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The Microbial Reduction of 2-Chloro-3-oxoesters

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Abstract: Several aliphatic or aromatic 2-chloro-3-oxoesters are stereoselectively reduced by yeast or fungal strains, affording in fair to good yield and high enantiomeric excess some of the respective 2-chloro-3-hydroxyester stereoisomers.

INTRODUCTION

The reduction of (±)-2-substituted 3-oxoesters by baker's yeast or other microorganisms has been already shown, in several examples, to give in high yield and excellent enantiomeric excess one single stereomer of the corresponding 2-substituted 3–hydroxyesters, making use of the fast spontaneous epimerization of the ester substrate in the incubation medium, coupled with the stereospecific reduction of a single enantiomer. Such a diastereoselective and enantiospecific preparation of general synthons, having two stereogenic centers, from a configurationally unstable racemic precursor, has been systematically explored and shown to give practical results with a series of variously substituted β-ketoester substrates, such as 2-methyl[1-8], 2-alkyl-[9-11], 2-hydroxy[9-12], 2-acetoxy[13], 2-sulfenyl[14,15], and 2-(acyl)amino-3-oxoalkanoates[16,17], in addition to various cyclic β-oxoesters derived from cycloalkanones[1,9,14,18-23]. The chemical counterpart of this method (the "dynamic kinetic resolution"), using catalytic hydrogenation of 2-substituted-3-oxoesters in the presence of BINAP- or chiral diphosphine-Ru(II) complexes, has been recently and concurrently developped[24-31].

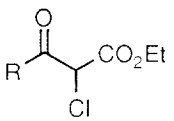
As a continuation of this work, we have been interested in examining the potential diastereo- and enantioselective microbial reduction of 2-chloro-3-oxoesters, in order to prepare from them asymmetric α,β-functionalized esters of high synthetic value[32]. For example, a direct nucleophilic substitution of 2-chloro-3-hydroxyesters with a nitrogen substituent could lead to stereospecific syntheses of α-amino β-hydroxy acids[33], such as the stereoisomers of 3-phenylserine, 3-hydroxyleucine, MeBmt, or various statine analogs. On the other hand, the conversion to the corresponding 2,3-epoxyesters[34], with various relative and absolute configurations, will afford most valuable synthons or key intermediates for the preparation of a number of optically active compounds of biological interest, such as 3-amino-2-hydroxyacids[35] (isothreonine, 3-amino-2-hydroxy-phenylbutyric acid, a constituent of bestatin, N-benzoyl-(2*R*,3*S*)-3-phenylisoserine, the side chain of Taxol), or (2*S*,3*S*)-Diltiazem® [36].

2-Halogeno-3-hydroxyesters (or acids) have been previously obtained in definite relative and/or absolute configuration by purely chemical methods: hydride reduction[37,38] or catalytic hydrogenation[31,39,40] of the corresponding 2-halogeno-3-oxoesters, nitrous halogeno-deamination of natural β-hydroxy α-amino acids[41],

addition of oxyhalogenated acids to α,β-unsaturated esters[42-51], eventually catalyzed by a peroxidasic enzyme[52-54], stereocontrolled aldolisation[55-62], Darzens[63] and Reformatsky[64,65] reactions, etc, eventually followed by classical[66] or enzymatic[67] resolution.

On the contrary, little data about the microbial reduction of 2-halogeno-3-oxoesters into isomeric 2-halogeno-3-hydroxyesters are available. Baker's yeast has been previously used to reduce (±)-ethyl 2-chloro-3-oxobutanoate to a 1:1 mixture of *syn* and *anti*-chlorohydroxyesters[68,69], but an estimation of the stereochemical purity of the products was not directly available, as the derived epoxyacids were crystallized as brucine salts. In a similar reaction, Hamdani *et al.*[70] have claimed the exclusive formation of the *syn* (2*R*,3*S*)-chlorohydroxyester. The reduction of 2-fluoro-3-oxoalkanoic esters by baker's yeast has been described[71], but the absolute configuration of the resulting predominating *syn* products has not been determined. A recent patent[72] has claimed the preparation of all four stereomeric ethyl 2-chloro-3-hydroxy-3-(4'-methoxy)phenyl propionates by reduction of the corresponding (±)-2-chloro-3-oxoester, using various microorganisms, bacteria, yeasts or fungi; the effective yields of the reduced products were generally poor, indicating low reduction rates and/or undesired conversion to other products.

On the other hand, the stereoselective reduction of (±)-3-chloro-2-oxoesters by baker's yeast has been recently described[73], affording essentially 2*S*-esters, but as a 1:1 mixture of *anti* and *syn* isomers, as expected accounting for an impossible epimerisation at position-3. Baker's yeast reduction of oxoesters substituted with chlorine in other positions have also been described[74].



1a: R= CH_3
b: R= CH_2-CH_2-CH_3
c: R= CH-$(CH_3)_2$
d: R= Ph
e: R= *p*-MeO-Ph
f: R= CH_2-O-CH_2Ph

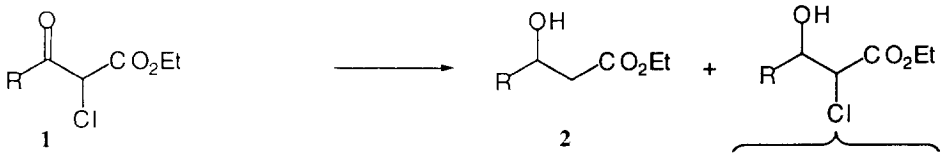
We report here our investigations about the stereospecific reduction of (±)-ethyl 2-chloro-3-oxo-3-alkyl and 3-aryl propionates **1a-f** by baker's yeast and several other fungal strains and the configuration of the resulting chlorohydroxyesters. The use of such synthons by stereospecific conversion to glycidic esters followed by nucleophilic opening of the epoxide ring, particularly illustrated by the synthesis of amino derivatives, will be the subject of a forthcoming paper.

