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Development of a Novel Chemoenzymatic Process for (*S*)-1-(Pyridin-4-yl)-1,3-propanediol

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ABSTRACT: We first developed a novel and efficient chemoenzymatic process to prepare (*S*)-1-(pyridin-4-yl)-1,3-propanediol, a vital HepDirect prodrug intermediate, from inexpensive and commercially available isonicotinic acid. Through this process, we provide a creative way to obtain the key chiral intermediate, *β*-hydroxyester, with ketoreductase (KRED) EA. After optimization of the process, we performed the reaction on a 100 g scale with a substrate concentration of up to 150 g/L, a yield of 93%, and an ee value of up to 99.9%. Additionally, we used a simple and effective $NaBH_4/MgCl_2$ reduction system to obtain (*S*)-1-(pyridin-4-yl)-1,3-propanediol with >99.9% ee and an 80% yield. This novel chemoenzymatic process has the potential to be a cost-effective and environmentally friendly process suitable for industrial use.

KEYWORDS: *(S)-1-(pyridin-4-yl)-1,3-propanediol, ketoreductase (KRED), chemoenzymatic process, HepDirect prodrugs, β-carbonyl ester,* $NaBH_4/MgCl_2$

1. INTRODUCTION

HepDirect prodrugs have attracted the attention of many researchers since the development of HepDirect technology by Erion et al.[1,2] These prodrugs are 1,3-diol cyclic phosphates that are designed to undergo an oxidative cleavage reaction in the liver, catalyzed by cytochrome P450 (CYP).[3−5] These prodrugs selectively release the monophosphate of a nucleoside (NMP) in CYP3A4-expressing cells (e.g., hepatocytes), while remaining intact in the plasma and extrahepatic tissues.[6,7] As a vital prodrug intermediate, (*S*)-1-(pyridin-4-yl)-1,3-propanediol (**1**) has found several applications in active compounds as potential pharmaceuticals, such as MB-07133, a phase III liver cancer prodrug[5] (Chart 1a). Additionally, other preclinical compounds exist that use this intermediate (Chart 1b−d).[8−10]

The existing synthesis methods of (*S*)-1-(pyridin-4-yl)-1,3-propanediol have drawbacks such as low enantiomeric purity, low yield, and inconvenient operation. In 2006, Boyer first reported the synthesis of (*S*)-1-(pyridin-4-yl)-1,3-propanediol using isonicotinaldehyde as the starting material via the aldol reaction, chiral separation, and $LiAlH_4$ reduction to obtain the target product, with an ee of 96% and an overall yield of 26%[5] (Scheme 1a). In this way, the use of LDA, EDCI, $LiAlH_4$, and column chromatography for purification at every step limited its applications in industries. In 2007, Erion selected 4-acetylpyridine as a starting material, and through ester condensation, asymmetric catalysis, and $NaBH_4$ reduction, the target compound was obtained but with a total yield of only 16%[11] (Scheme 1b). In this route, the expensive catalyst RuCl(*p*-cymene)[(*S*,*S*)-Ts-DPEN] (s/c 500:1) and the low ee value (87%) restricted its applications.

Ketoreductases (KREDs) catalyze reduction−oxidation (redox) reactions between ketones and alcohols. The biotransformation of ketones to chiral alcohols is carried out in the presence of cofactor NAD(P)H.[12] With the increasing use of KREDs, an increasing number of chiral intermediates are being synthesized by KREDs instead of chemical catalysts.[13,14] For example, Wei et al. reported an enzymatic process to synthesize (*S*)-2-chloro-1-(2,4-dichlorophenyl) ethanol, the chiral intermediate of luliconazole, by a novel KRED mutant, LK08, from *Lactobacillus kefiri*.[15] In 2004, Berendes Frank reported the bioreduction of ketone **4** to **5** using *Saccharomyces cerevisiae*, with a substrate concentration of 20 mmol/L (3.9 g/L) and an of ee 81% (the methyl ester derivative had an ee of 96%).[16] Herein, we aimed to develop a novel chemoenzymatic process for (*S*)-1-(pyridin-4-yl)-1,3-propanediol to overcome the shortcomings of existing routes. We first developed the bioreduction process using KRED EA to catalyze the transformation of substrate **4** to **5** with high efficiency (150 g/L substrate concentration, >99.9% ee, and 93% yield, Scheme 1c). Additionally, the starting material **2** is inexpensive and commercially available, and all reactions in our process are mild with a simple purification procedure. After optimization of the reaction parameters, the new route showed an overall yield of 55% with a product ee of >99.9% and purity of up to 99.5%. This route provides great potency in the industrial manufacturing of **1**.

2. RESULTS AND DISCUSSION

2.1. Optimization of Esterification (2 to 3). Initially, we carried out the esterification of isonicotinic acid with EtOH

