Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 505–511

TETRAHEDRON: *ASYMMETRY*

# Synthesis of all stereoisomers of cognac lactones via microbial reduction and enzymatic resolution strategies

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Received 16 January 2001; accepted 5 February 2001

**Abstract**—Both enantiomers of the diastereomeric cognac lactones have been synthesised using enzyme assisted reactions in the enantiodifferentiating step. This was accomplished by baker's yeast reduction of their precursors 3-methyl-4-oxononanoic acid and ester and by enzymatic hydrolysis of the latter. An inhibition of hydrolases by the products was observed. *Trans*-(+)-, *trans*-(−)-, *cis*-(+)- and *cis*-(−)-cognac lactones having 99, 88, 88 and 99% e.e., respectively, were thus obtained. Their CD spectra have also been studied. 

## 1. Introduction

The term *Quercus* lactones defines two pairs of diastereomeric γ-lactones (Fig. 1) having the same (*S*)-configuration at C-(4) and differing in the length of the aliphatic chain at C-(5); examples are the (−)-*cis*- and (+)-*trans*-whisky lactones **1** and **2** (R = *n*-$C_4H_9$)[1] and (−)-*cis* and (+)-*trans* cognac lactones **3** and **4** (R = *n*-$C_5H_{11}$). These lactones were isolated from different types of wood and they were identified as key flavours of aged alcoholic beverages such as whisky, brandy, wine and cognac.[2–4] A glucoside precursor of (−)-**1** was also isolated by Tanaka and Kouno[5] from the wood of *Platycarya strobilacea*.

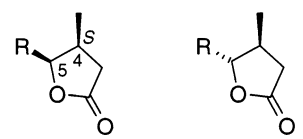
A variety of syntheses of racemic and enantiomerically pure whisky[6–10] and cognac lactones[6d,7,8,10–13] in both diastereomeric forms is reported in the literature. In particular, enantiomerically pure cognac lactones were synthesised starting from the chiral pool with natural products such as D-ribonolactone[11] and levoglucosenone,[8] using enzymes such as hydrolases in the enantiodifferentiating step of the synthesis,[7,12,13] or by asymmetric induction from a chiral auxiliary.[10]

The present study was aimed at the synthesis of both enantiomers of the diastereomeric cognac lactones **3** and **4** starting from their direct precursor, ethyl 3-methyl-4-oxononanoate **5**, by the use of reductive or hydrolytic enzymes in the enantiodifferentiating step.

## 2. Results and discussion

### 2.1. Synthesis of the substrates

The starting material, ethyl 3-methyl-4-oxononanoate **5** (Scheme 1), was prepared following the procedure used by Günther and Mosandl[9] for the synthesis of ethyl 3-methyl-4-oxooctanoate, namely by radical condensation of ethyl crotonate with hexanal. The corresponding 4-ketoacid **6**, obtained by basic hydrolysis of **5** and acidification of the resultant salt, was reduced with sodium borohydride under basic conditions to afford a 1:1 mixture of *cis*- and *trans*-cognac lactones **3** and **4**.[9]




(4*S*,5*S*)-(−)-**1** (4*S*,5*R*)-(+)-**2**
(4*S*,5*S*)-(−)-**3** (4*S*,5*R*)-(+)-**4**

R = *n*-$C_4H_9$, *n*-$C_5H_{11}$

**Figure 1.**

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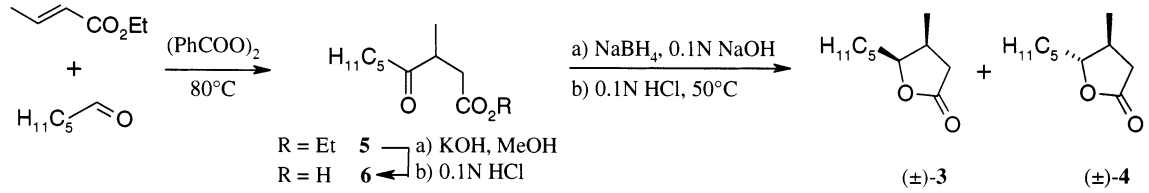
PII: S0957-4166(01)00059-3




