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Enzyme-catalysed improved resolution of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol

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Abstract

Resolutions of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1** using various enzymes were performed. Among them, *Pseudomonas fluorescens* resolved it with moderate stereoselectivity (*E*=13) and reacted faster with the (*S*)-enantiomer. To optimize enzyme-catalysed reaction conditions for the resolution, the effect of solvents and additives was studied. In *n*-hexane:ethyl acetate (9:1), both reaction rate and selectivity were high. When pyridine, potassium carbonate and molecular sieves were used as additives, the enantiomeric excess of the (*R*)-enantiomer was 99, 99 and 98% at 52–60% conversion, respectively. However, in diisopropyl ether, the enantiomeric excess of unreacted alcohol (*R*)-**1** was up to 99% at 70% conversion without additives.

1. Introduction

4-Cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1** is a valuable compound as the intermediate for manufacturing not only Ca^{++} antagonists but also α-blockers.[1] Verapamil, which is used extensively for the treatment of hypertension as a racemate, has one stereogenic center and can be synthesized from the compound **1** (Fig. 1). Recently, the pharmacological properties of (*R*)- and (*S*)-enantiomers of verapamil have been shown to be quite different. The (*S*)-enantiomer has greater effects on the slow calcium ion current and the (*R*)-enantiomer is useful in reversal of multidrug resistance in cancer chemotherapy.[2] Thus, attention has focused on methods to obtain the individual enantiomers. Among the various results concerning the synthesis of compound **2**, one is an asymmetric synthesis[3] using the synthon, (*S*)-1,2-propandiol, and there is a process via classical resolution of verapamil itself. An improved method[4] was reported using quinine and 4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropylbutanoic

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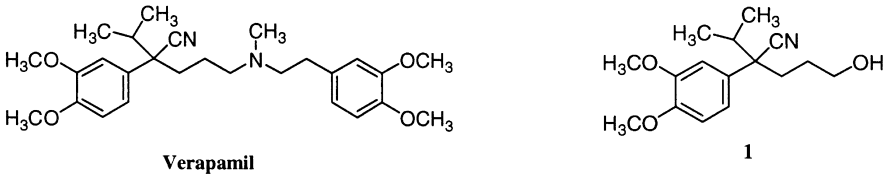


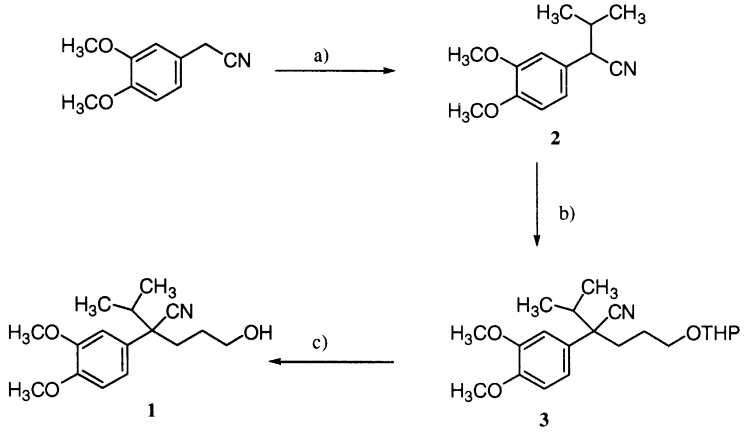
Figure 1.

acid. These methods appear to be tedious and involve long synthetic processes. To our best knowledge, there are no reports concerning the resolution of the compound **1** using enzymes.

Lipases as chiral catalysts play an important role in the synthesis of enantiomerically pure compounds by the resolution of racemates and have been used to catalyse the hydrolysis and esterification on a wide variety of substrates.[5] Although their usefulness is stressed, their application is limited to narrow fields because of their specificities. Many studies have been carried out in order to develop the stereospecific rules between lipases and substrates.[6] A simple rule comes out from the relative sizes of the substituents and some rules also include polarity or specific size restrictions for the two substituents. These rules seem to work very well for secondary alcohols, but not for primary or tertiary alcohols. Our target molecule is an uncommon structure for using enzymes and it cannot be predicted which enantiomer will react faster. In the previous work,[7] we used several lipases for resolving the similar structure to the compound **1**. As part of our continuing interest in the resolution of the quaternary chiral primary alcohol, we tried to get the compound **1** in enantiomerically pure form as the single isomers (*R*) or (*S*) using various enzymes and optimized resolution condition.

