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An efficient enzymatic aminolysis for kinetic resolution of aromatic α-hydroxyl acid in non-aqueous media

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ABSTRACT

A new and highly efficient enzymatic aminolysis approach for kinetic resolution of aromatic α-hydroxy acid in non-aqueous media has been developed. The corresponding α-hydroxyl acid ester was employed as the substrate, and commercially available *Candida antarctica* lipase B is used as the biocatalyst, anhydrous ammonia is the resolving agent. Reactions can be proceeded smoothly in organic solvent at ambient temperatures. High concentration of substrate is allowed due to the application of organic media and the products are obtained in yields of up to 49% with ee values of up to 99%, and with *E* value of >300, representing an appealing and promising protocol for large-scale preparations.

The demand for enantiopure α-hydroxy acids keeps increasing as they are valuable building blocks, auxiliaries and some are used as resolving agents in chemical and pharmaceutical industry for high value added substances.[1] There has been a plethora of methods developed for the preparation of chiral enantiomer of α-hydroxyl acid, mandelic acid, and its related derivatives.[2] Of them, the employment of lipase for kinetic resolution (KR) or dynamic kinetic resolution (DKR) of racemic α-hydroxyl acid has attracted considerable attention due to high regioselectivities of lipases and usually mild reaction conditions required for displaying their best activities.[3] In most developed procedures with lipase as the biocatalyst, substrate alkyl mandelic acid ester and its derivative are usually involved, and till nowadays, most of the KR reactions are proceeded in aqueous solution via selective hydrolytic cleavage of an ester bond (formed by a carboxylic group of an acid with hydroxyl group of an mandelic acid) to achieve the purpose of resolution. Nevertheless, these developed methods usually suffer from low substrate solubility, and low ee values of the resulted product, and some even suffer from treatment of serious downstream salt water drainage.[4,5] Recently, a high enantioselective lipase *Pseudomonas stutzeri* LC2-8 has been characterized with high enantioselectivity and organic solvent tolerance by He and his co-workers,[1b] with which, the desired product (*R*)-*O*-acetyl mandelic acid was obtained with excellent ee value of 99% and yield of 49% using vinyl acetate as both solvent and the acyl donor (Scheme 1a). Nevertheless, in his work, only one substrate mandelic acid was tested and evaluated, no attempt was made for extension of possible substrate spectrum for *Pseudomonas stutzeri* LC2-8. And also, Yang and his co-workers[6] have developed a chemical hydroxylation approach using oppolzer's sultam as chiral auxiliary to obtain chiral amide derivatives in excellent diastereoselectivities, the products could be yielded with drs of up to >20:1.

Previously we have developed an enzymatic approach for kinetic resolution of (*R*,*S*)-1-phenylethanol employing lipase/esterase as the biocatalyst through aminolysis of the corresponding (*R*, *S*)-1-phenylethyl acetate (Scheme 1b) to obtain both enantiomers,[4] the desired product (*R*)-1-phenylethanol was afforded in yields of up to >49% with ee values of up to 99%. The aminolysis reaction itself proved to be a better and more reliable way to obtain high enantiopure (*R*)-1-phenylethanol compared with conventional biohydrolytic or esterification approaches. Due to high solubility of substrate in organic media than in aqueous solution and excellent enantioselectivities of lipase, we would like to extend the possible substrate spectrum, for example, mandelic acid and its derivatives in non-aqueous medium.

Herein, we present an efficient kinetic resolution of mandelic acid and its derivatives catalyzed by lipase through the way of

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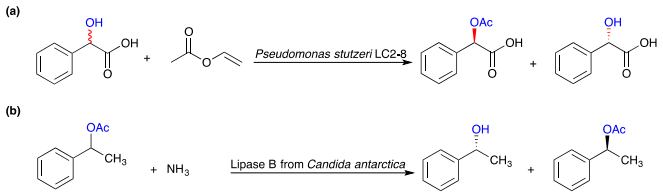
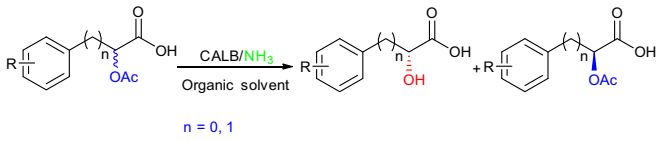


Scheme 1. Developed methods relating to KR of mandelic acid and aminolysis: (a) lipase *Pseudomonas stutzeri* LC2-8 catalyzed KR of mandelic acid; (b) lipase B from *Candida antarctica* catalyzed aminolysis for KR of phenethyl alcohol.



