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Stereodivergent Intramolecular Cyclopropanation Enabled by Engineered Carbene Transferases

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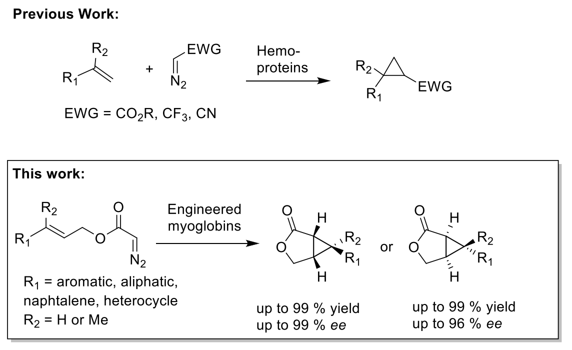
Supporting Information

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-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.

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 S,[4−6] 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-metal-catalyzed intramolecular cyclopropanations.[9−18] Despite this progress, the development of catalytic protocols for asymmetric intramolecular cyclopropanations involving an earth-abundant, inexpensive, and nontoxic 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 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, $-CF_3$, −CN), thus providing access to enantioenriched 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 thus 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.

Scheme 1. Biocatalytic Methods for Olefin Cyclopropanation



In initial studies, we discovered that wild-type sperm whale myoglobin (Mb) is able to catalyze the cyclization of *trans*-cinnamyl-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 (1*R*,5*S*,6*S*)-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

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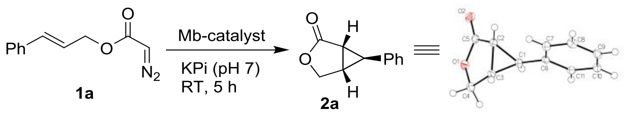
Table 1. Intramolecular Cyclopropanation of Cinnamyl 2-Diazoacetate (1a) with Mb and Variants Thereof[a]



entry	catalyst	OD_{600}	yield[b]	TON	*e.e.*
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[c]	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%)[e]	90	>99%

[a]Reaction conditions: 5 mM cinnamyl 2-diazoacetate (**1a**), 20 μM Mb variant (or C41(DE3) *E. coli* cells at indicated OD_{600}) in KPi buffer (50 mM, pH 7), 10 mM $Na_2S_2O_4$ (protein only), rt, 5 h in anaerobic chamber. [b]GC yield. [c]15 min reaction time. [d]Using 2.5 mM **1a**. [e]Isolated yield.

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 → 90–93% ee) and catalytic activity (32 → 163 TON) (Table 1; entries 2–3). Mb(H64V,V68A), a previously optimized Mb variant for *intermolecular* cyclopropanation,[21] showed higher activity (32 → 82 TON) but similar enantioselectivity compared to Mb (82% *ee*).

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 (1*R*,5*S*,6*S*)-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 min (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 19 possible 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 8). Using either catalyst, the intramolecular cyclopropanation reaction can be conveniently carried out in whole cells using *E. coli* cells expressing these Mb variants (entries 7 and 9). 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 (OD_{600}) 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)- and Mb(L29A,H64V,V68A)-catalyzed cyclization in whole-cell reactions on a semipreparative 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 and -donating groups were well tolerated, with high enantioselectivity being maintained across all of these substrates (90–99% *ee*; entries 1–8). This includes 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*) were also achieved for the intramolecular cyclopropanation of the sterically demanding naphtyl-based substrate **1j** (entry 9). Compared to Mb(H64V,I107S), 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 (1*R*,5*S*,6*S*) stereoselectivity across this diverse panel of substrates, as evinced from crystallographic analysis of **2a**, **2c**, and **2d** (Figures S4–S6) 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 (**1l**, **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 cyclopropane-γ-lactones in the presence of competing olefinic groups. In addition to **2m**, other (*Z*)-allylic diazoacetates (**1o**–**p**) could be efficiently cyclized with good enantioselectivity (88–89% *ee*), but moderate diastereoselectivity (Scheme S1). Methyl-substituted 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 site-saturation mutagenesis (a.k.a. ISM)[56] 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 (1*S*,5*R*,6*R*)-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/

