Title: Convenient enzymatic resolution of cis-6-benzyltetrahydro-1H-pyrrolo[3,4-b]pyridine-5,7(6H,7aH)-dione using lipase to prepare the intermediate of moxifloxacin

Author: Yuanyuan Li Anming Wang Yingqiang Shen Pengfei Zhang

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Research highlights:

(1) The optically pure (4a*R*,7a*S*)-**1** was successfully prepared efficiently using the enzymatic resolution.

(2) The target amine was obtained in conversion (49%) and an excellent degree of enantiomeric purity (>99%) when *Candida antarctica* lipase B (CAL-B) was combinated with phenyl allyl carbonates donors.

(3) Based on the enzymatic acylation, the chemo-enzymtic dynamic kinetic resolution for rac-cis-1 to obtain the amine (4a*R*,7a*S*)-**1** would be approached in the coming future when racemizing (4a*S*,7a*R*)-**1** by interchange between carboxide and enol.

Convenient enzymatic resolution of cis-6-benzyltetrahydro-1H-pyrrolo[3,4-b]pyridine-5,7(6H,7aH)-dione using lipase to prepare the intermediate of moxifloxacin

Yuanyuan Li[1], Anming Wang[1, 2]*, Yingqiang Shen[1], Pengfei Zhang[1]

[1]College of Materials, Chemistry and Chemical Engineering,

Hangzhou Normal University, Hangzhou 310036, P. R. China

[2]Research Center of Bioconversion and Biopurification, Ministry of Education,

Zhengjiang University of Technology, Hangzhou 310014, P. R. China

*Corresponding author at:

College of Material, Chemistry and Chemical Engineering,

Hangzhou Normal University, Hangzhou 310036, P. R. China

TEL: +86-571-28865978

FAX: +86-571-28865630

E-mail: waming@hznu.edu.cn; anmwang@hotmail.com

Abstact:

A convenient and efficient route has been successfully developed for preparing (4a*R*,7a*S*)-6-benzyltetrahydro-1H-pyrrolo[3,4-b]pyridine-5,7(6H,7aH)-dione through enzyme-mediated kinetic resolution processes under mild and environmentally acceptable conditions. Different reaction condition factors, including acyl agent, solvent, reaction temperature, and the concentration of Et_3N, were optimized in order to establish the optimal reaction conditions for the enzymatic kinetic resolution of secondary amines. It was demonstrated that acyl agent, the concentration of Et_3N or the type of solvent have a greater influence on the selectivity and rate of the asymmetric acylation reaction in the process of the enzymatic resolution, respectively. The Target product amine was obtained in good conversion (49%) and an excellent degree of enantiomeric purity (>99%) when *Candida antarctica* lipase B (CAL-B) and phenyl allyl carbonates were combinated to use in the tert-butyl methyl ether (TBME) medium. This method will be an alternative to the traditional route for producing the optically pure intermediate of moxifloxacin.

Key Words:

moxifloxacin; *Candida antarctica* lipase B; enantioselective; asymmetric acylation; dynamic kinetic resolution

1. Introduction

The optically pure (4a*R*,7a*S*)-6-benzyltetrahydro-1H-pyrrolo[3,4-b] pyridine-5,7(6H,7aH)-dione has attracted considerable attention because it is an important building blocks in synthesis of (4a*S*,7a*S*)-6-benzyloctahydro-1H-pyrrolo[3,4-b]pyridine, which is commonly used as a crucial intermediate in the preparation for moxifloxacin or its pharmacentically acceptable salts with antibacterial effectiveness [1-3]. Particularly, moxifloxacin is one of the most widely used fourth generation fluoroquinolones antibacterial agents with well tolerated drug developed by Bayer AG, which can exert their powerful antibacterial activity by interacting with two essential bacterial enzymes, DNA gyrase and DNA topoisomerase IV [4-6]. In the preparation of moxifloxacin, (4a*R*,7a*S*)-6-benzyltetrahydro-1H-pyrrolo[3,4-b]pyridine-5,7(6H,7aH)-dione ((4a*R*,7a*S*)-**1**) is the key chiral intermediate and is often recieved in the ways to split the chemically synthesized amine enantiomers such as recrystallization of diastereomeric salts [7]. (-)-2,3:4,6-di-O–Isopropyl-idene-2-keto-L-gulonic acid which is similar to tartaric acid has been reported to be well used in the separation of the above-mentioned enantiomers [8]. Unfortunately, there are some drawbacks in the chemical resolution of the enantiomer, such as harsh reaction conditions (high reaction temperature), poor crystallinity, low yield, moderate enantiomeric excesses and poor optical purity of products, etc. Additionally, a large number of organic solvents are required which may cause

a great waste and environmental pollution.

As the most common biocatalysis for enantioselective transformation, enzymatic catalysis possesses many remarkable advantages such as high enantioselectivity, mild reaction condition, small amount of organic solvent, and the ability to be recycled by the simple separation of the immobilized enzyme [9, 10]. Now, the methods for the resolution of chiral secondary amines have been developed by using the specific enzymes such as aminoacylases [11], proteases [12, 13] and amino oxidases [14, 15] which could be obtained by direct evolution. As known to all, lipases among the enzymes of synthetic interest usually were the most effective tool for the enzymatic resolution synthesis of enantiomerically pure compounds over the last two decades because of their outstanding merits of operability, low cost, and excellent tolerance of a broad range of racemic substrates [16-18]. Generally, most lipase-mediated kinetic resolution reactions involved acylation of chiral alcohols [19-23] or hydrolysis of esters [24-26], while in few reports the enzyme was used to prepare chiral primary and secondary amines despite the fact that gentle reaction conditions are required for the fomation of these nitrogenous compounds [27-31].

