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Regioselective Ring-Opening of Styrene Oxide Derivatives Using Halohydrin Dehalogenase for Synthesis of 4-Aryloxazolidinones

Nanwei Wan,* Jiawei Tian, Xiaoying Zhou, Huihui Wang, Baodong Cui, Wenyong Han, and Yongzheng Chen*

Key Laboratory of Biocatalysis & Chiral Drug Synthesis of Guizhou Province, Generic Drug Research Center of Guizhou Province, Green Pharmaceuticals Engineering Research Center of Guizhou Province, School of Pharmacy, Zunyi Medical University, Zunyi, 563000, China. * E-mail: nanweiwan@zmu.edu.cn; yzchen@zmu.edu.cn

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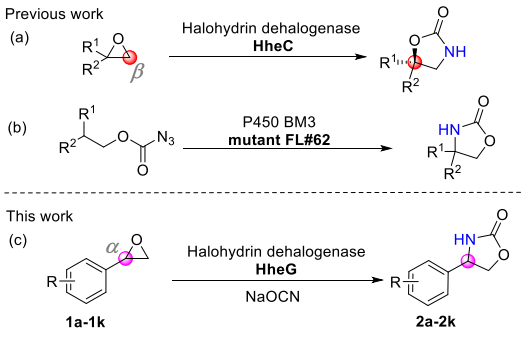
Abstract. A biocatalytic approach towards a range of 4-aryloxazolidinones is developed using a halohydrin dehalogenase from *Ilumatobacter coccineus* (HheG) as biocatalyst. The method is based on the HheG-catalyzed α-position regioselective ring-opening of styrene oxide derivatives with cyanate as a nucleophile, producing the corresponding 4-aryloxazolidinones in moderate to good yields. Synthesis of enantiopure 4-aryloxazolidinones is also achievable using chiral epoxide materials.

Keywords: Oxazolidinones; Halohydrin dehalogenase; Epoxides; Regioselectivity; Ring-opening

Heterocyclic compounds are not only key central skeletons of a wide range of biologically active compounds, but also serve as useful synthons in synthetic organic chemistry. Oxazolidinones are a class of oxygen and nitrogen containing heterocycles, which have received significant attention in medicinal chemistry due to their outstanding biological activity.[1] Oxazolidinones have been used as a new class of fungicides, antibacterials and antimicrobial agents.[2] In addition, oxazolidinones are valuable synthetic intermediates for a variety of organic transformations, for example, as precursors of β-amino acids.[3] 4-substituted oxazolidinones such as 4-ary substituted oxazolidinones[4] and 4-hydroxymethyl substituted oxazolidinones,[5] are one important type of oxazolidinone derivatives. Therefore many synthetic strategies have been designed for the formation of 4-substituted oxazolidinones.[5-6] However, these chemical methods usually suffer from the need for harsh reaction temperature, long reaction time, or the use of expensive catalysts.

Biocatalysis is an important green synthetic technology which have been widely used in the organic synthesis due to its mild reaction conditions.[7] Several efforts have been made on enzymatic catalysis for the synthesis of oxazolidinones. One enzymatic route was reported by Janssen and coworkers, which starts from epoxides using a halohydrin dehalogenase (HHDH) from *Agrobacterium radiobacter* (HheC) (Scheme 1a).[8] In this method, cyanate (OCN^-) is accepted by HheC as a nucleophile to ring-opening of epoxides at β-position, and the produced β-substituted alcohols are further converted to 5-substituted oxazolidinones undergoing spontaneous cyclization. Recently, Fasan and coworkers have successfully synthesized 4-substituted oxazolidinones via the P450-catalyzed cyclization of carbonazidates (Scheme 1b).[9] This is the first and unique report that synthesis of 4-substituted oxazolidinones via enzymatic catalysis. However, this method suffers from the low catalytic efficiency (turnover numbers <100) and the substrate carbonazidates are not easy to get. Compared with the P450-method, the HHDH-method exhibits relative high substrate tolerance and good enzyme stability. In addition, the epoxides are cheap and widely available materials. Because most of the characterized HHDHs show the high β-position regioselectivity in the ring-opening process of epoxides, synthesis of 4-substituted oxazolidinones using HHDH has not been reported until now.



Scheme 1. Biocatalytic routes for the synthesis of oxazolidinones.

