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Research Article

One-Pot Enzymatic–Chemical Cascade Route for Synthesizing Aromatic α-Hydroxy Ketones

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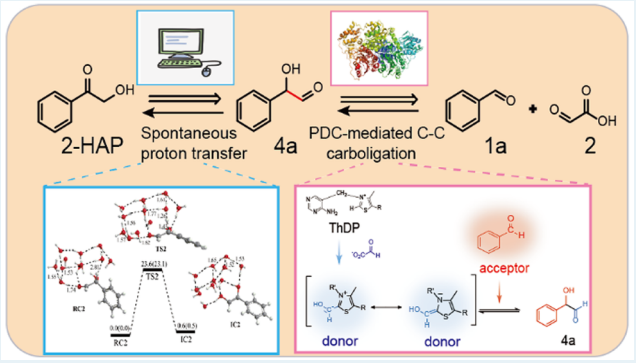
ABSTRACT: 2-Hydroxyacetophenone (2-HAP) is an important building block for the production of a series of natural products and pharmaceuticals; however, there is no safe, efficient, and economical method for 2-HAP synthesis. Here, a one-pot enzymatic-chemical cascade route was designed for synthesizing 2-HAP based on retrosynthetic analysis. First, a spontaneous proton-transfer reaction was designed using a computational simulation that enabled 2-HAP synthesis from the isomer 2-hydroxy-2-phenylacetaldehyde. A route for 2-hydroxy-2-phenylacetaldehyde synthesis was then constructed by introducing the unnatural substrate glyoxylic acid into a C−C ligation reaction catalyzed by *Candida tropicalis* pyruvate decarboxylase. Assembly and optimization of this enzymatic−chemical cascade route resulted in a final yield of 92.7%. Furthermore, stereospecific carbonyl reductases were introduced to construct a synthetic application platform that enabled further transformation of 2-HAP into (*S*)- and (*R*)-1-phenyl-1,2-ethanediol. This method of cascading spontaneous chemical and enzymatic reactions to synthesize chemicals offers insight into avenues for synthesizing other valuable chemicals.



KEYWORDS: *2-hydroxyacetophenone, spontaneous chemical reaction, pyruvate decarboxylase, glyoxylic acid, (S)-1-phenyl-1, 2-ethanediol*

INTRODUCTION

α-Hydroxy ketones are important compounds used to synthesize a series of natural products and pharmaceuticals[1−3] in the field of organic chemistry. Among these compounds, 2-hydroxyacetophenone (2-HAP) is a promising and cost-effective synthetic intermediate widely used as a building block for the synthesis of valuable larger molecules, such as α-ketoesters, *syn*-amino alcohols, and antitumor Schiff bases.[2,4−6] Additionally, 2-HAP is used to prepare optically pure chemicals, such as (*R*)- and (*S*)-1-phenyl-1,2-ethanediol, which can be used as medicine intermediates and liquid crystal materials, respectively.[7,8]

2-HAP can be produced using chemical and biocatalytic methods. The chemical synthesis methods mainly include photocatalytic oxidation of phenethyl alcohol, photocatalytic decarboxylative carbonylation of carboxylic acid,[9,10] oxidation of phenylglycol or terminal olefin by metal and nanometal catalysis,[11,12] and/or dehalogenation of bromoacetophenone catalyzed by microwave radiation.[13] Unfortunately, chemical methods include disadvantages such as harsh reaction conditions, high-priced substrates and catalysts, and technical challenges (such as catalyst stability and heavy metal recovery). By contrast, biocatalysis enables 2-HAP synthesis using a highly efficient and environmentally friendly approach. Currently, enzymatic synthesis of 2-HAP mainly include oxidation of α-aryl vicinal diols catalyzed by alcohol dehydrogenases[1] and C−C bond-formation reaction between benzaldehyde and formaldehyde catalyzed by benzaldehyde lyases.[14] However, the limiting factor of the former method is the high price of the starting substrate 1-phenylethane-1,2-diol, whereas that of the latter is the toxicity of the substrate formaldehyde and production of byproduct benzoin. Therefore, it remains necessary to develop a safer and more economical method for efficient synthesis of 2-HAP.

In this study, an enzymatic−chemical cascade route for 2-HAP synthesis was designed based on the catalytic mechanism of pyruvate decarboxylase (PDC)[15] and hybrid cluster-continuum (HCC) model calculation. The route for 2-HAP synthesis was constructed using benzaldehyde and glyoxylic acid as substrates by introducing PDC and a spontaneous proton-transfer reaction. Additionally, a route for synthesizing the optically pure compounds (*S*)- and (*R*)-1-phenyl-1,2-

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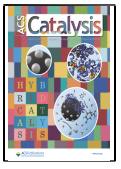

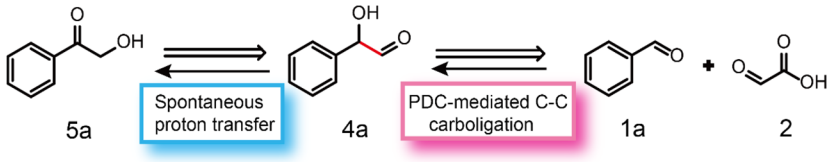

Figure 1. Retrosynthetic analysis of 2-HAP.

