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Deracemisation of β-hydroxy esters using immobilised whole cells of *Candida parapsilosis* ATCC 7330: substrate specificity and mechanistic investigation

Santosh Kumar Padhi, D. Titu, N. Ganesh Pandian and Anju Chadha*

Laboratory of Bioorganic Chemistry, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, India

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Abstract—Deracemisation of aryl substituted β-hydroxy esters by immobilised whole cells of *Candida parapsilosis* ATCC 7330 gave >99% ee and up to 75% yield of their corresponding (*S*)-enantiomers. Mechanistic investigation of the deracemisation reaction carried out using a deuterated substrate, ethyl 3-deutero-3-hydroxy-3-phenyl propanoate revealed that while the (*S*)-enantiomer remains unreacted the (*R*)-enantiomer undergoes enantioselective oxidation to its corresponding ketoester, which on complementary enantiospecific reduction gives the (*S*)-enantiomer in high yield and % ee.

1. Introduction

Optically pure β-hydroxy esters are important chiral synthons for the synthesis of numerous pharmaceuticals such as fluoxetine,[1] β-lactam antibiotics,[2] Tuckolide—an HMGCoA reductase-inhibitor[3] and dihydrokawain (a narcotic).[4] The amino derivative of a (*S*)-β-hydroxy ester is present in the side chain of Taxol®.[5] Ethyl 4-chloro-3-hydroxy butanoate is used in the preparation of L-carnitine, a nutraceutical.[6] Chiral β-hydroxy esters are also used as starting materials in the preparation of enantiomerically pure β-blockers, i.e., propranolol, alprenolol and 1-(isopropylamino)-3-*p*-methoxy-phenoxy-2-propanol.[7] The application of (*R*)-(−)-sodium β-hydroxy butanoate as a cerebral function improving agent on cerebral hypoxia, anoxia and ischeamia in mice and rats has been reported.[8] Both the enantiomers of ethyl 3-hydroxy butanoate and ethyl 3-hydroxy pentanoate are extremely useful in the synthesis of pheromones.[9] Optically pure β-hydroxy esters also play an important role in many biological reactions inside the human body.[10] With the varied applications of these molecules, there is much interest in their asymmetric synthesis. Asymmetric reduction and kinetic resolution are the two main biocatalytic approaches for the synthesis of these compounds. Asymmetric reduction of β-ketoesters by different microbial whole cells[11] and plant cells is known.[12] Engineered whole cells of baker's yeast are reported to carry out a highly stereoselective synthesis of α-unsubstituted and α-alkyl-β-hydroxy esters.[13] Kinetic resolution is widely used for the synthesis of optically pure β-hydroxy esters from racemic β-hydroxy esters.[14] The limited yield of each enantiomer (maximum 50%) and the formation of the 'unwanted' isomer are the major drawbacks in the resolution of a racemate. Deracemisation[15] is an attractive alternative approach to synthesise chiral β-hydroxy esters from their racemates in high ee and quantitative yield. Use of two-enzyme systems[16] and whole cells is known to deracemise secondary alcohols.[17] Azerad et al. used aged cultures of a local strain of *Geotrichum candidum* to deracemise ethyl 3-hydroxy butanoate into the (*R*)-enantiomer in 96% ee and 80% yield.[18] Nakamura et al. reported the stereoinversion of aliphatic β-hydroxy esters (methyl-3-hydroxy butanoate and methyl-3-hydroxy pentanoate) using *G. candidum* IFO 5767 to produce the (*R*)-enantiomers in 97–99% ee and 26–48% isolated yield.[15e] Deracemisation of β-hydroxy esters is restricted to the above examples, both of which use *G. candidum* for aliphatic β-hydroxy esters and the deracemised product is the (*R*)-antipode. Whole cells of *Candida parapsilosis* are a rich source of oxidoreductases[19] and different strains of this species are known to deracemise 1,2-diols[20] and 1,3-diols.[21] More recently, deracemisation of aryl and aryl substituted α-hydroxy esters by *C. parapsilosis* ATCC 7330 to the (*S*)-enantiomer [17] and asymmetric reduction of ethyl 4-chloro-3-oxo butanoate using genetically modified *C. parapsilosis* (IFO 1396) to the (*R*)-hydroxy ester in 99% ee and 95% yield[11c] were reported. We have previously reported the deracemisation of some racemic

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* Corresponding author. Tel.: +91 44 2257 4106; fax: +91 44 2257 4102; e-mail: anjuc@iitm.ac.in

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β-hydroxy esters using free and immobilised whole cells of *C. parapsilosis* ATCC 7330.[22] Here we report the deracemisation by immobilised *C. parapsilosis* ATCC 7330 (ICp) of 19 aryl and substituted aryl substrates with different electronic and steric effects. The substrate specificity of the biocatalyst and the investigated mechanism of deracemisation are discussed.

