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Preparation of optically pure (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates by *Candida parapsilosis* ATCC 7330 mediated deracemisation of the racemates

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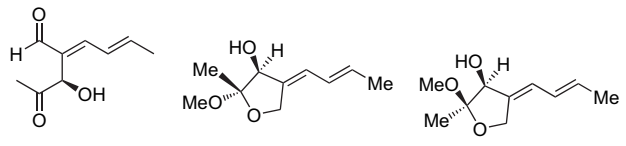
Abstract—Biocatalytic deracemisation of a range of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates using *Candida parapsilosis* ATCC 7330 resulted in pure (*S*)-enantiomers in yields of up to 80% and ee>99%.

1. Introduction

As a strategy to prepare optically pure organic molecules, deracemisation[1,2] has several advantages over resolution,[3,4] especially when a single biocatalyst[5,6] can be employed for the purpose. In continuation of our work on *Candida parapsilosis* ATCC 7330 mediated deracemisation of α- and β-hydroxy esters,[7–12] we now report here for the first time, the biocatalytic preparation of optically pure (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates. Though the syntheses of these optically pure (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates except for (3*E*,5*E*)-isopropyl-2-hydroxy-6-phenylhexa-3,5-dienoate are not reported yet, compounds containing this important subunit, i.e., β, γ, δ, ω unsaturated α-hydroxy carbonyl, have been found to have cytotoxic and antibacterial activities.[13,14] Some well known examples are avellaneol (**1**, Fig. 1), isolated from *Hypocrea avellanea*, which showed activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Mycobacterium smegmatis*,[13] and Lachnellins B (**2**, Fig. 1) and C (**3**, Fig. 1) isolated from submerged cultures of the *Lachnellula ascomycete* A,[14] which are inhibitors of malate synthase and show high cytotoxic and antimicrobial activities. These multifunctional chiral synthons are important building blocks in the synthesis of many bioactive molecules, e.g., 2(3*H*)-benzofuranone-3,5-dihydroxy[15] and an antitumour agent topotecin[16] in addition to other clinical applications.[17] Ruthenium catalyzed asymmetric reduction of (3*E*,5*E*)-isopropyl-2-oxo-6-phenylhexa-3,5-dienoate requires stringent conditions to give the hydroxy compound with an ee of only 7%.[18] Given the multifunctional groups on these molecules, it is not easy to design chemical methods for their preparation. Biocatalysts on the other hand, which are known for their specificity, can be used very effectively for such reactions as shown by the work reported herein. A series of (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates were prepared in high ee (up to >99%) and yields (up to 80%) using *C. parapsilosis* ATCC 7330, which deracemised these racemates to single enantiomers. Significantly, whole cells were used for this purpose thus obviating the need for added cofactors and elaborate enzyme purification protocols. We report here the synthesis of the racemic substrates, i.e., (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates, their chiral separation using chiral HPLC, optimization of the *C. parapsilosis* ATCC 7330 mediated deracemisation reaction for the representative substrate racemic (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate and substrate tolerance of the biocatalyst with structurally different (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates.



1 (Avellaneol) **2 (Lachnellins B)** **3 (Lachnellins C)**

Figure 1. Examples of β, γ, δ, ω unsaturated α-hydroxy carbonyl compounds.

Keywords: (3*E*,5*E*)-Alkyl-2-hydroxy-6-arylhexa-3,5-dienoates; Synthesis; Biocatalytic deracemisation; *Candida parapsilosis*.
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2. Results and discussion

2.1. Synthesis of substrates

Racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (Fig. 2) were synthesized by reported procedures to get high yields of the target esters. A representative scheme is shown in Scheme 1 and a detailed discussion follows. Aryl substituted cinnamaldehydes (**5a**–**5k**, Scheme 1) were prepared in good yields (60–70%) as per the reported method[19] and condensed with pyurvate.[20] (3*E*,5*E*)-2-Oxo-6-arylhexa-3,5-dienoic acids (**6a**–**6k**, Scheme 1) were obtained in 55–75% yields. As per the reported method,[21] (3*E*,5*E*)-ethyl 2-oxo-6-phenylhexa-3,5-dienoate (**7a**, Scheme 1) was synthesized via a two-step procedure (86% yield) consisting of the boron trifluoride-promoted reaction of 2-(trimethylsiloxy) acrylic esters with acetals followed by treatment with silica gel in benzene under reflux. In order to avoid these harsh reaction conditions and hazardous reagents, various (3*E*,5*E*)-alkyl-2-oxo-6-arylhexa-3,5-dienoates (**7a**–**7k**, Scheme 1) were prepared by esterification of the corresponding acids using a heteropoly acid[9,22,23] as a catalyst. Sodium borohydride reduction of 2-oxo esters[24] resulted in the corresponding racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8a**–**8k**, Scheme 1) in moderate to good yields (68–85%). Reduction of (3*E*,5*E*)-alkyl-2-oxo-6-arylhexa-3,5-dienoates (**7a**–**7k**, Scheme 1) using sodium borohydride is a simple method and it does not give the transesterified product, which was observed in the case of β-oxo esters.[25]

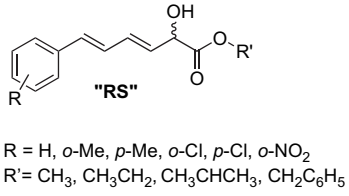


Figure 2.