However, lipase was seldom used to catalyze the kinetic acylation or alkoxycarbonylation of chiral cyclic secondary amines, even though they are useful building blocks for preparing many valuable products [32-41]. This may be due to the structure of the cyclic secondary amines which could cause the

steric hindrance of substrate access to the enzyme active site [13].

In this work, several commercially available lipases were screened to identify the ideal enzyme with the desired reactivity and enantioselectivity in the acylation of the cyclic secondary amine enantiomers (Scheme 1). The corresponding immobilized enzyme of screened lipase was further applied in the enzymatic process to isolate the intermediate (cyclic secondary amines) of moxifloxacin in an enantiomerically pure form. A comprehensive optimization of the reaction parameters in terms of the temperature, solvent and acyl donor was proceeded in order to obtain the high conversion rate and enantiomeric excesses in the chiral separation process of the racemic intermediate (rac-cis-**1**) of moxifloxacin.

2. Experimental

2.1. General

Racemic cis-6-benzyltetrahydro-1H-pyrrolo[3,4-b]pyridine-5,7(6H,7aH)-dione as a gift was given by Zhejiang Apeloa Kangyu Pharmaceutical Co., Ltd.. *Candida antarctica* lipase type B (CAL-B, Novozyme 435, ≥5,000 U/g)，*Candida antarctica* lipase type A (CAL-A, ≥5,00 U/g), *Candida antarctica* lipase (CAL, ≥1.5 U/mg), lipase from *Porcine pancreas* type II, (PPL, 30-90 units/mg protein using triacetin) and lipase from *Candida rugosa* type VII, (CRL, ≥700 unit/mg) were purchased from Sigma–Aldrich. rac-cis-**1** was stored over P_2O_5 at 0 °C. Chemical reagents were used as received from

commercial sources and used without further purification. Solvents were distilled over an adequate desiccant under nitrogen. Anhydrous solvents were stored over freshly reactivated 4 Å molecular sieves.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 MHz or Bruker 400 MHz spectrometer at ambient temperature with $CDCl_3$ or DMSO-d_6 as solvent unless otherwise noted and tetramethylsilane (TMS) was used as the internal standard. ^{1}H NMR data were reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = double– doublet, m = multiplet and br = broad), coupling constant (J values, Hz). ^{13}C NMR data were reported in terms of chemical shift (δ ppm). Mass spectra were determined using an Agilent 1100 series GC/MSD Trap instrument. High resolution mass spectra (HRMS) were determined using an Agilent 6210 TOF LC/MSD Trap instrument. Analytical thin layer chromatography (TLC) was performed on Merck precoated TLC (silica gel 60 F254) plates. Flash chromatography was performed using silica gel 60 (230–240 mesh). Measurement of the optical rotation was done in a Perkin–Elmer 241 polarimeter. High performance liquid chromatography(HPLC) analysis was carried out in a Waters 715 Ultra Wisp UV detector using a Daicel Chiralcel IC column (25 cm × 4.6 mm I.D.)(90% n-hexane/2-propanol; 1.0 mL/min; 254 nm; 35 °C for rac-cis-**1** and 80% n-hexane/ethanol; 1.0 mL/min; 254 nm; 35 °C for rac-cis-**3**). Retention times were as follows: for **1** [(4a*R*,7a*S*)-**1** 20.0 min, (4a*S*,7a*R*)-**1** 23.0 min], **3**

[(4a*R*,7a*S*)-**3** 12.8 min, (4a*S*,7a*R*)-**3** 15.0 min].

2.2. Chemical synthesis of cis-allyl 6-benzyl-5,7-dioxooctahydro-1H-pyrrol-o[3,4-b]pyridine-1-carboxylate rac-cis-3

Pyridine (14.5 uL, 0.18 mmol) was added to a solution of rac-cis-**1** (244 mg, 1mmol) in dry CH_2Cl_2(2.5 mL) in a nitrogen atmosphere and the mixture was cooled in an ice bath. Allyl chloroformate (130 uL, 1.1 mmol) was added dropwise and the system was allowed to warm up to room temperature. After stirring for 4 h, no starting material was detected by TLC analysis. The solvent was evaporated under reduced pressure and the residue obtained was purified by flash chromatography (10% EtOAc/hexane) to make 300 mg of carbamate rac-cis-**3** as a pale yellow oil (yield: 92%). Rf (10% EtOAc/hexane): 0.4; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29-7.36 (m, 5H), 5.93-5.94 (m, 1H), 5.22-5.38 (m, 3H), 4.66 (s, 4H), 3.92-4.03 (m, 1H), 2.77-3.02 (m, 2 H), 2.05 (s, 1H), 1.49 (s, 3H)；^{13}C NMR ($CDCl_3$, 100 MHz): δ 176.85, 174.89, 156.17, 135.51, 132.51, 128.81, 128.73, 128.09, 117.88, 66.78, 52.95, 42.44, 41.50, 38.87, 24.28, 20.52; GC-MS: (m/e, 328.1, M+); HRMS(ESI^+, m/z) calcd for $(C_{18}H_{20}N_2NaO_4)^+$ $(M + Na)^+$ 351.1315, found 351.1330. HPLC elution times: (4a*R*,7a*S*)-**3** 12.8 min, (4a*S*,7a*R*)-**3** 15.0 min; conditions: Daicel Chiralcel IC column (25 cm × 4.6 mm I.D.), eluent n-hexane/ethanol (80:20) at 1.0 mL/min flow rate.These data are in accordance with the values reported in the literature.