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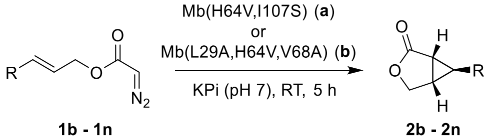
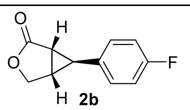
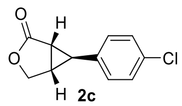
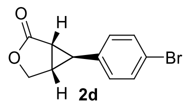
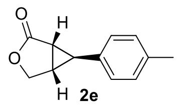
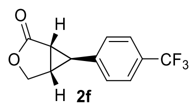
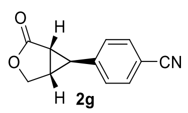
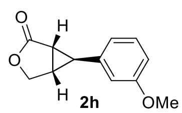
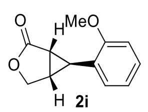
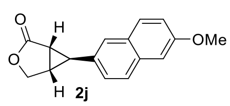
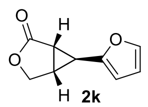
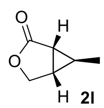
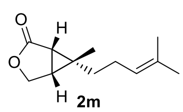
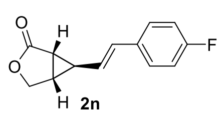
Table 2. Substrate Scope of Mb(H64V,I107S) and Mb(L29A,H64V,V68A)[a]



Entry	Product	Catalyst	Yield[b]	e.e.
1	 2b	a b	99% 95%	99% 96%
2	 2c	a b	83% 66%	97% 89%
3	 2d	a b	99% 85%	94% 80%
4	 2e	a b	62% 45%	95% 68%
5	 2f	a b	90% 55%	96% 70%
6	 2g	a b	77% 82%	90% 39%
7	 2h	a b	95% 70%	99% 74%
8	 2i	a b	99% 62%	99% 68%
9	 2j	a b	65% 23%	97% 81%
10	 2k	a b	71% 95%	79% 97%
11	 2l	a b	99% 99%	10% 52%
12	 2m	a b	87% 89%	5% 38%
13	 2n	a b	43% 68%	74% 51%

[a]Reaction conditions: 2.5 mM diazoacetate, Mb-expressing *E. coli* (OD_{600} = 40) in KPi buffer (50 mM, pH 7), 40 mL scale, rt, 3−5 h. [b]GC or SFC yield. See Table S5 for additional data.

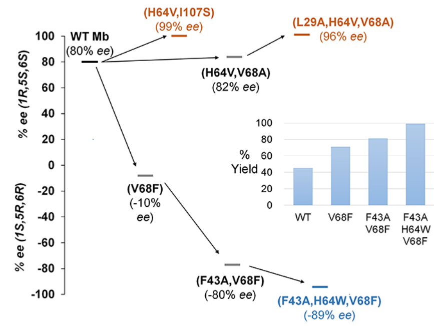
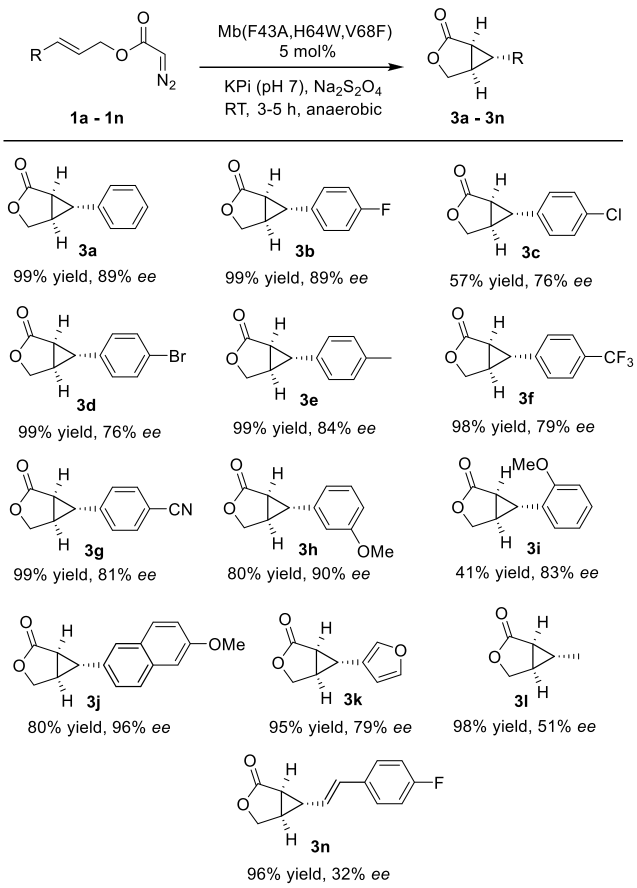


