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Chemoenzymatic and yeast-catalysed synthesis of diastereomeric ethyl γ-phenyl and γ-(*n*-pyridyl)paraconates

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ABSTRACT

The synthesis of γ-phenyl and γ-(*n*-pyridyl)paraconates was accomplished by chemical reduction of their respective ketodiester precursors followed by cyclisation of the resulting hydroxy diester intermediates. The *cis*- and *trans*-lactones thus obtained were separated and separately subjected to enzymatic hydrolysis with HLAP. The *cis*-lactonic esters had enantiomeric excesses ranging from 94% to 99%, while for the *trans*-isomers the ee's ranged from 80% to 93%. The same ketodiester precursors were subjected to reduction with a series of yeasts. The absolute configuration of *trans*-(−)-2-pyridyl paraconic acid was assigned by means of X-ray analysis of its hydrobromide salt, while the absolute configurations of the other lactones were determined via analysis of their respective CD curves.

1. Introduction

5-Oxo-tetrahydro-3-furancarboxylic acid derivatives, commonly known as paraconic acid derivatives, constitute a small class of highly substituted γ-butyrolactones, which are metabolites of mosses, lichens and fungi.[1] The first member of the series, paraconic acid **1**, namely 5-oxo-tetrahydro-3-furancarboxylic acid (Fig. 1), is non-natural, but it is an important intermediate in the synthesis of A-factor,[2] an inducer of the biosynthesis of streptomycin in inactive mutants of *Streptomyces griseus*, first isolated by Khokhlov et al.[3] in 1973. The esters of paraconic acid have industrial applications, especially in the perfume industry and solvent production.[4]

Besides the presence of a carboxylic group at the β-carbon atom, naturally occurring paraconic acid derivatives also bear an alkyl chain at the γ-carbon atom and a methyl[1e,5] or a methylene[1f,g] group at the α-position. Examples of natural paraconic acid derivatives are rocellaric acid (−)-**2**,[6] phaseolinic acid (−)-**3**[7] and methylenolactocin (−)-**4a**[8] (Fig. 1), while terebic acid (−)-**5**, 2-methyl paraconic acid (−)-**6** and 4-methyl paraconic acid (−)-**7** and their corresponding esters are examples of non-natural compounds, whose syntheses have been accomplished in our laboratories.[9]

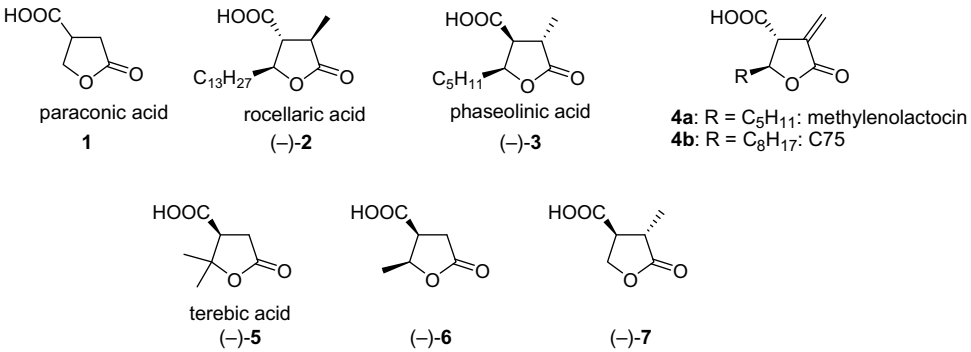

Figure 1. Examples of natural and non-natural paraconic acid derivatives.

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Recently, the unnatural compound **4b**, labelled C75,[10] was found to be an inhibitor of mammalian type I fatty acid synthase at the β-ketoacyl synthase (KAS) domain. It has been shown to have significant in vivo antitumour activity against human breast cancer xenografts.[11]

Within the frame of our studies on the synthesis of paraconic acid derivatives in diastereo- and enantiomerically pure forms, we turned our attention to γ-phenyl and γ-heteroaryl paraconic acids, which are uncommon in the literature. The heteroaryl group, 2-pyridyl, 3-pyridyl and 4-pyridyl were chosen: in fact these particular γ-butyrolactones, present as *cis*- and *trans*-diastereomers **8a**–**d** and **9a**–**d**, respectively (Fig. 2), seemed interesting as potentially biologically active compounds. For example, γ-pyridyl γ-butyrolactones have been shown to exhibit antihelminthic properties.[12]

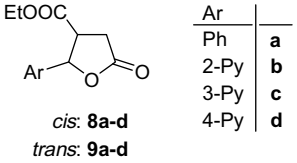


Figure 2. Ethyl γ-phenyl and γ-heteroarylparaconates **8a**–**d** and **9a**–**d**.

Within the biochemical methodologies which find frequent applications, also for the production of fine chemicals, are those which make use of microbial cells (e.g., yeast cells) as biocatalysts.[13] As an example, connected to the molecules presented in this work, the bioreduction of simple heteroaromatic ketones, such as 2-, 3- and 4-acetylpyridine by *Rhodococcus* sp.,[14] to the corresponding alcohols can be cited. These compounds are important building blocks for the synthesis of a wide range of pharmaceuticals, including the alkaloids akuamidine and heteroyohimidine,[15] and other biologically active compounds.

However, when the substrate contains different functionalities, the use of microbial whole cell systems can sometimes lead to undesired by-products, owing to the presence of various enzymes (located at the cytoplasm level or, more frequently, associated with some wall or membrane components) capable of catalysing side reactions, such as hydrolysis of an ester group,[16] accompanied by decarboxylation.[17] Moreover, one of the major problems in using whole cell yeasts is the presence of numerous alcohol dehydrogenases, which can be either (*R*)- or (*S*)-selective and hence make their use in organic synthesis difficult (especially when repeating experiments with a new batch of a yeast). For these reasons, the use of purified enzymes is often regarded as more profitable, in particular when hydrolases can be used, owing to their easy accessibility and low cost. This is the case for lactonic esters which have been successfully desymmetrised by this method.[5b,9a,b]

2. Results and discussion

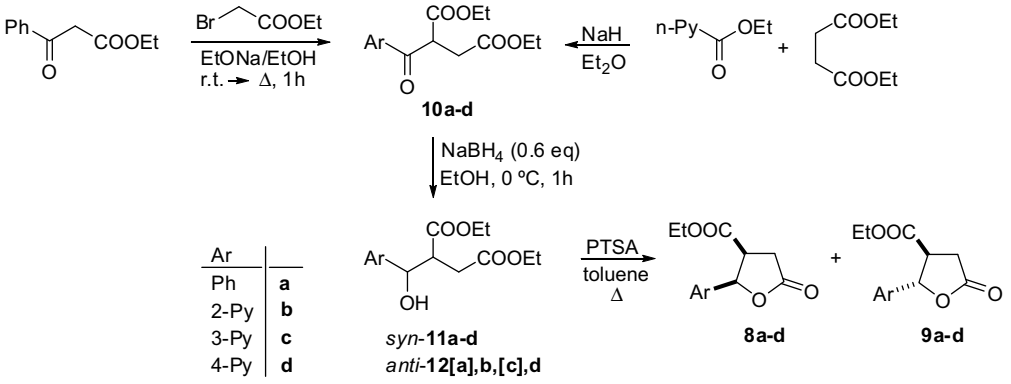
2.1. Synthesis of racemic substrates

The synthetic procedure leading to racemic *cis*- and *trans*-γ-aryl-β-ethoxycarbonyl-γ-butyrolactones **8a**–**d** and **9a**–**d**, respectively, involves the corresponding ketodiesters **10a**–**d** as intermediates (Scheme 1). As shown in Scheme 1, **10a**[18] was prepared by the reaction of ethyl benzoylacetate with ethyl bromoacetate under basic conditions,[19] while **10b**, **10c** and **10d** were synthesised by a Claisen condensation between diethyl succinate and the corresponding ethyl 2-, 3-, and 4-pyridylcarboxylates under basic conditions.[12] Subsequent reduction of the carbonyl group of **10a**–**d** with sodium borohydride afforded the corresponding hydroxy diesters **11a**–**d** and **12b**,**d**, as *syn*- and *anti*-diastereomers, respectively, in an admixture with their cyclisation products **8a**–**d** and **9a**–**d**. The reduction products **11a**–**d** and **12b**,**d** were not isolated but only identified in their respective crude reaction mixtures. Their cyclisation into the target molecules was accomplished either by refluxing in toluene, in the presence of PTSA as the catalyst, or by microwave irradiation. This latter procedure was necessary in particular for the 4-pyridyl derivative **11d** which was hard to cyclise.

The γ-phenyl and γ-heteroaryl-γ-butyrolactones prepared in this manner were mixtures of diastereomers, in which the *cis*-isomers **8a**–**d** prevailed over the *trans*-ones **9a**–**d**, their ratios, however, were dependent upon the time required for the lactonisation reaction to go to completion. After basic equilibration with DBU, all mixtures consisted of 80% of the *trans*-isomers and 20% of the *cis*-isomers. The mixtures were separated on column chromatography, and each isomer was subjected to enzymatic hydrolysis.

The geometry of the γ-phenyl derivatives **8a** and **9a** is known.[20] The assignment of the correct geometry to the other diastereomeric pairs followed from the results of the equilibration with DBU and from a comparison of the chemical shifts of H-2 and H-3 in their respective ^{1}H NMR spectra (Table 1). In all cases, the signals of the *trans*-substituted lactones resonated at higher field than those of the *cis*-isomers, in accordance with the literature data.[20,21]

Within the synthetic route leading to the desired optically active γ-lactones, the enantiodifferentiating process was applied either on the ketodiester precursors **10a**–**d**, by yeast reduction, or



Scheme 1. Synthesis of racemic **8a**–**d** and **9a**–**d**.

Table 1
Significant ^{1}H NMR data for the diastereomeric pairs **8a–d** and **9a–d**

γ-Lactone	H-2 δ, ppm	H-3 δ, ppm
8a	5.76	3.72
9a	5.66	3.32
8b	5.76	3.82
9b	5.75	3.80
8c	5.79	3.77
9c	5.67	3.34
8d	5.71	3.77
9d	5.66	3.26

on the final products **8a–d** and **9a–d**, by enzymatic hydrolysis of their ester group.

2.2. Yeast-catalysed bioreductions of ketodiesters 10a–d

The ketodiesters **10a–d** were submitted to reduction by using, as biocatalysts, both commercial *Saccharomyces cerevisiae* (Baker's yeast) biomass and by nine additional yeast strains, which are listed in Table 2.

