

# Stereodivergent Synthesis of Pyridyl Cyclopropanes via Enzymatic Activation of Pyridotriazoles

Satyajit Roy, Yining Wang, Xinyi Zhao, Thakshila Dayananda, Jia-Min Chu, Yong Zhang,\* and Rudi Fasan\*



Cite This: <https://doi.org/10.1021/jacs.4c06103>



Read Online

ACCESS |

Metrics & More

Article Recommendations

Supporting Information

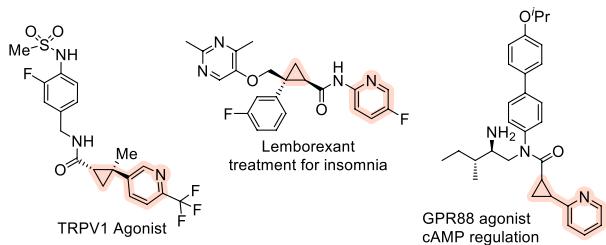
**ABSTRACT:** Hemoproteins have recently emerged as powerful biocatalysts for new-to-nature carbene transfer reactions. Despite this progress, these strategies have remained largely limited to diazo-based carbene precursor reagents. Here, we report the development of a biocatalytic strategy for the stereoselective construction of pyridine-functionalized cyclopropanes via the hemoprotein-mediated activation of pyridotriazoles (PyTz) as stable and readily accessible carbene sources. This method enables the asymmetric cyclopropanation of a variety of olefins, including electron-rich and electrodeficient ones, with high activity, high stereoselectivity, and enantiodivergent selectivity, providing access to mono- and diarylcyclopropanes that incorporate a pyridine moiety and thus two structural motifs of high value in medicinal chemistry. Mechanistic studies reveal a multifaceted role of 7-halogen substitution in the pyridotriazole reagent toward favoring multiple catalytic steps in the transformation. This work provides the first example of asymmetric olefin cyclopropanation with pyridotriazoles, paving the way to the exploitation of these attractive and versatile reagents for enzyme-catalyzed carbene-mediated reactions.

**R**ing systems are recurring structural motifs in small-molecule drugs and bioactive natural products.<sup>1–4</sup> Among them, the aromatic heterocycle pyridine represents the second most abundant ring structure found in pharmaceuticals (Figure 1a).<sup>5</sup> Owing to their peculiar conformational and configurational properties, cyclopropanes are key pharmacophores

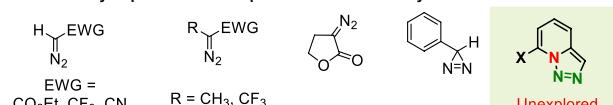
exploited for the design of bioactive molecules in the pharmaceutical and agrochemical industry alike (Figure 1a).<sup>5</sup> Accordingly, the fusion of a pyridyl moiety with cyclopropane rings can furnish highly valuable scaffolds for medicinal chemistry, as exemplified by the GPR88 agonist (Figure 1a) and other pharmacologically active compounds (Figure S1).

The transition-metal catalyzed cyclopropanation of alkenes with diazo compounds has represented a powerful strategy for the construction of enantioenriched functionalized cyclopropanes (Figure 1b),<sup>6–9</sup> including pyridyl cyclopropanes.<sup>10,11</sup> Over the past few years, we and other groups have shown that hemoproteins, such as myoglobin<sup>12–16</sup> and cytochromes P450s,<sup>17–19</sup> as well as artificial enzymes,<sup>20–26</sup> can provide efficient biocatalysts for abiological olefin cyclopropanation reactions. These biocatalytic approaches were shown to offer key advantages over chemo-catalytic methods in terms of chemo- and stereoselectivity, catalytic efficiency, and/or step economy for drug synthesis.<sup>27</sup> Using these engineered hemoproteins, a growing number of diazo compounds, including acyclic and cyclic diazoesters,<sup>28–30</sup> diazoacetonitrile,<sup>31,32</sup> trifluorodiazooethane,<sup>33,34</sup> diazoketones,<sup>15</sup> and diazophosphonates,<sup>35</sup> have been successfully applied for the asymmetric synthesis of functionalized cyclopropanes. Despite this progress, enzymatic strategies involving carbene precursor

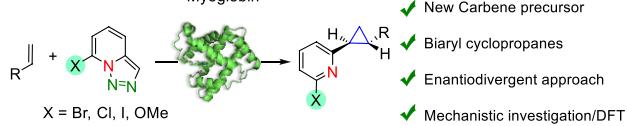
## a. Bioactive molecules containing pyridine-substituted cyclopropane



## b. Previously explored carbene precursors in biocatalysis



## c. This work

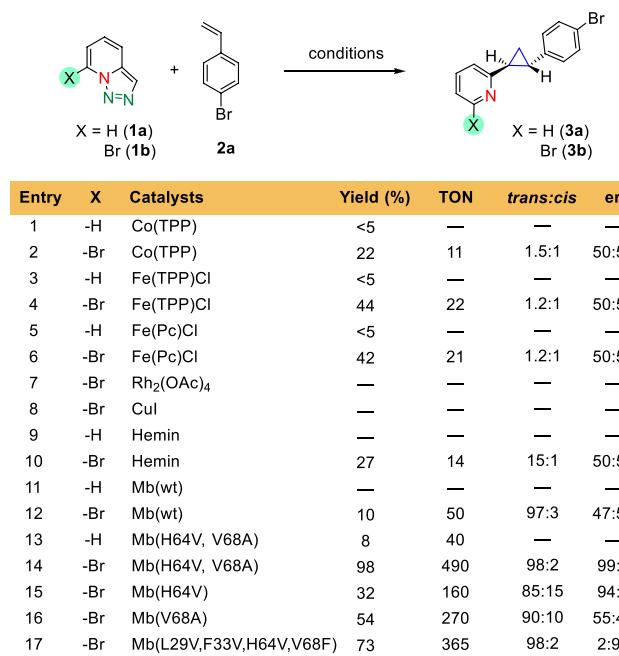


**Figure 1.** Representative bioactive molecules containing pyridine and cyclopropane rings and biocatalytic cyclopropanation with 1,2,3-pyridotriazole.