Figure 1. Evolutionary paths to stereodivergent intramolecular cyclopropanation biocatalysts.

H64W/V68F) to give enantioenriched **3a−n** in up to 96% *ee* and 41−99% yields (Scheme 2). In each case, Mb-

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



(F43A,H64W,V68F) exhibits opposite enantioselectivity compared to the (1*R*,5*S*,6*S*)-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 →

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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

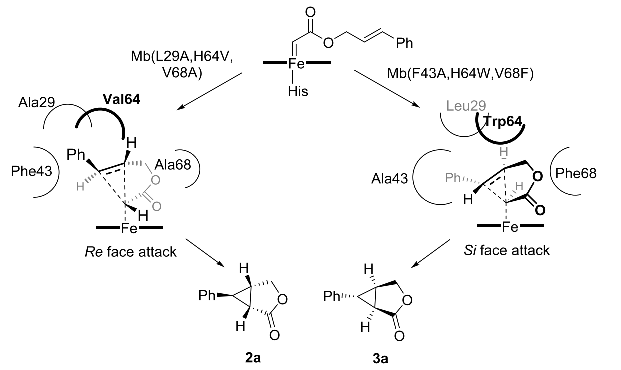


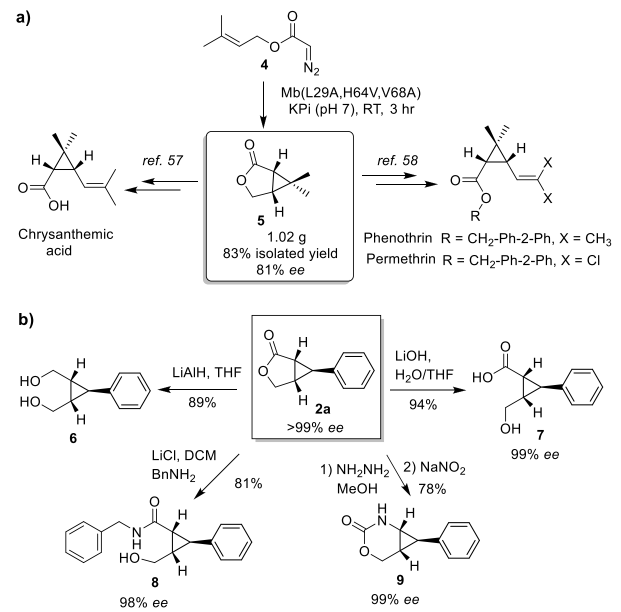
Figure 2. Stereochemical model for intramolecular cyclopropanation catalyzed by the stereodivergent Mb variants.

in the case of the (1*S*,5*R*,6*R*)-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* version[22] 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 **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 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 synthons for medicinal chemistry and total synthesis.[59] Illustrating this point, enantiopure **2a** produced with Mb(H64V,I107S) was reduced with $LiAlH_4$ 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, hydrazinolysis of **2a** followed by treatment with nitrous acid furnished the cyclopropane-fused urethane **9** in 78% yield. In all cases,

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



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

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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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Bisio, A.; Fontana, N.; Romussi, G.; Ciarallo, G.; De Tommasi, N.; Pizza, C.; Mugnoli, A. Clerodane diterpenoids from Salvia blepharophylla. *Phytochemistry* **1999**, *52*, 1535−1540.

(2) Opatz, T.; Kolshorn, H.; Anke, H. Sterelactones: New isolactarane type sesquiterpenoids with antifungal activity from Stereum sp IBWF 01060. *J. Antibiot.* **2008**, *61*, 563−567.

