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Deracemisation of aromatic β-hydroxy esters using immobilised whole cells of *Candida parapsilosis* ATCC 7330 and determination of absolute configuration by ^{1}H NMR

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Abstract—Deracemisation of aryl and substituted aryl β-hydroxy esters using immobilised whole cells of *Candida parapsilosis* ATCC 7330 yields the corresponding (*S*)-enantiomer in >99% enantiomeric excess and good yield (up to 68%). The absolute configuration of ethyl 3-(2,4-dichlorophenyl)-3-hydroxy propanoate and ethyl 3-hydroxy-5-phenyl-pent-4-enoate as determined by ^{1}H NMR using MTPA chloride was found to be '*S*'. The chemical shifts of the methoxy groups of the two diastereomeric MTPA esters were used as diagnostic signals to determine the absolute configuration.

1. Introduction

Enantiopure β-hydroxy esters are important chiral synthons[1] and are widely used in the preparation of many pharmaceutical intermediates such as L-carnitine,[2] fluoxetine[3] and inhibitors of HMG-CoA reductase[4] among others. In addition to routine chemical methods[5] for the synthesis of enantiomerically pure β-hydroxy esters, biocatalytic methods have been developed to a great advantage.[6–8] Biocatalytic approaches for the synthesis of these target molecules broadly involve (a) an asymmetric reduction[6–9] and (b) a kinetic resolution.[10] Both these methods can be carried out by commercially available enzymes and microbial whole cells. In the case of asymmetric reduction, the use of free whole cells is preferred over isolated enzymes in order to avoid the use of expensive cofactors and the immobilised whole cells are preferred over free cells as they are more robust and can be reused. The use of whole cells often results in the competitive action of multiple enzymes on the substrate molecules, which reduces the enantiomeric excess of the desired product. A solution to this problem was reported by Stewart et al. who engineered whole cells of *Escherichia coli* by expressing an oxido-reductase from baker's yeast for the reduction of β-ketoesters.[11] High enantiomeric excess of the product can also be achieved by kinetic resolution,[10] which is a widely used method. A maximum of 50% yield of each enantiomer and the unwanted enantiomer is the limitation of kinetic resolution. An alternative enantiomerically pure product in high ee and quantitative yield is deracemisation.[12] The use of isolated enzymes[13] for the deracemisation of mandelic acid and a two-biocatalytic system[14] for the stereoinversion of L-pantoyl lactone to its D-form are known. However, a single biocatalyst (whole cells) mediated deracemisation is more advantageous. Backvall and Huerta[15] used a ruthenium-catalyst along with *Pseudomonas cepacia* lipase in a chemo-enzymatic deracemisation of β-hydroxy esters. Deracemisation of ethyl 3-hydroxy butanoate using aged cultures of *Geotrichum candidum* resulted in the corresponding (*R*)-enantiomer in 96% ee and 80% yield.[16] Nakamura et al.[17] reported the stereoinversion of methyl 3-hydroxy butanoate and methyl 3-hydroxy pentanoate using *G. candidum* IFO 5767 to produce the corresponding (*R*)-enantiomer in 97–99% ee and 26–48% isolated yield. The deracemisation of β-hydroxy esters is restricted to the above examples, both of which use the same microbe, *G. candidum* to give the deracemised product, the (*R*)-antipode.

The activity of oxidoreductases in *Candida parapsilosis* is well illustrated by Kula et al.[8] Different strains of this species were later used for the deracemisation of 1,2-diols,[18] 1,3-diols[19] and α-hydroxy esters.[20] We have

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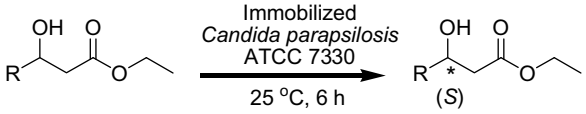
previously reported the deracemisation of some β-hydroxy esters using free cells of *C. parapsilosis* ATCC 7330.[21] Bearing in mind the advantages of using immobilised biocatalysts,[22] that is, easy separation, reusability of the cells, downstream processing, use of higher cell-concentration and stabilisation of several cell functions, we herein report the use of immobilised cells of *C. parapsilosis* ATCC 7330 for the deracemisation of a variety of β-hydroxy esters, thus illustrating the versatility of this biocatalyst. Experimental unequivocal proof that the (*S*)-enantiomer is formed on deracemisation is also included in this report.

