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Biocatalytically assisted preparation of antifungal chlorophenylpropanols

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Abstract—Fermenting baker's yeast converts 4′-chloropropiophenone **1** and 3′-chloropropiophenone **2** into enantiopure (*S*)-(−)-1-(4′-chlorophenyl)propan-1-ol (*S*)-**3** and (*S*)-(+)-1-(3′-chlorophenyl)propan-1-ol (*S*)-**4**, respectively. Application of inhibitors and organic solvents as additives enhanced the enantiomeric excesses. Enantiopure compounds (*R*)-(+)-1-(4′-chlorophenyl)propan-1-ol ((*R*)-**3**) and (*R*)-(+)-1-(3′-chlorophenyl)propan-1-ol (*R*)-**4** were prepared by lipase-mediated esterifications of the racemic alcohols. Maximum inhibition of the growth of the phytopathogenic fungus *Botrytis cinerea* was shown for the (*R*)-enantiomers.

1. Introduction

Botrytis cinerea is a grey mould that causes damage to economically important crops such as lettuce, carrots, tobacco, strawberries and grapes.[1] In southern Spain, this fungus principally affects strawberries and grapes, causing significant losses in the Sherry area, where *B. cinerea* is considered endemic.

In order to find substrates with antifungal properties against this fungus, we have undertaken a screening of compounds analogous to various phytoalexins.[2] As a result of these studies, we have found several different racemic secondary alcohols with skeletons similar to those of active compounds which exhibit fungicidal activity against *B. cinerea*, most notably (±)-1-(4′-chlorophenyl)propan-1-ol **3** and (±)-1-(3′-chlorophenyl)propan-1-ol **4**.

Since the absolute configuration of biologically active compounds is an important factor governing their activity, we were interested in devising a biocatalytic approach to the enantiomers of **3** and **4** in order to compare their antifungal activities. To produce the desired (*S*)-enantiomers, we undertook a reduction of the ketones using baker's yeast[3] since the known examples of this type of reduction have shown the (*S*)-configuration to be the prevalent enantiopreference. In contrast, our preparation of (*R*)-enantiomers was based on Kazlauskas' empirical rule[4] for the resolution of secondary alcohols in the enzymatic *O*-acetylation process by lipases.

We report herein on the biocatalytic preparation of 3′- and 4′-chlorophenylpropanols in both absolute configurations, as well as their antifungal properties against *B. cinerea*.

2. Results and discussion

2.1. Baker's yeast reduction

In light of the known ability of fermenting baker's yeast to reductively transform a variety of ketones into optically active alcohols with (*S*)-configuration (Prelog specificity family),[3] we decided to undertake a baker's yeast-mediated approach to enantiopure (*S*)-(−)-1-(4′- and 3′-chlorophenyl)propanols, (*S*)-**3** and (*S*)-**4**, using the corresponding ketones 4′-chloropropiophenone **1** and 3′-chloropropiophenone **2** as substrates. The results are shown in Table 1.

A major limitation of this methodology is the difficulty in extracting the product from the fermentation broth; consequently, often only moderate yields of products are obtained, presumably due to absorption of the starting material and/or product within the larger quantity of yeast cells.[5]

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Table 1. Results of baker's yeast-mediated reduction of 4′- and 3′-chloropropiophenones **1** and **2**

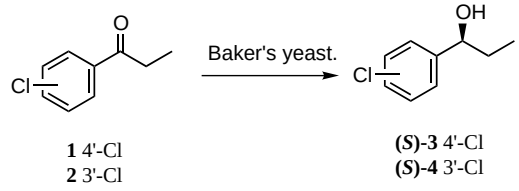
Substrate	Reaction time (h)	Substrate concentration (mM)	Allyl alcohol (mM)	Hexane (%)	Glucose (mM)	Conversion[a] (%)	e.e. (%)
1	24	5.93	–	–	505	9	81
	48	5.93	–	–	505	35	75
	48	13.20	–	–	265	14	77
	48	5.93	67	–	505	21	96
	48	5.93	67	–	1009	32	77
	48	5.93	67	1.2	505	17	92
	120	5.93	–	–	505	54	55
2	48	5.93	–	–	505	31	80
	48	13.20	–	–	265	13	86
	48	5.93	67	–	505	22	96
	48	5.93	67	–	1009	33	67
	48	5.93	67	1.2	505	26	94

[a] Conversion is the % of product in the recovered material.

