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Stereoselective Synthesis of Enantiopure Oxazolidinones via Biocatalytic Asymmetric Aminohydroxylation of Alkenes

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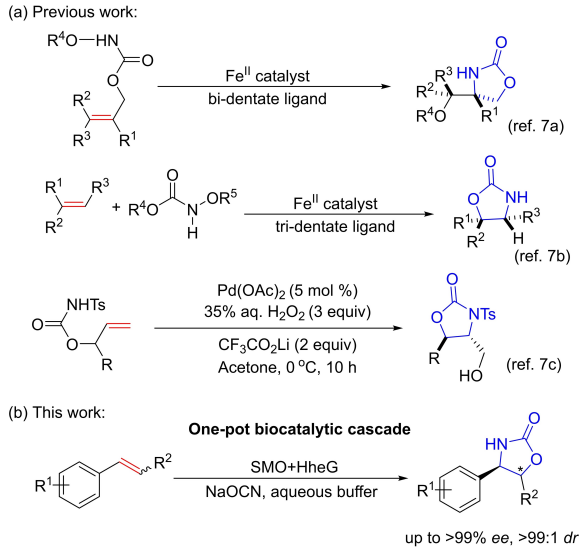
Abstract: Chiral oxazolidinones are of significance in both medicinal and synthetic chemistry, while preparing these compounds usually involves using expensive starting materials and harsh reaction conditions. Herein, a one-pot biocatalytic cascade process was developed for stereo- and regioselective aminohydroxylation of diverse alkenes by combining styrene monooxygenase and halohydrin dehalogenase, providing an approach to enantiopure oxazolidinones.

Keywords: Biocatalysis; Cascade; Oxazolidinones; Alkenes; Aminohydroxylation

Biocatalysis has shown persuasive advantages in organic transformations including, for instance, high chemo-, regio- and stereoselectivity. Enzymatic catalysis is widely recognized as a choice opportunity in organic synthesis, especially for chiral compounds manufacture. In recent years, the biocatalytic cascade that combining multi-enzyme reactions for a sequential biotransformation has emerged as a powerful toolbox leading to the generation of high-value-added chemicals from simple precursors.[1] Biocatalytic cascades are usually performed in one-pot system without isolating intermediates, and thus can improve product yield and reduce the amount of waste by saving reagents and resources. The development of new biocatalytic cascades for the synthesis of useful chemical scaffolds constitutes a hot topic in green chemistry and sustainable synthesis. As a result, many synthetic strategies based on biocatalytic cascades have been developed for production of diverse valuable compounds.[2]

Chiral oxazolidinones are important heterocycle compounds in organic transformations, which serve as not only chiral auxiliaries in asymmetric synthesis,[3] but also key synthons for the synthesis of naturally occurring amino acids.[4] More importantly, chiral oxazolidinones represent a privileged structural motif that present in many antimicrobials and antibiotics such as Linezolid.[5] Many synthetic approaches towards chiral oxazolidinones have been reported starting from various precursors such as epoxides, diols, cyclic enamido esters, chiral aziridines and chiral 1,2-amino alcohols.[6] However, these methods suffer from harsh reaction conditions and the using of chiral or functionalized substrates. Recently, Xu's and Liu's groups have reported the synthesis of oxazolidinones via metal-catalyzed inter- and intramolecular aminohydroxylation of alkenes (Scheme 1a),[7] while the catalytic stereoselectivity is absent in these methodologies.

