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Development of a Practical Enzymatic Process for Preparation of (*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol

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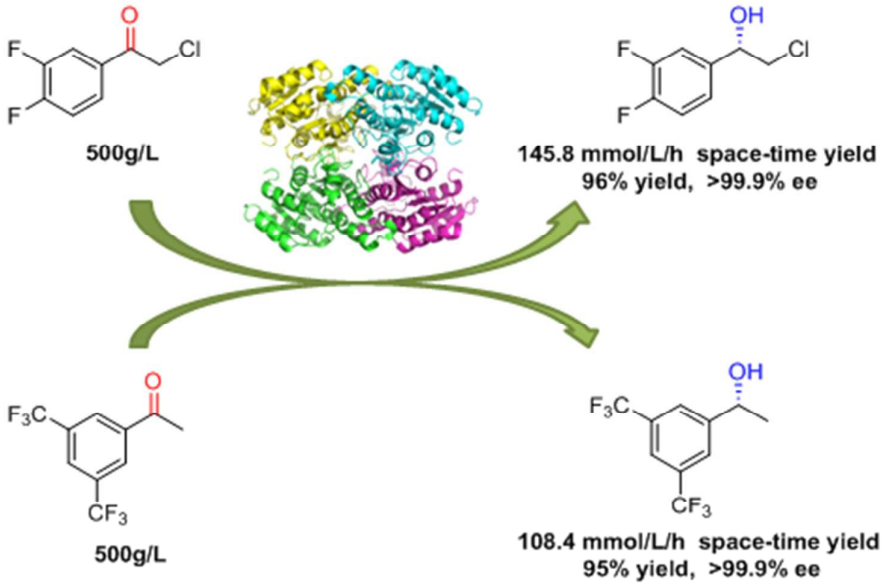
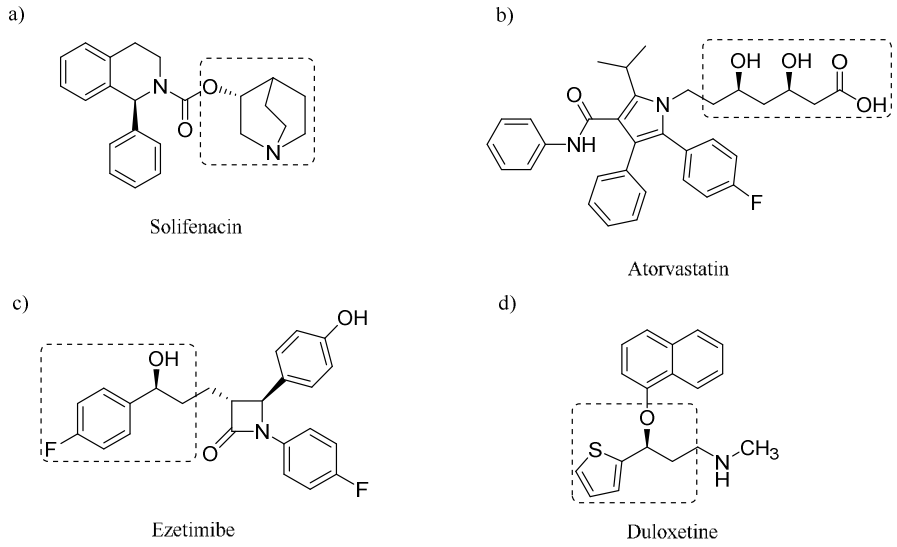

F
F
O
Cl
500g/L
OH
F
F
Cl
145.8 mmol/L/h space-time yield
96% yield, >99.9% ee
F3C
O
CF3
500g/L
F3C
OH
CF3
108.4 mmol/L/h space-time yield
95% yield, >99.9% ee

Abstract: (*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol (**1**) is a vital chiral intermediate for the synthesis of Ticagrelor—an effective treatment for acute coronary syndromes. A ketoreductase (KRED) KR-01 in our KRED library was screened out to transform 2-chloro-1-(3,4-difluorophenyl)ethanone (**2**) into the chiral alcohol **1**. During process optimization, the bioreduction procedure was performed at a substrate concentration of 500 g/L, giving a near 100% conversion with >99.9% ee. The product **1** was directly obtained by extraction and can be used for the synthesis of (1*R*, 2*R*)-2-(3,4-difluorophenyl)cyclopropanecarboxylic acid ethyl ester (**3**) with a yield of 98% and >99.9% de greatly simplifying the original process operation and reducing the safety risk. This process is green and environmentally sound with high productivity of biocatalysis and a space-time yield of 145.8 mmol/L/h. It has an opportunity to be very useful in industrial applications. Additional studies have indicated that KR-01 can also be used to prepare (*R*)‑1-(3,5-bis(trifluoromethyl)phenyl)ethanol (**4**) with a substrate concentration of 500g/L.

Keywords: Enzymatic; Bioreduction; Asymmetric synthesis; Industrial application; Keotreductase

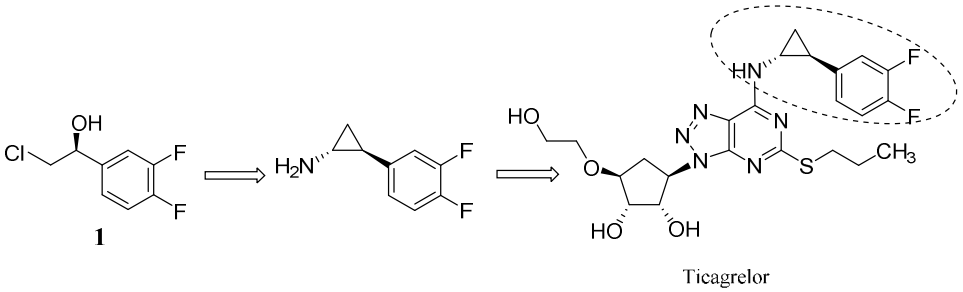
1. Introduction

The synthesis of enantiopure alcohols has attracted significant attention due to their broad versatility as key building blocks in the synthesis of pharmaceuticals.[1] For instance, (*R*)-3-quinuclidinol is an important intermediate of Solifenacin (Scheme 1). Other chiral alcohols are also key components in Atorvastatin, Ezetimibe and Duloxetine.[2-4]



Scheme **1.** Examples of pharmaceuticals that contain chiral alcohol building blocks.