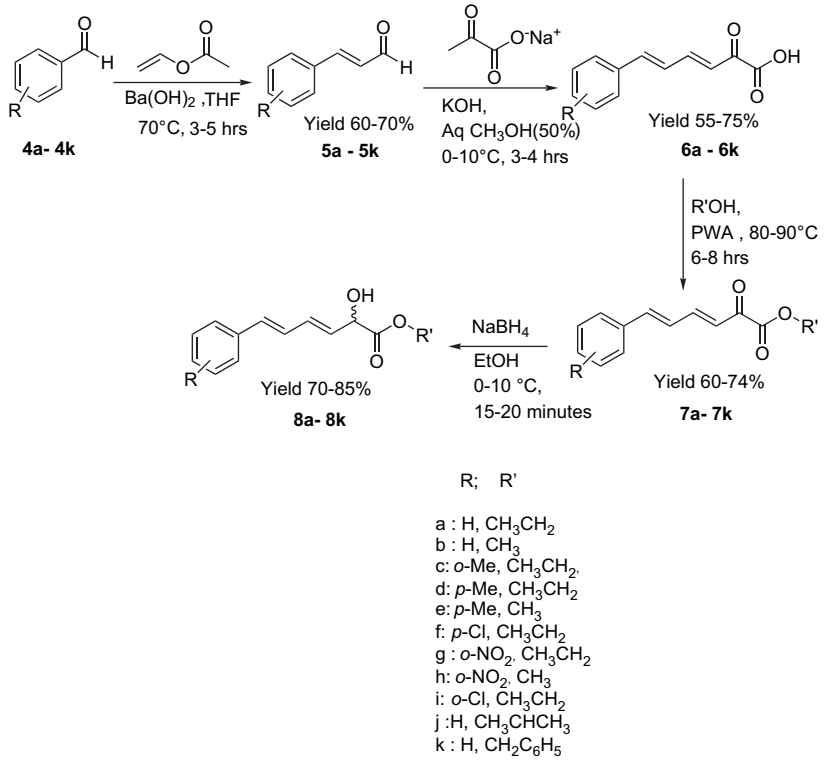
2.2. Chiral separation of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (8a–8k) by chiral HPLC

The direct separation of enantiomers of (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8a**–**8k**, Table 1) was achieved on a chiral stationary phase (CSP) column on HPLC. Compounds **8a**–**8c**, **8e**, **8g**, and **8j**–**8k** were resolved using a cellulose tris(3,5-dimethylphenyl)carbamate (Chiralcel OD-H) column and **8d**, **8f**–**8i** were resolved using a cellulose tris(4-methyl)benzoate (Chiralcel OJ-H) column. These CSPs have also been used to separate other α- and β-hydroxy esters.[9,12] Enantiomeric separation of **8a**–**8k**

Table 1. Chiral separation of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8a**–**8k**) by chiral HPLC

S. no	C. no[a]	Column used	Hex:IPA	Retention time (min)
1	**8a**	OD-H	98:2	20.3; 25.2
2	**8b**	OD-H	99:1	35.5; 40.5
3	**8c**	OD-H	98:2	20.6; 25.8
4	**8d**	OJ-H	98:2	25.9; 28.5
5	**8e**	OD-H	98:2	23.0; 24.8
6	**8f**	OJ-H	98:2	26.4; 30.6
7	**8g**	OD-H	97:3	36.7; 41.3
8	**8h**	OJ-H	97:3	46.5; 66.0
9	**8i**	OJ-H	98:2	22.2; 26.8
10	**8j**	OD-H	98:2	12.4; 15.2
11	**8k**	OD-H	98:2	20.2; 27.8

[a] C. no.—compound number.



Scheme 1. Synthesis of (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8a**–**8k**).

was achieved by varying the composition of hexane/isopropanol in the range of 99:1–97:3 and is reported here for the first time (Table 1).

2.3. Deracemisation of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (8a–8k) mediated by whole cells of *C. parapsilosis* ATCC 7330

Deracemisation is one of the most efficient methods to produce chiral synthons since it delivers 100% of a single enantiomer from its racemate.[1] Deracemisation of racemic *N*-(1-hydroxy-1-phenylethyl)benzamide to the (*R*)-enantiomer (98% ee) using whole cells of *Cunninghamella echinulata* NRRL 1384 is known.[26] Highly enantioselective conversion of the racemic 1-phenyl-1,2-ethanediol by stereoinversion involving a cofactor-dependent oxidoreductase system of *C. parapsilosis* CCTCC M203011 gave (*S*)-1-phenyl-1,2-ethanediol in 96.7% ee and 96% yield.[27] A combination of lipase and ruthenium metal was used for the dynamic kinetic resolution of racemic, secondary allylic alcohols, and α-hydroxy esters in high optical purity (94% ee) and high yield (76%),[28–30] in which ruthenium metal racemizes the unwanted enantiomers in situ.[31] Whole cells of *C. parapsilosis* ATCC 7330 have proven to be very effective biocatalysts for the deracemisation of α- and β-hydroxy esters as reported by us earlier.[9,12] We have also used this biocatalyst for asymmetric reduction of α-keto esters.[8] In the present study, *C. parapsilosis* ATCC 7330 has been employed to deracemise α-hydroxy esters with a conjugated diene system. Formation of the '*S*' enantiomers with high ee (up to >99%) and yields (up to 80%) reveal the wide substrate acceptance of this biocatalyst.

2.4. Optimization study for the deracemisation of (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (8a) using *C. parapsilosis* ATCC 7330

The representative dienoate used for optimizing the parameters for deracemisation was (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (**8a**, Scheme 2) and the product was monitored for its ee (%) and yield (%).

