Chemoenzymatic synthesis of the chiral herbicide: (*S*)-metolachlor

Liangyu Zheng, Suoqin Zhang, Fang Wang, Gui Gao, and Shugui Cao

Abstract: A chemoenzymatic approach for the production of (*S*)-metolachlor, one of the most widely used herbicides, has been developed. The starting material (*S*)-*N*-(2-ethyl-6-methylphenyl)alanine was obtained by the use of lipase-catalyzed hydrolytic kinetic resolution. Under the optimal conditions, the good activity and excellent enantioselectivity of lipase B from *Candida antarctica* (CAL-B, $E > 100$) are achieved in diethyl ether – water (15% *v*/*v*), which is about 9.7-fold more enantioselective than that in a pure buffered aqueous solution ($E = 12.1$). After a simple extraction procedure is used to separate the acid product from the remaining ester, the remaining ester is racemized, providing the basis for the continuous resolution process. Then (*S*)-metolachlor is synthesized by a simple chemical method using the enantiomerically pure (*S*)-acid.

Key words: (*S*)-metolachlor, herbicide, CAL-B, (*S*)-*N*-(2-ethyl-6-methylphenyl)alanine, resolution.

Résumé : On a mis au point une approche chimioenzymatique à la production du (*S*)-métolachlore, un des herbicides les plus utilisés. Le produit de départ, la (*S*)-*N*-(2-éthyl-6-méthylphényl)alanine, a été préparé en faisant appel à une résolution par hydrolyse cinétique catalysée par une lipase. Dans des conditions optimisées, la meilleure activité et l'excellente énantiosélectivité de la lipase B provenant de la *Candida antartica* (CAL-B, $E > 100$) sont obtenues en opérant dans un mélange d'éther éthylique et d'eau (15% *v*/*v*), une énantiosélectivité qui est environ 9,7 fois plus élevée que dans une solution aqueuse pure ($E = 12,1$). Après avoir utilisé une simple procédure d'extraction pour éliminer le produit acide et récupérer de l'ester résiduel, ce dernier peut être racémisé et servir dans un processus continue de résolution. La synthèse du (*S*)-métolachlore est ensuite complétée par une méthode chimique simple en utilisant l'acide (*S*) énantiomériquement pur.

Mots clés : (*S*)-métolachlore, herbicide, CAL-B, (*S*)-*N*-(2-éthyl-6-méthylphényl)alanine, dédoublement.

[Traduit par la Rédaction]

Introduction

As optically pure single enantiomers are often more specific targets and have fewer side effects than their corresponding racemates, there is a real driving force for the production of new drugs and agrochemicals with high optical purity (1). The herbicide metolachlor (*N*-(1′-methyl-2′-methoxyethyl)-*N*-chloroacetyl-2-ethyl-6-methylaniline) has been widely used for over 20 years with a main strength in the control of weeds following pre-emergence application. In addition, weed resistance has not been developed, making metolachlor an important option in weed-management strategies (2). Metolachlor comprises four stereoisomers, the isomerism of which is based on a combination of a chiral center in the aliphatic side chain and a chiral axis between the phenyl group and the nitrogen atom. Although the four stereoisomers differ in their ability to inhibit the growth of weeds, it is reported that the herbicidal activity is mainly influenced by the chiral center, the (*S*)-enantiomers (Fig. 1) providing higher herbicidal activity than the (*R*)-enantiomers (3). Consequently, it is recognized that enriching the isomeric ratio in favor of the (*S*)-enantiomer will increase the biological activity of the herbicide. Thus, providing excellent weed control at rates substantially lower than those for racemic metolachlor and showing a reduced risk of environmental contamination. Therefore, it is necessary to develop a new catalyst system to allow the production of enantiomercially enriched (*S*)-metolachlor on a commercial scale.

