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Communication

Stereodivergent Synthesis of Pyridyl Cyclopropanes via Enzymatic Activation of Pyridotriazoles

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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.

Ring systems are recurring structural motifs in small-molecule drugs and bioactive natural products.[1−4] Among them, the aromatic heterocycle pyridine represents the second most abundant ring structure found in pharmaceuticals (Figure 1a).[3] 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).[5] 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).

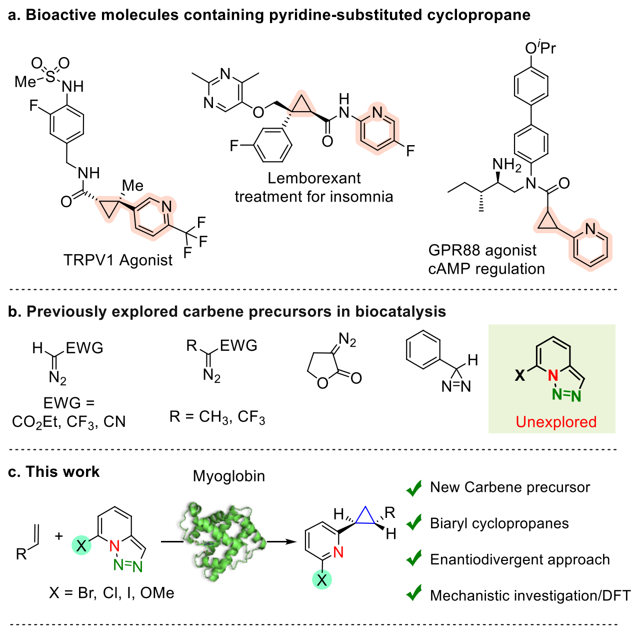

Figure 1. Representative bioactive molecules containing pyridine and cyclopropane rings and biocatalytic cyclopropanation with 1,2,3-pyridotriazole.

The transition-metal catalyzed cyclopropanation of alkenes with diazo compounds has represented a powerful strategy for the construction of enantioenriched functionalized cyclopropanes (Figure 1b),[6−9] including pyridyl cyclopropanes.[10,11] Over the past few years, we and other groups have shown that hemoproteins, such as myoglobin[12−16] and cytochromes P450s,[17−19] as well as artificial enzymes,[20−26] 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.[27] Using these engineered hemoproteins, a growing number of diazo compounds, including acyclic and cyclic diazoesters,[28−30] diazoacetonitrile,[31,32] trifluorodiazoethane,[33,34] diazoketones,[15] and diazophosphonates,[35] have been successfully applied for the asymmetric synthesis of functionalized cyclopropanes. Despite this progress, enzymatic strategies involving carbene precursor

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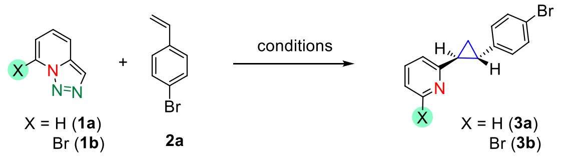

Entry	X	Catalysts	Yield (%)	TON	*trans:cis*	er
1	-H	Co(TPP)	<5	—	—	—
2	-Br	Co(TPP)	22	11	1.5:1	50:50
3	-H	Fe(TPP)Cl	<5	—	—	—
4	-Br	Fe(TPP)Cl	44	22	1.2:1	50:50
5	-H	Fe(Pc)Cl	<5	—	—	—
6	-Br	Fe(Pc)Cl	42	21	1.2:1	50:50
7	-Br	$Rh_2(OAc)_4$	—	—	—	—
8	-Br	CuI	—	—	—	—
9	-H	Hemin	—	—	—	—
10	-Br	Hemin	27	14	15:1	50:50
11	-H	Mb(wt)	—	—	—	—
12	-Br	Mb(wt)	10	50	97:3	47:53
13	-H	Mb(H64V, V68A)	8	40	—	—
14	-Br	Mb(H64V, V68A)	98	490	98:2	99:1
15	-Br	Mb(H64V)	32	160	85:15	94:6
16	-Br	Mb(V68A)	54	270	90:10	55:45
17	-Br	Mb(L29V,F33V,H64V,V68F)	73	365	98:2	2:98

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 μL scale using 20 μM 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.

