.[31b] $[\alpha]_D^{24}$=−11.21 (c=10, EtOH)]; $\Delta\varepsilon_{214}$=−1.4.

4.4.3. (±)-Tetrahydro-5-oxo-2-furancarboxylic acid n*-propyl ester* ***5c***

The crude reaction product was purified on silica gel (eluant: light petroleum:ethyl acetate, gradient from 95:5 to 80:20). 85% Yield, oil, IR (film) ν, cm^{-1}: 1780 (COO), 1746 (COO^{n}Pr); ^{1}H NMR, δ, ppm: 4.84 (m, 1H, CHO), 4.05 (t, 2H, CH_2O), 2.53–2.16 (m, 4H, CH_2CH_2), 1.63–1.14 (m, 2H, CH_2CH$_3$), 0.84 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 175.9 (s), 169.9 (s), 75.7 (d), 67.4 (t), 26.7 (t), 25.8 (t), 21.8 (t), 10.2 (q); MS (70 eV): 172 (8, [M$^{+\cdot}$]), 154 (22), 91 (43), 85 (100), 65 (20), 57 (16).

(*S*)-(+)-**5c** was obtained in 22% yield using PPL as the hydrolytic enzyme within 15–20 min: 97% e.e. (HRGC, γ-CDX, 10 min at 100°C, 3°C/min, 150°C); $[\alpha]_D^{25}$=+9.2 (c=1.00, MeOH); $\Delta\varepsilon_{212}$=+1.6.

(*R*)-(−)-**5c** was obtained in 17% yield (after purification on column) using HLAP as the hydrolytic enzyme within 15–20 min: 96% e.e.; $[\alpha]_D^{25}$ −9.8 (c=0.80, MeOH); $\Delta\varepsilon_{213}$=−1.5.

4.4.4. (±)-Tetrahydro-5-oxo-2-furancarboxylic acid i*-propyl ester* ***5d***

The crude reaction product was purified on silica gel (eluant: light petroleum:ethyl acetate, gradient from 95:5 to 80:20). 85% Yield; IR (film) ν, cm^{-1}: 1780 (COO), 1746 (COO^{i}Pr); ^{1}H NMR, δ, ppm: 5.11 (sept, 1H, CH(CH$_3$)$_2$), 4.89 (m, 1H, CHO), 2.70–2.45 (m, 3H), 2.30 (m, 1H), 1.30, 1.29 (2d, 6H, 2CH$_3$); ^{13}C NMR, δ, ppm: 174.4 (s), 169.4 (s), 75.9 (d), 69.9 (d), 26.7 (t), 25.8 (t), 21.6 (q), 21.5 (q); MS (70 eV): 129 (2, [M−C_3H_7]$^+$), 85 (100), 57 (10), 43 (49).

(*S*)-(+)-**5d** was obtained in 24% yield using PPL as the hydrolytic enzyme within 15–20 min: 95% e.e. (HRGC, β-CDX, 20 min at 80°C, 3°C/min, 150°C); $[\alpha]_D^{25}$=+10.0 (c=1.4, MeOH); $\Delta\varepsilon_{213}$=+1.7.

(*R*)-(−)-**5d** was obtained using HLAP as an enzyme within 15–20 min, 10% yield (after purification on column); m.p. 40–41°C, from light petroleum:ethyl acetate; 96% e.e.; $[\alpha]_D^{25}$=−10.6 (c=0.56, MeOH); $\Delta\varepsilon_{214}$=−1.6.

4.4.5. (±)-Tetrahydro-5-oxo-2-furancarboxylic acid n*-butyl ester* ***5e***

The crude reaction product was purified on silica gel (eluant: light petroleum:ethyl acetate, gradient from 95:5 to 80:20). 50% Yield; oil; IR (film) ν, cm^{-1}: 1780 (COO), 1746 (COOBu); ^{1}H NMR, δ, ppm: 4.85 (m, 1H, CHO), 4.11 (t, 2H, CH_2O), 2.54–2.21 (m, 4H, CH_2CO, CH_2CH$_2$CO), 1.56 (m, 2H, CH_2CH$_2$CH$_3$), 1.29 (m, 2H, CH_2CH$_3$), 0.85 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 175.9 (s), 169.8 (s), 75.7 (d), 65.6 (t), 30.3 (t), 26.6 (t), 25.7 (t), 18.8 (t), 13.5 (q); MS (70 eV): 186 (4, [M$^{+\cdot}$]), 131 (23), 87 (8), 86 (56), 85 (100, [M−COOBu]$^+$), 58 (17), 57 (60), 56 (24), 55 (20).

