Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 3501–3510

Baker's yeast-mediated reduction of ethyl 2-(4-chlorophenoxy)-3-oxoalkanoates intermediates for potential PPARα ligands

Maria Grazia Perrone,[a] Ernesto Santandrea,[a] Antonio Scilimati,[a,*] Vincenzo Tortorella,[a] Francesco Capitelli[b] and Valerio Bertolasi[c]

[a]*Dipartimento Farmaco-Chimico, Università di Bari, Via E.Orabona 4, 70125 Bari, Italy*
[b]*Istituto di Cristallografia (IC-CNR), Via Amendola 122/o, 70125 Bari, Italy*
[c]*Dipartimento di Chimica and Centro di Strutturistica Diffrattometrica, Università di Ferrara, Via Borsari 46, 44100 Ferrara, Italy*

Received 26 July 2004; accepted 20 August 2004

Abstract—Several 2-(4-chlorophenoxy)-3-oxoesters were prepared in fair to good yields and then reduced in the presence of baker's yeast to the corresponding alcohols having de's up to 92% and ee's >99%. The absolute configuration of nearly enantiomerically pure ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate was assigned by both comparison of the sign of the specific rotation and HPLC retention times of authentic samples prepared from threonines. Reduction of ethyl 2-(4-chlorophenoxy)-3-oxo-4-phenylbutanoate afforded only enantiomerically pure ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-4-phenylbutanoate (out of the four possible stereoisomers), whose absolute configuration was established by single crystal X-ray analysis. Furthermore, reduction of ethyl 2-methyl-2-(4-chlorophenoxy)-3-hydroxybutanoate with a quaternary stereogenic carbon (C_2) gave both of the two expected diastereoisomers with ee = 95% and 96%. Insight into the mechanism of baker's yeast-mediated reduction of prochiral ketoesters is also reported.

1. Introduction

The asymmetric reduction of prochiral ketones represents one of the most used methods for preparing chiral alcohols in very high enantiomeric excess.[1,2] This transformation can be accomplished by using various chiral reducing agents[3,4] or chirally modified boron- and aluminium-hydrides,[5,6] through enantioselective reduction by hydride transfer from carbon or nitrogen and by catalytic reduction with chiral transition metal complexes.[7–12]

Biotransformations have also extensively been used for the preparation of chiral alcohols.[13] In particular, lipase-mediated kinetic resolution of racemic alcohols,[14] asymmetric reduction of the carbonyl of a prochiral ketone by baker's yeast[15–18] and hydroxylation reaction[19] have widely been used to prepare enantiomerically pure alcohols.[20]

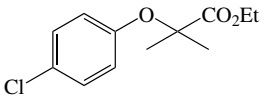
Baker's yeast is often chosen, with respect to other microorganisms with reducing activity, as it is easy to use and does not require the presence of expensive cofactors to exert its action, is not pathogenic, is versatile and has no environmental impact.

Herein baker's yeast has been used to prepare optically active clofibrate analogues. Fibrates constitute a widely used class of lipid-modifying agents. Treatment with fibrates results in a substantial decrease in plasma triglycerides and is usually associated with a moderate decrease in LDL and an increase in HDL cholesterol concentration.[21]

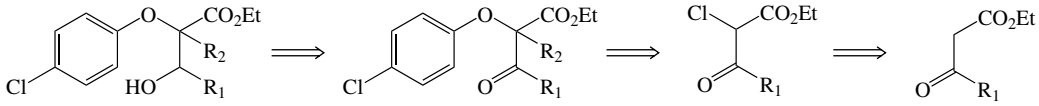
Over the course of our previous investigation, optically active, acyclic and cyclic (rigid) analogues of clofibrate, were prepared by asymmetric synthesis,[22] crystallization of their diastereomeric salts with chiral amines[23] and by kinetic resolution performed in the presence of lipases.[15,24] We have also investigated for a long time the pharmacological activity of clofibric acid derivatives in order to find and, possibly, dissociate the structural determinants of the different effects.[25–28] Hence, the first

* Corresponding author. Tel.: +39 080 5442762; fax: +39 080 5442231; e-mail: ascilimati@farmchin.uniba.it

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.08.027



Clofibrate



2 1

Figure 1. Clofibrate molecular structure and retrosynthesis of ethyl 2-(4-chlorophenoxy)-3-hydroxyalkanoates **2a–g**.

part of our study consisted of the synthesis of more functionalized optically active analogues of ethyl aryloxyalkanoates capable of eventually giving additional interactions with the target receptor ligand-binding domain.[29]

Such clofibrate analogues have been synthesized in their racemic form (Fig. 1) and pharmacologically evaluated, by a transactivation assay, as potential peroxisome proliferator-activated receptor isoform α (PPARα) agonists. Unfortunately, they were unable to interact with the PPARα ligand binding domain. Among the several reasons for a lack of activity, one could be that they were tested as a mixture of the four possible stereoisomers, these compounds having two stereogenic centres.

The last step of the previously used synthetic pathway consisted of the reduction of the carbonyl group with $NaBH_4$ (Fig. 1). The diastereomeric excesses obtained ranged between 2% and 60% (Table 1).

Herein, the results of the baker's yeast-mediated asymmetric reduction of 2-(4-chlorophenoxy)-3-oxoalkanoates **1a–g** to **2a–g** are reported. Such a method was selected in an attempt to prepare optically active or even better enantiomerically pure 2-(4-chlorophenoxy)-3-hydroxyalkanoates **2a–g** suitable for performing the bioassay.

2. Results and discussion

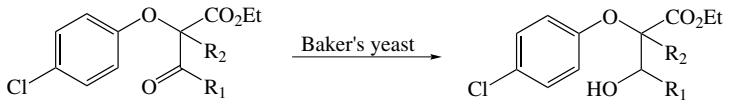
A number of racemic clofibrate analogues were synthesized[29] (Fig. 1) by reacting the chloroketoesters, prepared from commercially available ketones, with caesium 4-chlorophenate at 50 °C to give the 4-chlorophenoxyketoesters **1a–g**, which in turn were reduced to the corresponding desired alcohols **2a–g** by $NaBH_4$/methanol at 0 °C. Compound **1g** was prepared by treating **1a** with CH_3I/Cs_2CO_3. To prepare the same compounds **2a–g** in optically active form, the last step of Figure 1 was performed in the presence of baker's yeast/water at 30 °C. The results of the reduction of the prochiral ketoesters **1a–g** to the corresponding hydroxyesters **2a–g** by baker's yeast (Scheme 1) are summarized in Table 1.