2. Results and discussion

2.1. Deracemisation of racemic β-aryl and β-substituted aryl β-hydroxy esters

Deracemisation of aromatic β-hydroxy esters with immobilised whole cells of *C. parapsilosis* ATCC 7330 (ICp) was standardised using ethyl 3-hydroxy-3-phenyl propanoate as the standard substrate. The (*S*)-enantiomer of the product was obtained in 99% ee and 57% yield. To prove the generality of this deracemisation reaction with respect to the substrate structure, a variety of aryl substituted β-hydroxy esters were deracemised using ICp (Scheme 1), which resulted in the formation of their (*S*)-enantiomers in >99% ee and up to 68% yield, irrespective of the nature of substituents on the aromatic ring. Kinetic resolution of ethyl 3-hydroxy-5-phenyl-pent-4-enoate (Table 1, entry 7) by *Pseudomonas* sp. gave 30% conversion and 98% ee of the (*R*)-enantiomer.[23] Recently, Xu and Yuan[24] reported the *Candida rugosa* catalysed kinetic resolution of β-hydroxy-β-aryl-propionates, which also gave the (*R*)-enantiomer of the β-hydroxy esters. Using this method, ethyl 3-(2,4-dichlorophenyl)-3-hydroxy propanoate was obtained in 93.7% ee and 46% yield, ethyl 3-hydroxy-3-(4-nitrophenyl) propanoate in 91.6% ee and 43% yield and ethyl 3-hydroxy-3-(4-methylphenyl) propanoate in 94.5% ee and 42% yield. Apart from the above biocatalytic methods, asymmetric reduction using chiral catalysts are also reported. An (*o*-BINAPO)-Ru catalysed asymmetric hydrogenation of β-aryl and β-substituted aryl β-keto esters gave the corresponding (*R*)-enantiomer in 90–98% ee in 20 h reaction time, at 50 °C and 80 psi of H_2.[5a] A catalytic combination of (1*S*,2*R*)-ephedrine and a Ru-complex, for the asymmetric reduction of ethyl benzoylacetate and its aryl-substituted derivatives resulted in the corresponding (*S*)-enantiomers in 99% yield and 72–94% ee.[25] Another Ru (biaryl phosphine ligands) catalysed asymmetric hydrogenation of β-aryl β-keto esters gave 95–98% ee and 100% conversion in 20 h reaction time, at 65 °C and 30 atm of H_2.[26] The other synthetic methods reported have also used harsh reaction conditions and different metal catalysts. Substrates having different *para*-substituents (Table 1, entries 3, 4 and 5) on deracemisation gave 98–99% ee and 41–51% isolated yield of their corresponding (*S*)-enantiomers. Irrespective of the electronic nature of the substituents (methyl, chloro and nitro), the ee of the deracemised product remained very high (98–99%). The standard substrate with an *ortho* substituent (Table 1, entry 2) showed a poor ee (9%) of the product. The deracemisation of β-hydroxy esters by *C. parapsilosis* ATCC 7330 possibly follows a stereoinversion mechanism,[27] that is, enantioselective oxidation of one antipode to the keto-ester intermediate followed by a complementary enantioselective reduction of the intermediate ketoester to give a single enantiomer in high yield and ee. The poor ee in the case of ethyl 3-hydroxy-3-(2-methylphenyl) propanoate could be due to the steric hindrance and the hydrophobic nature of the *ortho*-substituent to the reaction centre. Ethyl 3-(2,4-dichlorophenyl)-3-hydroxy propanoate (Table 1, entry 6) on deracemisation gave 82% ee and 53% yield of its (*S*)-enantiomer. The absolute configuration of this molecule as determined by ^{1}H NMR using MTPA chloride is discussed here in detail. Interestingly, the ee of this *o*,*p*-disubstituted substrate (entry 6) was found to be slightly lower than the *para*-substituted substrates (entries 3, 4 and 5) and much higher than the *ortho*-substituted substrate (entry 2). The hydrophilic nature of the chloro substituent possibly favours deracemisation as opposed to the steric effect at the *ortho*-position in the case of substrate **6**. Deracemisation of ethyl 3-hydroxy-5-phenyl-pent-4-enoate (Table 1, entry 7) resulted in >99% ee and 28% yield of its (*S*)-enantiomer, the absolute configuration of which was also determined by ^{1}H NMR using MTPA



Scheme 1.

Table 1. Immobilised *Candida parapsilosis* ATCC 7330 mediated deracemisation of aryl and aryl substituted β-hydroxy esters

Entry	R	ee (%)	Yield (%)	$[\alpha]_D^{25}$	Abs Conf.
1	Ph	>99	57	− 50.1 (*c* 1.5, $CHCl_3$)[5a]	*S*[a]
2	*o*-MePh	09	68	Nd	Nd
3	*p*-MePh	98	51	−44.6 (*c* 1.2, $CHCl_3$)[5a]	*S*[a]
4	*p*-ClPh	99	42	−43.7 (*c* 1.38, $CHCl_3$)[5a]	*S*[a]
5	*p*-NO_2Ph	99	41	−59.5 (*c* 1.5, $CHCl_3$)[24]	*S*[a]
6	*o*,*p*-ClPh	82	53	−39.8 (*c* 1.5, $CHCl_3$)	*S*[b]
7	PhCH=CH	>99	28	−2.57 (*c* 2.7, $CHCl_3$)	*S*[b]

ee: Enantiomeric excess measured by HPLC; Nd: not determined.

[a] The absolute configurations were assigned on the basis of the sign of rotation.[5a,24]

[b] The absolute configurations were assigned by ^{1}H NMR using MTPA chloride.

chloride. The hydrolysed by-product (3-hydroxy-5-phenyl-pent-4-enoic acid) formed during the deracemisation of substrate **7** possibly due to the action of a hydrolase, accounts for the low yield of the product. The low yield of the deracemised product is also because the oxidised product which is a β-keto ester intermediate is hydrolysed and easily decarboxylated. Another deracemisation experiment was carried out starting with (*R*)-ethyl 3-hydroxy-3-phenyl propanoate. Chiral HPLC analysis of the product mixture indicates the inversion of configuration from (*R*)-**1** to (*S*)-**1**.[27] It is noteworthy that the use of different strains of *C. parapsilosis* reported so far for deracemisation reactions were limited to substrates with a single functional group, except in the case of α-hydroxy esters reported from our laboratory.[20]