2. Results and discussion

2.1. Mechanistic investigation of deracemisation

The different mechanisms by which deracemisation can be carried out are (i) re-racemisation and repeated resolution, (ii) dynamic kinetic resolution and (iii) stereoinversion.[15a] Kato et al. reported the deracemisation of α-alkyl carboxylic esters, which involves enantioselective esterification, epimerization and then hydrolysis.[15b] All these deracemisation mechanisms share a common stereochemical phenomenon, i.e., one enantiomer of the substrate retains its configuration throughout the deracemisation process and the other enantiomer crosses the plane of symmetry (or inversion of configuration) to become product.[15a] Literature reports on the use of different strains of *C. parapsilosis* in the deracemisation of secondary alcohols reveal that oxidoreductases actively participate in the deracemisation by stereoinversion.[15c–e,20] Deracemisation of α-hydroxy esters by whole cells of *C. parapsilosis* ATCC 7330 also occurs via stereoinversion.[17] Mechanistic investigation of the deracemisation of β-hydroxy esters was done using ethyl 3-hydroxy-3-phenyl propanoate (**1**, Table 1). The deracemised product of racemic ethyl 3-hydroxy-3-phenyl propanoate (**1**) on HPLC analysis (reverse phase) showed the presence of ethyl 3-oxo-3-phenyl propanoate, along with acetophenone and 1-phenyl ethanol, which led us to propose and investigate the stereoinversion mechanism for deracemisation of β-hydroxy esters. In order to prove the proposed mechanism of this deracemisation reaction, the following substrates were incubated with immobilised cells of *C. parapsilosis* ATCC 7330 and are discussed in detail.

2.1.1. Substrate (*R*)-ethyl 3-hydroxy-3-phenyl propanoate [(*R*)-1]. Optically pure (*R*)-ethyl 3-hydroxy-3-phenyl propanoate [(*R*)-**1**] was incubated with ICp for 6 h and the product was analysed at the end of every hour for the formation of (i) the (*S*)-enantiomer [(*S*)-**1**] using chiral HPLC and (ii) ethyl 3-oxo-3-phenyl propanoate (**2**) using reverse-phase HPLC. Chiral HPLC confirmed the presence of (*S*)-hydroxy ester (68%) at the end of 6 h. The ketoester (**2**) intermediate could be detected from 4 h onwards and was confirmed by comparison with a standard (Scheme 1).

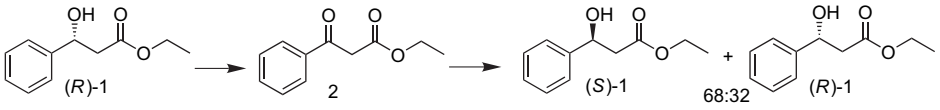
Oxidation of (*R*)-ethyl 3-hydroxy-3-phenyl propanoate [(*R*)-**1**] to the ketoester intermediate (**2**) followed by its reduction to [(*S*)-**1**] involves the loss of a methine proton. To prove the role of the methine proton in the stereoinversion, two experiments were carried out using ethyl 3-deutero-3-hydroxy-3-phenyl propanoate (**3**) and ethyl 3-hydroxy-3-phenyl butanoate (**4**) as the substrates.

2.1.2. Substrate ethyl 3-deutero-3-hydroxy-3-phenyl propanoate (3). Ethyl 3-deutero-3-hydroxy-3-phenyl propanoate (**3**) was incubated with ICp for 6 h and the resulting reaction mixture was analysed by ^{1}H NMR spectroscopy after work up. The ^{1}H NMR spectrum of the reaction mixture confirmed the presence of the starting reactant (deuterated, **3**) as well as its undeuterated counterpart (**1**) (dd at

Table 1. Deracemisation of β-hydroxy esters using immobilised whole cells of *Candida parapsilosis* ATCC 7330

Sub. no.	R	R′	*n*	ee%	Yield %	Abs. config.	$[\alpha]_D^{25}$ (This study)	$[\alpha]_D^{25}$ (Lit. reported)
1	Ph	Et	0	99	57	*S*	−50.1 (*c* 1.5, $CHCl_3$)	+43.7 (*c* 1.4, $CHCl_3$)[28]
7	*o*-MeC_6H_4	Et	0	09	68	Nd	Nd	
8	*p*-MeC_6H_4	Et	0	98	51	*S*	−44.6 (*c* 1.2, $CHCl_3$)	+44.7 (*c* 1.2, $CHCl_3$)[33a]
9	*p*-EtC_6H_4	Et	0	>99	42	*S*	−43.4 (*c* 1.2, $CHCl_3$)	Nr
10	*o*-$OMeC_6H_4$	Et	0	>99	75	*S*	−57.7 (*c* 1.8, $CHCl_3$)	−42.0 (*c* 2.30, $CHCl_3$)[33b]
11	*p*-$OMeC_6H_4$	Et	0	99	48	*S*	−43.7 (*c* 0.52, $CHCl_3$)	+39.4 (*c* 0.52, $CHCl_3$)[33a]
12	*p*-ClC_6H_4	Et	0	99	42	*S*	−43.7 (*c* 1.38, $CHCl_3$)	+44.2 (*c* 1.38, $CHCl_3$)[33a]
13	*m*-BrC_6H_4	Et	0	72	62	*S*	−21.4 (*c* 1.46, $CHCl_3$)	Nr
14	*p*-$NO_2C_6H_4$	Et	0	99	41	*S*	−59.5 (*c* 1.5, $CHCl_3$)	+23.1 (*c*, 1.0, $CHCl_3$)[28]
15	*m*-$NO_2C_6H_4$	Et	0	26	66	Nd	Nd	
16	1-Naphthyl	Et	0	00	63	—	—	
17	9-Anthranyl	Et	0	00	62	—	—	
18	Ph	Et	1	13	59	Nd	Nd	
19	Ph	Et	2	87	10	*S*	−2.35 (*c* 2.71, CH_2Cl_2)	+1.3 (*c* 1.0, $CHCl_3$)[33e]
20	Ph	Me	0	>99	48	*S*	−49.9 (*c* 1.0, $CHCl_3$)	−52.0 (*c* 1.0, $CHCl_3$)[33c]
21	Ph	*n*-Propyl	0	93	47	*S*	−51.6 (*c* 2.61, $CHCl_3$)	Nr
22	Ph	*n*-Butyl	0	67	71	*S*	−31.2 (*c* 3.78, $CHCl_3$)	+35.2 (*c* 3.78, $CHCl_3$)[33d]
23	Me	CH_2Ph	0	00	38	—	—	
24	Me	$CH_2CH{=}CHPh$	0	79	23	Nd	Nd	