RESULTS AND DISCUSSION

When we first attempted to reduce various 2-chloro-3-oxoesters **1a-e** by baker's yeast, at a substrate concentration of 1 g L^{-1}, we were surprised to obtain, in good yield and sometimes exclusively, reduced dechlorinated products **2** (Table 1). As 2-chloro-3-hydroxyesters were stable in these conditions, it was clear that an enzymic dechlorination mechanism of the chlorooxoesters was operating. Such a reaction has been previously described but in marginal amounts with some α-halogeno ketones (mostly iodo derivatives) and possibly attributed to a monoelectronic reduction mechanism involving NADH[75-77].

However, using higher substrate concentrations, a remarkable change in the reaction course was observed (Table 1), making the expected 2-chloro-3-hydroxyesters the main products of the reduction, and suggesting a deactivation of the dehalogenating activity in the presence of moderate amounts of the chlorooxoesters. Nakamura[78] has recently shown that a similar pretreatment of yeast with ethyl chloroacetate was able to modify the stereochemistry of the reduction products, by deactivating some of the dehydrogenase activities of the microorganism.

Table 1: Concentration-Dependent Reduction of (±)-2-Chloro-3-oxoesters by Baker's Yeast



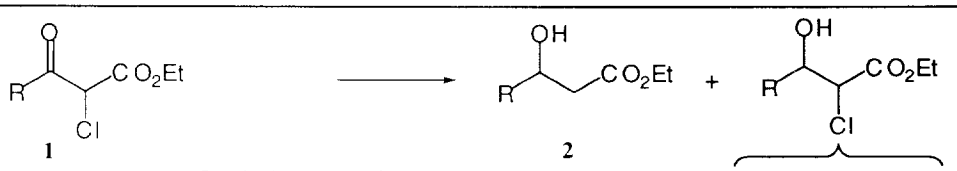
1 2

Substrate	g L^{-1}	Incubation time (hours)	Conversion (%)	(%)	*Syn*-3 % yield (% ee)	*Anti*-4 % yield (% ee)
1a (R= CH_3)	1	2	100	100	-	-
	2	2.5	100	40	38 (93)[a]	22 (70)[b]
	10	24	75	6	28 (88)	37 (85)
1b (R= *n*-C_3H_7)	1	4.5	95	95	-	-
1c (R= *i*-C_3H_7)	1	24	100	45[c]	-	-
1d (R= Ph)	1	7	100	100	-	-
	5	48	60	30	4[d]	17[e]
	10	144	23	7	3	6
1e (R=*p*-MeO-Ph)[f]	1	24	95	95	-	-

[a] (2*R*,3*S*)-ester. [b] (2*S*,3*S*)-ester. [c] beside 55% of dechlorinated ketoester [d] (2*S*,3*R*)-ester. [e] (2*R*,3*R*)-ester. [f] Methyl ester.

It was possible to make use of this feature in a practical way: after an initial treatment with ethyl 2-chloro-3-oxobutanoate (2 g L^{-1}), baker's yeast exhibited a greatly decreased dehalogenating activity and efficiently reduced added chlorooxoesters, even at 1 g L^{-1} concentration (Table 2). Anyway, excepted for the isopropyl derivative **1c**, diastereomeric and enantiomeric excesses of the hydroxychloroesters obtained were low, indicating a limited enantioselectivity (and stereospecificity) in the reduction by baker's yeast enzymes.

Table 2: Reduction of (±)-2-Chloro-3-oxoesters (1 g L^{-1}) by Baker's Yeast after Pretreatment[a] with Ethyl 2-chloroacetoacetate



1 2

Substrate	Incubation time (hours)	Conversion (%)	(%)	*Syn*-3 % yield (% ee)	*Anti*-4 % yield (% ee)
1a (R= CH_3)	3	100	5	35 (88)[b]	60 (85)[c]
1b (R= *n*-C_3H_7)	20	100	10	9 (86)[b]	72 (21)[c]
1c (R= *i*-C_3H_7)	20	100	14	-	86 (19)[d]
1d (R= Ph)	16	94	43	18 (68)[e]	33 (30)[d]

[a] Fresh baker's yeast was preincubated for 2 h in water at 27°C with ethyl 2-chloro-3-oxobutanoate (2 g L^{-1}). After centrifugation, the biomass was taken up in potassium phosphate buffer pH 6.0, and incubated with chloro oxoesters **1a-d** as indicated. [b] (2*R*,3*S*)-ester. [c] (2*S*,3*S*)-ester. [d] (2*R*,3*R*)-ester. [e] (2*S*,3*R*)-ester.

Other microorganisms were screened for their ability to reduce the same chlorooxoesters. Some significant results obtained with alkyl and aryl chlorooxoesters as substrates are given in Table 3 and 4 respectively. Yields, diastereomeric and enantiomeric ratios of the crude incubation products were determined by GC (see experimental part). Relative configurations were determined either by NMR techniques on purified

chlorohydroxyesters, or by GC analysis of incubation extracts. Absolute configurations of the reduction products were assigned from their specific rotation or the specific rotation of the corresponding dechlorinated hydroxyesters obtained by catalytic hydrogenation.