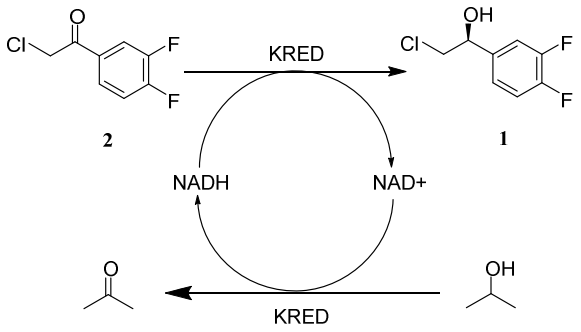
(*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol (**1**) is a valuable unit and is a vital chiral intermediate for the synthesis of Ticagrelor that has been developed as an effective treatment for acute coronary syndromes (Scheme 2).[5] The literatures show that **1** is generally synthesized though CBS-catalyzed reduction system.[5,6] Our team previously found that borane-THF (tetrahydrofuran) catalyzed via (*S*)-2-Me-CBS can reduce the substrate 2-chloro-1-(3, 4-difluorophenyl)ethanone (**2**) to the target product with 93-95% ee. Although chiral purity can be improved by recrystallization, the cost of (*S*)-2-Me-CBS and borane-THF makes the reaction inappropriate for industrial use. In addition, harsh reaction conditions and tedious post-processing also limit this method, implying that the synthesis of **1** remains to be further improved.



Scheme 2. A schematic diagram of the synthesis of Ticagrelor.

Biocatalysis is a popular 'green chemistry' concept with environmentally friendly reaction conditions and stringent stereoselectivity of enzymatic reactions. It is increasingly considered to be an ideal alternative to current chemical methods.[7] Various biocatalysts are available, including ketoreductases (KREDs), that can be used to prepare enantiopure alcohols with broad substrate specificity and high reactivity.[8] There are several successful cases in which KREDs were used in the synthesis of chiral alcohols on an industrial scale.[9]

Recently, Wu et al. synthesized **1** via a mutant reductase ChKRED20.[10] However, the maximum substrate concentration for this KRED was 200 g/L with a space-time yield of 52.5 mmol/L/h. To establish a better process for bioreductivity of ketone **2**, we screened our KRED library to search for a KRED with higher substrate concentration and excellent enantioselectivity. We noticed that the alcohol dehydrogenase from *Leifsonia* sp. Strain S749 (KR-01) had improved productivity toward the chiral alcohol **1** and was suitable for industrial scale production. Besides, it can be used to prepare enantiopure alcohols with other substrate. Herein, we report the processes of screening, applying, and optimizing the KR-01 in order to realize its industrial application for the bioreduction of ketone **2** (Scheme 3).



Scheme 3. Enzymatic reduction of ketone **2** with KR-01.

2. Results and Discussion

2.1. Screening of KREDs

To explore the potential biocatalyst for the asymmetric reduction of ketone **2** to alcohol **1**, 13 KREDs from our library were tested. Table 1 showed that almost all of the KREDs were active toward substrate **2** except for KR-04 and KR-06. Thereinto, KR-09, KR-05, KR-07 and KR-11 displayed relatively moderate reaction activity (>70%). Thus, KR-01 and KR-12 were selected due to their excellent conversion (>99%). Five of them showed the selectivity of (S)-enantiomer, and KR-01, KR-09 and KR-12 had high enantiomeric excess (>99%). Taking into account the conversion and enantioselectivity, we selected KR-01, KR-09 and KR-12 for further study.

Table 1. Screening of KREDs from our enzyme library

Entry	Enzyme	Conversion (%)[c]	Ee (%)[d]	Configuration
1	KR-01[a]	>99	>99	S
2	KR-02[b]	57.6	>99	R
3	KR-03[b]	3.6	91.4	R
4	KR-04[a]	N.D.	N.D.	N.D.
5	KR-05[b]	82.7	>99	R
6	KR-06[a]	N.D.	N.D.	N.D.
7	KR-07[b]	73.0	98.4	S
8	KR-08[a]	1.9	>99	R

9	KR-09[a]	85.8	>99	S
10	KR-10[a]	65.7	>99	R
11	KR-11[a]	75.1	91.9	S
12	KR-12[a]	>99	>99	S
13	KR-13[a]	32.3	96.8	R

Reaction conditions: (a) 10g/L ketone **2**, 20g/L wet cells, 0.5g/L NAD^+, 2eq IPA and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 1.5h at 25 °C and 220 rpm. (b) 10g/L ketone **2**, 20g/L wet cells, 0.2g glucose, 0.1g/L $NADP^+$ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 1.5h at 25 °C and 220 rpm. [c]Measured by HPLC. [d]Measured by chiral HPLC.

2.2. Further Optimization

2.2.1. Effect of substrate concentration

Substrate loading is key parameter for an industrial process for its influence on volumetric productivity.[1] The effect of substrate concentration (50 g/L – 500 g/L) on conversion was investigated. As shown in Figure 1, we conclude that nearly complete conversion was realized via KR-01 and KR-12 when the substrate concentration was lower than 200 g/L. The substrate with KR-09 was difficult to convert. However, when the substrate loading was up to 400 g/L, the conversions decreased to 88.4% and 80.2% by KR-01 and KR-12 respectively. There was no yield increase even at longer reaction time. In conclusion, KR-01 was selected due to its better catalytic ability.

