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Stereodivergent Intramolecular Cyclopropanation Enabled by Engineered Carbene Transferases

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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% enantiomeric 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-y-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 cvclopropyl-y-lactones of high value for medicinal chemistry and the synthesis of natural products.

Fused cyclopropyl-lactones are structural motifs found in many biologically active natural products (e.g., blepharolides, cedkathryn, laevinoids, sterelactones)^{1, 2} and synthetic compounds.³ In addition, they constitute versatile intermediates for the total synthesis of a diverse range of medicinally important compounds, including basiliolide B, ambruticin S⁴⁻⁶ and others.^{7, 8} Because of their high synthetic value, significant efforts have been devoted to developing methods for the preparation of these molecular scaffolds, in particular through transition metalcatalyzed intramolecular cyclopropanations.⁹⁻¹⁸ Despite this progress, the development of catalytic protocols for asymmetric intramolecular cyclopropanations involving an earth-abundant, inexpensive, and non-toxic metal like iron has been difficult, with success being reported thus far only with donor-acceptor diazo compounds.¹⁹

Engineered hemoproteins have recently emerged as promising biocatalytic platforms for carbene transfer reactions.²⁰⁻ ³⁷ Recently, our group have demonstrated that engineered myoglobins (Mb) are capable of catalyzing the stereoselective intermolecular cyclopropanation of vinylarenes in the presence of diazo compounds with varied α -electron withdrawing groups (-COOR, -CF₃, -CN), thus providing access to enantioenriched disubstituted cyclopropanes (Scheme 1).^{21, 22, 24, 25, 38} Engineered P450s^{20, 23, 26, 27} as well as artificial metalloenzymes^{32, 39-46} have also proven useful for promoting intermolecular cyclopropanation reactions. Despite this progress, biocatalytic strategies for intramolecular cyclopropanations have so far been elusive. In contrast to synthetic catalysts featuring an 'open active site', achieving this task using an enzyme is challenged by the need of orchestrating the intramolecular cyclopropanation reaction within the confined environment of a protein's active site. Furthermore,

the development of stereodivergent biocatalysts for a desired transformation remains an important yet challenging endeavor.^{47, 48} Here, we report the successful development of engineered myoglobin-based catalysts capable of promoting the intramolecular cyclopropanation of allyl α -diazoacetate derivatives with high stereocontrol and complementary enantioselectivity. This method provides efficient access to a variety of bicyclic cyclopropane-fused γ -lactones, in both enantiomeric forms and at a synthetically useful scale, for use as pharmacophores or as key intermediates for the synthesis of enantioenriched cyclopropane-containing molecules.





In initial studies, we discovered that wild-type sperm whale myoglobin (Mb) is able to catalyze the cyclization of transcinnamyl-2-diazoacetate (1a) to give 2a (Table 1). Despite its modest activity, which is comparable to that of free hemin (Table S1), Mb exhibits good enantioselectivity toward formation of the (1R,5S,6S)-configured intramolecular cyclopropanation product as determined by single crystal X-ray diffractometry (80% ee; Table 1, Entry 1). Compared to Mb, other hemoproteins including $P450_{BM3}$, cytochrome c, and catalase show negligible activity (0-2%) as well as lower enantioselectivity in this reaction (6-22% ee) (Table S1). 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 Val68 toward improving both enantioselectivity $(80 \rightarrow 90-93\% \text{ ee})$ and catalytic activity $(32\rightarrow 163 \text{ TON}; \text{ Entries } 2-3)$ (Table 1; Entries 2-3). Mb(H64V,V68A), a previously optimized Mb variant for intermolecular cyclopropanation,²¹ showed higher activity $(32 \rightarrow 82 \text{ TON})$ but similar enantioselectivity compared to Mb (82% ee).

and

 Table 1. Intramolecular cyclopropanation of cinnamyl 2-diazoacetate

 (1a) with Mb and variants thereof.^a

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Entry	catalyst	OD ₆₀₀	Yield ^b	TON	е.е.
1	Mb	-	13%	32	80%
2	Mb(L29A)	-	65%	163	93%
3	Mb(V68A)	-	65%	162	90%
4	Mb(H64V,V68A)	-	33%	82	81%
5	Mb(L29A,H64V,V68A)	-	99%	250	96%
6°	Mb(L29A,H64V,V68A)	40	74%	185	96%
7 ^d	Mb(L29A,H64V,V68A)	40	99%	90	96%
8	Mb(H64V,I107S)	-	78%	195	97%
9 ^d	Mb(H64V,I107S)	40	99% (83%)°	90	>99%

^a Reaction conditions: 5 mM cinnamyl 2-diazoacetate (**2a**), 20 μM Mb variant (or C41(DE3) *E. coli* cells at indicated OD₆₀₀) in KPi buffer (50 mM, pH 7), 10 mM Na₂S₂O₄ (protein only), r.t., 5 hours in anaerobic chamber. ^b GC yield. ^c 15 min reaction time. ^d Using 2.5 mM **2a**. ^c Isolated yield.

