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Asymmetric reduction of alkyl 2-oxo-4-arylbutanoates and -but-3-enoates by *Candida parapsilosis* ATCC 7330: assignment of the absolute configuration of ethyl 2-hydroxy-4-(*p*-methylphenyl)but-3-enoate by ^{1}H NMR

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Abstract—Enantioselective bioreduction of alkyl 2-oxo-4-arylbutanoates and 2-oxo-4-arylbut-3-enoates mediated by *Candida parapsilosis* ATCC 7330 resulted in the formation of the corresponding (*S*)-2-hydroxy compounds in high enantiomeric excesses (93–99%) and good isolated yields (58–71%). The absolute configuration of enantiomerically pure ethyl 2-hydroxy-4-(*p*-methylphenyl)but-3-enoate obtained by the reduction of the corresponding keto ester was assigned by ^{1}H NMR using Mosher's method.

1. Introduction

Enantiomerically pure alkyl 2-hydroxy-4-phenylbutanoates are important chiral building blocks,[1] particularly the β,γ-unsaturated α-hydroxy esters, as they can also lend themselves to stereoselective epoxidations, such as Sharpless epoxidation,[2] dihydroxylation[3] and amino hydroxylation.[4] These chiral molecules can be prepared by both chemical[5] and biocatalytic[6,7] methods. Enzymatic resolution,[7] a widely used method, results in 50% of the unwanted enantiomer unlike biocatalytic asymmetric reduction, which ideally gives only one enantiomer in high enantiomeric excess and chemical yield.[8] Isolated oxidoreductases, coupled with cofactors are known to reduce 2-oxo esters to enantiomerically pure 2-hydroxy esters,[9] but these enzymes in whole cells have the advantage of regenerating the cofactors viz NAD(P)H within the cell.[10] Among the whole cells, plant tissue culture,[11] bacteria[12] and yeast[13,14] have been used. These reactions either require too much time (10 days for *D. carota*) or result in lower chemical yields and enantiomeric excesses as seen in baker's yeast[13] and *Pseudonocardia thermophila* IFO 12133.[12] Purified carbonyl reductases from *Candida parapsilosis* DSM 70125 give highly stereoselective reductions of various aromatic and aliphatic ketones.[15] The involvement of oxidoreductases in deracemisation reactions via asymmetric reduction is also well explained.[16,17] Recently, we reported the deracemisation of racemic α- and β-hydroxy esters to the corresponding enantiomerically pure (*S*)-hydroxy esters in 62–85% chemical yields and 15–99% ees using whole cells of *C. parapsilosis* ATCC 7330.[17a,b] Herein we report for the first time the asymmetric reduction of α-oxo esters, specifically alkyl 2-oxo-4-arylbutanoates and 2-oxo-4-arylbut-3-enoates using the whole cells of *C. parapsilosis* ATTC 7330 into their corresponding 2-hydroxy esters in high conversion and high ee.

2. Results and discussion

2.1. Reduction of methyl and ethyl esters of 2-oxo-4-arylbutanoic acid

The enantioselective reduction of ethyl and methyl esters of 2-oxo-4-arylbutanoic acids **1a**–**f** and 2-oxo-4-arylbut-3-enoic acids **2a**–**f** using whole cells of *C. parapsilosis* ATCC 7330 provided the respective ethyl and methyl esters of (*S*)-2-hydroxy-4-arylbutanoic acids **3a**–**f** (Scheme 1A) and (*S*)-2-hydroxy-4-arylbut-3-enoic acids **4a**–**f** (Scheme 1B) in excellent enantiomeric excess (93–99%) and high isolated yields (58–71%). At the end of the reaction (i.e., after complete consumption of the α-keto