2.3. Typical procedure for the enzymatic kinetic resolution of racemic cis-6-benzyltetrahydro-1H-pyrrolo[3,4-b]pyridine-5,7(6H,7aH)-dione rac-

cis-1

Amine rac-cis-**1** (49mg, 0.2mmol), and phenyl allyl carbonate (89mg, 0.5mmol) were dissolved in dry TBME (2mL); 4 Å molecular sieves (100mg) and Et_3N (150uL, 1mmol) were added. The reaction was started by the addition of *Candida antarctica* lipase type B (CAL-B, Novozyme 435) (100 mg) and was shaken at 250 rpm on a rotary shaker at 45 °C for the necessary time to achieve a good kinetic resolution. The progress and ee (%) of the reaction were monitored by TLC and HPLC analysis. The reaction was stopped at close to 50% conversion by removal of the enzyme and molecular sieves and washed with TBME (2mL). The TBME organic layer was washed with 2 M HCl (2 × 5mL), and the combined acid was then washed with dichloromethane (2 mL). The pH of the aqueous layer was then adjusted to 9 with ammonia water (25%) and then extracted with dichloromethane (2 × 3mL). The organic phase was dried over Na_2SO_4 and evaporated to obtain (4a*S*,7a*R*)-**1** as a light yellow oil (20mg, yield: 41%, ee: 97%). The TBME layer was mixed with 2 M NaOH (5 mL), dried over $MgSO_4$, and evaporated to obtain carbamate (4a*R*,7a*S*)-**3** as a pale yellow oil (19mg, yield: 39%, ee: >99%).

2.4. Hydrolysis of carbamate (4a*R*,7a*S*)-3

A mixture of (4a*R*,7a*S*)-**3** (0.1mmol) and concentrated hydrochloric acid (1mL) was stirred refluxing at 110 ℃ for 5 h. After cooling to room temperature, 2mL H_2O was added into the mixture and washed with dichloromethane (2 × 2mL). The aqueous layer was then adjusted PH to 9 with

ammonia water (25%) and then extracted with dichloromethane (2 × 3mL). The organic phase was dried over Na_2SO_4 and evaporated to obtain the crude product (4a*R*,7a*S*)-**1** as a light yellow oil (22mg, yield: 92%, ee: >99%). $[\alpha]_D^{23}$ = -25.10 (C = 5, EtOH); GC-MS: (m/e, 244.1, M^+); ^{1}H NMR ($CDCl_3$, 500 MHz): δ7.25-7.36 (m, 5H), 4.64 (s, 2H), 3.82-3.84 (d, 1H, J=6.9Hz), 2.83-2.87 (dd, 1H), 2.76-2.80 (m, 1H), 2.63-2.68(m, 1H), 2.20(br s, 1H), 1.92-1.99 (m, 1H), 1.61-1.68 (m, 1H), 1.48-1.52 (m, 2H)；^{13}C NMR ($CDCl_3$, 100 MHz): δ177.94, 177.78, 135.84, 128.65, 128.40, 127.85, 55.39, 42.29, 42.12, 39.23, 23.02, 22.34. These data are in accordance with the values reported in the literature [1].

3. Result and discussion

All reactions were performed with the strict exclusion of water and molecular sieves were added to absorb any traces of moisture as well as the liberated alcohol. Primarily, the higher moisture of the reaction system was, the fewer available acylating agent remained and as a result the reaction rate was slow down. The adventitious hydrolysis of carbonate releases an acid, which could inhibit the enzyme activity or cause the substrate protonation that was unfavorable for reaction. It is worth mentioning that some activated esters are partly hydrolyzed for the presence of water in the seemingly dry enzyme preparation, always giving some acid in the reaction mixture. Thus, in all experiments triethylamine was used as the non-nucleophilic base to neutralize

the released acid to enhance the reaction rate and the enantioselectivity of the process.

3.1. Screen of enzyme

The enzymatic kinetic resolution of rac-cis-**1** was then carried out using lipase from different sources such as *Candida antarctica* lipase (CAL), *Candida antarctica* lipase A (CAL-A), *Candida antarctica* lipase B (CAL-B), *Porcine pancreas* lipase (PPL) and *Candida rugosa* lipase (CRL). Initially, we focused on the enzymatic acylation of rac-cis-**1** for enzyme screening to select the most suitable lipase. The enol esters were chosen as the acyl donor because of their fast rate of enzymatic acylation. The tautomerism of the enol to ketone means that the process is irreversible and the separation of product after the reaction is simple [36, 42]. Thus, vinyl acetate was used as the acyl donor in toluene. Some of these lipases were suited for catalyzing the synthesis of the amide, while the amine that remained was racemic and the reaction mixture became brown because high background reactions would occur when these enol esters were used as acylating agents. Moreover, many others activated esters, including 2,2,2-Trifluoroethyl butyrate and ethyl acetate, which are well-known in the enzyme-catalyzed acylation of alcohols and aliphatic amines [9], were also selected as acyl donors. Unfortunately, these acyl donors did not allow the formation of the corresponding amides for the chiral secondary amine (4a*R*,7a*S*)-**1** in our work. Based on the previous process that showed excellent results to the enzymatic resolution of chiral secondary amines, in which allyl

carbonates was used as alkoxycarbonylating reagent [12, 36, 38-41], we turned our attention to the study of the alkoxycarbonylation reaction of rac-cis-**1**.