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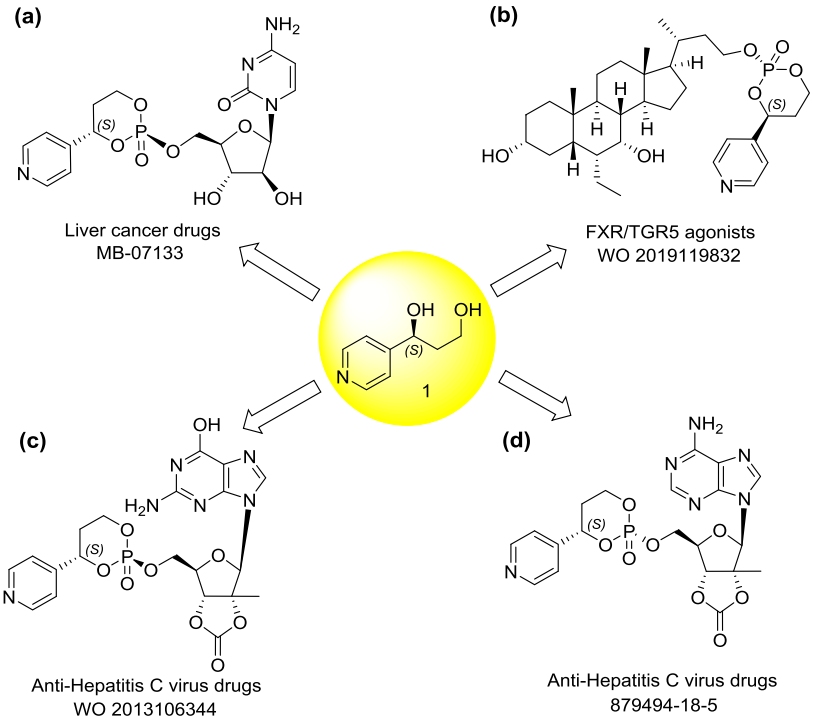
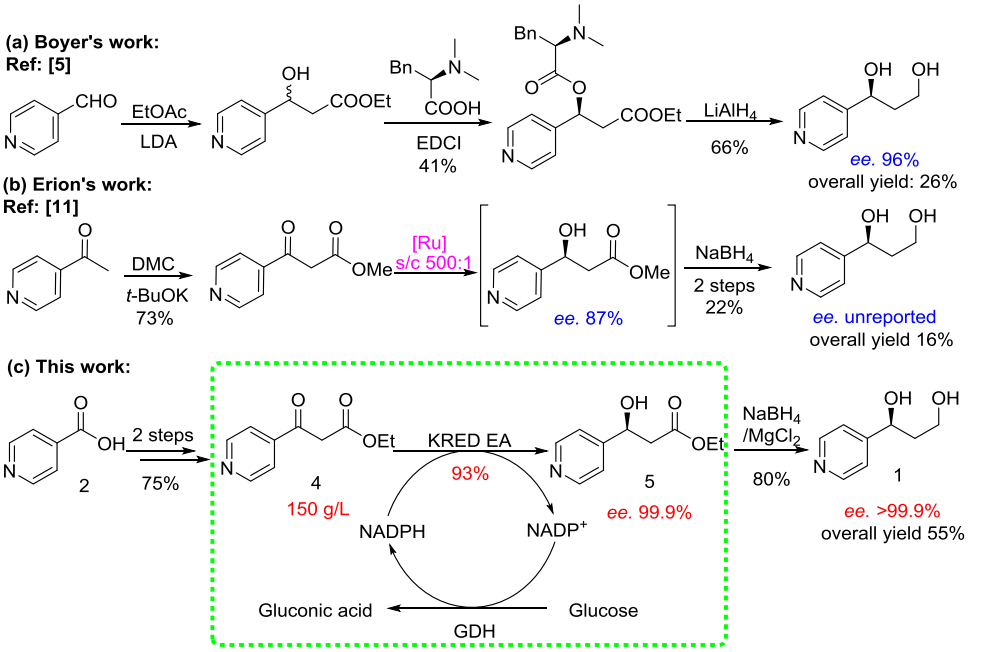
Chart 1. Application of (*S*)-1-(pyridin-4-yl)-1,3-propanediol 1 in different drugs: (a) liver cancer drugs, Chiva Pharmaceuticals & Ligand; (b) FXR/TGR5 agonists, Xi'An Biocare Pharma; (c) antihepatitis C virus drugs, Ligand; and (d) antihepatitis C virus drugs, Merck & Co. and Ligand



Scheme 1. Synthesis of (*S*)-1-(Pyridin-4-yl)-1,3-propanediol



using 1.0 equiv of H_2SO_4.[17] However, only 71% conversion was obtained after 15 h (Table 1, entry 1). Increasing the equivalents of H_2SO_4, we found that the reaction was significantly accelerated, and the conversion increased (entries 2−4). When the H_2SO_4 was increased to 1.5 equiv (entry 3), the reaction equilibrium was reached within 4 h, with 88% conversion, and the extension of time had no effect on the conversion. Although H_2SO_4 was increased to 2.0 equiv, we observed that the conversion was not significantly improved (entry 4). Moreover, the reaction required an equimolar amount of Na_2CO_3 to quench H_2SO_4 during the reprocessing operation. Considering the factors above, 1.5 equiv of H_2SO_4 was chosen for the reaction. The reaction was terminated at 4 h with an 88% isolated yield and a 99.7% purity (HPLC).

2.2. Optimization of Ester Condensation (3 to 4). Initially, we performed the ester condensation reaction using EtONa (1.5 equiv) and EtOAc under reflux conditions. EtONa was prepared using NaH with EtOH in situ[18] (Table 2, entry

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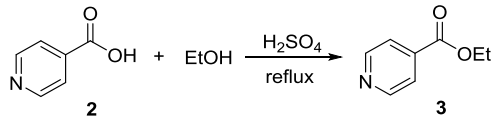
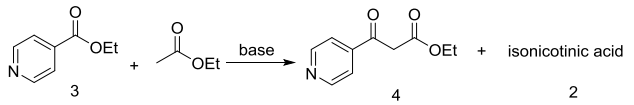
Table 1. Effect of H_2SO_4 Equivalents on the Reaction[a]



			HPLC (%)[b]	
entry	H_2SO_4 (equiv)	time (h)	2	3
1	1.0	15	28.7	71.3
2	1.2	12	14.8	85.2
3	1.5	4	12.0	88.0
4	2.0	4	10.1	89.9

[a]Reaction conditions: all experiments were carried out with 20 equiv of EtOH (10 V). [b]Composition of the product was measured by HPLC.