The enantioselectivities and conversions in the yeast reduction of the ketones **1** and **2** were investigated under conventional conditions with heat-treatment at 50°C for 30 min.[6] As shown in Table 1, after incubating **1** (1 g) in fermenting baker's yeast (250 g in 1 L tap water) for 48 h, a single transformation product was isolated as a colourless oil (35% conversion, $[\alpha]_D^{20} = -21$ (*c* 3, $CHCl_3$)) with 75% e.e. as shown by HPLC analysis using a Chiralcel OD column. This material was shown to be (*S*)-(−)-1-(4′-chlorophenyl)propan-1-ol (*S*)-**3** (Scheme 1). The (*S*)-configuration was determined by comparing the specific rotation with data from the literature.[7]

In the yeast reduction of 3′-chloropropiophenone **2**, the only detectable species present in the incubation mixture were the starting material and a colourless oil, (31% conversion, $[\alpha]_D^{20} = -27$ (*c* 5, $CHCl_3$)) with 80% e.e. by means of HPLC analysis on Chiralcel OD. This compound was shown to be (*S*)-(−)-1-(3′-chlorophenyl)propan-1-ol (*S*)-**4** (Scheme 1).

It has been shown that increasing the substrate concentration tends to increase the enantiomeric excess in yeast reductions,[8] presumably because the variation affects the kinetics of each enzyme (the K_m of the (*R*)-enzyme is smaller than that of the (*S*)-enzyme in baker's yeast). With this in mind, we increased the concentration of the substrate from 5.93 to 13.2 mM. The result was a slight increase in the e.e. of the (*S*)-enantiomer, but the conversion itself was diminished (see Table 1).



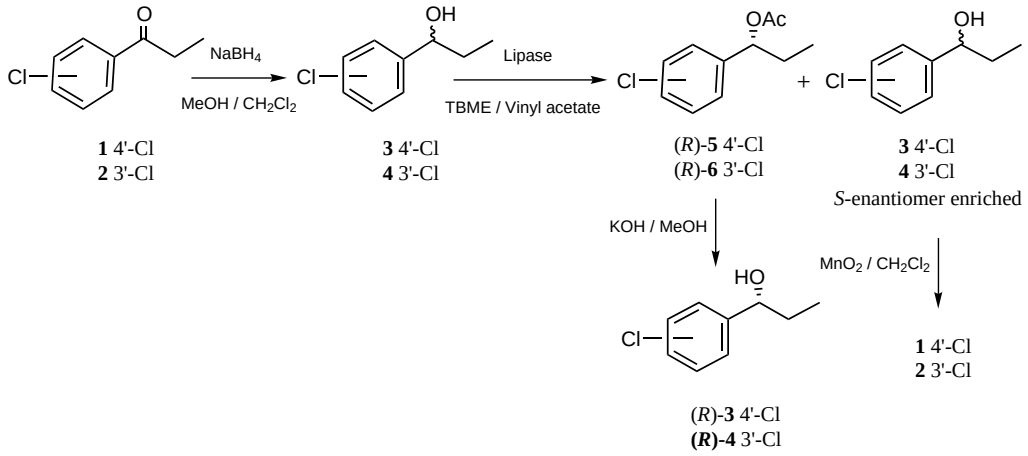
Scheme 1.

It has been reported that when the baker's yeast reduction of ketones is catalysed by certain enzymes, it produces (*S*)-enantiomers while catalysis with different enzymes produces (*R*)-enantiomers.[9] The inhibition of either type of enzyme may thus influence, if not control, the enantioselectivity of the reduction. We therefore tried to inhibit the reducing enzymes selectively by adding inhibitors such as allyl alcohol, as well as a small amount of organic solvent[10] to dissolve the substrate.

The use of allyl alcohol (67 mM) as an inhibitor led to a significant increase in enantioselectivity, but once again the conversion was diminished. While the addition of 1.2% hexane was not effective in the reduction of compound **1**, it did aid that of compound **2**. Finally, whereas a higher glucose concentration increased the conversions of the products, it led to a concomitant decrease in the enantioselectivity of the process (see Table 1).