As a general procedure, all the reductions reported in Table 2 were initially performed on a microscale level and the products were only identified spectroscopically. The most successful reaction was subsequently repeated at large-scale level in order to be able to purify the product.

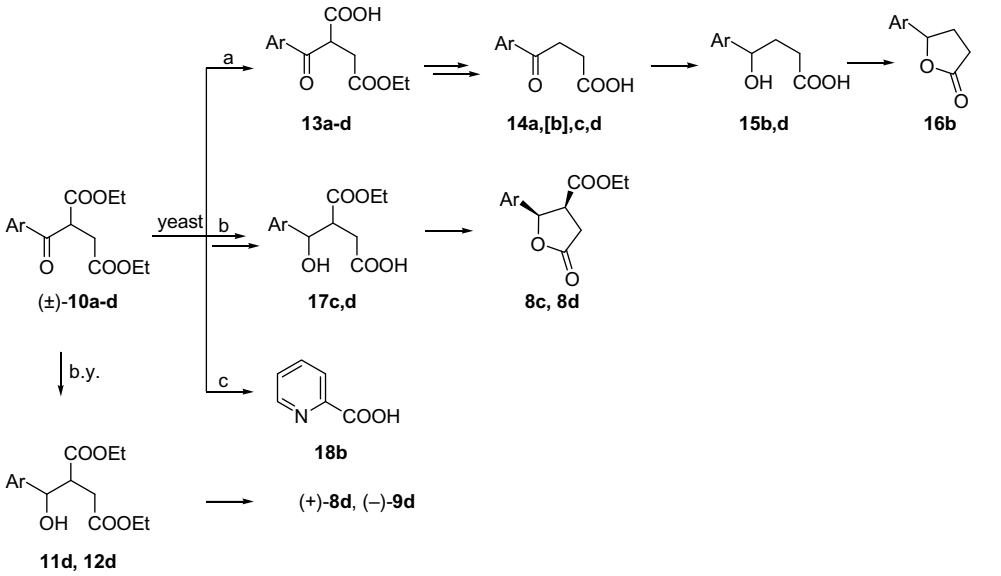
Reductions of the ketodiesters **10a–d** catalysed by the nine yeast strains were complicated by the high hydrolytic activity exhibited by all strains and by the decarboxylation reactions occurring on many intermediates. Scheme 2 summarises the possible pathways of the transformations involved, based on the nature of the products identified. It should be noted that the products containing a carboxy group have been identified as their respective methyl esters, although for the sake of clarity, they appear in Scheme 2 as carboxylic acid derivatives. In Section 4, they are given as methyl esters.

Pathway *a*, which is the most common for all yeast strains, involves the hydrolysis of the secondary ester group of **10a–d** to give the corresponding hemiesters **13a–d**, whose subsequent fate is decarboxylation and hydrolysis with formation of the γ-ketoacids **14a**,[**b**],**c**,**d**. In two cases, namely for **14b**, not identified, and **14d**, further reduction of the carbonyl group afforded the corresponding hydroxy acids **15b** and **15d**, the former also found as its lactonisation product, **16b** whose ee could not be evaluated.

Pathway *b*, which involves the hydrolysis of the primary ester group and reduction of the carbonyl group of the ketodiester, is followed only by compounds **10c** and **10d**, to give hydroxy

Table 2
Relative percentages of the products identified in the crude reaction mixtures after yeast-catalysed reduction of **10a–d**

Entry	Yeast strains	Products derived from yeast-catalysed reduction of **10a**, **10b**, **10c** and **10d** (rel. %)														
		10a		**10b**				**10c**				**10d**				
		13a	**14a**	**13b**	**15b**	**16b**	**18b**	**13c**	**14c**	**17c**	**8c**	**13d**	**14d**	**15d**	**17d**	**8d**
1	*Candida sake* DBVPG 6162	32	68	–	100	–	–	–	60	40	–	–	100	–	–	–
2	*Candida shehatae* DBVPG 6850	89	11	70	30	–	–	–	–	100	–	40	–	–	60 (*syn*)	–
3	*Debaryomyces nepalensis* DBVPG 7123	92	8	50	50	–	–	40	60	–	–	–	30	–	35 (*syn*)	35
4	*Hanseniaspora occidentalis* DBVPG 6798	78	22	–	–	–	–	–	100	–	–	–	37	63	–	–
5	*Kluyveromyces lodderae* DBVPG 6308	90	10	56	–	–	44	48	28	–	24	25	11	25	8 (*anti*) 25 (*syn*)	6
6	*Saccharomyces cerevisiae* DBVPG 6040	91	9	50	–	–	50	30	70	–	–	5	53	–	42 (*syn*)	–
7	*Saccharomyces exiguus* DBVPG 6469	14	86	100	–	–	–	20	80	–	–	–	24	34	16 (*syn*)	26
8	*Yarrowia lipolytica* DBVPG 6629	87	13	80	20	–	–	–	100	–	–	13	34	–	33 (*syn*)	20
9	*Yarrowia lipolytica* DBVPG 7070	82	18	33	47	20	–	–	100	–	–	–	34	30	36 (*syn*)	–



Scheme 2. General pathways of yeast-catalysed transformations.

hemiesters **17c** and **17d**, these can be easily converted into their corresponding ethyl γ-heteroarylparaconates (+)-**8c** and **8d**, both having a *cis*-geometry.

Finally, pathway *c* involves a retro Claisen reaction with the formation of the heteroaromatic acid and it is followed only by the 2-pyridyl derivative **10b**, which forms picolinic acid **18b**.

The detailed results for the transformations of **10a–d** are summarised in Table 2 for all yeast strains used. First of all it should be mentioned that no traces of the starting materials have been found in the crude reaction mixtures. This is probably due to the strong hydrolytic and decarboxylative activity of the yeasts.

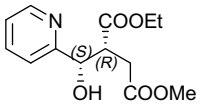
The best result was obtained with *Candida shehatae* DBVPG 6850 (entry 2), which chose route *b* exclusively; the resulting hydroxy hemiester **17c** was obtained with a *syn*-configuration and was isolated in 38% yield. Its chemical lactonisation furnished the corresponding γ-lactone (+)-**8c** with 99% ee. The same yeast, in the bioreduction of the 4-pyridyl derivative **10d**, led to the *syn*-hydroxy hemiester **17d** at 60% conversion.

As for the reduction catalysed by *S. cerevisiae* (Baker's yeast) biomass, only the ketodiester **10d** gave satisfactory results, leading to the corresponding hydroxy diesters *syn*-**11d** and *anti*-**12d** in a 7:3 ratio. After lactonisation, the corresponding γ-lactones (+)-**8d** with 99% ee and (−)-**9d** with 30% ee were obtained. Baker's yeast biomass reduction of **10c** led to the isolation of 10% of (+)-**8c** with 76% ee and 15% of (−)-**9c** with 94% ee, although in low yield, since the main product was the ethyl ketoester[22] of **14c** (75%). The remaining substrates **10a** and **10b** gave, as the only product, the corresponding ethyl ester[23] of **14a** (30%) and that[22] of **14b** (25%), respectively.

2.3. Enzymatic hydrolyses of lactones 8a–d and 9a–d

Horse liver acetone powder (HLAP) proved the most efficient for all the γ-substituted-γ-paraconic acid esters **8a–d** and **9a–d** prepared by chemical reduction of ketodiesters **10a–d** and subsequent acidic cyclisations (Scheme 1), since hydrolyses with Porcine Pancreatic Lipase (PPL) and α-chymotrypsin (α-CT) were not enantioselective. Prior to enzymatic hydrolyses, the amount of chemical hydrolysis was determined for all substrates. They were hydrolysed for about 10% when stirred in 0.1 M phosphate buffer at pH 7.4, for 12 h, giving a mixture of lactonic acid and lactonic ester, this latter was a consequence of the lactone ring opening and ring closure, under the reaction workup.

The workup of the enzymatic hydrolyses of the γ-pyridyl lactones deserves a comment. Usually, as in the case of the γ-phenyl-substituted γ-lactonic ester, after extraction at pH 7.4, which allows the isolation of the unreacted lactonic ester, the aqueous phase is acidified to pH 2, in order to separate the acidic product. In the present case, acidification was not possible, because of the presence of the pyridine ring. Therefore, the aqueous phase was evaporated, and the resulting crude reaction mixture was treated with trimethylsilyl chloride[24] in methanol in order to esterify the carboxy groups present. When the carboxy group was the result of ring opening, under the acidic esterification conditions, lactonisation partially occurred in all cases but one, namely in the case of the 2-pyridyl derivative **8b**, for which the hydroxy ethyl methyl diester **19b** was isolated (Fig. 3).



19b

Figure 3. Ethyl methyl hydroxydiester **19b**.

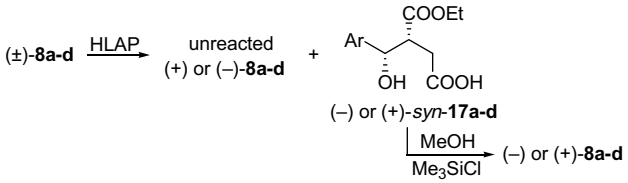
The results are summarised in Tables 3 and 4. As can be seen from Table 3, the *cis*-γ-lactones **8a–d** were hydrolysed regioselectively at the lactone group, whereas hydrolyses of the *trans*-γ-lactones **9a–d** (Table 4) were not regioselective, leading to mixtures of acids.

Since the reactions were stopped at high conversion values, both the unreacted lactonic esters **8a–d** and **9a–d** could be isolated with good enantiomeric excesses, the former ranging from 94% to 99%, and the latter from 80% to 93%. As a consequence, both the lactonic esters **8a–d** and **9a–d** (derived from lactonisation of the ring fission products *syn*-**17a–d** and *anti*-**17a–d**, respectively), and the lactonic acids **20a–d** (formed by hydrolysis of the ethoxycarbonyl group), were obtained with low enantiomeric excesses.

Only in the case of **9a** could the lactonic acid *trans*-(+)-**20a** and the lactonic ester (−)-**9a**, deriving from hydrolytic opening of the heterocycle, be easily separated (see Section 4). Interestingly, although the enantiomeric ratio was low, the two reactions exhibited opposite enantioselectivity: the enantiomer (+)-**9a** was hydrolysed at its ethoxycarbonyl group, whereas (−)-**9a** was hydrolysed at the lactonic group. When the same reaction was performed in a biphasic system (diisopropyl ether/buffer pH 7.4) hydrolysis took place at the alkoxycarbonyl group, although very slowly (8 d vs 1.5 h) and with poor selectivity ($E = 8$).

