

# A Diverse Library of Chiral Cyclopropane Scaffolds via Chemoenzymatic Assembly and Diversification of Cyclopropyl Ketones

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Cite This: *J. Am. Chem. Soc.* 2021, 143, 2221–2231

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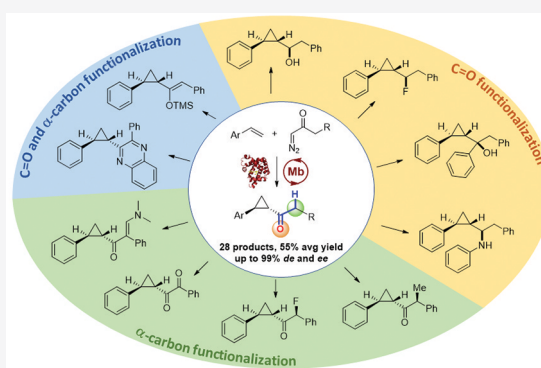
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**ABSTRACT:** Chiral cyclopropane rings are key pharmacophores in pharmaceuticals and bioactive natural products, making libraries of these building blocks a valuable resource for drug discovery and development campaigns. Here, we report the development of a chemoenzymatic strategy for the stereoselective assembly and structural diversification of cyclopropyl ketones, a highly versatile yet underexploited class of functionalized cyclopropanes. An engineered variant of sperm whale myoglobin is shown to enable the highly diastereo- and enantioselective construction of these molecules via olefin cyclopropanation in the presence of a diazoketone carbene donor reagent. This biocatalyst offers a remarkably broad substrate scope, catalyzing this reaction with high stereoselectivity across a variety of vinylarene substrates as well as a range of different  $\alpha$ -aryl and  $\alpha$ -alkyl diazoketone derivatives. Chemical transformation of these enzymatic products enables further diversification of these molecules to yield a collection of structurally diverse cyclopropane-containing scaffolds in enantiopure form, including core motifs found in drugs and natural products as well as novel structures. This work illustrates the power of combining abiological biocatalysis with chemoenzymatic synthesis for generating collections of optically active scaffolds of high value for medicinal chemistry and drug discovery.



## INTRODUCTION

Methods for the synthesis and structural diversification of pharmacophores and “privileged scaffolds” represent key assets in medicinal chemistry efforts directed at the discovery of new bioactive molecules and drugs.<sup>1,2</sup> Complementing purely synthetic approaches,<sup>3,4</sup> chemoenzymatic strategies have recently emerged for this purpose, whereby a selective enzymatic transformation is coupled to a chemical diversification step for functional elaboration of a target molecular scaffold. For example, regio- and stereoselective C–H hydroxylation by means of engineered and natural cytochrome P450s has been integrated with alcohol functionalization/interconversion chemistries for the chemoenzymatic diversification of natural product scaffolds.<sup>5–9</sup> As another example, halogenase enzymes have been exploited in combination with Pd-catalyzed cross-coupling chemistry for the diversification of aromatic compounds.<sup>10–12</sup> Other approaches have involved other classes of natural enzymes.<sup>13,14</sup> Despite this progress, these chemoenzymatic strategies for lead/scaffold diversification have thus far largely relied on enzyme-catalyzed transformations (e.g., hydroxylation, halogenation, and glycosylation) that occur in nature.

Over the past few years, engineered hemoproteins (myoglobin, cytochrome P450s, and cytochrome *c*)<sup>15–27</sup> as

well as artificial metalloenzymes<sup>28–35</sup> have been introduced for promoting abiological carbene transfer reactions. In particular, our group has reported that engineered variants of the oxygen-storage hemoprotein myoglobin (Mb) are capable of catalyzing the intermolecular cyclopropanation of olefins with ethyl  $\alpha$ -diazooacetate (EDA) with high catalytic activity and high stereoselectivity.<sup>16,36</sup> The reaction scope of these myoglobin-based biocatalysts was later extended to intramolecular cyclopropanation reactions<sup>37,38</sup> as well as to intermolecular cyclopropanations involving other types of carbene donor precursors such as trifluorodiazooethane and diazoacetone-trile.<sup>39,40</sup> More recently, biocatalytic strategies for olefin cyclopropanations involving other non-diazoester reagents have also emerged.<sup>41</sup>

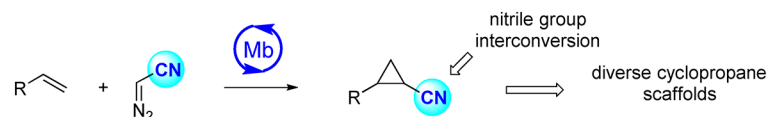
Given the relevance of optically active cyclopropane rings as pharmacophores in drug molecules and biologically active natural products,<sup>42</sup> we have recently begun to explore the

Received: September 3, 2020

Published: January 26, 2021



## a) Single-site chemoenzymatic diversification of nitrile-substituted cyclopropanes (ref. 40):



## b) Multi-site chemoenzymatic diversification of cyclopropyl diazoketones (this work):

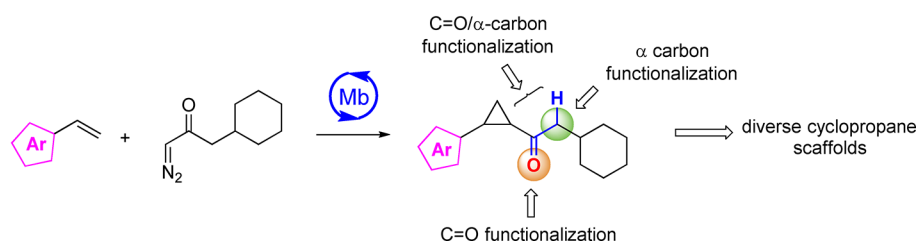
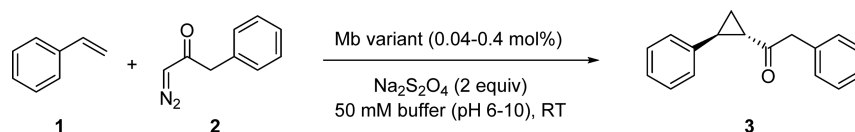


Figure 1. Strategies for chemoenzymatic assembly and diversification of cyclopropanes.