Nd: not determined; Nr: not reported.



Scheme 1.

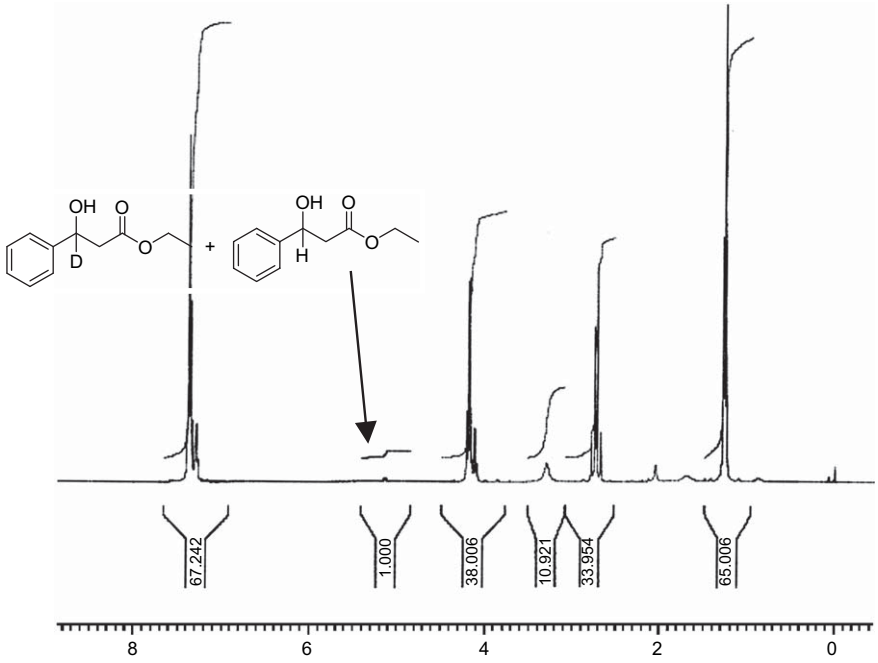
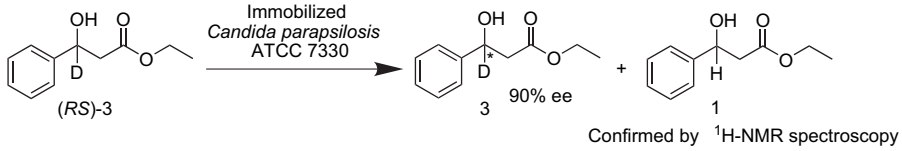


Figure 1. ^{1}H NMR of the deracemised product of ethyl 3-deutero-3-hydroxy-3-phenyl propanoate.



Scheme 2.

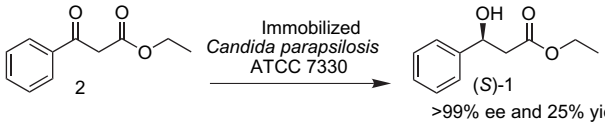
5.13 ppm, Fig. 1) as the products in the ratio of 94:6, respectively (Scheme 2).

The formation of the undeuterated product (**1**) is possible only by the reduction of the corresponding ketoester—ethyl 3-oxo-3-phenyl propanoate (**2**) detected earlier. The presence of the (*S*)-enantiomer in the product mixture (**3** and **1** in 94:6) in 90% ee indicates that the deuterated (*S*)-**3** is due to the retention of configuration of (*S*)-**3** from the racemic substrate while (*R*)-**3** is converted to (*S*)-**1** via the ketoester-**2**, which was detected earlier.

2.1.3. Substrate ethyl 3-hydroxy-3-phenyl butanoate (4). Ethyl 3-hydroxy-3-phenyl butanoate (**4**) when incubated with ICp for 6 h resulted in complete recovery of the starting racemate thus proving that the presence of a methine proton is mandatory for this deracemisation.

The experimental results described in Sections 2.1.2 and 2.1.3 unequivocally prove that ICp mediated deracemisation of β-hydroxy esters proceeds by a stereoinversion mechanism.