Finally, it should be underlined that enzymatic hydrolyses of *trans*-lactones proceeded faster than those of their *cis*-counterparts (1–3 h vs 16–39 h, respectively).

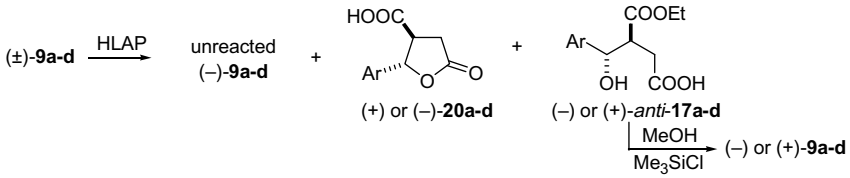
Table 3
Hydrolyses of compounds **8a–d** performed with HLAP



Substrate	E[a]	Conv.[b]	Time (h)	Unreacted ester		Lactonic ester from ring fission	
					ee[c] (%)		ee[c] (%)
8a	10	64	25	(+)-**8a**	94	(−)-**8a**	53
8b	13	65	16	(−)-**8b**	98	(+)-**8b**	53
8c	6	83	21	(+)-**8c**	99	(−)-**8c**	20
8d	12	68	39	(+)-**8d**	99	(−)-**8d**	46

[a] E-value was calculated from the formula containing the enantiomeric excesses of both the substrate and the product[25] $E = \frac{\ln[ee_P(1-ee_S)]}{\ln[ee_P(1+ee_S)]}$.
[b] Calculated.
[c] Determined by chiral HRGC.

Table 4
Hydrolyses of compounds **9a–d** performed with HLAP[a] at 80% conversion[b]



Substrate	Time (h)	Unreacted ester		Lactonic acid			Lactonic ester from ring fission		
			ee[c] (%)		ee[d] (%)	Rel. yield		ee[c] (%)	Rel. yield
9a	1.5	(–)-**9a**	93	(+)-**20a**	32	74	(–)-**9a**	37	26
9b	2	(–)-**9b**	80	**20b**[e]	47	75	(+)-**9b**	23	25
9c	1	(–)-**9c**	82	**20c**[e]	9	56	(+)-**9c**	73	44
9d	3	(–)-**9d**	89	**20d**[e]	11	40	(+)-**9d**	62	60

[a] *E*-values could not be determined, as the reactions were not regioselective.
[b] pH STAT value.
[c] Determined by chiral HRGC.
[d] Determined by chiral HRGC, after esterification as methyl esters.
[e] Not isolated.

2.4. Assignment of the absolute configuration of lactones 8 and 9

The ethyl γ-(2-pyridyl)paraconate (–)-**9b** with 80% ee was hydrolysed with 48% hydrobromide so that the corresponding hydrobromide salt (–)-**22b** was separated. Its single crystal X-ray analysis revealed the (2*R*,3*R*)-absolute configuration (Figs. 4 and 5).

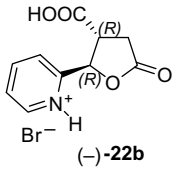


Figure 4. Hydrobromide salt (–)-**22b**.

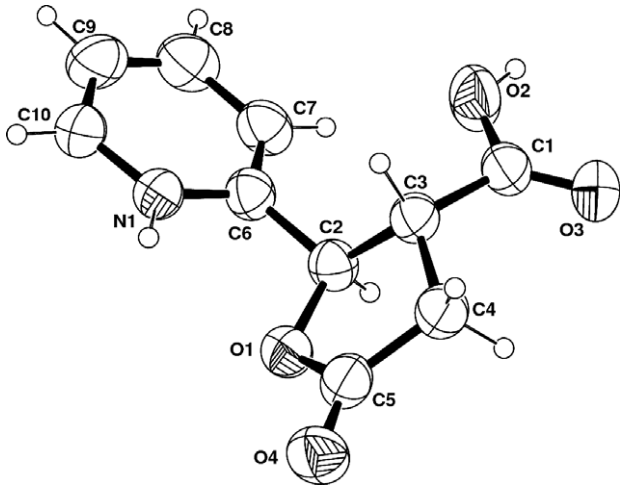


Figure 5. ORTEP diagram of the molecular cation of (–)-**22b**.

Since the ee of (–)-**22b** was only 80%, the X-ray structural determination was performed on two different crystals in order to guarantee the correctness of the assignment. The lactone conformation of the ring was an envelope type, with C(3) being displaced by 0.42 Å from the coplanar O(1)/C(2)/C(4)/C(5) atoms. The dihedral angle between the pyridine ring and the mean lactone plane was 58.1(1)°.

The absolute configuration of the other *trans*-lactones (–)-**9a**, (–)-**9c** and (–)-**9d** was determined by analysis of their CD spectra. In methanol they all showed a negative Cotton effect for the n→π* transition at about 210 nm (Fig. 6), as did (–)-**9b**. As a consequence, the (2*R*,3*R*)-configuration was attributed to all *trans*-lactones.

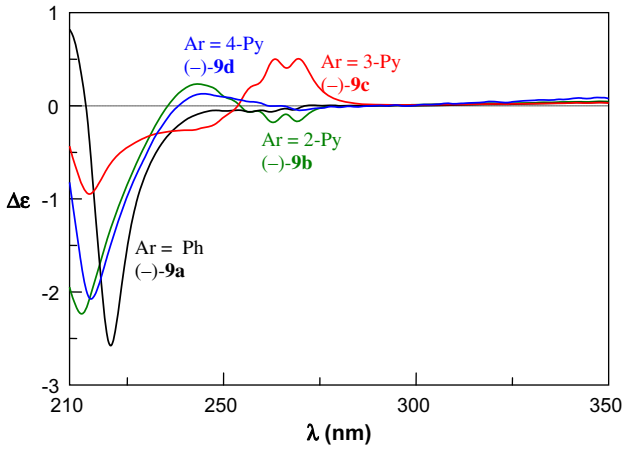


Figure 6. CD spectra of the *trans*-diastereomers (–)-**9a**, (–)-**9b**, (–)-**9c** and (–)-**9d**.

To determine the absolute configuration of *cis*-lactones **8a–d**, an equilibration reaction under basic conditions was performed on compound (+)-**8a**, which largely converted into its isomer (–)-**9a**. Since the reaction site was C-3, the absolute configuration of (+)-**8a** was assigned as (2*R*,3*S*). This attribution was then extended to the other *cis*-lactones (–)-**8b**, (+)-**8c** and (+)-**8d**, whose CD spectra exhibited the same positive Cotton effect at about 210 nm (Fig. 7).

3. Conclusions

The usual chemoenzymatic methodology was applied to the synthesis of enantiopure 2-phenyl and 2-(*n*-pyridyl) diastereomeric paraconic esters. This involves the chemical reduction of suitable β-ketodiesters followed by lactonisation and enzymatic resolution of the resulting lactonic esters by means of HLAP. A better route would be the direct diastereo- and enantioselective enzymatic reduction of the parent ketodiesters, followed by cyclisation of the resulting hydroxyl diester intermediates. With the availability of a series of yeasts, this strategy was adopted, with the aim of finding the most efficient one. This was found to be *C. shehatae*, which proved to only be effective on the ketodiester **10c**, eventu-

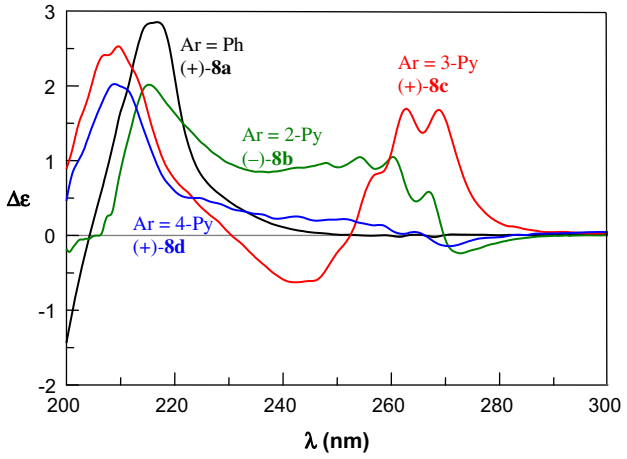


Figure 7. CD spectra of the *cis*-diastereomers (+)-**8a**, (−)-**8b**, (+)-**8c** and (+)-**8d**.

ally leading to lactone (+)-**8c** with high enantiomeric excess. Although this procedure may look difficult and tedious, the obtainment of the desired product is straightforward.

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT/IR 200 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton, 100.1 MHz for carbon), and on a Jeol EX-270 spectrometer (270 MHz for proton, 67.78 MHz for carbon) using deuteriochloroform as the solvent and tetramethylsilane as the internal standard. Chemical shifts are expressed in parts per million (δ). Optical rotations were determined on a Perkin Elmer Model 241 polarimeter. CD spectra were obtained on a Jasco J-710 spectropolarimeter (0.1 cm cell). GLC analyses were run on a Carlo Erba GC 8000 instrument and on a Shimadzu GC-14B instrument, the capillary columns being OV 1701 (25 m × 0.32 mm) (carrier gas He, 40 kPa, split 1:50, 150 °C for 2 min, 3 °C/min until 200 °C, 200 °C isotherm), a DiMePe β-cyclodextrin (25 m × 0.25 mm) (carrier gas He, 110 kPa, split 1:50, 150 °C isotherm) and a Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin (40 m × 0.25 mm) (carrier gas He, 180 kPa, split 1:100, 150 °C isotherm). Enzymatic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer, Copenhagen. Microwave reactions were performed in a CEM Discover MW reactor. Mass spectra were recorded on a ion trap instrument Finningan GCQ (70 eV). TLC's were performed on Polygram® Sil G/UV$_{254}$ silica gel pre-coated plastic sheets (eluent: light petroleum-ethyl acetate). Flash chromatography was run on silica gel for flash chromatography (BDH). Light petroleum refers to the fraction with bp 40–70 °C and ether to diethyl ether. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was purchased from Acros, and ethyl benzoylacetate, ethyl bromoacetate, diethyl succinate, ethyl 2-pyridylcarboxylate, ethyl 3-pyridylcarboxylate and ethyl 4-pyridylcarboxylate were purchased from Sigma–Aldrich.