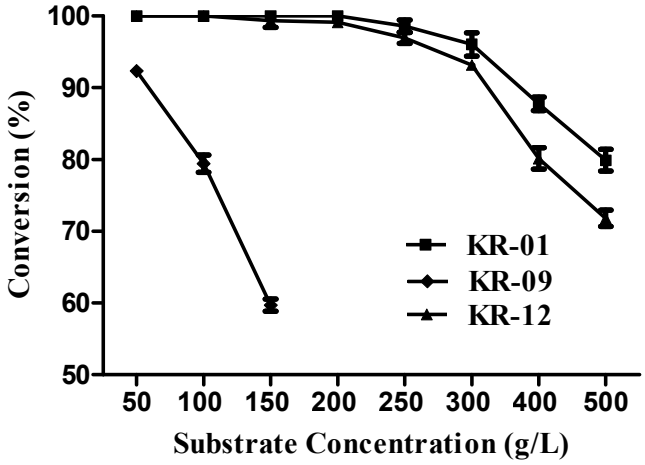


Figure 1. The effect of substrate concentration on the bioreduction of ketone **2**

Reaction conditions: various concentration of ketone **2**, 20g/L wet cells and 0.5g/L NAD^+, 2eq IPA and PBS buffer (100 mM , pH 7.0) in 2mL reaction system for 24h at 25 °C and 220 rpm.

Considering the low conversion rate with high substrate concentration (500 g/L), the process optimization of reaction conditions including co-solvents, temperatures, pH, catalyst loading and NAD^+ loading were subsequently performed.

2.2.2. Effect of co-solvents

In light of the poor solubility of ketone **2**, six organic solvents were chosen to improve the conversion rate in the water-mediated reaction system. Figure 2 shows that the addition of MeOH and IPA increased reaction activities; IPA was the best co-solvent giving an 18% improvement in conversion. Furthermore, IPA is a vital parameter for the enzymatic reaction due to its role as a co-substrate for cofactor regeneration.[11] Thus, IPA was an optimal co-solvent from this screening.

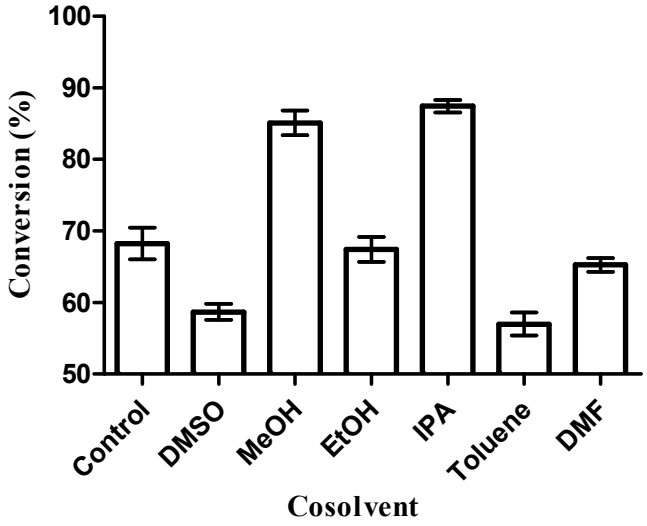


Figure 2. Comparison of the performance in different co-solvents

Reaction conditions: 500g/L ketone **2**, 100g/L wet cells and 0.5g/L NAD^+, 2eq IPA, 10% v/v co-solvent and PBS buffer (100 mM , pH 7.0) in 2mL reaction system for 24h at 25 °C and 220 rpm.

On the other hand, organic solvents in the reaction system may equally change the properties of the solvent including the polarity and dielectric constant. This would

change the three dimensional structure of protein and influence the catalytic activity of the enzyme.[12] Hence, we tried to confirm the optimum concentration by studying different proportions of IPA so as to maximize the solubility of substrate and drive the equilibrium to the desired product. As shown in Figure 3, the highest conversion was achieved at proportion of 3.5 eq IPA, and the enzyme activity dropped gradually with increasing levels of IPA. Therefore, 3.5 eq IPA was the optimal proportion that was chosen into application.

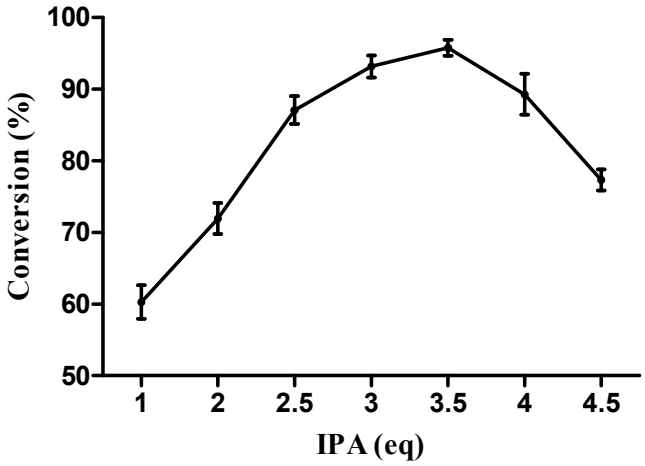


Figure 3. The effect of concentration of IPA on the activity of KR-01

Reaction conditions: the proportions of IPA study was performed with 500g/L ketone **2**, 100g/L wet cells and 0.5g/L NAD^+, 1~4.5eq IPA and PBS buffer (100 mM , pH 7.0) in 2mL reaction system for 24h at 25 ℃ and 220 rpm.

2.2.3. Effect of reaction temperature and pH

Reaction temperature and pH are the most critical influences on enzyme activity in the KRED-medicated reaction. Therefore, after an effective improvement in the conversion rate with high substrate concentration, the parameters (namely reaction temperature and pH) were subsequently explored to determine optimal reaction conditions for industrialization.

Temperature significantly influences both the conversion rate of the reaction and the stability of the protein. On the one hand an increase in temperature would lead to

an increase reaction rate. On the other, high temperature may denature the protein leading to rapid enzyme inactivation.[13] Thus, it is critical to find the appropriate reaction temperature.