Reduction rates depended upon the substrate concentration and the size of R_1. Substrate concentration (5.0 g/L for **1a**, 1.7 g/L for **1f**, 1.0 g/L for **1b–d** and **1g**) was chosen to have reasonable conversion rates. In the case of **1e**, a very small amount of product was formed (about 2% within 9 days) even at a substrate concentration of 0.05 g/L. Compound **1a** (R_1 = H) was the fastest reacting compound; within 3 h it was completely converted into the product **2a** with very high diastereoselectivity (de = 92%, vs 11% obtained in the reduction conducted with $NaBH_4$) with the major stereoisomer formed being

Table 1. Results of the reduction of **1a–g** performed in the presence of baker's yeast

Substrate	R_1	R_2	Substr. concn (g/L)	Reaction time (day)	De[a] (%) *syn* > *anti*	Ee_{syn}[b](%)	Ee_{anti}[b] (%)	Conversion[c] (%)
1a	H	CH_3	5.0	3 h	92 (11)	94	7	Quantitative
1b	H	C_2H_5	1.0	5	50 (7)	51	3	88
1c	H	*n*-C_3H_7	1.0	1.5	89 (22)	89	5	Quantitative
1d	H	*i*-C_3H_7	1.0	4	70 (25)	70	33	43
1e	H	*t*-C_4H_9	0.05	9	Nd[d] (8)	Nd[d]	Nd[d]	2
1f	H	C_6H_5	1.7	3	>99 (34)	>99	—	Quantitative
1g	CH_3	CH_3	1.0	2	2 (65)	>99	95	Quantitative

[a] Diastereomeric excesses of products **2a–g**, determined by ^{1}H NMR. In brackets are de values obtained reducing **1a–g** with $NaBH_4$.
[b] Enantiomeric excesses of **2a–g** determined by HPLC using a Daicel OD column packed with chiral stationary phase.
[c] Conversion = % substrate **1a–g** transformed into product **2a–g**, determined by ^{1}H NMR and GC analysis.
[d] Nd = not determined, due to low conversion (about 2%).



1 **2**

R_1 = H; R_2 = CH_3, C_2H_5, *n*-C_3H_7, *i*-C_3H_7, *t*-C_4H_9, C_6H_5
R_1 = R_2 = CH_3

Scheme 1. Baker's yeast reduction of **1a**–**g** to afford **2a**–**g**.

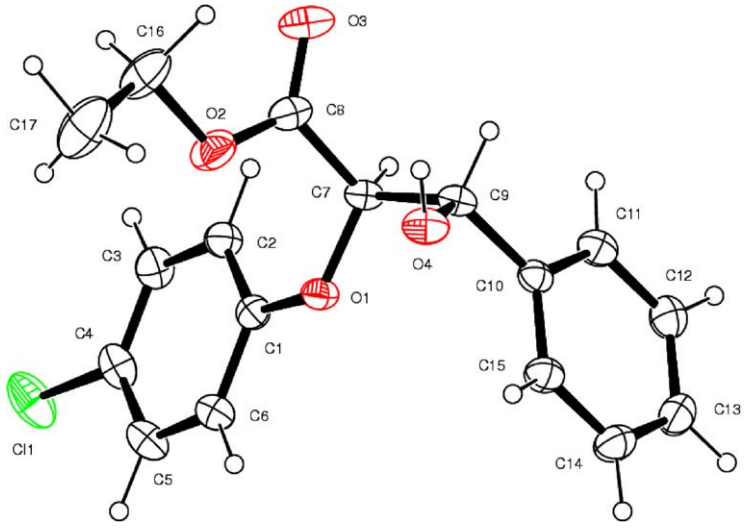


Figure 2. ORTEP view of the asymmetric unit with the atomic numbering scheme of ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-3-phenylpropanoate **2f**. Thermal ellipsoids probability level at 30%.

the *syn*-(2*R*,3*S*) with ee = 94%.[30] The reaction times to reduce **1b**–**g** (43%–100% extent of conversion) were higher than 24 h (1.5–5 days). Compound **1c** was completely converted in 1.5 days with good diastereo- and enantioselectivity (de = 89% and ee_{syn} = 89%, respectively).

Lower conversion (88% and 43%, respectively) and stereoselectivity (de = 50% and 70%, ee_{syn} = 51% and 70%, respectively) were obtained incubating **1b** and **1d** in the presence of baker's yeast.

Compound **1e** (R_1 = *t*-Bu) gave, as above mentioned, only 2% of alcohol **2e**. This very low extent of conversion does not seem to only be due to the steric hindrance exerted from the *t*-butyl group, but it also appears to be a consequence of different factors if the results obtained reducing **1e** and **1f** (R_1 = *t*-Bu and phenyl, respectively) are compared. In fact, a phenyl ring (**1f**) and a *t*-butyl (**1e**) have a similar Van der Waals volume: 45.84 cm^3/mol and 44.34 cm^3/mol, respectively.[31] Conversely for **1e**, which is almost unreactive under the conditions used, **1f** was completely reduced within 3 days and with the highest observed diastereo- and enantioselection [de >99% and ee_{syn} >99%, the (2*R*,3*S*) stereoisomer being only formed]. These results definitively suggest that baker's yeast-mediated bioreduction of compounds such as **1a**–**g**, are strongly affected by both electronic features and group size bonded to the prochiral carbonyl.

The absolute configuration of (2*R*,3*S*)-**2f** was established by a single crystal X-ray analysis (Fig. 2).[32–36]

3. Investigations into the mechanism of baker's yeast-mediated reduction of prochiral ketones 1a–g

All compounds **1a**–**f** are present in the reaction medium in both enol- and keto-forms as can be seen from their ^1H NMR spectra. The extent of enolization varies slightly from one compound to the other (40–60%).

Reduction of racemic α-substituted β-ketoesters poses a problem for diastereo- and enantioselection (the case of **1f** is depicted in Figure 3 as an example). Under the conditions of the yeast reduction, the β-ketoester group can enolize (see above), which for 2-substituted compounds, such as **1a**–**f**, results in racemization. Hence, racemic 2-substituted 3-oxoalkanoates **1a**–**f** can be converted under particular circumstances (see below) into a single enantiomer of the corresponding 2-substituted 3-hydroxyalkanoates **2a**–**f**. Reduction can in principle occur by enantiotopic face differentiating in the hydrogenation of the carbon–carbon double bond of the enol intermediate; in this case, in analogy to the reduction of an activated carbon–carbon double bond, *anti*-products should be expected.[37]

On the other hand, activated tetrasubstituted carbon–carbon double bonds are not usually reduced by baker's