4.2. Syntheses of racemic substrates

4.2.1. Diethyl 2-benzoylsuccinate 10a[18,19]

Ethyl benzoylacetate (9.6 g, 50 mmol) and ethyl bromoacetate (8.5 g, 106 mmol) were slowly added to a solution of EtONa (52 mmol) in EtOH (1.2 g of Na in 50 mL of EtOH) at 0 °C under stirring. The mixture was stirred overnight under an Ar atmosphere. Water was added until complete dissolution of NaBr, EtOH was removed under reduced pressure and the water solution was extracted with ether. The organic phase was washed with water containing a few drops of HCl and dried over anhydrous Na_2SO_4. Evaporation under reduced pressure afforded almost pure compound **10a** in quantitative yield. All spectroscopic data were in accordance with the literature.[18,19] MS, *m/z* 278 (4, M$^{+\cdot}$), 233 (15), 232 (15), 205 (52), 187 (33), 105 (100), 77 (34).

Ketodiesters **10b**, **10c** and **10d** were prepared according to the literature.[12]

4.2.2. Diethyl 2-pyridylcarbonylsuccinate 10b[12]

Yellow oil. IR (neat) 1732, 1716, 1579. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (1H, d, J = 4.8 Hz, Het H-6), 8.07 (1H, d, J = 7.7 Hz, Het H-3), 7.85 (1H, dt, J_1 = 7.7, J_2 = 1.1 Hz, Het H-4), 7.48 (1H, dd, J_1 = 4.8, J_2 = 7.7 Hz, Het H-5), 5.30 (1H, dd, part X of an ABX system, J_{AX} = 5.9, J_{BX} = 8.4 Hz, C*H*COOEt), 4.13 (4H, q, J = 7.3 Hz, 2 OC*H*$_2$CH$_3$), 3.12 (1H, part B of an ABX system, J_{AB} = 16.8, J_{BX} = 8.4 Hz, C*H*$_2$COOEt), 3.00 (1H, part A of an ABX system, J_{AB} = 16.8, J_{AX} = 5.9 Hz, C*H*$_2$COOEt), 1.22 (3H, t, J = 7.3 Hz, C*H*$_3$CH$_2$O), 1.13 (3H, t, J = 7.3 Hz, C*H*$_3$CH$_2$O). ^{13}C NMR, (100.1 MHz, $CDCl_3$) δ 195.7 (s, C=O), 171.2 (s, COO), 169.6 (s, COO), 152.1 (s, Het C-2), 148.9 (d, Het C-6), 136.9 (d, Het C-4), 127.3 (d, Het C-5), 122.4 (d, Het C-3), 61.3 (t, OCH$_2$CH$_3$), 60.9 (t, OCH$_2$CH$_3$), 48.5 (d, *C*HCOOEt), 32.9 (t, *C*H$_2$COOEt), 14.0 (q, CH$_3$), 13.8 (q, CH$_3$). MS, *m/z* 279 (7, M$^{+\cdot}$), 233 (56), 206 (29), 187 (33), 178 (19), 160 (100), 132 (14), 96 (12), 78 (14).

4.2.3. Diethyl 3-pyridylcarbonylsuccinate 10c[12]

Yellow oil. IR (neat) 1730, 1699, 1589. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (1H, d, J = 1.6 Hz, Het H-2), 8.81 (1H, dd, J_1 = 4.6, J_2 = 1.3 Hz, Het H-6), 8.31 (1H, dt, J_1 = 1.6, J_2 = 8.0 Hz, Het H-4), 7.45 (1H, dd, J_1 = 4.6, J_2 = 8.0 Hz, Het H-5), 4.82 (1H, dd, part X of an ABX system, J_{AX} = 5.9, J_{BX} = 8.6 Hz, C*H*COOEt), 4.15 (2H, q, J = 7.2 Hz, OC*H*$_2$CH$_3$), 4.13 (2H, q, J = 7.2 Hz, OC*H*$_2$CH$_3$), 3.20 (1H, part B of an ABX system, J_{AB} = 17.5, J_{BX} = 8.6 Hz, C*H*$_2$COOEt), 3.03 (1H, part A of an ABX system, J_{AB} = 17.5, J_{AX} = 5.9 Hz, C*H*$_2$COOEt), 1.23 (3H, t, J = 7.2, C*H*$_3$CH$_2$O), 1.16 (3H, t, J = 7.2, C*H*$_3$CH$_2$O). ^{13}C NMR, (100.1 MHz, $CDCl_3$) δ 193.2 (s, C=O), 179.9 (s, COO), 167.8 (s, COO), 153.5 (d, Het C-2), 149.9 (d, Het C-6), 136.1 (d, Het C-4), 131.5 (s, Het C-3), 123.5 (d, Het C-5), 61.9 (t, OCH$_2$CH$_3$), 61.0 (t, OCH$_2$CH$_3$), 49.5 (d, *C*HCOOEt), 32.9 (t, *C*H$_2$COOEt), 13.9 (q, CH$_3$), 13.7 (q, CH$_3$). MS, *m/z* 280 (4, M+1), 279 (5, M$^{+\cdot}$), 250 (31), 235 (83), 206 (100), 160 (34), 106 (84), 78 (63).

4.2.4. Diethyl 4-pyridylcarbonylsuccinate 10d[12]

Yellow oil. IR (neat) 1732, 1701, 1550. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (2H, d, J = 6.2 Hz, Het *H*-2 and *H*-6), 7.81 (2H, d, J_1 = 6.2 Hz, Het *H*-3 and *H*-5), 4.80 (1H, dd, part X of an ABX system, J_{AX} = 5.5, J_{BX} = 8.8 Hz, C*H*COOEt), 4.14 (2H, q, J = 7.1 Hz, OC*H*$_2$CH$_3$), 4.13 (2H, q, J = 7.1 Hz, OC*H*$_2$CH$_3$), 3.19 (1H, dd, part B of an ABX system, J_{AB} = 17.6, J_{BX} = 8.8 Hz, C*H*$_2$COOEt), 3.02 (1H, dd, part A of an ABX system, J_{AB} = 17.6, J_{AX} = 5.5 Hz, C*H*$_2$COOEt), 1.23 (3H, t, J = 7.1 Hz, C*H*$_3$CH$_2$O), 1.15 (3H, t, J = 7.1 Hz, C*H*$_3$CH$_2$O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 194.1 (s, C=O), 171.0 (s, COO), 167.7 (s, COO), 150.6 (2d, Het C-2 and C-6), 142.4 (s, Het C-4), 121.6 (2d, Het C-3 and C-5), 62.1 (t, OCH$_2$CH$_3$), 61.2 (t, OCH$_2$CH$_3$), 49.6 (d, *C*HCOOEt), 33.0 (t, *C*H$_2$COOEt), 14.0 (q, CH$_3$), 13.8 (q, CH$_3$). MS, *m/z* 279 (5, M$^{+\cdot}$), 233 (28), 206 (100), 187 (16), 177 (13), 160 (28), 152 (22), 106 (76), 78 (53).

4.3. Reduction of ketodiester 10a with sodium borohydride

Sodium borohydride (83 mg, 2.2 mmol) was slowly added to a solution of ketodiester **10a** (1.03 g, 3.7 mmol) in 7 mL of ethanol, under stirring at 0 °C for 1 h. At the end of the reaction, monitored by TLC, the solvent was evaporated under reduced pressure, water

was added, extracted with ether and dried over Na_2SO_4. Evaporation under reduced pressure afforded a mixture of the *syn*-hydroxyester **11a** and lactones **8a** and **9a** (27%, 60% and 13%, respectively, determined by ^{1}H NMR analysis of the crude reaction mixture). Conversion of **11a** into the corresponding lactone *cis*-**8a** was accomplished by refluxing in toluene with *p*-toluenesulfonic acid as the catalyst for 2 h. The mixture was extracted with a solution of $NaHCO_3$ (5%), dried over Na_2SO_4. Evaporation under reduced pressure afforded compounds **8a** and **9a** in the ratio of 4:1, respectively, as determined by ^{1}H NMR analysis. Equilibration of the mixture with DBU (2.5 equiv of DBU in CH_2Cl_2, stirred at room temperature for 24 h) changed the ratio to 1:4, respectively. The diastereomers **8a** and **9a** were then separated by flash chromatography (eluent: light petroleum–ethyl acetate, gradient from 9:1 to 4:1).

4.3.1. Diethyl (1′*R**,2*S**)-2-(hydroxymethylphenyl)butanedioate 11a

The following data were obtained from the ^{1}H NMR spectrum of the crude reaction mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.92 (1H, dd, J_1 = 5.1, J_2 = 7.3 Hz, C*H*OH), 4.17 (2H, q, J = 7.2 Hz, OC*H*$_2$CH$_3$), 4.08 (2H, q, J = 7.2 Hz, OC*H*$_2$CH$_3$), 3.20 (1H, m, C*H*COOEt), 3.19 (1H, d, J = 5.1 Hz, OH), 2.57 (1H, dd, part B of an ABX system, J_{AB} = 16.8, J_{BX} = 8.8 Hz, C*H*$_2$COOEt), 2.44 (1H, dd, part A of an ABX system, J_{AB} = 16.8, J_{AX} = 5.1 Hz, C*H*$_2$COOEt), 1.21 (6H, t, J = 7.2 Hz, 2C*H*$_3$CH$_2$O).

4.3.2. Ethyl *cis*-5-oxo-2-phenyltetrahydro-3-furancarboxylate 8a[20]

White solid; mp 74–76 °C, [lit.[20a] mp 74–75 °C], 0.52 g, 60% yield. All spectroscopic data are in accordance with the literature.[20] ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 175.1 (s, C-5), 169.6 (s, *C*OOEt), 135.2 (s, Ph), 128.8 (d, Ph), 128.4 (2d, Ph), 125.7 (2d, Ph), 81.2 (d, C-2), 61.2 (t, O*C*H$_2$CH$_3$), 46.5 (d, C-3), 31.6 (t, C-4), 13.5 (q, CH$_3$). HRGC (OV1701): t_R = 18.4 min.

4.3.3. Ethyl *trans*-5-oxo-2-phenyltetrahydro-3-furancarboxylate 9a[20]

Colourless oil, 0.37 g, 43% yield. All spectroscopic data are in accordance with the literature.[20] ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 174.2 (s, C-5), 170.7 (s, *C*OOEt), 138.0 (s, Ph), 128.9 (2d, Ph), 128.8 (d, Ph), 125.4 (2d, Ph), 82.2 (d, C-2), 61.9 (t, O*C*H$_2$CH$_3$), 48.7 (d, C-3), 32.2 (t, C-4), 14.1 (q, CH$_3$). HRGC (OV1701): t_R = 17.1 min.