As shown in Figure 4, KR-01 showed relatively high reaction conversion at temperatures ranging from 25℃ to 45℃; a temperature of around 35°C was the optimum temperature for the bioreduction. Additionally, we must consider the protein stability as well. Thus, further study was to determine the thermal stability of KR-01 at 25°C, 35°C, and 45°C. Figure 5 shows that the enzyme has better thermal stability at 25℃ and 35°C compared to 45℃. It maintained more than 90% residual activity after storage at 25°C or 35°C for 12 h, but the initial activities reduced by 73% at 45°C after 12 h storage. Therefore, the reaction temperature was set to be 35°C to balance reaction time and reaction activities.

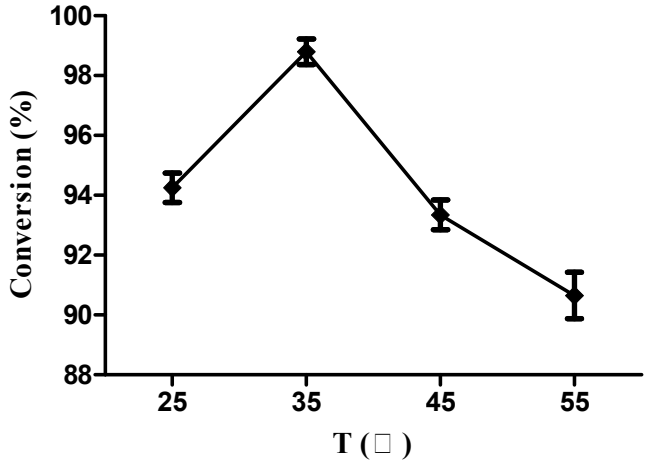


Figure 4. The effect of temperature on the activity of KR-01

Reaction conditions: the temperature study was performed with 500g/L ketone **2**, 100g/L wet cells and 0.5g/L NAD^+, 3.5eq IPA and PBS buffer (100 mM , pH 7.0) in 2mL reaction system for 24h at 220 rpm.

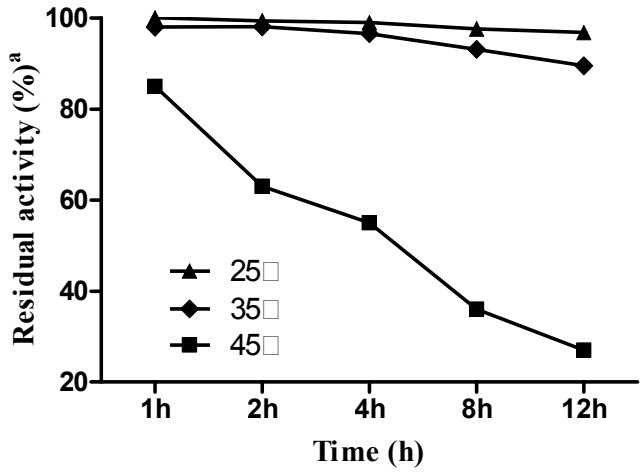


Figure 5. Activities of KR-01 to thermal treatment at 25℃, 35℃ and 45℃ respectively[b]

[a]The initial KR-01 activity of the wet cells was defined as 100%. [b]Reaction conditions: the wet cells were incubated at temperatures of 25℃, 35℃, 45℃ for different periods, respectively.

The pH significantly affects the dissociation of the key amino acids at the active center of the protein, so the choice of pH for enzyme-catalyzed reaction cannot be neglected.[14] The optimum value of pH was then explored in different buffer systems. As shown in Figure 6, no significant changes in conversion were observed from pH 5 to 8—a relatively broad range. The highest conversion of alcohol **1** was found at pH 6.0. Lower (<6) or higher (>6) pH led to a slight drop of the conversion. As a result, pH 6.0 was selected as optimum condition.

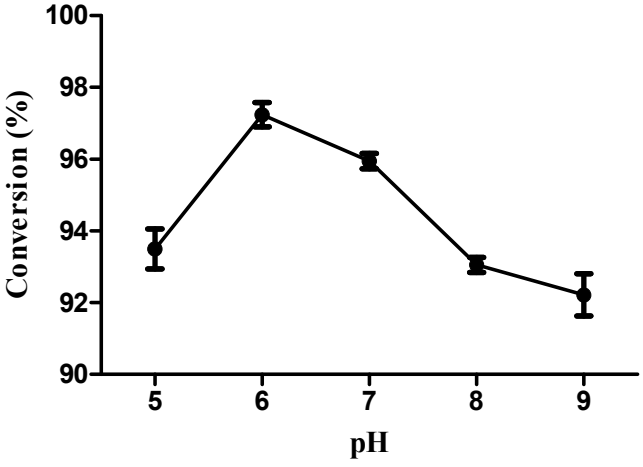


Figure 6. The effect of pH on the activity of KR-01

Reaction conditions: the pH study was performed with 500g/L ketone **2**, 100g/L wet cells 0.5g/L NAD^+ and 3.5eq IPA for 24h at 25 °C and 220 rpm in different PBS buffer, including100mM sodium citrate (pH=5 or 6), 100mM potassium phosphate (pH=7) and 100mM Tris–HCl buffers (pH=8 or 9).

2.2.4. Effect of biocatalyst loading

Under these conditions, chiral alcohol **1** was achieved at near 100% conversion in 10 h at 500 g/L substrate concentration. However, cost is important for a practical and efficient biocatalysis route suitable for industry. Thus, biocatalyst and cofactor loading were determined to minimize cost while maintaining complete conversion.