Table 1. Catalytic Activity and Stereoselectivity of Mb Variants for the Cyclopropanation of Styrene with 1-Diazo-3-phenyl-2-propanone (2)<sup>a</sup>

| entry           | catalyst       | catalyst loading (mol %) | [1] (mM) | [2] (mM) | buffer     | yield (%) (SFC) | TON  | d.e. (%) | e.e. (%) |
|-----------------|----------------|--------------------------|----------|----------|------------|-----------------|------|----------|----------|
| 1               | Mb             | 0.1                      | 20       | 20       | KPi pH 7   | trace           | n.d. | n.d.     | n.d.     |
| 2               | Mb(H64V)       | 0.1                      | 20       | 20       | KPi pH 7   | 1               | 10   | n.d.     | n.d.     |
| 3               | Mb(H64V)       | 0.1                      | 20       | 20       | KPi pH 6   | 1               | 10   | n.d.     | n.d.     |
| 4               | Mb(H64V)       | 0.1                      | 20       | 20       | KPi pH 8   | 2               | 20   | n.d.     | n.d.     |
| 5               | Mb(H64V)       | 0.1                      | 20       | 20       | NaBB pH 9  | 6               | 60   | >99      | >99      |
| 6               | Mb(H64V)       | 0.1                      | 20       | 20       | NaBB pH 10 | n.a.            |      |          |          |
| 7               | Mb(L29F,H64V)  | 0.1                      | 20       | 20       | NaBB pH 9  | n.a.            |      |          |          |
| 8               | Mb(L29S,H64V)  | 0.1                      | 20       | 20       | NaBB pH 9  | n.a.            |      |          |          |
| 9               | Mb(F43S,H64V)  | 0.1                      | 20       | 20       | NaBB pH 9  | 1               | 10   | n.d.     | n.d.     |
| 10              | Mb(F43I,H64V)  | 0.1                      | 20       | 20       | NaBB pH 9  | n.a.            |      |          |          |
| 11              | Mb(H64V,V68F)  | 0.1                      | 20       | 20       | NaBB pH 9  | n.a.            |      |          |          |
| 12              | Mb(H64V,V68A)  | 0.1                      | 20       | 20       | NaBB pH 9  | 8               | 80   | >99      | >99      |
| 13              | Mb(H64V,I107S) | 0.1                      | 20       | 20       | NaBB pH 9  | 1               | 10   | n.d.     | n.d.     |
| 14              | Mb(H64V,I107Y) | 0.1                      | 20       | 20       | NaBB pH 9  | n.a.            |      |          |          |
| 15              | Mb(H64A,V68A)  | 0.1                      | 20       | 20       | NaBB pH 9  | 12              | 120  | >99      | >99      |
| 16              | Mb(H64G,V68A)  | 0.1                      | 20       | 20       | NaBB pH 9  | 20              | 200  | >99      | >99      |
| 17              | Mb(H64G,V68A)  | 0.2                      | 10       | 10       | NaBB pH 9  | 33              | 160  | >99      | >99      |
| 18              | Mb(H64G,V68A)  | 0.4                      | 5        | 5        | NaBB pH 9  | 50              | 120  | >99      | >99      |
| 19              | Mb(H64G,V68A)  | 0.4                      | 10       | 5        | NaBB pH 9  | 80              | 200  | >99      | >99      |
| 20              | Mb(H64G,V68A)  | 0.4                      | 20       | 5        | NaBB pH 9  | >99             | >250 | >99      | >99      |
| 21              | Mb(H64G,V68A)  | 0.04                     | 20       | 5        | NaBB pH 9  | 40              | 995  | >99      | >99      |
| 22 <sup>b</sup> | Mb(H64G,V68A)  | 0.4                      | 10       | 2.5      | NaBB pH 9  | >99             | >250 | >99      | >99      |

<sup>a</sup>The reactions were carried out by using purified protein at the indicated reagents concentration in 50 mM potassium phosphate buffer (KPi) or 50 mM sodium borate buffer (NaBB) containing 10 mM sodium dithionite, overnight, under anaerobic conditions. Product yield and % *de* and *ee* as determined by chiral supercritical fluid chromatography (SFC) analysis. Reported values are mean values from  $n \geq 2$  experiments (SE < 15%) N.a. = not active. <sup>b</sup>Reaction time: 10 min (see Figure S1a for complete time-course reaction profile).

potential of combining enzyme-catalyzed cyclopropanation with postcyclization chemistries for diversification of the enzymatic cyclopropane product, i.e., through the interconversion of nitrile-substituted cyclopropanes (Figure 1a).<sup>37,40</sup> Expanding upon this concept, we envisioned diazoketones could represent a promising class of carbene donor reagents for this purpose, offering multiple opportunities for diversification of the cyclopropane product thanks to the versatile reactivity of carbonyls<sup>43</sup> along with the unique reactivity imparted by this

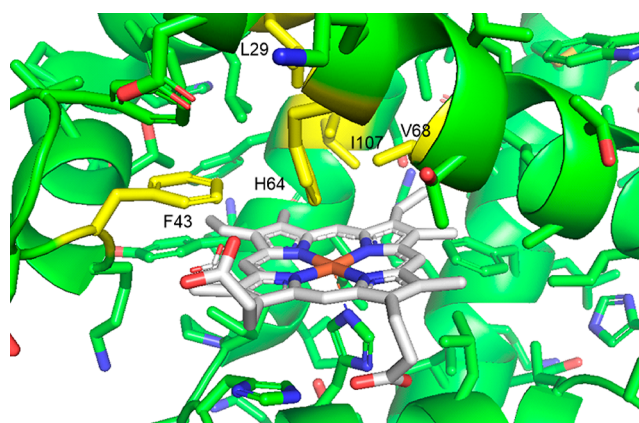
functional group to the adjacent bond and C–H site (Figure 1b).<sup>44–46</sup> Notably, diazoketones have remained largely underexploited in transition-metal-catalyzed carbene transfer reactions,<sup>47–51</sup> with only one isolated example of a highly enantioselective cyclopropanation involving an acceptor-only diazoketone reagent.<sup>52</sup> In addition, chemocatalytic protocols for asymmetric cyclopropanations with diazoketone typically require expensive and toxic metals (Ru, Rh) and synthetically complex ligands.<sup>47–49,52</sup> Here, we report the development of a

biocatalytic strategy for the highly diastereo- and enantioselective synthesis of cyclopropyl ketones using an engineered iron-containing protein. These enzymatic products can be readily diversified by chemical means to provide efficient access to a variety of structurally diverse cyclopropane scaffolds as valuable chiral building blocks for medicinal chemistry and/or natural product synthesis.

