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REGULAR ARTICLE

The enzymatic resolution of 1-(4-chlorophenyl)ethylamine by Novozym 435 to prepare a novel triazolopyrimidine herbicide

Yinjun Zhang | Feifei Cheng | Hongde Yan | Jianyong Zheng | Zhao Wang

Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou, China

Correspondence
Zhao Wang, Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Address No. 18, Chaowang Road, Hangzhou, China.
Email: hzwangzhao@163.com

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Abstract

The kinetic resolution of (R,S)-1-(4-chlorophenyl)ethylamine was accomplished using a commercial lipase from *Candida antarctica* (Novozym 435). The performance of this lipase was investigated for the enantioselective amidation of (R,S)-1-(4-chlorophenyl)ethylamine, leaving the target product (S)-1-(4-chlorophenyl)ethylamine in its unreacted form. The effects of various types of solvents and an acyl donor, the molar ratio of the substrate to the acyl donor, and the reaction temperature were studied. The optimum reaction conditions were found to result in amidation with methyl 2-tetrahydrofuroate at 40°C in methyl tert-butyl ether, with a substrate/acyl donor molar ratio of 1:2.4. The conversion rate of (R,S)-1-(4-chlorophenyl) ethylamine was 52%, with an enantiomeric excess of 99% towards the unreacted substrate in a reaction time of 22 hours. Finally, using optically pure (S)-1-(4-chlorophenyl)ethylamine as the raw material, the chemical synthesis of (S)-N-(1-(4-chlorphenyl)ethyl)-2-(5,7-dimethyl-[1,2,4]triazolo[1,5-a] pyrimidin-2-ylthio)acetamide, a novel triazolopyrimidine herbicide, was achieved, and the total yield and purity were 83.5% and 95.3%, respectively.

KEYWORDS

1-(4-chlorophenyl)ethylamine, amidation, lipase, Novozym 435, resolution

1 | INTRODUCTION

Chiral 1-phenylethylamine and its derivatives are important intermediates for the synthesis of chiral pesticides and pharmaceuticals.[1,2] Also, these compounds play critical roles during the process of chiral synthesis, such as in the form of chiral auxiliaries,[3,4] chiral resolving agents,[5] and base materials.[6] Therefore, the synthesis of optically active isomers of 1-phenylethylamine compounds has been a focus in recent years. (S)-1-(4-chlorophenyl) ethylamine ((S)-1) is a key intermediate for the synthesis of (S)-N-(1-(4-chlorphenyl)ethyl)-2-(5,7-dimethyl-[1,2,4] triazolo[1,5-a]pyrimidin-2-ylthio)-acetamide ((S)-4), an acetolactate synthase inhibitor, which is a new type of aromatic amide compound containing triazolopyrimidine structure developed by Zheng.[7] According to the preliminary study, this compound has high herbicidal activity, low residue, and a wide herbicidal spectrum, but is safe to gramineae.[8]

Preparation of chiral 1-phenylethylamine and its derivatives by enzymatic resolution has been proven a practicable universal method owing to its high catalytic efficiency, excellent stereoselectivity, and environmental friendliness.[1,9-11] For example, lipases from *Candida antarctica* and *Pseudomonas cepacia* were demonstrated to possess high enantioselectivity and catalytic efficiency

for the enantiomeric kinetic resolution of 1-phenylethylamine.[1,11] A new amidohydrolase from *Arthrobacter aurescens*, AcR5b, was found for deacetylating several *N*-acetyl-1-phenylethylamine derivatives with (R)-stereopreference.[12] Enzymatic enantioselective amidation of *p*-methoxy-1-phenylethylamine was achieved using immobilized lipases from *C. antarctica*.[13] In this study, the lipase from *C. antarctica* (Novozym 435) was screened for the stereoselective acylation of (R,S)-1 to obtain (S)-1 (Scheme 1A). Some factors affecting the resolution of (R,S)-1 were investigated, such as its organic solvent, acyl donor, molar ratio, and reaction temperature. Then, high optical purity of (S)-1 was utilized for the further synthesis of (S)-4 (Scheme 1B).