Scheme 2. Enzymatic aminolysis for kinetic resolution of mandelic acid ester and its derivatives.

aminolysis, ammonia is used here as the resolving reagent, and Lipase B from *Candida antarctica* (CALB) as the biocatalyst (Scheme 2). The reactions are proceeded in organic solvent, therefore, high concentrations of substrate are available as the substrates are more soluble in organic solvent than in aqueous solution, excellent ee values and high yields can be achieved. And also no downstream salt water (usually derived from buffer solution for biohydrolysis reactions) will be generated after reactions. Therefore, it is considered to meet some principles of green chemistry and therefore is more practical for large-scale preparations.

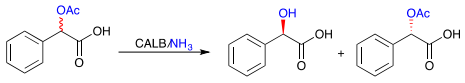
Initial investigations for suitable organic solvents were performed at a 5 mL scale using methyl 2-acet oxy-2-phenylacetic acid (R_1 = H, and R_2 = CH_3) as the model substrate, and CALB as the biocatalyst. A variety of organic solvents such as ethyl ester, *n*-hexane, *n*-octane, ethyl acetate, isopropyl ether, *iso*octane, and *cyclo*hexane were evaluated, and the results are summarized in Table 1. It was indicated that when reactions were proceeded in organic solvent isopropyl ether or isooctane, the desired product (*R*)-2-hydroxy-2-phenylacetic acid could be obtained in yields of up to 4% with ee values of up to 99%, and with *E* values of >300 and >200, respectively (Entry 2, 3, Table 1). Indicating that the above two organic solvents are well compatible with biocatalyst CALB when employed for aminolysis reactions using anhydrous ammonia. And in future investigations, all reactions will be processed in both isopropyl ether and isooctane.

Other reaction conditions such as temperatures and reaction times were also investigated. When enzymatic aminolysis reactions were proceeded in isopropyl ether at 25 °C, after 8 h, the desired product could be afforded in the yield of 43% (Entry 2, Table 1), and after 12 h, the product could be yielded in 49% (Entry 3, Table 1), indicating that a prolonged time will contribute positive effect on the conversion of substrate 2-acetoxy-2-phenylacetic acid (R_1 = H, and R_2 = CH_3) to the desired product (*R*)-2-hydroxy-2-phenylacetic acid. And when reactions were performed at higher temperatures, higher conversions of substrate could be achieved (Entry 6, 7, Table 1). Nevertheless, it is not the same with the resulted ee values, which decreased with increased reaction temperatures (Entry 3, 4, 8, Table 1), and the same happened to *E* values. Therefore, in future investigations, all reactions will be carried out at 25 °C, and reaction time 12 h.

With all the optimized conditions in hand, a variety of α-hydroxy acid esters were then subjected to the enzymatic aminolysis reactions, reactions were performed at 10 ml scale in isopropyl ether and isooctane, respectively. And the results are summarized in Table 2.

All reactions were proceeded smoothly in both isopropyl ether and *iso*octane. After reaction, the immobilized CALB can be easily removed simply by filtration, and the yielded products were condensed and purified. Results indicated that most of the ee values were obtained up to 99% with yields of up to 49% (Entry 1, 9, Table 2). The substituted group on aromatic ring can cause positive or negative effects on the conversions of substrates and ee values of the desired products, when *R* is H and –OCH_3, respectively, in the reaction time of 12 h applying isopropyl ether as the solvent, the conversions were 49% and 41% (Entry 1, 3, Table 2). Increased temperatures will cause negative effects on the resulted ee values, and also with *E* values, though conversions became higher (Entry 3, 4, 8, 9, Table 2). A higher concentration of racemic substrate was also tested, for a complete conversion of substrate, more loading of immobilized enzyme is needed, and the resulted product was afforded in 42% yield (isolated) and ee value of 99%. Compared with the chemical approach for chiral amides with good to excellent yields of 89%, this enzymatic aminolysis methodology can

Table 1
Investigation of reaction parameters using 2-acetoxy-2-phenylacetic acid as the model substrate[a]