Received: May 6, 2024

Revised: June 19, 2024

Accepted: June 21, 2024

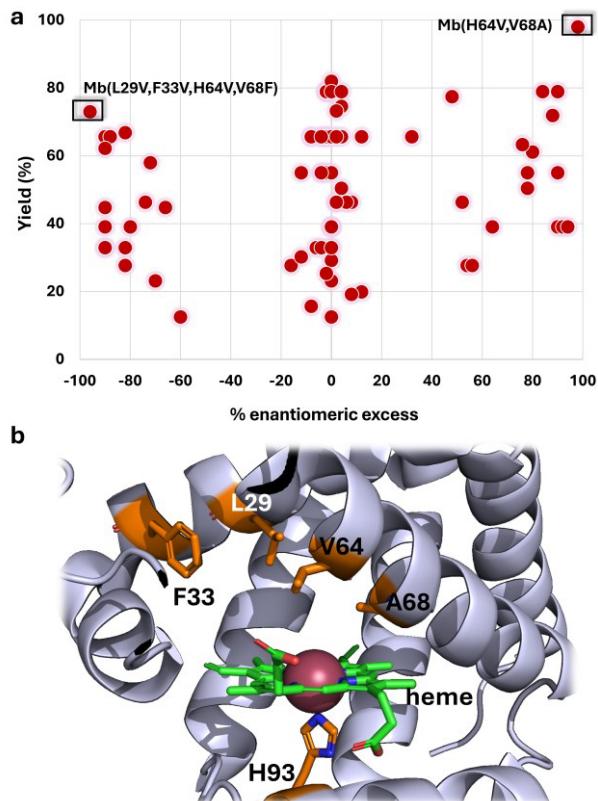


**Figure 2.** Cyclopropanation of **2a** with 1,2,3-pyridotriazoles (**1a** or **1b**). Transition metal catalyzed reactions were performed in DCM with 2 mol % catalyst (DMF for hemin reactions). Enzymatic reactions were performed at 400  $\mu$ L scale using 20  $\mu$ M biocatalyst, 10 mM **1a** or **1b**, 15 mM 4-bromostyrene (**2a**), 10 mM sodium dithionite, 3 h, r.t., and anaerobic conditions. Detailed reaction conditions are provided in Figure S2.

reagents beyond diazo compounds have remained largely unexplored, with the only recent exception of diazirines.<sup>36</sup>

Within our program focused on the development of abiological enzyme-catalyzed transformations, we became interested in 1,2,3-pyridotriazoles as potential reagents for hemoprotein-mediated carbene transfer reactions. These readily accessible and shelf-stable reagents are known to undergo a tautomeric equilibrium between the closed and open form,<sup>37,38</sup> thus providing a “masked” substitute for semistabilized heteroaryl diazo compounds.<sup>39</sup> Following pioneering reports on the use of 1,2,3-pyridotriazoles for rhodium-catalyzed Si–H insertion reactions,<sup>40</sup> these reagents have found utility in transannulations, insertions, ylide formation, and rearrangements for the synthesis of heterocycles.<sup>41–43</sup> More recently, 1,2,3-pyridotriazoles were also successfully employed for cyclopropanation reactions via *in situ* generation in combination with Co-porphyrin catalysts<sup>39,44</sup> or via light induced activation.<sup>45</sup> However, asymmetric variations of these reactions have so far remained elusive. Here, we report the development of a biocatalytic strategy for the activation of 1,2,3-pyridotriazoles and subsequent olefin cyclopropanation toward the stereoselective synthesis of pyridyl-functionalized cyclopropanes as well as diaryl cyclopropanes (Figure 1c), which are highly desirable structural motifs for medicinal chemistry and drug discovery (Figures 1a and S1).