2 | MATERIALS AND METHODS

2.1 | Materials

Novozym 435 was purchased from the China Headquarters of Novozymes (Beijing, China). (R,S)-4 (purity-99.8%) was provided by the pesticide institute of the Zhejiang University of Technology (Hangzhou, China). The standard sample of (R,S)-1 was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

2.2 | Enzymatic resolution of (R,S)-1

In a typical experiment, the reaction mixtures were composed of (R,S)-1 (0.7 mmol), acyl donor (200 μL), organic solvent (10 mL), and lipase (20 mg). The mixtures were incubated on a shaker at 200 rpm and 30°C for 24 hours. Reaction mixtures without lipase were also run to exclude any possible spontaneous nonenzymatic reactions. At appropriate intervals, the samples were withdrawn and filtered, then analyzed by high-performance liquid chromatography (HPLC).

2.3 | Synthesis of (S)-4

Chloroacetyl chloride (0.012 mmol) was slowly added to a solution of (S)-1 (0.01 mol) in 20 mL of acetone at 5°C for 30 minutes (Scheme 1B). The crystal of (S)-1-chloro-*N*-[1-(4-chlorophenyl) ethyl]acetamide ((S)-3) was precipitated while continuously stirring deionized water into the mixture. The filtered precipitate was washed with 18% hydrochloric acid to remove pyridine, and then washed with water until the pH became neutral.

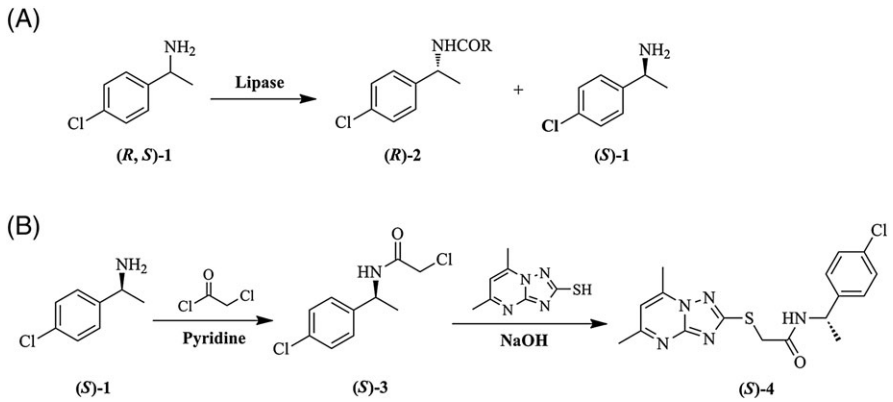
A total of 2.05 g of the 2-mercapto-5,7-dimethyl-1,2,4-triazolo [1,5-a] pyrimidine dissolved in 30 mL of 0.43 M sodium hydroxide reacted with 2.28 g (S)-3 dissolved in 100 mL of 95% ethanol for 30 minutes at a temperature of less than 25°C to prepare (S)-4. The product was precipitated through the addition of deionized water, and was washed successively with 3% hydrochloric acid, 5% sodium bicarbonate, and deionized water until the washing liquid became neutral. Following this, the product was dried at 50°C.

2.4 | Analytical methods

The conversion rate and enantiomeric excesses of (R,S)-1 ($e.e._s$) and (R,S)-2 ($e.e._p$) were measured by HPLC with a chiral CD-Ph column (250 mm × 4.6 mm, 5 μm; Daicel, Hyogo, Japan), the mobile phase was composed of methanol/0.5 M sodium perchlorate solution at a ratio of 90/10. The flow rate was 1 mL/minute; UV wavelength detection was performed at 254 nm. The enantiomeric excess was calculated according to the peak areas of (S)-enantiomer and (R)-enantiomer. The enantioselectivity (E) was calculated according to the following equation[14]:

$$e.e._s = \frac{[S1] - [R1]}{[S1] + [R1]} \times 100\% \quad (1)$$

$$e.e._p = \frac{[R2] - [S2]}{[S2] + [R2]} \times 100\% \quad (2)$$



SCHEME 1 A, The lipase-catalyzed resolution of (*R*,*S*)-1-(4-chlorophenyl) ethylamine ((*R*,*S*)-1). B, The synthesis of (*S*)-N-(1-(4-chlorphenyl)ethyl)-2-(5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-ylthio)acetamide ((*S*)-4).