Table 2. Effects of Base and Solvents on Ester Condensation[a]



				HPLC[d]		
entry	base (equiv)	solvent	time (h)	3	2	4
1[b]	EtONa (1.5)	none	17	23.3	4.6	71.1
2[b]	EtONa (3.0)	none	17	8.2	7.2	84.5
3[c]	*t*-BuONa (2.0)	THF	2	5.3	12.9	79.3
4[c]	*t*-BuOK (2.0)	THF	2	1.3	8.7	89.6
5[c]	*t*-BuOK (1.8)	THF	2	1.9	8.9	89.2
6[c]	*t*-BuOK (1.5)	THF	2	5.7	9.1	85.0

[a]Reaction conditions: all experiments were carried out on a 6 g scale. [b]Under reflux conditions with 2 equiv of EtOAc. [c]With 1.2 equiv of EtOAc and 2.0 M *t*-BuOK/*t*-BuONa solution in THF. [d]The product content was measured by HPLC.

1). However, we found that the product content was only 71% in HPLC. Even with an increased amount of EtONa to 3.0 equiv, the product content was only 84% in 17 h (entry 2). Moreover, we found that the purity of commercial EtONa was only 96−98%, which contains 2−4% NaOH. This resulted in the complete hydrolysis of **3**. Therefore, EtONa must be prepared using NaH or sodium with EtOH in situ, which limits its application. To overcome these drawbacks, we aimed to explore a commercially available base containing a lower amount of hydroxide. We tested *t*-BuONa and *t*-BuOK; *t*-BuOK showed better conversion and a higher product content in HPLC (entries 3 and 4). Experiments for various equivalents of *t*-BuOK showed that 1.8 equiv of *t*-BuOK was a suitable choice for product content and reagent consumption (entries 4−6). The hydrolysis of ethyl isonicotinate in the reaction occurred caused by KOH, which was probably generated by the weighing of *t*-BuOK in air. Water in THF and EtOAc also accounted for the hydrolysis. Attempts to use 2-MeTHF (lower water content) as the solvent instead of THF resulted in an extremely sticky reaction mixture; the reason for this may be the poor solubility of *t*-BuOK in 2-MeTHF (<1 mol/L) and the good solubility in THF (2 mol/L). Therefore, we decided to use THF as the reaction solvent. The optimum conditions are shown in entry 5.

Additionally, we developed a stable and easy-to-operate workup process to obtain a >99.5% pure product. Initially, we quenched the reaction with 2 N HCl to neutral and extracted the reaction mixture to obtain the product; however, the impurities and remaining raw materials were extracted together, resulting in the purity of the crude product being 95%. Further crystallization (by EtOH/H_2O 3:7) was necessary to obtain a product purity of >99.0%, but this led to yield loss and additional processing steps. Later, we were surprised to find that when the reaction was quenched with water, the unreacted ethyl isonicotinate was completely hydrolyzed, but the product remained stable. After the distillation of THF, the aqueous phase was gradually acidified to a pH of 7.5 with 37% HCl, and the product precipitated out with a purity of 99.6%. The simple precipitation method avoided extraction and recrystallization and enabled one to directly obtain products with a purity of 99.6% for the next reaction. When we performed the condensation with the optimized conditions on the 100 g scale, we found that the hydrolysis impurity level was reduced to 5%, and the product content was increased to 93% in HPLC. Finally, we obtained the product with an 86% isolated yield and a 99.6% purity (HPLC).

2.3. Optimization of Bioreduction Parameters (4 to 5). *2.3.1. Screening of KREDs for the Bioreduction of Ketone 4.* We found no report of a ketoreductase that can be used to catalyze substrate **4** to alcohol **5**. To identify new ketoreductases for the synthesis of alcohol **5**, 16 KREDs preserved in our laboratory were tested. Table 3 shows that seven KREDs were found to be active toward substrate **4**. KREDs 736, EA and 1500 showed stereoselectivities of ee >98% (Table 3, entries 3, 7, and 16), while KRED EA showed the highest stereoselectivity of ee 99.6% (entry 7). Although 1306 showed high activity toward ketone **4**, its ee value was only 16.5% (entry 15). Therefore, further study of KRED EA was carried out.

2.3.2. Optimization of the Substrate Concentration. Optimization of the reaction parameters not only improves the conversion efficiency but also minimizes production costs to meet industrial requirements. The substrate concentration is the first and most important factor that must be considered in industrial production. The substrate loading study was carried out with a concentration range of 25−200 g/L to investigate the catalytic ability. The results showed that the bioreduction can proceed completely with a substrate concentration lower than 100 g/L. However, the conversion was found to decrease sharply when the substrate concentration was increased to 150 g/L. In our previous research, we found that the pH and temperature are essential for improving the enzyme activity and the cosubstrate equivalent is a key factor for the biocatalytic process.[15,19] Thus, the process optimization for the reaction conditions, including the pH, temperature, cosubstrate loading, and catalyst loading, was subsequently performed (Figure 1).