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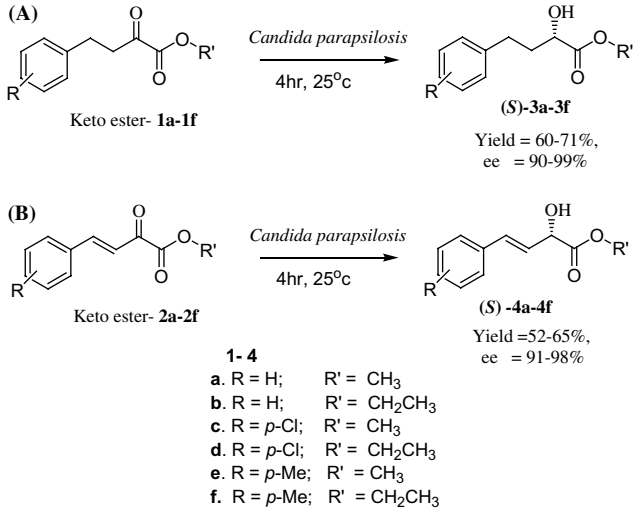


Scheme 1. Asymmetric reduction by *C. parapsilosis* ATCC 7330 of (A) alkyl 2-oxo-4-arylbutanoates **1a**–**f**; (B) alkyl 2-oxo-4-arylbut-3-enoates **2a**–**f**.

esters), the cells were filtered and the aqueous layer extracted with ethyl acetate. The enantiomeric excesses of the product 2-hydroxy esters were determined by chiral HPLC analysis. Chiral chemical catalysts for the preparation of **2a**,[5a] **2b**[5b] and **4a**[5d] have also been reported. Resolution of methyl 2-hydroxy-4-phenylbut-3-enoate **2a** using *Pseudomonas* sp. lipase[7b] resulted in the (*R*)-alcohol in 49% chemical yield and 88% ee while the (*S*)-acetate was obtained in 43% chemical yield and 98% ee. Asymmetric reduction of α-keto acids of esters **2a**–**d** using *Proteus vulgaris*[18] gave the corresponding enantiomerically pure α-hydroxy acids in 85–95% chemical yields and >97% ees. However, the reaction requires stringent anaerobic conditions. Preparation of enantiomerically pure (*R*)-alkyl and aryl 2-hydroxy-4-phenylbutanoates by baker's yeast mediated asymmetric reduction of the corresponding 2-oxo esters resulted in yields ranging from 80% to 90% and ees of >90%.[13b] This reaction involves the preincubation of baker's yeast with the additive phenacyl chloride and the enantiomeric excesses (48–99%) and chemical yields (18–99%) depending on the concentration of the additive.[13a] We have previously reported the asymmetric reduction of **1b** and **2a**–**f** using plant tissue culture cells of *Daucus carota*,[11,19] which resulted in the formation of the (*R*)-hydroxy ester. Herein we report the use of *C. parapsilosis* ATCC 7330 for biocatalytic asymmetric reduction of these compounds to give the enantiomerically pure (*S*)-2-hydroxy esters indicating different enantiospecificities of enzymes from different sources. *C. parapsilosis* ATCC 7330 mediated reduction of α-keto esters and their *p*-substituted derivatives **1a**–**f** and **2a**–**f** gave the corresponding α-hydroxy esters in good isolated yields (58–69%) and high ees (93–96%) (Table 1) regardless of the electronic properties of the substituents suggesting the wide range of substrates the enzyme can accept. In addition to high enantioselectivity, the biocatalyst displays chemoselectivity in that *C. parapsilosis* ATCC 7330 mediated reduction of C=O precedes that of C=C for compounds **2a**–**f**. Hence, unsaturated α-hydroxy esters, **4a**–**f** are obtained after 4 h, while saturated alcohols **3a**–**f** appear after 6–10 h. Thus, both the unsaturated hydroxy ester and the saturated hydroxy esters can be obtained by merely altering the time of the reaction. Notably, the chemical reduction of ethyl 2-oxo-4-phenylbut-3-enoate using sodium borohydride resulted in the formation of unsaturated diol while palladium/carbon gave the corresponding saturated α-hydroxy ester.[22]

An important consideration for a catalytic reaction is the reusability of the catalyst. In order to increase the robustness of whole cell biocatalysts they are often encapsulated in gels,[8b] which allows for easy separation of the biocatalyst and simplifies the downstream processing to separate the product. Even though alginated cells used in this study resulted in a slightly less yield over free cells, the same could be recovered and reused up to three cycles without loss in activity (Table 1).