Table 3: Reduction of 2-Chloro-3-oxo esters **1a-c** (1 g L^{-1}) by Yeasts and Fungi

			2-Chloro-3-hydroxyesters				
	Microorganisms	Incubation time (hours)	Yield (%)	Configuration *Syn/anti*	*Syn* (ee %)	*Anti* (ee %)	
1a	*Geotrichum candidum*	4[a]	75[b]	24:76	2*R*,3*S*	(81)	2*S*,3*S* (44)
	Rhizopus arrhizus ATCC 11145	48	66[b]	55:45	2*R*,3*S*	(70)	2*S*,3*S* (66)
1b	*Saccharomyces montanus* CBS 6772	24	30	14:86	2*S*,3*R*	(30)	2*R*,3*R* (15)
	Mortierella isabellina MMP108	24	26	13:87	2*S*,3*R*	(21)	2*R*,3*R* (92)
	Geotrichum candidum	48	30	2:98	nd[c]		2*R*,3*R* (53)
	Mucor plumbeus CBS 110-16	48	**46**	**6:94**	**2*S*,3*R***	**(88)**	**2*R*,3*R* (99)**
	Rhizopus arrhizus ATCC 11145	72	30	47:53	2*S*,3*R*	(97)	2*R*,3*R* (84)
	Sporotrichum exile QM 1250	24	48	27:73	2*S*,3*R*	(95)	2*R*,3*R* (67)
1c	*Pichia anomala* NRRL Y40	24	**30**	**74:26**	**2*R*,3*S***	**(95)**	**2*S*,3*S* (95)**
	Mortierella isabellina NRRL1757	24	18	63:37	2*R*,3*S*	(80)	2*R*,3*R* (19)
	Mucor plumbeus CBS 110-16	48	9	9:91	2*R*,3*S*	(15)	2*R*,3*R* (97)

[a] Substrate concentration: 2 g L^{-1} [b] determined by GC, without accounting for volatile by-products. [c] nd = not determined

Table 4: Reduction of 2-Chloro-3-oxo esters **1d-f** (1 g L^{-1}) by Yeasts and Fungi

			2-chloro-3-hydroxyesters				
	Microorganisms	Incubation time (hours)	Yield (%)	Configuration *Syn/anti*	*Syn* (ee %)	*Anti* (ee %)	
1d	*Rhodotorula glutinis* NRRL Y1091	2	**57**	**6:94**	nd[a]	**2*R*,3*R***	**(95)**
	Mucor plumbeus CBS 110-16	24	42	51:49	2*S*,3*R*	(98)	2*R*,3*R* (90)
	Mucor racemosus	24	**45**	**91:9**	**2*S*,3*R***	**(96)**	**2*R*,3*R* (71)**
	Sporotrichum exile QM 1250	24	**60**	**93:7**	**2*S*,3*R***	**(95)**	**2*R*,3*R* (74)**
1e	*Sporotrichum exile* QM 1250	24	nd[a]	**98:2**	**2*S*,3*R***	**(98)**	nd[a]
	Rhizopus arrhizus ATCC 11145	24	nd[a]	**98:2**	**2*S*,3*R***	**(98)**	nd[a]
1f	*Aspergillus niger* ATCC 9142	5	29	13:87	nd[a]	2*R*,3*R*	(70)
	Rhodotorula glutinis NRRL Y1091	1.5	43	12:88	nd[a]	2*R*,3*R*	(30)
	Mucor plumbeus CBS 110-16	6	37	15:85	nd[a]	2*S*,3*S*	(20)
	Mucor racemosus	6	44	22:78	nd[a]	2*R*,3*R*	(30)
	Geotrichum candidum	6	**39**	**4:96**	nd[a]	**2*S*,3*S***	**(90)**

[a] nd = not determined.

Beside dechlorination products, mostly found with alkyl substrates, decarboxylation products, mostly deriving from aryl substrates, were responsible for lower yields and formation of by-products such as α-chloroketones and α-chloroalcools. However with every substrate (excepted for **1a**), as emphasized in the tables, it was possible to select strains affording moderate to excellent yields of a major diastereomer with high enantiomeric excess.

Using the best strains identified in the screening experiments, it was possible to obtain, after flash chromatography, on a *1-5 g scale*, fair yields of several enantiomerically pure chlorohydroxyesters (Table 5), by optimization of the substrate concentration and incubation time, ensuring full conversion of the chlorooxoesters substrates. Both diastereomers could be separately obtained, as pure 3*R*-enantiomers from the ketoester **1d**.

Table 5: Preparative Microbial Reductions of Chlorooxoesters to Chlorohydroxyesters

					Syn		*Anti*	
		g.L^{-1}	(hours)	*Syn/anti*	Absolute configuration	yield	Absolute configuration	yield
1b	*M. plumbeus* CBS 110 16	1	24	1:9	-		**2*R*,3*R*** $[\alpha]_D^{20}$= +8.5 (c 1, $CHCl_3$) (>98% de, 92% ee)	50%[a]
1c	*P. anomala* NRRL Y40	1	30	1:1	**2*R*,3*S*** (97% ee)	[b]	**2*S*,3*S*** (26% ee)	[b]
1d	*R. glutinis* NRRL Y1091	5	72	2:98	-		**2*R*,3*R*** $[\alpha]_D^{20}$= –42 (c 1.5, $CHCl_3$) (96% de, 98% ee)	51%[a]
	M. racemosus	1	24	83:17	**2*S*,3*R*** $[\alpha]_D^{20}$= –3 (c 1.7, $CHCl_3$) $[\alpha]_{365}^{20}$= +7 (c 1.7, $CHCl_3$) (>98% de, 96% ee)	45%[a]	-	
1e[c]	*S. exile* QM 1250	1.5	24	98:2	**2*S*,3*R*** $[\alpha]_D^{20}$= +14 (c 1, MeOH) $[\alpha]_{365}^{20}$= +25 (c 1, $CHCl_3$) (96% de, 96% ee)	52%[a]	-	
	R. arrhizus ATCC 11145	0.55	24	98:2	**2*S*,3*R*** $[\alpha]_{365}^{20}$= +25 (c 1, $CHCl_3$) (96% de, 98% ee)	35%[a]	-	
1f	*G. candidum*	1	24	4:96	-		**2*S*,3*S*** $[\alpha]_{365}^{20}$= –4.5 (c 1.2, MeOH) (92% de, 90% ee)	34%[a]