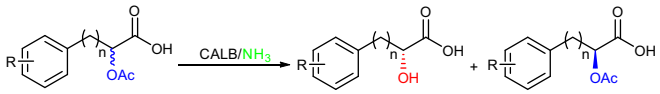
Entry	Solvent	Time (h)	*T* (°C)	Yield[b] (%)	ee[c] (%)	*E*[d] (%)
1	Ethyl acetate	15	25	32	93	12
2	Isopropyl ether	8	25	43	99	41
3	Isopropyl ether	12	25	49	99	>300
4	Isopropyl ether	6	35	41	97	27
5	Isopropyl ether	12	15	42	99	36
6	Ethyl ester	12	25	45	98	54
7	Ethyl ester	12	15	23	99	11
8	Isooctane	6	35	47	97	98
9	Isooctane	12	25	48	99	>200
10	Isooctane	15	15	29	99	14
11	*n*-Hexane	10	25	39	97	22
12	*n*-Hexane	15	15	26	98	11

[a] Conditions: Reactions were carried out on a 2 mL scale, immobilized *Candida antarctica* lipase B, 2 mg, organic solvent, 2 mL, substrate concentration, 20 mM. Except otherwise stated, ee values and conversions (conv) were determined by HPLC-analysis with a chiral OD-R column.

[b,c] Yields and ee values of mandelic acid were determined by HPLC analysis equipped with a Chiralcel OD-3R column (150 × 2.1, Daicel Chemical Industries, Ltd) at a wavelength of 254 nm at 30 °C.

[d] *E* values were calculated using the formula: $E = \ln[(1-c)(1-ee_p)]/\ln[(1-c)(1+ee_p)]$, $c = ee_s/(ee_s+ee_p)$.

Table 2
Results of aminolysis reactions[a]



Entry	Solvent	*R*	*n*	*T* (°C)	Yield[b] (%)	ee[c] (%)	*E*[d] (%)
1	Isopropyl ether	H	0	25	49	99	>300
2	Isopropyl ether	*o*-Cl	0	25	43	99	41
3	Isopropyl ether	*p*-OCH_3	0	25	41	99	32
4	Isopropyl ether	*p*-OCH_3	0	35	47	97	98
5	Isopropyl ether	H	1	25	40	99	29
6	Isooctane	H	0	25	48	99	>200
7	Isooctane	*o*-Cl	0	25	47	98	99
8	Isooctane	*p*-OCH_3	0	25	44	99	48
9	Isooctane	*p*-OCH_3	0	35	49	98	>200
10	Isooctane	H	1	25	45	99	58

[a] Conditions: Reactions were performed on a 10 mL scale, immobilized *Candida antarctica* lipase B, 2 mg, isopropyl ether/isooctane, 2 mL, substrate concentration, 20 mM. Except otherwise stated, ee values and conversions (conv) were determined by HPLC-analysis with a chiral OD column.
[b,c] Yields and ee values of aromatic α-hydroxyl acid were determined by HPLC analysis equipped with a chiral OD column (150 × 2.1, Daicel Chemical Industries, Ltd) at a wavelength of 254 nm at 30 °C.
[d] *E* values were calculated by the formula: $E = \ln[(1-c)(1-ee_p)]/\ln[(1-c)(1+ee_p)]$, $c = ee_s/(ee_s + ee_p)$.

provide an alternative to obtain chiral amides with ee values of up to 99%, though the yields cannot exceed 50%. Indicating that this approach can be applied for large-scale preparations especially for industrial purpose.

In summary, we have developed an efficient enzymatic approach for kinetic resolution of α-hydroxyl acid. The reaction can proceed in both isopropyl ether and *iso*octane smoothly at ambient temperatures, and anhydrous ammonia is employed as the separation reagent. Due to the application of organic solvent, the problems relating to low solubility of substrate are overcome as the substrate α-hydroxyl acids are more soluble in organic solvent than in aqueous solution. Simply by filtration, the biocatalyst (immobilized) can be removed. A scale-up reaction was also performed,[7] and the prove that this protocol is more suitable for large-scale preparations compared with conventional developed methods, representing an appealing and promising protocol for large-scale preparations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.10.054.

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7. *Scale-up kinetic resolution of mandelic acid ester:* a 250 ml round-bottom flask was charged with mandelic acid ester of 75 mmol, 150 mL isopropyl ether and immobilized lipase B from *Candida antarctica* 2 g, the flask was sealed and anhydrous ammonia was added through the surface of solution for about 3 h. The resulted mixture was stirred at 25 °C for another 9 h. Samples were taken at 1 h intervals, each time 20 μl of solution were used for analyzing. The separation procedure is the same as described in *Experimental section*. And the resulted crude residues were chromatographed on silica gel to afford the corresponding pure α-hydroxyl acid in an isolated yield of 41%.