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Preparation of optically pure alkyl 3-(hetero-2-yl)-3-hydroxypropanoates by *Candida parapsilosis* ATCC 7330 mediated deracemisation☆

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Abstract

Deracemisation of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates using whole cells of *Candida parapsilosis* ATCC 7330 resulted in the formation of the '*S*' enantiomer in high enantiomeric excess (up to >99% ee) and isolated yields (up to 75%).

Keywords: Deracemisation; Alkyl 3-(hetero-2-yl)-3-hydroxypropanoates; Hetero-2-yl; β-Hydroxy ester; *Candida parapsilosis* ATCC 7330

1. Introduction

Enantiopure alkyl 3-(hetero-2-yl)-3-hydroxypropanoates are industrially significant, in particular for the synthesis of important intermediates of pharmaceuticals like duloxetine, tetrahydropyrans, methyl 2-(hetero-2-yl(hydroxy)methyl)acrylate, methyl 2-(hetero-2-yl)-3-hydroxy-phenylpropanoate and heteroarylaminoalkanols [1–5] (Fig. 1). These key intermediates can be synthesized by using chemical [6,7] and biocatalytic methods [8,9]. Biocatalytic approaches for the synthesis of these target molecules broadly involves: (a) asymmetric reduction and (b) kinetic resolution. Microbial whole cells and commercially available enzymes can be used for both the processes. For asymmetric reduction, whole cells are preferred over isolated enzymes as the need for additional expensive cofactors is obviated. However the use of whole cells often results in the competitive action of multiple enzymes on substrate molecules which can reduce the enantiomeric excess of the desired product. To circumvent this limitation, engineered whole cells can be used for the biotransformation reaction [10]. The pure enzyme isolated from *Exiguobacterium* sp. F42 reduces ethyl 3-oxo-3-(2-thienyl)propanoate to optically pure (*S*)-ethyl 3-hydroxy-3-(2-thienyl)propanoate (>98% ee and 80% yield) [11]. Kinetic resolution of racemic ethyl 3-hydroxy-3-(2-furyl)propanoate gives high enantiomeric excess of the product but the yield of each enantiomer is limited to <50% [12,13] as expected in resolution. An alternative method to prepare enantiomerically pure products in high ee and quantitative yield is deracemisation [14]. Stereoinversion, as a mechanism for deracemisation is an efficient process [15]. Deracemisation of racemic aryl α- and β-hydroxy esters to the corresponding enantiomerically pure (*S*)-hydroxy esters in high chemical yields (up to 85%) and optical purity (up to >99% ee) by *Candida parapsilosis* ATCC 7330 is reported by our group [16–22]. Using the same biocatalyst, racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates were deracemised. We report here for the first time, the biocatalytic preparation of optically pure alkyl 3-(hetero-2-yl)-3-hydroxypropanoates by deracemisation. A series of optically pure alkyl 3-(hetero-2-yl)-3-hydroxypropanoates were prepared in high ee (up to >99%) and yields (up to 75%) using *C. parapsilosis* ATCC 7330.

2. Experimental

2.1. Materials and 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 from Hi-Media, Chennai. All substrates were synthesized using reported methods as mentioned in the Experimental Sections

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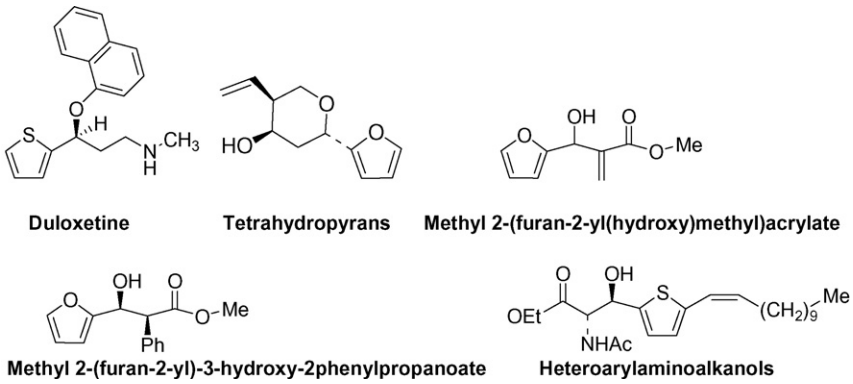
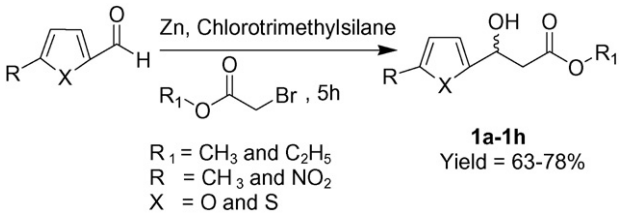