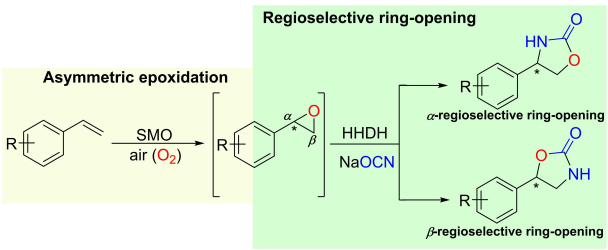
Given possessing promiscuous activity, halohydrin dehalogenase (HHDH) is considered as a promising biocatalyst in organic synthesis.[8] HHDHs can catalyze not only the dehalogenation of vicinal haloalcohols to the epoxides, but also the conversion of epoxides into *β*-substituted alcohols in the presence of several anion nucleophiles (*e.g.* CN^- and N_3^-). Importantly, several HHDHs have been identified to catalyze ring-opening of epoxides with cyanate (OCN^-), generating isocyanates and cyanate adducts. The unstable cyanate adduct is isomerized to the isocyanate, and then converted to oxazolidinone undergo spontaneous cyclization



Scheme 1. Strategies for the synthesis of oxazolidinones via aminohydroxylation of alkenes.

reaction.[9] Enzymatic synthesis of chiral oxazolidinones has been achieved based on the HHDH-catalyzed (dynamic) kinetic resolution of epoxides with cyanate.[9a,10] Styrene monooxygenase (SMO) is able to catalyze asymmetric epoxidation of styrene derivatives in high catalytic stereoselectivity and efficiency.[11] Several biocatalytic cascades involving SMO-catalyzed asymmetric epoxidation step have been developed to convert styrenes into many useful molecules.[12] In this study, we describe a biocatalytic cascade for stereo- and regioselective aminohydroxylation of styrenes to afford various enantiopure oxazolidinones in excellent optical purity (Scheme 1b).

Design of the biocatalytic cascade for aminohydroxylation of alkenes is showed in Scheme 2, which combines a stereoselective styrene monooxygenase (SMO) and a regioselective HHDH. The styrenes are transformed into chiral epoxide intermediates via SMO-catalyzed asymmetric epoxidation reaction, and then converted to chiral oxazolidinones by a subsequent regioselective ring-opening reaction catalyzed by

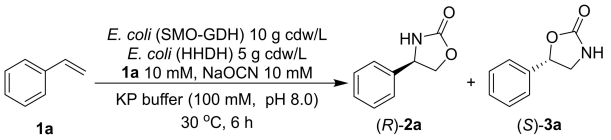


Scheme 2. Design of the biocatalytic cascade for the synthesis of chiral oxazolidinones via asymmetric aminohydroxylation of alkenes.

HHDH in the presence of cyanate. HHDHs are able to catalyze the *α*- and *β*-regioselective ring-opening of chiral epoxides, and thus would produce 4-aryloxazolidinones and 5-aryloxazolidinones, respectively (Scheme 2).

The recombinant strain *Escherichia coli* (SMO-GDH) strain co-expressing a SMO and a glucose dehydrogenase (GDH), and a range of *E. coli* (HHDH) strains were constructed and deposited in our laboratory (see Supplementary Information). Our investigations began with the screening of SMO-HHDH biocatalytic cascades for the model reaction of asymmetric aminohydroxylation styrene **1a**. The *E. coli* (SMO-GDH) exhibits high *S*-stereoselectivity in the asymmetric epoxidation of **1a** to afford chiral styrene oxide in >99% *ee*. By combining with *E. coli* (SMO-GDH), eleven recombinant *E. coli* (HHDH) strains were screened for the reaction of **1a** with NaOCN. Primary reactions were carried out on analytical scales in one-pot system. As shown in Table 1, styrene **1a** is not converted to oxazolidinones in the absence of HHDH (Table 1, entry 1). Although the dual-enzyme cascades that combining SMO and HHDHs produce 4-phenyloxazolidin-2-one (*R*)-**2a** and 5-phenyloxazolidin-2-one (*S*)-**3a** in high stereoselectivity, the catalytic efficiency and regioselectivity are evidently discrepant.