Increasing the biocatalyst loading is an efficient tool to increase the conversion rate and percent conversion, but inevitably increasing catalyst consumption.[15] Thus, the influence of biocatalyst dose was studied to find relationship between maximum conversion of substrate and utilization of enzyme. The additions of recombinant cells ranging from 10 g/L to 100 g/L were tested. A plot of reaction conversion versus biocatalyst loading was shown in Figure 7. Obviously, conversion steadily increased with increasing biocatalyst loading until to 50 g (wet)/L cells, but slightly increased when the cell amount was 100 g/L. Thus, to minimize the production cost, we reduced the dose of biocatalyst loading to 50 g (wet)/L cells, which was sufficient to achieve 99% conversion of substrate to alcohol **1** within 18 h.

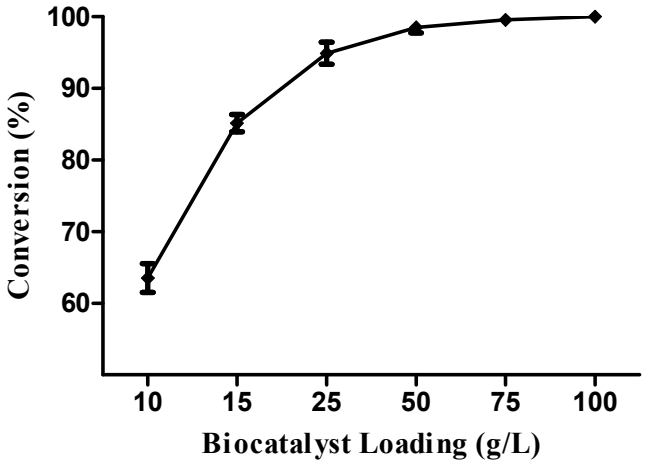


Figure 7. The dosages of biocatalyst loading on the influence of conversion

Reaction conditions: the biocatalyst loading study was performed with 500g/L ketone **2**, 10~100g/L wet cells and 0.5g/L NAD^+, 3.5eq IPA and PBS buffer (100 mM , pH 6.0) in 2mL reaction system for 18h at 35 °C and 220 rpm.

2.2.5. Effect of cofactor (NAD^+) loading

In terms of production cost, the dosage of NAD^+ was of equal importance for KRED-mediated reaction.[16] A series of experiments with different cofactor loading were performed to investigate the effect of NAD^+ concentration on reaction conversion. As shown in Figure 8, the conversions were not significantly different when NAD^+ increased from 0.05 g/L to 0.5 g/L. In addition, the reaction could progress without additional NAD^+ due to the *E. coli* intracellular release of NAD^+, but the reaction rate was low. Therefore, the addition of NAD^+ was necessary at high substrate concentration. Above all, when the production cost was considered, a concentration of 0.05 g/L of NAD^+ was applied.

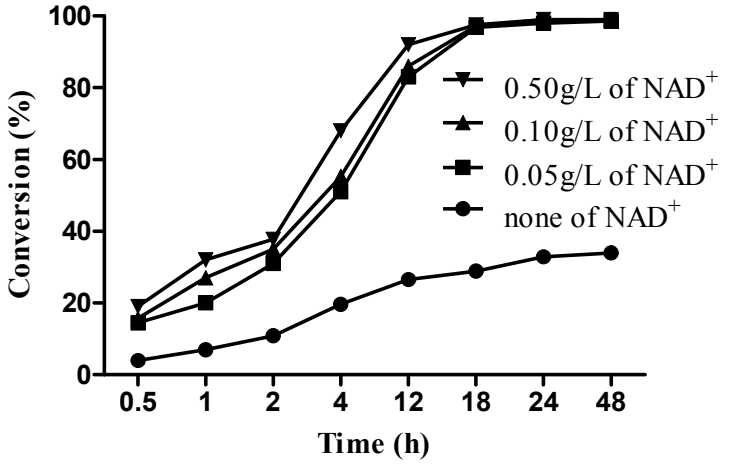


Figure 8. The effect of cofactor loading on the influence of conversion

Reaction conditions: the effect of different cofactor loading study was performed with 500g/L ketone **2**, 50g/L wet cells and 0~0.5g/L NAD^+, 3.5eq IPA and PBS buffer (100 mM , pH 6.0) in 2mL reaction system for 0.5~48h at 35 °C and 220 rpm.

2.3. Scale up the reaction

The reaction was carried out to 200 mL scale under the optimized conditions of 500 g/L substrate, 0.05 g/L NAD^+, 50 g/L biocatalyst, 3.5 eq IPA at pH 6.0 PBS buffer (100 mM) and 35°C. The time course for the bioreduction of ketone **2** to alcohol **1** is shown in Figure 9. The conversion reached >99% within 18 h. After completion of the reaction, the IPA and co-product of acetone were removed by evaporation under reduced pressure, and the product was separated by extraction. Upon isolation of desired product **1**, the yield was 96% with a HPLC purity of 98.9%; excellent stereoselectivity (ee>99.9%) was achieved without any further purification. Overall, the production process of our system met the basic requirements of an economically feasible process for industrial-scale production of optically pure alcohol **1**.