When phenyl allyl carbonate was selected as the alkoxycarbonylating reagent for an initial enzyme activity screening in order to increase the rate and selectivity, as shown in Table 1. PPL, CAL-A and CRL did not show any acylating activity, while CAL-B showed a high enantioselectivity (>99%) and a conversion of 4.56% to form the carbamate (4a*R*,7a*S*)-**3** after 120 h (entry 1). *Candida antarctica* lipase (CAL) was also used to catalyze the biotransformation but even lower conversions than CAL-B and moderate enantioselectivity were observed (entry 2). For CAL-B, the high enantioselectivity for the chiral secondary amine (4a*R*,7a*S*)-**1** was the key for the subsequent resolution of the racemic intermediate (rac-cis-**1**).

3.2. Effect of solvent and reaction temperature on the enzyme kinetic resolution

The effect of some solvents with different polarities, including dimethyl formamide (DMF), acetonitrile, ethanol, acetone, tetrahydrofuran, dichloromethane, tert-butyl methyl ether (TBME), diisopropyl ether and toluene, on the enzymatic kinetic resolution was investigated. As presented in Table 2, TBME was the most efficient medium in carrying out the enzymatic reaction. In this medium, both a higher conversion and a high enantioselectivity were obtained. The DMF with high solvent polarity may lead to enzyme denaturation through depriving the water from the surface of enzyme (entry 1).

Other solvents, such as acetonitrile, acetone, tetrahydrofuran, dichloromethane, and toluene led to low conversion (entries 2, 4-6, 9) and ethanol did not promote the product formation (entry 3). The catalytic capacity of the immobilized enzyme was essentially the same in moderate polar solvents, including TBME and isopropyl ether. The conversions in the two mediums were close to 50% along with excellent enantioselectivity. Nevertheless, the shorter reaction time was obtained in TBME, which may result from TBME's higher dissolving capacity to the substrate allowing the possibility of increasing the mass transfer. Thus, TBME is a good candidate for optimizing the reaction conditions of the enzymatic resolution of rac-cis-**1**. In order to improve the activity of enzyme, the effect of the temperature on the acylation was also examined (Table 2). The results showed that the highest conversion, reaction rate and ee_p values were observed when the temperature of the reaction mixture was at 45 ℃ (entry 11).

3.3. Effect of alkoxycarbonylating reagents and their concentrations on the conversion

Various alkoxycarbonylating reagents were introduced into the CAL-B catalytic reaction for the acylation the rac-cis-**1** in TBME(Scheme 2). The results in Table 3, showed that no corresponding amide product was formed when the diallyl carbonate **2**b was used as the alkoxycarbonylating reagent (entry 2). However, nearly 50% of the conversion rate was yielded using the

other three asymmetrical carbonates (entries 1, 3, 4). In addition, excellent enantioselectivities (>99%) were all observed when using these three efficient alkoxycarbonylating reagents. The appearance of the phenol by-product did not cause enzyme inhibition and it could be removed easily with a base wash at the end of the resolution. Noteworthily, the cost of the phenyl allyl carbonate **2**a is even lower than that of carbonates **2**c and **2**d.

As we well know, the concentration of the acyl donor is a key factor for the reaction rate. Thus, the effect of the amount of acyl donor on the enzyme catalytic reaction was investigated closely. The results were showed in Table 3. Originally, a small amount of carbonate was used in acylation of substrate in order to avoid any possible formation of by-products and reduce inhibitiory effects because of high concentrations of reactants. When the amount of **2**a was added up to 2.5 equivalents, around 50% of the conversion was obtained with excellent enantioselectivities (entry 1). However, the inhibitory effects at high concentration were observed and the conversion rate decreased distinctly (entries 6 and 7) when 5 and 10 equivalent amount of acyl donors were used.

3.4. Effect of the concentration of Et_3N on the acylation reaction rate

To furthuer optimise the reaction coditions, the effect of the concentration of Et_3N on the acylation reaction rate was developed (Figure 1). Interestingly, with the increase of the amount of extra Et_3N, the reaction became progressively faster, and both the enantiomeric excess (99%) and the

enantioselectivity (>200) remained high. In some papers, it was reported that the by-product acid could inhibit the enzyme or substrate protonation that was unfavorable for acylation reaction [43-45]. Thus, we speculated that the base has the ability to neutralize the by-product acid which was released from the adventitious hydrolysis of carbonate by water in the seemingly dry enzyme preparation.

3.5. Carbamate hydrolysis

The catalytic hydrolysis of acid and base are two classic approaches to hydrolyze carbamate. Here, common acid and base were used as the catalysts to hydrolyze the carbamate group. We found that no any (4a*R*,7a*S*)-**1** was obtained when the sodium hydroxide (2 M) base-catalyzed hydrolysis of the amide was under a high temperature. It was possible that excessive or improper hydrolysis through sodium hydroxide occurred and led to the hydrolysis of other amide chemical bond in the (4a*R*,7a*S*)-**3** instead of only the carbamate group. However, the proper and desired hydrolysis of the amide could be achieved by refluxing with concentrated hydrochloric acid (12 M), resulting in up to 92% high yields. Therefore, concentrated hydrochloric acid can effectively solve the problem that hydrolyzing the amide (4a*R*,7a*S*)-**3** to release the amine (4a*R*,7a*S*)-**1**.