**Scheme 1.**

### 2.2. Bioreductions of the ketoester 5 and ketoacid 6 with baker's yeast

Various reaction conditions were investigated for the bioreductions of the substrates **5** and **6**, basing the enzymatic reactions on those reported in the literature for bioreductions of ketoacids of varying chain length.[14–16] Significant results are listed in Table 1. Method A uses a slight modification of the procedure Günther et al. presented for the bioreduction of 3-methyl-4-oxooctanoic acid,[14] while method B uses slightly modified conditions of those reported by Utaka et al. for the reduction of 4-oxononanoic acid.[15] Nakamura reported the conditions used in method C,[16] which were originally used in the bioreduction of nitroketones.[17] These methods essentially differ in the substrate concentration, which is very low (4 mM for method A, 26 mM for method B and 50 mM for method C). Details are reported in the footnotes to Table 1.

The reaction was analysed at regular intervals and the results obtained at different reaction times show variations in the e.e. of the products. Bioconversions did not reach completion and therefore a variable amount of unreacted substrate with moderate e.e. was always recovered from the reaction medium. Bioconversions were diastereoselective only at a very early stage of the reaction when conversion was too low to be preparatively useful. Mixtures of diastereomers were always obtained.

While reduction of the ketoester **5** was better accomplished using method C, method A proved superior for ketoacid **6**. However, baker's yeast reductases showed an opposite enantiopreference in that the carbinolic carbon atom of the corresponding 4-hydroxyester intermediate had (*S*)-configuration while that of the 4-hydroxyacid intermediate was (*R*)-configured. As a consequence, bioreduction of ketoester **5** furnished the lactones *cis*-(−)-**3** and *trans*-(−)-**4**, while in the bioreduction of the ketoacid **6** the enantiomeric *trans*-(+)-**4** was the major reaction product. Analogous findings had already been reported by Utaka[15] for the baker's yeast reduction of 4-oxononanoic acid. It is also interesting to note that in the reduction of the 4-ketoacid **6** the lactone *trans*-(+)-**4**, initially formed as a pure enantiomer, completely racemised when the reaction time was prolonged for 15 days, evidently as a result of competition between reductases. In the absence of glucose no racemisation was observed and the *trans*-lactone (4*S*,5*R*)-(+)-**4** with >99% e.e. was obtained as the sole product. In this case, however, after 30% conversion, the reaction did not proceed any further.

Therefore, a correct choice of substrate and reaction times allowed the isolation of the lactones *cis*-(−)-**3** and

**Table 1.** Main results for baker's yeast reductions of compounds (±)-**5** and (±)-**6**

| Method | Ester (±)-**5** | | | | Acid (±)-**6** | | | |
|---|---|---|---|---|---|---|---|---|
| | Time (days) | Products e.e. (%) [rel. %] | | Unreacted ester e.e. (%) [rel. %] | Time (days) | Products e.e. (%) [rel. %] | | Unreacted acid e.e. (%) [rel. %] |
| A[a,14] | 10 | (−)-**3** >99 [9] | (−)-**4** 61 [14] | (±)-**5** 0 [77] | 3 | – | (+)-**4** >99 [36] | (+)-**6** 40 [64] |
| | 21 | (−)-**3** >99 [39] | (−)-**4** 50 [21] | (+)-**5** 44 [40] | 9 | 3 n.d. [5] | (+)-**4** 29 [81] | (+)-**6** 49 [14] |
| B[b,15] | 5 | – | – | (±)-**5** 0 [100] | 3 | – | – | (±)-**6** [100] |
| | 15 | (−)-**3** >99 [28] | (−)-**4** 85 [10] | (+)-**5** 26 [62] | 17 | – | – | (±)-**6** [100] |
| C[c,16] | 3 | (−)-**3** >99 [18] | (−)-**4** 76 [15] | (+)-**5** 21 [68] | 3 | – | (+)-**4** >99 [5] | (±)-**6** [95] |
| | 10 | (−)-**3** >99 [50] | (−)-**4** 83 [21] | (+)-**5** 45 [29] | 15 | – | (+)-**4** >99 [5] | (±)-**6** [95] |

[a] 3.6 mmol/L of substrate; 31 g raw baker's yeast/mmol of substrate; 112 g/L of glucose.
[b] 26 mmol/L of substrate; 5.6 g dry baker's yeast/mmol of substrate; 163 g/L of glucose.
[c] 50 mmol/L of substrate; 10 g raw baker's yeast/mmol of substrate.