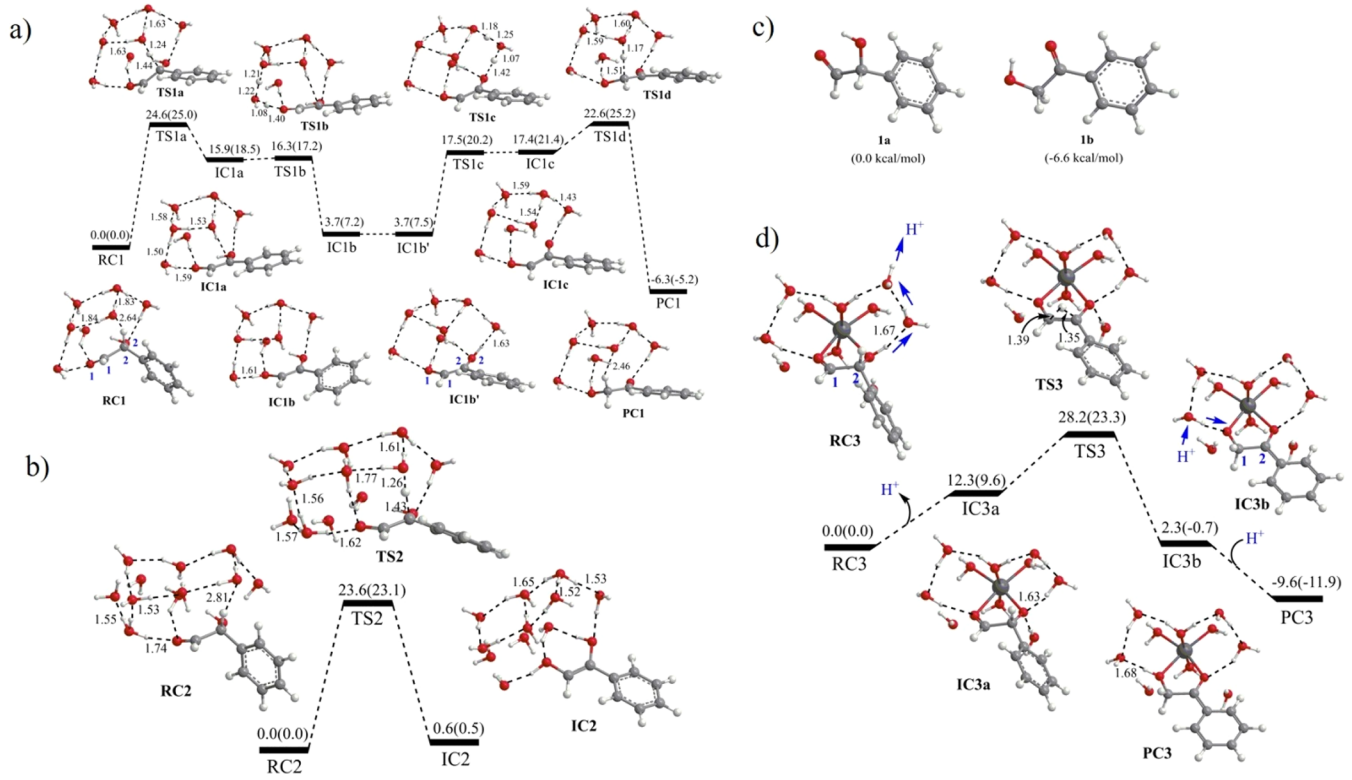

Figure 2. BMK/6-311++G(d,p) relative energies (in kcal/mol) for 4a → 5a conversion in the reaction solution. The relative energies are given as electronic energy first and then free energy (in parentheses) and shown along with B3LYP/6-31G(d)-optimized key species along the reaction pathway. (a) BMK/6-311++G(d,p) relative energies (in kcal/mol) for **4a** → **5a** conversion in aqueous solution. (b) BMK/6-311++G(d,p) relative energies (in kcal/mol) for the acid-catalyzed **4a** → **5a** conversion in aqueous solution. (c) B3LYP/B1-optimized structures of **4a** and **5a**. The calculated relative Gibbs energies (in kcal/mol) at the BMK/B2 level shown below the structures. (d) BMK/6-311++G(d,p) relative energies (in kcal/mol) for the Mg^{2+}-catalyzed **4a** → **5a** conversion in aqueous solution.

ethanediol was generated by cascading stereospecific carbonyl reductases.

RESULTS

Design of an Enzymatic−Chemical Cascade Reaction to Synthesize 2-HAP (5a). 2-HAP (**5a**) is an α-hydroxy ketone compound that contains a carbonyl group and a hydroxyl group and can be synthesized from its isomer 2-hydroxy-2-phenylacetaldehyde (**4a**) through keto−enol tautomerization via proton transfer. As shown in Figure 1, a chemical route was first designed to synthesize 2-HAP from **4a**. **4a** has a special sec-α-hydroxy ketone moiety that allows it to be synthesized from simple building blocks, such as benzaldehyde and formaldehyde, through the C−C bond-formation reaction catalyzed by aldolase. Moreover, **4a** can also be synthesized from benzaldehyde and glyoxylic acid via PDCs catalysis. Given the toxicity of formaldehyde, the route catalyzed by PDCs was second designed. Therefore, the enzymatic−chemical cascade reaction shown in Figure 1 involves a PDC-catalyzed C−C carboligation reaction with glyoxylic acid and benzaldehyde for the synthesis of **4a**, followed by **4a** conversion to 2-HAP (**5a**) by a proton-transfer reaction.

Exploring the Spontaneous Reaction of 4a to 5a. During this cascade reaction, the intermediate product **4a** is unstable and difficult to obtain, making it experimentally difficult to prove that **4a** can convert into **5a**. Therefore, the relative Gibbs energy of **4a** and **5a** was calculated, revealing that the relative energy of **5a** was lower than that of **4a**, theoretically indicating that **4a** can be spontaneous converted to **5a** (Figure 2c). This spontaneous reaction was then explored using an HCC model calculation under water-, acid-, and Mg^{2+}-catalyzed conditions, respectively, because this reaction occurs in an enzymatic reaction environment that includes water molecules, H^+, and Mg^{2+}.[15] Under water-catalyzed conditions (Figure 2a), transformations from **4a** to **5a** comprised two keto−enol tautomerization steps, where the proton was first transferred from C2−H to O1 (RC1 → IC1b), followed by transfer from O2−H to C1 (IC1b′ → PC1); therefore, the rate-determining step involved deprotonation at

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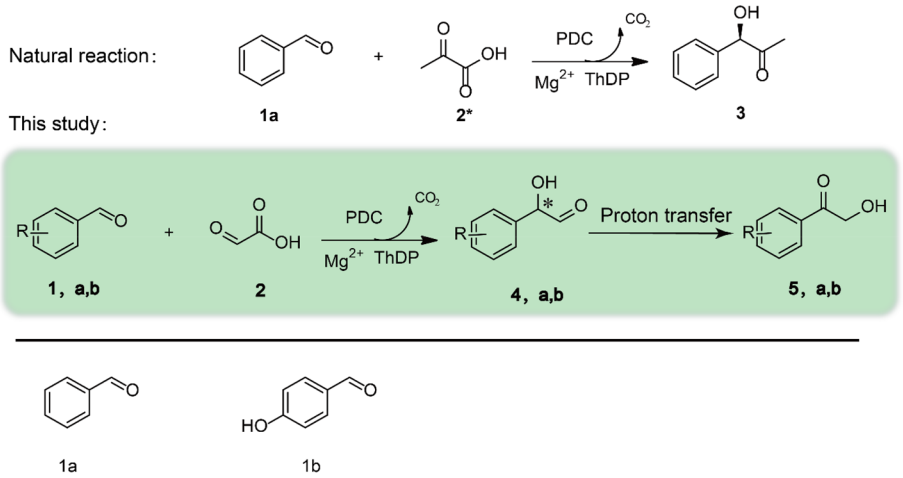
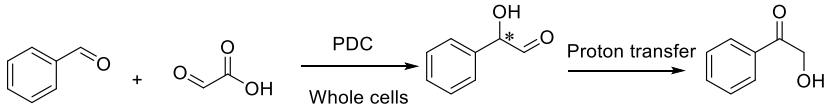


Figure 3. Natural reaction catalyzed by PDC and the proposed enzymatic−chemical cascade reaction.

Table 1. Summary of Different Pyruvate Decarboxylases Tested for 2-HAP Formation



entry	pyruvate decarboxylase	stains	yield[a] (%)
1	*Ct*PDC1	*Candida tropicalis* 1798	69.4
2	*Ct*PDC2	*Candida tropicalis* 1798	n.d.
3	*Ct*PDC3	*Candida tropicalis* 1798	n.d.
4	*Sc*PDC	*Saccharomyces cerevisiae* BY4741	47.4
5	*Cg*PDC1	*Candida glabrata*	41.8
6	*Cg*PDC2	*Candida glabrata*	n.d.
7	*Cg*PDC3	*Candida glabrata*	45.5
8	*Cu*PDC1	*Candida utiliz* 1767	30.6
9	*Cu*PDC2	*Candida utiliz* 1767	17.9
10	*Cu*PDC3	*Candida utiliz* 1767	53.1
11	*Cu*PDC4	*Candida utiliz* 1767	48.1
12	*Sp*PDC1	*Schizosaccharomyces pombe* 1056	39.4
13	*Sp*PDC2	*Schizosaccharomyces pombe* 1056	n.d.
14	*Sp*PDC3	*Schizosaccharomyces pombe* 1056	7.7
15	*Sp*PDC4	*Schizosaccharomyces pombe* 1056	n.d.
16	*Km*PDC	*Kluyveromyces marxiamus* 1056	44.6
17	*Pp*PDC	*Pichia pastoris* GS115	2.7

[a]Reactions were performed in 1 mL system with *E. coli* cells expressing the respective pyruvate decarboxylase, the screening conditions: 2 M MOPS buffer, pH 6.5, 5 mM $MgSO_4$, 0.5% DMSO, 5 mM thiamine diphosphate (ThDP), 5 mM benzaldehyde, 20 mM glyoxylic acid, 30 g/L whole cells, 30 °C, 200 rpm, and 24 h. HPLC analytics. Results are the average of duplicate reaction. n.d. not detectable.