(*S*)-(+)-**5e** was obtained in 22% yield using PPL as the hydrolytic enzyme; 94% e.e. (HRGC, γ-CDX, 150°C); $[\alpha]_D^{25}$=+7.5 (c=0.76, MeOH); $\Delta\varepsilon_{213}$=+1.2.

(*R*)-(−)-**5e** was obtained in 23% yield using HLAP as the hydrolytic enzyme; 53% e.e. $[\alpha]_D^{25}$=−4.2 (c=1.32, MeOH); $\Delta\varepsilon_{212}$=−0.74.

*4.5. General procedure for reductions of 2-oxodiesters **6a–e** and **9a–d***

4.5.1. Reduction with sodium borohydride

Sodium borohydride (1.0 mmol) was added over 30 min to a solution of the 2-oxodiester (2.0 mmol) in water or alcohol. The mixture was acidified to pH 2 with 2N HCl and extracted with dichloromethane. Elimination of the solvent left a colourless oil which was used without purification.

4.5.2. Reduction with baker's yeast

(a) Reduction in water: In a preparative run, 30 g of baker's yeast in 60 ml of water was preincubated at 50°C for 30 min, added to 3.0 mmol of the ketodiester and the mixture stirred at room temperature. The course of the reduction was checked by HRGC. At the end of the reaction, brine was added and the broth was continuously extracted for 48 h with diethyl ether. The organic phase was dried and evaporated and the product was purified by flash chromatography (eluant: light petroleum:ethyl acetate, gradient from 95:5 to 80:20).

(b) Reduction in benzene: The 2-oxodiester (0.5 mmol) and dry baker's yeast (5.0 g) in benzene (28 ml) and a buffer solution (0.1 M, 2 ml, pH 5) were stirred for the periods indicated in Table 3. The organic phase was dried on anhydrous Na_2SO_4 to give the product which was purified when necessary.

*4.5.3. (±)-Dimethyl 2-hydroxypentandioate **7a***

65% Yield; IR, ^{1}H NMR and MS data were identical with those reported in the literature.[8] ^{13}C NMR, δ, ppm: 174.9 (s), 173.5 (s), 69.3 (d), 52.6 (q), 51.6 (q), 29.3 (t), 29.2 (t).

(*S*)-(−)-**7a**: 16% Yield; 63% e.e. (β-CDX, 15 min at 110°C, 3°C/min, 150°C); $[\alpha]_D^{25}$=−4.5 (c=0.22, MeOH) [lit.[8] $[\alpha]_D^{25}$=−2.48 (neat)].

*4.5.4. (±)-Diethyl 2-hydroxypentandioate **7b***

74% Yield; IR, ^{1}H NMR and MS data were identical with those reported in the literature.[1d] ^{13}C NMR, δ, ppm: 174.6 (s), 173.1 (s), 69.4 (d), 61.8 (t), 60.5 (t), 29.6 (t), 29.3 (t), 14.1 (q).

(*S*)-(−)-**7b**: 18% Yield; 99% e.e. (determined on its trifluoroacetyl derivative, HRGC, γ-CDX, 100°C); $[\alpha]_D^{25}$=−4.9 (c=0.47, EtOH) [lit.[1d] for (*R*)-(+)-**7b** $[\alpha]_D^{20}$=+3.9 (c=0.3, EtOH)].

*4.5.5. (±)-Di-*n*-propyl 2-hydroxypentandioate **7c***

70% Yield; IR (film) ν, cm^{-1}: 3500 (OH), 1735 (COO); ^{1}H NMR, δ, ppm: 4.16 (m, 1H, C*H*OH), 4.08 (t, 2H, OCH_2), 3.97 (t, 2H, OCH_2), 2.80 (bs, 1H, OH), 2.40 (m, 2H), 2.10 (m, 1H), 1.87 (m, 1H), 1.65–1.50 (m, 4H), 0.89 (t, 3H, CH_3), 0.87 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 174.7 (s), 173.2 (s), 69.5 (d), 67.4 (t), 66.1 (t), 29.7 (t), 29.4 (t), 21.9 (t), 10.3 (q), 10.2 (q); MS (70 eV): 131 (11), 101 (19), 85 (100), 57 (23).