(*S*)-*N*-(2-ethyl-6-methylphenyl)alanine ((*S*)-**2**) is an important precursor in the synthesis of (*S*)-metolachlor, which is currently produced by chemical synthesis in large quantities (4). The chemical method presently used requires drastic reaction conditions that may cause racemization, decomposition, or side reactions. The high energy consumption required for a long reaction time at a high temperature is unfavorable especially for industrial processes (5). As alternative to such processes, a highly active biocatalyst could be used in a very efficient manner. Among various commercial enzymes, lipases are attractive in terms of availability, the fact that there is no need of cofactors, high stability, and activity in organic media (6–8). The resolution of racemates by enzyme is still the most important approach in the industrial synthesis of optically pure compounds (9,10), as it is often

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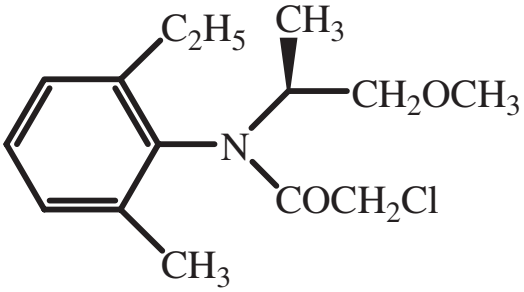
Fig. 1. Structure of (*S*)-metolachlor.



the most economical and convenient way to prepare enantiomerically pure compounds. The main disadvantage of a resolution process compared with an enantioselective synthesis is that the maximum theoretical yield is 50%. Therefore, racemization of the unwanted enantiomer is of critical importance for economically and environmentally acceptable resolutions. A combination of classical resolution processes with racemization to give asymmetric transformations will be necessary to keep up with the advances in asymmetric synthesis (11).

Herein, we first report a practical lipase-catalyzed hydrolytic kinetic resolution of (*S*)-**2**, the key intermediate for (*S*)-metolachlor. To obtain the higher enantiomercially pure (*S*)-acid, the catalytic properties of different lipases are compared, the conditions of lipase-catalyzed hydrolysis are optimized, and the remaining ester is racemized. Then, (*S*)-metolachlor is synthesized by a chemical method based on (*S*)-**2**.

Experimental

Materials

Pseudomonas sp. lipase (PSL) and *Candida cylindracea* A.Y. lipase (AYL) were purchased from Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). *Candida antarctica* lipase B (CAL-B) was kindly donated by Novo Nordisk Industries (Guangzhou, China). *Porcine pancreatic* lipase (PPL) was purchased from Shanghai Dongfeng biochemical reagent Co., Ltd. (Shanghai, China). *Candida lipolytic* lipase (CLL) was provided by Wuxi enzyme preparation plant (Wuxi, China). *Penicillium expansum* lipase (PEL) was provided by Nantong Pharmaceutical Co., Ltd. (Nantong, China). The authenticity of compounds prepared during the study was confirmed by spectroscopic analysis, including 300 MHz NMR (Mercury-300B, VARIAN, Palo Alto, USA), and GC–MS (Saturn 220, VARIAN, Palo Alto, USA). Reactions were routinely monitored on silica gel plates (Qingdao Haiyang Chemical Co., LTD., Qingdao, China) using UV light for detection of the spots. Optical rotation was measured with a WZZ-1S digital automatic polarimeter (Shanghai, China). All the organic solvents were reagent grade and used without further purification. Other reagents were all analytical grade or better.

Determination of conversion and enantiomeric excess (ee_p)

The analysis of the reaction mixtures and the determination of enantiomeric excesses of (*S*)-**2** were performed by capillary zone electrophoresis (P/ACE MDQ, Beckman, Fullerton, USA) with a 59 cm (49 cm to detector) × 50 μm i.d. eCAP^{TM} neutral capillary (Beckman, Fullerton, USA). The conversion of the reaction was determined by using 100 mmol/L triethylamine – acetic acid buffer (TEAA, pH = 5.5) as the background electrolyte. The enantiomeric excess of (*S*)-**2** was successfully analyzed in the buffer by using 40 mmol/L 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD, Beckman, Fullerton, USA) as a buffer additive. The analysis was performed with an applied voltage of –20 kV, and the absorbance was recorded at 200 nm.