(3) Ansiaux, C.; N'Go, I.; Vincent, S. P. Reversible and Efficient Inhibition of UDP-Galactopyranose Mutase by Electrophilic, Constrained and Unsaturated UDP-Galactitol Analogues. *Chem. - Eur. J.* **2012**, *18*, 14860−14866.

(4) Min, L.; Zhang, Y.; Liang, X. F.; Huang, J. R.; Bao, W. L.; Lee, C. S. A Biomimetic Synthesis of (±)-Basiliolide B. *Angew. Chem., Int. Ed.* **2014**, *53*, 11294−11297.

(5) Kirkland, T. A.; Colucci, J.; Geraci, L. S.; Marx, M. A.; Schneider, M.; Kaelin, D. E.; Martin, S. F. Total synthesis of (+)-ambruticin S. *J. Am. Chem. Soc.* **2001**, *123*, 12432−12433.

(6) Rogers, D. H.; Yi, E. C.; Poulter, C. D. Enantioselective Synthesis of (+)-Presqualene Diphosphate. *J. Org. Chem.* **1995**, *60*, 941−945.

(7) Reichelt, A.; Martin, S. F. Synthesis and properties of cyclopropane-derived peptidomimetics. *Acc. Chem. Res.* **2006**, *39*, 433−442.

(8) Tang, P.; Qin, Y. Recent Applications of Cyclopropane-Based Strategies to Natural Product Synthesis. *Synthesis* **2012**, *44*, 2969−2984.

(9) Doyle, M. P.; Forbes, D. C. Recent Advances in Asymmetric Catalytic Metal Carbene Transformations. *Chem. Rev.* **1998**, *98*, 911−936.

(10) Lebel, H.; Marcoux, J. F.; Molinaro, C.; Charette, A. B. Stereoselective cyclopropanation reactions. *Chem. Rev.* **2003**, *103*, 977−1050.

(11) Davies, H. M. L.; Denton, J. R. Application of donor/acceptor-carbenoids to the synthesis of natural products. *Chem. Soc. Rev.* **2009**, *38*, 3061−3071.

(12) Zhang, Z. H.; Wang, J. B. Recent studies on the reactions of alpha-diazocarbonyl compounds. *Tetrahedron* **2008**, *64*, 6577−6605.

(13) Lu, H. J.; Zhang, X. P. Catalytic C-H functionalization by metalloporphyrins: recent developments and future directions. *Chem. Soc. Rev.* **2011**, *40*, 1899−1909.

(14) Intrieri, D., Carminati, D. M., Gallo, E. In *Handbook of Porphyrin Science: Recent Advances in Metal Porphyrinoid-Catalyzed Nitrene and Carbene Transfer Reactions*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: 2016.

(15) Doyle, M. P.; Austin, R. E.; Bailey, A. S.; Dwyer, M. P.; Dyatkin, A. B.; Kalinin, A. V.; Kwan, M. M. Y.; Liras, S.; Oalmann, C. J.; Pieters, R. J.; Protopopova, M. N.; Raab, C. E.; Roos, G. H. P.; Zhou, Q. L.; Martin, S. F. Enantioselective Intramolecular Cyclopropanations of Allylic and Homoallylic Diazoacetates and Diazoacetamides Using Chiral Dirhodium(Ii) Carboxamide Catalysts. *J. Am. Chem. Soc.* **1995**, *117*, 5763−5775.

(16) Uchida, T.; Saha, B.; Katsuki, T. Co(II)-salen-catalyzed asymmetric intramolecular cyclopropanation. *Tetrahedron Lett.* **2001**, *42*, 2521−2524.

(17) Li, G. Y.; Zhang, J.; Chan, P. W. H.; Xu, Z. J.; Zhu, N. Y.; Che, C. M. Enantioselective intramolecular cyclopropanation of cis-alkenes by chiral ruthenium(II) Schiff base catalysts and crystal structures of (Schiff base)ruthenium complexes containing carbene, PPh3, and CO ligands. *Organometallics* **2006**, *25*, 1676−1688.