4.4. Reduction of the ketodiester 10b with sodium borohydride

To a solution of 1.20 g (4.3 mmol) of ketodiester **10b** in 10 mL of ethanol, 82 mg of $NaBH_4$ was added. The mixture was stirred for 30 min in an ice bath. At the end of the reaction, water was added and the solution was evaporated to dryness. The crude reaction mixture, consisting of the *syn*- and *anti*-alcohols **11b** (67%) and **12b** (20%), respectively, together with lactone **8b** (13%), was refluxed in toluene in the presence of *p*-toluenesulfonic acid (PTSA) for 15 h. The mixture was extracted with 5% $NaHCO_3$ solution, and the organic phase was dried over Na_2SO_4. Evaporation under reduced pressure afforded compounds **8b** and **9b** in the ratio of about 3:1, respectively, determined by ^{1}H NMR analysis. Equilibration of the mixture with some drops of DBU in refluxing toluene changed the ratio into 1:4, respectively. The diastereomers **8b** and **9b** were separated by flash chromatography (eluent: light petroleum–ethyl acetate, gradient from 3:1 to 3:2).

4.4.1. Diethyl (1′*R**,2*S**)-2-[(2-pyridyl)hydroxymethyl]-butanedioate 11b

The following data were obtained from the ^{1}H NMR spectrum of the crude reaction mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (1H, d, J = 4.8 Hz, Het *H*-6), 7.70 (1H, dt, J_1 = 7.4, J_2 = 1.5 Hz, Het *H*-4), 7.31 (1H, d, J = 7.4 Hz, Het *H*-3), 7.23 (1H, ddd J_1 = 4.8, J_2 = 7.4 Hz, Het *H*-5), 5.02 (1H, d, J = 4.0 Hz, C*H*OH), 4.65 (1H, br s, OH), 4.12 (2H, q, J = 7.1 Hz, OC*H*$_2$CH$_3$), 4.06 (1H, q, J = 7.1 Hz, OC*H*$_2$CH$_3$), 4.05 (1H, q, J = 7.1 Hz, OC*H*$_2$CH$_3$), 3.45 (1H, m, C*H*COOEt), 2.77 (2H, part AB of an ABX system, J_{AB} = 17.2 Hz, C*H*$_2$COOEt), 1.24 (3H, t, J = 7.1 Hz, C*H*$_3$CH$_2$O), 1.10 (3H, t, J = 7.1 Hz, C*H*$_3$CH$_2$O).

4.4.2. Diethyl (1′*R**,2*R**)-2-[(2-pyridyl)hydroxymethyl]-butanedioate 12b

The following data were obtained from the ^{1}H NMR spectrum of the crude reaction mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (1H, d, J = 3.7 Hz, C*H*OH), 3.34 (1H, dt, J_1 = 4.2, J_2 = 9.9 Hz, C*H*COOEt), 2.22 (1H, dd, J_1 = 4.2, J_2 = 17.0 Hz, C*H*$_2$COOEt).

4.4.3. Ethyl *cis*-5-oxo-2-(2-pyridyl)tetrahydro-3-furancarboxylate 8b

Colourless oil, 0.30 g, 30% yield. IR (neat) 1776, 1726, 1595, 1579. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (1H, dd, J_1 = 4.4, J_2 = 0.7 Hz, Het H-6), 7.71 (1H, dt, J_1 = 7.9, J_2 = 1.6 Hz, Het H-4), 7.38 (1H, d, J = 7.9 Hz, Het *H*-3), 7.25 (1H, ddd, J_1 = 4.4, J_2 = 7.9, J_3 = 0.9 Hz, Het H-5), 5.76 (1H, d, J = 8.0 Hz, H-2), 3.82 (3H, m, OC*H*$_2$CH$_3$ + H-3), 3.24 (1H, dd, J_1 = 7.0, J_2 = 17.6 Hz, H-4), 2.82 (1H, dd, J_1 = 8.8, J_2 = 17.6 Hz, H-4), 0.90 (3H, t, J = 7.1, C*H*$_3$CH$_2$O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 175.1 (s, C-5), 169.6 (s, *C*OOEt), 155.6 (s, Het C-2), 149.3 (d, Het C-6), 136.7 (d, Het C-4), 123.5 (d, Het C-3), 121.4 (d, Het C-5), 80.9 (d, C-2), 61.1 (t, O*C*H$_2$CH$_3$), 45.4 (d, C-3), 31.5 (t, C-4), 13.7 (q, CH$_3$). MS, *m/z* 235 (15, M$^{+\cdot}$), 162 (100), 118 (51), 108 (12), 96 (11). HRGC (OV1701): t_R = 19.0 min.

4.4.4. Ethyl *trans*-5-oxo-2-(2-pyridyl)tetrahydro-3-furancarboxylate 9b

Colourless oil, 0.35 g, 35% yield. IR (neat) 1776, 1726, 1595, 1579. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (1H, dd, J_1 = 4.0, J_2 = 1.0 Hz, Het H-6), 7.75 (1H, dt, J_1 = 7.7, J_2 = 1.8 Hz, Het H-4), 7.43 (1H, d, J = 7.7 Hz, Het H-3), 7.30 (1H, m, Het H-5), 5.75 (1H, d, J = 5.1 Hz, H-2), 4.25 (1H, q, J = 7.2 Hz, OC*H*$_2$CH$_3$), 4.24 (1H, q, J = 7.2 Hz, OC*H*$_2$CH$_3$), 3.80 (1H, dt, J_1 = 5.1, J_2 = 8.3 Hz, H-3), 2.97 (2H, apparent d, J = 8.3 Hz, part AB of an ABX system, H-4), 1.29 (3H, t, J = 7.2 Hz, C*H*$_3$CH$_2$O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 174.5 (s, C-5), 171.2 (s, *C*OOEt), 156.2 (s, Het C-2), 149.7 (d, Het C-6), 136.9 (d, Het C-4), 123.6 (d, Het C-3), 121.4 (d, Het C-5), 82.0 (d, C-2), 61.7 (t, O*C*H$_2$CH$_3$), 45.3 (d, C-3), 31.4 (t, C-4), 13.9 (q, CH$_3$). MS, *m/z* 235 (10, M$^{+\cdot}$), 190 (34), 162 (100), 145 (15), 134 (51), 118 (66), 106 (23), 96 (13), 95 (13), 79 (16). HRGC (OV1701): t_R = 18.2 min.

4.5. Reduction of the ketodiester 10c with sodium borohydride

The reduction was carried out as indicated for ketodiester **10b**. After the usual workup, the mixture of the *syn*-alcohol **11c** (77%) and lactones **8c** (18%) and **9c** (5%) was refluxed in toluene with *p*-toluenesulfonic acid for 15h. Compounds **8c** and **9c** were obtained in the ratio of about 4:1, respectively. Equilibration of the mixture with DBU (some drops of DBU in toluene refluxed under stirring for 5 h) changed the ratio to 1:4, respectively. The diastereomers were then separated by flash chromatography (eluent: light petroleum–ethyl acetate 3:7).

4.5.1. Diethyl (1′*R**,2*S**)-2-[(3-pyridyl)hydroxymethyl]-butanedioate 11c

The following data were obtained from the ^{1}H NMR spectrum of the crude reaction mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (2H, m, Het H-2 and H-6), 7.71 (1H, d, J = 7.8 Hz, Het H-4), 7.30 (1H, m, Het H-5), 5.03 (1H, d, J = 6.6 Hz, C*H*OH), 4.16 (2H, q, J = 7.1 Hz, OC*H*$_2$CH$_3$), 4.09 (2H, q, J = 7.3 Hz, OC*H*$_2$CH$_3$), 3.23 (1H, m, C*H*COOEt), 2.59 (2H, part AB of an ABX system, C*H*$_2$COOEt), 1.23 (3H, t, J = 7.1 Hz, C*H*$_3$CH$_2$O), 1.20 (3H, t, J = 7.3 Hz, C*H*$_3$CH$_2$O).

4.5.2. Ethyl *cis*-5-oxo-2-(3-pyridyl)tetrahydro-3-furancarboxylate 8c

Colourless oil, 0.40 g, 40% yield. IR (neat): 1788, 1731. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (2H, m, Het H-2 and H-6), 7.64 (1H, dt, J_1 = 1.7, J_2 = 7.9 Hz, Het H-4), 7.32 (1H, dd, J_1 = 7.9, J_2 = 4.9 Hz, Het H-5), 5.79 (1H, d, J = 7.7 Hz, H-2), 3.86–3.68 (3H, m, OCH_2CH_3 + H-3), 3.09 (1H, dd, J_1 = 4.6, J_2 = 17.6 Hz, H-4), 2.86 (1H, dd, J_1 = 8.8, J_2 = 17.6 Hz, H-4), 0.90 (3H, t, J = 7.1 Hz, CH_3CH_2O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 174.3 (s, *C*-5), 169.4 (s, *C*OOEt), 150.2 (d, Het C-2), 147.5 (d, Het C-6), 133.3 (d, Het C-4), 131.2 (s, Het C-3), 123.3 (d, Het C-5), 78.9 (d, C-2), 61.5 (t, CH_2O), 46.3 (d, C-3), 31.5 (t, C-4), 13.6 (q, CH_3). MS, *m/z* 236 (7, M+1), 235 (10, $M^{+\cdot}$), 191 (100), 178 (14), 161 (30), 149 (30), 134 (30), 118 (23), 108 (57), 106 (31), 80 (18), 78 (17). HRGC (OV1701): t_R = 22.4 min.

4.5.3. Ethyl *trans*-5-oxo-2-(3-pyridyl)tetrahydro-3-furancarboxylate 9c

Colourless oil, 0.50 g, 50% yield. IR (neat): 1789, 1731, 1596. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (2H, br s, Het H-2 and H-6), 7.73 (1H, d, J = 7.7 Hz, Het H-4), 7.28 (1H, br s, Het H-5), 5.67 (1H, d, J = 7.7 Hz, H-2), 4.24 (2H, m, OCH_2CH_3), 3.34 (1H, dt, J_1 = 9.5, J_2 = 7.7 Hz, H-3), 2.97 (2H, part AB of an ABX system, J_{AB} = 17.9 Hz, H-4), 1.28 (3H, t, J = 7.1 Hz, CH_3CH_2O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 173.5 (s, C-5), 170.1 (s, *C*OOEt), 150.4 (d, Het C-2), 147.4 (d, Het C-6), 133.7 (s, Het C-3), 133.2 (d, Het C-4), 123.7 (d, Het C-5), 80.0 (d, C-2), 62.2 (t, CH_2O), 48.5 (d, C-3), 32.2 (t, C-4), 14.1 (q, CH_3). MS, *m/z* 236 (14, M+1), 235 (17, $M^{+\cdot}$), 193 (100), 191 (51), 177 (26), 161 (50), 147 (25), 134 (32), 121 (20), 108 (33), 106 (57), 91 (14), 89 (15), 78 (27). HRGC (OV1701): t_R = 21.2 min.

