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Epoxide Hydrolase-Catalyzed Enantioselective Synthesis of Chiral 1,2-Diols via Desymmetrization of *meso*-Epoxides

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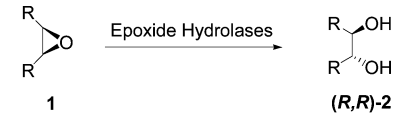
Enantioselective construction of chiral 1,2-diols has been the subject of extensive studies because of their importance in asymmetric synthesis.[1] Catalytic production of chiral diol derivatives via desymmetrization of *meso*-epoxides is an attractive route which simultaneously "constructs" two contiguous stereogenic centers and can furnish 100% theoretical yield of product.[2] Several asymmetric catalyst systems have been reported that promote this reaction using oxygen nucleophiles to generate 1,2-diol derivatives.[3] However, the lack of broad substrate scope, moderate enantioselectivity, and the need for expensive and sensitive metal catalysts have limited synthetic application of these systems.[4] Epoxide hydrolases (EHs) (EC 3.3.2.*x*), which catalyze epoxide ring opening by water, could offer a promising alternative strategy.[5] There have been reports of the discovery and application of microbial EHs, but examples of desymmetrization of *meso*-epoxides are scarce.[6,7] Here we report the discovery of a large and diverse set of microbial EHs and their utilization in the desymmetrization of *meso*-epoxides to produce chiral 1,2-diols.

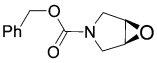
Since no single enzyme is likely to function optimally on all types of epoxides, the full potential of EHs as a synthetic tool will be realized only through creation of a library or collection of enzymes with differing substrate scopes. Traditional approaches to enzyme discovery by microbial cultivation have resulted in fewer than 20 reported microbial EHs, and fewer still that have been carefully studied. The generation of DNA libraries directly from environmental samples has been described previously.[8] To access the most diverse range of enzymes in nature, we screened our environmental libraries, which were created from numerous global habitats. To date, over 50 novel microbial EHs have been discovered using sequence-based and activity-based high-throughput assays.[9] All new EHs were found to be unique at the sequence level and were shown to possess the conserved aspartate residue that has been postulated as the active-site nucleophile attacking the epoxide ring in the first step of the catalysis.[10] Phylogenetic analysis showed that the newly discovered EH sequences are highly diverse and represent a wide range of sequence space.

Each EH in our library was overexpressed and stored as a lyophilized cell lysate. We first screened these enzymes for the desymmetrization of cyclic *meso*-epoxides. Under standard conditions, 2 mg/mL of substrate and 1 mg/mL of cell lysate were incubated in 20 mM phosphate buffer (pH 7.5) with 5% acetonitrile (v/v) at 22 °C. Five representative epoxides were examined, and for each substrate, multiple enzymes were found that catalyzed the formation of (*R*,*R*)-diol products. Several enzymes were characterized further, revealing that our EH library contained enzymes capable of hydrolyzing heterocyclic and alicyclic *meso*-epoxides of different ring sizes with both high rates and high enantioselectivities (Table 1). To demonstrate synthetic utility, entries 3, 4, and 5 (using BD9833) were carried out successfully on 0.1 g scale.[9]

Table 1. EH-Catalyzed Desymmetrization of Cyclic *meso*-Epoxides[a]



1 **(*R*,*R*)-2**

Entry	Epoxide	Epoxide Hydrolase	Spec. Act.[b]	TOF[c]	% ee
1		BD10721	9	5.0	90[d]
2		BD10332	0.1	0.07	81[d]
3		BD9884	0.2	0.1	93[e]
		BD9883	5	3.6	82[e]
4		BD9883	7	5.1	96[d]
5		BD9883	10	7.2	91[d]
		BD10721	0.2	0.1	95[d]

[a] Reactions were conducted under our standard conditions (see text). Determination of absolute configuration is described in the Supporting Information. [b] Specific activities were measured at 30 min time points and are expressed as μmol mg^{-1} min^{-1}. [c] TOF = turnover frequency, mol product/mol catalyst/sec. [d] Enantioselectivities were determined by chiral GC analysis. [e] Enantioselectivities were determined by chiral HPLC analysis.