Table 1. Screening of biocatalytic cascades for asymmetric aminohydroxylation of **1a**.[a]



Entry	HHDH	Conv. [%][b]	*ee* (*R*)-**2a** [%][c]	*ee* (*S*)-**3a** [%][c]	Regio. [**2a**:**3a**][c]
1	–	0	n.d.	n.d.	n.d.
2	HheA5	5.8	>99	>99	72:28
3	HheA10	7.0	94	>99	9:91
4	HheB3	9.8	>99	>99	26:74
5	HheB6	18.3	>99	>99	30:70
6	HheC	6.2	>99	99	82:18
7	HheD	6.5	>99	>99	72:28
8	HheD6	6.7	97	>99	82:18
9	HheE	15.3	98	>99	5:95
10	HheE5	11.0	97	>99	6:94
11	HheG	40.3	>99	>99	99:1
12	HheG2	7.0	>99	>99	99:1

[a] Reactions were performed in 5 mL KP buffer (K_2HPO_4–KH_2PO_4, 100 mM, pH = 8.0, 2% w/v D-glucose) containing 10 g cdw/L *E. coli* (SMO-GDH), 5 g cdw/L *E. coli* (HHDH), 10 mM **1a** and 10 mM NaOCN at 30 °C for 6 h.

[b] Conversions were calculated based on the product concentrations of **2a** and **3a**.

[c] Determined by chiral HPLC analysis. n.d. = not detected.

Most biocatalytic cascades exhibit low catalytic efficiency (conversion < 10%). The biocatalytic cascade SMO-HheB6 exhibits a relatively high catalytic efficiency, while the regioselectivity is unsatisfactory (**2a**:**3a** = 30:70, Table 1, entry 5). Though the cascades SMO-HheE and SMO-HheE5 show an excellent *β*-regioselectivity for the formation of (*S*)-**3a**, the catalytic efficiencies are not high (Table 1, entries 9–10). To our delight, the biocatalytic cascade SMO-HheG can convert **1a** to (*R*)-**2a** with the highest catalytic efficiency (40% conversion) as well as excellent *α*-regioselectivity (**2a**:**3a** = 99:1, Table 1, entry 11). The HheG from *Ilumatobacter coccineusis* has been characterized by Schallmey and coworkers,[13] which is able to convert cyclic epoxides by ring-opening with aizde or cyanide.[14] In addition, our recent work revealed that the HheG showed high *α*-regioselectivity but low enantioselectivity in the reaction of cyanate-mediated ring-opening of racemic styrene oxides.[9b] Based on the above cascade screening result, the SMO-HheG biocatalytic cascade was chosen to prepare chiral 4-aryloxazolidinones.

Due to the differences of enzyme characteristics such as pH and temperature dependence, condition matching is vital for maximizing the operating capacity of a multi-enzyme cascade system.[15] Therefore, we then systematically investigated reaction conditions of SMO-HheG cascade for high product throughput based on the model reaction. The aqueous-organic biphasic system (KP buffer: *n*-hexadecane = 8:1) dramatically decreased the conversion of **1a** down to 13% (data no shown). The result indicates that the biphasic system is unfavorable for this biocatalytic cascade, and thus the following study was carried out in aqueous system. The ratio of NaOCN to **1a** was investigated and the result was displayed in Figure 1a (See Table S1 for details). It can be seen that the yield of **2a** increases by increasing equivalence ratio and reaches up to 63% at the ratio of 4:1. Further enhancing the ratio do not cause obvious yield increase. Then cell loading of the two biocatalysts *E. coli* (SMO-GDH) and *E. coli* (HheG) was tested. As shown in Figure 1b (See Table S2 for details), when the *E. coli* (SMO-GDH) is used with 5 g cdw/L, increasing cell density of *E. coli* (HheG) from 5 to 10 cdw/L leads to improving the