## RESULTS AND DISCUSSION

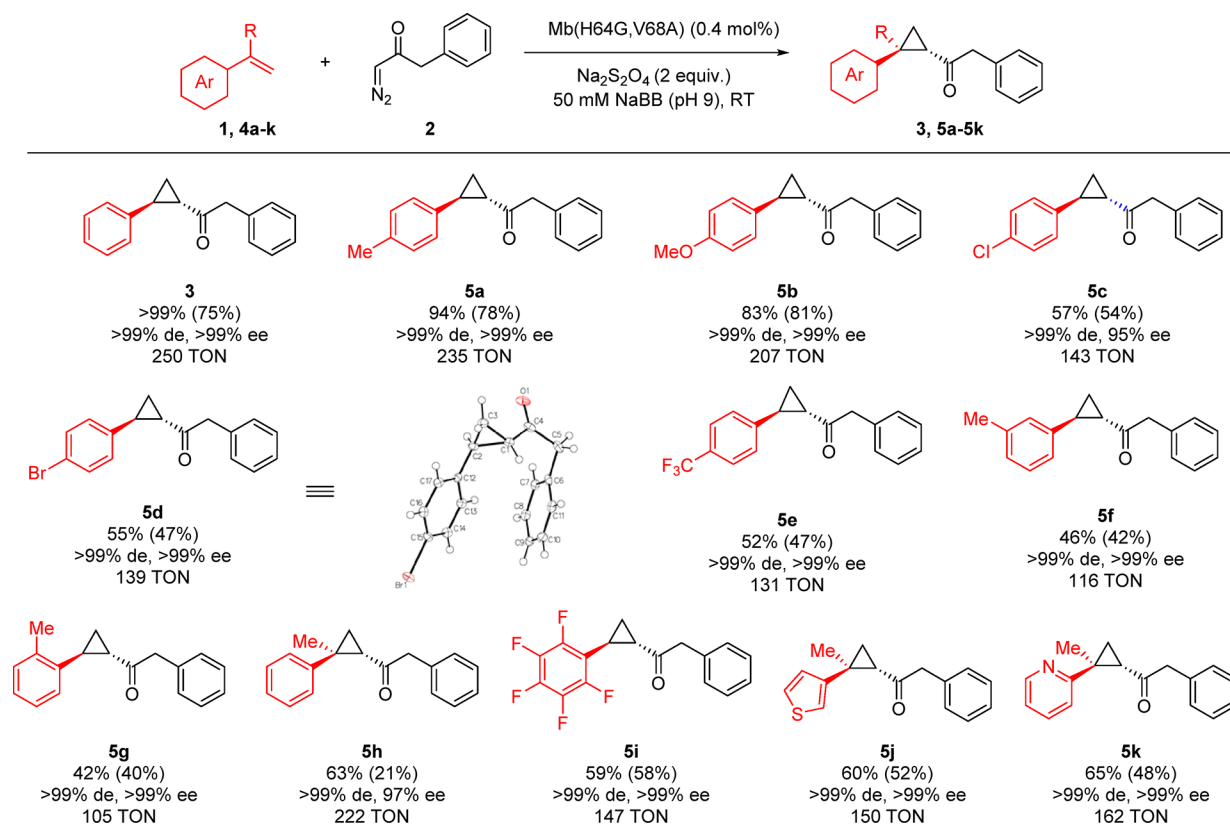
**Olefin Cyclopropanation with Benzyl Diazoketone.** In initial efforts toward developing this methodology, we targeted the cyclopropanation of styrene (**1**) in the presence of 1-diazo-3-phenylpropan-2-one (**2**) (referred to here as “benzyl diazoketone”) to give the keto-functionalized cyclopropane **3** (Table 1). We envisioned this reaction would offer multiple opportunities for chemoenzymatic diversification of the cyclopropanation product, namely, through variation of both the carbene donor and carbene acceptor reagent in the cyclopropanation reaction as well as through the functionalization of the carbonyl group and the  $\alpha$  C(sp<sup>3</sup>)-H site in the resulting cyclopropyl ketone product. To our knowledge, this reaction has never been reported using synthetic or biological carbene transfer catalysts. In addition, subjecting these reagents to commonly adopted organometallic catalysts for carbene transfer reactions such as Rh<sub>2</sub>(OAc)<sub>4</sub><sup>53</sup> and Co(TPP)<sup>54</sup> yielded no product or only trace amounts of **3** (Table S1). Similarly, various heme-containing proteins and enzymes, including wild-type sperm whale myoglobin (Mb), various cytochromes P450 (P450<sub>BM3</sub>, P450XplA, and P450BeZ), and cytochromes *c*, showed no detectable activity toward this reaction (Table S1). Despite the lack of activity of wild-type Mb, we previously found that mutations of the “distal” histidine residue (His64), such as H64V, can improve the reactivity of this metalloprotein toward non-native carbene transfer reactions,<sup>16</sup> an effect that has been attributed to an increased accessibility of the metal active center to the carbene donor reagents.<sup>55</sup> Promisingly, Mb(H64V) was found to exhibit low but detectable activity in the cyclopropanation reaction, producing **3** in 6% yield in sodium borate buffer at pH 9.0 (Table 1, entry 5). A sharp decrease in catalytic activity was evident at pH values below or above 9 (Table 1, entries 2–6). Furthermore, the Mb(H64V) variant was found to catalyze this reaction with excellent degrees of diastereo- and enantioselectivity (>99% *de* and *ee*). The latter results are in contrast with the modest enantioselectivity exhibited by this variant in the cyclopropanation of styrene in the presence of ethyl  $\alpha$ -diazooacetate as the carbene donor (10% *ee*).<sup>36</sup>

Using Mb(H64V) as background, we then tested a small library of Mb variants in which the shape of the distal heme cavity is systematically varied by mutating each of the “active site” residues (i.e., Leu29, Phe43, Val68, and Ile107; Figure 2) with an apolar amino acid of significantly different size (i.e., Leu29 → Ala/Phe: was mutated to Ala or Phe; Phe43 was mutated to Ile or Ser). This strategy has previously proven effective toward improving the efficiency of other Mb-catalyzed carbene transfer reactions such as the C–H functionalization of indoles.<sup>24</sup> While most of these mutations resulted in loss of catalytic activity (Table 1, entries 7–14), Mb(H64V,V68A) showed a slight improvement in activity (80 vs 60 turnovers or TON), while maintaining excellent diastereo- and enantioselectivity (>99% *de* and *ee*) (Table 1, entry 12). This variant was previously found to exhibit high activity and stereoselectivity for the cyclopropanation of styrene with EDA<sup>16</sup> and other



**Figure 2.** Distal heme pocket of sperm whale myoglobin (pdb 1VXA). The heme cofactor (gray) and neighboring residues are shown as stick models. The active site residues targeted for mutagenesis are labeled and highlighted in yellow.

small-sized acceptor-only diazo compounds such as trifluoro-diazoethane<sup>39</sup> and diazoacetonitrile.<sup>40</sup> Considering the significantly bulkier diazoketone reagent utilized in the present reaction, we envisioned that the latter could benefit from increasing the accessibility of the heme pocket through substitution of the “gating” residue at position 64 (Figure 2) with a small amino acid residue. Gratifyingly, a progressive enhancement in catalytic activity (8% (Val) → 12% (Ala) → 20% (Gly)) was observed upon reducing steric hindrance at this position through substitution of Val64 with alanine and glycine to give Mb(H64A,V68A) and Mb(H64G,V68A), respectively (Table 1, entries 15 and 16). Altogether, this structure–activity data showed a clear correlation between cyclopropanation activity with benzyl diazoketone **2** (TON) and the decreasing size of the amino acid at the “proximal” site (Gly > Ala > Val ≫ His). Upon optimization of the reaction conditions, the Mb(H64G,V68A)-catalyzed cyclopropanation reaction of styrene with **2** could be further optimized to produce **3a** in quantitative yield and with excellent stereocontrol (>99% *de* and *ee*; Table 1, entry 20). To further deconvolute the functional role of the mutations in Mb(H64G,V68A), the activity of a series of single mutants was investigated under these optimized reaction conditions (Figure S2). While confirming the beneficial effect of space-creating mutations at the level of the gating residue 64 (Gly > Ala = Val), these results also revealed a synergistic effect of the H64G and V68A mutations toward enhancing biocatalyst’s activity in this transformation, as judged based on the significantly higher TON exhibited by Mb(H64G,V68A) compared to Mb(H64G) and Mb(V68A) (>250 vs 24–32 TON; Figure S2). As noted for Mb(H64V) (Table 1, entries 3–6), the efficiency of the Mb(H64G,V68A)-catalyzed cyclopropanation reaction with diazoketone **2** was found to be pH dependent, showing an optimum at pH 9 (Table S3). This trend differs from that observed for other Mb-catalyzed carbene transfer reactions investigated previously, which showed either no pH dependence (e.g., indole C–H functionalization)<sup>24</sup> or optimal performance at neutral pH (e.g., benzofuran cyclopropanation).<sup>26</sup> Thus, the mildly alkaline conditions seem to be specifically beneficial for Mb-catalyzed olefin cyclopropanation with diazoketones. Using X-ray crystallography (*vide infra*), we determined the absolute configuration of the cyclopropanation product to be (1*S*,2*S*).