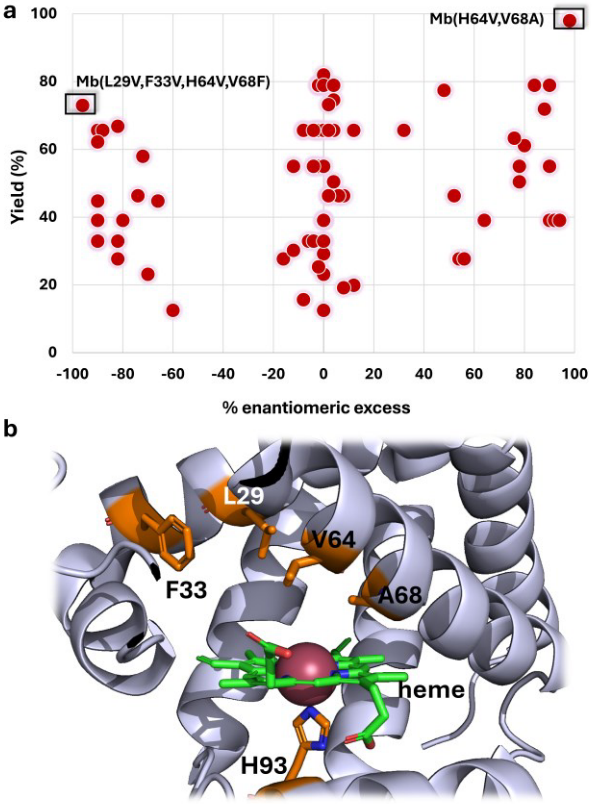

Figure 3. Engineered myoglobin catalysts. (a) Screening results of a diverse panel of engineered myoglobin variants for cyclopropanation of **2a** with **1b**. (b) Active site of Mb(H64V,V68A) (=Mb*) (PDB code 6M8F) displaying the heme cofactor (green) and selected active site residues (orange) as stick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

reagents beyond diazo compounds have remained largely unexplored, with the only recent exception of diazirines.[36]

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,[37,38] thus providing a "masked" substitute for semistabilized heteroaryl diazo compounds.[39] Following pioneering reports on the use of 1,2,3-pyridotriazoles for rhodium-catalyzed Si−H insertion reactions,[40] these reagents have found utility in transannulations, insertions, ylide formation, and rearrangements for the synthesis of heterocycles.[41−43] More recently, 1,2,3-pyridotriazoles were also successfully employed for cyclopropanation reactions via in situ generation in combination with Co-porphyrin catalysts[39,44] or via light induced activation.[45] 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,[12−16] 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 from commercial material (see SI for details). Similarly to hemin and common carbene transfer reagents such as Fe(TPP), Co(TPP), and $Rh_2(OAc)_4$, 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,[46] 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 1*S*,2*S*-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