2. Results and discussion

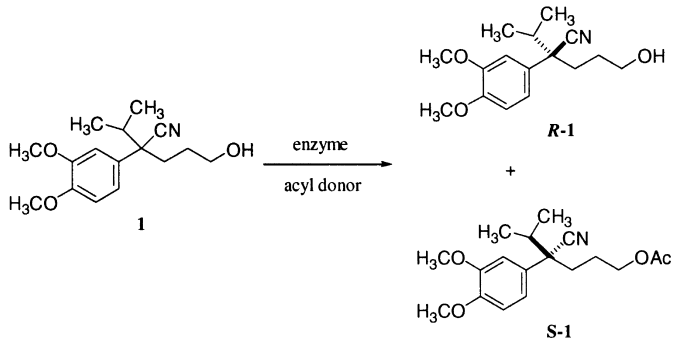
Compound **1** was prepared, as shown in Scheme 1, to submit to the enzyme reactions. We used it and its acetate **1**-**1** to obtain the pure enantiomers.



Scheme 1. (a) NaH, DMF, 2-bromopropane, 0°C→rt; (b) NaH, DMF, 3-tetrahydropyranyloxypropyl bromide, 0°C→rt; (c) 1N HCl, MeOH

We investigated the enzyme-catalysed transesterification between the alcohol **1** and an acyl donor (Scheme 2). As acyl donor, vinyl acetate and isopropenyl acetate were used to maintain irreversible conditions and they produced similar results. Thus, we used the reactions of the alcohol with vinyl

acetate in the presence of different commercially available enzymes. All of the reactions were carried out at 32–34°C and under anhydrous conditions. These results were obtained simply to screen for the appropriate enzymes when the used enzyme was 10% mass ratio to the substrate and the reaction time was within 24 h. Actually, we presumed Pig liver esterase (PLE), Porcine pancreas lipase (PPL), or *Candida rugosa* lipase (CRL) as the candidate enzymes for resolving compound **1** because the bulky stereogenic centers should be resolved by them. However, as shown in Table 1, only *Pseudomonas fluorescens* (LAK) and *Pseudomonas cepacia* (LPS) resolved it moderately and showed the same enantiomeric preference as the (*S*)-enantiomer.



Scheme 2.

Although none of the lipases gave a high enantioselectivity (E[8]=6.5 at best), LAK was selected as the best candidate for resolving the target molecule among the various enzymes. When it was used as mass equivalent, the conversion was 50% and the selectivity of a resolution (E) was a moderate 13. In the previous reports about the active site model for LAK, the primary and secondary alcohols were analysed by virture of the shapes and sizes of the hydrophobic binding sites and the predictive stereospecificity of their reactions worked very well. Also, the existence of a hydrophobic cleft in the active center of

Table 1
Lipase-catalysed resolutions of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1**

lipase[a]	Reaction time (h)	Conversion (%)	(*R*)-Alcohol ee (%)[b]	(*S*)-Acetate ee (%)	*E*
LPS[c]	2.5	41	22	24	2
LPS[d]	7	18	18	32	2.3
LPS-C	2.5	38	8	16	1.5
LPS-D	1	61	37	28	2.5
LAK	7	10	6	72	6.5
LAYS	24	0	-	-	-
CCL	1.5	27	8	8	1.3
LAS	24	0	-	-	-
PPL	24	0	-	-	-
CAL-B	5	68	0	0	0
LN	24	0	-	-	-

Reaction conditions : the enzyme was used 10 % mass equivalent to the substrate and acyl donor equivalent to it. Reaction mixtures were stirred at 32 ~ 34 °C for the given time. [a] lipases LPS (*Pseudomonas cepacia*), LPS-C (immobilized on carbon), LPS-D (immobilized on diatomaceous), LAK (*Pseudomonas fluorescens*), LAYS (*Candida rugosa*), LAS (*Aspergillus niger*) from Amano and PPL(porcine parcreas), CRL (*Candida rugosa*) from Sigma. CAL-B (*Candida antarctica*-B) and LN (mucor meihei) from Novo disk and Fluka, repectively. [b] ee = enantiomeric excess and detremined by HPLC using chiral capillary column. [c, d]obtained from Amano and Aldrich, respectively.