C2 (TS 1a) or protonation at C1 (TS 1d). Under acid-catalyzed conditions (Figure 2b), the deprotonation reaction occurred at C2, which was also the rate-determining step. Under Mg^{2+}-catalyzed conditions (Figure 2d), the reactions involved an H-shift from C2 to C1, which differed from both the water- and acid-catalyzed reactions. Furthermore, the Gibbs energy barriers of the water-, acid-, and Mg^{2+}-specific conversions were 25.2, 23.1, and 23.3 kcal/mol, respectively, suggesting the acid-catalyzed reaction as the most favorable.

Construction of the Enzymatic Reaction for 4a Synthesis. As shown in Figure 3, (*R*)-phenylacetylmethanol (**3**)[16] can be produced from benzaldehyde and pyruvate by PDC catalysis. Following this reaction mechanism, 17 PDCs were selected to catalyze the carboligation reactions of benzaldehyde (**1a**) with glyoxylic acid (**2**) for efficient synthesis of **4a**. These PDCs were overexpressed in *Escherichia coli* and then used as whole-cell catalysts to synthesize **4a** (because **4a** can spontaneously convert to 2-HAP, 2-HAP was detected as the target product) (Table 1). We found that the PDC from *Candida tropicalis* 1798 (*Ct*PDC1) exhibited the highest yield (69.4%) and this PDC was subsequently chosen as the catalyst for further experiments.

On the basis of the catalytic mechanism of PDC on natural substrates (Figure 4a), a putative formation mechanism of **4a** from **1a** with **2** was proposed (Figure 4b). First, the cofactor undergoes deprotonation to form a reactive ylide form, followed by nucleophilic attack by the ThDP C2 carbanion of the donor substrate **2** (Figure 2b, shown in blue) to produce a highly reactive intermediate (LThDP[a]). Second, the resonant carbanion/enamine forms of the postdecarboxylation intermediate 2-hydroxyethyl-ThDP (HEThDP^{-a}) are produced by LThDP[a] decarboxylate. Subsequently, the "acceptor" species

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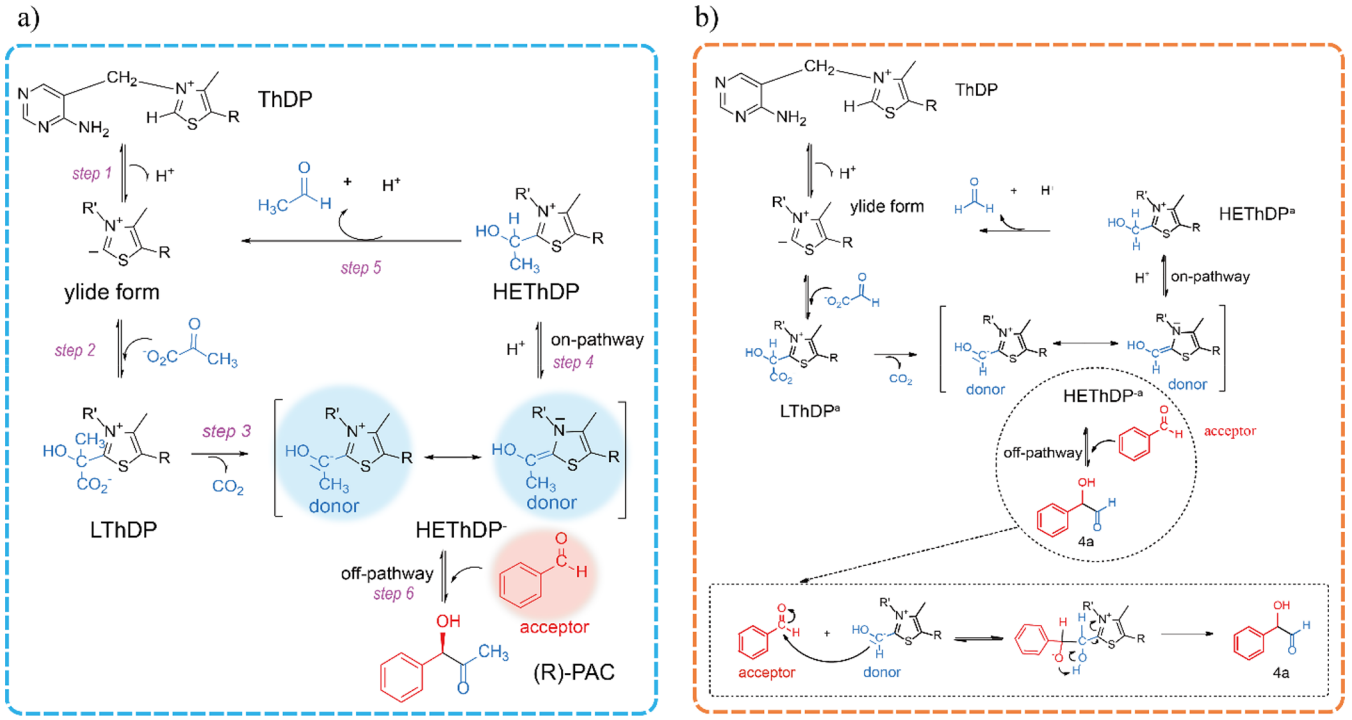

Figure 4. Mechanism of the natural reaction catalyzed by PDCs and the putative formation mechanism of **4a**. (a) The reaction mechanism involving natural substrates catalyzed by PDC.[18] The catalytic process of PDC can be subdivided into six elementary steps: (1) the cofactor is deprotonated to form a reactive ylide; (2) the ThDP C2 carbanion attacks the donor substrate carbonyl in a nucleophilic manner to yield the tetrahedral predecarboxylation intermediate 2-lactyl-ThDP (LThDP); (3) decarboxylation of LThDP generates the resonant carbanion/enamine forms of the postdecarboxylation intermediate 2-hydroxyethyl-ThDP (HEThDP$^-$); (4) if the second substrate is H^+, protonation at C2α of HEThDP$^-$ yields its conjugate acid HEThDP; (5) the C2α−OH group of HEThDP is ionized, and cleavage of its C2−C2α bond leads to liberation of the acetaldehyde product; and (6) if the second substrate is **1a**, the HEThDP$^-$ carbanion attacks the acceptor **1a** carbonyl group in a nucleophilic manner to yield (*R*)-PAC. (b) The putative formation mechanism of **4a**.