To our knowledge, there are no reports of microbial EHs that are able to desymmetrize bulky internal epoxides such as *cis*-stilbene oxide and its derivatives.[11] We therefore were pleased to find that 11 of our novel EHs were active on this substrate, and four afforded (*R*,*R*)-1,2-diphenyl-1,2-ethanediol with excellent selectivity (>96% ee). A significant difference in the specific activities of these enzymes toward *cis*-stilbene oxide was observed, with turnover frequencies ranging from 0.04 to nearly 5 per second (Table 2, entry 1). Interestingly, these four EHs share less than 35% sequence identity. To confirm synthetic utility and simplicity, 1.0 g of *cis*-stilbene oxide (250 mM) was treated with 109 mg of cell lysate containing 4.6 mg of BD8877, and after 48 h, (*R*,*R*)-1,2-diphenyl-1,2-ethanediol was isolated in 83% yield and 99% ee.[9]

Preliminary analysis of substrate scope was performed using one of our lead enzymes, BD8877, along with several substituted *cis*-stilbene oxides and dipyridyl analogues (Table 2, entries 2−8).[9] Substituents at the meta and para positions of the phenyl ring were well tolerated, as the enzymes catalyzed the hydrolysis of the 3- and 4-chloro analogues with high ee and at rates comparable to the parent substrate. Ortho substitutions, however, resulted in slower rates of hydrolysis. For the 2-chloro analogue, enantioselectivity remained high, although the specific activity was 300-fold lower than with *cis*-stilbene oxide (Table 2, entry 2). A 5-fold rate

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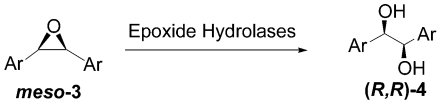
Table 2. EH-Catalyzed Desymmetrization of Aryl *meso*-Epoxides[a]

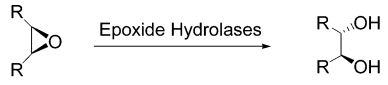


***meso*-3** **(*R,R*)-4**

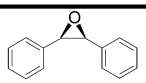
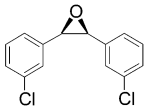
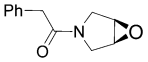
Entry	Epoxide (Ar =)	Epoxide Hydrolase	Spec. Act.[b]	TOF[c]	% ee[d]
1	C_6H_5	BD8877	9	4.9	99
		BD8876	0.2	0.1	99.5
		BD9300	0.2	0.1	96
		BD9883	0.05	0.04	99
2	2-Cl-C_6H_5	BD8877	0.03	0.02	98
3	2-F-C_6H_5	BD8877	2	1.1	80
4	3-Cl-C_6H_5	BD8877	14	7.6	98.5
5	4-Cl-C_6H_5	BD8877	4.7	2.6	>99.5
6	2-pyridyl	BD8877	30.5	16.5	99
7	3-pyridyl	BD8877	13.5	7.3	97
8	4-pyridyl	BD8877	0.5	0.3	98

[a] Reaction conditions as in Table 1. [b,c] See Table 1. [d] Enantioselectivities were determined by chiral HPLC analysis.

Table 3. (*S,S*)-Diols via Desymmetrization of *meso*-Epoxides[a]



1 **(*S,S*)-5**

Entry	Epoxide	Epoxide Hydrolase	Spec. Act.[b]	TOF[c]	% ee
1		BD9126	0.2	0.1	99[d]
2		BD9126	0.05	0.03	99[d]
4		BD10721	0.08	0.04	80[d]
5		BD10090	0.03	0.02	56[e]
6		BD10159	0.05	0.02	76[e]

[a] Reaction conditions as in Table 1. [b,c] See Table 1. Enantioselectivities were determined by chiral HPLC [d] or GC [e] analysis.

reduction also was observed for the 2-fluoro analogue, which suggests that steric hindrance may be primarily responsible for the rate decrease. BD8877 performed particularly well on dipyridyl substrates; the highest specific activity and ee were observed with bis(2-pyridyl) epoxide (entry 6). Reactions with substituted *cis*-stilbene oxide and dipyridyl epoxides were successfully performed at the 0.1 g scale with BD8877.[9]

To our knowledge, all previously reported microbial EHs have been observed to predominantly form (*R,R*)-diols from *meso*-epoxides.[7,12] We now have found in our EH library the first examples of (*S,S*)-selective enzymes for desymmetrization of *meso*-epoxides. As summarized in Table 3, these EH-catalyzed reactions exhibited moderate to excellent ee (56−99%), although reaction rates were generally ∼50−200-fold lower relative to those of (*R,R*)-diol-producing enzymes.

Enantioselective ring opening of *meso*-epoxides has proven to be challenging to accomplish through conventional chemical methods. We have demonstrated that a viable biocatalytic solution can be developed using a diverse set of microbial epoxide hydrolases that were discovered from nature. Enzymes were identified that are capable of selectively hydrolyzing a wide range of *meso*-epoxides and the corresponding chiral (*R,R*)-diols were furnished with high ee's and yields. Moreover, the first EHs providing access to complementary (*S,S*)-diols also were found. Given the high activity and selectivity, as well as broad sequence divergence and substrate scope, our expanding EH library is expected to find wide utility in the synthesis of a range of chiral 1,2-diols.

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Supporting Information Available: Materials and methods and amino acid sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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