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Regioselective Ring-Opening Reactions with Non-Natural Substrates using Engineered Halohydrin Dehalogenase

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PURPOSE OF THE ABSTRACT

Epoxides are important building blocks for chemical and pharmaceutical synthesis [1], and many of their ring-opening derivatives have been used as chiral building blocks or auxiliary agents such as chiral diols [2], halohydrins, glycinols [3], and Evans-type auxiliaries [4]. The conventional methods for epoxide ring-opening suffers from poor regioselectivity, which poses a challenge for the direct utilization of eopxide derivatives.

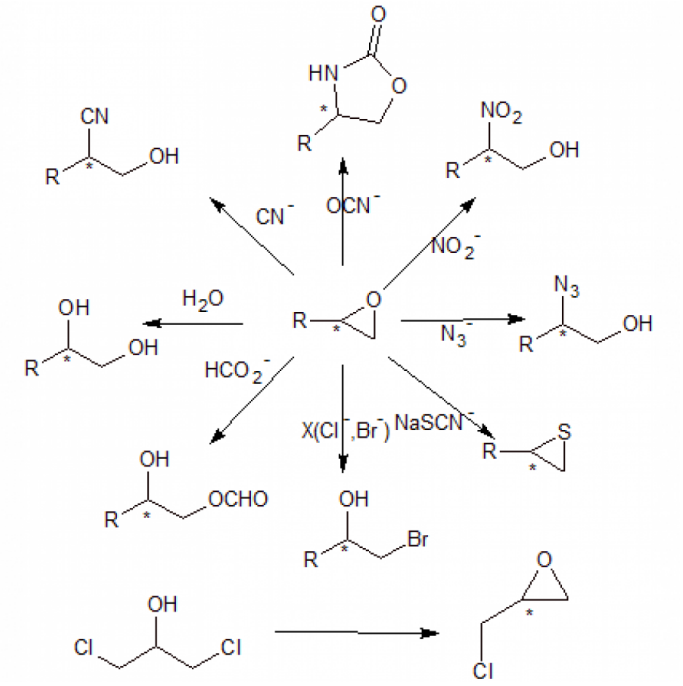
The oxazolidines are well known as Evans-type auxiliaries in the organic synthesis since 1981. A lot of medicine were developed by using oxazolidines as starting materials [4]. In industry, expensive amino acids are traditionally used as starting points for the above-mentioned chiral building blocks whose production involves NaBH4 reductions, phosgene/ethyl chloroformate cyclizations, and high pollutant emissions like hydrogen chloride, hydrogen sulfide, and methylbenzene [5,6]. These traditional approaches result in higher costs and pollution compared to epoxide dervatives [7–9].

Halohydrin dehalogenases (HHDH) are industrially relevant enzymes that catalyze the reversible dehalogenation of vicinal haloalcohols, yielding the corresponding epoxides [10]. In the reverse reaction, non-native nucleophiles like NaOCN [11] and NaSCN [12] may lead to the enzymatic SN2 ring-opening and spontaneous ring re-closing processes for desired products such as oxazolidines. As a result, such non-native reactions would give 100% conversion.

In recent research, many HHDHs were discovered to accept various non-native nucleophiles for ring-opening (Figure 1) [13]. Most of the ring opening reactions were triggered by nucleophiles at the beta carbon position (beta type), providing secondary alcohols as final products [3]. In nature, however, several HHDHs exhibit special regioselective alpha ring-opening reactions (alpha type), producing a new series of heterocyclic products [11]. Unfortunately, like other traditional catalysts for chemical ring opening [14], most of the wild-type (WT) enzymes had insufficient regio- and stereo-selectivity towards the desired alpha ring-opening products, leaving a large number of beta ring-opening by-products. At the same time, the process efficiency of HHDH-catalyzed ring-opening reactions is quite poor due to HHDHs' poor substrate tolerance and low catalyitc acitivity. Here, a novel HHDH has been engineered by computer-aided direct enzyme evolution in our enzyme engineering lab which enables highly regio- and stereo-selective synthesis of Evans-type auxiliary reagents and other chiral glycinols at high substrate loading. For our engineered HHDH variants, their substrate tolerance has been improved by 100fold [144 g/L] for full conversion, while the alpha ring-opening products have excellent chirality (ee >99%) with minimum by-products (<1%). Until now, our best HHDH variants were well suited for the SN2 ring-opening reactions with (R)-styrene oxide, giving 4-phenyl-2-oxazolidinone and (S)-styrene oxide, both in high enantiopurity.

From our research, a panel of HHDH variants was developed to enable chemoenzymatic synthesis of a series of high-value products, including chiral glycinols, halohydrins, epichlorohydrins and mandelic acid. These enzymatic synthesis routes will provide more economic and environmental-friendly technologies to the chemical industry.

FIGURES



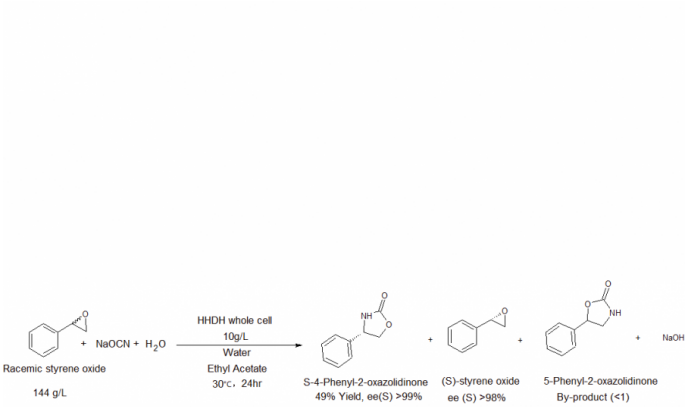


FIGURE 1

Figure 1

Reaction scopes of epoxides driven by HHDH and epoxide hydrolase

FIGURE 2

Figure 2

Scheme of enzymatic synthesis of 4-phenyl-2-oxazolidinone from styrene and NaOCN

KEYWORDS

Epoxides | Halohydrin dehalogenase | (S)-4-phenyl-2-oxazolidinone | Ring-opening

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