(*S*)-(−)-**7c**: 19% Yield; 33% e.e. (determined on its lactone derivative, HRGC, γ-CDX, 10 min at 100°C, 3°C/min, 150°C); $[\alpha]_D^{25}$=−5.5 (c=0.11, MeOH); $\Delta\varepsilon_{212}$=+0.4.

*4.5.6. (±)-Di-*i*-propyl 2-hydroxypentandioate* ***7d***

75% Yield; IR (film) ν, cm^{-1}: 3500 (OH), 1735 (COO); ^1H NMR, δ, ppm: 5.02 (quintet, 1H, CHO), 4.94 (quintet, 1H, CHO), 4.09 (m, 1H, C*H*OH), 2.96 (bs, 1H, OH), 2.43–2.28 (m, 2H), 2.07 (m, 1H), 1.84 (m, 1H), 1.19, 1.15 (2d, 12H, $4CH_3$); ^{13}C NMR, δ, ppm: 174.2 (s), 172.7 (s), 69.6 (d), 69.5 (d), 67.8 (d), 30.0 (t), 29.4 (t), 21.7 (4q); MS (70 eV): 144 (9), 130 (22), 103 (34), 85 (100), 57 (10), 43 (54).

(*S*)-(−)-**7d**: 9% Yield; 30% e.e. (HRGC, β-CDX, 15 min, 120°C, 3°C/min, 150°C); $[\alpha]_D^{25}$=−1.1 (c=1.4, MeOH); $\Delta\varepsilon_{213}$=+0.5.

(*R*)-(+)-**7d**: 17% Yield; 14% e.e.; $[\alpha]_D^{25}$=+0.5 (c=1.3, MeOH).

*4.5.7. (±)-Di-*n*-butyl 2-hydroxypentandioate* ***7e***[33]

77% Yield; IR (film) ν, cm^{-1}: 3500 (OH), 1735 (COO); ^1H NMR, δ, ppm: 4.16 (t+m, 3H, OCH, OCH_2), 4.05 (t, 2H, OCH_2), 2.52–1.85 (m, 4H, CH_2, CH_2CO), 1.65–1.30 (m, 8H, CH_2), 0.90 (t, 3H, CH_3), 0.91 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 174.7 (s), 173.1 (s), 69.4 (d), 65.6 (t), 64.4 (t), 30.5 (t), 30.4 (t), 29.6 (t), 29.3 (t), 19.0 (t), 18.9 (t), 13.6 (q), 13.5 (q); MS (70 eV): 261 (1, $[MH]^+$), 260 (0.8, $[M^{+\cdot}]$), 241 (1), 187 (5), 158 (16), 131 (29), 103 (33), 86 (14), 85 (100), 57 (33), 56 (23), 41 (33), 29 (39), 28 (21), 17 (30).

(*R*)-(+)-**7e**: 12% Yield; 42% e.e. (determined on its lactone derivative, HRGC, γ-CDX, 150°C), $[\alpha]_D^{25}$=+2.2 (c=0.76, MeOH).

4.5.8. Methyl 4-hydroxybutanoate ***8a***[34,35]

32% Yield; IR (film) ν, cm^{-1}: 3450 (OH), 1735 (COO); ^1H NMR, δ, ppm: 3.68 (s, 3H, OCH_3), 3.66 (t, 2H, CH_2O), 2.44 (t, 2H, CH_2CO), 1.87 (m, 2H, CH_2); ^{13}C NMR, δ, ppm: 174.3 (s), 61.8 (t), 51.6 (q), 30.6 (t), 27.5 (t); MS (70 eV): 118 (1, $[M^{+\cdot}]$), 88 (70), 87 (100, $[M-OCH_3]^+$), 85 (10), 74 (41), 68 (10), 60 (37), 59 (15), 55 (10).