2.2. Assignment of the absolute configuration of the deracemised products 6 and 7

The absolute configuration of substrates **1** and **3**–**5** was assigned based on the sign of specific rotation of their deracemised products in comparison with the literature values.[5a,24] Ethyl 3-(2,4-dichlorophenyl)-3-hydroxy propanoate **6** and ethyl 3-hydroxy-5-phenyl-pent-4-enoate **7** were the two substrates, which on deracemisation gave 82% and >99% ee, respectively, whose specific rotations are not reported in the literature and had to be determined. Several methods were used for the determination of absolute configuration of secondary alcohols, for example, X-ray crystallography (for which a crystalline sample is a prerequisite[28]), and NMR-techniques among others.[29] Assignment of the absolute configuration of β-hydroxy esters have also been reported using X-ray crystallography[28b] and ^{1}H NMR characterisation of MTPA-derivatives—directly[30] or with lanthanide shift reagents.[31] The secondary alcohol group of the β-hydroxy ester was derivatised with a chiral auxiliary of already known absolute configuration.[30] The first and most accepted derivatising chiral compound for secondary alcohols was MTPA or α-methoxy-α-(trifluoromethyl) phenylacetic acid, also known as Mosher's acid.[29b] Recently, the assignment of the absolute configuration of deracemised α-hydroxy esters using the Mosher's method was reported.[32] This method involves the derivatisation of the starting substrate with (*R*)- and (*S*)-MTPA chloride followed by the separation of the formed diastereomers and their characterisation by ^{1}H NMR. Herein, we report the assignment of the absolute configuration of deracemised β-hydroxy esters using a single enantiomer of Mosher's acid chloride.

The validity of Mosher's method for determining the absolute configuration of aryl β-hydroxy esters was first established by using known enantiomers of standard substrate-**1** viz. ethyl 3-hydroxy-3-phenyl propanoate. Racemic-**1**, (*R*)-**1** and (*S*)-**1** were treated with (*S*)-MTPA chloride (Scheme 2) to give the corresponding MTPA esters that is, MTPA esters of (*S*,*R*)- and (*R*,*R*)-**1**, (*R*,*R*)-**1** and (*S*,*R*)-**1**, respectively. The two diastereomers of MTPA ester of (*S*,*R*)- and (*R*,*R*)-**1** are represented in Figure 1a and b and the ^{1}H NMR of the three MTPA esters of (*RS*)-**1**, (*R*)-**1** and (*S*)-**1** are represented in Figure 2a–c, respectively.

According to Mosher's method, the chemical shift difference between any two similar protons of the β-hydroxy ester moiety (preferably the methylene protons for the present case) of the two diastereomers (Fig. 1a and b) are used to assign the absolute configuration. It can be inferred from (the ^{1}H NMR spectra) Figure 2a–c that the chemical shifts of the methylene protons of MTPA esters of racemate-**1** and that of a single enantiomer [(*R*) or (*S*)] are almost identical. However, closer examination of the ^{1}H NMR of these diastereomers

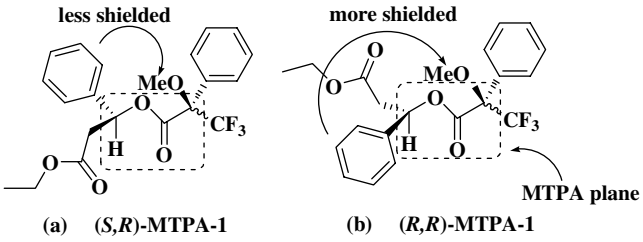
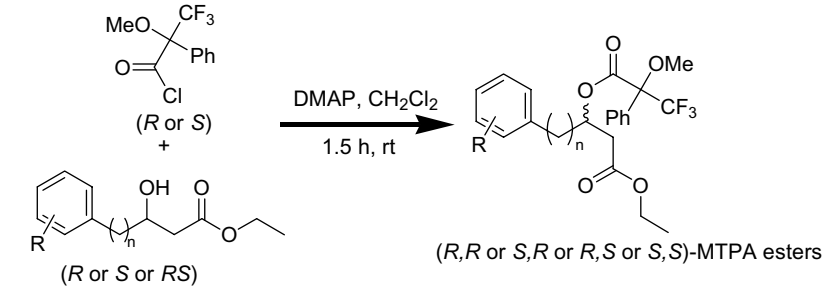


Figure 1. MTPA esters of (*S*,*R*)- and (*R*,*R*)-**1**.



R=H	n=0,	(*RS*)-**1**
R=H	n=0,	(*R*)-**1**
R=H	n=0,	(*S*)-**1**
R=*o*,*p*-diCl,	n=0,	(*RS*)-**6**
R=*o*,*p*-diCl	n=0,	**6**
R=H	n=2 (CH_2=CH_2),	(*RS*)-**7**
R=H	n=2 (CH_2=CH_2),	**7**

R=H	n=0,	MTPA -(*SR* and *RR*)-**1**
R=H	n=0,	MTPA -(*R*,*R*)-**1**
R=H	n=0,	MTPA -(*S*,*R*)-**1**
R=*o*,*p*-diCl,	n=0,	MTPA -(*S*,*S* and *R*,*S*)-**6**
R=*o*,*p*-diCl	n=0,	MTPA -**6**
R=H	n=2 (CH_2=CH_2),	MTPA -(*S*,*S* and *R*,*S*)-**7**
R=H	n=2 (CH_2=CH_2),	MTPA -**7**

Scheme 2.