(18) Xu, Z. J.; Fang, R.; Zhao, C.; Huang, J. S.; Li, G. Y.; Zhu, N.; Che, C. M. cis-beta-Bis(carbonyl) Ruthenium-Salen Complexes: X-ray Crystal Structures and Remarkable Catalytic Properties toward Asymmetric Intramolecular Alkene Cyclopropanation. *J. Am. Chem. Soc.* **2009**, *131*, 4405−4417.

(19) Shen, J. J.; Zhu, S. F.; Cai, Y.; Xu, H.; Xie, X. L.; Zhou, Q. L. Enantioselective Iron-Catalyzed Intramolecular Cyclopropanation Reactions. *Angew. Chem., Int. Ed.* **2014**, *53*, 13188−13191.

(20) Coelho, P. S.; Brustad, E. M.; Kannan, A.; Arnold, F. H. Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes. *Science* **2013**, *339*, 307−310.

(21) Bordeaux, M.; Tyagi, V.; Fasan, R. Highly Diastereoselective and Enantioselective Olefin Cyclopropanation Using Engineered Myoglobin-Based Catalysts. *Angew. Chem., Int. Ed.* **2015**, *54*, 1744−1748.

(22) Bajaj, P.; Sreenilayam, G.; Tyagi, V.; Fasan, R. Gram-Scale Synthesis of Chiral Cyclopropane-Containing Drugs and Drug Precursors with Engineered Myoglobin Catalysts Featuring Complementary Stereoselectivity. *Angew. Chem., Int. Ed.* **2016**, *55*, 16110−16114.

(23) Gober, J. G.; Rydeen, A. E.; Gibson-O'Grady, E. J.; Leuthaeuser, J. B.; Fetrow, J. S.; Brustad, E. M. Mutating a Highly Conserved Residue in Diverse Cytochrome P450s Facilitates Diastereoselective Olefin Cyclopropanation. *ChemBioChem* **2016**, *17*, 394−397.

(24) Tinoco, A.; Steck, V.; Tyagi, V.; Fasan, R. Highly Diastereo- and Enantioselective Synthesis of Trifluoromethyl-Substituted Cyclopropanes via Myoglobin-Catalyzed Transfer of Trifluoromethylcarbene. *J. Am. Chem. Soc.* **2017**, *139*, 5293−5296.

(25) Chandgude, A. L.; Fasan, R. Highly Diastereo- and Enantioselective Synthesis of Nitrile-Substituted Cyclopropanes by Myoglobin-Mediated Carbene Transfer Catalysis. *Angew. Chem., Int. Ed.* **2018**, *57*, 15852−15856.

(26) Brandenberg, O. F.; Prier, C. K.; Chen, K.; Knight, A. M.; Wu, Z.; Arnold, F. H. Stereoselective Enzymatic Synthesis of Heteroatom-Substituted Cyclopropanes. *ACS Catal.* **2018**, *8*, 2629−2634.

(27) Knight, A. M.; Kan, S. B. J.; Lewis, R. D.; Brandenberg, O. F.; Chen, K.; Arnold, F. H. Diverse Engineered Heme Proteins Enable Stereodivergent Cyclopropanation of Unactivated Alkenes. *ACS Cent. Sci.* **2018**, *4*, 372−377.

(28) Wang, Z. J.; Peck, N. E.; Renata, H.; Arnold, F. H. Cytochrome P450-catalyzed insertion of carbenoids into N−H bonds. *Chem. Sci.* **2014**, *5*, 598−601.

(29) Sreenilayam, G.; Fasan, R. Myoglobin-catalyzed intermolecular carbene N−H insertion with arylamine substrates. *Chem. Commun.* **2015**, *51*, 1532−1534.

(30) Tyagi, V.; Bonn, R. B.; Fasan, R. Intermolecular carbene S-H insertion catalysed by engineered myoglobin-based catalysts. *Chem. Sci.* **2015**, *6*, 2488−2494.

(31) Kan, S. B. J.; Lewis, R. D.; Chen, K.; Arnold, F. H. Directed evolution of cytochrome c for carbon-silicon bond formation: Bringing silicon to life. *Science* **2016**, *354*, 1048−1051.

