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

AUTHORS

Xinhang YANG / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Xiao LUO / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Fenshuai SUN / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Junxia ZHAO / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Kuifang HE / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Qinli PENG / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Ruimei HONG / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Marco BOCOLA / ENZYMASTER DEUTSCHLAND GMBH, NEUSSER STR. 39, DÜSSELDORF
Neha VERMA / ENZYMASTER DEUTSCHLAND GMBH, NEUSSER STR. 39, DÜSSELDORF
Hao YANG / ENZYMASTER (NINGBO) BIO-ENGINEERING CO, NO.333, SHIJI AVENUE NORTH SECTION, YINZHOU DISTRICT, NING BO
Thomas DAUSSMANN / ENZYMASTER DEUTSCHLAND GMBH, NEUSSER STR. 39, DÜSSELDORF
Osama MAHMOUD / ENZYMASTER DEUTSCHLAND GMBH, NEUSSER STR. 39, DÜSSELDORF
Corresponding author : Haibin CHEN / haibin.chen@enzymaster.com

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 epoxide derivatives.

Halohydrin dehalogenases (HHDH) are industrially relevant enzymes that catalyze the reversible dehalogenation of vicinal haloalcohols, yielding the corresponding epoxides[5]. In the reverse reaction, non-native nucleophiles like NaOCN[6] and NaSCN[7] 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.

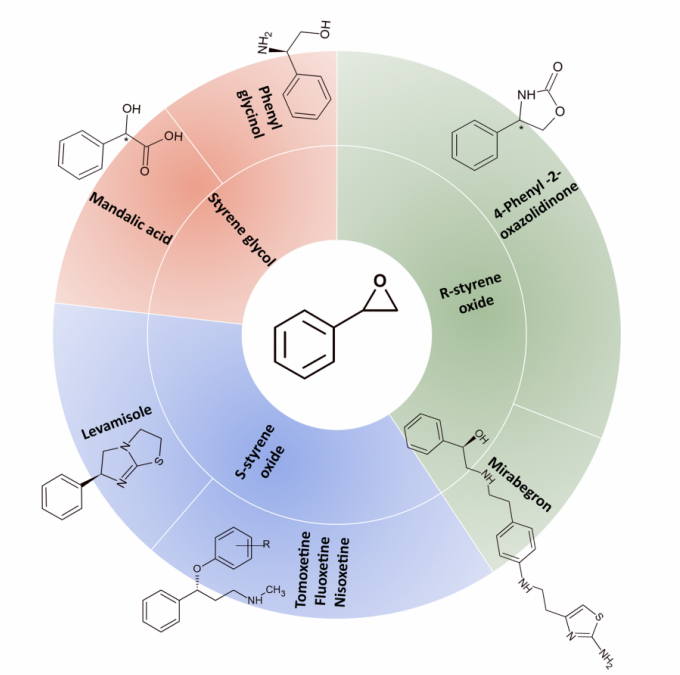
BioEngine® is an integrated directed enzyme evolution platform that offers the full-spectrum solution from enzyme discovery, enzyme engineering, process development, all the way to qualified product manufacturing. Powered with proprietary data collection as well as BioNavigator® toolbox and EM2L toolbox, Enzymaster's BioEngine® platform delivers biocatalytic solution effectively and efficiently.

From our research, 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 more than 100 fold for full conversion , while the alpha ring-opening products have excellent chirality with minimum by-products.

A panel of HHDH variants was developed to enable chemoenzymatic synthesis of a series of high-value products w/o assistance of epoxide hydrolase(EH), 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



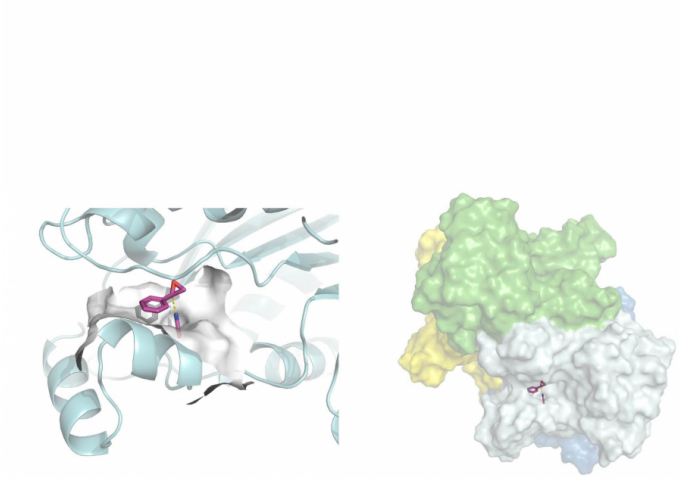


FIGURE 1

Product tree of styrene oxide

All potential medical intermediates and potential API products from styrene oxide are listed

FIGURE 2

Substrate docking results

The docking results from our BioEngine platform, leading to the library designs

KEYWORDS

Enzyme Evolutions | BioEngine | Halohydrin Dehalogenase | Styrene Oxide

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