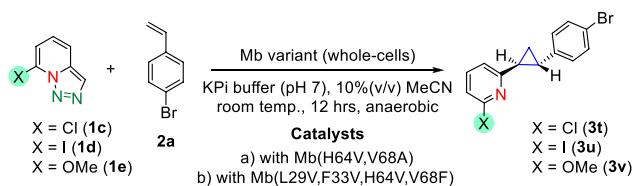
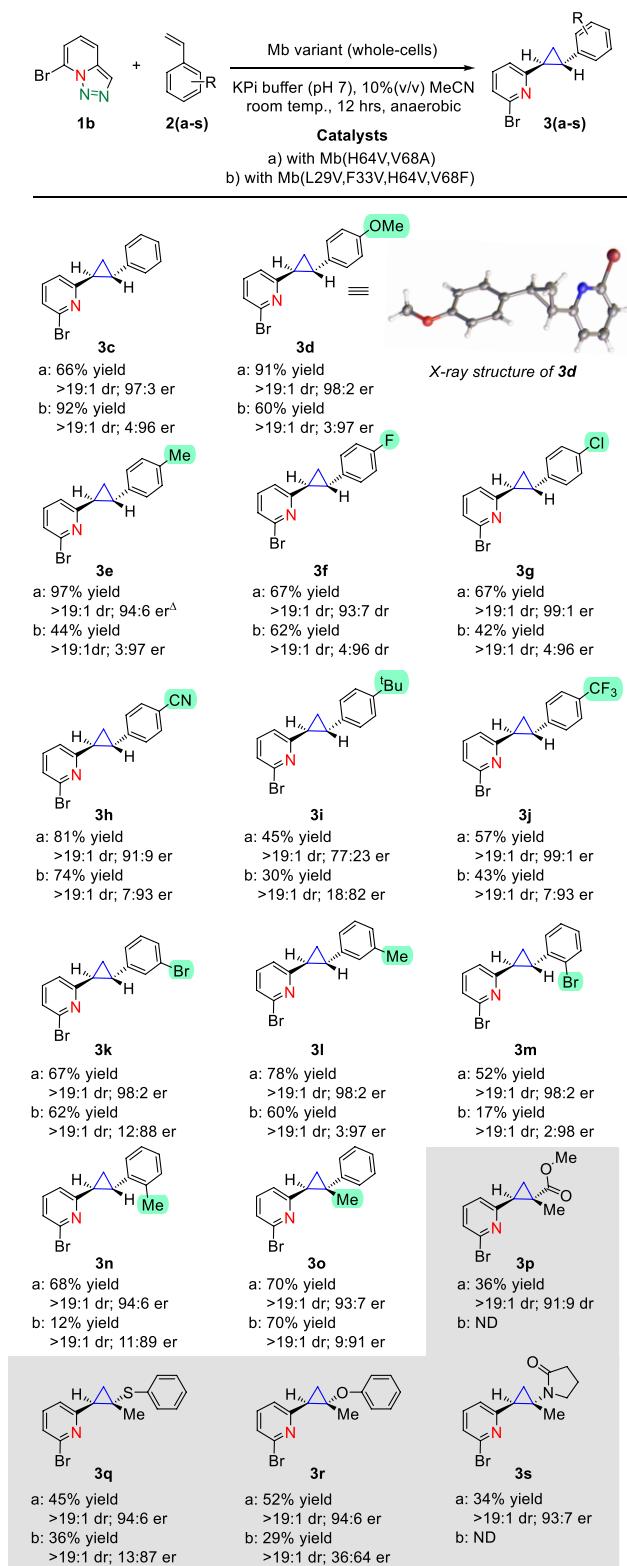
Given the reactivity of engineered myoglobins toward different types of cyclopropanation reactions,<sup>12–16</sup> we initially explored the reactivity of this protein toward cyclopropanation of 4-bromo-styrene in the presence of 1,2,3-pyridotriazole (PyTz) **1a** (Figure 2), which can be synthesized in one step



**Figure 3.** Engineered myoglobin catalysts. (a) Screening results of a diverse panel of engineered myoglobin variants for cyclopropanation of **2a** with **1b**. (b) Active site of Mb(H64V, V68A) (=Mb\*) (PDB code 6M8F) displaying the heme cofactor (green) and selected active site residues (orange) as stick models.

from commercial material (see SI for details). Similarly to hemin and common carbene transfer reagents such as Fe(TPP), Co(TPP), and Rh<sub>2</sub>(OAc)<sub>4</sub>, only trace amounts of the desired cyclopropanation product **3a** (<1–5% yield) was observed for wild-type (sperm whale) myoglobin (Mb) (Figure 2). Next, we investigated the same reaction in the presence of 7-bromo-1,2,3-pyridotriazole **1b** in reason of the known effect of halogen substituent at C7 toward shifting the pyridotriazole equilibrium toward the open form tautomer,<sup>46</sup> which was envisioned to potentially make it more available for activation by the hemoprotein. Gratifyingly, this modification resulted in Mb-catalyzed formation of the desired cyclopropane product **3b** with good *trans*-selectivity (97:3 dr), albeit only in modest yields (10%) and in a racemic form (6% ee) (Figure 2, entry 12).

To improve the activity and enantioselectivity of the biocatalyst, we screened a diverse panel of engineered myoglobin variants containing up to five active site mutations at positions 29, 43, 64, 68, and 107, as derived from prior protein engineering campaigns for developing Mb-based biocatalysts for different types of carbene transfer reactions. From this screening, multiple engineered variants with improved activity and selectivity toward the model reactions were identified (Figure 3a). Among them, Mb(H64V, V68A) (=Mb\*) (Figure 3b), emerged as the most efficient and stereoselective biocatalyst for formation of the 1S,2S-enantiomer of **3b**, affording this product in 98% yield, 98:2 d.r. (*trans*) and 99:1 enantiomeric ratio (e.r.) upon validation as purified protein (Figure 2, Entry 14). Deconstruction