(32) Sreenilayam, G.; Moore, E. J.; Steck, V.; Fasan, R. Metal substitution modulates the reactivity and extends the reaction scope of myoglobin carbene transfer catalysts. *Adv. Synth. Catal.* **2017**, *359*, 2076−2089.

(33) Tyagi, V.; Fasan, R. Myoglobin-Catalyzed Olefination of Aldehydes. *Angew. Chem., Int. Ed.* **2016**, *55*, 2512−2516.

(34) Tyagi, V.; Sreenilayam, G.; Bajaj, P.; Tinoco, A.; Fasan, R. Biocatalytic Synthesis of Allylic and Allenyl Sulfides through a Myoglobin-Catalyzed Doyle-Kirmse Reaction. *Angew. Chem., Int. Ed.* **2016**, *55*, 13562−13566.

(35) Weissenborn, M. J.; Low, S. A.; Borlinghaus, N.; Kuhn, M.; Kummer, S.; Rami, F.; Plietker, B.; Hauer, B. Enzyme-Catalyzed Carbonyl Olefination by the E. coli Protein YfeX in the Absence of Phosphines. *ChemCatChem* **2016**, *8*, 1636−1640.

DOI: 10.1021/jacs.9b02700
J. Am. Chem. Soc. 2019, 141, 9145−9150

(36) Vargas, D. A.; Tinoco, A.; Tyagi, V.; Fasan, R. Myoglobin-Catalyzed C-H Functionalization of Unprotected Indoles. *Angew. Chem., Int. Ed.* **2018**, *57*, 9911−9915.
(37) Chen, K.; Huang, X. Y.; Kan, S. B. J.; Zhang, R. K.; Arnold, F. H. Enzymatic construction of highly strained carbocycles. *Science* **2018**, *360*, 71−75.
(38) Tinoco, A.; Wei, Y.; Bacik, J.-P.; Carminati, D. M.; Moore, E. J.; Ando, N.; Zhang, Y.; Fasan, R. Origin of High Stereocontrol in Olefin Cyclopropanation Catalyzed by an Engineered Carbene Transferase. *ACS Catal.* **2019**, *9*, 1514−1524.
(39) Srivastava, P.; Yang, H.; Ellis-Guardiola, K.; Lewis, J. C. Engineering a dirhodium artificial metalloenzyme for selective olefin cyclopropanation. *Nat. Commun.* **2015**, *6*, 7789.
(40) Sreenilayam, G.; Moore, E. J.; Steck, V.; Fasan, R. Stereoselective Olefin Cyclopropanation under Aerobic Conditions with an Artificial Enzyme Incorporating an Iron-Chlorin e6 Cofactor. *ACS Catal.* **2017**, *7*, 7629−7633.
(41) Moore, E. J.; Steck, V.; Bajaj, P.; Fasan, R. Chemoselective Cyclopropanation over Carbene Y-H Insertion Catalyzed by an Engineered Carbene Transferase. *J. Org. Chem.* **2018**, *83*, 7480−7490.
(42) Key, H. M.; Dydio, P.; Clark, D. S.; Hartwig, J. F. Abiological catalysis by artificial haem proteins containing noble metals in place of iron. *Nature* **2016**, *534*, 534−537.
(43) Wolf, M. W.; Vargas, D. A.; Lehnert, N. Engineering of RuMb: Toward a Green Catalyst for Carbene Insertion Reactions. *Inorg. Chem.* **2017**, *56*, 5623−5635.
(44) Oohora, K.; Meichin, H.; Zhao, L. M.; Wolf, M. W.; Nakayama, A.; Hasegawa, J.; Lehnert, N.; Hayashi, T. Catalytic Cyclopropanation by Myoglobin Reconstituted with Iron Porphycene: Acceleration of Catalysis due to Rapid Formation of the Carbene Species. *J. Am. Chem. Soc.* **2017**, *139*, 17265−17268.
(45) Villarino, L.; Splan, K. E.; Reddem, E.; Alonso-Cotchico, L.; de Souza, C. G.; Lledos, A.; Marechal, J. D.; Thunnissen, A. M. W. H.; Roelfes, G. An Artificial Heme Enzyme for Cyclopropanation Reactions. *Angew. Chem., Int. Ed.* **2018**, *57*, 7785−7789.
