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Enantioselective resolution of methyl 2-chloromandelate by *Candida antarctica* lipase A in a solvent-free transesterification reaction

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Abstract

Enantiomerically pure 2-chloromandelic acid esters are important chiral building blocks for synthesis of a wide range of pharmaceutical products, such as an anti-thrombotic agent, (*S*)-clopidogrel. An efficient and novel process for resolution of methyl 2-chloromandelate was developed by using a lipase-mediated transesterification. Among 11 hydrolytic enzymes examined, *Candida antarctica* lipase A (CAL-A) showed the highest enantioselectivity and reaction rate toward methyl (*S*)-**2**-chloromandelate. Methyl (*R*)-**2**-chloromandelate was obtained in enantiomerically pure form (>99% ee) and 41% yield through the lipase-mediated resolution under a solvent-free condition. CAL-A maintained its catalytic activity during 13 cycles of repeated use without significant decrease in enantioselectivity, indicating that the method is economical and easy to scale-up for commercial production of methyl (*R*)-**2**-chloromandelate.

Keywords: *Candida antarctica* lipase A; Methyl (*R*)-**2**-chloromandelate; Transesterification; Solvent-free reaction; Vinyl propionate

1. Introduction

Enantiomerically pure 2-hydroxycarboxylic acids are currently being recognized as useful chiral intermediates and building blocks in pharmaceutical industry [1–3]. Among them, (*R*)-**2**-chloromandelic acid is the most preferred chiral building block for the industrial synthesis of the anti-thrombotic agent, (*S*)-clopidogrel, commercialized as a brand name, Plavix (Scheme 1) [2].

Previously, resolutions of (*R*)-**2**-chloromandelic acid were performed by diastereomeric crystallization [4,5] and enantioselective enzymatic hydrolysis of the corresponding nitrile compounds [6–8] and ester compounds [9–12]. However, they have some drawbacks for commercial production. The diastereomeric crystallization needs expensive resolving agents and results in low yield. The microbiological process using nitrilase also results in low yield and low turnover frequency toward *ortho*-substituted mandelonitriles [6]. An alternative synthetic route using hydroxynitrile lyase has been reported for the production of (*R*)-**2**-chloromandelic acid with good yield and enantioselectivity [7,8]. This process, however, includes the use of highly toxic HCN gas, which could need additional equipment for large-scale production. In the enzymatic resolutions using lipases and esterases, the concentration of substrates was low due to the low solubilities of the substrates in aqueous solutions [9,10]. This problem caused the low volumetric productivity of the reactors. There was a report for the preparation of (*S*)-mandelic acids by enantioselective degradation of racemates with an isolated *Pseudomonas putida* [13]. The microorganism showed high enantioselective activities on (*R*)-mandelic acid and its derivatives with *para*-substitution. However, either the enzymatic activity or the enantioselectivity was significantly decreased in case of *meta*- or *ortho*-substitutions. To overcome these problems, here, we employed a lipase-catalyzed reaction for the resolution of 2-chloromandelic acid. The lipase

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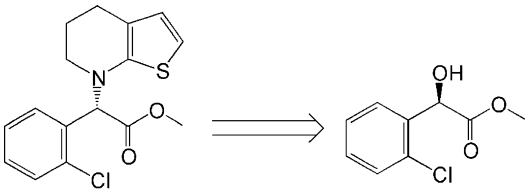


(S)-Clopidogrel (R)-1

Scheme 1. Retrosynthetic pathway for the synthesis of (*S*)-clopidogrel.

is a well-known biocatalyst for its high stability and activity in organic solvents [14,15]. However, no successful lipase-catalyzed resolution of methyl 2-chloromandelate has yet been reported, while there have been a couple of reports on the resolutions of mandelic acid and its derivatives [16–18]. In this report, we demonstrated a practical synthesis of optically active methyl (*R*)-**2**-chloromandelate (*R*)-**1** (>99% ee), using lipase A from *Candida antarctica* (CAL-A) under a solvent-free condition.