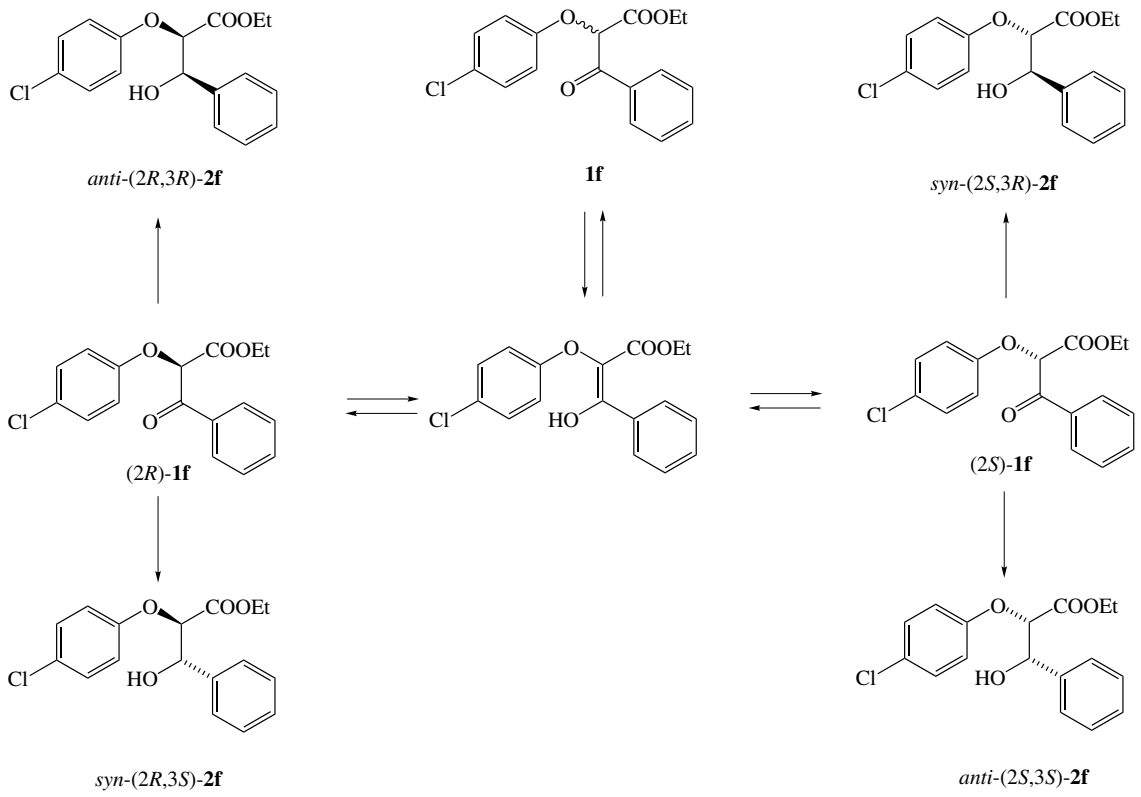
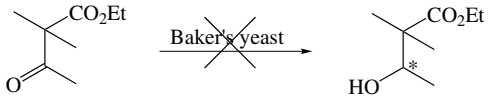


Figure 3. Possible reduction products of **1f** and its keto–enol equilibrium.

yeast, otherwise fast reduction of one of the two enantiomeric forms of the substrates, in its keto form, will drive the reaction towards the selective formation of only one out of the four possible stereoisomers, provided that difference in rate between racemization of the wrong enantiomer and reduction of the right enantiomer is large enough.[17]

In particular, in our case the *syn*-(2*R*,3*S*) is the major (**2a**) or the unique (**2f**) stereoisomer formed at least in the reduction of **1a** and **1f** for which the absolute configurations of the corresponding product, **2a** and **2f**, were established (Table 1).

Deol et al. while exploring this reaction,[37] in a study aimed at differentiating between these two possibilities, incubated the non-enolizable β-keto ester ethyl 2,2-dimethylacetoacetate in the presence of baker's yeast. However, it was not reduced (Scheme 2). This could suggest that the reduction of non-enolizable 3-oxoesters, such as ethyl 2,2-dimethylacetoacetate, does not occur, because the reaction should take place on the enol form. Unfortunately, this evidence is not proof enough for the proposed mechanism, because ethyl 2,2-dimethylacetoacetate might simply not be reduced probably for its non-acceptance in the enzyme catalytic site due to other factors.

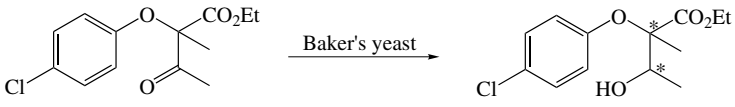


Scheme 2. Absence of reaction of ethyl 2,2-dimethylacetoacetate in the presence of baker's yeast.

The first evidence that the reduction might not undergo through the hydrogenation of the carbon–carbon double bond of the enol form comes from the reduction of compounds **1a**–**f** in which the main stereoisomer is the *syn*-form. This allowed us to hypothesize that the reaction proceeds through the reduction of the carbonyl.[17]

Additionally, the reaction of the non-enolizable 3-oxoester **1g** provides further evidence as the reduction took place and this supports the mechanism detailed in Figure 3.

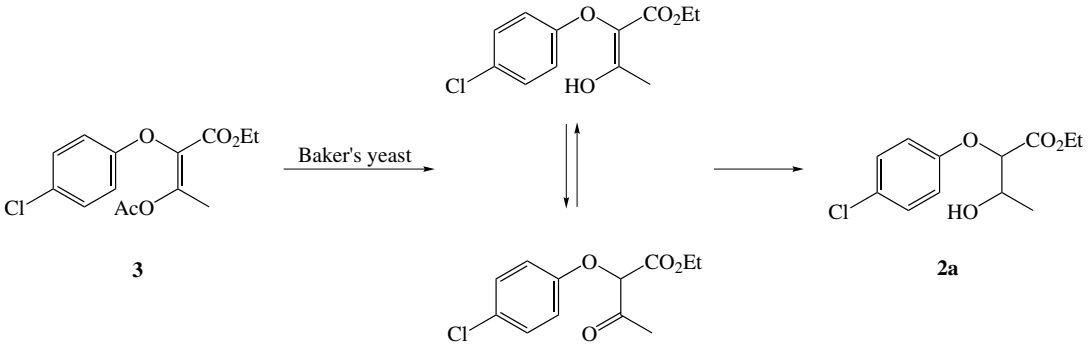
As expected, no diastereomeric excess (2%) was recorded because no interconversion between the enantiomeric forms is possible unlike for **1a**–**f**. In contrast, enantioselectivity is very high (ee >99% and 95%): only one enantiomer within each couple of possible stereoisomers is formed. Furthermore, the absence of diastereoselection observed, confirms that baker's yeast stereoselectivity is completely unaffected by the stereochemistry of the groups bound to the carbonyl.[38] In the reaction mixture, compound **1g** is racemic and each enantiomer is completely reduced to form the two almost enantiomerically pure stereoisomers **2g** (Scheme 3). This means that the newly formed stereogenic centre has the same absolute configuration both starting from



(*R*,*S*)-**1g** **2g**

quantitative yield; de = 5%
$ee_{major\ pair} > 99\%$, $ee_{minor\ pair} = 95\%$

Scheme 3. Reduction of ethyl 2-methyl-2-(4-chlorophenoxy)-3-oxobutanoate **1g** in the presence of baker's yeast.



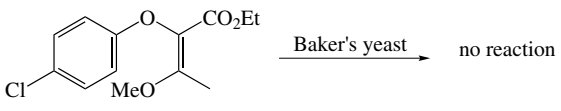
3 **2a**

Scheme 4. Reaction of **3** in the presence of baker's yeast.

(*R*)-**1g** and (*S*)-**1g**, otherwise we would have observed lower ee's and a higher de. Hence, considering the preferred direction of the hydride attack (in the presence of baker's yeast and on the base of the stereochemical outcome at C_3 of **2a** and **2f**, if it is the same reductase enzyme involved) for β-ketoesters, we should have prepared (2*R*,3*S*)- and (2*S*,3*S*)-**2g**.[39]

To support definitively the hypothesis that the reduction occurs on the carbonyl form, we have prepared **3** (the enol acetate of **1a)**. In the presence of baker's yeast, it gave the same results obtained incubating directly **1a**. This means that it was presumably first hydrolysed, by hydrolases contained in the yeast, and then reduced by the reductase enzyme to **2a** (Scheme 4). Once again, this finding represents further confirmation of the above proposed mechanism of the reduction reaction as the main products have a *syn*-conformation, namely that they could be not formed by direct reduction of the carbon–carbon double bond of the enol acetate.