2.3.3. Optimization of the pH and Temperature. The pH and temperature greatly influence the enzymatic activity. A pH tolerance study of the reaction system including KRED EA and GDH was carried out in the pH range of 4−9 (Figure 2a). The results showed that when the pH is below 5, the catalytic activity of the enzymes somewhat decreases, while above pH 7, the enzyme activity decreases drastically. The optimum pH is 6−7. As shown in Figure 2b, the optimum temperature for the reaction system was determined to be 35−40 °C. When the temperature was above 40 °C, the conversion was found to sharply decrease. Thus, the optimized temperature was chosen to be 35 °C.

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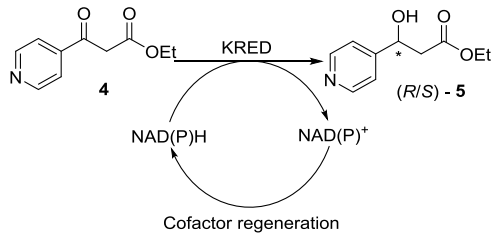
Table 3. Screening of KREDs from Our Enzyme Library



entry	enzyme	conversion (%)[e]	ee (%)[f]	configuration
1	223[a]	<5	N.D.	N.D.
2	500[a]	<5	N.D.	N.D.
3	736[a]	>99	98.5	*S*
4	740[a]	>99	95.1	*S*
5	774[b]	>99	97.8	*S*
6	CK03[b]	73	92.4	*R*
7	EA[b]	>99	99.6	*S*
8	787[c]	<5	N.D.	N.D.
9	1348[c]	<5	N.D.	N.D.
10	LSADH[c]	<5	N.D.	N.D.
11	LK08[c]	<5	N.D.	N.D.
12	701[d]	<5	N.D.	N.D.
13	902[d]	>99	94.6	*S*
14	1184[d]	<5	N.D.	N.D.
15	1306[d]	>99	16.5	*S*
16	1500[d]	>99	98.1	*S*

[a]Reaction conditions: 10 g/L ketone **2**, 20 g/L wet cells, 0.5 g/L NAD^+, 2 g/L GDH wet cells, 2 equiv of glucose in 2 mL of PBS buffer (100 mM, pH 7.0) for 24 h at 25 °C and 220 rpm. [b]10 g/L ketone **2**, 20 g/L wet cells, 0.5 g/L $NADP^+$, 2 g/L GDH wet cells, 2 equiv of glucose in 2 mL of PBS buffer (100 mM, pH 7.0) for 24 h at 25 °C and 220 rpm. [c]10 g/L ketone **2**, 20 g/L wet cells, 0.5 g/L NAD^+, 3 equiv of IPA in 2 mL of PBS buffer (100 mM, pH 7.0) for 24 h at 25 °C and 220 rpm. [d]10 g/L ketone **2**, 20 g/L wet cells, 0.5 g/L $NADP^+$, 3 equiv of IPA in 2 mL of PBS buffer (100 mM, pH 7.0) for 24 h at 25 °C and 220 rpm. [e]Measured by HPLC. [f]Measured by a chiral IC column with chiral HPLC.

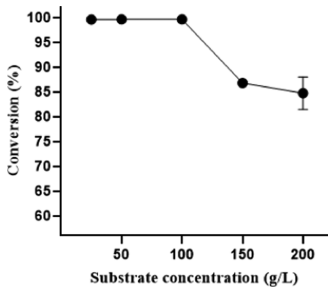


Figure 1. Effect of the substrate concentration on the bioreduction of ketone **2**. Reaction conditions: various concentrations of substrate ketone **4** (including 25, 50, 100, 150, and 200 g/L), 50 g/L EA wet cells, 10 g/L GDH wet cells, 2.0 equiv glucose and PBS buffer (100 mM, pH 7.0) in a 2 mL reaction system for 18 h at 25 °C and 220 rpm.

2.3.4. Optimization of the Cosubstrate Equivalents and Biocatalyst Loading. Cofactor regeneration is an essential step for the bioreduction reaction. Glucose has been well used as a substrate oxidized to gluconic acid by KREDs for the in situ regeneration of NADPH from $NADP^+$.[12] The glucose equivalents were set ranging from 1.1 to 3.0. As shown in Figure 2c, no significant influence on conversion was observed. Considering the conversion and glucose consumption, the glucose equivalent amount was chosen as 1.5 equiv. However, the conversion was merely 65−70% under the existing conditions, and thus, we increased the KRED EA wet cell dosage from 50 to 150 g/L. As shown in Figure 2d, the reaction can proceed completely with 100 g/L wet cells, and thus, 100 g/L wet cells was chosen.

2.3.5. Scale-Up of Bioreduction. With the optimized parameters for the bioreduction process (150 g/L substrate concentration, 100 g/L KRED EA wet cells, 1.5 equiv of glucose, 20 g/L GDH wet cells, 0.5 g/L $NADP^+$, 35 °C, and pH 7.0), the reaction was tested on a 10 g scale. In the reaction, the pH decreased gradually due to the generation of gluconic acid during the cofactor regeneration cycle. When we adjusted the pH using 10% Na_2CO_3, the addition of a base solution led to enzyme inactivation. We resolved this problem by the slow addition of 5% Na_2CO_3 to the reaction mixture. After we verified the process again on the 30 g scale, we performed the reaction on a 100 g scale. The reaction profile obtained is shown in Figure 3. No negative impact on the reaction was observed while operating on the 100 g scale. The product was obtained with a 99.9% ee, 99.5% purity, and 93% yield. The space−time yield of the bioreduction system reached 222 mmol/L/h, indicating that when required, this reaction can be scaled to larger quantities without the need for further optimization. A comparison of two asymmetric reduction systems is shown in Table 4. Our enzymatic process has the potential to be a cost-effective and environmentally friendly process suitable for industrial use.