2. Experimental

2.1. Chemicals and enzymes

2-Chloromandelic acid, vinyl acetate, vinyl propionate, vinyl benzoate, vinyl decanoate, vinyl pivalate, vinyl neodecanoate, isoprenyl acetate, acetone, hexane, ethyl acetate, diethyl ether, 1,4-dioxane, toluene and thionyl chloride were purchased from Aldrich (Milwaukee, USA). All these solvents were used without further purification. Other chemicals used were of analytical reagent grade. Immobilized lipase B from *C. antarctica* (Chirazyme L2 on carrier; 6.3 unit/mg), immobilized lipase A from *C. antarctica* (Chirazyme L5 on carrier; 2.8 unit/mg), immobilized lipase from *Rhizomucor miehei* (Chirazyme L9 on carrier; 24.9 unit/g), immobilized lipase from *Candida rugosa* (Chirazyme L3 on carrier; 0.592 unit/mg) were donated by Roche Diagnostics (Penzberg, Germany). The protease from *Bacillus subtilis* (2.5 AU/g) was gifted by Novozymes A/S (Bagsvaerd, Denmark). Lipases from *C. rugosa* (400 unit/mg), porcine pancreas (30 unit/mg), *Mucor javanicus* (300 unit/mg) and *Pseudomonas* sp. (25 unit/mg) were purchased from Sigma (St. Louis, USA). Lipases from *Pseudomonas stutzeri* (Lipase TL; 30 unit/mg) and *Pseudomonas cepacia* (Lipase SL; 60 unit/mg) were gifted by Meito Sangyo Co., Ltd. (Nagoya, Japan).

2.2. HPLC analysis

HPLC analysis was carried out with JASCO 880-PU instrument equipped with UV detector using Chiralcel ODH column for enantiomeric excess (250 mm × 4.6 mm, *n*-hexane/*i*-PrOH = 90/10, 0.5 mL/min). The conversion was calculated from the equation $c = ee_s/(ee_s + ee_p)$, where ee_s and ee_p represent the enantiomeric excess of the starting substrate ester and the diester product, respectively. Enantioselectivity (*E*) was also calculated by using the equation $E = \ln\{(1-c)(1-ee_s)\}/\ln\{(1-c)(1+ee_s)\}$ [19]. The retention times of the two pairs of enantiomers **1**, **2** are shown in parentheses (min): (*R*)-**1** (16.9), (*S*)-**1** (15.1), (*R*)-**2** (9.8) and (*S*)-**2** (9.1).

2.3. Preparation of methyl (RS)-2-chloromandelate

2-Chloromandelic acid (18.7 g, 0.1 mol) was dissolved in methanol (90 g). Thionyl chloride (13.5 g, 0.1 mol) was added dropwise at 0 °C for 10 min and the reaction mixture was refluxed for 4 h. After checking that the reaction was finished on TLC, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed twice with saturated sodium bicarbonate solution and then with water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give a pale yellow oil, methyl 2-chloromandelate (*RS*)-**1** (19.3 g, 96.2% yield).

2.4. Enzymatic transesterification of methyl (RS)-2-chloromandelate

In screening of enzyme, a solution of (*RS*)-**1** (40 mg) and vinyl propionate (120 mg) in toluene (0.4 mL) was stirred with an enzyme (3 mg) in a 1.5 mL screw-capped vial at 30 °C. Samples (30 μL) were withdrawn after 3 h and analyzed by HPLC.

In experiments to test the effect of acyl donors, a solution of (*RS*)-**1** (40 mg) and each activated ester (120 mg) in toluene (0.4 mL) was stirred with an enzyme (3 mg) in a 1.5 mL screw-capped vial at 30 °C. Samples (30 μL) were withdrawn after 4 h and analyzed by HPLC.

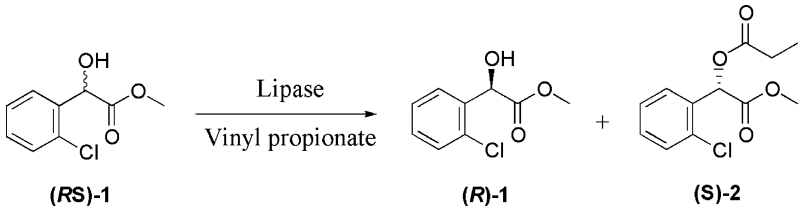
In experiments to test the effect of organic solvents, a solution of (*RS*)-**1** (40 mg) and vinyl propionate (120 mg) in each organic solvent (0.4 mL) was stirred with CAL-A (3 mg) in a 1.5 mL screw-capped vial at 30 °C. Samples (30 μL) were withdrawn after 3 h and analyzed by HPLC. The water contents of the organic solvents were in the range of 0.05–0.4%. The control reaction was carried out with a large excess of vinyl propionate at the same reaction condition.