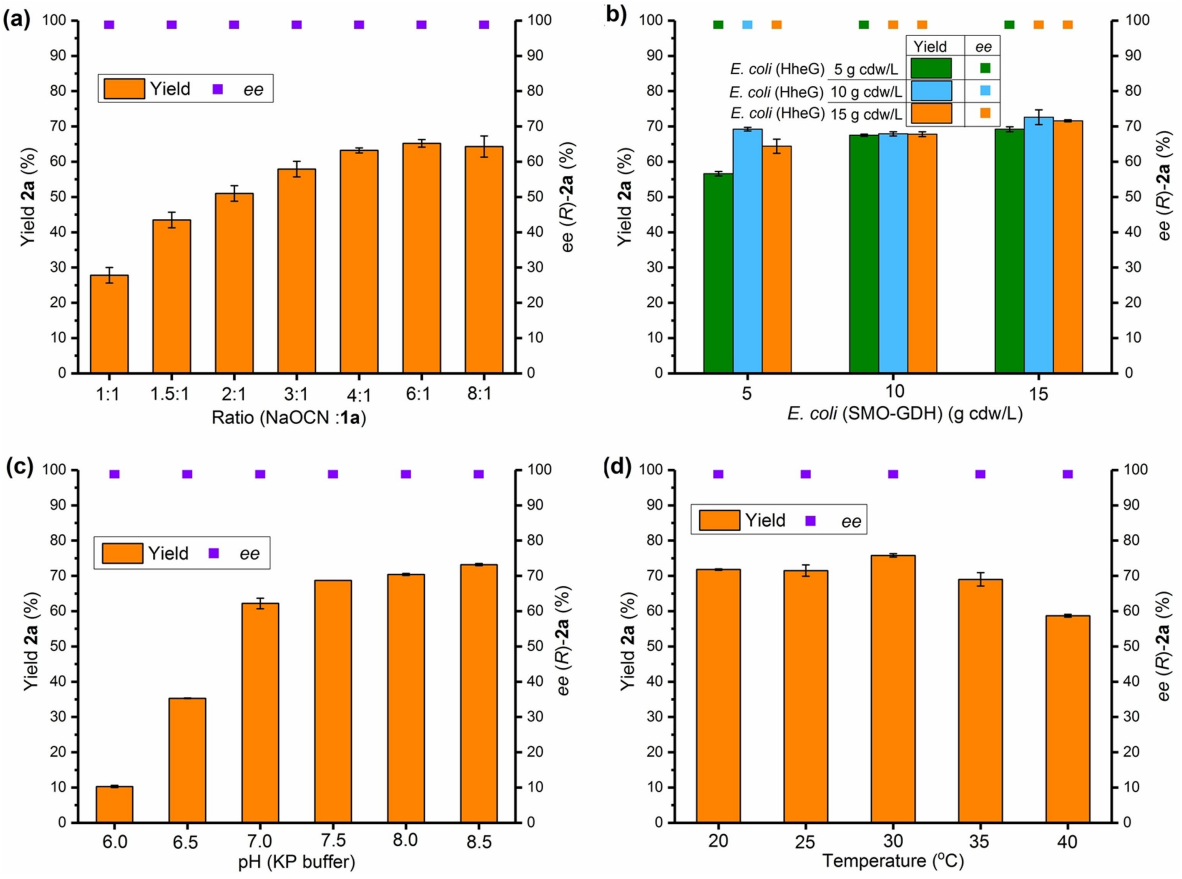
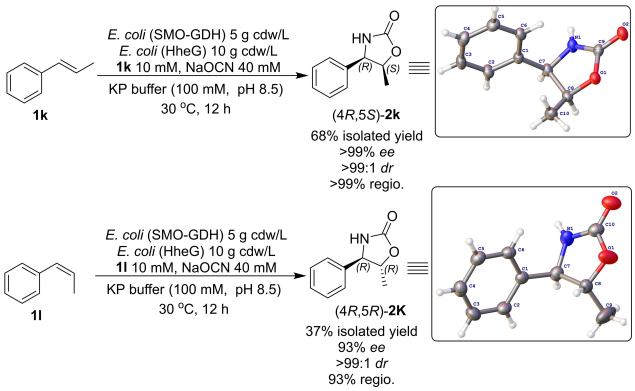