Pseudomonas cepacia has recently been postulated.[9] According to that, we predicted the (*R*)-enantiomer of the compound as the faster reacting one. In the other work, we had obtained (*S*)-2-butyl-2-cyano-2-phenylethanol using *Pseudomonas fluorescens* by the three-dimensional model,[6d] as shown in Fig. 2. However, in the resolution of compound **1** we obtained the opposite result as the (*S*)-enantiomer reacted faster. This result seems to obey the flat or non-flat hydrophobic pocket model.[6f] Thus, we suggest that this difference results from the molecular structure of the compound **1** as follows: (i) the reactive site is far from the stereogenic center (three bonds), (ii) the stereogenic carbon is quaternary, not secondary.

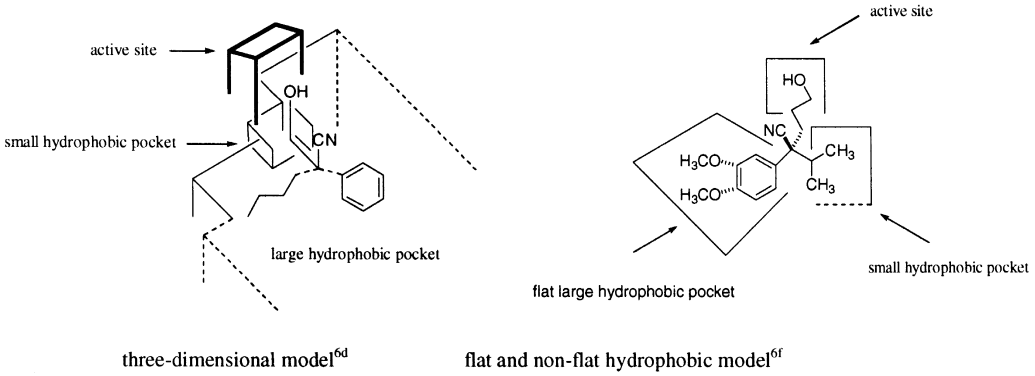


Figure 2. Two models suggested by the experimental results

Next, we tried to establish reaction conditions to increase the stereoselectivity. To do this, such factors as temperature effects,[10] water level,[11] solvent effect,[12] variation of the co-substrate and the additive[13] such as bases and metal salts in the irreversible transesterification were studied. We chose the two variables, solvent and additive. At first, we investigated the proper choice of the solvent. The effect of the solvent on substrate specificity and enantioselectivity of enzymes was well studied. In the enzyme reaction under the organic medium, when a substrate interacts with the enzyme's active center, water must be excluded.[14] Therefore, this phenomenon will affect reaction rate and reaction specificity in relation to solvent polarity.

As shown in Table 2, using ethyl acetate, 1,4-dioxane and *n*-hexane, the reaction proceeded smoothly within 10 h and the selectivities (*E*) were between 4.9 and 8.3. In polar solvents such as methanol, dichloroethane and acetonitrile, the reaction rate was slow and, especially in dichloroethane and methanol, the reaction did not proceed over 70 h. Among the above solvents, *n*-hexane as a non-polar solvent showed good results. In the case of diisopropyl ether, unreacted alcohol *R*-**1** was resolved in 99% ee. When *n*-hexane:ethyl acetate as co-solvent was used, the reaction rate increased and the selectivity (*E*) was 13. We assumed that this resulted from the solubility of the alcohol **1**. Thus, we chose the co-solvent system to increase the stereoselectivity in the range of 10–50% ethyl acetate:*n*-hexane solution. Finally, we studied the effect of the additives on the stereoselectivity. These results are shown in Table 3.