Fig. 1. Pharmaceuticals synthesized by optically pure alkyl 3-(hetero-2-yl)-3-hydroxy-propanoates.



Scheme 1. Synthesis of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates (**1a**–**1h**).

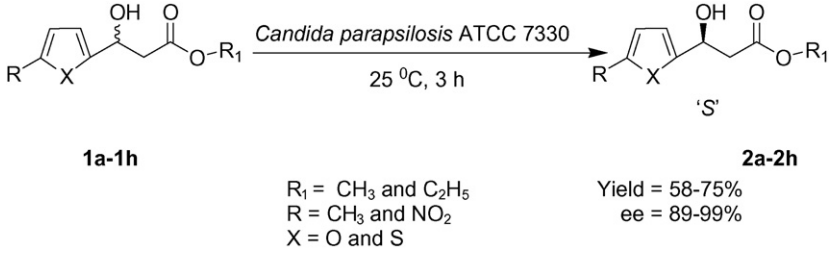
2.2, 2.3, 2.4 and 2.5 (Schemes 1 and 2). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on JEOL GSX400 and Bruker AV-400 spectrometers operating at 400 MHz and 100 MHz, respectively. Chemical shifts were expressed in ppm values using TMS as an internal standard. Infrared spectra were recorded on a Shimadzu IR 470 Instrument. Mass spectra were recorded on a Q TOF micromass spectrometer. The enantiomeric excess (ee %) was determined by HPLC analysis which was done on a Jasco PU-1580 liquid chromatograph equipped with PDA detector. The chiral columns used were chiralcel OB-H and chiralcel OJ-H (Daicel, 4.6 mm × 250 mm). The mobile phase used was hexane/isopropanol (93:7, 95:5 and 98:2) at a flow rate of 1ml/min and the absorbance was monitored using PDA detector at 254 nm. The formation of the keto ester as intermediate in the mechanistic study was detected by HPLC using a reverse phase column (R_t: 8.63 min; column: Sil C-18, solvent system: acetonitrile–water (50/50), flow rate: 1 ml/min, detector:PDA). Optical rotations were determined on an Autopal® digital polarimeter. TLC was done using Kieselgel60 F240 aluminium sheets (Merck 1.05554).

2.2. Synthesis of racemic ethyl 3 hydroxy-3-(furan-2-yl)propanoate (*1a*) [23]

A mixture of zinc powder (0.840 g, 13 mmol), tetrahydrofuran (10 ml) and chlorotrimethyl silane (0.2 ml) was heated up to 60 °C for 15 min under nitrogen atmosphere. Ethylbromoacetate (1.4 ml, 12.5 mmol) was subsequently added drop wise at 45 °C for 5 min. After stirring for 15 min, Furfural (0.8 ml, 10.5 mmol) was added at 20 °C. The reaction mass was stirred for 5 h at 25–30 °C which was then acidified to pH 2 with 20% dil. HCl and extracted with ethyl acetate (3× 10 ml). Organic phase was stirred with aq. NH_3 (5 ml) for 10 min, which was then separated, dried and concentrated under reduced pressure furnished the crude product which was purified by silicagel chromatography using hexane/ethylacetate (90:10) as solvent. Pale yellow liquid; IR(neat): 3430, 2942, 1750, 1372, 1264, 730 and 632 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.27 (t, J = 7.2 Hz, 3H), 2.80–2.94 (m, 2H), 3.32 (s, 1H), 4.18 (q, J = 7.2 Hz, 2H), 5.13 (dd, J = 4.0, 8.3 Hz, 1H), 6.28–6.30 (m, 1H), 6.34–6.36 (m, 1H), 7.38–7.40 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 14.08, 39.87, 60.87, 64.23, 106.21, 110.21, 142.16, 154.86, 171.81; HRMS[EI] Calculated for $C_9H_{12}O_4Na$: $(M + Na)^+$ = 207.0633 found $(M + Na)^+$ = 207.0632. The same procedure was followed for compounds **1b**–**1h** (Scheme 1 and Table 1).