Table 1. Microbial reduction of ethyl and methyl esters of 2-oxo-4-arylbutanoic acids **1a**–**f** and 2-oxo-4-arylbut-3-enoic acids **2a**–**f** using whole cells of *C. parapsilosis* ATCC 7330

Entry	Free cells of *C. parapsilosis* ATCC 7330					Entry	Immobilised cells of *C. parapsilosis*		
	Yield (%)	Ee (%)	$[\alpha]_D$ Values	Ref.	Abs. config.		Yield (%)	Ee (%)	Abs. config
3a	68	99	+32.2 (*c* 2.1, $CHCl_3$)	20	*S*	**3a**	63	97	*S*
3b	71	99	+21.4 (*c* 1, $CHCl_3$)	13a	*S*	**3b**	65	98	*S*
3c	65	95	+22.3 (*c* 0.57, $CHCl_3$)	18	*S*[b]	**3c**	60	96	*S*
3d	67	94	+17.5 (*c* 1, $CHCl_3$)	13a	*S*	**3d**	64	93	*S*
3e	68	93	+28.7 (*c* 1.05, $CHCl_3$)	—	Nd	**3e**	62	91	Nd
3f	69	93	+18.1 (*c* 0.6, $CHCl_3$)	—	*S*[c]	**3f**	64	90	*S*
4a	64	98	+67 (*c* 1, $CHCl_3$)	21	*S*	**4a**	54	96	*S*
4b	63	98	+21.4 (*c* 1.1, $CHCl_3$)[a]	13a	*S*	**4b**	55	97	*S*
4c	58	95	+55.5 (*c* 1.25, $CHCl_3$)	18	*S*[b]	**4c**	52	96	*S*
4d	59	96	+18.8 (*c* 1, $CHCl_3$)[a]	13a	*S*	**4d**	54	91	*S*
4e	65	95	+50.7 (*c* 1.1, $CHCl_3$)	—	Nd	**4e**	60	96	Nd
4f	62	94	+40.9 (*c* 1.2, $CHCl_3$)	—	*S*[c]	**4f**	61	93	*S*

[a] Specific rotation was taken after hydrogenation.
[b] Absolute configuration was assigned after hydrolysis.
[c] Absolute configuration was assigned by Mosher's method. Nd = not determined.

2.2. Role of the solvent in cofactor regeneration

Experiments done with ethanol and isopropanol resulted in complete conversion of the α-keto esters **1a**–**f** and **2a**–**f** into α-hydroxy esters **3a**–**f** and **4a**–**f**. This could be due to the regeneration of cofactors (NAD^+ or $NADP^+$)[23] by alcohol while in nonalcoholic solvents, such as hexane, tetrahydrofuran, dimethylformamide, benzene, dimethyl sulfoxide and chloroform, the reaction did not go to completion.

2.3. Assignment of the absolute configuration of 4f

The absolute configuration of compounds **3a**,**b**,**d** and **4a** was confirmed to be '*S*' by comparing their specific rotations with those reported.[13a,20,21] Dao et al. have assigned the absolute configuration of ethyl (*p*-methyl phenyl)butanoates **3f**[13a] by comparing the retention times of the corresponding unsubstituted, *p*-methoxy and *m*-chloro esters on chiral HPLC. However, a mere comparison of retention times is not a confirmation of absolute configuration. Herein, the absolute configuration of **4f** was determined by a detailed ^1H NMR study of the Mosher's derivative[24] of **4f**. (*R*)-MTPA esters of (*RS*)-**4b,** (*R*)-**4b,** (*S*)-**4b** were used as a reference for this study. (*R*)-MTPA esters of (*RS*)-**4f** and enantiomerically pure unknown **4f** were also prepared. A typical reaction is shown in Scheme 2. Enantiomerically pure (*R*)-**4b** was prepared according to the reported literature.[19] The structure of the diastereomers was confirmed by ^1H NMR spectroscopy as per the following discussion:

(a) Figure 1a and b represents the absolute configurations of diastereomers of MTPA-(*R*,*R*)-**4b** and MTPA-(*S*,*R*)-**4b** obtained from (*S*)-Mosher's acid chloride with (*R*)-**4b** and (*S*)-**4b**, respectively. Hence, the arrangement of the phenyl ring present in Mosher's acid moiety can be assigned as either *cis* or *trans* to the olefinic protons of (*R*)-**4b** or (*S*)-**4b**. The proton resonating upfield is due to the diamagnetic effect of phenyl ring present in the Mosher's acid.[25] In the ester MTPA-(*R*,*R*)-**4b** prepared from (*R*)-**4b** and (*S*)-MTPA Cl, the Ha (δ = 6.21–6.27 ppm) and Hb protons (δ = 6.67–6.71 ppm) are more shielded as compared to the Ha (δ = 6.28–6.34 ppm) and Hb (δ = 6.8–6.84 ppm) in MTPA-(*SR*)-**4b**, which is derived from (*S*)-**4b** and (*S*)-MTPA Cl. This is due to the diamagnetic effect of the phenyl ring of Mosher's ester as shown in Figure 1a and b. However, the diamagnetic effect of the phenyl ring on the protons of the $-OCH_2CH_3$ group in MTPA-(*RR*)-**4b** and MTPA-(*SR*)-**4b** is less as seen from the difference in the δ values (δ_{S-R} = <0.03 ppm and $-OCH_2$, δ_{S-R} = <0.02 ppm) due to a larger distance between the $-OCH_2CH_3$ moiety and the phenyl ring.

(b) An exact match in δ-values for protons Ha and Hb was seen in individual diastereomers MTPA-(*RR*)-**4b** and MTPA-(*SR*)-**4b** and a mixture of diastereomers [MTPA-(*R*,*R* and *S*,*R*)-**4b**] (Fig. 2). Therefore, the

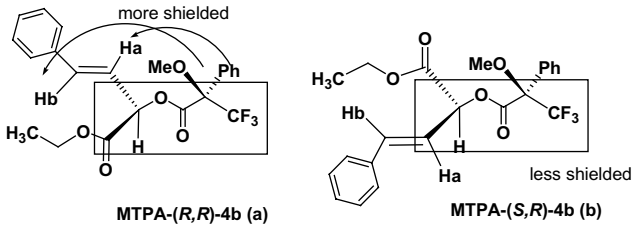


Figure 1. (*R*)-MTPA derivatives of (*R*,*R*)-**4b** and (*S*,*R*)-**4b**.

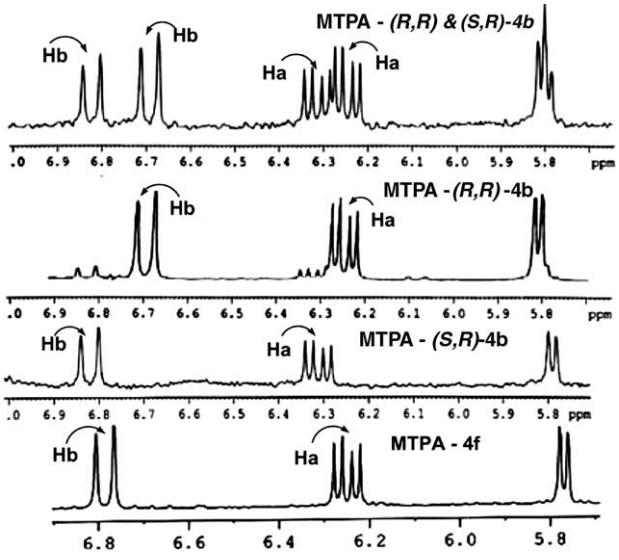
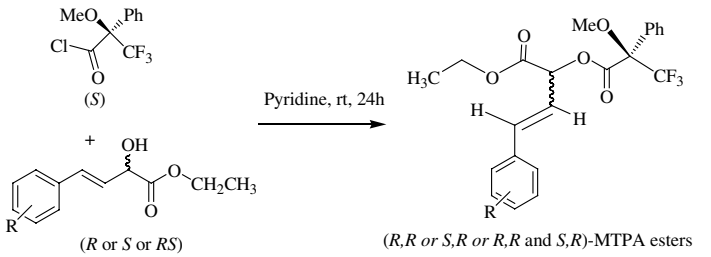


Figure 2. Signals of Ha and Hb in MTPA-(*R*,*R* and *S*,*R*)-**4b**, MTPA-(*S*,*R*)-**4b**, MTPA-(*R*,*R*)-**4b** and MTPA-**4f**.