*trans*-(+)-**4**, both with >99% e.e. When the reactions were repeated on a preparative scale, the former compound having >99% e.e. was isolated in 10% yield, and the latter with >99% e.e. in 33% yield after chromatographic separation.

### 2.3. Enzymatic hydrolysis of the 4-ketoester (±)-5

The racemic 4-ketoester (±)-**5** was then subjected to enzymatic hydrolysis with several commercially available hydrolases, including lipases, esterases and proteases (Section 4). Enzymic resolution with PPL of the cyanomethyl ester of **6** had already been reported.[18] After 1 hour, at 49% conversion, the corresponding 4-ketoacid (*S*)-(−)-**6** with 85% e.e. was isolated and the unreacted ester was also recovered with an e.e. of >98%.

Table 2 shows PPL, with an $E$[19] value of >100, to be the best enzyme in the hydrolysis of the ethyl ketoester **5**. In fact, at 21% conversion, the corresponding hydrolysis product (*S*)-(−)-**6** was isolated with 98% e.e. and in 13% yield, while the unreacted ester had an e.e. of 26%. Unfortunately, the reaction did not proceed to higher conversion, which is probably a result of inhibition of the enzyme by the product acid. Analogous inhibition has been observed by Francke[20] in the baker's yeast reduction of 5-oxodecanoic acid and more recently by Lencki and co-workers,[21] who were able to isolate the fatty acids produced by hydrolysis with *Bacillus roqueforti* only after continual extraction of the resulting acid from the reaction medium.

Hydrolysis of (±)-**5** with lipase PS was also satisfactory, leading to the corresponding ketoacid (*S*)-(−)-**6** with 93% e.e., but also in this case the reaction did not proceed to high conversion values. For the other enzymes investigated in this study $E$ values were too low to be useful for preparative purposes.

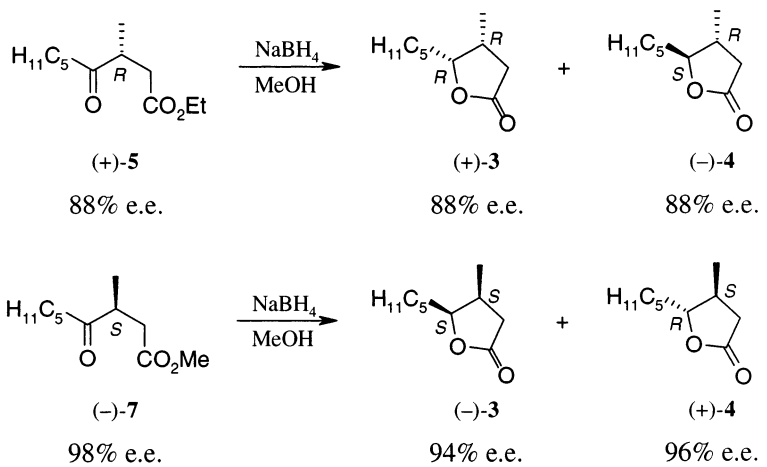
To circumvent the problem of enzyme inhibition by the ketoacid product, the unreacted ketoester (*R*)-(+)-**5** from either hydrolysis with PPL or the reduction with baker's yeast was recycled. After six consecutive hydrolysis procedures, ketoester (*R*)-(+)-**5** was recovered with 88% e.e. in 12% overall yield. This latter compound was reduced with sodium borohydride in ethanol, which afforded a 1:1 mixture of the lactones (4*R*,5*R*)-(+)-**3** and (4*R*,5*S*)-(−)-**4** having the same e.e. as the parent ketoester. Finally, ketoacid (*S*)-(−)-**6** with 98% e.e. was esterified with diazomethane and the resulting 4-ketoester (−)-**7** was reduced with sodium borohydride to yield the lactones (4*S*,5*S*)-(−)-**3** with 94% e.e., and (4*S*,5*R*)-(+)-**4** with 96% e.e (Scheme 2).