Figure 1. Investigation of SMO-HheG cascade conditions for asymmetric aminohydroxylation of **1a**. Reactions were carried out in 5 mL KP buffer (100 mM, 2% w/v D-glucose) containing *E. coli* (SMO-GDH), *E. coli* (HheG), NaOCN and 10 mM **1a** (See Tables S1–S4 for details). Analytical yields and *ee* of (*R*)-**2a** were determined by chiral HPLC.

yield up to 69%. However, further improving the cell density of *E. coli* (HheG) or/and *E. coli* (SMO-GDH) do not cause obvious yield increase. Subsequent investigation focused on the reaction pH of the biocatalytic cascade (Figure 1c), which reveals the acid environment tends to prevent the production of **2 a**. On the contrary, the alkali environment expedites the formation of **2 a**, giving 73% yield at pH 8.5. However, when the enzymatic cascade was performed in Tris-H_2SO_4 buffer at the pH ranging from 8.5 to 10.0, dramatic decreases in yield are observed (See Table S3 for details). These results demonstrate that both buffer pH and media influence the catalytic efficiency. Temperature investigation indicates SMO-HheG cascade tolerates a broad temperature profile of 20–35°C, and the maximum yield was obtained at 30°C (Figure 1d, see Table S4 for details). Performing the cascade at a higher temperature of 40°C results in a distinct yield decrease. Finally, the reaction course was investigated to show the maximum 75% analytical yield was obtained after reaction for 12 h (Table S5).

Under the optimized conditions, scope of the asymmetric aminohydroxylation of styrenes catalyzed by SMO-HheG cascade was investigated. As shown in Table 2, styrenes **1 a**–**1 j** are effectively converted into chiral 4-aryoxazolidinones **2 a**–**2 j** in 37–70% isolated yields. Importantly, asymmetric aminohydroxylation of **1 a**–**1 j** exhibits excellent stereo- and regioselectivity, affording the corresponding 4-aryoxazolidinones in 94–>99% *ee* and 94–>99% regioselectivity, respectively. It can be seen that styrenes with electron-withdrawing (F, Cl, Br) or electron-donating (Me) groups substituted on the phenyl are well tolerated by the biocatalytic cascade. Additionally, substrates with

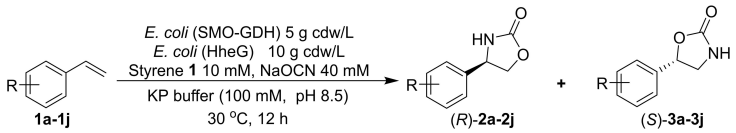


Scheme 3. Asymmetric aminohydroxylation of *β*-methylstyrenes **1 k**–**1 l** by the biocatalytic cascade. The *ee* and *dr* values were determined by chiral HPLC analysis. The regioselectivity was determined by ^{1}H NMR.

substituents on the *ortho*-, *meta*- or *para*-positions are all tolerated, albeit the *ortho*-substituted styrene **1 j** shows a relatively lower yield (Table 2, entry 10). Unfortunately, the biocatalytic cascade do not work well with the strong electron-withdrawing group substituted substrates (*e.g.* *para*-cyano and *para*-nitro styrenes), which is restricted by the SMO-catalyzed asymmetric epoxidation step (data not shown).

To expand the scope of SMO-HheG cascade, we next evaluated the reactivity of internal alkenes **1 k**–**1 l** under the standard reaction conditions (Scheme 3). Interestingly, asymmetric aminohydroxylation of *trans*-*β*-methylstyrene **1 k** also proceeds smoothly by the biocatalytic cascade, generating chiral 5-methyl-4-phenyloxazolidin-2-one (4*R*,5*S*)-**2 k** in 68% isolated

Table 2. Substrate scope of asymmetric aminohydroxylation of styrenes **1 a**–**1 j** by the biocatalytic cascade.[a]