One of the additive effects on stereoselectivity is deactivation of the enzyme. In the irreversible reaction using an acyl-donor, the acetaldehyde seems to be detrimental to the reaction rate and stereospecificity.[13] The mechanism of enzyme deactivation by aldehydes involves Schiff-base formation on lysine residues. Another additive effect is modification of the lipase local conformation as mentioned by Itoh et al.[15] These two aspects would explain the results as shown in Table 3. Although *Pseudomonas* lipases are less sensitive to aldehyde than the other lipases,[13] we investigated the aldehyde effect on LAK in the above reaction. Among the various additives, pyridine and potassium carbonate gave the (*R*)-enantiomer in up to 99% ee. This result is independent of solubility. In particular, it is interesting that molecular sieves also gave pure enantiomer. They are assumed to act by adsorption of the aldehyde or capturing water

Table 2
Solvent effects on the resolution of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1** using *Pseudomonas fluorescens*

Solvent	Reaction time (h)	Conversion (%)	(*R*)-Alcohol ee (%)	(*S*)-Acetate ee (%)	*E*
Benzene	24	17	14	66	2.3
Acetonitrile	50	6	6	75	7.4
Ethyl acetate	10	36	44	66	4.9
Tetrahydrofurane	52	6	4	76	7.6
Dichloroethane	70	0	-	-	-
1,4-dioxane	1.5	50	40	63	6.5
Methanol	70	0	-	-	-
n-Hexane	1.5	37	40	70	8.3
n-Hexane/ethyl acetae (9/1)	1.5	44	58	76	13
Diisopropyl ether	2	70	99	44	11

Reaction conditions : the enzyme and acyl donor were used mass equivalent to the substrate and mixtures were stirred at 32 ~ 34 °C for given time.

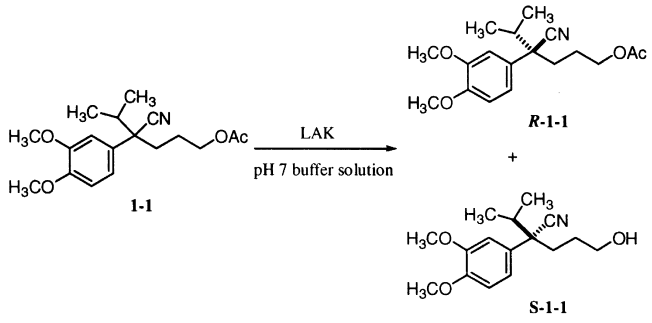
Table 3
Additive effects on the resolution of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1** using *Pseudomonas fluorescens*

Additives	Reaction Time (h)	Conversion (%)	(*R*)-Alcohol ee (%)	(*S*)-Acetate ee (%)	*E*
Triethyl amine(35μl)	3	30	37	82	14
Pyridine(35μl)	3	66	>99	52	15
Piperidine(35μl)	3	20	20	78	9.8
Piperazine(18mg)	3	14	14	76	8.4
Diisopropyl amine(35μl)	4	48	64	76	14
Imidazole(15mg)	21	30	12	52	3.6
Potassium carbonate(15mg)	2	67	>99	52	15
Lithium chloride(2.5M, 35μl)	25	7	6	32	2.1
Molecular sieve 4 Å (35mg)	2	63	>98	60	17
Water(35μl)	21	10	8	84	12
Sodium acetate(15mg)	2	45	62	74	12
18-Crown-6(10mg)[a]	1.5	70	88	89	49

Reaction conditions : the enzyme and acyl donor were used mass equivalent to the substrate and mixtures were stirred at 32 ~ 34 °C for given time. [a]used 2ml *n*-hexane as solvent.

molecules. As additive, when 18-crown-6 was used, the stereoselectivity (*E*) was best at 49. Additionally, the acetate **1-1** was hydrolysed in buffer solution using various enzymes, as shown in Scheme 3.

From screening enzymes for the resolution of **1-1**, *Pseudomonas fluorescens* was best and the fast reacting enantiomer was the (*S*) one. Its enantiomeric purity was up to 86% at 26% conversion when the enzyme was used in mass equivalent to the substrate **1-1**. When the second resolution of (*S*)-enantiomer was performed, its purity rose to 99%.