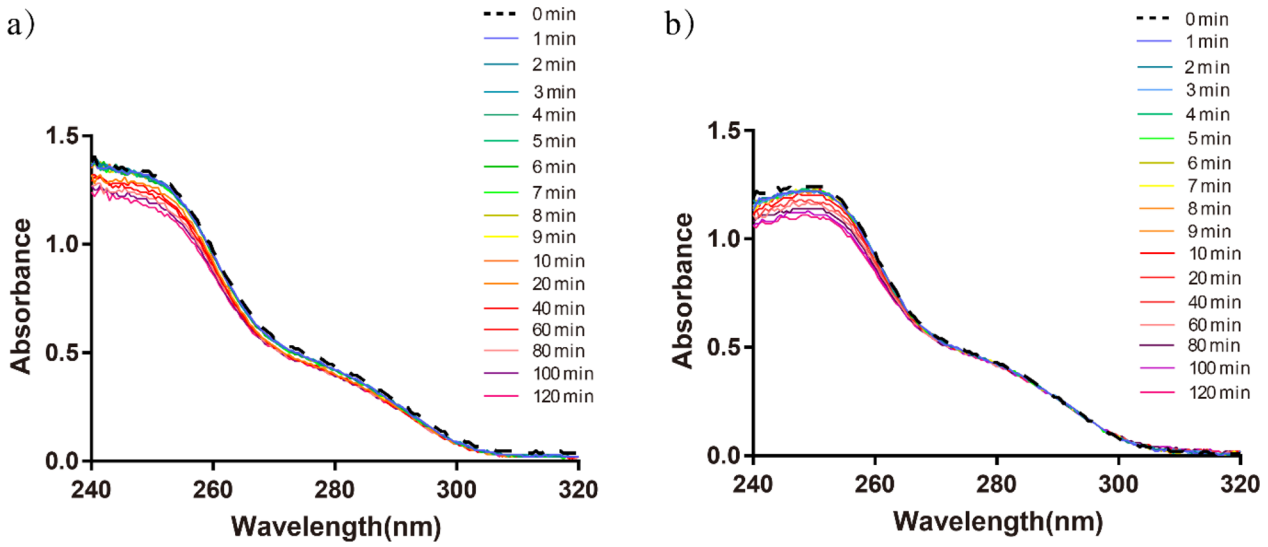

Figure 5. UV−vis spectra of **1a** reacting with the natural substrate pyruvic acid and the unnatural substrate **2**. (a) UV−vis spectra of reaction with the natural substrate pyruvic acid over the course of 2 h. (b) UV−vis spectra of reaction with the unnatural substrate **2** over the course of 2 h. Spectra were collected on a Shimadzu UV2550 spectrophotometer in a quartz cuvette with a 1 cm path length at 30 °C. Samples were prepared in a total volume of 1 mL with 10 μM of purified enzyme (with **1a** 1 mM, **2** 15 mM, Mg^{2+} 5 mM, 0.5% DMSO and ThDP 1 mM added, as needed) in 2.5 M MOPS buffer (pH 6.5).

1a (Figure 4b, shown in red) is attacked by the activated aldehyde intermediate (HEThDP^{-a}) and directly generates product **4a**. To confirm the formation mechanism of **4a**, the reaction-process curve was generated using UV−vis spectroscopy by first incubating 10 μM *Ct*PDC1 with 1 mM **1a** in 2.5 M MOPS buffer (Mg^{2+} 5 mM, 0.5% DMSO and ThDP 1 mM). At this point, the enzyme is still in the resting state[17] (Figure 5a and 5b, dotted lines), because when **1a** as the only substrate, there was no catalytic reaction occurred (Figure S1 of Supporting Information, SI). After addition of 15 mM

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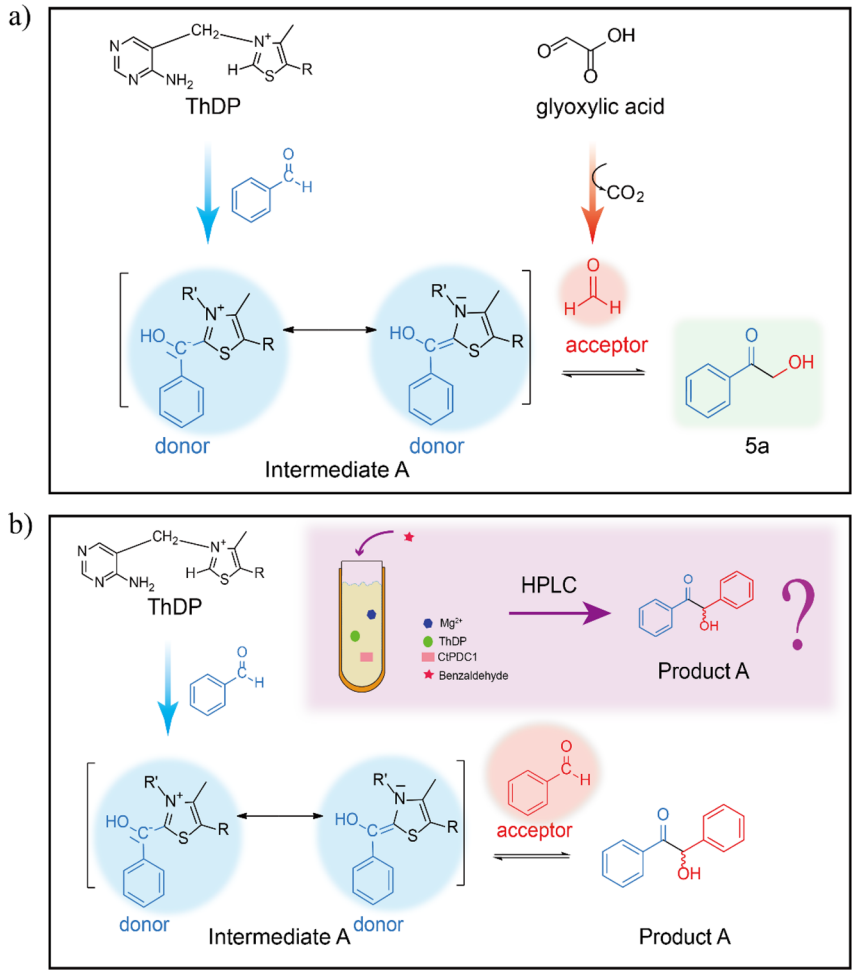

Figure 6. Putative mechanism and verification of donor−acceptor exchange caused by *Ct*PDC1. (a) Speculative mechanism of donor−acceptor exchange catalyzed by *Ct*PDC1. The cofactor ThDP is deprotonated to form a reactive ylide, followed by nucleophilic attack by the ThDP C2 carbanion of the donor **1a** (shown in blue) to yield intermediate A, which attacks an "acceptor" species formaldehyde (shown in red) formed by decarboxylation of **2** to produce the product **5a**. (b) Verification of the donor−acceptor exchange mechanism via *Ct*PDC1 catalysis.

pyruvic acid or **2**, no significant change in the curve was observed immediately; however, the absorbance at between 240 and 260 nm gradually decreased in 2 h (Figure 5). The trends of the spectral curves associated with the natural (Figure 5a) and unnatural (Figure 5b) reactions were determined as being the same, indicating similar reaction processes and transition states. This suggested that our proposed formation mechanism of **4a** from **1a** with **2** was correct.