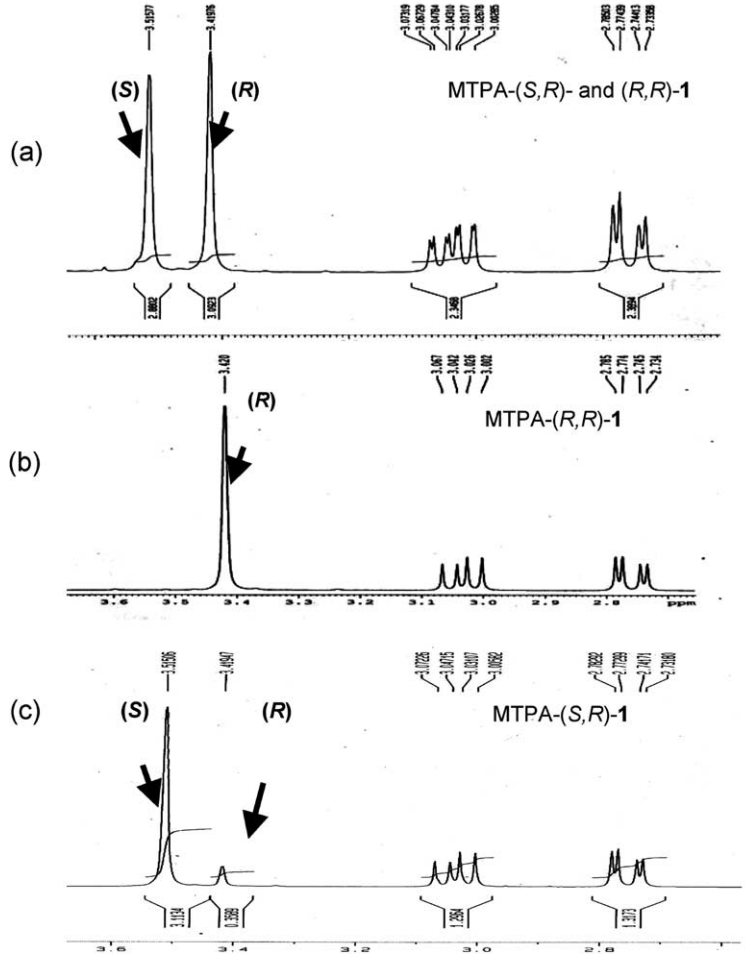
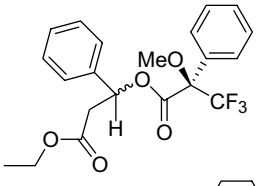
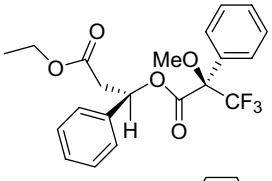
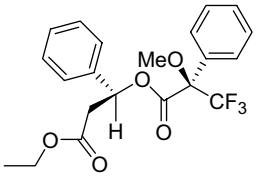


Figure 2. ^{1}H NMR of –OCH_3 and methylene in (a) MTPA-(*S*,*R*)- and (*R*,*R*)-**1**, (b) MTPA-(*R*,*R*)-**1** and (c) MTPA-(*S*,*R*)-**1**.

Table 2. Chemical shift (δ)-values in the ^{1}H NMR of MTPA esters of (*RS*)-**1**, (*R*)-**1** and (*S*)-**1** from Figure 2a–c

Entry	Product—Mosher ester	Abs Conf.	δ-values		
			OMe		Methylene[a]
MTPA-(*S*,*R*) and (*R*,*R*)-**1**		(*R*,*R*) and (*S*,*R*)	3.41 δ	3.51 δ	2.73–2.78 and 3.00–3.07 δ
MTPA-(*R*,*R*)-**1**		(*R*,*R*)	3.42 δ	—	2.73–2.78 and 3.00–3.06 δ
MTPA-(*S*,*R*)-**1**		(*S*,*R*)	—	3.51 δ	2.73–2.78 and 3.00–3.07 δ

[a] No difference in the chemical shift values was observed for all the above three cases.

[MTPA esters of (*S*,*R*)- and (*R*,*R*)-**1**, Fig. 1a and b] shows that the chemical shift difference between the methoxy signals of the two diastereomers is more significant than any other set of protons (Table 2).

This chemical shift difference of the methoxy signals is because in one structure, the methoxy group and the phenyl ring of the β-hydroxy ester are on the same side of the MTPA plane[29a] (Fig. 1b), while in the other diastereomer they are opposite to each other (Fig. 1a). The methoxy signals for the two diastereomers (Fig. 1a and b) appeared at 3.41 δ and 3.51 δ (Fig. 2a). The diamagnetic effect of the phenyl ring of the β-hydroxy ester is more pronounced in the structure where the methoxy group is on the same side of the phenyl ring (3.41 δ, shielded) as compared to the structure where the phenyl ring of the β-hydroxy ester and the methoxy group are on opposite sides of the MTPA plane (3.51 δ, deshielded). Consequently, the 3.41 δ methoxy signal should belong to the diastereomer (*R*,*R*)-MTPA-**1** (Fig. 1b) and 3.51 δ should belong to the diastereomer (*S*,*R*)-MTPA-**1** (Fig. 1a). This chemical shift difference of the methoxy signals is thus used to assign the absolute configuration of substrate **1** and subsequently for substrates **6** and **7**—a fact borne out by experiments carried out using (*S*)-MTPA chloride with (*R*)-**1** (authentic sample, >99% ee), which shows the methoxy signal at 3.42 δ (Fig. 2b) and (*S*)-MTPA chloride with standard (*S*)-**1** (authentic sample, 87% ee), which shows the methoxy signal at 3.51 δ (Fig. 2c). Thus, β-aryl-β-hydroxy propanoates lend themselves to this special observation where the chemical shift values of the methoxy signals can be used to determine the absolute configuration.

Having proved the absolute configuration of the known enantiomers of (*R*)-**1** and (*S*)-**1**, this method was used to assign the absolute configuration of the enantiomerically pure unknown enantiomers of **6** and **7** obtained after deracemisation. The MTPA-(*S*,*S*)- and (*R*,*S*)-**6** and MTPA-(*S*,*S*)- and (*R*,*S*)-**7** were prepared from (*R*)-MTPA chloride with (*R*,*S*)-**6** and (*R*,*S*)-**7**, respectively (Scheme 2, Figs. 3 and 5). MTPA-**6** and MTPA-**7** were also prepared using the (*R*)-MTPA chloride with the deracemised products of **6** and **7** (Scheme 2). The ^{1}H NMR analysis (Table 3) of Figure 4a shows a difference in the chemical shift between the two methoxy signals of MTPA-(*R*,*S*)- and (*S*,*S*)-**6** (Fig. 3a and b), which appears at 3.46 δ and 3.55 δ, respectively. The shielded methoxy signal (3.46 δ) belongs to the diastereomer in which the methoxy group and the aromatic ring of the β-hydroxy ester lie on the same side that is, MTPA-(*S*,*S*)-**6** (Fig. 3a). The 3.55 δ methoxy signal belongs to the MTPA-(*R*,*S*)-**6** (Fig. 3b). The methoxy signal for MTPA-**6** prepared as shown in Scheme 2 appears at 3.46 δ (Fig. 4b) (shielded region) and matches the methoxy group of MTPA-(*S*,*S*)-**6** (Fig. 4a). This confirms that MTPA-**6** has the same configuration as that of MTPA-(*S*,*S*)-**6** and the unknown pure enantiomer **6** prepared from the deracemisation reaction (Table 1) is the (*S*)-enantiomer.