Next, **4** (methyl enol ether of **1a**) was prepared, which in turn did not undergo hydrolysis during the incubation with baker's yeast under the same conditions used as for **1a**. No reaction took place after 6 days of incubation and the unmodified substrate was recovered from the reaction medium (Scheme 5).



4

Scheme 5. Absence of reaction of **4** in the presence of baker's yeast.

4. Conclusion

In summary, we have explored the reductive capabilities of baker's yeast towards compound **1a**–**g**. The results obtained show that this is a useful tool for preparing a set of optically active clofibrate analogues in good yields and with high enantiomeric excesses. In particular, the best results were obtained with compound **1g**, which was reduced with the highest diastereo- and enantioselectivity, and **1f**, whose reduction afforded only one enantiomerically pure isomer out of the four possible stereoisomers.

Efforts have also been made to clarify the baker's yeast-mediated reduction reaction mechanism, definitively demonstrating that baker's yeast-assisted bioreduction of such 3-oxoesters does not occur on the enol intermediate and, that the intermediate itself is not an essential condition for the reaction to occur.

Further investigations are currently in progress, aimed at finding different yeast strains capable of producing even better results in terms of yield and stereoselectivity in the reduction of **1a**–**g**, allowing the preparation of the four possible stereoisomers of **2a**–**g** as well.

5. Experimental

^{1}H NMR spectra were recorded in $CDCl_3$ on a VARIAN Mercury 300 MHz spectrometer and chemical

shifts are reported in parts per million (δ). Absolute values of the coupling constant (J) are reported. The extent of the enolization of **1a–f** was measured by ^{1}H NMR. IR spectra were recorded on a Perkin–Elmer 681 spectrometer. Reaction progress was monitored by TLC or GC analysis. Thin-layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Statocrom SIF, 60 F_{254}MERK); TLC spots were observed under ultraviolet light or visualized with I_2 vapour. Column chromatography was conducted using silica gel MERCK 60 (0.063–0.200 μm). GC analyses were performed by using a HP-5MS column (5% phenyl methyl siloxane; 30 m × 0.321 mm × 0.25 μm) on a Agilent 6850 SERIES GC SYSTEM. GC–MS analyses were performed on a HEWLETT PACKARD 6890–5793MSD, and microanalysis on a Elemental Analyzer 1106-Carlo Erba-instrument. Optical rotations were determined on a Perkin–Elmer model 341 polarimeter; determinations were performed in $CHCl_3$, $c = 1$ g/100 mL.

The ee's and absolute configurations of the reaction products were determined by HPLC analysis performed on a Perkin–Elmer 200 series with a UV/Vis detector 785A on a commercially available Chiralcel OD (Daicel) in isocratic conditions employing *n*-hexane/2-propanol = 98:2, flow rate 0.8 mL/min, $\lambda = 230$ nm.

All chemicals and solvents were purchased from Aldrich Chemical Co.

5.1. Baker's yeast-mediated reduction of ethyl 2-(4-chlorophenoxy)-3-oxoalkanoates 1a–g

5.1.1. Procedure A. Baker's yeast (2.5 g) was dispersed to give a smooth paste in tap water. Ethyl 2-(4-chlorophenoxy)-3-oxoalkanoate was then added and stirred at 37 °C and 250 rpm. The reaction progress was monitored by GC analysis and stopped at the time indicated in Tables 2–5. The reaction mixture was extracted several times with EtOAc. The extracts were dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. A yellow oil was obtained.

The reduction reaction was also carried out under slightly modified versions of procedure A: procedure B, C and D.

5.1.2. Procedure B. Same as procedure A, but the reaction mixture with sucrose was incubated at 30 °C and 250 rpm for 30 min before adding the substrate.

5.1.3. Procedure C. Same as procedure B, but the substrate was dissolved in EtOH.

5.1.4. Procedure D. Same as procedure A, but the substrate was dissolved in EtOH.

5.2. Ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoate 2a

From procedure A. Oil. $[\alpha]_D^{20} = +33.1$ (c 1.0, $CHCl_3$). De = 92%. Ee = 94%. IR (neat): 3600–3200, 3056,

Table 2. Results of the reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1a** to ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate **2a** by using the different reaction medium composition (procedures A–D)

Procedure	Reaction time	Yield (%)[a]	De (%)[b]	$Ee_{major\ (syn)}$ (%)[c]
A	5 days	93	92	94
B	5 days	99	90	95
C	3 h	83	92	95
D[d]	3 h	87	90	97

[a] Yields were determined on the product isolated by chromatography.
[b] Diastereomeric excesses were determined by ^{1}H NMR.
[c] Enantiomeric excesses were determined by HPLC. A *syn*-conformation was established by comparison with HPLC chromatograms of authentic samples prepared from threonines.[30]
[d] Procedure D was used to reduce **1b–f**, because it was faster than procedure A and B. Comparing, instead procedure C and D, it is evident that the presence of sucrose in the reaction medium did not produce (in this case) any effect. In fact, yields, diastereomeric excesses and enantiomeric excesses had almost the same values.

Table 3. Medium composition in the reduction reaction of ethyl 2-(4-chlorophenoxy)-3-oxoalkanoates **1a–g** by using procedure D

Compound	Substrate concentration (g/L)	Baker's yeast (g/L)	EtOH (%, v/v)	Reaction time (day)
1a	5	0.48	2	3 h
1b	1	0.33	6	5
1c	1	0.7	2	1.5
1d	1	0.7	2	4
1e	0.05	100[a]	2	9
1f	1.7	1.21	3.5	2
1g	1	0.2	6	2

[a] A further 100 g of baker's yeast were added to reaction medium after 2.5 days. Similarly, after 6 days.

Table 4. Medium composition of ethyl 2-methyl-2-(4-chlorophenoxy)-3-oxobutanoate **1g** reduction reaction by using different procedures

Procedure[a]	Baker's yeast (g)	Substrate (g)	H_2O (mL)	Sucrose (g)	EtOH (mL)
A	25	0.2	150	—	—
B	25	0.2	150	3.3	—
C	75	0.4	47	4.3	3
D	75	0.3	47	—	3

[a] For procedures A, B, C and D, see Section 5.1.