[a] after purification by flash chromatography. [b] not separated by repeated chromatography, 30% combined yield. [c] Methyl ester.

Only in the case of the chlorohydroxyester deriving from **1c**, both diastereomers were simultaneously obtained and could not be usefully separated. The 4-benzyloxy-chlorohydroxyester derived from **1f** was obtained with a high optical purity as the (2*S*,3*S*)-enantiomer, and constitutes a new and versatile multifunctionalized asymmetric synthon. The *syn* (2*S*,3*R*)-*p*-methoxyphenyl chlorohydroxyester derived from **1e** might represent a useful intermediate for an original synthesis of Diltiazem[79].

Experimental

Material and Methods: All chemical used in synthetic procedures were reagent grade or better. 3-Oxoesters were commercial products, except ethyl 3-oxo-4-benzyloxybutanoate which was prepared as previously described[20]. Solvent were dried using standard procedures. NMR spectra were recorded in $CDCl_3$ on a Bruker WM-250 instrument, at 250.13 MHz for ^1H and 62.9 MHz for ^{13}C. Chemical shifts were standardized to a $CHCl_3$ resonance of 7.24 ppm relative to TMS. GC-mass (GC-MS) data were obtained by electronic impact (EI) with a Hewlett-Packard 5890-II/5972 instrument equiped with a 30 m HP-1 capillary column. A Hewlett-Packard 5989A instrument equiped with a 25 m CPSilSCB (SE 30) capillary column was used for NH_3-chemical ionization (CI) mass spectra. IR spectra of thin films were recorded on a Perkin Elmer 783 spectrophotometer. Optical rotation were measured using a Perkin Elmer 241C spectropolarimeter, in a 1 dm cell. High resolution mass spectrometry (HRMS) was performed at the Spectrometry Center, University Pierre et Marie Curie, Paris. Elementary analyses were obtained from the CNRS Microanalysis Service, Gif-sur-Yvette. GC analyses were performed on capillary columns, DBWax (J &W Scientific, 30 m x 0.25 mm), BP 20 (SGE, 25 m x 0.22 mm),

BP 10 (SGE,, 15 m x 0.20 mm), and SE 30 (Pierce, 25 m x 0.20 mm), with a flame ionization detector and helium (1 kg cm^{-1}) as a carrier gas. Chiral HPLC was performed on a covalent-DNBPG Pirkle column (Baker, 4.6 mm x 25 cm) using a 1 $mL.mn^{-1}$ flow rate and UV detection at 254 nm.
Reactions were followed on Kieselgel $60F_{254}$ TLC plates, purchased from Merck. Visualization was achieved by UV inspection (254 nm), and by spraying with phosphomolybdic acid solution (5 g in 100 mL ethanol), or a solution of ammonium molybdate (6.25 g) and cerium sulfate (2.5 g) in concentrated H_2SO_4 (25 mL) and water (125 mL), followed by heating at about 120°C. Flash chromatography was realized either in glass column on Kieselgel 60 (Merck, 230-400 mesh), or using a Modulprep Jobin et Yvon system equiped with a 40 mm-diameter steel column, filled with Kieselgel 60H (Merck, 5-40 µm) at about 8 kg cm^{-1}pressure.

Determination of diastereomeric and enantiomeric excesses. Diastereomeric excesses were directly determined by GC on the adequate column (Table 6). Enantiomeric excesses were determined by GC after derivatisation of hydroxy compounds with (*S*)-O-acetyllactyl chloride (≥ 98% e.e.)[80] (see Table 6), except for the reduction product of **1f**, the (*S*)-O-acetyllactylester of which was analyzed by HPLC on a DNBPG chiral column.

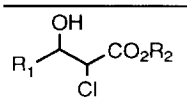
Preparation of 2-chloro-3-oxoesters. All chlorooxoesters, excepted ethyl 2-chloro-3-oxobutanoate (Aldrich), and methyl 2-chloro-3-(*p*-methoxyphenyl)-3-oxobutanoate (a gift from J.-A.Laffitte and J.-L.Seris, Elf-Aquitaine-GRL, Lacq, France), were prepared by the following method[81]. Sulfuryl chloride (117 mmol) was slowly added with shaking to a solution of 3-oxoester (78 mmol) in ethyl ether (150 mL) at 0°C. After 1 h at room temperature, the solvent was evaporated *in vacuo*, CH_2Cl_2 (100 mL) was added and the solution was washed with saturated aqueous Na_2CO_3 (100 mL). After drying upon $MgSO_4$ and solvent evaporation, the resulting oil was purified by distillation or flash chromatography.