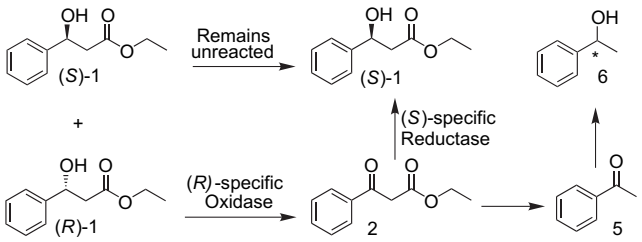
2.1.4. Reduction of ethyl 3-oxo-3-phenyl propanoate (2) using immobilised whole cells of *C. parapsilosis* ATCC 7330. Incubation of ethyl 3-oxo-3-phenyl propanoate with ICp resulted in the production of (*S*)-ethyl 3-hydroxy-3-phenyl propanoate [(*S*)-**1**] in >99% ee and 25% yield (Scheme 3) indicating the presence of a (*S*)-specific reductase in *C. parapsilosis* ATCC 7330.



Scheme 3.

In order to account for the low yield from ICp mediated reduction of **2**, the product mixture was analysed using reverse-phase HPLC, which confirmed the presence of ethyl 3-hydroxy-3-phenyl propanoate (**1**), acetophenone (**5**) and 1-phenyl ethanol (**6**). Formation of acetophenone could be due to decarboxylation of the hydrolysed[23] product of the ketoester intermediate (**2**) while 1-phenyl ethanol could be the reduced product of acetophenone accounting for ~15–20%, of the yield.[22b] Loss of another 10–15% of product can be attributed to the work up of the aqueous product mixture.[22c]

The scheme of reactions during deracemisation as proved from the above experiments can thus be represented as given in Scheme 4.



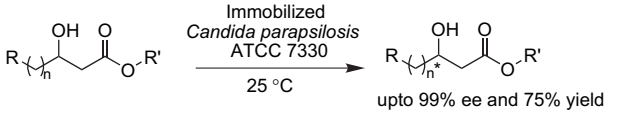
Scheme 4.

It is clear from Scheme 4 that, while the oxidation reaction is enantioselective and chemospecific, the reduction reaction is enantiospecific. The enantiospecificity of the reduction reaction is important as it is responsible for the high optical yield of the deracemised product. Notably, all earlier reported deracemisation reactions using different strains of *C. parapsilosis* were limited to substrates without multiple functional groups except in the case of α-hydroxy esters.[17] Thus, the appearance of the side products during deracemisation of β-hydroxy esters is not a surprise.

2.2. Deracemisation using immobilised whole cells of *C. parapsilosis* ATCC 7330

The number of biocatalytic methods for the preparation of chiral β-hydroxy esters is limited as compared to synthetic methods. Biocatalytic reduction of ketoesters of substrates **1**,[24] **18**,[25] **19**[26] and **20**[27] (Table 1) to their (*S*)-enantiomers has been reported in 87–97% ee and 42–87% yield. Lipase catalysed kinetic resolution of substrates **8**,[28] **11**,[28] **14**,[28] **18**,[25] **20**[25] and **22**[25] (Table 1) has also been reported in 86–94.5% ee and 42–48% yield. No suitable biocatalytic methods are reported for the preparation of substrates **7**, **9**, **10**, **12**, **13**, **15**, **16** and **21** (Table 1). Substrate **17** (Table 1) is a new compound and is used here for the first time for deracemisation.