Table 5. Results of ethyl 2-methyl-2-(4-chlorophenoxy)-3-oxobuanoate **1g** reduction reaction by using different procedures

Procedure	Reaction time (day)	Yield (%)[a]	De (%)[b]	$Ee_{major\ pair}$ (%)[c]	$Ee_{minor\ pair}$ (%)[c]
A	5	85	5	>99	96
B	4	80	2	90	81
C	8	20	3	97	96
D	8	21	2	96	95

[a] Yields were determined on the product isolated by chromatography.
[b] Diastereomeric excesses were determined by ^{1}H NMR.
[c] Enantiomeric excesses were determined by HPLC.[30]

2985, 2932, 2855, 1748, 1596, 1491, 1376, 1266, 1236, 1199, 1137, 1095, 1076, 1025, 1009, 826, 738 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, δ): 7.25–7.20 (m, 2H, aromatic protons); 6.85–6.80 (m, 2H, aromatic protons); 4.44–4.42 (d, $J = 4.94$ Hz, 1H, C*H*OC_6H_4Cl); 4.30–4.22 (qd, $J = 7.15$ Hz and 1.64 Hz, 2H of CH_2CH_3 completely overlapped to the signal of C*H*OH); 2.82–2.44 (br s, 1H, O*H*: exchanges with D_2O); 1.35–1.33 (d, $J = 6.45$ Hz, 3H, CH_3CHOH); 1.27–1.22 (t, $J = 7.15$ Hz, 3H, CH_3CH_2). ^{13}C NMR (76 MHz, $CDCl_3$, δ): 169.88 (1C, *C*O); 156.48 (1C, aromatic carbon); 129.68 (2C, aromatic carbons); 127.14 (1C, aromatic carbon); 116.80 (2C, aromatic carbons); 81.15 (1C, *C*HOC_6H_4Cl) 68.57 (1C, *C*HOH); 61.85 (1C, *C*H_2CH_3); 18.60 (1C, *C*H_3CHOH); 14.33 (1C, *C*H_3CH_2). GC–MS (70 eV) (*m*/*z*) (rel int.): 260 [M(^{37}Cl)$^+$, 6], 258 [M(^{35}Cl)$^+$, 19], 214 (19), 168 (9), 167 (8), 143 (32), 142 (8), 141 (100), 139 (15), 130 (16), 129 (10), 128 (49), 111 (10), 99 (7), 75 (10), 45 (7), 43 (9). Anal. Calcd for $C_{12}H_{15}ClO_4$: C, 55.81; H, 5.81. Found: C, 55.84; H, 5.83.

5.3. Ethyl 2-(4-chlorophenoxy)-3-hydroxypentanoate 2b

Oil. $[\alpha]_D^{20} = +5.2$ (*c* 1, $CHCl_3$). 32% Yield. De = 50%. Ee = 51% (of the major stereoisomer pair). IR (neat): 3432–3237, 3050, 2964, 2920, 2872, 1730, 1642, 1490, 1458, 1262, 1216, 1092, 1017, 800, 760 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.18–7.14 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 6.80–6.71 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 4.51–4.49 (d, $J = 4.54$ Hz, 1H, *CH*OC_6H_4Cl of one stereoisomer couple); 4.43–4.41 (d, $J = 3.98$ Hz, 1H, *CH*OC_6H_4Cl of the other stereoisomer couple); 4.20–4.13 (q, $J = 7.14$ Hz, 4H, CH_2OCO, 2H for each stereoisomer couple); 3.98–3.89 (m, 2H, *CH*OH, 1H for each stereoisomer couple); 2.40–2.00 (br s, 1H for each couple of stereoisomers, OH: exchange with D_2O); 1.62–1.57 (m, 4H, CH_2CHOH, 2H for each stereoisomer couple); 1.20–1.15 (t, $J = 7.14$ Hz, 6H, CH_3CH_2O, 3H for each stereoisomer couple); 0.99–0.92 (m, 6H, CH_3CH_2-CHOH, 3H for each stereoisomer couple). ^{13}C NMR ($CDCl_3$, δ): 170.00, 156.60, 129.72, 127.30, 116.84, 80.29, 79.88, 73.92, 73.92, 61.86, 61.78, 26.46, 25.78, 14.32. GC–MS (70 eV) (*m*/*z*) (rel int.) 274 [M(^{37}Cl)$^+$, 4], 272 [M(^{35}Cl)$^+$, 13], 216 (8), 214 (24), 168 (7), 143 (33), 142 (9), 141 (100), 139 (11), 130 (13), 129 (9), 128 (38), 111 (10), 99 (9), 75 (8), 57 (8), 43 (6). Anal. Calcd for $C_{13}H_{17}ClO_4$: C, 57.35; H, 6.25. Found: C, 57.36; H, 6.27.

5.4. Ethyl 2-(4-chlorophenoxy)-3-hydroxyhexanoate 2c

Oil. $[\alpha]_D = +2.0$ (*c* 1, $CHCl_3$). 53% Yield. De = 89%. Ee = 89% (of the major stereoisomer pair). IR (neat): 3500–3150, 3065, 2961, 2934, 2872, 1737, 1595, 1492, 1465, 1379, 1282, 1238, 1199, 1094, 1075, 1030, 825, 668 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.18–7.13 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 6.77–6.73 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 4.50–4.49 (d, $J = 4.40$ Hz, 1H, *CH*OC_6H_4Cl of the major stereoisomer couple); 4.42–4.40 (d, $J = 3.98$ Hz, 1H, *CH*OC_6H_4Cl of the minor stereoisomer couple); 4.19–4.10 (2q, $J = 7.14$ Hz, 4H, CH_2O, 2H for each stereoisomer couple); 4.09–3.95 (m, 2H, *CH*OH, 1H for each stereoisomer couple); 2.28–2.10 (br s, 1H for each couple of stereoisomers, OH: exchange with D_2O); 1.65–1.42 (m, 8H, CH_2CH_2CHOH, 4H for each stereoisomer couple); 1.27–1.22 (2t, $J = 7.14$ Hz, 6H, CH_3CH_2O, 3H for each stereoisomer couple); 0.94–0.82 (m, 6H, $CH_3CH_2CH_2$, 3H for each stereoisomer couple). ^{13}C NMR ($CDCl_3$, δ): 169.98, 156.53, 129.73, 116.89, 116.84, 80.61, 80.22, 72.35, 72.23, 61.93, 35.44, 19.00, 14.33, 14.08. GC–MS (70 eV) (*m*/*z*) (rel int.) 288 [M(^{37}Cl)$^+$, 4], 286 [M(^{35}Cl)$^+$, 12], 216 (11), 214 (32), 168 (8), 143 (37), 142 (13), 141 (100), 139 (11), 130 (14), 128 (38), 113 (10), 111 (11), 75 (8), 71 (1), 43 (11). Anal. Calcd for $C_{14}H_{19}ClO_4$: C, 58.74; H, 6.64. Found: C, 58.73; H, 6.61.