Enantiomeric ratio (*E*) of the hydrolysis of racemic *N*-(2-ethyl-6-methylphenyl) alanine methyl ester was calculated from the conversion (c) and enantiomeric excess (ee_p) of (*S*)-**2**, using the equation

$$E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$$

where $ee_p = (c_S - c_R)/(c_S + c_R)$, where c_S and c_R are concentrations of the (*S*)- and (*R*)-enantiomers, respectively (12). The absolute configuration of the enantiomers was established by comparison of the measured optical rotation with the literature data (4).

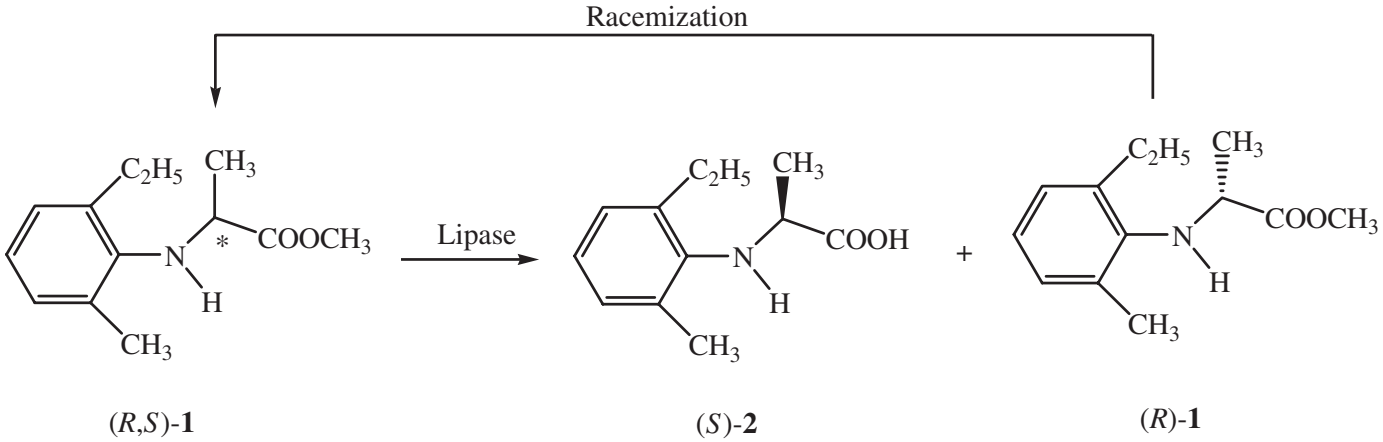
Preparation of (*R*,*S*)-*N*-(2-ethyl-6-methylphenyl) alanine methyl ester: (*R*,*S*)-1

The reaction mixture of 2-ethyl-6-methylaniline (8.4 mL, 60 mmol), $NaHCO_3$ (5.5 g, 65 mmol), and methyl 2-bromopropionate (180 mmol) was stirred under nitrogen atmosphere and slowly heated to 120–125 °C in 1 h. Then, the dark reaction mixture was continually kept at the same temperature for 18 h with stirring. After cooling, the reaction mixture was transferred into 30 mL of ice water and extracted with ethyl acetate. The ethyl acetate fractions were dried over anhydrous Na_2SO_4 and concentrated in a rotary evaporator at 40 °C. After normal work-up, the resulting ester was purified by column chromatography on silica gel using ethyl acetate – petroleum ether (1:5) as the eluant to furnish the corresponding ester. *N*-(2-ethyl-6-methylphenyl) alanine methyl ester (8.9 g, 67.2% yield): ^{1}H NMR ($CDCl_3$) δ: 7.02–6.96 (m, 2H, aromatic H), 6.88–6.83 (t, 1H, J = 7.2 Hz, aromatic H), 3.96–3.94 (q, 1H, J = 6.9 Hz, $CHCH_3$), 3.81 (s, 1H, NH), 3.66 (s, 3H, OCH_3), 2.69–2.66 (m, 2H, CH_2CH_3), 2.30 (s, 3H, aromatic CH_3), 1.38–1.35 (d, 3H, J = 6.9 Hz, CH_2CH_3), 1.26–1.21 (t, 3H, J = 7.5 Hz, $CHCH_3$).GC–MS *m*/*z* (%): 221(M^+, 25), 162 (100), 133 (30), 77 (11); GC: 98% area.