Conclution

In this work, a practical synthetic route was presented for the production of optically active secondary amine (4a*R*,7a*S*)-**1** through enzymatic kinetic resolution using the immobilized CAL-B. The optimal conditions for the enzymatic resolution is the combination of the immobilized CAL-B with phenyl allyl carbonates at 45℃ in TBME and a reaction mixture containing the substrate and Et_3N (molar ratio, 1:5). After the hydrolysis of (4a*R*,7a*S*)-**3**, the target product (4a*R*,7a*S*)-**1** was successfully obtained with a good conversion (49%) and an excellent degree of enantiomerical purity (99%). In conclusion, this proposed way proved to be simple, effective and more practical, which is expected to be an alternative to those previously described traditional methods in producing optically active secondary amine (4a*R*,7a*S*)-**1** after further scale-up experiment. In addition, (4a*S*,7a*R*)-**1**, which couldn't be acylated using CAL-B, could be isolated and further catalyzed to (4a*R*,7a*S*)-**1** to form the enantiomer mixtures again. Based on this transformation, our work will focus on the chemo-enzymtic dynamic kinetic resolution of rac-cis-**1** to obtain the amine (4a*R*,7a*S*)-**1** in the coming future.

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Figure legends

Scheme 1 Enzymatic kinetic resolution of rac-cis-**1** using phenyl allyl carbonate.

Scheme 2 Enzymatic kinetic resolution of rac-cis-**1** using different allyl carbonates. Solvent, TBME; molar ratio of amine versus carbonate, 1:2.5; reaction temperature, 45 ℃.

Figure Effect of the substrate-to-Et_3N molar ratio on the enzymatic resolution. Reaction conditions: rac-cis-**1** (0.2mmol); CAL-B (50mg/ml); phenyl allyl carbonate (0.5mmol); TBME (2 mL); 45 ℃; 250 rpm; 4 Å molecular sieve (100mg). (A) substrate-to-Et_3N molar ratio, 1: 0 ; (B) substrate-to-Et_3N molar ratio, 1:1; (C) substrate-to-Et_3N molar ratio, 1:5; (D) substrate-to-Et_3N molar ratio, 1:10.

Table captions

Table 1 Alkoxycarbonylation of rac-cis-**1** with phenyl allyl carbonate catalyzed by various lipases

Table 2 CAL-B enzymatic kinetic resolution of rac-cis-**1** with phenyl allyl carbonate using different solvents

Table 3 Enzymatic kinetic resolution of rac-cis-**1** with different carbonates

Table 1 Alkoxycarbonylation of rac-cis-**1** with phenyl allyl carbonate catalyzed by various lipases[a]

Entry	Enzyme	t (h)	ee_s (%)[b]	ee_p (%)[b]	c (%)[c]	E[d]
1	CAL-B	120	5	>99	5	>200
2	CAL	144	1	56	2	3

[a] Reaction conditions: rac-cis-**1** (0.2mmol); enzyme (50mg/ml); phenyl allyl carbonate (0.5mmol); Et_3N (150uL, 1mmol); toluene (2 mL); 37 ℃; 150 rpm; 4 Å molecular sieve (100mg).

[b] Calculated by HPLC.

[c] $c=ee_S/(ee_S+ee_P)$.

[d] $E= \ln[(1- c)*(1-ee_S)]/\ln[(1-c)*(1 + ee_S)]$.

Table 2 CAL-B kinetic resolution of rac-cis-**1** with phenyl allyl carbonate using different solvents[a]

Entry	Solvent	Log P	T (^{0}C)	t (h)	ee_s (%)[b]	ee_p (%)[b]	c (%)[c]	E[d]
1	Dimethyl formamide	-1.0	37	22	—	—	—	—
2	Acetonitrile	-0.33	37	22	7	>99	7	>200
3	Ethanol	-0.24	37	22	—	—	—	—
4	Acetone	0.29	37	22	2	>99	2	>200
5	Tetrahydrofuran	0.49	37	22	10	>99	9	>200
6	Dichloromethane	1.2	37	22	1	>99	1	>200
7	Tert-butyl methyl ether	1.35	37	22	90	>99	47	>200
8	Diisopropyl ether	1.9	37	58	87	>99	47	>200
9	Toluene	2.5	37	120	5	>99	5	>200
10	Tert-butyl methyl ether	1.35	30	22	69	>99	41	>200
11	Tert-butyl methyl ether	1.35	45	22	97	>99	49	>200

[a] Reaction conditions: rac-cis-**1** (0.2mmol); CAL-B (50mg/ml); phenyl allyl carbonate (0.5mmol); Et_3N (150uL, 1mmol); solvents (2 mL); 250 rpm; 4 Å molecular sieve (100mg).

[b] Calculated by HPLC.

[c] $c=ee_S/(ee_S+ee_P)$.

[d] $E= \ln[(1- c)*(1-ee_S)]/\ln[(1-c)*(1 + ee_S)]$.

Table 3 Enzymatic kinetic resolution of rac-cis-**1** with different carbonates[a]

Entry	Acyl donor	Ratio[e]	t (h)	ee_s (%)[b]	ee_p (%)[b]	c (%)[c]	E[d]
1	2a	1:2.5	22	97	>99	49	>200
2	2b	1:2.5	22	—	—	—	—
3	2c	1:2.5	22	93	>99	48	>200
4	2d	1:2.5	22	92	>99	48	>200
5	2a	1:1	22	53	>99	35	>200
6	2a	1:5	22	43	>99	30	>200
7	2a	1:10	22	26	>99	21	>200

[a] Reaction conditions: rac-cis-**1** (0.2mmol); CAL-B (50mg/ml); Et_3N (150uL, 1mmol); TBME (2 mL); 250 rpm; 4 Å molecular sieve (100mg).