5.5. Ethyl 2-(4-chlorophenoxy)-3-hydroxy-4-methylpentanoate 2d

Oil. $[\alpha]_D = +0.5$ (*c* = 1.0, $CHCl_3$). 20% Yield. De = 70%. Ee = 70% (of the major stereoisomer pair), ee = 33% (of the minor stereoisomer pair). IR (neat): 3500–3100, 2926, 2848, 1739, 1491, 1462, 1375, 1237, 1162, 1130, 1097, 953, 874, 800, 860 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.27–7.20 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 6.86–6.80 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 4.67–4.66 (d, $J = 3.29$ Hz, 1H, *CH*OC_6H_4Cl of the major stereoisomer couple); 4.63–4.61 (d, $J = 5.36$ Hz, 1H, *CH*OC_6H_4Cl of the minor stereoisomer couple); 4.28–4.21 (2q, $J = 7.14$ Hz, 4H, CH_2CH_3, 2H for each stereoisomer couple); 3.86–3.82 (t, $J = 5.36$ Hz, 1H, *CH*OH of the major stereoisomer couple); 3.75–3.72 (dd, $J = 3.29$ and 7.62 Hz, 1H, *CH*OH of the minor stereoisomer couple); 2.36–2.28 (m, 1H, *CH*$(CH_3)_2$ of one stereoisomer couple); 2.05–1.97 (m, 1H, *CH*$(CH_3)_2$ of the other stereoisomer couple); 1.70–1.50 (br s, 1H for each couple of stereoisomers, OH: exchange with D_2O); 1.29–1.22 (t, $J = 7.14$ Hz, 6H, CH_3CH_2, 3H for each stereoisomer couple); 1.09–1.03 (2d, $J = 6.73$ Hz, 6H, $(CH_3)_2$CH of the major stereoisomer couple); 1.00–0.92 (2d, $J = 6.73$ Hz, 6H, $(CH_3)_2$CH of the minor stereoisomer couple). ^{13}C NMR ($CDCl_3$, δ): 170.25, 156.00, 129.79, 127.15, 116.67, 78.54, 78.14, 70.78, 61.94, 31.21, 29.38, 27.44, 14.34. GC–MS (70 eV) (*m*/*z*) (rel int.) 288 [M(^{37}Cl)$^+$, 3], 286 [M(^{35}Cl)$^+$, 9], 216 (10), 214 (31), 168 (6), 143 (32), 141 (100), 130 (12), 128 (34), 113 (10), 111 (12), 75 (8), 71 (9), 43 (17), 41 (9). Anal. Calcd for $C_{14}H_{19}ClO_4$: C, 58.74; H, 6.64. Found: C, 58.74; H, 6.63.

5.6. Ethyl 2-(4-chlorophenoxy)-3-hydroxy-4,4-dimethylpentanoate 2e

Due to the small amount of **2e** formed, it was identified by comparing the GC–MS spectrum obtained when reducing **1e** by $NaBH_4$ and in the presence of baker's yeast. Oil. GC–MS (70 eV) (*m*/*z*) (rel int.) 302 [M(^{37}Cl)$^+$, 3], 300 [M(^{35}Cl)$^+$, 9], 227 (2), 216 (13), 214 (39), 168 (6), 143 (35), 141 (100), 139 (8), 130 (15), 128 (33), 111 (11), 57 (27), 41 (10).

5.7. Ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-3-phenylpropanoate 2f

Mp = 82.8–83.9 °C (by evaporation of ethyl ether), white solid. 33% Yield. De >99%. Ee >99%. $[\alpha]_D^{20} = +27.2$ (*c* 1.0, $CHCl_3$). IR (KBr): 3550–3250, 3225, 2955, 2926, 2845, 1733, 1596, 1582, 1490, 1450, 1375, 1261, 1235, 1216, 1192, 1093, 1050, 1026, 824, 760 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.50–7.16 (m, 7H, aromatic protons); 6.80–6.73 (m, 2H, aromatic protons); 5.17–5.15 (d, J = 5.50 Hz, 1H, *CH*OH); 4.69–4.67 (d, J = 5.50 Hz, 1H, *CH*OC_6H_4Cl); 4.11–4.04 (m, 2H, CH_2CH_3); 3.60–3.20 (br s, 1H, OH: exchange with D_2O); 1.09–1.05 (t, J = 7.14 Hz, 3H, CH_3CH_2). ^{13}C NMR ($CDCl_3$, δ): 169.47, 139.12, 129.73, 129.66, 128.73, 128.61, 126.93, 126.89, 117.19, 82.34, 81.45, 75.03, 74.38, 61.82, 14.17. GC–MS (70 eV) (*m*/*z*) (rel int.) 322 [M(^{37}Cl)$^+$, 0.1], 320 [M(^{35}Cl)$^+$, 0.2], 302 [M(^{35}Cl)$^+$ − 18, 0.3], 247 (2), 216 (22), 214 (68), 143 (34), 141 (100), 128 (20), 113 (16), 111 (25), 107 (19), 106 (27), 105 (36), 91 (14), 79 (16), 77 (49), 75 (15), 51 (16), 50 (10). Anal. Calcd for $C_{17}H_{17}ClO_4$: C, 63.25; H, 5.31. Found: C, 63.76; H, 5.33.

5.8. Ethyl 2-methyl-2-(4-chlorophenoxy)-3-hydroxy-butanoate 2g

(Tables 4 and 5). Oil. $[\alpha]_D$ = +1.1 (*c* = 1.0, $CHCl_3$) from procedure A. IR (neat): 3500–3100, 3062, 2983, 2936, 1736, 1593, 1490, 1443, 1376, 1239, 1094, 1047, 1012, 850, 823 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.21–7.18 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 6.85–6.82 (m, 4H, aromatic protons, 2H for each stereoisomer couple); 4.28–4.21 (q, J = 7.14 Hz, 4H, CH_2O-CO, 2H for each stereoisomer couple); 4.18–4.12 (q, J = 6.41 Hz, 2H, *CH*OH, 1H for each stereoisomer couple); 2.80–2.40 (br s, 1H for each stereoisomer couple, OH: exchange with D_2O); 1.46 (s, 3H, CH_3C_q of one stereoisomer couple); 1.40 (s, 3H, CH_3C_q of the other stereoisomer couple); 1.28–1.17 (m, 12H, 3H of CH_3CH_2 and 3H of CH_3CH for each stereoisomer couple, respectively). ^{13}C NMR ($CDCl_3$, δ): 172.55, 153.82, 129.48, 128.13, 121.27, 121.18, 85.80, 72.74, 72.47, 61.91, 16.85, 15.47, 14.27. GC–MS (70 eV) (*m*/*z*) (rel int.) 274 [M(^{37}Cl)$^+$, 3], 272 [M(^{35}Cl)$^+$, 8], 228 (23), 199 (13), 157 (11), 155 (34), 130 (34), 129 (13), 128 (100), 111 (9), 99 (19), 43 (36). Anal. Calcd for $C_{13}H_{17}ClO_4$: C, 57.35; H, 6.25. Found: C, 57.36; H, 6.27.

5.9. Synthesis of ethyl 2-(4-chlorophenoxy)-3-acetoxy-2-butenoate 3

Acetic anhydride (48 mL) was added dropwise to a solution of **1a** (2.6 g, 10.1 mmol) in pyridine (47 mL). The reaction mixture was stirred at room temperature, while monitoring the reaction progress by GC. After 15 h, ice was added. The mixture was extracted three times with ethyl acetate. The organic layer was washed three times with 1 M HCl, three times with a saturated solution of $NaHCO_3$ and once with water. The organic extracts were dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The product was isolated by chromatography (mobile phase: petroleum ether/ethyl ether = 7:3) as a mixture of the geometric isomers (*E*/*Z* = 70:30). Oil. 51% Yield.