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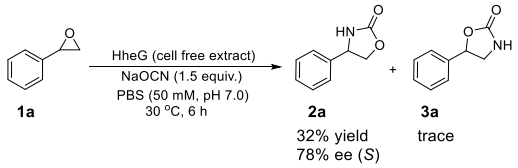
Recently, we have found that the HHDH from *Ilumatobacter coccineus* (HheG) showed high α-position regioselectivity in the azide-mediated ring-opening of styrene oxide derivatives, producing 2-azido-2-aryl-1-ols in good yields.[10] Herein, we further explored the HheG-catalyzed ring-opening reaction of styrene oxide derivatives using cyanates as a nucleophile and developed an alternative biocatalytic route to 4-aryloxazolidinones in moderate to good yields (Scheme 1c).

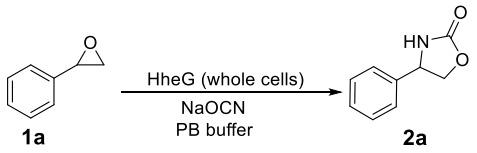
We began our investigation with an analytical reaction using styrene oxide **1a** as a model substrate (Scheme 2). The reaction was carried out in 5 mL PB buffer (Na_2HPO_4-KH_2PO_4, 50 mM, pH 7.0) containing 10 mM **1a**, 15 mM NaOCN and 3 mL cell free extract of *Escherichia coli* (HheG). After reaction at 30 °C for 6 h, the reaction mixture was extracted for chiral HPLC analysis. Compared to the synthesized (*R*, *S*)-**2a** and (*R*, *S*)-**3a**, the enzymatic reaction produced **2a** in 32% yield and trace of **3a**, which indicated HheG remained high α-position regioselectivity in the cyanate-mediated ring-opening of **1a**. Interestingly, HheG also exhibited moderate enantioselectivity in this reaction, giving 78% ee of (*S*)-**2a**. We also performed a control reaction using the *E. coli* cells in the absence of HheG as biocatalysts and found no production of **2a** or **3a**, which indicated spontaneous ring-opening of **1a** with cyanate did not occur in this reaction.



Scheme 2. HheG-catalyzed ring-opening of **1a** with cyanate.

Subsequently, the reaction conditions for the production of **2a** were investigated using the recombinant *E. coli* (HheG) whole cells as biocatalysts. We first evaluated the reaction pH of PB buffer. As showed in Table 1 (entries 1-5), the highest yield was found at pH 7.5 (entry 3). Though the maximum ee of **2a** was found at pH 6.0, the corresponding yield was very low. Investigation of reaction temperature was carried out next at the range from 25 to 40 °C in pH 7.5 PB buffer (entries 6-8). We found that the first choice of 30 °C was the optimum temperature for both the yield and ee of **2a** (entry 3 vs entries 6-8), which was in according with our previous study of HheG in the azide-mediated ring-opening reaction.[10] It was clear that increasing the temperature to 40 °C caused significant decrease in the yield (entry 8). The equivalence ratio of NaOCN to **1a** was examined in the range from 0.5 to 6 (entries 9-12). The yield increased with the increase of equivalence ratio and reached up to 64% at the ratio 3:1. When we further increased the ratio to 6:1, dramatic increase in the yield was not obtained (entry 12 vs entry 11). Reducing the equivalence ratio to 0.5:1 slightly improved the ee to 80%, but caused 20% decrease in the yield (entry 9 vs entry 3). Although we expected to synthesize enantiopure **2a** via kinetic resolution of **1a**, the enantioselectivity (*E* value) in the cyanate-mediated ring-opening process was really low to meet this goal. Hence, we chose the ratio 3:1 for this reaction with the consideration of the high yield. At last, a survey of cell density indicated the highest yield was obtained using 15 g cdw/L cells (entry 14). Higher cell density did not improve the yield (entry 15).

Table 1. Optimization of the reaction conditions for the production of **2a**.