$$C = \frac{e.e._s}{e.e._s + e.e._p} \times 100\% \quad (3)$$

$$E = \frac{\ln[(1-C)(1-e.e._s)]}{\ln[(1-C)(1+e.e._s)]} \quad (4)$$

where C represents the conversion ratio of (R,S)-1. $e.e._s$ represents the enantiomeric excess of the residual (R,S)-1. $e.e._p$ represents the enantiomeric excess of the product (R,S)-2. [R1] and [S1] are the peak areas corresponding to (R)-1 and (S)-1. [R2] and [S2] are the peak areas corresponding to (R)-2 and (S)-2, respectively.

The optical purity (S)-4 was determined by measuring the optical rotation at 589 nm through a polarimeter (NJ 07840, Rudolph, USA). The structural identification of (S)-4 was measured by ESI-MS (Waters, USA) and NMR (500 MHz). The yield was calculated at the practical and theoretical weight of (S)-4 subsequent to its drying. Finally, the purity of (S)-4 was detected by HPLC with Hypersil ODS2 (250 mm × 4.6 mm, 5 μm; Thermo Fisher Scientific, Germany). The mobile phase was composed of ethanol/water at a ratio of 60/40.

3 | RESULTS AND DISCUSSION

3.1 | Screening of the lipases

Screening is the key step for selection of suitable biocalysts.[15] Fifty commercial lipases were selected for selective amidation of (R,S)-1. Seven lipases displayed amidation activity and displayed a (R)-stereopreference, leaving the target product an (S)-enantiomer. The results are summarized in Table 1. Novozym 435 afforded high conversion and $e.e._s$ among the screened lipases, and the enantioselectivity was calculated with $E = 7.4$ from a conversion of 54.5%. Therefore, Novozym 435 was chosen for further study.

3.2 | The effects of organic solvents

It is well known that organic solvents can affect the solubility of substrates and the activity and enantioselectivity of enzymes[16]. Different organic solvents with log P values of from .46 to 3.76 were used to investigate the effects of solvents on the kinetic resolution of (R,S)-1. The results in Table 2 revealed that enzyme activity and enantioselectivity were not correlated with the log P values. Low conversions and low E values were observed in methylene chloride and chloroform. The high conversions of 77.8% and 85.5% were obtained in *n*-hexane and isopropyl ether, but the E values were both low. The other tested solvents afforded moderate conversions (41.5% to 56.4%), and the best performance was obtained in methyl *tert*-butyl ether (MTBE), with the highest E value being 7.8 and the conversion being 53.9%. Similar results were obtain in the enantioselective acylation of 2-phenylcycloalkanamines[17] and 1-(3′-bromophenyl)-ethylamine,[18] and MTBE seemed to be an excellent choice as a solvent in the lipase-catalyzed kinetic resolution of chiral amines. Finally, MTBE was selected as the optimum solvent for the following tests.

TABLE 1 The screening of commercial lipases

Enzyme	Source	$e.e._s$ (%)	$e.e._p$ (%)	Conversion (%)	E
Novozym 435	*Candida antarctica*	68.6	57.3	54.5	7.4
Lipozyme TLIM	*Thermomyces languginosa*	1.6	3.5	31.4	1.1
Lipase P	*Penicillium camemberti*	0.5	1.7	22.6	1.0
Lipase R	*Rhizopus niveus*	2.3	6.4	26.4	1.2
Amano lipase A	*Aspergillus niger*	2.2	6.7	24.6	1.2
Amano lipase AK	*Pseudomonas fluorescens*	1.6	4.7	25.2	1.1
Lipase VII	*Candida rugosa*	1.4	4.2	26.2	1.1

Reaction were carried out at 30°C, 200 rpm for 24 hours, including 100 μL (R,S)-1 (0.7 mmol), 200 μL isopropenyl acetate, 10 mL MTBE, and 20 mg lipase.