Assembly and Optimization of Chemoenzymatic Synthesis of 2-HAP. With **1a** and **1b** as substrate, the product **5a** and **5b** were produced by the enzymatic-chemical cascade reaction of *Ct*PDC1-catalyzed C−C carboligation reaction and spontaneous proton transfer. The relative molecular mass of **5a** and **5b** were 136.08 and 151.0124 (Figure S2), respectively, detected by GC−MS and LC−MS. The products were then separated and purified using preparative high-performance liquid chromatography and determined by ^{1}H and ^{13}C NMR spectroscopy, which revealed a CH_2-absorption peak for product **5** rather than a CH-absorption peak for product **4** (Figures S3a and S4a). These results demonstrated that the enzymatic−chemical cascade reaction successfully synthesized **5a** and **5b**.

There are two ways to verify whether the formation of **5a** was caused by donor−acceptor exchange catalyzed by *Ct*PDC1. The first way is to detect the presence of intermediate A by UV−vis spectroscopy and fluorescence spectroscopy (Figure 6a); however, given the speed of the reaction process, it was difficult to capture intermediate A. The second way is to determine whether **1a** becomes the donor substrate (Figure 6b). In theory, if **1a** is the donor substrate, it will react with the acceptor substrate **1a** to produce product B via C−C carboligation. In fact, in the reaction described, **1a** was the only substrate, and no product B was detected (Figure S1). Because **1a** was not used as the donor substrate, formation of **5a** was not a result of enzyme−catalyzed donor−acceptor exchange.

The transformation conditions of the enzymatic−chemical cascade reaction were then optimized (Figure 7), resulting in the following: **1a** 4.5 mM, **2** 15 mM, Mg^{2+} 5 mM, ThDP 1 mM, 0.5% DMSO, MOPS buffer 2.5 M, reaction temperature 30 °C, pH 6.5, and a 3.5-h reaction duration. The optimal conditions increased the titer of **5a** to 3.7 mM with a 82.7% yield (Figure 7). To further increase the production efficiency of **5a**, a homodimer structural model of *Ct*PDC1 in complex with two cofactors (ThDP) and two Mg^{2+} was generated via homologous modeling using *Kluyveromyces lactis* PDC (PDB ID: 2VJY; 70% identity) as the template. Docking of substrate **2** and natural substrate **2*** to the allosteric region of the model

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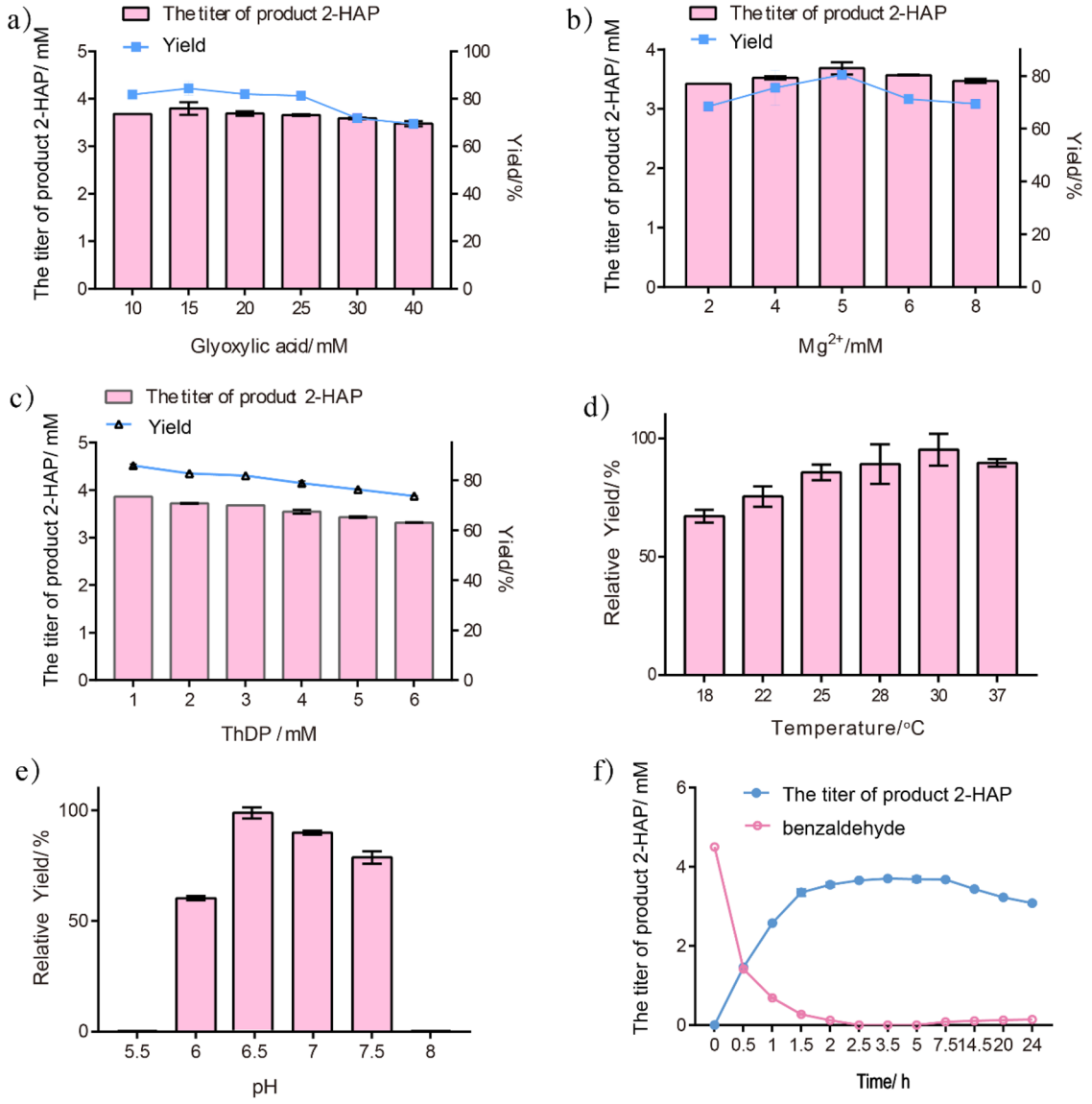

Figure 7. Optimization of transformation conditions of the enzymatic−chemical cascade reaction. Optimization of (a) **2** concentration, (b) Mg^{2+} concentration, (c) thiamine diphosphate (ThDP) concentration, (d) the reaction temperature, (e) reaction pH, and (f) reaction time. Initial conditions: **1a** 4.5 mM, **2** 20 mM, Mg^{2+} 5 mM, ThDP 5 mM, 0.5% DMSO, MOPS buffer 2.5 M, reaction temperature 30 °C, pH 6.5, and a 24-h reaction time.

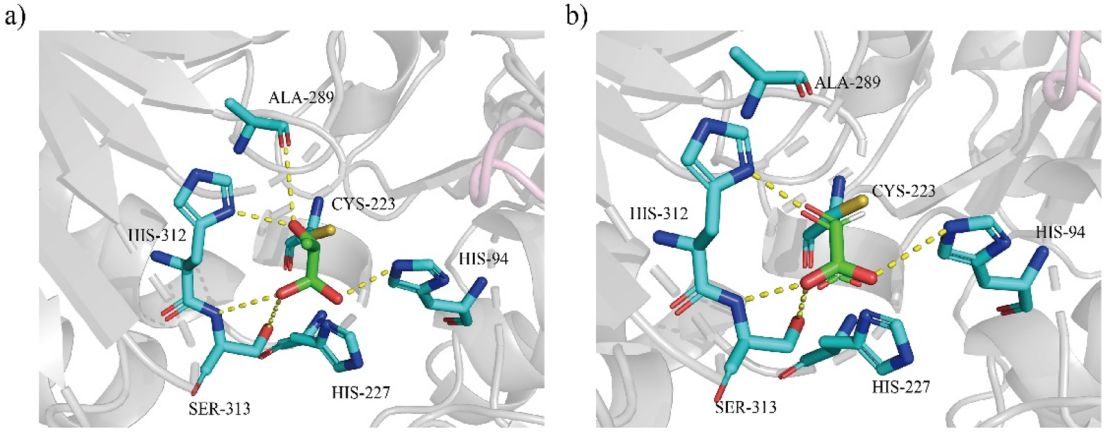

Figure 8. Docking of (a) pyruvic acid and (b) 2 substrates into the allosteric region of *Ct*PDC1.