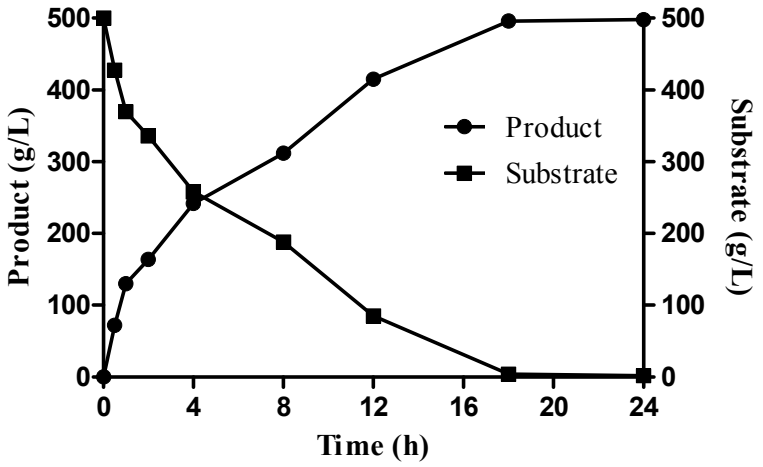


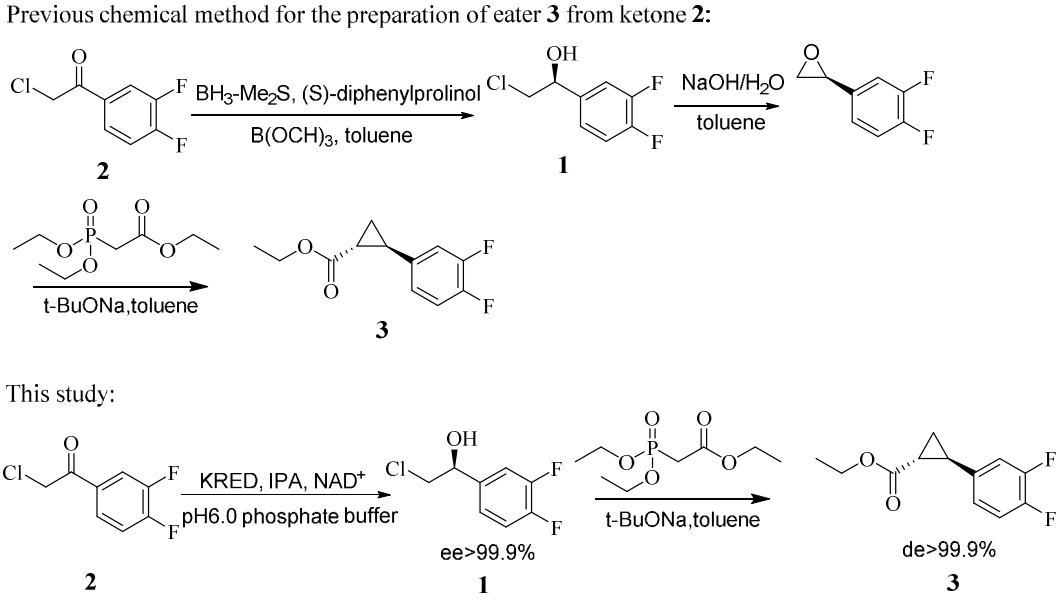
Figure 9. Time course for the bioreduction of ketone **2** to alcohol **1**

Reaction conditions: 500g/L ketone **2**, 50g/L wet cells and 0.05g/L NAD^+, 3.5eq IPA and PBS buffer (100 mM , pH 6.0) in 200mL reaction system for 24h at 35 °C and 220 rpm.

2.4. Synthesis of (1*R*,2*R*)-2-(3,4-difluorophenyl)cyclopropanecarboxylic acid ethyl ester (3)

Further studies found that chiral alcohol **1** can be obtained by extraction with toluene from an enzymatic reaction system and directly converted to **3** with a yield of

98% and >99.9% de. Compared with the current chemical synthetic method through CBS-catalyzed reduction system,[5] our study allows the reaction to avoid using toxic reagents and eliminates the cumbersome and dangerous quenching process. That is to say, we reduced the environmental impact, improved operational safety and simplified the original process operation. This study is of potential benefit for industrial applications (Scheme 4).

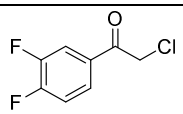
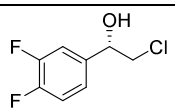
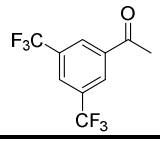
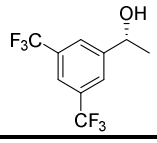


Scheme 4. Comparison of the synthetic method of ester **3** from ketone **2**

2.5. Synthesis of (*R*)-1-(3,5-bis(trifluoromethyl)phenyl)ethanol (4)

We were pleased to find that the selected KR-01 could also be applied to the preparation of alcohol **4** which is a vital chiral intermediate for the synthesis of Aprepitant. As shown in Table 2, the substrate 1-(3,5-bis(trifluoromethyl)phenyl)ethanone (**5**) can be converted almost completely in 500 g/L substrate loading with a space-time yield of 108.4 mmol/L/h. Furthermore, alcohol **4** obtained by KR-01 also showed high enantiomeric excess >99.9%. This exciting finding further demonstrated that the KR-01 shows potential for industrial application.

Table 2. The preparation of diverse chiral alcohols using KR-01[a]

Ketone	Product	Substrate Concentration	Conv. (%)[b]	ee (%)[c]	Conf.	Pharmaceutical
		500 g/L	>99	>99.9	S	Ticagrelor
		500 g/L	>98	>99.9	R	Aprepitant

[a]Reaction conditions: ketone **2** or **5** , 50g/L wet cells and 0.05g/L NAD^+, 3.5eq IPA and PBS buffer (100 mM , pH 6.0) for 18h at 35 °C and 220 rpm [b]Measured by HPLC. [c]Measured by chiral HPLC.

Thus far, the only bioreduction method for the synthesis of chiral alcohol **1** was reported by Wu et al. using a mutant of enzyme ChKRED20.[10] However, as shown in Table 3, the maximum substrate concentration for Wu's KRED can reach 200 g/L. In contrast, our reaction system showed that the bioreduction process achieved nearly complete conversion at a substrate concentration of 500 g/L with >99.9% ee giving a 2.8-fold increase in the space-time yield (145.8 mmol/L/h). Meanwhile, a much improved total turnover ratio (moles of product/moles of cofactor) of 34816 was obtained for this cofactor. Furthermore, the cofactor loading was only a quarter of the previous process. Lower costs are especially beneficial for industrial applications. Above all, this is the best result for the bioreduction of **2** to date.