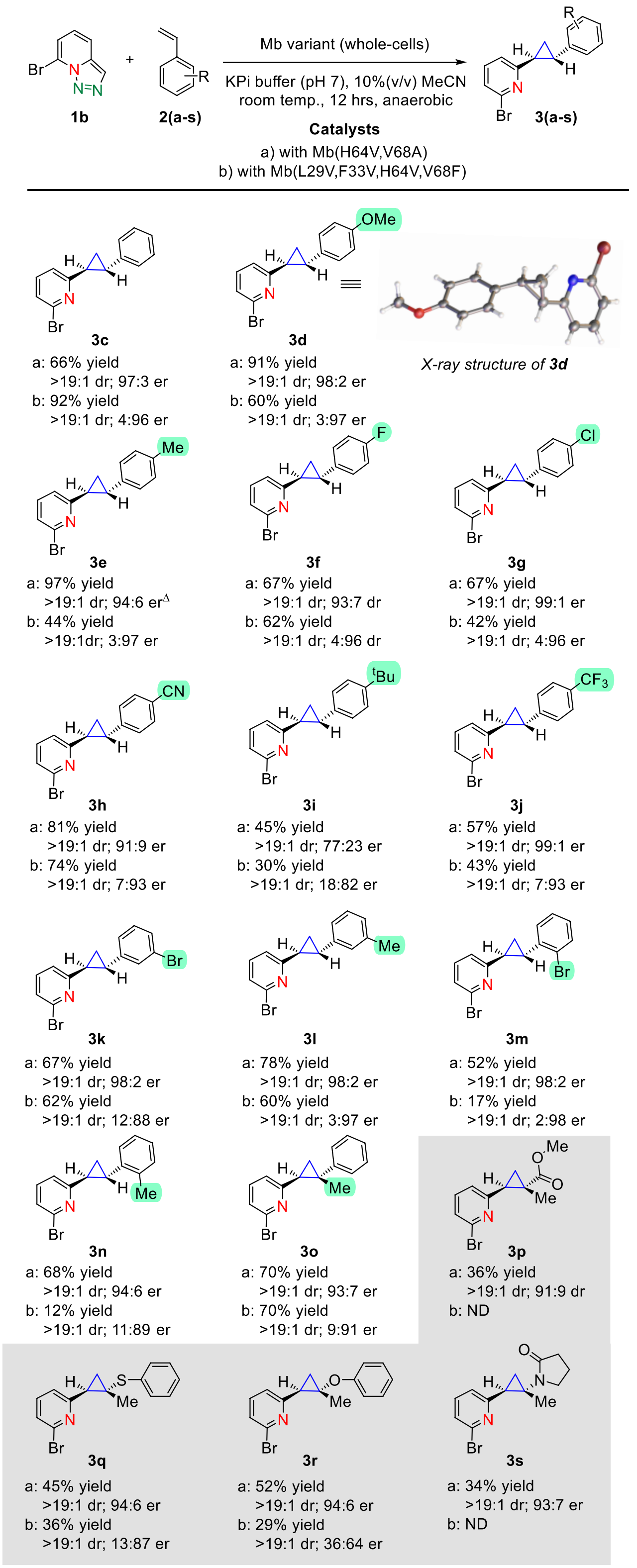

Figure 4. Substrate scope of Mb-catalyzed cyclopropanation with 7-bromo-1,2,3-pyridotriazole. Reaction conditions: Mb-expressing C41(DE3) *E. coli* cells, OD_{600} = 40, 10 mM **1**, 15 mM **2**, in KPi buffer (50 mM, pH 7), r.t., 12 h, anaerobic. ND = Not detected. $^{\Delta}$Enantiomers not fully resolved.

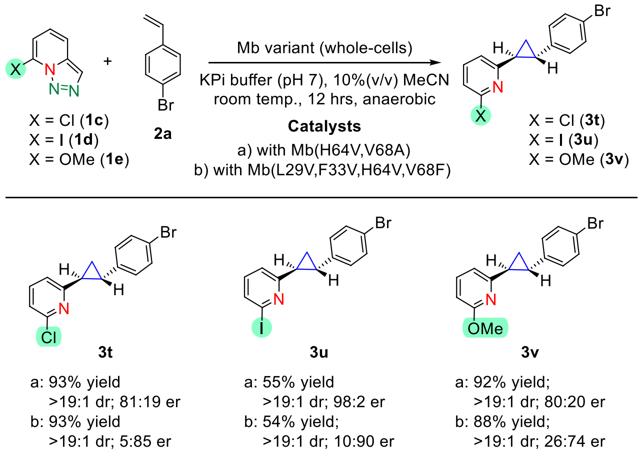

Figure 5. Mb-catalyzed cyclopropanation with different C(7)-substituted-1,2,3-pyridotriazoles. Reaction conditions are the same as those in Figure 4.

analysis of this variant showed that the H64V (Figure 3b) is primarily responsible for enhancing the stereoselectivity of the reaction (6% → 88% ee), whereas the V68A mutation is responsible for increasing activity (10% → 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.[12] 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 enantiocomplementary biocatalyst for this reaction (98:2 dr (trans); 2:98 e.r.; 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,[47] 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 d.r.; 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