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of *Ct*PDC1 resulted in substrates in approximately the same orientation (Figure 8); therefore, residues H94, C223, A289, 312H, and 313S, all of which showed interactions with the substrate in the allosteric region, were selected for site-directed saturation mutation. Among these mutations, *Ct*PDC1^{C223E} resulted in an increased yield of 92.7%, 10% higher than that of *Ct*PDC1^{WT}. This was a result of increases and decreases in k_{cat} and K_m (**2**) for *Ct*PDC1^{C223E} of 1.7 and −0.4, respectively, relative to *Ct*PDC1^{WT} (Table 2), suggesting increased affinity for the substrate.[19]

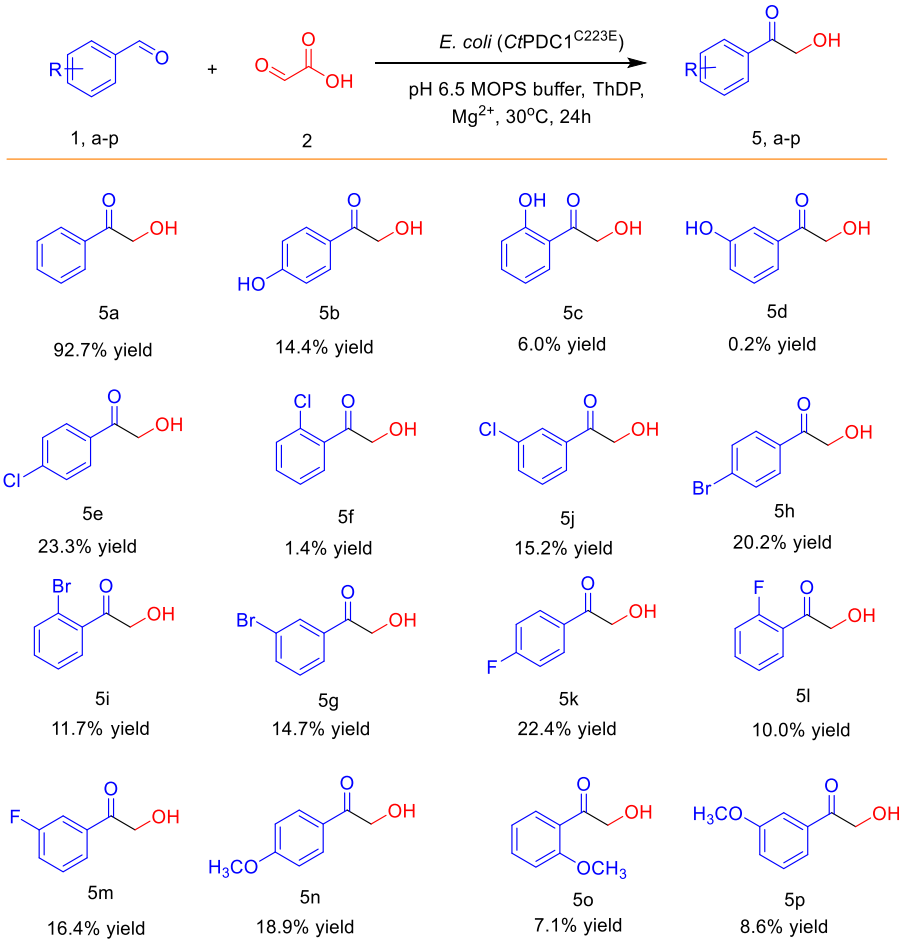
Table 2. Biochemical Characterization of *Ct*PDC1^{WT} and Mutants

enzyme	K_m (2) [mM]	k_{cat} [s^{-1}]	k_{cat}/K_m (2) [mM^{-1} s^{-1}]	yield [%]
*Ct*PDC1^{WT}	6.9 ± 0.22	96.4 ± 0.14	14.0	82.7
*Ct*PDC1^{C223E}	6.5 ± 0.16	98.1 ± 0.12	15.1	92.7

Since the property and position of any substituent in a benzene ring affect the nucleophilic addition reaction, the effects of the electron-withdrawing and donating substituents on reaction yield were investigated (Table 3). It was found that (1) *Ct*PDC1^{C223E} accepts various forms of ortho-, para-, or meta-substituted benzaldehyde as substrates and catalyzes the synthesis of the corresponding 2-HAP derivatives, though the yields are relatively low (0.2−23.3%). (2) The electron-withdrawing or donating substituents at the para position (Table 3 and **5b**, **e**, **h**, **k**, and **n**) result in relatively high yields relative to substrates of ortho- or meta-substituents. Among para-substituents, electron-withdrawing substituents (**5e**, **h**, and **k**) are more reactive than electron-donating ones (**5b** and **n**). (3) In general, the yields from substrates of meta-substituents were higher than those of substrates of ortho-substituents. In conclusion, the high yields from electron-withdrawing para-substituents might be due to the attraction of the electron-withdrawing groups on the benzene ring, resulting in less charge on α-C, which is favorable for the nucleophilic addition reaction.

Constructing the Cascade Reaction to Produce (*S*)- and (*R*)-1-Phenyl-1,2-ethanediol. *Ct*PDC1^{C223E} can convert **1a** and **2** to 2-HAP; therefore, the ability of 2-HAP to be used as a substrate to synthesize other higher-value products was determined. A cascade reaction was then designed with PDC, carbonyl reductase (SCR or RCR), and glucose dehydrogenase (GDH) to produce optically pure (*S*)- and (*R*)-1-phenyl-1,2-ethanediol [(*S*)- or (*R*)-PED] using **1a** and **2** as substrates (Figure 9). Additionally, based on the literature

Table 3. Substrate Scope of C−C Carboligation Reaction Catalyzed by *Ct*PDC1^{C223E}[a]



[a]Standard reaction conditions. Reactions were performed in 1 mL system: Substrate 1 (4.5 mM), glyoxylic acid (15 mM), *E. coli* (*Ct*PDC1^{C223E}) 30 g/L, Mg^{2+} 5 mM, ThDP 1 mM, 0.5% DMSO, MOPS buffer (2.5 M, pH 6.5), reaction temperature 30 °C, and 24 h. Yield determined via GC−MS with **5a** as a standard.