Therefore, both enantiomers of the diastereomeric cognac lactones in good enantiomeric purity were prepared by means of enzymatic resolution by hydrolases.

**Table 2.** Enzymatic hydrolysis of (±)-**5**[a]

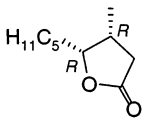
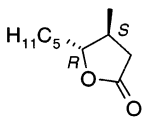
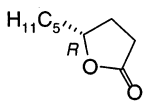
| Enzyme | $E$ | Reaction time | Conv. (%) | Unreacted 4-ketoester e.e. (%)[b] [yield (%)] | 4-Ketoacid e.e. (%)[c] [yield (%)] |
|---|---|---|---|---|---|
| Porcine pancreas lipase (PPL)[d] | >100 | 10 h | 21 | (*R*)-(+)-**5** 26 [67] | (*S*)-(−)-**6** 98 [13] |
| *Pseudomonas species* (PS)[e] | 30 | 13h 50 min | 10 | (*R*)-(+)-**5** 10 [80] | (*S*)-(−)-**6** 93 [13] |

[a] 1 mmol of (±)-**5** in 14 mL of 0.1 M phosphate buffer, pH 7.4.
[b] Determined by chiral HRGC.
[c] Determined by chiral HRGC of the corresponding methyl ester.
[d] 0.150 g of PPL.
[e] 0.033 g of lipase PS.




**Scheme 2.**

**Table 3.** UV and CD spectra of (4*R*,5*R*)-(+)-**3**, (4*S*,5*R*)-(+)-**4** and (*R*)-(+)-**8**[a]

| | UV | | CD | | |
|---|---|---|---|---|---|
| | $\lambda_{max}$ | $\varepsilon_{max}$ | $\lambda_{max}$ | $\Delta\varepsilon$ | Solvent |
|  (4*R*,5*R*)-(+)-**3** | 210<br>223 | 295<br>249 | 228 | +0.07 | *n*-Hexane |
| | 207<br>223 | 529<br>326 | 211 | –0.24 | Methanol |
|  (4*S*,5*R*)-(+)-**4** | 211<br>224 | 117<br>91 | 224 | +0.21 | *n*-Hexane |
| | 211<br>223 | 257<br>223 | 203<br>226 | –0.16<br>+0.13 | Methanol |
|  *R*-(+)-**8** | 218 | 165 | 215 | –0.21 | *n*-Hexane |
| | 208<br>217 | 222<br>206 | 211 | –0.52 | Methanol |

[a]The data are reported for those enantiomers having the same (*R*)- configuration at the γ-carbon atom for a better comparison.

### 2.4. Analysis of the circular dichroism spectra

The sign of the Cotton effect for the n→π* transition of the γ-lactone ring depends on both the electronic structure of the substituents[22] and the conformation of the ring, which in turn is related to the position of the ring substituents.[23] In general, γ-lactones having an alkyl group at the γ-carbon atom as the only substituent show a negative Cotton effect (methanol as the solvent) when the stereocentre has the (*R*)-configuration.[1] Table 3 reports the CD spectrum of (*R*)-(+)-**8**[15] as the reference compound. The *cis*-lactone (+)-**3** shows a smaller negative Cotton effect in methanol and therefore it seems that the methyl group at the β-position contributes positively to the sign of the effect (–0.24 versus –0.52). This effect is even larger for the *trans*-lactone, for which a second band at higher wavelength is present, attributable to a different conformation. It must be noted that, in methanol, solvation plays an important role. In fact in a non-polar solvent such as *n*-hexane the effect is completely reversed.

## 3. Conclusions

Using biotransformation procedures, all four cognac lactones have been obtained in high e.e. with comparable yields to those previously reported in the literature. Both of the enzymatic methods examined gave satisfactory results. Baker's yeast reductases showed a different enantiopreference for the ketoester to the ketoacid, while enzymatic hydrolyses were characterised by low conversion, probably a result of enzyme inhibition by the product acid.

