Stereodivergent Intramolecular Cyclopropanation Enabled by Engineered Carbene Transferases

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ABSTRACT: We report the development of engineered myoglobin biocatalysts for executing asymmetric intramolecular cyclopropanations resulting in cyclopropane-fused γ-lactones, which are key motifs found in many bioactive molecules. Using this strategy, a broad range of allyl diazoacetate substrates were efficiently cyclized in high yields with up to 99% enantioeneric excess. Upon remodeling of the active site via protein engineering, myoglobin variants with stereodivergent selectivity were also obtained. In combination with whole-cell transformations, these biocatalysts enabled the gram-scale assembly of a key intermediate useful for the synthesis of the insecticide permethrin and other natural products. The enzymatically produced cyclopropyl-γ-lactones can be further elaborated to furnish a variety of enantiopure trisubstituted cyclopropanes. This work introduces a first example of biocatalytic intramolecular cyclopropanation and provides an attractive strategy for the stereodivergent preparation of fused cyclopropyl-γ-lactones of high value for medicinal chemistry and the synthesis of natural products.

* Synthetic Methods for Olefin Cyclopropanation

**Scheme 1. Biocatalytic Methods for Olefin Cyclopropanation**

**Fused cyclopropyl-lactones are structural motifs found in many biologically active natural products (e.g., blepharolides, cedkathryn, laevinoids, sterelactones) and synthetic compounds. In addition, they constitute versatile intermediates for the preparation of fused cyclopropane-fused γ-lactones of high value for medicinal chemistry and the synthesis of natural products.**

**Used cyclopropyl-lactones are structural motifs found in many biologically active natural products (e.g., blepharolides, cedkathryn, laevinoids, sterelactones) and synthetic compounds. In addition, they constitute versatile intermediates for the preparation of fused cyclopropane-fused γ-lactones of high value for medicinal chemistry and the synthesis of natural products.**

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To develop a more efficient and selective biocatalyst for this transformation, we screened a panel of Mb variants (~40) featuring one to four mutations within the distal pocket of this protein (i.e., at positions Leu29, Phe43, His64, Val68, Ile107; Figure S1 and Table S2). These tests revealed the beneficial effect of large-to-small substitutions at the level of Leu29 and Phe43 (82% activity (32–40) compared to Mb (88% activity)). Gratifyingly, this variant enabled the intramolecular cyclopropanation,21 showed higher activity in the transformation of diazoacetates with aliphatic groups. In addition to cyclopropane-fused lactones in the presence of competing olefinic groups (88–99% ee), but moderate diastereoselectivity (Scheme S1). Methyl-substituted cinnamyl 2-diazoacetates and homoallylic diazoacetates could not be processed by the current biocatalyst (Scheme S2), defining targets for future catalyst development.

While challenging to obtain,17,48 stereocomplementary biocatalysts are key assets for the synthesis of drugs and complex molecules.22,49–55 To develop a stereodivergent biocatalyst for this reaction, wild-type Mb was subjected to iterative rounds of site-saturation mutagenesis (a.k.a. ISM) directed to the active site residues Leu29, Phe43, His64, Val68, and Ile107 (Figure S1). The resulting libraries were screened in whole cells using cinnamyl-2-diazoacetate (1a) as the substrate. Partial inversion of enantioselectivity was initially achieved via a Val → Phe mutation at position 68 (80% → 10−%); Figure 1). Progressive improvement of the desired (1S,5R,6R)-selectivity was then obtained through optimization of position 43 and 64 via two additional rounds of mutagenesis and screening. The resulting variant, Mb(F43A,H64W,V68F), catalyzes the intramolecular cyclopropanation of 1a to give 3a in 89% ee and quantitative yield (Table S4). To assess its substrate scope, this biocatalyst was then challenged with the panel of diazoacetate substrates described in Table 2. To our delight, all these substrates were converted by Mb(F43A/
H64W/V68F to give enantioenriched 3a−n in up to 96% ee and 41−99% yields (Scheme 2). In each case, Mb-
(F43A,H64W,V68F) exhibits opposite enantioselectivity compared to the (1S,5S,6R)-selective variants (Table 2), thus furnishing a stereodivergent catalyst for this reaction.

Upon mapping their mutations onto Mb structure (Figure S1), the stereocomplementary Mb variants clearly feature a distinct active site configuration. The mutations in Mb(L29A/H64V/V68A) expand the distal cavity in correspondence to the upper side of the pocket (Leu29 → Ala; His64 → Val) and the ring A/D side of the heme (Val68 → Ala). In contrast, Mb(F43A/H64W/V68F) features significantly increased steric occlusion at these positions (Leu29; His64 → Trp; Val68 →

**Table 2. Substrate Scope of Mb(H64V,I107S) and Mb(L29A,H64V,V68A)**

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*Reaction conditions: 2.5 mM diazoacetate, Mb-expressing *E. coli* (OD600 = 40) in KPi buffer (50 mM, pH 7), 40 mL scale, rt, 3−5 h.