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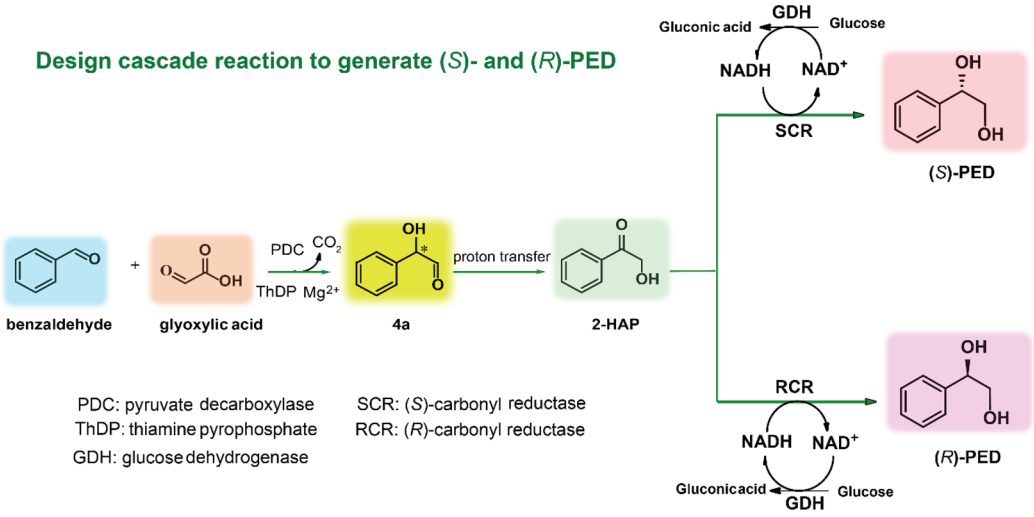

Figure 9. Biosynthesis of (*S*)-PED and (*R*)-PED using the respective engineered *E. coli* whole-cell catalysts.

mining and the enzyme activity, the NADH-dependent SCR from *Gluconobacter oxydans*, the RCR from *Bacillus subtilis* 168,[8] and *Bm*GDH from *Bacillus megatherium*[20] were used to construct engineered *E. coli* strains harboring pET-Deut-*Go*SCR-*Bm*GDH or pET-Deut-*Bs*RCR-*Bm*GDH plasmids (*E. coli* WL03 and *E. coli* WL04, respectively) (Figure S5 and Table 4).

Table 4. Specific Enzyme Activity and ee Values of Carbonyl Reductases

enzyme	specific enzyme activity (U/mg protein)	ee (%)
*Bs*RCR	0.39	>99.9
*Pp*RCR	1.28	33.5
*Go*SCR	0.53	>99.9

Evaluation of the transformation modes of the cascade reaction revealed two modes: a 1-pot 1-stage mode (*Ct*PDC1^{C223E} and *E. coli* WL03 participate in the reaction together) and a 1-pot 2-stage mode (*Ct*PDC1^{C223E} and *E. coli* WL03 participate in the reaction in order). The yield and enantiomeric excess (ee) values for the 1-pot 1-stage mode were 57.0% and 99.9%, respectively, whereas those for the 1-pot 2-stage mode were 73.7% and 99.9%, respectively. Additionally, the byproduct benzyl alcohol was identified in both modes, with the former mode producing 0.7 mM and the latter 0.1 mM. Therefore, the 1-pot 2-stage mode was chosen for the cascade reaction (Table 5).

Table 5. Comparison of the Two Transformation Modes

mode	yield (%)	ee (%)	byproduct (mM)
1-pot 1-stage	57.0	99.9	0.7
1-pot 2-stage	73.7	99.9	0.1

The 1-pot 2-stage cascade reaction for (*S*)-PED synthesis combines *Ct*PDC1^{C223E} and the (*S*)-selective *Go*SCR as catalysts. At the first stage, a nonisolated yield (92.7%, from 4.5 mM substrate) of 2-HAP was obtained in 3.5 h. In the second stage, the reaction was initiated by adding *E. coli* WL03, 0.2 mM NAD^+, and 6 mM D-glucose, resulting in substrate conversion of 79.5% and an ee value of 99.9% (24 h) (Figure 10a). The overall conversion of the cascade reaction was 73.7% with an ee value of 99.9%. The results showed that 26.3% of the 2-HAP intermediate accumulated during the reaction, which could not be further transformed. This indicated that the catalytic activity of *Go*SCR toward 2-HAP was the rate-limiting step of the cascade reaction.

The 1-pot 2-stage cascade reaction for (*R*)-PED synthesis combined *Ct*PDC1^{C223E} and *Bs*RCR as catalysts. For the subsequent reduction reaction, 60.4% substrate conversion of 60.4% and 99.9% ee value for 24 h was observed, with the overall conversion of the cascade reaction at 56.0% (Figure 10b). Additionally, 44.0% of the 2-HAP intermediate accumulated during the reaction. The results suggested that the reduction reaction remained the rate-limiting step; therefore, our future work will focus on improving the speed of this cascade reaction.

The enzymatic−chemical cascade route for preparative scale reactions with *E. coli* expressing *Ct*PDC1^{C223E} as a whole-cell biocatalyst (30 g/L, wet cells) was tested in a 100 mL reaction volume. Under optimal conversion conditions (2.5 M MOPS buffer [pH 6.5], 10 mM $MgSO_4$, 10 mM ThDP, 5% DMSO; benzaldehyde and glyoxylic acid were added in 20 mM and 60 mM batches every 2 h, respectively), this route converted the substrates benzaldehyde (100 mM, 10.6 g/L) and glyoxylic acid (300 mM, 22.2 g/L) to 12.3 g/L of 2-HAP after 24 h at 30 °C and 200 rpm, with a 90.0% conversion and 81% (996 mg) isolated yield, respectively. Additionally, preparative scale reactions were conducted on (*S*)-PED and (*R*)-PED. Subsequently, (*S*)-PED was obtained with a 71.2% (9.8 g/L) overall conversion, 99.9% ee value, and 63% (617 mg) isolated yield, and (*R*)-PED was obtained with a 55.4% (7.7 g/L) overall conversion, 99.9% ee value, and 60% (462 mg) isolated yield.

DISCUSSION

In this study, the design and evaluation of a cascade reaction combining enzymatic and spontaneous chemical reactions to produce 2-HAP were described. On the basis of retrosynthetic analysis, a spontaneous proton-transfer reaction was designed to generate a synthetic route for 2-HAP from **4a** through computational simulation. Furthermore, the unnatural substrate **2** was introduced into the C−C ligation reaction catalyzed by *Ct*PDC1 in order to construct the route for **4a** synthesis. Using 2-HAP as the starting substrate, routes to

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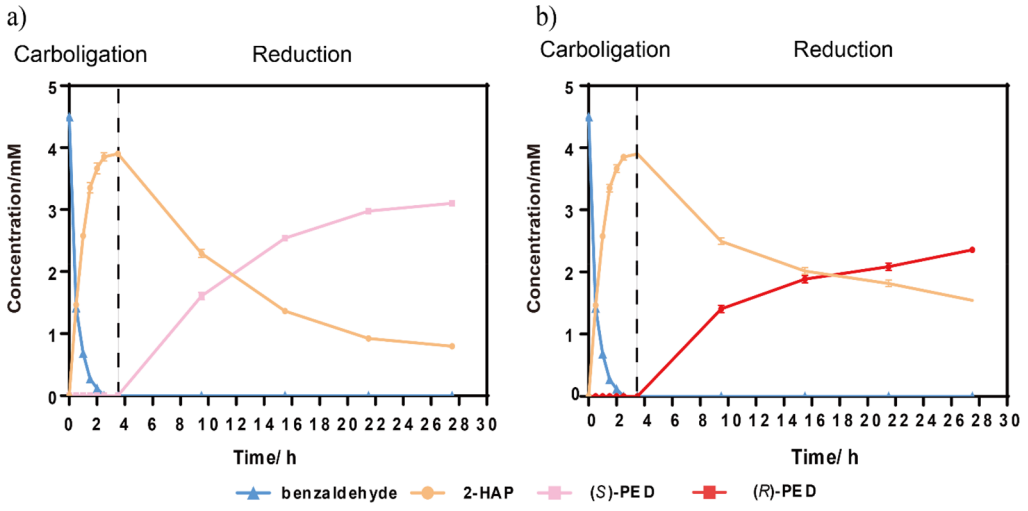