Scheme 3.

3. Conclusions

We resolved the (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1** to the (*S*)- and (*R*)-enantiomers using *Pseudomonas fluorescens* (LAK) among the various lipases. These enantiomers will be used to synthesize enantiopure (*S*)- and (*R*)-verapamil. This resolving efficiency on primary alcohol containing a quaternary stereogenic carbon can be extended to other similar structures and we will continue to study the reason for the discrimination in such a molecule.

4. Experimental

4.1. General

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer with TMS as internal reference. IR spectra were recorded on a MIDAC 101025 FT-IR spectrometer and optical rotation was measured on an Autopol® III polarimeter (Rudolph Research Co.). Low EI resolution mass spectra were determined on an HP GC 5972 and HP MS 5988A system at 70 eV. High pressure liquid chromatography for the determination of enantiomeric purity was performed on a Varian 9010 system with Varian 9050 UV detector at 254 nm and Chiralcel OD. The pH stat used was Metrohm 718 STAT Titrino with Metrohm electrode and Metrohm 722 stirrer. All reactions were carried out under an atmosphere of nitrogen or argon. Column chromatography was performed on Merck silica gel 60 (230–400 mesh). TLC was carried out using glass sheets pre-coated with silica gel 60 F_{254} prepared by E. Merck. All the commercially available reagent chemicals were obtained from Aldrich, Fluka and Tokyo Kasei Chemical Company and generally used without further purification. Solvents were distilled over appropriate drying materials before use.

4.2. Synthesis of 2-(3,4-dimethoxyphenyl)-3-methylbutyronitrile **2**

To a stirred suspension of sodium hydride (2.26 g, 54.6 mmol, 60% dispersion in mineral oil) in dried DMF (70 ml) was added dropwise (3,4-dimethoxyphenyl)acetonitrile (9.16 g, 51.7 mmol) in DMF (10 ml) over a period of 30 min at 0°C. After the mixture was stirred for another 30 min, 2-bromopropane (5.78 g, 47.0 mmol) was added at 15°C. The mixture was stirred at room temperature for 15 h. The reaction medium was quenched with ice cold water (200 ml) and extracted with diethyl ether (3×50 ml). The combined organic extracts were washed with saturated $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated to furnish 14 g of crude 2-(3,4-dimethoxyphenyl)-3-methylbutyronitrile **2**. Silica gel

column chromatography (*n*-hexane:ethyl acetate 10:1) provided the compound **2** (7.08 g, 69%) as clear liquid. Compound **2**: GC/MSD (m/e) 51, 63, 76, 90, 103, 115, 131, 146, 162, 176 (100), 189, 203, 219 (m^+); ^{1}H NMR (300 MHz, $CDCl_3$, ppm) 1.04 (d, J=6.7 Hz, 6H, -CH_3×2), 2.07–2.14 (m, 1H), 3.88 (s, 3H, -OCH_3), 3.90 (s, 3H, -OCH_3), 6.79–6.84 (m, 3H, aromatic CH).

4.3. Synthesis of (RS)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropylbutanol *1*

3-Tetrahydropyranyloxypropyl bromide was prepared from the commercially available 3-bromo-1-propanol (6.95 g, 50 mmol), 3,4-dihydro-2H-pyran (5.04 g, 5.47 ml, 60 mmol) and pyridinium *p*-toluene sulfonate as catalyst in CH_2Cl_2 (50 ml) for 3 h at room temperature. A solution was diluted with diethyl ether (50 ml), washed once with half-satd brine to remove the catalyst and concentrated to furnish 12.0 g of the crude compound 3-tetrahydropyranyloxypropyl bromide. Silica gel column chromatography (*n*-hexane:ethyl acetate 20:1) provided 10.5 g (94%) as clear liquid. GC/MSD (m/e) 223 (m^+), 196, 167, 151, 137, 123, 107, 93, 85 (100), 67, 56; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) 1.15–1.63 (m, 6H), 2.02–2.10 (m, 2H), 3.40–3.48 (m, 4H, -OCH_2×2), 3.76–3.81 (m, 2H), 4.53 (t, J=3.0 and 3.7 Hz, 1H, chiral CH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 19.8, 25.7, 30.9, 30.9, 33.2, 62.5, 65.1, 99.1.