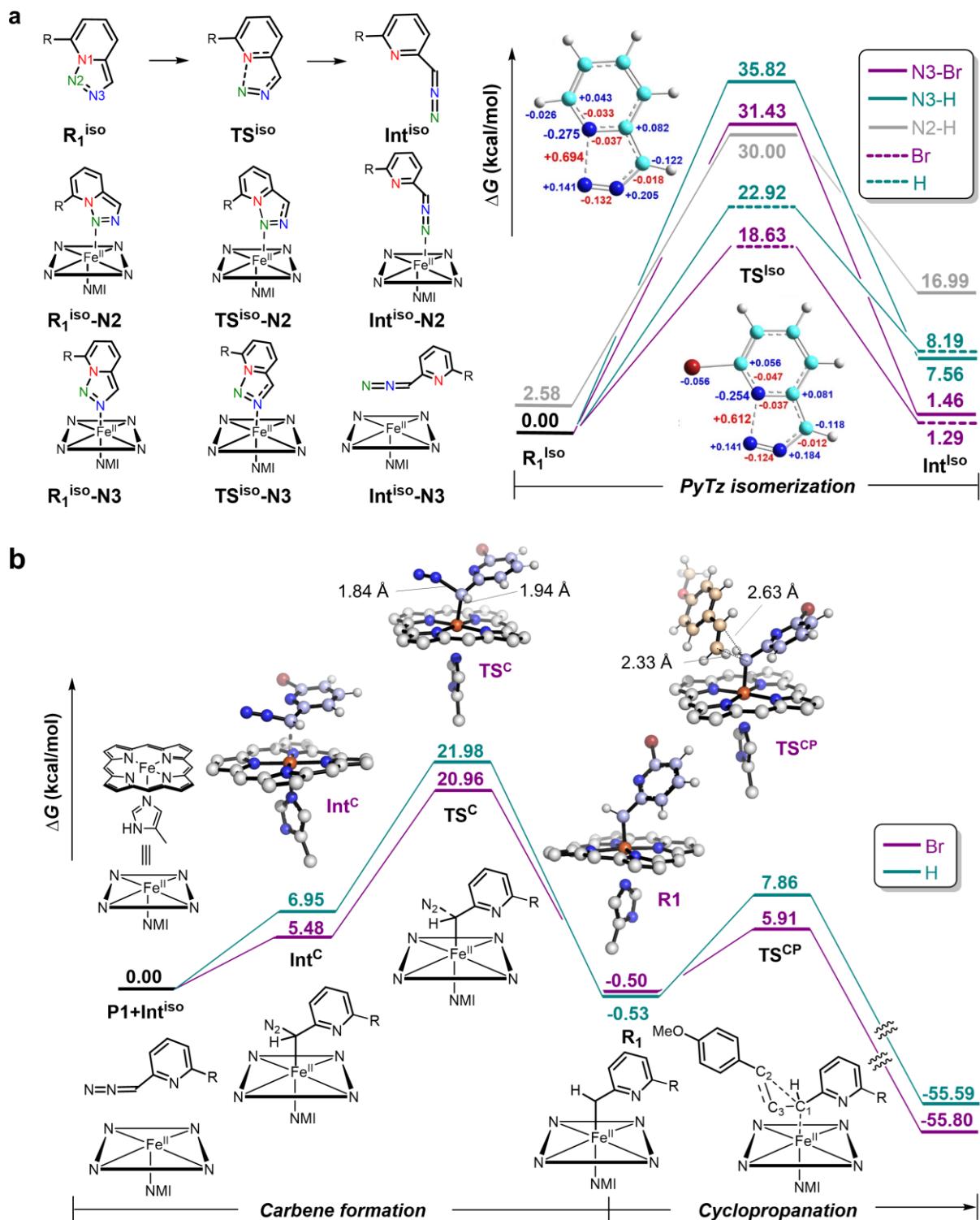


responsible for increasing activity (10%  $\rightarrow$  54%). This effect is in striking contrast to the effect of the same mutations for the cyclopropanation of styrene with EDA, where the V68A mutation was found to improve stereoselectivity, whereas the H64V mutation enhanced activity.<sup>12</sup> Notably, screening of the Mb library also revealed various variants with inverted enantioselectivity compared to Mb\* (Figure 3), from which Mb(L29V,F33V,H64V,V68F) was identified as the best enantioselective biocatalyst for this reaction (98:2 dr (trans); 2:98 er; Figure 2, Entry 17).

These experiments also revealed a background cyclopropanation activity for “empty” *E. coli* cells, producing 3b in racemic form. This activity was attributed to the *E. coli* hemoprotein YfeX,<sup>47</sup> as confirmed by testing of this protein in purified form (Figure S2). Whole cell transformations with Mb\* or Mb(L29V,F33V,H64V,V68F) showed comparable enantioselectivity to the same reactions with purified protein, indicating that the Mb-driven reaction largely outcompetes the basal activity of endogenous YfeX in *E. coli* cells.

After optimization of the reaction conditions (Figures S3–S6), the substrate scope for the Mb\*-catalyzed reaction was investigated across a diverse range of olefin substrates (Figure 4). A wide range of *para*-substituted styrenes containing both electrondonating and electronwithdrawing groups ( $-OMe$ ,  $-Me$ ,  $-I$ ,  $-Cl$ ,  $-F$ ,  $-CF_3$ ) were converted to the desired products 3c–3j in good to high yields (57–91%) and with high diastereo- and enantiocontrol (>19:1 dr.; 86–99% ee; Figure 4). *Ortho*- and *meta*-substituted styrenes as well as alpha-methylstyrene were also well accepted by the enzyme, furnishing 3k–3o in 67–78% yields and in high diastereo- and enantiomeric excess (94:6 to 99:1 er) (Figure 4). The generality of the methodology was then probed across olefin substrates beyond styrene derivatives. Notably, a variety of olefins, including *O/S*-allyl-(thio)phenols, acrylates, and vinyl amides, underwent Mb\*-catalyzed cyclopropanation to yield the desired products 3p–3s with high activity and selectivity (Figure 4). The reactivity of the enantiodivergent biocatalyst Mb(L29V,F33V,H64V,V68F) across the same set of substrates was also investigated. All of the substrates except two (2p, 2s) were transformed to the desired 1*R*,2*R*-cyclopropanes with good to high activity and stereoselectivity (up to 92% yield; >19:1 dr; 97:3 er; Figure 4), which demonstrated the broad

analysis of this variant showed that the H64V (Figure 3b) is primarily responsible for enhancing the stereoselectivity of the reaction (6%  $\rightarrow$  88% ee), whereas the V68A mutation is



**Figure 6.** DFT analyses. (a) Noncatalyzed and metal-coordinated PyTz isomerization pathways via N2 and N3 binding. R = H/Br. Key bond length changes (in red, unit: Å) and charge changes (in blue, unit: e) are shown. (B) Schematic free energy diagram for hemoprotein-catalyzed cyclopropanation of 4-methoxy-styrene with pyridotriazoles. See SI for details. NMI = 5-methyl-imidazole.

scope and reactivity of this pair of enantiocomplementary biocatalysts.