Biocatalytic hydrolysis of (*R*,S)-1 in aqueous buffer with organic compound

CAL-B (200 mg) and (*R*,*S*)-**1** (2.2 g, 10 mmol) were added to the aqueous phosphate buffer (100 mmol/L, pH 8.0) containing diethyl ether (15% *v*/*v*) 100 mL. The mixture was stirred at 25 °C and the pH was maintained using 0.1 mol/L NaOH solution. When the hydrolysis reached 49% conversion, a saturated solution of $NaHCO_3$ (100 mL) was added to the reaction mixture. The mixture was then extracted with ether (3 × 100 mL) to remove the unchanged ester. The aqueous mixture was then acidified to pH 5.5 with 0.1 mol/L HCl and was extracted again with ether (3 × 100 mL) to remove the acid product. Acid extracts were

Scheme 1. Lipase-catalyzed hydrolysis of (*R*,*S*)-**1** and chemical racemization.



dried over anhydrous Na_2SO_4 and evaporated to give (*S*)-**2** (1.0 g, ee_p > 98%).

Racemization of (*R*)-*N*-(2-ethyl-6-methylphenyl) alanine methyl ester: (*R*)-1

The solution of (*R*)-**1** (1.0 g, 4.5 mmol) in toluene (5 mL) was added to a mixture of *n*-butyraldehyde (0.32 g, 4.5 mmol) and benzoic acid (0.22 g, 1.8 mmol) under nitrogen atmosphere. After refluxing for 3 h, the mixture was cooled to room temperature and washed with 5% Na_2CO_3 (3×5 mL) and H_2O (5 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting products were purified by silica gel chromatography using ethyl acetate – petroleum ether (1:5) as the eluant to produce the corresponding (*R*, *S*)-**1** (0.9 g, 90% yield, *R*:*S* = 50:50).

Preparation of (*S*)-*N*-(1′-methyl-2′-hydroxyethyl)-2-ethyl-6-methylaniline: (*S*)-3

To a stirred solution of $NaBH_4$ (0.9 g, 23 mmol) in anhydrous THF (20 mL) under nitrogen atmosphere, a solution of (*S*)-**2** (ee_p > 98%, 1.9 g, 9 mmol) in anhydrous THF (10 mL) was added dropwise. Then, the concentrated H_2SO_4 (0.7 mL) was added slowly below 20 °C. The reaction mixture was stirred at room temperature overnight, and the reaction was quenched by slow addition of methanol (20 mL). After concentrating the mixture in vacuo, 5 mol/L sodium hydroxide (10 mL) was added, and the reaction mixture was heated under reflux for 3 h. The crude mixture was filtered and extracted with $CHCl_3$ (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give (*S*)-**3** (1.6 g, 92% yield): $[\alpha]^{20}_D$ +6.8° (*c* 2.0%, methanol), 97.1% ee_p based on $[\alpha]^{20}_D$ +7° (*c* 1.941%, methanol) reported in literature (4). GC–MS *m*/*z* (%): 193.1 (M^+, 13.2), 162.2 (100); GC: 90% area.

Preparation of (*S*)-*N*-(1′-methyl-2′-hydroxyethyl)-*N*-chloroacetyl-2-ethyl-6-methylaniline: (*S*)-4

To a solution of (*S*)-**3** (1.6 g, 8.3 mmol) and Na_2CO_3 (0.88 g, 8.3 mmol) dissolved in benzene (10 mL) under nitrogen atmosphere, chloroacetyl chloride (0.94 g, 8.3 mmol) was slowly added at 15–20 °C with efficient stirring. The reaction mixture was then stirred for 3 h at room temperature and filtered. The filtrate was washed with 10% HCl, 10% Na_2CO_3, and saturated NaCl. The organic phase was dried over anhydrous Na_2SO_4, and subsequent concentration under reduced pressure afforded the yellow oil (*S*)-**4** (2.1 g, 95% yield): $[\alpha]^{20}_D$ +11.6°(*c* 1.1%, methanol), 96.6% ee_p based on $[\alpha]^{20}_D$ +12° (*c* 1.166%, methanol) reported in literature (4). GC–MS *m*/*z* (%): 269.2 (M^+, 12), 162.2 (100), 133.1 (21); GC: 92% area.