2.4.1. Time course. The time of the deracemisation was typically 3 h, which is within the range (1.5–4 h) reported for the deracemisation of racemic alkyl 2-hydroxy-4-arylbut-3-enoates.[9] As can be seen from Figure 3, the reaction mixture was analyzed at regular time intervals of 30 min for 4.5 h. After 3 h (*S*)-**9a** was obtained in >99% ee and 80% isolated yield.

2.4.2. Effect of temperature. Most of the biotransformations carried out by resting cells of *C. parapsilosis* CCTCC M203011 (China Center for Type Culture Collection) were done at 30 °C[32,33] but deracemisation of racemic alkyl 2-hydroxy-4-arylbut-3-enoates by *C. parapsilosis* ATCC 7330 were reported at 25 °C from our own laboratory.[9]

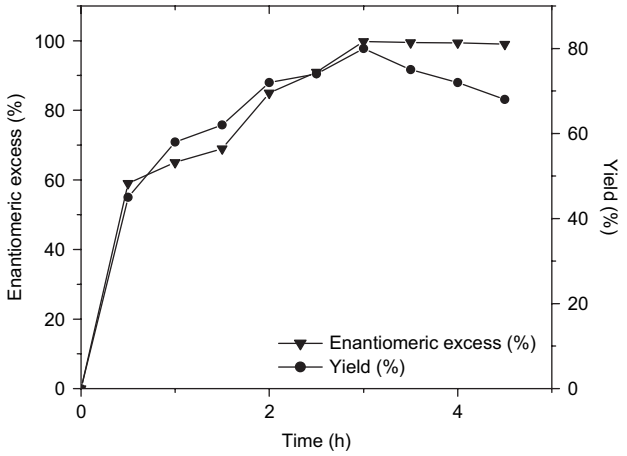


Figure 3. Time course of the deracemisation of (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (**8a**).

Deracemisation of (*RS*)-**8a** (3 mg ml^{-1}) by *C. parapsilosis* ATCC 7330 was carried out at 15, 20, 25, 30, 37, 40, and 45 °C under otherwise identical conditions (i.e., 1.0 g wet cells per 1 ml; 0.75 µl of ethanol for 3 h) (Fig. 4). The ee and isolated yield increased with increasing temperature from 15 °C (88% ee, 68% yield) to 25 °C (>99% ee, 80% yield) but decreased at 30 °C (96% ee, 74% yield) and 45 °C (8% ee, 58% yield). This variation in the ee with respect to temperature could be due to the different thermal stability of the ketoreductases encoded within the genome.[34] The deracemisation carried out at 25 °C gave the best results (yield ~81% and >99% ee).

2.4.3. Screening of solvents. The wet cell mass of *C. parapsilosis* ATCC 7330 used for deracemisation reactions is suspended in aqueous medium but the substrate being an organic molecule, has limited solubility in water. Therefore the substrate had to be dissolved in an organic solvent,

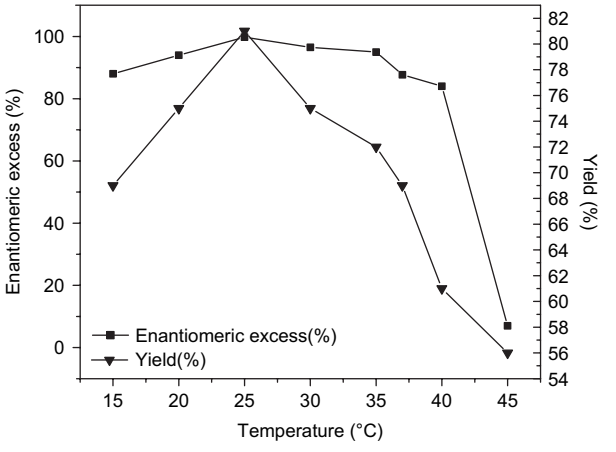
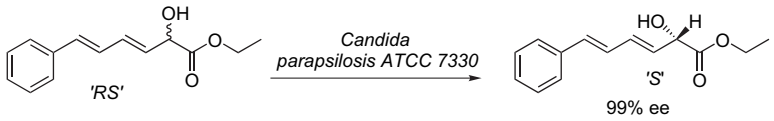


Figure 4. Effect of temperature in the deracemisation of (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (**8a**).



Scheme 2. Optimization study for (3*E*,5*E*)-ethyl-2-hydroxy-6-arylhexa-3,5-dienoate (**8a**).

preferably miscible with water. The effect of pure solvents (partition coefficient, log *P*) on the deracemisation of **8a** catalyzed by wet cells of *C. parapsilosis* ATCC 7330 was studied by monitoring the ee (%) and yield (%) of the product (Fig. 5). The reaction mixture of 3 mg of racemic substrate (**8a**) dissolved in 75 μl of pure solvent was added to 1.0 g of wet cells of *C. parapsilosis* ATCC 7330 suspended in 0.9 ml of distilled water and incubated for 3 h at 25 °C and 150 rpm. After completion of the reaction, product was extracted using ethyl acetate (1 ml) and ee was determined using chiral HPLC. The use of protic solvents like methanol (log *P* −0.74) and ethanol (log *P* −0.3) for dissolving the substrate for deracemisation resulted in good chemical (∼77–79%) and optical yields (>99% ee). However, in the case of solvents like benzene (log *P* 2.13), toluene (log *P* 2.5), and dichloromethane (log *P* 1.25), the ee of the product was poor (2–10%) as were the chemical yields (47–57%). In the case of solvents like diethylether (log *P* −0.01159), tetrahydrofuran (log *P* 0.49), and 1,4-dioxane (log *P* −0.42),