Next, the reactivity of both  $\text{Mb}^*$  and  $\text{Mb}^-(\text{L29V},\text{F33V},\text{H64V},\text{V68F})$  toward other 7-substituted 1,2,3-pyridotriazoles was analyzed.  $\text{Mb}^*$  is able to catalyze the cyclopropanation of 4-bromo-styrene in the presence of 7-Cl

(3t), 7-iodo (3u), and 7-methoxy- PyTz (3v) with good activity and high stereoselectivity (Figure 5). It also showed detectable activity on unsubstituted PyTz to give 3a in 8% yield (Figure 2, Entry 13), a transformation not viable using transition metal catalysts (Figure 2). Also for these reactions,

Mb(L29V,F33V,H64V,V68F) offers an enantiodivergent route to the corresponding diaryl cyclopropanes.

To gain insights into the mechanism of this reaction and enhanced reactivity of 7-Br-PyTz vs PyTz, we performed a quantum chemical study using models ([Fe(Por)(5-MeIm)]) and methods previously found to accurately predict spectroscopic and reactivity features of heme carbenes.<sup>48–53</sup> First, we analyzed the triazole isomerization pathway from the closed to the open form for both **1a** (R1<sup>Iso</sup>-H) and **1b** (R1<sup>Iso</sup>-Br) in the absence and in the presence of the heme catalyst. For the uncatalyzed pathway, the beneficial effect of the Br substitution is apparent from a decrease in  $\Delta G^\ddagger$  from 22.92 to 18.63 kcal/mol for the transition from the triazole to the diazole form (TS<sup>Iso</sup>-H vs TS<sup>Iso</sup>-Br; Figure 6a, Table S2). This effect can be partly attributed to the Br substitution lowering the N1–N2 bond length elongation by about 0.1 Å, which reduces the energy cost associated with cleavage of the N1–N2 bond required for tautomerization. Furthermore, the electron-withdrawing effect of Br helps stabilize the negative charge transfer to N1 in TS<sup>Iso</sup> (Figure 6a), further facilitating the process. Diazole **1a** is also thermodynamically more favorable than **1b** by 6.90 kcal/mol. Thus, consistent with previous reports,<sup>54,55</sup> the Br substitution facilitates ring opening by reducing both the kinetic barrier and thermodynamic reaction energy associated with it. In the presence of a heme catalyst, both N2 and N3 atoms are available for metal coordination. Accordingly, we considered three coordination modes, namely, terminal monocoordination ( $\eta^1$ ) via N2 or via N3 and side-on dual coordination ( $\eta^2$ ) via N2–N3. For PyTz, both  $\eta^1$ -N2 (called R1<sup>Iso</sup>-N2-H) and  $\eta^1$ -N3 complexes (called R1<sup>Iso</sup>-N3-H) are viable (Figure 6a), with the latter being more stable by 2.58 kcal/mol. In contrast, the  $\eta^2$ -(N2, N3) complex was found to be unstable with its optimization leading to the more stable  $\eta^1$ -N3 coordination mode. In contrast to PyTz, only the  $\eta^1$ -N3 complex (R1<sup>Iso</sup>-N3-Br) was found to be energetically viable for the 7-Br-PyTz interaction with the heme (Figure 6a), whereas the  $\eta^1$ -N2 mode is unstable due to steric repulsion between the Br substituent and the porphyrin ring. After isomerization, the N2 coordination mode (Int<sup>Iso</sup>-N2-H) forms an N-bound diazo-heme complex (Fe···N2 bond ~2 Å), whereas the N3 coordinated complex results in dissociated diazole and porphyrin. Interestingly, for the N3 coordination systems, the Br substitution in the PyTz reagent significantly lowers both the reaction barrier ( $\Delta\Delta G^\ddagger = -4.39$  kcal/mol) and the reaction energy ( $\Delta\Delta G = -6.10$  kcal/mol; Table S5) of the isomerization step, thus favoring opening of the triazole ring both kinetically and thermodynamically. Altogether, these results show that the 7-bromo substitution strongly favors tautomerization of PyTz to its open form, which becomes amenable to further activation for carbene transfer, both intrinsically (i.e., with no catalyst) and in the heme-catalyzed reaction. Within the latter, this substitution also favors the productive  $\eta^1$ -N3 coordination mode over the unproductive  $\eta^1$ -N2 mode, which leads to the end-bound diazo-heme complex previously determined to be unreactive toward formation of the reactive heme carbene.<sup>56</sup>

Next, we investigated the subsequent carbene formation and cyclopropanation pathway for both PyTz and 7-Br-PyTz, considering a concerted carbene transfer pathway based on previous analyses of cyclopropanations catalyzed by His-ligated heme/hemoproteins<sup>13,51</sup> (Figure 6b). These analyses revealed that the Br substitution exerts a favorable effect toward both carbene formation and cyclopropanation by lowering the

energy barriers associated with these steps by 1–2 kcal/mol (Figure 6b; Tables S8 and S11).

Thus, the 7-bromo substitution on PyTz was determined to facilitate all key steps of the transformation (i.e., isomerization, carbene formation, and cyclopropanation) and most significantly the rate-determining triazole ring opening step, providing a rationale for the experimentally observed higher reactivity of the enzyme with 7-Br-PyTz vs PyTz (Figure 2, Entries 13–14).