R= H, (***RS***)-**4b**
R= H, (***R***)-**4b**
R= H, (***S***)-**4b**
R= Me, (***RS***)-**4f**
R= Me, **4f**

R=H, MTPA-(*RR* and *SR*)-**4b**
R=H, MTPA-(*RR*)-**4b**
R=H, MTPA-(*SR*)-**4b**
R=Me, MTPA-(*RR* and *SR*)-**4f**
R=Me, MTPA-**4f**

Scheme 2. Synthesis of (*R*)-MTPA esters of (*R*,*S*)-**4b**, (*R*)-**4b**, (*S*)-**4b**, (*RS*)-**4f** and **4f**.

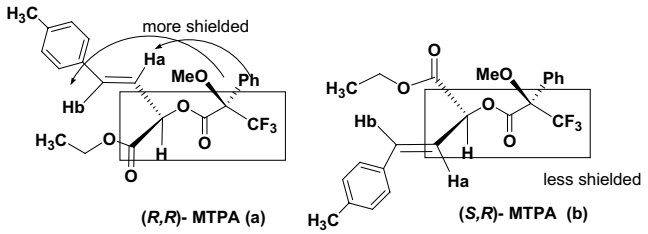


Figure 3. (*R*)-MTPA derivatives of (*R*,*R*)-**4f** and (*S*,*R*)-**4f**.

known (*R*)-**4b** and (*S*)-**4b**-ethyl 2-hydroxy-4-phenylbut-3-enoates were reconfirmed by ^{1}H NMR spectroscopy using Mosher's method. Having assigned the absolute configuration of known enantiomers of (*R*)-**4b** and (*S*)-**4b**-ethyl 2-hydroxy-4-phenylbut-3-enoates, this method was employed to establish the absolute configuration of the optically pure unknown enantiomer **4f**. The only structural difference between known enantiomers (*R*)-**4b** and (*S*)-**4b** and unknown enantiomer **4f** was the methyl substituent at the *para* position on the aromatic ring, which was assumed to have no effect on the protons under consideration for this study.

(c) The ^{1}H NMR spectrum of MTPA-**4f** (Ha = 6.22–6.28 ppm and Hb = 6.76–6.80 ppm) was compared with the ^{1}H NMR spectrum of the mixture of MTPA-(*R*,*R* and *S*,*R*)-**4f** (Ha = 6.22–6.28 and 6.15–6.21 ppm, Hb = 6.76–6.80 and 6.64–6.68). These δ-values for Ha and Hb protons match with the MTPA-*SR* (less shielded) as shown in Figure 3 indicating that the unknown optically pure enantiomer **4f** is '*S*'.

(d) The splitting pattern in the ^{1}H NMR spectra of MTPA-(*R*,*R* and *S*,*R*)-**4f** was similar to that obtained with the MTPA-(*R*,*R* and *S*,*R*)-**4b** and splitting pattern of MTPA-**4f** was similar to that obtained with MTPA-(*S*,*R*)-**4b** thus corroborating the (*S*)-configuration (Fig. 2). The absolute configuration of compound **3f** was determined by comparing the retention time on chiral HPLC of the corresponding saturated compound obtained after hydrogenating the unsaturated compound **4f**. In the case of the unsaturated compounds **4b** and **4d**, the absolute configuration was determined by hydrogenating and comparing them with **3b** and **3d**, respectively (Chiral HPLC). All these were found to be '*S*'. The specific rotation of the α-hydroxy acid obtained after the hydrolysis of ester **4c** was measured and compared with that reported in literature[18] based on which the absolute configuration was assigned as '*S*'. Thus, the reductase of *C. parapsilosis* ATCC 7330, which is known to play an important role in deracemisation of α-hydroxy esters[26] can be safely called the (*S*)-specific reductase for α-oxo esters.

3. Conclusion

We have established a convenient and simple procedure for the enantioselective reduction of alkyl 2-oxo-4-arylbutanoates **1a**–**f** and 2-oxo-4-phenylbut-3-enoates **2a**–**f** using whole cells of *C. parapsilosis* ATCC 7330 to give the (*S*)-2-hydroxy ester in good yields (58–71%) and high enantiomeric excesses (93–99%) under mild reaction conditions. The method is better than all the reported methods so far, as the biocatalyst shows high chemo-, and enantioselectivity towards β,γ-unsaturated and saturated α-hydroxy esters. With appropriate immobilisation techniques, this method can be suitably scaled up.