Preparation of (*S*)-*N*-(1′-methyl-2′-methoxyethyl)-*N*-chloroacetyl-2-ethyl-6-methylaniline: (*S*)-5

The reaction mixture of (*S*)-**4** (2.0 g, 7.4 mmol), 2,2-dimethoxypropane (1.69 g, 14.8 mmol), and *p*-toluenesulfonic acid (0.26 g, 1.1 mmol) dissolved in methanol (5 mL) was heated under reflux for 36 h. After cooling the reaction mixture to room temperature, methanol was evaporated under reduced pressure. The residue was taken up in ethyl acetate (10 mL), and the solution was washed with 10% Na_2CO_3 and water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residual oil was purified by column chromatography on silica gel using ethyl acetate – petroleum ester (1:6) as the eluant to furnish the corresponding (*S*)-metolachlor, (*S*)-**5** (1.4 g, 66% yield): $[\alpha]^{20}_D$ –8.2 ± 1° (*c* 2.1%, *n*-hexane), 91.1% ee_p based on $[\alpha]^{20}_D$ –9.0 ± 1° (*c* 2.073%, *n*-hexane) reported in literature (4). ^1H NMR ($CDCl_3$), δ: 7.24–7.13 (m, 3H, aromatic H), 4.22 (m, 1H, $CHCH_3$), 3.94 (dd, *J* = 4.2 and 9.4 Hz) and 3.75 (dd, *J*= 4.2 and 9.4 Hz) for one diastereomeric proton of $CHCH_2OCH_3$), 3.62–3.61 (s, 2H, CH_2Cl), 3.51 (dd, *J* = 4.2 and 9.4 Hz) and 3.49 (dd, *J* = 4.2 and 6.5 Hz) for one diastereomeric proton of $CHCH_2OCH_3$, 3.27–3.26 (d, 3H, OCH_3), 2.62–2.54 (m, 2H, CH_2CH_3), 2.26 (s, 1H, aromatic CH_3), 2.24 (s, 2H, aromatic CH_3), 1.28–1.23 (t, 3H, *J* = 7.5 Hz, CH_2CH_3), 1.17–1.14 (q, 3H, *J* = 7.0 Hz, $CHCH_3$). GC–MS *m*/*z* (%): 284 (M^+, 60), 252 (38), 250 (100), 218 (47), 178 (25), 162 (37), 73 (60); GC: 98% area.

Results and discussion

In a general experimental procedure, readily accessible racemic methyl ester **1** was used as a substrate in the identification of a suitable lipase for the enantioselective hydrolysis (Scheme 1), and the screening results were listed in Table 1. The lipase CAL-B was very active, and 48.2% of

Table 1. Lipase-catalyzed hydrolysis of (*R*,*S*)-**1**.

Lipase	Time (h)	Conversion (%)	ee_p (%)	*E* value	Major enantiomer
CAL-B	2	48.2	69.4	10.6	*S*-(–)
PSL	60	48.2	99.0	>100	*R*-(+)
PPL	72	1.7	99.0	>100	*R*-(+)
CLL	72	0.24	98.0	99.2	*S*-(–)
AYL	72	2.0	78.8	8.6	*S*-(–)
PEL	72	0.12	58.5	3.8	*S*-(–)

Note: Reaction conditions: buffer: 100 mmol/L phosphate buffer (pH = 8.0, 5.0 mL); substrate: 0.5 mmol; enzyme: 105 U; temperature: 37 °C.

Table 2. CAL-B-catalyzed hydrolysis of (*R*,*S*)-**1** in different aqueous-organic media.