Deracemisation of racemic ethyl 3-hydroxy-3-phenyl propanoate (**1**, Table 1) with ICp under optimised conditions[22b] gave its (*S*)-enantiomer in 99% ee and 57% isolated yield. In addition, a group of optically pure aryl and substituted aryl β-hydroxy esters was prepared using the ICp mediated deracemisation (Scheme 5) in order to study the substrate specificity of the biocatalyst. Deracemisation of substrates with electron donating (**8**, **9**, **11** and **12**, Table 1) and electron withdrawing (**14**, Table 1) substituents at the *para* position of the standard substrate resulted in the formation of their (*S*)-enantiomers in 98–99% ee and 41–51% yield. This indicates that the electronic nature of the substituents at the *para* position of the standard substrate does not affect the deracemisation. At the *ortho* position however, these substituents can obstruct the process of deracemisation due to their proximity to the reaction centre. Among the *meta* substituted substrates ethyl 3-hydroxy-3-(3-nitrophenyl) propanoate (**15**, Table 1) on deracemisation gave 26% ee and 66% isolated yield while ethyl 3-(3-bromophenyl)-3-hydroxy propanoate (**13**, Table 1) on deracemisation with ICp gave 72% ee and 62% isolated yield. The nitro group in the *meta* position lowers electron density in the aromatic ring, which could affect the removal of a hydride ion from the reaction centre, which we believe, is the initial step in this deracemisation reaction. The better optical yield in the case of *meta* bromo substituent than the *meta* nitro (**15**, Table 1) is because the bromo group does not have strong negative inductive effect as compared to the nitro group. Also the mesomeric effect is not active in the *meta* position for **15** (Table 1). Ethyl 3-hydroxy-3-naphthalen-1-yl-propanoate and ethyl 3-anthracen-9-yl-3-hydroxy-propanoate (**16**, **17**, Table 1) on ICp mediated deracemisation, were recovered in racemic form. The reaction centre in these molecules is sterically hindered due to the large size of the aromatic ring. Substrate **17** (Table 1), ethyl 3-anthracen-9-yl-3-hydroxy-propanoate in its racemic form is a new compound reported here for the first time and was used for deracemisation. β-Hydroxy esters with a spacer between the chiral centre and the phenyl ring, i.e., ethyl 3-hydroxy-4-phenyl butanoate and ethyl 3-hydroxy-5-phenylpentanoate (**18**, **19**, Table 1) on deracemisation gave 13% ee and 59% yield and 87% ee and 10% yield of the products, respectively. A similar study on the baker's yeast reduction[25] of the corresponding ketoesters of **1**, **18** and **19** (Table 1), indicates that with increasing number of carbon atoms between the keto group and the phenyl ring, the reduction proceeds at a faster rate. In this study substrate **1** without spacer and substrate **19** (Table 1) with two carbons as the spacer, undergoes deracemisation with high ee. The low yield of the product (**19**, Table 1) is due to hydrolysis of the ester group as a side reaction. The reasons for the poor ee of **18** (Table 1) are not clear at present. ICp mediated deracemisation of racemic β-hydroxy esters having ester moieties with increasing chain length (**1**, **20**–**22**, Table 1) resulted in a gradual decrease in the ee of the (*S*)-enantiomer product. High ee (>99%) of the product was obtained for methyl-3-hydroxy-3-phenyl propanoate (**20**, Table 1) with 48% yield. Phenylmethyl-3-hydroxy butanoate did not undergo deracemisation with ICp (**23**, Table 1). This molecule, with its aromatic ring on the opposite side of the reaction centre, is quite different from all the above substrates used for deracemisation. 3-Hydroxy-butyric acid 3-phenyl-allyl ester **24** (Table 1) on deracemisation resulted in 79% ee but a low isolated yield of 23%.



Scheme 5.

As evidenced from Table 1, the high ee (>99%) and high yield (up to 75%) of the products during the ICp mediated deracemisation of varied racemic β-hydroxy esters prove the broad substrate specificity of the biocatalyst.

Ethanol was used as the auxiliary solvent in the ICp mediated deracemisation to avoid the formation of side products due to transesterification except for substrates **20**, **21** and **22** (Table 1), where methanol, *n*-propanol and *n*-butanol were used, respectively.

3. Conclusion

The mechanism of ICp catalysed deracemisation of racemic β-hydroxy esters was proved to be via stereoinversion.

Experiments proving the mechanism showed that the (*S*)-enantiomer of ethyl 3-hydroxy-3-phenyl propanoate retained its absolute configuration and the (*R*)-enantiomer underwent an inversion of configuration via a ketoester intermediate, when incubated with ICp. Deracemisation of aryl and aryl substituted β-hydroxy esters by ICp gave >99% ee and up to 75% yield. High ee (98–99%) of the deracemised products was observed irrespective of the electronic nature of the substituents at the *para* position of the aromatic ring. A nitro group at the *meta* position of the aromatic ring decreases the ee of the deracemised product (26%). A methyl group at the *ortho* position of the aromatic ring decreases the ee of the deracemised product while a methoxy group does not. Substrates with polyaromatic rings did not undergo deracemisation. Increasing chain length of the ester moiety decreases the ee of the product.

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Bruker AV-400 spectrometer operating at 400 and 100 MHz, respectively. Chemical shifts are expressed in parts per million values using TMS as an internal standard. HPLC analysis was carried out on a Jasco PU-1580 liquid chromatograph with a PDA detector using Chiralcel-ODH and Chiralcel OJ-H chiral columns (Daicel, 4.6×250 mm). Optical rotations were recorded on a Jasco Dip 370 digital polarimeter. TLC was done on Kieselger $60F_{254}$ aluminium sheets (Merck 1.05554). The mobile phase was hexane/isopropanol, the proportion of solvents and the flow rate vary for different compounds.

4.2. Materials and methods

Racemic β-hydroxyl esters **1**, **7**–**11**, **14**, **15**, **18**, **23** and **24** were synthesised by sodium borohydride reduction of their corresponding ketoesters. The ketoesters of **1**, **11**, **14** and **15** were purchased from Fluka, Buchs SG, Switzerland; however, for substrates **7**, **8**, **9**, **10** and **18** the ketoesters were prepared by a literature known method.[29] The ketoesters of **23** and **24** were synthesised by a reported microwave irradiation method.[30] Substrates **12**, **13**, **16**, **17** and **19** were synthesised by Reformatsky reaction.[31] Substrates **20**, **21** and **22** were synthesised by our reported procedure,[32] i.e., sodium borohydride mediated reduction cum transesterification of the ethyl 3-oxo-3-phenyl propanoate. (*S*)-Ethyl 3-hydroxy-3-phenyl propanoate was prepared by baker's yeast reduction of the corresponding ketoester.[24] The (*R*)-ethyl 3-hydroxy-3-phenyl propanoate and the ketoester were purchased from Fluka, Buchs SG, Switzerland and used as such. Sodium borodeuteride was purchased from Sigma–Aldrich Chemical Co., Milwaukee, U.S.A. The deuterated hydroxy ester was prepared by the reduction of the corresponding ketoester with sodium borodeuteride. Acetophenone used as a standard was purchased from a local chemical company. 1-Phenyl ethanol was prepared by reducing acetophenone using sodium borohydride. Ethanol used in the biotransformation was of spectroscopic grade. *C. parapsilosis* ATCC 7330 was procured from American Type Culture Collection (ATCC 7330).