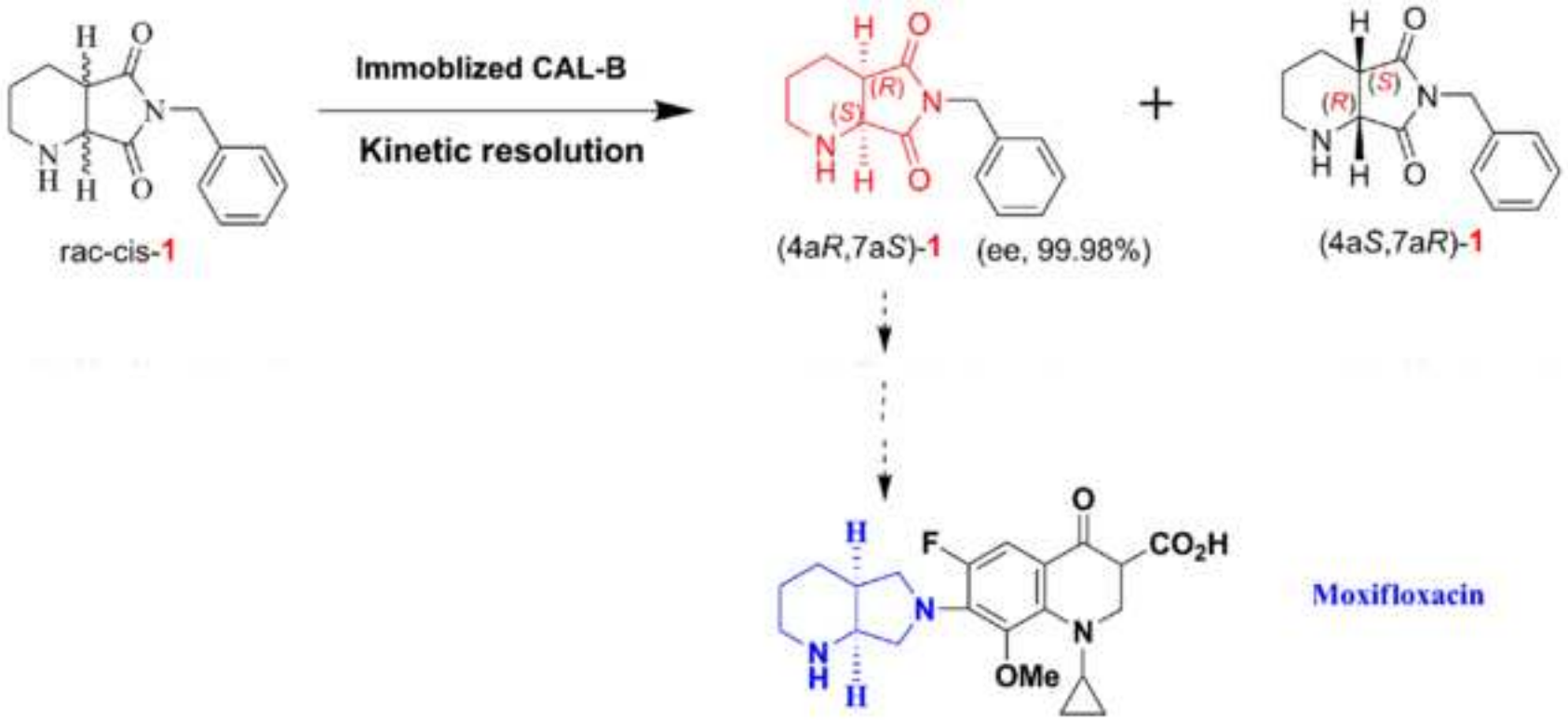
[b] Calculated by HPLC.

[c] $c=ee_S/(ee_S+ee_P)$.