Preparation of (±)-2-chloro-3-hydroxyesters. All hydroxychloroesters were obtained by chemical reduction of 2-chloro-3-oxoesters. Sodium borohydride (13.2 mmol) in water (20 mL) was slowly added to a solution of chlorooxoester (17.6 mmol) in THF (40 mL) at -10°C and the reaction mixture was stirred for 1 h at the same temperature. Acetic acid (3.2 mL), then ether (100 mL) were added. The solution was washed with water (80 mL), dried ($MgSO_4$) then concentrated in vacuo. The crude hydroxyesters mixture was separated and purified by flash chromatography. *Syn* chlorohydroxyesters were majorily obtained by this method, together with small amounts of *anti* esters. Both were identified by their difference in ^1H NMR coupling constants between -C*H*Cl- and -C*H*OH- signals (*anti*, 6-8 Hz; *syn*, 4-7 Hz).

Microorganisms and cultures .All cultures were maintained on agar slants containing (per litre), yeast extract (Difco) 5 g, Bacto-Peptone (Difco) 5 g, malt extract (Difco) 5 g, glucose 20 g and Bacto-agar (Difco) 20 g, stored at 4°C and subcultured before use. Fungi were purchased from the American Type Culture Collection (ATCC), Rockville, Md, USA, the Northern Regional Research Laboratories (NRRL), Peoria, Ill., USA, the Centraalbureau voor Schimmel Cultures (CBS), Baarn, the Netherlands, the Quartermaster Research and Development Center, U.S.Army, Natick, Mass., USA.(QM), or the Mycotheque of the Museum d'Histoire Naturelle (MMP), Paris, France. Some strains (no strain number) are from local origin. Screening experiments were realized by addition of the substrates (1 g L^{-1}) onto 65 h-old cultures of yeasts grown with orbital shaking (200 r.p.m.) at 27°C in 100 mL of a liquid medium containing (per litre), yeast extract (Difco) 5 g, Bacto-Peptone (Difco) 5 g, malt extract (Difco) 10 g, glucose 20 g. Fungi were similarly grown in a liquid medium containing (per litre), atomized corn steep (Solulys A, Roquette, France) 5 g, glucose 30 g, KH_2PO_4 1 g, K_2HPO_4 2 g, $NaNO_3$ 2 g, KCl 0.5 g, $MgSO_4$, $7H_2O$ 0.5 g, $FeSO_4$, $7H_2O$ 0.02 g. The fungal biomass was harvested by filtration, washed and resuspended in 0.2 M potassium phosphate buffer, pH 6.0, in a volume identical to that of the culture. Substrate (1 g L^{-1}) was added and incubation was continued at 27°C with orbital shaking. Samples (1-2 mL) were withdrawn at intervals, centrifuged, and the supernatants were microfiltered (0.45 µm). Aliquots of the filtrates were saturated with sodium chloride and extracted with ether-ethyl acetate (1:1) for GC analysis in the presence of known amounts of standards. Most transformations were continued until a total disappearance of substrate was observed (usually 2-3 days). Preparative experiments were similarly

performed, at a substrate concentration of 1-5 g L^{-1}, in several 1 L-amounts.

Table 6: GC Separation of Diastereomeric and Enantiomeric 2-Chloro-3-hydroxyesters Derived from **1a-e**.

		Diastereomeric chlorohydroxyesters Retention times (min)			(*S*)-O-acetyllactyl derivatives of enantiomeric chlorohydroxyesters				
		Column			Column	Retention times (min)			
R_1	R_2	(temperature)	*syn*	*anti*	(temperature)	2S,3S	2R,3R	2R,3S	2S,3R
Me	Et	BP20 (140°C)	7.1	7.5	SE30 (10 min at 150°C, then 150-180°C, 3°C/min)	18.8	19.0	19.7	20.0
n-Pr	Et	DBWax (120°C)	16.1	16.7	BP10 (140°C)	26.4	26.9	29.4	30.1
i-Pr	Et	DBWax (120°C)	11.8	11.8	BP10 (150°C)	14.7	15.2	17.1	17.9
Phe	Et	DBWax (220°C)	8.2	9.2	BP10 (180-200°C, 1°C/min)	16.5	16.8	18.0	18.2
p-MeO-Phe	Me	BP10 (200°C)	6.1	6.6	BP10 (200°C)	20.9	21.3	22.5	22.9
$PheCH_2OCH_2$	Et	BP10 (180°C)	14.9	14.9	SE30 (10 min at 170°C, then 170-200°C, 1°C/min)	36.3	36.3	37.4[a]	37.7[a]

[a] Undetermined absolute configuration.