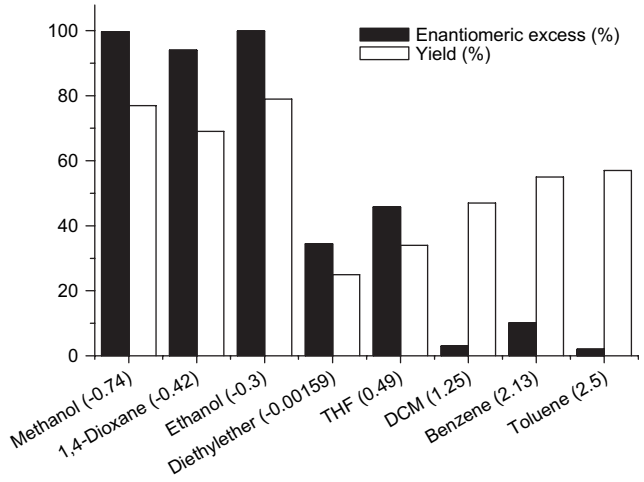


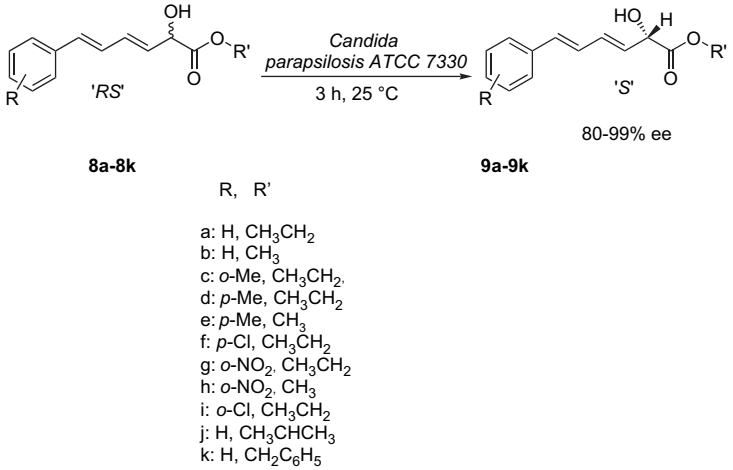
Figure 5. Screening of pure solvents for the deracemisation of (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (**8a**). [Experimental detail: 3 mg of racemic substrate (**8a**) dissolved in 75 μl of pure solvent was added to 1.0 g of wet cells of *C. parapsilosis* ATCC 7330 suspended in 0.9 ml of distilled water. Incubated for 3 h at 25 °C and 150 rpm. Product was extracted using ethyl acetate (1 ml) and ee was determined using Chiralcel OD-H column, solvent: hexane/isopropanol 98:2, flow rate: 1 ml min^{-1}].

deracemisation of **8a** resulted in moderate chemical (∼25–69%) and optical yields (34–94% ee). Optical and chemical yields of the deracemised product of **8a** therefore seem to increase as log *P* decreases. This is also true in the case of deracemisation of 2-hydroxy-4-aryl-butanoic esters mediated by *C. parapsilosis* ATCC 7330.[24] Alcohols are efficient solvents as they are also substrates for enzymes, especially alcohol dehydrogenases, which can regenerate cofactors. This is advantageous for the deracemisation process, which involves oxidation and reduction[35] and was also observed in the asymmetric reduction of ketones using secondary alcohols (2-propanol) using *Rhodococcus ruber* DSM 44541.[36] The solvent used for the biotransformations in this investigation was ethanol.

2.5. Substrate specificity

Under the optimized reaction conditions, racemic (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoates (**8a**–**8k**) were deracemized into their (*S*)-enantiomers on incubation at 25 °C for 3 h with whole cells of *C. parapsilosis* ATCC 7330 (Scheme 3) and the results obtained are shown in Table 2.

The most plausible pathway for the deracemisation of α-hydroxy esters involves stereoinversion mediated by oxidoreductases.[9a] Deracemisation of methyl and ethyl esters of aryl unsubstituted (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoates (**8a**–**8b**, Table 2) by whole cells of *C. parapsilosis* ATCC 7330 resulted in the optically pure (*S*)-enantiomers in excellent isolated chemical (73–80%) and optical yields (93–99% ee). These results show that the biocatalyst *C. parapsilosis* ATCC 7330 accepts these dienoates as well as the (*E*)-2-hydroxy-4-arylbut-3-enoic esters (98–99% ee and 74–75% yields) reported earlier.[9] Among the other aryl unsubstituted compounds with branched and benzyl esters, deracemisation of (3*E*,5*E*)-isopropyl-6-phenyl-2-hydroxyhexa-3,5-dienoate (**8j**, Table 2) results in the formation of the (*S*)-enantiomer in excellent ee (98% ee) and moderate chemical yield (65%) while aryl unsubstituted (3*E*,5*E*)-benzyl-6-phenyl-2-hydroxyhexa-3,5-dienoate (**8k**, Table 2) gives the deracemised product in only 42% ee and 51% yield. Introduction of electron donating methyl groups at *ortho* or *para* positions in (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoates (**8c**–**8e**, Table 2) causes a decrease in both isolated



Scheme 3. Deracemisation of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8a**–**8k**).