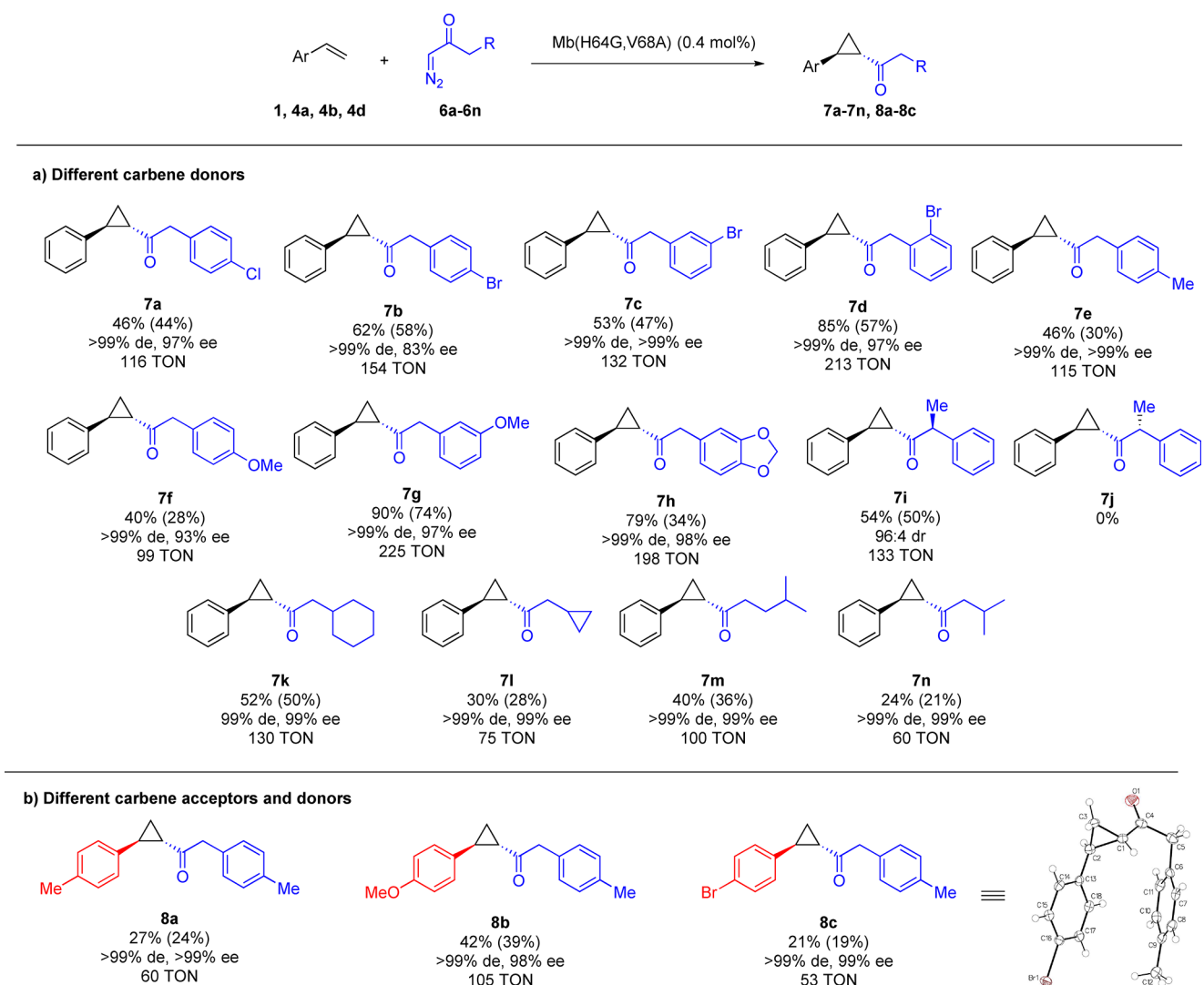
**Figure 3.** Substrate scope of Mb(H64G,V68A)-catalyzed cyclopropanation with diazoketone **2** in the presence of different olefin substrates. Reaction conditions: 20 mM olefin, 5 mM diazo compound, 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>, 20 μM Mb(H64G,V68A) in sodium borate buffer (50 mM, pH 9) containing 10% ethanol, room temperature, 12 h, anaerobic conditions. Product conversion and stereoselectivity was determined by chiral SFC using calibration curves with authentic standards, and yields of isolated products are reported in parentheses.

The high *trans*-(1*S*,2*S*) stereoselectivity exhibited by Mb(H64G,V68A) and Mb(H64V,V68A) in this reaction mirrors that of Mb(H64V,V68A) for the cyclopropanation of styrene with EDA,<sup>16</sup> suggesting a similar mechanism of catalyst-induced stereoselection.<sup>55</sup> Kinetic experiments showed that the Mb(H64G,V68A)-catalyzed reaction proceeds with an initial rate of 196 turnovers per minute (Figure S2), and it reaches completion within 10 min (Table 1, entry 22; Figure S1). Furthermore, the biocatalyst supports up to ~1000 total turnovers (TTN) under catalyst-limited conditions (Table 1, entry 21).

#### Analysis of Substrate Scope across Olefin Substrates.

To assess the substrate tolerance of the Mb(H64G,V68A) catalyst across the olefin reagent, the Mb variant was tested against a diverse set of styrene derivatives and other vinylarenes in the cyclopropanation reaction with diazoketone **2**. As summarized in Figure 3, these experiments showed that this variant can accept a range of *para*-substituted styrene derivatives (**4a–4e**) yielding the corresponding cyclopropyl ketones in enantiopure form (>99% *de* and *ee* for **5a,b,d,e**) or highly enantio-enriched form (95% *ee* for **5c**). Both electron-donating and electron-withdrawing groups were accepted with generally higher yields for the electron-rich styrenes (**5a,b**) compared to the electron-poor ones (**5c–e**). This trend is generally consistent with the electrophilic reactivity of the heme–carbene intermediate expected to mediate this reaction as determined for  $\alpha$ -diazooacetates.<sup>55,56</sup> Kinetic analysis of these reactions (Figure S2) showed indeed that electron-rich styrenes (176–190 TON min<sup>-1</sup> for **5a,b**) are cyclopropanated at faster rates than electron-poor ones (123–132 TON min<sup>-1</sup>