2.2. Lipase-mediated acetylations

In order to prepare the (*R*)-enantiomers of these compounds using commercially available lipases, we focused on the kinetic resolution of the racemic secondary alcohols **3** and **4** (prepared by reacting the commercial ketones **1** and **2**, respectively, with sodium borohydride) by means of an enantioselective transesterification reaction using vinyl acetate as an acyl donor and *tert*-butyl methyl ether as organic solvent. The lipases investigated converted the *R* enantiomers of **3** and **4** in accordance with Kazlauskas' empirical rule for secondary alcohols (Scheme 2).[4] Three different lipases were employed: lipase PS (from *Pseudomonas cepacia*), PPL (porcine pancreas lipase) and CRL (*Candida rugosa* lipase). Two of the enzymes tested, lipase PS and PPL, gave both compounds with high enantioselectivities under the initial testing conditions (77 h and 22°C). However, this enhanced selectivity was accompanied by a fairly low reaction rate. CRL was rejected as a possible mediator due to the low selectivity and slow conversions observed when it was used. The results are summarised in Table 2.



Scheme 2.

Table 2. Results of lipase-mediated acetylation of (±)-1-(4′-chlorophenyl)propan-1-ol, **3** and (±)-1-(3′-chlorophenyl)propan-1-ol, **4**

Cmpd.	Enz.	Reaction time (h)	Temp. (°C)	Conversion (%)	Acetylated product (*R*)		Alcohol recovered (*S*)		*E*
					e.e. (%)	Yield[a] (%)	e.e. (%)	Yield (%)	
3	PS	77	22	19	>99	38	23	79	250
	PS	100	22	25	>99	48	33	72	305
	PS	168	22	32	>99	66	47	63	317
	PS	190	22	44	81	82	64	54	18
	PS	77	48	25	98	47	32	71	248
	PPL	77	22	10	>99	24	11	86	>400
	PPL	100	22	13	>99	28	15	86	>400
	PPL	168	22	18	>99	34	22	78	>400
	PPL	190	22	21	>99	42	26	75	256
	PPL	240	22	28	71	45	28	73	8
	PPL	77	40	16	99	36	18	80	341
	CRL	77	22	7	72	9	5	92	6.5
	CRL	77	45	8	74	11	6	93	7.2
4	PS	77	22	14	>99	33	17	80	>400
	PS	100	22	17	>99	36	20	75	220
	PS	168	22	22	98	44	28	74	144
	PS	190	22	30	82	52	35	70	14
	PS	77	48	21	99	41	26	74	312
	PPL	77	22	3	>99	7	3	95	>400
	PPL	100	22	4	>99	10	4	93	>400
	PPL	168	22	6	98	14	6	91	117
	PPL	190	22	8	97	17	8	91	66
	PPL	240	22	18	96	22	11	87	53
	PPL	77	40	3	95	5	3	92	42
	CRL	77	22	2	73	4	2	92	6
	CRL	77	45	4	78	9	4	93	8

[a] Yield 100% at 50% conversion and after hydrolysis of the acetate derivatives.

In an attempt to increase the conversion rate, different reaction times were tested (Table 2). The best result was obtained with lipase PS after 168 h (e.e. >99%, conversion 32% for compound **3**).

In order to determine the optimal conditions for transesterification, we also studied the effect of temperature on the enantiomeric excesses and conversion rate. We found that transesterification at the optimal temperature of each enzyme increased the conversion and decreased the enantiomeric excesses, albeit only slightly (see Table 2). This indicates that temperature may accelerate the kinetics of the enzyme.