Based on this information, the beneficial L29A mutation was introduced into Mb(H64V,V68A) to give the triple variant Mb(L29A,H64V,V68A). Gratifyingly, this variant enabled the quantitative conversion of 1a into the (1R,5S,6S)-configured cyclopropane-fused γ -lactone 2a with high enantioselectivity (96%) ee) (Table 1, Entry 5). Moreover, about 74% product conversion was reached in merely 15 minutes (Table 1, Entry 6 and Figure **S2**). Following an alternative strategy for catalyst optimization, we also screened an 'active-site mutational landscape' library which samples all possible 19 amino acid substitutions at the active-site positions Leu29, Phe43, Val68, and Ile107 (Figure S1) in the Mb(H64V) background.²² From this library, Mb(H64V,I107S) was identified as another efficient and highly enantioselective catalyst (97% ee) for the synthesis of 2a from 1a (Entry 7). Using either catalyst, the intramolecular cyclopropanation reaction can be conveniently carried out in whole cells using E. coli cells expressing these Mb variants (Entries 6 and 8). Notably, in addition to quantitative conversion, the whole-cell reactions with Mb(H64V,I107S)-containing E. coli cells yielded 2a in high enantiopurity (>99% ee; Entry 9). A cell density (OD₆₀₀) of 40-60 was determined to be optimal for granting high conversion and high enantioselectivity in these biotransformations (Table S3). The whole-cell reaction with Mb(H64V,I107S) could be readily scaled up to enable the isolation of 180 mg of enantiopure 2a (>99% ee) in 83% isolated yield (Entry 9), thus demonstrating the scalability of the biocatalytic transformation.

To assess the substrate scope of these biocatalysts, a diverse panel of allylic diazoacetate derivatives were then subjected to Mb(H64V,I107S)or Mb(L29A,H64V,V68A)-catalyzed cyclization in whole-cell reactions on a semi-preparative scale (0.1 mmol) (Table 2). Substrates carrying para, meta, and ortho substituents on the phenyl group (1b-1i) were all efficiently processed by Mb(H64V,I107S), leading to the corresponding bicyclic products 2b-2i in good to quantitative yields (62-99%). Both electron withdrawing groups and electron donating groups were well tolerated, with high enantioselectivity being maintained across all of these substrates (90-99% ee; Entries 1-8). This include the ortho-substituted product 2i, indicating a good tolerance of this catalyst to steric hindrance in proximity to the olefinic bond. Good conversion and enantioselectivity (97% ee)

was also achieved for the intramolecular cyclopropanation of the sterically

Table 2. Substrate Mb(L29A,H64V,V68A).^a scope of

Mb(H64V.I107S) (**a**)

Mb(H64V,I107S)



Entr y	Product	Catal yst	TON	Yield	е.е.
1		a b	90 85	99% 95%	99% 96%
2		a b	75 60	83% 66%	97% 89%
3	O H Br	a b	90 77	99% 85%	94% 80%
4		a b	55 41	62% 45%	95% 68%
5		a b	82 50	90% 55%	96% 70%
6		a b	70 75	77% 82%	90% 39%
7		a b	85 64	95% 70%	99% 74%
8		a b	90 56	99% 62%	99% 68%
9	O H OMe	a b	60 21	65% 23%	97% 81%
10		a b	90 72	71% 95%	79% 98%
11		a b	90 90	99% 99%	10% 52%
12	H H 2m	a b	79 80	87% 89%	5% 38%
13	H 2n	a b	39 62	43% 68%	74% 51%

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^{*a*} Reaction conditions: 2.5 mM diazoacetate, Mb-expressing *E. coli* (OD₆₀₀ = 40) in KPi buffer (50 mM, pH 7), 40 mL-scale, r.t., 3-5 hours. ^{*b*} GC or SFC yield. See **Table S5** for additional data.

demanding naphtyl-based substrate 1j (Entry 9). Compared to Mb(H64V,1107S), Mb(L29A,H64V,V68A) displays a similarly broad substrate scope, albeit with overall reduced activity and/or enantioselectivity toward this set of substrates. Notably, both Mb variants exhibit a consistent (1R,5S,6S) stereoselectivity across this diverse panel of substrates, as evinced from crystallographic analysis of 2a, 2c and 2d (Figure S3-5) and the similar chromatographic behavior of the other products in chiral SFC or GC.