**5c,d**). At the same time, the cyclopropanation rate of electron-rich styrenes was comparable to that of styrene (Figure S2), indicating that while electronic effects are dominating, steric effects also play a role in influencing catalyst's reactivity toward these substrates. The results with **5f** and **5g** indicated that the catalyst exhibits good activity and excellent stereoselectivity (99% *de* and *ee*) also toward the cyclopropanation of *meta*- and *ortho*-substituted styrenes (Figure 3). The results across the **5a**, **5f**, and **5c** series indicated that substitutions at the *para* position are generally better tolerated than *ortho* and *meta* substitutions in terms of catalytic activity, while excellent stereoselectivity is retained in all cases. The Mb(H64G,V68A)-catalyzed cyclopropanation of electron-deficient pentafluorostyrene (**4i**) was also successful, producing **5i** in good yield and with excellent stereoselectivity (>99% *de* and *ee*). Notably, other challenging substrates such as the disubstituted  $\alpha$ -methylstyrene (**4h**) and thionyl- and pyridine-containing vinylarenes **4j** and **4k**, respectively, could be processed with good efficiency (60–65% SFC yields) to afford the corresponding trisubstituted cyclopropanes **5h**, **5j**, and **5k**, respectively, in high diastereomeric and enantiomeric excess (>99% *de* and 97–99% *ee*; Figure 3). Of note, substrates such as **4j** and **4k** are notoriously challenging substrates for transition-metal-catalyzed cyclopropanations due to the propensity of N- and S-containing heterocycles to bind and thus inhibit the activity of metal centers. Conversely, aliphatic olefins such as 1-octene and internal olefins such as cyclohexene did not participate in the reaction, defining the boundaries of the biocatalyst's scope with respect to the olefin substrate.



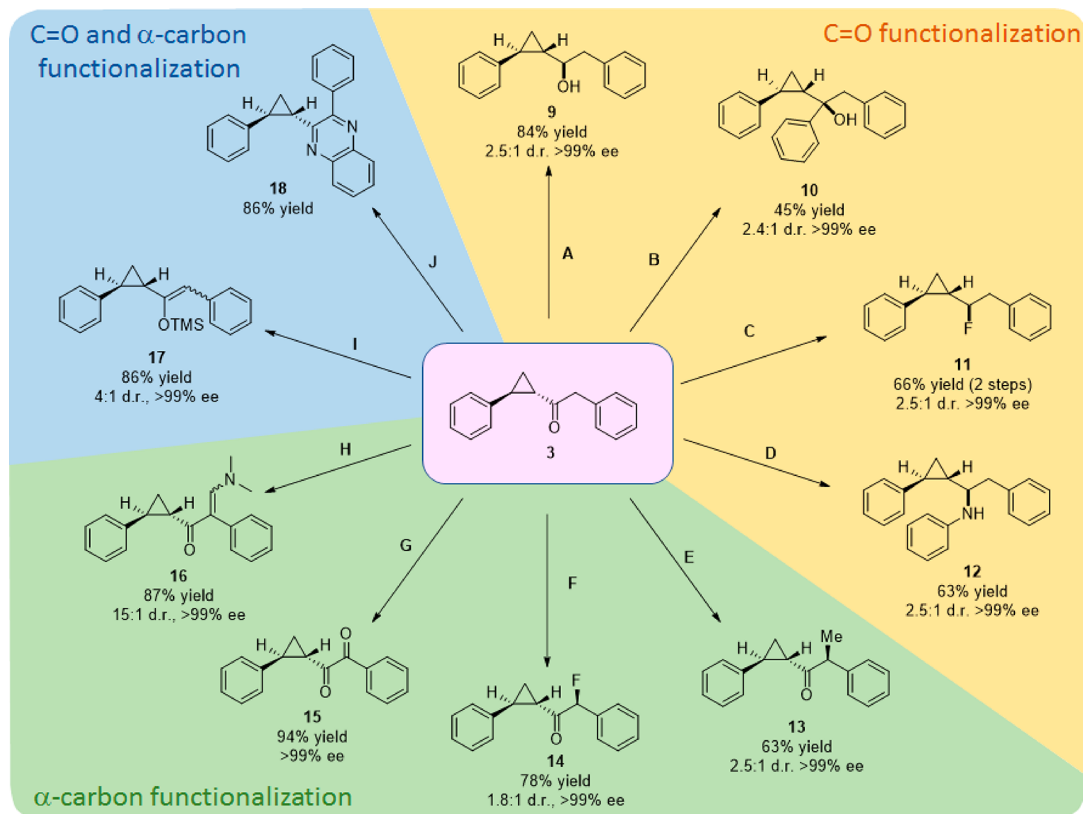
**Figure 4.** Substrate scope of Mb(H64G,V68A)-catalyzed cyclopropanation in the presence of different diazoketone reagents. Reaction conditions: 20 mM olefin, 5 mM diazo compound, 10 mM  $\text{Na}_2\text{S}_2\text{O}_4$ , 20  $\mu\text{M}$  Mb(H64G,V68A) in sodium borate buffer (50 mM, pH 9) containing 10% ethanol, room temperature, 12 h, anaerobic conditions. Product conversion and stereoselectivity was determined by chiral SFC (or chiral HPLC) by using calibration curves with authentic standards and yields of isolated products are reported in parentheses.

Crystallographic analysis of **5d** confirmed the (1*S*,2*S*) configuration of the cyclopropanation product (Figure 3, Table S3 and Figure S4), as it was anticipated based on the results with Mb(H64V,V68A) and its previously established stereopreference in the cyclopropanation of vinylarenes in the presence of other acceptor-only diazo compounds.<sup>16,39,40</sup> Using **5d** as reference, chiral chromatographic analysis of the other cyclopropane products indicated an identical enantioselectivity across all the other cyclopropanation products, denoting the generality and predictable stereoselectivity of the Mb(H64G,V68A) catalyst. All of the aforementioned reactions could be performed at the semipreparative scale (0.2 mol) enabling the synthesis of the desired cyclopropyl ketone products with an average isolated yield of 54%. Altogether, these results demonstrated the broad tolerance of the Mb(H64G,V68A) catalyst toward accepting a variety of vinylarene substrates.

#### Substrate Scope across Diazoketone Substrates.

Next, we assessed the performance of Mb(H64G,V68A) in the cyclopropanation reaction with styrene in the presence of different diazoketone reagents, as we envisioned this approach

could provide an additional means for diversification of the cyclopropyl ketone products (Figure 4a). To this end, a diverse set of diazoketones was prepared according to the Arndt–Eistert method<sup>57,58</sup> through reaction of the corresponding acyl chloride precursors with diazomethane generated *in situ* from diazald. This procedure yielded the desired carbene donor reagents in up to ~80% yield over two steps from readily available carboxylic acid precursors. Notably, a variety of benzyl diazoketone analogues containing *para*-, *meta*-, and *ortho*-substitutions (**6a–6g**) could be processed by the Mb(H64G,V68A) catalyst to afford the corresponding cyclopropanes **7a–7g** in up to 90% conversion, excellent *trans*-selectivity (>99% *de*), and very good to excellent enantioselectivity (83 to >99% *ee*) (Figure 4a). Interestingly, higher conversions and enantioselectivity were generally observed for the reactions involving the *ortho*- or *meta*-substituted diazo compounds compared to the *para*-substituted counterparts (e.g., **7d** vs **7b** and **7g** vs **7f**; Figure 4a) and regardless of the electronic effect of these substituents, suggesting a stronger influence of steric over electronic effects on the activity and selectivity of the biocatalyst upon variation of the diazo

Scheme 1. Diverse Cyclopropane Scaffolds via Diversification of Cyclopropyl Ketones<sup>a</sup>