Organic solvent	Log *P*	Time (h)	Conversion (%)	ee_p (%)	*E* value
None	—	2	32.5	78.7	12.1
Acetonitrile	–0.33	2	29.7	83.7	15.9
Acetone	–0.23	2	31.7	82.9	15.5
THF	0.49	2	28.2	82.8	14.6
Diethyl ether	0.85	2	19.6	87.8	19.0
DIPE	1.90	2	28.3	84.8	16.8
Toluene	2.50	2	17.4	84.3	13.9
Cyclohexane	3.20	2	27.1	82.1	13.7
n-Hexane	3.50	2	25.1	87.6	20.1
n-Heptane	4.00	2	23.1	86.5	17.9

Note: Reaction conditions: substrate: 0.5 mmol; CAL-B: 10 mg; organic compound/phosphate buffer (100 mmol/L, pH= 8.0, 5.0 mL) 1:9; temperature: 25 °C.

(*R*,*S*)-**1** was hydrolyzed only after 2 h at 37 °C; however, it displayed poor enantioselectivity (*E* = 10.6) towards the (*S*)-enantiomer of the acid. The *E* value of >100 and enantiomeric excess (ee_p) of >98% were obtained by lipase PSL and PPL, but they preferably produced the (*R*)-acid and needed longer reaction time. Other enzymes investigated in this work gave both low conversion and enantioselectivity, making them practically useless. As a result of its higher activity and stereospecificity towards the (*S*)-enantiomer, the lipase CAL-B was chosen from the enzymes screened for further study.

Initially we investigated the influence of the microenvironment on the CAL-B-catalyzed resolution and found that the enantioselectivity of CAL-B was still too low for a large-scale preparation (data not shown). This may be ascribed to the ability of CAL-B to catalyze the hydrolysis of both (*R*)- and (*S*)-enantiomers of substrate, thus leading to the low enantioselectivity of enzyme. According to this assumption, if we take measures to enantioselectively inhibit the reaction rate of CAL-B-catalyzed hydrolysis, and as a result, the (*S*)-enantiomer becomes overwhelmingly the faster reacting enantiomer, the enantioselectivity of CAL-B may be enhanced.

The strategy of enantioselective inhibition by adding an organic compound to the reaction medium to enhance the selectivity of lipase had previously been reported by our group (13). So, in this study, organic compounds with different hydrophobicities (log *P*) (14, 15) were added to the reaction system to observe their effect on CAL-B-catalyzed hydrolysis (Table 2). In all experiments, the biocatalytic system shows the same stereopreference for (*R*,*S*)-**1** giving (*S*)-acid. Also, the addition of organic compounds generally enhanced the enantioselectivity of CAL-B compared with that in a pure buffered medium, despite a decrease in enzyme activity.

According to the previously mentioned results, it is worthy to note that the use of diethyl ether and *n*-hexane as additives is preferred (*E* = 19.0 and 20.1, respectively). Herein, the effects of diethyl ether and *n*-hexane amounts on the activity and enantioselectivity of CAL-B were studied. The additive amount of the organic compound is an important parameter worthy of careful optimization because it may have a strong influence on enzyme activity and enantioselectivity. When diethyl ether was added to the reaction medium, the maximum *E* value of CAL-B was obtained at 15% (*v*/*v*) (*E* > 100, Fig 2), which was approximately 9.7-fold more enantioselective than that in the pure buffered medium. For *n*-hexane, the results differed and the maximum enantioselectivity of CAL-B was stabilized at the additive amount ranging from 10 to 15% (*v*/*v*) (*E* = 20.1, Fig 3). The conversion ratios of reactions for various amounts of organic compounds were also measured and it was found that the ratio decreased with an increase of additive amount of organic compound. Based on these results, we presume that diethyl ether is a stronger and more (*R*)-selective inhibitor than *n*-hexane.