In the diastereomers of MTPA-(*S*,*S*)- and (*R*,*S*)-**7** (Fig. 5a and b) the methoxy signals appeared at 3.52 δ and 3.56 δ (Fig. 6a and Table 3). This chemical shift difference between the methoxy signals is less because of the increased distance of the phenyl ring of the β-hydroxyl ester from the methoxy group in MTPA-(*S*,*S*)- and (*R*,*S*)-**7** as compared to the corresponding esters of **1** and **6**. The shielded methoxy signal (3.52 δ) should belong to the diastereomer in which the methoxy group and the phenyl ring of the β-hydroxy ester lie on the same side, that is, MTPA-(*S*,*S*)-**7** (Fig. 5a). In addition to these diagnostic signals, a difference in the chemical shift of the olefinc protons was also observed for both the diastereomers (Fig. 5a and b), which was used in characterising the individual diastereomers (Fig. 5a and b). The Ha signals appeared at 6.03–6.1 and 6.17–6.23 δ while the Hb signals appeared at 6.64–6.69 and 6.77–6.81 δ (Fig. 6a and Table 3) indicating that one pair of Ha and Hb are shielded (6.03–6.1 δ and 6.64–6.69 δ) as compared to the other pair, that is, Ha (6.17–6.23 δ) and Hb (6.77–6.81 δ) (Fig. 6a). According to Mosher's method this shielding is due to the diamagnetic effect of the phenyl ring of the Mosher's ester and hence, the shielded pair should belong to the diastereomer, where Ha and Hb of the β-hydroxy ester and the phenyl ring of the Mosher's ester lie on the same side, that is, MTPA-(*R*,*S*)-**7** (Fig. 5b) and the deshielded pair, Ha and Hb 6.17–6.23 δ and 6.77–6.81 δ belong to the diastereomer, that is, MTPA-(*S*,*S*)-**7** (Fig. 5a). The ^{1}H NMR analysis of MTPA-**7** revealed that the methoxy signal appeared at 3.52 δ, the Ha signal at 6.17–6.23 δ and the Hb signal at 6.77–6.81 δ (Fig. 6b), which exactly matches that of MTPA-(*S*,*S*)-**7** (Fig. 5a) as discussed

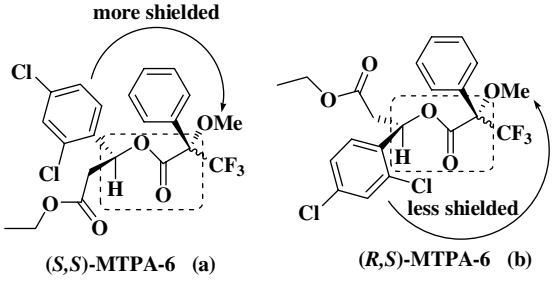


Figure 3. MTPA esters of (*S*,*S*)- and (*R*,*S*)-**6**.

Table 3. Chemical shift (δ)-values in the ^{1}H NMR of MTPA esters of (*R*,*S*)-**6** and **6** from Figure 4a and b and MTPA esters of (*R*,*S*)-**7** and **7** from Figure 6a and b

Entry	Abs Conf.	δ-values				
		OMe	OMe	Methylene	Hb	Ha
MTPA-(*S*,*S*)- and (*R*,*S*)-**6**	(*S*,*S*) and (*R*,*S*)	3.46 δ	3.55 δ	2.76–2.91 δ	—	—
MTPA-**6** (unknown)	(*S*,*S*) or (*R*,*S*)	3.46 δ	—	2.79–2.91 δ	—	—
MTPA-(*S*,*S*)- and (*R*,*S*)-**7**	(*S*,*S*) and (*R*,*S*)	3.52 δ	3.56 δ	2.68–2.75 and 2.81–2.9 δ	6.64–6.69 and 6.77–6.81 δ	6.03–6.1 and 6.17–6.23 δ
MTPA-**7** (unknown)	(*S*,*S*) or (*R*,*S*)	3.52 δ	—	2.68–2.73 and 2.81–2.87 δ	6.77–6.81 δ	6.17–6.23 δ