TABLE 2 The effects of organic solvents on the enantioselective amidation of (R,S)-1

Organic solvents	Log P	$e.e._s$ (%)	$e.e._p$ (%)	Conversion (%)	E
Tetrahydrofuran	0.46	18.3	25.8	41.5	2.0
Ethyl acetate	0.68	12.0	9.3	56.4	1.3
MTBE	1.29	68.9	58.9	53.9	7.8
Methylene chloride	1.41	6.0	26.3	18.6	1.8
Isopropyl ether	1.9	33.4	5.7	85.5	1.4
Chloroform	2.0	5.4	15.2	26.2	1.4
Toluene	2.74	34.9	34.3	50.4	2.8
n-Hexane	3.76	11.1	3.2	77.8	1.2

Reaction were carried out at 30°C, 200 rpm for 24 hours, including 100 μL (R,S)-1 (0.7 mmol), 200 μL isopropenyl acetate, 10 mL different organic solvents, and 20 mg Novozym 435.

3.3 | The effects of the acyl donor

The use of diverse acyl donors with differing chemical structures has been proven to have cause different results

in enzymes' acylation activity. The effects of the appropriate acyl donor can resemble very closely the "pocket" structures of enzymes that can fully expose enzymes' active centres.[19,20] As demonstrated in Table 3, methyl 2-tetrahydrofuroate had an obviously positive effect on the activity and enatioselectivity of Novozym 435, affording the highest $e.e._s$ (96.8%) and enantioselectivity ($E = 44.6$), with a conversion of 53.8%. Isopropenyl acetate also displayed good results, with a conversion of 54% and an E value of 7.8, but the isopropenyl acetate reaction had poor reproducibility. According to Gill's observation, extensive (up to 60%) by-product was formed with certain batches of isopropenyl acetate.[18] Miranda et al also found that an imine derivative formed between the free amine and propanone (derived from the isopropenyl ester).[21] Ethyl methoxyacetate, which is less reactive than isopropenyl acetate but much more reactive than simple esters, afforded a high conversion (52.9%) and moderate enantioselectivity ($E = 5.6$). The results were in agreement with the reported of Schmid et al. They assumed that the methoxy residue in the acyl part of the amide greatly enhances the reaction rate, compared to the acetate or the butyric residue, which is sterically almost equivalent.[22] Another enol ester, vinyl acetate, a widely used acyl donor for resolving chiral alcohols,[23] gave a low conversion rate (19.1%) and low E value (2.1). The results were similar to those reported for the lipase-catalyzed acylation of (R,S)-phenylethylamine and1-(3′-bromophenyl)ethylamine,[17,24] where vinyl acetate also had low E values. This may be due to the fact that the use of vinyl acetate resulted in nonselective nonenzymatic acetylation, as well as the formation of several side products.[17] Dimethyl succinate and diethyl succinate displayed low conversion and low enantioselectivity. But in the report of Gröger et al, an analog, diethyl malonates, served as a highly efficient acyl donor in the resolution of 1-phenylethyl amine and (R,S)-[1-(4-bromophenyl)]ethyl amine with E value >100.[25] This may be due to the differences of the nature of amines. Ethyl acetate and ethyl 3-hydroxybutyrate also gave low conversion and low enantioselectivity. Thus, methyl 2-tetrahydrofuroate was chosen as the acyl donor to employ in further experiments.