Preparative reduction of ethyl 2-chloro-3-oxohexanoate **1b**. The biomass of *M.plumbeus* CBS 110-16 (from 2 L of culture) was resuspended in the same volume of 0.2 M potassium phosphate buffer, pH 6 and **1b** (2 g, 10.4 mmol) was added as a solution in EtOH-Tween 80 (8:2, 20 mL). After a 24 h incubation, the suspension was filtered, and the filtrate was saturated with sodium chloride, filtered again with celite then extracted 3 times with EtOAc-ethyl ether (1:1). After drying and evaporation of solvents, the crude residue was flash chromatographed (cyclohexane-EtOAc, 9:1) to give **(2*R*,3*R*) ethyl 2-chloro-3-hydroxyhexanoate** (1.01 g, 50% yield). Retention time (DBWax, 120°C): 16.7 min; (*S*)-O-Acetyllactate (BP 10, 140°C): 26.9 min (see Table 6). Anal. for $C_8H_{15}ClO_3$, calc. C 49.36, H 7.76, O 24.65; found C 49.20, H 7.80, O 24.62. $[\alpha]_D^{20}$ + 8.5 (c 1, $CHCl_3$). IR cm^{-1}: 3460 (OH), 1740 (CO_2Et). ^1H NMR, δ ppm, J Hz: 0.93 (3H, t, J = 7.1, 6-$C\underline{H}_3$), 1.29 (3H, t, J = 7.1, $OCH_2C\underline{H}_3$), 1.35-1.70 (4H, m, 4- and 5-$C\underline{H}_2$), 2.43 (1H, d, J = 5.9, O<u>H</u>), 3.93-4.04 (1H, m, C<u>H</u>OH), 4.17 (1H, d, J = 6.7, C<u>H</u>Cl), 4.25 (2H, q, J = 7.1, $OC\underline{H}_2CH_3$). ^{13}C NMR, δ ppm: 13.79 (6-$\underline{C}H_3$), 13.94 ($OCH_2\underline{C}H_3$), 18.44 (5-$\underline{C}H_2$), 34.95 (4-$\underline{C}H_2$), 59.73 (<u>C</u>HCl), 62.22 ($O\underline{C}H_2CH_3$), 72.49 (<u>C</u>HOH), 168.77 (<u>C</u>O). EI.MS, m/z (relative abundance): 151(15) $[M-43]^+$, 124(29) $[M-70]^+$, 122(87) $[M-72]^+$, 94(100) $[M^+-100]^+$, 76(17) $[M-118]^+$, 71(18) $[M-123]^+$, 55(32) $[M-139]^+$.

Absolute configuration was determined by catalytic hydrogenation of the 2-chloro-3-hydroxyester (100 mg, 0.51 mmol) in EtOH (2 mL) in the presence of Et_3N (60 µL, 0.51 mmol) and 10% Pd on carbon (15 mg). After hydrogenation under atmospheric pressure during 1.5 h, filtration of the catalyst, and washing, the filtrate was added with ethyl acetate (10 mL) and diluted HCl (10 mL). The organic phase was dried, evaporated under reduced pressure, and purified by flash chromatography to give ethyl 3-hydroxyhexanoate (51 mg, 61% yield), which was derivatized with (*S*)-O-acetyllactyl chloride and compared by GC to the corresponding (±)- and (*R*)-(–)-hydroxyester previously obtained[82].

Preparative reduction of ethyl 2-chloro-3-oxo-4-methyl pentanoate **1c**. To a 65 h-old, 1L-culture of *P.anomala* NRRL Y40 was added **1c** (1 g, 5.2 mmol) in EtOH-Tween 80 (8:2, 10 mL) and incubation was continued for 30 h. The yeast cells were eliminated by centrifugation, and the supernatant was saturated with sodium chloride and extracted 3 times with EtOAc-ethyl ether (1:1). The organic phase was dried and evaporated under reduced pressure, and the residual oil was flash chromatographed (cyclohexane-EtOAc, 9:1) to give 303 mg (30%) of a 1:1 mixture of *syn*- and *anti*- ethyl 2-chloro-3-hydroxy-4-methyl pentanoate. Partially separated analytical samples were obtained by repeated chromatography. Retention time (DBWax, 120°C) 11.8 min. Anal. for $C_8H_{15}ClO_3$, calc. C 49.36, H 7.76, O 24.65; found C 49.23, H 7.81, O 24.65. IR cm^{-1}: 3480 (OH), 1740 (CO_2Et). *Syn*:, ^1H NMR, δ ppm, J Hz: 0.94 and 1.01 (6H, 2d, J = 6.8, 5- and 5'-$C\underline{H}_3$), 1.30 (3H, t, J = 7.0, $OCH_2C\underline{H}_3$), 1.83 (1H, m, 4-C<u>H</u>), 2.35 (1H, d, J = 6.8, O<u>H</u>), 3.71 (1H, ddd, $J_{CH-OH} = J_{CH-CH} = 6.8$, J_{CH-}

CHCl = 3.6, CHOH) 4.25 (2H, q, J = 7.0, OCH_2CH_3), 4.47 (1H, d, J = 3.6, CHCl). ^{13}C NMR, δ ppm: 13.98 (OCH_2CH_3), 18.04 and 18.95 (5- and 5'-CH_3), 31.31 (4-CH), 60.90 (CHCl), 62.44 (OCH_2CH_3), 77.09 (CHOH), 168.78 (CO). *Anti*, ^1H NMR, δ ppm, J Hz: 0.92 (3H, d, J = 6.7, 5-CH_3), 1.03 (3H, d, J = 7.0, 5'-CH_3), 1.30 (3H, t, J = 7.0, OCH_2CH_3), 2.06 (1H, m, 4-CH), 2.43 (1H, d, J = 6.0, OH), 3.79 (1H, ddd, J_{CH-OH} = 6.0, J_{CH-CH} = 4.4, $J_{CH-CHCl}$ = 7.6, CHOH), 4.20 (1H, d, J = 7.6, CHCl), 4.25 (2H, q, J = 7.0, OCH_2CH_3). ^{13}C NMR, δ ppm: 15.28 (OCH_2CH_3), 18.95 and 19.52 (5- and 5'-CH_3), 29.15 (4-CH), 56.77 (CHCl), 62.22 (OCH_2CH_3), 76.82 (CHOH), 169.27 (CO).

The (**2*R*,3*S***) and (**2*S*,3*S***) absolute configurations of respective *syn* and *anti* chlorohydroxyesters derived from **1c** were determined by combining the following data: enantiomeric excesses of the *syn* ester (97 %) and the *anti* ester (26%), determined by GC of their (*S*)-O-acetyllactate (Table 6), and enantiomeric ratio (*R/S* = 81:19) of the dechlorinated 3-hydroxyester obtained, as precedently described for the 2-chloro-3-hydroxyhexanoate, by catalytic hydrogenation of a mixed sample (*syn/anti* ratio = 51:49), and determined again by GC of (*S*)-O-acetyllactyl esters[82].