In conclusion, we report the first example of an enzymatic carbene transfer reaction involving stable and readily accessible pyridotriazoles as carbene precursors. Using engineered myoglobins, the asymmetric cyclopropanation of a variety of olefins, including both electron-rich and electrophilic ones, could be achieved with high efficiency and stereoselectivity, as well as enantiodivergent selectivity. This strategy provides direct access to a variety of optically active pyridine-containing mono- and diarylcyclopropanes as highly valuable scaffolds for medicinal chemistry. While the aryl bromine group in the **1b**-derived products furnishes a convenient handle for diversification (e.g., via aryl cross-coupling),<sup>57</sup> various 7-substituted PyTz reagents are compatible with the methodology. Computational analysis of the reaction mechanism provides a rationale for the experimentally observed reactivity trends, revealing a multifaceted role of the C(7)-Br substitution toward favoring all of the key steps implicated in this transformation. By introducing pyridotriazoles as a new class of carbene precursors amenable to enzymatic catalysis, this work paves the way for the exploitation of these versatile and attractive reagents for a variety of synthetically useful biocatalytic transformations.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.4c06103>.

Chiral GC and SFC chromatograms, synthetic procedures, compound characterization data, NMR spectra, and crystallographic data (PDF)

### Accession Codes

CCDC 2352089 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif), or by emailing [data\\_request@ccdc.cam.ac.uk](mailto:data_request@ccdc.cam.ac.uk), or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

## AUTHOR INFORMATION

### Corresponding Authors

Rudi Fasan – Department of Chemistry and Biochemistry, University of Texas at Dallas, Richardson, Texas 75080, United States; Department of Chemistry, University of Rochester, Rochester, New York 14627, United States; [orcid.org/0000-0003-4636-9578](https://orcid.org/0000-0003-4636-9578); Email: [Rudi.Fasan@utdallas.edu](mailto:Rudi.Fasan@utdallas.edu)

Yong Zhang – Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030, United States; Email: [yong.zhang@stevens.edu](mailto:yong.zhang@stevens.edu)

## Authors

**Satyajit Roy** — Department of Chemistry and Biochemistry, University of Texas at Dallas, Richardson, Texas 75080, United States;  [orcid.org/0000-0001-7374-4797](https://orcid.org/0000-0001-7374-4797)

**Yining Wang** — Department of Chemistry and Biochemistry, University of Texas at Dallas, Richardson, Texas 75080, United States

**Xinyi Zhao** — Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030, United States

**Thakshila Dayananda** — Department of Chemistry, University of Rochester, Rochester, New York 14627, United States;  [orcid.org/0000-0003-3726-7780](https://orcid.org/0000-0003-3726-7780)

**Jia-Min Chu** — Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/jacs.4c06103>

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was supported by the U.S. National Institute of Health grant GM098628 (R.F.). R.F. acknowledges chair endowment support from the Robert A. Welch Foundation (Chair, AT-0051). Y.Z. acknowledges support from the U.S. National Science Foundation (NSF) grant CHE-2054897. The authors are grateful to the UTD Center for High-Throughput Reaction Discovery & Synthesis supported by grant RR230018 from the Cancer Prevention and Research Institute of Texas.

## REFERENCES

- (1) Taylor, R. D.; MacCoss, M.; Lawson, A. D. G. Rings in Drugs. *J. Med. Chem.* **2014**, *57*, 5845–5859.
- (2) Shearer, J.; Castro, J. L.; Lawson, A. D. G.; MacCoss, M.; Taylor, R. D. Rings in Clinical Trials and Drugs: Present and Future. *J. Med. Chem.* **2022**, *65*, 8699–8712.
- (3) Ling, Y.; Hao, Z. Y.; Liang, D.; Zhang, C. L.; Liu, Y. F.; Wang, Y. The Expanding Role of Pyridine and Dihydropyridine Scaffolds in Drug Design. *Drug Des Devel Ther* **2021**, *15*, 4289–4338.
- (4) De, S.; Kumar S K, A.; Shah, S. K.; Kazi, S.; Sarkar, N.; Banerjee, S.; Dey, S. Pyridine: the scaffolds with significant clinical diversity. *RSC Adv.* **2022**, *12*, 15385–15406.
- (5) 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.
- (6) Charette, A. B.; Molinaro, C.; Brochu, C. Catalytic Asymmetric Cyclopropanation of Allylic Alcohols with Titanium-TADDOLate: Scope of the Cyclopropanation Reaction. *J. Am. Chem. Soc.* **2001**, *123*, 12168–12175.
- (7) Wei, B.; Sharland, J. C.; Lin, P.; Wilkerson-Hill, S. M.; Fullilove, F. A.; McKinnon, S.; Blackmond, D. G.; Davies, H. M. L. In Situ Kinetic Studies of Rh(II)-Catalyzed Asymmetric Cyclopropanation with Low Catalyst Loadings. *ACS Catal.* **2020**, *10*, 1161–1170.
- (8) Marcin, L. R.; Denhart, D. J.; Mattson, R. J. Catalytic Asymmetric Diazoacetate Cyclopropanation of 1-Tosyl-3-vinyl-indoles. A Route to Conformationally Restricted Homotryptamines. *Org. Lett.* **2005**, *7*, 2651–2654.
- (9) Chuprakov, S.; Kwok, S. W.; Zhang, L.; Lercher, L.; Fokin, V. V. Rhodium-Catalyzed Enantioselective Cyclopropanation of Olefins with N-Sulfonyl 1,2,3-Triazoles. *J. Am. Chem. Soc.* **2009**, *131*, 18034–18035.
- (10) Davies, H. M. L.; Townsend, R. J. Catalytic Asymmetric Cyclopropanation of Heteroaryldiazoacetates. *J. Org. Chem.* **2001**, *66*, 6595–6603.
- (11) Wang, X.; Ke, J.; Zhu, Y.; Deb, A.; Xu, Y.; Zhang, X. P. Asymmetric Radical Process for General Synthesis of Chiral Heteroaryl Cyclopropanes. *J. Am. Chem. Soc.* **2021**, *143*, 11121–11129.
- (12) 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.
- (13) 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.
- (14) Carminati, D. M.; Decaens, J.; Couve-Bonnaire, S.; Jubault, P.; Fasan, R. Biocatalytic Strategy for the Highly Stereoselective Synthesis of CHF<sub>2</sub>-Containing Trisubstituted Cyclopropanes. *Angew. Chem. Int. Ed.* **2021**, *60*, 7072–7076.
- (15) Nam, D.; Steck, V.; Potenzino, R. J.; Fasan, R. A Diverse Library of Chiral Cyclopropane Scaffolds via Chemoenzymatic Assembly and Diversification of Cyclopropyl Ketones. *J. Am. Chem. Soc.* **2021**, *143*, 2221–2231.
- (16) Siriboe, M. G.; Vargas, D. A.; Fasan, R. Dehaloperoxidase Catalyzed Stereoselective Synthesis of Cyclopropanol Esters. *J. Org. Chem.* **2023**, *88*, 7630–7640.
- (17) 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.
- (18) Wang, Z. J.; Peck, N. E.; Renata, H.; Arnold, F. H. Cytochrome P450-catalyzed insertion of carbeneoids into N–H bonds. *Chem. Sci.* **2014**, *5*, 598–601.
- (19) Chen, K.; Huang, X.; Kan, S. B. J.; Zhang, R. K.; Arnold, F. H. Enzymatic construction of highly strained carbocycles. *Science* **2018**, *360*, 71–75.
- (20) Srivastava, P.; Yang, H.; Ellis-Guardiola, K.; Lewis, J. C. Engineering a dirhodium artificial metalloenzyme for selective olefin cyclopropanation. *Nat. Commun.* **2015**, *6*, 7789.
- (21) Dydio, P.; Key, H. M.; Nazarenko, A.; Rha, J. Y. E.; Seyedkazemi, V.; Clark, D. S.; Hartwig, J. F. An artificial metalloenzyme with the kinetics of native enzymes. *Science* **2016**, *354*, 102–106.
- (22) 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.
- (23) 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.
- (24) Oohora, K.; Meichin, H.; Zhao, L.; Wolf, M. W.; Nakayama, A.; Hasegawa, J.-y.; 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.
- (25) Villarino, L.; Splan, K. E.; Reddem, E.; Alonso-Cotchico, L.; Gutiérrez de Souza, C.; Lledós, A.; Maréchal, J.-D.; Thunnissen, A.-M. W. H.; Roelfes, G. An Artificial Heme Enzyme for Cyclopropanation Reactions. *Angew. Chem., Int. Ed.* **2018**, *57*, 7785–7789.
- (26) Carminati, D. M.; Fasan, R. Stereoselective Cyclopropanation of Electron-Deficient Olefins with a Cofactor Redesigned Carbene Transferase Featuring Radical Reactivity. *ACS Catal.* **2019**, *9*, 9683–9697.
- (27) 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.