3.4 | The effects of the substrate concentration

Substrate concentration is a crucial factor that may affect enzyme activity or even result in substrate inhibition and affect the enantioselectivity of the reaction. Figure 1 displays the performance of Novozym 435 on the resolution of (R,S)-1 using different substrate concentrations. The results showed that high conversion (>50%) and high $e.e._s$ (>96%) were obtained when the substrate concentration was below 70 mM, and significant decreases of conversion and $e.e._s$ were observed when the substrate concentration was above 70 mM. When the substrate concentration was 70 mM, the $e.e._s$ and $e.e._p$ could reach 96.8% and 86.7%, with an E value of 54.6. Similar results were reported in the kinetic resolution of (R,S)-1-phenylethylamine, where an increase in substrate concentration also led to a less selective reaction.[26]

TABLE 3 The effects of acyl donors on the enantioselective amidation of (R,S)-1

Acyl Donor	$e.e._s$ (%)	$e.e._p$ (%)	Conversion (%)	E
Dimethyl succinate	11.6	41.1	22.0	2.7
Diethyl succinate	4.5	10.6	29.8	1.3
Isopropenyl acetate	68.2	58.1	54.0	7.8
Methyl 2-tetrahydrofuroate	96.8	71.6	53.8	44.6
Ethyl methoxyacetate	58.6	52.2	52.9	5.6
Ethyl acetate	17.0	30.2	36.0	2.2
Ethyl 3-hydroxybutyrate	5.2	14.7	26.1	1.4
Vinyl acetate	7.7	32.6	19.1	2.1

Reaction were carried out at 30°C, 200 rpm for 24 hours, including 100 μL (R,S)-1 (0.7 mmol), 200 μL different acyl donor, 10 mL MTBE, and 20 mg Novozym 435.

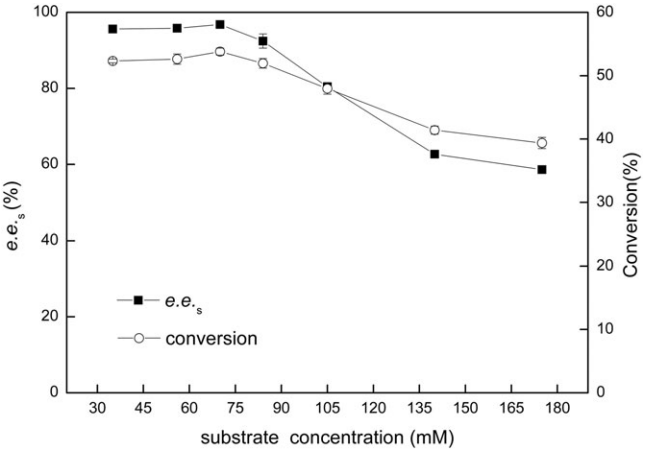


FIGURE 1 The effects of substrate concentrations on the enantioselective amidation of (*R*,*S*)-1. The reactions were carried out at 30°C, 200 rpm for 24 hours, and utilized different concentrations of (R,S)-1, 200 μL methyl 2-tetrahydrofuroate, 10 mL MTBE, and 20 mg Novozym 435

3.5 | The effects of the molar ratio of the substrate to the acyl donor

As shown in Figure 2, the conversion rate increased with an increase in the molar ratio of the substrate to the acyl donor. The $e.e._s$ also increased with an increase in the molar ratio of the substrate to the acyl donor, reaching a maximum value of 96.8% (ratio 1:2.4), but decreased subsequently with further increases in the molar ratio. The results were similar to the reports concerning the enantioselective acylation of (R,S)-phenylethylamine by Novozym 435, where the molar ratio of 1:4 gave the highest *E* value, and higher molar ratios resulted in a decrease in enantioselectivity.[24] Thus, considering the optical purity of the target product, a molar ratio of 1:2.4 was selected for the following experiments.

3.6 | The effects of temperature

The effects of temperature on reaction conversion rates and $e.e._s$ are described in Figure 3. There was a continuous increase in the conversion rate from 20°C to 40°C, and then a slight decrease from 40°C to 45°C. The $e.e._s$ showed an obvious increase from 20°C (85%) to 30°C (96.8%), a smooth increase from 30°C (96.8%)