2.5. Large-scale synthesis of methyl (R)-2-chloromandelate

CAL-A (4.8 g) was added to a solution of (*RS*)-**1** (500 g) and vinyl propionate (500 g). The resulting mixture was stirred at 30 °C for 18 h. The enzyme was filtered off and then the filtrate was concentrated. The residual oil was subjected to flash chromatography (hexane/ethyl acetate = 10/1) to give (*R*)-**1** as colorless oil (205 g, 41% yield); 99.3% ee by HPLC.

2.6. Recycling of immobilized CAL-A

The reaction was carried out in a 20 mL vial containing (*RS*)-**1** (1 g), vinyl propionate (1 g) and CAL-A (100 mg). The capped vial was incubated at 30 °C. The conversion was analyzed by HPLC. At the end of each cycle, the reaction mixture was removed by suction and then the enzyme was washed with 5 mL toluene to remove residual chemicals. The enzyme recovered was stored at 4 °C in toluene.



Scheme 2. Resolution of (*RS*)-**1** by enzymatic transesterification with vinyl propionate.

3. Results and discussion

For kinetic resolution of 2-chloromandelic acid esters, 10 different lipases and 1 protease among commercially available hydrolytic enzymes were tested for stereoselective transesterification activity toward methyl 2-chloromandelate (*RS*)-**1** with vinyl propionate (Scheme 2). The enzyme reactions were performed by incubating a racemic substrate and vinyl propionate with various enzymes at 30 °C in toluene. The toluene was used as a solvent due to its unreactivity in the reaction condition. The enantiomeric excess of the remaining ester substrate and the diester product was determined by HPLC on a Chiralcel ODH column. Of 11 hydrolytic enzymes, the lipase A from *C. antarctica* immobilized on carrier exhibited the highest conversion rate and enantioselectivity (Table 1). Other enzymes had, if any, very weak activities. Therefore, CAL-A was selected for further optimization reaction. In the final reaction mixtures, 2-chloromandelic acid, a possible hydrolytic product, was not detected.

It is generally known that the acyl group of acylating agents can affect the reaction rate and enantioselectivity in a lipase-catalyzed transesterification [16,20,21]. To examine the effect of acyl donors, we carried out the transesterification reaction of (*RS*)-**1** with various vinyl esters. The results in Table 2 indicated that the reaction rate increased with the alkyl chain length of acyl donor increasing, while the *E* value gradually decreased with increasing the length of alkyl chain.

To test the steric effect of acyl donor in transesterification reaction, we subsequently examined vinyl esters containing bulky side chains, such as vinyl benzoate, vinyl pivalate and vinyl neodecanoate, which has an aromatic ring, a *t*-butyl group and an isomeric side chain of vinyl decanoate, respectively. When we performed the enzymatic transesterification reactions using these bulky vinyl esters as acyl donors, all the reactions were completely inhibited presumably due to steric effects of acyl donors (Table 2). This result suggests that the steric effect is more crucial than alkyl chain length of the acyl donors in the lipase-catalyzed transesterification reaction. Previously, it was reported that the chain length of the fatty acid moiety of the enol esters significantly affected the conversion rate in *Pseudomonas* sp. lipase-catalyzed acylation of methyl (*RS*)-mandelate [16]; vinyl esters carrying a shorter alkyl chain served as better acyl donors than those carrying a longer alkyl chain. However, in CAL-A-catalyzed acylation in this paper, the conversion rate increased with the alkyl chain length [vinyl acetate (C2): 44.8%, vinyl propionate (C3): 62.9%, vinyl decanoate (C10): 71.0%], whereas enantioselectivity decreased [vinyl acetate (C2): 18.5, vinyl propionate (C3): 12.2, vinyl decanoate (C10): 5.1].