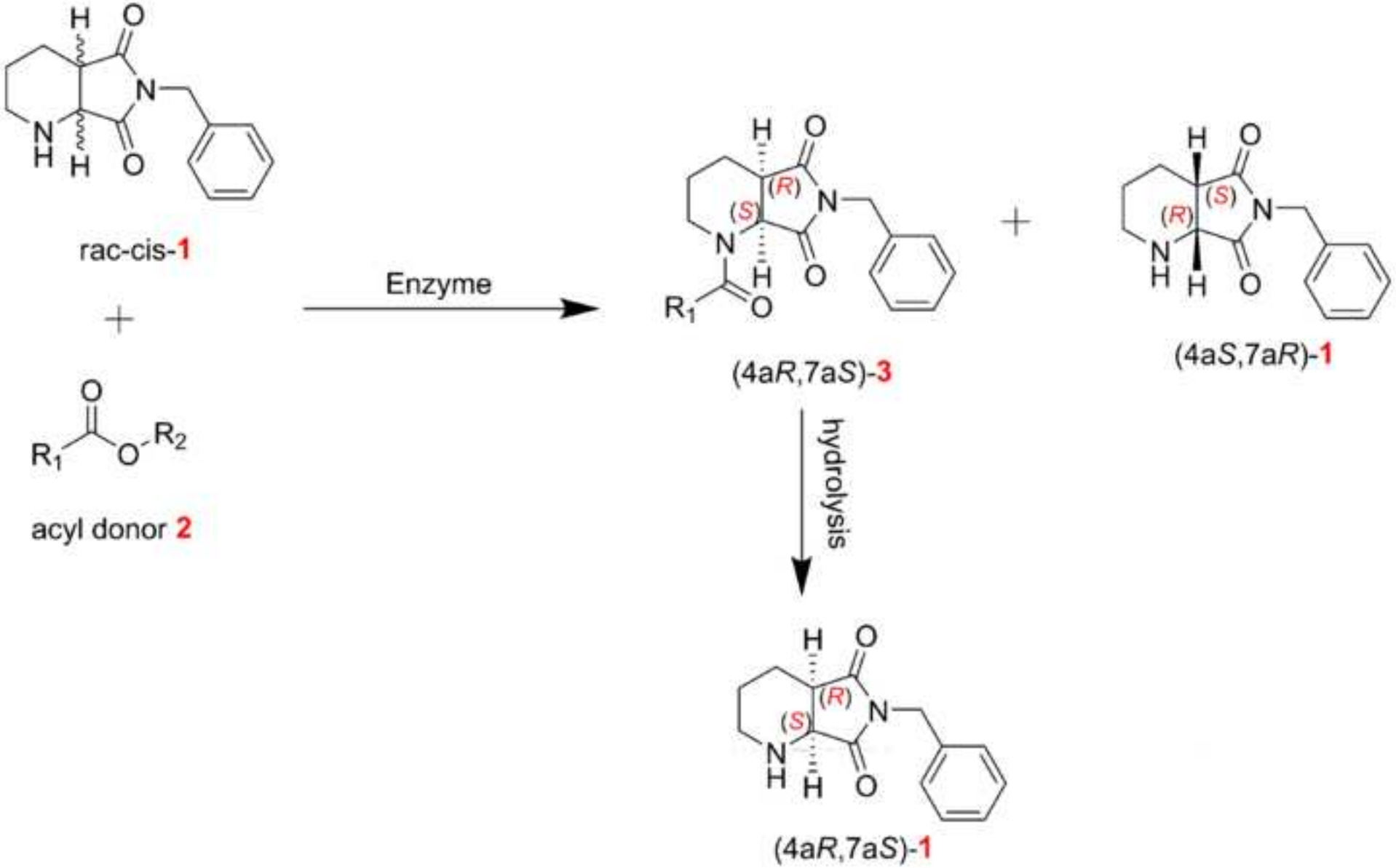
[d] $E= \ln[(1- c)*(1-ee_S)]/\ln[(1-c)*(1 + ee_S)]$.

[e] The ratio of amine/diallyl carbonate in molar.

*Graphical Abstract (for review)