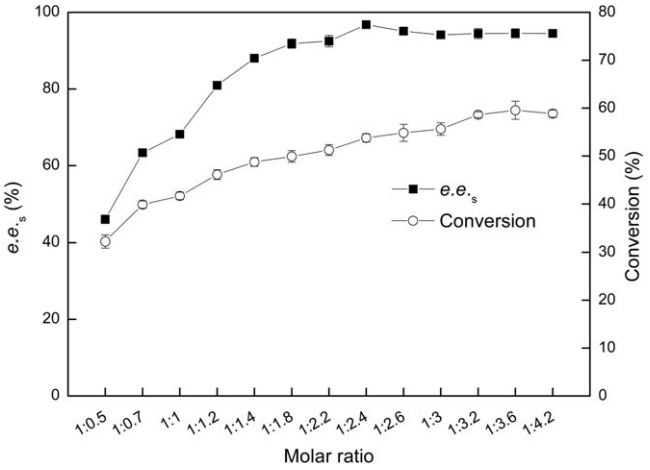


FIGURE 2 The effects of molar ratios of substrate to acyl donor on the enantioselective amidation of (R,S)-1. The reactions were carried out at 30°C, 200 rpm for 24 hours, and employed 100 μL (R,S)-1 (0.7 mmol), different amounts of methyl 2-tetrahydrofuroate, 10 mL MTBE, and 20 mg Novozym 435

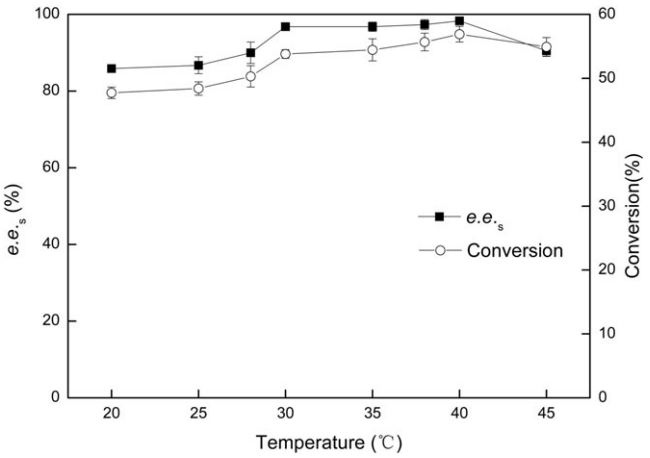


FIGURE 3 The effects of temperature on the enantioselective amidation of (R,S)-1. The reactions were carried out at 200 rpm at different temperatures for 24 hours; the experiment used 100 μL (R,S)-1 (0.7 mmol), 200 μL methyl 2-tetrahydrofuroate (1.7 mmol), 10 mL MTBE, and 20 mg Novozym 435

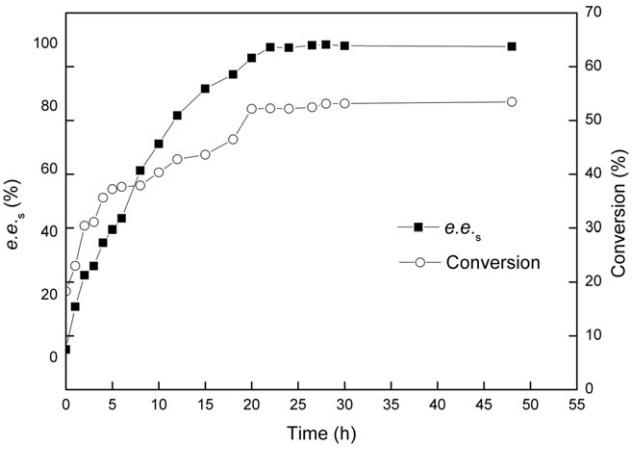


FIGURE 4 The time course for the enantioselective amidation of (R,S)-1. The reactions were carried out at 40°C and 200 rpm and entailed the use of 100 μL (R,S)-1 (0.7 mmol), 200 μL methyl 2-tetrahydrofuroate (1.7 mmol), 10 mL MTBE, and 20 mg Novozym 435