These results prompted us to investigate challenging substrates such as diazoacetates equipped with unactivated olefins (11, 1m) or multiple olefinic groups (1k, 1m, 1n). Notably, all these substrates were efficiently cyclized by either Mb variant to generate 2k-n in good to quantitative yields (68-99%; Entries 10-13). Interestingly, Mb(L29A,H64V,V68A) offered significantly higher enantioselectivity in the transformation of diazoacetates with aliphatic substituents compared to Mb(H64V,I107S), thus complementing its scope across this group of substrates. The results with 2n and the nerol derivative 2m also demonstrated the high regioselectivity of the biocatalysts toward formation of the cvclopropane-y-lactones in the presence of competing olefinic groups. In addition to 2m, other (Z)-allylic diazoacetates (10-p) could be efficiently cyclized with good enantioselectivity (88-89% ee), but modest diastereoselectivity (Scheme S1). Methylsubstituted cinnamyl 2-diazoacetates and homoallylic diazoacetates could not be processed by the current biocatalysts (Scheme S2), defining targets for future catalyst development.

While challenging to obtain,^{47, 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 sitesaturation mutagenesis (a.k.a. ISM)⁵⁶ directed to the active-site residue Leu29, Phe43, His64, Val68, Ile107 (**Figure S1**). The resulting libraries were screened in whole cells using cinnamyl-2diazoacetate (**1a**) as the substrate. Partial inversion of enantioselectivity was initially achieved via a Val \rightarrow Phe mutation at position 68 (80% \rightarrow -10%; **Figure 1**). Progressive improvement of the desired (1*S*,5*R*,6*R*)-selectivity was then obtained through optimi

Figure 1. Evolutionary paths to stereodivergent intramolecular cyclopropanation biocatalysts.



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 \rightarrow Ala;

His64 \rightarrow Val) and the ring A/D side of the heme (Val68 \rightarrow Ala). In contrast, Mb(F43A/H64W/V68F) features significantly increased steric occlusion at these positions (Leu29; His64 \rightarrow Ala; Val68 \rightarrow Phe), but an enlarged cavity at the level of the opposite side of the cofactor (i.e., ring B/D via Phe43 \rightarrow Ala).

zation of position 43 and 64 via two additional rounds of and screening. The resulting mutagenesis variant Mb(F43A,H64W,V68F), catalyzes the intramolecular cyclopropanation of 1a to give 3a in 89% ee and quantitative vield (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 3an in up to 96% ee and 41-99% yields (Scheme 2). In each case, Mb(F43A,H64W,V68F) exhibits opposite enantioselectivity compared to the (1R,5S,6S)-selective variants (Table 2), thus furnishing a stereodivergent catalyst for this reaction.

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



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 Mb active site was not only required 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**).

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Figure 2. Stereochemical model for intramolecular cyclopropanation catalyzed by the stereodivergent Mb variants.

Gem-dimethyl substituted cyclopropanes are found in several bioactive natural products, 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 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 **5** in 81% *ee* and 83% isolated yield (Scheme 3A). Further elaboration of this key intermediate via known methods^{57, 58} can furnish the pyrethroid natural products chrysanthemic acid, permethrin and phenothrin.

Scheme 3. Formal total synthesis of pyrethroid natural products (A) and chemoenzymatic synthesis of trisubstituted cyclopropanes (B).



The bicyclic lactones accessible through the present method also constitute versatile intermediates for affording chiral trisubstituted cyclopropanes, which are highly valuable synthons 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 3**). 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, hydrazinolyis of **2a** followed by treatment with nitrous acid furnish the cyclopropane-fused urethane **9** in 78% yield. In all cases, these transformations occur with minimal (**8**; 98% *ee*) to no erosion (**7**, **9**; 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 synthons 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.

ASSOCIATED CONTENT

Supporting Information

Supplementary tables, figures, experimental procedures, characterization data, and crystallographic data are available in the Supporting Information.

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Notes

The authors declare no competing financial interest.

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Graphic Table of Content

 N_2

R1 = aryl, alky, heterocycle

R² = H. Me

Mb(F43A H64W V68F

KPi (pH 7), RT

up to 99% yield

up to 96% ee

Mb(H64V.I107S)

KPi (pH 7), RT

up to 99%