*GC or SPC yield. See Table S5 for additional data.

**Scheme 2. Substrate Scope of (1S,5R,6R)-Selective Mb(F43A,H64W,V68F)**

![Scheme 2](image)
Phe), but an enlarged cavity at the level of the opposite side of the cofactor (i.e., ring B/D via Phe43 → Ala).

Based on these considerations, we propose a stereochemical model for the Mb(L29A/H64V/V68A)-catalyzed reaction whereby intramolecular attack to the re face of the carbene is favored by accommodating the ester group and phenyl group into the cavities created by V68A and L29A/H64V, respectively (Figure 2). This mode of attack is likely disfavored in the case of the (1S,5R,6R)-selective variant Mb(F43A/H64W/V68F) due to steric hindrance provided by the bulky Trp/Phe residues at positions 64/68, whereas attack to the si face of the carbene may be further facilitated by accommodating the phenyl group into the cavity created by the F43A mutation (Figure 2). While further computational and structural studies are warranted to probe these stereochemical models,38 it is instructive to observe how complete remodeling of the Mb active site was required not only for achieving stereodivergent selectivity in the intramolecular cyclopropanation reaction but also with respect to enantioselective Mb-based biocatalysts previously developed for the intermolecular version22 of this transformation (Table S4).

*gem-Dimethyl substituted cyclopropanes are found in several bioactive natural products and derivatives thereof, including the insecticide permethrin. To further demonstrate the synthetic utility of the present strategy, a large-scale biotransformation with Mb(L29A,H64V,V68A)-expressing E. coli cells was carried out in the presence of 1.5 g of 3-methylbut-2-en-1-yl 2-diazoacetate (4). This reaction enabled the stereoselective synthesis of dimethyl cyclopropane-3-oxabicyclo[3.1.0]hexan-2-one (Scheme 3a). Further elaboration of this key intermediate via known methods57,58 can furnish the pyrethroid natural product chrysanthemic acid, permethrin, and phenothrin.

The bicyclic lactones accessible through the present method also constitute versatile intermediates for affording chiral trisubstituted cyclopropanes, which are highly valuable synthetic synths for medicinal chemistry and total synthesis.59 Illustrating this point, enantiopure 2a produced with Mb-(H64V,I107S) was reduced with LiAlH4 to give the cis-hydroxymethyl-substituted cyclopropane 6 in 89% yield in a single step (Scheme 3a). On the other hand, alkaline hydrolysis of 2a or its treatment with benzyl amine in the presence of LiCl afforded the trisubstituted cyclopropanes 7 and 8 in 94% and 81% yield, respectively. Finally, hydrazinolysis of 2a followed by treatment with nitrous acid furnished the cyclopropane-fused urethane 9 in 78% yield. In all cases, these transformations occur with minimal (8; 98% ee) to no erosion (7; 99% ee) of enantiopurity (Scheme 3b).

In summary, the first example of biocatalytic intramolecular olefin cyclopropanation was accomplished through the engineering of myoglobin-based catalysts capable of offering high enantioselectivity as well as stereodivergent selectivity for the asymmetric construction of bicyclic cyclopropane-γ-lactones from allyl diazoacetates. These biocatalytic transformations can be performed in whole cells, at a gram scale, and they can be applied to gain stereoselective access to key intermediates for the synthesis of cyclopropane-containing natural products and a variety of highly valuable trisubstituted cyclopropane synths for medicinal chemistry and drug discovery. This work paves the way to the development of hemoprotein-based catalysts for other types of intramolecular carbene transfer reactions.

![Figure 2. Stereochemical model for intramolecular cyclopropanation catalyzed by the stereodivergent Mb variants.](image)

**Scheme 3. Formal Total Synthesis of Pyrethroid Natural Products (a) and Chemoenzymatic Synthesis of Trisubstituted Cyclopropanes (b)**

<table>
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<th>ASSOCIATED CONTENT</th>
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<td>Supporting Information</td>
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The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.9b02700.

- Supplementary tables, figures, experimental procedures, and characterization data (PDF)
- Crystallographic data for 2a (CIF)
- Crystallographic data for 2c (CIF)
- Crystallographic data for 2d (CIF)

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* A.L.C. and X.R. contributed equally to this work.
Notes
The authors declare no competing financial interest.

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