Table 2. Deracemisation of various racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8a–8k**) using *Candida parapsilosis* ATCC 7330

Entry	R	R′	ee (%)	Yield (%)	$[\alpha]_D^{25}$	Abs. Conf
8a	H	Et	>99	80	+92.0 (*c* 1.0, CH_3OH)[a,b]	*S*
8b	H	Me	93	73	+92.1 (*c* 1.0, CH_3OH)[a,b]	*S*
8c	*o*-Me	Et	82	65	+3.4 (*c* 1.0, $CHCl_3$)	Nd
8d	*p*-Me	Et	95	74	+2.7 (*c* 1.3, $CHCl_3$)	Nd
8e	*p*-Me	Me	87	72	+5.1 (*c* 1.1, $CHCl_3$)	Nd
8f	*p*-Cl	Et	>99	55	59.6 (*c* 1.2, CH_3OH)[a,b]	*S*
8g	*o*-NO_2	Et	90	66	Nd	Nd
8h	*o*-NO_2	Me	91	69	Nd	Nd
8i	*o*-Cl	Et	85	70	Nd	Nd
8j	H	*i*-pr	98	65	+90.0 (*c* 1.0, CH_3OH)[a,b]	*S*
8k	H	Benzyl	42	51	+93.2 (*c* 1.0, CH_3OH)[a,b]	*S*

Nd: Not determined.
[a] Ref. 37.
[b] Specific rotations were measured after hydrolysis.

chemical (65–74%) and optical yields (82–95% ee). This was also seen in aryl substituted (*E*)-2-hydroxy-4-arylbut-3-enoic esters, where the introduction of electron donating groups caused a decrease in optical yields (90–92% ee) and isolated chemical yields (68–70%). In aryl *ortho*-substituted (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoate (**8c**, Table 2), as compared to its *para* substituted counterpart (**8d**, Table 2), the chemical yield is marginally less (65%) probably due to prominent steric effects in the *ortho* compound. Introduction of a chloro group at the *ortho* position (**8i**, Table 2), which has both a mesomeric effect (+ve) and an inductive effect (−ve), gives marginally higher chemical (70%) and lower optical yields (85% ee) as compared to the *para* substituted dienoate (**8f**, Table 2) [chemical (55%) and optical yield (>99% ee)] as seen earlier in (*E*)-2-hydroxy-4-arylbut-3-enoic esters, which give chemical (65–68%) and optical yield (95–98% ee).[9] The presence of strong electron withdrawing nitro groups at the *ortho* position in the aromatic ring of dienoates (**8g–8h**, Table 2) produced the optically pure product in 66–69% yield and 90–91% ee as reported for *meta* substituted (*E*)-2-hydroxy-4-arylbut-3-enoic esters (93–95% ee and 69–70% yield).[9] Aryl substituted racemic (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoates (**8c–8i**, Table 2) give lower chemical yields (51–74%) and optical purity (42–99%) as compared to aryl unsubstituted dienoates (**8a–8b**, Table 2) (chemical yields 73–80% and optical purity 93–99%).

The deracemisation of racemic (3*E*,5*E*)-ethyl-6-phenyl-2-hydroxyhexa-3,5-dienoate (**8a**) competes with the formation of small amount (20%) of the corresponding (3*E*,5*E*)-6-phenyl-2-hydroxyhexa-3,5-dienoic acid (**10**, Scheme 4). A control experiment reveals that **10** is formed as a result of biocatalytic hydrolysis. Compound **10** was isolated and its specific rotation ($[\alpha]_D^{25}$ +92.0 (*c* 1.0, CH_3OH) was compared with the literature value[37] giving an ee of ~99% indicating that in all likelihood enzymatic hydrolysis follows deracemisation.

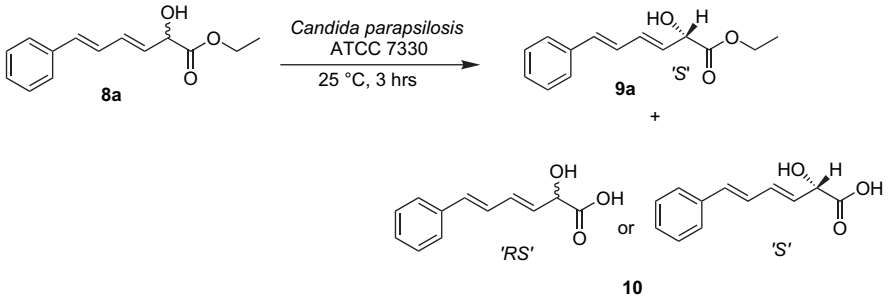
3. Conclusion

A convenient and simple procedure for the synthesis of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates from easily available starting materials with improvisations is reported. The synthesized racemic esters were resolved using chiral HPLC on chiral columns OD-H and OJ-H using hexane and isopropyl alcohol as eluents. Whole cells of *C. parapsilosis* ATCC 7330 were used to catalyze the deracemisation of aryl unsubstituted and substituted (3*E*,5*E*)-alkyl-2-hydroxy-6-aryl-hexa-3,5-dienoates to give the corresponding optically pure (*S*)-dienoates (42 to >99% ee and 51–80% yields). It has been found that *C. parapsilosis* ATCC 7330 mediated deracemisation of (i) aryl substituted (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoates that have either electron withdrawing or electron donating substituents at *ortho*/*para* positions in the aromatic ring give lower chemical yields (51–74%) and optical purity (42–99%) as compared to aryl unsubstituted dienoates (chemical yields 73–80% and optical purity 93–99%) and (ii) aryl unsubstituted and aryl substituted (electron releasing/withdrawing) (3*E*,5*E*)-alkyl-6-aryl-2-hydroxyhexa-3,5-dienoates give moderate to good isolated yields (51–80%) but moderate to excellent optical purity (42–99%) as compared to (*E*)-alkyl-2-hydroxy-4-arylbut-3-enoic esters (isolated yield 66–93% and ee 91–99%).[9]