<sup>a</sup>Reaction conditions: (A) sodium borohydride (1.5 equiv), MeOH, rt, 3 h; (B) phenylmagnesium bromide (3 equiv), CsCl<sub>2</sub> (3 equiv), THF, 0 °C, 2 h; (C) (1) NaBH<sub>4</sub> (1.5 equiv) MeOH, rt, 3 h, (2) XtalFluor-E (1.5 equiv), DBU (1.5 equiv), DCM, 0 °C to rt, overnight; (D) aniline (2 equiv), sodium borohydride (NaBH<sub>4</sub>) (3 equiv), THF, rt, overnight; (E) lithium diisopropylamide, MeI, THF, 0 °C to rt, overnight; (F) sodium dodecylbenzenesulfonate, SelectF, DCM, rt, overnight; (G) CuBr<sub>2</sub> (20 mol %), thiomorpholine (3 equiv), aerobic, DMSO, rt, overnight; (H) *N,N*-dimethylformamide dimethyl acetal, neat, 90 °C, overnight; (I) lithium diisopropylamide (1.1 equiv), trimethylsilyl chloride (1.2 equiv), Et<sub>2</sub>O, 0 °C to rt, overnight; (J) (1) copper(II) bromide (CuBr<sub>2</sub>) (20 mol %), thiomorpholine (3 equiv), aerobic, DMSO, rt, overnight, (2) *o*-phenylenediamine (1 equiv), potassium *tert*-butoxide (4 mol %), MeOH, rt, overnight.

reagent. Of note, a bulky carbene donor such as the benzodioxolane derivative **6h** was also readily converted by the hemoprotein to give **7h** in good yield and with excellent enantioselectivity (>99% *de*, 98% *ee*). Finally, the results with **7i** showed that the biocatalyst is also able to tolerate alpha substitutions in the diazo reagent. Using enantiopure (*S*)- $\alpha$ -methyl- $\beta$ -phenyl diazoketone **6i**, we produced the desired cyclopropyl ketone **7i** in 50% yield and with a (1*S*,2*S*,3*S*):(1*R*,2*R*,3*S*) diastereomeric ratio of 96:4 (Figure 4a), the latter result indicating that the Mb(H64G,V68A)-catalyzed cyclopropanation reaction has retained high stereoselectivity also in the presence of the  $\alpha$ -substituted diazo reagent. Interestingly, the same reaction in the presence of (*R*)- $\alpha$ -methyl- $\beta$ -phenyl diazoketone **6j** yielded no product, indicating that the Mb variant is enantioselective toward the chiral diazo reagent. Consistent with these results, a reaction with racemic ( $\pm$ )- $\alpha$ -methyl- $\beta$ -phenyl diazoketone produced **7i** in 28% yield and with a d.r. of 96:4, further demonstrating that the enzyme is indeed able to resolve the racemic diazo reagent by engaging only the (*S*)-enantiomer. As observed for the reaction with styrene (Table 1) and other vinylarenes (Figure 2), the Mb(H64G,V68A)-catalyzed reactions involving the different diazo compounds occur cleanly with no signs of carbene dimerization and with the desired product and unreacted substrate accounting for a total mass balance. In some cases,

trace amounts (<1%) of a reduced byproduct resulting from diazotization of the diazo reagent were observed. These results indicate that catalyst inactivation is the primary factor limiting product conversion under suboptimal reaction conditions (e.g., entry 15, Table 1) or in the presence of less favorable substrates (e.g., **5h** and **7f**).

To further explore the substrate scope of the reaction, a panel of different  $\alpha$ -alkyl-substituted diazoketones (**6k**–**6n**) was investigated. Notably, all of these diazoketone reagents were accepted by the Mb(H64G,V68A) catalyst, enabling the synthesis of the desired cyclopropanation products **7k**–**7n** in 21–50% isolated yields and with excellent diastereo- and enantioselectivity (99% *de* and *ee*; Figure 4a). Finally, we tested the ability of Mb(H64G,V68A) to accept combinations of substituted styrenes and substituted benzyl diazoketone reagents (Figure 4b). Albeit in moderate yields (21–40%), the Mb(H64G,V68A)-catalyzed cyclopropanation of various styrene derivatives in the presence of **6e** produced the desired disubstituted cyclopropyl ketones **8a**–**8c** with excellent degrees of diastereo- and enantioselectivity (>99% *de* and 98–99% *ee*; Figure 4b). Crystallographic analysis of **8c** confirmed the 1*S*,2*S* absolute configuration of the cyclopropane ring (Figure 4b, Figure S5, and Table S4), further highlighting the predictable and highly conserved stereoselectivity of this biocatalyst in these transformations. Together

with those discussed earlier (Figure 3), these results demonstrated the remarkable tolerance of the Mb-(H64G,V68A) biocatalyst toward accepting various diazoketone reagents to generate a diverse set of cyclopropyl ketones with high stereoselectivity.

**Diverse Cyclopropane Scaffolds via Chemoenzymatic Diversification of Cyclopropyl Ketones.** While remaining largely underexploited as pharmacophores, the optically active cyclopropyl ketones made accessible by using the present methodology are expected to constitute valuable building blocks for medicinal chemistry as  $\alpha$ -cyclopropyl ketone motifs are found in a number of bioactive molecules including the natural product bisgersolanolide<sup>59</sup> and approved drugs such as the larotaxel (anticancer)<sup>60</sup> and prasugrel (anticoagulant).<sup>61</sup> In addition to that, we envisioned that another key advantage of this biocatalytic strategy would derive from the opportunity to diversify the cyclopropanation product via chemoenzymatic means to obtain a variety of valuable chiral cyclopropane-based scaffolds (Figure 1b). To illustrate this point, enantiopure **3** produced from the Mb-catalyzed reaction was converted, through different chemistries, into a panel of structurally diverse cyclopropane-containing scaffolds, including  $\alpha$ -cyclopropyl alcohols, amines, and fluorides,  $\alpha$ -substituted cyclopropyl ketones, and diketones (Scheme 1). These compounds encompass both structural motifs found in medicinally active molecules and entirely new structures. As shown in Scheme 1,  $\alpha$ -cyclopropyl alcohol **9** could be readily obtained via reduction of the carbonyl group in **3** with sodium borohydride (84% yield, 2.5:1 d.r., >99% ee), whereas a nucleophilic addition reaction with phenylmagnesium bromide afforded the tertiary  $\alpha$ -cyclopropyl alcohol **10** (45% yield, 2.4:1 d.r., >99% ee; Scheme 1). Similar  $\alpha$ -cyclopropyl alcohol motifs are found in various members of eicosanoid-like families of natural products such as constanolactones, solandelactones, and halicholactones<sup>62,63</sup> as well as in synthetic drugs such as the triple reuptake inhibitor GSK1360707F.<sup>64</sup> Deoxofluorination of **9** in the presence of the nucleophilic fluorinating reagent XtalFluor-E afforded  $\alpha$ -cyclopropyl fluoride **11** (66% yield over two steps), whereas  $\alpha$ -cyclopropylamine **12** could be obtained in good yield (63%) via direct reductive amination of cyclopropyl ketone **3** in the presence of aniline (Scheme 1). An  $\alpha$ -cyclopropyl fluoride moiety is found in the anti-HIV agent lenacapavir,<sup>65</sup> whereas  $\alpha$ -cyclopropylamines are common pharmacophores in a variety of drugs, including the serotonin reuptake inhibitors milnacipran<sup>66</sup> and BMS-505130.<sup>67</sup> For all the aforementioned reactions, transformation of the carbonyl group introduces a third stereogenic center which could be formed with a significant (and comparable) degree of diastereoselectivity (~2.5:1 d.r.) as influenced by the chirality of the enzymatically assembled cyclopropane ring. Furthermore, in each case the two diastereomers could be separated and isolated as enantiopure entities (>99% ee).