We have also demonstrated that bioreduction with baker's yeast can be used together in sequence with enzymatic hydrolysis by various enzymes to afford the doubly transformed product.

## 4. Experimental

### 4.1. General

IR spectra were recorded on a Jasco FT/IR 200 spectrophotometer. $^1$H and $^{13}$C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton), using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Coupling constants and $W_H$s are given in Hz. Optical rotations were determined on a Perkin–Elmer Model 241 polarimeter. UV spectra were recorded on a UV–vis Perkin–Elmer Lambda 2 spectrometer. CD spectra were obtained on a Jasco J-700A 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) and a Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin (40 m×0.25 mm) (carrier gas He, 180 KPa, split 1:100) or DiMePe β-cyclodextrin (25 m×0.25 mm) (carrier gas He, 110 KPa, split 1:50). Enzymic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer Copenhagen. Mass spectra were recorded on a VG 7070 (70 eV) spectro-

meter. TLCs were performed on Polygram® Sil G/$UV_{254}$ silica gel pre-coated plastic sheets (eluant: light petroleum–ethyl acetate). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with bp 40–70°C and ether to diethyl ether.

### 4.2. Substrate synthesis

**4.2.1. Ethyl 3-methyl-4-oxononanoate 5**.[9] A mixture of ethyl crotonate (11.5 g, 0.1 mol) and hexanal (60.1 g, 0.6 mol) was heated at 80°C under $N_2$ in the presence of benzoyl peroxide (2.0 g, 80 mmol) for 9 h. Three further portions of benzoyl peroxide (2.0 g, 80 mmol) were added to the mixture regularly. The crude reaction mixture was then washed with saturated $NaHCO_3$ and the ketoester **5** was purified by flash chromatography (eluent: light petroleum–ether, gradient from 98:2 to 90:10). Oil. IR (neat), $cm^{-1}$: 1735 (O-C=O), 1715 (C=O, sh), 1187 (C-O); $^1H$ NMR, $\delta$, ppm: 0.87 (3H, t, $CH_3$), 1.12 (3H, d, $J$ 6.8 Hz, $CH_3CH$), 1.31 (7H, m, $(CH_2)_2$ and $CH_3CH_2O$), 1.57 (2H, quintet, $J_1=J_2=J_3=7.3$ Hz, $CH_2$), 2.26 (1H, dd, $J=17.0$, 8.8 Hz, H-2), 2.50 (2H, m, $CH_2$), 2.75 (1H, dd, $J=17.0$, 5.7 Hz, H-2), 2.98 (1H, m, H-3), 4.08 (2H, q, $CH_2O$); $^{13}C$ NMR, $\delta$, ppm: 13.9 (q), 14.1 (q, $CH_3CH$), 16.7 (q, $CH_3CH_2O$), 22.4 (t), 23.2 (t), 31.2 (t), 37.4 (t, C-5), 41.7 (t, C-2), 41.9 (d, C-3), 60.4 (t, $CH_2O$), 172.3 (s, C-1), 213.0 (s, C-4); $m/z$: 169 (33), 158 (32), 142 (16), 115 (25), 112 (37), 99 (100), 71 (78), 43 (92).