To a stirred suspension of sodium hydride (1.20 g, 25.0 mmol, 60% dispersion in mineral oil) in dried DMF (50 ml) was added dropwise the compound **2** (5.48 g, 25.0 mmol) in DMF (5 ml) at 0°C. After the mixture was stirred for 30 min, the above 3-tetrahydropyranyloxypropyl bromide (5.5 g, 25 mmol) was added at 15°C. The mixture was stirred at room temperature for 15 h. The reaction medium was quenched with ice cold water (200 ml) and extracted with diethyl ether (3×50 ml). The combined organic extracts were washed with saturated $NaHCO_3$, brine and H_2O, dried over $MgSO_4$ and concentrated to furnish 11 g of the crude compound **3**. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) 0.79 (d, J=6.7 Hz, 3H, -CH_3), 1.17 (d, J=6.6 Hz, 3H, -CH_3), 1.19–2.09 (m, 11H), 3.36–3.86 (m, 4H), 3.86 (s, 3H, -OCH_3), 3.88 (s, 3H, -OCH_3), 4.42–4.55 (m, 1H, chiral CH), 6.81–6.99 (m, 3H, aromatic CH).

To a solution of crude (*RS*)-**3** (11 g) in methanol (10 ml) was added 1N methanolic HCl (20 ml) at room temperature and stirred for 3 h. After evaporation of methanol, the residue was neutralized with satd aqueous $NaHCO_3$ and extracted with diethyl ether (3×50 ml). The combined organic extracts were washed with satd $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated to furnish 8.1 g of the crude (*RS*)-**1**. Silica gel column chromatography (*n*-hexane:ethyl acetate 10:1) provided 5.80 g (84%) as a clear liquid. Compound (*RS*)-**1**: GC/MSD (m/e) 277 (m^+), 234, 216 (100), 189, 185, 170, 146, 138, 115, 103, 77, 65, 51; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) 0.81 (d, J=6.8 Hz, 3H, -CH_3), 1.20 (d, J=6.6 Hz, 3H, -CH_3), 1.22–1.30 (m, 1H), 1.32 (s, 1H, -OH), 1.55–1.69 (m, 1H), 1.87–1.99 (m, 1H), 2.05–2.16 (m, 1H), 2.18–2.30 (m, 1H), 3.40–3.44 (m, 2H, -CH_2OH), 3.87 (s, 3H, -OCH_3), 3.89 (s, 3H, -OCH_3), 6.79–6.84 (m, 3H, aromatic CH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 18.8, 19.2, 29.1, 34.5, 38.1, 53.5, 56.1, 56.3, 62.2, 109.8, 111.4, 119.1, 121.8, 130.7, 148.5, 149.3; IR (neat, cm^{-1}) 3520 (OH), 3150 (aromatic CH), 2964 (aliphatic CH), 2234 (CN), 1592, 1520, 1465, 1456, 1375, 1258, 1152, 1026, 806, 768; HPLC analysis [column: Chiralcel OD (cellulose carbamate derivative), eluent: *n*-hexane:IPA 9:1, flow rate: 1.0 ml/min; detector: UV 254 nm]; retention time (min): 18.30 for (*R*)-enantiomer and 32.46 for (*S*)-enantiomer.

4.4. Synthesis of (RS)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropylbutyl acetate *1*-*1*

To a stirred solution of alcohol (±)-**1** (1.7 g, 6.14 mmol) in dried CH_2Cl_2 (10 ml) was added 4-*N*,*N*-dimethylaminopyridine (100 mg), pyridine (1.5 ml, 18.4 mmol) and acetic anhydride (1.74 ml, 18.4 mmol) at 0°C. After the mixture was stirred for 3 h at room temperature, the reaction mixture was poured