(28) Liu, Z.; Calvó-Tusell, C.; Zhou, A. Z.; Chen, K.; Garcia-Borràs, M.; Arnold, F. H. Dual-function enzyme catalysis for enantioselective carbon–nitrogen bond formation. *Nat. Chem.* **2021**, *13*, 1166–1172.

(29) Zhou, A. Z.; Chen, K.; Arnold, F. H. Enzymatic Lactone–Carbene C–H Insertion to Build Contiguous Chiral Centers. *ACS Catal.* **2020**, *10*, 5393–5398.

(30) Fasan, R.; Siriboe, M. G. Engineered Myoglobin Catalysts for Asymmetric Intermolecular Cyclopropanation Reactions. *Bull. Jpn. Soc. Coord. Chem.* **2022**, *80*, 4–13.

(31) 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.

(32) Xie, H.; Li, F.; Xu, Y.; Wang, C.; Xu, Y.; Wu, J.; Li, Z.; Wang, Z.; Wang, L. Vitreoscilla hemoglobin: a natural carbene transfer catalyst for diastereo- and enantioselective synthesis of nitrile-substituted cyclopropanes. *Green Chem.* **2023**, *25*, 6853–6858.

(33) Huang, X.; Garcia-Borràs, M.; Miao, K.; Kan, S. B. J.; Zutshi, A.; Houk, K. N.; Arnold, F. H. A Biocatalytic Platform for Synthesis of Chiral  $\alpha$ -Trifluoromethylated Organoborons. *ACS Cent. Sci.* **2019**, *5*, 270–276.

(34) Nam, D.; Tinoco, A.; Shen, Z.; Adukure, R. D.; Sreenilayam, G.; Khare, S. D.; Fasan, R. Enantioselective Synthesis of  $\alpha$ -Trifluoromethyl Amines via Biocatalytic N–H Bond Insertion with Acceptor-Acceptor Carbene Donors. *J. Am. Chem. Soc.* **2022**, *144*, 2590–2602.

(35) Ren, X.; Chandgude, A. L.; Carminati, D. M.; Shen, Z.; Khare, S. D.; Fasan, R. Highly stereoselective and enantiodivergent synthesis of cyclopropylphosphonates with engineered carbene transferases. *Chem. Sci.* **2022**, *13*, 8550–8556.

(36) Porter, N. J.; Danelius, E.; Gonen, T.; Arnold, F. H. Biocatalytic Carbene Transfer Using Diazirines. *J. Am. Chem. Soc.* **2022**, *144*, 8892–8896.

(37) Regitz, M. New Methods of Preparative Organic Chemistry. Transfer of Diazo Groups. *Angew. Chem. Int. Ed.* **1967**, *6*, 733–749.

(38) Regitz, M.; Arnold, B.; Danion, D.; Schubert, H.; Fusser, G.  $\alpha$ -diazoimine in der heterocyclensynthese. *Bull. Soc. Chim. Belges* **1981**, *90*, 615–631.