2.2.1. Methyl 3 hydroxy-3-(furan-2-yl)propanoate (*1b*) [25]

Pale yellow liquid; IR(neat): 3256, 2382, 1728, 1365, 1180, 1085, 1021, 931 and 453 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 2.77–2.81 (m, 2H), 3.63 (s, 3H), 5.05 (dd, J = 4.1 Hz, J = 8.5 Hz, 1H), 6.20–6.22 (m, 1H), 6.25–6.27 (m, 1H), 7.30–7.32 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 41.34, 51.92, 65.01, 105.04, 110.09, 143.32, 152.14, 172.15;



Scheme 2. Deracemisation of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates (**1a**–**1h**) using whole cells of *Candida parapsilosis* ATCC 7330.

Table 1
Synthesis of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates (**1a–1h**)

Entry	X	R_1	R	Yield (%)
1a	O	Et	H	70
1b	O	Me	H	65
1c	O	Me	Me	69
1d	O	Et	Me	63
1e	O	Et	NO_2	78
1f	S	Et	Me	70
1g	S	Et	H	72
1h	S	Me	H	68

HRMS[EI] Calculated for $C_8H_{10}O_4Na$: $(M+Na)^+ = 193.0477$ found $(M+Na)^+ = 193.0482$.

*2.2.2. Methyl 3-hydroxy-3-(5-methylfuran-2-yl)propanoate (**1c**) [27]*

Pale yellow liquid; IR(neat): 3481, 2451, 2384, 1767, 1321, 1235, 1280, 1040, 635 and 435 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 2.17 (s, 3H), 2.61–2.81 (m, 2H), 3.65 (s, 3H), 5.12 (dd, $J = 4.2$ Hz, $J = 8.2$ Hz, 1H), 6.0–6.02 (m, 1H), 6.28–6.30 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 13.48, 39.61, 51.91, 64.10, 106.0, 107.1, 152.1, 152.80, 172.3; HRMS[EI] Calculated for $C_9H_{12}O_4Na$: $(M+Na)^+ = 207.0633$ found $(M+Na)^+ = 207.0634$.

*2.2.3. Ethyl 3-hydroxy-3-(5-methylfuran-2-yl)propanoate (**1d**)*

Pale yellow liquid; IR(neat): 3430, 2361, 2984, 1728.5, 1371, 1275, 1180, 1085, 1021, 931 and 453 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.34 (t, $J = 7.2$ Hz, 3H), 2.32 (s, 3H), 2.81–2.98 (m, 2H), 4.23 (q, $J = 7.2$ Hz, 3H), 5.12 (dd, $J = 4$ Hz, $J = 8.8$ Hz, 1H), 5.93–5.95 (m, 1H), 6.18–6.20 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 14.08, 14.26, 41.34, 60.85, 64.05, 106.05, 107.08, 152.00, 152.80, 171.97; HRMS[EI] Calculated for $C_{10}H_{14}O_4Na$: $(M+Na)^+ = 221.0790$ found $(M+Na)^+ = 221.0793$.

*2.2.4. Ethyl 3-hydroxy-3-(5-nitrofuran-2-yl)propanoate (**1e**)*

Yellow liquid; IR(neat): 3460, 2983, 2336, 1713, 1530, 1496, 1351, 1235, 1177, 1016, 968, 810, 739 and 515 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.19 (t, $J = 7.2$ Hz, 3H), 2.78–2.92 (m, 2H), 4.13 (q, $J = 7.2$ Hz, 2H), 5.12 (dd, $J = 4$ Hz, $J = 8$ Hz, 1H), 6.51–6.53 (m, 1H), 7.31–7.32 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 14.05, 39.28, 61.39, 64.23, 109.68, 112.41, 151.63, 158.43, 171.32; HRMS[EI] Calculated for $C_9H_{11}NO_6Na$: $(M+Na)^+ = 252.0484$ found $(M+Na)^+ = 252.0491$.