4.6. Reduction of the ketodiester 10d with sodium borohydride

Reduction was carried out as indicated for ketodiester **10b**. After the usual workup, the mixture of the *syn*-alcohol **11d** (71%) and the lactones **8d** (22%) and **9d** (7%) was obtained. When the reaction was carried out at room temperature, a mixture of the *syn*- and *anti*-alcohols **11d** (65%) and **12d** (21%), in admixture with the lactones **8d** (8%) and **9d** (6%), was obtained. Lactonisation was completed by microwave irradiation in toluene for 5 min at 120 °C with a power setting of 250 W. Under these conditions, the *cis*- and *trans*-lactones **8d** and **9d**, respectively, were obtained in a 2:3 ratio. Equilibration of the mixture with DBU (some drops of DBU in toluene, under reflux for 5 h) changed the ratio to 1:4, respectively. The diastereomers **8d** and **9d** were then separated by flash chromatography (eluent: light petroleum–ethyl acetate 3:7).

4.6.1. Diethyl (1′*R**,2*S**)-2-[(4-pyridyl)hydroxymethyl]-butanedioate 11d

The following data were obtained from the ^{1}H NMR spectrum of the crude reaction mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (2H, d, J = 5.0 Hz, Het H-2 and H-6), 7.28 (2H, d, J = 5.0 Hz, Het H-3 and H-5), 4.98 (1H, d, J = 5.6 Hz, C*H*OH), 4.12 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.11 (2H, q, J = 7.1 Hz, OCH_2CH_3), 3.24 (1H, m, C*H*COOEt), 2.63 (2H, part AB of an ABX system, CH_2COOEt), 1.24 (3H, t, J = 7.1 Hz, CH_2CH_3), 1.16 (3H, t, J = 7.1 Hz, CH_2CH_3). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 172.9 (s, *C*OOEt), 171.3 (s, *C*OOEt), 150.3 (s, C-4), 149.8 (2d, C-2 and C-6), 121.2 (2d, C-3 and C-5), 72.6 (d, *C*HOH), 61.4 (t, OCH_2CH_3), 61.0 (t, OCH_2CH_3), 47.7 (d, C-2), 33.2 (t, C-3), 14.1 (q, OCH_2CH_3), 13.9 (q, OCH_2CH_3).

4.6.2. Diethyl (1′*R**,2*R**)-2-[(4-pyridyl)hydroxymethyl]-butanedioate 12d

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.22 (1H, d, J = 4.0 Hz, C*H*OH), 3.20 (1H, m, C*H*COOEt), 2.73 (1H, dd, J_1 = 9.5, J_2 = 16.8 Hz, CH_2COOEt), 2.40 (1H, dd, J_1 = 4.2, J_2 = 16.8 Hz, CH_2COOEt).

4.6.3. Ethyl *cis*-5-oxo-2-(4-pyridyl)tetrahydro-3-furancarboxylate 8d

Colourless oil, 0.40 g, 40% yield. IR (neat): 1793, 1726. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (2H, br s, Het H-2 and H-6), 7.27 (2H, br s, Het H-3 and H-5), 5.71 (1H, d, J = 7.7 Hz, H-2), 3.84–3.70 (3H, m, OCH_2CH_3 and H-3), 2.95 (2H, part AB of an ABX system, J_{AB} = 17.6 Hz, H-4), 0.90 (3H, t, J = 7.2 Hz, CH_3CH_2O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 174.1 (s, COO), 169.2 (s, *C*OOEt), 149.8 (2d, C-2 and C-6), 144.3 (s, C-4), 120.6 (2d, C-3 and C-5), 79.4 (d, C-2), 61.6 (t, OCH_2CH_3), 46.1 (d, C-3), 31.8 (t, C-4), 13.6 (q, CH_3). MS, *m/z* 235 (37, $M^{+\cdot}$), 207 (18), 192 (44), 176 (18), 161 (100), 147 (19), 134 (21), 128 (33), 117 (21), 108 (72), 106 (45), 100 (35), 91 (14), 79 (28). HRGC (OV1701): t_R = 22.2 min.

4.6.4. Ethyl *trans*-5-oxo-2-(4-pyridyl)tetrahydro-3-furancarboxylate 9d

Colourless oil, 0.50 g, 50% yield. IR (neat): 1792, 1732, 1602. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (2H, br s, Het H-2 and H-6), 7.31 (2H, d, J = 5.5 Hz, Het H-5 and H-3), 5.66 (1H, d, J = 7.3 Hz, H-2), 4.28 (1H, q, J = 7.0 Hz, OCH_2CH_3), 4.27 (1H, q, J = 7.0 Hz, OCH_2CH_3), 3.26 (1H, dt, J_1 = 7.3, J_2 = 9.0 Hz, H-3), 2.97 (2H, part AB of an ABX system, J_{AB} = 17.9 Hz, H-4), 1.32 (3H, t, J = 7.0 Hz, CH_3CH_2O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 173.4 (s, COO), 170.2 (s, *C*OOEt), 150.4 (2d, C-2 and C-6), 147.1 (s, C-4), 119.9 (2d, C-3 and C-5), 80.1 (d, C-2), 62.3 (t, OCH_2CH_3), 48.1 (d, C-3), 31.8 (t, C-4), 14.1 (q, CH_3). MS, *m/z* 235 (49, $M^{+\cdot}$), 206 (22), 194 (46), 193 (53), 178 (19), 161 (100), 147 (31), 132 (20), 117 (17), 107 (15), 105 (45), 79 (19). HRGC (OV1701): t_R = 21.1 min.

4.7. General procedure for the reduction of ketodiesters 10a–d catalysed by Baker's yeast biomass

Commercial *S. cerevisiae* (Baker's yeast) biomass (4 g) in 8 mL of water was preincubated at 50 °C for 30 min, then the ketodiester (0.36 mmol) was added and the mixture stirred at room temperature. The course of the reduction was monitored by HRGC. At the end of the reaction, 5% $NaHCO_3$ solution was added to deprotonate the pyridine rings after which the broth was extracted with diethyl ether. The organic phase was dried and evaporated. When the resulting hydroxy diesters did not cyclise in the reaction medium, the crude reaction mixture was refluxed in toluene with *p*-toluenesulfonic acid added and the product was purified by flash chromatography.

After 16 days and 30% conversion, ketodiester **10a** afforded the corresponding ethyl ester[23] of **14a.** Similarly, ketodiester **10b** afforded, after 16 days and 25% conversion, the corresponding ethyl ester[22] of **14b.** Conversely, compound **10c** was completely transformed after 5 days, giving the decarboxylated ethyl ester[22] of **14c** (75%), and lactones (+)-**8c** (10%) with 76% ee and (−)-**9c** (15%) with 94% ee, the latter two after lactonisation of the crude reaction mixture.

Bioreduction of ketodiester **10d** afforded, after 6 days and lactonisation of the crude reaction mixture, lactones (+)-**8d** (70%) with 99% ee and (−)-**9d** (30%) with 30% ee.

4.8. General procedure for the reduction of the ketodiesters 10a–d catalysed by yeast strains

The following procedure is described in detail for the pyridine derivatives **10b**–**d**, for which particular attention to the pH values must be paid, owing to a possible protonation of the nitrogen atom.

The yeast strains *Candida shehatae* DBVPG 6850, *Candida sake* DBVPG 6162, *Saccharomyces exiguus* DBVPG 6469, *Hanseniaspora occidentalis* DBVPG 6798, *Kluyveromyces lodderae* DBVPG 6308, *S. cerevisiae* DBVPG 6040, *Debaryomyces nepalensis* DBVPG 7123, *Yarrowia lipolytica* DBVPG 6629 and *Yarrowia lipolytica* DBVPG 7070

were used. All strains are conserved in the Industrial Yeast Collection DBVPG (www.agr.unipg.it/dbvpg). Aliquots (100 mL) of 48 h yeast cell suspensions (calibrated to A_{580} = 0.5) were used to inoculate 25 mL of minimal medium (yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1%). After 48 h of incubation on a rotary shaker (110 rpm) at 25 °C, the cells were harvested by centrifugation (4000*g*), washed, frozen at −80 °C and lyophilised. A preliminary screening was performed on 15 mg of the ketodiesters **10a–d** using 400 mg of lyophilised cells suspended in 12.5 mL phosphate buffer (pH 6.5) and incubated on a rotary shaker (110 rpm), at 25 °C, for 10 days. The reaction mixture was treated with 5% $NaHCO_3$ to deprotonate the pyridine ring, and the solution was extracted with ether, the ether phase was dried with sodium sulfate and analysed by NMR. The ^{1}H NMR spectrum of the crude mixtures, obtained from the ethereal extracts, indicated that no ester group was present and therefore the organic molecules should be present as salts. The mother liquors were centrifuged and the water phase, separated from the cells, was evaporated to dryness. The solid obtained was suspended in MeOH (0.4 mL), after which trimethylsilyl chloride (TMSiCl, 0.2 mL) was added and the solution was stirred overnight, in order to esterify all the carboxylic groups present. Methanol was evaporated, after which a solution of 5% $NaHCO_3$ was added, again to deprotonate the pyridine ring, and the residue was treated with ether, to extract the products in neutral forms. The crude reaction mixtures were analysed by ^{1}H NMR.

Compounds **13a**, **14a** and **16b** were identified as such, while **13b**, **13c**, **13d**, **14c**, **14d**, **15b**, **15d**, **17c**, **17d** and **18b** were identified as their respective methyl esters. Methyl esters of **13a–d** and **17d** were identified by comparison of their ^{1}H NMR spectra with their corresponding diethyl esters, ^{1}H NMR data of compounds **14a**[23] and **16b**[26] were in accordance with the literature.