The lipase-mediated esterification of these alcohols afforded the acetate derivatives (*R*)-**5** ($[\alpha]_D^{20}=+75$ (*c* 2.3, $CHCl_3$)) and (*R*)-**6** ($[\alpha]_D^{20}=+55$ (*c* 5, $CHCl_3$)), along with the unreacted alcohols **3** and **4**. After hydrolysis of (*R*)-**5** and (*R*)-**6** with potassium hydroxide in methanol, the enantiopure alcohols (*R*)-**3** ($[\alpha]_D^{20}=+27.3$ (lit.[11]

$[\alpha]_D^{20} = +28$)) and (*R*)-**4** ($[\alpha]_D^{20} = +30.5$ (lit.[12] $[\alpha]_D^{20} = +31$)) were obtained in high yield (>90%) and with very high enantiomeric excess (>99%). In both cases the (*R*)-configuration was determined by comparing the specific rotation with data reported in the literature.[11,12]

The unreacted alcohol derivatives **3** and **4**, which were enriched in the (*S*)-enantiomer during the esterification reactions, were oxidised with MnO_2 in order to recover the starting ketones 4′-chloropropiophenone **1** and 3′-chloropropiophenone **2** (Scheme 2). This oxidation thus serves to increase the efficiency of the process.

2.3. Antifungal assays

Having obtained the enantiopure compounds through the use of biological methods, we established their antifungal properties against the growth of *B. cinerea* using the 'poisoned food' technique.[13] The commercial fungicide Euparen® was used as a standard.

Compound (*R*)-(−)-1-(4′-chlorophenyl)propan-1-ol (*R*)-**3** exhibited the maximum percent of growth inhibition followed by its isomer (*R*)-(−)-1-(3′-chlorophenyl)-propan-1-ol (*R*)-**4**. Alcohol (*R*)-**3** was active even at 10 ppm; at 25 ppm it reduced the growth of the fungus by 70% after only 6 days. Compound (*R*)-**4** showed a slightly lower activity; at the same concentration it reduced fungal growth by 61% after 6 days. The corresponding (*S*)-enantiomers displayed a lower inhibitory activity on *B. cinerea*, with alcohol (*S*)-**4** being more active than its corresponding isomer (*S*)-**3**.

These results indicate that, at least for this class of compounds, the (*R*)-enantiomer produces a more pronounced antifungal effect. In addition, the relative position of the chloro substituent in the aromatic moiety was found to be of importance since the *para*-substituted compound is more active than the *meta*-substituted one.

It is worth noting that the acetyl derivatives (*R*)-**5** and (*R*)-**6** were inactive as antifungal agents, which indicates that the hydroxyl group plays an important role in the inhibitory mechanism.

3. Conclusions

Our attempt to use biological methods to obtain enantiomerically pure sample of **3** and **4** was successful. Moreover, because of their remarkable stability, the enzymes employed could actually be re-used. These methods, which use completely biodegradable biocatalysts, are therefore both environmentally acceptable and inexpensive.

The best *S* selectivity–conversion relationship for the reduction of ketones **1** and **2** was observed after heat-treatment of baker's yeast cells in the presence of allyl alcohol as an inhibitor. Under these conditions, treatment of **1** and **2** yielded compounds *S*-**3** and *S*-**4**, respectively, in 96% e.e. with 22% conversion. The addition of hexane at a level of 1.2% slightly enhanced the conversion for compound (*S*)-**3**.

Transesterification of racemic alcohols **3** and **4** with lipases from *P. cepacia* and porcine pancreas followed by hydrolysis of the acetate derivatives led to enantiomerically pure alcohols (*R*)-**3** and (*R*)-**4** with high *E* values. By prolonging the reaction times, the conversion was significantly improved. A longer reaction time was needed to obtain similar results with PPL.

Maximum inhibition against the growth of the phytopathogenic fungus *B. cinerea* was shown for compounds (*R*)-(+)-1-(4′-chlorophenyl)propan-1-ol (*R*)-**3** and (*R*)-(+)-1-(3′-chlorophenyl)propan-1-ol (*R*)-**4**. For this class of compounds, the (*R*)-enantiomer was found to have more pronounced antifungal properties.