*2.2.5. Ethyl 3-hydroxy-3-(5-methylthiophen-2-yl)propanoate (**1f**)*

Yellow liquid; IR(neat): 3464, 2978, 2307, 1736, 1368, 1155, 1029, 800 and 530 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.17 (t, $J = 7$ Hz, 3H), 2.35(s, 3H), 2.71–2.78 (m, 2H), 4.07 (q, $J = 7$ Hz, 2H), 5.16 (dd, $J = 4$ Hz, $J = 8$ Hz, 1H), 6.50–6.53 (m, 1H), 6.65–6.67 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 14.10, 15.21, 43.82, 61.23, 70.59, 125.36, 126.21, 139.51, 142.96, 173.12; HRMS[EI] Calculated for $C_{10}H_{14}O_3SNa$ $(M+Na)^+ = 237.0561$ found $(M+Na)^+ = 237.0559$.

*2.2.6. Ethyl 3-hydroxy-3-(thiophen-2-yl)propanoate (**1g**) [23]*

Pale yellow liquid; IR(neat): 3458, 2981, 1714, 1396, 1212, 1156, 1025, 851, 698 and 523.9 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.21 (t, $J = 7.2$ Hz, 3H), 2.81–2.98 (m, 2H), 4.09 (q, $J = 7$ Hz, 2H), 5.30 (dd, $J = 4$ Hz, $J = 8.1$ Hz, 1H), 6.88–6. 90 (m, 1H), 6.92–6.94 (m, 1H), 7.05–7.07 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 13.0, 42.1, 59.9, 65.5, 122.5, 123.7, 125.6, 145.2, 170.8; HRMS[EI] Calculated for $C_9H_{12}O_3Na$: $(M+Na)^+ = 223.0405$ found $(M+Na)^+ = 223.0405$.

*2.2.7. Methyl 3-hydroxy-3-(thiophen-2-yl)propanoate (**1h**) [25]*

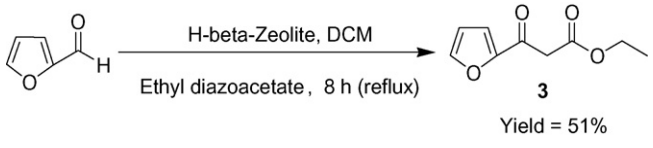
Pale yellow liquid; IR(neat): 3489, 2665, 2532, 1734, 1341, 1275, 1021, 931, 735, 645 and 453 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 2.76–2.81 (m, 2H), 3.72 (s, 3H), 5.12 (dd $J = 4.3$ Hz, $J = 8.0$ Hz, 1H), 6.85 (m, 1H), 6.88 (m, 1H), 7.15 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 42.9, 51.7, 65.7, 123.4, 124.6, 126.5, 146.3, 172.3; HRMS[EI] Calculated for $C_8H_{10}O_3SNa$: $(M+Na)^+ = 209.0290$ found $(M+Na)^+ = 209.0287$.

*2.3. Synthesis of ethyl 3-(furyl-2-yl)-3-oxopropanoate (**3**)*

Ethyl 3-(furyl-2-yl)-3-oxopropanoate **3** was prepared according to the literature reported method [24]. A solution of Furfural (0.96 g, 10 mmol) and Ethyl diazoacetate (1.71 g, 15 mmol) in dichloromethane (15 ml) containing 100 mg of H-β-zeolite was refluxed for 8 h under nitrogen atmosphere. After completion, the reaction mixture was filtered and the filtrate was concentrated under vacuum furnished the crude product. Ethyl 3-(furyl-2-yl)-3-oxopropanoate **3** (Scheme 3) was obtained in 51% yield after column purification using hexane/ethyl acetate (95/5) as solvent.

2.4. Growth conditions for C. parapsilosis ATCC 7330

C. parapsilosis ATCC 7330 was procured from ATCC 7330 which was grown in 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 4500 rpm for 15 min and subsequent washing with sterile water. The process was repeated thrice and the wet cells were used for biotransformation [22].



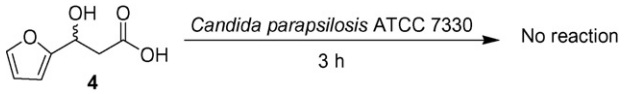
Scheme 3. Synthesis of ethyl 3-(furyl-2-yl)-3-oxopropanoate (**3**).