2.4. Optimization of Ester Reduction (5 to 1). In Erion's work, the methyl ester was reduced by $NaBH_4$ in BuOH, but the reaction required heating to 93 °C for several hours.[11] We tried to change the reduction system to a lower temperature and found that the combination of $NaBH_4$/EtOH could reduce the ester at a lower temperature (Table 5, entry 1). It has been reported that $NaBH_4$ could be applied to ester reduction with additives.[20] We found that after adding $MgCl_2$, $NaBH_4$ could reduce ester to alcohol quickly and completely (entry 2). $CaCl_2$ showed a slightly worse result (entry 3). However, the addition of $ZnCl_2$ led to a significant increase in side reactions (entry 4). Hence, $MgCl_2$ was chosen as the best additive. We found that the conversion decreased and the reaction rate slowed down when attempting to reduce the equivalents of $NaBH_4$ and $MgCl_2$ (entries 5−7). Additionally, we found that changing the temperature had no obvious influence on the reaction (entry 8). Therefore, we chose the reaction parameters to be 1.2 equiv of $NaBH_4$ and 5% of $MgCl_2$, 10 V EtOH, at 20−40 °C for 3 h.

During the workup process, there were two major problems: one was the quenching method and the other was the extraction efficiency. In Erion's work,[11] the reaction was quenched with 10% K_2CO_3 (aq), and the boron complex remained in the product (the purity of the product was unreported). Additionally, the workup process described was quite complicated. Moreover, the excellent water solubility of diol makes it extremely difficult to extract it from the aqueous phase. Aiming to solve the above problems, through experimental runs and a literature search, we developed an improved workup process. The reaction was quenched with 30% H_2SO_4 and stirred for 1−2 h to completely decompose the boron complex into the product. Then, the aqueous phase was adjusted to pH 8−9 with 20% NaOH; thus, the Na_2SO_4 was generated in situ to saturate the aqueous phase, which was proven to be a better salting-out reagent than NaCl.[21] Then,

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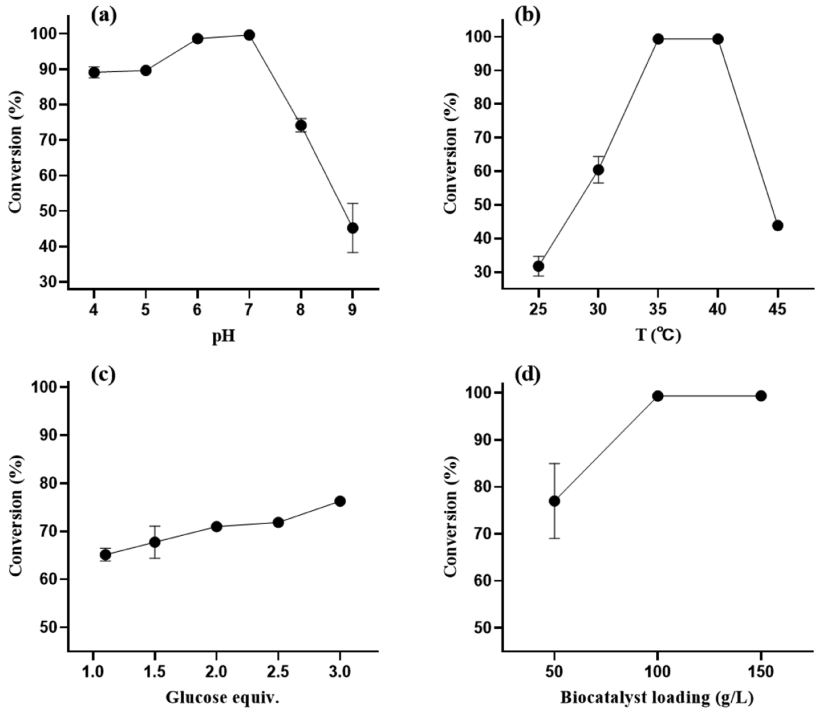

Figure 2. (a) Effect of the pH on the bioreduction system. Reaction conditions: the reactions were performed with 20 g/L ketone **2**, 20 g/L wet cells, 4 g/L GDH wet cells, 2.0 equiv of glucose in a 2 mL reaction system for 30 min at 25 °C and 220 rpm in different pH buffer solutions, including 100 mM sodium citrate (pH 4 and 5), 100 mM potassium phosphate (pH 6, 7, and 8), and 100 mM Tris−HCl buffers (pH 9). (b) Effect of the temperature on the bioreduction system. Reaction conditions: the temperature study was performed with 20 g/L ketone **2**, 20 g/L wet cells, 4 g/L GDH wet cells, 2.0 equiv of glucose, and PBS buffer (100 mM, pH 7.0) in a 10 mL reaction system for 30 min at 220 rpm. (c) Effects of the glucose equivalents on conversion by KRED EA wet cells. Reaction conditions: various equivalents of cosubstrate glucose (including 1.1, 1.5, 2.0, and 2.5 equiv), 150 g/L substrate ketone **2**, 50 g/L KRED EA wet cells, 10 g/L GDH wet cells, and PBS buffer (100 mM, pH 7.0) in a 2 mL reaction system for 6 h at 35 °C and 220 rpm. (d) Effects of the biocatalyst loading on conversion by wet cells. Reaction conditions: various KRED EA wet cells in the range of 50−150 g/L, 150 g/L substrate ketone **2**, 20 g/L GDH wet cells, and PBS buffer (100 mM, pH 7.0) in a 10 mL reaction system at 35 °C and 220 rpm.