Preparative reduction of ethyl 2-chloro-3-oxo-3-phenylpropionate **1d**.

a) The biomass of *M.racemosus* (from 2 L of culture) was resuspended in the same volume of 0.2 M potassium phosphate buffer, pH 6 and **1d** (2 g, 8.8 mmol) was added as a solution in EtOH-Tween 80 (8:2, 10 mL). After a 24 h incubation, the suspension was filtered, and the filtrate was saturated with sodium chloride, filtered again with celite then extracted 3 times with EtOAc-ethyl ether (1:1). After drying and evaporation of solvents under reduced pressure, the crude residue was flash chromatographed (cyclohexane-ether, 85:15) to give **(2*S*,3*R*) ethyl 2-chloro-3-hydroxy-3-phenylpropionate** (0.907 g, 45% yield). Retention time (DBWax, 220°C): 8.2 min; (*S*)-O-Acetyllactate (BP 10, 180-200°C, 1°C/min): 18.2 min (see Table 6). Anal. for $C_{11}H_{13}ClO_3$, calc. C 57.77, H 5.73, O 20.98; found C 57.55, H 5.82, O 20.74. $[\alpha]_D^{20} = -3$, $[\alpha]_{365}^{20} = +7$ (c 1.7, $CHCl_3$). IR cm^{-1}: 3460 (OH), 1740 (CO_2Et). ^1H NMR, δ ppm, J Hz: 1.12 (3H, t, J = 7.3, OCH_2CH_3), 2.94 (1H, d, J = 3.6, OH), 4.10 (2H, q, J = 7.3, OCH_2CH_3), 4.43 (1H, d, J = 6.5, CHCl), 5.12 (1H, dd, J_{CH-CH} = 6.5, J_{CH-OH} = 3.6, CHOH), 7.32-7.37 (5H, m, ArH). ^{13}C NMR, δ ppm: 13.8 (OCH_2CH_3), 62.2 (OCH_2CH_3), 62.9 (CHCl), 74.6 (CHOH), 126.6, 128.5, 128.7 and 138.1 (ArC), 167.9 (CO). CI.MS (NH_3), m/z (relative abundance): 228(100) $[M]^+$, 211(17) $[M-17]^+$, 194(43) $[M-34]^+$, 177(17) $[M-51]^+$.

b) To a 65 h-old, 1L-culture of *R.glutinis* NRRL Y1091 was added **1d** (5 g, 22.1 mmol) in EtOH-Tween 80 (8:2, 50 mL) and incubation was continued for 72 h. The yeast cells were eliminated by filtration with celite, and the filtrate was saturated with sodium chloride, filtered again, and extracted 3 times with EtOAc-ethyl ether (1:1). The organic phase was dried and evaporated under reduced pressure, and the crude residue was flash chromatographed (cyclohexane-ethyl ether, 85:15) to give **(2*R*,3*R*) ethyl 2-chloro-3-hydroxy-3-phenylpropionate** (2.57 g, 51%). Retention time (DBWax, 220°C): 9.2 min; (*S*)-O-Acetyllactate (BP 10, 180-200°C, 1°C/min): 16.8 min (see Table 6). Anal. for $C_{11}H_{13}ClO_3$, calc. C 57.77, H 5.73, O 20.98; found C 57.55, H 5.82, O 20.74. $[\alpha]_D^{20} = -42$ (c 1.5, $CHCl_3$). IR cm^{-1}: 3460 (OH), 1740 (CO_2Et). ^1H NMR, δ ppm, J Hz: 1.25 (3H, t, J = 7.3, OCH_2CH_3), 2.99 (1H, d, J = 5.1, OH), 4.23 (2H, q, J = 7.3, OCH_2CH_3), 4.36 (1H, d, J = 8.0, CHCl), 5.03 (1H, dd, J_{CH-CH} = 8.0, J_{CH-OH} = 5,1, CHOH), 7.33-7.39 (5H, m, ArH). ^{13}C NMR, δ ppm: 13.8 (OCH_2CH_3), 59.1 (CHCl), 62.3 (OCH_2CH_3), 75.2 (CHOH), 126.8, 128.4, 128.6 and 138.7 (ArC), 168.8 (CO). CI.MS (NH_3), m/z (relative abundance): 228(100) $[M]^+$, 211(17) $[M-17]^+$, 194(43) $[M-34]^+$, 177(17) $[M-51]^+$.

Absolute configurations of both chlorohydroxyesters were determined by catalytic hydrogenation, as described above, to give (*S*)-ethyl 3-hydroxy-3-phenylpropionate (60% yield), $[\alpha]_D^{20} = -44.8$ (c 1.6, $CHCl_3$) [lit.[83] −39.8 (c 1.5, $CHCl_3$)] from the *syn* , and –43.5 (c 1.2, $CHCl_3$) from the *anti* chlorohydroxyester.