2.5. A typical experimental procedure for the biotransformation

To a 250 ml conical flask containing 24 g of pelleted *C. parapsilosis* ATCC 7330 cells suspended in 22 ml of sterile distilled water, 72 mg (0.37 mmol) of ethyl 3-(furyl-2-yl)-3-hydroxypropanoate **1a** dissolved in 1.6 ml of ethanol was added. The reaction was carried out in a water bath shaker at 150 rpm and 25 °C for 3 h. After incubation, the reaction mixture was centrifuged at 5000 rpm for 10 min. The product formed was isolated using ethyl acetate (3× 30 ml) and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed by evaporation and the enantiomerically pure (*S*) ethyl 3-(furan-2-yl)-3-hydroxypropanoate **2a** was obtained as a pale yellow liquid after purification by silica gel column chromatography using hexane–ethyl acetate (90/10) as a mobile phase eluent. The ee was found to be 99% as determined using HPLC. The yield of the isolated product, (*S*) ethyl 3-(furan-2-yl)-3-hydroxypropanoate **2a** was 70% (50.5 mg). Racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates **1b**–**1h** was also used as substrates in the same manner (Scheme 2 and Table 1). Appropriate control experiments were carried out using: (i) cells in the reaction medium without the substrate and (ii) substrate in the reaction medium without cells. The HPLC profile of the first experiment revealed that nothing from the cells on extraction co-eluted with the product. The second control experiment indicated that the substrate remained unchanged in aqueous medium during the biotransformation.

3. Results and discussion

The deracemisation of ethyl and methyl esters of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates **1a**–**1h** (Table 1) by *C. parapsilosis* ATCC 7330 provided their optically pure corresponding (*S*)-esters **2a**–**2h** (Scheme 2, Table 2) in excellent enantiomeric excess (89 to >99%) and good isolated yields (58–75%). The introduction of electron donating 5-methyl group in the methyl and ethyl esters of 3-hydroxy-3-(furan-2-yl)propanoate **1c** and **1d** respectively (Table 1) and ethyl ester of 3-hydroxy-3-(thiophen-2-yl)propanoate **1f** gave yields 70–75%



Scheme 4. Deracemisation of 3-(furan-2-yl)-2-hydroxypropanoic acid (**4**) using whole cells of *Candida parapsilosis* ATCC 7330.

of the product enantiomer with good ee (94–99%). However in the case of electron withdrawing 5-nitro group, deracemisation of ethyl 3-hydroxy-3-(5-nitrofuran-2-yl)propanoate **1e** resulted in comparatively lower ee of 89% and an isolated yield of 58%. Thus the biocatalyst *C. parapsilosis* ATCC 7330 deracemises alkyl 3-(hetero-2-yl)-3-hydroxypropanoates **1a**–**1h** (Scheme 2, Table 1) and gives the enantiomerically pure products **2a**–**2h** (Scheme 2, Table 2) in good isolated yields (58–75%) and high ee (89 to >99%).

Deracemisation of racemic ethyl 3-(furan-2-yl)-2-hydroxypropanoate **1a** (Scheme 2, Table 1) results in the predominant formation of the (*S*)-enantiomer with a small amount (24%) of the corresponding 3-(furan-2-yl)-2-hydroxypropanoic acid **4** (72% ee) $\{[\alpha]_D^{25} = -17.5$ ($c = 1.0$ $CHCl_3$)$\}$ as a side product. Deracemisation of 3-(furan-2-yl)-2-hydroxypropanoic acid **4** (Scheme 4) did not proceed at all and the acid isolated after deracemisation of racemic ethyl 3-(furan-2-yl)-2-hydroxypropanoate **1a** was found to be optically active suggesting enzymatic hydrolysis follows deracemisation. The absolute configuration of the optically pure compounds **2a**, **2b** and **2h** was assigned to be '*S*' by comparing the specific rotation values with the literature data [25] and from the relative elution of peaks in an HPLC chiral column. Compounds **2a**, **2b**, and **2h** had identical elution profiles in chiral HPLC, that is, the '*R*' enantiomer is the early eluting enantiomer while the '*S*' enantiomer is the late eluting enantiomer (Table 3). The specific rotation values for the compounds **2c**–**2g** are reported here for the first time (Table 2) and from their elution profiles it seems likely that the deracemised product is the (S)-enantiomer. The mechanism of deracemisation of the racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates **1a**–**1h** (Scheme 2, Table 2) as seen in aryl α- and β-hydroxy esters [26], proceeds *via* a keto ester intermediate which is explained by a redox mechanism (stereoinversion) mediated by oxidoreductases.