To circumvent the possible inhibitory effect of acetaldehyde, which is released in the transesterification resolution with vinyl acetate as an acyl donor, we used isoprenyl acetate instead of vinyl acetate. However, the use of isoprenyl acetate resulted in lower conversion and enantioselectivity, suggesting that isoprenyl acetate is not an appropriate acyl donor for this resolution system (Table 2). Since the nature of the solvent can influence catalytic activity and enantioselectivity in a lipase-catalyzed reaction [22–24], we investigated several commonly used organic solvents for the transesterification resolution of methyl 2-chloromandelate using CAL-A as a catalyst. The results shown in Table 3 indicated that the conversion rates in non-polar solvents, such as toluene, cyclohexane and hexane

Table 1
Transesterification of (*RS*)-**1** with vinyl propionate catalyzed by different enzymes

Enzyme	Conversion (%)	ee_s[a] (%)	*E*
Immobilized *C. antarctica* lipase B	0	–	–
Immobilized *C. rugosa* lipase	9.6	8.5	9.8
Immobilized *C. antarctica* lipase A	57.3	90.8	15.9
Immobilized *Rhizomucor mehei* lipase	0	–	–
C. rugosa lipase	11.6	7.9	4.3
Porcine pancreatic lipase	10.2	5.9	3.4
M. javanicus lipase	7.8	4.6	3.5
Pseudomonas sp. lipase	3.6	3.0	9.3
P. stutzeri lipase	19.8	18.5	8.4
P. cepacia lipase	16.2	6.0	2.0
B. subtilis protease	0	–	–

All the reactions were conducted at least in triplicates at 30 °C for 3 h in toluene. The mean values are listed in the table and the standard deviations are generally 8% or less.
[a] The (*R*)-enantiomer was preferred in all cases.

Table 2
Effect of acyl donors on the transesterification of (*RS*)-**1** catalyzed by CAL-A

Acylating agent	Conversion (%)	ee_s[a] (%)	*E*
Vinyl acetate	44.8	65.7	18.5
Isoprenyl acetate	26.0	26.9	9.7
Vinyl propionate	62.9	94.8	12.2
Vinyl decanoate	71.0	85.3	5.1
Vinyl neodecanoate	n.d.	2.7	n.d.[b]
Vinyl benzoate	n.d.	2.0	n.d.
Vinyl pivalate	n.d.	1.2	n.d.

All the reactions were conducted at least in triplicates at 30 °C for 4 h in toluene. The mean values are listed in the table and the standard deviations are generally 8% or less.
[a] The (*R*)-enantiomer was preferred in all cases.
[b] n.d.: not determined since only one enantiomer of the ester could be detected due to low conversion.

Table 3
Effect of organic solvents on the transesterification of (*RS*)-**1** with vinyl propionate catalyzed by CAL-A

Solvent	Conversion (%)	ee_s[a] (%)	*E*
1,4-Dioxane	17.4	17.6	13.5
Acetone	18.1	17.4	10.1
Ethyl acetate	32.9	44.4	31.8
Diethyl ether	41.3	58.6	19.7
Toluene	57.3	90.8	15.9
Cyclohexane	61.5	96.9	16.0
Hexane	51.9	76.4	13.2
Vinyl propionate	47.4	75.3	25.2

All the reactions were conducted at least in triplicates at 30 °C for 3 h. The mean values are listed in the table and the standard deviations are generally 8% or less.
[a] The (*R*)-enantiomer was preferred in all cases.

were higher than those in polar solvents, such as 1,4-dioxane and acetone. However, there was no exact correlation between the *E* value and the polarity parameter (log *P* value) of the solvent in this reaction system. When the reaction was performed with a large excess of the vinyl propionate to make a solvent-free condition, the high enantioselectivity ($E = 25.2$) and good conversion rate were observed (Table 3). We chose the solvent-free system as an optimal condition for the resolution of (*RS*)-**1** since the high conversions in non-polar solvents resulted in decrease of the *E* values.

Considering all the results in terms of conversion rate, ee and *E* value, vinyl propionate was selected as not only an effective acyl donor, but also a good solvent for the transesterification resolution of (*RS*)-**1**.

Eliminating the need for using organic solvents is highly attractive for an industrial process since it can increase the volumetric productivity of the reactor, which results in higher space–time yields and lower investments in equipment and it can also prevent waste during chemical processes. To test if the solvent-free reaction described above can be applied to an industrial synthesis, a CAL-A-mediated large-scale reaction was carried out under the solvent-free condition. CAL-A (4.8 g) was added to a solution of (*RS*)-**1** (500 g) and vinyl propionate (500 g) and the reaction mixture was incubated at 30 °C. Within 3 h, the conversion rate and ee value reached 47 and 73%, respectively (Fig. 1), which are similar to those obtained previously in the small-scale reaction (Table 3). After 18 h reaction, enantiomerically pure (*R*)-**1** (99.3% ee) was obtained in a good yield (41%) and a high enantioselectivity ($E = 34.7$). These results showed that the scale-up process did not change the enantioselective nature of CAL-A enzyme toward (*RS*)-**1**.