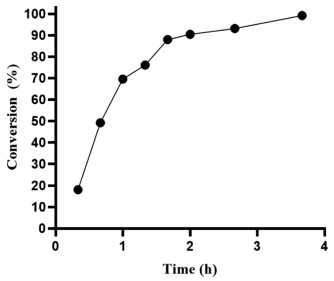

Figure 3. Time curve for the bioreduction of ketone **4** to alcohol **5**. Reaction conditions: 100 g of ketone **4** (150 g/L), 67 g of KRED EA wet cells (100 g/L), 14 g of GDH wet cells (20 g/L), 140 g of glucose (1.5 equiv), 0.33 g of $NADP^+$ (0.5 g/L), and 670 mL of PBS buffer (100 mM, pH 7.0) were added to a 2 L reaction vessel, and the reaction was carried out at 35 °C and 350 rpm.

EtOH was evaporated before extraction. Moreover, we also compared the extraction efficiencies of 2-MeTHF, THF, and *n*-BuOH, which revealed extraction efficiencies of 1:2:3 (HPLC). The TLC indicated that the *n*-BuOH could extract the product completely after two times, while the other solvents could not extract the product completely after four rounds of extraction. The organic phase was dried and evaporated under high vacuum. In the end, ethyl acetate was added to the sticky residue and evaporated again to remove the remaining *n*-BuOH to obtain a solid. The resulting solid was recrystallized with ethyl acetate to afford a white solid in an 80% isolated yield with a purity of up to 99.5% and an ee of >99.9%.

Table 4. Comparison of Two Asymmetric Reduction Systems[a]

entry	KRED EA	RuCl(*p*-cymene)[(*S*,*S*)-Ts-DPEN][c]
solvent	water	DMF
catalyst loading	0.66 kg (wet cells)	7.23 g (s/c 500:1)
catalyst cost	¥ 33	¥ 1040
cofactor[b] cost	¥ 66 (3.3 g)	0
time	3.5 h	16 h
yield	93%	not isolated (>100%)
purity	99.5%	unreported
ee	99.9%	87%

[a]The data were calculated by catalyzing 1 kg of substrate. [b]Referred to $NADP^+$. [c]Erion Mark D's work.[11]

3. CONCLUSIONS

In summary, we have designed and developed a new chemoenzymatic process to prepare a key HepDirect drug intermediate, (*S*)-1-(pyridin-4-yl)-1,3-propanediol. First, we proposed and optimized a process for efficiently constructing a *β*-carbonyl ester, a ketoreductase substrate, from inexpensive and commercially available isonicotinic acid. The yield of the two steps was 75%, and the purity of the product was up to 99.6%. Second, we developed an enzyme reduction process to

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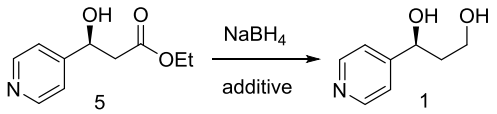
Table 5. Optimization of Ester Reduction[a] (5 to 1)



entry	$NaBH_4$ (equiv)	additive (equiv)	temperature (°C)	time (h)	HPLC[b] 1[c]	HPLC[b] 5	HPLC[b] impurities[d]
1	1.5	none	40	3	49.1	46.5	4.4
2	1.5	$MgCl_2$ (0.2)	40	1	99.6	0.2	0.2
3	1.5	$CaCl_2$ (0.2)	40	1	97.8	1.4	0.9
4	1.5	$ZnCl_2$ (0.2)	40	1	10.5	70.3	19.2
5	1.2	$MgCl_2$ (0.2)	40	1	99.3	0.4	0.3
6	1.0	$MgCl_2$ (0.2)	40	3	84.6	14.6	0.8
7	1.2	$MgCl_2$ (0.05)	40	3	99.4	0.2	0.4
8	1.2	$MgCl_2$ (0.05)	20	3	99.2	0.4	0.4

[a]Reaction conditions: experiments were carried out in EtOH, and $NaBH_4$ was added in two batches within 0.5 h. [b]The product content was measured by HPLC under 260 nm. [c]Contains **1** and a boron complex of **1**. [d]Structures of impurities not identified.

obtain a chiral alcohol intermediate with a 93% yield, 99.5% purity, and 99.9% ee. Finally, we found a simple and mild reduction system to obtain (*S*)-1-(pyridin-4-yl)-1,3-propanediol with an 80% yield, 99.5% purity, and 99.9% ee. The overall yield of this new route is up to 55%, and this novel chemoenzymatic process is both economic and environmentally friendly for industry applications.

4. EXPERIMENTAL SECTION

4.1. Materials and Methods. Isonicotinic acid **2** and glucose were purchased from Aladdin (China). The other chemicals were obtained commercially in analytical grade. Except for the reported enzyme, the other enzymes were purchased from the Huzhou Yihui Biological Technology Co., Ltd. (Zhejiang, China). The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AVANCE III 400 MHz spectrometer with a DMSO-d_6 solvent. The ^{1}H NMR chemical shift data were reported for δ (ppm) relative to tetramethylsilane as the internal standard, and the ^{13}C NMR chemical shift data were reported for δ (ppm) relative to DMSO-d_6. The reaction was monitored by HPLC (Waters H-Class), and the ee was determined by chiral HPLC (Dionex UltiMate 3000). Optical rotations were measured by a WZZ-2B automatic polarimeter.