These results give some indication of the structural modifications necessary if substrates of this type are to be further developed as selective fungal control agents for *B. cinerea*.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Perkin–Elmer 881 spectrophotometer. ^1H and ^{13}C NMR measurements were obtained on Varian Gemini 200 and Varian Unity 400 NMR spectrometers with $SiMe_4$ as internal reference. *J* values are reported in Hz. Mass spectra were recorded using a VG 12-250 and a VG Autospec spectrometer at 70 eV. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV–vis detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on Merck Kiesegel 60 F_{254}, 0.2 mm thick. Silica gel (Merck 9385) was used for column chromatography. Chemicals were products of Fluka or Aldrich. All solvents were freshly distilled. Baker's yeast was obtained from a local store. The following enzymes were used in this work: *C. rugosa* lipase (Sigma, Type VII, 950 U/mg), *P. cepacia* lipase (Amano Pharmaceuticals Co., Japan) and porcine pancreas lipase (Sigma, Type II). Enantiomeric excesses were determined by means of HPLC analyses on a chiral column (Chiralcel OD, Daicel, Japan): 254 nm, 0.8 mL/min, hexane:isopropanol (99:1) (*S*)-**3** $t_R = 46.3$ min, (*R*)-**3** $t_R = 52.2$ min, (*S*)-**4** $t_R = 49.7$ min, (*R*)-**4** $t_R = 55.3$ min.

4.2. Microorganism and antifungal assays

The culture of *B. cinerea* 2100 was obtained from the Colección Española de Cultivos Tipo (CECT), Facultad de Biología, Universidad de Valencia, Spain. The bioassays involved measuring the inhibition of the radial growth on an agar medium in a Petri dish.[12,13] The test compound was dissolved in ethanol to give final compound concentrations of 10–150 mg L^{-1}.

4.3. Conventional procedure for the baker's yeast transformation

A mixture composed of baker's yeast (250 g), D-glucose (100 g), and tap water (1 L) was stirred in a 2 L beaker at 50°C for 30 min, after which time the substrate (1 g, 5.93 mmol), which had been dissolved in the minimum amount of ethanol, was added dropwise. The reaction mixture was stirred for 2 days. At the end of this period, 1 L of ethyl acetate was added and the crude reaction mixture was filtered through a large Büchner funnel on a Celite pad, which was later washed with the same solvent. The aqueous phase was extracted twice with 0.5 L of ethyl acetate, the organic phase was dried over Na_2SO_4, and the solvent was then evaporated under reduced pressure to dryness. The residue obtained was purified by means of column chromatography.

4.4. Baker's yeast transformation at different substrate concentration

A mixture of baker's yeast (20 g), water (380 ml), and glucose (20 g) was stirred at 50°C for 30 min. The substrate (843 mg, 13.2 mmol/L) was added to the mixture and the reaction was allowed to stir for an additional 48 h. The work-up procedure was essentially the same as that described above. The results are listed in Table 1.

4.5. Baker's yeast transformation in the presence of several additives

A mixture of baker's yeast (250 g), water (1 L), and glucose (100 g, 0.505 mol or 200 g, 1.009 mol) was stirred at 50°C for 30 min. The substrate (1 g, 5.93 mmol) was dissolved in (8 ml, 67 mmol) of allyl alcohol and hexane (12 ml, 1.2%) and was then added dropwise to the mixture. The reaction was then stirred for 48 h. The work-up procedure was essentially the same as that described above. The results are shown in Table 1.

4.6. (*S*)-(−)-Chlorophenylpropanols (*S*)-3 and (*S*)-4

Baker's yeast reduction of **1** and **2** (1 g/L) yielded (*S*)-**3** and (*S*)-**4**, respectively.

(*S*)-**3**: $[\alpha]_D^{20}=-30.3$ (*c* 3, $CHCl_3$) (lit.[7] $[\alpha]_D^{20}=-28$); IR (film): 3364, 2965, 1492, 825; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.84 (3H, t, $J=7.5$ Hz, H-3), 1.50–1.85 (2H, m, H-2), 3.06 (1H, s, OH), 4.47 (1H, t, $J=6.7$ Hz, H-1), 7.15–7.30 (4H, m, $H_{arom.}$, $J=8.3$ Hz, $J=8.9$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.8 (q, C-3), 31.7 (t, C-2), 75.0 (d, C-1), 127.3 (d, $2C_{arom.}$), 128.3 (d, $2C_{arom.}$), 132.8 (s, C-1′), 142.8 (s, C-4′); *m*/*z* (EI): 172 (M^++2, 16), 170 (M^+, 50), 143 (82), 141 (100), 115 (59), 113 (85), 77 (80).