4.3. Culture medium

The composition of the media used for culturing *C. parapsilosis* ATCC 7330 was: YMB (yeast malt broth) [yeast extract (3 g L^{-1}), malt extract (3 g L^{-1}), peptone (5 g L^{-1}) and dextrose (10 g L^{-1}) at pH 6.5]. The yeast malt broth was sterilised in an autoclave at 121 °C and 15 lb kg^{-1} pressure for 20 min.

4.4. Cultivation of microorganism

The strain *C. parapsilosis* ATCC 7330 was routinely maintained in agar plates as well as slants (2.1% agar with the above mentioned culture media). Sub-culturing was performed every 12 weeks. The plates and slants were preserved at 4 °C.

4.5. Culture conditions

The pure culture of *C. parapsilosis* ATCC 7330 was inoculated with a loop, into the YMB media. A working volume of 50 mL (YMB media, after inoculation) in the 250 mL Erlenmeyer flasks was cultivated in an orbital shaker at 25 °C, 200 rpm. The cells were harvested by centrifuging the 44 h culture broth at 3750*g* followed by washing with sterile water. The process was repeated thrice and finally the wet biomass was used for biotransformation.

4.6. Immobilization

Immobilization was done following an earlier reported procedure.[22b]

4.7. Mechanistic investigation of deracemisation

In order to prove that the deracemisation follows a stereoinversion mechanism, different substrates were used with ICp. The details of these experiments are discussed below.

4.7.1. Substrate (*R*)-ethyl 3-hydroxy-3-phenyl propanoate [(*R*)-1]. To a suspension of 100 mL of immobilised *C. parapsilosis* ATCC 7330 in 50 mL of sterile distilled water taken in a 500 mL Erlenmeyer flask, 40 μL (210 μmol) of (*R*)-ethyl 3-hydroxy-3-phenyl propanoate (>99% ee) predissolved in 1 mL of absolute ethanol was added. The reaction mixture was incubated in a water-bath shaker for 6 h at 25 °C and 150 rpm. Aliquots were taken at different intervals of time (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0 h), extracted and analysed using both chiral HPLC (Chiralcel-ODH, hexane/isopropanol::95:05) and reverse-phase HPLC (Kromasil C-18 column, HPLC grade acetonitrile/water 40:60).

4.7.2. Substrate ethyl 3-deutero-3-hydroxy-3-phenyl propanoate (3). A suspension of 60 mL of immobilised beads of *C. parapsilosis* ATCC 7330 in 30 mL of sterile distilled water was prepared in a 500 mL Erlenmeyer flask. Ethyl 3-deutero-3-hydroxy-3-phenyl propanoate (15 μL, ~78 μmol) pre-dissolved in 375 μL of absolute ethanol was added to the above suspension. The reaction mixture was incubated in a water-bath shaker at 25 °C for 6 h at 150 rpm. Three similar experiments were carried out simultaneously to estimate the yield of the product. The reaction

mixture after the necessary work up was characterised using ^{1}H NMR. The reaction mixture was also analysed using chiral HPLC to determine the optical purity of the product.

4.7.3. Substrate ethyl 3-hydroxy-3-phenyl butanoate (4). To a suspension of 10 mL of immobilised *C. parapsilosis* ATCC 7330 in 5 mL of sterile distilled water taken in a 100 mL Erlenmeyer flask, 4 μL (21 μmol) of racemic ethyl 3-hydroxy-3-phenyl butanoate pre-dissolved in 100 μL of absolute ethanol was added. The reaction mixture was incubated in a water-bath shaker for 6 h at 25 °C and 150 rpm. After the incubation time, the reaction mixture was filtered to recover the immobilised beads and the filtrate was extracted with ethyl acetate (3×30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. This concentrated product was dissolved in 2 mL of hexane/isopropanol, 99:01 (HPLC grade), filtered through a 0.2-μm filter and analysed using chiral HPLC (Chiralcel-ODH, hexane/isopropanol::99:01). The chiral HPLC analysis (Chiralcel-ODH, hexane/isopropanol::99:01) indicated the presence of only the racemic substrate.

4.7.4. Reduction of ethyl 3-oxo-3-phenyl propanoate (2) using immobilised whole cells of *C. parapsilosis* ATCC 7330. The reaction was carried out as given in Section 4.7.3 using ethyl 3-oxo-3-phenyl propanoate as the substrate. The reaction mixture analysed by a reverse-phase HPLC (Kromasil C-18 column, HPLC grade acetonitrile/water 40:60) illustrated the presence of side products as shown in Scheme 4.