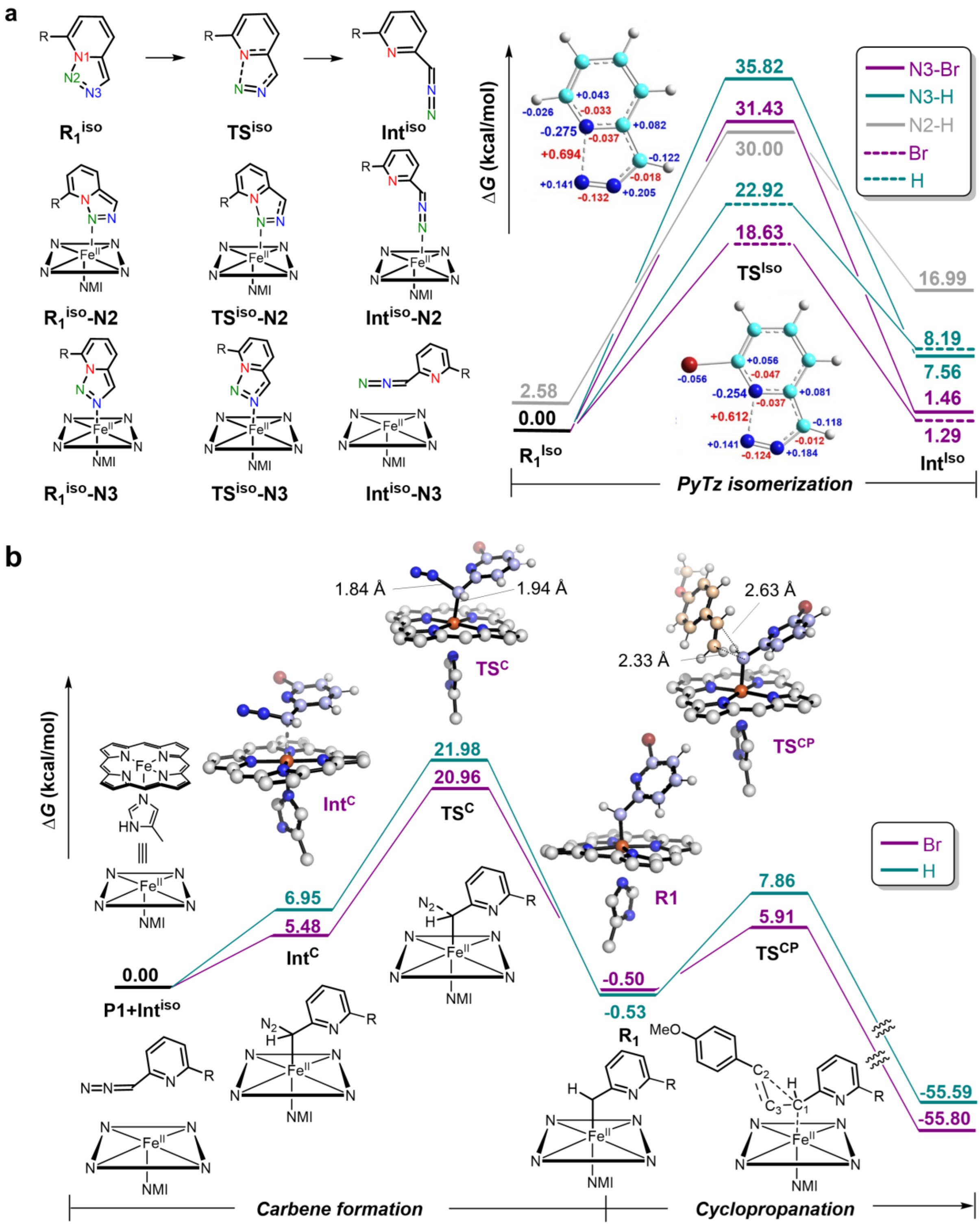

Figure 6. DFT analyses. (a) Noncatalyzed and metal-coordinated PyTz isomerization pathways via N2 and N3 binding. R = H/Br. Key bond length changes (in red, unit: Å) and charge changes (in blue, unit: e) are shown. (B) Schematic free energy diagram for hemoprotein-catalyzed cyclopropanation of 4-methoxy-styrene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

scope and reactivity of this pair of enantiocomplementary biocatalysts.

Next, the reactivity of both Mb* and Mb-(L29V,F33V,H64V,V68F) toward other 7-substituted 1,2,3-pyridotriazoles was analyzed. 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.[48−53] First, we analyzed the triazole isomerization pathway from the closed to the open form for both **1a** (R1$^{\mathrm{Iso}}$-H) and **1b** (R1$^{\mathrm{Iso}}$-Br) in the absence and in the presence of the heme catalyst. For the noncatalyzed 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$^{\mathrm{Iso}}$-H vs TS$^{\mathrm{Iso}}$-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$^{\mathrm{Iso}}$ (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,[54,55] 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 (η^1) via N2 or via N3 and side-on dual coordination (η^2) via N2−N3. For PyTz, both η^1-N2 (called R1$^{\mathrm{Iso}}$-N2-H) and η^1-N3 complexes (called R1$^{\mathrm{Iso}}$-N3-H) are viable (Figure 6a), with the latter being more stable by 2.58 kcal/mol. In contrast, the η^2-(N2, N3) complex was found to be unstable with its optimization leading to the more stable η^1-N3 coordination mode. In contrast to PyTz, only the η^1-N3 complex (R1$^{\mathrm{Iso}}$-N3-Br) was found to be energetically viable for the 7-Br-PyTz interaction with the heme (Figure 6a), whereas the η^1-N2 mode is unstable due to steric repulsion between the Br substituent and the porphyrin ring. After isomerization, the N2 coordination mode (Int$^{\mathrm{Iso}}$−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 η^1-N3 coordination mode over the unproductive η^1-N2 mode, which leads to the end-bound diazo-heme complex previously determined to be unreactive toward formation of the reactive heme carbene.[56]

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[13,51] (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 electrodeficient 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),[57] 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, or by emailing 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.

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Notes

The authors declare no competing financial interest.

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