Figure 10. 1-pot 2-stage cascade reaction from **1a** and **2** to (*S*)-and (*R*)-PED. Time courses of (a) (*S*)-PED and (b) (*R*)-PED synthesis using the 1-pot 2-stage mode. Reaction conditions: step 1:2.5 M MOPS (pH 6.5), 5 mM Mg^{2+}, 1 mM ThDP, 0.5% DMSO, 4.5 mM **1a**, 15 mM **2**, 30 g/L *E. coli* (*Ct*PDC1^{C223E}), 30 °C, 200 rpm, and 3.5 h; Step 2: pH 7, 0.2 mM NAD^+, 6 mM D-glucose, 30g/L *E. coli* WL03 or *E. coli* WL04, 2.5 M MOPS, 30 °C, 200 rpm, and 24 h.

produce (*S*)- and (*R*)-1-phenyl-1,2-ethanediol were also generated. The cascade reaction presented here represents a low-cost method for limiting byproduct production and the substrate toxicity associated with 2-HAP synthesis.

Here, spontaneous chemical reactions were used as a synthetic strategy in a cascade reaction to produce chemicals in order to (1) maximize reaction compatibility, (2) minimize the need for the reaction to be driven by an outside energy source, and (3) remove barriers to mass transfer. One example is a spontaneous ring-closing reaction using cascading asymmetric reductive amination to synthesize biaryl-bridged NH lactams under Ru-catalyzed conditions.[21] A second example is in the synthetic pathway of macromolecular natural products quinolone alkaloids catalyzed by dioxygenase AsqJ, a spontaneous nonenzymatic rearrangement and elimination reaction is required to achieve the synthesis of the final product.[22] Recently, chemical reactions have increasingly combined with biocatalysis to synthesize a series of high-value chemicals, including terpenes and their analogues (arabinomannan, glycoconjugates, and glycopeptide antibiotic analogues).[23−25] However, chemical−enzymatic cascade reactions are often limited by poor compatibility in areas, including temperature, solvent, pH, and reaction conditions,[26−29] that make it difficult to achieve one-pot, continuous catalysis under the same condition. In the present study, efficient and environmentally friendly synthesis of 2-HAP was achieved through introducing a spontaneous chemical reaction after *Ct*PDC1-catalyzed C−C ligation reaction, where the presence of H^+ and Mg^{2+} in solution promoted the spontaneous reaction. Furthermore, compared with chemical reactions requiring higher temperatures, high pressure, and specific light conditions,[13,26,30] this spontaneous chemical reaction does not require external energy to drive the transformation, which involves conversion of **4a** into 2-HAP via proton transfer, thereby promoting the concepts of "atom economy" and "green chemistry".[31,32] Furthermore, compared with other enzymatic−chemical cascade reactions involving two or more catalysts,[23,24,26−28,33] this spontaneous chemical reaction does not require an additional catalyst, which addresses the potential mass-transfer problem between the two catalysts.

In general, the natural substrates of PDCs are pyruvate and **1a**;[16] however, previous studies focused on expanding the catalytic availability of PDCs to include **1a** derivatives as substrates [i.e., PDC-mediated catalysis of the reaction of pyruvate with 2,5-dimethoxybenzaldehyd[34] and a halogenated **1a**[35] to produce 1-hydroxy-1-(2,5-dimethoxyphenyl)propan-2-one and the corresponding halogenated phenylacetylcarbinol]. In fact, pyruvate as the smallest keto acid substrate has multiple structural analogs. In the present study, **2** was used as a substrate for *Ct*PDC1 to promote its reaction with **1a**, resulting in the product **4a**. To the best of our knowledge, this is the first report using **2** as a smaller unnatural substrate to participate in a C−C carboligation reaction to produce aromatic α-hydroxy ketones. The expanding number of pyruvate-like substrates has led to increased numbers of aromatic α-hydroxy ketones synthesized by PDCs. Furthermore, biotransformations with *Ct*PDC1^{C223E} have a broad substrate scope, where *Ct*PDC1^{C223E} accepts various forms of ortho-, para-, or meta-substituted benzaldehyde as the substrate and catalyzes the synthesis of the corresponding 2-HAP derivatives. For the enzyme catalysis, expanding the substrate scope of a specific enzyme represents an efficient strategy for acquiring new products. For example, by expanding the substrate tunnel of *Cg*TD, the substrate scope of *Cg*TD could potentially show affinity for the bulky substrate phenylserine.[36] Additionally, a previous study produced a series of noncanonical amino acids by engineering tryptophan synthase to enlarge its substrate scope to include nitroalkane nucleophiles.[37]

The goal of this process was to insert a new reaction into a cascade reaction to promote synthesis of new products. A previous study described dihydroxylation of benzene catalyzed by P450-BM3 for synthesis of the natural product arbutin via a cascading glucosyltransferase reaction.[38] In the present study, stereospecific SCR and RCR were used to produce the optically pure compounds (*S*)- and (*R*)-1-phenyl-1,2-ethanediol, respectively. Additionally, under optimized conditions, the preparative scale transformations for the synthesis of (*S*)- and (*R*)-PED were achieved. This pathway has three

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characteristics: (1) it realizes the transformation process of "achiral compounds−prochiral compounds−chiral compounds" to synthesize high-value chemicals through chiral-group resetting;[39] (2) compared with the starting substrates 4-phenyl-1,3-dioxolan-2-one, styrene, and 2-HAP[40−42] for synthesis of (*S*)- or (*R*)-1-phenyl-1,2-ethanediol, it uses the achiral substrates **1a** and **2**, which are readily available, inexpensive, and represent sustainable alternatives for producing optically pure 1-phenyl-1,2-ethanediol; and (3) this one-pot enzymatic−chemical cascade reaction can incorporate other enzymes, such as transaminases, to synthesize high-value amino alcohols from **1a** and **2**.[43,44]

In summary, an enzymatic−chemical cascade synthetic pathway was designed combining *Ct*PDC1-catalyzed unnatural reaction and a spontaneous proton transfer for synthesizing 2-HAP. This method represents a platform for synthesizing aromatic α-hydroxy ketones from low-cost starting materials and offers an attractive strategy and reference for the synthesis of other chemicals.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscatal.0c04961.

Materials and methods; general, genetic constructions, optimized sequences, fermentation medium and conditions, enzyme purification, UV−Vis spectroscopy, activity assay, kinetic assay, biotransformation procedures, preparative scale biotransformation procedures, HPLC analysis, GC−MS analysis, isolation protocols, enzyme mutagenesis, and hybrid cluster-continuum (HCC) methodology; and additional tables, figures, and references (PDF)

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Notes

The authors declare no competing financial interest.

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