Entry[a)]	pH	T (°C)	NaOCN:**1a**	Cell Conc. (g cdw/L)	Yield **2a** (%)[b)]	ee (*S*)-**1a** (%)[c)]	ee (*R*)-**2a** (%)[c)]	*E* value[d)]
1	6.0	30	1.5:1	10	7	<5	85	13
2	7.0	30	1.5:1	10	16	19	77	9
3	7.5	30	1.5:1	10	37	50	72	10
4	8.0	30	1.5:1	10	29	43	71	9
5	8.5	30	1.5:1	10	21	39	73	9
6	7.5	25	1.5:1	10	34	48	62	7
7	7.5	35	1.5:1	10	17	24	70	7
8	7.5	40	1.5:1	10	14	12	69	6
9	7.5	30	0.5:1	10	18	15	80	11
10	7.5	30	2:1	10	49	75	57	8
11	7.5	30	3:1	10	64	88	47	7
12	7.5	35	6:1	10	68	92	31	5
13	7.5	30	3:1	5	56	79	53	8
14	7.5	30	3:1	15	78	86	24	4

15	7.5	30	3:1	20	70	83	20	3

[a] The reactions were performed in 5 mL PB buffer containing 10 mM **1a** and analyzed after 6 h. [b] The yields were determined by chiral HPLC using the calibration curve of racemic **2a**. [c] The ee values were determined by chiral GC or HPLC. [d] The *E* values were calculated from ee **1a** and ee **2a** using an online tool (http://biocatalysis.uni-graz.at/enantio/cgi-bin/enantio.pl).

Because the substrate concentration was an important indicator to evaluate a biocatalytic reaction, the reaction courses for the substrate concentrations from 5 to 50 mM were investigated under the optimized reaction conditions (Table 1, entry 14). As depicted in Figure 1, the reactions almost finished at 6 h. The yields did not increase obviously when the reaction time was prolonged to 12 h or 24 h. Stable yields (>75%) could be obtained at the substrate concentrations of 5-30 mM. Obvious decrease in the yield was observed when a higher substrate concentration of 50 mM was conducted for this reaction. With the comprehensive consideration of the yield and substrate concentration, the reaction was established to perform with epoxide concentration at 30 mM.

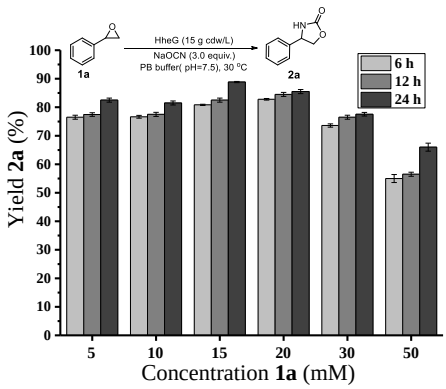
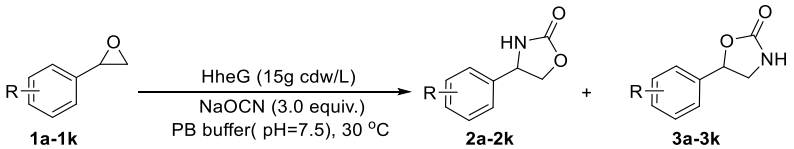


Figure 1. Reaction courses for the substrate concentrations from 5 to 50 mM. Analytic yields were determined by chiral HPLC with three parallel samples.

Following identification of this optimized catalytic system, we then started to investigate the scope of this process (Table 2). In general, all the tested styrene oxide derivatives **1a-1k** could be converted to the corresponding 4-aryloxazolidinones **2a-2k** in moderate to good yields (entries 1-11). As expected, the HheG exhibited good α-position regioselectivity in these ring-opening reactions, which was demonstrated by the ratio of 4-aryloxazolidinone **2** to 5-aryloxazolidinone **3**. It could be found that both *para*- and *meta*-substitutions on **1a** were well tolerated (entries 2-11 vs entry 1). The *para*-substituted epoxides gave relative higher yield and regioselectivity than that of the *meta*-substituted epoxides for the production of 4-aryloxazolidinones (entries 6-8 vs entries 2-4). The epoxides with the strong electron-withdrawing group or the bulk alkyl group on the aromatic ring resulted in moderate yields, while still retained good α-position ring-opening regioselectivity (entries 10-11). It is important to note that the 4-fluorophenyl substituted oxazolidinone **2f**, an important synthon for the synthesis of tyrosine kinase inhibitors, was usually prepared from the corresponding β-aminoalcohols and diethyl carbonate under high temperature (100-130 °C).[11] Using the enzymatic reaction, **2f** could be synthesized from the readily available epoxide **1f** in good yield under mild reaction condition (entry 6).

Table 2. Substrate scope for the synthesis of 4-aryloxazolidinones **2** catalyzed by HheG.