Table 2
Deracemisation of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates (**1a**–**1h**) into their optically pure enantiomers (**2a**–**2h**) using whole cells of *Candida parapsilosis* ATCC 7330

Entry	X	R_1	R	ee (%)[a]	Yield (%)	$[\alpha]_D^{25}$ $CHCl_3$	Lit value	Abs. conf.
2a	O	Et	H	99	70	−22.3 ($c = 1.0$)	−21.5 ($c = 1.1$)[b]	*S*
2b	O	Me	H	96	68	−25.6 ($c = 1.1$)	−24.1 ($c = 1.1$)[b]	*S*
2c	O	Me	Me	99	70	−5.33 ($c = 1.1$)	Nr	*S*[c]
2d	O	Et	Me	98	72	−4.53 ($c = 1.5$)	Nr	*S*[c]
2e	O	Et	NO_2	89	58	−16.6 ($c = 0.9$)	Nr	*S*[c]
2f	S	Et	Me	94	75	−9.12 ($c = 1.1$)	Nr	*S*[c]
2g	S	Et	H	98	62	−26.6 ($c = 1.0$)	Nr	*S*[c]
2h	S	Me	H	92	61	−24.6 ($c = 1.0$)	−25.0 ($c = 0.9$)[b]	*S*

[a] Enantiomeric excess was determined by using Chiral HPLC (column: chiracel OJ-H and OB-H; solvent: hexane:isopropanol, 98:2, 95:5 and 93:7; flow rate: 1 ml/min).
[b] Refer Refs. [9,25].
[c] Compounds **2c**–**2g** showed that the deracemised product had the same elution profile as compounds **2a**, **2b** and **2h**, that is, the '*R*' enantiomer is the early eluting enantiomer, while '*S*' enantiomer is the late eluting enantiomer (refer Table 3).

Table 3
Retention times of the enantiomerically pure alkyl 3-(hetero-2-yl)-3-hydroxypropanoates (**2a**–**2h**)

Entry	X	R_1	R	Elution of HPLC peaks		Column
				Retention time in min		
				Minor	Major	
2a	O	Et	H	13.6	16.5	OB-H
2b	O	Me	H	14.7	17.6	OB-H
2c	O	Me	Me	15.5	21.5	OB-H
2d	O	Et	Me	12.3	15.5	OB-H
2e	O	Et	NO_2	23.5	26.2	OJ-H
2f	S	Et	Me	10.2	12.5	OB-H
2g	S	Et	H	16.2	20.1	OB-H
2h	S	Me	H	15.7	18.3	OB-H

This was confirmed by the following experiment with ethyl 3-(furyl-2-yl)-3-hydroxypropanoate **1a** (Scheme 2, Table 1). The time course of the deracemisation of the racemic ethyl 3-(furyl-2-yl)-3-hydroxypropanoate **1a** (Scheme 2, Table 1) was monitored by HPLC using a reverse phase column. Aliquots of the reaction mixture were taken every 15 min for 3 h. The formation of keto ester intermediate was detected at 30 min, which was compared with standard keto ester **3**.

4. Conclusion

A highly enantioselective biocatalytic method was developed for deracemisation of racemic alkyl 3-(hetero-2-yl)-3-hydroxypropanoates (**1a**–**1h**) in to their corresponding optically pure (*S*)-enantiomers (**2a**–**2h**) in high ee (89 to >99%) and high yields (58–75%) using whole cells of *C. parapsilosis* ATCC 7330. The presence of electron donating groups in the hetero aryl β-hydroxy ester does not affect the yield and enantiomeric excess of this deracemisation reaction, whereas, the electron withdrawing group, $–NO_2$ resulted in slightly lower enantiomeric excess (89%) and yield (58%). Significantly, whole cells were used for this purpose thus obviating the need for added cofactors and elaborate enzyme purification protocols.

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