4.8.1. Methyl ester of 4-oxo-4-(3-pyridyl)butanoic acid 14c

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (1H, d, J = 1.8 Hz, Het H-2), 8.80 (1H, dd, J_1 = 1.5, J_2 = 4.8 Hz, Het H-4), 8.25 (1H, dt, J_1 = 2.0, J_2 = 8.1 Hz, Het H-6), 7.43 (1H, dd, J_1 = 4.8, J_2 = 8.1 Hz, Het H-5), 3.75 (3H, s, OCH_3), 3.33 (2H, t, J = 6.6 Hz, CH_2), 2.81 (2H, t, J = 6.6 Hz, CH_2).

4.8.2. Methyl ester of 4-oxo-4-(4-pyridyl)butanoic acid 14d

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (2H, dd, J_1 = 1.6, J_2 = 4.5 Hz, Het H-2 + H-6), 7.76 (2H, dd, J_1 = 1.8, J_2 = 4.5 Hz, Het H-3 + H-5), 3.72 (3H, s, OCH_3), 3.31 (2H, t, J = 6.6 Hz, CH_2), 2.80 (2H, t, J = 6.6 Hz, CH_2).

4.8.3. Methyl ester of 4-hydroxy-4-(2-pyridyl)butanoic acid 15b

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (1H, d, J = 4.4 Hz, Het H-6), 7.70 (1H, dt, J_1 = 7.7, J_2 = 1.7 Hz, Het H-4), 7.30 (1H, d, J = 7.7 Hz, Het H-3), 7.22 (1H, m, Het H-5), 4.80 (1H, dd, J_1 = 3.7, J_2 = 8.1 Hz, C*H*OH), 3.67 (3H, s, OCH_3), 2.56 (1H, m, H-2), 2.44 (1H, m, H-2), 2.22 (1H, m, H-3), 1.95 (1H, m, H-3).

4.8.4. Methyl ester of 4-hydroxy-4-(4-pyridyl)butanoate 15d

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (2H, d, J = 6.1 Hz, Het H-2 and H-6), 7.30 (2H, d, J_1 = 6.1 Hz, Het H-3 and H-5), 4.81 (1H, dd, J_1 = 4.2, J_2 = 7.7 Hz, C*H*OH), 3.69 (3H, s, OCH_3), 2.48 (2H, dt, J_1 = 4.2, J_2 = 6.9 Hz, H-2), 2.05 (2H, m, H-3).

4.8.5. Methyl ester of (3*S*,4*R*)-4-hydroxy-3-ethoxycarbonyl-4-(3-pyridyl)butanoic acid 17c

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (2H, m, Het H-2 + H-6), 7.71 (1H, d, J = 7.8 Hz, Het H-4), 7.30 (1H, dd, J_1 = 4.6, J_2 = 7.8 Hz, Het H-5), 5.03 (1H, d, J = 6.6 Hz, C*H*OH), 4.16 (2H, q, J = 7.1 Hz, OCH_2CH_3), 3.65 (3H, s, OCH_3), 3.23 (1H, m, C*H*COOEt), 2.59 (2H, part AB of an ABX system, CH_2COOMe), 1.20 (3H, t, J = 7.1 Hz, OCH_2CH_3).

4.9. Bioreduction of the ketodiester 10c catalysed by *C. shehatae* DBVPG 6850

Ketodiester **10c** (0.2 g, 0.7 mmol) and 5.44 g of lyophilised cells of *C. shehatae* DBVPG 6850 (obtained as reported above) suspended in 170 mL phosphate buffer (pH 6.5) were incubated on a rotary shaker (110 rpm) at 25 °C for 10 days. After incubation, the mixture was centrifuged. The water separated from the cells was washed three times with water. Water was cooled at −80 °C and lyophilised. To the crude reaction mixture (3.58 g), toluene (50 mL) and PTSA were added, after which the mixture was refluxed for 10 h, washed with 5% $NaHCO_3$ and dried over Na_2SO_4. After elimination of the solvent, the residue (0.063 g) was purified by flash chromatography and (+)-**8c** (0.04 g, 0.17 mmol, 24% yield) with 99% ee was isolated.

4.10. Enzymatic hydrolyses of the *cis*- and *trans*-γ-phenyl lactones 8a and 9a

Lactone **8a** or **9a** (0.160 g, 0.68 mmol) was dissolved in 2 mL of acetone, after which 0.1 M phosphate buffer at pH 7.4 (18 mL) and HLAP (0.150 g) were added under stirring. The course of the reaction was monitored with a pH-STAT, with continuous addition of 1.0 N NaOH. At high conversion values, the reaction mixtures were extracted with ether to separate the unreacted lactone (+)-**8a** (0.055 g, 35% yield, 94% ee) or (−)-**9a** (0.021 g, 13% yield, 93% ee). The mother liquors were acidified with 5% HCl to pH 2 and extracted with ether. From the hydrolysis of **8a**, the corresponding hydroxy half-ester *syn*-**17a** (0.098 g, 57% yield, 53% ee) was obtained. From the hydrolysis of **9a** a mixture of lactonic acid (+)-**20a** and lactonic ester (−)-**9a**, formed by ring fission, was obtained. This mixture was dissolved in ether, after which the organic phase was washed with a 5% solution of $NaHCO_3$, evaporation of the ether gave (−)-**9a** (0.021 g, 13% yield, 37% ee). The aqueous phase was filtered on Celite, acidified with 20% HCl to pH 2 and extracted with ether. Evaporation of the solvent gave (+)-**20a** (0.052 g, 37% yield, 32% ee).

4.10.1. Ethyl (2*R*,3*S*)-(+)-*cis*-5-oxo-2-phenyltetrahydro-3-furancarboxylate 8a

White solid, mp 102–103 °C, 0.056 g, 35% yield; $[\alpha]_D^{25} = +10.0$ (*c* 0.54, CH_3OH), $\Delta\varepsilon_{217}$ = +2.9 (CH_3OH), 94% ee, determined by chiral HRGC: (β-CDX): t_R = 151.3 min for the (−)-(2*S*,3*R*)-enantiomer, and t_R = 158.8 min for (+)-(2*R*,3*S*)-enantiomer.

4.10.2. (3*R*,4*S*)-4-Hydroxy-3-ethoxycarbonyl-4-phenylbutanoic acid 17a

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (5H, m, Ph), 5.0 (2H, vbr s, OH), 4.93 (1H, d, J = 7.3 Hz, C*H*OH), 4.16 (2H, q, J = 7.1 Hz, OCH_2CH_3), 3.18 (1H, m, C*H*COOEt), 2.54 (2H, part AB of an ABX system, J_{AB} = 17.2 Hz, CH_2COOEt), 1.20 (3H, t, J = 7.1 Hz, CH_3CH_2O). The hydroxy half ester *syn*-**17a** rapidly converted into (−)-**8a** on standing in $CDCl_3$, 53% ee by chiral HRGC.

4.10.3. Ethyl (2*R*,3*R*)-(−)-*trans*-5-oxo-2-phenyltetrahydro-3-furancarboxylate 9a

Colourless oil, 0.020 g, 13% yield, $[\alpha]_D^{25} = -55.3$ (*c* 0.58, CH_3OH), $\Delta\varepsilon_{221}$ = −2.7 (CH_3OH), 93% ee determined by chiral HRGC: (β-CDX): t_R = 111.5 min for the (+)-(2*S*,3*S*)-enantiomer, and t_R = 114.7 min for the (−)-(2*R*,3*R*)-enantiomer.

4.10.4. (2*S*,3*S*)-(+)-*trans*-5-Oxo-2-phenyltetrahydro-3-furancarboxylic acid 20a

White solid, mp 116–119 °C, 0.060 g, 37% yield; IR (Nujol): 3429, 1743. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (5H, m, Ph), 5.71

(1H, d, J = 6.7 Hz, H-2), 4.94 (1H, br s, O*H*), 3.40 (1H, ddd, J_1 = 6.7, J_2 = 8.3, J_3 = 9.5 Hz, H-3), 3.03 (1H, dd, J_1 = 8.3, J_2 = 17.9 Hz, H-4), 2.94 (1H, dd, J_1 = 9.5, J_2 = 17.9 Hz, H-4). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 175.3 (s, COO), 174.0 (s, COO), 137.7 (s, Ph), 129.0 (2d, Ph), 125.4 (d, Ph), 125.3 (2d, Ph), 82.0 (d, *C*-2), 48.2 (d, *C*-3), 31.9 (t, *C*-4). MS, *m/z* 206 (22, $M^{+\cdot}$), 164 (65), 160 (100), 145 (25), 132 (29), 115 (26), 105 (62), 79 (36), 77 (36). $[\alpha]_D^{25} = +19$ (*c* 0.2, CH_3OH), 32% ee. The enantiomeric excess of the acid **20a** was determined on its methyl ester.[27,21] Compound (+)-**20a** (2 mg) was dissolved in MeOH (1 mL) and trimethylsilyl chloride (TMSiCl, 20 μL) was added, the solution was stirred overnight, the solvent evaporated, after which CH_2Cl_2 (1 mL) was added to the residue. The solution was filtered and analysed by chiral HRGC: (β-CDX): t_R = 88.2 min for the (+)-(2*S*,3*S*) enantiomer, and t_R = 91.7 min for the (−)-(2*R*,3*R*) enantiomer.

4.11. Enzymatic hydrolyses of the *cis*- and *trans*-lactones 8b–d and 9b–d

The appropriate lactone (0.160 g, 0.68 mmol) was dissolved in 2 mL of acetone, 0.1 M phosphate buffer at pH 7.4 (18 mL) and HLAP (0.150 g) were added under stirring. The course of the reaction was monitored with a pH-STAT, with continuous addition of 1.0 M NaOH. At high conversion values, the reaction mixtures were extracted with ether to separate the unreacted lactones, namely (−)-**8b**, (+)-**8c**, (+)-**8d** and (−)-**9b**, (−)-**9c** and (−)-**9d**, respectively. The mother liquors were filtered on Celite, evaporated to dryness and the residue was treated with MeOH (8 mL) and trimethylsilyl chloride (TMSiCl, 0.4 mL), under stirring overnight. The solvent was evaporated, a 5% solution of $NaHCO_3$ was added, and the mother liquors were extracted with ether. The mixture of lactonic methyl and ethyl esters obtained was analysed by chiral HRCG. Only in the hydrolysis of **8b** the alcoholic precursor **19b** could be fully characterised as the ethyl methyl diester.