(39) Roy, S.; Das, S. K.; Chattopadhyay, B. Cobalt(II)-based Metalloradical Activation of 2-(Diazomethyl)pyridines for Radical Transannulation and Cyclopropanation. *Angew. Chem. Int. Ed.* **2018**, *57*, 2238–2243.

(40) Chuprakov, S.; Hwang, F. W.; Gevorgyan, V. Rh-Catalyzed Transannulation of Pyridotriazoles with Alkynes and Nitriles. *Angew. Chem. Int. Ed.* **2007**, *46*, 4757–4759.

(41) Akter, M.; Rupa, K.; Anbarasan, P. 1,2,3-Triazole and Its Analogues: New Surrogates for Diazo Compounds. *Chem. Rev.* **2022**, *122*, 13108–13205.

(42) Shen, R.; Yin, K.; Yu, M.; Li, X. Progress on Pyridotriazole Chemistry: Synthesis and Transformations. *Asian J. Org. Chem.* **2023**, *12*, No. e202200631.

(43) Das, S. K.; Roy, S.; Chattopadhyay, B. Transition-Metal-Catalyzed Denitrogenative Annulation to Access High-Valued N-Heterocycles. *Angew. Chem. Int. Ed.* **2023**, *62*, No. e202210912.

(44) Shang, Z.-H.; Zhang, Z.-X.; Weng, W.-Z.; Wang, Y.-F.; Cheng, T.-W.; Zhang, Q.-Y.; Song, L.-Q.; Shao, T.-Q.; Liu, K.-X.; Zhu, Y.-P. A Metal- and Azide-free Oxidative Coupling Reaction for the Synthesis of [1,2,3]Triazolo[1,5-a]quinolines and their Application to Construct C–C and C–P Bonds, 2-Cyclopropylquinolines and Imidazo[1,5-a]quinolines. *Adv. Synth. Catal.* **2021**, *363*, 490–496.

(45) Zhang, Z.; Yadagiri, D.; Gevorgyan, V. Light-induced metal-free transformations of unactivated pyridotriazoles. *Chem. Sci.* **2019**, *10*, 8399–8404.

(46) Bagheri, V.; Doyle, M. P.; Taunton, J.; Claxton, E. E. A new and general synthesis of  $\alpha$ -silyl carbonyl compounds by silicon–hydrogen insertion from transition metal-catalyzed reactions of diazo esters and diazo ketones. *J. Org. Chem.* **1988**, *53*, 6158–6160.

(47) Weissenborn, M. J.; Löw, 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.

(48) Khade, R. L.; Fan, W.; Ling, Y.; Yang, L.; Oldfield, E.; Zhang, Y. Iron Porphyrin Carbene as Catalytic Intermediates: Structures, Mössbauer and NMR Spectroscopic Properties, and Bonding. *Angew. Chem. Int. Ed.* **2014**, *53*, 7574–7578.

(49) Khade, R. L.; Zhang, Y. Catalytic and Biocatalytic Iron Porphyrin Carbene Formation: Effects of Binding Mode, Carbene Substituent, Porphyrin Substituent, and Protein Axial Ligand. *J. Am. Chem. Soc.* **2015**, *137*, 7560–7563.

(50) Khade, R. L.; Zhang, Y. C–H Insertions by Iron Porphyrin Carbene: Basic Mechanism and Origin of Substrate Selectivity. *Chem.—Eur. J.* **2017**, *23*, 17654–17658.

(51) Wei, Y.; Tinoco, A.; Steck, V.; Fasan, R.; Zhang, Y. Cyclopropanations via Heme Carbene: Basic Mechanism and Effects of Carbene Substituent, Protein Axial Ligand, and Porphyrin Substitution. *J. Am. Chem. Soc.* **2018**, *140*, 1649–1662.

(52) Vargas, D. A.; Khade, R. L.; Zhang, Y.; Fasan, R. Biocatalytic Strategy for Highly Diastereo- and Enantioselective Synthesis of 2,3-Dihydrobenzofuran-Based Tricyclic Scaffolds. *Angew. Chem., Int. Ed.* **2019**, *58*, 10148–10152.

(53) Khade, R. L.; Chandgude, A. L.; Fasan, R.; Zhang, Y. Mechanistic Investigation of Biocatalytic Heme Carbenoid Si–H Insertions. *ChemCatChem.* **2019**, *11*, 3101–3108.

(54) Blanco, F.; Alkorta, I.; Elguero, J.; Cruz, V.; Abarca, B.; Ballesteros, R. [1,2,3]Triazolo[1,5-a]pyridines. A theoretical (DFT) study of the ring–chain isomerization. *Tetrahedron* **2008**, *64*, 11150–11158.

(55) Abarca, B.; Alkorta, I.; Ballesteros, R.; Blanco, F.; Chadlaoui, M.; Elguero, J.; Mojarrad, F. 3-(2-Pyridyl)-[1,2,3]triazolo[1,5-a]pyridines. An experimental and theoretical (DFT) study of the ring–chain isomerization. *Org. Biomol. Chem.* **2005**, *3*, 3905–3910.

(56) Nam, D.; Bacik, J.-P.; Khade, R. L.; Aguilera, M. C.; Wei, Y.; Villada, J. D.; Neidig, M. L.; Zhang, Y.; Ando, N.; Fasan, R. Mechanistic manifold in a hemoprotein-catalyzed cyclopropanation reaction with diazoketone. *Nat. Commun.* **2023**, *14*, 7985.

(57) Johansson Seechurn, C. C. C.; Kitching, M. O.; Colacot, T. J.; Snieckus, V. Palladium-Catalyzed Cross-Coupling: A Historical Contextual Perspective to the 2010 Nobel Prize. *Angew. Chem. Int. Ed.* **2012**, *51*, 5062–5085.