We further posited that functionalization of the alpha C–H site in the enzymatic product **3** could furnish a viable route to gain access to other types of functionalized cyclopropane scaffolds. Accordingly, base-catalyzed  $\alpha$ -alkylation of **3** in the presence of methyl iodide could be applied to obtain cyclopropyl ketone **13** (63% yield; 2.5:1 d.r.; >99% ee; Scheme 1), whereas its  $\alpha$ -fluorination in the presence of electrophilic reagent Selectfluor in an emulsion<sup>68</sup> successfully afforded fluorinated cyclopropyl ketone **14** (78% yield; 1.8:1 d.r.; >99% ee; Scheme 1). Both of products were obtained as a separable mixture of two diastereomers in enantiopure form (>99% ee).

The configuration of the newly generated stereogenic center in **13** was assigned by direct comparison with the enzymatic product **7j** and that in **14** by analogy with **13**, since the two reactions are expected to exhibit similar facial selectivity during electrophilic attack of the enolate intermediate to generate the  $\alpha$ -functionalized ketone. Aerobic oxidation of **3** in the presence of copper bromide and thiomorpholine produced cyclopropyl-1,2-diketone **15** in high yield as a single stereoisomer (94% yield, >99% ee). While cyclopropane scaffolds **14** and **15** have never been obtained via cyclopropanation protocols before, both  $\alpha$ -fluoroketones and 1,2-diketones have represented key pharmacophores for the design of potent inhibitors of proteases<sup>69–71</sup> because of the ability of these moieties to mimic the tetrahedral intermediate and/or inactivate the nucleophilic residue mediating the cleavage of peptide bonds in these enzymes.<sup>69–71</sup> Subjecting **3** to an excess of dimethylformamide dimethyl acetal produced the  $\alpha,\beta$ -unsaturated ketone **16** in high yield (86%) as a mixture of enantiopure *Z* and *E* isomers (Scheme 1). Whereas **16** represents a novel cyclopropane-based scaffold per se,  $\alpha,\beta$ -unsaturated carbonyl derivatives have found a growing number of applications in the development of drugs acting as covalent protein/enzyme inhibitors.<sup>72,73</sup> Finally, other novel cyclopropane-based scaffolds could be obtained through functionalization of both the carbonyl group and  $\alpha$ -position. Enolization of **3** with lithium diisopropylamide (LDA) followed by enolate trapping with trimethylsilyl chloride generated the cyclopropane silyl ether **17** in 84% yield. On the other hand, subjecting diketone **15** to a double condensation reaction in the presence of phenylenediamine<sup>74</sup> readily afforded the cyclopropane-containing quinoxaline **18** in 66% yield over two steps from **3**. The latter chemoenzymatic route can provide access to diaryl-substituted cyclopropanes which are not readily accessible via transition metal-catalyzed carbene transfer.

## CONCLUSION

In summary, we have reported the development of a highly diastereo- and enantioselective biocatalytic strategy for the synthesis of keto-functionalized cyclopropanes. This represents the first example of a highly stereoselective protocol for olefin cyclopropanation with diazoketones involving a biocatalyst and iron-based catalyst. Notable features of the present strategy include a broad substrate scope and a consistently high stereoselectivity toward promoting this reaction in the presence of different vinylarenes and diazoketone reagents, as demonstrated through the synthesis of 28 different cyclopropyl ketone products by means of a single Mb-based catalyst. These compounds could be readily assembled and diversified by chemoenzymatic means to produce a collection of structurally diverse cyclopropane-based scaffolds (Scheme 1) useful for medicinal chemistry and other applications. This work paves the way to the application of diazoketones in other biocatalytic carbene transfer reactions, and it illustrates the power of combining abiological biocatalysis with chemoenzymatic synthesis for generating collections of optically active synthons of potentially high value for the synthesis of new bioactive molecules.

## EXPERIMENTAL DETAILS

**General Information.** All the chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, Alfa Aesar, ACS Scientific, and Acros) and used without any further purification,

unless otherwise stated. All dry reactions were performed under argon or nitrogen gas in flame-dried glassware with magnetic stirring by using standard gastight syringes, cannula, and septa.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured on a Bruker DPX-500 spectrometer (operating at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ ) or a Bruker DPX-400 (operating at 400 MHz for  $^1\text{H}$  and 105 MHz for  $^{13}\text{C}$ ).  $^{19}\text{F}$  was measured on a Bruker DPX-400 (operating at 375 MHz). TMS was used as the internal standard (0 ppm) for  $^1\text{H}$  NMR,  $\text{CDCl}_3$  was used as the internal standard (77.0 ppm) for  $^{13}\text{C}$  NMR, and trifluorotoluene served as the internal standard (−63 ppm) for  $^{13}\text{F}$  NMR. Silica gel chromatography purifications were performed by using AMD Silica Gel 60 230–400 mesh. Preparative thin layer chromatography was performed on TLC plates (1 mm thickness, Sigma-Aldrich).

**Protein Expression and Purification.** Wild-type sperm whale myoglobin and the engineered Mb variants were cloned and expressed in *E. coli* C41(DE3) cells as described previously.<sup>36</sup> Briefly, cells were grown in terrific broth (TB) medium (ampicillin, 100 mg L<sup>−1</sup>) at 37 °C (200 rpm) until OD<sub>600</sub> reached 1.0–1.2. Cells were then induced with 0.25 mM  $\beta$ -D-1-thiogalactopyranoside (IPTG) and 0.3 mM  $\delta$ -aminolevulinic acid (ALA). After induction, cultures were shaken at 180 rpm and 27 °C and harvested after 18–20 h by centrifugation at 4000 rpm at 4 °C. After cell lysis by sonication, the proteins were purified by Ni-affinity chromatography. The lysate was transferred to a Ni-NTA column equilibrated with Ni-NTA Lysis Buffer (50 mM KPi, 250 mM NaCl, 10 mM imidazole, pH 8.0). The resin was washed with 50 mL of Ni-NTA Lysis Buffer and then 50 mL of Ni-NTA Wash Buffer (50 mM KPi, 250 mM NaCl, 20 mM imidazole, pH 8.0). Proteins were eluted with Ni-NTA Elution Buffer (50 mM KPi, 250 mM NaCl, 250 mM histidine, pH 7.0). After elution, the proteins were buffer exchanged against 50 mM KPi buffer (pH 7.0) by using 10 kDa Centricon filters. Protein concentration in ferric form was determined by using  $\epsilon_{410} = 156 \text{ mM}^{-1} \text{ cm}^{-1}$  as the extinction coefficient.