Preparative reduction of methyl 2-chloro-3-oxo-3-(p-methoxyphenyl)propionate **1e**.

a) The biomass of *R.arrhizus* ATCC 11145 (from 0.5 L of culture) was resuspended in 0.4 L of 0.2 M potassium phosphate buffer, pH 6 and **1e** (methyl ester) (0.22 g, 0.9 mmol) was added. After 24 h incubation,

the suspension was filtered, and the filtrate was saturated with sodium chloride, filtered again with celite then extracted 3 times with EtOAc-ethyl ether (1:1). After drying and evaporation of solvents under reduced pressure, the crude residue was flash chromatographed (cyclohexane-EtOAc, 8:2) to give **(2*S*,3*R*) methyl 2-chloro-3-hydroxy-3-(*p*-methoxyphenyl)propionate** (77.6 mg, 35% yield). Retention time (BP 10, 200°C): 6.1 min; (*S*)-O-acetyllactate (BP 10, 200°C): 22.9 min (see Table 6). Anal. for $C_{11}H_{13}ClO_4$, calc. C 54.00, H 5.36, O 26.16; found C 54.38, H 5.31, O 24.03. $[\alpha]_D^{20}$ = +14 (c 1, MeOH) [lit.[72] +15.5], $[\alpha]_{365}^{20}$ = +25 (c 1, $CHCl_3$). ^1H NMR, δ ppm, J Hz: 2.86 (1H, s, O<u>H</u>), 3.65 (3H, s, $CO_2C\underline{H}_3$), 3.78 (3H, s, $OC\underline{H}_3$), 4.40 (1H, d, J = 4.5, C<u>H</u>Cl), 5.06 (1H, dd, $J_{CH\text{-}CH}$ = 5.5, C<u>H</u>OH), 6.86 (2H, d, J = 7.5, 2'-Ar<u>H</u>), 7.25 (2H, d, J = 7.5, 3'-Ar<u>H</u>). ^{13}C NMR, δ ppm: 52.9 ($CO_2\underline{C}H_3$), 55.2 ($O\underline{C}H_3$), 62.7 (<u>C</u>HCl), 74.1 (<u>C</u>HOH), 113.8, 127.8, and 130.1 (Ar<u>C</u>), 168.3 (<u>C</u>O).

b) The biomass of *S. exile* QM 1250 (from 1 L of culture) was resuspended in the same volume of 0.2 M potassium phosphate buffer, pH 6 and **1e** (methyl ester) (1.5 g, 6.2 mmol) in EtOH-Tween 80 (8:2, 10 mL) was added. After 24 h incubation, the suspension was filtered, and the filtrate was saturated with sodium chloride, filtered again with celite then extracted 3 times with EtOAc-ethyl ether (1:1). After drying and evaporation of solvents under reduced pressure, the crude residue was flash chromatographed (cyclohexane-EtOAc, 8:2) to give **(2*S*,3*R*) methyl 2-chloro-3-hydroxy-3-(*p*-methoxyphenyl)propionate** (0.786 g, 52% yield). All chromatographic and spectroscopic properties were identical to those described above.

Preparative reduction of ethyl 2-chloro-3-oxo-4-benzyloxybutanoate **1f**. The biomass of *G. candidum* (from 1 L of culture) was resuspended in 1 L of 0.2 M potassium phosphate buffer, pH 6 and **1f** (1 g, 3.7 mmol) in EtOH-Tween 80 (8:2, 10 mL) was added. After 24 h incubation, the suspension was filtered, and the filtrate was saturated with sodium chloride, filtered again with celite then extracted 3 times with EtOAc-ethyl ether (1:1). After drying and evaporation of solvents under reduced pressure, the crude residue was flash chromatographed (cyclohexane-EtOAc, 7:3) to give **(2*S*,3*S*) ethyl 2-chloro-3-hydroxy-4-benzyloxybutanoate** (342 mg, 34% yield). Retention time (BP 10, 180°C): 14.9 min; (*S*)-O-acetyllactate (HPLC on chiral column, hexane-iPrOH, 99:1): 25.9 min. HRMS for $C_{13}H_{17}ClO_4$, calc. 272.0815, found 272.0816. $[\alpha]_D^{20}$ = – 4.5 (c 1.2, MeOH). IR cm^{-1}: 3480 (OH), 1740 (CO_2Et). ^1H NMR, δ ppm, J Hz: 1.27 (3H, t, J= 7.1, $OCH_2C\underline{H}_3$) 2.84 (1H, d, J= 7.9, O<u>H</u>), 3.67 and 3.75 (2H, 2dd, $J_{CH\text{-}CH}$ = 9.9, $J_{CH\text{-}CHOH}$ = 3.5, $OC\underline{H}_2CHOH$), 4.22 (2H, q, J = 7.1, $OC\underline{H}_2CH_3$), 4.35 (1H, d, J = 7.9, C<u>H</u>Cl), 4.51 (3H, m, C<u>H</u>OH and $ArC\underline{H}_2$), 7.28-7.36 (5H, m, Ar<u>H</u>). ^{13}C NMR, δ ppm: 13.94 ($OCH_2\underline{C}H_3$), 55.76 (<u>C</u>HCl), 62.22 ($O\underline{C}H_2CH_3$), 69.42 ($O\underline{C}H_2CHOH$), 71.92 (CHOH), 73.59 ($Ar\underline{C}H_2$), 126.96, 127.95, 128.48 and 137.48 (Ar<u>C</u>), 168.60 (<u>C</u>O). EI.MS, m/z (relative abundance): 91(100) $[M\text{-}181]^+$, 65(14) $[M\text{-}207]^+$.
The (**2*S*,3*S***) absolute configuration of the chlorohydroxyester derived from **1f** was determined by catalytic hydrogenation, as described above, to give (*R*)-ethyl 3-hydroxy-4-benzyloxybutanoate (53 % yield), $[\alpha]_D^{20}$ = – 9.9 (c 1., MeOH); $[\alpha]_D^{20}$ = +10.1 (c 1.1, $CHCl_3$) [lit.[83] +8.0 (c 1.5, $CHCl_3$) ee= 71%]

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