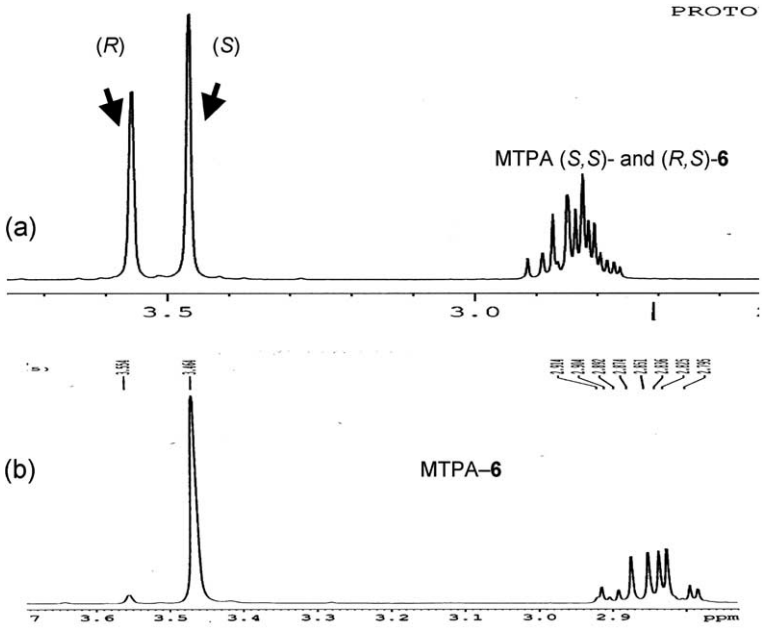


Figure 4. ^{1}H NMR of –OCH_3 and methylene in (a) MTPA (*S*,*S*)- and (*R*,*S*)-**6** and (b) MTPA-**6**.

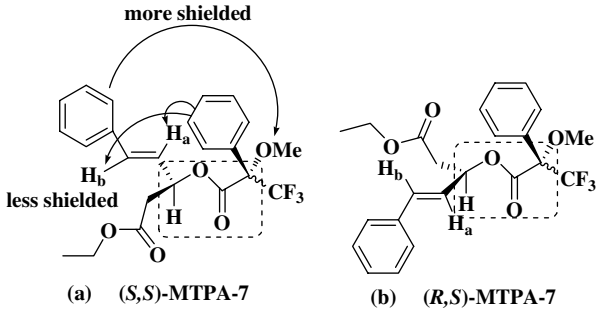


Figure 5. MTPA esters (*S*,*S*)- and (*R*,*S*)-**7**.

above. This proves that the unknown optically pure enantiomer **7** obtained from the deracemisation reaction (Table 1) is the (*S*)-enantiomer.

3. Conclusion

Deracemisation of ethyl 3-hydroxy-3-phenyl propanoate, the standard substrate, and aryl substituted β-hydroxy esters by immobilised whole cells of *C. parapsilosis* ATCC 7330 gave their corresponding (*S*)-enantiomer in 68% isolated yield and >99% ee. The nature and the position of the substituents on the aromatic ring affect the deracemisation. High ee (98–99%) and good isolated yield (41–51%) of product was observed with substrates having different *para* substituents on the aromatic ring irrespective of their electronic nature. A hydrophobic substituent present at the *ortho*-position of the standard substrate obstructs the process of deracemisation while the substrate with an *ortho*, *para*-dichloro substitution is poorly affected (82% ee). The absolute configuration of the deracemised products as determined by ^{1}H NMR using an enantiomerically pure MTPA chloride was found to be *S*. The chemical shift difference between the methoxy signals of the two diastereomers of the MTPA esters (substrates **1**, **6** and **7**) were used to assign the absolute configuration of the aromatic β-hydroxy esters.

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 ppm 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 OD-H 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 varies for different compounds. Ethyl 3-oxo-3-phenyl propanoate and ethyl 3-oxo-3-(4-nitro phenyl) propanoate were bought from Fluka, Buchs SG, Switzerland. All other chemicals used were of analytical grade and glass distilled.

4.2. Synthesis of racemic β-hydroxy esters

Racemic β-hydroxy esters (Table 1, entries 1 and 5) were synthesised by the sodium borohydride reduction of the corresponding β-keto esters. Ethyl 3-oxo-3-(4-methylphenyl) propanoate and ethyl 3-oxo-3-(2-methylphenyl) propanoate were synthesised using a reported method.[33] The keto esters were reduced using sodium borohydride,

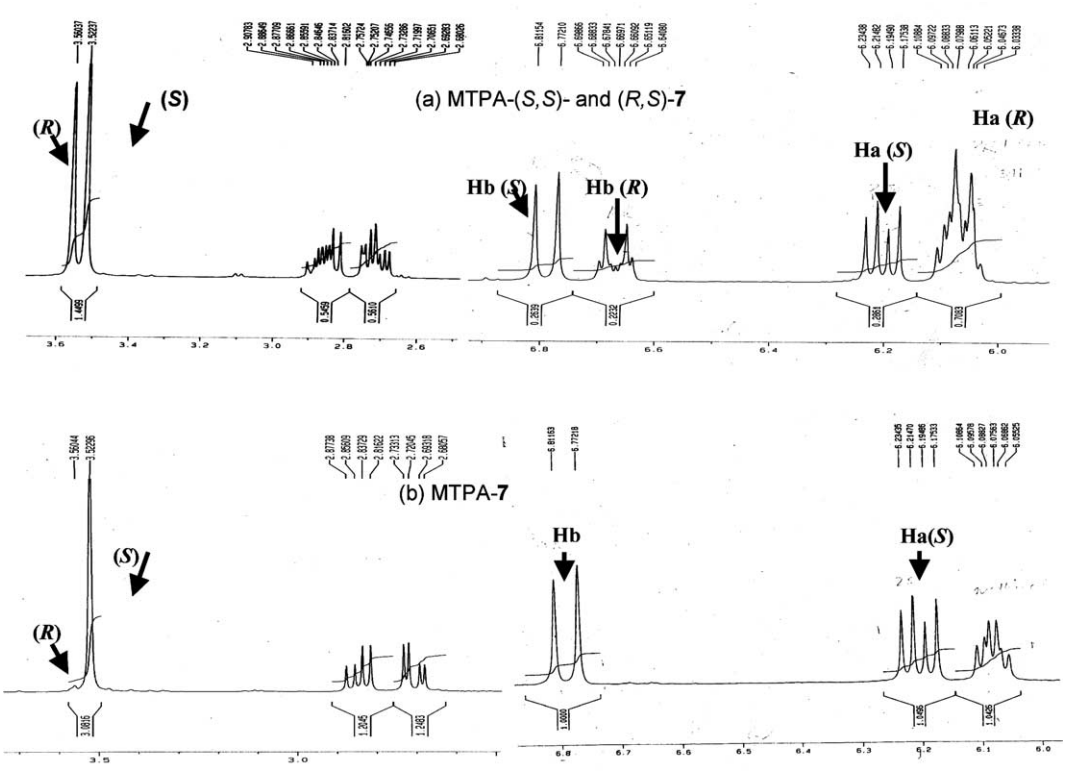


Figure 6. ^{1}H NMR of –OCH_3, methylene, Ha and Hb in (a) MTPA-(*S*,*S*)- and (*R*,*S*)-**7** and (b) MTPA-**7**.