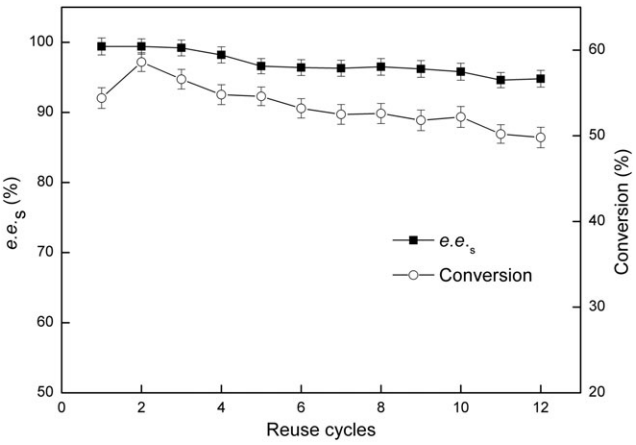


FIGURE 5 The reuse cycles of immobilized Novozym 435 on the preparation of (S)-1. The reactions were carried out at 40°C at 200 rpm for 22 hours, and entailed 100 μL (R,S)-1 (0.7 mmol), 200 μL methyl 2-tetrahydrofuroate (1.7 mmol), 10 mL MTBE, and 20 mg Novozym 435

to 40°C (98%), and then an obvious decrease from 40°C to 45°C (90%). The highest $e.e._s$ was found at approximately 98% at 40°C, with a conversion rate of 57%. Thus, a reaction temperature of 40°C was selected for the subsequent experiments.

3.7 | The time course for the enantioselective amidation of (R,S)-1

The time course for the enantioselective amidation of (R, S)-1 at the optimized reaction conditions was next investigated. The results are presented in Figure 4. There was a marked tendency for the conversion, together with the $e.e._s$ to rise as the reaction time increased to 22 hours. At this reaction time, the final $e.e._s$ reached about 99% with a conversion rate of 52%.

3.8 | Reusability of immobilized lipase in the preparation of (S)-1

Compared with free enzymes, there are several advantages for using immobilized enzymes, which include their stability of conformation, easy separation, and reusability.[27,28] As shown in Figure 5, the $e.e._s$ was maintained at a high level (>95%) following the first 12 batches. Although the conversion rate slightly decreased as the