After CAL-B-catalyzed hydrolysis finished under the optimized conditions, the enantiomercially pure (*S*)-**2** (>98% ee_p) and the remaining ester (*R*)-**1** were extracted with ether. The two compounds can be separated easily by partitioning in ether or water. This simple extraction–partition procedure is especially convenient and suitable for a large-scale operation. The remaining ester can be recycled by chemical racemization, which is achieved through treatment with a mixture of *n*-butyraldehyde and benzoic acid dissolved in toluene (Scheme 1). The aldehyde-catalyzed racemization of

Fig. 2. Effect of different volume fractions of diethyl ether on the conversion and enantioselectivity of CAL-B-catalyzed hydrolysis of (*R*,*S*)-**1**.

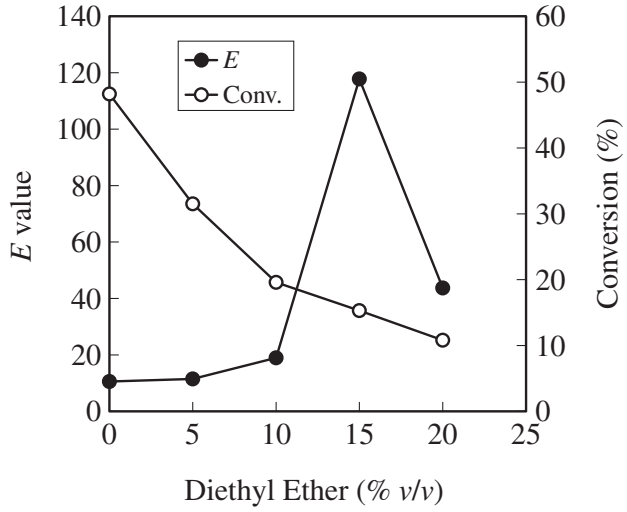
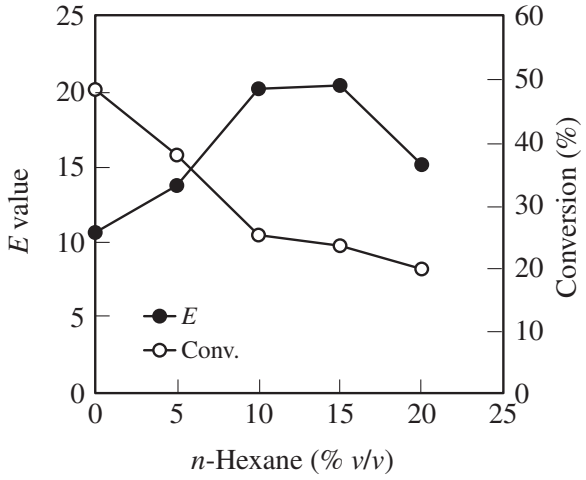
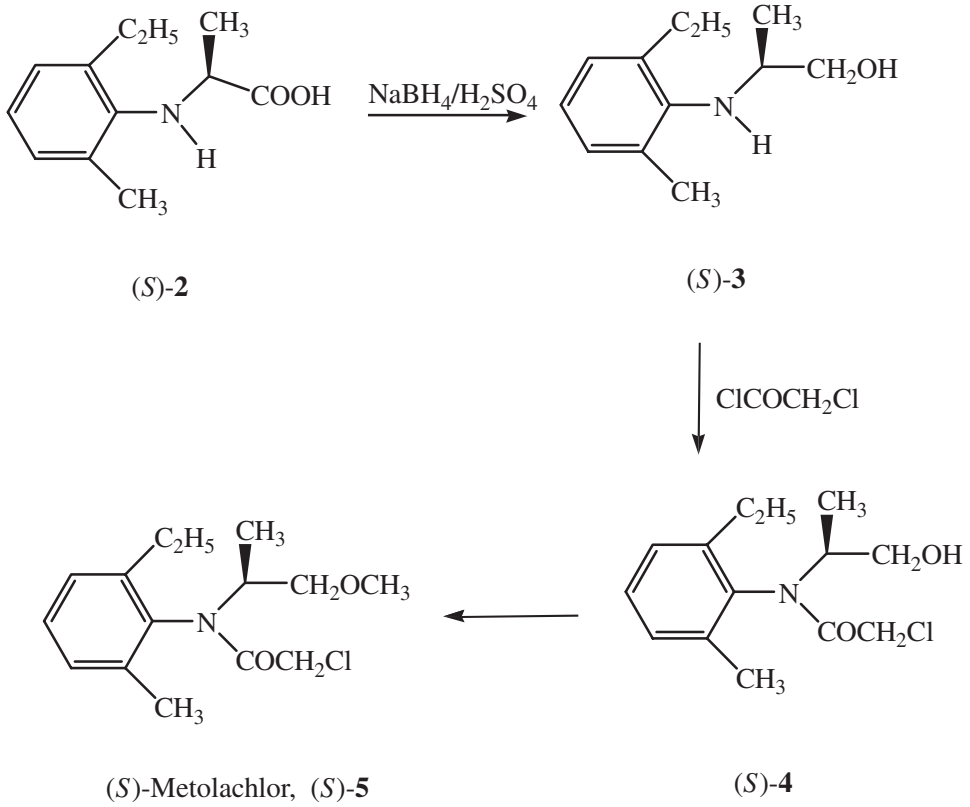