(46) Hayashi, T.; Tinzl, M.; Mori, T.; Krengel, U.; Proppe, J.; Soetbeer, J.; Klose, D.; Jeschke, G.; Reiher, M.; Hilvert, D. Capture and characterization of a reactive haem-carbenoid complex in an artificial metalloenzyme. *Nature Catal.* **2018**, *1*, 578−584.
(47) Reetz, M. T. Biocatalysis in Organic Chemistry and Biotechnology: Past, Present, and Future. *J. Am. Chem. Soc.* **2013**, *135*, 12480−12496.
(48) Li, G. Y.; Wang, J. B.; Reetz, M. T. Biocatalysts for the pharmaceutical industry created by structure-guided directed evolution of stereoselective enzymes. *Bioorg. Med. Chem.* **2018**, *26*, 1241−1251.
(49) Turner, N. J. Deracemisation methods. *Curr. Opin. Chem. Biol.* **2010**, *14*, 115−121.
(50) Mugford, P. F.; Wagner, U. G.; Jiang, Y.; Faber, K.; Kazlauskas, R. J. Enantiocomplementary Enzymes: Classification, Molecular Basis for Their Enantiopreference, and Prospects for Mirror-Image Biotransformations. *Angew. Chem., Int. Ed.* **2008**, *47*, 8782−8793.
(51) Feske, B. D.; Kaluzna, I. A.; Stewart, J. D. Enantiodivergent, biocatalytic routes to both taxol side chain antipodes. *J. Org. Chem.* **2005**, *70*, 9654−9657.
(52) Wu, Q.; Soni, P.; Reetz, M. T. Laboratory Evolution of Enantiocomplementary Candida antarctica Lipase B Mutants with Broad Substrate Scope. *J. Am. Chem. Soc.* **2013**, *135*, 1872−1881.
(53) Zhang, K.; Shafer, B. M.; Demars, M. D., 2nd; Stern, H. A.; Fasan, R. Controlled oxidation of remote sp3 C-H bonds in artemisinin via P450 catalysts with fine-tuned regio- and stereoselectivity. *J. Am. Chem. Soc.* **2012**, *134*, 18695−18704.
(54) Koszelewski, D.; Grischek, B.; Glueck, S. M.; Kroutil, W.; Faber, K. Enzymatic Racemization of Amines Catalyzed by Enantiocomplementary omega-Transaminases. *Chem. - Eur. J.* **2011**, *17*, 378−383.
(55) France, S. P.; Aleku, G. A.; Sharma, M.; Mangas-Sanchez, J.; Howard, R. M.; Steflik, J.; Kumar, R.; Adams, R. W.; Slabu, I.; Crook, R.; Grogan, G.; Wallace, T. W.; Turner, N. J. Biocatalytic Routes to Enantiomerically Enriched Dibenz[c,e]azepines. *Angew. Chem., Int. Ed.* **2017**, *56*, 15589−15593.
(56) Reetz, M. T. Laboratory Evolution of Stereoselective Enzymes: A Prolific Source of Catalysts for Asymmetric Reactions. *Angew. Chem., Int. Ed.* **2011**, *50*, 138−174.
(57) Arlt, D.; Jautelat, M.; Lantzsch, R. Syntheses of Pyrethroid Acids. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 703−722.
(58) Mandal, A. K.; Borude, D. P.; Armugasamy, R.; Soni, N. R.; Jawalkar, D. G.; Mahajan, S. W.; Ratnam, K. R.; Goghare, A. D. New Synthetic Route to (1r)-Cis-(−)-Permethrin, (1r)-Cis-(+)-Cypermethrin and (1r)-Cis-(+)-Deltamethrin (Decis) from (+)-3-Carene. *Tetrahedron* **1986**, *42*, 5715−5728.
(59) Talele, T. T. The "Cyclopropyl Fragment" is a Versatile Player that Frequently Appears in Preclinical/Clinical Drug Molecules. *J. Med. Chem.* **2016**, *59*, 8712−8756.

DOI: 10.1021/jacs.9b02700
J. Am. Chem. Soc. 2019, 141, 9145−9150