4.8. Typical procedure for the deracemisation using immobilised whole cells of *C. parapsilosis* ATCC 7330 (ICp)

Racemic β-hydroxy esters (504 μmol, 96 μL) dissolved in an appropriate amount of solvent (0.06% v/v, ethanol/methanol/*n*-propanol/*n*-butanol) were added to a suspension of 250 mL of ICp in 125 mL of distilled water, equally distributed in four 250 mL Erlenmeyer flasks. The biotransformation was carried out for 6 h at 25 °C and 150 rpm in a water-bath orbital shaker. The beads were filtered and the filtrate was extracted using ethyl acetate, dried over anhydrous sodium sulfate and concentrated. The crude product after column purification (30:70::ethyl acetate/hexane, for substrate **1**) was analysed by chiral HPLC to determine the optical purity of the deracemised product. The standard substrate **1** under identical conditions gave 99% ee and 57% yield. Appropriate control experiments with the reaction mixture containing all the components except (i) racemic β-hydroxy ester and (ii) the immobilised whole cells of *C. parapsilosis* ATCC 7330, established the optical purity of the product and the chemical yield. Deracemisation of other aromatic β-hydroxy esters (**7**–**24**, Table 1) was carried out following the same procedure.

4.9. Spectroscopic characterisation of the deracemised products

4.9.1. (*S*)-Ethyl 3-hydroxy-3-phenyl propanoate (1). Colourless oil; spectroscopic data identical to that reported in literature,[22a] $[\alpha]_D^{25}$ −50.1 (*c* 1.5, $CHCl_3$). HRMS (ESI): found 217.0837, $C_{11}H_{14}O_3Na$ $[M+Na]^+$ requires 217.0837.

4.9.2. Ethyl 3-hydroxy-3-(2-methylphenyl) propanoate (7). Colourless oil; spectroscopic data identical to that reported in literature.[22a]

4.9.3. (*S*)-Ethyl 3-hydroxy-3-(4-methylphenyl) propanoate (8). Colourless oil; spectroscopic data identical to that reported in literature,[22a] $[\alpha]_D^{25}$ −44.6 (*c* 1.2, $CHCl_3$). HRMS (ESI): found 231.1004, $C_{12}H_{16}O_3Na$ $[M+Na]^+$ requires 231.0997.

4.9.4. (*S*)-Ethyl 3-(4-ethylphenyl)-3-hydroxy propanoate (9). Colourless oil; $[\alpha]_D^{25}$ −43.4 (*c* 1.2, $CHCl_3$). IR ν_{max} (neat): 3455, 3059, 2966, 1733, 1514, 1372, 1269, 1116, 1037, 965, 890, 871, 833, 691 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (t, *J*=7.6 Hz, 3H), 1.24 (t, *J*=7.1 Hz, 3H), 2.63 (q, *J*=7.5 Hz, 2H), 2.7 (ddd, *J*=16.2, 9.1, 3.8 Hz, 2H), 3.3 (s, 1H), 4.16 (q, *J*=7.1 Hz, 2H), 5.09 (dd, *J*=9.1, 3.8 Hz, 1H), 7.17 (d, *J*=8.0 Hz, 2H), 7.27 (d, *J*=8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 15.5, 28.5, 43.4, 60.7, 70.2, 125.7, 127.9, 139.9, 143.81, 172.3. HRMS (ESI): found 245.1161, $C_{13}H_{18}O_3Na$ $[M+Na]^+$ requires 245.1154.

4.9.5. (*S*)-Ethyl 3-hydroxy-3-(2-methoxyphenyl) propanoate (10). Colourless oil; spectroscopic data identical to that reported in literature,[34a] $[\alpha]_D^{25}$ −57.7 (*c* 1.8, $CHCl_3$).

4.9.6. (*S*)-Ethyl 3-hydroxy-3-(4-methoxyphenyl) propanoate (11). Colourless oil; spectroscopic data identical to that reported in literature,[22a] $[\alpha]_D^{25}$ −43.7 (*c* 0.52, $CHCl_3$).

4.9.7. (*S*)-Ethyl 3-(4-chlorophenyl)-3-hydroxy propanoate (12). Colourless oil; spectroscopic data identical to that reported in literature,[34a] $[\alpha]_D^{25}$ −43.7 (*c* 1.38, $CHCl_3$). HRMS (ESI): found 251.0457, $C_{11}H_{13}O_3ClNa$ $[M+Na]^+$ requires 251.0451.

4.9.8. (*S*)-Ethyl 3-(3-bromophenyl)-3-hydroxy propanoate (13). Pale yellow liquid; spectroscopic data identical to that reported in literature,[34b] $[\alpha]_D^{25}$ −21.4 (*c* 1.46, $CHCl_3$). HRMS (ESI): found 294.9946, $C_{11}H_{13}O_3BrNa$ $[M+Na]^+$ requires 294.9942.

4.9.9. (*S*)-Ethyl 3-hydroxy-3-(4-nitrophenyl) propanoate (14). Pale yellow liquid; spectroscopic data identical to that reported in literature,[22a] $[\alpha]_D^{25}$ −59.5 (*c* 1.5, $CHCl_3$).