(*S*)-**4**: $[\alpha]_D^{20}=-27.4$ (*c* 5, $CHCl_3$); IR (film): 3362, 2966, 1492, 825; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (3H, t, $J=7.5$ Hz, H-3), 1.60–1.90 (2H, m, H-2), 2.04 (1H, s, OH), 4.56 (1H, t, $J=6.4$ Hz, H-1), 7.15–7.35 (4H, m, $H_{arom.}$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.9 (q, C-3), 31.9 (t, C-2), 75.3 (d, C-1), 124.1 (d, $1C_{arom.}$), 126.1 (d, $1C_{arom.}$), 127.5 (d, $1C_{arom.}$), 129.6 (d, $1C_{arom.}$), 134.2 (s, C-1′), 146.6 (s, C-3′); *m*/*z* (EI): 172 (M^++2, 9), 170 (M^+, 29), 143 (81), 141 (100), 115 (48), 113 (84), 77 (96).

Chiral HPLC analysis of (*S*)-**3** and (*S*)-**4** showed them to have e.e. of 96%.

4.7. Racemic alcohols 3 and 4

Reduction of 4′- and 3′-chloropropiophenone **1** and **2** (20 g, 0.12 mol) with sodium borohydride (7.5 g, 0.2 mol) in methylene chloride:methanol 1:1 (350 ml) at room temperature gave (±)-1-(4′- and 3′-chlorophenyl)propan-1-ol **3** and **4** (19.8 g, 93% and 19.1 g, 94%). The ^{1}H NMR spectra of these products were in agreement with those found in the literature.[11,12]

4.8. General procedure for enzyme-mediated acetylations

A mixture of the racemic alcohol **3** or **4** (1 g, 0.006 mol), lipase (1 g), and vinyl acetate (4 ml) in *tert*-butyl-methyl ether (15 ml) was stirred at room temperature. The residue obtained upon evaporation of the filtered reaction mixture was chromatographed on a silica gel column and eluted with hexane:ethyl acetate (95:5). The first eluted fractions provided the acetate derivative and the last eluted fractions afforded the unreacted starting material. Detailed results of the enzyme-mediated acetylations are reported in Table 2.

4.9. (*R*)-(+)-1-(4′- and 3′-Chlorophenyl)propyl acetates (*R*)-5 and (*R*)-6

After a reaction time of 168 h, lipase PS-mediated acetylation of racemic chlorophenylpropanols **3** and **4** (1 g, 0.006 mol) gave enantiopure acetate derivatives (*R*)-**5** (0.375 g, 33%) $[\alpha]_D^{20}=+75$ (*c* 2.3, $CHCl_3$) and (*R*)-**6** (0.250 g, 22%) $[\alpha]_D^{20}=+55$ (*c* 5, $CHCl_3$).

(*R*)-**5**: IR (film): 2972, 1738, 1238, 822; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.84 (3H, t, $J=7.3$ Hz, H-3), 1.60–2.00 (2H, m, H-2), 2.05 (3H, s, $COCH_3$), 5.59 (1H, t, $J=6.7$ Hz, H-1), 7.15–7.35 (4H, m, $H_{arom.}$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.8 (q, C-3), 21.2 (q, $COCH_3$), 29.1 (t, C-2), 76.6 (d, C-1), 127.9 (d, $2C_{arom.}$), 128.5 (d, $2C_{arom.}$), 133.5 (s, C-1′), 139.0 (s, C-4′), 170.3 (s, CO); *m*/*z* (EI): 214 (M^++2, 3), 212 (M^+, 8), 185 (M^++2−29, 6), 183 (M^+−29, 19), 172 (M^++2−$COCH_3$, 4), 170 (M^+−$COCH_3$, 11), 143 (28), 141 (98), 127 (21), 125 (65), 117 (100), 77 (32).