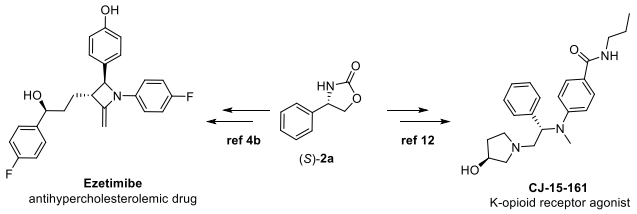


Entry[a]	Substrate	Product	R	Ratio **2**:**3**[b]	Yield **2** (%)[c]	ee **2** (%)[d]
1	**1a**	**2a**	H	91:9	77	31(*S*)
2	**1b**	**2b**	*m*-F	85:15	66	9(-)
3	**1c**	**2c**	*m*-Cl	84:16	65	7(-)
4	**1d**	**2d**	*m*-Br	80:20	46	8(-)
5	**1e**	**2e**	*m*-CH_3	92:8	49	<5(-)
6	**1f**	**2f**	*p*-F	87:13	67	30(-)
7	**1g**	**2g**	*p*-Cl	87:13	70	<5(-)
8	**1h**	**2h**	*p*-Br	90:10	58	20(-)
9	**1i**	**2i**	*p*-CH_3	92:8	47	15(-)
10	**1j**	**2j**	*p*-CN	91:9	32	52(-)
11	**1k**	**2k**	*p*-$(CH_3)_3$	91:9	29	6(-)

12[e)]	(*R*)-**1a**	(*S*)-**2a**	H	ND	84	>99(*S*)
13[e)]	(*S*)-**1a**	(*R*)-**2a**	H	ND	62	>99(*R*)

[a)] Reaction conditions: PB buffer (50 mM, pH 7.5) 30 mL, cell density 15 g cdw/L, epoxides conc. **1a-1k** 30 mM, NaOCN conc. 90 mM, reaction temperature 30 ˚C, reaction time 12 h. [b)] Determined by ^{1}H NMR. [c)] Isolated yield. [d)] Configuration were defined using commercial enantiopure (*R*)-**2a** and (*S*)-**2a**. The ee values were determined by chiral HPLC. [e)] Epoxides conc. 15 mM, NaOCN conc. 45 mM. ND = not detected.

When the chiral epoxides (*R*)-**1a** was used in the reaction, the optically active (*S*)-**2a** was obtained in 84% yield and >99% ee (entry 12). Replacement of (*R*)-**1a** with the opposite enantiomer (*S*)-**1a** also gave enantiopure (*R*)-**2a** in good yield (entry 13). What should be emphasized was that (*S*)-**2a** was a key intermediate for the synthesis of antihypercholesterolemic drug ezetimibe[4b] and K-opioid receptor agonist CJ-15161 (Scheme 3).[12] These results indicated enantiocomplementary synthesis of chiral 4-aryloxazolidinones from chiral epoxides was practicable for this enzymatic method.



Scheme 3. Synthetic applications of (*S*)-**2a** in chiral drugs.

In conclusion, we conducted a detailed study of the HheG catalyzed ring-opening of styrene oxide derivatives with cyanate. Our study has achieved for the first time biocatalytic synthesis of 4-aryloxazolidinones from cheap and readily available epoxides. This synthetic route represents a great improvement compared to the method via P450-catalyzed cyclization of carbonazidates. In addition, synthesis of enantiopure 4-aryloxazolidinones was also achievable from chiral epoxide materials.

Experimental Section

General procedure for the synthesis of 4-aryloxazolidinones 2a-2k

Expression and preparation of the recombinant *E. coli* cells (HheG) was carried out using the previously reported protocol.[10] To a 30 mL (25 mL for **1j**, 20 mL for **1k**) suspension of 15 g cdw/L *E. coli* cells (HheG) in 50 mM PB buffer (pH 7.5) was added solid NaOCN to a final concentration of 90 mM. Then the epoxide **1** was added to a final concentration of 30 mM using 300 μL of dimethyl sulfoxide as co-solvent. The reaction mixture was stirred at 250 rpm and 30 ºC. After reaction for 12 h, the mixture was extracted using ethyl acetate (2 × 30 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated by rotary evaporation. The residue was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate = 1:1) to afford the desired product 4-aryloxazolidinone **2**.

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