Table 3. Comparison of existing biocatalytic processes for the preparation of **1**

Parameters	ChKRED20 mutant L205A	KR-01
Solvent system	25%Water/75%IPA	30%Water/70%IPA
Cofactor (g/L)	0.2	0.05
Concentration of ketone **2** (mM)	1049.5 (200g/L)	2623.7 (500g/L)
Concentration of alcohol **1** (mM)	1049.5	2623.7
Reaction time (h)	20h	18h
Conversion (%)	>99	>99
Space–time yield (mmol/L/h)	52.5	145.8
Turnover number (mol/mol)	3481	34816
Enantiomeric excess (%)	>99	>99.9
References	(Zhong-LiuWu et al. 2017)	This study

3. Conclusion

A practical, efficient and environmental bioreductive process for the preparation of the chiral alcohol **1** has been developed by using KR-01. We have optimized the reaction with KR-01 to a higher degree than that achieved previously, making the reaction more applicable for industrial use. Besides, this study simplifies the original process operation and reduces the safety risk. We also found that KR-01 can be used to prepare alcohol **4** with a 500 g/L substrate concentration.

4. Experimental Section

4.1. Materials and Methods

Ketone **2** and **5** were Energy Chemical® (China), IPA was Sinopharm Chemical Reagent Co.,Ltd. All other reagents were obtained commercially and used without additional purification. All the KRED genes (the codon-optimized open reading frames encoding proteins) were synthesized by Bioligo (Shanghai). ClonExpress® Entry One Step Cloning Kit used for molecular cloning was from Vazyme Biotech (Nanjin, China) and other enzymes from Takara (Dalian, China). BCA Protein Assay Kit was purchased from cwbiotech (Beijin, China).

The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Arance III 400 MHz spectrometer. The solvents used were $CDCl_3$. The ^{1}H-NMR chemical shift datas were reported on δ (ppm) relative to internal standard tetramethylsilane (TMS) and the ^{13}C-NMR chemical shift datas were reported on δ (ppm) relative to $CDCl_3$. The reaction was monitored by HPLC and the ee was determined by chiral HPLC using the conditions listed below. The HPLC analyses were recorded on a Dionex UItiMate 3000 HPLC instrument.

4.2. Cloning and Expression

The KREDs used here are shown in Table **4**. The codon-optimized genes of KR-01, KR-04, KR-06, KR-08, KR-09, KR-10, KR-11, and KR-13 were cloned into a

pET-22b(+) vector. A KR-05 plasmid containing the KR-05 and BmGDH (from *Bacillus megaterium*) genes had a common T7 promoter, and an individual ribosome-binding site (RBS) region was inserted into pET-22b(+) by ClonExpress® Entry One Step Cloning Kit. Other plasmids, namely KR-02, KR-03, KR-07, and KR-12, were also performed by ClonExpress® Entry One Step Cloning Kit. All plasmids were transformed into *E. coli* BL21 (DE3) cells for expression. The transformed cells cultured at 37°C in 50 ml LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) containing 50 ug/mL kanamycin. When OD600 reached 0.6-0.8, 0.1 mM IPTG was added in fermentation medium and cultivation was continued at 25°C overnight. The cells were then harvested by centrifugation (4000 g, 4°C, 30 min) and stored at -20 °C.

Table 4. KREDs used in this study

Enzyme	Strain	References/Genbank Number
KR-01	*Leifsonia* sp. Strain S749	AB213459
KR-02	*Exiguobacterium* sp. F42	17
KR-03	*Streptomyces bingchenggensis*	WP043488748.1
KR-04	*Microbacterium luteolum* JCM 9174.	AB733448
KR-05	*Chryseobacterium* sp. CA49	KC342003
KR-06	*Rhodococcus ruber*	WP017681535.1
KR-07	*Debaryomyces hansenii* CBS767	XM459695
KR-08	*Corynebacterium* sp. ST-10	18
KR-09	*Streptomyces cyaneogriseus*	CP010849.1
KR-10	*Lactobacillus kefir*	19
KR-11	*Lactobacillus kefir*	19
KR-12	*Lactobacillus kefir*	20
KR-13	*Rhodococcus ruber* DSM 44541	WP043801412.1

4.3. General Protocol for the synthesis of (*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol (1)

The 1g (500g/L) of substrate was dissolved in the solution of 1.4mL (3.5 eq) IPA and 0.6mL PBS buffer (100 mM, pH 6.0). The 0.1mg (0.05g/L) of NAD^+ and 0.1g (50g/L) of KR-01 (wet cells) were added. The reaction was shaken at 220 rpm shaker (35°C) for 18h. Subsequently, the reaction was extracted with 1mL DCM (×3), and

the organic layers was combined and dried over Na_2SO_4. The conversion and ee were determined by HPLC.

HPLC analysis was performed using a BDS C18 column (4.6×250 mm, 5 μm, Elite, Dalian), with a mobile phase of 60% water and 40% ACN (v/v). The substrate and product were detected at 210 nm, and the retention time was 8.7 min (**1**) and 13.4 min (**2**).

Chiral HPLC was performed using a CHIRALPAK IC (4.6×250 mm, 5 μm, DAICEL, Shanghai) column with a mobile phase of 95% Hex-0.1% TFA and 5% IPA. The product was detected at 260 nm, and the retention times of enantiomers were 10.2 min (**1**) and 11.4 min, respectively..