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 done on a Jasco PU-1580 liquid chromatograph equipped with a manual injector (20 μL) and a PDA detector. The columns used were Chiralcel OD-H and Chiralcel OJ-H (Daicel, 4.6 × 250 mm). The enantiomeric excesses (% ee) of **3a**–**f** and **4a**–**f** were determined by HPLC analysis. The eluent used was hexane/isopropanol (98:2) at a flow rate of 1 mL min^{-1} and the absorbance monitored using a PDA detector at 254 nm. Optical rotations were determined on a Jasco Dip 370 digital polarimeter. TLC was done on Kieselger 60 F_{254} aluminium sheets (Merck 1.05554). All starting materials were prepared according to literature procedures.[27]

4.2. Culture medium of the microorganism

The yeast, *C. parapsilosis* (ATCC 7330) was grown in YMB medium (60 mL) in 250 mL Erlenmeyer flasks. The flasks were incubated at 25 °C with a shaking speed of 200 rpm for 40 h. The cells were pelleted down by centrifugation at 3214*g* for 15 min, washed with distilled water and used for the biotransformation reaction.

4.3. Determination of conversion and enantiomeric excess of the product

Keto ester **1b** (0.39 mmol, 80 mg) dissolved in ethanol (2 mL) was added into an Erlenmeyer flask containing the cells (20 g wet wt) in water (40 mL) and stirred on an orbital shaker at 150 rpm for 4 h at 25 °C. The conversions were determined by HPLC using a Sil-C18 column (size 4.6 × 250 mm) by comparing the retention time of the product obtained with that of the keto ester (reactant) and standard hydroxy ester (expected product). The eluent used was acetonitrile and water (85:15). The absence of keto ester **1b** by HPLC was taken to indicate that all the keto ester was consumed at which point the contents of the flask were transferred into a centrifuge tube. The broth was centrifuged (3214*g* for 30 min) and the supernatant decanted and filtered. The filtrate (40 mL) was extracted with ethyl acetate (2 × 20 mL), the organic phase separated, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography on silica gel (mesh size 100–200) using 5% ethyl acetate–hexane, to give **3b** as colourless oil (0.313 mmol, 65 mg, 71%). The ees of the hydroxy esters **3a**–**f** and **4a**–**f** were deter-

mined by chiral HPLC using Chiralcel OD-H and OJ-H column, respectively, using hexane/isopropanol (98:2) as the mobile phase. The other keto esters, **1a**,**c**–**f** and **2a**–**f** were also reduced by employing the same procedure and afforded the (*S*)-α-hydroxy ester in excellent ee (93–99%). The ^{1}H and ^{13}C NMR (400 and 100 MHz) of the methyl and ethyl esters of 2-hydroxy-4-arylbutanoic acid and 2-hydroxy-4-arylbut-3-enoic acids **3a**,**b**,**d**,**f** and **4a**–**f** were identical to those reported earlier.[13a,18–20]

4.4. Asymmetric reduction of α-keto esters using immobilised cells of *C. parapsilosis* ATCC 7330

Keto ester **1b** (0.39 mmol, 80 mg) dissolved in ethanol (2 mL) was reduced using alginated beads of *C. parapsilosis* ATCC 7330 (20 g, wet weight) in 40 mL of water. The reaction mixture was incubated at 25 °C for 4 h with a shaking speed of 150 rpm. The crude product was purified as mentioned for free cells to give **3b** as a colourless oil (0.25 mmol, 52 mg, 65%). Although the (*S*)-alcohol was formed in all the reductions using alginated cells, the ee and yield of the product was slightly lower than that obtained using free cells. The recovered cells were reused for up to three cycles without any loss in activity.