4.5.9. Ethyl 4-hydroxybutanoate ***8b***[34]

34% Yield; IR (film) ν, cm^{-1}: 3450 (OH), 1735 (COO); ^1H NMR, δ, ppm: 4.05 (q, 2H, OCH_2), 3.59 (t, 2H, CH_2OH), 2.35 (t, 2H, CH_2CO), 1.8 (m, 2H, CH_2CH_2OH), 1.17 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 173.9 (s), 61.9 (t), 60.4 (t), 31.0 (t), 27.6 (t), 14.1 (q); MS (70 eV): 132 (2, $[M^{+\cdot}]$), 115 (1), 102 (30), 88 (60), 87 (100, $[M-OEt]^+$), 74 (33), 72 (13), 68 (20), 61 (18), 60 (33), 56 (13).

4.5.10. n-*Propyl 4-hydroxybutanoate* ***8c***

20% Yield; IR (film) ν, cm^{-1}: 3450 (OH), 1735 (COO); ^1H NMR, δ, ppm: 3.96 (t, 2H, CH_2OH), 3.60 (t, 2H, CH_2O), 2.36 (t, 2H, CH_2CO), 1.84–1.53 (m, 4H), 0.86 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 174.1 (s), 66.1 (t), 61.9 (t), 31.0 (t), 27.7 (t), 21.9 (t), 10.3 (q); MS (70 eV): 129 (7, $[M-OH]^+$), 128 (7, $[M-H_2O]^{+\cdot}$), 101 (100), 85 (90), 73 (20), 55 (54).

4.5.11. i-*Propyl 4-hydroxybutanoate* 8d[34]

14% Yield; IR (film) ν, cm^{-1}: 3450 (OH), 1735 (COO); ^1H NMR, δ, ppm: 4.99 (m, 1H, CH), 3.65 (t, 2H, CH_2OH), 2.37 (t, 2H, CH_2CO), 2.25 (bs, 1H, OH), 1.85 (quintet, 2H, CH_2), 1.21 (d, 6H, $2CH_3$); ^{13}C NMR, δ, ppm: 173.5 (s), 67.8 (d), 62.0 (t), 31.4 (t), 27.7 (t), 21.7 (q); MS (70 eV): 147 (28, MH^+), 131 (7), 119 (24), 116 (16), 101 (51), 89 (49), 76 (100).

4.5.12. n-*Butyl 4-hydroxybutanoate* ***8e***

15% Yield; IR (film) ν, cm^{-1}: 3450 (OH), 1735 (COO); ^1H NMR, δ, ppm: 4.01 (t, 2H, CH_2OH), 3.60 (t, 2H, OCH_2), 2.36 (t, 2H, CH_2CO), 2.50 (bs, 1H, OH), 1.81 (quintet, 2H, CH_2), 1.54 (quintet, 2H,

CH_2), 1.30 (sextet, 2H, CH_2), 0.86 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 174.4 (s), 64.7 (t), 62.1 (t), 31.3 (t), 30.8 (t), 27.9 (t), 19.3 (t), 13.9 (q); MS (70 eV): 160 (0.5, $[M^{+\cdot}]$), 87 (100, $[M-BuO]^+$), 85 (11), 74 (20), 69 (19), 60 (20), 57 (30), 56 (71), 45 (16), 43 (40), 42 (13), 41 (43).

4.5.13. Dimethyl 2-hydroxybutandioate ***10a***[36]

The title compound was purchased from Aldrich: HRGC (Carbowax, 100°C for 5 min, 3°C/min up to 200°C; β-CDX, 120°C for 20 min, 3°C/min up to 150°C) retention time 9.33 min for (*R*)-(+)-**10a**, 9.85 min for (*S*)-(−)-**10a**.

4.5.14. Diethyl 2-hydroxybutandioate ***10b***[26,37]

HRGC (Carbowax, 100°C for 5 min, 3°C/min up to 200°C; β-CDX, 120°C for 20 min, 3°C/min up to 150°C) retention time 16.19 min for (*R*)-(+)-**10b**, 16.56 min for (*S*)-(−)-**10b**; IR and ^{1}H NMR data were in accordance with literature.[26,37] ^{13}C NMR, δ, ppm: 173.3 (s), 170.4 (s), 67.2 (d), 61.4 (t), 60.9 (t), 38.6 (t), 13.7 (q); MS (70 eV): 145 (10), 117 (100), 89 (39), 71 (74).