(*R*)-**6**: IR (film): 2971, 1738, 1236, 785, 696; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (3H, t, $J=7.5$ Hz, H-3), 1.70–2.00 (2H, m, H-2), 2.09 (3H, s, $COCH_3$), 5.62 (1H, t, $J=6.7$ Hz, H-1), 7.15–7.35 (4H, m, $H_{arom.}$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.7 (q, C-3), 21.0 (q, $COCH_3$), 29.2 (t, C-2), 76.4 (d, C-1), 124.7 (d, $1C_{arom.}$), 126.5 (d, $1C_{arom.}$), 127.8 (d, $1C_{arom.}$), 129.6 (d, $1C_{arom.}$), 134.2 (s, C-1′), 142.5 (s, C-3′), 170.2 (s, CO); *m*/*z* (EI): 214 (M^++2, 3), 212 (M^+, 9), 185 (M^++2−29, 5), 183 (M^+−29, 16), 172 (M^++2−$COCH_3$, 38), 170 (M^+−$COCH_3$, 100), 143 (18), 141 (70), 127 (24), 125 (70), 117 (75), 77 (39).

4.10. (*R*)-(+)-1-(4′- and 3′-Chlorophenyl)propan-1-ol (*R*)-3 and (*R*)-4

Treatment of acetate derivatives (*R*)-**5** and (*R*)-**6** (0.110 g, 0.52 mmol) with potassium hydroxide (0.1 g, 1.8 mmol)) in methanol solution (35 ml) at room temperature afforded enantiopure alcohol derivatives (*R*)-**3** (79 mg, 91%) and (*R*)-**4** (83 mg, 94%) showing $[\alpha]_D^{20}$ ((*R*)-**3**) = +27.3 (*c* 2.4, $CHCl_3$) (lit.[11] $[\alpha]_D^{20} = +28$) and $[\alpha]_D^{20}$ ((*R*)-**4**) = +30.5 (*c* 1, $CHCl_3$) (lit.[12] $[\alpha]_D^{20} = +31$), respectively.

(*R*)-**3**: IR (film): 3374, 2966, 1492, 825; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.84 (3H, t, $J = 7.3$ Hz, H-3), 1.55–1.80 (2H, m, H-2), 2.38 (1H, s, OH), 4.49 (1H, t, $J = 6.7$ Hz, H-1), 7.15–7.30 (4H, m, $H_{arom.}$, $J = 8.1$ Hz, $J = 8.6$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.9 (q, C-3), 31.8 (t, C-2), 75.1 (d, C-1), 127.3 (d, $2C_{arom.}$), 128.4 (d, $2C_{arom.}$), 132.9 (s, C-1′), 142.9 (s, C-4′); *m*/*z* (EI): 172 (M^++2, 4), 170 (M^+, 14), 143 (55), 141 (100), 115 (61), 113 (95), 77 (87).

(*R*)-**4**: IR (film): 3364, 2966, 1432, 784, 698; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92 (3H, t, $J = 7.5$ Hz, H-3), 1.60–1.90 (2H, m, H-2), 2.06 (1H, s, OH), 4.58 (1H, t, $J = 6.7$ Hz, H-1), 7.15–7.40 (4H, m, $H_{arom.}$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.9 (q, C-3), 31.9 (t, C-2), 75.3 (d, C-1), 124.1 (d, $1C_{arom.}$), 126.1 (d, $1C_{arom.}$), 127.5 (d, $1C_{arom.}$), 129.6 (d, $1C_{arom.}$), 134.3 (s, C-1′), 146.6 (s, C-3′); *m*/*z* (EI): 172 (M^++2, 7), 170 (M^+, 24), 143 (47), 141 (85), 115 (68), 113 (92), 77 (100).

The ^{1}H NMR spectra of these enantiopure compounds were in agreement with those of the racemic mixtures **3** and **4**. The two materials were shown to possess >99% e.e. upon chiral HPLC analysis.

4.11. 4′- and 3′-Chloropropiophenones (1 and 2)

The recovered unreacted alcohol derivatives **3** and **4** (5 g, 0.029 mol) were oxidised with manganese(IV) oxide (1.5 equiv.) in methylene chloride solution (50 ml) at room temperature. After purification on a silica gel column, eluting with hexane:ethyl acetate (9:1), the derivatives afforded the 4′- and 3′-chloropropiophenones **1** and **2** (97% yield). The ^{1}H NMR spectra of these compounds were in agreement with those of the commercial products.

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