ethanol and the corresponding racemic β-hydroxy esters were used for deracemisation. Ethyl 3-hydroxy-3-(4-chlorophenyl) propanoate, ethyl 3-(2,4-dichlorophenyl)-3-hydroxy propanoate and ethyl 3-hydroxy-5-phenyl-pent-4-enoate were synthesised by Reformatsky reaction.[34] All the above-synthesised racemic β-hydroxy esters were characterised by ^{1}H and ^{13}C NMR and compared with the literature reported data (Table 4).

4.3. Microorganism and cultivation

C. parapsilosis ATCC 7330 was procured from ATCC and was grown in a yeast malt broth medium (50 mL) in 250 mL Erlenmeyer flasks incubated at 25 °C, 200 rpm. The cells were harvested by centrifuging the 44 h culture broth at 3214*g* for 15 min and washed with sterile water. The process was repeated thrice and the wet cells were used for preparing the immobilised beads.

4.4. Immobilisation of biocatalyst

In a 500 mL Erlenmeyer flask containing sterilised water (204 mL), sodium alginate (4.08 g, 2% w/v) was added and the suspension was stirred at 50 °C for 1 h. Wet cells of *C. parapsilosis* ATCC 7330 ($1.7 \times 24 = 40.8$ g) obtained after centrifugation were suspended in 43.2 mL (1.8×24) of distilled water and added to the above mixture. A homogeneous suspension was obtained after stirring for another hour. This uniform slurry was added dropwise to a pre-chilled $CaCl_2$ aqueous solution (2% w/v), which resulted in the formation of beads of average 1.5–2.0 mm diameter (240–250 mL). The beads were kept in $CaCl_2$ aqueous solution for 12 h and then thoroughly washed [3×300 mL of distilled water] and stored at 10–15 C before use in biotransformation. 40.8 g wet cells (from 24 flasks) of *C. parapsilosis* gave 240–250 mL of ICp. 1.7 g of wet cells from one culture flask contributes ~10 mL of ICp.

Table 4. HPLC and NMR characterisation of the deracemised products

Sub. no. (Table 1)	HPLC			NMR—Ref. (^{1}H and ^{13}C)
	Column used	Solvent (Hex: IPA)	Ret. time (min)	
1	Chiralcel OD-H	95:05	20.4 (*S*) and 25.7 (*R*)	21
2	Chiralcel OJ-H	90:10	15.5 (minor); 19.1 (major)	5a
3	Chiralcel OJ-H	98:02	125.3 (*R*) and 134.8 (*S*)	5a
4	Chiralcel OJ-H	99.5:0.5	198.1 (*R*) and 215.8 (*S*)	5a
5	Chiralcel OJ-H	95:05	77.7 (*R*) and 84.0 (*S*)	24
6	Chiralcel OJ-H	99.5:0.5	41.4 (*R*) and 50.8 (*S*)	24
7	Chiralcel OD-H	95:05	33.5 (*R*) and 48.6 (*S*)	

4.5. Deracemisation

Racemic β-hydroxy esters (504 μmol, 96 μL) dissolved in the appropriate amount of ethanol (0.04% v/v) was 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 reaction mixture extracted using ethyl acetate, dried over anhydrous sodium sulfate and concentrated. The crude reaction mixture after column purification was analysed by chiral HPLC to determine the enantiomeric excess of the deracemised product. Appropriate control experiments with the reaction mixture containing all the components (i) racemic β-hydroxy ester (ii) except the immobilised whole cells of *C. parapsilosis* established the optical purity of the product and the chemical yield.

4.6. Determination of the reusability of ICp

To a suspension of 250 mL of freshly prepared ICp in 125 mL of distilled water was added a solution of 504 μmol of ethyl 3-hydroxy-3-phenyl propanoate pre-dissolved in 2400 μL of ethanol. The reaction was carried out at 25 °C and 150 rpm for 6 h. After filtering the ICp, the reaction mixture was extracted with ethyl acetate, dried over anhydrous sodium sulfate and concentrated. The crude reaction mixture after column purification was analysed for enantiomeric excess by chiral HPLC. The filtered beads were thoroughly washed in distilled water and stored at 10–15 °C. This procedure was repeated by reusing the same ICp to determine the recyclability of these beads by calculating the isolated yield and the ee of the product.

A gradual decrease in activity was observed with increasing number of cycles (Table 5). The activity of the reaction was determined by the ee of the deracemised product that is, ethyl 3-hydroxy-3-phenyl propanoate. The immobilised cells could be re-employed thrice after first use with a loss of 5% activity. A loss of ~10% activity was observed for fifth to seventh cycle beyond which the loss is ~15%. The gradual decrease in deracemisation activity could be due to cell leakage through the immobilisation matrix.