Scheme 1


rac-cis-1
+
acyl donor 2
Enzyme
(4aR,7aS)-3
+
(4aS,7aR)-1
hydrolysis
(4aR,7aS)-1

Scheme 2

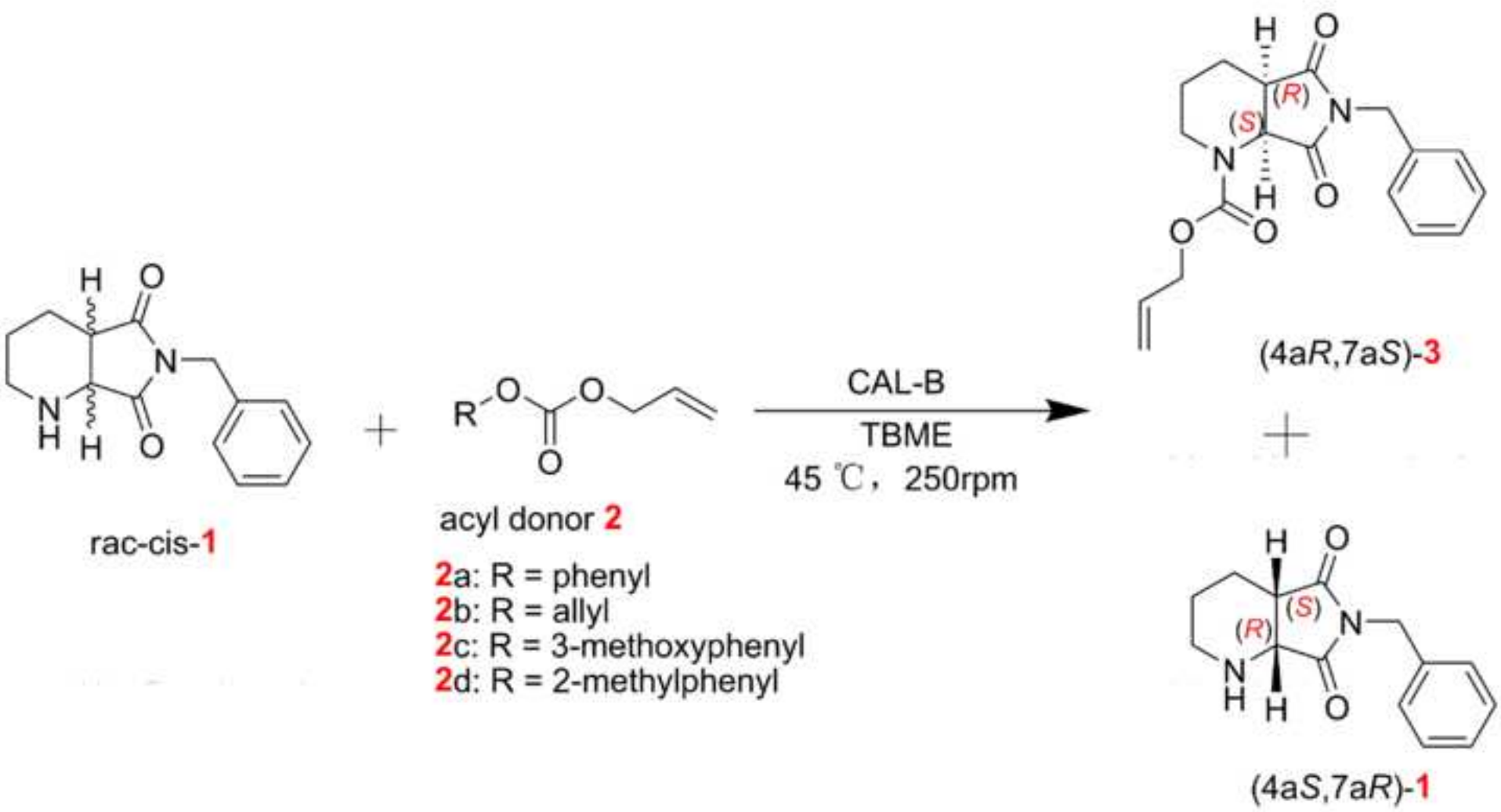
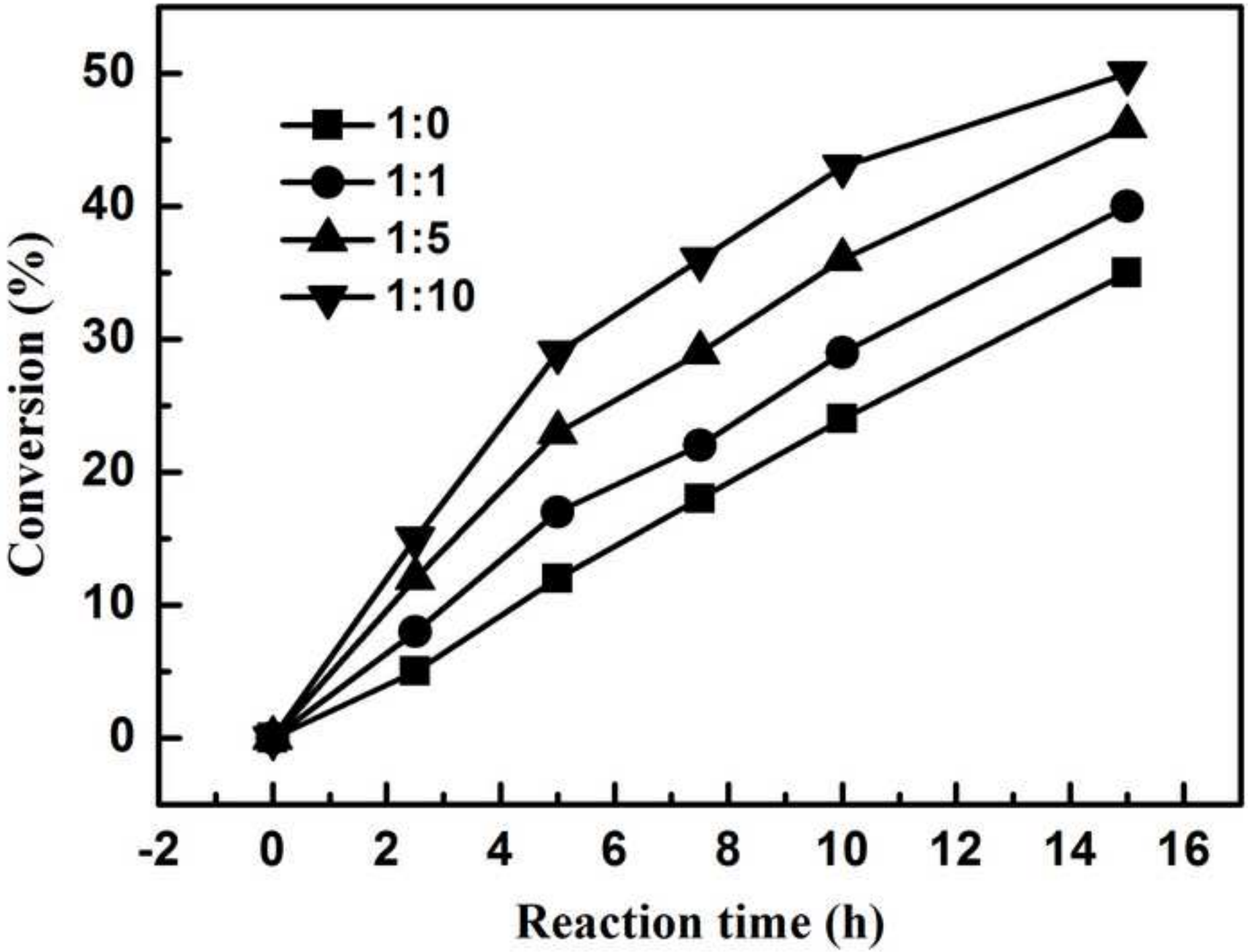

rac-cis-1
acyl donor 2
2a: R = phenyl
2b: R = allyl
2c: R = 3-methoxyphenyl
2d: R = 2-methylphenyl
CAL-B
TBME
45 ℃， 250rpm
(4aR,7aS)-3
(4aS,7aR)-1

Figure 1


Conversion (%)
Reaction time (h)
1:0
1:1
1:5
1:10
50
40
30
20
10
0
-2
0
2
4
6
8
10
12
14
16