4.4. Scale-Up of the KRED Reaction for the synthesis of (*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol (1)

The reaction was performed in a 500 mL Erlenmeyer flask containing 60 mL phosphate buffer (100 mM, pH 6.0), 140 mL IPA, 100 g ketone **2**, 0.01 g NAD^+, and 10 g wet cells. The reaction was carried out at 35°C and agitated by overhead stirring for 18 h. The reaction mixture was then extracted with 200mL DCM (×3), and the DCM layers were combined. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, obtaining 97.0g oil (yield 96%). Spectral data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (ddd, *J* = 10.6, 7.6, 2.2 Hz, 1H), 7.20 – 7.12 (m, 1H), 7.12 – 7.07 (m, 1H), 4.90 – 4.83 (m, 1H), 3.71 (dd, *J* = 11.3, 3.5 Hz, 1H), 3.59 (dd, *J* = 11.3, 8.5 Hz, 1H), 2.80 (d, *J* = 3.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.68 (dd, *J* = 26.1, 12.6 Hz), 149.20 (dd, *J* = 26.0, 12.6 Hz), 137.24 – 136.93 (m), 122.26 (dd, *J* = 6.5, 3.7 Hz), 117.58 (d, *J* = 17.5 Hz), 115.37 (d, *J* = 18.2 Hz), 73.00 (d, *J* = 1.3 Hz), 50.64 (s). $[\alpha]_{589}^{25}$ +31.325 (*c* 1.0, CH_3OH). HRMS *m/z* $[M]^+$ calcd for $C_8H_7F_2ClO$: 192.0153, found: 192.0157

4.5. Synthesis of (1*R*,2*R*)-2-(3,4-difluorophenyl)cyclopropanecarboxylic acid ethyl ester (3)

The t-BuONa (169.4g, 3.5eq) and toluene (500mL) were charged into a reaction vessel. Subsequently, triethyl phosphonoacetate (282.3g, 2.5eq) was added to the mixture with stirring during which the internal temperature should be controlled below 20°C. After finishing, the temperature was set to 40°C. The organic layer that was extracted from enzymatic process by toluene (600mL), contained about 97g alcohol **1** (1eq), was added dropwise to the mixture with keeping the internal temperature below 45°C. After addition, stirring was continued for 24h at 60°C. After cooling to room temperature, the mixture was washed with H_2O, and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the product **3** as oil (111.4g, yield 98%). Spectral data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (dt, *J* = 10.1, 8.4 Hz, 1H), 6.89 (ddd, *J* = 11.3, 7.4, 2.2 Hz, 1H), 6.86 – 6.81 (m, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.52 – 2.43 (m, 1H), 1.84 (ddd, *J* = 8.6, 5.3, 4.2 Hz, 1H), 1.62 – 1.58 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.26 – 1.21 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.03 (s), 151.63 (d, *J* = 12.8 Hz), 150.45 (d, *J* = 12.6 Hz), 149.16 (d, *J* = 12.8 Hz), 148.00 (d, *J* = 12.7 Hz), 137.30 (dd, *J* = 5.8, 3.7 Hz), 122.45 (dd, *J* = 6.1, 3.5 Hz), 117.28 (d, *J* = 17.3 Hz), 115.27 (d, *J* = 17.7 Hz), 61.00 (s), 25.26 (d, *J* = 1.4 Hz), 24.22 (s), 16.99 (s), 14.33 (s). $[\alpha]_{589}^{20}$ -226.553 (*c* 0.9, CH_3OH). HRMS *m/z* $[M]^+$ calcd for $C_{12}H_{12}F_2O_2$: 226.0805, found: 226.0802

HPLC analysis was performed using a phenomenex Gemini 5u C18 110A column (4.6×250 mm, 5 μm). The mobile phases consisting of A (0.1%TFA-H_2O) and B (0.1%TFA-ACN) were used with the gradient mode. The substrate and product were detected at 260 nm. Initial gradient starts with 20% of B and at 15 min it was set to 80%. The ratio had been set to 20% at 35.1 min which continued up to 40 min. The retention time was 16.8 min (**3**) and 12.3 min (**1**).

The chiral HPLC was performed using a CHIRALPAK IC-3 (4.6×250 mm, 5 μm, DAICEL, Shanghai) column with a mobile phase of 99% Hex-1% IPA. The product was detected at 260nm, and the retention times of diastereoisomers were 9.6 min (**3**) and 10.5 min, respectively.

4.6. Synthesis of (*R*)-1-(3,5-bis(trifluoromethyl)phenyl)ethanol (4)

The reaction was performed in a 500 mL Erlenmeyer flask containing 60 mL phosphate buffer (100 mM, pH 6.0), 140 mL IPA, 100 g ketone **5**, 0.01 g NAD^+, and 10 g wet cells. The reaction was carried out at 35°C and agitated by overhead stirring for 18 h. The reaction mixture was then extracted with 200mL DCM (×3), and the DCM layers were combined. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, obtaining 95.7g off-white solid (yield 95%). Spectral data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 2H), 7.79 (s, 1H), 5.04 (qd, J = 6.5, 4.0 Hz, 1H), 2.08 (d, J = 3.8 Hz, 1H), 1.55 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.36 (s), 132.43-31.44 (q), 127.58 (s), 125.75 (d, J = 2.6 Hz), 124.87 (s), 122.16 (s), 121.46 (dt, J = 7.7, 3.8 Hz), 119.46 (s), 69.44 (s), 25.52 (s). $[\alpha]_{589}^{25}$ +23.377 (*c* 1.0, DMF). HRMS *m/z* $[M]^+$ calcd for $C_{10}H_8F_6O$: 258.0479, found: 258.0471.

HPLC analysis was performed using a BDS C18 column (4.6×250 mm, 5 μm, Elite, Dalian). The mobile phases consisting of A (H_2O) and B (ACN) were used with the gradient mode. The substrate and product were detected at 210 nm. Initial gradient starts with 10% of B and at 20 min it was set to 80%. The ratio had been set to 10% at 30 min which continued up to 36 min. The retention time was 22.8 min (**5**) and 22.2 min (**4**).

The chiral HPLC was performed using a CHIRALPAK IB-3 (4.6×250 mm, 3 μm, DAICEL, Shanghai) column with a mobile phase of 97% Hex-0.1% TFA and 3%

IPA. The product was detected at 260nm, and the retention times of enantiomers were 7.7 min and 8.3 min (**4**), respectively.

Asssociated Content

Supporting Information. ^{1}H NMR, ^{13}C NMR, HRMS spectrogram, specific rotation and chiral HPLC chromatogram of compounds **1, 3, 4**.

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