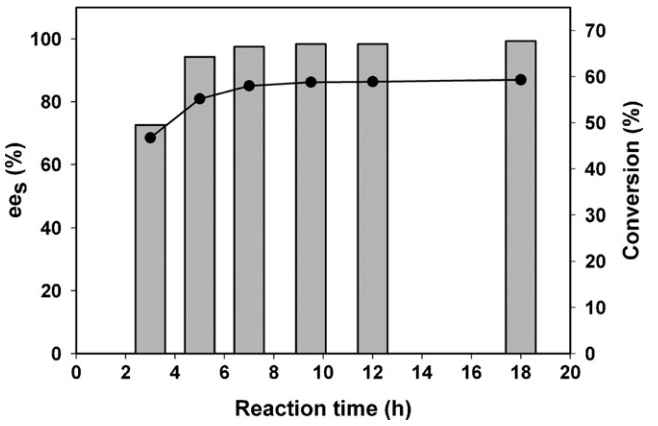


Fig. 1. Large-scale synthesis of methyl (*R*)-**2**-chloromandelate. Transesterification of (*RS*)-**1** (500 g) with vinyl propionate (500 g) was performed using CAL-A (4.8 g) as a catalyst at 30 °C for 18 h. Grey bars mean ee_s values and open circles (◯) mean conversion percent.

One of the main advantages of immobilized lipases is their easy recovery for reuse, which lowers the production cost. To take advantage of this, the transesterification reaction catalyzed by CAL-A was performed repeatedly. Interestingly, CAL-A maintained its catalytic activity during 13 cycles without any significant decrease in reaction rate and enantioselectivity (Fig. 2). Taking all these results together, the CAL-A-catalyzed resolution described in this paper could be an efficient method for an industrial synthesis of methyl (*R*)-**2**-chloromandelate.

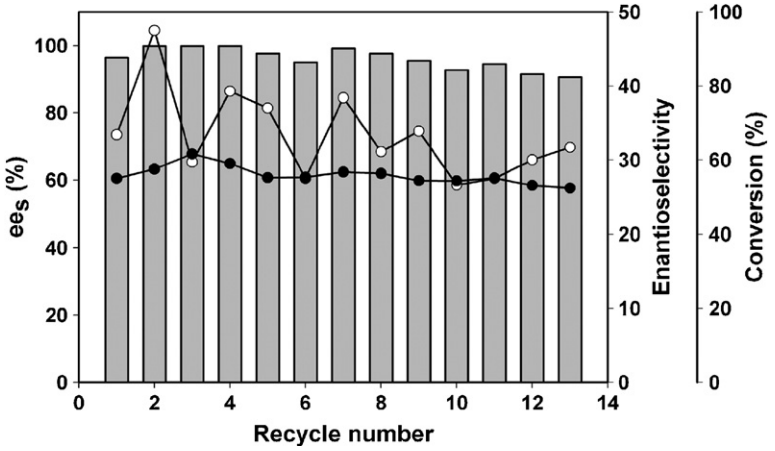


Fig. 2. Recycling of CAL-A in the transesterification reaction of (*RS*)-**1** with vinyl propionate. Transesterification was carried out repeatedly in a vial containing (*RS*)-**1**, vinyl propionate and CAL-A. At each cycle, the enzyme was washed and reused. Grey bars mean ee_s. Open circles (◯) and closed circles (●) mean enantioselectivity and conversion percent, respectively.

4. Conclusion

We have developed an efficient solvent-free enzymatic process for methyl (*R*)-**2**-chloromandelate, the key intermediate of clopidogrel, through the CAL-A-catalyzed transesterification reaction using vinyl propionate as an acyl donor. Under the optimized condition, enantiomerically pure methyl (*R*)-**2**-chloromandelate (>99% ee) was obtained in good yield (41%) and high enantioselectivity ($E = 34.7$). Furthermore, the immobilized CAL-A can be reused 13 times without any significant loss of enantioselectivity and reactivity. The methods described here is economical and easy to scale-up for commercial production of methyl (*R*)-**2**-chloromandelate.

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