5.9.1. (*Z*)-3. (Table 6). IR: (neat): 3106, 3069, 2984, 2937, 1770, 1724, 1663, 1593, 1488, 1369, 1304, 1215, 1180, 1090, 1068, 1012, 829 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.25–7.22 (m, 2H, aromatic protons); 6.97–6.94 (m, 2H, aromatic protons); 4.14–4.06 (q, J = 7.14 Hz, 2H, OCH_2CH_3); 2.25 (s, 3H, CH_3CO); 1.99 (s, 3H, $CH_3C{=}C$); 1.14–1.09 (t, J = 7.14 Hz, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$, δ): 168.73 (1C, CH_3CO); 161.31 (1C, *C*OOEt); 152.68 (1C, C_{Ar}); 151.61 (1C, C_{olefin}); 133.90 (1C, C_{Ar}); 129.82 (1C, C_{Ar}); 123.17 (1C, C_{olefin}); 116.61 (1C, C_{Ar}); 61.32 (1C, OC_2CH_3); 21.07 (1C, CH_3CO); 17.13 (1C, $CHs_3C{=}C$); 14.15 (1C, CH_3CH_2). GC–MS (70 eV) (*m*/*z*) (rel int.) 300 [M(^{37}Cl)$^+$, 1], 298 [M(^{35}Cl)$^+$, 4], 258 (35), 257 (15), 256 (100), 212 (7), 211 (7), 210 (21), 148 (10), 147 (85), 142 (28), 139 (78), 128 (7), 111 (l9), 75 (l4), 43 (89).

5.9.2. (*E*)-3. (Table 6). IR (neat): 3106, 3069, 2984, 2937, 1770, 1724, 1663, 1593, 1488, 1369, 1304, 1215, 1180, 1090, 1068, 1012, 829 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.21–7.18 (m, 2H, aromatic protons); 6.86–6.83 (m, 2H, aromatic protons); 4.17–4.10 (q, J = 7.14 Hz, 2H, OCH_2CH_3); 2.40 (s, 3H, CH_3CO); 2.05 (s, 3H, $CH_3C{=}C$); 1.14–1.09 (t, J = 7.14 Hz, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$, δ): 167.62 (1C, CH_3CO); 163.04 (1C, *C*OOEt); 156.09 (1C, C_{Ar}); 151.61 (1C, C_{olefin}); 133.01 (1C, C_{Ar}); 129.55 (1C, C_{Ar}); 127.58 (1C, C_{olefin}); 116.97 (1C, C_{Ar}); 61.55 (1C, OCH_2CH_3); 20.75 (1C, CH_3CO); 17.92 (1C, $CH_3C{=}C$); 14.15 (1C, CH_3CH_2). GC–MS (70 eV) (*m*/*z*) (rel int.) 300 [M(^{37}Cl)$^+$, 2], 298 [M(^{35}Cl)$^+$, 7], 258 (38), 257 (16), 256 (100), 212 (9), 211 (12), 210 (24), 148 (9), 147 (80), 141 (26), 139 (69), 128 (9), 111 (18), 75 (l4), 43 (72) (Tables 6 and 7).

5.10. Synthesis of ethyl 2-(4-chlorophenoxy)-3-methoxy-2-butenoate 4

To a solution of **1a** (1 g, 3.91 mmol) in dry *N*,*N*-dimethylformamide (4 mL) kept at room temperature under an

Table 6. Medium composition of ethyl 2-(4-chlorophenoxy)-3-acetoxy-2-butenoate **3** reduction reaction by using different procedures

Procedure[a]	Baker's yeast (g/L)	Substrate (g/L)	EtOH (mL)	Reaction time
B	150	3.5	—	2 days
D	400	5	12.5	20 h

[a] For procedures A, B, C and D, see also Section 5.1.

Table 7. Medium composition of ethyl 2-(4-chlorophenoxy)-3-methoxy-2-butenoate **4** reduction reaction by using different procedures

Procedure[a]	Baker's yeast (g/L)	Substrate (g/L)	EtOH (mL)	Reaction time
D	800	5	12.5	6 days

[a] For procedures A, B, C and D, see also Section 5.1.

N_2 atmosphere, Cs_2CO_3 (1.273 g, 3.91 mmol) was added. CH_3I (2.5 mL, 39.06 mmol) in dry *N*,*N*-dimethylformamide (8 mL) was then added dropwise. The solution was stirred at room temperature. Reaction progress was monitored by TLC (mobile phase: petroleum ether/ethyl ether = 8:2). After 22 h, ethyl ether was added and the mixture obtained, washed three times with saturated $Na_2S_2O_3$ and three times with saturated $NaHCO_3$. The extracts were dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The product was isolated by chromatography (mobile phase: petroleum ether/ethyl ether = 8:2) as a colourless oil. 5% yield (Table 7). IR (neat): 3050, 2922, 2856, 1706, 1627, 1593, 1486, 1456, 1381, 1273, 1223, 1137, 1098, 1006, 826 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 7.22–7.19 (m, 2H, aromatic protons); 6.85–6.82 (m, 2H, aromatic protons); 4.15–4.08 (q, $J = 7.14$ Hz, 2H, OCH_2CH_3); 3.75 (s, 3H, OCH_3); 2.50 (s, 3H, $CH_3C{=}C$); 1.15–1.10 (t, $J = 7.14$ Hz, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$, δ): 165.28 (1C, $COCH_3$); 160.65 (1C, *C*O); 157.11 (1C, C_{Ar}); 129.45 (2C, C_{Ar}); 126.63 (1C, C_{Ar}); 120.55 (1C, *C*COOEt); 116.30 (2C, C_{Ar}); 60.88 (1C, OCH_2CH_3); 56.37 (1C, OCH_3); 14.59 (1C, CH_3CH_2O); 14.34 (1C, CH_3CHOCH_3). GC–MS (70 eV) (*m*/*z*) (rel int.) 272 [$M(^{37}Cl)^+$, 34], 270 [$M(^{35}Cl)^+$, 100], 225 (17), 199 (11), 197 (32), 175 (33), 169 (18), 167 (11), 162 (43), 161 (10), 147 (15), 141 (14), 139 (45), 137 (28), 125 (14), 115 (27), 113 (12), 111 (32), 103 (12), 99 (11), 85 (11), 75 (38), 57 (21), 43 (70).

6. X-ray analysis

To establish the absolute configuration at C (7) and C (9) in an unambiguous manner, suitable crystals were grown and subjected to single-crystal X-ray analysis, using a Nonius Kappa CCD area detector diffractometer equipped with a fine focus sealed graphite-monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). Data for ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-3-phenylpropanoate were collected at 293(2) K. Data reduction and cell refinement were carried out with the programs DENZO[32] and COLLECT.[33] The structure was solved by the direct methods procedure of SIR97,[34] while the refinement processes were carried out on a full matrix least squares technique using SHELXL-97.[35] Detailed crystal data and geometrical parameters are deposited in the Supporting Information (cif file).[36] The asymmetric unit of ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-3-phenylpropanoate with the atomic numbering scheme is depicted in Figure 2.

Pertinent crystallographic data for ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-3-phenylpropanoate: $C_{17}H_{17}ClO_4$, $M_r = 320.76$ g cm^{-3}, monoclinic, space group: $P2_1$, $a = 10.4419$ (1), $b = 5.5948$ (1), $c = 13.5736$ (4) Å, $\beta = 97.163$ (1)°, Cell volume = 786.79 (4) $Å^3$, $Z = 2$, $T = 293$ (2) K, $\rho_c = 1.354$ g cm^{-3}, $\mu = 0.258$ mm^{-1}, θ range = 2.33°–30.01°, *hkl* indices $-14 \leqslant h \leqslant 14$, $-7 \leqslant k \leqslant 7$, $-19 \leqslant l \leqslant 18$, reflections (measured) = 12123, reflections (unique) = 4426, reflections (unique [$F_o > 2\sigma\{|F_o|\}$]): 3355, $R_{int} = 0.032$, 267 parameters, R_1/wR_2 (all data): 0.0665/0.1129, R_1/wR_2 ($I > 2\sigma(I)$): 0.0420/0.0992, Flack parameter = −0.04 (6), largest diff. peak/hole: 0.430/−0.501 e $Å^{-3}$.