**4.2.2. 3-Methyl-4-oxononanoic acid 6**.[18] A mixture of ketoester **5** (0.490 g, 2.29 mmol) and 90% KOH in methanol (3.7 mL, 4.6 mmol) was stirred at room temperature for two days. After evaporation of the solvent, water was added and the mixture acidified with HCl. The mixture was extracted with diethyl ether and the organic extract dried over $Na_2SO_4$, filtered and the filtrate evaporated to afford acid **6** as a colourless oil (0.417 g, 98%). IR (neat), $cm^{-1}$: 3150 (OH), 1740 (O-C=O), 1715 (C=O), 1280 (C-O); $^1H$ NMR, $\delta$, ppm: 0.87 (3H, t, $CH_3$), 1.14 (3H, d, $J=7.3$ Hz, $CH_3CH$), 1.23–1.34 (4H, m, $(CH_2)_2$), 1.57 (2H, quintet, $J_1=J_2=J_3=$ 7.3 Hz), 2.32 (1H, dd, $J=17.0$, 5.4 Hz, H-2), 2.50 (2H, m, 2 H-5), 2.81 (1H, dd, $J=17.0$, 8.9 Hz, H-2), 2.96 (1H, m, H-3), 10.0 (1 H, br s, OH); $^{13}C$ NMR, $\delta$, ppm: 13.9 (q), 16.6 (q), 22.4 (t), 23.2 (t), 31.3 (t), 36.6 (t, C-5), 41.0 (t, C-2), 41.7 (d), 178.3 (s, C-1), 213.0 (s, C-4); $m/z$: 130 (15), 115 (11), 112 (15), 99 (82), 71 (56), 43 (100).

**4.2.3. Synthesis of the lactones 3 and 4**.[9] To a solution of the ketoester **5** (3.85 g, 18 mmol) in methanol (5 mL) was added solid NaOH (0.78 g, 19.5 mmol) and the mixture stirred at room temperature for 3 h. After evaporation of the solvent, 1N NaOH (1.8 mL) was added and the mixture was heated at 50°C. Sodium borohydride (0.680 g, 18 mmol) in 0.1N NaOH (7.6 mL) was added dropwise. At the end of the reaction the mixture was acidified with concentrated HCl and heated for a further two hours. After the usual work-up a 1:1 mixture of the two diastereomers **3** and **4** was obtained in 68% yield, which were separated by flash chromatography. The first product was evaporated to afford *trans*-4,5-dihydro-4-methyl-5-pentyl-2(3*H*)-furanone **4**. All spectroscopic data are in accordance with those reported in the literature.[24] The second band to elute was evaporated to afford *cis*-4,5-dihydro-4-methyl-5-pentyl-2(3*H*)-furanone **3**. IR (neat), $cm^{-1}$: 1779 (O-C=O), 1212 (C-O); $m/z$: 170 (1), 101 (17), 99 (100), 83 (24), 71 (35), 70 (14), 56 (20), 55 (26), 43 (44), 42 (44), 41 (32). $^1H$ and $^{13}C$ NMR data are in accordance with the literature.[12]

**4.2.4. Synthesis of methyl 3-methyl-4-oxononanoate 7**.[18] The acid **6** (0.100 g, 0.54 mmol) was esterified with diazomethane to give the title compound **7**. IR (neat), $cm^{-1}$: 1735 (O-C=O), 1715 (C=O), 1187 (C-O); $^1H$ NMR, $\delta$, ppm: 0.87 (3H, t, $J=6.8$ Hz, $CH_3$), 1.12 (3H, d, $CH_3CH$), 1.31 (4H, m, $(CH_2)_2$), 1.57 (2H, quintet, $J_1=J_2=J_3=7.3$ Hz, $CH_2$), 2.26 (1H, dd, $J=17.0$ and 8.8 Hz, H-2), 2.50 (2H, m, $CH_2$), 2.75 (1H, dd, $J=17.0$, 5.7 Hz, H-2), 2.98 (1H, m, H-3), 4.08 (3H, s, $CH_3$); $^{13}C$ NMR, $\delta$, ppm: 13.9 (q), 16.6 (q, $CH_3CH$), 22.4 (t), 23.2 (t), 31.2 (t), 36.6 (t, C-2), 41.0 (t, C-5), 41.9 (d, C-3), 51.6 (q, $CH_3$), 172.8 (s, O-C=O), 213.0 (s, C=O).

### 4.3. General procedure for baker's yeast reductions

Method A: To a stirred suspension of raw baker's yeast (56 g) in phosphate buffer (0.1 M, pH 7.4, 500 mL) was added glucose (56 g); the suspension was stirred for 30 min and the 4-keto ester **5** or 4-ketoacid **6** (1.8 mmol) was added at room temperature. The reaction was monitored by HRGC. At the end of the reaction, brine was added and the broth was continuously extracted with ether for 48 h. The organic phase was dried and evaporated.