Entry	R	Substrate	Product	Yield **2** [%][b]	*ee* **2** [%][c]	**2**:**3**[c]
1	H	**1 a**	(*R*)-**2 a**	60	>99	98:2
2	*p*-F	**1 b**	(*R*)-**2 b**	62	>99	97:3
3	*p*-Cl	**1 c**	(*R*)-**2 c**	61	>99	94:6
4	*p*-Br	**1 d**	(*R*)-**2 d**	66	99	95:5
5	*p*-CH_3	**1 e**	(*R*)-**2 e**	66	94	>99:1
6	*m*-F	**1 f**	(*R*)-**2 f**	63	>99	94:6
7	*m*-Cl	**1 g**	(*R*)-**2 g**	64	>99	97:3
8	*m*-Br	**1 h**	(*R*)-**2 h**	56	>99	97:3
9	*m*-CH_3	**1 i**	(*R*)-**2 i**	70	>99	>99:1
10	*o*-F	**1 j**	(*R*)-**2 j**	37	99	94:6

[a] Reactions were performed in 50 mL KP buffer (100 mM, pH = 8.5, 2% w/v D-glucose) containing 5 g cdw/L *E. coli* (SMO-GDH), 10 g cdw/L *E. coli* (HheG), 10 mM **1** and 40 mM NaOCN at 30 °C for 12 h.
[b] Isolated yields.
[c] The *ee* and regioselectivity were determined by chiral HPLC analysis.

yield, >99% *ee*, >99:1 *dr* and >99% regioselectivity. In addition, chiral (4*R*,5*R*)-**2k** is produced from *cis*-*β*-methylstyrene **1l** in 37% yield, 93% *ee*, >99:1 *dr* and 93% regioselectivity. Absolute configurations of the products (4*R*,5*S*)-**2k** and (4*R*,5*R*)-**2k** were determined by single-crystal X-ray diffraction analysis.[16] These results indicate the chiral 4,5-disubstituted oxazolidinones that containing two chiral centres can be prepared by the biocatalytic cascade, and thus further highlight the methodology for asymmetric aminohydroxylation of alkenes. Remarkably, chiral 4,5-disubstituted oxazolidinones have been proved to be the potential anticancer agents against lung and prostate cancer cells.[17]

In summary, we have developed a one-pot biocatalytic cascade for the asymmetric aminohydroxylation of alkenes by combining SMO and HheG. The combined dual-enzyme cascade SMO-HheG is compatible and work well, enabling various styrenes to be converted into enantiopure oxazolidinones with good stereo- and regioselectivity. Additionally, inexpensive alkene, dioxygen and cyanate were used as starting resources.

Experimental Section

General procedure for biocatalytic asymmetric aminohydroxylation of styrenes: 50 mL KP buffer (K_2HPO_4-KH_2PO_4, 100 mM, pH 8.5, 2% w/v D-glucose) containing resting cells of 5 g cdw/L *E. coli* (SMO-GDH) and 10 g cdw/L *E. coli* (HheG) were added to a 250 mL centrifuge tube. To this solution, 0.5 mmol alkene **1a**–**1l** was added to a final concentration of 10 mM using DMSO as cosolvent. Then 2.0 mmol NaOCN was added to a final concentration of 40 mM, and the mixture was vibrated at 30°C for 12 h. The reaction mixture was then extracted with ethyl acetate (3×100 mL), and the organic phases were separated by centrifugation, combined, dried over anhydrous Na_2SO_4 and evaporated at reduced pressure. The resulting mixture was purified by flash chromatography (petroleum ether: ethyl acetate = 5:1, dichloromethane: ethyl acetate = 50:1 ~ 10:1) to afford enantiopure oxazolidinones **2a**–**2k**.

Acknowledgements

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[16] The crystallographic data for (4*R*,5*S*)-**2k** (CCDC number: 2073424) and (4*R*,5*R*)-**2k** (CCDC number: 2073425) have been deposited in The Cambridge Crystallographic Data Centre. These data can be obtained free of charge from the website (www.ccdc.cam.ac.uk/data_request/cif.) For details about the crystallographic data for (4*R*,5*S*)-**2k** and (4*R*,5*R*)-**2k**, see Tables S6 and S7 in the Supporting Information.
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COMMUNICATIONS

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