*4.5.15. Di-*n*-propyl 2-hydroxybutandioate* ***10c***[38]

Oil; IR (film) ν, cm^{-1}: 3460 (OH), 1720 (COO); ^{1}H NMR, δ, ppm: 4.44 (1H, dd, J_1 4.4, J_2 6.3, C*H*OH), 4.09 (2H, t, J 6.8, OCH_2), 2.76 (2H, dd, J_1 4.4, J_2 11.4, CH_2CO), 1.61 (4H, m, CH_2CO), 0.87 (6H, 2t, CH_3); ^{13}C NMR, δ, ppm: 173.3 (s), 170.4 (s), 67.3 (t), 67.2 (d), 66.3 (t), 38.6 (t), 21.7 (2t), 10.1 (q), 10.0 (q); MS (70 eV): 159 (11), 131 (38), 117 (8), 89 (100), 71 (28).

*4.5.16. Di-*i*-propyl 2-hydroxybutandioate* ***10d***[37c,d]

Oil; IR (film) ν, cm^{-1}: 3460 (OH), 1720 (COO); ^{1}H NMR, δ, ppm: 4.98 (2H, m, C*H*$(CH_3)_2$), 4.37 (1H, dd, J_1 4.4, J_2 5.9, C*H*OH), 2.72 (1H, dd, J_1 4.4, J_2 16.1, CH_2CO), 2.66 (1H, dd, J_1 5.9, J_2 16.1, CH_2CO), 1.18 (12H, d, CH(CH_3)$_2$); ^{13}C NMR, δ, ppm: 172.8 (s), 169.8 (s), 69.6 (d), 68.3 (d), 67.2 (d), 38.9 (t), 21.4 (2q), 21.54 (q), 21.51 (q); MS (70 eV): 159 (5), 131 (18), 117 (20), 89 (100), 71 (24).

4.5.17. Methyl 3-hydroxypropanoate ***11a***[39]

All spectroscopic data were in accordance with the literature.[35,40]

4.5.18. Ethyl 3-hydroxypropanoate ***11b***[41]

Oil; IR (film) ν, cm^{-1}: 3500 (OH), 1735 (COO); ^{1}H NMR, δ, ppm: 4.10 (q, 2H, O$CH_2$$CH_3$), 3.78 (t, 2H, CH_2OH), 3.50 (bs, 1H, OH), 2.51 (t, 2H, CH_2CO), 1.20 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 172.0 (s), 60.7 (t), 58.1 (t), 36.7 (t), 14.0 (q). MS data were in accordance with the literature.[42]

4.5.19. n*-Propyl 3-hydroxypropanoate* ***11c***

Oil; IR (film) ν, cm^{-1}: 3500 (OH), 1735 (COO); ^{1}H NMR, δ, ppm: 3.97 (t, 2H, J 6.8, OCH_2), 3.77 (t, 2H, J 5.8, CH_2OH), 2.48 (t, 2H, J 5.8, CH_2CO), 1.56 (m, 2H, $CH_2$$CH_3$), 0.84 (t, 3H, J 7.6, CH_3); ^{13}C NMR, δ, ppm: 172.9 (s), 66.2 (t), 58.1 (t), 36.7 (t), 21.8 (t), 10.2 (q).

4.5.20. i*-Propyl 3-hydroxypropanoate* ***11d***[43]

Oil; IR (film) ν, cm^{-1}: 3500 (OH), 1735 (COO); ^{1}H NMR, δ, ppm: 5.11 (m, 1H, C*H*$(CH_3)_2$), 3.91 (t, 2H, J 5.6, CH_2OH), 2.59 (t, 2H, J 5.6, CH_2CO_2), 1.30 (d, 6H, J 6.3, 2CH_3); ^{13}C NMR, δ, ppm: 173.0 (s), 68.7 (d), 58.2 (t), 36.9 (t), 21.7 (q), 21.9 (q).

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