4. Experimental

4.1. General methods

C. parapsilosis ATCC 7330 was purchased from American Type Culture Collection, Manassas, VA 20108, USA. All chemicals used for media preparation were purchased locally. All substrates were synthesized using the given schemes. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a JEOL GSX400 and Bruker AV-400 spectrometers operating at 400 MHz. Chemical shifts are expressed in parts per million values using TMS as an internal standard. Infrared spectra were recorded on a Shimadzu IR 470 Instrument. Mass spectra were recorded on a Q TOF micromass



Scheme 4. Partial hydrolysis of the substrate (3*E*,5*E*)-ethyl-2-hydroxy-6-arylhexa-3,5-dienoate (**8a**) during deracemisation.

spectrometer. The ee (ee%) was determined by HPLC analysis. HPLC analysis was done on a Jasco PU-1580 liquid chromatograph equipped with PDA detector. The chiral columns used were Chiralcel OD-H and Chiralcel OJ-H (Daicel, 4.6×250 mm). The solvent used was hexane/isopropanol (99:1, 98:2, and 97:3) at a flow rate of 1 ml min^{-1} and the absorbance was monitored using a PDA detector at 254 nm. Optical rotations were determined on an Autopal® digital polarimeter. Melting points were determined on Toshniwal melting point apparatus and are uncorrected. TLC was done using Kieselgel 60 F_{254} aluminum sheets (Merck 1.05554).

4.2. Synthesis of (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (8a–8k)

Reduction of (3*E*,5*E*)-ethyl-2-oxo-6-phenylhexa-3,5-dienoate (**7a**) (0.17 mmol, 400 mg) was carried out with sodium borohydride (0.17 mol, 6 mg) using ethanol as solvent (5 ml) for 20 min at 0–10 °C. After the reaction was complete the reaction mixture was neutralized with dil HCl, which was extracted with ethyl acetate (3×10 ml). The collected organic fractions were dried and concentrated. Compound (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (**8a**) was obtained as a yellow solid in 81% yield (326 mg, 0.14 mmol) after column purification using hexane/ethyl acetate (95:5) as solvent. The same procedure was followed for compounds **8b**–**8k** (Table 1).

4.3. Deracemisation of racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (8a–8k)

4.3.1. Culture medium for the growth of *C. parapsilosis* ATCC 7330. Cells of the yeast, *C. parapsilosis* ATCC 7330 were grown as reported earlier[9] and harvested after 44 h and used for the deracemisation reaction.

4.3.2. A typical procedure for the deracemisation reaction of 8a using whole cells of *C. parapsilosis* ATCC 7330. Deracemisation of racemic (3*E*,5*E*)-ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (**8a**) was done as reported earlier except for the quantity (1.0 g ml^{-1} wet weight) of biocatalyst *C. parapsilosis* ATCC 7330 and the time course of the reaction (3 h).[9] After incubation, the products were extracted into ethyl acetate, concentrated, and analyzed for enantiomeric purity. Appropriate control experiments were carried out using (i) cells in the reaction mixture without the substrate and (ii) substrate in the reaction mixture without cells. The HPLC profile of the control experiments revealed that (i) nothing from the cells on extraction co-eluted with the product and (ii) the substrate did not yield any products in water. In order to determine the isolated chemical yield, the deracemisation of **8a** was carried out with 72 mg of the substrate. The other racemic (3*E*,5*E*)-alkyl-2-hydroxy-6-arylhexa-3,5-dienoates (**8b**–**8k**) were also used as substrates in the same manner.

4.4. Analytical data for the substrates (8a–8k)

4.4.1. (3*E*,5*E*)-Ethyl-2-hydroxy-6-phenylhexa-3,5-dienoate (8a). Yellow solid; mp 37 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.3 (t, J=6.9 Hz, 3H), 3.06 (br s, 1H), 4.2 (q, J=6.9 Hz, 2H), 4.74 (d, J=5.3 Hz, 1H), 5.83 (dd, J=15.6, 5.3 Hz, 1H), 6.57 (dd, J=15.6, 10.4 Hz, 1H), 6.58 (d, J=15.6 Hz, 1H), 6.75 (dd, J=15.6, 10.4 Hz, 1H), 7.23–7.40 (5H, m); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.1, 62.2, 71.0, 126.4, 127.5, 127.7, 128.6, 129.0, 132.5, 133.8, 136.9, 173.2; IR ν_{max} (KBr): 3367, 2986, 2939, 1741, 1732, 1638, 1448, 1289, 1092, 1054, 989 cm^{-1}; HRMS (ESI): found 255.1007, $C_{14}H_{16}O_3Na$ $[M+Na]^+$ requires 255.0997.

4.4.2. (3*E*,5*E*)-Methyl-2-hydroxy-6-phenylhexa-3,5-dienoate (8b). Yellow solid; mp 33 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.25 (br s, 1H), 3.80 (s, 3H), 4.77 (d, J=5.6 Hz, 1H), 6.19 (dd, J=15.2, 5.6 Hz, 1H), 6.59 (dd, J=15.2, 10.7 Hz, 1H), 6.61 (d, J=15.2 Hz, 1H), 6.77 (dd, J=15.2, 10.7 Hz, 1H), 7.22–7.39 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 50.9, 60.4, 71.1, 126.5, 127.4, 127.8, 128.6, 128.9, 134.0, 136.8, 173.4; IR ν_{max} (KBr): 3558, 2920, 2350, 2335, 1772, 1742, 1507, 1460, 974, 761, 700 cm^{-1}; HRMS (ESI): found 219.1012, $C_{13}H_{15}O_3$ $[M+H]^+$ requires 219.1021.