into cold 10% aq. hydrochloride and extracted with diethyl ether (3×30 ml). The combined organic extracts were washed with saturated $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated to furnish the compound **1-1** as crude 4 g. Silica gel column chromatography (*n*-hexane:ethyl acetate 4:1) provided the pure compound **1-1** as a pure liquid (1.80 g, 92%). Compound **1-1**: GC/MSD (m/e) 319 (m^+), 277, 258, 235, 216 (100), 189, 174, 138, 128, 115, 91, 77, 51; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) 0.81 (d, J=6.8 Hz, 3H, -CH_3), 1.20 (d, J=5.0 Hz, 3H, -CH_3), 1.29–1.33 (m, 1H), 1.65–1.79 (m, 1H), 1.80–1.91 (m, 1H), 2.03 (s, 3H, -$COCH_3$), 2.06–2.13 (m, 1H), 2.14–2.25 (m, 1H), 3.89 (s, 3H, -OCH_3), 3.91 (s, 3H, -OCH_3), 4.00 (t, J=6.3 Hz, -CH_2CO-), 6.85–6.91 (m, 3H, aromatic CH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 18.9, 19.3, 21.2, 25.4, 34.8, 38.2, 53.5, 56.2, 56.3, 64.1, 109.8, 111.5, 119.0, 121.4, 130.5, 148.7, 149.4, 171.3; IR (neat, cm^{-1}) 3150 (aromatic CH), 2968 (aliphatic CH), 2234 (CN), 1732 (C=O), 1592, 1520, 1465, 1456, 1375, 1258, 806, 768, 636; HPLC analysis [column: Chiralcel OD (cellulose carbamate derivative); eluent: *n*-hexane:IPA 9:1; flow rate: 1.0 ml/min; detector: UV 254 nm]; retention time (min): 13.34 for (*R*)-isomer and 15.00 (*S*)-isomer.

*4.5. Enzymatic esterfication of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol **1***

To a stirred solution of alcohol (*RS*)-**1** (100 mg, 0.36 mmol) in dried *n*-hexane:ethyl acetate (9:1, 10 ml) was added the enzyme (mass equivalent to substrate) and vinyl acetate (31 mg, 0.36 mmol) at 32–34°C. When the conversion of the reaction reached around 44%, the enzyme was removed by filtration and washed with diethyl ether. The combined organic layer was concentrated to afford an oily residue, which was chromatographed on silica gel column with *n*-hexane:ethyl acetate (10:1) system to give the corresponding ester (*S*)-**1** and unreacted (*R*)-**1**. The isolated (*S*)-acetate was hydrolysed with 1.2N methanolic KOH to afford (*S*)-**1**. The enantiomeric excess of (*S*)-**1** was 76% while that of (*R*)-**1** was 58%.

When each of the resolved acetate (*S*)-**1** and the unreacted alcohol (*R*)-**1** was re-resolved under the same conditions at the 40% and 50% conversions, their enantiomeric excesses were 96.6 and >99%, respectively. (*R*)-(+)-**1**: $[\alpha]_D^{29}$ +16.92 (c=1.77, $CHCl_3$); >99% ee; (*S*)-(−)-**1**: $[\alpha]_D^{29}$ −16.26 (c=1.15, $CHCl_3$); 96.6% ee.

*4.6. Enzymatic hydrolysis of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropylbutyl acetate **1-1***

To a stirred suspension of acetate (*RS*)-**1-1** (100 mg, 0.31 mmol) in phosphate buffer (8 ml, pH 7) was added the enzyme (mass equivalent to substrate) at room temperature. The pH of the reaction was kept at 7.0 by 0.01N aqueous NaOH via a syringe pump interfaced with a pH controller. When the conversion of the reaction was 26%, the reaction mixture was extracted with ethyl acetate (20 ml×2). The combined organic layer was concentrated to afford an oily residue, which was chromatographed on silica gel column with *n*-hexane:ethyl acetate (10:1) system to give the corresponding alcohol (*S*)-**1-1** and unreacted acetate (*R*)-(+)-**1-1**. The isolated (*R*)-**1-1** was hydrolysed with 1.2N methanolic KOH to afford the alcohol. The enantiomeric excesses of the alcohol and acetate were 86% and 44%, respectively. When the acetate of the reacted alcohol (*S*)-**1-1** was hydrolysed again under the same conditions, its optical purity was up to 99%.

Acknowledgements

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