Method B: To a stirred suspension of dry baker's yeast purchased from Sigma Aldrich (2.8 g) in water (19 mL) was added glucose (3.1 g); the suspension was stirred for 30 min and the 4-keto ester **5** or 4-ketoacid **6** (0.5 mmol) was added at room temperature. The reaction was monitored by HRGC. At the end of the reaction, brine was added and the broth was continuously extracted with ether for 48 h. The organic phase was dried and evaporated.

Method C: To a stirred suspension of raw baker's yeast (5 g) in water (10 mL) at room temperature was added the 4-keto ester **5** or the 4-ketoacid **6** (0.5 mmol). The course of the reaction was monitored by HRGC. At the end of the reaction, brine was added and the broth was continuously extracted with ether for 48 h. The organic phase was dried and evaporated.

**4.3.1. Baker's yeast reduction of the ketoester 5**. The reaction was carried out on ketoester **5** (0.642 g, 3 mmol) according to method C. Since the reaction stopped at low conversion values additional raw baker's yeast (15 g) in water (20 mL) was added. The reaction was extracted after 31 days. The organic phase was dried and evaporated. Separation by flash chromatography (eluent: gradient of petroleum ether–ethyl acetate from 0% up to 3%) afforded the lactone *cis*-

(4*S*,5*S*)-(−)-**3** with 99% e.e. (10% yield) and the lactone *trans*-(4*R*,5*S*)-(−)-**4** with 57% e.e. (5% yield). The lactone (−)-**3** was further purified by bulb to bulb distillation, at 120°C (4 mmHg).

*cis*-(4*S*,5*S*)-4,5-Dihydro-4-methyl-5-pentyl-2(3*H*)-furanone (−)-**3**: 99% e.e. $[\alpha]_D^{25}$ −69.5 (*c* 0.42, MeOH); lit.[10] $[\alpha]_D^{25}$ −78.2 (*c* 0.2–1.0, MeOH); $[\alpha]_D^{25}$ −65.2 (*c* 0.25, $CHCl_3$); lit.[12] $[\alpha]_D^{25}$ −73 (*c* 1, $CHCl_3$); $\Delta\varepsilon_{211}$ +0.24 (MeOH); $\Delta\varepsilon_{228}$ −0.07 (*n*-hexane).

**4.3.2. Baker's yeast reduction of the ketoacid 6**. The reduction was performed on the ketoacid **6** (0.380 g, 1.8 mmol) according to method A. After five days, at 54% conversion, saturated $NaHCO_3$ solution was added to separate the unreacted acid and the mixture was extracted with ether. From the ethereal solution the *trans*-lactone (+)-**4** (0.100 g, 33% yield) was obtained after purification by flash chromatography (eluant: gradient of petroleum ether–ethyl acetate from 0% up to 3%).

*trans*-(4*S*,5*R*)-4,5-Dihydro-4-methyl-5-pentyl-2(3*H*)-furanone (+)-**4**: The lactone was purified by a bulb to bulb distillation, 120°C, 6 mmHg; >99% e.e.; $[\alpha]_D^{25}$ +73.0 (*c* 0.2, MeOH), $[\alpha]_D^{25}$ +78.9 (*c* 0.18, $CH_2Cl_2$), lit.[6d] $[\alpha]_D^{25}$ +82.2 (*c* 0.71, MeOH); lit.[11] $[\alpha]_D^{20}$ +48.3 (*c* 0.79, $CH_2Cl_2$), lit.[8] $[\alpha]_D^{23}$ +83.2 (*c* 0.69, MeOH) and lit.[7] $[\alpha]_D^{25}$ +75 (*c* 1.0, $CH_2Cl_2$), e.e. 96%. $\Delta\varepsilon_{203}$ −0.16, $\Delta\varepsilon_{226}$ +0.13 (MeOH); $\Delta\varepsilon_{224}$+0.21 (*n*-hexane).