4.9.10. Ethyl 3-hydroxy-3-(3-nitrophenyl) propanoate (15). Pale yellow liquid; IR ν_{max} (neat): 3451, 3073, 2986, 2972, 2938, 1990, 1713, 1538, 1463, 1380, 1346, 1304, 1277, 1226, 1203, 1182, 1073, 1043, 1007, 939, 914, 859, 816, 734, 692, 622, 566 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.26 (t, *J*=7.1 Hz, 3H), 2.75 (m, 2H), 3.77 (s, 1H), 4.19 (q, *J*=7.1 Hz, 2H), 5.25 (m, *J*=5.2 Hz, 1H), 7.88 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0, 43.0, 61.1, 69.2, 120.7, 122.6, 129.4, 131.7, 144.7, 148.4, 171.8. HRMS (ESI): found 262.0692, $C_{11}H_{13}NO_5Na$ $[M+Na]^+$ requires 262.0691.

4.9.11. Ethyl 3-hydroxy-3-napthalen-1-yl propanoate (16). Colourless oil; spectroscopic data identical to that reported in literature,[34a] HRMS (ESI): found 267.0999, $C_{15}H_{16}O_3Na$ $[M+Na]^+$ requires 267.0997.

4.9.12. Ethyl 3-anthracen-9-yl-3-hydroxy propanoate (17). Pale yellow liquid; IR ν_{max} (neat): 3486, 3049, 2983, 1707, 1621, 1447, 1332, 1282, 1174, 1077, 1105, 1024, 963, 892, 736, 422 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27 (t, J=7.2 Hz, 3H), 3.18 (ddd, J=16.8, 10.5, 3.0 Hz, 2H), 3.38 (s, 1H), 4.22 (q, J=7.1 Hz, 2H), 6.74 (dd, J=10.4, 2.4 Hz, 1H), 7.47 (m, 4H), 7.98 (d, J=8.2 Hz, 2H), 8.4 (s, 1H), 8.66 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 41.7, 61.0, 67.2, 124.6, 124.8, 125.8, 128.5, 129.1, 129.3, 131.6, 132.34, 172.6; HRMS (ESI): found 317.1170, $C_{19}H_{18}O_3Na$ $[M+Na]^+$ requires 317.1154.

4.9.13. Ethyl 3-hydroxy-4-phenyl butanoate (18). Colourless oil; spectroscopic data identical to that reported in literature.[34b]

4.9.14. (*S*)-Ethyl 3-hydroxy-5-phenyl pentanoate (19). Colourless oil; spectroscopic data identical to that reported in literature,[34b] $[\alpha]_D^{25}$ −2.35 (*c* 2.71, CH_2Cl_2).

4.9.15. (*S*)-Methyl-3-hydroxy-3-phenyl propanoate (20). Colourless oil; spectroscopic data identical to that reported in literature,[22a] $[\alpha]_D^{25}$ −49.9 (*c* 1, $CHCl_3$).

4.9.16. (*S*)-*n*-Propyl 3-hydroxy-3-phenyl propanoate (21). Colourless oil; $[\alpha]_D^{25}$ −51.6 (*c* 2.61, $CHCl_3$). IR ν_{max} (neat): 3453, 3064, 3032, 2969, 2880, 1732, 1494, 1455, 1394, 1356, 1269, 1195, 1058, 915, 761, 700, 609 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, J=7.4 Hz, 3H), 1.62 (sextet, J=7 Hz, 2H), 2.69 (ddd, J=16.1, 8.9, 3.8 Hz, 2H), 3.56 (s, 1H), 4.03 (t, J=6.6 Hz, 2H), 5.14 (dd, J=7.7 Hz, 1H), 7.28 (m, 5H) ^{13}C NMR (100 MHz, $CDCl_3$) 10.3, 21.8, 43.4, 66.3, 70.2, 125.6, 127.6, 128.4, 142.7, 172.3. HRMS (ESI): found 231.0997, $C_{12}H_{16}O_3Na$ $[M+Na]^+$ requires 231.0997.

4.9.17. (*S*)-*n*-Butyl 3-hydroxy-3-phenyl propanoate (22). Colourless oil; spectroscopic data identical to that reported in literature,[28] $[\alpha]_D^{25}$ −31.2 (*c*, 3.78 $CHCl_3$).

4.9.18. Methyl phenyl 3-hydroxy butanoate (23). Colourless oil; spectroscopic data identical to that reported in literature.[34c]

4.9.19. 3′-Phenyl-prop-2′-enyl 3-hydroxy butanoate (24). Pale yellow liquid; IR ν_{max} (neat): 3445, 3069, 3023, 2979, 2937, 1731, 1578, 1494, 1449, 1372, 1275, 1224, 1147, 1039, 965, 886, 750, 693, 634, 607, 540 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.23 (d, J=6.3 Hz, 3H), 2.49 (ddd, J=16.5, 8.2, 4.1 Hz, 2H), 3.17 (s, br, 1H), 4.21 (m, 1H), 4.75 (d, J=6.4 Hz, 2H), 6.26 (dt, J=15.9, 6.4 Hz, 1H), 6.64 (d, J=15.9 Hz, 1H), 7.3 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.5, 42.8, 64.2, 65.2, 122.7, 126.6, 128.3, 128.5, 134.5, 136.0, 172.6. HRMS (ESI): found 243.0997, $C_{13}H_{16}O_3Na$ $[M+Na]^+$ requires 243.0998.

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