4.11.1. Ethyl, methyl (1′*S*,2*R*)-2-(2-pyridyl)hydroxymethylbutandioate 19b

Oil, 0.064 g, 40% yield. IR (Nujol) 3462, 1793, 1726, 1596. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (1H, d, J = 4.8 Hz, Het H-6), 7.70 (1H, t, J = 7.0 Hz, Het H-4), 7.31 (1H, d, J = 8.0 Hz, Het H-3), 7.24 (1H, m, Het H-5), 5.02 (1H, d, J = 3.7 Hz, C*H*OH), 4.68 (1H, br s, O*H*), 4.05 (2H, q, J = 7.0 Hz, O*C*H_2CH$_3$), 3.67 (3H, s, O*C*H_3), 3.46 (1H, m, *CH*COOEt), 2.70 (2H, part AB of an ABX system, J_{AB} = 17.2 Hz, CH_2COOEt), 1.09 (3H, t, J = 7.0 Hz, CH_3CH$_2$O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 172.5 (s, COO), 172.1 (s, COO), 158.9 (s, Het C-2), 148.1 (d, Het C-6), 136.7 (d, Het C-4), 122.8 (d, Het C-3), 121.0 (d, Het C-5), 72.9 (d, *C*HOH), 60.7 (t, O*C*H$_2$CH$_3$), 51.8 (q, CH$_3$), 48.0 (d, *C*HCOOEt), 32.5 (t, C*H*$_2$COOMe), 13.9 (q, CH$_3$). On standing in $CDCl_3$ solution compound **19b** easily converted into (+)-**8b** with 53% ee.

4.11.2. Ethyl (2*R*,3*S*)-(−)-*cis*-5-oxo-2-(2-pyridyl)tetrahydro-3-furancarboxylate 8b

Colourless oil, 0.056 g, 35% yield. $[\alpha]_D^{25} = -7.3$ (*c* 0.49, CH_3OH), $\Delta\varepsilon_{215}$ = +2.0 (CH_3OH), 98% ee, determined by chiral HRGC: (β-CDX): t_R = 161.8 min for the (+)-(2*S*,3*R*)-enantiomer, and t_R = 175.8 min for the (−)-(2*R*,3*S*)-enantiomer.

4.11.3. Ethyl (2*R*,3*S*)-(+)-*cis*-5-oxo-2-(3-pyridyl)tetrahydro-3-furancarboxylate 8c

Colourless oil, 0.056 g, 35% yield. $[\alpha]_D^{25} = +14.7$ (*c* 0.6, CH_3OH), $\Delta\varepsilon_{209}$ = +2.6 (CH_3OH), 99% ee, determined by chiral HRGC: (β-CDX): t_R = 276.4 min for the (−)-(2*S*,3*R*)-enantiomer, and t_R = 291.6 min for the (+)-(2*R*,3*S*)-enantiomer.

From the mother liquors (−)-**8c** with 20% ee was obtained.

4.11.4. Ethyl (2*R*,3*S*)-(+)-*cis*-5-oxo-2-(4-pyridyl)tetrahydro-3-furancarboxylate 8d

Colourless oil, 0.016 g, 10% yield. $[\alpha]_D^{25} = +12.6$ (*c* 0.35, CH_3OH), $\Delta\varepsilon_{210}$ = +2.0 (CH_3OH), 99% ee, determined by chiral HRGC: (β-CDX): t_R = 287.7 min for the (−)-(2*S*,3*R*)-enantiomer, and t_R = 319.6 min for the (+)-(2*R*,3*S*)-enantiomer.

From the mother liquors, (−)-**8d** with 46% ee was obtained, in admixture with (−)-**9d** with 12% ee in about 1:1 ratio, after lactonisation under microwave irradiation.

4.11.5. Ethyl (2*R*,3*R*)-(−)-*trans*-5-oxo-2-(2-pyridyl)tetrahydro-3-furancarboxylate 9b

Colourless oil, 0.064 g, 40% yield. $[\alpha]_D^{25} = -52.8$ (*c* 0.49, CH_3OH), $\Delta\varepsilon_{213}$ = −2.3 (CH_3OH). Compound (−)-**9b** was obtained with 80% ee determined by chiral HRGC: (γ-CDX): t_R = 150.2 min for the (−)-(2*R*,3*R*)-enantiomer, and t_R = 153.2 min for the (+)-(2*S*,3*S*)-enantiomer.

From the mother liquors treated as indicated above, a mixture of (+)-**9b** with 23% ee and the corresponding methyl ester with 47% ee was obtained.

4.11.6. Ethyl (2*R*,3*R*)-(−)-*trans*-5-oxo-2-(3-pyridyl)tetrahydro-3-furancarboxylate 9c

Colourless oil, 0.038 g, 24% yield. Compound (−)-**9c** was obtained with 82% ee. From the mother liquors, a mixture of (+)-**9c** with 73% ee and the corresponding methyl ester with 9% ee was obtained.

Lactonic ester (−)-**9c** with 82% ee was submitted to further enzymatic resolution in order to increase the enantiomeric excess. $[\alpha]_D^{25} = -51.6$ (*c* 0.5, CH_3OH), $\Delta\varepsilon_{215}$ = −1.0 (CH_3OH), 94% ee, determined by chiral HRGC: (β-CDX): t_R = 220.7 min for the (+)-(2*S*,3*S*)-enantiomer, and t_R = 226.8 min for the (−)-(2*R*,3*R*)-enantiomer.

4.11.7. Ethyl (2*R*,3*R*)-(−)-*trans*-5-oxo-2-(4-pyridyl)tetrahydro-3-furancarboxylate 9d

Colourless oil, 0.044 g, 27% yield. $[\alpha]_D^{25} = -48.7$ (*c* 0.23, CH_3OH), $\Delta\varepsilon_{215}$ = −2.2 (CH_3OH). Compound (−)-**9d** was obtained with 89% ee, determined by chiral HRGC: (β-CDX): t_R = 208.1 min for the (+)-(2*S*,3*S*)-enantiomer, and t_R = 216.3 min for the (−)-(2*R*,3*R*)-enantiomer.

From the mother liquors, a mixture of (+)-**9d** with 62% ee and the corresponding methyl ester with 11% ee was obtained.

4.12. (2*R*,3*R*)-(−)-*trans*-5-Oxo-2-(2-pyridyl)tetrahydro-3-furancarboxylic acid hydrobromide (−)-22b

To compound (−)-**9b** (0.1 g, 0.4 mmol) with 80% ee, water (2 mL) and 48% HBr (0.150 mL) were added, after which the solution was refluxed for 30 min and the solvent was evaporated. After three weeks at room temperature, crystals of pure (−)-**22b** were collected and analysed by X-ray crystallography. The ^{1}H NMR spectrum of the crude reaction mixture showed that acidic conditions completely hydrolysed the ester function and opened the lactone ring for 20%, giving the corresponding hydroxy diacid which was identified spectroscopically.

Compound (−)-**22b**: Colourless crystals, mp 149–152 °C, $[\alpha]_D^{25} = -23.4$ (*c* 0.47, H_2O) for the 4:1 mixture of (−)-**22** and the corresponding hydroxy diacid. ^{1}H NMR (400 MHz, D_2O) δ 8.59 (1H, d, J = 5.5 Hz, Het H-6), 8.42 (1H, t, J = 8.0 Hz, Het H-4), 7.97 (1H, d, J = 8.0 Hz, Het H-3), 7.85 (1H, t, J = 6.8 Hz, Het H-5), 5.93 (1H, d, J = 8.4 Hz, H-2), 3.64 (1H, q, J = 8.8 Hz, H-3), 2.95 (2H, part AB of an ABX system, J_{AB} = 17.8 Hz, H-4). ^{13}C NMR (100.1 MHz, D_2O + one drop of CD_3OD as the reference) δ 177.2 (s, COO), 173.7 (s, COO), 152.0 (s, Het C-2), 147.9 (d, Het C-4), 143.0 (d, Het C-6), 127.8 (d, Het C-5), 125.8 (d, Het C-3), 78.7 (d, C-2), 47.0 (d, C-3), 31.9 (t, C-4).

4.12.1. (1′*R*,2*R*)-2-[(2-Pyridyl)hydroxymethyl]butanedioic acid hydrobromide

^{1}H NMR (400 MHz, D_2O) δ 8.53 (1H, d, J = 5.8 Hz, Het H-6), 8.42 (1H, t, J = 8.0 Hz, Het H-4), 7.90 (1H, d, J = 8.0 Hz, Het H-3), 7.82 (1H, t, J = 6.9 Hz, Het H-5), 5.49 (1H, d, J = 4.0 Hz, C*H*OH), 3.27 (1H, m, dt, J_1 = 4.7, J_2 = 9.4 Hz, C*H*COOH), 2.62 (1H, dd, J_1 = 9.4, J_2 = 17.2 Hz, CH_2COOH), 2.16 (1H, dd, J_1 = 4.7, J_2 = 17.2 Hz, CH_2COOH). ^{13}C NMR (100.1 MHz, D_2O + one drop of CD_3OD as the reference) δ 176.1 (s, COO), 175.2 (s, COO), 155.6 (s, Het C-2), 147.9 (d, Het C-4), 141.6 (d, Het C-6), 127.2 (d, Het C-5), 125.8 (d, Het C-3), 69.8 (d, C-2), 48.1 (d, C-3), 30.6 (t, C-4).

4.13. X-ray single crystal analysis of compound 22b·H_2O

Diffraction data were collected at room temperature on a Nonius DIP-1030H system with Mo-Kα radiation (λ = 0.71073 Å). Cell refinement, indexing and scaling of the data sets were carried out using DENZO and SCALEPACK.[28] The structure was solved by direct methods and Fourier analyses,[29] and refined by the full-matrix least-squares method based on F^2.[29] All calculations were performed using the WINGX System, Ver 1.70.01.[30]

4.13.1. Crystal data

$C_{10}H_{12}BrNO_5$; M = 306.12, orthorhombic; space group $P2_12_12_1$, a = 7.390(3), b = 8.928(3), c = 18.503(4) Å, V = 1220.8(7) Å^3, Z = 4, ρcalcd = 1.666 g/cm^3, μ(Mo-Kα) = 3.376 mm^{-1}, F(000) = 616. Final R = 0.0361, wR_2 = 0.0787, S = 0.989 for 163 parameters and 14059 reflections, 2669 unique [R_{int} = 0.0481], of which 1858 with $I > 2\sigma(I)$, max positive and negative peaks in ΔF map 0.425, −0.432 e Å^{-3}. Absolute structure parameter 0.028(15).[31]

Crystallographic data (excluding structure factors) for **22b** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 679550. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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