Fig. 3. Effect of different volume fractions of *n*-hexane on the conversion and enantioselectivity of CAL-B-catalyzed hydrolysis of (*R*,*S*)-**1**.



Scheme 2. Synthesis of (*S*)-metolachlor from enzymatically prepared (*S*)-**2**.



amino acids and derivatives is the most prominent example of how to derive a functional group connected to a chiral center and affect optical stability. Herein, we also investigated the effects of other aldehydes including formaldehyde, acetaldehyde, propionaldehyde, *n*-valeraldehyde, and benzaldehyde on the racemization, and found that the experimental results were similar. However, considering the economic factor and the boiling point of the aldehyde, we selected *n*-butyraldehyde as the racemic reagent. Very similar results were also reported by Park et al. (6).

The chemical synthesis of (*S*)-metolachlor was then carried out using enantiomercially pure (*S*)-**2** as a substrate via a number of pathways, which included reduction, chloroacetylation, and etherification (Scheme 2). The reduction of (*S*)-**2** to (*S*)-**3** is conveniently carried out in an inert solvent. Suitable reducing agents are lithium aluminium hydride and

sodium borohydride, but the selectivity of lithium aluminium hydride towards the functional group of the compound is poor and it is rather expensive. Thus, it is preferable to use sodium borohydride in the form of a complex, especially as a sodium borohydride – tetrahydrofuran complex. The conversion of (*S*)-**3** into (*S*)-**4** is carried out in an inert solvent by the reaction with a chloroacetylating agent, such as chloroacetyl chloride, in the presence of a base. Suitable bases may be alkali metal carbonates, triethylamine, and pyridine. Suitable inert solvents may be hydrocarbons, such as hexane, cyclohexane, benzene, toluene, and chlorobenzene. In this work, Na_2CO_3 and benzene were selected as the base and inert solvent, respectively. The reaction was carried out at a low or moderately elevated temperature. The etherification of the 2-hydroxy group of (*S*)-**4** was carried out by heating the reaction mixture, dissolved in methanol, in the presence of a strong acid at a reflux temperature. A strong acid suitable for the reaction is *p*-toluenesulfonic acid. Then (*S*)-metolachlorwas obtained with the optical purity of 91.1% ee_p. Compared with racemic metolachor, (*S*)-metolachlor has a markedly superior herbicidal action against weeds without increasing phytotoxicity towards cultivated plants.

Conclusion

We successfully developed a chemoenzymatic procedure for the synthesis of enantiomerically pure (*S*)-metolachlor. We took advantage of the higher activity and excellent enantioselectivity of lipase CAL-B, chemical racemization of the remaining ester, and chemical reactions including reduction, chloroacetylation, and etherification. The method is easy to perform with standard equipment and can be widely applied. At the same time, we found that organic compounds strongly influenced the selectivity of CAL-B. Diethyl ether, in particular, was found to achieve the highest enantioselectivity of CAL-B ($E > 100$) in the hydrolysis of (*R*,*S*)-**1**. Further work in this area, including the repeated use of enzymes to decrease the enzymatic cost and scale-up results of the process, is being done in our laboratory and will be reported soon.

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