4.4.3. (3*E*,5*E*)-Ethyl-2-hydroxy-6-(*o*-methylphenyl)hexa-3,5-dienoate (8c). Yellow solid; mp 68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (t, J=6.8 Hz, 3H), 2.3 (s, 3H), 3.14 (br s, 1H), 4.27 (q, J=6.8 Hz, 2H), 4.79 (d, J=5.3 Hz, 1H), 6.18 (dd, J=15.6, 5.3 Hz, 1H), 7.04–7.19 (m, 4H), 7.12 (d, J=15.6 Hz, 1H), 7.27 (dd, J=15.6, 10.4 Hz, 1H), 7.51 (dd, J=15.6, 10.4 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.1, 21.1, 71.3, 126.2, 126.8, 127.0, 127.5, 128.4, 128.9, 129.5, 129.7, 130.4, 133.4, 134.4, 173.0; IR ν_{max} (KBr): 3465, 3060, 2981, 2932, 1732, 1693, 1594, 1486, 1460, 1374, 1264, 1136, 1083, 1023, 974, 861, 738, 701 cm^{-1}; HRMS (ESI): found 247.1333, $C_{15}H_{19}O_3$ $[M+H]^+$ requires 247.1334.

4.4.4. (3*E*,5*E*)-Ethyl-2-hydroxy-6-(*p*-methylphenyl)hexa-3,5-dienoate (8d). Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, J=7.0 Hz, 3H), 2.33 (s, 3H), 3.9 (br s, 1H), 4.26 (q, J=7.0 Hz, 2H), 4.76 (d, J=5.3 Hz, 1H), 5.83 (dd, J=15.2, 5.3 Hz, 1H), 6.57 (d, J=15.2 Hz, 1H), 6.66 (dd, J=15.2, 10.7 Hz, 1H), 6.74 (dd, J=15.2, 10.7 Hz, 1H), 7.12 (d, J=7.9 Hz, 2H), 7.28 (d, J=7.9 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.1, 21.1, 61.5, 71.1, 124.5, 126.5, 126.6, 128.5, 129.3, 132.7, 133.4, 137.7, 173.4; IR ν_{max} (KBr): 3477, 2981, 2923, 1731, 1597, 1513, 1446, 1371, 1259, 1207, 1079, 1018, 858, 716, 488, 451, 438, 411 cm^{-1}; HRMS (ESI): found 247.1335, $C_{15}H_{19}O_3$ $[M+H]^+$ requires 247.1334.

4.4.5. (3*E*,5*E*)-Methyl-2-hydroxy-6-(*p*-methylphenyl)-hexa-3,5-dienoate (8e). Yellow solid; mp 77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.28 (s, 3H), 2.95 (br s, 1H), 3.74 (s, 3H), 4.69 (d, J=6.0 Hz, 1H), 5.73 (dd, J=15.2, 6.0 Hz, 1H), 6.51 (dd, J=15.2, 10.8 Hz, 1H), 6.50 (d, J=15.2 Hz, 1H), 6.65 (dd, J=15.2, 10.8 Hz, 1H), 7.05 (d, J=8.0 Hz, 2H), 7.22 (d, J=8.0 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 21.2, 52.9, 71.1, 126.4, 127.6, 128.2, 128.5, 129.3, 129.5, 132.9, 137.8, 173.7; IR ν_{max} (KBr): 3432, 2359, 1732, 1677, 1578, 1513, 1439, 1279, 1080, 1020, 811 cm^{-1}; HRMS (ESI): found 255.0999, $C_{14}H_{16}O_3Na$ $[M+Na]^+$ requires 255.0997.

4.4.6. (3*E*,5*E*)-Ethyl-2-hydroxy-6-(*p*-chlorophenyl)hexa-3,5-dienoate (8f). Yellow solid; mp 90 °C; ^{1}H NMR

(400 MHz, $CDCl_3$) δ: 1.28 (t, J=6.8 Hz, 3H), 3.09 (br s, 1H), 4.26 (q, J=6.8 Hz, 2H), 4.76 (d, J=5.3 Hz, 1H), 5.83 (dd, J=15.2, 5.3 Hz, 1H), 6.57 (d, J=15.2 Hz, 1H), 6.66 (dd, J=15.2, 10.6 Hz, 1H), 6.74 (dd, J=15.2, 10.6 Hz, 1H), 7.12 (d, J=7.9 Hz, 2H), 7.28 (d, J=7.9 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.1, 62.1, 71.0, 124.5, 126.4, 126.6, 128.5, 129.3, 132.7, 133.8, 134.2, 173.4; IR ν_{max} (KBr): 3482, 2989, 2936, 2365, 2335, 1742, 1597, 1483, 1392, 1263, 1088, 822 cm^{-1}; HRMS (ESI): found 289.0618, $C_{14}H_{15}O_3ClNa$ $[M+Na]^+$ requires 289.0607.