4.2. Cloning and Expression. The codon-optimized genes of CK03, EA, LSADH, LK08, and GDH (GenBank accession number: AFQ56330.1) were cloned into a pET-28a(+) vector using the ClonExpress Entry One Step cloning kit. The resulting plasmids were transformed into *E. coli* BL21(DE3) cells. The resulting recombinant *E. coli* was cultured at 37 °C in LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) containing 50 μg/mL kanamycin. When the optical density of the culture measured at a wavelength of 600 nm reached 0.6−0.8, 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the fermentation medium. The cultivation was continued overnight at 25 °C. Then, the cells were harvested by centrifugation (4000*g*, 4 °C, and 30 min) and stored at −20 °C for further use (Table 6).

4.3. Culture of *E. coli*/pET-28a(+)-EA Cells in a 10 L Bioreactor. *E. coli*/pET-28a(+)-EA cells were precultured overnight in 150 mL of LB medium containing 50 μg/mL kanamycin at 37 °C. Then, the seed culture was transferred to a 10 L bioreactor containing 7 L of fermentation medium (24 g/L yeast extract, 12 g/L soybean peptone, 3 g/L NaCl, 5 g/L glycerol, 2 g/L K_2HPO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 50 μg/mL kanamycin, and pH 7.0). When the optical density of the culture measured at a wavelength of 600 nm reached 3.0, the cultures were cooled to 25 °C, and 168 mg of IPTG was added to induce the expression for 20 h. Then, the recombinant *E. coli*/pET-28a(+)-EA cells were harvested by centrifugation (4000*g*, 30 min, 4 °C) and stored at −20 °C. The *E. coli*/pET-28a(+)-GDH cells were prepared by the same method.

Table 6. KREDs Used in This Study[a]

entry	enzyme	strain	references/genbank number
1	223		purchased
2	500		purchased
3	736		purchased
4	740		purchased
5	774		purchased
6	CK03	*Chryseobacterium* sp. CA49	KC342003
7	EA	*Exiguobacterium* sp. F42	Wada et al.[22]
8	787		purchased
9	1348		purchased
10	LSADH	*Leifsonia* sp. strain S749	AB213459
11	LK08	*Lactobacillus kefir*	Wei et al.[15]
12	701		purchased
13	902		purchased
14	1184		purchased
15	1306		purchased
16	1500		purchased

[a]The purchased enzyme was provided by the Huzhou Yihui Biological Technology Co., Ltd.[23]

4.4. Synthesis of Ethyl Isonicotinate 3. First, 123 g of isonicotinic acid (1.0 mol) was placed into a 2 L four-necked flask, followed by the addition of 0.92 kg (20 mol) of absolute ethanol and mechanical stirring at 200 rpm. Then, 147 g (1.5 mol) of concentrated H_2SO_4 (98%) was added to the mixture dropwise. Heating was turned on after the addition of H_2SO_4, and the solution was refluxed at 80 °C. The reaction was monitored by HPLC and terminated after 4 h when the ethyl isonicotinate content was 88−89%. Then, the heating source was removed and the inner temperature was below 30 °C. Next, 500 mL of water was added, and 159 g (1.5 mol) of sodium carbonate was added in batches. After ethanol was

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evaporated under reduced pressure, the mixture was extracted with ethyl acetate (2 × 300 mL). The organic phase was washed with brine (200 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Finally, 128 g of crude product was obtained as a colorless liquid with a yield of 88% and an HPLC purity of 99.7%. HPLC (method 1): the product ester **3** and starting material **2** in the reaction solution were analyzed using a Waters BEH C18 column (2.7 × 50 mm^2, 1.7 μm), a flow rate of 0.4 mL/min, 35 °C, mpA = 10 mM KH_2PO_4 pH 4.4, mpB = ACN. Gradient: 0−2.4 min, 5−65% B; 2.4−3.25 min, 65% B; and 3.3−4.5 min, 5% B. UV at a wavelength of 220 nm. RT, 2.2 min (**3**), 0.3 min (**2**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86−8.69 (m, 2H), 7.87−7.74 (m, 2H), 4.35 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.59, 150.72, 136.99, 122.45, 61.56, 13.96.

4.5. Synthesis of Ethyl 3-oxo-3-(pyridin-4-yl)-propanoate 4. First, 650 mL of THF and 141 g of *t*-BuOK (1.26 mol) were added to a 1 L four-necked glass, followed by mechanical stirring at 300 rpm; the temperature was controlled at 0 °C. Then, a mixture of 105.8 g (700 mmol) of ethyl isonicotinate **3** and 74.0 g (840 mmol) of ethyl acetate was added dropwise to the *t*-BuOK/THF solution within 30 min with the temperature maintained below 10 °C. Once this mixture was added, the reaction temperature was increased to room temperature for 2 h; HPLC showed that the reactant was less than 1%. Then, 300 mL of water was added to quench the reaction, followed by concentration to remove THF. The aqueous phase was acidified by 37% HCl to a pH of 7.5, and the ketone precipitated out as the pH became lower. Then, the mixture was cooled to 0−5 °C for 2 h to precipitate crystals under stirring conditions. The crystals were filtered and washed with cool water, followed by drying in an air-drying oven to a constant weight to obtain 116.3 g of a white granular solid with a yield of 86%. Melting point: 50.7−51.3 °C. HPLC purity 99.6%. HPLC (method 1): RT, 2.0 min (**4**), 2.2 min (**3**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89−8.75 (m, 2H), 7.85−7.80 (m, 2H), 4.26 (s, 2H), 4.12 (q, J = 7.1 Hz, 2H), 1.16 (t, J = 7.1 Hz, 3H). Ketone/enol = 2.6:1. ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.81, 167.22, 150.88, 141.70, 121.34, 45.61, 13.93.