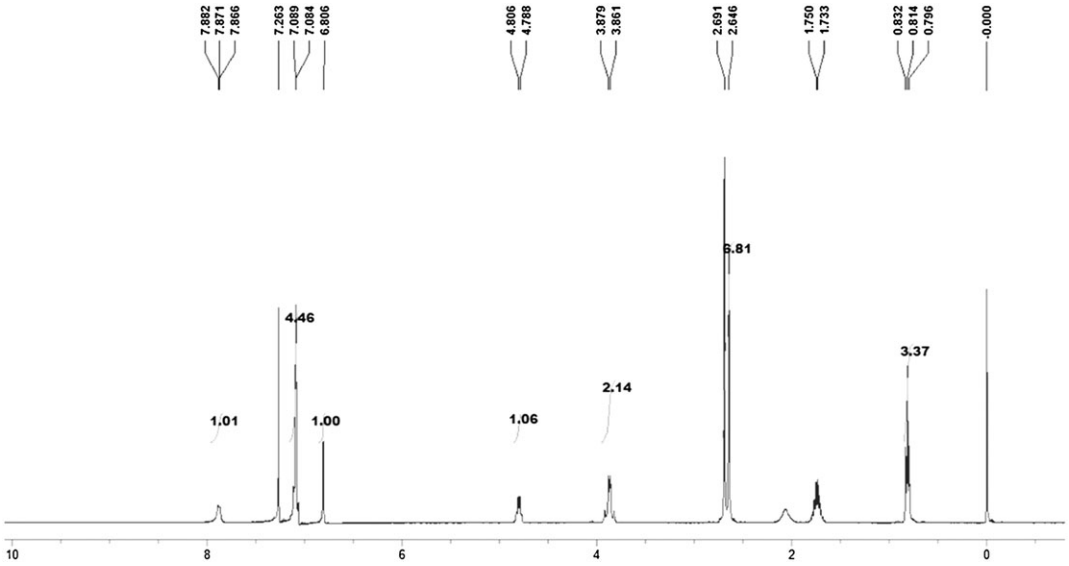


FIGURE 6 ^{1}H NMR spectra of (S)-4

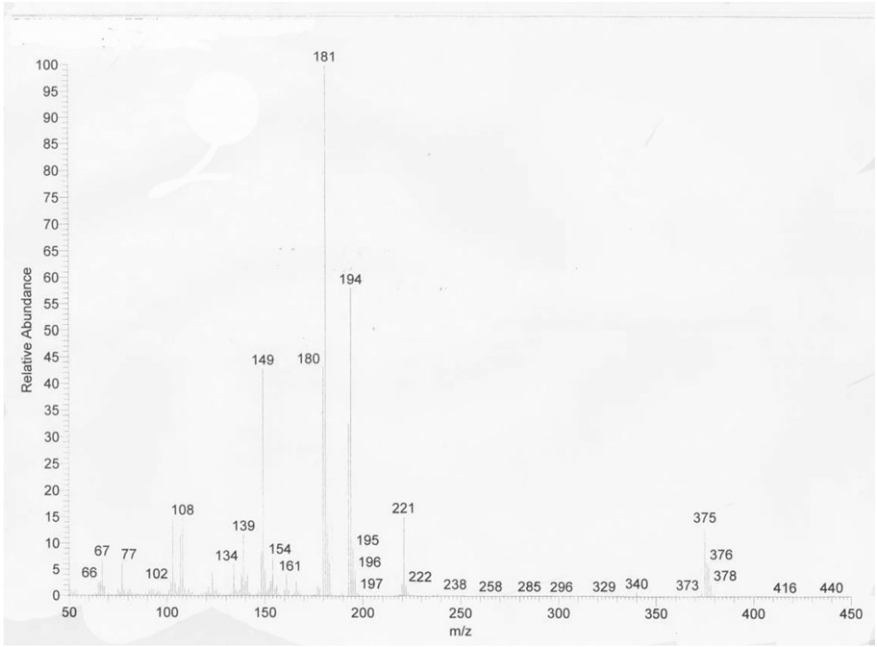


FIGURE 7 Mass spectrogram of the product (S)-4

batches progressed, the residual activity was greater than 90% following 12 reuse cycles. Therefore, the immobilized Novozym 435 had an improved application in the preparation of (S)-1.

3.9 | Synthesis of (S)-4

The chemical synthesis of (S)-4 was developed from (S)-1. First, (S)-3 was synthesized through the chemical acylation of (S)-1. Then, (S)-3 was used to obtain (S)-4 through its reacting with 2-mercapto-5,7-dimethyl-1,2,4-triazolo[1,5-a]pyrimidine. Under the appropriate conditions, the target, (S)-4, was acquired, and the total yield and *e.e.* values were 83.5% and 95.3%, respectively.

(S)-4 was white solid. Formula: $C_{17}H_{18}ClN_5OS_1H$. Melting point: 179~181°C. ^{1}HNMR (500 MHz, $CDCl_3$) (Figure 6): δ: 1.41~1.42 (d,3H), 2.65 (s,3H), 2.67 (s,3H), 3.79~3.91 (m,2H), 5.01~5.05 (m,1H), 6.79 (s,1H), 7.07~7.13 (m,4H), and 7.78~7.79 (d,1H). MS (ESI) m/z (Figure 7): 375 $[M + H]^+$.

4 | CONCLUSION

The asymmetric amidation of (R,S)-1 by Novozym 435 was achieved with MTBE. Methyl 2-tetrahydrofuroate was proven an ideal acyl donor, with the molar ratio of substrate to acyl donor being 1:2.4. When the reaction was carried out at 40°C for 22 hours, the enantiomeric excess of (S)-1 was able to reach as high as 99%, with a conversion rate of 52%. Therefore, a green and highly efficient protocol for the asymmetric resolution of (R,S)-1 was developed. Using the obtained (S)-1 as the raw material, (S)-4 was successfully synthesized with an optical purity of 95.3%.

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ORCID

Yinjun Zhang http://orcid.org/0000-0001-8504-1036
Jianyong Zheng http://orcid.org/0000-0002-9821-1062

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