Acknowledgements

Work carried out under the framework of the National Project 'Progettazione, Sintesi e Valutazione Biologica di Nuovi Farmaci Cardiovascolari' was supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, Rome). Thanks are also due to the University of Bari and CNR-Istituto di Chimica dei Composti OrganoMetallici-ICCOM/Sezione di Bari (Italy).

References

1. Wills, M.; Hannedouche, J. *Curr. Opin. Drug Discov. Dev.* **2002**, *5*, 881–891.
2. Procter, G. *Asymmetric Synthesis*; Oxford University Press, 1996.
3. Corey, E. J.; Helal, C. J. *Angew. Chem.* **1998**, *37*, 1986–2012.
4. Fontaine, E.; Namane, C.; Meneyrol, J.; Geslin, M.; Serva, L.; Roussey, E.; Tissandiè, S.; Maftouh, M.; Roger, P. *Tetrahedron: Asymmetry* **2001**, *12*, 2185–2189.
5. Chen, W.-Y.; Lu, J.; Shen, Z.-X.; Lang, J.-P.; Zhang, L.-F.; Zhang, Y.-W. *Chinese J. Chem.* **2003**, *21*, 192–195, Chem. Abstr. 138:305760.
6. Hu, J.-B.; Zhao, G.; Ding, Z.-D. *Angew. Chem., Int. Ed.* **2001**, *40*, 1109–1111.
7. Vinogradov, M. G.; Gorshkova, L. S.; Pavlov, V. A.; Mikhalev, O. V.; Chel'tsova, G. V.; Razmanov, I. V.; Ferapontov, V. A.; Malyshev, O. R.; Heise, G. L. *Russ. Chem. Bull.* **2000**, *49*, 460–465, Chem. Abstr. 133:252092.
8. Hu, X.-M.; Liu, J. Wuhan *Univ. J. Nat. Sci.* **1999**, *4*, 205–210, Chem. Abstr. 132:107497.
9. Knowles, W. S.; Sabacky, M. J. *J. Chem. Soc., Chem. Commun.* **1968**, 1445–1446.
10. Knowles, W. S.; Sabacky, M. J.; Vineyard, B. D. *J. Chem. Soc., Chem. Commun.* **1972**, 10–11.
11. Kagan, H. B.; Dang, J. P. *J. Am. Chem. Soc.* **1972**, *94*, 6429–6433.
12. Carneiro, J. W. M.; Oliveira, C. S. B.; Passos, F. B.; Aranda, D. A. G.; Souza, P. R. N.; Antunes, O. A. C. *J. Mol. Catal. A: Chem.* **2001**, *170*, 235–243.
13. Margitfalvi, J. L.; Tfirst, E. *J. Mol. Catal. A: Chem.* **1999**, *139*, 81–95.
14. Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659–2681.
15. Ferorelli, S.; Franchini, C.; Loiodice, F.; Perrone, M. G.; Scilimati, A.; Sinicropi, M. S.; Tortorella, P. *Tetrahedron: Asymmetry* **2001**, *12*, 853–862, and references cited therein.
16. Di Nunno, L.; Franchini, C.; Scilimati, A.; Sinicropi, M. S.; Tortorella, P. *Tetrahedron: Asymmetry* **2000**, *11*, 1571–1583.
17. Servi, S. *Synthesis* **1990**, 1–25.
18. Roberts, S. M. *Biocatalysts for Fine Chemicals Synthesis*; John Wiley & Sons: Chichester, 1999.
19. Faber, K. *Biotransformations In Organic Chemistry*, 2nd ed.; Springer: Berlin, 1995.
20. Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformation in Preparative Organic Chemistry*; Academic: San Diego, 1989.
21. Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J. C. *Circulation* **1998**, *98*, 2088–2093.

22. Bettoni, G.; Ferorelli, S.; Loiodice, F.; Tangari, N.; Tortorella, V.; Gasparrini, F.; Misiti, D.; Villani, C. *Chirality* **1992**, *4*, 193–203.
23. Loiodice, F.; Longo, A.; Bianco, P.; Tortorella, V. *Tetrahedron: Asymmetry* **1995**, *6*, 1001–1011.
24. Chiaia Noya, F.; Ferorelli, S.; Franchini, C.; Scilimati, A.; Sinicropi, M. S.; Tortorella, P. *Il Farmaco* **1996**, *51*, 293–296.
25. Conte-Camerino, D.; Tortorella, V.; Ferrannini, E.; Bryant, S. H. *Arch. Toxicol.* **1984**, *7*, 482–484.
26. Feller, D. R.; Kamanna, V. S.; Newman, H. A. I.; Romstedt, K. J.; Witiak, D. T.; Bettoni, G.; Bryant, S. H.; Conte-Camerino, D.; Loiodice, F.; Tortorella, V. *J. Med. Chem.* **1987**, *30*, 1265–1267.
27. Bettoni, G.; Loiodice, F.; Tortorella, V.; Conte-Camerino, D.; Mambrini, M.; Ferrannini, E.; Bryant, S. H. *J. Med. Chem.* **1987**, *30*, 1267–1270.
28. Esbenshade, T. A.; Kamanna, V. S.; Newman, H. A. I.; Tortorella, V.; Witiak, D. T.; Feller, D. R. *Biochem. Pharmacol.* **1990**, *40*, 1263–1274.
29. Perrone, M. G.; Santandrea, E.; Dell'Uomo, N.; Giannessi, F.; Milazzo, F. M.; Sciarroni, A. F.; Scilimati, A.; Tortorella, V. *Eur. J. Med. Chem.* **2004**, in press.
30. Sample standards were prepared from threonines first transformed into the corresponding chlorohydrins and then allowed to react with caesium 4-chlorophenate (*Tetrahedron: Asymmetry* **2004**, submitted for publication).
31. Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441–451.
32. Otwinowski, Z.; Minor, W. In *Methods in Enzymology, Macromolecular Crystallography. Part A*; Carter, C. W., Sweet, R. M., Eds.; Academic Press; Southwestern Medical Centre at Dallas, HKL DENZO and Scalepack: USA, 1997; Vol. 276, p 307.
33. COLLECT. Nonius, 1998. KappaCCD Server Software. Nonius B. V., Delft. The Netherland.
34. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
35. Sheldrick, G. M. SHELXL-97. Program for the Refinement of Crystal Structures. University of Gottingen, Germany (1997).
36. Crystallographic data (excluding structure factors) for ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxy-3-phenylpropanoate have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-241644. Copies of available material can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
37. Seebach, D.; Roggo, S.; Maetzke, T.; Braunschweiger, H.; Cercus, J.; Kreiger, M. *Helv. Chim. Acta* **1987**, *70*, 1605–1615.
38. Deol, B. S.; Ridley, D. D.; Simpson, G. W. *Aust. J. Chem.* **1976**, *29*, 2459–2467.
39. Kitazume, T.; Koayashi, T. *Synthesis* **1987**, 187–188.