**Enzymatic Reactions with Purified Protein.** Substrate screening reactions were performed at a 400  $\mu\text{L}$  scale by using 20  $\mu\text{M}$  myoglobin, 20 mM styrene, 5 mM diazoketone, and 10 mM sodium dithionite inside an anaerobic chamber. In a typical procedure, a buffered solution containing the myoglobin variant was carefully charged to a vial inside an anaerobic chamber. After the catalyst solution was diluted with sodium borate buffer (50 mM, pH 9.0), 20  $\mu\text{L}$  of sodium dithionite solution (200 mM stock solution in 50 mM pH 9 sodium borate buffer) was added. Reactions were initiated by addition of 20  $\mu\text{L}$  of styrene (from 400 mM stock solution in ethanol), followed by the addition of 20  $\mu\text{L}$  of diazoketone (from 100 mM stock solution in ethanol). The reaction mixture was stirred for 16 h at room temperature inside an anaerobic chamber. The reaction mixtures were added with 20  $\mu\text{L}$  of internal standard (50 mM benzodioxole in ethanol), followed by extraction with 400  $\mu\text{L}$  of dichloromethane. For determination of product yield, diastereomeric excess, and enantiomeric excess, the extraction solutions were analyzed by chiral SFC, chiral GC, or chiral HPLC as described in the Supporting Information (see the Analytical Methods section in the Supporting Information and Figures S3 and S4). Time-course experiments (Figure S1) and kinetic experiments (Figure S2) were performed in a similar manner by using 10  $\mu\text{M}$  enzyme, 10 mM styrene 1, 2.5 mM diazoketone 2, 10 mM  $\text{Na}_2\text{S}_2\text{O}_4$  in 50 mM sodium borate buffer (pH 9.0) with 10% EtOH, room temperature, and an anaerobic atmosphere. The reactions were quenched with 3 M HCl at the different time points, followed by extraction and chiral SFC analysis as described above.

**Reactions with Whole Cells.** Whole cell experiments were performed at a 400  $\mu\text{L}$  scale by using 370  $\mu\text{L}$  of *E. coli* cells expressing Mb(H64V,V68A), 20 mM styrene derivative, and 5 mM diazoketone inside an anaerobic chamber. In a typical procedure, cells suspended in potassium phosphate buffer (50 mM, pH 7.2) with 10% glycerol were charged to a vial inside an anaerobic chamber. After the catalyst solution was diluted with potassium phosphate buffer (50 mM, pH 7.2) with 10% glycerol, reactions were initiated by addition of 20  $\mu\text{L}$  of styrene (from 400 mM stock solution in ethanol), followed by the

addition of 20  $\mu\text{L}$  of diazoketone (from 100 mM stock solution in ethanol). The reaction mixture was stirred for 16 h at room temperature inside an anaerobic chamber. The reactions were prepared for chiral SFC analysis by adding 20  $\mu\text{L}$  of internal standard (50 mM benzodioxole in ethanol) to the reaction mixture, followed by extraction with 400  $\mu\text{L}$  of dichloromethane. The TON for the whole-cell reactions was calculated based on Mb concentration in the reaction mixture as measured via UV–vis spectroscopy ( $\epsilon_{410} = 156 \text{ mM}^{-1} \text{ cm}^{-1}$ ) after cell lysis.

**General Procedure for Synthesis of Diazoketones 2 and 6a–6n (Procedure A).** The diazoketone reagents were prepared from the corresponding carboxylic acids according to the following procedure. To a flame-dried round-bottom flask, carboxylic acid (1.0 mmol) was dissolved in DCM (1.0 mL) under argon with a drop of dimethylformamide as catalyst. After dropwise addition of thionyl chloride (1.5 equiv), the reaction was stirred for 2 h at room temperature. Solvent was removed in vacuo and used in the next step without further purification. Synthesis of diazoketones from acetyl chlorides was performed using a diazomethane generator (Aldrich) with System 45 compatible connection. *Caution: diazomethane is a toxic and explosive gas, and it must be handled with great caution at all times!* In the outer tube of the diazomethane generator, acetyl chloride (0.3 mmol) was dissolved in Et<sub>2</sub>O (3.0 mL), and in the inner tube, diazald (5.7 equiv) was suspended in carbitol (1.0 mL). Once the reaction mixture was immersed in an ice bath, aqueous KOH (37%, 1.5 mL) was injected dropwise via a syringe into the inner tube. After stirring for 3 h at 0 °C, silicic acid (0.15 g) was added to the inner tube to quench any unreacted diazomethane. Solvent in the outer tube was removed, and the reaction mixture was subjected to column chromatography to yield diazoketones 2 and 6a–6n. See the Supporting Information for compound characterization data.

**General Procedure for Preparative-Scale Enzymatic Synthesis of Cyclopropanation Products 3, 5a–5k, 7a–7n, and 8a–8c (Procedure B).** To a round-bottom flask, 18 mL of 20  $\mu\text{M}$  myoglobin in argon-purged sodium borate buffer (50 mM, pH 9.0) and 70 mg of sodium dithionite were added under argon. The reaction was initiated by addition of 1 mL of 400 mM alkene solution in ethanol (20 mM final concentration), followed by addition 1 mL of 100 mM diazoketone solution in ethanol (5 mM final concentration). The reaction was stirred for 16 h at room temperature under argon. After extraction with DCM (3  $\times$  50 mL), organic layers were combined and dried over  $\text{NaSO}_4$ . The solvent was removed in vacuo, and the reaction mixture was subjected to column chromatography to yield cyclopropanes 3, 5a–5k, 7a–7n, and 8a–8c. See the Supporting Information for compound characterization data.

**Racemic Standards.** Analytical amounts of racemic products were prepared from 400  $\mu\text{L}$  scale cyclopropanation reactions with 1 mM Fe(TPP)Cl (10 mol %), 20 mM olefin, 10 mM diazoketone, and 10 mM sodium dithionite in toluene:H<sub>2</sub>O:EtOH (8:1:1). In a typical procedure, a mixture containing 40  $\mu\text{L}$  of sodium dithionite (100 mM stock solution in distilled H<sub>2</sub>O) and 300  $\mu\text{L}$  of toluene was degassed by sparging with argon for 5 min in a sealed vial. A separate vial containing 20  $\mu\text{L}$  of Fe(TPP)Cl (20 mM stock solution in toluene) was carefully degassed in a similar manner. The two solutions were then mixed together via a cannula. Reactions were initiated by addition of 20  $\mu\text{L}$  of olefin (400 mM stock solution in ethanol), followed by the addition of 20  $\mu\text{L}$  of diazoketone (200 mM stock solution in ethanol) with a syringe, and the reaction mixture was stirred for 16 h at room temperature under positive argon pressure. The product was isolated via preparative TLC (hexanes:EtOAc 9:1), extracted with dichloromethane, and analyzed by chiral SFC, GC, or HPLC as described in the Supporting Information.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.0c09504>.



Tables and figures, chiral GC and SFC chromatograms, synthetic procedures, compound characterization data, and NMR spectra (PDF)

Crystallographic data for **5d** and **8c** (ZIP)

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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was supported by the U.S. National Institutes of Health Grant GM098628. The authors are grateful to Dr. William Brennessel for assistance with crystallographic analyses. MS and X-ray instrumentation are supported by U.S. National Science Foundation Grants CHE-0946653 and CHE-1725028.

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