4.6. Synthesis of Ethyl (*S*)-3-Hydroxy-3-(pyridin-4-yl)propanoate 5. First, 670 mL of PBS buffer (100 mM, pH 7.0) was added to a 2 L four-necked flask, followed by mechanical stirring at 350 rpm. Then, 140 g (776 mmol) of glucose, 14 g of GDH wet cells, 67 g of EA wet cells, 100 g of ketone **4** (518 mmol), and 0.33 g of $NADP^+$ were added, respectively. The reaction vessel was placed in a 35 °C water bath. During the reaction, the pH value was closely monitored using a pH meter. A 5% Na_2CO_3 solution was added dropwise (slowly) to keep the pH level between 5.7 and 7.0. After 3.5 h of reaction, HPLC analysis showed that the product content was 99.6%, at which point the reaction was terminated. The pH was adjusted to 7.5; the mixture was extracted with 2-MeTHF (600 mL × 2); and the organic layer was washed with saturated NaCl (400 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Finally, 94.0 g of product was obtained as a light-yellow oil with a yield of 93%, an HPLC purity of 99.5%, and an ee value of 99.9%. HPLC (method 2): the different parameters compared to method 1 are as follows: mpA = 10 mM KH_2PO_4 pH 6.0, UV wavelength of 260 nm. RT, 1.7 min (**5**), 2.2 min (**4**). Chiral HPLC for **5**: DAICEL IC (4.6 × 250 mm^2, 5 μm), mobile phase: 25% IPA in hexane (V/V) isocratic. UV wavelength of 254 nm. RT: 11.0 min (*S*), 13.0 (*R*). $[\alpha]_D^{25}$ = −30.3° (*c* 1.00, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55−8.49 (m, 2H), 7.42−7.36 (m, 2H), 5.80 (d, J = 5.0 Hz, 1H), 5.00 (dt, J = 9.1, 4.6 Hz, 1H), 2.76−2.54 (m, 2H), 1.17 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.28, 153.23, 149.40, 120.96, 68.18, 59.93, 43.50, 14.02.

4.7. Synthesis of (*S*)-1-(Pyridin-4-yl)-1,3-propanediol 1. First, 60.0 g (307.3 mmol) of (*S*)-3-hydroxy-3-(pyridin-4-yl)propanoate **5** was added to a 1 L four-necked flask. Then, 600 mL of absolute ethanol was added, followed by mechanical stirring. Next, 1.46 g (15.4 mmol) of anhydrous $MgCl_2$ was added slowly, with the inner temperature controlled to 20 °C. Then, 13.95 g (123 mmol) of $NaBH_4$ was added in batches within 0.5 h. The reaction was monitored by HPLC until the raw material was <0.5%. Next, 150 mL of 30% H_2SO_4 was added to quench the reaction, the mixture was stirred for 1 h, and the boron complex decomposed to the product completely. Next, 20% NaOH was added to adjust the pH to 8.5. The resulting salts were then filtered, and the ethanol was removed under reduced pressure. The aqueous phase was extracted with *n*-BuOH (240 mL × 2). The combined organic phase was dried and evaporated under a high vacuum to obtain a sticky colorless oil. EtOAc (240 mL) was added and evaporated again, and then, EtOAc (180 mL) was added and heated to reflux for 0.5 h to obtain a clear solution, followed by gradual cooling to 0−5 °C for 1 h to precipitate the crystals. Subsequently, filtration was carried out, and the obtained white powder was washed with EtOAc (45 mL). The solid was then dried in an air-drying oven to a constant weight to obtain 38.1 g (307.3 mmol) of a white powder with a yield of 80%. Melting point: 98.1−99.3 °C. ee >99.9%. HPLC (method 2): RT, 0.5 min (**1**), 1.7 min (**5**). Chiral HPLC for **1**: DAICEL IC (4.6 × 250 mm^2, 5 μm), mobile phase: 15% EtOH in hexane (V/V) isocratic. UV at a wavelength of 254 nm. RT: 9.8 min (*S*), 11.7 (*R*). $[\alpha]_D^{25}$ = −51.5° (*c* 1.00, MeOH).[5] ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51−8.43 (m, 2H), 7.35−7.28 (m, 2H), 5.45 (d, J = 4.9 Hz, 1H), 4.68 (dt, J = 7.6, 5.2 Hz, 1H), 4.57 (t, J = 5.2 Hz, 1H), 3.48 (ddt, J = 39.6, 10.7, 5.3 Hz, 2H), 1.78−1.57 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.17, 149.28, 120.90, 68.17, 57.51, 41.83.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00403.

Chiral HPLC chromatograms of compounds **1** and **5** and NMR data of compounds **1**, **3**, **4**, and **5** (PDF)

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Notes

The authors declare no competing financial interest.

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