### 4.4. Enzymatic hydrolysis

For (±)-**5** (1 mmol) in phosphate buffer (0.1 M, pH 7.4, 14 mL), the following enzymes were used: Porcine pancreatic lipase (PPL, 0.150 g), lipase from *Pseudomonas fluorescens* (Amano AK, 29,000 U/g, 1.0 g), lipase from *Pseudomonas* species (Amano PS, 30,000 U/g, 0.033 g), lipase from *Candida cylindracea* (CCL, 943 U/mg, 0.160 g), lipase from *Aspergillus niger* (Amano AP 12, 120,000 U/g, 0.019 g), lipase from *Mucor miehei* (MML, Lipozyme, 0.400 g), lipase from *Candida antarctica* (Novozyme 435®, 7000 U/g, 0.180 g), porcine liver acetone powder (PLAP, 0.230 g), horse liver acetone powder (HLAP, 0.900 g), pig liver esterase (PLE, 19 U/mg, 0.055g), α-chymotrypsin (α-CT, 51.8 U/mg, 0.019 g) and *Bacillus subtilis* (39.1 U/mg, 0.076 g).

To a solution of the ketoester **5** in phosphate buffer (pH 7.4, 14 mL) was added the enzyme under vigorous stirring. The course of the reaction was monitored with a pH-stat, with continuous addition of 1.0N NaOH. At 20% conversion, the reaction mixture was extracted with ether to separate the unreacted ketoester. The mother liquors were acidified with 5% HCl to pH 2 and extracted with ether. The organic phase was dried on $Na_2SO_4$ and treated with diazomethane to esterify the carboxylic group before the HRGC analysis.

Hydrolysis of **5** carried out with PPL gave, after 20% conversion, (*S*)-(−)-**6** in 13% yield. (*S*)-(−)-**6**: 98% e.e.; $[\alpha]_D^{25}$ −32 (*c* 0.55, MeOH).

Esterification of (*S*)-(−)-**6** with diazomethane afforded the corresponding methyl ester derivative (*S*)-(−)-**7**. (*S*)-(−)-**7**: 98% e.e.; $[\alpha]_D^{25}$ −31 (*c* 1.3, THF), lit.[18] $[\alpha]_D^{25}$ −30.1 (*c* 2.9, THF), 85% e.e.; after six subsequent cycles the ester (*R*)-(+)-**5** was obtained with 88% e.e. (*R*)-(+)-**5**: $[\alpha]_D^{25}$ = +16.3 (*c* 0.75, MeOH).

### 4.5. Chemical reduction of 5 and 7 with $NaBH_4$

To a solution of $NaBH_4$ (0.5 mmol) in the corresponding alcohol (0.5 mL), the ketoester (1 mmol) was added under stirring. At the end of the reaction, the solvent was evaporated and after addition of saturated NaCl the crude reaction mixture was extracted with ether. The organic phase was dried over anhydrous $Na_2SO_4$.

**4.5.1. Reduction of ethyl 3-methyl-4-oxononanoate 5 with $NaBH_4$.** To a solution of $NaBH_4$ (12.2 mg) in ethanol (0.32 mL), the ketoester (*R*)-(+)-**5** (88% e.e.) (0.137 g, 0.64 mmol) was added. After 1 h a 1:1 mixture of the two lactones *cis*-(4*R*,5*R*)-(+)-**3** and *trans*-(4*R*,5*S*)-(−)-**4** both having 88% e.e. was obtained in 87% yield.

**4.5.2. Reduction of methyl 3-methyl-4-oxononanoate 7 with $NaBH_4$.** To a solution of $NaBH_4$ (10.5 mg) in methanol (0.32 mL), ketoester (*S*)-(−)-**7** (98% e.e.) (0.110 g, 0.55 mmol) was added. After 24 h a 1:1 mixture of the lactones *cis*-(4*S*,5*S*)-(−)-**3**, having 94% e.e., and *trans*-(4*S*,5*R*)-(+)-**4**, having 96% e.e., was obtained in 78% yield. Unreacted ketoester **7** (90% e.e.) was also recovered in 5% yield.

## Acknowledgements

Financial support from the M.U.R.S.T., C.N.R. (Rome) and the University of Trieste is gratefully acknowledged.

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