Table 5. Determination of the reusability[a] of immobilised whole cells of *Candida parapsilosis* ATCC 7330

Cycle no.	ee (%)	Yield (%)	% Loss of activity
1	99	57	0
2	96	56	3
3	94	59	5.1
4	94	55	5.1
5	89	57	10.1
6	87	56	12.1
7	87	49	12.1
8	84	55	15.1

[a] The substrate used was ethyl-3-hydroxy-3-phenyl propanoate for the deracemisation reaction.

4.7. Preparation of MTPA esters

To a solution of racemic ethyl 3-hydroxy-3-phenyl propanoate (10 μL, 0.054 mmol) in CH_2Cl_2 (0.85 mL), Dimethylamino pyridine (26 mg, 0.214 mmol) and (*S*)-(+)-α-(trifluoromethyl) phenyl acetyl chloride (20 μL, 0.107 mmol) were added at room temperature. Water was added to the reaction mixture after 1.5 h. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by preparative thin layer chromatography (*n*-hexane/EtOAc = 95:05) to give the product (16 mg, 73% yield). The product was characterised by ^{1}H NMR spectroscopy. The MTPA esters of (*R*)-**1**, (*S*)-**1**, (*RS*)-**6**, **6**, (*RS*)-**7** and **7** were also synthesised by this method.

4.8. ^{1}H NMR characterisation of the MTPA derivatives

(i) MTPA-(*S*,*R*)- and (*R*,*R*)-**1**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.18–1.23 (2t, 6H, 2 × –OCH$_2$C*H*$_3$), 2.73–2.78 and 3.00–3.07 (dd and d of dd, 4H, 2 × –C*H*$_2$CO), 3.41 (s, 3H, –OC*H*$_3$), 3.51 (s, 3H, –OC*H*$_3$), 4.04–4.12 (2q, 4H, 2 × –OC*H*$_2$CH$_3$), 6.33–6.44 (2 dd, 2H, –C*H*OCO), 7.26–7.71 (m, 20H, 4 × C$_6$*H*$_5$–).

(ii) MTPA-(*R*,*R*)-**1**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.16–1.19 (t, 3H, –OCH$_2$C*H*$_3$), 2.73–2.78 and 3.00–3.06 (d of dd, 2H, –C*H*$_2$CO), 3.42 (s, 3H, –OC*H*$_3$), 4.02–4.08 (q, 2H, –OC*H*$_2$CH$_3$), 6.4–6.44 (dd, 1H, –C*H*OCO), 7.26–7.71 (m, 10H, 2 × C$_6$*H*$_5$–).

(iii) MTPA-(*S*,*R*)-**1**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.21–1.24 (t, 3H, –OCH$_2$C*H*$_3$), 2.73–2.78 and 3.00–3.07 (d of dd, 2H, –C*H*$_2$CO), 3.51 (s, 3H, –OC*H*$_3$), 4.04–4.09 (q, 2H, –OC*H*$_2$CH$_3$), 6.33–6.37 (dd, 1H, –C*H*OCO), 7.23–7.69 (m, 10H, 2 × C$_6$*H*$_5$–).

(iv) MTPA-(*S*,*S*)- and (*R*,*S*)-**6**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.18–1.26 (2t, 6H, 2 × –OCH$_2$C*H*$_3$), 2.76–2.91 (m, 4H, 2 × –C*H*$_2$CO), 3.46 (s, 3H, –OC*H*$_3$), 3.55 (s, 3H, –OC*H*$_3$), 4.05–4.19 (2q, 4H, 2 × –OC*H*$_2$CH$_3$), 6.64–6.73 (2 dd, 2H, –C*H*OCO), 7.08–7.72 (m, 16H, 2 × C$_6$*H*$_5$–, 2 × –*o*,*p*-diClC$_6$*H*$_3$).

(v) MTPA-**6**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.18–1.25 (t, 3H, –OCH$_2$C*H*$_3$), 2.79–2.91 (d of dd, 2H, –C*H*$_2$CO), 3.46 (s, 3H, –OC*H*$_3$), 4.04–4.12 (q, 2H, 2 × –OC*H*$_2$CH$_3$), 6.69–6.73 (dd, 1H, –C*H*OCO), 7.24–7.72 (m, 8H, C$_6$*H*$_5$–, –*o*,*p*-diClC$_6$*H*$_3$).

(vi) MTPA-(*S*,*S*)- and (*R*,*S*)-**7**: ^{1}HNMR (400 MHz, $CDCl_3$): 1.17–1.26 (2t, 6H, 2 × –OCH$_2$C*H*$_3$), 2.68–2.75 and 2.81–2.9 (2d of dd, 4H, 2 × –C*H*$_2$CO), 3.52 (s, 3H, –OC*H*$_3$), 3.56 (s, 3H, –OC*H*$_3$), 4.04–4.15 (2q, 4H, 2 × –OC*H*$_2$CH$_3$), 6.03–6.1 (m, 2H, –C*H*OCO), 6.03–6.1 and 6.17–6.23 (2 dd, 2H, PhCH=C*H*–CH–), 6.64–6.69 and 6.77–6.81 (2d, 2H, PhC*H*=CH–CH–), 7.28–7.52 (m, 20H, 4 × C$_6$*H*$_5$–).

(vii) MTPA-**7**: ^{1}HNMR (400 MHz, $CDCl_3$): 1.17–1.25 (t, 3H, –OCH$_2$C*H*$_3$), 2.68–2.73 and 2.81–2.87 (d of dd, 2H, –C*H*$_2$CO), 3.52 (s, 3H, –OC*H*$_3$), 4.03–4.11 (q, 2H, –OC*H*$_2$CH$_3$), 6.05–6.1 (m, 1H,

–C*H*OCO), 6.17–6.23 (dd, 1H, PhCH=C*H*–CH–), 6.77–6.81 (d, 1H, PhC*H*=CH–CH–), 7.28–7.52 (m, 10H, $2 \times C_6H_5$–).

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