4.5. Preparation of MTPA-(*R*,*R* and *S*,*R*)-4b

To a solution of racemic ethyl 2-hydroxy-4-phenylbut-3-enoate (*RS*)-**4b** (10 mg, 0.048 mmol) in pyridine (100 μL) was added (*S*)-MTPA chloride (20 mg, 0.0794 mmol) and the solution allowed to stand at room temperature for 24 h. The reaction mixture was then washed with dil HCl, followed by aqueous Na_2CO_3. The product was extracted using ethyl acetate (4 × 1 mL) and concentrated to obtain the crude product, which was purified by preparative TLC using hexane and ethyl acetate (95:5) as mobile phase eluent. The product was characterised by the ^{1}H NMR spectroscopy. The MTPA esters of (*R*)-**4b**, (*S*)-**4b**, (*RS*)-**4f** and **4f** were also prepared by this procedure (Scheme 2).

(i) **MTPA-(*R*,*R* and *S*,*R*)-4b**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.28–1.35 (2 t, 6H, 2 × –OCH_2CH_3), 3.59 (s, –OCH_3), 3.7 (s, –OCH_3), 4.2–4.37 (2q, 4H, 2 × –OCH_2CH_3), 5.78–5.81 (2d, 2H, 2 × Ph–CH=CH–C*H*), 6.21–6.27 (dd, 1H, Ph–CH=C*H*–CH), 6.28–6.34 (dd, 1H, Ph–CH=C*H*–CH), 6.67–6.71 (d, 1H, Ph–C*H*=CH–CH), 6.80–6.84 (d, 1H, Ph–C*H*=CH–CH) and 7.3–7.66 (m, 20H, 4 × C_6H_5–).

(ii) **MTPA-(*R*,*R*)-4b**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.29–1.32 (t, 3H, –OCH_2CH_3), 3.7 (s, –OCH_3) 4.26–4.31 (q, 2H, –OCH_2CH_3), 5.79–5.81 (d, 1H, Ph–CH=CH–C*H*), 6.21–6.27 (dd, 1H, Ph–CH=C*H*–CH), 6.67–6.71 (d, 1H, Ph–C*H*=CH–CH) and 7.27–7.66 (m, 10H, 2 × C_6H_5–).

(iii) **MTPA-(*S*,*R*)-4b**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.28–1.29 (t, 3H, –OCH_2CH_3), 3.595 (s, –OCH_3), 4.23–4.27 (q, 2H, –OCH_2CH_3), 5.78–5.80 (d, H, Ph–CH=CH–C*H*), 6.28–6.34 (dd, 1H, Ph–CH=C*H*–CH), 6.80–6.84 (d, 1H, Ph–C*H*=CH–CH) and 7.3–7.66 (m, 10H, 2 × C_6H_5–).

(iv) **MTPA-(*R*,*R* and *S*,*R*)-4f**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.23–1.32 (2t, 6H, 2 × –OCH_2CH_3), 2.33 (s, 3H, –CH_3Ph), 2.34 (s, 3H, –CH_3Ph), 3.59 (s, 3H, –OCH_3), 3.696 (s, 3H, –OCH_3) 4.22–4.30 (2q, 4H, 2 × –OCH_2CH_3), 5.78–5.789 (2d, 2H, 2 × –CH_3 Ph–CH=CH–C*H*), 6.15–6.21 (dd, 1H, –CH_3Ph–CH=C*H*–CH), 6.22–6.28 (dd, 1H, –CH_3Ph–CH=C*H*–CH), 6.64–6.68 (d, 1H, –CH_3Ph–C*H*=CH–CH), 6.76–6.80 (d, 1H, –CH_3Ph–C*H* =CH–CH) and 7.13–7.66 (m, 18H, 2 × $CH_3C_6H_4$–, 2 × C_6H_5–).

(v) **MTPA-(*S*,*R*)-4f**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.23–1.27 (t, 3H, –OCH_2CH_3), 2.34 (s, 3H, –CH_3Ph), 3.59 (s, 3H, –OCH_3), 4.22–4.28 (q, 2H, OCH_2CH_3), 5.76–5.78 (2d, 2H, –CH_3 Ph–CH=CH–C*H*), 6.22–6.28 (dd, 1H, –CH_3Ph–CH=C*H*–CH), 6.76–6.80 (d, 1H, –CH_3Ph–C*H*=CH–CH) and 7.13–7.66 (m, 9H, $CH_3C_6H_4$–, C_6H_5–).

Acknowledgements

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