4.4.7. (3*E*,5*E*)-Ethyl-2-hydroxy-6-(*o*-nitrophenyl)hexa-3,5-dienoate (8g). Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, J=7.3 Hz, 3H), 3.04 (br s, 1H), 4.20 (q, J=7.3 Hz, 2H), 4.69 (d, J=5.3 Hz, 1H), 5.89 (dd, J=15.1, 5.3 Hz, 1H), 6.57 (dd, J=15.1, 10.7 Hz, 1H), 6.66 (dd, J=15.1, 10.7 Hz, 1H), 6.98 (d, J=15.1 Hz, 1H), 7.28 (t, J=7.8 Hz, 1H), 7.46 (t, J=7.8 Hz, 1H), 7.55 (d, J=7.8 Hz, 1H), 7.81 (d, J=7.8 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.3, 65.5, 71.1, 124.9, 128.1, 128.2, 128.3, 131.9, 132.1, 132.6, 132.8, 133.1, 148.0, 173.1; IR ν_{max} (neat): 3501, 1734, 1644, 1521, 990, 784, 740 cm^{-1}; HRMS (ESI): found 300.0841, $C_{14}H_{15}NO_5Na$ $[M+Na]^+$ requires 300.0848.

4.4.8. (3*E*,5*E*)-Methyl-2-hydroxy-6-(*o*-nitrophenyl)hexa-3,5-dienoate (8h). Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.53 (br s, 1H), 3.86 (s, 3H), 4.70 (d, J=5.5 Hz, 1H), 5.89 (dd, J=15.1, 5.5 Hz, 1H), 6.57 (dd, J=15.1, 10.6 Hz, 1H), 6.69 (dd, J=15.1, 10.6 Hz, 1H), 6.99 (d, J=15.1 Hz, 1H), 7.53–7.63 (m, 3H), 7.94 (d, J=8.3 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 52.4, 72.1, 124.5, 127.9, 128.2, 129.3, 130.4, 130.7, 132.0, 132.8, 137.9, 146.7, 181.9; IR ν_{max} ($CHCl_3$): 3672, 1732, 1685, 1396, 1005, 962, 790 cm^{-1}; HRMS (ESI): found 286.0691, $C_{13}H_{13}NO_5Na$ $[M+Na]^+$ requires 286.0691.

4.4.9. (3*E*,5*E*)-Ethyl-2-hydroxy-6-(*o*-chlorophenyl)hexa-3,5-dienoate (8i). Yellow solid; mp 85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27 (t, J=6.9 Hz, 3H), 3.14 (br s, 1H), 4.25 (q, J=6.9 Hz, 2H), 4.79 (d, 1H, J=5.5 Hz), 6.70 (dd, J=15.8, 5.5 Hz, 1H), 7.12 (d, J=15.8 Hz, 1H), 6.57 (dd, J=15.8, 10.3 Hz, 1H), 6.72 (dd, J=15.8, 10.3 Hz, 1H), 7.04–7.19 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.2, 21.5, 71.3, 126.8, 127.0, 127.5, 128.4, 128.9, 129.5, 129.7, 130.4, 133.4, 134.4, 173.0; IR ν_{max} (KBr): 3444, 2982, 2361, 2093, 1732, 1667, 1607, 1472, 1441, 1371, 1130, 1084, 978, 860, 676, 587, 466 cm^{-1}; HRMS (ESI): found 267.0626, $C_{14}H_{16}O_3Cl$ $[M+H]^+$ requires 267.0631.

4.4.10. (3*E*,5*E*)-Isopropyl-2-hydroxy-6-phenylhexa-3,5-dienoate (8j). Yellow solid; mp 38 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (d, J=11.2 Hz, 6H), 3.07 (br s, 1H), 4.70 (d, J=5.3 Hz, 1H), 5.11 (sep, J=11.1 Hz, 1H), 5.84 (dd, J=15.6, 5.3 Hz, 1H), 6.59 (dd, J=15.6, 10.7 Hz, 1H), 6.59 (d, J=15.6 Hz, 1H), 6.78 (dd, J=15.6, 10.7 Hz, 1H), 7.21–7.43 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 21.6, 70.1, 71.0, 126.1, 127.6, 127.7, 128.6, 129.6, 129.2, 132.3, 136.9, 172.8; IR ν_{max} (neat): 3521, 3062, 3029, 2982, 2936, 2556, 1730, 1454, 1375, 1278, 906, 862, 755, 700, 485, 451, 412 cm^{-1}; HRMS (ESI): found 247.1339, $C_{15}H_{19}O_3$ $[M+H]^+$ requires 247.1334.

4.4.11. (3*E*,5*E*)-Benzyl-2-hydroxy-6-phenylhexa-3,5-dienoate (8k). Yellow solid; mp 118 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.09 (br s, 1H), 4.79 (d, J=5.4 Hz, 1H), 5.23 (q, J=9.7 Hz, 2H), 5.85 (dd, J=15.6, 5.4 Hz, 1H), 6.59 (dd, J=15.6, 10.7 Hz, 1H), 6.75 (dd, J=15.6, 10.7 Hz, 1H), 6.57 (d, J=15.6 Hz, 1H), 7.21–7.39 (m, 10H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 67.7, 71.7, 126.5, 126.9, 127.4, 127.8, 128.3, 128.5, 128.6, 128.7, 132.7, 134.0, 134.9, 136.9, 173.1; IR ν_{max} (KBr): 3418, 3063, 2936, 1957, 1887, 1731, 1581, 1496, 1385, 1270, 1210, 970, 847, 750, 496, 452, 413 cm^{-1}; HRMS (ESI): found 317.1140, $C_{19}H_{18}O_3Na$ $[M+Na]^+$ requires 317.1154.

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